

AutoGate Allergy Diagnostic Demo

Version 2

Because this demo serves as a first lesson in using AutoGate it has more detailed explanations than the other demos on the basics of using AutoGate.

The de-identified experiment teaches the use of AutoGate for diagnostic purposes. It teaches

- Labeling an experiment, running auto compensation
- Gating a base line sample
- Applying this sample's gating to other samples
- Running a report that measures the degree of difference between the base line and other samples
-

The next demo illustrates using AutoGate for discovery purposes.

<http://cgworkspace.cytogenie.org/GetDown2/demo/bCellMacrophageDiscoveryDemo.pdf>

1 Setup the demo experiment

1.1 Click the “Open AutoGate demo experiment” option



Start new AutoGate experiment

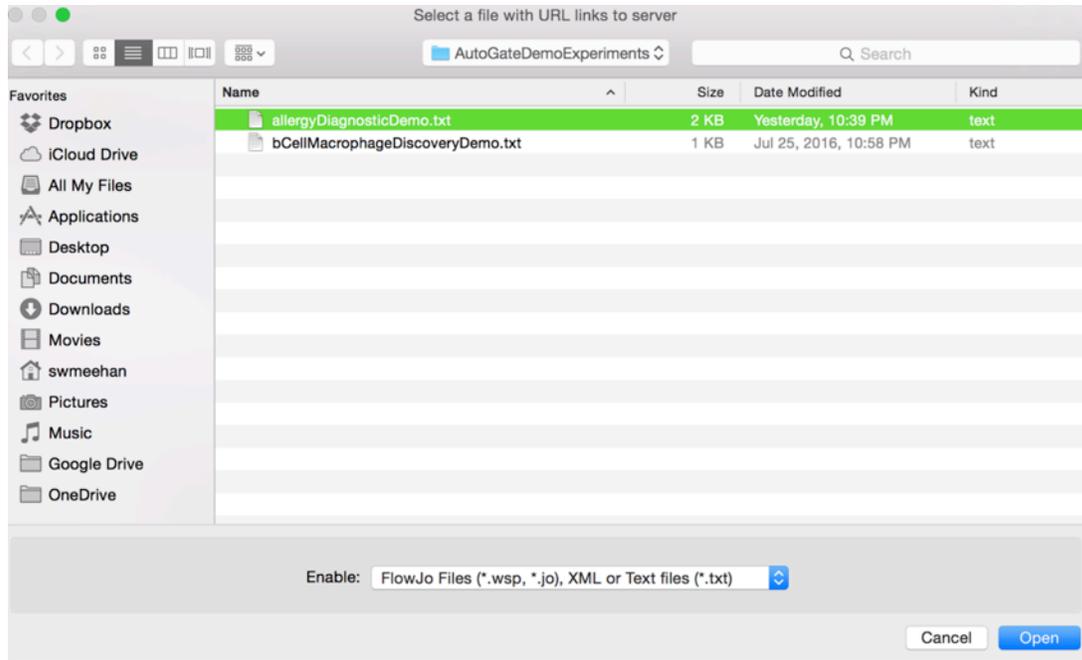
Open existing AutoGate experiment

Open AutoGate demo experiment

Click to open a pre-packaged demo experiment

Exit AutoGate

AutoGate responds by showing this file selection window



1.2 Choose “allergyDiagnosticDemo.txt”

If you have already run this demo once then you will see the gating tree next.

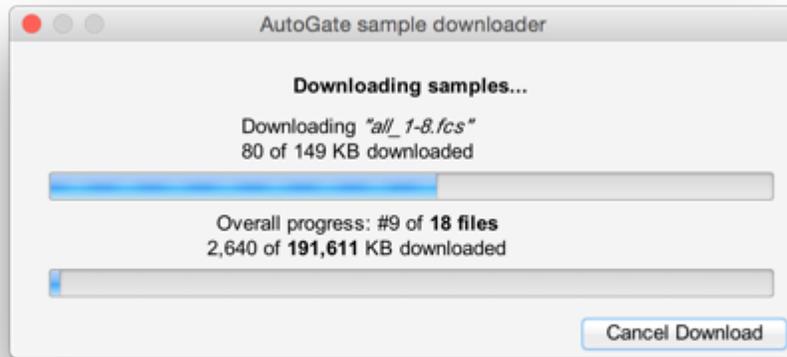
1. Follow the steps for re-running the experiment

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

2. Skip to step 1.3 below

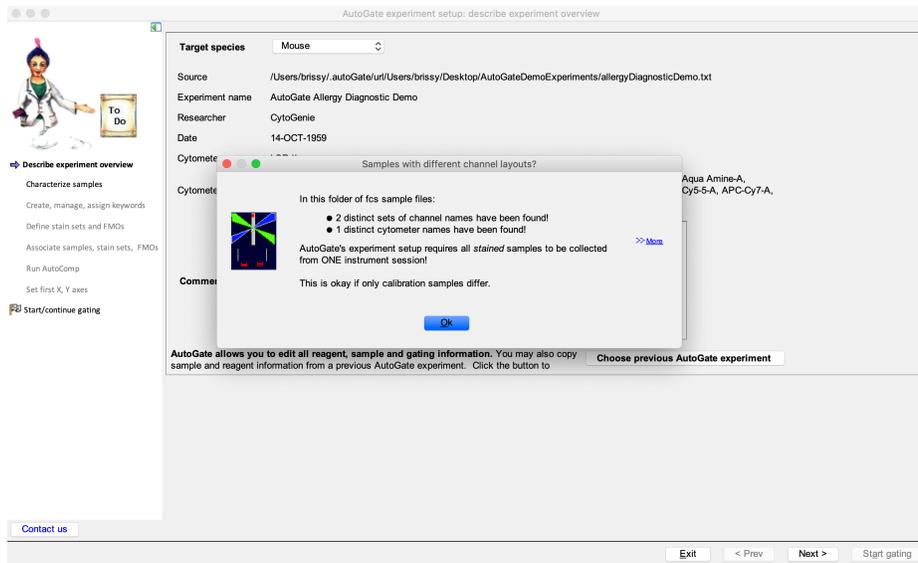
AutoGate responds by

- Downloading the demo experiment’s samples from the cytogenie.org server

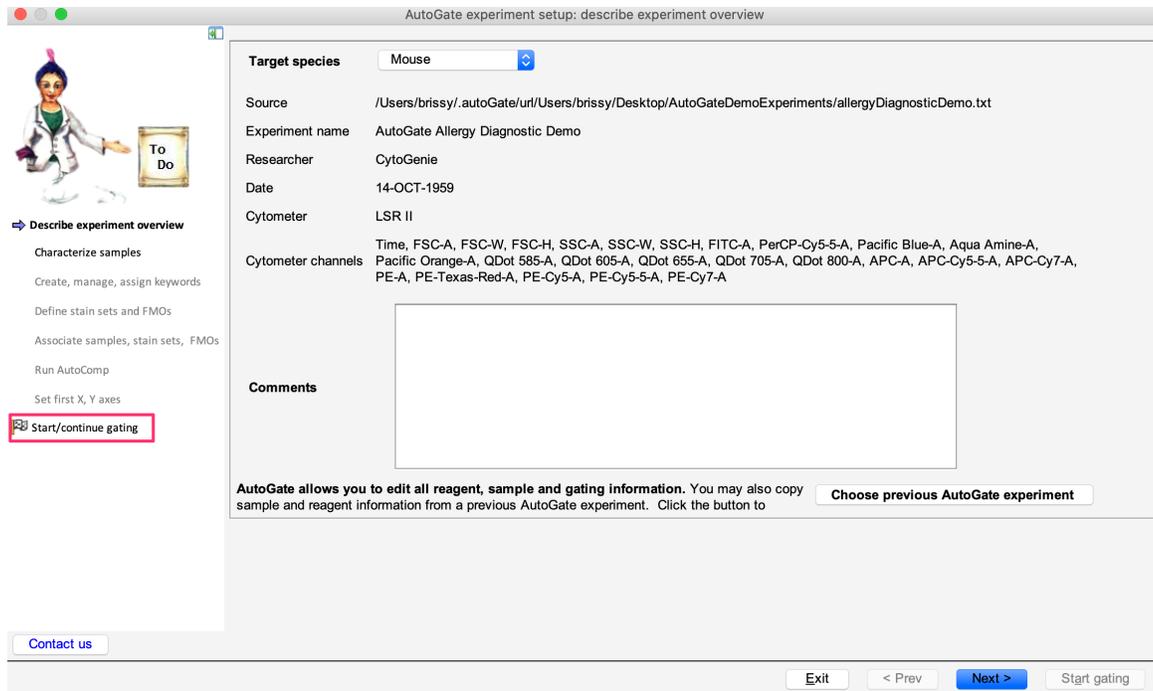


AutoGate keeps these downloaded samples in an internal cache. After a period of non use AutoGate removes them to conserve your local disk space. When you need these samples AutoGate automatically downloads them again.

- Opening it's experiment setup window with a genie cartoon.
- Alerts that experiment has data from different channel layouts. Click OK to proceed



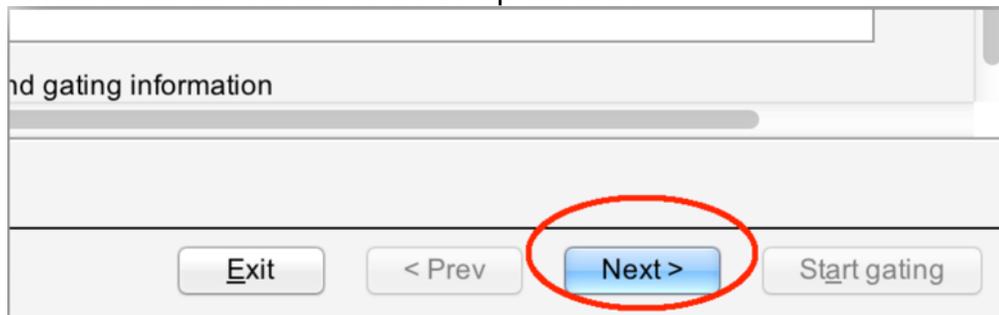
- The left side of this window is a “to do list” of 7 tasks which prepare/confirm the experiment’s reagent labels, sample labels and the compensation. You can fast track this tasks by clicking **Start/continue gating**



- The left side of this window is a “to do list” of 7 tasks which prepare/confirm the experiment’s reagent labels, sample labels and the compensation.

