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Katrin Philipp, Florian Lemke, Matthias C. Wapler, Nektarios Koukourakis, Ulrike Wallrabe, Jürgen W. Czarske

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Axial scanning and spherical aberration correction in confocal microscopy employing an adaptive lens

Katrin Philipp^a, Florian Lemke^b, Matthias C. Wapler^b, Nektarios Koukourakis^a, Ulrike Wallrabe^b, and Jürgen W. Czarske^a

^aTechnische Universität Dresden, Laboratory for Measurement and Sensor System Techniques, Helmholtzstraße 18, 01069 Dresden, Germany

^bLaboratory for Microactuators, Department of Microsystems Engineering-IMTEK, University of Freiburg, Georges-Köhler-Allee 102, 79110 Freiburg, Germany

ABSTRACT

We present a fluid-membrane lens with two piezoelectric actuators that offer versatile, circular symmetric lens surface shaping. A wavefront-measurement-based control system ensures robustness against creeping and hysteresis effects of the piezoelectric actuators. We apply the adaptive lens to correct synthetic aberrations induced by a deformable mirror. The results suggest that the lens is able to correct spherical aberrations with standard Zernike coefficients between 0 μm and 1 μm , while operating at refractive powers up to about 4 m^{-1} . We apply the adaptive lens in a custom-built confocal microscope to allow simultaneous axial scanning and spherical aberration tuning. The confocal microscope is extended by an additional phase measurement system to include the control algorithm. To verify our approach, we use the maximum intensity and the axial FWHM of the overall confocal point spread function as figures of merit. We further discuss the ability of the adaptive lens to correct specimen-induced aberrations in a confocal microscope.

Keywords: adaptive optics, tunable lenses, confocal microscopy, aberration correction

1. INTRODUCTION

The need to shift the measurement volume in relation to a specimen under investigation is a long-standing objective in the imaging community particularly for microscopic applications. Conventionally, a motorized stage system is used to move either the specimen itself or optical elements such as the objective. Due to an increased number of applications that require scanning rates in the kHz range, piezo driven movement of the objective became the norm for high-speed depth-resolved measurements. However, this comes with its own challenge: The use of advanced adaptive optics approaches, such as aberration correction^{1–4} and scattering compensation^{5,6} is hampered by moving the objective, as the required mapping from a beamforming device onto the objective varies with the same rate as the focal plane movement. In contrast, adaptive lenses enable inertia-free axial scanning of the sample. Here, the axial position of the objective is fixed and, thus, a beamforming device can be mapped statically onto the back-aperture of the objective. As a consequence, electrically tunable lenses have been employed for axial scanning in several microscopic techniques such as confocal microscopy,^{7–10} 2photon microscopy,^{11,12} structured illumination microscopy,^{13–18} SPIM^{19,20} as well as standard widefield microscopy.^{21–23} A remaining challenge, however, is that the static elements of the microscope can only be optimized for a single design focal length of the tunable lens. Hence, standard tunable lenses induct aberrations to the optical system when operated at different focal lengths. To overcome this problem, we propose a hybrid actuation adaptive fluid-membrane lens with tunable spherical aberrations. An additional support structure ensures the minimization of mechanical forces to the glass-membrane. By using two piezo-actuators placed on top and below the membrane, two degrees of freedom are available to adjust the lens surface. To compensate the creep- and rate-dependent hysteresis effect of the piezoactuators, we implement a control system based on phase measurements. The adaptive lens including the control system is characterized in an aberration correction setup and in a confocal microscope.

Further author information: (Send correspondence to K.P.)
K.P.: E-mail: katrin.philipp@tu-dresden.de

2. ADAPTIVE LENS AND CONTROL SYSTEM

2.1 Adaptive Lens

The adaptive lens consists of a $50\text{ }\mu\text{m}$ thin glass membrane, which is glued in-between two piezo rings (Fig. 1). This piezo-glass sandwich seals a fluid chamber that is filled with transparent paraffin oil. The piezo rings enable a direct deformation of the glass membrane which leads to a change of the focal length of the lens depending on the curvature of the membrane. As shown in Fig. 1, one contracting and one expanding piezo ring lead to a bending deformation with an approximately parabolic lens surface (Fig. 1 left) in comparison to two contracted rings that yield a buckling deformation with a more hyperbolic lens surface (Fig. 1 right). In this way one can not only control the focal power of the lens but as well its spherical behavior (see [24] for a more complete description of the lens and [25] for the general principle). Fig. 2 shows a photograph of the adaptive lens in a custom-build mount.

Figure 1. Illustration of lens curvature with one expanding and one contracting piezo ring (bending mode, left) and both piezo rings contracting (buckling mode, right) [24].

