

# BD LSRII Startup & QC Procedure



The BD LSRII at the WIBR is equipped with 5 lasers (355, 405, 488, 561 & 638 nm). All researchers must be trained by the flow cytometry core facility staff to be granted access to book and operate independently. All WIBR core facility cytometers are available for use to trained users 24/7. This SOP is to followed by all trained researchers.

## Instrument Startup & QC

*Mon-Fri, all analyzers in the facility will be turned on and QC'd by core facility staff every morning by 9AM if they are being used that day. If the instrument is being turned on outside of working hours (Mon-Fri 9AM-5PM), the following steps must be taken:*

### I. Fluidics Check

Sheath fluid and waste levels need to be visually inspected. The sheath fluid (1xPBS) should be at least halfway full and the waste (biological material + bleach) should be no more than half full.

*Note:* The FACS flow supply system is not used.

### II. Turn on Computer

Once the computer is powered on, log in as follows:

**Username:** Operator

**Password:** Welcome#1

### III. Turn on Cytometer

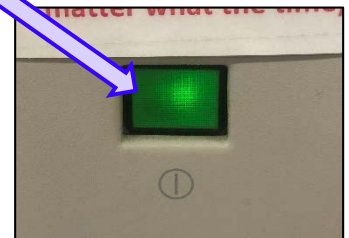
Cytometer is powered on through the main power button on front panel of the instrument.

### IV. Clean

The first user of the day is responsible for cleaning the instrument. Run each of the following at a flow rate of high for 5 minutes: 10% bleach, FACSRinse, H2O. Once complete, put the instrument in standby on low.



Waste



**Run:** Pressurizes the tube, allowing for sample to be acquired.


**Standby:** Tube not pressurized.

**Prime:** Drains and refills the flow cell with sheath fluid. Prime is only done with no tube on the SIP and the arm to the side.

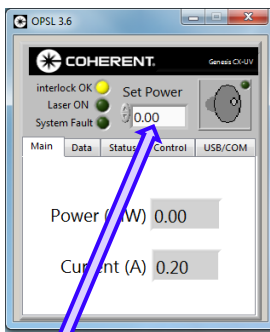
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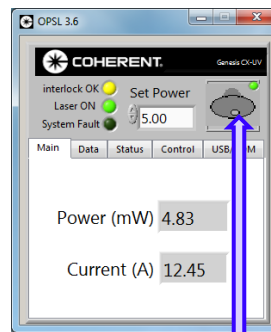
## V. Turn on UV Laser

Open Coherent Connection software. 

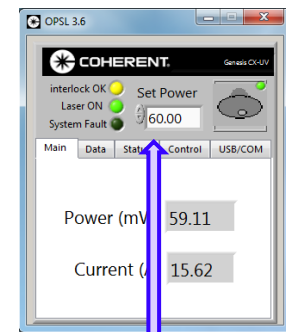
- Type 5mW in "Set Power" text box.
- Click Power toggle button and wait for power readout to go to ~5mW.
- Set laser power to 60mW and select "Enter" on the keyboard. Power readout should go up to ~60mW.



a




b

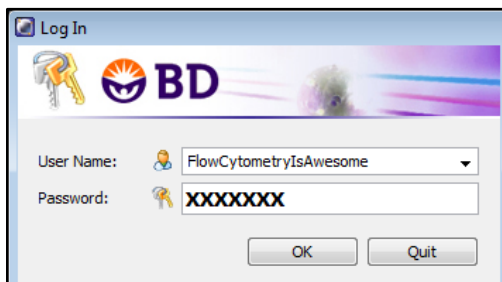


c

## VI. Log into Diva

Open BD FACSDiva software and log in with your credentials. 

Note: Credentials are specific for each researcher and cannot be shared.

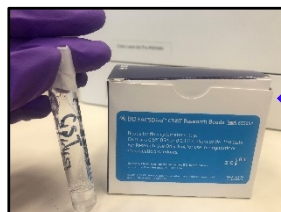


**Username:** *FullName*  
**Password:** *userspecified*

## VII. Instrument QC

After the instrument cleaning is complete, optical QC should be carried out to validate instrument performance.

- Locate the pre-made tube of CS&T beads in the refrigerator in room 429. If no tube is present, prepare a fresh aliquot by combining one drop of vortexed CS&T beads (Cat# 655051) with 350  $\mu$ L of 1xPBS.

