Dresdner Berichte zur Messsystemtechnik

Herausgeber: Prof. Dr.-Ing. habil. Jürgen Czarske

14

Katrin Philipp

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SHAKER VERLAG

MST
Professur für
Mess- und Sensorsystemtechnik

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Band 14

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Shaker Verlag
Düren 2019

Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at http://dnb.d-nb.de.

Zugl.: Dresden, Techn. Univ., Diss., 2019

Printed in Germany.

ISBN 978-3-8440-7062-0
ISSN 1866-5519

Shaker Verlag GmbH • Am Langen Graben 15a • 52353 Düren
Phone: 0049/2421/99011-0 • Telefax: 0049/2421/99011-9
Internet: www.shaker.de • e-mail: info@shaker.de

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.