# A simple device for multiplex ELISA made from melt-extruded plastic microcapillary film

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# Supplementary Data

## Supplementary Methods

### Optical Properties of MCF and Single Capillaries

MCFs containing 10 or 19 parallel microcapillaries were produced from a range of different thermoplastic materials as listed in Table S1 by a novel melt-extrusion process described in patents GB2408961, EP1691964, JP2007514566 and US2009011182 and Hallmark *et al* {Hallmark, 2005 #154}. The individual cylindrical FEP microcapillaries, used to compare with the optical properties of FEP MCF, with dimensions listed in Table S2, were from Upchurch Scientific (Oak Harbor, WA, USA). The optical properties of MCF fabricated from FEP and a range of other thermoplastic materials with varying optical properties (Table S1) and two sizes of commercial microcapillaries produced from FEP (Table S2) were compared. Samples were washed with PBS-T and then filled either with PBS-T or a fully-converted OPD substrate solution and scanned with a HP ScanJet 4050 Photo Scanner in transmittance mode. RGB images were acquired at a resolution of 1,200-3,200 dpi, and post-processed with ImageJ software[27]. A profile across the MCF of optical intensity was plotted for RGB images and the blue channel that showed maximum absorbance by the yellow converted OPD.

### Protein Binding within FEP MCF capillaries

Initial experiments to demonstrate the effective adsorption of antibodies or protein antigens onto the internal surface of FEP MCF capillaries were conducted by measuring the amount of mouse IgG bound using AP-anti-mIgG and the fluorescent substrate FDP. The internal capillary wall of FEP MCF shows high hydrophobicity due to the hydrophobicity of FEP combined with a high surface roughness with a contact angle with water of $123.8 \pm 1.6$ degrees estimated with Laplace-Young equation using hydrostatic equilibrium height. Individual capillaries within 5 meters of FEP MCF were coated by adsorption with a series of concentrations of mouse IgG in PBS ranging from 0-100,000 ng/ml, followed by washing and blocking. Replicate 50-mm long pieces taken from either end of the 5m length of FEP MCF were filled and incubated with 1:1,000 dilution of anti-mIgG-AP and incubated for 1 hour at room temperature. Each piece was then extensively washed with PBS-T and FDP buffer followed by filling with 20μM FDP substrate, incubation at room temperature for 10 minutes, and imaging with a Leica TCS SP5 broadband confocal fluorescence microscope (Leica, Milton Keynes, UK) using an excitation wavelength of 480 nm and measuring emission from 500 to 540 nm. A z-stack series was scanned at different heights for each capillary and then the maximum fluorescence from each stack series projected in the z-axis using LAS AF post-processing software (Leica, Milton Keynes UK). A profile of fluorescence signal (converted to grey scale) across the MCF section was then plotted using ImageJ software[27]. The height, *h*, of the fluorescence signal within each capillary was then plotted as a function of the input concentration of mouse IgG used for coating.

## Supplementary Results

### Adsorption of proteins inside FEP MCF capillaries

The adsorption of antibodies onto the surface of capillaries within a long MCF was initially studied using a simple direct ELISA that detected bound mouse IgG using AP-anti-mIgG plus the substrate FDP (Fig. S1A). Antibodies adsorbed on the internal super-hydrophobic surface of a FEP MCF was detected with a fluorescent signal (Fig. S1B) which was quantified by determining peak fluorescence height (Fig. S1C). Individual capillaries were coated with varying concentrations of mIgG. The fluorescent signal was proportional to the concentration of IgG in the solution for concentrations below 800ng/ml, whereas for concentrations of IgG of 800 ng/ml or higher there was no further increase in mIgG on the surface of the capillary (Figs S1B,C). To test if antibody was depleted by adsorption along the length of a long capillary, capillaries within a 5m length of FEP MCF were coated, a piece was taken from the inlet and output, and bound mIgG levels was compared by quantifying fluorescent signal. Even when the input mIgG concentration was limiting, at 32-160ng/ml, equal fluorescence was detected at the outlet and inlet, indicating even coating along the length of the 5m capillary (Fig. S1D). These initial experiments demonstrated the ease of adsorbing antibodies and antigens inside FEP MCF, and proved the feasibility of the production method outlined in Fig. 1 for producing batches of identical devices capable of

multiple analyte detection in the parallel array of capillaries. Similar adsorption was seen with other proteins, and adsorbed mIgG was also detected using anti-mouse HRP with the colourimetric substrate OPD imaged using a flatbed scanner (data not shown). Protein adsorption was also measured by directly coating capillary walls with enzyme-antibody conjugates, and adsorption inside the capillaries of MCF extruded from a range of different thermoplastics was observed – albeit with poor optical clarity – suggesting that protein binding did not require any particular surface characteristic of FEP (data not shown).

In this experiment (Fig. S1), each microcapillary was coated with varying concentrations of antibody, and it was observed that: i) there is a linear increase of the signal with the antibody concentration for low mouse IgG concentrations, with the signal converging to a plateau for high concentration which clearly shows a typical Langmuir isotherm and antibody adsorption to the FEP material in a monolayer; ii) this plateau, presumed to be due to surface saturation, was observed for mouse IgG concentrations greater than just under1 µg/ml.

