Features of free software packages in flow cytometry: a comparison between four non-commercial software sources

Fazel Sahraneshin Samani · Jodene K. Moore ·
Pardis Khosravani · Marzieh Ebrahimi

Received: 9 March 2013 / Accepted: 15 June 2013 / Published online: 10 July 2013
© Springer Science+Business Media Dordrecht 2013

Abstract Flow cytometers designed to analyze large particles are enabling new applications in biology. Data analysis is a critical component of the process FCM. In this article we compare features of four free software packages including WinMDI, Cyflogic, Flowing software, and Cytobank.

Keywords Data analysis · WinMDI · Cyflogic · Flowing software · Cytobank

Introduction

Flow cytometry is a versatile analytical platform, capable of high speed, quantitative measurements of cells, other organisms, and particles. This technology allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of up to thousands of particles per second. Therefore, it is

routinely used in the diagnosis of health disorders, in basic research, and in clinical practice.

Regardless of instrument type, lab situation, type of experiment, etc., data analysis is a critical component of the process, because the output and interpretation of flow cytometry experiments can have different empirical and even clinical outcomes, depending on subjective strategies for gating used during the analysis. Many factors affect the ability to perform sound and reproducible data analysis. One is having a robust software package that can perform comprehensive post-acquisition analysis and graphic representation of the data. Many companies are working to develop cutting-edge software for flow cytometry data analysis.

In 2008, the International Society for Analytical Cytology (ISAC) and Data Standards Task Force (DSTF), in collaboration with computational statisticians, bioinformaticians, software developers, and instrument manufacturers, created the “Minimum Information about a Flow Cytometry Experiment” (MIFlowCyt) (Lee et al. 2008). In 2012, a database of flow cytometry experiments, named Flow Repository (2), was developed. This database can query and download data that are collected and annotated according to the MIFlowCyt standard. In addition, Mario Roederer recently proposed supplementary guidelines that were deemed to have sufficient experimental method details for publications in print, and presentation of data on slides (Alvarez et al. 2010; Roederer et al. 2004).

F. Sahraneshin Samani (✉) · P. Khosravani ·
M. Ebrahimi

Department of Stem Cells and Developmental Biology,
Cell Science Research Center, Royan Institute for Stem
Cell Biology and Technology, ACECR,
P.O. Box 19395-4644, Tehran, Iran
e-mail: fazel.sahraneshin@yahoo.com

J. K. Moore
Department of Systems Biology, Harvard Medical School,
Boston, MA, USA

Most software designed for flow cytometry data analysis can be classified according to general operating characteristics, i.e., compatibility with computer operating systems, and functional characteristics, referring to the capabilities of various analysis and graphical representation tools. Thus, users have several choices of software that can be selected on the basis of their particular type of experiment, analysis needs, and endpoints for graphical data output.

In this article we compare features of four free software packages including WinMDI, Cyflogic, Flowing software, and Cytobank. To evaluate each of these programs a panel of 10 different CD markers was surveyed in 100 umbilical cord blood samples.

Materials and methods

We prepared mononuclear cells (MNC) of umbilical cord blood with Ficoll gradient density ($d: 1.077 \text{ g/cm}^3$, Amersham Biosciences, Uppsala, Sweden) (Shayan et al. 2012). Cells were stained at 1×10^6 cells with antibodies for FCM staining: Anti-CD56/RPE, Anti-CD3/FITC-CD19/RPE, Anti-CD14/FITC, Anti-CD38/FITC, Anti-CD34Class III/FITC, Anti CD16/RPE, Anti-CD45RO/RP, Anti CD41/FITC, Anti HLA-DR/RPE (all from DAKO; Hamburg, Germany at 4°C and in the dark. After two washes with PBS-(Sigma-Aldrich, St. Louis, MO, USA) the cells were re-suspended in PBS containing 1 % paraformaldehyde and immediately analyzed using an unmodified BD FACSCalibur (Becton–Dickinson, San Jose, CA, USA) flow cytometer with a 488 nm laser and the signal was captured by a band pass filter BP-585/42 for PE and 530/30 for FITC. Unstained cells, single fluorochrome stained cells, and cells stained as fluorescence-minus-one (FMO) controls were used to set-up the machine. The data generated were then analyzed by the software listed above, in order to produce comprehensive tables detailing both common and unique features of each software package. WinMDI, developed by Joe Trotter, was downloaded from <http://facs.scripps.edu/software.html>. Flowing Software, developed by Perttu Terho, was downloaded from <http://www.flowingsoftware.com>. Cyflogic, developed by CyFlo Ltd, was downloaded from www.Cyflogic.com. Cytobank, developed by Nikesh Kotecha, Jonathan Irish, and Peter Krutzik, was downloaded from <http://cytobank.org>.

