Guidelines for Setting up an Acousto-Optic Lens 3D laser scanner on an existing galvanometer-based two-photon microscope

The SilverLab Microscope development team 16/01/2016

Introduction

The Silver Lab compact configuration Acousto Optic Lens (AOL) can be used to convert a galvanometer-based two photon microscope into a 3D AOL scanned microscope. To date we have converted two conventional Prairie Technologies Ultima microscopes (Fernandez-Alfonso et al., J. Neurosci. Methods, 2014). The optical transmission efficiency and significant chromatic dispersion of the AOL mean that care must be taken to minimise optical loss in the complete optical path and that a stand-alone pre-chirper is usually necessary to compensate for the temporal dispersion. The design process and details are outlined here. The same design process can also be used with a 'pure' AOL microscope with a custom optical relay replacing the galvanometers scan lens and tube lens. Indeed, we have built several such AOL microscopes using modified Scientifica slice scopes. This document assumes that the galvanometer mirrors are simply left in their central zero position for set up and use.

Overall System

The overall system is shown in **Figure 1**.

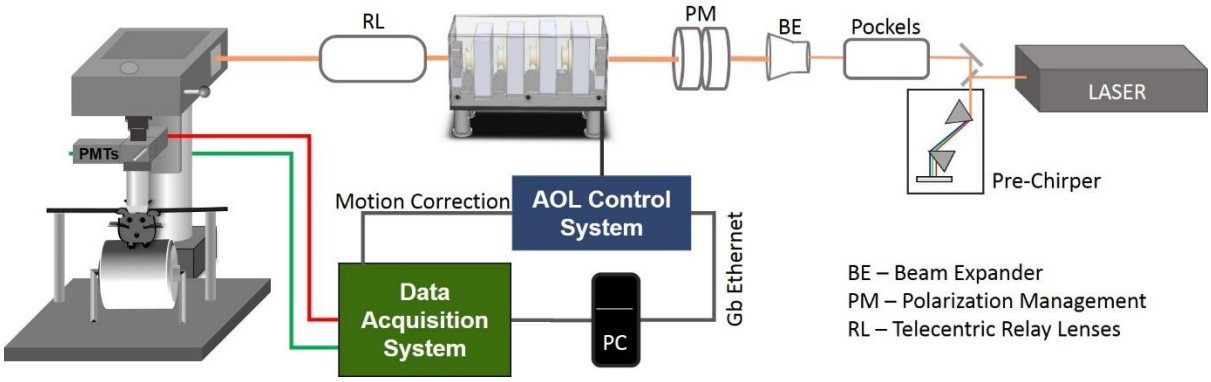

Figure 1: Basic layout of main components of AOL 3D laser scanning two photon microscope.

Acousto-Optic Lens 3D Two-Photon Microscope Parts List

1. Acousto-Optic Lens (Nadella et al., Nature Meth. 2016)	
1.1	Compact Acousto-Optic Lens (AOL)
1.2	AOL Controller
2. Data Acquisition System – National Instruments	
2.1	NI PXIe-1073 Integrated MXIe, 5 Periph Slot, 1 Port PCIe, 3m Cable
2.2	PXIe-7966R FlexRIO FPGA Module (Virtex 5 SX95T, 512MB RAM)
2.3	NI PXI-6733 with 8 16-Bit Waveform Analog Outputs, 8 D I/O lines, and 2 counter/timers
2.4	NI PXIe-6537 High-Speed Digital I/O (50 MHz, 32 Channels, Selectable Voltage of 2.5, 3.3, or 5 V, PXI Express)
2.5	NI 5772 (-02) 2-Channel, 1.6 GS/s, 12-Bit, DC-Coupled, 350 MHz Antialias Filter Digitizer Adapter Module for NI FlexRIO
2.6	BNC-2110 Noise Rejecting, Shielded BNC Connector Block
2.7	Cable, Type SH68-68-EP, Shield Cable, 1m
2.8	SHC68-C68-D4 Shielded Single-Ended Cable, 1 m
2.9	SMB-2163 Single-Ended Digital I/O Accessory (Rack-Mountable)
3. Optics and Mechanics – THORLABS	
3.1	Relay Optics
3.2	Mirrors – Dielectric mirrors/ Hard coated Silver Mirrors
3.3	Mechanical Mounts (Cages, Lens, Mirrors etc)
3.4	Half wave Plates
3.5	Quarter wave Plates
4. Other	
4.1	80 MHz Femtosecond Laser with >100 fs pulse width and output power of 2W at 920 nm (numerous suppliers)
4.2	Pre-chirper – Custom pre-chirper from APE-Berlin
4.3	Pockels cell – ConOptics / AOM – AA OptoElectronic
4.5	Galvanometer based two photon microscope - Microscope Head, Slider mirror, excitation and emission filters, PMT mounts etc.
4.6	Objectives – Olympus/Nikon/Leica
4.7	GaAsP PMTs – Hamamatsu
4.8	PreAmps (Femto Model No. DHPCA-100)
4.9	Microscope alignment laser
5. Software (SilverLab GitHub Repository)	
5.1	AOL 3D scanner firmware
5.2	3D imaging software LabVIEW or MATLAB versions

