

Sort protocol on the influx cytometer

This isn't meant to replace the Help function available on Software. It's fantastic! This is only meant to quickly offer an overview of important common functions.

Step 1- Generating Gates around the populations of cells you intend to sort

- Chose a Gate "shape"
- Segregate populations

Step 2- Chose a sort stringency

- Drop size
- Coincidence acceptability
- Quality

Step 3- Purpose of sort (Downstream application!)

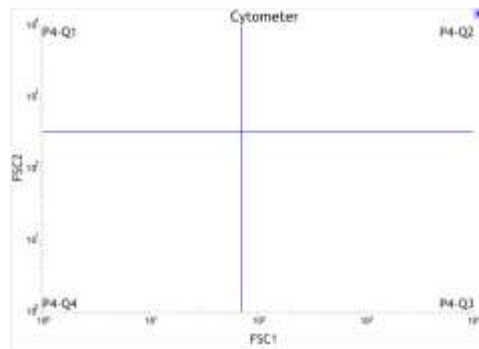
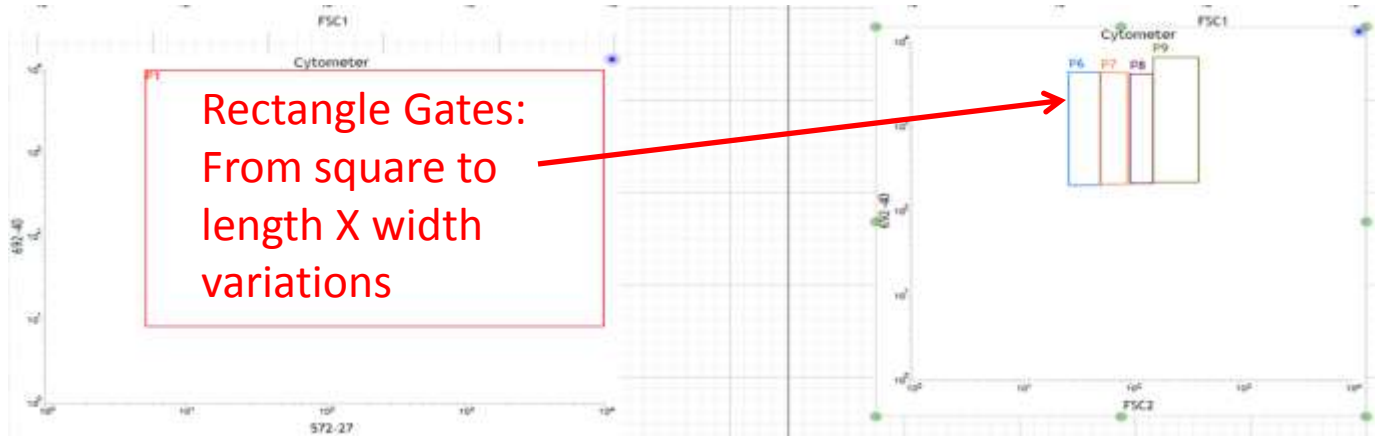
- Preparations and Precautions
- Sheath Fluid
- Vessel prep

Step 4- Chose vessel into which to sort

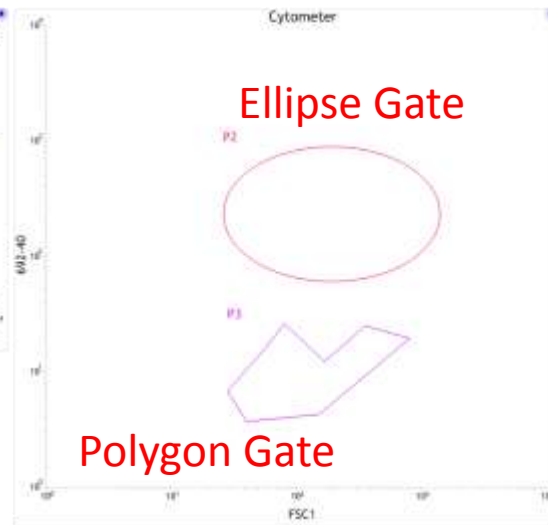
- Tubes
- Plates

Sort protocol on the influx cytometer

Step 1- Generating Gates around the populations of cells you intend to sort
-Chose a Gate "shape"



