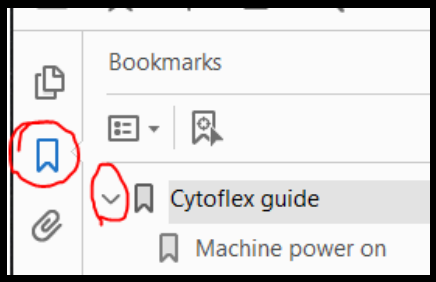
Viewing this guide in acrobat reader

Use the bookmarks button for quick access to sections within the guide.

Use to expand a section.



Cytoflex guide

Machine power on

1/ Check levels of sheath and waste bottles

2/ Turn on cytometer from power point

3/ Wake PC (press left arrow on keyboard)

4/ Log into Windows account

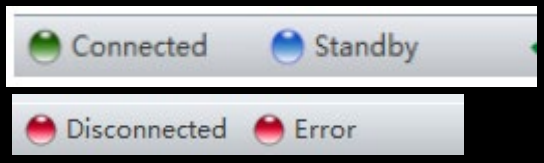
U: Cytoflex

P: Password

5/ Start cytometer software; CytExpert

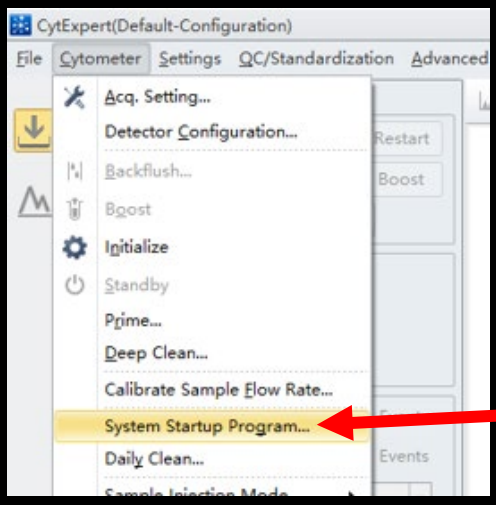
6/ Check that cytometer is connected (status bar bottom left)

If not connected: check machine is turned on; check USB connection between computer and cytometer



System start up: first user of day

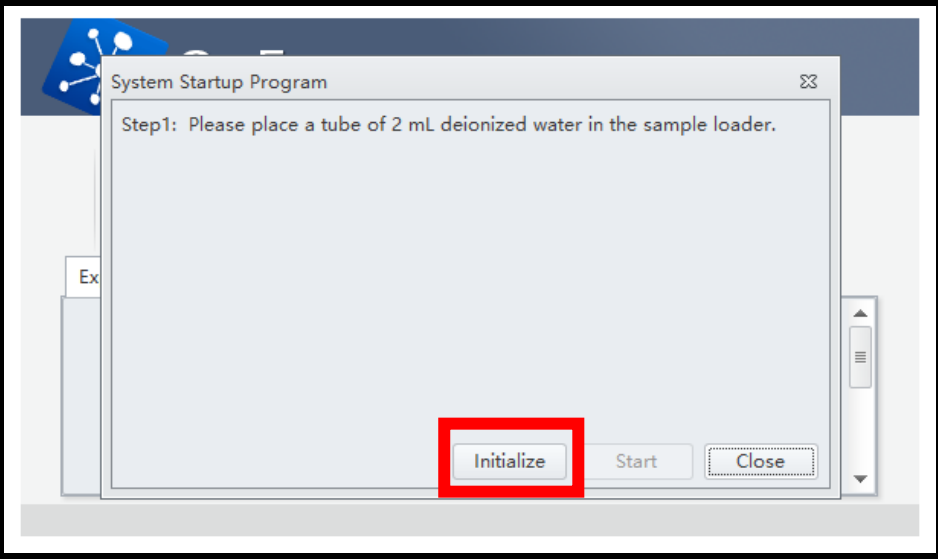
Cytometer menu > “System Startup Program”

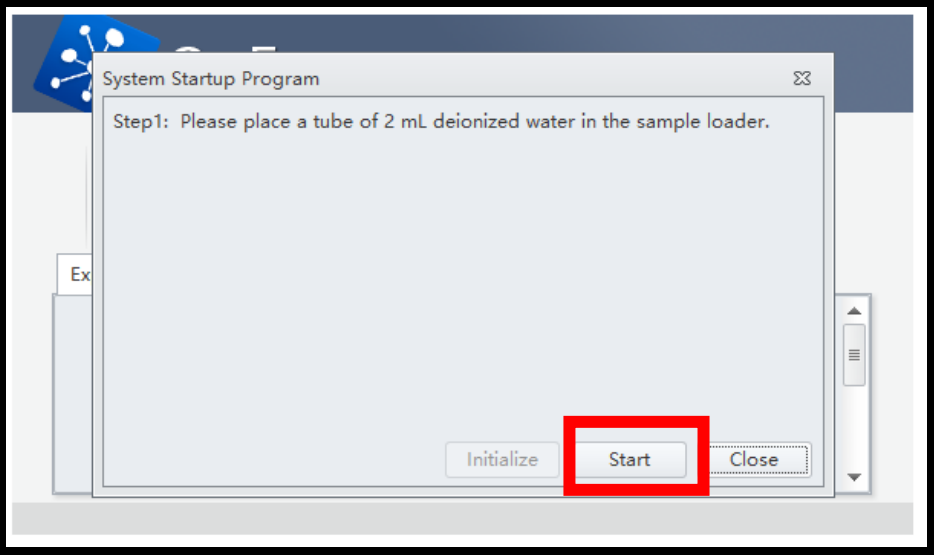


Tube arm (front right of cytometer) can be freely moved (if tube not being run).

Load a full tube of water into tube arm.

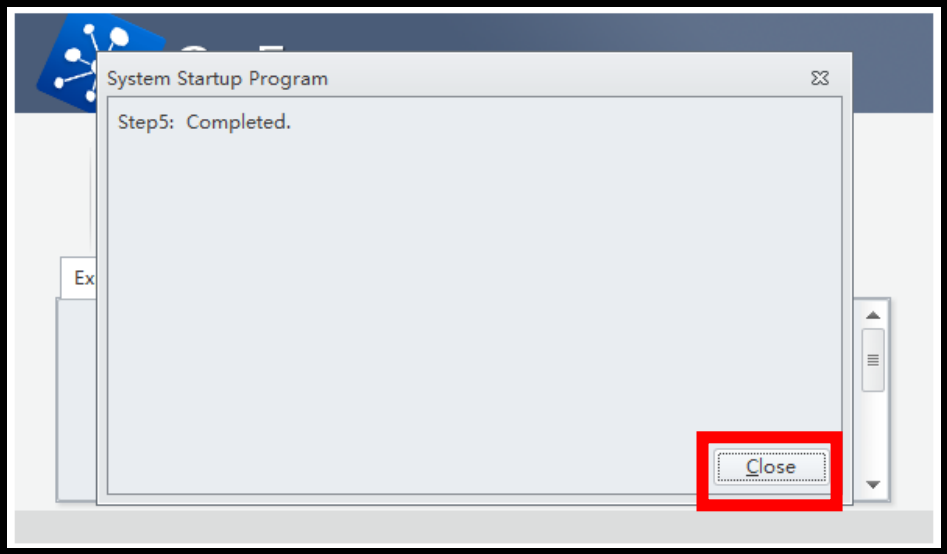
Click “Initialize”





Then click “Start”

Startup will take 10 minutes.



Click "close"

Daily QC

1) QC beads

Beads are in the fridge underneath the centrifuge.

