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A Modular Multi Electrode Array System for Electrogenic Cell Characterisation and Cardiotoxicity Applications

By

Olivia M. Flaherty

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of

Doctor of Philosophy

June 2012

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Certificate of Originality

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Acknowledgements

I would like to thank Professor Andrew West and Professor Paul Conway for the opportunity to complete this research and for their guidance. Thanks must also be extended to Professor Chris Denning, Dr David Anderson, Miss Divya Rajamohan and Dr David Hutt for their invaluable expertise contributions, and to Dr Xiaoyun Cui for her constant professionalism, and for her friendship.

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This thanks is most importantly extend to my supporters outside of “the bubble”, to Leah, Vincent, Katie, Sophie, Lisa and Sharon.

This thesis is dedicated to the memory of Gloria Ademokun.

"To accomplish something great, we must not only act, but we must also dream."
Anatole France (1844-1924).

Abstract

Multi electrode array (MEA) systems have evolved from custom-made experimental tools, exploited for neural research, into commercially available systems that are used throughout non-invasive electrophysiological study. MEA systems are used in conjunction with cells and tissues from a number of differing organisms (e.g. mice, monkeys, chickens, plants). The development of MEA systems has been incremental over the past 30 years due to constantly changing specific bioscientific requirements in research. As the application of MEA systems continues to diversify contemporary commercial systems are requiring increased levels of sophistication and greater throughput capabilities.

This research has identified current requirements of MEA systems. Generic system requirements, and application specific requirements, have been identified and prioritised. Product design specifications were drafted and a novel MEA biochip concept was designed. Manufacturing approach experimentation occurred until a suitable prototype of the biochip concept was produced. This first generation of prototype was tested by MEA system users at the University of Nottingham. User feedback and testing outcomes resulted in development of a second design concept that was also manufactured and tested.

The research presented in this thesis identifies areas within the field of MEA technology application where design changes can bring significant benefits to MEA system users, especially to stem cell-derived cardiomyocyte MEA system users. This thesis presents novel MEA biochip design concepts designed to address specific needs of MEA system users. The prototypes that were manufactured and tested have demonstrated promise within this application domain. Further work is required to achieve robust signal acquisition from the designs presented. The results of prototype testing have also been shared internationally at conference and through journal publications.

Keywords: Multi electrode array, Stem cell-derived cardiomyocyte, Biosensor fabrication.

Publications arising from this work

Flaherty, O.M., Cui, X., Rajamohan, D., Anderson, D., Hutt, D., Denning, C., Conway, P.P. and West, A.A. (2011) **Fabrication of a Novel Multi-Electrode Array (MEA) Biochip Using Polyester Insulated Electrodes with Microwell Features for Cardiomyocyte Analysis.** Proceedings of the 18th European Microelectronics Packaging Conference, 12-15 Sept 2011.

Flaherty, O.M., Cui, X., Rajamohan, D., Hutt, D., Denning, C., Conway, P.P. and West, A.A. (2011) **A Multi-Electrode Array (MEA Biochip with Excimer Laser-produced Micro-well Features.** *The Journal of the Institute of Circuit Technology, 5(1),* 5-9.

Flaherty, O.M., Cui, X., Rajamohan, D., Hutt, D., Denning, C., Conway, P.P. and West, A.A. (2012) **A multi-electrode array (MEA) biochip with excimer laser produced microwell features.** *Circuit World: The International Journal of Circuit Technology, 38(1),* 30-37.

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Abbreviations

A – Activity/Activities

ACSF – Artificial Cerebrospinal Fluid

ADP – Adenosine Diphosphate

AP – Action Potential

ATP – Adenosine Triphosphate

BPR – Business Process Re-engineering

CIMOSA – Computer Integrate Manufacturing Open System Architecture

COI – Channel of Interest

CVD – Chemical Vapour Deposition

D – Domain

DIV – Days in Vitro

DMEM - Dulbecco's Modified Eagle's Medium

DP – Domain Process

DTI – Department of Trade and Industry

E-beam – Electron Beam

ECM – Extra Cellular Matrix

EM – Enterprise Model/Modelling

FP – Field Potential

G1 – Prototype One

G2 – Prototype Two

GUI – Graphical User Interface

hESC – human Embryonic Stem Cell

HoQ – House of Quality

IC – Integrated Circuit

LBM – Laser Beam Machining

LCD – Liquid Crystal Display

LPCVD – Low Pressure Chemical Vapour Deposition

LU – Loughborough University

MEA – Multi (or Micro) Electrode Array

MEMS – Microelectromechanical System(s)

OTFT – Organic Thin Film Transistors (OTFT)

P – Process/Processes

PCB – Printed Circuit Board

PDS – Product Design Specification

PECVD – Plasma Enhanced Chemical Vapour Deposition

PGA – Pin Grid Array

PLED – Polymer Light Emitting Diodes

PNS – Peripheral Nervous System

QFD – Quality Function Deployment

QMD – Quality Management System

R – Radiator

RF – Radio Frequency

S – Spiral

SC – Stem Cell

SC-CM – Stem cell- derived Cardiomyocyte

SCSI – Small Computer Standard Interface

SOP – Standard Operating Procedure

UoN – University of Nottingham

V1 – Version One

V2 – Version Two

WTSI – Wellcome Trust's Sanger Institute

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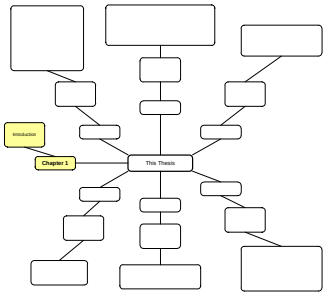


Chapter One

Introduction

This thesis presents research that was conducted to understand contemporary Multi Electrode Array (MEA) system application and to define requirements that would lead to a novel system design. This research also presents the work conducted in the design, manufacture and testing of resulting novel prototype devices.

1.1 Motivation

This research is focused on the development of a novel Multi Electrode Array (MEA) System. MEA systems are tools that allow bioscientists to study electrogenic tissues *in vitro* by detecting and capturing electrical activity. Understanding this activity aids characterisation of cells and understanding of the functioning of that particular tissue.

Technical development of the MEA system could: (1) enhance usability, (2) improve signal acquisition, (3) increase the volume of meaningful data that is extracted per test, and (4) reduce

time spent setting-up, using and maintaining systems. This thesis presents research undertaken during the definition of design-and-build objectives that were used to specify and prototype an MEA system-based solution that holds potential to be superior in functionality to current commercially available alternatives.

1.2 Current MEA system employment

Early research focused on protocols documented in literature that described employment of MEA systems. Author MEA system users were from institutions all over the world allowing identification of the following generic global similarities. Each culture used in conjunction with an MEA is prepared, maintained, used and disposed of manually by hand. The cell source(s) of interest must be set-up in an MEA biochip in such a manner that is amenable to MEA systems. The user must maintain the cells, set-up the MEA system, run the experiments (often timely), dispose of the cells, clean the system, and perform an appropriate analysis of the data.

Currently trends across MEA system-based research utilise high data sampling rates (>10 kHz), resulting in generation of vast amounts of data, very little of which is used in analysis. Current methods of MEA system employment, and the consequent post experiment analysis, is highly skilled and user intensive.

1.3 The research

The fundamental concern of this research was to investigate the feasibility of an MEA system that was less intensive to use. It was intended that a reduction in the overall system and experiment complexity could be brought about through this work, resulting in a more intuitive and user-friendly MEA system.

1.4 This thesis

This compilation of research demonstrates derivation of specific contemporary user requirements that have resulted in the development of product design specifications that have led to novel MEA biochip designs. Logical, previously validated research approaches have been used to create opportunities to gather information pertaining specifically to this research domain that is relevant and correct.

The hypothesis (**theory)** driving forward this research is laid out in the initial research proposal (Appendix A). The author of this thesis then derived information (**questioned**) relating to current MEA systems and areas of research in which the systems are employed (Chapter 2). Once a basic understanding of the domain and its current needs were ascertained (Chapters 3 and 4) work was conducted to design and manufacture solution devices (Chapter 5) that better address the identified needs. **Observations** of users before and during testing were made (Chapters 4 and 6). Relevant **data** were generated and appropriate **analyses** performed (Chapter 6). The outcome of this process has resulted in the **proposal** of further work that needs to be pursued in this research and design domain (Chapter 7). This research cycle is depicted in Figure 1.1.

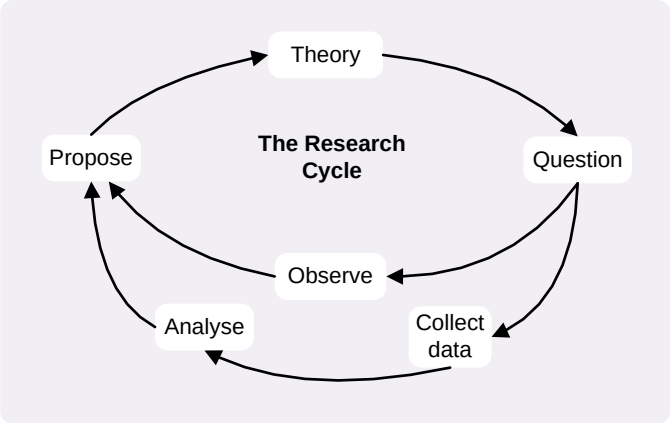


Figure 1.1: The Research Cycle.
[Adapted from How to Research by Blaxter et al, 2000.]

Information relating to the manufacturing approaches that were implemented during prototype development is reported into this thesis. Initial testing of the prototypes manufactured is presented. Suggestions of future avenues for this research to pursue that are of high relevance are included in the conclusions of this thesis.

The method of research presentation used in this thesis is presented in Figure 1.2 and the content of each chapter is summarised in Figure 1.3.

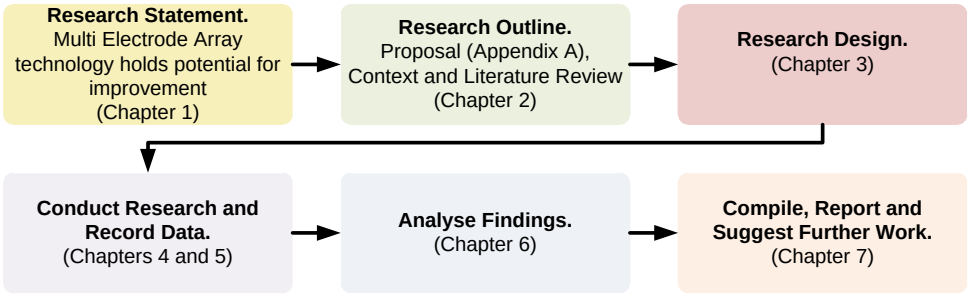


Figure 1.2: Structure of the research presented in this thesis

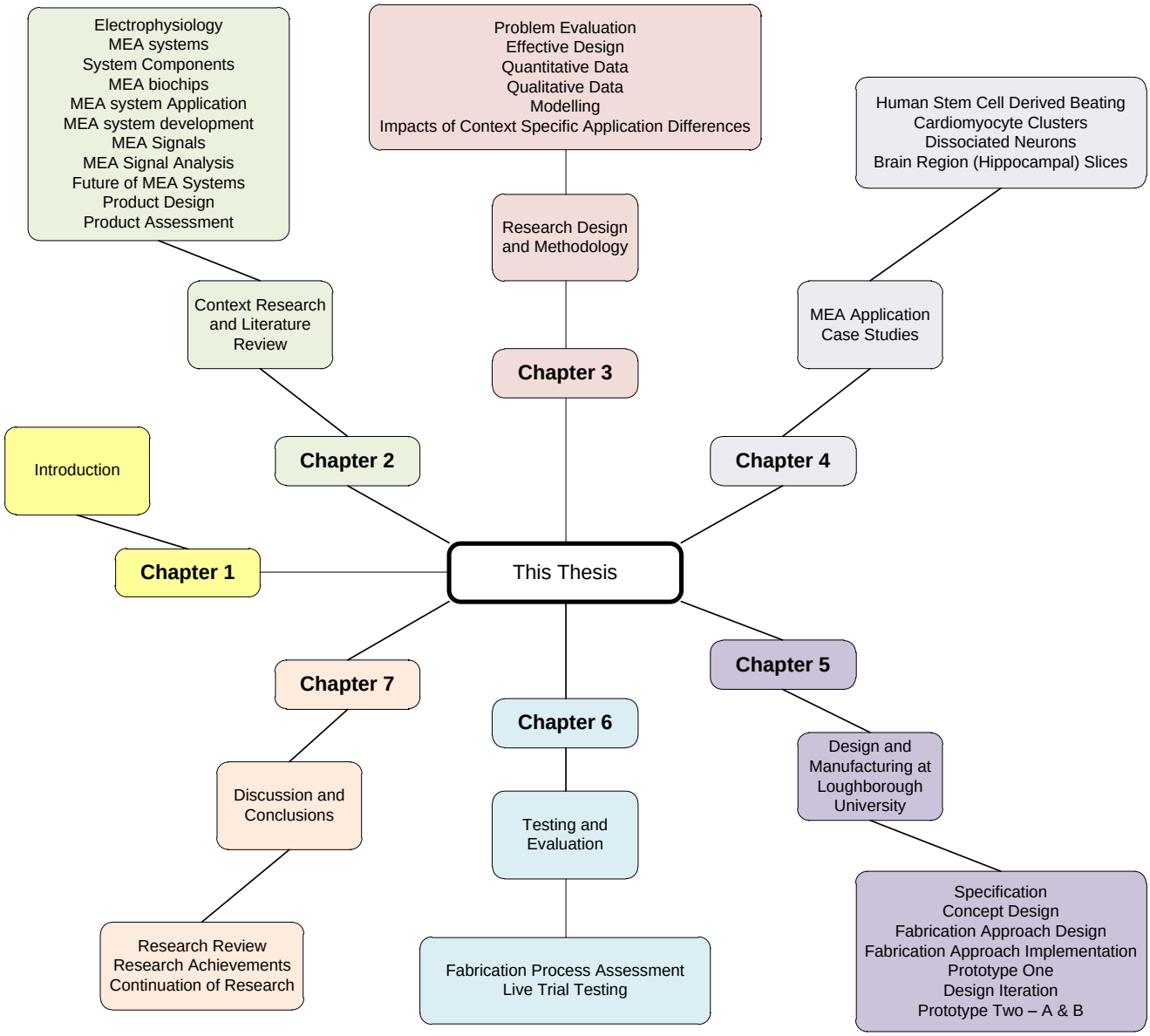


Figure 1.3: A summary of the chapter content of this thesis.

1.5 Research Question Answers

This research has considered the following questions:

1. What is the research problem?

Multi electrode array systems have developed into a confusing array of different configurations and system types, each matched to the subtly differing requirements of varying electrogenic tissue and cell preparations. Commercially available systems require further development before truly catering for the diverse plethora of contemporary user needs.

2. What is the motivation for completing this research?

New knowledge continues to be derived using these MEA systems that relates to cellular functioning and genetic variation. However, MEA system employment is both slow and costly. Systems better suited to contemporary research needs that are scalable would contribute significantly to accelerating bioscientific understanding and discovery.

3. What is the current situation in this research domain?

MEA system development has increased in pace over the past fifteen years. A global industry exists that is presently led by seven leading companies. In addition to this industry much technical advancement is being made by universities and academic institutions using MEA systems for various research purposes around the world.

Facets that limit MEA system application that can be improved upon to better suit the increasingly specific requirements of MEA-based research are: 1) MEA systems are still not suited to humidified, incubated environments, 2) MEA systems still take up a relatively large amount of space, and 3) throughputs are low and so the amount of valuable data extracted collected is limited following significant time and labour outlays.

4. How does this thesis address identified research objectives?

The content of this thesis identifies and addresses objectives by including:

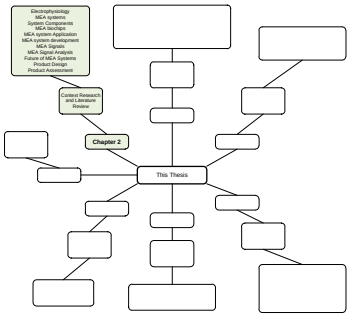
1) a thorough review of the subject area (Chapter 2),

2) a description and justification of the processes employed in eliciting user requirements (Chapter 3),

3) an investigation of MEA systems in use (Chapter 4),

4) the generation of solution concepts and investigations into manufacturing possibilities for those concepts (Chapter 5), and,

5) the testing and evaluation of resultant prototypes (Chapter 6). The testing outcomes are also discussed, conclusions draw and further work is suggested (Chapter 7).



Chapter 2

Context Research

This chapter contains relevant research relating to MEA systems and their applications. Key topics are:

- the physiology of a cell,
- an introduction to electrophysiology,
- multi electrode array systems and their components,
- how MEA systems are used across bioscientific disciplines,
- a brief summary of MEA system history, and of how systems have and are likely to continue to develop,
- the different signal types detected from neural and cardiac cell types and features of interest within those signals,
- how recordings made using an MEA are analysed and used by the bioscientist,
- a prediction of the direction of future MEA system development.

Product design and innovation management techniques are also included as this research has been of a design-and-build nature.

The integration of engineering and biology is more profoundly needed and *in vitro* Micro or Multi Electrode Array (MEA) systems are just one example of a technological tool combining living biological samples with sophisticated engineering (Wang et al, 2009a). Further development of MEA

systems is required to optimise this tool for its numerous applications. The structure of this chapter is demonstrated in Figure 2.1.

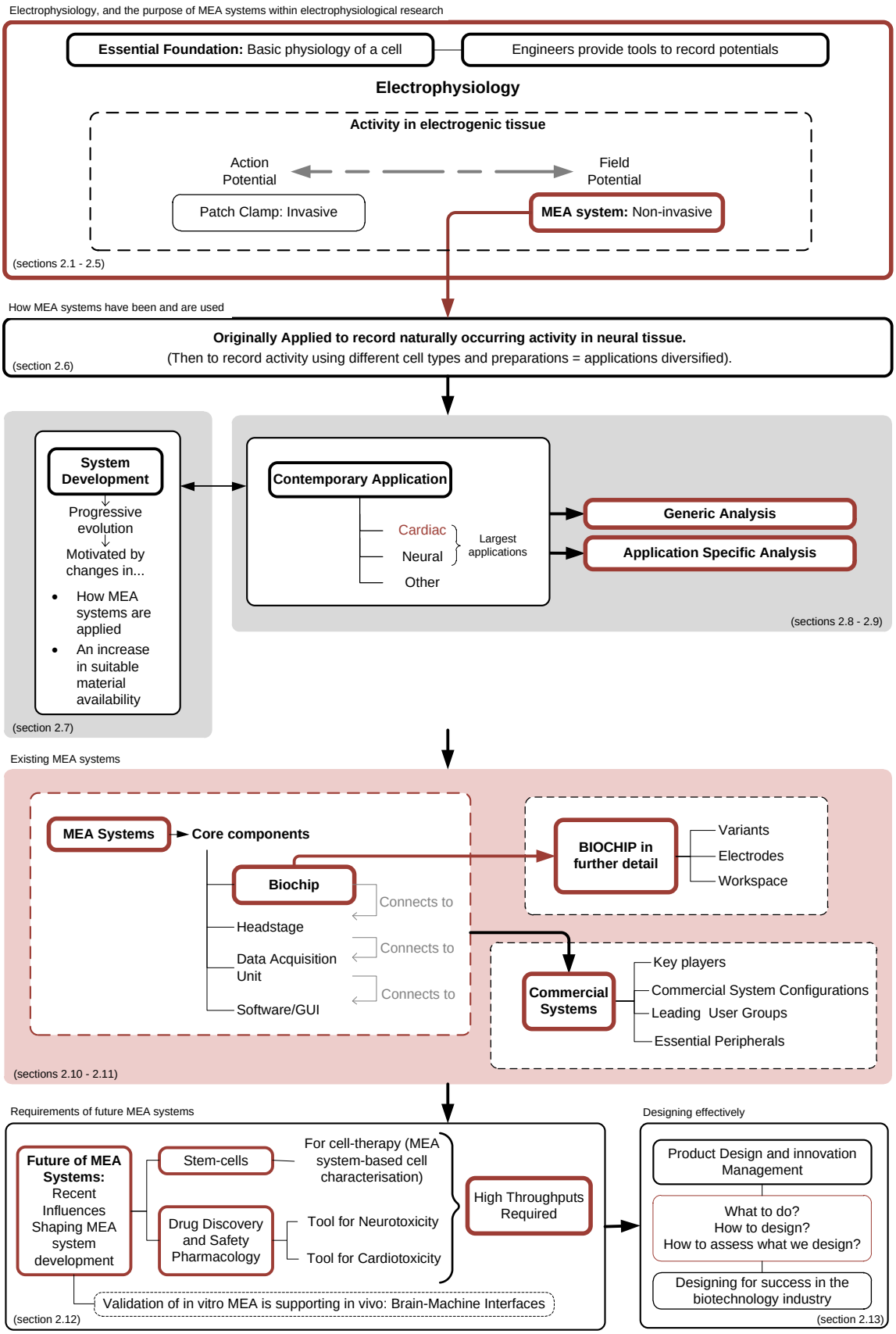


Figure 2.1: Chapter 2 Schematic

An MEA system is used in bioscientific settings as a sensor to detect, record and quantify electrochemical fluctuations as they occur through either a single electrogenic cell (grown over a microscopic electrode) or through samples of electrogenic tissue (grown or placed over several microscopic electrodes). An understanding of the fundamentals of cell physiology and the electrophysiological activity of single and multiple cells is demonstrated in this chapter (sections 2.1 – 2.4).

Two avenues exist for bioscientists wishing to assess electrophysiological activity of cells: clamping techniques (in the contemporary form, patch clamp) or MEA system techniques. Clamping techniques are described as invasive because they damage and ultimately kill the cell of interest. Non-invasive alternatives use an MEA system that does not require destruction of the cells during use. The fundamental difference between the two techniques is that clamping records the actual action potential (AP) as it occurs through an individual cell, and an MEA system records the field potentials, which are ion current fluctuations through the matter around the cells and close to the detecting electrode.

A brief history relating to the evolution of MEA systems and how they have been developed over the last decade is included to support identification of current trends in, and possible future avenues of, component and system development (section 2.5 - 2.6). Latest developments are described in terms of what motivated the development (e.g. the user), how the development was realised (e.g. how it is manufactured), and what materials were used (e.g. for the microelectrode tip, tracking, insulation).

Understanding of the activity that occurs on an MEA biochip at the complex cell-electrode interface has been pursued (section 2.6.6). A number of theoretical models of the interactions that occur at this interface have been collated for comparison and reference. This aspect was investigated to consider how a signal is physically detected and transmitted through existing MEA systems.

The differing ways in which MEA systems are presently used has been presented and generic features of MEA analysis routines are described. Neural and cardiac applications are summarised and the future of MEA system application for higher throughputs via semi-automated or automated systems in these domains, as speculated by leading academic and industrial authorities, is considered (sections 2.7 - 2.8).

MEA systems are made up of a chain of components that are integrated to provide appropriate sensitivity to detect microvolt signals that occur in electrogenic tissues. Manufacturers of commercial MEA systems maintain close relationships with academic development to keep up-to-date with the various ways in which systems are being applied.

The research outlined in this thesis has been focused on designing and manufacturing a novel MEA biochip. Therefore, current state-of-the-art MEA biochip variants have been identified and are presented (section 2.10). The microelectrodes and workspace layouts (key distinguishers for this component) show large degrees of variation across collated MEA biochip models.

Understanding ascertained by completing this review has been integrated during the requirements definition and design of prototypes. Methods were considered from a product design point of view when assessing the success of prototypes. Innovation management and design for the biotechnology domain have been explored to support effective design and future product compliance within this field (section 2.12).

Answers to the following research questions have been pursued and relevant information is contained in this chapter:

1. **In what context(s) are multi electrode array systems used, what are the core components and how do they differ between systems? What is the current state-of-the-art?**

1. **How are MEA systems applied in research, how has that application changed since their introduction and are there any trends identifiable in development?**

2. **What are the key factors that will influence and limit design possibilities and where are the biggest challenges associated with this work?**

3. **How do applications using different cellular preparations differ and how are the signals that are recorded used?**

4. **What is expected of future MEA systems?**

5. **How can product innovation be managed throughout this research and how can developers design and assess prototypes appropriately?**

2.1 The Cell

Every living organism is comprised of either a cell or a collection of cells. A cell can be referred to as the “basic unit of life” (Allen and Cowling, 2011). Each cell is a separate entity enclosed by a surface membrane. Cells interact with the environment around them to extract nutrients and energy for growth and maintenance, and most cells are capable of replicating themselves. It has been suggested that a human is composed of about 100 trillion cells (Allen and Cowling, 2011).

2.1.1 Fundamental Cell Physiology

There are numerous types of cells within multi-cellular organisms, each of which has its own “blueprint”. A generic representation of the microscopic internal functional entities inside a cell that are responsible for metabolism (organelles) is demonstrated below (Figure 2.2). Organelles are built from proteins and each one has a particular function or functions. Collectively organelles (i.e. ribosomes, mitochondria, Golgi apparatus, endoplasmic reticulum, centriole), in collaboration with the surrounding cytoplasm, are responsible for the cell’s metabolism (Starr et al, 2010).

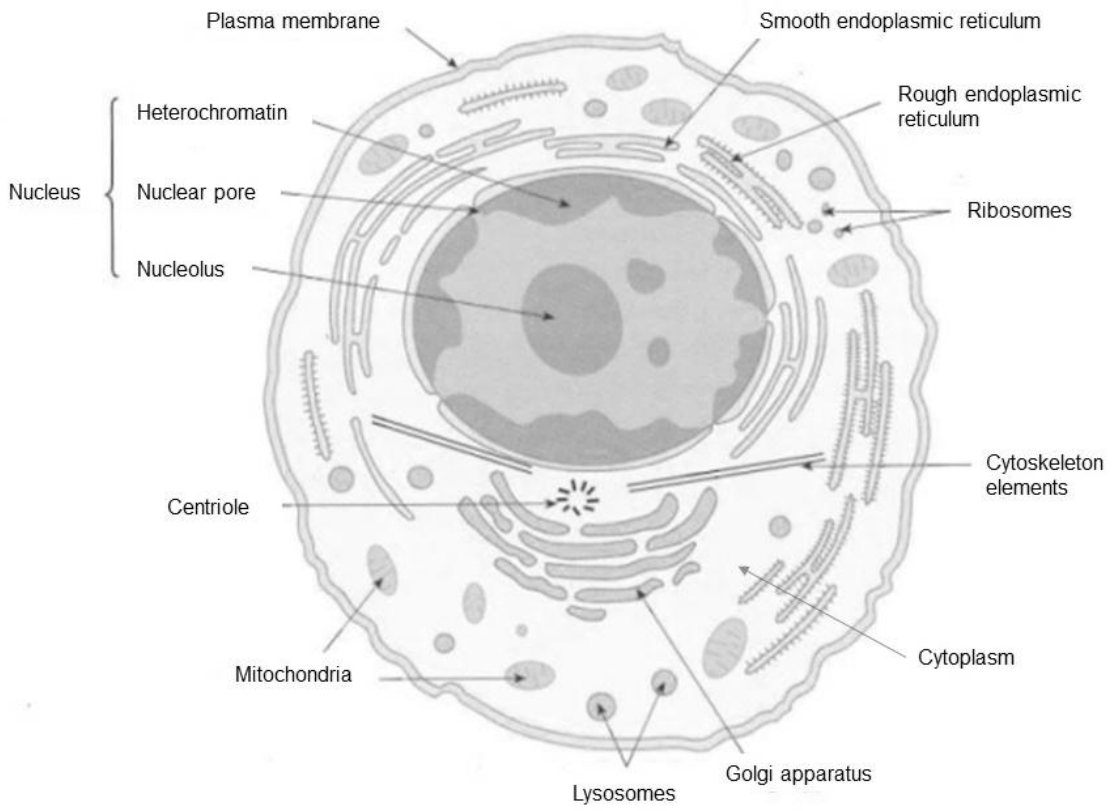


Figure 2.2: Structure of a mammalian cell.
[Adapted from Pocock and Richards, 1999.]

Organelles (see Appendix B) exist suspended in a jelly-like fluid called cytoplasm that provides water, sugars, ions and proteins (Figure 2.2). The cytoplasm and organelles are all contained within a phospholipid membrane. All cells are enclosed by this membrane that consists of two layers of phospholipids (fat molecules). Membranes in animal cells have proteins embedded that facilitate the movement of molecules from the cell's surrounding environment into the cell. Exchanges between a cell and its surroundings, the extracellular matrix, are selectively controlled by these proteins. It is the ion fluctuations into and out of cells through the cell membrane that are detected by MEA systems in the form of field potentials (see section 2.4.2.2).

2.2 The Neuron

The nervous system in humans is a complex structure consisting of billions of interconnecting nerve cells, neurons. Numerous variants of neuron make up the nervous system and they can be classified according to their structure (e.g. number of dendrites, size, and degree of myelination) and function (e.g. direction of signal, effect on other neurons firing rates, discharge patterns, neurotransmitters produced). MEA systems were originally developed for neural research applications.

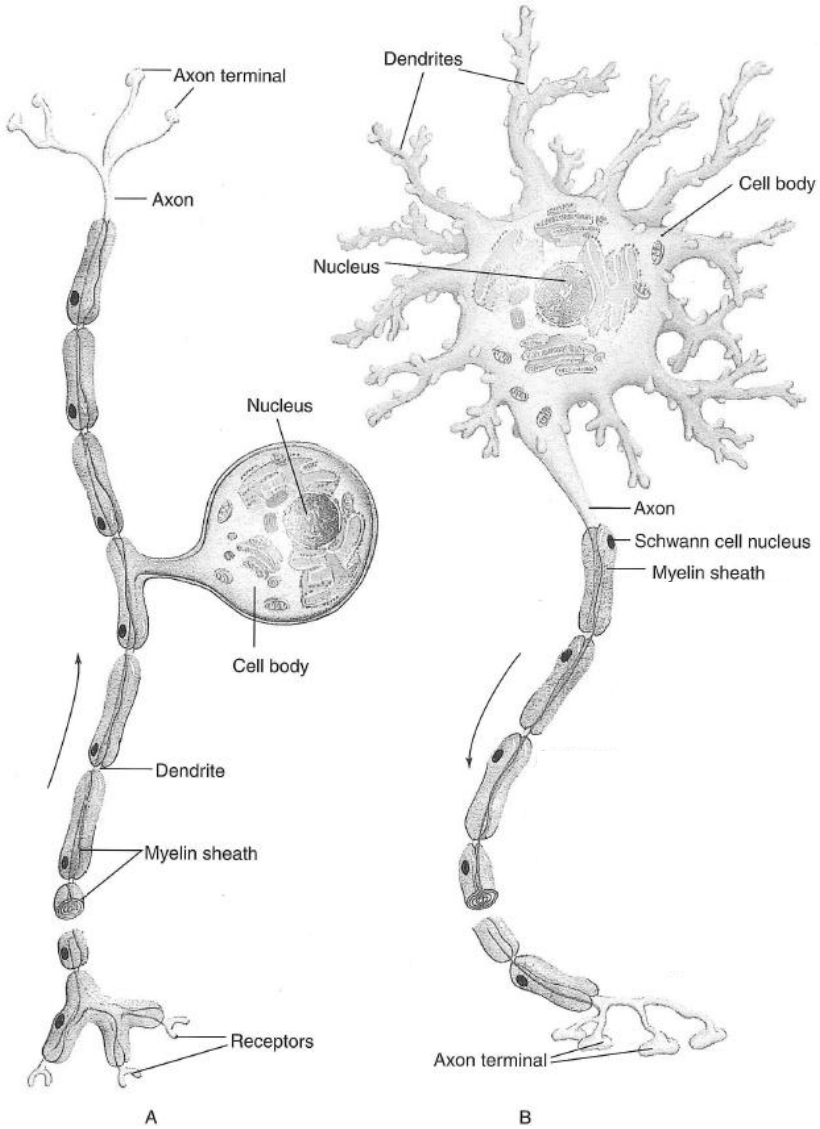


Figure 2.3: Neuron Structure.
(A) A typical sensory neuron. (B) A typical motor neuron.
[Adapted from Scanlon and Sander, 1997.]

The primary function of a neuron (Figure 2.3) is information transfer. Two descriptions are used to describe the types of signalling used for this information transfer; intracellular signalling, that is from one part of a neuron to another part, and intercellular, from one neuron to another neuron (Levitan

and Kaczmarek, 2002). The signal (electrical impulse) that is transmitted through and between neurons is called an Action Potential, AP (see section 2.4.1). Neurons consist of a soma (cell body), an axon, several dendrites and synapses.

The soma contains the nucleus and therefore the DNA, and typically a high concentration of the other organelles necessary for metabolism (e.g. mitochondria, endomembrane system organelles). The axon is identifiable as the longest and thinnest projection coming from the soma. The movement of charged ions through membrane channels along the length of the axon generates an electrical impulse, the AP. Axons in the human brain can be less than 1mm in length, whereas others extending from the spinal cord to muscles in limbs can measure over 1m in length (Roberts, 2010).

The axons of most neurons, especially those found in the peripheral nervous system (PNS) are wrapped in an insulating layer called a myelin sheath. Schwann cells make up this sheath encapsulating the axon in short sections, serving to insulate the signal and also resulting in an increased speed of transmission of that impulse. Small gaps between the Schwann cells, called Nodes of Ranvier, are responsible for the increased conduction velocities along neurons. The nodes form specialised areas where ion channels and specific proteins are concentrated (Pavelka and Roth, 2005).

Dendrites are small protrusions most commonly originating from the soma, but in some cases from elsewhere on the cell, that are typically thicker and shorter than the axon. Dendrites are often branched resulting in what is called the dendritic tree. The purpose of dendrites is to receive and integrate information from other nerve cells. The distal (away from the body) ends of dendrites are called receptors. Where neurons transmit impulses on to other neurons, no physical contact actually occurs. Synapses are minute spaces present between one neuron and another. Chemicals that are collectively classified as neurotransmitters, of which there are currently >100 identified, are released from a site referred to as the synaptic knob, present at the axon terminal, that diffuse rapidly across the minute gap to receptor sites on the dendrites of another neuron (Clark, Boutros and Mendez, 2010). Stimulation of the receptor site by the neurotransmitter generates the electrical impulse that travels along the length of the neuron via the axon.

The brain has ~10^{11} neural cells. Each cell can have 10000 connections. Neurons communicate information around an organism's body via APs through a network of nerves. Some basic nervous system dimensions and numbers are incorporated in Table 2.1.

Table 2.1: Typical values for neurons in the human nervous system.

Node of Ranvier	4µm
Axon diameter	1-20µm
Soma diameter	5-20µm
Nerve diameter	Several mm
Brain neural cells	10^{11}
Connections per brain cell	10^{4}
Membrane resting potentials:	
Resting potential of Na^+	+55mV
Resting potential of K^+	-102mV
Resting potential of Cl^-	-76mV

[Adapted from Rutten, 2002.]

2.3 The Cardiomyocyte

The mammalian heart beats automatically at regular intervals. This rhythmic contractile activity is due to the electrical activity of the myocardium driven by the generation of action potentials in individual cardiomyocytes which propagates activity through the myocardium (Niwa and Nerbonne, 2010). Cardiomyocytes are specialised muscle cells that connect forming myocardial tissue via intercalated disks (Figure 2.4). MEA systems are suited to recording from cardiac cell types (Reppel et al, 2004). The specific cell of interest within the cardiac tissue is the cardiomyocyte. Cardiomyocytes are the sole generators of contractile force within a mammalian heart's myocardium (Lee and Terracciano, 2010). They have the unique ability to contract spontaneously without the need for nervous excitation.This research focuses on this cell type from Chapter 5 onwards.

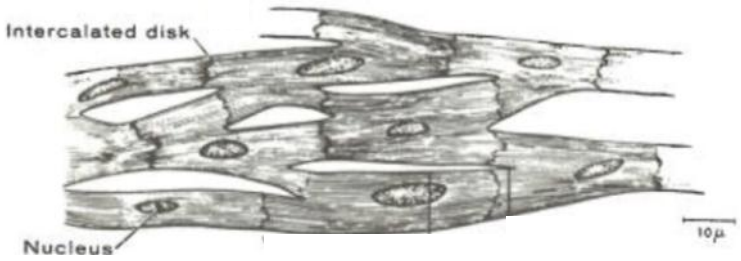


Figure 2.4: A diagram of the light microscope appearance of cardiomyocytes as myocardial tissue.
[Adapted from Stapleton, 1983.]

2.3.1 Cardiomyocyte contraction

The contraction of cardiomyocytes is also associated with an action potential. These action potentials reflect the coordinated functioning of voltage-gated ion channels carrying depolarising (Na^+ and Ca^+) and repolarising (K^+) ions. Numbered phases (0-4) are used to characterise a cardiomyocyte AP when recorded via a patch clamp (Figure 2.5).

Phase 4 is the resting state of the cell. When the cell membrane is electrically stimulated, permeability of the membrane alters. At Phase 0 Na^+ channels open and sodium ions flow into the cardiomyocyte. The transverse potential of the cell membrane reduces. Membrane potential rapidly reaches 0mV and transiently becomes positive. This phase is depolarisation.

Phase 1 is start of repolarisation. Na^+ channels have been inactivated and a transient current moves the membrane potential back towards 0mV.

Phase 2 is mediated by slow Ca^{2+} channels that first began to open when the potential was approximately -40mV, but that remain open much longer than the Na^{+} channels. With low permeability to K^{+} the potential is maintained at around 0mV. This period is known as the plateau. Ca^{2+} channels begin to deactivate gradually and K^{+} channels open, beginning phase 3.

Phase 3 occurs towards approximately -10mV when the K^{+} permeability increases. At about -60mV the K^{+} channels are most activated, accelerating a return to the resting potential of approximately -90mV.

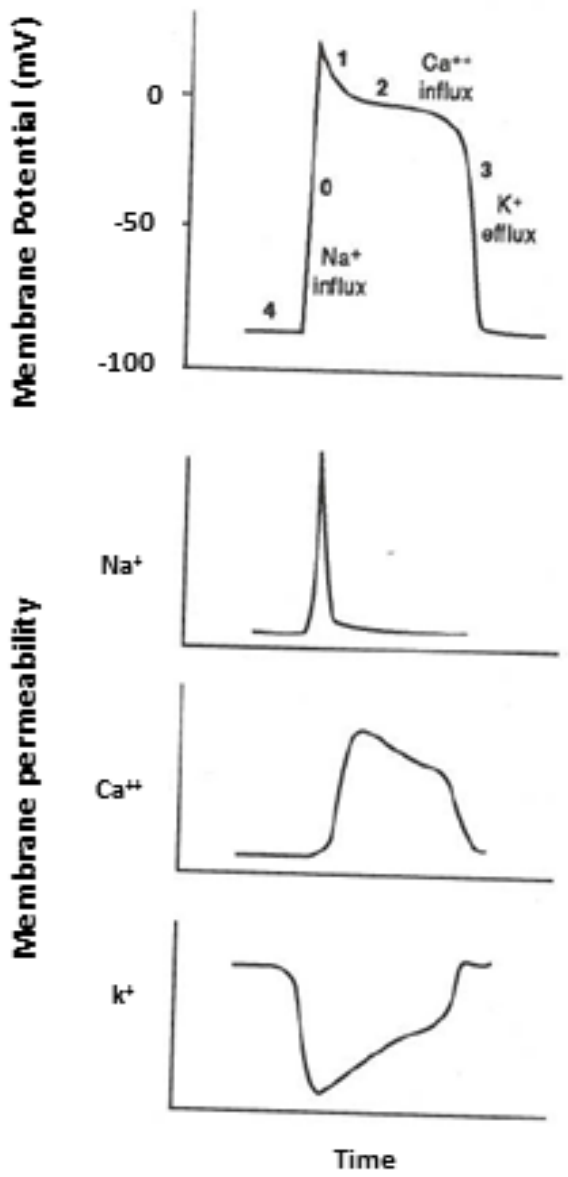


Figure 2.5: An action potential (patch clamp) and the relative ion fluctuations for Na^{+}, Ca^{2+} and K^{+}. The resting potential of the cell is represented by phase 4 of the action potential.

[Adapted from Lilly, 1993.]

The action potential characteristics are also related to the position of the cell in the heart (Figure 2.6).

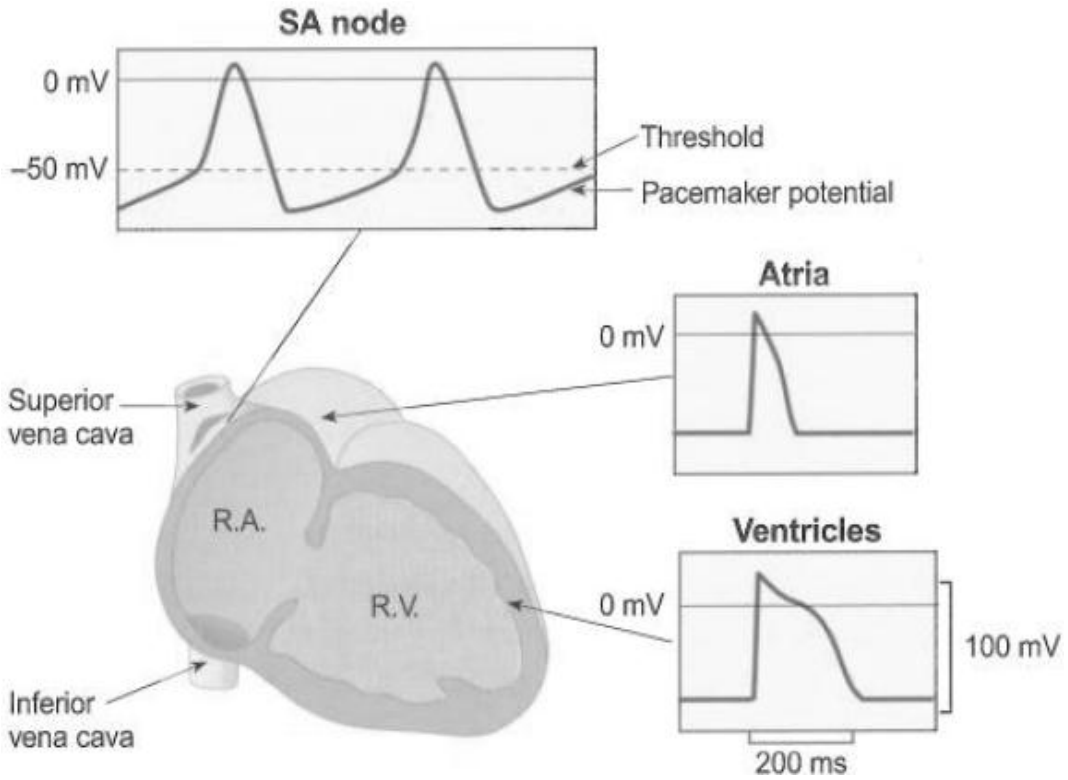


Figure 2.6: The characteristics of action potentials through cardiomyocytes and the corresponding region of the heart.

[Adapted from Pocock and Richards, 1999.]

2.3.2 Stem-cells into cardiomyocytes

Differentiation of stem cells into cardiac cell types has attracted significant attention in recent years and is an especially strong area of interest in the regenerative medicine domain (Braam et al, 2009).

A major design challenge that accompanies interest in using stem cell-derived cardiomyocytes in MEA culture for drug candidate testing is largely associated with the immaturity of stem cell differentiation techniques. Generally, differentiation of pluripotent stem cells into beating embryoid body's results in mixtures of ventricular-like, atrial-like and pacemaker-like cardiomyocyte cells (Gupta et al, 2010). This variation in cardiomyocyte phenotype has been identified and characterised in patch clamp studies of APs (He et al, 2003; Mummery et al, 2003). Specific differentiation protocols affect the ratios of cell phenotypes found in EB cultures (Braam et al, 2009). MEA and patch clamp tools are currently being applied in parallel, to understand and characterise the cells produced.

2.4 Electrophysiology

"Electrophysiology is the branch of physiology that deals with the electrical phenomena associated with physiological processes, especially in nerve and muscle; also the electrophysiological characteristics of a process, tissue, or disorder" ("Electrophysiology," Oxford English Dictionary). The study of electrophysiology involves functional and physiological responses of electrogenic (electrically active) cells, tissues and organs across an assortment of organisms from molluscs (i.e. snails (Merz and Fromherz, 2002)) to mammals (i.e. rats (Suzuki et al, 2004a) and humans (Shibasaki, 2008)). MEA systems exist specifically as a tool to support electrophysiological investigation.

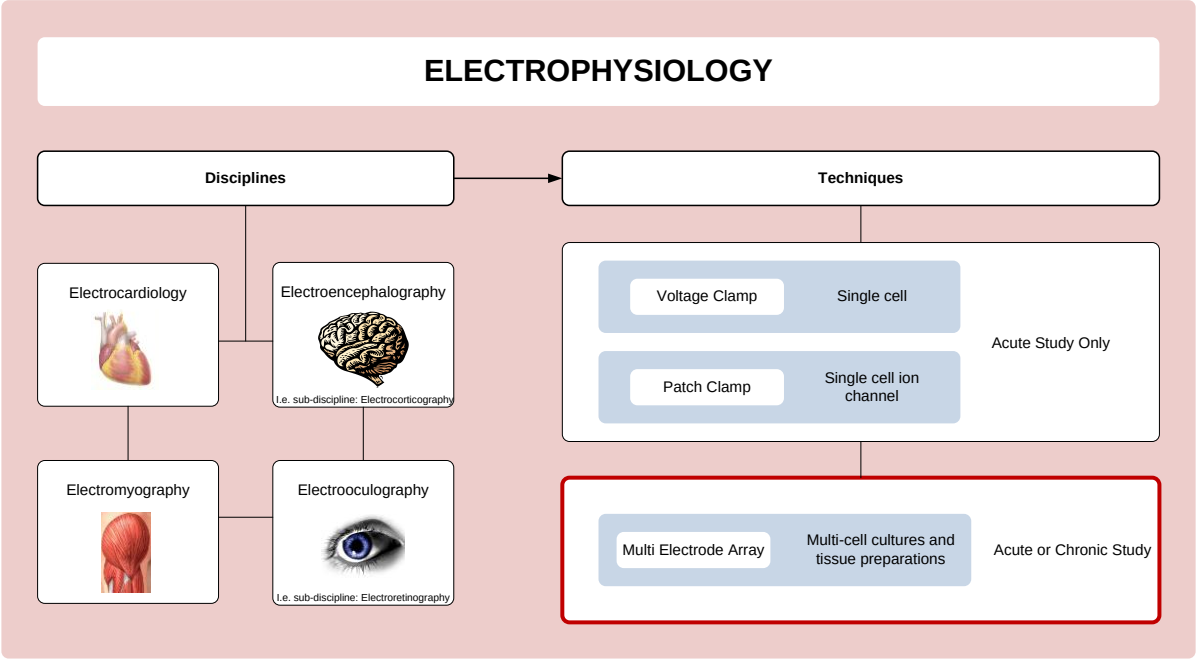


Figure 2.7: Disciplines of electrophysiology and techniques that can be used to detect and record electrogenic activity.

Mammalian electrophysiology has been divided into a number of disciplines (Figure 2.7) that are most commonly studied as research domains in themselves. Electrocardiography is electrophysiological study of the heart, electroencephalography is electrophysiological study of the brain, electromyography is electrophysiological study of muscles and electrooculography is the electrophysiological study of the eye(s).

As a large proportion of electrophysiology interacts with the brain these separated disciplines overlap greatly sharing a vast number of commonalities (e.g. such as action potentials that have originated from neurons of the central nervous system). To distinguish further regions of electrophysiological study these disciplines have in some cases been further defined into sub-disciplines. For example electrocorticography is the specific study of the cerebral cortex (Ray et al,

2008), and electroretinography is the specific study of the retina within the eye. These sub-disciplines fall into electroencephalography and electrooculography respectively. The underlying electrical occurrence fundamental to all of these disciplines and sub-disciplines is the Action Potential (AP) (Kaplan and Nguyen, 2011).

2.4.1 Action Potentials

Electrical potentials exist across many different biological cells due to different distributions in ionic concentrations between intracellular and extracellular spaces (Horsman, Conway and Yeager, 1985). Excitable cells are those that "are capable of generating rapidly changing electrochemical impulses at their membranes. In most instances, these impulses can be used to transmit signals along nerve or muscle membranes" (Guyton and Hall, 2000). The instance of one of these impulses is referred to as the occurrence of an Action Potential (AP) (Figure 2.8). Action potentials are defined as long-distance electrical signals (e.g. up to 1m), that are a brief (~1-500ms depending on the cell), rapid 0.1 to 100m/sec (Matthews, 2001), and large (100mV) change in membrane potential, during which the potential actually reverses so that the inside of the cell transiently becomes more positive than the outside (Sherwood, 2001). APs propagate along the length of a cell in a non-diminishing manner (Martinsen and Grimnes, 2008) meaning that the signal will not weaken as it passes through the cell's membrane.

To initiate an AP the cell membrane potential must alter (depolarise) from a resting potential of about -70mV beyond a threshold of about -55mV. Depolarisation from the resting potential towards the threshold potential occurs comparatively slowly. When the potential reaches the threshold explosive depolarisation occurs. Membrane potential increases rapidly towards about 30-40mV and then rapidly reverses, allowing the potential to drop again (repolarise) towards the resting potential. Hyperpolarisation, which is a brief fluctuation below the resting potential (~-10-20mV), often occurs as the ion changes responsible for repolarisation overshoot briefly (Figure 2.8). The entire period (~1-400ms (Matthews, 2001)) during which the potential changes from resting level, to its peak and returns back to resting is defined as the action potential. If the initial threshold (-55mV) is not reached during depolarisation no AP will take place. Thus, this threshold is in some texts also referred to as the critical all-or-none point (Plowman and Smith, 2008).

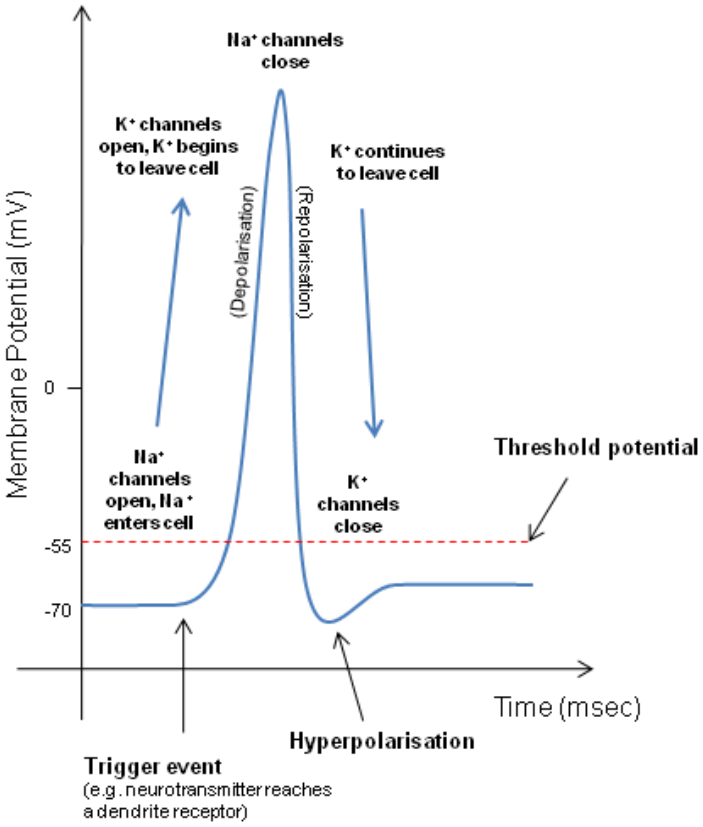


Figure 2.8: An action potential (neuron).

Neurons exhibit the fastest APs, lasting 1 to 100ms. In muscular tissues the APs are slower (e.g. cardiomyocytes ~400ms). APs are often referred to in electrophysiology as spikes (Dai et al, 2010). Collections of APs occurring at about the same time or in very close succession (within a few ms) are referred to as bursts (Figure 2.39).

Potassium ions (K^+) are most responsible for the resting potential of excitable cells. This is due to the cell membrane at rest demonstrating increased permeability to K^+ than to sodium ions (Na^+). When an AP occurs the permeability of the membrane to K^+ and Na^+ ions changes rapidly, resulting in ion currents that are detectable as overall potentials. In brief, an action potential begins with a stimulus that prompts the Na^+ channels to open, allowing an influx of sodium ions into the cell. The result of this influx is that the membrane of the cell becomes positive. As the positivity of the membrane increases, K^+ channels open, allowing K^+ ions to flow out of the cell. The Na^+ channels close, ceasing the inflow of positive charge. As the K^+ channels remain open the outflow of positive charge continues, resulting in a rapid drop in membrane potential. When the cell's membrane potential reaches its original resting potential the K^+ channels close.

The occurrence of action potentials can be detected and quantitatively measured using electrophysiological techniques developed over the past 60 years. These techniques are either

termed invasive, using voltage or patch clamp technology (Levitan et al, 2000), or non-invasive using MEA systems (Raoux et al, 2011).

2.4.2 Capturing action potentials

The following is a brief introduction to the electrophysiology techniques developed for action potential detection, recording and analysis (Figure 2.9).

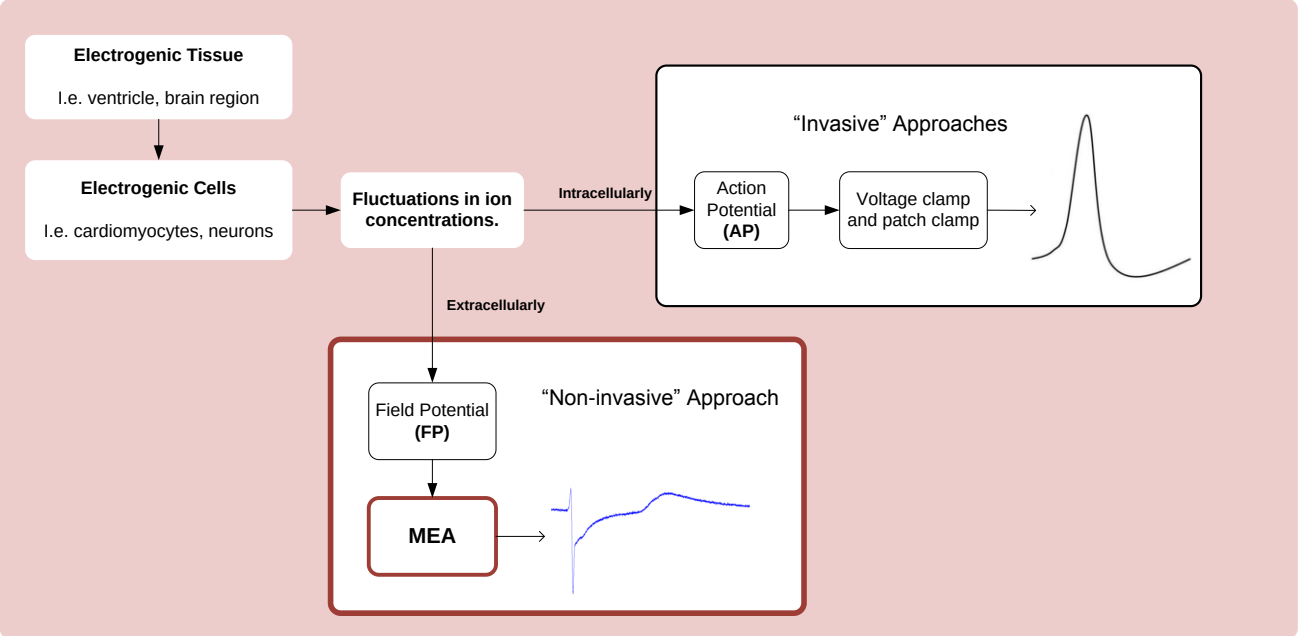


Figure 2.9: Electrophysiology techniques.

2.4.2.1 Invasive Voltage and Patch Clamp Techniques – intracellular recording.

Patch clamp techniques (Figure 2.10) are a progression of previous voltage clamp techniques used through the 1950's for the measurement of ion currents through individual neuron membranes (Boulton et al, 1990). The patch clamp technique was developed at around the end of the 1970's (Pine, 1980), introducing a greater level of sophistication to single cell characterisation by facilitating the assessment of ion movement through individual ion channels in cell membranes.

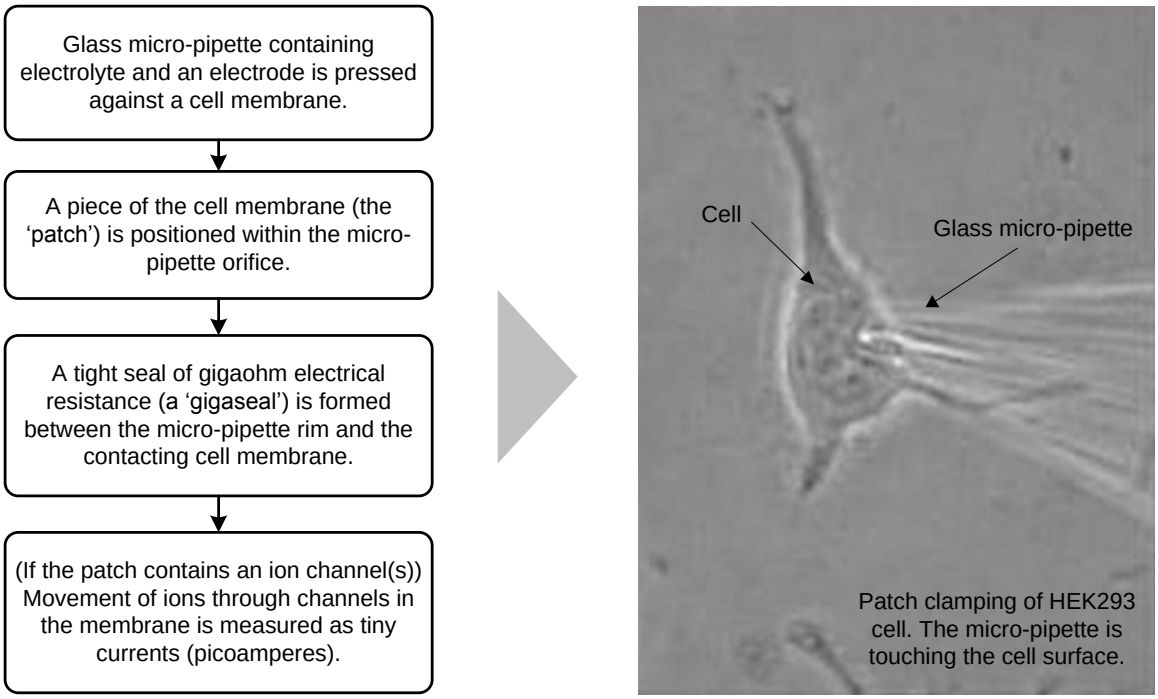


Figure 2.10: The patch clamp technique.
HEK = human embryonic kidney
[Constructed with reference to www.sophion.com and Molnar and Hickman, 2007].

Voltage and patch clamp techniques are the most mature techniques used for analysing the electrophysiology of cells. They were fundamental in defining single ion channel communication between and within cells. The electrodes used are typically glass micropipettes with open tips. The tip opening is usually less than 1µm. These micropipettes are filled with a solution that is conductive and connected to a voltmeter. The AP (described in some texts as the "transmembrane voltage") of the cell can then be recorded (Purves et al, 2000). For original voltage clamp recording techniques two electrodes were required, one micropipette tip records the membrane voltage, the other passes a current to maintain the membrane at a constant "command" voltage (Matthews, 2003). For patch clamp measurements only one electrode is required, the tip of which is placed against the cell membrane and suction is applied creating a high resistance "gigaseal" (typically >1GΩ) (Mathes et al, 2009)) around a very small area (~>1µm^2), or "patch", of the cell membrane. Both of these techniques are described as invasive as they firstly require the cells to be dissociated out of the tissue of origin. Then secondly, the individual cell of interest is placed into a specific conductive solution (that is not the same as in vivo conditions) and is attached to, or punctured by, the glass micropipette electrode for recording. Due to these conditions of pinching or puncturing, patch clamp recordings must be taken within a few hours as the cells utilised are damaged and will die (Osorio and Delmas, 2011).

The action potentials through the cell membrane also result in ion current fluctuations in the extracellular fluid and matrix that surrounds the cell. This fluctuation, called an extracellular Field Potential (FP), can be detected using microelectrodes embedded at the centre of an MEA biochip.

2.4.2.2 Non-invasive Multi Electrode Array Technique – extracellular.

The detection of electrical activity occurring in a living sample using MEA systems is based upon the presence of membrane voltage gradients, or potentials, not only through cells but also through the surrounding extracellular matter and media. These potentials, referred to as field potentials (FPs), are a direct consequence of a flow of ions into or out of the fluid media as the result of cellular activity (Ray et al, 2008). When measured using an MEA system, ion current changes occurring over the microelectrodes (FPs) can be visualised in terms of both space and time, and recorded. This is due to the distributed nature of the micro electrodes underneath the sample of cells.

The use of MEA systems for recording electrogenic activity in *in-vitro* preparations is now well established and validated (Heuschkel et al, 2002; Wagenaar, DeMarse and Potter, 2005). MEA system alternatives do not presently exist and so this *in-vitro* technique is unique in its ability to capture (non-invasively) electrical signals from living matter (Stett et al, 2003). In vitro MEA systems serve as an intermediary technology between acute patch clamp measurement and the concurrently evolving field of chronic *in-vivo* MEA recording (Caspi et al, 2009).

In-vitro MEA systems also offer the facility to detect, measure and analyse particular parameters thought to be significantly influenced by, or the result of, particular genetic combinations (Valor et al, 2007; Chong et al, 2011). Genetic mutation investigations utilise tissues dissected out of specifically bred animals. The breeding of animals for laboratory testing is labour intensive and expensive (Jurga, Forraz and McGuckin, 2010). It has been described that complementary *in-vitro* MEA recording and analysis techniques help to overcome the limited availability of "significant collections of genetically interesting in vivo subjects" by making better use of the tissue and cells that are available (Kobel and Lutolf, 2010). For example, a brain region from one mutant mouse can be cut into very fine slices and studied on multiple in-vitro MEA biochips.

Another advantage brought about by MEA systems has been the facility to take repeated measurements from the same culture of cells or tissue over prolonged periods of time (Gross and Pancrazio, 2006). Some cases have reported results taken from one culture over periods of up to 3.5 months (Dubois-Dauphin et al, 2010). MEA systems serve too as powerful tools for evaluating the

dynamics and differences both in spontaneous activity and in activity evoked by exposure to external stimuli (e.g. drugs or electrical stimulation) (Johnstone et al, 2010). This combination of advantages described thus far has resulted in the suggestion that to further exploit MEA systems, adaptations that will allow an increase in throughput for chemical screening are now required to support the fields of safety pharmacology and drug discovery (Denning and Anderson, 2008; Braam et al, 2009; Yla-Outinen et al, 2010).

MEA recorded data, particularly from dissociated cell cultures and stem cell derived cell cultures, show significant amounts of variation in terms of SNR, signal size and waveform shapes from culture to culture (Shahid et al, 2009; Cohen et al, 2011). These variations can be attributed to innate differences in the cultures themselves (e.g. cell size) and to the differing cell types under investigation. Cell orientations, spatial distribution of occurring activity, and the timing of that activity is also unique to each culture (Lappalainen et al, 2010). Various other user group specific factors such as MEA biochip pre-processing procedures (e.g. the use of coatings such as laminin or Matrigel) also affect signal detection (Graham et al, 2009).

2.4.3 Cardiac Electrophysiology

Ion current flows responsible for cardiomyocyte contractions are detectable as action potentials (AP) and field potentials (FP) using electrophysiological recording techniques. Cardiomyocyte studies conducted on an MEA system detect the extracellular FPs that can be correlated to both patch clamp AP recordings and whole heart electrocardiogram (ECG) contraction signals (see Figure 2.11).

Across medicine and the pharmaceutical industry a significant emphasis has been placed on understanding the action of chemical substances used as prescription drugs on the natural contracting rhythm of the human heart (Braam et al, 2010). This follows unforeseen interactions of some commercially available drugs that have been recalled due to risks posed to human life, such as Vioxx (Martinez et al, 2004; Goenka et al, 2010). MEA systems are currently receiving much attention as a tool in helping to screen substances at pre-clinical stages of development. The parameter of most interest is called the QT-interval. It has been demonstrated that the correlation of this parameter *in vitro* (detectable on MEAs) with *in vivo* signals can be exploited to understand further this parameter that if altered *in vivo* can result in death of the animal or human.

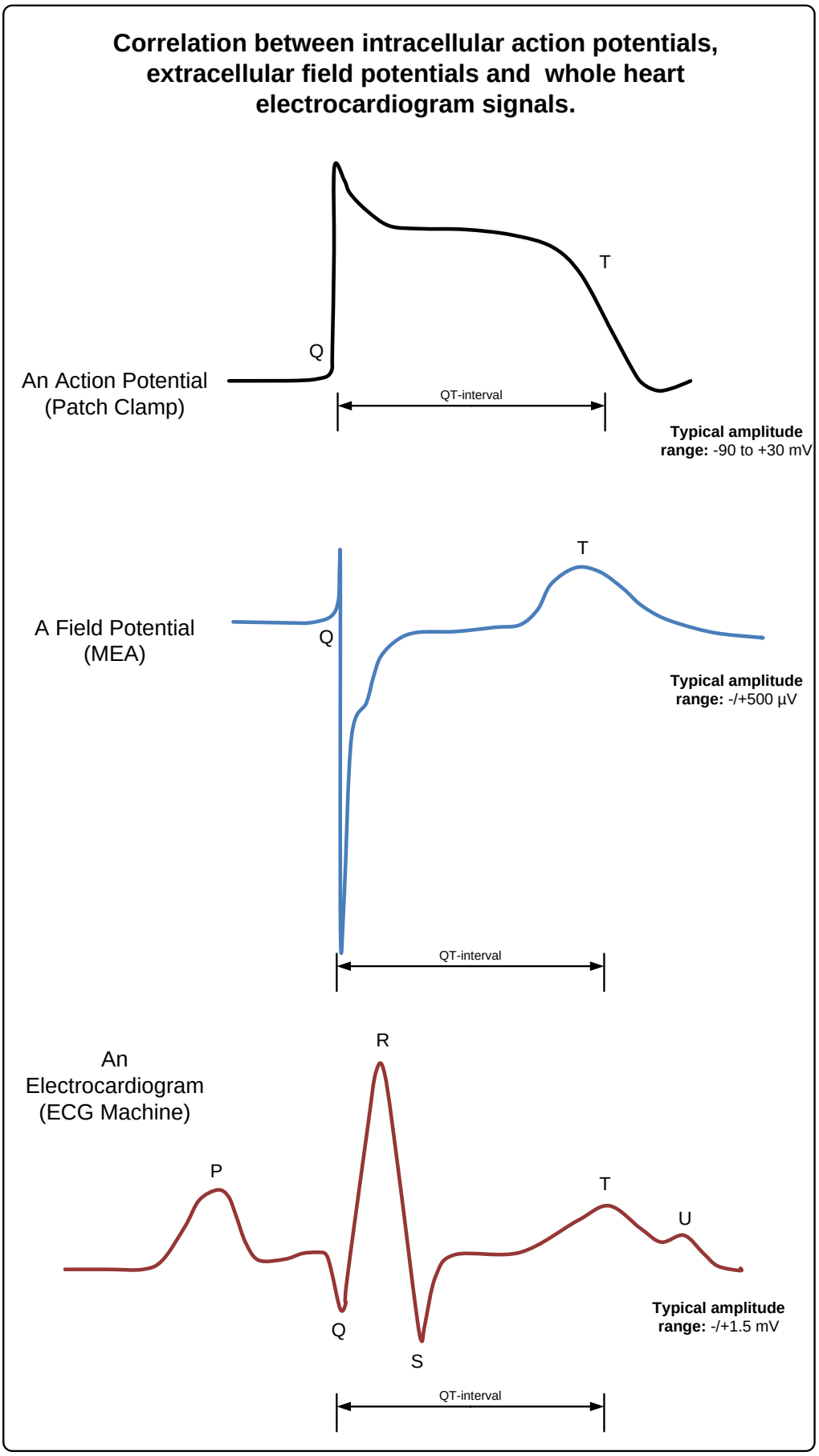


Figure 2.11: The correlation between AP, FP and ECG signals.

2.4.3.1 Characterisation and Identification of Normal Cardiac Activity

The normal electrophysiological behaviour of the heart is determined by ordered propagation of excitatory stimuli that results in rapid depolarisation and slow repolarisation of various excitable cell types across the heart (Sadek et al, 2008). The resulting contraction ejects blood from the heart forming the basis of the mammalian circulatory system (Sherwood, 2006).

The excitable cells that make up the myocardium (heart muscle) receive stimuli originating from a region of the heart referred to as the sinoatrial (SA) node. The SA generates a wave of excitation that spreads rapidly through both atria reaching the atrioventricular (AV) node. The AV node is positioned between the atria and the two ventricles of the heart. The AV stimulates a bundle of conducting fibres, the Bundle of His, that spreads excitation down into the muscle of each ventricle through a further network of fibres, the Purkinje fibres (Starr and McMillan, 2007). This set route of stimulation is essential for normal cardiac function and can be detected and monitored through the use of an electrocardiogram (ECG) (Houghton and Gray, 1997).

The ECG is used for identification of disorders of cardiac rhythm (e.g. torsade's de pointes), diagnosis of heart abnormalities (e.g. myocardial infarction) and to provide clues to the presence of other generalized disorders of the body (e.g. electrolyte disturbances) (Houghton and Gray, 1997).

The muscle mass of different regions of the heart (atria and ventricles) differs so the electrical change that accompanies contraction correlates to those differences. The contraction of the atria produces a smaller wave (P wave) of electrical excitation when compared to that detected when the ventricles contract (QRS complex). It is the presence of these waves that allows ECG signals to be described in terms of the wave of excitation as it spreads through the heart. Different parts of the detected signal are classified using an alphabetical description. The letters P, Q, R, S, T and occasionally U (see Figure 2.11) are used to describe all ECG signals (Hampton, 2008).

Each wave represents either depolarisation or repolarisation of a certain region of the heart. Changes (e.g. presence, duration, amplitude) in waves can be used to detect and diagnose abnormalities.

Prolongation of the QT interval is one abnormality that can cause cardiac arrhythmia and consequently death (Stett et al, 2003). Pharmaceutical companies must assess all new cardio-active substances for adverse effects on the QT interval. QT interval can be detected in whole heart and in stem-cell derived beating cluster recordings (Figure 2.11), which can be of use when screening a substance for unwanted cardiac interaction (Halbach et al, 2003).

2.5 Original MEA system Application

The development of MEAs began more than 35 years ago (Gross et al, 1977; Wolpaw and Wolpaw, 2012). MEA system use has increased rapidly over the past twenty years following successful system uptake across research applications during the 1990's (Connolly et al, 1990; Jimbo and Kawana, 1992: Owens et al, 1995). Planar MEA biochips were the first type to be introduced and have slowly but steadily gained the highest degree of popularity amongst neuroscientists (Morin et al, 2005) later to be adopted by bioscientists working with other electrogenic cell types. MEA biochips for the first time enabled chronic monitoring of excitable cells in-vitro. Early work using these devices was able to monitor neural cultures for periods of several months capturing the exhibition of slowly altering field potentials and rapidly occurring spikes. For the first time neuroscientists were able to view the "plasticity" of neural networks over much more meaningful durations. Plasticity is the capacity of the nervous system to change during development and when learning new skills, establishing new memories and responding to injury (Purves et al, 2001). By capturing plasticity using MEAs neuroscientists have been able to understand more about how the mammalian brain works (Frey et al, 2009).

The microelectrodes in an MEA can detect signals from neurons up to ~100µm away in a culture or within a tissue slice. The work of Egert et al. (2002a) has also suggested that in monolayer cultures typically the majority of signal sources are within a radius of about 30µm from the microelectrode centre. The current 8x8 arrangement of 60 electrodes (where one electrode missing at each corner) is believed to offer "good spatial and temporal resolution" for most network based neurological investigations (Stett et al, 2003).

2.5.1 Stimulating with an MEA system

MEA biochips have been used to record from cells and also provide electrical stimulation. Stimuli can be input to a culture electrically by application of either current or voltage impulses through the microelectrodes (Merrill et al, 2005; Kopanitsa, Afinowi, and Grant, 2006; Herrmann and Stett, 2010). It is generally well understood that across neuroscience MEA application observations have shown that neurons respond differently to altered stimulation protocols (Figure 2.12, Wagenaar et al, 2004).

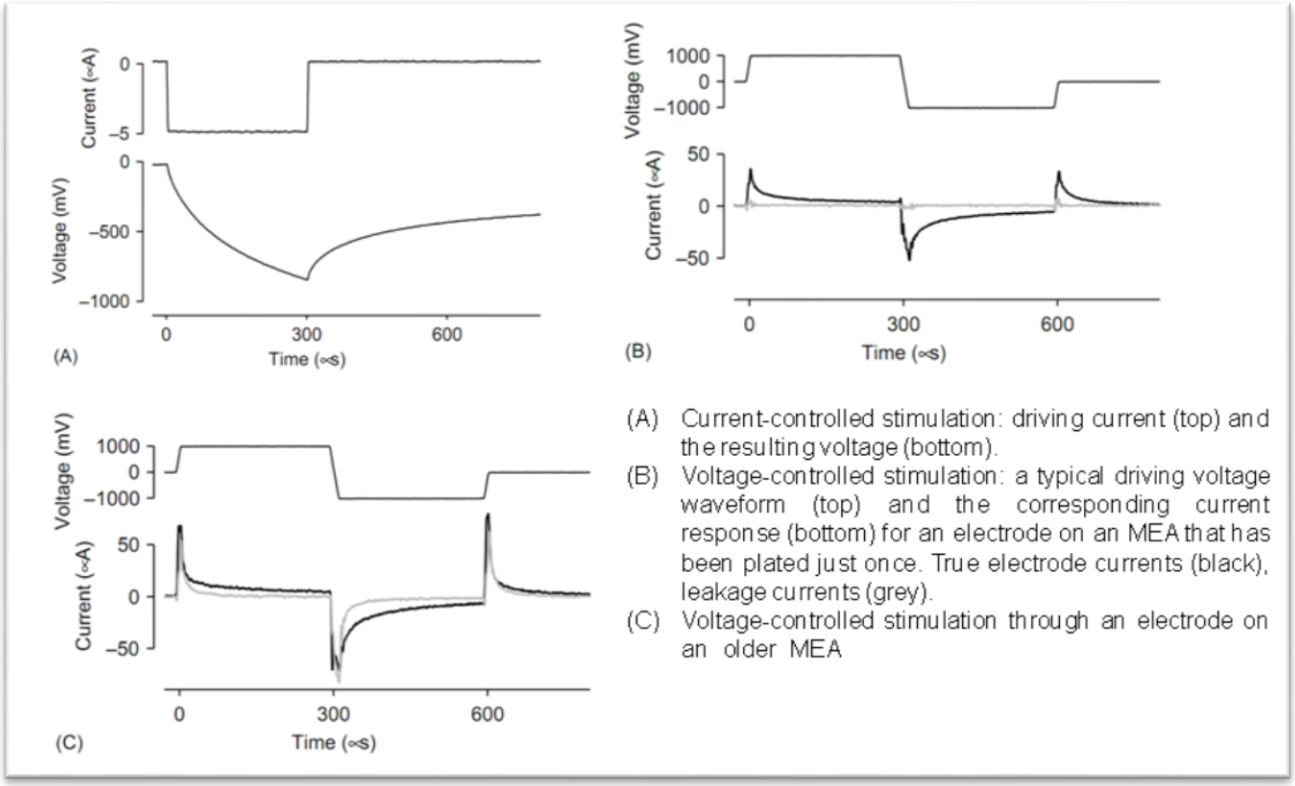


Figure 2.12: Example stimulation waveforms.
[Adpated from Wagenaar et al, 2004.]

When stimuli protocols are employed through a specific electrode(s) to a target region of cells the membrane polarisation of those cells is affected by an external voltage gradient in the surrounding media. The efficacy of stimulation protocols depends upon effective spreading of the input pulse at the cell-electrode interface and through the local region of the culture (Fejti et al, 2006). Descriptions of stimulation experiments across academic publications usually describe protocols in terms of the charge injected through the electrode(s) per pulse (Erickson, 2010; Ide et al, 2010). When charge is injected with current pulses the amplitude and duration of the pulse will be important in defining the protocol, but where voltage pulses are injected the tissue resistance and the capacity of the cell-electrode interface is also important. Most manufacturers of MEA biochips have investigated the “charge-injection capacity”. This is a value given in technical data sheets specifying the limit to which the biochip’s electrodes can be charged before irreversible damage may occur at the electrode surface.

Important factors both users and developers alike must be aware of relating to stimulation include:

1. Recording during the period of the stimulation is not possible as the electrodes saturate.
2. Injection of stimuli creates artefacts across most channels during and immediately after stimulation due to saturation of the electrodes. This has been addressed across the MEA

domain and is typically overcome by specific signal processing or specialist hardware design (Morin et al, 2005).

3. Care must be taken when selecting the input limit (i.e. 4V). If selection is too large harm may be inflicted upon cells or to the microelectrodes (Morin et al, 2005). The voltage at the microelectrodes should always be controlled to be as low as possible to prevent unnecessary electrochemical damage at the electrode surface. Inappropriately designed stimulation protocols may cause damage to the tissues under investigation or to the electrode in-use (Merrill, Bikson and Jefferys, 2005).

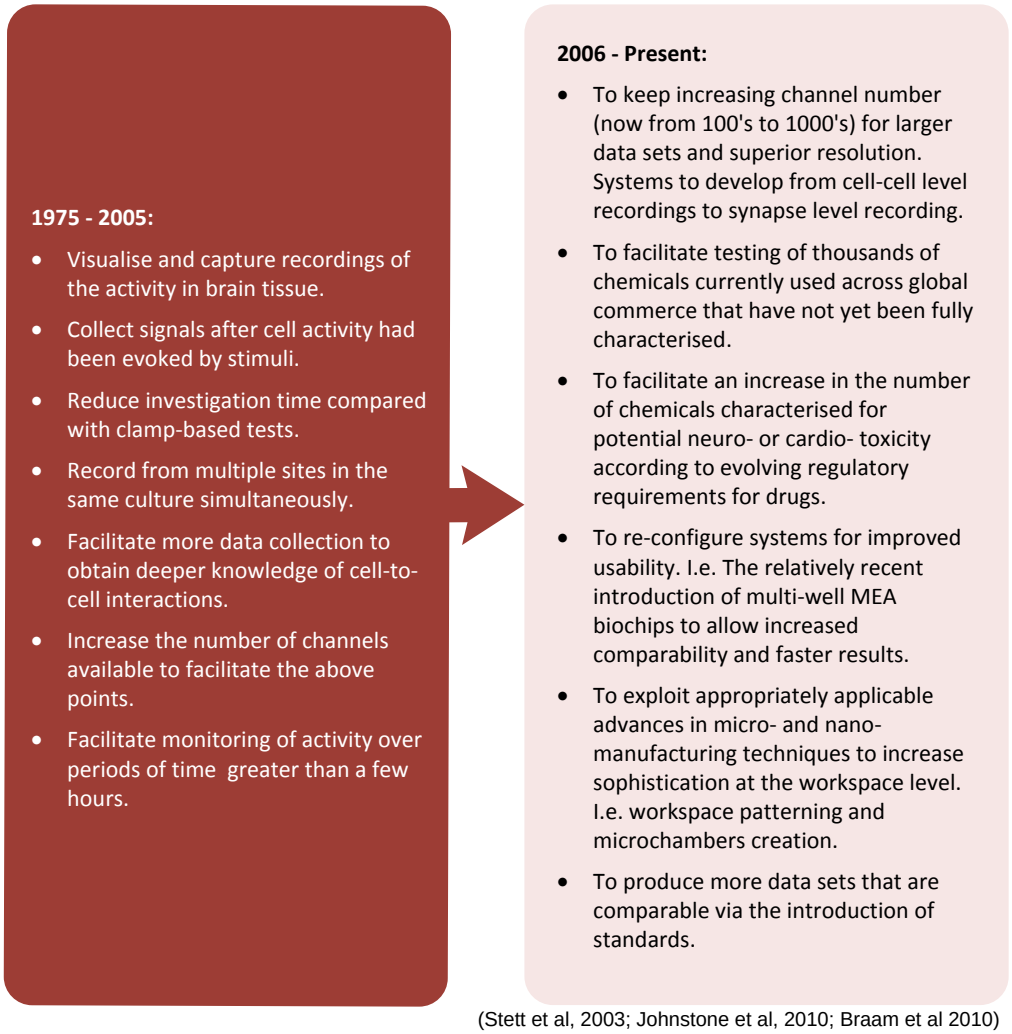
2.5.1.1 Artefact suppression

A recognised limitation of recording signals while using stimulation protocols is that when stimulation is applied it takes time (t = 50-100ms) for the electrodes to recover (Brown et al, 2008). The presence of a stimulation artefact results in a problem specifically associated with stimulation delivery and the microelectrode's ability to retain appropriate recording capability during and straight after a pulse is input. Blanking circuits have been introduced for users of MEA systems who wish to stimulate their cultures or slices and record straight after (Zrenner et al, 2010; Biodo et al 2010). These circuits are integrated into the headstage and work by decoupling all of the microelectrodes during the stimulation. The microelectrodes are then returned to their normal recording states prior to the resumption of spike activity in the culture, approximately 40-160msec after stimulation (Wagenaar, Pine and Potter, 2004).

2.6 MEA System Development

Improvements in both electronics and electrogenic cell culture methods have brought about increased uptake of MEA systems for electrophysiological-based study in both academia and industrial settings (Johnstone et al, 2010). Original development was centred on simply recording signals from collections of neurons. Natural incremental advances from these early experiments to meet ever evolving user needs has resulted in improved systems and differing methods of employment.

Objectives that have driven the developments of the first twenty-five years following the MEA biochip's introduction, with more recent requirements that have been made in the last five to ten years are listed in Figure 2.13.



(Stett et al, 2003; Johnstone et al, 2010; Braam et al 2010)

Figure 2.13: Past and present MEA development objectives.

This research presented in this thesis has been focused on design-and-build efforts addressing two distinct priorities: (A) the MEA biochip, and (B) the supporting system and required software (Figure 2.14). This thesis is centred on priority A. Generation of concepts and production of prototypes for testing are documented in this thesis (Chapter 5).

Design details were identified pertaining to each priority and research was conducted relating to each design detail prior to design of the concepts for manufacture and prototype testing. For the MEA biochip material suitability, overall chip and workspace configuration, required throughputs and overall usability were considered. The cell-electrode interface is a topic still under investigation that has been addressed in this research (section 2.6.6). For the underlying system and software, the requirements, usability and throughput were considered with the intention to re-configure the MEA system to facilitate a reduction in the time required for analysis.

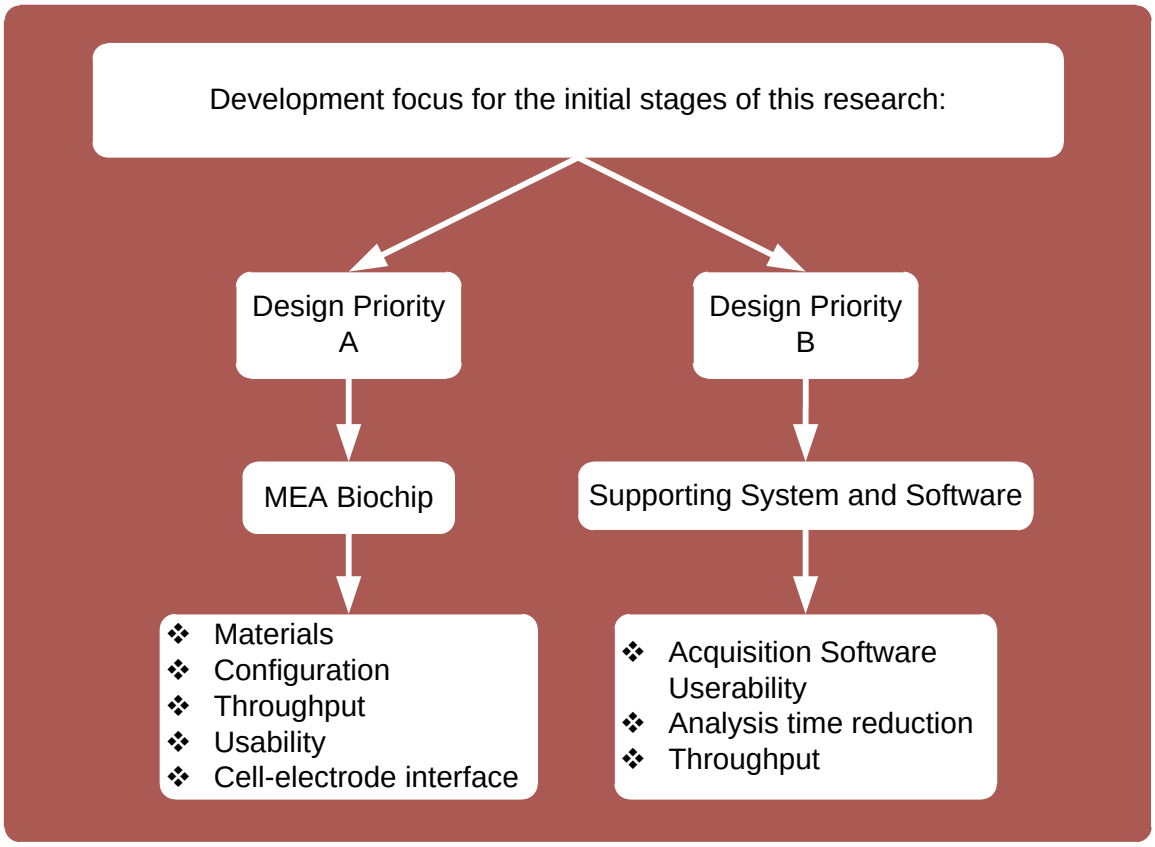


Figure 2.14: Priorities of development used in this research.

2.6.1 Industrial Case Study

As the identified innovation leaders in this field, Multi Channels Systems (MCS) have progressed the development of their systems as documented into the following series of diagrams. This section presents an industrial case study.

Study of MCS's MEA systems development shows system iterations launched over the past three years since this research began. The standard MEA-60 system configuration, available in October 2008 and those that have been developed since are depicted. The frequency range across all MCS systems is 2 – 50kHz.

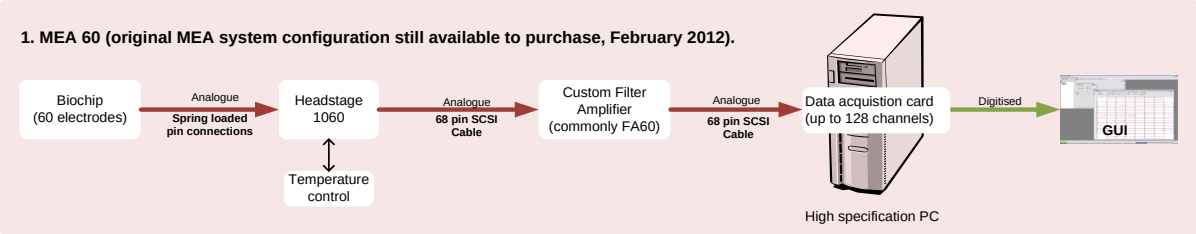


Figure 2.15: The MEA 60. Multi Channels System's original standard system. With a specialist PC housing the 128 channel data acquisition card, running a Windows operating system.

The MEA 60 system (Figure 2.15) consists of an MEA60 biochip connected via an MCS headstage 1060 (section 2.9.1.2) to a personal computer (PC). The headstage unit connects via a standard 68-pin SCSI cable to an FA60 Filter Amplifier (FA). The FA60 is sold and configured as part of the headstage configuration but exists as a separate unit that sits between the headstage and the MC_Card. The MC_Card is a PCB housed inside a PC that serves as the data acquisition unit for the system, converting the analogue signals to digital streams for visualisation and analysis.

The MEA 120 system (Figure 2.16) can also be described as the MEA 60 system in a twofold configuration. The only alteration is the addition of an MEA_Switch unit. The MEA_Switch is a separate unit that connects between the FA60 of each headstage into the MC_Card.

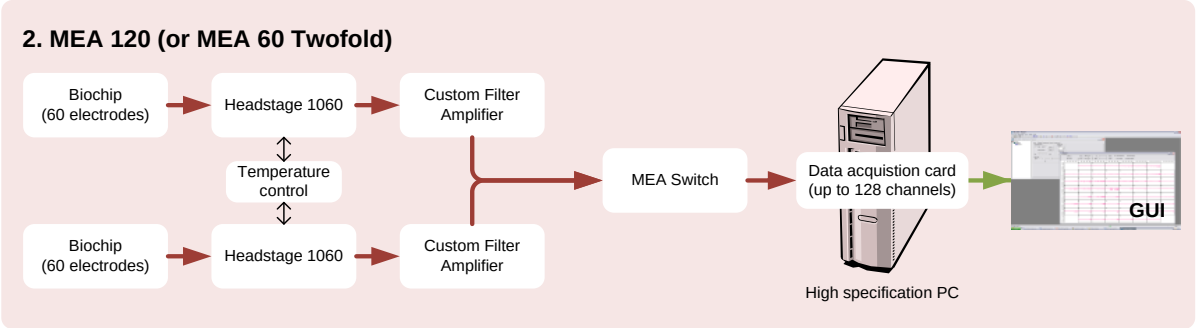


Figure 2.16: MEA 120. A representation of how the original MEA-60 can be scaled up using a device called the MCS MEA switch to accommodate two MEA biochips at one time. This allowed the data that could be captured per trial to be doubled from 60 to 120 channels.

The MEA_Switch can also accommodate input from two further headstage units allowing the MEA 120 system to also be scaled up into a twofold configuration (Figure 2.17). The number of channels that the MEA_Switch can process remains the same, so using the accompanying MEA_Switch software to turn on and off microelectrodes, the user must select up to 120 of the available 240 microelectrode channels to monitor.

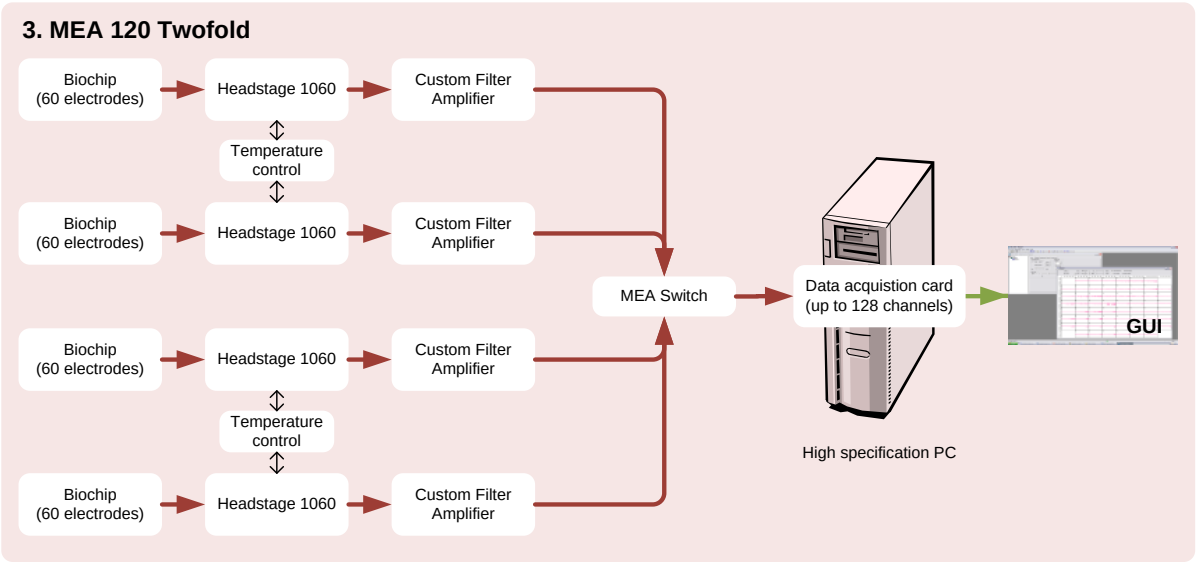


Figure 2.17: MEA 120 Twofold. How the same MEA switch device can scale the system to accommodate four MEA biochips. The number of recordable channels remains 120. It's intended to allow groups to capture more useful data using the equivalent number of channels (if their application resolution requirements permit). A reduction in the number of recordable channels per MEA biochip is required so either smaller areas at high resolution or larger areas at lower resolutions across the workspace must be defined.

Developments up to this point allowed scientists to capture more data in one set-up, while concurrently allowing opportunity for increased throughput if an appropriate channel compromise is accommodated by the testing protocol. USB 2.0 connections (480Mb/s) have been employed in a new data acquisition unit that integrates with existing MCS system components (Figure 2.18 &

Figure 2.19) allowing increased flexibility in terms of data acquisition volume and the physical location of where the MEA system is set-up and on which PC the data is acquired and stored.

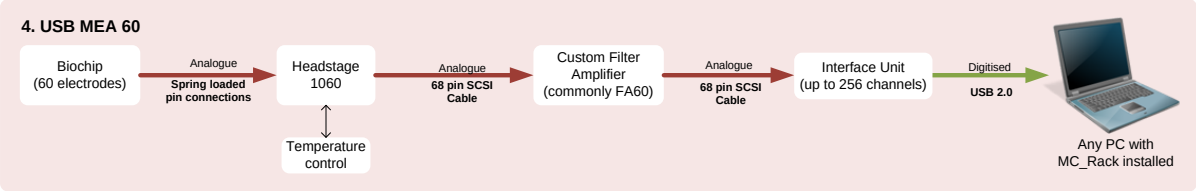


Figure 2.18: USB MEA60. A visualisation of the first MEA-60 USB system.

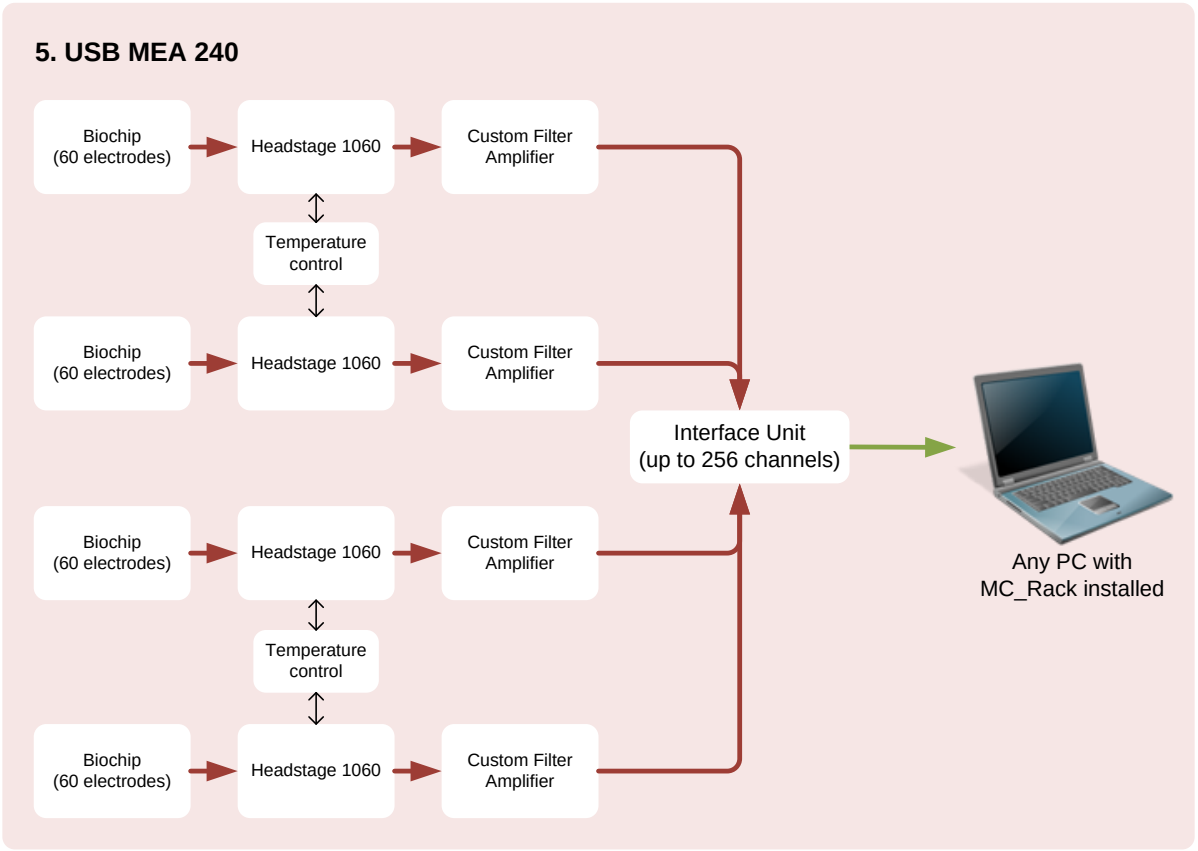


Figure 2.19: USB MEA 240. The USB interface unit integrated with four MEA biochips. In this instance the recordable channel and resolution compromises of previous systems is eliminated.

In the second quarter of 2010 a new generation of MEA system was launched by MCS. The model of the system was called USB MEA2100-60-HS (Figure 2.20). The headstage unit was redesigned to house more of the core technology compactly into one unit, i.e. the custom filter amplifiers (FA) and stimulation units were integrated together into one unit. The new headstages, called Headstage 2100-60-HS, also offer the facility to house up to two MEA biochips side-by-side (Appendix B).

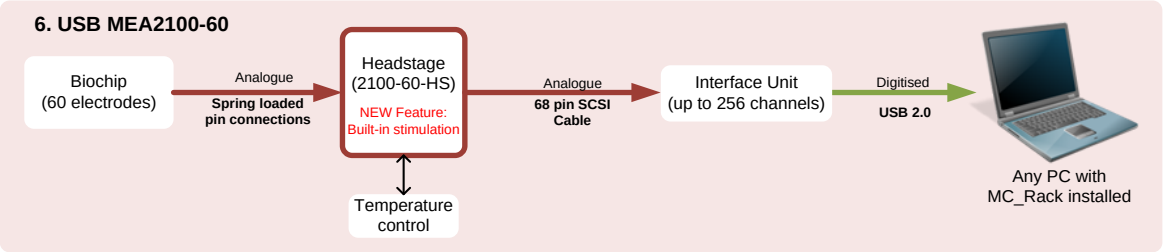


Figure 2.20: USB MEA 2100-60. The MEA 2100 system in a 60 electrode MEA format. Two 60 electrode MEAs can also be used in this one unit positioned side-by-side is desired.

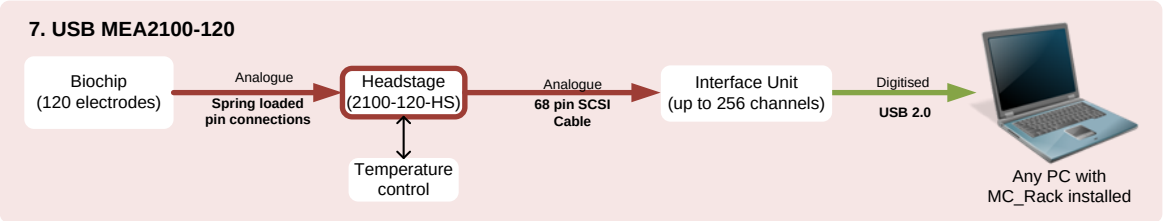


Figure 2.21: USB MEA 2100-120. The MEA 2100 system headstage connectors can also be adjusted to allow contact to biochips with >60 electrodes, in this configuration 120.

The alternative configurations of the MEA2100 systems available are documented in figures Figure 2.20, Figure 2.21 and Figure 2.22.

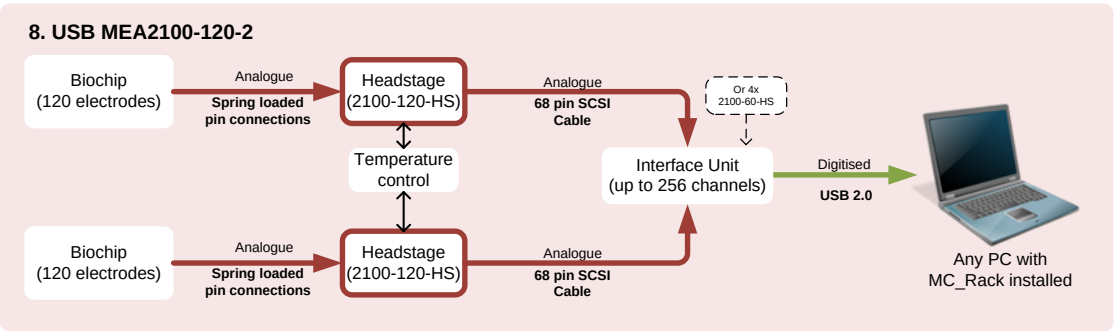


Figure 2.22: USB MEA 2100-120-2. Two 120 electrode chips can also be accommodated in one experiment via the use of two 2100 headstage units.

The most recent models released to the market by MCS have been called “integrated MEA systems”. These configurations integrate the headstage and interface unit into one box that plugs in to an acquisition PC via USB 2.0.

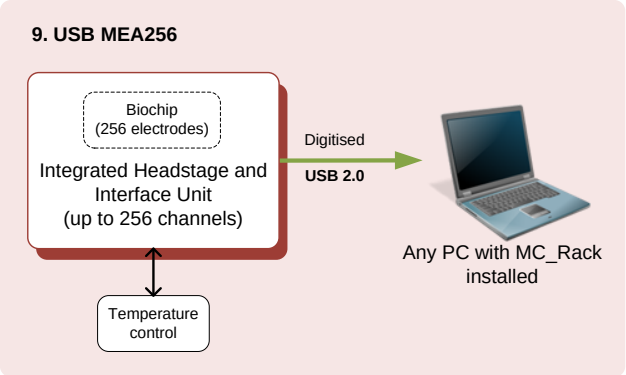


Figure 2.23: USB MEA 256. The current state-of-the-art for neuron applications. This configuration allow MEA biochips that arrange 256 electrodes (16x16) into one biochip to be recorded from in a simple three piece system; the headstage, acquisition unit and PC. The headstage in this unit is not the 2100 but again has been redesigned into one compact unit.

This unit (Figure 2.23) also accommodates a single biochip with up to 252 contact pads. This system can record 252 channels at sampling rates of up to 40kHz. The other 4 channels are additional analogue for units such as oscilloscopes or signal splitters. These units are regarded as the current state-of-the-art in MEA systems with other major competitors developing equivalents.

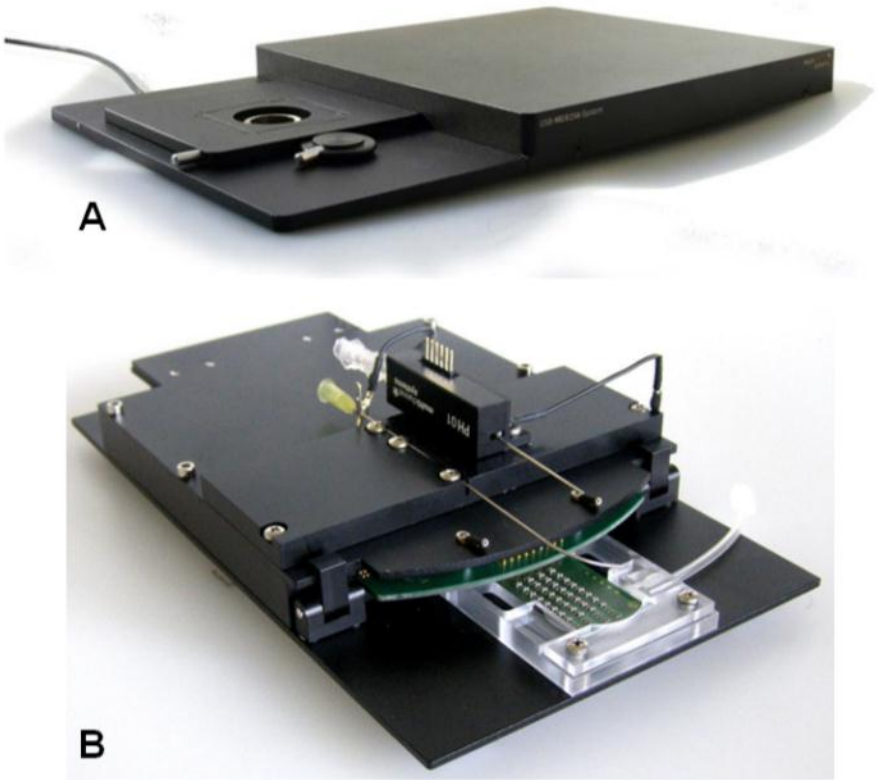


Figure 2.24: The most recent configuration of USB MEA256 systems available from Multi Channels Systems. A) The USB-MEA256 System. B) The USB-MEA32-STIM4 System.

The integrated unit (as of July 2011) is available in two distinctly different forms. The first unit (Figure 2.24A) would be classified as the new standard unit that accommodates one MEA biochip of

up to 256 electrodes depending upon user preference. The second (Figure 2.24B) has been adapted to accommodate the perforated, perfusion specific MEA biochips (Figure 2.25) developed specifically for longer term slice studies.

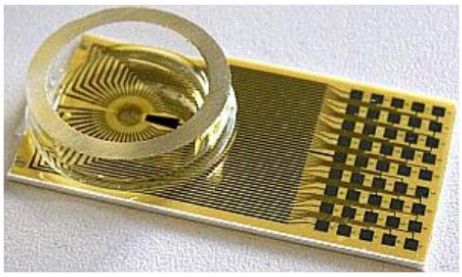


Figure 2.25: A perforated MEA biochip manufactured by Multi Channels System for use in the USB-MEA32-STIM4 system.

2.6.2 System Design Limitations

To address the complexity of developing a complete MEA system three naturally occurring design domains were defined: (i) the biochip, (ii) the supporting network of electronics and (iii) the software(s) used to drive the system. It is also essential to communicate design influencing factors to support overall system success. These additional influences are those that dictate how a system is applied and have consequently motivated much of MEA system development in the past (Figure 2.26).

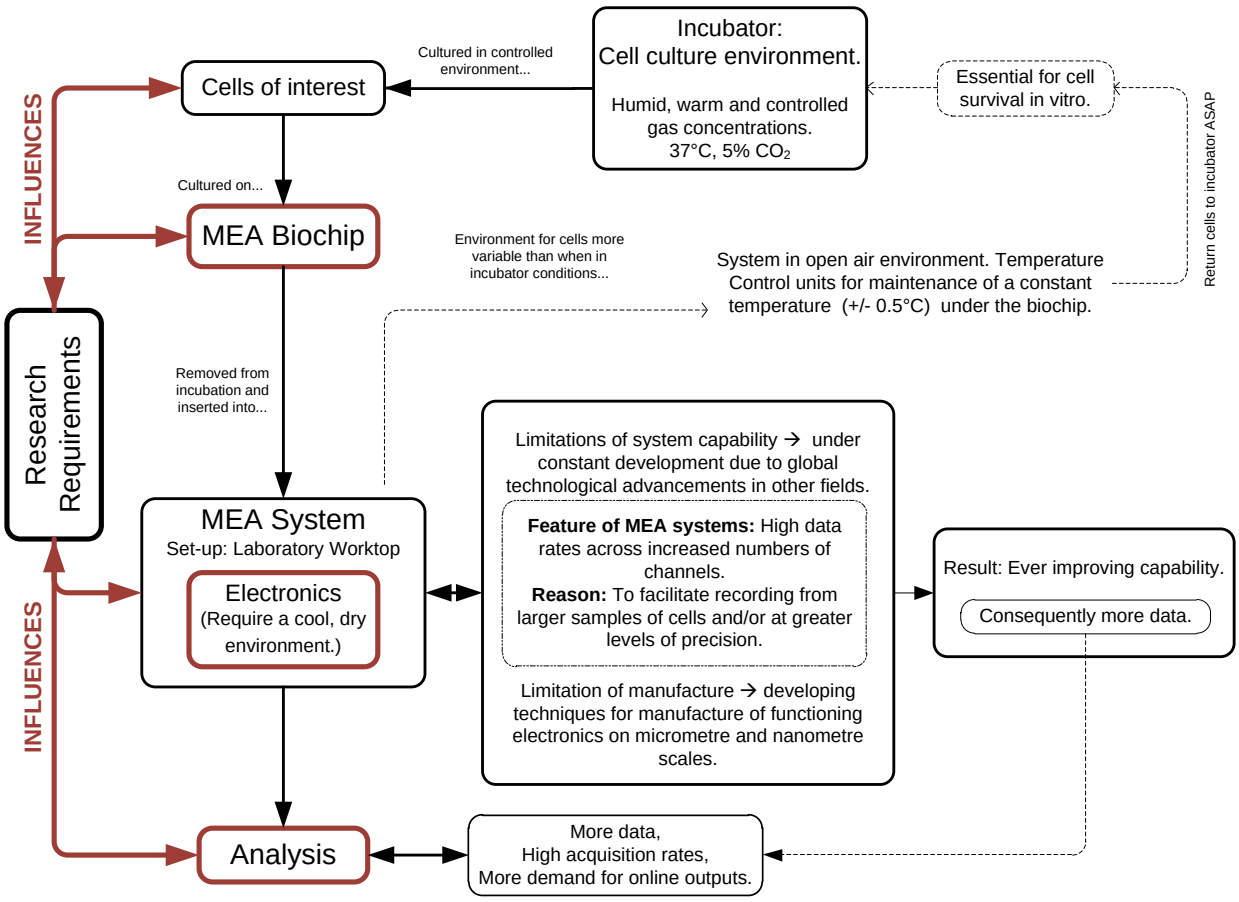


Figure 2.26: Factors influencing MEA system design. The areas highlighted in red show the three distinct areas of MEA design where incremental development occurs.

The cell type under investigation affects the type of MEA biochip that a user will select as certain types of biochip make it easier to detect appropriate signals (e.g. 3D electrodes used in slice applications). The type of biochip coupled with the cell type affects the settings of the required underlying electronics as the signal detected will also vary (e.g. slice applications demonstrate higher amplitudes than cultured cells). The software must then provide appropriate support for the cell type selected (i.e. in terms of required sampling frequency), the biochip of choice (i.e. in terms of accommodating the layout and number of channels), the settings of the underlying electronics and the analysis requirements of the user.

Continually evolving MEA systems on the market today are the result of development that has been motivated and driven by the desire to acquire as much information as possible from the cells of interest (*increased useful data*). This results in *increased complexity* during signal acquisition and analysis, the outcome is that there is an *increased understanding* of the subject. Consequently new questions arise based on this new knowledge (new research questions) and further assessment is required, often involving an *increased level of sophistication* to acquire the data (Figure 2.27).

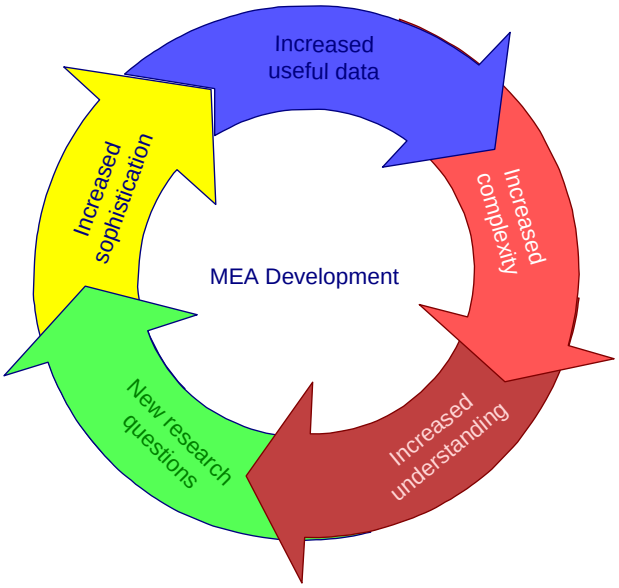


Figure 2.27: The MEA motivation-development cycle.

2.6.3 MEA system local environment

The incubated cell culture environment in which living cells are contained is critical to their survival and currently MEA systems are a long way from being optimised for this facet of their use. Heated headstages and set-ups with microscope integration have been introduced (Morin et al, 2005) but these initial MEA systems developed have not been suited to long-term humidified, incubated environments.

Control over the temperature (~37°C) and humidity (e.g. slow evaporation rates) of the environment local to a cell in culture is more critical for culture users than slice users. This is because slices are exploited in short studies and not required for the periods of days to months (Egert et al, 2002a). Successful survival of living samples in-vitro is dependent upon provision of an environment that mimics in-vivo as closely as possible. Temperatures at which cultures are maintained is matched to that of the in-vivo region of the body from which that cell type originates, and culture medium content is precisely controlled over defined durations by control of evaporation and gas exchange by incubators (Mather and Roberts, 1998). A consequence of this need to provide a controllable environment is that MEA systems in their current state are sub-optimal in design. Since the early 2000's some research groups have experimented with methods and specialised tooling specifically with the intention of allowing MEAs containing living contents to be left in MEA headstages for

longer under conditions that are better suited to cell survival. Examples of this include the use of dry incubators to reduce disturbances to set-ups and support long-term investigations in which the cells are monitored continuously and left untouched throughout the trial. The first research group to use dry incubators also needed to provide a way to reduce evaporation of the culture media. They developed a specialist lid for MEAs allowing them to subsist better in a non-humidified environment (Potter and DeMarse, 2001).

In 2005 Morin et al stated that "establishment of an experimental set-up suitable for very long-term monitoring of neuronal networks cultured on planar MEAs is becoming an absolute requirement in order to design truly significant experiments". This statement suggests that MEA systems were still not optimised and supports the on-going development of MEA systems that can be left in environments more suited to cell culture minimising human interventions after seeding. There is however, yet to be a suitable commercially available solution.

2.6.4 Evolution of the motivations driving system development

Since Jerome Pine first reported MEA employment in 1980 (Pine, 1980) numerous research groups have designed their own MEA biochips, headstages, amplifiers and even complete systems to match more closely their own needs (Jimbo et al, 2003; Chen et al, 2008; Clark et al, 2009; Hwang et al, 2009; Shimada et al, 2011). The motivations that have driven MEA system development have varied from group to group but were roughly summarised by Stett et al (2003) as having been:

i. to gain more information about the interactions that occur between cells,
ii. to reduce the time required to run an experiment by simultaneously recording from multiple sites of the same culture,
iii. to allow monitoring of the changes of electrical activity that occur over time (e.g. changes in burst durations, changes in waveform amplitudes).

To acquire significant data from MEA systems an appropriate throughput capability is required, as is the ability to analyse the data in an efficient manner.

An area that would benefit user applications the most is the increase of throughput via a parallel improvement of available signal analysis techniques (Johnstone et al, 2010; Braam et al, 2010; Natarajan et al, 2011) as well as rapidly and efficient identification of toxicity (Tanaka et al, 2009).

In addition, MEA systems that are simpler to use (e.g. require shorter set-ups, allow more instances to collect data per test) are also required (Robinette et al, 2011). Cheaper fabrication of biochips and system components will be essential to provide affordable MEA systems for the demanded higher throughputs of the future (Rolston et al, 2009). There are also expectations that systems need to provide continuous or integrated imaging capability in addition to the core electrophysiological monitoring (Shew et al, 2010). In 2010 Johnstone et al described the needs that are now motivating developers as:

i. need for better understanding of the tens of thousands of chemicals currently used in commerce,
ii. need to increase number of chemicals characterised for potential toxicity,
iii. need to reduce the time,
iv. need to reduce cost,
v. need to reduce the number of animals used in contemporary tests.

Early MEA system development focused upon elements demonstrated in Figure 2.28. The upshot of this focus and the consequent development has been that the elements that are depicted as secondary and tertiary design priorities (see Figure 2.28) have become more significant to the overall success of the whole system in application.

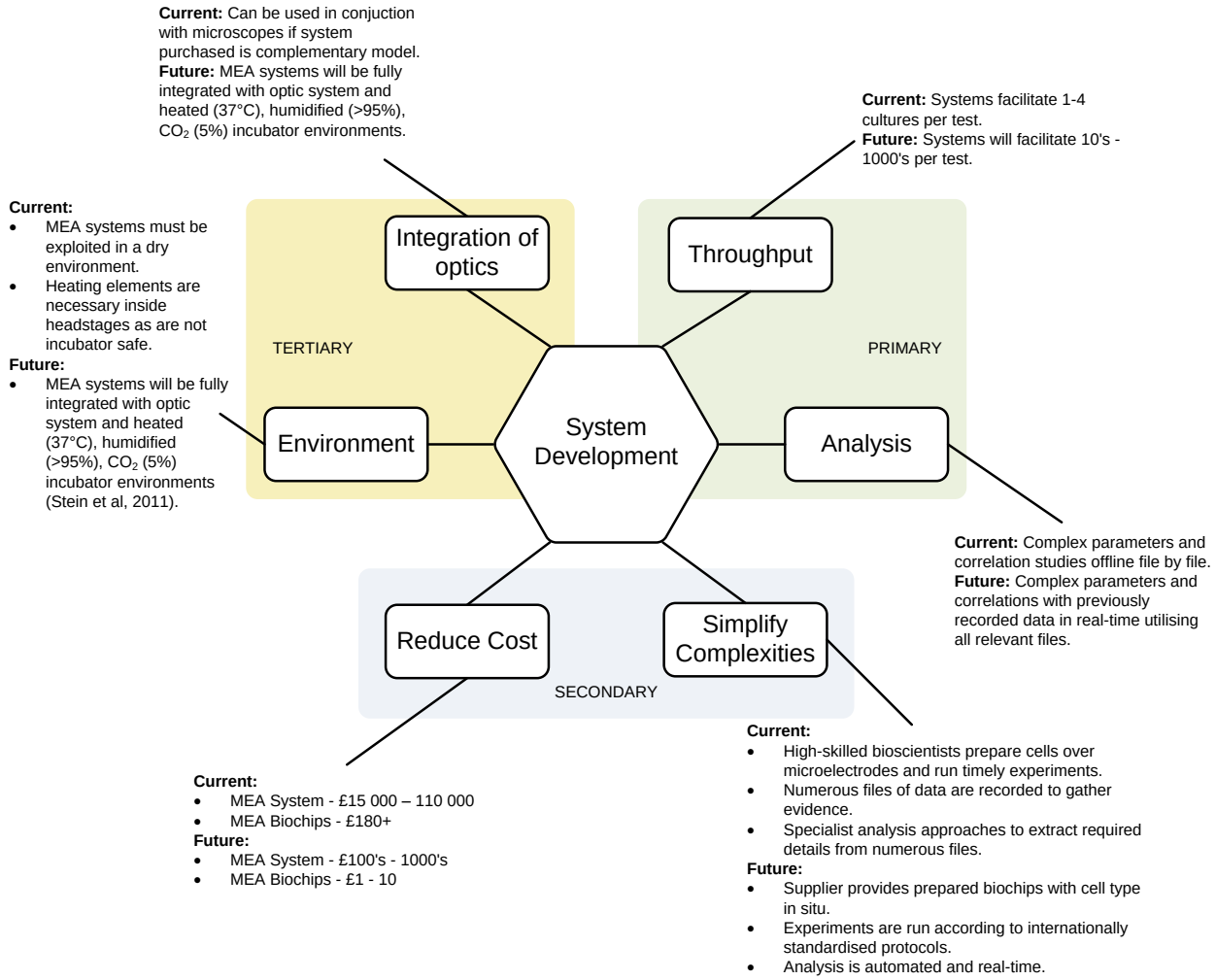


Figure 2.28: MEA system development focuses and their prioritisation.

With regard to the increased emphasis upon environment and integration of optics, presently, the ability to record from cells and to ensure their viability throughout prolonged testing depends upon 1) maintaining an environment that ensures cell viability, and 2) providing optics that allow visual inspection of the cells (Li et al, 2008). These are important influences on MEA system design that do not necessarily appear as essential design parameters as they do not directly influence the data that is collected. Nonetheless, without adequate design for these requirements novel MEA solutions will fail to find support amongst scientist users and applications.

The points highlighted describe motivations of developers, as well as cost and timing concerns associated with employment of this technology on the larger scales currently being demanded. The cell source is also a significant area for designers as new cell sources thought to be more suited to specific applications are being successfully derived and validated that may require different biochip

parameters or system settings. For example MEA biochips specifically for slices (perforated MEA, Figure 2.25) are being developed that connect to headstages that have different gains (x550) as when compared to a gain setting used in a headstage that is for cultured neurons (x1100) (Kopanitsa et al, 2006).

2.6.5 Manufacturing Approach and Materials Advancement

Microfabrication is a general term used for describing the construction of microstructures ranging in size from a few to hundreds of micrometres (Kobel and Lutolf, 2010). MEA biochip developers have experimented with microfabrication approaches in line with their emergence as valid manufacturing capabilities over the past few decades (He et al, 2000), and the use of microfabrication techniques to construct specialised tools for stem cell biology is now becoming common place (Lutolf, Gilbert and Blau, 2009). This uptake of micrometre scale tooling has been motivated by a combination of lower reagent consumption, high throughput capacities and shorter analysis times (Maerkl, 2009).

2.6.5.1 Microfabrication

There are three clear schools of thought amongst those who exploit microfabrication approaches for MEA biochip advancements:

1. To make more electrodes, smaller and positioned closer together (Aziz et al, 2007; Charvet et al, 2009) for greater detail of activity in recorded data.
2. To incorporate growth pathways or patterning into biochip workspaces using specific biocompatible materials (Wonhee et al, 2010; Kunze et al, 2010).
3. To design MEA biochips and electrode configurations to fit specifically to physiological tissues or regions of interest (i.e. in-vitro MEAs for stimulation of murine hippocampal slices (Gholmieh et al, 2006), or ex-vivo Langendorff heart recordings via flexible MEAs (Stett et al, 2003)).

Arrays with hundreds of microelectrodes at a high spatial density (i.e. 256 - 4096 microelectrodes) are being encouraged as results obtained to-date indicate that improved spatial precision will allow the simultaneous recordings from numerous dissociated cells without need for skilful control of positioning cells. The possibility to measure potentials at a synaptic level would also be more likely and that would be highly exploitable by neuroscientists working to understand the functioning of the brain (Berdondini et al, 2009).

2.6.5.2 Rapid Prototyping

Modern technologies such as rapid prototyping techniques are also being explored within this domain for their suitability to produce customised arrays (Claverol-Tinture et al, 2005). The work of Morin et al (2005) is an example of a new method of fabrication for microelectrode arrays based on rapid prototyping concepts (Figure 2.29). The outcomes of this work have shown promise as cells were generally found to grow readily in the specific microchannels formed (in PDMS).

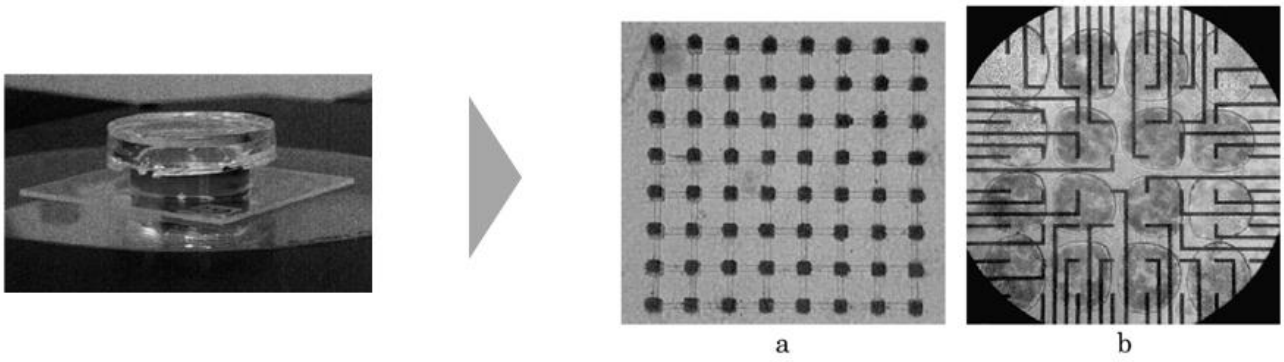


Figure 2.29: New method of fabrication for microelectrode arrays based on rapid prototyping concepts using photolithographic masks printed on OHP sheets and WL5370 (a photo-patternable silicon from Dow Corning, Michigan, USA). (a) Commercial pMEA (Panasonic Medprobe) fitted with patterns with 50µm^2 chambers and 25 µm-wide channels. Electrodes are 50µm^2 and give the scale of the picture. (b) Custom pMEA fitted with larger patterns: chambers are 600µm in diameter and channels are 30µm wide. Wiring tracks are 50 µm wide and give the scale of the image.

[Adapted from Morin et al, 2005.]

2.6.5.3 Substrates for manufacture.

Flexible MEAs that can be curved around tissue and organ structures have also been fabricated (Myllymaa et al, 2008; Lacour et al, 2010). These devices are most suited to *in vivo* applications but material and manufacturing techniques have also been applied to produce devices for *ex vivo* applications such as brain slice study (Boppart et al, 1992). The recent emergence of MEAs made on thin glass substrates, allowing superior optical monitoring (Multi Channels Systems, 2011) has also supported the aforementioned need to incorporate adequate optical characteristics into the biochip and headstage design.

2.6.5.4 Biomaterials

Discussion across design of items for cell culture seeks to investigate the sensitivity of different cell types to biophysical factors (e.g. stiffness and density of the growth substrate) in culture (Vickerman et al, 2008; Chou et al, 2009; Gilbert et al, 2010). This questioning has come about concurrently with the development of, and increased application of, soft biomaterials (such as soft hydrogels), intended to mimic the elastic properties of the living tissue in which the cells in culture would have

originated (such as that of the brain when investigating the mechanisms of neuron differentiation), as cell culture substrates in micro-fabricated platforms (Flaim et al, 2005; Cordey et al, 2008).

2.6.6 The Cell-Electrode Interface

The interpretation of MEA recordings has been challenged by a lack of a definitive detailed electrical understanding of the cell-electrode interface (Jenker et al, 2001). Numerous theoretical models have been considered similar to the one demonstrated in Figure 2.30 (Stett and Kindervater, 2008).

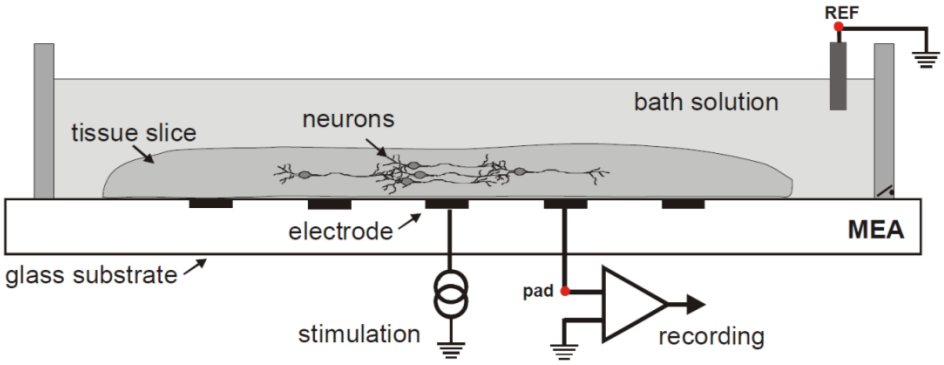


Figure 2.30: Stimulation and recording of electrical activity in tissue slices with a planar MEA biochip. Substrate-integrated microelectrodes can be used for both stimulation and recording.
[Stett and Kindervater, 2008.]

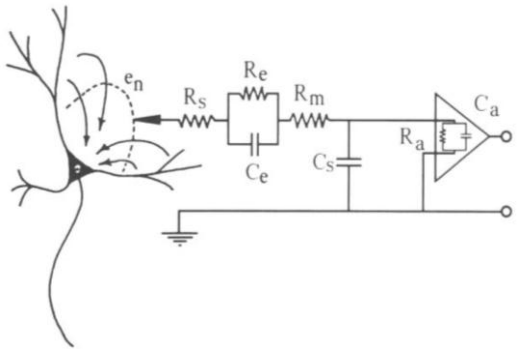
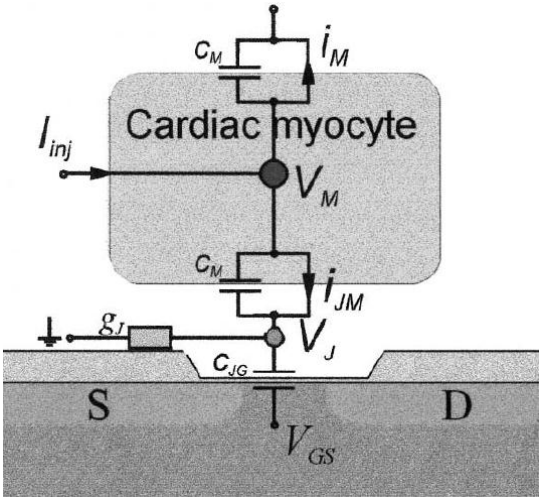
This interface is particularly important to understand if applications require electrical stimuli to be delivered to the cells in culture because the conditions for extracellular stimulation are affected by a delicate balance of interface geometry and ion channel dynamics in the target cell's membrane (Kopanitsa et al, 2006). The region of depolarisation is known to depend upon the cell-electrode interface in terms of orientation and extracellular matrix (ECM) meaning that the signal shapes recorded from a cell sample may have been different if the same sample where placed on a different MEA biochip in a slightly different position (Buitenweg et al, 2002).

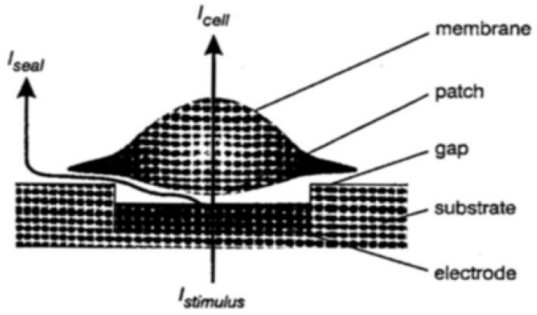
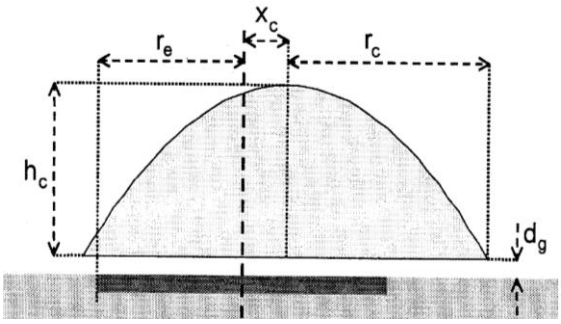
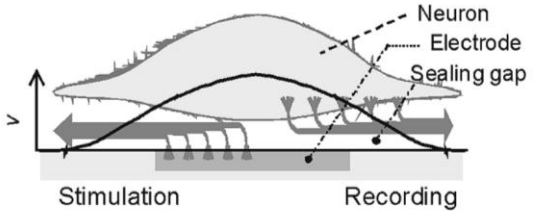
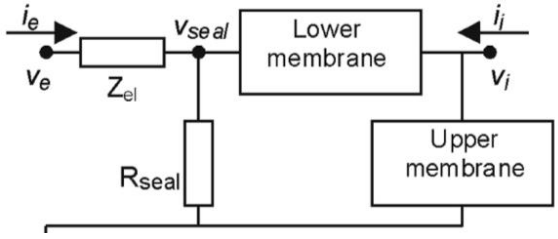
There is still no single complete theoretical description of the interface that occurs between a cell (or tissue) and a planar metallic microelectrode that has been universally accepted. It has been suggested that the work of Buitenweg et al (2003) is the best so far as it is the first example of a model that attempts to incorporate the ion channel influences on local membrane properties. Finite Element Analysis (FEA) models studying both the electrical properties of the contact between a passive cell membrane (Buitenweg et al, 2002) and of a membrane containing voltage-gated ion channels (Buitenweg et al, 2003) over a planar electrode has been constructed allowing theoretical consideration of different influences on parameters that are not presently possible to physically

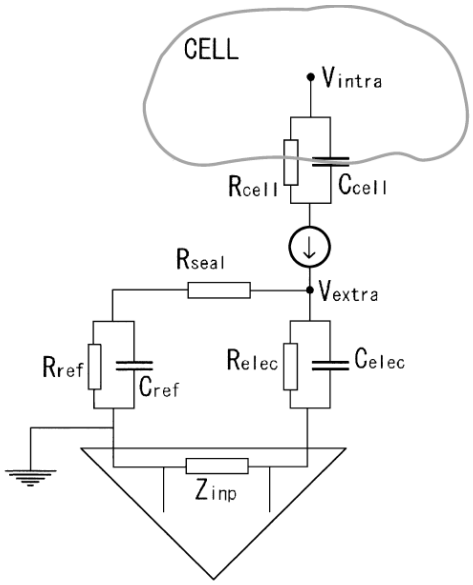
measure. The results of this work offered theoretical spatial and temporal information about the combined electrical behaviour of the extracellular space, the electrode-electrolyte interface and the cell membrane but additional considerations and reformulations where suggested as further work.

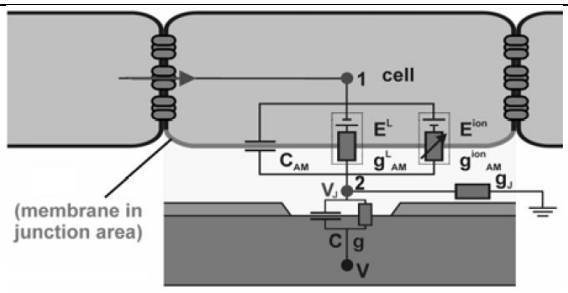
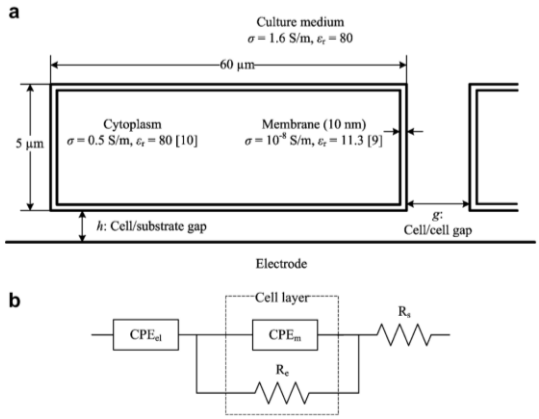
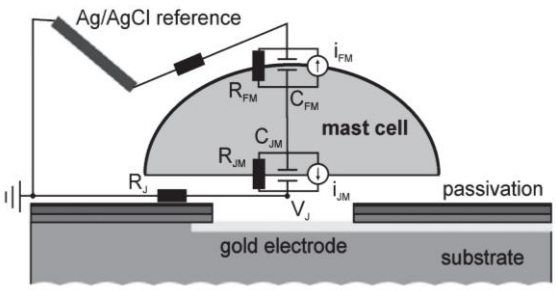
A collection of the state-of-the-art cell-electrode models are provided in Table 2.2 addressing neurons and cardiomyocytes. The coupling between the cell and electrode is a critical parameter in determining the quality of the acquired signal, in terms of both the signal shape and amplitude (Grattarola and Martionoia, 1993). All cell-electrode interface models consist fundamentally of a cell, an electrode and the seal between the two, commonly refered to as the seal resistance. The seal resistance, the electrode parameters and the shunt capacitance of the interface are all documented as having a combined influence over the overall signal acquisition (Nisch et al, 1994; Buitenweg et al, 1998; Yeung et al, 2008). Note: the discriptions accompanying each diagram are as stated in the referenced article.

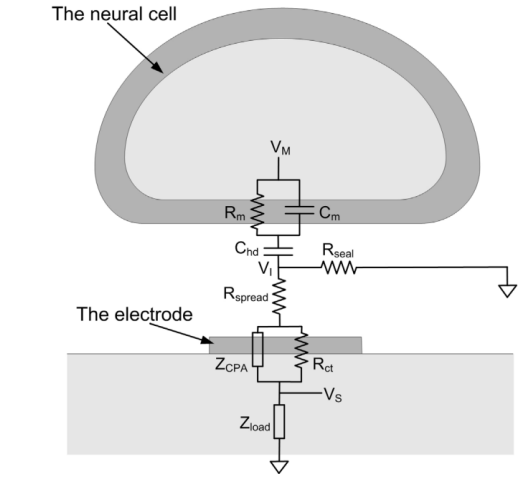
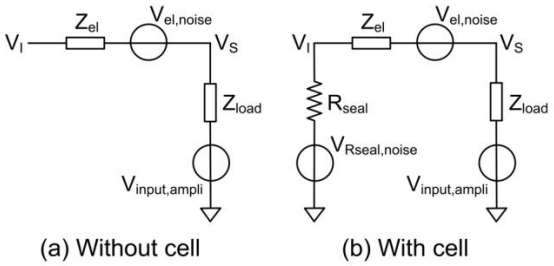
Table 2.2: Examples of proposed models of cell-electrode interfaces in MEA biochips.

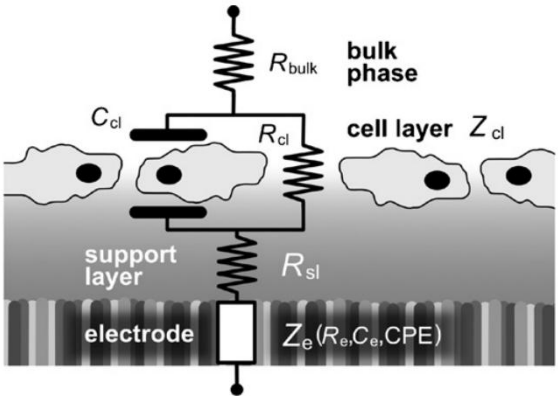
Authors and Year	Paper and model diagram	Supporting notes
Nisch et al, 1994	**A thin film microelectrode array for neuronal activity in vitro.** 	Where: e_n – isopotential line of neuron R_s - resistance of saline between metallic interface and ground electrode R_e and C_e - resistance and capacitance of the double layer R_m - Resistance of the metallic portion of the electrode C_s - shunt capacitance to the ground R_a and C_a - input resistance and capacitance of the amplifier
Ingebrandt et al, 2001	**Cardiomyocyte-transistor-hybrids for sensor application.** 	Where: g_J – cleft of electrolyte between the membrane and the gate (seal conductance), C_{JG} the specific capacitance of the gate, C_M - specific membrane capacitance, V_J extracellular voltage, i_{JM} - current through the membrane, V_M - intracellular voltage
Buitenweg et al, 1998, 2002, 2003	**Measurement of the sealing resistance of cell-electrode interfaces in neuronal cultures using impedance spectroscopy (1998).** **Extracellular Stimulation Window Explained by a Geometry-Based Model of Neuron-Electrode**	

	Contact (2002). **Geometry-based Finite Element Modelling of the Electrical Contact Between a Cultured Neuron and a Microelectrode (2003).**  (1998)  (2002)  (2003a)  (2003b)	1998: Geometry of the neuron-electrode interface. 2002 & 2003b: R_{seal}- the current path through the gap between the bottom of the cell and the surface of the substrate. r_c- circular soma radius. r_e- electrode radius. d_g- sealing gap thickness. 2003a: The neuron-electrode interface in extracellular stimulation and recording. Due to current densities, arising from the neuronal membrane of the electrode, a potential distribution exists in the sealing gap which modifies the membrane potential (stimulation) or can be probed by the electrode (recording). 2003b:Electrical equivalent lumped circuit which is commonly used as a model of the neuron-electrode contact.

Morin et al, 2005	**Investigating Neuronal Activity with Planar Microelectrode Arrays: Achievements and New Perspectives.** 	For planar electrode, where: C, Ionic layer capacitance; Rs, spreading resistance; Rt, charge transfer resistance; Zw, Warburg impedance; Zp, impedance associated with porosity; Rc, resistance of interconnects; Cp, capacitance to electrolyte; Cc, coupling capacitance between interconnects; Cs, capacitance to substrate; Vintra, Intracellular potential; Vextra, extracellular potential; Rseal, sealing resistance (a measure of the quality of the contact between cell membrane and electrode surface); Relec and Celec, resistance and capacitance of the recording electrode, respectively; Zinp, input impedance of the amplifier; Rref and Cref, resistance and capacitance of the reference electrode, respectively; Rcell and Ccell, resistance and capacitance of the cell membrane, respectively.
Sommerhage et al, 2006	**Simulation of extracellular recorded cardiac action potentials.**	"The standard Point-Contact-Model for cardiac myocytes". This model can distinguish inputs for different ion currents.

		
Cho and Thielecke, 2008	**Electrical characterisation of human mesenchymal stem cell growth on microelectrode.** 	(a) Schematic model of cell monolayer on electrode for FEM simulation (not scaled), σ and εr: conductivity and dielectric constant. (b) Equivalent circuit. CPE_{el} – constant phase element for electrode impedance. R_e – extra cellular resistance. CPE_m – constant phase element for impedance of cell membrane. R_s – spreading resistance.
Yeung et al, 2008	**The Use of Microelectrode Array (MEA) to Study Rat Peritoneal Mast Cell Activation.** 	Where: R_j - seal resistance typical distance values for the membrane from surface lie in range of 10s of nm to 100nm (ref171) resulting in values of 1-10MΩ. V_j - contact point. This model is for mast cells that have slower signals than neurons and cardiomyocytes so capacitive components have been excluded.

Joye et al, 2008 and 2009	**An electrical model of the Cell-Electrode Interface for high-density Microelectrode Arrays (2008). A Cell-Electrode Interface Noise Model for High-density Microelectrode Arrays (2009).**   	Point-contact model of the cell-electrode interface (not to scale). Z_{CPA} – constant phase angle impedance (represents the interface capacitance in parallel with a charge transfer resistance). R_{ct} – charge transfer resistance. R_{spread} – spreading resistance. R_{seal} – sealing resistance. Z_{load} – load impedance of the cell-electrode interface system. C_{hd} - cell membrane-electrolyte capacitance R_m and C_m - membrane resistance and capacitance. Equivalent cell-electrode interface noise model for (a) no cell and (b) one cell lying on top of the electrode. Z_{el} – the electrode impedance. $V_{el,noise}$ – electrode noise voltage. $V_{input,ampli}$ – the input referred noise of the amplification stage of a CMOS-based MEA. $V_{rseal,noise}$ – seal resistance noise voltage.
Krinke et al, 2009	**A microelectrode-based sensor for label-free in vitro detection of ischemic effects on cardiomyocytes.**	Where: Z_e – impedance of the MEA system, comprising of an RC circuit in parallel (R_e, C_e) and an additional constant phase element (CPE, A, n) in series. R_{sl} – resistance of the support layer for cell adhesion. Z_{cl} – impedance of the cell layer

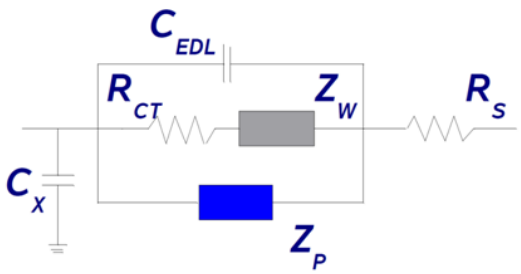
		constituting of an RC circuit in parallel (R_c,C_{cl}). R_{bulk} – resistance between the cell layer and the large counter electrode.

2.6.6.1 Cellular sealing and signal-to-noise ratio.

A number of different attempts to clarify and understand the influences of the "sealing" that occurs between a cell and its underlying electrode have been published, aiming to help interpretations of amplitudes and waveform shapes recorded by MEA systems. This literature also suggests that the signal-to-noise ratio of recordings increases if a cell completely "seals" an electrode, thus enhancing signal quality (Hofmann et al, 2011). The stimulation pulse required to evoke particular responses also decreases when the stimulating electrode is completely covered by the cell (Bove et al, 1995). By decreasing the sealing gap using adhesion promotors significant improvements can also be made to the quality of the signals acquired. Matrigel™ in an example of such an adhesion promotor shown previously to favourably support human embryonic stem cells (hESCs) (Braam et al, 2008).

2.6.6.2 Simplified interface circuitry

The following models (Figure 2.31 and Figure 2.32) offer a simplified summary of the theoretical models collated in Table 2.2.



Component	Value
C_{EDL}	125 pF
R_{CT}	3 MΩ
Z_W	$(1-j)0.39 / \sqrt{f}$
Z_P	$(1-j)5e6 / \sqrt{f}$
R_S	7 KΩ
C_X	2.5 pF

Figure 2.31: Cell-electrode model and calculated values of components of the interface model. C_{EDL} – capacitance of Electrical Double Layer, R_{CT} – charge transfer resistance, Zw – Warberg impedance, Zp – impedance of the porous surface, Rs – spreading resistance, Cx – parasitic capacitance.

[Adapted from Al-Gayam et al, 2010.]

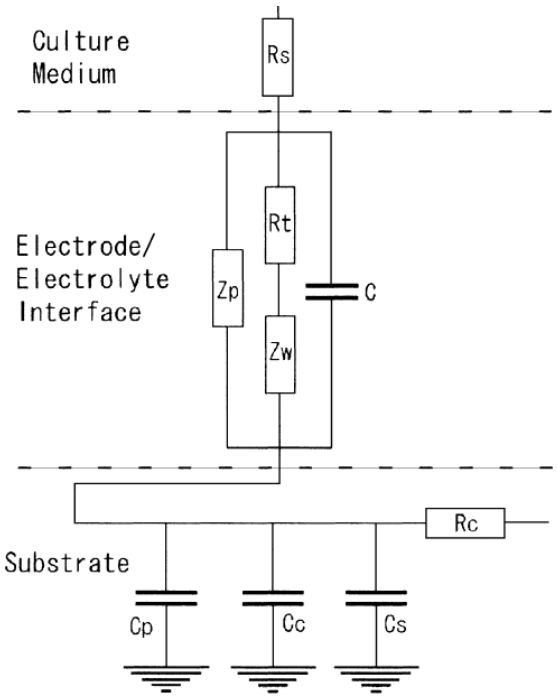


Figure 2.32: Equivalent circuit for a planar microelectrode array element. Where C - Ionic layer capacitance, Rs - spreading resistance, Rt - charge transfer resistance, Zw - Warburg impedance, Zp - impedance associated with porosity, Rc - resistance of interconnects, Cp - capacitance to electrolyte, Cc - coupling capacitance between interconnects, and Cs - capacitance to substrate.

[Adapted from Kovacs (1994) by Morin et al (2005).]

2.7 Contemporary Application

MEA technology is most commonly applied in cardiac and neural applications (Proceedings of MEA Meeting 2008). Other tissue types are discussed in this document as niche applications due to a lesser volume of published work validating protocols and findings (section 2.7.3).

It is possible to use MEA technology with cells that have been dissociated from animal tissues or differentiated from various stem cell sources (Charlesworth et al, 2006). Cells can either be in the form of cultures or thin tissue slices dissected directly from animal subjects. The data yielded can be analysed to provide information for a number of differing research problems that may be considered to be within the fundamental research domain, for example “what happens electrically when this cell contracts?” or, within an applied research domain, such as “will this drug applied at high concentrations induce heart rhythm abnormalities?”

The cell source used will result in differing application strategies in terms of how the cells are positioned and cultured to ensure a recordable signal. For example, when seeding beating cardiomyocyte clusters that have been differentiated from stem cells the challenges in terms of positioning those cells over the electrodes differs from that of positioning a heart slice dissected from a chicken embryo. Where in both cases a close cell-electrode interface is required, but in practical terms the achievement of this is pursued differently (see Chapter 4).

