Research Article Vol. 57, No. 5 / 10 February 2018 / Applied Optics 1019

applied optics

Accurate estimation of the illumination pattern's orientation and wavelength in sinusoidal structured illumination microscopy

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Received 6 September 2017; revised 31 December 2017; accepted 31 December 2017; posted 5 January 2018 (Doc. ID 305681); published 2 February 2018

Structured illumination microscopy is able to improve the spatial resolution of wide-field fluorescence imaging by applying sinusoidal stripe pattern illumination to the sample. The corresponding computational image reconstruction requires precise knowledge of the pattern's parameters, which are its phase (ϕ) and wave vector (p). Here, a computationally inexpensive method for estimation of p from the raw data is proposed and illustrated with simulations. The method estimates p through a selective discrete Fourier transform at tunable subpixel precision. This results in an accurate p estimation for all the illumination patterns and subsequently improves the superresolution image recovery by a factor of 10 around sharp edges as compared to an integer pixel approach. The technique as presented here is of major interest to the large variety of custom-build systems that are used. The feasibility of the presented method is proven in comparison with published data.

OCIS codes: (100.6640) Superresolution; (180.2520) Fluorescence microscopy; (100.2000) Digital image processing; (100.2650) Fringe analysis.

https://doi.org/10.1364/AO.57.001019

1. INTRODUCTION

Wide-field fluorescence microscopy is limited in its maximum spatial resolution to what is commonly known as the Abbe diffraction limit [1]. The diffraction limit is a result of the finite range of spatial frequencies that a microscope's objective can capture depending on its aperture. The 2D image formation under plain illumination can be formulated as a convolution ($\otimes$), giving $D(\mathbf{r}) = [S(\mathbf{r})I] \otimes h(\mathbf{r})$, with the acquired image D, the sample S, the plain illumination intensity I, the point spread function (PSF) h and the spatial coordinate $\mathbf{r}$. In the Fourier transform of this expression, the convolution is replaced by a multiplication and vice versa $\tilde{D}(\mathbf{k}) = [\tilde{I}(\mathbf{k}) \otimes \tilde{S}(\mathbf{k})]\tilde{h}(\mathbf{k})$, where tilde ($\sim$) indicates the Fourier transform, $\mathbf{k}$ is the Fourier space coordinate or spatial frequency, and $\tilde{h}$ is called the optical transfer function (OTF), which has the property of limiting the highest spatial frequency of the sample represented in the image to a cutoff frequency $\|\mathbf{k}\|_2 \leq k_{\max}$ and thus the maximum resolution of the image. Introducing non-uniform illumination of the sample will mix high spatial frequency information lying beyond the OTF's support into its passband [2]. In structured illumination microscopy (SIM), this idea can be used by implementing a sinusoidal stripe pattern illumination. It is described by a wave vector $\mathbf{p} = p_x\hat{k}_x + p_y\hat{k}_y$, with the unit vectors $\hat{k}_x$ and $\hat{k}_y$, that determines a pattern angle

$$\gamma = \arctan(p_y/p_x), \tag{1}$$

a fringe spacing of

$$L = (p_x^2 + p_y^2)^{-1/2}, \tag{2}$$

and a phase ϕ yielding the pattern's shift along the wave vector. Computational image reconstruction upon raw data acquisition may now separate spatial frequency components shifted from their original position into the passband and those naturally lying within the passband [3]. Relocating shifted components to their original position eventually results in a larger information content in the Fourier domain and equivalently in a higher resolution of the reconstructed image. For a single SIM reconstruction, multiple raw images of the sample with different illumination patterns are needed. In the 2D case, a typical configuration of three different illumination wave vectors $\mathbf{p}$ (with constant fringe period and three different orientations) and three different phases ϕ each yielding a total of nine images can be used to reconstruct one image. Image reconstruction in SIM for the described stripe pattern illumination is well known and can be derived from the more general 3D case [4]. Software