Figure 2. Left: Photograph of the adaptive lens with custom-build mount. Right: schematic drawing of adaptive lens.

2.2 Control System

The creeping and hysteresis effects of the piezoelectric actuators make it necessary to control the actuation process based on wavefront measurements. The wavefront is determined using digital holography in an off-axis Mach-Zehnder-configuration. A Zernike composition of the wavefront phase is conducted. Initial actuation voltages are determined based on a previously recorded look-up-table between actuation voltages and defocus as well as spherical aberration Zernike coefficients. The actuation voltages are then applied to the adaptive lens and the Zernike coefficients of the wavefront are obtained again. The actuation voltages are then refined iteratively based on the gradient of the look-up-table. Usually two to five iteration cycles were sufficient.

3. CONFOCAL MICROSCOPY

As spherical aberrations are the dominating contribution to deteriorating imaging quality and resolution, we apply the proposed lens in a confocal microscope for axial scanning and spherical aberration correction. The maximum intensity and the full width half maximum (FWHM) of the confocal point spread function (CPSF) serve as figures of merit, whereby high maximum values and small FWHM are the desired outcome.

3.1 Experimental setup

As shown in Fig. 3, the adaptive lens is placed in a conjugate plane of the objective lens in a custom-build confocal microscope via a lens relay system. An aspheric lens with a numerical aperture of 0.5 is used as the objective lens. A DPSS laser with a wavelength of 532 nm is used in the microscope. A optional white LED illumination path and a camera is used to enable easier specimen handling and locating.

Figure 3. Experimental setup, for description see text.

We extend the microscope with an additional Mach-Zehnder-interferometer for phase measurements to control the adaptive lens. The adaptive lens together with the detection pinhole serves as a spherical aberration correction sensor as will be further described in Section 3.3.

3.2 Axial scanning with spherical aberration correction

In order to characterize axial scanning and spherical aberration capability of the lens, we perform a-scans by moving a glass-plate along the optical axis and acquiring the depth-position dependent intensity of the PMT. The Mach-Zehnder-Interferometer in off-axis configuration is used to acquire simultaneously the spherical aberration coefficient via Zernike decomposition of the retrieved phase.

Fig. 4 shows exemplary A-Scan measurement data. It can be seen, that different spherical aberration coefficients of the adaptive lens at a given focal length lead to different shapes of the axial CPSF. Plotting the maximum intensity and the FWHM of the CPSF at a fixed axial position suggest that there is an optimal spherical aberration coefficient for each axial position in terms of axial resolution.

3.3 Towards correction of specimen-induced aberrations in confocal microscopy

The Zernike sensor introduced by M. Neil et al.²⁶ is based on deliberately inserting negative and positive bias aberrations to the wavefront to be measured. The difference of the signals obtained behind a detection pinhole serves as the response curve to determine the corresponding Zernike coefficient of the wavefront. This Zernike sensor can be elegantly implemented in a confocal microscope by only inserting a beamforming device into the microscope,²⁷ since a confocal microscope already includes a pinhole that can be reused for the Zernike sensor.

However, in these pioneering and also the many following works, a beamforming device that is (almost) symmetric in terms of Zernike coefficients is used. In contrast, the adaptive lens introduced in this paper

Figure 4. a) Selection of acquired A-Scans. Solid lines correspond to measurement data and dashed lines indicate the Lorentz-fit that has been used for extracting the Full width half maximum (FWHM) and maximum intensity of the confocal point spread function (CPSF) b-d) maximum intensity of (CPSF) depending of the spherical aberration of the adaptive lens. e-f) FWHM of CPSF. Same marker type and color in different subplots indicates same underlying measurement series.

is not only asymmetric in terms of spherical aberration coefficients but also supports mainly negative spherical aberration coefficients. In our talk, we will discuss the consequences of this difference and also possible approaches to address these challenges.

4. SUMMARY AND CONCLUSION

In this contribution, we proposed an adaptive lens for simultaneous axial scanning and spherical aberration correction. In a separate experimental setup, we applied the adaptive lens to correct synthetic aberrations induced by a deformable mirror. The results suggest that the lens is able to correct spherical aberrations with standard Zernike coefficients between $0\ \mu\text{m}$ and $1\ \mu\text{m}$, while operating at refractive powers up to about $4\ \text{m}^{-1}$.

The adaptive lens was also used to simultaneously perform axial scanning and spherical aberration tuning in a confocal microscope. The maximum intensity of the CPSF was increased by a factor of about three to four and the FWHM could be decreased by a factor of about two to three (depending on axial position).

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