SH800z User Guide

Initial Startup

1. Check fluidics - empty waste, refill sheath. Check DI H2O (door on the left side of the instrument) and EtOH.
   a. Sheath tank need to be depressurized to open - use vent on top of cap.
2. Turn on compressor (button on back right). Open the blue valve.
3. Power up instrument and computer
4. Log into Windows (fcm/fcm), log into SH800 Software
   a. Do not log into software until instrument is in "Standby" mode
5. To proceed to sorting, follow screen prompts:
   a. Scan chip QR code
      i. Verify correct chip information is displayed
   b. Open top cover (verify compressor is on first!)
   c. Remove old chip and load new chip (Sony and chip size should face you)
      i. Leave empty sleeve with QR code in top cabinet
   d. Select lasers (this can be modified later)
   e. Proceed through fluidics check
      i. Verify that liquid is dripping from sample line (if not perform cleanings)
      ii. Verify droplet shape is stable (bouncing, morphing - if so, debubble)

Chip alignment/Sort calibration

*Can check box to skip sort calibration if only analyzing.
1. Load Calibration beads and click OK.
   a. May need to swap out sample tube holder
   b. Minimum volume: 0.5mL
   c. Concentration: stock concentration, do not dilute
2. Click OK to proceed after completion. If setup fails, can retry.
   a. Note at which stage failure occurred for troubleshooting.

Experiment Setup

1. Change experiment name
   a. Recommended just to add something descriptive at start of existing name.
2. Measurement settings:
   a. Select colors used
   b. Add marker labels
3. Activate lasers needed if not yet done.
4. Click Create!
5. Select compensation wizard or not:
   a. If using Comp wizard, follow all on-screen directions. Be sure gating is correct for each control and that you select whether the Negative is the same as your controls or not (ie Negative is cells and Comp controls are beads - very important!).

i. Comp wizard will only be helpful if you have good controls!

ii. All voltages must be set correctly before attempting to compensate. Recommended to put up Negative to find FSC/SSC voltages and get populations on scale (from low end). Then stop/unload and look at positively stained tube/tubes to ensure nothing is off scale and your populations are properly resolved.

b. If not using Comp Wizard you can select the already created tube and begin adding the plots you would like to see. For manual compensation you should be looking at a combination of all colors versus all colors (for a 6-color experiment that is 15 plots!). See not 5.a.ii above about setting voltages before comping.

- Tubes must be 'Assigned' to begin acquisition. This happens automatically with the first tube and when you hit "Next tube". Double clicking or right click and selecting "assign" should work as well.
- Tubes cannot be overwritten. Make a new tube to record more cells (files can be combined in FlowJo later on).
- Only one sort per tube in the software - sort information is saved with the tube. To sort separate aliquots from the same sample you must unload, create a new tube and then reload.
- To change the 'worksheet settings' of a previous tube you can copy the settings from the tube you want and paste it to other or right click and select 'apply to all'.
- Options are usually available on the menu ribbon (that changes depending on what you've clicked on). You can also usually right click for most options.

Exporting data
1. To export FCS files, right click on the experiment name in the Experiment Manager, select Export FCS Files.
2. To save the image of the worksheet, right click on the worksheet area away from plots, select Copy Worksheet Image, and paste the image into a powerpoint or Paint.

Cleaning and shutdown

- Automatic cleaning and shutdown:
  a. In the cytometer tab, click Hardware and Software Shutdown button.
  b. When prompted, perform bleach cleaning using 10 mL of 10% bleach in a 15 mL tube. Put the tube in before you click Start.
  c. When prompted, perform DI water cleaning using 12 mL of DI water in a 15 mL tube when prompted, have the option "Leave DI water in lines" checked.
  d. When prompted, click Shutdown and OK.
  e. Log out of the software.
  f. Turn off the compressor, close the blue valve.

- Manual cleaning and shutdown (this option allows you to either take your chip with you or leave the instrument calibrated with the current chip for someone else to use within one hour):
    a. In the cytometer tab, click Bleach Cleaning button.
    b. Load 10 mL of 10% bleach in a 15 mL tube. Click Start.
    c. Perform DI water cleaning using 12 mL of DI water in a 15 mL tube when prompted, have the option "Leave DI water in lines" checked.
    d. If taking the chip with you, click Exchange chip in the cytometer tab. Scan the chip sleeve when prompted. Take the chip out. Press the Power button on the instrument to shut it down. Turn off the compressor, close the blue valve. Log out of the software.
    e. If leaving the instrument calibrated for someone else, check to keep the calibration settings when prompted. Log out of the software. Keep in mind that the calibration will expire if not used within one hour.

Plate sorting

1. Attach the plate support arm to the collection stage with the screw. Mount the plate holder on top of it with the A1 mark facing forward. Leave the lid on the plate for alignment.
2. In the sort control panel, choose the type of plate used.
3. Click Sort Settings. In the Plate Acquisition tab, select a combination of wells to look at during the test sort and click Start. Examine the position of each puddle. Make sure that the plate is exactly in the same position before continuing.
4. Select well A1. Adjust the aim with the arrows. Repeat for wells A12 and H12.
5. Run a test sort again. Adjust the aiming again, if needed.
6. If the puddle positions are satisfactory, click the Plate Sorting Settings tab. Specify:
    - whether you are doing an index sort (recording separate data for each individual sorted cell);
    - direction of sorting;
    - sort gate;
    - sort mode (single cell for most plate sorts);
    - Cell size;
    - Stop count (how many cells to deposit in a well).
7. Select which wells to use, click Add.
8. Close the sort settings.
9. Resume the sample flow; click Sort.
10. Once the plate is finished, click Continue if you want to perform the same sort on another plate; click Finished if you don't.