UNIVERSITY of GUELPH
Advanced Analysis Centre

Molecular & Cellular Imaging Facility

Sony FACS SH800Z

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Fluorescence Assisted Cell Sorting
- Sony SH800Z -

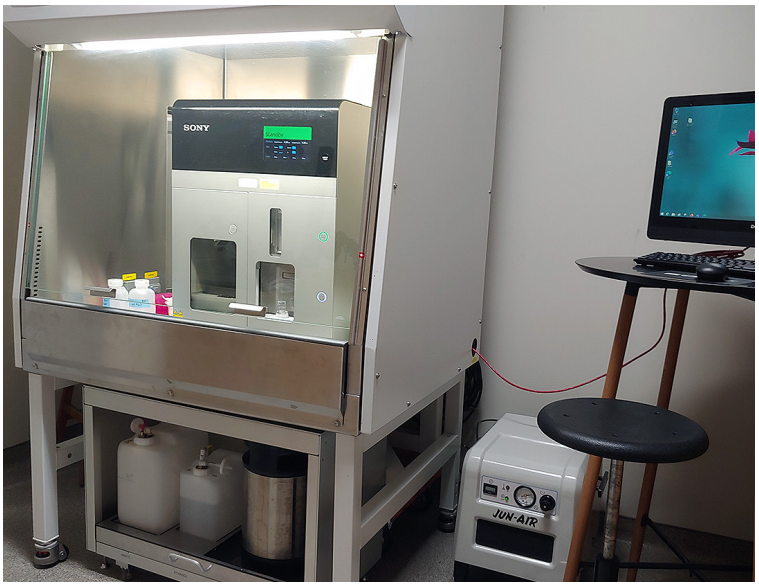
The SH800Z cell sorter permits analysis and sorting of a wide range of cell sizes and applications using 70 µm, 100 µm, or 130 µm microfluidics sorting chips. In addition, the software provides an assistive mode for large cells to improve sorting performance. It supports sorting into tubes (0.5, 1.5, 5, 15 ml) as well as high throughput sorting and precise deposition of cells into 6, 12, 24, 48, 96 and 384-well plates or PCR plates.

The optical design offers 4 collinear excitation lasers (405 nm, 488 nm, 561 nm and 638 nm) with one common beam spot for particle detection, 2 light scatter detectors (forward scatter and back scatter), and 6 fluorescence detectors. The six PMTs enable detection of fluorescence signals from any laser based on filter selection.

Temperature control pre-sort: 5 ˚C or 37 ˚C; post-sort: 5 ˚C

The software generates FCS 3.0 and FCS 3.1 files that also can be exported to third party analysis tools.

The custom BCC300AMS Class II biosafety cabinet provides protection for personnel and products.



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User Guidelines

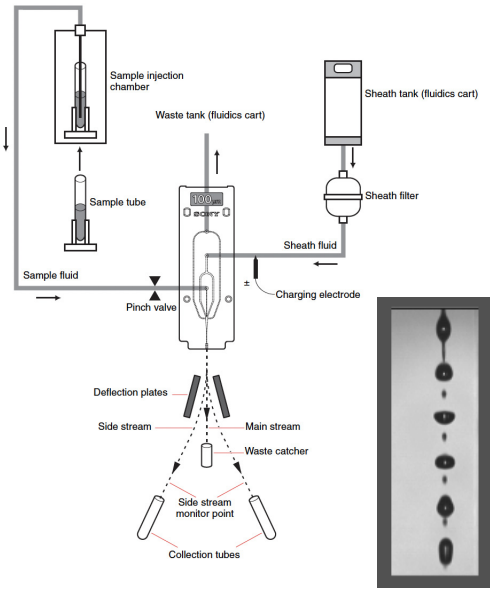
1. When you begin to use these facilities please provide a Billing Authorization Sheet including the supervisor's signature and the Trust Fund Account number.
2. Before any unsupervised access is granted, users must enroll in supervised training sessions during which they will review with the confocal manager how to operate the equipment properly and safely. The time required for the training sessions will vary depending upon the user's demonstrated competency with the equipment. Billing will be at the "Training" fee rate.
3. The instruments are booked in the GryphMail calendar. Unused booked sessions will be charged at 50% of the regular hourly rate and 100% if the instrument was left on overnight.
4. For any planned after hours use of the confocal system, please make arrangements with the confocal manager for access to the hallways.
5. Users are expected to bring all their own supplies including pipets, tips, collection tubes, buffer and media, etc. The facility provides sorting chips for a fee.
6. Files saved to computer hard drives must be removed as soon as possible. All computer hard drives will be cleared on a regular basis - it is the users' responsibility to manage their own data files. USB keys are not allowed on any of the instrument computers! The facility offers a variety of other options for data transfer.
7. Please notify the confocal manager immediately of any problems that you encounter with the equipment - it is essential that we work together in taking care of the facility. Improper care of the equipment will result in rejection of access to the facility.

Flow Cytometry Basics

A flow cytometer is made up of three main systems: fluidics, optics, and electronics.

- The fluidics system transports particles in a stream to the laser beam for interrogation.
- The optics system consists of lasers to illuminate the particles in the sample stream and optical filters to direct the resulting light signals to the appropriate detectors.
- The electronics system converts the detected light signals into electronic signals that can be processed by the computer. For some instruments equipped with a sorting feature, the electronics

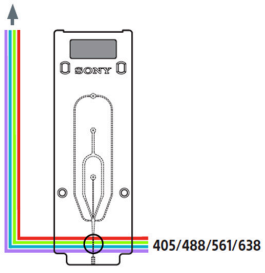
Basic principle of a cell sorter



After the sample is loaded, the pressure inside the injection chamber forces the sample fluid up through the sample probe and the sample line. It enters the sorting chip through the sample fluid inlet port via a pinch valve which opens/closes the sample line to control sample flow. The sample pressure determines the speed of the sample flow, which indirectly controls the distance between cells in the sample fluid flow.

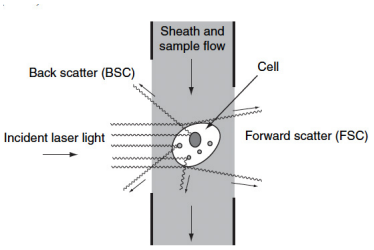
A transducer causes the sorting chip to vibrate at an ultrasonic frequency as fluid flows through the chip. These vibrations cause the fluid flow to be ejected in a jet of uniform droplets from the nozzle. At a point a short distance beneath the sorting chip, called the breakoff point, the fluid flow breaks into a very regular stream of uniform droplets. If the last attached droplet satisfies the sorting criteria, the droplet containing the cell receives an electrostatic charge just before the fluid flow reaches the breakoff point from a charging electrode located near the sheath fluid inlet on the sorting chip and retains the electrical charge after it breaks off from the fluid flow. Unwanted droplets are left uncharged and are collected by the waste catcher.

Hydrodynamic focusing



Sample fluid and sheath fluid are forced into the sorting chip using air pressure. The sample fluid is injected into the center of the sheath fluid stream, and surface tension and laminar flow keep both streams separated (hydrodynamic focusing of the sample fluid within the sheath fluid). The relative difference in pressure between the sheath fluid flow and the sample fluid flow forces the diameter of the sample fluid core to vary. When sample pressure is lowered, the diameter of the sample fluid core decreases, “focusing” the sample fluid into a narrow core stream. When the sample pressure is raised, the diameter increases with a corresponding increase in the event detection rate. The highest measurement resolution is obtained when the sample fluid flow is focused into a single-file stream of cells moving past the optical detection point at low sample flow pressure, maximizing the exposure to excitation laser energy that each cell receives. The diameter of the channel in the sorting chip narrows as it approaches the nozzle at the bottom of the chip, increasing the flow speed while maintaining the relative proportion between the sheath and sample fluid.