Amber, bead bottle; and glass tube for running on the Flex

"cytoflex ready to use daily QC fluorospheres"

If glass tube is empty:

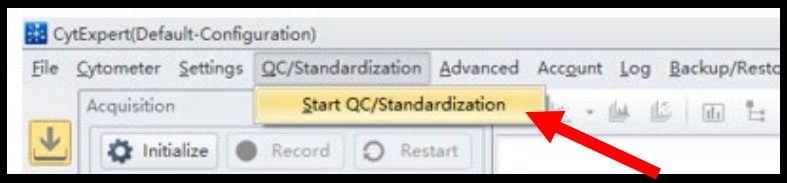
Vortex bead bottle

Add 6 drops to a new glass tube (from blue box next to Flex)

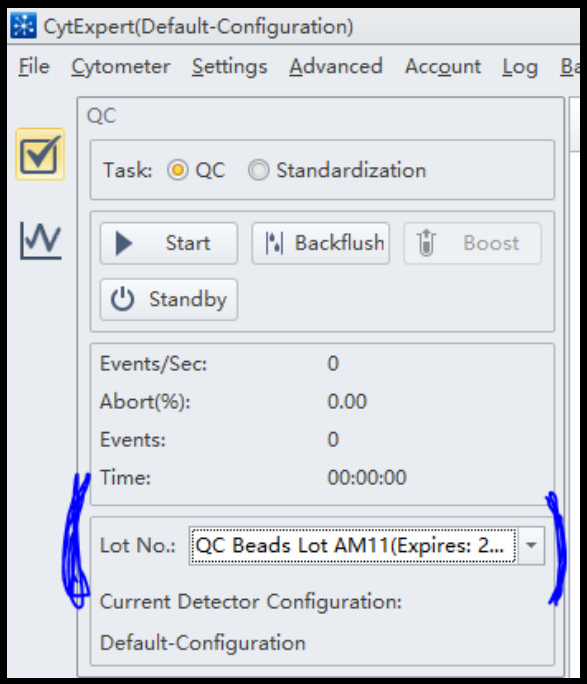
Used tube in pipette tip box lid

2) Load QC bead tube

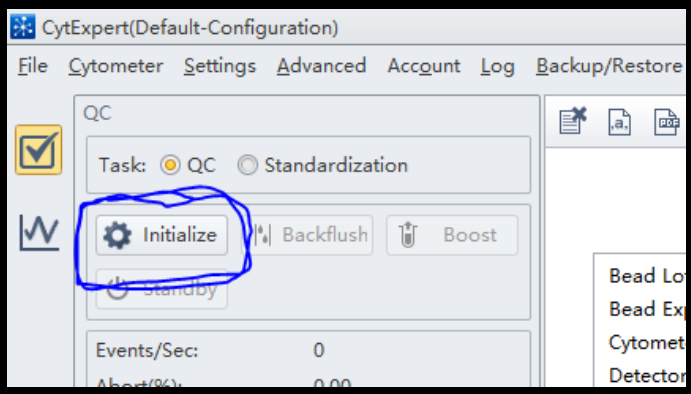
3) QC/Standardisation menu > Start QC/Standardisation



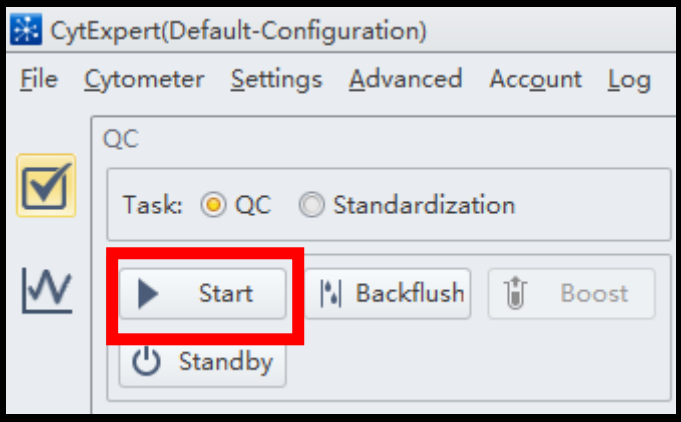
4) Set the current bead lot



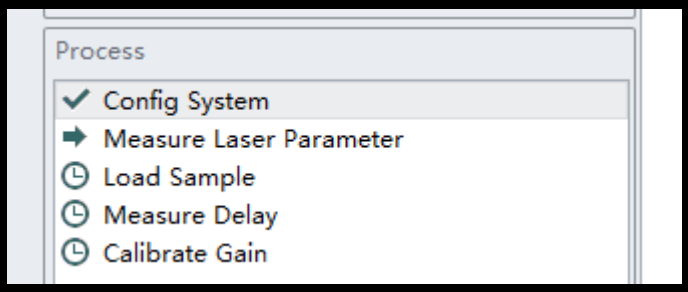
5) Initalise the cytometer (if in standby)



6) Press start



7) Cytometer will perform pre-checks (1 minute) and then start QC (30 seconds)



8) QC report

Things of interest:

Global pass/fail [Blue box]

Parameter pass/fail [red box]

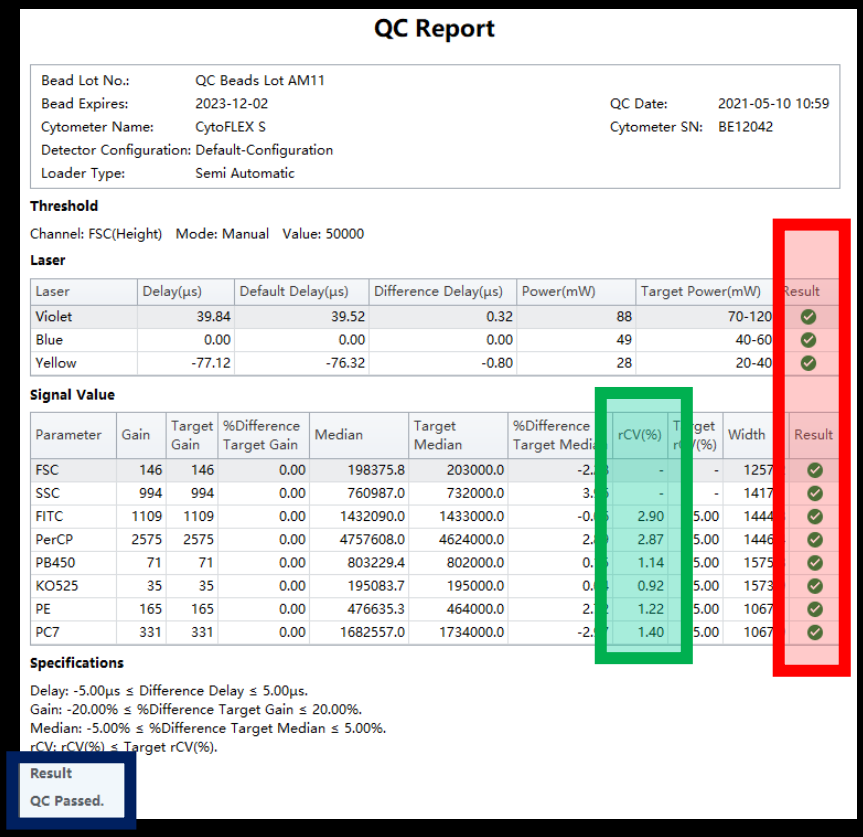
Parameter rCVs (variance) [Green box]

To try if QC fails:

Ask machine minder for help

Run a wash tube, then try QC again

Remake QC beads, then try QC again



QC Report

Bead Lot No.:	QC Beads Lot AM11		
Bead Expires:	2023-12-02	QC Date:	2021-05-10 10:59
Cytometer Name:	CytoFLEX S	Cytometer SN:	BE12042
Detector Configuration:	Default-Configuration		
Loader Type:	Semi Automatic		

