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MPAPASS - Gating flow cytometry multiplex data

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bjnwkmfe

Translational Nanobiology Section

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ABSTRACT

This is collection contains the protocols required for each step in the mpapass software pipeline for performing stitched multiplex analysis. This is one of a number of protocols in the pipeline for using the mpapass software package and is applicable to the latest release of the software.

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PROTOCOL CITATION

Joshua A Welsh, Sean Cook, Jennifer Jones 2020. MPAPASS - Gating flow cytometry multiplex data. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bjnwkmfe

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MATERIALS TEXT

FlowJo

DISCLAIMER:

This protocol summarizes key steps for a specific type of method, which is one of a collection of methods and assays used for EV analysis in the NCI Translational Nanobiology Section at the time of submission of this protocol. Appropriate use of this protocol requires careful, cohesive integration with other methods for EV production, isolation, and characterization.

Importing the Files into FlowJo

1 Import the desired files into the FlowJo workspace using either the Add Samples (red box) button under the FlowJo tab (blue box), or simply drag the desired files into FlowJo.

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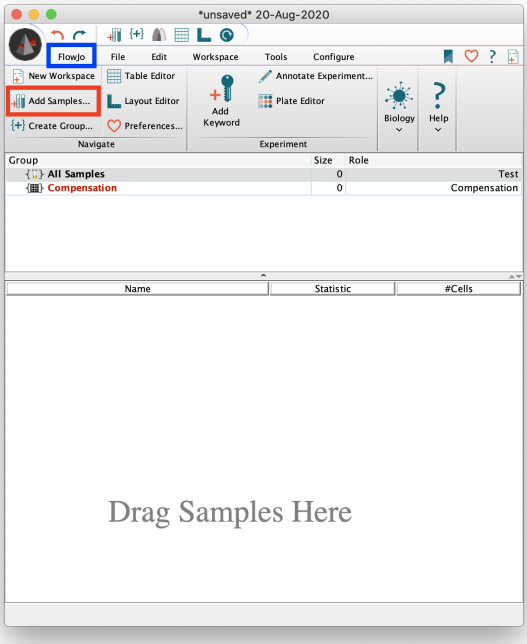
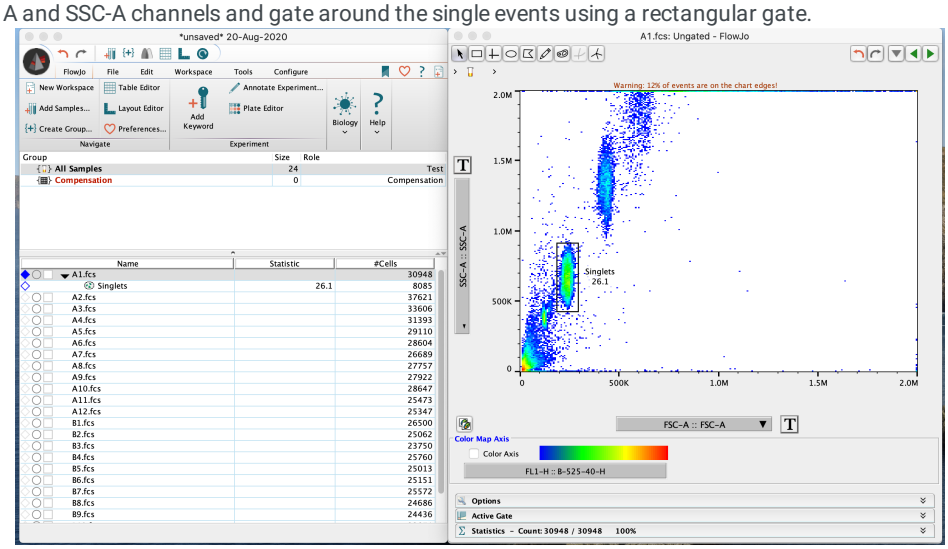


It is recommended that the fluorescence parameters be calibrated into standard units of 'molecules of equivalent soluble fluorophore' (MESF) to aid in reproducibility of data. This can be done using FCMPASS software in combination with commercially available MESF beads. See the FCMPASS software for further information at https://nano.ccr.cancer.gov/fcmpass

Gating the Bead Populations

2 Double-click on any of the samples in order to bring up a scatter plot. Change the parameters of the scatter plot to FSC-A and SSC-A channels and gate around the single events using a rectangular gate.



