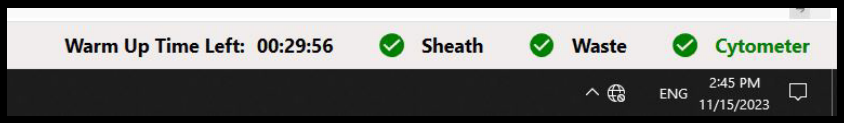
Northern Lights user manual

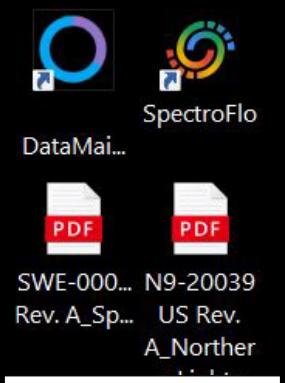
Basic user guide

Click titles to return to this page

Start-up

NL has a 30 min warm up time. Timer starts after opening a template





Spectroflo icon and PDF user manual

1) Power on the cytometer. Power button on the left-hand side of the cytometer.

   Windows password: Welcome#1

2) Open the SpectroFlo software
3) Log in

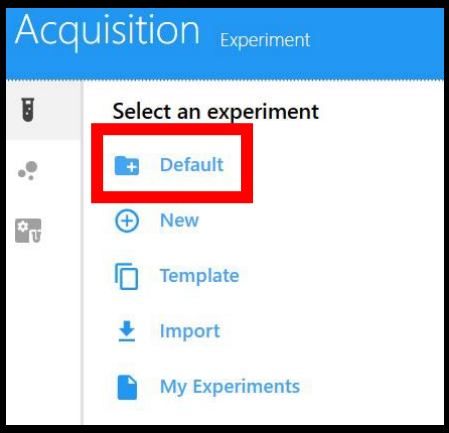
   Start typing your name in the username field; mouse-click to select user account
   Password: first letter of first name; entire last name; all lowercase

   QC/startup account U: QC_startup | P: CytekNL

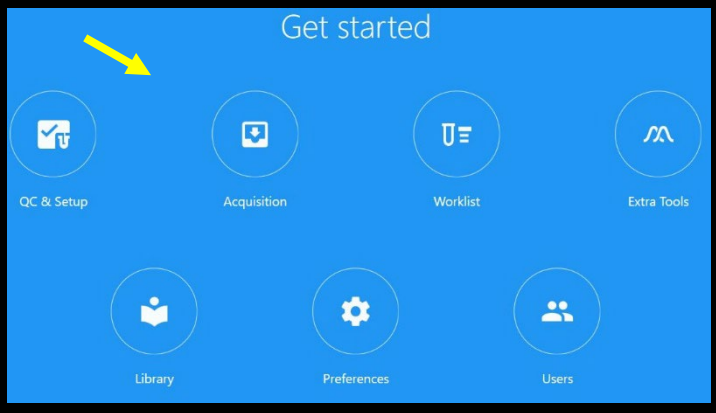
Run water during warm up

1) Click the [acquisition] icon on the welcome screen
2) Select the [Default] experiment layout
3) Remove the water tube from the sample probe
4) Load a tube with 2.5mL milliQ water
5) Set the flow rate [high]
6) Click on [start ▶] and run water as the cytometer warms up
7) (approx. 25 minutes).

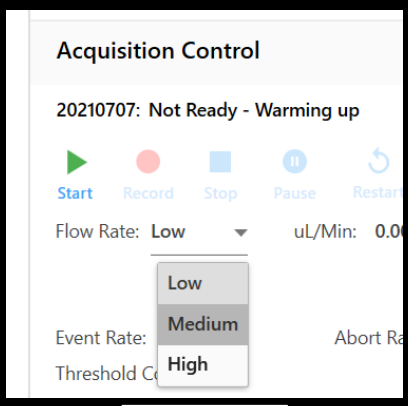
⚠ when changing sample tubes, wait until the SIT flush is complete.



New experiment

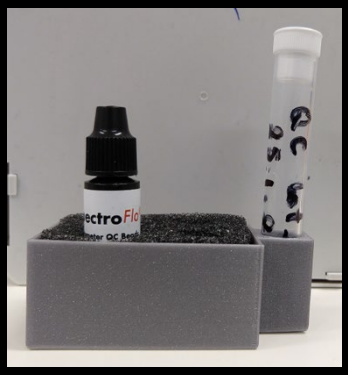


Welcome screen



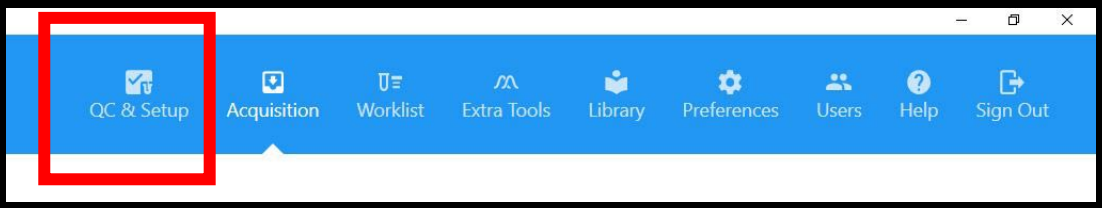
Flow rate

Daily QC

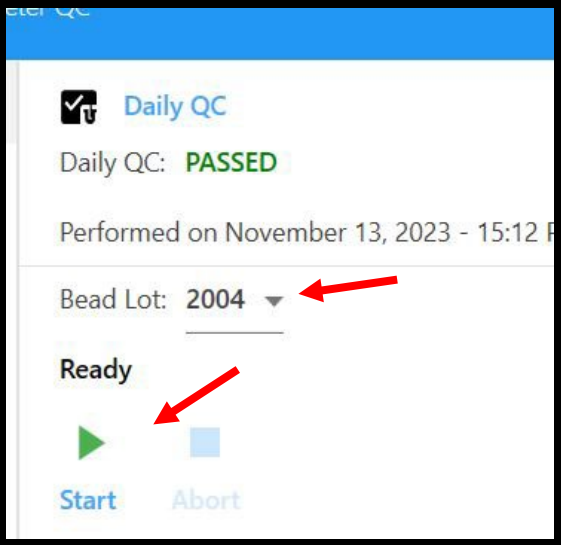


1) SpectroFlo QC Beads are in the fridge in 9-52 (chem prep)
    a. Check if there is already a 5 mL tube made up (need >80 µL for a QC run)
    b. *If need to prepare a new bead tube*, Vortex bead bottle and add one drop to 300 µL milliQ water in a 5 mL tube. Vortex tube.