Light and Signal Generation



Light Scatter

When laser light hits a cell passing the optical detection point in the sorting chip, light is scattered in all directions. Light scattered in the forward direction is called forward scatter (FSC). Light scattered to the sides and back toward the light source is called side scatter (SSC) or back scatter (BSC).

In general, forward scatter can provide information about the size of the cell or can indicate the state of living cells. Back scatter can provide information about the internal detail of the cell, indicating complexity, granularity, and irregularities in a cell.

Fluorescence

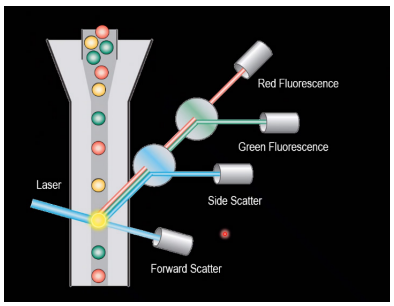
When laser light hits a cell passing the optical detection point in the sorting chip, the excitation wavelength causes fluorochromes to fluoresce in the sample, producing fluorescent light (FL). Fluorochromes emit high-level fluorescent light when excited by lasers at specific wavelengths and low-level fluorescent light at non-specific wavelengths.

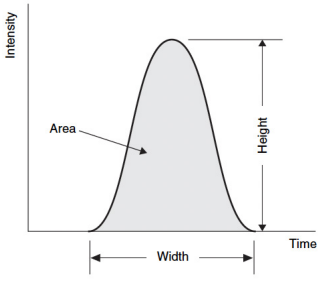
Pulse shape analysis

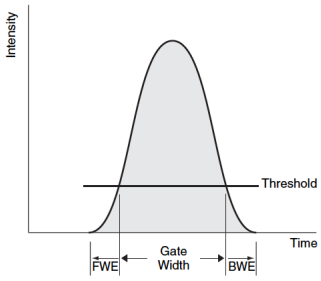
The laser lines are combined into a collinear beam and the detection module takes the light collected from the objective lens module and separates it into up to eight channels, comprising FSC, BSC, and six channels of fluorescent colour light (FL1 to FL6). These fluorescence signals are separated by PMTs (photomultiplier tubes) that convert the light energy into an electrical signal. The detection module is basically an array of dichroic longpass filters (LPF) and bandpass optical filters (BPF); LPF reflect light below a certain wavelength and transmit light above this wavelength, BPF transmit light in a narrow bandwidth and block all other wavelengths, i.e. they are quite specific.

When a cell passes through the laser beam, scattered and/or fluorescent light is detected and an electronic signal (= pulse) is generated. Analysis of the shape of this pulse (area/height/width) can give information about the size and shape of the cell.

When sorting or analyzing cells, you can set a threshold level for the pulse height (intensity) to discriminate between real events and background noise or debris. The threshold level for a channel sets the minimum light detector output that is required to record an event.







Sony FACS SH800Z: General Information & Care

Optical Configuration

Below is a table with the options for fluorescent markers.
For panel design, select one dye from each FL channel. Dyes from the same FL channel cannot be run together.
Each dye's circle label indicates its excitation laser. FSC and BSC scatter is detected with the 488-nm laser.

405	488	561	638		
FL1 450/50	**FL2 525/50**	**FL3 600/60**	**FL4 665/30**	**FL5 720/60**	**FL6 785/60**
BV421	AmCyan	BV570	BV650	BV711	BV785
Alexa Fluor® 405	BV510	Zombie Yellow™	7-AAD	PE-Cy™5.5	PE-Cy™7
Zombie Violet™	T-Sapphire	LIVE/DEAD® Yellow	PE-Cy™5	PerCP-Cy5.5	APC-Cy7
LIVE/DEAD® Violet	Pacific Orange™	BV605	PerCP	PerCP-eFluor® 710	APC-Alexa Fluor® 750
DAPI	Zombie Aqua™	PE	APC	APC-Cy5.5	APC/Fire™ 750
Pacific Blue™	LIVE/DEAD® Aqua	PE-Texas Red®	Cy5	Alexa Fluor® 700	Zombie NIR™
mCFP	EGFP	PE/Dazzle™594	Alexa Fluor® 647		LIVE/DEAD® NIR
BFP	CFSE	PI	LIVE/DEAD® Far Red		
SYTOX™ Blue	FITC	LIVE/DEAD® Red	mPlum		
Hoechst 33342	Alexa Fluor® 488	Zombie Red™			
	SYBR® Green	mOrange			
	EYFP	tdTomato			
	mVenus	DsRed/mRFP			
	mCitrine	mCherry			
	LIVE/DEAD® Green	Alexa Fluor® 594			
	Zombie Green™	mKate			

Sample Preparation, Controls, and Set-up

Sample preparation is very important and depends on what kind of experiment you want to run.
You can bring your sample either in media for the cells or in FACS buffer (1x PBS with 2% FCS and 2mM EDTA). To keep live cells happy it is useful to work swiftly and at a steady temperature (samples can be run at 4 ˚C, RT, or 37 ˚C; collection is possible at 4˚C or RT). It is strongly recommended to filter the cells no more than 30 minutes before running them on the flow cytometer: 40-70 µm nylon mesh is perfect, there are also specific filter tubes with cell strainer (Falcon) available – Corning 352235.

Make cells as concentrated as possible – it's always easier to dilute. However, if cells are too concentrated, the sample line will clog up. **Recommended concentration: 5-9 x 10^6**

Include a viability stain as control to avoid analyzing/sorting dead cells.
If you are planning to sort your cells, please bring the relevant collection tubes/plates, each tube and each well prefilled with an appropriate amount of collection media (this depends on the amount of cells to be collected and the downstream protocol).
Please bring the following controls (depending on your experiment):

negative control without any marker
individual positive control for each fluorescent marker

Sony FACS Start-up and Sample Set-up

System Startup and Software Startup Window

The cell sorter will already be started, cleaned, and all fluids topped-up when you arrive for your session. This is done by staff and a set-up fee will be charged per day. Each sorting chip is valid for 24 hours, the sorting chips are sold by the facility at cost and will be included in the monthly invoice. In case several groups use the cell sorter during the day, the set-up fee and sorting chip cost are split equally.

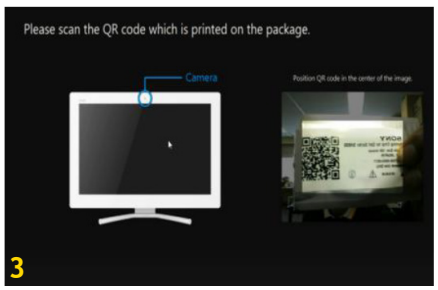
Upon your arrival, you will see the login screen (Fig. 1).

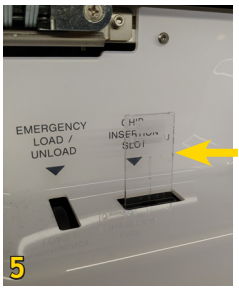
Chip and Sorting Calibration

This calibration is necessary for each sorting chip and it is valid until the software is closed. If someone else is scheduled after you, you can select 'Keep calibration for the next user' but it is only held for up to 60 minutes.

