CyTOF2 Sample Acquisition: Super Sampler* (CyTOF software version 6.0.626)

*Amended from Sampler Manual created by Zach Bjornson

Because CyTOF computer is on a private network, you will be bombarded with error windows reporting an inability to access network functions. In each of these instances, simply chose the “Continue” option.

This protocol provides instructions for disconnecting the default Loop System embedded in the CyTOF instrument and instead using the SuperSampler device to process samples through the CyTOF. The SuperSampler is recommended for samples in which one wants more than 200,000 data points per sample.

A. Bead preparation (Recommended that Calibration/Normalization beads be added to samples as an internal Standard, can just as easily be replaced or supplemented with normalization beads of your choosing- EQ Four Element Cal Beads- Cat#201078: Ce140/142, Eu 151/153, Ho 165, Lu 175/176)
   1. Note that your panel MUST contain channels for Ce140, Eu 151, Eu 153, Ho165, and Lu 175 regardless of their designation
   2. Vigorously shake CyTOF Calibration Beads bottle
   3. I will prepare a dilute mixture, 1:10 in fresh MilliQ nanopure water each day
      a. Beads will be at approximately 3e4 beads per mL

B. Log In to your User account on the Windows log in page
   1. SUNET User name and FACS Facility password
   2. Open the CyTOF Software package located on the desktop
   3. Close all CyTOF sub windows (“Status” Window will open on Startup)
   4. Select the “About” option on the CyTOF software, and Login as Administrator
      a. User: Administrator; Password: donuts
   5. Immediately close the About tab
      a. All sub-windows in the CyTOF software must be closed or else an error will occur
   6. Disable the CyTOF syringe pump
      a. Open the Control Panel and select the Devices tab
      b. Click on the drop-down selector for Syringe Pump and hit ESC on your keyboard to clear the selection.
      c. Click “Set Configurations” to initiate the Syringe Pump Shutdown.
      d. Click OK on the resultant prompt to acknowledge that background processes will be restarted.
7. Open the Status and Monitor Windows
   a. Shrink both windows to reduce footprint on the physical monitor

![Status Tab](image1)
![Monitor Tab](image2)

8. Open the Super Sampler software (Located in each users Documents folder).
   a. Place the Green tubing from the SuperSampler (Right Port) into the CyTOF Source water bottle
   b. Disconnect blue sample line from the CyTOF grounding nut and let this dangle
   c. Click “Initialize.” The syringe pump will home itself.
9. Begin a cleaning cycle
   a. Place the orange sample inlet tubing into a tube containing 1:1 CyTOF wash solution:3% nitric acid
      i. Stored in a 50mL falcon tube on the sample tube rack for your convenience (I prepare a new batch every 2-3 weeks)
   b. Place the blue sample line from the SuperSampler into a waste container.
      i. Ensure the tubing is secure so as to not spray acid or detergent.
   c. Click “Clean” in the software.
      i. Cycle takes approximately 4-5 minutes. Display will go to IDLE when done
   d. Place the orange sample inlet tubing into a tube containing MilliQ water
   e. Stored in a 50mL falcon tube on the sample tube rack for your convenience
      i. should have current day’s date
   f. Click “Clean” in the software.
      i. Cycle takes approximately 4-5 minutes. Display will go to IDLE when done
   g. Reattach the Sampler’s white nut to the CyTOF grounding nut.
   h. Set the Sampler to run for 1.0 mL
i. Press START on the SuperSampler Software
j. This will rinse the Sample Capillary line for a short period.
k. In the CyTOF Acquisition Panel’s Control Tab, Press RE-PREVIEW periodically to ensure you see 1) no events, 2) no background signal from contaminated channels.
l. Initially, it might look awful. Be patient… it will improve gradually
m. Press STOP on the Sampler Software
n. Press RESET on the Sampler Software
o. The Sampler is now ready to go

C. Set up Acquisition Parameters and Sample Introduction

1. Click the “Acquisition” Icon on the CyTOF home menu bar
2. Right click in the “Gray” Analyte table to select a pre-existing template or to create a new one.

