

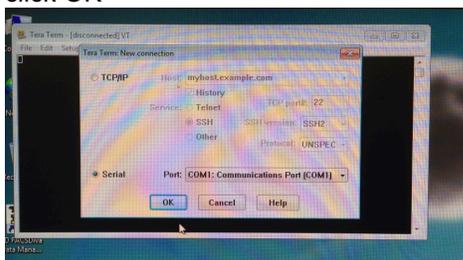
# FACSAria II SORP QUICK REFERENCE GUIDE

## INSTRUMENT:

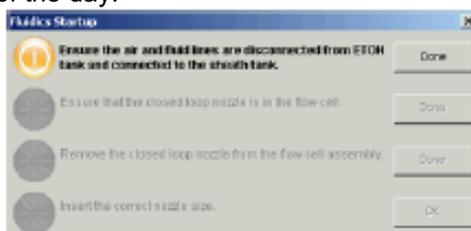
1. The computer is left on at all times.

*Note: If not Username: Admin Password: BDIS*

2. Ensure the flow cell access door is raised and the sort chamber door is open. You will want to check the plates to make sure they are dry and clean before you turn on the stream. The door is equipped with a laser interlock that block the laser light when the door is open. *Only close the cover after the stream is on.*
3. Unlock the screen with your PPMS account (UTSW username and password)
4. Turn on chiller unit if you want your samples chilled and attached the tube holder adapter you want to sort into
5. Check sheath and waste levels.  
If emptying waste, add 0.5L household bleach + ~500µL of Anti Foam (Sigma) to the new waste tank.
6. Launch Tera Term, Click on *Serial* and make sure the Port is on COM1 and click OK



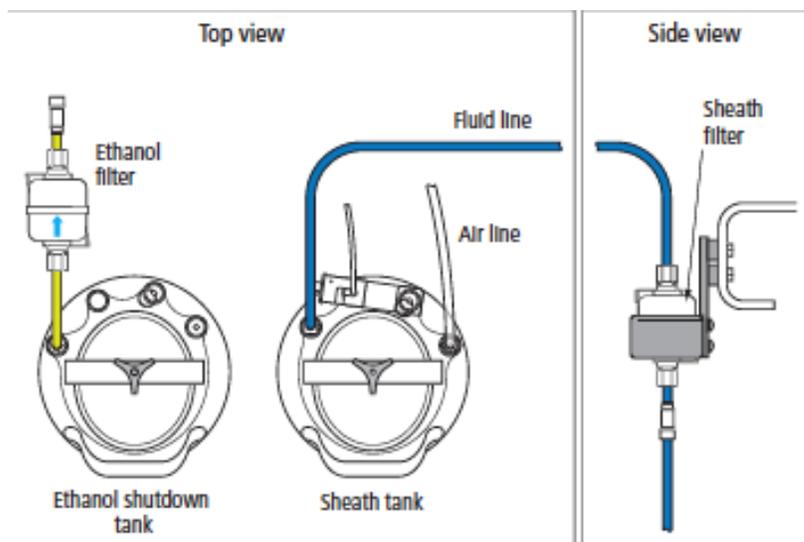
7. Turn on cytometer (Green button-left side), Verify lasers are on (white button)
8. Then Launch BD coherent 3  
(make sure all lasers windows are there and lasers have their power ON)
9. Allow ~20 minutes for lasers to warm-up. Continue while waiting.
10. Launch FACSDiva Software and log in at the prompt with your ID and password.
11. Once connected, Cytometer>Fluidics Startup (with the close nozzle) if you are the first user of the day.



12. Verify that the air and fluid lines are disconnected from the ethanol tank

First user of the day

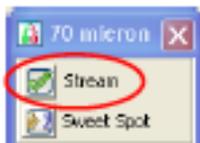
First user of the day



13. Replace the close nozzle with the appropriate one (if analysis use 70 $\mu$ m)  
4 sizes are currently available: 70 $\mu$ m, 85 $\mu$ m, 100 $\mu$ m, and 130 $\mu$ m. Fragile cells do better with a larger nozzle but Sorting speed would be faster with the 70 $\mu$ m nozzle.

