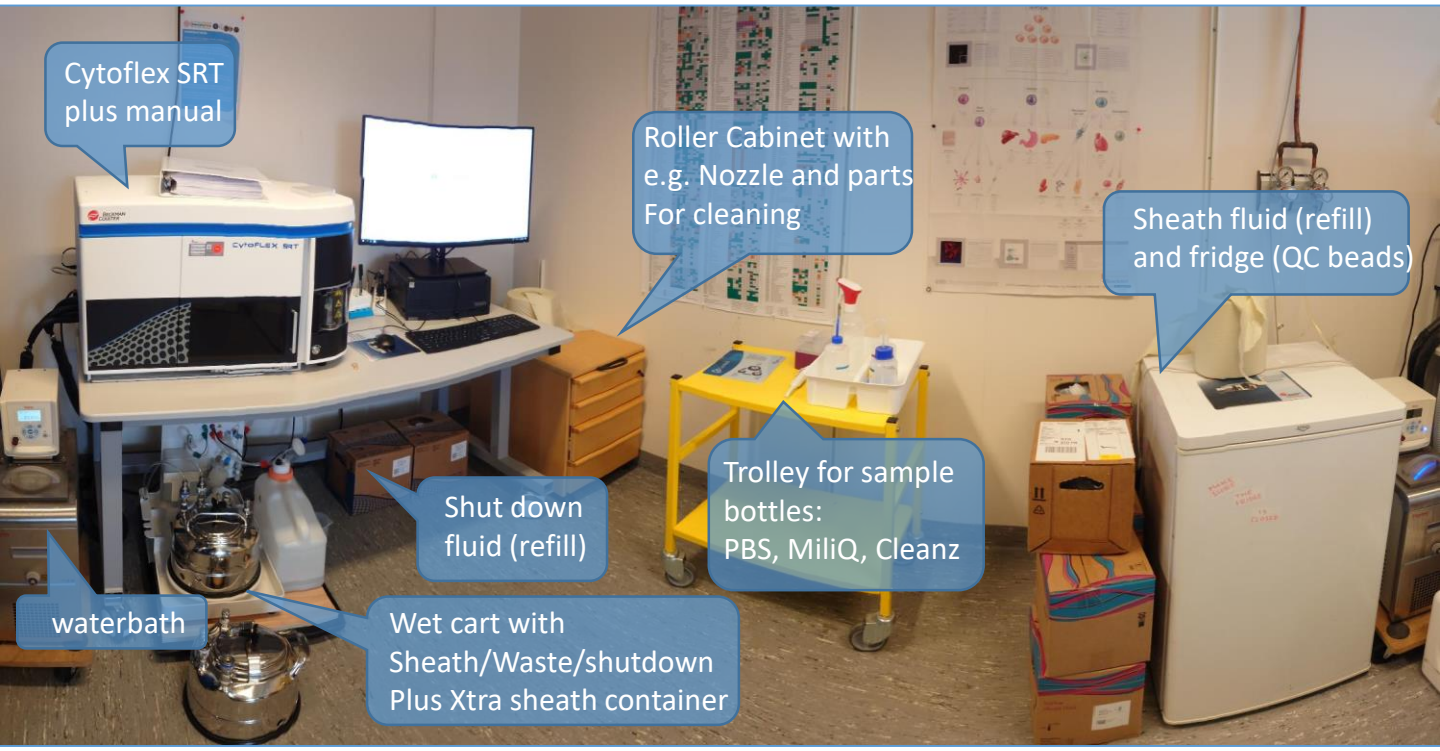
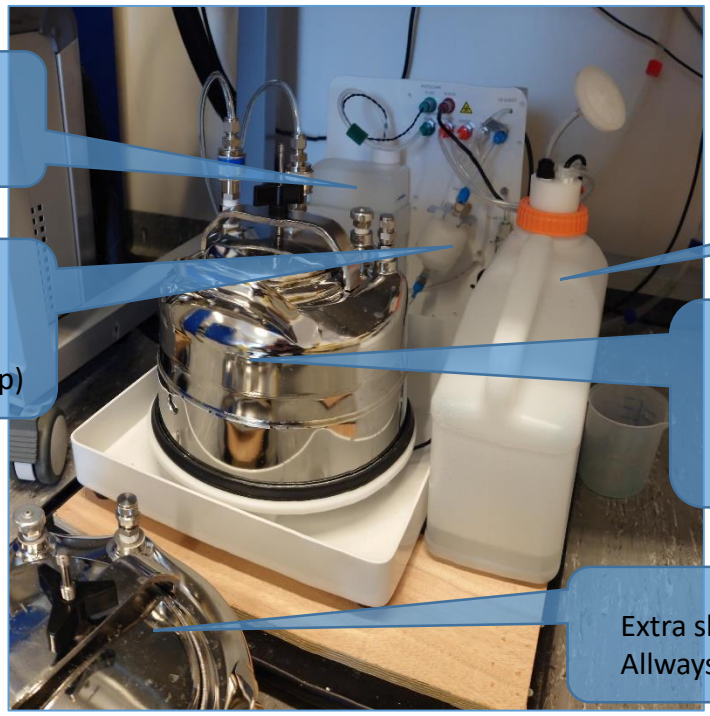
Overview of Instrument



