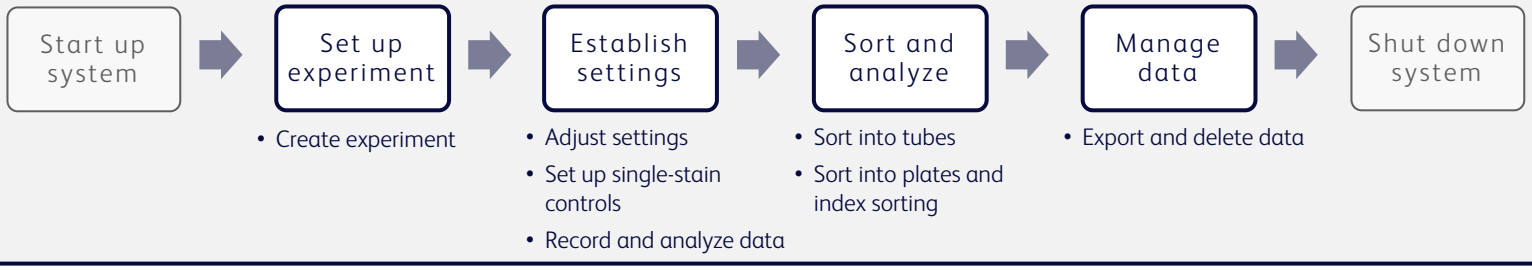
Day 4 targeted workflow

5-Color PBMC sort



Before you begin: Start up the system and run the daily startup procedure. Collect the job aids listed above. You will use those to guide you through this workflow.

Objective: To isolate T cell/monocyte doublets with high purity.

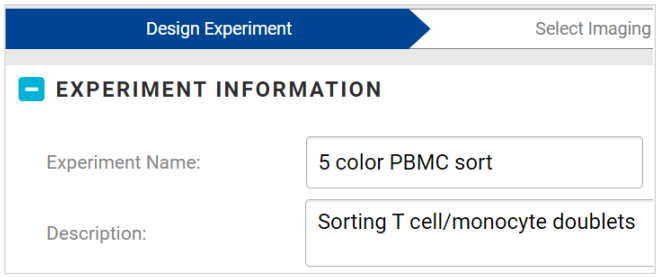
Sample description: Peripheral blood mononuclear cells (PBMC), ranging from 5 to 14 µm, were stained with the listed antibodies and fixed for biosafety. Single-stain controls were prepared with beads using the same antibodies. An unstained control for beads and cells is also included.

Fluorochrome	Label	Excitation/emission (nm)
BUV805	CD45	355/805
BB515	CD3	488/515
PE-CF594	CD14	488/612
RB780	CD19	488/780
APC-H7	HLA-DR	628/782

1. **Create experiment.**

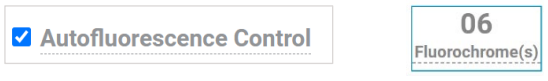
   a. Create a new blank experiment.

   b. Enter experiment name and description.



   c. Add fluorochromes and labels to match those listed in the table above.

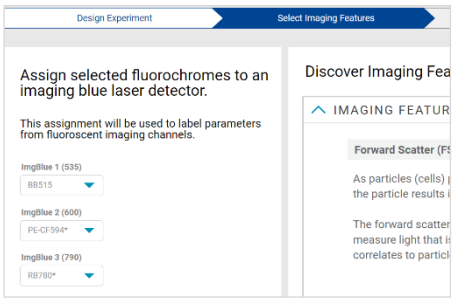
   d. Include an autofluorescence control.



Create experiment, continued

e. Assign fluorochrome(s) to the appropriate imaging detector(s).

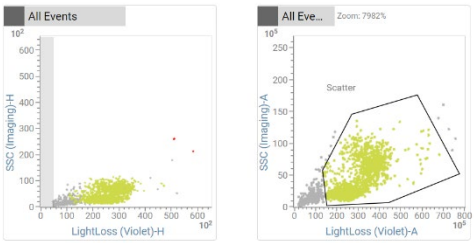
f. (Optional) Use the carousel to explore the imaging features and determine which you might be interested in using.



