What You Will Need

All those who use the FCCF are required to go through biosafety training specific to the facility. This training takes approximately 15-20 minutes and must be done before any types of samples are brought into the facility. This training takes place a few days before instrument training or sorting.

Users can find a list of consumables to use in the facility that are available in the stockroom at the following link: http://facs.bio.indiana.edu/protocols.html (scroll down on the website to get to the consumables portion).

Anyone using the facility must bring their sample(s) in a sealed plastic container, and wear appropriate Personal Protective Equipment (PPE) **after** entry into the BSL2 area of the facility. No open-toed shoes may be worn and the entire foot should be covered. Legs should be completely covered. Also, if a user wishes to wear a skirt or similar item of clothing, tights or a similar leg covering should be worn if the item of clothing does not cover the whole leg. No hats are allowed in the facility, and scarves and jacket hoods should be completely covered by the lab coat. This is for Biosafety purposes.

PPE includes the following items:

- Safety Glasses
- Lab Coats
- Gloves

The facility supplies cloth lab coats that are cleaned by autoclaving and washing. If you desire to bring your own lab coat, please bring a **disposable** one.

The facility supplies gloves (Xceed Nitrile – powder free), however, if the user has a different preference, they may bring their own gloves to the facility. Gloves may not be removed from the Flow Facility and are to be disposed of in the biohazard bags that are located in the facility. Safety glasses may be kept in a designated drawer in the facility OR may be cleaned with ethanol and removed from the facility.

Please bring a **flash drive** or other data storage device to transfer your data after analysis. This is very important as data files will be purged at regular intervals as not to slow down the analysis and sorting software.

Sample prep

For analysis-only samples

To collect data for analysis only (no sorting), researchers should bring their samples in a 5 ml tube (specifics listed at the afore-mentioned link). Samples should be filtered before being brought to the facility through a filter with no greater than a 40um pore size. Please know the approximate size of your cell before coming to the facility, as large cells, or cell aggregates may clog the cytometers. Samples should be brought in no less than 0.5ml and no greater than a 3ml volume at a concentration of 1x10^6 – 1x10^7 cells per ml. It is recommended that the cells be resuspended in 1x PBS, but other options may be possible. Cells should **NOT** be resuspended in any viscous medium or medium containing any more than 1% FBS or BSA as this can also clog the cytometer.

For single stained cells, an unstained control sample is needed. For cells stained with two or more dyes, an unstained and single stained (one for each color) controls are needed to set up compensation (spectral overlap). Other controls may also be needed depending on the experiment. For unfixed samples, it is recommended that a dye such as propidium iodide, DAPI, or 7AAD be added to your sample to distinguish between live and dead cells.

If you are working with antibodies, it is highly recommended that you **TITRATE** your antibodies. This allows detection of any background staining or separation problems caused by excess antibody in your sample, and can help determine which concentration of antibody works best for your cell type.

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For sorting samples

To sort samples, researchers have a few options on how to bring their samples. Samples can be brought in 1ml or 5ml round-bottom or 15ml conical tubes. Samples should be filtered before being brought to the facility through a filter with no greater than a 50um pore size. Please know the approximate size of your cell before coming to the facility, as large cells, or cell aggregates may clog the cytometers. Also, the concentration of your samples will affect how many events (cells) of the sample you will be able to recover. This is also dependent upon the percentage of cells of interest in the population, which also determines how long it will take to sort a certain number of cells for a population of interest. It is suggested that samples be brought *in no less than* **500ul** volume and at a concentration of 1x10^7 – 1x10^8 cells per ml. It is recommended that the cells be resuspended in 1x PBS, but other options may be possible (please ask). Cells should **NOT** be resuspended in any viscous medium or medium containing any more than 1% FBS or BSA.

When recovering the cells for sorting, cells may be *collected* by the following methods:

· Slides – frosted or non-frosted
· Multi-well plates (6 - 384-wells)
· 1ml tubes (we have been able to work with eppendorf tubes as well – please cut tops)
· 5ml tubes
· 15ml tubes

To help with cell viability, plates and tubes should contain the medium that is preferred by the cells/populations you are interested in collecting. This may be any type of medium, including those containing sucrose, FBS, BSA, etc. In order to prevent cells from sticking to the sides of the tubes, it is recommended to coat the entire inner surfaces of the tubes with the collection medium of your choice and/or serum (FBS, BSA, etc) for at least 30 minutes. Coated surfaces as well as an increase amount of serum in your collection media may help increase cell viability. For unfixed samples, it is recommended that a dye such as propidium iodide, DAPI, or 7AAD be added to your sample to distinguish between live and dead cells.

If possible for your particular cell population – we **highly recommend you add antibiotics** to your collection medium, especially if you are planning on culturing your cells after collection. While we are able to perform "aseptic" sorting, it is not possible to sort in a 100% sterile environment. Due to the variety of organisms sorted in the facility, we do not add antibiotics to the sheath fluid. However, we take precautions to keep the sorting environment as contaminant-free as possible by sterilizing instrumentation and tools, aerosol management system, wearing PPE, etc. Also, it is important to make sure that the samples come to the facility as sterile as possible.

For single stained cells, an unstained control sample is needed. For cells stained with two or more dyes, an unstained and single stained (one for each color) controls are needed to set up compensation (spectral overlap). Other controls may also be needed depending on the experiment.

If you are working with antibodies, it is highly recommended that you **TITRATE** your antibodies. This allows detection of any background staining or separation problems caused by excess antibody in your sample, and can help determine which concentration of antibody works best for your cell type

If you have any questions or concerns, please do not hesitate to contact the FCCF.

Christiane Hassel
IU Bloomington
Flow Cytometry Core Facility
Jordan Hall 029

http://facs.bio.indiana.edu/
812-855-7101
chassel@indiana.edu