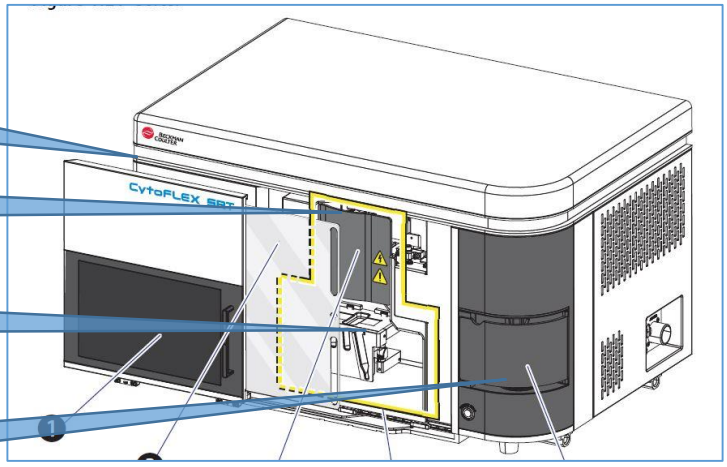


You will find and leave the instrument (if not otherwise agreed)

- In daily shut down mode
- With a sheath container filled for at least 2.5h sorting
- An extra sheath container, filled, but not coupled to the instrument
- Nozzle, side stream illuminator, deflection plates plates clean
- Waterbath off
- Nozzle, clean, in its plastic box, in cabinet
- Table and instrument clean

→ check the log book for sheath info

Overview of Instrument

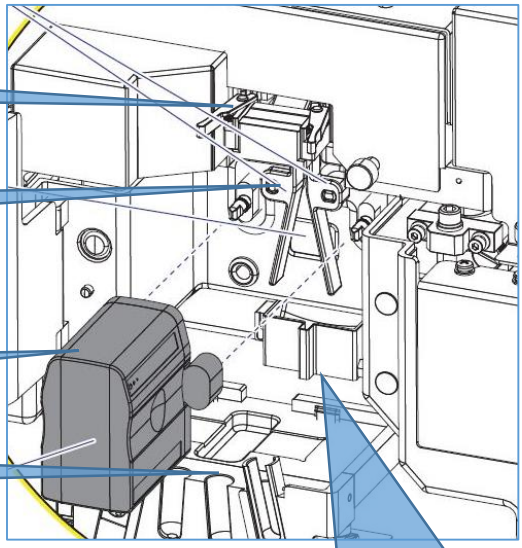


Slide door to Sort vessel chamber

Swing door (black) to Sort chamber

Tray for Sort vessel

Swing door (transparant) to sample chamber



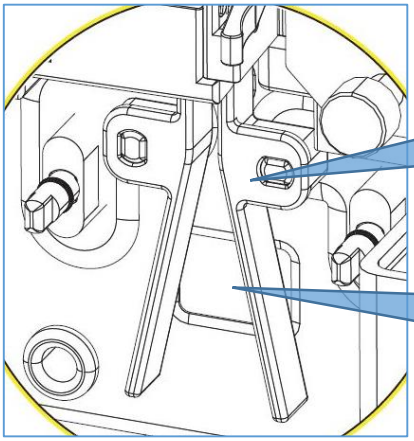
Insert for nozzle, nozzle lift and clamps

Deflection plates

Side Stream illuminator (here: removed)

Tray for sort vessel

Waste drawer



Deflection plates
Can be removed for ccleaning

One of the windows

CHECK that the windows of the Side stream Illuminator and those ´behind´ the Deflection plates acre clean
Use 70% EtOH + Qtips to clean

The 100 µm Nozzle

The Cleaned & dry Nozzle placed in its plastic container. Container placed in top compartment of cabinet. 2 spare nozzle available in compartment´s back.
BioVis´standard nozzle found in front of compartment.

Nozzle with rubber O-ring

Nozzle holder, **contains electronics**

The Sample chamber

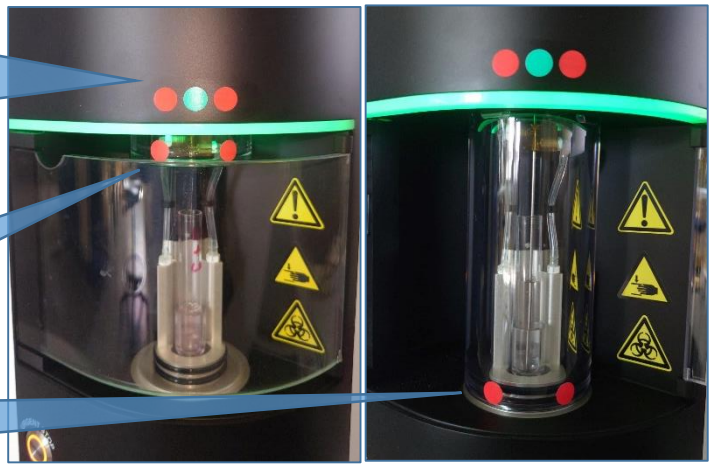
Do not open the transparant door of the sample chamber when the sample chamber (marked by 2 red dots) is moving up or down

Opening the door, whilst the moving, will generate a ”full stop error” and the whole repertoire of debubbling, QC and drop delay is needed thereafter (15min)

You can open the chamber door when the green light is NOT blinking.

