Remote focusing in confocal microscopy by means of a modified Alvarez lens

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Key words. Alvarez lens, confocal microscopy, diffractive optics, fast tunable lens, image scanning microscopy, life cell imaging.

Summary
Alvarez lenses are actuated lens-pairs which allow one to tune the optical power by mechanical displacement of subelements. Here, we show that a recently realized modified Alvarez lens design which does not require mechanical actuation can be integrated into a confocal microscope. Instead of mechanically moving them, the sublenses are imaged onto each other in a 4f-configuration, where the lateral image shift leading to a change in optical power is created by a galvo-mirror. The avoidance of mechanical lens shifts leads to a large speed gain for axial (and hence also 3D) image scans compared to classical Alvarez lenses. We demonstrate that the suggested operation principle is compatible with confocal microscopy. In order to optimize the system, we have drawn advantage of the flexibility a liquid-crystal spatial light modulator offers for the implementation. For given specifications, dedicated diffractive optical elements or freeform elements can be used in combination with resonant galvo-scanners or acousto-optic beam deflectors, to achieve even faster z-scans than reported here, reaching video rate.

Introduction
Life cell imaging is striving for fast 3D-scans in order to provide high spatiotemporal resolution of volumetric data. This is important because certain dynamics of living specimens such as the activities of muscle cells or the firing of neurons occur at short timescales (Göbel et al., 2007; Cella Zanacchi et al., 2011; Jones et al., 2011; Planchon et al., 2011; Botcherby et al., 2012).

Apart from the life-sciences, the ability to steer a focus rapidly in three dimensions is also desirable for the investigation of materials, profilometry or machine vision applications. While lateral 2D-scans can achieve video rate by employing resonant scanners or acousto-optic deflectors, a sufficiently fast modulation of the axial focus position is still challenging. Partly in response to this specific demand, in recent years several technologies have been developed to tune the optical power of lenses (Berge, 2005; Ren et al., 2006; Bernet et al., 2013) or Micro-Electro-Mechanical System mirrors (Qi et al., 2004; Shain et al., 2017). Some of these systems, like the tunable polymer-filled lenses (Ren et al., 2006; Jabbour et al., 2014; Nakai et al., 2015) or lenses based on electro-wetting (Berge, 2005) are now available. However, these systems possess a minimal response time of more than several milliseconds, which is inadequate for high-speed recording. Only acousto-optic lenses (Kaplan et al., 2001; Mermillod-Blondin et al., 2008; Konstantinou et al., 2016), whose phase front modulations are generated by ultrasonic waves, can achieve refocusing rates above 100 kHz. The main drawbacks of these lens types are that they can either be only operated in an oscillating mode (such as the tunable acoustic gradient index (TAG) lens) or have a fixed focusing speed which is determined by the velocity of the travelling acoustic wave (Konstantinou et al., 2016). In contrast, the modified Alvarez lens of this work enables both, arbitrary tuning of the optical power with a response time in the submillisecond range as well as oscillating focus tuning with a frequency of up to 10 kHz (realized by resonant galvo-scanners).

The tunable lens system used here is optimized for high refocusing speed, as has already been demonstrated in an earlier paper (Bawart et al., 2017). It is based on a general principle that was invented by Luis Alvarez in the 1960s (Alvarez, 1964), where modulation of the optical power is achieved by shifting two complementary cubic phase plates in opposite direction (see Fig. 1). The resulting ‘lens’ generates a parabolic phase profile that scales linearly with the lateral displacement of the plates. Due to the parabolic phase profile of the Alvarez lens, it is mainly suited for low-NA applications (spherical phase profiles are not realizable). We would like to emphasize, however, that this restriction is shared by almost all remote focusing methods. An exception is the system devised by the Wilson group (Botcherby et al., 2007), which uses two objective lenses in a specific configuration.
The Alvarez lens in its original form has found application in numerous scientific and technical devices (Humphrey, 1974; Humphrey, 1976; Mukaiyama et al., 1994; Rege et al., 2004; Simonov et al., 2006; Van Der Heijde, 2006; Spivey, 2008).

Modified Alvarez lens

In the modified Alvarez lens, the two sublenses are not in close vicinity but are instead imaged onto each other via a 4f-lens-configuration (see Fig. 2A). Additionally, a beam deflecting element is placed into the pupil plane of the telescope (Fourier plane) where a beam deflection manifests itself as a lateral shift of the image of sublens 1.