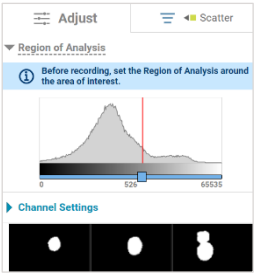
2. Adjust settings.

a. Load the sample tube.

b. Adjust the plot zoom, scatter gains, threshold, and Scatter gate to encompass the cells.

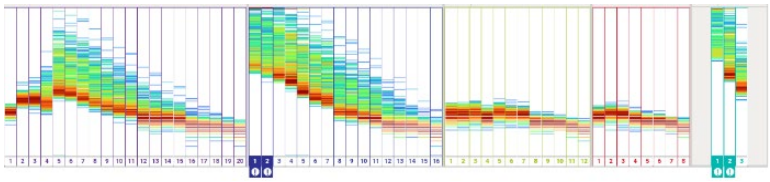


c. Adjust the Region of Analysis properly for the Scatter gate.



d. View the spectral plot and if any detectors are saturated, lower the gains.

e. Unload the tube.

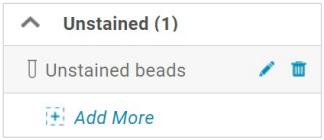


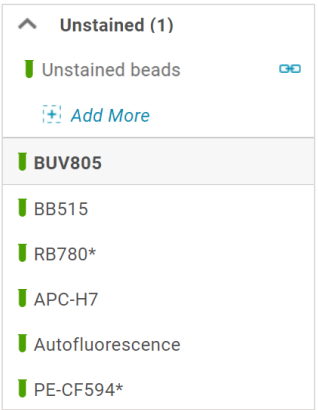
3. Set up single-stained controls.

a. Add an Unstained control and name it.

b. Ensure that the Region of Analysis is set correctly for the beads.

c. Record data for each bead control tube.





d. Ensure that the Region of Analysis is set correctly for the autofluorescence control (cells). Record data.

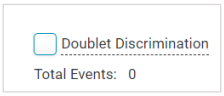
e. Adjust plot scaling and gate positions as needed and click **OK** to confirm each control.

f. Verify that the Raw Mode indicator disappears when all tubes have been confirmed.

4. Record and analyze data.

a. Load the sample tube.

b. Clear the doublet discrimination checkbox.

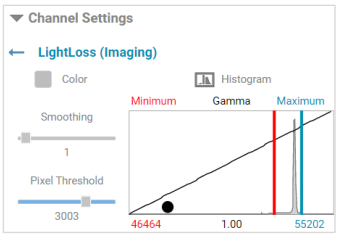


c. Adjust the plot zoom and scatter gate to encompass cells.

d. Use the image wall to adjust settings for detectors of interest:

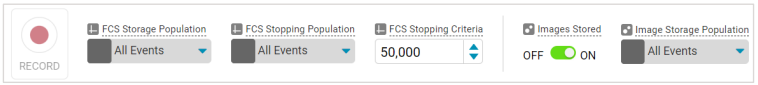
   i. Adjust **Region of Analysis**, if needed.

   ii. Adjust **Pixel Threshold** for each imaging detector.



e. Enter 50,00 events to record. Toggle on the Images Stored switch.

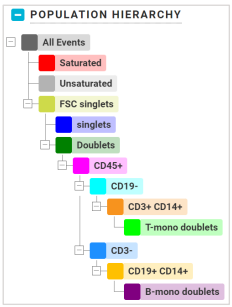
f. Record and name the data file.



g. Create new plots to view populations of interest.

h. Gate the appropriate populations. Rename the gates.

i. Use the image wall to adjust the channel settings for each imaging detector.



