Protocol for alignment of SPIM laser launch

Laserlaunch and AOTF

Alignment of optical elements in the laser launch should maximize the transmittance of light through the AOTF. Achieving a high transmission rate for all wavelengths in the AOTF is difficult, as transmission of one often comes at the expense of another. In principle, a high overall transmittance is most desirable.

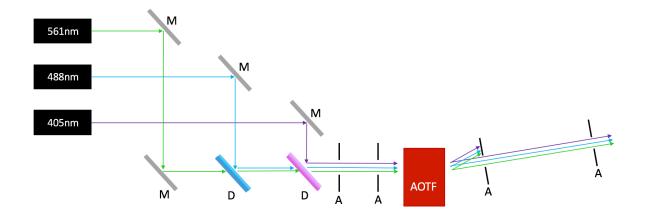
Due to chromatic aberrations, it is advisable to use intermediate colors as a baseline for alignment. Because of the inferior chromatic correction at shorter wavelengths, the 405nm should be avoided as a reference. Moreover, the 488nm laser is currently the most used while it is also the one with the lowest power. We will thus optimize alignment for that laser.

Building the laser launch (Lasers, mirrors, dichroic mirrors, cover box)

1. Set the laser launch as shown in figure 1. Lasers must be placed in chromatic order. To ensure alignment, it is important that each beam is reflected at two reflecting surfaces before reaching the AOTF. Dichroic mirrors are high-pass filters.

Note: If a red laser is needed, replace the second mirror on the optical path of the 561 laser for a dichroic.

2. Place two apertures between the laser launch and the AOTF. Set them to guide the beam at a straight line roughly perpendicular to the AOTF. Close the apertures and align the 488nm laser by maximizing the intensity with the aid of the powermeter.



3. Connect the AOTF to the digital synthesizer and the computer. Turn it on. The AOTF Control software is written in LabView. Upon initialization, it will request the COM port for the AOTF. Use port COM9.

The software allows the simultaneous control of up to 3 laser lines. The user can tune the frequency and the power of phonons in the AOTF to modulate transmission. Refer to the manufacturer's datasheet for standard values for common lasers. These, however, may not be the optimal values, so fine tuning is advisable.

- 4. Set the AOTF to the optimal frequency for the 488nm laser.
- 5. Inside the AOTF, the crystal is not necessarily perpendicular to the housing. It is desirable that light reaches the crystal perpendicularly.

The output of the AOTF consists of two beams, one for each transmitted mode. We will discard the beam that is most strongly deviated from the original path as it is not possible to ensure that all wavelengths will follow the same path in this mode. Note that the sum of the intensities of the two modes will roughly correspond to the intensity of the beam at the input.

Rotate the AOTF gently and slightly to ensure maximum transmittance on the desired mode. Fix the AOTF. Record the resulting output intensity.

- 6. Remove the apertures before the AOTF, they are no longer necessary as it is not possible make the beams coincide in path both before and after the AOTF. You may leave one of aperture in case you want to modulate the size of the beam entering the AOTF (although that is rarely needed).
- 7. Set the AOTF to transmit another wavelength. Adjust the path of that laser by means of its mirror and dichroic (once a laser is aligned you may not change the orientation of its mirror and dichroic again!). Maximize the intensity at the output. Do so for all lasers.
- 8. Verify that all laser lines have good transmission. It is often the case that one wavelength reaches great transmission at the expense of the others. Consider the regular illumination needs for the experiments conducted in the microscope and try to optimize the output accordingly. If the output for one or some of the beams is not satisfactory, return to step 5 and realign.
- 9. Once you have achieved satisfactory intensities for all wavelengths, set the AOTF to transmit for the 488 solely. Place one aperture 4cm after the AOTF (which will block the undesired output mode). Fix the powermeter after it. Close the aperture to a pinhole and move it gently until transmission is maximized. Fix the aperture to

the table with a forkclamp. Place a second aperture 20m from the first, move the powermeter after this one and repeat the process keeping both apertures closed. Once transmission is maximized, fix the second aperture.

These apertures are extremely important for the further alignment so they should not be moved.

10. For each of the other lasers, set the AOTF to transmitting it solely, and fine-tune the orientation of mirrors and dichroics to maximize the intensity at the powermeter after the two closed apertures. By doing this, you will guarantee that all the wavelengths leave the AOTF following the same path.