*Note: Fusion nozzle are label MS (Aria II nozzle are label M) do not switch nozzle between instruments.*

14. When you change nozzles select the correct instrument configuration in Cytometer > View Configuration
15. Turn on the stream

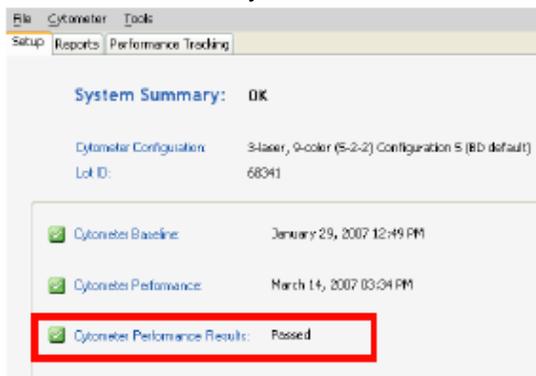


16. Close the flow cell access door
17. Check the stream quality (position, satellite drop resolution)

**CST:**

Run CD&T if they haven't been run on the same day for the configuration you chose

1. Cytometer > CS&T.
2. Choose the right configuration (nozzle size you going to use)
3. Verify lot number. Run.



*Note 1: Failed CS&T is NOT acceptable.*

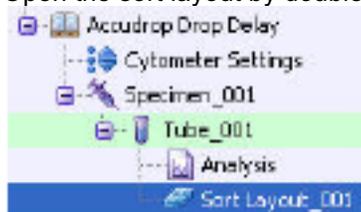
*Note 2: 1 drop CS&T beads in 300uL*

First user of the day

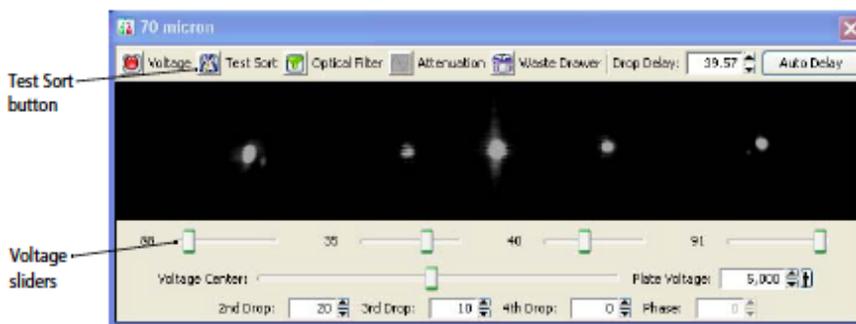
**ACCUDROP (only if sorting):**

*Note: Before beginning this procedure, make sure the stream is stable and the Sweet Spot is on.*

1. Create an experiment from the Accudrop Drop Delay template.
2. Expand Specimen\_001 and Tube\_001.
3. Set the current tube pointer to Tube\_001.
4. Open the sort layout by double-clicking it.



5. Turn on the Voltage in the Side Stream window.
6. Select Test Sort in the Side Stream window.



7. Adjust the micrometer dial to obtain the brightest bead spots.
8. Adjust the 2<sup>nd</sup> drop in the Side Stream window until the cleanest center stream is observed.
9. De-select Test Sort in the Side Stream window.
10. Prepare the Accudrop Bead Tube using 2 drops of beads / 0.5 ml isotonic diluent.
11. Place the Accudrop Bead Tube in the sample chamber.

**Using Auto Drop Delay**

12. Load the Accudrop Bead Tube.
13. Adjust the flow rate to achieve these values of events per second
  - 70µm = 1,000 to 3,000
  - 85µm = 800 to 2,000
  - 100µm = 600 to 1,500
  - 130µm = 400 to 1,200
14. Turn on the Voltage in the Side Stream window.
15. Engage the Optical Filter in the Side Stream window.
16. Select Sort on the Sort Layout window.
17. Select Cancel to keep the tubes covered
18. Click on Auto delay button in the Side Stream windows.
19. Select Start Run in the Auto Drop Delay dialog.
20. Monitor the Auto Drop Delay dialog for progress. A message appears when the process is completed.