Quadrant
gates



Ellipse Gate

Polygon Gate

Sort protocol on the influx cytometer

Step 1- Generating Gates around the populations of cells you intend to sort
-Hierarchy



Mother Gates- Contains a population of cells by which all other cells are defined
-i.e. All Events is the Mother of all other gates

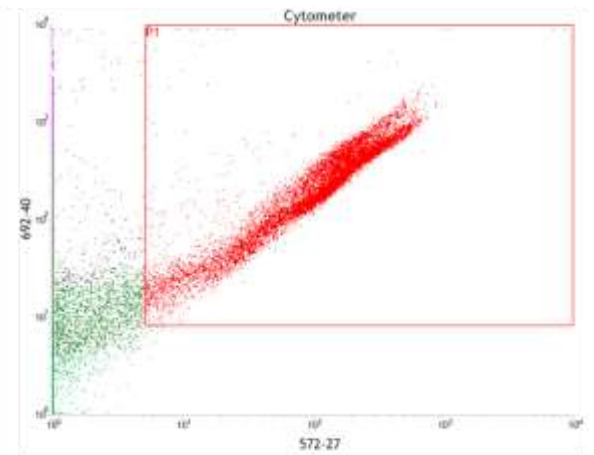
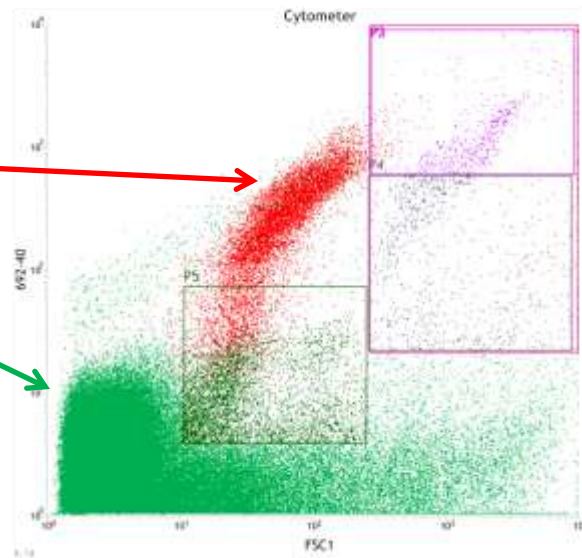
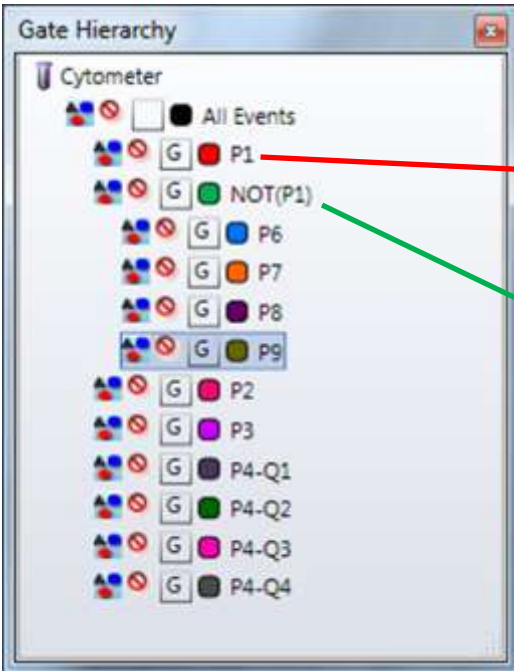
Daughter Gates- Cells contained in subset populations found within the Mother Gate populations

Boolean Gates- (False or NOT gates) Used to define cells who do not have characteristics of a previously defined "gated population."

