BD Aria Fusion Standard Operation Protocol – Basic Operation

A. Laser

1. Check if the laser needed is turned *ON.*

   * Turn off laser(s) not required may improve cell viability

   **** Blue Laser MUST be turned ON.**

B. User Login

1. Login to FACSDiva Software with your username and password.
   *If you do not have account, please contact our staff for assistance.

2. Click *Use CST Settings* if the window below pops up.

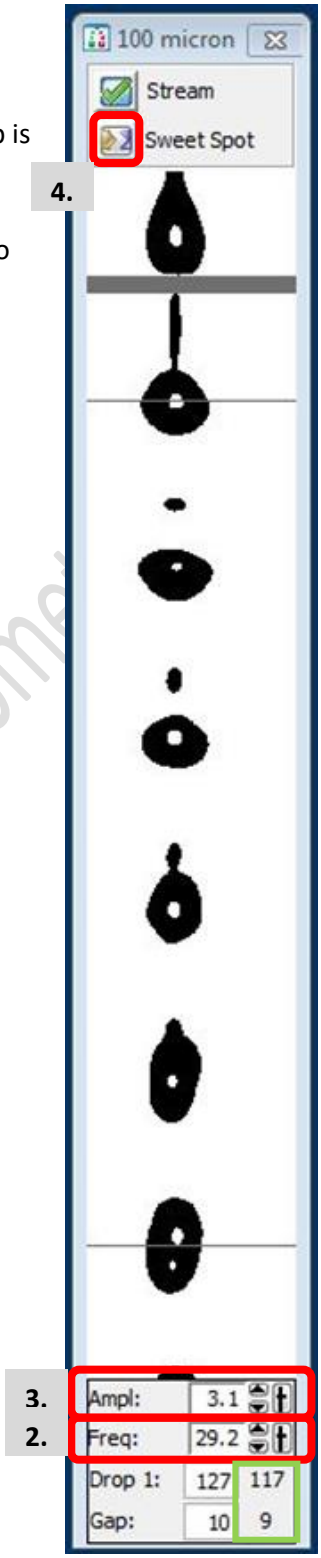
C. Stream Optimization

1. Go to 100 micron window (upper monitor)

2. Adjust the *Freq* (Starting Value 29.2) so the "neck" of a drop is formed

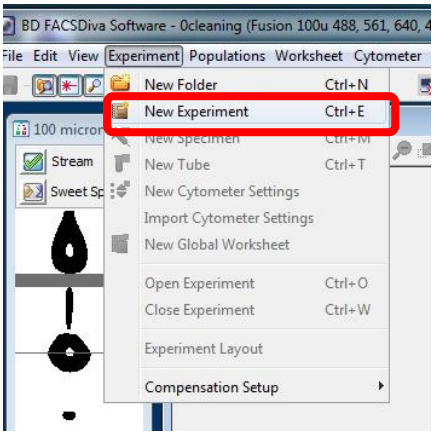
3. Adjust the *Ampl* (Starting Value 3.0) so the Drop 1 is close to 120 and Gap is close to 10

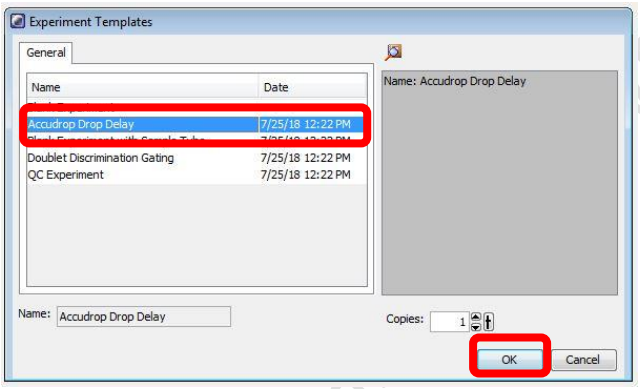
4. Turn ON the *Sweet spot*



D. Accudrop Delay Assay

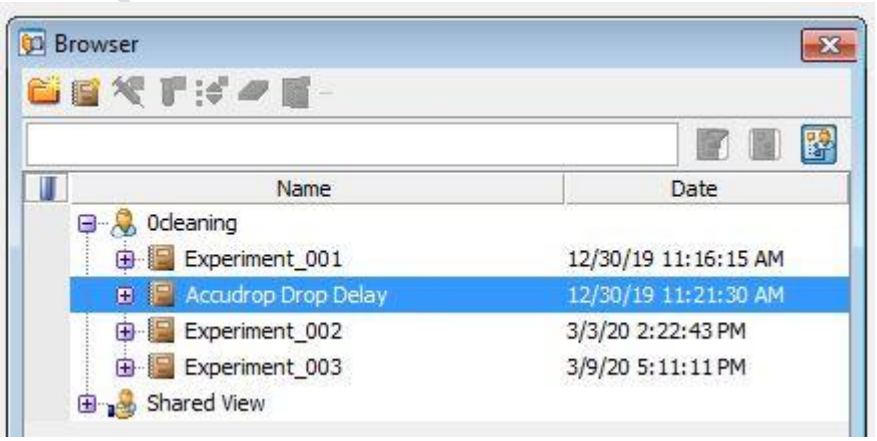
1. To Import Accudrop Drop Delay template, click *Experiment > New Experiment*. Select Accudrop Drop Delay, then click *OK*



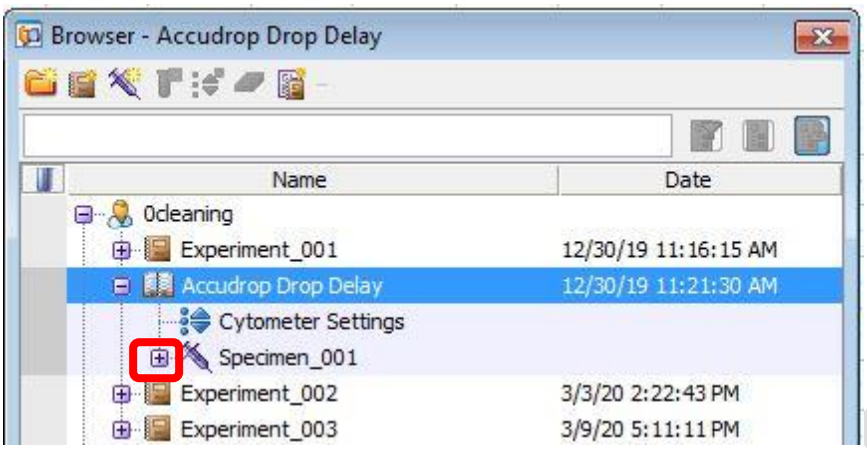


- OR -

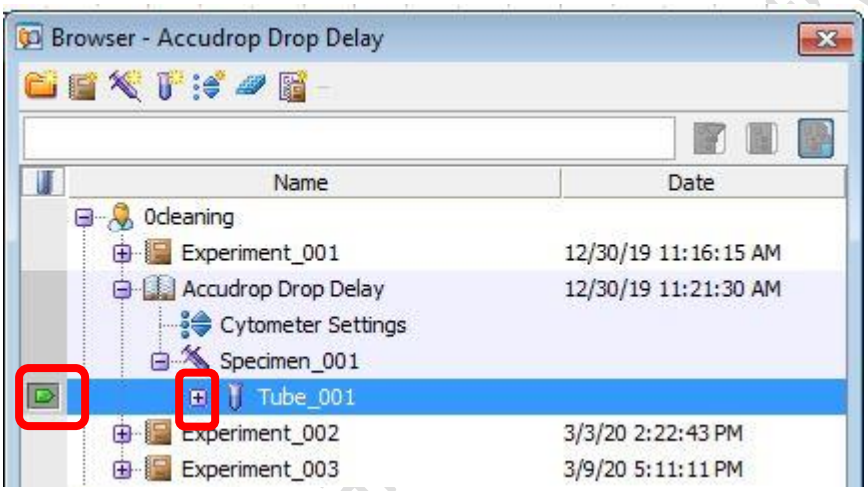
2. To open existing Accudrop Drop Delay experiment, double click *Accudrop Drop Delay* on the Browser window



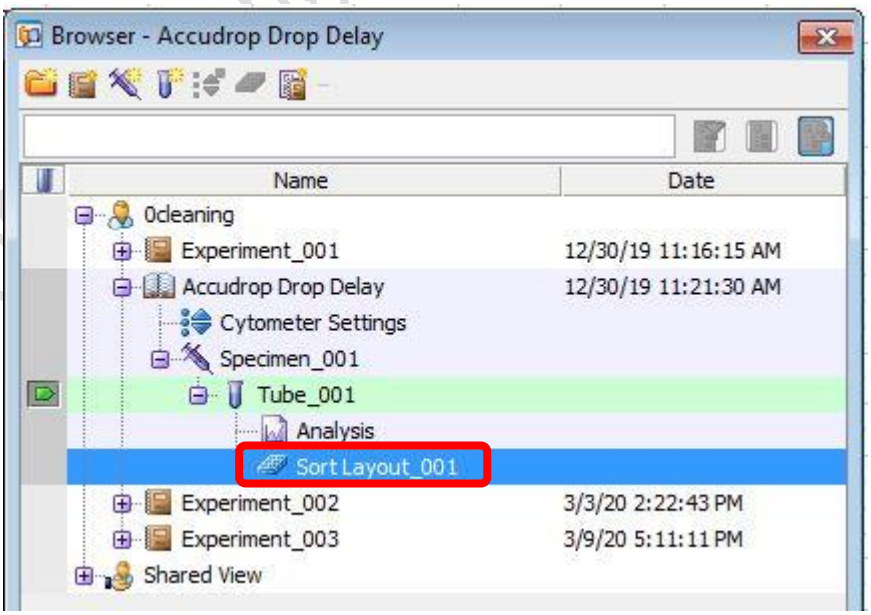
3. Expend Specimen_001



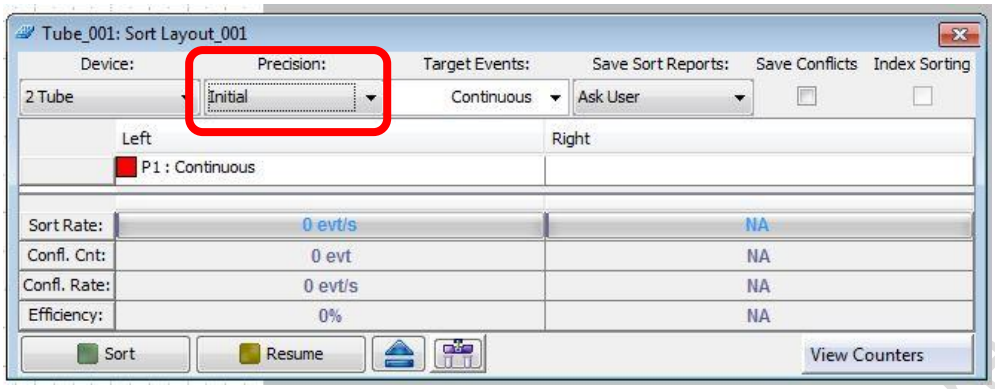
4. Click the tube pointer and expend Tube_001



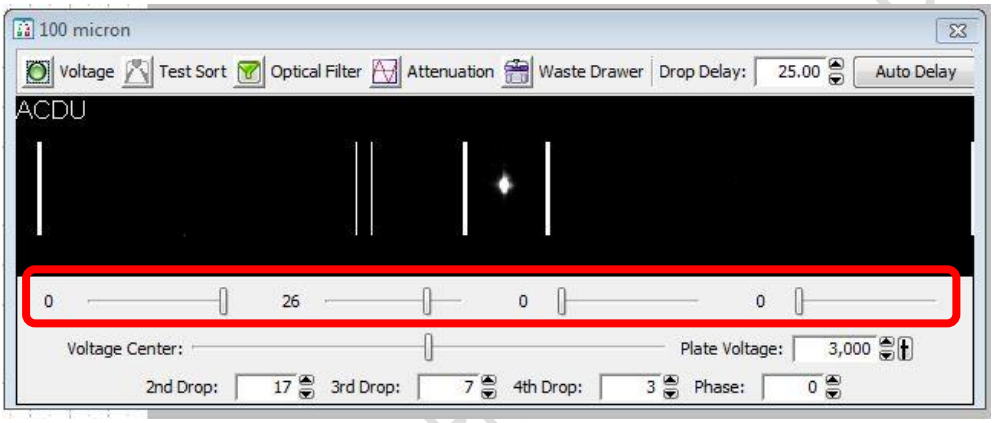
5. Double click Sort Layout_001. Sort Layout window will pop up



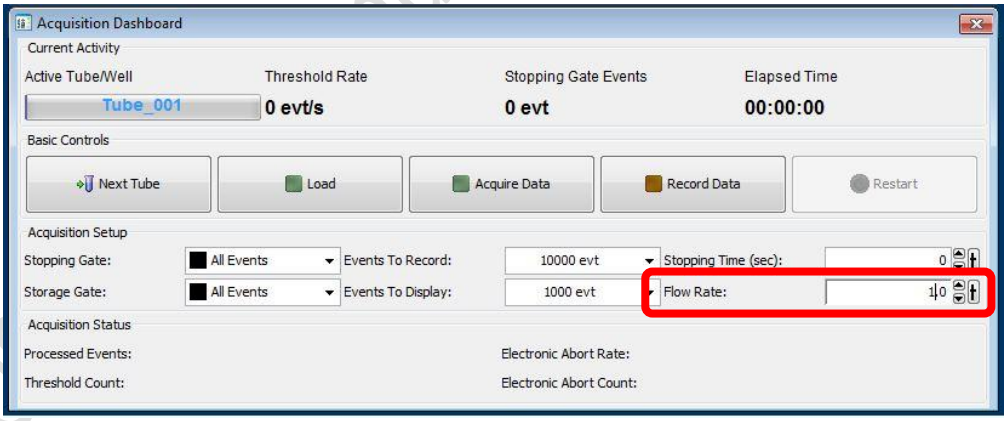
6. Go to Sort Layout Window, Select *Precision > Initial*



7. Go to 100 micron window (lower monitor), set the slider reading as 0 - 26 - 0 - 0



8. Go to Acquisition Dashboard window, Set *Flow rate* to 1.0



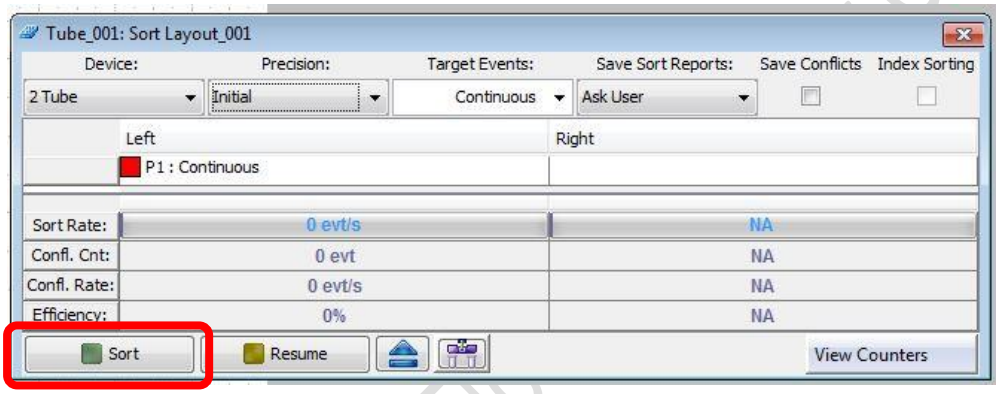
9. Close upper flow cell access door.

