CyTOF2 Sample Acquisition (CyTOF software version 6.0.626)

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. A “Status” Window will open on Startup
   4. Click the “Monitor” tab on the CyTOF home window
   5. Shrink both windows to reduce footprint on the physical monitor
C. Set up Acquisition Parameters and Sample Introduction

1. Click the “Acquisition” Icon on the CyTOF home menu bar
2. Right click in the Analyte table to select Experimental parameters

<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>

a. There should be several offering of saved Analyte templates
   i. Highlight your experimental Template
   ii. Press “Select template” button
b. For new template
   i. Scroll down to the bottom of the Templates Table
   ii. Click an empty “name” cell at the bottom and enter a reference name
   iii. Click the Periodic table and Highlight and select the Elements and the corresponding Isotope to be included in your experiment
   iv. Press “OK” to populate the table on the left
   v. Press “Select Template”

3. In the Acquisition Tab of the Acquisition window, specify a pathway and file name to save the FCS file
   a. CyTOF data should be stored in the E-drive folder named “CyTOF_Data”
      i. It is recommended that you create a user specific folder with “Data” with your initials and the date in the folder name

4. In the Acquisition Tab of the Acquisition window, set up Acquisition Parameters
   a. Acquisition time: depends on user
      i. Samples flow rate is ~0.045 mL/min over a 520 µL loop volume
         a. Single loop, I set to 650 sec to be safe
         b. Two loops = 1300 sec; add 650 each subsequent loop
         c. Unknown time or targeted events should be set to 9999 sec
   b. Acquisition delay: 30-40 sec
      i. Allow time for samples to make way to detector
   c. Detector Stability delay: 10 sec
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 75/10 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. Ideally, one would like a final volume mixture at between 3e5 and 8e5 cells per mL
   2. Bring the volume of sample to 600 µL volume increments using diluted bead mixture
      a. Add directly into tubes containing pelleted samples and mix well
   3. Pass the cell/bead mixture through a blue cap tube with a 35um mesh
   4. Collect the cell/bead mixture with a 1mL syringe at 600 µL per push
5. Inject ~600 µL of sample into the sample injection port

   <Critical> Ensure that no bubbles remain in the sample line. <Critical>

![Sample Injector port](image)

6. In the Acquisition Tab of the Acquisition window, select the “Control” tab

![Acquisition window](image)

7. Click “Run” - Make note of the Active Sample loop on the Syringe Pump Panel
   a. Data can be viewed in the plot view with raw data visible on acquisition view of
      Time of Flight for each Panel Component versus Push Number

8. Once Complete a Plot View Window will open with an opportunity to preview the data

9. Return to the Acquisition Tab of the Acquisition Window to specify a new file name for
   your next sample. (See Section C3)

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E. Cleaning between samples (Normally 2 sample loops may be run consecutively followed by 2 washes)
   1. MilliQ water wash

   a. Inject 1.5 mL of MilliQ water into the injection port
   b. Click the “Switch Valve” Icon to switch sample loops
   c. Wait 2-5 minutes (0.100 mL) to allow MilliQ water to run through
   d. Inject 1.5 mL of MilliQ water into the injection port to fill the second loop
   e. Click the “Switch Valve” Icon to switch sample loops
   f. Wait 2-5 minutes (0.100 mL) to allow MilliQ water to run through
   g. In the “Acquisition” Tab window select the “Control” tab
   
   h. Click “Preview” to check background currently being detected
   i. If background is low (see image), proceed to the next sample (Part A)
   j. If background is high, proceed to Part 2 Wash Solution

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2. Wash Solution (As necessary or after your last sample)
   a. Inject 1.5 mL of Wash Solution into the injection port
   b. Click the “Switch Valve” icon to switch sample loops
   c. Wait 2+ minutes (0.100 mL) to allow HF Wash Solution to run through
   d. Inject 1.5 mL of Wash Solution into the injection port
   e. Click the “Switch Valve” icon to switch sample loops
   f. Wait 2+ minutes (0.100 mL) to allow HF Wash Solution to run through
   g. Repeat steps a-f with MilliQ water
   h. As before, in the “Acquisition” Tab window select the “Control” tab
   i. Click “Preview” to check background the first loop
   j. Click “Preview” again to check background in the second loop.
   k. Repeat Part 2 as necessary
Data Collection at the End of the Day

F. 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.

G. 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 Checkln 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

H. 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

I. 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 computer/plasma failure

J. Manually restart the computer (in case of a frozen computer or BSoD- Otherwise SKIP)
   1. Hold down power button on the front panel for a hard shutdown
   2. Press the button again to restart the computer
   3. Once restarted, log in to your User account on the Window’s log in page
      a. SUNET User name and FACS Facility password (First name, First Letter CAP)
   4. Open the CyTOF Software package located on the desktop

K. Restarting the Plasma
   1. If in the midst of an Acquisition (not a computer crash), please Press STOP in the
      Acquisition window “Control” tab. Doing this will save your data file for concatenation
      later.
   2. 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
   3. Click the “Control Panel” Tab in the CyTOF Main Menu to open the control panel
   4. Click the “Plasma” Tab to open the Plasma control
   5. Click “Start Plasma”
      a. Plasma Start up takes about 5 minutes
      b. Do a manual valve switch to ensure your sample is in the inactive loop.
      c. In the event of a computer failure, an .imd file has been saved containing your
         incomplete run. We will need to work with DVS/Fluidigm to “complete” this file
         to preserve your data.

L. Resume Acquisition as described in Part C
   1. Note that you will need to do a “Run” to return to the loop containing your sample.

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