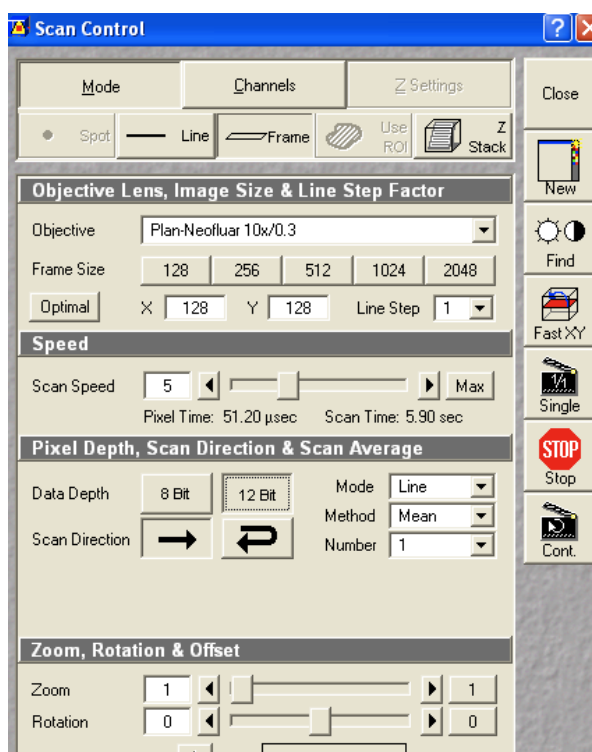


Reflections of the key LASER lines on your confocal laser scanning microscope (CLSM) will be used to test the wavelength accuracy of your spectral detector. A mirror slide will be provided for this standard test.

1. Under the **Microscope Control** window, put a 10x magnification objective lens in place (or other low magnification lens).
2. Place the mirror slide on the stage with the coverslip facing the objective. Focus on the edge of the mirror via the microscope.
3. Choose the **Lambda Mode** under the **Configuration Control** window.
4. In the same window, put an 80/20 mirror (**NT80/20**) into the light path.

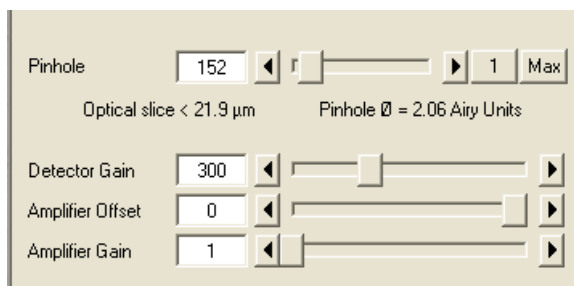
5. Adjust the range of wavelengths (under **lambda mode** menu) to cover the range of available lasers.
6. Do not select the 405 nm LASER because the detector does not measure wavelengths below about 420 nm.
7. Under the **Scan Control** menu in the **mode** tab, set the parameters to the following (see table below and image, right):

Parameter	Setting
<b>Frame Size</b>	128x128
<b>Scan Speed</b>	5-9
<b>Frame Averaging</b>	1
<b>Bit Depth</b>	12 Bit
<b>Zoom Factor</b>	1.0

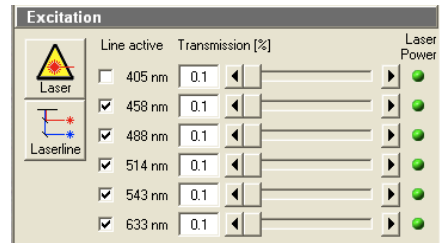


8. Under the **Scan Control** within the **channels** tab set the parameters as follows (slight adjustments may need to be made):