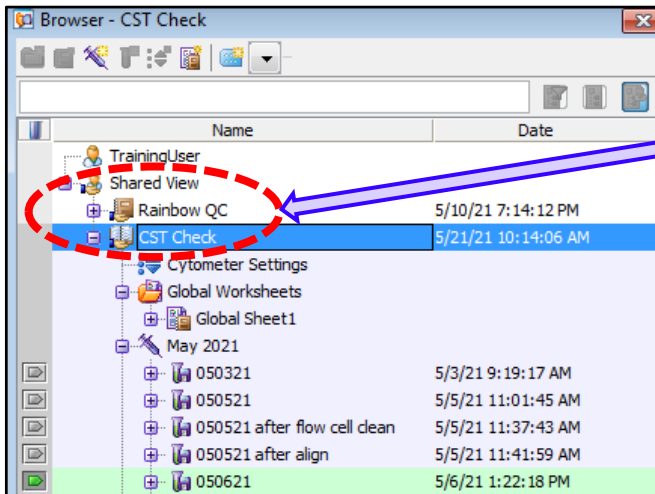


All tubes must be labeled with date of preparation, "CST" and lot number from stock vial.

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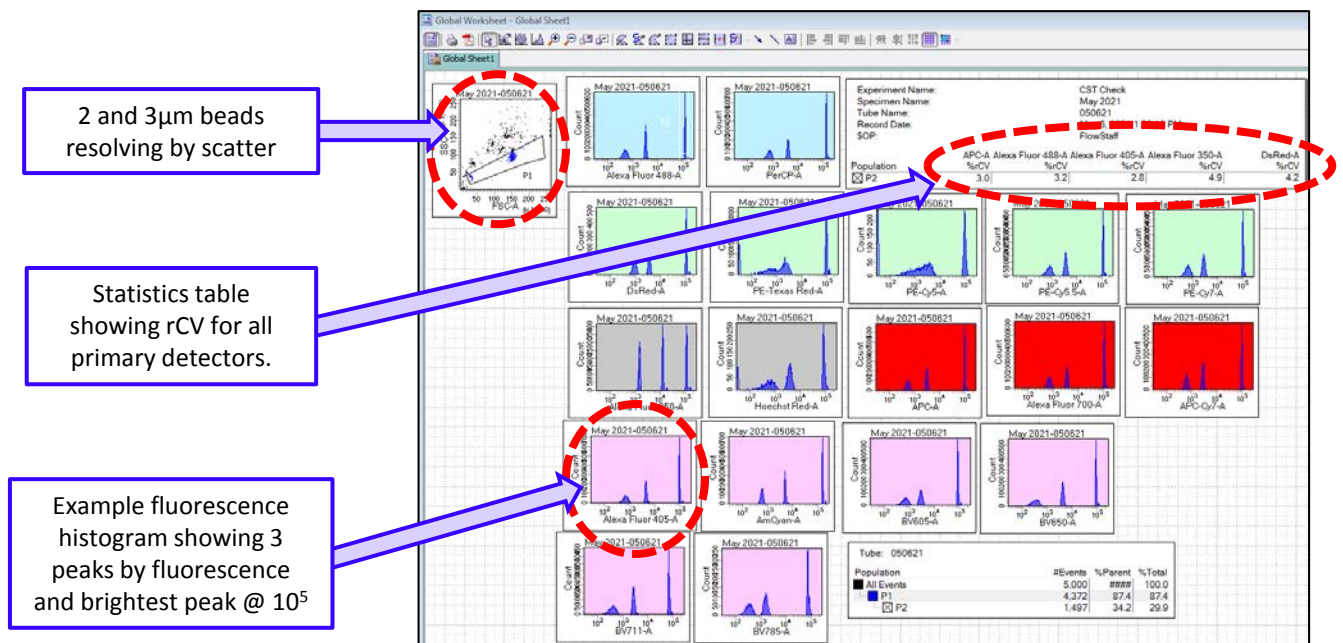
b) From the browser expand on the shared view and open the “CST Check” experiment. Create a new tube and name it with the date in the following format: “MMDDYY”.



Experiment book must be opened (by double clicking) in order to see or acquire/record any data.

c) Vortex and load the CS&T beads onto the cytometer and select “run” and “low”. In Diva, from the acquisition dashboard, select “acquire” followed by “record”.

*Note:* 5,000 events from gate P1 should be recorded.



d) Inspect the beads for the following:

- Two populations appear by scatter
- Three populations appear in each fluorescent detector, with the brightest peak at 10<sup>5</sup>
- rCV of the primary detector for each laser line is ≤ 6.0

*Note:* If any results are out of specifications, please contact flow cytometry core staff for guidance.