1.3 Click the Next button

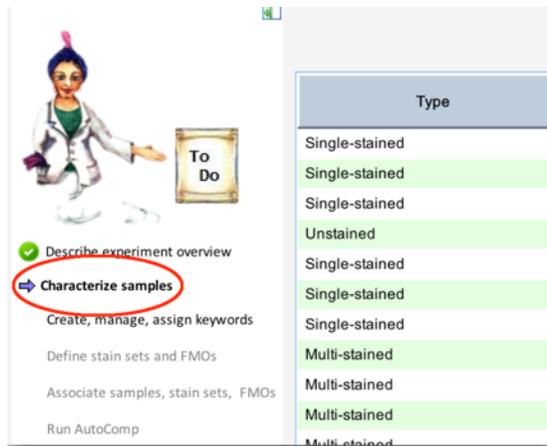
This is found at the bottom left of the setup window.



AutoGate responds by:

Showing a list of the samples found for the experiment.

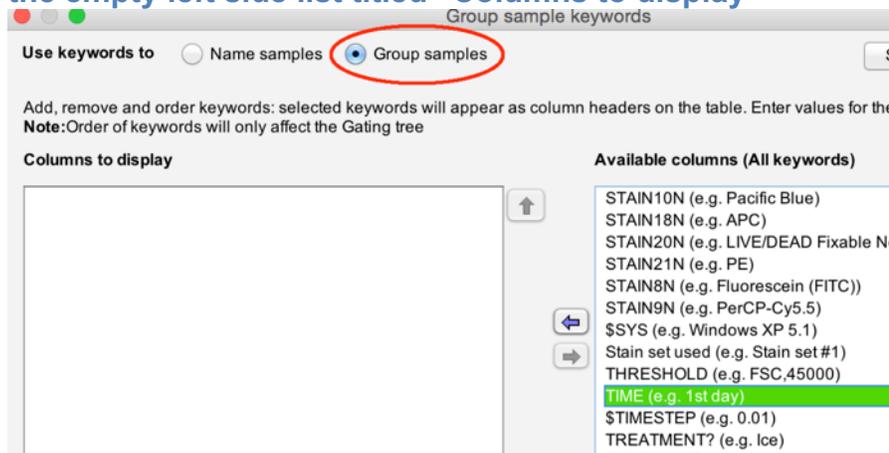
1.4 Click the task “Create, manage, assign keywords”



AutoGate responds by popping up the keywords window

1.4.1 Ensure the “Group samples” radio button is selected at the top left of the window.

1.4.2 Drag the keyword TIME from the list on the window ‘s right side to the empty left side list titled “Columns to display”

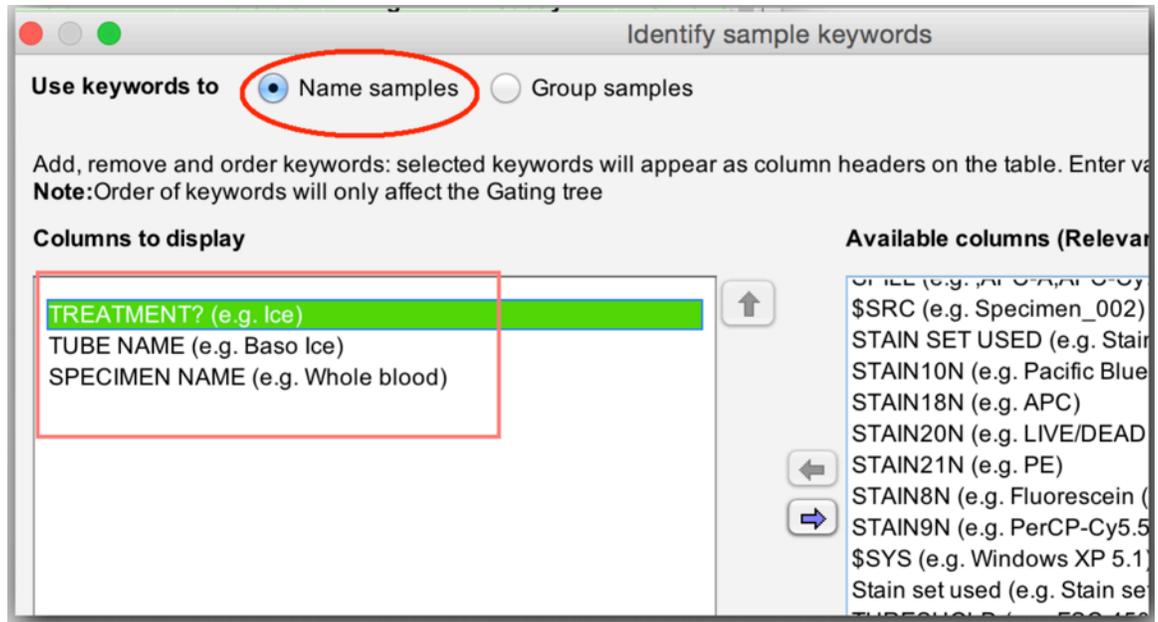


This adds the TIME column to the sample type table and re-organizes it with a navigation tree

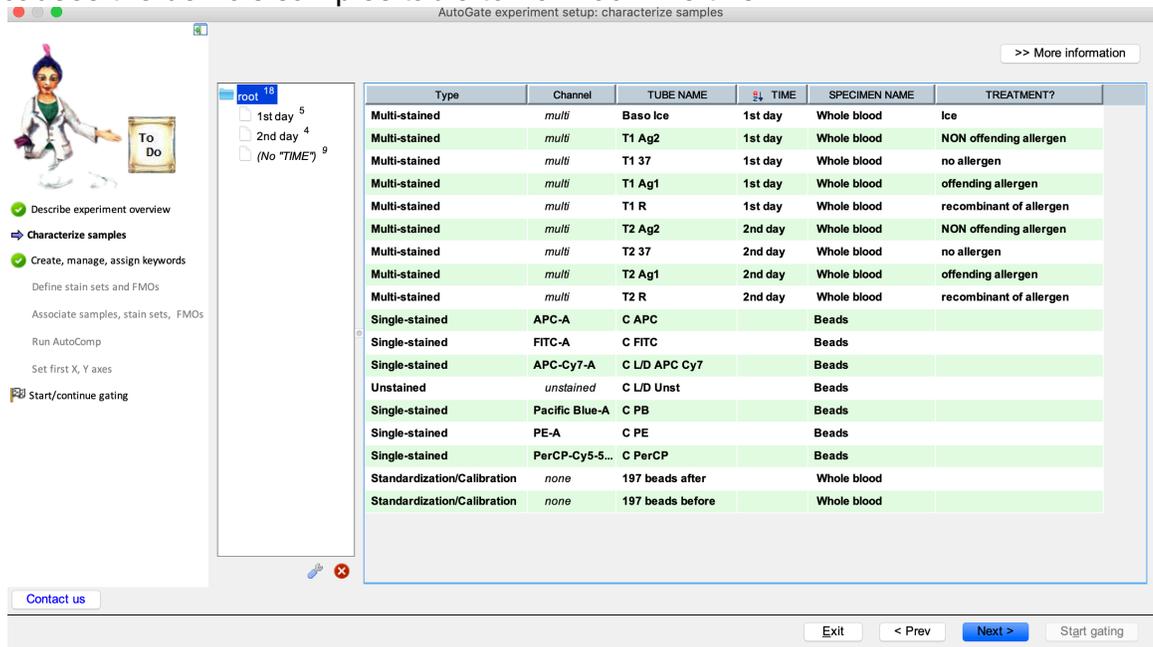
1.4.3 Ensure the “Name samples” radio button is selected at the top left of the window

1.4.4 Drag the keyword “TREATMENT” to the left side list “Columns to display”

1.4.5 Click the up arrow button to ensure TREATMENT is the first keyword



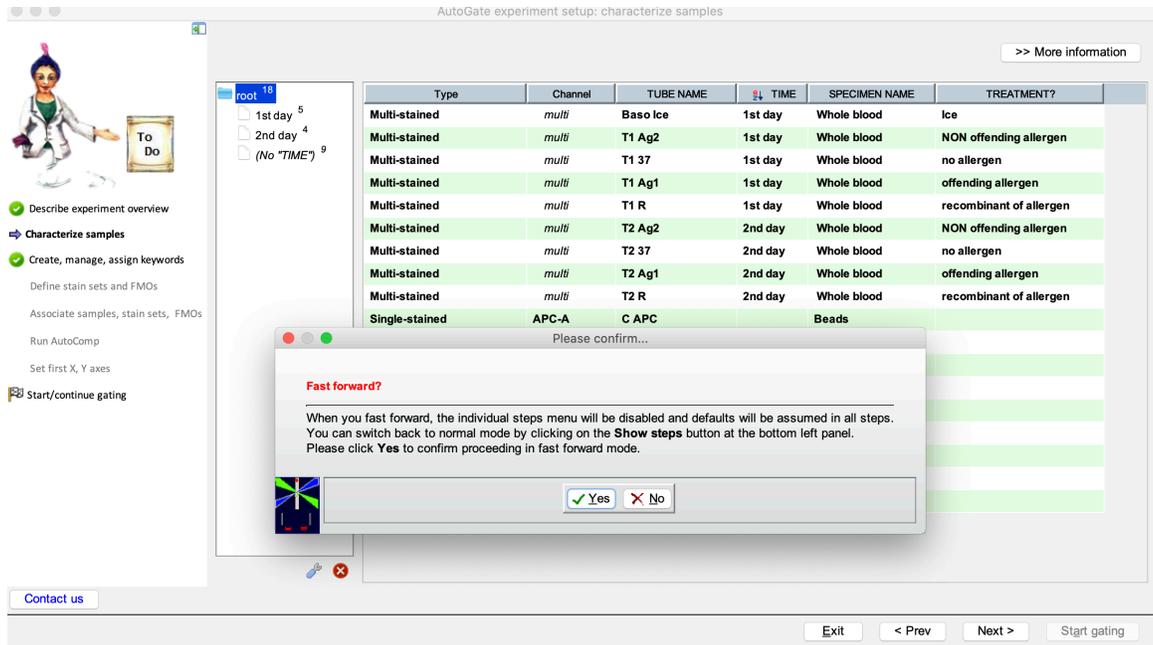
This causes the demo's samples table to now look like this.



1.5 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.