**Determining maximum Absorbance values in capillaries within MCF from scanned images**

Scans were postprocessed using ImageJ as follows. A profile across the MCF of optical intensity, averaged along a 2mm section of the test strip was plotted for the blue channel, which showed maximum response for the yellow coloured converted OPD substrate. Examples of the images obtained are shown in Fig. S3. A high grey level (maximum of 255) means low absorbance, whereas low grey levels (minimum of 0) represent high light absorbance by the solution. The signal response on each capillary was measured by taking the peak-to-valley height, $h$ from the profile plot, and then converted to absorbance by calculating Abs = $-\log((255-h)/255)$. The extinction coefficient measured for the flatbed scanner and microplate reader were similar (data not shown); to directly compare the signal response in the FEP MCF and microwell plates the absorbance values were normalised to units of cm$^{-1}$ by dividing by the maximum path length of 0.02 cm for the FEP MCF capillaries or 0.3 cm to the microwell plate. For the FEP MCF strips, the mean value for Abs/cm was taken just for the 8 central capillaries and plotted against anti-HB CAg concentration; the outer two capillaries were disregarded due to their reduced diameter compared to the central 8 capillaries (caused by the extrusion process) which returned lower Abs/cm values, however these channels could be utilised simply by correcting for optical distance. Furthermore, in some cases edge refraction by the semi-circular edges of the film caused high noise that interfered with outer capillary signal detection (Fig. S2A); this could be eliminated by modifying the extrusion die geometry to separate the outer capillaries further from the sides of the film.

**Optical properties of FEP MCF**

The schematic cross section of FEP MCF highlights the flat top and bottom surfaces of the film (Fig. S2A top), in contrast to the single capillaries which have a round section (Fig. S2B, C top). From the images and intensity profiles, the FEP MCF containing buffer alone was completely transparent apart from grey lines along either edge of the film (Fig. S2A). These lines were caused by refraction of the transmitted light by the rounded edges and varied in intensity depending on positioning within the scanner; for example in Fig. S2A, the left edge appears darker than the right edge, with a wider and more intensely grey peak. When OPD was filled into all capillaries, however, intense yellow lines were seen within the film (Fig. S2A). This represented strong blue light absorbance, as seen by the dark grey lines and strong absorbance peaks in the blue colour channel (Fig. S2A, bottom). The effects of edge refraction are again clear, with the yellow signal of the outermost left capillary merging with the grey edge absorbance, although the outermost right capillary can be clearly separated from the grey edge line.

Apart from the outermost two capillaries (because of the semi-circular edges of the film), therefore, the FEP MCF has excellent transparency for optical interrogation; in contrast, when single capillaries were imaged, refraction effects from the rounded sides dominated the images (Fig. S2B, C). Firstly, for a thin walled capillary, strong grey lines were seen at both edges when filled with buffer, once again with varying intensity depending on orientation (Fig. S2B). These lines merged with the yellow OPD signal, and the peak edge refraction absorbance reached greater than 60% of the peak blue absorbance of the OPD. Note that the internal diameter of this thin-walled capillary is greater than the thick-walled capillary or the FEP MCF (Table S2), giving a greater pathlength and therefore greater blue light absorbance of OPD. In order to attempt to separate the edge refraction effect from the absorbance within the capillary, a single FEP capillary with a thicker wall was tested (Fig S2C). Although this geometry clearly separated the capillary absorbance (yellow line) from the edge refraction (grey edges), the increased thickness of FEP resulted in far higher background absorbance, thereby limiting the signal to noise ratio.

To determine the importance of the optical properties of the thermoplastic material used - as opposed to the geometry - MCF samples extruded from different transparent thermoplastics with varying optical properties (Table S1) were imaged alongside FEP MCF. Whereas FEP MCF filled with buffer was transparent (Fig S2A),

other MCF with refractive indexes higher than water gave strong optical distortion, resulting in appearance of strong and variable grey lines along all the capillaries (Fig. S2D-H). Although some yellow colour is visible when these capillaries were filled with OPD, the strong intensity of grey signal resulting from refraction by the internal curved walls of the capillaries prevented accurate detection of OPD within the capillaries. This problem was not compensated by splitting RGB channels since in all cases the grey refractive signal for each capillary gave blue absorbance with buffer that was as strong as the OPD absorbance. This distortion was clearly caused by the difference in refractive index between the liquid filling the capillaries and the plastic film, since it was not observed when MCF extruded from ethylene vinyl acetate (refractive index of 1.48, Table S1) with glycerol (refractive index 1.47) was scanned (data not shown). Thus EVA MCF filled with glycerol gave almost as clear images as FEP MCF filled with aqueous buffers.