-i.e. NOT(P1) is a boolean gate of P1
(visually represented on the next slide)

Sort protocol on the influx cytometer

Step 1- Generating Gates around the populations of cells you intend to sort
-Segregate populations



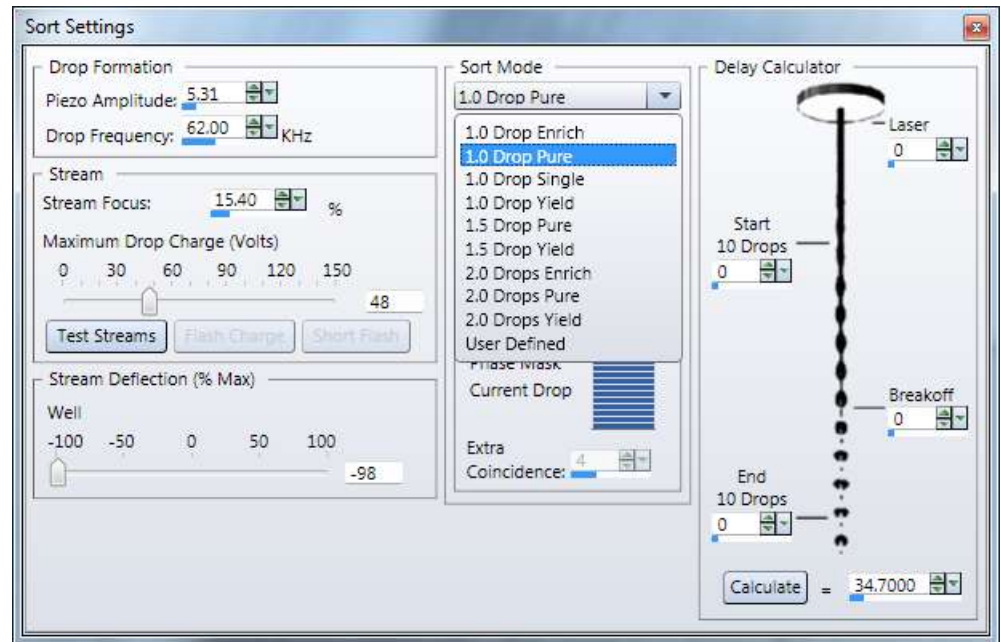
P1 (graphically represented on the right in red) is the primary gated population by which all other populations are defined. All red dots represent a cell that possesses . The green represents all cells "Not P1." Gate P9 (Olive Green) would sort all in its boundaries except the Red.

Sort protocol on the influx cytometer

Step 2- Chose a sort stringency
-Drop Count

Drop count- how many cells to sort to ensure a particle is sorted.

1. 1.0 will ensure only one droplet is sorted regardless of the position of the particle in that droplet
2. 1.5 will ensure the sorting of two drops if consecutive particles have at least one cell positioned near the edge of a drop.
3. 2.0 will sort two consecutive drops if a cell is near the boundary of one drop.