2.7.1 Cardiac MEA Application and This Research.

MEA systems are used to characterise cardiomyocyte preparations for both fundamental (e.g. understanding cell-cell communication) and applied (e.g. chemical entity effects on a cell type) research purposes. For this research, focus has been placed upon the stem-cell derived cardiomyocyte preparations used by the collaborating institute, the University of Nottingham (UoN), in applied cardio-active substance research. The main topic of interest at the UoN is in the investigation and measurement of QT-interval changes following addition of chemical substances (e.g. drugs and drug candidates) and combinations of chemical substances.

The cell sources used were human embryonic and human skin cell-induced pluripotent stem cells. From these stem cell lines cardiomyocytes were differentiated *in vitro* that could be used to monitor substance effects via correlations that have been identified (Denning and Anderson, 2008) between field potentials from these cells and ECG signals recorded from human hearts.

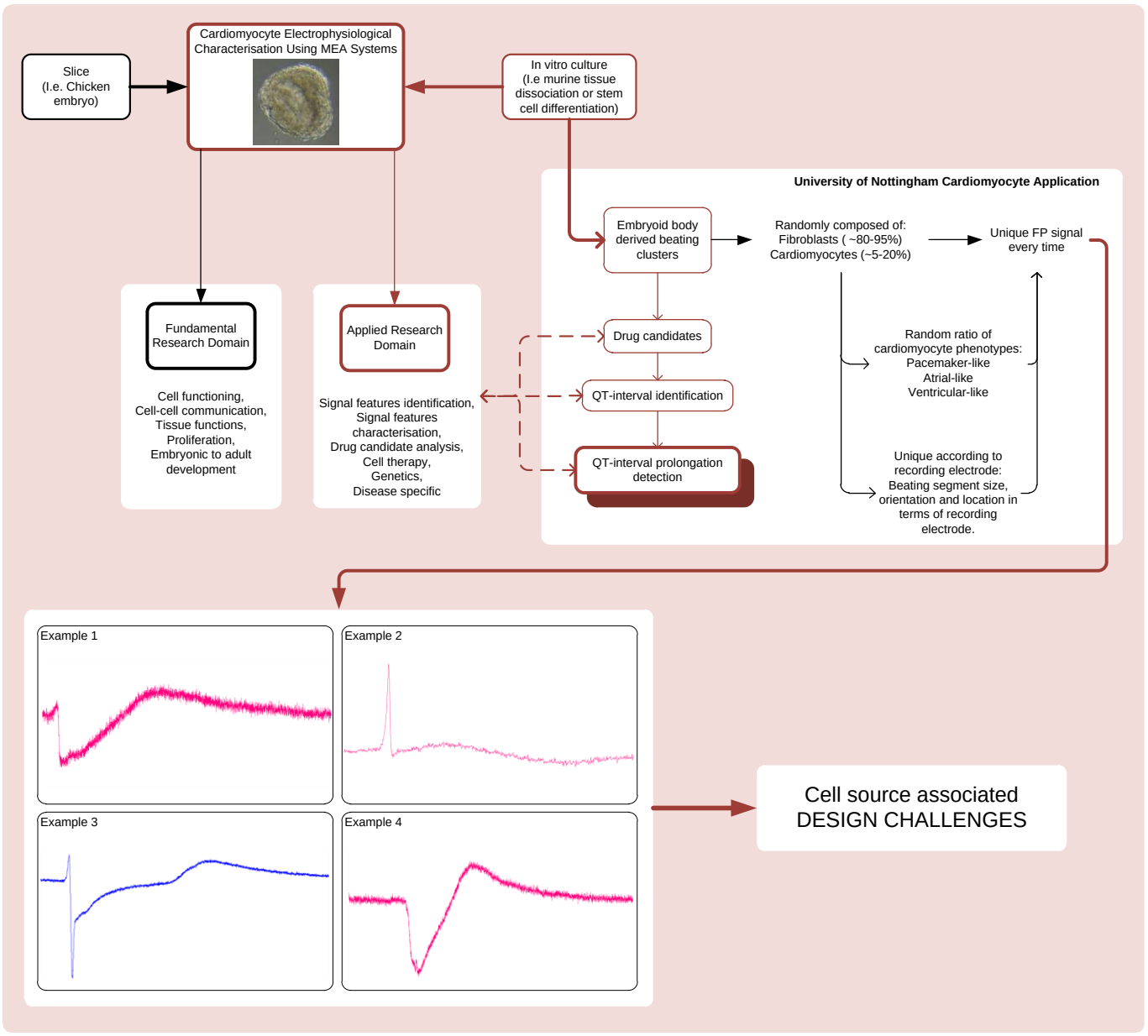


Figure 2.33: Contemporary application of MEA systems for cardiac and specifically stem cell-derived cardiomyocyte based research.

The research conducted at UoN has been limited by the variability that exists in stem cell derived cardiomyocyte clusters. The beating cardiomyocyte clusters that are dissected out and used for recording are typically composed of <20% cardiomyocytes. The inherent variability of these clusters results in a different MEA recording for each cluster as no two clusters are the same. Examples of four signals captured from four different beating cardiomyocyte clusters seeded over an MEA workspace are demonstrated in Figure 2.33.

2.7.1.2 International guidelines

Over the last 10-15 years the QT-interval has been under investigation by the pharmaceutical industry and global regulatory bodies (Braam et al, 2010). Major losses in the region of hundreds of millions dollars have been incurred on drugs developed and marketed that have later had to be withdrawn due to unforeseen interference with the human QT interval (Frantz, 2007).

At around the start of the millennium, in attempts to prevent QT interval altering drugs from making it to the market, the International Conference on Harmonisation's Global Cooperation Group, comprised of representatives from Europe, Japan and the USA, produced two guidelines to be used by all parties involved in drug development. The document S7B (Appendix B) endorses nonclinical methodologies of QT interval assessment and the document E14 contains the clinical equivalents. It is intended that together these two documents will contribute to a better understanding of the link between results derived in a laboratory setting and QT prolongations as they actually occur in humans. In terms of MEA system application the document S7B is of greatest relevance as it emphasises the need to assess all new chemical entities, intended as cardiac therapies or otherwise, for any alteration to cardiac ventricular repolarisation.

2.7.1.3 Using stem cell-derived cardiomyocytes

Each time a new beating cardiomyocyte cluster signal is detected and visualised using MEA technology a unique contraction waveform is identifiable. This is due to the novel composition of the beating cluster. The resting potentials (signal baseline) of the cells also alters throughout development towards maturity. In early embryos it is typically at around -40 to -50mV. This changes progressively as the cells develop toward adulthood to approximately -75 to -85mV. The maximum rate of rise of an AP upstroke velocity also increases during this growth period from about 20V/sec to about 200V/sec. Consequently, the same beating cluster will show differing signal waveform characteristics as it matures (Banach et al, 2003; Marin-Garcia, 2011). Comparisons have shown hESC-derived cardiomyocytes are morphologically relatively immature but that they do contain the appropriate ion channels and signalling pathways previously been shown to be modified by specific cardiac drugs (Kohl et al, 2005; Harding et al, 2007).

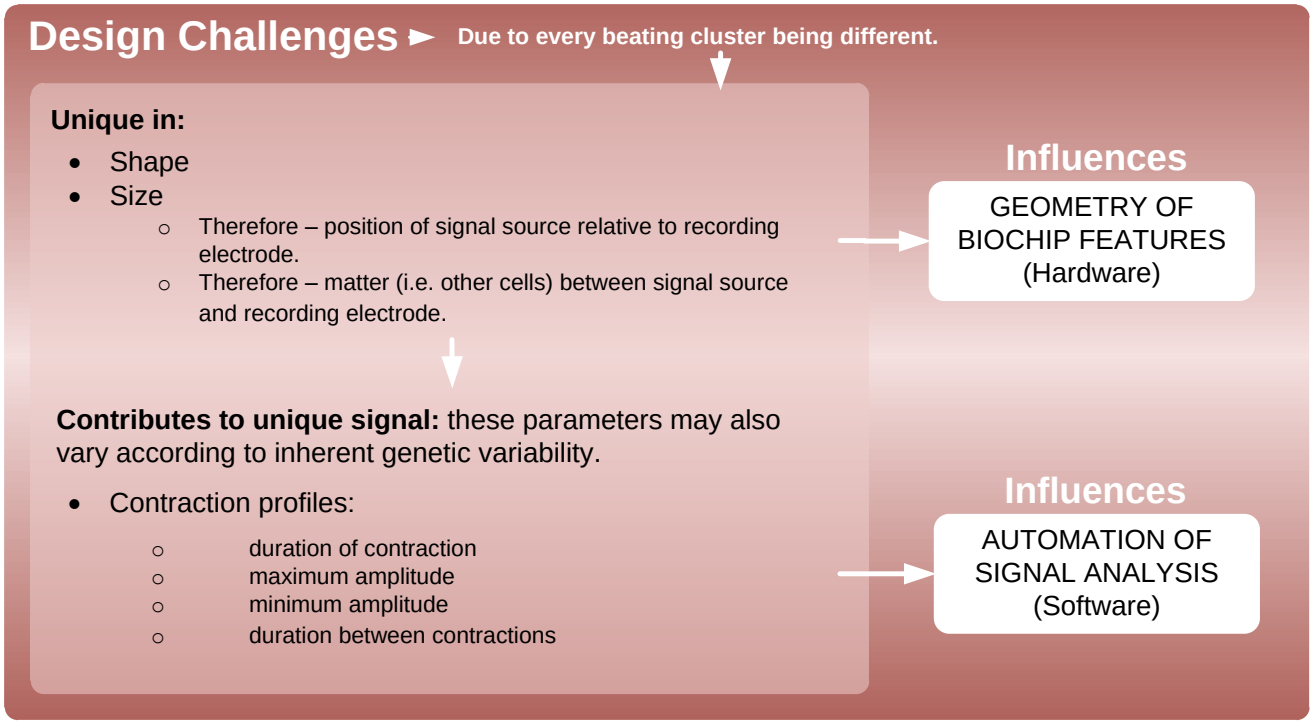


Figure 2.34: Current design challenges in the cardiac domain.

The design challenges that are introduced due to unique nature of each beating cardiomyocyte cluster were collated in Figure 2.34 for this research.

2.7.1.4 Cell therapy

In-depth understanding of under lying cellular mechanisms of cells could provide the most promising route toward their optimisation for cell therapy. To obtain such information by trial and error would take significant time and resources and expose patients to substantial risk. A methodical characterisation is required that also accommodates overall therapy safety. MEA systems will support safe methodical electrogenic cell characterisation.

2.7.1.5 Commercial QT measuring MEA systems

The first commercial system and enterprise to address the needs of MEA QT-interval assessment were QT-Screen by MCS, Germany and the QTempo service conceived and offered by ReproCELL, Japan.

In the QT-Screen system (Appendix B) a ninety-six well plate specifically adapted to record from beating cardiomyocyte clusters has been the focus of design efforts. The format aims to seed beating clusters of single cardiomyocyte cells to each well for standardised QT interval analysis.

Note: employment of a 96-well plate could be very labour intensive depending upon the preferred laboratory protocols of the user. No independent publications that have employed this system have yet been identified.

The QTempo offers a service whereby functional assays are used to detect functional waveform responses. Standard MEA systems (MCS MEA60) are used and analysis is performed as part of the service, resulting in results provided to the relevant stakeholders in the form of portable document format (.pdf) reports. ReproCELL's QTempo business model is included in Figure 2.35.

	Receiving patient tissue	Generation of pluripotent cell strains	Differentiation into cardiomyocyte	Measurement and analysis of cardio-toxicity
Patent		• Composition of culture medium		• High throughput measurement instrument • Composition of measurement medium
Tangible asset		• Standard protocol • Culture reagents such as medium, detachment solution, and stock solution	• Standard protocol	• Measurement instrument (generic) • Analysis software (generic)
Know-how	• Patient tissue collection and inspection • Biological product safety management	• Characterization of obtained hES/iPSCs • Cell strain selection method • Undifferentiated cell culturing method	• Strain-dependent differentiation method	• Differentiated cell seeding method
External network		• Supply agreement of hESC strains • Licensing agreement with two academic institutions	• Licensing relationship (differentiation method) • Advisory contract with four experts	• Patent / tangible asset licensing agreements from business partners

Figure 2.35: Business system QTempo.

[Adapted from Sengoku et al, 2010.]

2.7.2 Neural

Originally developed for neural applications MEA systems have been developed to suit specifically the needs of a plethora of neurological investigation domains. A brief summary of slice and culture applications in shown below (Figure 2.36).

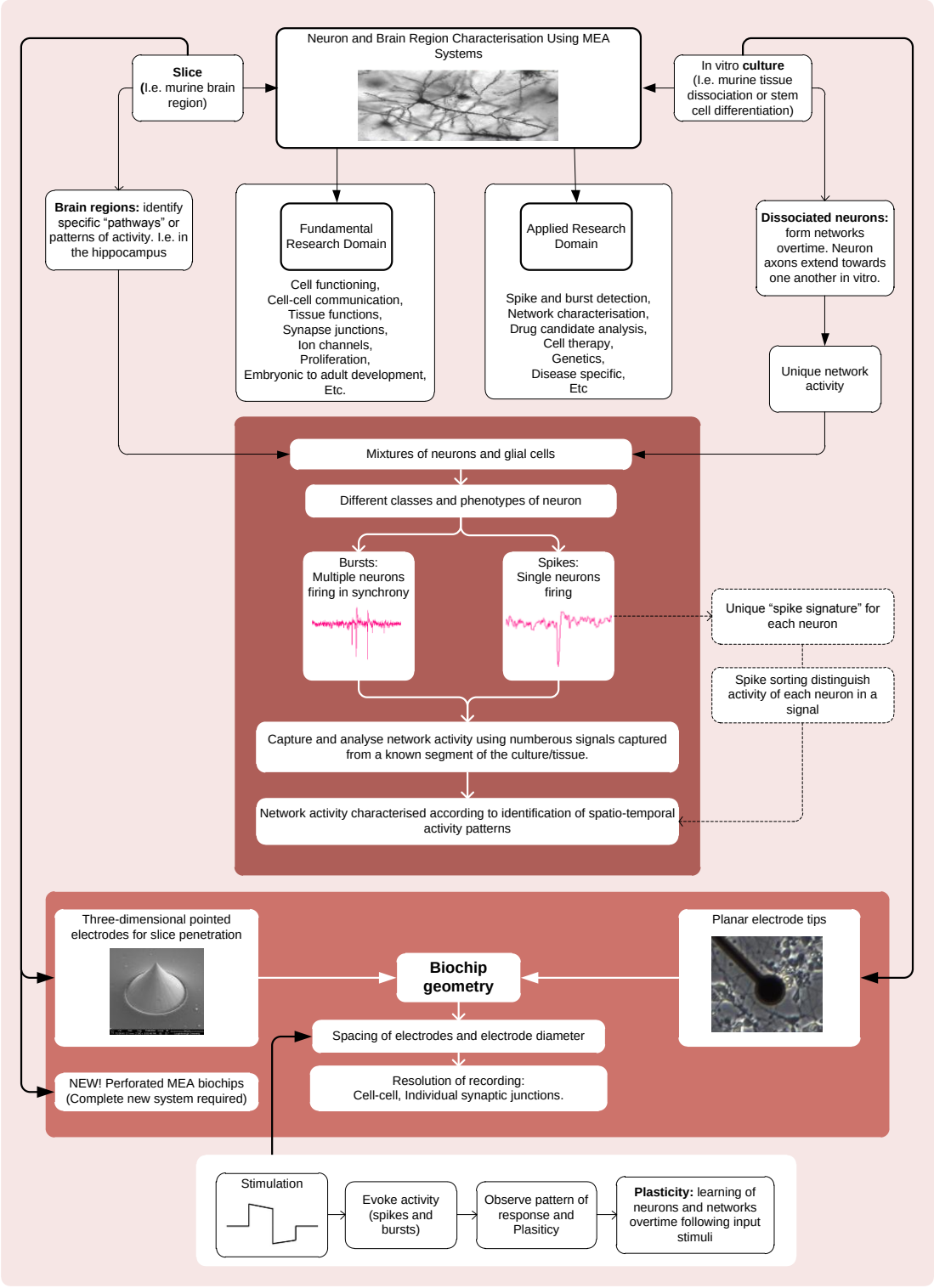


Figure 2.36: Contemporary application of MEA systems for neurology based research.

The patterns of electrical activity that occur in the brain are complex and rapid (Hill et al, 2010). There is still much that is unknown about the mechanisms that underpin the functioning of the mammalian and specifically the human brain (Hinton, 2002).

MEA systems were conceived and initially built by neuroscientists (Thomas et al, 1972). Neural tissue can be studied on MEA biochips in one of two forms of preparation (Potter, 2001). Preparations are

either dissociated cells cultured over the MEA biochip's surface or are thin slices precisely anchored in place over the microelectrode array. The cell preparation will influence which type of MEA biochip is best suited to use.

Planar, 3D, perforated planar and CMOS biochips have been introduced, resulting in improved signal quality, longer testing times (e.g. minutes to hours) and ever greater degrees of resolution (e.g. from cell-cell towards synapse-synapse). Work of neuroscientists is moving deeper into how neural cells communicate, by shifting from the study of individual cells towards individual synapses (Berdondini et al, 2009).

2.7.2.1 Stimulation studies

MEAs have been used in experiments aiming to decipher and comprehend the cellular mechanisms of learning and memory. Electrical stimuli have been used to induce activity and monitor plasticity. Stimuli and the tissue responses have been recorded and observed using MEA systems (Ide et al, 2010; Li et al, 2010), with particular brain regions showing reproducible results on comparable set-ups (Boehler et al, 2012).

2.7.2.2 Cell preparations

Dissociated neurons can be sourced from brains or brain regions that have been dissected out of animals or that have been differentiated from a stem cell source. Dissociated neurons can then be dispensed over a treated MEA workspace where a unique neural network will develop *in vitro*. These cultures can be maintained for periods of months (Dubois-Dauphin et al, 2010). Slices can be used if anchored securely over an MEA workspace. These preparations are usually maintained for periods of hours. In slice preparations, pathways of activity that already exist in tissue can be observed and interacted with via targeted stimuli input. In dissociated cultures the part of the neuron that successfully survives the dissociation process is the soma (neuron's main cell body). Axons and dendrites are damaged and break off during centrifuge stages. The somas are suspended in media. A small sample of the suspension is examined and the number of cells counted so that an approximately controlled quantity of cells can be seeded per MEA. This process allows culture monolayers if desired. Axons and dendrites re-grow towards one another creating unique networks that start to spontaneously interact.

2.7.2.3 Tissue composition, spike signatures and spike sorting

Both slice and dissociated cultures will be made up of a mixture of cell types. Glial cells will be present in all cases as they are essential for neuron survival. The activity that occurs through the neural samples is referred to as spikes and bursts. A key feature of the spikes, that collectively also make up bursts, is that each spike recorded on an MEA will be unique. Therefore each neuron is described as exhibiting its own spike signature. The signature is due to physical differences between the neurons and its unique position in relation to the recording electrode (Quian Quiroga, 2012).

Specialist algorithms have been developed (Quian Quiroga et al, 2004; Pereda et al, 2005) that can separate out spike signatures thus allowing individual neurons to be identified and monitored. This process is called “spike sorting” and is regarded a fundamental first step in the majority of analysis routines for MEA system recorded neural data. Deeper degrees of understanding require on-going monitoring of network activity to identify patterns and determine the significance of those patterns.

2.7.3 Other/niche

Other tissue application domains where MEA technology has been previously demonstrated with some success include duodenum tissue (Lammers et al, 1993), stomach tissue (Nakayama et al, 2006), peritonea cells (Yeung et al, 2008), olfactory epithelium cells (nasal tissue) (Liu et al, 2010) and pancreas cells (Raoux et al, 2011) investigations (Figure 2.37).

Potentials exist in “non-electrogenic” tissues that alter slowly. Slow field potentials that occur across muscle tissues of the gastrointestinal tract of small mammals have been successfully detected using MEA technology (Lammers et al, 1993; Nakayama et al, 2006). Note: Should MEA technology be adopted by gastrointestinal research on larger scales, these types of studies will require systems that can record for prolonged durations without disturbance.

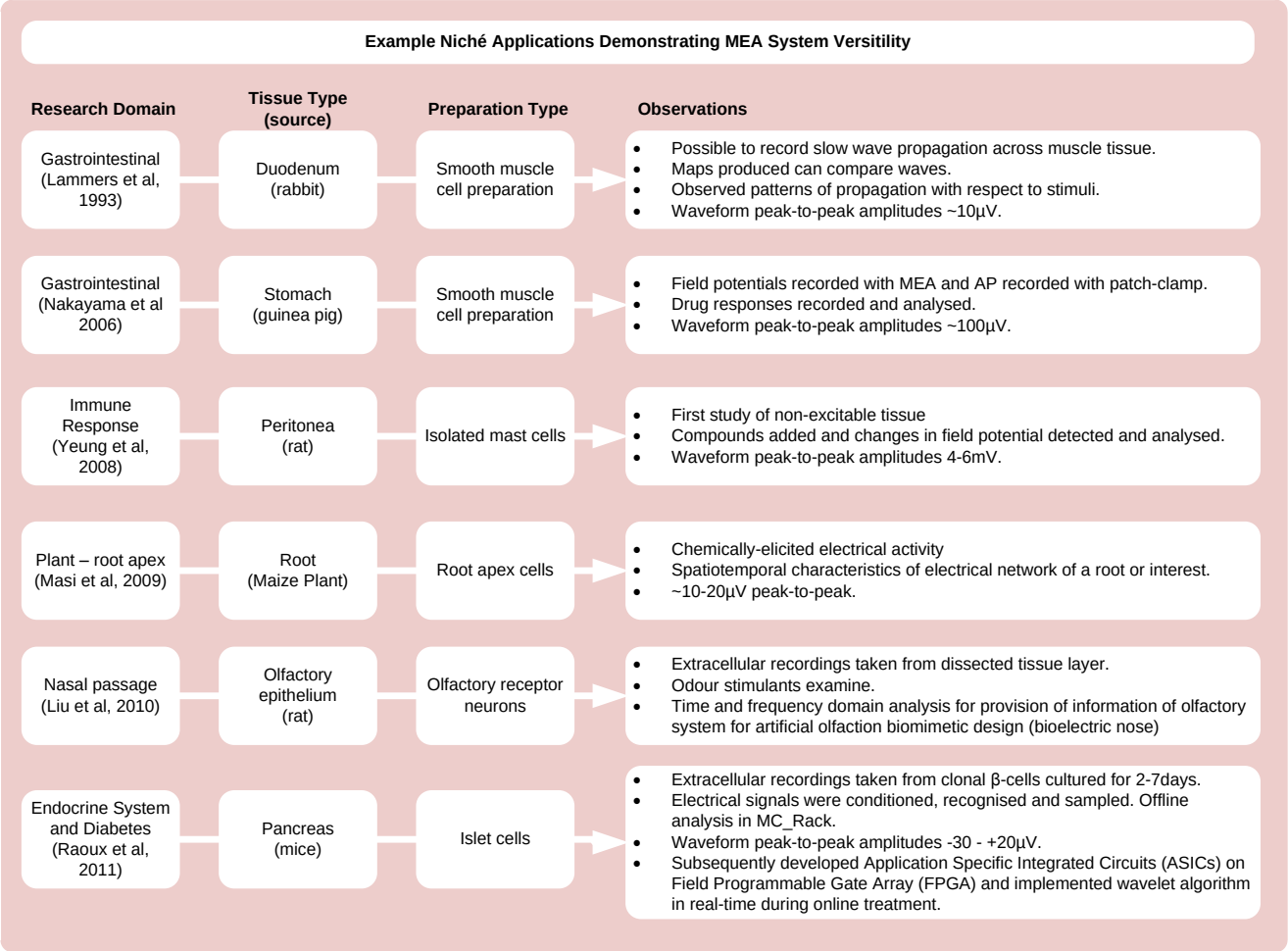


Figure 2.37: Domains where MEA technology has been successfully applied to record field potential fluctuations over time.

The electrical response of mammalian mast cells has been demonstrated (Yeung et al, 2008). Resting potential changes were observed in these cells showing successful application of an MEA system to collect data from cell samples that do not spike or burst.

With regard to tissues of the nervous system, it is not just the study of neurons that have been demonstrated on MEA systems. Receptor cells also produce detectable signals and have been studied using MEA technology to demonstrate and derive knowledge concerning activity so as to aid the development of a biomimetic device that hopes to recreate the functionality of real organs (Liu et al, 2010).

Non-mammalian cells have also been used on MEA systems (Masi et al, 2009). The electrical activity that occurs at the apex of a maize plant has also been detected and recorded for investigation.

2.8 Generic Analysis

The analysis of MEA signals is a broad and diverse field of expertise however in all cases the generic processes involve signal detection, recording and knowledge extraction (see Figure 2.38).

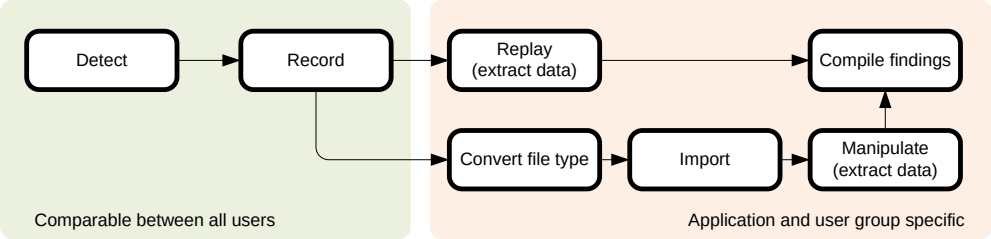


Figure 2.38: The elements of detection and analysis that are comparable between users and that are specific according to application and research group.

Addressing the area of MEA signal analysis involves determining, what is output as a signal and what are the features identifiable within those signals. All signals captured contain a roughly consistent flat line referred to as the baseline. This baseline typically carries high frequency noise components and stays roughly consistent at the resting potential of the cells/tissue (Prentice et al, 2011). The high frequency noise, present in varying degrees in all MEA recordings, can be attributed to noise generated by items in the surrounding environment (such as lights, nearby PCs or electrical monitoring devices, e.g. microscopes). The field potentials occurring across the living sample are identifiable when the signal line diverts from the baseline and beyond the noise. This typically occurs rapidly, resulting in what is called a spike or a series of spikes called a burst (Figure 2.39), or depending on the application, can be identified more gradually as either a positive or negative fluctuation of the baseline from initial value and back towards it (Figure 2.39).

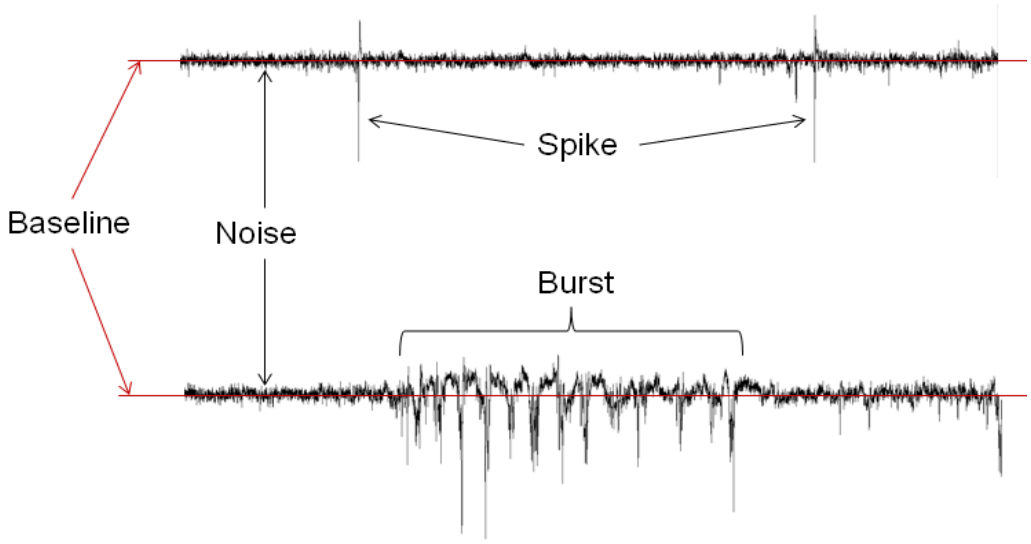


Figure 2.39: Two channels of neural data. Each corresponds to a single microelectrode. Spike and burst features are distinguishable and baseline noise identifiable.

MEA systems present potentials to human users as a voltage value to an accuracy of a few microvolts (μV). Spikes that occur above the noise level are identifiable visually on the graphical user interfaces that are provided with MEA system acquisition software. These interfaces are essential for users who must ensure that their preparations are active, and that the contacts between the cells and the microelectrodes permits recordings of a quality that is reliable and valid as a source of data.

In some cases the user may focus their attention on specific channels of data within the array, or alternatively they may be interested in the spatiotemporal activity over the entire array. In all cases the data acquisition software must accommodate the user's needs.

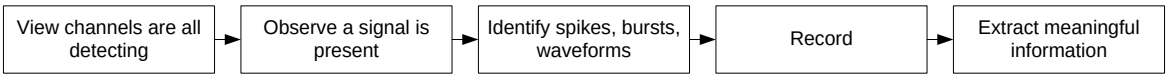


Figure 2.40: Basic fundamentals of MEA system use.

In Figure 2.40 basic user interaction with MCSs MC_Rack software is depicted with the purpose of demonstrating core functionalities required of all acquisition and analysis software. It is important to highlight that experimental outcomes vary from application to application according to the research questions of the scientist user groups.

All users will prepare their MEA biochip with a living biological sample of interest. A designed protocol will be used for testing. A reliable signal is required before executing an experiment. All users will insert the biochip into the system's headstage and wait for a few minutes to observe signals on an interface with a grid that corresponds to the microelectrode layout (Figure 2.41). Different experiments will quantify and assess different features within the signal shapes so users will initially observe signals prior to recording to ensure the feature(s) of interest is (are) identifiable. Once satisfied with signal presence and quality the user will conduct the experiment recording data as appropriate. The user will then analyse the recorded data in a fashion that best identifies and quantifies that feature(s) of interest.

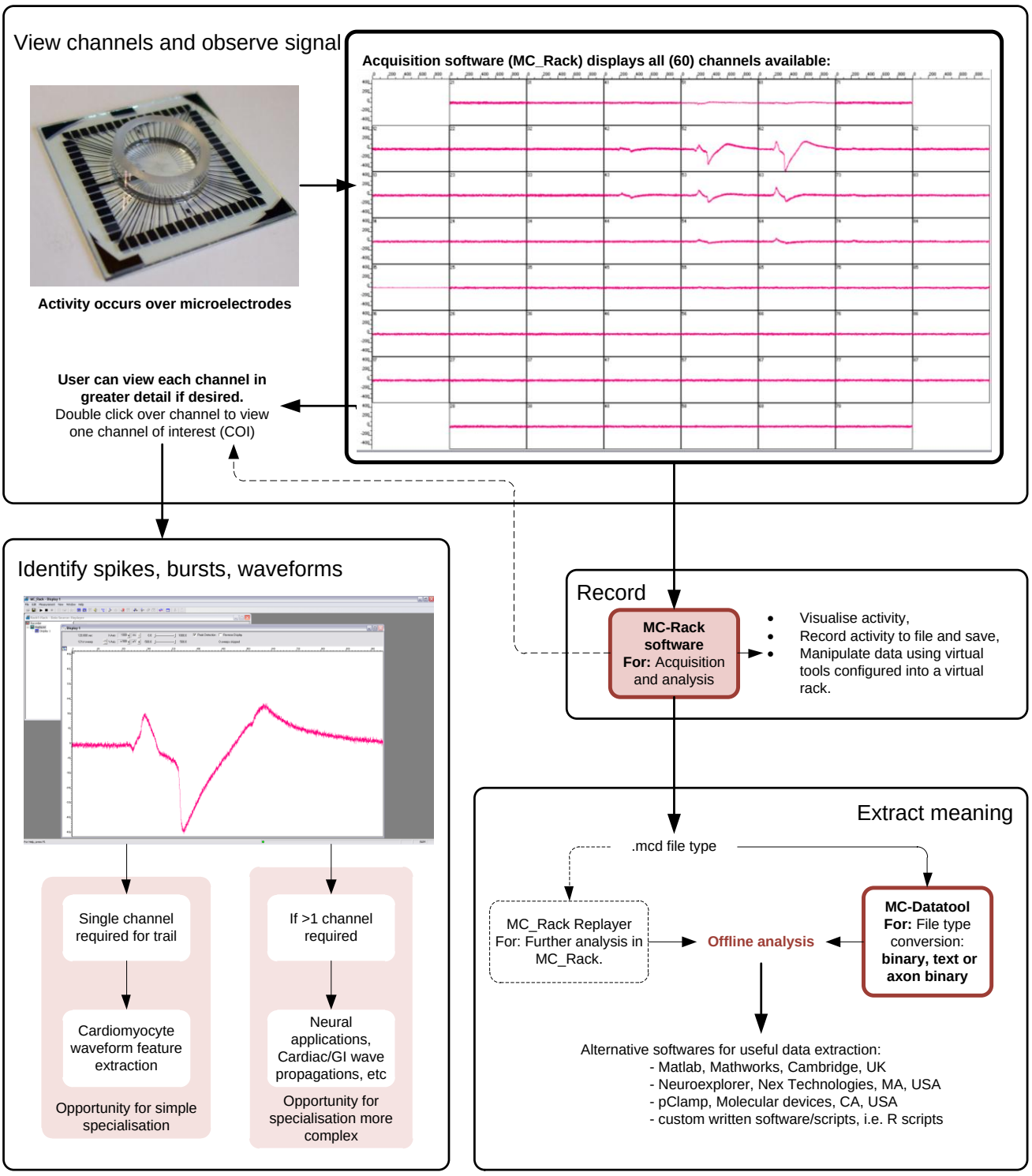


Figure 2.41: Analysis routine commonalities. The details of outputs will vary according to the preference of the user(s).

2.9 MEA Systems

The core components of an MEA system are demonstrated in series in Figure 2.42 comprising of a cell generating an AP that is detected by a nearby voltage probe that is embedded into a circuit on a biochip that connects to an amplifier, filter and computer. The computer stores and displays the detected signals for the human user to view and analyse.

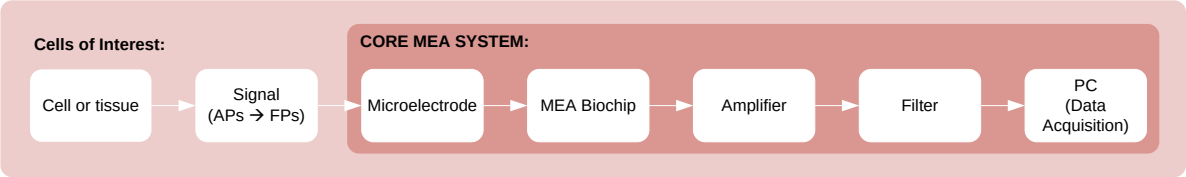


Figure 2.42: Core components of an MEA system.

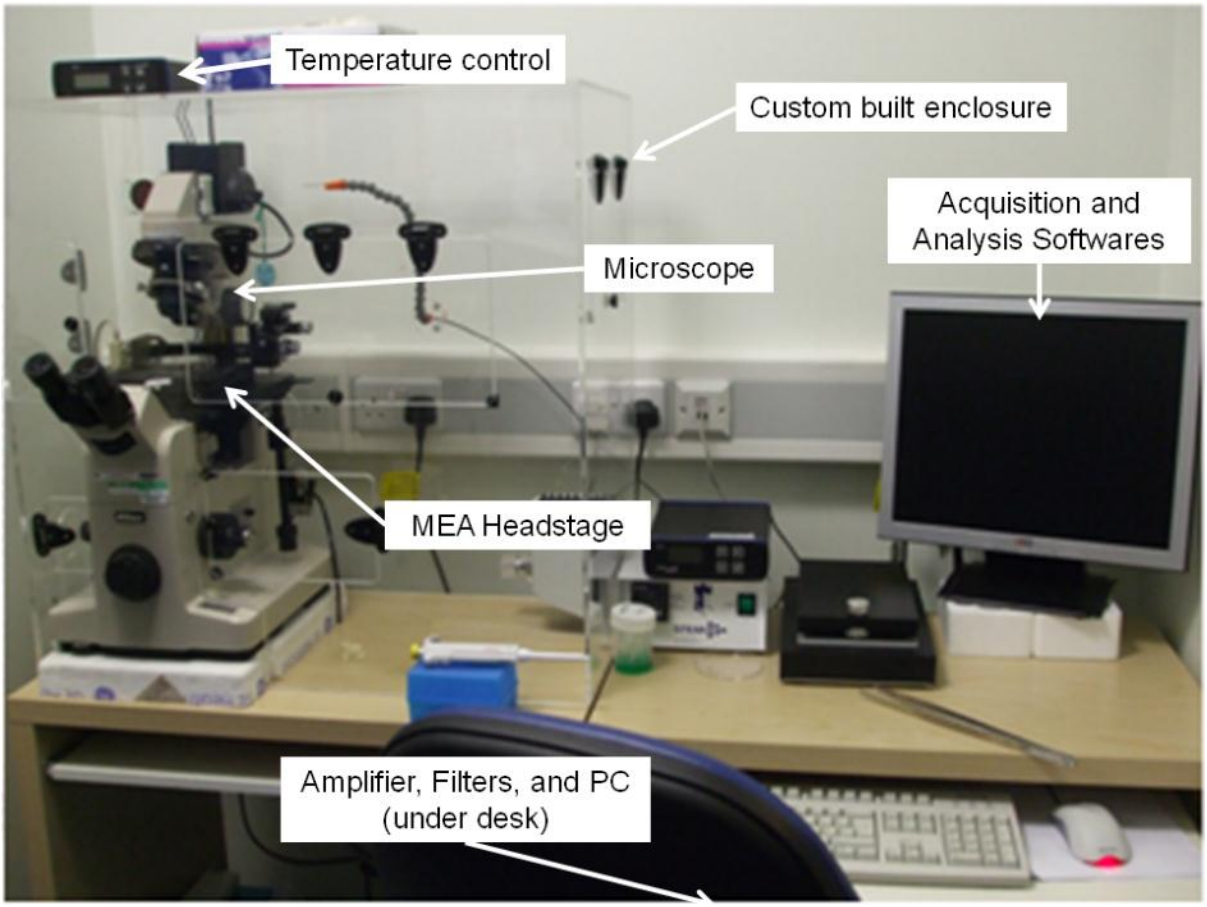


Figure 2.43: A Multi Channels Systems MEA60 System in a bioscientific laboratory.

A typical MEA system set-up in a bioscientific research laboratory at the University of Nottingham is illustrated in Figure 2.43. Specialist housing or units like that seen in Figure 2.43 are custom-made to serve as enclosures for the headstage to improve atmospheric conditions (e.g. temperature (37^{o}C), humidity (>95%), CO_2 concentration (5%)) around the cells while in the headstage (Figure 2.47 and Figure 2.48). The unit also aims to reduce the possibility of contamination occurring as this particular

research group does not use MEA biochip lids. The need for an enclosing unit is also something that must also be factored into decision making prior to installation as space for the system within a laboratory environment may be limited.

Not all research groups use their MEA system continuously and at times a system can remain unused for periods of weeks to months. This occurs due to the varying demands of the overall research question being addressed. The issue of portability of MEA systems has yet to be debated on a broad scale but recent discussion of “lab-in-a-box MEA systems” suggested that portability is one aspect that could be drastically improved upon (Fejti et al, 2006). This has been acknowledged in recently launched commercial systems as reconfigurations of the MEA60 set-ups into new plug-and-go units using USB connections have emerged (section 2.9.2.1).

2.9.1 System components

A brief description of the main units and critical components making up MEA system set-ups are shown in Figure 2.44. It is essential that developers looking to build a complete MEA system understand and can manufacture parts that integrate seamlessly. Microelectrodes must be manufactured to a high quality on a base substrate that can be insulated by specifically biocompatible materials. The insulation material must also accommodate a water-tight seal (between the glass or polymer ring that will form the media well) with an adhesive or sealant that is non-toxic and that will withstand periods of up to months in humid environments. A headstage must also be constructed that will reliably connect to the contact points on the MEA biochip. Amplifiers must be sufficiently close to that point of contact to ensure the signals reach the analogue-to-digital converters (ADCs) and required filters without too much noise interference from background 50Hz and radio frequency sources. A means of temperature control with sufficient accuracy (+/- 0.5^{o}C) must also be incorporated into the headstage to control the temperature local to where the cells and cell media will be positioned. This is especially important for longer recordings (e.g. for periods of months). Appropriate connections must be made between the headstage and PC. A PC must be programmed to run the MEA system and interface to software constructed for signal acquisition and manipulation.

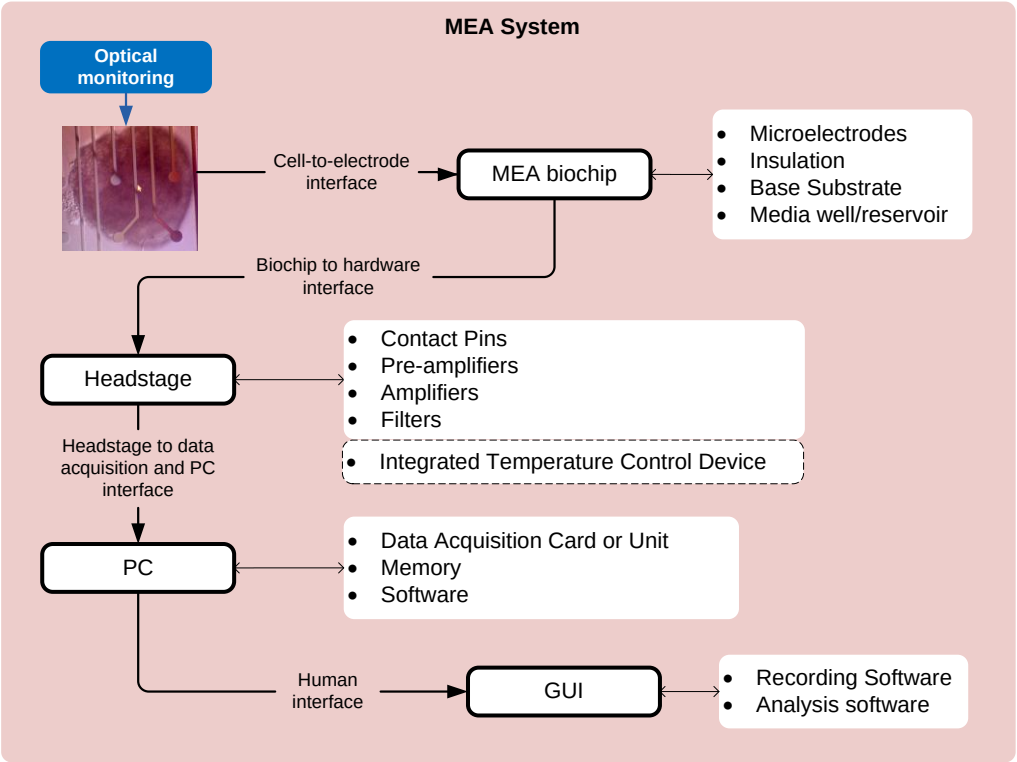


Figure 2.44: An MEA system set-up, important interfaces and sub-components applying to each main component.

The key interfaces that must be understood are cell-electrode, biochip to headstage hardware (electronics), headstage hardware to PC and PC to user.

2.9.1.1 The MEA Biochip

Typically the shape of an MEA biochip is square (i.e. Multi Channels Systems and Ayanda Biosystems are 49mm x 49mm), with the electrode array embedded at its centre (Figure 2.45). Each electrode is connected to a contact pad by a thin strip of conductive tracking. Contact pads fan-out around the periphery of the biochip in line with the layout of spring-loaded contact pins held in the MEA headstage unit. The local area of the biochip surface where the electrode tips are located is referred to as the workspace as it is only cells or tissue over this area that electrical activity can be recorded from.

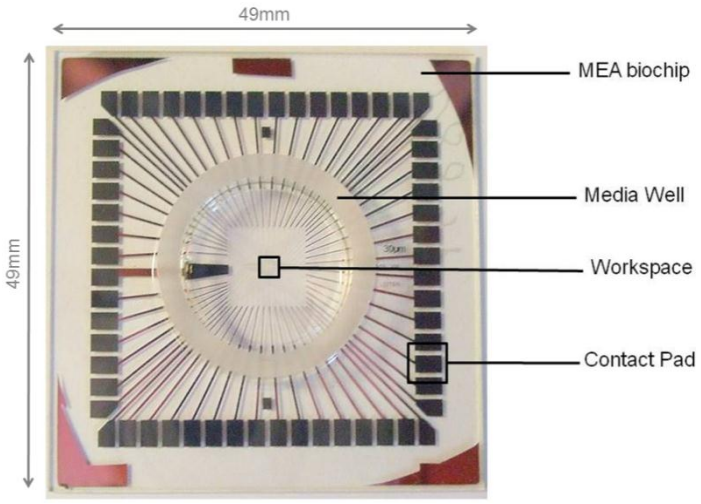


Figure 2.45: A typical 60 electrode MEA biochip. This model is manufactured by Multi Channels Systems. Electrode diameter is 30µm with inter-electrode distances of 100µm.

The standard MEA biochip containing 60 electrodes is arranged in an 8 x 8 grid array. The corners of the grid array are not filled with an electrode (see Figure 2.46). Above and around the workspace is a volume of space contained by an adhered ring. The ring is made from glass or plastic, forming the media well. A known volume of cell media will be added to this well to provide the living cells with both the nutrients that they require for *in-vitro* survival and to provide a means of removing waste products excreted.

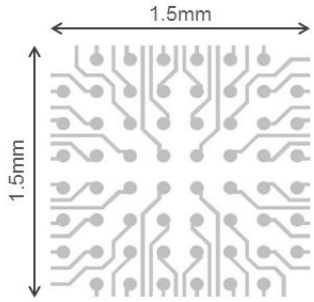


Figure 2.46: A schematic of the electrode arrangement in a typical 60electrode MEA biochip workspace. Inter-electrode distances vary between models (see Figure 2.65).

2.9.1.2 The Headstage

The headstage is the unit that supports the biochip and houses the initial interconnect pins and pre-amplifiers. A range of headstage specifications (section 2.9.2.1) are available according to the MEA biochip configuration required and the cellular signal source of interest (e.g. brain slices, stem cell derived cardiomyocytes). Multi Channels Systems typical 60-channel headstage amplifier consists of a circuit board that integrates initial preamplifiers and filter amplifiers in one single unit. The dimensions of this unit are 165 x 165 x 19mm. The manufacturing approach used to create the circuit within is surface mounted device (SMD) technology (MCS MEA Amplifier for Inverse

Microscopes Manual, 2010). MEA headstages require amplification as close to the signal source as possible (Chu et al, 2006). This is due to the FPs being in the range of micro-volts thus the best signal-to-noise ratio (SNR) attainable is required.

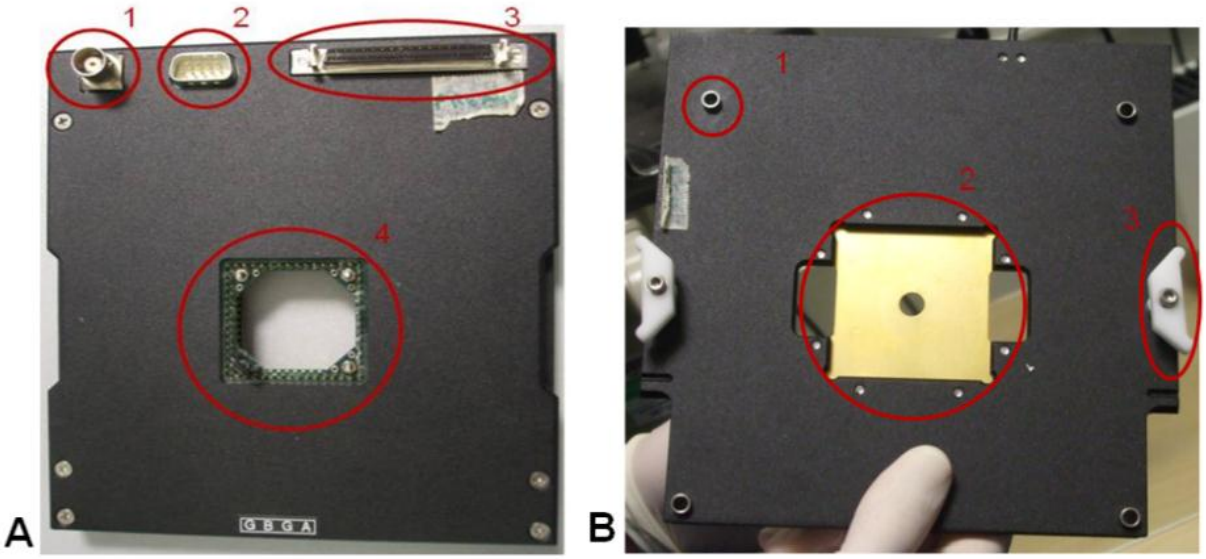


Figure 2.47: A Multi Channels Systems (MCS) Headstage Model, "MEA1060-Inv-BC-Standard". Views are from the top. A) The top plate houses the contact pins and headstage circuitry. 1. Digital Input/Output (I/O), 2. Serial I/O (used for the MEA_Select tool where two headstages are used in parallel), 3. 64-pin Connector to pre-amp and filter unit (FA60), 4. Opening to where the MEA biochip well is enclosed. B) The bottom plate. 1. One of four female connection alignment recessions. 2. Contains a brass plate that is heated by an external temperature control unit. The brass plate in this instance has a hole at its centre to accommodate and inverted microscope set-up. 3. Turning clasps used to hold the two parts of the device closed.

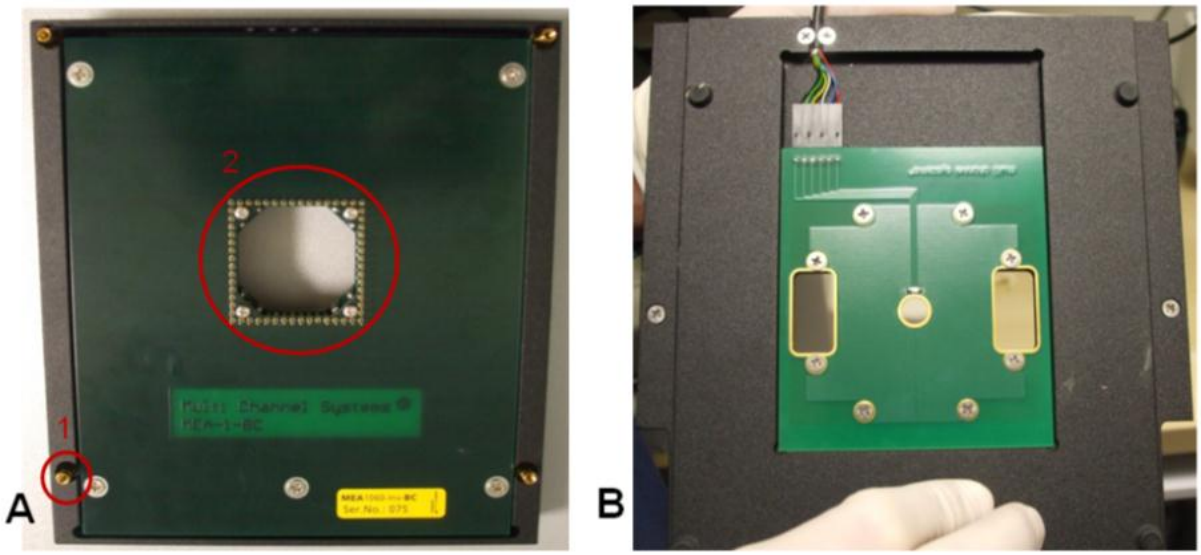


Figure 2.48: The corresponding undersides of the MCS MEA1060-Inv-BC-Standard headstage (fig. 2.47). A) The underside of the top plate. 1. One of four male connection alignment pins. 2. Spring loaded gold pin interconnections. B) The underside of the bottom plate demonstrating a recession in which the induction board is situated for heating the brass plate surface above.

A number of headstages are now available with blanking circuits built-in to enhance recording properties during stimulation experiments (see section 2.5.1.1). Specialist adapters (i.e. physical connectors to the headstage of biochip) are also available that connect directly onto the headstage contact pins that are designed for certain stimulating protocols. Users can also have an adapter custom made (by MCS) to meet more individual requirements (e.g. most common for specialist stimulation experiments).

In some instances up to four headstages can also be linked to one PC for simultaneous investigation of four independent MEA biochips. This is done with MCS systems through the addition of a hardware device called an MEA switch.

Figure 2.49: The 68-pin small computer standard interface (SCSI) cable type used for connecting MEA components in MCS in-vitro MEA systems.

In original MCS MEA-60 systems a small computer standard interface (SCSI) cable type (Figure 2.49) is used to connect the headstage to further filters, an MEA switch and the data acquisition card. The data acquisition card that is housed within a PC is based on PCI-bus technology allowing up to 128 channels to be sampled at a frequency between 2kHz up to 50kHz. Therefore, if four headstages are connected to one PC via an MEA switch system, only 120/240 (50%) of the electrodes in the four MEA biochips will be recordable.

Each headstage also has three analogue channels and a digital port for connection to other peripheral devices , such as additional oscilloscopes (MCS MEA Manual, 2010).

2.9.1.3 Data acquisition card or unit

Original MEA-60 systems connect from the headstage and filters, or MEA switch, directly into a data acquisition card (Figure 2.50) that is housed in a specially built PC operating an XP or Windows 7 operating system.

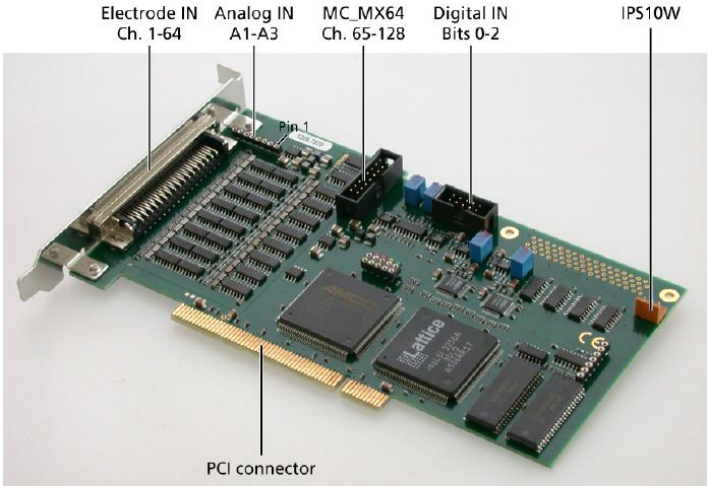


Figure 2.50: An MCS MC_Card removed from PC

In more recent years new compact acquisition systems have appeared on the market that connect to the PC using USB 2.0 cables. This has made MEA systems more portable in the sense that the acquisition unit no longer needs to be housed in a desktop computer and so any computer with MC_Rack software (the Multi Channels System acquisition software) installed. In some cases this can help user groups with many MEA system users to manage the data produced more effectively. Groups can divide up the memory load simply onto each individual user's PC. Scientists are then also responsible for their own recording and analysis, and the adequate protection and back-up of their data. It is this feature that has brought MEA technology a step closer to being a tool that any user can plug in to and operate intuitively. However, conversely this potential division of memory, and thus data, may not be ideal for groups where users need to share centralised data.

2.9.1.4 System software and GUI

All commercially available MEA systems are provided with a specialist acquisition software allowing initial experiment set-up and data acquisition.

The acquisition software used in conjunction with Multi Channels Systems system is called MC_Rack and it is used to record or replay corresponding multi channels data files (.mcd extension). Upon opening an instance of MC_Rack the user interface (Figure 2.51) presents a grey work bench area that is filled with an appropriate virtual rack of tools. The tools (Figure 2.52) used to record and extract information from an .mcd file varies according to the cell preparation and the nature of the experiment being conducted.

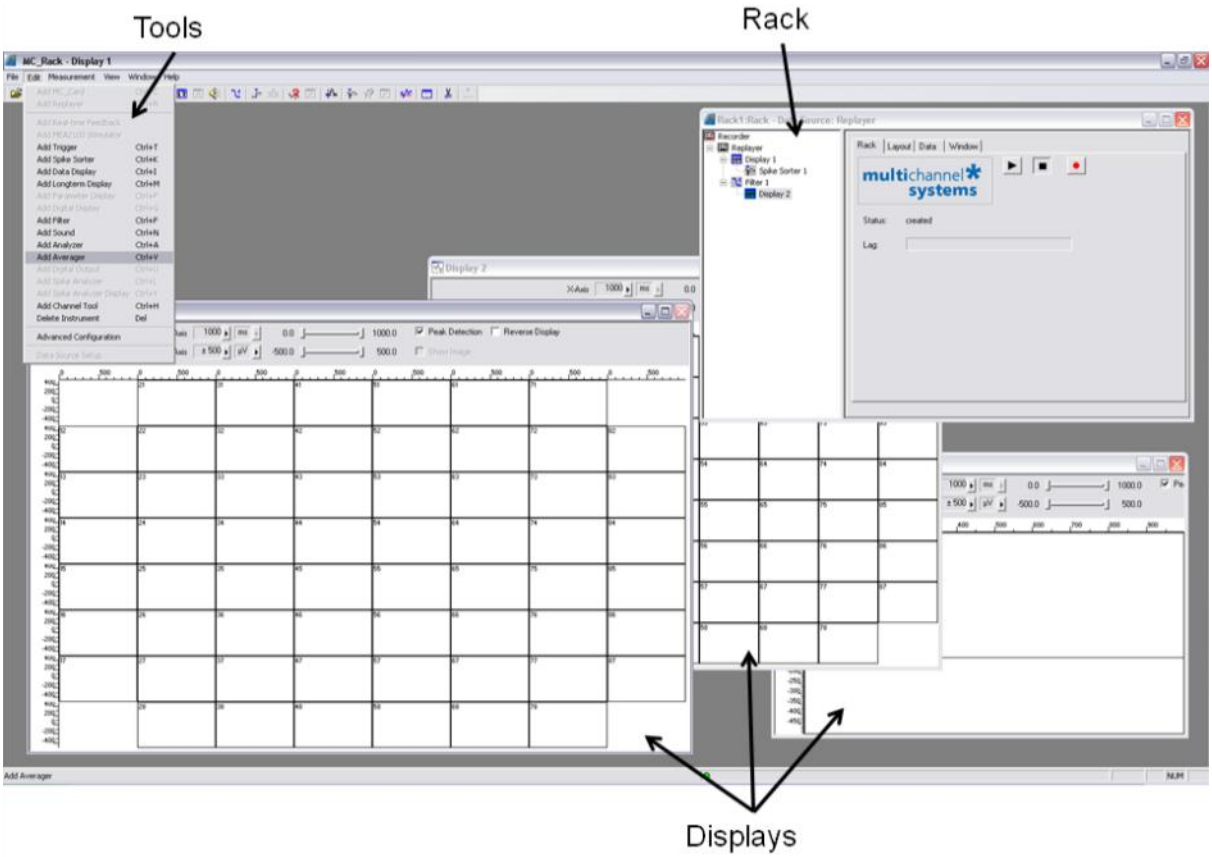


Figure 2.51: The user interface upon opening a previously constructed recording rack in MC_Rack.

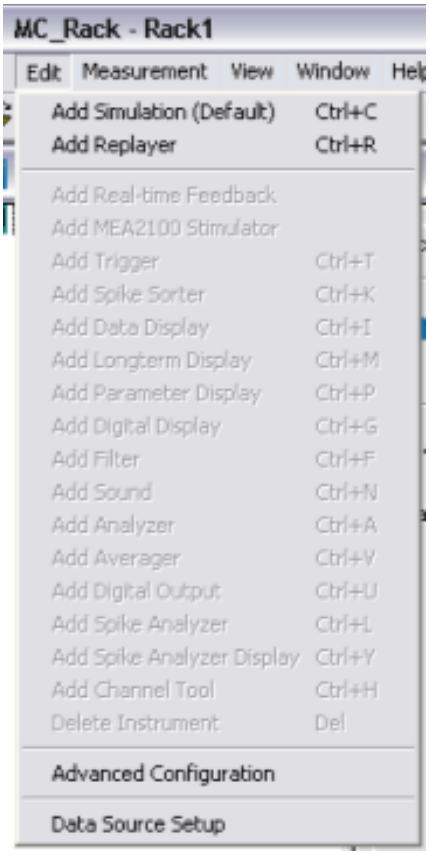


Figure 2.52: The tools available in MC_Rack.

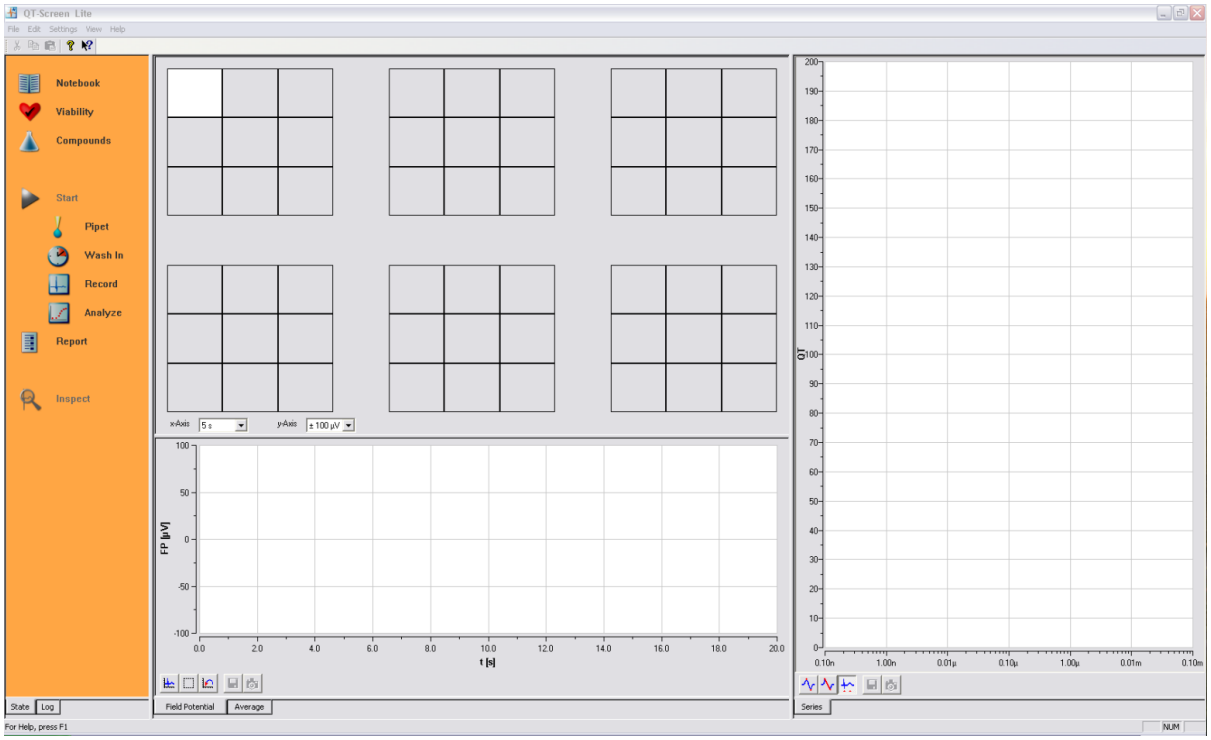


Figure 2.53: The user interface for the MCS 96-well QT-Screen systems acquisition software, called QT-Screen.

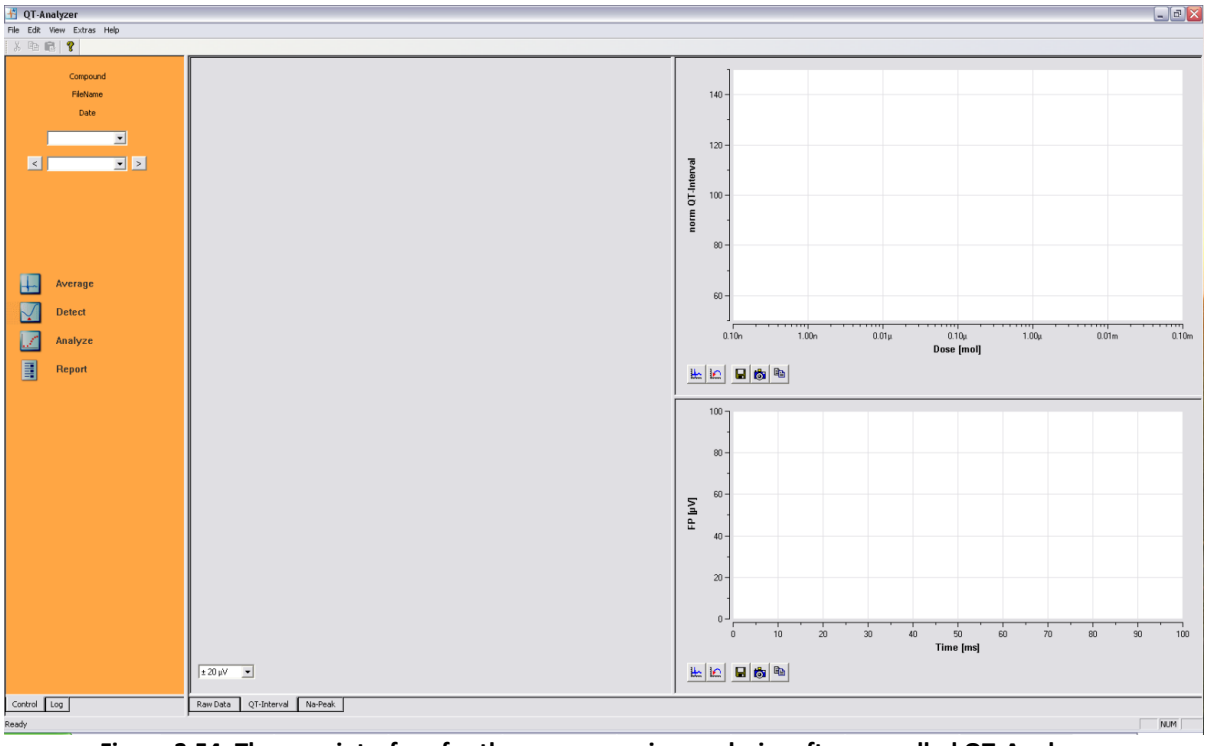


Figure 2.54: The user interface for the accompanying analysis software, called QT-Analyser.

The graphical interface of the MCS QT screen's system software is shown in Figure 2.53 and Figure 2.54. The QT-screen is a specialist system for use with cardiomyocyte cells types in chemical entity screening. Separate software has been provided for recording (QT-Screen) and analysis (QT-Analyser). The interfaces of the two software system components are complementary to one another. This differs from MC_Rack and other comparable generic MEA software (e.g. neuroexplorer) where one software tool acquires and analyses MEA data.

2.9.1.4.1 Racks

The virtual instruments used in signal acquisition and manipulation in MCS's MC_Rack systems are built up into custom configured lists, called "racks", consisting of logically ordered software tools. These racks, once constructed, can then be saved with the recorded data for both reference and for later re-use. Saving racks into folders with the recorded data also creates a working record of the settings and parameters used in both the acquisition and analysis (if MC_Rack is used for analysis). For example, in Figure 2.55 all of the data (.mcd files) for a particular test has been created in one appropriately labelled folder. The settings used to record those data are also recorded by saving the rack to the folder. In this example one rack was used to record the data, and another was used to extract information from those data.

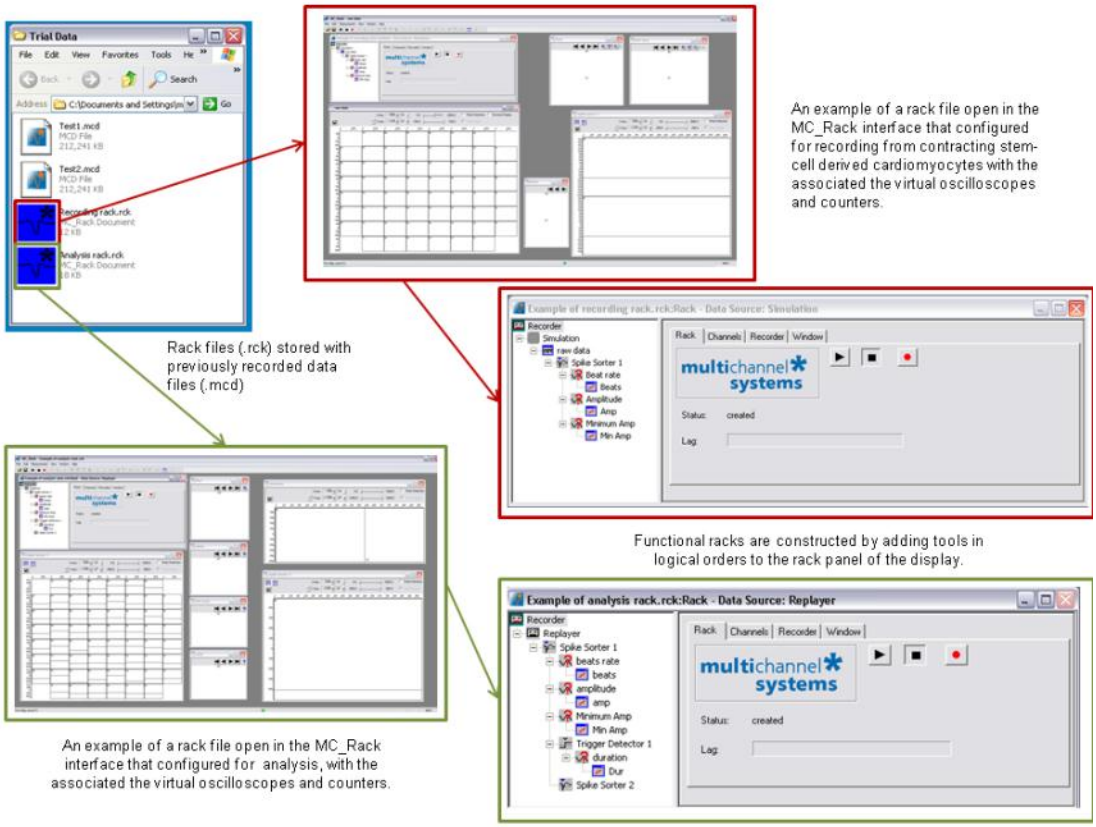


Figure 2.55: The storage of acquisition and analysis racks constructed in MC_Rack are clearly distinguishable when stored in amongst recorded multi channels data files (.mcd files).

An alternative analysis route, exploited by many MEA system users is to utilise other software for analysis stages. A software tool that has been widely exploited by MEA system users across applications is called NeuroEXplorer from Nex Technologies, Massachusetts, USA (Kapucu et al, 2009; Tye et al, 2010; Robinette et al, 2011). The interface differs greatly to that of the MC_Rack program (Figure 2.56).

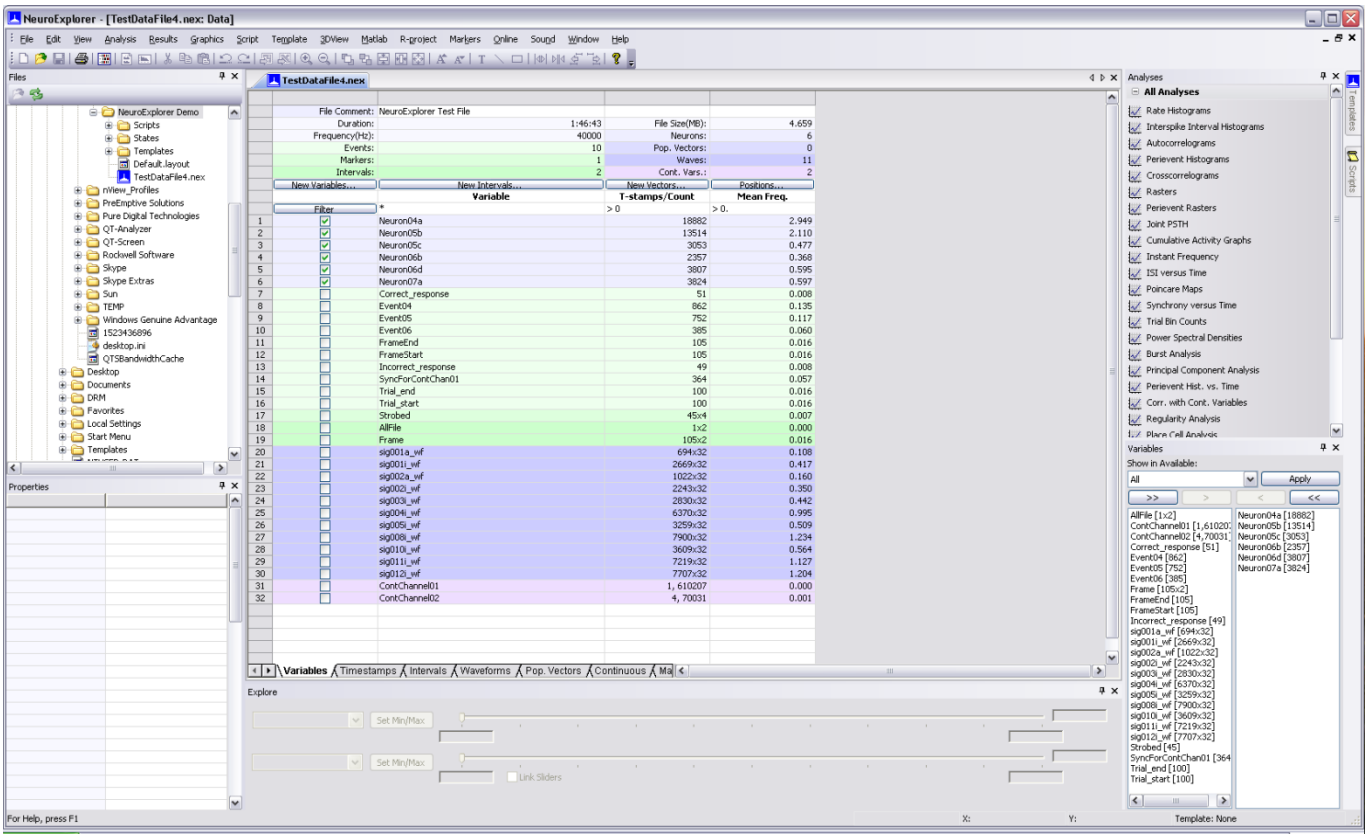


Figure 2.56: The user interface upon opening Nex Technologies' neurophysiological data analysis software NeuroEXplorer (Version 4.088).

The analysis capabilities built into NeuroEXplorer software is limited (e.g. can only process one file at a time). As a result a number of specialist analysis software tools are also available for more detailed analysis of MEA data (e.g. that can analyse a common feature of multiple files at one time). This is also the analysis software provided with much of Plexon's electrophysiology hardware. NeuroEXplorer is compatible with a diversity of MEA recorded data due to the built-in facility to import data files from all of the major MEA data acquisition software (e.g. Spike-2, Cortex, SciWorks). The following data acquisition data formats were supported by NeuroEXplorer at the time of writing:

- **Alpha Omega's data acquisition software Alpha map** which is used with Axion Biosystems MEA devices as well as Alpha Omega's other data acquisition hardware (www.alphaomega-eng.com).

- **Cambridge Electronic Design's Spike-2 software** (www.ced.co.uk). Cambridge, UK.
- **The Cortex software available from the US National Institute of Mental Health Laboratory of Neurophysiology** for data acquisition and experimental control (www.cortex.salk.edu). Bethesda, Maryland, USA.
- **Cyberkinetics data acquisition systems** as demonstrated in and exploited for the highly publicised Brain Gate project (www.cyberkinetics.com). Cyberkinetics is a spinout from Brown University, Rhode Island, USA.
- **DataWave Technology's SciWorks** core software for data acquisition and analysis. (www.dwavetech.com). Colorado, USA.
- **HEKA Elektronik's numerous data acquisition software** (formerly Instrutech). (www.heka.com). Germany, Canada and USA.
- **Multi Channel Systems acquisition and analysis software MC_Rack** (www.multichannelsystems.com). Reutlingen, Germany.
- **Neuralynx's Cheetah Data Acquisition Software** (www.neuralynx.com). Montana, USA.
- **Plexon's MEA Sort Client primary MEA software** interface (www.plexon.com). Texas, USA.
- **R.C. Electronics data acquisition systems** (www.rcelectronics.com). California, USA,

NeuroEXplorers functionality is described in Figure 2.57.

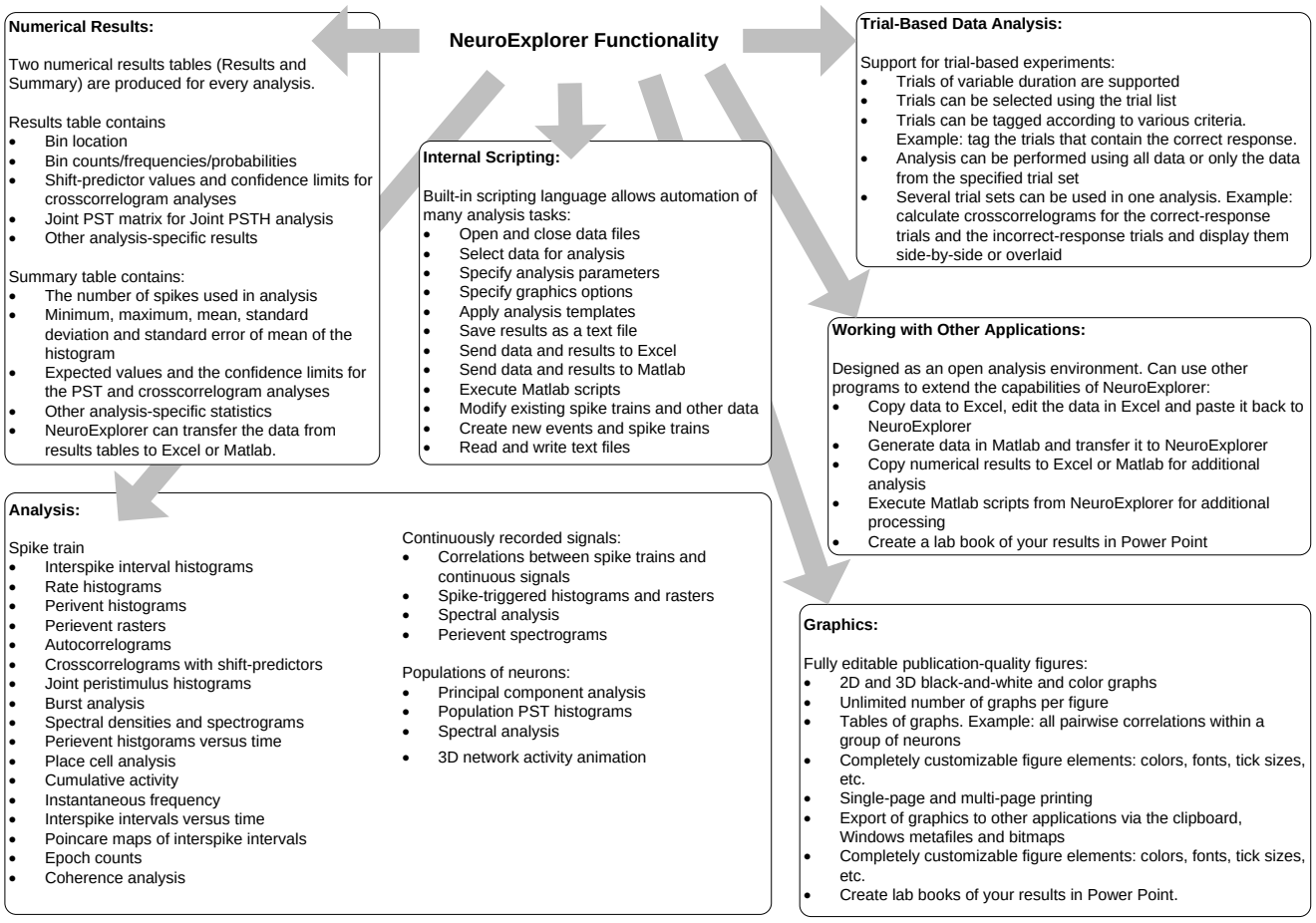


Figure 2.57: NeuroEXplorer Functionality.

[Source: Nex Technologies, 2011.]

A user can dictate how many channels to record from, at what frequency, for how long and, if desired, which features from the pre-processed raw signal they wish to keep as the resultant data files. For example, only the spike traces and their timestamps from a raw signal that cross a set threshold may be retained from a recording at 25kHz to reduce the overall file size.

2.9.2 Commercially available systems

A number of commercial enterprises have created an MEA industry where MEA systems are available for special order purchase. Due to the varying needs of scientist user groups around the world these systems cannot be described as being available "off-the-shelf". User groups must approach manufacturers with their requirements so that they can be advised of the system configuration that is best matched to their needs. Once the required configuration has been agreed upon the purchasing user group can acquire anything from a full MEA system down to the bare minimum components of a system (e.g. a two-fold system with perfusion cannulas, stages, micromanipulators or a basic MEA-60 with just headstage amplifiers and custom-PC) depending on their budget. The most prominent industrial players and the global region in which they are based are listed in Figure 2.58.

Figure 2.58: The key providers of commercial MEA systems worldwide.

The number of commercially available MEA systems is increasing (currently ~7 manufacturers with 1-2 systems) with the leading manufacturers providing both the hardware and software solutions. MEA development has been driven by the evolving needs of electrophysiological research, and as such a number of academic-industrial collaborations exist between the world-leading suppliers and

world-leading user groups. Industrial-industrial collaborations are also apparent between the smaller industrial players.

Example Collaborations:

- **Potter Lab – Multi Channels Systems (Academic-Industrial).** The Potter Lab at Georgia Tech (formerly CalTech) developed specialised lids for MEA biochips to prevent contamination and maintain local environment around cells (Potter and DeMarse, 2001). These lids are now available commercially via MCS (http://www.multichannelsystems.com/products-mea/product-details/products/189/ala-mea-mem.html).
- **Ayanda Biosystems - Multi Channels Systems (Industrial-Industrial).** Ayanda Biosystems manufacture MEA biochips only. Previously held a strong collaboration with MCS so the design configurations of Ayanda Biosystems MEA biochips is complimentary to early MCS headstages.
- **Ayanda Biosystems – BioLogic (Industrial-Industrial).** At the MEA Meeting 2010 a new collaboration was demonstrated with the bioMEA system manufactured by BioLogic of Claix, France (www.bio-logic.info) following previous collaboration with MCS.
- **Multi channels Systems - NMI Natural, Germany - Medical Sciences Institute at the University of Tubingen, Germany (Industrial-Academic-Academic).** A large amount of MCS research and development activity stems from the work of these two academic institutes.
- **3-Brain – Plexon (Industrial-Industrial).** The world's first CMOS-based commercial MEA system was developed by a company called 3-Brain who announced a collaborative relationship with electrophysiology key players Plexon in 2010.

2.9.2.1 System Configurations

Multi Channels Systems provide several system configurations that centre on the number of channels and type of data acquisition unit. Competing enterprises have launched a number of equivalent systems but fail to exhibit a similar number of varying model configurations (Table 2.3). *Inv = Design is suitable for use with an inverted microscope. BC = Headstage contains a blanking circuit (for use with stimulation protocols. Up = Design is not suitable for an inverted microscope set up. Where -2- occurs implies two MEA biochips can be used through the same acquisition hardware simultaneously. E = system is supplied with the enhanced perfusion equipment included.*

Table 2.3: Commercially available configuration and their manufacturers.

Channels	Systems	Manufacturer
60	**MEA60-Basic Systems**	Multi Channels Systems
	MEA60-Inv-System	
	MEA60-Inv-System-E	
	MEA60-Inv-BC-System	
	MEA60-Inv-BC-System-E	
	MEA60-Up-System	
	MEA60-Up-System-E	
	MEA60-Up-BC-System	
	MEA60-Up-BC-System-E (£34533 – February 2012)	
	MEA60-2-Systems	
	MEA60-Inv-2-System	
	MEA60-Inv-2-System-E	
	MEA60-Inv-2-BC-System	
	MEA60-Inv-2-BC-System-E	
	MEA60-Up-2-System	
	MEA60-Up-2-System-E	
	MEA60-Up-2-BC-System	
	MEA60-Up-2-BC-System-E	
	MEA2100-System	
	MEA2100-60-System	
	MEA2100-60-System-E (£42558 – February 2012)	
	MEA2100-60-2-System	
	MEA2100-60-2-System-E	
	USB-Systems	
	USB-ME64-System	
	USB-MEA60-System	
	USB-MEA60-Twofold System	
	MED64	Alpha Med Sciences
	MED64 4-sample system	
	The Muse (€42000 –June 2010)	Axion Biosystems
	MEA Workstation	Plexon
120	**MEA120-2-Systems**	Multi Channels Systems
	MEA120-Inv-2-System	
	MEA120-Inv-2-System-E	
	MEA120-Inv-2-BC-System	
	MEA120-Inv-2-BC-System-E (£48671 –February 2012)	
	MEA120-Up-2-System	
	MEA120-Up-2-System-E	
	MEA120-Up-2-BC-System	
	MEA120-Up-2-BC-System-E	
	MEA2100-System	
	MEA2100-2x60-System	

	MEA2100-2x60-System-E	
	MEA2100-120-System	
	MEA2100-120-System-E	
	MEA2100-2x60-2-System	
	MEA2100-2x60-2-System-E	
	MEA2100-120-2-System (£45919 – February 2012)	
	MEA2100-120-2-System-E	
	USB-ME128-System	
	USB-MEA120-Twofold system	
96	**MEA96-Well System**	Multi Channels Systems
256	**USB-MEA240-Fourfold System (£73571 – February 2012)**	Multi Channels Systems
	USB-MEA256-System (£52642 – February 2012)	
768	**The Maestro** (€142000 – June 2010)	Axion Biosystems
4096	**BioCAM-4096**	3 Brain

[Correct 31st May 2011]

The fundamental the differences between the systems can be described in terms of the number of electrodes through which recordings can be made simultaneously. Each electrode results in a channel of data, so the number of electrodes is the number of channels. By offering systems with an increased number of channels the flexibility of the MEA system is improved (in certain applications). For example, users can take recordings from larger cultures grow over larger surface areas to observe the activity of increased, and therefore more complex, neural networks, or, they may use a greater number electrodes positioned in a tighter grid array in attempts to identify the activity of individual synapses as opposed to entire cells, or, they may use biochips with multi-well layouts that have improved resolution (more electrodes) within each well to facilitate more cellular samples per test.

There are also systems and biochips used in some published literature that are not commercially available but that have been built by researchers to cater for specific needs or problems (e.g. need to position electrodes in specific geometries or desire to grow cells into or through certain structures) (Gholmieh et al, 2006; Oka et al, 1999; Dworak and Wheeler, 2008; Wang et al, 2009b; Zhang et al, 2009). Example devices are demonstrated in the following figures (Figure 2.59 to Figure 2.63).

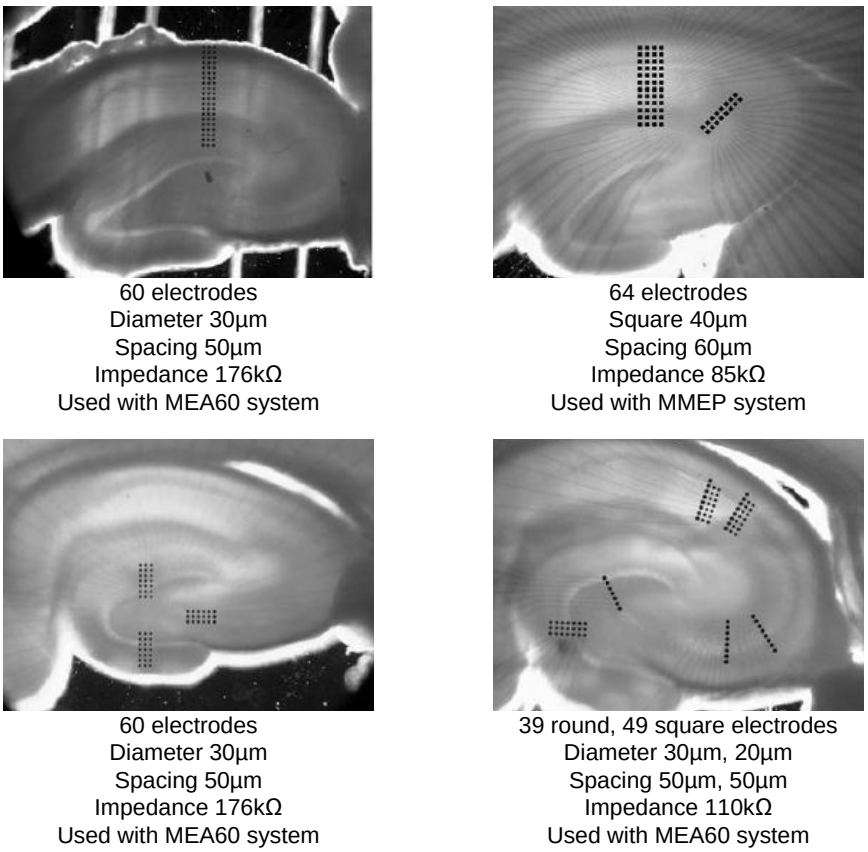


Figure 2.59: An example of an MEA biochip for targeted brain slice stimulation and recording. ITO tracking, Au microelectrode tips.

[Source: Gholmieh et al, 2006.]

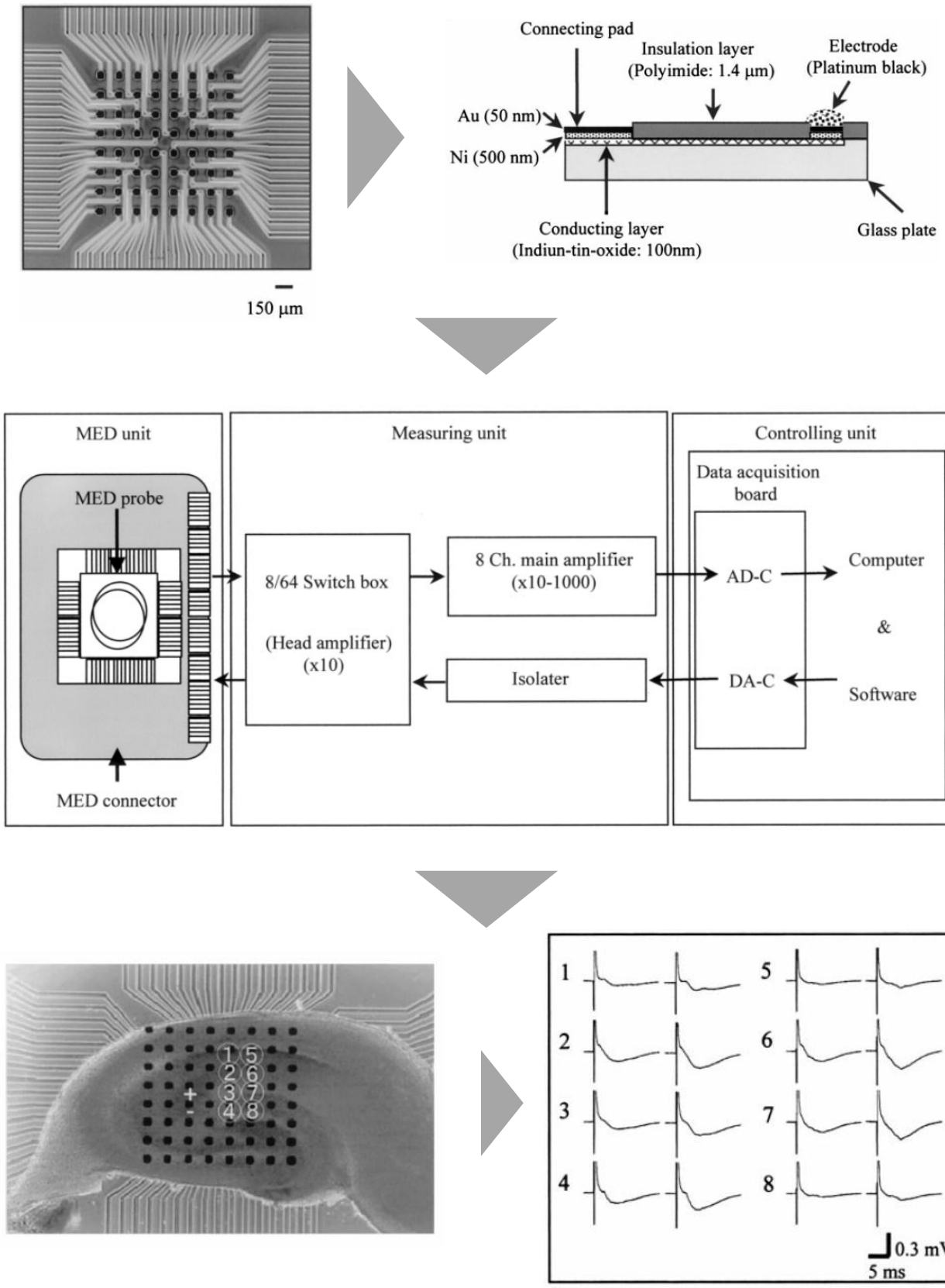


Figure 2.60: An example of a novel MEA biochip and MEA system for use specifically in hippocampal slice studies.

[Source: Oka et al, 1999.]

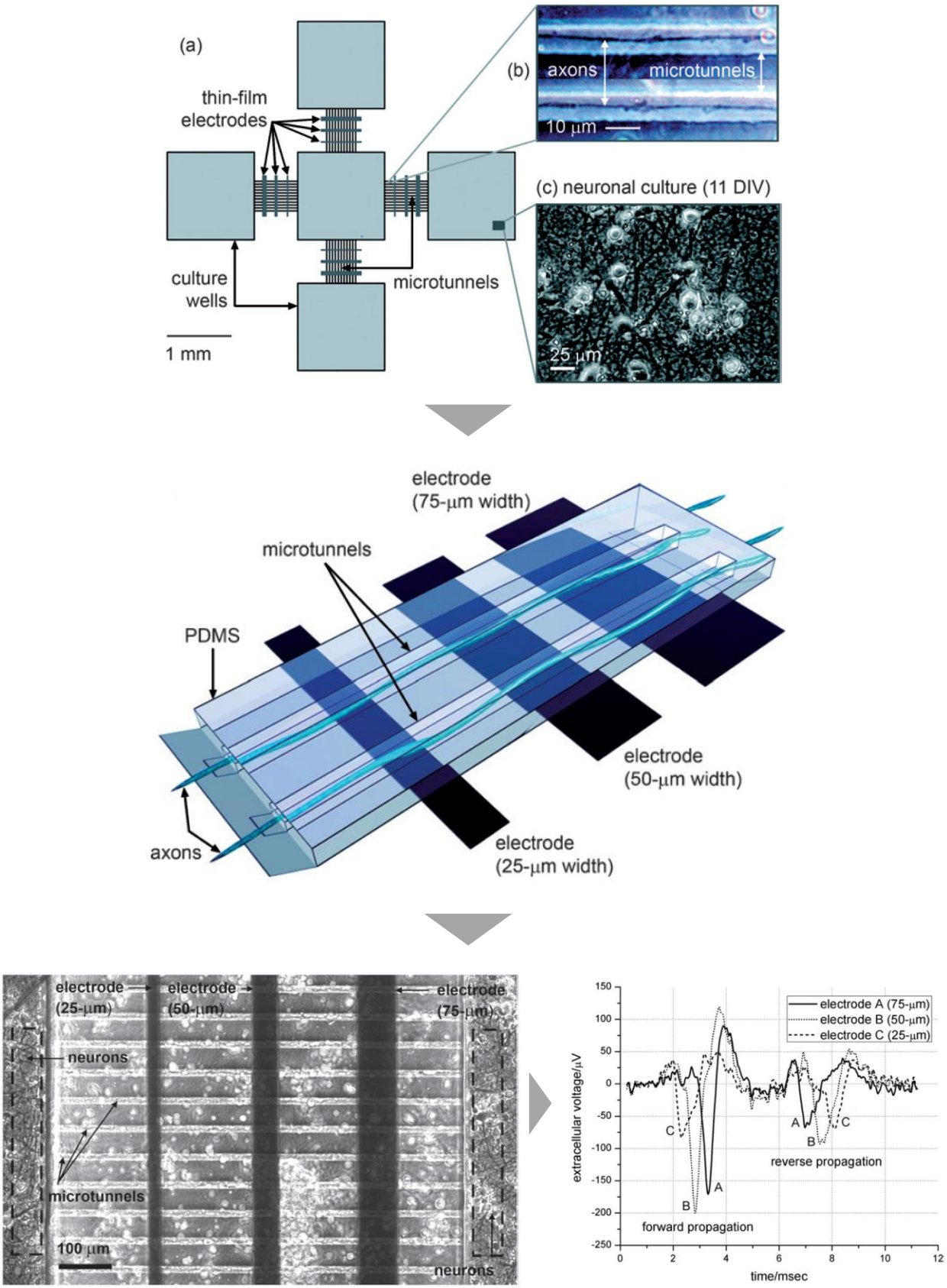


Figure 2.61: An example of a biochip for highly selective recordings of axonal signals.
[Source: Dworak et al, 2008.]

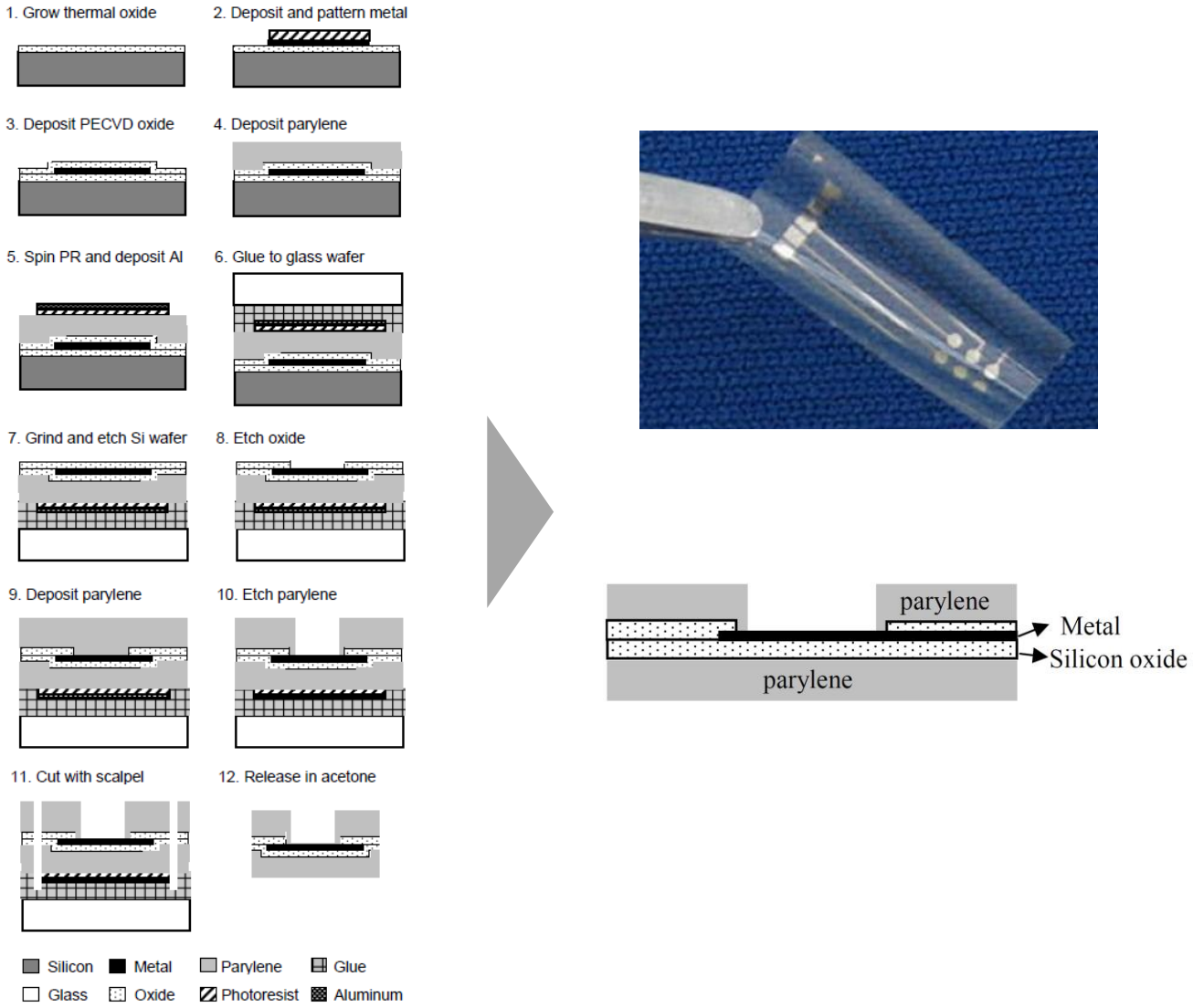
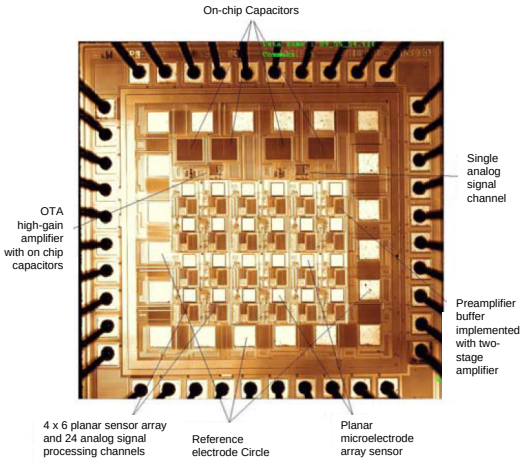
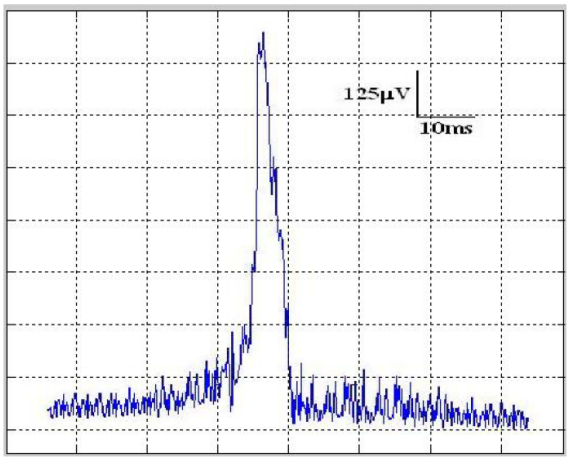


Figure 2.62: An example of the manufacturing approach for a novel flexible MEA made by researchers at Philips Research, Netherlands.

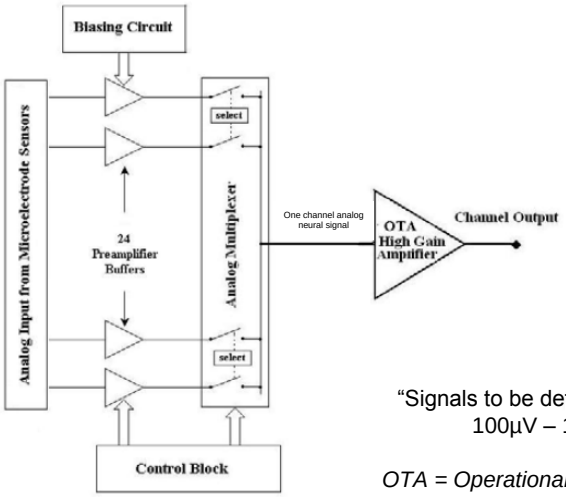
[Source: Wang et al, 2009b.]





4 x 6 Microelectrode sensor array pixels = 24 analogue signal processing channels.

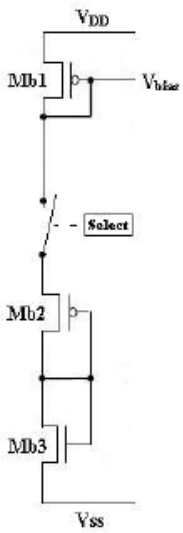
Electrical testing conducted using emulated neural signals.



Architecture of the sensing biochip.

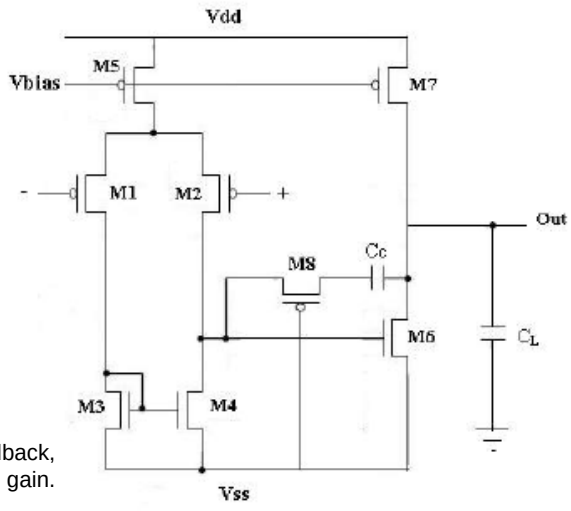
"Signals to be detected are typically of order 100µV – 1mV peak-to-peak."

OTA = Operational transconductance amplifier.



Biasing circuit for preamplifier buffers.

Schematics of the preamplifiers.



Preamplifier with resistive feedback, configured with 20dB gain.

Figure 2.63: A 24-channel mixed signal CMOS integrated biochip and buffers, amplifiers and control/interface unit schematics.

[Source: Zhang et al, 2009.]

2.9.2.1.1.1 International Research Interest

Every two years the world's only conference for MEA users and developers, MEA Meeting, occurs in Reutlingen, Germany. It is at this event that the most up-to-date technical advances are showcased alongside presentation of developments in MEA system application. The following table (Table 2.4) shows how the division of interrelating MEA-specific research has been done for the past 3 meetings and is planned for the next meeting.

Table 2.4: Lists of MEA Meeting conference categories. These lists demonstrate how MEA related research is categorised, expanding and diverse

MEA Meeting 2006	MEA Meeting 2008	MEA Meeting 2010	MEA Meeting 2012 (Planned)
Neural Dynamics and Plasticity	Neural Dynamics and Plasticity	Neural Dynamics and Plasticity	Analysis of neural dynamics and plasticity & Information coding in neural networks
Retinal Signalling	Retinal Signalling	Retinal Signalling	Applications in Systems Neuroscience (brain slices, retina, spinal cord, others)
Signal Analysis and Statistics	Signal Analysis and Data Mining	Signal Analysis, Statistics and Software	Signal analysis and statistics: (standardization and validation of multi-electrode recordings)
Heart	Heart	Heart	Heart: Electrophysiology and pharmacology in primary and stem cell-derived cardiac myocyte cultures
Pharmacology, Toxicology, Drug Screening	Pharmacology, Toxicology, Drug Screening	Pharmacology, Toxicology, Drug Screening	Pharmacology, toxicology, drug screening (with MEA-based assays)
Advances in culture, recording and stimulation techniques	CMOS-based array technology; Advances in culture, stimulation and recording	Electrodes, Surfaces and Set-ups	New materials and MEA design; Advances in MEA fabrication and instrumentation

	techniques.		
Cellular Sensors	Neuronal Engineering	Stem Cell Derived Neuronal Networks	Molecular engineering and analysis of cellular and sub-cellular neuron properties
	Neurostimulation and Neuroprosthetics	Electrical Stimulation, Implants and Robotics	Electrical stimulation of single cells and of neural tissue
		Culture Techniques	Cell and tissue culture including microfluidics
		Active Arrays and Electronics	Multi-electrode probes for in - vivo applications
		Plant Cells	

The amendment of categories through the years shows continuing interest in neural, retina, heart, and pharmacological developments. Signal analysis is also a consistent area of interest. In addition to that the trend towards development for cell culture and *in vivo* influences is emerging.

2.9.2.2 Cutting edge MEA Biochip Re-design

New generations of biochips are emerging (Braeken and Prodanov, 2010). For example, a recent advance in biochips specifically suited to slice applications has emerged that allows perfusion of media and chemical entities throughout prolonged testing (Motamedi et al, 2011). These biochips are called perforated MEAs (pMEA) (Figure 2.65).

Three dimensional arrays are also under development in the USA as the next step forward in in vitro recreation of natural neural conditions for controlled studies (Musick et al, 2009). The concept behind such a biochip is production of electrode sites at locations within the three-dimensional volume of space over and above the base substrate (Musick et al, 2009). A prototype of one concept developed by established researchers in the field (Figure 2.64) consists of a stack of individually patterned layers. Chambers are formed through the stacked structure defining a 3D region of microtunnels through which neurons are grown. Along the walls of these microchambers and tunnels are exposed gold electrodes that allow both recording and stimulation. Silicon elastomer microfluidic layers serve as an “artificial vasculature for nutrient supply and aeration” and are

incorporated to provide media to the neurons in situ as well as to serve as insulation between microelectrodes (Musick et al, 2009).

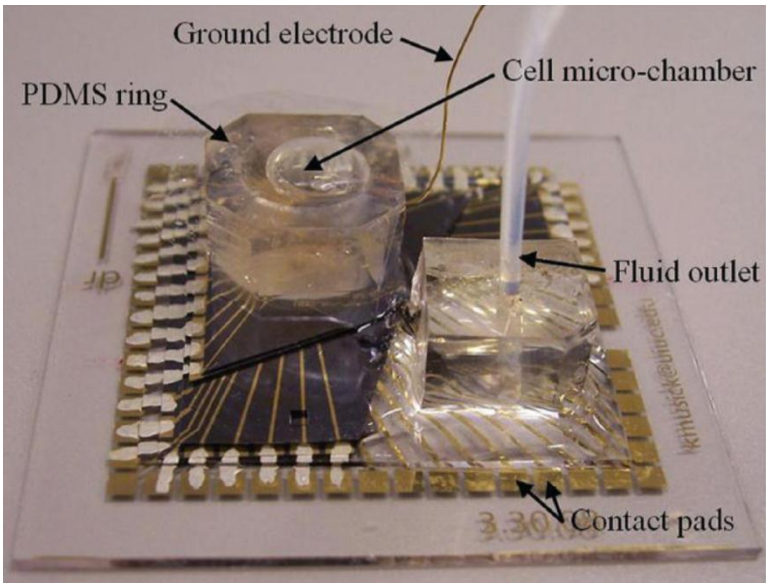


Figure 2.64: A fully assembled 3D array with a layered approach manufactured by the University of Florida's Neuroengineering laboratory (Musick et al, 2009).

2.10 The MEA biochip

MEA biochips are delicate consumables that due to their micrometre scale features can be easily damaged by micromanipulators or cleaning protocols. Nevertheless, biochips are intended to be multiple use products (~10-30 uses) if cared for appropriately (MCS MEA Manual, 2010). Gentle cell removal and cleaning (i.e. enzymatic rinses, plasma cleaning, UV sterilisation) can allow repeated use before electrode degradation occurs. Johnstone et al (2010) described the MEA biochip as "quite economical" at "$10-30 per use". There are a number of different MEA biochip configurations available from industrial vendors (see Figure 2.65) and some facility to make custom biochips also exists if scientists require a particular workspace design configuration for their research needs.

2.10.1 Microelectrodes

The microelectrode quality is critical to the success of each MEA biochip manufactured. Knowledge of material options, electrode geometries, electrode spacing, signal-to-noise ratios and insulation types is required.

Microelectrode Materials: Traditionally MEA biochips were made using gold (Au) for the microelectrodes, tracking and contact pad material (Wise et al, 1970). Gold is relatively chemically inert so serves well as a conductive, non-toxic electrode material. Due to the relatively high impedance of pure Au compared to Au alloys later techniques used platinised gold to improve the Signal-to-Noise ratio (SNR) (Breckenridge et al, 1995). Alternative material types were required for the electrode tips, tracks and contact pads as the platinised Au electrodes were shown to be more unstable over prolonged use due to a greater degree of degradation (Fejti et al, 2006).

New fabrication approaches have become available to MEA biochip manufacture, resulting in changes to the standard material used at the microelectrode tip, tracking and contact pad. Titanium nitride (TiN) microelectrode tips and contact pads are now the most common variant (Figure 2.65) due to superior electrical properties (see Table 2.5), with titanium (Ti), or Indium tin oxide (ITO) used for tracking tracks. Au is used for cheaper MEA biochips that have larger electrodes at greater pitches (e.g. ecoMEA from MCS). Other leading manufacturers, namely Ayanda Biosystems and Axion Biosystems, provide gold (Au) and platinum (Pt). Plasma-enhanced chemical vapour deposition

(PECVD) is an example of a manufacturing technique exploited for the production of these microelectrodes (De Asis et al, 2009).

Table 2.5: Electrical properties of microelectrodes (microelectrode surface area = 80µm^2)

Material	Safe Charge Injection Limit (mC/cm^2)	Charge Capacity (mC/cm^2)	Z_f=1kHz (kΩ)
Au	3.7	5.7	2900
TiN	23	42	150

[Adapted from Janders et al, 1996.]

Titanium nitride (TiN) was introduced to MEA biochip manufacture in the 1990's (Janders et al, 1996). MCS currently produce a range of configurations using TiN microelectrode tips because of the superior electrical properties. TiN has been adopted in part due to its high nanoporosity, which results in electrodes with higher capacitances values when compared to previous Au tips. A high capacitance not only results in improved SNR but also reduces undesirable faradaic processes that if allowed to occur may harm the cells in situ (Janders et al, 1996). The biocompatibility of TiN is also now well documented (Huang et al, 2005). Interesting properties that make it suitable for microelectrode tips include reduced levels of bacteria on TiN surfaces (Scarano et al., 2003) and improved cell adhesion (Groessner-Schreiber et al., 2002; Cyster et al, 2004) when compared to Ti. TiN is also stable in aqueous solutions, and has high abrasion and corrosion resistances (Watari et al, 2004).

Indium tin oxide (ITO) is optically transparent and has been found to have good biocompatibility (Zhang and Oyama, 2005). ITO has been demonstrated as a viable material for creation of recording and stimulating biochip elements allowing systems that could monitor network activity without limiting optical observation of the network during culture (Gross et al, 1995).

Carbon Nantotubes (CNT): As well as good chemical and thermal stability (Gambazzi et al, 2010) carbon nanotubes has exceptional biocompatibility (Harrison and Atala, 2007), are ultra-light weight, high mechanical strength, large surface area and excellent electrical properties (Keefer et al, 2008; Gabay et al, 2007) which are all ideal for the MEA application environment.

Multi-well MEAs

Number of wells: 4, 6, 9
Electrode arrangement: 4x (3x5), 6x (3x3), 9x (6x5) recording with 9x (2x1) stimulating
Inter-electrode distances: 200, 300, 500, 1000µm
Electrode material: TiN
Tracking material: Ti or ITO
Electrode diameter: 30, 50µm electrodes
Electrode impedances: 30µm diameter = 30-50 kΩ
Electrode shape: Planar
Insulation material: Si_3N_4,
Base substrate: Glass
Base substrate thickness: 1mm.
Temperature compatibility: 0 - 125°C.

Electrodes split into groups/patterns.
Groups separated by varying distances.

Where electrodes: Electrode 10µm with Spacing 30µm. Principle = allows the activity of single neuron to be picked up by a number of electrodes. Allows "multi-dimensional fingerprint-like patterns" for identification of single cells more precisely (Fejti et al, 2006)

High-density MEAs

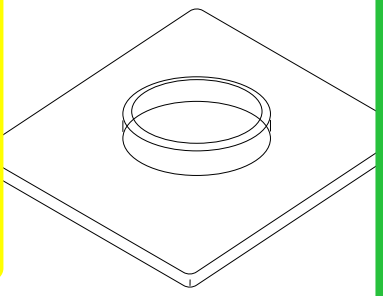
Electrode arrangement: 16x16
Inter-electrode distances: 60, 100, 200µm
Electrode material: TiN
Tracking material: ITO
Electrode diameter: 10 , 30µm electrodes
Electrode impedances: 30µm diameter = 30-50 kΩ, 10µm diameter = 250-400 kΩ.
Electrode shape: Planar
Insulation material: Si_3N_4, Kapton, SU-8.
Base substrate: Glass
Base substrate thickness: 1mm.
Temperature compatibility: 0 - 125°C.

Spatial resolution for conduction velocity calculation and synaptic delay measurability.
100µm spacing in centre, 200µm further out.
Workspace ~2.8 x2.8mm

"Eco" MEAs

Electrode arrangement: 8x8, 2x(5x6)
Inter-electrode distances: 100,200,500 µm
Electrode material: TiN or Au
Tracking material: ITO or Au
Electrode diameter: 10, 30, 100µm electrodes
Electrode impedances: 30µm diameter = 30-50 kΩ, 10µm diameter = 250-400 kΩ, 10010µm diameter = ~30 kΩ
Electrode shape: Planar
Insulation material: Si_3N_4, Kapton, SU-8.
Base substrate: Glass or Kapton
Base substrate thickness: ~1mm.
Temperature compatibility: 0 - 125°C.

MEA Biochip Variants



"Thin" MEAs

Electrode arrangement: 8x8, 2x(5x6)
Inter-electrode distances: 30, 100, 200µm
Electrode material: TiN
Tracking material: ITO tracks
Electrode diameter: 10 or 30µm electrodes
Electrode impedances: 30µm diameter = 30-50 kΩ, 10µm diameter = 250-400 kΩ
Electrode shape: Planar
Reference electrode: Electrode 15
Insulation material: Si_3N_4
Base substrate: Thin glass supported on ceramic.
Base substrate thickness: Thin glass - 180µm, ceramic - ~800 µm
Temperature compatibility: 0 - 125°C
Reason for selection: superior optics.

Flexible MEAs

Electrode arrangement: 9x8, 6x6, 10x2 +4
Inter-electrode distances: 300, 625, 750µm.
Electrode material: TiN or Au
Tracking material: Au
Electrode diameter: 30, 50, 80, 100µm electrodes
Electrode impedances: ~50 kΩ
Electrode shape: Planar
Insulation material: Si_3N_4
Base substrate: Polyimide (2611 foil or Kapton)
Base substrate thickness: ~1mm.
Temperature compatibility: 10 - 40°C (2611 foil),
0 - 125°C (Kapton).
Reason for selection: For in vivo and semi-intact preparations.

Standard MEAs with 60 electrodes

Electrode arrangement: 8x8 or 6x 10
Inter-electrode distances: 100, 200, 500µm
Electrode material: TiN
Tracking material: Ti or ITO tracks
Electrode diameter: 10 or 30µm electrodes
Electrode impedances: 30µm diameter = 30-50 kΩ, 10µm diameter = 250-400 kΩ
Electrode shape: Planar
Reference electrode: Electrode 15
Insulation material: Si_3N_4
Base substrate: Glass
Base substrate thickness: ~1mm.
Temperature compatibility: 0 - 125°C

Perforated MEAs

Electrode arrangement: 8x8, 6x10
Inter-electrode distances: 200µm (8x8), 100µm (6x10)
Electrode material: TiN
Tracking material: Ti
Electrode diameter: 30 (record), 50 (stimulate) µm electrodes
Electrode impedances: 30-50 kΩ
Electrode shape: Planar
Insulation material: Polyimide
Base substrate: Ceramic or glass carrier with Polyimide film perforated centre
Base substrate thickness: 1mm.
Temperature compatibility: 10 - 50°C.

Different arrangements exist where specific stimulation electrodes are integrated.

For slice recording. Positioning and fixation.
Tight contact – for good SNR
By application of negative pressure.
Additionally the same openings can be used to perfuse the slice from the underside.

Addresses concern - penetrating 3D electrodes could damage the tissues natural circuitry.

Figure 2.65: A summary of state-of-the-art commercially available MEA biochips. Microelectrode shapes, materials, diameters, impedances and temperature compatibility can all be compared quickly.

2.10.1.1 Electrode Profile

Microelectrodes may have a planar profile (Figure 2.66) to allow a monolayer of cells (Figure 2.67B) to be grown on top of them (Nam et al, 2004) or may alternatively be shaped into three-dimensional cones (Figure 2.68) for slice preparations (Figure 2.67A). The "3D tips" are designed to penetrate through the outer layers of cells damaged during slicing process to the healthy cells deeper into the slice (geometry information can be viewed in Table 2.6). The outcome is larger signal amplitudes due to better cell-electrode coupling with the inner undamaged cells (Heuschkel et al, 2002).

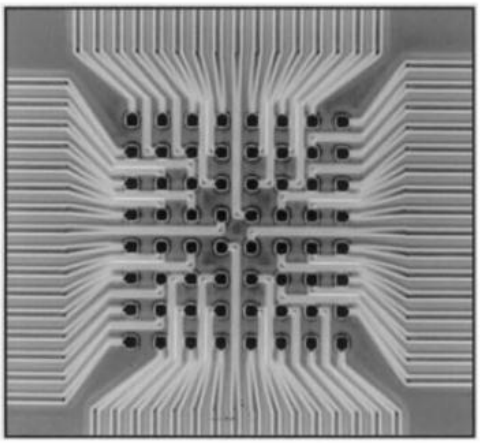


Figure 2.66: A planar MEA. Electrode tips 50 x 50µm.

[Source: Oka et al, 1999.]

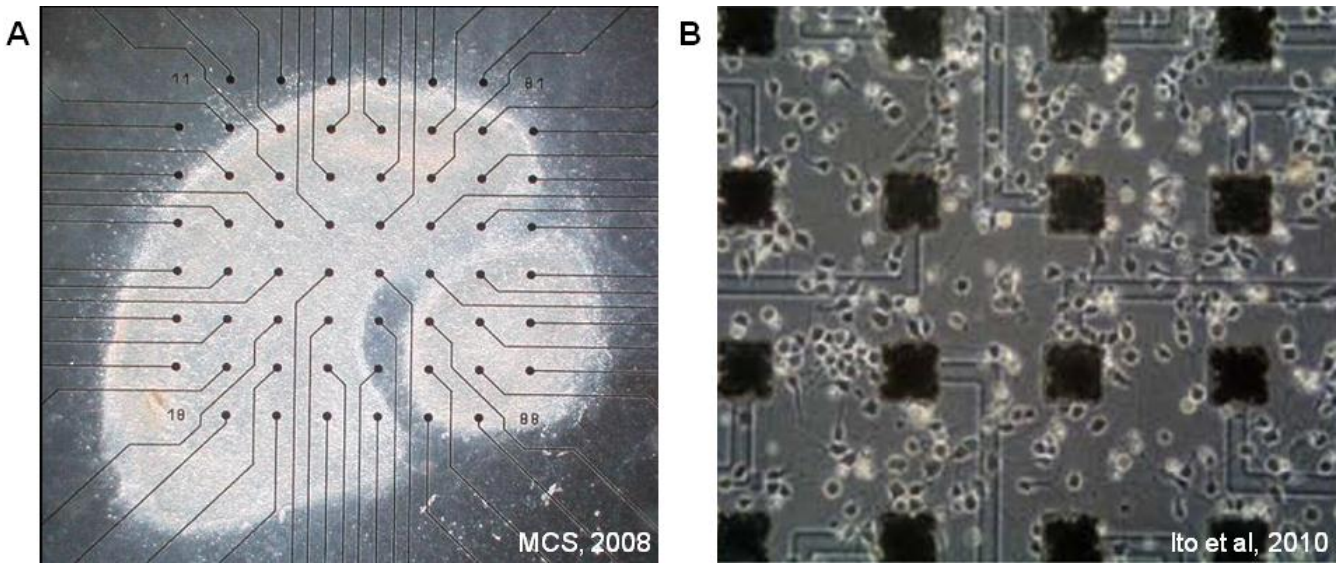


Figure 2.67: Neural cell MEA applications. A) Hippocampus slice positioned over 3D microelectrodes. B) Dissocaited neurons cultured over planar electrodes (~1000 cells per mm^2).

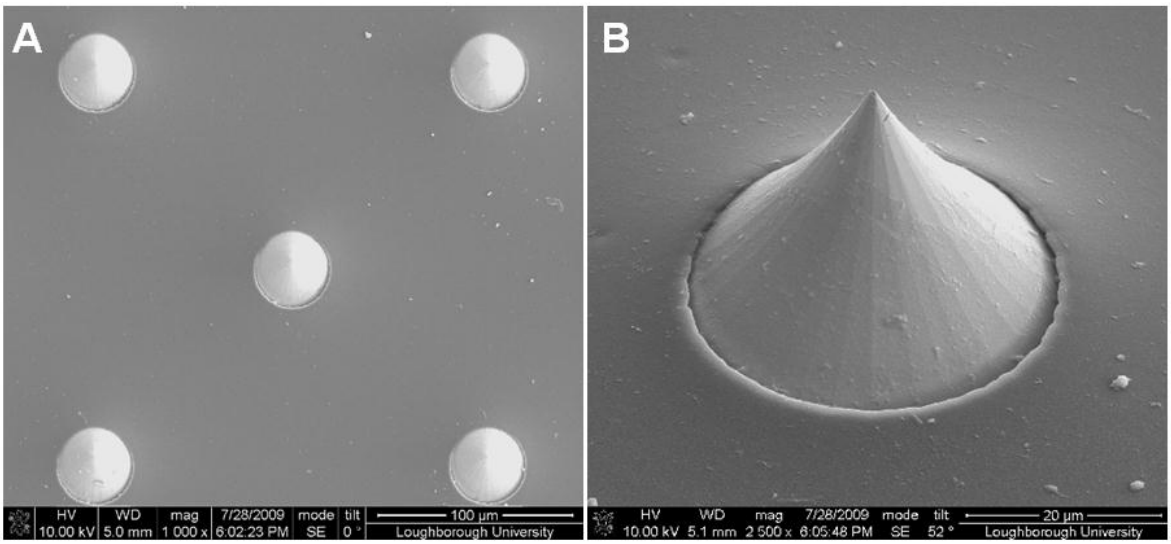


Figure 2.68: A scanning electron microscope (SEM) image of a 3D MEA biochip manufactured by Ayanda Biosystems intended for use with tissue slice applications. Model MEA60 V5. A) A top view of 5 microelectrodes diameter 30µm, magnification x1000. B) A closer look at one 3D electrode, magnification x2500.

Table 2.6: Example of 3D microelectrode geometries in MEA biochips.

Author	Diameter (µm)	Height (µm)	Comment
Held et al, 2010a	30	50	Cylindrical pillar, Gold
Vazquez et al, 2010	~30	≥50	"Scalloped" cone and pillar
Held et al, 2010b	≥2	~5	For intracellular recording on MEA, Silver
Chu et al, 2005	30	50-70	Cylindrical pillar

2.10.1.2 Signal-to-noise ratio

Large electrode surface areas result in an increase in capacitance (Janders et al, 1996). High capacitance is ideal where low noise is required (Adl and Paekerar, 2008). High capacitance is especially relevant in applications that inject electrical stimuli via the microelectrodes, allowing more reliable stimulation protocols to be employed (Meacham et al, 2008).

Typically the standard microelectrode diameter is 30µm. Noise levels have been documented at around less than +/- 10µV of the baseline when sampled at 25kHz. It is important to be aware of the compromises that are interrelated in MEA biochip design. The size of the electrode, the resulting signal-to-noise ratio, and the probability of recording a signal all vary between research applications (Morin et al, 2005). In terms of design, larger electrodes results in bigger capacitances. This reduces impedance values of the electrodes (good for stimulation protocols) but has a detrimental effect on

the signal-to-noise ratio. For detection of cellular signalling the signal-to-noise ratio needs to be as high as possible to ensure that the field potential is distinguishable from the noise.

2.10.1.3 Electrode layout and spacing for signal detection

There is an increasing demand for specific electrode layouts based on specifications laid out by scientists in pursuit of particular biological questions (Fejti et al, 2006). Biochips have been successfully manufactured that deviate from the traditional 8 x 8 grid array design (e.g. Figure 2.65). Previously the size and spacing of the electrodes was considered to be of critical importance when seeking to guarantee signal detection (from neurons). Preparation techniques such as controlled application of surface treatment agents (such as Poly-D-lysine (Soussou et al, 2007)) prior to cell seeding (or plating) are used to enhance attachment and control positioning of the cells over the workspace region of the MEA's surface.

Some particularly representative examples of physical design changes (Figure 2.69) that have been made to biochips in attempts to improve controlled cell positioning for signal detection and quality include the micro-machining of silicon wafers with integrated electrodes to produce "cages designed to imprison neurons" (Maher et al, 1999; Erickson et al, 2008) and production of micro-chambers and micro-channels made from agarose material around and between electrodes to "trap neural cells" (Suzuki et al, 2004b). Awareness of these designs supported research that has delivered novel ideas and findings to this area.

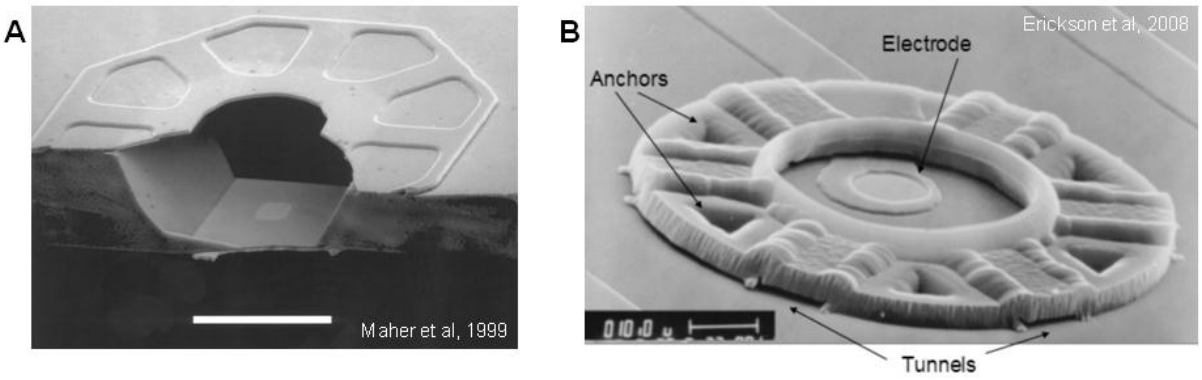


Figure 2.69: "Caged neuron" MEA biochips. A) Scanning electron micrograph (SEM) of a neurochip cleaved to reveal the cross section. The octagonal structure on the top surface is the silicon nitride canopy. The radial raised bars of the canopy are tunnels through which neural processes can grow. The walls are silicon. The floor of the well is a suspended film of silicon nitride. The square centred in the floor of the well is a gold electrode. Scale bar: 20 µm. B) SEM of the "neurocage" design. A neuron is placed in the central chimney region, near the electrode. Axons and dendrites are free to grow though the tunnels to synapse with other neurons. The cage is made out of 4 µm parylene, a biocompatible polymer. Low-stress silicon nitride insulates the gold electrode and leads. Scale bar: 10 µm.

2.10.1.4 Insulation

Insulation materials are applied over the upper surface of the MEA biochips everywhere except at the microelectrode tips and the contact pads to isolate the conductive material from the external environment and culture medium (Heuschkel et al, 2002). This insulation material effectively serves as the underlying cell culture substrate. Biocompatibility is therefore important for all materials selected for application as insulators as they are exposed to the cellular environment within the media well area. The insulator must also be suitable for use in conjunction with pre-treatments, such as poly-D-lysine or Matrigel™, and post-treatments, such as trypsin, industrial methylated spirit (IMS) and plasma cleaning protocols. Materials that have been successfully adopted for this purpose include silicon nitride (Si_3N_4), polyimide, silicon resin, polyacrylamide and SU-8.

In many cases the insulation surface is pre-treated with specific biological molecules prior to cell seeding to promote cell adhesion (Berichevsky et al, 2009). Treatments (e.g. poly-lysine, laminin, polyethyleneimine, and Matrigel™) are applied in minute quantities (e.g. 4µl per 50 biochips) prior to cell seeding via dispersion in solutions. These treatments have also been experimented with for enhancing signal capture reliability through localised, controlled application to precise locations over and between electrodes where cell attachment and growth are most desired (Brewer, Boehler and Wheeler, 2006; Worz et al, 2008; Goto et al, 2010).

2.10.1.5 Most Recent Areas of Microelectrode Development

The recent areas of MEA biochip are listed in Table 2.7.

Table 2.7: MEA biochip areas of development.

Area of Development	Development	Publication and Research Institution(s)
Surface engineering of biochip for controlled cell positioning and/or growth	"3D Scaffold" geometry or microchannels	Musick et al, 2009 – University of Illinois and University of Florida.
	Patterned surface treatments	Kim et al, 2010 - Seoul National University, Seoul, Republic of Korea /Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea.

		Dworak et al, 2008 – University of Illinois.
	PDMS microchannels and Wells	Nam et al, 2010 - Department of Bio and Brain Engineering, KAIST, Daejeon, Korea.
	Agarose and hydrogel microchannels and wells	Harbodt et al, 2010 - University of Tübingen, Reutlingen, Germany /Multi Channel System MCS GmbH, Reutlingen, Germany/ TILL Photonics GmbH, Gräfelfing, Germany.
	Laser	
Electrode Surface	Carbon nanotubes (CNT)	Gambazzi et al, 2010 - Lab. of Neural Microcircuitry, Brain Mind Institute, EPFL, Lausanne, Switzerland /Dept. Pharmaceutical Sciences, Univ. Trieste, Italy /Naturwissenschaftliches und Medizinisches Institut (NMI), Reutlingen, Germany /Dept. Biomedical Sciences, Univ. Antwerp, Universiteitsplein 2, Wilrijk, Belgium.
		Koester et al, 2010a – University of Rostock, Germany.
	Nano-structures	Koester et al, 2010b - University of Rostock, Germany / University of

	Pores	Freiburg.
Electrode Geometry	Pillars and 3D cones for stimulation	Held et al, 2010a – NMI Reutlingen, Germany. Vazquez et al, 2010 – Technical University of Denmark.
	Cavities	Baaken et al, 2010 - University of Freiburg, Germany
	Microneedles	Saito et al, 2010 – University of Tokyo. Gunning et al, 2010 - University of Glasgow, Glasgow, UK /AGH University of Science and Technolgy, Krakow, Poland /University of Indiana, Bloomington, IN, USA /University of California Santa Cruz, Santa Cruz, CA, USA /SLAC National Accelerator Laboratory, Menlo Park, CA, USA
Electrode Material	CNT	Gabriel et al, 2010; Bongard et al, 2010 - Instituto de Microelectronica de Barcelona, Spain /Institute of Biomedical Research August Pi y Sunyer, Barcelona, Spain /Institut Catala de Recerca i Estudis Avancats, Barcelona, Spain.

		Fuchsberger et al, 2010; Stamm et al 2010 - University of Tuebingen, Reutlingen, Germany /University of Trieste, Trieste, Italy.
		Held et al, 2010b - NMI Reutlingen, Germany.
	Solid silver (Ag) Microneedles	Gobbels et al, 2010 - RWTH Aachen University, Germany
	Iridium Oxide	Colombo et al, 2010;Gosso et al, 2010 – Ulm University, Germany /NIS Centre Italy.
	Boron-doped nanocrytalline diamond	Ryynanen et al, 2010 (polystyrene) – Tempere University of Technology, Finland Kang et al, 2010 – KAIST, Daejeon, Korea.
	Polymer Coatings	Gautam and Narayan, 2010 - Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur P.O., Bangalore, India.

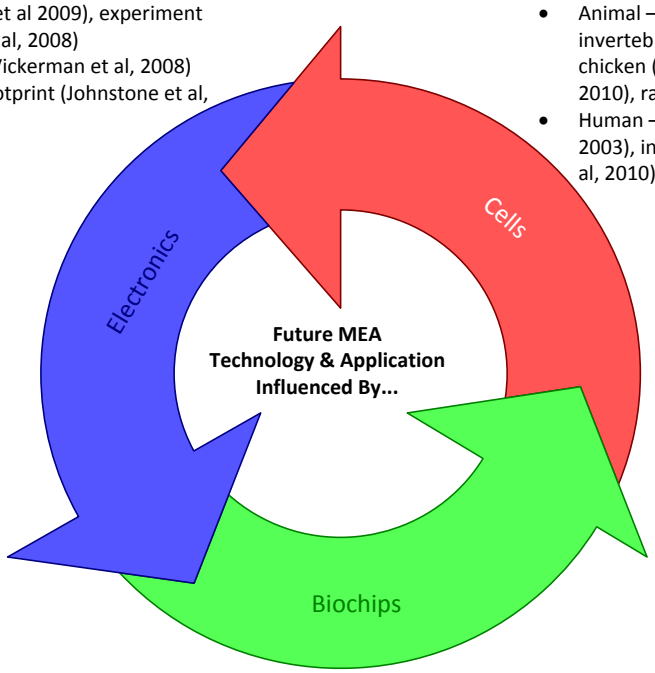
2.11 Future

In vitro MEA systems currently allow a number of different cell types and sources to also be analysed in different ways, increasing not only convenience for user groups but also the diversity of applications to which these systems can be applied. Figure 2.70 illustrates factors that are influencing the design and manufacture of MEA systems.

Types:
- Neurons (Hill et al, 2010)
- Cardiomyocytes (Denning and Anderson, 2006)
- Other – smooth muscle cell (Nakayama et al, 2008), mast cell (Yeung et al, 2008), Islet cells (Raoux et al, 2001), Plant root apex (Masi et al, 2009).

Sources:
- Animal – monkey (Lehmann et al, 2010), invertebrate (Merz and Fromherz, 2002), chicken (Jones et al, 2011), mice (Biodo et al, 2010), rat (Hill et al, 2010).
- Human – embryonic stem cell (Banach et al, 2003), induced pluripotent stem cells (Gupta et al, 2010)

Evolution of technology:
- Through increased throughput (Breier et al, 2010)
- Increased Sophistication – analysis (Brown et al, 2004), system (Charvet et al 2009), experiment protocols (Vickerman et al, 2008)
- Increased automation (Vickerman et al, 2008)
- Reduced system size/footprint (Johnstone et al, 2010)



Improve specialisation for cell types and preparations:
- Biochips for brain region research (Gholmieh et al, 2006)
- Biochips for general slice preparations (Boppart et al, 1992)
- Biochips for beating cardiomyocyte clusters (Flaherty et al, 2012)
- Biochips for controlled neural network propagation (Dworak et al, 2008)

Improve fabrication capabilities:
- Manufacture biochips that allow more recording channels (Berdondini et al, 2009)
- Manufacture smaller electrode geometries to facilitate improved resolution over equivalent contact area (Aziz et al, 2009).

Pursue novel sensor/electrode materials:
- Carbon nanotubes for better electrical connection with cells (Bongard et al, 2010)
- ITO for improved optical parameters (Jahnke et al, 2009)

Figure 2.70: Future MEA technology application will be directly influenced by a combination of developments across cells, biochips and electronics.

There is promise for MEA systems to support assessment of cells for cell therapies, and in new drug discovery and safety pharmacology. A need to screen compounds in their native environments on larger scales, rapidly is required. Increased sophistication of attention to new chemical entity treatments prior to in-vivo studies could potentially eliminate earlier those candidates that will fail at a later stage (Stett et al, 2003), therefore reducing revenue lost.

The following schematic (Figure 2.71) demonstrates potential cell sources that have been successfully coupled to MEA technology and how application of cells types is changing. The combination of patch clamp and MEA technologies have been validated as valuable methods of assessing electrophysiological characteristics of the cell types described. Systems will continue to evolve with the needs of the differing cell sources and electrophysiological characterisation requirements of fundamental research, cell therapy development and pharmacology settings.

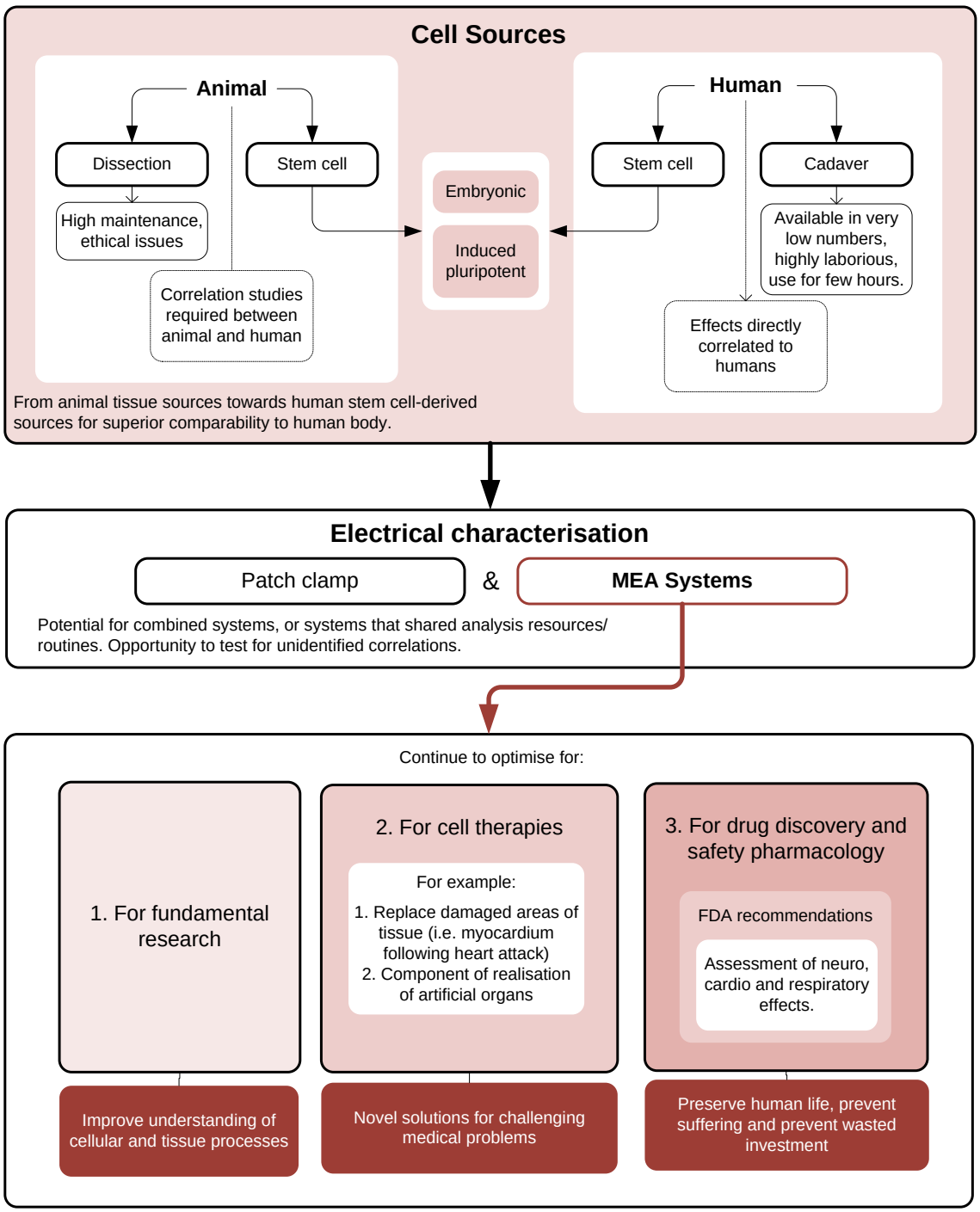


Figure 2.71: How cell sources and electrical characterisation technologies will combine serve future research, medical and pharmaceutical industry needs.

2.11.1 Cell types/sourcing

Much of the work published that has used MEA technology has studied cells and tissues from animal sources such as mice (Biodo et al, 2010), monkeys (Lehmann et al, 2010) and chickens (Jones et al, 2011). Animal cell sources can be genetically mutated (Bazelot et al, 2012) relatively simply and are available in greater quantities (Johnstone et al, 2010) than living samples directly from humans.

Safety pharmacology applications still require animal tissue based testing but future intentions aim to reduce or eradicate the need for animal testing by adjusting testing to cells sourced from human stem cells (Johnstone et al, 2010). This source is intended to show a closer correlation of responses observed in *in vitro* testing to human centred *in vivo* stages at clinical trials. At present work must be conducted following animal based trails to correlate findings to likely human tissue/system responses.

2.11.1.1 Human Stem Cell Sources

Human stem cells can be acquired by different means. Embryonic stem cell lines have been established using discarded In Vitro Fertilisation (IVF) embryos (Thomson et al, 1998). This source of stem cells is globally controversial due to a number of ethical debates centred on the destruction of human embryos. Alternatively induced pluripotent stem cells have recently emerged (Takahashi et al, 2007) which derive stem cells from human adult somatic cells (already differentiated cells taken from an adult donor). A somatic cell can be any cell that contributes to the formation of an organism, such as a skin, bone or blood cell.

Stem cell extraction and passaging from human embryos is illustrated in Figure 2.72. Stem cells are transferred from a pre-implantation stage embryo into a culture dish containing an appropriate culture medium. The stem cells divide and spread over the surface of the culture dish. In some cases a feeder layer is used to support the stem cells *in vitro*. Mouse cells are used to create a sticky surface to which the stem cells can attach. These feeder cells also release nutrients into the culture medium.

If the plated cells survive, divide and multiply enough to crowd the culture dish they are gently re-plated into several culture dishes. The process of re-plating (known as sub-culturing) is repeated many times over several months. Each re-plating (sub-culturing) cycle is referred to as a passage.

Once a the embryonic stem cells have proliferated in cell culture for a prolonged period of time without differentiating , they are confirmed to be pluripotent, and they have not developed genetic abnormalities they are referred to as a stem cell line.

The process of establishing stem cell lines is inefficient so a new line is not established every time embryonic stem cells are needed. At any stage in the passaging process batches of cells can be frozen and shipped to other stem cell users (The National Institutes for Health Resource for Stem Cell Research, 2012).

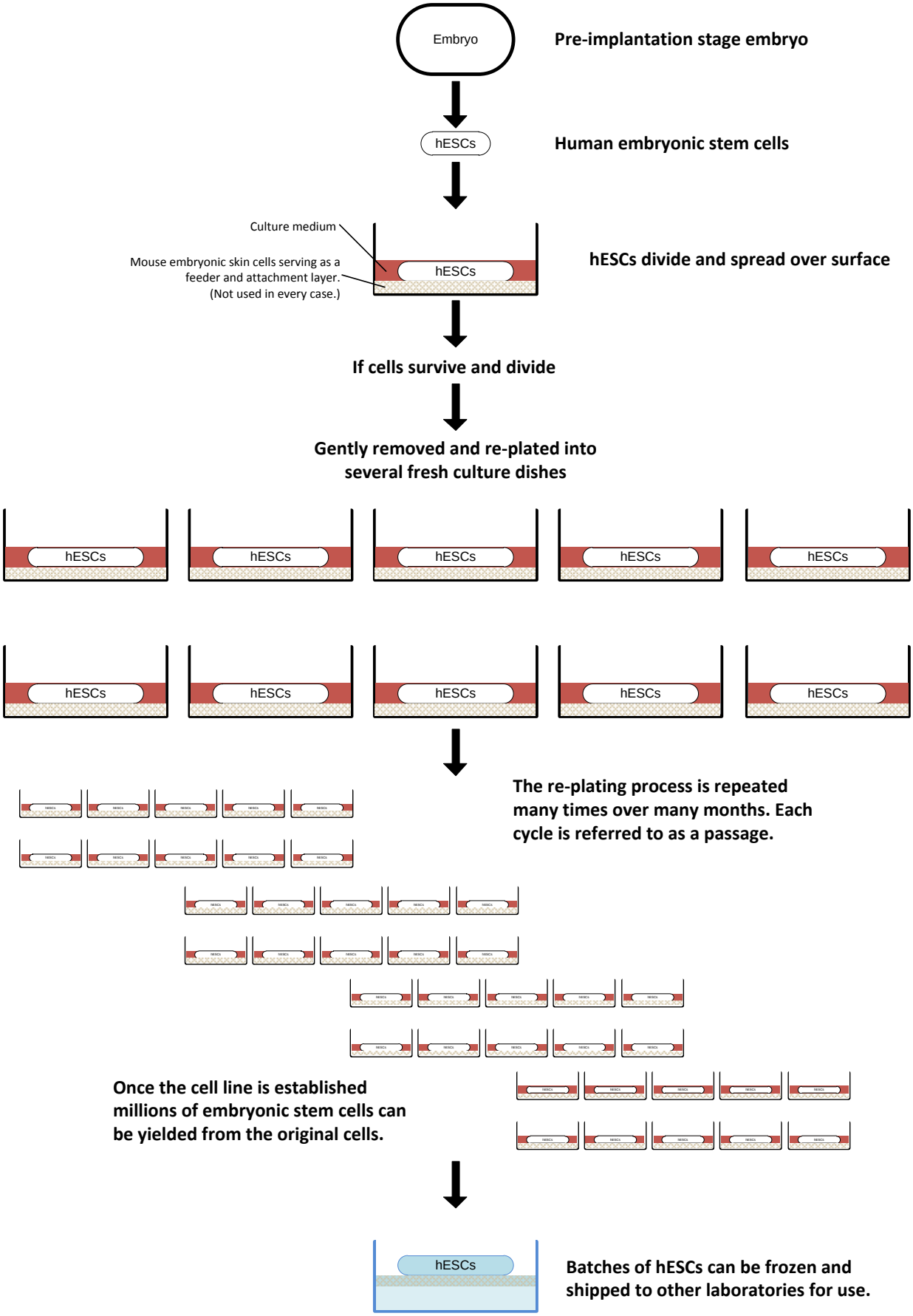


Figure 2.72: Stem cell passaging and line establishment.

