Imaging and Flow Cytometry Core

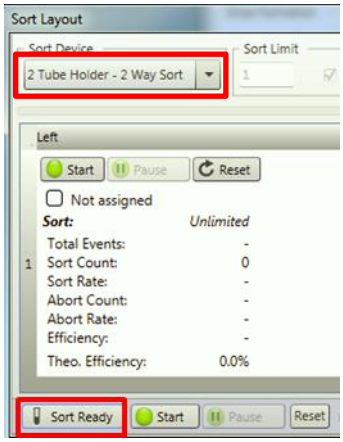
BD Influx Standard Operation Protocol - Basic Operation

Optimizing the drop phase

1. Verify the 2-tube holder with dummy 15ml tube is in place on the sort stage

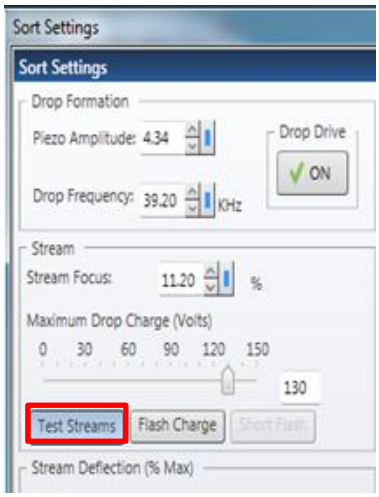
2. Select '**2 tube holder – 2 way sort'** as the sort device in the *Sort Layout* pane.

3. Click '**Sort Ready'** in the *Sort Layout* pane to place the dummy in place.

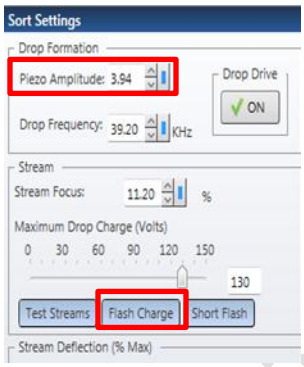


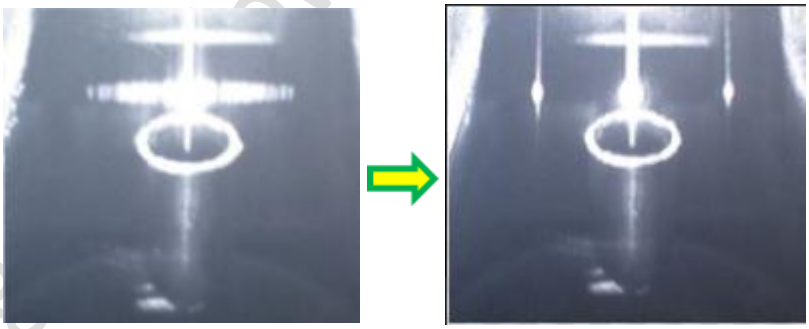
4. Close the deflection plates and press '**Plate**' to charge them
   Caution: Risk of electric shock if touch the deflection plates when they are charged.

5. Confirm the sort chamber door is closed. Click '**Test Streams**' in the *Sort Setting* pane to initiate test sort.



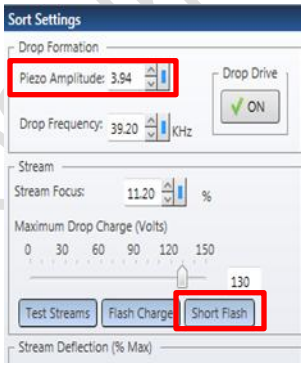
6. Click '**Flash Charge**' and adjust the **Piezo Amplitude** so that the side streams are maximally deflected with no fanning.





Fanning | No fanning

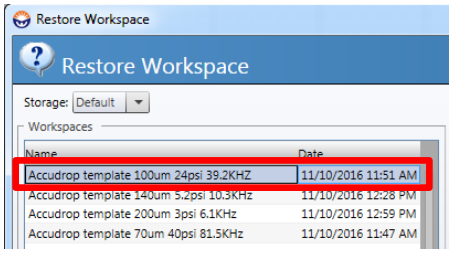
7. Click '**Short Flash**' and fine tune the **Piezo Amplitude** so that the side streams are maximally deflected with no fanning.



