AutoGate B cell and Macrophage Discovery Demo

Version 2, Updated June 2019

This demo teaches the use of AutoGate for discovery purposes. It assumes you have first been through our first demo, which teaches using AutoGate for diagnostic purposes: http://cgworkspace.cytogenie.org/GetDown2/demo/allergyDiagnosticDemo.pdf

This second demo is based on the publication

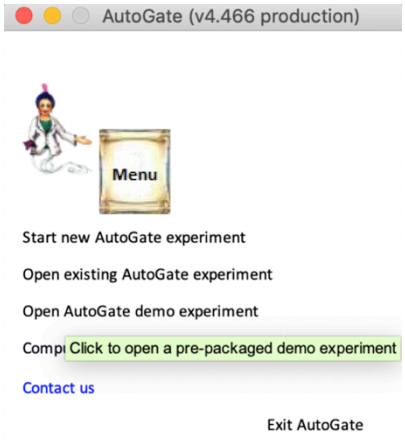
Two physically, functionally, and developmentally distinct peritoneal macrophage subsets
Eliver Eid Bou Ghosn, Alexandra A. Cassado, Gregory R. Govoni, Takeshi Fukuhara, Yang Yang, Denise M. Monack, Karina R. Bortoluci, Sandro R. Almeida, Leonard A. Herzenberg, and Leonore A. Herzenberg.
PNAS; Feb 9, 2009; vol. 107 no. 6

The full article is at: http://cgworkspace.cytogenie.org/GetDown2/demo/Ghosn.pdf

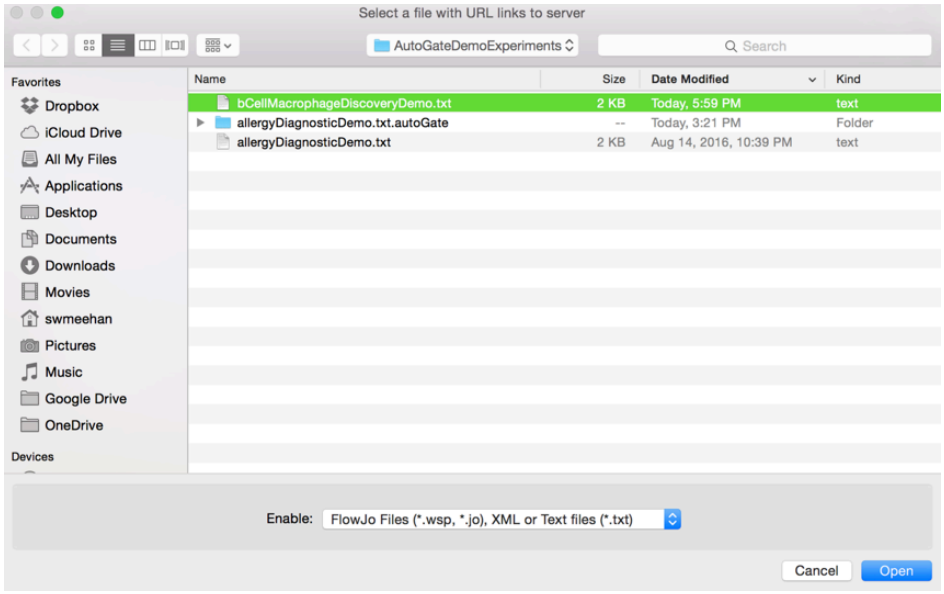
The demo teaches how to reproduce the publication's gating strategies and then discover more subsets beyond it.

1 Set up the experiment

1.1 Click "Open AutoGate demo experiment" on the main window



AutoGate responds by showing you the bundled demos copied to your Desktop under the folder AutoGateDemoExperiments.



2 Pick bCellMarcrophageDiscoveryDemo.txt

If this is your first time with this demo experiment then AutoGate's experiment set up wizard must run.

If you have already setup this experiment then you will see the gating tree window.

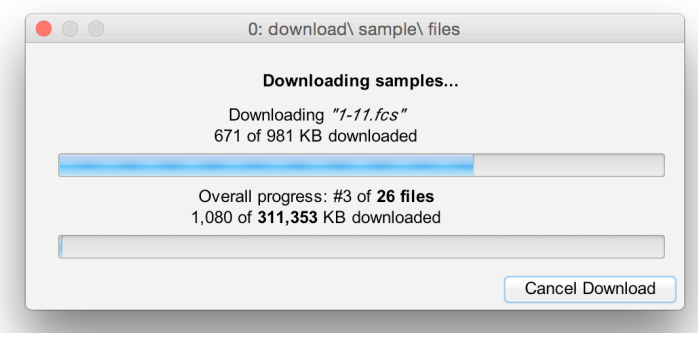
To re-run the experiment setup for this demo follows the steps for re-running the experiment at:

http://cgworkspace.cytogenie.org/GetDown2/demo/allergyReRunningSetup.pdf

Then skip to step 3.1 below

2.1 Allow sample files to download

The first time you open this demo experiment AutoGate downloads all files from the server. These files must be present to compute the bi-exponential's W parameter using AutoGate's "Democratic W" algorithm which sets the correct size of linear region of the biexponential scale for stain parameters.



2.2 Click “Start/continue gating” at the bottom of the task list.

For this experiment you do not need to step through each setup task. This is because AutoGate’s required setup information was entered correctly during data collection with BD’s DiVa software.

More details on each of these setup tasks are at

http://cgworkspace.cytogenie.org/GetDown2/demo/bCellSetupTasks.pdf

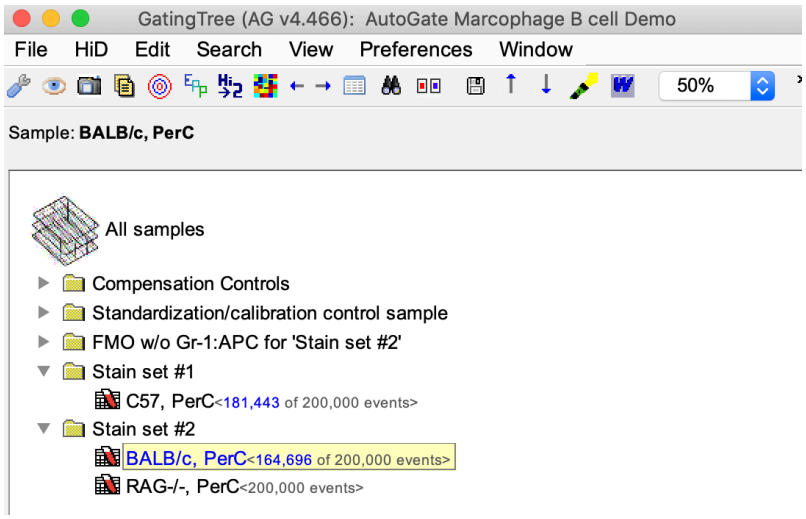
AutoGate responds by

- Checking all of the information found in the samples
- Running our automatic compensation (AutoComp) if a feasible set of compensation controls are found
- Halting only when problems are found; for example, non plausible labels for compensation controls.

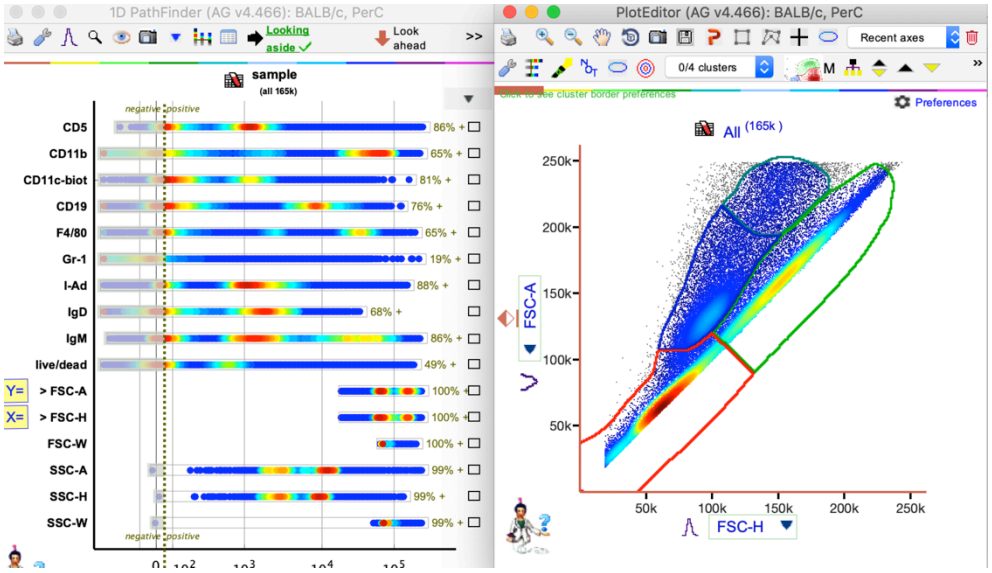
3 Building the macrophage gating sequence

3.1 Open a sample

Double click on the BALB/c sample in the 2nd stain set

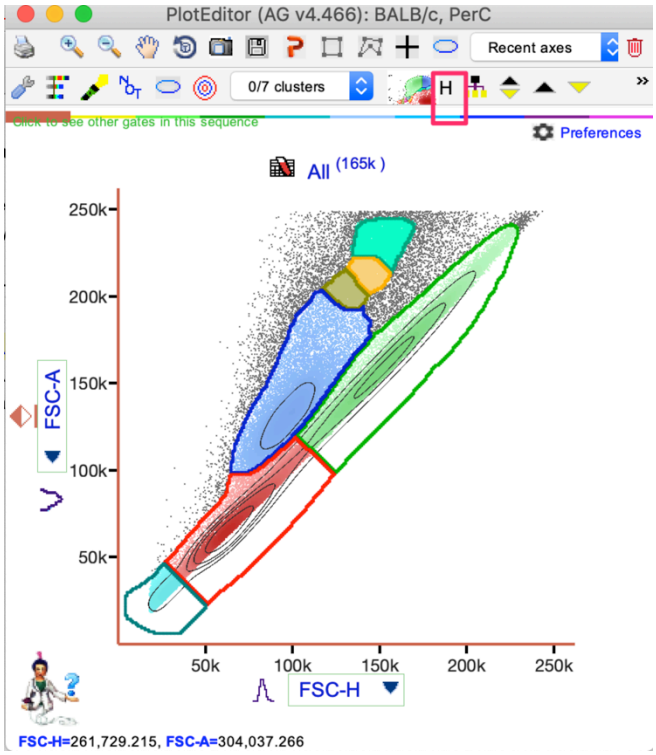


AutoGate responds by opening the plot editor window using the singlet seeking X and Y parameters you configured in the experiment setup.

