Development and application of a ray-based model of light propagation through a spherical acousto-optic lens

Geoffrey J. Evans, Paul A. Kirkby, K. M. Naga Srinivas Nadella, Bóris Marin, and R. Angus Silver
Department of Neuroscience, Physiology and Pharmacology, University College London, Gower Street, London, WC1E 6BT, UK

Abstract

A spherical acousto-optic lens (AOL) consists of four acousto-optic deflectors (AODs) that can rapidly and precisely control the focal position of an optical beam in 3D space. Development and application of AOLs has increased the speed at which 3D random access point measurements can be performed with a two-photon microscope. This has been particularly useful for measuring brain activity with fluorescent reporter dyes because neuronal signalling is rapid and sparsely distributed in 3D space. However, a theoretical description of light propagation through AOLs has lagged behind their development, resulting in only a handful of simplified principles to guide AOL design and optimization. To address this we have developed a ray-based computer model of an AOL incorporating acousto-optic diffraction and refraction by anisotropic media. We extended an existing model of a single AOD with constant drive frequency to model a spherical AOL: four AODs in series driven with linear chirps. AOL model predictions of the relationship between optical transmission efficiency and acoustic drive frequency including second order diffraction effects closely matched experimental measurements from a 3D two-photon AOL microscope. Moreover, exploration of different AOL drive configurations identified a new simple rule for maximizing the field of view of our compact AOL design. By providing a theoretical basis for understanding optical transmission through spherical AOLs, our open source model is likely to be useful for comparing and improving different AOL designs, as well as identifying the acoustic drive configurations that provide the best transmission performance over the 3D focal region.

1. Introduction

Spherical acousto-optic lenses (AOLs) are dynamic diffractive devices that can focus and steer an optical beam with high speed and precision. AOLs have applications in high-speed, 3D two-photon functional microscopy, and are increasingly popular in neuroscience [1–6]. The advantages of an AOL over a galvanometer-based system for laser scanning are the ability to shift the focus axially as well as laterally and high speed, inertia-free jumping between 3D spatial positions. This makes AOLs well-suited for imaging rapid neuronal signalling distributed in 3D space using activity-dependent fluorescent indicators.

* a.silver@ucl.ac.uk.

OCIS codes: (230.1040) Acousto-optical devices; (180.6900) Three-dimensional microscopy; (170.2655) Functional monitoring and imaging.
AOLs were initially conceived as cylindrical lenses [7] that used two acousto-optic deflectors (AODs), oriented to have their acoustic waves travelling in opposite directions (anti-parallel). Additionally, [7] proposed that a spherical AOL could be constructed from two orthogonal cylindrical AOLS, as is possible with long-focal-length conventional lenses. Three spherical AOL designs, based on two orthogonal cylindrical AOLS, have been developed: Two use relay optics between AODs resulting in a long optical path length [5, 8]. In contrast, our compact AOL design utilizes closely spaced AODs without relay optics in-between [9]. An advantage of the compact design is it can be added to an existing two-photon microscope with relative ease.

The focal position of an AOL is controlled by driving each AOD’s transducer with either constant or linearly chirped frequencies. The deflection angle $\theta$ of a light ray passing through an AOD depends on the acoustic frequency $F$, the optical wavelength in vacuum $\lambda$ and the acoustic velocity $V$ [8]:

$$\theta = \frac{\lambda F}{V} \quad (1)$$

When driven with constant frequencies, each AOD in an AOL deflects the optical beam by an angle proportional to the acoustic drive frequency—Fig. 1(a). When linearly chirped drives are applied they produce a gradient of acoustic frequencies across the AOD. This adds curvature to the optical wavefront because the light rays are diffracted by different angels across the AOD aperture—Fig. 1(b). Thus the strength of focussing increases with the steepness of the frequency ramp. Because acoustic waves propagate across the AOD, the drive frequency at the transducer must be continuously decreasing (or increasing) in order to maintain a constant frequency gradient. However, AODs operate within a finite frequency range, which imposes a ceiling on the duration of a linear chirp. To overcome this limitation and allow points to be imaged for an indefinitely long sequence of short intervals, AODs are driven with a series of frequency ramps (sawtooth) instead of a single, long, linear chirp. The ramp gradients for each of the four AODs in the compact AOL are calculated using the drive equations derived in [9].

A detailed discussion of the design and operating principles of our compact AOL and 3D two-photon AOL microscope (AOLM) is given in [9], which we briefly summarise here. Referring to the schematic of our AOLM in Fig. 2, the key features are as follows. A femtosecond laser beam passes through a double pass prism-based prechirper before reaching the compact AOL. The prechirper compensates for the temporal dispersion introduced by the four TeO$_2$-based AODs that make up the spherical AOL and the other optical components in the microscope. The AOL deflects and adds curvature to the optical wavefront, which is subsequently relayed to the back focal plane of the microscope objective by two 4f systems. The position of the focal point depends on the deflection and curvature added by the AOL. The green and red light emitted by the fluorophores (green lines in Fig. 2), following two-photon excitation, is collected by the microscope objective, separated from the near infra-red excitation light (red lines in Fig. 2) by a dichroic mirror and detected by a photo-multiplier tube.
The size of an AOLM’s imaging volume is of practical importance and depends on many factors. For example, a continuum of AOL drive configurations are possible for focusing at the same point. However, it is not feasible to experimentally explore all these factors in a brute force fashion and quantitative predictions have been difficult due to the lack of a theoretical model. To date, predicting AOL performance has relied on models of single AODs, the thin lens equation and geometrical considerations [5, 7–9]. The most detailed model of a single AOD was developed in [10] and relies on intensive numerical computations. Approximate closed form expressions for AOD diffraction efficiency and angular deflection are provided in [11]. The model developed in [12] extends [11] and considers two orthogonally arranged AODs. All three of these models assume the AODs are driven with constant frequency and this means they cannot be used to simulate a spherical AOL with finite focal length because focusing requires four AODs to be driven with linear frequency chirps.

