#### Cytometry Data Analysis in FlowJo V10



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#### **Outline-Part I**

- What is FlowJo?
- Navigating the V10 Workspace
- Graphs, Gating and Ancestry
- The Table Editor
- The Layout Editor
- Batching and Exporting Graphics





#### What is FlowJo?

- An integrated environment for viewing and analyzing flow cytometry data.
- Uniformly analyze whole experiments encompassing many related samples.
- Sophisticated tools allow generation of graphs and statistical reports, driving discovery of biological mechanisms.



#### The Leading Analysis Software





#### The FlowJo V10 Workspace

• A graphical interface to organize your data.

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Ribbon Tabs and Bands

#### Groups and Group Analysis

#### Samples and Sample analysis



#### **Ribbons, Tabs and Bands**

• Ribbon organization allows easy visual navigation of workspace functions.



- Tabs group similar Bands together.
- Bands group similar Actions together.



#### **Customizing Ribbons**

#### Click on the Ribbon icon to configure.



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#### **Importing Data**

Two possible methods:

## Drag and drop into samples pane Click Add Samples button

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### **Samples and Sample Analysis**

- Displays the sample list and associated analysis of the currently selected group.
- Statistic and #Cells columns are displayed by default. Additional information can be displayed as columns. (Workspace Tab → Add Keywords or Configure Tab → Edit Columns)

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 Double click on a sample to open a Graph Window and add gates.

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### **The Graph Window**

• Facilitates data visualization and gating.



- Several different plot types are available to display flow data.
- Click on the Options Menu below the graph image and select Graph Type from the dropdown menu.



#### **Graph Display Options**





#### **Gating tools**

• Are located at the top left in a Graph Window.

Gating Tools



- Gates can always be modified or removed, so don't be shy.
- Explore the gating options and pick what works best for you.



#### **Biexponential Transformation**

- to Better Visualize Data After Compensation



- Transformation is a bi-exponential display tool by introducing linear scales into your axes to better visualize your data.
- It does not change your data!

Herzenberg et. al. Nature Immunology 2006



Comp-PerCP-A:: CD45RA

Q3 12.1%

102

#### **Transforming Data**

- Your data may initially look 'squished'.
- Click the Transformation button **T** and Select Customize Axis... to change the visual display.



# Gating: Define Population(s) of Interest (POI)

#### **\*Gating basic**

- Gate on population of interest and gate out population not of interest
- Gate out debris, dead cells, doublets/cell aggregates, Focus on POI
- Is both objective and subjective

#### **\*Gating tools**

- Hierarchical gating



#### **Gating Strategy**



Plot on FSC&SSC

#### Gate out dead cells



#### **Doublets Discrimination**

What are doublets and why are they harmful?

- Doublets are two cells stick together
- Doublets can lead to higher background and false positive and especially harmful for cell cycle analysis.



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#### **Hierarchical Gating**

- When you create a gate on a sample, FlowJo shows you this gate (subset population) as a genealogical tree.
- The the subset below is the child, subset above is the parent.





#### **The Table Editor**

- A tool for creating statistical reports.
- Click on the Table Editor icon.
- Drag populations from sample to Table Editor.



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### With in Table Editor

• Again, the Table Editor has its own customizable Ribbon with Tabs and Bands to organize actions.

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• Specify the group you wish to batch, and how to iterate the batch process, then in the Output band, specify where you want the batch output to go.



## **The Layout Editor**

- A tool for creating graphical reports.
- Click on the Layout Editor icon.



Drag populations from a sample to Layout Editor.





### **Working in Layout Editor**

 Similar to the Workspace, the Layout Editor has its own customizable Ribbon with Tabs and Bands to organize actions.

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Text and Draw Tools

 Try clicking on the different tabs to see what types of actions are available.



#### Within Layout Editor

- Graphs can be organized and re-formatted.
- Statistics, keywords, text and even shapes or objects can be added to illustrate your analysis.



 We encourage you to explore the tools and display features available to improve the visualization of your data.



## **Working in Layout Editor**

- Click once on a graph or object to select it.
- Double Click a graph to change its properties.





- Right click the graph for even more options.
- Hold down shift and click on multiple graphs to select and edit their properties simultaneously.

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#### Batch Analysis of Layout Editor Graphics

- Batch operations perform repetitive analysis on multiple samples, applying the layout to an entire set of samples.
- Within the Layout Editor Tab, Look for the Create Batch Report icon.





#### Batch Analysis of Layout Editor Graphics

 Specify the group you wish to batch, and how to iterate the batch process (ex. by sample or keyword), then specify where you want the batch output to go. Finally, click on

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**Iteration Criteria** 

#### **Outline – Part II**

- Compensation
- Templates
- Special Analysis Platform:
  - Cell cycle
  - Proliferation (CFSE)
  - Cell Kinetics (Calcium flux)



#### Compensation

• Compensation corrects for spillover between fluorochrome emission spectra.



Compensation is essential for multicolor panels



#### **Compensation Controls-To Correct Spectra Spillover**



Slide credit to Dr. Holden Maecker Stanford University

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#### **Three Rules of Compensation**

- First, there must be a single stained control for every parameter in the experiment!
- In Addition, there are three *rules* for 'good' compensation controls.
  - 1. Controls need to be at least as bright or brighter than any sample the compensation will be applied to.
- 2. Background fluorescence should be the same for the positive and negative control.
- 3. Compensation controls MUST match the exact experimental fluorochrome.



#### **Compensation I**

 Select a Compensation Group in the groups window, then click in the task bar.



Compensation



## **Compensation II**

- Then fills in the positive and negative.
- Choose options from the dropdown lists for each parameter.
- Double click preview graphs to modify properties.

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APC-Cy7-A	D4	Bead Comps_APC-Cy7_F05.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/APC-Cy7-A+
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PE-TxRed-A	CD14_19	Bead Comps_PE-TR_F07.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/PE-TxRed-A+
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PE-A	KIR3DL1_S1	Bead Comps_PE_F08.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/PE-A+
APC-A	CD107alpha	Bead Comps_APC_F10.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/APC-A+
FITC-A	IFNgamma	Bead Comps_FITC_F11.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/FITC-A+
AARD-A	Dead	Cell Comps_AARD Comp1_C12.fcs	Cell Comps_Unstained Cells 2_B12.fcs:Size	Size/AARD-A+



#### **Compensation III**

#### • View Matrix... to Modify, Apply, Save or Preview







#### **Workspace Templates**

- Allows saving all analysis reports in your workspace without data.
- Streamlines repetitive analysis of multiple runs using the same staining panel(s).

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#### **Special Analysis Platforms**



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FlowJo University
 Webinars





#### **Additional Training Resources**

- Webinars on basic and advanced features of FlowJo, held on the 1<sup>st</sup> and 3<sup>rd</sup> Thursday of each month.
- Webinar Schedule can be found at <u>http://www.flowjo.com/webinars/</u>
- Technical Documentation for V10 can be found at <u>http://docs.flowjo.com/</u>
- The Daily Dongle provides tips, tricks and answers to common questions.
   <u>http://flowjo.typepad.com/</u>







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