# Fast-physical optics modeling of two-photon fluorescence microscopy with 3D-structured illumination

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We perform a fast-physical optics modeling of two-photon fluorescence microscopy with 3D-structured illumination in the context of field tracing. We analyze the inhomogeneity of the 3D-structured illumination pattern and the temporal focusing, which should be accounted for in image processing.

## 1 Introduction

Recent advancements in microscopy techniques open up new possibilities towards imaging single live cells in deep tissue with high resolution. Structured illumination microscopy (SIM) [1] is one of the fluorescence imaging techniques that can tackle this challenge. However, in the case of thick samples the SIM technique suffers from out-of-focus fluorescence background signal, which significantly reduces the signal-to-noise ratio. To overcome this obstacle, it has been suggested to combine with two-photon excitation [2]. For the analysis and optimization of this kind of high-NA microscopy system, the physical optics modelling is required that includes coherence, aberration and interference effects. In this work, we perform a fast-physical optics modeling of the real microscopy system [3] in the context of field tracing [4]. The Local Plane Interface Approximation (LPIA) algorithm [5, 6], a free-space-propagation algorithm and the Fourier Modal Method (FMM) [7] are all connected. We analyze the inhomogeneity of the 3D-structured illumination pattern and the temporal focusing in the focal region, based on the aberration of the real lens system.

## 2 Theoretical Background

The 3D-structured illumination and the temporal focusing are investigated separately in $y$ and $x$ directions respectively.
The schematic of 3D-structured illumination is shown in Fig. 1 (a). The structured illumination is achieved via interfering three coherent beams in the focal region. The fully vectorial modeling is performed in the framework of field tracing as shown in Fig.1 (b) [8]. We trace the field through different optical elements in different regions by different operators via switching between different domains with use of the Bi-directional operators denoted as $\mathcal{B}$ in space domain, as $\tilde{\mathcal{B}}$ in spatial-frequency domain, in order to maximize the accuracy and minimize the numerical effort.

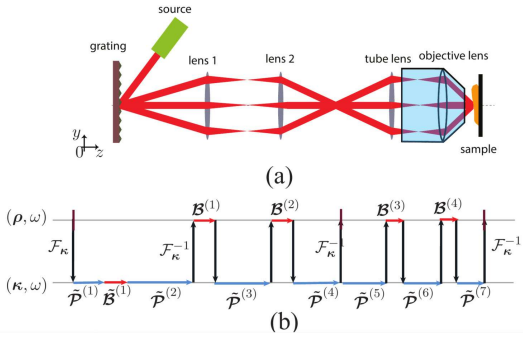


***Fig. 1*** *(a). Schematic of the microscopy system with 3D-structured illumination. Collimated laser beam illuminates the grating. Then the diffracted beam goes through the lenses including the high-NA objective lens to form the illumination pattern in the focal region. (b). Field tracing diagram.* $(\rho,\omega)$ *denotes the operator is in spatial domain* $(\kappa,\omega)$ *denotes the operator is in spatial-frequency domain.*

In Fig.1 (b), we generate the electric field in space domain, and then propagate the field by free space operator $\tilde{\mathcal{P}}_1$ in $\kappa$-domain which can be obtained rigorously by Spectrum of Plane Wave(SPW). When the field is diffracted from the grating, we trace it by $\tilde{\mathcal{B}}_1$ operator which is calculated rigorously by FMM in $\kappa$-domain. Then it is propagated again by free space operator. We make an assumption in this case that field which enters into the lens is in the homeomorphic zone which was sometimes referred to geometric zone [9]. Therefore, the homeomorphic inverse Fourier transform [9] can be applied to accelerate to whole modeling procedure. Then, the LPIA is applied to propagate the field through the curved interfaces of the lens in space domain. A homeomorphic Fourier transform is followed to obtain the field in $\kappa$-domain. We repeat the procedure to obtain the field in the focal region.
The schematic of the temporal focusing is shown in Fig. 2 (a). The temporal focusing is achieved via correlation of different spectrum in the focal region. The full modeling is analogous to the one for the 3D-structured illumination except additional (inverse)