To develop a quantitative description of optical transmission through a compact AOL we have developed an experimentally constrained model using a novel ray-based approach. This has allowed us to systematically explore how the different drive configurations affect optical transmission efficiency over 3D space and thus determine the imaging volume. Our model-based simulations indicate that imaging volume depends sensitively on how lateral deflection is divided between the first two AODs (X1 and Y1) and the last two AODs (X2 and Y2), which we have confirmed experimentally with a 3D two-photon AOLM.

2. Methods

2.1. Acousto-optic lens configuration

For the compact AOL design considered in this paper, the acoustic directions of the four AODs were (in the order that the optical beam passed through them) +x, +y, −x, −y, where x, y, z are the axes of our lab reference frame. The AODs are referred to X1, Y1, X2, Y2 respectively. The first two AODs (X1 and Y1) were wide transducer designs (about 3 mm) and the second two (X2 and Y2) were narrow (about 1 mm).

The AODs were made from paratellurite (TeO2), chosen for its slow shear acoustic mode which has a very high acousto-optic figure of merit [13]. To make use of the slow acoustic mode, all of the AODs had the transducer normal to ⟨110⟩ in the basis of the AOD’s crystal lattice vectors and the aperture was normal to the crystal’s optic axis ⟨001⟩ in the crystal lattice vector basis. The AODs had dimensions of 20 × 20 × 8 mm and were manufactured by Gooch and Housego.

The input optical beam was aligned with the z-direction (lab frame) and each AOD in the AOL was aligned at its Bragg angle (the angle for peak diffraction efficiency) corresponding to 39 MHz drive frequencies and 920 nm optical wavelength. There were narrow gaps between the AODs (5 cm from centre-to-centre) containing polarising units used to convert the optical polarisation to near-circular as required for optimal coupling into the desired AOD modes. The drive equations used were derived in [9].

Opt Express. Author manuscript; available in PMC 2016 March 18.
Lateral displacement of the focus by a spherical AOL can either be constant for the duration of a ramp, with a series of ramps causing the focus to jump discontinuously between points (pointing mode), or it can change continuously such that the focal point has a velocity (scanning mode). In this paper we have considered pointing mode exclusively. To achieve a constant lateral displacement over the duration of a ramp, the centre frequency of each acoustic drive ramp was offset. These offsets can be seen by comparing Fig. 3(a) with Figs. 3(b) and 3(c). The paths taken by the purple, cyan and orange rays in Fig. 3(d) (artificially coloured; all of 800 nm wavelength) correspond to the AOL being driven with the ramps shown in Figs. 3(a), 3(b) and 3(c), respectively. Note that the cyan and orange rays focus at the same point despite taking different paths through the AOL, illustrating that there is some freedom when choosing acoustic drive frequencies.

2.2. The base ray

We define the base ray [14], in the context of AOLs, as the ray that passes through the centres of all the AODs at the instant in time when the drive ramp centres coincide with the AOD centres (the AODs are laterally offset relative to each other in order for this to be possible). The base ray enables us to understand the degree of freedom available when choosing drive ramp centre frequencies. In Fig. 3(d) the base rays are drawn as solid lines whilst the neighbouring rays are dashed. Note the offset between the input and output base ray height corresponding to the lateral AOD offsets.

To drive an AOL, first the linear chirps are calculated from the drive equations, and then the centre frequencies are determined (the frequencies at the centre of the drive ramp). Our procedure for determining the centre frequencies is to trace the base ray through the AOL. At each AOD, the acoustic frequency, \( F \), encountered by the base ray is by definition the centre frequency of that AOD’s drive ramps. This enabled us to directly relate the AOL’s lateral focal displacement to the drive ramp centre frequencies.

AODs are manufactured to have a particular optimal frequency, \( F_0 \). For our AODs, \( F_0 = 39 \) MHz. By Eq. (1) this frequency corresponds to an optimal deflection angle, \( \theta_0 \), where

\[
\theta_0 = \frac{\lambda F_0}{V} \quad (2)
\]

By applying Eq. (1) to the base ray, we determined the relationship between the centre frequencies of each AOD (\( F_{X1}, F_{Y1}, F_{X2}, F_{Y2} \)) and the two lateral deflection angles, \( \phi_x \) and \( \phi_y \) (measured in radians from the centre of the last AOD to the focal point):

\[
\phi_x = \frac{\lambda}{V} \left[ (F_{X2} - F_0) \left( 1 + \frac{s}{z} \right) - (F_{X1} - F_0) \left( 1 + \frac{3s}{z} \right) \right] \quad (3)
\]

\[
\phi_y = \frac{\lambda}{V} \left[ (F_{Y2} - F_0) - (F_{Y1} - F_0) \left( 1 + \frac{2s}{z} \right) \right] \quad (4)
\]
where $s$ is the separation between the AODs, $z$ is the distance from the last AOD to the focal point. We have implicitly assumed that the AODs are operating in the $-1$ diffraction mode and small angle approximations are made throughout this paper.

Equations (3) and (4) for the spherical AOL are equivalent to two orthogonal cylindrical AOLs offset axially by distance $s$, the first aligned in the $x$-direction and the second in the $y$-direction. When the AODs in an AOL are all driven at $F_0$, there is no focusing ($z = \infty$) and the lateral angle is zero ($\phi_x = 0, \phi_y = 0$), in agreement with Eqs. (3) and (4). This is shown at the top of Fig. 3(e) where the base ray is first deflected down by $\theta_0$ at X1 and then back up by $\theta_0$ at X2 such that it is parallel with its initial direction.

Eq. (3) has two free parameters $F_{X1}, F_{X2}$ so there is a degree of freedom when choosing the centre frequencies for a cylindrical AOL. This can be understood as follows: if the drive frequency of the first AOD in a cylindrical AOL increases then increasing the drive frequency of the second will counteract this change by deflecting the base ray in the opposite direction. Eq. (4) also has a degree of freedom and therefore there are two degrees of freedom when choosing the centre frequencies for a spherical AOL.