*Note: The drop delay value will remain fixed for subsequent sort experiments. It will not need to be adjusted again until the drop break off moves from the selected point aligned with on the Sort Layout window.*

**FOR A NEW ANALYSIS EXPERIMENT: USE COMP BEADS and/or SINGLE STAINED CELLS:**

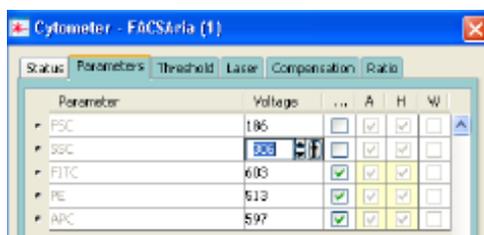
*For a new experiment without application setting ever being done*

1. Open a new experiment "Experiment>New Experiment.  
Select the experiment titled "Blank Experiment".
2. Click cytometer settings. Delete all parameters then insert experiment specific fluorophores in the order you will run them for compensation purposes.
3. Create a new specimen by clicking the New Specimen button on the Browser toolbar
4. Expand the new specimen.
5. Place the Acquisition Pointer on Tube\_001.

**Adjusting Area Scaling**

6. Select the H checkbox in the "Parameters" tab to select height for each parameter.
7. In the Browser, right click Global Sheet1 and select Apply Analysis Template
8. In the Template dialog, select the **Area Scaling** worksheet and click OK
9. Adjust histograms and statistics according to fluorophores used (Use one fluorophores per laser).
10. Install an unstained sample onto the loading port and click **Load** in the acquisition dashboard.
11. Adjust the FSC and SSC voltages to place the particles on scale.

Note: Keep record of FSC and SSC voltages, if using compensation beads.



12. Adjust the P1 gate around the population of interest in the FSC vs SSC plot
13. Click the **Laser** tab in the **Cytometer** window and adjust the FSC Area Scaling factor until the FSC-A signal matches the FSC-H signal.
  - Increase the area scaling factor if the FSC-A signal is lower than the FSC-H
  - Decrease the area scaling factor if the FSC-A signal is higher than the FSC-H



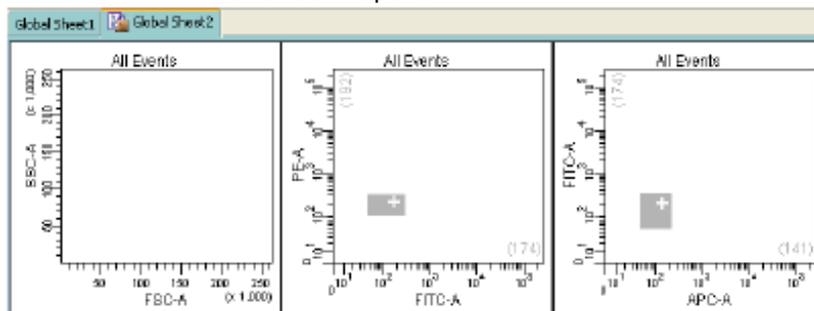
14. Unload the unstained tube.
15. Load the compensation tubes

Note: Adjust FSC PMT voltage in the parameter window if you are using compensation beads.

16. In the **Laser** tab adjust area scaling for each laser (being used) with appropriate fluorophores until the "A" signal matches the "H" signal.
17. In the Inspector, clear the checkbox for height for all parameters. **(Uncheck the H!!!)**

### Optimizing PMT Voltages

- Right-click Cytometer Settings > Application Settings > Create Worksheet.  
A sheet titled "Global Sheet2" opens.



- Load the unstained control sample onto the cytometer.

*Note: If beads have been used, enter FSC and SSC voltage recorded earlier under the parameters tab*

- Under the Parameters tab enter FSC and SSC voltages recorded earlier.
- Adjust the FSC threshold.
- Adjust the appropriate fluorescence voltage to center the negative population in the grey zone and around the + sign. Do not record.

*Note: However, lowering PMT voltage from the setting established by CS&T might affect the resolution of dim populations.*

- Load the fully stained sample to verify that all fluorescent parameters are on scale.  
If a positive population is off scale, lower the PMT voltage for that parameter until the positive population can be seen entirely on scale
- Unload the fully stained sample from the cytometer.
- Right-click Cytometer Settings > Application Settings > Save.
  - Name the experiment including your initials and the date.
  - Click "Yes" to apply the optimized threshold, if applicable.
  - The newly saved Application Settings can be found under Cytometer > Catalogs to view or delete when no longer in use.
  - The settings are now optimized for this new experiment and are relative to current CST.