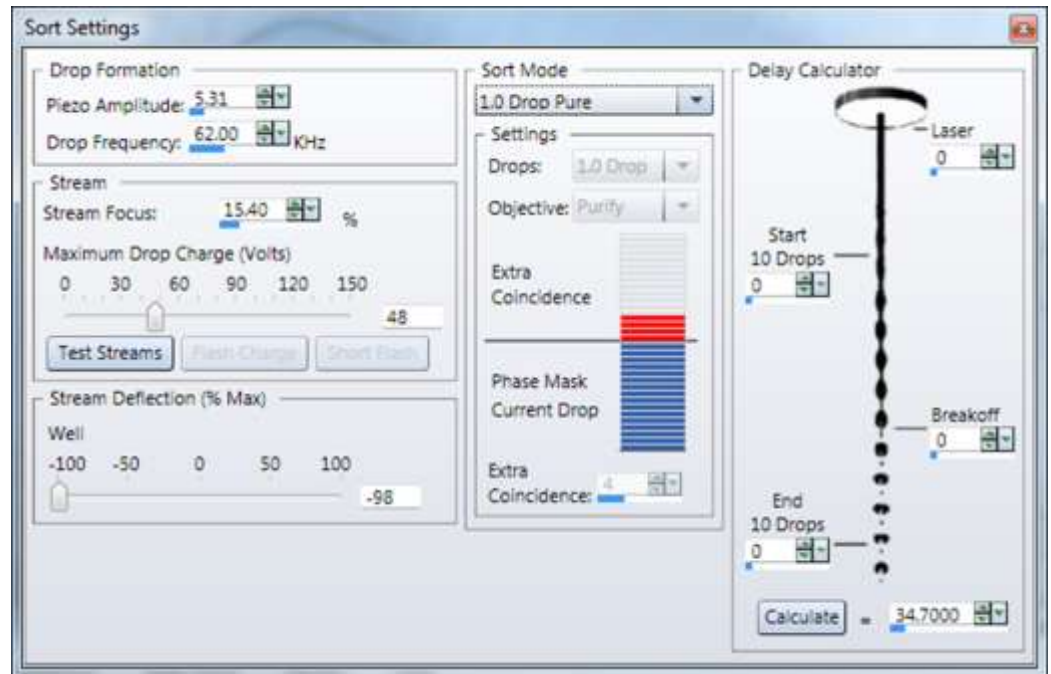
Sort protocol on the influx cytometer

Step 2- Chose a sort stringency
-Coincidence

Coincidence- Phenomena of more than one cell being present in a droplet

Blue defines the area in which a droplet may contain a cell and be sorted.

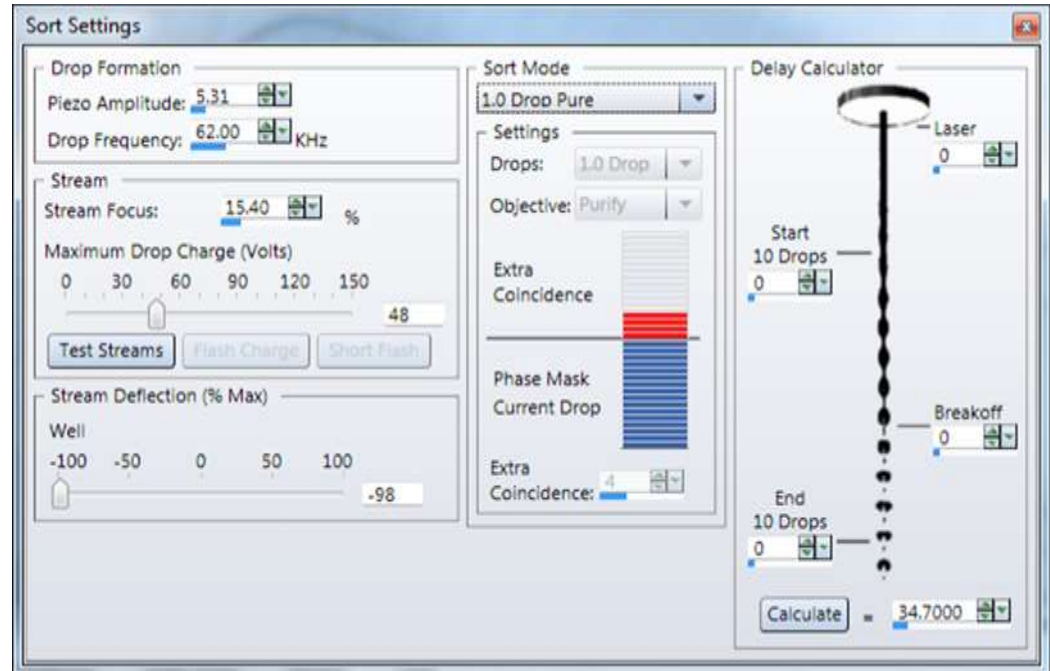
Red defines the area in the next droplet in which the presence of a cell will abort.



Sort protocol on the influx cytometer

Step 2- Chose a sort stringency
-Quality (Pure)

