Linearly modulated multi-focal diffractive lens for multi-sheet excitation of flow-driven samples in a light-sheet fluorescence microscope

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Abstract. Light-sheet fluorescence microscopy (LSFM) with single light-sheet illumination enables rapid 3D-imaging of living cells. In this paper we show the design, fabrication and characterization of a diffractive optical element producing several light sheets along an inclined tube for applications in flow-driven imaging. The element, which is based on a multi-focal Fresnel zone plate and a linear grating, generates in combination with a refractive cylindrical lens five thin light sheets of equal intensity.

1 Introduction

The approach of light-sheet fluorescence microscopy (LSFM) enables 3D-imaging of many types of samples [1–3]. 3D cell cultures are cultivated, stored and characterized in tubes on the droplet based platform of pipe based bioreactors [4], offer the opportunity of using the sample movement for the scanning mechanism providing a z-stack of images in LSFM platforms [5]. The generation of not only one but a series of equal intensity light-sheets enables multi-plane imaging [6, 7], which can effectively reduce the aberrations caused by the mechanical motion of the system and speed. It also enables velocity and correlation measurements.

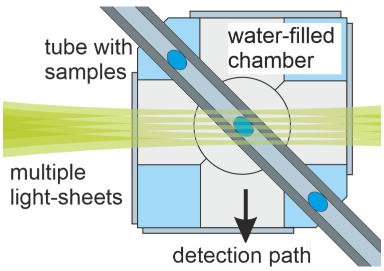

Figure 1. Schematic of the measurement configuration with tube-guided samples illuminated with multiple light sheets. The inclination of the tube of 45° offers the option of a 90°-detection between illumination and detection path.

2 Design, Fabrication and Measurement Results

For the generation of several equal-intensity light-sheets the combination of a multi-focal Fresnel zone plate with a linear grating is optimized. The result is a multi-focal diffractive lens (MFL) which plays the dual role of a multi-focal diffractive lens, as a multi-plane defocuser, and a beam splitting linear grating based on a detour phase approach. The optimized nonlinear phase values in each period affect the energy distribution in the diffraction orders [8, 9] leading to an efficient equal-intensity array of focus spots. Additionally, a refractive cylindrical lens is required to obtain the desired light-sheet distribution.

The transmission function of the MFL is given by

$$T_{\mathrm{MFL}}(\rho,\theta) = \sum_{s=-\infty}^{\infty} c_s \exp\left[is\left(\frac{\pi\rho^2}{\lambda f} + \frac{2\pi\rho\cos\theta}{d}\right)\right], \quad (1)$$

where ρ and θ denote the polar coordinates in the input plane. f and d are the focal distance of the MFL and the period of the linear grating. The coefficients c_s depend on the optimized phase values assigned to each area of the MFL to assure a uniform energy distribution for

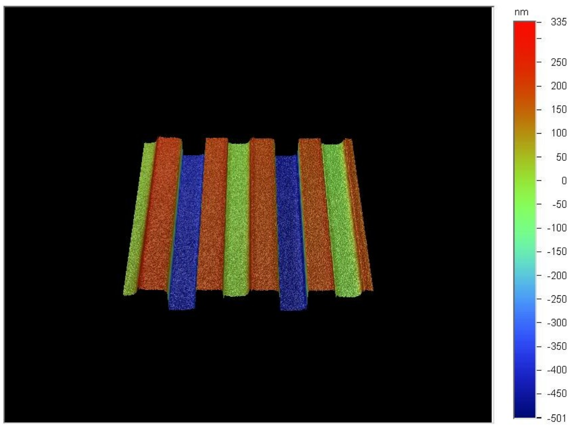

Figure 2. Measurement of the profile of the etched element using white light interference microscopy.

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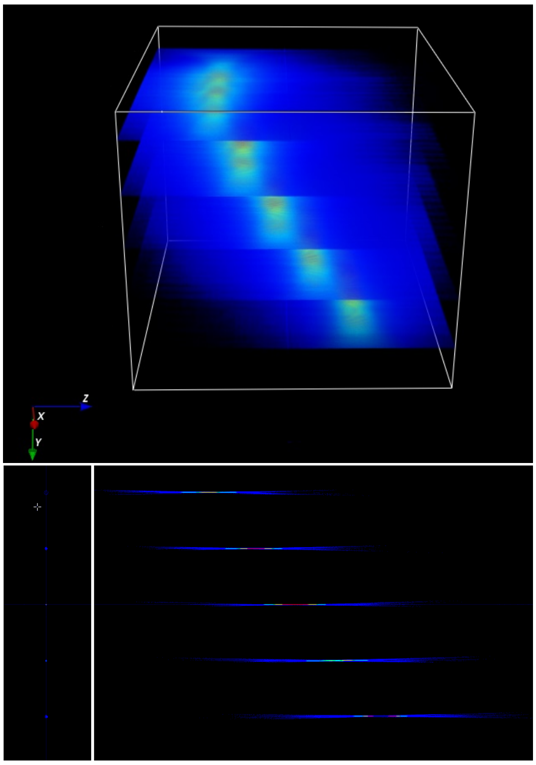

Figure 3. Cube of 6 mm x 6 mm x 6 mm showing the five light-sheets in 3D and cross section.

the generated diffraction orders [8, 9]. The transmission function includes an order-dependent focusing power and a linear phase. This introduces a change of direction leading to an orientation of the focal spots along a desired line coincident with the specimen trajectory within the tube. The general structure of the designed MFL is a slightly curved grating with four phase levels, whereas two levels are equal. Two different elements were designed for the generation of five light-sheets with distances of 1 mm and 0.25 mm to each other. The phase function was adapted for the illumination within a block of water as the aqueous samples are guided in a FEP-tube immersed in a water chamber. The refractive index of FEP is very close to the one of water.

The elements were fabricated in the clean room by means of maskless lithography. Two mask layers were calculated from the phase levels with pixel sizes of 250 nm $\times$ 250 nm. The required etching depths were converted from the phase difference values. For the wafer material of fused silica and a wavelength of 532 nm the etching depths were calculated to be 196.3 nm and 617.8 nm, respectively. Reactive ion etching was used for the fabrication process [10]. The achieved etch depths were measured with white light interference microscopy. The measured profile is shown in Fig. 2 With 194 nm and 628 nm they are close to the design values.

The light distribution behind the fabricated MFL in combination with a cylindrical lens was measured plane by plane and reconstructed. Fig. 4 gives the light distribution in 3D and a cross section. The efficiency varies only slightly between the orders. The performance of the element was evaluated by considering the whole optical setup. Figure 4 shows a qualitative impression by showing the tube illuminated with the five light sheets.

3 Conclusion

We successfully designed, fabricated and characterized a DOE which generates in combination with a cylindrical lens five light sheets of equal intensity along a guiding tube for multi-plane LSFM-imaging.

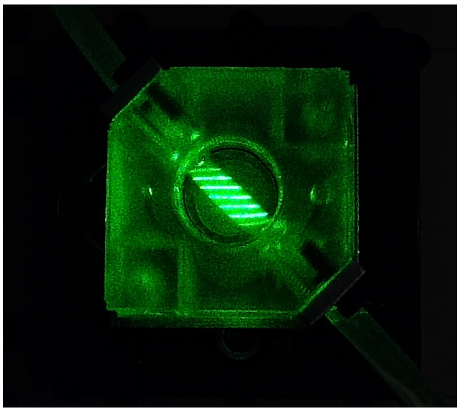

Figure 4. Measurement chamber with inserted FEP-tube filled with water and illuminated by the multiple light sheets.

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