



User's Guide to the CSULB Luminex MUSE Cell Analyzer

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Emergency contact

In case of questions or emergencies, please contact:

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User's Guide to the CSULB Luminex MUSE Cell Analyzer

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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 MUSE Cell Analyzer uses miniaturized fluorescent detection and microcapillary technology to deliver accurate, precise, quantitative cell analysis. Laser-based fluorescence detection of each cell event can evaluate up to 3 cellular parameters – cell size (forward scatter) and 2 colors (detected in the red and/or yellow channels).

The system uses a microcapillary and miniaturized optics, which occupy one-tenth the space of a typical cytometer. A green diode laser is used for excitation, and a uniquely designed series of retro-reflective lenses provide maximum light capture and sensitivity.

One major advantage is that the Muse features a highly intuitive touchscreen interface that allows simple step-by-step operation, so easy that no flow expertise is required to run assays. The touchscreen prompts you through simple on-screen instructions and guides you through sample loading to simple setting adjustments to results—in just a few steps.

If you are being trained on the MUSE Cell Analyzer it is assumed that you have a basic understanding of the technique of flow cytometry. If you are new to the concept of flow cytometry, 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 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 MUSE Cell Analyzer Users Guide video prior to requesting training.

A copy of the official Luminex User Guide is also available in printed form, next to the MUSE Cell Analyzer.

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Essential system specifications

Lasers:

532nm – Green laser

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

Detection:

Red or Yellow fluorescence. The Muse. Open Modules can be used for one- or two-color assays and can be applied to a variety of experiments such as the study of extracellular and intracellular expression of protein, as well as the screening and analysis of red fluorescent proteins. The modules allow researchers to stain samples with their own fluorochrome-conjugated antibodies, dyes, or other reagents that are excited by a 532-nm laser. The yellow parameter uses a detection channel with 576/28 emission and can be used for the detection of fluorochromes such as Phycoerythrin (PE), Cy3, Alexa Fluor. 555, and Dylight. 550. The red parameter uses a detection channel with 680/30 emission and can be used for the detection of fluorochromes such as PE-Cy5, 7-AAD, and Propidium Iodide (PI).

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 cell analyzer. Appropriate personal protective equipment (PPE) including gloves, lab coat and eye protection are required during operations. The waste bottle should contain 10ml fresh bleach prior to each use.
- **LASER SAFETY.** This device is considered a Class I laser product that does not produce injurious laser emissions accessible to the operator under normal operating conditions. Within the enclosed system is a higher power Class 3B potentially injurious laser operating at 532nm (maximum output power of 23mW). Light shields within the instrument enclose the path of laser radiation, and the instrument enclosure provides secondary protection from laser radiation. NEVER attempt to remove equipment housing components or attempt to perform any internal maintenance as this may result in hazardous exposure to laser radiation.
- **ELECTRICAL SAFETY.** Turn off the power to the system before removing the flowcell.
- **CHEMICAL SAFETY.** Some of the solutions used by the cell analyzer are hazardous. Bleach and the ICS Cleaning Fluid used as sanitizers are corrosive. Appropriate personal protective equipment (PPE) including gloves, lab coat and eye protection are required during operations.
- **REMOVING FILES FROM THE MUSE CELL ANALYZER.** The only safe way to remove files from the MUSE is by copying them to a **virus-free USB drive**. Copy the files and transfer them to your own computer in a timely manner. Files older than 1 month old are fair game for deletion.

- KEEP THE AREA CLEAN. The MUSE Cell Analyzer resides in the Fraser lab. Please be respectful of the space, and tidy up after yourself. 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

Luminex MUSE Cell Analyzer Kits

A number of kits that have been optimized for analyzing your cells with the MUSE Cell Analyzer are available: <https://www.luminexcorp.com/flow-cytometry-kits-and-reagents/>

Please follow the manufacturer's recommendations and protocols for these kits. *Kits include:*

[Muse Count & Viability Kit 40 mL](#)

[Muse Count & Viability Kit \(200X\)](#)

[Muse Annexin V & Dead Cell Kit](#)

[Muse Cell Cycle Kit](#)

[Muse Cell Dispersal Reagent](#)

[Muse Caspase-3/7 Kit](#)

[Muse MultiCaspase Kit](#)

[Muse Autophagy LC3-Antibody Based Kit](#)

[Muse Mitopotential Kit](#)

