



User's Guide to the CSULB Sony Cell Sorter SH800

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Emergency contacts

In case of questions or emergencies, please contact:(in this order):

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2. **Michelle Giffin** (Dept. Biol. Sciences, 562-985-4038)
3. A **Sony technician** (see information on side of Biosafety cabinet in MLSC 222)

After hours, if you have exhausted the troubleshooting procedures in this user guide, please contact Dr. Fraser by email with your phone number, and she will call you back ASAP.

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INTRODUCTION

Flow cytometry is a powerful high-throughput technique to measure multiple parameters in 10s to 100s of thousands of individual cells per sample. The parameters consist of FSC (forward scatter, equivalent to cell size), SSC/BSC (sides-scatter or back-scatter, both of which are equivalent to cell complexity/granularity) and FL (fluorescence). The fluorophores that can be detected are dependent on the excitation and emission spectra of the lasers and filters used. Our Sony SH800 has 3 lasers (405nm, 488nm and 565nm), and 6 filters, which means a total of 6 different fluorescent 'channels' can be observed for each cell. The Sony SH800 is also a cell sorter, which is capable of isolating a subpopulation of cells with specific parameters and "sorting" them into a collection chamber after data acquisition. **If you are being trained on the SH800 it is assumed that you have a basic understanding of the technique of flow cytometry and cell sorting.** If you are new to the concept of flow cytometry and/or cell sorting, please watch the Introduction to Flow Cytometry videos on the CSULB Research Training beachboard site, or available through the CSULB BUILD program. This video includes useful tips on setting up your flow cytometry experiment, and data analysis. Additional useful links are found in Section I at the end of this document. You will **be quizzed** on your knowledge of flow cytometry and experimental set up before undergoing training. The questions you need to be able to answer are given at the end of this document (Section J). You will also need to provide proof that you have taken the CNSM BSL-2 online training module. Please contact the CNSM safety office if you require more information about this: <http://www.csulb.edu/colleges/cnsm/safety.html>. You should also watch the Flow Cytometry Users Guide video prior to requesting training.

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Essential system specifications

Lasers:

405nm – Violet laser

488nm – Blue laser

561nm – Green laser

Information on fluorophore excitation and emission frequencies is available on websites listed in the “links” section I of this guide.

Nozzle Sizes:

70uM: best for small cells, less aggregated: for cells approx. 0.5 – 12 uM.

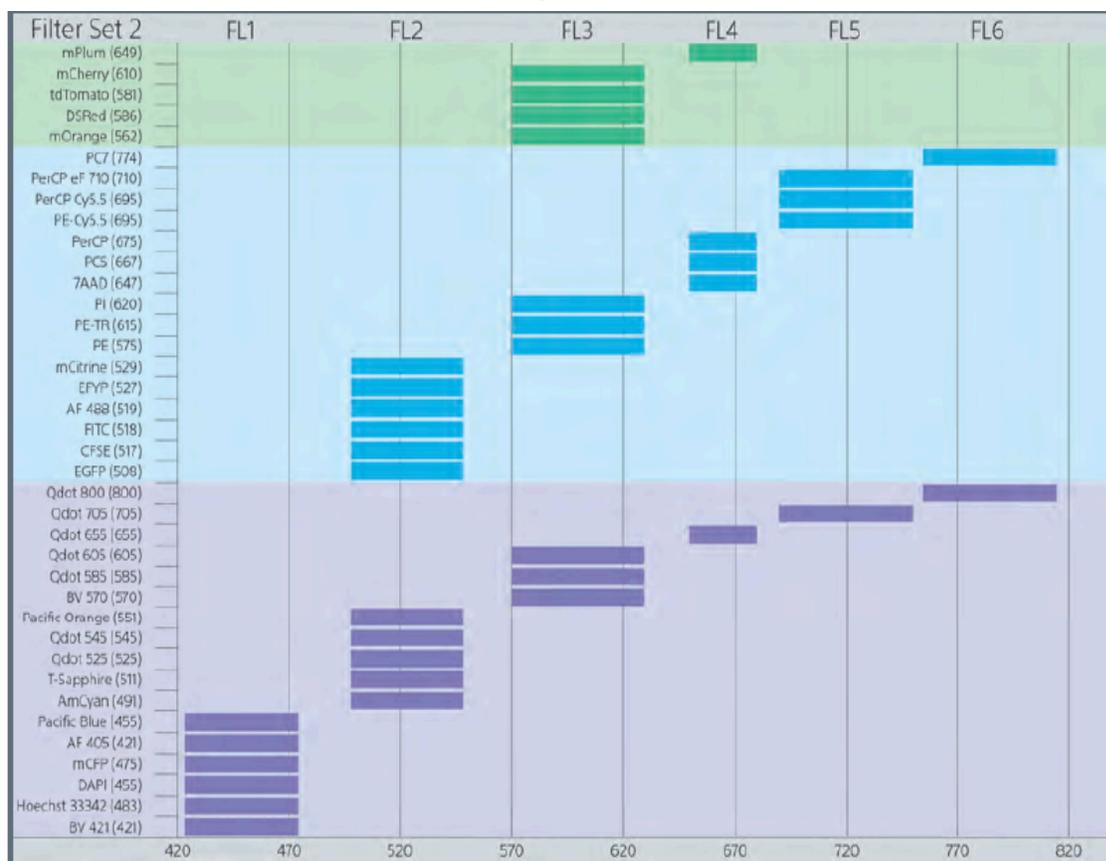
100uM: this is the ‘usual’ size, without excessive clumping. For cells approx. 7 – 17uM