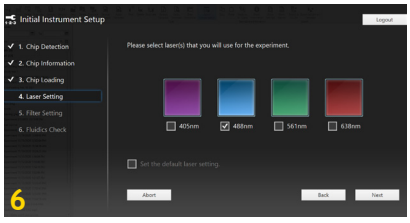
1) Log into the software with your user name and password.
2) Once you log into the software, you will be guided through the chip and sorting calibration. This initial calibration is run through a wizard and will prompt what needs to be done.
First, you need to scan the QR code on the sorting chip (Figs. 2,3 - package provided by staff) and, when prompted, open the top hood of the cell sorter and exchange it for the cleaning chip (Figs. 4,5).

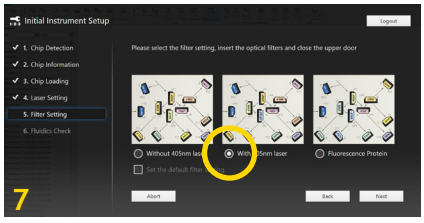
Important: The cleaning chip can be used several times - please place it back into the package in the top hood!



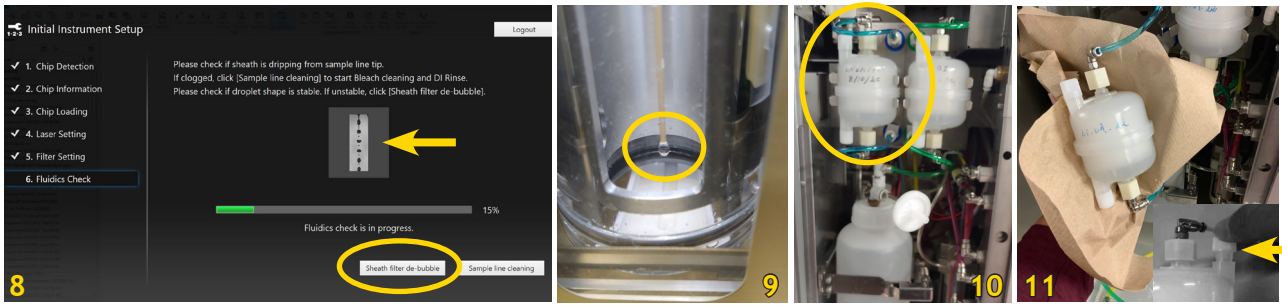


3) Select the lasers you need for your experiment (Fig. 6) and confirm the default optical layout (Fig. 7, do not change it!). The 488 laser always needs to be selected!!





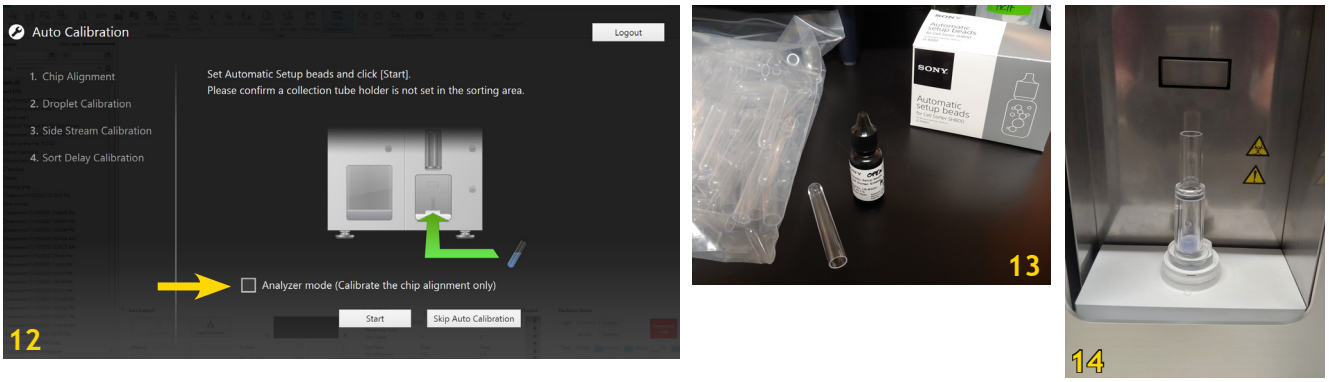
4) The system will now perform a fluidics check (Fig. 8, takes about 5-10 minutes):
Select ‘Start’ and wait; the system will do 10 chip de-bubbles. Follow the instructions on the screen. If the image of droplets is unstable or blurry (Fig. 8, arrow), please select ‘Sheath Filter De-Bubble’ (Fig. 8, circle) and follow the instructions (see below). Also check if there are drops coming from the sample probe (Fig. 9). Observe the sample probe and if no drops are formed, select ‘Clean Sample Line’ (Fig. 8) and follow the instructions.



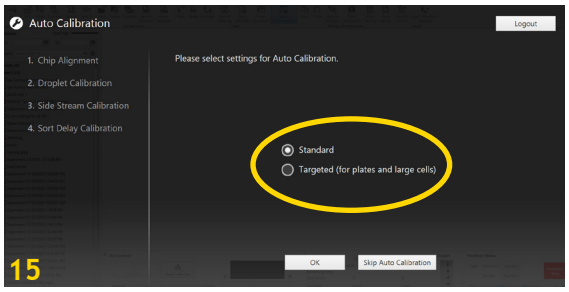
Sheath filter de-bubble (Figs. 10, 11):
Start the wizard and wait. The system will do several sheath filter de-bubbles. Once this is completed, pull the sheath filter out of the bracket (don’t disconnect!, Fig. 10); cradle it in paper towels in your hand, hold at 45 degrees with the valve on the top side, and knock 3x hard on the side wall (above middle) – then open the valve to vent and vacate air pockets (Fig. 11); wipe up any spillage and return filter into the bracket; check that lines are not kinked but mobile. Check the live stream image of the droplets to make sure they are stable. Click OK.

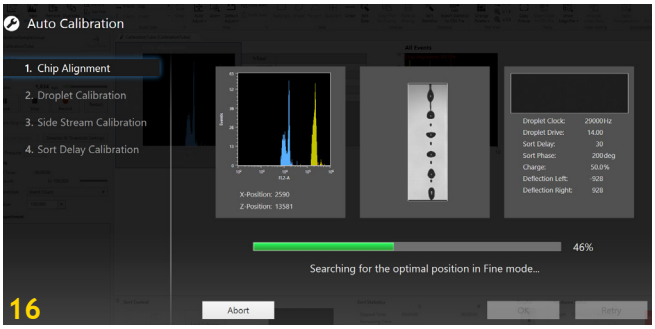
Clean Sample Line:
This will guide you through Bleach and DI water rinses, exactly as during the logoff or shutdown procedure (see page 18).

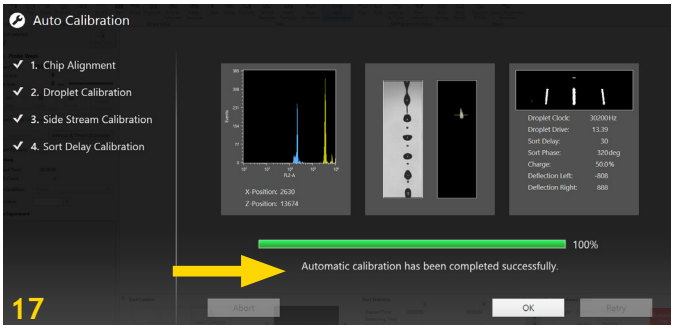
5) Once the Fluidics check is completed, the autocalibration wizard will start (Figs. 12-17) – autocalibration can take 15-20 minutes, depending on the nozzle and whether sorting calibration is included or not (100 µm sorting calibration takes about 20 minutes).
6) If you run a sample without subsequent sorting, select ‘Analyzer Mode’ (Fig, 12, arrow).
7) Use 8 (Analyzer Mode) or 12 (Sorting Mode) drops of undiluted AutoSetup Beads (in fridge) and drop into the 5ml polyethylene tube (clear), use the 5ml tube holder; make sure to shake the bottle vigorously before dispensing the beads; never dilute the suspension; use the bottle completely up before opening a new one (Figs. 13, 14).