8. Click '**Test Streams**' again to turn off.

Accudrop Test

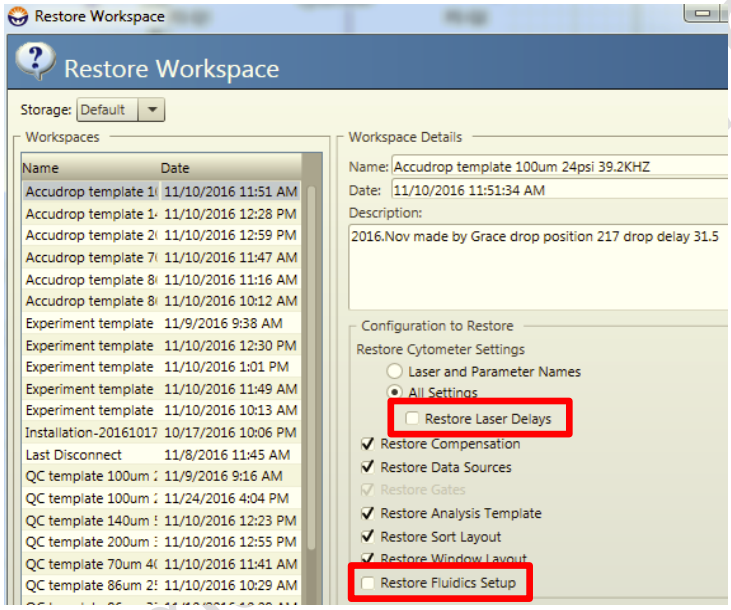
1. Click *File* > *Restore* > *Workspace*. The following window will appear.



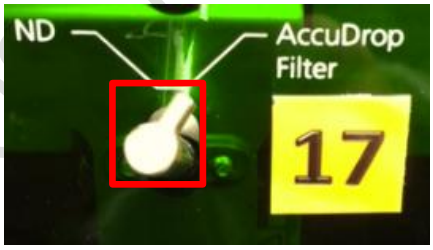
Storage: Choose **Default**

Choose the Accudrop template according to the nozzle size.

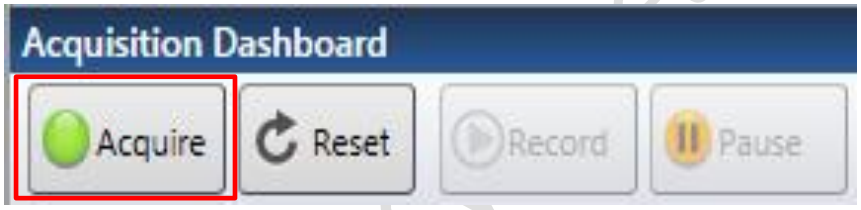
2. Uncheck "**Restore Laser Delay**" and "**Restore Fludics Setup**"



3. Turn the **ND** filter knob to **AccuDrop** filter.



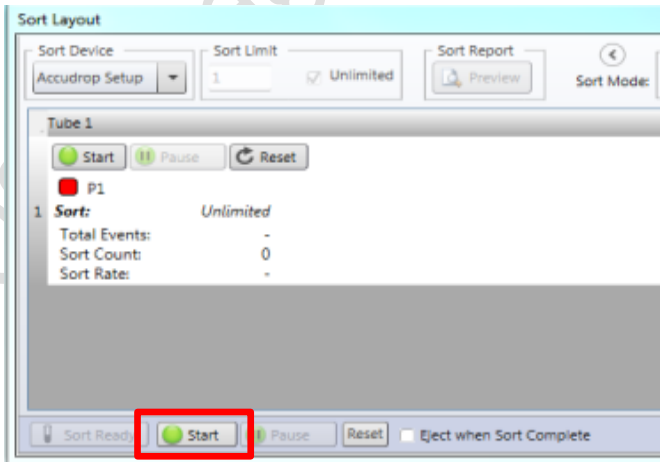
4. Load a tube of AccuDrop beads, press '**Sample**' button beside the sample loading port and click '**Acquire**'on the *Acquisition Dashboard*.



5. Adjust the **Sample Offset** to reach an event rate of between 1000 to 3000 events per second. (For 100um, event rate should be around 2000 events per second)

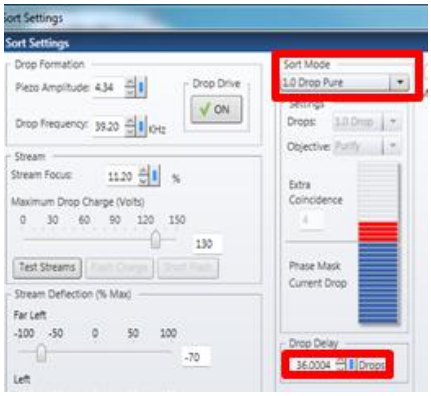
6. Check if '**Plate**' button is on. Turn it on if needed.

7. Click '**Start**' in the *Sort Layout* pane.

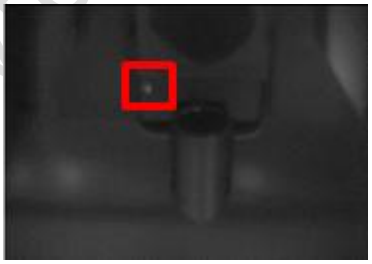


8. Choose **Sort Mode 2.0 drop Enrich** in the *Sort Settings* pane.

9. Adjust the '**Drop Delay**' until the left stream is the brightest while nearly no fluorescent signals can be detected in the mainstream position.



Wrong Drop Delay



Optimal Drop Delay

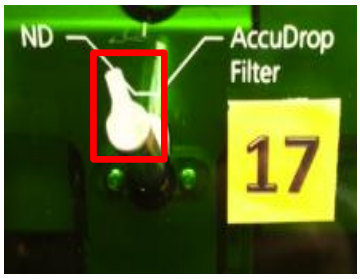
10. Choose **Sort Mode 1.0 drop Enrich** in the *Sort Settings* pane and repeat Step 7 to fine tune.