### 2.3. Pair deflection ratio

In a cylindrical AOL, the relative angular deflections of the base ray at the first ($\delta \theta_1$) and second ($\delta \theta_2$) AODs is given by

$$\delta \theta_1 = \theta_0 - \theta_1 = \frac{\lambda (F_0 - F_1)}{V}, \quad \delta \theta_2 = \theta_2 - \theta_0 = \frac{\lambda (F_2 - F_0)}{V} \tag{5}$$

where the sign difference is due to the AODs being anti-parallel and the units are radians. These angular deflections are shown in Fig. 3(e).

To express the degree of freedom in terms of the frequencies $F_1, F_2$ experienced by the base ray, we define the pair deflection ratio (PDR) as the ratio of the base ray’s deflection at the first AOD to the deflection at the second AOD:

$$\text{PDR} = \frac{\delta \theta_1}{\delta \theta_2} = \frac{F_0 - F_1}{F_2 - F_0} \tag{6}$$

A spherical AOL has a PDR value for each of its two orthogonal cylindrical AOLs:

$$\text{PDR}_x = \frac{F_0 - F_{X1}}{F_{X2} - F_0}, \quad \text{PDR}_y = \frac{F_0 - F_{Y1}}{F_{Y2} - F_0} \tag{7}$$

To calculate drive frequencies for chosen values of PDR$_x$ and PDR$_y$, we solved Eqs. (3), (4) and (7) simultaneously for $F_{X1}, F_{Y1}, F_{X2}, F_{Y2}$.
We have explored how the performance of a compact AOL varies with different values of PDR using our ray-based computer model, and have compared these predictions with experimental measurements from an AOLM.

2.4. Computer model

We developed a computer model of a spherical AOL [15], where each of the four AODs is treated similarly to the approach developed in [12]. Our AOL model has extended the work of [12] in three important respects as detailed in Table 1. Time-varying drive frequencies were handled by dividing the optical beam into rays. At every AOD, each ray was approximated as a plane wave interacting with a constant frequency acoustic wave. The frequency of the acoustic wave was taken as the local, instantaneous frequency at the ray’s point of incidence with the AOD. The justification for this is that the optical field behaves locally as a plane wave [16], and the diffraction angle for each ray depends only on the local, instantaneous acoustic frequency [7]. Each ray can then be treated at each AOD with the model detailed in [11]. Thus, by dividing up our beam into \( N \) rays, we effectively replaced each non-constant drive frequency AOD with \( N \) (smaller) AODs with constant drive frequencies. For the purposes of this work we have found \( N = 25 \) to be a good trade-off between precision and computation speed.

For a given focal position, our model calculates the drive frequencies across each AOD for a sequence of times. To model light propagating through an AOL, a bundle of rays is generated and passed through each AOD. The energy of each ray is calculated after each AOD. We used our model to predict the two-photon fluorescence over a region of space by summing the energy of the rays after the last AOD and squaring the total to account for the quadratic dependence of two-photon excitation on optical power. This left an unknown constant of proportionality, which we removed by normalising all model predictions of fluorescence so that the peak has a value of 1.

Our computer model of an AOL is conceptually simple: rays propagate through each of the AODs and the intervening spaces of the AOL. For each AOD we modelled the following three processes

- Refraction into the AOD, accounting for the anisotropy of the AOD.
- Acousto-optic diffraction inside the AODs: calculation of the diffracted optic wavevector, diffraction efficiency and secondary diffraction effects (the diffracted wave rediffracting).
- Refraction out of the AOD, again, accounting for the anisotropy of the AOD.

To calculate the refraction in and out of each AOD, we used the values for refractive indices and optical activity for TeO\(_2\) given in [17]. For acousto-optic diffraction, the wavevector of the diffracted optic wave is calculated from the sum of the incident optic wavevector and acoustic wavevector. We calculated diffraction efficiency, \( \eta \), between neighbouring modes using Eq. (8) as described in [11]:

\[ \eta = \frac{1}{1 + \left( \frac{2 \sin^2 \theta}{\sin^2 \theta + \frac{1}{N}} \right)} \]
where $\lambda$ is the optical wavelength in vacuum; $n_o$ is the ordinary refractive index; $n_e$ is the extraordinary refractive index; $p$ is the elasto-optic coefficient; $S$ is the sound amplitude; $\Delta k$ is the wavevector mismatch (the diffracted optic wavevector plus the acoustic wavevector subtract the incident optic wavevector); and $L$ is the effective width of the transducer. The value of the elasto-optic coefficient, $p$, was taken from Appendix E of [11].

Following Section 2.5 in [12], we made approximations to account for secondary diffraction into the $-2$ mode by using the equation:

$$
\eta_1 = \eta_1 (1 - \alpha \eta_2)
$$

where $\eta_1$ is the diffraction efficiency into the $-1$ mode, corrected for secondary diffraction; $\eta_1$ is the diffraction efficiency from the $0$ mode into the $-1$ mode calculated using Eq. (8); $\eta_2$ is the simplified diffraction efficiency from the $-1$ mode into the $-2$ mode calculated using Eq. (8); $\alpha$ is a parameter that determines the strength of the secondary diffraction, estimated from experimental data and discussed in the next section.

Our model does not include polarisation effects. We neglect these because the optical polarisation inside the AODs depends only on the ray directions relative to the optic axis and these will not vary much. Our calculations indicate that ray angles to the optic axis (inside the AOD crystal) will vary between $2^\circ$ and $0^\circ$ with the input polarisation optimised for $1^\circ$ and output polarisation optimised for $0.6^\circ$. Using Fig. 1 of [18] we find the actual polarisation of rays varies between perfectly circular and $90\%$ ellipticity. The input polarisation for each AOD is configured to be nearly circular ($>95\%$ ellipticity). The polarisation mismatch will therefore be insignificant.

