Future Flow Cytometry Functionality

3/3/14

Flow Cytometry Setup
To-Do:

- Add ability to look at proteins using antibodies
- Add ability to look at different dyes/stains

In StarCellBio, we will need to format the setup table like the microscopy setup table where we can have a drop down menu to select type of analysis and conditions. The exception is that the Flow Cytometry setup table also needs to still have the live/dead radio buttons. Depending on which radio button is selected, then different options will be available in the Analysis drop down menu. For example, if using live cells with the dye PI, then the researcher will assess which cells in the population are alive and which are dead. If the researcher uses fixed cells with the dye PI, then the researcher will assess DNA content of the cells.

Options for Flow Cytometry Analysis are: Dye/Stain, Antibody-labeling
Options for Conditions: We currently only have PI for the Dye/Stain option. Other options will be able to be selected in the future using radio buttons (like microscopy).

Note: Can do more than one type of analysis using each sample. This means that you can add multiple analyses and that the setup table should be built to function like the microscopy setup table.

Flow Cytometry Data
Different types of data:

- Show data for each protein/dye as a histogram

We will need to decide whether each protein/dye is in a separate small-tabbed window or if the user can switch between looking at different proteins/dyes by changing the X-axis with a drop down menu.

Things to think about:

- Using dot plots instead of histograms to:
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    - Show forward scatter vs side scatter data as a dot plot. This should appear for each sample in the first small tabbed window.
    - Show data for looking at up to two proteins at the same time - (or one protein compared to a dye or FSC/SSC) - as a dot plot

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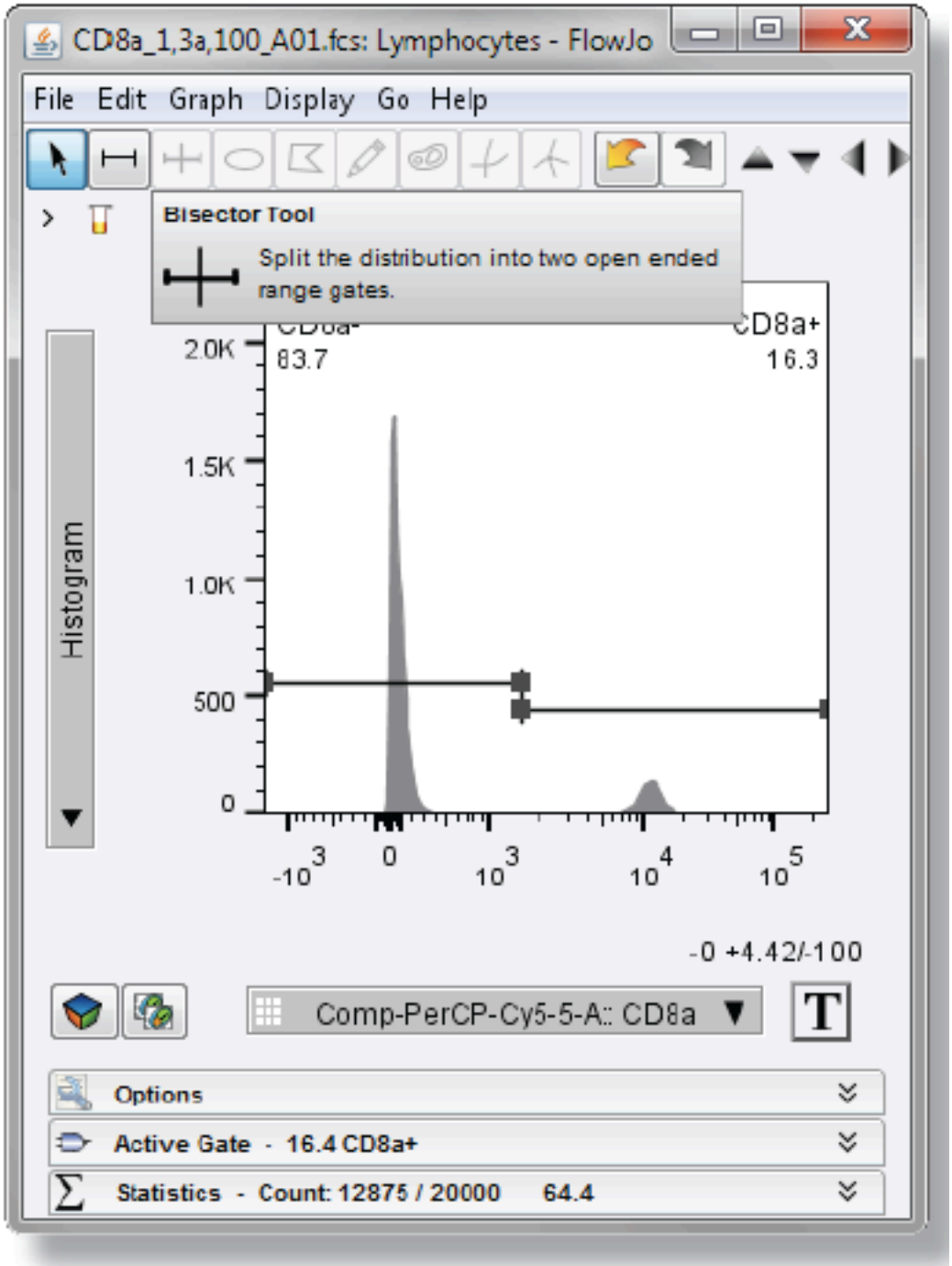
Flow Cytometry Analysis Tools
We currently have a single histogram tool. We would like to add in another additional analysis tool, right now with possibly two more later when we incorporate dot plots. We will have Jamie draw icon(s) for us for the new tools.