[Muse Oxidative Stress Kit](#)

[Muse Nitric Oxide Kit](#)

[Muse Ki67 Proliferation Kit](#)

[Muse Human CD4 T Cell Kit](#)

[Muse Human CD8 T Cell Kit](#)

[Muse Human B Cell Kit](#)

[Muse H2A.X Activation Dual Detection Kit](#)

[Muse EGFR-RTK Activation Dual Detection Kit](#)

[Muse PI3K Activation Dual Detection Assay Kit](#)

[Muse MAPK Activation Dual Detection Assay Kit](#)

[Muse Bcl-2 Activation Dual Detection Assay Kit](#)

[Muse Multi-Color DNA Damage Kit](#)

[Muse PI3K/MAPK Dual Pathway Activation Kit](#)

[Muse Malaria *P.f-P.v.* Detection Kit](#)

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:

You must use 1.5ml microtubes without a lid (or cut off the lid).

Fixed samples: Where possible, biohazardous samples should be fixed for analysis. Usually 1-4% paraformaldehyde in PBS. You should 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 Calcium or Magnesium to your buffers. They can form salt crystals in the tubing, and clog the machine.

Temperature: The appropriate temperature varies between cell types and staining conditions. For analysis only, usually samples are kept on ice.

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

Number of cells: Optimal results are attained with cell concentrations between 1×10^4 to 1.2×10^6 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 do not overlap in their emission spectra.
3. Advanced – chose combinations with low spectral overlap, and low photobleaching. 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

Please check with Dr. Fraser (Deborah.Fraser@csulb.edu) prior to using the instrument to check it is available. Include the day and time you will be using it in your email.

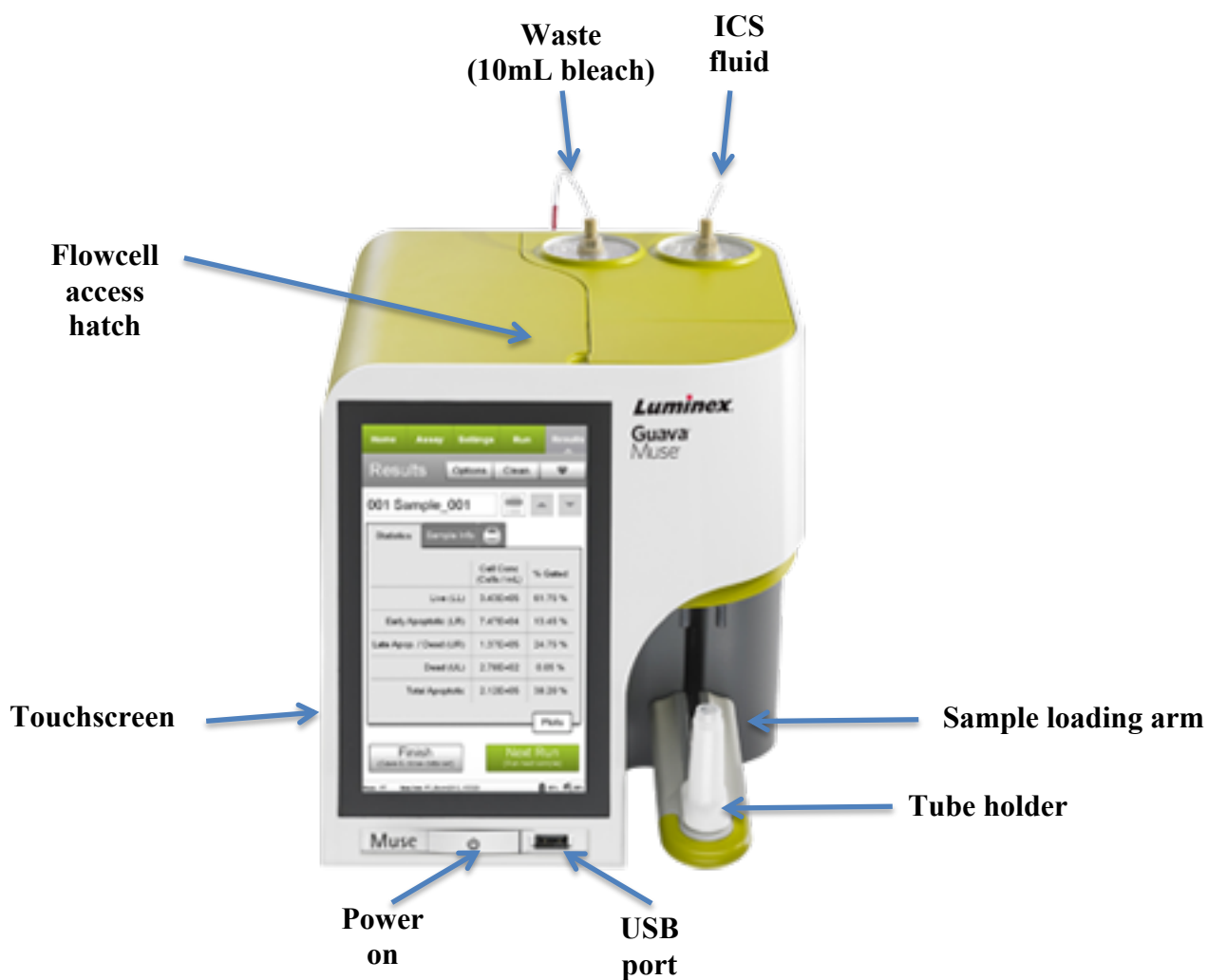
Please note: only authorized users, who have undergone the training may use this machine.

