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High-contrast 3D image acquisition using HiLo microscopy with an electrically tunable lens

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ABSTRACT

We present a HiLo microscope with an electrically tunable lens for high-contrast three-dimensional image acquisition. HiLo microscopy combines widefield and speckled illumination images to create optically sectioned images. Additionally, the depth-of-field is not fixed, but can be adjusted between widefield and confocal-like axial resolution. We incorporate an electrically tunable lens in the HiLo microscope for axial scanning, to obtain three-dimensional data without the need of moving neither the sample nor the objective. The used adaptive lens consists of a transparent polydimethylsiloxane (PDMS) membrane into which an annular piezo bending actuator is embedded. A transparent fluid is filled between the membrane and the glass substrate. When 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 $1/f = (-24 \dots 25) \text{ 1/m}$. As the NA of the adaptive lens is only about 0.05, a fixed high-NA lens is included in the setup to provide high resolution. In this contribution, the scan properties and capabilities of the tunable lens in the HiLo microscope are analyzed. Eventually, exemplary measurements are presented and discussed.

Keywords: Electrically tunable lens, Speckle, Fluorescence Microscopy, HiLo Microscopy

1. INTRODUCTION

Recently, adaptive lenses are used as an attractive alternative to conventional techniques for axial scanning in wide field microscopy,¹ confocal microscopy^{2,3} and light sheet microscopy.⁴ While wide field microscopy provides video rate image acquisition, it lacks optical sectioning. Confocal microscopy and light sheet microscopy are based on illuminating only a small spot or a thin sheet, respectively. As a result optical sectioning is achieved. However, at confocal microscopy this comes at the cost of scanning speed, because axial and lateral scanning is required for imaging. In contrast, light sheet and structured illumination microscopy require no lateral scanning. However, light sheet microscopy heavily relies on delivering a well-defined illumination pattern into the specimen. HiLo microscopy⁵ uses speckle-patterned illumination for optical sectioning. It exploits the fact, that the speckle contrast is high for in-focus components and low for out-of-focus contributions. Consequently, the local speckle contrast is used as a weighting function of the in-focus and out-of-focus components. By applying an adaptive lens for axial scanning, we present a microscope that is capable of three-dimensional data acquisition.

The experimental setup including the adaptive lens as well as the fundamentals of HiLo microscopy are described in section 2. The experimental results are listed in section 3. Finally, a conclusion is given in section 4.

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2. MATERIALS AND METHODS

2.1 Microscope Setup

The microscope setup is depicted in Fig. 1. A laser diode with a wavelength of 532 nm is coupled into a multi mode fiber (MMF) generating the speckles through its modes. The multi mode fiber is attached to a magnetic metal sheet (MS). The fiber can be vibrated using an alternating magnetic field generated by a coil with iron core (MC). As a result, uniform illumination is generated when vibrating the fiber, since the camera exposure time is significantly larger than the period time of the speckle motion. In contrast, a speckle patterned illumination is generated when the electric field is turned off. After passing the fiber, the laser beam is focused through a polarizing beam splitter (PBS) into the objective lens system consisting of an adaptive lens (AL: $NA \approx 0.05$, $1/f_{AL} = -25 \dots 25 \text{ m}^{-1}$) and an objective lens (OL: $NA = 0.55$, $f = 4.5 \text{ mm}$) using the lenses L1 and L2. The reflected light is then imaged onto a camera using lens L3. In order to suppress reflexes caused by the adaptive lens a polarizing beam splitter and a $\lambda/4$ plate is used.

Figure 1. A laser diode is coupled into a multi mode fiber that can be moved through an alternating magnetic field. After propagating through the fiber, the light passes a lens system (L1 and L2) and a polarizing beam splitter (PBS). The light is then focused into the objective lens system consisting of an adaptive lens (AL) and an objective lens (OL). A $\lambda/4$ plate is used to suppress reflexes caused by the adaptive lens. Reflected light is then imaged onto a camera (CCD) using lens L3.

2.2 Adaptive Lens

The adaptive lens used for axial scanning is based on a lens concept described by J. Draheim et al.^{6,7} 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 $1/f = -23.6 \dots 25.2 \text{ m}^{-1}$, see Fig. 2 for refractive power against the applied piezo voltage.

