1) Check the level of the sheath tank (1), water (2) and waste (3) before you start.

2) Turn on the compressor (4) (button in the back) and the machine (5)

3) Login in the computer, start the TIMER (self service), and open the Sony spectral program (SP6800 Spectral).
4) When you open the program will pop out a message for Prime the machine >> select START. If the message doesn't appear automatic, go to CYTOMETER tab and PRIMING.

5) After to Prime, go to the tab CYTOMETER and run MANUAL CLEANING with contrad 5% first, then bleach 30% and water.
Repeat to clean with bleach and water
6) Go to the tab PREPARATION >> select PREPARATION tab on the left side >> Then select EXPERIMENT TEMPLATE >> BLANK TEMPLATE >> CREATE EXPERIMENT
7) It will open a new screen, on the tab **ACQUISITION** select **SAMPLE GROUP**. It will generate the tube 1.
8) Organize your work space: right click and remove lines of grid, add 2 new spectrum graphics and put the axis as wavelength
Change the FSC-A for the color that you are working
9) Select the tube 1, rename it as UNSTAINED and insert the tube in the holder on the machine. Click in PREVIEW to find your cells/beads adjusting the SSC-A and FSC-A:

![Image of flow cytometry software interface showing the tube selection and gate adjustment process.]

10) Put the gate in your cells/beads while you are seeing in Preview, then show the gate A on all the graphics.

![Image of flow cytometry software interface showing the gate application on the various graphics.]
11) Once that the gate is set for your cells/beads click on **ACQUIRE** to record.

![Image of flow cytometry software interface](image1)

12) After record the first tube (unstained), the next tube will appear automatic: rename (first color, ex. PE), adjust the voltage (arrow) when you are in **PREVIEW**, and then, **ACQUIRE**.

**Adjust the PMT voltage on a full stained sample.**

![Image of flow cytometry software interface with PMT adjustment](image2)
13) After record all the fluorochromes/antibodies, add them to the COLOR PALETTE one by one.
14) After add all the fluorochromes, select the **unstained Tube** (arrow 1). Make a square gate in any of the channel (arrow 2), then ask to show this gate (unstained) on the histogram.

Select the linear gate on the histogram for the negative cells (arrow 3). On **UNMIXING**, double click and assign Negative Spectrum for the unstained and for each fluorochrome (arrow 4).
15) Select the next tube with fluorochrome and make a square gate in the highest pick on channel or wavelength graphic corresponding to the channel/wavelength used for the specific fluorochrome. Then, show this gate (i.e. PE-Cy7) on the histogram. Select the linear gate on the histogram for the positive cells. On UNMIXING, double click in the fluorochrome and assign the positive gate for the specific color (i.e. B).

16) Repeat the steps on the item 15 for each individual color (i.e. PacBlue)
17) After assign the Positive Spectrum for each fluorochrome go to >>> CALCULATE >>> APPLY
18) Save the Normalized Reference Spectra to the Spectrum Library

19) To proceed the Analysis you need to close your experiment ???

20) To shutdown the machine, go to CYTOMETER tab and select HARDWARE SHUTDOWN >> DAILY CLEAN and proceed the cleaning with bleach and water.
21) gkdngngk