Electronics and Wiring Diagram:

Figure 2 shows detailed wiring diagram for the AOL Microscope. PXI chassis (NI PXIe-1073) interfaced with PC using MXI card. Chassis consists of three cards:

1. PXIe7966R along with NI-5772 data acquisition card to send triggers to the AOL controller and simultaneously acquire data in two channels at 800 MHz. This card also communicates with controller for real time motion correction through a serial cable (HDMI). AOL radio frequency (RF) drives are generated from FPGA based AOL controller based on user defined parameters from the PC.
2. PXI 6733 Analog output card to generate analog signals for PMTs, Hard shutter, galvos and other peripherals.
3. PXI 6373 digital input output card to trigger treadmill encoder, cameras and other peripherals.

AOL controller receives user defined scan parameters from PC via Gigabit Ethernet Cable. It also generates analog signals required for Pockels cell/ AOM controller to modulate the laser power. The controller generates a clock signal to synchronise the data acquisition and scanning. It receives an input trigger from data acquisition card (NI 5772) to trigger RF generation for the acoustic drives to the 4 crystals making up the AOL (X1, Y1, X2, Y2) based on user defined scan parameters. Real time motion correction information is transferred using serial communication using a HDMI cable between DAQ card and AOL controller.

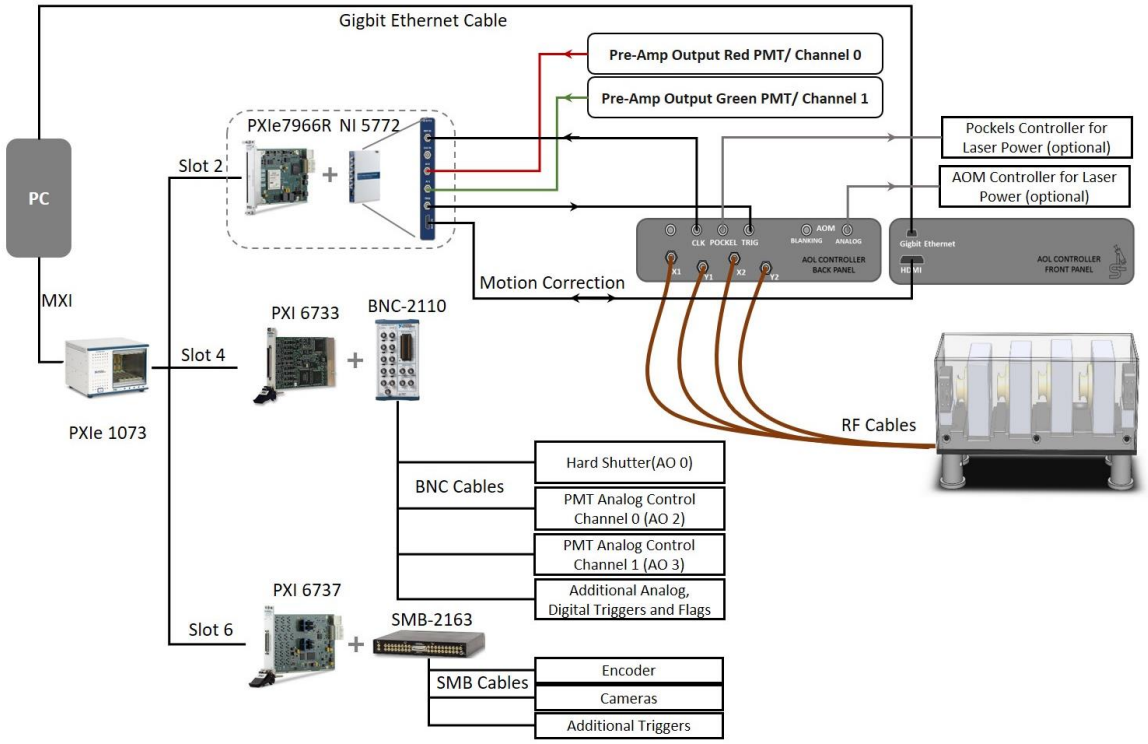

Figure 2: AOL Microscope wiring diagram.