2) Click on the QC tab in the blue ribbon, top right

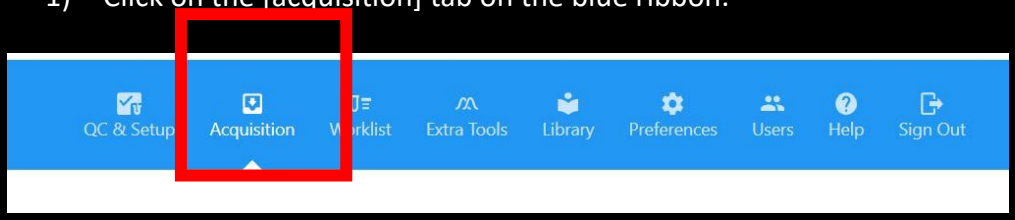


3) Load 5 mL bead/milliQ tube on the cytometer
4) Check bead lot
    a. Bead lot on the side of bead bottle
5) Click start
6) QC will take 5 minutes
    a. After QC finishes, return bead bottle and bead/milliQ tube (if >100 µL) to fridge



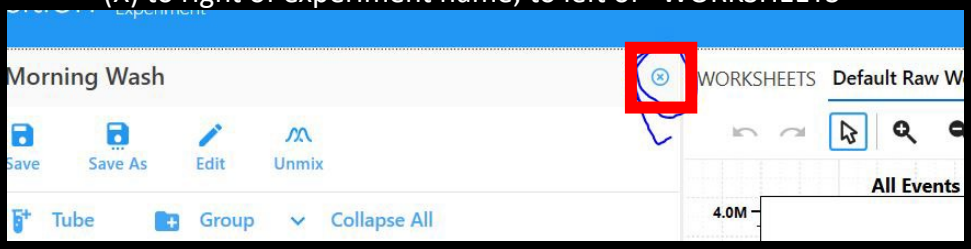
New experiment

1) Click on the [acquisition] tab on the blue ribbon.



2) Close the wash experiment.

(X) to right of experiment name; to left of "WORKSHEETS"

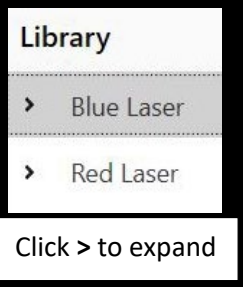


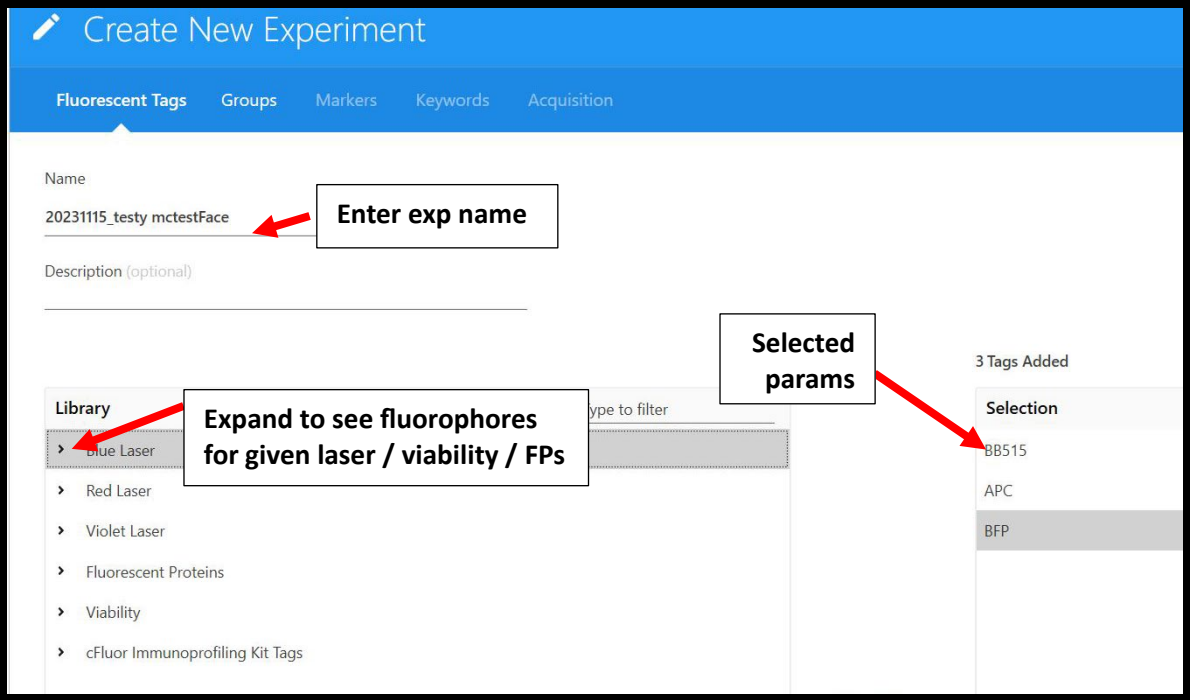
3) Select [New] experiment layout 

4) Enter exp name (YYYYMMDD_exp name)

5) Choose parameters

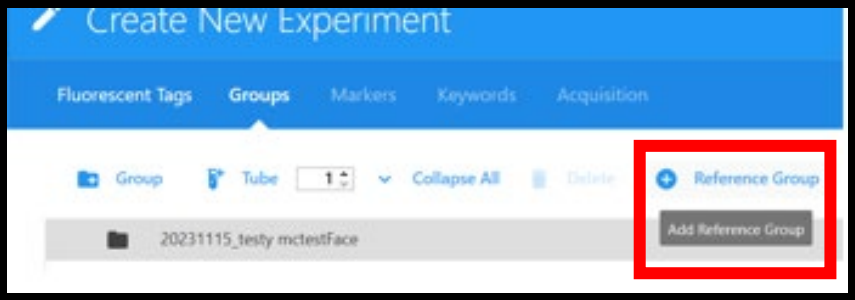
Expand library subgroup and double-click on fluorophore needed
If not there, use the closest fluor





6) Click [next] (bottom right)