### 2.5. Constraining the computer model with experimental measurements of AOD properties

Because AOD optical transmission has a complex dependence on the transducer properties, we constrained our model with experimental measurements. To do this we recorded the optical power transmitted through an individual AOD over a range of incidence angles and drive frequencies.

To constrain the effective transducer width, $L$, we measured the dependence of diffraction efficiency on relative incidence angle for both narrow and wide transducer AODs, as shown in Fig. 4 (see Section 2.6 for description). We then inferred $L$ from the separation between the efficiency minima. The values of $L$ that gave the best match to the measured separation in the minima were $L = 1.15$ mm for the narrow transducer and $L = 3.25$ mm for the wide transducer.
We calculated the efficiency with which radio frequency (RF) electronic drive signals were converted into acoustic power (RF-acoustic coupling) by the AOD transducers over a range of drive frequencies, by fitting single AOD model predictions to experimentally measured efficiencies at the (frequency dependent) Bragg angle (see Section 2.6). Two wavelengths were used (800 nm and 909 nm) in order to check our calculated acoustic power was independent of optical wavelength. Holding the RF power at 1.5 W, we measured the $-1$ diffraction mode efficiency over a range of drive frequencies (Fig. 5 green and blue dots) and determined the acoustic powers that gave the best fit to model predictions (magenta triangles). We divided our inferred acoustic drive powers by the the RF power (1.5 W) to find the RF-acoustic coupling. The RF-acoustic coupling (magenta line) was interpolated between each experimentally measured frequency using a cubic spline and the interpolated coupling was then used in the model to calculate the $-1$ (blue and green lines) and $-2$ (red and cyan lines) diffraction efficiencies. This tuning procedure is weakly dependent on the value chosen for $\alpha$, which determines the diffraction efficiency into the $-2$ mode (red and cyan lines). We experimentally measured the $-2$ mode diffraction efficiency (red and cyan dots) and chose the value of $\alpha$ that gave the best fit. We found $\alpha = 0.5$.

Fig. 5 shows the RF-acoustic coupling efficiency data for the narrow transducer AODs. We also measured the properties of the wide transducer AODs and used the same process to infer the RF-acoustic coupling profile. Having found values for $L$, $\alpha$ and the RF-acoustic coupling for both AOD designs, we had constrained our spherical AOL model. The source code of our model (written in Python 2.7) is available online [15].

### 2.6. Experimental setup for measuring AOD efficiency

We used two distinct experimental set-ups. The first was for measuring AOD diffraction efficiency into the $-1$ mode and incidence angles. This setup was used to produce Fig. 4. The second was to measure diffraction efficiencies into multiple modes at two different wavelengths (but not incidence angle) and was used to produce Fig. 5.

The optical set up used to measure diffraction efficiency and incidence angle (Fig. 4) consisted of a red (785 nm) and a green laser (632 nm), which allowed us to simultaneously measure the orientation of an AOD and the power of the diffracted (or non-diffracted) beams — Fig. 6(a). A gimbal (Thorlabs GMB1) was used to control and measure angles. Rotating the gimbal by an angle, $\Theta$, deflected the green reflected beam (dashed) by $4\Theta$ and the red beam by $2\Theta$. When the AOD was rotated by an angle $\Theta$, the green focus on the screen shifted corresponding to the angle $2\Theta$. The gimbal could then be counter-rotated by $\Theta/2$ to cancel out the $2\Theta$ shift and the angle $\Theta/2$ could be read off the gimbal.

To measure the RF drive power, a power meter (Diamond Antenna SX-200) was used to measure the power transmitted to the AOD’s transducer. In all experiments, the RF power was fixed at 1.5 W at all drive frequencies. The angles in Fig. 4 were measured relative to one another and the peak was aligned to the Bragg angle from the model. The polariser ensured the input beam to the AOD was circularly polarised (the optimal polarisation for our AODs is near-circular).
The second setup, used to measure the diffraction efficiency into several modes (Fig. 5) at two different wavelengths (800 nm, 909 nm), was a simplification of Fig. 6(a) where the beam splitter was removed and the beam passed into the power meter via a spatial filter used to switch between diffraction modes. An unusual feature of this experiment was that we adjusted the incidence angle for each frequency to obtain the maximum diffraction efficiency. This was done to avoid frequency-biasing the efficiency. We used a Chameleon Ultra II (Coherent Inc.) laser for this experiment.

3. Results

3.1. Simulations of single AOD diffraction efficiency

Having tuned our model we used it to calculate how the diffraction efficiency of wide and narrow transducer AODs varied as a function of incidence angle and drive frequency for 920 nm optical wavelength and 1.5 W RF drive power—Figs. 6(b) and 6(c). The high efficiency region (white band) was less broad for the wide transducer AOD than for the narrow transducer, indicating that there was a smaller acceptance angle (range of incidence angles with high diffraction efficiency), as expected from previous work [9].

The simulations for the wide transducer AOD, shown in Fig. 6(b), had a high peak efficiency of over 85%. The Bragg angle (i.e. the angle corresponding to peak efficiency) increased with drive frequency and the angular range of high efficiency (acceptance angle) was under 1° and nearly constant between 20 and 50 MHz. For an incidence angle of around 2°, the drive frequency is limited to under 50 MHz. Below 28 MHz there is a 50% reduction in efficiency due to poor RF-acoustic coupling.

For the narrow transducer AOD, the simulations shown in Fig. 6(c) predicted that the acceptance angle was 2° at 39 MHz. However, the peak efficiency (in agreement with Fig. 5) was lower than that for the wide transducer AOD, being under 60%. As drive frequency increased, the acceptance angle narrowed and the Bragg angle increased. The broad dip in efficiency at around 28 MHz corresponds to the measured drop in transducer efficiency found experimentally (see Fig. 5).

3.2. Predictions of cylindrical AOL performance

Combining our diffraction efficiency simulations for wide and narrow transducer AODs together enabled us to identify drive configurations that maximised the transmission efficiency of a cylindrical AOL (a pair of anti-parallel AODs). Here, we describe how the pair deflection ratio (PDR; the ratio of lateral deflection between the first and second AODs) affects the size of lateral deflection that can be achieved whilst maintaining high transmission efficiency.