PC and Software:

Computer Requirements: Operating System: Windows 10; RAM: 4GB (atleast) ; Monitor resolution: 1920 x 1080; Data drive throughput: 750MB/s supported by the motherboard's front serial bus; Data drive size: >1TB recommended

AOL firmware, Imaging Software and documentation is available on GitHub:

https://github.com/SilverLabUCL/SilverLab-Microscope-Software

Overall System Design

The AOL two-photon microscope has 6 major optical sub units (**Figure 3**), Laser, Pre-chirper, beam expander, AOL, relay optics and scan lens, tube lens and objective lens together with galvanometers if present.

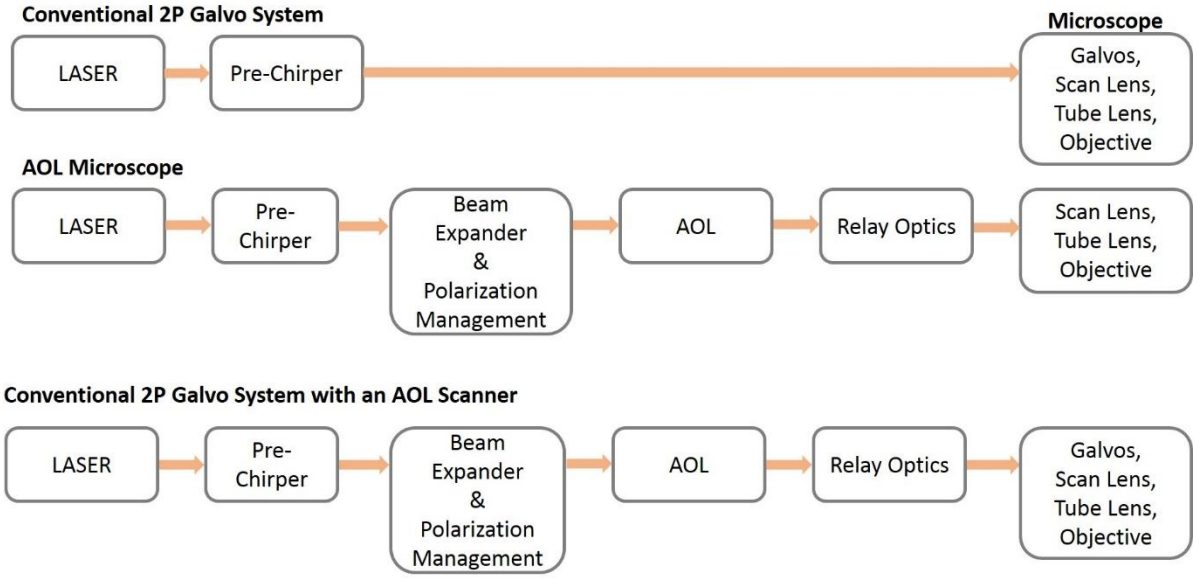

Figure 3: Optical subunits of a conventional Galvo system, an AOL Microscope and AOL Scanner as an add-on module to conventional 2P Galvo System.

Laser: We have found that 80 MHz femtosecond lasers with 100- 150 fs pulse widths have a spectral bandwidth that gives acceptable chromatic aberration (Ref. P. A. Kirkby et al, Optics Express 2010). Given the optical power budget from laser to specimen (table below), for deep tissue imaging of Ca^{2+} in vivo requiring up to 150 mW at the specimen, a laser with an output of at least 2 W at 920 nm is needed. To check whether the targets can be met, the system designer needs to have information the optical loss of each element in the optical path. An example of typical power budget would be:

Component	Transmission %	Power Level mW
Laser output at e.g. 920nm		2200
Pockel cell	95%	2090
Pre-chirper	80%	1672
Beam Expander	95%	1588
AOL	25%	397
Relay to microscope	95%	377
Microscope to Back aperture	70%	264
Objective	70%	185
Specimen		185

Pre-Chirper: The Silver Lab compact AOL consists of four 8 mm thick Tellurium Dioxide AOD crystals in series (Nadella et al., Nature Methods 2016). This introduces a significant extra chromatic dispersion, which needs compensation with a pre-chirper. Unfortunately, this extra dispersion may take the total dispersion of the AOL microscope over the maximum compensation of some commercial 2-photon lasers with integrated pre-chirpers. This then necessitates the use of an

external standalone pre-chirper such as those available from APE (femtoControl-APE Berlin). Figure 4 shows the dispersion as a function of laser wavelength. The dispersion of the AOL at 920nm is 13,000 fs^2 and here it is assumed that the Pockels cell was 8000 fs^2 and the rest of the microscope and relay optics 5000 fs^2. The pre-chirper path length between the two prisms for this dispersion is approximately 112 cm.