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Fourier transforms are performed from/to time domain to/from spectral domain shown in Fig. 2 (b)

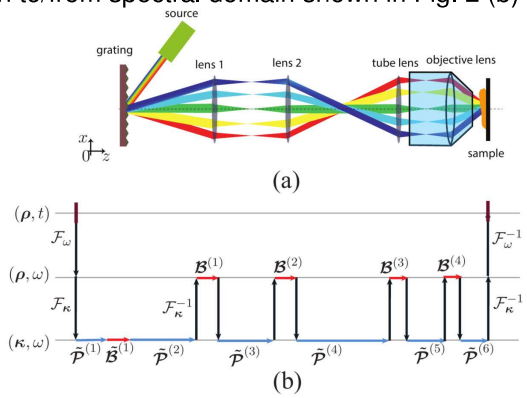


***Fig. 2*** *(a). Schematic of the microscopy system with temporal focusing. Collimated pulse laser illuminates the grating. Then the diffracted beam goes through the lenses including the high-NA objective lens to have the temporal focusing. (b). Field tracing diagram. Ananolgous to Fig. 1 (b).* $(\boldsymbol{\rho}, t)$ *denotes the operator is in time domain.*

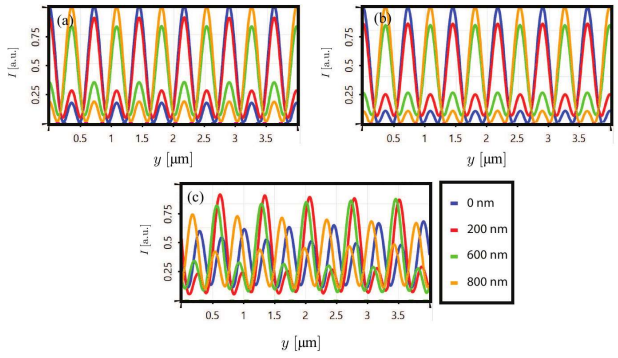


***Fig. 3*** *(a). The interference pattern assuming an ideal system. (b). The interference pattern by the real lens with perfect alignment. (c). The interference pattern by the real lens with lateral misalignment of the objective lens of* 450 µm*. Note that different colors represent the different defocused distance.*

## 3 Numerical Results

Results are obtained via the software VirtualLab Fusion [10]. The wavelength is 780 nm for 3D-structured illumination, and 780 nm of center wavelength of 100 fs pulse laser for temporal focusing. The grating is blazed grating with blazed angel 12°. The lens 1,2 and tube lens are from Thorlab (AC254-200-B). The objective lens is water immersion from Nikon (US Patent: 7889433B2). We show the 3D-structured interference pattern in Fig. 3. They are obtained within several seconds. The inhomogeneity defined the same as in [8] is 0 for both ideal lens and real lens with perfect alignment. But it increase to 0.3 when the lateral misalignment is 450 µm shown in Fig.3 (3). The temporal focusing is shown in Fig. 4 obtained within half a minute. The FWHM of the intensity is $\sim$ 4.5 µm and $\sim$ 5.4 µm for both ideal lens and real lens with perfect alignment. It increases to $\sim$ 7.5 µm.

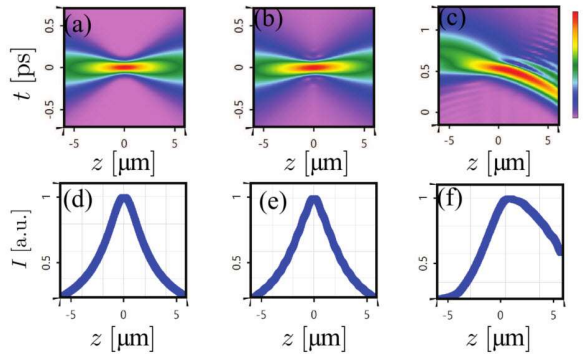


***Fig. 4*** *(a-c). The field in the focal region at the center point by ideal lens, real lens with perfect alignment and real lens with lateral misalignment of the objective lens of* 1.5 mm *respectively. (d-f). The corrensponding intensity to Fig.4 (a-c).*

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