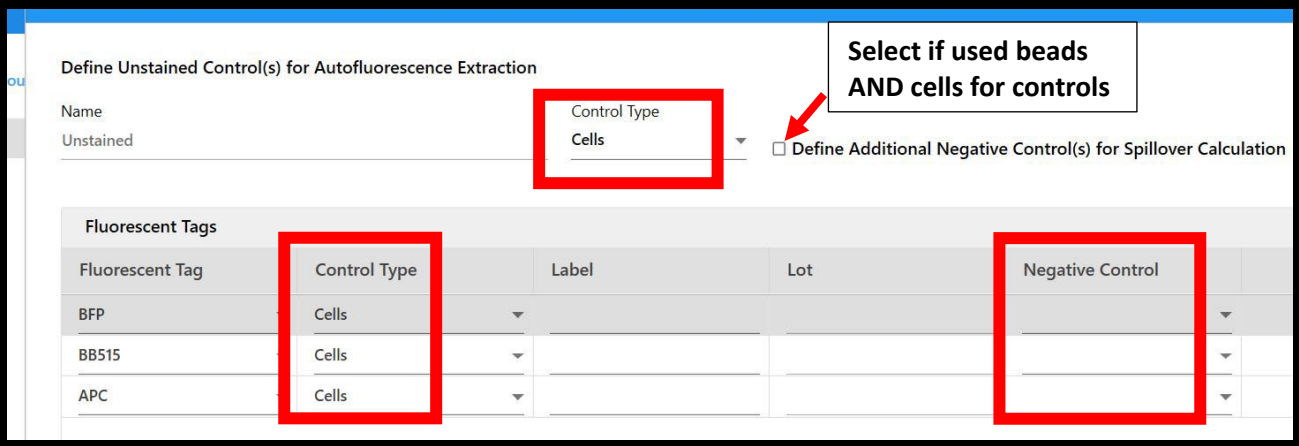
7) Add a reference group (single stain controls)



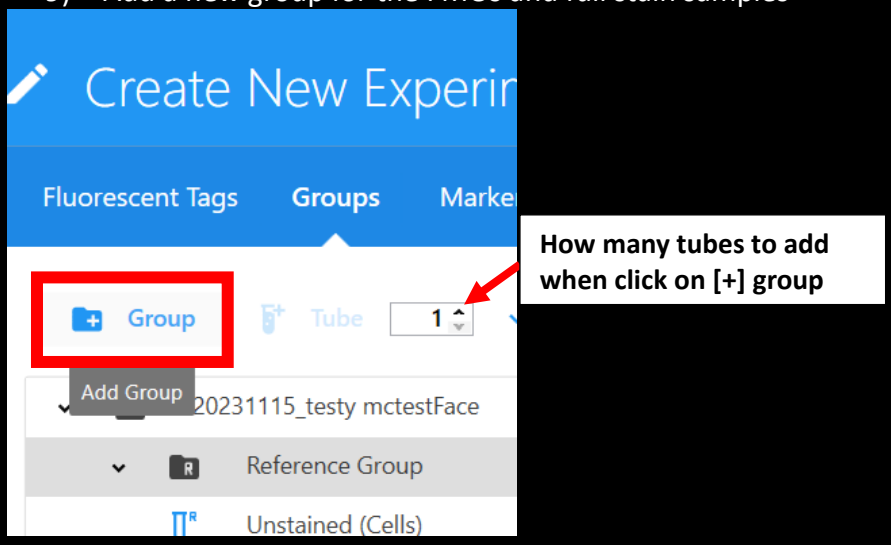
8) Setup single stain controls

Set cells or beads.

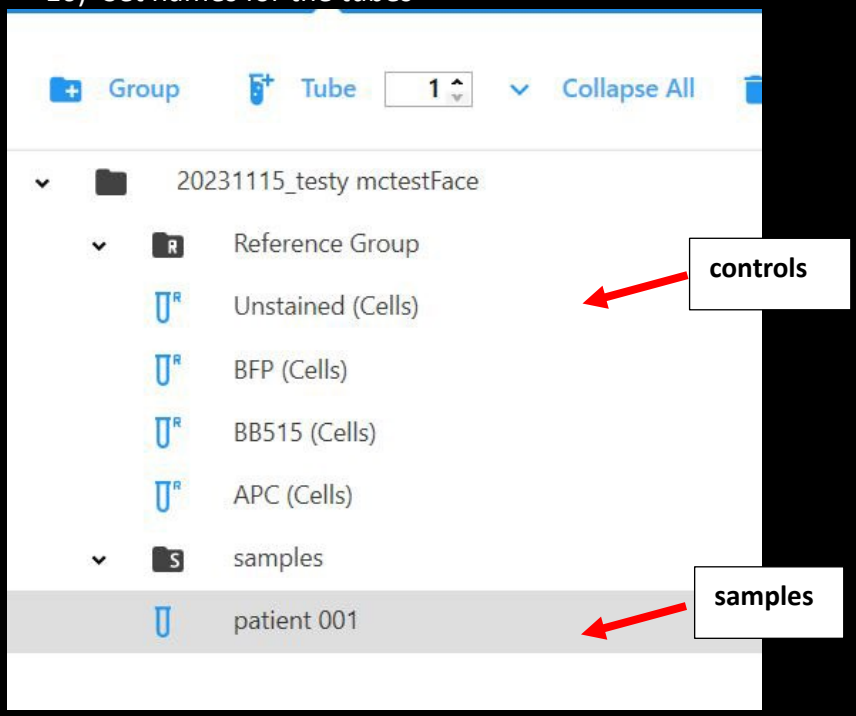
Set [negative control] for fluors if these controls do not have an “internal negative population (ie, CD45 or cells that are 100% GFP+).