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

Please bring with you:

Your samples in 1.5mL tubes with removable lids

Equipment Overview (MLSC 222)



GETTING STARTED

A. Fill out the user log book

We use this to keep track of usage of the instrument, 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. Check Fluid Levels of ICF

Fill the cleaning solution bottle with ICF at the start of each day, and as needed. Do not allow the bottle to empty. This will pull air into the fluid system and require that you prime the system with water.

1. Unscrew the tubing from the top of the cleaning solution bottle.
2. Press down on the cleaning solution bottle to release it from the unit. The bottle will pop up slightly allowing you to remove it.
3. Unscrew the cap.
4. Fill the bottle to the fill line with Guava ICF.
5. Replace the cap and reinstall the bottle in the instrument. Align the shower icon on the bottle with the notch/line on the instrument. Press down on the bottle to engage it.
6. Reconnect the fluid line to the top of the bottle

C. Empty the waste bottle

Empty the waste bottle at the start of each day, and as needed.

1. Unscrew the tubing from the top of the waste bottle.
2. Press down on the waste bottle to release it from the unit. The bottle will pop up slightly allowing you to remove it
3. Carefully unscrew the cap.
4. Empty the contents down the sink with copious amounts of water running.
5. Rinse the bottle with water.
6. Add 10 mL of bleach to the empty waste bottle.
7. Replace the cap and reinstall the bottle in the instrument.
Align the trash can icon on the bottle with the notch/line on the instrument. Press down on the bottle to engage it.
8. Reconnect the fluid line to the top of the bottle.

D. Switching On

1. Press the Power on button on the instrument
2. Log in as 'Student'.
 - a. Password = 1234

E. Run a Complete System Clean

Run the Complete System Clean feature to clean the unit at the beginning and end of each day and between assays if a thorough cleaning is needed. You can also run this cleaning cycle to prime the fluid system or if you suspect there is air in the fluid lines. *Always ensure the cleaning solution bottle is filled with ICF whenever you run the Complete System Clean.* Use the Reset Fluid Levels option to reset the status indicator for the waste bottle and cleaning solution bottle back to 100% and 100% after you have emptied the waste bottle and filled the cleaning solution bottle. It is important to reset the fluid levels each time you fill and empty the bottles so that the instrument can accurately determine the amount of fluid in each bottle.

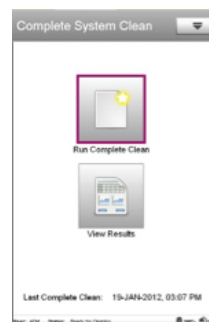
1. Select **Muse System Cleaning** under Essential Tools at the main menu. This feature is also available by selecting **Clean** from the System Check screen or any assay screen, then selecting **Complete System Clean** from the list of cleaning protocols.

2. Select **Complete System Clean**.



3. Select **Run Complete Clean**.

Displays the last time a complete system clean was performed. This means I can check if you performed the clean!



4. Load a full tube of Guava ICF on the unit and select **Run**.



5. When the ICF cycle is complete, the system prompts you for a tube of DI water. Load a full tube of DI water on the unit and select **Continue**.



