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Optimization of the imaging properties of a light sheet fluorescence microscope (LSFM) by illumination with Talbot carpets

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LSFM is a method for 3D-characterization of biological samples. Fluorescent light is captured perpendicularly to the light sheet. Scattering of the excitation light limits the axial resolution and the penetration depth. We investigate the self-reconstructing properties of so called Talbot carpets to improve the imaging properties.

1 Introduction

In a light sheet fluorescence microscope (LSFM) a fluorescing sample is illuminated with a thin sheet of light which can be generated statically by a cylindrical lens or dynamically by scanning a beam, e.g. a Gaussian beam or a Bessel beam. The emitted fluorescence light is then imaged orthogonally to the illumination path through a detection arm onto a 2D detector. By a relative movement of the sample through the set-up an image stack can be recorded and assembled to a 3D-picture of the sample. Scattering and absorption events lead to artifacts in the illumination distribution and to a non-uniform spreading of the illumination [1]. Bessel beams are known to have self-reconstructing properties and have been exploited to improve the axial resolution and penetration depth [2].

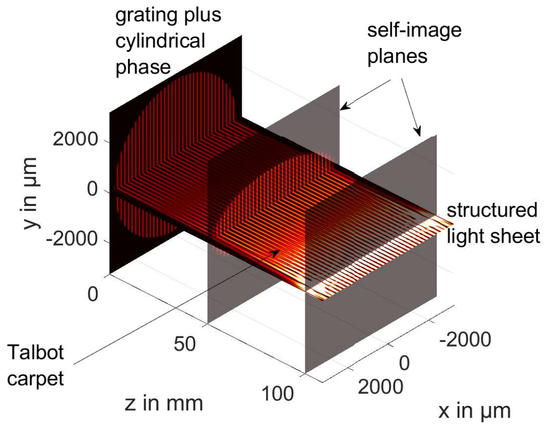

Fig. 1 *Light distribution behind a grating combined with the phase function of a cylindrical lens. The chart is overexposed for a better visibility of the pattern in the different planes.*

Structured illumination has also been investigated to remove background blur and to increase image contrast [3, 4, 5]. The illumination with Talbot patterns combines both approaches as these light distributions show lateral periodicity in certain planes and show moreover self-reconstructing properties.

2 Talbot effect

The Talbot effect is an interference effect occurring directly behind diffraction gratings leading to typical light distributions depending on the diffraction efficiencies of the orders. A regular pattern is formed in propagation direction, the so called Talbot carpet. For an amplitude grating with period p illuminated with a plane wave of wavelength λ, self-images occur at certain distances $z_T = 2p^2/\lambda$. Fig. 1 shows the light distribution behind a grating in combination with a cylindrical lens of a focal length corresponding to a multiple of the Talbot distance forming a structured light sheet.

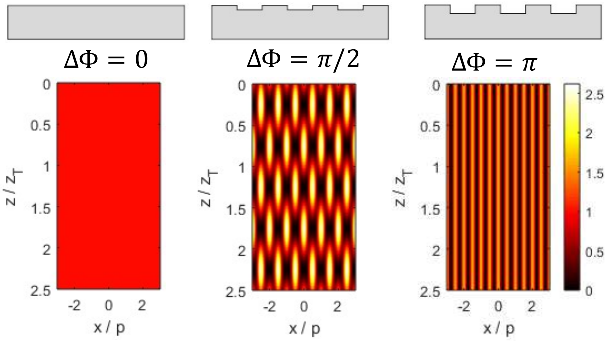

Fig. 2 *Intensity distributions with no grating and phase gratings of $\pi/2$ and π phase shift.*

The intensity distribution in a light sheet is important as it directly influences the magnitude of the fluorescence signal. In light critical applications it is important to keep the light for signal generation and concentrate it within the bright stripes of the pattern. We studied the light distributions behind the phase gratings quantitatively with increasing groove depths resulting in phase shifts of $\Delta\Phi = 0 ... \pi$. The resulting patterns for three values of $\Delta\Phi$ are shown in Fig. 2. The consideration of the peak value and the

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contrast showed that a phase shift of $\Delta\Phi = \pi/2$ is the best choice, giving the 2.5-fold peak signal compared to a non-structured light sheet.

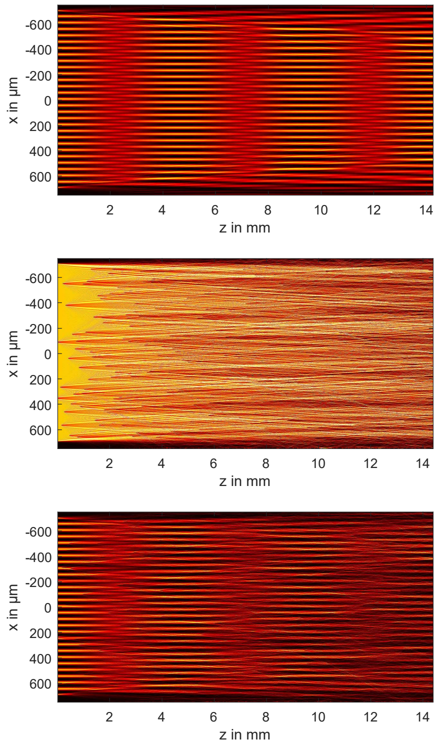

***Fig. 3** Undisturbed Talbot carpet, scattering distribution from particles and combined light distribution.*

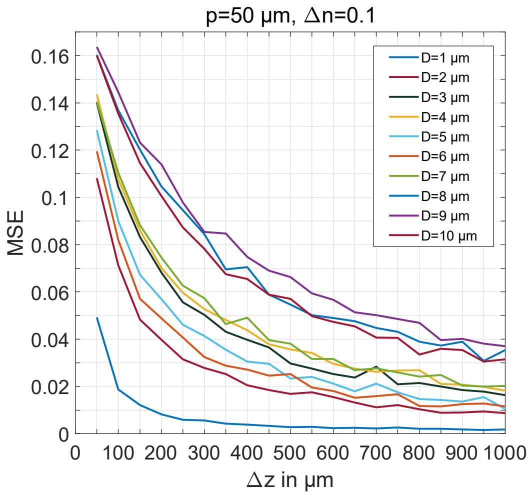

***Fig. 4** Mean square error between the undisturbed and the disturbed Talbot carpet.*

3 Light sheet degradation by scattering

Scattering events change the Talbot pattern [6], but as it is an interference phonomenon all of the orders transport some redundant information. The grating diffraction and scattering was modelled employing a Fresnel propagation algorithm. The particles were implemented as disks representing the phase function of a sphere having a different refractive index than the surrounding medium. Fig. 3 shows the calculated undisturbed Talbot carpet, the distribution from particle scattering and the combined light distribution representing the Talbot carpet within a scattering medium. As an evaluation criterion we considered the mean square error between disturbed and non-disturbed Talbot carpets. Fig. 4 shows the results for particle diameters between 1 and 10 µm related to the axial distance between randomly distributed particles. The lower the particle diameter and the particle density in the sample, the less is the error between the distributions.

4 Conclusion and Outlook

We showed that phase gratings with a phase delay of $\pi/2$ give a pattern with good contrast and maximum light in the bright stripes. We considered the impact of scattering particles and used the mean squared error between undisturbed and disturbed Talbot carpets to evaluate the self-reconstructing properties. The next steps will be a more quantitative analysis and comparison with Bessel beams as well as an experimental verification of the model.

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