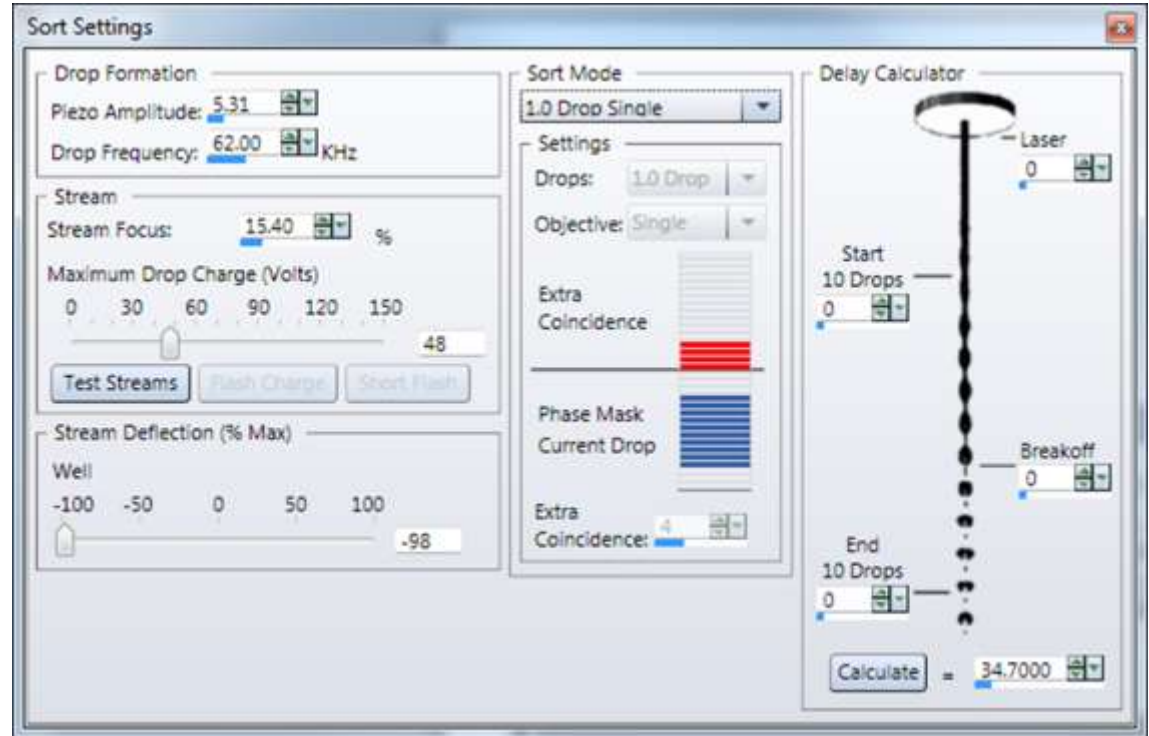
Pure- Highly selective sort mode that will take a cell within the entire phase excluding the presence of a cell in the following drop.



Sort protocol on the influx cytometer

Step 2- Chose a sort stringency
-Quality (Single)

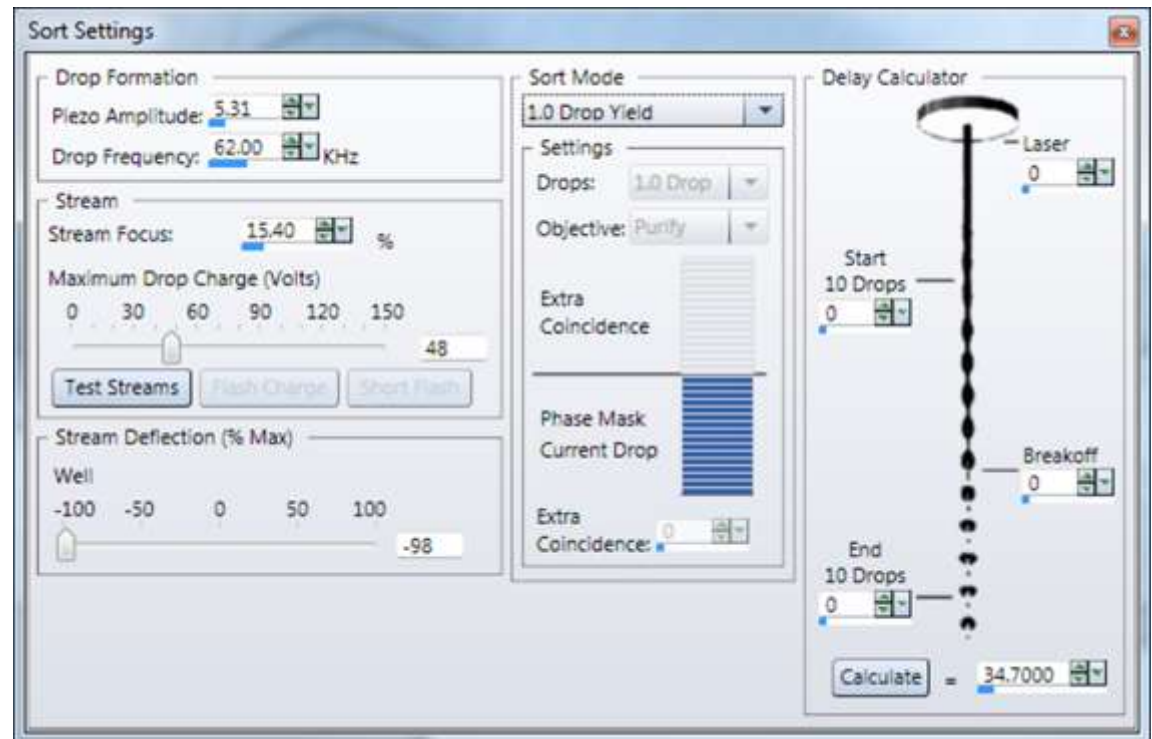
Single- Most selective sort mode that will take a cell only within the center of a drop and excluding the presence of a cell in the following drop.