11. Click '**Stop**' in the *Sort Layout* pane and '**Stop**' on the *Acquisition Dashboard*.

12. Press '**Plate**' to turn off the deflection plates.

13. Unload the tube and press '**Backflush**'. Allow the flush for at least 5 seconds.

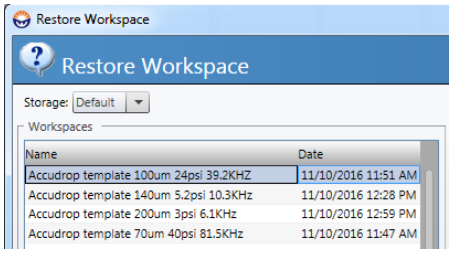
14. Turn the **Accdrop filter** knob back to **ND** filter position.



Attention: If you would like to further culture your collected cells, it is recommended to clean the sample line with FACSClean for 8 minutes at 27.5psi followed by MilliQ Water of same conditions after the Accudrop Test. BSC hood is recommended to be turned on.

Experimental Set up

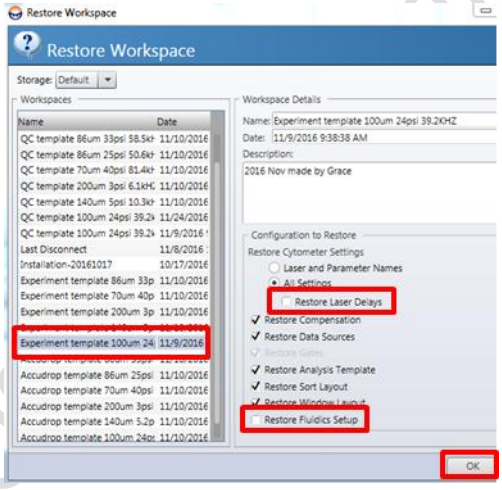
1. Click *File* > *Restore* > *Workspace*. The following window will appear.



Storage: Choose **Default**

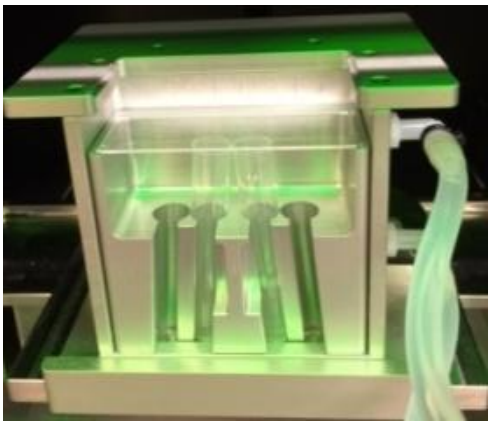
Choose the Experiment template <u>according to the nozzle size</u>.

2. Uncheck "**Restore Laser Delay**" and "**Restore Fludics Setup**". Click '**OK**'.

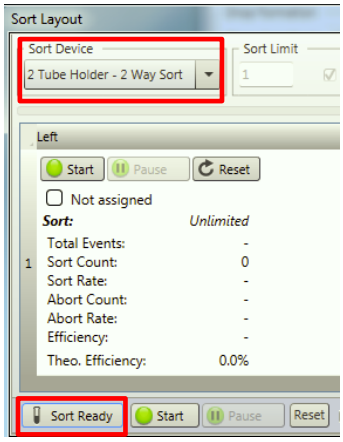


3. Place the desired collection device and dummy tubes into the sort chamber and connect the tubes to the ports if cold condition is needed. Place a slide on top of the dummy tubes.