10. Load a tube of Accudrop beads (1 mL of PBS + 1 drop of stock) on the sample stage

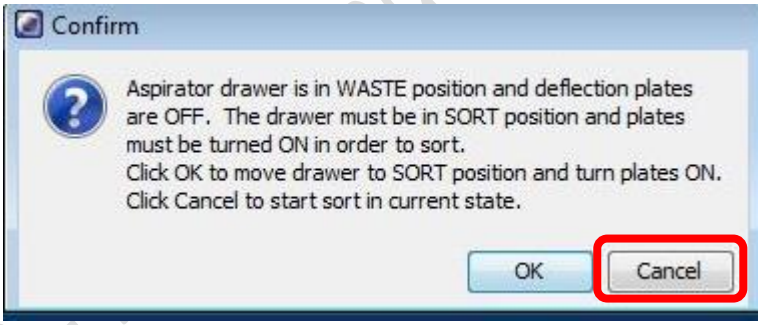
11. Go to Acquisition Dashboard, Click *Load*

12. Adjust *Flow rate* if needed to obtain threshold rate constantly 1000-1500 events per sec

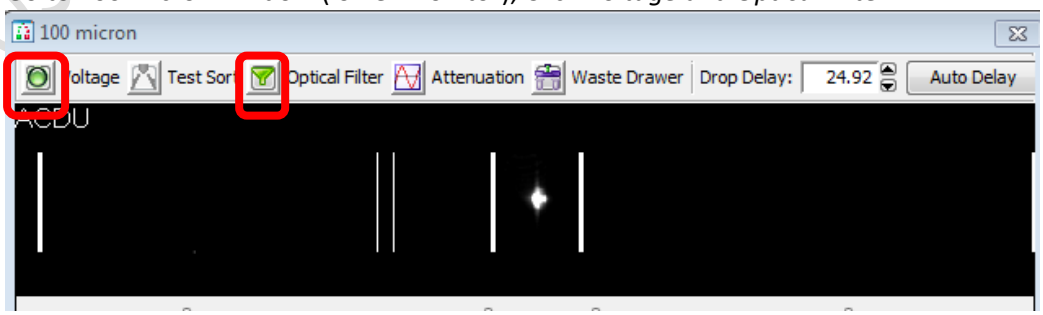
13. Go to Sort Layout window, click *Sort*



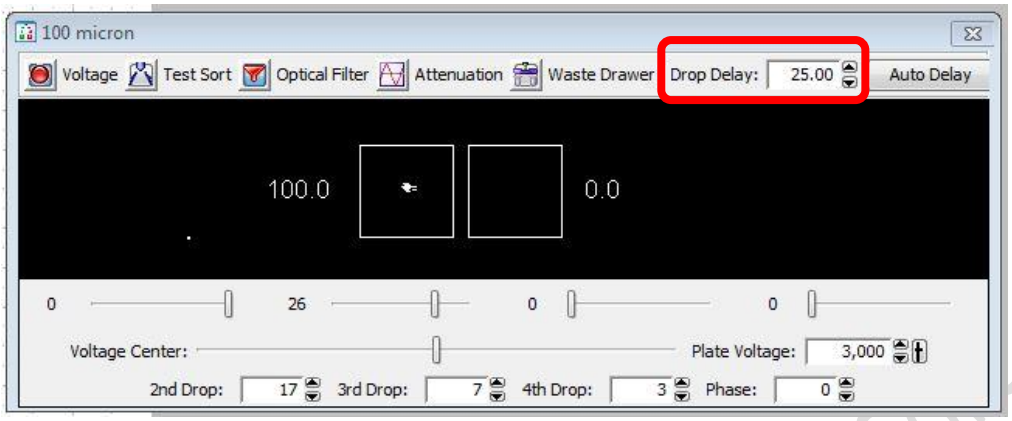
14. Click *Cancel* on the confirm window



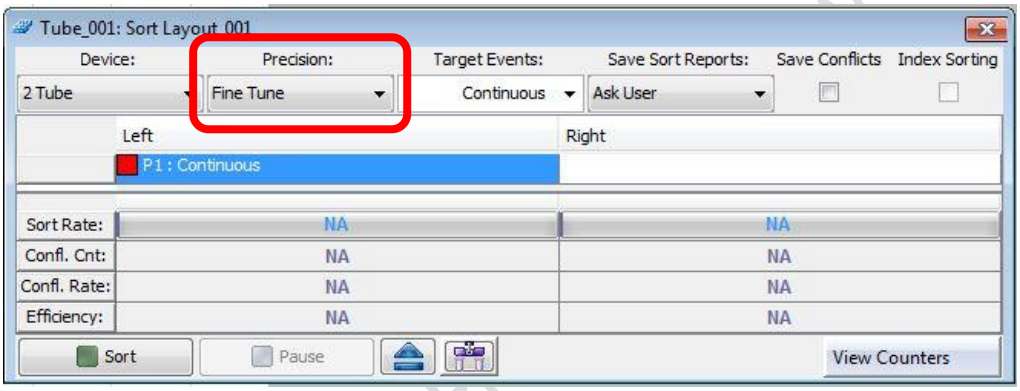
15. Go to 100 micron window (lower monitor), Click *Voltage* and *Optical Filter*



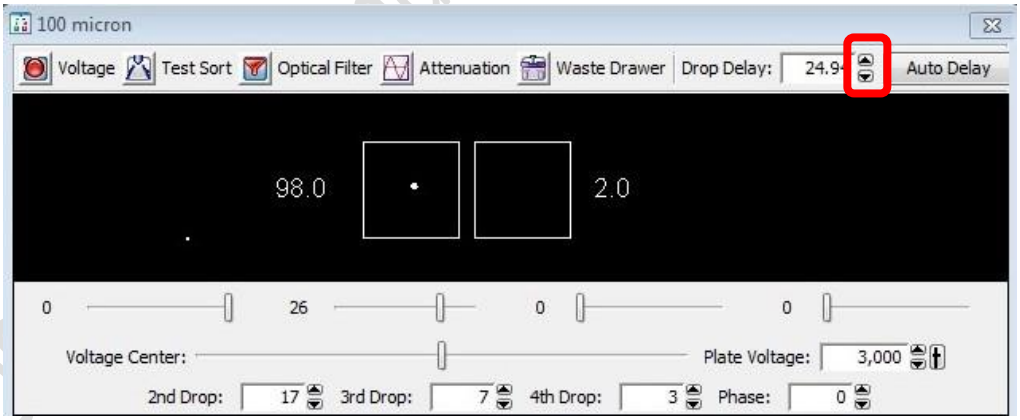
16. Adjust *Drop Delay* value (starting value: 25.00) so that the reading on the left reach 100



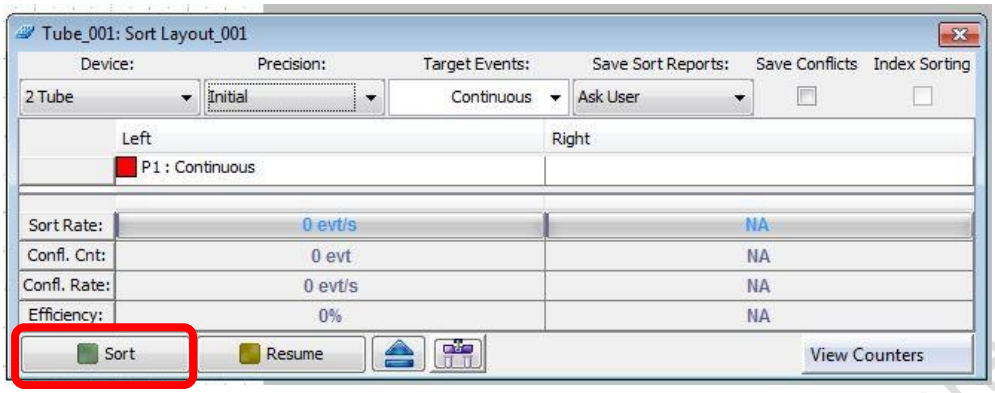
17. Go to Sort Layout window, Select Precision > Fine Tune



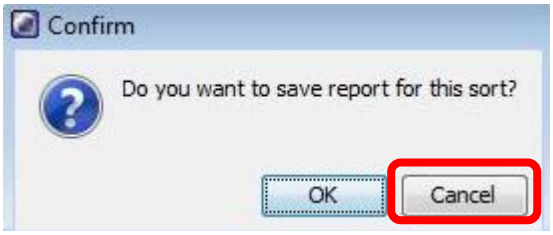
18. Adjust *Drop Delay* value **bit by bit** so that the reading on the left reach >97



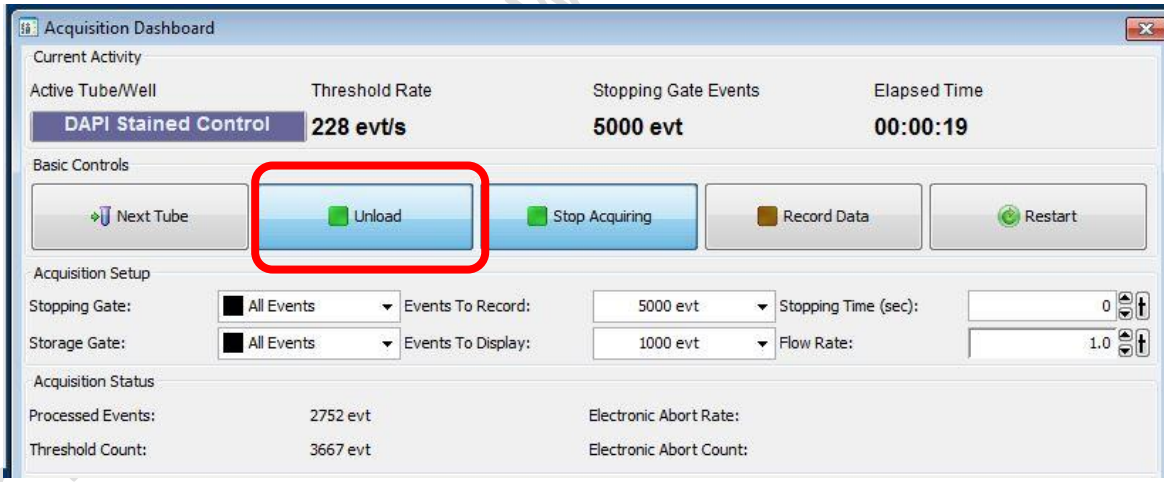
19. Go to Sort Layout Window, Click Sort again.



20. Click *Cancel* on the confirm window



21. Go to Acquisition Dashboard, Click *Unload*

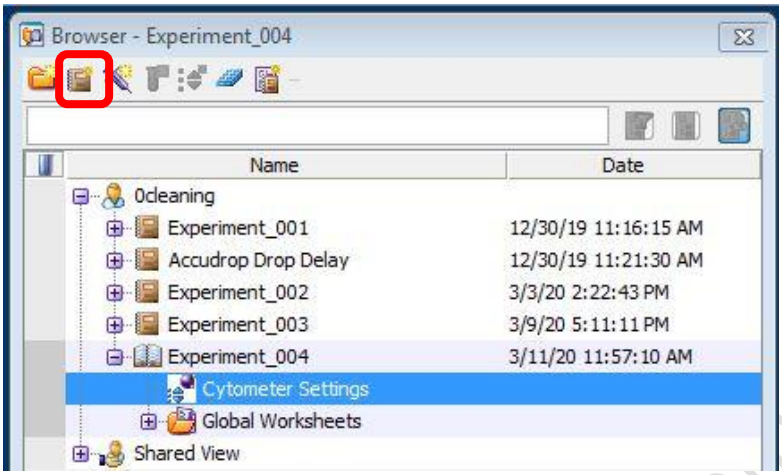


22. Return the Accudrop beads to 4 degree refrigerator

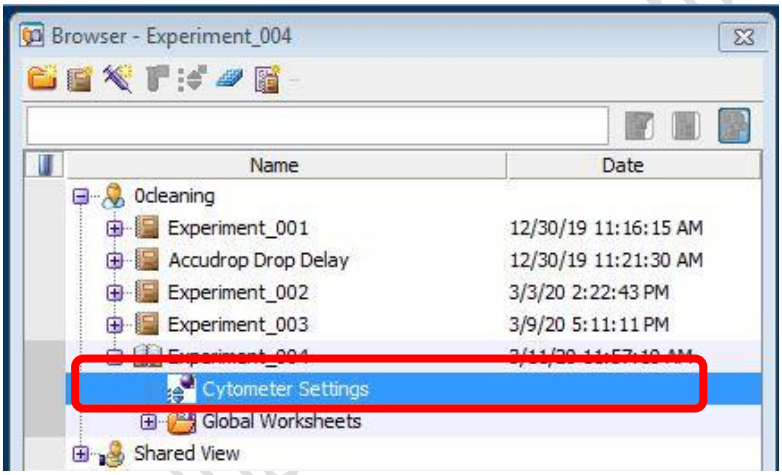
E. Experiment Setup

1. Setup New Experiment

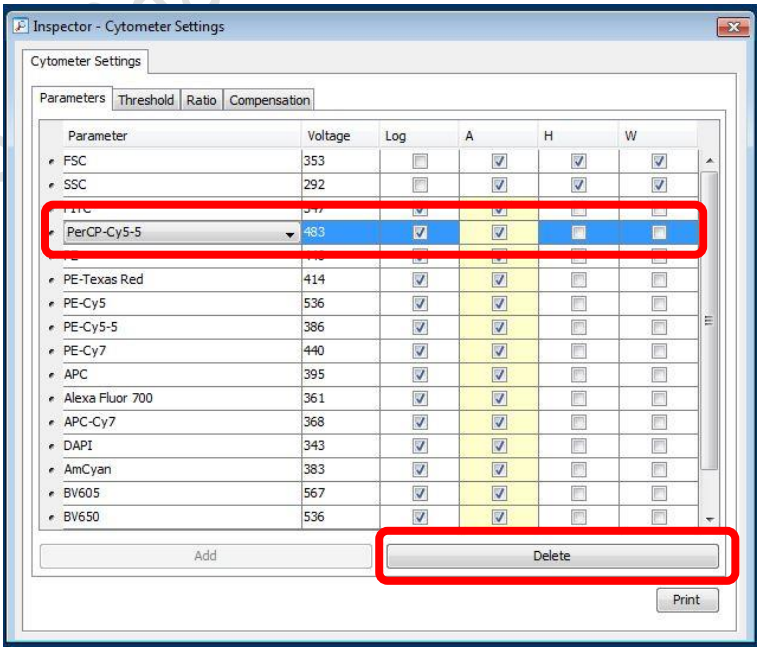
1.1 Go to Browser, Click *New Experiment icon*. A new experiment will be created



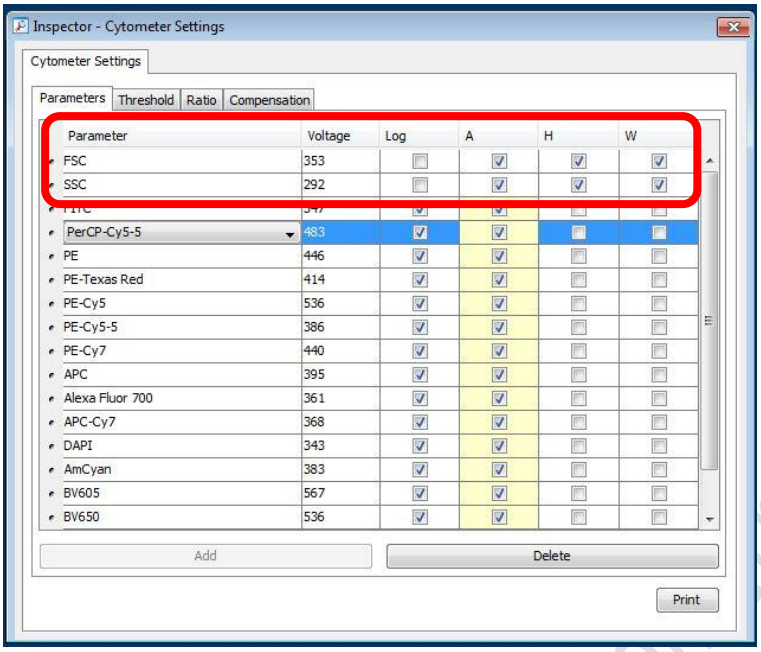
1.2 Click *Cytometer Settings* under the newly created Experiment



1.3 Go to Inspector Window, highlight **unwanted** channels and click *Delete*.



1.4 Check H and W boxes of FSC and SSC



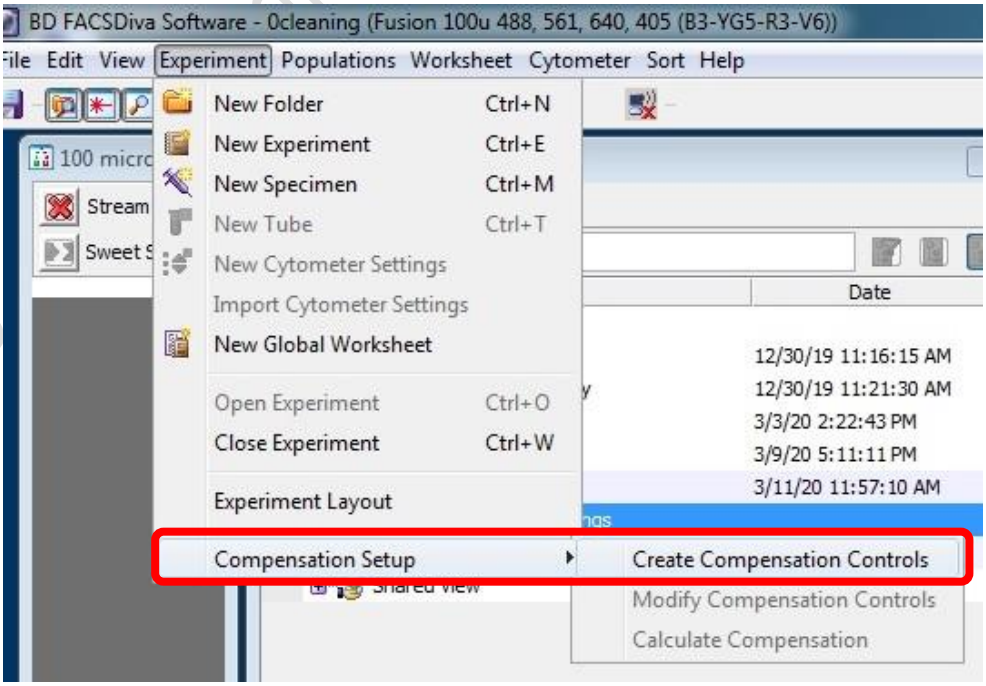
1.5 Keep *Log* boxes of FSC and SSC **unchecked**

1.6 Keep *Log* boxes of all fluorescence channels **checked**

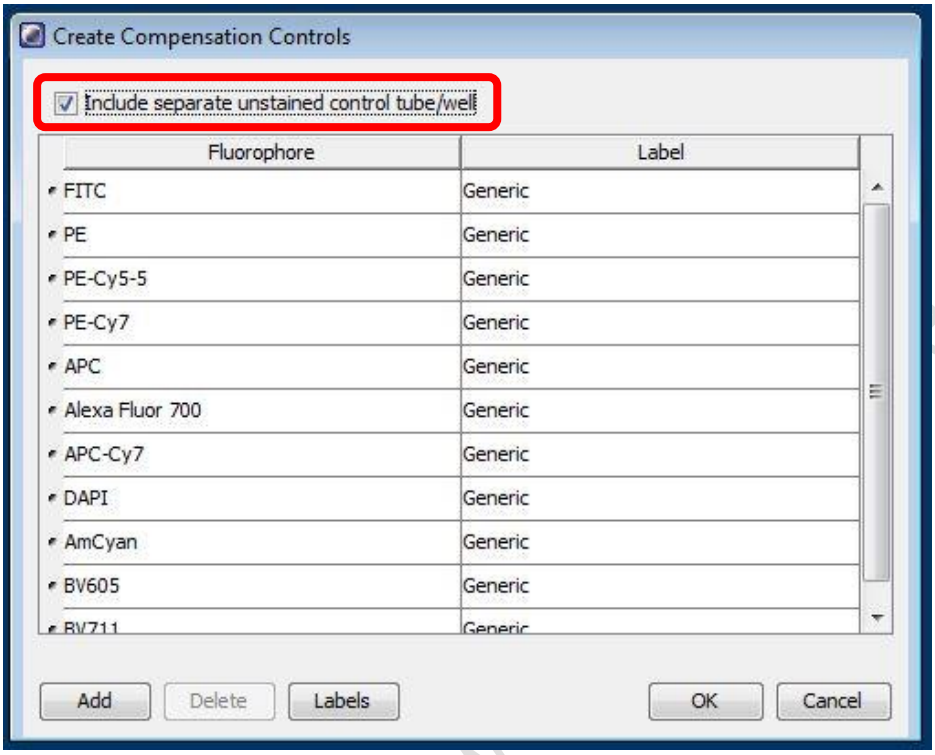
* If you are doing **cell cycle or DNA content** analysis, please keep **log box** of your DNA specific fluorescence channel **unchecked**.

