Start up system
- Log into network and PC
- Turn on ICU
- Turn on 'LASER DO NOT ENTER' light; check wall unit is on, then use remote to turn on/off
- Go to 1st box (contains 2 diode lasers) and turn key from standby to on position (check the current) and press 'shutter open' button
- Check enough de-ionised water in cooler, then turn on with the white rocker switch
- **Oscilloscope**; turn power button on
- **Conoptics**; turn power on
- Go to **Mira controller box** and move central switch to CW position
- Wait for 15-30 minutes before tuning the laser
- Put fluorescent sample on microscope
- Turn knob on RHS of scope to **A** = eyepiece, find field of view and focus, then send signal to PMT (turn knob to **C** = confocal scanning)
- Check slider inside hot box is set to third position TRANS/CONFOCAL
- Log into Lasersharp as usual

Look at readout on **Mira controller** box; this shows Total Power Output if unit is switched to CW (top bar = course readout, bottom bar = fine readout)
Middle switch is PEAK RESET; flip this switch to the right to reset power output reading
RH is relative humidity; check this is under 10%

Look at the **oscilloscope**; should see a narrow peak that is the power output
Should also see 3 rows of dots, top row spaced at 10nm, 2nd row spaced at 1nmm, bottom row?
Should also see a 'Jumping dot' that indicates wavelength (e.g. 820nm)
Move the peak (using controls on main Laser) over this jumping dot, to check the wavelength
Keep PRE-TRIG on
May sometimes need to switch to HI GAIN
Turn the oscilloscope gain knob to make peak bigger/smaller (not laser gain!)
To change the wavelength of the actual laser, need to turn knobs on the top of Mira laser box
In the Mira laser box, the light bounces back and forth between the two sets of mirrors that have to be aligned; the slit cuts out the light completely.

Pump Alignment
Tweak H & V pump alignment knobs very slightly to maximise power output (e.g. to about 450) then press RESET to zero the display output.
Tweak H & V knobs on mirror1 very slightly to maximise power output

Tuning the laser
Open V knob on mirror2 to open the slit clockwise all the way. Notice how the power output increases and the peak on the oscillator increases.
Tune the output with the tall tuning knob, and the oscilloscope peak will disappear out of range. It's best to align the Mira using 800-830nm, even if you intend to use a different wavelength for your experiment.

Turn prism knob clockwise to drop power, then anti-clockwise to restore power to max.
Go back to V knob on mirror2, close it down (anticlockwise) and observe the power output; then stop this V knob down to half of max power output.
Tweak the H knob on mirror2 to maximise power output.
Open up V knob on mirror2 again a little bit

On Mira Controller, move CW switch to ML (centre position)
This mode locked position shows the vibration modes
The frequency is determined by the length of the cavity

The idea is to lock together all the vibration modes, hence 'mode lock'
The prism helps achieve mode lock, but need to tune the laser too
The crystal RI changes as the high intensity pulse goes through it.
As the pulse goes through the crystal, the crystal focuses the pulse to an elliptical shape.
Closing the slit (V knob of mirror2) cuts out all the CW; gradually close anticlockwise until CW all gone!
ML (=mode lock) will actually hold this state in place; you can switch back to CW and it will probably hold OK, but ML is safer.
Increase the gain on the oscillator to see the output properly.
Use the slit (V knob of mirror2) to control output; too far open and you get CW back (don't want this), too far closed and the power drops off.

Pulse Width

At 800nm the pulse width may be around 200 femtosecs, which is fine.
At 960nm you can get it a bit shorter. Go to prism knob on Mira and turn anti-clockwise to get a narrower pulse (narrower pulse looks wider on oscilloscope – counter-intuitive!)
To get the pulse as narrow as possible, turn the prism knob anticlockwise, whilst gradually closing the slit (V knob on Mirror2) to keep out CW. Alternate these knobs to get the pulse as narrow as possible (The pulse width increases inside the microscope optics).

Microscope
- Pull the 'hooked' slider (on RHS under hotbox) out until it clicks.
- NB: This slider should always be pulled out, unless you are viewing specimen down binoculars with DIC
- Turn the knob on RHS to 'C' position
- Move the slider inside the hotbox to the 3rd position

Lasersharp
Create a new method:
- One panel
- Mira set to 10%
- PMT2 (because red emission)
- Blocking filter open
- HQ590/70 emission filter
- Mira (800)
- Tick signal enhancing lens (because not confocal)
- Tick transmission selector
- 1st dichroic 100% det 2/3
- 2nd dichroic 100% det 2
- SETCOL loaded in pane
- Gain 10-20
- Iris open (because not confocal)
- Start with Mira off, then gradually increase to 2% (the Mira is output is 100x other lasers)
- Do offset as usual then reduce Mira to about 1.4 until hardly any red
- Check focus on microscope is optimal

Final Alignment
BCU = beam conditioning unit (unit at rear of microscope)
This contains the pockel cell that controls power determined by MIRA slider in Lasersharp
BCU has more mirrors; gently tweak the two uppermost knobs (see diagram) to direct more power into the objective whilst scanning the sample (turn the computer monitor around so you can see). Try to get the brightest image but not uneven. Leave the other two knobs alone.

The beam expander in the BCU is tied to the objective setting in Lasersharp
[To see this login as administrator, then go to Tools/system setup/sytem configuration/collimator]

Some of the objectives are not setup; best advice is to always use 10x or 60x setting in Lasersharp.

<u>Turning off the Mira</u>
Leave everything on if someone else is using the system within 2hrs, otherwise:
- Switch from MD to CW on Mira controller
- Press 'shutter open' switch
- Turn the key to standby on the diodes box
- Turn off the chiller
- Turn off the pockel cell driver
- Turn off the oscilloscope
- Leave the little box on top of the oscilloscope on (as it shows the last tuning of the laser)
- Turn off the laser warning light, hide the remote in the drawer