2.3 HiLo Microscopy

The advantage of HiLo microscopy as proposed by Lim et al.⁸ over standard wide field microscopy is, that it provides optical sectioning. HiLo microscopy uses uniformly illuminated and speckle-patterned illuminated images $I_u(\vec{r})$ and $I_s(\vec{r})$ in order to extract the in-focus components of an image. The high-frequency (as Hi in

Figure 2. The refractive power of the adaptive lens depending on the applied voltage.

HiLo), in-focus components $I_{\text{Hi}}(\vec{r})$ are extracted by applying a simple highpass filter to the uniform illumination image, i.e.

$$I_{\text{Hi}}(\vec{r}) = \text{HP} \{I_{\text{u}}(\vec{r})\}, \quad (1)$$

since high-frequency components are naturally optically sectioned.⁹ The low-frequency, in-focus components are extracted by weighting the uniform image $I_{\text{u}}(\vec{r})$ with the speckle-contrast $C_{\text{s}}(\vec{r})$, since the speckle contrast decreases with increasing defocus,¹⁰ i.e.

$$I_{\text{Lo}} = \text{LP} \{C_{\text{s}}(\vec{r}) \cdot I_{\text{u}}(\vec{r})\} \quad (2)$$

with the low pass filter LP. In order to remove object-induced contrast, the speckle contrast is calculated over the difference image $I_{\Delta}(\vec{r}) = I_{\text{s}}(\vec{r}) - I_{\text{u}}(\vec{r})$ as

$$C_{\text{s}} = \frac{\langle \sigma_{\Delta}(\vec{r}) \rangle_A}{\langle I_{\text{u}}(\vec{r}) \rangle_A} \quad (3)$$

with the standard deviation $\sigma_{\Delta}(\vec{r})$ over a subimage of area size A . The HiLo image is eventually obtained by

$$I_{\text{HiLo}}(\vec{r}) = \eta I_{\text{Lo}}(\vec{r}) + I_{\text{Hi}}(\vec{r}), \quad (4)$$

whereas the scaling factor η ensures a smooth transition between high- and low-frequency components.

3. RESULTS

The axial scanning capability is demonstrated using an optical mirror as specimen. At a fixed voltage, the mirror is moved along z -direction and the intensity signal at the camera is integrated. As depicted in Fig. 3 the intensity signal peak from the camera image moves with the voltage applied to the piezo of the adaptive lens. Consequently, axial scanning is achieved by tuning the adaptive lens.

As depicted in Fig. 4, the total tuning range amounts to about $800 \mu\text{m}$. The shape and the height of the intensity peaks are stable between -35 V and 15 V , enabling reliable operation in this voltage range. Low and high applied voltages lead to an over- and under-illumination of the aperture of the adaptive lens, respectively. Consequently, the peaks are broadened and secondary peaks appear. These effects can be minimized by decreasing the distance between the adaptive and the objective lens. However, the minimal distance is limited due to geometrical constraints of the $\lambda/4$ -plate (including mounting). Furthermore, a decreased distance between adaptive and objective lens leads to a decreased tuning range. Consequently, a trade-off between tuning range

Figure 3. The integrated intensity of HiLo images of an optical mirror are determined along axial direction z . The intensity maximum and thus the focal plane of the microscope moves with varying voltage. Consequently, axial scanning is achieved using the adaptive lens.

Figure 4. Axial displacement z in dependency of the applied voltage at the electrically tunable lens. The total scanning range amounts to about $800\ \mu\text{m}$.

and axial resolution has to be made for the desired application. In summary, the scanning behavior is stable in the main voltage range between $-35\ \text{V}$ and $15\ \text{V}$ and, thus, provides stable tuning over about $500\ \mu\text{m}$.

Exemplary measurements are conducted on a aluminum sample that consists of several steps of height $10\ \mu\text{m}$. The distance between adaptive and objective lens is adjusted to meet the requirements of this sample. As can be seen in Fig. 5 optical sectioning is achieved. At an applied voltage of $5\ \text{V}$ the lower step (right side in images) is in-focus, whereas at an applied voltage of $6.2\ \text{V}$, the upper step is in-focus. The uniformly illuminated images contain both in-focus and out-of-focus contributions. In contrast, in the HiLo images the out-of-focus contributions are (almost) rejected. This demonstrates the optical sectioning of the HiLo measurement principle.