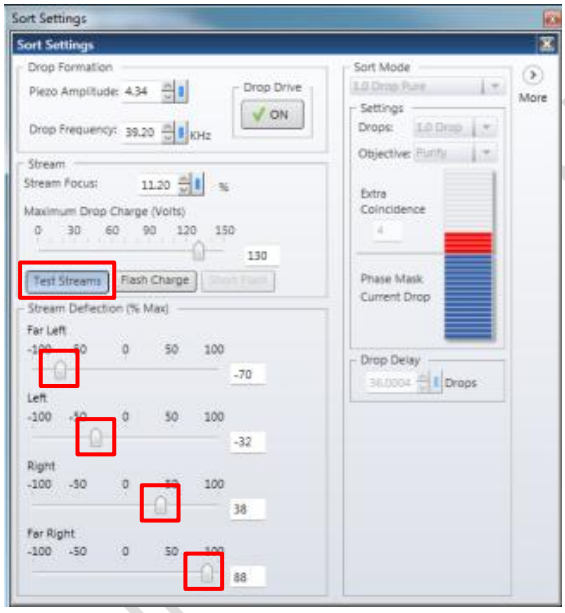
Imaging and Flow Cytometry Core

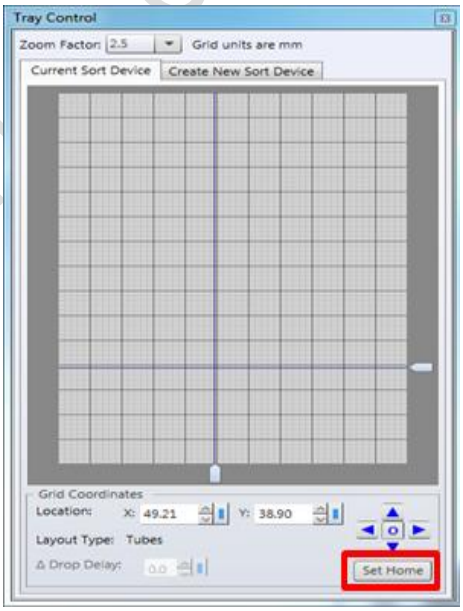


4. Choose the appropriate **Sort Device**. Click '**Sort Ready**' to move the collection device in place.



5. Press '**Plate**' to charge deflection plates. Double click "**Test Streams**" to place a drop of sheath fluid on the slide to see if the position is correct. Adjust the deflection angle of the side streams using the slider and/or adjust the tray position if needed.





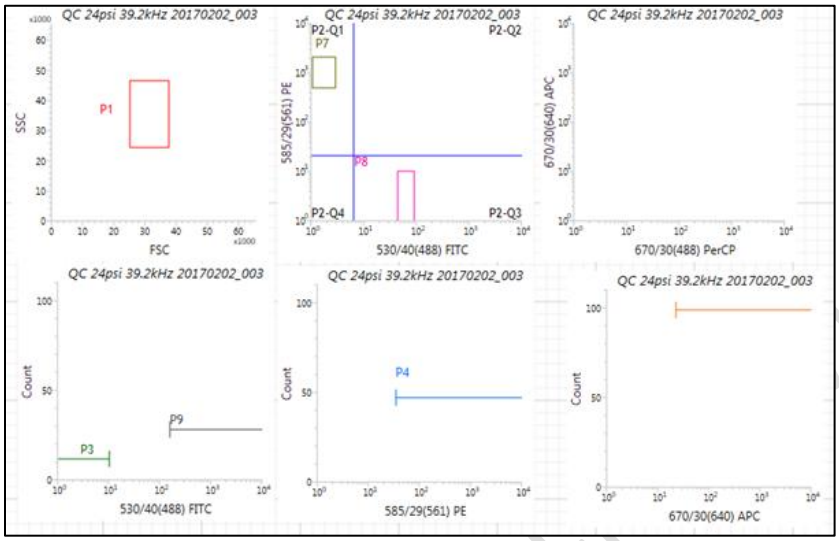
6. Remove the slide and Click '**Test Streams**' to verify if the side streams can enter the dummy tubes. Fine tune if needed.

7. Click '**Set Home**' in the *Tray Control* pane if the tray position has been modified.

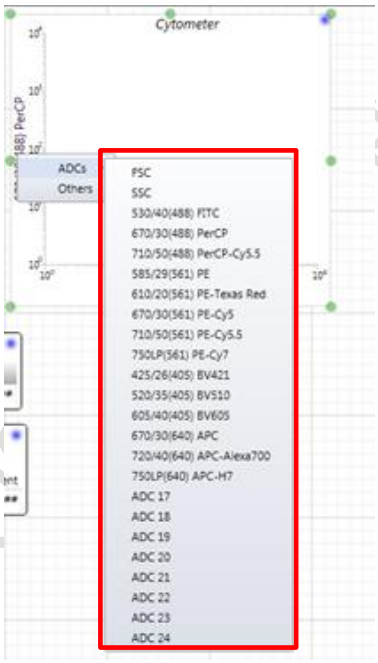
8. Press '**Plate**' to turn off the deflection plates.

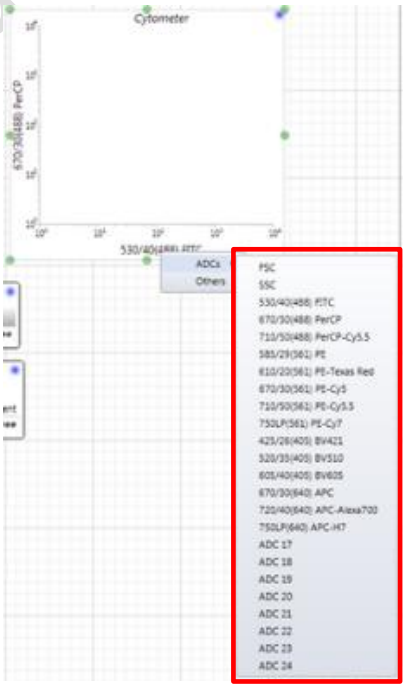
9. Replace the dummy tubes with the collection tubes that contain enough amount of medium or collection buffer.