This deflection can also be accomplished by a rotatable mirror. Then, only a single Alvarez sublens is required which is imaged onto itself (see Fig. 2B). When the mirror is rotated, the image of the phase plate is shifted laterally, resulting in the phase profile of a parabolic lens with an optical power proportional to the displacement $x_0$ (see Eq. (1)). Incidentally, the centre of the resulting lens moves away from the optical axis by half of the lateral shift $x_0$ (beam ‘walk-off’, see Fig. 2A). In case the lens is used for a multidimensional confocal image scan, this offset can be compensated by a subsequent beam deflection unit (which scans, e.g. in x-direction).

The optical power $D$ of the Alvarez lens is calculated as follows (see (Bawart et al., 2017) for derivation):

$$ D = \frac{A_0}{\pi} x_0 \text{ for } |x_0| < L. $$

Here, $\lambda$ describes the wavelength and $A$ is a design parameter of the lens, which depends on the curvature of the lens and thus determines its maximally attainable optical power. In case of a diffractive sublens, $A$ depends on its minimal feature size $p$ (which is – in case of an SLM – the pixel size) and its maximal radius $r_{\text{max}}$:

$$ A_{\text{max}} = \frac{\pi}{p \cdot r_{\text{max}}^2}. $$

The lateral lens shift $x_0$ depends on the mechanical galvo-mirror rotation angle $\alpha$ and on the focal length $f_A$ of the lenses in the 4f-configuration:

$$ x_0 = f_A \tan(2\alpha). $$

In the previous publication, it was shown that a circular aperture (with a diameter of $2L/3$; $L$ being the x-dimension of the Alvarez sublens) should be placed in a plane conjugated to the Alvarez sublens in order to optimize the adjustable optical power range (see Fig. 3). This limits the maximal sublens shift to $L/3$.

Alternatively to an additional beam deflection unit, the ‘walk-off’ can also be cancelled by guiding the modulated light through another 4f-lens-configuration with an additional galvo-scanner in its Fourier plane (see Fig. 3). If this second 4f-setup is equipped with lenses of half the focal length ($f_B = f_A/2$) the lateral shift $x_1$ of the modulated beam generated by the galvo-scanner is also half of the previous shift $x_0$ and of opposite sign:

$$ x_1 = f_B \tan(-2\alpha) = -\frac{f_A}{2} \tan(2\alpha) = -\frac{x_0}{2}. $$

Therefore, this second shift exactly compensates the beam ‘walk-off’ illustrated in Figure 2. The same strategy has been used for an all-optical implementation of ‘pixel-reassignment’ (Roth et al., 2013), a resolution-improving confocal imaging technique (Sheppard, 1988; Müller & Enderlein, 2010).

Experimental setup

We integrate the modified Alvarez lens into a reflection confocal microscope for enabling fast axial scans, as shown in Figure 4.

In case the Alvarez sublens is realized with a diffractive optical element (DOE) or a refractive phase plate, the Alvarez setup shown in Figure 3 can directly be integrated into a confocal microscope. In our proof-of-principle experiment, the phase modulation was actually realized by a reflective phase only SLM for visible light (Hamamatsu X10468-07, Hamamatsu.

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1. Humphrey, 1974.
2. Humphrey, 1976.
3. Mukaiyama et al., 1994.
4. Rege et al., 2004.
5. Simonov et al., 2006.
6. Van Der Heijde, 2006.
7. Spivey, 2008.
Fig. 2. Outline of modified Alvarez lens configurations: (A) Alvarez lens where the sublenses are imaged onto each other via a 4f-configuration. As shown in the subfigure, the centre of the quadratic phase profile appears off the optical axis by an amount proportional to the deflection angle. (B) Folded concept of a modified Alvarez lens where only a single sublens is required, since it is imaged back onto itself by reflection at a rotatable mirror.

Folded setup with “walk-off“-correction:

Fig. 3. ‘Walk-off’-correction in a modified Alvarez lens by guiding the modulated beam through a second 4f-setup with lenses of halffocal lengths and onto the galvo-mirror in its pupil plane. The numbered arrows in the sketch serve for better traceability of the beam paths.
Fig. 4. Scheme of a confocal microscope for axial- or 3D image scans. The illumination light is guided via a beam splitter (BS) towards the tunable (Alvarez-) lens where it is modulated and finally focused by the microscope objective onto the sample. The reflected light again enters the tunable lens where it experiences the same optical power and is thus collimated towards the tube lens. This lens focuses the light onto a pinhole with attached photomultiplier tube (PMT).