The analysis tools are as follows:

1. Draw two segments at the same time (using a modified histogram tool) - called a "bisector gating tool" in FlowJo.

This is more likely used for histogram plots, but can also be used for dot plots.

How does this work in StarCellBio?
To make this, the user will select the tool, click on the graph to start one end of the bisector gate, click again at the dividing "middle" point (although it doesn't have to be exactly in the middle), and then click again at the opposite end of the bisector gate. The appropriate part(s) of the gate will need to appear with each click. The percentage of cells within each gate will be displayed on the graph itself. In addition, the data can be displayed in a table with the percentage of cells in each of the two gates. The user can delete one set of (2) gates at the same time.



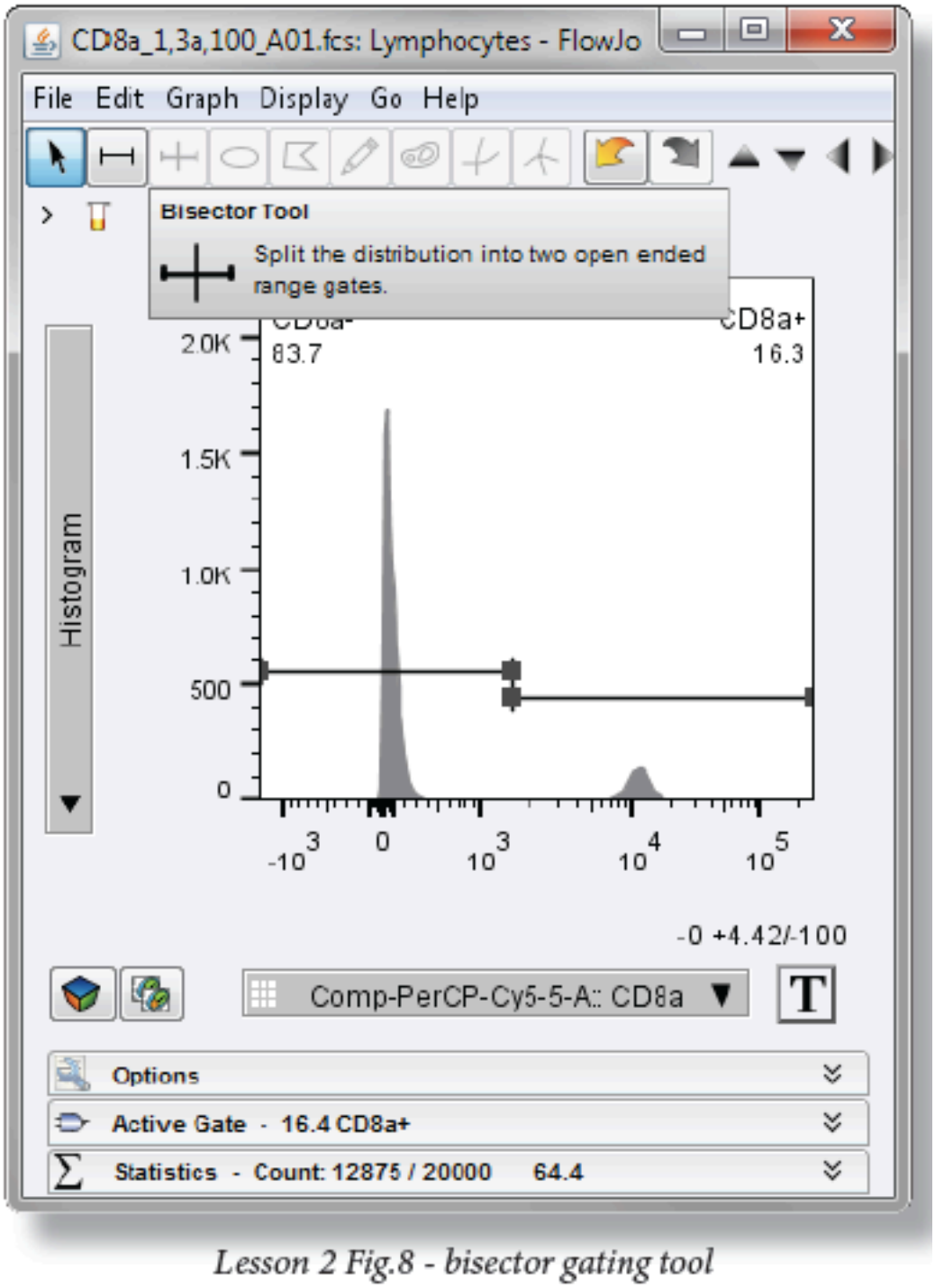
Lesson 2 Fig.8 - bisector gating tool

2. For Later - Divide the graph into 4 quadrants and the percentage of cells within each quadrant will be displayed. This is the third icon from the left.

This is the most relevant for dot plots, not histogram plots.

How does this work in StarCellBio?

The user will select the appropriate analysis icon, then when the user hovers over the graph, the two intersecting lines will appear. The intersection point will be directly underneath the cursor and will move as the cursor moves. To "set" the location of the intersecting lines, the user will click on the portion of the graph where the intersecting lines