10. Create **plots** on the *Worksheet* according to your experimental design.

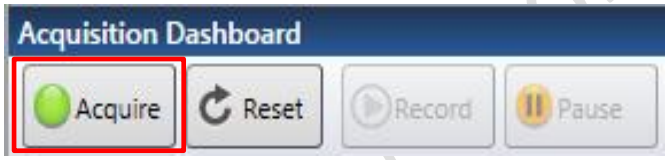


11. Right click at X and Y axes label and use ADCs to choose your parameters accordingly.



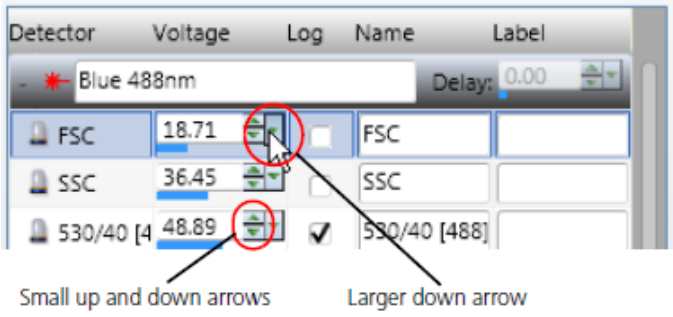


12. Load a tube of you sample, press '**Sample**' button beside the sample loading port and click '**Acquire**'on the *Acquisition Dashboard*.

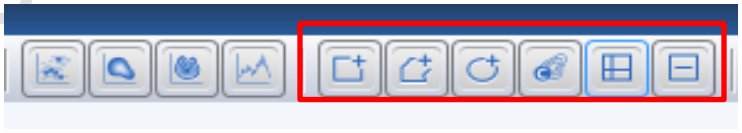


13. Adjust the **Sample Offset** and set the sample pressure to around 25psi**.**

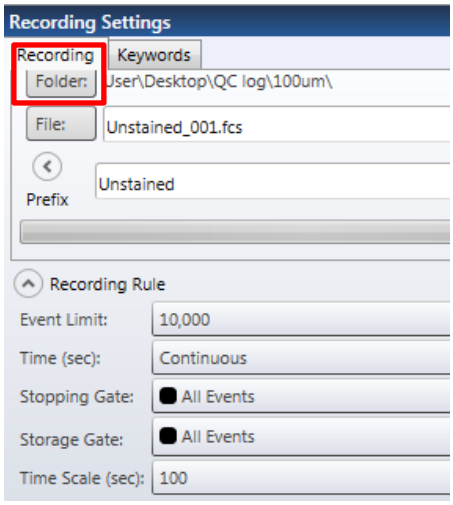
14. Adjust the corresponding PMT voltage using the **slider** on the corresponding axis in a plot or in the *Cytometer* Settings pane.



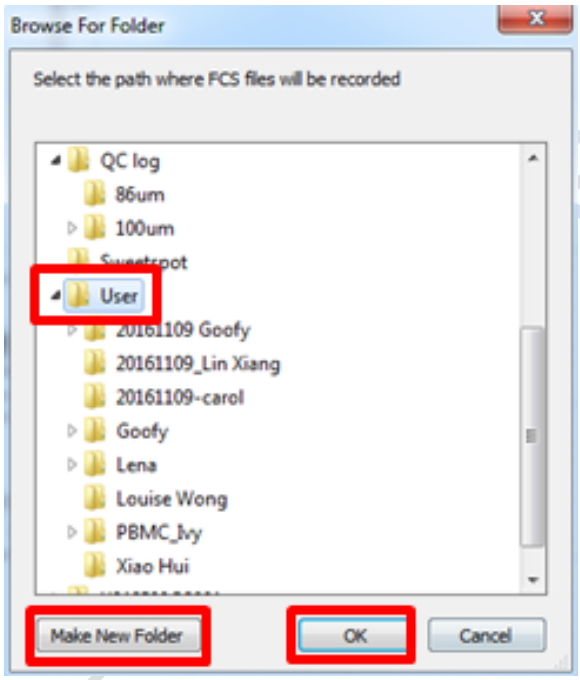
15. Create **Gates** according to your plot type and experiment design. Make sure the Hierarchy of the gates is correct.



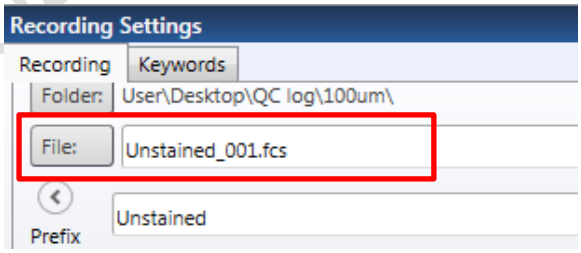
16. To record FCS file, click the '**Folder**' button in the *Recording Settings* pane.



