Cytoflex User Guide

Starting up (For Plate Mode, See Page 5)

1. Check the sheath fluid level every time you use the cytometer. This ensures that you do not run out of sheath fluid during an experiment. Replenish fluids if needed as follows:
   - Unscrew and remove the cap from the sheath tank.
   - Fill with Cytoflex sheath fluid. (The cytoflex uses MilliQ filtered water)
   - Replace tank onto the instrument.
   - Replace the cap.
2. Check the waste container, empty if necessary and add a small amount of bleach to inactivate pathogens.
   - Unscrew and remove the cap from the waste tank.
   - Empty the waste into the sink. Use eye protection and avoid splashing.
   - Add a small amount of bleach into the bottom of the tank.
   - Replace the tank onto the instrument.
   - Replace the cap.
3. Turn on the cytometer with switch on the back of the instrument.
4. Turn on the computer.
5. Log in to the computer as "facsusers" with password "facsusers".
6. Open CytExpert software using the shortcut. Log in with your SUNetID. Initial password is "password". You will be prompted to create your own password at your first login.
7. Check the bottom left of the screen to verify that the Cytometer is connected. If the computer was already on, you may have to initialize the instrument through the software.
8. Check to see if the instrument is set up in tube or plate mode. If not in the mode you need, refer to "Changing to Tube Mode **from Plate Mode**" section below to switch the instrument to tube mode.
9. From the Cytometer Menu, select System Startup Program. The Startup program takes approximately 8 minutes.
10. Click Initialize and place a tube of 2 mL deionized water in the sample loader. Click Start.
11. System will initialize and prime. At the end of priming, the remainder of the time the system will be warming up appears. Close to quit when warm up is finished.

Changing to Tube Mode from Plate Mode

1. In the Cytometer menu select Sample Injection Mode. Select Semi Auto for running tubes.
2. A Restart warning prompt will appear on the screen. Select OK.
3. Turn the cytometer's main power switch off.
4. Turn the valve counter-clockwise until it stops, a "T" will be visible.
5. Turn the cytometer's main power switch on.
6. Refer back to Starting up section, step 9, to continue starting up.

Checking Cytometer performance

1. Before QC, turn Cytometer to Standby mode by selecting Cytometer menu > Standby, prime the FlowCell by choosing Cytometer menu > Prime.
2. From the QC menu, select Start QC.

3. Prepare the QC sample. Use Cytoflex Daily QC Fluorospheres. Dilute as follows: 1 drop beads to 200ul deionized water.
4. Select the correct bead Lot ID from the menu (bead lot is printed on each Bead vial).
5. If loader arm is not out, click Initialize to move the loader arm out.
6. Install tube onto the cytometer loading port.
7. **Click Start. The performance check takes approximately 2 minutes to complete. Ensure that the events/sec rate is >100.**
8. Once the performance check is complete, View Report.
9. Verify that the performance passed. There will be a green checkbox next to each passed parameter and it says QC passed at the bottom of the report.
10. If any parameters did not pass, ask the Cytometry staff for help troubleshooting.
11. Select "Close QC Standardization" from the File menu.

Creating your experiment

1. View the Detector Configuration in the Cytometer menu to verify that the detector configuration will work for your experiment.
2. Select OK to close the Detector Configuration screen.
3. Create a new experiment. From the file menu, select New Experiment.
4. Use the default path as the location for your saved data, rename the experiment as you see fit, and save the experiment (.xit).

Creating New Experiment settings

1. Select Set Channel in the Settings menu.
2. Uncheck all parameters you will not be using, apply labels if you desire. Unselected parameters will not be saved into the data files. Select Apply to all empty tubes, and OK. Select Close.
3. Select Acq Settings in the Acquisition Window. The gain adjustment window will appear. Click the Default button to use the optimal gain settings for the Cytoflex. Use the Width Tab to select FSC Width for doublet discrimination.
4. Create a worksheet to view your cells as they are acquiring and recording. There are many types of plots to use. Right click on plots to change properties. Axis ranges can be adjusted also. Click the title of the plot to display gated events or make combo gates using the commands and, or, and not.
5. Use the Population hierarchy tool to bring up a population hierarchy.
6. Add a tube to the experiment.
7. Choose the Slow sample flow rate in the Experiment Window to start.
8. Install the unstained cells tube onto the cytometer.
   Note: If the Cytometer is in Standby mode, you need to hit Initialize in the Acquisition Window before you install the tube onto the cytometer.
9. Optimize the FSC and SSC gains to place the population of interest on scale.
10. Install the all color stained cells tube onto the cytometer.
11. Verify that the positive populations are on scale. If a positive population is off scale, first try adjusting the number of decades shown on the plot. If a population is still off scale, lower the gain for that parameter until the positive population is entirely on scale. Note that lowering gain settings below the default settings for fluorescence channels may decrease resolution on that channel.