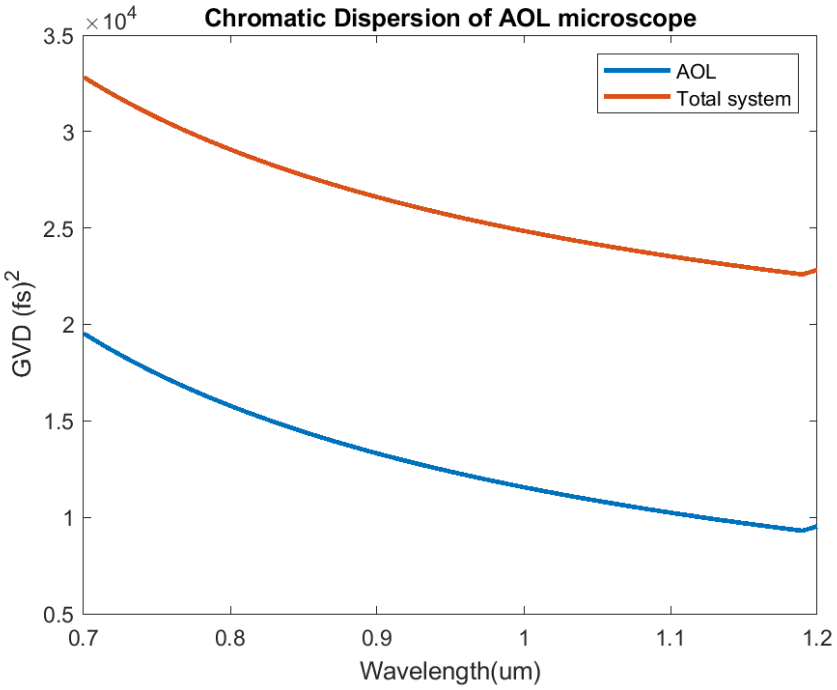

Figure 4: Calculated dispersion vs wavelength for the AOL and a complete AOL microscope system

The system designer also requires information to be able to control all interlocks, shutters, galvos, XYZ stages and other peripherals from LabVIEW software of the AOL.

Optical path requirements

The systems should be capable of easily accepting the input beam from a remote scanning device like an AOL.

Operating wavelength

The AOL operates at its highest efficiency over a range of about 100 nm, without any mechanical adjustment. Although the peak efficiency can be set at any desired wavelength, we typically set up the AOLs for 875 nm so it still has high efficiency at 920 nm for GCaMP6 fluorescent indicators and high enough efficiency for imaging at 800 nm (the Ca^{2+} independent wavelength) without any adjustment of the AOL or optical path. A typical AOL efficiency vs wavelength is shown in **Figure 5**.

