

Troubleshooting...

The Fluorescence Light Path

Make sure:

1. The **port selector** is pointed to the **eyepieces**.
2. Your **eyepiece filter** is **synchronized** with the excitation filter. (Rotate your eyepiece filter to the correct position, or if it's already there, rock it out of position and then back in.)
3. The **arc lamp** is **ON**.
4. The **Neutral Density** filter is letting through enough light for you to see the sample. (Try 100%.)
5. The **slider** that holds the UV-blocking filter is **seated** in position. (Slide the UV-blocking filter out of position if imaging a UV-excited dye like DAPI, or if using the laser.)
6. The **Field-Stop aperture** is not blocking your field of view. Push it IN to OPEN the aperture (IX71).
7. The **correct dichroic** is in place.
8. The **shutter** on the **dichroic wheel** is **open**.
9. You are viewing the sample with the **eyepiece optics** set to **O** (Open).
10. *The **excitation shutter** is **open**. Press the EX button on the joystick keypad. You should be able to see the light coming through, although DAPI will be hard to see.
- 11.

*If the shutter will not stay open, be sure the eyepiece filter wheel is set in a detent position.

Troubleshooting...

Focus Drift

5 things that may be causing a focus drift problem:

1. The **temperature** in the room/environmental chamber is not stable. The microscope and stage will expand/contract 1 μ m (?) for every 0.1 degree C temperature shift.
2. The **sample is moving**
 - a. It is not completely attached to the coverslip.
 - b. It is alive!
 - c. There is an air current in the room.
 - d. It is being lifted by the objective. (Not enough working distance.)
3. The **objective turret is full** and the weight of the objectives slowly lowers the turret over time.
 - a. The coarse focus knob is too loose. Tighten this by tightening the thin inner ring of the right-hand focus knob (IX71).
 - b. Be sure to put on the coarse focus lock for a long term experiment.
 - c. Try locking the fine focus knob with a set-up similar to this: (insert picture here.)
 - d. Remove other objectives from the turret.
4. The **coverslip is flexing**, usually due to not being sealed to the slide.
5. **Vibration**
 - a. Large vibrations in the floor
 - b. Small vibrations on the air table -- from another piece of equipment, e.g. environmental chamber heater.

Troubleshooting...

QLM Laser Setup

7 things to check if you don't see your laser beam firing:

Make sure:

1. The **key** on the laser chassis is in the **ON** position.
2. The **port selector** is directing light to the **camera**.
3. The **laser** is **Active** in the Settings | QLM Tab.
4. The FOM **mirror** is **IN** (horizontal).
5. The **UV-blocking filter** is **OUT** of the light path.
6. The **beam expander turret** is clicked into a **detent** position.
7. The **dichroic wheel shutter** is **OPEN**.

2 things to check if your laser is not firing where you aim:

1. Make sure the **laser is centered**
 - a. Move to a part of your sample where there are no cells, only media.
 - b. Go to the QLM Settings Tab
 - c. Turn on Laser Spot Alignment crosshairs.
 - d. Click “Continuous” to start the laser firing continuously.
 - e. Rotate the black knobs at the base of the Beam Expander Turret to center the laser spot in the crosshairs.
 - f. “Stop” the continuous laser fire. Turn off the crosshairs.
2. Make sure you have the **correct objective selected** in the Resolve3D window, and that you have checked the “Auxiliary Mag” box if you are using the 1.6X optivar.