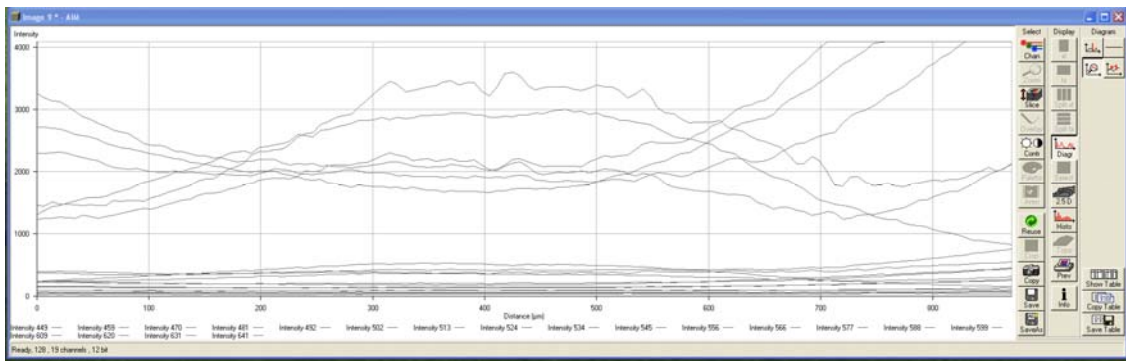
Parameter	Setting
<b>Pinhole</b>	1-2 Airy Units
<b>Detector Gain</b>	200-400
<b>Amplifier Offset</b>	>0
<b>Amplifier Gain</b>	1.0



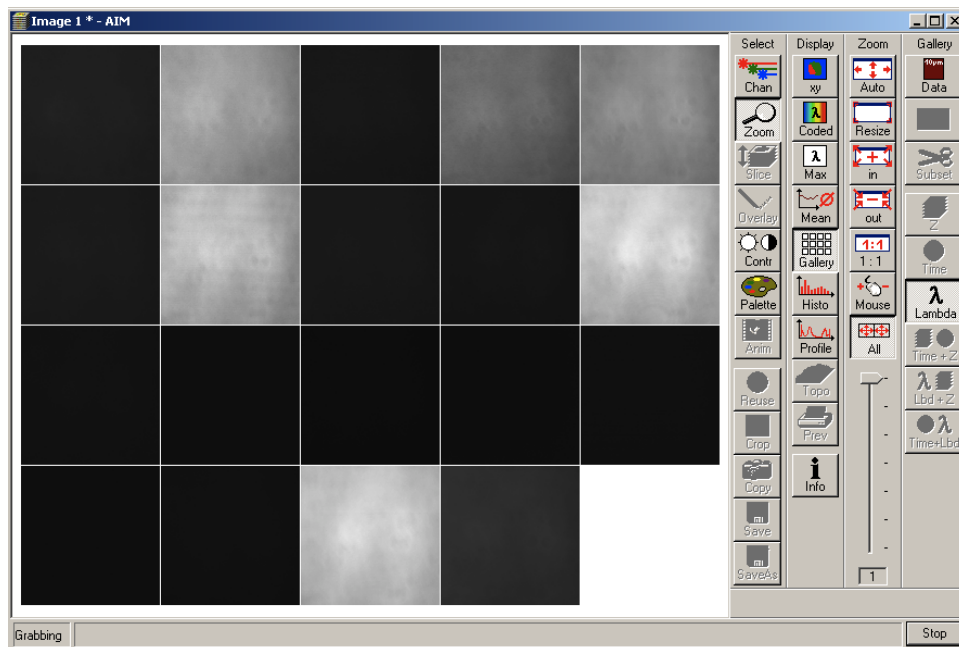
9. Under the **Channels** tab in **Scan Control**, check off the lasers being tested (all lasers except the 405nm).
10. Set the laser power for each laser line to give an intensity signal of 2000-3000 for a 12-bit image (150-200 for an 8-bit image).



