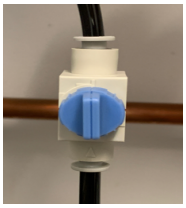
Sony MA900 Cell Sorter

70 and 100 µm chips are currently in use
130 chips are being updated release planned 2020



On

First user of the Day Start Up Procedure:

If the Instrument is on and calibrated skip to page 7

1. **Turn on the air pressure (counterclockwise) to the instrument, this must be done first. The blue valve should be inline as pictured above. It is clearly marked and located on the left back wall area of the instrument**

2. First clean the sample and sort chambers with Cavicide. Do not spray inside the chamber as it may dirty the window optics that monitor the side streams. Soak a kim wipe and then clean all the surfaces inside the chamber except the sort plates. The sort plates can only be cleaned with clean water or 70% ethanol. To clean the plates carefully remove one plate by undoing the thumb screws. Leave the sort plate inside the sort chamber to prevent accidentally dropping it. Working inside the sort chamber, wipe both plates in one direction with alcohol swipe from top to bottom. Clean the base of the sort chamber, point out floor drain area to clean.
3. Fill sheath tank to below the upper weld line. Check the pressure release ring on top of the tank to be sure it is down in the groove on the top of relief valve.
4. Empty the waste tank, add bleach to maintain 10% for biosafety decontamination. Hold the metal fittings in the cap stable and turn the outer cap only to remove. Make sure the filter on the waste cap points up and is not allowed to get wet. There is a beaker on the floor to place it in to keep it oriented in the correct position.
5. Staff generally fills the the water, 70% ethanol and 10% bleach tank levels. Users need the sheath full and waste empty to run.
6. Power up the MA900.

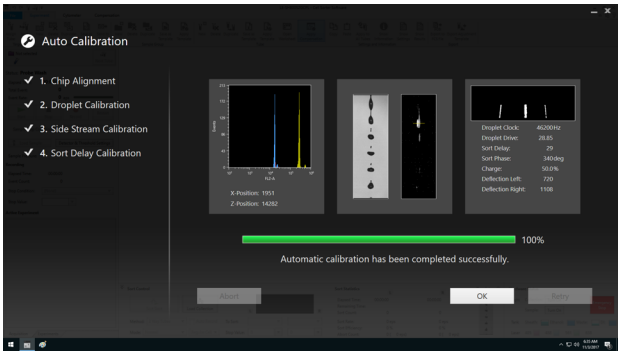
7. The Aerosol Management System is unique for this system. It is only activated to clear aerosols for 30sec-1 minute before opening the collection chamber. The unit is NOT ON during the sort. ONLY activated briefly before opening the sort chamber. Demonstrate on/off functions of the Buffalo unit and use of the foot pedal controls.
8. *Once the system has been setup, in order to empty the waste or fill sheath you must put the MA900 in "standby" mode. Directions for putting the machine in standby are located on page 6*. If you fail to do this it will result in having to recalibrate the system and a significant loss of time.
9. Log in to Windows. Account: guest1, password: guest.
10. Once the MA900 is in Standby, start the Cell Sorter Software.
11. Log in with your username and password.

Automated Alignment and Sort Setup (30 minutes) Read all prompts and respond

ATTENTION: QC takes 30-40 minutes, to help assist in passing clean the plates, and aspirator. During the fluidics start up select clean sample line, and sheath filter de-bubble prior the QC is recommended.

