Supplementary information

**Current trends in flow cytometry automated data analysis software**

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Supplementary Table 1. Software identified in literature survey, ranked according to number of citations.

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| --- | --- | --- | --- | --- | --- |
| **Rank** | **Software name** | **Number of citations** | **Abbreviation** | **Purpose** | **Reference** |
| 1 | viSNE | 294 | visualization tool for high-dimensional single-cell data based on the t-Distributed Stochastic Neighbor Embedding (t-SNE) algorithm | Visualisation of high-dimensional single-cell data via dimensionality reduction | (1) |
| 2 | SPADE | 236 | Spanning-tree progression analysis of density-normalized events | Visualisation of high-dimensional cytometry data by downsampling, clustering and a minimal spanning tree | (2) |
| 3 | t-SNE | 194 | t-Distributed Stochastic Neighbor Embedding | Dimensionality reduction for visualisation | (3) |
| 4 | Phenograph | 156 |  | Model cellular phenotypes | (4) |
| 5 | FLAME | 107 | Flow analysis with automated multivariate estimation | Identify cell populations by multivariate mixture modelling | (5) |
| 6 | Citrus | 87 | Cluster identification, characterization, and regression | Identification of stratifying cellular subpopulations | (6) |
| 7 | FlowSOM | 75 | Self-organizing map | Clustering data into self-organizing maps and visualisation by minimal spanning trees | (7) |
| 8 | DensVM | 70 | Density-based clustering aided by support vector machine | Cell population identification and classification | (8) |
| 8 | flowMeans | 70 |  | Cell population identification by k-Means based clustering | (9) |
| 10 | ACCENSE | 66 | Automatic Classification of Cellular Expression by Nonlinear Stochastic Embedding | Identification of cell subpopulations through t-SNE dimensionality reduction and density-based partitioning | (10) |
| 10 | Wanderlust | 66 |  | Developmental trajectory detection | (11) |
| 12 | FLOCK | 63 | FLOw Clustering without K | Cell population identification by density-based clustering | (12) |
| 13 | flowClust | 58 |  | Cell population identification by multivariate t-mixture modelling with Box-Cox transformed data | (13) |
| 14 | flowMerge  | 53 |  | Cell population identification using flowClust and a cluster merging algorithm | (14) |
| 15 | X-Shift | 50 |  | Exploration of single-cell data by clustering (K-nearest neighbour density estimate) and visualisation by divisive marker trees and force-directed layouts | (15) |
| 16 | SamSPECTRAL | 48 |  | Cell population identification by spectral clustering and sampling | (16) |
| 17 | flowPeaks | 42 |  | Cell population identification by K-means clustering and density peak finding | (17) |
| 17 | OpenCyto | 42 |  | Mimicking manual gating based on hierarchical automated gating pipelines | (18) |
| 19 | Mixture model | 41 |  | Cell population identification by mixture modelling | (19) |
| 20 | HPDGMM | 31 | Hierarchical Dirichlet Process Gaussian Mixture Model  | Rare event detection and cell subset alignment across multiple samples | (20) |
| 21 | flowDensity | 26 |  | Mimicking manual gating based on cellular density distributions | (21) |
| 22 | SWIFT | 24 | Scalable Weighted Iterative Flow-clustering Technique | Identification of rare cell populations based on Gaussian mixture model-based clustering | (22,23) |
| 23 | HSNE | 16 | Hierarchical Stochastic Neighbor Embedding | Visual exploration of the hierarchy in cytometry data | (24) |
| 24 | Misty Mountain | 15 |  | Cell population identification by density contour clustering | (25) |
| 25 | COMPASS  | 14 | Combinatorial Polyfunctionality Analysis of Single Cells | Identification of cell subsets correlated with clinical outcomes | (26) |
| 25 | FlowFP | 14 | Fingerprinting for Flow Cytometry | Generation of multivariate distribution 'fingerprints' | (27) |
| 25 | immunoClust  | 14 |  | Cell population identification by iterative model-based clustering | (28) |
| 28 | JCM | 12 | Joint Clustering and Matching | Cell population identification and matching across a batch of samples | (29) |
| 29 | flowType/RchyOptimyx | 11 |  | Cell population identification by partitioning and correlation with clinical outcomes | (30) |
| 30 | ASPIRE | 10 | Anomalous sample phenotype identification with random effects | Identification of anomalous samples with random effects | (31) |
| 30 | DeepCyTOF | 10 |  | Cell classification by deep learning | (32) |
| 32 | AutoGate | 9 |  | Sequential selection of cell subsets and visualisation | (33) |
| 33 | FloReMi | 8 | Flow Density Survival Regression Using Minimal Feature Redundancy | Survival time prediction | (34) |
| 34 | CCAST  | 7 | Clustering, Classification and Sorting Tree | Isolation of homogenous subpopulations | (35) |
| 35 | flowLearn | 6 |  | Identification and quality checking of cell populations | (36) |
| 36 | ACDC | 5 | Automated Cell-type Discovery and Classification | Cell population discovery and classification | (37) |
| 37 | Competitive SWIFT  | 4 | Scalable Weighted Iterative Flow-clustering Technique | Sample comparison by competitive clustering | (38) |
| 37 | SPADE 3 | 4 | Spanning-tree progression analysis of density-normalized events | Visualisation of high-dimensional cytometry data by downsampling, clustering and a minimal spanning tree | (39) |
| 39 | cytometree | 2 |  | Cell population identification based on a binary tree algorithm | (40) |
| 39 | DAFi | 2 | Directed Automated Filtering and Identification of cell populations | Cell population identification based on recursive data filtering and clustering | (41) |
| 39 | diffcyt | 2 | Differential discovery in high-dimensional cytometry via high-resolution clustering  | Differential discovery analysis | (42) |
| 39 | FlowVIEW | 2 |  | Quantification of cell populations via a supervised learning approach | (43) |
| 39 | LDA | 2 | Linear discriminant analysis | Prediction of cell populations | (44) |
| 44 | ECLIPSE | 1 | Elimination of Cells Lying in Pattern Similar to Endogeneity | Identification of disease-specific cells | (45) |
| 44 | NPflow | 1 | Bayesian Nonparametrics for Automatic Gating of Flow-Cytometry Data | Cell population identification by model-based clustering | (46) |
| 44 | PSM with GemStone | 1 | Probability State Modeling | Cell population identification via a probability-based approach | (47) |
| 44 | SOPHE | 1 | Second order polynomial histogram estimators | Cell population identification by data binning  | (48) |
| 48 | PHATE | 0 | Potential of heat diffusion for affinity-based transition embedding | Dimensionality reduction for visualisation | (49) |
| 48 | SIC  | 0 | Subset Identification and Characterisation | Subset identification and characterisation pipeline | (50) |
| 48 | SigClust | 0 | Signature based Single-Cell Clustering | Cell population identification using phenotypic signatures | (51) |
| 48 | UMAP | 0 | Uniform Manifold Approximation and Projection | Dimensionality reduction for visualisation | (52) |