2. Setup Compensation (for Multi-colour panel)

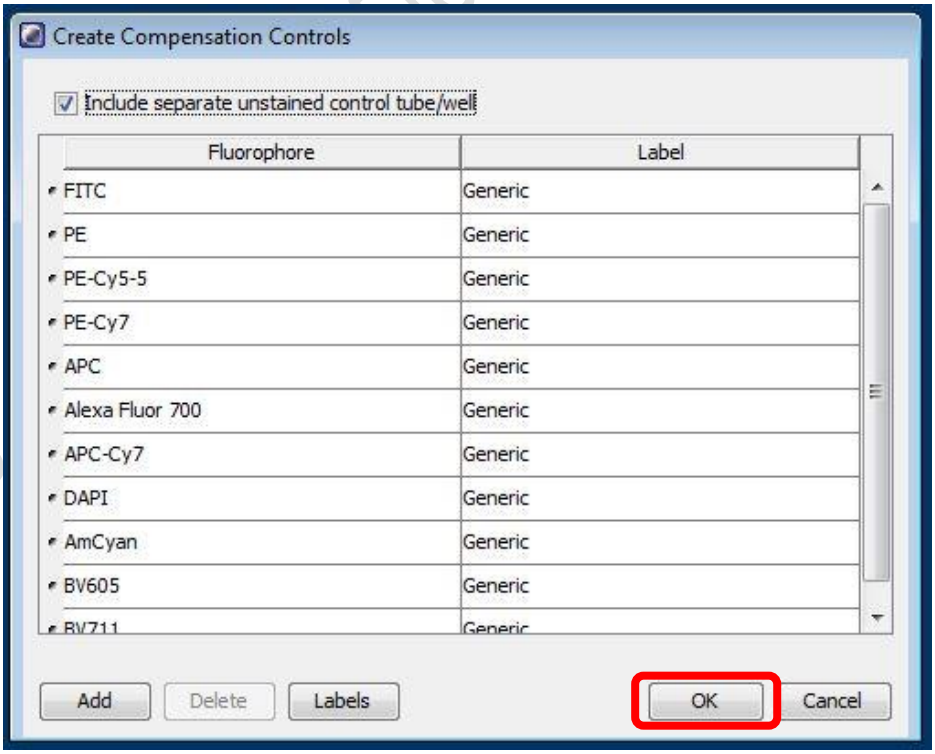
2.1 Click *Experiment > Compensation Setup > Create Compensation Control*



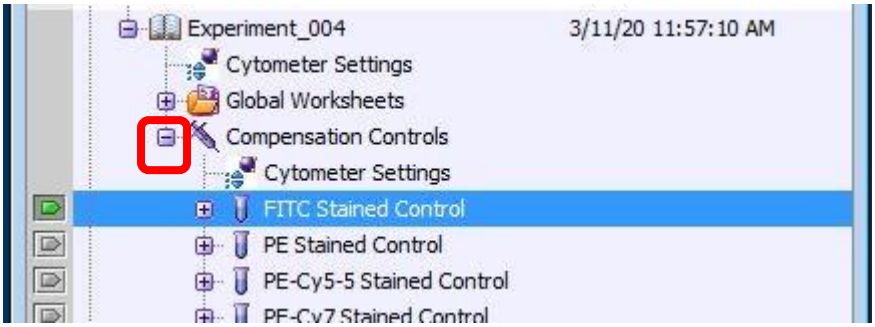
2.2 If any one of your single stain controls is known to be 100% positive, i.e. no negative population, check the box “Include separate unstained control tube/well”.



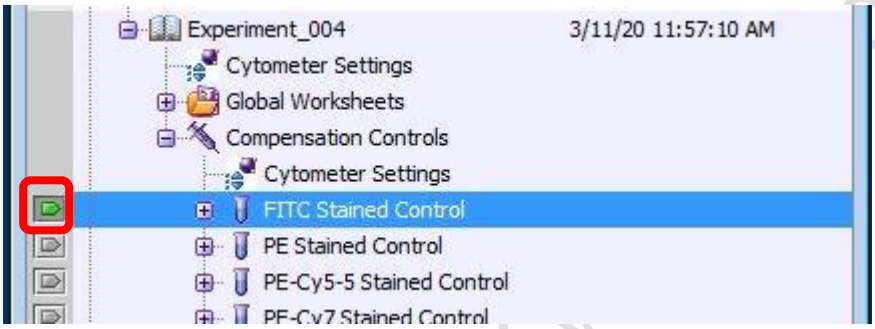
2.3 Click *OK*



2.4 Expand the Compensation Control Specimen



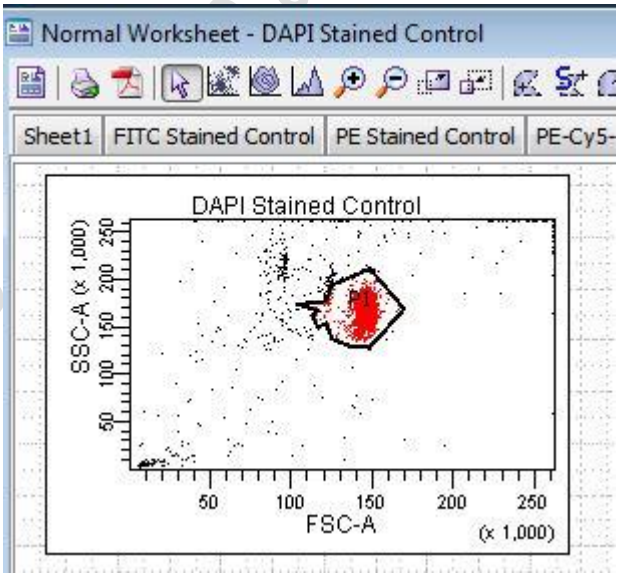
2.5 Click the tube pointer of the first tube



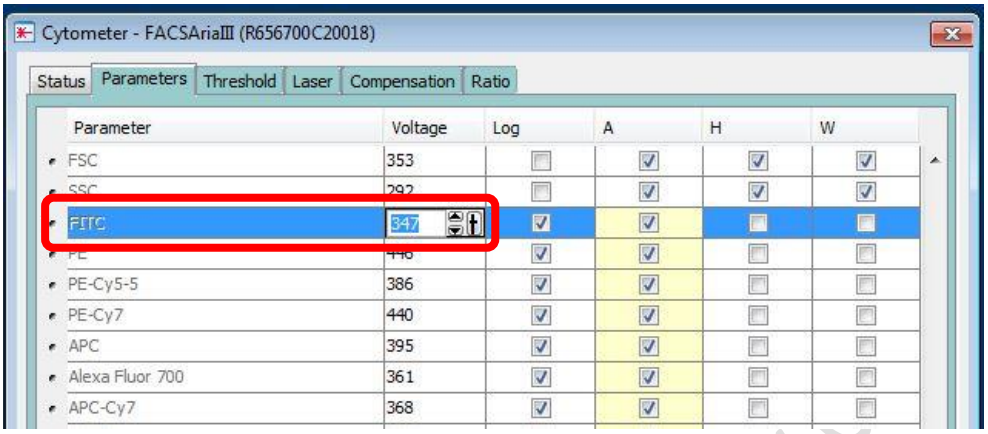
2.6 Load the single stain controls on the sample stage according to the tube label, i.e. run FITC single stain when the tube pointer is pointing at “FITC Stained Control”

2.7 Go to Acquisition Dashboard, Click *Load*.

2.8 Go to Normal Worksheet, move the P1 gate to include major cluster. Adjust FSC and SSC PMT Voltage if needed

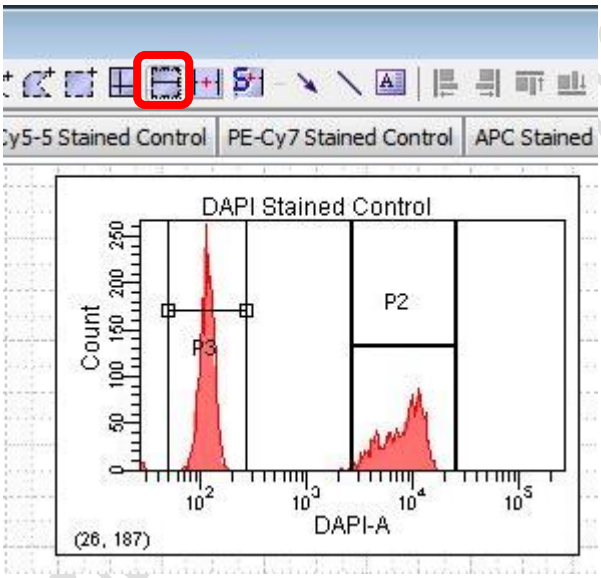


2.9 Go to Cytometer window, Fine tune the corresponding fluorescence PMT voltage to have best separation of negative and positive peak

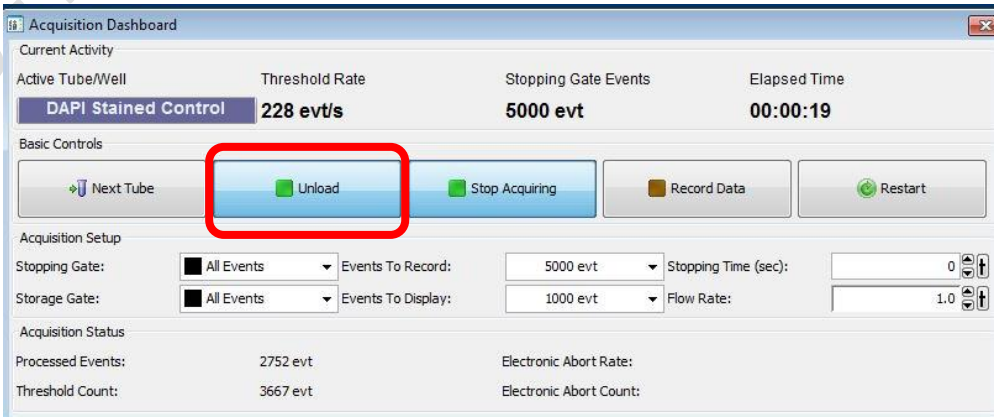


2.10 Move the interval gate (P2) to include the positive peak

2.11 Use interval gate to gate out negative peak (P3)



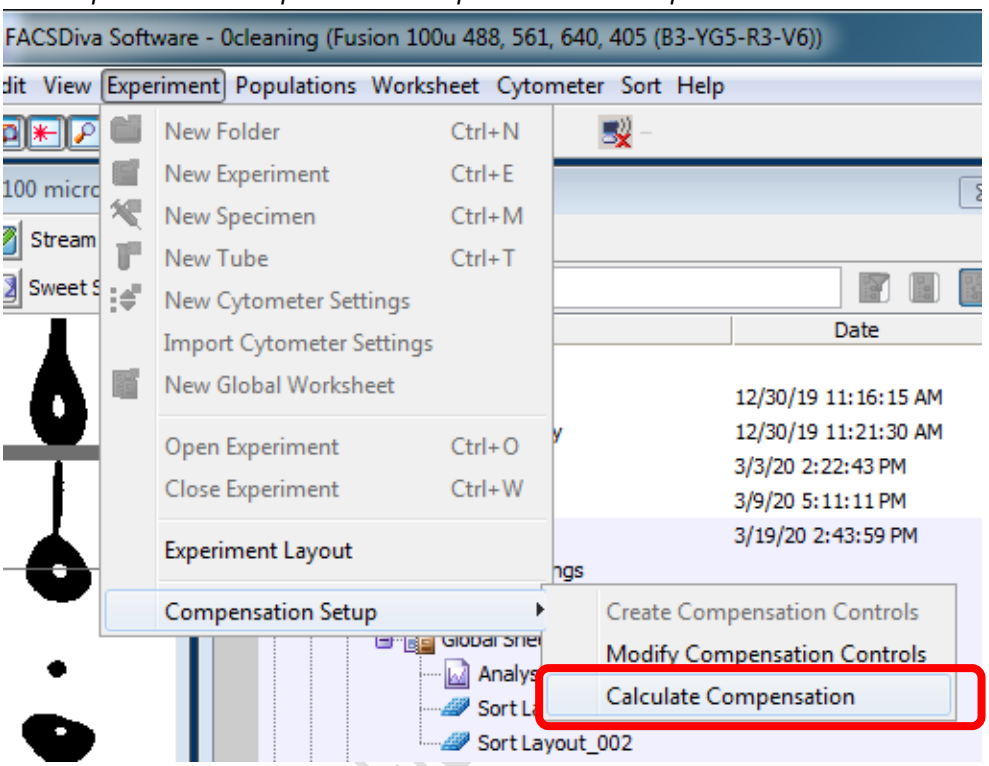
2.12 Go to Acquisition Dashboard, Click *Unload*. *DO NOT Record Data at this point



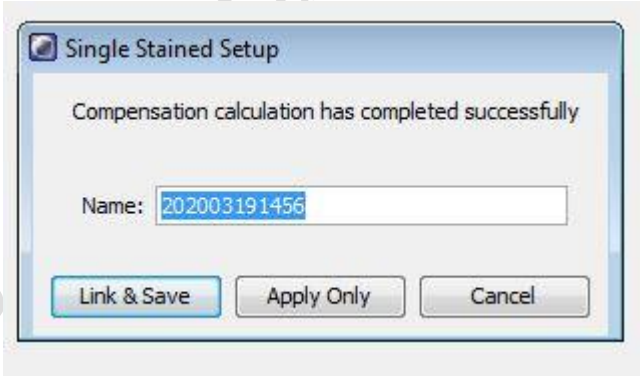
2.13 Repeat step 2.5 – 2.12 with all the single stain controls.

2.14 After optimising the PMT voltage of ALL the fluorescence channel, load each single stained control and click *Record Data* for ALL single stain controls

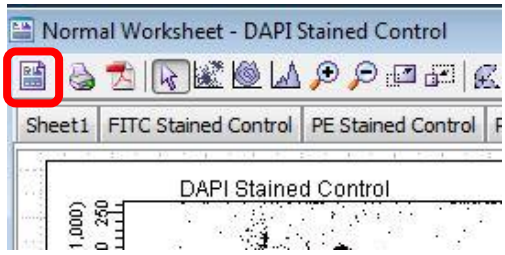
2.15 Click *Experiment > Compensation Setup > Calculate Compensation*



2.16 Click *Link and Save* for the most stringent practice, i.e. cannot adjust PMT voltage anymore **OR** Click *Apply Only* for some flexibility on PMT voltage adjustment of your samples.