Click Yes to fast forward

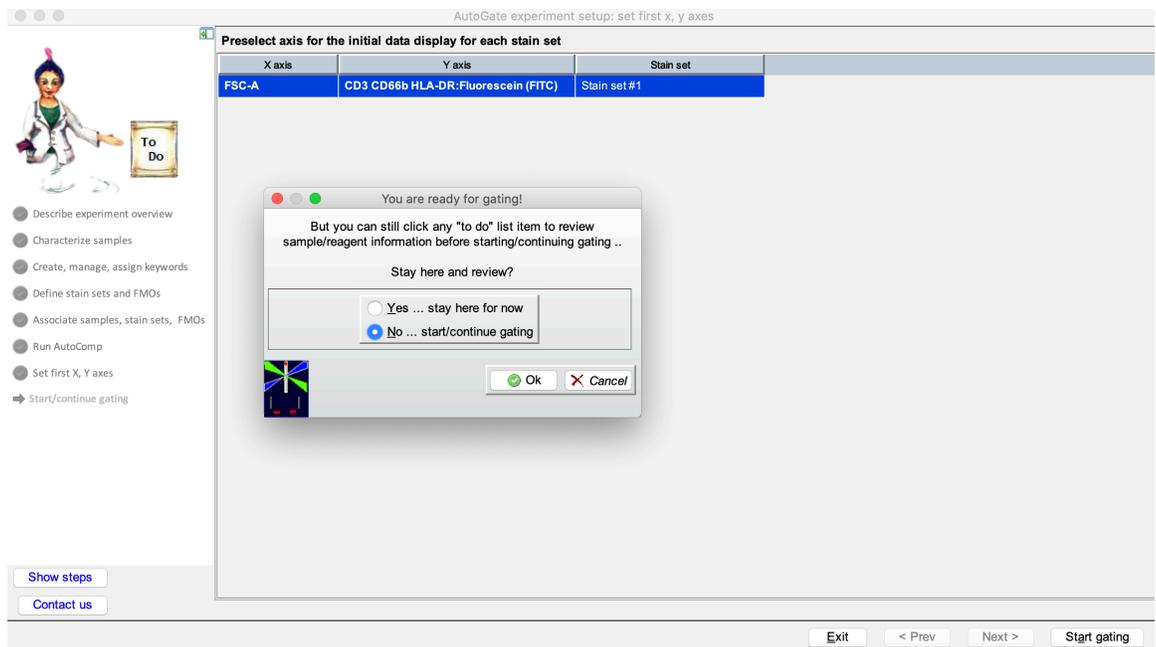


More details on each of these setup tasks are at

<http://cgworkspace.cytogenie.org/GetDown2/demo/allergySetupTasks.pdf>

AutoGate responds by

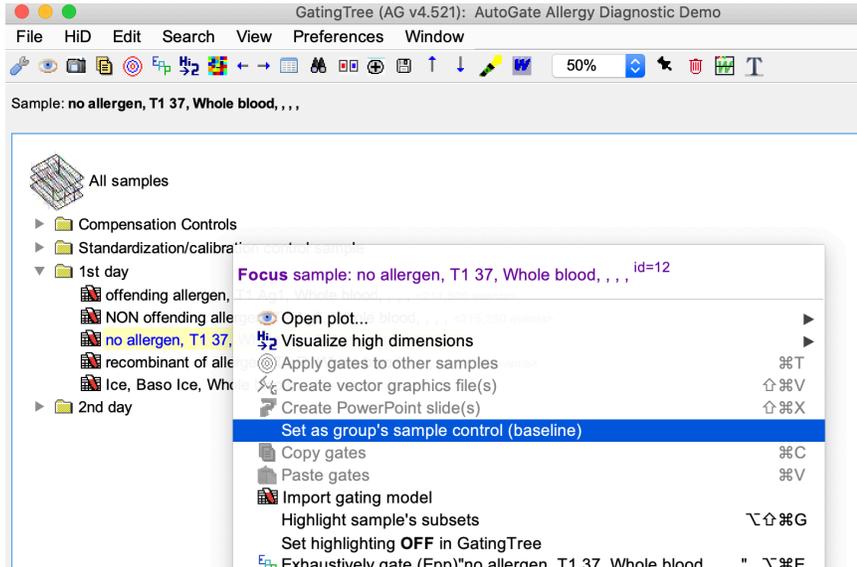
- Checking all of the information found in the samples
- Running our automatic compensation (AutoComp) if a feasible set of compensation controls are found
- Halting only when problems are found; for example, non plausible labels for compensation controls.



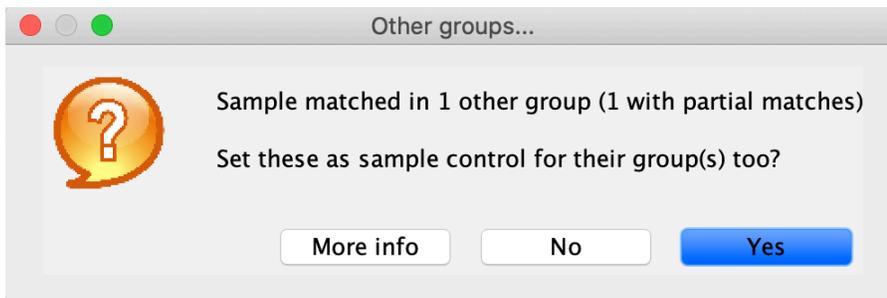
2 Gate the experiment

2.1 Define the allergy gating model for the base line sample

2.1.1 Select the base line and declare it such



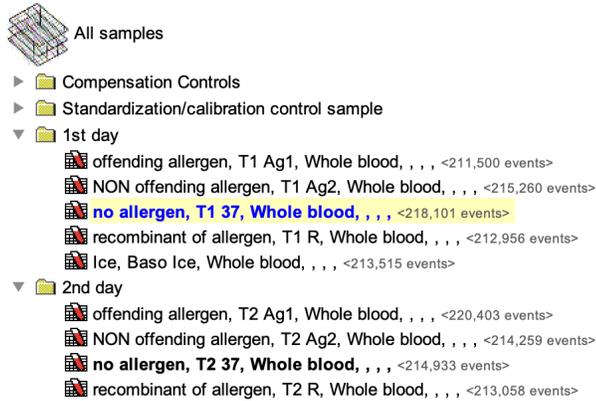
AutoGate responds by noticing that a sample in the 2nd day group has a similar treatment and thus asks this question



2.1.2 Click the Yes button

This sets the base line in both time groups as the sample with a value of "no allergen" for the keyword treatment.

AutoGate responds by highlighting both samples in bold to indicate that they are base line samples

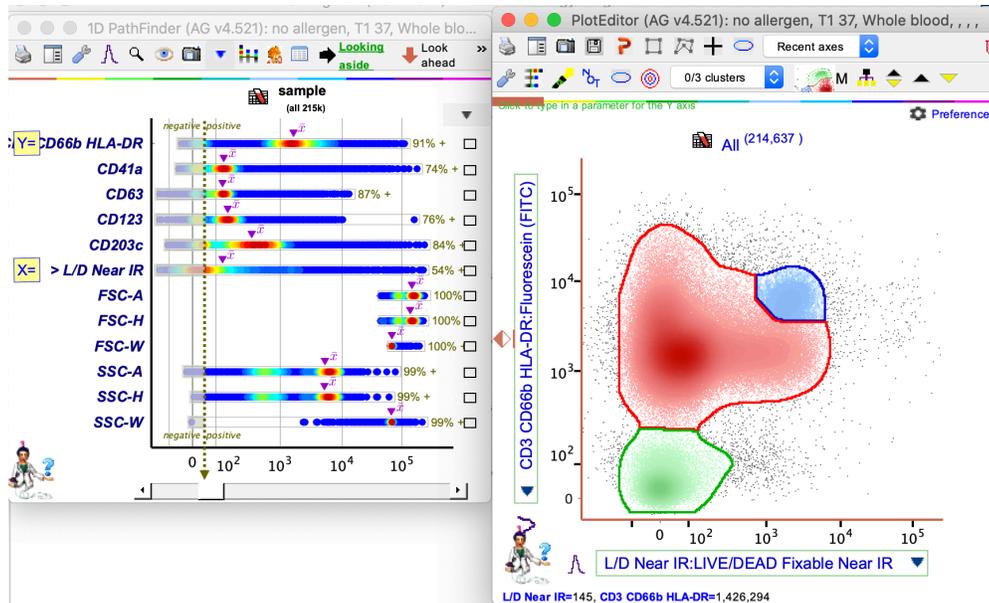


2.1.3 Open the plot editor window for the first base line sample

Double click on the sample “no allergen, T1 37, Whole blood” under the folder “1st day”.

AutoGate responds by

- Opening the plot editor window.
- Computing the bi-exponential's W (width) parameter to set the log/linear region of the scale as accurately as possible. It does this by analyzing EVERY sample which uses the same compensation matrix. This **only happens** the **first** time that AutoGate opens a sample for each compensation matrix in an experiment.
- Setting the X and Y axis to defaults automatically found during experiment setup
 - X=Live/dead marker
 - Y=dump channel
- Clustering the data for all of the sample's cells and the X and Y markers
- Showing each cluster by a different color



AutoGate's cluster analysis is based on this publication.