In this example, the A1.fcs sample was used to gate for singlets.

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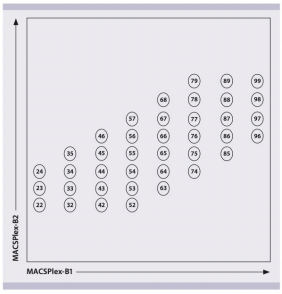
3 Double-click on the newly gated Singlets population to open a new scatter plot. Change the parameters to the FITC-A and PE-A channels, and a pattern similar to the figure below should be seen.

There are supposed to be 39 distinct bead populations, however only 38 can be seen due to the merging of the 89 and 99 bead populations.

image.png

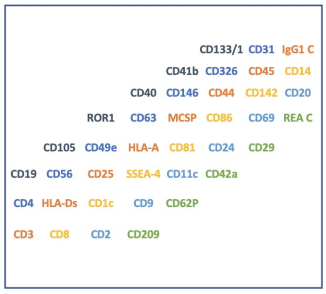
4 Each of the distinct populations corresponds to a antibody coated bead that can be determined from the bead legend below:



No.	Antibody	Isotype
22	CD3	mIgG2a
23	CD4	mIgG2a
24	CD19	mIgG1
32	CD8	mIgG2a
33	HLA-DRDPDQ	REA
34	CD56	REA
35	CD105	mIgG1
42	CD2	mIgG2b
43	CD1c	mIgG2a
44	CD25	mIgG1
45	CD49e	mIgG2b
46	ROR1	mIgG1κ
52	CD209	mIgG1
53	CD9	mIgG1
54	SSEA-4	REA
55	HLA-ABC	REA
56	CD63	mIgG1κ
57	CD40	mIgG1κ
63	CD62P	REA
64	CD11c	mIgG2b

No	Antibody	Isotype
65	CD81	REA
66	MCSP	mIgG1
67	CD146	mIgG1
68	CD41b	REA
74	CD42a	REA
75	CD24	mIgG1
76	CD86	mIgG1
77	CD44	mIgG1
78	CD326	mIgG1
79	CD133/1	mIgG1κ
85	CD29	mIgG1κ
86	CD69	mIgG1κ
87	CD142	mIgG1κ
88	CD45	mIgG2a
89	CD31	mIgG1
96	REA Control	REA
97	CD20	mIgG1
98	CD14	mIgG2a
99	mIgG1 control	mIgG1

Table 1.1: Overview of surface marker antibodies used for the MACSPlex Exosome Kit.



5 Gate each of the bead populations using a elliptical gate and label the populations according to the bead legend in step 4. For the merged population, gate around the entire population--later the population wil be separated.

image.png

Separating the Merged Population

6 On select cytometers it has been found that some lots of Miltenyi exosome multilpex beads do not adequately separate populations 89 and 99. If this is not the case proceed to the next section.

The FlowJo workspace should now have subpopulations under the Singlet population for each bead population as well as the merged population.

Click on the sample used to gate the bead populations (the highlighted A1.fcs sample in the figure below) and then navigate to the tools tab (blue box).

Click on Derive Parameters (red box) which will bring up a new window.