will be. The percentage of cells within each quadrant will then appear on the graph itself. Each quadrant should be labeled as 1, 2, 3, 4 (clockwise from upper left) and then the data can be displayed in a table on the right. Technically, a user may be able to draw more than one set of quadrants. The user can delete one set of (4) quadrants at a time.

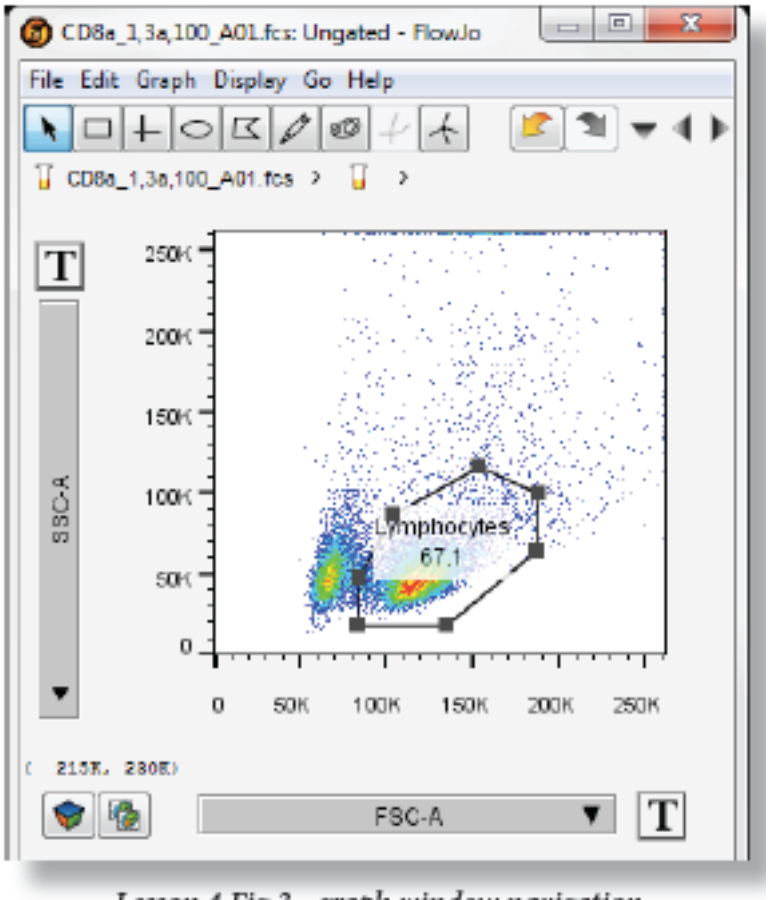


Lesson 2 Fig.8 - bisector gating tool

3. For Later - Draw "gates" around specific populations of cells using an "oval" or "polygon" shaped tool. These are the fourth and fifth icons from the left in the image above.

This is most relevant for dot plots, not histogram plots.

How does this work in StarCellBio?
The user will select the appropriate analysis icon, then click in the graph. For the oval icon, the user will click and drag until the oval is the desired size. The user should also be able to move the oval by clicking on the border of the oval once it is drawn and dragging it into position. For the polygon icon, the user will click for each point on the polygon shape until the area is "closed" (the user will need to double click to "close" the shape). The user can also move the polygon by clicking and moving the shape into position. The data is displayed within the shape on the graph itself (see screenshot). The gate can also usually be named. More than one gate can be drawn. We should display this data in a table with the name of the gate and the percentage of cells within the gate. The user can delete each gate as desired.