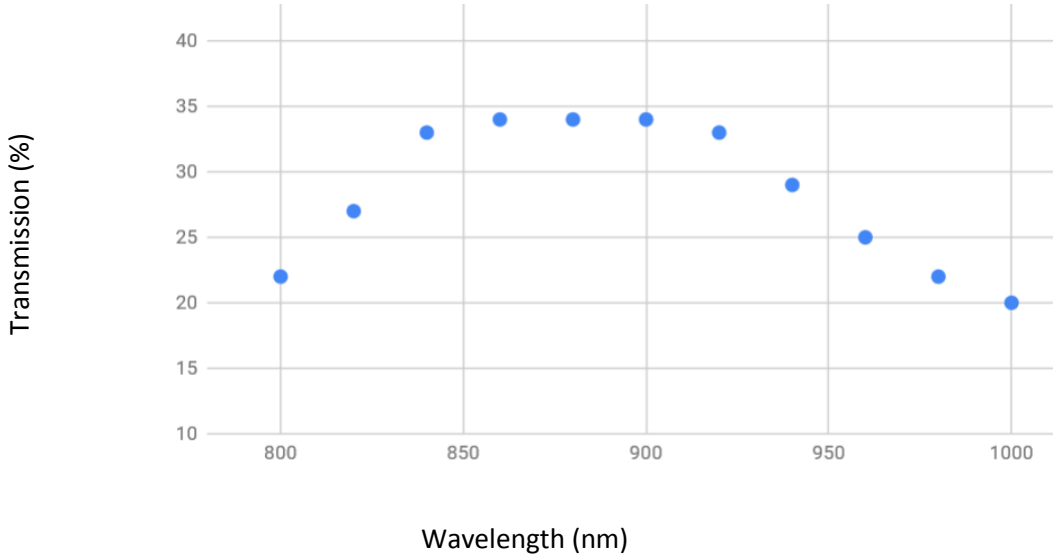

Figure 5. AOL optical transmission efficiency as a function of wavelength for an AOL aligned for maximum efficiency at 875 nm. Peak efficiency can be set during the construction of the AOL. We have tested operation over the range 800 nm – 1055 nm to date.

Coupling the AOL scanner into the optical train

The AOL 3D laser scanner is a compact remote focusing device that sits on the optical bench behind the microscope (**Figure 6**). The telecentric optical relay between the AOL and the microscope must be able to couple the 15 mm diameter beam with a scanning angle of ±1 degree (output of the AOL) into the microscope. This is expected to use one or two telecentric relays in series and suitable mirrors near the input and output iris planes of the telecentric relays. The magnification 'M_1' of this relay will be chosen to provide the right size input iris diameter 'D= M_1 x 15 mm' to the relay optics and galvanometer mirrors of the microscope itself. The scan angle at this aperture is ±(1/M_1) degrees. The input iris of the microscope is defined as an aperture conjugate with the back aperture (BA) of the microscope objective. If there are galvanometers this is expected to be either at the first galvanometer for the case of a galvanometer microscope with telecentric relays between the X and Y galvanometers (e.g. the Scientifica Hyperscope) or half way between the X and Y galvanometers if they are close coupled. Thus, the complete optical system projects the suitably magnified image of the last AOD of the AOL onto the back aperture of the microscope objective.

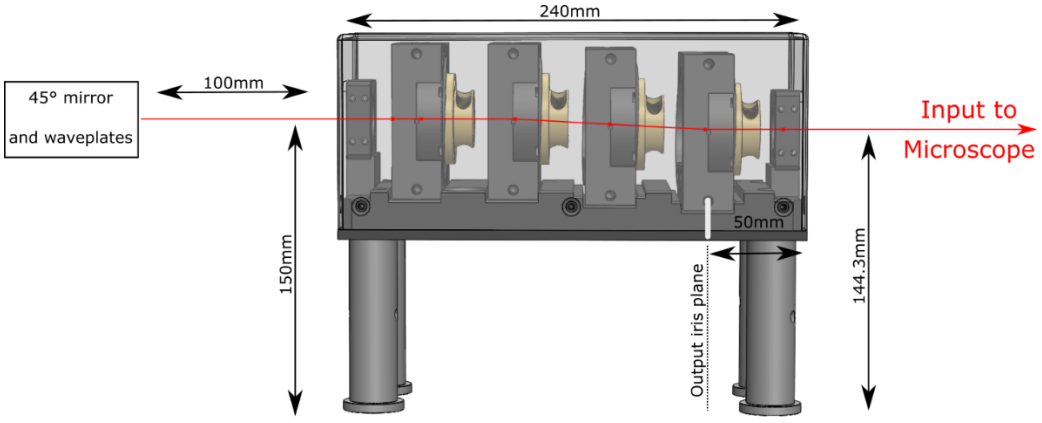

Figure 6: Side view of the AOL showing key measurements for alignment with the input beam and the following relay to the microscope

The output of the AOL relay should have an aperture diameter D chosen to project through the microscope relay(s) onto the back aperture of the objective with a diameter D_{BA} to give the desired NA focussed laser beam onto the specimen. Note that in the absence of any apertures the Field of View of the AOL microscope is entirely fixed by the apparent NA of the projected output aperture of the AOL on the back aperture of the microscope objective, irrespective of the combination of relay lenses that link the two (Kirkby, P.A., "The development of a high speed 3D 2-photon microscope for neuroscience", 2010 doctoral thesis, UCL; https://discovery.ucl.ac.uk/id/eprint/20463/). For an Olympus 20X NA=1 objective, we use D_{BA} =12 mm diameter, which underfills the BA of the objective and gives an effective laser beam NA of 0.6 to 0.7. For an Olympus 40X NA=0.8 objective, we use a D_{BA} = 8 mm diameter, which fills the BA of the objective and thus gives an effective NA of 0.8. If the microscope relay(s) have a magnification 'M_2', the input aperture diameter D needs to be D = D_{BA}/M_2. The magnification 'M_1' of the AOL relay is therefore given by

$$M_1 = D_{BA}/(15 \text{ mm} \times M_2)$$

The design requires the precise position of the input iris plane and a 3D model of the microscope body in order to design the opto-mechanics of the input relays. If there is any optics before this input aperture the design needs information about e.g. focal length of the lens or position of any mirrors at the entrance of the microscope.