In our design of cylindrical AOL, the first AOD (X1) is the wide transducer design and the second (X2) is the narrow. The red dashed lines labelled by PDR value on Fig. 6(c) show how incidence angle to and drive frequency of X2 vary with lateral focal position, $\varphi_x$, at $z = \infty$. PDR = 0 and PDR = $\infty$ correspond to fixed incidence angle and fixed drive frequency, respectively. For PDR = 1, the line runs perpendicular to the ridge of high efficiency, while the value of $-0.4$ runs along the ridge, keeping largely within the high efficiency region. The
drive frequency of X1 also depends on PDR but the angle of incidence to X1 is fixed (at 2.1° for 920 nm optical wavelength).

A simplistic strategy to drive a cylindrical AOL would be to maintain optimal diffraction efficiency on X2. This requires PDR = −0.4 for our design of AODs. As an example, the green and blue dots in Figs. 6(b) and 6(c) show the points corresponding to the same focal position but with different PDR values (blue, 0; green, −0.4). As can be seen from the figure, PDR = 0 in this example has greater diffraction efficiency than PDR = −0.4 due to X1 losing efficiency sharply above 45 MHz (Fig. 6(b) green dot). Two points can be drawn from this example. Firstly, for our AOL, we can achieve larger lateral deflections with PDR = 0 than PDR = −0.4 whilst maintaining high transmission efficiency. Secondly, it would be beneficial if the efficiency ridge in Fig. 6(b) was vertical, which could be achieved by using an acoustically-rotated AOD design.

Our model also predicted that second order diffraction into the −2 mode occurs in X2 at certain drive frequencies and incidence angles. This effect is unwanted because it produces observable second-order ‘ghost’ images. A second-order ‘ghost’ image that appears when the X2 drive frequency is F will be identical to the ‘proper’ image found when the X2 drive frequency is 2F. Consequently, these faint ‘ghost’ images of objects appear displaced from their correct positions. Figure 6(d) shows the relationship between the ratio of the second order to the first order efficiency as a function of the incidence angle and drive frequency. The yellow line marks the AOD’s second-order boundary, below which second order diffraction becomes significant. To block second-order ‘ghost’ images we added a polariser, but at the expense a reduction in the AOL’s optical transmission efficiency in the region of 20%. An alternative would have been to avoid the second-order region of Fig. 6(d) by carefully choosing drive frequencies, but this would have restricted the field of view.

All the results discussed in this section for X1 [X2] in a cylindrical AOL apply equally to both X1 and Y1 [X2 and Y2] in a spherical AOL.

3.3. Spherical AOL field of view dependence on PDR

To understand how AOL performance varies with PDR we compared the predictions from our AOL model with experimental measurements of AOL efficiency using a 3D two-photon AOLM. To do this we predicted the intensity of two-photon fluorescence at z = ∞, An AOL focal position at z = ∞ corresponds to our microscope focusing at z_M = 0, where z_M is the axial position after an infinity corrected microscope objective relative to its nominal focal plane. To approximate the relationship between z and z_M for the post-AOL optical arrangement we used z_M = 140/z where z_M is in micrometres and z is in metres.

Fig. 7 compares the simulated fluorescence intensity (normalised) and experimentally measured intensity of a uniform fluorescent sample over the field of view (expressed as the x-axis and y-axis deflection angles, ϕ_x and ϕ_y) for a wide range of PDR values at an excitation wavelength of λ = 920 nm. The region of efficiency (ROE; white areas) predicted by our AOL model agreed closely across the full range of PDR values spanning +5 to −5. The peak fluorescence intensities are all at the centre because that point (ϕ_x = 0, ϕ_y = 0, z =
∞) corresponds to the AODs all being driven at the optimal frequency, $F_0$, and is independent of PDR.

Comparison of the top two rows shows that the ROE is bell-shaped and its area progressively increases as the PDR is reduced from +5 to 0.5. However, for PDR = 0 the shape of the ROE became more complex both in the model and in the experimental measurements (Fig. 7). The horizontal and vertical troughs at around −1 degree correspond to the efficiency dip for the narrow transducer seen in Fig. 5 at around 28 MHz. The central region is extended in the x-axis more than in the y-axis so the image does not have a diagonal line of symmetry. This was due to X2 and Y2 being aligned at slightly different angles and we confirmed this by observing X2 or Y2 could be rotated to restore the diagonal line of symmetry to the ROE (data not shown).

We extended our investigation to values below PDR = 0 to test the validity of our model. These results show close agreement between the results from the AOL model and the experimental measurements in this region. The value of −1 is missing because it is not physically achievable due to perfect cancellation of the X1 and X2 deflection. From Fig. 7 we anticipated that a PDR value between 0 and 0.5 would be best and found PDR = 0.3 gave the widest central ROE.

### 3.4. Dependence on axial position of spherical AOL region of efficiency

We next examined whether our AOL model could also predict the ROE when the AOL was used to focus above or below the nominal focal plane of the objective. This required driving each AOD with linearly chirped drive frequencies to add positive or negative curvature to the optical wavefront (PDR is defined at the temporal centre of a drive ramp so it is unaffected by chirps). Figure 8 compares the normalised predicted fluorescence intensity (top row) and experimentally measured fluorescence intensity (bottom row) over the field of view in three different focal planes. We use PDR = 0.3 here for its wide ROE. The shape of the ROE in the model and experimental measurements changed only subtly with axial position. The ROE expanded in the positive x-direction as z increased in both the model and experiment.

