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Investigation of aberration correction and axial scanning in microscopy employing adaptive lenses

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Investigation of aberration correction and axial scanning in microscopy employing adaptive lenses

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Abstract

Adaptive lenses allow for compact, fast and inertia-free axial scanning and therefore are increasingly employed in numerous microscopic techniques, such as confocal microscopy, two-photon microscopy, structured illumination microscopy and light sheet microscopy. However, these complex optical systems can only be dimensioned for one specific focal length of the tunable lens. When the lens is used for axial scanning, not only the focus position is axially moved, but additional aberrations are induced leading to a deteriorating spatial resolution due to focal spot broadening. The placement of the tunable lens into the optical system in a non-imaging way, which is often done either by geometrical constraints or to increase the axial tuning range, magnifies this effect even further.

In the scope of this thesis, methods to model, minimize and actively compensate these induced aberrations were investigated with a focus on confocal microscopy. As an example of a camera-based microscope, additionally a novel hybrid illumination microscope employing a tunable lens for fast volumetric measurements was developed and characterized. In a confocal microscope, the use of a second tunable lens in the detection path to compensate aberrations due to the non-imaging placement of the tunable lens for axial scanning of the focus is discussed. While this approach was found to be sufficient for specific configurations, spherical aberrations due to incomplete illumination of the objective lens or due to the tunable lens itself cannot be corrected. For this purpose, a novel bi-actuator adaptive lens was used to manipulate the wavefront with an additional degree of freedom. A control strategy was developed for the bi-actuator adaptive lens to allow the independent tuning of defocus and induced spherical aberrations. For axial scanning in free space with a confocal microscope, the diffraction-limited range was increased by a factor of almost two from 78 µm to 150 µm by spherical aberration correction. Beyond that, the additional degree of freedom of the bi-actuator adaptive lens was used to compensate specimen-induced aberrations. As a result, the axial resolution at measurements inside a phantom specimen was increased by a factor of up to 3 and the specimen-induced aberrations were corrected to propagation depths up to 340 µm. To demonstrate the procedure in biological specimens, the bi-actuator lens was used for spherical aberration correction at measurements of zebrafish embryos with reporter-gene-driven fluorescence in the thyroid gland resulting in increased contrast and enhanced fluorescence signal. Due to the improved optical sectioning, substructures of the thyroid follicles were observable, which were not visible without the spherical aberration correction.

While the presented methods for aberration correction employing the bi-actuator adaptive lens were realized at the example of a confocal microscope, they can be employed in several microscopic techniques. In particular, point-scanning techniques such as two-photon and Brillouin microscopy are expected to benefit from this approach. Spherical aberration correction employing the bi-actuator adaptive lens promises to bridge a gap in the currently available adaptive optics toolset.

Contents

Symbols

Notation	Description
A_n^m	coefficients of Zernike composition of optical path length $\Delta = A_n^m \Delta_n^m$
$E(\vec{r}, t)$	wave field
P	refractive power $P = 1/f$
R_{curv}	radius of curvature of a lens
U	field distribution of wave
V_1	first actuation voltage of bi-actuator adaptive lens
V_2	second actuation voltage of bi-actuator adaptive lens
V_{TL}	actuation voltage of tunable lens
V_{max}	maximum actuaton voltage of bi-actuator adaptive lens
V_{min}	minimum actuation voltage of bi-actuator adaptive lens
$V_{\text{p, ref}}$	reference for voltage of differential pressure sensor integrated in tunable lens in control system
V_{p}	voltage of differential pressure sensor integrated in tunable lens
$\Delta\kappa$	spatial bandwidth of an imaging system
$\Delta x_{\text{s,proj}}$	backprojected pixel size of reconstructed image from lateral scanning
Δx_{s}	pixel size of reconstructed image from lateral scanning
Δz	axial resolution of a microscope
Δ	optical path length difference
Ω	pupil solid angle
Φ	phase of a wave
α_n^m	coefficients of Zernike composition of optical path length $\Phi = \alpha_n^m \Psi_n^m$
$\gamma_{\downarrow\uparrow}$	fixed focus trajectory in the actuation category of bi-actuator lens with V_1 decreasing and V_2 increasing ($[\downarrow\uparrow]$) actuation category
$\gamma_{\uparrow\downarrow}$	fixed focus trajectory in the actuation category of bi-actuator lens with V_1 increasing and V_2 decreasing ($[\uparrow\downarrow]$) actuation category
κ	wavenumber κ
$\mathcal{E}(\vec{\kappa}, \nu)$	radiant wave field
AU	Airy unit
CSF	coherent spread function (for monochromatic fields)
NA	nuerical aperture
OPL	optical path length
OTF	optical transfer function (for incoherent intensities)
PSF	point spread function (for incoherent intensities)

Notation	Description
jinc	jinc-function
sinc	$\mathrm{sinc}(x) = \sin(\pi x)/\pi x$
ν	temporal frequency of a wave field
FWHM_z	axial resolution of a microscope
$\vec{\kappa}$	wave vector $\vec{\kappa} = (\kappa_x, \kappa_y, \kappa_z) = (\vec{\kappa}_\perp, \kappa_z)$, Fourier conjugate variable of position vector $\vec{r}$
$\vec{\rho}$	two-dimensional, lateral position vector $\vec{\rho} = (x, y)$
$\vec{k}$	angular wave vektor $\vec{k} = 2\pi\vec{\kappa}$
$\vec{r}$	three-dimensional position vector $\vec{r} = (x, y, z) = (\vec{\rho}, z)$
c	propagation velocity of light in vacuum
d_{induced}	induced propagation distance by a tunable lens in an axial scanning system that is propagated twice in opposing direction
d_a	aspheric description of a lens surface
d_p	polynomial description of a lens surface
f	focal length
n	refractive index of a medium
r_{Airy}	radius of first minimum in Airy diffraction pattern; lateral resolution according to Abbe/Rayleigh criterium
$r_{\mathrm{ph,proj}}$	backprojected pinhole radius (to sample plane)
r_{ph}	pinhole radius
t	time
u	axial optical unit
v	radial optical unit
λ	wavelength
λ_{em}	wavelength of emitted light
λ_{ex}	excitation wavelength
$\overline{\lambda}$	mean wavelength of emission and excition wavelength

Acronyms

Notation	**Description**
[↓↑]	actuation category of bi-actuator lens with V_1 decreasing and V_2 increasing
[↑↓]	actuation category of bi-actuator lens with V_1 increasing and V_2 decreasing
FWHM	full width half maximum
AO	Adaptive optics
DM	deformable mirror
DMD	digital micromirror device
DOF	depth of field
EDOF	extended depth of field
ETL	electrically tunable lens
EWOD	electrowetting on dielectrics
GFHT	Quasi-Fast Hankel Transform
HS sensor	Hartmann-Shack wavefront sensor
LC	liquid cristal
OCT	optical coherence tomography
OPD	optical path difference
PDMS	Polydimethylsiloxan
PID	Proportional-Integral-Differential
PMT	photomultiplier tube
PSD	Proportional-Summation-Difference
SLM	spatial light modulator
SNR	signal-to-noise ratio
TL	tunable lens