2.11.1.1.1 Cells for cell-based therapies

Cell therapies are amongst the most appealing applications for stem cells. Cardiomyocytes derived from stem cells are an example of a cell type currently under examination as potential cell for therapy (Xu et al, 2006).

A common mechanism of development of heart failure is the loss of ventricular cardiomyocytes (Lee and Terracciano, 2010). It is hoped that they may in the future contribute to restoration of normal heart function by replacing damaged cardiomyocytes in damaged tissue (Braam et al, 2008). This is an on-going area of research that is believed to hold much promise and early experiments using skeletal myoblasts were transplanted with the intention of replacing damaged cells. Results showed modest improvements (Taylor et al, 1998) but later repetitions found that cells appear to remain mechanically and electrically isolated from the recipient myocardium (Rubart et al, 2004). Bone-marrow derived cells have also been attempted (Orlic et al, 2001), showed encouraging results, but these results have not been replicated by others attempting to reproduce them (Murry et al, 2004). Interestingly all groups measured improvement in whole heart function.

2.11.1.1.2 Drug discovery and safety pharmacology.

Recent work has revealed pharmacological sensitivities that can be identified and characterised using MEA systems (Bettencourt et al, 2008; Ham et al, 2008). Any possibility to improve decision making and reduce timelines and attrition rates would provide enormous benefits to the process of drug discovery and development (Braam et al, 2008). The current work of several laboratories is testing the same set of compounds using a standard approach to demonstrate that chemical entities can be identified and categorised consistently based on observations of their toxicity pathways (Johnstone et al, 2010). A 5 year European Union (EU) funded project called ACuteTox took place between 2005-2010. This project involved working to integrate toxicity screening approaches so that standard assessments of toxicity can be conducted (Bal-Price et al, 2008).

2.11.1.1.2.1 Neurotoxicity

"MEAs are high content platforms that can provide detailed information regarding changes in function of networks of neurons exposed to test compounds" (Johnstone et al, 2010). At present regulatory authorities (e.g. Organisation of Economic Cooperation and Development, U.S. Environmental Protection Agency) use solely animal *in vivo* methods for both adult and developmental neurotoxicity testing. Until now "no *in vitro* approaches for evaluating the neurotoxic hazard of compounds have been formally validated." The main problem in the development of a test

strategy with a predictive capacity for neurotoxicity is the complexity of action on the human nervous system. Mechanisms underlying the effects of compounds in the central nervous system (CNS) and the peripheral nervous system (PNS) are so diverse that it is currently impossible to cover all these mechanisms with one single model and using a small set of end points (Bal-Price et al, 2010).

Previous work has used spike rates or average spike rate for neurons in a network as one parameter to describe actions of drugs or chemicals even though even more sophisticated assessment is possible on MEAs. The possibility to determine concentration-response curves using neural activity as a neuronal specific endpoint has been suggested and early demonstrations presented (Lenk and Priwitzer, 2011).

2.11.1.1.2.2 Cardiotoxicity

Effects on the hERG channel present in the cell membranes of cardiomyocytes, which governs the I_{Kr} current, can be blocked by drugs resulting in cardiotoxic effects. The hERG channel has a major role in cardiac repolarisation. Interference at the hERG channels affects the length of action potentials and consequently the QT interval on an ECG. In humans a prolongation to anything greater than about 440-460ms may result in life threatening arrhythmias. Identification of QT interval changes and specifically its prolongation has led to the withdrawal of some drugs from the global market.

The QT interval on an ECG corresponds to the total time of ventricular depolarisation. It's now part of standard pre-clinical evaluation of all new drug candidates to assess risk of delayed repolarisation and prolonged QT interval (Braam et al, 2008).

2.11.2 Medium to High Throughput Screening

The need for higher throughputs throughout future MEA system applications is summarised in Figure 2.73. MEA technology application is presently time consuming and labour intensive. Increasing the throughput capability of systems will allow opportunities to speed up experiments and to improve experiment efficiencies. Development of new MEA technologies will improve reproducibility of results in fundamental research by providing larger more detailed data sets, applicable cell therapies development, and especially in drug discovery and safety pharmacology settings. This combined with automation may be key to convincing the pharmaceutical industry to

use planar MEA technologies for drug discovery and high-throughput drug screening (Morin et al, 2005).

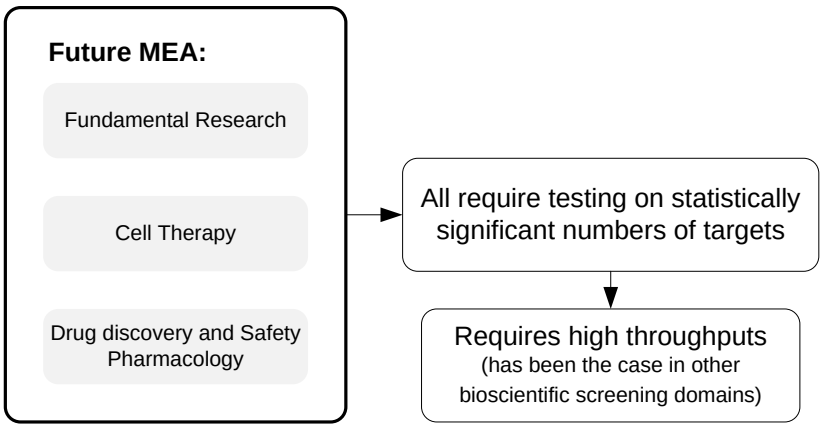


Figure 2.73: Future MEA technology will be required with high throughput capability across application domains.

2.11.3 MEA for a Brain Machine Interface

The emerging field of neuroprosthesis aims to restore some of the lost neural function by selective electrical stimulation of specific sensory or motor pathways. A key element in this research field is the interfacing of the electronics that will deliver the stimuli with the nervous tissue. Development of penetrating MEAs for *in vivo* applications is concurrently evolving drawing upon over lapping scientific principles and microfabrication techniques. The active tips in these penetrating MEAs provide selective access to small populations of neurons or nerve fibres. These MEAs also provide scientists with an ability to study spatiotemporal activity and information processing in a living nervous system, findings which may form the basis of future therapies. This technology is still in its infancy but current proof of principle studies argue much potential (Normann, 2007).

2.12 Product Design and Assessment

Each time a novel product or new iteration of an existing product is developed, appropriate assessments must be carried out on that product to ensure that it delivers performance according to initial specifications. A design process incorporating innovation management principles relevant to MEA technology development is illustrated in Figure 2.74.

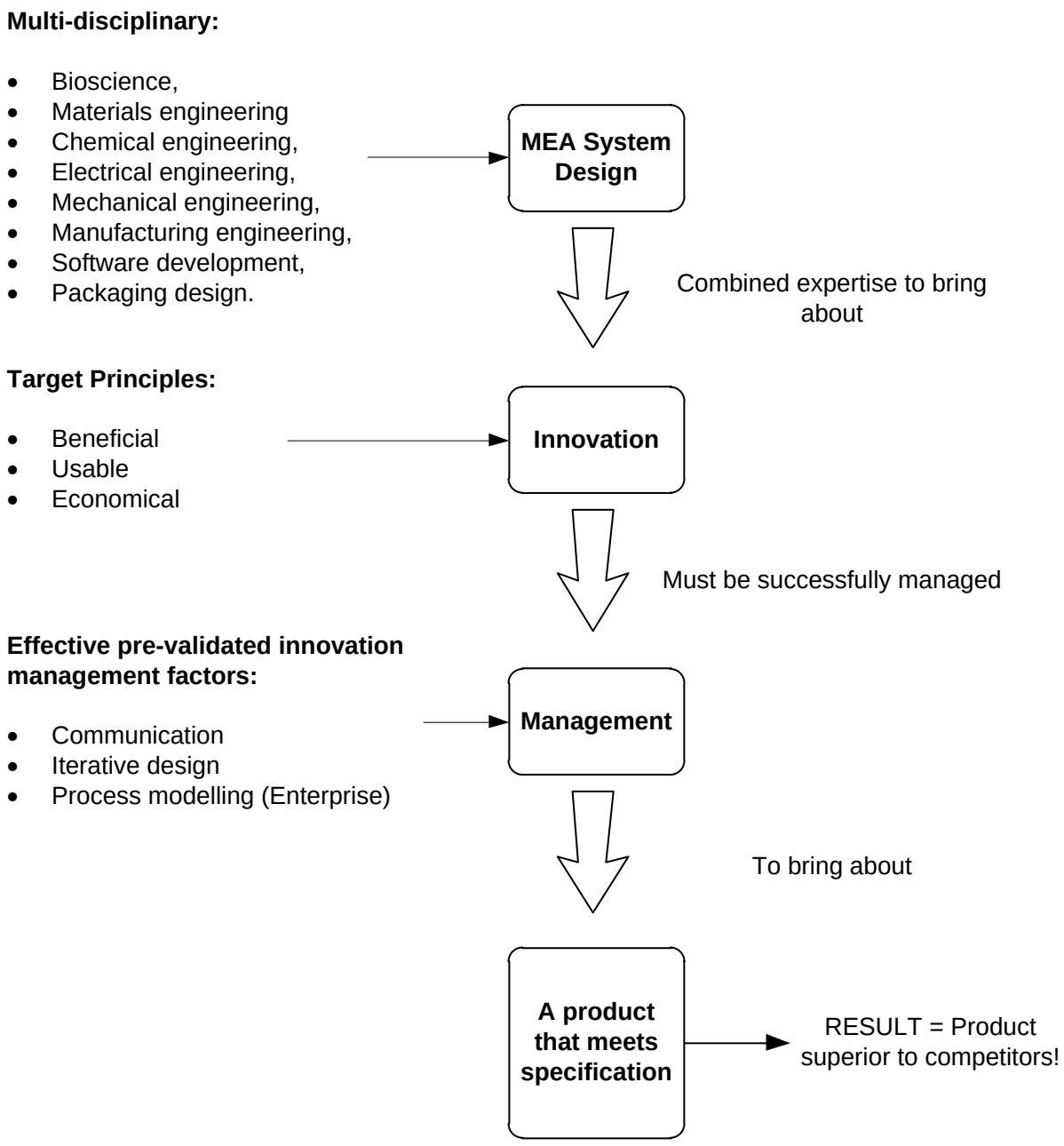


Figure 2.74: Innovation management principles.
[Adapted from Le Corre and Mischke, 2005.]

2.12.1 Product design

A new product must be designed with features that distinguish it from other alternatives (Saffer, 2010). The research and design process must identify and realise potential product differentiators that will motivate consumers to purchase this product over competing equivalents. Distinguishers must be decided upon during the concept design stages to ensure adequate inclusion into the final product. In the case of MEA technology an existing industry and assortment of products already exist so distinguishers will be particularly important for intended large scale system user uptake. Work to introduce a new device or system will depend upon marketable distinguishers that offer real value to current and future system users. Before realisation planning can take place, a final design must be conceived. The design must be original if a unique benefit is to be brought to system users. The process of manufacture must then also be designed to realise the final concept as physical devices. Figure 2.75 describes concept design stages common to all design problems that lead to a final design, and the corresponding inclusion of those stages relating to this thesis.

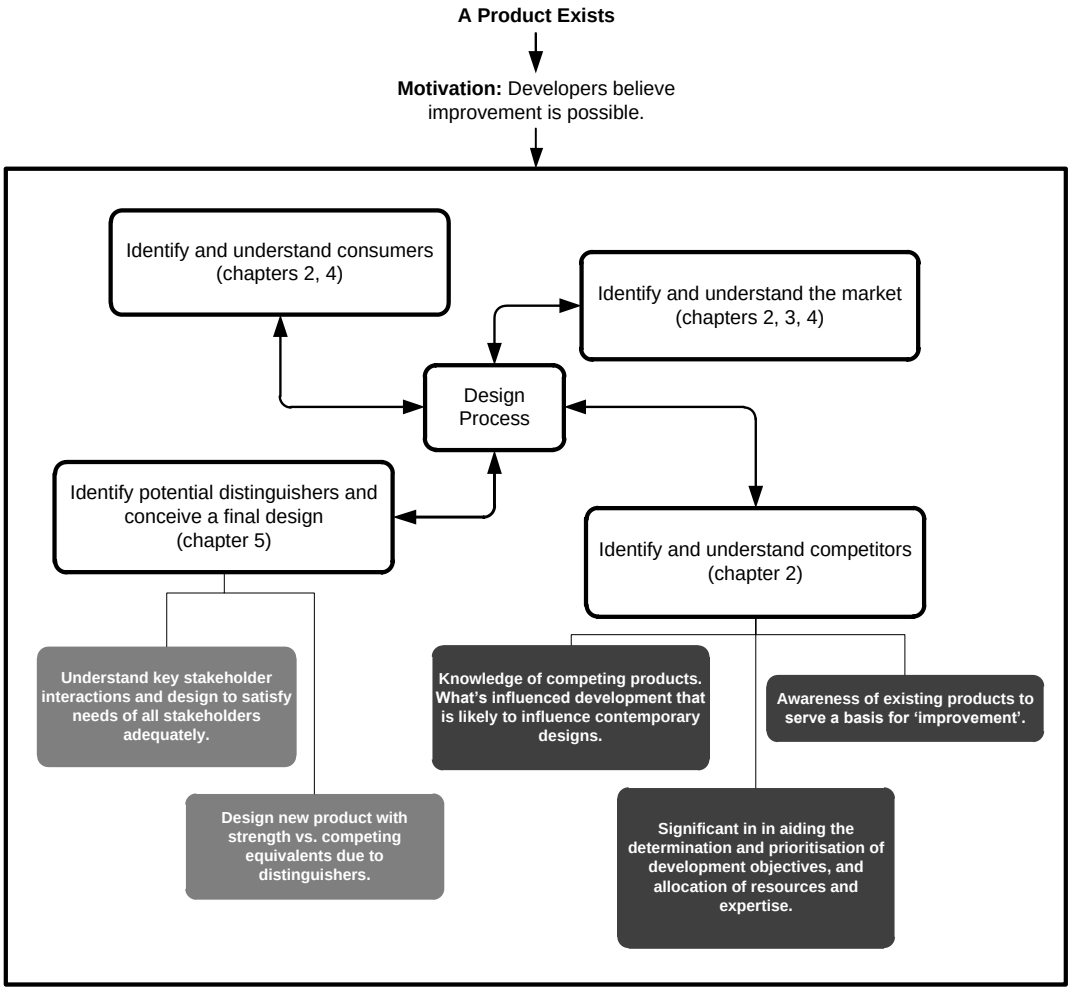


Figure 2.75: Product design research principles.

2.12.2 Innovation management

Strategic business approaches are employed across industries in order to identify: (1) customers and markets to be served, (2) competitors, and (3) potential competitive strengths of a new product or redesign (Saffer, 2010). A framework for evaluating products and potential opportunities can be constructed via appropriate product planning (see Chapter 3), influencing product success from a number of angles. For example, if considering a product's positioning relative to competing products, appropriate identification of distinguishers and product differentiators must be incorporated into the product development. Awareness of competing products in the sense of how they have been, and how they continue to be developed is another angle that aids the determination and prioritisation of development priorities for new products. Resource allocation for product development and product deployment should also be contemplated and distributed appropriately in planning stages. This can be done by utilising information derived from forward planning (Schattenberg et al, 2005).

Management principles that can be applied to innovation include: (1) manage time from start to finish, (2) map out objectives and allocate resources appropriately, (3) pilot tests prior to full scale commencement, (4) identify and understand key figures and institutes, (5) embrace techniques and technologies that will be required to facilitate innovation and communicate output early on, and (6) share responsibility appropriately between innovators (Stark, 2011).

Early identification of key stakeholders in a product's lifecycle serves as a good way to assess major interactions that are likely to occur during all product development stages, as well as throughout the product's life in service (Finkbeiner, 2011). The stakeholders in this field and their interactions with one another can be seen in Figure 2.76. Relevant interaction details that are part of the overall impacts that may affect stakeholder parties that must be considered if changes are made to this particular product are depicted in this diagram.

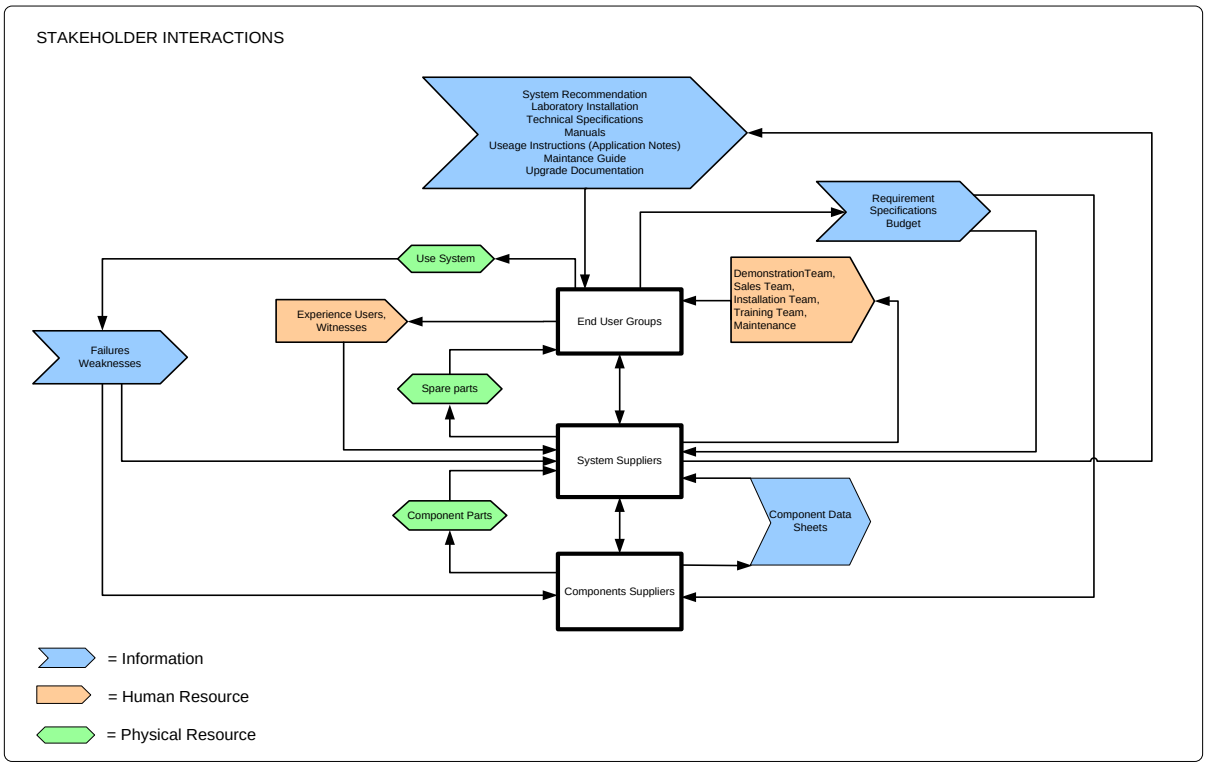


Figure 2.76: The major human involvements, physical parts and information flows that result from MEA technology in use.

Research at Cambridge University's Centre for Technology Management has outlined a five-step process for making a business case for new technologies (see Figure 2.77 (IfM Briefing, 2009)). These steps have been taken into account during this work so as to ensure a final product that brings significant value to the real end-users. The five steps are concisely defined in terms of this project as follows:

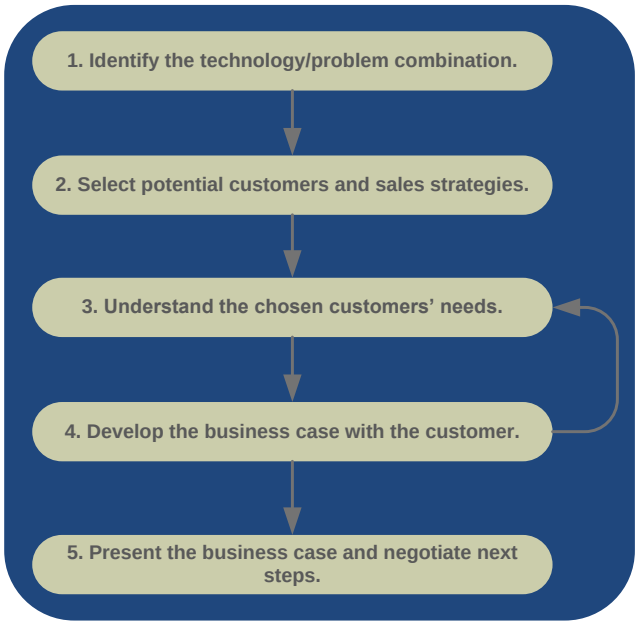


Figure 2.77: Five steps for making a business case for new technologies.
[Source: IfM Briefing 2009].

1. **Identify the technology/problems combination:** MEA systems exist in a relatively immature state and are presently expensive to purchase. In addition to this, systems are inherently complex in both design and user interaction. The scales of employment of MEA technology in scientific trials is limited by hardware capability constraints and a lack of appropriately sophisticated analytical tools.

2. **Select potential customers and sales strategies:** MEA-systems are sold on a global scale. Early employment of this technology has been for addressing specific research questions; the needs of which are becoming ever more diverse. Application of MEA-systems within a range of research domains has highlighted potential for next generation exploitation of this technology on greater scales within the pharmacology industry (Meyer et al, 2004).

3. **Understand the chosen customer needs:** Systems that meet the specific needs of three end user cases have been explored. Proper identification and understanding of the needs of each case is essential if systems are to be designed to meet true user needs (see Chapter 5).

4. **Develop the business case with the customer:** A final system that can be exploited economically must not only be what a high number of end users will desire, but it must also be a viable purchase that is affordable, maintainable and fully exploitable by individuals with differing skill sets. Input from the current user groups assists in ascertaining what these "desires" will be and what level of financial output will be acceptable to differing groups around the world.

5. **Present the business case and negotiate next steps:** This step is covered in Chapter 7.

These steps have been utilised throughout this product development and the information derived from them is presented in this thesis.

2.12.3 Biomedical industry

In the last decade the biomedical industry has been the fastest growing sector of the US economy. Annual spending on healthcare in the USA increased from $75 billion in 1970 to $2.2 trillion in 2007, and estimates are at $4.3 trillion for 2018 (Turchetti et al, 2010). A positive correlation exists between the adoption of new medical technologies and the increased overall healthcare costs. The current global economic climate is resulting in increasing pressure on technology developers to produce medical technologies that are more cost effective. The role of biomedical device testing and evaluation is thus concurrently evolving to ensure that in products delivered at a lower cost overall safety is maintained. These evaluation processes also influence adopters decision to acquire, implement and apply these new technologies.

The modelling technique used in this research seeks to improve efficiencies by identifying weaknesses or bottlenecks that can be improved through technical solutions. The CIMOSA technique has previously been used in a clinical setting to model the protocols employed in providing care to patients in a hospital (Staccini et al, 2001). This work highlighted inefficiencies that could be tackled through the development of new management methods and technological devices. The CIMOSA modelling technique has not been reported as having been used to assess protocols used in a bioscientific cell culture setting before. In the case of this research this method of user requirement elicitation is novel.

2.13 Chapter Summary

The following list is a summary (Figure 2.78) of the key points identified and understood from initial context research and literature reviewed.

- MEA systems are tools developed for evaluating tissues and cells non-invasively.

- Traditionally MEA technology has facilitated testing of cells for longer durations than invasive patch-based recording techniques. In addition to this whole samples of tissue can be studied improving upon previous single cell methods.

- Numerous tissue types can be examined using MEA systems. In more recent years some cases using non-electrogenic tissues where a resting potential could be detected have been successfully demonstrated.

- It is possible to detect and capture signals by careful and specific preparation that varies according to tissue type and source.

- Signal traces captured using MEA systems differ vastly between cell types, the form of cell preparation and the testing protocols employed.

- MEA systems offer opportunities to exploit tissues from genetically mutated animal candidates more thoroughly and on larger scales; helping to provide better value genetics research.

- Different types of stimuli can be applied to the cells in controlled and repeatable ways.

- Analysis of signals is often via a custom routine that has been developed by the bioscientist's research group, institution or collaborators.

- An MEA system consists of a number of interconnected complex components.

- Each component must be designed to fulfil its own function as well as be complementary to all of the other interdependent components in the system. Where necessary some

components must be designed and manufactured to be specifically configurable to the particular cellular preparations and/or applications.

- A number of software packages can be adopted to meet the ever evolving needs of analysis; usability of these software could be improved.

- A number of system configurations are commercially available from a small number of vendors. Systems must be custom ordered and configured to a specification that specifically suits the intended application(s) of users.

- MEA biochips exist in a number of configurations to suit the needs of the differing cellular preparations.

- In addition to differing configurations biochips are manufactured in a range of materials to help to accommodate differing budgets and applications.

- Microelectrodes have evolved over the past thirty years due to improved sophistication of microfabrication and manufacturing techniques.

- A number of research groups around the world are working to improve and further optimise MEA biochip designs. Current trends in design support re-configuration of MEA biochips toward cell type and application specialisation and away from a standard biochip for all.

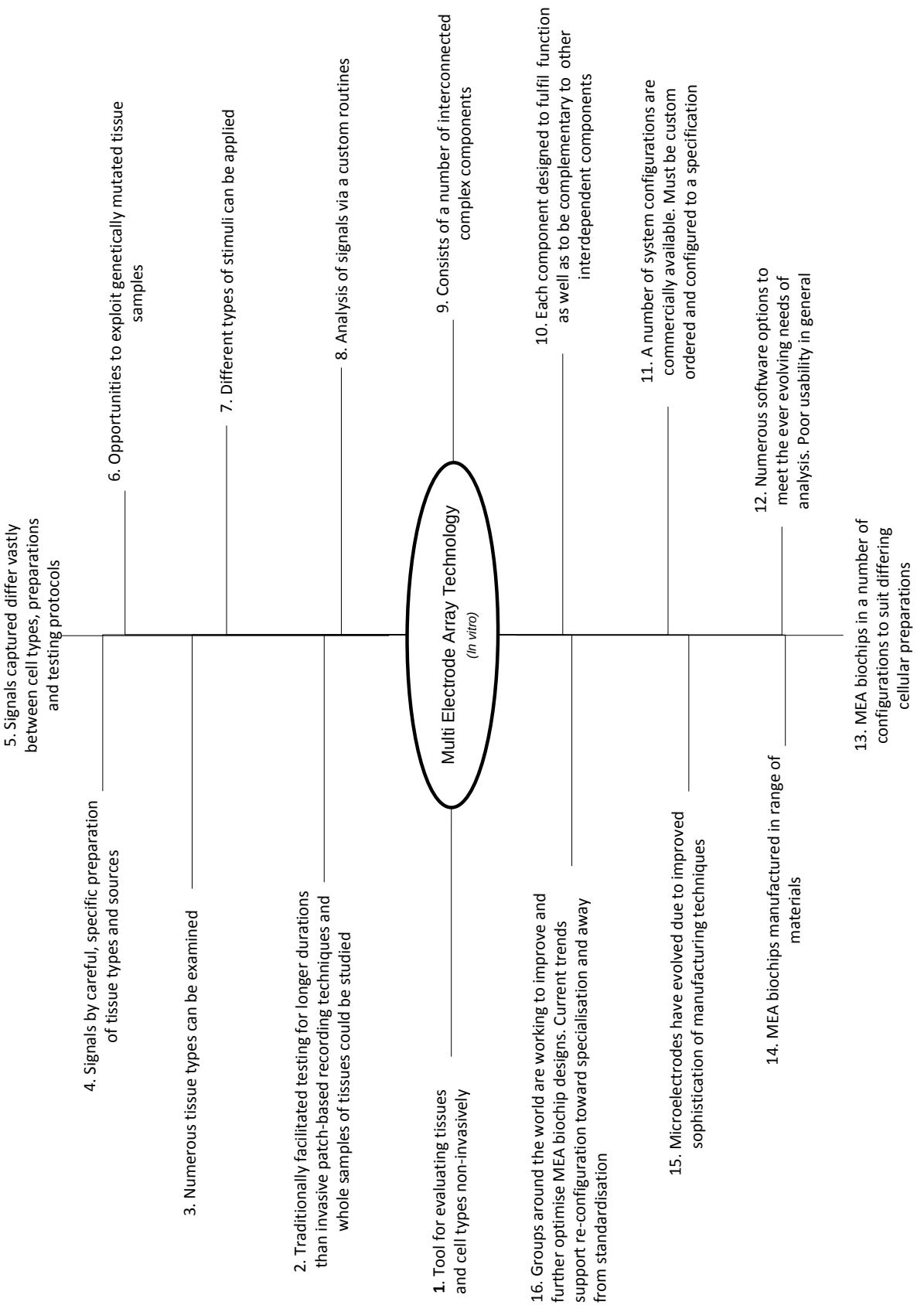


Figure 2.78: A summary of the findings of Chapter 2.

2.14 Research Question Answers

The following address the research questions originally defined at the start of this chapter.

1. **In what context(s) are multi electrode array systems used, what are the core components and how do they differ between systems? What is the current state-of-the-art?**

Used to:

Multi electrode array systems are employed to detect and capture the electrical signalling occurring in electrogenic tissues and cells. Research characterising various cell types and seeking to understand specific responses can be conducted using these systems to detect, monitor and record changes of specific ionic concentrations over time. These systems are predominantly applied to record activity occurring through neural and cardiac tissues, or more specifically through neuron and cardiomyocyte cells.

Used in:

MEA technology is a tool that is suitable for both fundamental and applied research. For example MEA-based studies can be used to investigate fundamental research questions such as “how do neurons grow in vitro?” or “at what stage in development do neurons start communicating spontaneously?”, and applied research questions such as “how does this chemical entity effect activity in this neural network?”.

Used by:

MEA systems are also employed in the most part by small teams of research scientists working with a specific cell type that is prepared or cultured for recording in a particular way. For example, some MEA user scientists will prepare slices of tissue that will be anchored tightly over the microelectrodes for recording, whereas others will culture neurons dissociated from tissue and cultured over the microelectrodes surface. These differences in preparation will result in differences in signal detection that results a need to allow for system configurability.

MEA systems are comprised of a **biochip**, interconnecting into a unit referred to as a **headstage**, that usually houses specialist **amplifiers** and **filters** that interconnects to a **data acquisition system** and **PC**. An acquisition and basic analysis **software** is usually supplied with MEA systems by the manufacturers. A number of other peripheral tools support MEA system application. For example

appropriate **optical tooling** makes it possible for bioscientists to work with microscopic cell populations.

Different system configurations exist to meet differing needs of users. In essence the configuration used will depend on the cell type and the intended preparation of that cell type that is to be investigated. The model of MEA biochip will also be selected according to the cell preparation and intended nature of the investigation being conducted. The amplifiers will provide appropriate degrees of gain to suit the anticipated signal and filters will be configured to removed noise components and enhance signal features of specific interest. The settings in the acquisition unit and software will be dependent on the requirements of the users with regard to how they intend to analyse and interpret the data collected.

Current state-of-the-art in MEA systems is demonstrated by Multi Channels Systems as the USB MEA256 system (section 2.6.1) and their USB-MEA32-STIM4 system. The USB MEA256 system can be considered that most advanced generally applicable MEA system, suited to all cell types and preparations. The USB-MEA32-STIM4 system is the first of new range of systems optimised specifically for slice studies. This system has perforated MEA biochips and an option for automated perfusion through the biochip and system while in use.

The competing MEA system vendor Axion Biosystems, USA, intends to offer the new state-of-the-art system in the form of the “Maestro” system. This system will incorporate 768 recording electrodes into varying multi-well biochip configurations but has yet to appear on the market or in published literature.

The relatively immature vendor, 3 Brain, offers the world’s first commercially available CMOS based MEA. This tool holds particular promise for neurological studies as the fine resolution of the 4096 sensors offers superior resolution. A limitation of this system however is the restricted optical inspection of cells once cultured on the array; for studies where optical inspection is essential and resolution is less important this system is unlikely to be adopted.

2. **How are MEA systems applied in research, how has that application changed since their introduction and are there any trends identifiable in development?**

Initially MEA systems were developed by neuroscientists to facilitate recordings of the electrical activity in neural tissue with the intention to bypass the necessity to physically damage the cellular sample as is the case for early clamp-based electrophysiological recording techniques. For the first

time MEA tools allowed scientists to record several areas of activity simultaneously from a culture of cells. More has been learnt about the functioning of the mammalian brain due to early MEA-based testing and as more has been understood new questions requiring greater and greater degrees of resolution have been required.

As a consequence of the need for greater resolution the number of channels that can be recorded from has also increased resulting in larger data sets if desired or required.

The facility to record responses to stimuli has been demonstrated across differing cell types and preparations. Both chemical and electrical stimuli have been successfully delivered at non-lethal doses to cells on MEA biochips.

So as more application compatibility has been demonstrated and understood, new questions have been asked and development of system components to suit this increasingly specialised questioning has resulted. A dominant trend in MEA development has been to facilitate more channels to record from in each single MEA biochip. In addition to this, the configuration of the available electrodes has also been explored on numerous occasions.

3. **What are the key factors that will influence and limit design possibilities and where are the biggest challenges associated with this work?**

Influence:

The cell types used influence design and introduce diverse requirements. Literature sources report a broad selection of cell sources with predictions of moves from animal sources towards stem cells. Global research and development is especially strong in areas that offer high value returns, such as, drug identification and development, and pursuit of understanding how mammalian and human brains function. This may influence how systems develop if requirements in these fields are particularly specialised and strong.

To understand complex research questions with thousands of variants that must be explored experiment scales required are large.

Greater system applicability has led to greater system uptake across a plethora of bioscience settings. This has and will continue to identify new design priorities. In addition to this, if MEA systems are employed in larger numbers, economies of scale will improve, possibly reducing the cost to research using such systems.

Most importantly developers must try to anticipate the future needs of users based on findings and observations of contemporary experiments.

Limit:

Certain applications, such as spatiotemporal neural network investigations require high sampling frequencies (~25kHz). Developments will be limited by computing technology capabilities. As appropriately priced hardware develops that is capable of managing hundreds to tens of thousands of channels sampling at these high rates in real time MEA developers will continue to merge these tools into systems.

Manufacturing techniques and resolutions available will continue to limit and facilitate what features shapes and sizes in micrometre and nanometre ranges can be made to an appropriately high quality using the biocompatible materials suited to the applications.

Development of MEA systems uses top of the range technologies and highly skilled expert knowledge resulting in high development costs.

The humid, warm environments suited to *in vitro* cell culture environments is not suited to electronic devices.

Analysis capabilities must evolve with the hardware technologies to ensure that the increased data acquisition is adequately supported to allow users to reliably and robustly extract meaning and therefore value from the system.

Greatest challenges:

The development of systems globally applicable to diverse applications is complex and requires exceptional organisation, communication and management. Opportunities for and actions towards standardisation where possible would support development of high tech products in the future that are delivered to markets in a timely manner.

4. **How do applications using different cellular preparations differ and how are the signals that are recorded used?**

Cultured:

Cells that are cultured over MEA workspaces are typically dissociated from an animal or stem cell source into a separated cell suspension. The MEA biochips surface will have been treated with a

specific growth protein prior to seeding of the suspended cells to ensure that close attachment occurs over the microelectrode tips. Typically a less dense covering of cells is achieved in these samples as when compared to a slice placed on top. Monolayers (layers of cells that are one cell thick) are also possible by careful control of the volume of cells dispensed into the MEA biochips media well.

Attachment is important for signal detection, especially from immature cells. Signal amplitudes are typically in the region of tens to a few hundred microvolts (μV).

Slice:

Slices are made of a dissected organ and placed over the microelectrode array. In some cases careful positioning of anatomical landmarks may be required to study specific network pathways. Slices are anchored using commercially available weights or custom built micromanipulators to ensure tight cell-electrode coupling. Microelectrode tips used for slices are typically 3D in profile to penetrate through the outer layer of cut-through damaged cell matter. Signals captured are typically larger than from cultures.

Stimulation experiments are common where groups use slices to study existing naturally developed networks or tissue responses.

Neurons:

A neuron action potential is triggered by a sufficient chemical or stimuli at a synapse. The action potential travels along the length of neuron in a few milliseconds. The corresponding field potentials (FPs) that are detected by nearby microelectrode tips are seen as spikes in the resting baseline data. Where several spikes occur at the same time or within a few milliseconds of one another the occurrence is referred to as a burst (see Figure 2.39).

Cardiomyocytes:

Cardiomyocytes contract in a rhythmic, roughly simultaneous manner, producing FP waveforms that are detectable when coupled to an MEA. These waveforms are the combination of the APs from all of the contracting cells present (which can vary in phenotype). Signal spikes, or waveforms, are much longer in duration than those recorded from neurons typically lasting several hundred milliseconds.

5. **What is expected of future MEA systems?**

Present applications are moving towards targeted testing of cellular characteristics or chemical entities that require repeated experiment repetitions to ensure reliable and valid findings. Therefore it can be predicted that the trend to increase the number of useful channels per experiment will continue from the now hundreds of channels available into systems offering thousands and tens of thousands channels. Further re-configuration of biochips and systems will be required to facilitate this trend that will depend on cutting edge micro and nano-fabrication technologies.

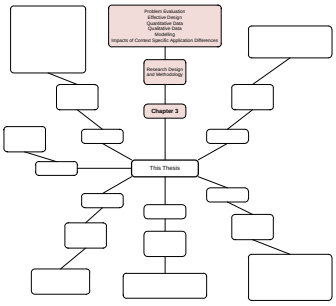
System usability must also improve to facilitate methods in which user scientists can manage and maintain larger numbers of cell cultures in conjunction with needs of future MEA biochips and systems.

6. **Can product innovation be managed throughout this research, and how can developers design and assess prototypes appropriately?**

Innovation management is required to steer design efforts towards a product possessing distinguishers that offer the best value to target users.

This research requires inclusion of expertise from a number of overlapping disciplines to bring about a product that is usable, realistic and beneficial. The multidisciplinary team required must communicate effectively to prevent confusion that may otherwise be detrimental to efficient new product development. Employment of pre-validated modelling techniques allow thorough understanding of target user populations as well as offering a clear and comprehensive communication medium that is suited to multidisciplinary research.

Throughout concept design and design development appropriate methods of prototype assessment must be agreed upon to ensure a final product that meets specification and is superior to competing equivalents.



Chapter Three

Requirement Capture Methodology

To tackle a research and design problem a strategy must be employed that controls how designs evolve and that available resources are exploited (Blaxter et al, 2000). A structured approach was sought for the research contained in this thesis to ensure research objectives were managed effectively, to ensure the delivery of research outcomes. The following research questions are addressed in this chapter:

1. **What can be done to ensure that a thorough evaluation of the field of multi electrode array application and system production is performed, to support the identification of robust end-user requirements in this research area?**

2. **Can formalised product design approaches be implemented to support the conception of effective component and system designs?**

3. **What kinds of information will be required to support effective design and manufacture of prototypes?**

4. **What formalised approaches should be used to represent and communicate research inputs and outputs?**

The logical order taken by this research is demonstrated in Figure 3.1. Research conducted and documented in this thesis has been based upon the research methods described in the following sections.

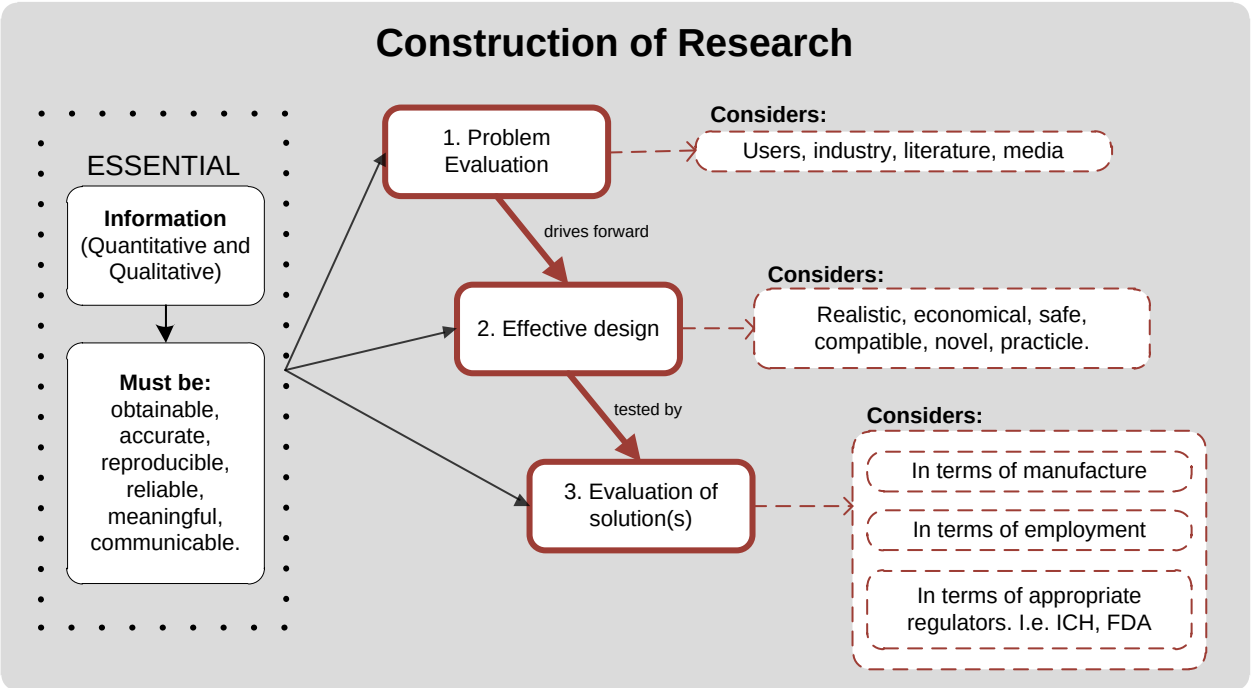


Figure 3.1: Construction of this research.

Problems encountered by current MEA system users are evaluated and the resulting solution designs driven forward by product design specifications constructed to provide effective solutions to current needs. There are many ways of designing, executing and analysing research (Edwards and Talbot, 1999), hence a mix of previously validated research methods have been used to evaluate the outputs of this work prior to physical prototyping and testing.

3.1 Evaluation of the Problem

This research contains and draws upon information gathered using group meetings, one-on-one interviews, literature reviews, e-mail communications and enterprise modelling of MEA application domains (Figure 3.2). Information concerning the context of the research problems identified (user requirements) has been collated and summarised, highlighting problems as identified by the system stakeholders.

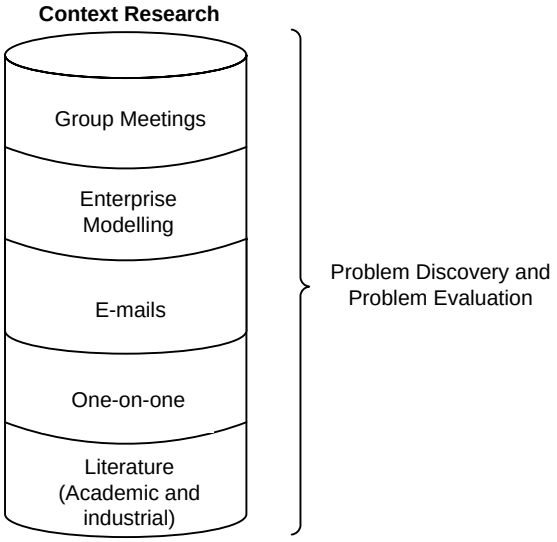


Figure 3.2: Research approaches brought together by this project to thoroughly evaluate problems faced by MEA technology users across three different applications.

Key points identified through literature review as critical to the success of this research are listed below (Figure 3.3).

A) Strategy: To design a product that will be successful a strategic design approach should be used.

B) Existing MEA Systems: MEA systems are complex and exist in a number of different configurations (section 2.9.2.1). Each commercially available system configuration has a number of potentially different settings that have to be adjusted upon system installation according to the intended application (i.e. amplification gain settings, number of channels required for recording).

C) Applications: MEA technology is mainly used in brain and cardiac research domains, resulting in generic but also application specific needs. Any new system must maintain a comparable level of flexibility as is present in commercial systems, to permit experimental application of the system with other tissue types.

D) Data Outputs: Data output formats and the approaches used to analyse those data vary between applications. There are nonetheless some commonalities that can be roughly defined as "standards" to work with, in terms of basic output requirements of any new or replacement software analysing system.

E) Data Analysis: MEA system users analyse data post-recording according to the needs of their research. The level of sophistication of analysis that is possible offline (post-recording) is not possible online (while recording). This has led to a lack of comparability across published results as MEA users do not disclose full details of analysis routines in published results. The outcome of this has been calls for steps towards standardisation to be made (Smith et al, 2007a).

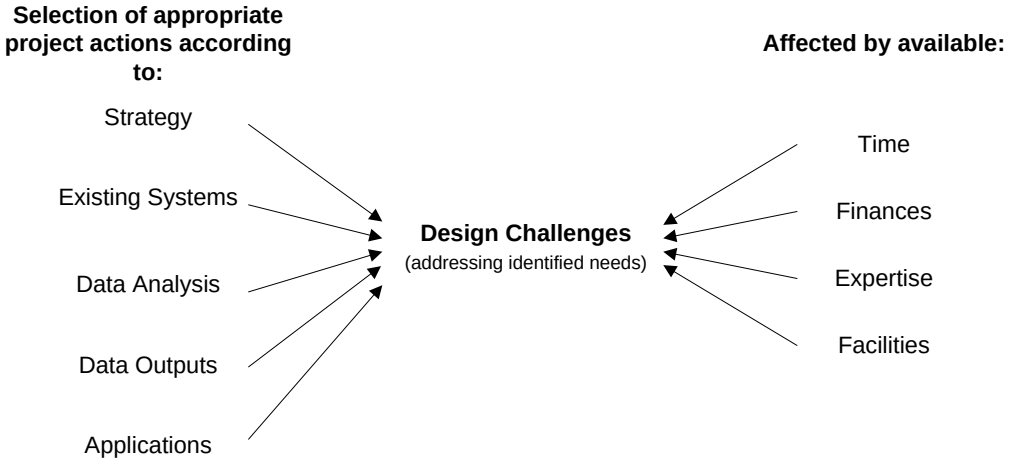


Figure 3.3: Design challenge influencers.

In this research design targets are defined in terms of the technology component or sub-component being designed (system needs), and also in terms of the application domain (user needs), i.e. heart or brain, dissected slice or stem cell-derived. A number of complex design (e.g. high frequency real-time systems) challenges are associated with the development of MEA systems and system components (Rolston et al, 2009) that have to be refined into design specifications. The goal is to select the most appropriate approaches to address these specifications depending on capabilities in terms of expertise, time, facilities available, and financial constraints. Strategies employed to

ascertain relevant user and systems requirement information pertaining to the major application domains drew upon three current MEA system applications (see Chapter 4).

A well-structured and previously validated modelling approach was adopted to represent relevant information and functionality while observing and interviewing the collaborating MEA system users. The enterprise modelling (EM) techniques utilised enabled a deeper understanding of current MEA system employment protocols to be determined than if literature alone had been used.

3.1.1 Drafting Product Design Specifications

Regular interview, meeting and email conversations followed initial shadowing of the MEA user groups studied during the construction of enterprise models. Three product design specifications (PDS) have resulted from what was uncovered using the multi-method combination of research approaches. A generic MEA system PDS (Figure 3.5) that can be considered as an industry-wide PDS (PDS1), an intermediary PDS that focuses on the specific needs of the collaborating users in this project (PDS2), and a specific cardiomyocyte application PDS (PDS3) have all been defined (Appendix C). Within the specification documents, defined points can be classified as system-requirement-centred or user-requirement-centred.

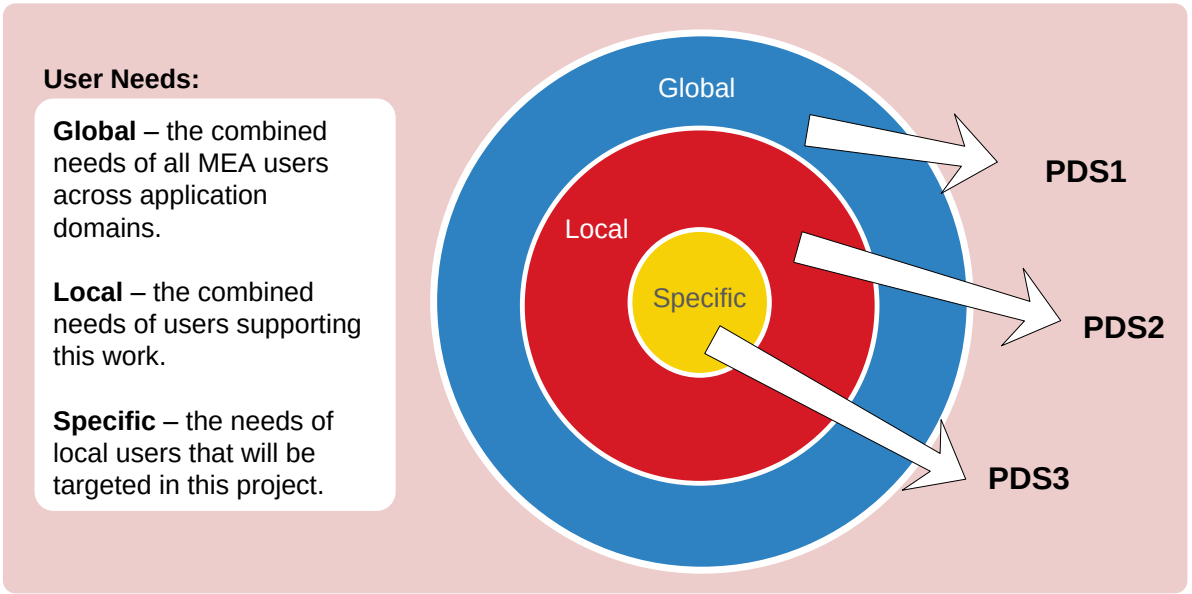


Figure 3.4: Product Design Specification Formats.

3.1.2 Design Objective Prioritisation

The overall industry-wide needs of MEA systems from a global point of view were address in PDS1. PDS2 is an evolution of the industry-wide PDS1, following a review and specialisation process introducing needs identified by enterprise modelling as specific to project collaborators. Therefore, PDS2 defines the longer term targets of the project. Within PDS3, realistic design requirements were laid out that allowed targets to be set with respect to time, cost, facility and expertise available at the time. Solutions that are realistic and that would bring the best value to MEA system users in the time scale of this research were targeted.

Note: This research has focused efforts primarily on PDS3. All PDS documents are contained in Appendix C.

3.1.2.1 PDS 3

This product design specification incorporates specific features required by the University of Nottingham end users (cardiomyocyte applications) with global and local points that are of high importance to system success.

Nottingham University users have specified needs for the biochip to be re-designed in accordance with their specific application needs. They have also specified the direction in which software changes should be made in order to support their analysis requirements more effectively than current protocols. It is the needs of the Nottingham users that this project will primarily focus time and resource on:

Biochip:

- **Modify well maintaining commercial interface configuration.**
  Comments: that will interface with the current MCS MEA60 System in their lab.
- **Alter well dimensions to be close to those of a 35mm culture dish.**
  Comments: internal dimensions 35mm diameter, 10mm height, culture area 8.8cm^2, media volume 3ml.
- **Incorporate a light-transmissible lid.**
  Comments: to prevent evaporation.
- **Interior of well must allow for potential etching or patterning.**
- **Re-usable.**
  Comments: therefore must withstand sterilisation using UV light and 70% ethanol.
- **16 micro-wells.**

Comments: in any pattern.

- **Microwells as far apart as possible.**

  Comments: at least 5mm in from the edge of the dish.
- **Microwells 500µm diameter,**
- **with a maximal depth of 250µm,**
- **and a slightly curved shape.**

  Comments: cardiomyocyte clusters at time of seeding vary between 200-500µm in diameter.
- **Electrode in centre of well flush to the surface.**
- **Microwells with as large a surface area as possible.**
- **Made from biocompatible material(s).**

Software:

- **Intuitive, simple-to-use software.**
- **Output results in real-time**

  Comments: while maintaining the current feature of optional post-processing if desired.
- **Features to allow patch clamp files to be analysed in the same software.**

  Comments: WinEDR software format suggested.
- **Facility to average all traces for a given treatment.**
- **Facility to compare averaged traces.**
- **Increased detail at individual waveform level.**

  Comments: **QT-period** identification and comparison emphasised as most important feature for automated extraction. Other waveform elements of interest detailed in the MEA Specification document by the UoN.
- **Adjustable, automatic pre-processing.**
- **Automatic thresholding** at the time of recording.

System:

- **Sample rates of at least 2-25kHz.**
- **Grounded.**
- **Parts that can be sterilised**

  Comments: If the entire system is to go in an incubator the electronics must be sealed in a suitable casing for sterilisation as incubators are sterile environments.

- **Input capabilities limited appropriately.**

  Comments: to protect/prevent damage to cells or tissue.

- **Appropriate pre-processing and amplification.**
- **Facilitate/support a constant cell environment.**

  Comments: I.e. temperature, humidity.

- **Allow easy access**

  Comments: for users to perform culture maintenance and run perfusions systems. (Perfusion systems are ideal MEA system components as they support long term experiments (especially those using slices) and the balanced distribution of nutrients and/or chemical entities throughout the culture media during system use. In certain experiments perfusion systems are essential for the execution of particular testing protocols.)
- **Support visual inspection of the cells.**
- **Simple to learn.**
- **User-friendly interfaces.**

3.2 Effective Design

A number of previously formalised design approaches or methodologies exist (e.g. design for failure (Hojnacki, 1992), or design for sustainability (Dimson, 1996)) that could be suitable for supporting conception and the creation of a successful product.

3.2.1 Design methods

Product design specifications used in conjunction with approaches incorporating principles of iterative design, user centric design, design for manufacture, design for failure, and cost effective design are valid product design approaches (Pugh, 1991). The key point is that for any product to be successful it must be designed with the users in mind.

3.2.2 Communication

Careful communication of user needs to interdisciplinary team members has been used throughout this project to support the design of a system that will:

(1) address real user needs,

(2) that can be manufactured,

(3) at a reasonable cost, and,

(4) in an appropriate period of time.

3.3 Derivation of Information

A multi-method approach has been taken throughout this research (Figure 3.5) to derive the required design information (Blaxter et al, 2000). Management of information compiled from multiple research methods involves a process of sorting, labelling (or coding), reducing and summarising. It is important to reduce size and scope of the information that is collected so that meaning can be subtracted from it. The summarised information that remains then consists of the important and significant information that relates specifically to particular development objectives. In this research enterprise models and PDS documents have been used to collate and condense research information of differing types into useful summaries that have been used to draw out the design and manufacture objectives.

Multi-method research involves the integration of several research methods (e.g. one-one interviews, group brainstorming, observations) to determine information about the research topic. Research can be desk-based, involving the use of literature and media such as brochures and websites, or field based, involving activities to physically identify and attain information by means of some sort of pursuit (i.e. observing a human interacting with the system under investigation). Both desk- and field-based methods have been used throughout this research.

The information gathered by multi-method approaches is a mixture of qualitative (i.e. learning about how a user feels about the device being researched (high-value qualitative information)) and quantitative (i.e. gathering numerical data relating to the frequency of use of the device (high-value quantitative information)) (Figure 3.5).

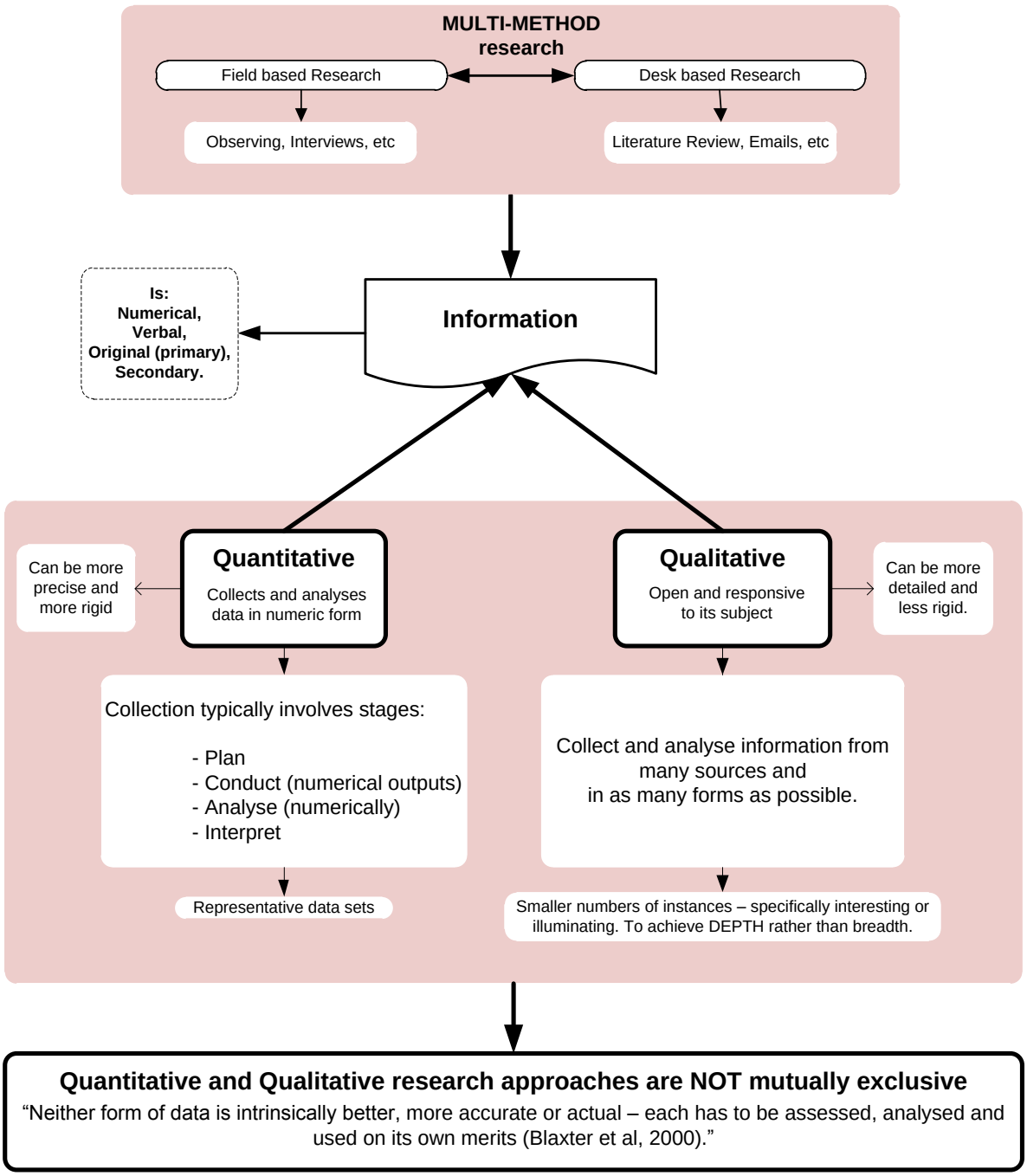


Figure 3.5: Research in pursuit of useful information.

3.3.1 Flexible Planning

Flexible planning strategies involve acting opportunistically as opposed to following a fixed pre-planned schema (Wallace, 1991; Schattenberg et al, 2005). Flexible planning was incorporated into this research so that appropriate amendments to objectives could be made as new information was ascertained, and to enable essential compromises to be accommodated during production of prototypes as necessary. This approach was particularly relevant as the project involved several

variables (e.g. number of recording channels required, electrode geometry required) resulting in the need for design alteration to facilitate progress towards achieving objectives.

3.3.2 Enterprise Modelling

To understand the needs of target MEA user groups in detail, enterprise modelling (EM) was adopted and used as a tool to support the derivation of relevant, up-to-date information.

3.3.2.1 Process Modelling of Workflows

EM is a variant of process modelling (Huertas-Quintero, 2010). Process modelling (also referred to as process mapping) is described as a way to "represent graphically the transactions and stories that make up a business" and it helps to complete an analysis of a process or processes (Jacka and Keller, 2002). EM is one of a number of methodologies that have evolved to support design, analysis and re-design of **workflows**, which in its entirety is a procedure known as Business Process Reengineering (BPR). A workflow is "the sequence of processes through which a piece of work passes from initiation to completion".

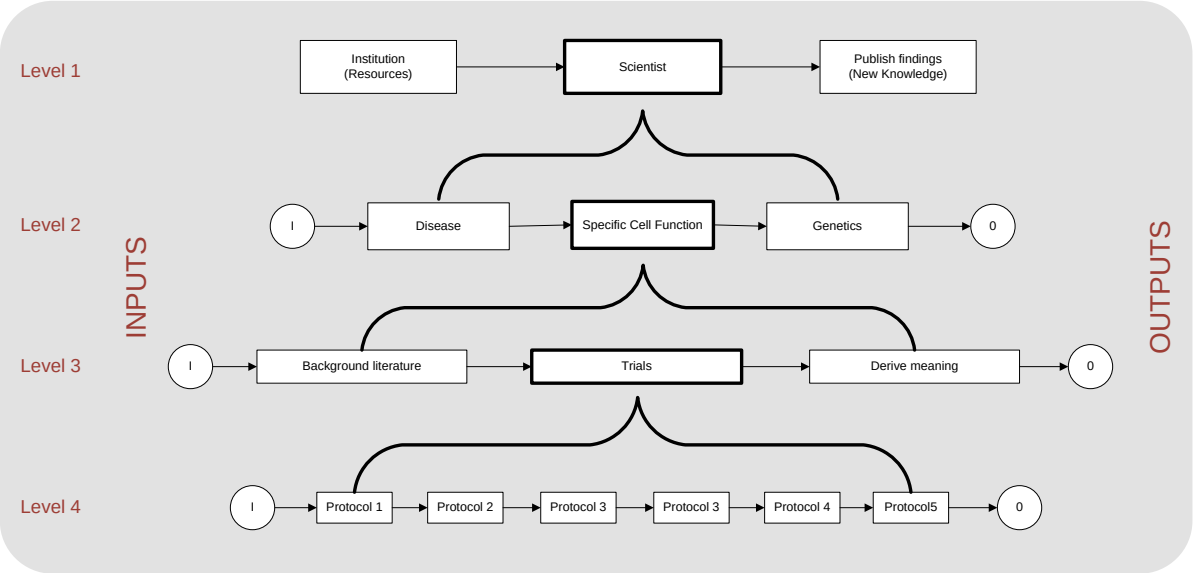


Figure 3.6: The high-level "drill down" associated with bioscientific research.

BPR is used by both large and small organisations alike (Childe et al, 1995), as a way of characterising processes that are carried out in particular areas (**domains**) of their business. A thorough understanding achieved using BPR can influence overall improvements in the enterprise's efficiency. BPR is a top down technique (see Figure 3.6). In terms of this project the EM feature of BPR serves as

a pre-validated method of identifying and understanding potential or existing bottlenecks in processes that can be addressed with protocol amendment, product alteration or a combination of both.

3.3.2.2 Static Enterprise Modelling (AS-IS)

The starting point for conducting BPR is to view the system under scrutiny as it currently is (Kawalek, 1995; McHugh et al, 1995). Static models are generated identifying the major processes (workflows) undertaken to achieve a particular enterprise goal. The main process or processes of interest are then modelled to a greater extent as sets of smaller, more detailed individual workflows that collectively represent the whole (Peppard and Rowland, 1995). This allows the modeller to capture and document each and every activity exactly as it is currently completed. Therefore, these static workflow models are also referred to as “AS-IS” models (Petersen et al, 2010).

Of the four levels of “drilling down” shown in Figure 3.6, the EMs derived and documented in this project pertain specifically to levels 3 and 4 as levels above do not directly influence project outputs. Thus the greater details of these levels have been modelled in the case studies completed (Chapter 4). The “AS-IS” EMs that were constructed represent the entire duration of, and approach to, using a standard MEA system for different types of bioscientific investigations. A variety of information types have been amalgamated into one clear visual format in these models.

In many cases, static “AS-IS” models can be used as a basis for drafting “TO-BE” processes, allowing experimentation of potential alterations to a workflow prior to any real-life execution of that change taking place. Therefore, any failing or loss that would have otherwise occurred as the result of implementing a particular change is identified and rectified before ever being made in reality. The result is a vast reduction in the amount of physical reengineering that was previously relied upon in past “trial-and-error” workflow improvement approaches (McHugh et al, 1995).

3.4 Modelling to Understand Users and Represent Information

Models are representations that allow complex processes or systems to be represented in a more manageable format (Johansson et al, 1993). Through the thought processes used in the construction of models, different types of knowledge can be generated, formalised and used to benefit the organisation (DTI, 2002; DTI , 2004). It is important to be aware of *who* is doing *what*, *how* they are doing it, *where* and *when* is it done, and what is achieved by doing it. Knowledge of these elements allows identification and extraction of requisite details of a system. As a consequence a better understanding of the important complexities and interactions involved in critical workflows is achieved (Hunt, 1996). It is these complexities and interactions that ultimately impact upon overall process or system redesign. In this research MEA system user workflows (e.g. activities to prepare MEA biochips, activities to run tests, activities to clean MEA biochips) have been defined as the workflows that are most critical to successful novel MEA system and component design.

3.5 Modelling Architecture Selection

A number of previously defined and applied reference architectures exist (e.g. IDEF - Integration Defintion for Function Modelling (Kim et al, 2003), GRAF - Graphs with Results and Activities Interrelated integrated methodology (Doumeingts et al, 2000), Petri nets (Peterson, 1977), CIMOSA - Computer Integrated Manufacturing Open System Architecture (Vernadat, 1998)). Specific modelling architectures are used to provide structured models that are comparable. By using a previously validated reference structure the EMs are constructed in a consistent manner allowing straightforward, reliable comparisons to be made (Monfared, 2000). The CIMOSA-based approach has been exploited in both academic and industrial fields (Zwegers and Gransier, 1995; Pierard, 1995; Ong, 2004; Mullane et al, 2010) and is the reference architecture chosen for this thesis. CIMOSA was also selected due to its greater level of versatility when compared to alternative methods (see Figure 3.7).

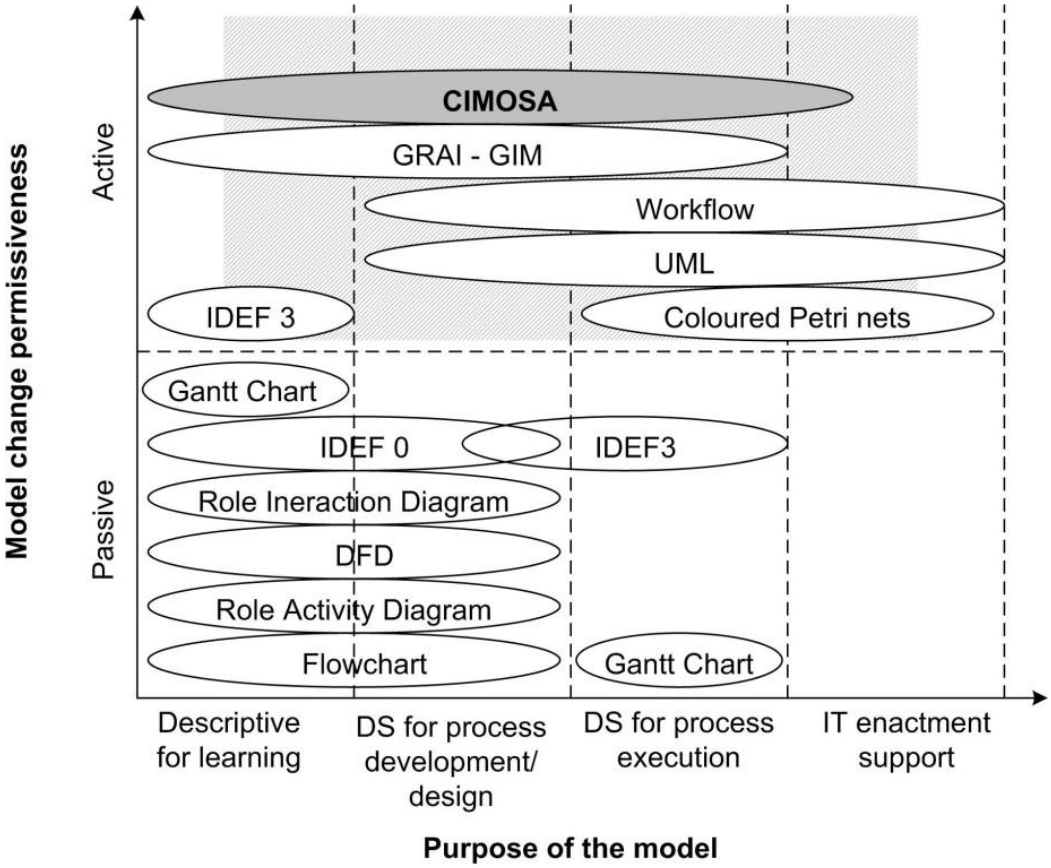


Figure 3.7: Comparison of available modelling techniques.
[Adapted from Augilar-Saven, 2004]

CIMOSA offered greater scope for learning, design, and development execution, which are all of importance to this research (Figure 3.7). CIMOSA was applied so that systematically generated sets

of EMs could be used to collectively represent all of the available knowledge relating to MEA system employment protocols and manufacturing processes used in this research. Multiple EM sets across three different settings by each partner user group permitted a more detailed understanding of generic MEA system user needs.

3.5.1 One Architecture for All

Throughout this thesis representation of physical bioscientific protocols and human-MEA system interaction, and manufacturing approach documentation, has been presented using a single EM architecture (CIMOSA).

Process representation is a sub-set of CIMOSA enterprise modelling (Monfared, 2000) that suited the requisites of the required workflow examination in this research.

Careful construction of sets of EMs was chosen as a single method to depict and visually communicate physical protocols and the human-MEA system user interaction. The single architecture offered a structured means of allowing the overall use of an MEA System to be broken down in terms of:

(i) information inputs (e.g. cell type under investigation, cell preparation type),
(ii) information outputs (e.g. number of MEAs sterilised and pre-treated per batch, number of data files recorded per experiment),
(iii) resources consumed (e.g. volumes of media/solutions required per MEA biochip per experiment, high-skilled human dependant tasks, additional essential tooling),
(iv) time required (e.g. time taken to set-up experiment, time to run experiment, time in terms of human labour, time taken to analyse), and
(v) the dependencies on the human users (e.g. by-hand positioning of cells over microelectrodes, visual observation of cell viability prior to experiments, visual assessment of cell-substrate attachment quality).

3.6 The CIMOSA-based Modelling Solution

CIMOSA supports system decomposition into:

1. an enterprise (workflow) modelling framework,
2. an integrating infrastructure,
3. an (computer) integrated (manufacturing) system lifecycle.

(Adapted from Vernadat, 1996.)

A number of fundamental concerns are incorporated into the CIMOSA approach. The multi-dimensional nature of the approach is highlighted in Figure 3.8.

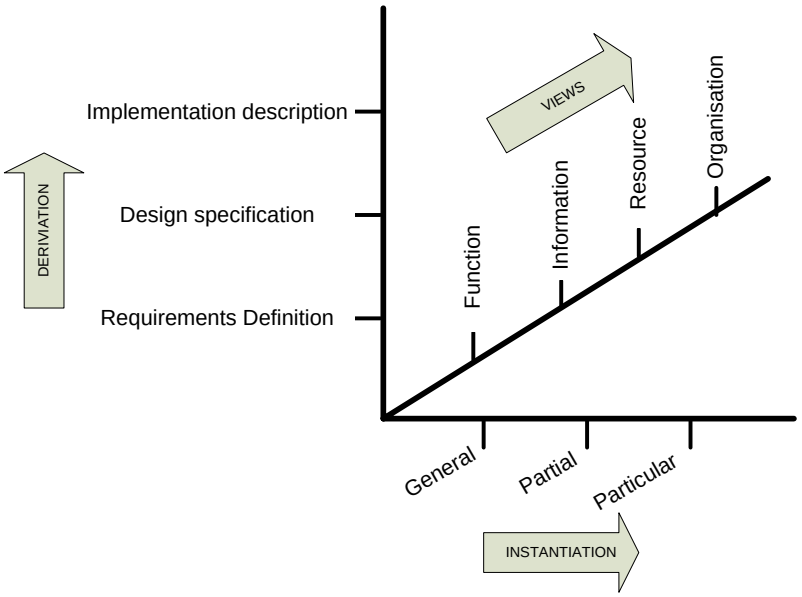


Figure 3.8: The concerns of the CIMOSA framework. Additional concerns can be added as required.
(Adapted from Vernadat, 1996).

3.6.1 CIMOSA Flexibility

When generating enterprise models using CIMOSA four different views are integrated to facilitate full description of workflows within an overall system (Figure 3.8). The views are divided into the function, information, resource and organisation views:

- Function – is an activity-centred view. Models demonstrate activities that make workflows happen to reach endpoints and produce outputs.
- Information – information enters and exits functions.

- Resources – are required to make functions happen and can be either human or physical.
- Organisation – functions are ordered to ensure inputs become outputs during workflows.

The DERIVATION dimension covers the lifecycle aspect of the system, from requirements definition to design specification and implementation.

The INSTANTIATION is concerned with the level of granularity of the respective views (i.e. from the generic to the particular aspects of a domain).

3.6.2 Specific CIMOSA Architecture Adaptation

Application of CIMOSA relies upon representing processes and activities using constructs (see Table 3.1). Constructs have been adapted to improve the communicability of models generated in this research.

Table 3.1: Constructs applied in this research.

Constructs:	Constructs with regard to MEA system research:
Events	Extraction of electrophysiological signals
Domains	In research and pharmaceutical industry laboratories
Domain Processes	Laboratory standard operating procedures (SOPs) and good practice
Business Processes	Application and laboratory specific protocols
Enterprise Activities	Activities involved in carrying out SOPs and relevant protocols
Functional Operations.	Activity details. This is where activities may be made up of a further set of activities, requiring a further drill down to ascertain all relevant information. Relevant information is included as various function operations.

These constructs have been applied in modelling overall system functionality and user group behaviour in this work. A collection of pre-defined diagrams need to be adopted to enable the details of the domain to be represented in terms of these constructs.

3.6.3 Diagram Structure Type

The specific types of diagram used in this work are based on Monfared's (CIMOSA-based) Modelling Approach (MMA). This approach has been applied because it has been well represented within academic literature as a technique that can be used effectively to represent real processes and activities (Ong, 2004; Dong, 2006; Mullane et al, 2010), and it has been previously validated by Loughborough University (Monfared et al, 2002; Aguiar, 1995).

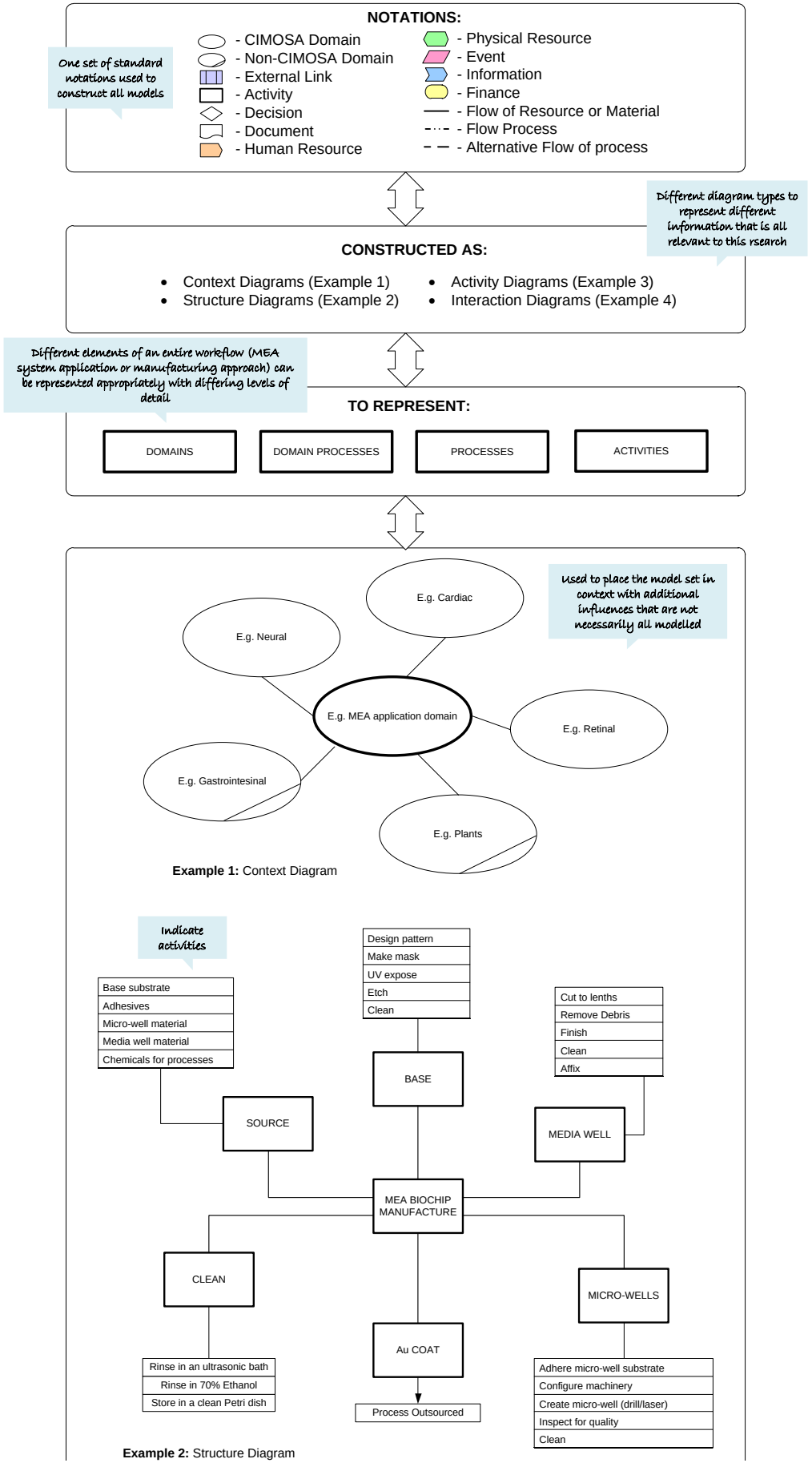
A set of four basic representational diagrams that focus on the function view are required (Figure 3.9) (Monfared, 2000). Representations are decomposition-based breaking down workflows into distinct units that enable information (e.g. timing, cost, resource utilisation) to be readily derived.

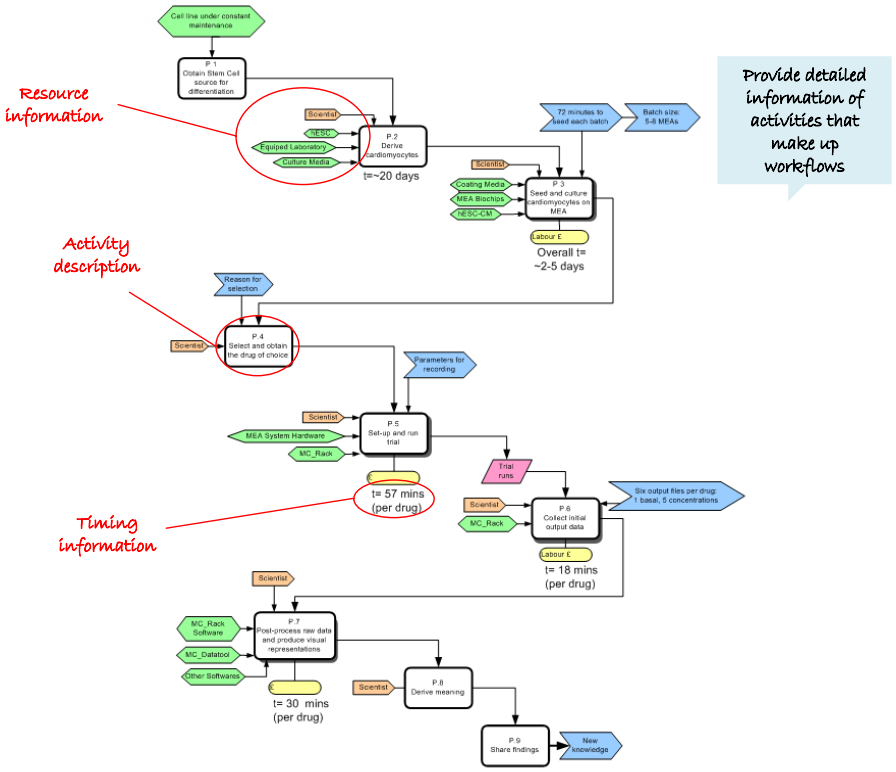
The context-, interaction-, structure- and activity-centred diagrams have been constructed (see Chapters 4 and 5) to provide a set of "views" of real bioscientific laboratory protocols and manufacturing approaches at varying levels of abstraction. The MMA modelling notations used to represent the different processes are documented in Figure 3.9. The four diagram types allow visual representation of all workflow processes in a manner that ensures derivation of clear and comparative information. The details contained within these diagrams serve as integrated sources of information. The information has been derived and exploited for the definition of user and system requirements, and for prioritisation of actions to meet those requirements.

Context diagrams are used to define the high level of the workflows of an enterprise that are under investigation. For example, a research institute is comprised of a number of different areas of expertise that combine to produce new knowledge. Within an area of expertise different aspects are interrelated that are in themselves different areas of investigation (Figure 3.6). Distinctions between areas of expertise and differing investigations can be readily visualised using context diagrams.

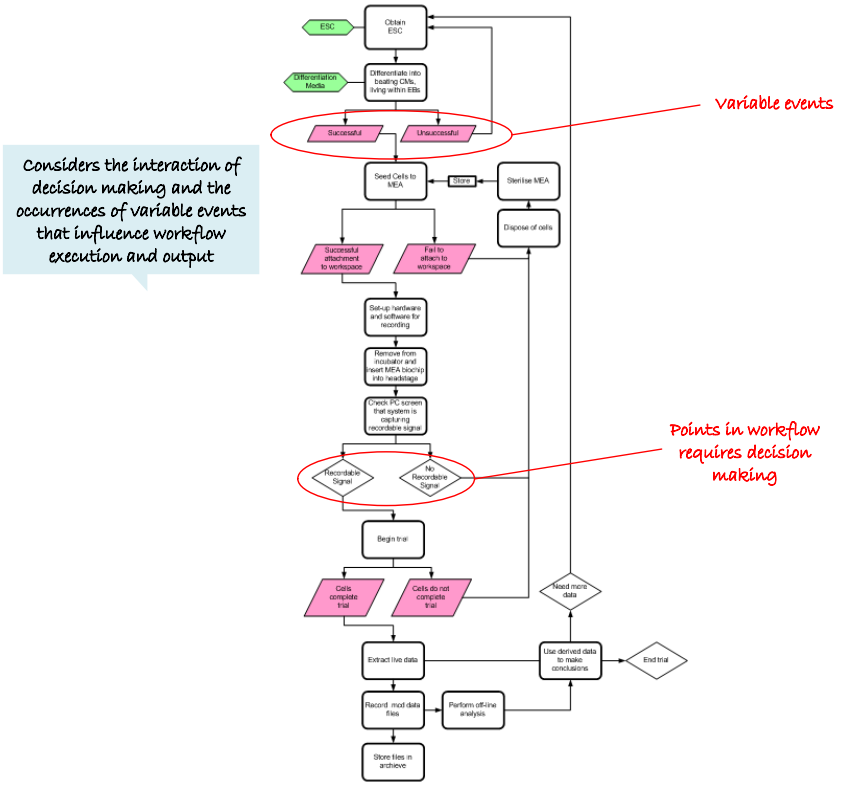
Structure diagrams indicate activities that make up workflows.

Interaction diagrams can be used to demonstrate complexities (e.g. elements where outputs of one activity may vary, therefore resulting in alternative ensuing activities) that might exist within or between workflows.


NOTATIONS:
One set of standard notations used to construct all models
- CIMOSA Domain
- Non-CIMOSA Domain
- External Link
- Activity
- Decision
- Document
- Human Resource
- Physical Resource
- Event
- Information
- Finance
- Flow of Resource or Material
- Flow Process
- Alternative Flow of process
Different diagram types to represent different information that is all relevant to this rsearch
CONSTRUCTED AS:
Context Diagrams (Example 1)
Structure Diagrams (Example 2)
Activity Diagrams (Example 3)
Interaction Diagrams (Example 4)
Different elements of an entire workflow (MEA system application or manufacturing approach) can be represented appropriately with differing levels of detail
TO REPRESENT:
DOMAINS
DOMAIN PROCESSES
PROCESSES
ACTIVITIES
Used to place the model set in context with additional influences that are not necessarily all modelled
E.g. Cardiac
E.g. Neural
E.g. MEA application domain
E.g. Retinal
E.g. Gastrointesinal
E.g. Plants
Example 1: Context Diagram
Indicate activities
Base substrate
Adhesives
Micro-well material
Media well material
Chemicals for processes
Design pattern
Make mask
UV expose
Etch
Clean
Cut to lenths
Remove Debris
Finish
Clean
Affix
SOURCE
BASE
MEDIA WELL
MEA BIOCHIP MANUFACTURE
CLEAN
Au COAT
MICRO-WELLS
Rinse in an ultrasonic bath
Rinse in 70% Ethanol
Store in a clean Petri dish
Process Outsourced
Adhere micro-well substrate
Configure machinery
Create micro-well (drill/laser)
Inspect for quality
Clean
Example 2: Structure Diagram



Example 3: Activity Diagram



Example 4: Interaction Diagram

Figure 3.9: The diagram types used in this research.

[Adapted from Monfared, 2002.]

All diagrams are constructed from the notations shown (Figure 3.9) allowing clear visual representation of the various details of domains, domain processes, processes and/or activities modelled.

3.6.4 Model Set Organisation and Comparability

To represent a system in detail using CIMOSA requires a number of differing models to be constructed. Careful organisation of these models into sets helps to define distinct modules within certain settings and can reduce overall system complexity, thereby improving information manageability (Smith, 2007b).

The CIMOSA constructs are used to model domains, domain processes, processes and activities. A domain is a region that the modeller will choose to focus upon at its highest level (e.g. MEA system application in neuroscience). **Domains (D)** are broken down into sets of **Domain Processes (DP),** which are broken further down into **Processes (P)**, that are made up of series of **Activities (A).**

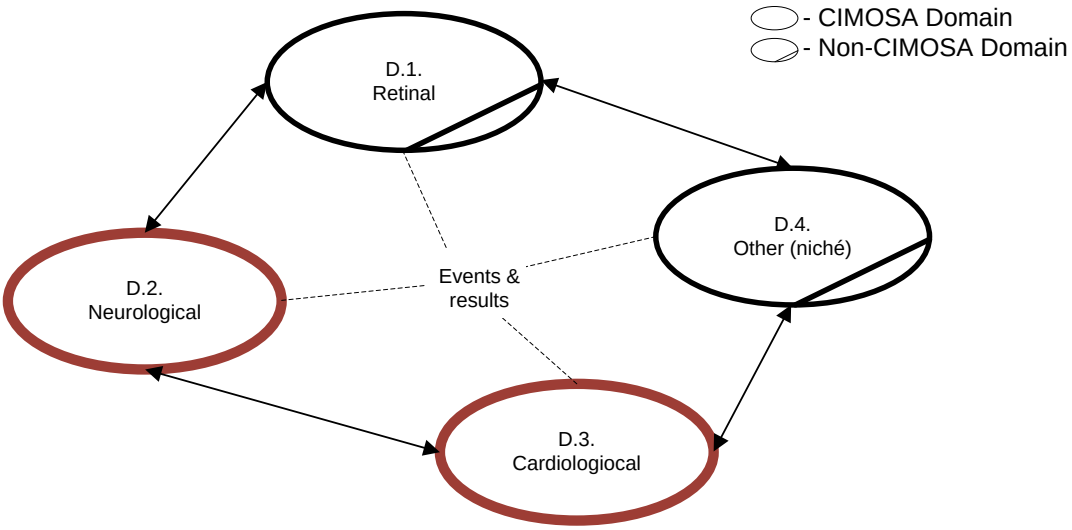


Figure 3.10: A context diagram identifying the main research domains that are currently exploiting MEA systems.

Decomposition serves a functional purpose. Domains interact with one another by the exchange of events and results. In bioscience, for example, researchers from different domains publish findings obtained using multi-electrode array systems, this information can be of use to other MEA user groups, potentially altering workflows across domains (Figure 3.10). For example, a group working in the pharmaceutical industry with brain slices on MEA systems exploits findings derived by

researchers that are studying disease in dissociated neurons on MEA systems. The result is a change the pharmaceutical group's protocols or workflows.

In Figure 3.10 the niche domain (D.4) has been incorporated to represent the application of MEA technology in low volume novel domains such as nasal tissues (Liu et al, 2010) and the gastrointestinal tract which are fields that may expand in the future depending on the outcomes of these early investigations.

In this case domain processes are the processes involved in using an MEA system independent of the research question under investigation. Each domain process (DP) is an end-to-end process. Modelling of DPs provides overviews of the sequence of activities that are carried out by the collaborating laboratories. DPs have defined starting conditions and finishing points that together provide a measurable or quantifiable end-result that can then be used by the bio-scientist. The domain processes delineated for this work are demonstrated in Figure 3.11. Specific model sets incorporating details of each partner user group's workflows have been constructed (Chapter 4).

Activity diagrams are detailed descriptions of a domain process in terms of functionality. The CIMOSA architecture defines an enterprise activity (in this case adapted to an Activity) as a set of elementary actions requiring resources and time for execution. Activity models are sets of elementary processing steps executed by one functional entity, in this case the MEA-system user.

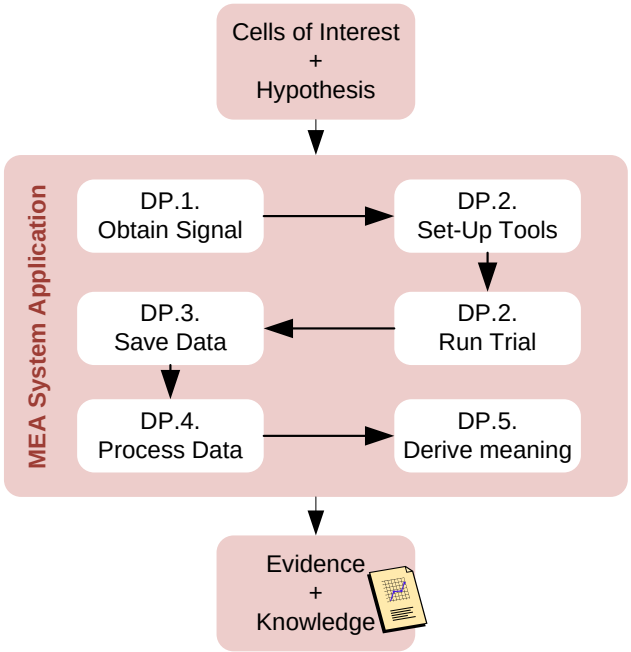


Figure 3.11: The flow through generic domain processes demarcated for this work.

3.6.5 Application of the Modelling Approach

Research domains where MEA technology has been applied (Chapter 2) have been separated into domains (see Figure 3.12). Within each domain MEA-systems are employed using different protocols to best suit the varying needs of each application. Differing application **domains (D)** consist of **domain process (DP)** details. For example, approaches employed when conducting trials using acute neural slices differ significantly from when using chronically cultured neurons. For more detail see Chapter 4.

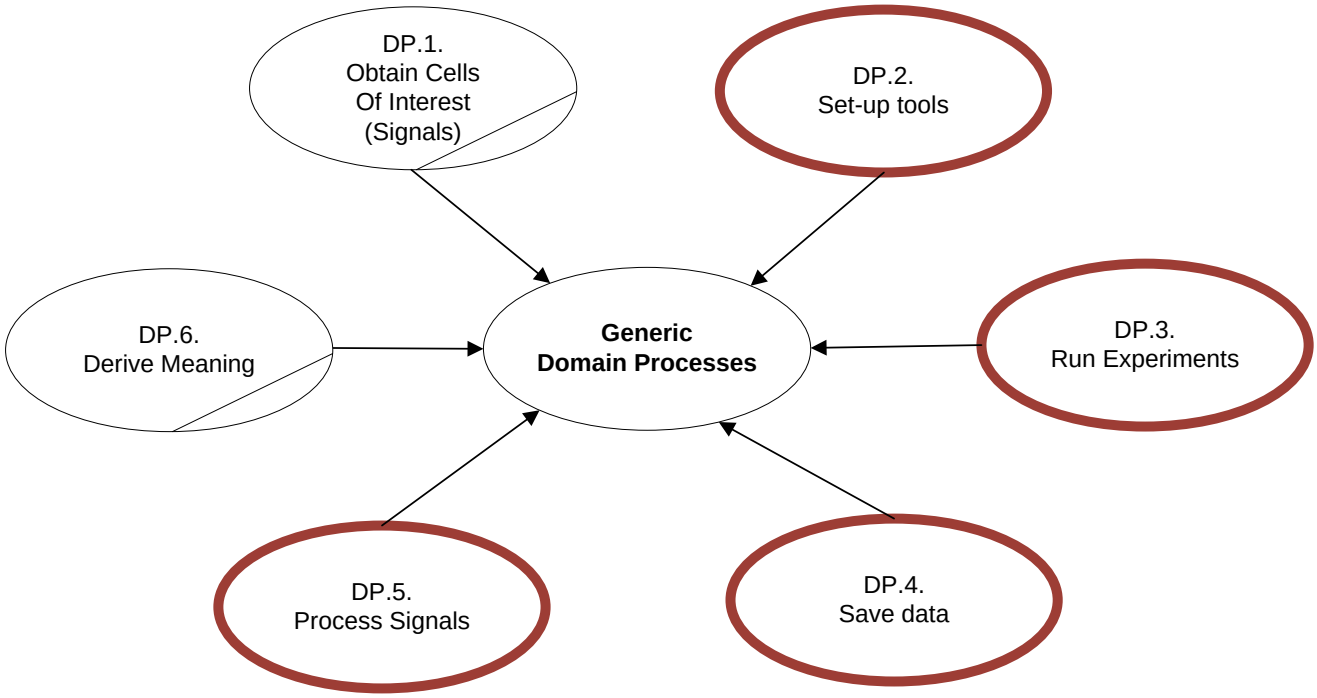
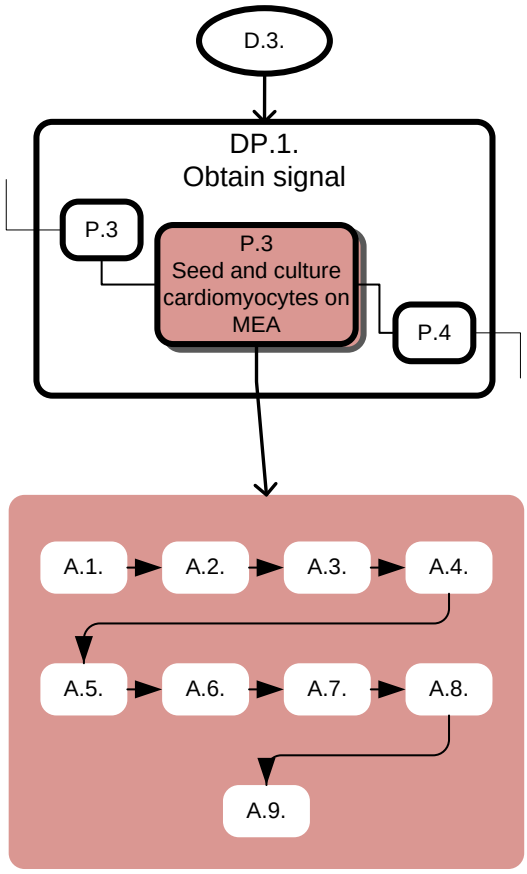


Figure 3.12: Domain processes generic to MEA system application into a context diagram. Domain processes that have been identified for modelling as part of this work are highlighted in red.

Unique domain specific models were constructed following the initial model's structure to provide increased levels of detail for each individual case study application.

Context diagrams have been used to demonstrate each domain process as a distinct flow of activities using the activity diagrams constructed using detailed knowledge derived from protocol analysis and interview.



Domain (D):
CARDIOLOGICAL RESEARCH

Domain Process (DP):
OBTAIN SIGNAL
(Generic to all MEA applications)

Process (P):
CULTURE CELLS
(Application specific)

Activities (A):
A.1 DIVIDE MATRIGEL,
A.2 THAW MATRIGEL,
A.3 DILUTE INTO BASE,
A.4 DISPENSE ON MEA,
A.5 LEAVE AT RT FOR ~45 MINUTES,
A.6 ASPIRATE MEDIUM,
A.7 ADD FRESH MEDIUM,
A.8 SEED CELLS,
A.9 INCUBATE WELL.
(Application specific)

Figure 3.13: The decomposition of a Domain into its Domain Processes, then into individual Processes, and then into the Activities involved in carrying out one of those processes.

Figure 3.13 is a representation of how domains, domain processes, processes and activities decompose deeper and deeper into these cases of MEA-system use. An activity is the elementary unit within the architecture. A process is usually comprised of more than one activity. Activities are carried out in flowing routines to bring about a result or output. For example the activities (A.1. to A.9.) could be, A.1. Divide up Matrigel upon delivery and store in freezer, A.2. Thaw Matrigel prior to use, A.3. Dilute Matrigel in base media, and so on, depending upon the user application.

3.6.6 Successful Application

The CIMOSA modelling approach was successfully applied in this research setting due to the flexibility of the modelling constructs. MEA-system workflows are almost certainly unique to every institution and research group that uses an MEA system at this time. In addition to the clear institutional/research group differences it was possible to observe how individual preferences within groups at the same institute, working to the same guidelines, resulted in subtle differences in protocols (workflows).

3.7 Identification of Influential Application Differences

Multi-method approaches to gather data for case studies that are of a comparable nature, such as those documented in Chapter 4, highlighted key differences between model sets (Figure 3.14). Research methods were selected and applied that suitably addressed collection of appropriate information for each set.

The number of workflow stages required varied between MEA system user groups and their applications (e.g. the number of activities in sterilisation, cell seeding, experiment execution). Flexibility of the applied modelling architecture allowed models that differed to large extents while remaining comparable. The cell type used and type of preparation of that cell type resulted in substantial differences in MEA-system workflows. All three user groups modelled used different cell preparations that are used in conjunction with an MEA system in different ways (Figure 3.14).

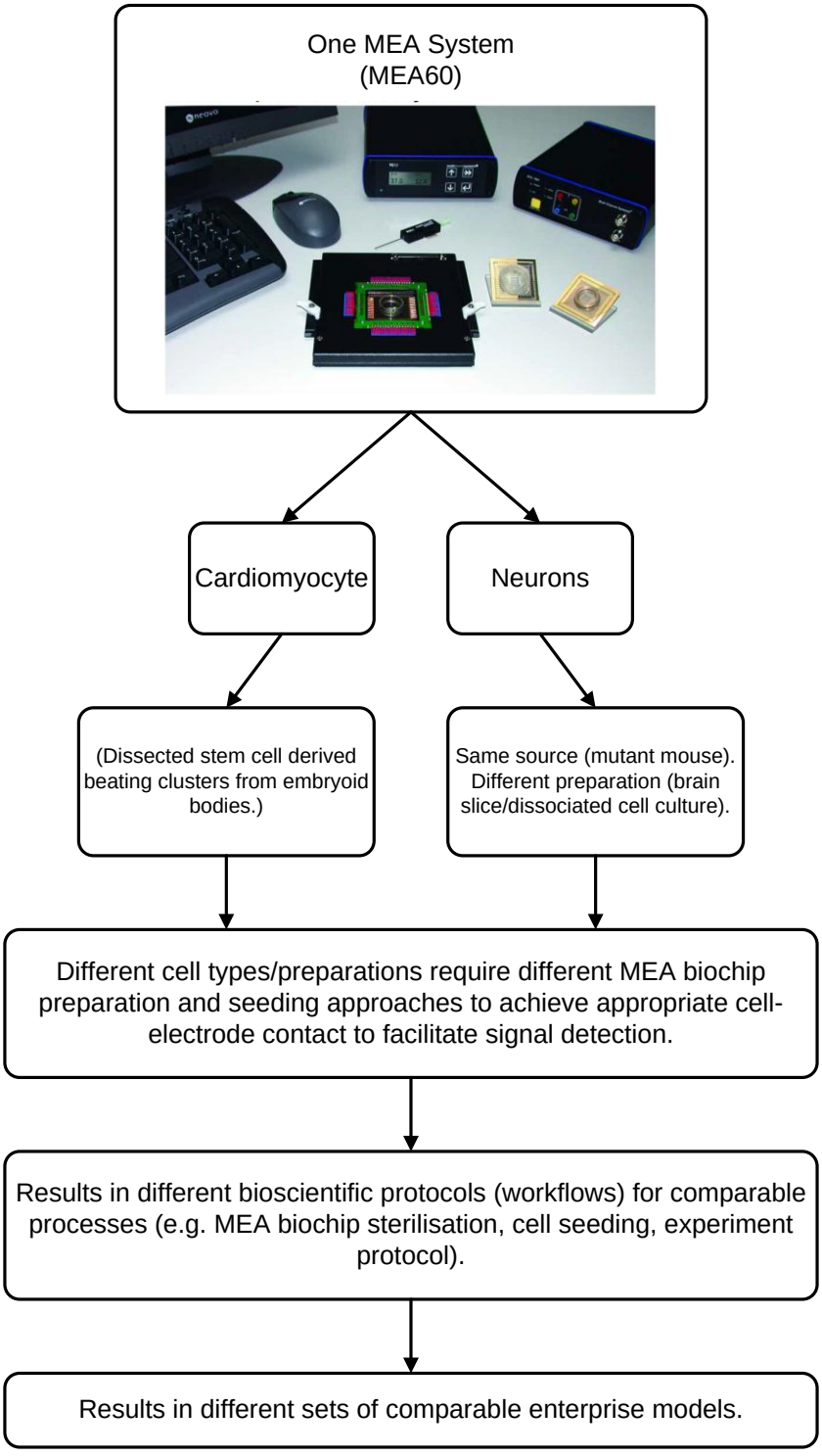


Figure 3.14: Derivation of different workflows using the same MEA system.

The University of Nottingham user group apply MEA systems in the electrical characterisation of stem cell derived cardiomyocyte (SC-CM) cells derived from one of a number of maintained stem cell lines. The cardiomyocytes are differentiated from human-derived stem cells into microscopic clusters of beating cardiomyocyte cells. The beating cluster, referred to by users as “beaters”, are dissected out of the original culture by hand and seeded into an MEA biochip over the array of

microelectrodes. In contrast, the scientist users at the Sanger Institute use two distinctly different instances of the same cell type from the same source type (mutant mice); brain slice and dissociated neuron culture. The dissociated neuron culture user (modelled in section 4.3) applies MEA technology to cultures where a dissected section of tissue is initially obtained from specifically genetically mutated mice and the cells are the dissociated from one another using specific enzymatic agents resulting in a suspension of single cells. It is these suspended cells that are separated (by centrifuge) and seeded into MEA biochips. Cells prepared in this manner are then allowed a period of attachment and re-growth in incubation, during which time new networks of activity will propagate. The brain slice user also uses brains taken from specifically mutated mice but in this instance the slices are left intact in their natural networks. Slices are then simply placed on top of the electrode array and gently secured in place. The protocols (workflows) undergone by each instance described differ, the result of which is that the three sets of EMs are unique, possessing differing timing and resource consumption information.

3.8 Evaluation of Solutions

The findings of the research conducted for this project are presented through this thesis and in research publications. Discussions of the areas of this research that must be evaluated (Figure 3.15) are included in Chapters 6 and 7. The following points have been defined for evaluating the research presented and for discussion in the closing chapters of this thesis as they are considered essential areas of evaluation specific to this research context.

1. The effectiveness of the data collection methods.
2. The design approach and prototype manufacture.
3. The likelihood of product acceptance - that the designed solution will be accepted and adopted by real MEA system users.

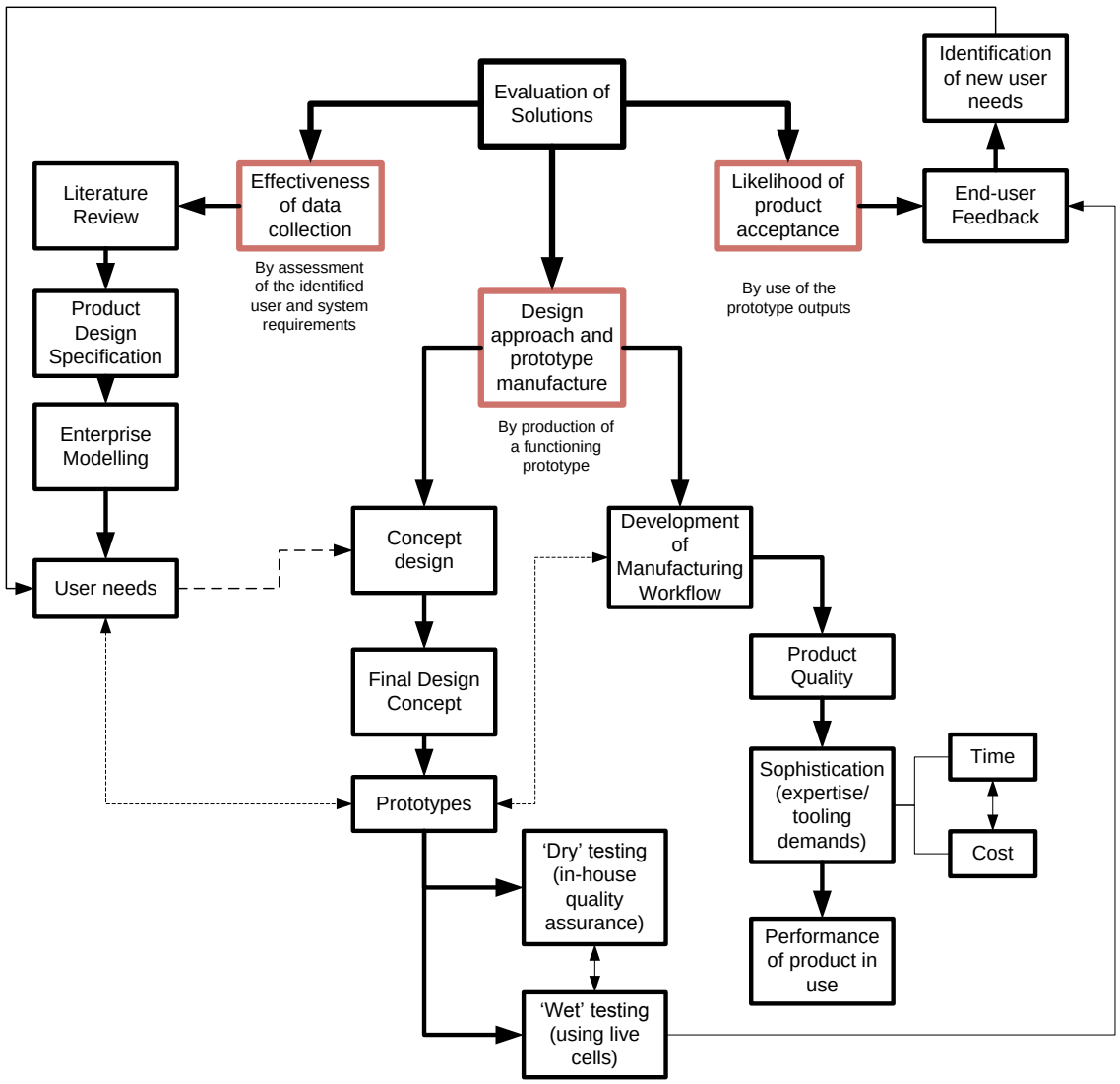


Figure 3.15: Defined areas of research evaluation for this project.

3.8.1 The effectiveness of data collection methods

An assessment of the techniques that have been used for gathering, archiving, communicating and exploiting information and data collected throughout this project is required.

Compilation of a literature review, product design specification drafting and enterprise modelling research techniques have been used in both user and system requirements (Chapter 5) definition and during the concept design stages. Physical prototypes were constructed to meet the requirements identified that have been tested by end-users. Feedback from users relating to the "in-use characteristics" of the solution devices was sought and is described in Chapter 6.

3.8.2 The design approach and prototype manufacture

An assessment of how design concepts were conceived and produced, and of how physical prototypes manufactured of those concepts performed in both “dry” (without living content) and “wet” environment have been carried out and documented in Chapter 6.

Testing and evaluation of prototypes was conducted with regard to MEA biochip integrity and also in a system integrated MEA system fashion.

Planned configurations were pre-defined for testing and evaluation of prototype devices prior to commencing manufacture:

- Prototype biochip(s) in commercial hardware (e.g. hardware: headstage, amplifier, ADC) with commercial software.
- Prototype biochip(s) in commercial hardware with novel software.
- Prototype biochips(s) in novel hardware with novel software.
- Novel software with commercial MEA biochip in commercial hardware.
- Novel software with commercial MEA biochip in novel hardware.
- Novel hardware connecting to commercial MEA biochip and commercial software.

The likelihood of product acceptance was considered throughout this research. An assessment of how the user felt using the prototypes was pursued (Chapter 7) during this research.

3.9 Summary

In research and development, set approaches are used to support identification of issues or areas to be targeted for improvement, and to provide focus to the consequential derivation of appropriate solutions. The major objective of this research has been to produce a novel state-of-the-art multi-electrode array system. A summary diagram of the content of this chapter has been included (Figure 3.16).

- Research was constructed around three distinct project stages: (1) Problem evaluation, where short- and mid-term targets were defined; (2) Effective design, where the defined targets were addressed, resulting in the output of prototypes (solutions), (3) Evaluation of solutions, where prototypes were tested and evaluated.
- During problem evaluation, felicitous context research facilitated the identification of up-to-date user and system requirements and product design specification documents were consequently constructed. Context research was conducted logically and strategically in order to attain a crucial understanding of the research domain, existing MEA system technologies and different types of MEA system application. Producers of cutting-edge technologies in the field of MEA system application were identified as both competitors and domain leaders. It was understood from conducting strategically focused early research that different application of the same technology leads to considerably different data outputs and new knowledge contributions. New knowledge is excogitated based on quantified measurements extracted from those differing data types.
- Through effective design, solution devices (prototype MEA biochips and system) were sought that would match user expectations at the same time as being economically viable and industrially competitive. Qualitative and quantitative information was called upon throughout decision making as evidence to support design decisions. Information and data was gathered using a multi-method research approach that integrated both desk-based and field-based findings to provide a detailed view of the design challenges (user and system requirements). It was identified that communication of relevant information between researchers and project stakeholders was important. A process modelling methodology was selected and exploited as a tool for documenting and communicating MEA system applications and specific application differences. The same previously validated and internationally standardised modelling framework was also used to model manufacturing

workflows as they were conceived and developed allowing simple monitoring of critical manufacturing details (e.g. time, resource input) as manufacturing workflows evolved.

- Evaluation of the outputs of this research was conducted in terms of the physical prototype solutions demonstrated. The evaluation was conducted to address how well the research approaches used identified user and system requirements (Chapter 5), as well as how this research can be of use to others (Chapter 7).

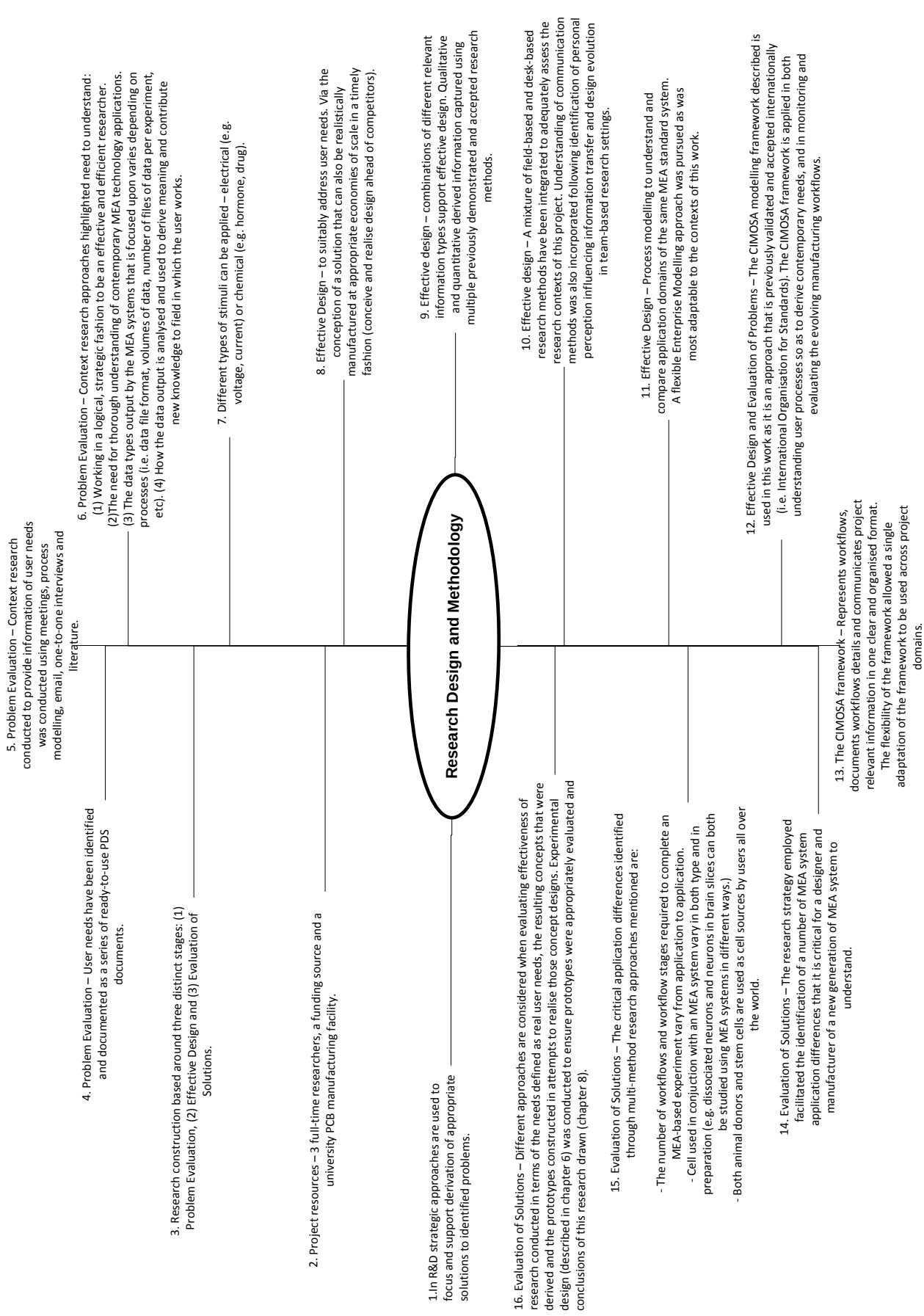


Figure 3.16: A summary of the findings of Chapter 3.

3.10 Research Questions Answered

The following statements address the research questions originally defined at the start of this chapter.

1. **What can be done to ensure a thorough evaluation of the field of multi electrode array application and system production is performed so as to support the identification of real end-user requirements in this research area?**

It was intended as part of this research that real end-user requirements would be identified to support the composition of a design specification that would be used throughout design and manufacture of a new state-of-the-art MEA system. Identification of user requirements depended upon appropriate understanding of how users apply MEA systems and where they encounter problems or issues that could be tackled through component and/or system redesign. Up-to-date research was pursued so as to identify current requirements that would ensure the evolving solutions would be classed as novel and state-of-the art.

Thorough understanding of the field of MEA system technology use was sought. In addition to this it was recognised that a number of MEA system components depend upon recently developed manufacturing techniques. To ascertain appropriate knowledge with regard to both MEA system use and system component manufacture a strategic multi-method approach to research was engaged. This involved using a number of well-established, previously validated research techniques (Figure 3.2) to gather a broad range of information and relevant data.

2. **Can formalised product design approaches be implemented to support conception of effective component and system designs?**

A number of design approaches are described and formalised in design and innovation literature. For example, design for manufacture, design for failure, user-centred design and sustainable design are all examples of terms used to describe design processes with particular emphasis on a particular aspect of design (Pugh, 1991). Design for manufacture and user-centred design foci have been most significant in this research.

Formalised modelling (via CIMOSA enterprise modelling) of existing MEA systems as they are currently used, as well as during prototype manufacture also supported communication of necessary component and system details (e.g. MEA biochips electrode geometries used with

which cell type and preparation, what gains are set in the detection hardware) used throughout design conception and iteration.

3. **What kinds of information will be required to support effective design and manufacture of prototypes?**

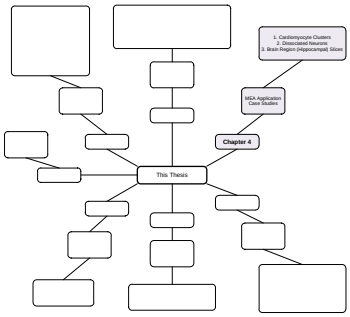
A combination of qualitative (i.e. user preference and opinion) and quantitative information (i.e. timing and resource data) have been used to support PDS drafting and the consequent design of prototype solutions. Varying information has been collected from different sources using both desk-based and field-based research to provide a balanced and broad collection of information.

Effective concept generation and user-centred design was supported by well-informed identification of user and system requirements. Derivation of design specifications relied upon literature sources, observations, meetings, documents, emails and one-to-one interviews.

Effective design for manufacture was supported by identification of information relating to current manufacturing approaches for similar existing devices (e.g. how commercially available MEA biochips are made, what manufacturing approaches offer a means to produce feature desired using materials necessary), as well as communication of timing and cost information relating to the novel manufacturing approaches being developed.

4. **What formalised approaches should be used to represent and communicate research inputs and outputs?**

Enterprise models have been used extensively throughout this research to document and display a range of input types (i.e. material, human labour). Conference paper and journal papers have been produced and published to communicate research findings in the form of prototype design and manufacture, testing and validation. In addition to this, this thesis presents the research from start to finish in one document.



Chapter Four

Case Studies and Requirement Identification

"A case study is an account or description of a situation, or sequence of events, which raises issues or problems for analysis and solution" (Heath, 2002). Case studies have been used in this research to support problem, and user and system requirements identification.

To consider the differing domains in which MEA systems are applied (i.e. in the neural research domain or the cardiac research domain), and to understand the ways in which MEA systems are used within those domains (i.e. differences between use in slice-based experiments and dissociated cell culture-based experiments with the neural research domain), three research institute-based case studies have been pursued and are presented in this chapter. Note: the domains of MEA system application and the ways in which systems are used within those domains is continually evolving and diversifying (section 2.7).

Case studies contained within this chapter have supported identification of user and system requirements across application domains. The following research questions are addressed through the work presented in this chapter:

1. **How are MEA systems currently employed by users that are working with different cell sources and cell types?**

2. **What are the major differences between the applications examined?**

3. **Can any system or component changes that have been identified as requirements by this research offer value to users, and of those changes, which ones can be realised by this research?**

The overall population of MEA system users is diverse so samples of representative users are discussed in this thesis. The population of MEA users has been divided into population samples depending upon the cell of interest (e.g. neuron, cardiomyocyte). Two cases from the neural tissue-based sample and one from the cardiac tissue-based sample are presented (Figure 4.1).

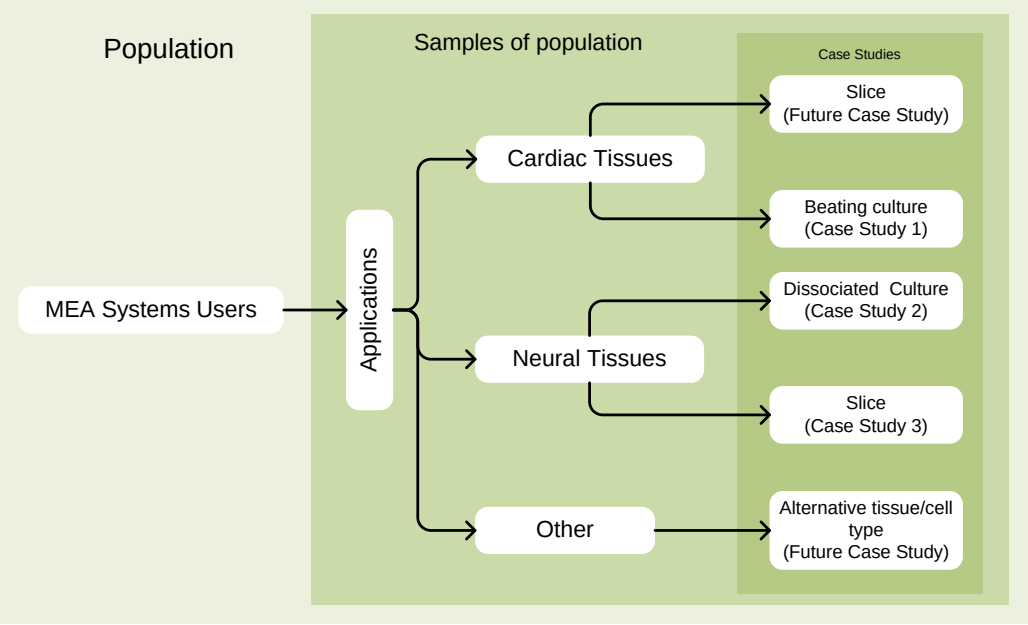


Figure 4.1: MEA system applications and corresponding case studies.

The MEA system user groups that have partnered this work are:

1. **The Wolfson Centre for Stem Cells, Tissue Engineering and Modelling (STEM)** at The University of Nottingham's Centre for Biomolecular Sciences
   Group leader: Professor Chris Denning.
   Major project contacts: Dr David Anderson and Miss Divya Rajamohan.
   Website:
   http://www.nottingham.ac.uk/news/expertiseguide/experts/d/professorchrisdenning.aspx

2. **Genes to Cognition Research Group** at the Wellcome Trust's Sanger Institute (moved to University of Edinburgh November 2011).
   Group leader: Professor Seth Grant.
   Major project contacts: Dr Paul Charlesworth and Dr Maksym Kopanitsa.
   Website: http://www.genes2cognition.org

4.1 Case Study Development

The method used to develop the presented case studies is illustrated in Figure 4.2. Data collection took place through desk-based research methods resulting in Chapter 2. Field-based research was required to gather the requirement specific information that was structured as enterprise models. As understanding of the application and user requirements of each application increased additional supplementary information was gathered and integrated into the case studies.

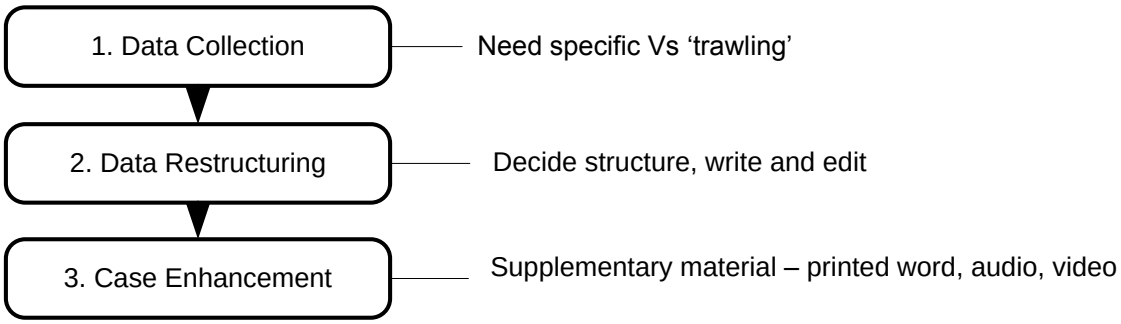


Figure 4.2: The case study development process.

[Adapted from: Heath, 2002.]

The enterprise modelling (EM) architecture used is described in Chapter 3. EM was applied to create structured sources of information that supported problem understanding, as well as up-to-date user and system requirement identification, definition and prioritisation.

4.1.1 Case Study Commonalities

Across the MEA system user population commonalities exist: i) obtain and/or culture appropriate cells over MEA electrodes, ii) record signals that these cells produce, and iii) analyse the signals to derive understanding so as to contribute new knowledge. The remainder of this Chapter demonstrates user group specific information that was collected and constructed into enterprise models centred on identifying application specific differences and problems associated with MEA system's use.

4.1.2 Information for Requirement Identification

Information derived during observations of MEA system users has identified user requirements that are presented at the end of each case study. The information that is combined in the construction of each EM was initially obtained via passive observation of user workflows (bioscientific protocols) in each context. Further detail was captured though interaction between the modeller (thesis author) and the system users, and also with the physical workflows themselves (e.g. the modeller gained hands on experience of the protocols with the users). Meetings were also held prior to and post modelling to plan what to observe and to collect supplementary information as required after observation. Reference to laboratory standard operating procedure (SOP) documents written by the UoN and Sanger Institute users were also referred to for additional details. Examples of these documents are contained in Appendix D.

4.1.2.1 Modelling Identifiers and Process Relevance

Processes and activities of each case study are presented as overall workflows using the CIMOSA architecture and modelling constructs. This approach enables the identification and definition of MEA system use into elements. Alphanumeric identifiers are used to note individual actions and to group actions into processes (see Figure 3.13). For example, the abbreviation P.1 represents process one, the first process of the overall workflow, and the abbreviation A.1 is used to represent the first activity carried out in order to complete that process.

So in the forthcoming figure, Figure 4.4, process one could be “Obtain stem cells” which in this case is out of the immediate scope of this project but is a vital process that all project stakeholders need to be aware of. Process two in the same model, “Derive cardiomyocytes”, is also out of the scope of this project but is a focal area of research for the UoN group and must be documented in the model. Process three, “Seed and culture cardiomyocytes” is the first process that has direct relevance to the focus of this research and is dependent upon successful completion of P.1 and P.2. Process three is the first to have been modelled in further detail as drill down models and this is usually represented in the model by the presence of a shadow under the shape in which the words “P.3. Seed and culture cardiomyocytes” are contained. Processes four, five, six and seven are also relevant to this research so have been modelled in further sub-models too, again indicated by shadowing. Processes eight (P.8 –Derive meaning) and nine (P.9 – Share findings) are not directly relevant so have also not been modelled beyond this point.

The identifiers are constant throughout all three of the ensuing case studies.

4.2 Case Study One: Stem cell-derived Cardiomyocyte Application

There are areas of cardiological research where using MEA systems to record electrogenic activity as it occurs in cardiomyocyte cells or across cardiac tissues can serve as tools that are used to contribute new knowledge (Caspi et al, 2009). Examples of such areas include: investigations of the mechanisms of arrhythmia, which is irregularity of the heartbeat (Jacquir et al, 2008); investigation of specific membrane channels present in cells of the myocardium (heart muscle) (Law et al, 2010), and investigation of responses to pharmacological substance testing (Caspi et al, 2009).

Jacquir et al (2008) studied monolayers of cardiomyocytes cultured over an MEA. Basal conditions were observed. Proarrhythmic conditions (the new or more frequent irregularity of cardiac contractions) were then induced by using the MEA electrodes to deliver high frequency electrical stimulations to the cardiomyocytes. The response of cells to anti-arrhythmic drugs was then observed and the implications discussed.

Law et al (2010) studied the effect of potassium channel openers (a selection of cardio-active drugs that facilitate transport of ions through potassium channels) by using an MEA system to facilitate observation of metabolic inhibition of cardiomyocyte cells. Parameters of cardiac activity captured from the system were beat frequency and field potential amplitudes and durations. This work supports the use of MEA systems for long- and short- term monitoring of metabolic inhibition of cultured cardiomyocytes and suggested that models of the heart on MEA systems could serve as a reliable platform for discovery and study of cardioprotective drugs.

Caspi et al (2009) examined the possibility of combining single cell electrophysiology (see section 2.4.2.1) with MEA recordings as a novel model for complete electrophysiological drug screening. Anti-arrhythmic substances were examined to observe known responses using the MEA system. Alterations in field potential durations, and consequently QT-intervals, were observed and discussed to support MEA systems use in cardio-active substance screening.

The context diagram in Figure 4.3 is an example of the highest high level of CIMOSA model created in this research case study. Figure 4.3 shows domains that can be autonomous research fields in their own right but that are also interdependent as they are all associated with the use of stem cell-derived cardiomyocytes (SC-CM) for research. It is of use to this research to be aware of advances in the other fields depicted as they each contribute to an increased understanding across cardiological

research. Developments in one area (e.g. culturing of a stem cell line to meet a demand) may potentially influence developments in another field too (e.g. use MEA tools to extract useful electrophysiological data from SC-CM sources). Figure 4.3 also places electrophysiological data into context with overall research and development in the SC-CM research domain.

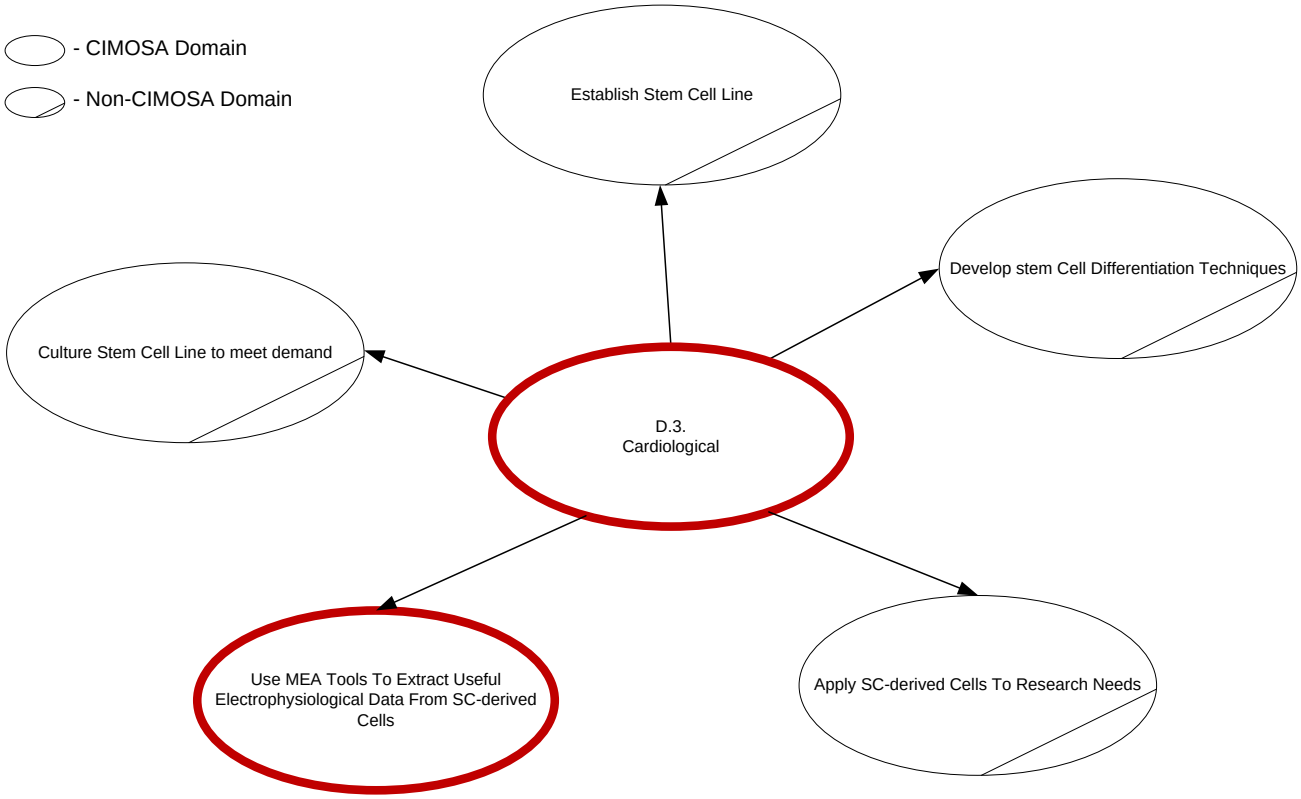


Figure 4.3: A context diagram depicting the interdependent research fields that are implemented contributing to successful stem cell-derived cardiomyocyte research.

4.2.1 Case Study Context

This case study is focused on an application that uses MEA systems to extract useful electrophysiological data from stem cell derived cardiomyocytes as they are exposed to various chemical entities.

Bioscientific protocols have been defined by University of Nottingham (UoN) MEA system users to facilitate this application.

Enterprise models (EMs) in this section communicate information relating to how MEA systems are presently used by the UoN and what current biochip and system requirements have been identified.

4.2.2 Modelling of the University of Nottingham SC-CM Application

The ensuing EMs document the protocols employed in a specialist cell culture laboratory environment at the UoN. Early construction of a high-level application summary model (Figure 4.4) outlined the overall workflow used in this cardiomyocyte-centred MEA application. The workflow is broken down into processes that are elements with clear start and end points. Further "drill down" models were constructed of processes that were relevant to MEA system interaction to ensure collection of detailed knowledge of all relevant processes.

4.2.2.1 Overview

The UoN workflow used to detect, record and analyse electrical field potential signals from stem cell-derived cardiomyocytes has been summarised as (Figure 4.4):

- Process One (P.1.) – Obtain stem cells
- Process Two (P.2.) – Obtain cardiomyocytes from those stem cells
- Process Three (P.3.) – Seed and culture the cardiomyocytes over the electrodes of an MEA biochip
- Process Four (P.4.) – Select and obtain substance(s) to be investigated
- Process Five (P.5.) – Set-up and run experiment
- Process Six (P.6.) – Collect data output
- Process Seven (P.7.) – Analyse output offline
- Process Eight (P.8.) – Derive meaning from values ascertained through analysis
- Process Nine (P.9.) – Share findings to contribute new knowledge to the research community

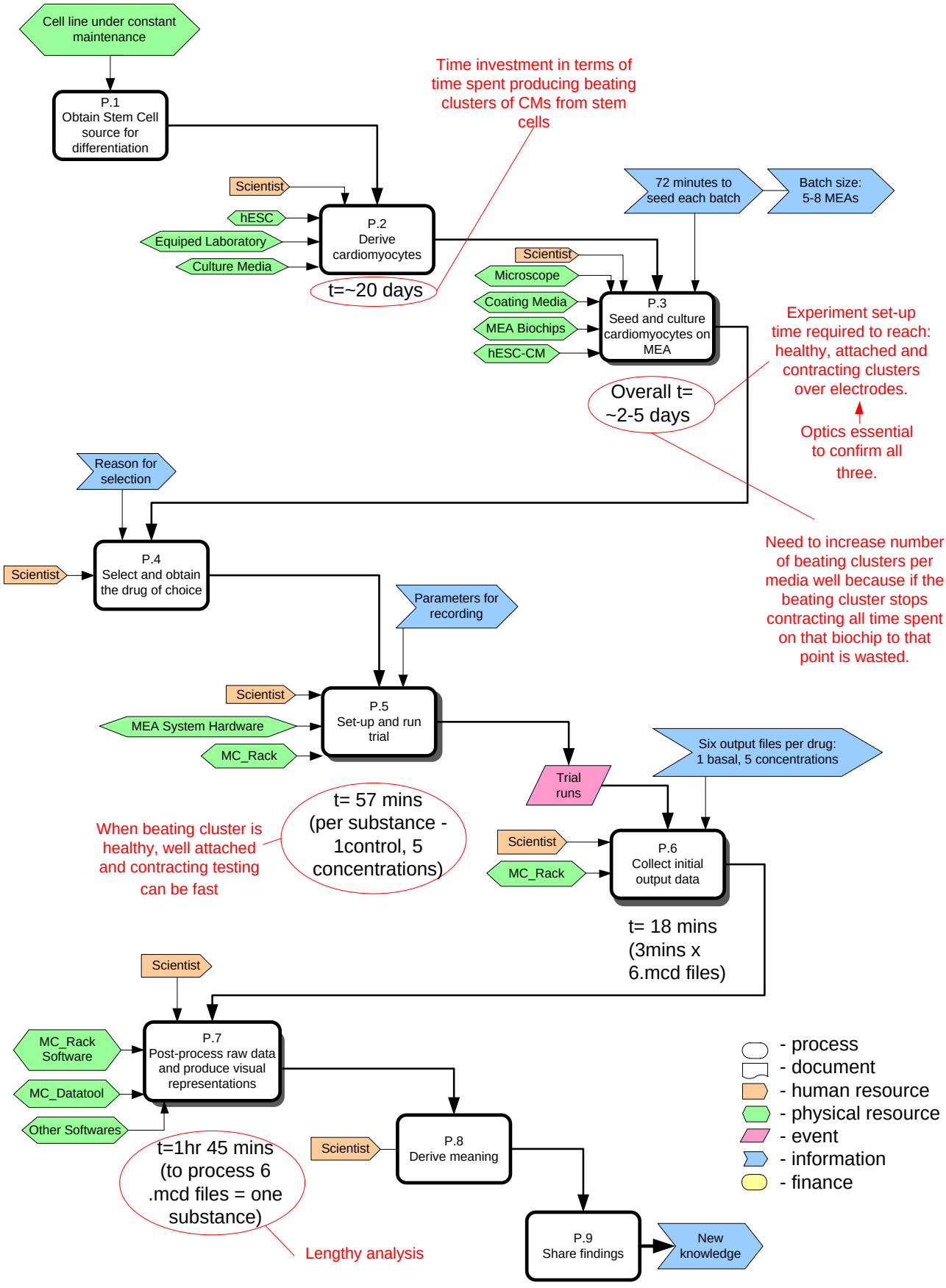


Figure 4.4: An activity diagram of the overall workflow of processes implemented by UoN MEA system users.

To complete an investigation from start to finish takes a total of approximately 22-25 days.

The protocols of most interest to this research are those where the MEA system or a component of the system has been physically interacted with in some way. These processes are identified and modelled further to capture relevant detail. Shadows are used under modelling constructs (e.g. P.3, P.5, P.6 and P.7) to indicate where further models have been constructed to incorporate details relevant to this research.

User and system requirements identified during the observation and the construction of the overall workflow used for this application are:

1. To ensure as many beating clusters as possible make it from differentiation to the end of testing through facilitating appropriate handling and cell culture conditions.
2. To increase the number of beating clusters per media well, thereby improving the volume of output per test and to improve efficiencies of the MEA biochip preparation process.
3. To ensure outputs are of comparable or better quality than current.
4. To ensure tests can be conducted with comparable or better ease and speed.

4.2.2.1.1 University of Nottingham processes

The following models document each process that is MEA system or component relevant.

Process Three – Seed and culture cardiomyocytes on MEA Biochip

Process three (Figure 4.5) is the preparation of each MEA biochip (workspace and media well) to a standard suitable for beating cluster attachment and survival in vitro. This requires sterilisation and, depending upon the user preference, treatment of the surface with an appropriate attachment matrix, which in this case is e.g. Matrigel. The beating clusters of cardiomyocytes are seeded into the MEA biochips, one at the centre of each workspace and therefore per media well.

Once seeded, the MEA biochips are moved from the culture hood into the incubator where the environment is controlled at a steady 37°C with a CO_2 concentration of 5% (which is required to control acidity levels within the culture media) and left undisturbed for a period of at least sixteen hours. The exact incubation period differs, depending upon the individual scientist and the duration of time that each individual beating cluster takes to attach adequately to the underlying attachment matrix or surface.

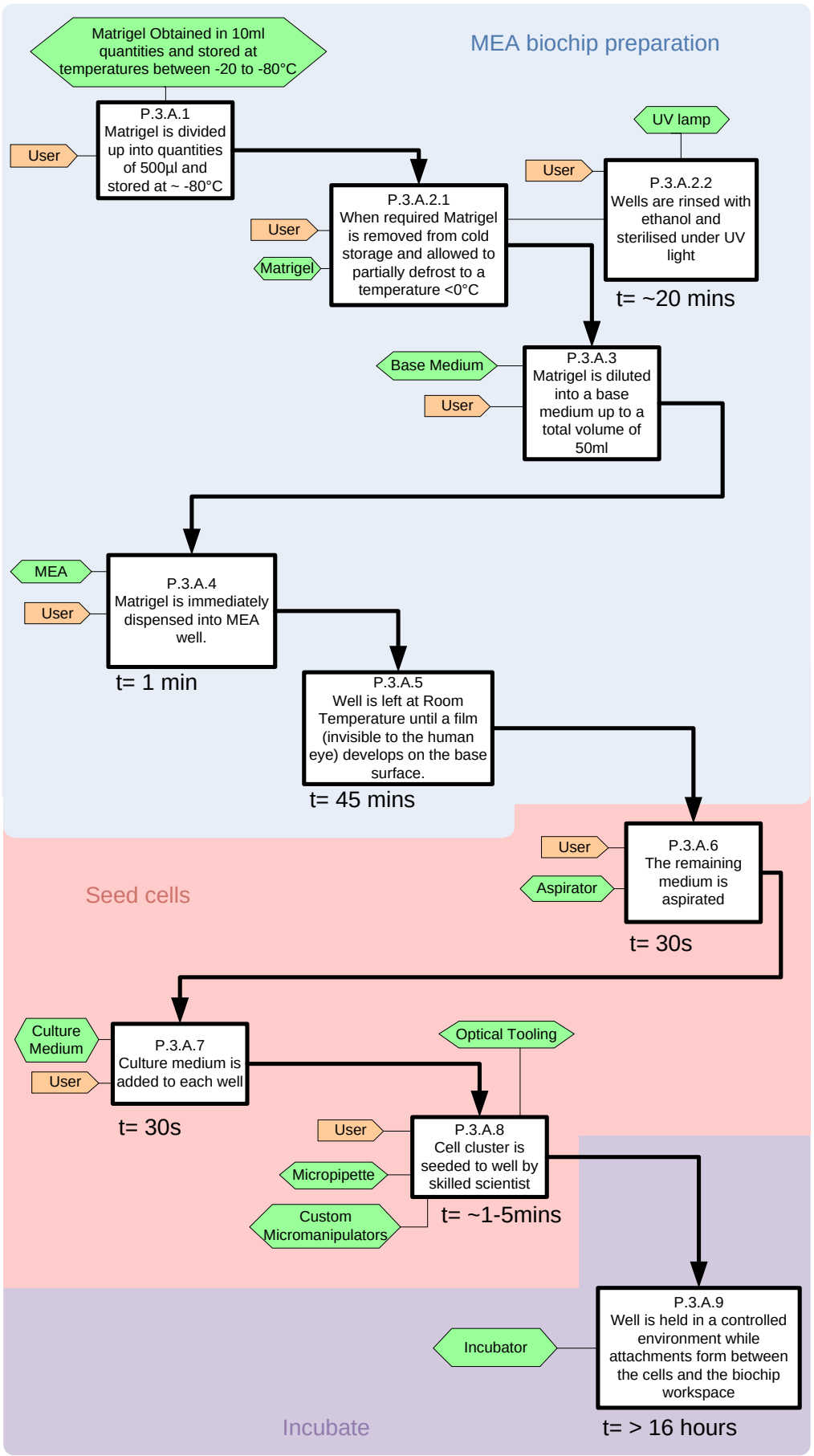


Figure 4.5: An activity diagram depicting the activities carried out to complete process three.

User and system requirements identified:

1. Sterilisation of the biochip takes 20 minutes. Treatment of the workspace surface with Matrigel takes a further 1 hour and 45 minutes.
2. Attachment of the contracting cell cluster requires at least 16 hours. UoN users have described occasions where, due to the contracting nature of the sample, the beating cluster has moved away from the microelectrodes prior to attachments forming with the surface. On these occasions no signal is detected, so the time spent preparing the biochip to this point has been wasted. In addition to this, if the beating cluster of cardiomyocytes is to be re-used, more time is lost dissecting it out of the MEA for re-seeding onto a newly prepared MEA biochip. A biochip that ensures attachment over electrodes every time is required.

Note: ~10% of cardiomyocyte clusters seeded onto an MEA biochip complete the testing process from initial seeding to full data capture (Personal Communication, Dr David Anderson).

Process Four – Select and obtain drug

Process four, select and obtain the drug of choice, is user dependant and is out of the scope of this project in terms of delivering improvements to the MEA system.

Process Five – Set-up and run trial

Process five (Figure 4.6), set-up and run trial, is when the MEA system is physically put to into use. Signals are detected and recorded into files that contain data for all of the 60 microelectrodes. The files are called multi channels data (.mcd) files. The response of the cardiomyocytes to chemical treatments added during recording is of primary interest to UoN users in this case study.

User and system requirements identified:

1. Every stage of each test is carried out manually by the user. Automation of the process or of parts of this process (i.e. automatic perfusion of the culture media and substances that are under investigation) would increase convenience to users and reduce the likelihood of human error.

