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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. Computer workstation

4. PROCEDURE:

4.1. Instrument Startup

Note: During weekdays, if Users have booked time, Flow Core Staff will perform start-up on the Melody and check for sorting on each of the sort devices using 8-peak beads.

Fluidics Startup

1. Ensure waste container is empty/near empty prior to sorting
    a. If waste container is empty, add 1 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 autoclaved sheath (More sheath is available on shelves by the BSL2 sorters)
3. Open the valve for the house compressed air (copper line behind monitor), check that pressure gauge is reading ~95 psi.
4. Turn on Melody by clicking on the power button on the front of the instrument
5. Log into the workstation using your ONYEN
6. Open and instrument software “FACSChorus”
7. Open the sorting chamber to check if closed loop nozzle is inside flow cell

*Note: When inserting the Closed-loop Nozzle assure that it is secure, the o-ring is facing up, and the nozzle is flat (not askew). Misalignment or clogging of the closed-loop nozzle during Start-up or Shutdown will result in leakage during the system flushes. Monitor these steps carefully.

*Note: If needed connect the water bath connections to the proper collection container. Use setpoints to control the temperature of the collection tubes

8. 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

*Note: Once the process starts it **CAN NOT** be stopped. Double check all fluidic tank connections!

Flow Cell Clean

1. Next Click on “Flow Cell Clean”
    a. Load a tube of 3 mL of DI H_2O in loading chamber and start the cleaning process

Sort Nozzle

1. Once finished remove closed loop nozzle and insert 100 um nozzle
2. Click the prompt to turn the stream on
    a. If the stream display doesn’t open right away click on the “Stream” status button on the bottom left of the software to open the pop-up; clicking “Start Stream” in the pop-up window will similarly attempt to start the stream
    b. If any issues occur find a flow core staff member for assistance

Cytometer Setup (CS&T)

1. Once the stream is running click on “Cytometer Setup (CS&T)”

*Note: if optical layout is changed you will need to click on “Optical Configuration”, and change the filter sets

    a. Verify bead lot number is the same as the lot number in the software
        i. Lot number can be found on CS&T bottle

**Note:* Diluted CS&T beads are stored in the Melody room in the fridge; if there are insufficient diluted beads or they are >1 week old make fresh CS&T beads by vortexing the CS&T bottle, then add 1 drop stock beads to the 5 mL tube and add 300 uL of sheath

2. Insert CS&T beads in loading chamber
    a. Loading chamber door, sorting chamber door, and front sliding door panels must be shut
3. Click on “Run Cytometer Setup” this starts an automated routine

Drop Delay

**Note:* Diluted Accudrop beads are stored in the Melody room in the fridge; if there are insufficient diluted beads or they are >1 week old make fresh Accudrop beads by vortexing the bottle, then add 1 drop stock beads to the 5 mL tube and add 600 uL of sheath (Beads should be a dim aquamarine color).

    a. Once finished click continue and insert Accudrop beads in loading chamber
1. Click on “Run Drop Delay” and click continue
2. If no issues have occurred, Instrument Startup is now finished