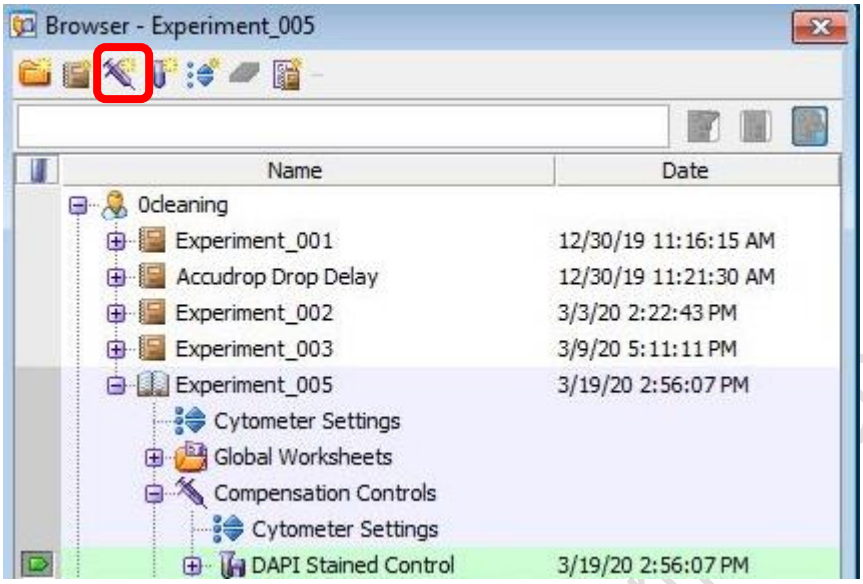


2.17 Switch Normal worksheet to Global worksheet

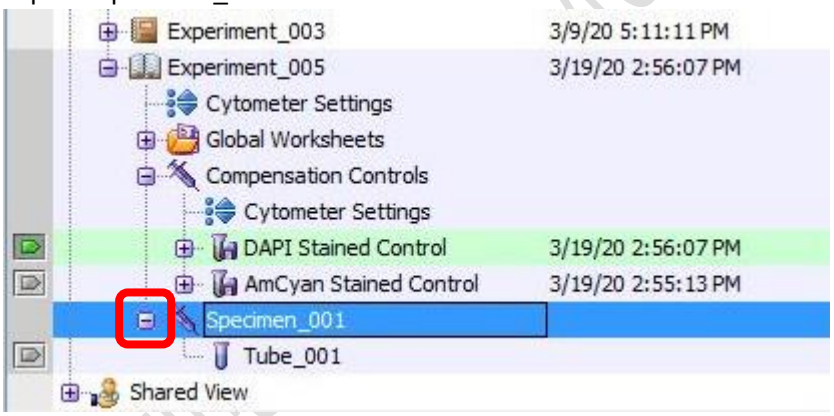


3. Setup Plots and Tables

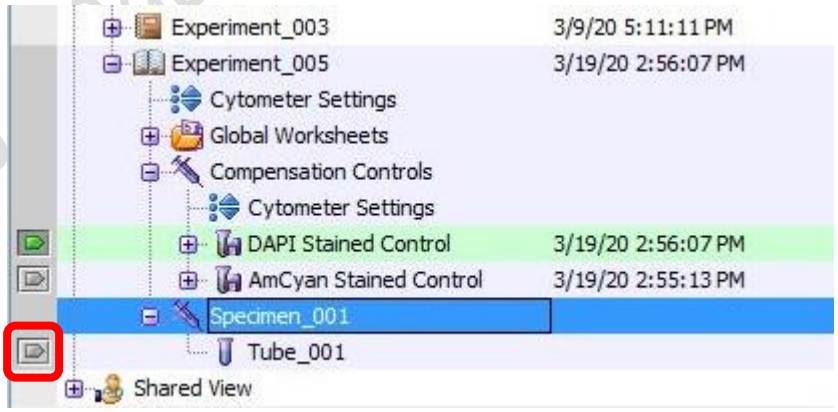
3.1 Go to Browser, Click *New Specimen icon*



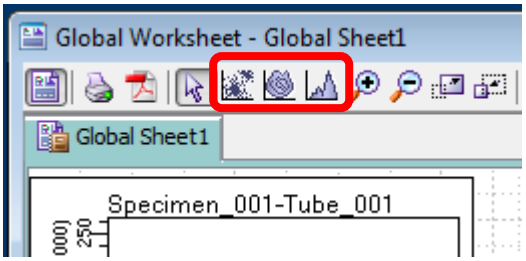
3.2 Expand Specimen_001



3.3 Click the tube pointer of Tube_001



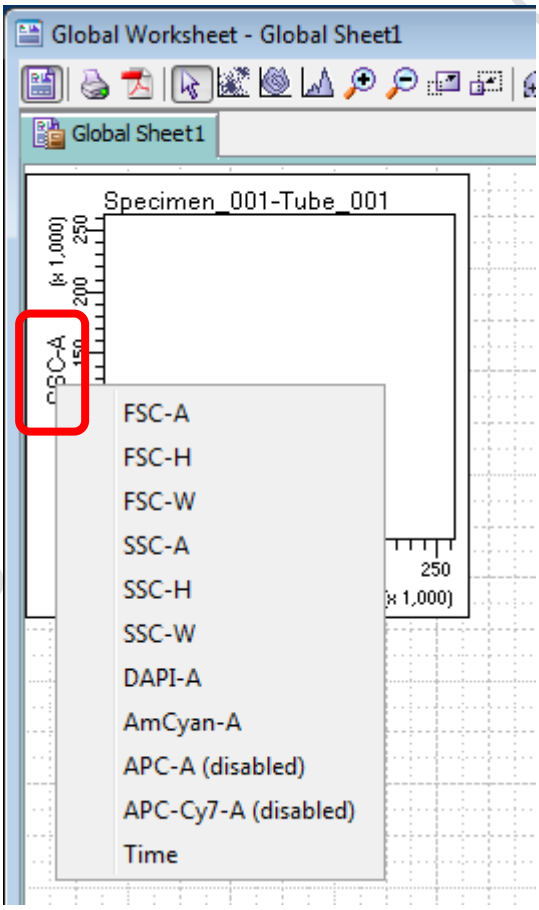
3.4 Go to Global Sheet Window, Click the graph type icon



Icon	Type
	Dot Plot
	Contour Plot
	Histogram

3.5 Click on the blank area of Global Worksheet window to create a new plot.

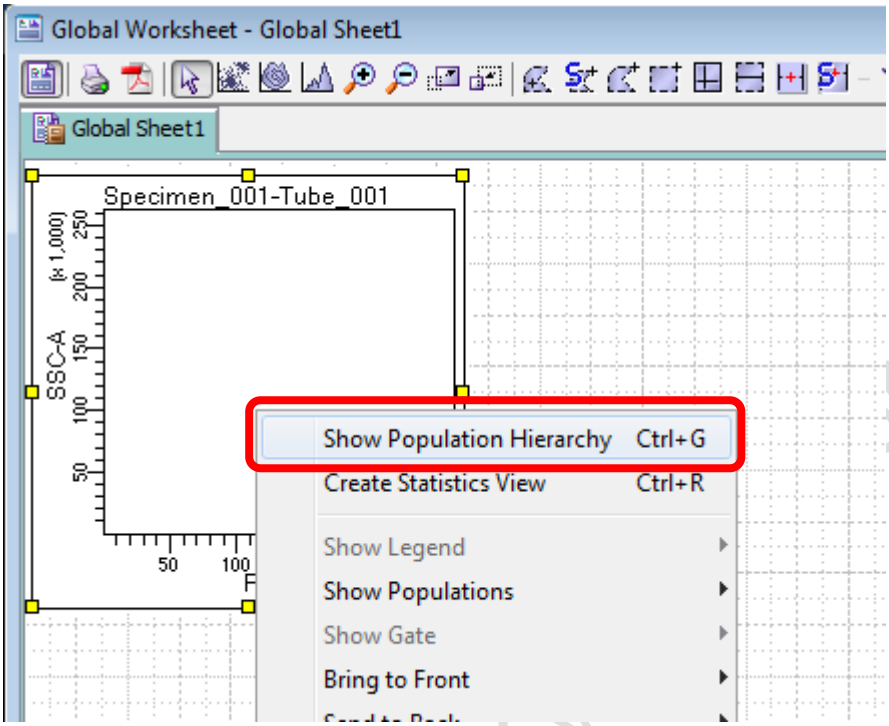
3.6 Mouse over the axis label and right click. Select the parameters of interest from the list.



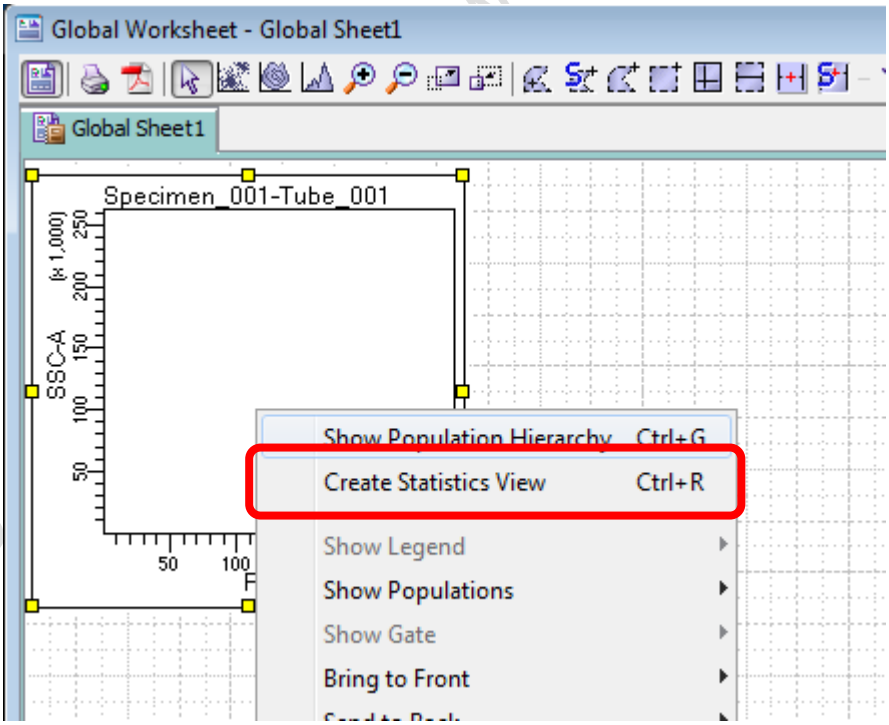
3.7 Repeat step 3.4 – 3.6 until all plots needed is created.

*** Essential Plots: FSC-A VS SSC-A; FSC-H VS FSC-W; SSC-H VS SSC-W**

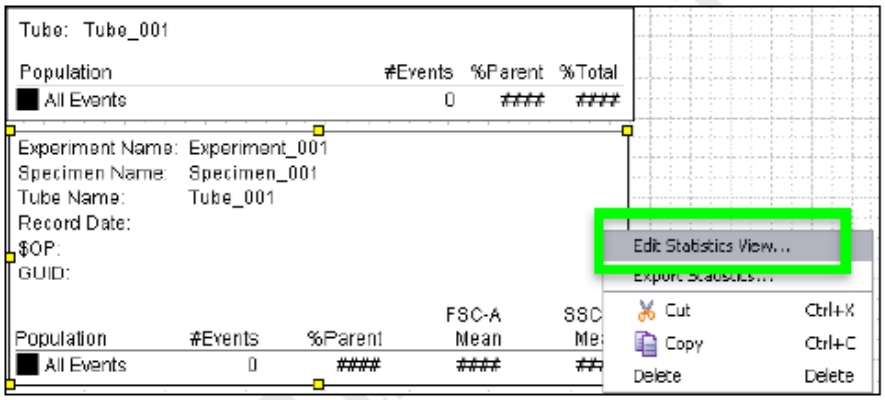
3.8 Click on any plot and right click. Click *Show Population Hierarchy*



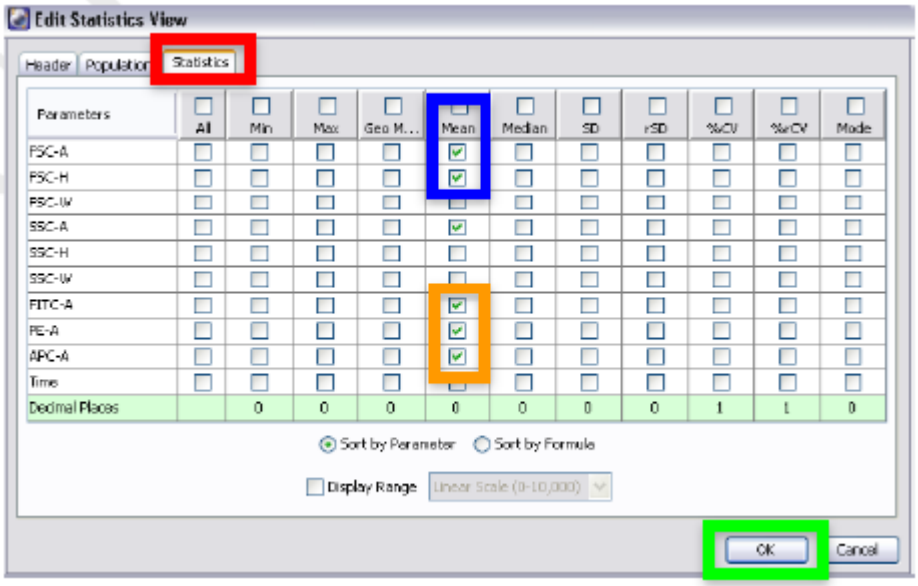
3.9 Click on any plot and right click. Click *Create Statistics View*



3.10 Click on Statistics View table and right click, Click *Edit Statistics View* to select statistics of interest to be shown in the table.



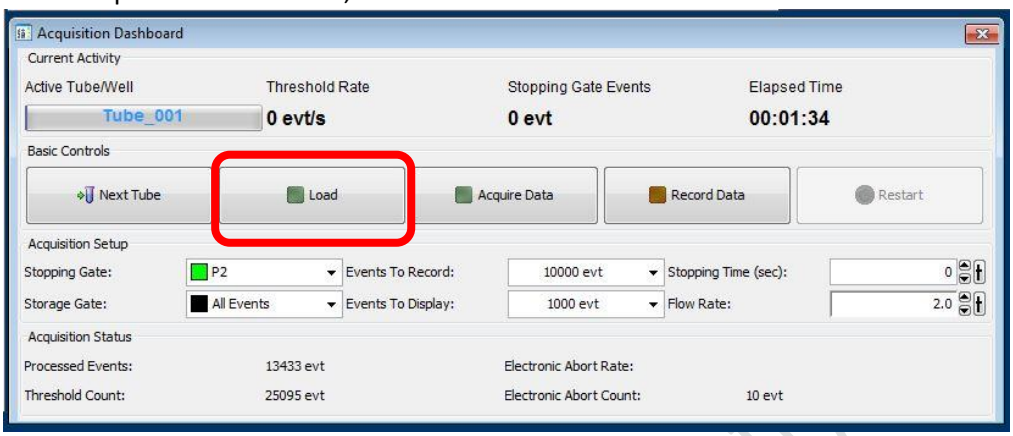
3.11 Click *Statistics* Tab, check the boxes of interested statistics and then click *OK*



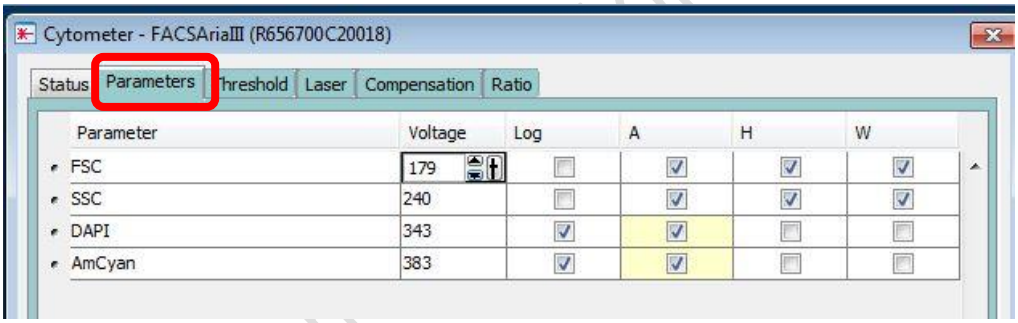
4. Sample Acquisition

4.1 Load your sample on the sample stage

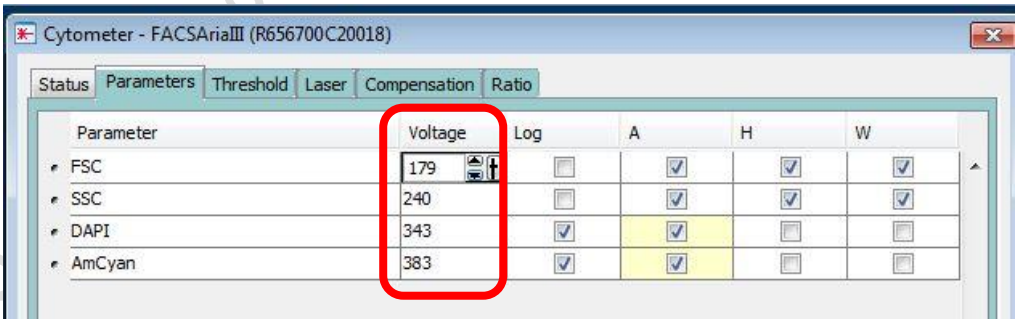
4.2 Go to Acquisition Dashboard, Click *Load*



4.3 When you start seeing dots appear on the plot, Go to Cytometry window and Click *Parameters*

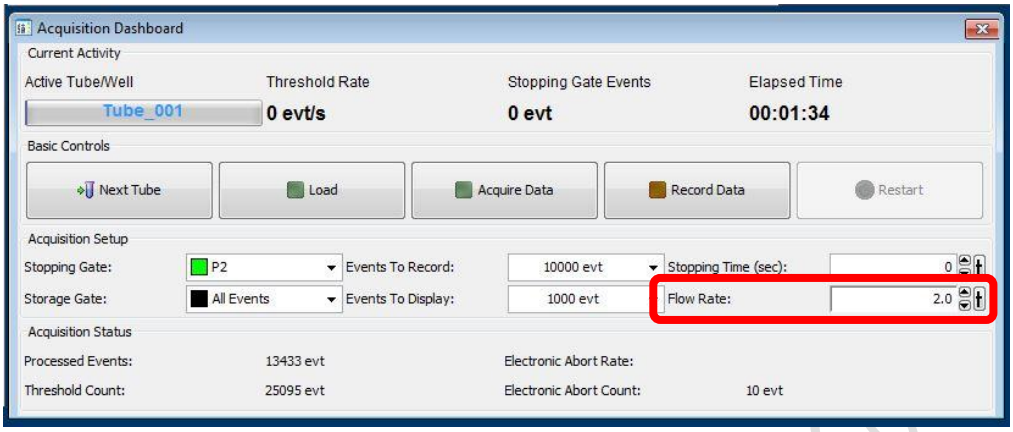


4.4 Adjust PMT Voltage accordingly



Channel	Suggested Voltage range for mammalian cells
FSC	180-300 *If you sample cell size is too big to visualise with FSC voltage 180, you may change the FSC ND filter from 1.5 to 2.0
SSC	230-330
Fluorescence	300-850

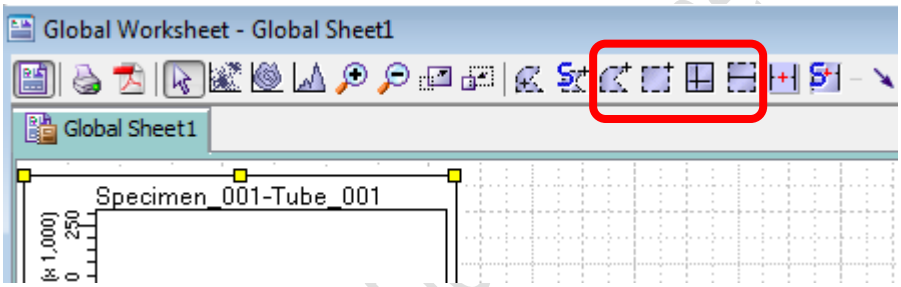
4.5 Adjust *Flow rate* if needed (optimum Threshold rate 2000 – 5000 evt/s)