Results and discussion

The results of the software evaluation are summarized in Table 1. The software was ranked on the basis of applications and features available in the software. We categorized the features into the following groups: common analysis tools and graphical data representation tools. In addition, we listed general characteristics of each software package. All features and characteristics of the software described in this paper reflect the current versions of each software package, downloaded from the above mentioned web sites.

To evaluate the features of each software package, we utilized the data generated from the 10 CD Markers panel used on the cord blood samples. An identical gating strategy was utilized for the analysis of the data in each program that only 2 CD markers are presented (Fig. 1). To simplify presentation of our findings, we describe attributes of each software, based on its general characteristics, capabilities of its analysis tools and graphical data representation tools (Table 1).

WinMDI

WinMDI was one of the first non-commercial, analytical software packages available to flow cytometrists. It was released in the 1990s; it has basic features for analysis and graphical presentation of flow cytometry data, and is compatible with Windows OS. One common problem that users encounter with WinMDI is unexpected freezing of the program. This can happen at any step prior to having saved the data, resulting in loss of analysis performed up to that point. Also, this software is limited to analysis of fcs 2.0 files.

Cyflogic

Cyflogic, released in 2008, is more advanced than WinMDI and has special features like post-acquisition compensation, crash recovery, savable work sheets, and 3D plots that are easy to manipulate. Its limitations include Mac OS incompatibility and software crashes when multiple plots are drawn. We rate this as semi-powerful software.

Flowing software

Flowing software, released in 2009, is even more sophisticated than Cyflogic due to its advanced

Table 1 Comparison of the features four different software according to General software characteristics, Analysis tools and Graphical data representation tools

Features		WinMDI Version 2.9	Flowing Software Version 2.5.1	Cylogic Version 1.2.1	Cytobank Version 2-6-1-1
General software characteristics	Developed in	1993	2009	2008	2010
	Current version release date	06.19.2000	10.10.2012	12.17 2010	11.02.2012
	Windows OS	95/98/2000	Vista/XP/7	XP/Vista/7	Web-based
	Compatible with Mac OS	×	×	×	Web-based
	Program size	2027 Kb	755 Kb	2.62 MB	Web-based
	Java	×	×	×	✓
	Tutorial film available	×	✓	×	✓
	Tutorial PDF available	✓	×	✓	✓
	Crashes with multiple plots	✓	×	×	×
	Sudden unpredictable restarts	✓	×	×	✓
	Sharing with collaborators	×	×	×	✓
	User friendly	+	+++	++	+++
	FCS version(s) allowed	FCS 2.0	FCS2.0 FSC3.0	FCS2.0 FSC3.0	FCS2.0 FSC3.0
Analysis tools	Reporting	✓	✓	✓	✓
	Direct export to Excel	×	✓	✓	✓
	Copy/paste to PowerPoint slides	×	×	×	✓
	Batch processing	×	✓	✓	✓
	Formula generation	×	✓	×	×
	Floating quadrants	×	✓	×	×
	Image collector (well/plate)	×	✓	×	✓
	Image collector	×	✓	✓	✓
	Types of gates	Polygon Ellipse Quadrant	Polygon	Polygon	Polygon Ellipse Quadrant
	Hierarchical Gating	×	×	×	✓
	Kinetics Analysis	✓	✓	✓	✓
				Except Median	
	Cell cycle analysis with modeling algorithms	×	×	×	×
	Proliferation Analysis	×	×	×	×
	Intuitive software	✓	✓	✓	✓
	Single click plot manipulation	×	×	×	✓
	Save worksheet	×	✓	✓	✓
Graphical data representation tools	Intensity plot	×		×	×
	Density plot	✓	✓	✓	✓
	Counter plot	✓	×	×	
	Dot plot	✓	✓	✓	✓
	Histogram	✓	✓	✓	✓
	Overlay histogram	Difficult	Very easy	Easy	Very easy
	3D plot manipulation	Difficult	×	Easy	×

Table 1 continued

Features	WinMDI Version 2.9	Flowing Software Version 2.5.1	Cytflogic Version 1.2.1	Cytobank Version 2-6-1-1
High-content analysis	×	96 well plates	×	Unlimited
Lin/log axis change	×	✓	✓	×
Parameter Labels	✓	✓	✓	×
Change event number displayed	✓	✓	×	×
Virtual gain	×	✓	×	×

✓ designates features that are available in the software

×

+ denotes the grade of the available characteristics

Fig. 1 Schematic images of data analysis using WinMDI, Cytflogic, Flowing Software and Cytobank. The arrow indicates the progress of developed software with the aforementioned features

analysis tools. High-throughput data analysis, batch processing, calculation tools, an image collector, floating quadrants, post-acquisition compensation, and intensity plots constitute unique features of this software. Limitations include lack of contour and 3D plots, cell cycle modeling, and incompatibility with Mac OS (Table 1).