<CRITICAL> MAKE IT A HABIT TO DOUBLE CHECK YOUR ANALYTE PARAMETERS. DATA WILL NOT BE SAVED IN EITHER THE .IMD OR THE .FCS FILE FORMATS WITHOUT BEING ADDED TO THE ANALYTE ACQUISITION PARAMETER FOR EACH EXPERIMENT. <CRITICAL>
3. For Pre-existing templates, highlight your template from the database list
   a. Press “Select template” button

4. For new template
   a. Scroll down to the bottom of the Templates database
   b. Highlight the “New Template” entry and enter a custom name for your panel
   c. Click the Periodic table and select all elements and the corresponding Isotopes to be included in your experimental panel
      i. Press “OK” to populate the table on the left
   d. Press “Select Template”
5. In the Acquisition tab of the Acquisition window, specify a pathway and file name to save your data
   a. CyTOF data should be stored in the E-drive folder named “CyTOF_Data”
      i. Create a user specific folder named with your initials and the date
6. In the Acquisition Tab of the Acquisition window, set up Acquisition Parameters
   a. Acquisition time: depends on user
      i. 9999sec - in practice we will reset runs approx. every hour
   b. Acquisition and Detector Stability delay
      i. Super Sampler: 0 sec and 10 sec
         a. Acquisition doesn’t begin until manually set by user.
7. In the Analysis Tab of the Acquisition window, setup data defining parameters
   a. Enter a value for Maximum/minimum event duration in push units
      i. Default is 150/10 push units
   b. Enter a value for “Target Events” or leave at 0 for Unlimited

---

D. Sample Preparation with beads

1. Perform a cell count on each sample prep to determine the cell concentration
   a. Final concentration should be roughly 5e5 cells per mL in a final volume that is calculated in increments of 250uL plus a dead volume of 100uL.
2. Final volumes should be created using the diluted bead mixture provided and prepared each morning.
3. Pass the cell/bead mixture through a blue cap tube with a 35um mesh
   a. Super Sampler: Collect your cell/bead mixture in a tube of choice (5mL to 50mL) puncturing the lid of the tube with needle point forceps
4. Sample introduction:
   a. In the Sampler software, enter your sample volume.
   b. Place the orange inlet tube through the sample cap stopping near the bottom of your sample tube (approximately < 2-5 mm from bottom).
   c. Good idea to tape the line down to prevent movement
   d. Press START in the Sampler software.
5. In the Acquisition Tab of the Acquisition window, select the “Control” tab
   a. Click “Run”
   b. Raw data will be visible acquisition via the Time of Flight presented as Panel Component versus Push Number

6. Data collection will continue until you STOP data collection in the CyTOF or you set a predetermined STOP value for events
8. Press RESET to return the remainder of your sample to your source sample tube
9. Once Complete a Plot View Window will open with an opportunity to preview the data

E. Cleaning between samples (Non-Patient Samples)
1. Place the orange sample inlet tubing into a tube containing MilliQ water (Should have current day’s date)
2. Disconnect the white nut from the CyTOF grounding nut and place into a waste container (50mL centrifuge tube taped to the CyTOF instrument housing underneath the sampler)
3. Click “Clean” in the software. (Cycle takes approximately 4-5 minutes.)
4. Reattach the Sampler’s white nut to the CyTOF grounding nut.
5. Set the SuperSampler to run for 1.0mL
6. Press RUN on the Sampler Software
7. In the CyTOF Acquisition Panel’s Control Tab, Press RE-PREVIEW periodically to ensure you see 1) no events, 2) no background signal from contaminated channels.