12. Go to the threshold tab and adjust the FSC threshold if necessary to remove unnecessary signal noise.
13. You now have your gain and threshold settings. Save these settings as follows: In the Acq Settings window, choose Export.
14. Continue with compensation as necessary.

Compensation

1. Ensure that you have saved a file of the correct gain settings first. (see above)
2. Create a compensation experiment by selecting New Compensation in the File menu. Use the default file path and select Save.
3. Select the channels requiring compensation and the sample type. The unstained negative control tube can be selected if needed.
4. Select OK. A compensation control experiment is generated.
5. Import the gain settings of your experiment and apply to all tubes. This will ensure that the compensation samples are run with the same settings as your experiment.
6. Install the unstained cells tube onto the cytometer and select Run. Adjust the number of decades shown on the plot so that the population of interest is on scale. Move the FSC SSC gate so that it encloses the desired population. Right-click on the gate and select Apply to All Tubes.
7. Select Record. Unload the tube.
8. Install the next tube onto the cytometer.
9. If necessary, move the positive gate in the plots so that it encloses the positive population. Select Record. Repeat for all compensation tubes.
10. Select Compensation Calculation in the Settings menu.
11. The matrix displays. Click Save as to export the matrix and specify where to save it. It is handy to save it in your experiment folder.

Recording Sample Data

1. Open your previously created experiment (From the File menu, choose Open Experiment. It should be listed as your "experiment name.xit".)
2. If compensation was performed import it (using the compensation icon in the tube window) and apply to all tubes. From the compensation matrix window, choose Import > Compensation. Select Compensation Matrix in the Settings menu. Select one of the following:
    - Import compensation matrix and transform it with current gains.
    - Import compensation matrix.
    - Import compensation matrix and gain.
   Select Import compensation matrix. (Note: If you adjust your gain after compensation is preformed, you can import compensation and transform it with current gains.) If you have multiple tubes, make sure to apply compensation to all tubes. Select OK.
3. Add as many tubes to the experiment as necessary. Rename them by right clicking on them.
4. Adjust the sample flow rate in the Experiment Window.
5. Click the first tube, install the tube, load it and record it.
   You may need to initialize to bring the tube holder out again.
6. Continue for all tubes you wish to run.

Data Export

1. Navigate to your experiment in My Documents.
2. Save a copy of your experiment to "C:\FACSData".
3. Go to the Desktop.
4. Open the "Data Upload" program.
5. A command terminal will open.
6. Follow the on-screen instructions:
   a. Type your SUnetID, hit Enter;
   b. Type "yes" when asked, if your SUnetID was typed correctly, then hit Enter;
   c. Confirm you are uploading the correct experiment, then hit Enter when prompted.
7. Leave the program to run until it closes on its own. DO NOT CLOSE THE PROGRAM EARLY, OR TURN OFF THE COMPUTER. Otherwise your upload will be cancelled.
8. (First Upload Only) You will receive an email from a FACS Core staff member containing a link and password to your data. This information will be used to access all subsequent uploads.

Preparing for The Next User or Shutting down

1. Select Daily Clean from the Cytometer menu.
2. Load a tube of 2mL FlowClean solution into the sample holder and select Run. Cleaning time should be at least 3 minutes.
3. Repeat with a tube of 2mL DI water for at least 5 minutes and select Run.
4. After cleaning process is completed close the Daily Clean window.
5. Exit the Cytoflex software.
6. If there are other users right behind you, hit the Start menu of the computer, choose Switch User or Log off, leave the Cytometer and computer on.
7. If the next user is 4 hours or more behind you or if you are the last user of the day, turn off the cytometer and computer when finished.