Cytobank

The Cytobank effort started in 2010. It is a web-based application that analyzes and stores all flow cytometry files on a central server and is compatible with both

Windows and Mac platforms. Anyone can access and analyze their experiments, view or edit experiments and figures, and drill down through the underlying data. Scientists can upload a folder of fcs files, create publication-quality figures, and then generate a URL link to the figures and raw data files (Kotecha et al. 2010). Some other novel features of this software include heat maps, phosphorylation analysis, hierarchical gating, and the ability to share results and data with collaborators all over the world (Table 1). One of the most convenient features of this software is single-click plot manipulation. Dot plots can be converted to contour plots or density plots with one click. However,

limitations of this software include the requirement for Internet access, and the utilization of the java interface, which may present problems for some users (Table 1).

Conclusion

The progress of the science of cytometry, and specifically flow cytometry, requires advances in analytic software. The use of free software is common among researchers. Regardless of being commercial or non-commercial, the software should be user-friendly, provide a comprehensive variety of plots, statistical analysis, publication-quality graphical outputs, a tutorial film or PDF, and simple data export to Excel or PowerPoint. We compare various features of four different free software packages, including WinMDI, Cyflogic, Flowing Software, and Cytobank (Table 1). These analysis programs share many common features that are adequate for performing routine data analysis, however, only Flowing Software and Cytobank have the capability of performing high-throughput analysis. In addition, Cytobank takes advantage of web-based technology, promoting the sharing of data and global collaborations. In the future, software designers, in collaboration with ISAC, should develop guidelines that would include necessary features for a comprehensive, intuitive and free analysis software with the ability to perform graphic data presentation of publication quality, easily export statics and figure into Excel and PowerPoint, and containing cell cycle modeling and proliferation

analysis tools, similar to ModFit software, the gold standard for cell cycle analysis. Also, the ability to display and manipulate parameter PMT voltages, draw curly gates, and convert data into bar charts would be additional advantageous features for users. The arrow indicates the progress of developed software with aforementioned features.

References

- Alvarez DF, Helm K, Degregori J, Roederer M, Majka S (2010) Publishing flow cytometry data. *Am J Physiol Lung Cell Mol Physiol* 298:L127–L130. doi:[10.1152/ajplung.00313.2009](https://doi.org/10.1152/ajplung.00313.2009)
- Kotecha N, Krutzik PO, Irish JM (2010) Web-based analysis and publication of flow cytometry experiments. *Curr Protoc Cytom.* 53:10.17.1–10.17.24. doi:[10.1002/0471142956.cy1017s53](https://doi.org/10.1002/0471142956.cy1017s53)
- Lee JA, Spidlen J, Boyce K, Cai J, Crosbie N, Dalphin M, Furlong J, Gasparetto M, Goldberg M, Goralczyk EM, Hyun B, Jansen K, Kollmann T, Kong M, Leif R, McWeeney S, Moloshok TD, Moore W, Nolan G, Nolan J, Nikolich-Zugich J, Parrish D, Purcell B, Qian Y, Selvaraj B, Smith C, Tchuvatkina O, Wertheimer A, Wilkinson P, Wilson C, Wood J, Zigon R, Scheuermann RH, Brinkman RR (2008) MIFlowCyt: the minimum information about a Flow Cytometry Experiment. *Cytometry A J Int Soc Anal Cytol* 73:926–930. doi:[10.1002/cyto.a.20623](https://doi.org/10.1002/cyto.a.20623)
- Roederer M, Darzynkiewicz Z, Parks DR (2004) Guidelines for the presentation of flow cytometric data. *Methods Cell Biol* 75:241–256
- Shayan N, Ebrahimi M, Beiki B, Janzamin E (2012) A non-rotational, computer-controlled suspension bioreactor for expansion of umbilical cord blood mononuclear cells. *Biotechnol Lett* 34:2125–2131. doi:[10.1007/s10529-012-0993-5](https://doi.org/10.1007/s10529-012-0993-5)