

Core staff pre-training checklist:

- Assign project to training session in PPMS
 - Change user rights to autonomous
 - Request HSRB badge access if needed
 - Share ISX training folder on OneDrive with user
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1. Turn on ISX, large computer, and small computer
2. Check fluids before turning on instrument:
 - a. Check SpeedBeads – used to keep the autofocus and camera synchronized during the sample run.
 - b. Check fluid levels; containers are in order in cabinet under instrument
 - c. If necessary add bleach to waste tank and empty
3. Login info (Windows if needed and PPMS)
4. Launch INSPIRE software
5. Load default template
6. Start up with ASSIST (~30 mins), can log out of PPMS during warm-up
 - a. Check 'Run ASSIST after initialization'
 - b. Colors indicate pass or failure – check the date to see if assist has completed
 - c. Tests that fail – try re-running test 2 or 3 times, as it may eventually pass
 - d. Re-run all calibrations and tests if a test or calibration initially failed and then passed
 - e. After completing ASSIST, close the Calibrations Window
7. Inspire User Interface (3 areas):
 - a. Image gallery
 - b. Analysis/Work area for graphs – where features are displayed
 - c. Instrument control panel
8. Status info along bottom of window
9. Image Gallery (detailed)
 - a. Selecting populations to view
 - b. Pausing display and scrolling

10. Analysis Area (detailed)

- a. Setting up graphs and plots
- b. Drawing regions and adjusting properties

11. Instrument Control Panel

- a. Sample
 - i. Volume to be loaded is between 20 – 200 uL
 - ii. Volume and time status
 - iii. Return option
- b. File Acquisition
 - i. Setting file name (auto numbers samples, number appended to file name)
 - ii. Files collected with BF off will be appended with noBF in file name, EDF
 - iii. Setting file path – Documents folder
 - iv. Set number of events to collect
 - v. Acquire means record
- c. Illumination
 - i. Turn lasers and brightfield illumination on and off – BF can be moved but will always be paired
 - ii. Set laser powers
 - iii. Select scatter channel depending on fluorochromes used: 6 or 12
- d. Select magnification (40X default)
 - i. EDF (spot counting/punctate imagery)
- e. Fluidics
 - i. SpeedBeads running (can be paused)
 - ii. Set speed – inversely related to sensitivity

12. Menu Bar

- a. Select 'Generate FCS file'
- b. Templates

13. Acquisition order (Experimental sample -> Comp controls)

- a. Load default template
- b. Experimental sample first with brightest stains to set the sensitivity
 - i. Sample can be from 20-200 uL
 - ii. Turn on BF and SSC (~ 5 mW) and appropriate lasers
 - iii. Select channels to be collected by clicking on a channel name in Image gallery (BF channel 1 and 9, SSC channel 6 by default)
 - iv. Look at Scatterplot of Area (X axis) versus Aspect Ratio (Y axis) of Brightfield (channel 1 and 9) for doublets
 - v. Draw region around SpeedBeads, cells and doublets
 - vi. Check for saturation and set laser powers

1. Set laser powers so that each fluorochrome has Raw Max Pixel Intensities between 100 – 4000 counts
2. Can also look at intensity in plots (background subtracted)
3. At least a full log shift in fluorescence should be achieved
4. Data quality is enhanced when the brightness levels of all probes excited off a single laser are balanced to within a log of each other
- vii. Use Gradient RMS to look at events that are focused
- viii. Do not make changes after establishing settings on experimental sample
- ix. Record min of 1000 events for experimental samples
 1. Sample should be in PBS with 1-2% protein
 2. Filter
 3. 2-3K events ideally
 4. Sample concentration: 20 million per mL (1 million in 50 uL)
- c. Run Compensation controls with Wizard or manually
 - i. Turn BF and SSC off
 - ii. Open up all channels so that they are all collected
 - iii. Collect 500 – 1000 cells for each comp control

14. Shut down instrument

- a. Fill Rinse, cleanser, Sterilizer and Debubbler bottles if necessary
- b. Empty waste bottle
- c. Remove any tubes from uptake ports
- d. Click Shutdown (~40 mins)
- e. Transfer data
- f. Logout of PPMS and walk away (instrument will power down itself)

15. Troubleshooting

- a. If software freezes, force quit INSPIRE software