9) Add a new group for the FMOs and full stain samples



10) Set names for the tubes



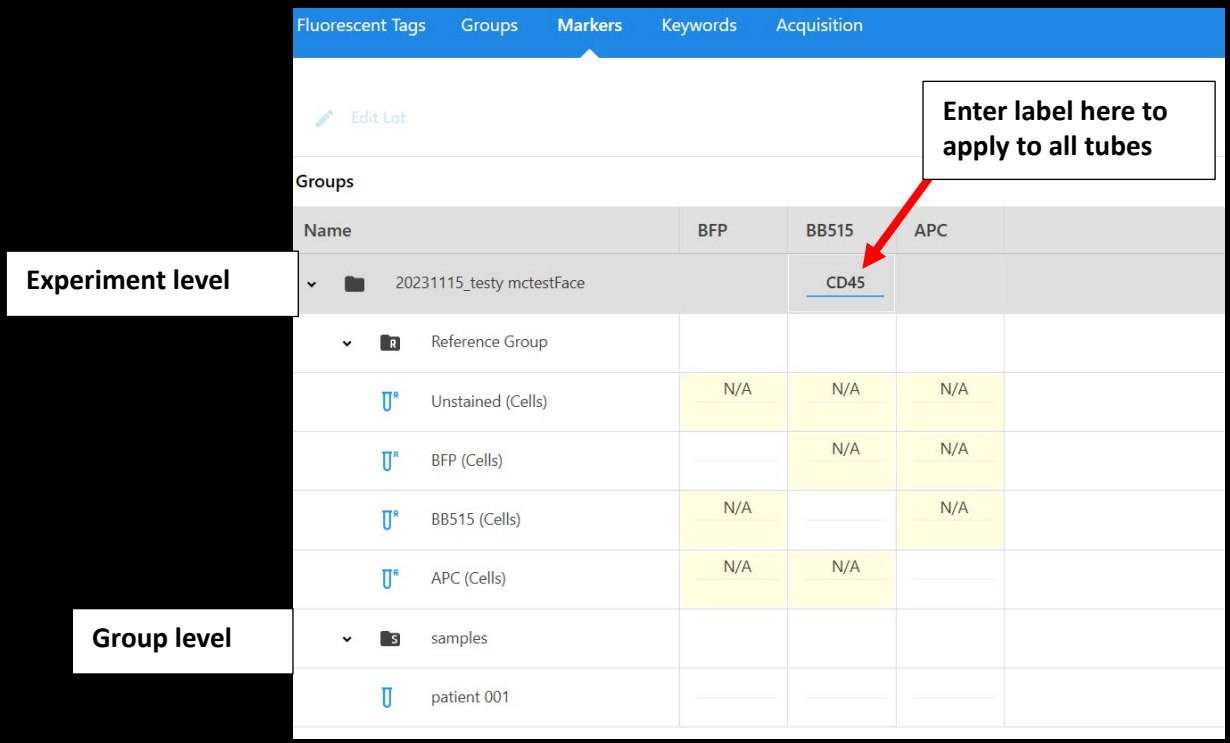
11) Click [next]

12) Set marker names

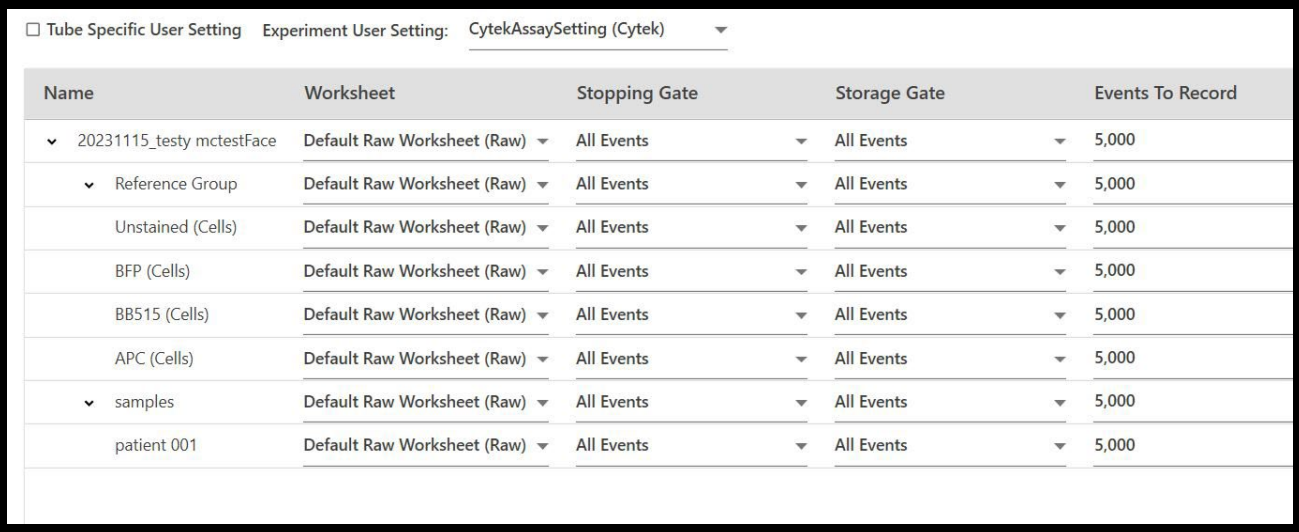
Click on the box at the experiment level (see below) to set the name for all tubes (in the image below, did not press [enter] yet, so it hasn't applied label to all.

Can also set labels at the group level

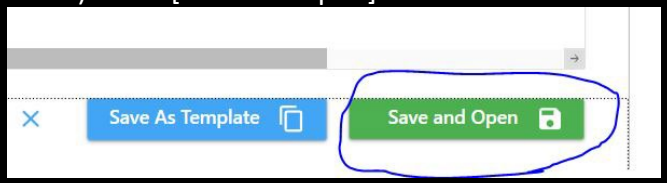
Will not appy to reference controls



13) Click next
14) Keywords (can leave blank)
15) Click [next]
16) Set stopping event number, volume, or time.
    Again, can set at a global level, group level, or individual tubes



17) Click [save and open]



Sample acquisition

Final protocol checks

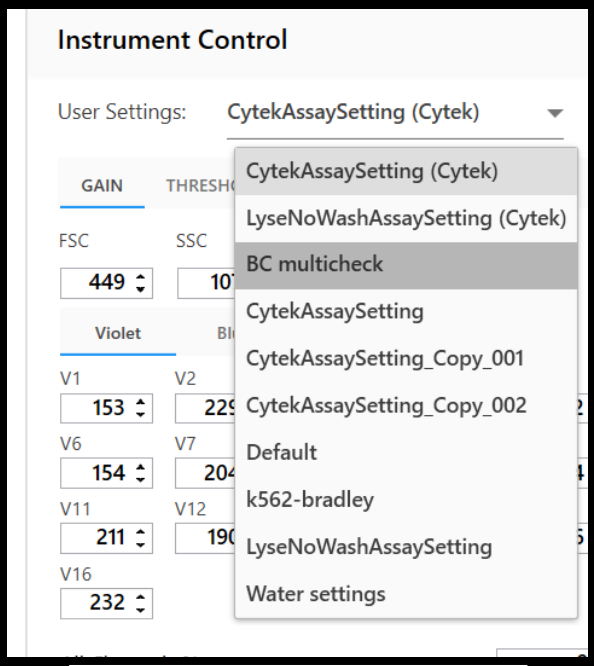
- Flow rate (can not change mid run; requires [pause]
- User settings (loads fluorescence and scatter gains and threshold)
- ⚠ DO NOT change individual channel gains. If signal too high or low, change via the "all channel %" setting.

