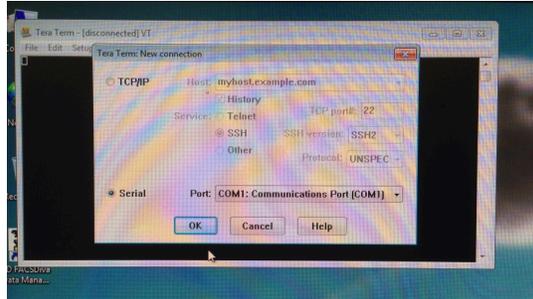# FACSCanto RUO Special Order
QUICK REFERENCE GUIDE

INSTRUMENT:

1. The computer is left on at all times.

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

2. Unlock the screen with your PPMS account (UTSW username and password)
3. Launch Tera Term, Click on *Serial* and make sure the Port is on COM1 and click OK

4. Turn on cytometer (Green button-left side), and launch FACSDiva Software.
5. Allow ~30 minutes warm-up for lasers. Continue while waiting.
6. Check sheath and waste levels. If emptying waste, add 1L household bleach and 500uL anti-foam reagent to the new waste tank.
7. Perform Fluidics Start-Up (~7 min.) (Cytometer>Fluidics Start Up)

CST:

1. Cytometer > CS&T.
2. Verify lot number.
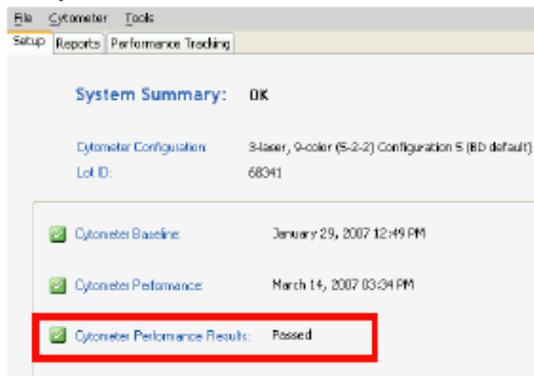
3. Choose between load the tube manually or not
4. Run.

Note: Failed CS&T is NOT acceptable.

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

First user of the day

**FOR A NEW EXPERIMENT: USE COMP BEADS 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*.
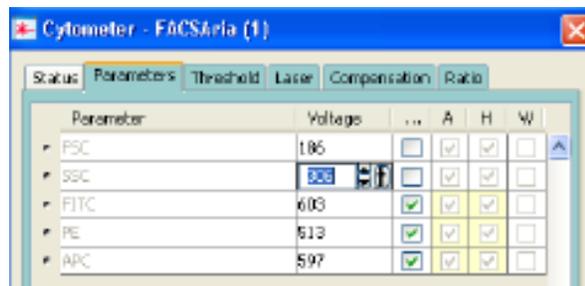
12. Adjust the P1 gate around the population of interest in the FSC vs SSC plot
13. Click the **Laser** tab in the **Cytometer** window and adjust the FSC Area Scaling factor until the FSC-A signal matches the FSC-H signal (A in the +10% of H value).
    - Increase the area scaling factor if the FSC-A signal is lower than the FSC-H
    - Decrease the area scaling factor if the FSC-A signal is higher than the FSC-H

14. Remove the unstained tube.
15. Load the comp beads tubes and in the parameter window adjust the FSC PMT voltage.

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 H checkbox in the Inspector window.

Optimizing PMT Voltages

18. Right-click Cytometer Settings > Application Settings > Create Worksheet.
    A sheet titled "Global Sheet2" opens.
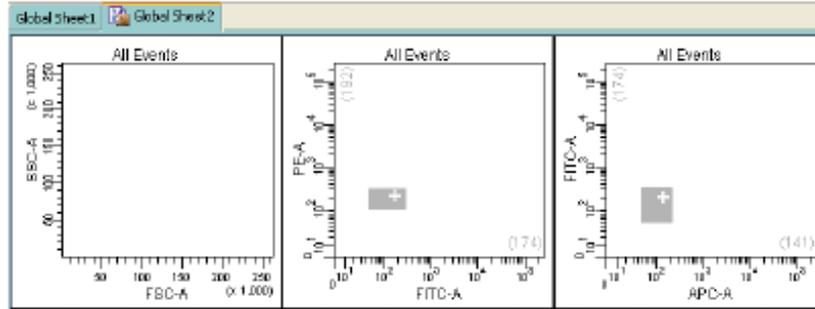
19. Load the unstained control sample onto the cytometer.

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

20. Adjust the FSC threshold if needed.
21. 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.

22. 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
23. Unload the fully stained sample from the cytometer.
24. If you are going to run the same type of experiments later on –same cells, same staining-; Right-click Cytometer Settings > Application Settings > Save.
    a) Name the experiment including your initials and the date.
    b) Click "Yes" to apply the optimized threshold, if applicable.
    c) The newly saved Application Settings can be found under Cytometer > Catalogs to view or delete when no longer in use.
    d) 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 (~every 6 months).

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

1. Import the experiment template or create a blank experiment
2. Right-click Cytometer Settings > Application Settings > Apply
3. Select your application setting and Ok -if necessary click on Overwrite and Yes- If the confirm dialog appears.
    "Cytometer Settings" is now called "Application Settings" in the Browser window

CALCULATING COMPENSATION

25. Create compensation tubes including a tube for unstained cells/beads under Experiment > Compensation Setup > Create. Add or change labels for tandems.
26. Acquire and record unstained and single stained compensation samples.
27. Check the gates for all tubes, excluding outlier events as needed.
28. Calculate compensation under Experiment > Compensation Setup > Calculate Compensation. Name Compensation Set-Up. Choose Link & Save only if you are running a similar experiment during the same CST period (24hours). Otherwise select Apply Only.
29. 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.

IF USING SINGLE TUBES:

   a. Make sure the probe is in "**single tube**" mode
      (tube guide OFF, and probe on upper position)
   b. Run tubes.
   c. Record data.

IF USING CAROUSEL:

   a. Carousel > carousel setup. Under the "carousel ID" tab select the carousel # and under the "Export folder" click to select the location for the Data file to be exported (FCS files are exported during experiment acquisition. Once recorded, each tube is exported).
   b. Select appropriate **Run setting** and **Tube pressurization error handling** parameters and click ok when finished.
   c. Make sure the probe is in **"Carousel mode"** is on when using the carousel
      (tube guide ON and probe on down position
   d. Click on Run Carousel.

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

EXPORTING DATA:

1. **For single tubes**: Export > experiment to your computer folder.

   For Carousel: Make sure that FCS files have been exported to your designated FCS folder on the 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. **For single tubes**: Clean the fluidics:
   50% Contrad - 5 min., 10% Bleach – 5 min., Shutdown – 5 min.

   For Carousel: Clean the fluidics:
   50% Contrad - 5 min., 10% Bleach – 5 min., Shutdown – 5 min.

2. Wipe down workspace.
3. Check sheath and waste levels.
4. Logout of FACS DiVa
5. Logout of PPMS

DAILY SHUTDOWN:

6. **For single tubes**: Clean the fluidics:
   50% Contrad - 5 min., 10% Bleach – 5 min., Shutdown – 5 min.

   For Carousel: Clean the fluidics:
   50% Contrad - 5 min., 10% Bleach – 5 min., Shutdown – 5 min.

7. Performs "Fluidics Shutdown" (Cytometer>Fluidics Shutdown)
8. Wipe down workspace.
9. Check sheath and waste levels.
10. Turn off FACS DiVa
11. Turn off cytometer.
12. Logout of PPMS