* If you perform sorting, DO NOT set flow rate > 5.0 or threshold rate > 5000 event/s

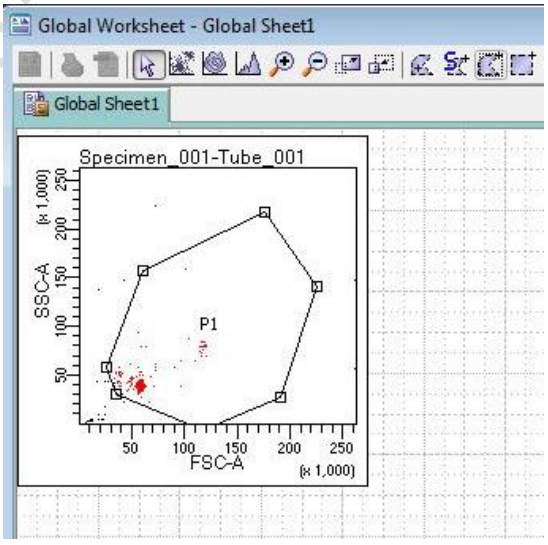
5. Create Gates

5.1 Go to Global Sheet Window, Click the type of gate needed

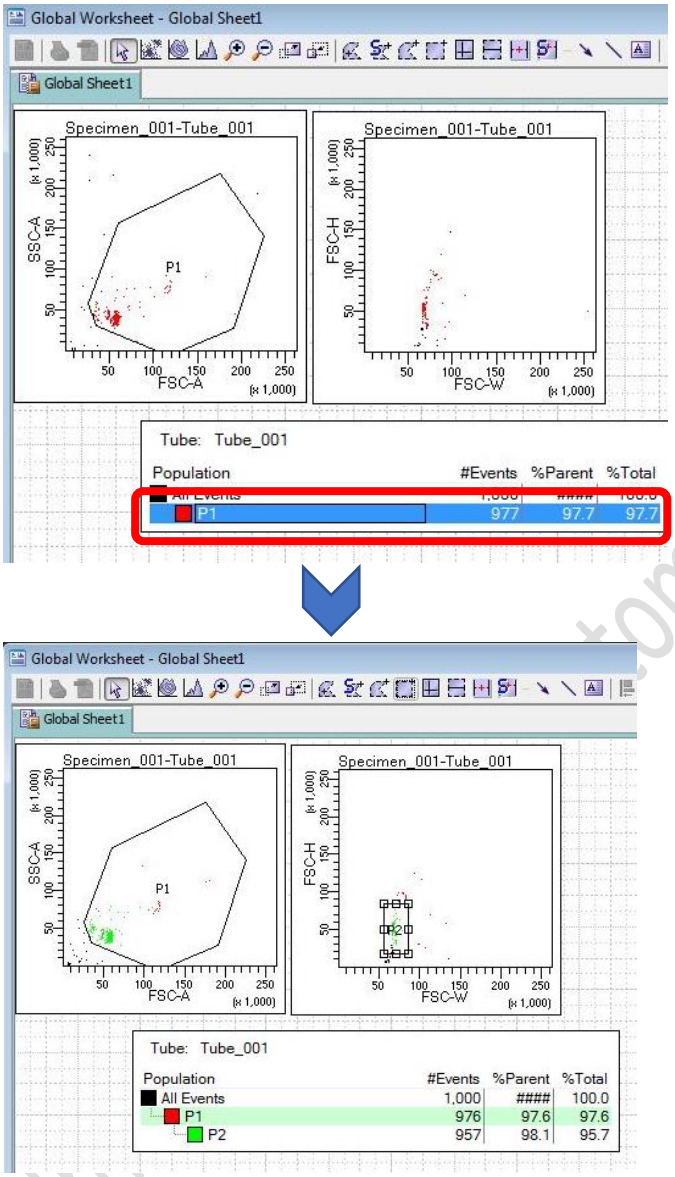


Icon	Type
	Polygon Area Gate
	Rectangle Area Gate
	Quantrad Gate
	Interval Gate

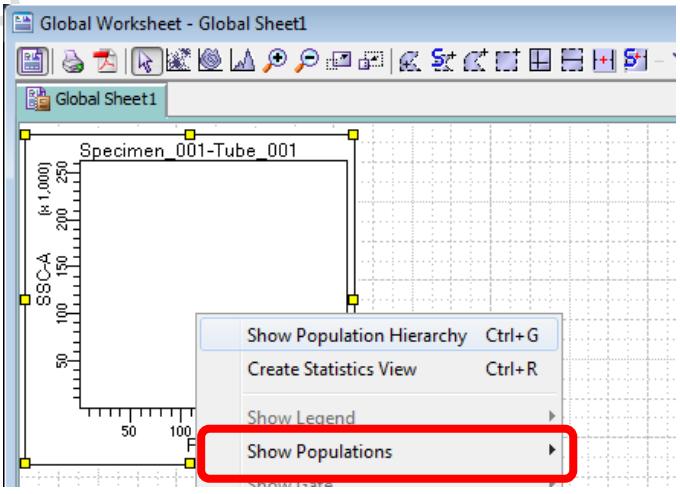
5.2 Draw the gate on the plot of interest to gate out target cluster /peak



5.3 If you want to create a new population out of particular parent population, highlight the parent gate on the hierarchy table first and then create the gate.

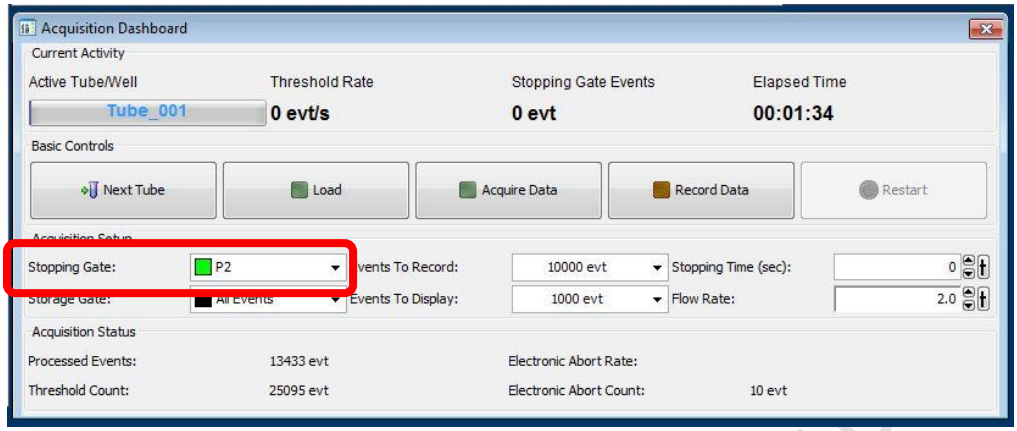


5.4 Click on target plot and right click, Click *Show Population* > *Target population* to visualize the target population ONLY in that plot.

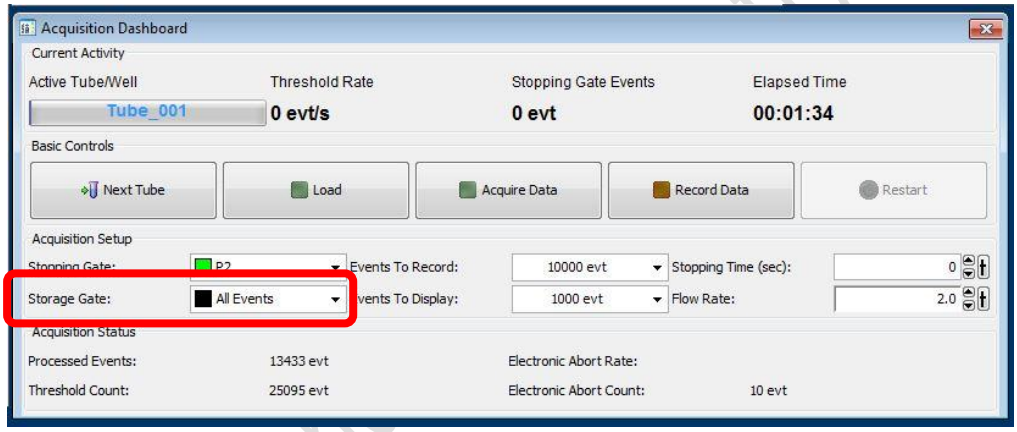


6. Data Recording

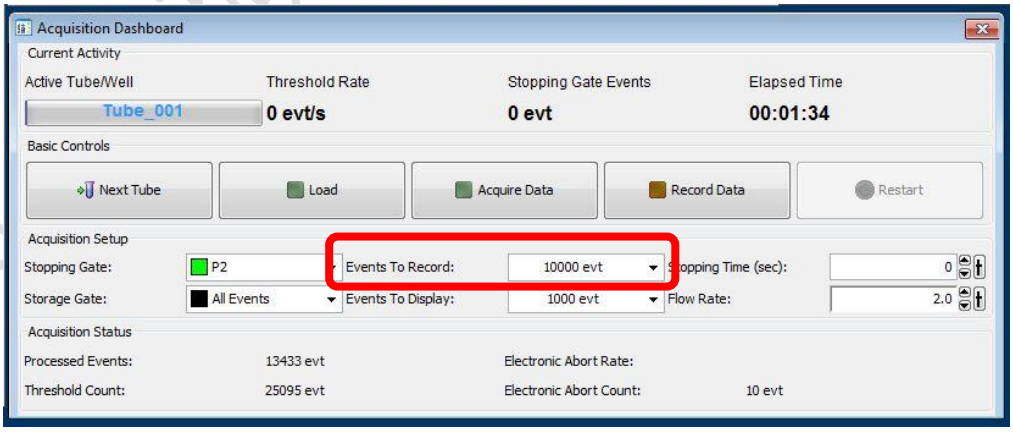
6.1 Go to Acquisition Dashboard, set Stopping gate to singlet gate or live cell gate



6.2 Set the Storage gate to All Events



6.3 Set Events To Record, i.e. events number out of stopping gate to be recorded (suggested number 10,000 – 50,000)



6.4 if the sample is Unload or Acquisition is stopped, Click *Load* or *Acquire Data*

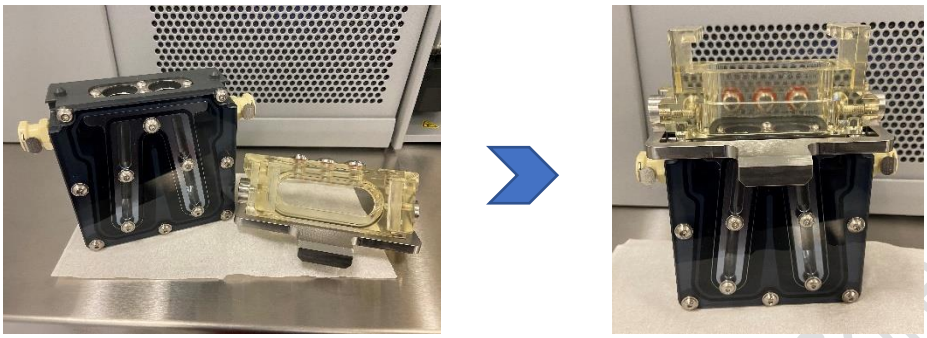
6.5 Click *Record Data*

6.6 Click *Next Tube* to create a new sample

F. Sort Device Alignment

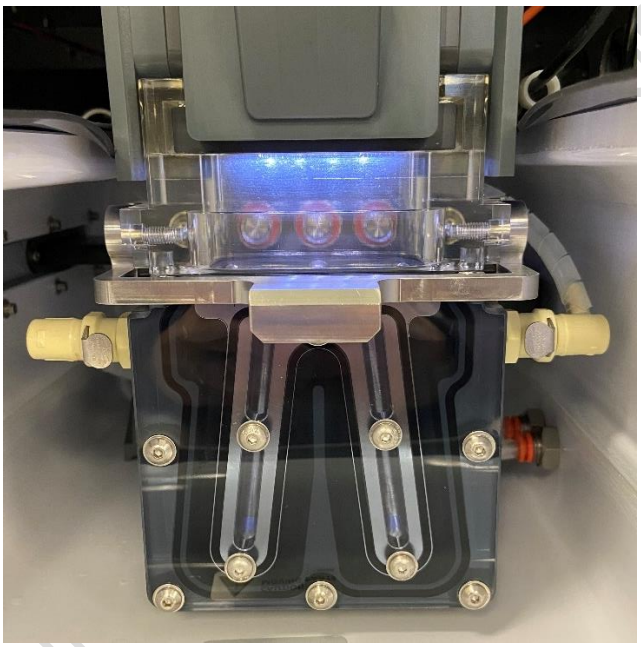
1. Tube Holder

1.1 Assemble the tube holder as picture below



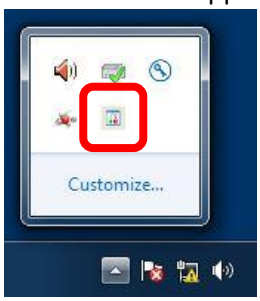
1.2 Put dummy tubes into the position

1.3 Slide the tube holder right under the sort chamber. Plug in water tubing if cooling is needed.

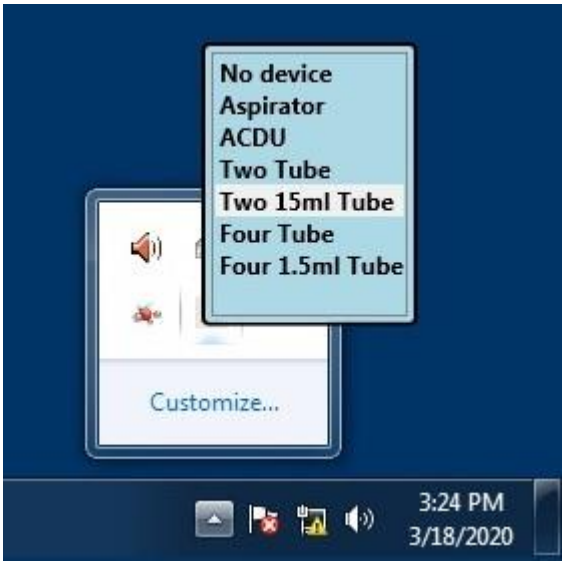


1.4 Click *Select Device* icon on the lower right corner of the upper monitor

OR



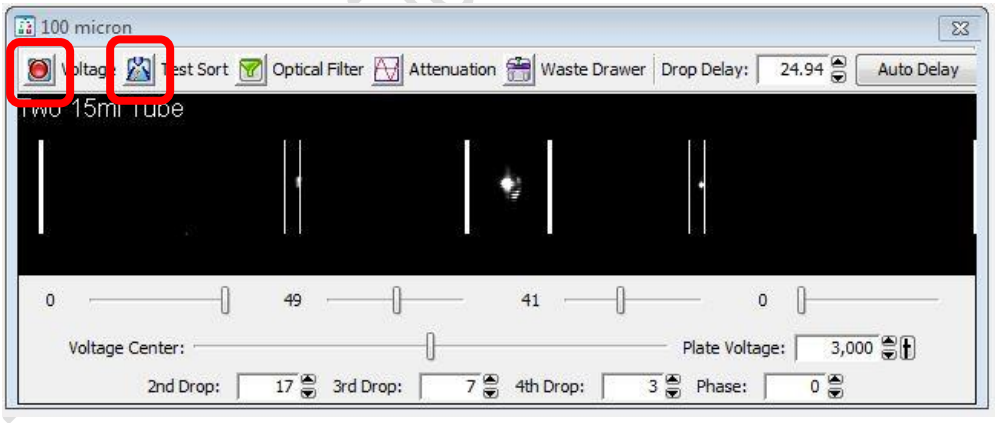
1.5 Select collection device of interest



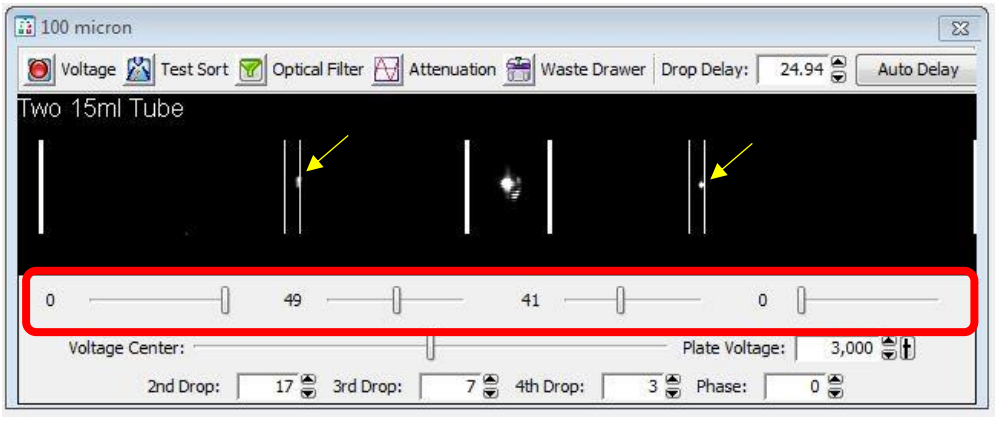
1.6 Go to 100 micron window (lower monitor). Adjust the Slider

Device	Suggested Slider reading
2-tube 15 ml	0 – 49 – 41 – 0
4-tube 1.5ml / 2.0ml	71 – 30 – 25 – 68
4-tube 5 ml	80 – 30 – 25 – 74

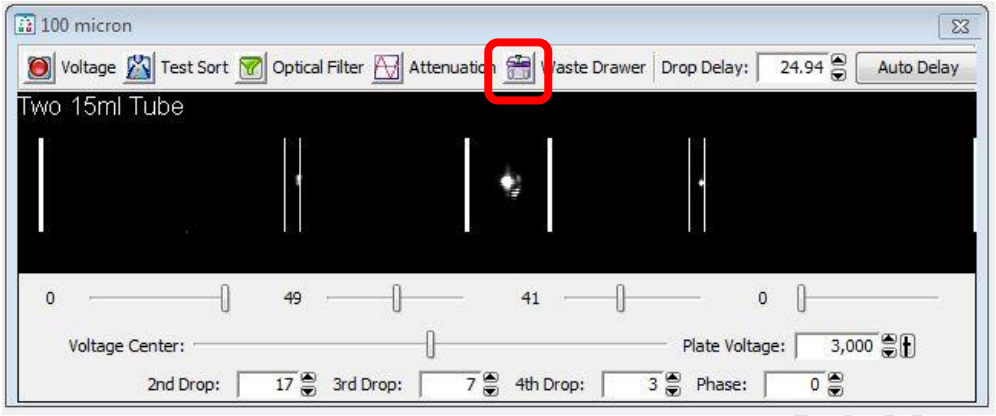
1.7 Click *Voltage*. Wait 2 seconds and then Click *Test Sort*.



