

## BD Influx Standard Operation Protocol - System Startup

Before turning on the system, please make sure the sheath tank is full (~6 kg) and waste tank is empty (only containing bottom filled layer of bleach).

### BSC Hood (Optional):

1. Turn on the **light** of the BSC hood for a convenient working environment
2. Close the screen of BSC hood
3. Turn on the **Blower** and/or **Aerosol Management System** buttons if clean sorting environment is required or biohazardous samples are to be run on the machine

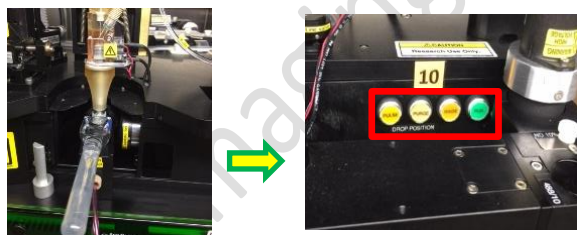


### System Start Up:

1. Turn on the “**Main Power**” (Label No. 1)
2. Turn on “**Fluidics Power**” and press the “**ILLUM**” button (Label No. 2)
3. Turn On “**Air compressor**” (gauge must show 20 psi) (Label No.3 )
4. Turn On “**Chiller Power**” and press the “**Arrow**” button (Label No. 4)
5. Turn On the “**Air Pump**” (gauge must show between 5-10 Hg) of waste tank (Label No. 5)
6. Turn on the **power and key switch of each laser** that is required and warm up for about 20 minutes (blue laser is compulsory to be turned on) (Label No. 6)
7. Turn on **Air Switch** at **bottom left** side of Influx body (Label No.7)
8. Turn on the Computer (in the cabinet) and Log in to “**Admin**” account, password “**BDIS**” (twice beep sound will be heard if the server has connected with the instrument successfully) (Label No.8 and 9).

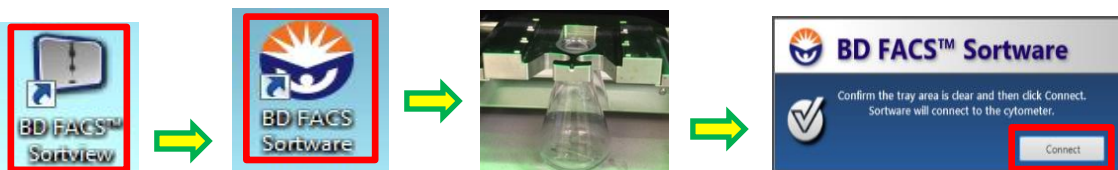


10. Place the flush bucket and de-bubble reservoir under the nozzle assembly. Fill in MilliQ water and start the de-bubbling process with **Purge/ Pulse** modes until all the bubbles are removed followed by turning on the stream with **“Rinse”** button. Remove the tube from the sample line and press **“Backflush”** until sheath solution dripping from the sample line. Press backflush again to stop. De-bubble the sheath filter by loosen the cap.

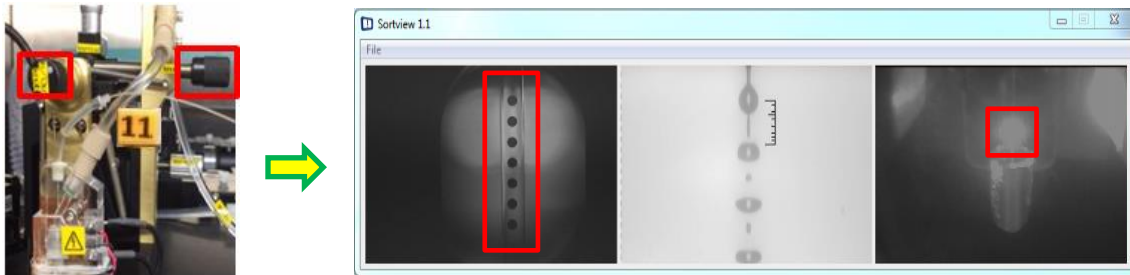


11. Press **“Run”**. Remove the de-bubble reservoir and flush bucket.

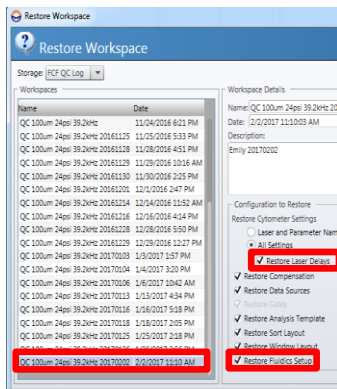
12. Double click **BD FACS Sortview** and **BD FACS Software**. Make sure the flask is in place and then click **Connect**.