130uM: better for large, aggregated or clumpy cells. Up to 22uM

Filters:

6 fluorescent parameters can be detected at one time (FL1 – FL6).

The standard combination of filters is configured as follows:



Please note: if you need to use combinations of fluorophores that emit in the same FL channel (e.g. as configured, mCherry and PE are both going to be detected in FL3 channel), it may be possible to reconfigure the filter settings to accommodate this. Please discuss your needs with Dr. Fraser and **do not** attempt to alter filters without additional training.

PRECAUTIONS

• **BIOSAFETY.** This unit is used for the sorting of materials including those that may be biohazardous including human cell lines and microbial pathogens. Operators must complete CNSM (Biosafety Level 2 (BSL-2) training prior to using the sorter. Appropriate personal protective equipment (PPE) including gloves, lab coat and eye protection are required during operations. If you are sorting BSL-2 agents, be sure that

- The SH800 sort door panel is fully closed
- The biosafety cabinet is on and properly operating
- The biosafety hood sash is at the indicated safe working height
- The biosafety hood side door is fully closed

In addition, if you are sorting into a 96-well plate:

- AMS Control button on biosafety hood must be switched ON to create a vacuum to remove aerosols in case of a clog. Detailed instructions for dealing with clogs during sorting are included in Section H (troubleshooting).

• **OPENING FRONT CHIP ACCESS DOOR.** If you try to open the SH800 front chip access door (where the chip is loaded) and the compressor is off, you will **break this machine!!!** **ONLY open this door if you are certain the compressor is ON!**



• **LASER SAFETY.** This device is considered a Class I laser device or one that does not produce injurious laser emissions accessible to the operator under normal operating conditions. Within the enclosed system are higher power Class 3B potentially injurious lasers. The user is protected from harmful laser emissions by the equipment housing and the sorting chamber door interlock switch. NEVER attempt to remove equipment housing components or disable the sorting chamber door interlock.

• **ELECTRICAL SAFETY.** The deflection plates inside the sorting chamber are charged at an extremely high voltage. Touching them when charged may result in a serious injury via electric shock. DO NOT touch the deflection plates while the power is connected to the cell sorter.

• **CHEMICAL SAFETY.** Some of the solutions used by the cell sorter are hazardous and under pressure in the system fluid tanks and associated lines. Bleach used as a sanitizer is corrosive. Ethanol is flammable. Open flames and other ignition sources should never be placed near the main unit or fluids cart below the biosafety cabinet. Appropriate personal protective equipment (PPE) including gloves, lab coat and eye protection are required during operations.

- REMOVING AND REPLACING FILTERS. **Don't do this.** If you know ahead of time that you need a different configuration, please ask Dr. Fraser and she will train you to swap out the filters.



- REMOVING FILES FROM THE SH800 COMPUTER. Anti-virus programs interfere with the SH800 software. Thus we have to be careful not to introduce viruses to the system. This is why the computer is not, and should not be, connected to the internet. The only safe way to remove files from the computer is by copying them to a **virus-free USB drive**. Copy the files, transfer them to your own computer. Note; please also eventually delete them from the common computer. Files older than 1 month old are fair game for deletion.

- KEEP THE AREA CLEAN. The cell sorter resides in the Fraser lab. Please be respectful of the space, and tidy up after yourself. There can be lots of waiting time in cell sorting, time when you will be twiddling your thumbs. If you need food or drink, do it *outside*— **no food or drink in MLSC 222!** Also, please limit your specimen preparation in the lab. You are welcome to use our gloves, pipets, 70% ethanol and paper towels as needed, but make sure to leave the room clean when you're finished.

