Core staff pre-training checklist:

- Assign project to training session in PPMS
- Change user rights to autonomous for both instruments
- Add lab login to Diva software (on both instruments) if none exists
    - Set to automatically export FCS files after recording in User Preferences -> Export
- Request HSRB badge access if needed

Two instruments (same configuration) available: Winship C-5027 and HSRB E-362

- Separate badge access request if using the A3 in Winship (instructions on ECFCC website)
    - https://sites.winship.emory.edu/facilities
- First user of the day/instrument off: power on UV, warm-up period of 20 minutes is required
- Only BD falcon tubes may be used (VWR #352054)

Startup/Fluidics

- PPMS login
- Windows (BDAdmin) login
- Startup if first user/instrument off:
    - Green button on right side of instrument
    - UV laser (355nM) must be turned on and set to 60mW
        - 20min warmup period
        - Minimize Coherent connection
- Check fluid levels (alarm will sound if sheath tank is empty or waste is full)
    - Sheath tank and plenum
    - Waste tank
    - Don't silence alarm
    - Hit restart then hold prime button down until the light goes off
    - Don't turn off FACSFlow supply system
- Bleed out air in the bubble filter
- Instrument control
    - Sample injection port (SIP)
        - Must use Falcon brand polystyrene tubes
        - Check for cracks
    - Acquisition control panel
        - Standby and Run
            - Run means the sample is running regardless of what is going on in the software
        - Flow rate – don't use high button
            - Fine adjustment – keep between 250-350
        - Prime – avoid using unless you get a clog
            - Run di water after for 5 minutes if prime is used
        - Don't touch the mode button - OK if flashing

Instrument Setup

- Open FACSDiva software
    - Lab login
    - No password
    - CST settings
        - Adjusts voltages and laser delays based on the daily morning QC
    - Make a folder with your name
- Open experiment from template or create new
    - Always rename experiments to include a date
- Instrument settings
    - Detectors and voltages
        - FSC and SSC
        - Doublet discrimination
        - Channel names based on excitation laser and bandpass emission filter
            - Delete all fluorescent parameters and then add each one
            - Configuration on printout and ECFCC website
        - Voltrations
        - Stain index

Compensation controls

- Experiment menu -> create comp controls
    - Comp controls specimen is created automatically
    - Universal negative option
        - Provides a worksheet that displays all parameters at once
        - Run single stains first to make sure all are on scale and in correct detector
        - Bring beads or cells on scale in scatter plot
        - Apply gate to all compensation controls (if using all beads or all cells)
    - May need to titrate Abs for the Symphony due to its high sensitivity
- Record compensation tubes
    - Adjust scatter gate and positive gates – software should find the positive population but confirm gating and manually move gate to the peak fluorescence if necessary
    - Keep universal negative or modify compensation to remove
- Calculate compensation
    - Experiment menu -> calculate compensation
    - Apply only

Experiment setup

- Adding specimens to experiment (syringe icon)
    - Provides way of organizing tubes
    - File naming

    - Adding tubes
- Global vs Normal worksheets
- Add dot plots
    - Change parameters in plots to reflect fluorochromes being used
- Experiment layout
    - Create labels
    - Set acquisition stopping parameters
        - Acquire enough events to be statically significant
        - Can also set stopping parameters based on gates drawn (i.e. 100,000 singlets)

Acquisition

- Voltages
    - FSC and SSC voltages can be adjusted, especially between running beads and cells
    - Fluorescence voltages cannot be adjusted after compensation
- Acquire/Record
    - Restart button
    - Refresh rate of events on worksheet
    - Append vs overwrite and associated files
    - Gating, changing gate color
    - Show population hierarchy
    - Probe backflush between samples

Cleaning/shutdown

- Creating cleaning specimen from template
    - Run on high and record tubes
    - Do not overfill cleaning tubes with fluid
    - Okay if there are events in cleaning solution (doesn't mean it's dirty)
    - Instrument in standby and water on probe when finished cleaning
    - Shutdown if last user of the day

Exporting data

- Data automatically exported to PC
    - Desktop -> FCS folder
    - Transfer to OneDrive while cleaning
    - No flashdrives
    - Data stays on PC for one month
    - Experiments stay on PC for one month – create templates

Troubleshooting

- Fluid level isn't moving in tube
- Cytometer isn't connected
    - Power cycle instrument and computer
    - Windows password
    - Turn UV laser back on

Resources and PPMS

- Symphony resources on ECFCC website
- Booking through PPMS
    - 24 hour cancellation policy
    - Rescheduling/deleting time
- Incident reporting
- Requesting assistance