8) For the Sorting Calibration, the 100 µm nozzle has 2 optional settings: ‘Standard’ (for regular cell suspensions and 2-way sorting) or ‘Targeted’ (for amorphic or large cells or to do plate sorting) – select which is needed (Fig. 15).







9) Automatic Calibration for cell sorting automatically runs through the following steps (Figs. 16, 17):

Chip alignment
Droplet calibration
Side stream calibration
Sort delay calibration

‘Analyzer Mode’ will only perform the chip alignment!

In the final calibration screen (Fig. 17), make sure it says ‘Calibration completed successfully’ and then click ‘OK’ - the Experiment window will open and the instrument is now ready for analysis and sorting.

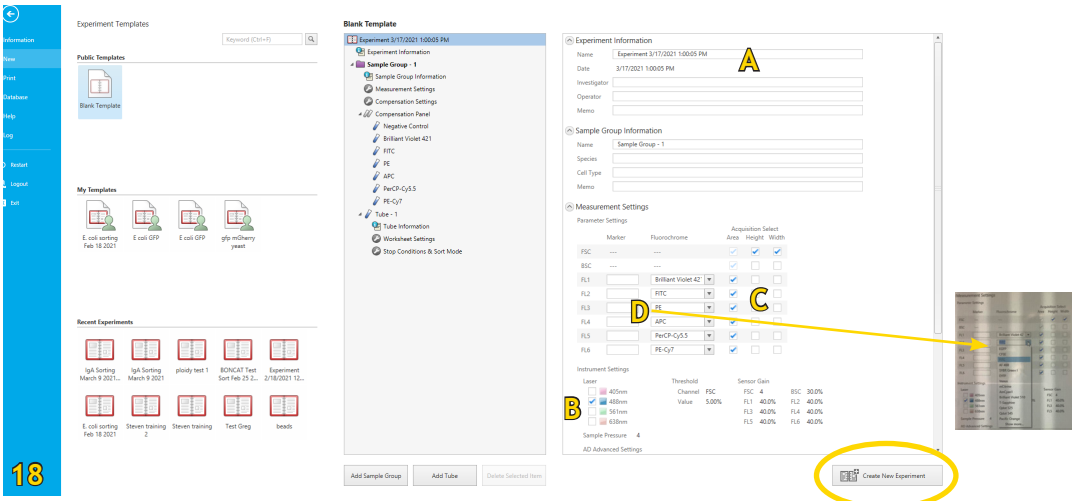
Important: Should there be any problem with the chip and/or sorting calibration, please let staff know immediately!

Experiment Window

In the Experiment window, you can select a file name (Fig 18A) and note other details about your experiment.

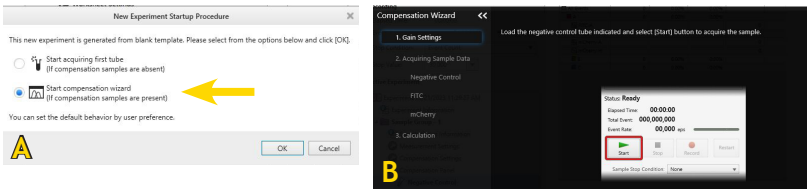
Laser Selection and Activation

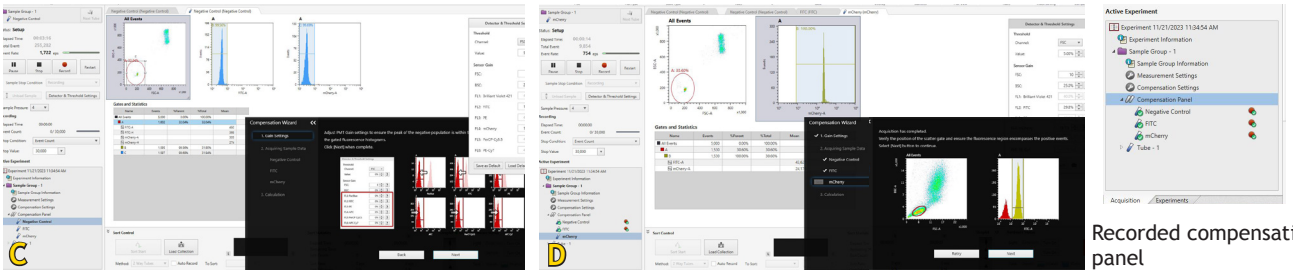
You need to select the lasers (Fig. 18B) and the detection channels (Fig. 18C). In the drop down menu beside each channel, you find a list of specific dyes/fluorescent proteins that help you with the selection (Fig. 18D).
Once you have selected all parameters, click ‘New Experiment’ (Fig. 18, circle).



Compensation Wizard

If your sample contains more than one fluorescent marker, the software will offer to run a compensation wizard before you start acquiring your data (Fig. 19A).
The Compensation Wizard will be initiated at the start of the experiment: it will prompt for and run through all controls (negative, each fluorophore separately), and finally calculate a compensation matrix for your specific set of markers (Figs. 19B-E).
You can choose not to follow the wizard but then you will need to do this on your own or compensate manually after data acquisition (not recommended for new users!).