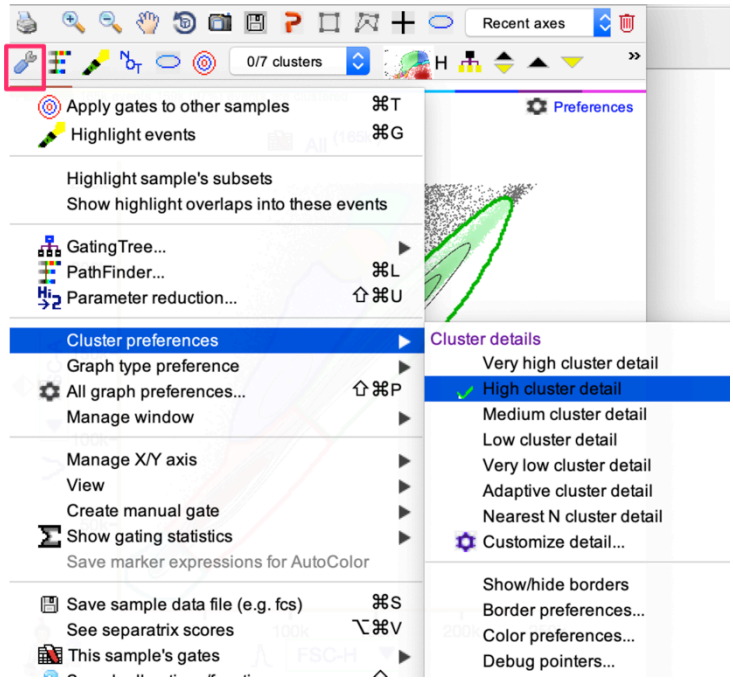


3.1.1 Confirm high detail for cluster preference

Click the cluster detail button on the toolbar to confirm your level of cluster detail is high.



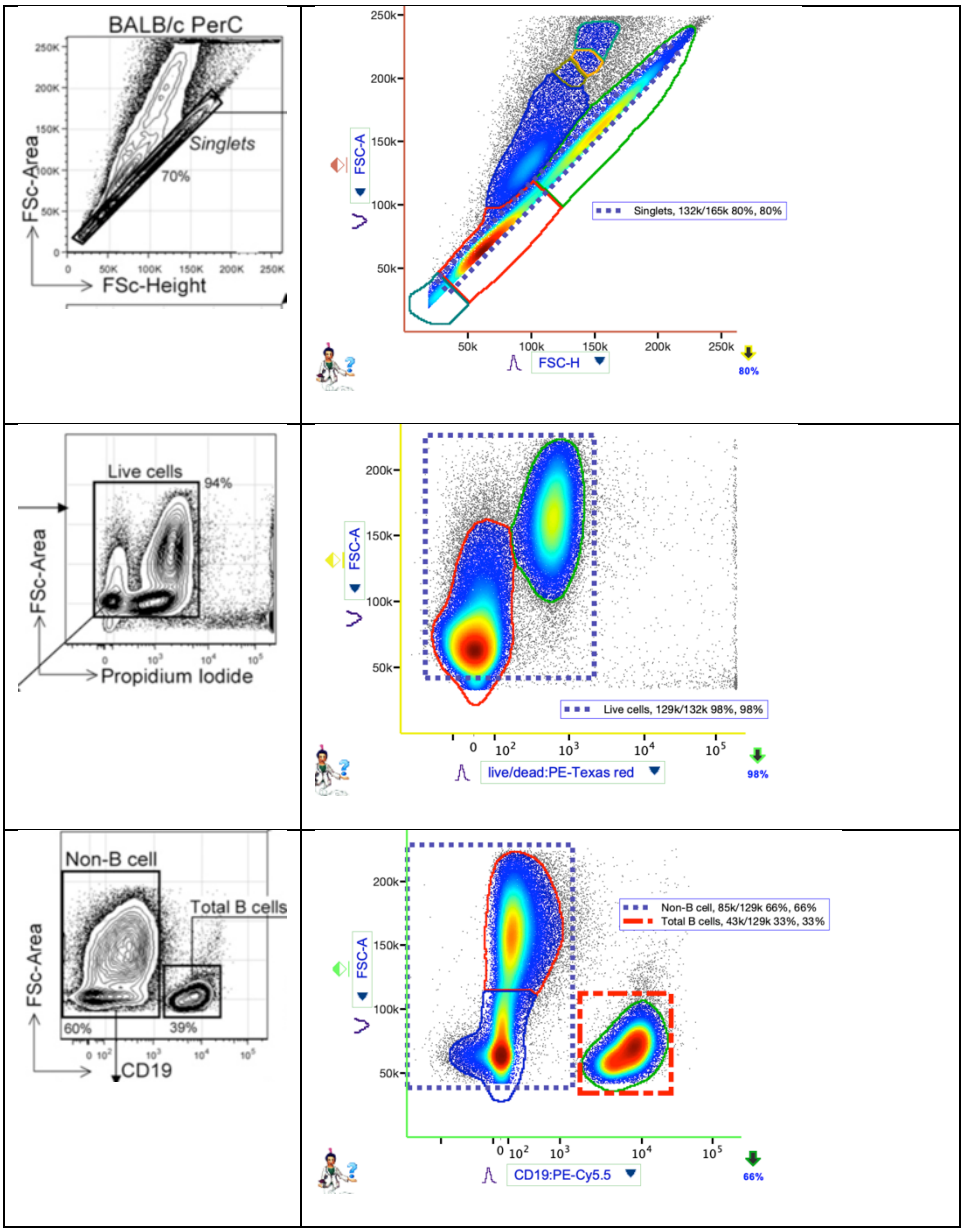
You can also use the *Tools* (highlighted below) menu to confirm your level of cluster detail is high.

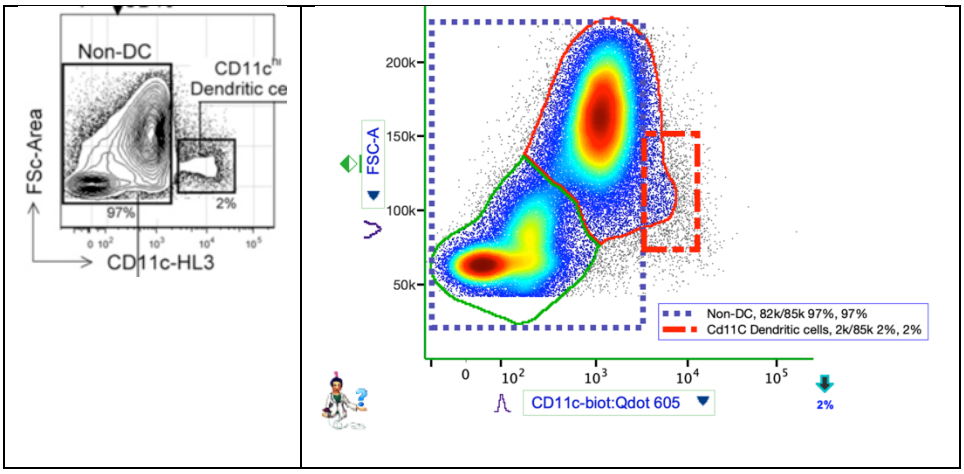


3.2 Create the parent gates of the macrophage plot

Use AutoGate's plot editor window as described in the previous tutorial to create the 4 parent gates shown above in Eliver's publication. You can create each gate without drawing manual gates and instead by selecting clusters. No manual gates are required. When selecting multiple clusters you hold the command key down if using a MAC or the Ctrl key down if using Windows.

When done your gating tree should like this. The demo samples are similar but not identical to Eliver's publication's.





3.2.1 Alter display preferences

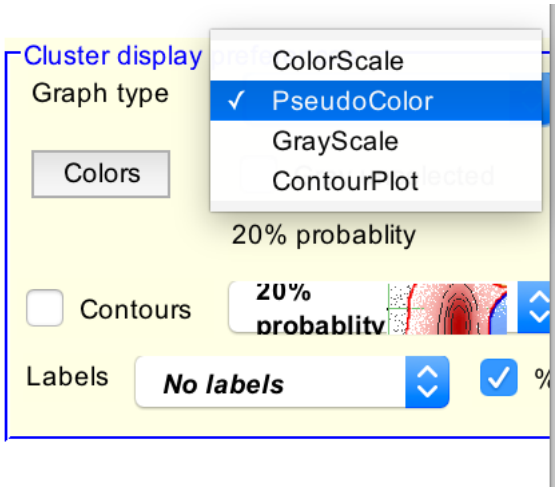
This graph type so far has been AutoGate's default Cluster Color graphing that

- Gives each cluster has a different color
- Highlights densities within the cluster by shading the color.

For the rest of this tutorial we switch to FlowJo's popular/conventional pseudocolor graph for both the plot editor window and the publication graphics

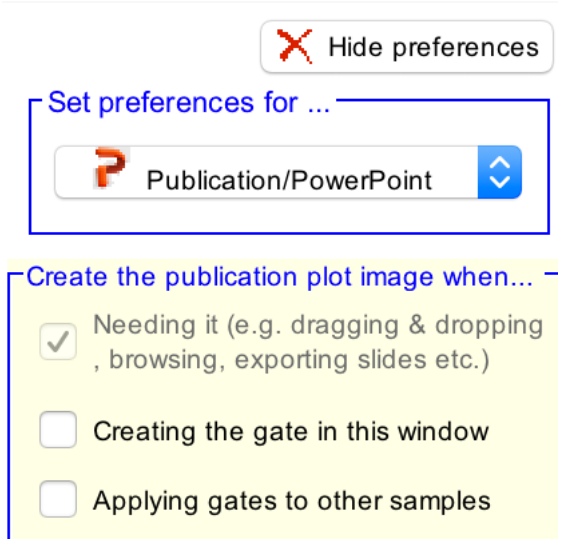
3.2.1.1 Click the Preferences button

3.2.1.2 Change the graph type at the bottom right to PseudoColor



3.2.1.3 Set "Set preferences for" to "Publication (e.g. Powerpoint)"

This is at the top right of the window

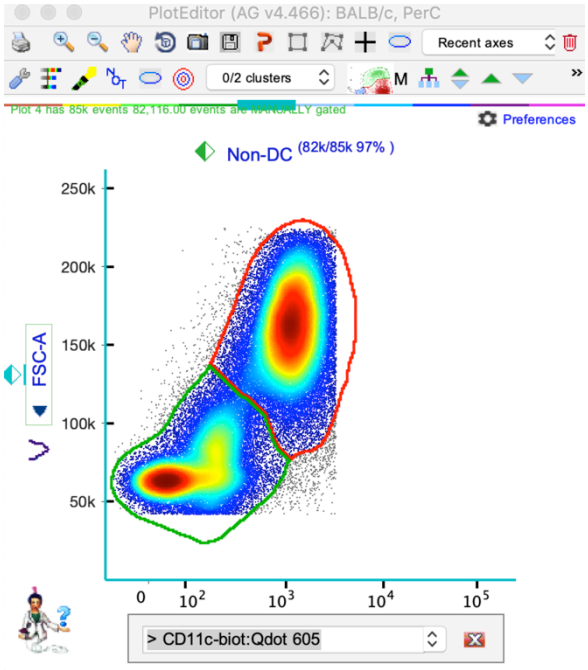


3.2.1.4 Change the graph type at the bottom right to PseudoColor

3.2.1.5 Change the “Hide preferences” button

3.2.2 Change the cluster detail to medium

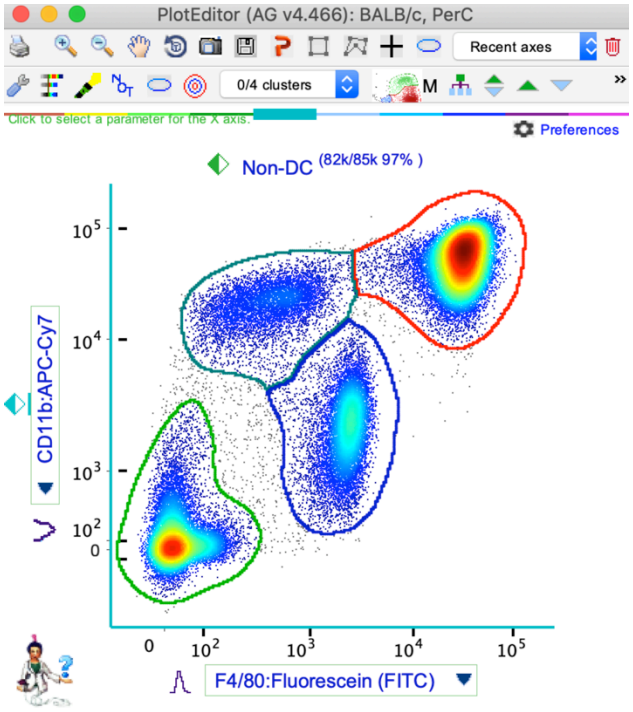
Click on the cluster detail button on the toolbar and select Medium from the dropdown.