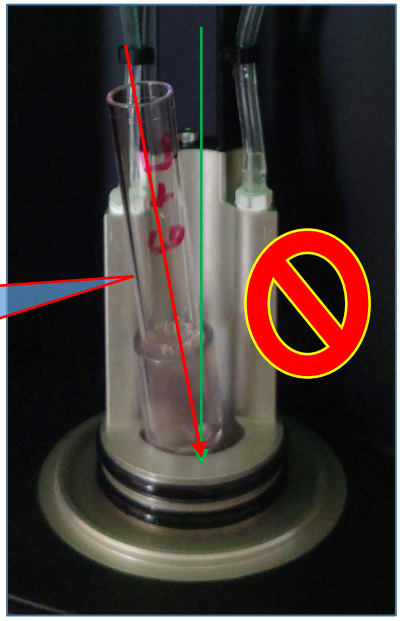
To put more attention the the green light bar, three dots were added, 2 red flanking one green. The green light will also blink when sample is been taken up. The door can be opened as well under that condition.

Sample chamber can be up, for exchanging tubes, or down, sealing the chamber for Run/Record/Sort.



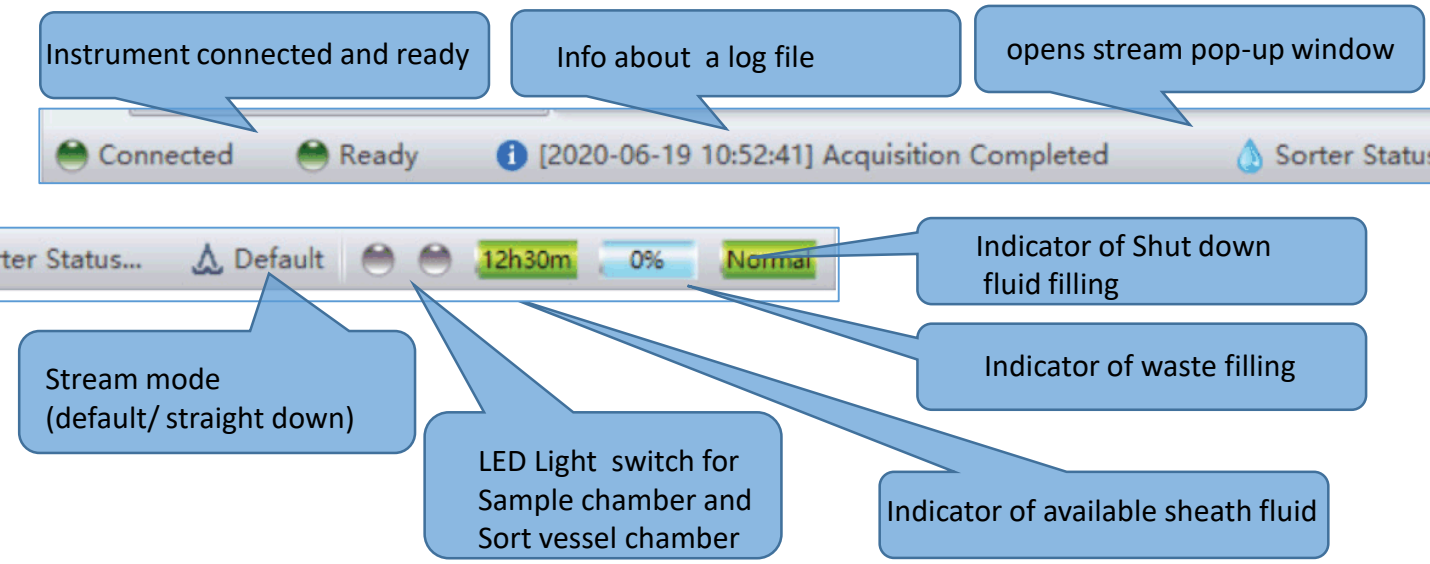
Make sure the tube is upright not tilted.

The tube holder is hold in place by a magnet (and can be taken out). Do not load the sample with a tiled tube
Make also sure your tube does not have lid



Overview of Software

The lower bar of the Software gives important information



Menues of the software

File	Cytometer	QC/Standardization	Sorting	Settings	Advanced
New Experiment...	System Startup...	Start QC/Standardization	Sort Calibration	Set Channel	Delay Setting
New Experiment from Template...	System Shutdown...	Close QC/Standardization	Manual Drop Delay	Set Label	Laser Setting
New Experiment from FCS...	Long Term Shutdown...		Manual Side Stream Calibration	Set Customized Paramete	Event Rate Setting
New Compensation	Turn On		Sorter Status	Compensation Matrix	Sort Guardband Setting
Open Experiment	Turn Off		Side Stream Monitor	Compensation Library	Default Amplitude Setting
Open Compensation	Backflush		Stream Mode Switch	Events Display Setting	Collection Device Library
Convert CytExpert Experiment	Sheath Filter De-bubble		Reset Cyclone	Language Setting	Sort Mode Library
Save	Flow Cell De-bubble		Tube Position Setting	Options	Maintenance
Save As	Daily Clean...				Bubble Detector Calibration
Save As Template	Flow Cell Clean...				Sheath Tank Scale Reset
Import FCS File	Aseptic Clean...				
Export FCS File	Reset Sample Chamber				
Recent	Acq.Setting...				
Recent Template	Acq.Setting Catalog...				
Recent Compensation	Detector Configuration				
Exit	Sample Flow Parameter Adiustment				
	Cytometer Information..				