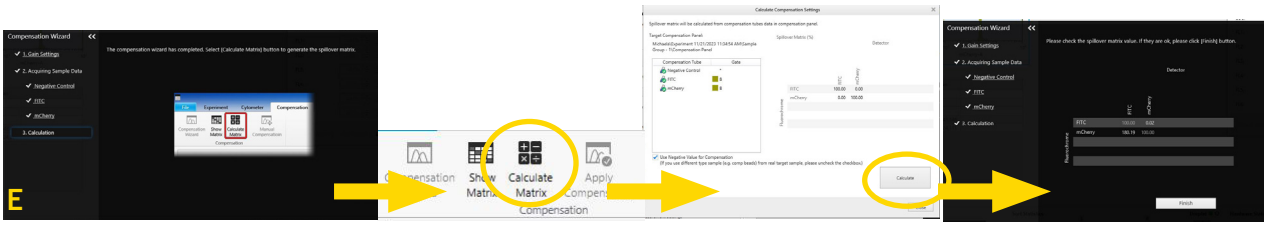




Negative Control

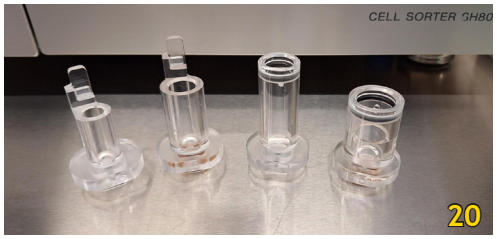
Single Positive Control

Recorded compensation panel



Cell Sorter Data Acquisition

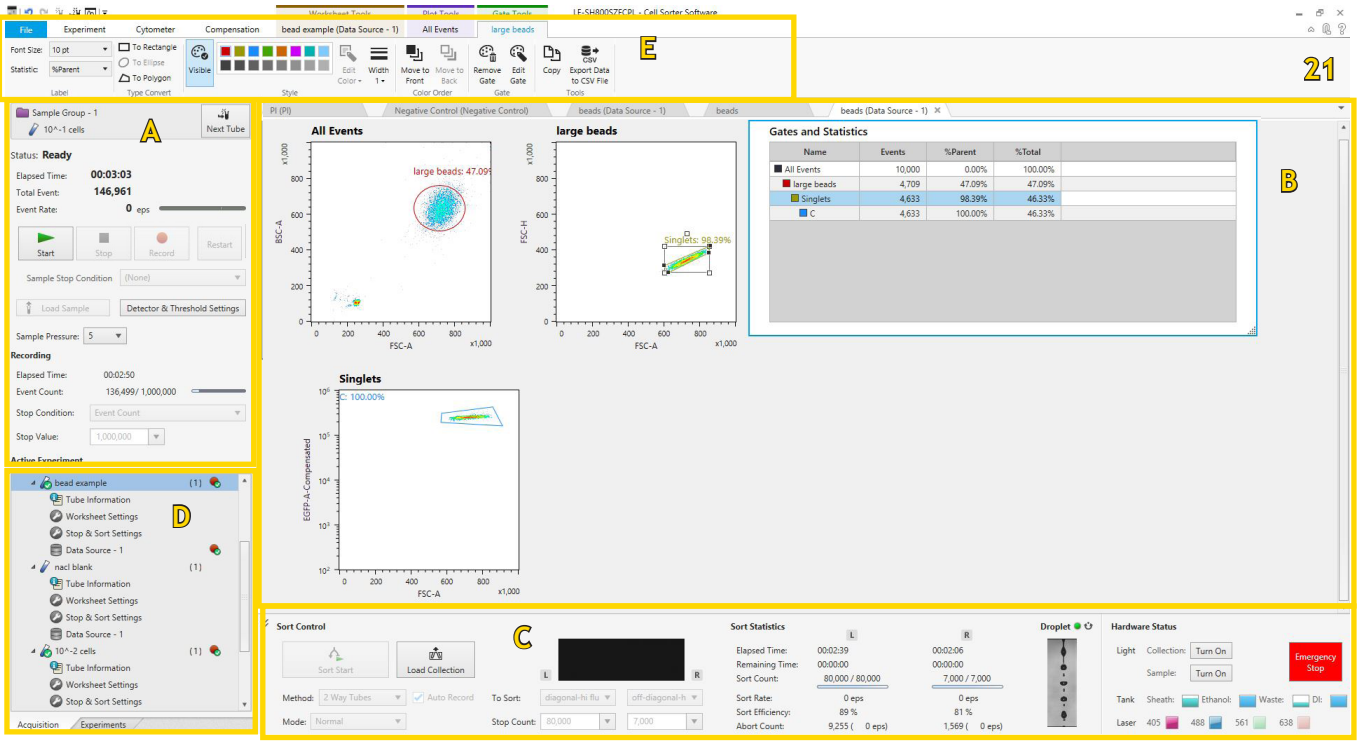
Place your sample in the correct sample holder (Fig. 20) - if you use a microcentrifuge tube, make sure its lid is clamped into the lip of the holder.



Once the samples are in the instrument, switch off the BSC lights as much as possible.

Control Interface

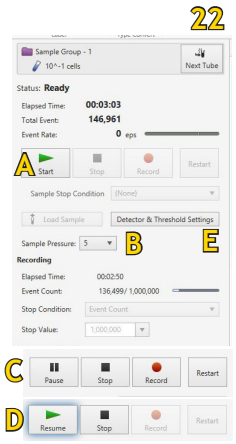
Once you select ‘New Experiment’ (and possibly completed the Compensation Wizard), the Control Interface opens (Fig. 21).



It consists of the following areas

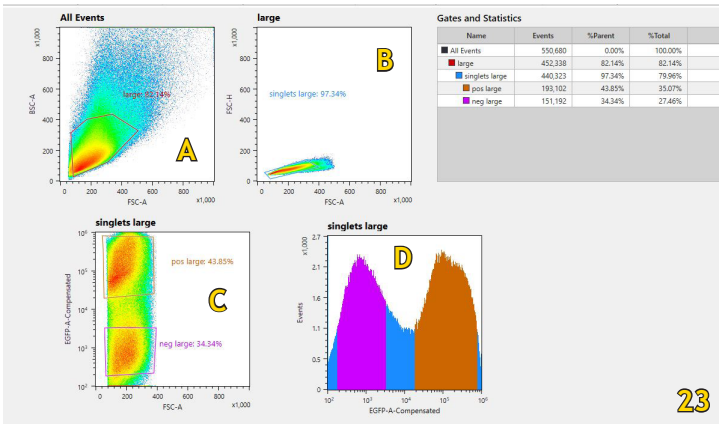
A - Flow and Detection controls
B - Data Acquisition with Scatter Plots
C - Sort Parameters and Controls (not visible if ‘Analyzer mode’ was selected)
D - Tubes (= samples)
E - Other controls

Sample Set-up



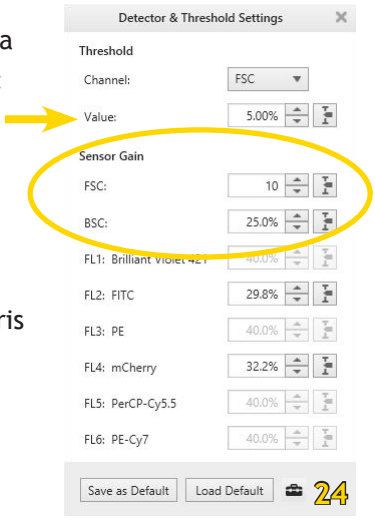
Load the sample into the sample chamber and select 'Start' (Fig 22A)
The instrument will move the sample up so that the sample line immerses into your tube.
There's an initial boost (=maximum sample pressure) to force the liquid through the sample line and chip.
The sample pressure will then be lowered and the sample flow is steady after approx. 40-60 seconds.
Don't start recording or sorting before!
The default sample pressure is 4 - you can decrease or increase it, depending on the registered events per second (eps, Fig. 22B), your cells, and your sorting preferences (see page 4, Hydrodynamic Focusing).
Always select 'Restart' (Fig. 22C) after changing parameters and defining a gate to clear the previous events to avoid confusion.
Never select the 'Stop' button to pause sample flow - it will withdraw the tube and assume this sample is finished. Always select the 'Pause' button and then click 'Resume' to continue (Fig. 22C, D).
Important: Data will only be saved if you press the red 'Record' button (see below).

Gating and Scatter Plots



Before you can analyze or sort your cells, you need to set all parameters correctly and define the populations to be sorted/analyzed. This is called 'Gating'.

By default, the first scatter plot is already loaded: PLOT 1 (Fig. 23A) is the light scatter (FSC-A/BSC-A, usually linear scale) to determine gain and threshold settings to make sure the cells of interest are well displayed on the plot; when you see (or don't see) the events accumulating in a certain area on the plot, open the Detector & Threshold Window (Figs. 24 and 22E):



- Adjust FSC gain (value 1-16) to move the events of interest towards the middle region of the x-axis scale
- Adjust BSC gain (value 0-100%) as needed to move the events of interest towards the centre of the y-axis scale
- Adjust the Threshold settings for FSC BSC (value 0-100%) to eliminate excess debris at the low x- and y-axis scales
- After restart, move the gate around the cells of interest and adjust its size.