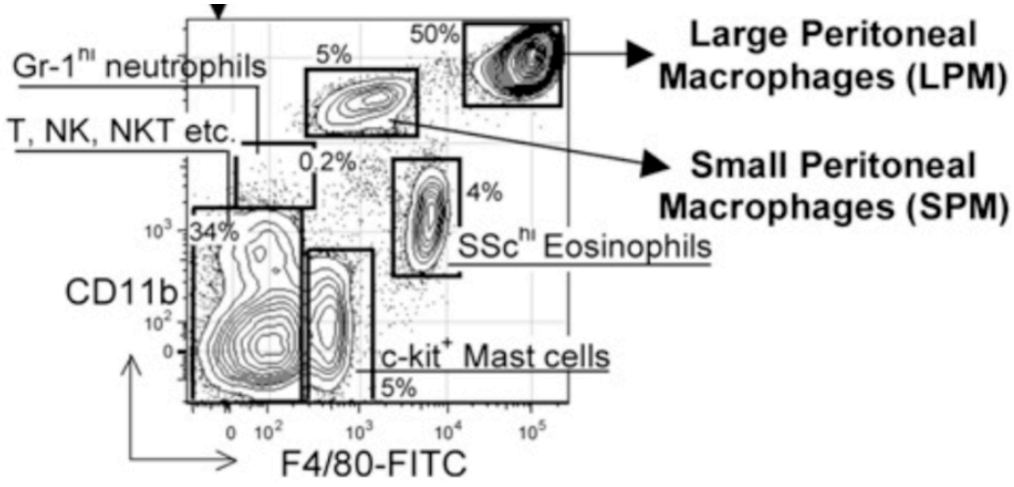
3.2.3 Restrict the Non-DC plot

3.2.4 Set X=F4/80 and Y=CD11b

AutoGate responds with this data projection.



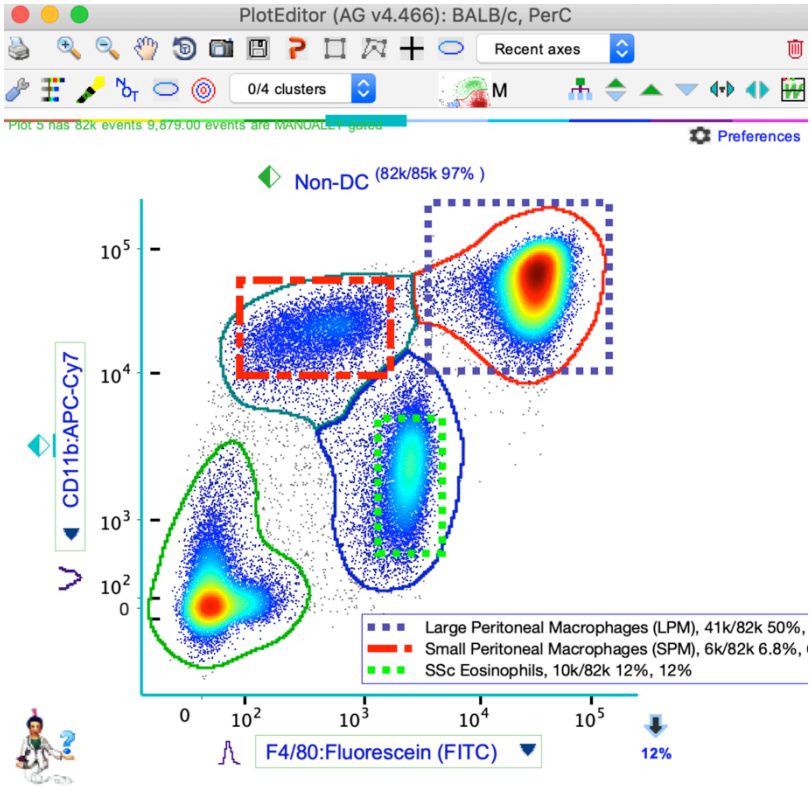
Here is the equivalent gate for the similar used by Eliver's publication.



3.2.5 Create top right 3 gates

You can create each of the 3 gates at the top right of Eliver's plot using cluster picks as illustrated in the previous tutorial. As you pick each cluster you can name it without thereby restricting the plot by NOT clicking on the down arrow but clicking on the bottom

right rectangle that says ”Subset”. You can also select the “Rename gate” menu item of the right click menu.



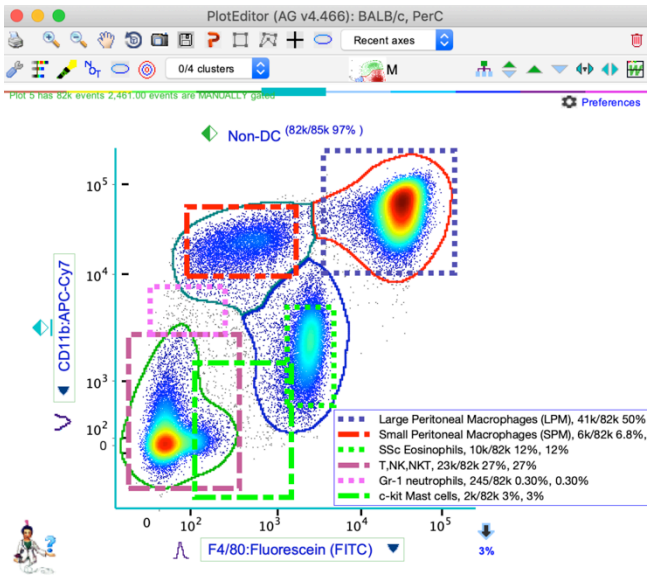
3.2.6 Create bottom left 3 gates

The bottom left 3 gates must be created using the conventional manual gating tools. This does not mean clusters cease to be relevant to this part of the gating model. When you apply a gating model with manual gates to either an ungated sample or another part of the gating tree, AutoGate re-positions the manual gates by first matching clusters in their neighborhood.

3.2.6.1 Select the rectangle gate tool

3.2.6.2 Draw Eliver’s Gr-1+ neutrophils gate

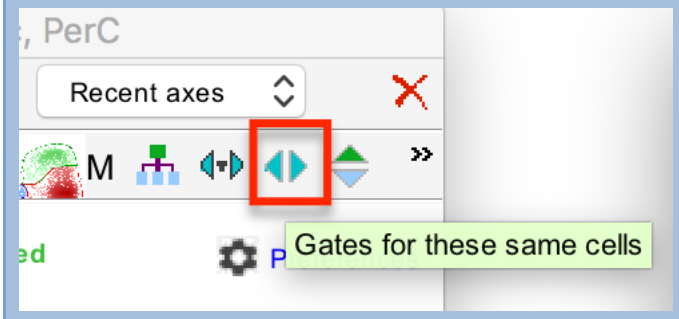
Draw it just as you do in FlowJo. When done you release the mouse and *AutoGate responds by* asking you for the gate’s name.



You now have Eliver's 6 gates.

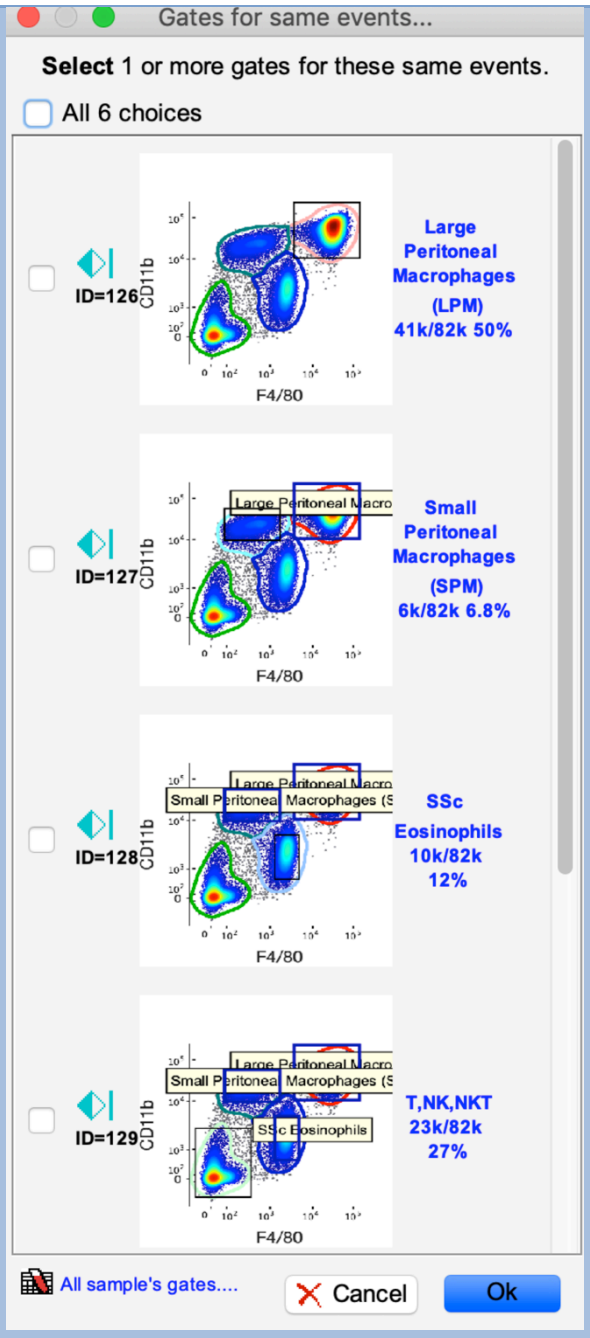
3.2.6.3 View all gates for same events in the plot editor window