Fig. 5. (A) Section through the acquired PMT signal of the axial responses for two different axial (z-) mirror positions (at a distance of 10 μm, specified by objective shift). The size of the pinhole (0.1 mm) is chosen to be about 1.7 times the size of the Airy-disc (i.e., 60 μm). From the centre of mass positions of these intensity distributions, the scaling in the z-direction and thus a full-width-half-maximum (FWHM-) value of 9.4 μm can be derived (FWHM-average for both mirror positions). The ideal mirror response is shown in red and has a FWHM-value of 10.3 μm. (B) Axial responses at 6 further mirror positions are shown in addition to the original 2. For higher optical powers of the modified Alvarez lens the detected signal strength decreases and the influence of aberrations gets more prominent. The stated numbers reflect the percentage of the signal strength in relation to the maximal peak.

with our modified Alvarez setup and for lateral scanning of the beam.

Experimental results

Determination of the mirror response of a confocal Alvarez lens

To characterize the confocal Alvarez setup, its reflection response to a mirror is measured in a x-z-scan. Each pixel of the scan-image displays the light intensity detected by the PMT for a specific galvo-scan position (angle). In case of a mirror such a scan resembles a thickened line (see inset in Fig. 5A) whose profile reflects the axial resolution when scanning through interfaces. In Figure 5 experimentally determined sections through these profiles for several axial (z-) mirror positions are shown.

For central mirror locations (where the optical power of the modified Alvarez lens is low), the detected axial responses correspond well with the theoretically predicted distributions (calculated by scalar beam propagation; see red curve in Fig. 5A). In particular, the extent of the responses is in the range of the diffraction limit (FWHMexp = 9.4 μm ≲ 10.3 μm = FWHMsim). The fact that the experimental FWHM is slightly better than the simulated one is most likely due to inaccurate identification of the scaling-factor between the adjusted and measured mirror position. The scaling-factor was determined as follows: The step size (in z-direction) in the inset of Figure 5(A) was evaluated by shifting the microscope objective for 10 μm and determining the distance of the mirror response,
e.g. by cross-correlation of the responses (mirror responses were recorded within a short time to minimize position drift of the objective). The positioning accuracy of the objective is in the range of 100 nm and thus significantly below 1 μm (i.e. below the deviation between the measured and theoretical FWHM-value of the mirror response = 0.9 μm). From the step size, the FWHM-value of the mirror response itself can be calculated (by interpolation).

For higher optical powers, aberrations get more pronounced and the detected integral signal falls off considerably. To detect the outermost mirror responses depicted in Figure 5(B) (curves in green), a sublens shift \( x_0 \) of around 2.8 mm is required (i.e. a mechanical galvo twist angle of 0.16°, see Eq. (3): \( f_A = 500 \) mm). As the step response (0.1°) of the used galvo-scanner is 250 μs, the lens system could be driven with a frequency of approximately 1 kHz in this configuration. In our actual experiment the Alvarez lens was effectively tuned with a frequency in the order of 10 Hz. To increase scanning speed, the required electronic triggering implementation would have to be more sophisticated. The amount of lens displacement \( x_0 \) to produce a certain axial focus shift, depends, on the one hand, on the lens design parameter \( A \) which is chosen to be half of the maximal value \( A_{\text{max}} \) (see Eq. (2); i.e. \( A = 7.854 \cdot 10^8 \text{m}^{-1} \)), and on the other hand, on the properties of the employed microscope objective (20×, NA = 0.4). The largest occurring sublens displacement in our experiments is 4.4 mm (i.e. less than the maximal displacement 5.33 mm = \( L/3 \)) and therefore the scanning range is 114 μm (see Fig. 5B).

One reason for the signal drop at larger defocus values is the reduced diffraction efficiency of the SLM at the edge of the hologram due to higher phase gradients in these regions (there the phase pattern features merely 4 levels with a diffraction efficiency of around 70%). This effect gets more pronounced for higher optical powers since hologram areas with large phase gradients (lower diffraction efficiencies) represent an increasing portion of the effective lens area (see Fig. 2). Nevertheless, our simulations (based on Fourier propagation of the electric field) reveal that the associated overall efficiency modulation is in the range of less than 10% (due to the small sublens shifts).