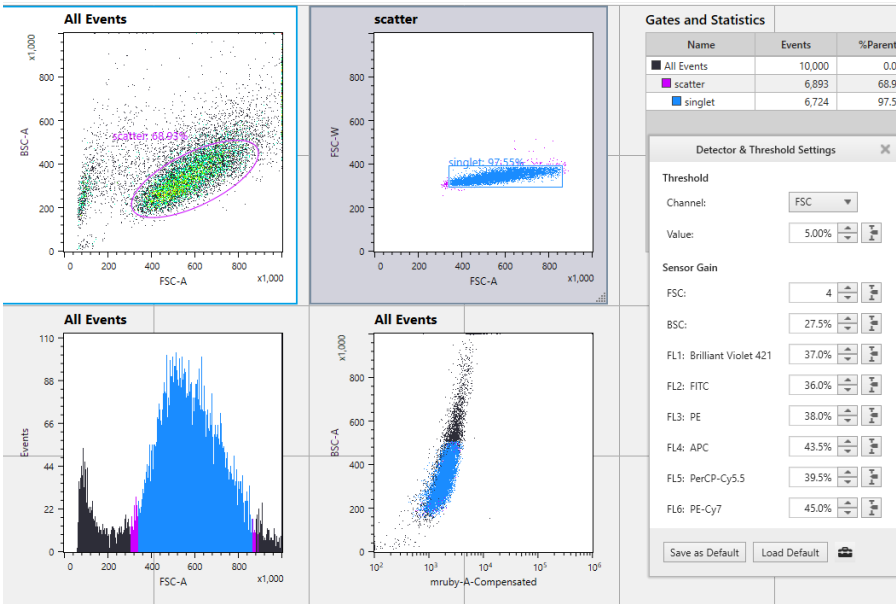
- Side streams may not form if the plates are not clean and dry
- If sample line is dirty it may not deliver adequate beads to pass QC
- Air in sheath filter will cause instability of the stream and cause failure of sort parameters
- Full sheath tank promotes stability

1. After log in, you are prompted to scan and load the new chip – Chips are good for 24h and the previous day's chip can be reused if <24h has passed. If continuing use on the current chip, simply open the instrument front and reload the current chip once it is ejected. Load/exchange the chip following the onscreen instructions. Write date and time on the package if using a new chip.
   i. To load a chip – feed the chip until the soft stop, then **gently** engage the last ¾ inch demonstrate.
   ii. Select the desired lasers**, the 488 nm laser is mandatory select other desired lasers.**
   iii. Select the Standard filter configuration for the instrument (this is the system not your application setting).
   iv. Follow prompts on screen to check sample line for back flush.
2. During the fluidics start up select the Sheath Filter De-bubble option and Sample line cleaning (this will do a 10% Bleach and Sterile DiH20 clean for 8-10 minutes) available on the bottom of the screen during start up before running the QC beads.
3. Before you run the beads **check to be sure the plates are clean and dry**. Occasionally they will get wet during the fluidics startup and if they are not cleaned and dry it may cause a failure in your QC and a significant loss of time.
4. **Run Autocalibration**. **NO CAP on tube.**
   i. Mix the Sony beads vial gently and dispense about 1 ml into a tube (can be polystyrene or polypropylene)**,** place 1 ml of Sony beads in the correct 5ml tube holder on machine. When prompted, load the calibration beads. **Make sure the tube goes to the bottom of the tube holder.**
   ii. Selected the Targeted Steam option.
   iii. Step one of the calibration aligns the chip and finds the target fluorescent sensitivity and sets the laser delay. This takes approximately 10 minutes. Steps 2-4 fine tune the stream profile, side streams, drop charge and sort delay. This takes approximately 30 minutes.



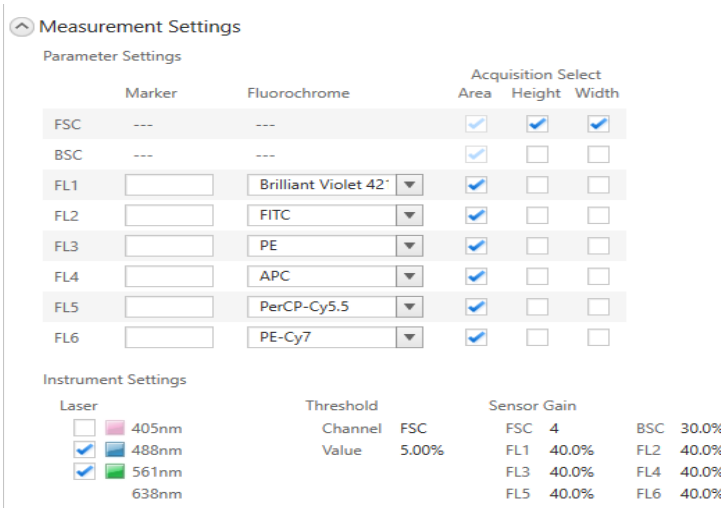
   iv. If autocalibration fails, check the log file for failure explanation, check that the plates are clean and dry, do a sheath filter de-bubble.

v. If this does not resolve the issue, check the optical filters, sheath tank seal, check front cytometer pane for liquid levels. Text or call staff for assistance.