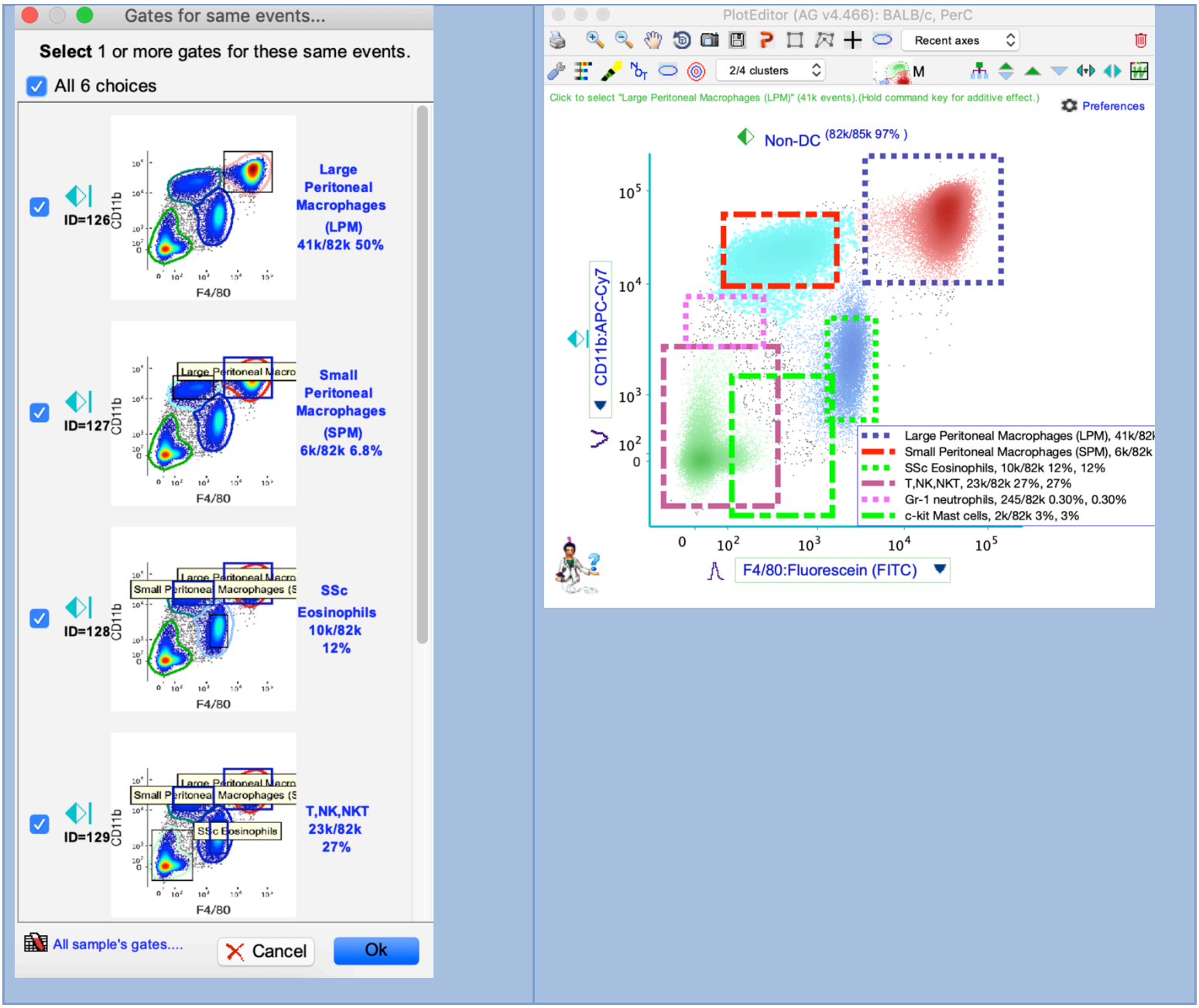
Click the left/right arrow button on the right side of the toolbar.	***AutoGate responds with***



Select all 6 gates and press OK

AutoGate responds with



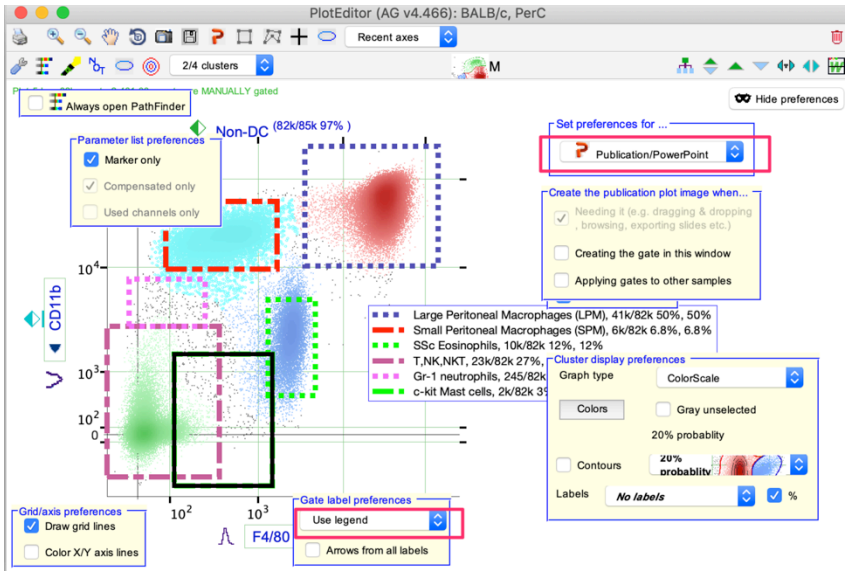


3.2.6.4 Set this view as the “publication” view

3.2.6.4.1 Click the preferences button

3.2.6.4.2 Select Publication/PowerPoint for “Set preferences for”

3.2.6.4.3 Select “Use legend” for Gate label preferences

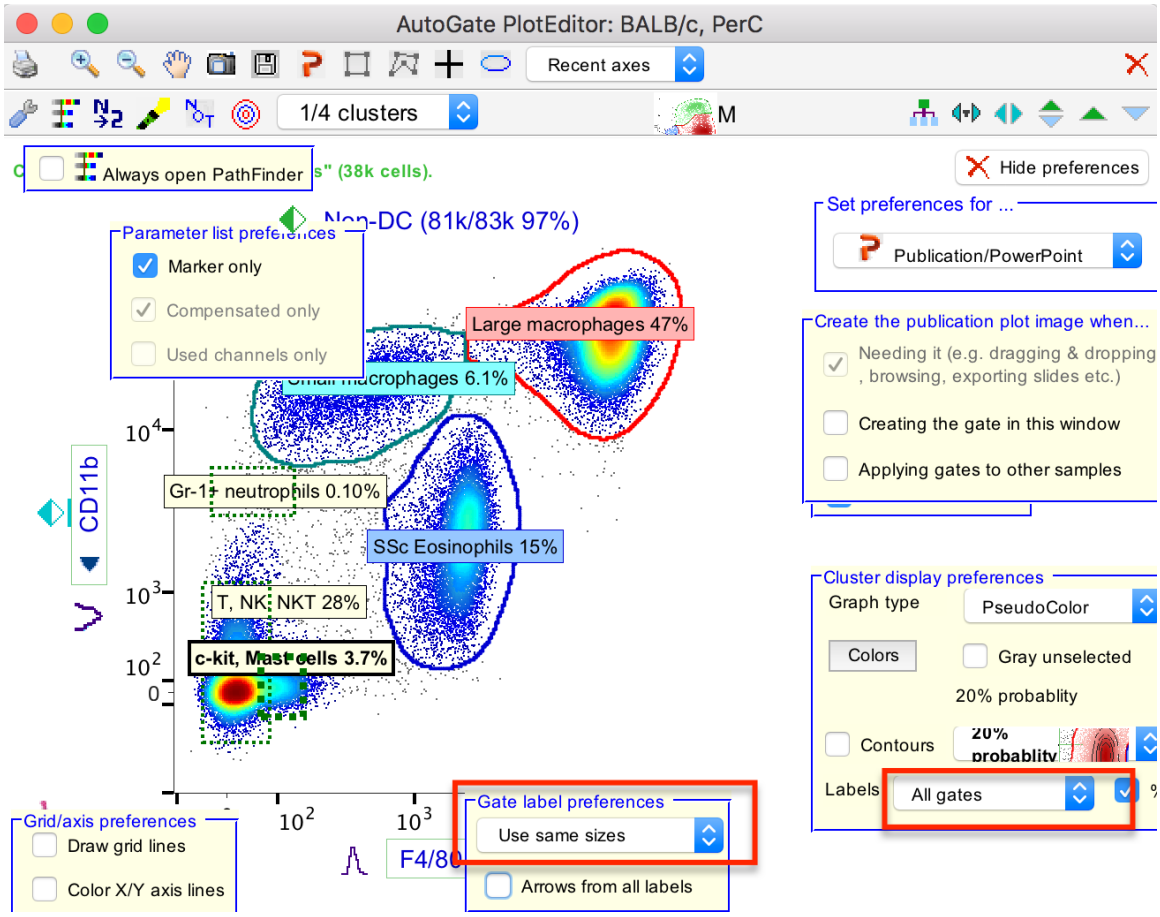

PlotEditor (AG v4.466): BALB/c, PerC
Recent axes
2/4 clusters
Always open PathFinder
Hide preferences
Parameter list preferences
Marker only
Compensated only
Used channels only
Non-DC (82k/85k 97%)
Set preferences for ...
Publication/PowerPoint
Create the publication plot image when...
Needing it (e.g. dragging & dropping , browsing, exporting slides etc.)
Creating the gate in this window
Applying gates to other samples
CD11b
F4/80
Large Peritoneal Macrophages (LPM), 41k/82k 50%, 50%
Small Peritoneal Macrophages (SPM), 6k/82k 6.8%, 6.8%
SSc Eosinophils, 10k/82k 12%, 12%
T,NK,NKT, 23k/82k 27%,
Gr-1 neutrophils, 245/82k
c-kit Mast cells, 2k/82k 3
Cluster display preferences
Graph type
ColorScale
Colors
Gray unselected
20% probablity
Contours
Labels
No labels
%
Grid/axis preferences
Draw grid lines
Color X/Y axis lines
Gate label preferences
Use legend
Arrows from all labels