Ballpark user-settings (derived from cytek assay settings)

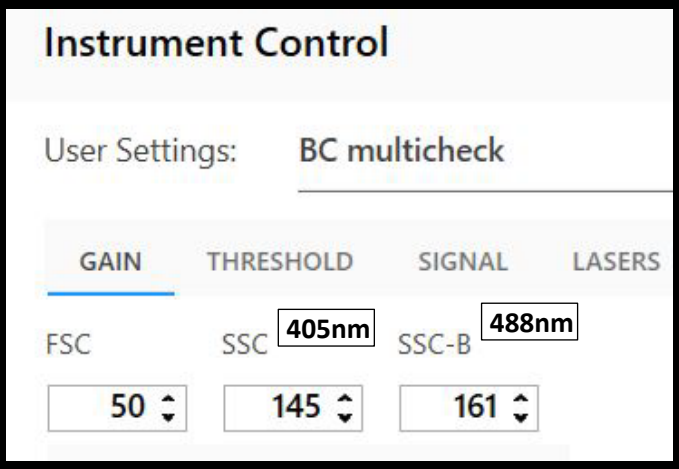
	leuk (human)	Cell lines
FSC	50	20
SSC (violet / blue)	145 / 160	60 / 60
Threshold	FSC, 350,000	FSC, 350,000
V1	45	20
B1	1090	50
R1	80	20

Use the all channel % option to set V1, B1 and R1 to the numbers above. This will also set the other channels V2-V16, B2-B14, R2-R8 to the appropriate gain (relative to % change).

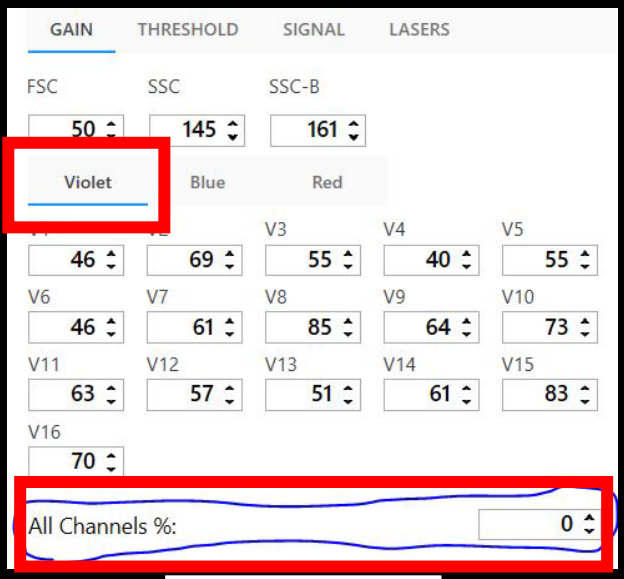
Changing 'all channel %', increases or decreases all the gains of that laser line by the number (percentage) entered. The % value is relative to the loaded user settings. So, If you save these settings, the % value will be set to 0, with any changes from then being applied to the newly saved gains.



Choosing predefined user settings



Setting FSC and SSC



Change all gains for the selected laser

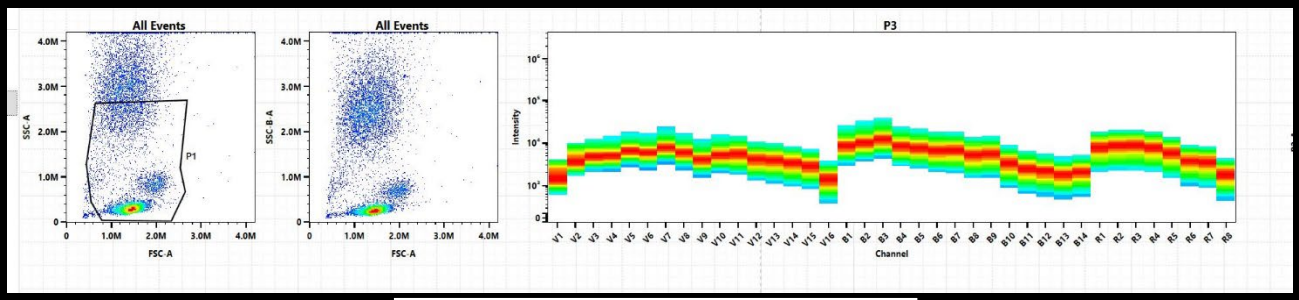
Threshold settings

1) Load unstained sample, this will be the first tube in the group.

If you want, you can load a full stain tube to check if the fluorescence signal is on scale. Then switch back to the unstained to record data for that tube.

2) Press [Start ▶]

Make sure that scatter and snake looks ok (ballpark: set lowest channel (of each laser near 0)



Scatter and snake for unstained PBMCs

3) Press [Record] to save data for that tube

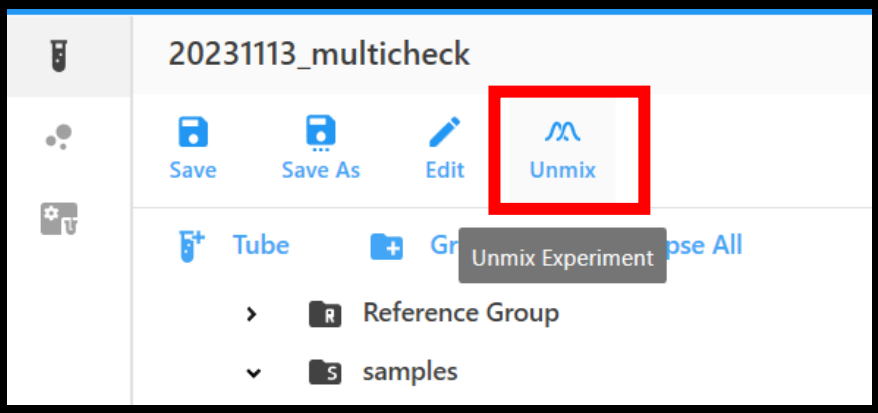
Once finished, the selected tube will go down the line of the group.
You can manually change tubes by left-clicking on them.