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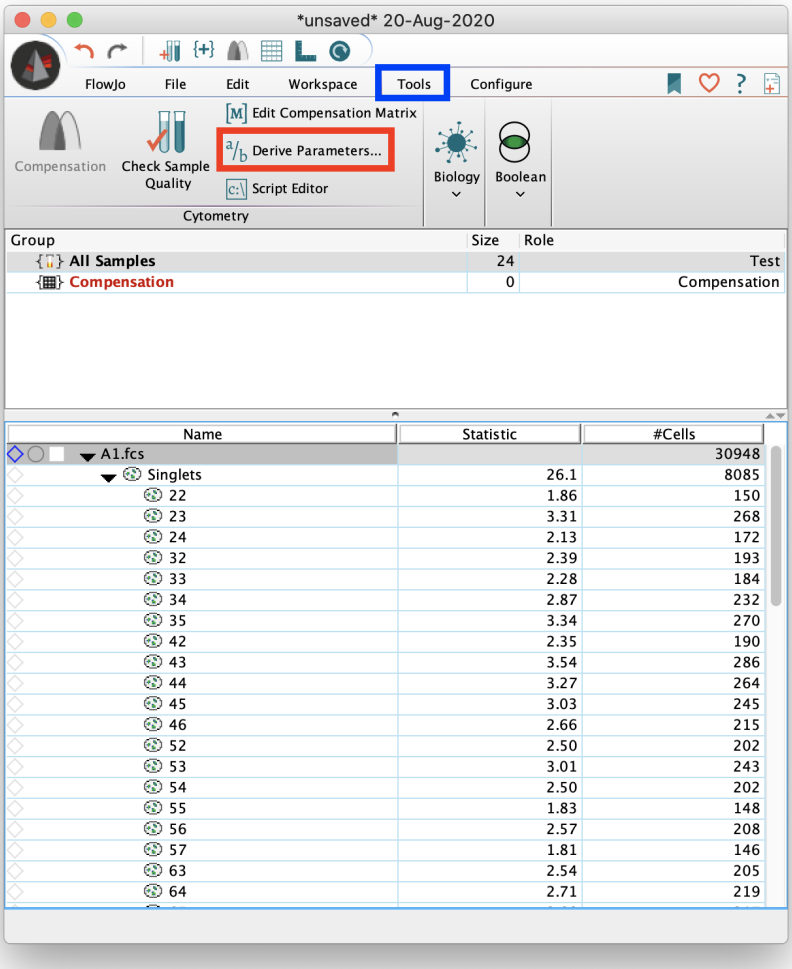

7 Creating the Derived Parameter:

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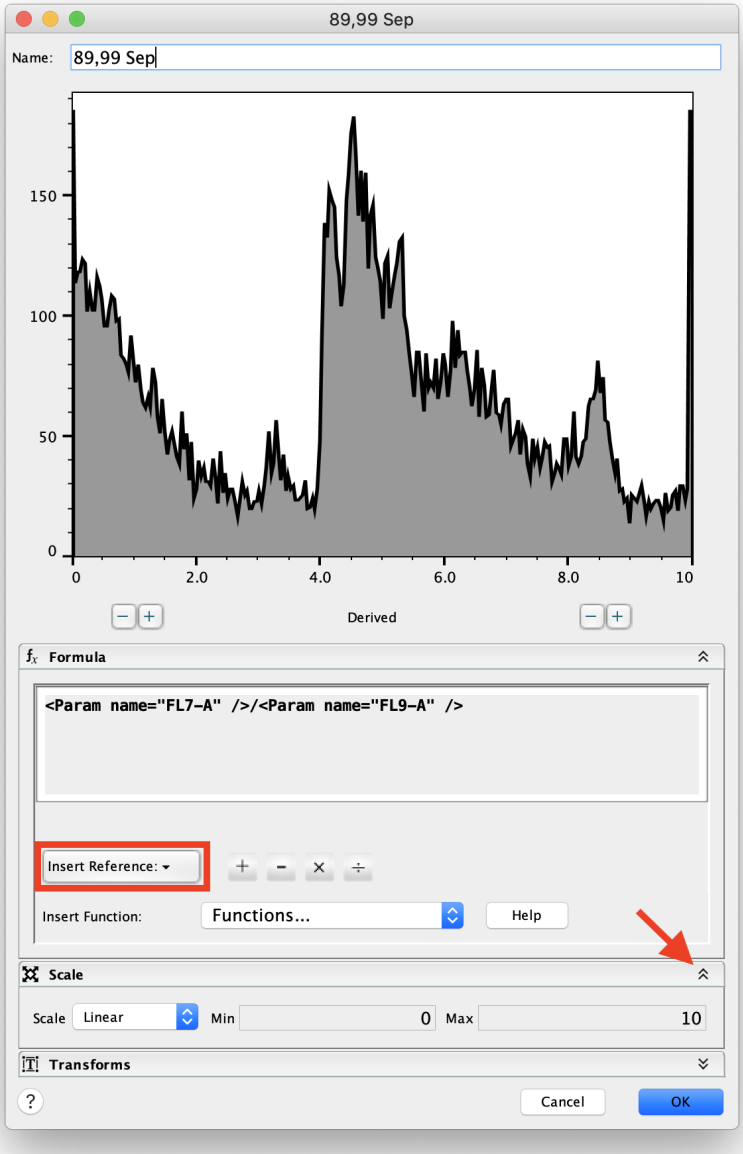