3.2.6.4.4 Use your mouse to drag the legend to the bottom

3.2.6.5 Change the “publication” view to show each gate label separately

3.2.6.5.1 Select “Use same sizes” for Gate label preferences

3.2.6.5.2 Select “All gates” for label

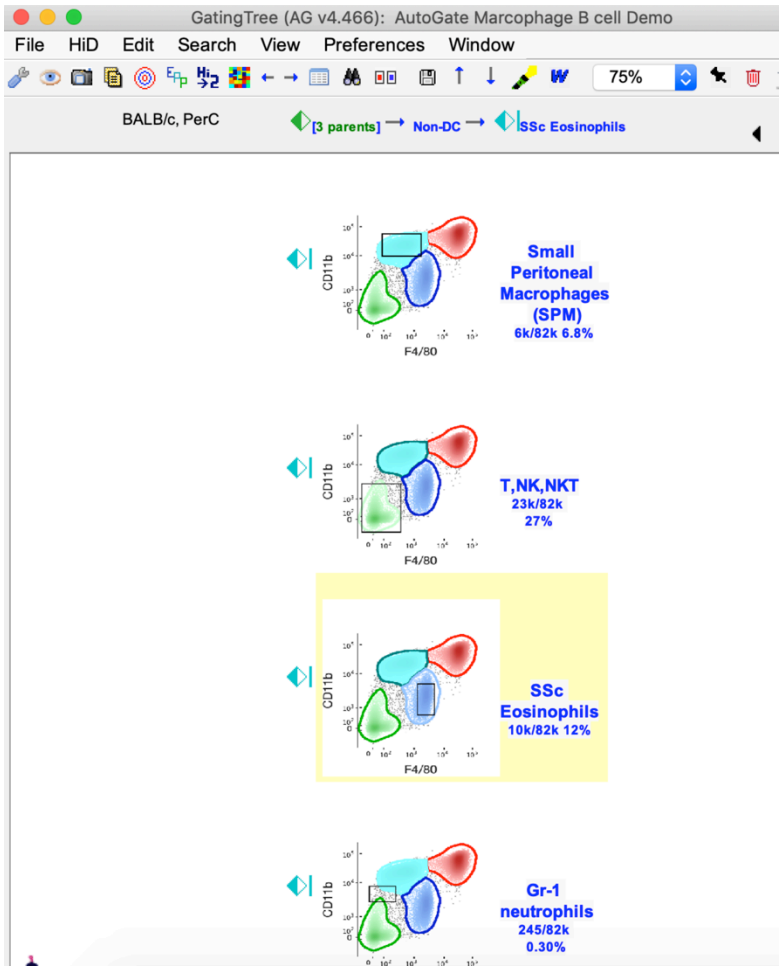


3.2.6.5.3 Use your mouse to move each gate label into the desired position

3.2.6.6 Click the Hide preferences at the top right to close the preferences view

4 Discover new subsets

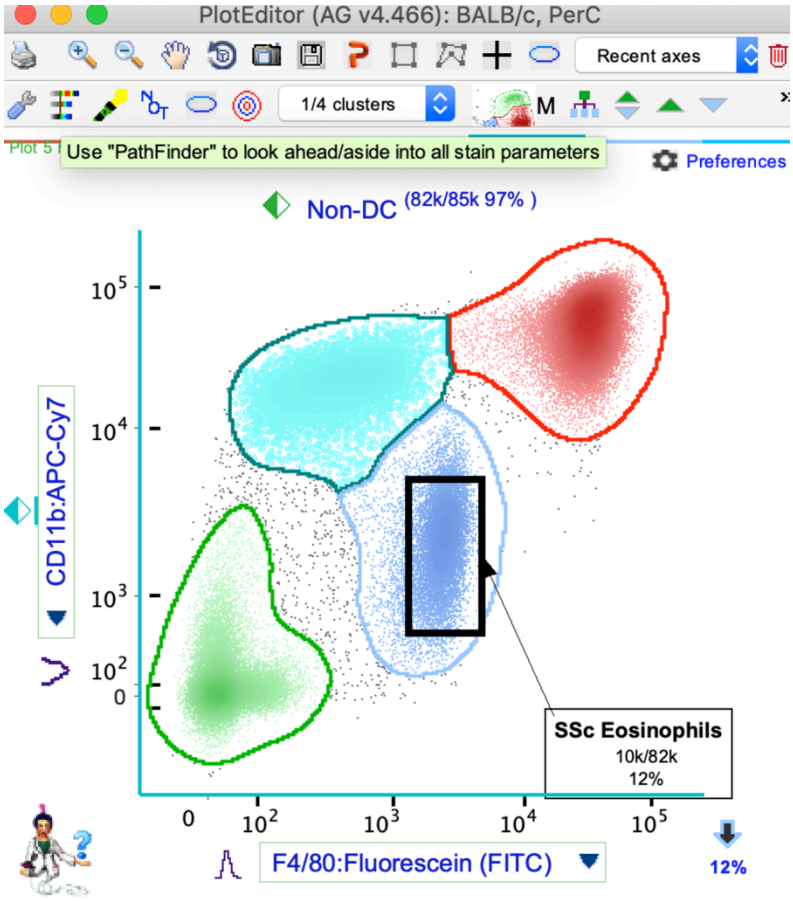
Select the SSc Eosinphils gate in the gating tree window



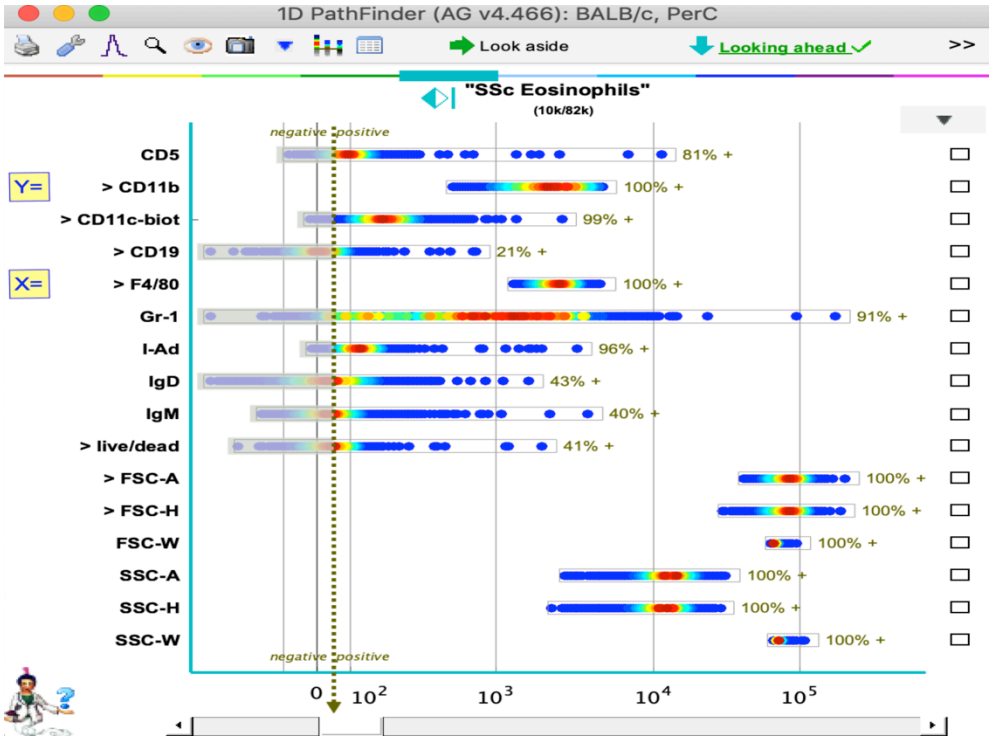
AutoGate responds by focusing the plot editor window on this gate.

4.1 Use “Path finder” to look ahead into all stain parameters

Activate AutoGate’s look ahead by clicking on the toolbar button with the horizontal pseudo color bars.



AutoGate responds with this window.



The “Look ahead” window

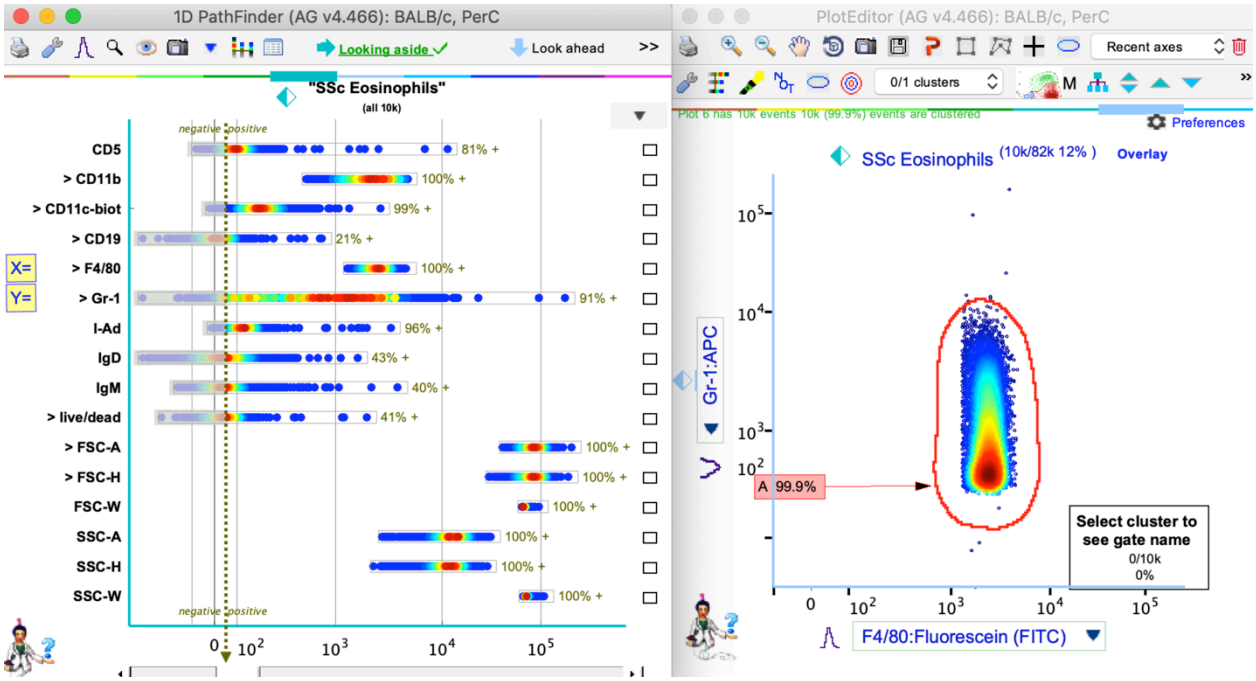
- Associates with a specific plot editor window
- Shows the staining on all parameters for selected cells or for all cells in the associated plot editor window.
- Depicts each parameter with a horizontal bar that uses pseudo color’s convention of showing where staining occurs most.
- Uses a vertical dashed line to indicate a user definable threshold for positive staining. It defaults to 10^3 but you can use the slider to set it any where on the scale. AutoGate indicates the percent cells above that line at the right side of each parameter’s horizontal bar.
- Uses bold font for labels of parameters unused in the current gating sequence and normal font with a “>” prefix for labels of parameters used in the current gating sequence.

Look ahead shows SSc Eosinophils cells have only one unused parameter with significant positive staining: Gr-1.