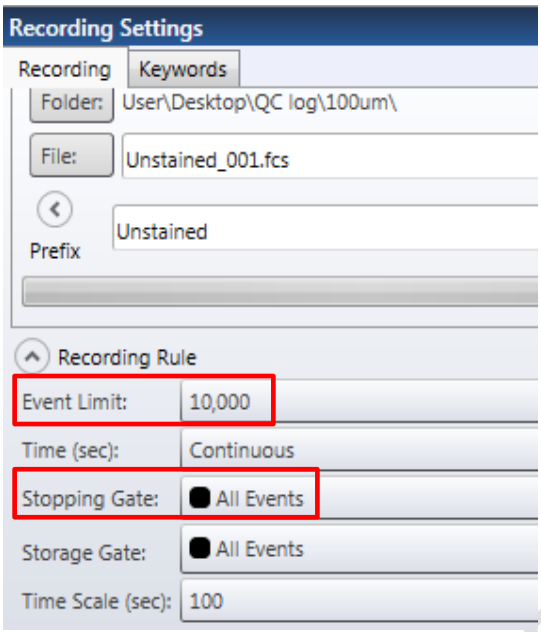
17. Select '**User**' folder and create yourself a new folder if you do not have a folder yet.



18. Key in the sample name in the **File** box.

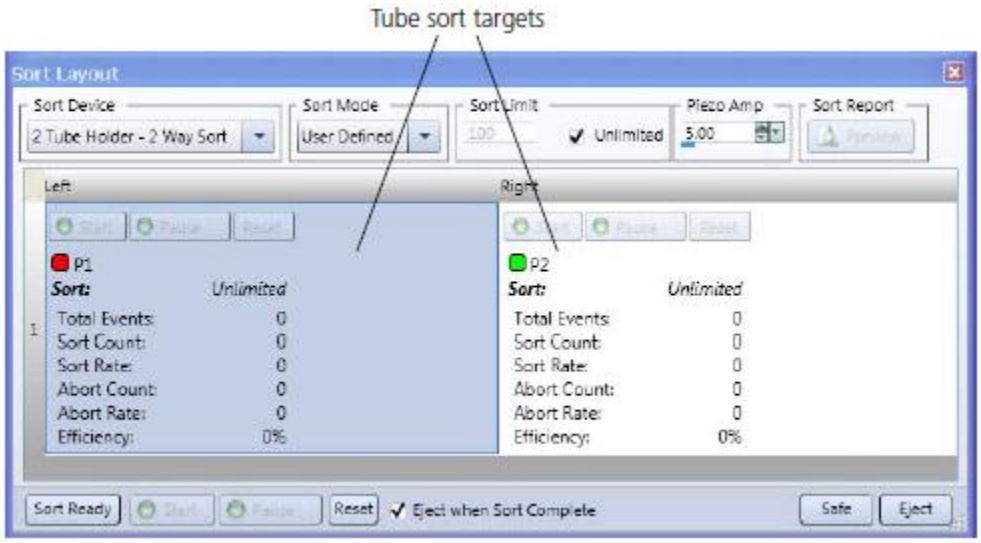


19. Set the number of events to record (**Event Limit**) and select the appropriate **stopping gate** (e.g. 10,000 events of P3).

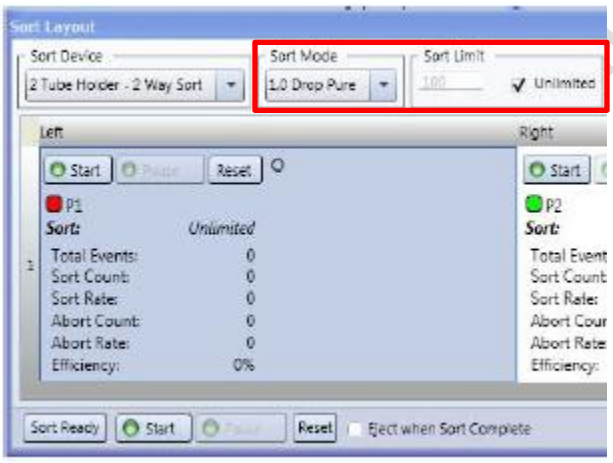


20. Click '**Record**' on the *Acquisition Dashboard*.

21. Right click at the corresponding tube position box (**Left/ Right** in the Diagram) in the *Sort Layout* pane. **Assign** the population to be collected.

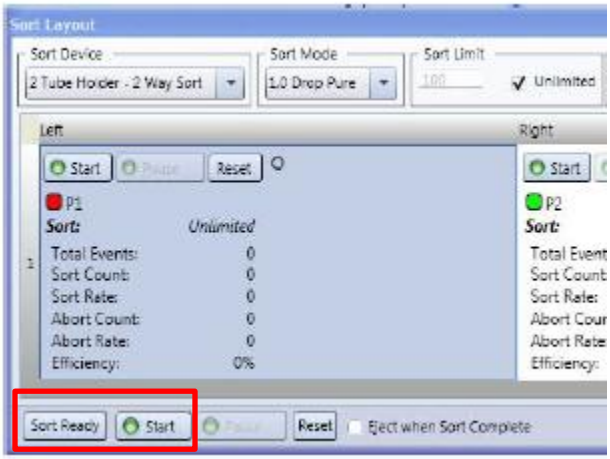


22. Select the appropriate **Sort Mode.** Set a **Sort Limit** if needed by **unchecked Unlimited** and Key in the target value.