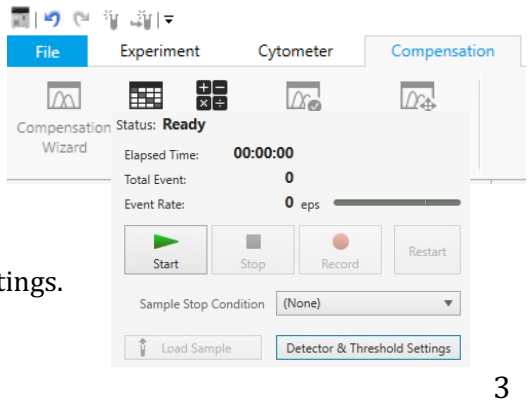
Experiment Setup

1. **Filter all samples including controls 40u mesh.**
2. Select the experiment you would like to use, or the Blank Template.
3. On the upper right, change the experiment name.
4. Fill in other information for your experiment.
5. Check or uncheck the fluorescent parameters that are needed and name the parameters if you wish.
6. Turn on/off lasers for your experiment. Be sure that the 488nm laser is on.
7. Click Create New Experiment in the lower right of the monitor.



When running a compensation matrix

1. There are 2 methods for compensation available the wizard and manual.
2. Choose to start compensation wizard if you are sorting with multiple fluorochromes and have prepared single color controls.
3. Click OK to continue
4. Flow rate is controlled by sample pressure setting.
5. Follow the wizard's instructions and run the negative control and adjust the FSC and BSC in Detector & Threshold settings to get the populations on scale. Briefly load a fully stained sample at this point (do not record it) and verify that none of the positive populations are off scale. If they are, lower the PMT value to get them back on scale.
6. Then reload the negative control and record. Adjust the gate such that it is around the correct scatter population.
7. Acquire the single-stained controls, adjust the gate around the positive populations, then click Calculate Matrix. The shape of the record button changes from circular to square when data is being recorded.
8. Click Finish to end the Wizard.



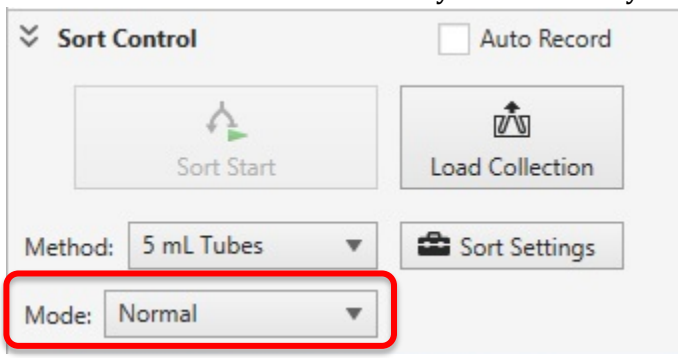
- If no compensation is necessary, select Detector & Threshold Settings.
- Adjust FSC gain and BSC gain such that your cells are on scale.

- Acquire enough events for the sample to be sorted so that you can set gates. Pause the acquisition.
- Create plots on the worksheet. Double click inside gates to create gated plots or change the gates by clicking on the gate name at the top of a plot. Be sure to include a singlet gate based on FSC-A vs FSC-H or FSC-H vs FSC-W
- Adjust gate settings for populations of interest.
- Set the value to record to 5,000 or 10,000 events. If the population is rare, record more events.

9. Start making graphs and approximate gates for your experiments. Created elements, plots and approximate gates before collecting data will allow them to be available in all the tubes. If you forget you can copy the worksheet elements to the following tubes.