4.2 Change the Y axis to Gr-1

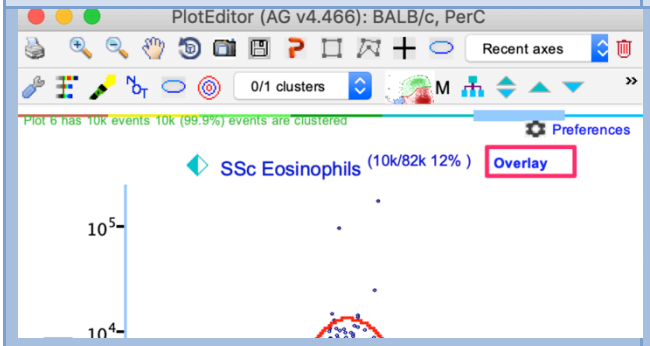
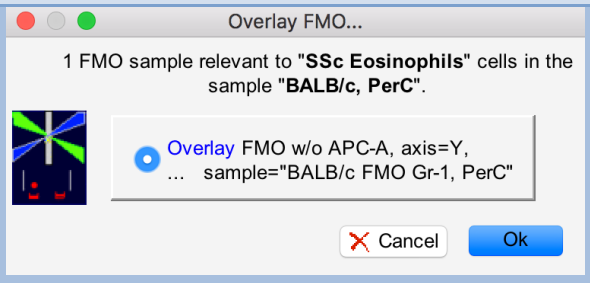
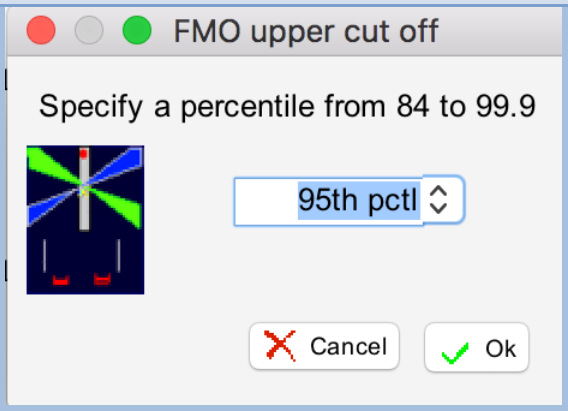
The look ahead window lets you do this quickly by dragging the Y axis label on to the Gr-1 pseudo color bar.

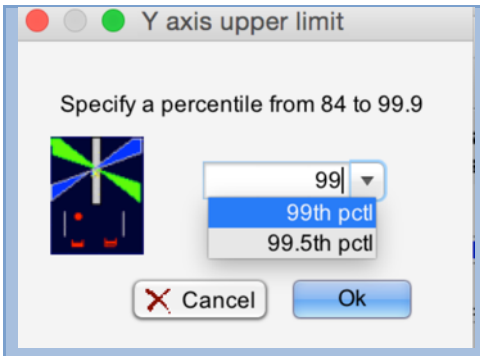
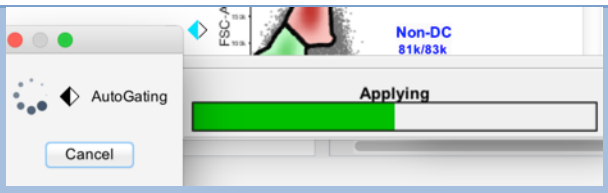
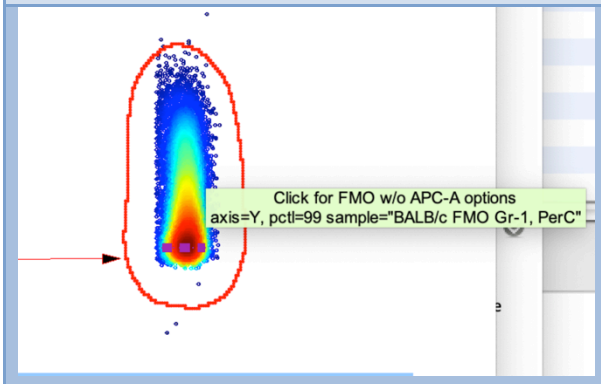
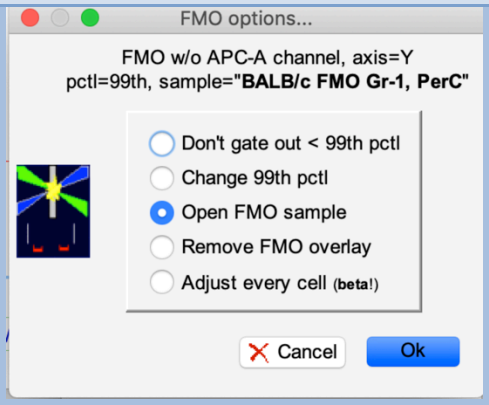
AutoGate responds by restricting the plot editor window to the 10k of cells in the SSc Eposinophils gate.

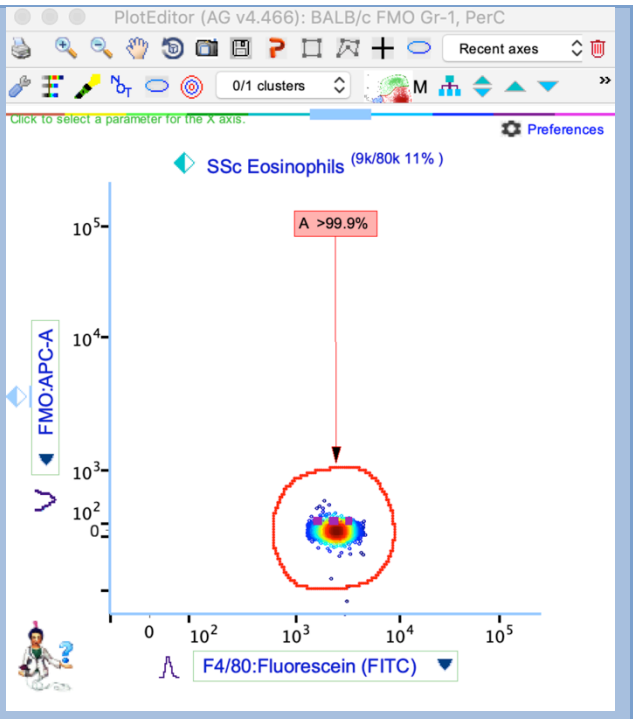
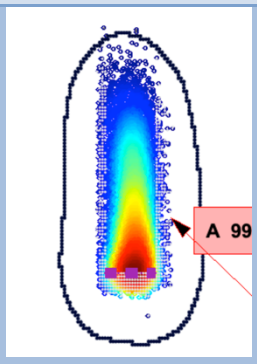


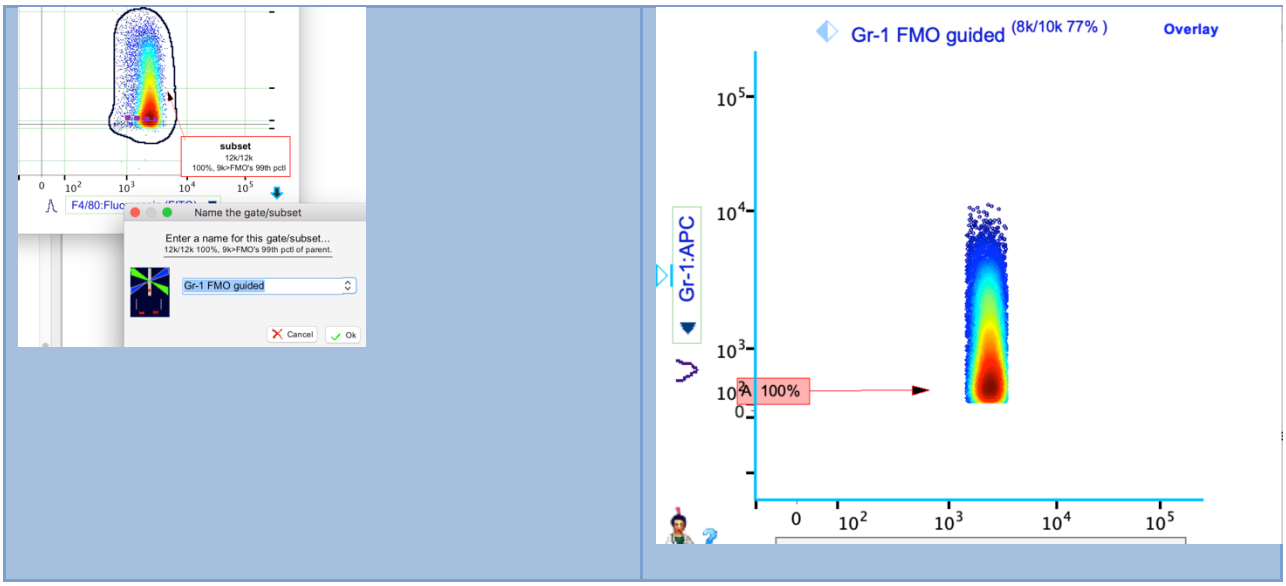
4.3 Use an FMO to create a gate

We want to choose only those cells that we are sure are stained by the Gr-1 reagent. To do this we can use the sample which uses all the same reagents as this sample *except the Gr-1 reagent on the APC-A parameter.* The staining for this sample was designed to highlight the true staining of the Gr-1 reagent.

Click the "Overlay" button at the top right of the plot editor window	***AutoGate responds with***
	
Click the OK button on the Overlay FMO window	*AutoGate responds with*
	
Enter 99th percentile and then click OK	*AutoGate responds by* creating the same gating sequence in the FMO sample for SSc Eosinophils and then draws the 99th percentile line

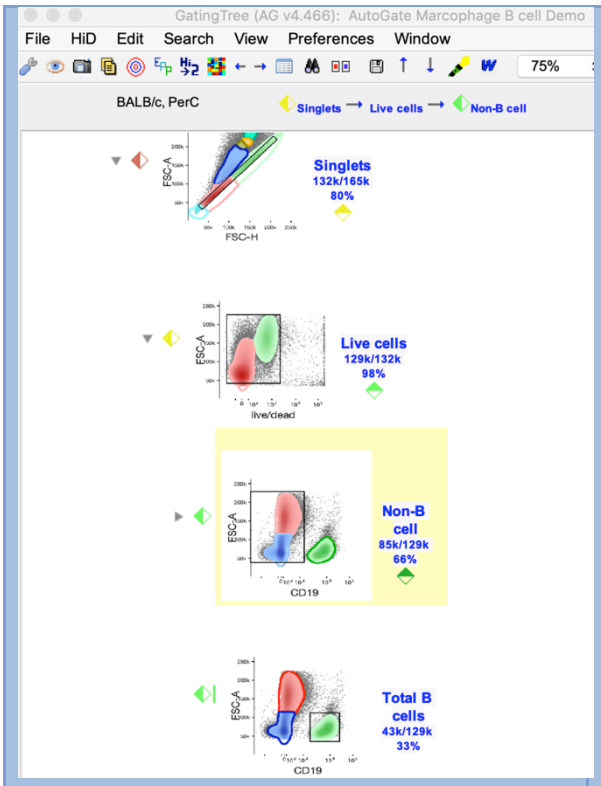
<table>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>Click on the purple 99th percentile line computed in the FMO sample</td><td>AutoGate responds with these choices</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>Choose “Open FMO sample”</td><td>AutoGate responds by opening a separate plot editor window for the equivalent cells in the FMO sample</td></tr>
</table>

