Day 3 targeted workflow

GFP imaging sort

Start up system → **Set up experiment** → **Establish settings** → **Sort and analyze** → **Manage data** → Shut down system

- Set up experiment: Create experiment
- Establish settings: Adjust settings; Set up single-stain controls; Record and analyze data
- Sort and analyze: Sort into plates and index sorting
- Manage data: Export and delete data

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 sort cells based on the quality and location of eGFP expression.

Sample description: HEK 293T cell line (human embryonic kidney), ranging from 11 to 15 um, was transfected to express GFP, stained with a fixable viability dye, and fixed for biosafety. Single-color controls and an unstained control are included.

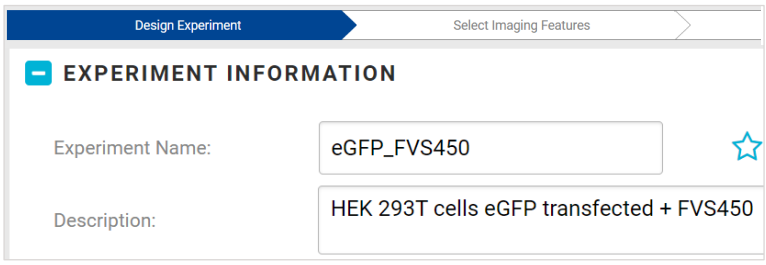
Fluorochrome	Label	Excitation/emission (nm)
FVS450	Viability	405/450
eGFP		488/510

1. **Create experiment.**

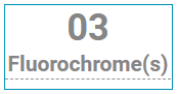
   a. Create a new blank experiment.

   b. Enter experiment name and description.

   c. Select eGFP and FVS450. Enter Viability as the label for FV450



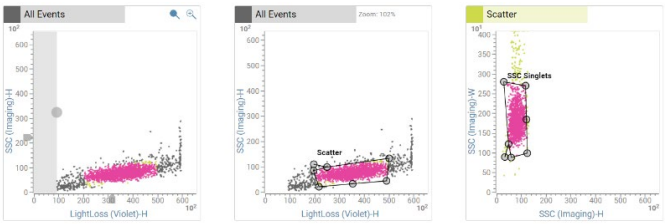
   d. Include an autofluorescence control.



   e. Assign eGFP to the appropriate imaging detector.

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

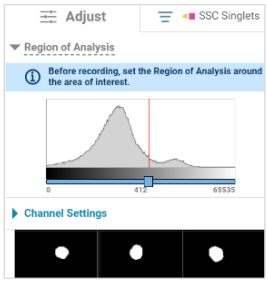
2. Adjust settings.



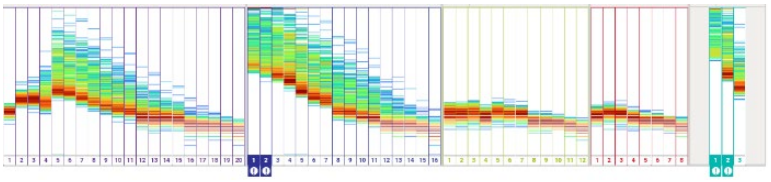
a. Load the sample tube.

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

c. Adjust the Region of Analysis properly for the SSC Singlets.



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



e. Unload the tube.

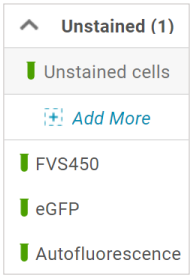
3. Set up single-stained controls.

a. Add an Unstained control and name it.

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

c. Record data for each control tube.

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



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

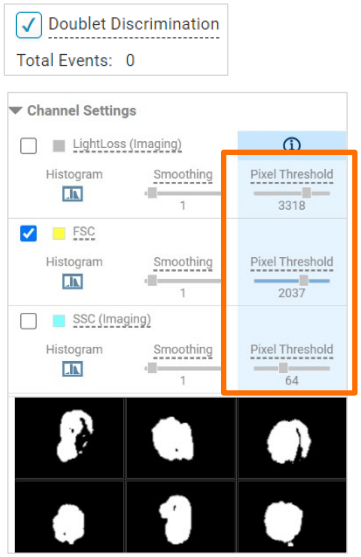
4. Record and analyze data.

a. Load the sample tube.

b. Verify doublet discrimination is selected.

c. Adjust the plot zoom and scatter and singlets gates 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.

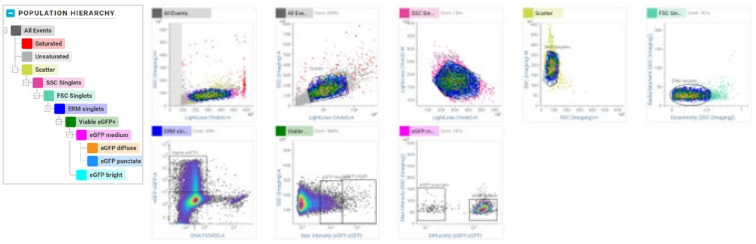
View data, continued

e. Toggle on the Images Stored switch and enter 10,000 events to record.