Then double-click in the gate of PLOT 1 to create a second scatter plot, depending only on this population.

PLOT 2 (Fig. 23B) is light scatter area vs height (FSC-A/FSC-H, linear scale) for Doublet Elimination of the gated population in plot 1. You can change the parameters of an axis by clicking on it (Fig 25A).

FSC-H = max. intensity (peak) of forward light scatter

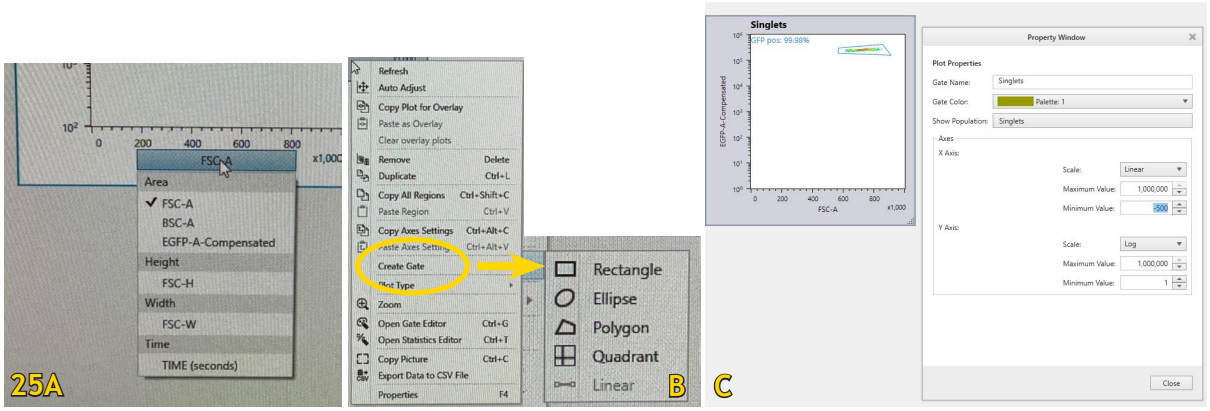
FSC-A = total area forward light scatter signal

For single cells, the ratio of FSC-A to FSC-H is approximately 1; for cell aggregates, the ratio of FSC-A to FSC-H < 1

Find the diagonal and eliminate pixels below 1 by gating

To create a gate, right-click in the plot and select 'Create Gate' (Fig 25B) - in the submenu select 'polygon' - left-click to set markers around your population and end by double-clicking to close the gate.

Double-click in this gate to create a third scatter plot (and possibly more), depending only on this population.



PLOT 3 (Fig. 23C) is Fluorescence (FL1/FL2, log scale) or Fluorescence vs light scatter (FL1/FSC-A, log and linear scale, respectively) of the gated population in plot 2.

If you define a log scale, set the maximum value to 100,000 or 1,000,000 and also define a negative area to -500 (Fig. 25C). You can access the Plot 'Property Window 'by right-clicking on the plot and selecting 'Properties', Fig. 25B).

Adjust the Fluorescence PMT values (Sensor Gain FL1-6, Fig. 24) as needed to maximize separation of the FL-negative and FL-positive populations if you haven't done this already in the compensation wizard.

Repeat this for all fluorescent markers/maker pairs and until all necessary parameters are set.

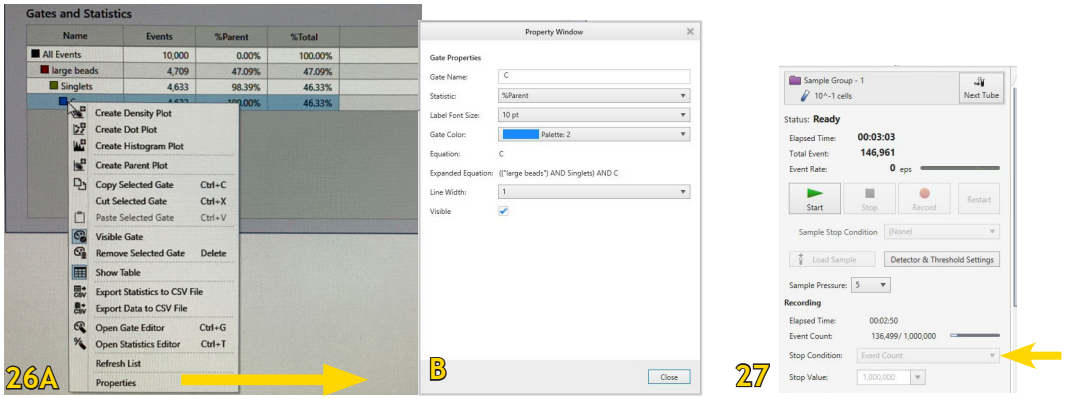
For certain applications (i.e. ploidy measurements), you can also create a 'Histogram Plot' (Fig. 23D) - here only one parameter (fluorescence) is displayed: the intensity is plotted on the x-axis with its corresponding number of events shown on the y-axis.

Continue with Data Acquisition or Cell Sorting.

Data Acquisition

Once all parameters and gates are set correctly, pause the stream and select the acquisition conditions.

First, make sure you have created gates around all populations that you want to analyze. You can rename the gates if you right-click on the population, select 'Properties' (Fig. 26A) and change the parameters in the pop-up window (Fig. 26B). Now you need to record your data to save them for analysis.



Recording Stop Conditions

There are 3 options for stop conditions of recording (Fig. 27, arrow):

Event count - Gated event count - Elapsed Time

Select which one is most appropriate for your experiment and define the amount of events or the time that you want to record. Press ‘Resume’ and then ‘Record’ (Fig. 22C, D) - all previous data points will automatically be deleted).

Important: Keep and eye on the event rate. If it slows down significantly, you need to perform a manual Bleach and DI Water rinse (Figs. 39 & 40, page 18).

Cell Sorting into Tubes

If you want to sort your cell populations, pause the stream and select the acquisition and sorting conditions. First, make sure you have created gates around all populations that you want to analyze.

Place your tubes in one of the collection tube holders (Fig. 28A) and place this on the metal plate in the sorting chamber (Fig 28B, arrow).

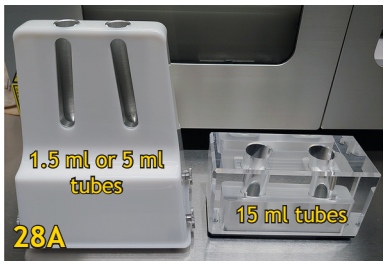
Select ‘Load Collection’ (Fig. 29A).

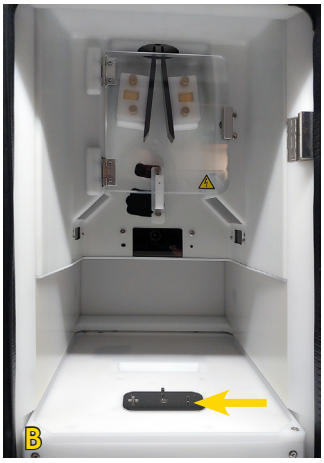
Define which gate will be sorted into the right tube, which one in the left tube, and the ‘Stop Condition’ (Fig. 29, circle), i.e., how many cells should be sorted into each tube.

