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Jürgen Czarske, Katrin Philipp, Nektarios Koukourakis

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Structured illumination 3D microscopy using adaptive lenses and multimode fibers

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Structured illumination 3D microscopy using adaptive lenses and multimode fibers

Jürgen Czarske*[a], Katrin Philipp[a], Nektarios Koukourakis[a]

[a]Laboratory for measurement and sensor system technique, Technische Universität Dresden, Helmholtzstraße 18, 01069 Dresden;

ABSTRACT

Microscopic techniques with high spatial and temporal resolution are required for in vivo studying biological cells and tissues. Adaptive lenses exhibit strong potential for fast motion-free axial scanning. However, they also lead to a degradation of the achievable resolution because of aberrations. This hurdle can be overcome by digital optical technologies. We present a novel High-and-Low-frequency (HiLo) 3D-microscope using structured illumination and an adaptive lens. Uniform illumination is used to obtain optical sectioning for the high-frequency (Hi) components of the image, and nonuniform illumination is needed to obtain optical sectioning for the low-frequency (Lo) components of the image. Nonuniform illumination is provided by a multimode fiber. It ensures robustness against optical aberrations of the adaptive lens. The depth-of-field of our microscope can be adjusted a-posteriori by computational optics. It enables to create flexible scans, which compensate for irregular axial measurement positions. The adaptive HiLo 3D-microscope provides an axial scanning range of 1 mm with an axial resolution of about 4 microns and sub-micron lateral resolution over the full scanning range. In result, volumetric measurements with high temporal and spatial resolution are provided. Demonstration measurements of zebrafish embryos with reporter gene-driven fluorescence in the thyroid gland are presented.

Keywords: three dimensional microscopy, adaptive optics, speckle pattern evaluation

1. INTRODUCTION

Structured illumination has become an important tool in optical devices (see e.g. [1] for review on applications), such as improving the resolution in light microscopy [2, 3]. There are numerous optical devices like spatial light modulators, gratings and deformable mirrors for the generation of structured illumination. While adaptive elements like deformable mirrors and spatial light modulators offer several advantages such as flexibility, they are also cost-intensive and lead to bulky optical setups. As a consequence, static elements like gratings may be a more suitable solution for compact, cost-effective devices. However, this static optical elements do not allow to switch between structured and uniform illumination as is required for some microscopic techniques.

We propose an alternative approach using a multimode fiber for the generation of both structured, speckle-patterned, and uniform illumination. As a demonstration of the capabilities of the multimode fiber-based illumination-unit, we implement it in a 3D microscope. Demonstration measurements of zebrafish embryos with reporter gene-driven fluorescence in the thyroid gland are presented.

2. ILLUMINATION GENERATION EMPLOYING A MULTIMODE FIBER

As illustrated in Fig.1a, the structured illumination, having a light wavelength of 532 nm, is generated using a multimode fiber with a diameter of $a = 1$ mm and a numerical aperture of $NA = 0.5$. The minimal speckle size results in

$$\Delta s_{min} = \frac{\lambda}{2NA} = 532 \, nm. \tag{1}$$

The theoretical number of modes is

$$N \approx \left(\frac{a}{\Delta s_{\min}}\right)^2 = 4 \cdot 10^6. \tag{2}$$

The corresponding speckle pattern is shown in Fig. 1a at the bottom.

In order to generate a uniform illumination, a metal sheet was attached to the multimode fiber as illustrated in Fig. 1b. Applying an alternating magnetic field leads to a vibration of the multimode fiber and, thus, a uniform illumination due to temporal averaging of the camera as shown in Fig. 1b at the bottom.
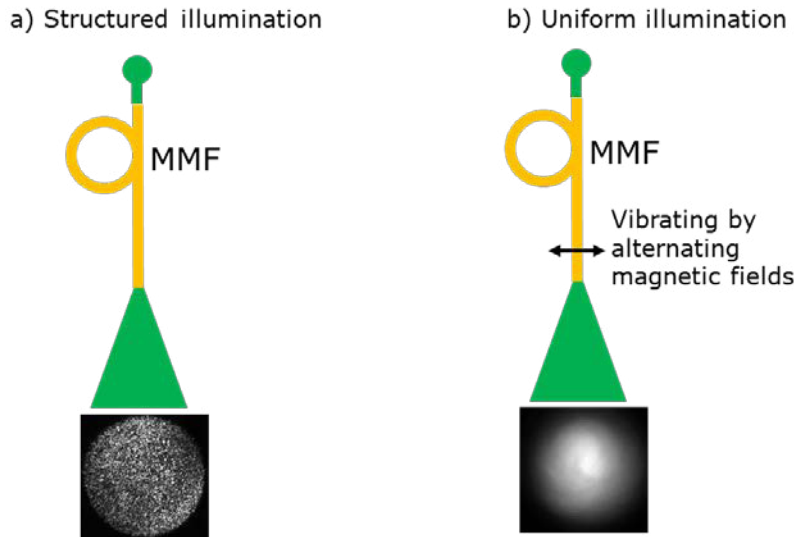

Fig.1. Illustration of illumination generation. a) Structured illumination as a superposition of the modes of the multimode fiber. b) Uniform illumination results as temporal averaging when the multimode fiber is vibrating.

