Core staff pre-training checklist:

- Assign project to training session in PPMS
- Change User rights to Novice after the first training for both instruments.
- Change user rights to autonomous after second training for both instruments.
- Add lab login to SpectroFlo software (on both instruments) if none exists
- Request HSRB badge access if needed

Two Aurora's (same configuration) available: Winship C-5027 and HSRB E-362B

- Separate badge access request if using the Aurora in Winship (instructions on ECFCC website) https://sites.winship.emory.edu/facilities
- First user of the day and instrument is off: warm-up period of 20 minutes is required

Logging in/initializing software

- PPMS
- Launch SpectroFlo software
    - Login: XXX-Lab
    - Password: facs123 (
    - Passwords posted on front of instrument
    - Acquisition -> opens Acquisition experiment menu

Physical components of cytometer

- Power button
- Visibly check fluid levels on sheath and waste tank (software indicators are not reliable)
    - Replace sheath when low and/or waste tank is full, leave empty for staff to refill
    - Spare sheath tanks
    - Sheath tank – detach line by releasing at the quick disconnect first and then unscrew lid and place on new sheath tank
    - Waste tank – unscrew, place in holder, pour waste down sink, and add bleach to empty tank

Basics of spectral flow cytometry

- Rather than measuring fluorescence in a single bandpass filter, an entire spectrum of fluorescence is collected for every dye used across a detector array
- 4 laser system (no UV)
- Number of detectors: 48
- Detector arrays off each laser (naming convention – excitation laser, detector #) e.g. YG4

Setting up an Experiment

- Select “New experiment”
- Name Experiment
    - Experiment name character limit: 50 characters
    - Include date
- Assigning fluorochromes to experiment
    - Choose based on laser excitation or type name of fluorochrome to filter
    - Notify staff to add any fluorochromes not currently in software
- Groups: Creating reference controls for unmixing
    - Unmixing ≠ compensation but achieves the same thing
    - Autofluorescence extraction
        - Set up an unstained cells reference control for autofluorescence extraction
        - Needs to be the same type of cells in your sample and treated the same way as your sample
        - Group-specific unstained controls
    - Reference controls are single color spectral controls (same as comp controls)
        - May be cells (recommended) or beads
        - If necessary, add additional unstained controls and assign to each reference control
    - Labels
- Groups: Experimental samples
    - Add as many tubes as you have samples
    - Create cleaning group w/two tubes: rename tubes to bleach and water
- Labels - can be set at experiment, group or tube level
- Keywords - typically not used – for tracking in clinical labs (core staff can add if requested)
- Acquisition
    - Worksheets (raw or unmixed can be assigned to each group)
    - Acquisition stopping criteria based on:
        - # of events based on stopping gate
        - Storage gate
        - Stopping time
        - Stopping volume
    - Number of events to collect for reference controls
        - depends on sample type and marker
    - 2,000 events minimum in negative and positive populations
    - For experimental samples, collect enough events of your rarest population to ensure statistical significance
    - Cleaning tubes
        - Set stopping gate to 1,000,000
        - Set stopping time to 180 seconds (3 minutes)

Acquisition

- Sheath, Waste, Cytometer and Loader status in software
    - Check cytometer is connected (green)
    - If using Plate Loader, power should be on (green)
- Raw worksheet orientation
    - Full spectrum histogram corresponds to individual, color-coded histograms showing individual detectors
    - Gated vs ungated
- Editing experiment
- Flow rates: low/med/high
    - uL/min should not be less than 5
    - Adjusting SIT height/SIT calibration
- Manual SIT flush
- Clean flow cell - stubborn clogs
- Events to display
- Instrument settings
    - Start w/Cytek Assay settings
    - SSC detected from two different lasers
    - Fluorescent detector gains
        - Gains set based on QC every morning
        - Gains are optimized for best resolution (signal to noise ratio)
        - Anything off scale -> titrate Abs and use less dyes
        - Lower fluorescent gains as a last resort
            - Start on excitation laser for fluorochrome being acquired
            - Decrease all detector gains the same amount
    - Reference controls
        - Always run in Raw worksheet
        - Preview all reference controls first before recording
            - Adjustments to FSC and SSC (more sensitive) and B-SSC will probably be necessary between beads and cells
            - Make any adjustments to fluorescent detector gains
            - SIT extends down into sample during acquisition and retracts otherwise – give it time to retract before removing tube
            - Reference spectral properties on the printout or using Cytek's spectra viewer to confirm the fluorochrome in each reference control

Live unmixing

- Check box for autofluorescence extraction
- Adjust gates for unmixing – adjust in 3 places
    - Hold "ctrl" down to adjust scatter gate

    - Check interval gates includes negatives and positives
    - Check correct peak channel is selected (brightest peak on Y axis)
- Select “Live Unmixing” (not Unmix, Save and Open)

Unmixed Worksheets

- Renaming, Save, Save as
- Editing experiment – assigning unmixed worksheet to groups/tubes
- Drawing plots
    - Drawing regions and applying gates
    - Setting acquisition limits based on new gates
    - Creating stats/population hierarchy
    - Editing plot properties – axis scaling
    - Changing parameters on plots
        - AF parameter

Cleaning

- Run on high
- Record tubes
- Water tube stays on SIT
- Check instrument schedule on PPMS
    - Last user of the day performs Fluidics Shutdown
    - 3 mL fluid in each tube
    - Power button on side of instrument

Data

- Two types of FCS files are exported automatically as files are recorded
    - Raw
    - Unmixed
- Shortcut to data folder on Desktop (E drive)
- Edit experiment name in SpectroFlo, not in Windows file explorer
- Use One Drive to transfer data - no flash drives (bookmarked in Chrome)
- Data and experiments are safe for one month

Saving and Templates

- 3 places to save – [add character limits]
    - Experiment – Save as (template) and Save
        - 50 Character Limit for Experiment Name
    - Worksheets
        - 30 Character Limit for user settings Name
    - User Settings – Save as (still be updated based on daily QC)
        - 30 Character Limit for user settings Name

Booking/PPMS

- Emory ID and pw login
- Select instrument, time, then “book”
- Can cancel up to 24 hours in advance of booking for free
- Can move time around on same day
- Requesting assistance – need 48-72 hours advance notice
- Incident reporting
    - Use low or medium severity
    - Zoom/remote assistance
    - Troubleshooting guide

ECFCC website

- Aurora resources
- Cytek webpage (including spectral viewer)