PLANNING YOUR EXPERIMENT

Sample Preparation Tips:

Avoiding clumps:

Single cell suspensions are required for optimal staining. Aggregated cells will clog the flow cytometer. If you are running “clumpy” samples, make sure you filter them through a 40-100µM cell strainer before running flow cytometry. Flowmi pipet tip cell strainers (Belart.com, H136800040) or Falcon filter-top tubes (Fisher 08-771-23) are ideal for this purpose.

Sample tubes:

Standard FACS tubes are 12x75mm polypropylene or polystyrene round bottom tubes. You can also use 1.5ml microtubes, or 15ml conical tubes for larger samples.

Fixed samples: Where possible, biohazardous samples should be fixed for analysis. Usually 1-4% paraformaldehyde in PBS. However, if you are sorting cells for subsequent culture you will want live cells. In this event, make sure the AMS Control button on the biosafety hood is switched ON to create a vacuum to remove aerosols in case of a clog. Detailed instructions for dealing with clogs during sorting are included in Section H (troubleshooting). In addition, check that your fluorophore is not sensitive to fixation.

Unfixed samples: Avoid using buffer containing phenol red during your run as it can increase background fluorescence. PBS or HBSS are usually used to run samples. Some people add 1% BSA to keep their cells happier in solution, preventing clumping and non-specific antibody binding. 0.05% sodium azide can also be added to prevent shedding or internalization of antibodies. Sodium azide is highly toxic. Use appropriate safety measures. Do not add azide if you want to sort live cells after analysis as it will kill your cells. *Please note:* If you are running samples at non-mammalian physiological osmolarity (e.g. marine) you will need to prepare around 5-10L sheath fluid consisting of buffered saline and 5 mM EDTA. The ready-use sheath fluid provided has a salt concentration of 150mM NaCl. Do not add Calcium or Magnesium to your buffers. They form salt crystals in the tubing, and clog the machine.

Temperature: The appropriate temperature varies between cell types and staining conditions. For analysis only, many samples are more stable on ice. For sorting live cells, they may be happier at room temperature.

Light: Fluorescently stained samples should be kept dark until ready to analyze.

Number of cells: If possible aim for >250,000 cells at approximately 0.1-1 million cells/ml

Multicolor flow: If you plan on staining your samples with multiple colors, there is an art to combining color combinations. The links in section J should help. Some things to consider:

1. The brightness of your fluorochromes – use the brightest fluorochrome for the least expressed protein and dimmest for the most highly expressed proteins.
2. Make sure the fluorochromes will be detected in different channels of the SH800 (see chart on page3).
3. Advanced – chose combinations with low spectral overlap, low photobleaching and that use a different excitation laser. This will maximize your signal and reduce background and bleed-through.
4. Set up appropriate controls. At very least you will need:
negative control (unstained) sample

single-stained positive controls – one for each antibody-fluorophore you are using. Use the sample that is most likely to be most positive for that marker

BEFORE YOU START

You must sign up to use the flow cytometer ahead of time using the online reserve calendar

<http://web.csulb.edu/depts/biology/calendar/>

Click on the “Cell Sorter” option in the top banner

Select the day you would like to book on the calendar, and then fill in the start time using the 24-hour clock (for example 09:00 is 9am, 13:00 is 1pm). Select duration. The options are 1-4 hours. Include your name, lab PI, and email in the sign up.

Please note: only authorized users, who have undergone the training may use this machine.

Violation of this policy will lead to restricted lab access to instrument use except for the lab PI.

Please bring with you:

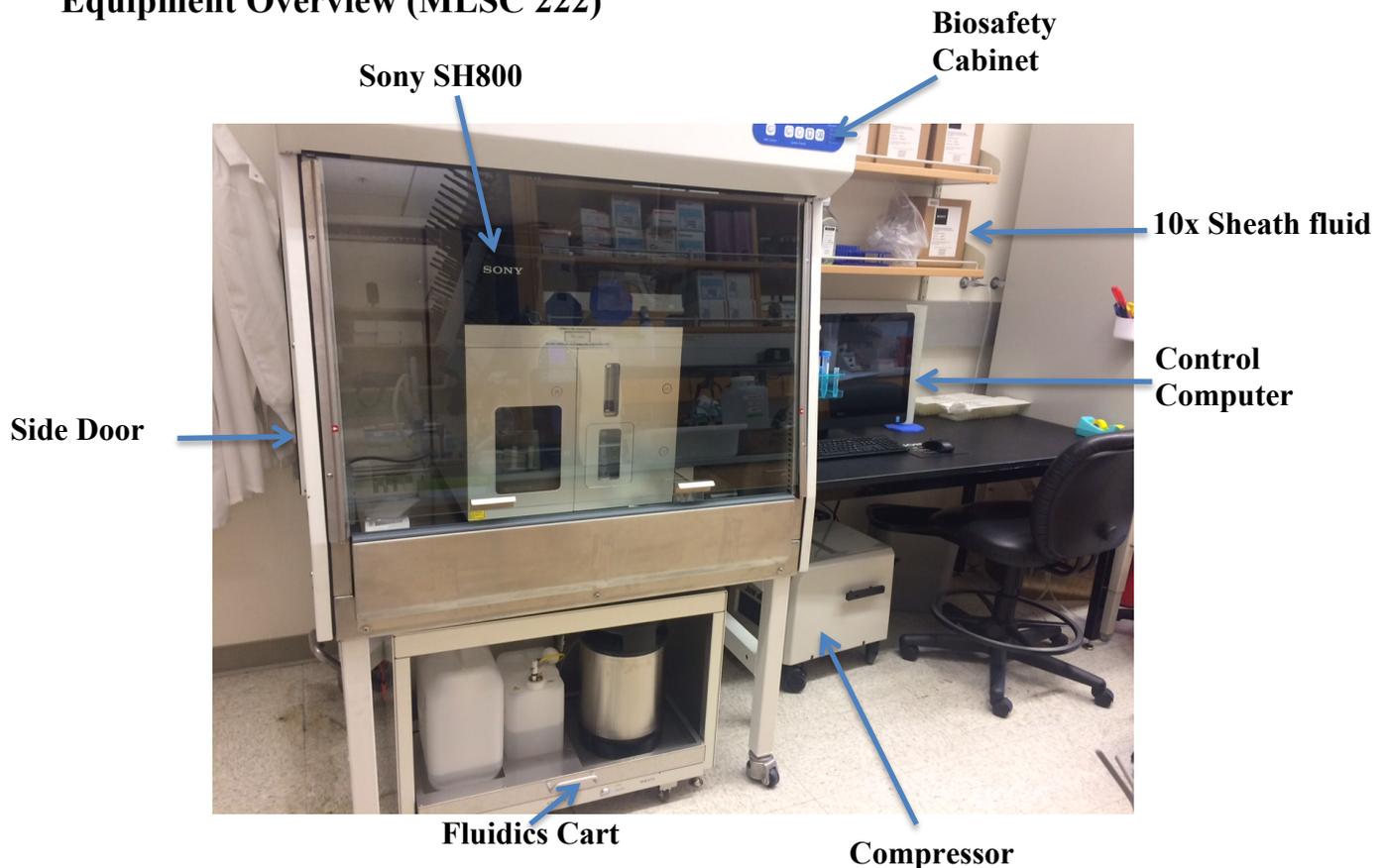
Your samples

At least 1L bleach

PBS/sample buffer in case you need to dilute your samples (~10ml)

10% bleach solution for shutdown (10ml in a 15ml conical tube)

Equipment Overview (MLSC 222)



GETTING STARTED

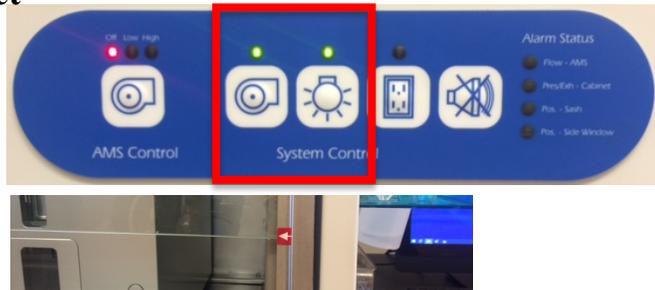
A. Fill out the user log book

We use this to keep track of usage of the chips, and the labs that are using the machine. If you do not fill out the log, your access to the machine will be rescinded.

B. Turning on the biosafety cabinet

In this order:

1. Switch on the **blower**
2. Switch on the **light** (if needed)
3. Open front sash to red arrows



C. Check Fluid Levels

The waste, 70% ethanol and sheath fluid tanks are on the cart under the biosafety hood



Please be gentle when removing and replacing tanks. The cart contains sophisticated balances to measure volume in the tanks. **Do not drop the tank back onto the cart when full!**

The **water bottle** is inside the side door of the SH800, and can be accessed via the side door of the biosafety cabinet.



As needed:

1. Fill Sheath Tank

Twist the **pressure release valve** (top loop) a half turn to release pressure

Lift handle

Push down lid and twist to remove

If levels are low (below half full), prepare 4L sheath fluid

Using the 4L Measuring cylinder found behind you, mix:

400mL of 10x sheath fluid

Add 3.6 L milliQ water from dispenser at sink (just press the blue button to dispense)

Add to tank, and replace lid, closing both the handle and the pressure loop.