Details of how to align the AOL to the microscope

This section describes a procedure that the Silver Lab uses for aligning the AOL to the microscope. It assumes that the AOL will be coupled with a telecentric relay into e.g. the first galvanometer mirror of the microscope. The AOL input replaces the input laser beam in the galvanometer only microscope.

WARNING – The AOL polarisers can be damaged if laser power density above a few W/cm^2 is put in the input. It is safe at up to 4 W with full 15 mm aperture. It is easy to damage the polarisers if an unexpanded (e.g. 3 mm) multi Watt beam is inadvertently directed into the AOL input.

PROCEDURE

1. Check the power budget is sufficient for the application.

2. Carryout the detailed design of the optical relay according to section entitled coupling the AOL scanner into the optical train. Keep in mind that after the Ti:Sapphire laser and pre-chirper comes the beam expander that increases the beam diameter to the 15 mm required by the AOL. The relays between the last AOD of the AOL and the back aperture of the microscope objective should be telecentric, i.e. these two surfaces are conjugate. There is a white mark on the AOL showing the position of the last AOL (see **Figure 6**).

3. The AOL module is engineered to a high level of precision, so that the input and output are parallel to the optical table and straight edge placed against the support pillars. So, the axis of the input beam to the AOL should be horizontal to the optical bench within 1 milliradian (the angle 1mm subtends at 1m, 1mrad=1/17th of a degree). Also when the AOL is pushed against a straight edge about 30 mm long to align it parallel to the bolt holes on the bench, the input and output axis of the AOL is then parallel to the straight edge to within 1mrad. This makes insertion or replacement of AOLs straightforward once the input beam is aligned.

