Title

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Considerations for Two Beam Interference on Excitation and Emission Light Paths for Structured Illumination Microscopy

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Abstract The use of adaptive optics correction for structured illumination microscopy requires spatial sinusoid patterns in the sample obtained via two beam interference. We simulate the effect of AO correction on both excitation and emission paths, and propose a better SIM microscope based that considers the special requirements for SIM. **OCIS codes:** (110.0180) Imaging systems; (100.6640) Image processing; (110.1080) Imaging systems.

1. Introduction

The light microscope offers biologists the advantage *in vivo* imaging, while suffering from a reduced resolution due to constraints imposed in the optical domain. The hard limit on resolving power was given by Abbe as the diffraction limited resolution, which is roughly one-half wavelength of the light used in the imaging system. Further constraints on resolution are imposed by the optical system, image capture, and sample-induced aberrations. Super-resolution imaging can relax the constraint on resolution.

Structured Illumination Microscopy (SIM) is an optical transfer function (OTF)-based super-resolution technique that increases the OTF support region in Fourier space via spatial frequency mixing. In particular, the optical imaging system acts as a low-pass filter on the image of the biological sample, which constrains the OTF to low spatial frequencies. By optically mixing the biological sample with SIM fringes, high spatial frequency information from the sample is mixed down into the pass band of the microscope.

In addition to super-resolution, SIM techniques can also be applied to 3D imaging systems as a method of optical sectioning. This technique, known as sectioning SIM, does not rely on integral transformations in the least. Rather, sectioning SIM is a method for rejecting out-of-focus light that comes from axial regions above and below the focal plane within the sample. In either case, SIM requires that a high-contrast, high-fidelity SIM pattern be projected into the biological sample. Typically, SIM patterns are generated in the sample via two-beam interference, which is obtained by projecting the +/-1 diffractive orders of light from a diffractive grating onto the back aperture of the microscope objective.

To address the issue of light aberrations, adaptive optics (AO) has been introduced into microscopy. AO has been successful in removing some aberrations induced by the sample and the optical system. Most AO systems place the corrective element at a plane conjugate to the pupil plane of the microscope. Recalling the fact that the pupil plane is the Fourier dual of the focal plane in the sample, we note that the Fourier transform of the SIM fringes are impulse-like functions at the pupil plane. Therefore, it is not possible to make AO corrections on the SIM fringes in the excitation path of the microscope, since the two diffractive orders are too small to be affected by wavefront correction. Several authors have noted that lower order aberrations, those which can be represented by lower order Zernike polynomials, do not have a large effect on the generation of SIM fringes in the sample, so that AO correction on the emission path of light is sufficient.

In this work, we introduce a framework for modeling the wavefront aberrations of both excitation light and emission light within the context of SIM. We show that in some cases, AO correction is sufficient on the emission path of light alone, while in other cases it will be necessary to add AO correction on the excitation path of light. We use the Fourier beam propagation method as a convenient way to model the two-beam interference effect that is utilized to SIM pattern generations. This method allows us to model phase aberrations present in the excitation light path as linear combinations of Zernike polynomials. We choose the Zernike mode representation for aberrations since it is widely used in AO corrective systems. The rest of this paper is as follows: In the Methods section, we discuss the Fourier beam propagation method for modeling light traveling in the forward excitation path, and the autocorrelation of the pupil function to represent the light interaction with the OTF in the emission path. In the Results section, we show the simulations. We conclude with a discussion of our results, along with a proposed method for wavefront correction on excitation light for SIM fringes.

1. Methods

Here we introduce the simulation framework for this study. The light fields that cause the two-beam interference patterns present in SIM are represented as complex exponentials with wave number **k** based on the wavelength of light, in this case 488nm. Thus, the electric field **E** is obtained by creating a matrix

$$E = \exp\left(i \sin(\theta) \cos(\alpha)\, k_x \boldsymbol{x} + i \sin(\theta) \sin(\alpha)\, k_y \boldsymbol{y} + i \cos(\theta)\, k_z\right), \quad (1)$$

ith azimuthal angle α on the objective lens (0, π), beam angle θ as defined by the numerical aperture of the objective lens (in this case, NA=1), and **k** the wave number with *x*, *y*, and *z* components. The direction of propagation in the *z* direction.

With this representation of the EM fields for both beams, we can mathematically obtain the two-beam interference pattern by decomposing the fields into their polarized components and adding pairwise. To obtain the real-valued intensity of the field, ***I***, the *x*, *y*, and *z* components of ***E*** are multiplied by their complex conjugates and then summed:

$$\boldsymbol{I} = \sum_{n=x,y,z} E_n \cdot E_n^* \quad . \quad (2)$$

To represent the propagation these electromagnetic fields from the objective lens into the sample, we note that the distance travelled can be represented by a number oscillations of the field. In other words, we have a phase delay, which can be represented in the Fourier domain with a complex exponential:

$$\mathcal{F}\{\boldsymbol{E}\} \cdot e^{-ikz_0}, \quad (3)$$

with the result of having traveled a distance of z_0-nm being the inverse Fourier transform of (3). We can now obtain the two beam inteference pattern with Equation (2), which gives us the SIM fringes in the object plane.

To address the field aberrations, we can adjust our model so that the initial electric field is scale by a subset of the pupil function, which is in turn has a Zernike polynomial representation. Wavefront aberrations are induced by the sample and coverslip/mounting media index of refraction mismatch; these aberrations are in fact variations of the optical path distance (OPD) across the beam as it travels from the objective lens to the focal plane in the sample. Using Zernike polynomials is a convenient way to capture the OPD variation across the beam for reasons discussed above.

