

NLO/Multiphoton Laser Reference Sheet

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8 April, 2014

First and foremost: be mindful of what you are doing at all times. This is a very powerful infrared laser. You cannot see the light produced by it and will not blink to protect your vision.

PREPARATION

- Obtain the room key from 6.053 (James Springfield's office) as the door to Confocal2 *must* remain locked whilst a multiphoton experiment is running.
- Place the lanyard on this key around your neck so you cannot lock yourself out of the room.
- Obtain the laser safety goggles and the key for the Chameleon multiphoton laser from the cabinet outside of 6.053.

STARTUP

- Lock the door to the room.
- Inspect the scope to be sure that all covers are in place, that there is no oil where it should not be, no medium spilled on the scope, etc.
- Switch the microscope remote control on.
- Insert the key into the Chameleon and turn the laser *On*.
- Log in to the computer and start *ZEN 2009*.

LASER SETUP

- Place your sample on the stage and find focus using a visible wavelength – focus on one small, distinct object.
- **Make sure you are wearing laser safety goggles.**
- Turn the Chameleon laser on in ZEN. This opens the shutter on the Chameleon laser.
- In the *Channels* menu, ensure that the 790 laser is selected and set the wavelength to about 900nm (if you are working with FITC- or EGFP-like fluorophores). It may take up to 30 seconds for the laser to tune to the desired wavelength – wait until the laser power supply reads *Modelocked* before continuing.
- Set laser power to 1%.
- Set the main beam splitter (MBS) to KP 650 or something similar.
- Set gain and offset as usual.
- *Snap* an image.
- If an image appears:
 - You may increase the laser power, but *do not* raise it above 4% at this time.
 - Without adjusting the focus, locate the spot on which you had focused previously.
 - Go to the *Maintain* tab and find the *Adjust Pinhole and Collimator* menu.
 - Return to the *Acquisition* tab and click the *Continuous* acquisition button.
 - Return to the *Maintain* tab, adjust the collimation to obtain the brightest image, and store the current position.
- Fine-tune the excitation wavelength to obtain the best signal. This process can be somewhat random.
 - Change the wavelength and check the front of the laser housing to see that it is modelocked.
 - Scan an image and check the results – are they better or worse?
 - If the results are worse, change the wavelength in the other direction.
 - If the results are better and very bright, turn down the laser power and check the image quality.
 - If the image quality is still poor, change the wavelength and scan a new image.
 - If you get a completely white screen at any point, turn the laser power down significantly.
 - This whole process takes much trial and error.

SHUT DOWN

- Turn the key on the front of the Chameleon to *Standby* and remove the key
- Unlock the door to the room, and return the keys and laser safety goggles.