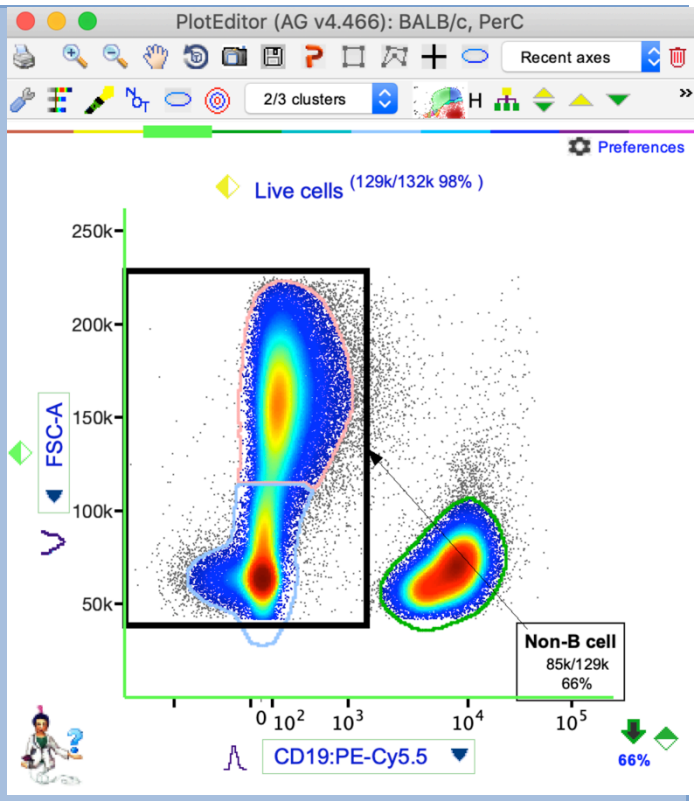
	
Select the one cluster in the original fully stained sample	*AutoGate responds by* selecting only those cells that are • Above the 99th percentile • Within the chosen cluster
	
Click the restrict down arrow button at the bottom left of the plot editor window and name the gate "Gr-1 FMO guided"	*AutoGate responds by* restricting the plot to only those cells above the 99th percentile line



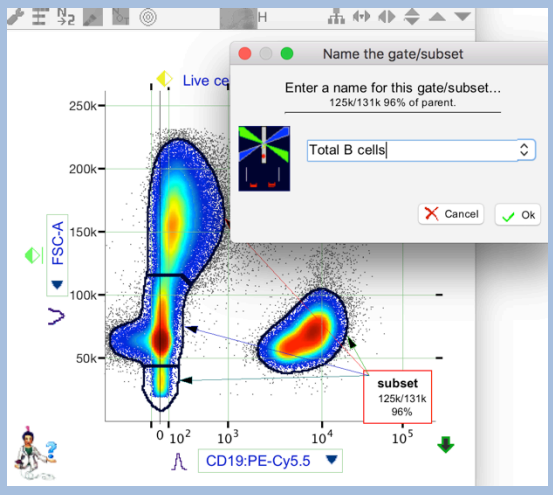
5 Build Eliver's B cell gating sequence

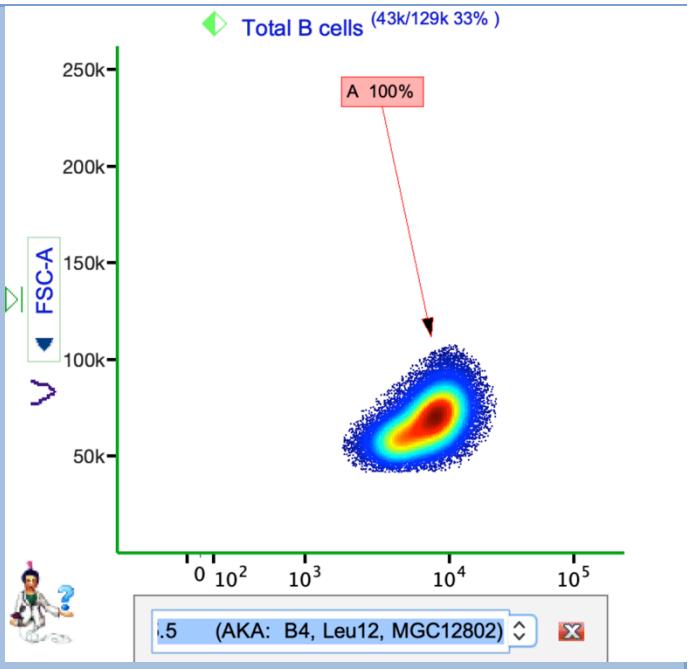
Select the Non-B cells gate in the BALB/c Perc sample and double click.	***AutoGate responds by*** **opening the plot editor window**



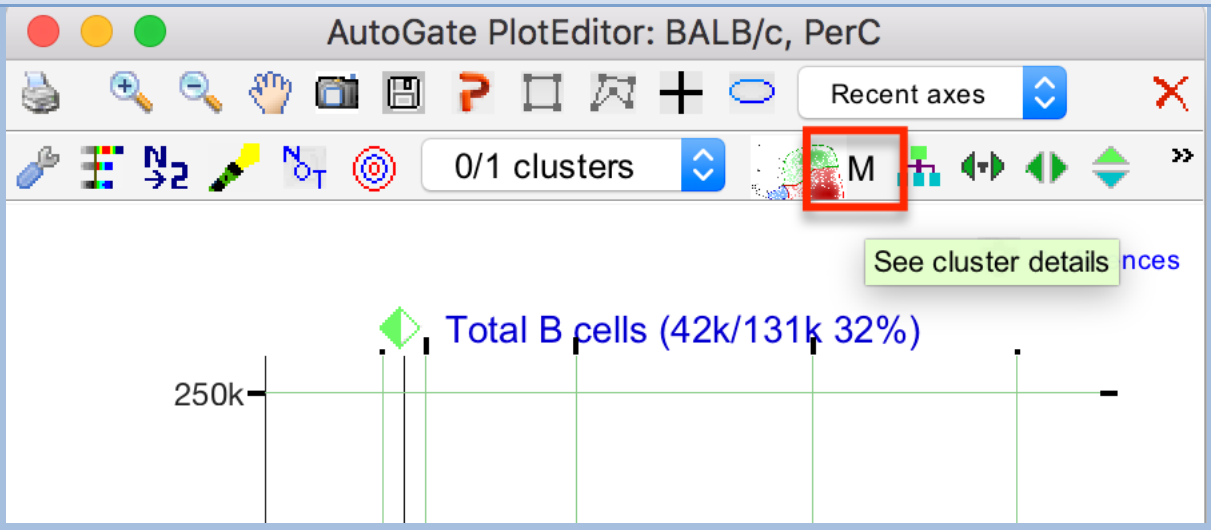


Double click the right most cluster and name the gate “Total B cells” **(This didn’t restrict the plot to the selected cluster. Hence, changed as below)** **Single click the right most cluster and click the down Arrow to name the gate “Total B cells”**	*AutoGate responds by* restricting the plot to the selected cluster



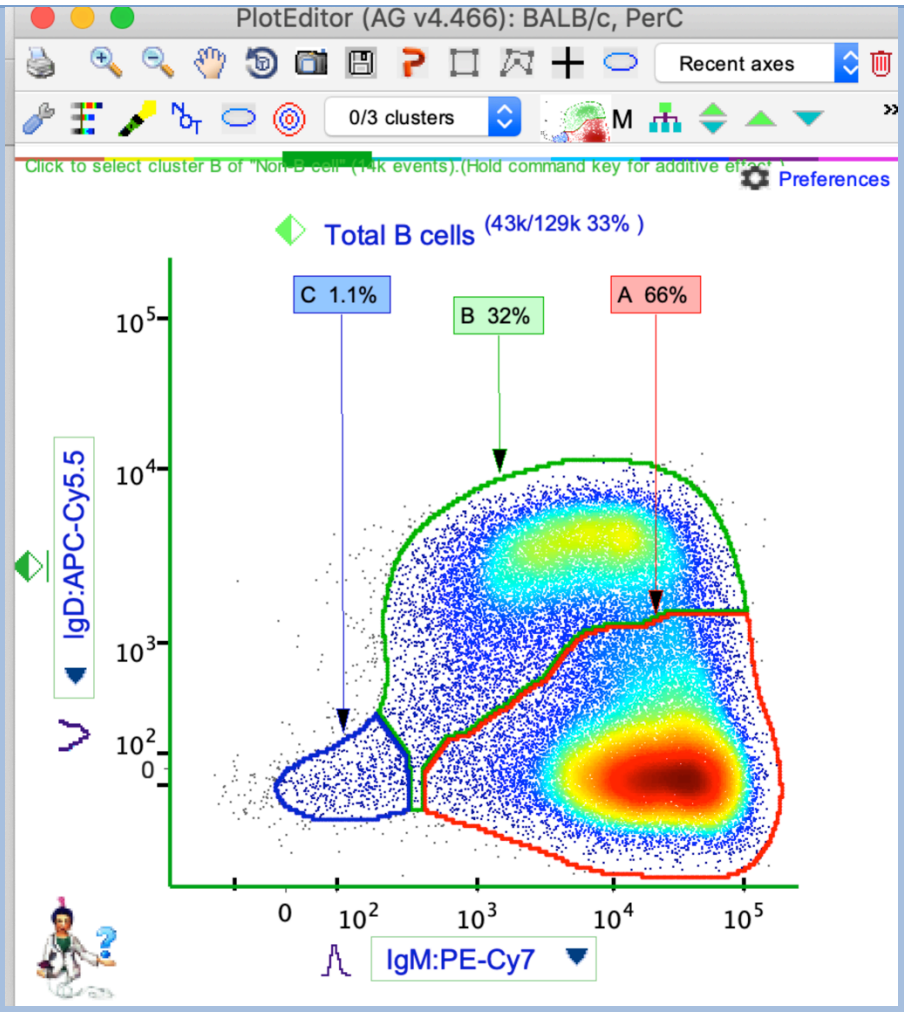


Confirm you have medium clustering detail

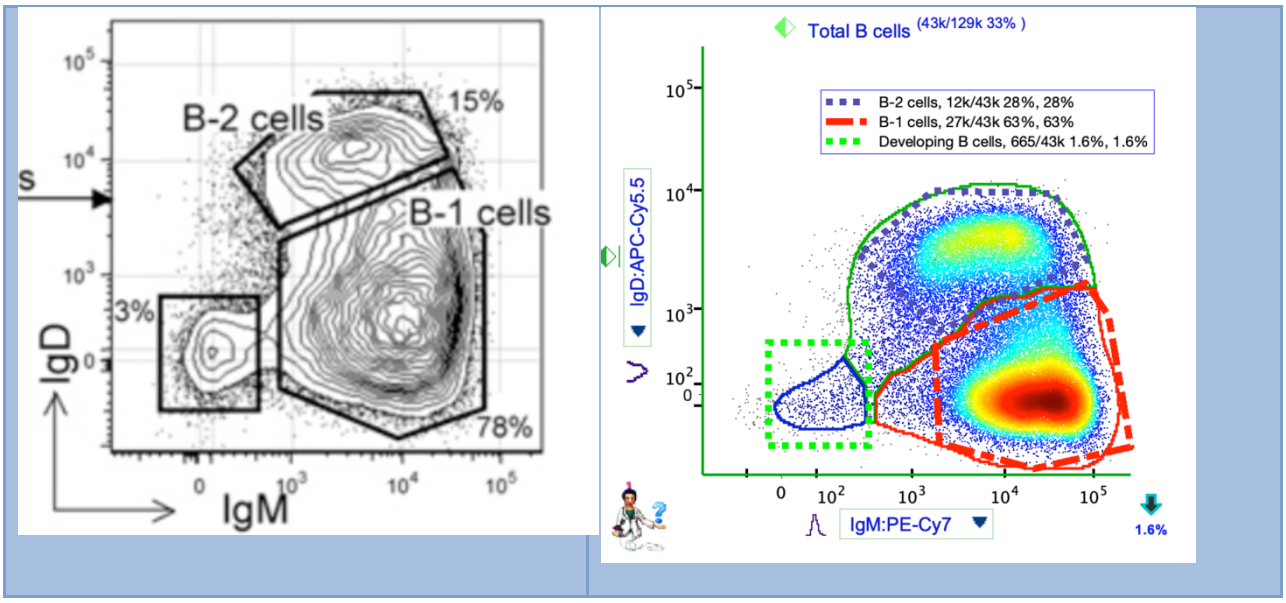


Set the parameters to X=IgM and Y=IgD

AutoGate responds by clustering the data



Create each of the gates published by Eliver (see below) and then show all gates in same plot	*AutoGate responds by* showing the gates