3. THREE-DIMENSIONAL MICROSCOPY

3.1 Hybrid illumination microscopy for optically sectioned image acquisition

As depicted in Fig. 2, the adaptive hybrid-illumination microscope [4] employing an electrically tunable lens [5] is an extended version of a conventional widefield microscope. It can be used as a fluorescence microscope by placing an additional wavelength filter in front of the camera or as a conventional light microscope without the filter. Instead of a single illumination source, we us the illumination unit described in the previous section.

Therefore, we obtain a uniformly illuminated image with intensity distribution $I_u(n_x, n_y)$ and a speckle-patterned illuminated image with intensity distribution $I_S(n_x, n_y)$, whereas n_x and n_y denote the pixel index in horizontal and vertical direction, respectively. We now reconstruct the high- and low-frequency components $I_h(n_x, n_y)$ and $I_l(n_x, n_y)$ of the optically sectioned image separately as introduced by Lim *et al.* [6]. For the reconstruction of the high-frequency component, we make use of the fact, that high-frequency objects are already optically sectioned. Thus,

$$I_{Hi}(n_x, n_y) = HP(I_u), \tag{3}$$

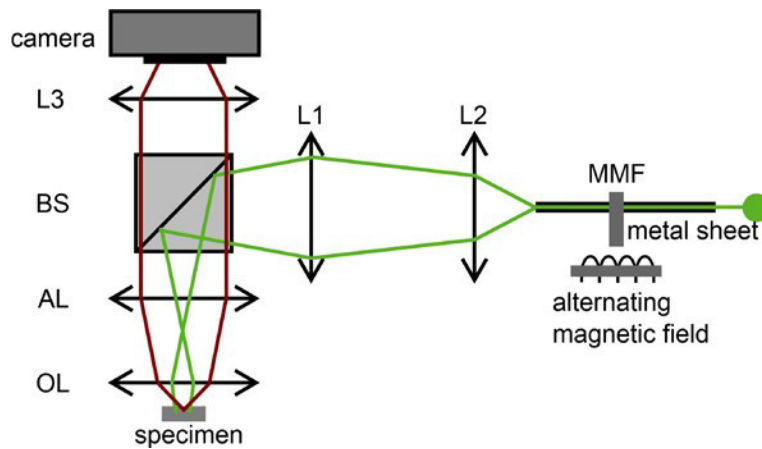with the two-dimensional Gaussian high-pass filter HP.

Fig.2. Experimental setup of the adaptive hybrid-illumination wide-field microscope employing an electrically tunable lens.

To obtain the low-frequency component, we exploit that the speckle-patterned illuminated image is only sharp as long it is in-focus and gets blurred for out-of-focus components of the specimen. Hence, we use the local contrast $C_S = \frac{<\sigma_\delta>_w}{<I_\sigma>_w}$ of the differential image $I_\delta(n_x, n_y) = |I_S(n_x, n_y) - I_u(n_x, n_y)|$ as a weighting function of the in-focus contributions. The standard deviation is denoted by σ_δ and $<\cdot>_w$ denotes the local mean over a window of size w. Consequently, the low-frequency component is determined by

$$I_{Lo}(n_x, n_y) = LP(C_S \cdot I_u),\qquad(4)$$

with the opposite two-dimensional Gaussian low-pass filter LP. Finally, we obtain the optically sectioned image by a weighted superposition of the low- and high frequency image as

$$I_{HiLo}(n_x, n_y) = I_{Lo}(n_x, n_y) + \eta \cdot I_{Hi}(n_x, n_y),\qquad(5)$$
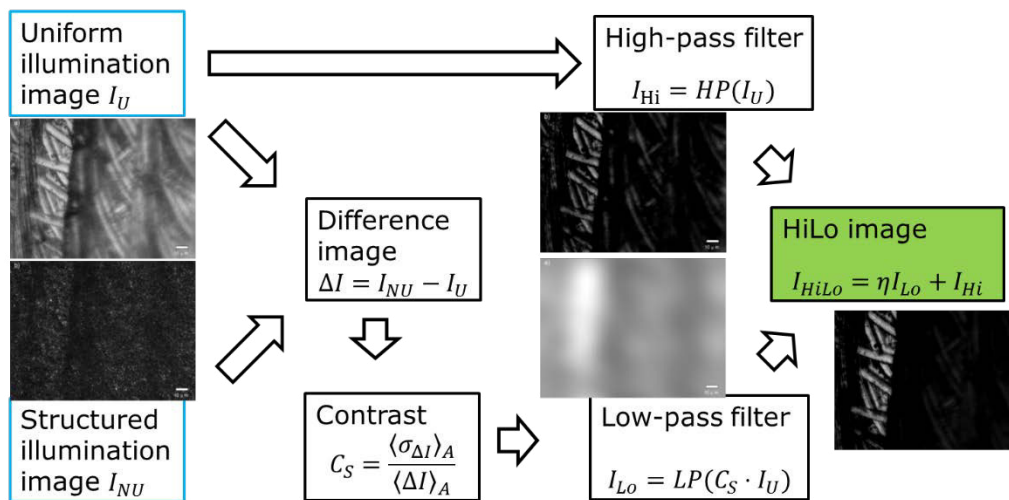
with the weighting constant η.

