

1. Turn on the cytometer and the computers (Linux and Windows)
2. Login with your Agendo Credentials:
Username – **Your email** Password - **Your Agendo's password**
3. Launch **ISX** (INSPIRE) software
4. Check the beads and buffer containers level and ensure the waste tank is empty
5. Select **Startup** and check the **ASSIST** calibration option (*it takes around 45 min*)
*If ASSIST was already performed that day by another user or by UCF but is showing a yellow or red status, select **Calibrate with ASSIST** from the menu and re-run the failed test(s)*
6. Start running your samples
 - a. If you already have a template
 - i. Select **File** and load your experiment template (.ist file)
 - ii. Under **File Acquisition**, write the sample name and choose the directory of the file (**USERS DATA** folder shortcut available in the Desktop)
 - iii. Specify the number of events to count and the population to collect
 - iv. Press **Load** and load an aliquot of your sample
 - v. Press **Acquire** to record
 - vi. When finished, press **Return** to recover your sample
 - b. If you do not have a template
 - i. Under **Illumination** option, turn on the appropriate lasers that will be used for every fluorochrome
 - ii. Under **Fluidics**, set the flow rate to **Low Speed, High Sensitivity**
 - iii. Press **Load** and load an aliquot of your sample
 - iv. Adjust image brightness by performing two steps:
 - 1) Select **Display Settings** and click on **Full Scale** for each channel to visualize the full dynamic range of fluorescence intensity detected by the camera. If the channel is saturated, the peak will be out of scale even in **Full Scale**
 - 2) Adjust the laser power to maximize the brightness of each channel, avoiding saturation
 - v. Under **Magnification & EDF**, choose the appropriate Magnification for your experiment

- vi. Create **Scatter Plots** and or **Histograms** and draw **Regions** to identify the cell populations of interest and define a gating strategy (we recommend starting with a Area_M01 vs Aspect Ratio_M01 scatter plot to select the desired population and exclude beads)
- vii. Compensate data using the **Compensation Wizard**
 - 1) Load the single colors
 - 2) For each single color, confirm if the correct channel is selected
 - 3) Set the file name and press acquire to save the data
 - 4) After all single colors are recorded, press exit to save matrix
- viii. Under **File Acquisition**, write the sample name and choose the directory of the file (**USERS DATA** folder shortcut available in the Desktop). Define the number of events to record and the population to collect
- ix. Chose file format (**.rif** – analyse on IDEAS, **.fsc** – analyse on FlowJo)
- x. Press **Acquire** to record
- xi. When finished, press **Return** to recover your sample
- 7. Go to **File** and select **Save Template** to save an experiment template in the **USERS SETTINGS** folder shortcut available in the Desktop
- 8. Copy your data to the server
- 9. When you are finished
 - a. If you are the last user of the day, shut down the system by selecting **Shutdown**. The system will sterilize in 40 min and automatically shut down the cytometer and computers
 - b. If you are not the last user of the day, close the INSPIRE software and log off from your account