Please note: if the tank is really low, make a full 10L of sheath fluid, by adding 1 full bottle of 10x sheath fluid (=1L) + 9L milliQ water



NOTE: even if you don't need to fill sheath tank, you still need to close the pressure loop!

2. Empty Waste Tank (*every time you use the instrument*)

Empty all contents of waste down sink with copious amounts of water

Add 1L bleach to tank using the dedicated bleach cylinder, on the desk

Replace lid

3. Fill 70% Ethanol Tank (*only required if performing instrument sterilization*)

Fill tank from the carboy of 70% ethanol behind you

If carboy is empty, Michelle Giffin in MLSC 100 will refill the empty carboy with 95% ethanol. Just ask her to fill to the first line.

Note: Ethanol refills can take 1-2 days. Please plan accordingly.

Then, you can fill with MilliQ water to the second line to make 70%.

Gently swirl bottle to mix.

4. Fill diWater Bottle (*every time you use the instrument*)

unclip bottle and disconnect tubing by pushing in **quick release valve**

Fill with MilliQ water from dispenser at sink

Replace bottle and reconnect tubing



D. Switching On

1. Power on the computer if it is not already on. Do not open the software yet.

Computer password is fcm

2. Power on the compressor (under the table). Just flip the **switch** to on. It will make a noise so you will know you are successful!



3. Wait at least a full minute for the system to pressurize. This is a good time to prepare your calibration beads (see pg.11)
4. Turn on the instrument by pressing the front **'Power' button**. When it has initialized, the front screen will read 'Standby'. Then, proceed to:
5. Open the SH800 software. The login screen should appear.



E. Starting Up

On the Login screen you have 3 options:

1. Shutdown - did you change your mind?! You can shut down the machine at this point.
2. Maintenance

Ethanol cleaning: to be followed if you are concerned about sanitizing the lines (e.g. you may want to do this before sorting cells that will be subsequently cultured)

WasteA - Use this option if you see in the logbook that the machine hasn't been used in over a month.

Follow the onscreen instructions to flush the lines

Sample line exchange – use this option if you are using your own sample line

This is for labs running non-mammalian organisms (yeast, etc)

Follow the onscreen instructions for this

3. Login – most of the time you go straight to login

Username: user1

Password: (none)

The machine will take you through the following step-by-step:

NOTE: please wear clean gloves to keep yourself and our machine free of contamination.

1. Chip Detection. You will need to scan the QR code on the sorting chip package. Note, once it has been scanned this chip can only be used in the following 24 hours. Since chips are around \$40 each, this should not be done until you are sure your samples are good, and are ready!
2. Chip Information. Check the chip information is correct, and click next
3. Chip Loading. Load the new chip into the slot. Discard the old chip into biohazard trash bin
 - Chip should be loaded with writing facing towards you
 - Push gently on the chip until you feel the machine start to pull it down automatically
 - Click next when the chip has loaded
4. Laser Setting. Select which lasers to switch on – this will depend on the excitation wavelength of the fluorophores you are using (if in doubt, check all of them)

5. Filter Setting. Check filters are set according to the onscreen diagram. If not please ask Dr. Fraser to assist in returning them to this setting. Don't forget to close the access door
6. Fluidics Check. Machine will go through a fluid check. If you do not see sheath fluid dripping from the sample tip of sample probe, follow onscreen instructions to perform a sample line cleaning as prompted.

Auto Calibration

There are 3 options on this page:

Skip Auto Calibration

Not recommended!

Analyzer Mode

If you are not sorting cells, only analyzing them, this is a quicker start up procedure that skips full calibration and only calibrates chip alignment. However, if you are sorting cells you must run the full calibration.

Calibration

Prepare Calibration Beads:

Prepare a new 12x75mm FACS tube with a few drops (0.5-1ml) of calibration bead solution, from the fridge in MLSC 222. Be sure to vortex the stock container before use- the bead solution should be blue in color. Label it "calibration beads".

Load tube with beads into 5ml holder in machine and Press OK to calibrate.

Then calibration – takes about 5-10 minutes

Chip Alignment, Droplet Calibration, Side Stream Calibration, Sort Delay Calibration

Are all performed automatically

Calibration Errors

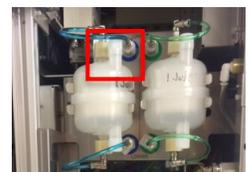
If there is a problem with calibration, the machine will give you an error message. It is usually caused by air in the filters, which can be fixed via a troubleshooting wizard. Follow the directions on the screen. Briefly:

Cytometer tab → sheath filter debubble

When directed by the computer, find the sheath filter in the side door of the cytometer, on the LEFT (blue tubing). Tap/hit the filter several times.