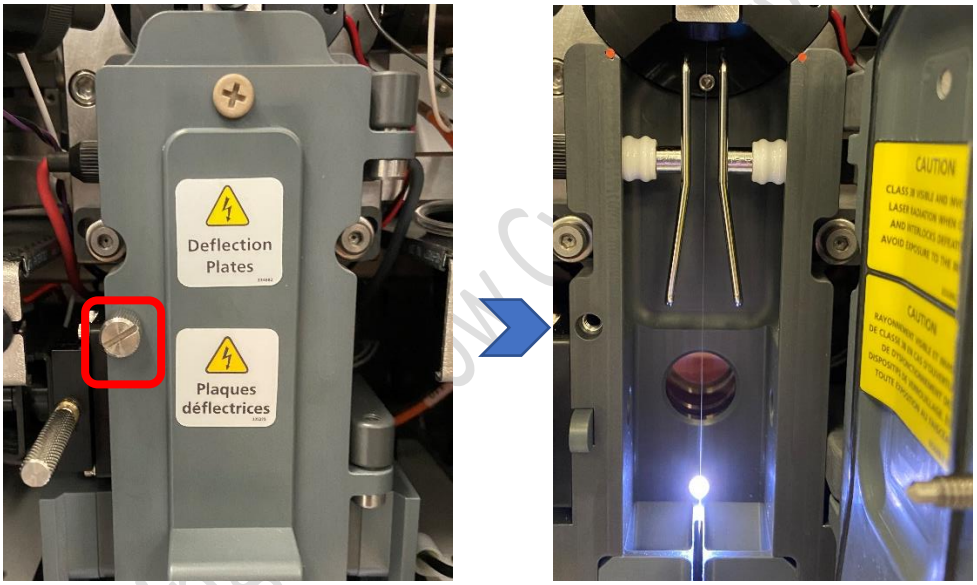
1.8 Adjust the slider so the side stream dot lines within the target lines



1.9 Click *Waste Drawer*

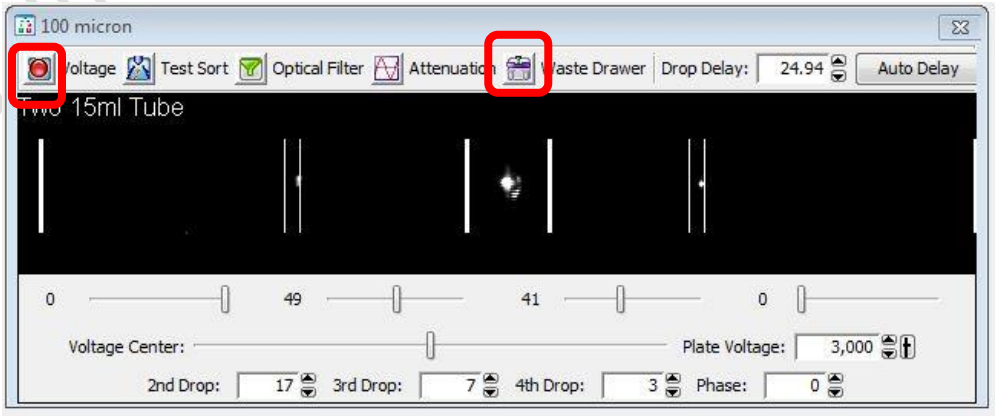


1.10 Open the sort chamber door



1.11 Check if the side streams can entre the dummy tubes. Adjust the slider if needed

1.12 Click *Waste Drawer* and then *Voltage*

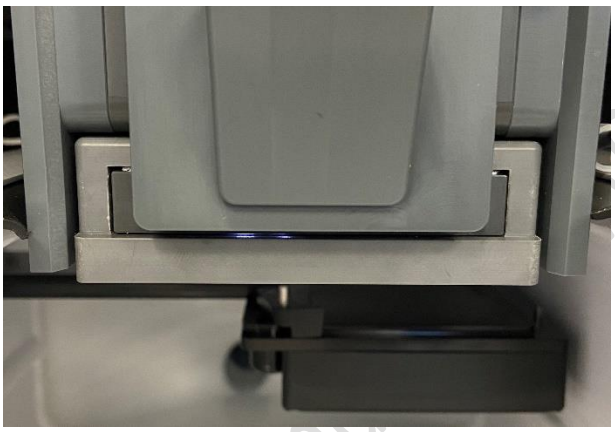


1.13 Close the sort chamber door

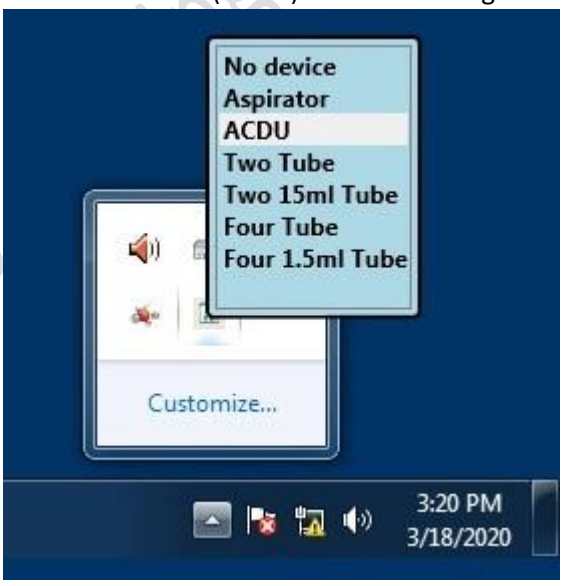
1.14 Slide out the tube holder and Put collection tubes into position

2. ACDU (Culture plate)
   2.1 Slide the ACDU adaptor right under the sort chamber

ACDU adaptor



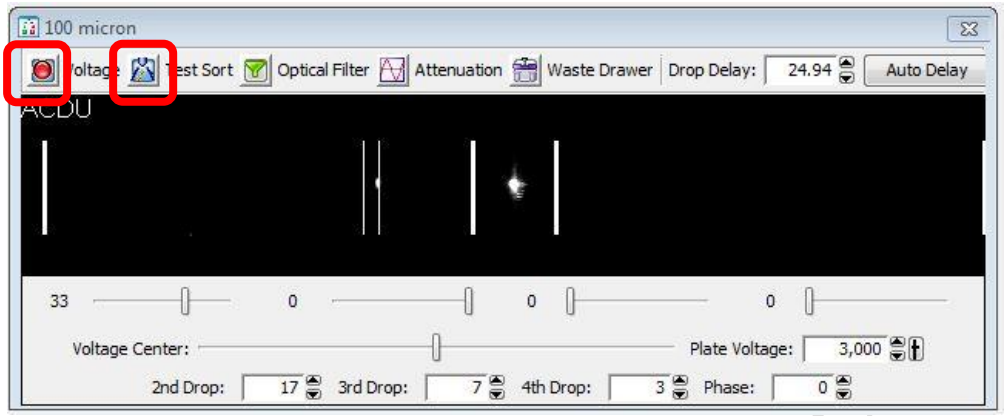
   2.2 Select the device (ACDU) on the lower right corner of the upper monitor



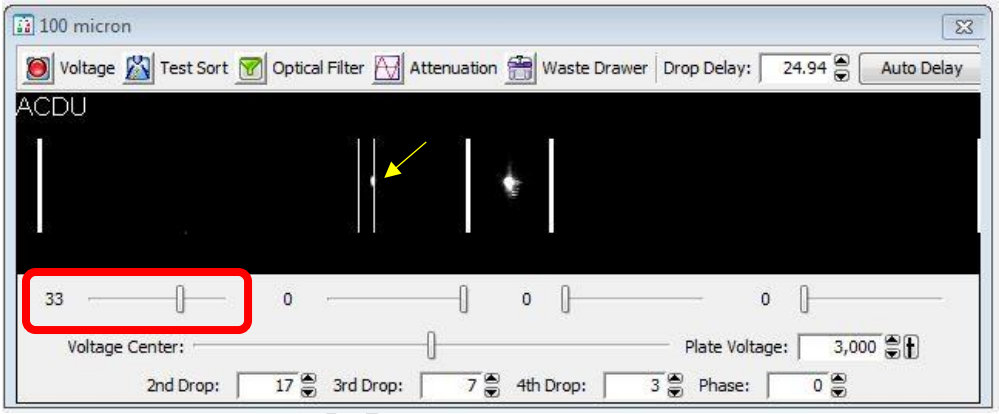
   2.3 Go to 100 micron window (lower monitor). Adjust the Slider

Device	Suggested Slider reading
96-well plate	33 – 0 – 0 – 0

2.4 Click *Voltage*. Wait 2 seconds and then Click *Test Sort*

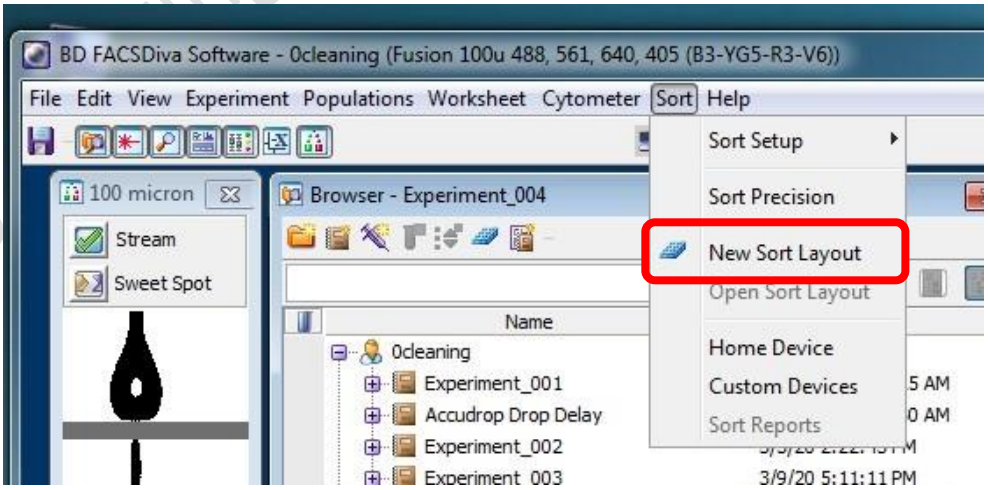


2.5 Adjust the slider so the side stream dot lines within the target lines

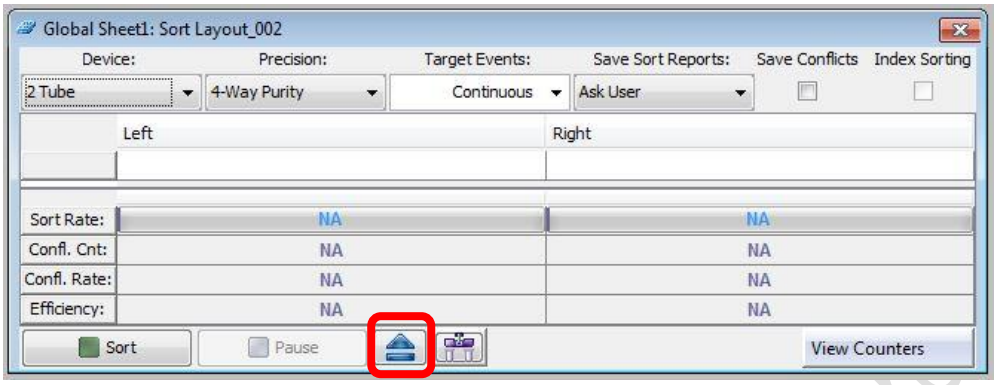


2.6 Click *Voltage* again to stop test sort

2.7 Click *Sort > New Sort Layout*

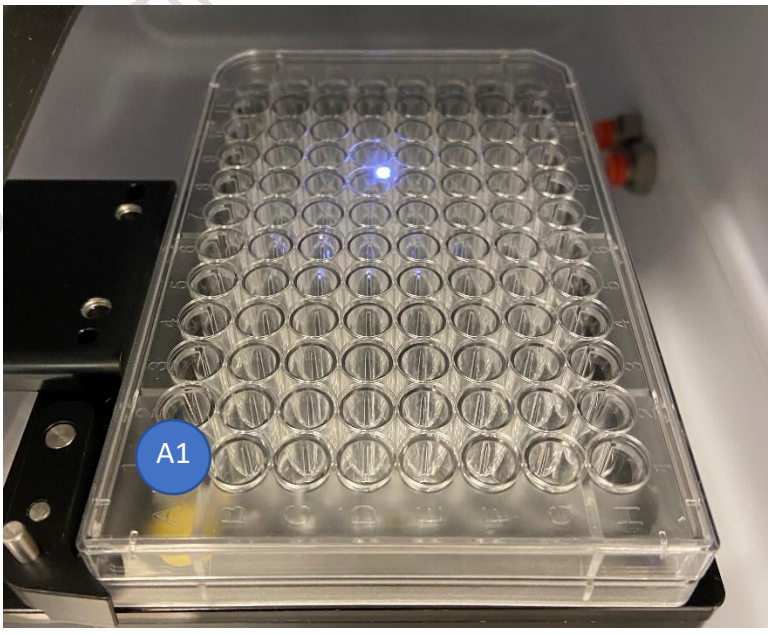


2.8 Click the *Eject* button on the Sort Layout window

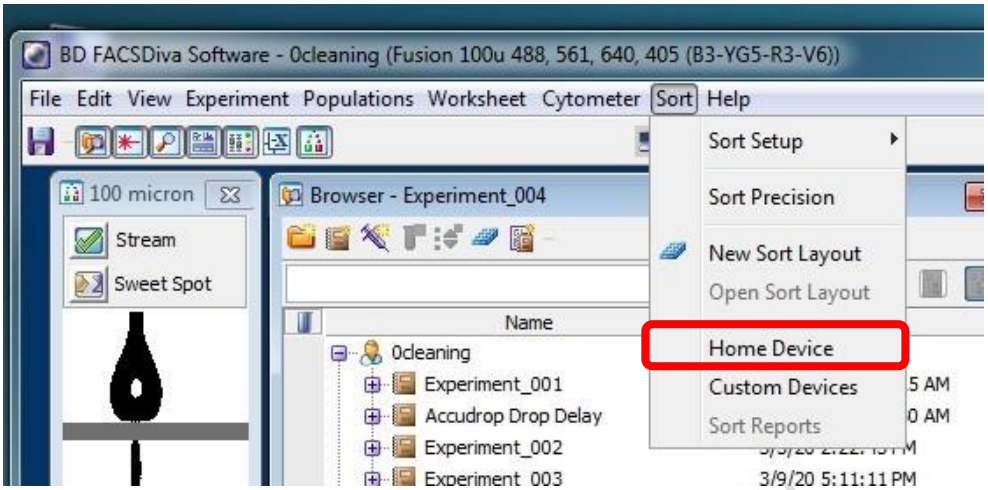


2.9 Plug in water tubing if cooling of the ACDU stage is needed

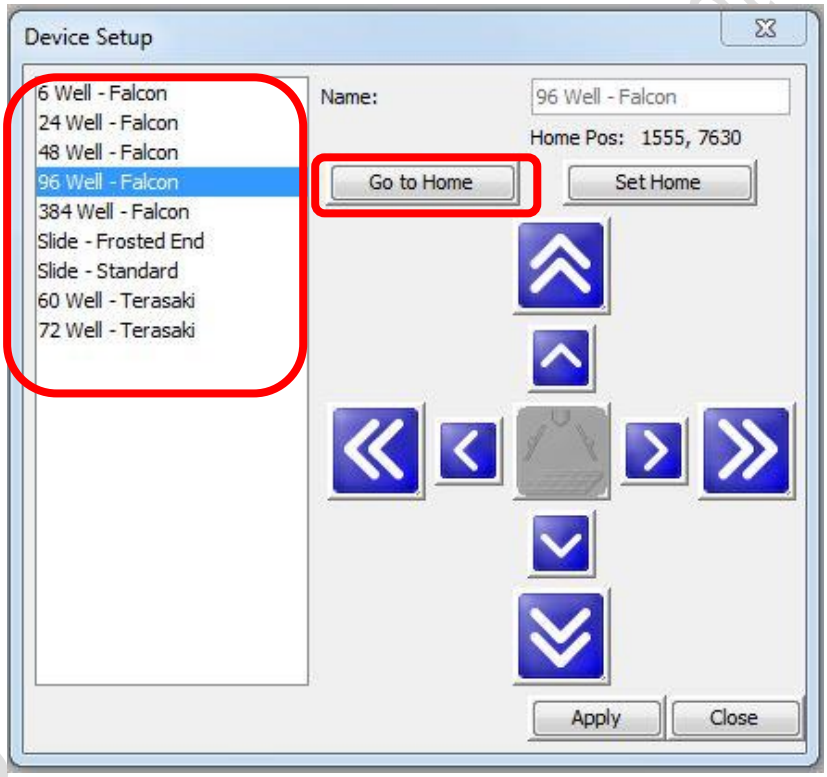
2.10 Load a dummy plate on the ACDU stage with A1 on the outer left corner