Figure 3. Illustration of the HiLo mechanism.

3.2 Axial scanning employing an electrically tunable lens

The adaptive lens used for axial scanning is based on a lens concept described by J. Draheim *et al.* [5]. The lens consists of a transparent polydimethylsiloxane (PDMS) membrane into which an annular piezo bending actuator is embedded. Between the membrane and the glass substrate a transparent fluid is filled. When being actuated, the piezo generates a pressure in the lens which deflects the membrane and thus changes the refractive power. This technique enables a large tuning range of the refractive power between $1/f = -(23.6 \ldots 25.2)$ m^{-1}, see Fig. 4 for refractive power against the actuation voltage.
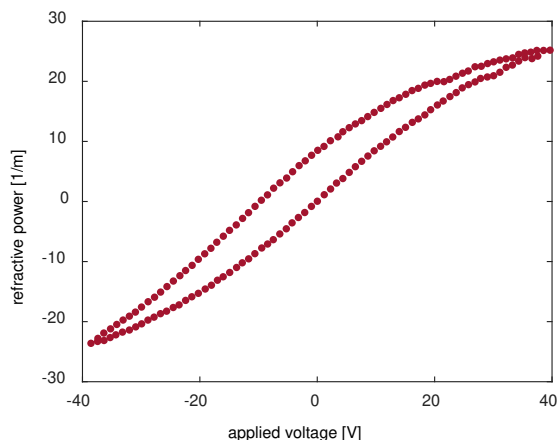

Fig.4. Tuning range of adaptive lens as a function of the applied actuation voltage.

4. RESULTS

An USAF 1951 test chart was imaged as illustrated in Fig. 5a. Element 6 of group 7 can be resolved clearly, as illustrated in the line profiles in Fig 5b and Fig. 5c. Consequently, the lateral resolution is at least 2 µm.
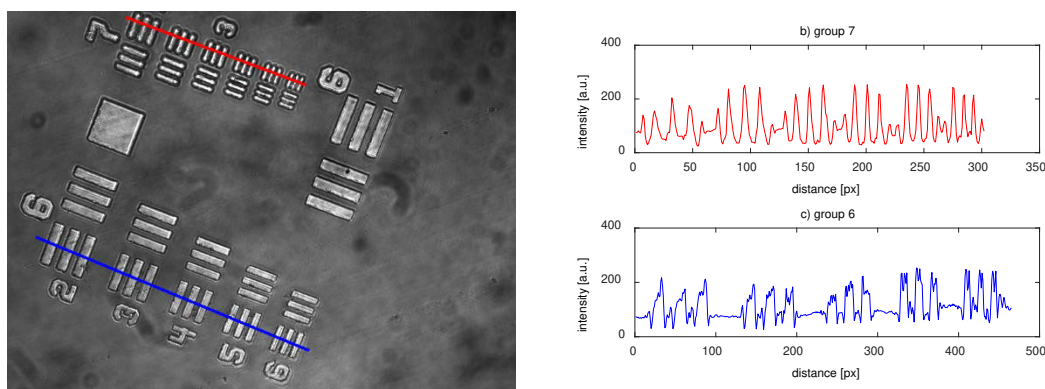

Figure 5. a) Uniform illumination image of group 6 and 7 of USAF 1951 test chart with 800x600 pixels resolution. b) and c) Intensity profile along red (b) and blue (c) lines corresponding to elements of group 7 and group 6, respectively [7].

The axial resolution was previously determined experimentally [4] as about 4 µm over the full scanning range.

As an example of a biological specimen, we investigated zebrafish embryos with reporter gene-driven fluorescence in the thyroid gland, as shown in Fig. 6. The HiLo image (Fig. 6b) provides better optical sectioning and, thus, improved contrast to the uniform illumination image (Fig. 6a).

Figure 6. (a) Uniform illumination and (b) HiLo image of zebrafish thyroid.

5. SUMMARY AND OUTLOOK

We implemented an illumination unit based on a multimode fiber that enables both uniform and structured illumination. In general, multimode fibers with digital optical technologies enable versatile systems [8, 9]. A three-dimensional microscope for volumetric measurements was demonstrated employing this multimode-fiber based illumination unit as well as an adaptive lens for axial scanning. Demonstration measurements at an USAF 1951 test chart as well as at zebrafish embryos with reporter gene-driven fluorescence in the thyroid gland were performed.

ACKNOWLEDGEMENTS

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