Sort protocol on the influx cytometer

Step 2- Chose a sort stringency
-Quality (Yield)

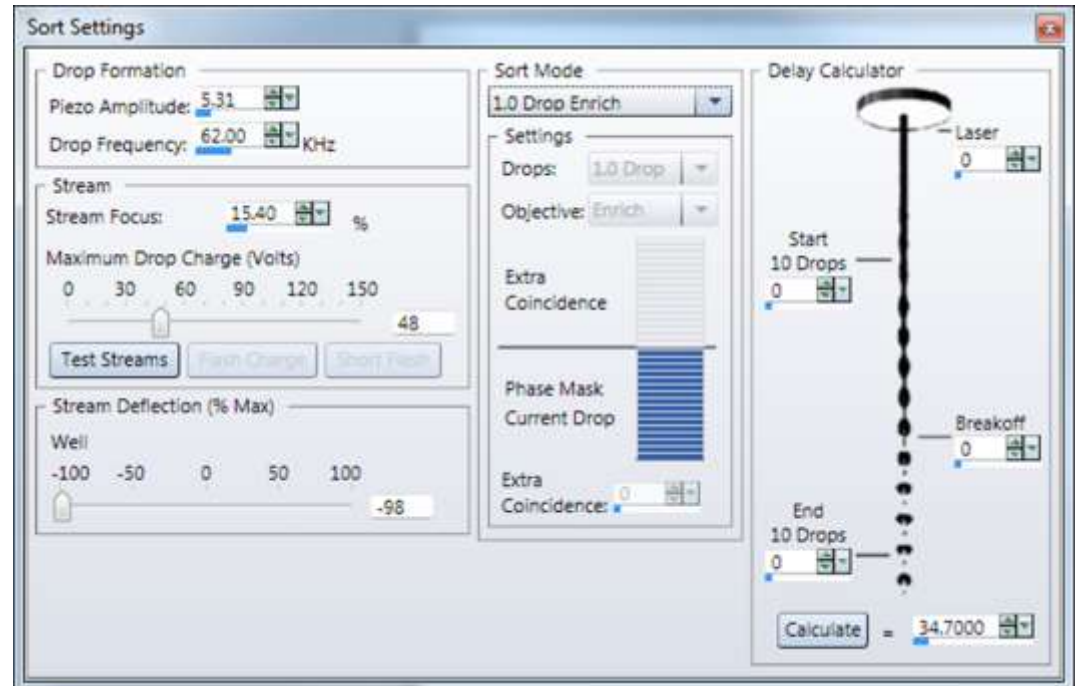
Yield- Moderately selective in that this sort mode will take cells within the entire drop phase and will not exclude drop sorts from consecutive drops but will exclude coincidence in all instances.



Sort protocol on the influx cytometer

Step 2- Chose a sort stringency
-Quality (Enrich)

Enrich- Sorts cells regardless of its location in the droplet, and regardless of whether another cell is present in that droplet. Also will sort consecutive drops in this mode .



Sort protocol on the influx cytometer

Step 3- Purpose of sort (Downstream application!)

-Precautions and Preparations

Molecular Biology- 1L 5% bleach rinse of the sheath and samples lines, 12L nanopure water rinse of the sheath and samples lines, Autoclaved sheath reservoir, freshly prepared sheath fluid, UV disinfecting of the sort chamber.

Culture- Autoclaved sheath reservoir, freshly prepared sheath fluid, UV disinfecting of the sort chamber.