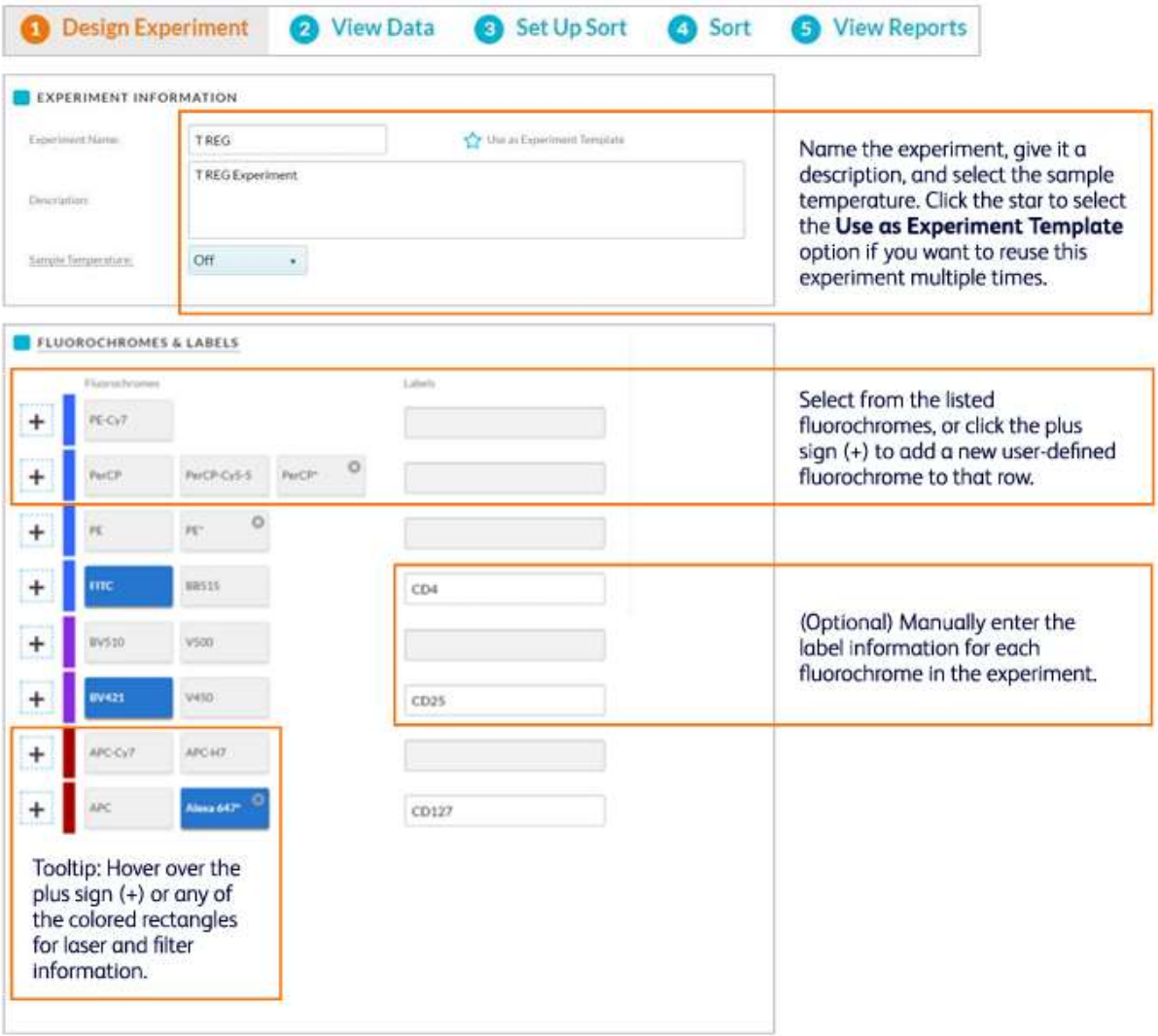
Post Start-up Log Out

**Note:* During Morning Start-up staff will perform test sorts with 8-peak beads using the 2-way, 4-way and plate devices. Following successful completion of these test sorts staff will select “Log Out” (left menu) and then in the next menu select “Log Out” again. This will leave the Stream on and the System ready to sort.
**Note:* If the Melody has been started-up is on and running then Users can move directly to Experiment Start-up below.

4.2. Experiment Setup

Design Experiment

1. Click on “Design Experiment” on the top left
    a. If you will use this experiment again click the star to select the **Use as Experiment Template** option.
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



View Data

1. Click on the "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. Set the speed to 1 (out of 100), Load a sample, and Adjust the scatter or fluorescence voltages by using the sliders on the graph axes while running a sample
        i. When hovering over a biexponential plot axis solid black triangles will appear these can be clicked to adjust the scaling.
    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
2. Compensation:

*<u>*Note*</u>: The FACSMelody can have default spillover values created using BD Fluorescence control (FC) beads. Currently this approach is not appropriate and is not used because it does not meet the needs of the current user base.

    a. If your experiment utilizes fluorescent signals with significant spill-over a compensation matrix will need to be calculated.
    b. Click on "Data Sources > Update Compensation"
    c. Select all the fluorochromes in your experiment
        i. You can use a single universal negative control (unstained sample) or select a negative population that is present in your single stained samples. *Consult Flow Core staff if you have questions about setting up your compensation experiment.*
    d. Run and record each single stain sample
    e. After all controls have been run adjust gates to identify the positive populations

Set Up Sort

1. Click on "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

*<u>*Note*</u>: if using plates, place the splash shield in FIRST before selecting the plate type in the Set Up Sort Tab

2. Click on the tube then click on the gated population you would like to sort on the population hierarchy on the left

Sort

*<u>*Note*</u>: The Melody has sample agitation and a sample light available. The core recommends running the agitation at max (300 rpm). Utilize the light to help monitor the level of sample.