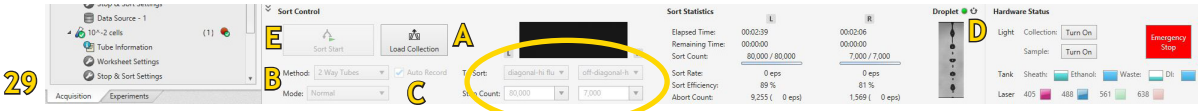
Define the sorting mode (Fig. 29B)

Select ‘Auto record’ (Fig. 29C).

When you’re sorting, keep an eye on the droplet breakoff control window (Fig. 29D). Green light indicates all is okay, orange indicates instabilities, and red means the stream is interrupted.

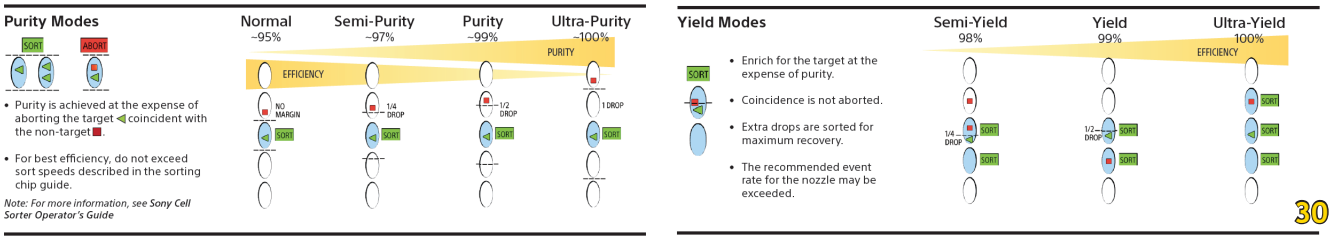






Sorting Modes

Depending on your requirements, you select a ‘Purity’ mode, or a ‘Yield’ mode (Fig. 30). This depends on your downstream application. Purity selects strictly for positive events, yield maximizes the yield of your sorting (if purity is not so much of concern).



Purity Yield

Select which one is most appropriate for your experiment and define the amount of events or time that you want to record. Press ‘Resume’ and then ‘Sort and Record Start’ (Fig. 29E) - all previous data points will automatically be deleted.

After the sort, run a sample evaluation:

Create 2 new plots (FL1/FL2 or FL1/FSC-A)

Run clean sheath fluid or clean PBS until there are no more event counts (<2 events/sec)

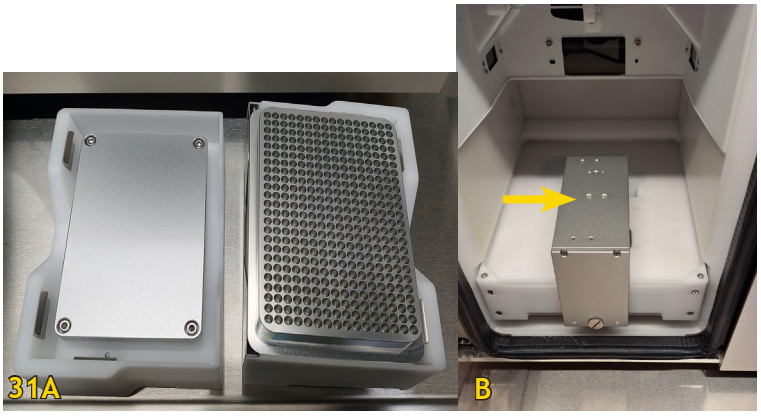
Select one of the sorted samples and either take an aliquot or the full sample to evaluate purity; let run until you have 100-500 events – this should give you enough data points to evaluate the purity.

Run clean sheath fluid or PBS again

Select the second sorted sample and repeat as above

Important: Keep and eye on the event rate. If it slows down significantly, you need to perform a manual Bleach and DI Water rinse (Figs. 39 & 40, page 18).

Cell Sorting into Plates



If you want to sort your cell populations into well plates for downstream applications, the same steps as above apply: pause the stream and select the acquisition & sorting conditions and make sure you have created gates around all populations that you want to sort.

You can sort into regular 6- to 96-well plates, into 384-well plates, or 96-well PCR plates.

Select the appropriate holder for your plate (Fig. 31A) and place your plate with the lid on in the plate holder on the arm in the sorting chamber (Fig. 31B, arrow). Select ‘Load Collection’ (Fig. 29B).

The next step is to calibrate the plate and then define which populations (gates) are sorted into which wells, and how many (see below).

Define the sorting mode (Figs. 30,32). Besides Purity and Yield, plate sorting also enables 3 Single Cell Modes:

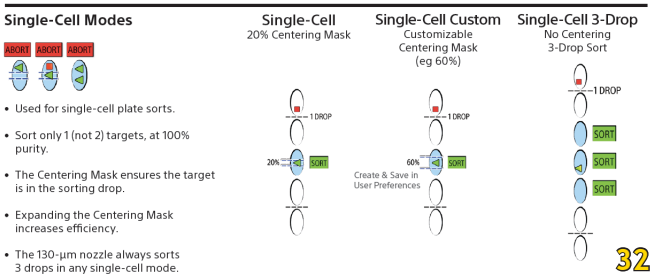
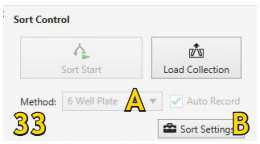


Plate Alignment



In the 'Sort Control' panel, select your well format (Fig. 33A) and then click on 'Sort Settings' (Fig. 33B).
In the 'Plate Adjustment' tab (Fig. 34A) select 'Four Corners and Center Well' (recommended) and select the number of droplets to be deposited on the plate lid (arrow). Click 'Start'

Once the test is finished, remove your plate and check the location of the droplet puddles (Fig. 34B).

Select a corner target well and adjust the droplet puddle position to the well's centre (Fig. 34C). Repeat with another corner well if necessary.
Wipe away puddles and repeat the alignment.
Once the puddles are in the centre of the wells, you can start sorting.

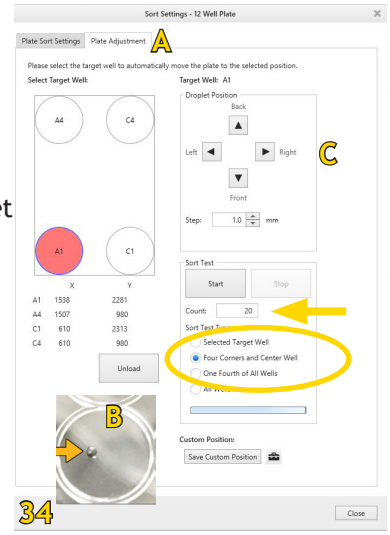
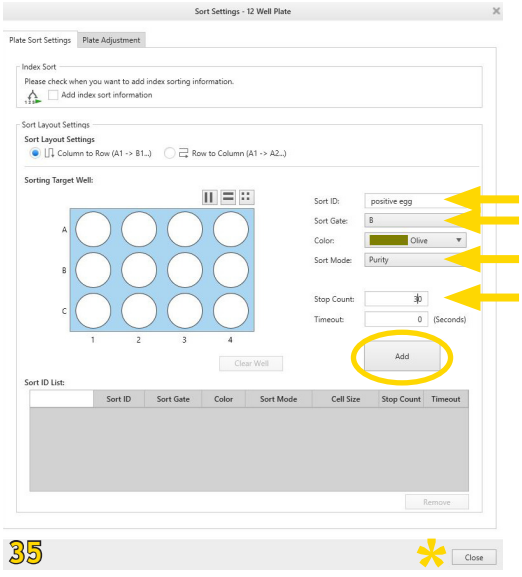


Plate Sorting

Remove the plate lid from your plate in the sorting chamber and close the door.
It the tab 'Plate Sort Settings' (Fig. 35), highlight the desired wells for each population, enter the name of your population, select the 'Sort Gate', confirm the 'Sort Mode', and enter the 'Stop Count' (how many cells per well).