The remaining signal loss can most likely be attributed to the imperfect alignment of the reflected beam with the centre of the galvo-mirror (in lateral and axial direction), because then the described ‘walk-off’ correction is not perfect. Hence the focus of lens C modulates proportional to the optical power in both, the lateral direction (due to the imperfect imaging properties of lens C) as well as in axial direction (due to the axial out of focus position of the galvo-mirror). As the pinhole in front of the PMT exhibits a diameter of 0.1 mm, it is possible that even a small focal ‘walk-off’ leads to a reduction of detected signal. Additionally, the reflected signal inherently inherits a ‘walk-off’ proportional to the optical power, which also lowers the signal. This fact can be understood by following the pathway of the reflected light from the sample through the modified Alvarez setup shown in Figure 7 towards the PMT. One immediately recognizes that the sequential order of optical components the reflected light travels through differs from the order of components for the illumination light. The reflected signal first impinges on the galvo-mirror (not on the SLM – as the illumination light) where it is deflected and thus shifted by an amount of \(-x_0/2\) on the SLM surface. There the light reflects and is guided again to the galvo-mirror which causes a deflection that results in a relative displacement of the imaged phase plate by \( x_0 \). Therefore, the modulated, reflected beam is always shifted off the optical axis by an amount of \( x_0/2 \). For all but a perfect tube lens C, this results in a lateral focal shift at the detector. Obviously, one could also go through the effort to guide the reflected beam through a second, adapted, Alvarez setup in order to avoid this ‘walk-off’. 

**Imaging of a diffractive surface with the confocal Alvarez lens**

In a second experiment, a certain surface part along a line (see Fig. 6A) of a DOE (Bernet et al., 2013) is scanned with the setup shown in detail in the Appendix. This DOE consists of fused silica (with a refractive index of \( n = 1.46 \) at \( \lambda \)). In the scanned area the surface represents a four-level stepped structure with a step height of 350 nm corresponding to a maximal structure depth of 1.051 μm (verified by atomic force microscopy data). The result of the x-z-scan is shown in Figure 6(B).

The x-z-scan of the DOE surface in Figure 6(B) shows good agreement with the expected height structure of the diffractive element (continuous white line). The axial response of the individual steps is approximately diffraction limited (around 1 μm compared to the ideal FWHM-value of 997 nm). As we operate the lens with little optical power, there are almost no brightness modulations between the individual structure levels noticeable (compared to the mirror responses depicted in Fig. 5B).

In our proof-of-principle experiments, the phase modulation of the Alvarez sublens is generated by an SLM which drastically limits the optical power and therefore the axial scanning range. DOEs, in contrast, would offer an approximately 20 times higher optical power range (due to the 20 times smaller feature size) and are thus much more apt for dynamic measurements. Furthermore, SLMs possess diffraction efficiency of less than 90%, and since the light passes the SLM-hologram four times, this has a great impact on the overall efficiency. Both disadvantages could be avoided by employing Alvarez lenses produced as dedicated DOEs or as refractive freeform elements.

To avoid aberrations in our modified Alvarez lens it has to be ensured that the diffractive phase pattern is imaged exactly onto itself (which we have achieved to a good degree). In the case of refractive phase plates, the relatively large thickness leads to aberrations which can be corrected to a certain degree by adapting their surface functions. In case of
thin DOEs (our SLM represents a thin diffractive element to a good approximation), a parabolic phase front is actually generated, which is free of aberrations for paraxial beams and low NAs.

Conclusions and outlook

We have proposed and demonstrated the applicability of a modified Alvarez lens not requiring mechanical actuation for fast $z$-scanning in confocal microscopy. Currently, using a LC-SLM as phase modulator to emulate the Alvarez-lens we have achieved an optical power range of $\pm 0.45$ diopters with a potential scanning speed of around 1 kHz. We have determined the response of a mirror as well as imaged the surface of a stepped DOE. For low optical powers, a diffraction limited resolution is attained, for larger powers aberrations get more prominent and moreover the response signal drops off considerably.