7.1 In this new window, at the top, name the derived parameter 89,99 Sep.

7.2 Using the Insert Reference button (red box), choose the V-525-A channel. A string of characters will appear in the text box above.

Click on the division button next to the Insert Reference button.

Once again using the Insert Reference button (red box), now choose the V-660-A channel.

A plot similar to the figure above should be now be seen

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7.3 Click on the collapse/open arrows for the Scale tab (denoted by a red arrow).

Choose the Linear scale and set the Min to 0 and the Max to 10.

Now click OK, and the derived parameter should appear beneath the chosen sample.

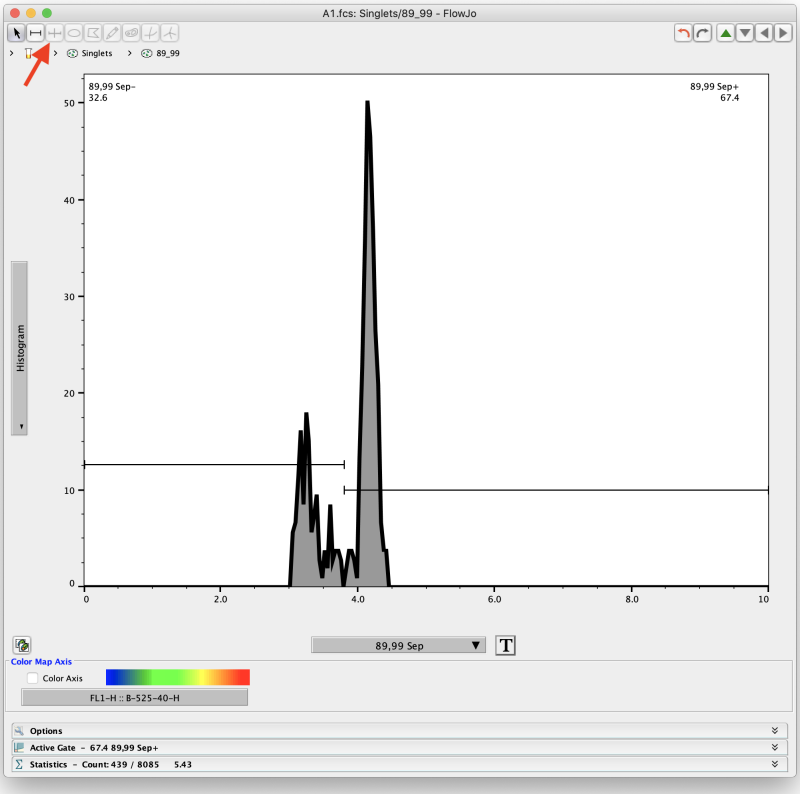
8 Gating the Merged Population:

8.1 Click on the 89_99 subpopulation to open a new scatter plot.

Choose 89,99 Sep derived parameter from the dropdown menu on the X-axis and the histogram option on the Y-axis.