Cytoflex Plate Mode

Starting up

1. Check the sheath fluid level every time you use the cytometer. This ensures that you do not run out of sheath fluid during an experiment. Replenish fluids if needed as follows:
    - Unscrew and remove the cap from the sheath tank.
    - Fill with Cytoflex sheath fluid. (The cytoflex uses MilliQ filtered water.)
    - Replace tank onto the instrument.
    - Replace the cap.
2. Check the waste container, empty if necessary and add a small amount of bleach to inactivate pathogens.
    - Unscrew and remove the cap from the waste tank.
    - Empty the waste into the sink. Use eye protection and avoid splashing.
    - Add a small amount of bleach into the bottom of the tank.
    - Replace the tank onto the instrument.
    - Replace the cap.
3. Turn on the cytometer with switch on the back of the instrument.
4. Turn on the computer.
5. Log in to the computer as "facsusers" with password "facsusers".
6. Open CytExpert software using the shortcut. Log in with your SUNetID. Initial password is "password". You will be prompted to create your own password at your first login.
7. Check the bottom left of the screen to verify that the Cytometer is connected. If the computer was already on, you may have to initialize the instrument through the software.
8. Check to see if the instrument is set up in tube or plate mode. If not in the mode you need, refer to "Changing to Plate from Tube Mode" section below to switch the instrument to plate mode.
9. From the Cytometer Menu, select System Startup Program. The Startup program takes approximately 8 minutes.
10. Select Plate Type. Add 250ul of deionized water to default A1, A2, A3 wells (or choose wells for deionized water with mouse. Click Set as Deionized Water. Make sure you Set as Empty Wells to default wells).
11. Load plate on plate holder. Click start. Click Ok to confirm plate is properly on.
12. System will initialize and prime. At the end of priming, the remainder of the time the system will be warming up appears.
13. Once startup is done, remove plate and click close.

Changing to Plate from Tube Mode

1. In the Cytometer menu select Sample Injection Mode. Select Plate Loader for running plates.
2. A Restart warning prompt will appear on the screen. Select OK.
3. Turn the cytometer's main power switch off.
4. Turn the valve clockwise until it stops, a "P" will be visible.
5. Turn the cytometer's main power switch on.
6. Refer back to Starting up section, step 9, to continue starting up.

Checking Cytometer performance

1. Before QC, turn Cytometer to Standby mode by selecting Cytometer menu > Standby, prime the FlowCell by choosing Cytometer menu > Prime.
2. From the QC menu, select QC start.
3. Click Plate Settings and a plate map appears. Select desired Plate Type. Click on a well to choose where you will place the QC sample. Select Ok.
4. Prepare the QC sample. Use Cytoflex Daily QC Fluorospheres. Dilute as follows: 1 drop beads to 200ul deionized water.
5. Place 200ul QC to the well. Select Eject. Place plate on plate holder. Select Load.
6. Select the correct bead Lot ID from the menu. (Bead lot is printed on each Bead vial) .
7. **Click Start. Confirm Plate position. Select OK. The performance check takes approximately 2 minutes to complete. Ensure that the events/sec rate is >100.**
8. Once the performance check is complete, View Report.
9. Verify that the performance passed. There will be a green checkbox next to each passed parameter and it says QC passed at the bottom of the report.
10. If any parameters did not pass, ask the Cytometry staff for help troubleshooting.
11. Select "Close QC Standardization" from the File menu.

Creating your experiment

1. View the detector Configuration in the Cytometer menu to verify that the detector configuration will work for your experiment.
2. Select OK to close the Detector Configuration screen.
3. Create a new Experiment. From the file menu, select New Experiment.
4. Use the default path as the location for your saved data, rename the experiment as you see fit, and save the experiment (.xit).

Creating New Experiment Settings

1. Click the Plate icon in the Tube Window.
2. From the Add Plate dropdown select Add plate.
3. The add plate window appears. Select Plate Type and Sampling Sequence. Click OK.
4. Click and drag your mouse to highlight the desired wells for your setup samples.
5. Select the Set As Sample Wells icon and a window opens.
6. Select Channel Tab.
7. Uncheck all parameters you will not be using, apply labels if you desire. Unselected parameters will not be saved into the data files.
8. Under the Group section, enter create group name as Control Setting. Click OK when done.
9. If desired, you can rename a well by right clicking on the well and selecting Set Naming Rules. For example, renaming A1's Prefix to Unstained.
10. Pipette samples onto plate. Click Initialize. Click Eject to eject the loader. Place plate onto loader and click Load.
11. Select Acq Settings in the Acquisition Window. The gain adjustment window will appear. Click the Default button to use the optimal gain settings for the Cytoflex. Use the Width Tab to select FSC Width for doublet discrimination.
12. Create a worksheet to view your cells as they are acquiring and recording. There are many types of plots to use. Right click on plots to change properties. Axis ranges can be adjusted

also. Click the title of the plot to display gated events or make combo gates using the commands and, or, and not.

