The FACSymphony has five lasers, and these lasers are controlled via the **BD Coherent Connection software**. Laser wavelengths and maximum output powers are as follows:

Laser	FACSymphony
UV 355 nm	60 mW
Vio 405 nm	100 mW
Blue 488 nm	200 mW
YG 561 nm	200 mW
Red 640 nm	140 mW

For new experiments, it is recommended to start with all lasers at their maximum output power and only decrease the power when needed (see Laser Power and Fluorescence below). As the lasers retain their settings also across a system reboot, you have to check the laser settings before every measurement session to make sure that the lasers are set appropriately for your experiment.

Using the BD Coherent Connection Software

Starting the Software

The software starts automatically when starting the PC. If not, or if it has been closed since then, start it via the short cut on the desktop or via the start menu.

Upon starting the software, you should see all five lasers.
Note: It might take a little while for all lasers to show up in the software!

If only three lasers show up in the software, the instrument PC was already running before you started the FACSymphony. To connect the remaining lasers:

1. Shut down the FACSymphony.
2. Shut down the instrument PC.
3. Start the FACSymphony.
4. Wait for a couple of seconds.
5. Start the instrument PC.

Starting the UV laser

For laser safety regulation reasons, the 355 nm UV laser does not start automatically when the instrument is started. The laser will be set to start, but the output power will be (around) 0 and a red symbol on the lower right will light up as well as the "LASER FAULT" message at the bottom left.

To start the 355 nm UV laser:

1. Click "Laser STOP".
2. Click "Laser START".
3. Set the laser to the desired output power (see section on Laser Controls below).

Laser Controls

- Laser START: turns the laser on. Laser is on when Laser START is green and the message at the bottom left says LASER ON.
- Laser STOP: turns the laser off. Laser is off when Laser STOP is green and the message at the bottom left says LASER OFF.
- Setting the laser output power **manually** (only operate the laser at or below its stated maximum output power (see table above):
    - Type a number in the field on the lower left and press "Enter".
      OR
    - Move the slider to the desired position.

- Checking the laser output power:
  The large dial ("laser speedometer") in the middle and the mW number in it display the measured actual output power of the laser. It should correspond to the set power on the left. After setting a new output power, it usually takes several seconds for the laser to adjust. Note: the actual laser power reaching the flow cell will be lower (typically 10 – 30% loss).

Using Preset Laser Configurations

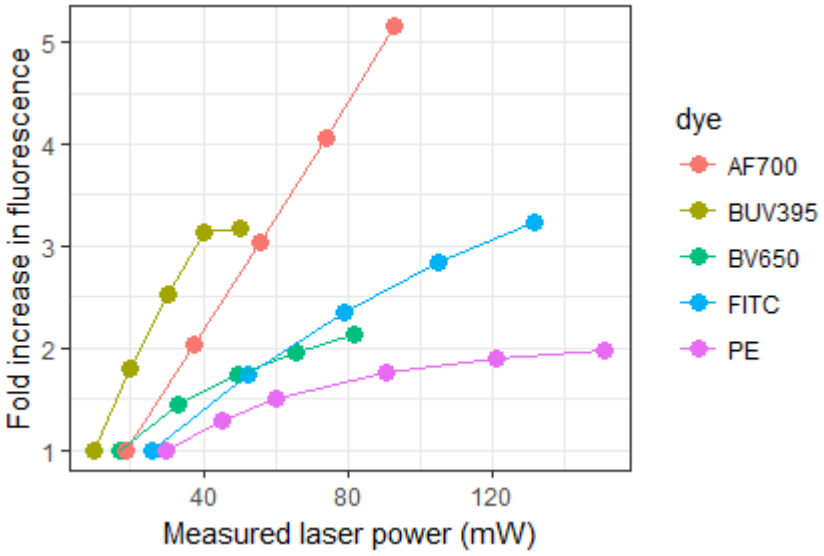
For convenience, laser settings can be saved as configurations. To set all lasers to their recommended maximum output powers, open the "Lasers and Configurations" bar on the left and double click on the "All laser full power" configuration.

You can save your own configurations by clicking on the "Save Config As…" button after making your desired settings. **Please take care not to overwrite existing configurations by using the "Save Config" button.**

Laser Power and Fluorescence

By increasing the laser power, you increase the stream of photons with which you illuminate your sample when it passes through the flow cell. The higher the photon stream, the higher the chance that a fluorophore passing that stream is excited. Thus, more laser power equals (potentially) more fluorescence signal, and therefore higher sensitivity and resolution.

Due to the fluorescence lifetime (the average time it takes a fluorophore to emit a photon after it has been excited), there is a limit on the number of photons we can obtain from a fluorophore during the short time we observe the cell in the laser focus. As soon as we near that limit, we start to saturate the dye and a further increase in laser power will only marginally increase the collected fluorescence. As the fluorescence lifetime is different for each dye, the saturation behavior will be different for each dye too. The following figure shows the dependency of collected fluorescence on the input laser power for five dyes. Only one dye, Alexa Fluor 700, shows a linear increase of fluorescence across the tested laser power range. For PE, we see that a 5-fold increase in laser power only results in a 2-fold increase in measured fluorescence intensity. This illustrates that not every dye will profit the same from higher laser powers.



With higher laser powers, we also increase the risk of adverse photo physical effects like bleaching and triplet state formation that will decrease the amount of collected fluorescence. Test experiments showed that bleaching could be observed with the high laser powers on the FACSymphony. However, we have no reports that bleaching affected actual experiments.

By now, we have only come across two reasons to lower the laser power:

- Extremely bright fluorescence signals.
  If you have extremely bright fluorescence signals that would require you to lower detector voltages to very low levels (at which detector noise contribution increases), you might get better results by decreasing the laser power instead.
- High levels of cellular autofluorescence.
  Autofluorescence does not seem to reach saturation at the current laser powers. Meaning that you can have cases in which your dye already has reached saturation but the autofluorescenc levels keep increasing. In these cases the higher laser powers would effectively decrease your resolution instead of increasing it. So if you have highly autofluorescent cells, test if lower laser powers result in higher resolution.

When adjusting the laser power, keep in mind that decreasing the laser power affects all dyes exited by that laser.