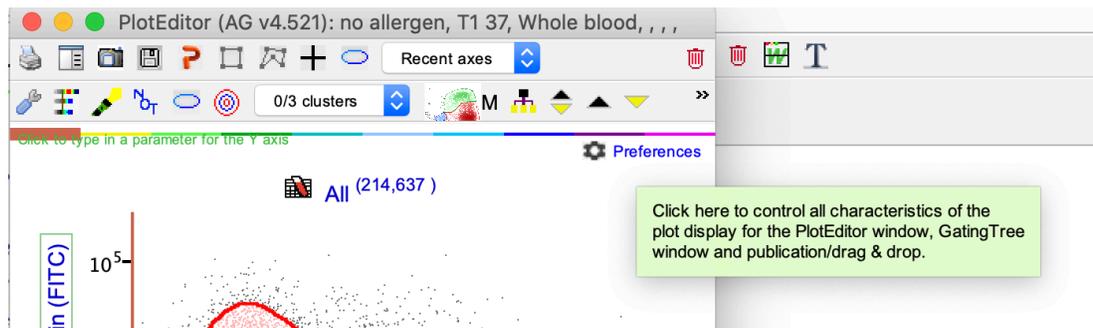
<http://cgworkspace.cytogenie.org/GetDown2/demo/dbm.pdf>

2.1.4 Match the preferences used by this tutorial

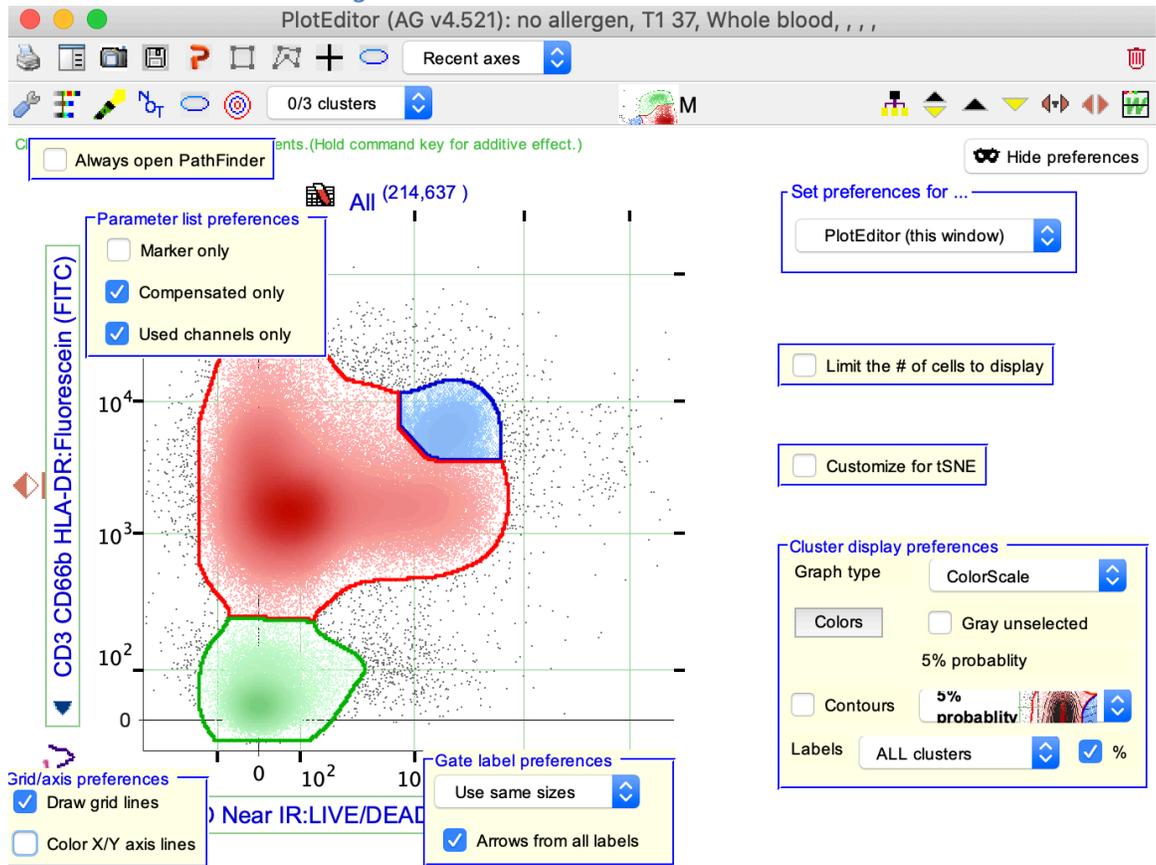
This is optional, it allows you to see the same things on your screen as are shown here.

2.1.4.1 Click the preferences button

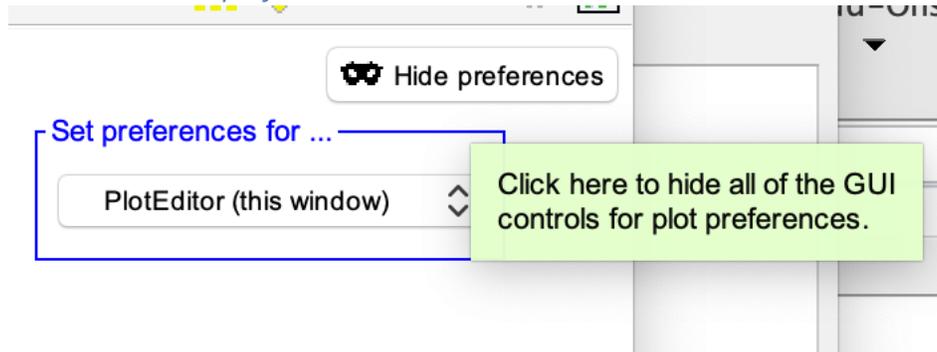
This button is at the top right of the plot editor window.



2.1.4.2 Ensure these visual settings

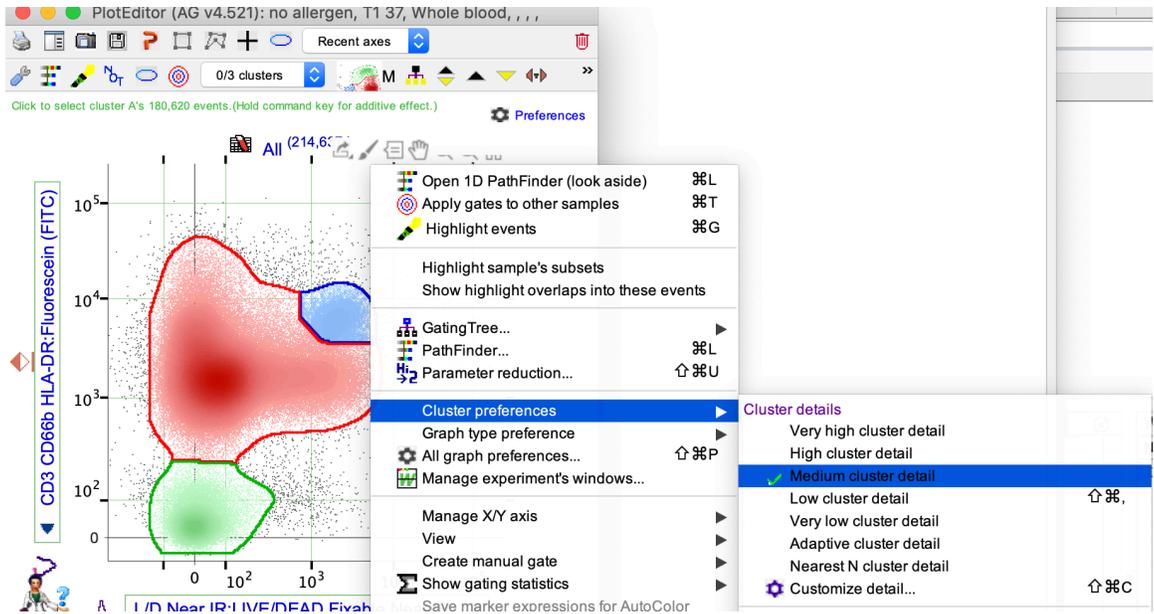


2.1.4.3 Click the "Hide preferences" button



2.1.4.4 Ensure these cluster detail preferences

Activate the right click menu



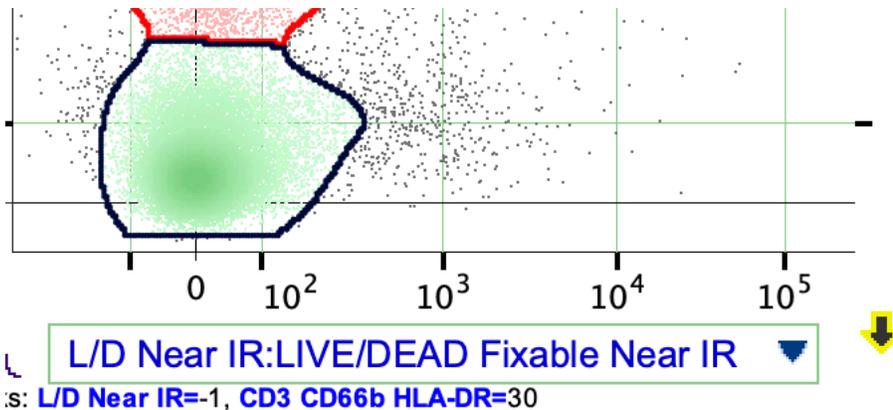
2.1.5 Create the first “Live Dumped” gate of the allergy gating sequence

2.1.5.1 Click on the green cluster.

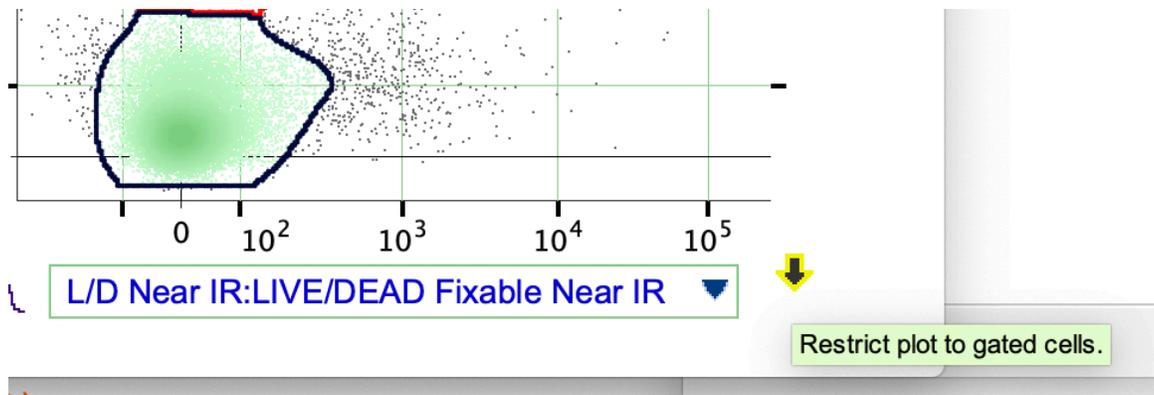
Using your mouse, hover over the green cluster and click. These are live cells low on the dump channel.