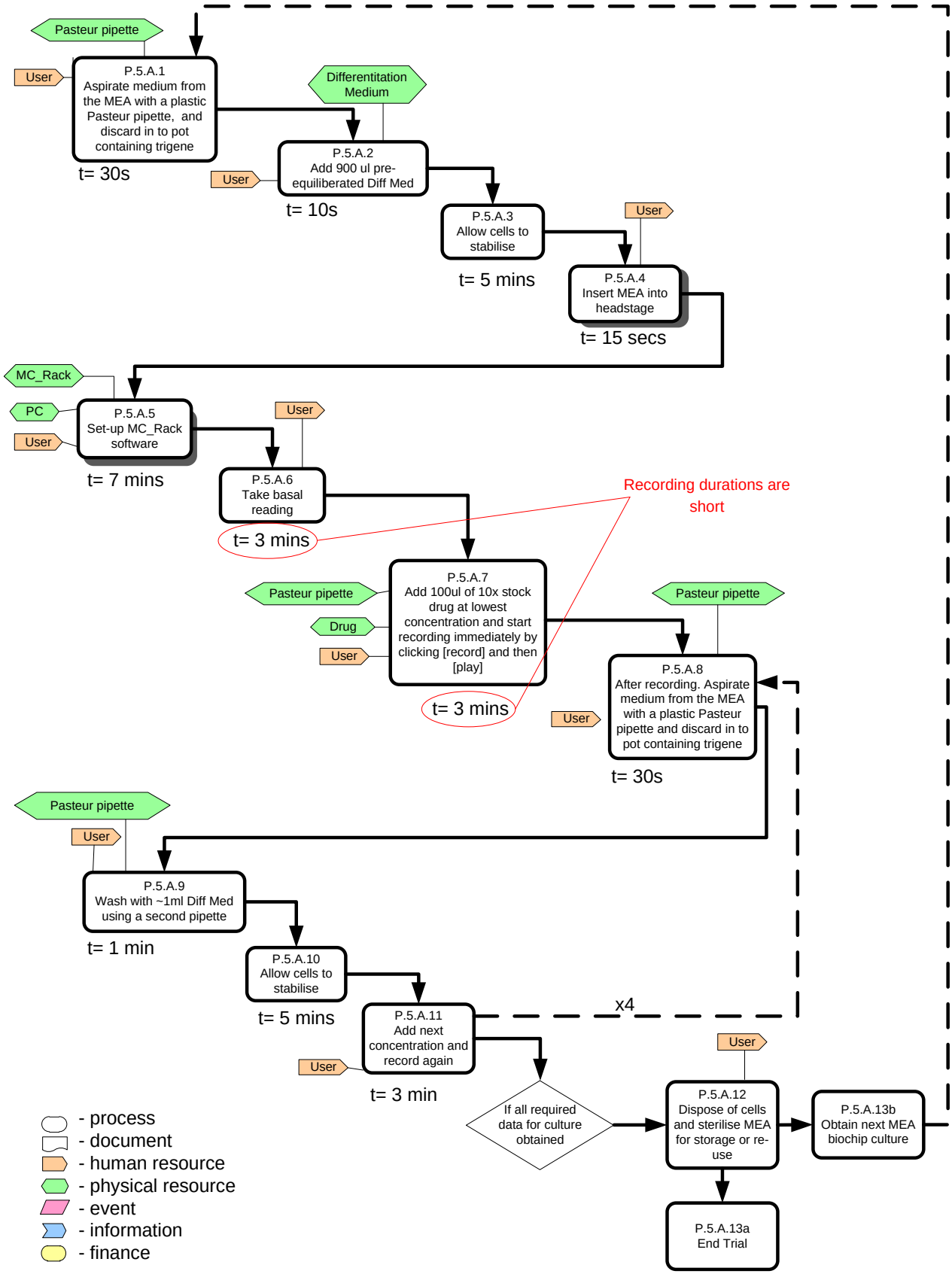


Figure 4.6: An activity diagram depicting the activities carried to complete process five.

Process Six – Collect initial output data

Process six, collect initial output data (Figure 4.7), uses the MC-Rack re-player function to re-run recorded raw data files through a previously configured rack that has been specified to output specific **parameters of interest**.

Parameters of interest

For this case study those parameters were:

- **Beat rate – number of contractions per minute**
- **Average peak-to-peak amplitude (per minute) – µV**
- **Average minimum amplitude (per minute) – µV**
- **Average duration between beats (per minute) – ms**
- Average QT-interval (per minute) – ms (see process seven)

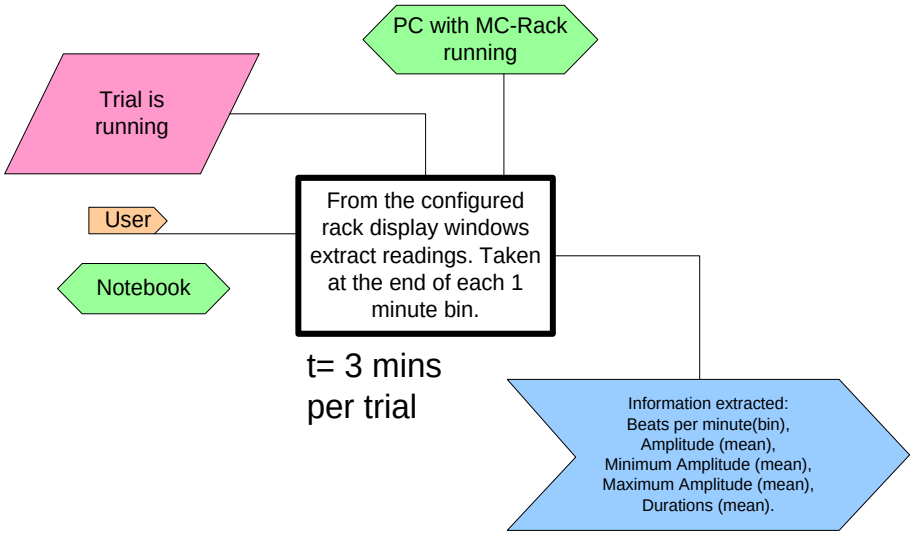


Figure 4.7: An activity diagram depicting process six, the initial data extraction using the replayer function in MC_Rack.

Process six captures the parameters identified in bold type above. Average QT- interval is extracted by process seven.

Process six takes a little over three minutes per file to perform. Six files are recorded per cell cluster, per test (1 basal, 5 during treatment with different concentrations of a substance). The data used is from the final two minutes of each three minute recording and is from a single channel. Values for each parameter for the signal occurring on the channel selected are documented by hand into a spreadsheet. The channel used is the “channel of interest” throughout the analysis (P.6 and P.7).

This process can be carried out during testing (online) but is most often done after the recordings have been made (offline) so as to reduce the likelihood of human error during the live experiments.

User and system requirements identified:

1. Automated extraction of basic parameters including beat per minute, amplitude values and durations of and between waveform features (i.e. duration between beats, duration of QT-interval).

Process Seven – Post-process raw data and produce visual representations of data

Process seven, post process the raw data and produce representations, is documented in Figure 4.8.

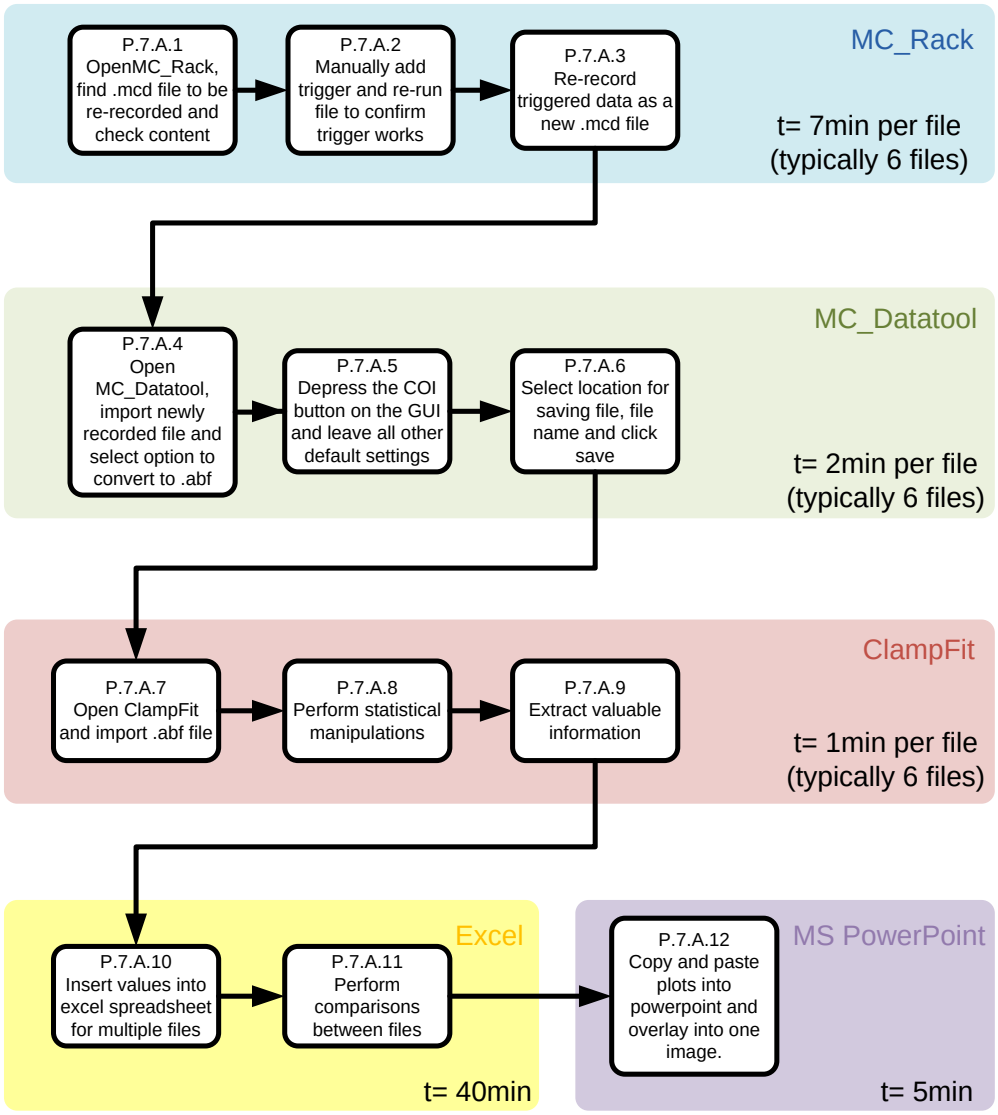


Figure 4.8: An activity model of process seven as it is currently performed.

In this application analysis of the raw signals recorded is conducted offline, assessing the influence of a substance or substances on a known feature of the cardiac waveform that is called the QT-interval. Process 7 takes a significant amount of time to perform

The shape of signals varies between cell clusters (see Figure 2.33). Consequently user perception of where the Q feature starts and T feature ends is currently a variable.

The parameter of interest extracted by this process is:

- **Average QT-interval duration (per minute) – ms (process seven)**

This is a critical parameter of drug development and safety pharmacology that is considered as part of new substance development prior to any clinic-based testing (section 2.11.1.1.2).

The following further drill down models demonstrate how QT interval is extracted in further detail in way that is repeatable by different users of the same research group.

Further drill down modelling

Activity models have been used to document further details of each protocol. In this section the drill down models generated for this case study are presented and additional requirements identified are noted.

Process Five

Activities pursued to complete action P.5.A.4 are shown in Figure 4.9, insert MEA into headstage, into defined in the higher-level model of process five (Figure 4.6).

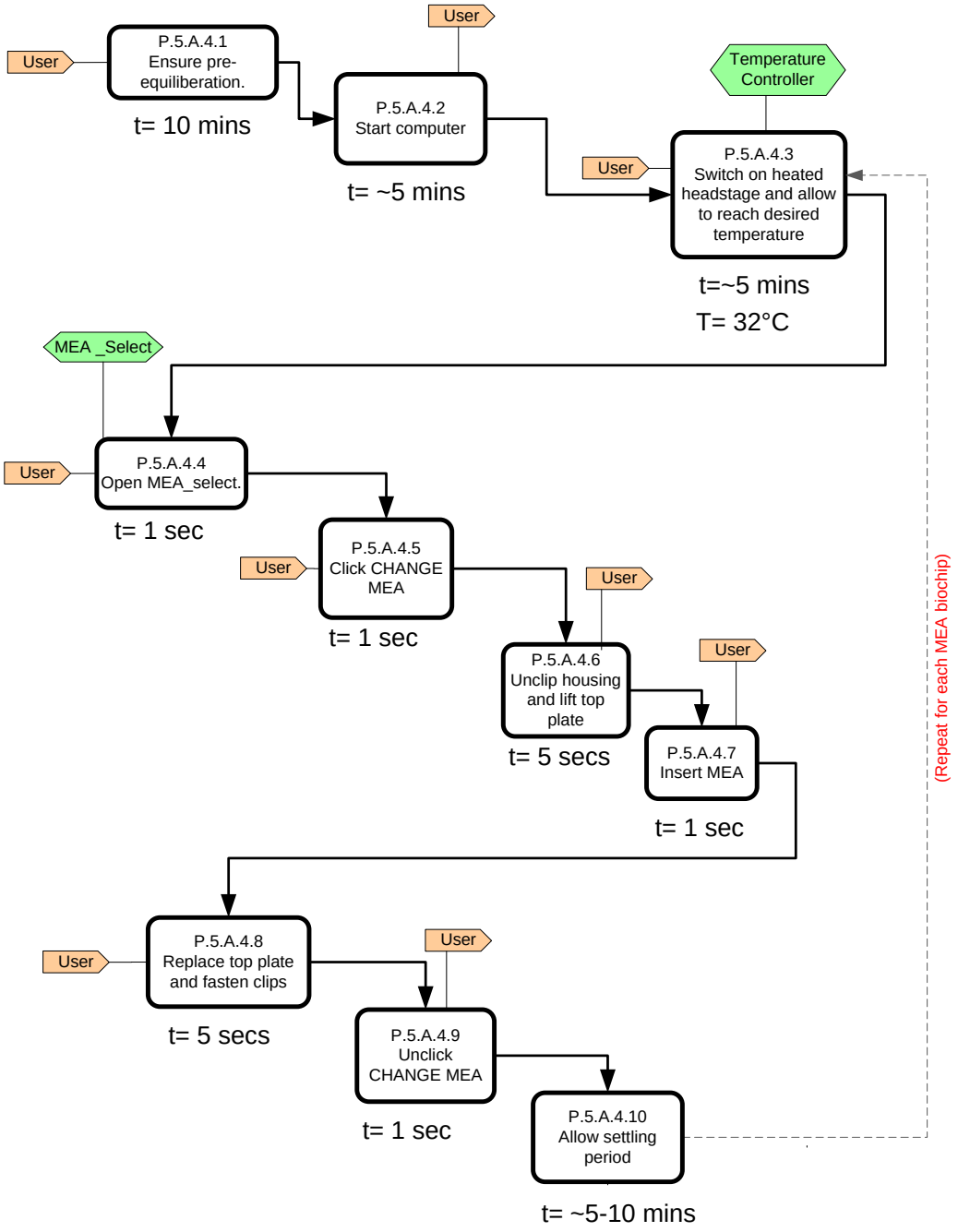


Figure 4.9: The low-level activities carried out to complete activity four or process five.

P.5.A.4.3 to P.5.A.4.10 (Figure 4.9) are repeated where >1 seeded MEA biochip is tested. P.5.A.4, insert MEA into headstage, takes approximately 10-15 minutes per biochip due to the need to allow a settling period. In this example an experiment may be repeated on five cell clusters. Therefore P.5.A.4 repeated five times adds ~50 minutes to the overall test duration. This research has been able to highlight that if five cell clusters were seeded into one MEA biochip and independent contractions maintained, then at least 40 minutes could be saved per experiment.

User and system requirements identified:

1. Support system electronics that can facilitate more seeded beating clusters of cardiomyocytes per execution of a test automatically.

The activities involved in P.5.A.5 are demonstrated in Figure 4.10, the initial software set-up at the start of the experiment. When a new analysis routine is constructed a new rack may be constructed that can be saved and re-used for subsequent relevant tests (section 2.9.1.4.1). Within this model P.5.A.5.3 has been modelled further as this aspect may be adjusted by users between individual MEA biochips in a complete testing cycle using several MEA biochips.

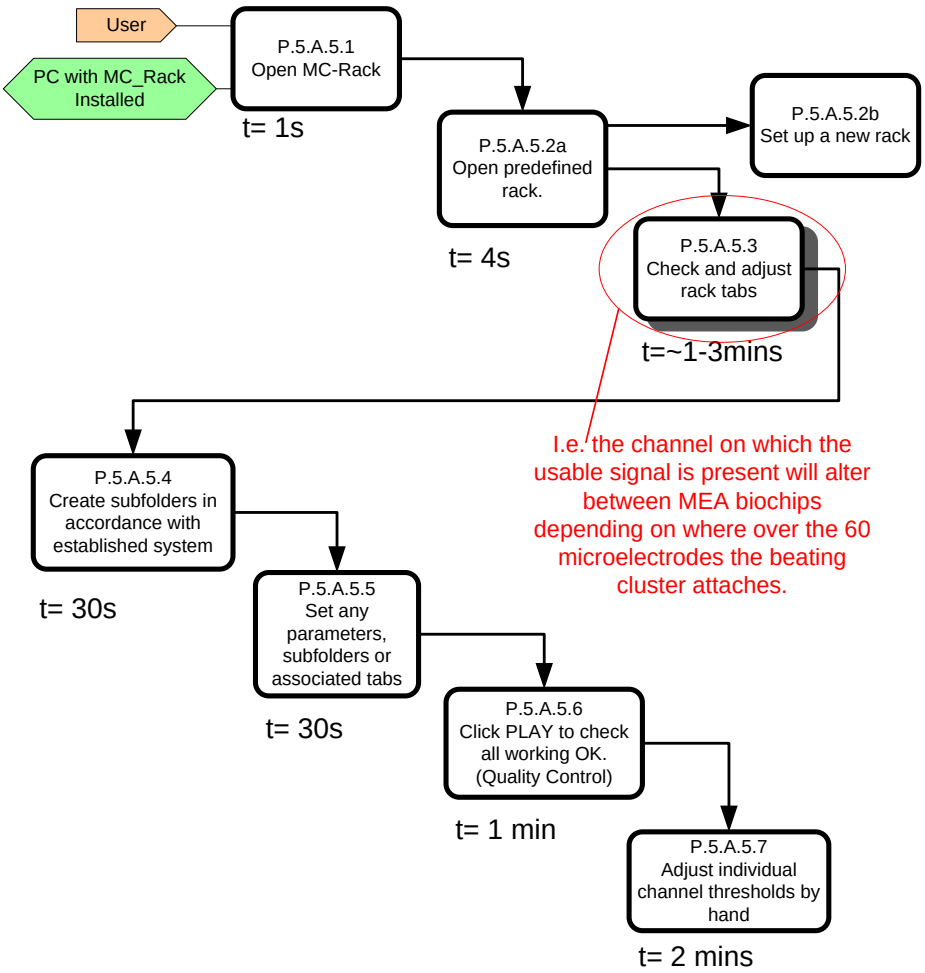


Figure 4.10: The low-level activities carried out in order to complete activity five of process five.

The following models incorporate further details of how the software is used in a visual manner. The software was described by users as complicated and lacking intuitive ease of use. For a novel software solution to be implemented a thorough knowledge of how current software is interacted with was sought by the author of this thesis. Figure 4.11 shows the steps taken to set-up the analysis rack used.

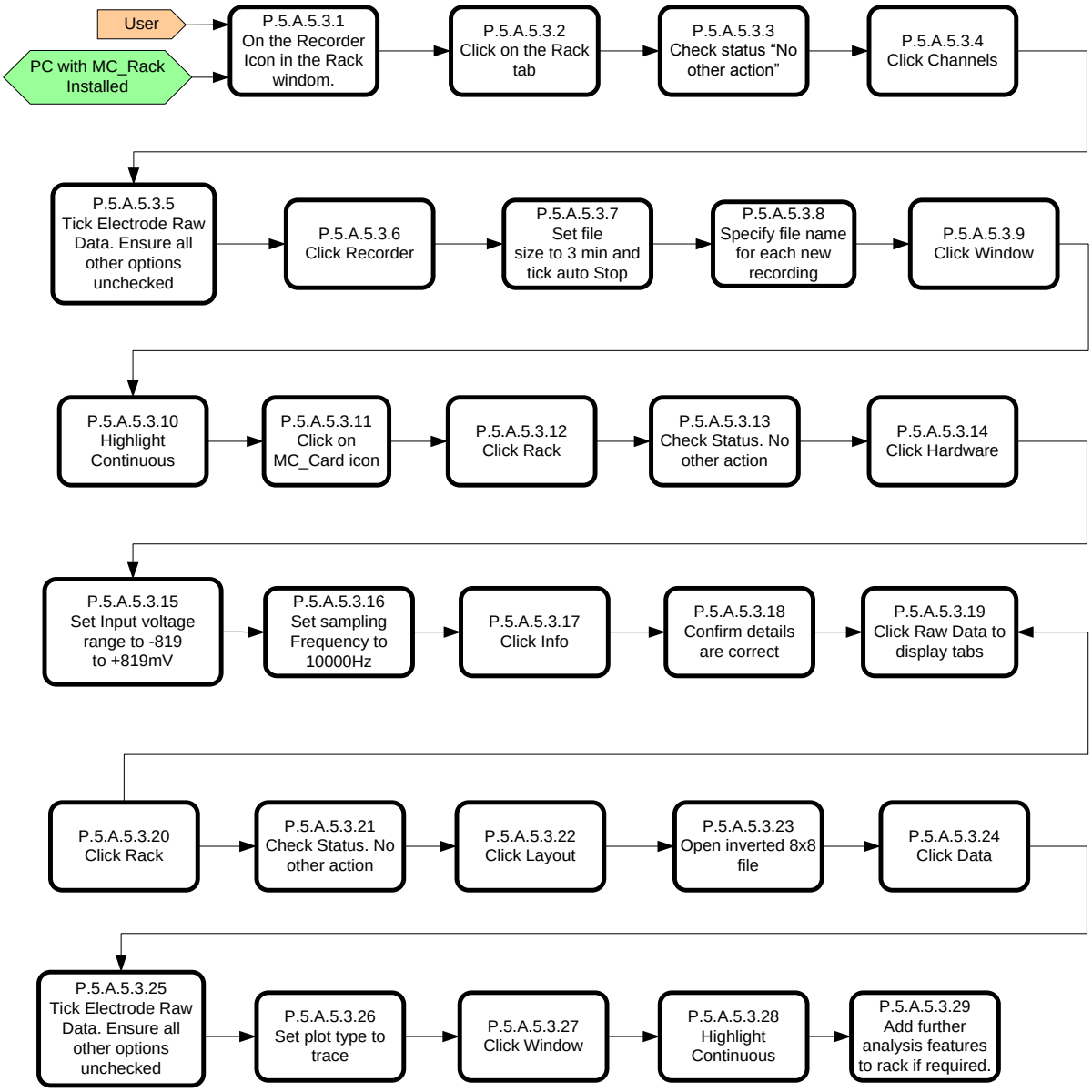
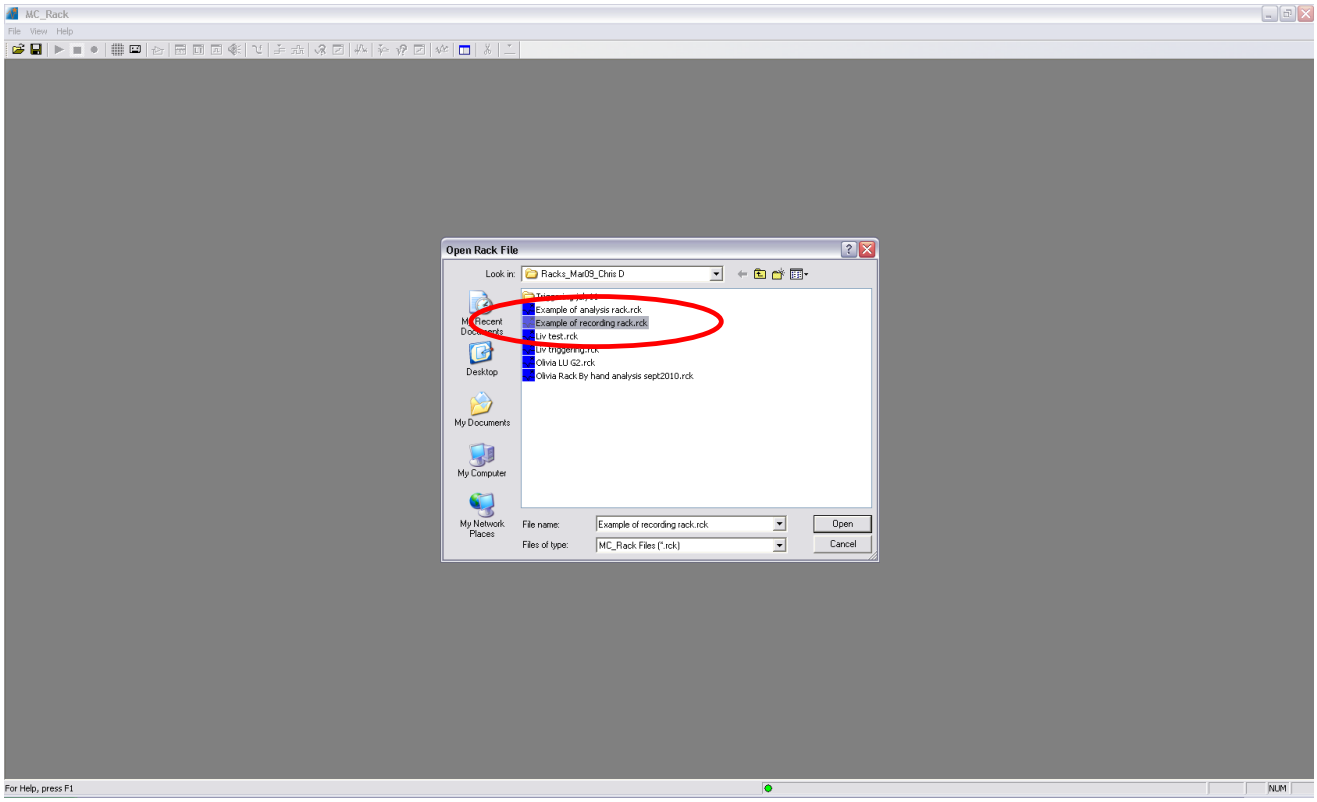
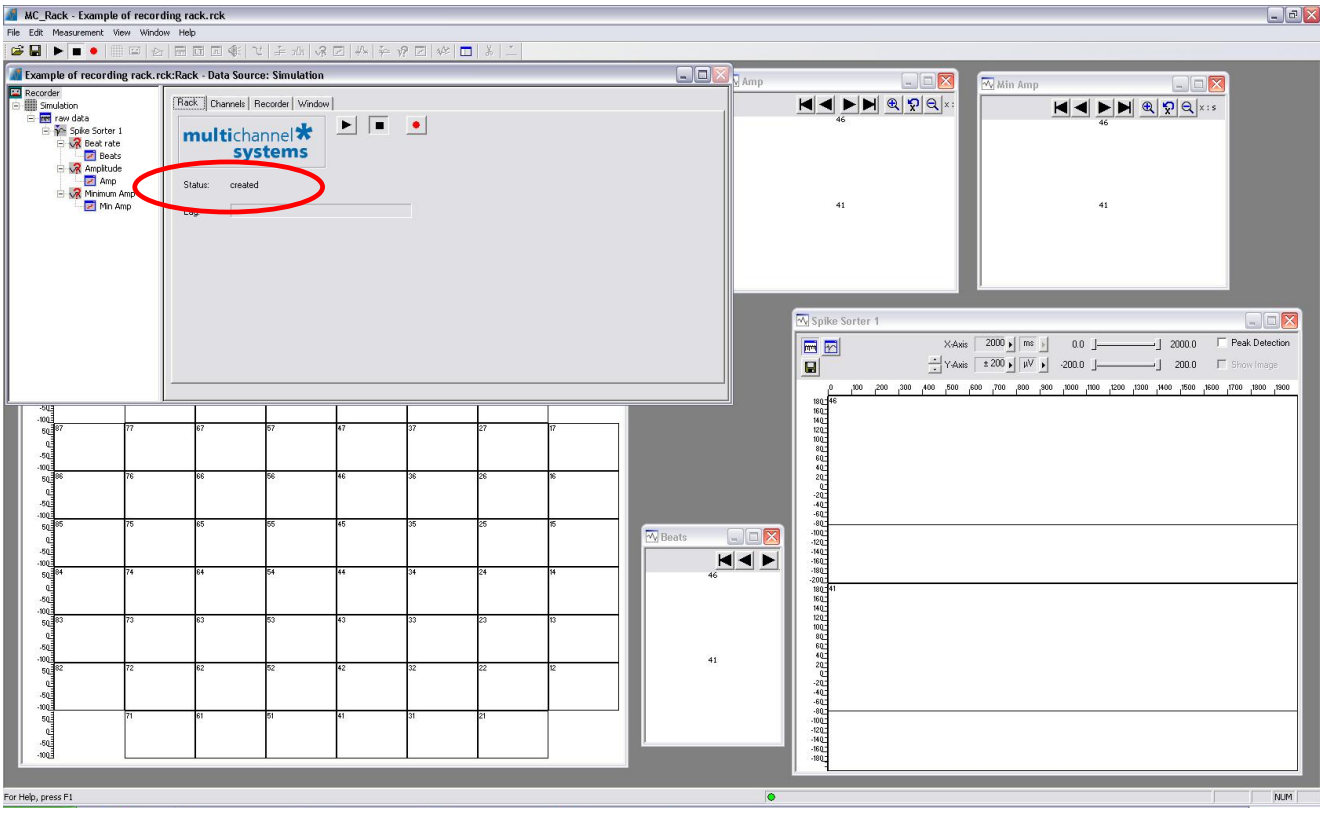


Figure 4.11: The low level activities carried out to complete A.5.3 of process five. (This process also the same for activity 2b of process five.)

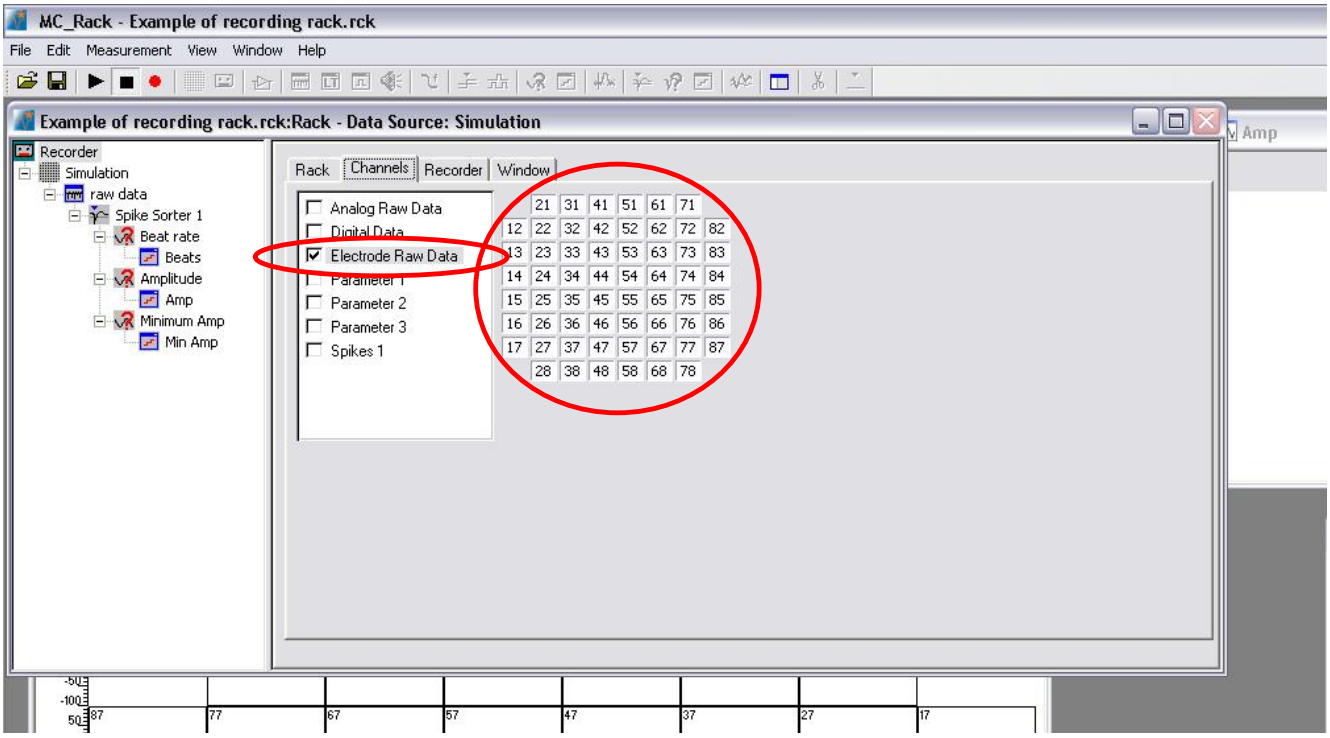
Settings are adjusted in the rack tabs prior to testing, via a series of repetitive click tasks. It is this element of software interaction that UoN users have described as complex and lacking intuition. This aspect of set-up is entirely user dependant so it must be carried out correctly if the recordings taken are to be useful. Figure 4.12 shows what the rack and its tabs in this instance look like.



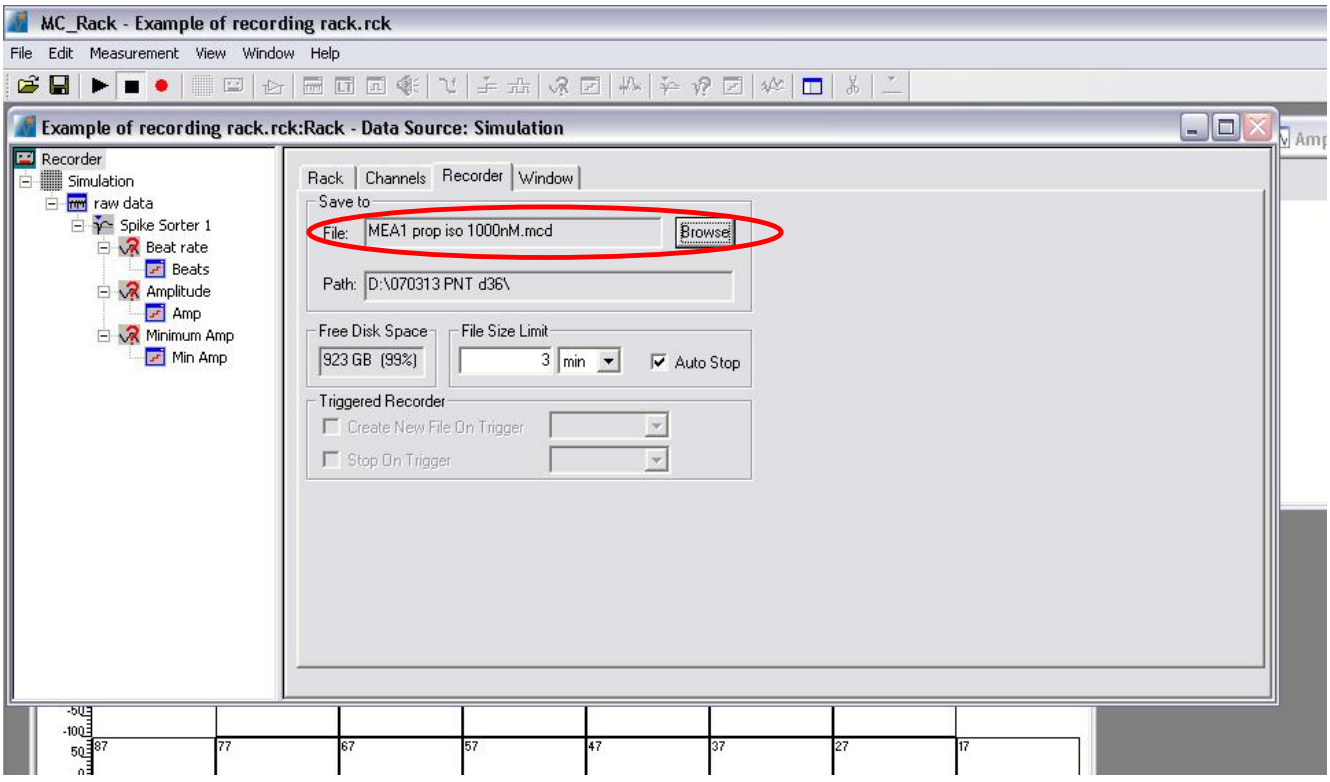
Open the previously created .rck file



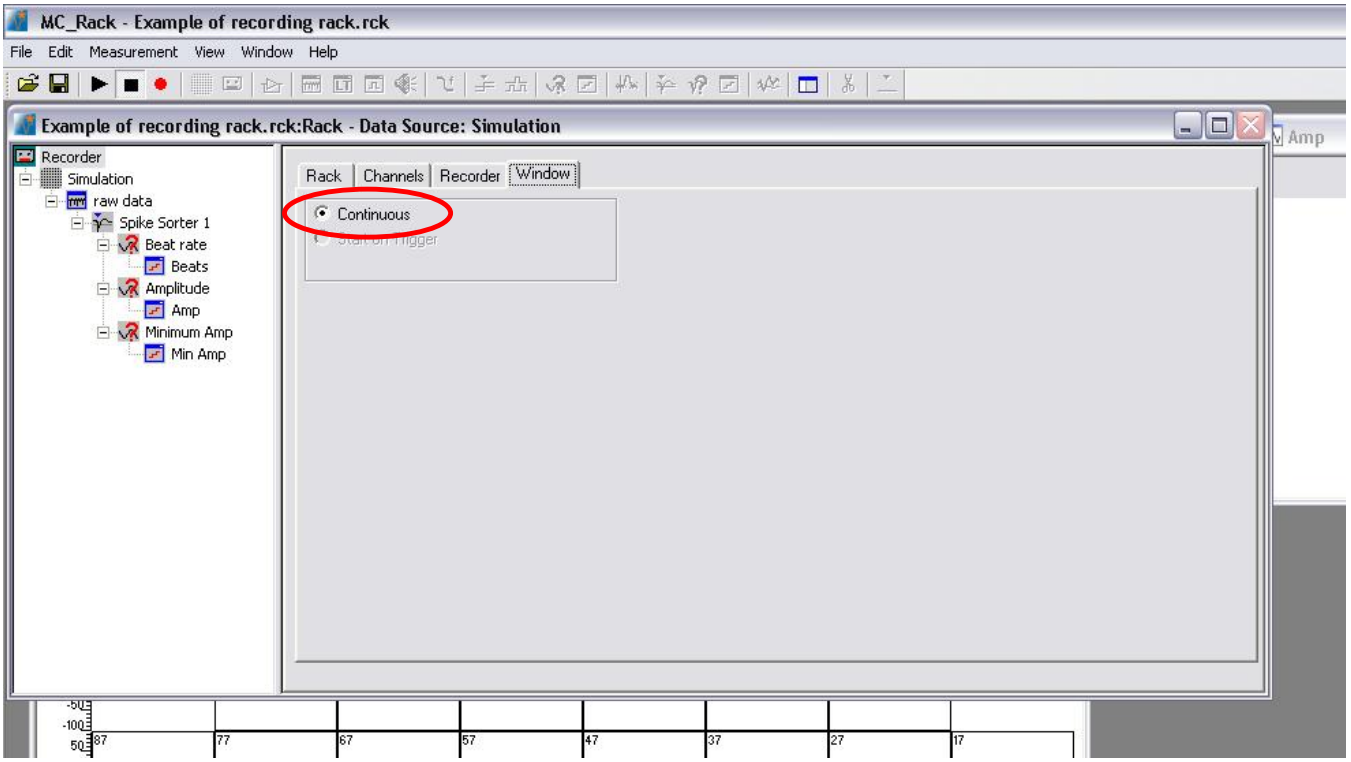
Check status of the rack



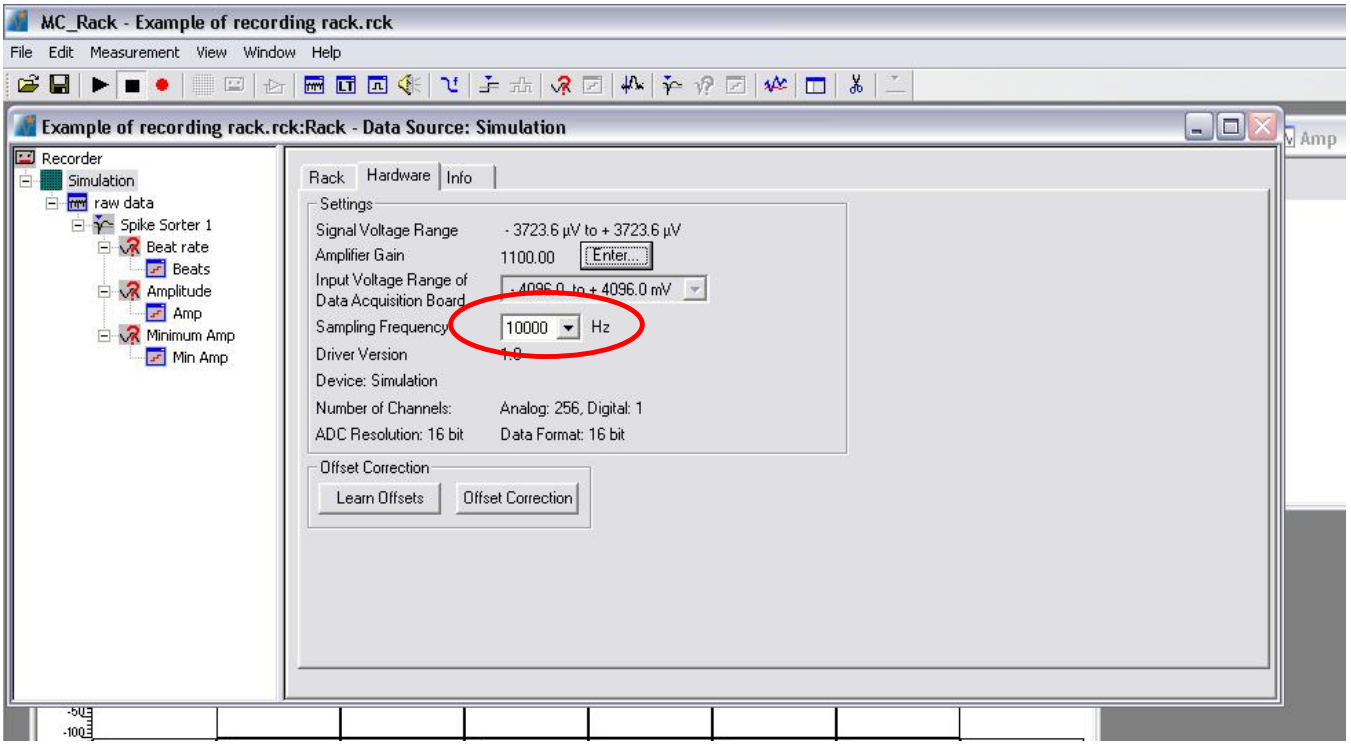
Adjust channels to be recorded



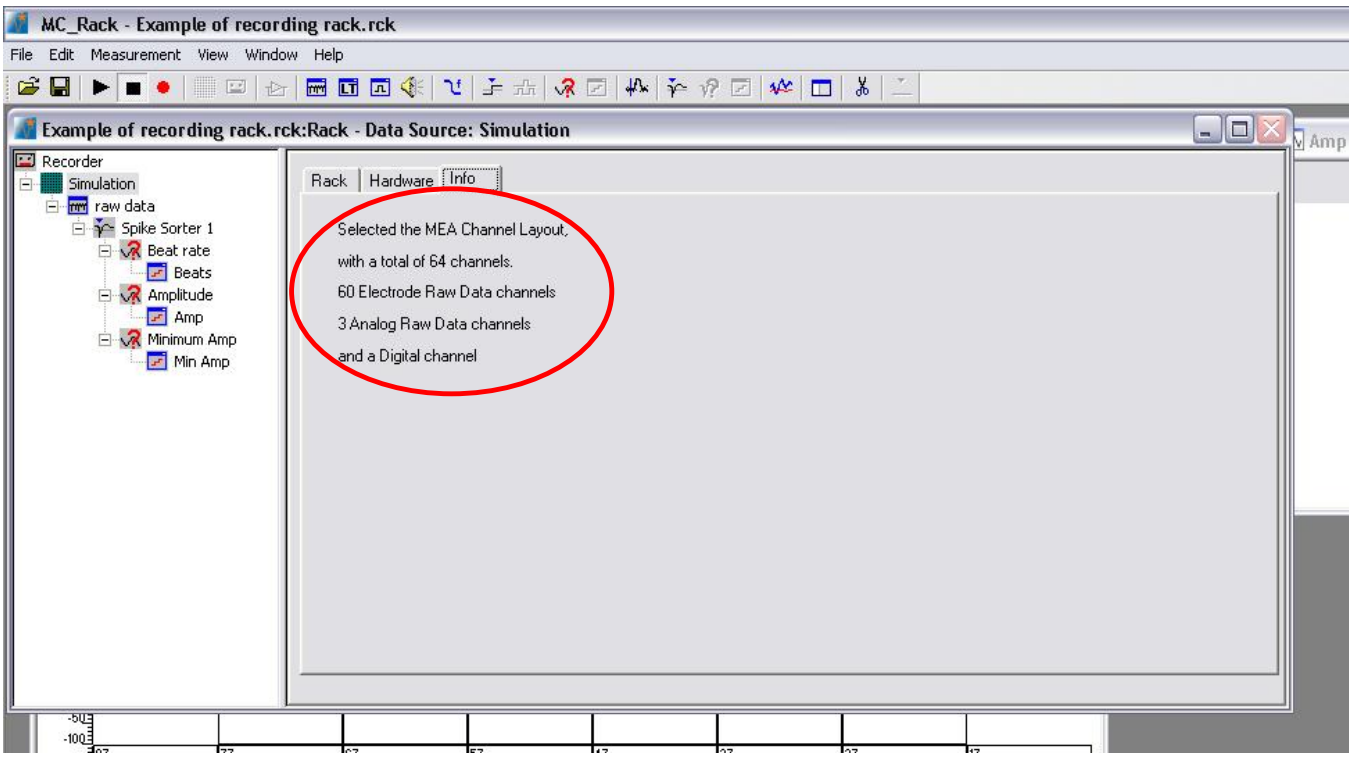
Define the file that is to be created



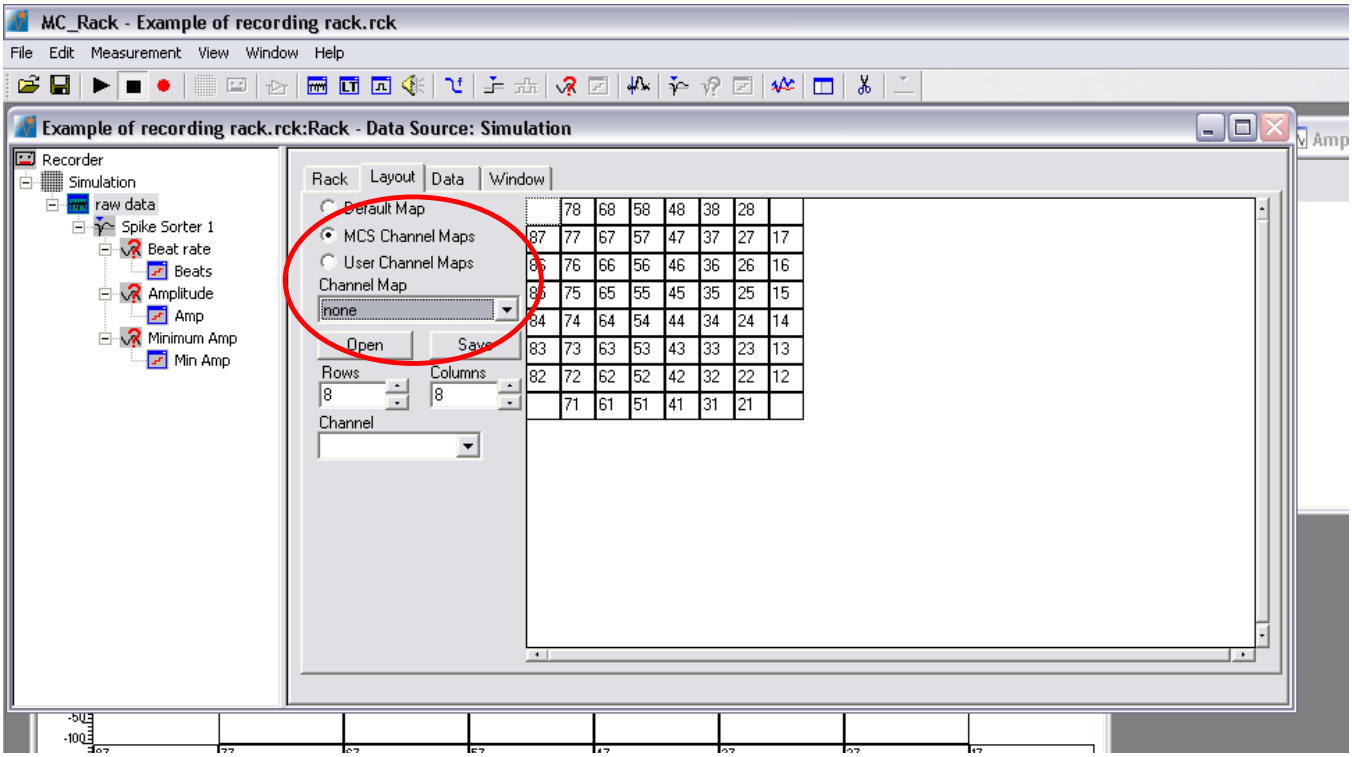
Adjust window setting to continuous



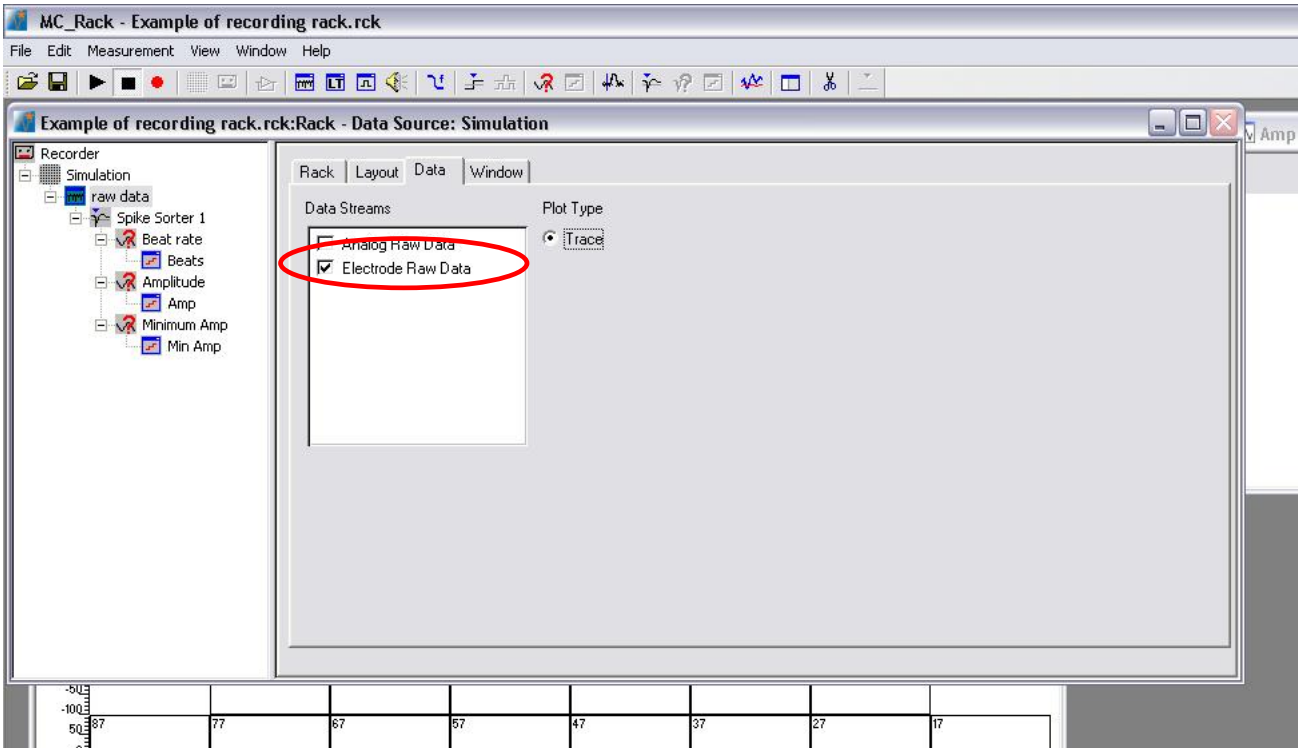
Set hardware (the MC_Card) settings



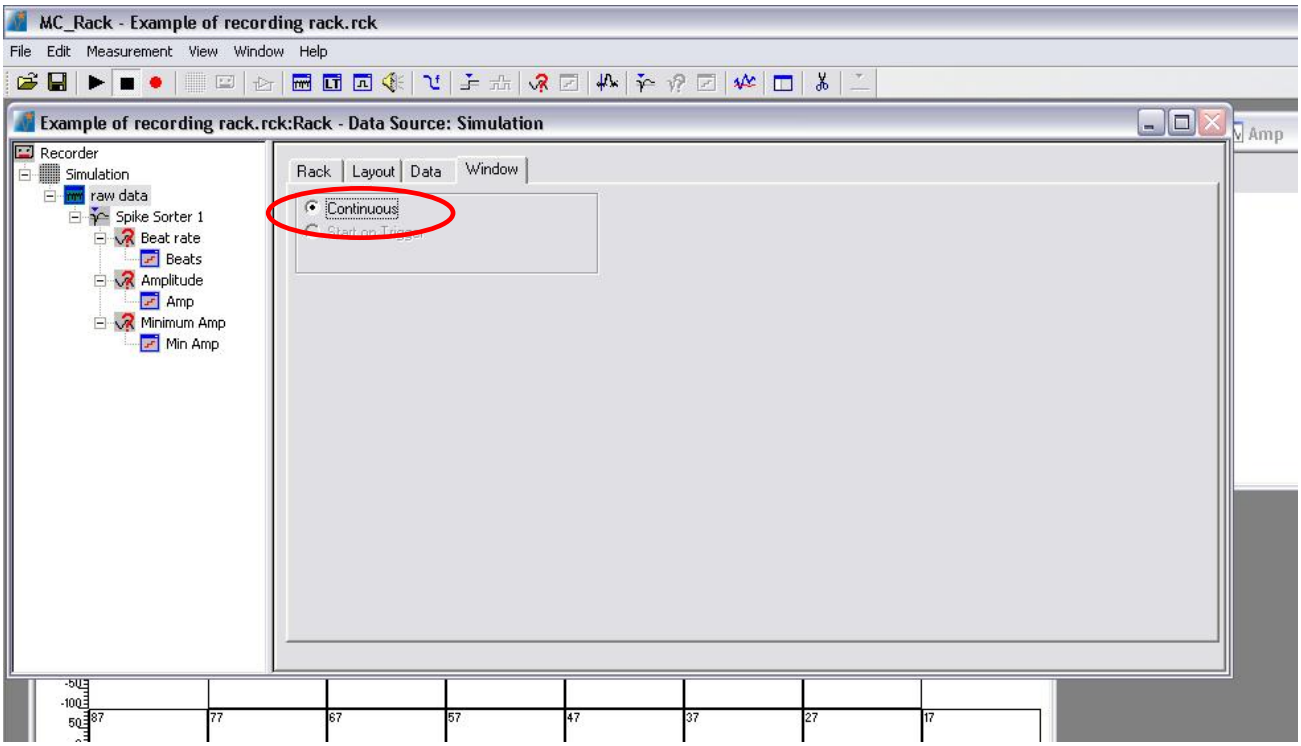
Confirm details



Set the layout map for the ensuing tools



Set data



Set window to continuous

Figure 4.12: Setting up the recording rack for the UoN cardiomyocyte MEA system application.

User and system requirements identified:

1. Software that has a greater level of automation. Should be more intuitive and less complex.

Process Seven – QT-interval data collection

The process used to extract the QT-interval are documented in greater detail in the figures Figure 4.13 to Figure 4.21.

MC_Rack

The MC_Rack replay function is used to re-record the original raw data files that have been recorded and used to extract other basic parameters into "triggered files". A triggered file breaks the continuous stream of raw data into individual windows of data that each contain a single contraction waveform (SC-CM cluster field potential).

The process required to add a trigger is:

- A.1.1 – A.1.3: Add Replayer > Replay file tab > Select file > Slow replay rate to ~1.0x.
- A.1.4 – A.1.5: Add display > Press play to run file in fast mode to confirm signal present and COI.
- A.1.6: Return to replayer> adjust replay speed further if required.
- A.1.7 – A.1.9: Double click on display over COI> Right click> Turn on "display crosshairs"> Use cross hairs to decide where threshold should be set and approximately how long each beat is in duration. Note values on a piece of paper.

- A.2.1 – A.2.5: Add Trigger Detector > Select Trigger tab> Set channel to COI> Set Level to appropriate position for threshold (decided using cross hairs on replayer display)> Set dead time to point beyond end of last wave feature and before start of next wave > choose for trigger to be on the positive or negative slope of the spike.
- A.2.6 – A.2.9: Add Display to trigger detector > Layout> Select COI in Channel drop down menu> Data > Check "Electrode Raw Data" and "Trigger 1" > Window> Start on Trigger> Drop down Trigger to "trigger 1".
- A.2.10 – A.2.11: Return to configuring replayer
    - Set start time to begin 181 seconds from end (last three minutes of recording.
    - Replay file. Ensure the Trigger detector display is showing each window overlaying and not the raw data stream.

- A.3.1 – A.3.6: Configure recorder:
    - Channels > Select channels to export
    - Recorder>Set file name and saving location
    - Window> Check “Start on Trigger” > Set start time to before wave begins>Select appropriate window extent by measuring using cross-hairs feature of MC_Rack>Leave Trigger 1.
- A.3.7: To start rerecording the triggered file>On the main display press down the red “recording” spot button> Press play.

If the settings are not correct during the re-recording of the data with the trigger the file will fail to convert in the MC_Datatool software.

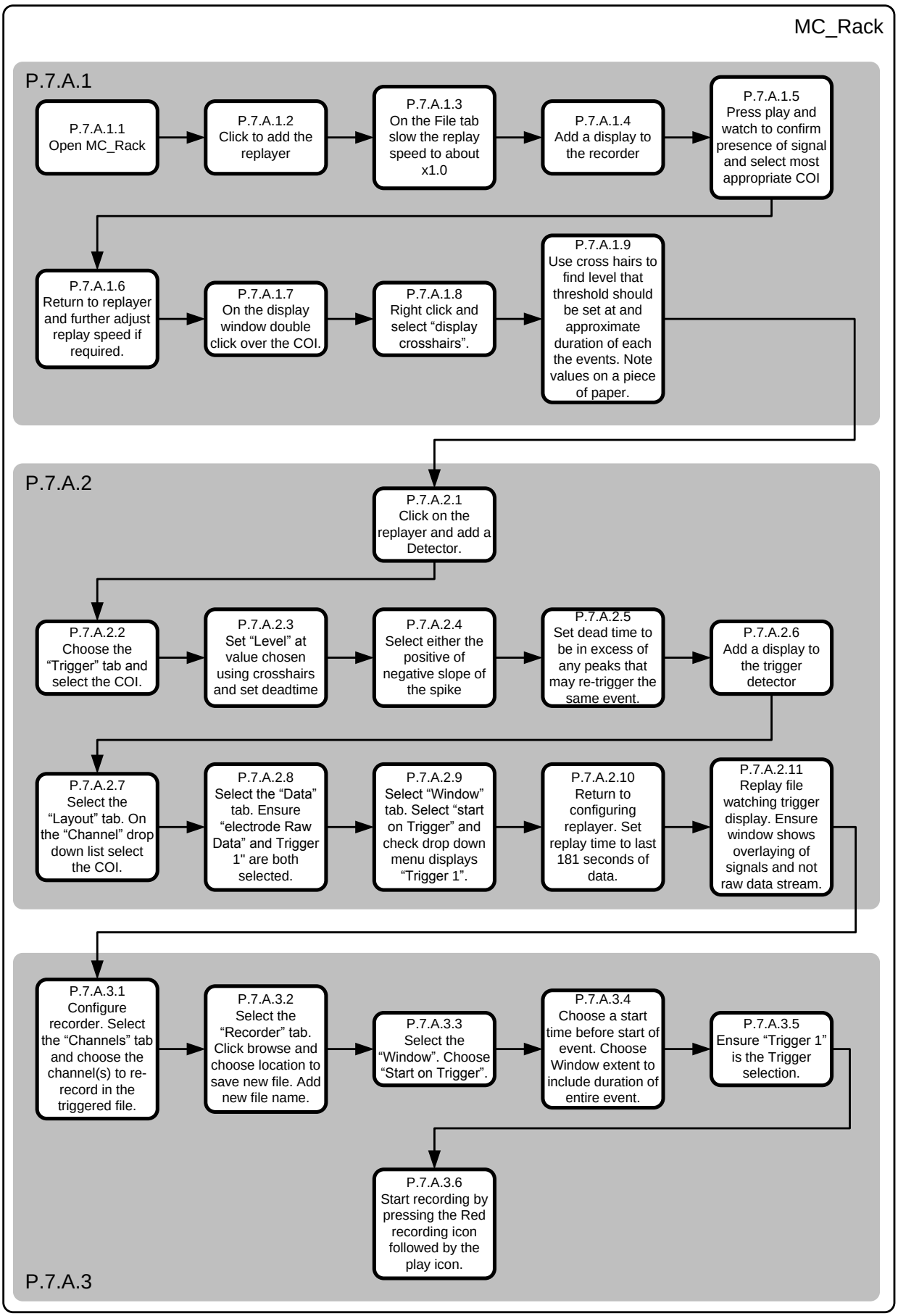


Figure 4.13: The low level activities of activities 1 to 3 of process seven.

MC_Datatool

The MC_Datatool is a specialist software tool that specifically converts .mcd files into alternative formats that can be imported into other software. The MC_Datatool supports conversion to binary (.bin), text (.txt) and axon binary (.abf) file types. In this case users convert the raw .mcd files into .abf files, which is required to import MEA system data into the Clampfit software (Molecular Devices, Sunnyvale, CA, USA) used in the ensuing stages of the analysis (Figure 4.14).

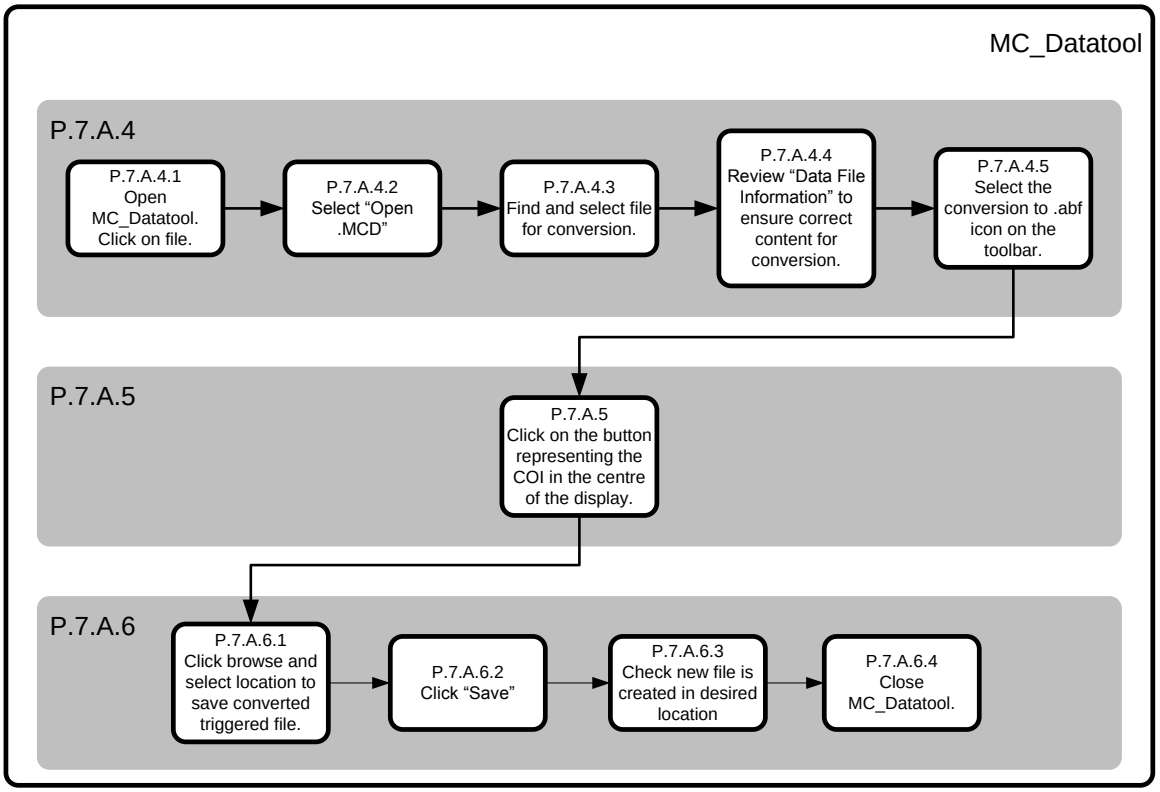


Figure 4.14: The low level activities of activities 4 to 6 of process seven.

The process required to convert from .mcd to .abf is:

- A.4.1 – A.4.3: Open MC_Datatool>File>Open MCD>Select file for conversion from .mcd (multi channels data) to .abf (axon binary file).
- A.4.4: Check Data File Information.
- A.4.5: Select the button on the main toolbar that looks like this  .
- A.5: Click on the button in the middle of the display that shows the channel of interest.
- A.6.1 – A.6.3: Click Save.
- A.6.4: Close the MC_datatool.

Clamp Fit

Clampfit (pCLAMP v10) is a specialist electrophysiology data acquisition and analysis software that regards itself as the “gold standard” software for this electrophysiology application. This software has been developed recording and analysis of patch clamp data. In this application all of the waveforms in one triggered file (lasting 181 seconds) are averaged and the QT-interval is extracted by hand using drag-and-drop cross-hairs (Figure 4.15).

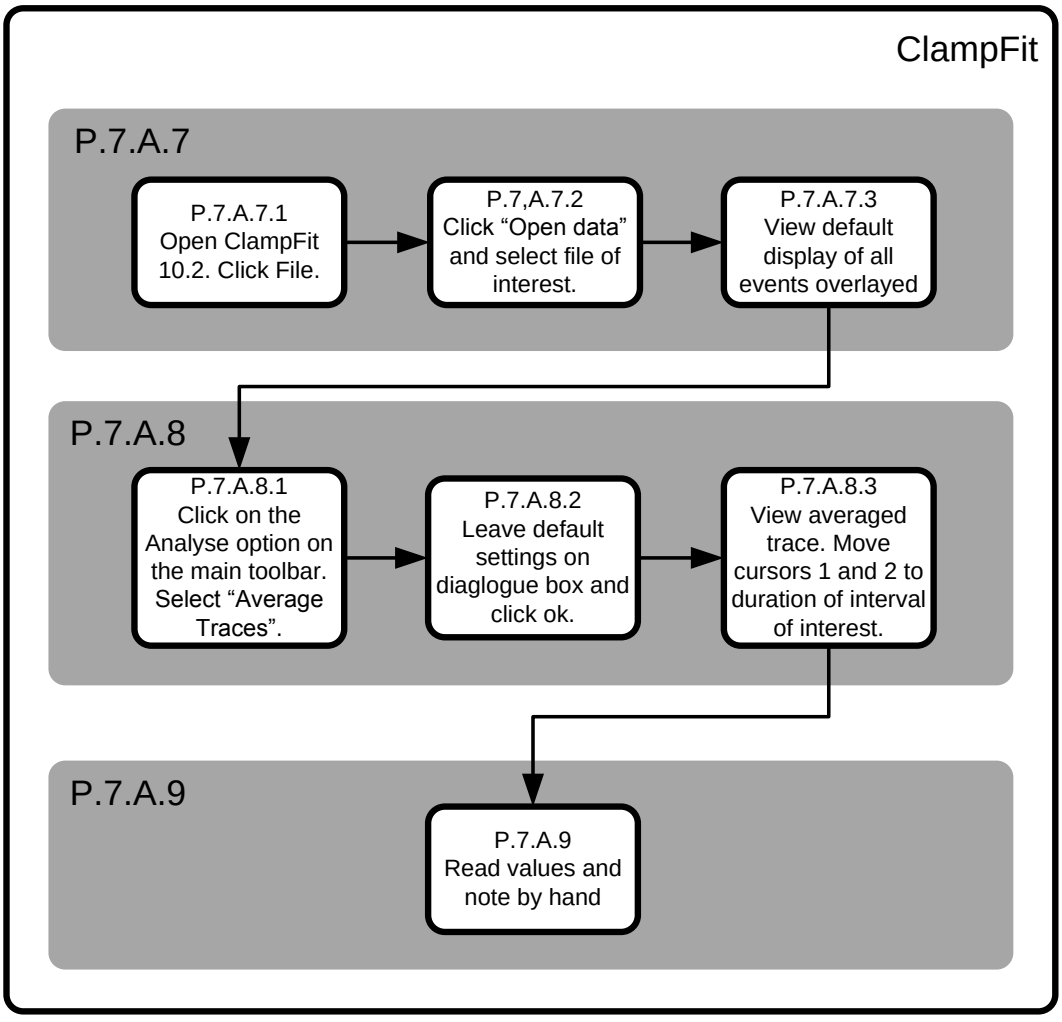


Figure 4.15: the low level activities of activities 7 to 9 or process seven.

The process required to average the waveforms and capture the QT-interval is:

- A.7.1: Open Clamp Fit 10.2
- A.7.2: File>Open Data> Double click .abf of interest.
- A.7.3: View all windows captured overlayed (Figure 4.16).

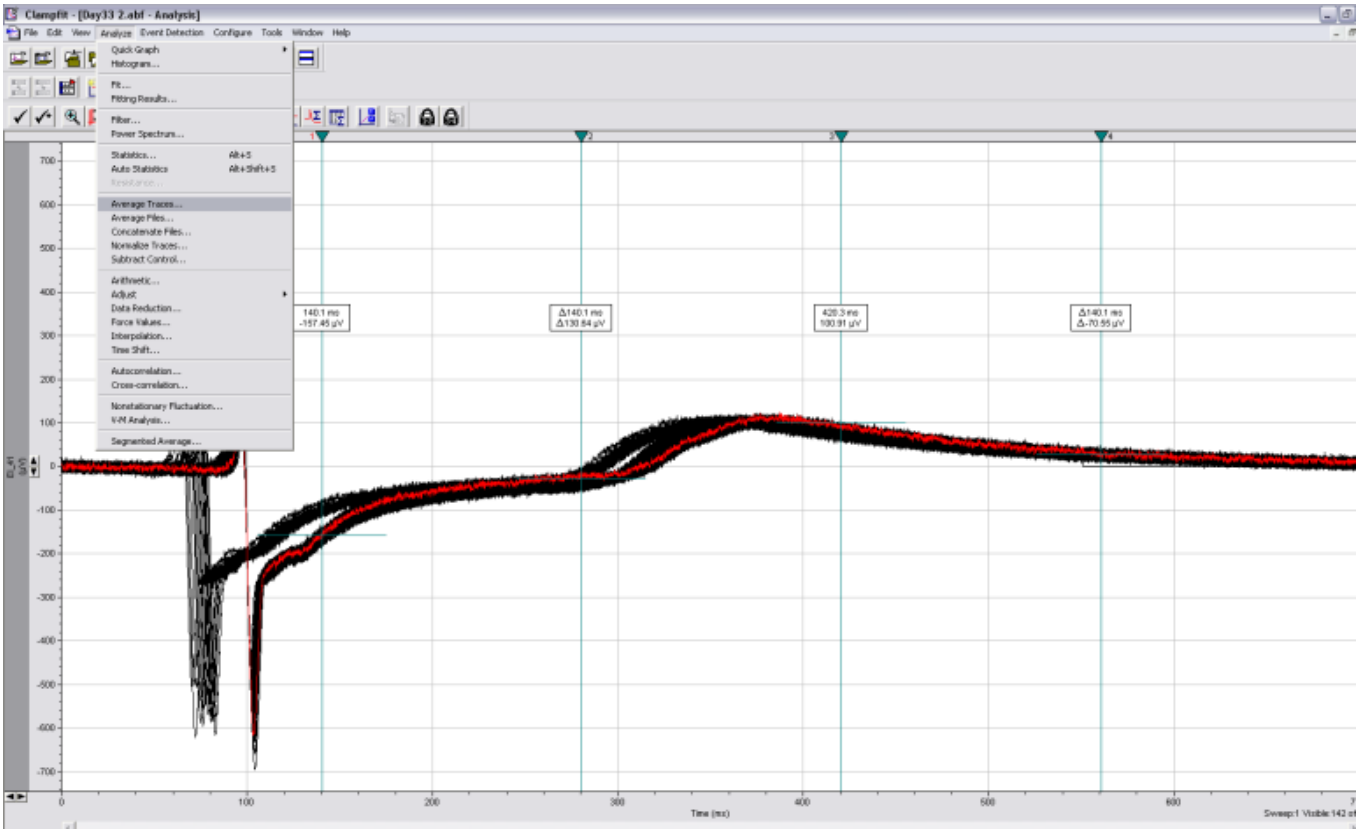


Figure 4.16: The ClampFit graphical user interface with the analyse tab selected.

- A.8.1: Analyse> Average traces
- A.8.2: Average Traces Button> click  > Following Dialogue box appears>click OK (Figure 4.18).
- A.8.3: View averaged trace> Move cursors at top of display to read off timing information and amplitude values (Figure 4.18).

- A.9: Note values by hand for insertion.

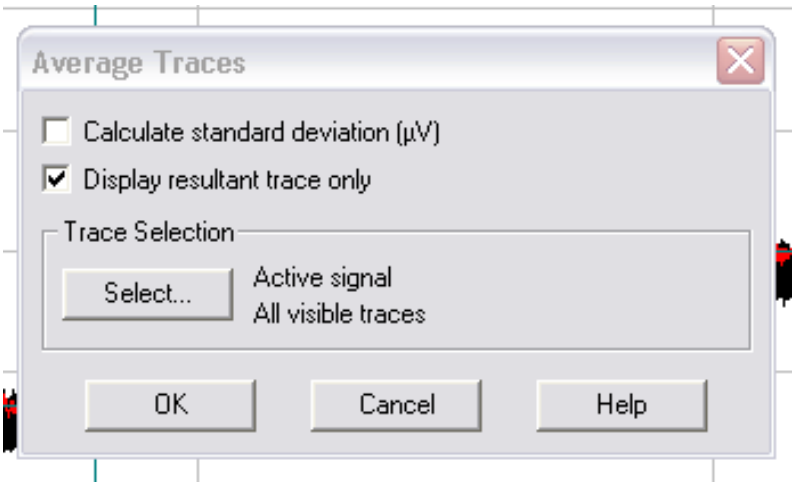


Figure 4.17: The average traces panel in the ClampFit software.

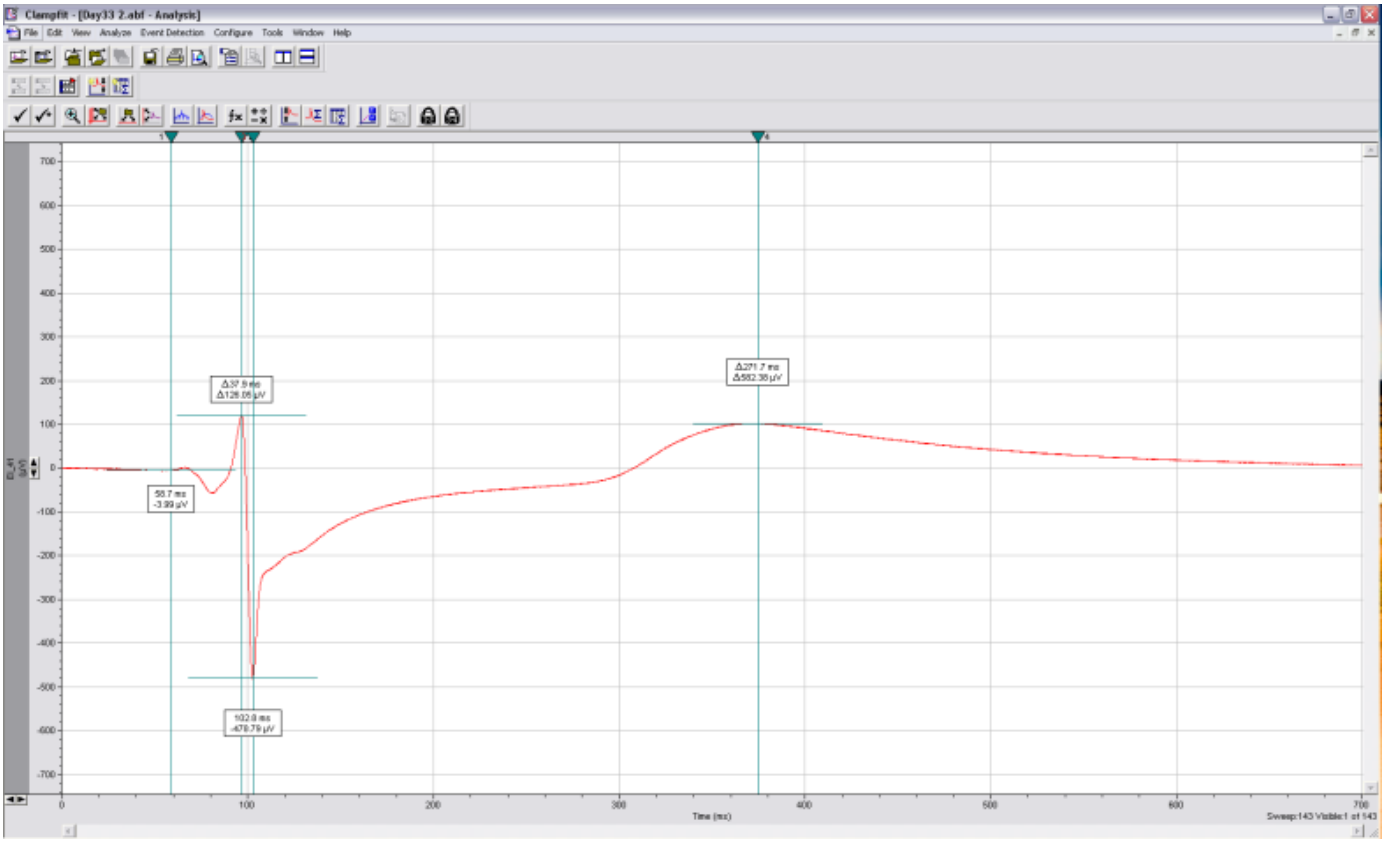


Figure 4.18: The averaged trace with click-to-drag cross-hairs that are accompanied by a panel displaying the time (ms) and amplitude (µV) of the selected data point.

Additional analysis facility: The facility to extract statistical information regarding the file is also available. This is not fully exploited by users in this specific application but the outputs of this feature are considered useful by the bioscientist user.

The process of accessing this statistical information is:

- Click Analyse>Statistics…>Select preferences from panel demonstrated in Figure 4.19.
- Click OK
- To view results minimize the display to see the results spreadsheet (Figure 4.20).
- Extract required data by hand and input into excel.

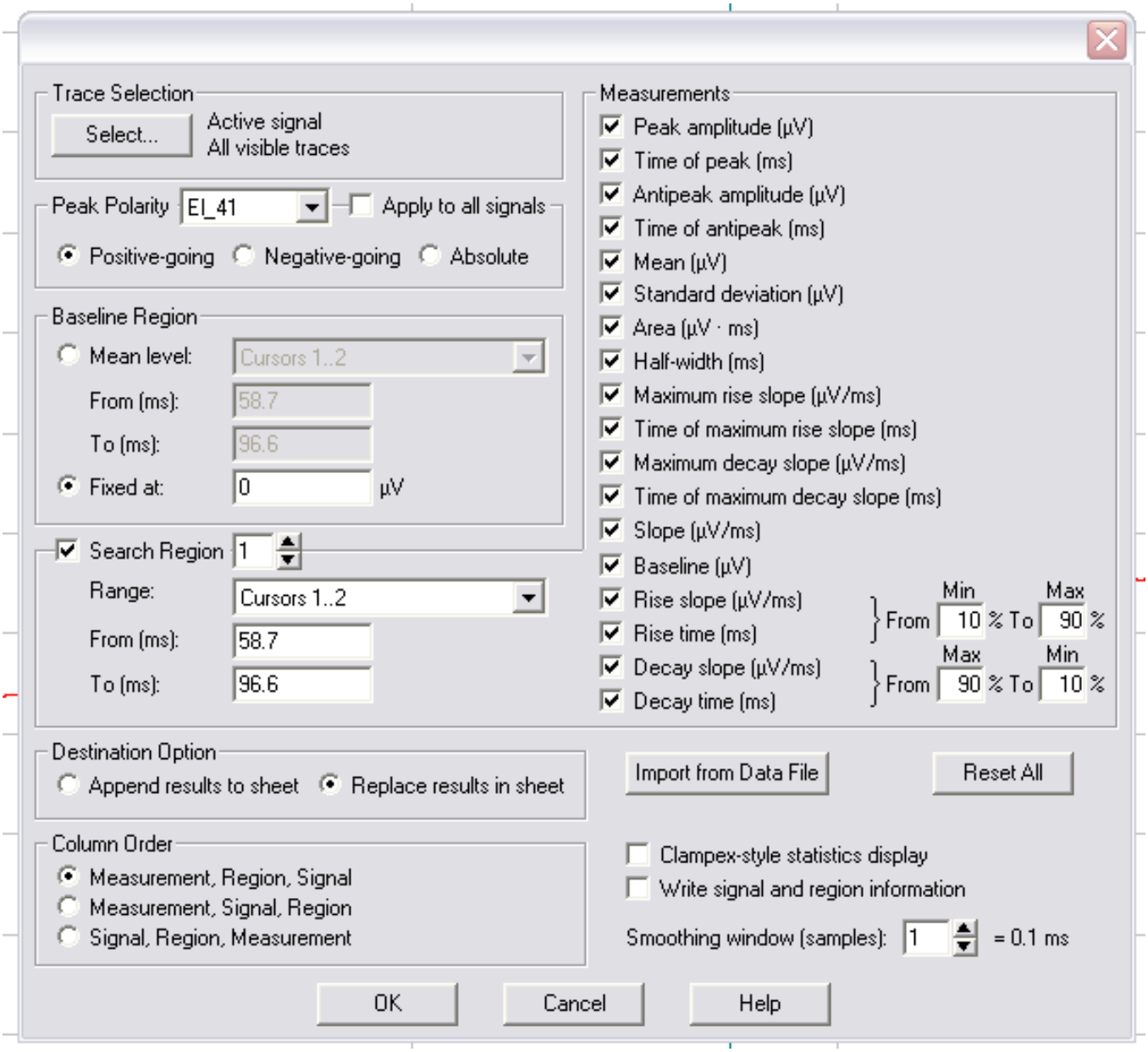


Figure 4.19: Statistical tools available in ClampFit.

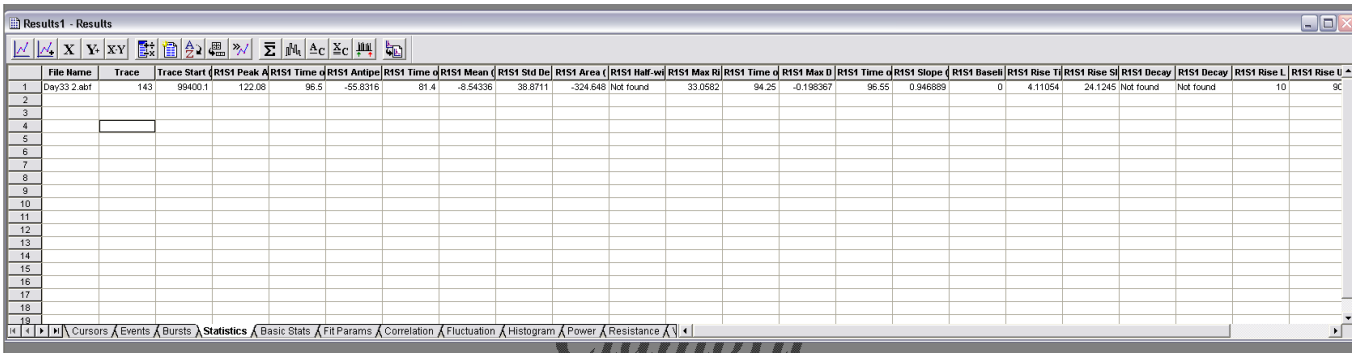


Figure 4.20: The resulting spreadsheet from the panel shown in figure 4.20.

Excel and Powerpoint

The remaining stages are Microsoft Office based (Figure 4.21). Values are collated, correlated and presented as combined images to compare the cellular response to different concentrations, different substances and different combinations of substances.

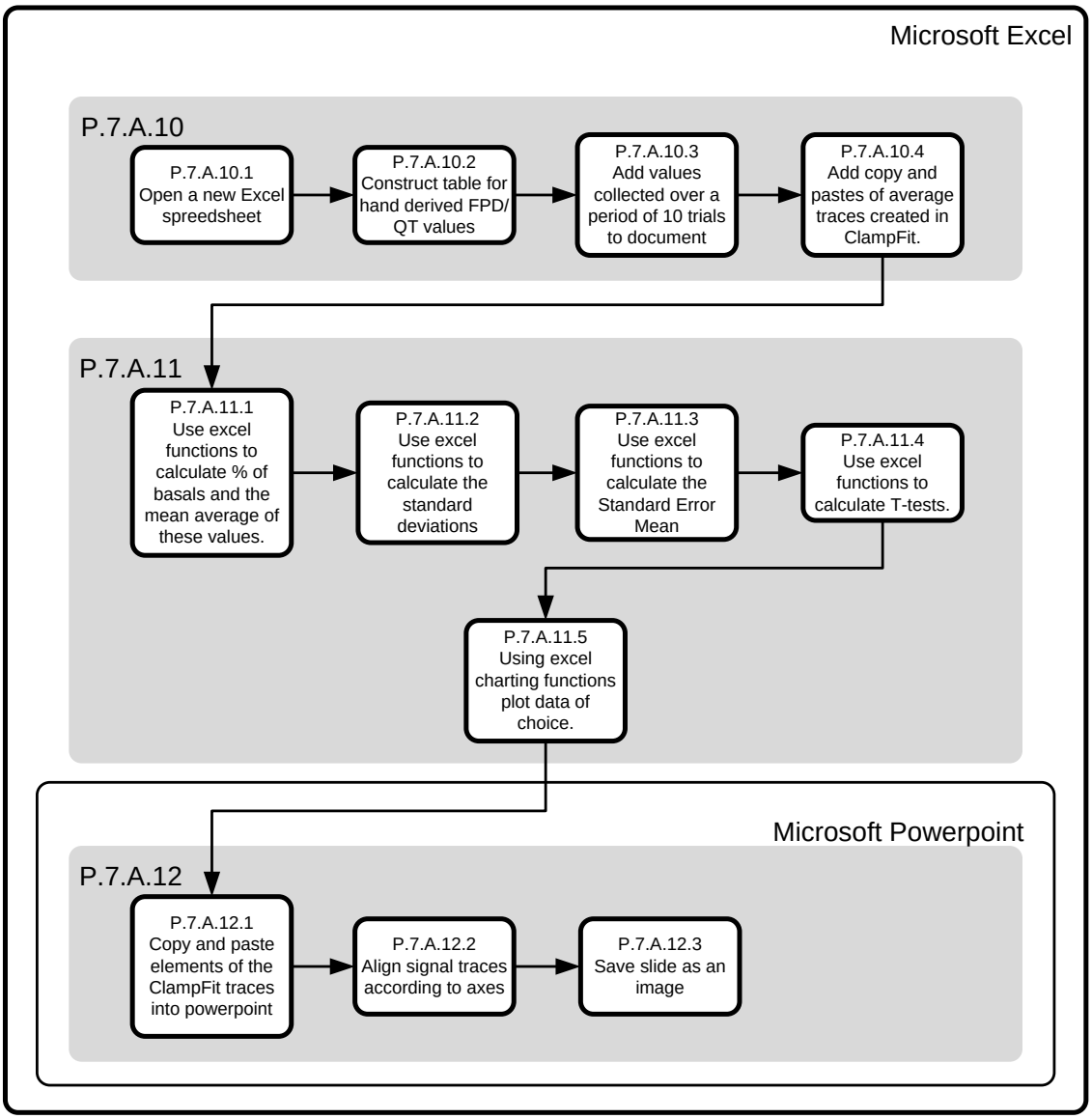


Figure 4.21: The low level activities completed to carry out activities of process seven.

Examples of the outputs from this stage can be seen in Appendix D.

User and system requirements identified from process seven drill down models:

1. Introduce automatic triggering or remove need for triggering.
2. Automatic QT-interval identification.
3. Facility to compare QT-interval values between different data files (data mining).

4.2.2.2 UoN Requirements summary

Through carrying out this case study the following requirements have been identified as required by this specific application. The identified needs that are addressed by this research (compliment PDS 3) are marked in bold. Other needs identified are to be addressed by further work.

1. To ensure as many beating clusters as possible make it from differentiation to the end of testing through facilitating appropriate handling and cell culture conditions.
2. **To increase the number of beating clusters per media well thereby improving the volume of output per test and to improve efficiencies of the MEA biochip preparation process.**
3. **To ensure outputs are of comparable or better quality than current.**
4. **To ensure tests can be conducted with comparable or better ease and speed.**
5. Removal of the need to sterilise and treat attachment surfaces would significantly reduce preparation time. (Comment: This could be addressed through further consideration of production using low cost, disposable materials supporting production of MEA biochips that can be sold in a pre-treated, sterile package and thrown away after use. The facility to print suitably biocompatible, conductive material onto transparent polystyrene substrates will facilitate this in the future. Application of dehydrated artificial extracellular matrix materials could also be considered in line with current experimental applications of comparable substances across this research domain if required.)
6. **A biochip that ensures attachment over electrodes every time is required.**
7. Every stage of each test is carried out manually by the user. Automation of as many processes or of parts of processes (i.e. perfusion of culture media and investigated substances during testing or automated software configuration) would increase convenience to users and reduce the likelihood of human error.
8. Automated extraction of basic parameters including as beat per minute, amplitude values and durations of and between waveform features (i.e. duration between beats, duration of QT-interval).
9. **System which automatically facilitates more beating samples per test.**
10. Software that has a greater level of automation which is intuitive and less complex.
11. Introduce automatic triggering or remove need for triggering.
12. Automatic QT-interval identification.
13. Facility to compare QT-interval values between different data files (data mining).

4.3 Case Study Two: Neural Cell Culture Application

Neurologists have spent centuries investigating the workings of the human brain (Finger, 1994). Several different types of neural cells have been identified (Hao and Young, 2009) within complex structures throughout the brain (Halloway, 1967). Neurons communicate by the propagation of action potentials (APs) (see section 2.4.1). Neural APs propagate from cell to cell across synapses, which are physical gaps (20-40nm) between the axon terminal of one cell and the dendrites of another (see section 2.2). Minute volumes of chemical substances, collectively known as neurotransmitters, are released across a synapse resulting in a shift in ionic charge all the way along the membrane of the neuron. This change in ionic charge is the AP, which is recorded by MEA systems in the form of field potentials. Observing spikes and bursts of APs (see section 2.4.2) using MEA systems is contributing to furthering understanding of the brain.

4.3.1 Case Study Context

MEA systems are used by neuroscientists at the Sanger Institute to study synaptic function via the monitoring of spatial and temporal aspects of the electrical activity in neural tissue simultaneously (Chiappalone et al, 2008; Teemu et al, 2008).

The networks studied in this case study are grown over the MEA biochip microelectrodes from somas (cell bodies, see section 2.2) of neurons that have been dissociated (chemically separated into single cells) from dissected samples of brain tissue. While in culture new axons and synapses grow out from the somas forming new neural networks. Neural cells dissociated from samples of brain tissue (typically murine sourced) have been used in research investigating network development (Chippalone et al, 2006), learning and plasticity (Broccard et al, 2009), memory (Marom and Shahaf, 2002) and degenerative diseases, such as Alziemer's disease or dementia (Gortz et al, 2004).

Chippalone et al (2006) cultured networks from dissociated neurons over MEA biochip workspaces. Spontaneous activity was monitored during development. Results of correlations between recordings taken at regular intervals demonstrated the development of rich patterns of activity over the electrode array. These patterns changed as the network matured.

Broccard et al (2009) used cultures of dissociated neurons to investigate learning and plasticity.

Marom and Shahaf (2002) investigated the formation of neural activity groups that was defined as learning and the conservation of those activity groups as memory using dissociated neurons grown over an MEA.

Gortz et al (2004) investigated neuropharmacological effects of homocysteine (a substance connected to the occurrence of dementia and Alzheimer's) on neuronal networks developed from dissociated neurons.

4.3.2 Modelling of the Chronic Neural Cell Culture Application

Specifically bred mice are used to source genetically specific brain tissue in the application presented in this case study. Spontaneous network activity is recorded that is no way induced by any form of chemical or electrical stimuli. All of the activity observed is associated with the natural development of the neural network *in vitro*. The MEA system allows the neuroscientist to visualise and quantify the growth and formation of these functional networks over time.

Neurons are dissociated from a genetically mutated mouse brain and seeded over the electrode array. They are then and left in incubation for ~6 days before recording. Spiking activity (the presence of APs) can be observed in a newly developing neural network after about 3 days in vitro (DIV). The neuroscientists in this case study have observed the development of hundreds of these unique neural networks over several years to create a bank of data files. Comparison of hundreds of data files is carried out using statistical analysis written as R scripts. Particular parameters are calculated and correlated automatically by the scripts, for example, spiking frequency and the duration of bursts.

4.3.2.1 Overview

The EMs in this case study document the bioscientific protocols employed by the Genes to Cognition research laboratory at the Wellcome Trust's Sanger Institute (WTSI) when studying dissociated neurons in culture. An overview of the application (Figure 4.22) was constructed to separate the workflow used into distinct processes that encompassed all of the scientific protocols used from the start of an MEA system-based experiment to its completion.

The overall workflow used to in the MEA system application of this case study is:

- Process One (P.1.) – Breed genetically mutated mice
- Process Two (P.2.) – Kill mice, dissect brain region of interest, dissociated desired cells
- Process Three (P.3.) – Prepare MEAs
- Process Four (P.4.) – Seed and culture cells
- Process Five (P.5.) – Set-up and run trials
- Process Six (P.6.) – Condition data and analyse
- Process Seven (P.7.) – Derive meaning
- Process Eight (P.8.) – Share findings

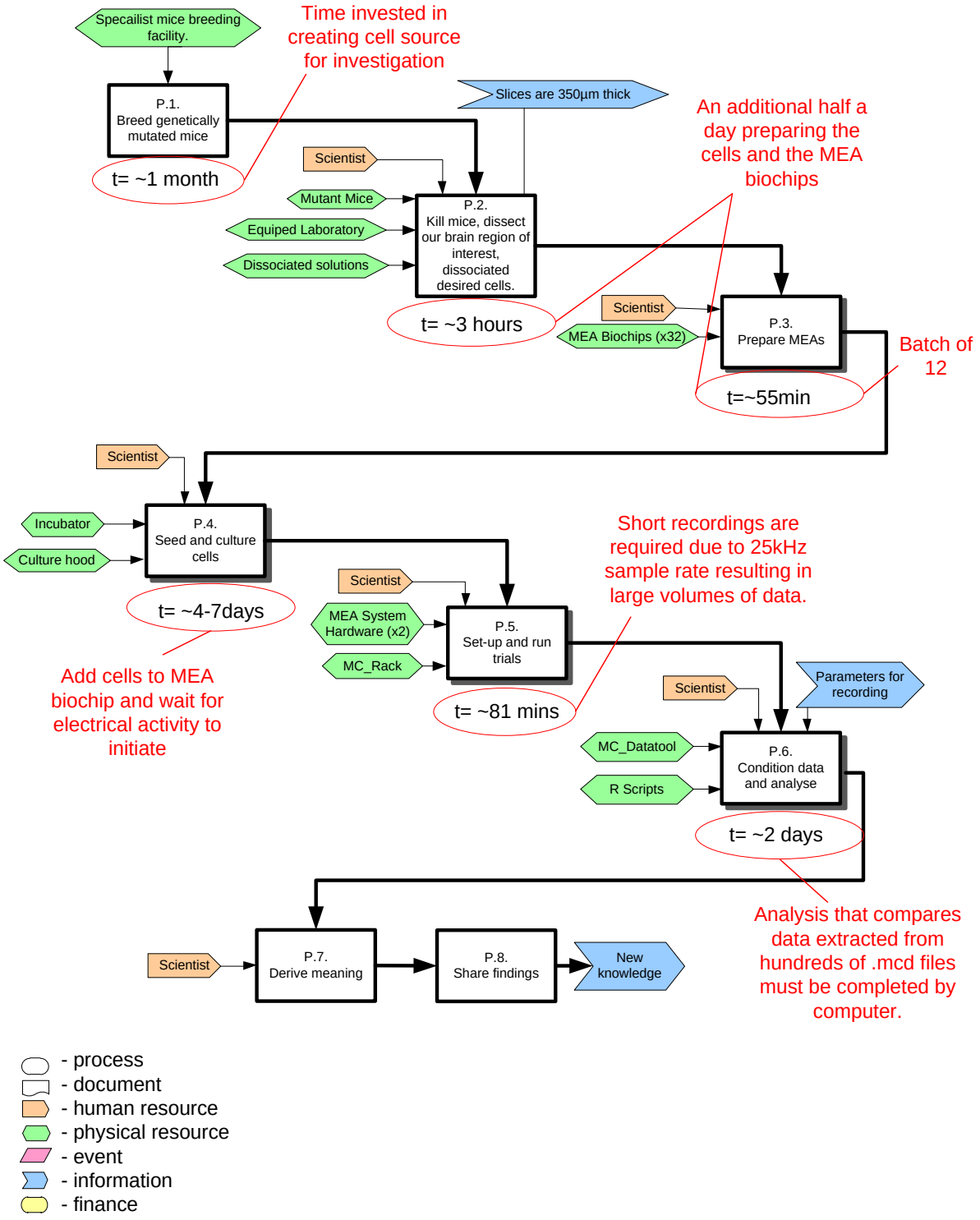


Figure 4.22: An activity diagram of the overall workflow carried out by the chronic neural cell culture users at the Sanger Institute.

The processes of interest to this research were those where the MEA system or its components are interacted with. These processes are identified in modelling using shadows: P.3, P.4, P.5, and P.6. The shadowing indicates that further modelling has taken place in order to extract all details relevant to understanding the system in use, and to support user and system need identification.

User and system requirements identified from construction of Figure 4.22:

1. To guarantee recordings are obtained from cells due to the high cost of the source.
2. To improve surface properties of MEA biochip to reduce or eliminate the need for special cell type specific workspace surface treatments.
3. To provide a system that allows longer or continuous recording.
4. To provide a system that correlates new data with previously recorded data automatically and quickly.

4.3.2.1.1 Chronic Culture (Neurons) Sanger Institute Users Processes

Process Three – Prepare MEAs

In carrying out process three in this instance, the MEA biochips are removed from storage and treated in a plasma cleaner. The workspace is treated for improved cellular attachment by applying a small amount of Poly-D-lysine using a suspended solution. After a two minute settling period the contents of the well are aspirated out and reverse osmosis water is used as a rinse. The Neurobasal medium is then added to the well and a small amount (4µl) of Laminin is dropped exactly over the workspace. A specially made zero-evaporation lid is placed over the well. All of the biochips prepared in that batch are then placed into a Petri dish and inside an incubator at 32°C with 5%CO_2 and left for >45minutes (Figure 4.23).

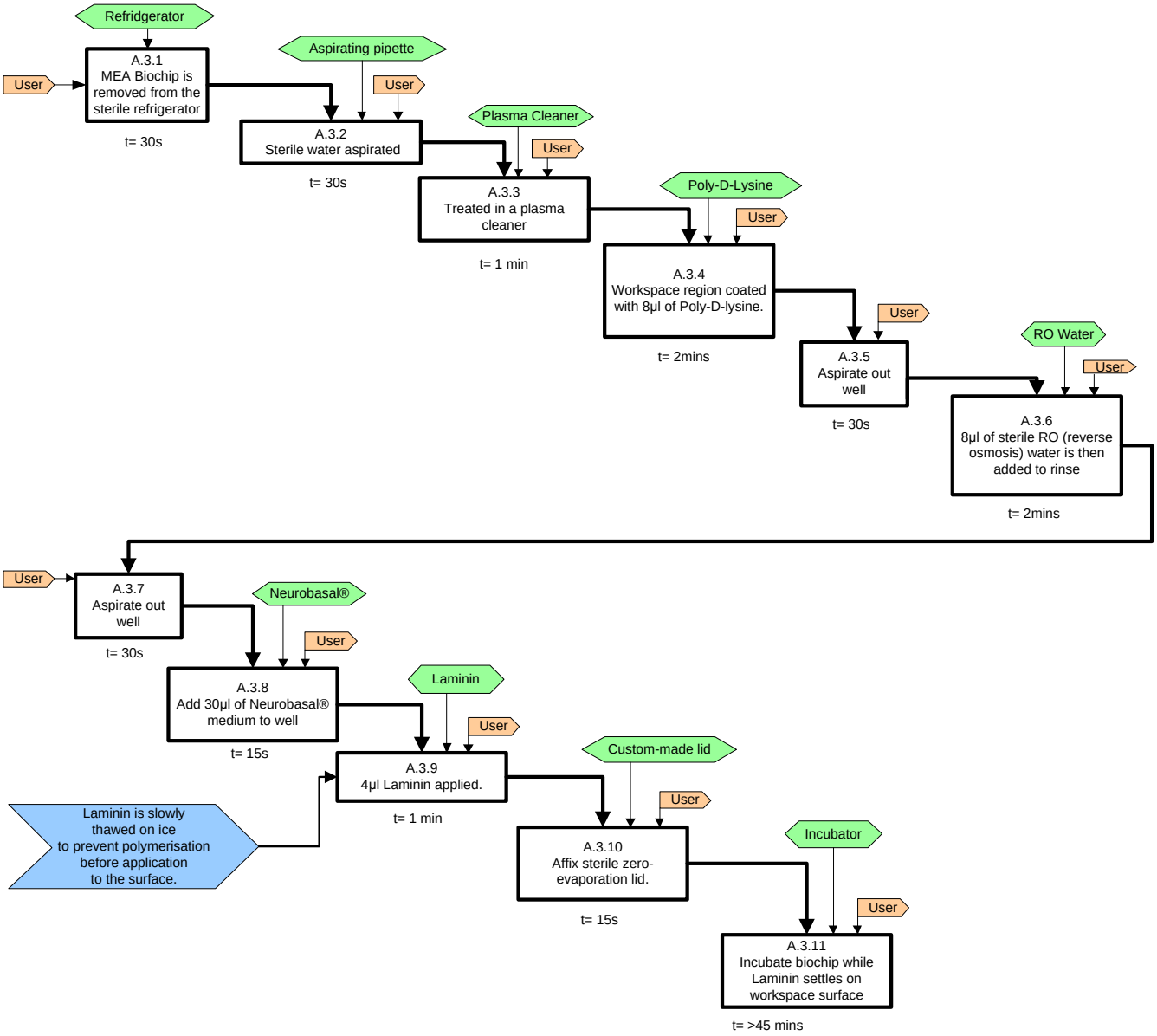


Figure 4.23: An activity model depicting process three, Prepare MEAs.

User and system requirements identified:

1. Remove the need to apply specific treatment the MEA surface.

Process Four – Seed and culture cells

Process four involves dispensing 21µl of the previously prepared neural cell suspension over the MEA workspace (Figure 4.24). The user must set-up the required items in a culture hood, refresh the culture media and dispense the cells into the centre of the media well. A lid is placed over the MEA to prevent media evaporation and contamination. Labels are added and the biochip use is logged manually by-hand in a notebook. Cells are visualised under an inverted light microscope to check for adequate dispersion over the workspace area. Seeded MEA biochips are carefully moved into incubation where they are left for >3 days.

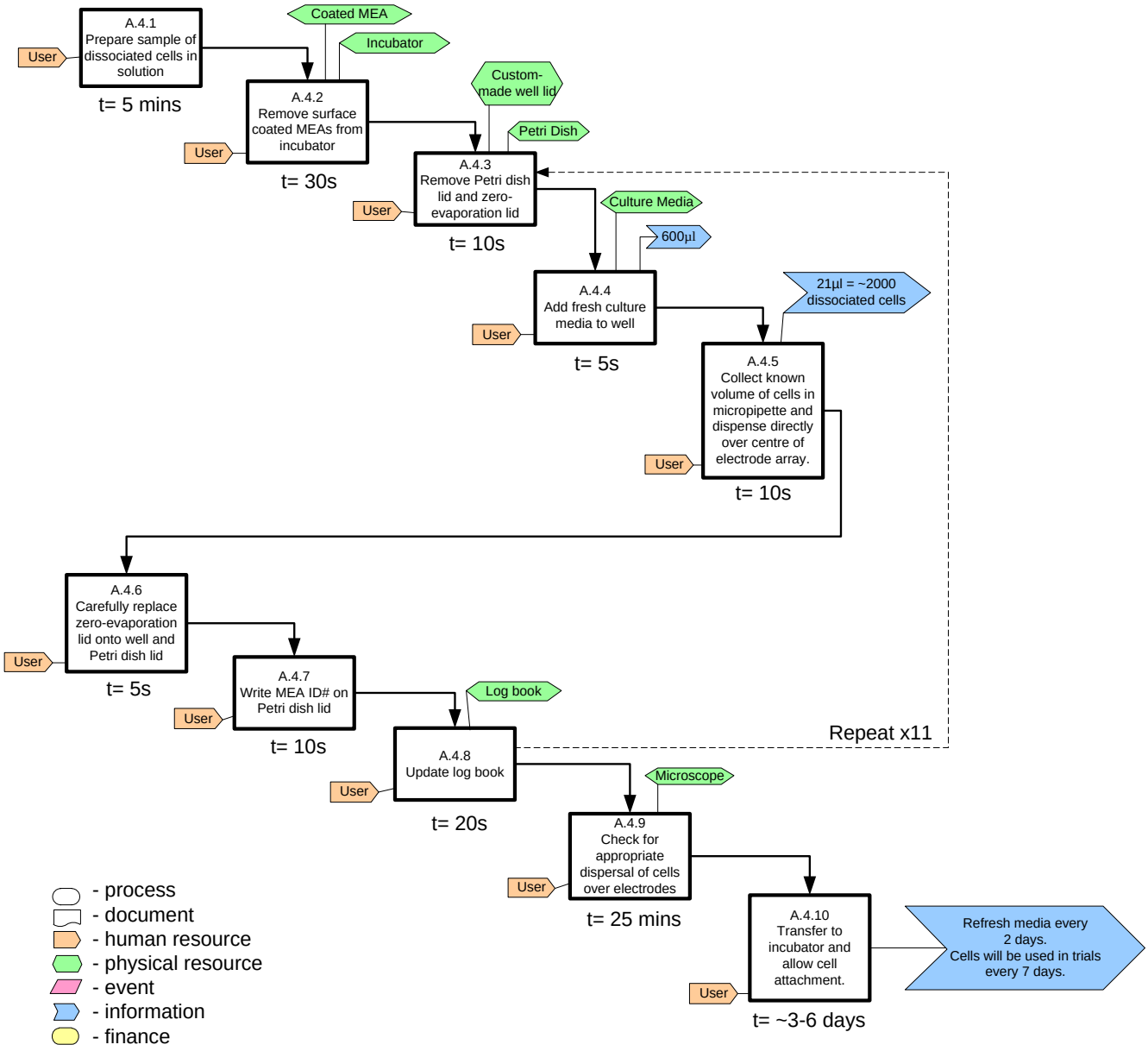


Figure 4.24: An activity model of process four, seed and culture cells.

User and system requirements:

1. To provide an automatic or electronic based method of recording MEA biochip use. Possibly creating facility the to insert into data file metadata for future reference.

Process Five – Set-up and run trial

Process five is the stage at which the full MEA system is employed to record the field potential data that is of value to the user application (Figure 4.25).

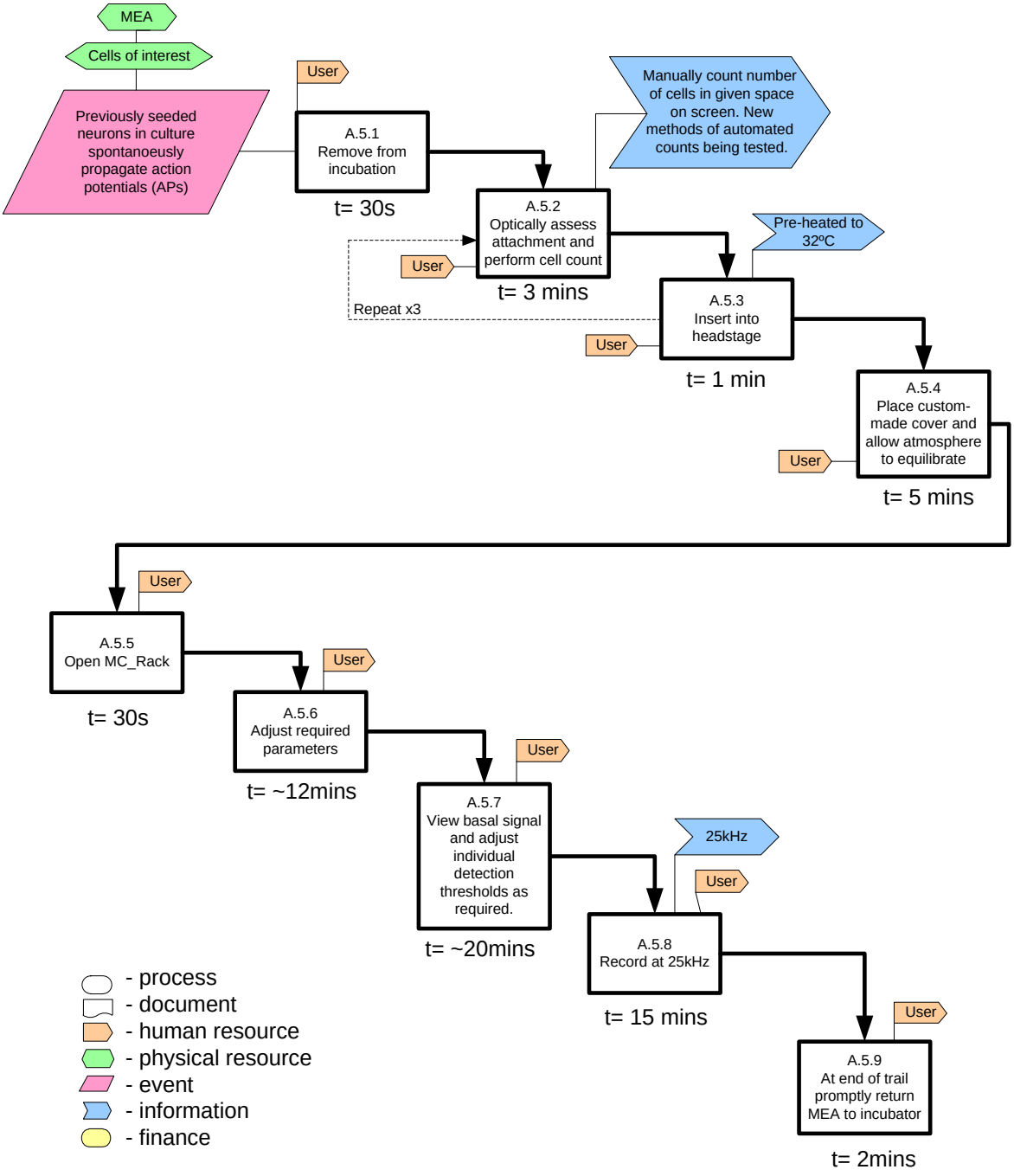


Figure 4.25: an activity model of process five, set-up and run trials.

To record the cellular signalling that is analysed in this application the user removes the MEA biochip from incubation, visually assesses the cells under a microscope to ensure health and attachment to the workspace area, and places it into a pre-heated MEA system headstage (see section 2.9.1). The configuration of system used in this instance is the MEA 60 two-fold system. Two instances of this configuration are used at the same time by the user observed for this work, Dr Paul Charlesworth, so up to four MEA biochips are recorded from simultaneously.

When all four headstages are set-up the user opens two instances of a previously configured and saved recording rack in the MC_Rack software. The user checks all 60 channels of each MEA to see where spiking activity is occurring using the graphical user interface (GUI). For every channel where bursts or spikes (see Figure 2.39) are present the threshold bar for that channel is adjusted by-hand to ensure the spike amplitude(s) exceeds the threshold required for detection. A recording of 15 minutes is taken. The MEA biochips are then returned to the incubator where they will remain in culture until the next testing day.

User and system requirements:

1. A system where the MEA biochips do not need to be removed from the incubator.
2. A system that can record continuously.

Process Six - Condition data and analyse

This process (Figure 4.26) involves the conversion of the data files generated, multi channels data (.mcd), into multi channels time (.mct) files using NeuroExplorer software (Nex Technologies, Massachusetts, USA). The .mct file then contains only timing information pertaining to the spikes recorded in the original .mcd file. Noise and periods of inactivity are discarded, thereby reducing the file size. These .mct files are linked to the statistical scripting software, “R”, by inserting the file name into a condition table that exists in Microsoft Excel. Once the condition tables are complete a full automatic analysis is run using a custom written “R script”. The outputs generated by these scripts are compiled directly into a report format that contains the data tables and plots of correlations generated by the R script. These reports are created as portable document format (.pdf) files. The content of the reports is then manually manipulated for presentation by entering useful sections into the data analysis and graphing software OriginPro (OriginLab Corporation, Massachusetts, USA).

User and system requirements:

1. Remove the need to convert file types by facilitating The facility to produce statistical outputs through the recording software
2. Statistical outputs in real-time.
3. The facility to automatically produce plots.

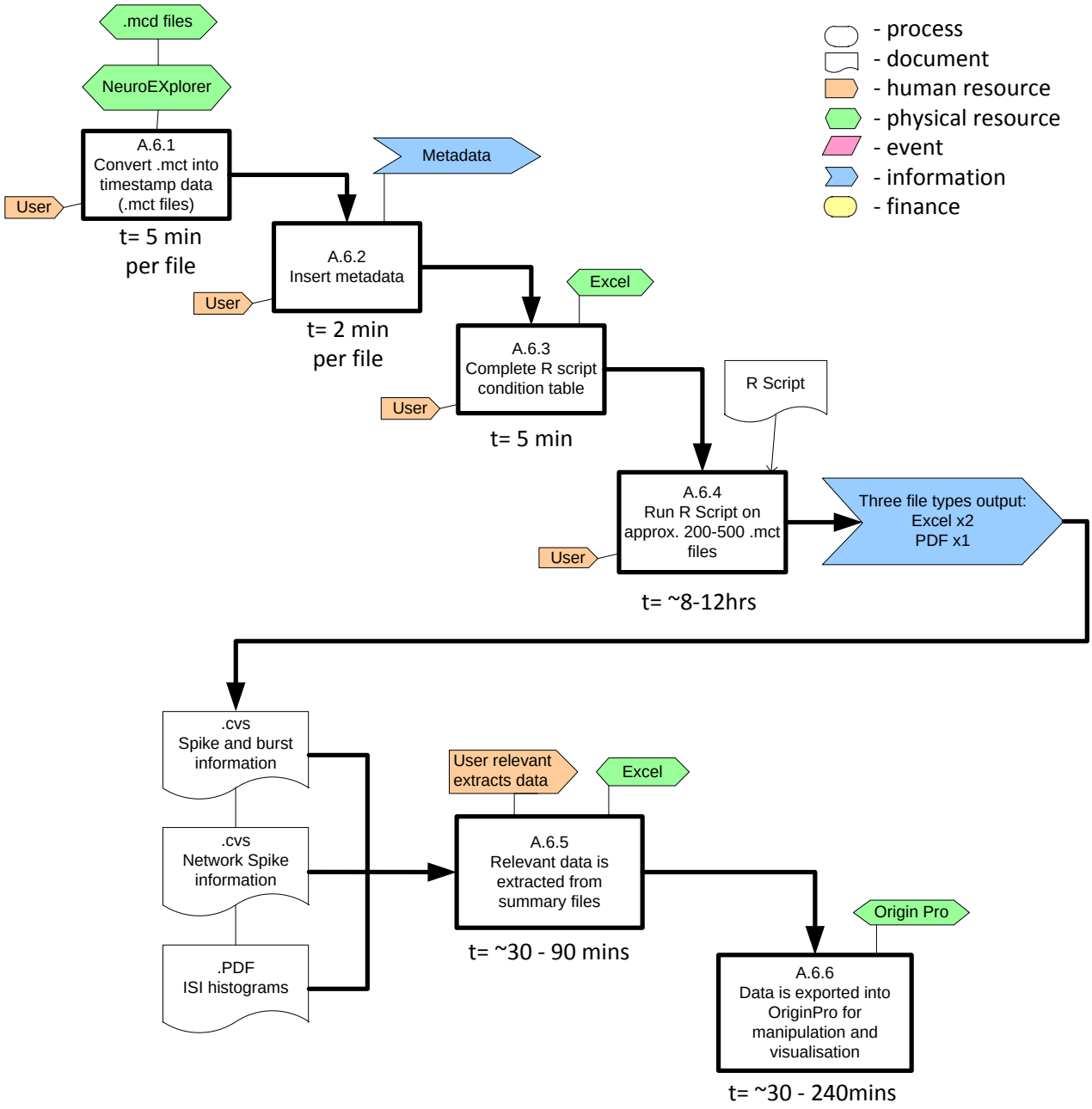


Figure 4.26: An actvity model of process 6, condition data and analyse.

4.3.2.1.2 MEA biochip cleaning protocol

Applications presented in case study two (chronic neural network culture) and in case study three (acute brain slice) clean the biochips following use in the same manner. The process used to clean MEA biochips after use, in preparation for storage before re-use, is shown in Figure 4.27.

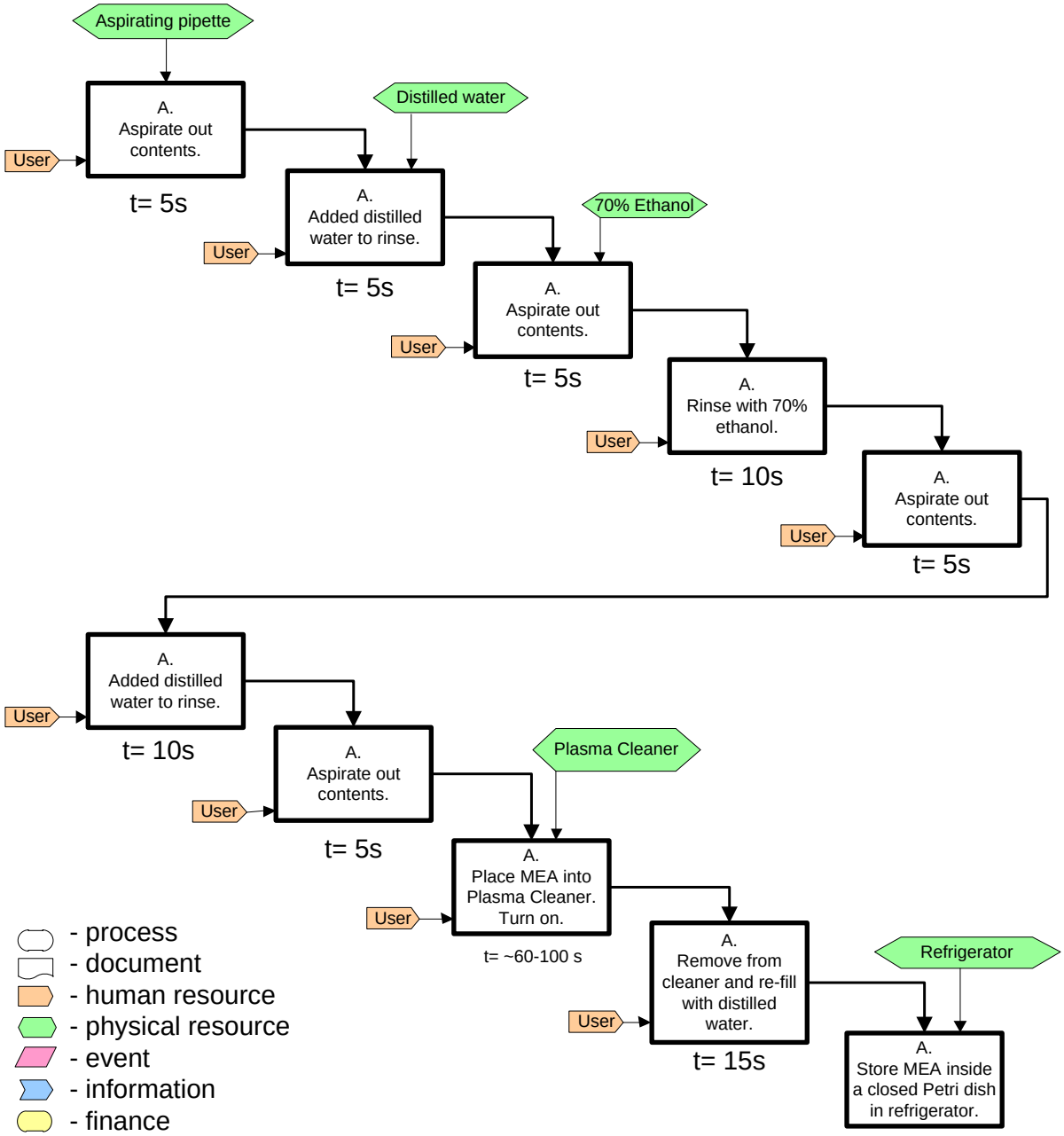


Figure 4.27: The cleaning protocol employed by the Sanger Institute applications presented in case study two and three.

User and system requirements identified:

1. Remove need to clean MEA biochips or make process automated.

4.3.2.2 Chronic Culture (Neurons) Requirements summary

Through carrying out this case study the following requirements have been identified that are specific to this application.

1. To guarantee recordings are obtained from cells due to the high cost of the source.
2. To improve surface properties of MEA biochip to reduce or eliminate the need for special cell type specific workspace surface treatments.
3. To provide a system that allows longer or continuous recording.
4. To provide a system that correlates new data with previously recorded data automatically and quickly.
5. Remove the need to apply specific treatment to the MEA surface.
6. To provide an automatic or electronic based method of recording MEA biochip use. Possibly creating facility to insert into data file metadata for future reference.
7. A system where the MEA biochips do not need to be removed from the incubator.
8. A system that can record continuously.
9. Remove the need to convert file types by facilitating The facility to produce statistical outputs through the recording software
10. Statistical outputs in real-time.
11. The facility to automatically produce plots.
12. Remove need to clean MEA biochips or make process automated.

The requirements that have been identified through this case study are to be addressed by further work.

4.4 Case Study Three: Brain Slice Application

This application differs from the applications presented in case studies 1 and 2 in the following ways:

- it investigates responses of brain slices to electrical stimuli delivered via a microelectrode.
- cell sources are not cultured inside an incubator. Slices are used for recording on the same day that they are dissected; they are then disposed of at the end of testing.
- the MEA biochip used with the MEA-60 system the 3D 8x8 grid array, whereas for case study 1 and 2 the planar 8 x 8 array is used.

The Multi Channels MEA-system requires a specialist system called a Stimulus_II that injects electrical stimuli into slices though user designated microelectrodes.

For this application the positioning of the brain slice over the electrode array is imperative as stimulus pulses must be delivered to precise anatomical locations in the brain slice. Controlled positioning of stimulus delivery is required to evoke the particular network pathway under investigation.

4.4.1 Case Study Context

The application observed for this case study electrically stimulates known signal pathways that are present in the hippocampus. The response of neurons at particular locations is recorded and analysed. Slices tested are genetically different. The user in this application is investigating the ability of the slices to learn responses to the same stimulation protocol, and then is correlating differences that are observed between the different genetic combinations assessed.

4.4.2 Modelling of the Acute Brain Slice Application

Modelling the processes employed in this application has resulted in the identification of application specific user requirements.

4.4.2.1 Overview

An overview of the workflow used in this application is documented in Figure 4.28.

The overall workflow used to in the MEA system application of this case study is:

- Process One (P.1.) – Breed genetically mutated mice
- Process Two (P.2.) – Kill mice, dissect out brain region of interest, prepare slices

- Process Three (P.3.) – Prepare MEAs
- Process Four (P.4.) – Position slices, set-up software and run trails
- Process Five (P.5.) – Convert data and analyse
- Process Six (P.6.) – Derive meaning
- Process Seven (P.7.) – Share findings

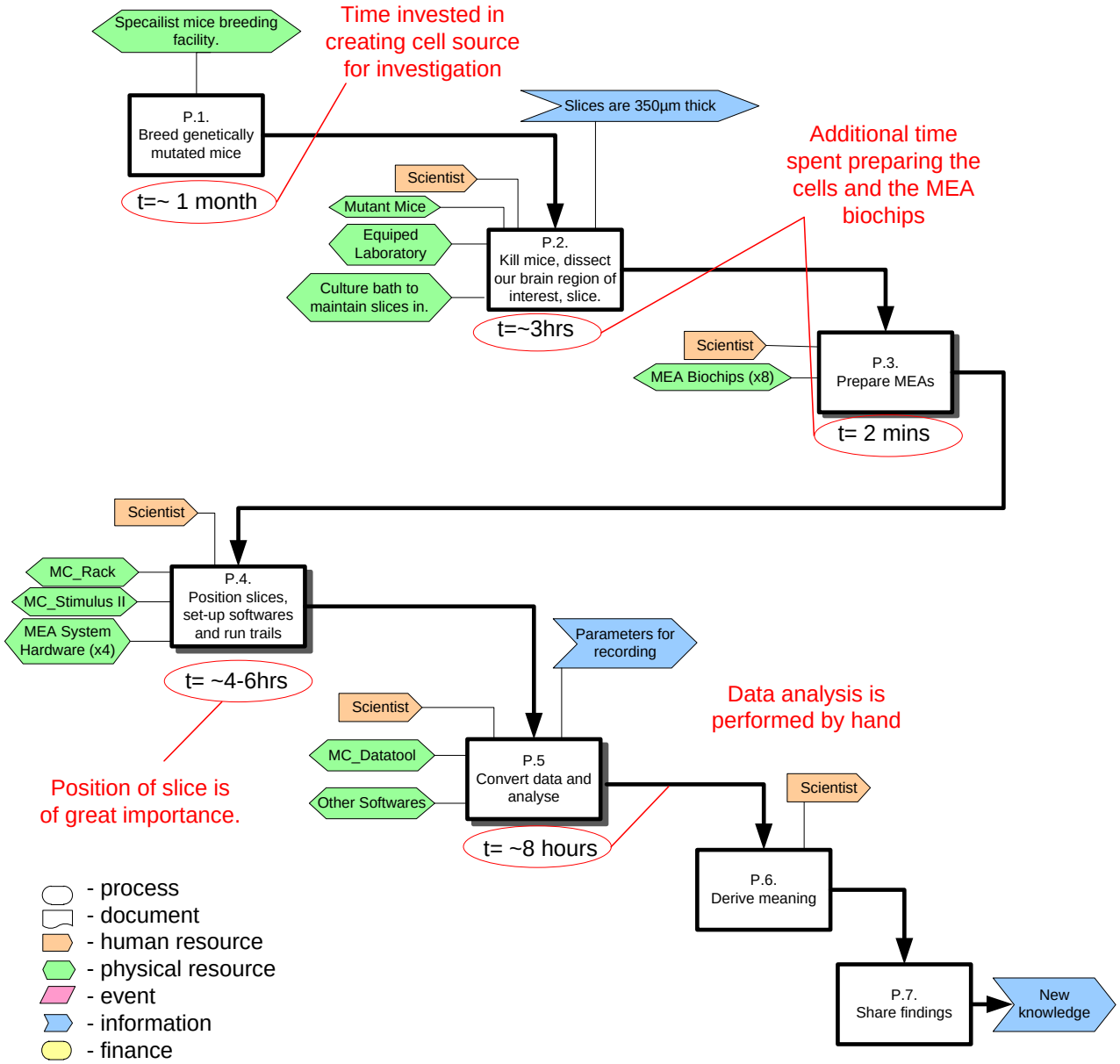


Figure 4.28: An activity model of the overall workflow implemented by the acute brain slice Sanger Institute users.

Genetically appropriate mice are bred and the brain region of interest, in this case the hippocampus, is dissected out. The hippocampus is cut into slices of a thickness ~300-350µm and stored in a special brain slice chamber (Warner Instruments, Connecticut, USA). Slices are placed into MEA biochips and stimulated. Recordings are taken of the stimuli delivery and the consequential responses that are analysed offline to examine the plasticity exhibited by that particular genetic combination.

User and system requirements identified:

1. Biochips that make hippocampal slice positioning easier and quicker.
2. Automated data analysis.

4.4.2.1.1 Acute Brain Slice Sanger Institute Users Processes

Process Three – Prepare MEAs

The activities carried out to complete process three are shown in Figure 4.29.

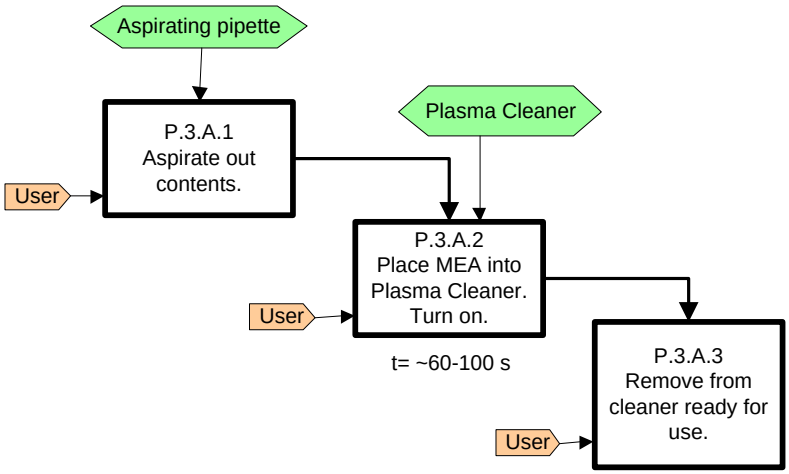


Figure 4.29: An activity diagram of MEA biochip preparation for brain slice application.

While in storage the MEA biochip media well is filled with distilled water. This water is removed prior to placing the MEA biochip into the plasma cleaner. After ~60-100s inside the plasma cleaner the biochip is ready for use in this particular application.

Users are very satisfied with this process and did not highlight any problems or suggest any improvement.

Process Four – Position slices, set-up software and run trial

Process four describes the steps executed from inserting the slice into the media well to recording the useful data (Figure 4.30).

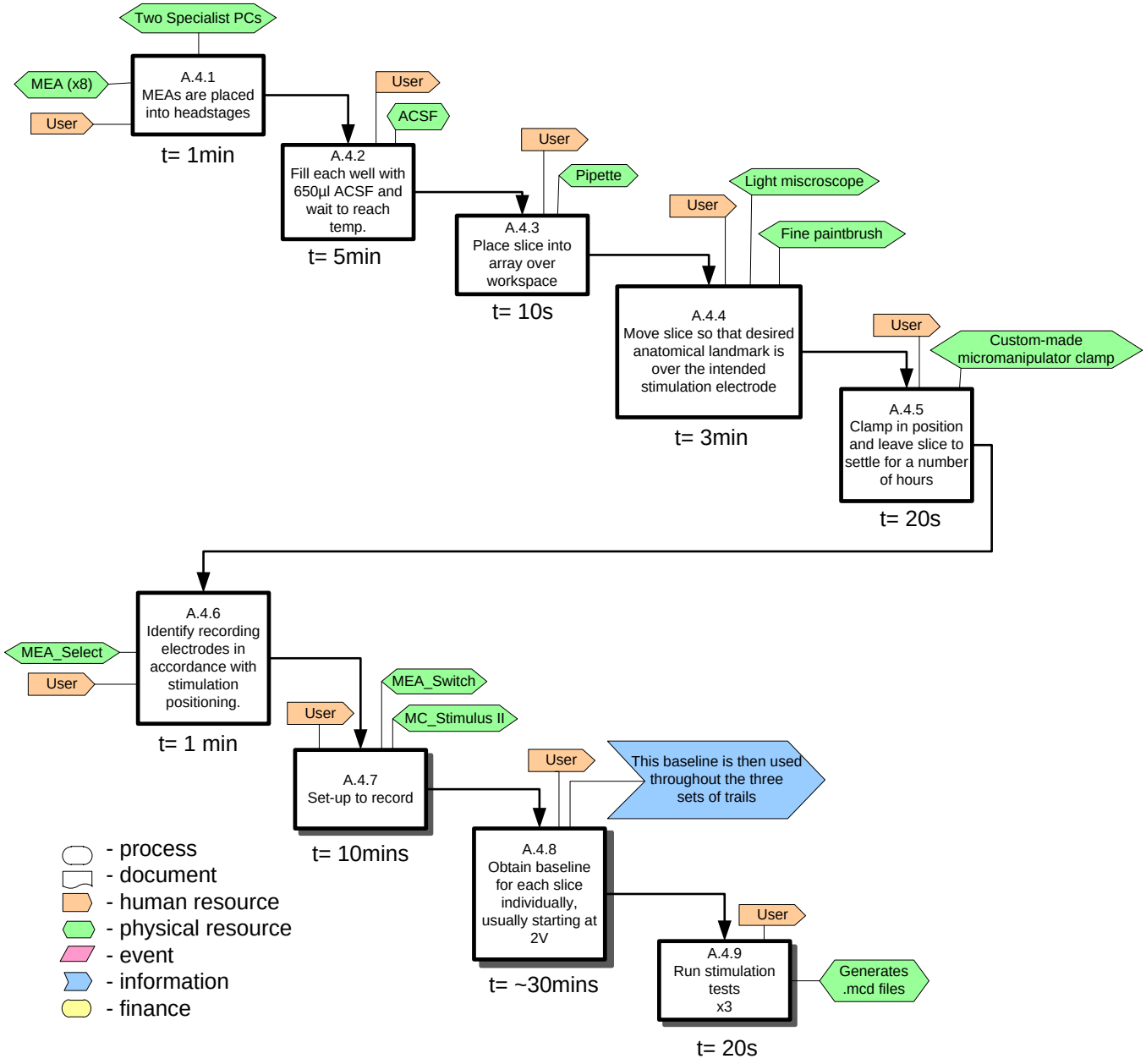


Figure 4.30: An activity model depicting activities carried out in order to complete process four.

MEA biochips are inserted into the headstage and filled with artificial cerebrospinal fluid (ACSF) prior to addition of the hippocampus slice. Slices are carefully positioned by eye through a light microscope and gently clamped in position using a home-made mesh. The intended recording electrodes are observed according to the anatomy of the slice and assigned in the software. Base line readings are taken of the response of the slice to a 2V pulse. The full stimulation protocol

(Table 4.1) is executed generating .mcd files containing the useful data, and when complete the slices are disposed of.

Table 4.1: The stimulation protocol delivered by this application user:

Stimulation Type	Frequency of delivery	Voltage
Biphasic stimulations (positive/negative, 100µs/a phase)	2 trains of 100 pulses at 100Hz	– 2V up to a maximum of 4.2V

The system configuration used in this application involves 8 MEA system headstages in two four-fold configurations. Two stimulus generators are used. The operator sets-up up all of the MEA biochips and the software, and the stimulation protocols are the delivered together using two separate computers.

User and system requirements identified:

1. MEA biochips that offer better slice positioning.
2. One system that can facilitate ≥8 MEA biochips at one time.
3. A stimulus generator that can deliver protocols to >4 MEA biochips at one time.

Process Five –Convert data and analyse

Process Five also explains analysis at the very highest level (Figure 4.31).

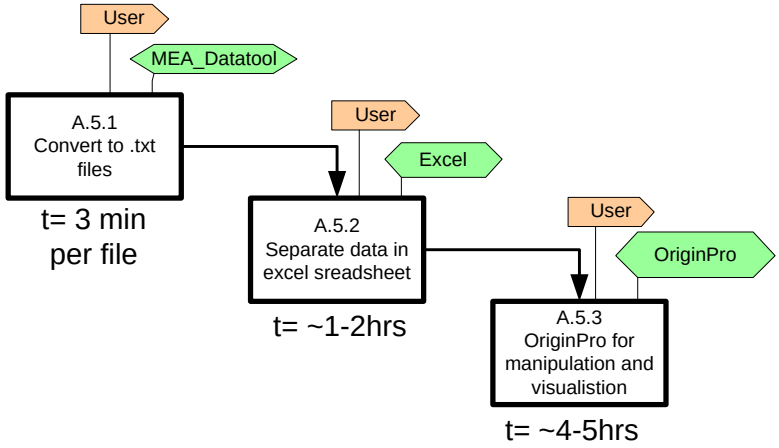


Figure 4.31: An activity model of process five.

One .mcd data file is generated per stimulus generator. Therefore two files are recorded per system per stimulus protocol repetition. So in this application 8 .mcd files (2 baseline, 6 stimulated) contain

the data used to investigate the response of the hippocampus under investigation. Each file is converted into a text (.txt) file using the MEA_Datatool software. The text file is imported into Microsoft Excel and relevant data extracted by-hand and inserted into OriginPro for further manipulation and presentation.

User and system requirements identified:

1. Analysis features in the recording software.
2. Analysis in real-time.

Further drill down modelling

Additional activity models have been generated for this case study to incorporate relevant information that provided better understanding. Additional needs collated by addressing/including deeper details are noted.

Process Four

The protocol undergone for checking the set-up and configuring the recording software is documented in Figure 4.32.

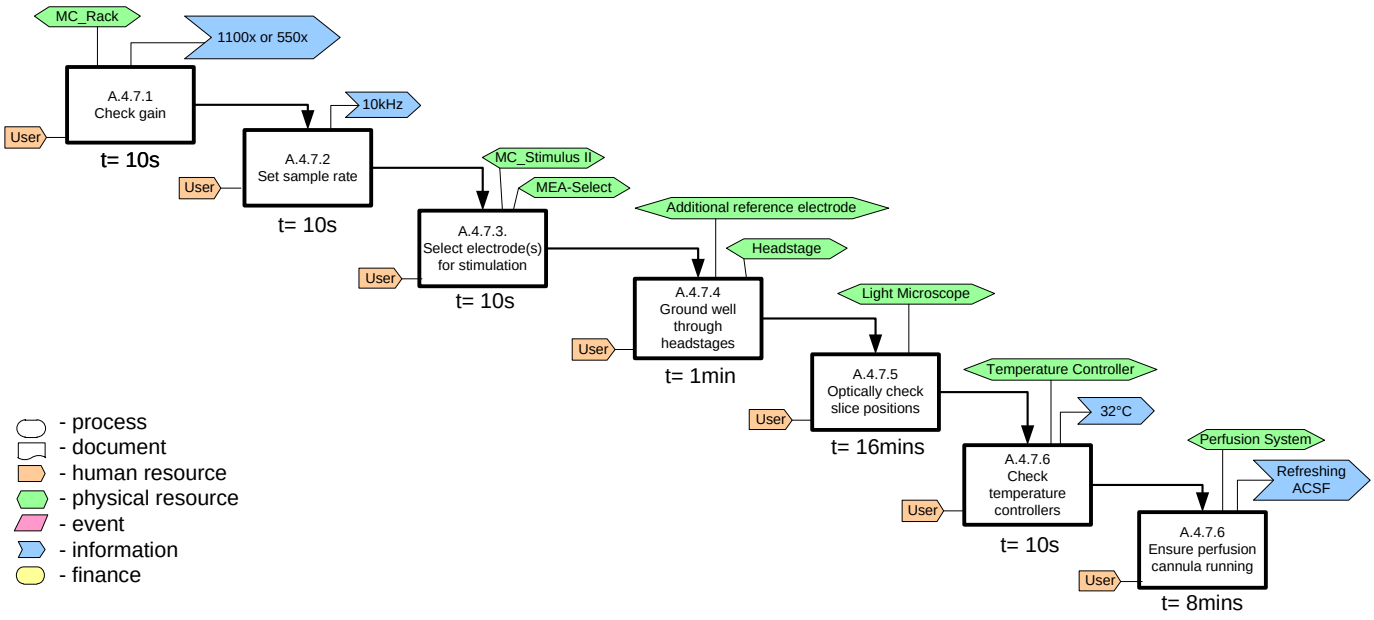


Figure 4.32: A model of the activities carried out in order to complete process four, activity seven, check set-up and configure recording software.

User and system requirements identified:

1. Predefined stimulating electrodes facilitated by anatomically precise microelectrode positioning.
2. Automated slice position checking.
3. Integrated perfusion system.

The protocol undergone for obtaining at baseline recording from each slice is documented in Figure 4.33.

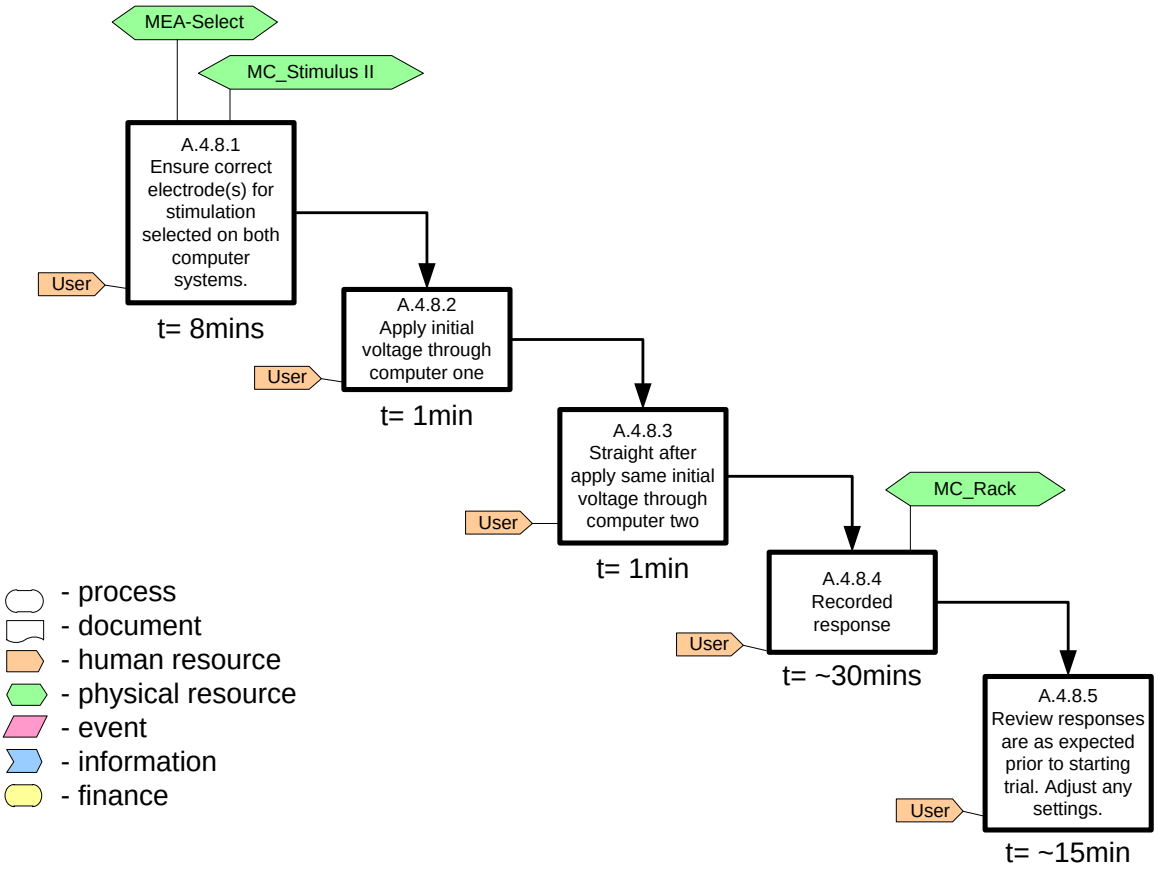


Figure 4.33: A model of the activities carried out in order to complete process four, activity eight, obtain baseline for each slice.

User and system requirements identified:

1. Automatic baseline recording feature.

The protocol undergone for delivering the stimulations is documented in Figure 4.34.

The stimulation protocol delivered using the MC_Stimulus II software for this process is documented in the aforementioned Table 4.1.

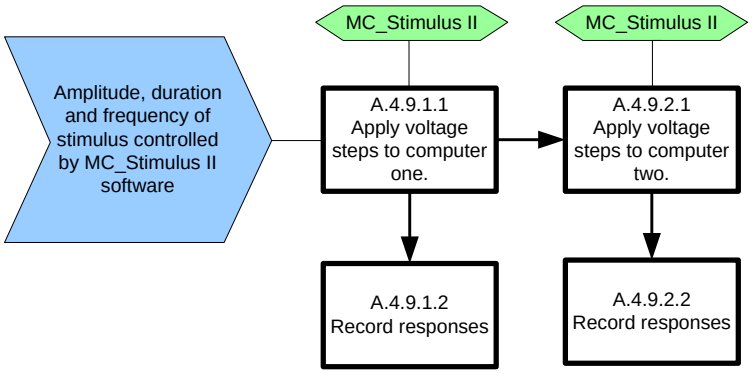


Figure 4.34: A model of the activities carried out in order to complete process four, activity nine, run stimulation tests.

User and system requirements identified:

1. System that can apply defined stimulation protocol to all >4 MEA biochips.
2. System with lists of stimulation protocols that the user can select without needing to adjust.

4.4.2.2 Acute Brain Slice Requirements Summary

Through carrying out this case study the following requirements have been identified that are specific to this application.

1. Biochips that make hippocampal slice positioning easier and quicker.
2. Automated data analysis.
3. MEA biochips that offer better slice positioning.
4. One system that can facilitate ≥8 MEA biochips at one time.
5. A stimulus generator that can deliver protocols to >4 MEA biochips at one time.
6. Analysis features in the recording software.
7. Analysis in real-time.
8. Predefined stimulating electrodes facilitated by anatomically precise microelectrode positioning.
9. Automated slice position checking.
10. Integrated perfusion system.
11. Automatic baseline recording feature.
12. System that can apply defined stimulation protocol to all >4 MEA biochips.
13. System with lists of stimulation protocols that the user can select without needing to adjust.

The requirements that have been identified through this case study are to be addressed by further work.

4.5 Summary

Accounts of the sequences of activities that are required by three different MEA system applications were documented to support identification of user and system requirements for this research.

The applications observed and presented in this chapter as case studies were:

1. Stem-cell derived Cardiomyocyte Application
2. Neural Cell Culture Application
3. Brain slice Application

The case studies were developed thoroughly and methodically using research approaches documented in Chapter 3.

All MEA systems require the user to source cells. The MEA biochip must be made sterile and, depending upon the application, treated before the living cells can be added. The durations that cellular samples spend on an MEA biochip's surface before testing can be from ~15 minutes to several months depending upon the preparation. The user will insert the MEA biochip into the full MEA system when appropriate for recordings of the electrical activity in the cells above to be made. These recordings are then analysed offline in all of the case applications. Following experimentation and recording the cells are discarded and the MEA biochips are cleaned in preparation for future re-use.

A summary of all of the requirements derived from these case studies is contained in Figure 4.35.

The case studies differ from one another significantly. This research has chosen to address the requirements derived through case study one. Consideration and prioritisation of those needs is presented in Chapter 5.

The requirements identified through case studies two and three are suggested as further work following achievements of the design targets defined in Chapter 5.