*Note 1: Application settings are relevant until a new baseline is performed.*

*Note 2: Even though the saved experiment template contains Application Settings, these Application Settings are not automatically synchronized with current CS&T every time a new experiment is created from the template*

*Note 3: When you return to perform a similar experiment and you want the setting to be relevant to previous runs.*

#### **For a new experiment with recalling an existing application setting**

- Import the experiment template or create a blank experiment
- Right-click Cytometer Settings > Application Settings > Apply
- Select your application setting and Ok -if necessary click on Overwrite and Yes-If the
- Confirm dialog appears and you have a new set of compensation tubes, click "Set all compensation to zero" and proceed with step 9 above. Alternatively, you can keep compensation values from a previous run or "Duplicate Experiment Without Data", but in the latter two examples the settings won't be relative to the current CS&T.

## **CALCULATING COMPENSATION**

26. Create compensation tubes including a tube for unstained cells/beads under Experiment > Compensation Setup > Create. Add or change labels for tandems.
27. Acquire and record unstained and single stained compensation samples.
28. Check the gates for all tubes, excluding outlier events as needed.
29. Calculate compensation under Experiment > Compensation Setup > Calculate Compensation. Name Compensation Set-Up. Choose Link & Save.
30. Turn FSC voltage down to the original noted value when switching from compensation beads to cells.

## **ACQUISITION:**

1. Place the Acquisition Pointer on Tube\_001.
2. Click to go back on Global Worksheet
3. In the compensation Tab, make sure "enable compensation" is selected
4. Create a new Global Worksheet (#3) by click on the button in the browser window  
Develop the Global Worksheet with:
  - a. Create your plots (do plots, contour plots and/or histograms), stats (Median Fluorescence Intensity, CV, etc...) and Population Hierarchy for your analysis.
  - b. Create gates (Polygonal gates, squares, quadrants or intervals) to analyses correctly your data
5. Add additional tubes to the experiment.
6. Experiment > Experiment Layout.
  - a. In Labels Tab  
Change tubes names and the axis labels.
  - b. In Keyword Tab  
Add Patient ID & Sample ID.
  - c. In Acquisition Tab.  
Click Global Worksheet column header then select Global Sheet 3 on the Global Worksheet pull-down tab.  
Select the number of events to acquire as well as the stopping gate by selecting the appropriate column header first.
7. Set up the global worksheet and turn on the bi-exponential display for the fluorescent log plots.
8. Run a fully stained sample and setup gates.
9. Run tubes.
10. Record data.

*Note 1: Export the Experiment Template to the hard drive for future use: File > Export > Experiment Template. Name, etc...*

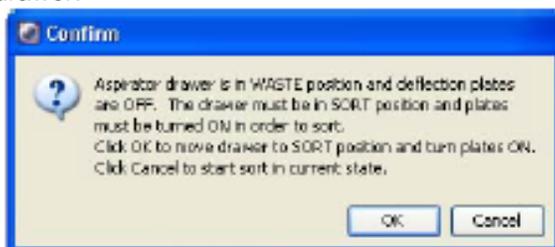
*Note 2: Even though the saved experiment template contains Application Settings, these Application Settings are not automatically synchronized with current CS&T every time a new document is created from the template. When you return to perform a similar experiment and you want the settings to be relevant to previous runs, import the experiment template, right-click Cytometer Settings > Application Settings > Apply. If the Confirm dialog appears and you have a new set of compensation tubes, click "Set all compensation to zero" and proceed with step 9 above. Alternatively, you can keep compensation values from a previous run or "Duplicate Experiment Without Data", but in the latter two examples the settings won't be relative to the current CST.*

## **SORTING**

1. Create a new sort layout by clicking the New Sort Layout button on the Browser toolbar.
2. Make appropriate entries in the Sort Layout windows
  - a. Select the collection Device from the Device menu.
  - b. Change the precision mode to Purity (two tubes), 4-Way Purity (four tubes), or Single Cell (plate or slide)
  - c. Specific numbers of cells to be sorted into each tube if you like, otherwise, just leave it as "continuous".
  - d. Select a Save Sort Report option: Save None, Save all, or Ask User
3. Assign the sorting gates to be applied for each deflected stream



4. Insert a collection device with prepared tubes.
5. Click Acquired and then click the Sort button in the Sort Layout. Click OK to open the drawer.