AutoGate responds by

- Drawing a black border around the green cluster to signify user’s selection.
- Indicating “Subset” on the right side to signify an unnamed gate exists

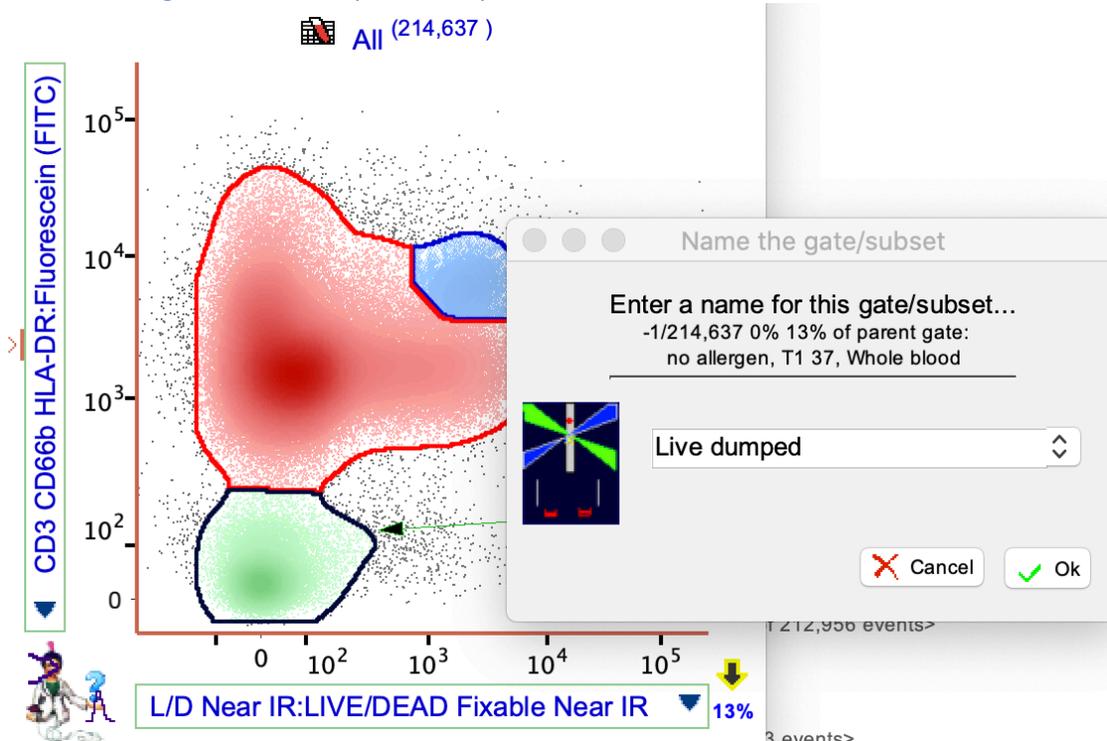


2.1.5.2 Click the down arrow button



AutoGate responds by asking you to name the gate.

2.1.5.3 Name the gate "Live dumped" and press the Ok button



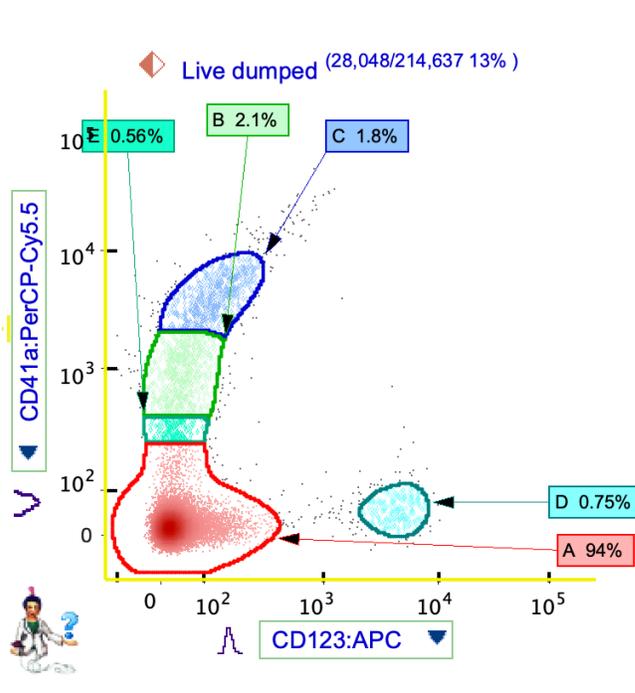
AutoGate responds by restricting the plot window to ONLY the cells/events within your chosen cluster.

2.1.5.4 Set the next plot to X=CD123 and Y=CD41a

To set the axes you click on the down arrow button next to the X and the Y axis labels you activate a drop down of choices.

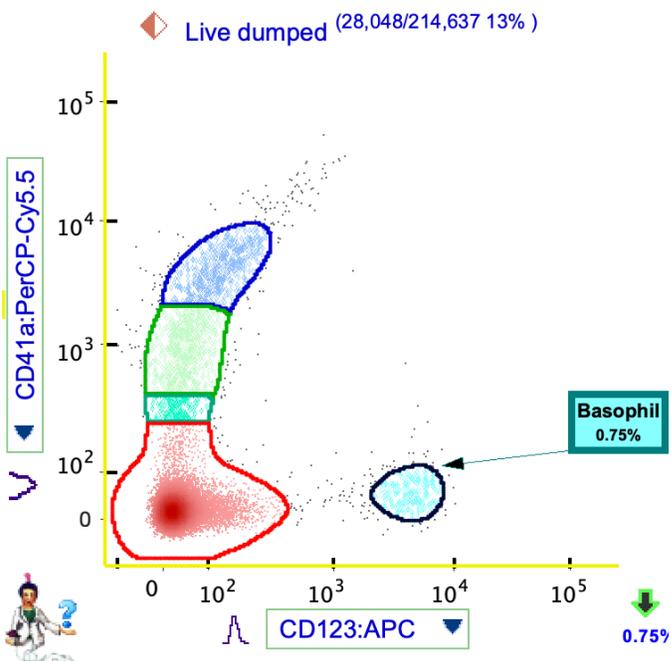
AutoGate responds by

- Clustering the cell subset for the new X and Y parameter settings
- Rendering this 2D projection of the current data set.

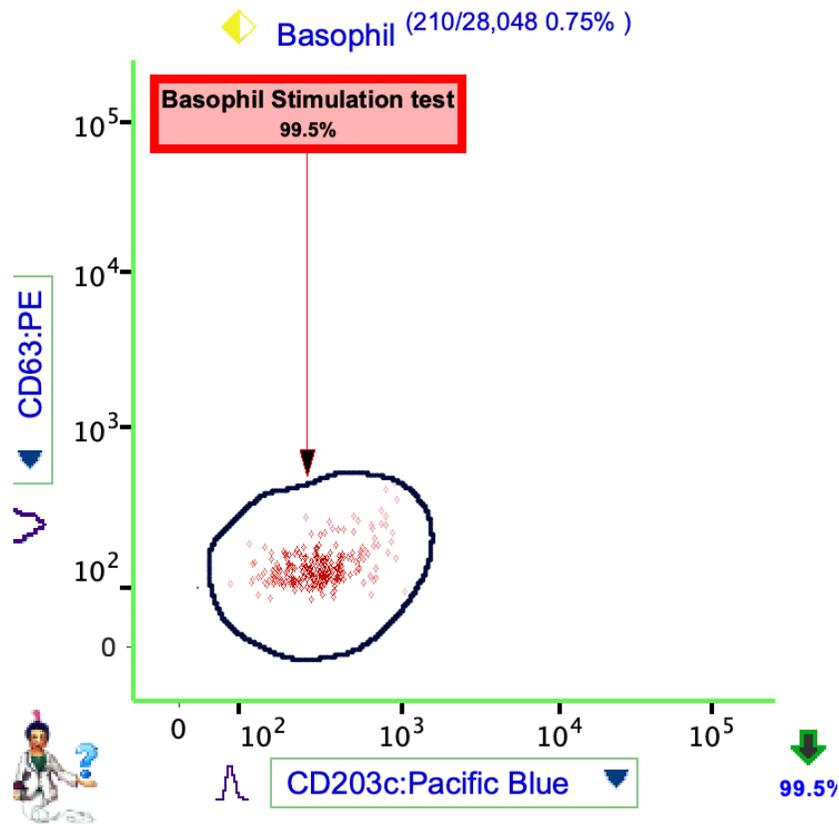


2.1.6 Create the Basophils gate

Use the same actions as when you created the previous "Live dumped" gate.



2.1.7 Create the gate for testing basophils stimulation

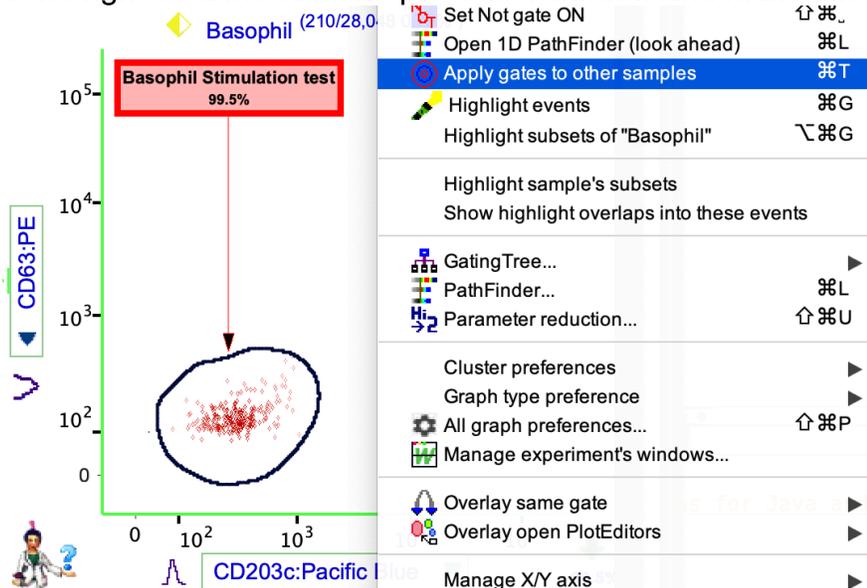


Use the same actions as when you created the previous “Basophils” gate.

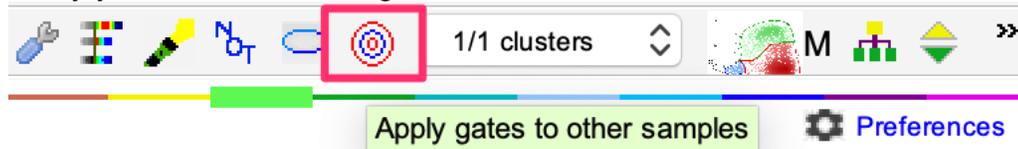
2.2 Apply the gating model created for the base line sample to all other samples

2.2.1 Apply gates to other samples

Activate the right click menu in the plot editor window and make this choice.