When the system cleaning procedure is complete, the Cleaning Log appears. Each entry in the log represents one system cleaning procedure. The list shows the user who performed the cleaning, the date and time the cleaning was performed, whether the cleaning was completed or aborted, and the type of cleaning (Complete System Clean or Extreme Clean).

F. Running a System Check

Run a System Check at the start of each day that you use the instrument to ensure that it is performing properly. Three replicates of the System Check Bead sample are acquired. The results are averaged to determine if they are within the expected range.

Run a Complete System Clean at the start of each day and before performing the System Check procedure.

A cleaning cycle will prime the fluid system and remove bubbles that may have formed in the tubing.

1. Prepare a 1:20 dilution of System Check Beads. Refer to the Muse® System Check Kit User's Guide for information. (usually 20uL beads in 380ul diluent). This kit is kept in the Fraser refrigerator.

2 Select System Check under Essential Tools at the main menu to display the System Check screen.

3 A message appears prompting you to check the fluid levels in the cleaning and waste bottles. Check the fluids, then click Close.

Note: Always remember to reset the fluid levels when you fill the cleaning solution bottle and empty the waste bottle.

The first time you run the procedure, enter the bead lot number, expiration date, and check code.

- Enter the Bead Lot # and press Done on the keypad.
- Touch the calendar icon in the Exp. Date field to select the expiration date. Touch outside the calendar to close it.
- Touch the Check Code field and enter the code. All values are required and can be found on the information card that comes with the bead kit. Once you enter this information, it will remain in the software. Each time you run the procedure, check the information to ensure it is accurate. Update the values when a new lot number of System Check Beads is used, if necessary.

4. Mix the tube of prepared beads and load it on the system.

5. Select Run.

6. The system performs a prime, then acquires the first replicate. The progress bar and fly wheel provide indicators as to the status of acquisition. The progress bar is divided into three sections—one for each replicate. If the fly wheel is turning but the progress bar is not advancing, the fluid system may be clogged or the beads may have settled to the bottom of the tube. If the beads settled, select Abort, unload the tube and mix. Then reload and select Run System Check again.

7. Remove the tube and vortex it to resuspend the beads.

8. Load the tube and select Run. The system acquires the second replicate.

9. Repeat steps 7 and 8 to acquire the third replicate. Upon completion, the system displays a PASS/FAIL result and the Particles/mL value for each replicate, as well as the average.

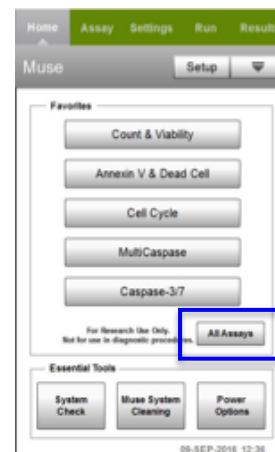
10. If any result for Particles/mL falls outside 10% of the expected value, the result is outside the acceptable range and appears in red. ***If the procedure fails, touch the Help button (?) to display troubleshooting information.***

G. Running Samples

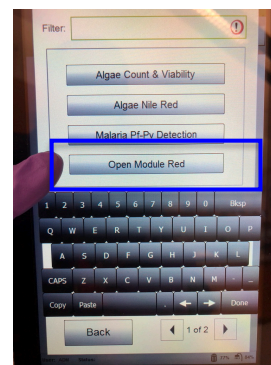
1. Select the assay you wish to run from the list of Favorites on the main menu.

If the assay does not appear under Favorites, select **All Assays**. The Assay Search screen appears. All assays are grouped into four categories: Cell Health, Cell Signaling, Immunology, and Other.

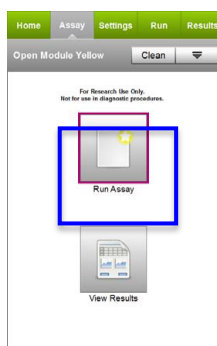
If you are running a Kit-based assay, follow the instructions of the kit, and on screen.