8. Press STOP on the Sampler Software
9. Press RESET on the Sampler Software
10. Proceed to your next Sample (Step C5)

F. Final Cleaning or between Patient Samples
1. Place the orange sample inlet tubing into a tube containing 1:1 CyTOF wash solution:3% nitric acid
   a. Stored in a 50mL falcon tube on the sample tube rack for your convenience (I prepare a new batch every 1-2 weeks)
2. Disconnect the white nut from the CyTOF grounding nut and place into a waste container.
   a. Ensure the tubing cannot fall out and spray acid or detergent.
3. Click “Clean” in the software. (Cycle takes approximately 4-5 minutes.)
4. Place the orange sample inlet tubing into a tube containing MilliQ water
   a. Stored in a 50mL falcon tube on the sample tube rack for your convenience (should have current day’s date)
5. Click “Clean” in the software. (Cycle takes approximately 4-5 minutes.)
6. Reattach the Sampler’s Blue Sample line to the CyTOF grounding nut.
7. Set the Sampler to run for 1.0mL
8. Press RUN on the Sampler Software
   a. This will rinse the Sample Capillary line for a short period.
9. In the CyTOF Acquisition Panel’s Control Tab, Press RE-PREVIEW periodically to ensure you see 1) no events, 2) no background signal from contaminated channels.
   a. Initially, it might look awful! Be patient... it will improve gradually
10. Press STOP on the Sampler Software
11. Press RESET on the Sampler Software
12. Proceed to your next Sample (Step C5)
G. Restoring CyTOF 2 plumbing and software configuration to the default Loop System
   1. Close all sub-windows in the CyTOF software.
   2. Remove the green SuperSampler line from the CyTOF water source bottle and return the “loop” system Green water line to the CyTOF water source bottle.
   3. Open the Control Panel window and select the Devices tab.
      a. Re-select the Syringe Pump settings in the from the dropdown menu.
         i. Select “Hamilton…”
         ii. Enter device setting, COM4
   4. Click “Set Configurations” to initiate the Syringe Pump Restart.
      a. Click OK on the resultant prompt to acknowledge that background processes will be restarted.
   5. Disconnect the white nut from the CyTOF grounding nut and place it in the waste container.
   6. Place the super sampler Green water line in a 50mL centrifuge tube labeled MilliQ water.
   7. Place the “cleaned” Orange Sample Line in the same 50mL centrifuge tube as the Green water line.
   8. Close the SuperSampler Software
Data Collection at the End of the Day

H. Normalization
1. Open the FCS Analysis Tab on the top panel of the CyTOF software
2. Use the Browse button to load files into the “Source File”
3. Under the Normalization Feature select the Bead Lot associated with the beads used in your sample from the “Normalization Beads” pull down window
4. Press the Start Button at the bottom of the FCS Analysis Window
5. The Output files can be found in the folder containing your Source files each annotated with an additional “_1” at the end of the file name.

I. Data Collection (The FACS facility is a USB flash drive free zone)
1. Minimize the CyTOF software
2. Transfer your data files from the E:Drive to the C:Drive folder “CyTOF data”
3. Open “FACS Data CheckIn” found on the Desktop
4. A Window will open asking you to “Select an Experiment”
   a. The data folder you created in Part C3ai will be highlighted
   b. Select and Press OK
5. A window will open asking you to “Select an investigator”
   a. Your last and first name should appear at the top
   b. Select and Press OK
6. A last window will enable you to add additional email addresses to the FACS Data CheckIn alert system
   a. Press OK
7. You will be prompted that data will be available from a remote server shortly
   a. Data takes approximately 15 minutes to transfer
   b. An email will prompt you to the completion of the transfer

J. Log Out (Should there be a User after you)
   1. Close CyTOF (and all other programs that may be open on the desktop)
   2. **Log Out of your windows account to end that day’s accounting**

K. Retrieving data (From anywhere, worldwide)
   1. Go to the Shared FACS Facility web site: http://facs.stanford.edu
   2. Expand from the left frame the Data Management header to reveal the Data Archive link
      a. Click the Data Archive link
      b. When Prompted enter your SUNET ID and password
         i. You’ll need a Web Authenticator to proceed
      c. When prompted enter the Username: flowjo, Password: 314159
   3. You should now have access to all your generated FCS files from today’s session
In the event of a plasma failure

L. Restarting the Plasma
   1. If in the midst of an Acquisition, please Press STOP in the Acquisition window “Control” tab. Doing this will save your data file for concatenation later.
   2. Press STOP on the SuperSampler software window
      a. Press RESET to return your remaining sample to your source tube
   3. Click the “About” Tab on the CyTOF home window
      a. Select “Login” into administrator user account
         i. Enables access to the “Control Panel”
         ii. Administrator / donuts
      4. Click the “Control Panel” Tab in the CyTOF Main Menu to open the control panel
      5. Click the “Plasma” Tab to open the Plasma control
      6. Click “Start Plasma”
         a. Plasma Start up takes about 5 minutes
   M. Resume Acquisition as described in Part C5
      1. You will need to make a new file name for the second part of your sample collection.
         This data can be concatenated with the previous data file once data collection is complete.

Ask for help if you have any questions: Brandon, cell (775) 722 4672.