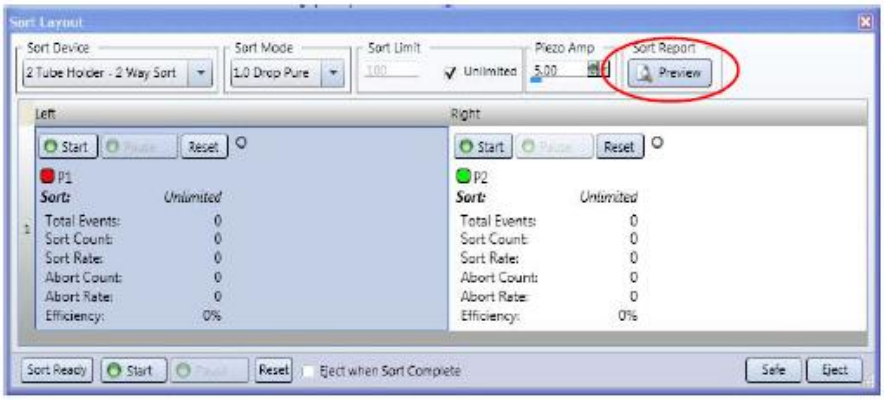
23. Press '**Plate**' to charge the deflection plates.

24. Click '**Sort Ready**' and '**Start**' in the Sort Layout pane to initiate cell sorting.



25. Click '**Pause**' if you want to pause the sort. The Sort Count will continue upon clicking '**Resume**'

26. Click '**Stop**' to complete your sort. Click '**Preview**' to view the **Sort Report**. Report can be saved as PDF file if needed.



27. Click '**Stop**' on the *Acquisition Dashboard*. Unload your sample and press '**Backflush**'. Allow the flush for at least 5 seconds.

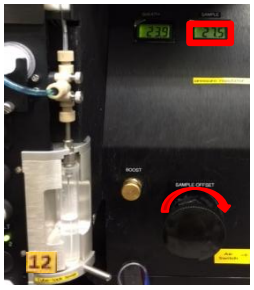
28. Continue with other samples or proceed to cleaning procedure.

Imaging and Flow Cytometry Core

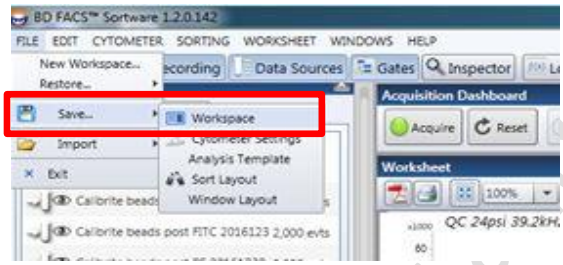
System Cleaning and Data Saving:

1. Load a tube of 3 ml FACS Clean Solution and set the sample pressure to 27.5 psi by adjusting the sample offset. Press '**Sample**' and let the solution run **for 5 minutes**. Unload the tube and Press '**Backflush**'.

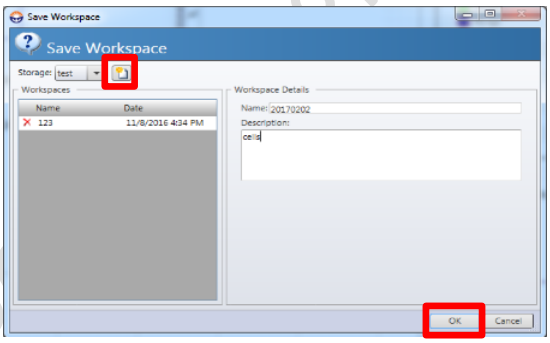
2. Repeat Step 1 with 3 ml FACS Rinse Solution and MilliQ water respectively. Leave the tube of water on the sample port when finish. DO NOT backflush after cleaning with water.