Tube sorting setup

1. Select the desired sort mode. Purity or Semi-Purity is recommended.



2. Set the sort gates. Indicate the number of cells to be sorted or leave the value at 0 to sort continuously.

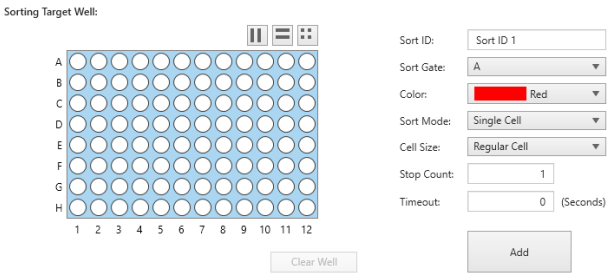
Sort Statistics	Far Left	Left	Right	Far Right
Sort Gate:	C	D	E	F
Elapsed Time:	00:00:00	00:00:00	00:00:00	00:00:00
Remaining Time:				
Sort Count:	0	0	0	0
Sort Rate:	0 eps	0 eps	0 eps	0 eps
Sort Efficiency:	0 %	0 %	0 %	0 %
Abort Count:	0 (0 eps)	0 (0 eps)	0 (0 eps)	0 (0 eps)

3. To check trajectory of your side streams, load a targeting tube with tape or parafilm on top so you can see the drop deposited in your sample collection holder. Place sample collection holder in sort collection chamber.
4. In the cytometer ribbon select the black toolbox labelled settings. Select Advanced Settings tab near the bottom, in the stream window bottom left, select Load Collection, then hit Start to deposit fluid and check stream trajectory, adjust if necessary, by clicking the arrows on the right. Once targets are defined load collection tubes with media for sort.
5. Place the collection tube holder into the collection area and set collection tubes.
6. Click “Next Tube” to create a new tube.
7. Click “Load Collection”.
8. Make sure flow rate is stable, click “Start” to load sample tube and start acquiring.
9. Hit “Sort & Record Start” to sort and save data for the sample.
10. Run the sample pressure at a maximum of 6 during sorting.
11. From the Cytometer tab, you can adjust temperature control for the sample and collection, chamber lights and agitation.
12. Monitor the collection tubes change them when they are full. Turn on Aerosol management unit for 60 seconds before opening the chamber. The unit must be **off** for sorting.

13. To change tubes: Pause the sample, Unload Collection, switch tubes, load collection and the sort will continue.
14. To stop the sort, press the black Stop button. On/off aerosol management. The Aerosol Management System on this unit it is **ONLY** on when you are removing a sample after sorting, or to clean the unit after a clog. Turn on the unit using the power button bottom left front. Hit the foot pedal on the floor to activate suction. Leave the unit evacuating for 30-60 seconds. **THE UNIT IS OFF WHEN YOU ARE SETTING UP AND SORTING.** It is only active when the door is to be opened after BSL-2 sorting or in event of a clog. Turn off unit immediately.

Sorting into a Plate

1. Install splash guard, plate stand and plate holder (stored in refrigerator).
2. Change the Sort Method to 96 or 384-well plate.
3. Start the sample, then Pause when enough cells are seen to set sorting gates. Pause the sample and set the gates.
4. Click on Sort Settings to open the 96 well sort setup and plate adjustments
5. Load a plate with the cover on.
6. Select the Plate Adjustment tab.
7. Choose either to deflect empty drops of sheath fluid, or to run the sample (preferred).
8. Select '4 corners and center well'
9. If running the sample, select the gate to use (typically the singlets).
10. Click 'Start' and the MA900 will sort 50 drops into the corner wells and a center well.
11. When it finishes, Pause the sample and Unload the plate (automatic if the sample is not running). You can remove the plate to check the drop positions over the wells. Note the adjustments needed, and re-load the plate. Click on each corner one at a time, and adjust the drop position. The default adjustment of 1 mm can be changed down to 0.1 mm if desired. Once the adjustments are finished, click 'Start' again.



12. Remove the plate to be sure that the adjustments are correct, re-do if necessary.
13. Select the wells to be sorted:
    - In the Plate Sort Settings tab, highlight the wells to be sorted. Select the gate and Stop Count, then click Add.
    - Check the Index Sort box if desired.
14. Remove the plate lid.
15. Click 'Index Sort & Record Start'.
16. When sort is finished, you will have the option to continue sorting an additional plate. Click continue if you wish to sort additional plates with the same sample. You will have the opportunity to change sort criteria. Selecting "no" will require that you unload/reload the sample. On/off aerosol management
17. Remove the plate holder (store in refrigerator) and splash guard.
18. To analyze index sort data, click on the Worksheet Tools and 'Analyze Index Data'

***If the waste needs to be emptied or sheath filled after the auto calibration has already been done, it will need to be put in standby mode**. If you do not put the machine in standby mode and attempt to empty the waste, the stream will shut off and you will have to run the auto calibration again.*