The CytExpert Software will not be explained here anyfurther
Please check the manual for CytExpert to get to know how to Set up an experiment

Start-up 10 min

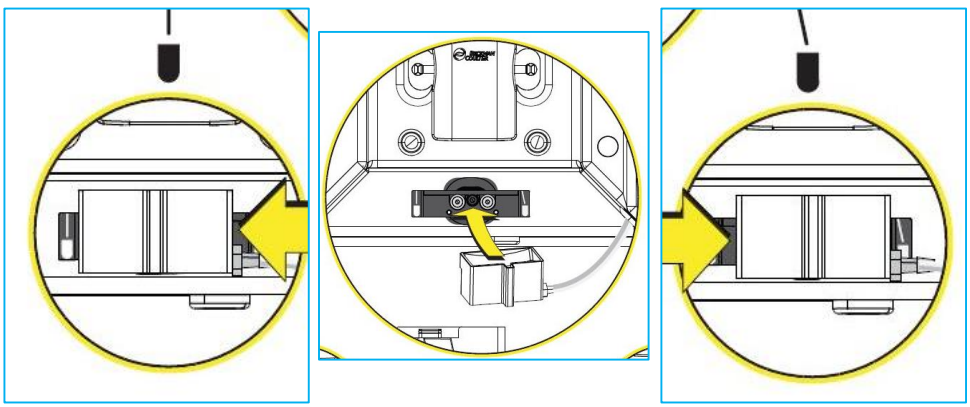
Prerequisite:

A) Instrument has two Sheath tanks, one coupled to Instrument , one refilled for later use
B) Waste is empty
C) Shut down fluid is not empty
D) Deflection plates ad illuminator are clean

Decision point: Decide whether you want a plate or tube sort .

- This can be done later, but will need a Standby and debubble.
- The lower bar of the software gives you important information, in wich sort mode the doftware is.
- Please cross check with the actual position of the waste drawer.
- Changes of sort mode must be done in software AND the waste drawer
- Sortmode change Software: Sorting>Stream mode switch (pop-up window instruction)
- Sortmode change Instrument: STRAIGHT DOWN (LEFT), DEFAULT (RIGHT)

Straight Down Sort	Default Sort
• Straight down modus • Centre stream is used for sort • Sorted drops lets are NOT charged • 96 deep well and 384 only supported by straight down modus	• Side stream modus • Side stream droplets will be charged • Up to four side streams are used for sort • Tube sorts • plate sort up to 96 well plate



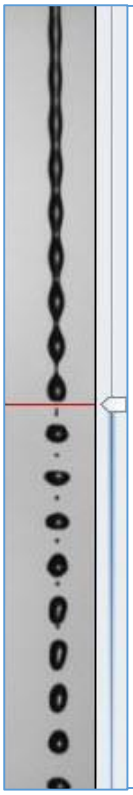
Following the pop up window instructions carefully drag out the waste drawer (magnet holding)
And place it to the left or right for straight down or default sort set up

Having a mismatch between Software and Hardware
Will cause flooding (the instrument) and errorneous sort

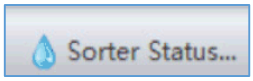
Start-up 10 min

Prerequisite:

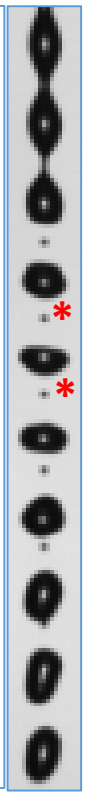
A) Instrument has two Sheath tanks, one coupled to Instrument , one refilled for later use
B) Waste is empty
C) Shut down fluid is not empty
D) Deflection plates ad illuminator are clean



1) Switch ON: Computer andf Waterbath
2) CytExpert > Cytometer > Turn ON



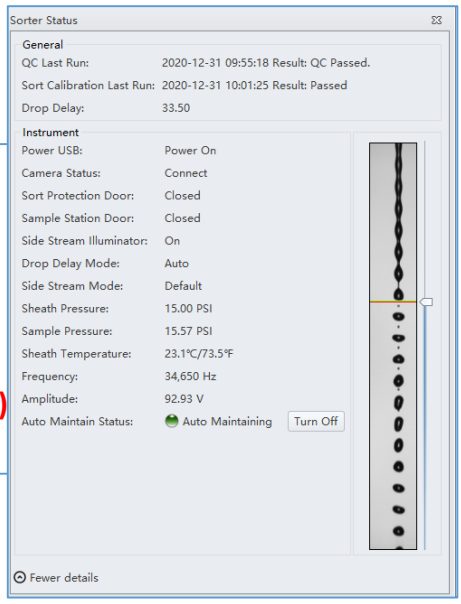
3) Have the sorter status open to see stream
4) >Cytometer>Start up (Follow instructions of pop up window)
5) After start up : do twice a >Cytometer> Sheath filter de-bubble
6) Followed by check of any air bubbles in filter
7) Followed by twice cytomter >Flow Cell Debubble
8) Check stream, satellite droplets* fuse to droplets (often top)
9) QC> QC/Standardization >Start QC > Run QC /drop delay *
10) Drop delay separately done via >Sorting>Sort Calibration
11) You still can do the debubbling from here
12) Run Droplet break off point
13) Mark the green line of break off point with red line to indicate postion changes/retain