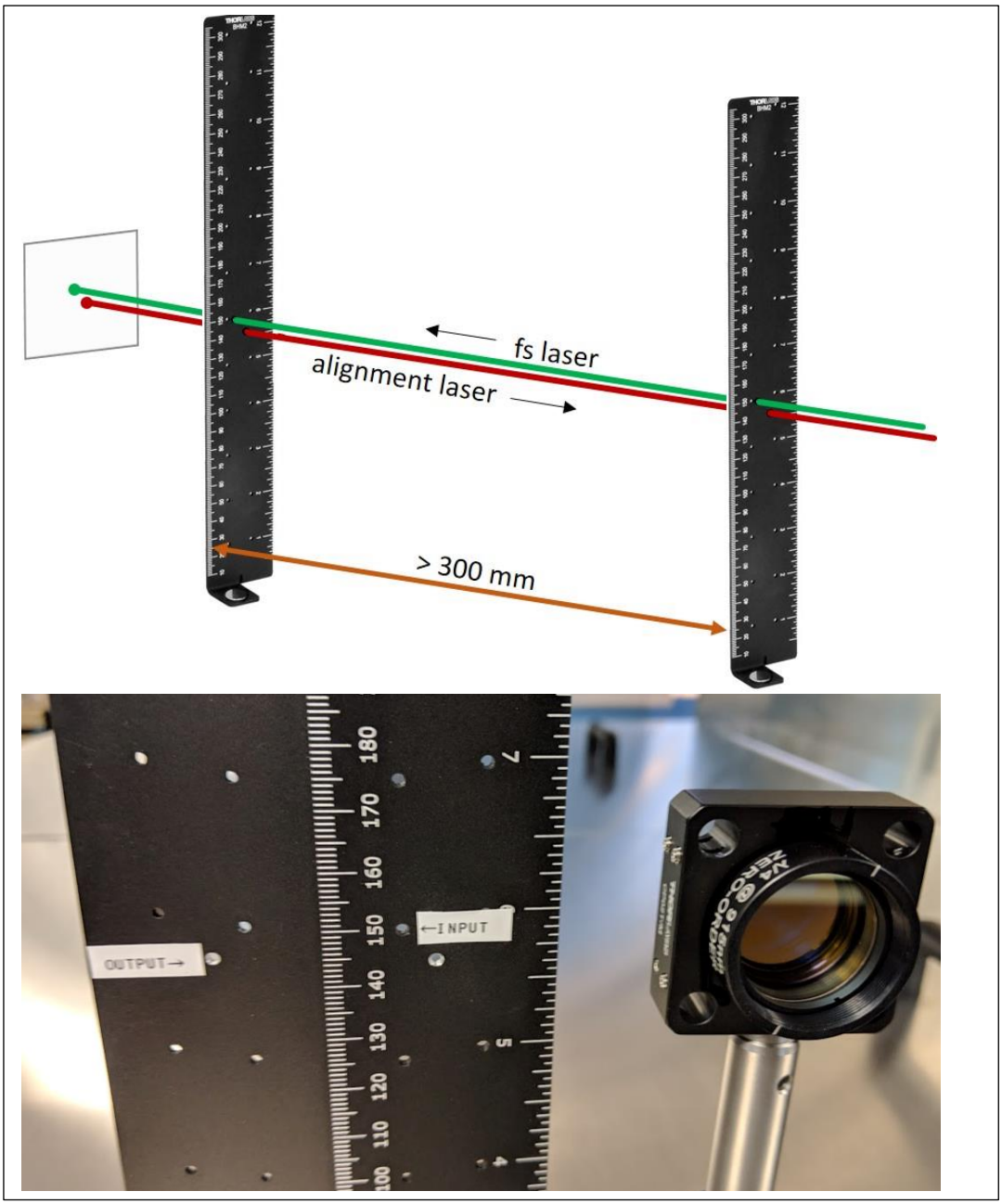

Figure 7 ***top***: Alignment rulers are used to align the input and output beams of the AOL prior to its insertion in the optical path, arrows indicate laser beam direction. ***Bottom*** , two rules with alignment holes, on the right is the reference analyser used to set input polarisation.

4. Two rulers are required for the alignment of the input and output beams with holes at the precise heights 150 mm and 144.3 mm (**Figure 7**). The input beam axis is 150 mm above the optical bench. As it passes through the AOL the beam is displaced down and to the right by 5.7 mm horizontally and 5.7 mm vertically as shown in **figure 6**. The output beam is exactly parallel to the input beam when the AOL is driven with 4 equal frequencies. (This beam is labelled as input to microscope on **Figure 6**.) These are the displacements for a centre frequency of 39 MHz at an operating wavelength of 920 nm. Using the two alignment rulers the input beam can be aligned with the two holes at 150 mm and the holes marked output can be aligned with a back propagating visible alignment beam generated by an alignment laser plugged into the objective mount of the microscope (microscope alignment laser).

5. It is recommended that a 45^0 alignment mirror 100 mm in front of the entrance to the AOL is used (**Figure 8**). This leaves plenty of space for power meters and the required waveplates before the AOL and allows the precise input beam angle to be adjusted

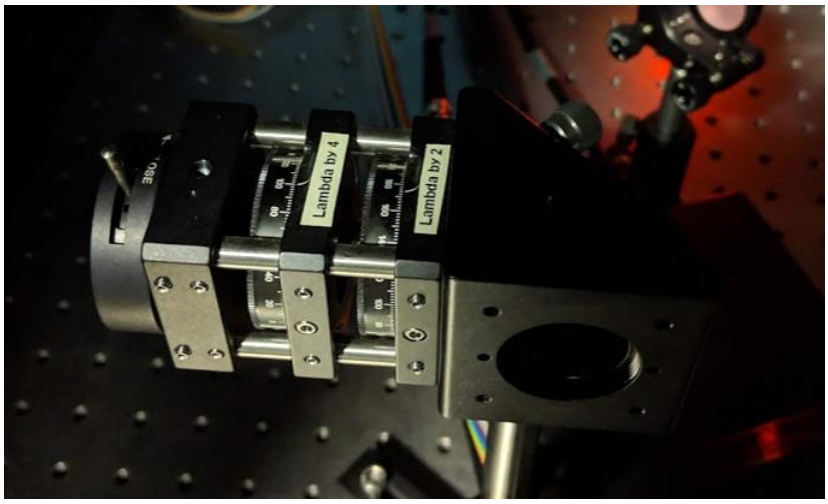

Figure 8 Final 45^o mirror after beam expander and the half wave and quarter wave plates to adjust the polarisation of the input to the AOL.