*<u>*Note*</u>: The Flow Core staff recommends that you monitor the sort efficiency and the sample rate. Try to maintain an efficiency >90% and an event rate of 2000-5000 events/sec. Running at lower speeds may improve the sort efficiency.

1. Click on the “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. While sorting the core recommends recording a data file of the sort.
    d. Once sample has run all volume, reached sort limitation, or you have collected the desired number of cells unload sample
    e. Change sort report name and click “OK”

*<u>*Note*</u>: The Melody allows Index sorting this allows you to sort cells onto a plate or slide and indexes the well or slide location to the collected parameters for that cell.

View Reports

1. Go to “View Reports” on the top right of the software
    i. Click on “Export Report” to all reports

2. Export FCS files
    a. On View Data > Data Sources Click **Export FCS Files**. Move exported FCS files to your folder in the "J" Drive

*Note: Screenshots of recorded data files can be made by printing the screen or using the Snipping Tool.

4.3. Cleaning between Users on the same day

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. Log Out of the Software (Left Menu) > In the pop-up about shutting down simply select Log Out again, then close the software (top right 'X'), and log off windows.

*Note: This procedure will leave the instrument clean, but on with the stream running.

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. Turn off the stream
4. Click on "Cytometer" in the left tab
5. Perform "Daily Shutdown" with 1.5% BD Detergent Solution. The software walks you through the following steps:
    a. Remove the 100um Nozzle and insert the Closed-loop Nozzle

*Note: When inserting the Closed-loop Nozzle assure that it is secure, flat, and not askew. Mis-alignment or clogging of the closed-loop nozzle during Start-up or Shutdown will result in leakage during the system flushes. Monitor these steps carefully.

    b. Assure that there is sufficient room in the waste container (<75% full)
    c. Load a 3mL tube of 1.5% BD Detergent Solution.
6. Wipe down the deflection plates with a lightly damp kimwip
7. Log out of your account and exit from the software
8. Sign out of windows account
9. Turn off the Melody and close the valve to shut off the house compressed air.

4.5. General Notes and Troubleshooting

1. If changing optical layouts, you must double check and re-run CS&T
    a. Change filters before starting the software if possible
2. Do NOT force open the 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 or polypropylene tubes
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
7. CS&T beads are: 1 drop with 300 uLof sheath
8. Accudrop beads are: 1 drop with 600 uL of sheath
9. Most troubleshooting issue can be resolved by turning off/on the Melody and chorus software

4.6. Cleaning and Maintenance

1. Change Sheath filter every 6 months
2. Recommended to store nozzle dry. If needed, sonicate in DI water
3. Flow Core staff will run an Extended Term Shutdown with Ethanol during instrument start-up to help avoid instrument contamination.

5. REFERENCES:

FACSMelody User Guide
FACSMelody QuickStart 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	Roman Bandy
UT SOP010.3	5/30/24	Clarified steps. Made it clear to use BD detergent. Added more troubleshooting lines.	Roman Bandy	
UT SOP010.4	12/13/2024	Minor Updates, Training Notes. New link for QuickStart Guide	Bob Immormino	
UT SOP010.5	10/1/2024	Minor Updates, Staff Startup	Bob Immormino	

Training Notes

Instrument Startup (Performed by Staff on Weekdays): Check Sheath and Waste levels. Turn on the compressed air, Login to workstation with onyen, Open instrument software; FACSChorus. Work through the automated start-up routines; Daily Start-up, Flow Cell Clean, Sort Nozzle, CS&T, and Drop Delay.
Create an Experiment: Open a new Experiment or a previously saved Experiment Template. On the View Data tab create a gating scheme. While running a sample at low speed adjust the PMT voltages using the sliders on the plots. If needed run the Update Compensation routine to create a compensation matrix.
Sorting: On the Set Up Sort tab, select the tube or plate type and assign the gated population to be sorted. Try to maintain a high sort efficiency (>90%) and an event rate of 2000-5000 evt/sec.
Export Data: On the View Data tab under Data Sources click **Export FCS Files**. On the View Reports tab click **Export Report** to export the sort reports. Temporarily store files on the J:drive (\\ad.unc.edu\med\microbiology\Groups\Flow Facility). The J:drive will be cleared automatically every month
Clean-up: FACSClean, diH_2O, Log Out if there is another user OR Daily Shutdown with 1.5% BD Detergent Solution