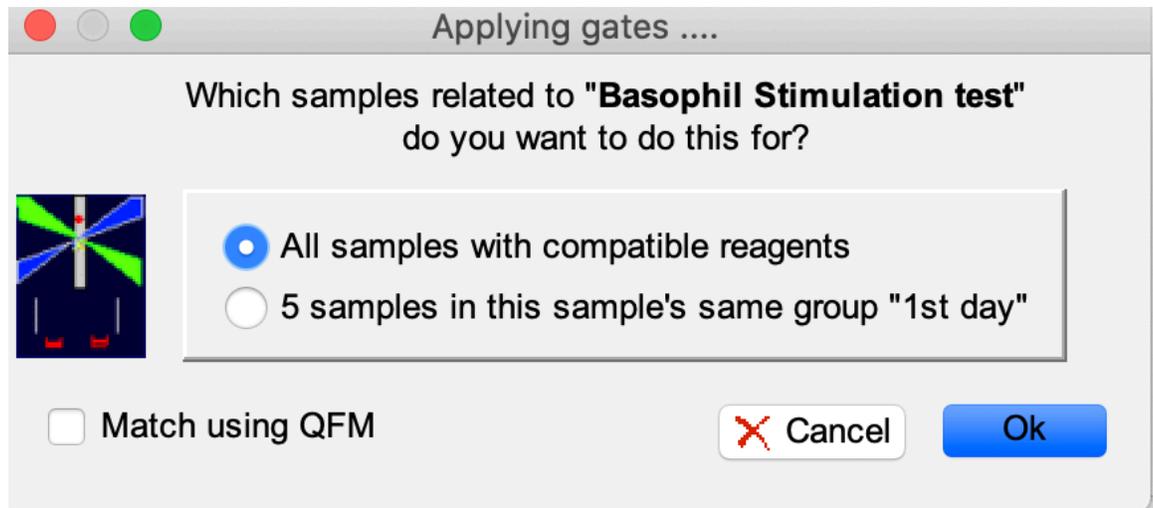


Alternatively you can click the target button on the toolbar.

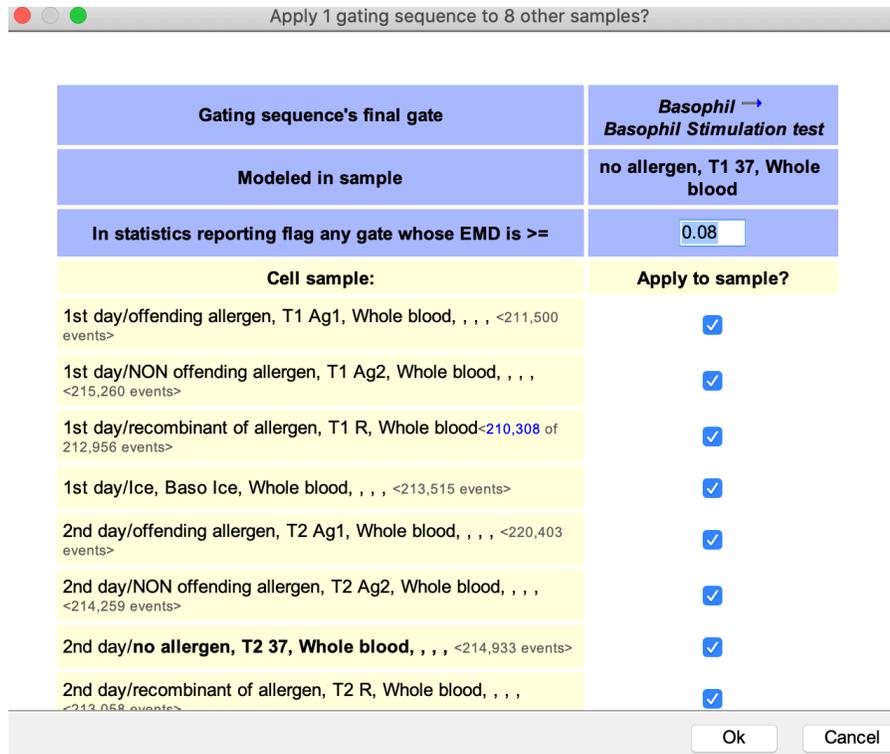


2.2.2 Choose all compatible samples

Click the Ok button for this window



2.2.3 Review the next window



The top entry field in the above window is the earth mover's distance (EMD) threshold. By default AutoGate sets it to 0.08. The purpose of this is only visual - to tell AutoGate to highlight any sample that is greater than or equal to this threshold. AutoGate does not pre-determine this threshold. Instead, the researcher must arrive at an appropriate threshold by testing multiple experiments with AutoGate's EMD measurement. The default of 0.08 was chosen after looking at several patients for the current basophil allergy of assay.

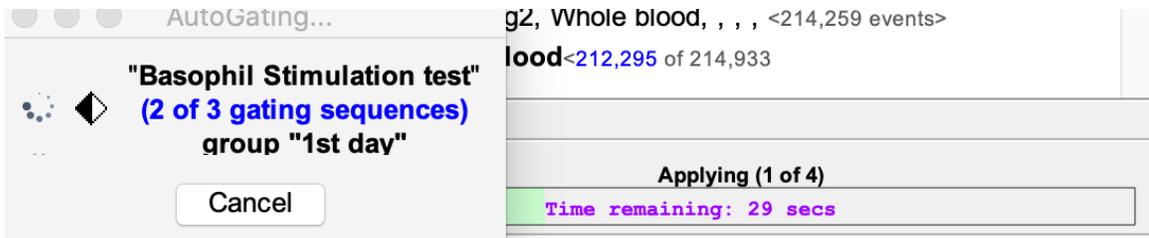
The logic in the earth mover's distance for biological change quantification is described in this publication.

<http://cgworkspace.cytogenie.org/GetDown2/demo/emd.pdf>

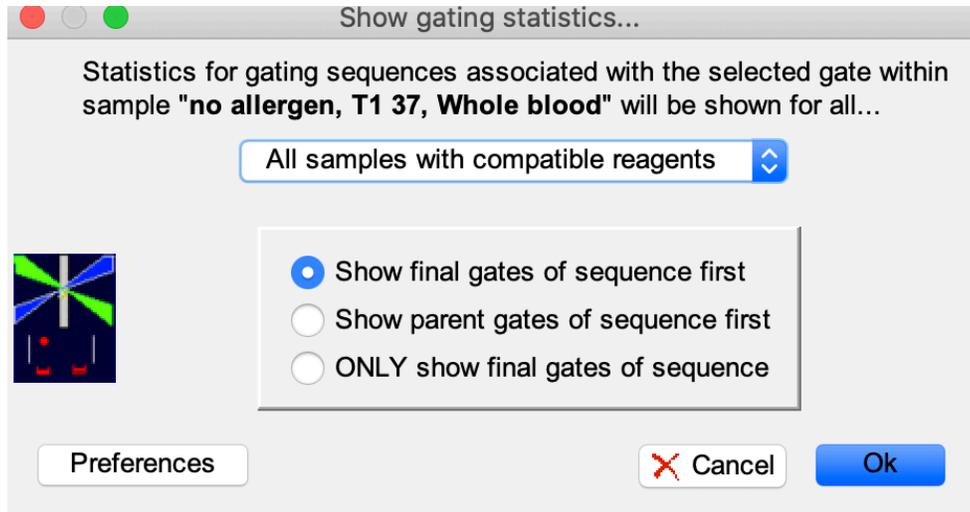
2.2.4 Click the Ok button

AutoGate responds by

- 1) Applying the gating model in the
 - a) "No allergen" sample for the 1st dy group to the no allergen sample in the 2nd day group
 - b) "No allergen" sample for the 1st day group to all other samples in the 1st day group
 - c) "No allergen" sample for the 2nd day group to all other samples in the 2nd day group



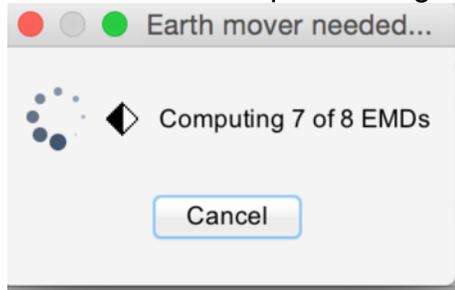
2) Asking you to confirm the format of the output report



2.2.5 Choose “Show final gates of sequence first”

AutoGate responds by

1) Quantifying the degree of difference/change between each sample’s final gate “Stimulated basophils” using the earth mover’s distance statistic (EMD)



2) Showing the final results with this window

If a gate surpasses the EMD threshold AutoGate puts a check mark in column 1 and makes the font of the EMD column bold and italics.