These results clearly demonstrated that simple optical detection of colourimetric ELISA substrates by transmission imaging is feasible in FEP in the form of an MCF, but not possible either in single FEP capillaries or in MCF extruded from other thermoplastics with inferior optical properties. Three dimensional reconstruction of the capillary volume using from a z-stack also clearly demonstrated the absence of any optical distortion when imaging fluorescent dye solutions within FEP MCF using laser scanning confocal microscopy (NFR and ADE, unpublished observations).

**Cost of MCF devices**

The typical density of FEP MCF is 5g/m, with a market cost for pelleted FEP material in the range of £80/kg. Using this procedure, a 10-plex, 50mm long MCF FEP test strip can be produced for less than £0.10, reduced to £0.02 if the length of test strip is further reduced to 10 mm (the minimum length required for signal detection is 2 mm as mentioned above in materials and method section). Clearly, this cost only reflects one part of complete device manufacturing; however, device cost is clearly unlikely to be limited by the manufacturing cost of this core microchannel element.

# Supplementary Tables

**Table S1.** Geometry of MCF thermoplastic materials tested in the flatbed scanner

| | Thermoplastic polymer | Mean capillary diameter (µm) | Number of capillaries | Polymer Refractive Index |
|---|---|---|---|---|
| **FEP MCF** | Fluorinated ethylene propylene copolymer | 206 | 10 | 1.34 |
| **EVA MCF** | Ethylene vinyl acetate | 142 | 19 | 1.48 |
| **EVOH MCF** | Ethylene vinyl alcohol | 109 | 19 | 1.51-1.52 |
| **LLDPE MCF low-voidage** | Linear low-density polyethylene | 167 | 19 | 1.51 |
| **COC MCF** | Cyclic olefin copolymer | 119 | 19 | 1.53 |
| **LLDPE MCF high-voidage** | Linear low-density polyethylene | 200 | 13 | 1.51 |

**Table S2.** Geometry of individual commercial FEP capillaries tested

| Capillary | Material | O.D. (mm) | I.D. (mm) |
|---|---|---|---|
| FEP1-32x0.016 | FEP | 0.794 | 0.406 |
| FEP1-16x0.008 | FEP | 1.59 | 0.203 |

# Supplementary Figure Legends

**Figure S1.** Evaluation of protein immobilization and signal detection within FEP MCF capillaries.

**A** Schematic representation of the direct assay to measure antibody immobilisation. **B** Fluorescence signal in a representative section of the FEP MCF, coated with increasing concentrations of mouse IgG and quantified using a confocal fluorescence microscope. **C** Plot of mean fluorescent intensity signal across FEP MCF. **D** Plot of peak height, h, of the fluorescence signal generated at each initial concentration of mouse IgG for the inlet and

outlet of a 5m long reel of FEP MCF indicating no depletion of the IgG along the length of the reel. This data is representative of 2 similar binding tests.

**Figure S2.** Comparison of optical properties of MCF extruded from FEP and other thermoplastics, and individual FEP capillaries, imaged using a flatbed scanner.

Two pieces of each material were washed in PBS-Tween solution, filled with (1) PBS-T or (2) fully converted OPD substrate and scanned in transmitted mode at 3,200 dpi with a HP ScanJet 4050 Photo Scanner. **A** Top: diagram representing cross section geometry of MCF. Middle: RGB image of the capillary array, showing an averaged intensity plot across the film underneath. Lower: blue channel split from RGB images, with averaged intensity ploy underneath. **B and C** Cross-section diagram, RGB and blue channel images and intensity plots for either thin wall, small o.d./i.d. ratio (**B**) or thick wall, high o.d./i.d. ratio (**C**) individual capillaries made from FEP. **D-H** Images of MCF extruded from plastics with different refractive index than water; details of MCF thermoplastic, size and optical properties are given in Table S1.

**Figure S3.** Examples of images of singleplex and multiplex colorimetric and fluorescent ELISA conducted in FEP MCF.

**(A-B)** Singleplex colorimetric ELISA images in FEP MCF devices. **A** Assay schematic. **B** OPD detection in FEP MCF devices at increasing concentrations of anti-HB CAg. The top row shows the profile plot of the scanned RGB images that were then split into Red, Green and Blue channels, and the peak-height in the grey plot was used as a measure of OPD signal intensity. Data are representative of more than 4 repeat assays.

**(C-D)** Multiplex fluorescent ELISA in FEP MCF devices. **C** Assay schematic. **D** The 10 capillaries in a reel of a FEP MCF were individually coated with the following antigens: (a) negative (buffer) control, (b) positive control mouse IgG, (c) antigen FLAG peptide, and (d) Hepatitis B Core antigen, in the pattern indicated, and then trimmed in individual 50-mm sections for testing the indicated samples: (1) buffer, (2) anti-HB CAg, and (3) anti-FLAG. The fluorescence signal was imaged after 15 min by confocal microscopy. A representative plot of the fluorescence detected is shown for each sample, and each capillary is scored to indicate positive or negative signal. Similar results were seen with replicate test strips, and similar findings were observed in a repeat experiment with a different pattern of detection channels and antigens.