Threshold

Channel: FSC(Height) Mode: Manual Value: 50000

Laser

Laser	Delay(μs)	Default Delay(μs)	Difference Delay(μs)	Power(mW)	Target Power(mW)	Result
Violet	39.84	39.52	0.32	88	70-120	✓
Blue	0.00	0.00	0.00	49	40-60	✓
Yellow	-77.12	-76.32	-0.80	28	20-40	✓

Signal Value

Parameter	Gain	Target Gain	%Difference Target Gain	Median	Target Median	%Difference Target Medi	rCV(%)	T… get r…(%)	Width	Result
FSC	146	146	0.00	198375.8	203000.0	-2.	-	-	1257	✓
SSC	994	994	0.00	760987.0	732000.0	3.	-	-	1417	✓
FITC	1109	1109	0.00	1432090.0	1433000.0	-0.	2.90	5.00	1444	✓
PerCP	2575	2575	0.00	4757608.0	4624000.0	2.	2.87	5.00	1446	✓
PB450	71	71	0.00	803229.4	802000.0	0.	1.14	5.00	1575	✓
KO525	35	35	0.00	195083.7	195000.0	0.	0.92	5.00	1573	✓
PE	165	165	0.00	476635.3	464000.0	2.	1.22	5.00	1067	✓
PC7	331	331	0.00	1682557.0	1734000.0	-2.	1.40	5.00	1067	✓

Specifications

Delay: -5.00μs ≤ Difference Delay ≤ 5.00μs.
Gain: -20.00% ≤ %Difference Target Gain ≤ 20.00%.
Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.
rCV: rCV(%) ≤ Target rCV(%).

Result

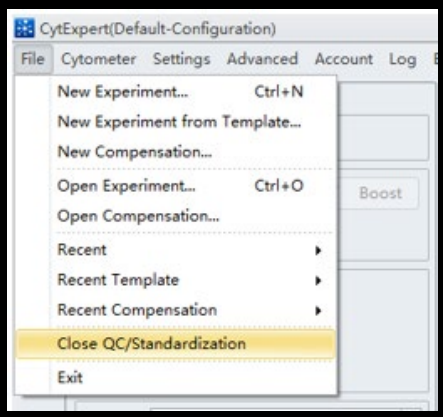
QC Passed.

9) Close QC

File menu > Close QC/Standardisation

Return glass QC tube to fridge; or discard in pipette-box-lid, if empty.

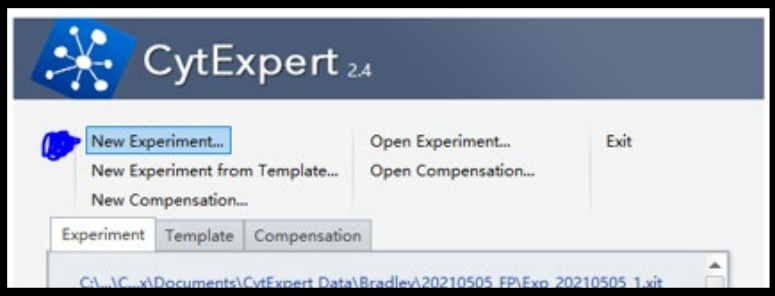
Cytometer ready for running samples



Running a new experiment

If you require fluorescence compensation; follow the compensation chapter first

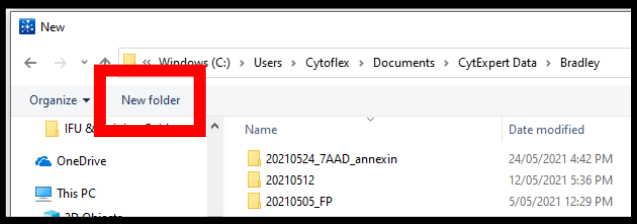
Click "new experiment"



Save the experiment in your folder

Your account is setup to default to a folder with your user name.

1/ click on “New folder”

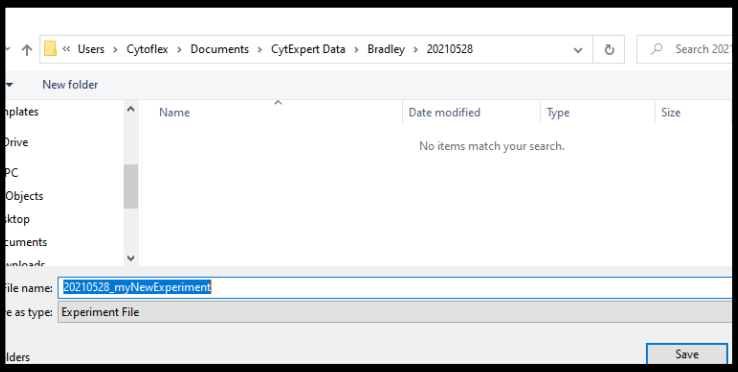


2/ Name the folder with today’s date: YYYYMMDD (e.g, 20210512)

3/ Press enter. Double click the newly created folder to open it.

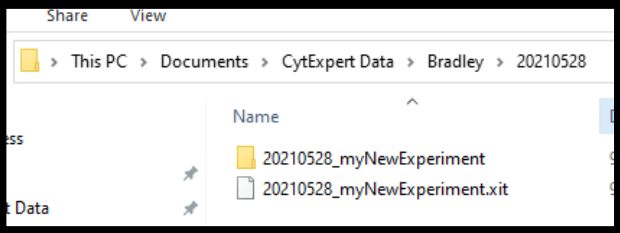
4/ Enter a name for the experiment (or leave the default name)

5/ Click “Save”



In the folder with today’s date, there will be a folder named after the experiment name, as well as a .XIT file.

Your data files will be saved in the folder. The XIT file is the experiment layout file. You need both for offline analysis



Set parameters for experiment

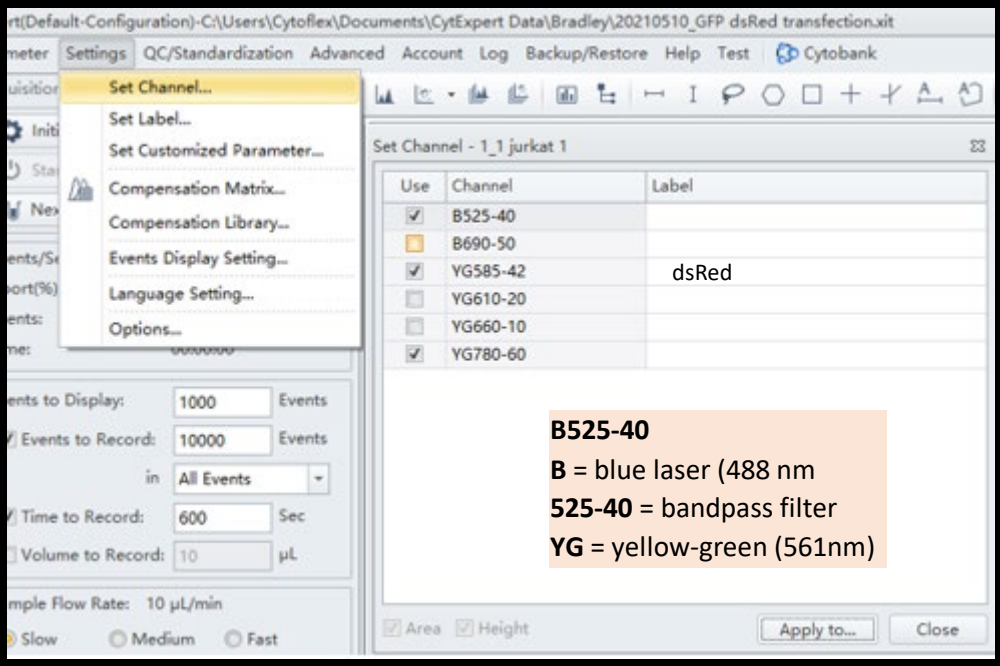
Settings > Set Channel

This brings up a window to select channels.