QC / drop delay is not explained in this manual,
but will be shown in hands-on session. → Automatic /Manual way

Have the Sorter Status window always open
- **Check that Auto Maintain is ON**
- Monitor that break off point is overlaying with the green line

In case the stream must be stopped and restarted
- Check that break off point is back at position marked by green line
- IF NOT: RUN **>Sorting>Sort Calibration (Pop up window instructions)**
- Auto-Maintain



Setting up Experiment and Sorting

Explanation of Sort modes

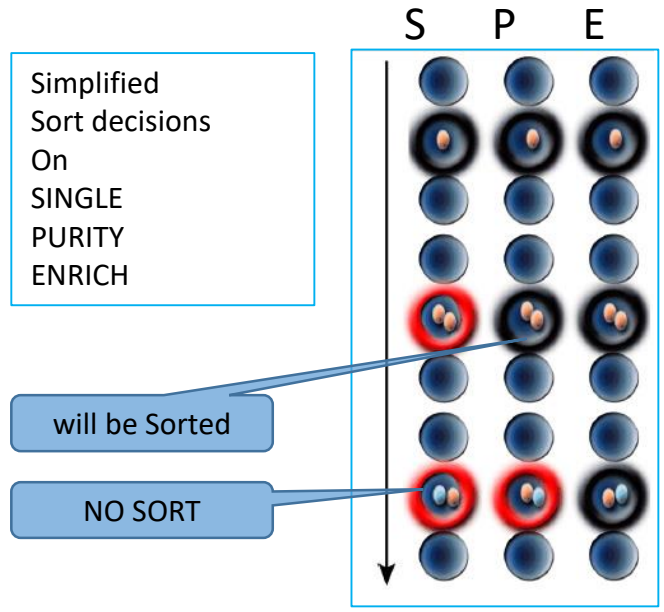
Single Mode - when having only one event per drop is the most important aspect of the sort.
Purity Mode - when the purity of the sort is the most important.
Purity1-2 Mode - when the purity of the sort is the most important. Purity 1-2 Mode is more inclusive than Purity Mode.
Enrich Mode - when recovery is the most important aspect of the sort. With Enrich, all positive events (an event that falls within the sort logic) are sorted
NOTE
Beckman Coulter recommends the Purity 1-2 Mode for sorting macro-particles.

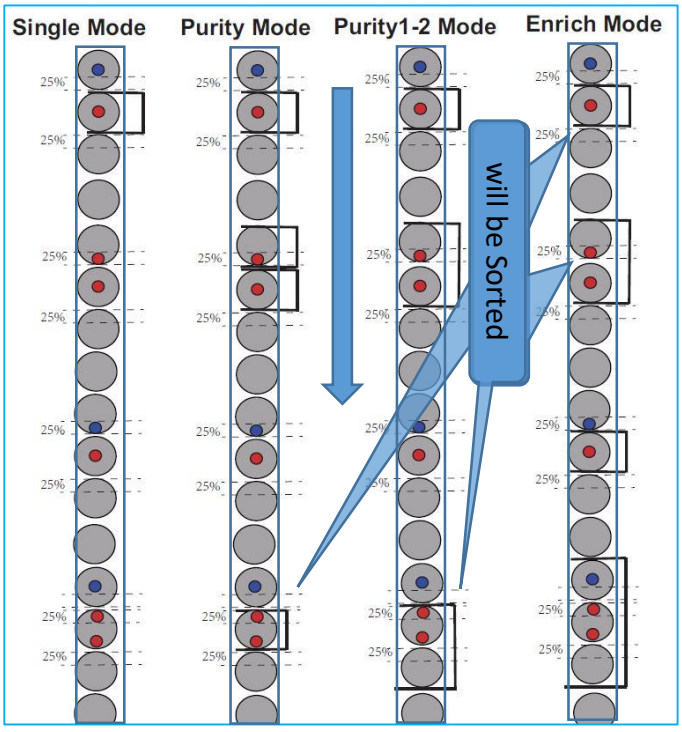
Image to the right illustrates actually the adjacent stream BEFORE the sort droplets appear. Direction of Flow is indicated. **Target** population is red dot, **contamination** is blue. IFan event is not in the centre of the droplet, it might end up in the preceeding or trailing droplet. Depending on sort mode, such "just in case" droplet (preceding or trailing) is sorted as well .
Single mode: no contaminant gets sorted. Stricly one drop let is sorted, target needs to be droplet-centered, and only ONE target event per droplet. Is accepted.contamination should not end up in such "target"-droplet
Purity mode: Kind of a " wider Single mode". Target can be 1 an more per droplet, no contaminant will be sorted.
Purity 1-2 mode: No contaminant gets sorted. Preceding/trailing droplets will get sorted.
Enrich mode: Contaminant will end iup in sort.When ever a target can get sorted it will get sorted, irresoective whether or not a contaminant is present.

Each sorted droplet will be charged in DEFAULT mode. In STRAIGHT down mode all non-sorted droplets are charged.