To put the machine in standby mode:

1. From the cytometer ribbon, select "settings" (a)
2. On the settings panel, select "advanced settings"

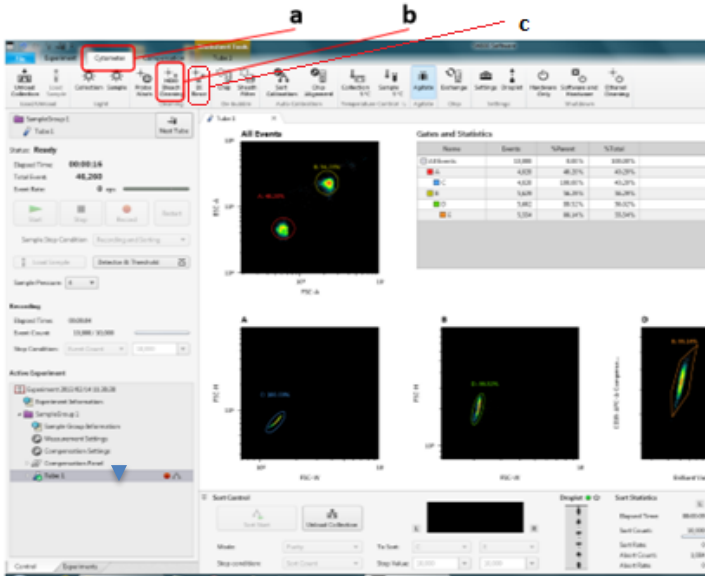
3. On the “pressure options” tab, select “standby” (b)
4. Once you have replaced the tank after emptying and adding bleach, or filled the sheath tank select “ready” (c)

Data Export

1. FCS file export: right click on the Experiment (scroll up past all the sample tubes to the top of the experiment), and select Export as FCS Files, or in the Experiment tab, select Export FCS Files from the ribbon.
2. The … button lets you browse to the appropriate folder.
    1. click export → click close
3. A PDF of the experiment layout, including sort setup and a screenshot of the gates can be saved by clicking custom print (in the Worksheet Tools Ribbon).
4. Experiment export: From the File menu, select Database. Click on the experiment to export, then on the arrows to move it to the export window on the right.
5. The … button lets you browse to the appropriate folder C:\Facsdata.
6. This saves the entire experiment and allows you to save the data, the plots and the gates. It is recommended that you do this export in order to have a full backup of the experiment. It can then be deleted and imported back into the database if necessary.
7. On the desktop use the data upload icon to upload your experiment to box, the same procedure for all our cytometers is used.
8. Check the scheduler to see if you are the last user

Clean up

If NOT, the last user of the day:

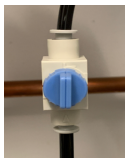


1. From the Cytometer tab, select Bleach Clean. Prepare 15 ml tube with 10 ml of 10% bleach. Place on tube holder and proceed with cleaning. This will take 6 minutes, and approximately 7 ml of bleach will be run through the sample tubing and chip.
2. From the Cytometer tab, select Shutdown Rinse. Prepare a 15 ml tube with 12 ml of Di water. Place the tube on the holder and proceed with the rinse. This will take another 6 minutes.
3. Log out of the software.
4. When the box comes up asking if you are sure you want to log out, check the box that says “Keep sort calibration.” If you do not check the box, the next person will have to run the auto calibration again before sorting.
5. If there is more than a 2 hour gap between log-ins, the machine will shut the stream off and the auto calibration will have to be run again at the next log.

Shut Down

1. If the last user, from the Cytometer tab (a), select Hardware and Software Shutdown (b). The cleaning wizard will start. Prepare a 15 ml tube with 12 ml of 10% bleach. Place on tube holder and proceed with cleaning. This will take 6 minutes, and approximately 7 ml of bleach will be run through the sample tubing and chip.

2. After the bleach cleaning is finished, you will be prompted to prepare a 15 ml tube with 12 ml of Di water. Place the tube on the holder and proceed with the rinse. This will take another 6 minutes.
3. Select Shutdown to power down the instrument and you will also be logged out of the software.
4. Turn off the air pressure with switch on wall (Rotate valve to horizontal position not in line with the fitting).