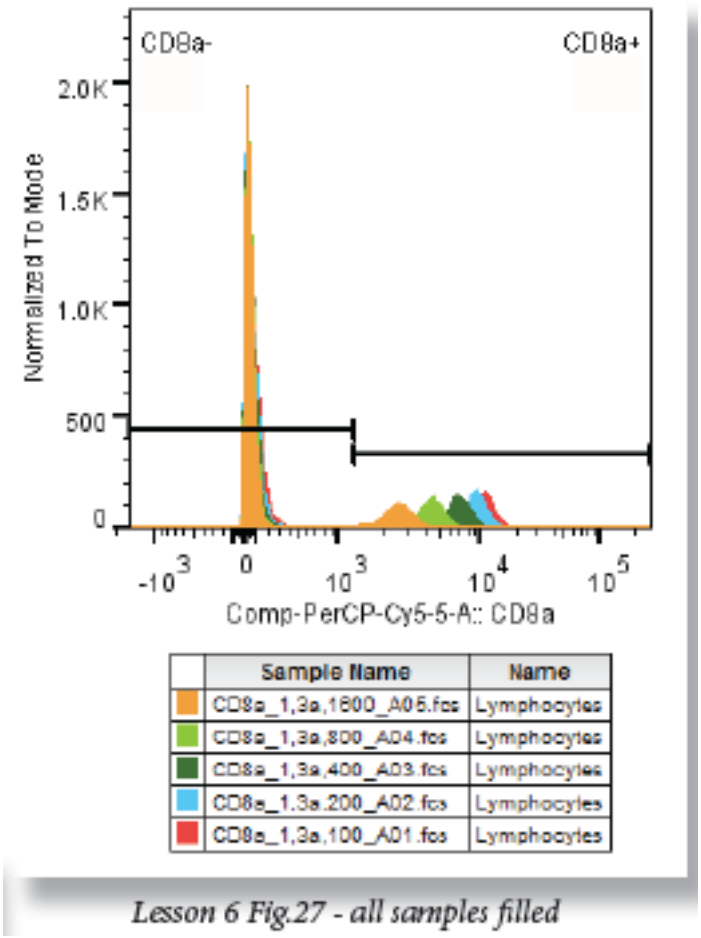
Lesson 4 Fig.3 - graph window navigation

Additional Analysis Functionality
1. Add in the ability to overlay histograms (Discussed in FlowJo's Basic Analysis User Guide starting on page 26).

- Would need to have the ability to turn on this feature and do this special kind of analysis.
- The user would need to select the data that he/she would like to overlay. In FlowJo, we usually drag the desired plots into another plot, but I'm not sure how to do this in StarCellBio considering

that all the samples are in the samples window, but each dye/protein/etc is in a different tab. Perhaps a drop down menu would work for the user to select the necessary data?

- There should be the ability to overlay somewhere between 3 and 5 histograms - depending on what you think is possible.
- Each histogram would be automatically color-coded and displayed in a table with the color-coded information.
- The user would then have access to the same histogram analysis tools (single gate and bisector gate) as for regular histogram analysis. Although if the user selects the single gate option (which is currently the only available analysis tool), we will no longer color-code the area indicated by a histogram. We will have to use a black line and label nearby the name of the histogram and the % of cells contained within the histogram.
- This is what overlaid histograms look like with the bisector gate analysis:



Lesson 6 Fig.27 - all samples filled

Flow Cytometry Functionality Development Timeline

Top Priority

1. **Develop the bisector gate analysis tool**
2. **(Low priority - Convert current histogram tool into color-coded lines indicating ranges (rather than vertical dotted lines that indicate and color-code entire segments))**

Second Priority

1. Update flow cytometry setup to make it more like the microscopy setup functionality. This will enable users to analyze proteins by antibody-labeling and will allow users to perform more than one type of analysis at the same time. Even though we are building in the functionality for users to perform multiple analyses on the same sample, we will limit their analysis to only looking at the data with histograms and at one dye/stain or one protein at a time.
2. Think about whether different types of flow cytometry analyses will be available in different small tabbed windows or in one window but accessible by selecting different options in a drop down menu on the X-axis

Third Priority

1. Develop histogram overlay tool

Fourth Priority

1. Develop ability to look at dot plots, including FSC vs. SSC and looking at more than one protein at the same time. In this case, the user will need to be able to select the protein/dye/FSC/SSC/histogram options using drop down menus on the x- and y-axes.
2. Develop the quadrant analysis tool
3. Develop the ability to draw oval/polygon gates.
4. Develop the ability for the user to analyze protein/dye/FSC/SSC/histogram options for only those cells contained within one gate. An example of this is that you may select cells instead of debris on the FSC vs. SSC plot and then only analyze "real" cells for protein expression and/or dye/stains.