BioVis is using guard band 25%, for best purity. 15% is also available (0.1% error)

Simplified
Sort decisions
On
SINGLE
PURITY
ENRICH





Setting up Experiment and Sorting

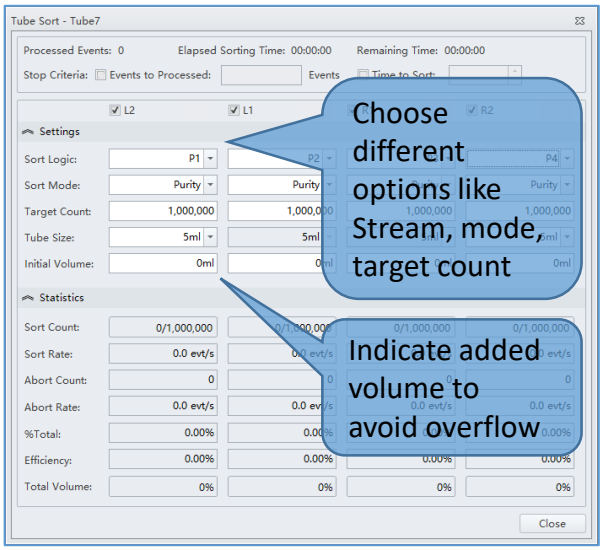
CytExpert is considered are selfexplanatory software, so only brief infor will be given here.

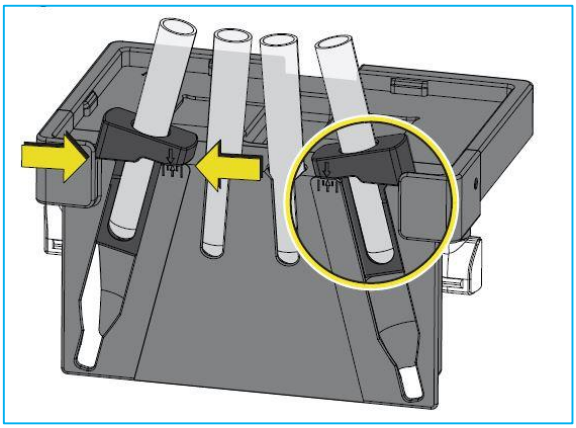
The CytExpert Software will not be explained here anyfurther Please check the manual for CytExpert to get to know how to Set up an experiment

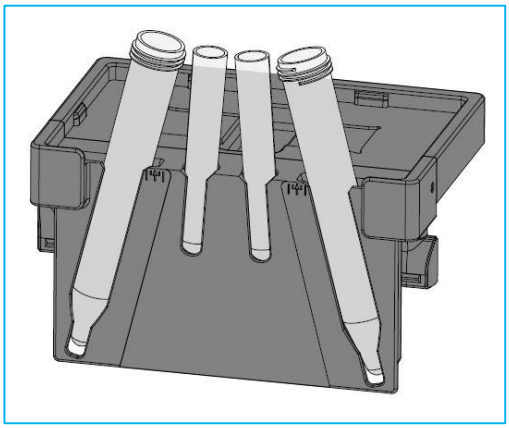
1. Set up an Compensation experiment (or have a matrix ready to apply to actual Experiment)
2. Set up the experiment with plots and gates
3. Open the ”Acquistion set up” window and load the default 100 file, to have all values on 100
4. Tubes (in software) with data cannot be used setting up sort
5. Have an empty tube to apply sort set up

Sorting gates can only by
8 steps deep
into the gatingHierarchy
Autogates cannot be used
For Sort gates

Check that droplets enter the sort tubes in case alignment is needed:
Sorting > **Tube Position Setting**
More alignment options are manually over the outer tube inserts







Sort Stream Predence
Place most precious and/or rare events in outer stream (L2 or R2) for maximum purity.
Place sorting events of high abundency or larger size (above 15µm) to inner streams (L1 or R1)

Setting up Experiment and Sorting

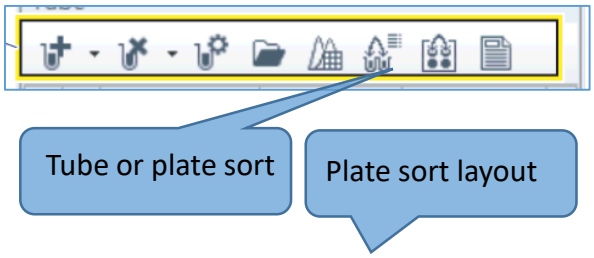
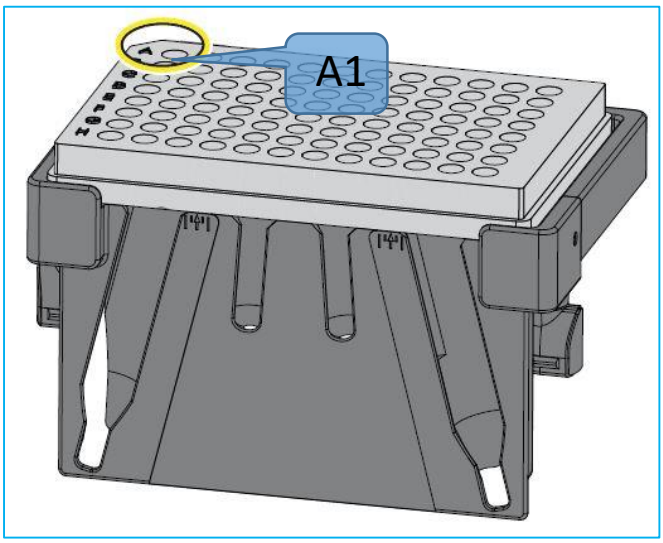
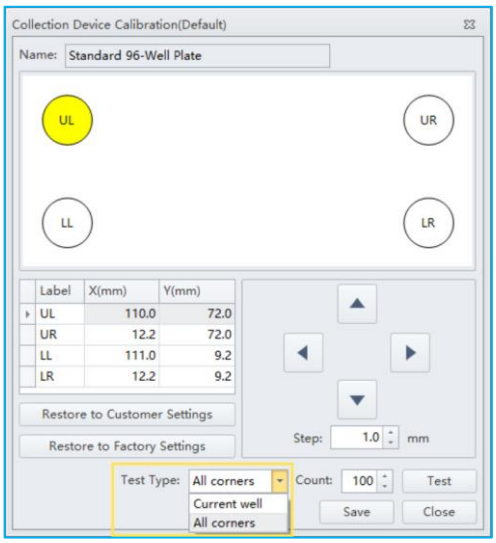