5. Sort.

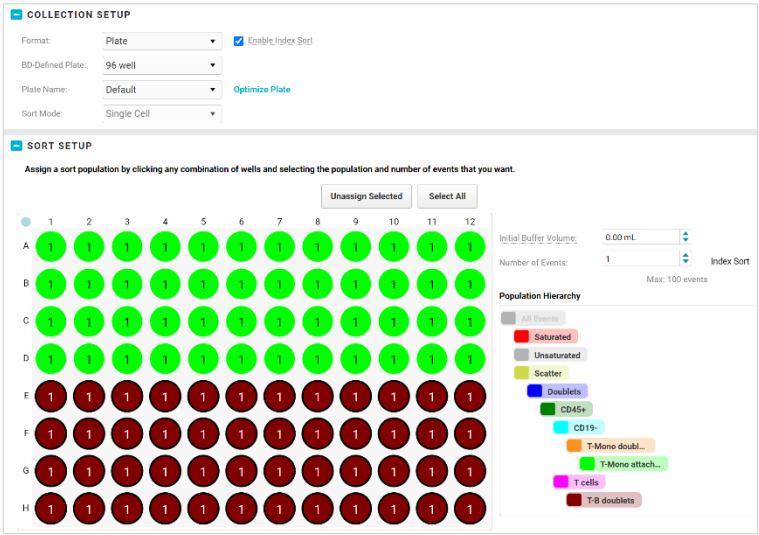
a. Make appropriate selections in the Collection Setup panel.

b. Click **Optimize Plate** and verify plate alignment.

c. Assign populations to wells.

d. Assign the target event count for each tube/well.

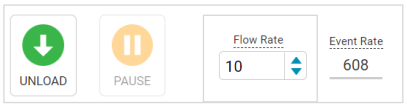
e. Install your sort collection device and close the sort block door.



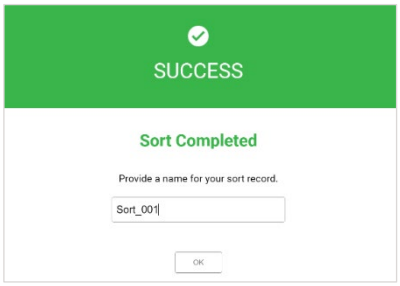
Sort, continued

f. Start the sort.

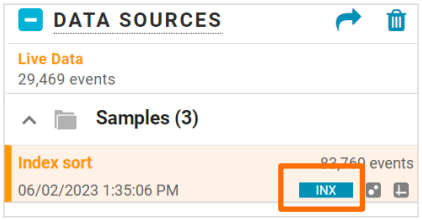
g. Monitor the sort as it progresses. If needed, adjust the flow rate.



h. When sorting finishes or is stopped, name the sort report.



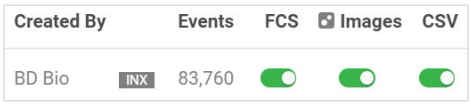
i. (Optional) Review index sort data in the Index Sort View.