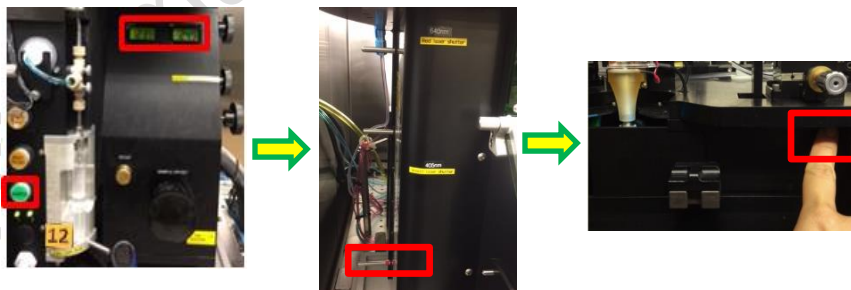
13. Start aligning the stream by adjusting the **In/Out, Left/Right, Horizontal** and the **Focus** knobs until a sharp image of the stream and are obtained (left image of Sortview) and the stream lines at the centre of the waste cup (Right image of Sortview).



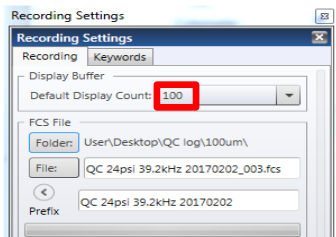
14. Select the latest QC workspace for 100um nozzle in “**FCF QC Log**” Storage folder. Make sure the box of “**Restore Laser Delay**” and “**Restore Fluidics Setup**” is checked and Click “**OK**”.



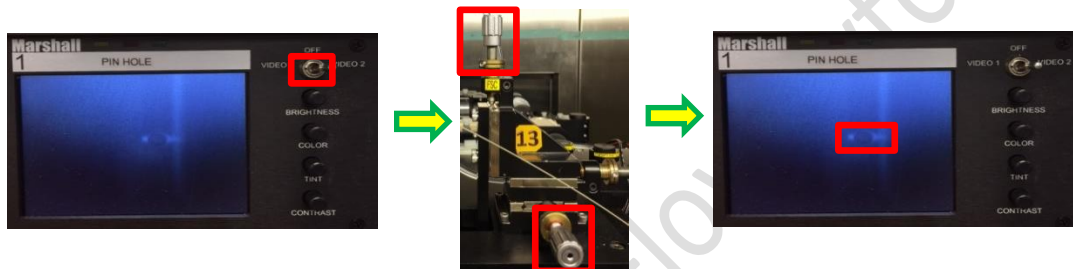
15. Adjust the sample flow rate, sample pressure > sheath pressure 0.5 psi. Load the diluted Rainbow beads and press “**Sample**”. Open the Blue Laser shutter by pulling out the corresponding rod switch. Close the door in front of the nozzle and activate laser by presenting your finger onto the sensor.



16. Click **Acquire** in the Software. Set the “**Display event rate**” to 200.

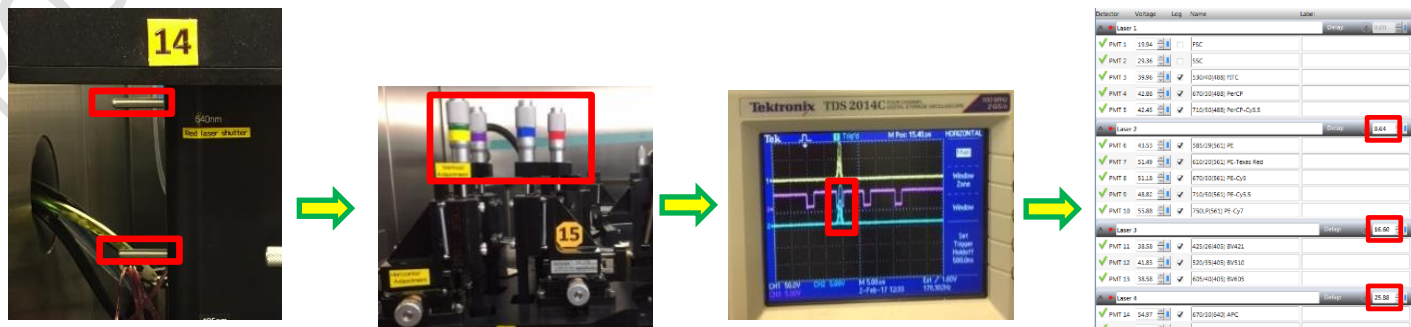


17. Switch the video channel to **Video 2** on the Oscilloscope. Start aligning the horizontal and vertical knobs corresponding to Forward Scatter to the pinhole and stream by referring to the fluorescent signals exciting by blue laser.