⚠ when changing sample tubes, wait until the SIT flush is complete.

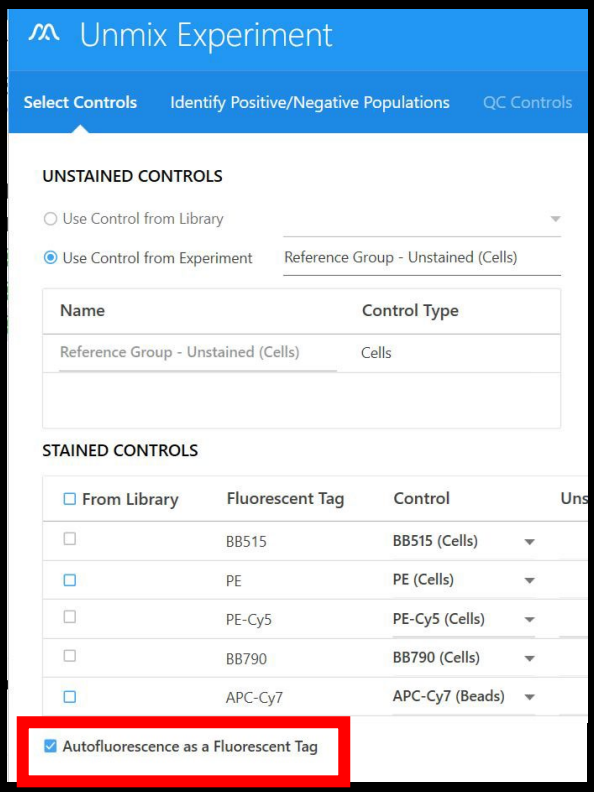
4) Run/record all reference controls and FMO/full-stains

Unmixing

1) Click unmix

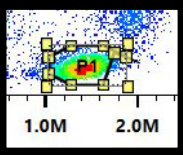


2) In the new window, click the auto-fluorescence button.



3) [next]

4) Set the scatter gate appropriately and then set the positive and negative populations for each fluorophore.

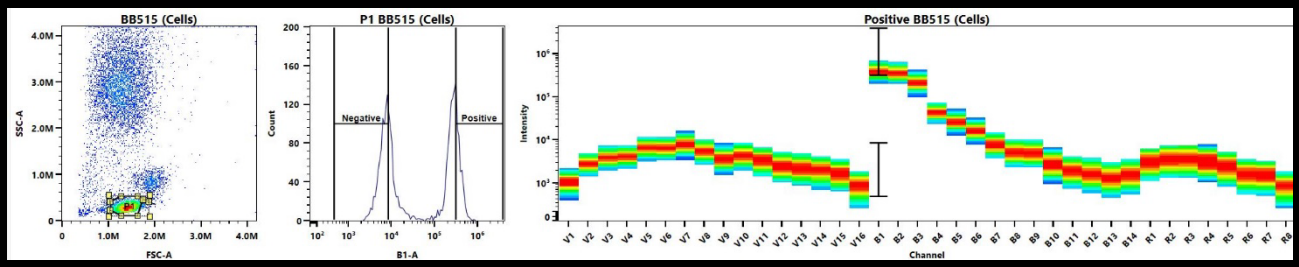


FSC/SSC: Tight selection around cells of interest.

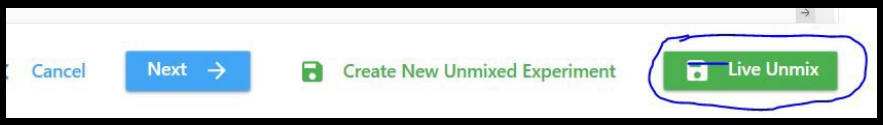
Positive peak: Mode and greater, or brightest 500-1000 events

Negative peak:

You can manually change the channel used for setting the gates by dragging the “error bar” on the snake graph to another channel



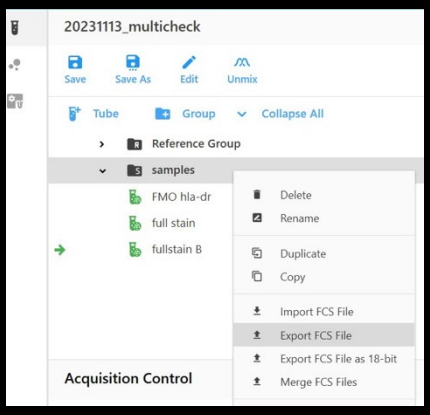
5) Select the [live unmix] button to apply unmixing to this experiment (the non reference group samples).

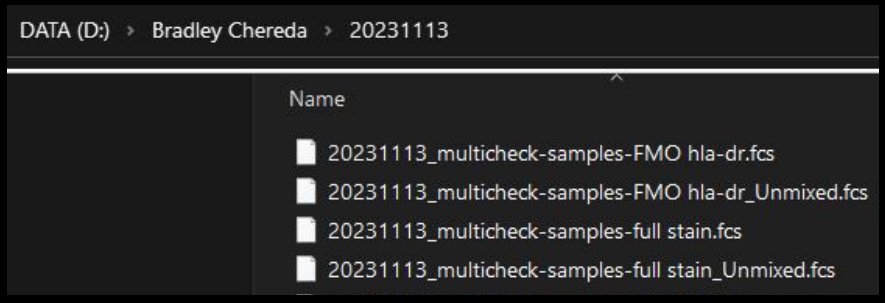


Exporting data

Export files to open in FCS express or flowJo.