Unscrew the **white screw** on top of the sheath filter A HALF TURN and wait until sheath fluid bubbles out the top

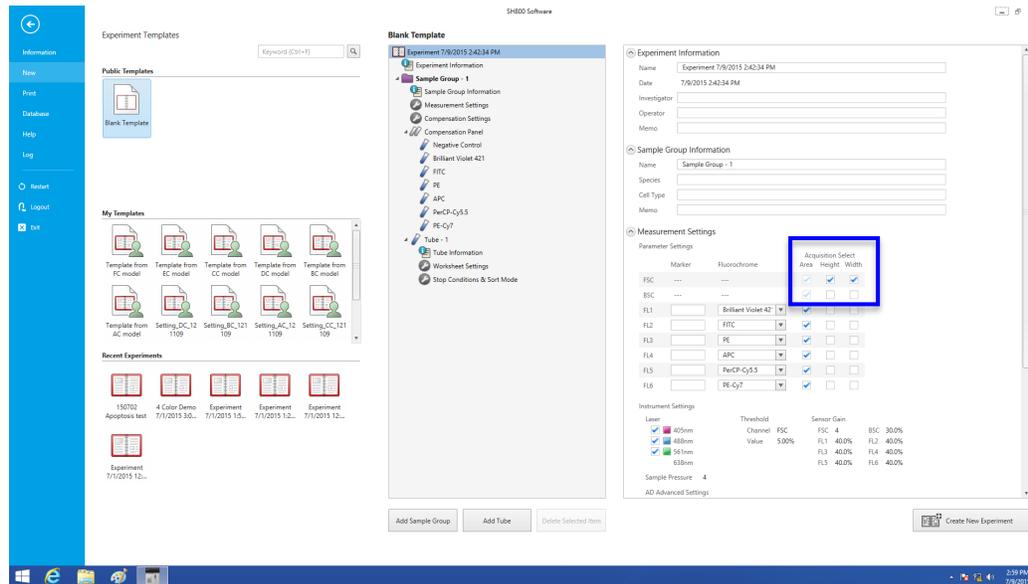
Tighten the screw back up, and replace the filter in the machine (without pinching tubing). Clean up any liquid.



F. Running Your Samples

A general outline is given, however the specifics will vary according to your individual needs and the samples you are running.

When you start you will see this screen giving you the option to open up a previously saved template, or “Set up new experiment”

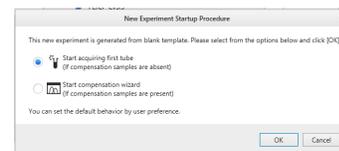


New users should input Experiment Information on the right:
 Fill out investigator and any other information you want to include (Species Cell Type etc.)
 Fill out Parameter Settings under Measurement Settings
 Include the markers you are using (antibody targets) and select the fluorochromes you will use to detect them.
 Under Acquisition, its good to check **Area, Height and Width** for both FSC and BSC for sorting, to make sure you can select a population with even properties of size and granularity

Click: “Create New Experiment”

Option:

Either select: Start acquiring first tube (if no compensation required. E.g. you are running a single color experiment only)
 Or, for multicolor flow, select: Compensation Wizard



Compensation Wizard: follow onscreen instructions to run compensation
 You will need a negative control sample
 Single-stained positive control samples for all your fluorophores

Note: make sure you are using the correct tube holder for the tubes you are using. The options are 12x75mm holder, 1.5ml holder, 15ml holder.

Acquisition Worksheet:



Experiment Tab

Assign tube to assign first tube

can enter tube information if you want, by double clicking on Tube Information in the Active Experiment box

Set up:

Start by running your unstained, negative control to see FSC and BSC (size and granularity properties) of cells, and gate out debris/ unwanted populations if necessary as follows:

Vortex sample, then Load sample into correct tube holder, and into machine

Press **start**

Open **Detector&Threshold Settings**

Alter Threshold to exclude as much debris from your sample counts as possible

Alter Gain to bring cell populations off axes and to define populations. Lower is better.

Draw a rectangle or **Ellipse** or Polygon Gate to select a subpopulation of cells (e.g. "live cells" gate) if required

Double clicking inside gates will bring up a subpopulation **gated graph**. This can be altered to display a dot plot (measuring 2 parameters) or a **histogram** (choose one parameter on x-axis versus Events on Y-axis). In the example below, **A** is a subpopulation, or gated population of All Events.

Press **Record** to start saving data.