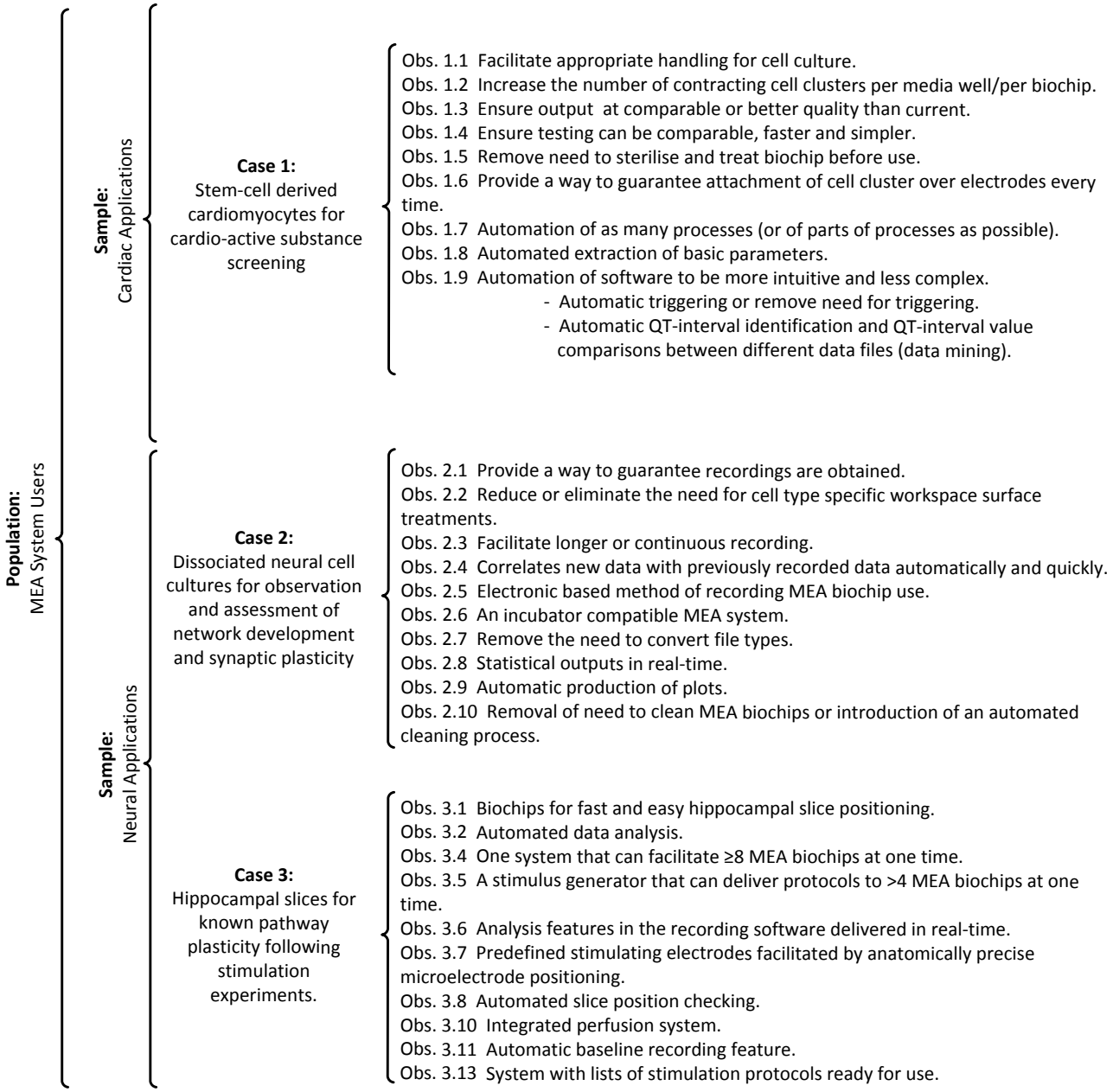


Figure 4.35: Case study observed requirements

[Format adapted from: Gerring, 2007.]

4.6 Research Questions Answered

The following statements address the research questions originally defined at the start of this chapter.

1. **How are MEA systems currently employed by users that are working with different cell types and cell sources?**

The overall process stages are the same irrelevant of the application. This research has focused on the following common stages:

1. Preparing the MEA biochip to the point of use,
2. Insertion of the MEA biochip in the MEA system for recording of electrogenic activity of the cells within the MEA biochip,
3. Extraction of meaning from those data.

The details of the activities undertaken at each stage differ between applications resulting in significant differences between the case study model sets generated.

2. **What are the major differences between application domains and application types within those domains?**

Each application domain differs significantly due to the differing nature of the cells that are used, and the form in which those cells are used. For example, case study one and two differ most significantly due to case study one's exploration of the cardiomyocyte cell type and case study two's exploration of the neuron. Different cell types result in different requirements. Case studies two and three both seek to understand neural functions but use different formats of cells sources (individual cells grown in culture versus slices) which also have different requirements.

The major differences identified through case study construction are:

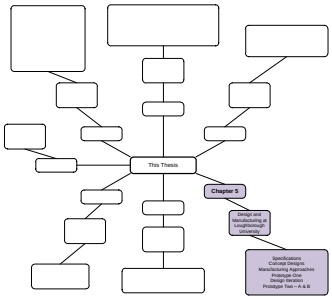
- The MEA biochip can be altered to suit the form in which the cells are to be observed. For example the planar style microelectrode grid array is well suited to cultured neurons, but for slices a 3D electrode profile is more suitable so as to enable closer detection of the field potentials generated from the live cells away from the sliced surface. For the cardiomyocyte

application this work has defined a requirement to alter the MEA biochip to better suit the beating clusters produced from the stem cells.

- The signals that are recorded across each of the case study applications differ significantly. This is attributed back to the different cell types and the format in which those cells are presented to the microelectrodes. Consequently the signal features that are of interest in each case study differ.
- The system settings (e.g. amplifier gain, sampling frequency) differ for each application. The cultured neurons sample at 25kHz, the brain slice application at 10kHz and the cardiomyocyte application at 2kHz.

3. What system or component changes have been identified as required?

This question is addressed by the summary section (section 4.5) and Figure 4.35.



Chapter Five

Requirements, Concept Design, and Manufacturing Approach

In the preceding chapters research has:

- Described current MEA system technology and its application (Chapters 2 and 4) ,
- Demonstrated the research methods selected and implemented to identify and define contemporary user requirements and the needs of next generation systems (Chapters 2, 3 and 4),
- Presented three real end user case studies conducted to support the understanding of practical realities associated with MEA system application in differing settings. Case studies also demonstrated the diversity of applications and identified application specific user requirements that have been targeted by this work (Chapter 4).
- Derived and described three product design specifications created by this research to drive the definition of objectives (Chapter 3). The product design specification that is specifically addressed by the remainder of this thesis is PDS 3.

The work in this chapter has been carried out to address the following research questions:

1. **What current and future user and system requirements have been derived from literature reviews, commercial system assessment and case studies that can be realistically addressed by this research? How does this research prioritise requirements?**

2. **How can this research project meet the defined and targeted requirements?**

3. **What manufacturing solutions are suitable for production of the proposed solution concept?**

4. **What are the critical manufacturing outcomes with regard to the proposed solution? What is the benchmark or gold standard to which the novel biochip will be compared?**

Contemporary MEA biochip design modifications are (see section 2.9.2.2 and Figure 2.65):

i) Reductions in microelectrode tip geometries; to facilitate more electrodes, with denser distributions, to assist closer coupling to cells and more detailed data collection.

ii) Provision of specifically patterned surfaces using growth proteins or materials with hydrophobic characteristics, or growth pathways through polymers or hydrogels; used for the controlled and/or structured proliferation of cells in-vitro.

iii) Provision of microelectrode layouts specifically suited to certain physiological geometries or experimental requirements; to improve ease of set-up and experiment result consistency and repeatability.

These modifications have not occurred sequentially but concurrently through academic necessity (MEA Meeting 2010, Reutlingen, Germany). Each development has been made possible due to technical advances in microelectronics and microelectromechanical systems (MEMS) manufacturing (Khoshnoud and de Silva, 2012). This research has investigated how existing electronics fabrication techniques could be exploited to produce new generations of MEA biochip that are better suited specifically to the cardiomyocyte application documented in case study one.

5.1 Design Rationale

The initial aims of this research presented a vision for a new MEA system that is compatible with continuous operation within a humidified incubator with integrated optics for neural applications (Appendix A). The areas that guided realistic prioritisation of early research objectives are (Figure 5.1): (1) existing systems, (2) MEA system application, (3) manufacturing possibilities, and (4) the pursuit of novelty, were of most significance to the production of successful prototype devices.

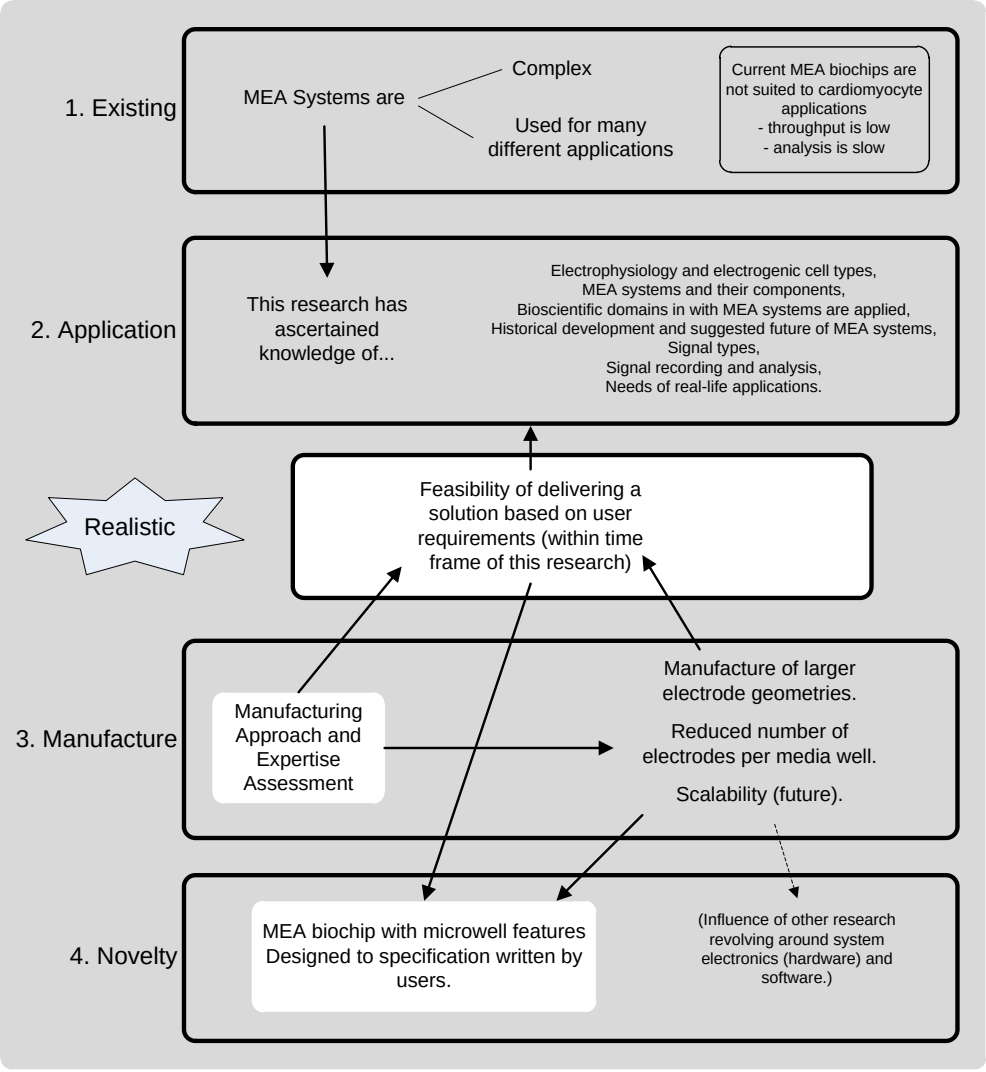


Figure 5.1: Design Rationale.

PDS documents were generated to target the overall research objective to create a novel MEA system. These documents considered research targets from differing levels (section 3.3.1): general (e.g. needs of all MEA systems), partial (e.g. needs identified from all case study users) and specific (e.g. needs specific to cardiomyocyte beating cluster applications). The design targets focused upon

in this thesis were specific in nature to address the needs of the stem cell-derived cardiomyocyte (SC-CM) application domain.

Prior to action on any design targets an assessment of manufacturing facilities and the expertise available was carried out (see section 5.4.1.1). Manufacturing facilities immediately available for prototyping were limited to producing circuitry resolutions >~100µm. Smaller feature sizes were not possible during early stages of concept prototyping.

Chapters 5 and 6 of this thesis describe research conducted to develop a novel MEA biochip that is optimised specifically for stem-cell derived cardiomyocyte applications.

5.2 MEA Biochip Specification

The research targets addressed in the following concepts are based on a specification document derived from University of Nottingham (UoN) MEA system users defining application specific requirements (Appendix C).

The following specification points were set as design deliverables for this research incorporating user requirements from UoN with generic and partial PDS points identified as of high in importance to system success (see PDS 1 & PDS 2).

Biochip:

- **Modify the media well while maintaining commercial interface configuration.**
  Comments: that will interface with the current MCS MEA60 System in their lab.
- **Alter the media well dimensions to be close to those of a 35mm culture dish.**
  Comments: internal dimensions 35mm diameter, 10mm height, culture area 8.8cm^2, media volume 3ml.
- **Incorporate a light-transmissible lid.**
  Comments: to prevent evaporation.
- **Interior of well must allow for potential etching or patterning.**
- **Re-usable.**
  Comments: therefore must withstand sterilisation using UV light and 70% ethanol.
- **16 micro-wells.**
  Comments: in any pattern.
- **Microwells as far apart as possible.**
  Comments: at least 5mm in from the edge of the dish.
- **Microwells 500µm diameter,**
  - **with a maximal depth of 250µm,**
  - **and a slightly curved shape.**

  Comments: beating clusters at time of seeding vary in size and shape (Figure 5.1) between 200-500µm in diameter.
- **Electrode in centre of well flush to the surface.**
  Comments: To support early attachment of the beating cluster to the surface.
- **Microwells with as large a surface area as possible.**
  Comments: To support early attachment of the beating cluster to the surface.

- **Made from biocompatible material(s).**

  Comments: Essential for cell culture.

System:

- **Sample rates in the range of 2-25kHz.**

  Comments: Current commercial systems 2-50kHz though few applications utilise >25kHz.
- **Electrically grounded.**

  Comments: Essential for provision of reference channels and electrical safety.
- **Parts that can be sterilised**

  Comments: If the entire system is to go in an incubator the electronics must be sealed in suitable casing for sterilisation as incubators are sterile environments.
- **Input capabilities limited appropriately.**

  Comments: to protect/prevent damage to cells or tissue.
- **Appropriate pre-processing and amplification.**

  Comments: Noise reduction or removal. Commercial gains: Slice ~x550, Culture ~x1100.
- **Facilitate/support a constant cell environment.**

  Comments: I.e. temperature, humidity, etc
- **Allow easy access**

  Comments: for users to perform culture maintenance and run perfusions systems.
- **Support visual inspection of the cells.**
- **Simple to learn.**
- **User-friendly interfaces.**

UoN users specified requirements for a biochip design that would complement their cellular preparation, stem cell-derived beating cardiomyocyte clusters (Figure 5.2), more appropriately than the most suitable commercial biochip currently used; which is a standard planar 60 electrode 8x8 array (Figure 2.45).

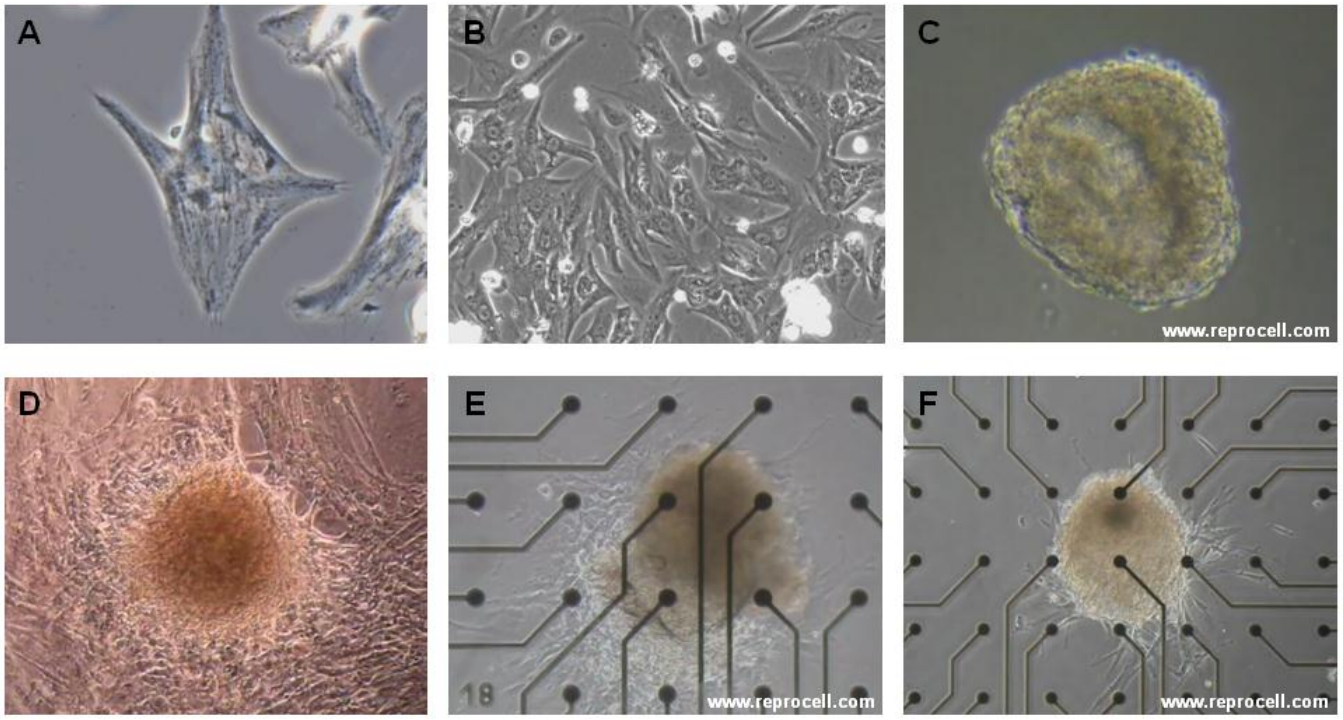


Figure 5.2: Stem cell-derived cardiomyocyte cells. A) A single cardiomyocyte in culture. B) A monolayer of cultured cardiomyocytes. C) An example of freshly dissected 'beating cluster', consisting of fibroblast cells and cardiomyocytes. D) An example of the attachment that forms between a beating cluster and the substrate. Fibroblast cells are the predominant cell type present in a cluster and are responsible for the attachment of the cluster to the base substrate. E & F) Examples of beating clusters seeded over MEA biochip microelectrodes. (Scale: electrode tips are 30µm in diameter.)

5.2.1 New Product Development and Quality Assurance

To consider how the aforementioned requirements influence one another and affect design and manufacturing decisions a Quality Function Deployment (QFD) methodology was employed.

QFD is a part of internationally standardised quality management system (QMS) guidance (ISO 9000:2005; ISO 9001:2008). QMS guidance was produced to specifically support the achievement of customer satisfaction through the appropriate execution of planning, design and manufacture (Justham and West, 2008) making this guidance relevant to this research.

QFD methods are applied during investigation and definition of user requirements, and also while translating those requirements ("user desires") are translated into technical requirements. QFD has been successfully demonstrated in diverse application domains, such as hospitality and finance, as well as in engineering (Shahin and Chan, 2006; Justham and West, 2008), and as such was deemed suitable for the needs of this work.

5.2.1.1 The House of Quality Interrelationships Matrix

An enterprise can improve its productivity, control costs and remain competitive through application of QFD (Madu, 2006). The primary planning tool available in QFD is the house of quality (HoQ) matrix (Besterfield et al, 2011). A HoQ matrix is completed to logically examine a number of factors that influence a product's quality in terms of meeting customer or user expectations. User requirements, technical requirements, requirement interrelationships, planning for competitiveness and design and manufacture targets, are all incorporated into one structure (Figure 5.3).

Within the matrix user requirements are identified and prioritised (see section 5.2.1.1.1). Existing products are compared and evaluated, identifying potential areas of greatest pay-off (see section 5.2.1.1.2). Technical characteristics required in order to respond to the user requirements are identified (see section 5.2.1.1.3), and relationships between the customer requirements and technical characteristics are considered (see section 5.2.1.1.4 and 5.2.1.1.5). Product development targets are defined using scores generated by all of these aspects together (Barkley and Saylor, 2001).

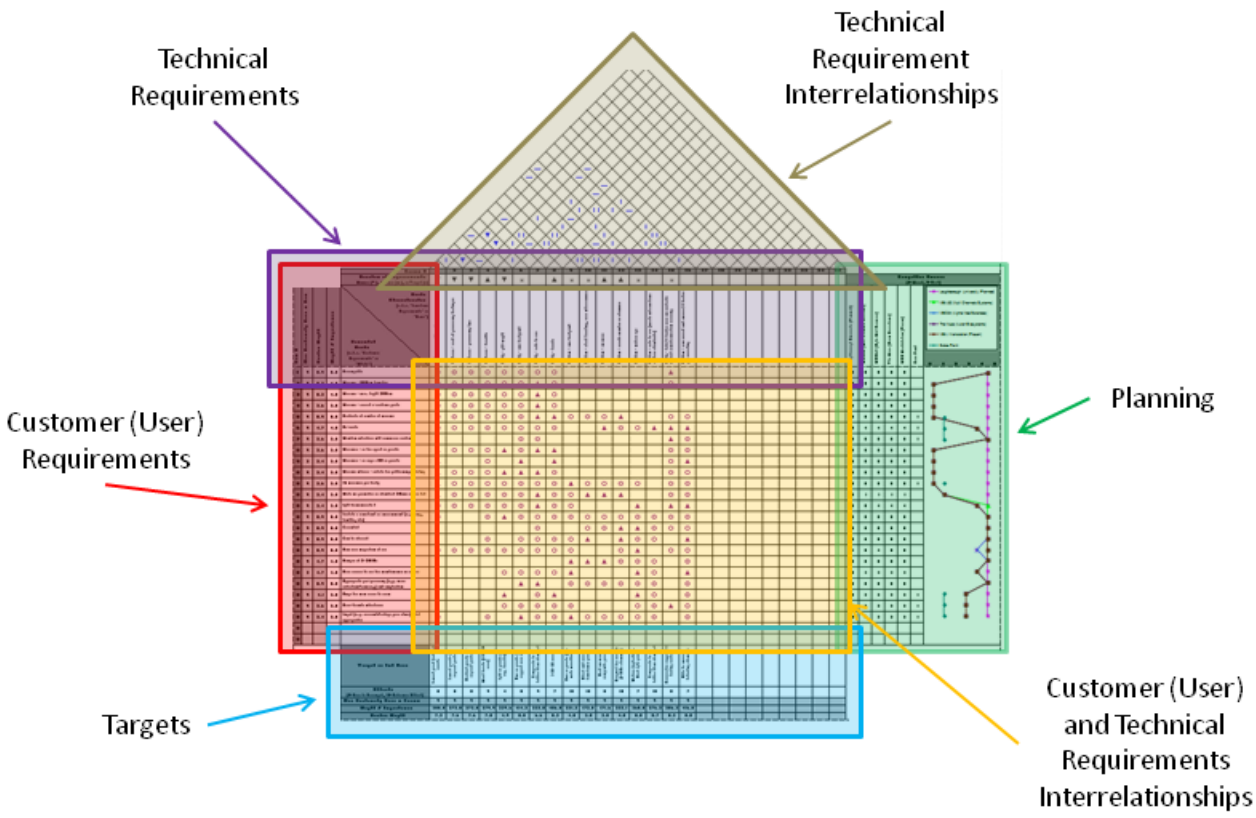


Figure 5.3: The QFD House of Quality Matrix.

The complete HoQ used in this research can be viewed in Appendix E. The spread sheet template was sourced from QFD (Quality Function Deployment) Online (2010).

5.2.1.1.1 User Requirements

The requirements from PDS 3 were listed and given an importance weighting by a system user (Figure 5.4). This panel of the matrix represents the “voice of the customer” (Zhang and Wang, 2011). Weightings were given assigning a number between zero and five to each requirement; five indicated most important/essential and zero indicated not important.

Weight / Importance	Demanded Quality (a.k.a. "Customer Requirements" or "Whats")
5.0	Biocompatible
4.5	Microwell - 500um diameter
4.5	Microwell - max. depth 250um
3.0	Microwell - curved x-sectional profile
5.0	Electrode at centre of microwell
4.0	Re-usable
3.0	Maintain interface with commercial system
3.0	Microwells - as far apart as possible
2.0	Microwells - as large a SA as possible
2.0	Microwell interior - suitable for patterning/etching
3.0	16 microwells per biochip
2.0	Media well geometry as standard 35mm culture dish
2.0	Light transmissable lid
5.0	Facilitate a constant cell environment (e.g temp, humidity, etc)
5.0	Grounded
5.0	Can be sterilised
5.0	Allow visual inspection of cells
4.0	Samples at 2-25kHz
4.0	Easy access to cells for maintenance in culture
5.0	Appropriate pre-procesing (e,g. noise reduction/removal,) and amplification
3.5	Simple for new users to learn
3.0	User-friendly interfaces
2.0	Input (e.g. current/voltage pulse stimuli) limited appropriately

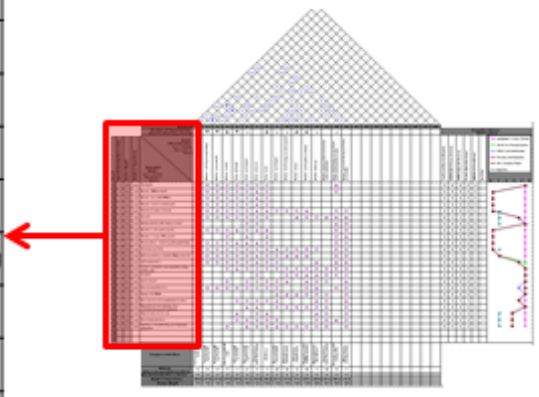


Figure 5.4: HoQ User requirements

Requirements were also listed in groups, biochip-centred and system-centred (Figure 5.5), for clarity.

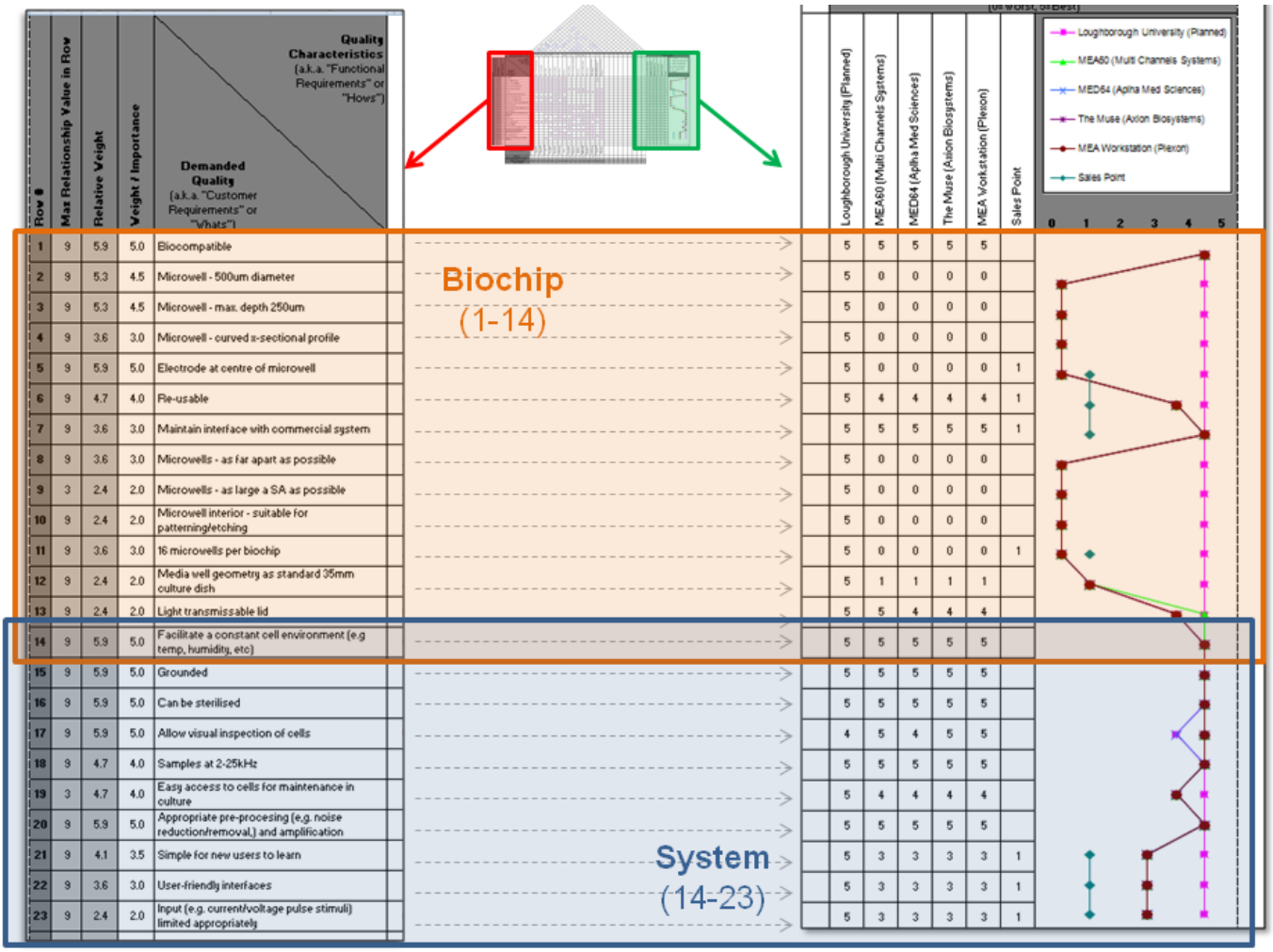


Figure 5.5: HoQ Biochip- and system-centred user requirements.

5.2.1.1.2 Product Planning

The product planning panel (Figure 5.6) correlates the listed user requirements with user perception of how existing products perform in terms of those requirements.

This panel of the matrix serves to demonstrate current user satisfaction and to highlight areas where improvements may provide the greatest pay-back when compared to existing products. Sales points have been used to add to weight requirements that could be heavily exploitable in marketing the product according to current user trends (Chapter 2).

Combined scores relating to each user requirement have been calculated by multiplying previously defined user importance weighting by the improvement (0 = poor, 5= best) and sales point scores (0 = neutral, 1 = current sales point).

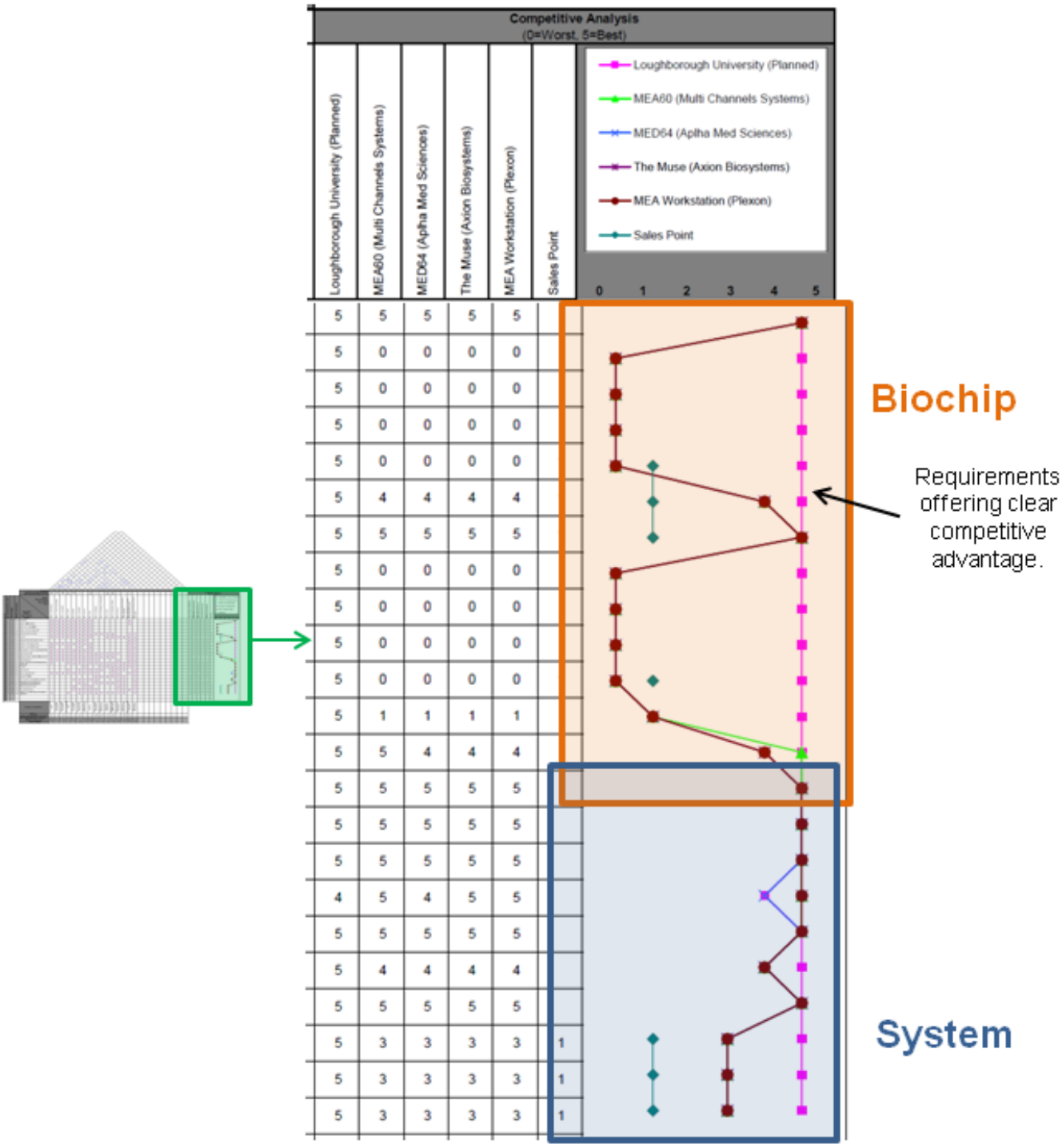
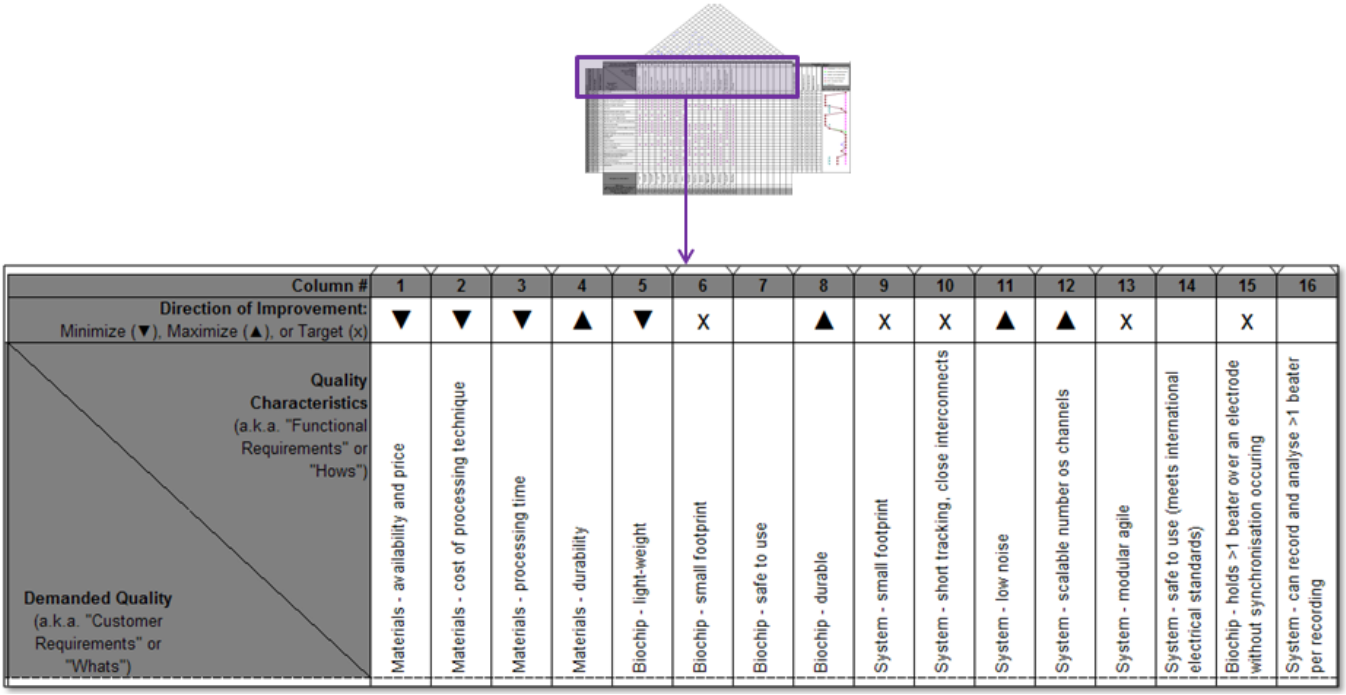


Figure 5.6: HoQ Product Planning and Competitive Analysis

This panel identified that an MEA biochip that meets the biochip-centred user requirements defined at the start of this chapter will better meet the needs of users of SC-CMs and this will offer good competitive advantage. The system-focused requirements that were assessed did not offer as clear an advantage.

5.2.1.1.3 Technical Requirements

It is in this panel (Figure 5.7) the engineering requirements ("voice of the company") are described. Technical requirements that are perceived as important in meeting the specified user requirements were listed and analysed. An additional row at the top of the panel illustrates the direction of the intended change of each characteristic to achieve the desired improvement.



Column #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Direction of Improvement: Minimize (▼), Maximize (▲), or Target (x)	▼	▼	▼	▲	▼	x		▲	x	x	▲	▲	x		x	
Quality Characteristics (a.k.a. "Functional Requirements" or "Hows") / Demanded Quality (a.k.a. "Customer Requirements" or "Whats")	Materials - availability and price	Materials - cost of processing technique	Materials - processing time	Materials - durability	Biochip - light-weight	Biochip - small footprint	Biochip - safe to use	Biochip - durable	System - small footprint	System - short tracking, close interconnects	System - low noise	System - scalable number os channels	System - modular agile	System - safe to use (meets international electrical standards)	Biochip - holds >1 beater over an electrode without synchronisation occuring	System - can record and analyse >1 beater per recording

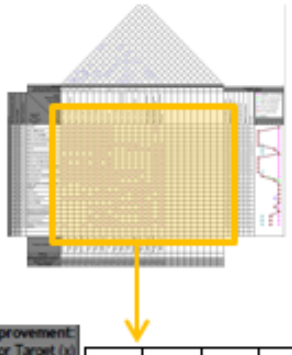
Figure 5.7: HoQ Technical Requirements

This panel highlighted:

- Materials are required at the lowest possible prices, while facilitating processing techniques that are as low cost and fast as possible.
- The selection of the materials for biochip construction should be such that the highest durability available for each sub-component (e.g. interconnection point, insulator, electrode surface).
- The weight of the biochip should be low so as to ease both handling and transportation.
- The overall size of the MEA biochip should be small to promote future biochip footprints that are as small as practically possible. Initial interconnect design had to be kept the same as commercial equivalents so as to meet the user specified requirement to connect with commercial systems.
- A flexible design that facilitates a vertical interconnection layout should be aimed for so as to support system foot print and module size reduction.

5.2.1.1.4 Requirement Interrelationships

This panel (Figure 5.8) forms the main body of the matrix and its purpose is to translate the user requirements into technical requirements. Combined consideration of user and technical requirements resulted in the definition of design and manufacturing targets used in this work.



Θ Strong Relationship
O Moderate Relationship
▲ Weak Relationship

Direction of Improvement: Minimize (▼), Maximize (▲), or Target (x) / Quality Characteristics (a.k.a. "Functional Requirements" or "Hows") / Demanded Quality (a.k.a. "Customer Requirements" or "Whats")	Makeitdo - availability and price	Makeitdo - cost of processing technique	Makeitdo - processing time	Makeitdo - durability	Biochip - light-weight	Biochip - small footprint	Biochip - safe to use	Biochip - durable	System - small footprint	System - short tracking, close interconnects	System - low noise	System - scalable number of channels	System - modular style	System - safe to use (meets international electrical standards)	Biochip - holds >1 beaker over and decks without synchronisation/occurring	System - can record and analyse >1 beaker per recording
Biocompatible	Θ	Θ	Θ	O	O	O	O	O							▲	
Microwell - 500um diameter	Θ	Θ	Θ	O	O	O	▲	O							O	
Microwell - max. depth 250um	Θ	Θ	Θ	O	O	O	▲	O								
Microwell - curved x-sectional profile	Θ	Θ	Θ	O	O	O	▲	O								
Electrode at centre of microwell	O	Θ	Θ	O	O	Θ	▲	▲	O	Θ	O	▲			O	O
Re-usable	Θ	O	O	Θ	O	O	O	Θ			▲	Θ	O	▲	▲	▲
Maintain interface with commercial system						Θ	Θ								▲	Θ
Microwells - as far apart as possible	▲	O	O	O	▲	Θ	▲	▲							Θ	Θ
Microwells - as large a SA as possible	▲			O		▲		▲							O	▲
Microwell interior - suitable for patterning/etching	▲	O	O	O	▲	▲	▲	Θ							Θ	O
16 microwells per biochip	▲	O	O	O	O	Θ	O	Θ	▲	O	O	Θ	O		Θ	Θ
Media well geometry as standard 35mm culture dish	▲	Θ	Θ	Θ	Θ	Θ	▲	Θ	O	▲	▲	▲			Θ	Θ
Light transmissable lid	Θ	Θ	Θ	Θ	Θ	Θ	▲	Θ	O				▲		▲	▲
Facilitate a constant cell environment (e.g temp, humidity, etc)	O			Θ	▲	Θ	O	Θ	Θ	O	O	O	Θ	Θ	Θ	Θ
Grounded							Θ			O	Θ	▲	▲	Θ	O	O
Can be sterilised				Θ		O	O	Θ	O	▲		▲	Θ	O		▲
Allow visual inspection of cells	Θ	Θ	Θ	Θ	Θ	O	O	O	O			O	▲		O	O
Samples at 2-25kHz									▲	▲	▲	Θ	Θ	O		Θ
Easy access to cells for maintenance in culture					O	O	O	O	▲				▲	O		▲
Appropriate pre-processing (e.g. noise reduction/removal) and amplification						▲	▲		Θ	Θ	Θ	Θ	Θ	Θ		Θ
Simple for new users to learn					▲		Θ	▲					▲	O		Θ
User-friendly interfaces					O	Θ	Θ	Θ	Θ				O	Θ	▲	O
Input (e.g. current/voltage pulse stimuli) limited appropriately	O			Θ		▲	Θ	Θ	▲	O	O	O	O	Θ		Θ

Figure 5.8: HoQ Interrelationships

In this grid (Figure 5.8) symbols were used to represent interrelationship significance in terms of product success. Correlations between each user-technical requirement were considered and rated using a four point scale (high significance, medium significance, low significance, no significance). These ratings then corresponded to a score that was agreed prior to completing the matrix (e.g. high – 9, medium – 3, low – 1, none – 0). This panel guided decision making when compromises where required. The strength of relationships could be visually referred to with ease. The scores generated in this panel also contributed to the final weighting calculation of the design and manufacture targets derived.

5.2.1.1.5 Technical interrelationship impedance or support

The "roof" of the matrix looked at how previously defined and scored technical requirements either supported or impeded one another (Figure 5.9). Consideration was made of whether improving one requirement would cause either an improvement or deterioration of the other requirement.

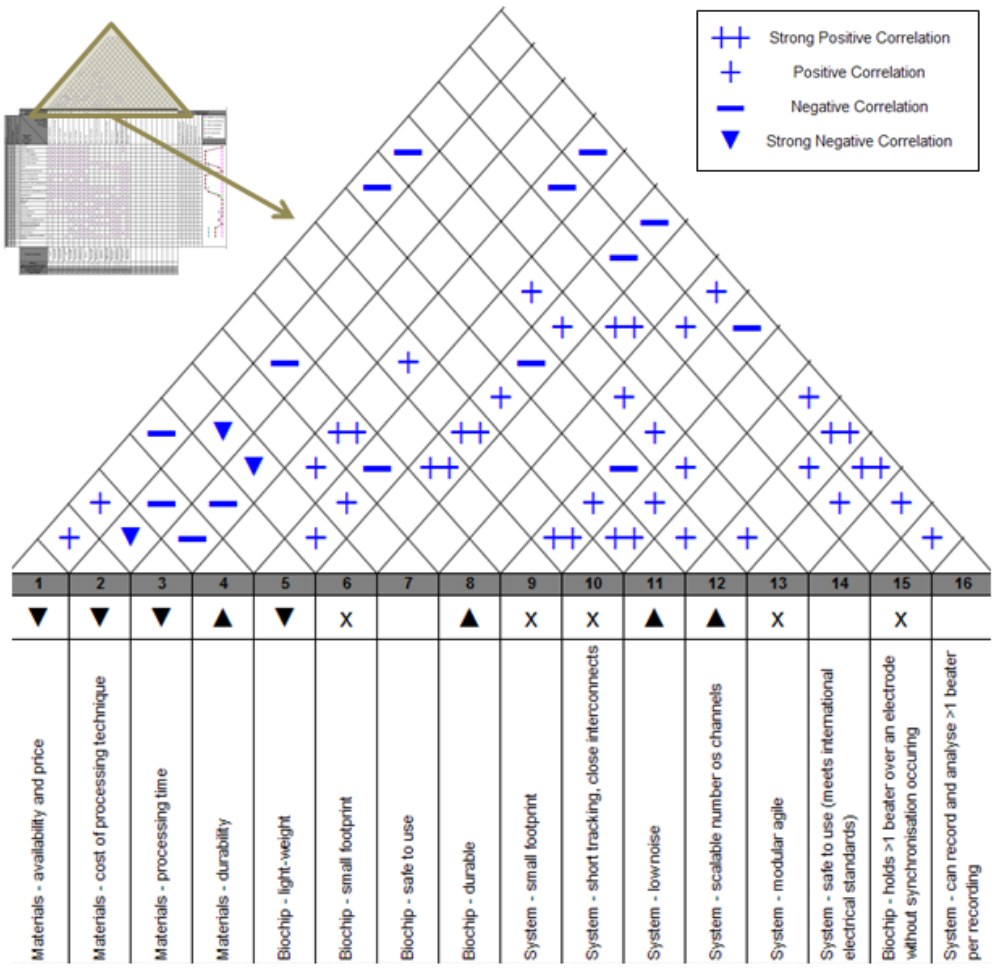


Figure 5.9: HoQ Technical Requirement Interrelationships

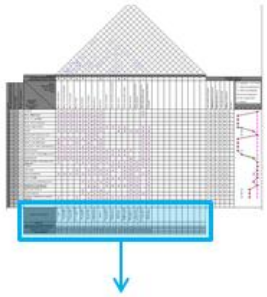
Where deterioration was identified a "-" symbol is inserted and where improvement was identified a "+" symbol was inserted. The level of positive or negative interactions was indicated by adding strength to the indicators:

- + = weak positive,
- ++ = strong positive,
- - = weak negative,
- ∇ = strong negative).

This panel was used during concept design to identify positive interrelationships (e.g. increasing the number of recording channels would support the ability of the biochip and system to record and analyse from more than one beater at a time. Negative interactions were also identified and given attention in attempts to seek an innovative solution that would not introduce new problems where compromises had to be made (i.e. in selection of material and manufacturing processes).

5.2.1.1.6 Targets

This was the final panel of the HoQ to be completed (Figure 5.10). The targets panel summarises the conclusions drawn from completing the matrix. Within this panel the relative importance of each technical requirement in meeting the specified user requirements was calculated from the weightings contained in the other panels. Design and manufacture targets were set and the difficulty of achieving those targets quantified and considered prior to action.



Target or Limit Value	Lowest priced (suitable) available	Lowest possible (@ required quality)	Quickest possible (@ required quality)	Most durable (>10-15 uses)	Light as possible (@ req. durability)	Small as possible (to support scale up)	Comparable to or better than standard	>10-15 uses	Small as possible (Fits inside incuabtor)	Short and close as functionally possible	Best low noise compents possible	Designed for scalability (>100's channels)	Modules (updrades). Best agility poss.	Comparable to or better than standard	Successfully support >1 beating cluster	Ability to analyse all detecting channels
Difficulty (0=Easy to Accomplish, 10=Extremely Difficult)	5	8	5	9	4	8	9	7	10	10	8	10	7	10	5	7
Max Relationship Value in Column	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Weight / Importance	355.0	372.8	372.8	379.9	239.6	414.2	322.5	406.5	221.3	172.8	171.6	233.1	268.0	276.3	256.2	416.0
Relative Weight	7.3	7.6	7.6	7.8	4.9	8.5	6.6	8.3	4.5	3.5	3.5	4.8	5.5	5.7	5.3	8.5

Figure 5.10: HoQ Targets

The outputs from this panel (Figure 5.52) are ordered in Figure 5.11.

	Materials - availability and price	Materials - cost of processing technique	Materials - processing time	Materials - durability	Biochip - light-weight	Biochip - small footprint	Biochip - safe to use	Biochip - durable	System - small footprint	System - short tracking, close interconnects	System - low noise	System - scalable number os channels	System - modular agile	System - safe to use (meets international electrical standards)	Biochip - holds >1 beater over an electrode without synchronisation occuring	System - can record and analyse >1 beater per recording
Target or Limit Value	Lowest priced (suitable) available	Lowest possible (@ required quality)	Quickest possible (@ required quality)	Most durable (>10-15 uses)	Light as possible (@ req. durability)	Small as possible (to support scale up)	Comparable to or better than standard	>10-15 uses	Small as possible (Fits inside incuabtor)	Short and close as functionally possible	Best low noise compents possible	Designed for scalability (>100's channels)	Modules (updrades). Best agility poss.	Comparable to or better than standard	Successfully support >1 beating cluster	Ability to analyse all detecting channels
Difficulty (0=Easy to Accomplish, 10=Extremely Difficult)	5	8	5	9	4	8	9	7	10	10	8	10	7	10	5	7
Max Relationship Value in Column	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Weight / Importance	355.0	372.8	372.8	379.9	239.6	414.2	322.5	406.5	221.3	172.8	171.6	233.1	268.0	276.3	256.2	416.0
Relative Weight	7.3	7.6	7.6	7.8	4.9	8.5	6.6	8.3	4.5	3.5	3.5	4.8	5.5	5.7	5.3	8.5
	6	5	5	4	11	2	7	3	13	14	15	12	9	8	10	1

Figure 5.11: Priority of defined development targets according to HoQ matrix score.

The highest importance score (416.0) was obtained by the requirement “ability to analyse all detecting channels”. Although this requirement is therefore prioritised as priority 1 this requirement could not be directly addressed in conjunction with the following biochip-centred requirements. Highly scoring biochip-centred requirements could be addressed in conjunction with one another so

were tackled in this research. Table 5.1 shows prioritised targets in terms of the HoQ weightings. The combined biochip-centred requirements scored more highly than the system-centred targets justifying this research's pursuit to design and manufacture a novel MEA biochip for this particular application domain.

Table 5.1: Prioritised targets with discussion. *Blue panels = biochip, orange panels = system.*

HoQ Priority	Target	Discussion
1	The system must possess the ability to analyse data collected from all of the detecting channels.	This is an essential system requirement. All existing and future MEA systems possess this feature. The biochip produced must also support this requirement.
2	Biochip must be as small as possible (supporting scale up).	Small biochips improve practicality for bioscientist users by allowing batch sterilisation, preparation, cleaning and storage. The area to work with inside culture hoods is limited. Small biochips facilitate scaling up by better suiting the limiting dimensions of incubators. (In this application cells must be cultured over the microelectrodes prior to recording.)
3	Biochip to withstand greater than 10-15 uses.	Present day commercial biochips degrade with use and it is recommended by manufacturer's that they are replaced after every 10-15 uses. To improve value and convenience (3-6 weeks delivery) to customers biochips that can be used >15 times are desired.
4	Materials used to be the most durable possible (i.e. support production of a device that is suited to at least 10-15 uses).	Current degradation of biochips is attributed to the delicate nature of the materials and feature size, as well as detrimental consequences of stimulation protocols. Materials that do not degrade with use are required.
=5	Use of manufacturing processes that are of lowest possible cost while producing device at required quality.	A number of manufacturing processes are combined to produce an MEA biochip. There are choices in which combination of processes are employed at each stage of device manufacture. Compromises are made between speed: cost: quality. Decisions are also influenced by expertise: tooling: funding. Low cost approaches are favoured.
=5	Use of manufacturing processes that produce devices to required quality as quickly as possible	MEA biochips are developing rapidly (chapter 2) so processes that can produce devices quickly and efficiently are favoured so as to realise a suitable device as soon as possible. In addition to that processes that take a long time to execute in this manufacturing domain may be high-skilled and high cost.
6	Materials selected to be lowest priced available	Materials used to manufacture current commercial MEA biochips (i.e. glass, gold, titanium nitride) are relatively high cost. Where the in use and manufacturing properties of two

		materials is comparable the price of the material must be a priority. Compromises between function: quality: cost: processing.
7	Safety of the biochip (i.e. when in use, when in storage).	Must be comparable to or better than standard commercial equivalents. The new device must not pose any additional risks.
8	Safety of the system (i.e. when in use, when in storage).	Must be comparable to or better than standard commercial equivalents. The new device must not pose any additional risks.
9	System should be modular in design to accommodate easy upgrade of components.	Due to the diversity of MEA application types a system that can be adapted to meet the needs of either different applications or of future needs of contemporary applications is desired. Chapter 2 demonstrates the diversification of systems into application specific units due the need for different hardware settings. Modular design to facilitate maximum component and sub-component cross over is desired which will also increase efficiency in terms of production.
10	Successfully support >1 beating cluster	For the novel biochip to be competitive with a commercial equivalent it must support the attachment and recording from 2 or more stem cell-derived beating cardiomyocyte clusters per media well.
11	Light-weight	Biochips must be light-weight to support ease of use and transportation but without any compromise to durability.
12	Designed for scalability	In order to compete with state-of-the-art commercial systems the novel system is required to support hundreds of channels.
13	Small as possible (system)	Competitive advantage can be gained by producing a system that is a compact and small as possible. Current commercial systems are about the size of a shoe box. A system that is comfortably accessible while operating inside an incubator is desired.
14	Interconnects and tracking that is as short and close together as functionally possible	In order to facilitate production of a low noise, small MEA system components and sub-components should be design with close interconnects and short lengths of tracking.
15	Best low noise components possible	The components sourced to build system units should be specified with low noise characteristics as a high priority if affordable.

5.2.2 Section Summary

This research has sought to identify and prioritise targets for design and manufacture of a novel device. The QFD methodology's HoQ matrix resulted in a prioritised list of targets used in concept design, prototype manufacture and testing.

The targets focused on by the work in this thesis were:

- Produce a biochip to support >1 beating cluster of SC-CMs at one time, providing >1 channel of usable data per test repetition.
- Provide a biochip that safely interconnects with an existing MEA system that is small, lightweight and can withstand repeated use (at least 15 times).
- Manufacture a biochip using the best quality possible, lowest priced materials that complement fast and low cost production.
- Design a biochip flexibly to allow potential future scalability, whilst also featuring low noise design properties.

5.3 MEA Biochip Concept Design

The specification provided by the UoN and targets identified through the HoQ matrix highlighted potential competitive advantage in biochip-centred user requirements over system-centred requirements (section 5.2.1.1). Biochip-centred requirements were used to generate a design concept suited specifically to stem-cell derived cardiomyocyte beating cluster cell preparations.

A number of manufacturing approaches were conceived for manufacturing the novel design, and due to varying degrees of success, were progressively adapted until a prototype of a satisfactory quality meeting the specified requirements was achieved.

5.3.1 Critical MEA Biochip Components

An MEA biochip is comprised of two components: (1) a patterned base substrate and (2) a media well (Figure 5.12). The media well is usually a ring of material adhered to the base substrate so as to enclose the surface area of the biochip that supports the microelectrode tips, which is referred to as the workspace (see Figure 2.46). The volume of space within the media well is filled with a liquid cell culture media (e.g. Neurobasal, DMEM) when in use.

Design of an MEA biochip requires appropriate consideration of the techniques applicable for the manufacture of: i) a base substrate, with integrated microelectrodes capable of detecting microvolt sized amplitudes and appropriate contacts for external interconnects; and ii) a media well that reliably adheres to ensure a water-tight product that can be used for periods of hours to months in incubated conditions.

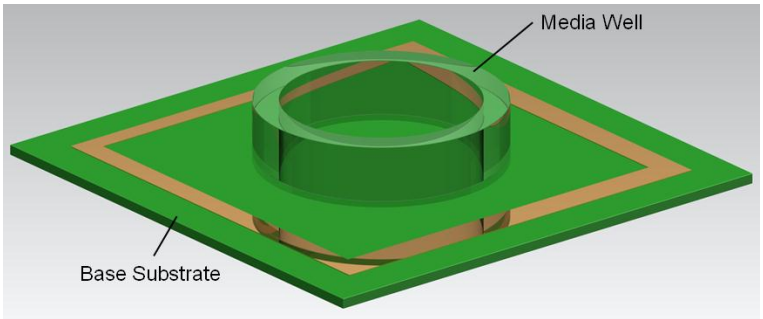


Figure 5.12: The two main components in MEA biochip design and manufacture.

All commercially available MEA biochips have an insulating material applied over the upper, patterned surface of the base substrate. The insulation layer ensures only the tips of the microelectrodes are exposed to the cellular environment and media reducing noise and protecting the underlying circuitry. Insulation is described in section 2.10.1.4.

5.3.2 The Concept

Throughout the process of designing a suitable manufacturing approach the fundamental design concept for the novel MEA biochip remained consistent. A recess of ~200-250µm, with a diameter of 500µm at each electrode site, to accommodate a single beating cardiomyocyte cluster directly over an electrode (see Figure 5.13) was required.

An inter-electrode distance (pitch) of ~5mm was maintained across designs to accommodate the user requirement to keep beating clusters far enough apart so as to keep contractions independent from one another while in the same culture media. It has been observed by UoN users that beating clusters of cardiomyocytes that are in close proximity will gradually exhibit synchronised contractions.

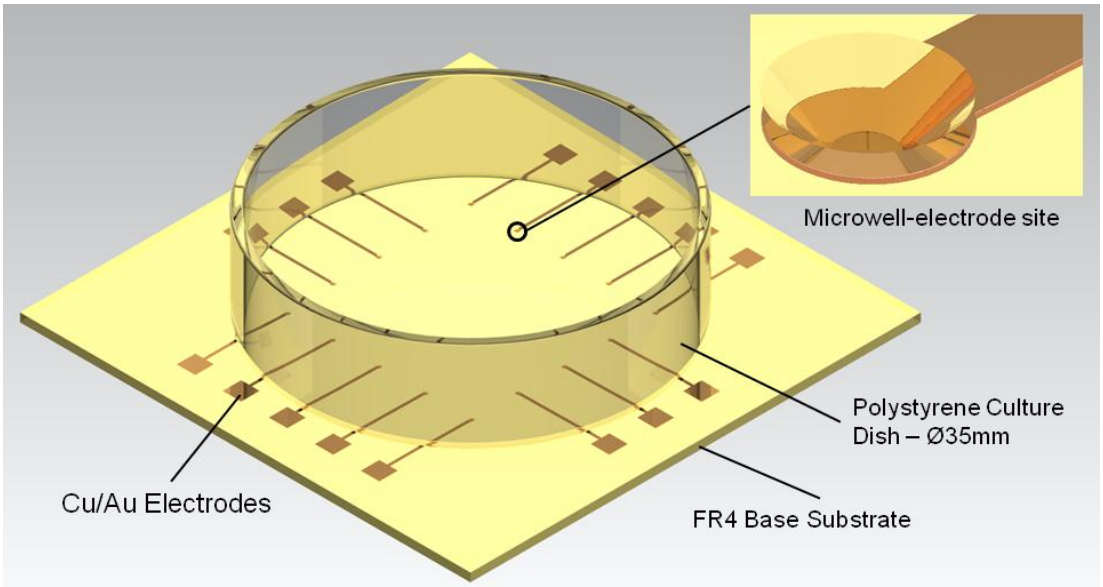


Figure 5.13: A computer aided design of the original MEA concept for beating cluster cardiomyocyte applications at the University of Nottingham.

The geometry of the peripheral interconnect design was kept consistent with commercial equivalents produced by Multi Channels Systems and Ayanda Biosystems (see Figure 5.14) so as to facilitate the connection with the commercial system headstage (e.g. in this case MCS MEA60) as specified by the UoN user group.

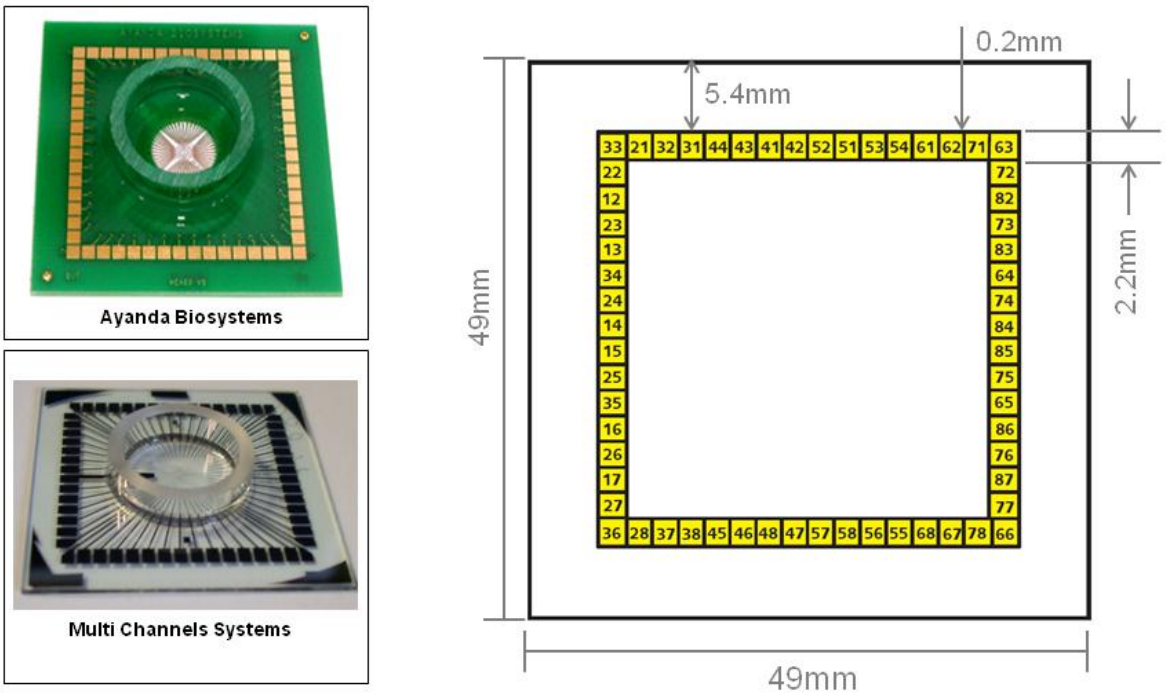


Figure 5.14: Interconnection geometry of Ayanda Biosystems and Multi Channels Systems standard 60 electrode MEA biochips. Contact pads are represented by yellow squares. The numbering on each pad represents the channel that the pad corresponds to in the recording and analysis software.

5.3.3 Section Summary

A biochip specifically suited to SC-CM beating cluster cell source applications does not yet exist and is required. A microwell feature over each electrode is desired by users to increase the number of beating clusters accommodated per biochip. Underlying circuitry must be appropriately insulated using materials that are biocompatible and that can withstand wet and warm (~37°C) conditions for periods of days to months.

Media wells are essential around the area of the electrodes as the SC-CMs require a constant supply of cell culture medium, which in this application is Dulbecco's Modified Eagle's medium (DMEM).

The peripheral interconnect layout was to be kept consistent with commercial designs.

The user specified microwell feature at each electrode is completely novel in its size, shape and suggested material. This novel feature brings new advantages to the user by facilitating easier cell positioning during the seeding process, improving signal quality as cells will attach directly over the larger surface area of the larger diameter electrode, and creating the facility to process more than one beating cluster per MEA biochip.

5.4 Manufacturing Approach Selection

A design concept was conceived to meet the prioritised biochip-centred design and manufacture targets. Manufacturing approach and materials suited to production of the design concept were considered (Figure 5.15) with respect to possibilities the materials or techniques offer, limitations that might be of significance, costs, environmental factors and scalability for manufacture of the likely quantity of biochips required, both separately and in association.

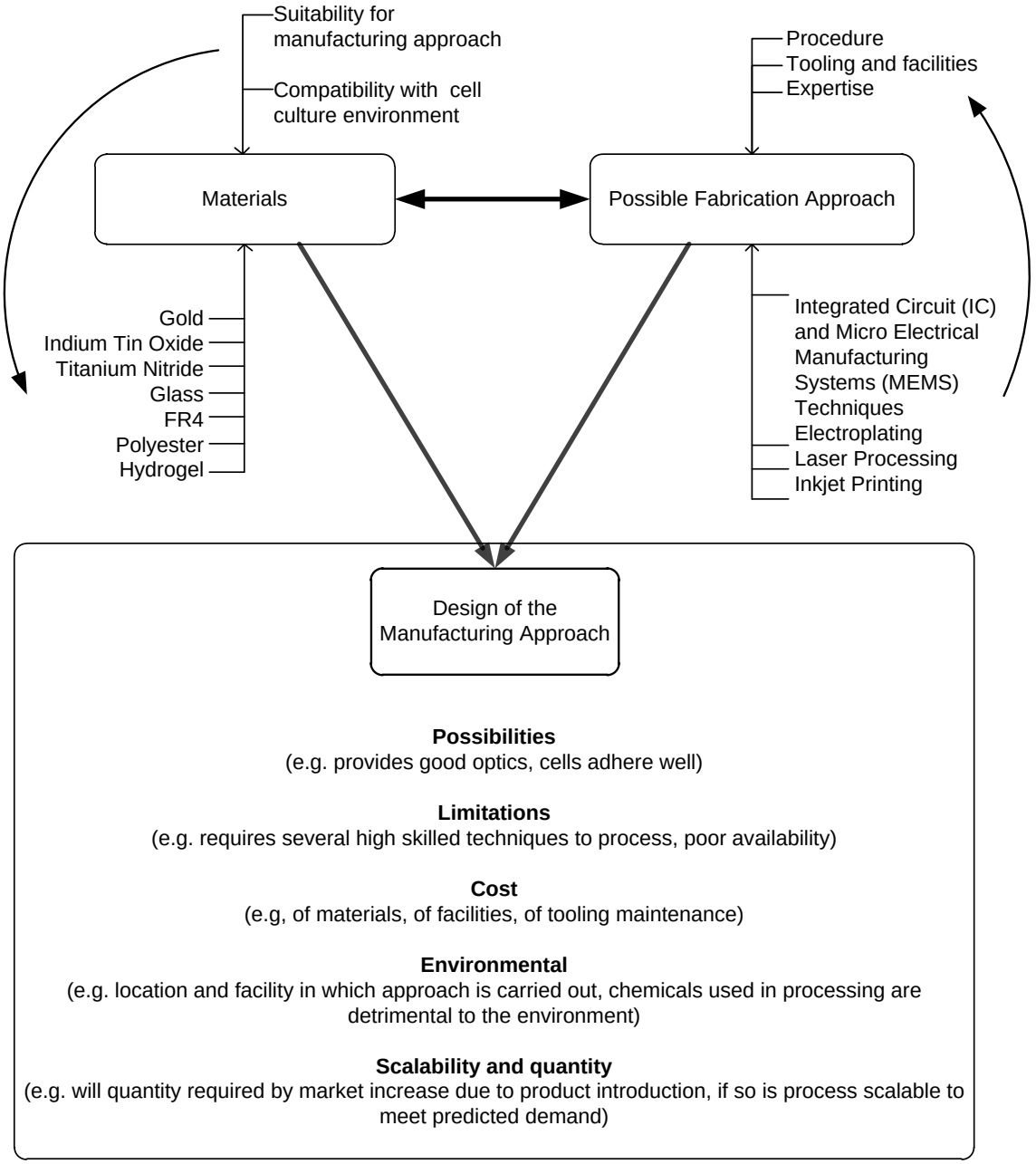


Figure 5.15: Manufacturing approaches conceived were described in terms of what was possible, what materials were suitable and specific advantages and disadvantages posed by each approach.

Possibilities and limitations: Manufacturing technology was evaluated to determine what would be possible and where limitations of techniques might rule them out with regard to identified potential manufacturing techniques (section 5.4.1) in terms of:

i) the physical procedures required to execute that technique;
ii) the tooling and facilities those procedures required;
iii) the feature sizes and geometries attainable using that technique;
iv) the level of expertise available to ensure necessary procedures could be carried out effectively and safely.

These considerations are described in Table 5.3.

This research demands the use of materials that suit biosensor applications. Only materials that were biocompatible and suited to incubated conditions were considered. Materials selection priority was such that materials chosen would be compatible with:

1. the needs of the cell culture environment;
2. the manufacturing approach.

Costs: Associated costs that would impact upon the manufacture and thus the final unit price of the MEA biochip and/or system were taken into account. The scale on which the novel MEA biochip could be manufactured was an influence as it is essential that manufacturers balance the scale of production and supply to the actual market need (Fisher et al, 1994).

Environment: Environmental concerns are now a major consideration for all parties involved in manufacturing electrical or electronic products (Toyasaki et al, 2011). Disposable devices would be ideal for MEA system user groups as they could vastly reduce demands on users in terms of sterilisation and preparation. However, the ability to reclaim the high cost raw materials (such as gold or ITO) used in fabrication would be an environmental concern if large quantities were manufactured and sold.

Quality and scalability: Current commercial manufacturing uses approaches that are carried out in batches. Scalable approaches are desired as the demand for devices in this application area will initially be low during research to validate the stem cell-derived cardiomyocyte as a reproducible

and reliable cell source. If substance screening is validated using these cells it is hypothesised that the pharmaceutical industry will require assays that process 100's to 1000's beating clusters per instance.

5.4.1 Possible Fabrication Approaches

A number of existing microelectronics manufacturing approaches were identified as potentially suitable for the manufacture of the novel MEA biochip concept:

- Integrated Circuit and Micro Electrical Manufacturing Systems (MEMS) Techniques
    - Photolithography
    - Thin Film Deposition
    - Etching (wet)
- Electroplating
- Laser Machining
- Additive Manufacturing (Inkjet Printing)

[Source: Campbell, 1996.]

The following sections describe each approach and the ways in which that approach is suited to the manufacture of the concept.

5.4.1.1 Integrated Circuit and Microelectromechanical Systems (MEMS) Techniques

Microelectromechanical systems (MEMS) are combinations of electrical and mechanical systems with manufactured parts that are less than 1mm in length (Meyer, Bischoff and Feltrin, 2009). The manufacture of MEMS devices draws upon many batch processing technologies that are used in the manufacture of various integrated circuit (IC) devices (Figure 5.16).

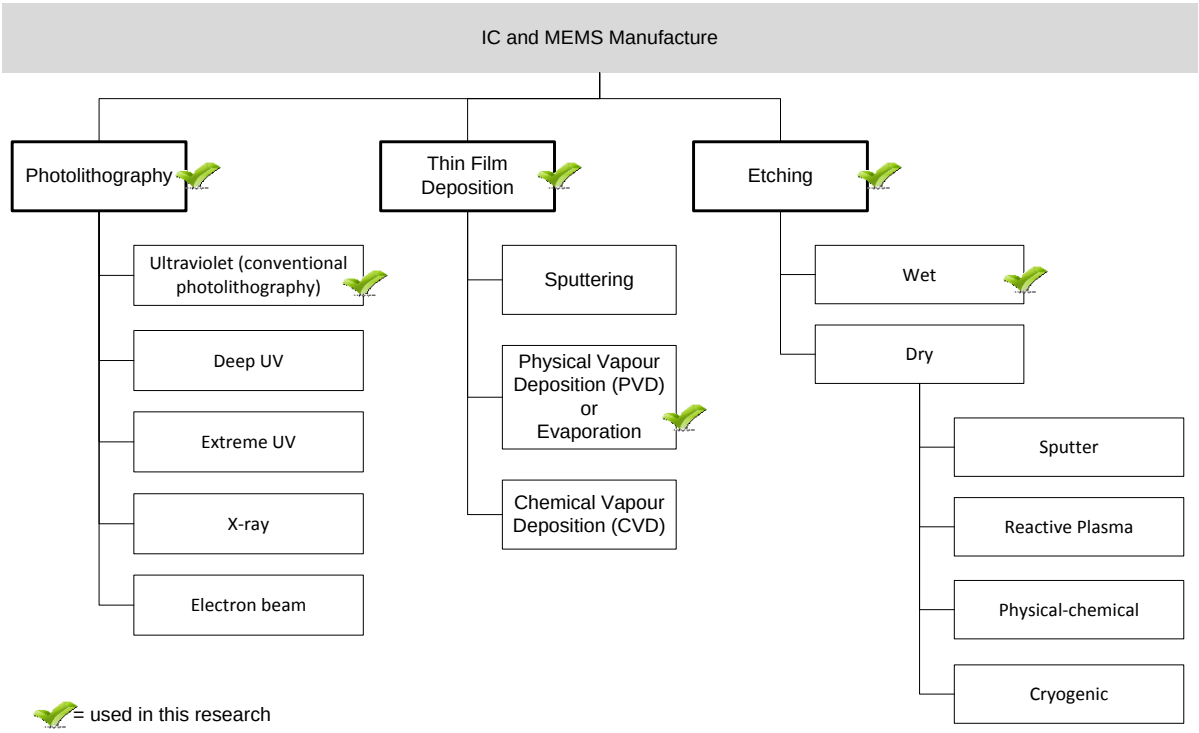


Figure 5.16: Processes used for the manufacture of Integrated Circuits (IC) and Microelectromechanical Systems (MEMS) that are applicable to this research.

This research utilised UV photolithography, e-beam PVD and wet etching throughout the described approaches.

Technique suitability was considered in terms of possible feature size, process complexity, tooling sophistication, facility access and expertise availability (Table 5.2 and Table 5.3), contributing to decision making during manufacturing approach selection and implementation.

Table 5.2: Suitability and accessibility states used in Table 5.3.

Consideration	**Possible States**
Feature Size	≥100µm, <100µm, ≤30µm
Process Complexity	Low, Moderate, High
Tooling Sophistication	Low, Moderate, High
Facility Access	Available, Limited Access, No Access
Expertise Access	Available, Limited Access, No Access

Table 5.3: Technique suitability with regard to this research

Technique	Consideration	State	Comments
IC and MEMS (Conventional photolithography and etching @ LU)	Feature Size	≥100µm	Facility and expertise is readily available at low cost to the research. Proof-of-principle parts can be manufactured in a few days.
	Process Complexity	Low	
	Tooling Sophistication	Low	
	Facility Access	Available	
	Expertise Access	Available	
IC and MEMS (Clean room based thin film deposition @ HWU)	Feature Size	≤30µm	Access can be booked month in advance. Process is time consuming. Additional expertise required to supervise.
	Process Complexity	Low	
	Tooling Sophistication	Moderate	
	Facility Access	Limited Access	
	Expertise Access	Limited Access	
IC and MEMS (Clean room based photolithography and etching @ HWU)	Feature Size	<100µm	Access can be booked month in advance. Variable results as done by hand. Time consuming.
	Process Complexity	Moderate	
	Tooling Sophistication	Moderate	
	Facility Access	Limited Access	
	Expertise Access	Limited Access	
Electroplating	Feature Size	<100µm	Low throughput. Time consuming. Facility to perform batches of ~4 biochips. Electroplating parameters experimental.
	Process Complexity	Moderate	
	Tooling Sophistication	Moderate	
	Facility Access	Available	
	Expertise Access	Limited Access	
Laser Machining	Feature Size	≤30µm	CO_2 and Excimer laser facilities available. High cost. Expertise available.
	Process Complexity	High	
	Tooling Sophistication	High	
	Facility Access	Available	
	Expertise Access	Available	

Additive Manufacture (Inkjet Printing)	Feature Size	≤30µm	Available through collaborative efforts with Printed Electronics Limited. Au inks on glass not optimised.
	Process Complexity	Moderate	
	Tooling Sophistication	High	
	Facility Access	Limited	
	Expertise Access	Limited	

HWU= Heriot Watt University

Conventional photolithography facilities were readily available to this research. Photolithography is used to produce commercial MEA biochips (Heuschkel et al, 2002). This technique has been exploited in this research in clean and non-clean environments.

5.4.1.1.1 Photolithography.

Photolithography has been used in all manufacturing approaches presented in this thesis for the production of the electrode patterns on the upper surface of the MEA biochip base substrates. The exact procedure executed and the materials used differ slightly between manufacturing approaches to suit the needs of concept iterations. However, the overall principles of manufacture via photolithography are consistent (Figure 5.17). The feature sizes possible using different types of lithography (Table 5.4) support the suitability of photolithography for MEA manufacture. The smallest feature sizes present in commercial MEA biochips (section 2.10) is 10µm. Therefore UV photolithography was confirmed as suitable for manufacture of all base substrate electrode patterns where material selection is complementary.

Table 5.4: General characteristics of lithography techniques.

Technique	**Wavelength (nm)**	**Finest feature size (nm)**
Ultraviolet (photolithography)	365	350
Deep UV	193	190
Extreme UV	10-20	30-100
X-ray	0.1-1	20-100
Electron beam	-	80

[Source: Dupas et al, 2004.]

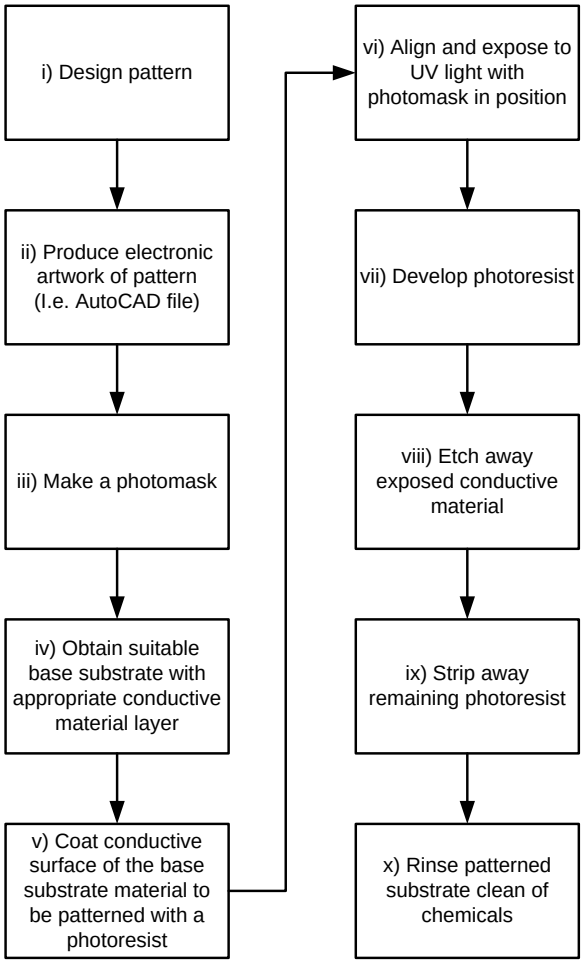


Figure 5.17: Step-by-step photolithography procedure overview. The details at each stage may differ depending upon intended geometries and resolution of the pattern being transferred.

As seen in Figure 5.17, the stages of photolithography are as follows:

The photolithography process involves developing a design concept (i) and an electronic file, called an artwork (Figure 5.18), of that design constructed (ii). A number of CAD and electronic design software are suited to this purpose (e.g. AutoCAD, DipTrace, CadSoft EAGLE). From this file a photomask of the pattern is made (iii). A photomask can be made in either a positive or negative format to suit the photoresist that it is to be manufacture micro electrode array used in conjunction with.

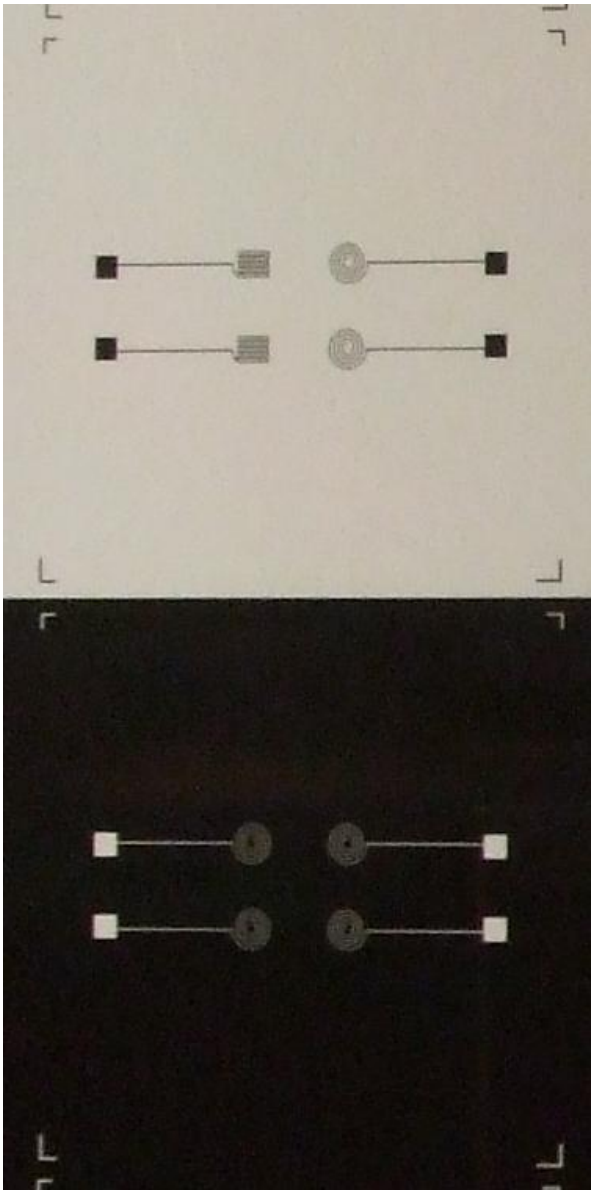


Figure 5.18: Artworks produced for this research in a format to suit either positive or negative photoresists.

A base substrate is selected (iv) that has been previously coated or laminated with a conductive material (e.g. copper, Figure 5.19) is processed to produce the desired conductive pattern on its surface using the photomask. Designers select the conductive material and the thickness of the film (laminated layer) of that material that is required to give appropriate resistivity to the conductive pattern being produced. The process of transferring the pattern from the photomask to the substrate is the photolithography stage of the MEA biochip manufacture. The most common form of lithography (Campbell, 2001), and the type used in this research is optical (photo) lithography. Other types of lithography used in the production of microelectronics include x-ray lithography, electron-beam lithography and ion beam lithography (Kalpakjian and Schmid, 2008).

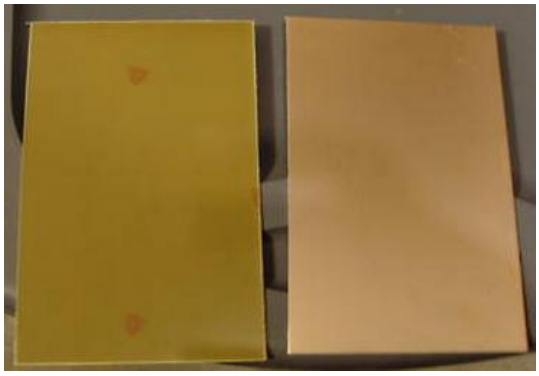


Figure 5.19: Copper laminated FR4 used as base substrate for manufacturing approaches 1, 2 and 3, and consequently Prototype 1.

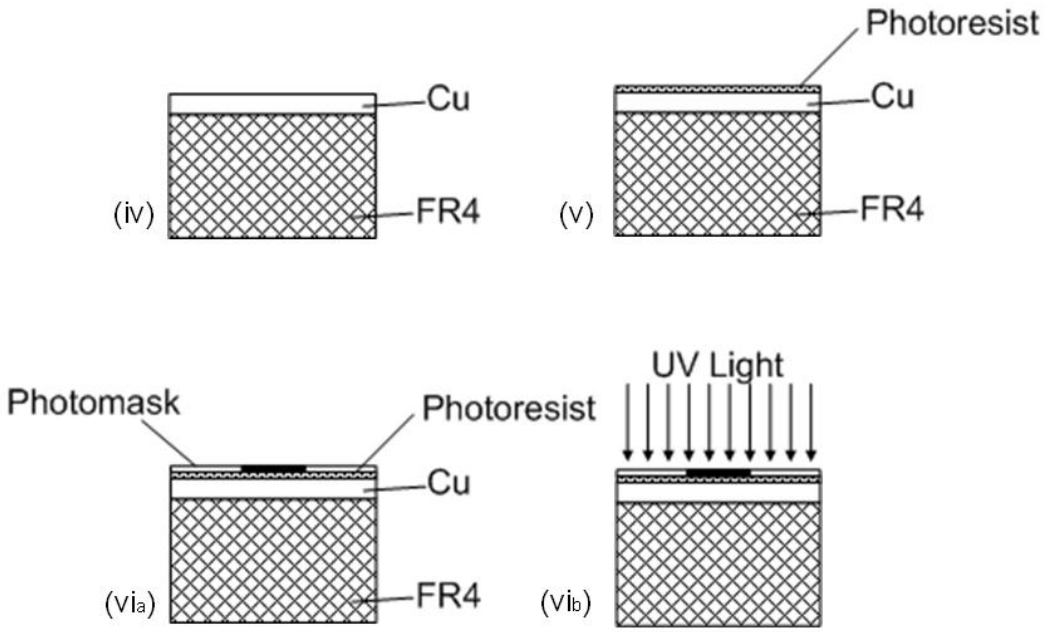


Figure 5.20: Photolithography Stages

A photosensitive material called photoresist is thinly and evenly deposited (e.g. by lamination, spin coating, sputtering) over the conductive surface that is to be processed (v). Photoresists are available in various forms (i.e. liquid, film) that can be classified into positive or negative resists (Figure 5.20). The photoresist coated substrate is positioned with the photomask tightly over the top and given a controlled exposure to UV light (vi). The exposed areas of resist undergo a photochemical reaction resulting in some areas of photoresist being resistant to a developing solution and other areas being easily dissolved by the same solution.

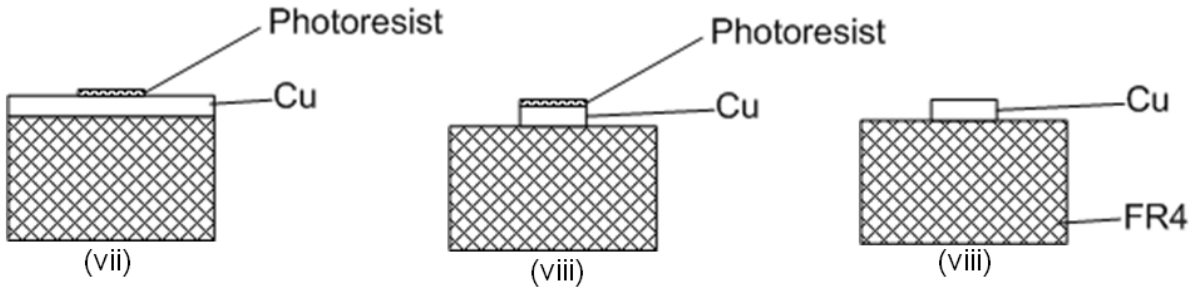


Figure 5.21: Continued photolithography stages.

The exposed samples are developed to remove areas of dissolvable photoresist (vii) resulting in patterned areas of developed photoresist and exposed conductive material. The exposed conductive material is etched away by submerging the sample in an appropriate acidic formulation (viii). When all of the exposed conductive material has been removed the semi-prepared substrates are removed from the acid and immediately rinsed clean under water.

The desired conductive pattern has now been created (Figure 5.21, viii and Figure 5.22) on the substrate surface (viii). Remaining photoresist is removed using an appropriate alkali solution and samples are rinsed clean again (x) and dried.

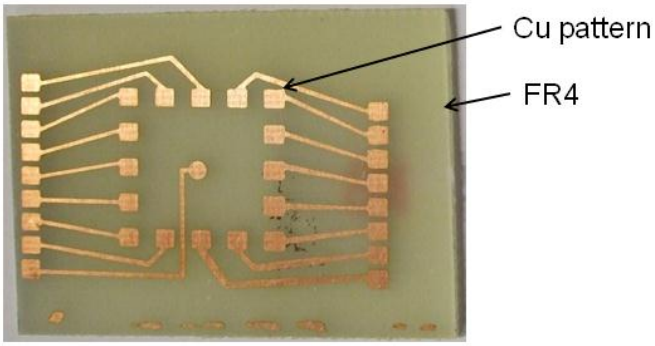


Figure 5.22: An example of a patterned substrate made during this research by photolithography.

There are two kinds of photoresist used in photolithography: positive and negative (Figure 5.23).

In the case of positive resists the area of the resist that is exposed to UV light under goes chemical changes that result in those areas becoming soluble to a particular developing solution. Therefore when the area that has been exposed to UV light is submerged in developer the photoresist covering that area is removed.

In the case of negative resists the area of resist that is exposed to UV light polymerises to more resistant to being dissolved by the developing solution. Therefore it is the unexposed areas of the negative resist that are removed.

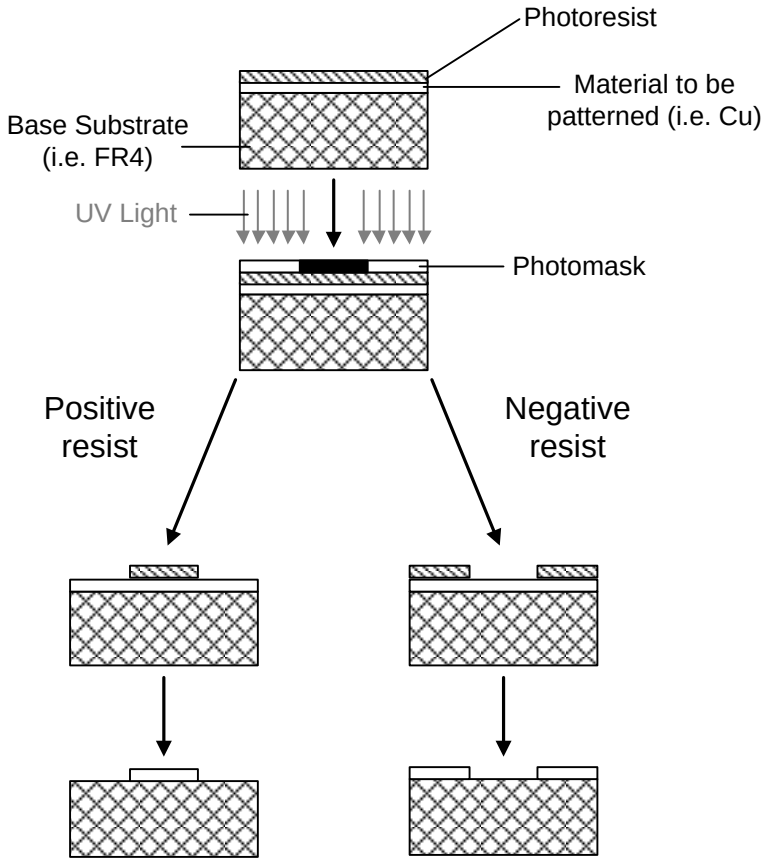


Figure 5.23: Positive and negative photoresists.

Photoresists that have been used in this research are negative Alpha 940 and positive AZ 9260.

5.4.1.1.2 Thin Film Deposition

Films of various materials are thinly deposited over substrate materials for various microelectronics manufacturing requirements, such as patterning, protecting or insulating. The type of material depends upon the function required of the layer and the properties of the material. In the case of conductive films used for circuitry, materials such as titanium, copper and gold, (used in this research) are usually selected for their suitably low resistivity, current carrying ability and suitability for connection.

Thin films may be deposited via a number of deposition techniques. Techniques vary in terms of pressure, temperature and vacuum systems required. The simplest and oldest method of thin film deposition is evaporation. Sputtering and chemical-vapour deposition techniques are also available for film deposition and have not been used in the research presented in this thesis but may be suited to future work.

Physical Vapour Deposition (PVD)/ Evaporation

Involves heating (via a hot filament or electron beam) metal in vacuum to the point of vaporisation (e.g. for Au temperature of vaporisation =~2800^{o}C). The vaporised (or evaporated) metal forms a thin layer on the surfaces of substrates contained within the deposition chamber.

Electron beam deposition was used in this research. Detailed description of this approach is included in section 5.8.2.1).

Sputtering

Involves bombarding a target with high-energy ions (typically Ar^{+}) in a vacuum. As the ions strike the target material atoms are knocked off and deposited on wafers mounted in the system. Sputtering gives a highly uniform coverage (Ueda et al, 2006). Advanced sputtering approaches have been developed using radio-frequency power sources (RF sputtering) and magnetic fields (magnetron sputtering).

Sputtering was not used in this research but may be useful to future work.

Chemical Vapour Deposition (CVD)

In CVD the film of metal covering a substrate is deposited by way of the reaction and/or decomposition of gaseous compounds. There are two different set-ups:

Low pressure chemical vapour deposition (LPCVD): operates at low pressures and is capable at higher production rates than atmospheric pressured CVD. The low pressure approach also provides superior uniformity with lower consumption of carrier gases.

Plasma-enhanced chemical vapour deposition (PECVD): involves processing wafers in an RF plasma containing source gases. The advantage of this process is that it can operate at relatively low temperatures.

These approaches may exploited in future work.

Thin film deposition and this research

Thin film deposition stages for the materials used in this project were carried out commercially in the case of FR4 based prototypes. Cu laminated FR4 is readily available. For the glass based prototypes thin film deposition processes were carried out as part of this research. The specific process used in this work was e-beam deposition of Ti and Au at very low pressure.

5.4.1.1.3 Etching

Once a photoresist pattern has been created on the surface the next stage is to transfer that image into the relevant layer of material (e.g. Cu, Ti) underneath. Etching is used to remove the conductive material and is classified as either dry or wet (Campbell, 1996).

Wet etching involves immersion of the photoresist patterned substrate into an etchant solution. The etchant reacts with the exposed areas removing the material from the substrate into the solution.

Dry etching involves the acceleration of reactive ions towards the base substrate in a low pressure system. Dry etching usually provides improved directionality when compared to wet etching (Kalpakjian and Schmid, 2010) but is typically more expensive (Wilkinson and Rahman, 2004). There are four variants of dry etching: (i) sputter, (ii) reactive plasma, (iii) physical-chemical and (iv) cryogenic. Dry etching has not been used in this research.

This research called upon wet etching of Cu (sections 0, 0, 0), Ti (section 0) and Au (section 0) through various manufacturing approaches designed.

5.4.1.1.4 Clean rooms

Clean room facilities are necessary for the production of IC and MEMS devices due to the scale features produced. Clean rooms serve to remove potentially damaging particles from the manufacturing atmosphere (e.g. dust, smoke, perfume, bacteria). There are varying levels of cleanliness of clean room defined into internationally standardised classes. The system of classification refers to the number of particles within a cubic foot of air that are greater than 0.5µm. Most clean rooms used for microelectronics manufacturing range from class 1 to 10. The size and number of particles are used to classify clean rooms, demonstrated in Figure 5.24.

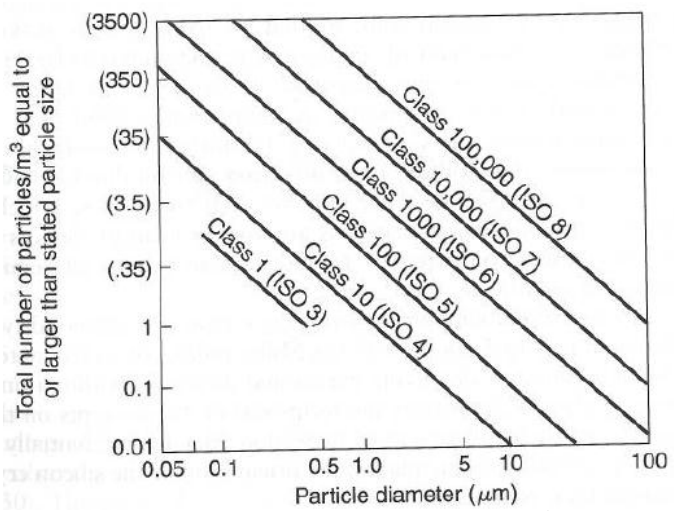


Figure 5.24: Particle size and concentrations of particle per m^3 for clean room classes.
[Source: ISO 14644-1:1999]

A class 1000 clean room was used by this research for the manufacturing approach described in section **Error! Reference source not found.**.

5.4.1.2 Electroplating

Electroplating is the process of coating an article with metal by means of electrolysis (Oxford English Dictionary, 2012). The article that is being plated is referred to as the cathode and the metal being used as the plating material is called the anode. This research has both implemented (section 0) and outsourced (section 0) electroplating.

The electroplating process can be described as follows:

1. Metal ions from the anode are discharged by means of a potential (usually from an external power source) or are delivered in the form of metal slats.
2. The metal ions dissolve into solution.
3. The metal ions move to the cathode where they are deposited on its surface.

[Adapted from Kalpakjian and Schmid, 2010]

Copper (Cu) is commonly the coated material where electroplating is used. Cu has been electroplated with gold (Au) in this research to meet biocompatibility requirements. A typical electroplating process involves the application of a DC voltage between the article that is to be plated (cathode), and a source of the material that is to be deposited (anode). Both the cathode and the anode are submerged in a conductive electrolyte solution. Application of voltage results in the

metal ions of the anode migrating to the cathode. Upon reaching the cathode's surface the ions lose their charge and are consequently deposited on the surface.

Plating can be controlled to produce high quality end results. Appropriate selection of a combination of variables is required for controlled plating. Those variables are:

- the electrolyte and concentration of its various dissolved components,
- the temperature of the electroplating bath,
- the electrical voltage and current.

Variables are interrelated increasing process complexity and making the process of control a more challenging problem (DeGermo, Black and Kohser, 1999). Investigation of appropriate control of the above variables has been a challenge encountered by this research.

5.4.1.2.1 Surface preparation for electroplating

Preparation of the surfaces prior to plating is especially important to quality of the final surface. Holes, scratches, and defects must be removed if a smooth end result is to be achieved. Degreasing, cleaning and pickling can be used to ensure a chemically clean surface prior to plating.

5.4.1.2.2 Part geometry in electroplating

The geometry of the part to be plated also influences the final surface shape and finish. Plated metal tends to be preferentially attracted to corners and protrusions and as such design recommendations exist for parts to be electroplated (Figure 5.25).

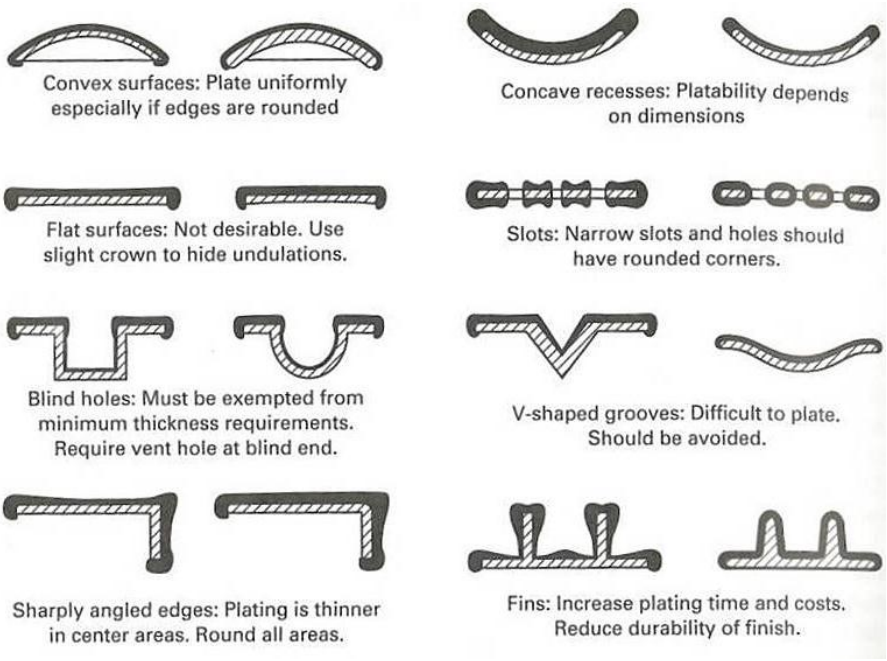


Figure 5.25: Design Recommendations for Electroplating Operations. (Source: DeGarmo, Black and Kosher, 1999)

Electroplating has been used in this research to pursue a manufacturing approach based on via (through) hole filling approaches used in layered PCB construction (section 5.6.1). The geometry of the feature electroplated in section 5.6.1 is a blind hole.

5.4.1.3 Laser-beam Machining

Laser-beam machining (LBM) uses various sources of focused, high-density energy as tools that can melt and vaporise a target's material in a controlled manner (Dubey and Yadava, 2008). LBM can be used to process metallic and non-metallic materials. In this research lasers are used in conjunction with non-metallic materials. Different lasers are advised for different purposes (Table 5.5).

Table 5.5: General applications of lasers in manufacturing.

Application	**Laser Type**
Cutting	
Metals	Pulsed CO_2, Continuous Wave CO_2, Nd:YAG, ruby
Plastics	Continuous Wave CO_2
Ceramics	Pulsed CO_2
Drilling	
Metals	Pulsed CO_2, Nd:YAG, Nd:glass, ruby
Plastics	Excimer
Marking	
Metals	Pulsed CO_2, Nd:YAG
Plastics	Excimer
Ceramics	Excimer
Surface Treatment	Continuous Wave CO_2
Welding	
Metals	Pulsed CO_2, Continuous Wave CO_2, Nd:YAG, Nd:glass, ruby, Diode
Plastics	Diode, Nd:YAG

CO_2 = Carbon dioxide, Nd:YAG = neodymium: yttrium-aluminium-garnet, Nd:glass = neodymium: glass.
[Source: Kalpakjian and Schmid, 2010.]

The outcomes of LBM are affected by the physical parameters of the surface of the material that is to be processed. Reflectivity and thermal conductivity influence LBM outcome. The lower the reflectivity and thermal conductivity the more efficient the process (Kalpakjian and Schmid, 2008).

Gas streams: Many lasers are used in conjunction with gas streams (e.g. oxygen, nitrogen, argon) to leave oxide free surfaces and edges. Gas streams also have the important function of blowing away molten and vaporised material from the surface of the workpiece.

Carbon dioxide (CO_2) laser: The CO_2 laser is one of the most efficient and powerful lasers used industrially for cutting and welding. The laser consists of 10-20% carbon dioxide, 10-20% nitrogen, a few per cent hydrogen, and the remaining helium (Hummel, 2011).

The smallest comparable features that can be manufactured using a CO_2 laser have been 12.6µm (Longsine-Parker and Han, 2012) supporting the use in this application setting.

Excimer lasers: Are used across materials processing, medical devices and research and development settings (Csele, 2004). The term Excimer laser does not describe a single device but a family of lasers (Table 5.6) that possess comparable output characteristics (Hecht, 1992). Excimer systems work by using pulses of short wavelengths such as ArF (193nm), KrF (248nm), XeCl (308nm) and XeF (351nm). The pulses are short in duration (3-10 ns) and high in energy. The pulses interact rapidly with the sample material resulting in material ablation at the surface (Lee and Wu, 2007). Excimer lasers have been reported in use for the fabrication of components with feature sizes between 0.05µm -1mm (Gower, 2001).

Table 5.6: The excimer laser family gas mixtures.

Excimer Species	Halogen	Inert gas	Balance
KrF	0.2% fluorine	5% krypton	Helium
	0.1% fluorine	2% krypton	Neon
ArF	0.23% fluorine	14% argon	Helium

	0.1% fluorine	4% argon	Neon
XeF	0.39% fluorine	0.75% xenon	Helium
	0.15% fluorine	0.35% xenon	Neon
XeCl	0.06% hydrogen chloride	1.5% xenon	Helium
	0.06% hydrogen chloride	1.5% xenon	Neon

[Source: Csele, 2004.]

The excimer laser type used in this research was KrF (section **Error! Reference source not found.**).

5.4.1.4 Additive Manufacturing (Inkjet Printing)

Inkjet printing is used in manufacturing because of its high speed and accuracy (Fahad, 2011). The material processed in inkjet printing presently exhibit poor mechanical properties. The functionality of printed products is therefore limited.

Inkjet printing has however been applied across a diversity of applications including industrial, textile, bio-medical, and electronics manufacturing.

Electronic applications of inkjet printing include (Fahad, 2011):

i. Manufacture of printed circuit boards (PCB),
ii. Manufacture of polymer light emitting diodes (PLED)
iii. Manufacture of colour filters for liquid crystal displays (LCD)
iv. Manufacture of organic thin film transistors (OTFT).

Inkjet printing is classified into two categories: Continuous Inkjet Printing (CIJ) and Drop-on-Demand Inkjet Printing (DOD).

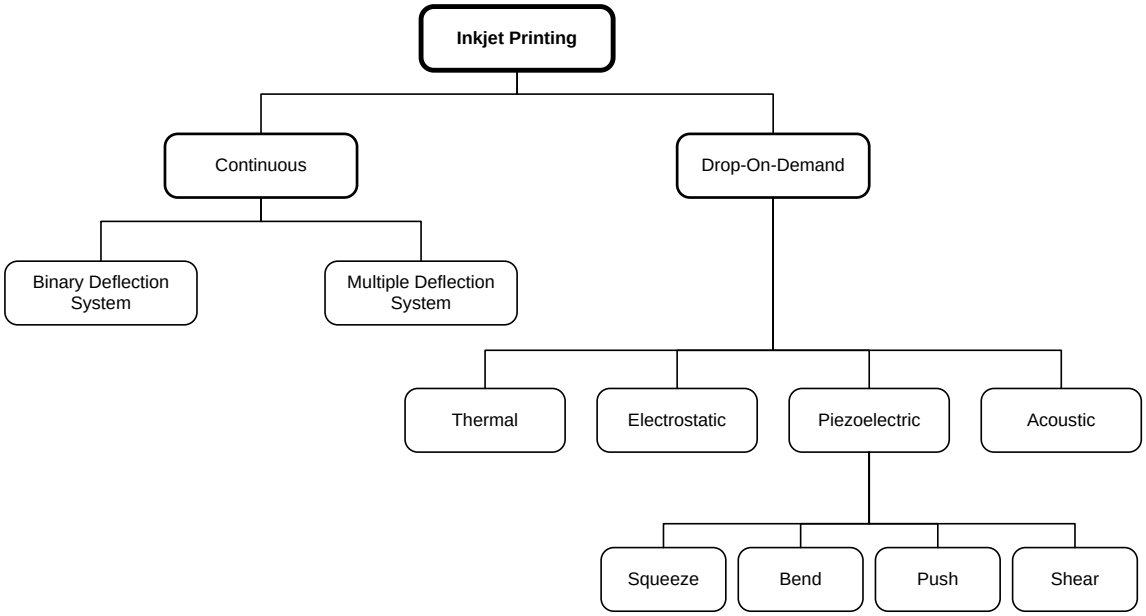


Figure 5.26: Categories of Inkjet Jet Printing.

[Source: Le, 1998]

CIJ is the provision of a steady stream of droplets onto the substrate at a controlled rate. These droplets are produced and controlled by the continuous vibration of a piezoelectric crystal inside the printer head. The vibration creates a pressure wave inside the chamber where the ink is held forcing a droplet out of the nozzle (Fahad, 2011).

DOD sends a signal to the nozzle only when needed. DOD is categorised according to the actuation system used: electrostatic, acoustic, thermal and piezoelectric.

A wide variety of materials have been printed using inkjet technology (e.g. polymers, organic solvents, ceramic suspensions, nanoparticle materials, molten metal, biological materials).

Properties that will influence the final quality of the print include:

- Surface tension
- Viscosity
- Molecular weight
- Concentration of the polymer (in polymer based suspensions or solutions).

Inkjet printing is promising in electronics manufacture due to the ability to pattern high-purity electrically functional materials without the need for a mask (Sekitani et al, 2008). It is the facility to print PCBs that was pursued as an avenue of interest to this research. The feasibility of using inkjet printing was considered in collaboration with PEL Printed Electronics Limited (Invotec Group, Tamworth, UK). Further details are presented in section 5.9.

5.4.2 Selection of manufacturing Approaches for Implementation

The major factors (Figure 5.27) influencing which manufacturing approaches should be implemented were materials suitability, fabrication technique suitability, resolutions and geometries limitations, suitability for sterilisation and cleaning, and robustness.

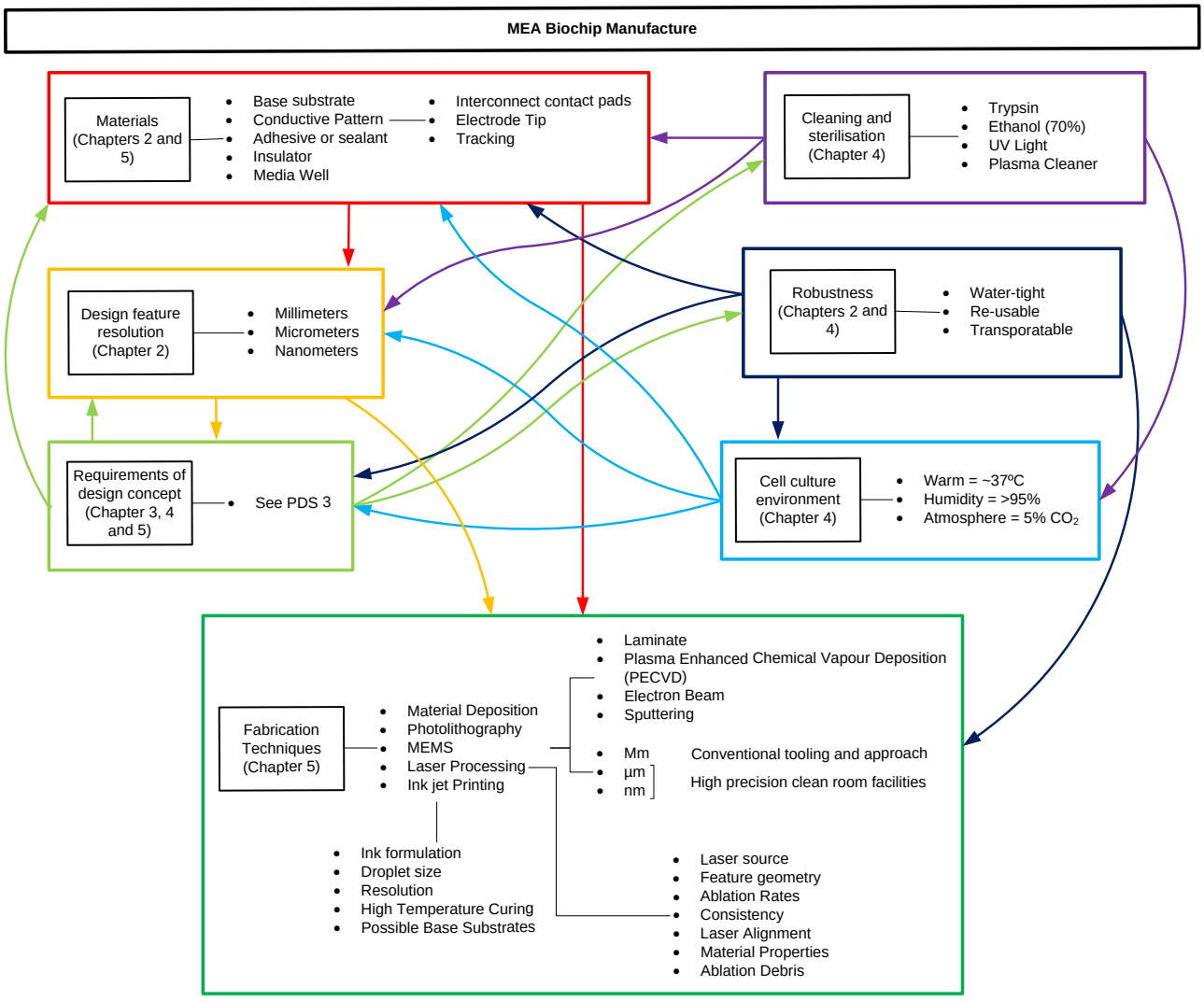


Figure 5.27: Factors Influencing Manufacturing Approach Selection.

Note: The MEA biochip must be made to be **robust** enough to withstand repeated use, exposure to prolonged periods in humidified incubation and the repeated cleaning and sterilisation procedures associated to that use without failure.

5.4.2.1 Materials Selection

Appropriate material selection for MEA biochip sub-components was based upon the materials demonstrated in comparable applications, material properties, processing technique suitability, availability and costs.

5.4.2.1.1 Conductive Pattern and Electrode Tips

Copper, gold, platinum, titanium, ITO and TiN were considered as potential materials for the conductive pattern and electrode tips of the novel MEA biochips designed and manufactured during this research.

Gold: Is used for areas of the electrode surface that are exposed to the cellular environment throughout derived design concepts as a wealth of literature reviewed had previously demonstrated its successful application in numerous similar biochip designs (Nam et al, 2004; Held et al, 2010a). Au is biocompatible (Chen, 2011) and was favoured over ITO and TiN because the processing of Au using photolithography methods is more mature with a vast amount of published information available to support experimentation. Suitable facilities and access to expertise (PMD Plating, Coventry) supported the use of Au during this project.

Au was selected for use in all of the manufacturing approaches that were designed by this research as the cell-electrode surface interface material.

Indium tin oxide (ITO or tin-doped indium oxide): Is a semiconductor made from a mixture of typically 90% indium oxide (In_2O_3) and 10% tin oxide (SnO_2) and is exploited in the manufacture of a number of contemporary electronic devices (e.g. flat panel displays, solar cells). ITO exhibits good transparency, low electrical resistance and excellent surface adhesion (Damiani and Mansano, 2007).

ITO also demonstrates good physical and chemical stability (Eisgruber et al, 1999) making it a suitable candidate material for transparent tracking in MEA biochip manufacture. Thin films of ITO are required in MEA manufacture. A selection of techniques can be used to deposit films of ITO: (i) Thermal evaporation deposition, (ii) Direct current (dc) and radio frequency (rf) magnetron sputtering, (iii) Electron-beam evaporation, (iv) Spray Pyrolysis, (v) Chemical Vapour Deposition (CVD), (vi) Dip coating, or (vii) Pulsed Laser Deposition (Singh, 2006). Of these, sputtering is one of

the most versatile methods with the advantage of providing uniform thin films reproducibly (Singh, 2006).

The research presented in this thesis has not used ITO in designs but future design iterations may be suited to this material.

Titanium Nitride (TiN): A surface is coated in TiN by reactive sputtering and CVD with titanium tetrachloride as the metal source and either nitrogen gas or ammonia gas as a source of nitrogen (Pierson, 1996). TiN is ideal for MEA biochip electrodes as it is biocompatible, stable in aqueous solutions, and has high abrasion and corrosion resistances (Watari et al, 2004)

The research presented in this thesis has not used TiN in designs but future design iterations may be suited to this material.

Electrode Material Combinations: Combinations of metals are use in manufacture to reduce costs and improve quality and performance. In this research the combinations Cu/Au and Ti/Au were used for the conductive patterns and electrodes of the prototype biochips produced.

Cu/Au combinations were used to reduce costs in prototype one. Ti/Au was used in prototype two A and B as Au on glass required an initial seed layer of Ti to ensure high quality coating on the glass surface.

Using thin film deposition, glass substrates were coated with titanium (Ti) as a seed layer and then gold (Au).

5.4.2.1.2 Base Substrate and Media Well

The polymer-based material FR4 was used in initial prototyping (sections 5.6.1, 5.6.2, and 5.6.3) as it was suitable for manufacturing approaches available and the FR4 and Cu layer were designed such that there would be no contact with the live matter. Glass was also used as a base substrate and media well material. Glass was selected due to its superior optical qualities as when compared to transparent and translucent plastics (Thibaud et al, 2005), as well as its suitability and durability for repeated processing and sterilisation. In addition to that glass is also bio-inert making it suitable for chronic cell culture.

5.4.2.2 Bioscience Influences

Selection and implementation of the varying manufacturing approaches has also been influenced by the bioscientific application environment. General observations identified during case study construction influenced decision making.

An example of an influence from observation of the bioscientific environment in which the device is to be applied that may have affected component geometry decisions or compromises is that the amount of space in a tissue culture hood. This space is limited and the specific tooling used during cell seeding is of particular dimensions. Awareness of these factors (that will influence how the user interacts with the biochip) helps to make appropriate design compromises when defining component geometries.

Influences that were specific to using SC-CMs were also central to design decisions. An example of an observation that influenced material selection was awareness of UoN users' observations that SC-CMs attach differently to different materials. During material selection certain materials were favoured over others if they were suited to the manufacturing workflow under development (e.g. UoN users have observed that polyester can facilitate better cell attachment than glass which is also supported by the work of Jiao and Cui (2007) who demonstrated the importance of the surface interface on attachment using osteoblast cells).

In the initial biochip design novelty was favoured over optical assessment capability. This compromise was made by UoN users; a high quality signal for analysis was considered more important than good optical assessment.

5.4.3 Section Summary

A number manufacturing approaches can be used to produce the feature geometry specified in PDS 3. Decisions relating to which approach to pursue in the following section of this chapter are based upon material suitability, facilities availability, expertise, time and reducing the cost of manufacture.

This research called upon combinations of the listed manufacturing techniques while attempting to manufacture the concept designed to meet the needs of real users:

- MEMs approaches – in the form of UV photolithography, physical vapour deposition and etching
- electroplating
- laser machining
- inkjet printing

5.5 Manufacturing Approach Implementation

A design concept was conceived (Figure 5.13) using PDS3 and attention was directed toward planning a method of manufacture. An overview of the manufacturing approaches planned and implemented during this research is described in Figure 5.29. The manufacturing approaches were designed and implemented as workflows (Table 5.7). A period of concurrent manufacturing development occurred until a suitable prototype was achieved (section 5.6.3).

The prototypes were tested using live SC-CMs, and initial user feedback collected which led to a re-evaluation of the MEA biochip design concept, resulting in a different second generation design concept.

Clean room based manufacture was required to produce a finer conductive pattern in the second MEA biochip design concept. The new design, concept 2, was manufactured with two different electrode iterations, A and B (Figure 5.28, section 5.8). These prototypes were also tested using live SC-CMs (Chapter 6).

The remainder of this chapter describes how each manufacturing approach was planned and implemented, and the general success of each approach in terms of critical manufacturing outcomes. Detailed results of each manufacturing approach are presented in Chapter 6.

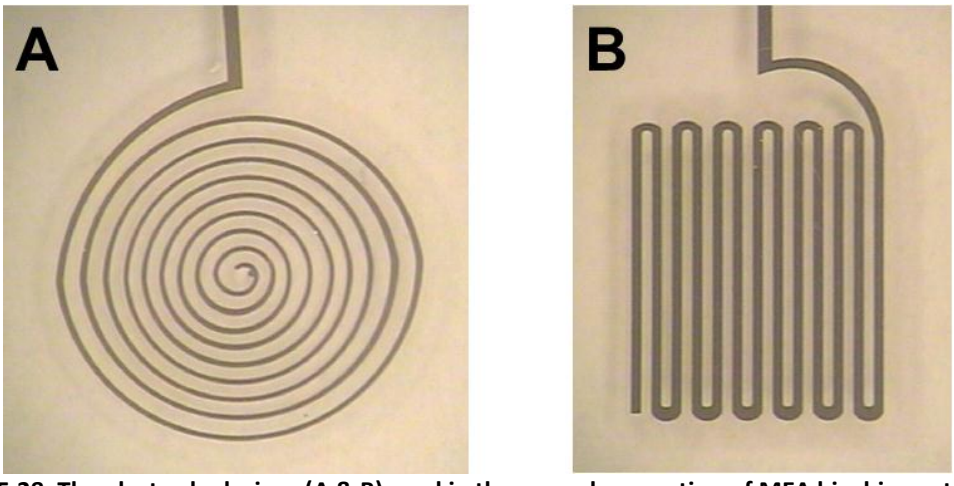


Figure 5.28: The electrode designs (A & B) used in the second generation of MEA biochip prototypes.

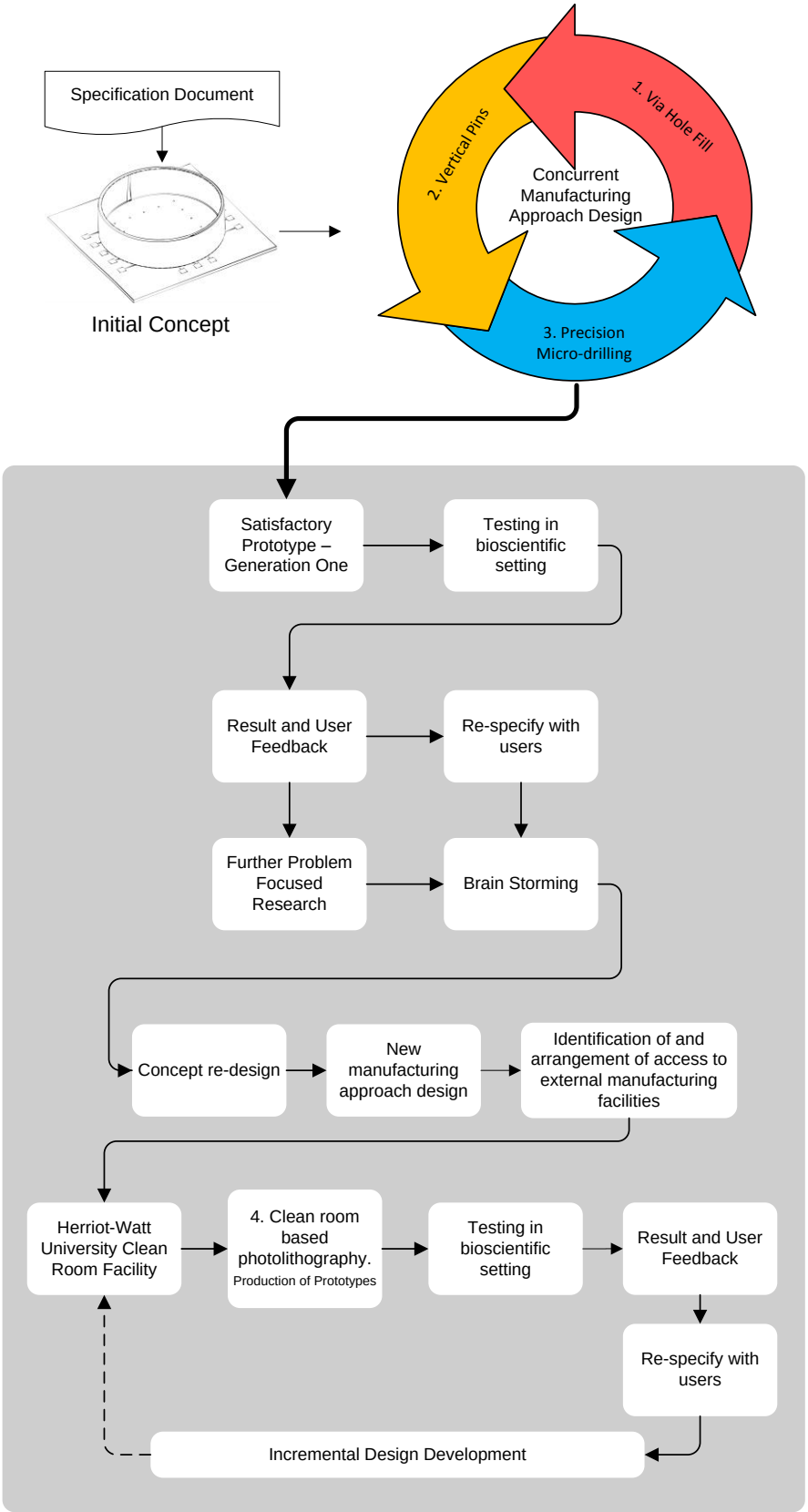
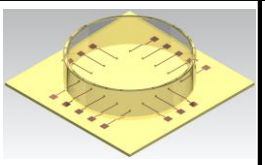
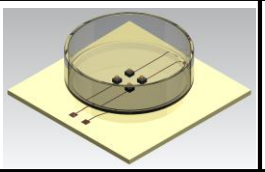
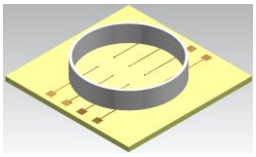
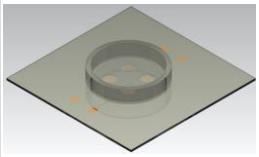


Figure 5.29: The design cycle used throughout this work. Three initial manufacturing approaches were designed and progressively adapted until a prototype of satisfactory quality was achieved for testing. Following feedback from that testing a new approach was adopted for the next generation of prototype.

5.6 Prototype One Manufacturing

Four manufacturing approaches were implemented by this research with differing levels of success. Each manufacturing approach described in the following text was implemented successively, and for a time concurrently, building upon the successes and failures of the previous approach until a satisfactory prototype was achieved. Table 5.7 summarises each approach explored and visualises the planned outcome.

Table 5.7: A brief summary of manufacturing approaches designed and implemented in pursuit of functioning prototypes.

Approach	1. Via Hole Fill	2. Vertical Pins	3. Micro drilling	4. Clean room based Photolithography
Concept Design				
Progress	Failed at manufacture	Failed at manufacture	Generation One Prototype	Generation Two Prototypes A and B
Section in this thesis	5.6.1	5.6.2	5.6.3	5.8

Where an approach is investigated via a number of tangents the details of those tangents are also described. The success of the manufacturing approach in terms of production of a prototype is stated in Table 5.7. Manufacturing approach success was considered and compared in terms of the following critical component outcomes (Table 5.8).

Table 5.8: Outcomes critical to successful manufacture of an MEA biochip. These critical outcomes were also prioritised with respect to interdependencies.

Priority	Critical Manufacturing Outcome
1	Produce satisfactory base substrate
2	Produce satisfactory media well
3	Facilitate adequate attachment of the media well and base substrate components
4	Produce a satisfactory electrode site for cell-electrode interfacing
5	Produce an appropriate micro-well geometry around electrodes
6	Produce enough sites so as to allow assignment of a reference
7	Produce an MEA biochip that allows appropriate optical inspection of living samples

5.6.1 Manufacturing Approach One: Via hole filling (Electroplating).

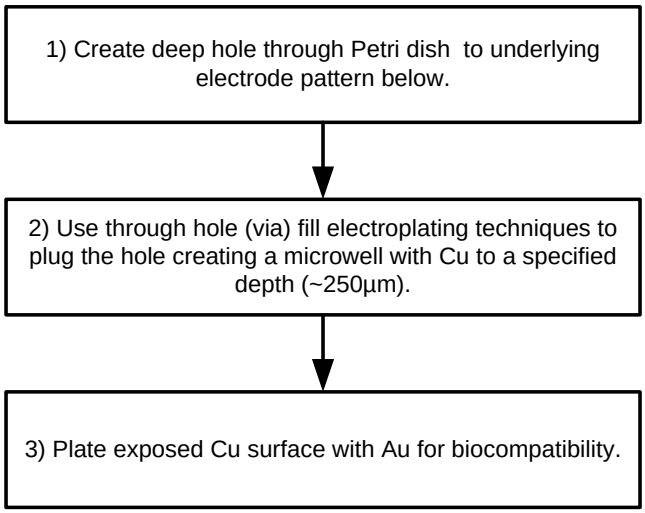


Figure 5.30: The intention of the via hole fill manufacturing approach.

The first manufacturing approach exploited a well-defined and extensively demonstrated process used throughout printed circuit board (PCB) manufacturing. An electroplating process is used to fill "vias" and "through holes" in layered printed circuit boards (PCBs). The same principle was utilised as a method of filling 500µm diameter holes drilled through a 35mm diameter Petri dish previously adhered over an underlying electrode pattern. Each hole was to be filled to a controlled depth, resulting in a recession ~250µm deep. Electroplating was used to fill the hole/ a filling tolerance of ~10µm was aimed for. The intention was to create a microwell feature into which the beating cluster would be seeded and "held" during the physical attachment period. The intention of this manufacturing approach is seen in Figure 5.30 and Figure 5.31.

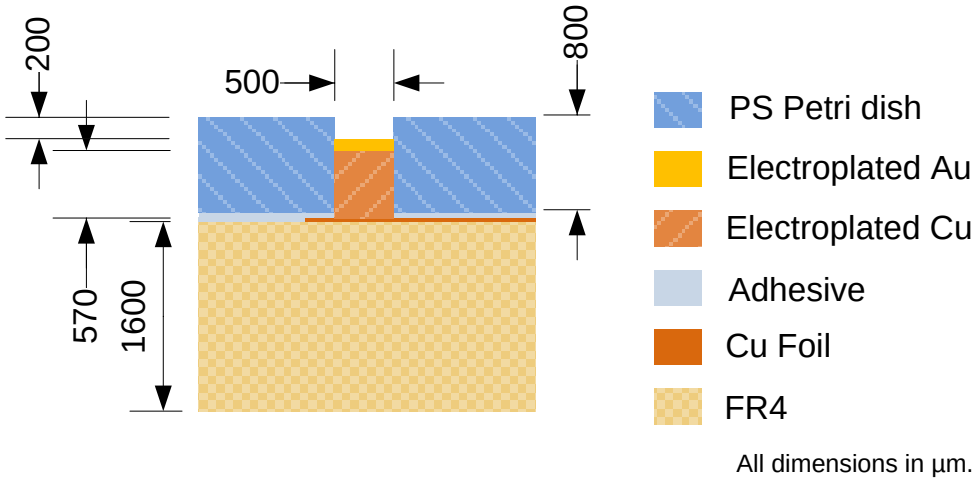


Figure 5.31: A schematic of the intended cross-section though the electroplated microwell.

5.6.1.1 Manufacturing Approach One Description

On overview of this manufacturing approach can be seen in Figure 5.32. The CIMOSA modelling technique described in Chapter 3 and demonstrated in Chapter 4 has been used to construct these manufacturing approach models.

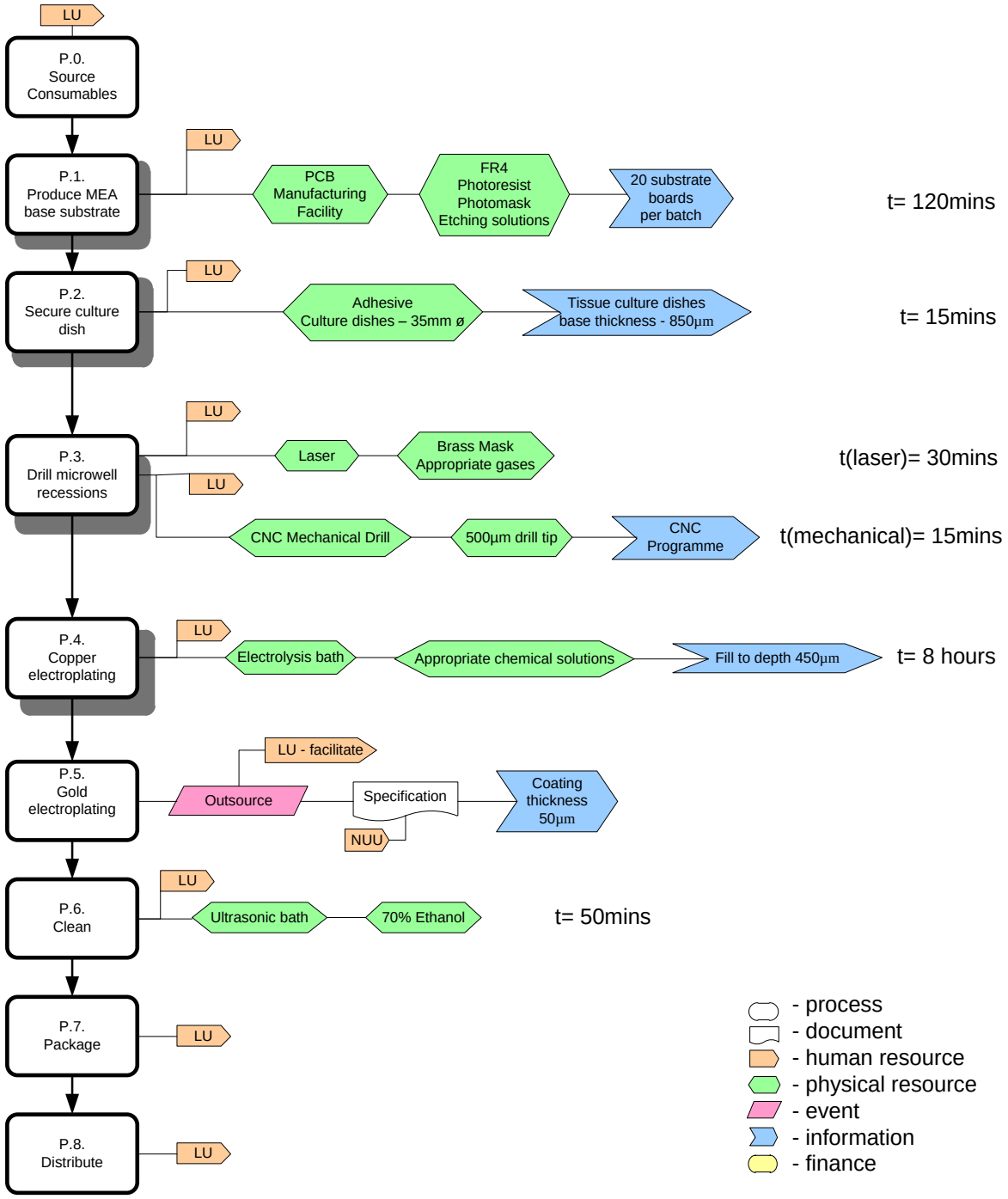


Figure 5.32: A CIMOSA activity diagram representing the major processes attempted for the via hole filling manufacturing workflow design.

The processes physically implemented for this manufacturing approach are:

- P.1. – production of base substrate,
- P.2. – securing of media well (35mm culture dish),
- P.3. – drilling of the microwell recessions,
- P.4. – copper electroplating processes (via hole filling).

Process One – Production of base substrate

Base substrates were produced using photolithography (section 5.4.1.1.1). The precise approach implemented for this process is documented in the activity model below (Figure 5.35).

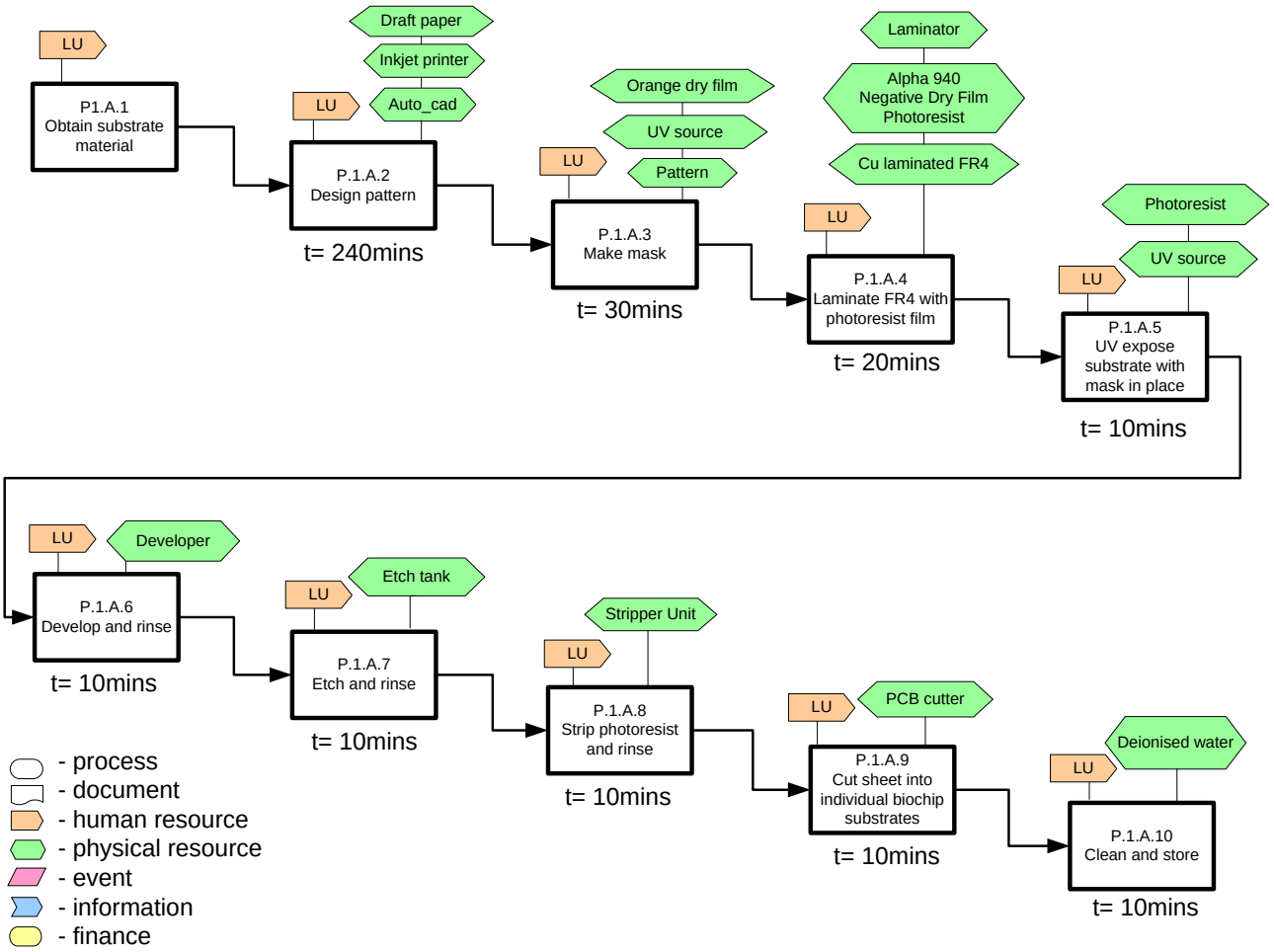


Figure 5.33: A CIMOSA activity diagram of the photolithography process used to manufacture Cu patterned base substrates for the novel MEA biochip design.

For the activities A.1 - A.3 the materials required for this approach were purchased and a conductive pattern was designed using AutoCAD software (Autodesk, Inc., CA, USA). The pattern was printed onto to draft paper using a deskjet printer. The printed pattern was transferred to orange dry peel film by UV exposure resulting in a photomask to scale (Figure 5.34).

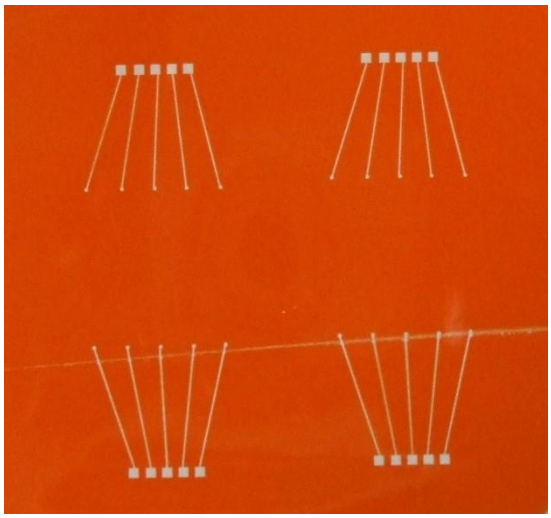


Figure 5.34: An orange dry peel film photomask used in the manufacture of base substrates for the via fill approach.

During activity A.4, an A4 sized sheet of Cu coated (35µm) FR4 (Figure 5.19) was laminated by hand with a negative dry film photoresist, Alpha 940 (Figure 5.35) on one side by passing through an Albyco PhotoPro33 laminator (Figure 5.36) set at 115°C. A roll through speed of 350mm/min was used to ensure a uniform covering.

Figure 5.35: Aplha 940 negative dry film photoresist.

During activity A.5, the photomask was then secured over the laminated substrate and the combined sheet exposed to UV light using a Parker Graphics UV exposure unit (Figure 5.37). Exposures were 9 seconds in duration at full power.

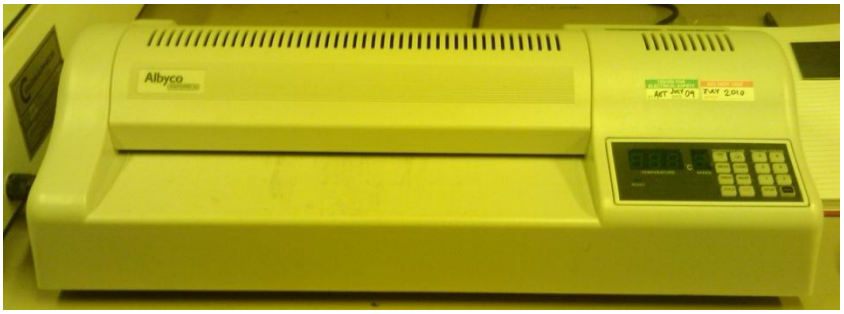


Figure 5.36: The Albyco laminator used to cover the FR4 substrates with the negative dry film photoresist.

Figure 5.37: The Parker Graphics UV exposure unit used for UV exposure.

During activity A.6, the exposed sheets were passed through a film developing tank (Figure 5.38). A Roller speed setting 6 (~0.2m/min) was used to pass the sheets through jets of the alkaline K_2CO_3 developing solution (1% potassium carbonate). The temperature of the solution was 35°C. Developed sheets were rinsed under tap water and dried before etching.

Figure 5.38: The dry film developing unit.

To etch the exposed areas of Cu, as occurs in A.7, the developed sheets were passed through a Mega Electronics etching machine (Figure 5.39) containing a ferric chloride solution. The substrate sheets were rinsed under tap water and dried.

Figure 5.39: The Mega Electronics etching machine.

Throughout activities A.8 – A.10 the developed photoresist was then stripped from each sheet using the stripping well portion of a Circuitape Circuit 4 Processor (Figure 5.40). Sheets were rinsed under jets of distilled water following stripping. The resulting patterned sheets were cut into individual biochip substrates by hand (Figure 5.41).

Figure 5.40: The Circuitape Circuit 4 Processor used for photoresist stripping following Cu etching.

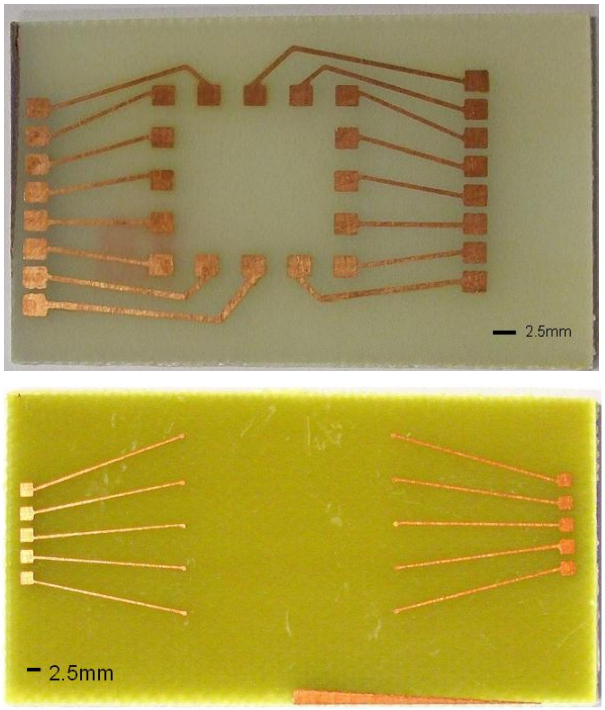


Figure 5.41: Individual patterned FR4 substrates manufactured for Via hole filling approach experimentation. Top: Larger resolutions used during initial process experimentation. Bottom: Ensuing finer resolutions.

Process Two - Securing of media well

An example of the commercially available Petri dishes utilised in experiments is seen in Figure 5.42.

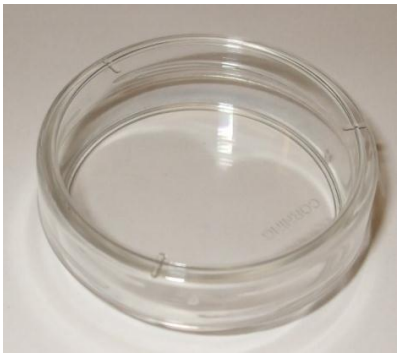


Figure 5.42: A 35mm diameter Petri dish made by Corning, USA.

The process used to secure the commercially available 35mm diameter Petri dish is documented in Figure 5.43. This process was completed by-hand for speed and convenience as small batches were required to experiment with critical approach parameters (e.g. the electroplating parameters were more important to spend time on than precise positioning of the Petri dish at this point).

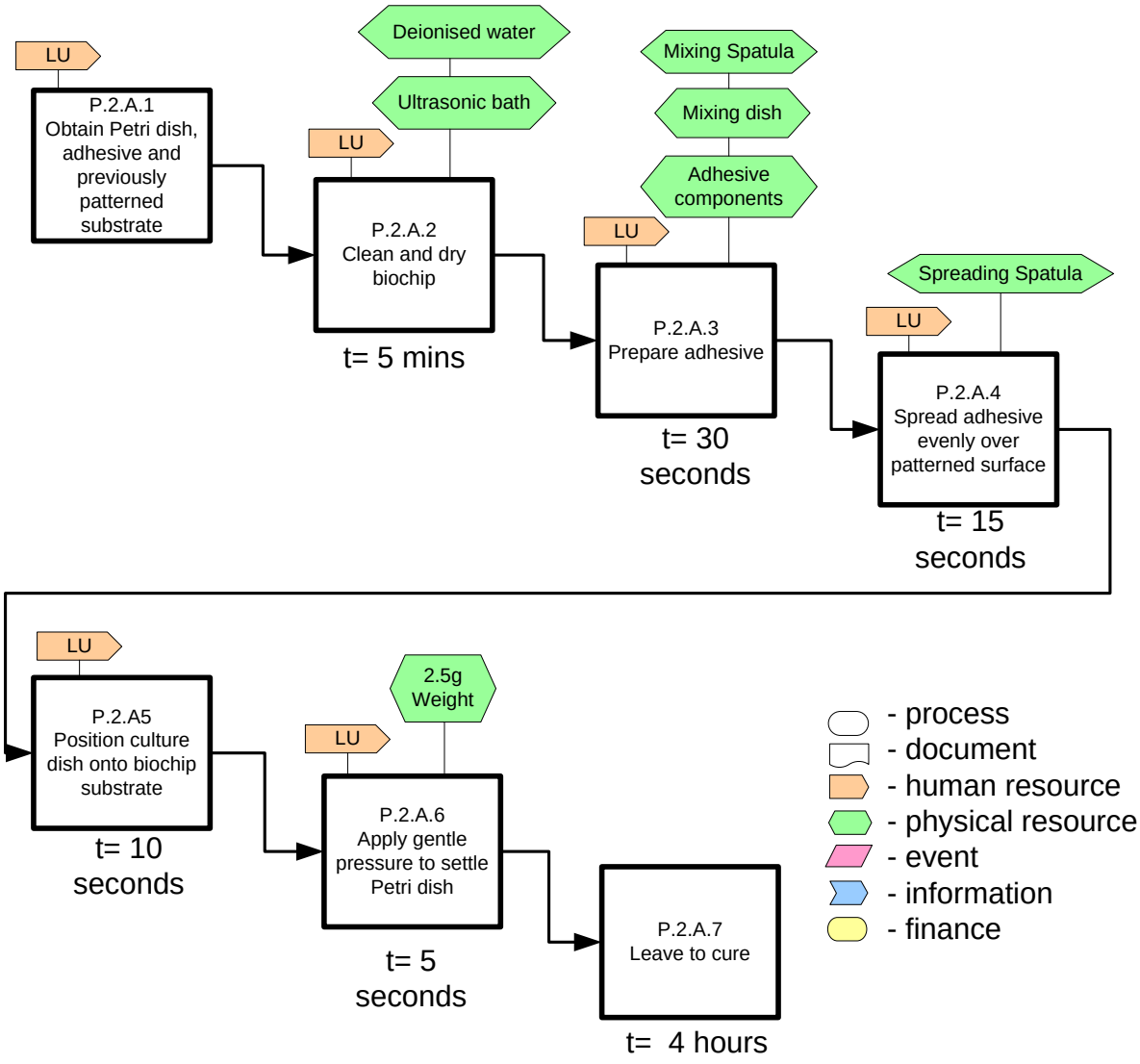


Figure 5.43: The activities (carried out by-hand) to secure the Petri dish onto the base substrate.