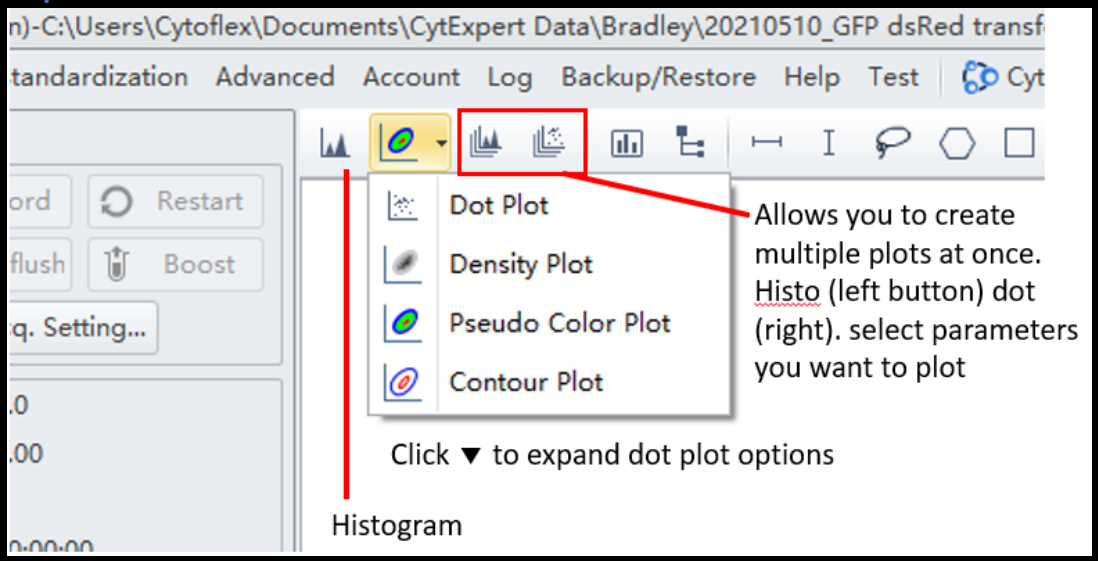
Deselect the channels you don't need.

You can set a label for the channel (e.g, CD45, GFP, Tubulin). Single left click on an appropriate label

Click the close button (only need to use "apply to" if you already created multiple tubes)

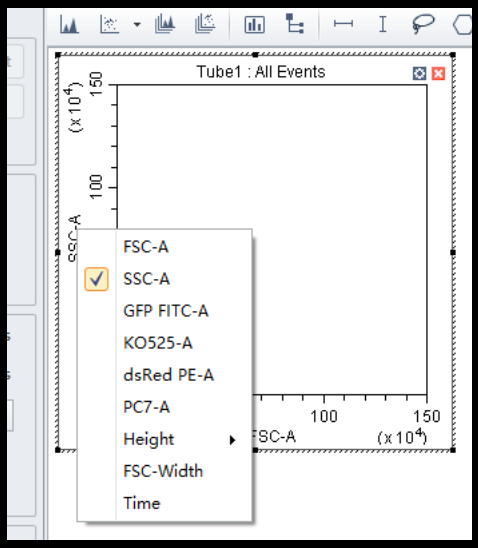


Create plots

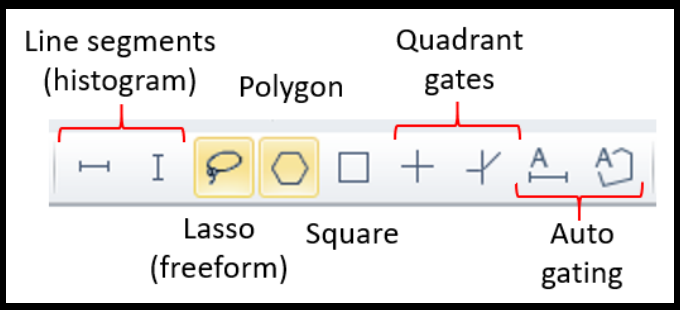


Select parameters for plot

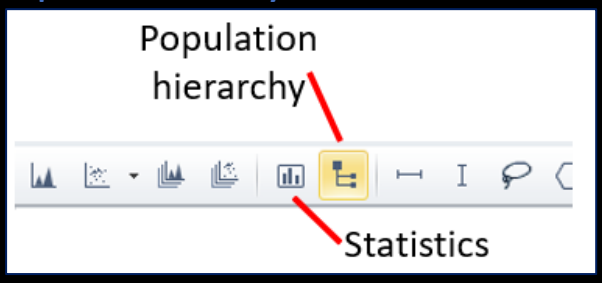
Left click the parameter name on a plot to select the channel you want



Draw gates



Population hierarchy and statistics

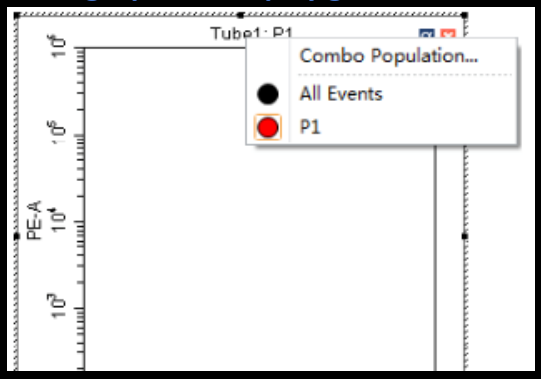


Population hierarchy:

Change gate name, colour and hierarchy

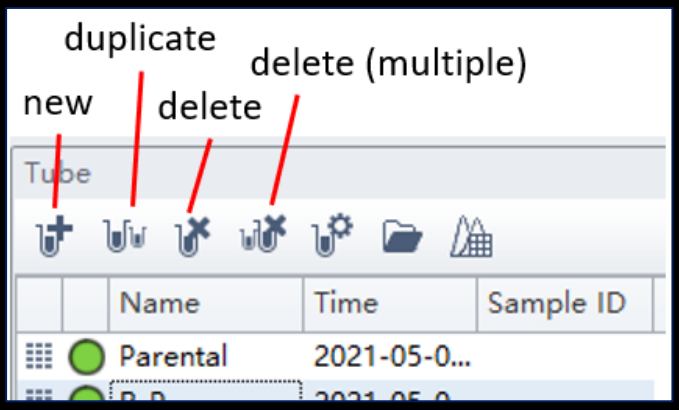
Display statistics (right click statistics box to change displayed stats and populations)

Setting a plot to display gated events



Left click a plot's title to set the gate to display.