Slide Preparation- Freshly prepared sheath fluid

Analytical Measurements- Autoclaved sheath reservoir, freshly prepared sheath fluid.

Sort protocol on the influx cytometer

Step 3- Purpose of sort (Downstream application!)

-Sheath Fluid

Flow cytometer:

Sheath Fluid- We use a preservative-free, 8X concentrate sheath fluid (Biosure, inc, Cat# 1020) prepared fresh prior to sorting for cells to be incorporated into a FACS to molecular biology work flow using nano-purified, distilled water to dilute to a 1X working solution.

Filtered Seawater- 0.2 μm filtered seawater may be used as a sheath fluid especially when isolating microorganisms from seawater samples for culture.

Nanopure water- Distilled may be used as Sheath fluid but cannot be used to sort.

Sort protocol on the influx cytometer

Step 3- Purpose of sort (Downstream application!)

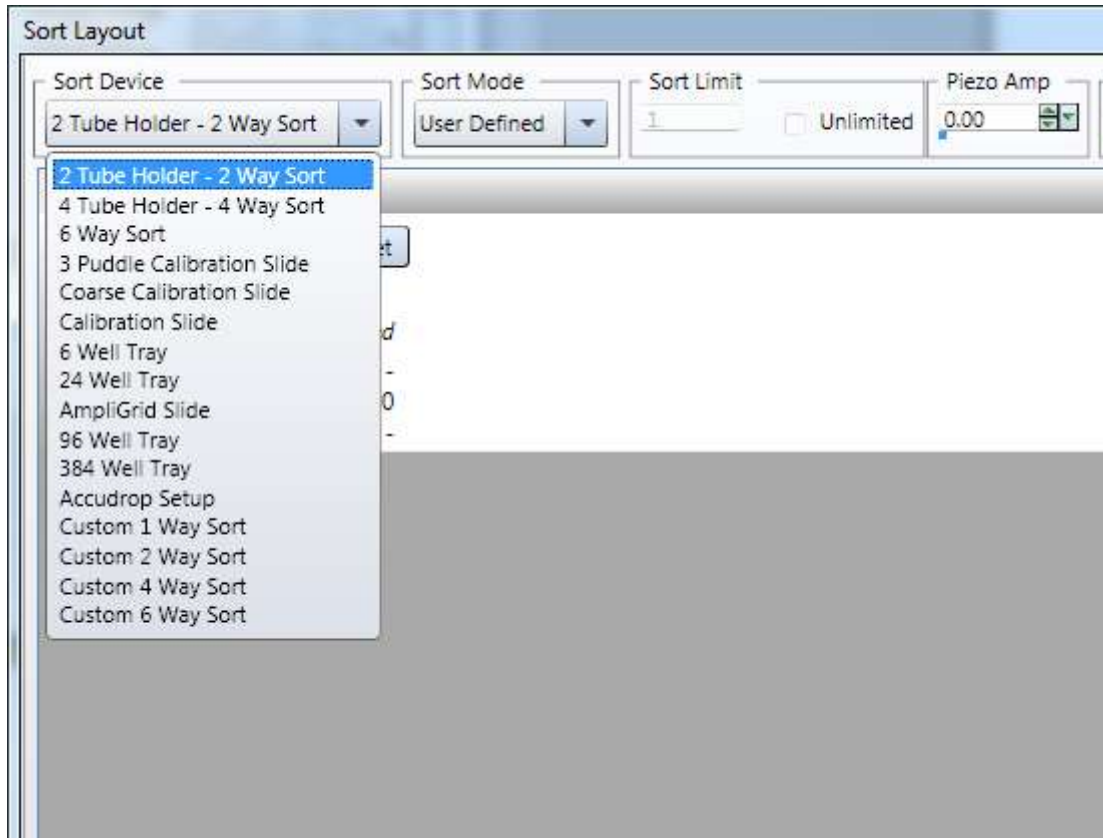
-Vessel prep

- FACS product can be sorted into a variety of different tubes.
 - Large sorts (>5,000 cells; 1.5mL micro-centrifuge tubes in 20 to 100 mL 5kD filtered, nano-purified, distilled water or TE buffer)
 - Moderate sorts (100 to 5,000 cells; 0.2mL reaction tubes (individual or strips of 8) in 10 to 20 mL 5kD filtered, nano-purified, distilled water)
 - Small sorts (1-100 cells; 0.2mL reaction tubes (strips of 8, or 96 and 384 well plates) in 5 to 10 mL 5kD filtered, nano-purified, distilled water)
- Reaction tubes (both PCR and optical tubes for qPCR) are purchased sterile and, DNase and RNase free.
- All reaction tubes are exposed to 10 minutes of UV light prior to the addition of water or TE buffer.
- Reaction tubes are stored capped until ready for sorting