## **THINGS YOU SHOULD KNOW ABOUT SORTING**

1. Highest expressed populations in the main sample should be in the left and/or right and more rare populations should be in far left and/or far right in the collection tubes device. (3-1-2-4)
2. Gates change in real time. If you adjust your sort gates in your template, they are immediately applied to the Aria II SORP
3. When the Sweet Spot notices there is a problem with the stream, it will pause the sort, but not the Acquisition. So if there is a major problem with the stream, it may run through your whole sample without sorting any events
4. If the nozzle is clogged: open the access door, replace the nozzle with the close nozzle, clean flow cell with beach and Shutdown solution, sonicate nozzle in DiH2O
5. If the line is clogged: Cytometer>Cleaning Modes>Sample Line Backflush...

## **EXPORTING DATA:**

1. Export > experiment to your computer folder.
2. Connect to your online tools (= Cytobank.org with your personnel username and password) to upload the folder on this online server.

### **CLEANING BETWEEN USERS:**

1. Open the flow cell access door
2. Turn off the stream
3. Remove the ceramic nozzle and put it in its box
4. Install the integrated closed-loop nozzle



5. Cytometer>Cleaning Modes>Clean Flow Cell. When prompted, install a tube containing approximately 3mL of Windex. X2
6. Repeat twice the step 4 and 5 with Shutdown solution
7. Wipe down workspace.
8. Check sheath and waste levels.
9. Logout of PPMS

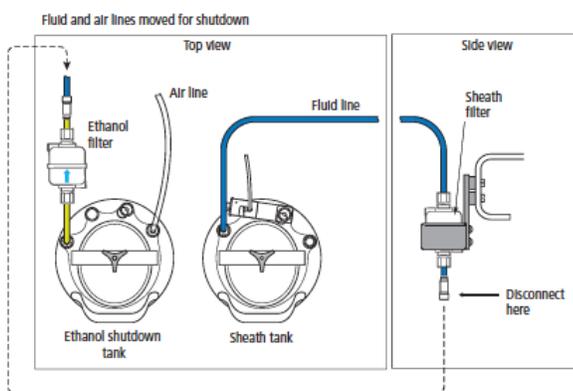
### **DAILY SHUTDOWN:**

10. Open the flow cell access door
11. Turn off the stream
12. Remove the ceramic nozzle and put it in its box
13. Install the integrated closed-loop nozzle
14. Cytometer>Cleaning Modes>Clean Flow Cell. When prompted, install a tube containing approximately 3mL of Windex. X2
15. Repeat twice the step 4 and 5 with Shutdown solution
16. Wipe down workspace.
17. Check sheath and waste levels.
18. Quit Laser software (BD Coherent 3)
19. Then Quit FACSDiva software
20. Turn off cytometer main power
21. Vent the air pressure from the sheath tank by pulling up on the vent ring
22. Logout of PPMS

### **WEEKEND SHUTDOWN:**

1. Open the flow cell access door
2. Turn off the stream
3. Install the integrated closed-loop nozzle. Click Done
4. Cytometer>Cleaning Modes>Clean Flow Cell. When prompted, install a tube containing approximately 3mL of Windex. x2
5. Check Ethanol Shutdown tank and waste levels.
6. Select Cytometer > Fluidics Shutdown
  - Connect the air and the fluid line to the stainless steel ethanol shutdown tank.
  - a. Disconnect the air line from the sheath tank and connect it to the airport on the ETOH shutdown tank
  - b. Disconnect the fluid line from the bottom side of the sheath filter and connect it to the ethanol filter on the ETOH shutdown tank
  - c. Click Done

*Note: Keep the sheath filter attached to the sheath tank. Do not run ethanol through the sheath filter*



7. When prompted, install a tube containing 3mL of *Shutdown solution* on the loading port, the click Done
8. Quit Laser software (BD Coherent 3)
9. Then Quit FACSDiva software
10. Turn off cytometer main power
11. Vent the air pressure from the sheath tank by pulling up on the vent ring
12. Logout of PPMS