Press **STOP** (black) to end

Sample Pressure can be increased to speed up if desired (8 is recommended max)

Stop Condition is set at 100,000 cells by default, but can be altered. At least 10,000 is recommended for regular samples. At least 1000 is recommended for rare cells

The screenshot displays the Sony SH800 Cell Sorter software interface. On the left, the 'Sample Group - 1' panel shows 'Status: Ready', 'Elapsed Time: 00:00:08', and 'Total Events: 4,975'. Below this are 'Start', 'Stop', and 'Record' buttons, and a 'Detector & Threshold Settings' dialog box. The main area features two plots: 'All Events' (a scatter plot of BSC-A vs FSC-A with a red gate at 2.54%) and 'A' (a histogram of Brilliant Violet 421-A-Co...). On the right, the 'Gates and Statistics' table is visible:

Name	Events	%Parent	%Total
All Events	4,975	0.00%	100.00%
A	1,619	32.54%	32.54%

At the bottom, the 'Sort Control' panel includes 'Sort Start' and 'Load Collection' buttons, 'Sorting Method: 2 Way Tubes', and 'Stop Value' fields. The 'Sort Statistics' panel shows 'Elapsed Time: 00:00:00' and 'Sort Count: 0'. The 'Hardware Status' panel shows 'Light Collection: Turn On' and 'Sample: Turn On'.

SORTING

Open sort door and put in required holder



2-way tube holder (line up 3 holes)



Plate holder – can be pre-chilled in freezer. Must put in plate holder attachment to hold plate. Can sort into PCR plate or regular 96-well plate

Controls to set up the sort are on the bottom of the screen :

This close-up screenshot focuses on the bottom control panels of the software. The 'Sort Control' panel has 'Sort Start' and 'Load Collection' buttons highlighted with a green box. The 'Sort Statistics' panel shows 'Elapsed Time: 00:00:00' and 'Sort Count: 0'. The 'Droplet' panel, highlighted with a blue box, shows a 'Droplet' icon and a 'Droplet' label. The 'Hardware Status' panel shows 'Light Collection: Turn On' and 'Sample: Turn On'.

First check **Droplet ID**. It should not be moving. If you see pulsing or movement you will not get a good sort. If this happens, try recalibrating the machine. The green light means go ahead. If flashing it is adjusting, so wait for it to turn solid green to start.

Press **load Collection**

Choose method (2 way tubes or plates)

Choose how many cells to sort if required

Press START to run the samples



Press **sort start** when you're happy your sample is running well, and your population is there
The sort will continue until you reach your max cell# or until you press STOP



When finished, remove your tube/plate from the collection port.

Save and export your data to FCS file by:

Highlighting the experiment in the Active Experiment box

Click **Export to FCS** file in the Experiment Tab

Save data in your Lab's folder. Do not save directly on desktop it **will** be deleted.

FCS files can be imported into this software on your own computer, or used in FlowJo for analysis.



G. Shutting Down

You will need

15mL tube of 10% bleach (10ml)

15mL tube of MilliQ water (12ml)

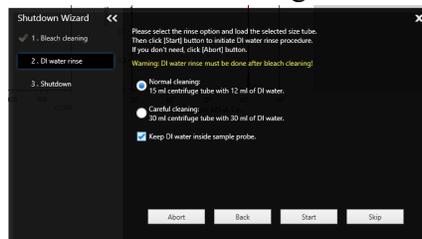
Cytometer tab → **Software and Hardware shutdown**



ALWAYS perform the bleach cleaning and water rinse before you shut down. **Do not skip this.**

So, press **START (not SHUTDOWN)** on shutdown wizard.

Select Normal Cleaning Mode



(unless you have been having issues with clogs, where a “Careful cleaning” is advised)

The software will walk you through bleach and water rinses. At the end of the wizard, the software and the machine will shut down

Make sure the front chip access door is closed before you...



Chip Access Door is CLOSED

Turn off the compressor

Release the pressure on the sheath tank by turning the pressure release valve a quarter turn

Switch off the biosafety cabinet and light, and close the sash

Please take all your samples with you, and leave the bench clean and tidy.

H. Troubleshooting

Follow these procedures first.

If you have tried all of these, and are still having issues, then ask Dr. Fraser.

Last resort, call the Sony technical support number on the side of the biosafety cabinet.

Problems during Analysis Only:

Problems running a sample, or washing the lines?