Sort protocol on the influx cytometer

Step 4- Chose vessel into which to sort

-Variety of choices although we've limited our sorts vessels predominantly to tube and plate options

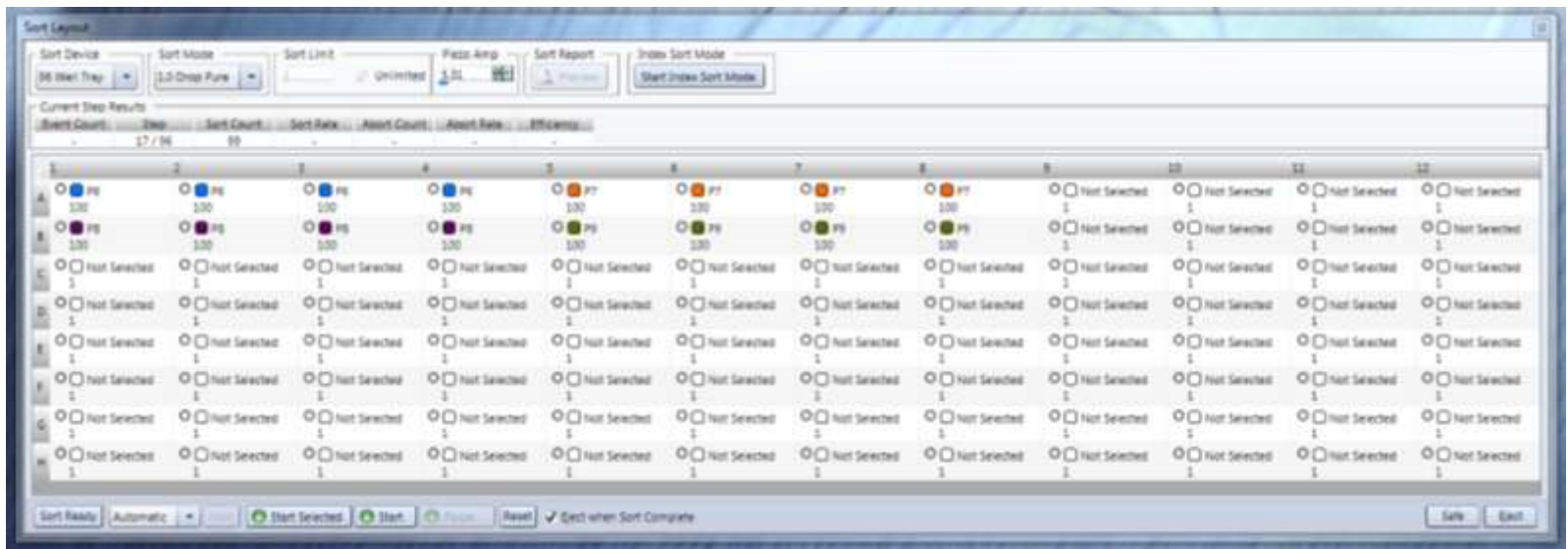


Sort protocol on the influx cytometer

Step 4- Chose vessel into which to sort
-Plates

Molecular biology applications- 96-well and 384-well plates
Cell Culture- 6-well, 24-well, 48-well and 96-well plates

A cell per well number must be defined if you're planning to sort into multiple wells.



Sort protocol on the influx cytometer

Step 4- Chose vessel into which to sort

-Two tubes

Molecular biology applications- WGA into 1.5mL tubes; often at 10,000 cells or greater sort concentrations.

Cell Culture- 5 mL round bottom culture tubes, normally containing 1-3 mL of media

One may sort between 1 and an infinite number of cells per tube per sort.