1) Right click sample group
2) Select [export FCS]
3) Save on D:\ in your folder
4) Raw and unmixed files exported. Open the unmixed file in analysis software



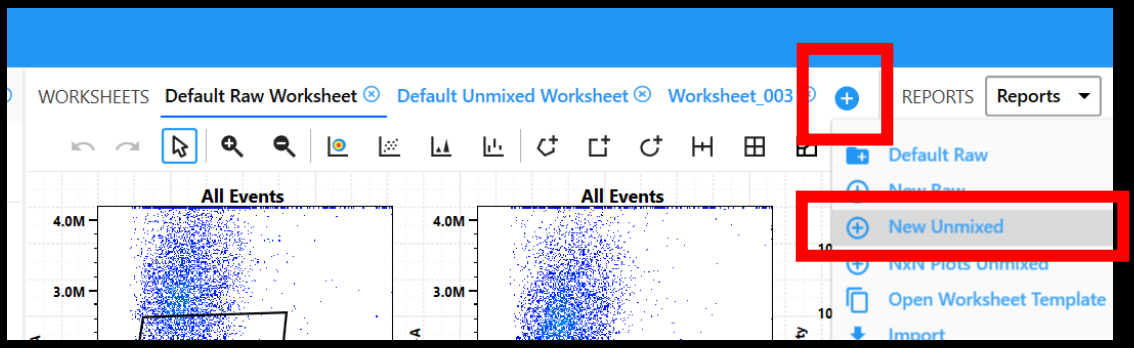


Analysis

You can export the unmixed data to FCS express or plot data in spectroFlo.

1) Create a new mixed worksheet

   Clicking the (+) to the left of the [REPORTS]
   Select [new unmixed]

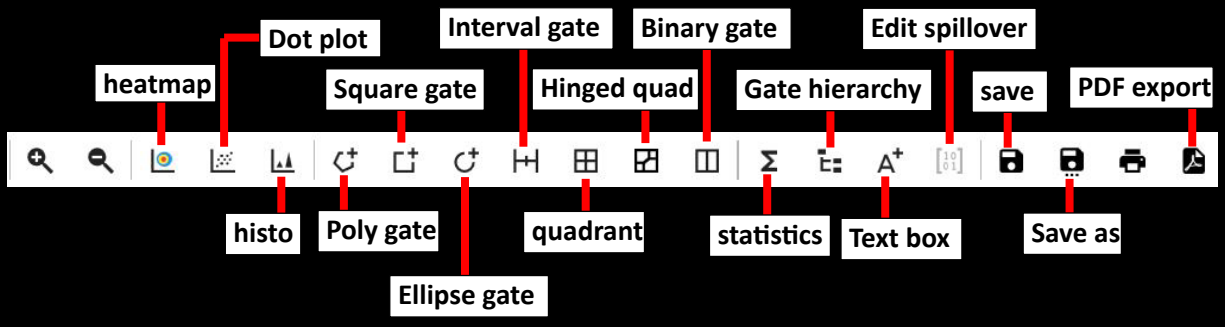


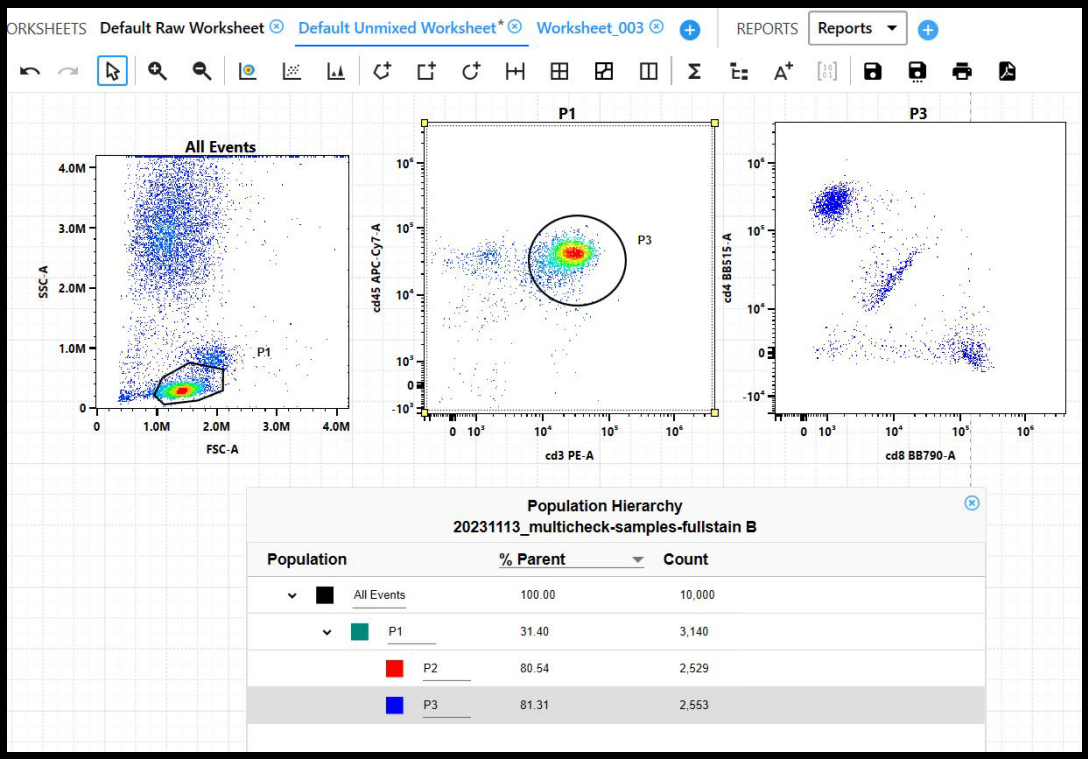
2) Create plots and gates

   To change the parameter, right click the plot axes

   To set a gate to a plot, right click the plot > properties > set the gate

   The properties window also allows bi-ex limits, dot size.





Population Hierarchy
20231113_multicheck-samples-fullstain B

Population	% Parent	Count
All Events	100.00	10,000
P1	31.40	3,140
P2	80.54	2,529
P3	81.31	2,553

Standby wash

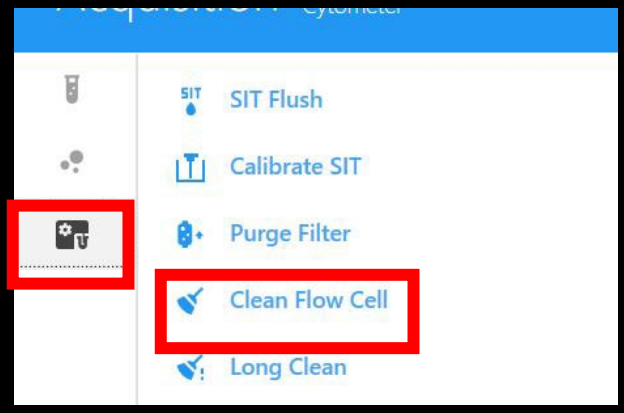
1) Prepare 2 tubes
    a. 2.5mL sodium hyperchlorite
    b. 2.5mL water

2) Click the third icon on the left (cytometer)

3) Click [clean flow cell]

4) Follow on-screen instructions.