Statistics for 1 gating sequence(s) associated with Basophil Stimulation test

row #	TIME	TREATMENT?	TUBE NAME	SPECIMEN NAME	Size	Basophil → Basophil Stimulation test				All → Live dumped			Live dumped → Basophil			
						Clusters picked	Model gate	EMD	Events	Clusters picked	% of parent	Events	Clusters picked	% of parent	Events	
1	1st day	offending allergen	T1 Ag1	Whole blood	208,841 of 211,500				0.161	352		6.6%	13,803		2.6%	355
2	1st day	NON offending allergen	T1 Ag2	Whole blood	212,609 of 215,260				0.018	273		9.8%	20,781		1.3%	279
3	1st day	no allergen	T1 37	Whole blood	214,637 of 218,101			n/a	n/a	209		13%	28,048		0.75%	210
4	1st day	recombinant of allergen	T1 R	Whole blood	210,308 of 212,956				0.059	273		9.6%	20,263		1.4%	276
5	1st day	ice	Baso Ice	Whole blood	210,528 of 213,515				0.118	212		9.4%	19,887		1.1%	212
6	2nd day	offending allergen	T2 Ag1	Whole blood	216,854 of 220,403				0.153	187		14%	30,842		0.61%	189

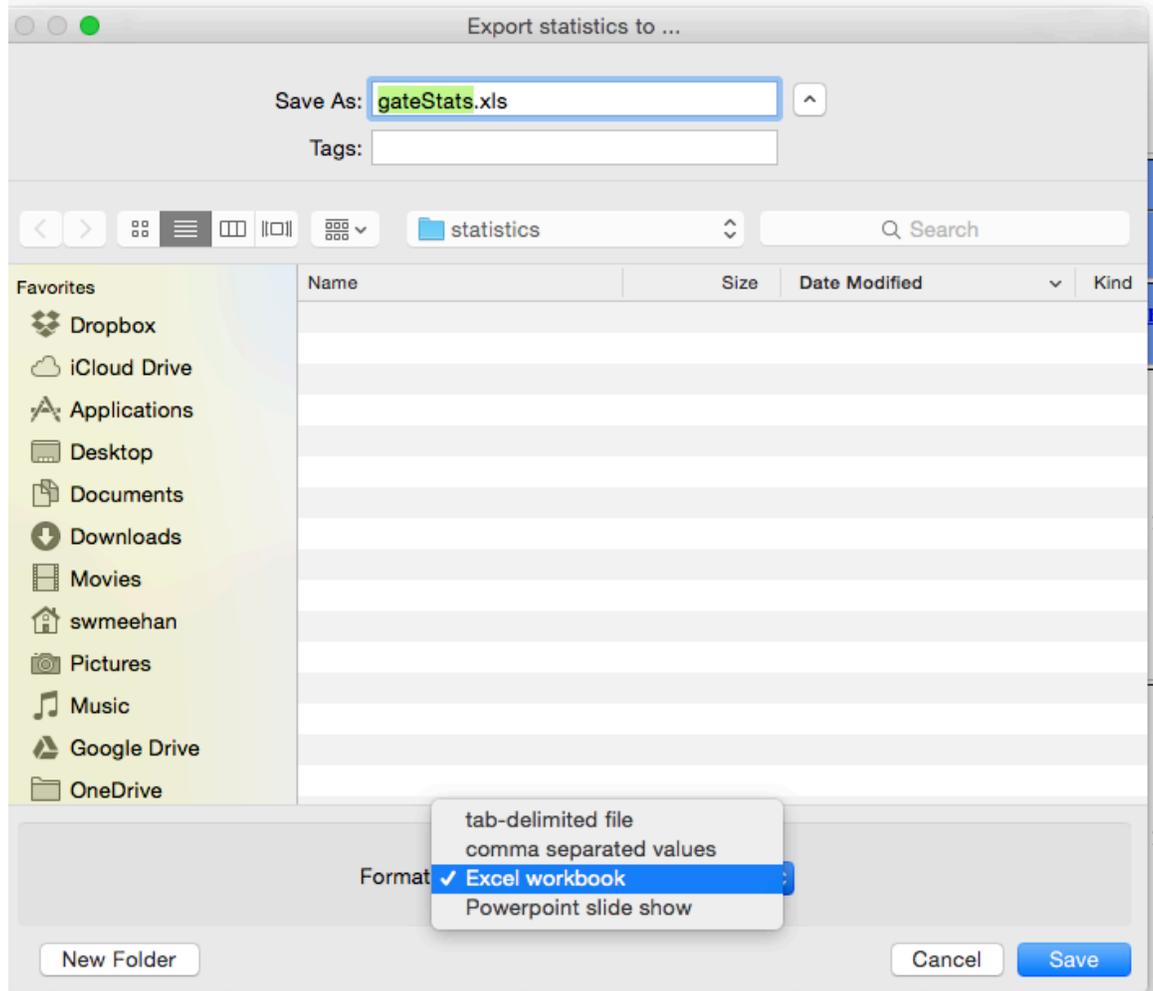
2.3 Export the experiment report to Excel

2.3.1 Click the Export button

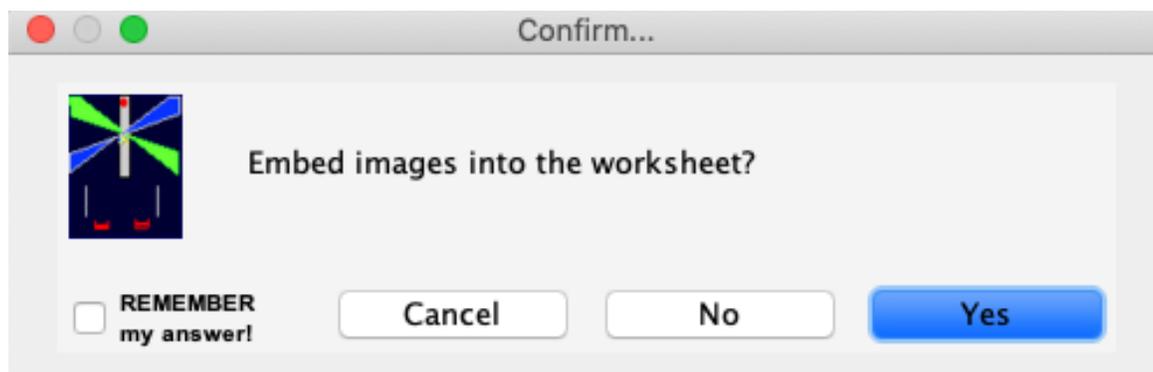
AutoGate responds by showing a file system window for you to indicate the name and format of the export file.

2.3.2 Select the Excel workbook export format and click the Save button

AutoGate will export to a straight text file (comma or tab delimited) or powerpoint or excel.

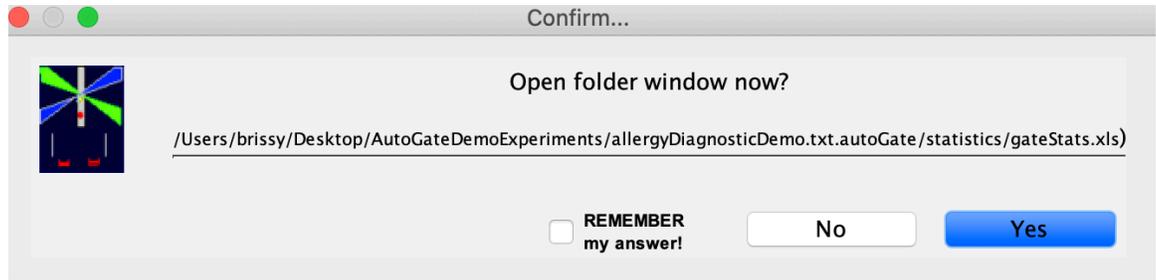


AutoGate responds by asking if you want the gate images embedded



2.3.3 Click the Yes button to embed the gate images

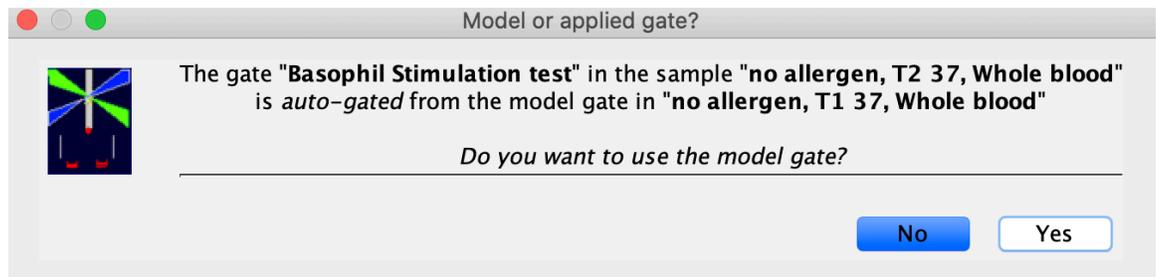
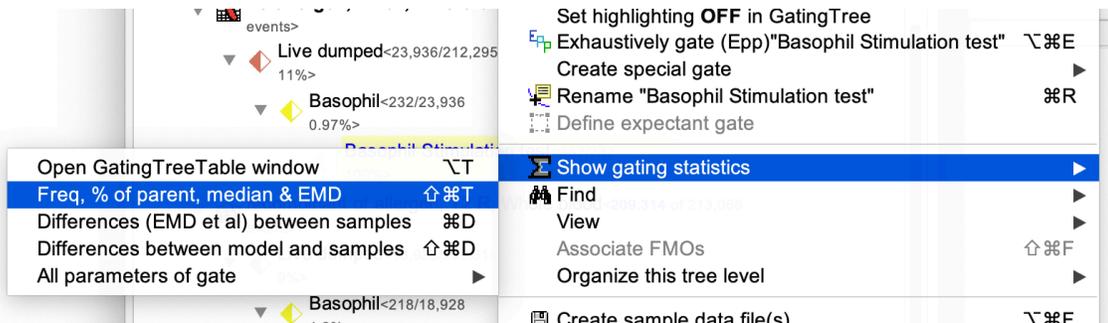
AutoGate responds by creating the workbook. The location of the work book file gateStats.xls is in the following sub folder of your Desktop.



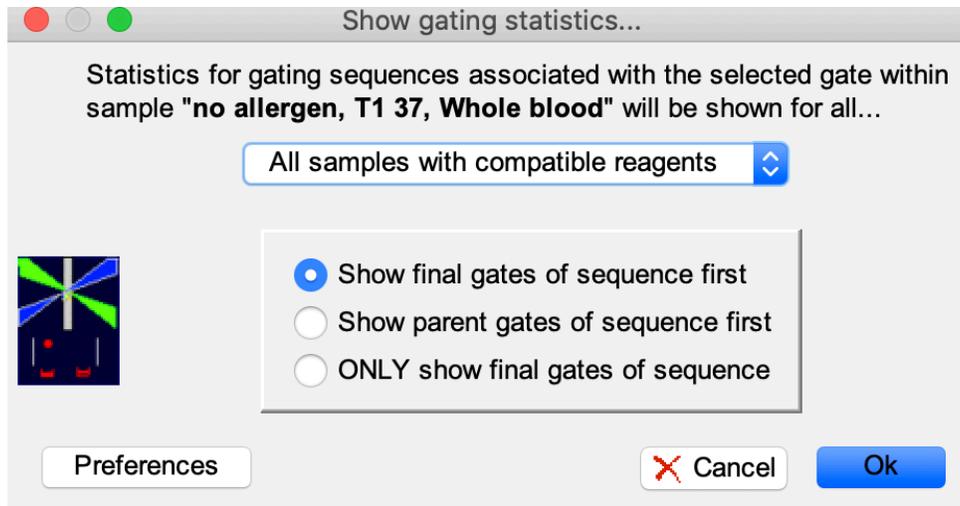
If you always wish to open the workbook when you create it then, set the preferences accordingly.