Tube setup



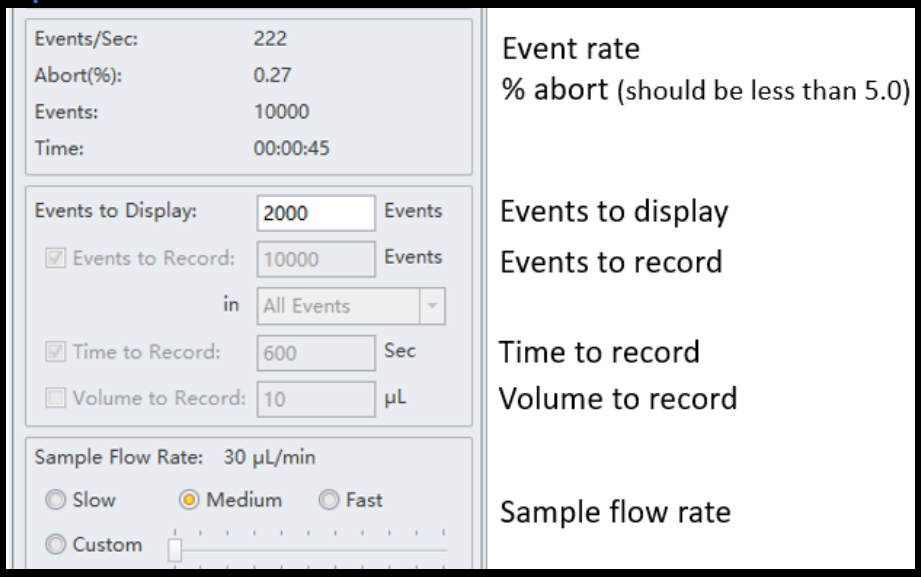
Edit tube name by double left click, or right click and ‘rename’

Any changes to a tube are not carried over to existing tubes. This includes gain settings, and gate changes.

Recommend to create one tube. Set up the plots, gates and gains. Once everything is setup, clicking on the new tube button will keep all the settings from the selected tube.

Sample (data) acquisition

Acquisition options



Running a sample tube

- Load sample tube

  Make sure lid is off

- Initialize (if required)
- Click run button to acquire data

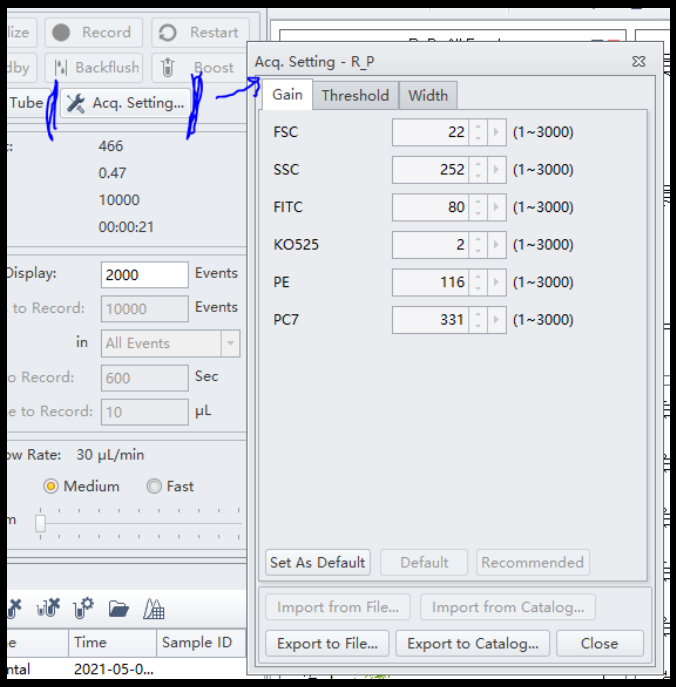
  When you click run, you will begin saving data straight away and until you press "stop"
- Set channel gains (see next)
- Click record to acquire data according to time/event/volumetric stop-conditions

  i.e, recording 10,000 events for a particular sample.
- The button on the front of the cytometer is the load button and will function like clicking on "record"

Changing gains (voltage)

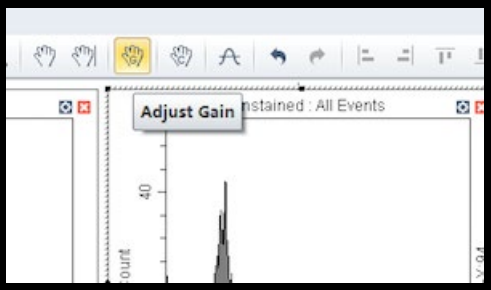
On the Cytoflex: gain is the same as “voltage”

There are two ways to change a channels gain:



Click Acq. Setting (before or during run)
Change gain value
Can also import values from a file

While a sample is running:



Click the hand icon with a G (toolbar) while sample is running. Use hand tool to drag data on a plot to set desired gain setting.

Applying gains to other (subsequent) tubes

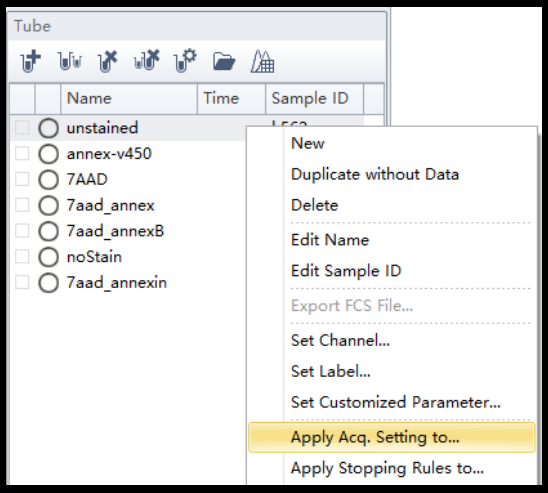
Setting up the gain for one tube will not apply to already existing tubes.

Right click the tube with desired gain settings

Select “apply acq. Setting to…”

This will display all the tubes in the experiment.

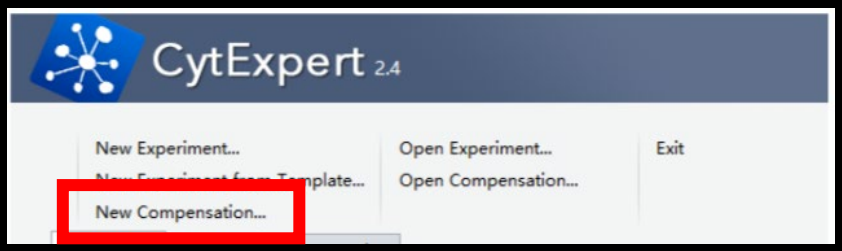
Use the tick-boxes to select which tubes to apply the gains settings to.



Compensation experiment

On the cytoflex, compensation controls are run in a separate experiment.

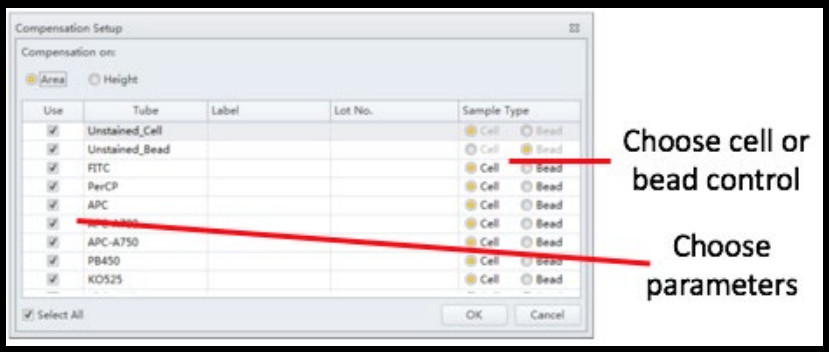
Make a new compensation experiment



Save location: your data folder > in a folder with today's date

Eg, CytExpert Data/Bradley/20210513/

Setup compensation controls



The software will automatically generate the required plots and tube layout

Acquire data

See Sample Acquisition chapter for detailed information on sample acquisition

1/ Select unstained control tube in software

2/ Load unstained control tube on loader-arm

3/ Click "run"

4/ Set FSC/SSC gains (voltage) and set negative fluorescent peak to approx. 10^2

Clicking "run" will save data, the data will refresh whenever you make a change to the gain value

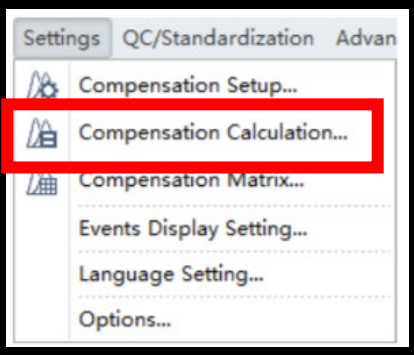
5/ Stop the run when desired, or click "record" to record set amount of events

6/ Set up gating to include beads/cells, and brightest fluorescence signal

7/ Acquire data for all controls

Calculate compensation

Settings > Compensation calculation



A compensation matrix window will appear.