A plot similar to the figure below should be seen.

8.2 Click on the Bisector Tool (red arrow) to gate the two bead populations. Make sure to choose a point where the two populations are distinctly separated.



8.3 The populations should be relabeled by right clicking the populations in the FlowJo workspace. The upper population should be labeled 99 while the lower population should be labeled 89.

Applying the Gates to Each Sample

9 In the FlowJo workspace, highlight all the gated populations and the derived parameter.

Now drag them into All Samples Group above (red arrow).

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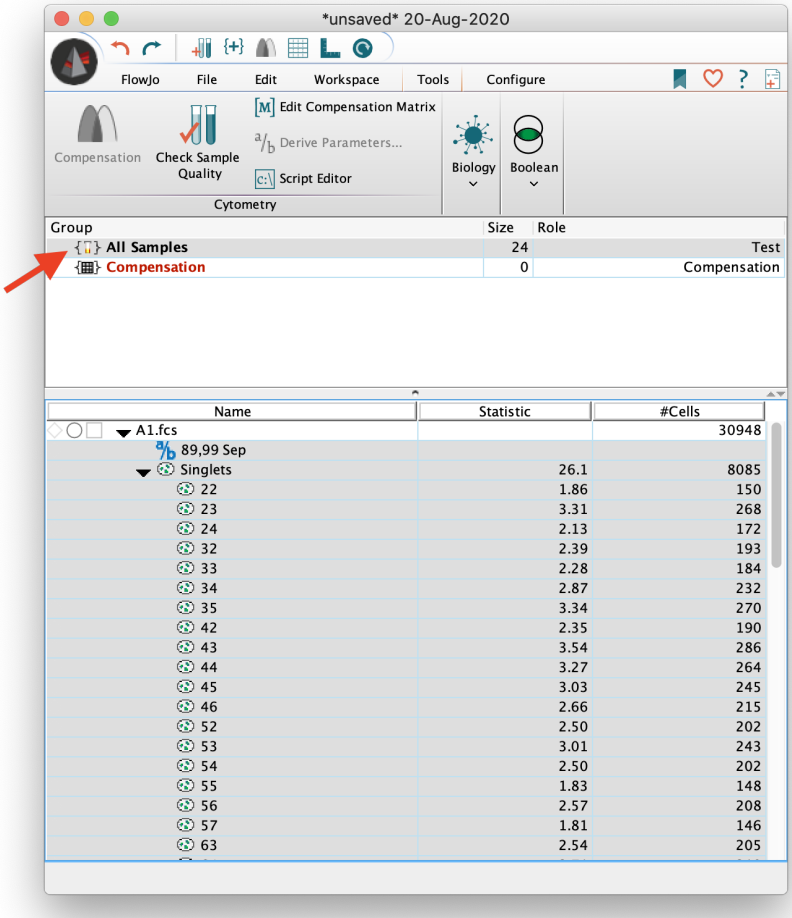
All files in the FlowJo workspace should now be gated exactly how we gated our chosen sample. This will be reflected in the FlowJo workspace as all samples should now have the same populations.



Exporting the Files

10 Now that all our samples have been gated, it is time to export the data from FlowJo into .csv files.

For any of the samples select all the bead populations to export, then under the Edit tab (blue box), click the Select Equivalent Nodes button to select all bead populations from every sample to export.