The ratio of fluorescence peaks from left to right in Fig. 8 for the model was 0.78 : 1 : 0.86, and for experiment was 0.55 : 1 : 0.70. The decrease in intensity as the focus moves away from the nominal focal plane is likely to arise from two factors. The first is that the microscope objective introduces spherical aberration as the focus shifts axially away from $z_M = 0$ m so the point spread function deteriorates, changing the fluorescence excitation. This effect is present in the experimental measurements but not in the model. The second effect, which is present in both model and experiment, is that AODs are driven with linearly chirped frequencies to shift the axial position. The gradient of the chirp is proportional to the distance from the nominal focal plane. As the distance increases, the range of frequencies across each of the AODs increases and progressively blurs the ROE, reducing the peak.

### 3.5. Dependence of fluorescence imaging volume on PDR

In order to evaluate how AOLM performance depends on the PDR value we imaged a uniform fluorescence preparation at a range of different focal depths using the AOLM. The volumes are shown as z-stacks in Figs. 9(a)–9(c). In Fig. 9(a) we use a large PDR value of 5,
corresponding to X1 and Y1 contributing most of the lateral deflection. The central plane 
\(z_M = 0\) is brightest and the area of the bright regions narrows slightly at the bottom. Figure 9(b) is for PDR = 0.3, corresponding to X2 and Y2 contributing most of the lateral deflection. The central plane is brightest again and the shape of the bright regions is nearly constant throughout the volume. Figure 9(c) is for PDR = −2, corresponding to X2 and Y2 contributing a negative amount of the lateral deflection and X1 and Y1 having to contribute over 100% of lateral deflection to compensate. The area of the bright regions is small in comparison and increases with \(z_M\).

From a series of these z-stacks we calculated the dependence of the AOLM imaging volume on PDR value, where we defined the imaging volume boundary as 60% of peak fluorescence intensity. The dependence of imaging volume on PDR is shown in Fig. 9(d). We observed a peak in imaging volume at PDR = 0.3 and a sharp fall in the vicinity of −1 (−1 is not physically possible as mentioned in Section 3.3). These results demonstrate the sensitivity of AOL performance on PDR and confirm our model’s prediction that using X2 and Y2 to contribute the majority of the lateral deflection is advantageous for our AOL design.

4. Discussion

We have developed an experimentally constrained ray-based model of light propagation through a spherical AOL that incorporates refraction and acousto-optic diffraction, and is able to handle chirped acoustic frequency drives for each of the four AODs. The close match between AOL model predictions and experimental results from a 3D two-photon AOL microscope (AOLM) confirm that the model reproduces the main properties of light propagation through the AOL over a wide range of acoustic centre frequencies and linear frequency chirps. We have used the AOL model to explore how AOL optical transmission depends on the amount of deflection contributed by the first and second AOD of each X or Y anti-parallel pair. The AOL field of view predicted by our model depends on the choice of this pair deflection ratio (PDR) and we find that a value of 0.3 maximised the volume for our compact AOL design. Measurements with a 3D two-photon AOLM confirmed these theoretical predictions and also revealed a peak in AOLM imaging volume for PDR = 0.3.

By providing a theoretical framework for understanding light propagation through a spherical AOL, our open source approach provides a powerful tool for improving the design and control of this rapidly developing 3D microscope technology.

Our ray-based approach builds on classical models of AODs, by generalising the drive frequency to include chirped drives and by combining AODs of different spatial orientations in series to form a spherical AOL. Our model was tuned to simulate a compact AOL [9], using AODs which had the optic axis normal to the aperture and the transducer aligned with the slow shear acoustic mode. In our model, the RF-acoustic coupling properties of our wide and narrow transducer AODs were experimentally inferred and we demonstrated that our model was able to correctly account for the wavelength-dependence of diffraction efficiency between 800 nm and 920 nm (Fig. 5). The RF-acoustic coupling inferred for our narrow transducer AOD has a similar form (frequency scale reduced) to the theoretical calculation shown in [12] Fig. 7 and is consistent with the transducer having a resonance around 40 MHz, which was specified in the design. Examining the field of view for different PDR
values was a rigorous test of our model, because each PDR value corresponds to a different cross-section through the frequency-incidence angle plane for the narrow transducer AOD—red lines in Fig. 6(c).

The model confirms the benefits of several of the design features of our current compact AOL design. For example, the diffraction efficiencies of the AODs X2 and Y2 depend on optical incidence angle (Fig. 4). Because the incidence angle of rays into X2 and Y2 vary with lateral focus deflection and focal length, a large acceptance angle is needed for a large imaging volume and so a narrow transducer AOD design is favourable [9]. In contrast, the incidence angles for X1 and Y1 are both fixed and the main requirement for X1 and Y1 are high peak efficiencies. A wide transducer AOD design requires less RF drive power to achieve high diffraction efficiency, which makes wide transducer designs suitable for the first two AODs in the AOL.

Additionally, our model and experimental measurements show that the way in which the AODs are driven strongly affects the size of the volume that can be imaged with an AOLM. The largest AOL field of view occurred at PDR = 0.3, which implies that deflecting predominantly with the last two AODs provides the best transmission efficiency characteristics for our compact AOL design. Fixing the value of PDR is a new simple rule that we can use to achieve a near-optimal field of view. Alternatively, optimal AOD drive frequency can be determined on a voxel by voxel basis. However, this ‘optimised frequency limits’ approach [19] is far more complicated to implement than simply fixing the PDR value.

In this study we have explored the properties of a spherical AOL in pointing mode (AOL focus is stationary). However, PDR is also applicable to other imaging modes including raster scanning mode where the focus moves continuously in y and is stationary in x. In this case, PDR = 0.3 for the X1,X2 pair would provide the largest imaging volume. The y-scan would be centred at $\phi_y = 0$ and so the Y1,Y2 pair would be driven at the optimal frequency, $F_0$, irrespective of PDR value.

A large field of view was achieved by the substantially more complex AOL design of [5]. The design of [5] differed to the compact design in that the AODs were acoustically-rotated, and a telecentric relay was used between Y1 and X2. They reported using only X2,Y2 to produce lateral deflections, which is PDR = 0 in the terminology introduced in this paper. Given the differences between the AOL designs, this seems reasonable and certainly not inconsistent with our results. It would be possible to extend our model to the acoustically-rotated AOD designs used in [5] by accounting for the following differences: large acoustic walk-off angle, thicker crystals (30 mm [5] versus 8 mm), and higher drive power (20 W [5] versus 1.5 W). Further to these extensions, experimental measurements for the AODs would be needed to constrain the model.