2.3.3.1 Choose show gating statistics from the view menu of the gating tree window

Ensure you have selected the desired gate in the tree first.

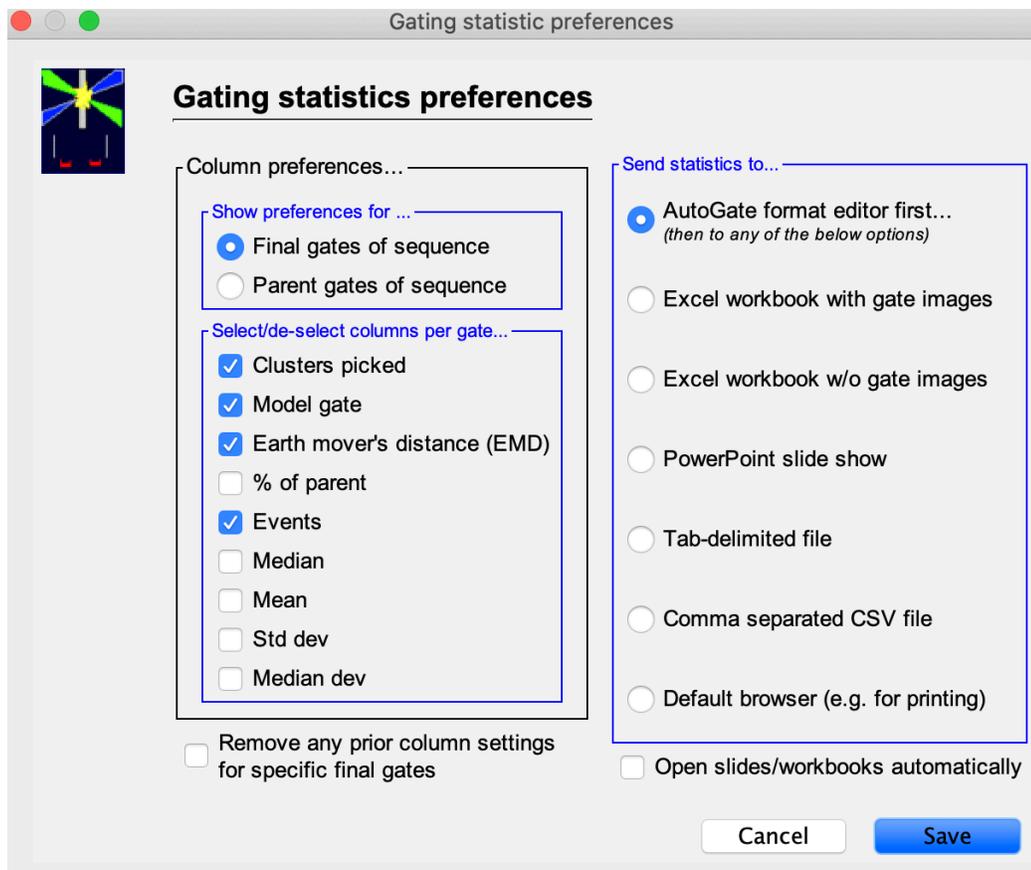


AutoGate responds by showing this window



2.3.3.2 Click the Preferences button

AutoGate responds by showing the “Gating statistics preferences” window. Select the bottom right checkbox “Open slide/workbooks automatically”.



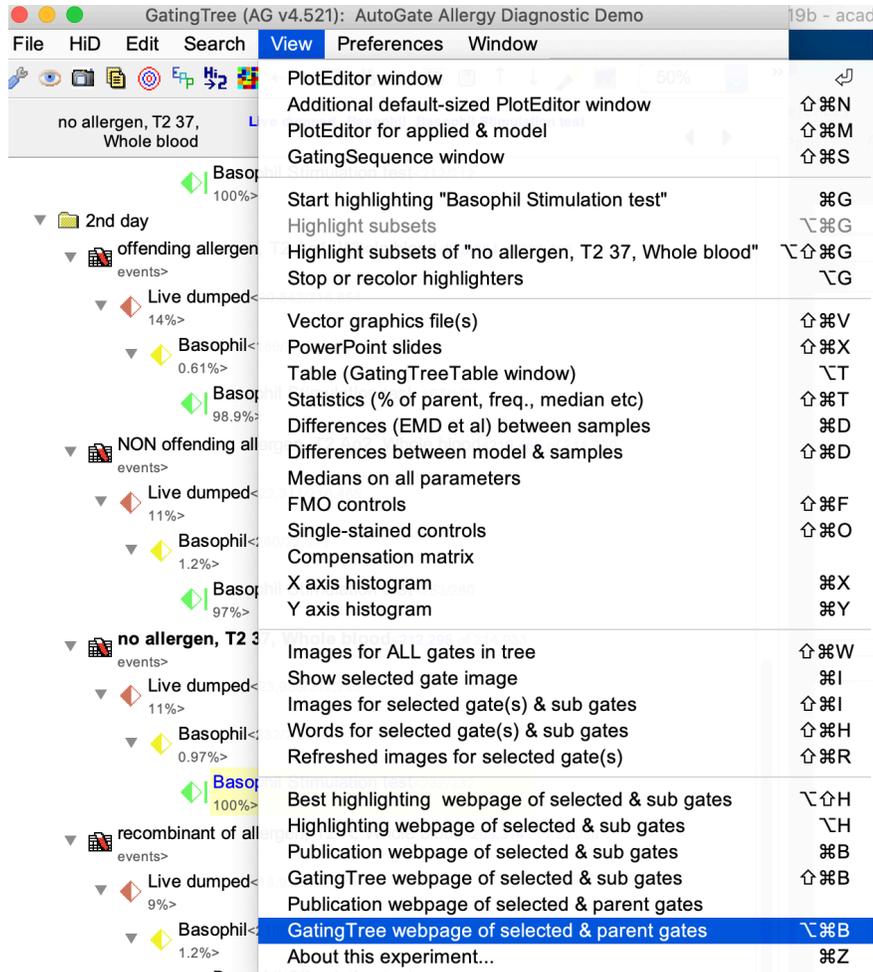
Click [here](#) to download the workbook produced by the author of this tutorial.

<http://cgworkspace.cytogenie.org/demo/allergyDiagnosticDemo.xls>

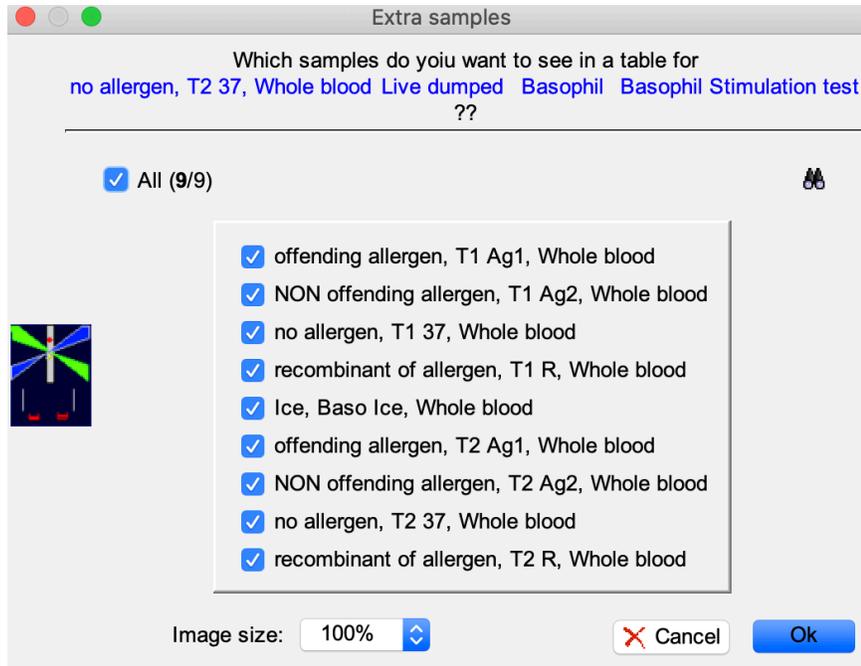
2.4 Show gate images for all samples in a table

2.4.1 Choose the appropriate browse menu item on the View menu of the gating tree window

2.4.2 Ensure you have selected the appropriate gate first.

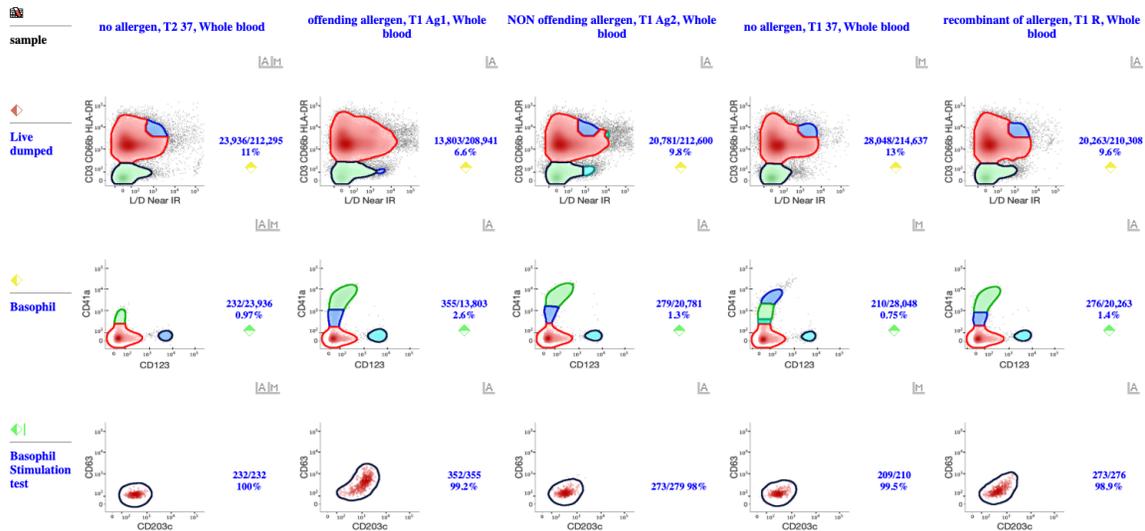


AutoGate responds by showing this window.



2.4.3 Click the Ok button for the “Extra samples” window

AutoGate responds by showing this in your default browser. You can convert this to a PDF or other things using your browser’s tools.



This is tested with Internet Explorer and Safari browsers.