During activities A.1 – A.7, the materials required were gathered and the biochip components cleaned. The two part adhesive was mixed in a small disposable vessel and an approximate amount added to the centre of the biochip substrate. The Petri dish carefully positioned over the base substrate and a gentle, even force was applied to spread the adhesive between the dish and substrate. A circular 25g weight was placed into the Petri dish and the part was left for ~24hours to cure.

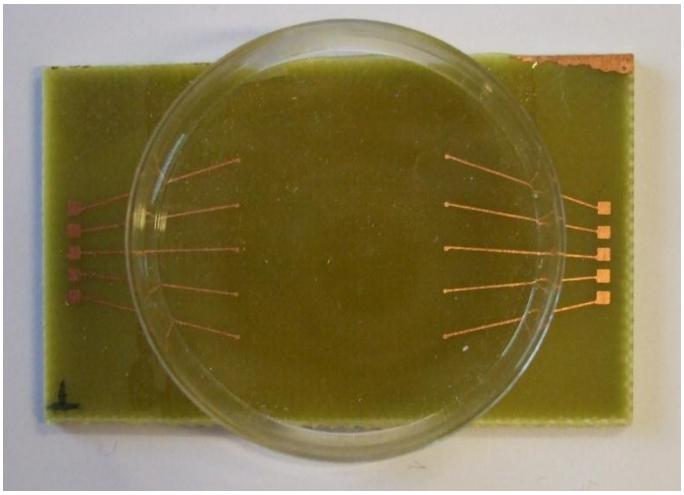


Figure 5.44: A biochip base substrate with Petri dish adhered prior to drilling.

The adhesive used in the proof-of-principle tests described was transparent epoxy-based resin (Figure 5.44), Epoxicure™, manufactured by Buehler (Dusseldorf, Germany). The recommended cure time at room temperature, 6 – 8 hours, was exceeded in all devices to ensure the resin was completely cured prior to further processing. The ratio of resin to hardener was 5 parts resin to 1 part hardener.

Process Three - Drilling of microwell recessions

The process used to create the holes that would subsequently be filled to a controlled depth to result in microwells was investigated using two different methods (Figure 5.45). Computer numerically controlled (CNC) drilling and CO_2 laser beam machining (LBM) approaches are described.

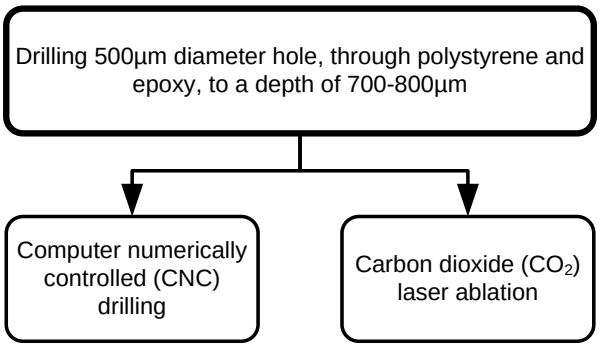


Figure 5.45: Two approaches investigated for the creation of the microwell recessions.

Drilling specification:

- 16x 500µm Ø holes through the polystyrene dish and adhesive to the underlying Cu surface,

- in a square arrangement, spaced 5mm apart (Figure 5.46).

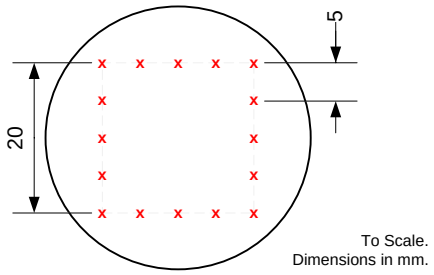


Figure 5.46: The drilling diagram for CNC processing.

Process Three by Computer Numerically Controlled (CNC) Drilling: The CNC drilling procedure (Figure 5.48) exploited to produce holes through a polystyrene Petri dish and epoxy resin to an underlying Cu electrode pattern was carried out by high-skilled workshop technicians to aforementioned specification (Figure 5.46) using a Hurco VM1 3 axis milling machine (Figure 5.47).

Figure 5.47: The Hurco VM1 CNC milling machine used in this research.

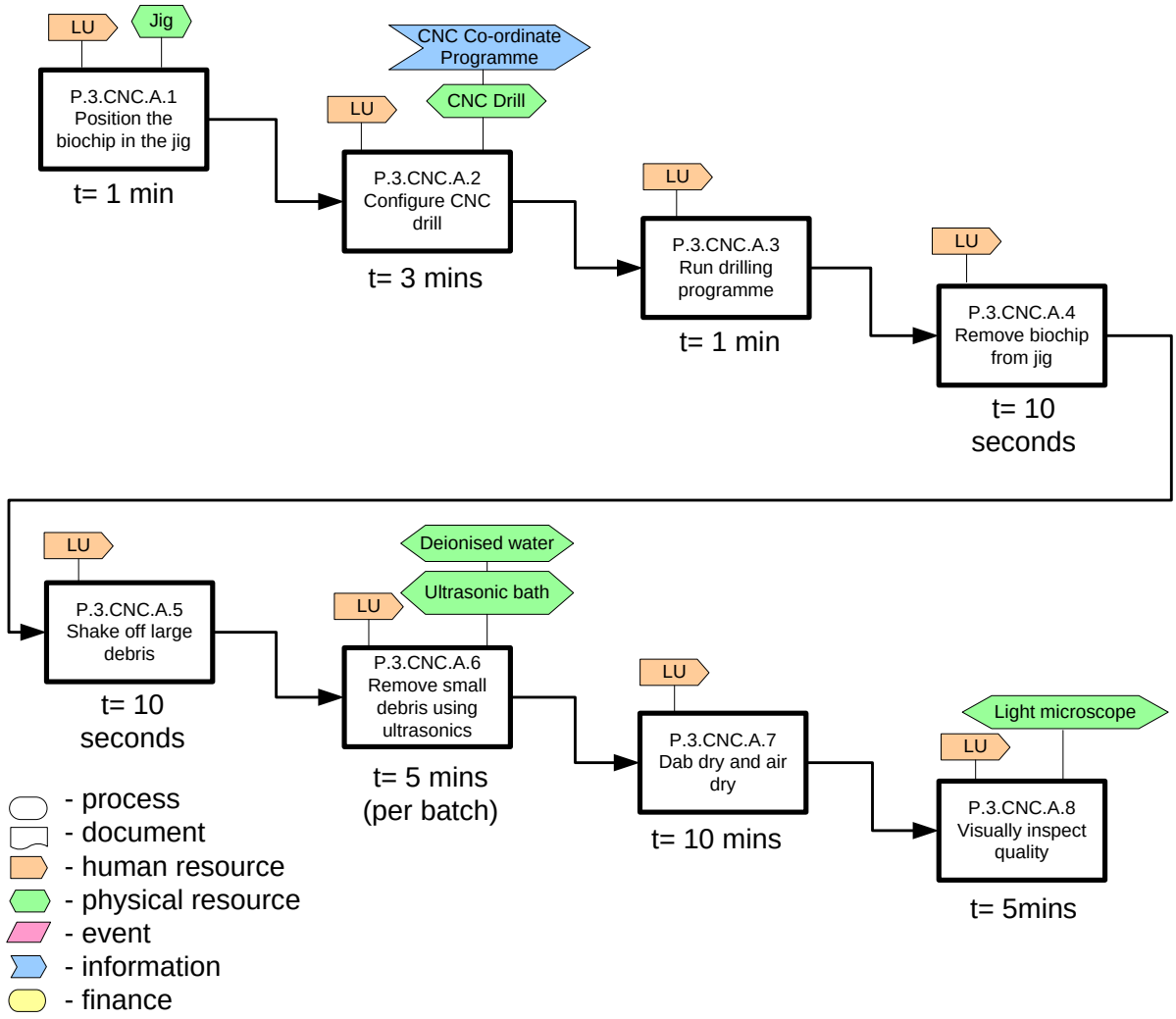


Figure 5.48: The CNC drilling process used to drill through the polystyrene culture dish and epoxy resin to the underlying Cu pattern.

In activities CNC.A.1 – CNC.A.4, a biochip with Petri dish secured was positioned inside the drilling machine and held in place using a jig. The CNC drilling programme was configured and allowed to run. The biochip was removed and these steps repeated for each biochip in a batch of 20.

During CNC.A.5 – CNC.A.8, large debris was shaken off the biochips. To remove smaller debris that may have been inside the 500µm diameter holes the biochips were submerged in deionised water in an ultrasonic bath for 5 minutes. Samples were dried and the holes were inspected using light microscopy.

Process Three by CO_2 Laser Ablation: The carbon dioxide laser-based process required precise positioning of the substrates in the system such that the co-ordinates of the pins measured and

entered into the lasers program were drilled in the precise location required to within a tolerance of 5 - 10µm. Activities carried out are depicted in Figure 5.49.

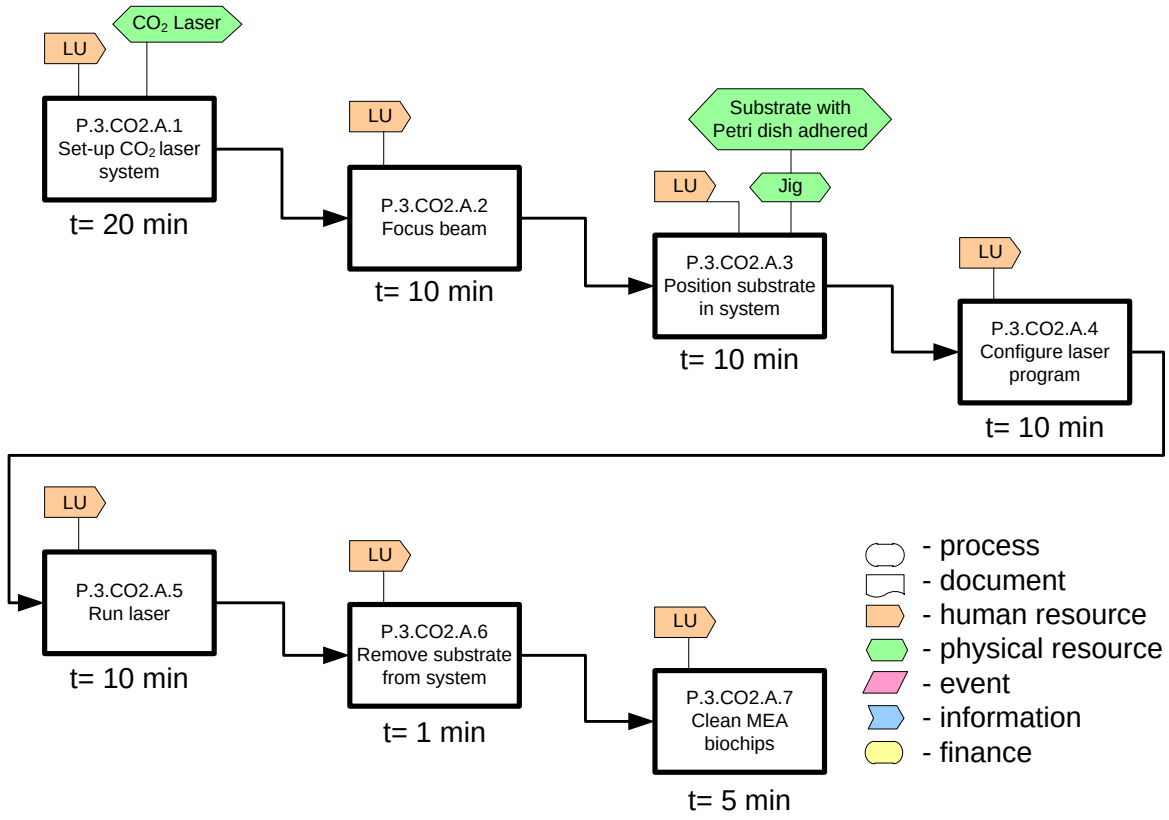


Figure 5.49: The CO_2 laser process used to drill through the polystyrene culture dish and epoxy resin to the underlying Cu pattern.

Activities CO_2.A.1 - CO_2.A.2 were the start of drilling where the CO_2 laser was set-up and the beam focused. The typical diameter of the focused beam throughout this process was 0.2 – 0.3mm. The pulse repetition rates used were varied across samples in pursuit of approach optimisation. The observations and outcomes of this drilling method is detailed in Chapter 6.

During activities CO_2.A.3 - CO_2.A.7, a biochip was positioned onto the headstage inside the laser unit and secured in place with adhesive tape. The laser program was configured by zeroing the system and providing the appropriate drilling co-ordinates. The laser program was run. The MEA biochip substrate was removed from the laser system and cleaned.

(Activities A.3 – A.6 were repeated for each biochip. MEA biochip substrates were processed in a batches of 10.)

The laser parameters for this approach were:

- Wavelength: 10.6µm
- Pulse energy: 10V
- Focused beam diameter: 0.2-0.3mm

The laser configurations were tested for this method (Table 5.9):

Table 5.9: The CO_2 laser repetition rates tested for manufacturing approach one.

Pulse-repetition rate	Number of passes	Comments
500/30	2	No hole
500/35	2	Observed holes not through PS to Cu. Measured depths between 0.535 - 0.674 µm.
500/40	2	Not through
500/50	2, 3	Not through. For three passes hole depths between 0.756 – 0.800µm. Sooty deposits.
500/65	3	Not through. Sooty deposits.
500/75	3	Not through Sooty deposits.
500/85	3	Not through. Sooty deposits.
500/95	3	Not through. Sooty deposits.

This approach resulted in sooty deposits around the holes. The Petri dish was distorted around the holes which were irregular in shape. Debris material also coated the surrounding surface of the Petri dish so samples from the CO_2 laser drilling method were not carried forward to the electroplating stage.

Process Four – Copper electroplating

The following CIMOSA model (Figure 5.50) represents the electroplating stage implemented for this research using only samples that had been drilled mechanically using the CNC process.

In activity A.1, two electrolyte solutions were tested. One was commercially available through-hole plating solution (Electroposit 1300) and the other was a recipe made in-house.

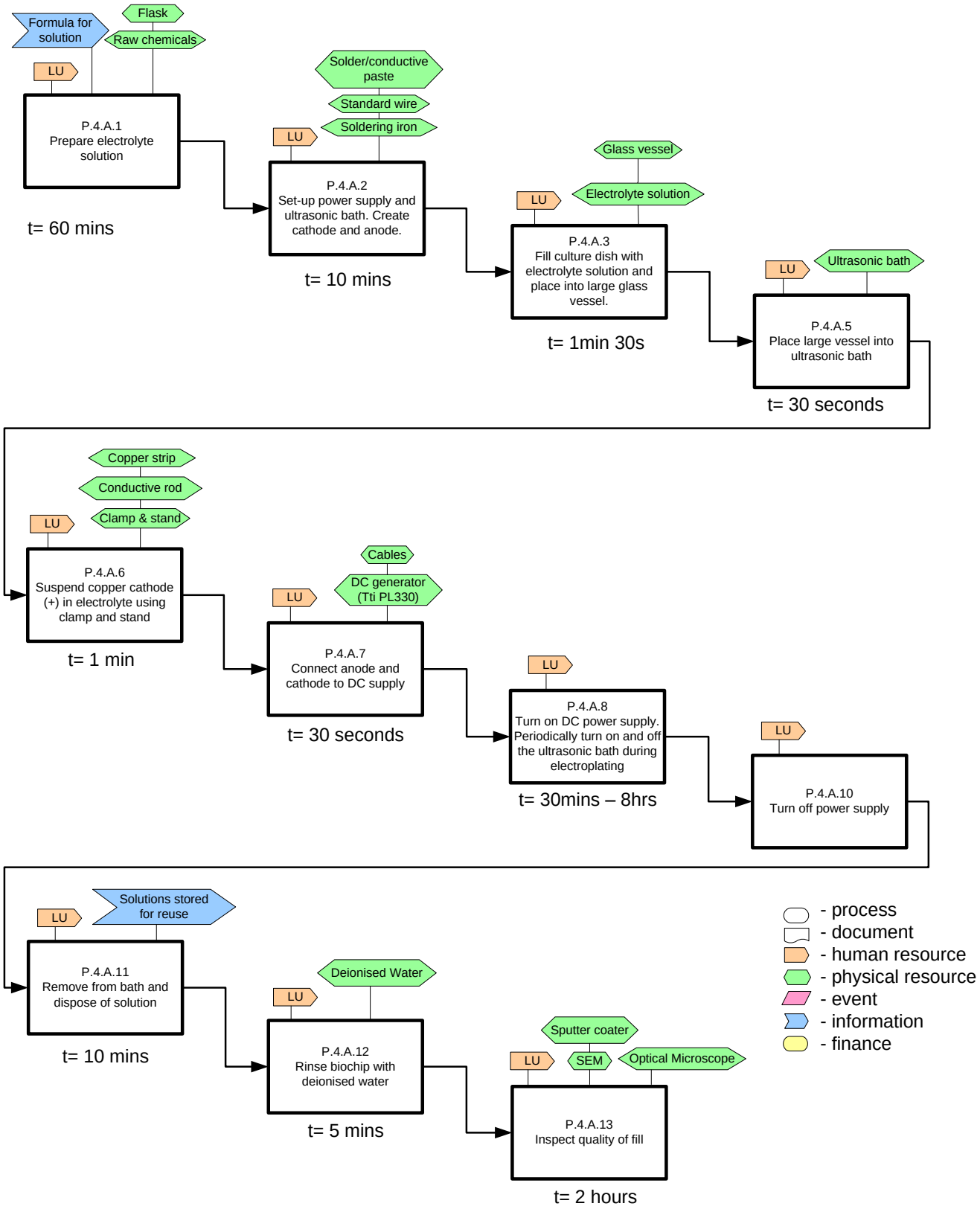


Figure 5.50: The electroplating process implemented by manufacturing approach one.

Home-made electroplating solution

The electrolyte solution mixed by hand for initial electroplating experimentation failed to produce a filling suitable for this work (section 6.1.1).

The formula of the electrolyte used was:

22.4g Copper (II) sulphate hydrate ($CuSO_4$) • $5H_2O$: 0.26 mol/dm^3

42.61ml Sulphuric acid (H_2SO_4): 2.0 mol/dm^3

357.4ml Deionised water

This recipe is for 400ml of electrolyte which was the quantity mixed for each test.

Operating conditions used:

Temperature: Room temperature 18-20^oC

Agitation: Regular agitation by ultrasonic vibration.

Ventilation: Electroplating took place in a well-ventilated laboratory.

Commercial Solution

The Electroposit™ 1300 acid copper solution (The Dow Chemical Company, USA) is a single component additive that is specifically designed to improve reliability in via and through-hole plating in PCB manufacture.

As the Electroposit solution is a commercial product the formula is unavailable. The mixture is however described as > 95% water and <5% inorganic salts.

The advised operating conditions were adhered to while using this solution:

Temperature: advised 20-25^oC, used 20 ^oC.

Agitation: advised moderate, used stirring and occasional ultrasonic bath.

Ventilation: advised to work in a ventilated space, used a ventilated laboratory.

The results of electroplating were improved by using this solution. The outcomes were however not up to the standard required of this application (see section 6.1.1).

Activities A.2 – A.3 of Figure 5.50 demonstrate the electroplating set up. The equipment used was set-up and a cathode and anode created using electrode probes and a copper sheet (Figure 5.51). The electrolyte solution was added to a glass vessel into which the biochip was submerged vertically. The areas of the biochip that were not to be plated (tracking and peripheral interconnect points not insulated by the Petri dish) were previously covered in protective insulating tape and the anode connection made using solder paste.

A.4 – A.10: Place the glass vessel into an ultrasonic bath. Suspend the copper cathode in the electrolyte close to the Petri dish connect both the cathode and the anode to the DC Supply TTi PL 330 unit. Begin electroplating by switching on the power supply. Turn on the ultrasonic bath every thirty minutes for five minutes to dislodge any air bubbles. After 7-8 hours switch off the power supply.

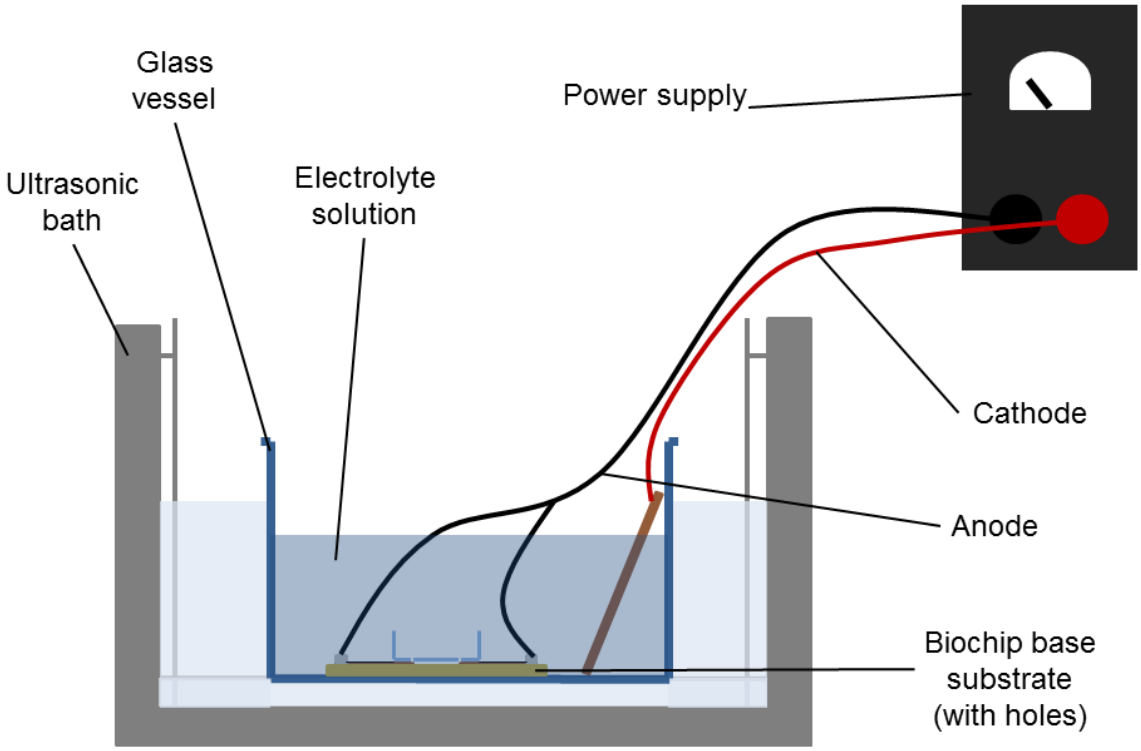


Figure 5.51: The electroplating set-up used for the via hole filling manufacturing approach.

A.11 – A.13: Remove biochip from the solution and rinse under deionised water. Prepare samples for quality inspection.

Cross-sections of the holes filled using this approach are contained in section 6.1.1.

5.6.1.2 Manufacturing Approach One Summary

A 35mm diameter Petri dish was adhered over a prepared Cu patterned base substrate using an epoxy resin. Drilling of 16, 500mm diameter holes, 5mm apart was computer-numerically controlled (CNC) allowing hole depth controlled to a tolerance of a few micrometres. The tolerance for this aspect is important to prevent detrimental removal of the underlying Cu pattern that was essential for successful electroplating. The drilled holes were ~800µm in depth. A conventional via hole filling (electroplating) procedure was adopted to fill the 500µm.

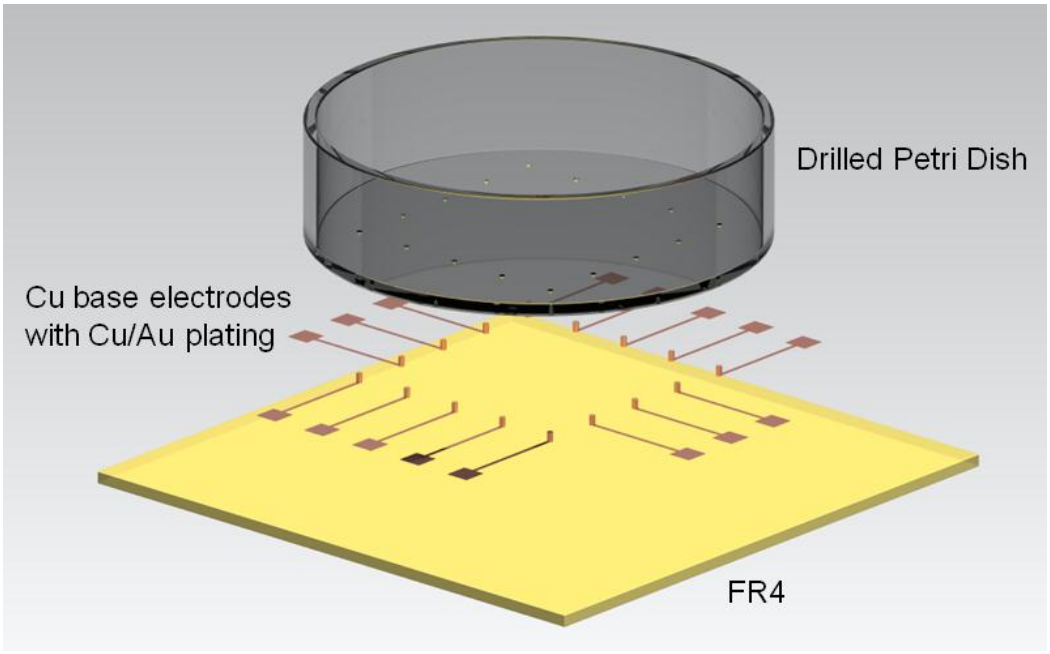


Figure 5.52: An exploded view of the amended original concept according to the possibilities of the initial design of a manufacturing approach using via hole filling techniques.

Copper (Cu) filling to a depth of ~600µm was aimed for with Gold (Au) to be plated on top to seal in the Cu, creating a cell-friendly surface. An exploded diagram depicts the intended Cu filling of the drilled holes (Figure 5.52).

This approach initially plated too slowly using the home-made electrolyte. When the electrolyte was replaced for a commercial equivalent the quality of the outputs improved but the presence of air bubbles in all samples plated was a continuing problem.

5.6.1.3 Evaluation of Manufacturing Approach One

The via hole filling approach produced the following outcomes.

Table 5.10: The outcomes of the via hole filling approach.

Priority	Critical Manufacturing Outcome	Approach achieved?
1	Produce satisfactory base substrate	Yes
2	Produce satisfactory media well	Yes
3	Facilitate adequate attachment of the media well and base substrate components	Yes
4	Produce a satisfactory electrode site for cell-electrode interfacing	No
5	Produce an appropriate micro-well geometry around electrodes	No
6	Produce enough sites so as to allow assignment of a reference	No
7	Produce an MEA biochip that allows appropriate optical inspection of living samples	No

Further description of the results of this approach are contained in section 6.1.1.

5.6.2 Manufacturing Approach Two: Vertical Pins.

An alternative manufacturing approach was developed in parallel with the on-going experimentation of the via hole fill approach.

The vertical pin approach was considered as a potential avenue for developing an MEA biochip that has vertical as opposed to presently commercially available horizontal interconnections. A metallic pin was sought to serve as the electrode tip that could be mounted such that it would protrude through the culture dish into a drilled microwell recession to a controlled depth, thus filling the hole to result a ~200-250µm microwell feature with an electrode at the bottom (Figure 5.53).

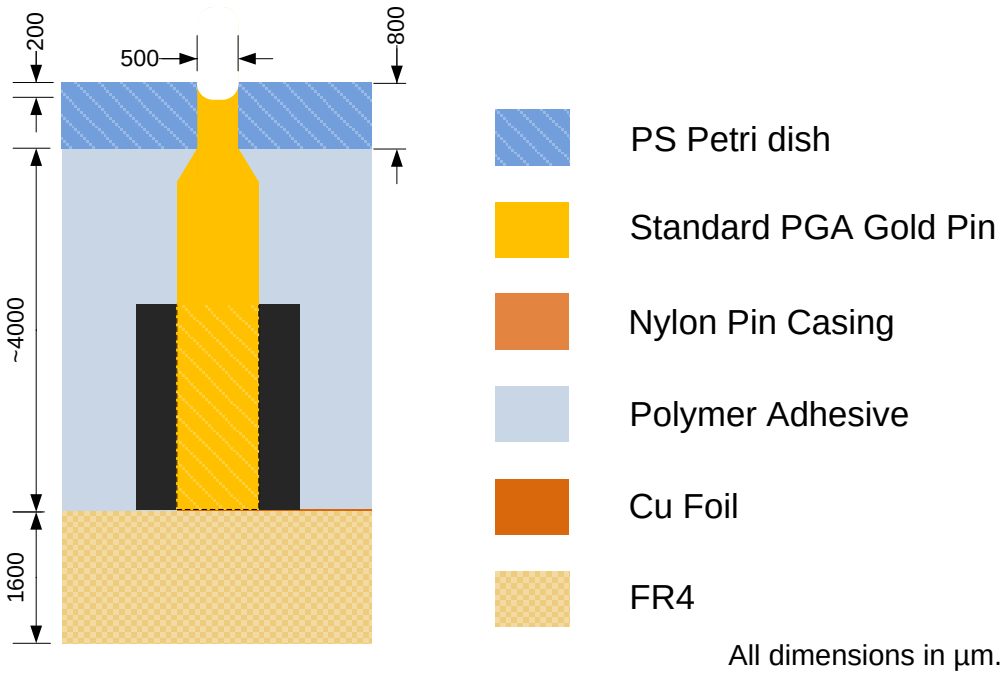


Figure 5.53: A schematic of the intended cross-section at the microwell-electrode site for the Gold Pin manufacturing approach.

Vertical interconnects are described in the original novel system vision. The intention of vertical connections is to support the pursuit to reduce the overall footprint of the MEA system. Moving the interconnect points away from the biochip periphery would be novel in *in vitro* MEA biochips and the use vertical pin interconnects would enhance the feasibility of creating a system where the electronics are contained in a compact package underneath the MEA biochip while *in situ*.

5.6.2.1 The Pins

The pins used in early approach experimentation were sourced from pin grid arrays, PGA (Figure 5.54) as they are easy to obtain quickly and at low cost.

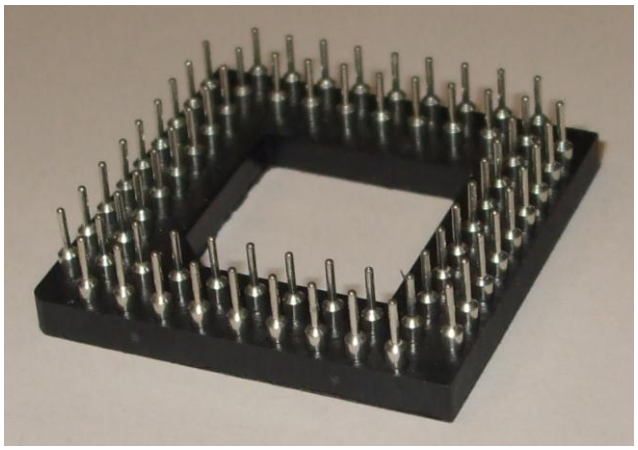


Figure 5.54: A standard 68-pin gate array.

The pins used were made of beryllium copper core, encased by a tin pin protrusion that is plated in 100µm nickel. It was intended that once the overall approach was approved the pins used would be replaced with the more expensive Au equivalents so as to introduce biocompatibility.

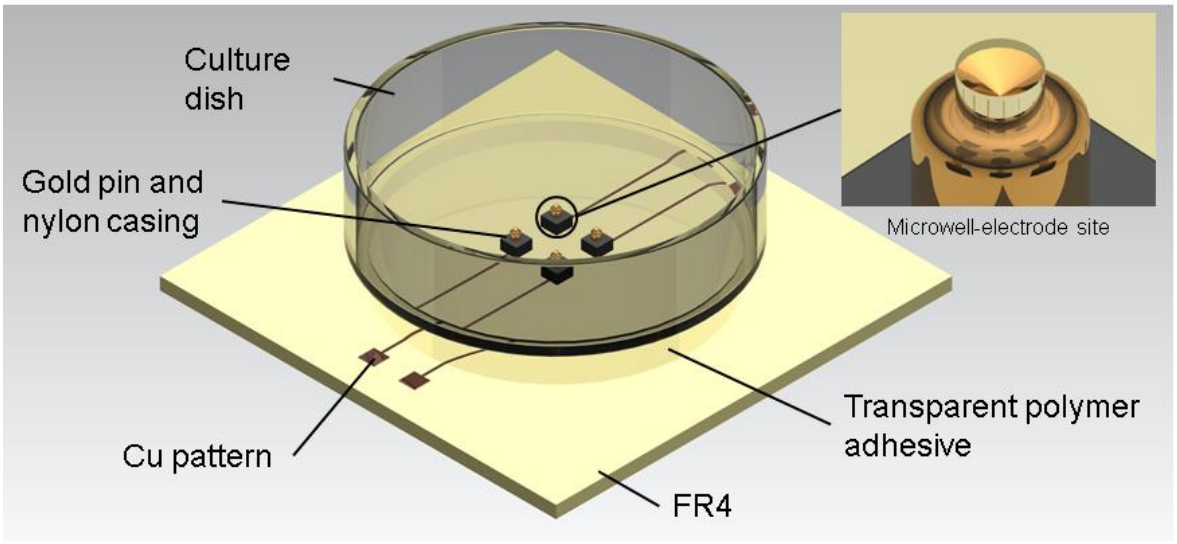


Figure 5.55: A visualisation of the PGA Gold Pin concept and the microwell-electrode site.

The new design concept (Figure 5.55) connects the vertical interconnect points to a patterned FR4 base substrate to accommodate proof-of-principle testing in an existing MEA system.

5.6.2.2 Manufacturing Approach Two Description

Processes implemented for this manufacturing approach are (Figure 5.56):

- P.1. – Make patterned MEA base substrate
- P.2. – Drill Petri dish
- P.3 – Produce electrode in microwell

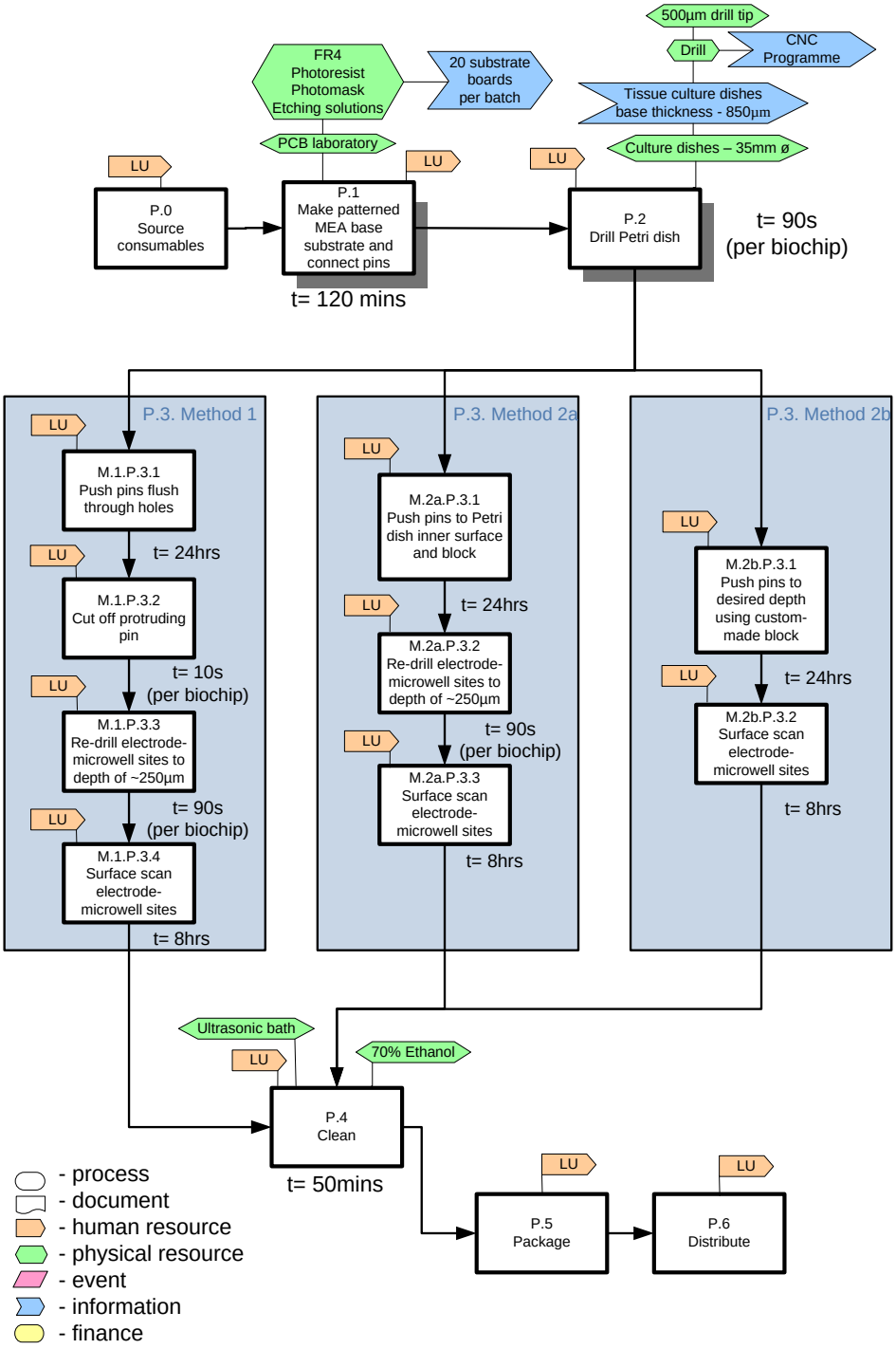


Figure 5.56: A CIMOSA activity diagram representing the major processes attempted for the Gold Pins manufacturing workflow.

Process One – Make patterned MEA base substrate

For details of the UV photolithography method used to manufacture the base substrate see section 0.

To connect the sections of pins (four pins per section) tin (Sn) paste was used to create a conductive interface between the vertical pins and the underlying Cu patterned FR4 substrate. In future designs the patterned base substrate is not intended to be a necessary feature of the design as vertical pins are intended to connect straight in the system electronics below.

Process Two - Drill Petri dish.

The Hurco VM1 3 axis milling machine was used to drill holes in the Petri dishes that would align with the spacing of the PGA pins. PGA pin alignment was measured using a Zygo 3D touch probe and co-ordinates calculated for the CNC program.

Process Three - Produce electrode in microwell

For this process attention was paid toward how to locate the pins in the correct position. Fundamentally two methods were considered (Figure 5.57). Both methods considered for securing the pins were intended to hold the pins in position in a robust, water-tight manner at a controlled depth, allowing the pins to create electrode tips over which contracting SC-CM clusters could be seeded.

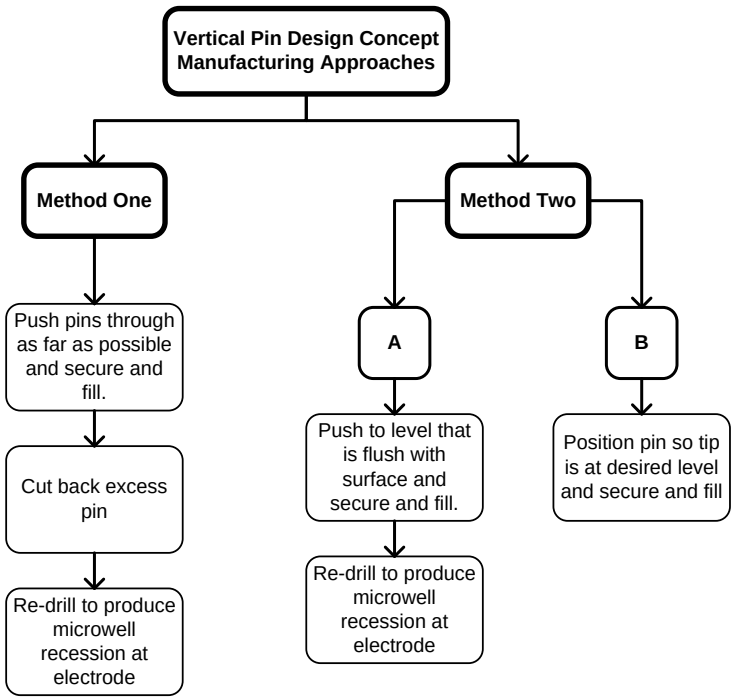


Figure 5.57: The different methods experimented with for the vertical pin approach.

1. Push pin through pre-drilled hole in Petri dish firmly to furthest point, fix in position using a filling and sealing material, cut-off the protruding part of the pin and drill the pin back down to a level that is ~200-250µm from the culture dishes surface.
2. Position pin precisely in pre-drilled hole to a controlled depth, control adhesion and filling around the pin to hold in correct position. The manufacturing approach did not progress past this point during this research as alternative approaches yielded prototypes for testing (see sections 5.6.3 and 5.9).

Method 1 above requires a precise re-drilling procedure to ensure the first drilling of holes aligns exactly with the second. The drilling was to be carried out using the CNC procedure documented in section **Error! Reference source not found.**.

Method 2 required a device to facilitate controlled pin positioning during the securing process, ensuring that the pin tip (serving as the electrode tip) is 200-250µm from the inner surface of the Petri dish. This approach contemplated whether to position pins level with culture dish surface and drill back slightly (method 2A, Figure 5.58) or, to position the pin tip at the correct depth, removing the second drilling process (method 2B, Figure 5.59).

Method Two A

A circular, perfectly flat mild steel weight was placed into the Petri dish over the holes. The transparent adhesive, Araldite Instant Clear, was injected all around the pins and left for 24 hours to harden. A second drilling over each pin to a depth of 200-250µm was then to be carried out to create a concave pin tip and therefore microwell.

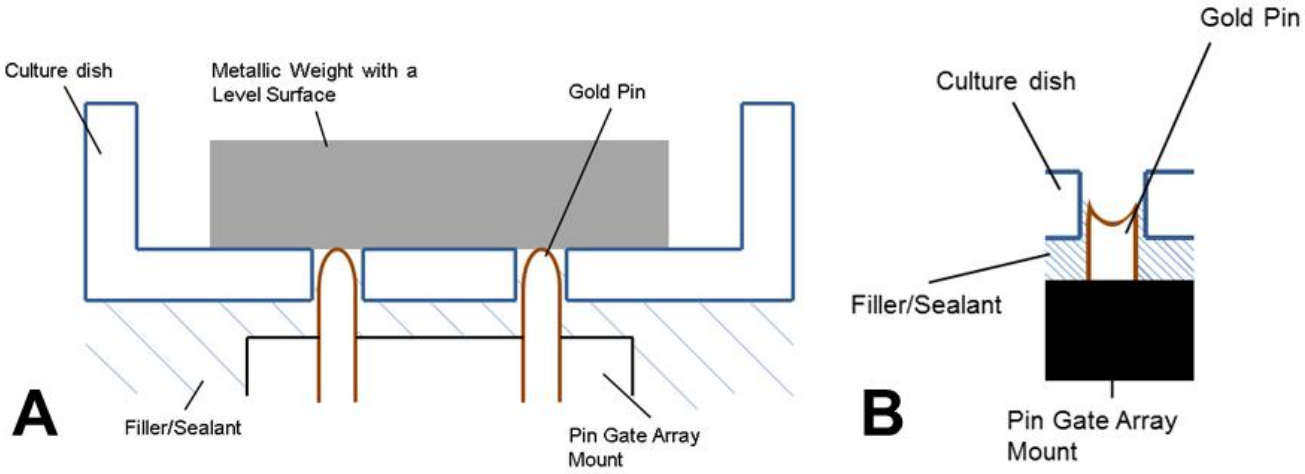


Figure 5.58: Method 2A. A) Secure pin tips flush with the inner surface of the Petri dish. B) Drill back 200-250µm to create a microwell.

Method Two B

A custom-made block with precisely aligned nipples was to be used to hold pins to the desired depth during filling and sealing. This method was not practically implemented by this research as an alternative manufacturing method produced prototypes for testing prior to manufacture of the nippled block.

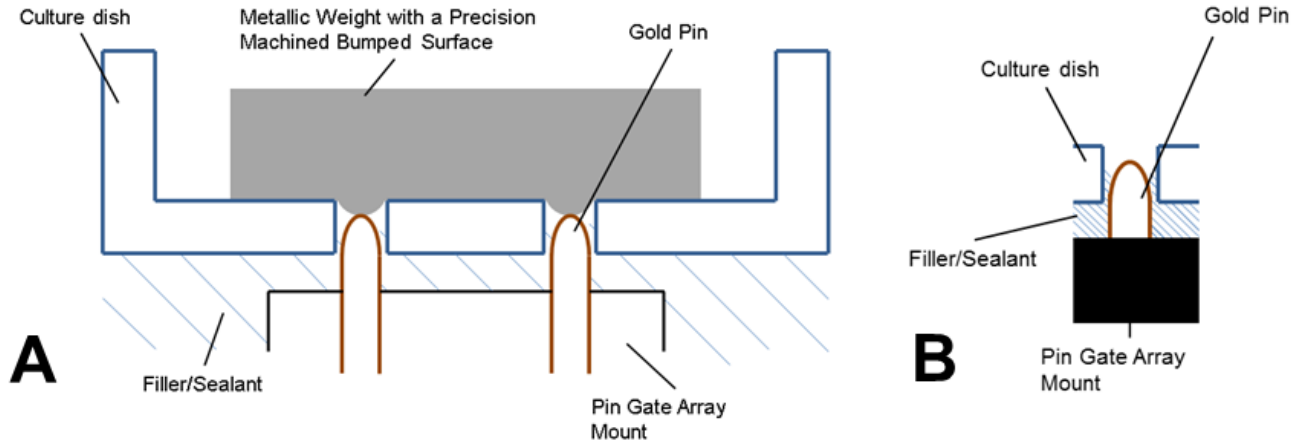


Figure 5.59: Method 2B. A) Secure pin tips at desired depth using nippled block. B) Remove block leaving pins in place.

5.6.2.3 Manufacturing Approach Two Summary

A section of mounted pins were adhered to a previously made patterned base substrate. Holes were drilled into a commercially available 35mm diameter Petri dish. The intention was to mount the Petri dish over the pins in a controlled manner. Two methods were considered for ensuring the provision of a microwell that the specified meets user requirements. One method considered a second drilling of pins after mounting. Another considered precise positioning so as not to require a second drilling procedure. This approach failed to yield a prototype. The outcomes are summarised in following section and described in greater detail in Chapter 6.

5.6.2.4 Evaluation of Manufacturing Approach Two

This approach was under investigation concurrently with manufacturing approaches one and three. The outcomes are summarised Table 5.11 and described in greater detail in Chapter 6.

Table 5.11: The outcomes of the gold pins approach.

Priority	Critical Manufacturing Outcome	Approach achieved?
1	Produce satisfactory base substrate	Yes
2	Produce satisfactory media well	No
3	Facilitate adequate attachment of the media well and base substrate components	No
4	Produce a satisfactory electrode site for cell-electrode interfacing	No
5	Produce an appropriate micro-well geometry around electrodes	No
6	Produce enough sites so as to allow assignment of a reference	No
7	Produce an MEA biochip that allows appropriate optical inspection of living samples	No

Further results of this approach are contained in section 6.1.2.

5.6.3 Manufacturing Approach Three: Micro Drilling

As the Via Fill and Gold Pin approaches were being optimised a third alternative approach was also conceived and concurrently investigated.

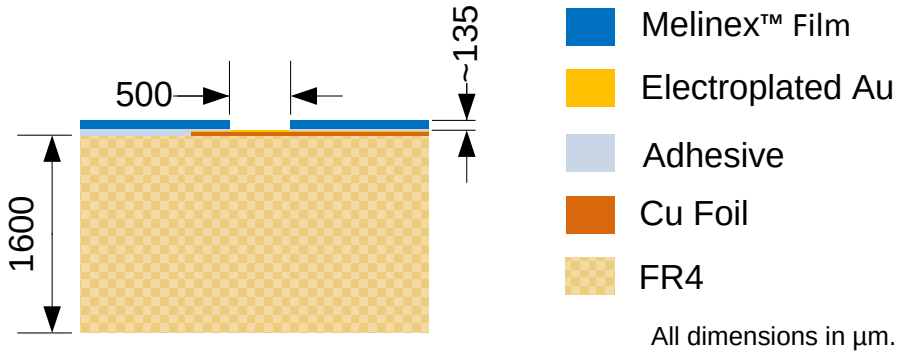


Figure 5.60: A schematic of an intended cross-section of the microwell-electrode site for the micro-drilling approach.

This manufacturing approach was the first of the three approaches under development to yield a prototype suitable for presentation to and testing by our external collaborating user group at the UoN. A cross-section schematic of the microwell feature produced using this approach is represented in Figure 5.60.

This approach aimed to create a microwell over each electrode site by carefully drilling a 500µm hole through a layer of insulating material (Figure 5.61). This process was implemented with glass and polyester coverslip materials. Outcomes of implementation are included in section 6.1.3.

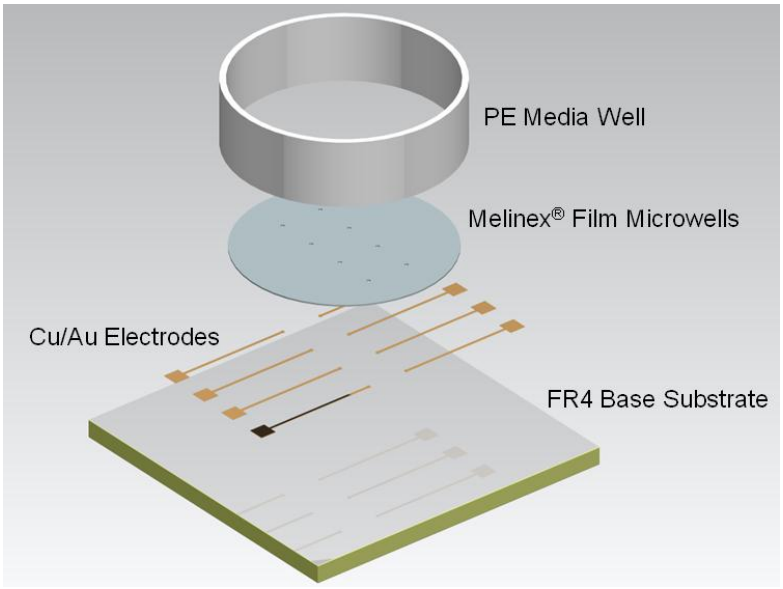


Figure 5.61: An exploded view of the micro drilling concept where the Melinex film is drilled through over the conductive pattern to form a microwell over each electrode.

5.6.3.1 Manufacturing Approach Three Description

All of the processes of this manufacturing approach were implemented (Figure 5.62).

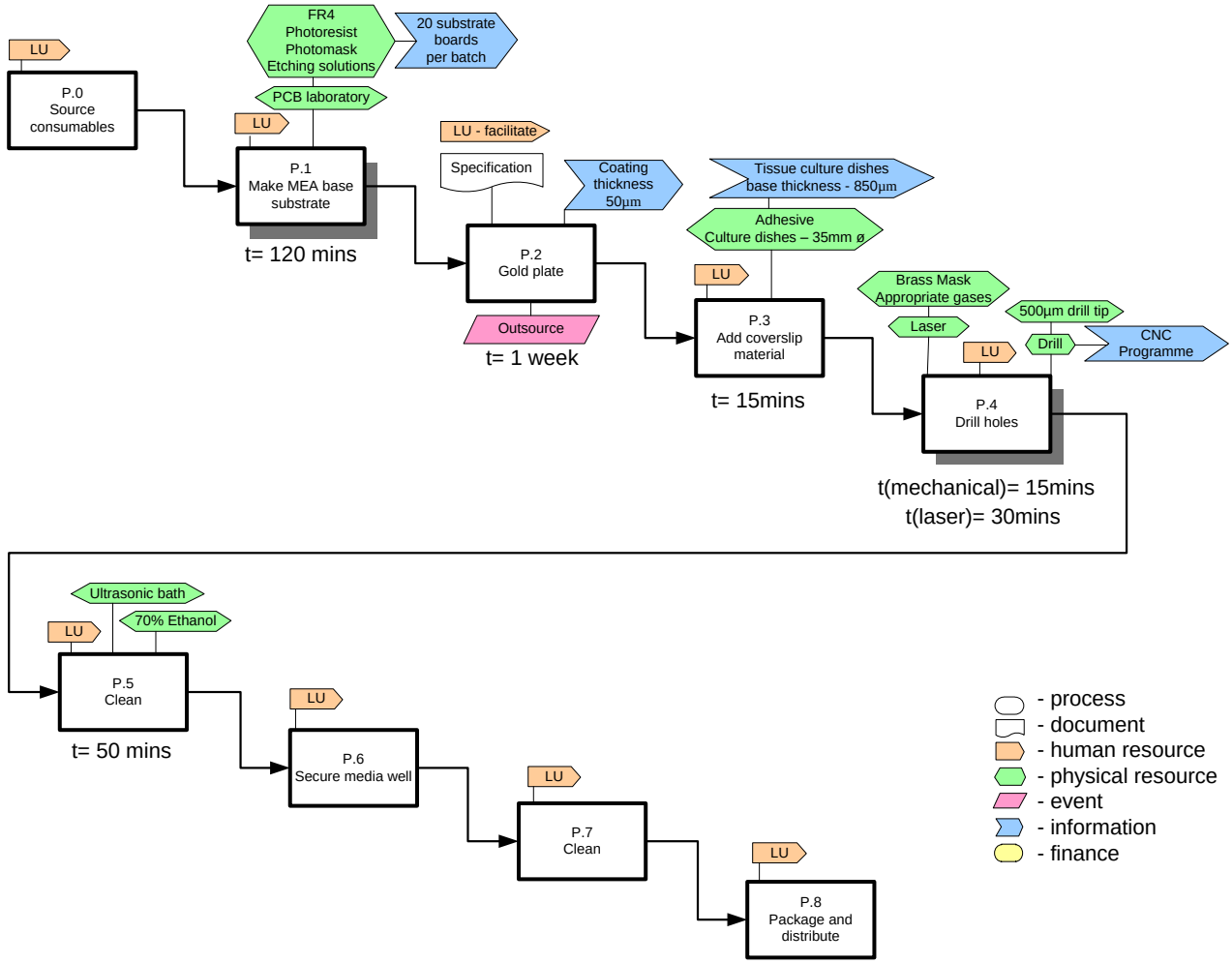


Figure 5.62: A CIMOSA activity diagram representing the major processes attempted for the micro drilling manufacturing workflow.

- P.0. – Source consumables
- P.1. – Make patterned MEA base substrate
- P.2. – Gold plate copper base substrate
- P.3 – Adhere coverslip material
- P.4 – Drill holes
- P.5 – Clean
- P.6 – Secure media well
- P.7 – Clean
- P.8 – Package and distribute for testing

Process One – Make MEA base substrate

The process used to make the base substrate pattern is described in section 0.

Process Two – Gold plate base substrates

Base substrates were outsourced to PMD Group, Coventry, UK for Au plating. The Au coating was approximately 3-5µm thick.

Process Three – Add coverslip material

Coverslip material was employed as a growth surface and insulator due to depth problems encountered when using the 35mm diameter Petri dish. The Petri dish was base was also relatively thick (~800µm) in comparison to coverslips. Therefore the hole in the Petri dish (plus adhesive) was too deep to meet user specified microwell depth. Filling wasn't producing satisfactory results so an alternative approach was to alter the insulating material and media well. Coverslip coverglass material is available in thicknesses from 150 to 220µm. These thicknesses plus adhesive held the potential to create a well simply by drilling through them. Glass and polyester coverslips were tested. A specialist film, Melinex, was also commercially available as a specifically treated cell culture substrate material in sheets of thicknesses 125 - 350µm. Being available in sheet form offered advantages over coverslips that are available in standard geometries, or alternatively if custom made are high cost.

Process Four – Drill holes

CNC drilling and Excimer laser ablation were considered for precision drilling of holes through the insulating material to the underlying electrode pattern (Figure 5.63). These methods were both implemented with polyester and glass coverslips and Melinex film. The results for each material investigated are discussed in section 6.1.3.

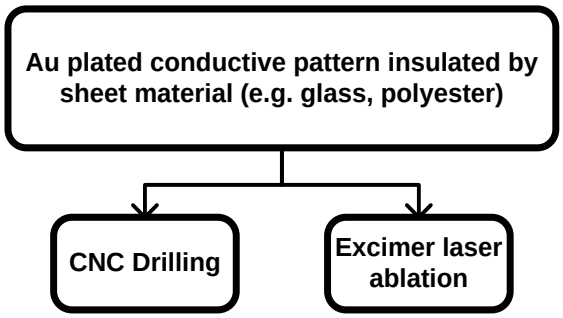


Figure 5.63: The two methods of drilling the 500µm diameter holes required to produce the microwell features.

The CNC drilling approach detailed in section **Error! Reference source not found.** using the Hurco VM1 3 axis milling machine was used to create 500µm diameter holes over each electrode.

This approach damaged the surface of electrodes resulting in exposed copper and was therefore not appropriate for further investigation.

An Excitech Lambda Physik LPX 100i Excimer laser, consisting of a short-pulse laser source (KrF, wavelength 248nm) and an Aerotech positioning system was used to ablate 500µm diameter holes at each electrode site (Figure 5.64).

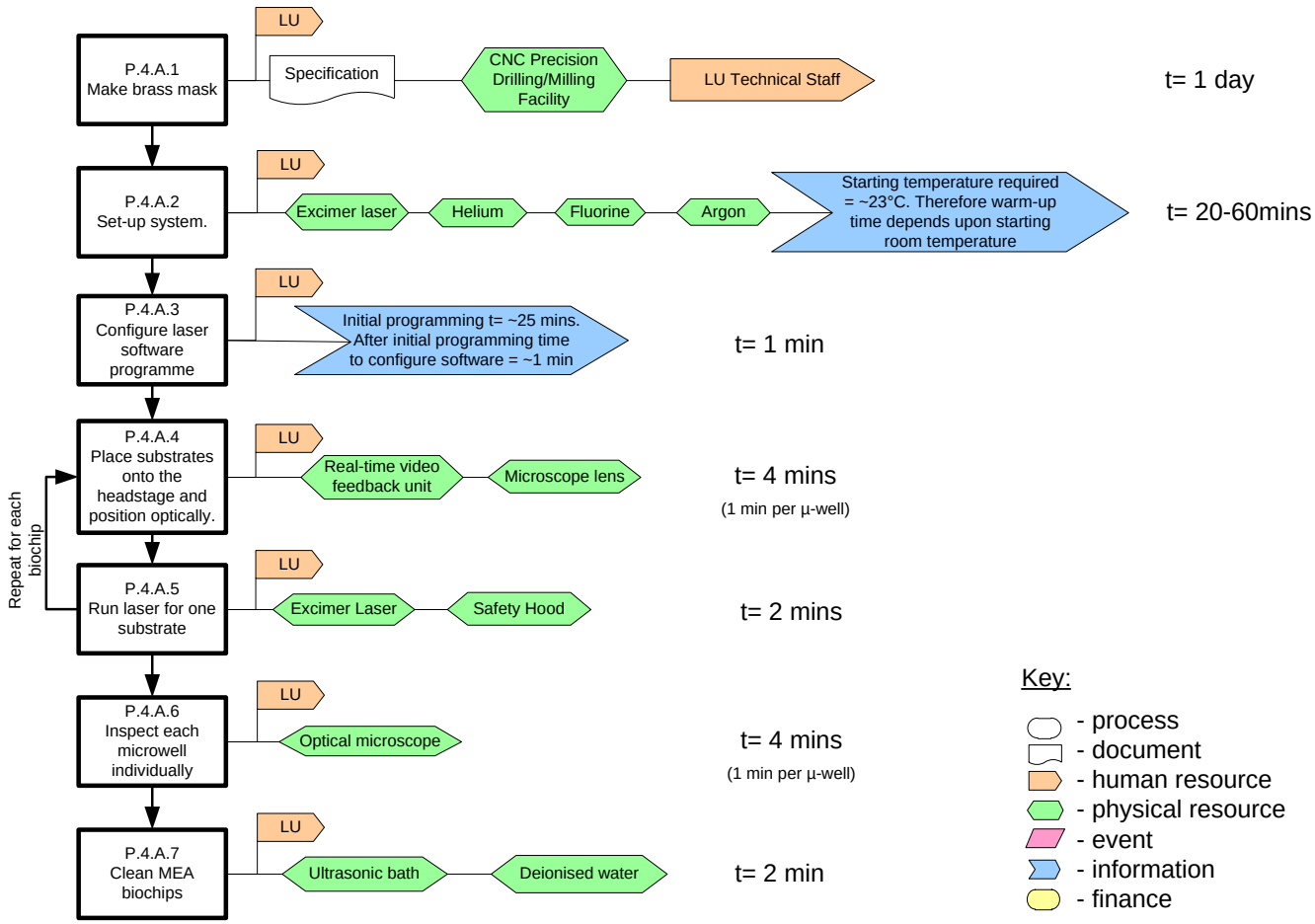


Figure 5.64: An activity diagram of the Excimer laser process implemented by this research.

During activities A.1 – A.3, a mask was made by drilling a 5mm diameter hole into a brass plate. The brass plate was inserted into the laser system during set-up. A program was written and configured to control the laser source and positioning system.

During A.4 – A.5, biochip substrates were positioned on the positioning system's headstage one at a time and the laser program run. These two activities were repeated until a batch consisting of between 4 to 10 samples (depending on success of previous sample) had been machined. Where the

processed showed to be repetitively breaking or causing damage to the sample, testing was ceased. The program was adjusted to testing different degrees of exposure. Figure 5.65 demonstrates the system and the system while carrying out this process. The results of this activity using three different test materials are included in this thesis in section 6.1.3.2.

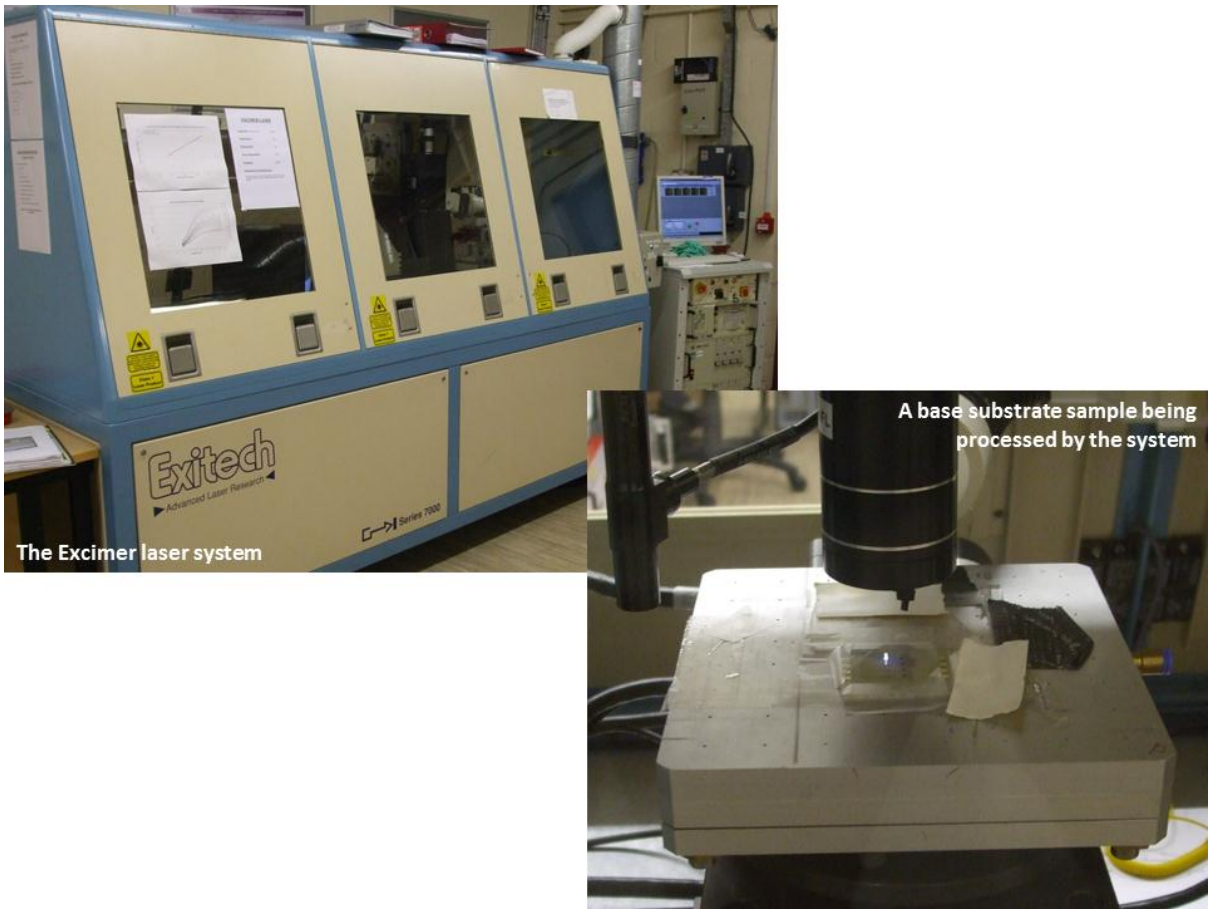


Figure 5.65: The Excimer laser system used in this research with example of a base substrate being processed.

For activities A.6 –A.7, each hole of each biochip was inspected optically. If samples demonstrated 8 good holes they were cleaned and progressed to the next process.

Process Five - Clean

Samples were placed into glass vessel containing deionised water. The glass vessel then was placed into an ultrasonic bath for 50 minutes to remove any debris from the ablated holes.

Process Six - Secure media well

Two adhesives were used to secure a PE and glass ring to the surface of the insulating Melinex™ film. The results of adhesive testing for this prototype are contained in section 6.1.3.

Process Seven – Clean

The cleaning process was completed by submerging the completed biochips in 70% ethanol in an ultrasonic bath for 2 minutes, removing and leave to dry.

Process Eight – Package and distribute for testing

Packaging for storage and transportation exploited 90mm diameter Petri dishes. Biochips were secured to the surface using adhesive tape and the lid placed over the top. Lids were seal with adhesive tape to prevent dust contamination and prototypes were delivered to the UoN for testing.

5.6.3.2 Manufacturing Approach Three Summary

This approach created a microwell in the insulating material over each electrode (see Figure 5.66). An FR4 base substrate that had been patterned in Cu and outsourced for a thin layer of Au to be electroplated on top was insulated using a layer of cell friendly material (e.g. polyester, polystyrene, glass) with a thickness comparable to the specified microwell depth. An excimer laser process successfully removed selective areas of insulator over the underlying gold electrodes.

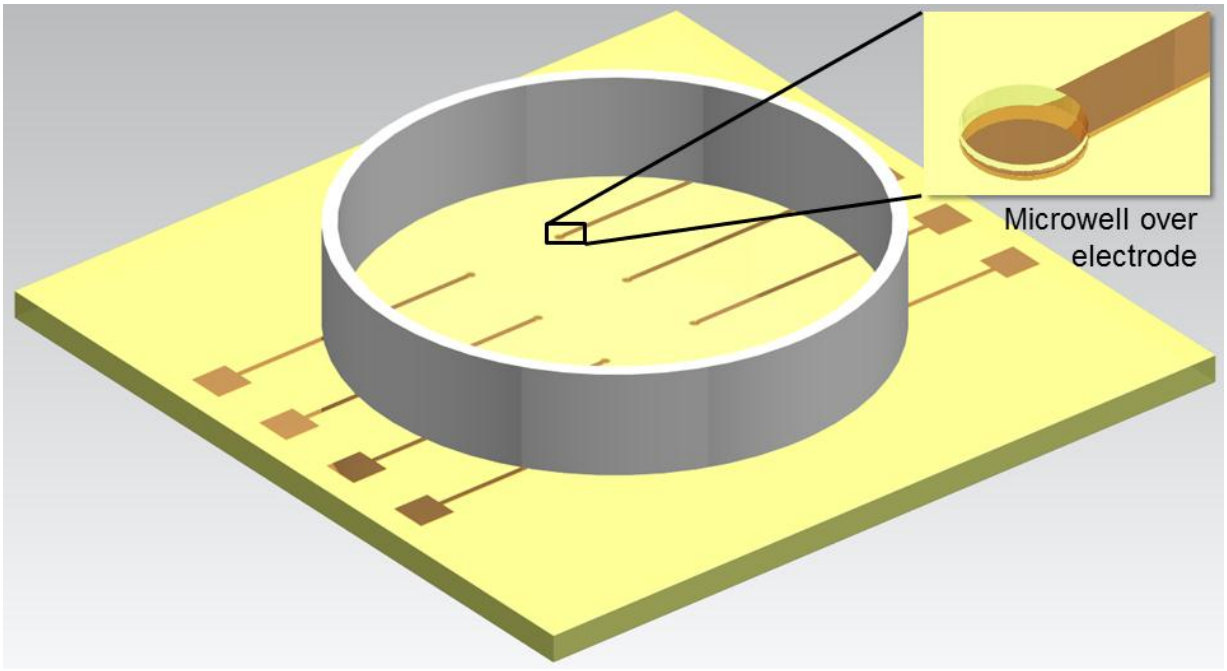


Figure 5.66: A CAD model of the concept and microwell site for the micro drilling manufacturing approach.

The approach exploiting an excimer laser produced results of a satisfactory quality for testing with live SC-CM cells. Live testing is incorporated into this thesis in Chapter 6.

5.6.3.3 Evaluation of Manufacturing Approach Three

The micro drilling approach produced the following outcomes.

Table 5.12: The outcomes of the micro drilling (Excimer laser) approach.

Priority	Critical Manufacturing Outcome	Approach achieved?
1	Produce satisfactory base substrate	Yes
2	Produce satisfactory media well	Yes
3	Facilitate adequate attachment of the media well and base substrate components	Yes
4	Produce a satisfactory electrode site for cell-electrode interfacing	Yes
5	Produce an appropriate micro-well geometry around electrodes	Yes
6	Produce enough sites so as to allow assignment of a reference	Yes
7	Produce an MEA biochip that allows appropriate optical inspection of living samples	No

This manufacturing approach resulted in the first prototype (Figure 5.67) available for testing. Further description of the results of this approach are contained in section 6.1.3.

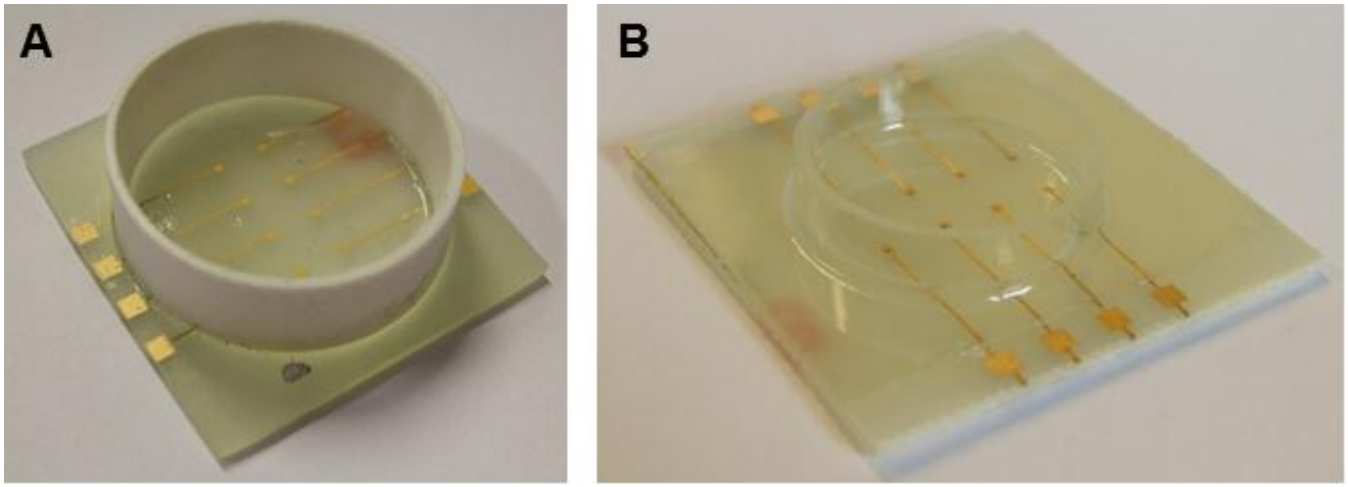


Figure 5.67: Prototype One. A) Made using 33mm diameter opaque polyester media well ring, adhered using epoxy resin. B) Made using 22mm diameter glass ring, adhered using silicon sealant.

5.7 Prototype One Design Iteration

Prototype one underwent a period of testing that was conducted by MEA users in a cell culture laboratory at the University of Nottingham, UK. Testing was conducted in small batches. Feedback was collected through observation of the prototype in use, and through interviews following testing. Early feedback led to sub-component alteration of the prototype.

Further testing that ensued following sub-component amendment resulted in total MEA biochip re-design. Testing is described fully in section 6.2.1.

5.7.1 Early testing of prototype one.

Problem 1: The media well geometry specified by the user was excessive. The media well was too deep so in retrospect the end users specification was incorrect.

Problem 2: The initial feedback (received during testing of the first batch of prototypes, Figure 5.68) was that the optics where too restricted when compared to using to the commercial standard.

Problem 3: The beating cluster was difficult to position into the microwells. It was suggested that this was due to the combination of the reduced lighting of the culture media, the large media well wall height, and the geometry of the microwell or size of the beating culture used in this particular instance.

Problem 4: No beating clusters attached inside a microwell. Contractions could not be observed. No signals were recorded.

Action: The opaque polyester ring used as the media well (Figure 5.67,A) was replaced with a glass alternative of smaller dimensions (diameter 22mm, height 6mm) to improve lighting for optical enhancement and to improve access to the microwells. Further testing using the amended prototype (Figure 5.67,B) was conducted.

Problem 4: The adhesive used to secure the Melinex™ film (insulating and microwell material) to the base substrate and the media well to the Melinex™ film showed signs of having absorbed some culture medium after use. A pink residue was present in each microwell and around the inner seal of the media well to Melinex film joint.

Action: Replace adhesive with a non-absorbent alternative.

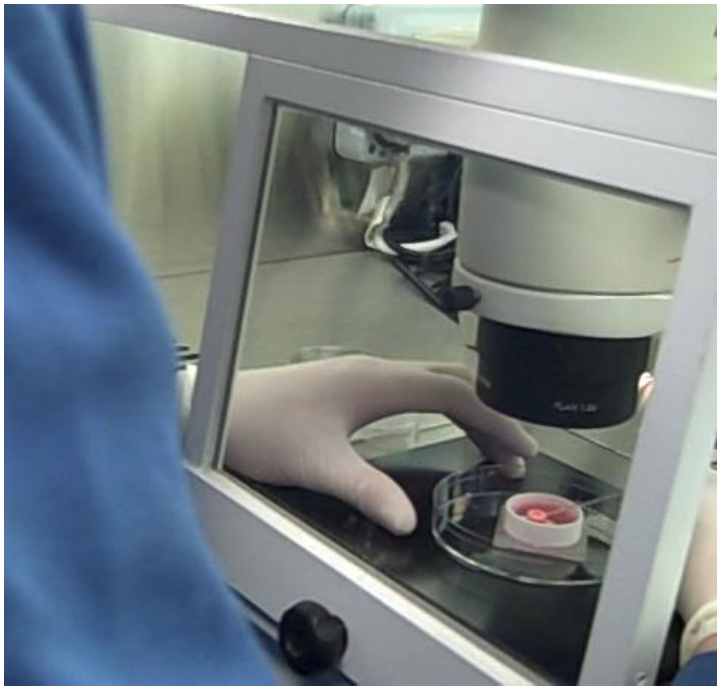


Figure 5.68: Seeding of a beating cluster of stem cell-derived cardiomyocytes into prototype one.

5.7.2 Ensuing testing of prototype one.

An example of the alterations made to the first prototype design following the first batch of tests can be seen in Figure 5.67. This amended version of prototype underwent further testing and the following problems were identified and actions taken as solutions.

5.7.2.1 Problems and solutions

Each problem identified through the wet testing conducted at the UoN is documented in Table 5.13. The implications of the problem are briefly presented from the users' point of view and the actions decided upon to address each problem is described.

Problem 1: Scientists could still not confirm whether clusters were contracting or were adequately attached due to the limited optics.

Action: Replace the translucent base substrate with a transparent alternative.

Problem 2: Although seeding of cells into microwells was more successful a number of seeded beating clusters did not attach inside the microwell.

Action: Alter microwell geometry.

Problem 3: No signals were recorded from any of the MEA biochip prototypes where beating clusters were growing inside a microwell.

Action: Investigate cell-electrode interface. Amend electrode design to improve likelihood of good cell-electrode interface formation. Investigate if detection system parameters are appropriately complimentary for detection of the microvolt signals. Adjust detection system parameters if appropriate.

Problem 4: The media well joint between the glass ring and the Melinex™ film failed during second use.

Action: Investigate suitable alternative and replace the adhesive.

5.7.3 MEA Biochip Re-design

A number of problems were identified with the first concept that impact upon the success of the design concept and the manufacturing approach used to construct the prototype. The MEA biochip design was revised. Actions that were considered necessary by this research to produce a final device that would meet the user needs are collated and Table 5.13.

Table 5.13: Problems associated with prototype, the implications each problem has for the user and actions to solve each problem.

Problem	Implication for user	Action to solve
Difficulty getting beaters to attach inside the microwells	Cells must attach very close to, or on, the electrode surface for a signal to be detected.	Improve ease of cell positioning by increasing the size of the microwell. Cloning rings (viable in Figure 5.68) are used to aid cell positioning. The new microwell geometry specification was diameter 800µm to 4mm, height 200 - 250µm.
The level of optical inspection was not suitable for confirming the degree of attachment of the beating cluster to the surface or if the	The clusters of cells that are derived from stem cell in this way contract spontaneously. Clusters may stop contracting at any time for currently unknown reasons. A signal will only be detected from a	Change the base substrate to glass to suit the inverted microscope facilities at the testing laboratory.

cluster is contracting.	beating cluster. The routine prior to setting up an MEA based test using cardiomyocytes involves visually confirming that cells to be recorded from are both attached to the MEA surface and are contracting. Cells are also checked visually during testing.	
Where a beating cluster is seen to be inside the microwell no signals have been recorded from any of the devices tested.	The signals recorded contain the information of use to the scientist. Without signals this device fails.	Investigate possibility of short circuits in the prototype device. Investigate alternative electrode geometries that offer greater likelihood of beating cluster attachment.

5.7.4 Section Summary

The prototype devices manufactured using manufacturing approach three – micro drilling – were tested in wet trails using live stem cell-derived cardiomyocyte beating clusters. Problems were identified with:

- adhesive
- well size
- well material
- micro-well geometry
- base substrate transparency
- signal capture

Actions to address the identified problems (Table 5.13) were incorporated together into an improved design concept. Design concept 2 and the required manufacturing approach to produce prototypes for testing are presented in the following section.

5.8 Prototype Two Manufacturing

An improved concept design was conceived to address the following actions defined as required of this research:

1. Improve ease of cell positioning by increasing the size of the microwell. Cloning rings (viable in Figure 5.68) are used to aid cell positioning. The new microwell geometry specification was diameter 800µm to 4mm, height 200 - 250µm.
2. Change the base substrate to glass to suit the inverted microscope facilities at the testing laboratory.
3. Investigate why no signals were detected. Consider the possibility of short circuits in the prototype device. Investigate alternative electrode geometries that offer greater likelihood of beating cluster attachment. Consider if detection system was appropriately matched.

5.8.1 Generation Two Concept Design

The actions are present in the concept design alteration in the following ways.

1. Improve ease of positioning – The purpose of the microwell feature is to provide a perimeter around the area of MEA biochip's surface where the electrodes are located. This perimeter is intended to ensure that the beating clusters containing cardiomyocyte cells, which move due their contracting nature, attach over an electrode every time. There can also be additional movement of the beating cluster from the original position on which it has been positioned due the fact that the MEA biochip must be moved by hand from the culture hood where seeding takes place, into an incubator. A larger perimeter has been specified to ensure the beating cluster of cells can settle and attach within it. Consequently the electrode geometry was increased.
2. Change the base substrate – The area of micro electrode array workspace for all commercially available MEA biochips is made from glass. The use of glass for this part of the device allows good quality visual inspection of the cellular sample. This work has used glass to allow visual confirmation that cell samples seeded are contracting before testing and to allow verification that the beating cluster is attached to the base substrate. The electrode geometry has been amended to facilitate optics.
3. Investigate why no signals were detected – The conductivity of the surrounding culture media (DMEM) was assessed and impedance of the electrodes compared to a previously demonstrated commercial equivalent. The electrode geometry was altered in two variants

to ensure that an increased number of beating clusters seeded would attach to the electrode surface.

5.8.1.1 MEA biochip Concept 2

The re-designed MEA biochip is demonstrated in Figure 5.69. Two different electrode geometries were considered to address the question – will altering the electrode geometry influence signal detection? Both variants of electrode were manufactured in exactly the same way at the same time using a clean room based UV photolithography process. The patterned MEA biochip substrates were outsourced to Fondazione Filarete, Milan, Italy, for microwell creation using a novel process currently under development.

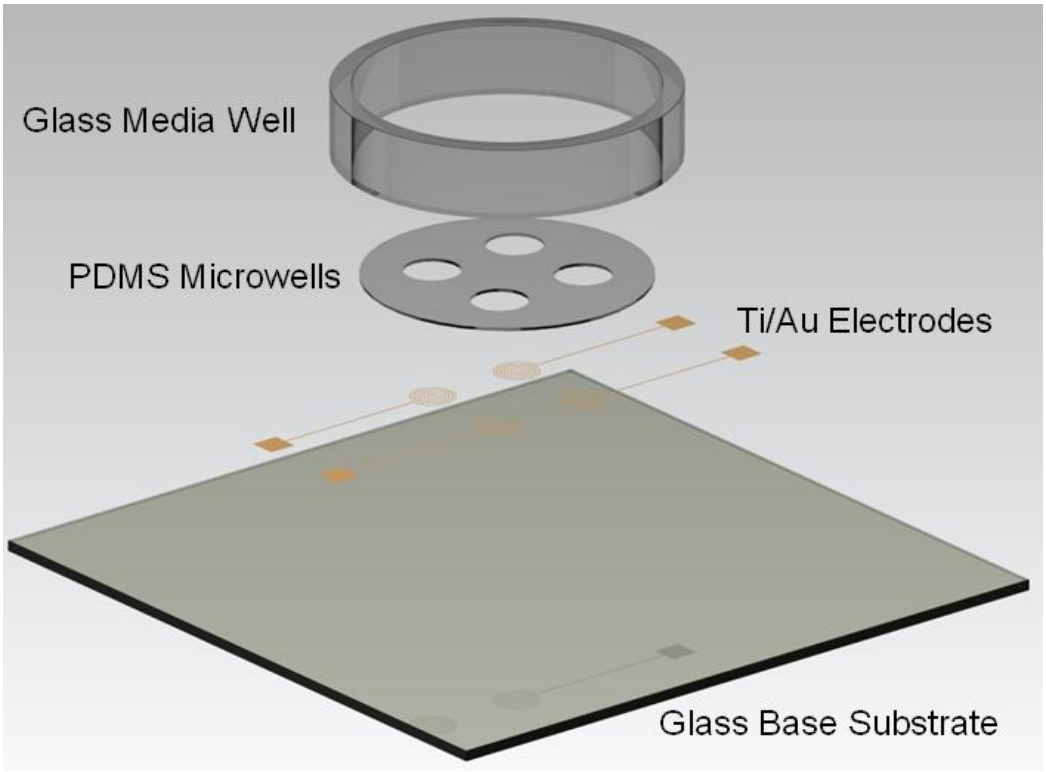


Figure 5.69: An exploded view of MEA biochip design concept 2.

The results for both A and B versions of this chip in live testing were the same. User feedback showed slight variation as the user commented that the radiator (B) pattern was easier to use as the cloning ring appeared to catch on the spiral design, making movement more awkward. Details of testing and results are contained in Chapter 6.

5.8.1.1.1 A – Spiral electrode

The spiral shaped electrode was designed to the following specification:

- Spiral diameter: 3.5mm

- Width of track from contact pad to spiral: 150µm
- Width of spiral tracking: 80µm
- Spacing between spiral tracking: 150µm
- Contact pad: 2mm x 2mm
- Largest gap between tracking (at spiral centre): <300µm

The position of the contact pads matches commercial equivalents so that the biochip can be tested in the headstage of a commercial MEA system (MCS MEA-60). The artwork generated for this electrode pattern is below (Figure 5.70).

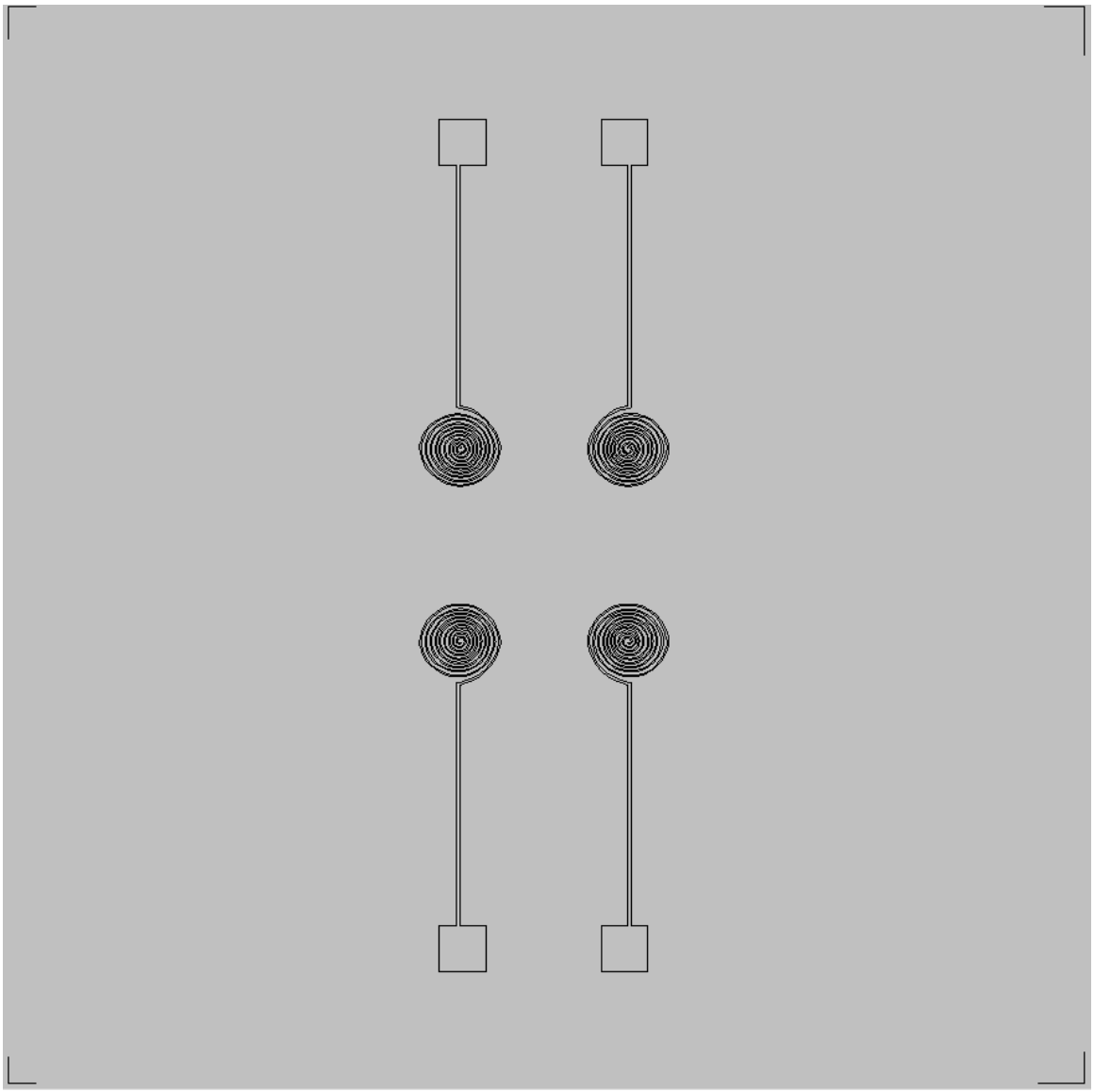


Figure 5.70: Artwork generated for the spiral electrode design.

5.8.1.1.2 B – Radiator Electrode

The radiator shaped electrode was designed to the following specification:

- Radiator dimensions: 2.5mm x 2.75mm
- Width of track from contact pad to radiator: 150µm
- Width of radiator tracking: 100µm
- Spacing between tracking: 100µm
- Contact pad: 2mm x 2mm
- Largest gap between tracking (at spiral centre): <300µm

The position of the contact pads matches commercial equivalents so that the biochip can be tested in the headstage of a commercial MEA system (MCS MEA-60). The electrode pattern can be seen Figure 5.71.

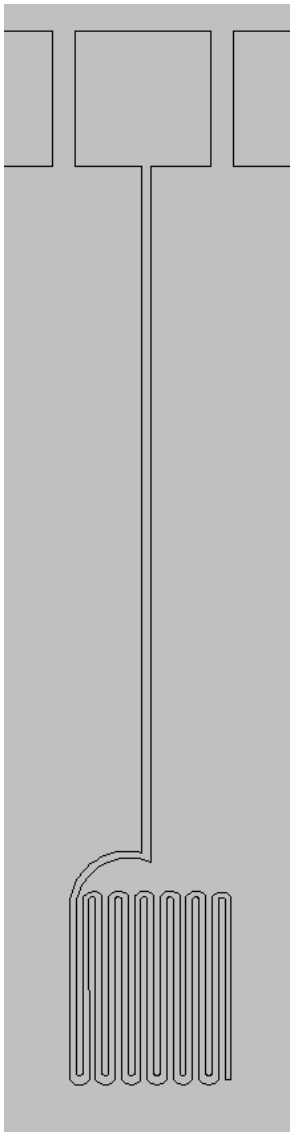


Figure 5.71: The radiator shaped electrode.

5.8.1.1.3 Microwell

The material that was selected for insulation and microwell formation in this design was polydimethylsiloxane (PDMS). PDMS is a lower cost material than traditional micro-fabrication materials such as silicon and glass (Jo et al, 2000). PDMS is also chemically inert, thermally stable, and simple to handle (Mata et al, 2005) and has been successfully demonstrated in biomedical microdevices (Fujii, 2002; Yabuta et al, 2003). The application of the PDMS onto the glass substrates was carried out by a collaborating research group at Fondazione Filarete, Milan, Italy using a plasma surface activation technique to adhere hand stamped PDMS discs over the biochip surface (Figure 5.72).

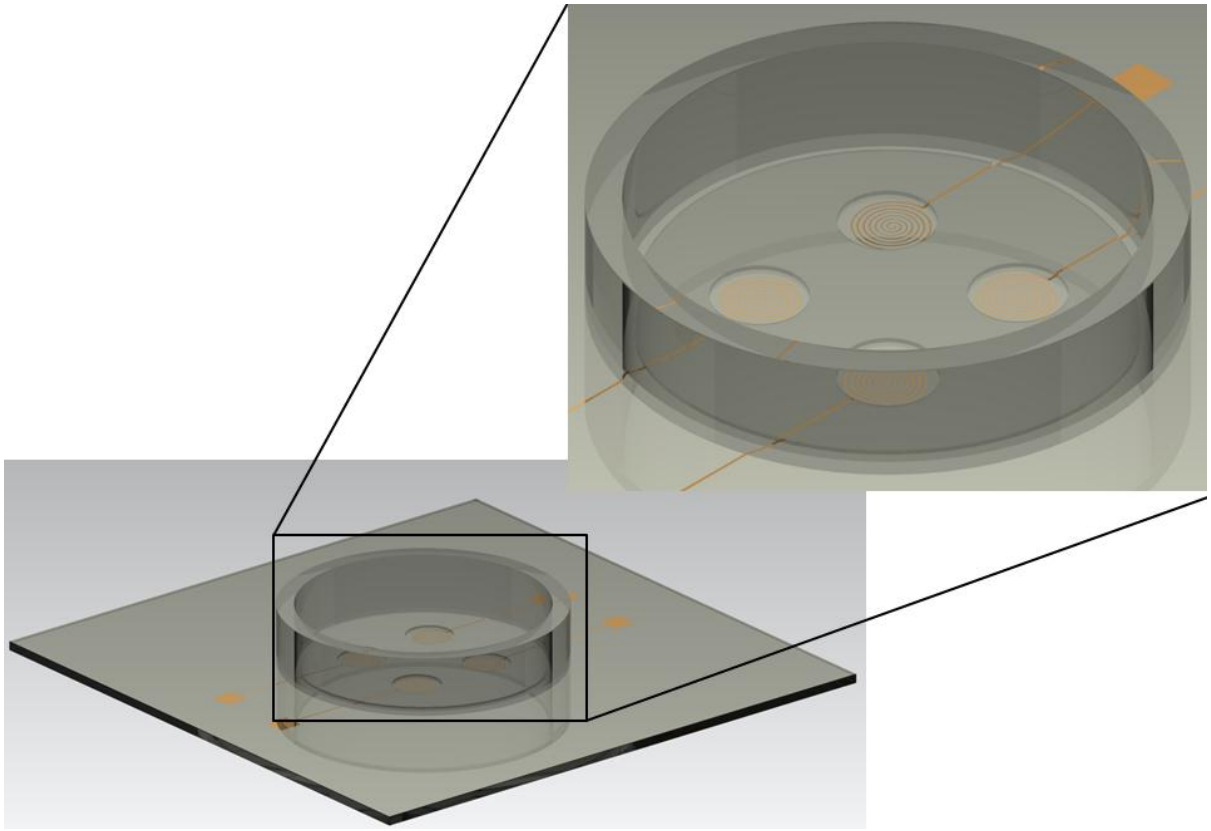


Figure 5.72: A CAD representation of the intended design concept 2 with PDMS microwells.

The results of this approach are presented in section 6.2.2.

5.8.2 Manufacturing Approach Four: Clean Room Based Photolithography

All of the processes of this manufacturing approach were implemented (Figure 5.73).

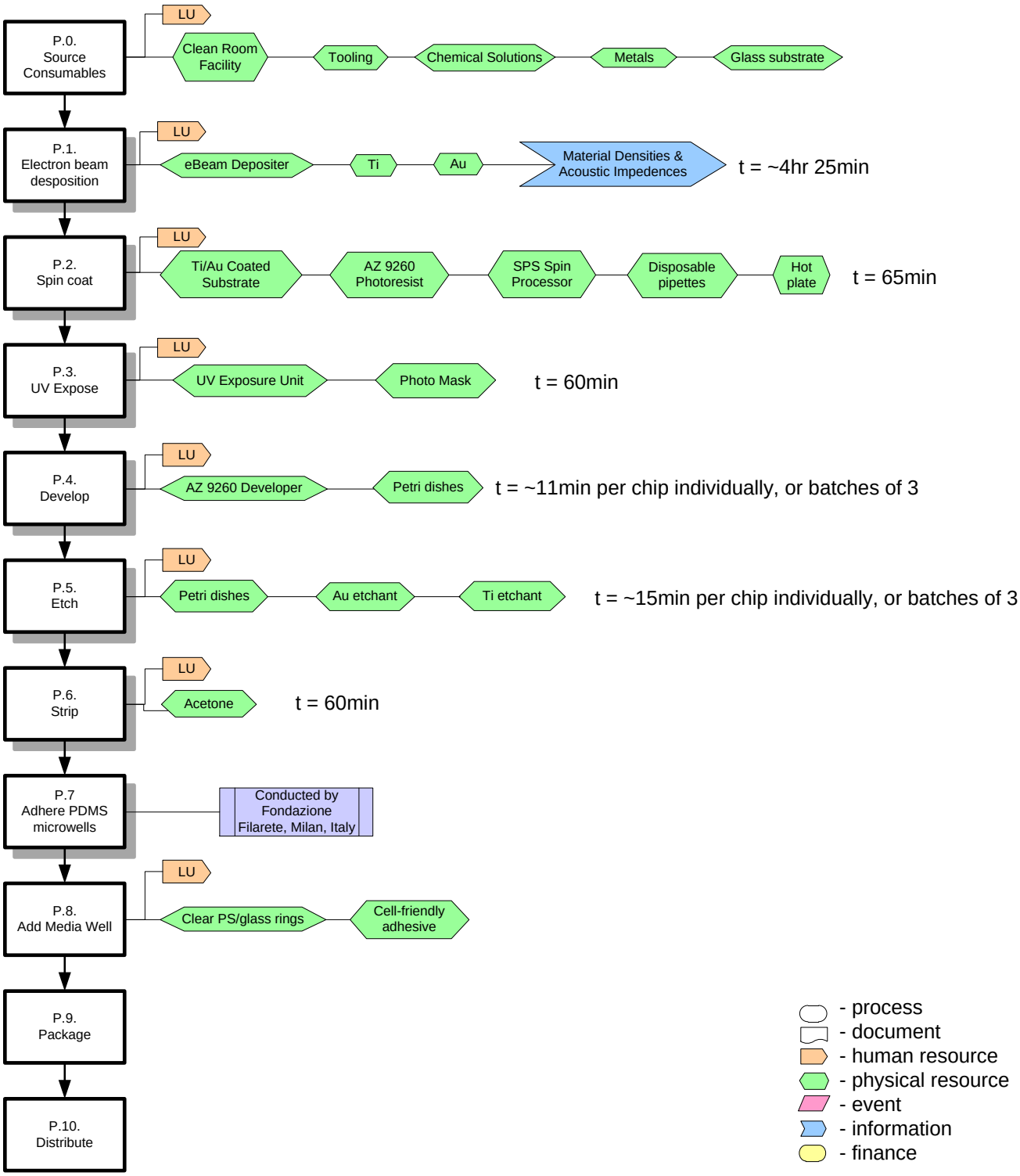


Figure 5.73: An activity diagram depicting the major processes used in clean room based MEA biochip manufacture.

All of the processes were successfully implemented resulting in the production of prototype biochips.

P.1. – Use electron beam deposition to coat glass slides with titanium and then gold.

P.2. – Spin coat the Ti/Au coated glass substrates with photoresist

P.3. – Expose the photoresist coated samples to UV light with patterned photomask in place.

P.4. – Develop the exposed photoresist

P.5. – Etch the exposed areas of the Au and Ti layers

P.6. – Strip the remaining photoresist to expose Au pattern

P.7. – Adhere PDMS microwells

P.8. - Add a media well to each biochip

P.9. – Package for transportation

P.10. – Distribute for testing

Process One – Electron beam deposition

An electron beam deposition system was used for this process (Figure 5.74). The process used to make the base substrate pattern is described in Figure 5.73. Additional drill down models of each activity can be viewed in Appendix E. Coating was done using batches of five base substrates. Substrates were coated in Ti then Au while inside the system.

Figure 5.74: Electron beam deposition system.

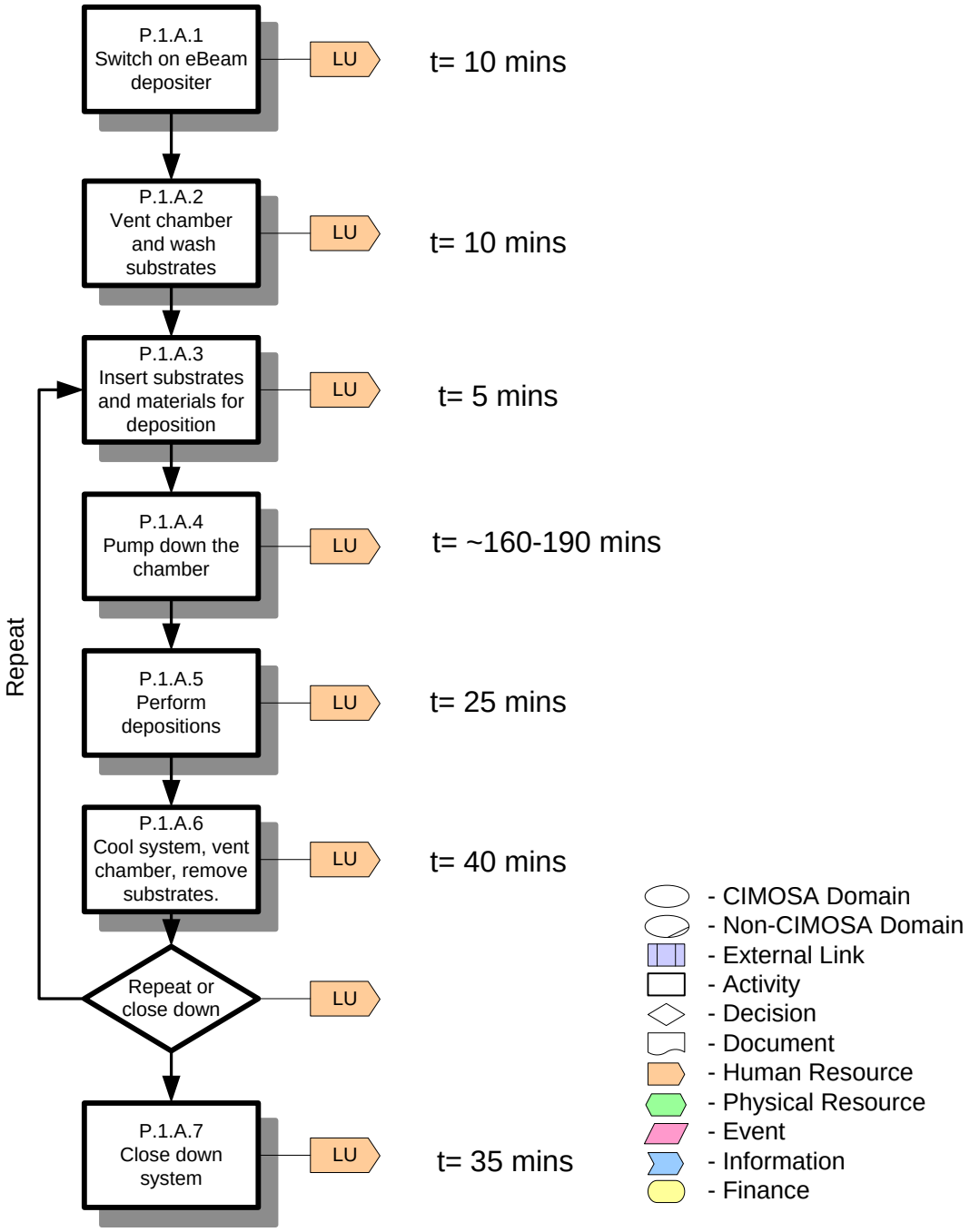


Figure 5.75: The process used to make the base substrate pattern for the clean room based manufacturing approach.

In activities A.1 – A.3 (Figure 5.74) the deposition was set-up by switching on the electron beam (e-beam) deposition system and venting the chamber up to atmospheric pressure. The chamber was opened and the glass wafers that have the glass base substrates secured (Figure 5.76) were placed inside on to the turn table (Figure 5.77).

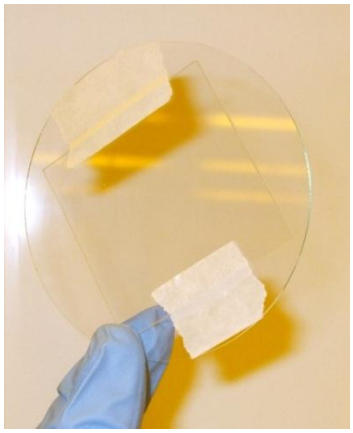


Figure 5.76: Glass base substrate secured to glass wafer for mounting in the e-beam deposition system chamber.

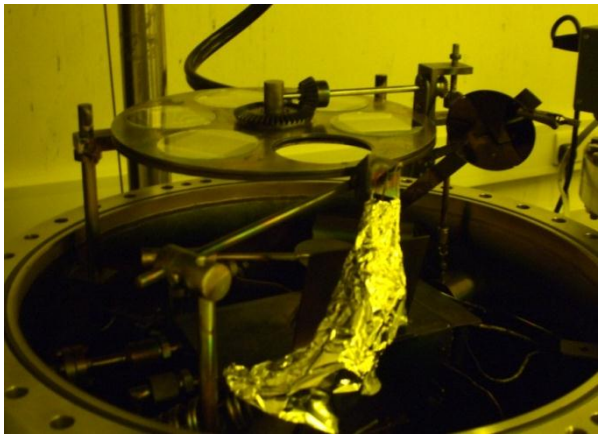


Figure 5.77: The turn table inside the chamber that allows sample to be rotated directly over the metallic source as it is heated by the electron beam.

For activities A.4 – A.6, the chamber was closed and pumped down to low pressure. Ti deposition was performed first. Then the source crucible was rotated to the Au and Au was deposited over each substrate. When completed the system was allowed to cool and vented back to atmospheric pressure. The chamber was opened and the coated glass substrates removed (Figure 5.78).

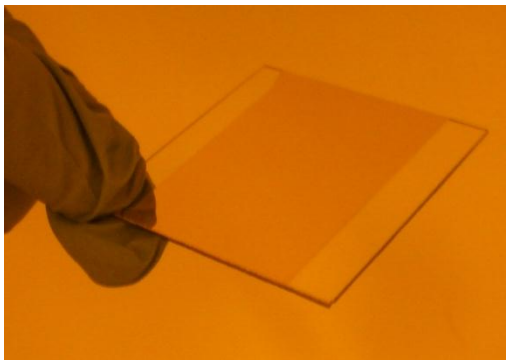


Figure 5.78: A Ti/Au coated substrate.

For system shut down, A.7, the chamber was closed, pumped down and switched off.

Process Two – Spin coat

The negative photoresist AZ 9260 was applied to each substrate by hand using a spin coating machine. Figure 5.79 shows the processes used.

Figure 5.79: The activities carried out for spin coating of the glass base substrates.

For A.1 the SPS spin processor 150 (Figure 5.80) was switched on and allowed to initialise up to the programming options.

Figure 5.80: The SPS spin processor 150 used in this manufacturing approach.

Table 5.14: The Spin coating program used to apply AZ 9260 photoresist.

Step One		**Step Two**	
Time	60	**Time**	40
RPM	400	**RPM**	2400

During activities A.2 – A.4 the substrate to be coated was positioned as centrally as possible onto the chuck. The vacuum was switched on to hold substrate in position. Using a disposable pipette 2.5ml of the AZ 9260 photoresist was dispersed onto the substrate in the centre (Figure 5.81) while taking care not create any air bubbles in the resist.

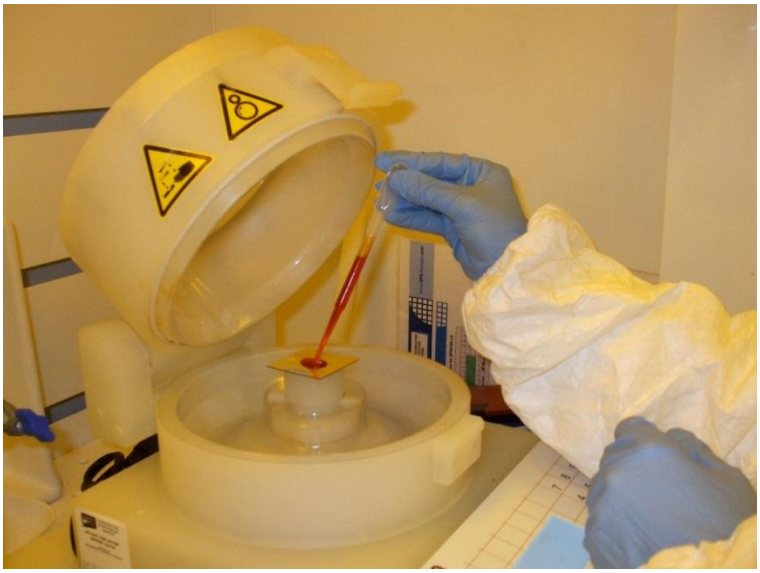


Figure 5.81: Application of AZ 9260 onto a Ti/Au coated base substrate.

For activities A.5 – A.8 the coating program was allowed to run. When the program was complete the lid was opened, the vacuum switched off and the coated substrate removed. Substrate where then left for 15 minutes before baking on a hot plate (Figure 5.82) at 100^{o}C for 3 – 4 minutes.

Figure 5.82: The hot plate and temperature control unit used in this manufacturing approach.

Process two was repeated for each individual base substrate.

Process Three – UV expose

Each photoresist coated substrate was exposed one-by-one in a UV exposure machine (Figure 5.83).

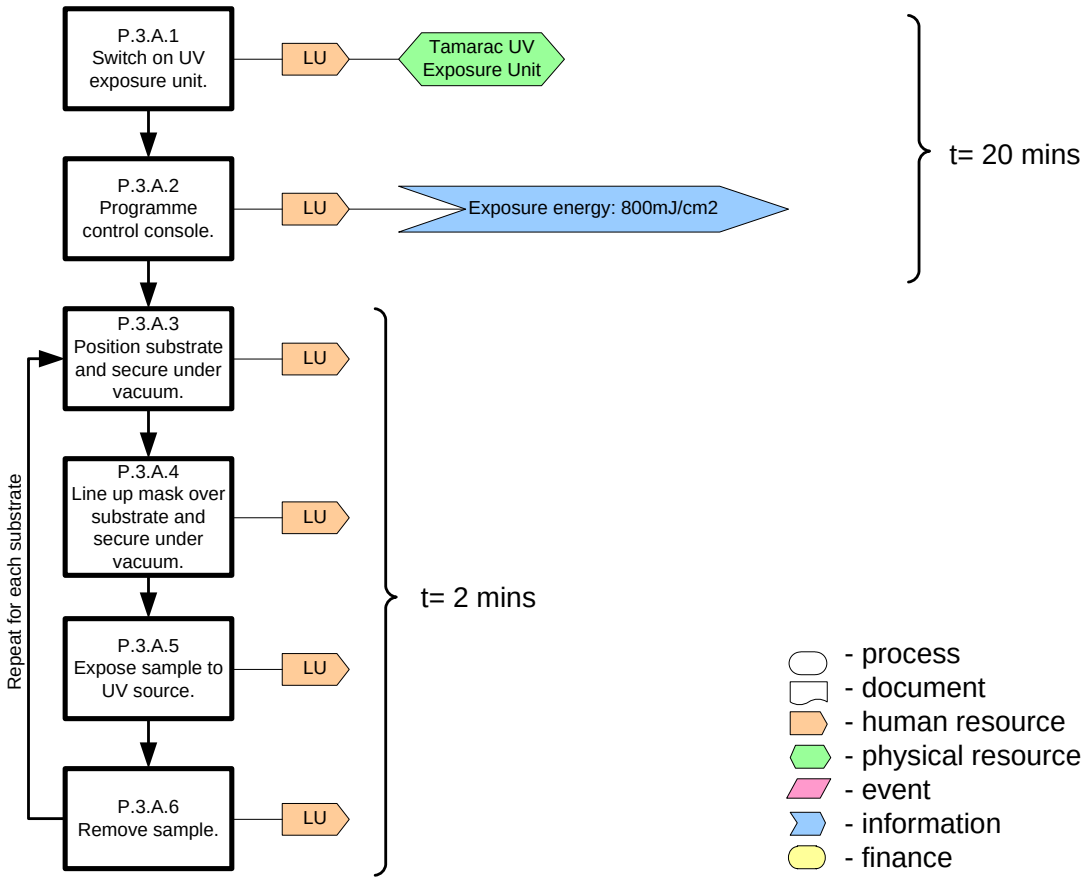


Figure 5.83: An activity model of clean room based UV exposure.

For activities A.1 – A.2 of process three the UV exposure unit (Figure 5.84) was switched on, allowed to initialise and programmed to exposure for a given period (30s) at a set energy (800mJ/cm^2).

Figure 5.84: The UV exposure system.

For activities A.3 – A.6 the AZ 9260 coated substrates were positioned on the system's stage. The acetate photomask (printed by JD Photo-Tools Ltd, Oldham, UK) was carefully lined up over the substrate by hand and a vacuum switched on to secure. The exposure program was run. When complete the substrate was removed from the system. This process was repeated for each base substrate.

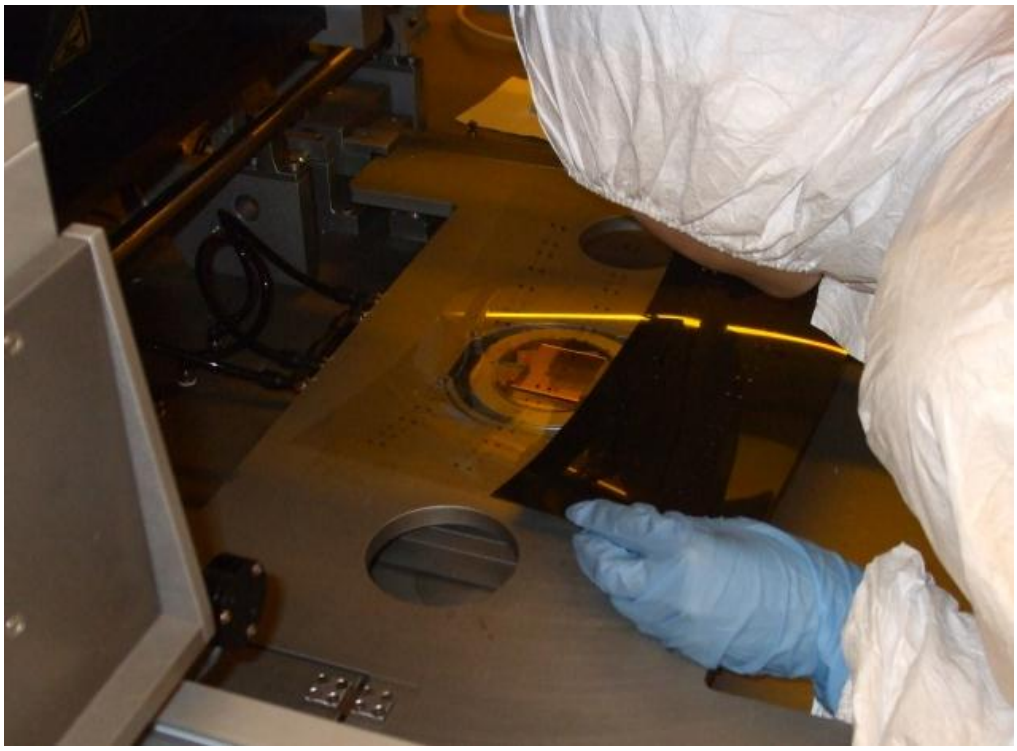


Figure 5.85: A coated substrate being positioned in the UV exposure system with the acetate photomask in place.

Process Four – Develop

The following model (Figure 5.86) described the activities carried out to develop the exposed substrates.

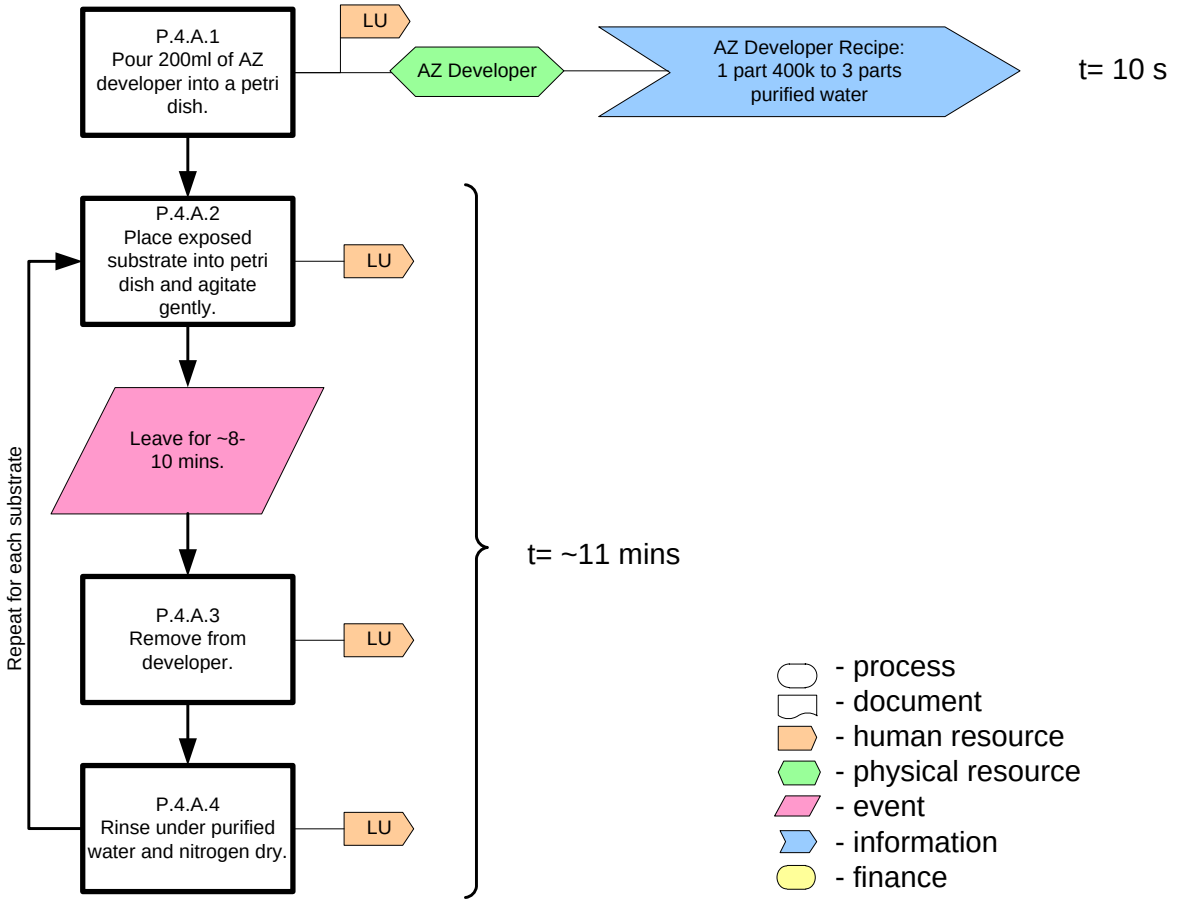


Figure 5.86: Developing the UV exposed base substrates.

For activities A.1 – A.4, a glass Petri dish was filled with 200ml of AZ developer, 1 part AZ® 400K with 3 parts purified water. The substrate was placed into the Petri dish and gently agitated to move the solution over the surface. The substrate was left in the developer (Figure 5.87) for 8-10 minutes until all of the exposed resist had been removed. The substrate was removed from the solution and rinsed under purified water. To dry the sample a pressurised stream of nitrogen gas was moved over the surface.

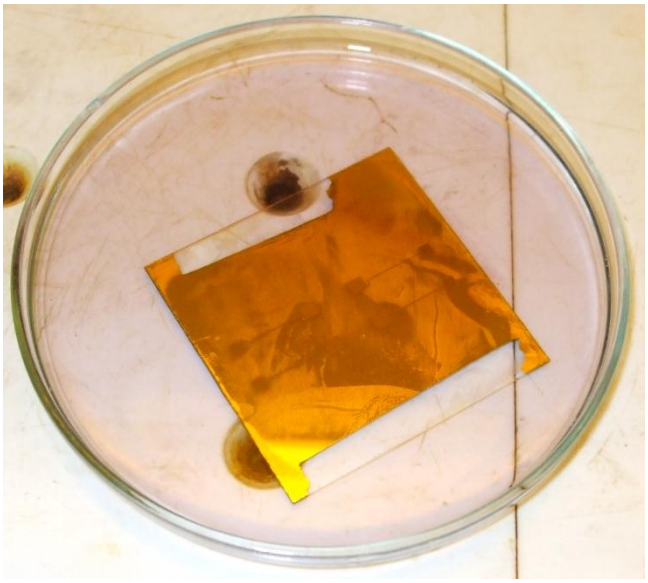


Figure 5.87: A base substrate in AZ developer. The pattern can be seen as the exposed areas of photoresist are dissolved.

Process Five – Etch

Etching was a two part process (Figure 5.88). The gold material was etched using a solution of nitric acid mixed with hydrochloric acid (1: 3 parts respectively). The titanium layer was then etched using a solution of hydrofluoric acid (HF) with hydrogen peroxide in deionised water (1: 1:20 parts respectively).

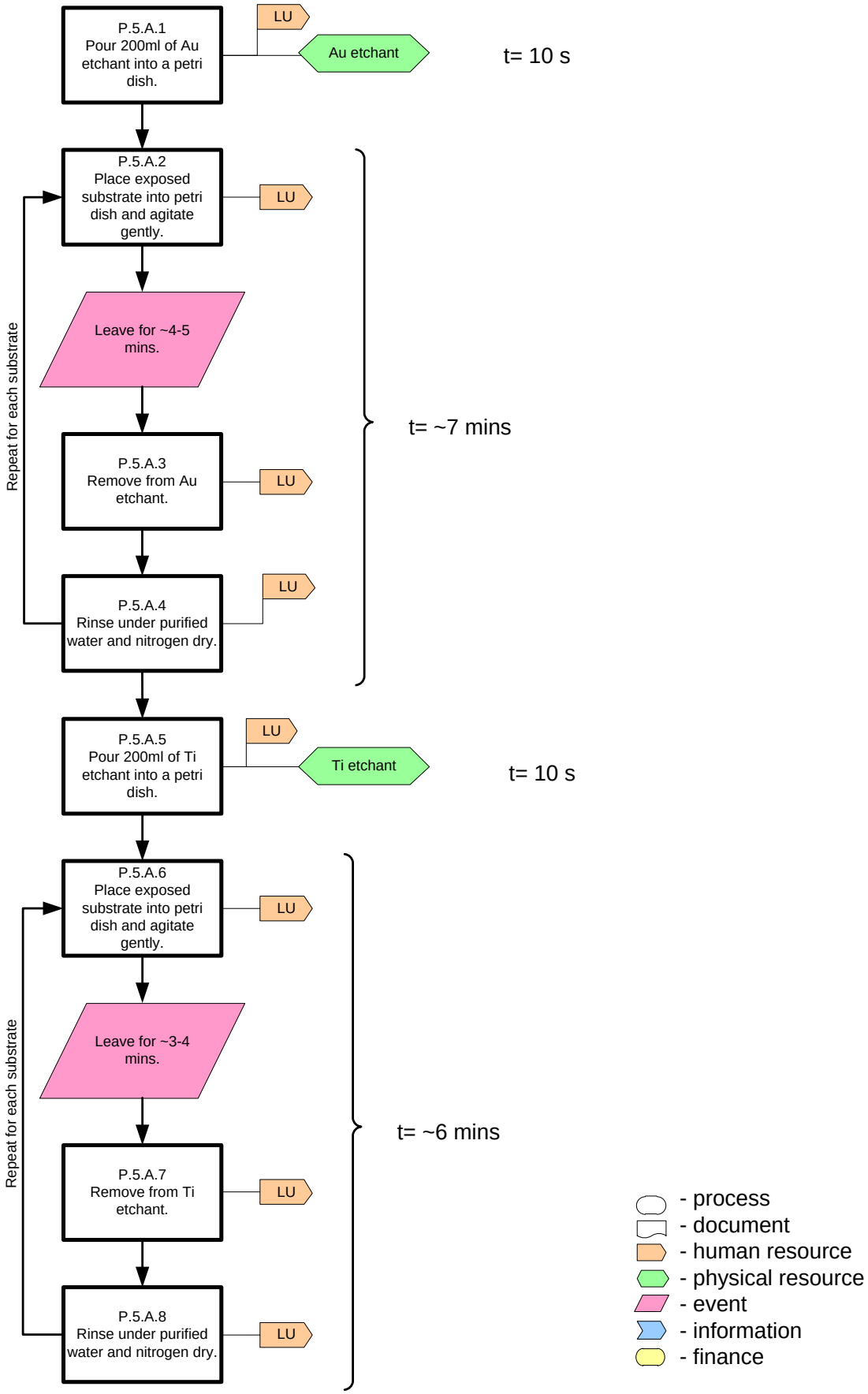


Figure 5.88: An activity diagram of the etching activities carried out for the clean room manufacturing approach.

To complete activities A.1 – A.8 200ml of the gold etchant solution was poured into a glass Petri dish and 200ml of the titanium etchant was poured into a polystyrene petri dish. The base substrate was placed into the etchant and gently agitated until the exposed metal was removed. The substrate was rinsed under purified water and dried under the nitrogen gas stream between etches, and again after etching. See Figure 5.89 for images of the process etching conducted.

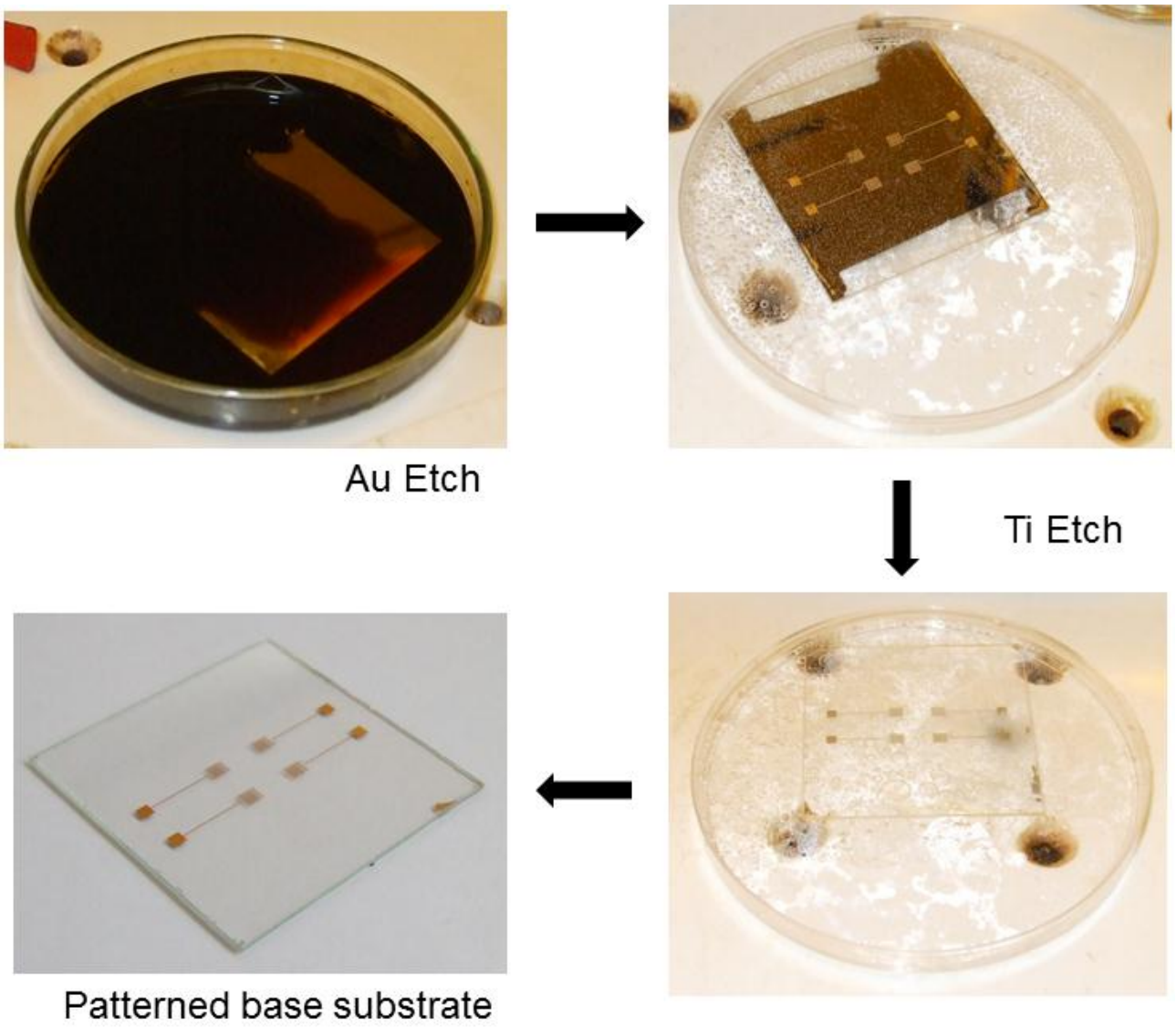


Figure 5.89: The etching of design concept 2 base substrates.

Process Six – Strip

The process used to strip the remaining photoresist from the patterned substrate is documented in Figure 5.90. Substrates were clean using acetone manually in a one-by-one fashion. Each one was them rinsed and dried using nitrogen gas.

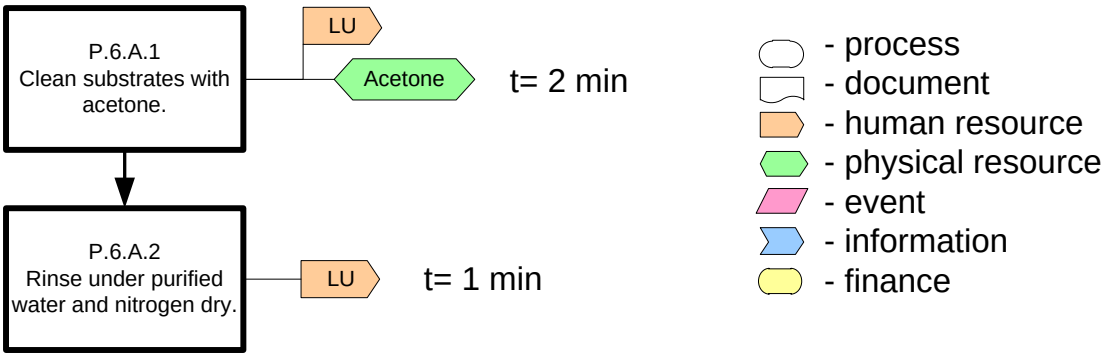


Figure 5.90: The photoresist stripping process for the clean room-based manufacturing approach.

Process Seven – Adhere PDMS microwells

The PDMS microwell and insulating material was applied to the prepared base substrate (Figure 5.91) by researchers at Fondazione Filarete, Milan.

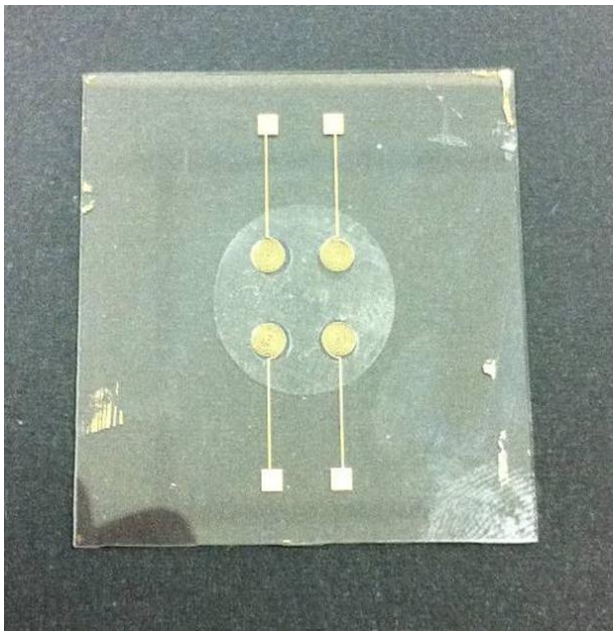


Figure 5.91: A base substrate with the PDMS microwells adhered.

Process Eight - Add media well

The media well attachment was conducted using the same process described in section 0.

Processes 9 and 10 – package and distribute are the same as those described in section 0.

5.8.3 Manufacturing Approach Four Summary

This manufacturing approach was completed inside a class 1000 clean room at Heriot Watt University, Edinburgh, UK. UV photolithography tooling was used to produce patterns in Ti/Au of resolutions down to 80µm. Two pattern variants were manufactured as one batch. A total of 24 base substrates were patterned. After a quality inspection the base substrates suitable for testing were constructed into MEA biochips for wet testing at the University of Nottingham.

5.8.4 Evaluation of Manufacturing Approach Four

The clean room based photolithography approach produced the following outcomes.

Table 5.15: The outcomes of the micro drilling (Excimer laser) approach.

Priority	Critical Manufacturing Outcome	Approach achieved?
1	Produce satisfactory base substrate	Yes
2	Produce satisfactory media well	Yes
3	Facilitate adequate attachment of the media well and base substrate components	Yes
4	Produce a satisfactory electrode site for cell-electrode interfacing	Yes
5	Produce an appropriate micro-well geometry around electrodes	Yes
6	Produce enough sites so as to allow assignment of a reference	Yes
7	Produce an MEA biochip that allows appropriate optical inspection of living samples	Yes

This manufacturing approach resulted in the second generation of prototype available for testing (Figure 5.92). Further description of the results relating to this manufacturing approach and the prototypes tested are contained in section 6.2.2.

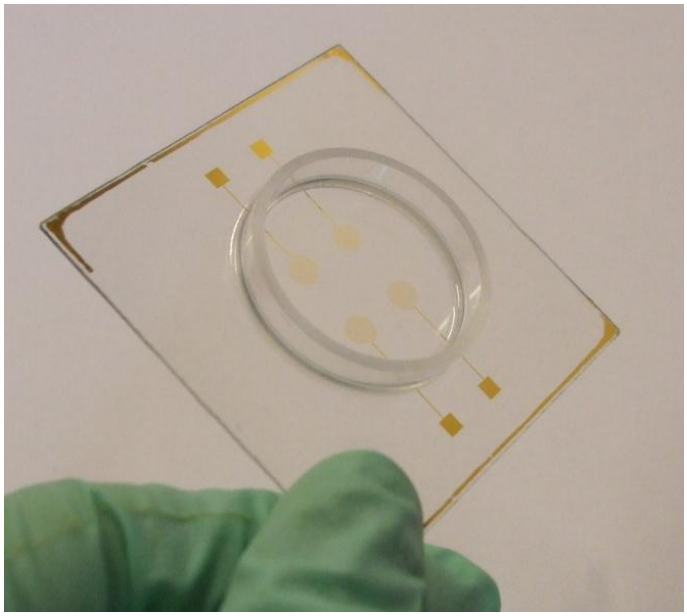


Figure 5.92: The second generation prototype, with spiral shaped electrodes.

5.9 Inkjet Printing Investigation

In addition to the manufacturing approaches, described the feasibility of using inkjet printing manufacturing techniques to make the base substrate component was investigated. The investigation of this approach was conducted by Printed Electronic Limited of the Invotech Group (Tamworth, UK). Attempts were made to print the design concept 2 pattern directly on to glass using an Au ink. The results of this investigation are demonstrated here in Figure 5.93 and Figure 5.94.

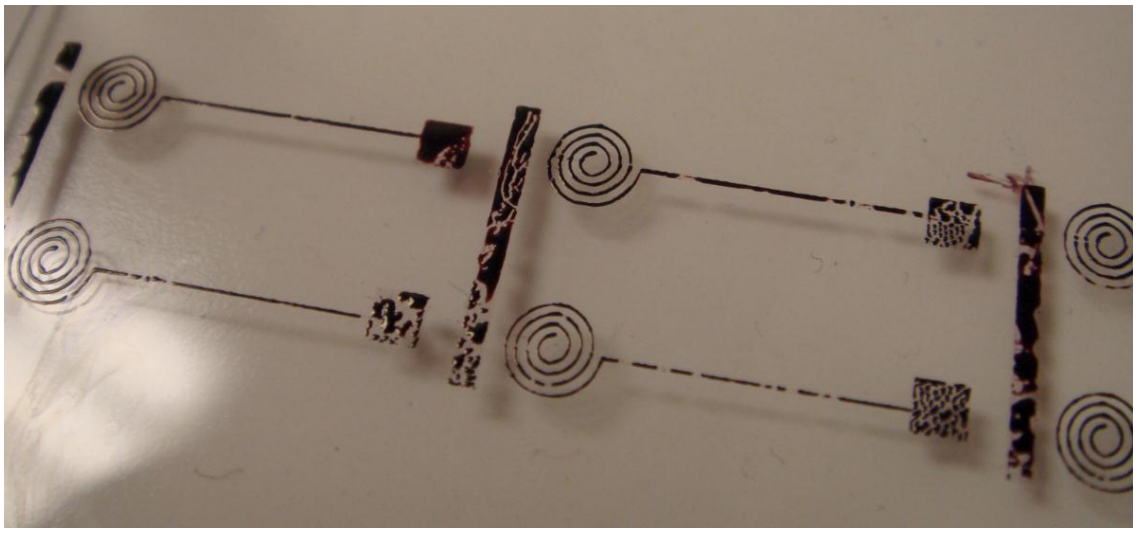


Figure 5.93: The design concept 2 pattern printed using a gold ink.

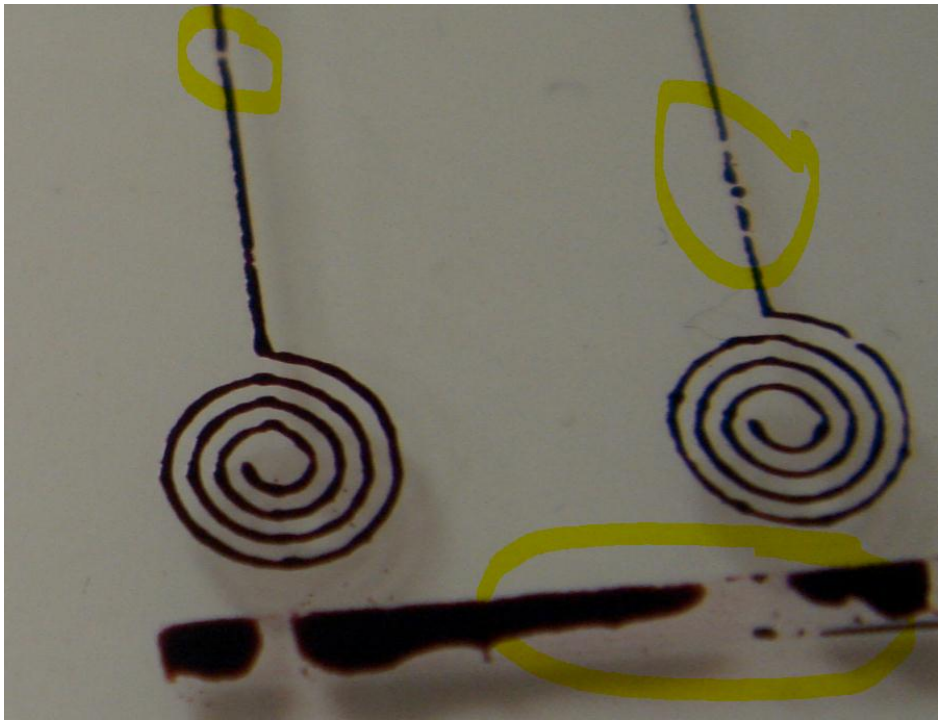


Figure 5.94: Examples of the failed adhesion (highlighted within yellow circles) of the Au ink to the surface of the glass.

The findings of this work imply that inkjet printing might be a viable manufacturing technique for devices such as MEA biochips in the future if ink formulations can be suited to the necessary base substrate materials. The key benefit that inkjet printing offers is fast (e.g. patterns as illustrated in Figure 5.94 can be produced in <15 minutes) and flexible production of conductive parts. A potentially limiting factor that would require appropriate consideration if this technique were to be implemented in the future would be that printed inks show poor mechanical properties so may not be suited to the cleaning protocols required by the bioscience environment and re-use facility demanded by consumers purchasing these high cost consumables.

5.10 Summary

The rationale used to guide design and manufacture target definition considered both the original aim of the research, to produce a novel MEA system (described in Appendix A), and also existing systems in terms of what would be novel and realistic. Existing systems were considered in terms of capabilities they offer users, how components of existing systems are manufactured, and how systems are used.

An application specific design specification was created during this research and used to generate targets that were prioritised into design and manufacture deliverables.

The prioritisation of targets intending to produce a solution device that would meet the defined user requirements was made using a house of quality matrix that assessed interrelationships and influences present between user requirements and the technical requirements brought about by the solution concept designed.

Two novel concepts were designed and manufactured to meet the prioritised targets.

In the pursuit of prototype manufacture a number of possible manufacturing techniques were considered and implemented to differing extents. The results of prototypes manufactured during this research are presented and discussed in the following chapters.

The requirements, rationale, concept design, manufacturing and prototype success that have been presented through this Chapter are detailed in Figure 5.95.

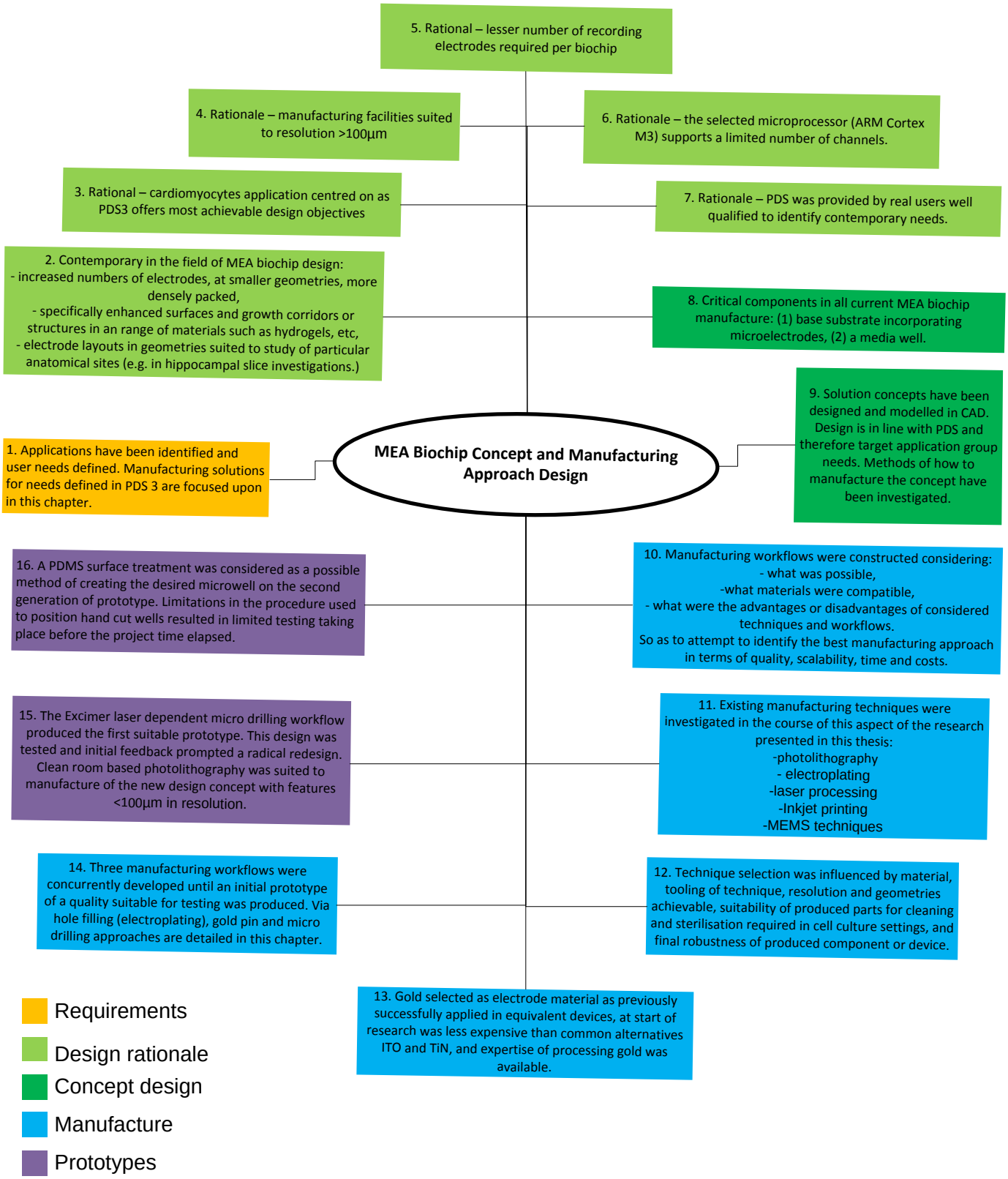


Figure 5.95: A summary of the contents of chapter 5.

5.11 Research Questions Answered

1. **What current and future user and system requirements have been derived from literature reviews, commercial system assessment and case studies that can be realistically addressed by this research? How does this research prioritise requirements?**

User requirements identified and documented throughout Chapters 2, 3 and 4 have been prioritised using the quality function deployment house of quality (HoQ) method. The HoQ considered interrelationships and influences present between both user and technical requirements. The following targets were prioritised and addressed by this research:

- Biochip must be as small in size as possible
- Biochip must be re-useable at least 10 – 15 times
- Materials used in manufacture must be durable
- Manufacture of the device must be through techniques that are as low cost as possible
- Manufacturing techniques must be as fast as possible
- Materials utilised must be as low cost as possible
- Prototype devices must be safe
- Solution biochips must support >1 beating cluster per media well
- The biochip must be lightweight

2. **How can this research project meet the defined and targeted requirements?**

This research constructed product design specification documents to collate the numerous needs identified through this research (section 5.2). User requirements were assessed and prioritised targets used to specify and create solution design concepts (section 5.3). Investigation took place of how to manufacture the design concepts into prototypes for testing (section 5.4 – 5.9). Prototypes manufactured were tested and the results in terms of successfully meeting targets are discussed in Chapters 6 and 7.

3. **What manufacturing solutions are suitable for production of the proposed solution concept?**

A number of existing microelectronics manufacturing approaches were identified as suitable for the manufacture of the novel MEA biochip concept:

- Integrated Circuit and Micro Electrical Manufacturing Systems (MEMS) Techniques
  - Photolithography
  - Thin Film Deposition
  - Etching
- Electroplating
- Laser Machining
- Additive Manufacturing (Inkjet Printing)

The ways in which each method has been implemented in pursuit of prototype manufacture are described in this chapter.

The success of the above approaches implemented in this work are listed in Table 5.16.

Table 5.16: A summary of successes of the manufacturing approaches implemented.

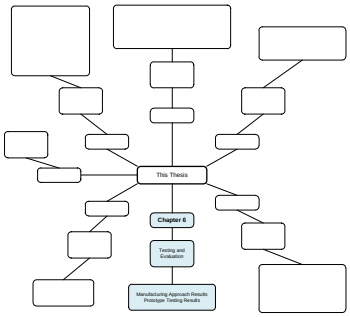
Approach	Successful / Unsuccessful
IC and MEMS Techniques - Photolithography - Thin Film Deposition - Etching	Successful Successful (sections **Error! Reference source not found.**) Successful (section 5.8) Successful (section 5.6 and 5.8)
Electroplating	Unsuccessful (section 5.6.1)
Laser Machining	Successful (section 5.6.3) – Excimer laser Unsuccessful (section 5.6) – CO_2 laser
Additive Manufacturing (Inkjet Printing)	Unsuccessful (section 5.9)

4. **What are the critical manufacturing outcomes with regard to the proposed solution? What is the benchmark or gold standard to which the novel biochip will be compared?**

Manufacturing approaches were assessed during implementation through achievement of the following critical manufacturing outcomes:

- Produce satisfactory base substrate
- Produce satisfactory media well
- Facilitate adequate attachment of the media well and base substrate components
- Produce a satisfactory electrode site for cell-electrode interfacing
- Produce an appropriate micro-well geometry around electrodes
- Produce enough sites so as to allow assignment of a reference
- Produce an MEA biochip that allows appropriate optical inspection of living samples

The benchmark and gold standard device for the target application type, stem cell-derived cardiomyocyte beating clusters, is the same. A typical planar microelectrode array, arranged in an 8x8 grid array with ~200µm spacing is available for all MEA manufacturers and is presently the most suited MEA biochip for this particular cell source. Novel prototypes are compared to this device.