Off

Quick Start Instructions if the machine is already set up.

When the previous user has already setup the chip and left the instrument on for you. When the last user logged out they must have clicked on "Keep autocalibration for next user". The machine is already calibrated for you.

1. Login to the software with your username and password. Create your experiment or select a template you have saved. Select desired lasers and parameters and label fluorophores.

2. If the Waste and Sheath fluid levels are low (check the instrument panel), it is recommended that you refill them now. To do so, you should first put the instrument in "Standby" mode. This turns off the stream and depressurizes the instrument. (Cytometer tab > Settings > "Advanced setting" > "Pressure Options" > "Standby" button). Do not close this dialog box.

3. IN STANDBY MODE: Empty the waste container and refill the sheath fluid. Be sure to depressurize the sheath fluid tank by disconnecting the air pressure hose. It has a snap-fit connection. For the waste container, hold the fitting stationary and twist the plastic white cap. Do not tilt the waste container fitting. Keep the filter pointed so it does not get wet. Put it inside the beaker to the side. This prevents liquid from entering and clogging the air filter.

4. IN STANDBY MODE: Clean the sample and sort chambers with Cavicide. The sort plates can only be cleaned with sterile water or 70% ethanol. To clean the plates carefully remove one plate by undoing the thumb screws. Leave the sort plate inside the sort chamber to prevent accidentally dropping it. Working inside the sort chamber, wipe both plates in one direction with sterile water or alcohol swipe top to bottom. Clean the sides and floor of the sort chamber where the waste goes. The side windows need to be cleaned for side stream evaluation demonstrate cleaning these windows.

5. IN READY MODE: Put the machine back into "Ready" mode. In the same "Pressure Options" dialog box, click on "Ready" button. The machine will now start re-pressurizing.

6. Run the bleach cleaning (Cytometer tab > "Bleach Clean" button on the ribbon). Follow the prompts. Clean with 15mL conical.

7. Run the DI Water clean (Cytometer tab > "DI Rinse" button on the ribbon). Follow the prompts. Clean with 15mL tube. You're now good-to-go.

8. (OPTIONAL) While cleaning is running, you can start making graphs and approximate gates for your experiments. Created elements, plots and approximate gates before collecting data will allow them to be available in all the tubes. If you forget this step you can copy the worksheet elements to the next tube.

9.The Aerosol Management System for this unit is ONLY on when you are removing a sample after sorting or to clean the unit after a clog. Turn on the unit using the power button bottom left of unit. Hit the foot pedal on the floor to activate suction. Leave the unit evacuating for 30-60 seconds. **THE UNIT IS OFF WHEN YOU ARE SETTING UP AND SORTING.** It is only active when the door is to be opened after BSL-2 sorting or in event of a clog, then turn it off immediately