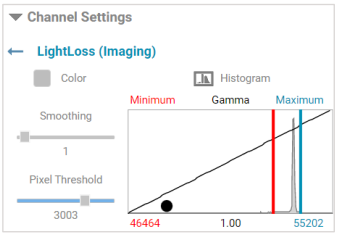
f. Record and name the data file.

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.

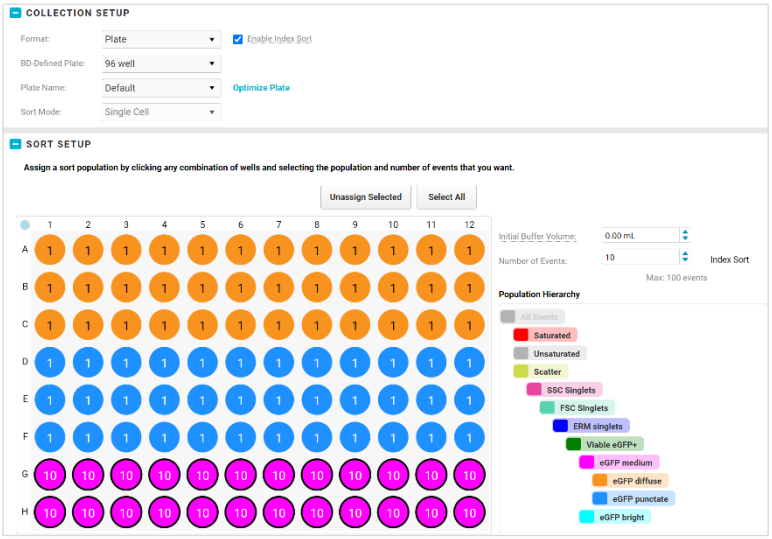
a. In the Collection Setup panel, select **Plate**, **96 well**, **Default**, **Single Cell**, and **Enable Index Sort**.

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

c. Assign populations to wells.

d. Assign 1 as the target event count for the eGFP diffuse and eGFP punctate wells. Assign 10 as the event count for the eGFP medium wells.

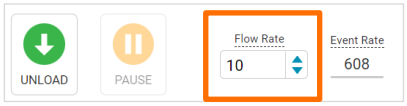
e. Install your collection device and close the sort chamber door.



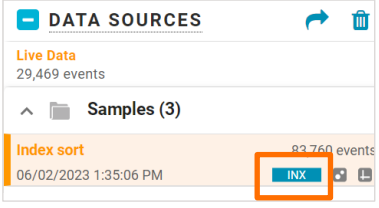
f. Start the sort.

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

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



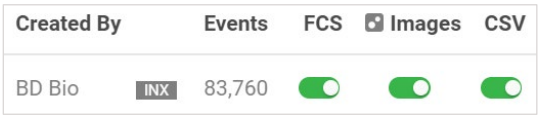
i. (Optional) Review index sort data in the Index Sort View.