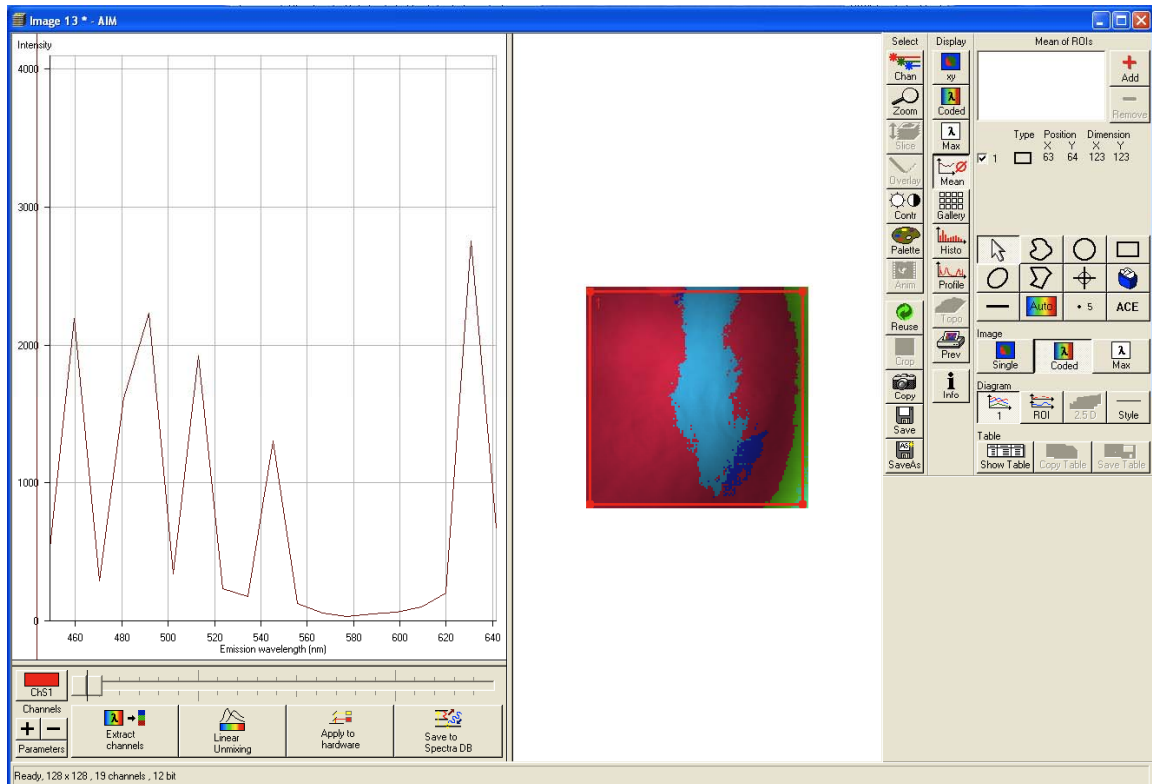
11. To aid in setting the intensity units, start a **continuous scan**, and in the **image** window under the **display menu**, select the **diagram** view. Change the percentage of power to the laser lines and/or the detector gain to adjust the intensities to within 2000-3000. If this view is not available use an ROI measurement.



12. Use the **palette** and check off **range indicator** to ensure that there are no saturated pixels (red) within the Lambda stack. (See **gallery** view on **continuous** mode).



13. Collect a Lambda stack of images (single). You may get interference patterns in the images from the laser reflections. This is normal. Notice how the periodicity of the interference pattern gets larger as you move to longer wavelength lasers.
14. After the picture has been taken, select **mean** from the **display** menu. Draw a region of interest over the entire image. Verify that the laser peaks are falling within 10-15 nm of the expected values. Notice the emission peaks at the wavelengths that correspond to the laser lines. The parameters should be set to only ChS1. Click on **show table** and then click on **save table** to log the data.



15. The text values for the spectra can be saved as a text file and imported into Excel.
16. Name the file with your name and the name of the confocal platform you collected the data on. Send the text file (.txt or .xls) to the ABRF-LMRG at [abrf.lmrg@gmail.com](mailto:abrf.lmrg@gmail.com).