13. Use the Population hierarchy tool to bring up a population hierarchy.
14. Adjust the sample flow rate in the Experiment Window.
15. Select the all color stained cells well and click run.
16. Optimize the FSC and SSC gains to place the population of interest on scale.
17. Verify that the positive populations are on scale. If a positive population is off scale, first try adjusting the number of decades shown on the plot. If a population is still off scale, lower the gain for that parameter until the positive population is entirely on scale. Note that lowering gain settings below the default settings for fluorescence channels may decrease resolution on that channel.
18. Go to the threshold tab and adjust the FSC threshold if necessary to remove unnecessary signal noise.
19. Click stop once done with optimizing.
20. You now have your gain and threshold settings. Save these settings as follows: In the Acq Settings window, choose Export to file. Continue with compensation as necessary.

Compensation

1. Ensure that you have saved a file of the correct gain settings first. (see above)
2. Create a compensation experiment by selecting New Compensation in the File Menu. Use the default file path and select Save.
3. Select Plate Type and Sampling Sequence.
4. Select the channels requiring compensation and the sample type. The unstained negative control well can be selected if needed.
5. Assign the well locations.
   a. Select the fluorochrome on the left.
   b. Select the desired sample well location for the fluorochrome on the right.
   c. Click Set As Sample Well.
   d. Repeat Steps a-c for each fluorochrome.
6. Select OK. A compensation control experiment is generated.
7. Import the Gain Settings of your experiment saved above. This will ensure that the compensation samples are run with the same settings as your experiment.
8. Click Eject to load plate. Click Auto Record to record compensation data.
9. Adjust the number of decades shown on the plots so that the population of interest is on scale. Move the FSC SSC gate so that it encloses the desired population. If necessary, move the positive gate in the plots so that it encloses the positive population.
10. Select Compensation Calculation in the Settings menu.
11. The matrix displays. Click Save as to export the matrix and specify where to save it. It is handy to save it in your experiment folder.

Recording Sample Data

1. Open your previously created experiment (From the File menu, choose Open Experiment. It should be listed as your "experiment name.xit".). If desired, continue adding additional sample wells to existing control setting plate. If not, refer to Creating New Experiment Setting section to create a new plate.

2. If compensation was performed import it (using the compensation icon in the tube window) and apply to all wells. From the compensation matrix window, choose Import > Compensation. Select Compensation Matrix in the Settings menu. Select one of the following:
    - Import compensation matrix and transform it with current gains.
    - Import compensation matrix.
    - Import compensation matrix and gain.

    Select Import compensation matrix. (Note: If you adjust your gain after compensation is preformed, you can import compensation and transform it with current gains.) Select OK.
3. Adjust the sample flow rate in the Experiment Window.
4. Highlight wells for your samples and select Set As Sample Wells.
5. Setting Sample Wells:
    a. Enter name in the prefix box.
    b. Select the desired Mix & Backflush setting.
    c. Enter Group name in the name box.
    d. Select sample well color using the color dropdown.
    e. Select desired acquisition setting under the acquisition setting tab.
    f. Set compensation under the Compensation Matrix tab. Import the matrix from where you saved it to.
    g. Enter Events to Record or Time to Record under Stopping Rule Tab.
6. When done with setting up Set as Sample Wells window, click ok.
7. Select the wells that you want to auto record. Right click the wells and choose auto acquisition. Select Auto Record in the Experiment Window to record plate. Select Record to record one well at a time manually.

Data Export

1. Navigate to your experiment in My Documents.
2. Save a copy of your experiment to "C:\FACSData".
3. Go to the Desktop.
4. Open the "Data_Upload" program.
5. A command terminal will open.
6. Follow the on-screen instructions:
    a. Type your SUnetID, hit Enter.
    b. Type "yes" when asked, if your SUnetID was typed correctly, then hit Enter.
    c. Confirm you are uploading the correct experiment, then hit Enter when prompted.
7. Leave the program to run until it closes on its own. DO NOT CLOSE THE PROGRAM EARLY, OR TURN OFF THE COMPUTER. Otherwise your upload will be cancelled.
8. (First Upload Only) You will receive an email from a FACS Core staff member containing a link and password to your data. This information will be used to access all subsequent uploads.

Preparing For The Next User or Shutting down

1. Select Daily Clean from the Cytometer menu. The Daily Clean window appears. The plate loader stage automatically ejects.
2. Select Plate Type. Select 3 wells for FlowClean and set as cleaning solution. Select 5 wells for deionized water and set as deionized water. Note: If not using pre-selected wells, highlight them and select "Set As Empty Well".

3. Add 250ul of FlowClean (blue) to wells and 250 ul of deionized to wells.
4. Click Start after loading the plate.
5. Click Close after cleaning procedure finish. Exit the Cytoflex software.
6. If there are other users right behind you, hit the Start menu of the computer, choose Switch User, leave the Cytometer and computer on.
7. If the next user is 4 hours or more behind you or if you are the last user of the day, turn off the cytometer and computer when finished.