Although our ray-based model is able to reproduce the experimental data from our AOL, one limitation is that it is unable to calculate the microscope illumination point spread functions (PSF). The design of AODs we used had the benefit of introducing very little optical aberration into the system. Consequently, the ray-based model was well-suited to our
system. For modelling an AOL which produces significant optical aberration, it would be necessary to calculate the PSF in order to accurately predict two-photon excitation fluorescence. To do this, our model could be extended to calculate aberrations geometrically by using its ray-tracing capability. Alternatively the wave optics AOD model of [10] could be extended to a spherical AOL, which would naturally calculate PSFs, but it would be far more complex and would be computationally intensive.

We have developed an open source ray-based model of a spherical AOL [15]. This provides a theoretical framework that will help to improve the design and control of AOLs, thereby improving the performance of fast 3D two-photon imaging.

Acknowledgments

This work was funded by the ERC (294667), the UCL impact PhD programme and the Wellcome Trust. RAS is in receipt of a Wellcome Trust Principal Research Fellowship in Basic Biomedical Science (095667). BM is supported by the Brazilian agency CAPES. We thank George Konstantinou, Victoria Griffiths and Chiara Baragli for help setting up the experiments; Duncan Farquharson and Alan Hogben from the UCL Biosciences mechanical engineering workshop for the design and fabrication of mechanical components and Antoine Valera for comments on the manuscript.