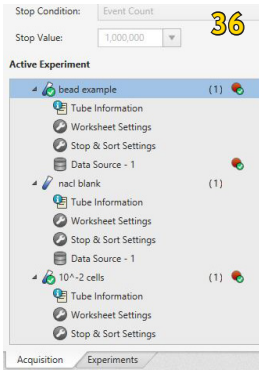
Click 'Add' to create the 'Sort ID'
Repeat these steps for each population. Click 'Close'
Double-check your gates and acquisition settings
Select 'Auto Record' and click 'Sort & Record Start'.
When completed, click 'Finish' to end your session or 'Continue' to sort into another plate.



Important:

Keep and eye on the event rate. If it slows down significantly, you need to perform a manual Bleach and DI Water rinse (Figs. 39 & 40, page 18).

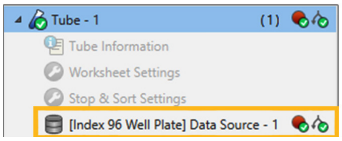
Data management



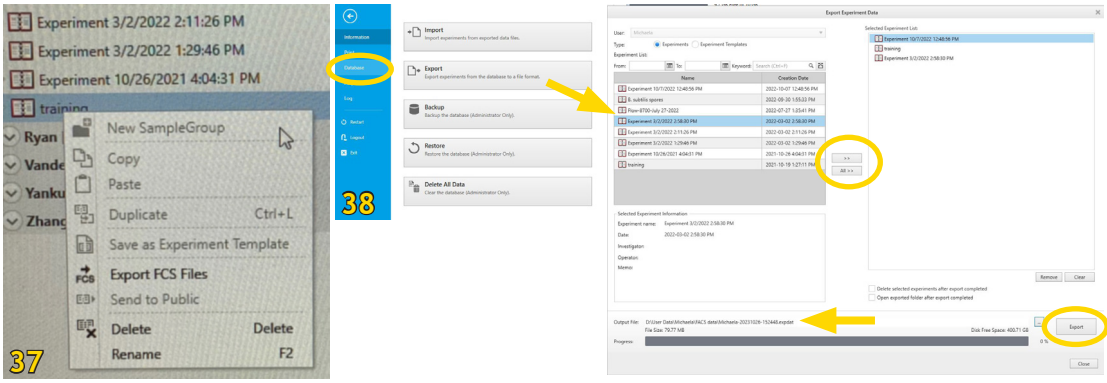
Your current recorded data files are stored in the database under the ‘Acquisition‘ tab (Fig. 36). Older eperiments can be accessed in the ‘Experiments ‘ tab.

You can save your data as FCS files - this is a general data format that can be read by third party flow cytometry analysis programs. In the database (Fig. 36), right-click on your experiment, select ‘ Export FCS Files’ (Fig 37).

You can also export them as ‘expdat’ files - this format is needed to import your files into the Sony Cell Sorter software on the image analysis compter of the facility.
Select ‘File’ - ‘Database’ - ‘Export’ - Select the files you want to export and define the destination folder - Click ‘Export’ (Fig. 38).



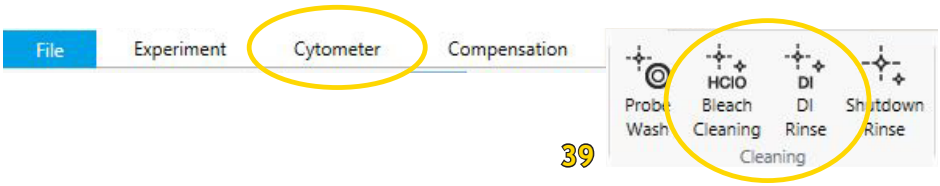
Files can be transferred via OneDrive.



Shutdown

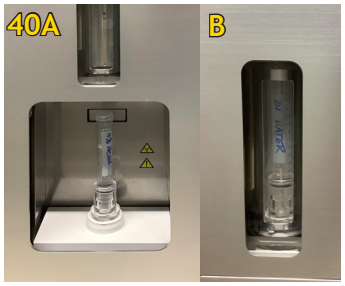
1-Hour-Rule:

Don't do a full instrument shutdown if someone is booked within the next hour after you finish.
There is an option to log off the software but 'Keep calibration for next user' - do this if someone else is booked within the next hour. You then need to do a manual bleach cleaning and MilliQ rinse:



Partial Shutdown

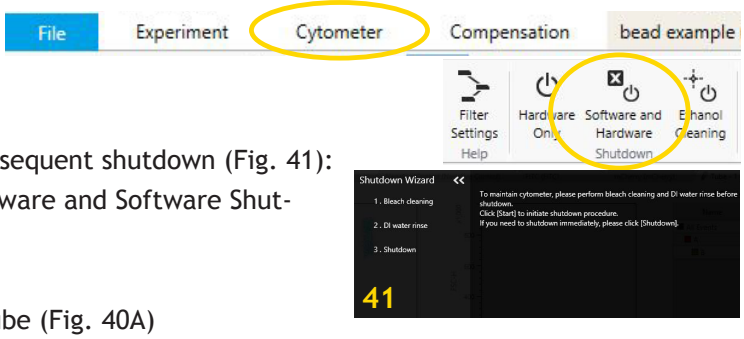
Remove your sample
Wipe the sample chamber briefly with Kimwipe and 70% EtOH
Perform a manual bleach cleaning and MilliQ rinse (Fig. 39):
Select 'Cytometer' - 'Maintenance' - 'Bleach Cleaning' (and follow instructions) and once this is finished 'DI Rinse' (and follow instructions).
The cleaning steps require
10 ml of fresh 10% bleach in 15 ml Falcon tube (Fig. 40A)
12 ml of MilliQ in 15 ml Falcon tube (Fig. 40B)

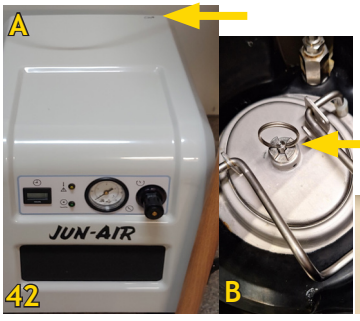


Select 'File' - 'Log out' and select 'Keep calibration settings for the next user'

Full Shutdown

Remove your sample
Wipe the sample chamber briefly with Kimwipe and 70% EtOH
Perform bleach cleaning and MilliQ rinse with subsequent shutdown (Fig. 41):
In the software menu, select 'Cytometer' - 'Hardware and Software Shutdown' and follow the instructions.
The cleaning steps require
10 ml of fresh 10% bleach in 15 ml Falcon tube (Fig. 40A)
12 ml of MilliQ in 15 ml Falcon tube (Fig. 40B)





Once the Flow Cytometer has switched off, turn off compressor (Fig. 42A)
Wipe sorting chamber briefly with Kimwipe and 70% EtOH
Wipe inner BSC cabinet surface with cloth and 70% EtOH
Release air pressure from the sheath tank by lifting the ring and turning it 90° so that it sits elevated to vent (Fig. 42B, arrow).
Switch off lights and airflow of the BSC (Fig. 42C).