

Estimation of Phase Shifts in Structured Illumination for High Resolution Imaging

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Abstract: The application of structured illumination for enhanced resolution requires accurate knowledge of phase shifts in the sinusoidal illumination. This work proposes a method to estimate random, unknown phase shifts and subsequent image reconstruction.

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1. Summary

The numerical aperture of an imaging system and the imaging wavelength limit the resolution. But this limit holds only for uniform, on-axis illumination with single-lens geometry and linear absorption and emission. Structured illumination breaks the constraint of uniform illumination to achieve superresolution. Structured illumination has been used in fluorescence imaging both for finer axial resolution and sectioning [1,2] and lateral superresolution [3-8]. Our work involves lateral resolution enhancement.

Multiple images of an object with a sinusoidally patterned illumination are taken. The Fourier spectrum of each sinusoidally illuminated image contains three overlapping replicas of the object Fourier spectrum – two shifted versions carrying superresolution information and one unshifted version. Three or more such images are taken. Each image differs in the phase shift of the sinusoidal illumination. These images are used to segregate the three overlapping object spectra. The knowledge of the phase shift in the sinusoidal illumination is critical for this processing. Any inaccuracy in this knowledge leads to artifacts in the reconstruction. Once separated, the spectra are combined appropriately to form a composite image. Similar composite images, obtained by rotating the sinusoidal pattern 60° and 120°, are combined to get an image which can have resolution as much as twice the diffraction limit.

Microscopes usually employ piezo-actuators to introduce known phase shifts of fixed step sizes in the sinusoidal illumination. But this is often impracticable, such as when imaging randomly moving cells *in vivo*. The ability to estimate these phase shifts in post-processing along with image registration would enable the application of this superresolution technique to many such areas of dynamic imaging. We use a Fourier based approach for the estimation of these phase shifts [8]. We assume random and completely unknown phase shifts. Our simulations show that our approach gives us good accuracy in estimating these phase shifts. We propose an improved algorithm for estimating the phase shifts to achieve even greater precision. We also propose an algorithm to reconstruct images with lateral superresolution using random phase shifts in the structured illumination images.

2. References

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