Try to debubble the water filter, as follows:

Cytometer tab → **probe wash (see below)**

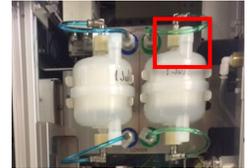
Find the water filter in the side door of the cytometer, on the RIGHT (green tubing)

Unscrew the **white screw** on top of the water filter

Tap/hit the filter 5-10 times

(note: you will not hear a loud hissing from the cytometer unlike sheath filter debubble)

Tighten the screw back up, and replace the filter in the machine (without pinching tubing)



If you get a Clog (often diagnosed by observing a change in droplet view)

Cytometer tab → **chip debubble**

Press chip de-bubble a few times. Gets rid of most clogs

If this doesn't work, try a sheath debubble

Then perform a water rinse

Cytometer tab → **sheath filter debubble**

Find the sheath filter in the side door of the cytometer, on the LEFT (blue tubing)

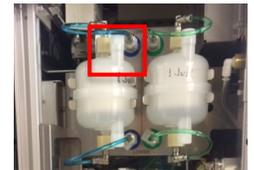
Unscrew the **white screw** on top of the sheath filter and wait until sheath fluid

bubbles out the top

Tap/hit the filter until you hear a loud hissing from the cytometer

Tighten the screw back up, and replace the filter in the machine (without pinching tubing)

Cytometer tab → **DI rinse**



Problems during Sorting:

Sample Path Clog. If the data rate decreases to near zero, but the droplet formation is normal (as seen in the Droplet Viewer), then the sample path is clogged. This is easily remedied:

1. Do not click on stop
2. Click on pause. This will pinch the sample line and stop the sample flow.
3. Click on unload sample
4. Go to the cytometer tab and click on Probe Wash
5. Watch the sheath fluid drip from the sample probe. If needed, repeat the Probe Wash until the sheath fluid drips at a normal rate, indicating the sample path is clear.
6. Inspect the sample in the sample tube. If needed, filter the sample to remove clumps, do not just reload
7. Re-mount the sample tube
8. Click on Load Sample
9. Click on Resume
10. Data should appear again within 90 seconds. Sorting will resume automatically

11. At end of sort and AFTER THE MACHINE HAS BEEN SHUT DOWN, wipe detection plates and interior of sorting chamber with kimwipe wetted with 70% ethanol

Nozzle Clog. If the data rate decreases to near zero and the droplet formation is disrupted (as seen in the Droplet Viwer), then the chip nozzle is clogged. This is rare, but easy to fix:

1. The software should have already detected the problem and stopped the sort automatically. If not, click on Stop.
2. Go to the Cytometer tab and click on Sheath Filter De-Bubble
3. Perform de-bubble as described above
4. When the pressure has returned to normal, look at the droplet formation image in the droplet Viewer. If needed, repeat the Sheath Filter De-bubble until the droplet formation appears normal.
5. Go to the Cytometer tab and click on Sort Calibration. Follow the software prompts.
6. Return to your experiment, click on next tube, and reinitiate the sort.
7. At end of sort and AFTER THE MACHINE HAS BEEN SHUT DOWN, wipe detection plates and interior of sorting chamber with kimwipe wetted with 70% ethanol

As a last resort try a chip exchange (you will have to use another new chip).



I. Useful Links

The following links describe flow cytometry, and designing a good experiment. Feel free to forward suggestions of any others you find to be useful, so I can continue to build the list.

Introduction to Flow Cytometry for beginners:

1. "Introduction to Flow Cytometry" video and powerpoint by Dr. Deborah Fraser (Available on CSULB CNSM Research Training webpage, and through CSULB BUILD)
2. Video training introductory course: http://www.bdbiosciences.com/us/support/s/itf_launch
3. online book: <http://www.coulterflow.com/bciflow/practical/book/index.html>
4. pdf: http://users.path.ox.ac.uk/~nrust/a_beginners_guide_to_flow_cytometry.pdf

Fluorophore / fluorochrome Selection

Note: there may be some bias from companies that produce their own fluorophores! Here are my favorites:

1. <https://www.lifetechnologies.com/content/dam/LifeTech/migration/en/filelibrary/cell-tissue-analysis/pdfs.par.13383.file.dat/fluorophore-selection-guide-flow-cytometry.pdf>
2. <http://www.bdbiosciences.com/us/s/spectrumviewer>
3. <https://www.ebioscience.com/media/pdf/literature/multicolor-flow-cytometry-tech-resource-guide.pdf>
4. http://www.biolegend.com/multicolor_staining

J. Test Questions

You will be expected to be able to answer the following questions **before** you can begin in-person training on the Sony SH800:

1. What is flow cytometry?
2. What type of samples will you be running on the SH800?
3. How big are your cells in μM ?
4. Which nozzle size should you use? (70 μM , 100 μM , 130 μM ?)
5. What fluorophores will you be using to stain your samples?
6. What lasers will you need to excite those fluorophores?
7. Which FL channels will you use to detect your fluorescence?
8. What will your single stained controls be?
9. What will you need to bring with you to use the machine?