Plate alignment is needed to ensure that droplets hit the wells
>Advanced > **Collection Device Library**.
Then select **Calibrate** from the Collection Device Library window. Follow instructions of Pop-up windoe.





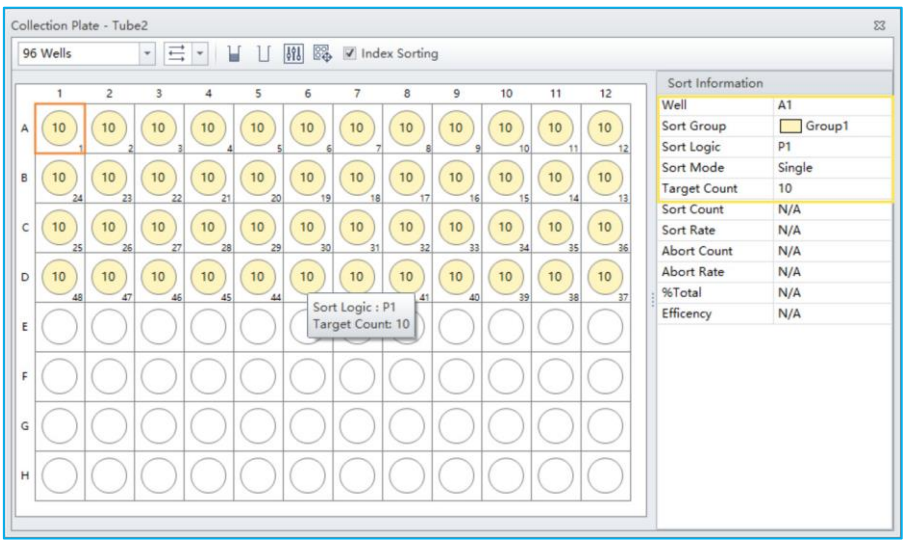
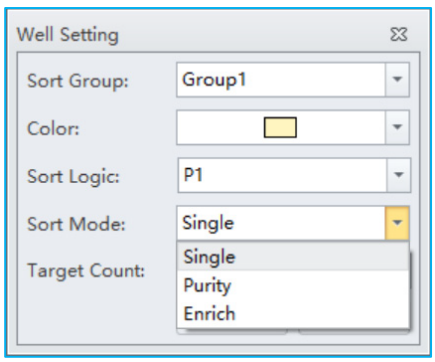
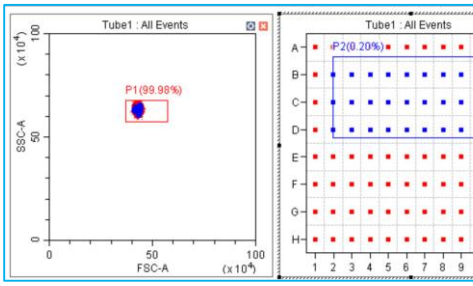


Plate sort layout is self explanatory,. Hover over icons And drop down menue to Get more information
One can group sort set ups, to Have them reused.



In case one plate holds sorts of rare events and abundant – make sure to sort the anbundant before the rare events, as more sample will be taken up to ”find” a rare event



Index Sorting selected: entire index sort data (max 30min) is recorded. Lets you also monitor which cells have ended up in the well (contaminant included in case…) Special gating are applied over the wells for that purpose.
Use the drawing Index sorting tool

Exporting Data and Sort reports

Export your data different options
- >File>Export FCS file
- Data to pdf
- Sort reports
- Statistic reports

Keeping it clean

For proper sorting the instrument must be able to take up sample, have a stable stream (with no satellite droplets) and must be able to properly charge droplets .