2. If you are running your own red or yellow-stained samples, select Open Module Red or Open Module Yellow

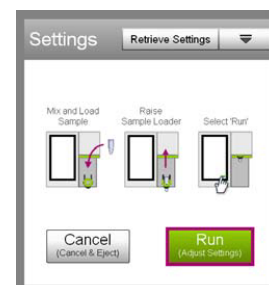


3. Select RUN ASSAY

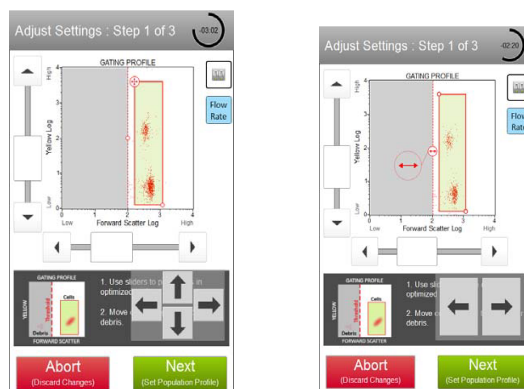


3. Adjust the instrument settings.

Load a negative or isotype control to adjust the instrument settings and select Run (Adjust Settings).



4. Fine tune the settings for the dot plot (Forward Scatter Log vs Yellow Log or Forward Scatter Log vs Red Log), if necessary.



Use the Flow Rate button () if you want to adjust the flow rate.

Check the desired box to set the flow rate to Very Low (0.12 $\mu\text{L/s}$), Low (0.24 $\mu\text{L/s}$), or Medium (0.59 $\mu\text{L/s}$). The default flow rate is Medium.

Follow instructions on the screen to set up controls, and run samples.

The software displays results immediately after each sample is acquired. The Yellow vs Red (or Red vs Yellow) dot plot results include sample information, percentage of the cells in each quadrant, and mean fluorescence intensity (MFI) of yellow fluorescence and red fluorescence for the cells in each quadrant. Results can be displayed without plots or with plots.

Results from each run are stored in a data file, as well as its corresponding spreadsheet (CSV) file. The spreadsheet file contains the following statistics:

- Sample Number
- Sample ID
- Total number of cells collected
- Total number of cells in the counting gate
- Dot plot: Count, concentration, percentage, mean and median intensity for yellow fluorescence and red fluorescence in each quadrant.
- Histogram: Count, concentration, percentage, mean and median intensity for yellow fluorescence of yellow-negative and -positive cells.

Retrieving & Exporting Data Files

You can open an existing file for review or analysis. You can open only your files; not files from other users, unless they are in the Public folder.

You can open an existing data set and append data to this file.

1. Select the assay from the main menu, then select **View Results** from the assay screen.

The Retrieve Data Set screen appears.



2. Select the file and then select **Retrieve**.

The data set opens and the last sample in the set is displayed in the Results screen. Use the sample list button to view all the samples in the data set. Or, use the scroll arrows to scroll through the list of samples.

3. Select “options”

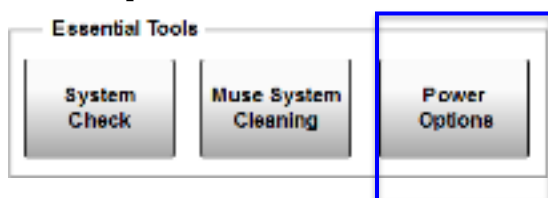
4. Export data to USB. (For analysis with FlowJo software, export as FCS format).

H. Shutdown Procedure

1. Run the **Complete System Clean** procedure at the end of the day before shutting down the unit (see section E). If you ran whole blood samples, run an Extreme Clean instead.
2. Leave the tube of DI water on the sample loader.

◆ **WARNING:** Do not leave a tube of Guava ICF, bleach, or any other cleaning agent loaded on the instrument overnight or for an extended period of time. Prolonged exposure to strong oxidizing agents will damage the flow cell. Always leave a fresh tube of DI water on the system when shutting it down. Change the tube of water regularly to ensure it is clean and free of particles.

3 Select **Power Options** from the main menu under Essential Tools.



4 Select **Power Off** to turn off the system.



I. Troubleshooting

Follow these procedures first.

Run a Complete System Clean

Run a System Check

Consult the printed official Luminex MUSE Cell Analyzer Manual (next to MUSE)

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

Last resort, call the Luminex technical support.

J. 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 on MUSE Cell Analyzer by Dr. Deborah Fraser (Available on CSULB CNSM Research Training webpage, and through CSULB BUILD)
3. Video training introductory course: http://www.bdbiosciences.com/us/support/s/itf_launch
4. online book: <http://www.coulterflow.com/bciflow/practical/book/index.html>
5. 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