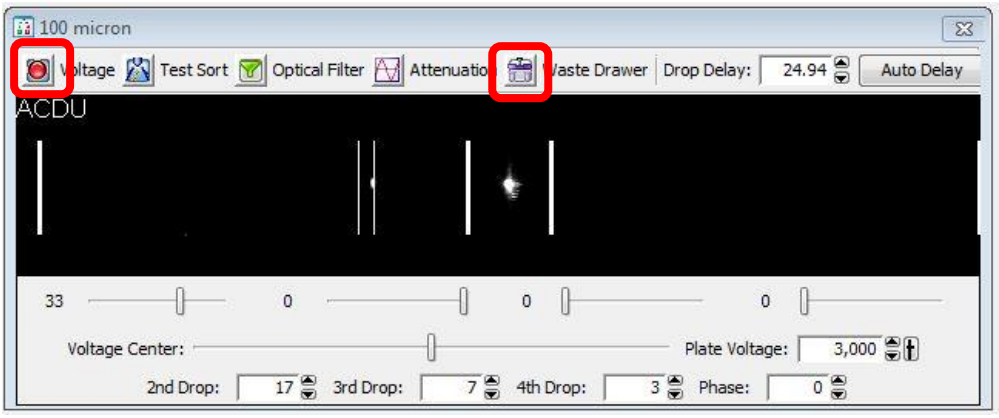
2.11 Click *Sort > Home Device*



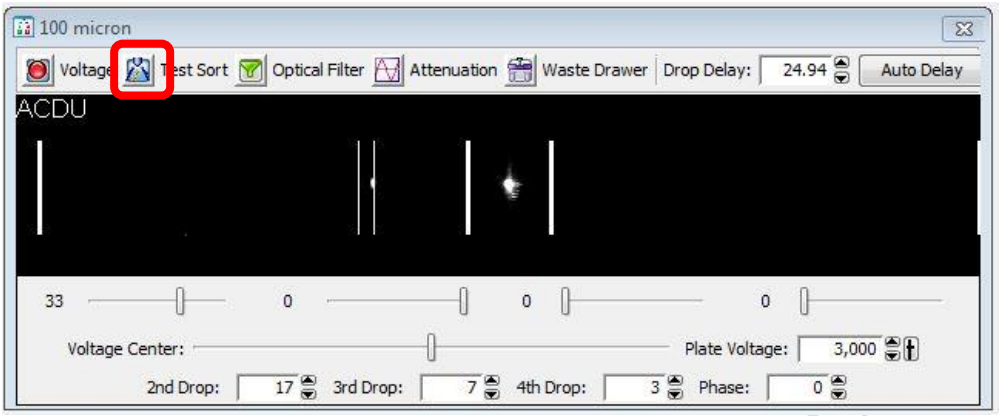
2.12 Select plate type and then click *Go to Home*



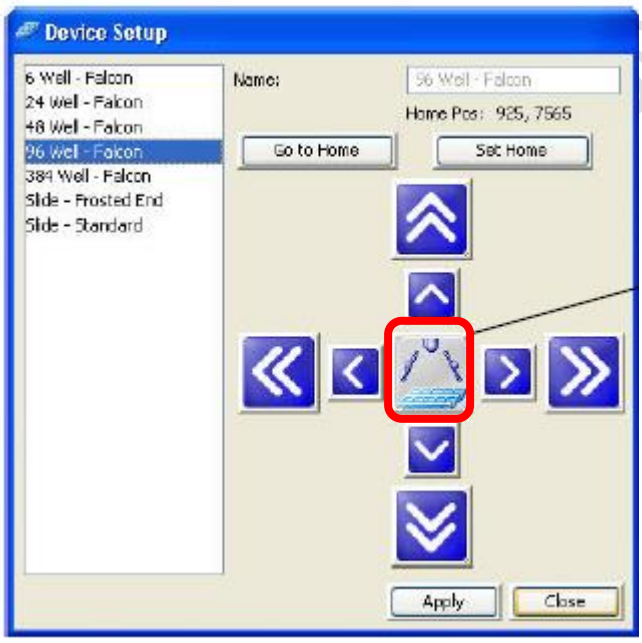
2.13 Go to 100 micron window (lower monitor), Click *Voltage* and *Waste Drawer*



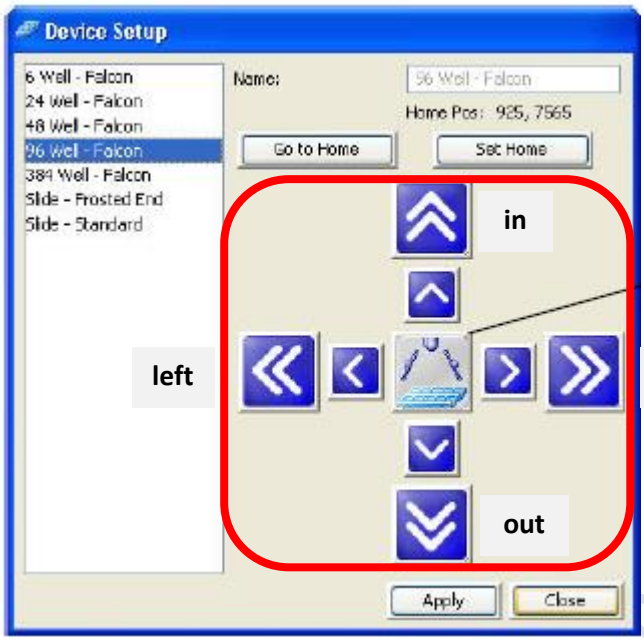
2.14 Double Click *Test sort* to shot a small drop of sheath on the dummy plate cover



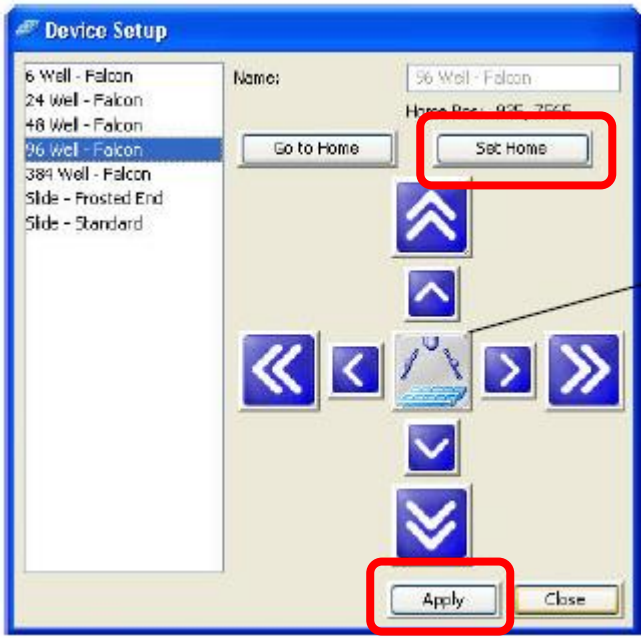
- OR –



2.15 Move the stage accordingly in order to shot the drop of sheath on A1 position



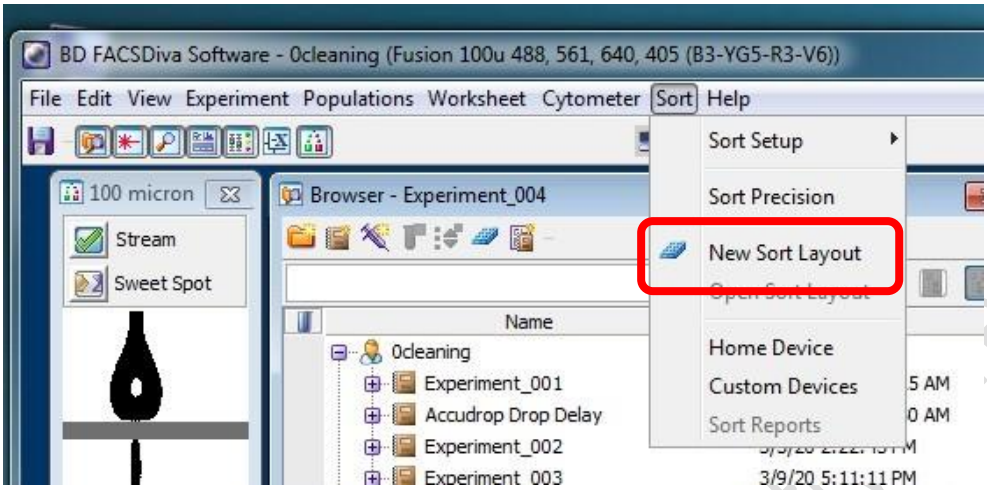
2.16 Go to Home Device Window, Click *Set Home* and *Apply*



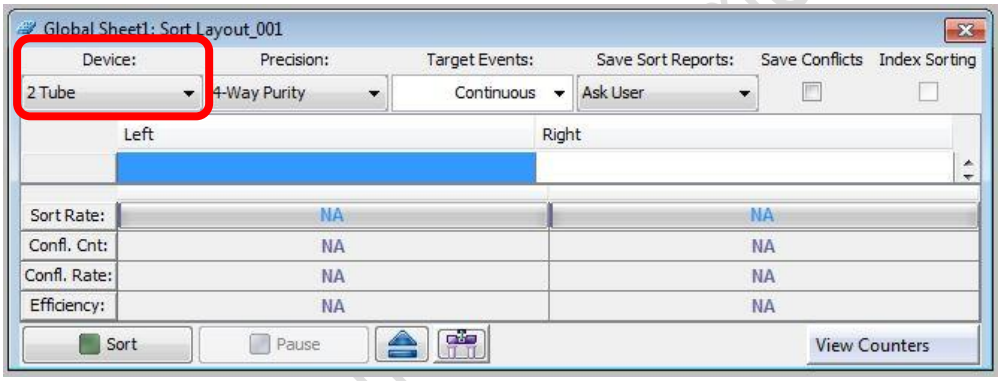
2.17 Remove Dummy plate from the stage and Load the collection plate

G. Sort Setup

1. Click Sort > *New Sort Layout*

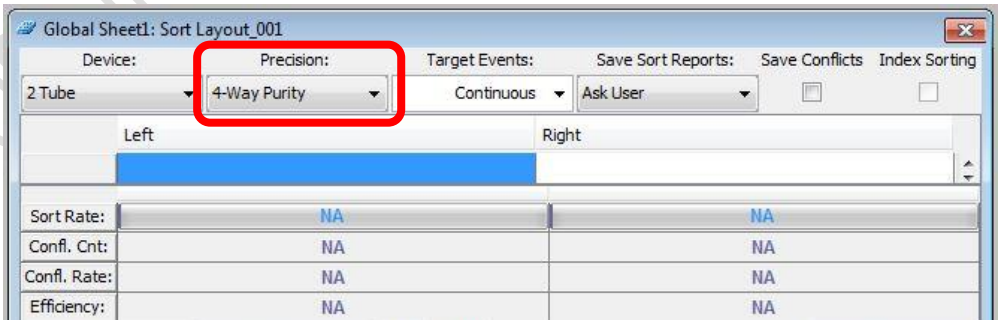


2. Select appropriate *Device*:



Name	Supported Device
2 tube	2-way 15 mL, 5.0 mL, 2.0 mL, 1.5mL
4 tube	4-way 5.0 mL, 2.0 mL, 1.5 mL
96-well Falcon	96-well culture plate

3. Select appropriate *Precision*:

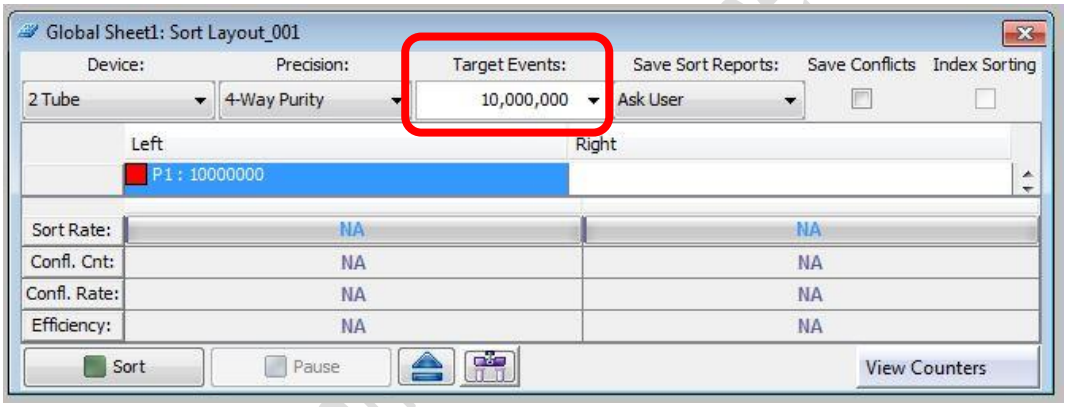


Name	Suitable Application
Purity	Sorting target population higher than 20%
Yield	Sorting target population less than 20%
Single Cell	Single cell sorting into 96-well plate / Single cell sequencing
4-way Purity	4-way sorting target population higher than 20%

4. Assign target population to position by *clicking the position > Add > Target gate*

5. Input Target Events (sorting will stop when the sorted cell number reached the target event) for each target population if needed.

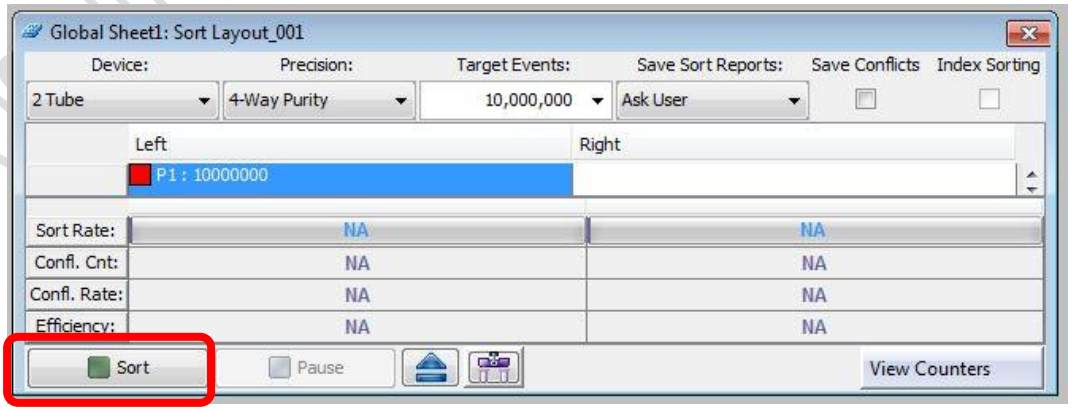
   Select *Continuous* for unlimited number.



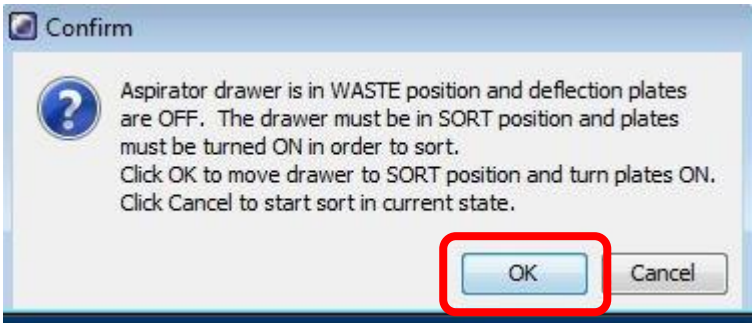
6. Load your Sample onto the sample stage.

7. Go to Acquisition Dashboard window, click *Load*

8. Go to Sort layout window, Click *Sort*



9. Click *OK* on Confirm window

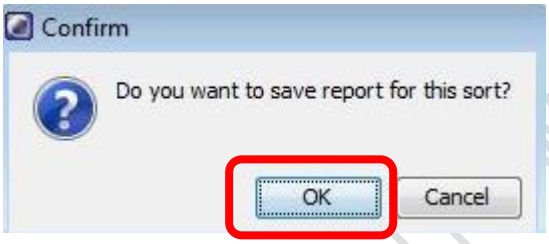


10. During the sort keep **monitoring Threshold Rate and Drop 1 value**

11. Click *Pause* if you wish to pause the sort and replace new collection tube. Click *Resume* after finish replacement.

12. Click *Sort* to end the sort

13. Click *OK* to save sort report



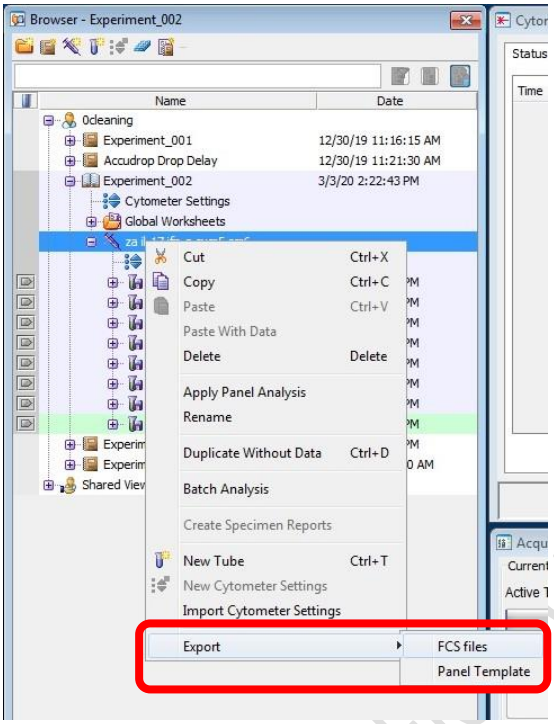
14. Go to Acquisition Dashboard window, click *Unload*.