5) Log out of SpectroFlo



Shutdown

1) Click the third icon on the left (cytometer) > fluidics shutdown

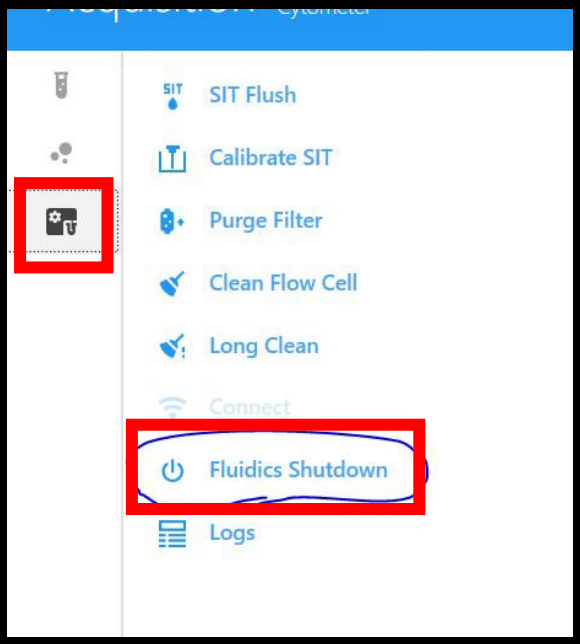
2) Prepare 3 tubes (2.5 mL):
    a. Bleach (1:1 bleach : water)
    b. Water
    c. Contrad (1:3 contrad : water)
    d. Water, can refill the second tube.

3) Follow on screen instructions

4) When finished, software will instruct to power down cytometer (power button on side of machine)

5) Log out of SpectroFlo

6) Leave PC on, it will go into standby.



Sheath and waste

Waste tank full

First, empty decontaminated tank (on preparation table)

1) Using the biocan-holder, take the waste tank to the chemical preparation room.
2) Turn on water tap
3) Unscrew waste tank lid and decant into the sink.
4) Return to 9-50 with the empty waste tank and biocan-holder.

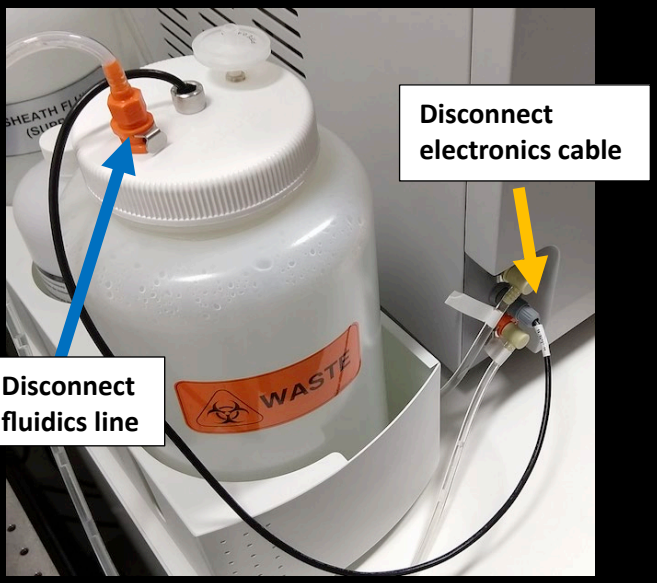
Second, decontaminate the now full waste tank

5) Unplug fluidics line on the waste tank lid (quick connect)
6) Unplug electronics cable from the cytometer body (black wire)
7) Move the waste tank to the preparation table behind the cytometer, put it in the biocan holder.
8) Add 350 mL sodium hypochlorite to decontaminate waste

   /!\ Do NOT throw out hypochlorite bottles; they will be reused.

Connect empty waste tank to cytometer

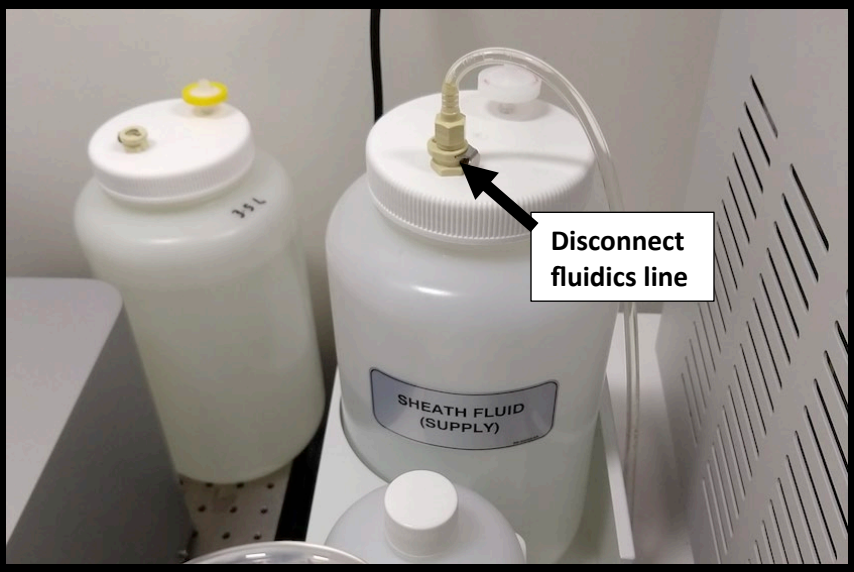
9) Put empty waste tank in the holder
10) Attach the fluidics and electronics lines.
11) Resume your cytometer run.



Decontaminating waste tank, placed in the biocan-holder

Sheath tank empty

1) Disconnect fluidics line from the sheath tank lid.
2) Remove empty sheath tank from the holder
3) install the full sheath tank in the holder and connect fluidics line.
4) Refill empty sheath tank. Use the chemical preparation room Satorius system.
5) Unscrew lid and fill tank with 3.5 L of milliQ water.
   Biocan holder available to aid in transporting between the chem prep room and 9-50.
6) 6/ Place the newly filled sheath tank to the left of the in-use one.



(left) place filled sheath tank here (right) installed shealth tank