Flow Cytometry analysis software

Limited freeware available

- Flowing software
  - http://www.flowingsoftware.com
- Redmatter
  - https://www.redmatterapp.com/#/home
- Cyflogic
  - http://www.cyflogic.com/
- FCSalyzer
  - https://sourceforge.net/projects/fcsalyzer/

Remark

- Freeware is OK for very basic analysis
Flow Cytometry analysis software

Open source programs available

- Bioconductor:
  - open-source, community-driven project based on the statistical programming language R
  - tools for next-generation DNA sequencing and mass spectrometry, high-throughput screening and gene expression
  - More than 40 packages related to flow cytometry
    - flowCore, flowViz, flowStats
    - FlowSOM: algorithm developed at IRC

https://www.r-project.org/
http://opencyto.org/

Remark

- Some of the developed tools are available as plugin in FlowJo
  http://docs.flowjo.com/d2/plugins/installing-plugins/
  http://exchange.flowjo.com/

Commercial analysis software

Manufacturer software

- FACS DIVA software       BD Biosciences
- FACS Suite              BD Biosciences
- Summit software         Beckman Coulter
- Kaluza                  Beckman Coulter
- All kinds of other acquisition software Partec, Miltenyi, …

Third party software designers:

- Cytobank http://www.cytobankinc.com/
- FCS Express 5 http://www.denovosoftware.com/
- Flowlogic http://www.inivai.com/
- Infinicyt http://www.infinicyt.com/
- Flowjo http://www.flowjo.com/
- …
Why FlowJo X at VIB?

- Very intuitive program and easy to learn the basics
- Easy to produce high quality graphs for publishing
- For the moment it is the preferred software program by the flow community
- Historic reason: big FlowJo usergroup at VIB-UGent Center for Inflammation Research
- Centralized licensing at VIB-IRC
- We centralized the licenses at the VIB level so we can get better prices for all VIB scientists
- Licenses are distributed via the VIB Bioinformatics Core

Source: http://flowjo.typepad.com/the_daily_dongle/page/1/
Installing FlowJo

How to install Flowjo

Before January 2016 we can offer FlowJo V9 for a price of 175k per activation per year. Be aware that you need to have a valid activation number. If you want to use the software for research, please contact FlowJo before the offer is lifted.

Some things you need to follow:

2. Install FlowJo.
3. Running the FlowJo, license information will appear shortly for further instructions if the licensing is successful.
5. If you've not yet registered your computer, enter your username and password.
6. Click Next and complete the license agreement.
7. Select the installation options and click Next.
8. Click Finish and restart FlowJo.
9. The software is now installed on your system.

FlowJo X

FlowJo X History

- First written by Mario Roederer and Adam Treister
- 1997 first commercial Flowjo version (Mac) released by Treestar
- 2010 release of Flowjo versioning
  - running on iOS only and still gets updates
- 2012 release of Flowjo X
  - first cross-compatible Flowjo version based on another architecture (available for Windows, Linux and iOS)
- Small software company based in Ashland Oregon (USA)
- Since 2013 Mike Stadnisky became CEO and Treestar became Flowjo LLC
FlowJoX on the internet

• Very good training movies on You Tube channel
  • https://www.youtube.com/user/FlowJoMedia

FlowJoX on the internet

• FlowJo blog: “The daily dongle”
  • http://flowjo.typepad.com/
FlowJoX on the internet

- Flowjo tutorials available
  - https://www.flowjo.com/learn/flowjo-university/flowjo

FlowJoX on the internet

- Flowjo 10.2 manual
  - http://docs.flowjo.com/

- Twitter
  - https://twitter.com/FlowJoNow
  - https://twitter.com/immflection
Flowcytometry info on the internet

- Google+ community
- Purdue Flow email list
  - http://www.cyto.purdue.edu/hmarchiv
- Chromocyte website
  - https://www.chromocyte.com/

Flow cytometry info on the internet

Howard Shapiro: Practical Flow Cytometry

Flow cytometry companies
  - All good informative websites
  - Lots of them have spectraviewers
  - Protocols available
  - Info on fluorochromes
  - Webinars
  - CD marker info
Flow Companies

- BD biosciences
  - http://wwwbdbiosciencescom/eu/home
- Biolegend
  - http://wwwbiolegendcom/
- ThermoFisher Scientific
- Miltenyi
- Tonbo biosciences
  - http://wwwtonbobiocom/
- ...

Flow Companies

BD biosciences
- http://wwwbdbiosciencescom/eu/home
Flow Companies

Biolegend
  • http://www.biolegend.com/

Flow Companies

ThermoFisher Scientific
  • https://www.thermofisher.com/be/en/home.html
Flow Companies

Miltenyi


Flow Companies

Tonbo biosciences (VWR)

Panel design for flow cytometry

- Know the configuration of your flow cytometer
- Most difficult part of multiparameter flow cytometry
  - Consider matching Bright colors with Low expression markers
  - Consider matching Dim colors with High expression markers
- Reduce data spread
  - Check resolution impact matrix (BD biosciences)
- Reduce spillover
  - Compensation presentation
- Lots of info online (e.g. Fluorofinder, BD biosciences, ...)
- Optimized Multicolor Immunofluorescence Panels (OMIP): Cytometry part A
Panel design tools

- Fluorofinder
  - https://fluorofinder.com/
  - Access to all catalogues of the major antibody suppliers
  - IRC Flow Core: all our cytometer configurations are online

Fluorofinder
Panel design tools

- BD biosciences (NEW)
  - http://www.bdbiosciences.com/eu/slgps/

Cytometer configuration
Flow essentials

• Path to reliable data:

1. Know (and optimize) your instrument
2. Know (and optimize) your panel
3. Know (and optimize) your reagents
4. Know (and rely on) your controls
5. Know (and annotate) your acquisition
6. Know (and validate) your data files
7. Know yourself (and plan your experiments accordingly)

By Dr. Adrian Smith, Sydney ACF

Flow essential controls

• 5 colour exp?
  - Bring unstained cells
  - Bring 5 single stained cell controls (voltage setting)
  - Bring 5 single stained bead controls (compensation calculation)
  - Use FMO controls = Fluorescence Minus One
  - Isotype controls are highly debatable...
About data

• Garbage in = Garbage out
  ▶ Bad sample preparation
  ▶ Wrongly titrated antibodies
  ▶ Not optimized panel design
  ▶ Bad voltage settings
  ▶ No single stain controls
  ▶ No gating controls (Fluorescence Minus One)

• Label your samples as good as possible during acquisition
  ▶ Give your experiment a name
  ▶ Label your antibodies+fluorochromes
  ▶ Label your samples

Flow Data file standards

FCS 3.0 and FCS 3.1
• All the raw data collected by flowcytometers is saved according to the international standards
• All parameters are saved LINEAR
• Gating strategy is never saved within a file
• Acquisition compensation matrix is also included
• Lots of keywords are saved within file

More info?
• http://www.flowjo.com/v8/html/fcskeys.html
• ISAC website (International Society for Advanced Cytometry)
Let’s start!