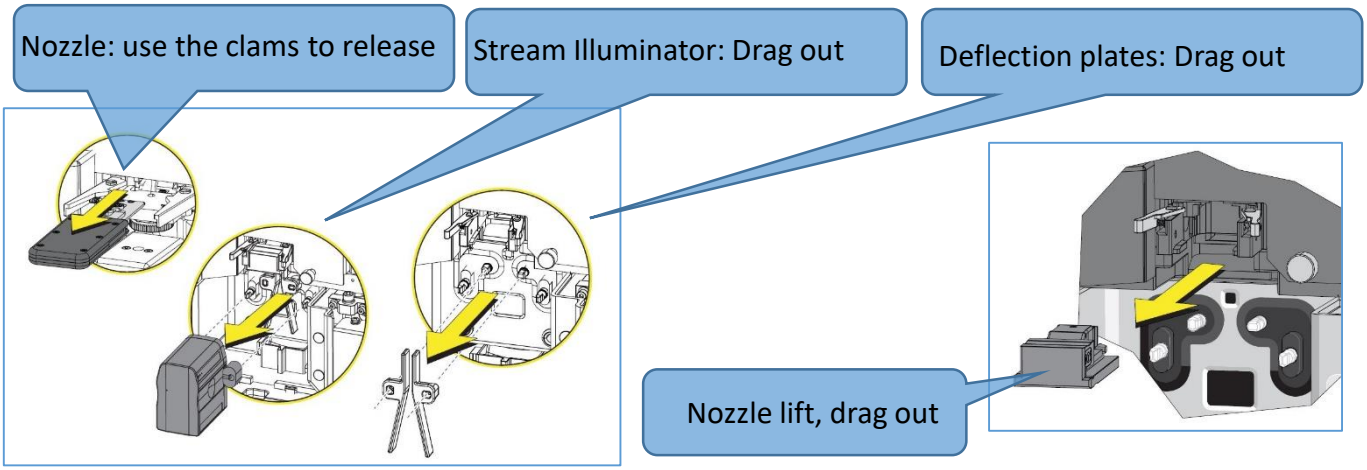
- Clogs and bubbles → **Debubble function** , Cytometer> Sheath Filter / Flow Cell debubble
- Dirty deflection plates → open sort chamber remove illuminator and plates
- Dirty camera windows → open sort chamber remove illuminator
- Clogged sample line I → low or no events, BACKFLUSH,
- Clogged Sample line II → Run Cleanz full speed,

When sorting is done

1. RUN CLEANZ in your experiment, followed by MiliQ water : No events of your sample must appear
2. Never leave the instrument without DAILY CLEAN, Cytometer > Daily Clean **3 min each**

Shut Down

1. RUN **DAILY CLEAN** >Cytometer>Daily Clean run , minimum **3 min** for each step
2. RUN **SHUT DOWN**, follow shut down procedure, Clean all necessary part
3. When done, **Switch OFF instrument** , Cytometer> Switch Off and **Waterbath**



Leaving the instrument to next customer on same day

Instrument is clean and on Standby

Some extra information

When **handling**

The instrument

- Dont open the sample door chamber when Sample chamber unit is moving

This cause way to much trouble ;)*

the sheath container

- make sure the lid is tight
- filling it up only until upper welding line

Pop-up windows

- Certain pop-up block the software
- Press hide button on the pop up to access software
- Find pop up window info in lower left of software

BACKFLUSH : Cytometer> Backflush

Clean the nozzle by

- Spray with EtOH
- Dab dry
- Use a syringe with MiliQ
- Place syringe tip on top of Nozzle O ring
- Press water through nozzle
- Stream visible
- Dab dry

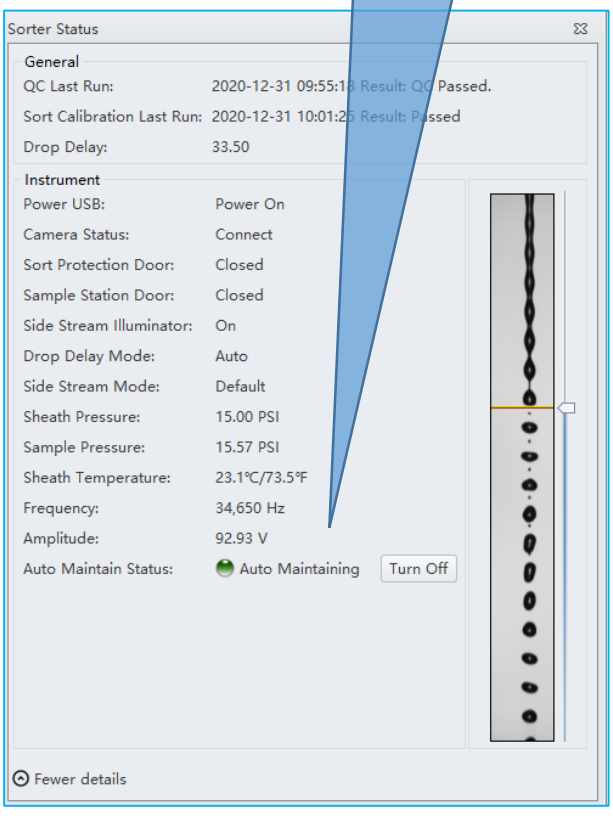
After start ups or Stream issues

- Check for bubbles in sheath filter
- Cytometer > Sheaht filter debubbling 2x
- Cytometer > Flow Cell debubbling 2x
- Proceed with drop delay >Sorting>Sort Calibration

*** Sample Chamber door**

Do not open it if sample chamber, which seals the sample holder is in move
Opening the door, whilst the moving, puts instrument into Standby
Usually the full repertoire of Debubbling, QC and drop delay is needed thereafter

IMPORTANT to have activated



Once system is ready, always make sure the Window ”Sorter Status” shows
That ”Auto Maintain” is on to monitor the stream automatic