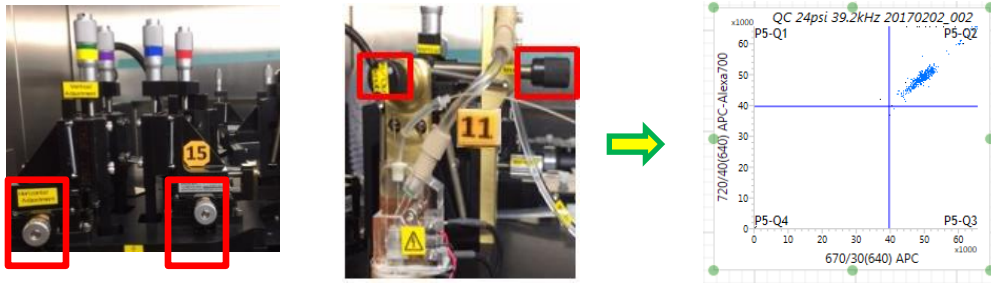


18. Open the shutters of the other lasers, preferably with this sequence: “Red” → “Violet” → “Yellow-green”. Open the shutter of each laser and highlight each dot plot in the Software by referring to its corresponding channel. Fine adjusting the vertical knob of each laser to fit its designated pinhole and signal reaches maximum, followed by optimizing the “**Laser Delay**” to make sure the signal of each peak is located at the center of it corresponding bucket which shown on the oscilloscope (applies to all lasers except Blue laser).

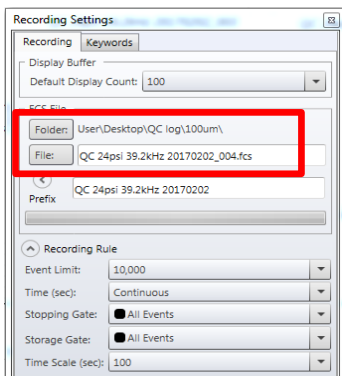
- a. Blue Laser: vertical knob → 1<sup>st</sup> pinhole
- b. Red Laser: vertical knob → 7<sup>th</sup> pinhole
- c. Violet Laser: vertical knob → 5<sup>th</sup> pinhole
- d. YG Laser: vertical knob → 3<sup>rd</sup> pinhole



19. Slightly adjust the horizontal knob and the stream alignment knob to achieve highest signal strength and tightest CV.



20. When all the alignments have been done, select QC Log folder to record QC FCS file

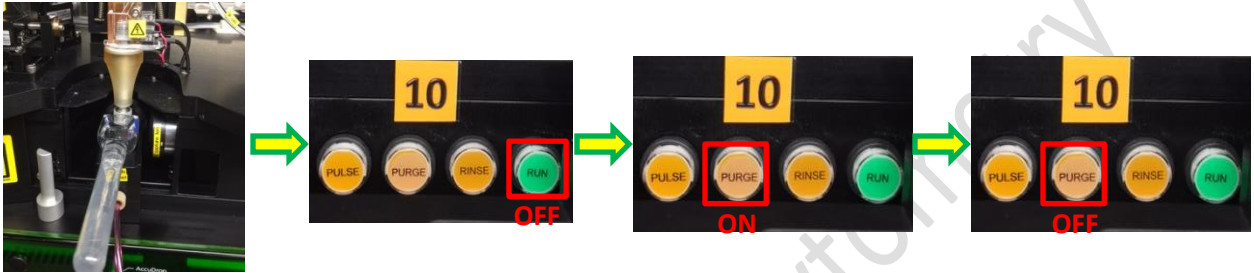


21. Save workspace in FCF QC Log folder.

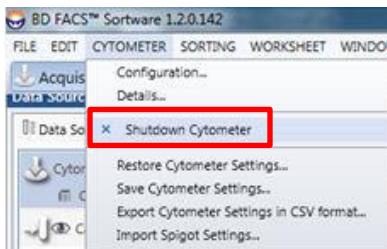


**System Shutdown:**

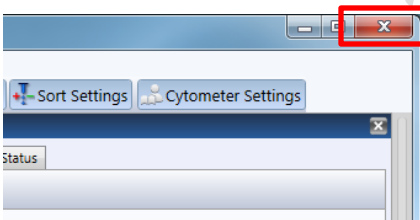
1. Clean the sample line with standard cleaning procedure. Leave the tube of MilliQ water on the sample port.
2. Turn the key switch of the lasers to begin the cooling down process.
3. Place the flush bucket and de-bubble reservoir under the nozzle. Fill up de-bubble reservoir with MilliQ water. Press “Run” button to stop the stream and press “Purge” to fill up the flow cell with water for 1 minute. Press “Purge” to stop.



4. Go to “Cytometer” and click “Shutdown Cytometer”.



5. Close the Software software and shutdown the computer.



6. Turn off the follow items in order stated:  
Air switch (7) → Lasers Power of Blue Laser (6) → Air pump (5) → Chiller (4) → Air compressor (3) → Fluidics Power and ILLUM (2) → Main Power (1)
7. Disconnect waste line and air pump line and empty the waste tank. Put the waste tank on the side of the sink.
8. Refill Sheath Tank with PBS.
9. Decontaminate any spills if necessary. Clean the sort chamber by wiping internal area and deflection plates with MilliQ water.
10. Remove the sorting device and install the waste flask inside the sorting chamber.