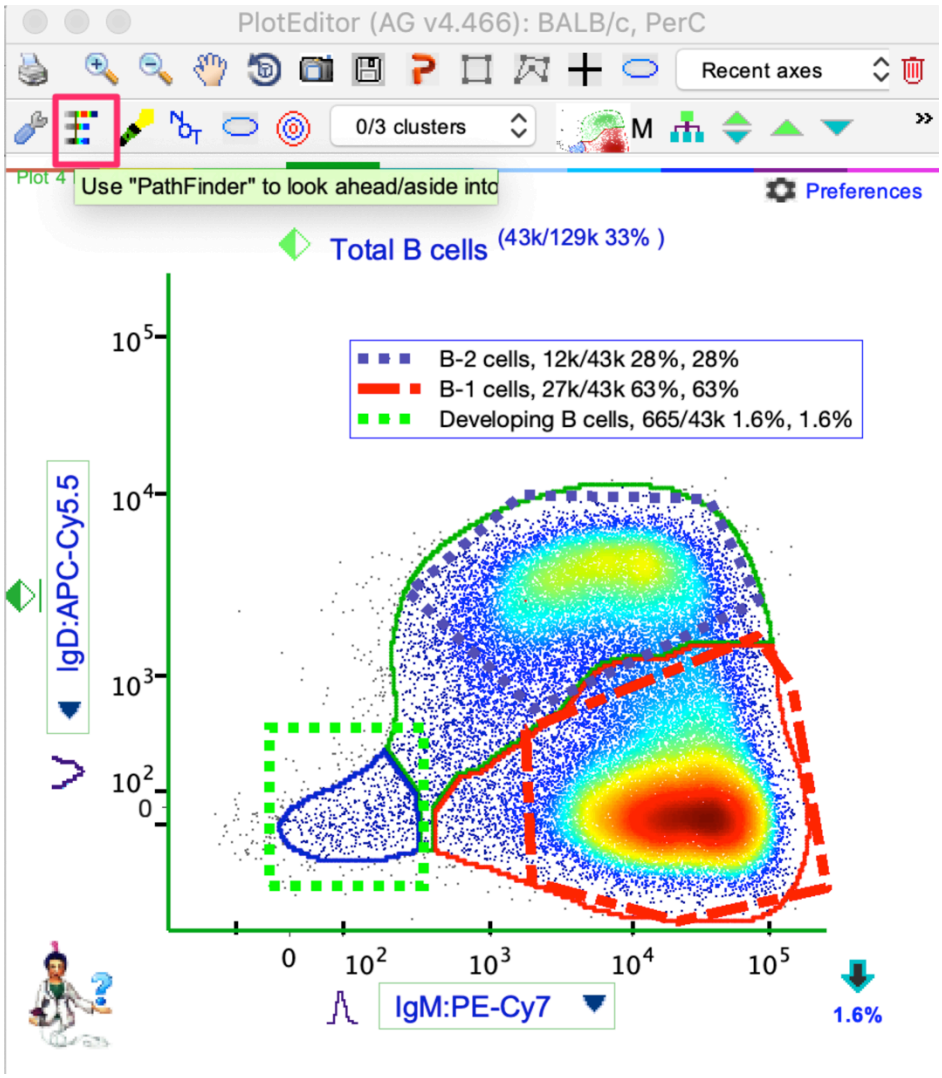


6 Look for new subsets for each B cell subset

Make sure the plot editor window is showing the gate for B-1 cells, or B2-cells or Developing cells.

6.1 Activate Path finder to look ahead

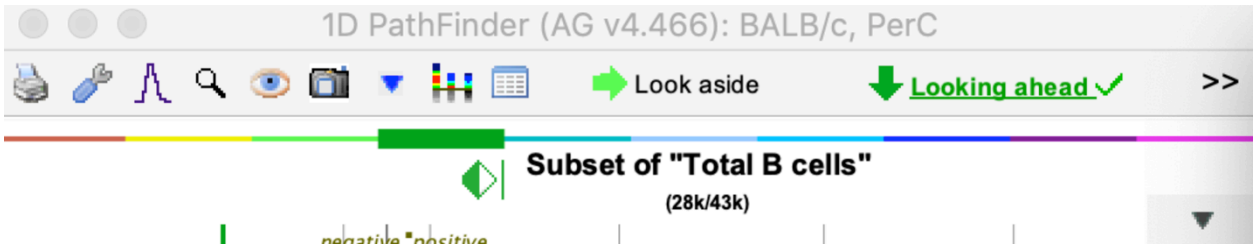
You do this by clicking either on the toolbar or on the right click menu.



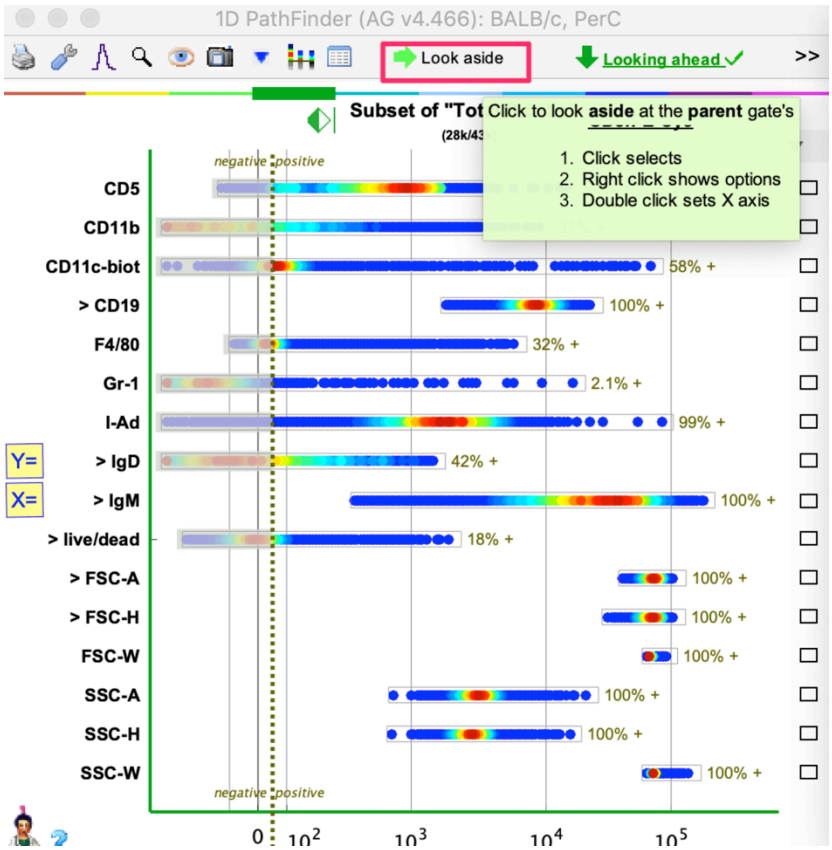
6.2 Activate Path finder

The look ahead mode only examines cells in the selected gate (B-1) of the plot editor window. To see the staining for all cells in the window you either de-select the gate in the plot editor window or in the look ahead window you click the disabled right arrow.

Click the button for **Show navigation buttons**

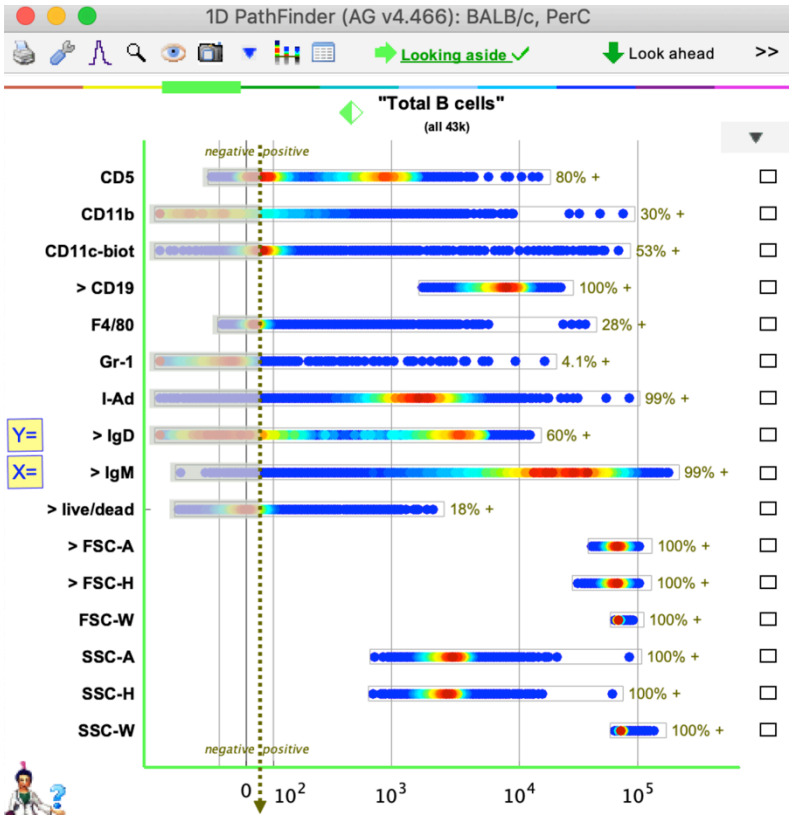


Then click the “Look aside” button



AutoGate responds by showing the staining for the I B cells parent gate.

Of the unused reagents (bold font) there are 2 with some positive staining: CD5 and I-Ad. To see this more clearly, you can hide all the used gates by clicking the green check mark for each on the right side.


1D PathFinder (AG v4.466): BALB/c, PerC
Looking aside
Look ahead
>>
"Total B cells"
(all 43k)
negative positive
CD5
80% +
CD11b
30% +
CD11c-biot
53% +
> CD19
100% +
F4/80
28% +
Gr-1
4.1% +
I-Ad
99% +
Y=
> IgD
60% +
X=
> IgM
99% +
> live/dead
18% +
> FSC-A
100% +
> FSC-H
100% +
FSC-W
100% +
SSC-A
100% +
SSC-H
100% +
SSC-W
100% +
negative positive
0
10^2
10^3
10^4
10^5