1. Turn on the cytometer, the server and the computer
2. Login with your Agendo Credentials:
   Username – **Your email** Password - **Your email's password**
3. Launch **ISX** software
4. Check the buffer containers level and ensure the waste tank is empty
5. Select **startup with the ASSIST** calibration check *(it takes around 45 min)*
6. Select the **File menu** and load a default template or an experiment template
7. Press **Load** and load an aliquot of a stained sample
8. Under **Illumination** option, turn on the appropriate lasers for each fluorochrome
9. Adjust the laser power to maximize brightness and prevent saturation (blue dots on the cells images)
10. Create **dot plots** and regions to identify the cell populations
11. Set the acquisition parameters
12. Chose file format (**.rif** – analyse on IDEAS, **.fsc** – analyse on FlowJo)
13. Compensate data
    a. Select the **Compensation Wizard**
        i. Load the single stains
        ii. For each single confirm if the correct channel is selected
        iii. Set the file name and press acquire to save the data
        iv. After all singles recorded press exit to save matrix
    b. **Manually** compensate data
        i. **Turn off** Brightfield and SSC, **turn on** all channels
        ii. Collect **1000 events** for each single color
        iii. (The final compensation will be set on IDEAS Software)
14. Turn on the Brightfield and SSC and confirm that the lasers you need are on
15. Record all your experiment samples
16. Go to **File menu** and select **Save Template** to save an experiment template
17. Shut down the system by press the **Shutdown button**. The system will sterilize in 40 min and automatically the ImageStream, computer and server will shut down