Chapter Six

Manufacturing and Prototype Testing Results

This chapter presents the findings of this research in terms of the manufacturing approaches implemented, and the subsequent testing of the resulting prototype MEA biochips.

The work in this chapter answers the following research questions:

1. **What has this research done to produce a solution device to meet the requirements identified?**

2. **How were the manufactured prototypes tested, and what were the outcomes of those tests?**

3. **Has a suitable alternative to current the standard commercial biochips used in cardiomyocyte cluster application been realised?**

6.1 Results of Manufacturing Approaches

Each manufacturing approach presented in Chapter 5 is described in the following sections.

6.1.1 Via Hole Filling Approach

The via hole filling manufacturing approach produced the following MEA biochip outcomes (Table 6.1).

Table 6.1: The outcomes of the via hole filling approach.

Priority	Critical Manufacturing Outcome	Approach achieved?
1	Produce satisfactory base substrate	Yes
2	Produce satisfactory media well	Yes
3	Facilitate adequate attachment of the media well and base substrate components	Yes
4	Produce a satisfactory electrode site for cell-electrode interfacing	No
5	Produce an appropriate micro-well geometry around electrodes	No
6	Produce enough sites so as to allow assignment of a reference	No
7	Produce an MEA biochip that allows appropriate optical inspection of living samples	No

Prototype produced for testing: No

6.1.1.1 Base substrate

The base substrates were manufactured using a UV photolithography approach. The lowest feature resolution produced in manufacturing was ~ 100µm. Figure 6.1 shows the first batch of MEA base substrates manufactured at Loughborough University using standard FR4 board with a 35µm Cu foil cladding.

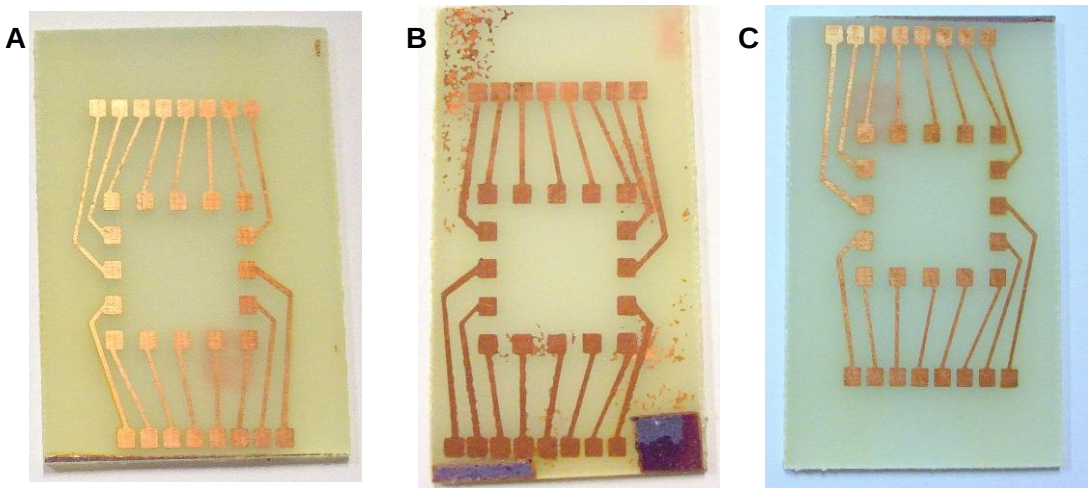


Figure 6.1: An example of three base substrates patterned by hand for experimentation during the development of manufacturing approach one.

The initial interconnect contact pads and electrode sites patterned were 2mm x 2mm. Tracking was 500µm wide. Large electrode sites reduced the precision required of early drilling process experimentation while providing samples for electroplating testing. Samples A, B, and C (Figure 6.1) demonstrate the variation in patterning quality where substrates were processed in the same way. Sample B particularly demonstrates occurrences where areas of speckled Cu remained on the substrates. The consequence of this speckling of conductive material over the substrate is significant if it results in short circuits.

The quality of base substrates patterned was variable throughout manufacture. Factors that influenced the variability of samples were: the manual positioning of the photomask and UV exposure system set up; the presence of dust contamination in the atmosphere during UV exposure; and the concentrations of the etchants used, varying between processing of different batches due to numerous facility users. Improved control over these variables resulted in achievement of an optimum manufacturing process for base substrates (Figure 6.2) that was used by manufacturing approaches one to three (Chapter 5).

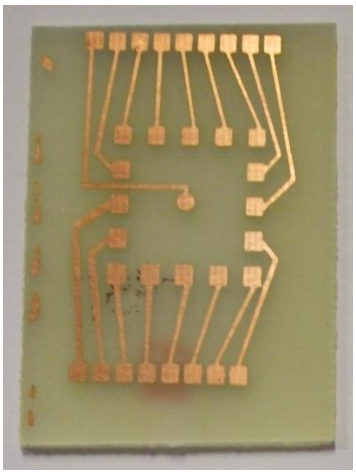


Figure 6.2: A suitably patterned base substrate containing 16 electrode sites and 1 reference electrode site at the centre.

In the demonstrated samples (Figure 6.1 and Figure 6.2) the outer geometries of the boards are random. Samples were cut by-hand for speed and convenience as these base substrates were for experimental use only. As the patterning process was optimised smaller electrode and track dimensions ensued for base substrates and biochip dimensions were more tightly controlled.

6.1.1.2 Media well selection and attachment to the base substrate

To accommodate the specified 35mm diameter media well dimensions commercially available Petri dishes were selected. The Petri dishes required a small amount of pre-processing to make them suitable for application. The bottom surface of the dish has a small lip to aid stacking. This lip was polished down using a very fine grade of wet and dry paper producing a flat surface. A flush contact between the Petri dish surface and the Cu patterned base substrate was achieved.

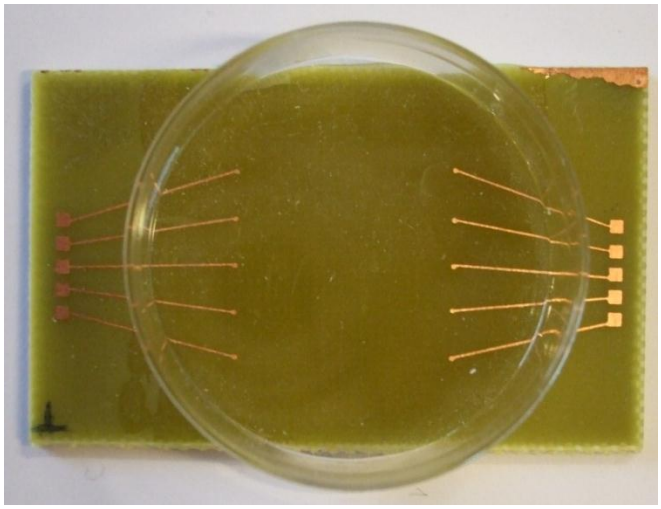


Figure 6.3: A base substrate with a 35mm Petri dish adhered. Scale: Electrode tip diameter is 500µm.

A transparent epoxy resin suitably affixed the Petri dish over the Cu pattern as demonstrated in Figure 6.3. A cylindrical 25g weight was placed into the Petri dish during curing to ensure good contact.

6.1.1.3 Satisfactory electrode sites and microwell geometry

Each prepared substrate required individual positioning inside a CNC drilling machine (Figure 5.47) due to biochip-biochip variation.

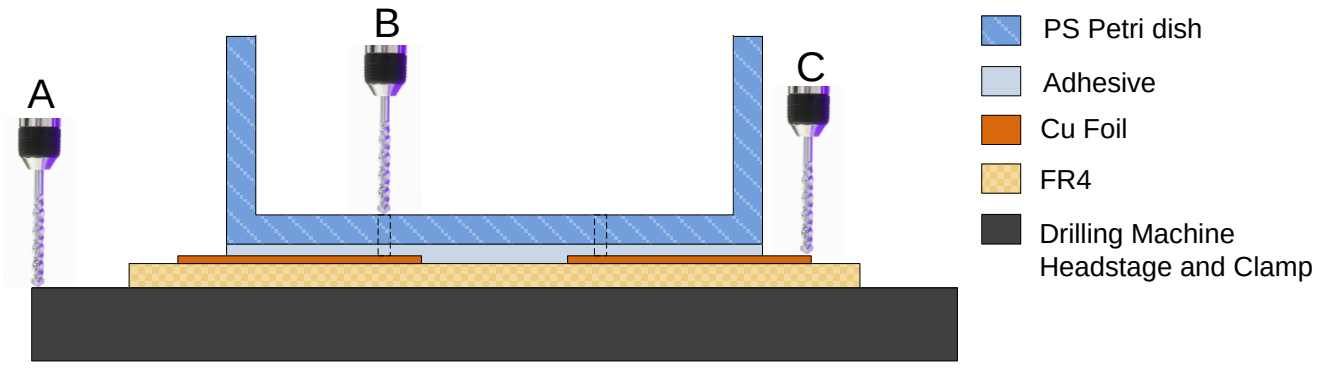


Figure 6.4: The zeroing positions considered for this process.

A problem encountered during the CNC drilling process related to the by-hand, relatively imprecise positioning of the Petri dish on the base substrates and base substrates on the drilling stage. Base substrates placed on the level stage did not sit level due to warping of the FR4. Figure 6.4 shows three positions (A, B and C) considered as zeroing points for the CNC drilling program.

The most significant issue encountered during drilling was in controlling the tolerance of the drilling depth in the z-axis. The base substrates had each been cut by-hand and were of differing dimensions. A standard jig was also not able to guarantee that the substrates were positioned in exactly the same position each time. The Petri dishes had also been adhered to the base substrate by-hand resulting in differing thicknesses of adhesive between the two surfaces on differing prototype samples. The consequence of this was that the drilling in the z-orientation could be either too deep, resulting in detrimental removal of the underlying Cu, or not deep enough, therefore not reaching the Cu.

Zeroing position A –This was not used as substrates were different shapes and were positioned by-hand each time.

Zeroing position B – This position was considered to provide greater z-axis control due the variations in adhesive thickness. By drilling from the surface through to the plane on which the Cu was located would improve drilling success. In this case, where base substrates sat level on the drilling stage surface adequately, controlled depth of drilling was achieved. However, a number of the substrates did not lay flat resulting in holes that were of differing depths. A number of holes were either too deep, resulting in detrimental removal of the underlying Cu pattern, or not deep enough to reach the Cu.

Zeroing position C – This position was considered as a method of preventing imprecise drilling on the z-axis but was not implemented due to time constraints.

Substrates failed to progress beyond the drilling stage due to the following drilling process defects:

- Holes too deep
- Holes not deep enough
- Holes were not completely over an electrode.

For the substrates where drilling was successful, to minimise costs of material consumption, initial electroplating was Cu. A final thin layer of Au was plated on top to seal in the Cu from the living cells and culture media. The depth of holes through the Petri dish and adhesive to the under lying Cu were approximately 780-850µm. The Cu layer was intended to be ~550-600µm deep to create a

recess over each electrode that would serve as the microwell into which a single beating cluster of cardiomyocytes would be placed. Unfortunately, this approach failed to fill adequately due to air bubbles lodged in the drilled holes during electroplating (Figure 6.5 and Figure 6.6).

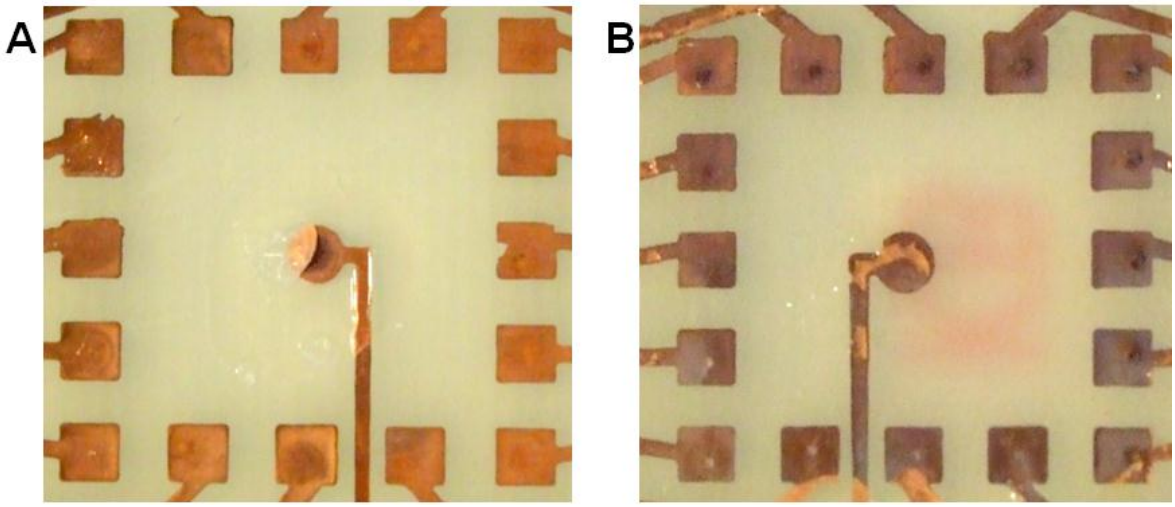


Figure 6.5: Images of the electrode sites and attempted microwell filling. Petri dishes and adhesive were carefully removed to reveal the extent of filling.

Initial drilling and electroplating was performed using the pattern design demonstrated in Figure 6.2. Different electroplating solutions were tested (section 0). The commercial solution, Electroposit™1300 (Figure 6.6), produced better results than the home-made alternative (Figure 6.5). To tackle the problem of air bubbles in the drilled holes the electroplating process was conducted in an ultrasonic bath. Improvements were made but bubbles inside the holes persisted (Figure 6.6).

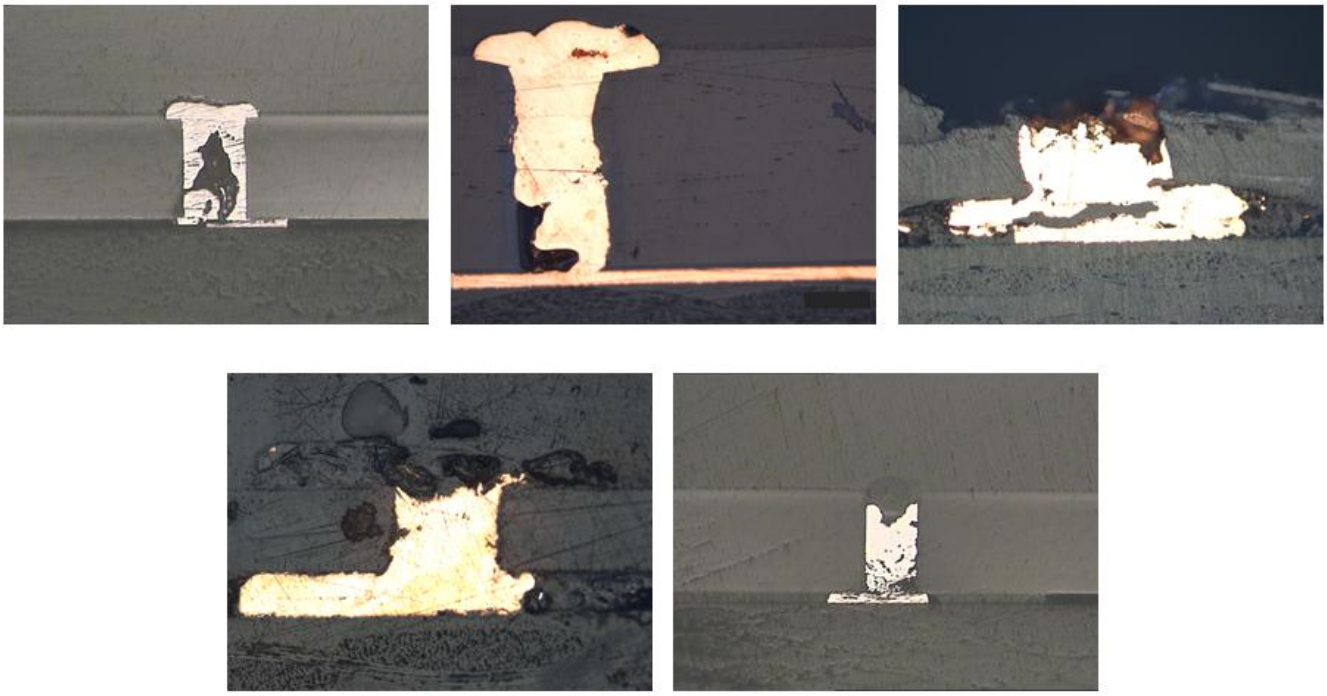


Figure 6.6: Cross-sectional micrograph images of the microwells filled during manufacturing approach implementation.

Control over the depth of the filling was variable. The surface of the holes that did fill was also not level or smooth which was not suitable for the intended application where consistent microwells are required that can be thoroughly cleaned.

6.1.2 Gold Pins Approach

The gold pins manufacturing approach produced the following outcomes (Table 6.2).

Table 6.2: The outcomes of the gold pins approach.

Priority	Critical Manufacturing Outcome	Approach achieved?
1	Produce satisfactory base substrate	Yes
2	Produce satisfactory media well	No
3	Facilitate adequate attachment of the media well and base substrate components	No
4	Produce a satisfactory electrode site for cell-electrode interfacing	No
5	Produce an appropriate micro-well geometry around electrodes	No
6	Produce enough sites so as to allow assignment of a reference	No
7	Produce an MEA biochip that allows appropriate optical inspection of living samples	No

Prototype produced for testing: No

6.1.2.1 Base substrate

The base substrates were manufactured using the photolithography process previously described. The only difference was that the artwork was adjusted to produce a pattern of contact points that matched the alignment of the pins held in sections of PGA (Figure 6.7). The sections of pins were cut by-hand and successfully connected to the base pattern using a conductive tin solder paste.

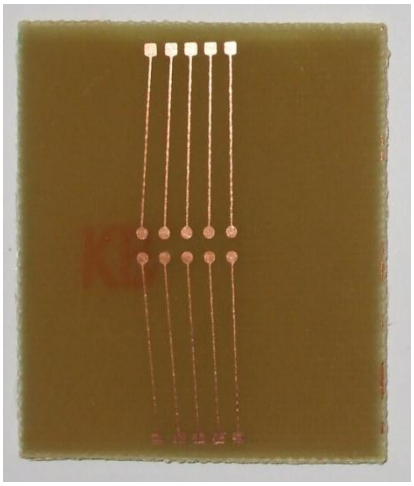


Figure 6.7: The adjusted pattern of contact points used for the gold pin manufacturing approach to ensure prototypes could connect to a commercial MEA system for testing.

6.1.2.2 Media well drilling and attachment of the media well to the conductive pins

Base substrates were paired with Petri dishes that had been drilled with hole configurations matching the PGA vertical pin geometry. Petri dishes were secured in position using clear epoxy resin (Figure 6.8). Two methods were explored for securing the Petri dishes in position (section 0). For methods where a second drilling process was intended, imprecise positioning of the Petri dish resulted in difficulty repeating the drilling process. Method 2B was not implemented so no results are presented.

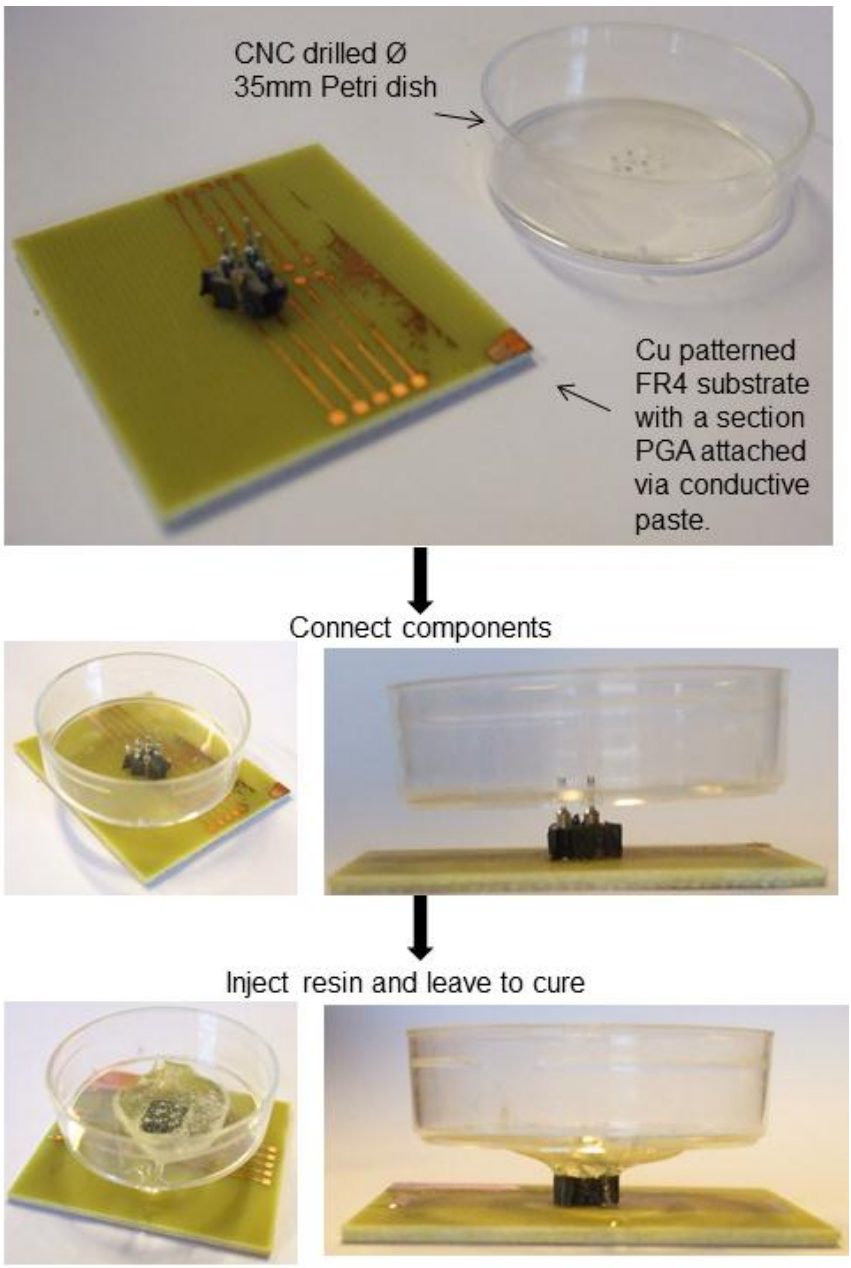


Figure 6.8: Securing the previously drilled Petri dish to the base substrate that has PGA pins connected to each electrode site.

Problems with this approach were:

- For method 2B a precision manufactured nippled blocker was required.
- Ensuring a water-tight seal every time without encapsulating the electrode tip or protruding out of the hole was difficult to control.
- Where the PGA attachment was not at exactly 90° there were differences in pin location that were significant when drilling for the second time. (The re-drilling process was difficult to align due to the imprecise construction of the biochips, described in 5.6.2.2).

While this approach was being explored manufacturing approach 3 (micro drilling) was also being developed. Manufacturing approach 3 provided a prototype for testing before this approach was developed any further.

6.1.3 Micro Drilling Approach

The micro drilling manufacturing approach produced the following outcomes (Table 6.3).

Table 6.3: The outcomes of the micro drilling approach

Priority	Critical Manufacturing Outcome	Approach achieved?
1	Produce satisfactory base substrate	Yes
2	Produce satisfactory media well	Yes
3	Facilitate adequate attachment of the media well and base substrate components	Yes
4	Produce a satisfactory electrode site for cell-electrode interfacing	Yes
5	Produce an appropriate micro-well geometry around electrodes	Yes
6	Produce enough sites so as to allow assignment of a reference	Yes
7	Produce an MEA biochip that allows appropriate optical inspection of living samples	No

Prototype produced for testing: Yes

6.1.3.1 Base Substrate

Base substrates were manufactured in Cu and outsourced to PMD Group, Coventry, UK for Au plating. Eight electrode sites were incorporated per biochip, of which one (not seeded with cells) required assignment as a reference electrode in the detection and recording software.

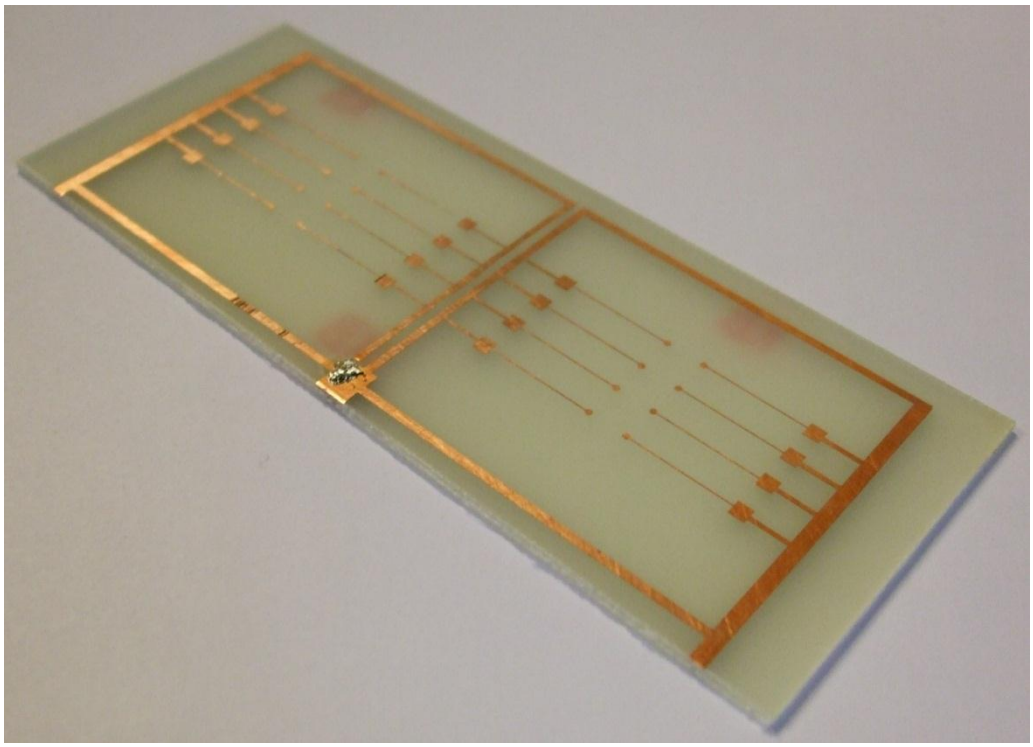


Figure 6.9: The Cu pattern produced for manufacturing approach three. Design compliments connections for Au plating.

6.1.3.2 Production of microwell features

Three materials were tested during this manufacturing approach development as insulator and consequent microwell material: glass coverslips, PE coverslips and Melinex PET film. Two methods were investigated for drilling microwells into these materials: a mechanical CNC drilling technique and an Excimer laser ablation technique.

CNC Drilling: Glass and PE cover slips were drilled using the Hurco VM1 CNC milling machine described in section **Error! Reference source not found.**. The glass cover slips shattered for all attempts at drilling. The PE coverslips were successfully processed using this approach but difficulties arose relating to the control of the exact depth of the hole (as described in section 6.1.1.3). This process damaged the underlying metallic surface on test samples due to warping of the FR4 base material and the variation of the thickness of the epoxy adhesive. The holes produced were also distorted in shape.

Excimer laser ablastion: Glass and PE coverslips were initially investigated. The PE samples were ablated at much lower energies than the glass equivalents. All of the glass coverslips used in testing broke with handling so glass was eliminated from testing. To overcome the dimensional limitation of standard coverslips an alternative film, Melinex™, was tested. Melinex is a polyethylene terephthalate (PET) film available as A4 sized sheets of varying thicknesses (12-350µm) that had been used previously in cell culture as a growth substrate (Laskarakis, Georgiou and Logothetidis, 2010). Melinex™ is treated on both sides producing a surface that is well-suited to cell culture. The Melinex™ film was ablated successfully to produce microwells meeting the specification.

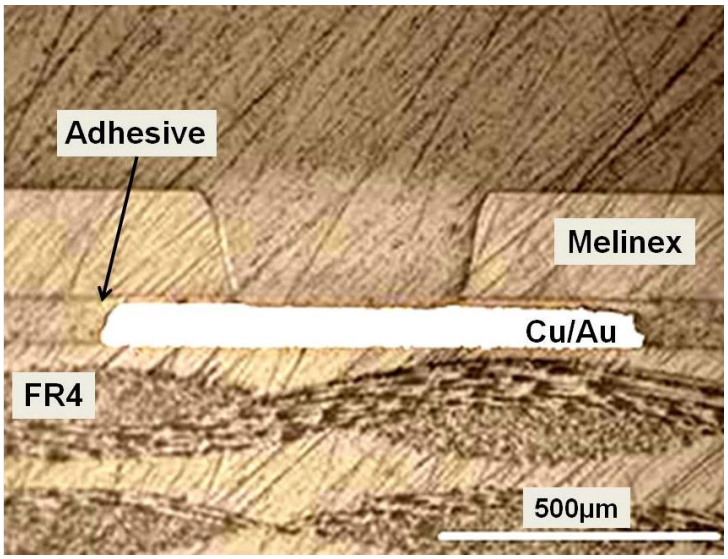


Figure 6.10: A cross sectional micrograph of the microwell ablated over an electrode through Melinex™ to form a microwell that meets the specification.

6.1.3.3 Media Well

Media wells were made by cutting standard PE tubing into rings (height 10mm) and adhered to the Melinex surface to complete construction (Figure 5.67). During trials the user testing the devices recommended that this sub-component was changed to a transparent material and was reduced in height. The PE tubing material was replaced with transparent tubing cut to heights of 6mm.

6.1.4 Clean Room Based Photolithography

The clean room based photolithography manufacturing approach produced the following outcomes (Table 6.4).

Table 6.4: The outcomes of the clean room based photolithography approach.

Priority	Critical Manufacturing Outcome	Approach achieved?
1	Produce satisfactory base substrate	Yes
2	Produce satisfactory media well	Yes
3	Facilitate adequate attachment of the media well and base substrate components	Yes
4	Produce a satisfactory electrode site for cell-electrode interfacing	Yes
5	Produce an appropriate micro-well geometry around electrodes	Yes
6	Produce enough sites so as to allow assignment of a reference	Yes
7	Produce an MEA biochip that allows appropriate optical inspection of living samples	Yes

Prototype produced for testing: Yes

6.1.4.1 Base Substrate

The base substrates were successfully manufactured using the processes described in section **Error! Reference source not found.**.

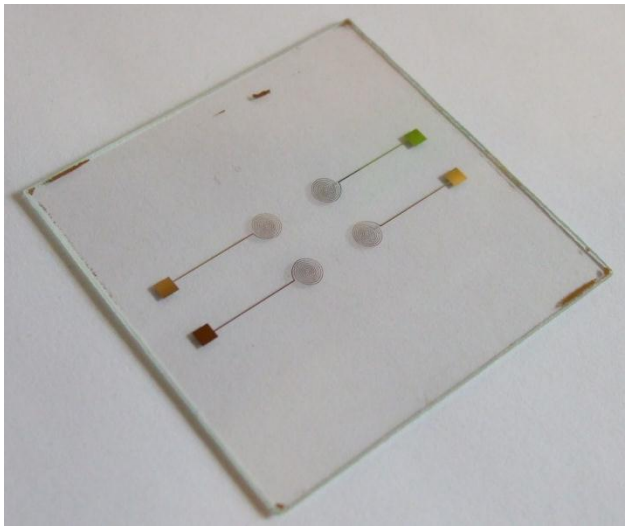


Figure 6.11: A patterned glass substrate made using clean room based photolithography.

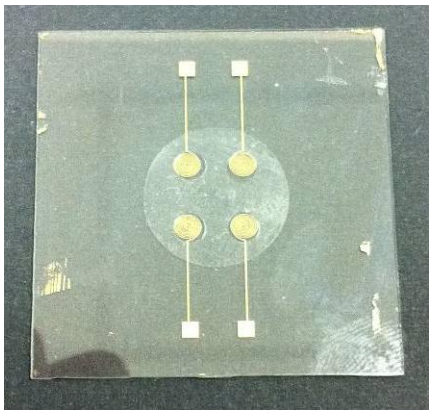
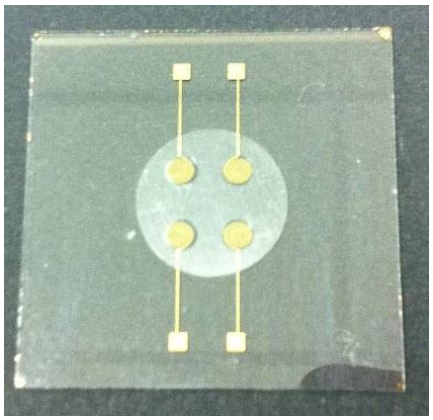
6.1.4.2 Microwell

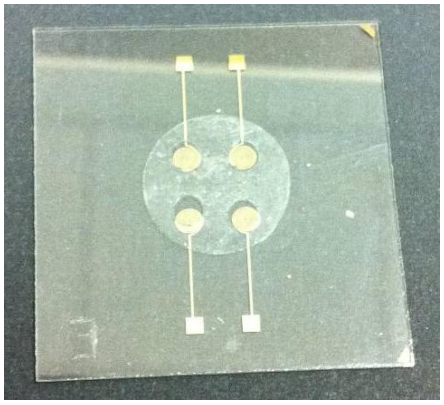
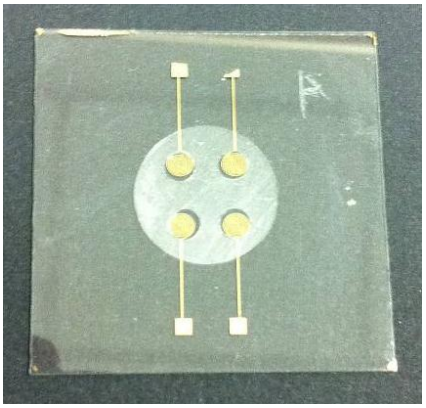
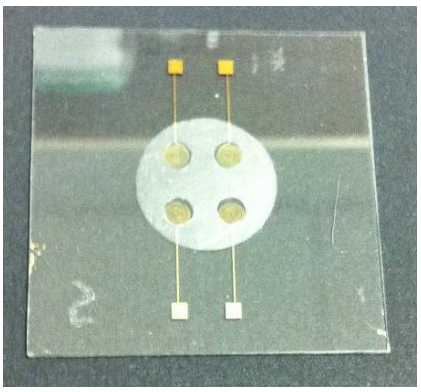
Microwells were created on five sample substrates by researchers at Fondazione Filarete, Milan, Italy, for testing of a PDMS alternative microwell and insulating material. The microwell geometry was re-specified in this design to a diameter of 4mm and depth between 150-250µm. The 4mm diameter was specified to compliment the dimensions of the cloning rings that are used to control beating cluster position during seeding. The electrode geometries are also intended to cover the

surface area within these large microwells with the intention of achieving 100% attachment of all clusters seeded over the electrode.

Five circles, 19mm in diameter, were stamped out of sheets of PDMS (that differed in thickness) by-hand. Four microwell holes, 4mm in diameter, were stamped by-hand out of each circle. A stencil was placed underneath during stamping for accurate positioning of microwell holes. The PDMS samples were then activated using a plasma treatment and placed over the glass samples. This positioning (Table 6.5) was also carried out by-hand, resulting in variations in alignment of the circle microwells over the electrodes on each sample. Samples 1, 2 and 5 were used in prototype testing at the UoN.

Table 6.5: The five base substrates that had PDMS samples adhered over the electrode space.

Sample Number	Thickness of PDMS	PDMS on the base substrate
1	150 μm	
2	240 μm	

3	240 µm	
4	250 µm	
5	240 µm	

6.1.4.3 Media wells

Glass rings, 21mm inner diameter x 24mm outer diameter x 6mm height, were sourced as bespoke parts from MDC Vacuum Limited, Sussex, UK, and adhered to samples using transparent epoxy resin (Figure 6.12). The epoxy resin was replaced by a silicon sealant during testing as the epoxy-glass interface resulted in leaks.

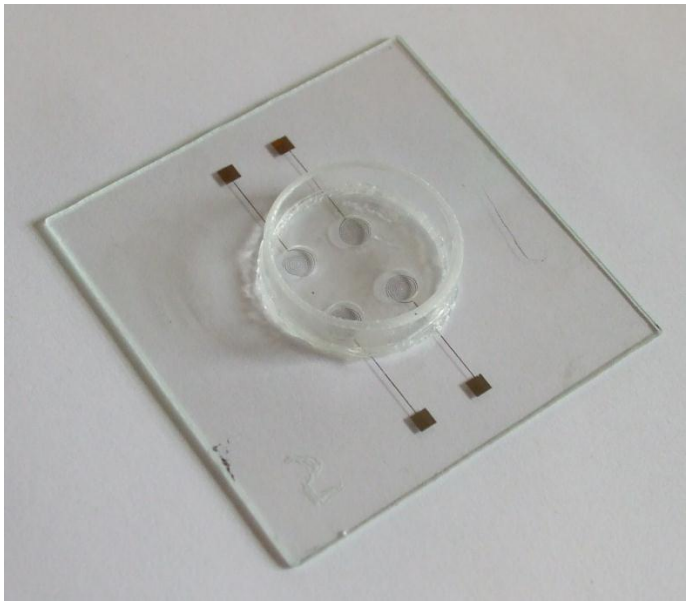


Figure 6.12: A complete PDMS coated MEA biochip.

6.2 Prototype Testing

The manufactured prototypes were tested by experienced UoN bioscientists using stem cell-derived cardiomyocytes as described in case study one (see section 4.2). A limited number (20) of beating cardiomyocyte clusters were made available for testing by the UoN group due to the time intensive and experimental nature of their differentiation. Testing was completed in small batches (2 – 4 biochips) over a period of several months. Iterative design improvements were implemented as tests revealed manufacturing or prototype flaws. The following sections of this chapter describe the outcomes of testing using living matter (contracting cardiomyocyte clusters) from the perspective of user satisfaction (users can position cardiomyocyte clusters as desired) and functionality (data can be collected biochips for analysis).

6.2.1 Prototype One Testing

An overview of the entire procedure used to test the first generation of MEA biochip prototypes is incorporated in Chapter four, Figure 4.4.

6.2.1.1 User interaction and cluster seeding testing

The following images (Figure 6.13 - Figure 6.21) demonstrate the process of cell seeding used. This was the first point of interaction between a user and prototype device allowing observations to be made and feedback to be captured.

Figure 6.13: Sterilisation procedure for the first batch of tests, soaking in industrial methylated spirit (IMS).

The space in which the bioscientist works can also be seen in these figures facilitating better understanding of the constraints imposed on design by the environment in which the MEA biochips are used.

Figure 6.14: MEA biochip is left to dry inside the laminar flow culture hood.

Figure 6.15: MEA biochip is rinsed with phosphate buffered solution (PBS).

Matrigel™ treatment was not used for generation 1 prototype testing as the microwell was intended to eradicate the need for surface treatments.

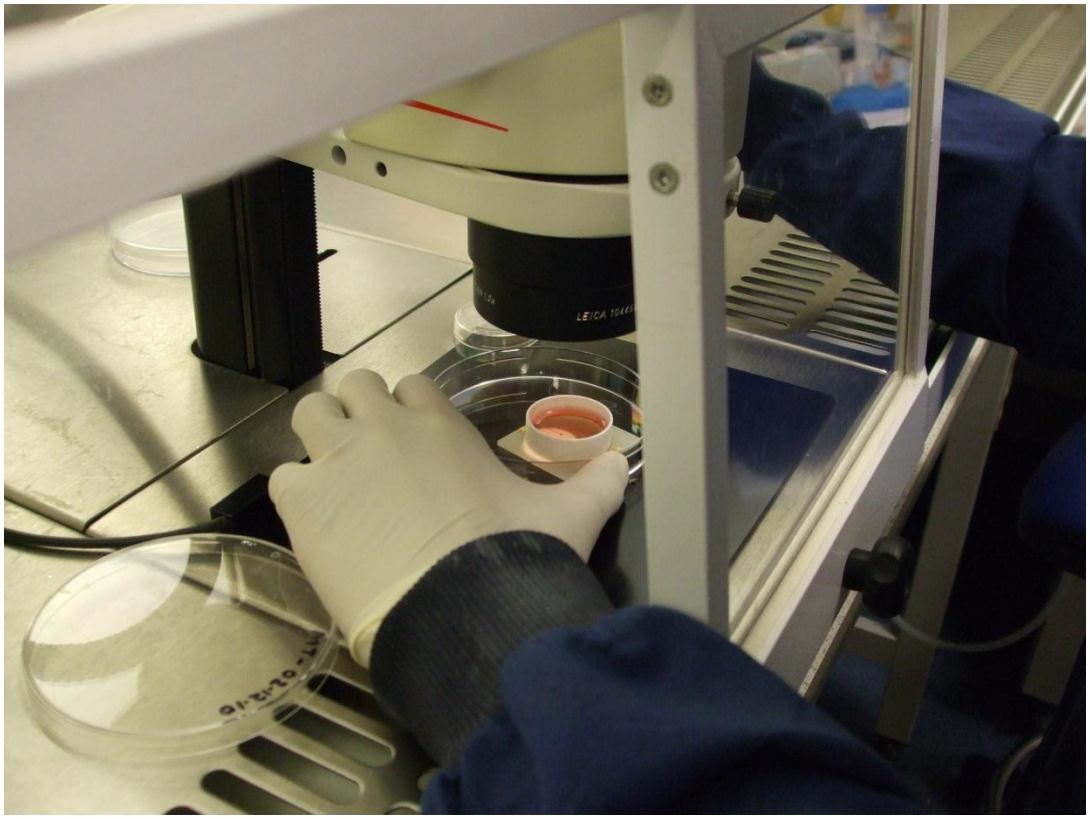


Figure 6.16: The MEA biochip was filled with Dulbecco's Modified Eagle Medium (DMEM) and microwells were checked for air bubbles.

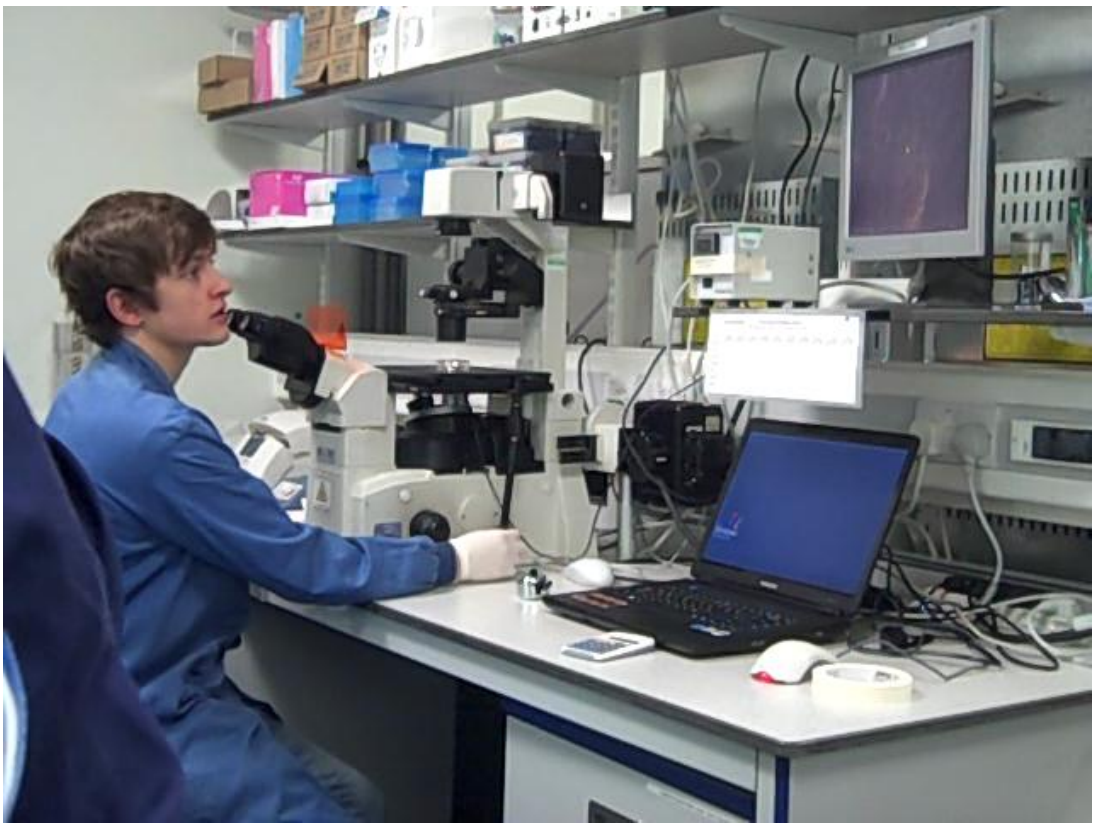


Figure 6.17: Dr Anderson locates the stem cell-derived clusters of cardiomyocytes within the embryoid body (EB) and confirms cells are contracting.

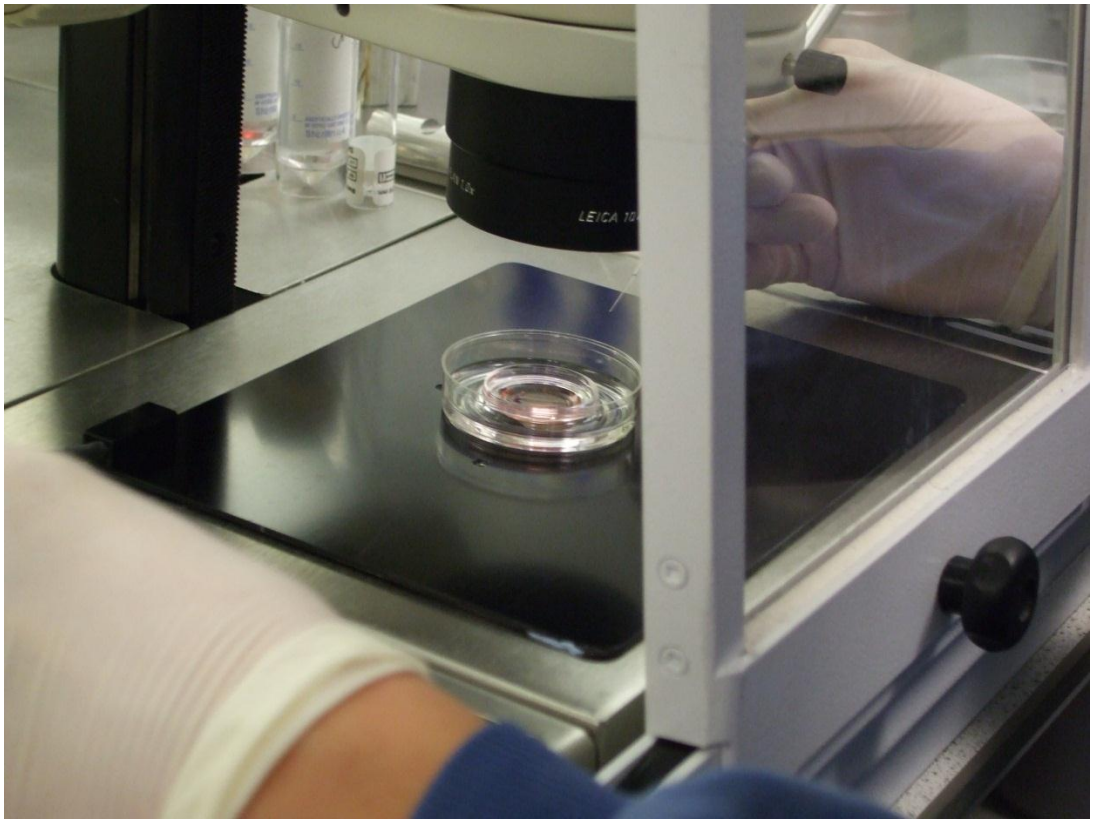


Figure 6.18: The contracting cardiomyocyte cluster is identified under the culture hood integrated inverted microscope. A stem cell knife is used to carefully dissect the contracting area of cells from the EB.

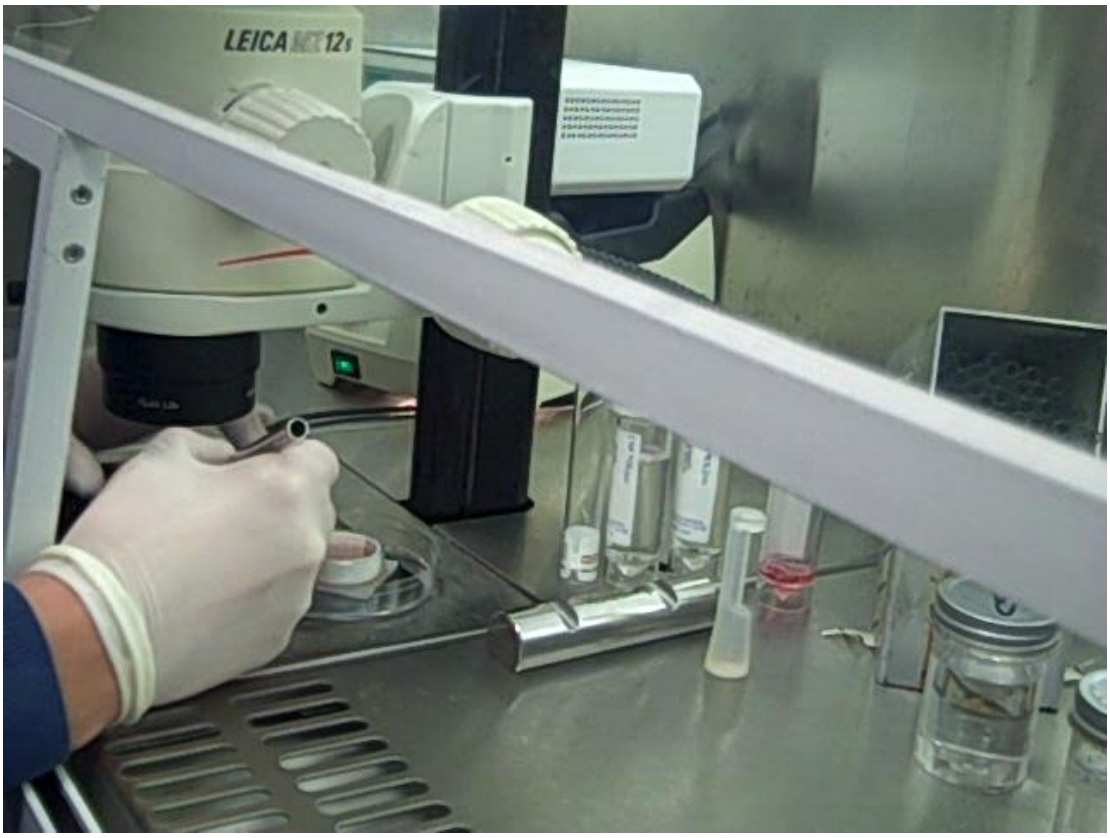


Figure 6.19: The dissected area is drawn into the stem cell knife's tip, held and transferred into the MEA biochip.

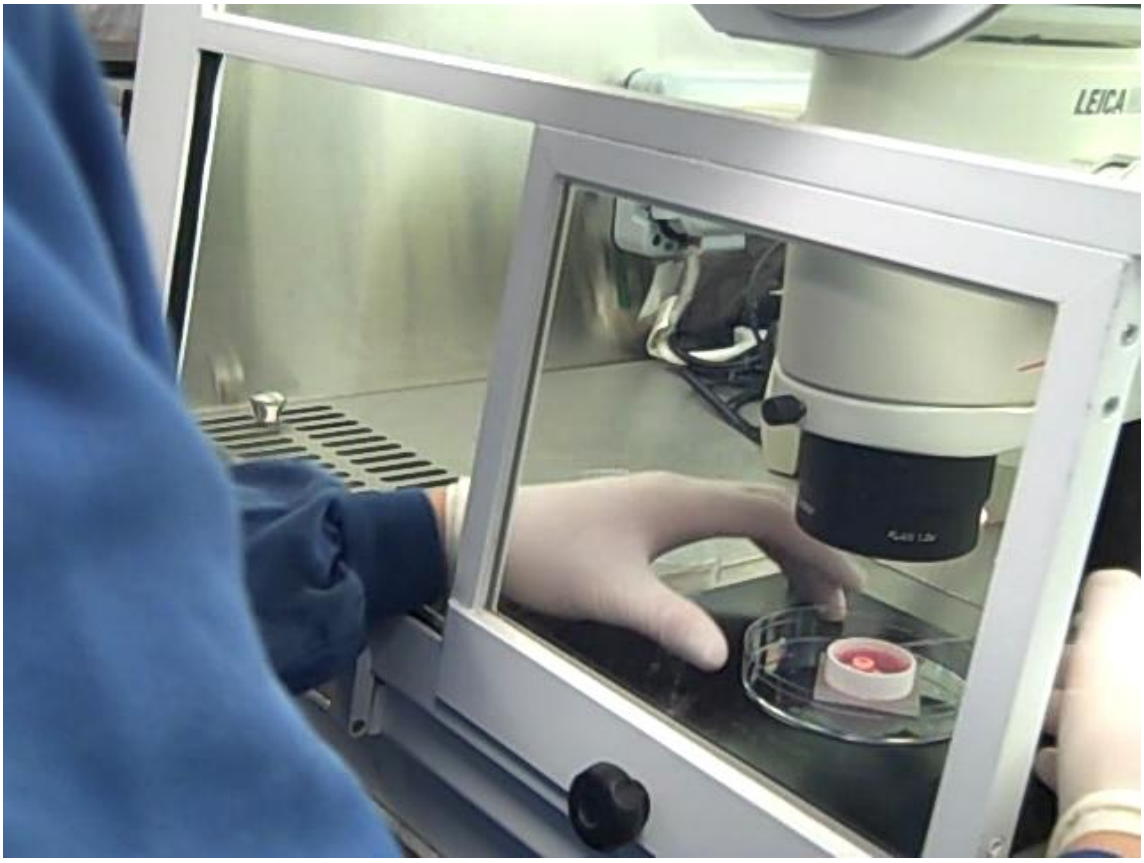


Figure 6.20: A cloning ring was used to help move released cardiomyocyte clusters into the microwells.

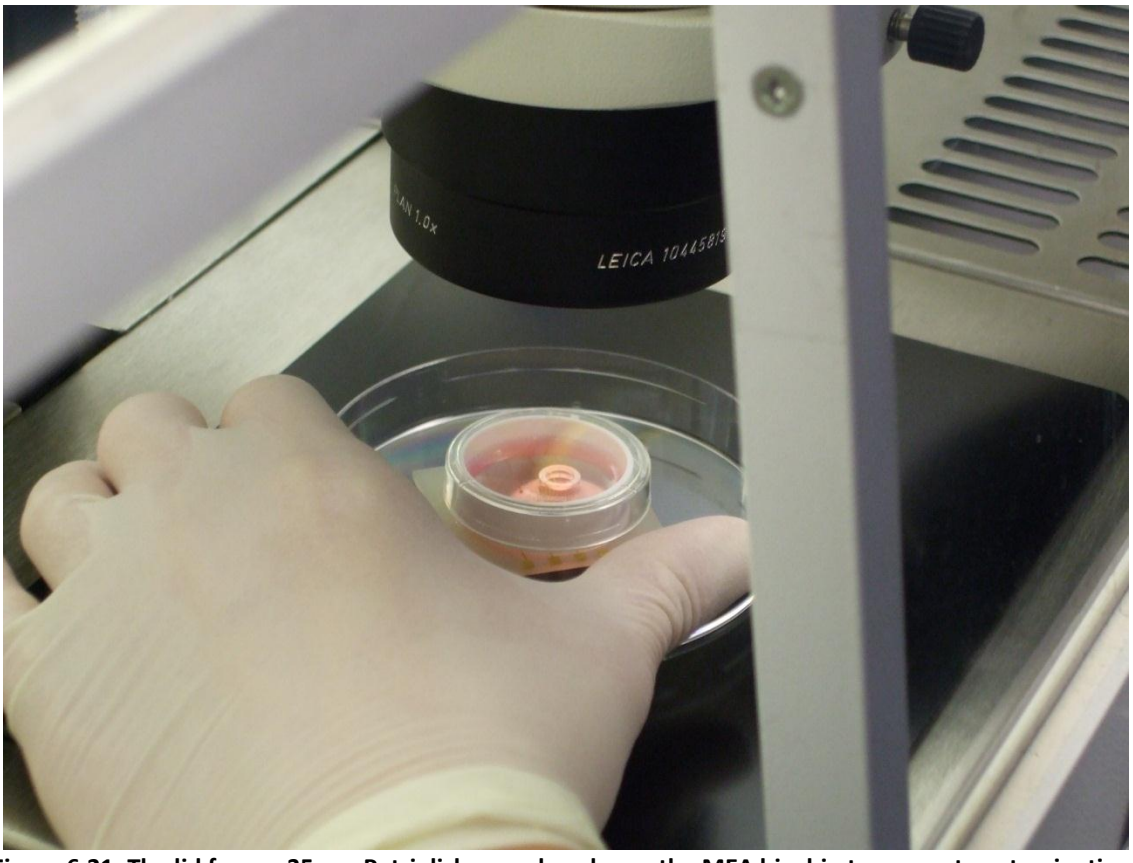


Figure 6.21: The lid from a 35mm Petri dish was placed over the MEA biochip to prevent contamination.

6.2.1.2 Signal capture testing

After a period in culture (varied between 3-12 days) the MEA biochips were inserted into a commercially available MEA system to test signal detection. The Multi Channels System MEA-60 was used for testing of all seeded prototypes (Figure 6.22).

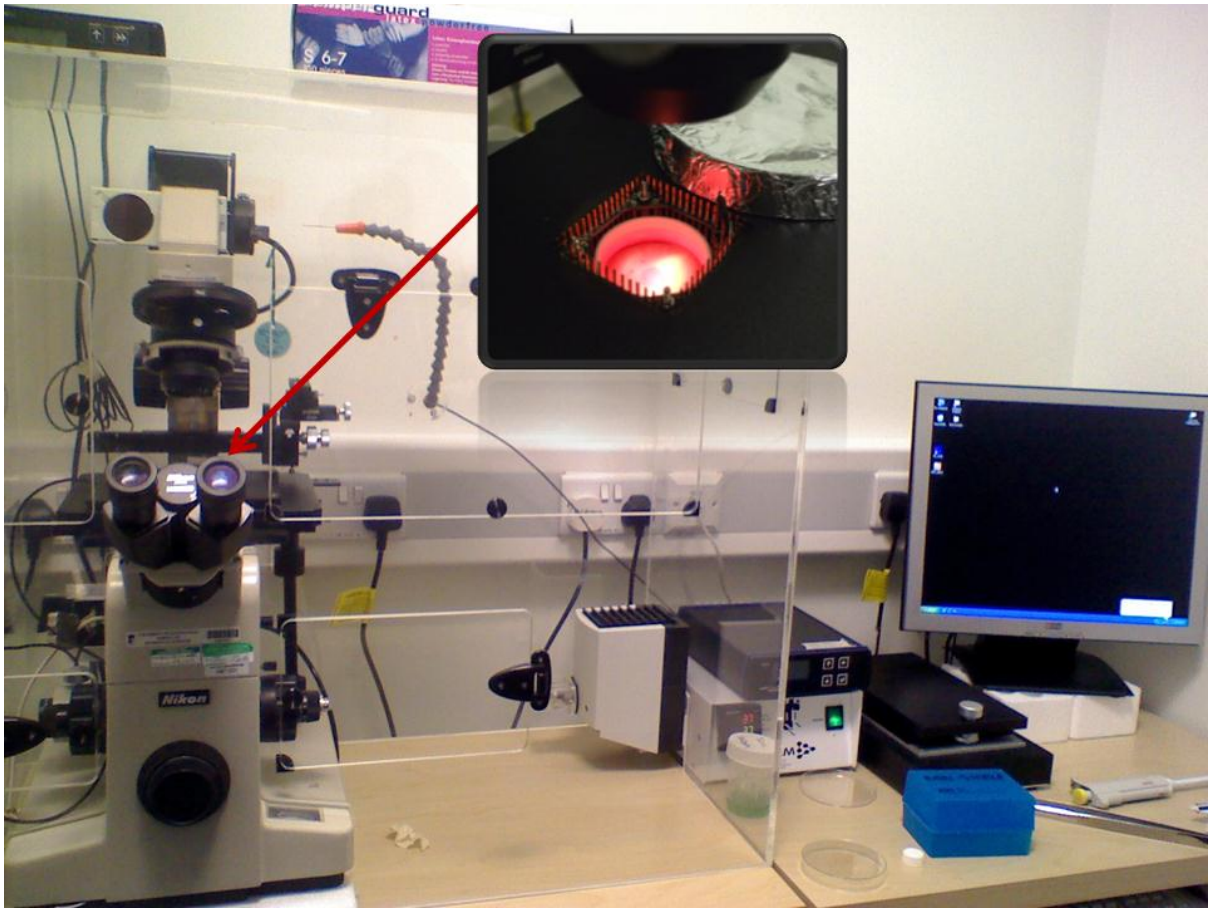


Figure 6.22: A first generation prototype in the MEA60 system.

A control MEA biochip was used prior to each testing session to ensure the MEA system was functional. A commercial MEA biochip seeded with a one cardiomyocyte cluster of the same age, and that had been treated and seeded in the same way, was used as the control biochip device.

Each prototype biochip tested was removed from incubation, visually assessed under a light microscope, and if contamination free, was inserted into the pre-heated (37^{o}C +/-0.5^{o}C) MEA system headstage (Figure 2.47). The findings for each individual test conducted are contained in Table 6.6.

During testing stages the lead contact changed from Dr Anderson (DA) to Miss Rajamohan (DR). The protocols used to test biochips remained consistent due to adherence to laboratory standard operating procedure documents (Appendix D).

Table 6.6: The tests conducted using the generation one prototypes made using the micro drilling manufacturing approach.

Scientist/ Test number	Biochip	Cells monitored optically	Contamination free	Attachment to a surface	Attachment in microwell	Contracting before insertion	Signal detected	Contracting following removal
DA 1	G1 V1a	No (Figure 6.24)	Contaminated	Yes	No	-	-	-
DA 2	G1 V1a	No (Figure 6.25)	Contaminated	Yes	Yes	-	-	-
DA 3	G1 V1b	No	Contaminated	No	-	-	-	-
DA 4	G1 V1b	No	Contaminated	Yes	No	-	-	-
DR 5	G1 V1a	No	Yes	Yes	No (Figure 6.26)	-	-	-
DR 6	G1 V1b	No	Yes	Yes	Yes (Figure 6.27)	Unknown	No	Unknown
DR 7	G1 V2a	No	Yes	Yes	Yes	Unknown	No	Unknown
DR 8	G1 V2b	No	Yes	Yes	No	-	-	-
DR 9	G1 V2a	No	Yes	Yes	Yes	Unknown	No/ No	Unknown
DR 10	G1 V2b	No	Yes	Yes	Yes	Unknown	No/ No	Unknown

DA= Dr David Anderson, DR= Miss Divya Rajamohan, G1= Prototype one, V1= opaque media well, V2= transparent media well, a,b= prototype identifier.

Where two results exist the first relates to use in the MEA60 system and the second relates to use in the custom-built system.

Where V1 and V2 are included this represents the media well material change described in section 6.2.1.

6.2.1.3 Discussion of tests

6.2.1.3.1 Cleaning

The first sterilisation process used a five minute IMS soak, which was inadequate and led to contamination. For all following tests a 20 minute exposure to UV light (Figure 6.23) with an IMS soak was used. Contamination did not re-occur throughout remaining tests.

Figure 6.23: A culture hood undergoing UV exposure for sterilisation.

6.2.1.3.2 Optical monitoring of cells

Optical assessment of the seeded cardiomyocyte clusters was not possible using the inverted microscope systems installed in the UoN laboratory. The biochip prototypes were assessed to establish whether shadowing could be used to identify the cell clusters, electrode tips or tracking. Figure 6.24 and Figure 6.25 demonstrate observations made. Defects that can be seen in these figures are damage that occurred to the base substrates underlying surface during manufacturing. Tracking, electrodes and cell clusters cannot be identified.

Figure 6.24: An image of G1 V1a MEA biochip positioned with electrode tips directly under the microscopes field of view.

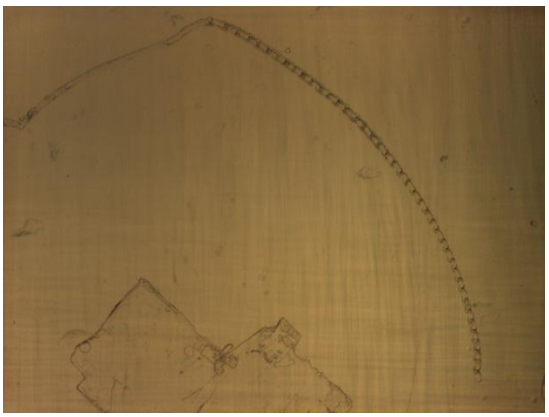


Figure 6.25: An image of G1 V2a positioned with an electrode tip directly under the microscopes field of view.

At the request of the UoN the media well material was changed from white PE (G1 V1) to transparent polystyrene (PS) (G1 V2) (see Figure 5.67) to improve illumination within the media well. This change did not permit users to ascertain if the seeded clusters were beating. The height of the media well was also reduced from 10mm to 6mm to improve ease of access while under the microscope during cell seeding process.

6.2.1.3.3 Microwell

End users reported that it was “difficult” and “awkward” to position the cardiomyocyte clusters into the microwells respectively. The cardiomyocyte clusters used in testing ranged in size and shape, varying between 200 -500 µm in diameter or length. Ten cell clusters were seeded directly over ten microwells throughout G1 tests, eight of which formed attachments to the Melinex surface, and four of which attached inside the microwells and were used for signal tests. Figure 6.26 demonstrates a cardiomyocyte cluster that was initially positioned over the centre of the microwell that, due to its contracting nature, formed its attachments with the base substrate away from the microwell and electrode. Figure 6.27 shows a cardiomyocyte cluster that is attached inside a microwell.

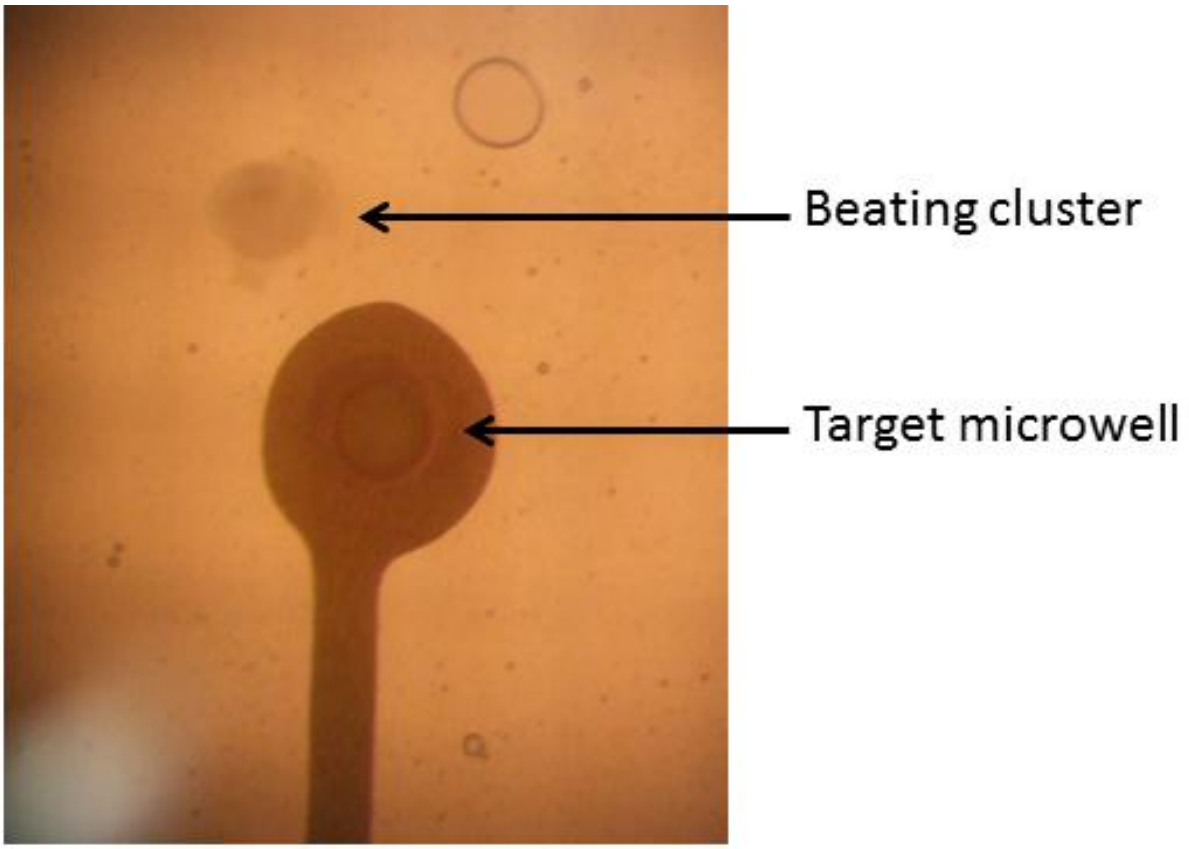


Figure 6.26: An example of a cluster of cardiomyocytes attached to the Melinex surface away from the microwell. Scale: Target microwell 500µm in diameter.

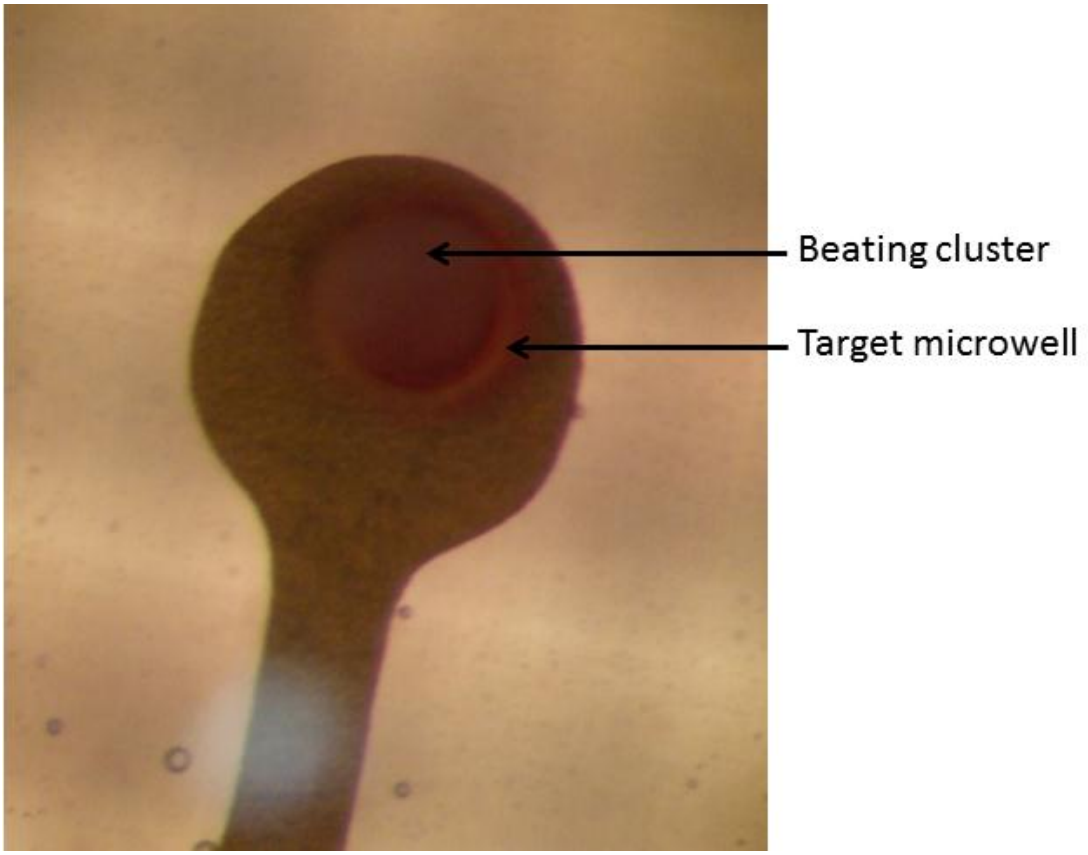


Figure 6.27: An example of a cardiomyocyte cluster attached inside a microwell. Scale: Target microwell 500µm in diameter.

6.2.1.3.4 Signal detection

Each cardiomyocyte cluster attached inside a microwell over an electrode was tested for signal detection. In every case no signal was detected.

A total of ten cell clusters were seeded over a microwell, four of which attached inside the microwell and therefore over an electrode. It was not possible to confirm whether or not these clusters were contacting prior to or following tests due to the optical constraints imposed by material choices. The importance of being able to confirm visually that the cardiomyocytes were contracting at the start of tests was emphasised so it was agreed that a re-design would take place to exploit a transparent base substrate that would allow optical assessment.

Two MEA systems were used for signal detection testing of this prototype. The described MCS MEA60 system and an experimental, custom-made system, that is currently under development (Appendix F). Neither system detected a signal from any of the prototypes.

6.2.2 Prototype Two Testing

The second concept design (see section 5.7.3) was manufactured to provide an alternative prototype intended to address problems highlighted in initial tests of the first prototype (see section 5.7.1). This second generation (G2) of prototype was made using clean room based photolithography (see section **Error! Reference source not found.**) on glass substrates. The results of the testing using cardiomyocyte clusters are presented in the following sections.

6.2.2.1 User interaction and cluster seeding testing

The second generation of prototypes were cleaned, seeded and maintained in culture in the same way as the first generation (G1) prototypes (Figure 6.28).

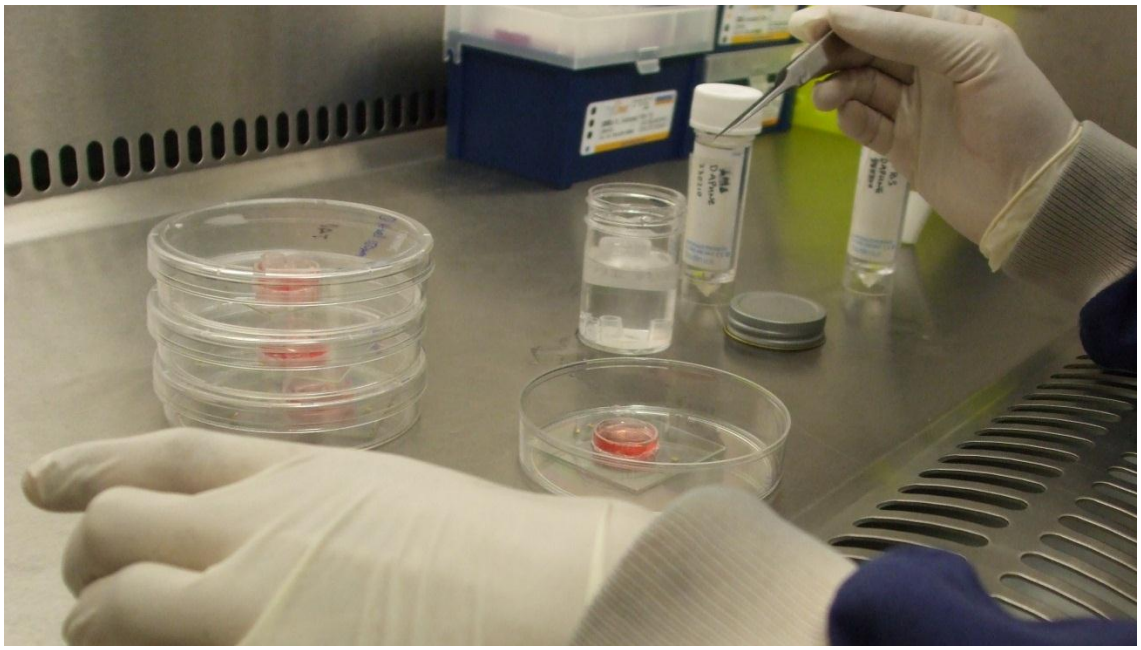


Figure 6.28: The seeding of second generation prototypes.

Improvements in the testing of this generation of prototype as when compared to testing of the first generation were:

- Suited to cleaning
- Adequate optical assessment
- Improved media well height
- Greater ease of cardiomyocyte cluster positioning in the microwell over the centre of the underlying electrode.

6.2.2.2 Signal capture testing

The MCS MEA60 system used (Figure 6.29) to test the first generation prototype was also used to test all of the generation two prototypes (Table 6.7). Three of the G2 prototypes successfully seeded with cardiomyocyte clusters over the electrodes were also connected to the custom-made MEA system (see Appendix F, Figure 6.30).

No signals were detected from either MEA system from any of the G2 prototypes tested.

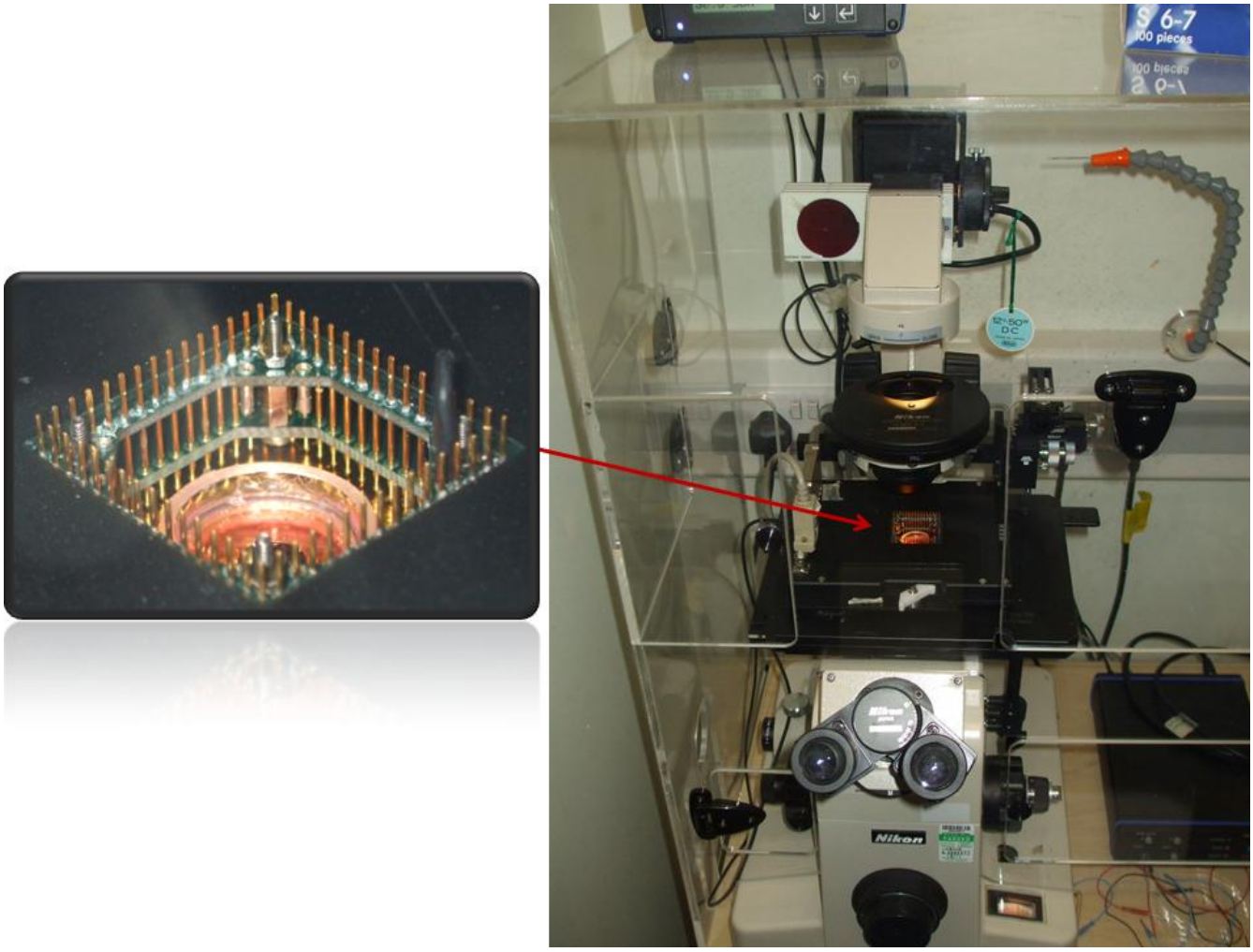


Figure 6.29: A second generation prototype in the MEA 60 system.

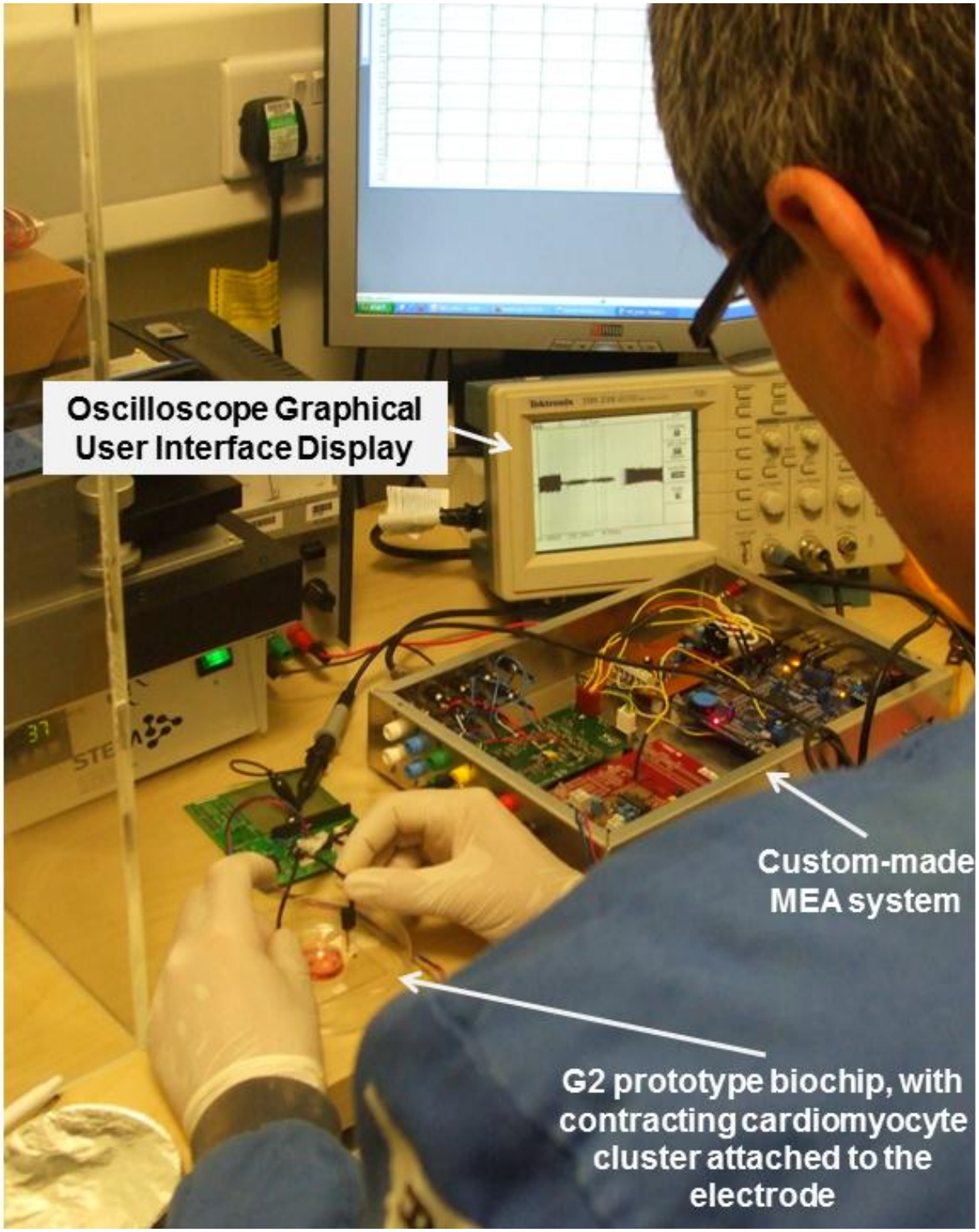


Figure 6.30: A second generation prototype being tested with the custom-made MEA system.

Table 6.7: The tests conducted using the generation one prototypes made using the micro drilling manufacturing approach.

Scientist/ Test number	Biochip	Cells monitored optically	Contamination free	Attachment to a surface	Over electrode	Contracting before insertion	Signal detected	Contracting following removal
DR 1	G2 S n/m a	Yes	Yes	Yes	Yes (Figure 6.31B) *Leaked*	Yes	No	Yes
DR 2	G2 S n/m b	Yes	Yes	Yes	Yes *Leaked*	No	-	-
DR 3	G2 R n/m c	Yes	Yes	Yes	Yes. Lost in handling. *Leaked*	-	-	-
DR 4	G2 R n/m d	Yes	Yes	Yes	Yes (Figure 6.31D) *Leaked*	Yes	No	Yes
DR 5	G2 S n/m	Yes	Yes	Yes	Yes (Figure 6.33)	Yes	No	Yes
DR 6	G2 S w/m	Yes	Yes	Yes	Yes (Figure 6.33)	Yes	No	Yes
DR 7	G2 S w/m	Yes	Yes	No	No (Figure 6.34)	No	-	-
DR 8	G2 R n/m	Yes	Yes	Yes	Yes (Figure 6.34)	Yes /Yes	No /No	Yes/Yes
DR 9	G2 S w/m	Yes	Yes	Yes	Yes	Yes /Yes	No /No	Yes /Yes
DR 10	G2 S w/m	Yes	Yes	Yes	No	No	-	-

DR 11	G2 S w/m	Yes	Yes	Yes	Yes	Yes/ Yes	No/ No	Yes/ Yes
DR 12	G2 S w/m	Yes	Yes	Yes	Yes	Yes	No	Yes
DR 13	G2 S w/m	Yes	Yes	Yes	Yes	Yes	No	Yes
DR 14	G2 S w/m	Yes	Yes	Yes	Yes	Yes	No	Yes

DR= Miss Divya Rajamohan, G2 = Design concept two, S= spiral shaped electrode design, R= radiator electrode design, n/m = no microwell, w/m = with PDMS microwell

Where two results exist the first relates to use in the MEA60 system and the second relates to use in the custom-built system.

The spiral (S) and radiator (R) electrode designs are described in section 5.8.1.1.

6.2.2.3 Discussion of tests

Cleaning: All G2 prototypes were cleaned using a 20 minute UV light exposure and 5 minute IMS soak. There were no instances of contamination during generation two tests.

Optical monitoring of cells: The new design facilitated good optical inspection of cells. It was now possible to confirm if cardiomyocyte clusters were contracting prior to and after testing in the two MEA systems.

6.2.2.3.1 Media Well

The first media wells employed were glass rings (21mm inner diameter x 24 outer diameter x 6mm height) adhered using epoxy resin. The G2 prototypes leaked (Figure 6.31) and the epoxy showed signs of absorption of the culture media (Figure 6.32).

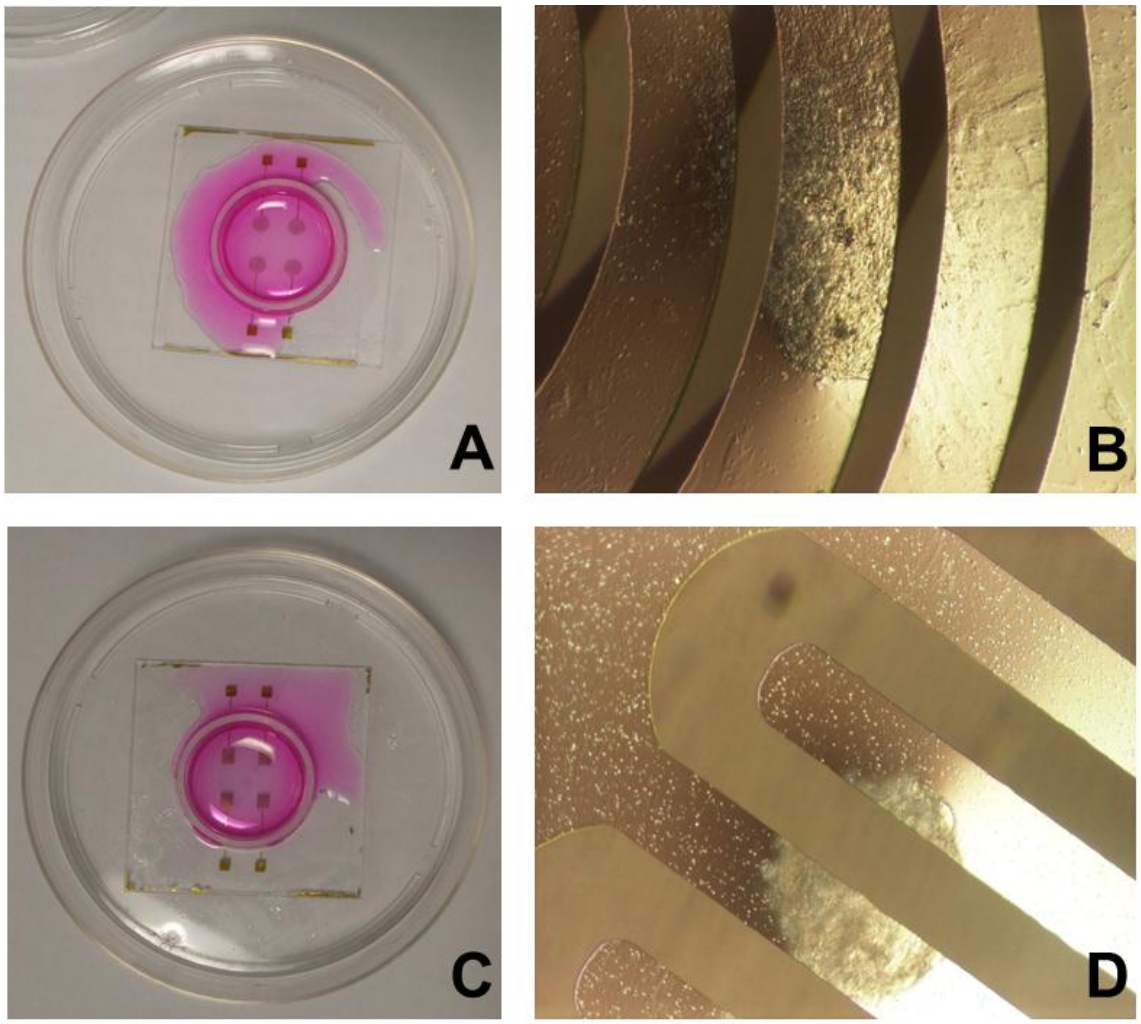


Figure 6.31: The first batch of tests during which the G2 prototypes leaked. A) Spiral electrode biochip prototype. B) The cardiomyocyte cluster attached to the spiral electrode in the biochip demonstrated in A. Scale: Electrode tracking width 80µm. C) Radiator electrode biochip prototype. D) The cardiomyocyte cluster attached to the radiator electrode in the biochip demonstrated in C. Scale: Electrode tracking width 100µm.

For biochips shown on Figure 6.31, the attached beating clusters were still contracting and so were tested for signals before retrieval and re-seeding.

In Figure 6.31 the prototype shown in image B has not had a Matrigel treatment applied to the surface. The cardiomyocyte cluster shows good attachment to the glass/electrode surface. Matrigel has been used on the prototype shown in image D, in case cardiomyocyte clusters had failed to attach to the glass/electrode so as to ensure a signal test could be conducted. It was also noted that if prototypes could function reliably without the need for a Matrigel pre-treatment, further savings could be brought to the user, adding an additional competitive advantage to the novel MEA biochip over the existing commercial standard.

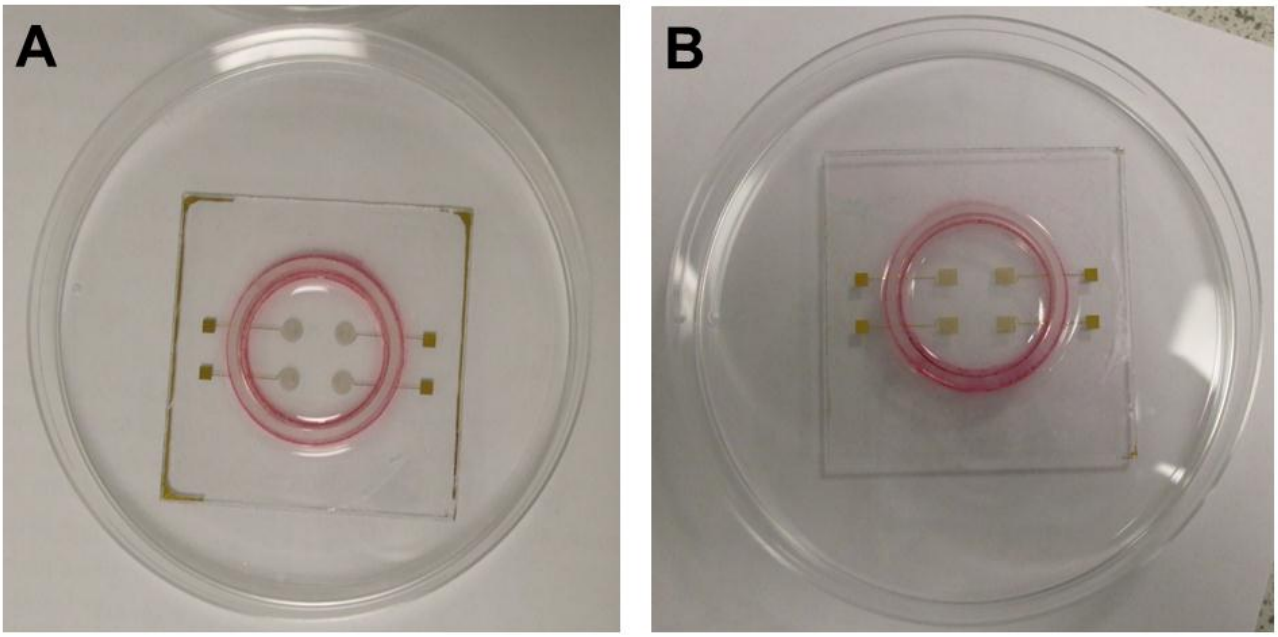


Figure 6.32: Examples of absorption of culture media by the adhesive used to secure the media well. A) A spiral electrode prototype. B) A radiator electrode prototype.

The epoxy resin used to secure the media wells was changed to Geocel aquarium grade silicon sealant (Geocel Ltd, Plymouth, UK). The Geocel silicon was first tested at Loughborough University by securing glass rings and un-patterned glass substrates (Figure 6.33). The sealant was tested as it would be used during UoN testing prior to incorporation into prototypes: DMEM was left in the media wells for 10 days, the samples were then rinsed with deionised water and treated with trypsin (used at UoN to remove the cellular matter and to breakdown the Matrigel), followed by an IMS soak and 20 minute UV light exposure. The samples were then re-filled with DMEM for 14 days. No leaking occurred across any of the samples so the silicon sealant was used for remaining prototypes.

The Geocel sealant prevented leaks and did not show signs of culture media absorption following use in trials with living matter at the UoN. Cells seeded in prototypes made using this sealant did not show any adverse effects after up to three weeks in culture implying that the biocompatibility of the sealant is suited to this application.

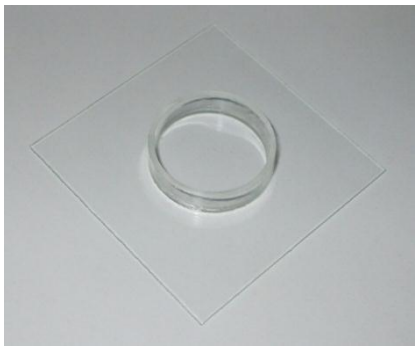


Figure 6.33: An un-patterned glass substrate used to test adhesives.

6.2.2.3.2 Microwell

PDMS was selected to replace the Melinex™ film for the insulation and microwell material as it was believed that PDMS would encourage cell adhesion to the glass/electrode surface as opposed to the surrounding areas of the biochip or the microwell walls.

PDMS possesses a natural tendency that inhibits cell adhesion (De Silva et al, 2004) so it was believed that if, due to the contracting nature of the cells the cluster moved to the edge of the microwell, then they would preferentially form their attachments with the underlying glass/electrode substrate as opposed to the wall of the microwell or the insulating material as occurred in first generation prototypes.

To test attachment to PDMS two cell clusters were seeded per prototype. One cluster was seeded the glass/electrode substrate, at the centre of the electrode; and the other at the centre of the media well on the PDMS surface. The results of this test are described in Table 6.7 and can be seen in Figure. The amount of movement that occurred between initial positioning and attachment to the underlying surface can also been seen.

The clusters over the electrodes attached to the underlying substrate surface. The clusters positioned over the PDMS demonstrated no attachment. Where Matrigel had been used on some samples a lighter attachment was observed between the cell cluster and the PDMS than was apparent between the other cell cluster and the glass/electrode substrate in the same prototype at the same time.

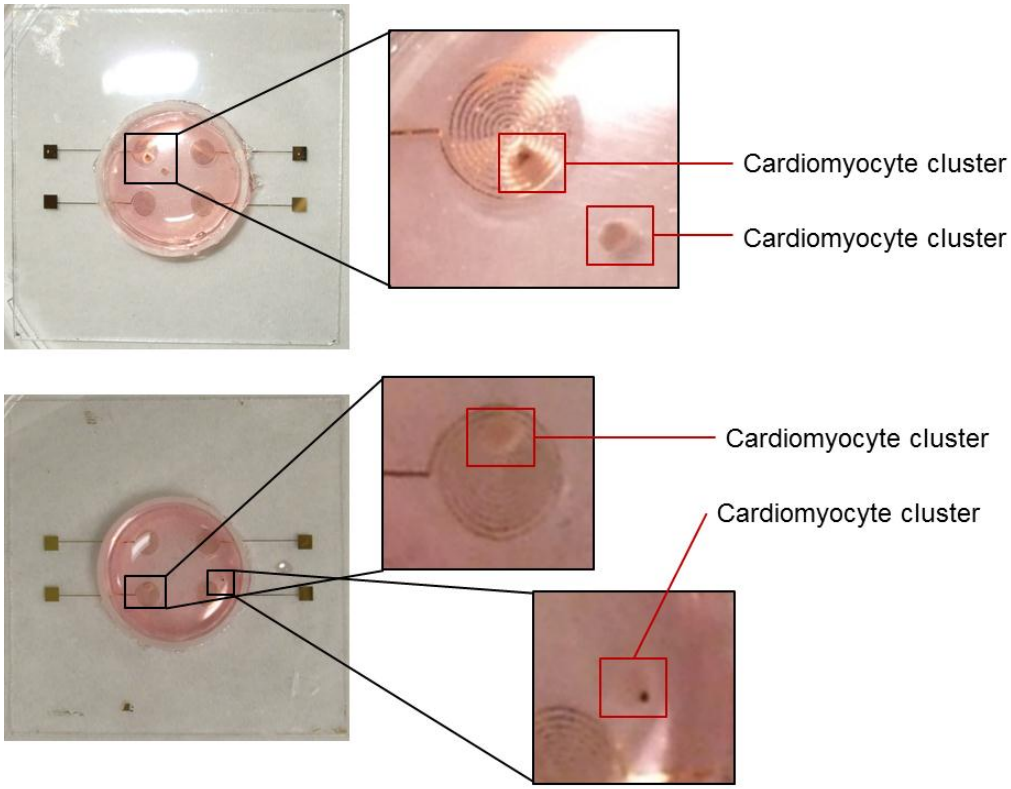


Figure 6.34: Second generation prototypes with PDMS microwells, with cardiomyocyte clusters. Beating clusters over electrodes were initially positioned at the centre of the corresponding electrode. The beating clusters on the PDMS were positioned at the centre of the media well.

6.2.2.3.3 Signal Detection

Ten beating clusters (differing size diameter ~150 - 500µm) attached directly over the electrodes on second generated prototypes that were used to test for signal detection (Figure 6.34 and Figure 6.35). No signals were detected (Table 6.7). Clusters were seen to be contracting and well-attached to the base substrate in all cases prior to, and following, insertion into the MEA systems.

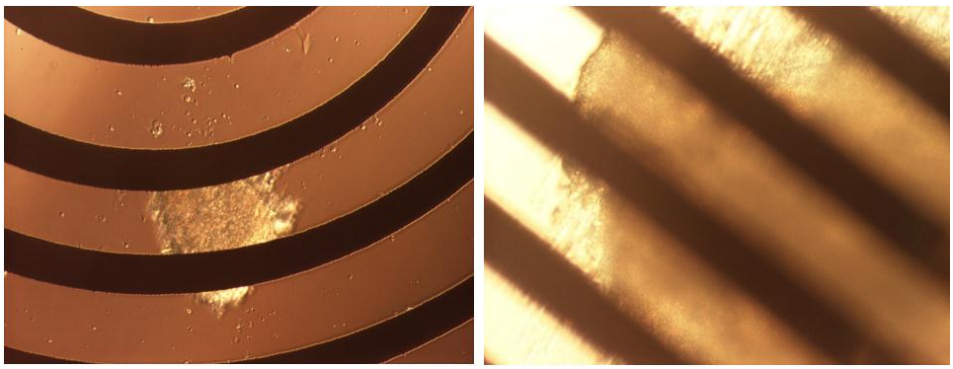


Figure 6.35: Examples of more attached beating clusters over second generation prototype electrodes. Left: Spiral electrode. Right: Radiator electrode.

6.2.3 Signal detection investigation

To investigate why both prototypes failed to show signals when tested in two different MEA systems the following impedance investigation was conducted to compare the prototype electrodes manufactured characteristics to those in the commercial standard.

6.2.3.1 Impedance Measurements

A Wayne Kerr Principle Component Analyser (PCA) (Figure 6.36) was used to measure the impedance of electrodes in the prototypes and a commercially available MEA biochip. Measurements were taken from each MEA biochip while containing a physiological saline solution that was confirmed to have comparable conductivity to the culture media used in this MEA application, Dulbecco's Modified Eagle Medium (DMEM) (Figure 6.37). The measured conductivity of each solution is contained in Table 6.8.

Figure 6.36: The Wayne Kerr Principle Component Analyser used for impedance measurements.

Figure 6.37: The DMEM and saline solutions used during impedance measurements.

Table 6.8: Conductivity of solutions used in impedance tests.

Solution	**mS**
Saline Solution	17.6
DMEM	17.3-18.07

Table 6.9: PCA Impedance Measurement parameters.

Internal Bias	Off
	150mVac
Measurement	AC

6.2.3.1.1 Prototype One

Ten values were recorded for six frequencies, using the principle component analyser unit shown in figure 6.36, for each of the connections indicated in Figure 6.38 (A, B and C). The analyser's operational set-up as described in table 6.9. Average impedance values for each frequency are displayed in Figure 6.38.

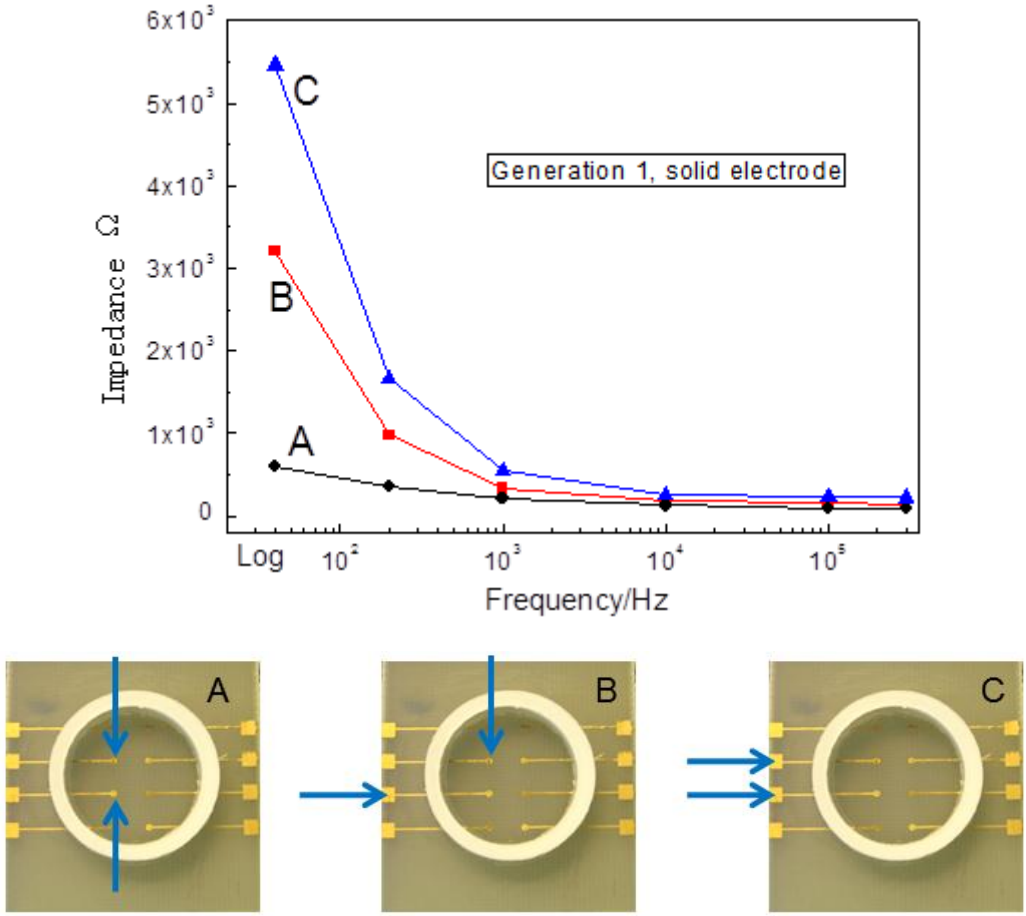


Figure 6.38: Impedance results for the solid 1mm diameter Cu/Au electrodes on an FR4 base substrate in prototype generation 1.

6.2.3.1.2 Prototype Two

Ten values were recorded for six frequencies for each of the connections indicated in Figure 6.39(A, B and C). The analyser was set-up as described in Table 6.9. Average impedance values for each frequency are displayed in Figure 6.39.

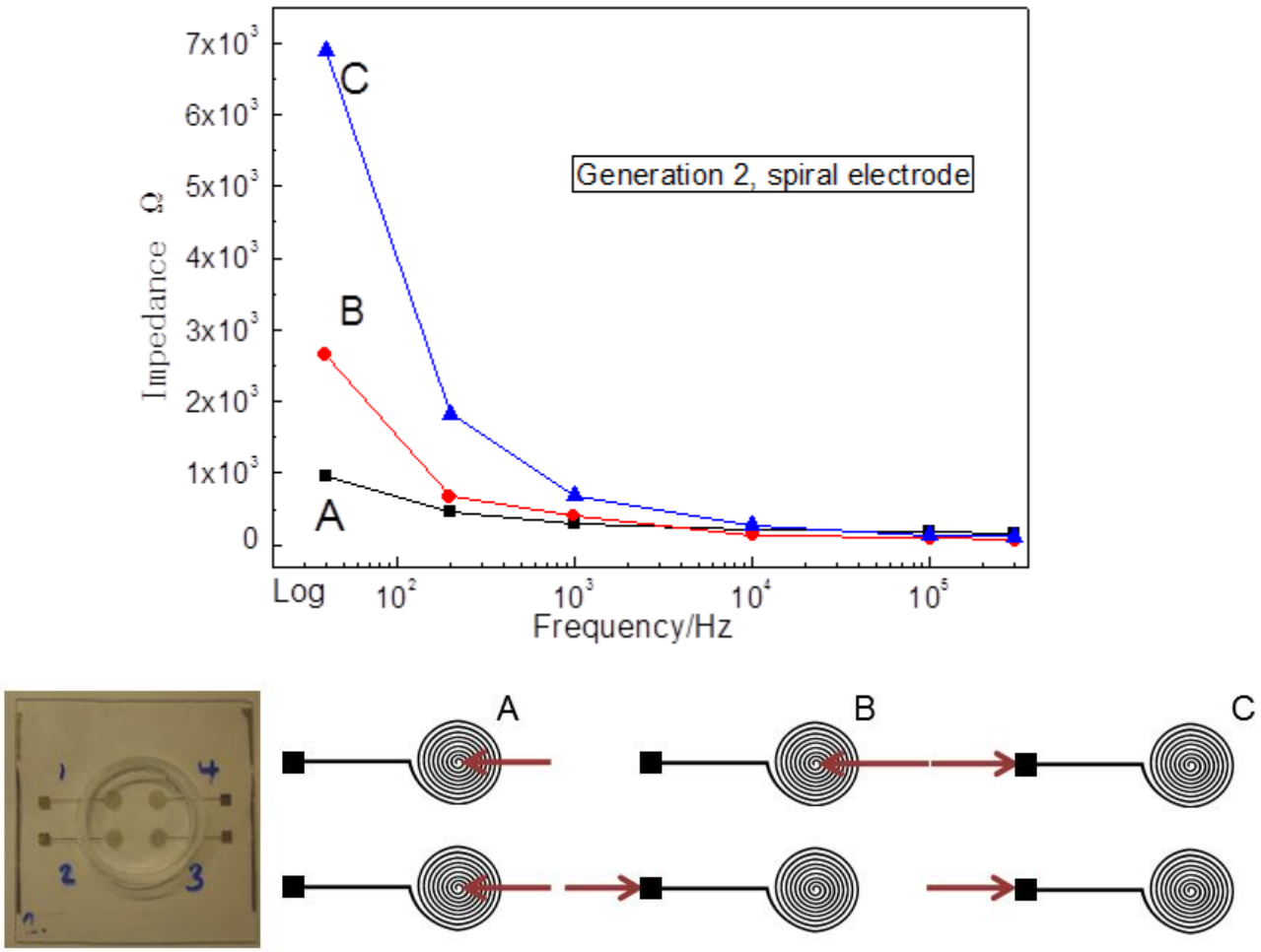


Figure 6.39: Impedance results for the spiral shaped Ti/Au electrodes on the glass base substrate prototype generation 2.

6.2.3.1.3 Commercial MEA biochip

Ten values were recorded for six frequencies for each of the connections indicated in Figure 6.40 (A and B). The analyser was set-up as described in Table 6.9. Average impedance values for each frequency are displayed in Figure 6.40.

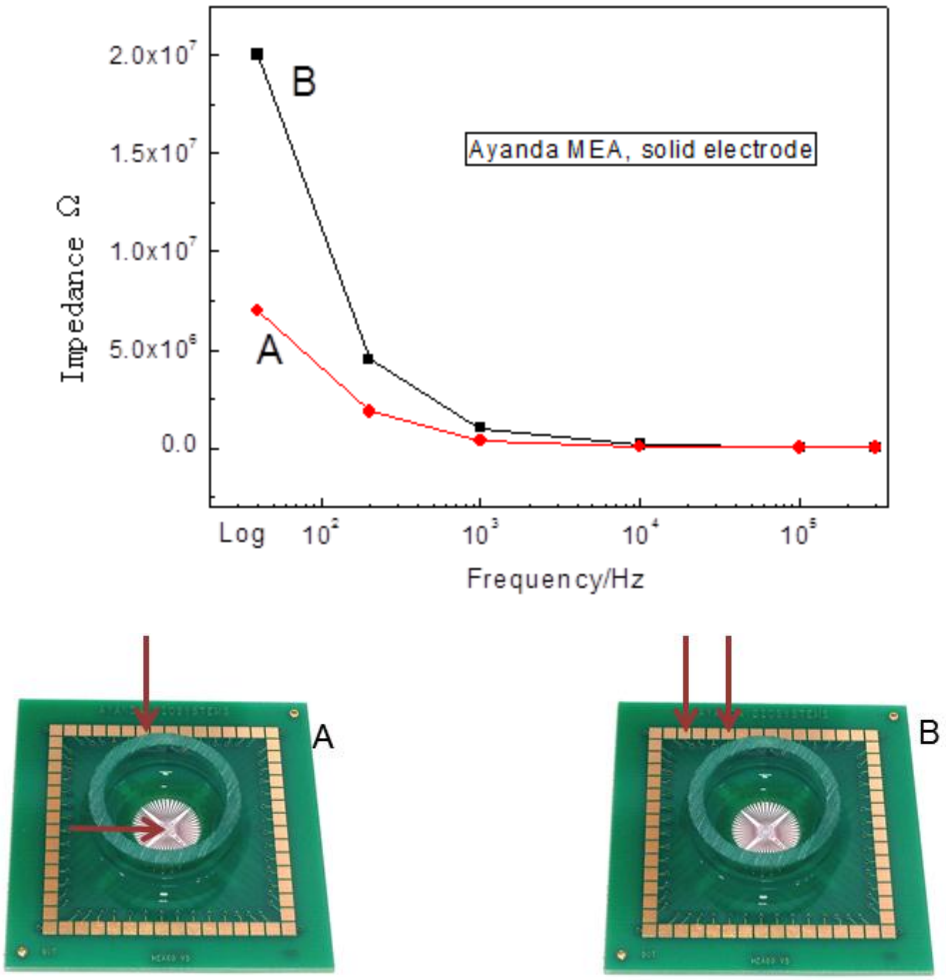


Figure 6.40: Impedance results for a commercially available 60 electrode planar MEA biochip.

6.2.3.1.4 Discussion

The hypothesis used during the electrode design throughout this research was:

- Greater electrode tip surface area results in lower impedance, which would result in improved signal detection.

Impedance measurements were made using two probes, a sensing probe and counter probe (the reference), connected to the principle component analyser in Figure 6.36. The measurements taken demonstrate that the impedance of the electrode change depending on: (1) the frequency of the input, and (2) the location of the sensing probes on the MEA biochip.

The measurement locations that are comparable between the biochips shown in Figure 6.38, Figure 6.39 and Figure 6.40 are seen in Table 6.10.

Table 6.10: Comparable impedance measurement locations

Measurement Location Description	Prototype One	Prototype Two	Commercial Standard
Electrode tip – Electrode tip	A	A	Not possible with probes available
Electrode tip – interconnect contact pad	B	B	A
Interconnect contact pad – interconnect contact pad	C	C	B

It was not possible to measure the impedance of the electrode tip – electrode tip in the commercial standard as the probes used were too large to adequately touch one 30µm diameter tip without contacting another adjacent electrode tip.

The highest impedance values observed from these measurements were from the interconnect contact pad – interconnect contact pad locations. This recording location is directly comparable to the MEA systems recording interconnections (through spring loaded pins) during the live testing conducted.

Impedance values were also observed to be highest at low frequencies. The recording frequency used at the UoN during MEA system employment is 2000Hz. The value used in measurement that compares closest to this frequency was 1000Hz. Therefore the impedance values observed at the interconnect contact pad – interconnect contact pad locations while using a frequency of 1000Hz

were believed to be closest to the impedance values during the live prototype testing conducted. These values were compared and it was confirmed that both of the prototype devices produced provided electrodes with significantly lower impedances than the commercial standard.

A summary of these conclusions is represented in Figure 6.41.

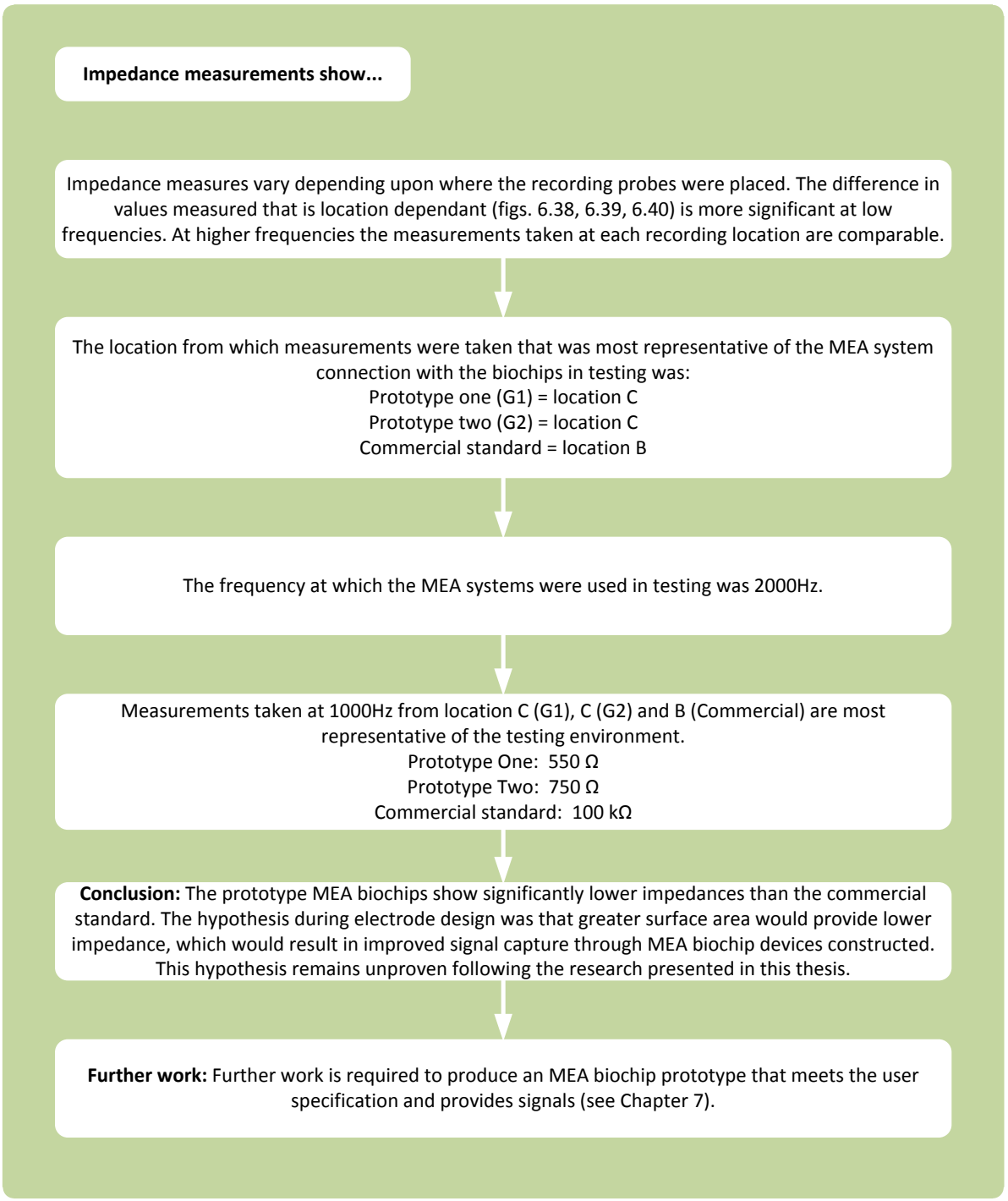


Figure 6.41: Impedance measurements conclusion

6.3 Summary

This chapter has demonstrated the results of: (1) the manufacturing approaches implemented during this research, (2) testing of prototypes manufactured using live stem cell-derived cardiomyocyte clusters, and (3) the work conducted in order to characterise impedance of prototype electrodes compared to those of a commercial standard.

Manufacturing

- Four manufacturing approaches were implemented demonstrating different problems.
- Two different prototypes resulted from manufacturing approach experimentation.
- Testing of the first generation of prototype with live cardiomyocyte clusters influenced the design and manufacture of the second generation of prototype.

Testing using cardiomyocyte clusters

- Prototype MEA biochips were tested using the exact protocols used in real application of the commercial standard MEA biochip.
- Problems encountered relating to the first generation prototype were:
    - The initial cleaning procedure employed failed and the seeded cells were lost due to contamination.
    - The media well material was opaque limiting illumination of the area to be seeded inside the biochip.
    - The media well height made access (for cluster seeding) while under the microscope more difficult.
    - The base substrate material and electrode geometry resulted in restricted optical monitoring of the cardiomyocyte clusters once seeded inside the MEA biochip.
    - The microwells geometry resulted in difficulty during cluster seeding. It was observed that larger microwell geometries may be more suitable due to the differing sizes of the cardiomyocyte clusters.
    - No signals were observed from the microwells that had cardiomyocytes successfully attached inside.

- Improvements implemented in the second generation of prototype:
    - Media well height was reduced so that it was easier to access the area inside more easily during cardiomyocyte cluster seeding.
    - The glass base substrate and altered electrode geometry allowed monitoring of the clusters after seeding and throughout testing. This allowed confirmation that the clusters were contracting prior to and following signal detection tests.
    - The glass/electrode surface exhibited appropriate cardiomyocyte cluster attachment over the electrodes.
    - PDMS used as the microwell and insulating material demonstrated poor cell attachment making it a more suitable material than the Melinex™ used in the first generation of prototype.
    - The large microwells improved ease of cluster positioning during seeding.
- Problems encountered relating to the second generation prototype were:
    - The media wells leaked and epoxy demonstrated signs of culture media absorption.
    - No signals were observed from the microwells that had cardiomyocytes successfully attached inside.
- First and second generation prototypes were tested in two different MEA systems, one commercially available system and one custom-made system. No signals were captured from any of the tested prototypes tested in either MEA system.

Impedance comparison

- Various impedance measurements were made of each prototype and of the commercial standard used in testing as the control MEA biochip.
- Prototype electrodes were shown to have significantly lower impedance as when compared to the commercial standard. It was hypothesised that this would result in improved signal detection. This hypothesis was disproved through the live testing conducted.
- It is thought that two factors may have influenced the failed signal detection tests: (1) the detection electronics (MEA system configurations) were inappropriately matched to the electrode's properties, or (2) a better understanding is required of the cell-electrode interface to ensure electrode properties match the physics of signal transmission.

Figure 6.42 summarises the contents of this chapter.

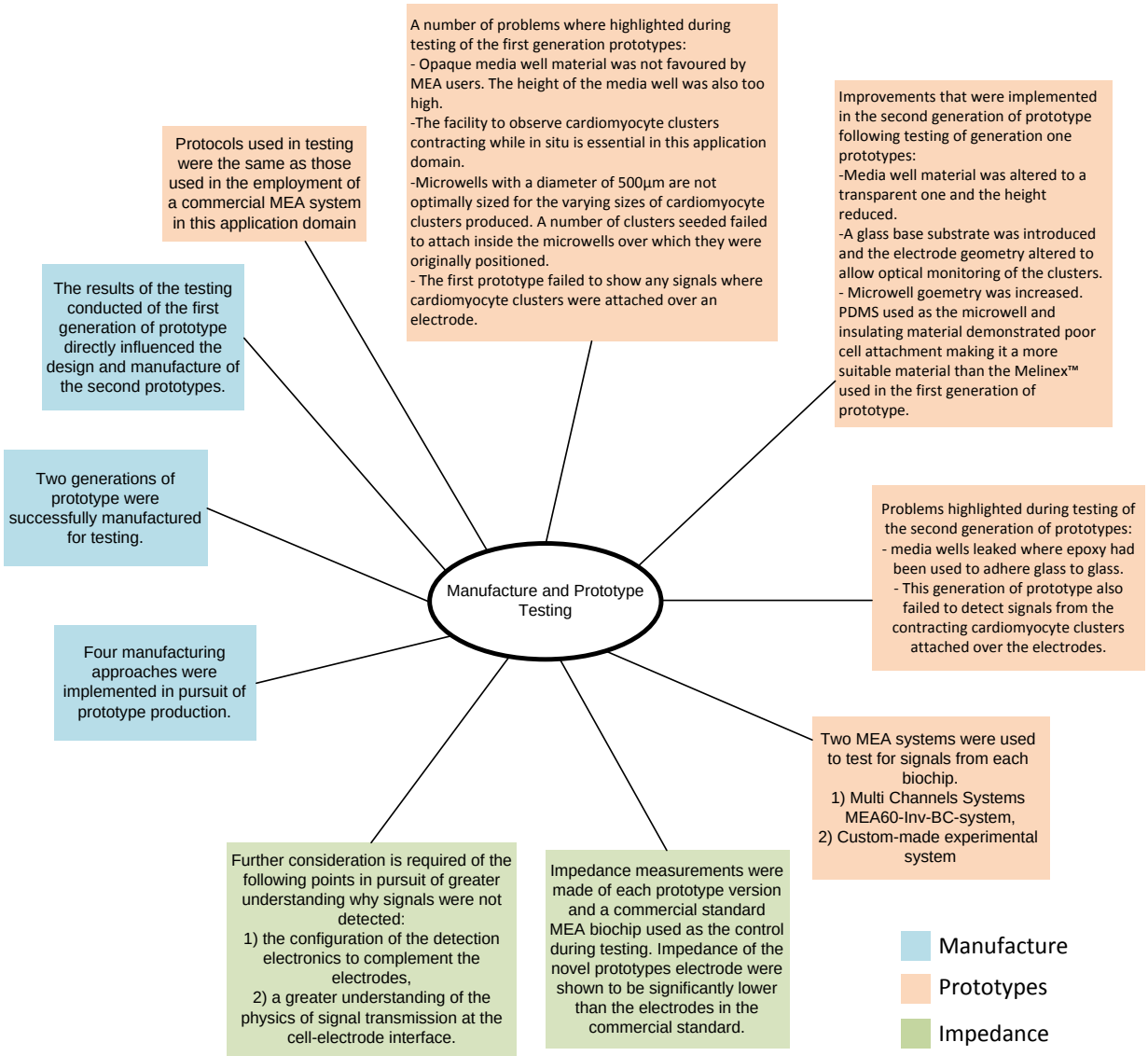


Figure 6.42: A summary of the contents of Chapter 6.

6.4 Research Questions Answered

1. **What has this research done to produce a solution device to meet the requirements identified?**

Following user requirement identification (Chapter 4) a design concept was drafted for a prototype solution device that would meet the user needs (Chapter 5). In attempts to manufacture the design concept a number of manufacturing approaches were implemented (Chapter 5).

The problems that arose during manufacture (section 6.1) were strategically targeted until a prototype that was suitable for testing was manufactured. The resulting first generation (G1) of prototype was tested in a bioscientific laboratory by skilled MEA system users. The testing protocol was equivalent to the protocols used in real MEA system application when using stem cell-derived cardiomyocyte clusters. The results of testing conducted (section 6.2.1) led to a concept re-design with the intention to produce a prototype better suited to additional user requirements highlighted in G1 tests.

The new concept design was manufactured using a different manufacturing approach and materials combinations resulting in a second generation (G2) of prototypes suitable for testing in the cardiomyocyte cluster bioscientific application environment. The results of G2 testing (section 6.2.2) highlighted further issues with design that must be addressed before arriving at a suitable solution MEA biochip device.

2. **How were the manufactured prototypes tested, and what were the outcomes of those tests?**

Prototypes were tested for user satisfaction and functionality. The ease of cardiomyocyte cluster positioning, and optical monitoring were considered as user satisfaction metrics and biocompatibility and signal detection were considered as functionality metrics.

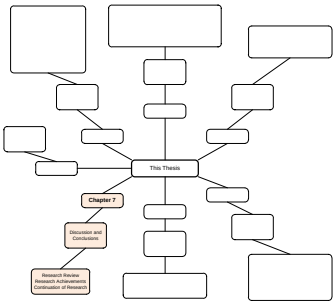
First generation prototypes exhibited poor cardiomyocyte cluster positioning and poor optical characteristics. The materials used were demonstrated to be suitably biocompatible but no signals were detected through the electrodes.

Second generation prototypes exhibited good cardiomyocyte cluster positioning and good optical monitoring, satisfying user requirements in terms of these essential metrics. The base substrate, electrode, and microwell and insulation materials were demonstrated to be appropriately biocompatible. The signal detection function however failed, resulting in overall prototype failure.

3. **Has a suitable alternative to current the standard commercial biochips used in cardiomyocyte cluster application been realised?**

The prototype devices manufactured and tested during this research do not provide a solution device that would be equivalent or superior to the commercially available MEA biochips used in this MEA system application domain.

Further work is suggested as required to provide a suitable solution device (see Chapter 7).



Chapter Seven

Conclusions and Recommendations

Early research evaluated the research problem using literature and MEA system user observations so that effective design would result in a manufacturable concept that is well suited to testing with living tissue. The intention of this research was to realise a new design that would meet the identified (Chapters 4 and 5) and prioritised (Chapter 5) requirements.

Chapters 2, 3 and 4 present the context research that was compiled and used to gain a thorough understanding of current MEA system components, applications, developmental trends and user requirements.

Requirements were identified that were separated into global generic requirements, local collaborator specific requirements, and application of interest specific requirements, through the use of three progressively specific product design specification (PDS) documents (Appendix C).

A diversity of requirements were identified, that due to limited resources and the time scale of this research, could not all be addressed in a single PDS. One user application was focused upon for the remainder of the research conducted. The stem cell-derived cardiomyocyte cluster application was selected because a PDS containing realistic and achievable design objectives was drafted by MEA system users that could be immediately focused upon (PDS 3).

PDS 3 contained requirements that were analysed and prioritised using an internationally standardised quality management system methodology (HoQ Matrix, see section 5.2.1.1). This methodology led to a prioritised list of design objectives that supported the design and manufacture of a device that offers best novelty and competitive advantage.

A novel MEA biochip concept was designed that was intended to specifically address issues relating to the positioning of the contracting cardiomyocyte clusters over the microelectrodes. The design was manufactured and tested (section 6.2.1). Feedback gained during testing of this first concept (generation one prototypes) led to a revised second novel MEA biochip design (generation two prototypes) that sought to meet the original user requirements targeted in PDS 3.

Prototypes were successfully manufactured that met a number of the PDS design objectives. However, the overall functionality of both generations of prototype failed in tests using living cardiomyocyte clusters. Contraction field potentials could not be visualised using detection hardware available.

In summary research in this thesis was conducted to:

1. Investigate the feasibility of designing and manufacturing of a novel MEA system
2. Identify current user and system requirements
3. Develop solution concepts and manufacture solution prototypes
4. Test manufactured prototype devices

7.1 Research Review

Numerous research questions arose and were addressed throughout the course of this research. Each research question is addressed at the end of each chapter.

Knowledge and understanding of MEA systems has been ascertained through this research in terms of:

1. System components (Chapter 2),
2. How systems are used (applications) (Chapters 2, 3 and 4),
3. How system use has changed since their introduction approximately 35 years ago (Chapters 2, 4 and 6).

Experience has been gained of:

1. Conducting context research and requirement identification, definition and prioritisation (Chapter 2),
2. PDS construction (Chapter 3),
3. Enterprise modelling (Chapter 4),
4. Concept design (Chapter 5),
5. Implementation of manufacturing techniques (Chapter 5),
6. Testing of prototype devices using stem cell derived cardiomyocyte clusters (Chapter 6).

While seeking to address the throughput issue stated in Chapter 1 the following novelty has been presented in this thesis:

1. A novel electrode layout has been introduced with the specific intention to support >1 cardiomyocyte cluster in one media well.
2. A microwell feature has been introduced to limit the mobility of the contraction cardiomyocyte clusters immediately after seeding and during the attachment period.
3. The biochip and microwell features have been manufactured using a manufacturing approach that is novel in this domain (e.g. Melinex coupled with excimer laser ablation for first generation prototypes, and PDMS over novel electrode geometries for second generation prototypes).

This novelty has been designed to be transferable to future systems that will be humidified incubator compatible and also more compact.

7.2 Research Achievements

This research identified MEA system requirements from three perspectives (global, local and application specific). This research then developed novel MEA biochip concepts that were application specific. The novel design concepts were successfully manufactured as prototypes using a combination of approaches not used in this manufacturing domain before. Testing of the prototype devices was conducted, validating the manufacturing approaches and the varying material combinations exploited.

Contributions have been made to academic research through presentations and publications.

The findings of this research have also emphasised an importance of complimenting MEA biochip electrode design and detecting system configuration to facilitate adequately support biochip functionality.

7.3 Continuation of Research

Recommendations for further work in the continuation of this research are as follows:

1. Implement alterations to the custom-made system currently under development and re-test existing prototypes for signal detection.
2. Reconfigure the electrode and microwell design to provide smaller electrode geometries while still providing a microwell that adequately sized to contain cardiomyocyte clusters of varying sizes (~200-800µm).
3. Consider packaging of all components developed such that they are suited to long periods within the incubated environments essential during cell culture.
4. Address the remaining unaddressed requirements cardiac application requirements presented in Figure 7.1.
5. Address the remaining unaddressed requirements neural application requirements presented in Figure 7.1.

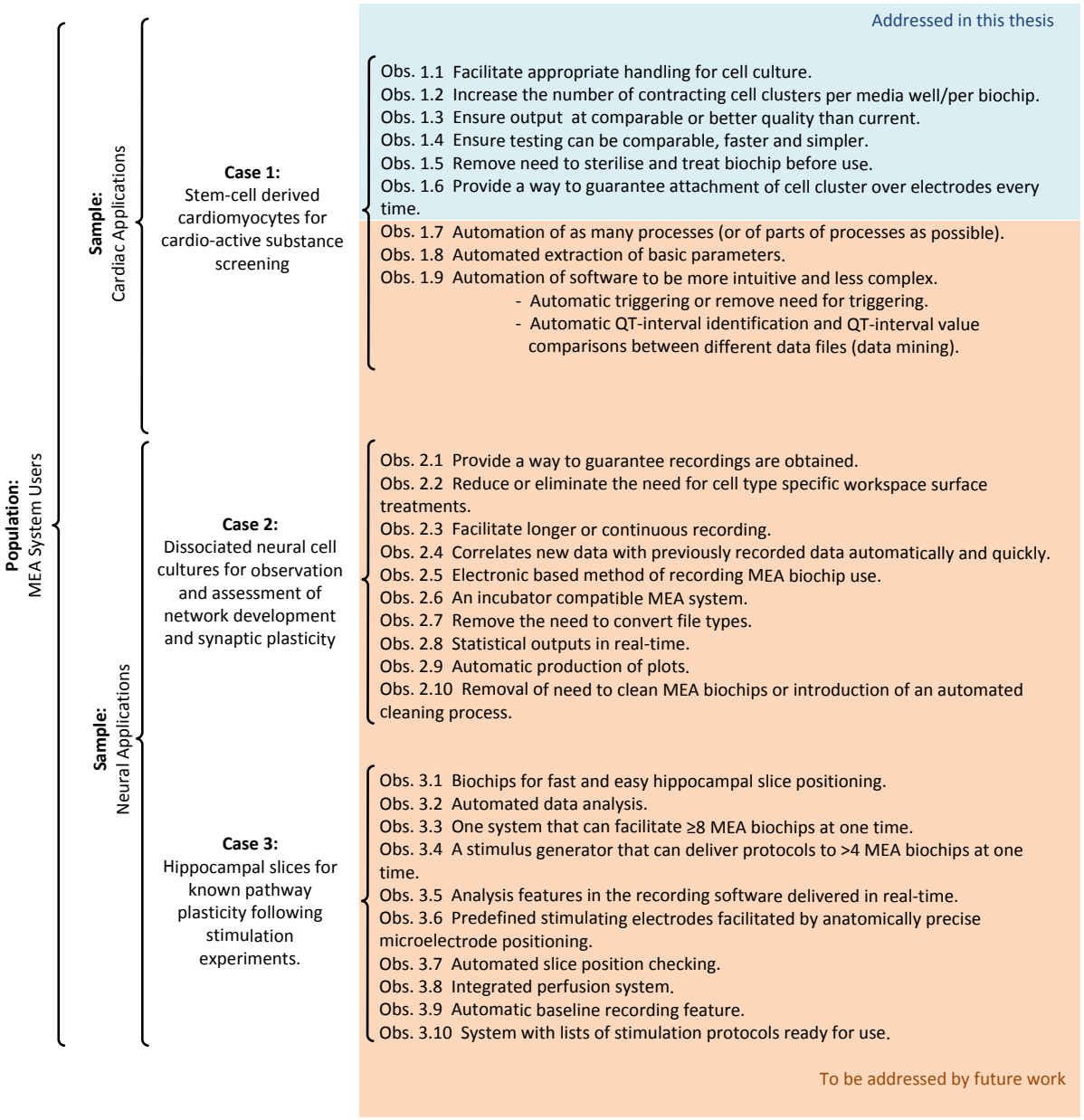


Figure 7.1: Case study observed requirements. Those addressed in this thesis (blue box) and those still to be addressed are identified (orange box).

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Appendix A

- Research Proposal

Research Proposal

Cell Friendly Electrophysiological Characterisation of Cells and Cell Therapies using Novel Modular Multi Electrode Array Systems

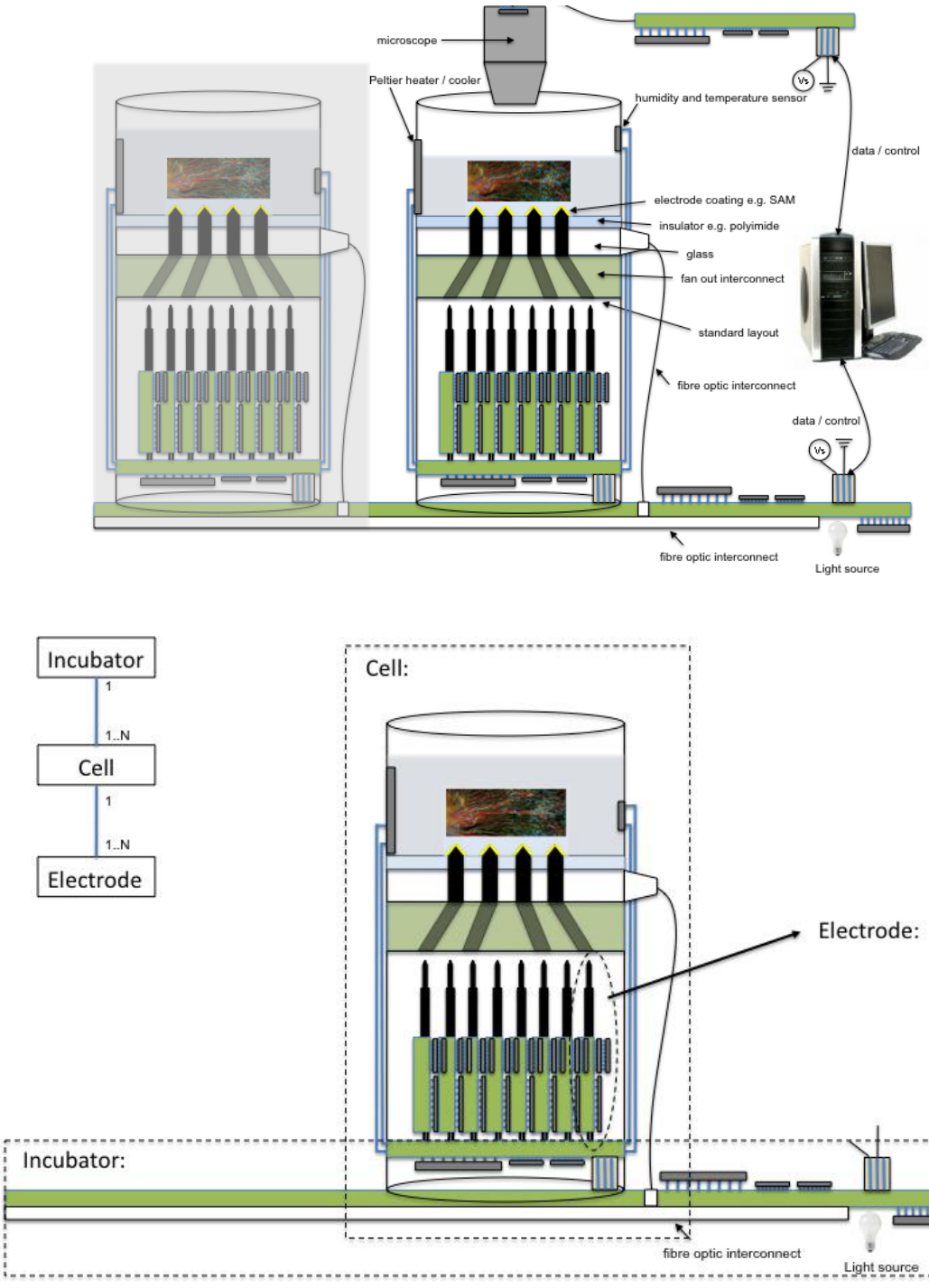
Research:

The objective of this research is to show the feasibility of scalable cell friendly electrophysiological characterisation using a novel modular multi electrode array system.

The work will research a novel physical architecture for each sample well module supporting both electrical and optical requirements via vertical electrical interconnect and the integration of novel optical waveguide technology to support integrated electrical and optical monitoring capabilities. Signal processing, sensor integration and control logic for both stimulation and recording will be realised as software web services. Depending on requirements, appropriate encapsulation of the module and its rechargeable power units, measurements can proceed without ever needing to remove the cells from the cell friendly but electronics harsh environment of the incubator/manufacturing system.

Configuration of the hardware is envisaged to encompass the possibility for end user design of electrode: (i) geometry (i.e. 2D, 3D size and shape), (ii) layout and separation, (iii) structure (i.e. surface porosity, central through holes to enable possible vacuum attachment to electrodes) and (iv) bio-compatible surface coatings. These capabilities will support better cell integration with the electrodes ((i), (iii) above), closer mapping recording layout to the physical cell architecture ((ii) above) and better longevity of cells ((iv) above).

The aim of this proposal is to support (eventually via a case database of best practice) the end user in being able to determine his own appropriate shape, diameter, separation, insulation and coating to obtain the best signal to noise ratio for the cells under investigation. If this is unknown best case defaults for other successful applications will be available for start-up trials. Configuration of software will support the tuning of, for example, system logic, signal processing and signal pattern recognition based upon the requirements. Post processing software will include the detailed visualisation and analysis of the output of the system based upon the detailed requirements. There are significant challenges to the successful outcome of this research, primarily the requirement to integrate optical monitoring, integrate control, signal processing and sensors all within a scalable wireless package of the size of a well that can operate robustly within the cell culture environment and have 2D and 3D electrode geometries of relevance.



A.1: System concept – 2D

Appendix B

- Key Organelles
- Cell Characterisation Methods
- Headstage containing 2 MEA biochips
- S7B
- QT-Screen Brochure
- Appendix B References

Key cell organelles

Nucleus: Every cell contains a nucleus of which the contents are contained by a membrane. The nucleus contains the genetic material for the cell (the DNA) which is protected from the constant metabolic activity occurring in the cytoplasm.

Endomembrane system: This consists of multiple organelles, the endoplasmic reticulum, vesicles and Golgi apparatus. The Golgi bodies and endoplasmic reticulum organelles modify proteins, and synthesise lipids as required by the cell for metabolism. The vesicles basically transport, store or digest substances.

Mitochondria: Mitochondria are 2-6µm in length and 0.6µm in diameter (Pocock and Richards, 1999) and are responsible for converting adenosine triphosphate (ATP) into energy. When one of the three negatively charged phosphate groups present in ATP is removed by hydrolysis a more stable molecule, adenosine diphosphate (ADP) results. The change from a less stable to more stable molecule results in the release of energy. This hydrolysis occurs within mitochondria and it is this energy that is used for all cell activities (Goldberg, 2010).

Cytoskeleton: The cytoskeleton is made from proteins in the form of filaments (micro and intermediate) and microtubules. The cytoskeleton serves to provide support to the cell and gives it shape. Cytoskeletal elements interact with the cell membrane (Doherty and McMahon, 2008) and aid in movement of cell parts or of the whole cell.

Bioscientific Cell Characterisation Techniques

Optical assessment: Visual assessment of cell, tissues and their components using light microscopy is limited due to various cellular components (e.g. mitochondria) possessing similar optical densities (Cormack, 2001). In addition a single culture may contain several thousand cells making it difficult to distinguish one cell from another. Skilled cell culture experts rely upon visual methods to assess culture viability (e.g. staining) and to quantify cell populations in culture (e.g. to monitor growth through calculation of population multiplication/depletion).

Staining: Difficulties viewing cells and their particular cellular components are over come through the use of a range of selectively absorbed dyes or markers. There are dozens of variants of histological stains that reveal tissue components by either colouring them selectively or by increasing their optical densities to different extents (Cormack, 2001). Stains differ according to the sample that is to be observed and what the bioscientist is interested to see. A list of the 50 most commonly used stains in cell culture-based research is incorporated in table B1.

Table B1: Standard histology staining procedures. [Source: Florida State University College of Medicine, 2012.]

Acid Fast Stain (for mycobacteria)	Colloidal Iron Stain	Gram (Modified Brown-Brenn) Stain	Methyl Green Pyronin (MGP) Stain	Sudan Black B Stain (for lipochrome)
Acid Fast Stain	Congo Red Stain	Gridley's Stain for ameba	Mucicarmine Stain	Sudan Black B Stain (for fat)
Alcian Blue Stain	Copper Stain	Grimelius Argyrophil Stain (Pascual's Method)	Nissl Stain	Trichrome Stain - Masson's method
Alcian Blue-PAS Stain (PAB)	Elastic van Gieson Stain	Grocott's Methenamine Silver (GMS) Stain	Oil Red O Stain	Trichrome Stain - microwave method
Hyaluronidase Digestion for Alcian Blue	Elastic - Weigert's resorcin-fuchsin method	Holzer's Glial Fiber Stain	Orcein Stain	Thioflavin S Stain (for amyloid in tissues)
Alizarin Stain for calcium	Modified Elastic van Gieson Stain	Hortega's Pineal Stain	Periodic acid-Schiff Stain (PAS)	Modified Thioflavin S Stain (for senile plaques)
Auramine-Rhodamine Stain (fluorescent)	Fontana-Masson Stain for melanin	Iron Stain (Prussian blue)	Periodic acid-Schiff, digested Stain (PAS-D)	Toluidine Blue Stain (for mast
Bielschowsky Stain (for senile	Melanin Bleach		PTAH Stain	
	Fraser Lendrum Stain		Reticulin Stain	
	Giemsa (Modified		Spirochete Stain (Steiner & Steiner	

plaques) Bile Stain Bodian's Stain	May-Gruenwald) Stain (for hematopoietic tissues) Giemsa Stain (for Helicobacter)	Iron Stain (Turnbull's blue) Jones' Silver Stain Luxol Fast Blue (LFB) Stain	method)	cells) Urate Crystal Stain VonKossa Stain for calcium

Monitoring growth and multiplication/depletion: Cells can be counted using a tool called a haemocytometer (Lund et al, 1958; Wiedemann et al, 2011). This is small tile or specimen slide with a microscopic grid etched into the glass. Samples from a culture are prepared to a known concentration in a solution with water. A controlled quantity of the mixed solution is dispersed over the grid and cells are counted by eye. Calculations are used to estimate the number of cells per ml of solution and then corrections are made for dilution to establish populations. This process is time consuming and user dependant. There is an advantage to using a haemocytometer, and that is that dead cells can be identified by eye and eliminated from the count.

An electronic alternative such as a Coulter counter can also be utilised which is faster than using a haemocytometer, but cannot distinguish between live and dead cells (Strober, 2001).

Recent technologies intending to offer automated cell counting with the in-built ability to distinguish and disregard dead matter include the CASY and xCELLigence systems from Roche (Sussex, United Kingdom). The CASY system is designed for overall cell culture quality control (e.g. cell concentration determination, cell viability, cell volume, cell aggregation and cell debris) (Roche Media Release, March 2011). The xCELLigence system is “for label-free (e.g. stain free) and real-time monitoring of cell viability” (Ke et al 2011).