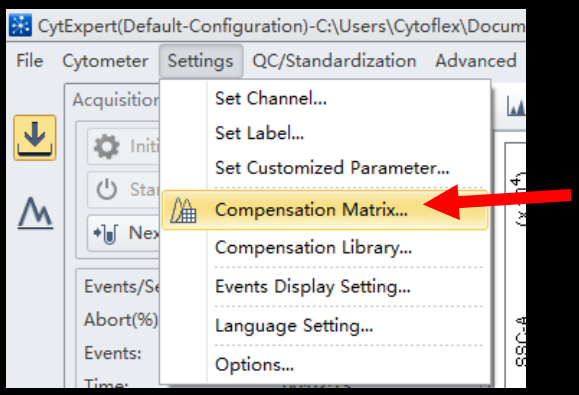
- Save the compensation settings into today's folder.
- Close the compensation experiment.
- Create new experiment for your samples.

Apply compensation

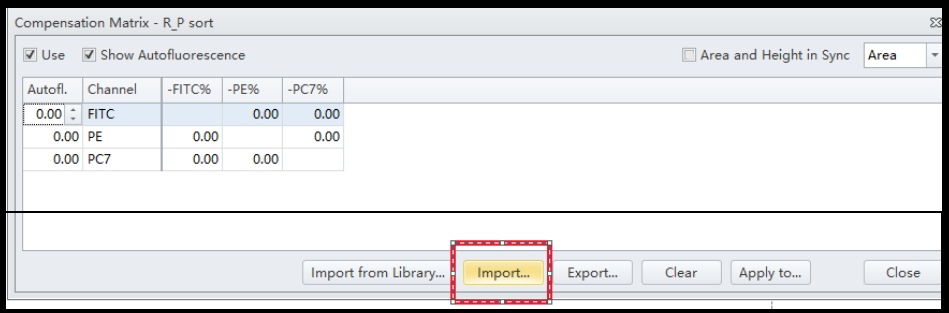
1/ Open or create experiment for running your samples

2/ Set up tubes

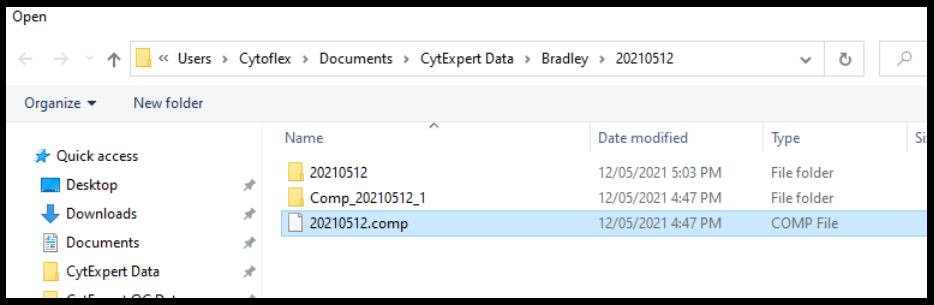
3/ Settings > Compensation Matrix



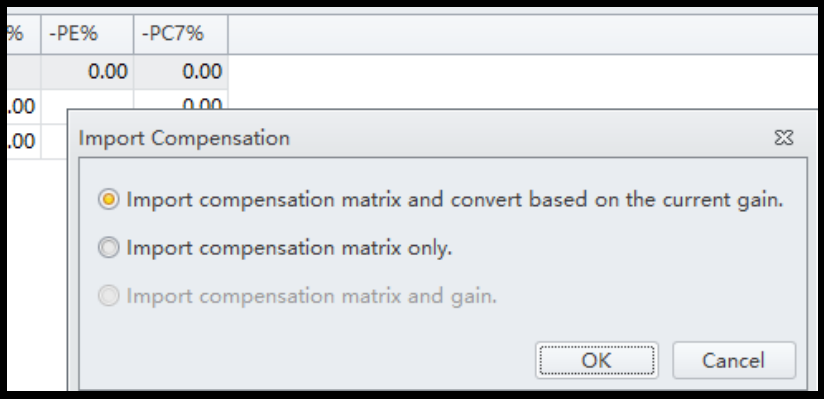
4/ Import compensation file



5/ Find appropriate comp file and open it



6/ Choose option



Import comp matrix and convert based on current gain.

CytExpert converts compensation values based on the difference in gains between the compensation experiment and the current experiment (if gains are different)

It is good practice to use the same gains for compensation controls and samples (with exception for FSC and SSC if using beads).

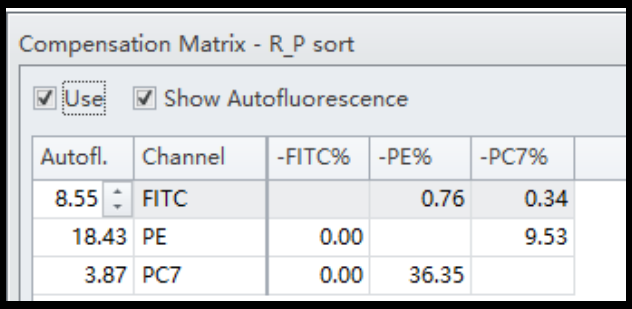
Import compensation matrix only

CytExpert will import compensation values from the comp-file to this experiment without adjusting for any changes in gains between the two.

Import compensation matrix and gains [disabled if tube has data acquired]

CytExpert will import the gains and compensation values from the comp file to this experiment

7/ Compensation values will be applied in matrix



8/ Acquire data for experimental samples

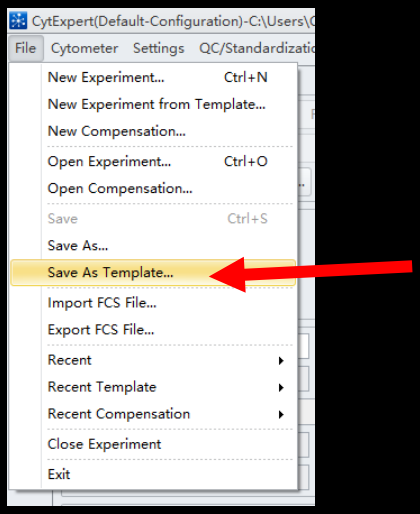
Using a previous experiment layout

Save a previous experiment as a template.