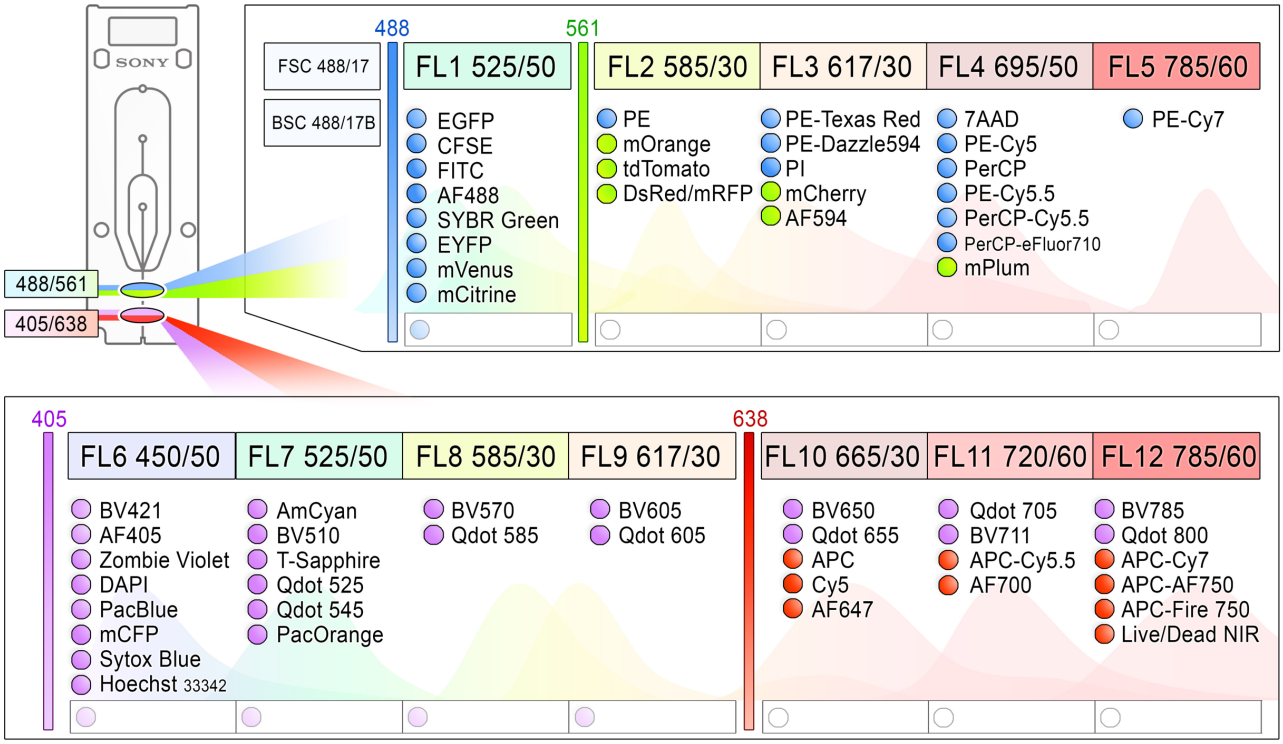
Please pick only **ONE** dye per column~~umn.~~

Fluorochrome		FL1 525/50	FL2 585/30	FL3 617/30	FL4 695/50	FL5 785/60
EGFP		●				
FITC		●				
Alexa Fluor® 488		●				
EYFP		●				
mCitrine		●				
CFSE		●				
PE			●			
dsRed			●			
tdTomato			●			
mCherry				●		
PE-Texas Red®				●		
PE/Dazzle 594				●		
Propidium Iodide				●		
mPlum					●	
7-AAD					●	
PE-Cy™5					●	
PerCP					●	
PE-Cy5.5					●	
PerCP-Cy5.5					●	
PerCP-eFluor 710					●	
PE-Cy7						●

	FL6 450/50	FL7 525/50	FL8 585/30	FL9 617/30	FL10 665/30	FL11 720/60	FL12 785/60
BD Horizon Brilliant Violet (BV421)	●						
Alexa Fluor® 405	●						
DAPI	●						
Pacific Blue™	●						
mCFP	●						
Hoechst 33342	●						
AmCyan		●					
BV510		●					
BV570			●				
BV605				●			
BV650					●		
BV711						●	
BV785							●
APC					●		
Alexa Fluor® 647					●		
APC-Cy5.5						●	
Alexa Fluor® 700						●	
APC-Cy7							●
APC-Alexa® Fluor 750							●

SONY MA900 FLUOROCHROME SELECTION GUIDE

For 4-laser, 12-color models LE-MA900F/FP



Legend: Sony MA900 cell sorter can be equipped with up to 4 lasers. Two laser beam spots interrogate particles sequentially, with each spot containing up to 2 lasers (model dependent). 488 & 561 lasers are on Spot 1, while 405 & 638 lasers are on Spot 2. For panel design, select one dye from each FL channel of each beam spot. Dyes from the same FL channel cannot be run together. Each dye's circle label indicates its excitation laser. Empty rectangles aid in panel design. FSC & BSC scatter is detected with 488 laser. Follow Compensation Wizard for spillover compensation.