Biomechanical properties and microforce generation: It has been shown that there are links between the structure, mechanical properties, phenotype behaviour and function of cells in microenvironments that are believed to have implications in human health and the biotechnology industry (Addae-Mensah and Wikswo, 2008). Scientists seeking to understand forces exerted in cell culture environments have created numerous techniques for investigating the mechanical and microforce properties of cells *in vitro*. The deformation of cells in culture has been addressed using tools that generate compressive or tensile forces, shear forces, bending forces, twisting forces, or a

combination of forces. The following table (table B2) describes methods that have been classified as either exerting forces actively or recording forces passively to calculate force generation in cells.

Table B2: Techniques applied in the study of mechanical and microforce generation of cells *in vitro*, classified as either passive or active. [Adapted from Addae-Mensah and Wikswo, 2008].

Passive		Active	
Technique - *Principle*	Example publications	Technique – *Principle*	Example publications
Elastic Substratum Method – *Wrinkling patterns developed in artificial flexible sheets are used to infer cell traction forces.*	Harris et al, 1980; Danowski, 1989.	Atomic force microscopy *(AFM) - Relative deformation of a cantilever tip and substrate (cell) is used to estimate forces.*	Hoh and Schoenenberger, 1994; Radmacher et al, 1996; Mathur et al, 2001.
Flexible Sheets with Embedded Beads – *Displacements of beads within flexible sheets are used to infer cell traction forces.*	Lee et al, 1994; Dembo and Wang, 1999; Munevar et al, 2001; Butler et al, 2002.	Micropipette Aspiration – *Gentle suction is applied to a micropipette attached to a cell.*	Jones et al, 1999; Alexopoulos et al, 2003; Chu et al, 2004.
Flexible Sheets with Micropatterned Dots or Grids – *Deformation of grid or dot patterns is used to infer cell traction forces.*	Balaban et al, 2001.	Optical Tweezers – *Dielectric beads of high refractive index are moved using laser beams.*	Henon et al, 1999; Dao et al, 2005.
Micromachined Cantilever Beam – *Horizontal deflection of cantilever with attachment pad is used to infer traction force.*	Galbraith and Sheetz, 1997.	Micromachined Force Sensors and Actuators – *Movable parts are fabricated in silicon and various methods such as piezo actuation are used to move them.*	Yang and Saif, 2005; Serrell et al, 2007.
Array of Vertical Microcantilevers – *Horizontal deflection of individual vertical microcantilevers is used to infer traction forces.*	Tan et al, 2003; Addae-Mensah et al, 2007; du Roure et al , 2004; du	Shear Flow Methods – *Enclosed chambers with inlet and outlets for fluid flow are used to subject cells to fluid shear stress.*	Dong et al, 1999; Civelek et al, 2002; Ainslie et al, 2005; Leyton-Mange et al,

	Roure et al , 2005;		2006.
		Stretching Devices – *The flexible membrane is attached to structures that enable the membrane to be stretched.*	Wang et al, 2000; Zhuang et al, 2000.
		Carbon Fibre- Based Systems – *Carbon fibres are attached to directly to a cell and controlled mechanically using feedback systems.*	Yasuda et al, 2001; Nishimura et al, 2004; Iribe et al, 2007.
		Magnetic tweezers/magnetic twisting cytometry – *Magnetised ferromagnetic or superparamagnetic beads are moved by weaker directional magnetic fields/gradients.*	Bausch et al, 1999; Chen at al, 2001; Lele et al, 2007.

Electrical Characterisation: Cells and tissues can be characterised in terms of electrical activity. The details of which are described throughout Chapter 2.

Multi Channels System MEA2100-System Headstage

This headstage is available in two configurations to house one or two MEA biochips (B.1).

B.1: The two available variants of the MEA2100 headstage.

Guidance for Industry

S7A Safety Pharmacology Studies for Human Pharmaceuticals

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

ICH
July 2001

Guidance for Industry

S7A Safety Pharmacology Studies for Human Pharmaceuticals

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Guidance for Industry[1]

S7A Safety Pharmacology Studies for Human Pharmaceuticals

This guidance represents the Food and Drug Administration's current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.

I. INTRODUCTION (1)[2]

A. Objectives of the Guidance (1.1)

This guidance was developed to help protect clinical trial participants and patients receiving marketed products from potential adverse effects of pharmaceuticals, while avoiding unnecessary use of animals and other resources.

This guidance provides a definition, general principles, and recommendations for safety pharmacology studies.

B. Background (1.2)

Pharmacology studies have been performed worldwide for many years as part of the nonclinical evaluation of pharmaceuticals for human use. There have been, however, no internationally accepted definitions, objectives or recommendations on the design and conduct of safety pharmacology studies. (Note 1)

The term ***safety pharmacology studies*** first appeared in ICH *M3 Timing of Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals* and *S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* as studies that should be conducted to support use of therapeutics in humans (1, 2). Details of the safety pharmacology studies, including their definition and objectives, were left for future discussion.

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[1] This guidance was developed within the Expert Working Group (Safety) of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document has been endorsed by the ICH Steering Committee at *Step 4* of the ICH process, November 8, 2000. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan, and the United States.

[2] Arabic numbers reflect the organizational breakdown in the document endorsed by the ICH Steering Committee at Step 4 of the ICH process, November 8, 2000.

C. Scope of the Guidance (1.3)

This guidance generally applies to new chemical entities and biotechnology-derived products for human use. This guidance can be applied to marketed pharmaceuticals when appropriate (e.g., when adverse clinical events, a new patient population, or a new route of administration raises concerns not previously addressed).

D. General Principle (1.4)

It is important to adopt a rational approach when selecting and conducting safety pharmacology studies. The specific studies that should be conducted and their design will vary based on the individual properties and intended uses of the pharmaceuticals. Scientifically valid methods should be used, and when there are internationally recognized methods that are applicable to pharmaceuticals, these methods are preferable. Moreover, the use of new technologies and methodologies in accordance with sound scientific principles is encouraged.

Some safety pharmacology endpoints can be incorporated in the design of toxicology, kinetic, and clinical studies, while in other cases these endpoints should be evaluated in specific safety pharmacology studies. Although adverse effects of a substance may be detectable at exposures that fall within the therapeutic range in appropriately designed safety pharmacology studies, such effects may not be evident from observations and measurements used to detect toxicity in conventional animal toxicity studies.

E. Definition of Safety Pharmacology (1.5)

Pharmacology studies can be divided into three categories: primary pharmacodynamic, secondary pharmacodynamic, and safety pharmacology studies.

For the purpose of this document, safety pharmacology studies are defined as those studies that investigate the potential undesirable pharmacodynamic effects of a substance on physiological functions in relation to exposure in the therapeutic range and above. (See Note 2 for definitions of primary pharmacodynamic and secondary pharmacodynamic studies.)

In some cases, information on the primary and secondary pharmacodynamic properties of the substance contributes to the safety evaluation for potential adverse effects in humans and should be considered along with the findings of safety pharmacology studies.

II. GUIDANCE (2)

A. Objectives of Studies (2.1)

The objectives of safety pharmacology studies are (1) to identify undesirable pharmacodynamic properties of a substance that may have relevance to its human safety, (2) to evaluate adverse pharmacodynamic and/or pathophysiological effects of a substance observed in toxicology and/or clinical studies, and (3) to investigate the mechanism of the adverse pharmacodynamic effects observed and/or suspected. The investigational plan to meet these objectives should be clearly identified and delineated.

B. General Considerations in Selection and Design of Safety Pharmacology Studies (2.2)

Since pharmacological effects vary depending on the specific properties of each test substance, the studies should be selected and designed accordingly. The following factors should be considered (the list is not comprehensive).

1. Effects related to the therapeutic class of the test substance, since the mechanism of action may suggest specific adverse effects (e.g., proarrhythmia is a common feature of antiarrhythmic agents)

2. Adverse effects associated with members of the chemical or therapeutic class, but independent of the primary pharmacodynamic effects (e.g., antipsychotics and QT prolongation)

3. Ligand binding or enzyme assay data suggesting a potential for adverse effects

4. Results from previous safety pharmacology studies, from secondary pharmacodynamic studies, from toxicology studies, or from human use that warrant further investigation to establish and characterize the relevance of these findings to potential adverse effects in humans

During early development, sufficient information (e.g., comparative metabolism) may not always be available to rationally select or design the studies in accordance with the points stated above; in such circumstances, a more general approach in safety pharmacology investigations can be applied.

A hierarchy of organ systems can be developed according to their importance with respect to life-supporting functions. Vital organs or systems, the functions of which are acutely critical for life (e.g., the cardiovascular, respiratory, and central nervous systems), are considered to be the most important ones to assess in safety pharmacology studies. Other organ systems (e.g., the renal or gastrointestinal system), the functions of which can be transiently disrupted by adverse pharmacodynamic effects without causing irreversible harm, are of less immediate investigative concern. Safety pharmacology evaluation of effects on these other systems may be of particular importance when considering factors such as the likely clinical trial or patient population (e.g., gastrointestinal tract in Crohn's disease, renal function in primary renal hypertension, immune system in immunocompromised patients.).

C. Test Systems (2.3)

1. General Considerations on Test Systems (2.3.1)

Consideration should be given to the selection of relevant animal models or other test systems so that scientifically valid information can be derived. Selection factors can include the pharmacodynamic responsiveness of the model, pharmacokinetic profile, species, strain, gender and age of the experimental animals, the susceptibility, sensitivity, and reproducibility of the test system and available background data on the substance.

Data from humans (e.g., in vitro metabolism), when available, should also be considered in the test system selection. The time points for the measurements should be based on pharmacodynamic and pharmacokinetic considerations. Justification should be provided for the selection of the particular animal model or test system.

2. Use of In Vivo and In Vitro Studies (2.3.2)

Animal models as well as ex vivo and in vitro preparations can be used as test systems. Ex vivo and in vitro systems can include, but are not limited to: isolated organs and tissues, cell cultures, cellular fragments, subcellular organelles, receptors, ion channels, transporters and enzymes. In vitro systems can be used in supportive studies (e.g., to obtain a profile of the activity of the substance or to investigate the mechanism of effects observed in vivo).

In conducting in vivo studies, it is preferable to use unanesthetized animals. Data from unrestrained animals that are chronically instrumented for telemetry, data gathered using other suitable instrumentation methods for conscious animals, or data from animals conditioned to the laboratory environment are preferable to data from restrained or unconditioned animals. In the use of unanesthetized animals, the avoidance of discomfort or pain is a foremost consideration.

3. Experimental Design (2.3.3)

a. Sample Size and Use of Controls (2.3.3.1)

The size of the groups should be sufficient to allow meaningful scientific interpretation of the data generated. Thus, the number of animals or isolated preparations should be adequate to demonstrate or rule out the presence of a biologically significant effect of the test substance. The sample size should take into consideration the size of the biological effect that is of concern for humans. Appropriate negative and positive control groups should be included in the experimental design. In well-characterized in vivo test systems, positive controls may not be necessary. The exclusion of controls from studies should be justified.

b. Route of Administration (2.3.3.2)

In general, the expected clinical route of administration should be used when feasible. Regardless of the route of administration, exposure to the parent substance and its major metabolites should be similar to or greater than that achieved in humans when such information is available. Assessment of effects by more than one route may be appropriate if the test substance is intended for clinical use by more than one route of administration (e.g., oral and parenteral) or where there are observed or anticipated significant qualitative and quantitative differences in systemic or local exposure.

D. Dose Levels or Concentrations of Test Substance (2.4)

1. In Vivo Studies (2.4.1)

In vivo safety pharmacology studies should be designed to define the dose-response relationship of the adverse effect observed. The time course (e.g., onset and duration of response) of the adverse effect should be investigated, when feasible. Generally, the doses eliciting the adverse effect should be compared to the doses eliciting the primary pharmacodynamic effect in the test species or the proposed therapeutic effect in humans, if feasible. It is recognized that there are species differences in pharmacodynamic sensitivity. Therefore, doses should include and exceed the primary pharmacodynamic or therapeutic range. In the absence of an adverse effect on the safety pharmacology parameters evaluated in the study, the highest tested dose should be a dose that produces moderate adverse effects in this or in other studies of similar route and duration. These adverse effects can include dose-limiting pharmacodynamic effects or other toxicity. In practice, some effects in the toxic range (e.g., tremors or fasciculation during ECG recording) may confound the interpretation of the results and may also limit dose levels. Testing of a single group at the limiting dose as described above may be sufficient in the absence of an adverse effect on safety pharmacology endpoints in the test species.

2. In Vitro Studies (2.4.2)

In vitro studies should be designed to establish a concentration-effect relationship. The range of concentrations used should be selected to increase the likelihood of detecting an effect on the test system. The upper limit of this range may be influenced by physico-chemical properties of the test substance and other assay specific factors. In the absence of an effect, the range of concentrations selected should be justified.

E. Duration of Studies (2.5)

Safety pharmacology studies are generally performed by single-dose administration. When pharmacodynamic effects occur only after a certain duration of treatment, or when results from repeat dose nonclinical studies or results from use in humans give rise to concerns about safety pharmacological effects, the duration of the safety pharmacology studies to address these effects should be rationally based.

F. Studies on Metabolites, Isomers, and Finished Products (2.6)

Generally, any parent compound and its major metabolites that achieve, or are expected to achieve, systemic exposure in humans should be evaluated in safety pharmacology studies. Evaluation of major metabolites is often accomplished through studies of the parent compound in animals. If the major human metabolites are found to be absent or present only at relatively low concentrations in animals, assessment of the effects of such metabolites on safety pharmacology endpoints should be considered. Additionally, if metabolites from humans are known to substantially contribute to the pharmacological actions of the therapeutic agent, it could be important to test such active metabolites. When the in vivo studies on the parent

compound have not adequately assessed metabolites, as discussed above, the tests of metabolites can use in vitro systems based on practical considerations.

In vitro or in vivo testing of the individual isomers should also be considered when the product contains an isomeric mixture.

Safety pharmacology studies with the finished product formulations should be conducted only for formulations that substantially alter the pharmacokinetics and/or pharmacodynamics of the active substance in comparison to formulations previously tested (i.e., through active excipients such as penetration enhancers, liposomes, and other changes such as polymorphism).

G. Safety Pharmacology Core Battery (2.7)

The purpose of the safety pharmacology core battery is to investigate the effects of the test substance on vital functions. In this regard, the cardiovascular, respiratory, and central nervous systems are usually considered the vital organ systems that should be studied in the core battery. In some instances, based on scientific rationale, the core battery should be supplemented (see section H (2.8)) or need not be implemented (see also section I (2.9)).

The exclusion of certain tests or explorations of certain organs, systems or functions should be scientifically justified.

1. Central Nervous System (2.7.1)

Effects of the test substance on the central nervous system should be assessed appropriately. Motor activity, behavioral changes, coordination, sensory/motor reflex responses and body temperature should be evaluated. For example, a functional observation battery (FOB) (3), modified Irwin's (4), or other appropriate test (5) can be used.

2. Cardiovascular System (2.7.2)

Effects of the test substance on the cardiovascular system should be assessed appropriately. Blood pressure, heart rate, and the electrocardiogram should be evaluated. In vivo, in vitro and/or ex vivo evaluations, including methods for repolarization and conductance abnormalities, should also be considered. (Note 3)

3. Respiratory System (2.7.3)

Effects of the test substance on the respiratory system should be assessed appropriately. Respiratory rate and other measures of respiratory function (e.g., tidal volume (6) or hemoglobin oxygen saturation) should be evaluated. Clinical observation of animals is generally not adequate to assess respiratory function, and thus these parameters should be quantified by using appropriate methodologies.

H. Follow-up and Supplemental Safety Pharmacology Studies (2.8)

Adverse effects may be suspected based on the pharmacological properties or chemical class of the test substance. Additionally, concerns may arise from the safety pharmacology core battery, clinical trials, pharmacovigilance, experimental in vitro or in vivo studies, or from literature reports. When such potential adverse effects raise concern for human safety, these should be explored in follow-up or supplemental safety pharmacology studies, as appropriate.

1. Follow-up Studies For Safety Pharmacology Core Battery (2.8.1)

Follow-up studies are meant to provide a greater depth of understanding than, or additional knowledge to, that provided by the core battery on vital functions. The following subsections provide lists of studies to further evaluate these organ systems for potential adverse pharmacodynamic effects. These lists are not meant to be comprehensive or prescriptive, and the studies should be selected on a case-by-case basis after considering factors such as existing nonclinical or human data. In some cases, it may be more appropriate to address these effects during the conduct of other nonclinical and/or clinical studies.

a. Central Nervous System (2.8.1.1)

Behavioral pharmacology, learning and memory, ligand-specific binding, neurochemistry, visual, auditory, and/or electrophysiology examinations

b. Cardiovascular System (2.8.1.2)

Cardiac output, ventricular contractility, vascular resistance, the effects of endogenous and/or exogenous substances on the cardiovascular responses

c. Respiratory System (2.8.1.3)

Airway resistance, compliance, pulmonary arterial pressure, blood gases, blood pH

2. Supplemental Safety Pharmacology Studies (2.8.2)

Supplemental studies are meant to evaluate potential adverse pharmacodynamic effects on organ system functions not addressed by the core battery or repeated dose toxicity studies when there is a cause for concern.

a. Renal/Urinary System (2.8.2.1)

Effects of the test substance on renal parameters should be assessed. For example, urinary volume, specific gravity, osmolality, pH, fluid/electrolyte balance, proteins, cytology, and blood chemistry determinations such as blood urea nitrogen, creatinine, and plasma proteins can be used.

b. Autonomic Nervous System (2.8.2.2)

Effects of the test substance on the autonomic nervous system should be assessed. For example, binding to receptors relevant for the autonomic nervous system, functional responses to agonists or antagonists in vivo or in vitro, direct stimulation of autonomic nerves and measurement of cardiovascular responses, baroreflex testing, and heart rate variability can be used.

c. Gastrointestinal System (2.8.2.3)

Effects of the test substance on the gastrointestinal system should be assessed. For example, gastric secretion, gastrointestinal injury potential, bile secretion, transit time in vivo, ileal contraction in vitro, gastric pH measurement, and pooling can be used.

d. Other Organ Systems (2.8.2.4)

Effects of the test substance on organ systems not investigated elsewhere should be assessed when there is a reason for concern. For example, dependency potential or skeletal muscle, immune, and endocrine functions can be investigated.

I. Conditions Under Which Studies Are Not Necessary (2.9)

Safety pharmacology studies may not be needed for locally applied agents (e.g., dermal or ocular) where the pharmacology of the test substance is well characterized, and where systemic exposure or distribution to other organs or tissues is demonstrated to be low.

Safety pharmacology studies prior to the first administration in humans may not be needed for cytotoxic agents for treatment of end-stage cancer patients. However, for cytotoxic agents with novel mechanisms of action, there may be value in conducting safety pharmacology studies.

For biotechnology-derived products that achieve highly specific receptor targeting, it is often sufficient to evaluate safety pharmacology endpoints as a part of toxicology and/or pharmacodynamic studies; therefore, safety pharmacology studies can be reduced or eliminated for these products.

For biotechnology-derived products that represent a novel therapeutic class and/or those products that do not achieve highly specific receptor targeting, a more extensive evaluation by safety pharmacology studies should be considered.

There may be additional exceptions where safety pharmacology testing is not needed, for example, in the case of a new salt having similar pharmacokinetics and pharmacodynamics.

J. Timing of Safety Pharmacology Studies in Relation to Clinical Development (2.10)

When planning a safety pharmacology program, section I (2.9) should be reviewed to determine whether or not specific studies are recommended.

1. Studies Prior to First Administration in Humans (2.10.1)

The effects of a test substance on the functions listed in the safety pharmacology core battery should be investigated prior to first administration in humans. Any follow-up or supplemental studies identified as appropriate, based on a cause for concern, should also be conducted. Information from toxicology studies adequately designed and conducted to address safety pharmacology endpoints can result in reduction or elimination of separate safety pharmacology studies.

2. Studies During Clinical Development (2.10.2)

Additional studies may be warranted to clarify observed or suspected adverse effects in animals and humans during clinical development.

3. Studies Before Approval (2.10.3)

Safety pharmacology effects on systems listed in section H (2.8) should be assessed prior to product approval, unless not warranted, in which case this should be justified. Available information from toxicology studies adequately designed and conducted to address safety pharmacology endpoints, or information from clinical studies, can support this assessment and replace safety pharmacology studies.

K. Application of Good Laboratory Practice (GLP) (2.11)

It is important to ensure the quality and reliability of nonclinical safety studies. This is normally accomplished through the conduct of the studies in compliance with GLP. Due to the unique design of, and practical considerations for, some safety pharmacology studies, it may not be feasible to conduct these in compliance with GLP. It has to be emphasized that data quality and integrity in safety pharmacology studies should be ensured even in the absence of formal adherence to the principles of GLP. When studies are not conducted in compliance with GLP, study reconstruction should be ensured through adequate documentation of study conduct and archiving of data. Any study or study component not conducted in compliance with GLP should be adequately justified, and the potential impact on evaluation of the safety pharmacology endpoints should be explained.

The safety pharmacology core battery should ordinarily be conducted in compliance with GLP. Follow-up and supplemental studies should be conducted in compliance with GLP to the greatest extent feasible. Safety pharmacology investigations can be part of toxicology studies; in such cases, these studies would be conducted in compliance with GLP.

Primary pharmacodynamic studies do not need to be conducted in compliance with GLP.

Generally, secondary pharmacodynamic studies do not need to be conducted in compliance with GLP. Results from secondary pharmacodynamic studies conducted during the compound selection process may contribute to the safety pharmacology evaluation. When there is no cause for concern (e.g., there are no findings for the safety pharmacological endpoint or the chemical or therapeutic class), these studies need not be repeated in compliance with GLP. In some circumstances, results of secondary pharmacodynamic studies may make a pivotal contribution to the safety evaluation for potential adverse effects in humans, and these are normally conducted in compliance with GLP.

III. NOTES (3)

1. General pharmacology studies have been considered an important component in drug safety assessment. General pharmacology studies were originally referred to as those designed to examine effects other than the primary therapeutic effect of a drug candidate. Safety pharmacology studies were focused on identifying adverse effects on physiological functions. All three regions have accepted data from general pharmacology studies (Japan and EC) or safety pharmacology studies (USA) in the assessment of a marketing application. The Japanese Ministry of Health and Welfare (MHW) issued the *Guideline for General Pharmacology* in 1991. In this MHW guideline, general pharmacology studies include those designed to identify unexpected effects on organ system function and to broaden pharmacological characterization (pharmacological profiling). However, there has been no internationally accepted definition of the terms ***primary pharmacodynamics***, ***secondary pharmacodynamics*** and ***safety pharmacology***. The need for international harmonization of the nomenclature and the development of an international guidance for safety pharmacology has been recognized.

2. Studies on the mode of action and/or effects of a substance in relation to its desired therapeutic target are primary pharmacodynamic studies. Studies on the mode of action and/or effects of a substance not related to its desired therapeutic target are secondary pharmacodynamic studies (these have sometimes been referred to as part of general pharmacology studies).

3. There is no scientific consensus on the preferred approach to, or internationally recognized guidance on, addressing risks for repolarization-associated ventricular tachyarrhythmia (e.g., Torsade de Pointes). A guidance (S7B) will be prepared to present some currently available methods and discuss their advantages and disadvantages. Submission of data to regulatory authorities to support the use of these methods is encouraged.

IV. REFERENCES (4)

1. ICH *M3 Timing of Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals* (FDA, 1997).
2. ICH *S6 Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals* (FDA, 1997).
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Guidance for Industry

S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

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Guidance for Industry[1]

S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals

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I. INTRODUCTION (1, 1.1)[2]

This guidance describes a nonclinical testing strategy for assessing the potential of a test substance to delay ventricular repolarization. The guidance includes information concerning nonclinical assays and integrated risk assessments. The assessment of the effects of pharmaceuticals on ventricular repolarization and proarrhythmic risk is the subject of active investigation. When additional data (nonclinical and clinical) are accumulated in the future, they will be evaluated and this guidance might be revised.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

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[1] This guidance was developed within the Expert Working Group (Safety) of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document has been endorsed by the ICH Steering Committee at *Step 4* of the ICH process, May 2005. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan, and the United States.

[2] Arabic numbers reflect the organizational breakdown in the document endorsed by the ICH Steering Committee at Step 4 of the ICH process, May 2005.

Contains Nonbinding Recommendations

A. Background (1.2)

The QT interval (time from the beginning of the QRS complex to the end of the T wave) of the electrocardiogram (ECG) is a measure of the duration of ventricular depolarization and repolarization. QT interval prolongation can be congenital or acquired (e.g., pharmaceutical-induced). When ventricular repolarization is delayed and the QT interval is prolonged, there is an increased risk of ventricular tachyarrhythmia, including torsade de pointes, particularly when combined with other risk factors (e.g., hypokalemia, structural heart disease, bradycardia). Thus, much emphasis has been placed on the potential proarrhythmic effects of pharmaceuticals that are associated with QT interval prolongation.

Ventricular repolarization, determined by the duration of the cardiac action potential, is a complex physiological process. It is the net result of the activities of many membrane ion channels and transporters. Under physiological conditions, the functions of these ion channels and transporters are highly interdependent. The activity of each ion channel or transporter is affected by multiple factors including, but not limited to, intracellular and extracellular ion concentrations, membrane potential, cell-to-cell electrical coupling, heart rate, and autonomic nervous system activity. The metabolic state (e.g., acid-base balance) and location and type of cardiac cell are also important. The human ventricular action potential consists of five sequential phases:

- phase 0: The upstroke of the action potential is primarily a consequence of a rapid, transient influx of Na^+ (I_{Na}) through Na^+ channels.
- phase 1: The termination of the upstroke of the action potential and early repolarization phase result from the inactivation of Na^+ channels and the transient efflux of K^+ (I_{to}) through K^+ channels.
- phase 2: The plateau of the action potential is a reflection of a balance between the influx of Ca^{2+} (I_{Ca}) through L-type Ca^{2+} channels and outward repolarizing K^+ currents.
- phase 3: The sustained downward stroke of the action potential and the late repolarization phase result from the efflux of K^+ (I_{Kr} and I_{Ks}) through delayed rectifier K^+ channels.
- phase 4: The resting potential is maintained by the inward rectifier K^+ current (I_{K1}).

Prolongation of the action potential can result from decreased inactivation of the inward Na^+ or Ca^{2+} currents, increased activation of the Ca^{2+} current, or inhibition of one or more of the outward K^+ currents. The rapidly and slowly activating components of the delayed rectifier potassium current, I_{Kr} and I_{Ks}, seem to have the most influential role in determining the duration of the action potential and thus the QT interval. The human ether-a-go-go-related gene (hERG) and KvLQT1 gene encode pore-forming proteins KCNH2 and KCNQ1 that are thought to represent the α-subunits of the human potassium channels responsible for I_{Kr} and I_{Ks}, respectively. These α-subunit proteins can form hetero-oligomeric complexes with auxiliary β-subunits (i.e. MiRP and MinK gene products), which have been speculated to modulate the gating properties of the channel proteins. The most common mechanism of QT interval prolongation by pharmaceuticals is inhibition of the delayed rectifier potassium channel that is responsible for I_{Kr}.

Contains Nonbinding Recommendations

B. Scope of the Guidance (1.3)

This guidance extends and complements the ICH guidance on *S7A Safety Pharmacology Studies for Human Pharmaceuticals*. This guidance applies to new chemical entities for human use and marketed pharmaceuticals when appropriate (e.g., when adverse clinical events, a new patient population, or a new route of administration raises concerns not previously addressed). Conditions under which studies are not called for are described in ICH S7A.

C. General Principles (1.4)

Principles and recommendations described in ICH S7A also apply to the studies conducted in accordance with the present guidance. In vitro I_{Kr} and in vivo QT assays described in sections II.C.1 and C.2 (2.3.1 and 2.3.2) when performed for regulatory submission should be conducted in compliance with good laboratory practice (GLP). Follow-up studies described in section II.C.5 (2.3.5) should be conducted in compliance with GLP to the greatest extent feasible.

In vitro and in vivo assays are complementary approaches; therefore, according to current understanding, both assay types should be conducted.

The investigational approach and evidence of risk should be individualized for the test substance, depending on its pharmacodynamic, pharmacokinetic, and safety profiles.

II. GUIDANCE (2)

A. Objectives of S7B Studies (2.1)

The objectives of studies are to: (1) identify the potential of a test substance and its metabolites to delay ventricular repolarization, and (2) relate the extent of delayed ventricular repolarization to the concentrations of a test substance and its metabolites. The study results can be used to elucidate the mechanism of action and, when considered with other information, estimate risk for delayed ventricular repolarization and QT interval prolongation in humans.

B. Considerations for Selection and Design of Studies (2.2)

Nonclinical methodologies can address the following:

- Ionic currents measured in isolated animal or human cardiac myocytes, cultured cardiac cell lines, or heterologous expression systems for cloned human ion channels
- Action potential parameters in isolated cardiac preparations or specific electrophysiology parameters indicative of action potential duration in anesthetized animals
- ECG parameters measured in conscious or anesthetized animals
- Proarrhythmic effects measured in isolated cardiac preparations or animals

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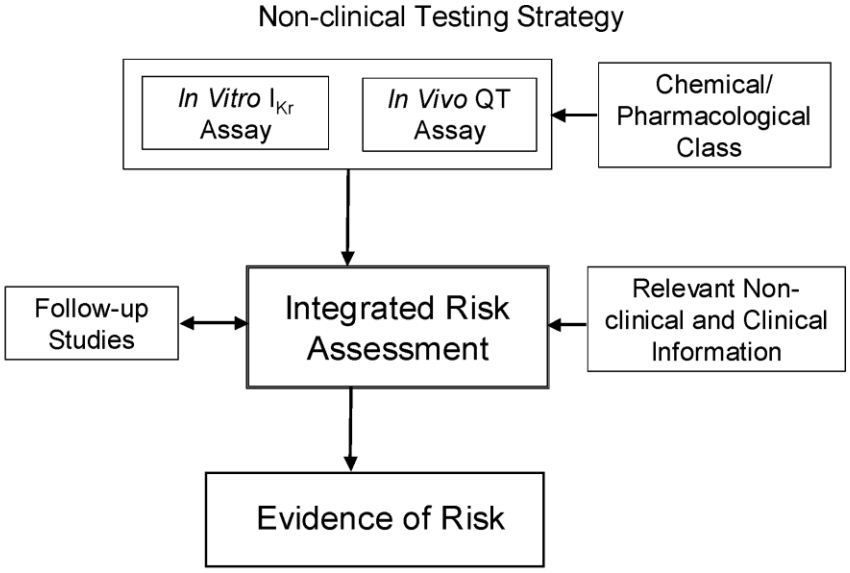
As indicated above, these four functional levels can be investigated by in vitro and/or in vivo methods. Findings from the functional levels listed above are considered useful and complementary.

In vitro electrophysiology studies can explore potential cellular mechanisms that might not be evident from in vivo data. Changes in other cardiovascular parameters or effects on multiple ion channels can complicate interpretation of data. Complementary assessments in other systems can address this issue. Although delay of repolarization can occur through modulation of several types of ion channels, inhibition of I_{Kr} is the most common mechanism responsible for pharmaceutical-induced prolongation of QT interval in humans.

In vivo models that possess the full complement of molecular, biochemical, and physiological systems can also be informative with regard to the response in humans to the test substance. Carefully designed and conducted in vivo studies allow evaluation of parent substance and metabolites and can enable estimation of safety margins. In vivo ECG evaluations provide information on conduction properties and noncardiac influences (e.g., autonomic nervous system tone). Studies of action potential parameters provide information on the integrated activity of multiple ion channels in the heart.



C. Nonclinical Testing Strategy (2.3)

The following sections describe a general nonclinical testing strategy for assessing risk for delayed ventricular repolarization and QT interval prolongation that is pragmatic and based on

currently available information. The figure illustrates the component elements of the testing strategy, but not specific test systems or their designs.

1. In Vitro I_{Kr} Assay (2.3.1)

An in vitro I_{Kr} assay evaluates the effects on the ionic current through a native or expressed I_{Kr} channel protein, such as that encoded by hERG (see section III.B (3.1.2)).

2. In Vivo QT Assay (2.3.2)

An in vivo QT assay measures indices of ventricular repolarization such as QT interval (see section III. C (3.1.3)). This assay can be designed to meet the objective of both ICH S7A (cardiovascular core battery study) and S7B. This will reduce the use of animals and other resources.

3. Chemical/Pharmacological Class (2.3.3)

Consideration should be given to whether the test substance belongs to a chemical/pharmacological class in which some members have been shown to induce QT interval prolongation in humans (e.g., antipsychotics, histamine H-1 receptor antagonists, fluoroquinolones). This factor should, where appropriate, influence the choice of reference compound(s) and be included in the integrated risk assessment.

4. Relevant Nonclinical and Clinica lnformation (2.3.4)

Additional information for the integrated risk assessment can include results from:

- Pharmacodynamic studies
- Toxicology/safety studies
- Pharmacokinetic studies, including plasma levels of parent substance and metabolites (including human data if available)
- Drug interaction studies
- Tissue distribution and accumulation studies
- Postmarketing surveillance

5. Follow-up Studies (2.3.5)

Follow-up studies are intended to provide greater depth of understanding or additional knowledge regarding the potential of test substance for delayed ventricular repolarization and QT interval prolongation in humans. Such studies can provide additional information concerning potency, mechanism of action, slope of the dose-response curve, or magnitude of the response. Follow-up studies are designed to address specific issues, and as a result, various in vivo or in vitro study designs can be applicable.

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In circumstances where results among nonclinical studies are inconsistent and/or results of clinical studies differ from those for nonclinical studies, retrospective evaluation and follow-up nonclinical studies can be used to understand the basis for the discrepancies. Results from follow-up studies can be a significant component of an integrated risk assessment.

Relevant nonclinical and clinical information along with the following should be considered in the selection and design of follow-up studies:

- Use of ventricular repolarization assays that measure action potential parameters in isolated cardiac preparations (see section III.B (3.1.2))
- Use of specific electrophysiological parameters indicative of action potential duration in anesthetized animals (see section III.C (3.1.3))
- Repeated administration of test substance
- Selection of animal species and gender(s)
- Use of metabolic inducers or inhibitors
- Use of concurrent positive control substances and reference compounds (see section III.A (3.1.1))
- Inhibition of other channels not previously evaluated
- Measurement of electrophysiological parameters at multiple time points
- Confounding effects in conscious animals that limit the interpretation of data such as test substance-induced effects on heart rate or autonomic tone, or toxicities such as tremor, convulsion, or emesis

6. Integrated Risk Assessment (2.3.6)

The integrated risk assessment is the evaluation of nonclinical study results including the results from follow-up studies and other relevant information. The integrated risk assessment should be scientifically based and individualized for the test substance. Such an assessment can contribute to the design of clinical investigations and interpretation of their results. When available, the integrated risk assessment should be included in the Investigator's Brochure and the Nonclinical Overview (ICH M4). Depending on the stage of drug development, the integrated risk assessment should also consider:

- Assay sensitivity and specificity
- Potencies of test substance in S7B assays relative to reference compound(s)
- Relationship between the exposures associated with an effect on repolarization and those eliciting the primary pharmacodynamic effect in the nonclinical test species or the proposed therapeutic effect in humans
- Contribution of metabolites to QT interval prolongation as well as metabolic differences between humans and animals

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7. Evidence of Risk (2.3.7)

Evidence of risk is the overall conclusion from the integrated risk assessment for a test substance to delay ventricular repolarization and prolong QT interval in humans.

D. Timing of S7B Nonclinical Studies and Integrated Risk Assessment in Relation to Clinical Development (2.4)

Conduct of S7B nonclinical studies assessing the risk for delayed ventricular repolarization and QT interval prolongation prior to first administration in humans should be considered. These results, as part of an integrated risk assessment, can support the planning and interpretation of subsequent clinical studies.

III. TEST SYSTEMS (3)

Considerations for Test Systems (3.1)

This section provides an overview of methodologies currently used to assess the potential for a test substance to delay ventricular repolarization and to prolong QT interval. The following should be considered in selecting the most appropriate test systems.

- Assay methodology and experimental endpoints are scientifically valid and robust.
- Assays and preparations are standardized.
- Results are reproducible.
- Endpoints/parameters of the assays are relevant for assessing human risk.

A. Use of Positive Control Substances and Reference Compounds (3.1.1)

A sub-maximally effective concentration of a positive control substance should be used to demonstrate the responsiveness of in vitro preparations for ion channel and action potential duration assays and should be included in every study. In the case of in vivo studies, positive control substances should be used to validate and define the sensitivity of the test system, but need not be included in every study.

For test substances belonging to a chemical/pharmacological class that is associated with QT interval prolongation in humans, the use of concurrent reference compound(s) (member(s) of the same class) in in vitro and in vivo studies should be considered to facilitate ranking the potency of the test substance in relation to its comparators.

B. In Vitro Electrophysiology Studies (3.1.2)

In vitro electrophysiology studies can provide valuable information concerning the effect of a test substance on action potential duration and/or cardiac ionic currents. These assays have an important role in assessing the potential for QT interval prolongation and elucidating cellular mechanisms affecting repolarization. In vitro electrophysiology studies employ either single cell

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(e.g., heterologous expression systems, disaggregated cardiomyocytes) or multicellular (e.g., Purkinje fiber, papillary muscle, trabeculae, perfused myocardium, intact heart) preparations. Heterologous expression systems, where human ion channel protein(s) are expressed in noncardiac cell lines, are used to assess the effects of a test substance on a specific ion channel. Disaggregated myocytes are technically more challenging than the expression systems but have the advantage of being suitable for assessing effects on both action potential duration and ionic currents. Although single cell preparations are more fragile, they minimize diffusional barriers to the site of action. Multicellular preparations are stable test systems to study action potential duration. The analysis of parameters for each phase of the action potential such as V_{max} for phase 0 (I_{Na}), APD_{30} or APD_{40} for phase 2 (I_{Ca}) and "triangulation" for phase 3 (I_K) can be useful to investigate the effects on specific channels responsible for these phases. In addition, some parameters derived from the Langendorff preparation have been reported to provide information regarding proarrhythmic risk.

Tissue and cell preparations for in vitro assays are obtained from different laboratory animal species including rabbit, ferret, guinea pig, dog, swine, and occasionally from humans. The ionic mechanisms of repolarization in adult rats and mice differ from larger species, including humans (the primary ion currents controlling repolarization in adult rats and mice is I_{to}); therefore, use of tissues from these species is not considered appropriate. Species differences in terms of which cardiac ion channels contribute to cardiac repolarization and to the duration of the action potential should be considered in selecting a test system. When native cardiac tissues or cells are used, the characteristics and source of the preparation should be considered because the distribution of ion channel types varies according to the region and type of cell.

Test substance concentrations for in vitro studies should span a broad range, covering and exceeding the anticipated maximal therapeutic plasma concentration. Ascending concentrations should be tested until a concentration-response curve has been characterized or physicochemical effects become concentration-limiting. Ideally, the duration of exposure should be sufficient to obtain steady-state electrophysiological effects, unless precluded by the viability of the cell or tissue preparation. The duration of exposure should be indicated. Appropriate positive control substances should be used to establish the sensitivity of the in vitro assay system.

Factors that can confound or limit the interpretation of in vitro electrophysiology studies include the following:

- The testing of high concentrations of the test substance can be precluded by limited solubility in aqueous physiological salt solutions.
- Adsorption to glass or plastic or nonspecific binding to the test matrix can reduce the concentration of the test substance in the incubation or perfusion medium.
- Test substance concentrations can be limited by cytotoxic or physicochemical attributes of the test substance that disrupt cell membrane integrity so that electrophysiological endpoints cannot be obtained.
- Cardiac cells and tissues have limited capacity for drug metabolism; therefore, in vitro studies using the parent substance do not provide information on the effects of metabolites. When in vivo nonclinical or clinical studies reveal QT interval prolongation that is not

consistent with data from in vitro studies using the parent substance, testing metabolites in the in vitro test systems should be considered.

New technologies for potassium channel assays are being developed. Novel ion channel activity assays can be useful in preliminary screening of test substances to identify lead candidates. It is important to demonstrate concordance between conventional and new technologies before adopting new technologies for regulatory purposes.

Competition binding protocols, in which test substances are studied for their ability to displace a radiolabeled hERG channel blocker from a cell line expressing hERG, are used. However, competition for radioligand-binding sites provides no information on agonistic or antagonistic effects of the test substance on I_{Kr}. Moreover, this assay will not identify test substances that bind to hERG at sites other than the radioligand binding sites. Based upon these potential limitations, this assay is not considered a substitute for voltage clamp assays described above.

C. In Vivo Electrophysiology Studies (3.1.3)

Intact animal models allow investigation of ventricular repolarization or associated arrhythmias where integrated effects on the full complement of ion channel and cell types are assessed. Also, potential neuronal and hormonal influences on the pharmacodynamic effect of the pharmaceuticals are present in animals.

The QT interval of the ECG is the most commonly used endpoint to gauge effects of a test substance on ventricular repolarization. In specialized electrophysiology studies, information regarding the ventricular repolarization (e.g., monophasic action potential duration and effective refractory period) can also be obtained from in vivo models. Additional safety parameters of interest, including blood pressure, heart rate, PR interval, QRS duration, and arrhythmias, can be assessed simultaneously.

The QT interval and heart rate have an inverse, nonlinear relationship, which varies among species and between animals within a species. Thus, a change in heart rate exerts an effect on QT interval, which can confound the assessment of the effect of the test substance on ventricular repolarization and the QT interval. There are two important situations where there is variability in heart rate among animals: one is due to difference in autonomic tone, and the other is due to effects of test substances on heart rate. Therefore, the interpretation of data from in vivo test systems should take into account the effect of coincident changes in heart rate. Ideally, QT interval data obtained after administration of a test substance should be compared with control and baseline data at similar heart rates. When the heart rate variability is not due to the test substance, it can be reduced by acclimatization, or the use of anesthetized animal models. When the effects are due to a test substance, the most common approach is to correct the QT interval for heart rate (QTc) using formulae such as Bazett or Fridericia. The choice of heart rate correction formula should be justified with data from the test system. When differences in heart rate between treatment and control are large, the correction formulae may not be effective for assessing risk of QT interval prolongation. An alternative approach is to maintain a constant heart rate using cardiac pacing. An analysis of QT/RR relationship, including correction of the QT interval using formulae for individual animals, may be more appropriate.

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Laboratory animal species used for in vivo electrophysiology studies include dog, monkey, swine, rabbit, ferret, and guinea pig. The ionic mechanisms of repolarization in adult rats and mice differ from larger species, including humans (the primary ion currents controlling repolarization in adult rats and mice is I_{to}); therefore, use of these species is not considered appropriate. The most appropriate in vivo test systems and species should be selected and justified.

The dose range should be in accord with that discussed in ICH S7A and, whenever feasible, should include and exceed the anticipated human exposure. The dose range can be limited by animal intolerance to the test substance (e.g., emesis, tremor, or hyperactivity). For studies designed to relate the extent of delayed ventricular repolarization to concentrations of the parent test substance and its metabolites, controlled exposure via constant intravenous infusion can be used. Monitoring exposure to the test substance and metabolites (see ICH S3A) provides opportunities to interpret dose- and concentration-response data and to design follow-up studies, if appropriate.

Factors that should be considered in conducting studies and interpreting the results include the following:

- Data acquisition and analysis methods
- Sensitivity and reproducibility of the test systems
- Dosing period and measurement points
- Heart rate and other effects that confound interpretation of QT interval data
- Inter-species and gender differences (e.g., cardiac electrophysiology, hemodynamics, or metabolism of pharmaceuticals)
- Pharmaceuticals that have effects on several ion channels, yielding complex dose-response relationships that could be difficult to interpret

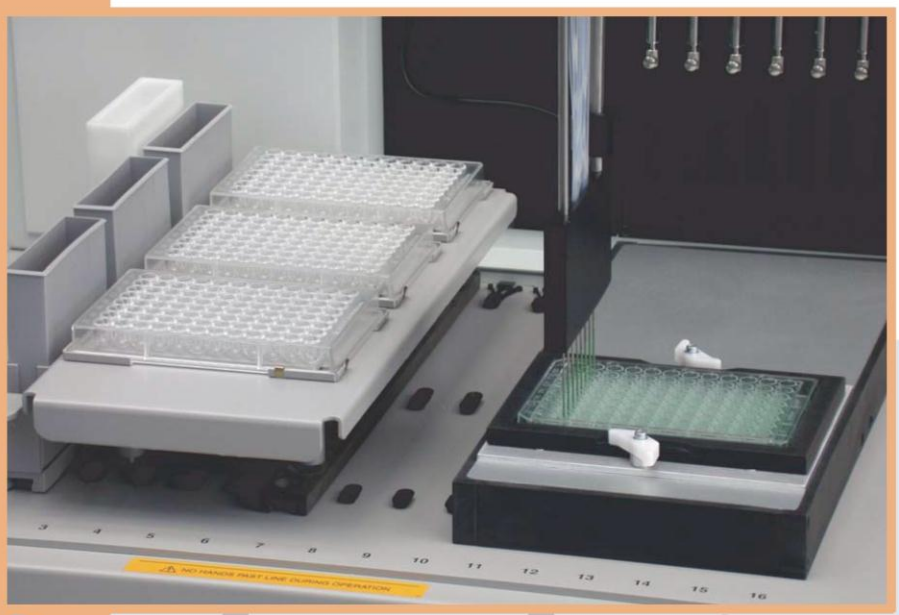
D. Simulated Pathological Conditions and Arrhythmias (3.1.4)

The precise relationship between test substance-induced delay of ventricular repolarization and risk of proarrhythmia is not known. Directly assessing the proarrhythmic risk of pharmaceuticals that prolong the QT interval would be a logical undertaking. Indices of proarrhythmic activity (e.g., electrical instability, temporal and/or spatial dispersion of refractoriness, reverse use-dependency, changes in action potential configuration) and animal models might have utility in assessing proarrhythmia. Interested parties are encouraged to develop these models and test their usefulness in predicting risk in humans.

multichannel systems

QT-Screen*

Automated Cardiac Electrophysiology
for
Drug Profiling and Safety Screening



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Advantages

* Automated detection of drug-induced delayed ventricular repolarization (QT-prolongation):

  - assay based on primary cardiac myocytes
  - parallel recording on 96 channels
  - high throughput
  - 21 CFR Part 11 software compliance

* QT-Screen supervises your experiment:

  - online signal quality check
  - automated analysis
  - compound saving strategy
  - automated generation of concentration series

* Direct data export to database:

  - QT-interval duration
  - multiparameter analysis
  - sample traces
  - arrhythmogenic activity
  - concentration-response curve
  - environmental parameters

* Throughput:

hands-on time per run	5 min
time per run	45 min
compounds per day	100
data points per day	up to 8.000
cost per datapoint	0,16 USD

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Signals

Field potentials are measured by extracellular microelectrodes.
This allows the recording of the composite cardiac action potential, because all components of the action potential are reflected in the field potential.
The interpretation is very similar to the electrocardiogram.
Whereas the QRS complex in the ECG reflects ventricular depolarization, the opening of sodium channels in ventricular cardiomyocytes is revealed as a rapid upstroke.
The repolarization is visible as a "T-Wave" in both cases.

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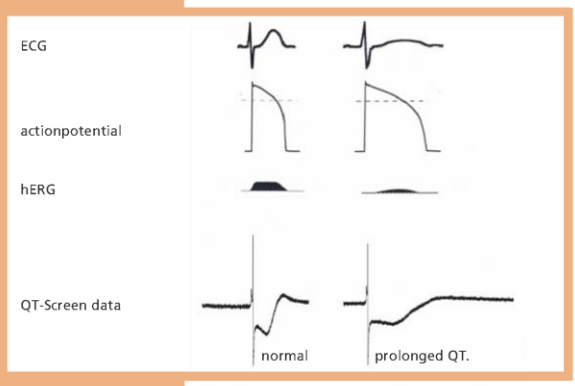
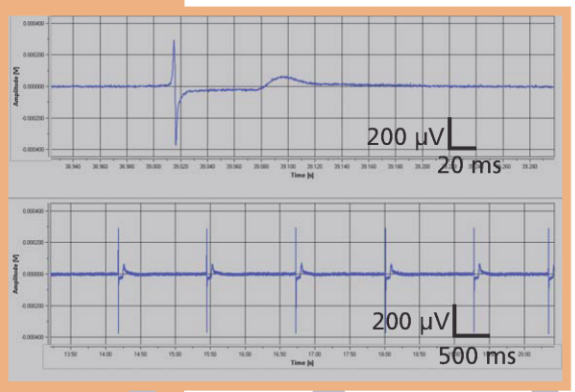


In the figure, data recorded from ECG, intracellular recording of action potential, and voltage-clamp assay of HERG channels, under control conditions and conditions inducing prolonged QT, are aligned with data recorded from QT-Screen under similar conditions.



In the upper panel the signal display window of the QT-Screen software shows the shape of a single cardiac action potential (recorded as field potential).
In the lower panel the same channel is displayed on a compressed timescale.
Each recorded channel can be displayed in this window with a simple mouseclick. The scaling of both axes can be adjusted.
The signals displayed show the typical time course of ECG recordings :
rapid depolarisation, sustained plateau and a repolarisation wave.

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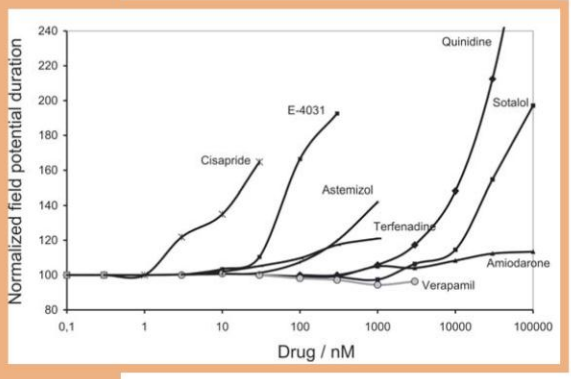
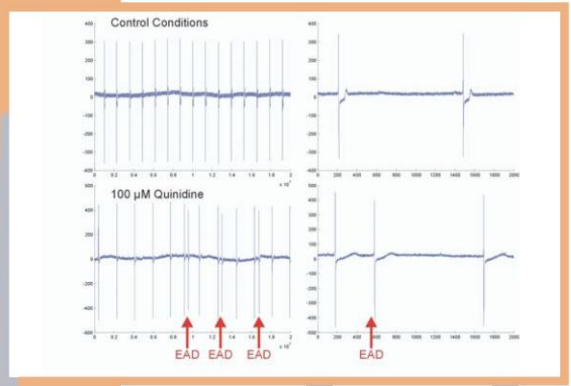
Results

A variety of drugs known to prolong the QT interval in the ECG, along with appropriate negative controls, have been tested with the QT-Screen.
The results are in agreement with results obtained by classical repolarization assays or patch-clamp studies. All known positives also yield positive results in the QT-Screen; all negative controls yield negative results. Tested drugs include:

- Cisapride
- E-4031
- Sotalol
- Quinidine
- Astemizol
- Terfenadine
- Amiodarone
- Verapamil



For all drugs tested, the effects are in agreement with the published data recorded with various standard assay systems.
Verapamil is particularly interesting, because it yields a false positive in assays based on hERG-transfected cells.
Because the QT-Screen is based on cardiac myocytes, Verapamil yields no effect.



Unlike assays based on transfected cells, the QT-Screen, employing cardiac myocytes, directly measures the arrhythmogenic activity of tested compounds.
In the lower figure, early afterdepolarization evoked action potentials appear as a result of the Quinidine-induced AP prolongation.
The upper panel displays similar recordings under control conditions; the right panels display the data on an expanded time scale.

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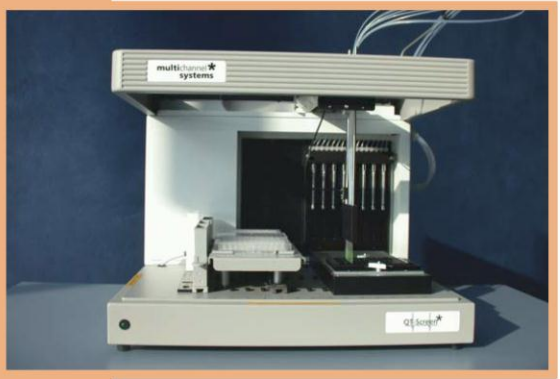
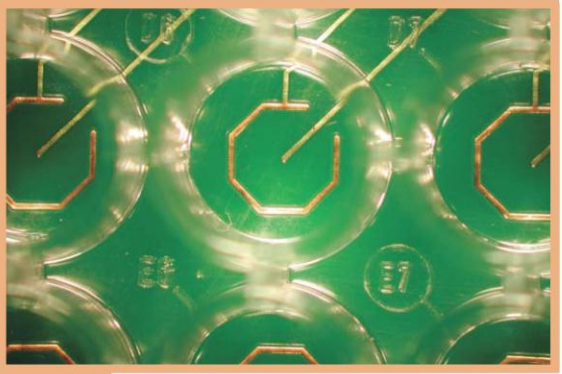
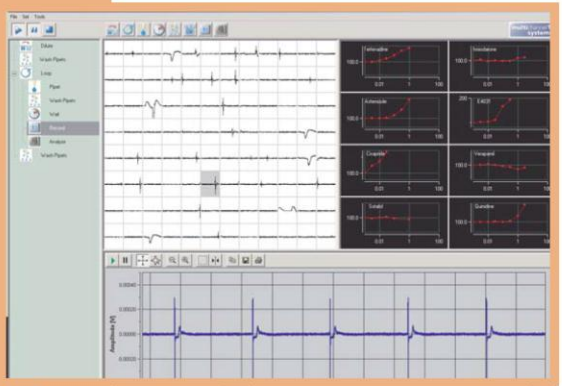
Technology



QT-Screen is the world's first fully automated system for high-throughput QT prolongation screening and combines the advantages of superior extracellular recording and reliable data acquisition technology with standardized-liquid handler technology.
Concentration series are generated automatically from 96 well compound plates. Starting with the lowest concentration, 8 to 10 concentrations of 8 different compounds are tested in parallel and in replicates of 12.



Cardiac myocytes are easily plated directly into disposable 96 well QT-plates. After 2-3 days in culture, the cell cultures begin to beat spontaneously.
Each well includes a recording, reference, and stimulating electrode. All materials are tested for excellent biocompatibility. The low cost of consumables insures low operating costs.



With the user-friendly, flexible graphical user interface, designing recording sequences, and implementing custom quality checks and compound saving strategies are easy. Fluid delivery, real-time monitoring, and online dose response analysis are all computer-controlled.
The wide range of analytical tools available include algorithms for calculating corrected QT intervals and identifying tachycardia and bradycardia. Results are entered automatically into a standard database.

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Appendix C

- PDS 1
- PDS 2
- PDS 3

Product Design Specifications

British standard BS 7373-3:2005 outlines the primary elements of a Product Design Specification (PDS); these elements have been considered in the context of this project and given a high, low or medium priority according to this work.

PDS1

The following points can be described as the "gold standard" for this project area.

Product Design Specification for MEA Systems (in accordance with BS 7373-3:2005).

1st June 2009

O.M.Flaherty

a. Aesthetics.

Priority: LOW

Comments: are of relatively low importance to this product.

b. Company constraints.

Priority: HIGH

Comments: the development of this novel system is to be completed by three full time researchers utilising limited facilities and funding.

c. Competitors.

Priority: HIGH

Comments: this is an area of industry that is becoming highly competitive. There is a lot of interest globally in how this technology can be developed and adapted (MEA Meetings 2008 and 2010).

d. Cost of product.

Priority: HIGH

Comments: this technology is relatively high-cost in terms of both purchasing and setting it up and also in its employment.

e. Customer.

Priority: HIGH

Comments: this system is aimed at researchers across a number of biological disciplines as well as towards the pharmacology industry. Ability of designs to meet their needs will dictate success of this work.

f. Disposal.

Priority: MEDIUM

Comments: There are currently no specific regulations governing the disposal of MEA systems. The disposal of biochips and systems is likely to require similar processing facilities to electrical products regulated by the European Waste Electrical and Electronic Equipment directive (WEEE). Reclaimability of materials to some degree would be highly desirable.

g. Documentation.

Priority: MEDIUM

Comments: the production of appropriate documentation or manuals to for users will be required and should be developed alongside the technology at the appropriate time.

h. Environment.

Priority: MEDIUM

Comments: the environment in which the system is to be used will be defined in literature. Environmental issues such as power consumption and recycling will be considered further into the project once prototyping design in underway.

i. Ergonomics.

Priority: LOW

Comments: modular design will be utilised to make the novel system superior to present systems from an ergonomic perspective. Current systems are not portable and elements that humans interact with are relatively lightweight so this aspect is not a pressing priority at this time.

j. Installation.

Priority: MEDIUM

Comments: it is not intended that specialist installation will be required as is the case for current commercially available systems.

k. Legal.

Priority: LOW

Comments: systems used in drug development will need to be robust and incorporate appropriate quality control to ensure legal aspects are fully are addressed. Regulators will specify details as required.

l. Life (shelf/storage).

Priority: MEDIUM

Comments: products will initially be made to order according to demand.

***m. Life (service*).**

Priority: HIGH

Comments: systems used for chronic studies will be used for months at a time. Systems must be robust enough not to fail during use. Biochip parts must be re-usable at least 10 times, as is the case for commercial equivalents.

n. Life span of product.

Priority: HIGH

Comments: System parts must be comparable or better in this element than the equivalent part in commercial systems. Biochips = 10 uses, Full system = 5-15 years.

o. Maintenance.

Priority: HIGH

Comments: the novel system should require a lesser degree of maintenance than present systems. Time savings potentially brought about by design changes must not be cancelled out by a need for increased maintenance.

p. Manufacturing facility.

Priority: HIGH

Comments: manufacturing capabilities at Loughborough University will be exploited. If specialist facilities are required appropriate collaborations will be pursed.

q. Market constraints.

Priority: LOW

Comments: this product is intended to compete globally with a number of different variants across a number of disciplines.

r. Materials.

Priority: HIGH

Comments: novel materials may be experimented with. All materials used for biochip preparation will be bio inert.

s. Packing.

Priority: LOW

Comments: will be investigated for each component. Must be able to be sterilised and ideally suited to an incubator environment. To be addressed once the initial core components have been designed, prototyped and validated.

t. Patents.

Priority: MEDIUM

Comments: have been investigated and will be kept up-to-date with regular searches throughout the duration of the project so as to ensure Loughborough University's designs do not breach other existing patents.

u. Performance.

Priority: HIGH

Comments: all system parts must function robustly. Modes of failure must should be considered during design.

v. Politics.

Priority: LOW

Comments: are of low importance to this project and product area at this time.

w. Processes.

Priority: HIGH

Comments: have been identified from the user perspective that must not be lengthened but can be shortened. Manufacturing processes will be addressed in more detail in future work according to design decisions.

x. Quality and reliability.

Priority: HIGH

Comments: to avoid overall product failure quality and reliability must be proven to be equivalent or superior to commercial equivalents at the time of launch.

y. Quantity.

Priority: MEDIUM

Comments: will be predicted in future work.

z. Safety.

Priority: HIGH

Comments: will be of high importance throughout manufacture and prototype testing. The final product must be safe to use and if it fails, it must fail in a safe way. Use must pose zero risk to the user.

aa. Shipping.

Priority: LOW

Comments: will be considered at the end of the project

bb. Size.

Priority: MEDIUM

Comments: systems will be designed to be as small and compact as realistically possible.

cc. Standards Specifications.

Priority: MEDIUM

Comments: shall be investigated in more detail in future work to ensure parts and the final system meet any standards identified at the time.

dd. Testing.

Priority: HIGH

Comments: shall be of major importance and will be conducted throughout development.

ee. Time scales.

Priority: HIGH

Comments: this project is funded for a two years during which time results must be generated.

ff. Weight.

Priority: MEDIUM

Comments: will not be a major consideration in design.

The following points are in addition to the standard PDS points listed above. The following factors are design elements incorporated into the PDS as needs identified of users generically that must be addressed by designers in this industry as a whole.

MEA technology specific needs identified by users in literature and meetings (broad MEA application):

gg. **Insulation for cell-to-substrate adhesion.**

Priority: MEDIUM

Comments: Find a highly hydrophilic insulator that is biocompatible and more economical (either in labour or cost) than laminin, Matrigel™, poly-d-lysine, etc.

hh. **Opportunity to anchor tissue slices.**

Priority: MEDIUM

Comments: slices need to be securely but gently secured in place.

ii. **Higher density of electrodes.**

Priority: HIGH

Comments: create opportunity to record from larger areas or to a greater precision without compromising the intensity of stimulus that you can pass between the electrodes.

jj. **One contained system.**

Priority: MEDIUM

Comments: ideally all testing operations and protocols executed via one interface located within full view or as a part of the hardware set-up.

kk. **Easy to sterilise.**

Priority: HIGH

Comments: must withstand current good practice procedures for sterisation.

ll. **Scalable.**

Priority: HIGH

Comments: current trends in this industry is for more electrodes and thus data to be required. Suggestions that this technology should be used on larger scales would be complemented by automation of elements.

mm. **Frequency.**

Priority: HIGH

Comments: maintain or increase current facility to record at rates between 2kHz and 50kHz. The ability to change frequency of from 0 – a 1000's Hz is required. It must be easy for the user to do this.

nn. **Analysis features enhancement.**

Priority: HIGH

Comments: A new system will be accompanied by an acquisition software that must match or be better than current equivalents. Post processing should be designed out by writing enhanced capability into a single acquisition and analysis software.

oo. **Archiving and data accumulation.**

Priority: MEDIUM

Comments: ability to automatically analyse hundreds or thousands of trail files irrelevant of time of collection quickly is required to make sense of the data being captured.

pp. **Recording limitations.**

Priority: MEDIUM

Comments: users do not currently record all of the data but future systems ideally should (Kopanitsa et al, 2006). The ability to record from every electrode every time without having to be overly concerned about consumption of disk space.

qq. **Constant evaluation.**

Priority: MEDIUM

Comments: allow recording and simultaneous analysis 24 hours a day, 7 days a week.

rr. **Automatic logging.**

Priority: LOW

Comments: need improved ways to log which exact pieces of equipment have been used in the creation of each file. Incorporating where and when files were recorded and by whom.

ss. **Non-invasive.**

Priority: HIGH

Comments: must be non-invasive to cells.

tt. **Interface.**

Priority: HIGH

Comments: user-friendly interface is essential to guarantee scientist acceptance and uptake of the system. Simple “grab and drop” controls are ideal. Interface MUST be simple as users do not want to spend much time learning how to use new software. Must be quick to learn. Users would like option boxes that they simply check, click “go” and it gives requested values. It will be suitable if this feature has to be carried out offline as currently it does not exist.

uu. **Perfusion.**

Priority: MEDIUM

Comments: allow integrated control of perfusions systems.

vv. **Auto-thresholding.**

Priority: HIGH

Comments: shifting of preset functions in a rack. Many windows in MC_Rack must currently be done one by one, by hand. Automatic threshold setting feature where the system sets thresholds according the data coming in.

ww. **Wave elements.**

Priority: HIGH

Comments: New systems need to incorporate more functions that will measure actual wave elements automatically. Currently much measurement is done by hand after data has been collected.

xx. **Simplify employment.**

Priority: HIGH

Comments: improved methods for seeding of cells. I.e. potentially eradicate the need for coating. MEA with incorporated recession to aid in placement of cells.

yy. **Data flexibility.**

Priority: MEDIUM

Comments: The facility to add markers/flags to the data set online and offline.

zz. **Quality control**

Priority: HIGH

Comments: components that reach users must perform consistently.

PDS 2

July 2009

The following points are additional to the points discussed in PDS1. These requirements have been specifically emphasised by users at The Wellcome Trust's Sanger Institute and Nottingham University's Centre for Biomolecular Sciences during early group meetings.

- System must sample at 10-25kHz.
- Hardware must be grounded.
- Hardware biosensor surfaces inside the media well must be made from biocompatible material(s).
- All hardware parts must be contained in a casing that can be sterilised or must themselves be suited to sterilisation.

  Comments: If the entire system is to go in an incubator the electronics must be sealed in suitable casing for sterilisation as incubators are sterile environments.

- Input capabilities must be limited in hardware.

  Comments: to protect/prevent damage to cells or tissue.

- Hardware headstage unit and amplifiers must have appropriate pre-processing and amplification to allow for spike identification.
- System must allow cell environment to be kept constant.

  Comments: I.e. temperature, humidity, etc.

- System must allow appropriate human access

  Comments: for culture maintenance and perfusions systems.

- System must incorporate a visual inspection method.
- New software must allow thresholds to be configured to data at the time of recording.
- Software must incorporate adjustable and ideally automatic pre-processing.
- Software must allow for appropriate post-processing facilities if not incorporated in.
- Software must be simple for users to learn.
- Software must incorporate a user-friendly graphical user interface.

PDS3

This product design specification lays down exactly what Loughborough University's team have been working on, incorporating specific points from a document developed by Nottingham University users (cardiomyocyte applications) with global and local points that are of high importance to system success.

Nottingham University users have specified needs for the biochip to be re-designed in accordance with their specific application needs. They have also specified the direction in which software changes should be made in order to support their analysis requirements more effectively than current protocols. It is the needs of Nottingham Users that this project will primarily focus time and resource on.

Biosensor PDS:

- **Modify well maintaining commercial interface configuration.**

  Comments: that will interface with the current MCS MEA60 System in their lab.
- **Alter well dimensions to be close to those of a 35mm culture dish.**

  Comments: internal dimensions 35mm diameter, 10mm height, culture area 8.8cm^2, media volume 3ml.
- **Incorporate a light-transmissible lid.**

  Comments: to prevent evaporation.
- **Interior of well must allow for potential etching or patterning.**
- **Re-usable.**

  Comments: therefore must withstand sterilisation using UV light and 70% ethanol.
- **16 micro-wells.**

  Comments: in any pattern.
- **Microwells as far apart as possible.**

  Comments: at least 5mm in from the edge of the dish.
- **Microwells 500µm diameter,**
- **with a maximal depth of 250µm,**
- **and a slightly curved shape.**

Comments: beating clusters at time of seeding vary between 200-500µm in diameter.

- **Electrode in centre of well flush to the surface.**
- **Microwells as large a surface area as possible.**
- **Made from biocompatible material(s).**

Software PDS:

- **Intuitive, simple-to-use software.**
- **Output results in real-time**

  Comments: while maintaining the current feature of optional post-processing if desired.
- **Features to allow patch clamp files to be analysed in the same software.**

  Comments: WinEDR software format suggested.
- **Facility to average all traces for a given treatment.**
- **Facility to compare averaged traces.**
- **Increased detail at individual waveform level.**

  Comments: **QT-period** identification and comparison emphasised as most important feature for automated extraction. Other waveform elements of interest detailed in the MES Specification document by NU below.
- **Adjustable, automatic pre-processing.**
- **Automatic thresholding** at the time of recording.

System:

- **Sample rates of at least 2-25kHz.**
- **Grounded.**
- **Parts that can be sterilised**

  Comments: If the entire system is to go in an incubator the electronics must be sealed in suitable casing for sterilisation as incubators are sterile environments.
- **Input capabilities limited appropriately.**

Comments: to protect/prevent damage to cells or tissue.

- **Appropriate pre-processing and amplification.**
- **Facilitate/support a constant cell environment.**

  Comments: I.e. temperature, humidity, etc.

- **Allow easy access**

  Comments: for users to perform culture maintenance and run perfusions systems.

- **Support visual inspection of the cells.**
- **Simple to learn.**
- **User-friendly interfaces.**

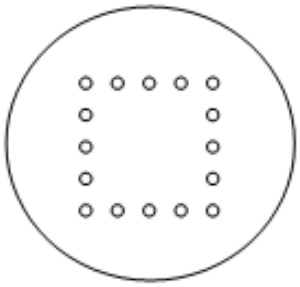
The MEA Specification Document Drafted for LU by Nottingham University Users.

MEA Specification Document

Top Part

- Initially, what would be most advantageous to us would be a modified well system, into which we could seed our beating clusters, which could attach to our current MEA hardware with the detailed specifications
- Housing of wells will ideally be similar to dimensions of a 35mm dish to allow required volume of culture medium (internal dimensions 35mm diameter, 10mm height, culture area 8.8cm², media volume 3ml), should have a lid to prevent evaporation of media, and should be made of a light transmissible material that can be etched/patterned to aid cell attachment

- Housing should be re-usable, and UV and 70% ethanol treatable for sterilisation
- 16 wells in any pattern (4x4 or square is ok if its easier)
- Wells should be as far apart as possible, but at least 5mm in from the edge of the dish

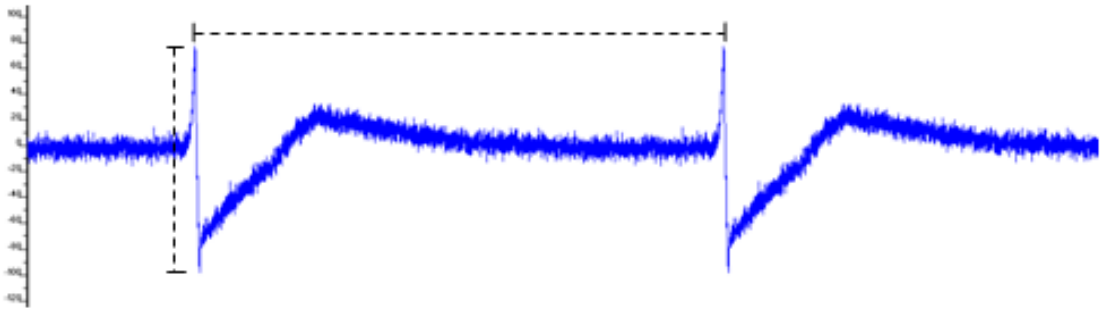


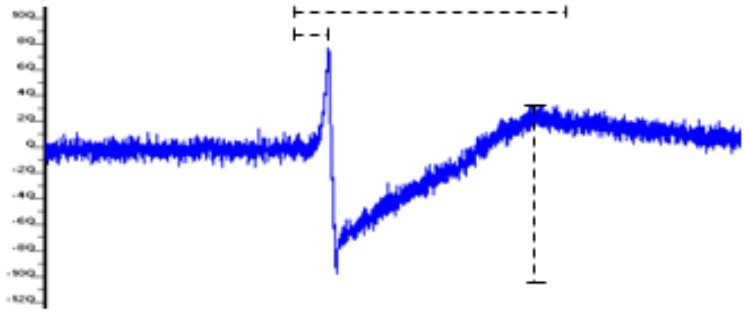
- Dissected beating clusters vary in size from 200-500 µm in diameter so well shape should be slightly curved with a diameter of 500 µm and a maximal depth of 250 µm

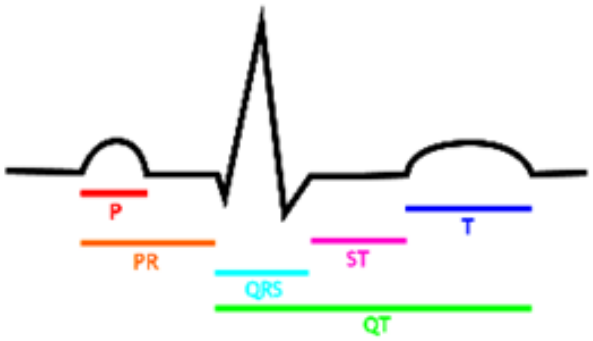
- Electrode should be central in the well, should be flush to the surface of the well (i.e. not protruding) and should cover as large a surface area as possible

Software

- software should be intuitive and easy to use
- outputs should be provided in real time, as well as be available for offline analysis, and compatible with Excel or Prism
- software should be able to analyse patch clamp data files in the WinEDR format
- ideally average between traces produced from same treatments and compare between traces produced from different treatments
- waveform analysis required...





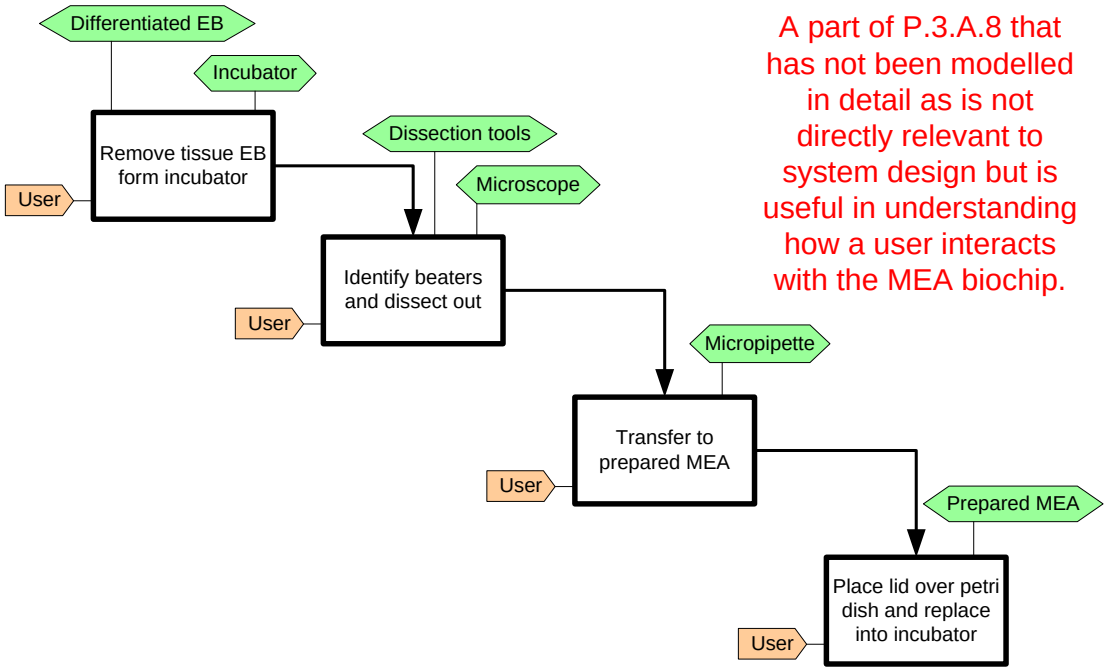


Appendix D

- Case Study Timing Data
- Standard Operating Proceedure Examples
- QT-interval Analysis Output Example

Case study timing data

In some cases variation of certain user dependant protocols resulted in timing data that is represented as a range (i.e. ~1-3mins). Timing information is represented in this manner where skilled dependencies exist in MEA system application. An example of such a situation is demonstrated in the figure below (D.1).



D.1: An example of an activity where timing information was skilled user dependant.

The above figure accounts for the variable time given by UoN users for P.3.A.8 in Chapter 4, Figure 4.6. This phase of the protocol differs depending upon the naturally formed geometry of the newly differentiated EB, and also depending upon the skill level of the individual scientist that is performing the dissecting task.

Example University of Nottingham SOP Documents

SOP for Multielectrode array (MEA) use and pharmacological testing on hESC cardiomyocytes

Preparing the MEA

1) Prepare beating EBs by forced aggregation. Use beating EBs of ideally >20 days of differentiation.
    a. Use suitable cardiac selected MEFs for BGK-conditioned medium
    b. Use suitable cardiac selected serum for Diff Medium (= 78% DMEM, 20% cardiac serum, 1% NEAA, 1% GlutaMax, 10uM β-ME
2) Prepare MEA (**all in class II hood**)
    a. If MEA is being reused, treat with 0.25% trypsin for at least 16 hrs
    b. Wash with PBS x 2
    c. Place in 70% ethanol for 5 mins
    d. Allow to dry for ~10' in a fresh Petri dish
    e. UV treat for 30'
    f. Matrigel treat MEA
        i. use 1ml of Matrigel and allow to polymerise for 45' in the hood.
    g. Aspirate and add 1ml PBS
    h. Aspirate and add 1ml of Diff Med
    i. Equiliberate in incubator for > 2hrs
3) Position up to 4 beating areas on the electrodes of the MEA under the stereo scope. Ensure they are sufficiently spaced so not touching each other.

CARE NOT TO DAMAGE ELECTRODES WITH PIPETTE OR STEM CELL KNIFE (MEAs cost ~£300 each!!!)

4) Allow to attach for ~2hrs on the heated stage in the hood.
5) Check attachment and move (very gently) to incubator to fully attach overnight
6) To pre-equiliberate Diff Med, add sufficient volumes to T75 flask(s) and place in incubator overnight (MEA recordings next day)

N.B. Although not common practice for normal culture, add pen / strep to Diff Med as current MEA recording conditions are non-sterile

MEA setup

7) Ensure sufficient Diff Med to T75 flask(s) has been pre-equiliberated (see above)
8) Start computer
9) Switch on heated stage
10) Open [MEA select] on desktop
    a. Click [change MEA]
        i. This reduces electrical interference to subsequent recordings
    b. Unclip housing and lift top plate
    c. Insert MEA
        i. reference strip = black strip to right hand side = electrode 15
    d. Replace top plate
    e. Fasten clips
    f. Cover with Petri dish
    g. Unclick [change MEA]
11) Open [MC_Rack] on desktop
    a. For a previous used recording rack open D:/Profile Examples/Example of recording rack. If this rack is used, jump to click [play] several steps below
    b. If setting up from scratch, proceed to next step
12) Establish subfolder system as follows
    a. Recorder
        i. MC_Card
            1. Data display (rename to 'raw data')
13) Set parameters in subfolders and associated tabs as follows
    a. Recorder tabs
        i. Rack – no action
        ii. Channels – tick only Electrode raw data
        iii. Recorder
            1. specify file name for each new recording
            2. Set file size to 3 min and tick auto Stop
        iv. Window – highlight Continuous
    b. MC_Card tabs
        i. Rack – no action
        ii. Hardware
            1. Input voltage range -819 to +819mV
            2. Sampling Freq 10000Hz
        iii. Info – no action
    c. Display (raw data) tabs
        i. Rack – no action
        ii. Layout – open inverted 8x8 file
        iii. Data
            1. check only electrode raw data
            2. Plot type – trace
        iv. Window – highlight continuous
14) Click [play] to check all working OK.
    a. N.B. some parameter adjustments CANNOT be changed when system is playing or recording

MEA data capture

15) With a plastic Pasteur pipette, aspirate medium and discard in to pot containing trigene
    a. ***take care not to damage beaters or MEA***
16) Add 900 ul pre-equiliberated Diff Med
17) Allow cells to stabilise for 5 min
18) Click [recorder]
    a. Click recorder tab
    b. Click [browse]
    c. Assign file name
19) Add 100ul of 10x stock drug (e.g. isoprenaline) and start recording immediately by clicking [record] and then [play].
    a. This will record for 3min and then auto stop.
20) With one plastic Pasteur pipette, aspirate medium and discard in to pot containing trigene
21) Using a second plastic Pasteur pipette, wash with ~1ml Diff Med
    a. Using a second pipette only for washing should reduce chance of drug carry over between treatments
22) Add 900 ul pre-equiliberated Diff Med
23) Go to step highlighted in red above

Drug concentrations (Make all drug dilutions on day of use)

Agent	Company	Code	Quantity	Price
Isoprenaline (Isoproterenol)	Tocris	1747	100mg	£35

Weight out xxx and dilute into YYY of ZZZ (water??)

Make 10x stocks at	10^{-8}M (10nM)	10^{-7}M (100nM)	10^{-6}M (1000nM)	10^{-5}M (10000nM)
Using 100ul into 900ul Diff Med gives working conc of	10^{-9}M (1nM)	10^{-8}M (10nM)	10^{-7}M (100nM)	10^{-6}M (1000nM)

Propranolol	Sigma	P0884	1g	£12

Weight out xxx and dilute into YYY of ZZZ (water??)

Make 10x stocks at	10^{-6}M (1000nM)
Using 100ul into 900ul Diff Med gives working conc of	10^{-7}M (100nM)

Finishing

24) Ensure locations of different beaters on the MEA are recorded in you lab book in a diagrammatic form for future reference
25) Photograph beaters on MEA
    a. if necessary also take video recordings using digital camera on tripod (see Chris for details)
26) Transfer each beater from MEA to eppendorf tube
    a. Alternative 1, place beater in MatTek dish for immunos
    b. Alternative 2, disaggregate and seed to MatTek dish for immunos
27) Snap freeze in Liq N.
28) Store at -80^{o}C
29) Wash MEA twice with PBS
30) Add 1ml of 0.25% trypsin and incubate for at least 16hrs at 37oC. This:
    a. releases any cells still attached to the MEA
    b. digests the Matrigel matrix.
31) MEA can be reused following the instructions in 'Preparing the MEA' above

Play back and analysis (Off-line analysis)

32) Open [MC_Rack] on desktop
    a. For a previous used analysis rack open D:/Profile Examples/Example of analysis rack. If this rack is used, jump to Replay File several steps below
    b. If setting up from scratch, proceed to next step
33) Establish subfolder system as follows
    a. Replayer
        i. Spike sorter
            1. Analyser (set name to Beat rate)
                a. Parameter Display (set name to beats)
            2. Analyser (set name to Amplitude)
                a. Parameter Display (set name to Amp)
            3. Analyser (set name to Minimum Amp)
                a. Parameter Display (set name to Min Amp)
34) Set details of each folder as follows
    a. Recorder
        i. This tab does not need to be adjusted
    b. Replayer
        i. Rack – no action
        ii. Replayer
            1. Adjust Replay Speed to ensure spike are being analysed properly in the display windows
            2. Each file should be 3 min long. Set the start time to 60s
            3. Use Start / Pause button
        iii. Replay File – use to open file to be analysed
        iv. File info – no action
        v. Buffer info – no action
    c. Spike sorter
        i. Rack – no action
        ii. Settings
            1. Pre trigger = 20ms
            2. Post trigger = 100ms
            3. Dead time = 100ms
        iii. Channels – check Electrode Raw Data
        iv. Detection – Threshold channel 12; -119.9uV negative slope; Std Dev -3
        v. Sorting – no spike sorting
        vi. Layout – Use to define electrode that will appear in the display windows
    d. Beat rate
        i. Rack – no action
        ii. Channels – check only spikes
        iii. Analyser
            1. Window means (No. of windows) = 1
            2. Check only Number
        iv. ROI – continuous 2 min bins
    e. Beats
        i. Rack – no action
        ii. Layout – use to define electrodes to be analysed
        iii. Data
            1. check Parameter 1 and Number

2. Highlight Number

iv. Colour – no action

v. Ranges

1. Set X and Y axis to min = 0; max = 100

f. Amplitude

i. Rack – no action

ii. Channels – check only Electrode Raw Data

iii. Analyser

1. Window means (No. of windows) = 1
2. Check only Peak-Peak Ampl.

iv. ROI – continuous 1 min bins

g. Amp

i. Rack – no action

ii. Layout – use to define electrodes that will appear in the display windows

iii. Data

1. check Parameter 2 and Peak-Peak Ampl
2. Highlight Number

iv. Colour – no action

v. Ranges

1. Set X axis to min = 0; max = 100s
2. Set Y axis to min = -800; max = 800uV

h. Minimum Amp

i. Rack – no action

ii. Channels – check only Electrode Raw Data

iii. Analyser

1. Window means (No. of windows) = 1
2. Check only Minimum

iv. ROI – continuous 1 min bins

i. Min Amp

i. Rack – no action

ii. Layout – use to define electrodes that will appear in the display windows

iii. Data

1. check Parameter 3 and Minimum
2. Highlight Number

iv. Colour – no action

v. Ranges

1. Set X axis to min = 0; max = 100s
2. Set Y axis to min = -800; max = 800uV

35) Open file for play back from replayer > Replay File tab (browse)

36) Go to Replayer > Replayer tab and set start time at 60s

37) Click start

a. Ensure thresholds on spike sorter are detecting spikes correctly

b. Move thresholds by drag & drop

38) Record data (Can pause run if time is needed to record data)

a. One reading / electrode will be produced for **beats** and represents beats in 120s

b. Two readings / electrode will be produced for **Amp** (record both) and represent bin 1 = 60-120s and bin 2 = 120-180s

c. Two readings / electrode will be produced for **Minimum Amp** (record both) and represent bin 1 = 60-120s and bin 2 = 120-180s

MATRIGEL ALIQUOTS AND COATING

PREPARATION OF MATRIGEL

Available from BD Biosciences, Growth Factor Reduced (GFR) Matrigel™ Matrix is a solubulized basement membrane preparation extracted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in ECM proteins (Greenlee et al., 2004; see www.bdbeurope.com). Its major component is laminin, followed by collagen IV, heparan sulfate proteoglycans, and entactin. Matrigel is frozen at -20°C, liquid at 0°C and polymerises at room temperature to produce biologically active matrix material. This resembles the mammalian cellular basement membrane and can provide a physiologically relevant environment for studies of cell morphology, biochemical function, migration or invasion, and gene expression (Greenlee et al., 2004). It is also an effective substrate for the culture of hESCs in feeder-free conditions.

MATRIGEL ALIQUOTS

1. Matrigel will arrive in a 10ml pot frozen on dry ice. Transfer immediately to -80°C until ready to make aliquots as described below.
2. To aliquot, place Matrigel pot in a large beaker of ice overnight in the fridge. The beaker must still contain ice by the following morning otherwise the Matrigel will start to polymerise. Also place a 5 ml pipette, 20 x 0.5 ml eppendorf tubes and an eppendorf tube rack at -20°C

   Note: *All plastics must be thoroughly chilled and all procedures must be carried out swiftly, otherwise polymerization will occur while aliquotting Matrigel.*

3. Using the chilled pipette and tubes, prepare 0.5ml aliquots of Matrigel and store immediately at -80°C

   Note: *Matrigel can also be stored at -20°C. However, many 20°C freezers undergo freeze / thaw cycles that could adversely affect Matrigel. Therefore, we store Matrigel aliquots at -80°C.*

PREPARING MATRIGEL-COATED CULTURE VESSELS

1. To prepare Matrigel-coated flasks and dishes, place 50 ml cold DMEM base medium (direct from fridge) into a tube. Also take a 0.5 ml aliquot from the -80°C freezer.
2. Using a P1000 pipette, draw up cold medium and use this to defrost the aliquot of Matrigel.
3. Transfer the Matrigel to the 50ml tube and mix well.
4. Immediately add 0.2ml / cm^2 of diluted Matrigel to flasks and dishes (i.e. 5ml diluted Matrigel / T25 flask; scale volumes according to culture vessel surface area).
5. Allow to polymerise for 45 min in the hood or overnight in the fridge

Note: *There should be no visible changes when the diluted Matrigel polymerises because the Matrigel layer will form as a very thin, transparent layer bathed in excess diluting DMEM. If blobs of Matrigel are visible, the diluting or coating process was not carried out swiftly enough and premature polymerization occurred.*

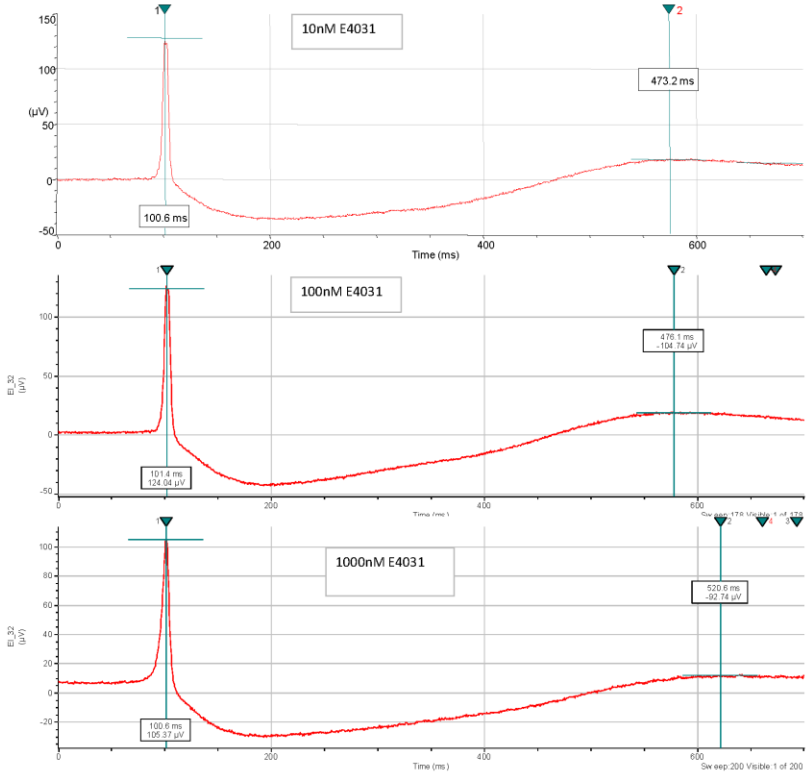
6. Matrigel-coated vessels can be stored in the fridge for ~ 1 month. Flasks should have the caps fully tightened and dishes should be sealed with parafilm to prevent evaporation of the bathing DMEM diluent

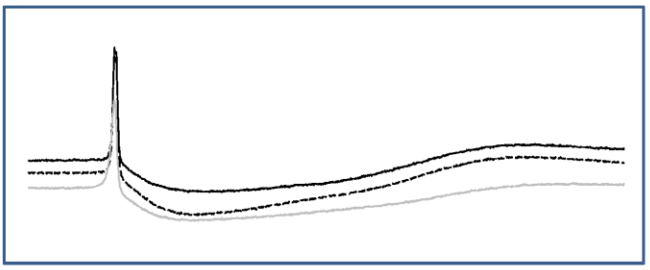
   Note: *The biological properties of Matrigel are only maintained when wet. It is critical that evaporation during storage is minimized. We also use a spirit level to ensure the shelves of the fridge are level so parts of the Matrigel-coated vessels do not dry out.*

QT-interval Analysis Output Example

		basal		1nM	%	10nM	%	100nM	%	1000nM	%	
06/04/2010	channel switched after 1nM		100			474	**100**	476	**100.4219**	520	**109.7046**	
14/04/2010	no shift		100									
15/04/2010	no shift		100									
15/04/2010		205	100	205	**100**	215	**104.878**	215	**104.878**	220	**107.3171**	15
17/04/2010		272	100	271	**99.63235**	278	**102.2059**	290	**106.6176**	405	**148.8971**	133
17/04/2010		345	100	379	**109.8551**	405	**117.3913**	411	**119.1304**	420	**121.7391**	75
11/12/2009		238	100	297	**124.7899**					255	**107.1429**	17
17/12/2009		360	100			365	**101.3889**			455	**126.3889**	
27/04/2010		255	100	254	**99.60784**	250	**98.03922**	310	**121.5686**	305	**119.6078**	50
29/04/2010		250	100	255	**102**	262	**104.8**	318	**127.2**	310	**124**	60
				AVG	**105.9809**		**104.1005**		**113.3028**		**120.5997**	**58.33333**
				STDEV	**10.01326**		**6.353936**		**10.74245**		**13.75457**	**43.61498**
				SEM	**4.087896**		**2.401562**		**4.385589**		**4.862973**	**17.80574**
				TTEST	**0.073396**		**0.056071**		**0.001284**		**0.000207**	
				n=	**10**							

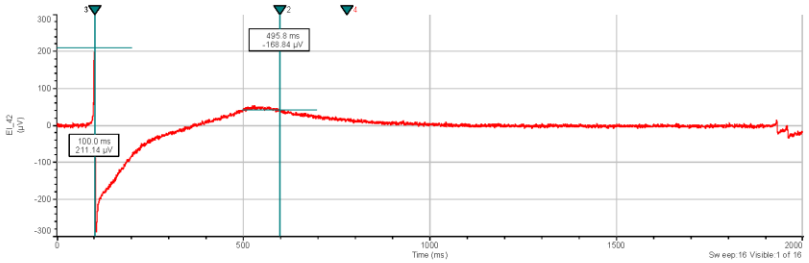
experiment conducted in NT buffer solution (2mM CaCl), switched recording electrode so basal and 1nM not relative to later recordings



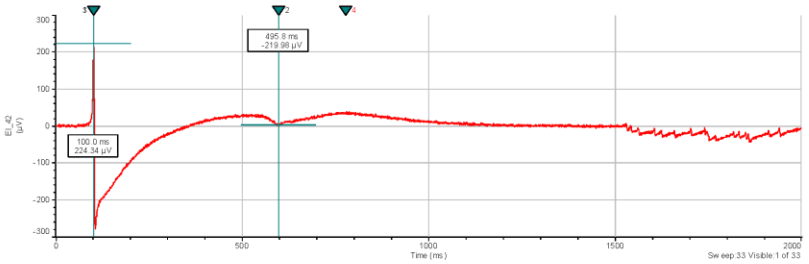


experiment conducted in NT buffer solution (2mM CaCl), switched recording electrode so basal and 1nM not relative to later recordings

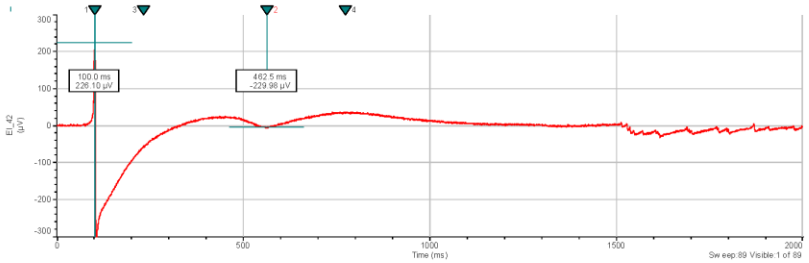
basal



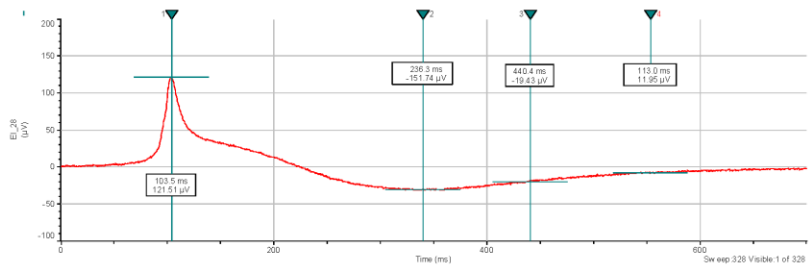
1nM



1000nM



basal



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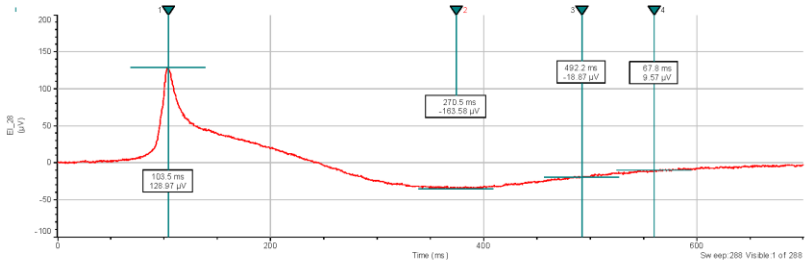
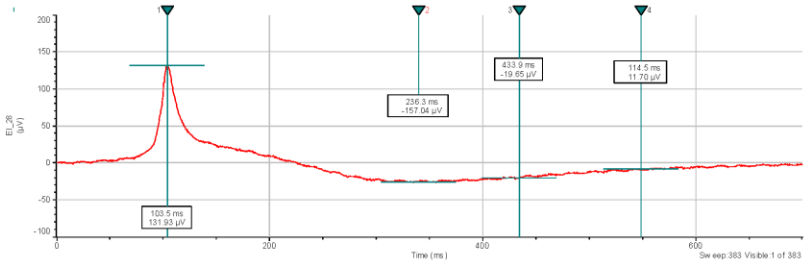


100nM



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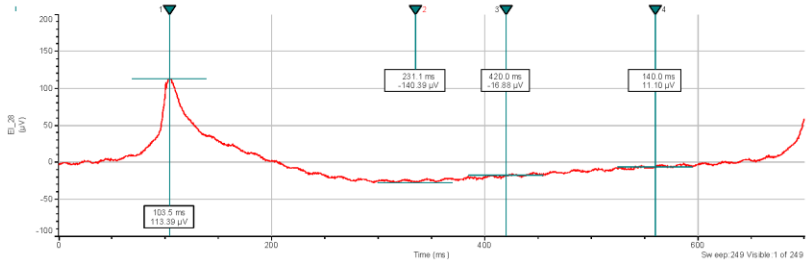

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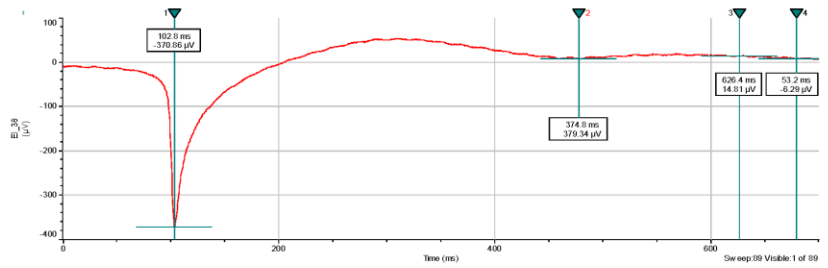

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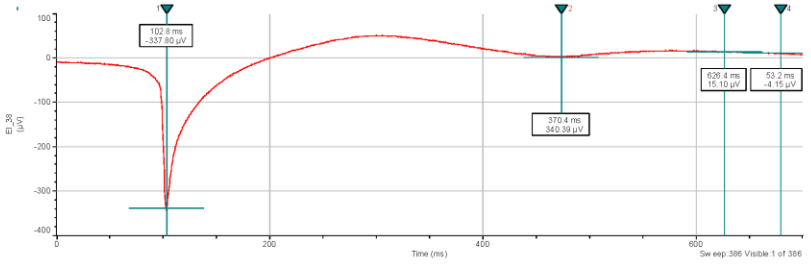

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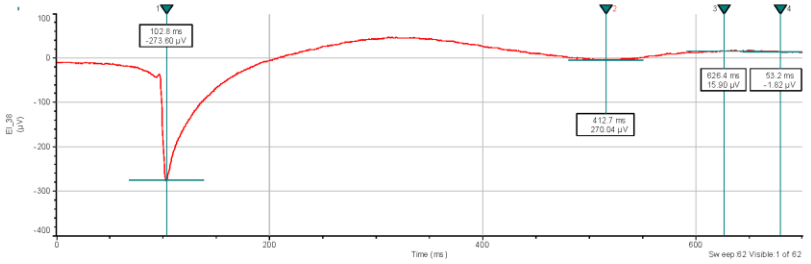

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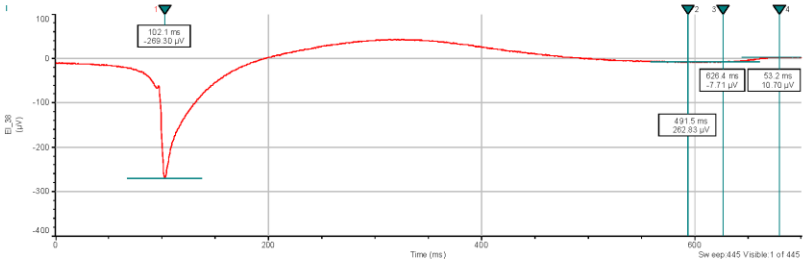

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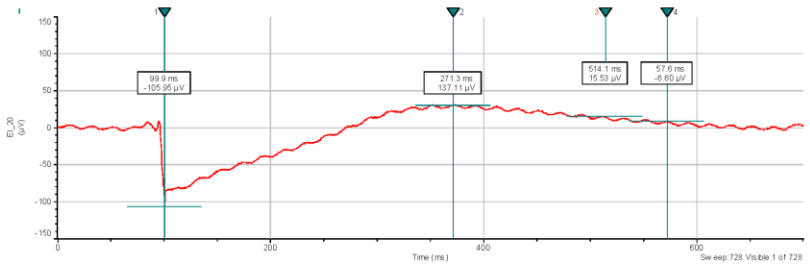

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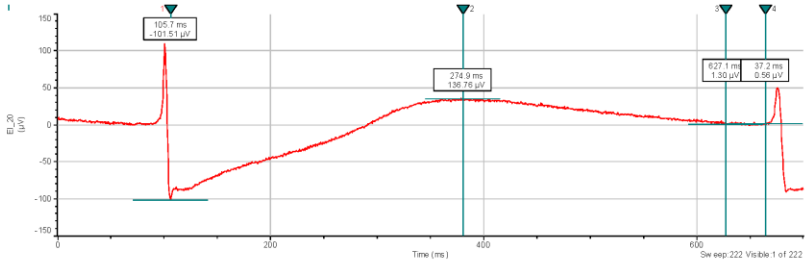

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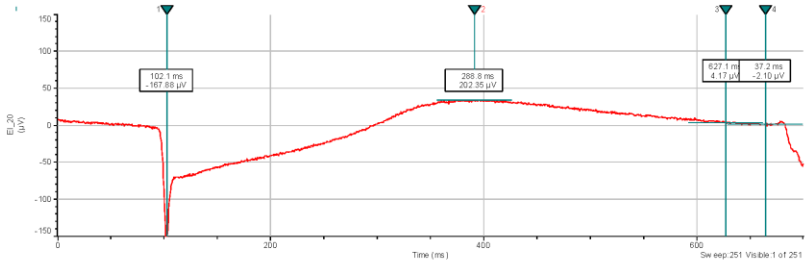

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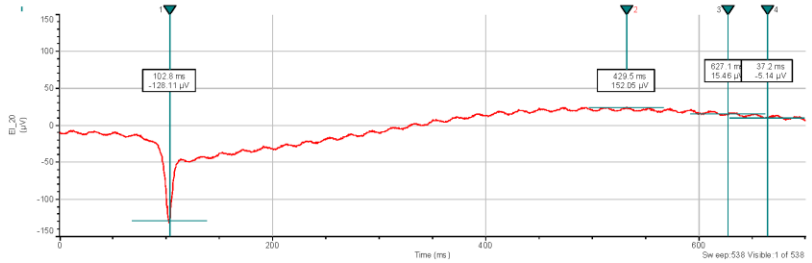
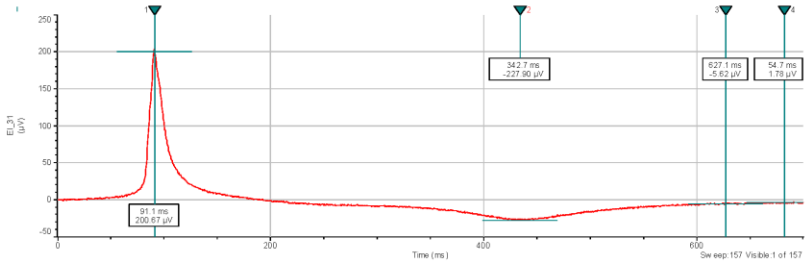


basal



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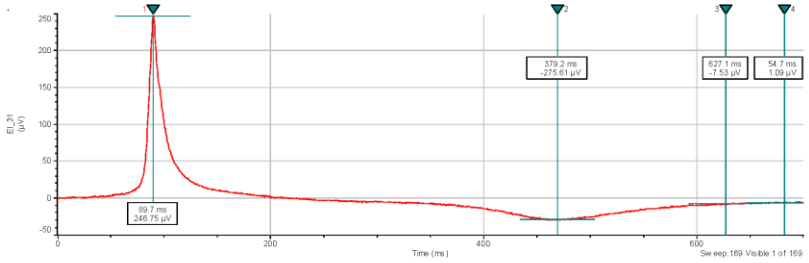

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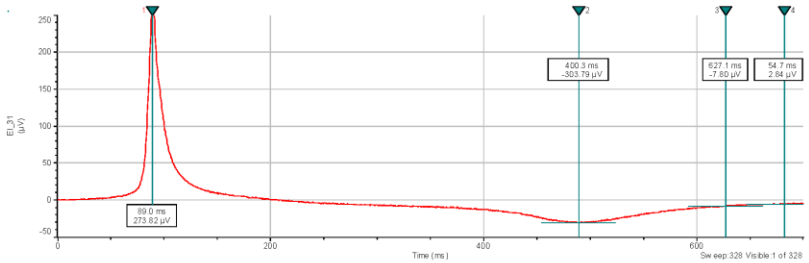
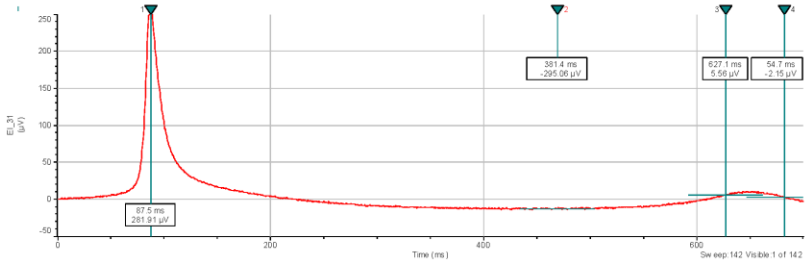


100nM



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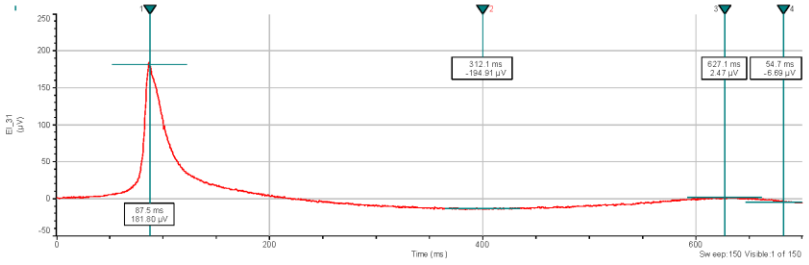
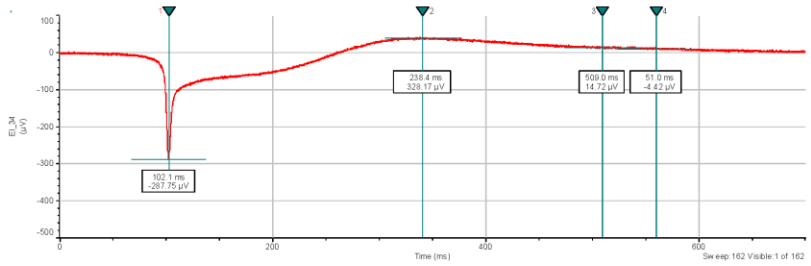


basal



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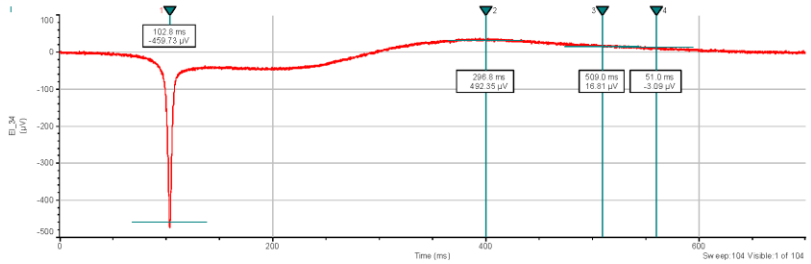
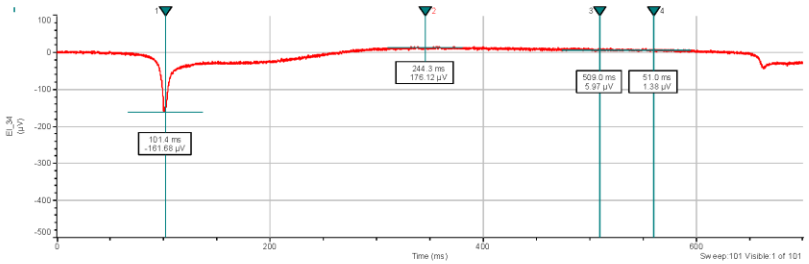
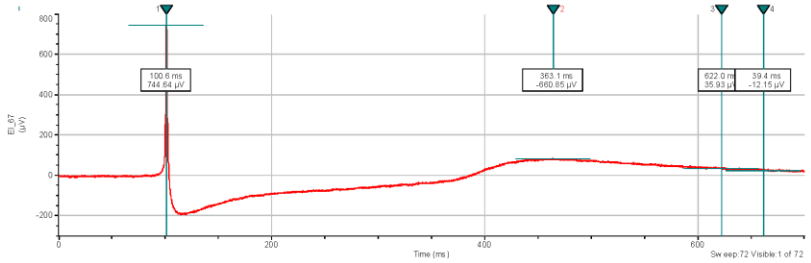


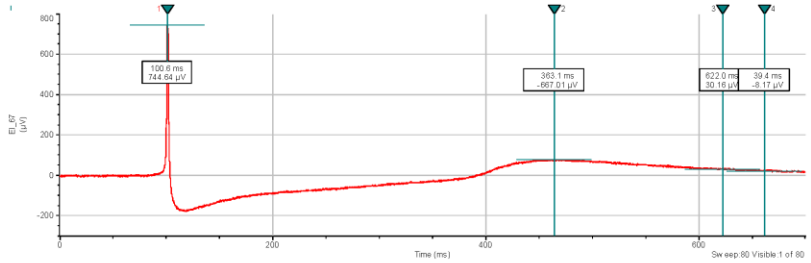
2uM



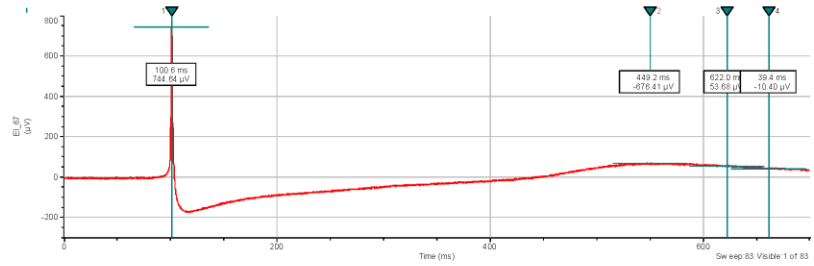
basal



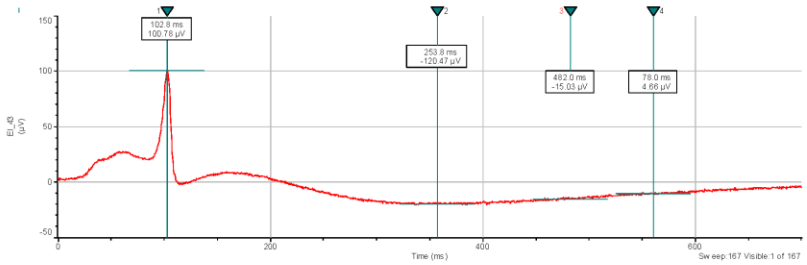
20nM



2uM



basal



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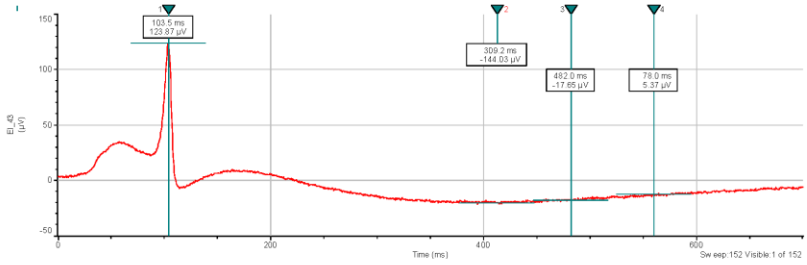

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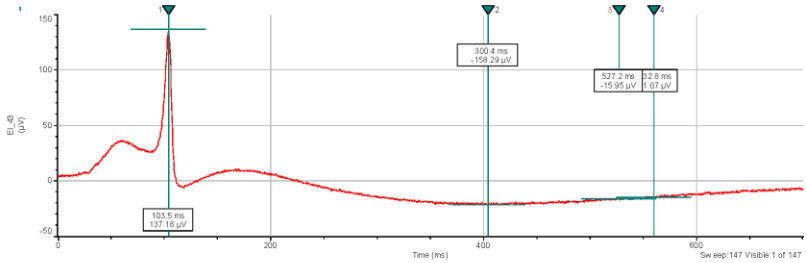
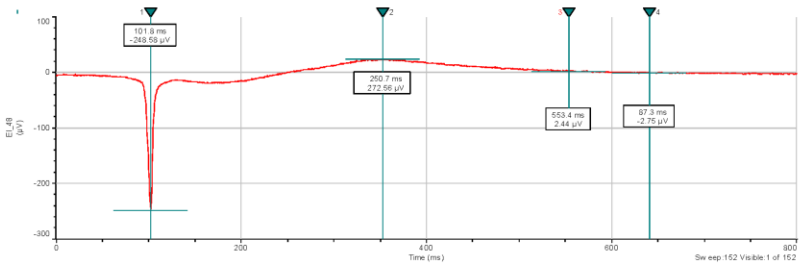
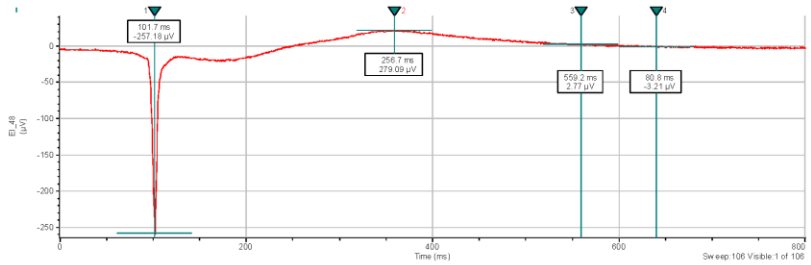


basal



1nM



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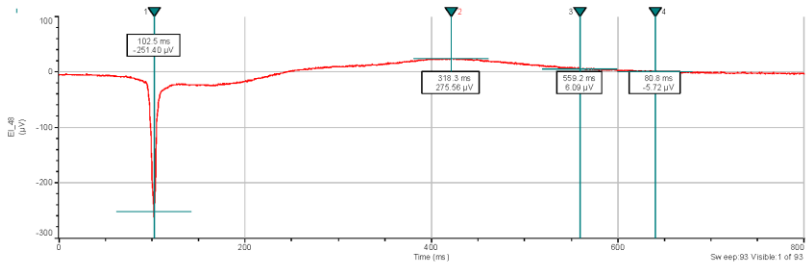
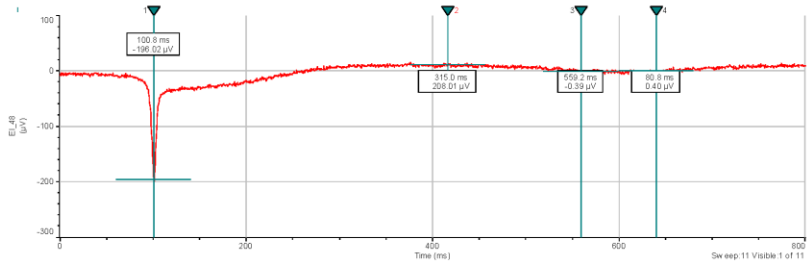
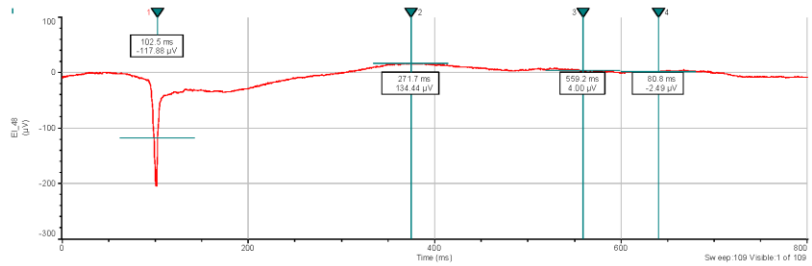


1000nM



washout



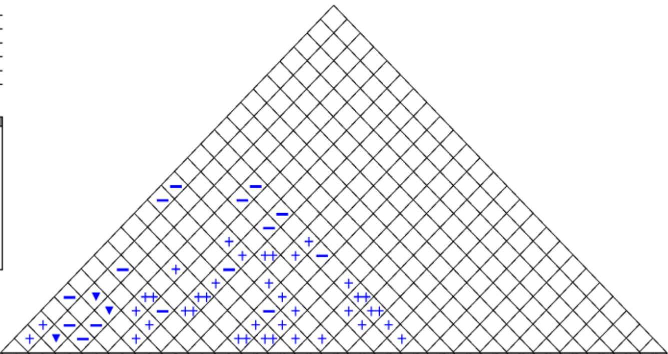
Appendix E

- House of Quality Matrix
- Additional e-beam deposition models

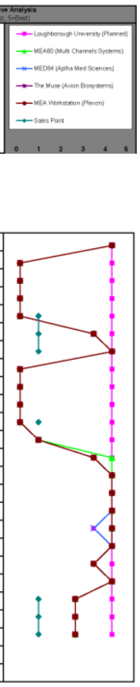
House of Quality Matrix

Title: MEA House of Quality
Author: Olivia M Flaherty
Date:
Notes:

Legend		
Θ	Strong Relationship	9
O	Moderate Relationship	3
▲	Weak Relationship	1
++	Strong Positive Correlation	
+	Positive Correlation	
−	Negative Correlation	
▼	Strong Negative Correlation	
▼	Objective Is To Minimize	
▲	Objective Is To Maximize	
X	Objective Is To Hit Target	



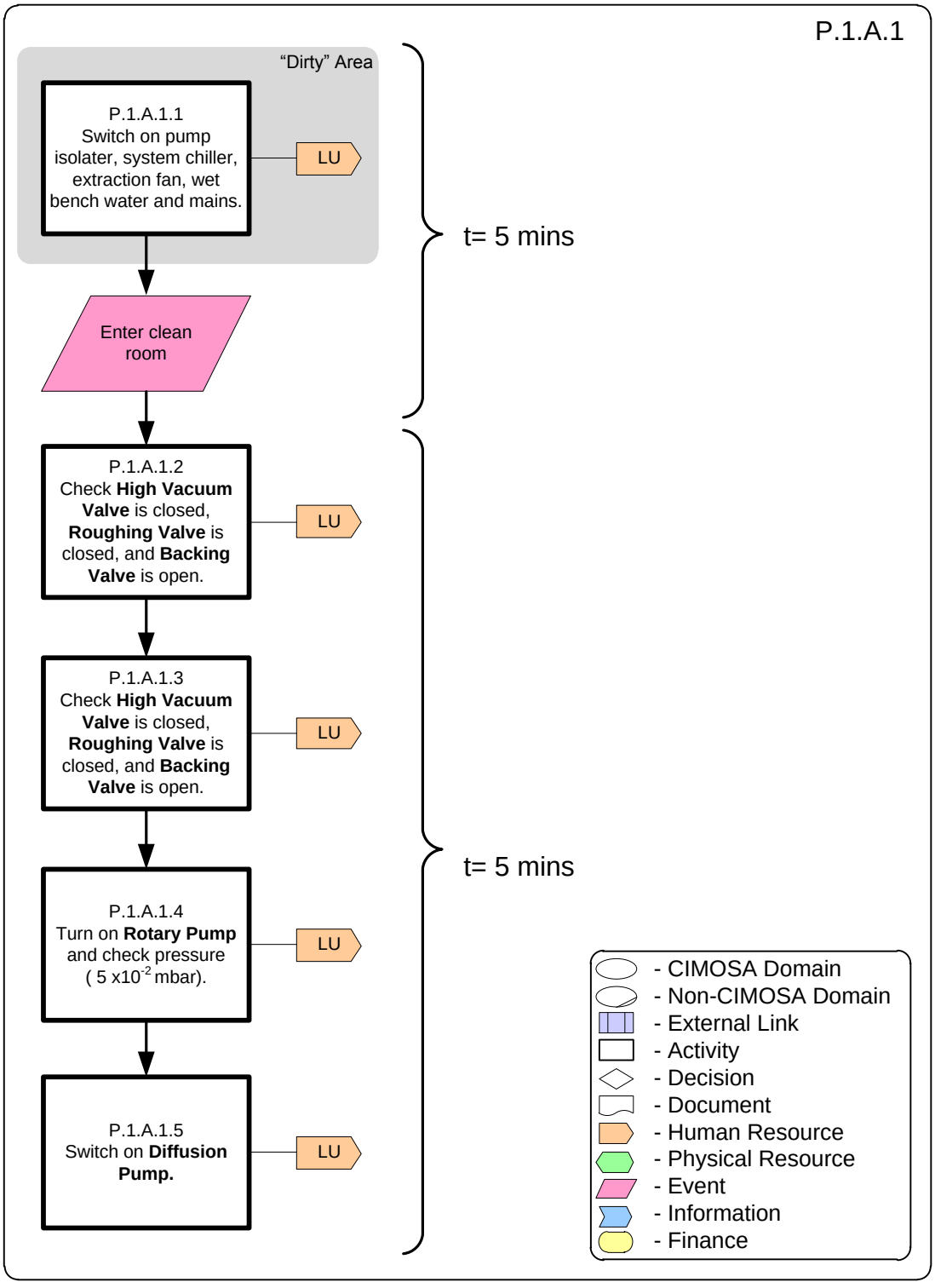
Row #	Max Relationship Value in Row	Relative Weight	Weight / Importance	Demanded Quality (a.k.a. "Customer Requirements" or "Whats") / Quality Characteristics (a.k.a. "Functional Requirements" or "Hows")	1 Materials - availability and price	2 Materials - cost of processing technique	3 Materials - processing time	4 Materials - durability	5 Biochip - light-weight	6 Biochip - small footprint	7 Biochip - safe to use	8 Biochip - durable	9 System - small footprint	10 System - short tracking, close interconnects	11 System - low noise	12 System - scalable number os channels	13 System - modular agile	14 System - safe to use (meets international electrical standards)	15 Biochip - holds >1 beater over an electrode without synchronisation occuring	16 System - can record and analyse >1 beater per recording	Loughborough University (Planned)	MEA60 (Multi Channels Systems)	MED64 (Aplha Med Sciences)	The Muse (Axion Biosystems)	MEA Workstation (Plexon)	Sales Point
				Direction of Improvement: Minimize (▼), Maximize (▲), or Target (x)	▼	▼	▼	▲	▼	X		▲	X	X	▲	▲	X		X		Competitive Analysis (0=Worst, 5=Best)					
1	9	5.9	5.0	Biocompatible	Θ	Θ	Θ	O	O	O	O	O							▲		5	5	5	5	5	
2	9	5.3	4.5	Microwell - 500um diameter	Θ	Θ	Θ	O	O	O	▲	O							O		5	0	0	0	0	
3	9	5.3	4.5	Microwell - max. depth 250um	Θ	Θ	Θ	O	O	O	▲	O									5	0	0	0	0	
4	9	3.6	3.0	Microwell - curved x-sectional profile	Θ	Θ	Θ	O	O	O	▲	O									5	0	0	0	0	
5	9	5.9	5.0	Electrode at centre of microwell	O	Θ	Θ	O	O	Θ	▲	▲	O	Θ	O	▲			O	O	5	0	0	0	0	1
6	9	4.7	4.0	Re-usable	Θ	O	O	Θ	O	O	O	Θ			▲	Θ	O	▲	▲	▲	5	4	4	4	4	1
7	9	3.6	3.0	Maintain interface with commercial system						Θ	Θ								▲	Θ	5	5	5	5	5	1
8	9	3.6	3.0	Microwells - as far apart as possible	▲	O	O	O	▲	Θ	▲	▲							Θ	Θ	5	0	0	0	0	
9	3	2.4	2.0	Microwells - as large a SA as possible	▲			O		▲		▲							O	▲	5	0	0	0	0	
10	9	2.4	2.0	Microwell interior - suitable for patterning/etching	▲	O	O	O	▲	▲	▲	Θ							Θ	O	5	0	0	0	0	
11	9	3.6	3.0	16 microwells per biochip	▲	O	O	O	O	Θ	O	Θ	▲	O	O	Θ	O		Θ	Θ	5	0	0	0	0	1
12	9	2.4	2.0	Media well geometry as standard 35mm culture dish	▲	Θ	Θ	Θ	Θ	Θ	▲	Θ	O	▲	▲	▲			Θ	Θ	5	1	1	1	1	
13	9	2.4	2.0	Light transmissable lid	Θ	Θ	Θ	Θ	Θ	Θ	▲	Θ	O				▲		▲	▲	5	5	4	4	4	
14	9	5.9	5.0	Facilitate a constant cell environment (e.g temp, humidity, etc)	O			Θ	▲	Θ	O	Θ	Θ	O	O	O	Θ	Θ	Θ	Θ	5	5	5	5	5	
15	9	5.9	5.0	Grounded							Θ			O	Θ	▲	▲	Θ	O	O	5	5	5	5	5	
16	9	5.9	5.0	Can be sterilised				Θ		O	O	Θ	O	▲		▲	Θ	O		▲	5	5	5	5	5	
17	9	5.9	5.0	Allow visual inspection of cells	Θ	Θ	Θ	Θ	Θ	O	O	O	O			O	▲		O	O	4	5	4	5	5	
18	9	4.7	4.0	Samples at 2-25kHz									▲	▲	▲	Θ	Θ	O		Θ	5	5	5	5	5	
19	3	4.7	4.0	Easy access to cells for maintenance in culture					O	O	O	O	▲				▲	O		▲	5	4	4	4	4	
20	9	5.9	5.0	Appropriate pre-procesing (e.g. noise reduction/removal,) and amplification						▲	▲		Θ	Θ	Θ	Θ	Θ	Θ		Θ	5	5	5	5	5	
21	9	4.1	3.5	Simple for new users to learn					▲		Θ	▲					▲	O		Θ	5	3	3	3	3	1
22	9	3.6	3.0	User-friendly interfaces					O	Θ	Θ	Θ	Θ				O	Θ	▲	O	5	3	3	3	3	1
23	9	2.4	2.0	Input (e.g. current/voltage pulse stimuli) limited appropriately	O			Θ		▲	Θ	Θ	▲	O	O	O	O	Θ		Θ	5	3	3	3	3	1
24																										
25																										
				Target or Limit Value	Lowest priced (suitable) available	Lowest possible (@ required quality)	Quickest possible (@ required quality)	Most durable (>10-15 uses)	Light as possible (@ req. durability)	Small as possible (to support scale up)	Comparable to or better than standard	>10-15 uses	Small as possible (Fits inside incubator)	Short and close as functionally possible	Best low noise components possible	Designed for scalability (>100's channels)	Modules (upgrades). Best agility poss.	Comparable to or better than standard	Successfully support >1 beating cluster	Ability to analyse all detecting channels						
				Difficulty (0=Easy to Accomplish, 10=Extremely Difficult)	5	8	5	9	4	8	9	7	10	10	8	10	7	10	5	7						
				Max Relationship Value in Column	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9						
				Weight / Importance	355.0	372.8	372.8	379.9	239.6	414.2	322.5	406.5	221.3	172.8	171.6	233.1	268.0	276.3	256.2	416.0						
				Relative Weight	7.3	7.6	7.6	7.8	4.9	8.5	6.6	8.3	4.5	3.5	3.5	4.8	5.5	5.7	5.3	8.5						



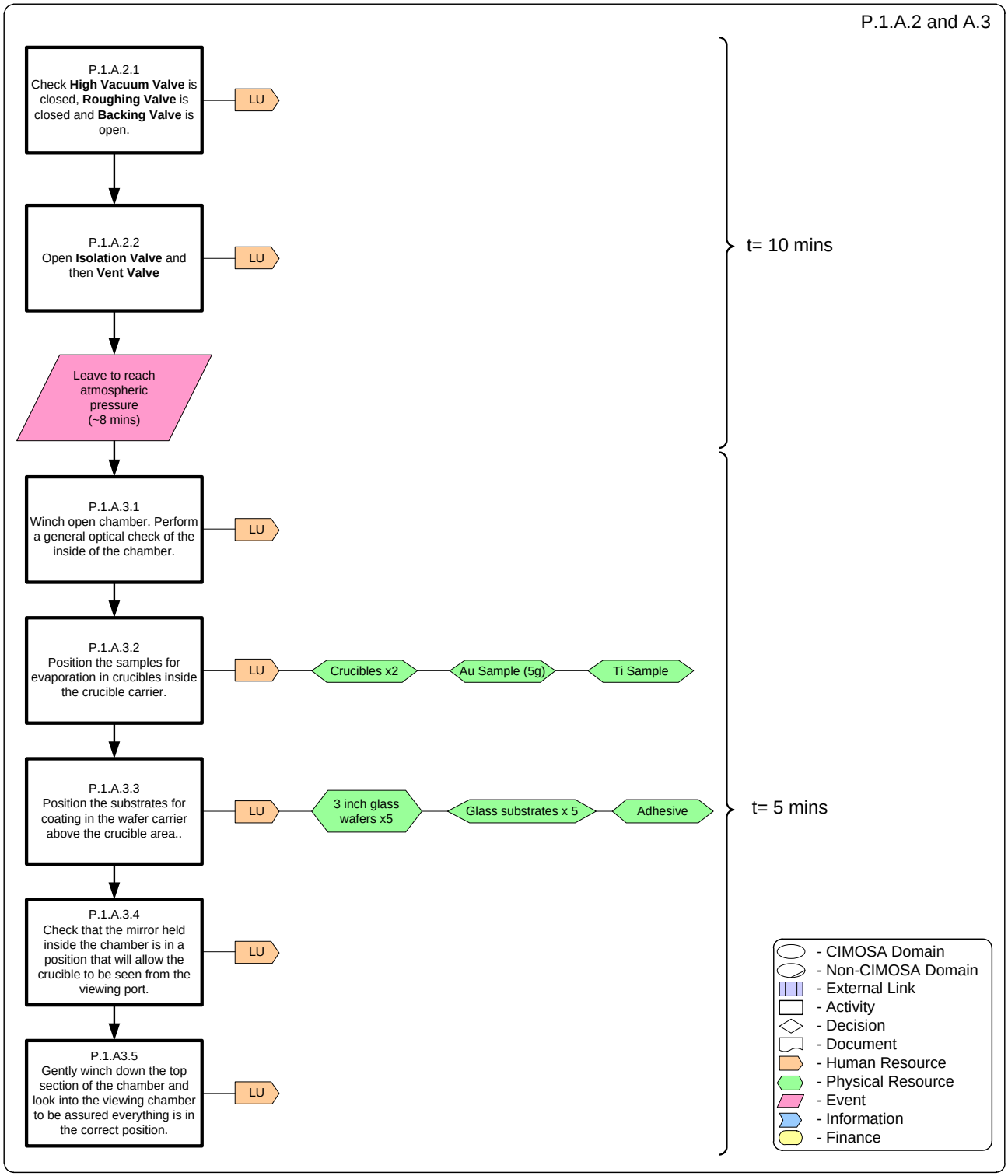
Powered by QFD Online (http://www.QFDOnline.com)

Additional drill down models for Process One of the Clean Room Based Photolithography Manufacturing Approach

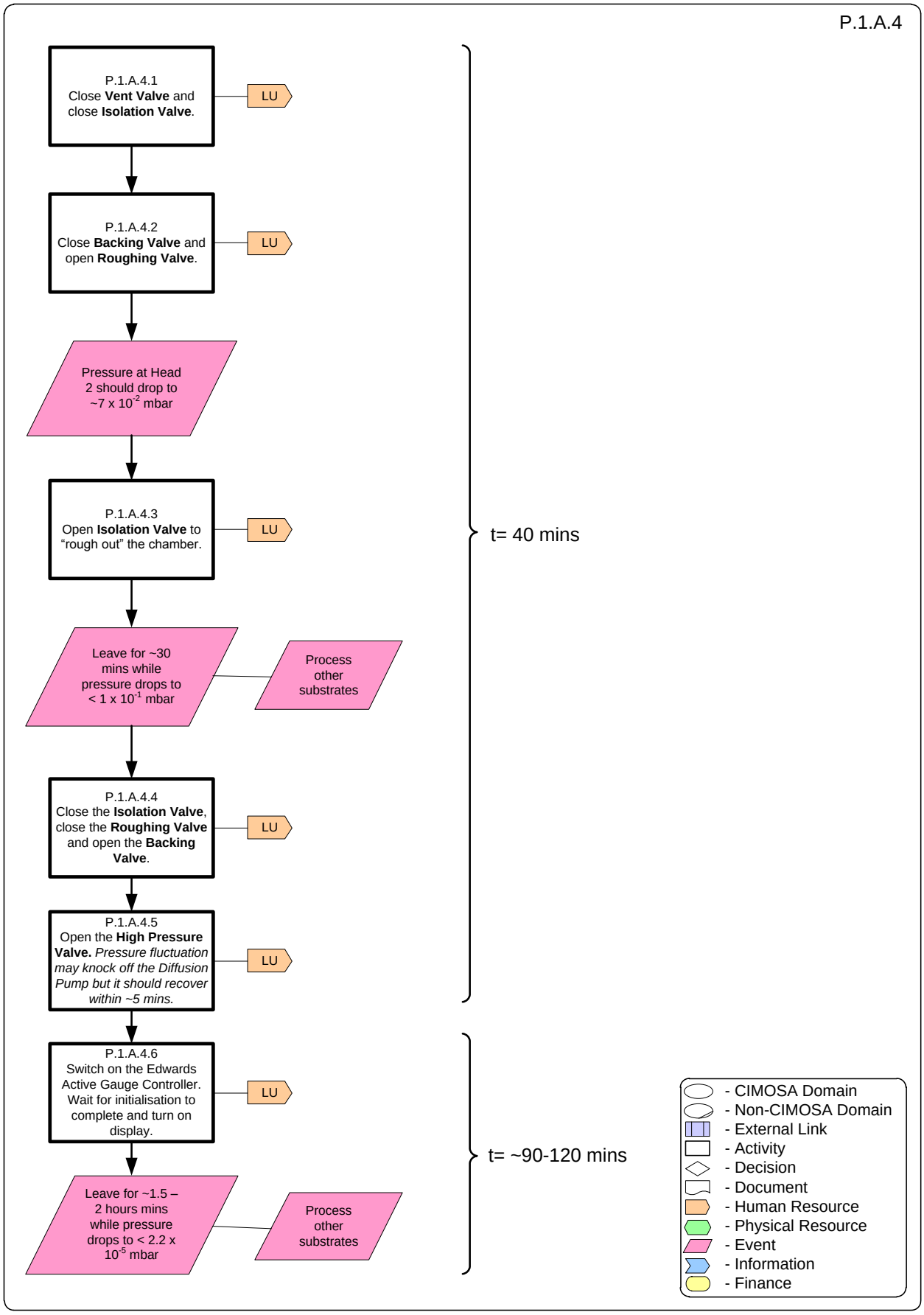
The following interaction models (E.1 – E.4) captured and communicated further detail of the e-beam deposition used to coat the glass substrates with Ti and Au.



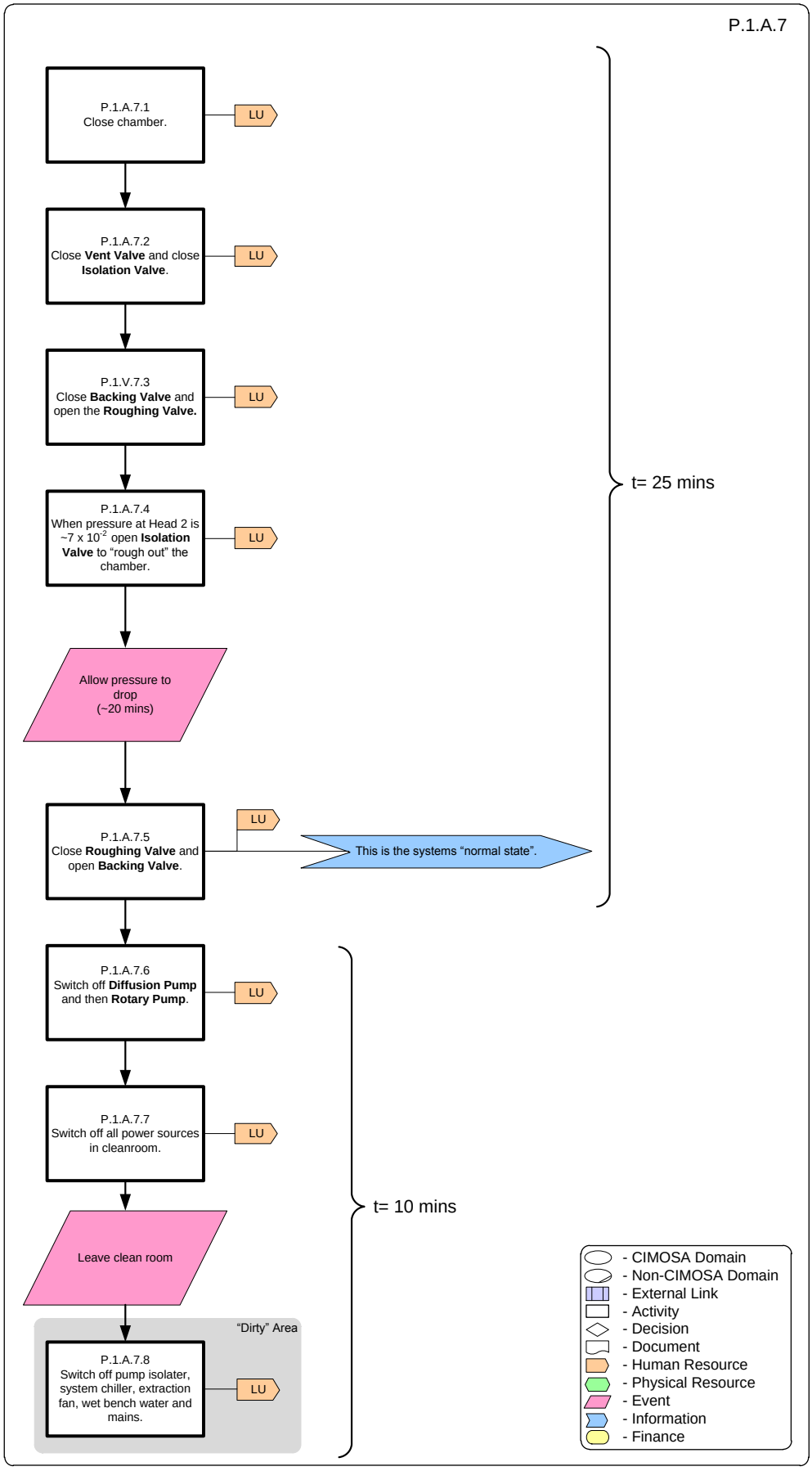
E.1: Process One (Electron beam deposition), Activity One (Switch on e-beam depositing system).



E.2: Process One (Electron beam deposition), Activity Three (Insert substrates and materials for deposition).



E.3: Process One (Electron beam deposition), Activity Four (Pump down the chamber).



E.4: Process One (Electron beam deposition), Activity Seven (Close down system).

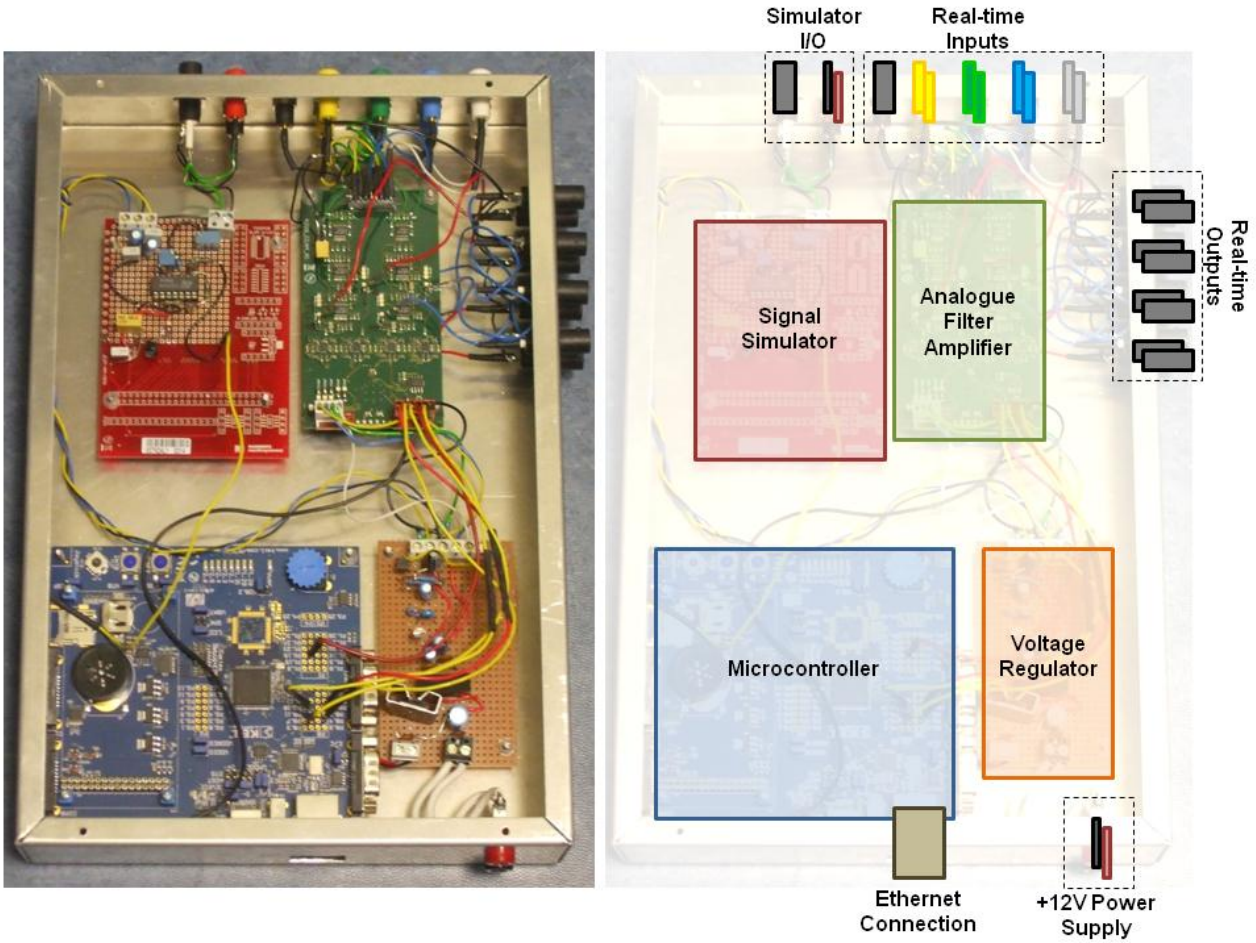
Appendix F

- Custom-made supporting electronics

The Supporting Electronics and Software Solution

Additional research overlapping with this work also specified supporting electronics for a new MEA system (F.1). Hitex Development Tools, Coventry UK, manufactured the prototype to filter and amplify signals with complimenting embedded real-time signal processing capability.

A signal simulator was incorporated into the unit to test the surrounding electronics that would not be required in systems that were to be supplied to an end user.

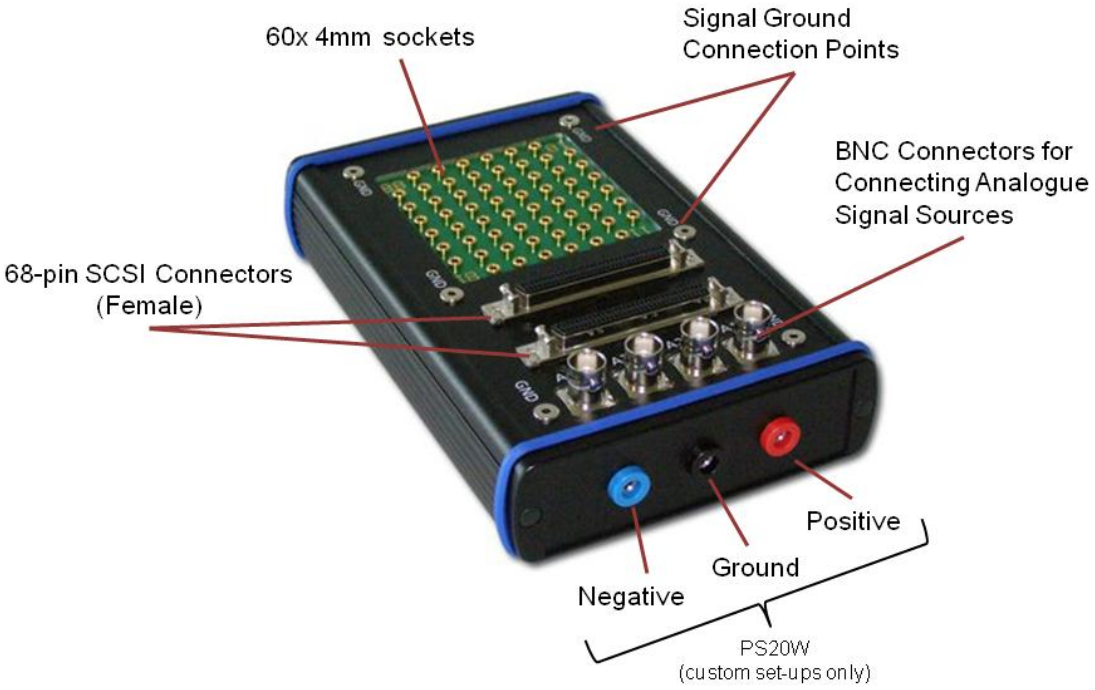


F.1: The Loughborough University Supporting Electronics Prototype.

The custom-made supporting electronics was designed to be powered by a +12V continuous power supply.

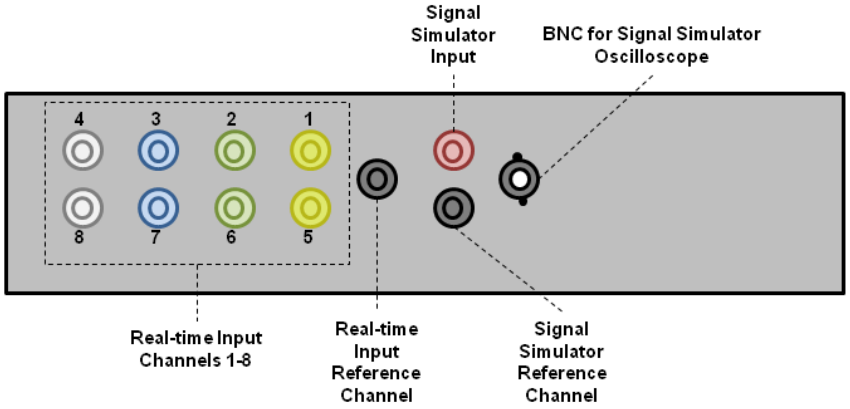
In order to consider the level of amplification present in the MCS system presently exploited by project collaborators an MCS MEA Signal Divider (SD) was purchased (F.2). The MEA-SD is inserted into the MEA 60 system between the MEA 1060 amplifier and the MC_Card also using 68-pin SCSI cables (see figure below). Comparing values of signals visualised in MC_Rack with those visualised on

an oscilloscope connected to the signal divider it is possible to understand the level of amplification in the MCS headstage.



F.2: An MCS MEA Signal Divider for connection between the MCS 1060 headstage amplifier and the MC_Card. The supply voltage inputs are ordinarily not used for this device. A low-noise isolated power supply with 21W power and ±7V output voltage (PS20W) can be used for custom setups where the IPS10W power supply (usually integrated into the PC) is not connected. Input supply voltage can range from 6V to 12V in such cases.

The custom-made electronics connects up to 8 channels of signals from a live culture in an MEA biochip. The connections for both the live inputs and the simulated inputs, used in pre-test system and software validation, are demonstrated in the figure below (F.3).



F.3: The Loughborough University Real-time Inputs and Signal Simulator Connections.

The outputted filtered and amplified signals from the custom-made system can be visualised through connection of an appropriate device to the inputted channels corresponding BNC connector (F.4).

F.4: The Loughborough University Real-time Outputs via BNC connection.

The ARM Cortex-M3 microprocessor was state-of-the-art in January 2009 when hardware specifications were drafted. A top of the range microcontroller was selected to facilitate the intention to develop improved processing of neural signals in real-time (a long-term objective of the project). The contracted company (Hitex, Coventry UK) defined the maximum number of recording I/Os as 8 channels per microcontroller. System components designed would be scaled accordingly. Therefore 4, 8, 10 and 16 channel layouts were investigated throughout MEA biochip design iteration.