References and links

1. Chiovini B, Turi GF, Katona G, Kaszás A, Pálfi D, Maák P, Szalay G, Szabó MF, Szabó G, Szadai Z, Káli S, Rózsa B. Dendritic spikes induce ripples in parvalbumin interneurons during hippocampal sharp waves. Neuron. 2014; 82(4):908–24. [PubMed: 24853946]
2. Froudarakis E, Berens P, Ecker AS, Cotton RJ, Sinz FH, Yatsenko D, Saggau P, Bethge M, Tolias AS. Population code in mouse V1 facilitates readout of natural scenes through increased sparseness. Nat. Neurosci. 2014; 17(6):851–7. [PubMed: 24747577]
3. Fernández-Alfonso T, Nadella KMNS, Iacaruso MF, Pichler B, Roš H, Kirkby PA, Silver RA. Monitoring synaptic and neuronal activity in 3D with synthetic and genetic indicators using a compact acousto-optic lens two-photon microscope. J. Neurosci. Methods. 2014; 222:69–81. [PubMed: 24200507]
4. Cotton RJ, Froudarakis E, Storer P, Saggau P, Tolias AS. Three-dimensional mapping of microcircuit correlation structure. Front. Neural Circuits. 2013; 7:151. [PubMed: 24133414]
5. Katona G, Szalay G, Maák P, Kaszás A, Veress M, Hillier D, Chiovini B, Vizi ES, Roska B, Rózsa B. Fast two-photon in vivo imaging with three-dimensional random-access scanning in large tissue volumes. Nat. Methods. 2012; 9(2):201–8. [PubMed: 22231641]
6. Duemani Reddy G, Kelleher K, Fink R, Saggau P. Three-dimensional random access multiphoton microscopy for functional imaging of neuronal activity. Nat. Neurosci. 2008; 11(6):713–20. [PubMed: 18432198]
7. Kaplan A, Friedman N, Davidson N. Acousto-optic lens with very fast focus scanning. Opt. Lett. 2001; 26(14):1078–80. [PubMed: 18049525]
8. Reddy GD, Saggau P. Fast three-dimensional laser scanning scheme using acousto-optic deflectors. J. Biomed. Opt. 2005; 10(6):064038. [PubMed: 16409103]
9. Kirkby PA, Nadella KMNS, Silver RA. A compact acousto-optic lens for 2D and 3D femtosecond based 2-photon microscopy. Opt. Express. 2010; 18(13):13721–45. [PubMed: 20588506]
10. Mihajlik G, Barócsi A, Maák P. Complex, 3D modeling of the acousto-optical interaction and experimental verification. Opt. Express. 2014; 22(9):10165–80. [PubMed: 24231720]
11. Xu, J.; Stroud, R. Acousto-Optic Devices: Principles, Design, and Applications. Wiley; 1992.
12. Maák P, Jakab L, Barócsi A, Richter P. Improved design method for acousto-optic light deflectors. Opt. Commun. 1999; 172(1–6):297–324.
13. Warner AW, White DL, Bonner WA. Acousto-optic light deflectors using optical activity in paratellurite. J. Appl. Phys. 1972; 43(11):4489.
14. Buchdahl, HA. An Introduction to Hamiltonian Optics. Courier Dover Publications; 1993.
15. Evans, GJ. Ray-based AOL model. github.com/SilverLabUCL/aol_model
16. Born, M.; Wolf, E. Principles of Optics: Electromagnetic Theory of Propagation, Interference and Diffraction of Light. CUP Archive; 1999.
17. Uchida N. Optical properties of single-crystal paratellurite (TeO2). Phys. Rev. B. 1971; 4(10): 3736–3745.
18. Yano T, Watanabe A. Acousto-optic figure of merit of TeO2 for circularly polarized light. J. Appl. Phys. 1974; 45(3):1243.
19. Kirkby, PA.; Nadella, KMNS.; Silver, RA. Methods and apparatus to control acousto-optic deflectors. (WO2011131933). 2011.
Fig. 1.
Action of a single acousto-optic deflector (AOD) on an optical beam (rays shown in red). Acoustic wavefronts shown in blue and optical wavefronts shown as dashed lines. (a) AOD transducer is driven with a constant frequency. The optical beam is uniformly deflected with no change in curvature (parallel rays remain parallel). (b) AOD transducer driven with a linear chirp (acoustic frequency changes linearly with position and time). The centre ray experiences the same local frequency as in (a) and is deflected by the same angle. However, the top ray experiences a higher frequency so it is deflected more and the bottom ray experiences a lower frequency so it is deflected less. The net effect is to introduce curvature (i.e. focus the beam) as well as deflection. The curvature is proportional to the frequency gradient.
Fig. 2.
Schematic diagram of a 3D two-photon AOL microscope (AOLM). Mirrors and lenses shown in grey. All other components coloured and labelled. The crossed mirrors to the right of the laser are at different elevations. The laser is a femtosecond pulsed laser suitable for two-photon excitation. The prechirper comprises two prisms and a mirror used to add temporal dispersion to the laser beam. The intensity of the laser beam is controlled by the Pockels cell. The AOL is shown as four AODs (X1, Y1, X2, Y2) with their acoustic directions indicated in black. Polarising units placed before the AODs are not shown. The beam is relayed by two 4f systems from the AOL to the back focal plane of the microscope objective. The position of the focal point inside the imaging volume is precisely and rapidly controlled by the AOL. Green lines indicate two-photon fluorescence that is detected by the photo-multiplier tube (PMT). The mirror to the right of the objective is dichroic.
Fig. 3.
(a)–(c) Sequences of acoustic frequency ramps used to drive the four AODs that make up an AOL. The colour indicates which AOD the ramp is for: X1 blue; Y1 red; X2 green; Y2 black. The ramps cause the AOL to focus at (a) (0,0,1); (b) (0.01,0,1); (c) (0.01,0,1) m. Note the resulting focal lengths of the AOL are the same ($z = 1$ m) for (a)–(c) because the ramp gradients are unchanged. (d) Comparison of light ray paths through the AOL (purple, cyan and orange) when driven with the ramps shown in (a), (b) and (c), respectively. Note the cyan and orange rays take different paths but focus at the same point. The short grey lines
indicate AOD z-positions. (e) Base ray passing through a pair of anti-parallel AODs to illustrate Eqs. (5) and (6).
Fig. 4.
Tuning the AOD transducer width parameter in our model by fitting experimentally measured separations between efficiency minima. Model simulations following tuning (solid lines) and experimental measurements (dots) for AOD diffraction efficiency into the $-1$ mode against incidence angle. Plots are for two different AODs with transducer widths of (a) 1.15 mm and (b) 3.25 mm. Optical wavelength was 785 nm; acoustic frequency 39 MHz; RF drive power 1.5 W.
Fig. 5.
Tuning the RF-acoustic coupling and second-order diffraction coefficient, $\alpha$, in our model for the narrow transducer AODs. The diffraction efficiencies of $-1$ and $-2$ modes measured at the $-1$ mode Bragg angle (adjusted for each frequency) are plotted against drive frequency, for two different wavelengths. The green and blue dots are experimentally measured efficiencies into the $-1$ mode at 800 nm and 909 nm optical wavelengths respectively. The cyan and red dots are experimentally measured efficiencies into the $-2$ mode at 800 nm and 909 nm optical wavelengths respectively. The magenta triangles are the conversion efficiency of RF drive signal into acoustic power (RF-acoustic coupling) inferred from the experimental measurements by our model. The magenta line is used by the model to interpolate the RF-acoustic coupling between the magenta triangles. The green, blue, cyan and red lines are the model predictions, using the inferred RF-acoustic coupling, corresponding to the experimentally measured quantities.
Fig. 6.
Experimental set-up and single AOD simulations. (a) Schematic diagram of the
experimental set-up for measuring the incidence angle and efficiency of an AOD. Optical
paths of red and green laser light shown as solid lines. Reflected green beam shown as
dashed line. Components are coloured as follows: beam splitter, yellow; lenses, blue;
mirrors, dark grey. (b) Simulated diffraction efficiency of a wide transducer AOD into the −1
mode. (c) Simulated diffraction efficiency of a narrow transducer AOD into the −1 mode.
Dashed red lines mark out different PDR values if the AOD was used as X2 (or Y2) in an
AOL. (d) Simulated diffraction efficiency of a narrow transducer AOD into the −2 mode as a
fraction of the −1 mode efficiency. Optical wavelength was 920 nm in (b)–(d). The dotted
yellow lines in (c) and (d) indicate the AOD’s second-order boundary. Blue and green dots
on (b) and (c) indicate frequency and incidence angle for X1 and X2 in a cylindrical AOL
focussed at $\phi_x = 1^\circ$, $z = \infty$ with PDR = 0 and PDR = −0.4 respectively.
Fig. 7.
Comparison of simulated fluorescence intensity over the field of view (model; first and third rows) with experimentally measured fluorescence intensities (experiment; second and fourth rows) for the PDR values 5, 2, 1, 0.5, 0, −0.5, −2, −5 (shown in bold). Fluorescence intensity values were normalised to the peak value. Excitation wavelength 920 nm. Each plane is described by the AOL’s lateral deflection of the optical beam, x-angle and y-angle—see Eqs. (3) and (4).
Fig. 8.
Comparison of normalised fluorescence intensity from experiment and simulations for PDR = 0.3. Three focal planes are shown: $z = -0.5$ m, $z = \infty$, $z = 0.5$ m. The optical wavelength was 920 nm.
Fig. 9.
AOLM z-stacks for (a) PDR = 5, (a) PDR = 0.3 and (b) PDR = −2. Fluorescence intensity in arbitrary units. (d) Relationship between AOLM imaging volume and PDR. AOLM imaging volume boundary defined as 60% of peak fluorescence. The peak is at PDR = 0.3. The yellow, green and blue dots identify the volume shown in three z-stacks (a)–(c) on the plot (d). Optical wavelength 920 nm.
Table 1
Comparison of our computer model with the model detailed in [12].

| Maák 1999 Model | Our Model          |
|-----------------|--------------------|
| 2 AODs          | 4 AODs             |
| constant frequency | time-varying frequency |
| efficiency only | efficiency and ray tracing |