H. Data Export

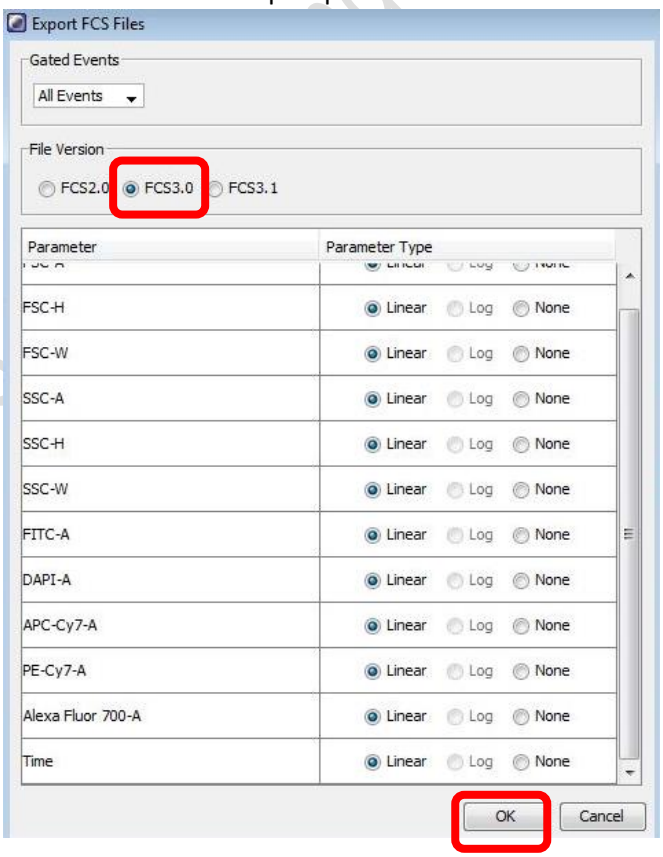
1. FCS file

1.1 Go to Browser window, Select the Tubes / Specimen of interest.

1.2 Right Click over the selection and click *Export > FCS file*

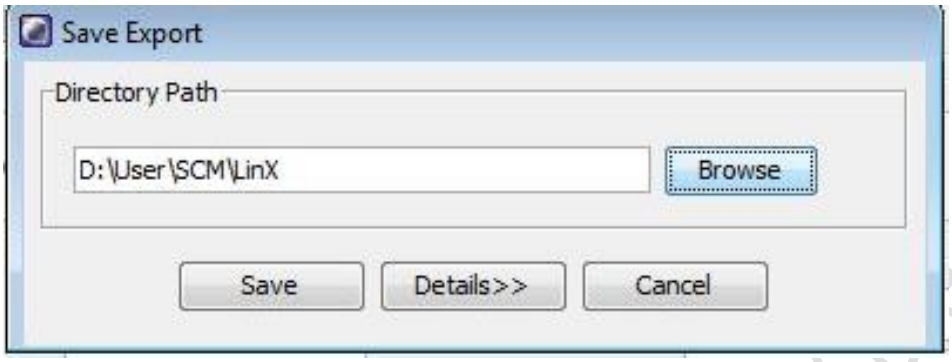


1.3 Select *FCS 3.0* and keep all parameters Linear. Click *OK*



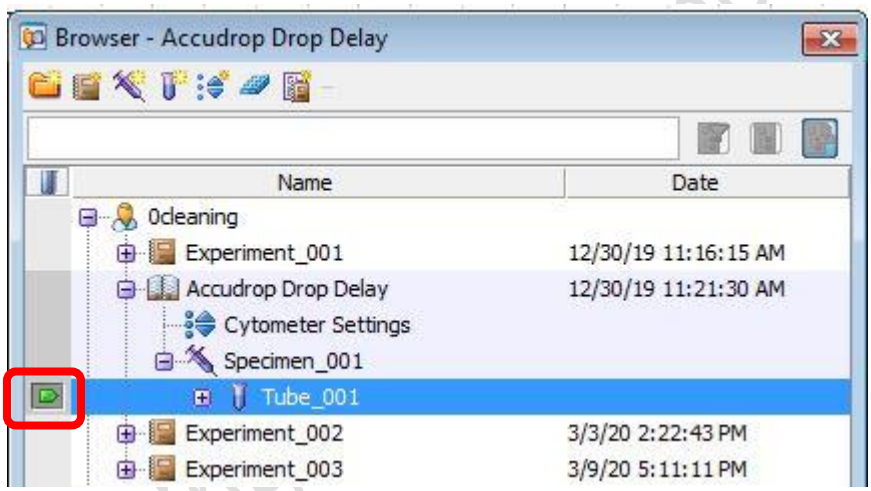
1.4 Click *Browse* to choose the destination (D:/User/Department/PersonalFolder)

1.5 Click *Save*



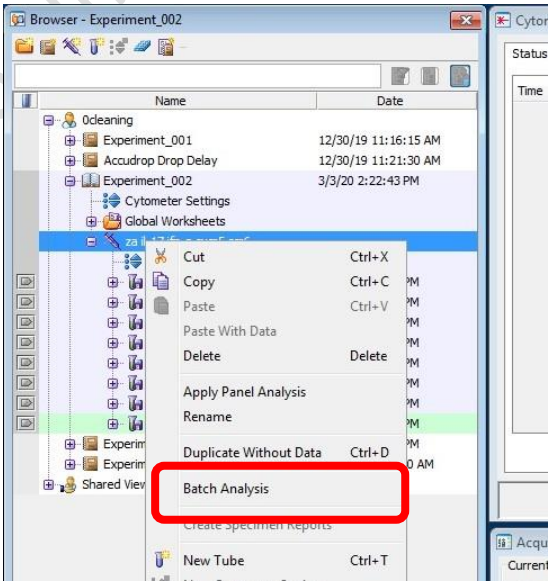
2. PDF file

2.1 To export pdf of multiple tubes, Click the tube pointer of any tubes

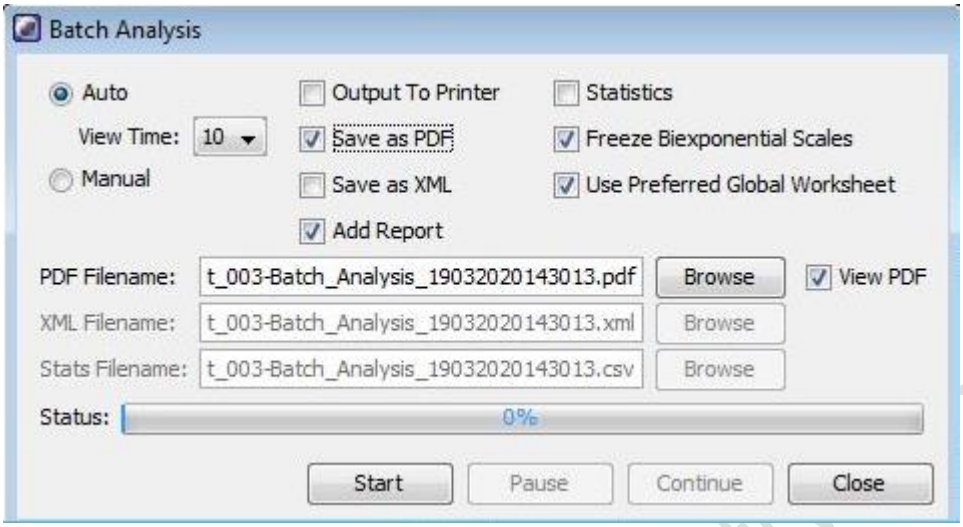


2.2 Select the wanted tubes/ Specimen.

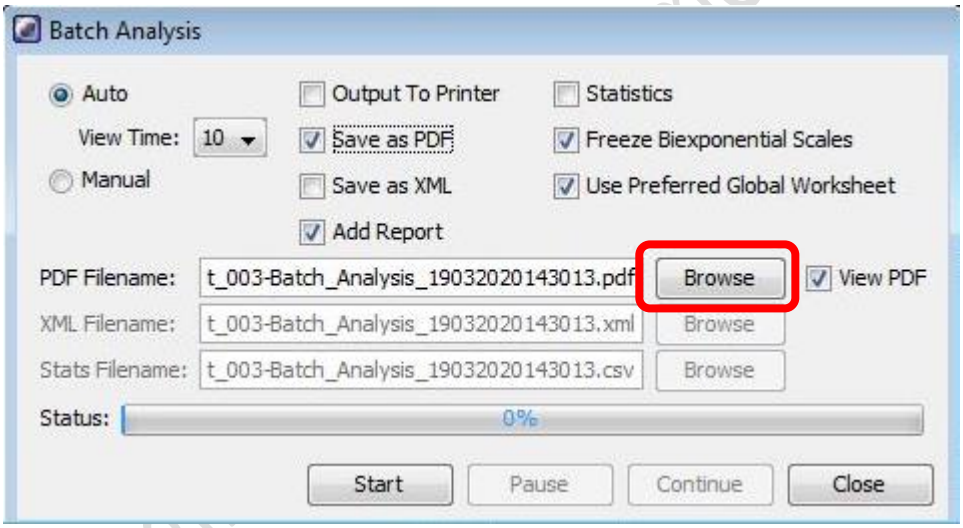
2.3 Right Click over the selection and click *Batch Analysis*



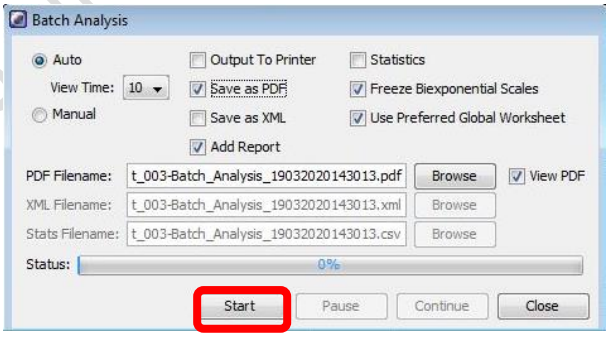
2.4 Check the boxes as picture below

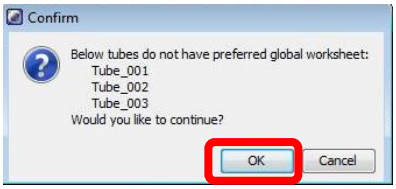


2.5 Click *Browse* to choose the destination (D:/User/Department/PersonalFolder)



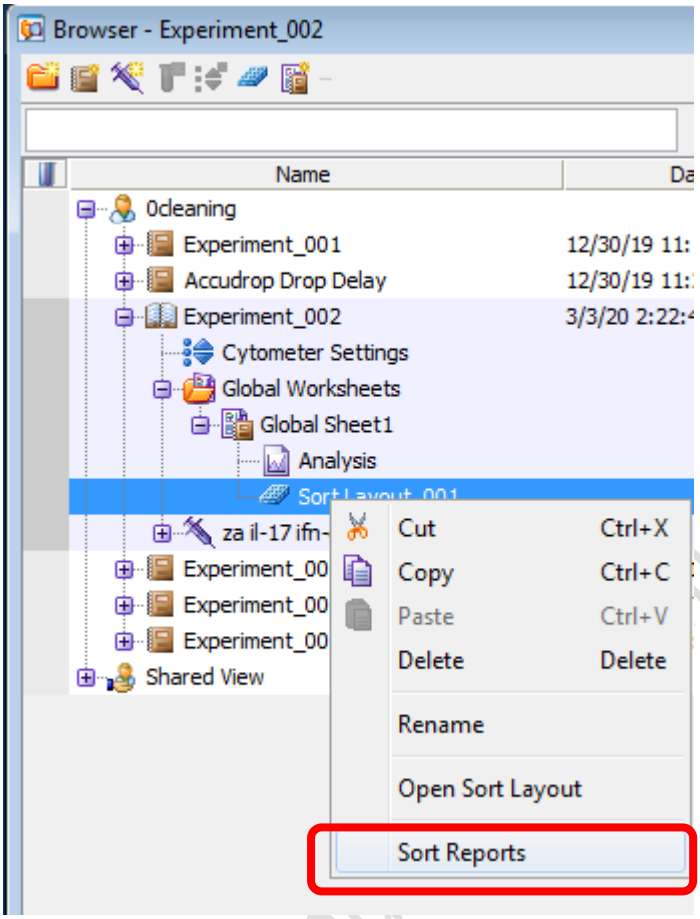
2.6 Click *Start* and then *OK*.



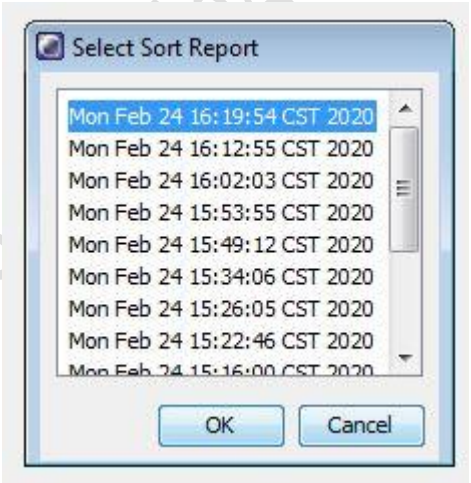


3. Sort Report

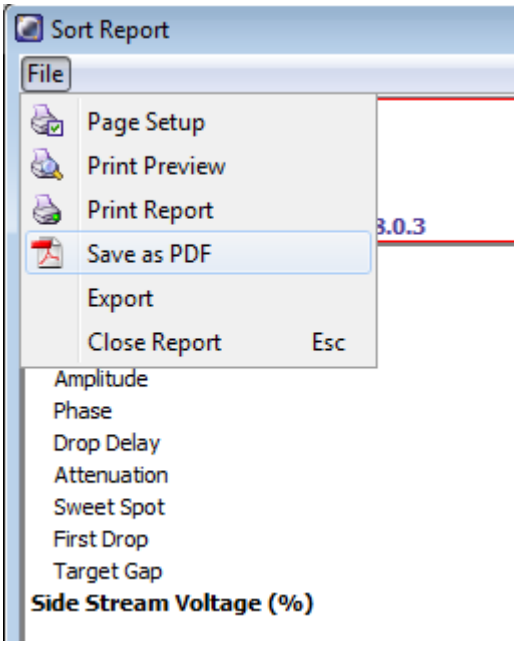
3.1 To export sort report, select *Sort Layout* and then right click. Click *Sort Reports*



3.2 Select the sort from the list and then click *OK*



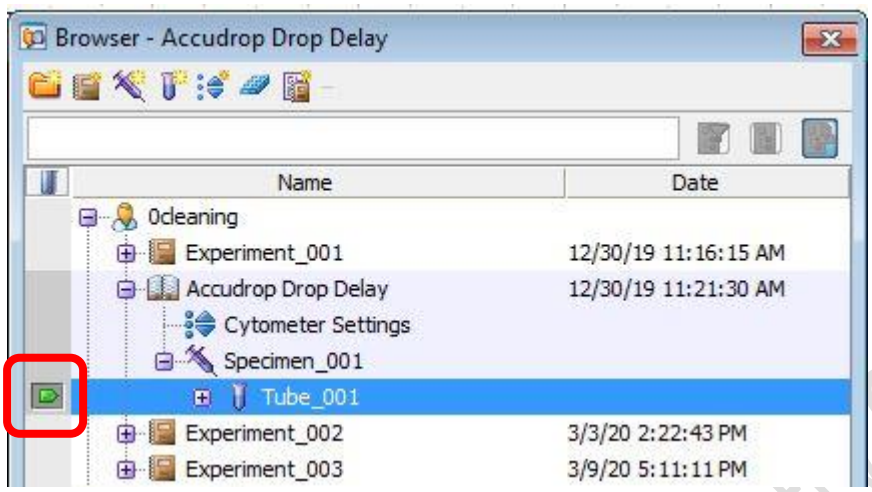
3.3 Click *File > Save As PDF*



3.4 Choose the destination (D:/User/Department/PersonalFolder) and click *Save*

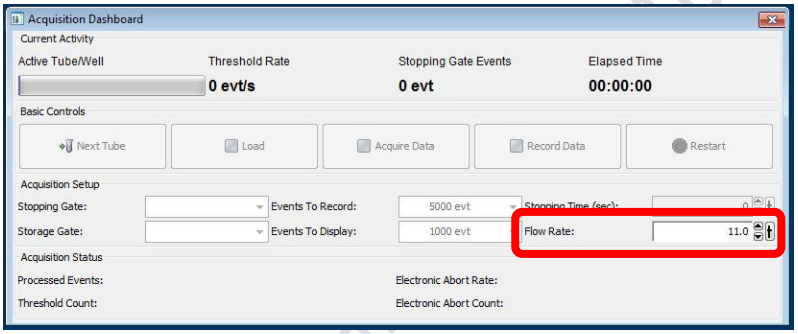
I. Cleaning

1. Go to Browser window, click the tube pointer of any tube



2. Load a tube of 2 mL of cleaning solution No. 1 (FACSClean) on the sample stage

3. Go to Acquisition Dashboard window, set *Flow rate* to 11.0



4. Click *Load*.

5. Acquire the solution for **5 minutes**.

6. Click *Unload*.

7. Load a tube of 2 mL of cleaning solution No.2 (FACSRinse) on the sample stage

8. Repeat step 3-5.

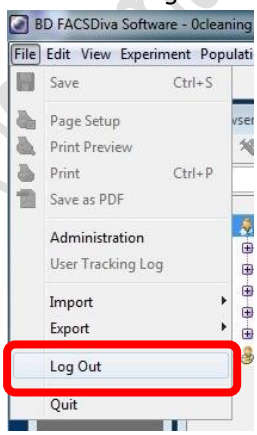
9. Load a tube of 2 mL of cleaning solution No.3 (MilliQ water) on the sample stage

10. Repeat step 3-5.

J. User Logout

1. **TURN OFF** the ***Sweet Spot***

2. Click *File > Logout*



3. Open the upper flow cell access door of the system before you leave.