1. OBJECTIVE/PURPOSE:

This SOP will serve as an operations guide to the BD FACS Melody cell sorter.

2. RATIONALE:

Every Instrument in the core should have clear, precise guidelines on how to operate the instrument.

3. EQUIPMENT:

1. BD FACMelody
2. Jun-Air
3. AD-connected Computer

4. PROCEDURE:

4.1. Startup

1. Ensure waste container is empty/near empty prior to sorting
    a. If waste container is empty, add 2 inches of bleach to the waste container
    b. Empty waste in the large waste container provided by EHS
2. If needed, refill sheath tank with filtered sheath
3. Turn on “Jun-Air” by flicking the switch on the back, wait for pressure to reach ~90 on pressure gauge
4. Turn on Melody by click on power in-front of machine
5. Turn on the monitor, and log in using AD credentials
6. Open and set up sorting software “FACS Chorus Melody”
7. Open sorting chamber to check if closed loop nozzle is inside flow cell
    a. If 100 um nozzle is inside the flow cell please inform flow core staff
8. Disconnect air and fluidic lines from ethanol tank and insert them into appropriate connectors on the sheath tank, if necessary
9. In the software click on “Fluidics Startup”
    a. Click on “Run Daily Fluidics Startup” or “Run Extended Fluidics Startup” and click on “Start” in the pop-up window
    b. Note: Once the process starts it CAN NOT be stopped. Double check all fluidic tank connections!
10. Next Click on “Flow Cell Clean”
    a. Load a tube of 3 mL of DI H_2O in loading chamber and start process
11. Once finished remove closed loop nozzle and insert 100 um nozzle
12. Turn on water bath in wanted. Use setpoints to control the temperature of the collection tubes

4.2. Quality Control

1. Turn on the stream by clicking on the “Stream” tab on the bottom left of the software, and clicking “Start Stream” in the pop-up window

   a. If any issues occur find a flow core staff member for assistance
2. Click on “Cytometer Setup (CS&T)”
   a. Note: if optical layout is changed you will need to click on “Optical Configuration”, and change the filter sets
   b. Verify bead lot number is the same as the lot number in the software
3. Insert CST beads in loading chamber
   a. Loading chamber door, sorting chamber door, and front sliding door panels must be shut
4. Click on “Run Cytometer Setup”
   a. Once finished click continue and insert Accudrop beads in loading chamber
5. Click on “Run Drop Delay” and click continue
6. If no issues have occurred set up is now finished

4.3. Experiment Setup

1. Click on “Experiment” on the top left
2. Click “New Experiment” on the top left of the software
   a. Change name
   b. Select appropriate fluorochromes and labels. Users can add user-defined fluorochromes as needed
3. Click on the “2 View Data” tab on the top right
   a. Add the desired plots in the “PLOTS” section by click the “+” sign
   b. By default, the software will populate a FSC vs. SSC and 2 singlet plots, but this can be turned off by unchecking the “Doublet Discrimination” box
   c. Adjust voltage by using sliders on the graphs while running a sample
   d. Adjust Threshold by adjusting the grey gate on the first FSC-H x SSC-H plot. NOTE: thresholding debris out is not always the best option for sorting. Ask staff for details.
      i. To change trigger channel, change the x-axis on the threshold plot
4. Compensation:
   a. Click on “Update Compensation”
   b. Select single stain control
   c. Run and record each single stain sample
   d. After all control have been ran arrange gates over positive controls
5. Click on “3 Set Up Sort” tab on the top right
   a. Select the tube/plate you will sort into, the volume/plate type, and the number of events you will collect
   b. Place the selected tube/plate in the sort collection device
   c. NOTE: if using plates, place the splash shield in FIRST before selecting the plate time in the Set Up Sort Tab
6. Click on the tube then click on the population hierarchy on the left
7. Click on the “4 Sort” tab on the top right
   a. Place sample in loading chamber and click “Load Sample”
   b. Click on “Start sort” in sort status

c. Once sample has ran all volume or reached sort limitation, unload sample
d. Change sort report name and click “OK”
e. Go to “View Reports” on the top right of the software
    i. Click on “Export Report” to all reports

4.4. Shutdown Procedure

1. In your experiment, load up 3 mL of FACS Clean and run on Flow Rate 100 for 5 minutes
2. Load up 3 mL of DIH_2O and run on Flow Rate 100 for 5 minutes
3. Click on “Cytometer” in the left tab
4. Perform “Daily Shutdown”
5. Wipe down plates with lightly damp kimwip
6. Export FCS files
    a. Move exported FCS files to your folder in the “J” Drive
7. Log out of your account and exit from the software
8. Sign out of windows account
9. Turn off the “Jun-Air” and the Melody

4.5. General Notes and Troubleshooting

1. If changing optical layouts, must double check and re-run CS&T
    a. Change before starting software if possible
2. Do NOT force open sort chamber door when sample is loaded. If opened, force the stream off, wait for sample to be ejected. Restart stream, re-do drop delay
3. Run CS&T with 1.0 ND filter
    a. Other filters available if needed
4. Recommended Event rate should be 2000-5000 events per second
    a. Generally, 10 million cells per mL concentration
5. The instrument can only take sample from 5 mL polystyrene tubes
    a. Polypropylene is too thick to fit in the chamber
6. Collection Tube options:
    a. Slide
    b. Tubes
        i. 5.0 mL
        ii. 2.0 mL
        iii. 1.5 mL
    c. Plates
        i. 6 wells
        ii. 12 wells
        iii. 24 wells
        iv. 96 well
            1. Culture
            2. PCR
        v. 384 wells

4.6. Cleaning and Maintenance

1. Change Sheath filter every 6 months

2. Recommended to store nozzle dry. If needed, sonicate in DI water

5. REFERENCES:

FACSMelody User Guide
On-Site BD User Training

6. REVISIONS:

SOP version Number	**Date**	**Tracked Changes** (clearly list changes made and why)	**Primary Reviewer**	**Secondary Reviewer**
UT SOP010.2	3/17/2023	Added troubleshooting section. General Formatting	Ayrianna Woody	