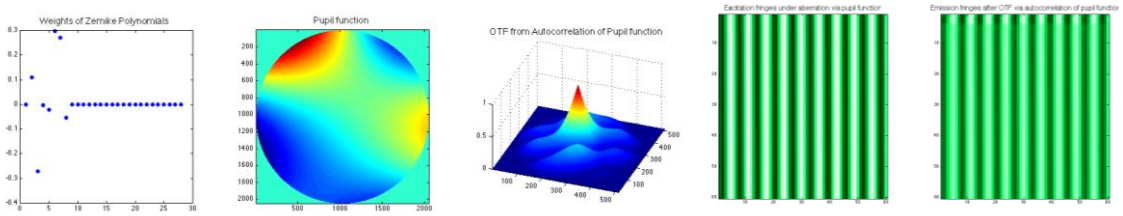
We choose the simulate the aperture of the objective lens as a large Zernike polynomial with a matrix of 2048x2048 pixels with a circular subset showing the active portion of the back aperture of the objective. The two interfering beams are represented as subsets of the aperture matrix with radially opposing locations, a size of 256x256 pixels, and an offset from the edge of the aperture matrix by 40 pixels. In this way both the aperture and the two interfering beams are represented by Zernike polynomials in a way that is faithful to actual SIM techniques.

Having produced the two-beam interference patterns in the sample by Equation (2), we can now simulate the effect of emission light under the effect of the OTF. There are two ways to simulate the OTF of the microscope. One of which is to calculate the Fourier dual of the PSF. This involves either theoretically calculating the PSF given the wavelength of light and the photonic qualities of the beam (single photon vs multi-photon laser source), or measuring the PSF directly on a diffraction limited object.

The other to obtain the OTF is to use the autocorrelation of the pupil function. In our case, since we have already represented the pupil function as a linear combination of Zernike modes, it makes sense to use this second method. Thus, we are are able to simulate the effect of optical modulation of the SIM fringes under the OTF of the microscope, and in this way obtain a simulation of the image which the science camera observes of the SIM fringes under both excitation and emission aberrations.

2. Results and discussion

We used the simulation framework to show two different types of aberrations. One type, shown in Figure 1 consists of low-order, low-magnitude aberrations in terms of Zernike bases. Figure 1(e), we see that the SIM fringes visible at the science camera have a good modulation depth, and are rotationally aligned with the azimuthal angle, which in this case corresponds to a “vertical” alignment.

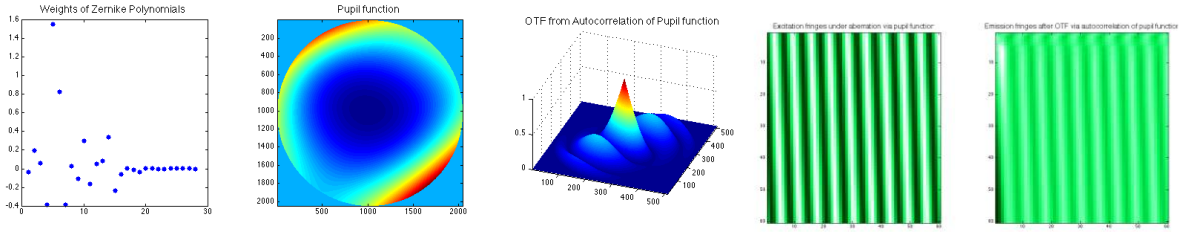


(a) (b) (c) (d) (e)

Figure 1: When the aberrations present at the pupil plane can be represented by lower order Zernike polynomials, the resulting SIM pattern at the science camera are acceptable.

On the other hand, in Figure 2 we see the simulation of high-order, high-magnitude aberrations. Figure 2(d) shows that the SIM fringes in the sample are in a different azimuthal orientation, while the bottom right panel shows the fringes under the effect of the OTF suffer from poor modulation depth. Naturally, this is bad for both super-resolution and sectioning SIM. In the former, the effect of high-order, high magnitude aberrations are seen to change the location of the delta function in reciprocal space, which causes difficulties in the SIM reconstruction. Under the effect of the OTF, we can see weak modulation depth, which inhibits the spatial frequency mixing effect upon which the super-resolution SIM reconstruction is based. For sectioning SIM, the azimuthal angle of the fringe patterns is not as important, if they remain constant throughout the imaging process. The modulation depth, however, is problematic, since it is not able to fully reject out of focus light.

In terms of AO correction, most AO systems will be successful in correcting for the OTF of the microscope, since the AO correction is conjugated to the pupil plane. This will correct for the poor modulation depth seen in Figure 2(e). However, the change of azimuth angle will not be addressed by this AO correction. In fact, there is a whole class of aberrations for which the excitation fringes undergo degradation. This is because the two-beam interference effect is degraded while light travels from the objective lens to the sample and passes through aberrating media.



(a) (b) (c) (d) (e)

Figure 2: With the presence of higher order Zernike mode aberrations at the pupil plane, the resulting SIM patterns are severely degraded by the time the light reaches the science camera. Not only are the spatial frequency values shifted in the Fourier plane (not shown), the fringes suffer from severe contrast reduction. The resulting SIM reconstruction from such aberrations would not be successful.

A better SIM system will place another AO correction on one of the interference beams at a plane that is conjugated to an aberrating layer. It is sufficient to have correction on a single beam, not both. This is because the two-beam interference effect arises from mutual phase coherence. Therefore, it is sufficient to match the wavefronts so that they are phase-coherent, rather than require a perfectly corrected wavefront on both beams. The precise location of the corrective element should be conjugate to an aberration plane in the sample, if that plane is known. AO systems in both astronomy and microscopy have be developed in multiconjugate AO. Correction for the wavefront in the detection path should be implemented as required by the biological tissue or sample.