In order to determine the lateral resolution of the microscope, group 6 and 7 of an USAF 1951 test chart are investigated with the microscope. As can be seen in Fig. 6, all elements of both groups are fully resolved. Hence,

Figure 5. Uniformly illuminated images with a) 5 V and b) 6.2 V applied voltage at the adaptive lens. The HiLo images with c) 5 V and d) 6.2 V provide optical sectioning in contrast to the uniform illumination images.

Figure 6. a) Uniform illumination image of group 6 and 7 of USAF 1951 test chart with 800x600 pixels resolution. Intensity profile along elements of b) group 7 and c) group 6. The red and blue lines, respectively, mark the position where the intensity profile is taken.

the lateral resolution of the microscope is at least $2.2\ \mu\text{m}$. The magnification of the microscope is about 17.

4. CONCLUSION

A proof-of-concept HiLo microscope employing an electrically tunable lens for axial scanning was presented. The relation between applied voltage at the adaptive lens and the axial displacement was investigated. With applied

voltages between -40 V and 40 V , a total axial scanning range of $800\text{ }\mu\text{m}$ was achieved, of which the central range of about $500\text{ }\mu\text{m}$ at voltages between -35 V and 15 V is fully stable. The lateral resolution was determined to be at least $2\text{ }\mu\text{m}$. The axial scanning range of the microscope is adjustable by changing the distance between adaptive lens and objective lens. Eventually, a aluminum step specimen was investigated with a modified setup. As a result, optical sectioning ability was proven. Future works will focus on the extension of the microscope for fluorescence measurements and the improvement of axial resolution. Additionally, we aim to increase the measurement rate by using a CMOS camera and a faster electrically tunable lens.

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REFERENCES

- [1] Nakai, Y., Ozeki, M., Hiraiwa, T., Tanimoto, R., Funahashi, A., Hiroi, N., Taniguchi, A., Nonaka, S., Boilot, V., Shrestha, R., Clark, J., Tamura, N., Draviam, V. M., and Oku, H., "High-speed microscopy with an electrically tunable lens to image the dynamics of in vivo molecular complexes," *The Review of scientific instruments* **86**, 013707 (jan 2015).
- [2] Koukourakis, N., Finkeldey, M., Stürmer, M., Leithold, C., Gerhardt, N. C., Hofmann, M. R., Wallrabe, U., Czarske, J. W., and Fischer, A., "Axial scanning in confocal microscopy employing adaptive lenses (CAL)," *Opt. Express* **22**(5), 6025–6039 (2014).
- [3] Duocastella, M., Vicidomini, G., and Diaspro, A., "Simultaneous multiplane confocal microscopy using acoustic tunable lenses," *Opt. Express* **22**, 19293–19301 (aug 2014).
- [4] Fahrbach, F. O., Voigt, F. F., Schmid, B., Helmchen, F., and Huisken, J., "Rapid 3D light-sheet microscopy with a tunable lens," *Optics express* **21**, 21010–26 (sep 2013).
- [5] Lim, D., Ford, T. N., Chu, K. K., and Mertz, J., "Optically sectioned in vivo imaging with speckle illumination HiLo microscopy," *Journal of biomedical optics* **16**, 016014 (jan 2011).
- [6] Draheim, J., Schneider, F., Burger, T., Kamberger, R., and Wallrabe, U., "Single chamber adaptive membrane lens with integrated actuation," in [*Optical MEMS and Nanophotonics (OPT MEMS), 2010 International Conference on*], 15–16 (aug 2010).
- [7] Schneider, F., Draheim, J., Kamberger, R., Waibel, P., and Wallrabe, U., "Optical characterization of adaptive fluidic silicone-membrane lenses," *Opt. Express* **17**, 11813–11821 (jul 2009).
- [8] Lim, D., Chu, K. K., and Mertz, J., "Wide-field fluorescence sectioning with hybrid speckle and uniform-illumination microscopy," *Opt. Lett.* **33**, 1819–1821 (aug 2008).
- [9] Mertz, J., [*Introduction to Optical Microscopy*], Roberts (2010).
- [10] Goodman, J. W., [*Speckle phenomena in optics: theory and applications*], Roberts and Company Publishers (2007).