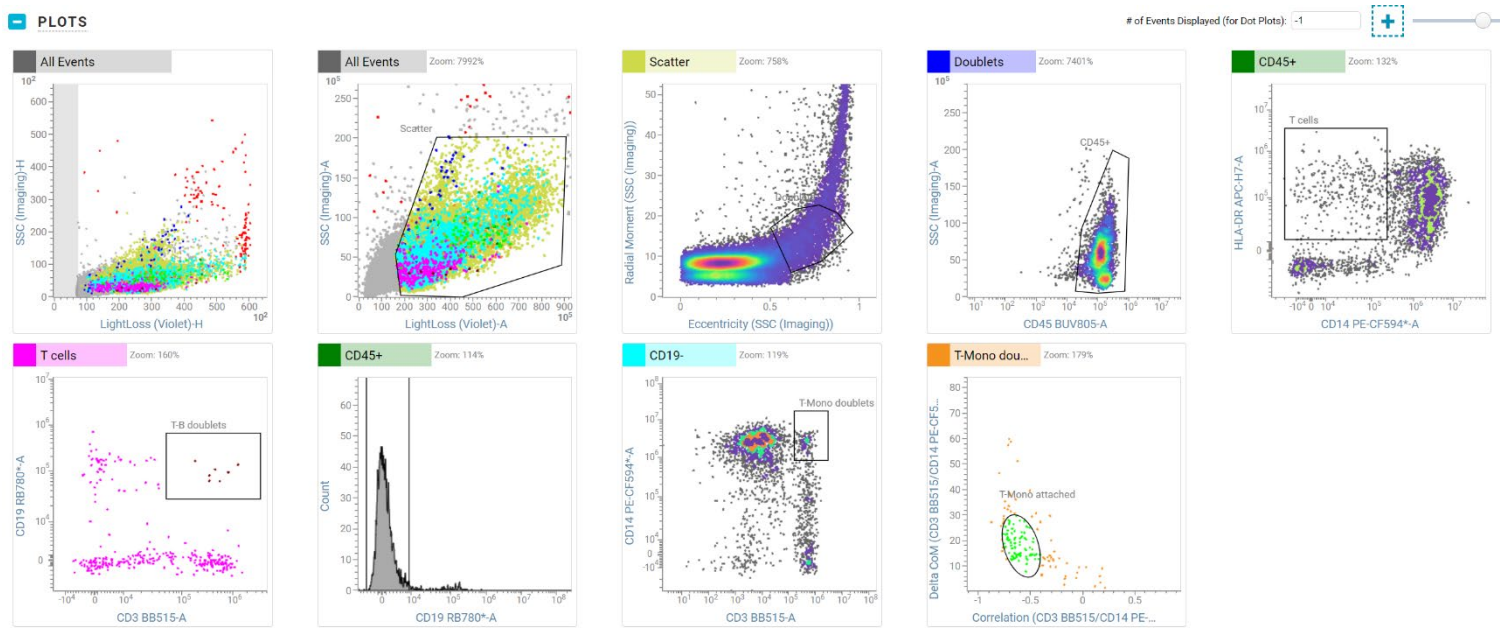
6. Export and delete data.

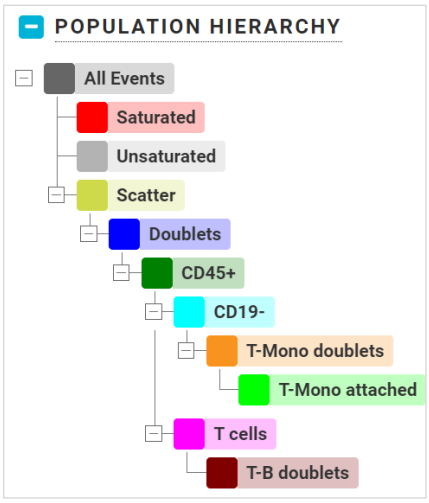
a. Export the report as a PDF.

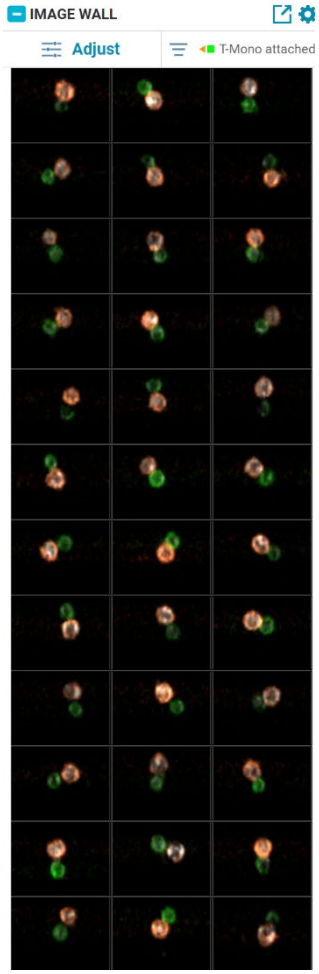
b. Export data from the experiment.



5-Color PBMC sort example data







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