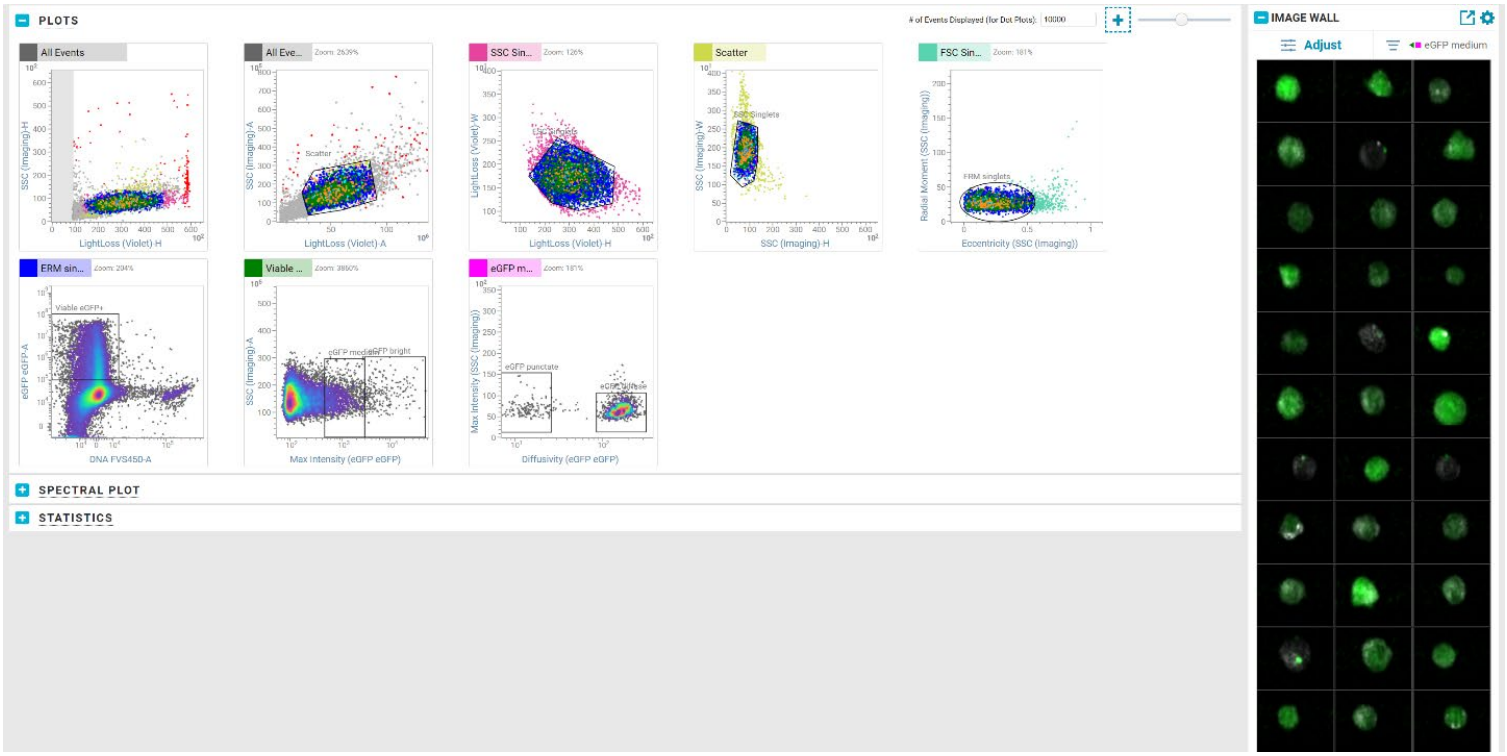
6. Export and delete data.

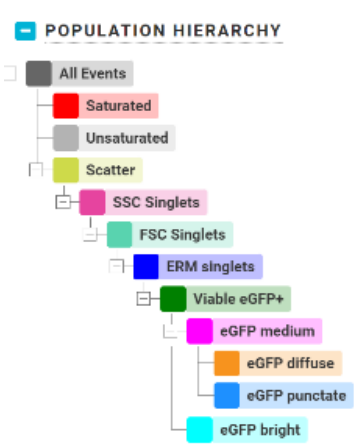
a. Export the report as a PDF.

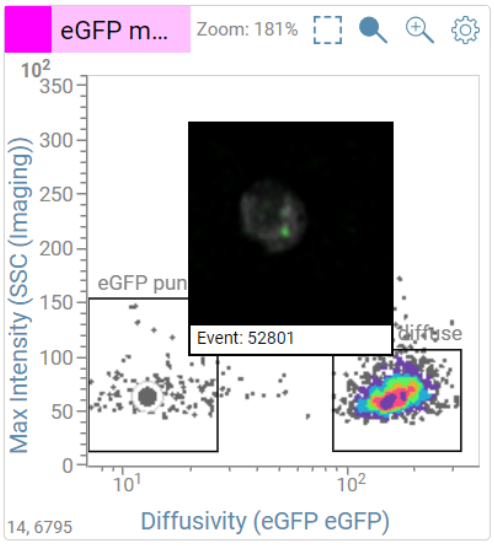
b. Export data from the experiment.

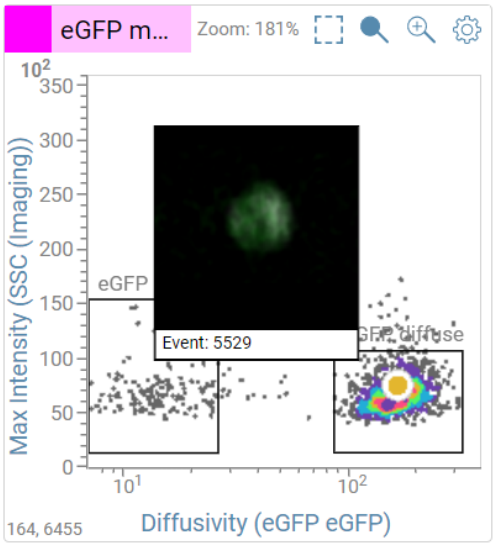


GFP imaging sort example data









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BD Life Sciences, San Jose, CA, 95131, USA

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