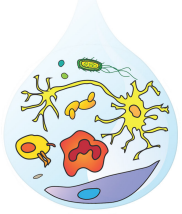
SONY MA900 NOZZLE GUIDE

PART #	LE-C3207	LE-C3210	
NOZZLE SIZE	70 um	100 um	130 um

Mode		Standard	Targeted	
DROP Frequency (EMPTY DROPS / SECOND)	~50K drops/sec	~30K drops/sec	~22K drops/sec	~12K drops/sec
MAX SUGGESTED* DATA RATE (TOTAL EVENTS / SECOND)	12K eps	6K eps	<4K eps	2.5K eps
DROP VOLUME	1.5 nL	3.5 nL		7.9 nL
SORTED EVENTS / 1mL (OF SORTED VOLUME)	~700K	~300K		~130K

*When sorting for purity, do not exceed the indicated data rates

APPLICATION



- Fastest sort speed
- Limited to small particles
- Runs at highest pressure

- Most common nozzle for various cell types
- Targeted Mode reduces side-stream fanning & improves targeting precision of larger cells with uneven geometry into tubes or multi-well plates

- Gives best cell viability
- Runs at lowest pressure
- Slowest sort speed
- Can sort any cell size under 25um

E. Coli, RBC, Yeast, Lymphocytes, Bone Marrow, CHO, HEK-293T, Macrophages, ES cells, Neurons, Fibroblasts, Cancer Cell Lines, Cultured Stem Cells

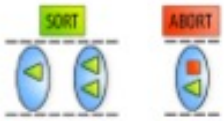
FSC Gain	16	8	4	0.5
PARTICLE SIZE (Nozzle size must be 5 to 7x larger than particle size)	<10um		<18um	<25um

MA900 Software auto-calibrates optimal Clock and Drive for each individual chip

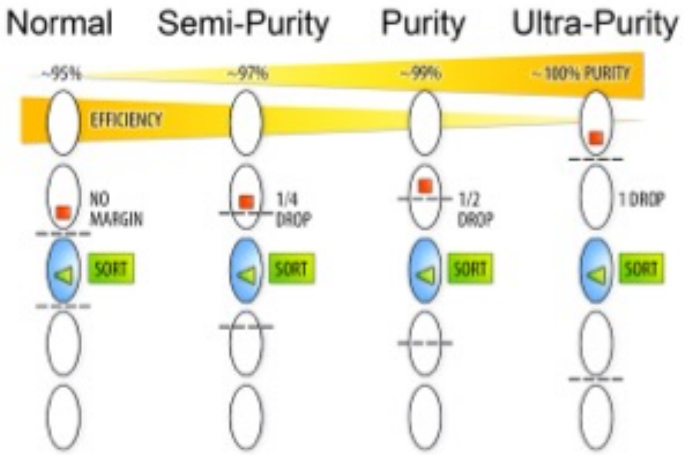
PRESSURE	40 psi	20 psi		9 psi
FREQUENCY (Clock)	42-52 kHz	27-31 kHz	21-23 kHz	12 kHz
AMPLITUDE (Drive)	30-50%	10-20%	30-50%	30-50%
DROPLET PROFILE				

SONY MA900 SORTING MODES

PURITY MODES:

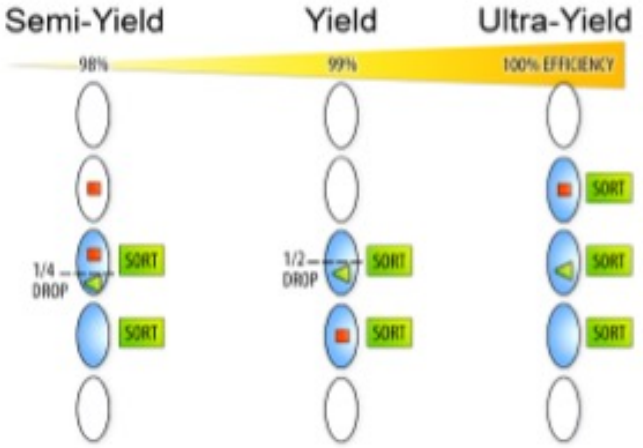


- Purity is achieved at the expense of aborting the target ◀ coincident with non-target ■
- For best efficiency, do not exceed sort speeds described in the nozzle selection guide



YIELD MODES:

- Enrich for the target at the expense of purity
- Coincidence is not aborted
- Extra drops are sorted for maximum recovery
- You may exceed nozzle sort speed



SINGLE CELL MODES:

- Used for single-cell plate sorts
- Sort only 1 (not 2) targets, at 100% purity
- Centering Mask ensures target is in the sorting drop
- Expanding the Centering Mask increases efficiency
- 130um nozzle always sorts 3 drops in any single-cell mode