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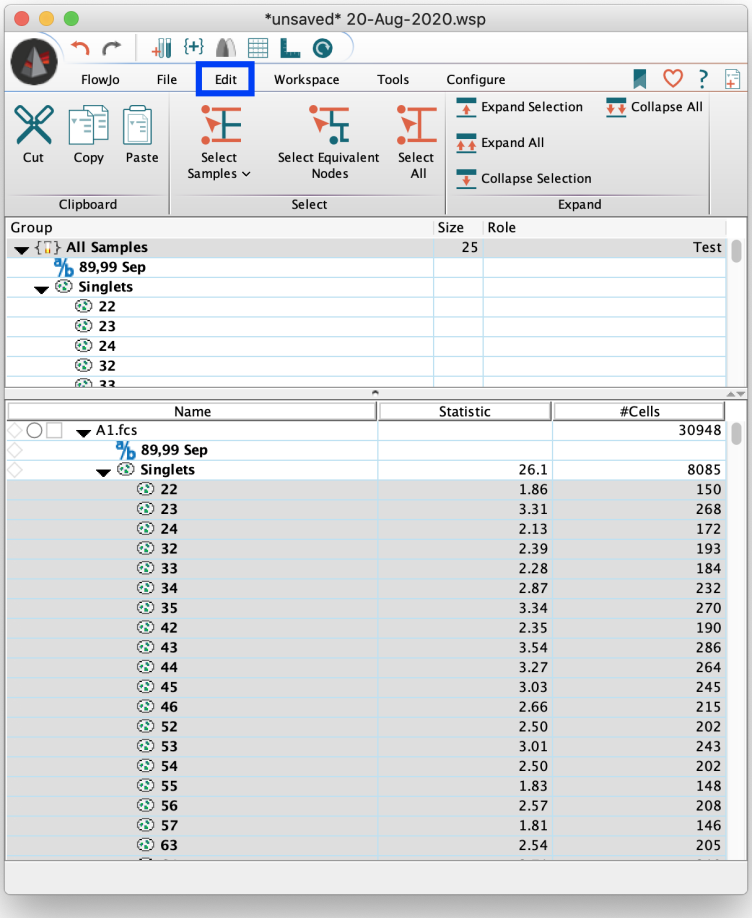

11 Navigate to the file tab and click on the Export/Concatenate Populations button.

12 Change the format to CSV - Scale Values.

Uncheck 'Include Header'

Change the Destination to desired folder.

Click on Custom Set of Parameters and then the View/Edit button directly next to it. Choose the APC parameter from the pop-up list.

Finally click Export to generate the .csv files in the destination folder.

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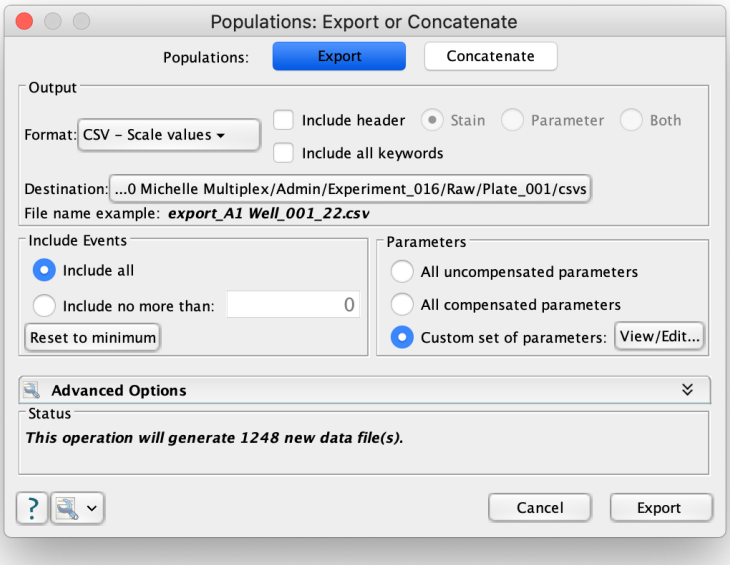

13 In preparation for the MPAPASS software, you will have to manually rename the 99 bead population from the export suffix of 89_99_99.csv to simply _99.csv

Repeat if this occurs with the 89 bead population.

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