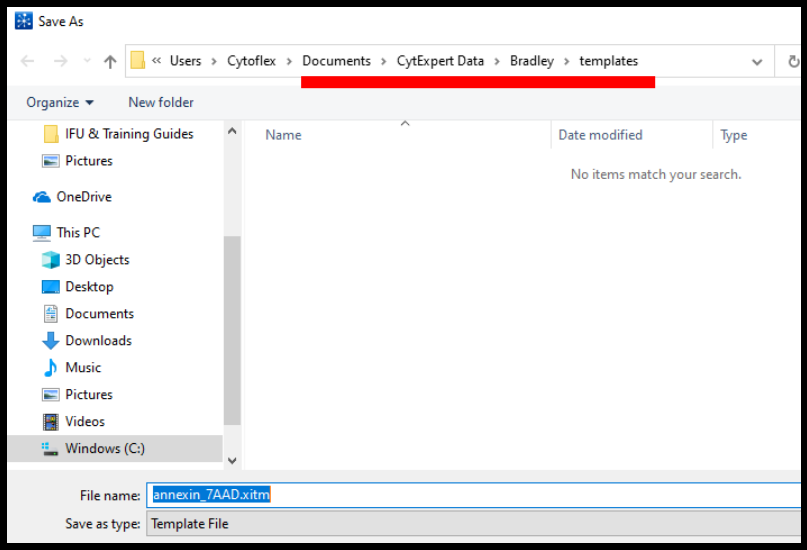
Create a new experiment using the template file

Setup the new experiment as required

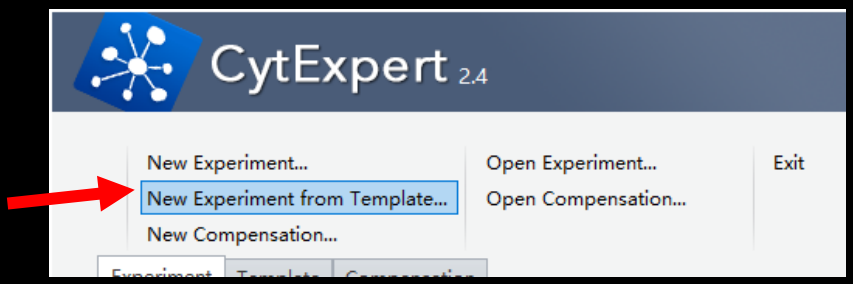
1/ Save experiment you want to use as a template



2/ Save the template file in a templates folder in your data-folder



3/ Close experiment. Then at the main screen choose “new experiment from template”

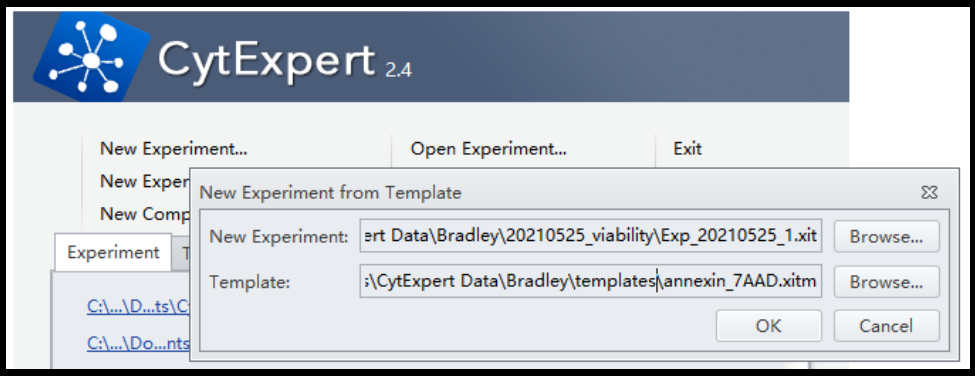


4/

New experiment: Create a new experiment. Save in a new folder in your data-folder

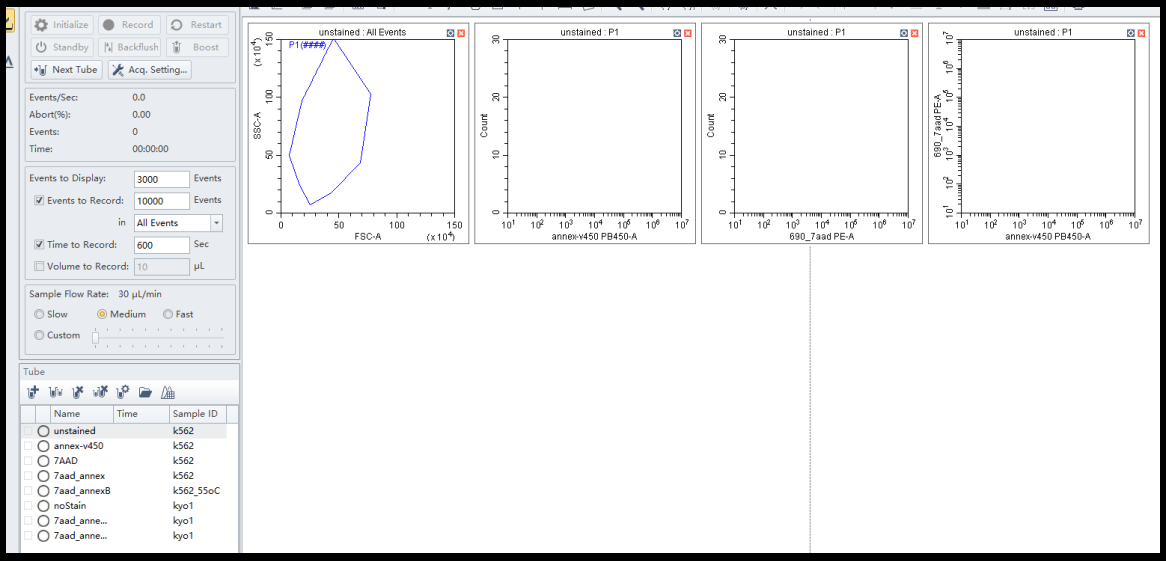
Template: Select the template you want to use

Then click “OK”.



5/ A new experiment will be created with plots, gates, tube layout and acquisition settings from the template.

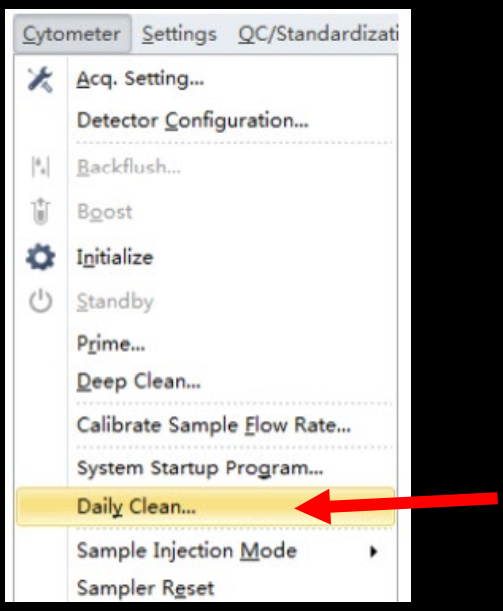
Setup tube layout and check acquisition settings are appropriate for the experiment you are now going to run.



Cytometer clean

At the end of your run:

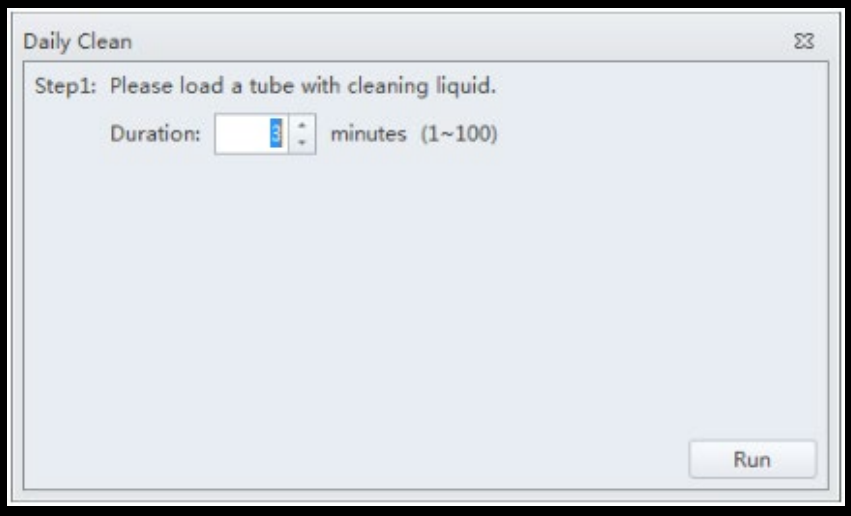
1/ Cytometer > daily clean



2/ Insert tube with **2mL FACS clean**

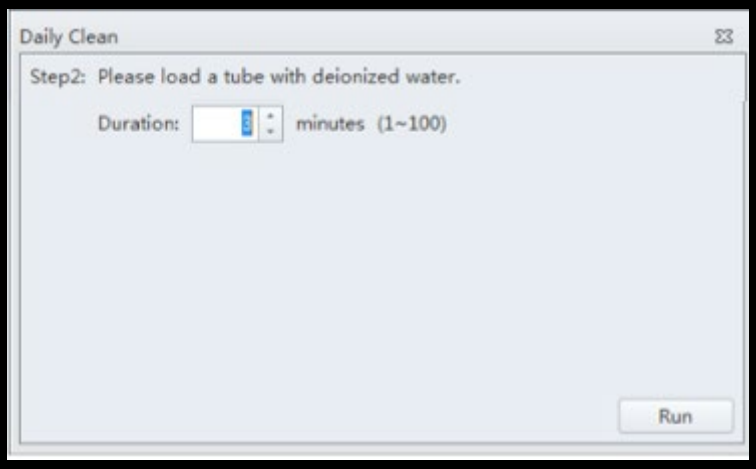
1% Bleach found in dispenser bottle, between the Flex and Fortessa.

3/ Set duration to 3 minutes; run



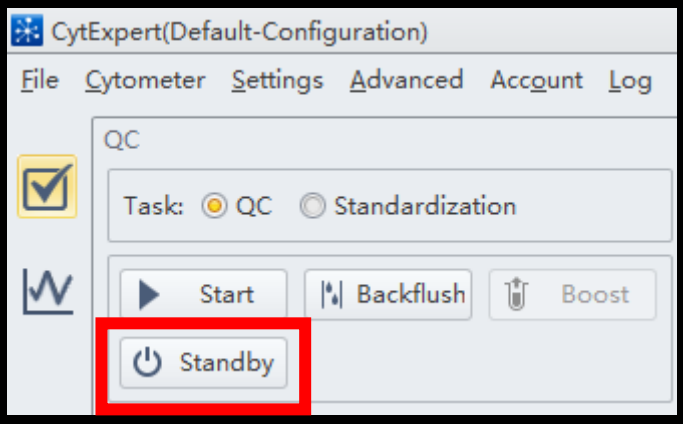
4/ Insert tube with DI water

5/ Set duration to 3 minutes; run



6/ Close window

7/ Put Cytoflex into standby



8/ Exit software

9/ If last user of the day: turn off Cytoflex at the powerpoint

Refill Sheath

Sheath tank is 4 litres

 **DO NOT use Isoflow** on the cytoflex, this solution will damage the cytometer

Use:

Cytoflex sheath fluid,

Sartorius water from 10 litre dispenser, or,

Sartorius water unit (in adjacent room 9-51)

1/ Put Cytoflex in standby

2/ Unscrew sheath line

3/ Fill bottle

4/ Replace sheath line

5/ Re-initialize cytoflex

Empty waste

Sheath tank is 4 litres

It will be emptied into a 5L waste tank, prefilled with 400 mL hypochlorite

1/ Put Cytoflex in standby

2/ Unscrew waste line

3/ Pour waste bottle into 10L waste tank labelled cytoflex.

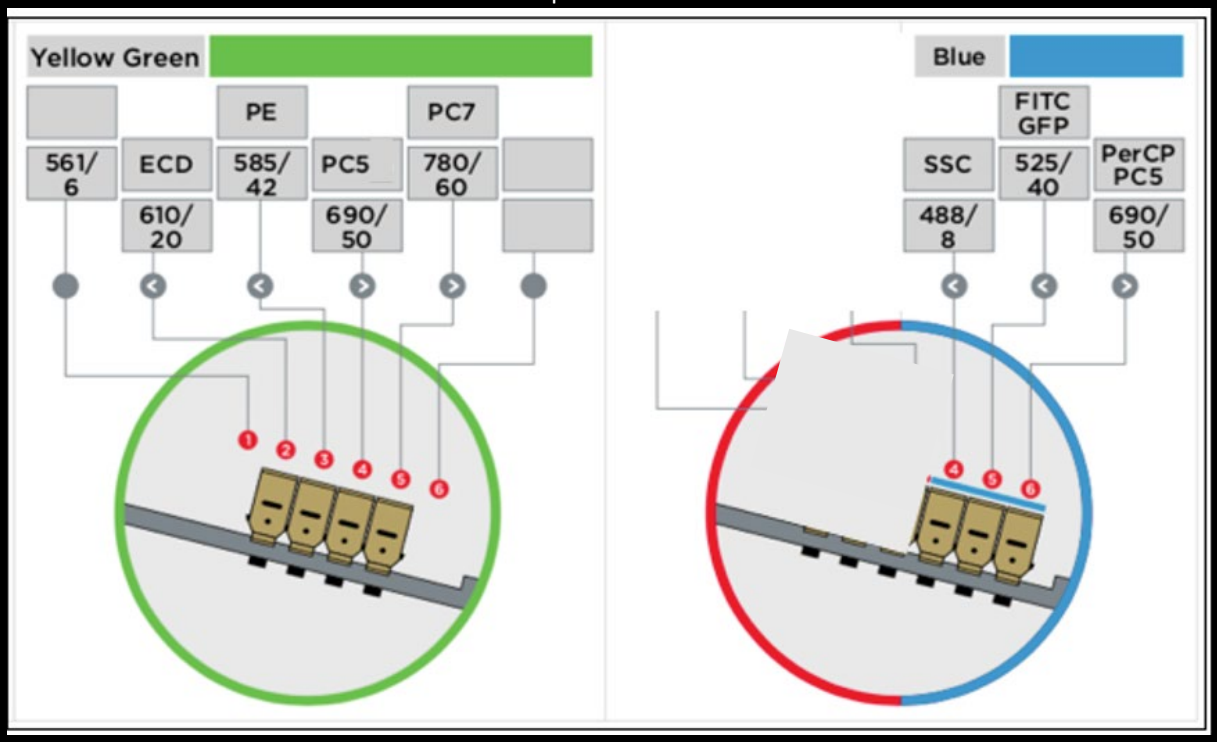
4/ Place 10L tank on the protecta pad next to the spill kit

5/ Replace 4L waste tank and line

6/ Re-initialize cytoflex

Instrument Optics

Blue - 2 | Yellow-Green - 4



Gain+	Blue	Ex: 488 nm
400	B 525/40	GFP; FITC; Alexa 488; BB515; CFSE; YFP; Sytox Green
300	B 690/50	RB705, PerCP; BB700; PerCP-Cy5.5; *7-AAD**; *PI**
	Yellow-Green	Ex: 561 nm
300	YG 585/42	PE; dsRed; dTomato; Alexa 546, 555; Sytox Orange
300	YG 610/20	PE-CF594; NovaFluor 610; PI; Alexa 568, 594; mCherry; 7-AAD; PE-TexasRed; Sytox Orange
300	YG 690/50	RY703; PE-Cy5; novaFluor 660; mPlum; *APC*^
400	YG 780/60	PE-Cy7; RY780; *miRFP703*;
	Spare filters	610/20; 660/10; 712/25; 780/60

* 7-AAD and PI: 561-610/20 is the optimum laser/filter

^ APC: can be detected on 561-660/10, but only for abundant antigens. Will be very dim

\+ recommended minimum gain setting