Supplementary Table 2. Survey questions and answer response choices.

|  |  |
| --- | --- |
| Q1 | In a typical week, how many hours do you spend analysing (gating) flow cytometry data on a computer?* Over 30 hours
* 20-30 hours
* 10-20 hours
* 1-10 hours
* Less than 1 hour
 |
| Q2 | How often do you use automated flow cytometry data analysis software to identify cell populations?* Never – I only use manual gating to identify cell populations.
* Rarely – I mainly use manual gating, but occasionally use automated tools.
* Sometimes – I split my analysis equally between manual and automated cell population identification.
* Usually – I mainly use automated tools, but occasionally use manual gating.
* Always – I use automated tools for all my data analysis.
 |
| Q3 | Which software do you use for manual cell population identification? (Check all that apply)* BD FACS Diva
* BD FACS Canto
* BD FACSuite
* BD CellQuest
* FCS Express
* FlowJo
* FlowLogic
* Infinicyt
* Kaluza
* Navios
* VenturiOne
* WinList
* Other (please specify)
 |
| Q4 | Which software do you use for automated cell population identification? (Check all that apply)(Answer choices as in Supplementary Table 1) |
| Q5 | Which automated data analysis software are you aware of, but do not currently use? (Check all that apply)(Answer choices as in Supplementary Table 1) |
| Q6 | When using automated data analysis software, which of the following factors is most important to you?(Answer choices for each factor: Not at all important/ Not so important/ Somewhat important/ Very important/ Extremely important)* Appearance of software
* Availability of software
* Compatibility with other software
* Level of technical support
* Seen in literature
* Software data output quality
* Software reputation
* Software speed
* Cost
* Other (please specify)
 |
| Q7 | Please select the automated cell identification tool you are most familiar with. (Answer choices as in Supplementary Table 1) |
| Q8 | Please mark your response about the software in Q7 to the following statements:(Answer choices for each statement: Strongly disagree/ Disagree/ Neither agree nor disagree/ Agree/ Strongly agree)1. I think that I would like to use this software frequently.
2. I found the software unnecessarily complex.
3. I thought the software was easy to use.
4. I think that I would need the support of a technical person to be able to use this software.
5. I found the various functions in this software were well integrated.
6. I thought there was too much inconsistency in this software.
7. I would imagine that most people would learn to use this software very quickly.
8. I found the software very awkward to use.
9. I felt very confident using the software.
10. I needed to learn a lot of things before I could get going with this software.
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Supplementary figures



Supplementary Figure 1. Software citations by computational method. Citations by algorithm class, showing unsupervised methods with graphical user interfaces (GUIs) only.



Supplementary Figure 2. Results of a survey of clinical laboratories on the use of automated flow cytometry software.

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