The method has the potential to perform volume scans at video rate: By slightly adapting the setup, the galvo-scanner can be substituted by an acousto-optic beam deflector to further increase axial scanning speed. Moreover, the SLM, which was used because of its flexibility in developing and optimizing the approach, can be replaced by a DOE of substantially smaller structure size (e.g. 0.5 $\mu$m), which will significantly increase the available $z$-scan range. If a refractive freeform element is used as an Alvarez element, white light applications become accessible without fundamental limits concerning the light throughput of the setup. With these extensions the approach will become ideally suited for the investigation of dynamic objects in low-NA confocal microscopy.

Appendix

In order to keep the paper conceptually simple, we have summarized the specific details that are only necessary for the implementation with a LC-SLM in this Appendix. The complication arises from the polarization sensitivity of the SLM which prevents one from using both polarization channels in a straightforward way: Using a nonpolarizing beam splitter as shown in the original setup (Fig. 3) 50% of the illumination light modulated by the Alvarez lens is reflected towards the laser source/detector. But the light reflected by the sample is also be directed to the laser source/detector as it travels exactly the same path as the illumination light but in opposite direction. Since both signals possess the same polarization, they are not separable by the beam splitter of the confocal setup shown in Figure 4 (which decouples light of the illumination path from light of the detection path). Consequently, modulated light of the illumination path would enter the detector.

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In order to prevent this, the nonpolarizing beam splitter of our original setup of Figure 3 has to be avoided. Our solution to the problem is shown in Figure 7, where the galvo-mirror does not back-reflect the impinging light.

In the amended setup, laser light with a wavelength of 640 nm is guided through a 50/50 beam splitter and a mirror to illuminate the entire active SLM surface (phase structure visible in Fig. 2). A polarizer in the beam path prepares the linear polarization required for the phase-only SLM. In an experimental fluorescent microscopy implementation, it would be more convenient to place the polarizer between SLM and beam splitter to also align the polarization of the (possibly) depolarized beam coming from the sample (e.g. in case of fluorescence measurements or if high-NA objective lenses are employed). The depolarization of the returning beam is responsible for a further efficiency loss in case a modified Alvarez lens is operated by an SLM. The lenses A₁ and A₂ with same focal length \( f_A = 500 \text{ mm} \) form the first 4f-configuration to image the phase structure onto itself. The galvo-mirror (Cambridge Technology 6220H, Bedford, MA, USA; with broadband antireflection-coating) is situated in the Fourier plane of the phase plate, thus a rotation of the mirror leads to a lateral displacement of the imaged sublens and hence to a change in optical power of the composite system. The required dimension of the galvo-mirror depends on the maximum phase gradient of the Alvarez sublens as it determines the maximal diffraction angle and thus the extent of the Fourier image. For a first-order diffractive lens (with a maximal phase shift of \( 2\pi \)), this gradient is characterized by the minimal structure size \( s = 80 \mu \text{m} \) in our case). The employed galvo-mirror offers a clear aperture of 10 mm which is enough for our setup as can be seen by calculating the diameter of the Fourier image:

\[
d_r = 2 f_A \tan^{-1}(\lambda/s) = 8 \text{ mm}
\]

In order to correct for the beam ‘walk-off’, the modulated laser beam passes another 4f-configuration consisting of lenses \( B_1 \) and \( B_2 \) with the same focal length \( f_B = f_A / 2 \). This 4f-setup is designed such that the same galvo-mirror is situated again in the Fourier plane. An aperture with a diameter equal to the minimal overlap \( d = 2L/3 = 10.7 \text{ mm} \) is located at a distance \( f_B \) from lens \( B_2 \) (conjugated to the SLM) to block unmodulated light and consequently aberrations resulting from variations of the effective lens area. The modulated light passes through several 4f-lens-configurations until it reaches the microscope objective where it is focused onto a reflective sample. The light is reflected all the way back towards the laser source. The setup can also be used for fluorescence imaging, however, in this case a freeform Alvarez element is advisable, due to the dispersion of diffractive structures. Before the reflected light reaches the source it is branched off at the 50/50 beam splitter and focused on the pinhole of the photomultiplier (Hamamatsu H5784-20, Hamamatsu, Japan) via a 400-mm lens. The diameter of the pinhole is selected to be 0.1 mm in order to (approximately) meet the lateral extension of the focus of the tube lens \( d_T \equiv 60 \mu \text{m} \).
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