6. The AOL requires a right hand circular polarisation to give maximum efficiency. To generate this polarisation a half wave and quarter wave plate are required after the final input beam alignment mirror. You are provided with a waveplate and polariser as shown in **figure 7, bottom right.** We refer to this as a reference analyser set up. It accurately blocks the input beam when the input beam's polarisation is optimal. It is straight forward, once the input beam is aligned to input to the AOL, to place this reference analyser in front of a power meter and adjust the angle of the half wave and quarter wave plates to reduce the transmitted optical power down to a minimum typically 1-2% of the input power. The polarisation is then optimal for maximum efficiency from the AOL.
The main Thorlabs components of the 45degree mirror waveplates and final iris are as follows :-

Thorlabs 45 degree mirror and waveplate unit		
Part Number	Part	Quantity
BBE1-E03	Elliptical mirror	1
KCB1E/M	Mirror mount	1
ER1.5-P4	Pack of 4 cage rods	1
CRM1/M	Cage rotation mount	2
CP02T/M	Cage plate	1
WPH10M-915	Half wave plate	1
WPQ10M-915	Quarter wave plate	1
SM1T2	coupler	1
SM1D25	Iris diaphragm 25 mm	1

7. Once all the parts are in place for coupling into and out of the AOL the most important part is aligning the input and output beams sufficiently well that diffraction limited point spread function is obtained beneath the objective of the microscope. To achieve this the light must

not zig zag through the lenses but must remain parallel to the system axis at all times. In general, where possible, it is best to use cage mounts and removable lenses in e.g. the beam expander and telecentric relay so that a small unexpanded beam can be aligned along the axes of the cage mounts before inserting the lenses. **Note** as mentioned earlier that it is important to use low power pilot beams to avoid damaging polarisers.

8. Use an axial visible alignment laser to send a beam backwards from the position of the objective. Align back though the microscope checking the alignment beam is axial right through the system (centre the galvanometer mirrors) and through the telecentric relay between the microscope and the AOL. Place the two sets of alignment rules (**Figure 7**) to define the required position of the axis of the AOL approximately at the input and output positions of the AOL. Adjust the lateral position of the rules so that when the alignment beam passes through both output alignment holes, the horizontal axis of the alignment beam is parallel to the appropriate bolt holes on the optical bench. The reference beam position and angle is now set.

9. Adjust the position and angle of the low power pilot beam from the femtosecond laser to pass through the two 'input' holes on the alignment rules. This may well involve adjusting both the position and angle of the 45 degree mirror and preceeding beam expander before the AOL.

10. Next put the lenses into place in the telecentric relay and beam expander. Check that the full diameter beams are central to the pilot alignment beam and readjust as necessary.

11. Use your reference analyser and a power meter to set the two waveplates to the correct angle to give optimum polarisation into the AOL. Use the iris of the beam expander to make a low power density input alignment beam. The AOL can now be inserted.

12. These two alignment beams can now be aligned with a fluorescent target screwed into the input and output cage units of the AOL. This positions the AOL correctly. To enable easy replacement of the AOL, firmly clamp a straight edge to the optical table just touching two of the AOL support pillars so that the AOL can be repeatedly repositioned with its axis correctly aligned to the input and output beams.

13. Drive the AOL with four RF Drives at 39 MHz. The AODs are in order X1, Y1, X2, Y2. Set the software to drive at constant frequency of 39 MHz. The pre-set RF powers are 2 W,2 W, 3 W, 3 W respectively. You may need an RF power meter if there are problems. Contact UCL before use to learn about adjusting the preset drivers in the AOL Driver Unit.

14. Check with a fluorescent target that the AOL is transmitting at maximum efficiency. By pulling the front leg and then the back leg of the AOL support pillars, the AOL can be slightly misaligned by rotation on the optical table. Check that the efficiency is maximum with the pillars pushed against the straight edge. If not fine tune the angle of the straight edge.

15. With the aperture after the beam expander set to 15 mm use a power meter check that the overall efficiency of the AOL at 920 nm is over 25%.

16. The narrow beam from the apertured down beam expander can now be used to check the alignment through the AOL and through the microscope. Check that the beam out of the AOL is axial in the input to the telecentric relay and central in a fluorescent target set in place of the final objective.

17. Check that when the aperture before the AOL is opened up to 15 mm, the beam in the fluorescent target is central and circular, and that it is the correct design diameter for the objective lens. If there are problems, then any of the items from 8 onwards may have to be repeated.

18. Next the Microscope is put into imaging mode and the image quality tested first with pollen grains then with sub-micrometer beads to test point spread function.

19. First image with a large field of view so that brightness is falling off at the edges. Check that it is brightest in the centre of the field of view. If the image is clearly brighter to one side or up and down, then fine tune the angle of the final mirror before the AOL.

20. Your AOL 3D two photon microscope is now ready to use.