3. Click *File* > *Save* > *Workspace* to save workspace for next time.



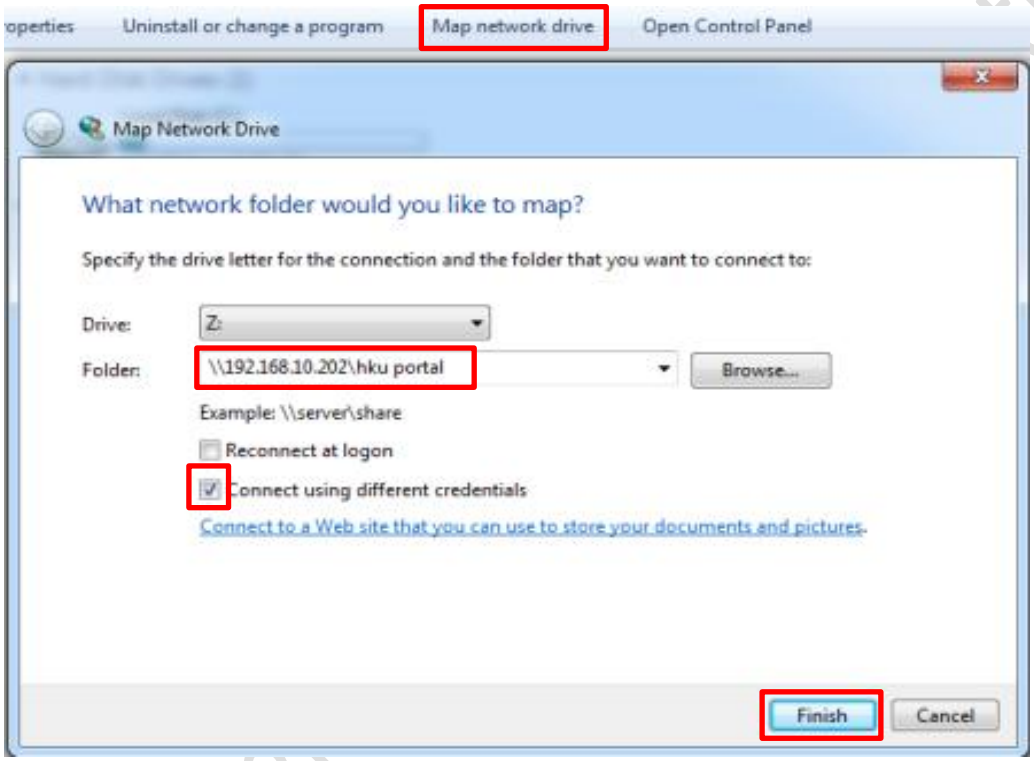
Click the icon next to the drag list to **create a New Folder** for yourself. Fill in Workspace Details and click '**OK**' to save.



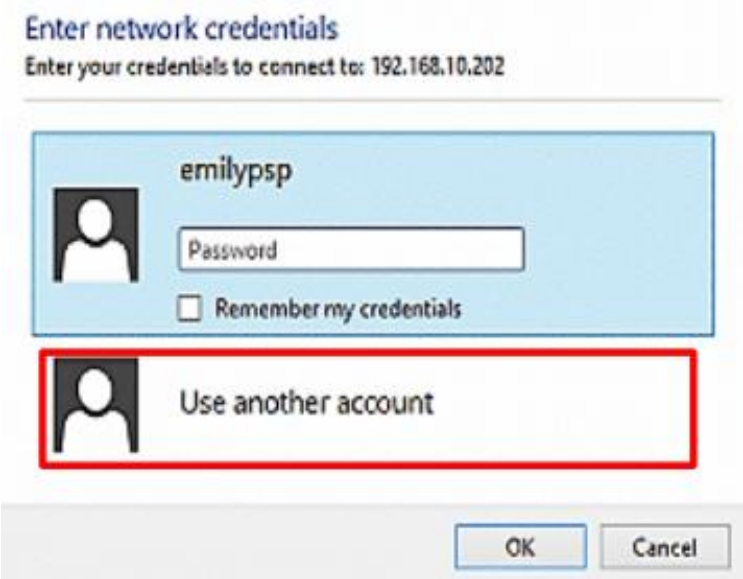
4. You may save PDF file by clicking '**PDF symbol**' for each sample and save it in your own folder in the '**User'** folder on **Desktop**.

Data Transfer:

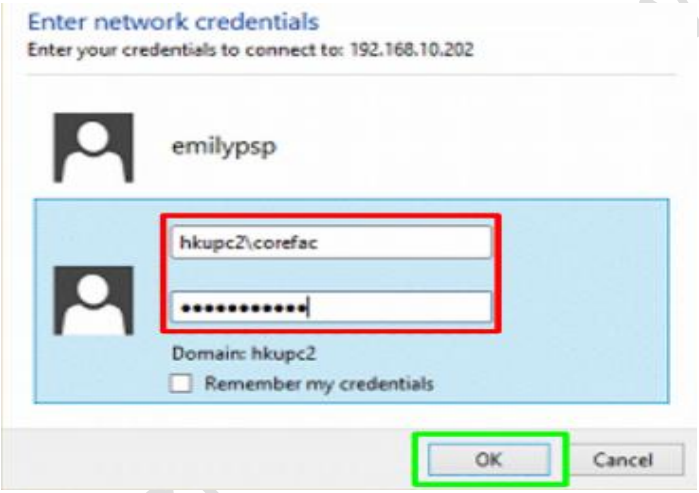
1. Double click '**Computer**' icon on desktop.
2. Click '**Map Network Drive**'. Key in **\\192.168.10.202\HKU Portal ID** at the Folder. Check '**Connecting using different credentials**' and Click '**Finish**'.



3. Another window would popup automatically. Click '**Use another account**'.



4. Enter **hkupc2\HKU Portal ID** and your portal password. Click '**OK**'.



5. Copy and Paste your data from your folder to the server folder.

6. Disconnect the Network Drive when finish.

Imaging and Flow Cytometry Core