

# Phase Shift Estimation in Structured Illumination Imaging for Lateral Resolution Enhancement

Sapna A. Shroff, James R. Fienup, David R. Williams

Department of Electrical and Computer Engineering, Institute of Optics, Center for Visual Science  
University of Rochester, Rochester, NY – 14627, USA  
sapna@optics.rochester.edu

**Abstract:** Lateral resolution enhancement using structured illumination imaging requires accurate knowledge of phase shifts in the sinusoidal illumination on the object. We discuss a method to estimate these phase shifts and the resulting image reconstructions.

©2007 Optical Society of America

**OCIS codes:** (170.0180) Microscopy; (170.2520) Fluorescence microscopy; (050.5080) Phase shift

## 1. Introduction

The lateral resolution of an imaging system is limited by the numerical aperture of the system. This limits the ability to resolve finer details of the object. Structured illumination has been used to enhance the lateral resolution of images in microscopy [1–5]. The object is illuminated by a sinusoidal pattern. Several images are taken with the phase of the sinusoidal illumination shifted by a small amount in each. Images reconstructed from these can have as much as twice the resolution achieved by conventional forms of imaging for the same numerical aperture. The resolution of the image increases with the spatial frequency of the sinusoid, up to a fraction of two.

The accurate reconstruction of images in this technique requires very good knowledge of the phase shifts in the sinusoidal illumination in each image. Most previous implementations require prior knowledge of these phase shifts. Hence they demand expensive precision actuation equipment and cumbersome calibrations of the instrument in order to ensure the accuracy of these known phase shifts. Most reconstruction methods also demand equal step sizes of the phase shifts.

We investigate the possibility of estimating randomly introduced phase shifts, with no prior knowledge. We propose a post-processing method that can easily be incorporated into existing structured illumination setups with no additional hardware. Our technique should permit the use of inexpensive actuation equipment with no calibration. It should also support the imaging of moving objects such as *in vivo* retinal imaging. We also discuss here the reconstruction of images with these random phase shifts and artifacts in the reconstructed image in case of inaccuracy or imprecision of such phase estimates.

## 2. Incoherent illumination, incoherent image equation

We assume that a completely incoherent image of a grating, of spatial frequency  $f_o$  along the x axis and contrast  $m$ , is projected on the object producing a sinusoidal fringe illumination. We assume that the illumination and imaging optical transfer functions,  $\mathcal{H}$ , are identical. But this analysis could easily be extended to dissimilar imaging and illumination paths.  $N$  images are taken of this object, each having a different unknown phase shift,  $\phi_n$ , of the sinusoidal illumination. The spectrum (Fourier transform) of each image is given by [2,6],

$$\begin{aligned} \mathcal{G}_{in}(f_x, f_y) = \mathcal{H}(f_x, f_y)\mathcal{H}(0, 0)\mathcal{G}_g(f_x, f_y) + \frac{m}{2}\mathcal{H}(f_x, f_y)\mathcal{H}(f_o, 0)\mathcal{G}_g(f_x - f_o, f_y)e^{i\phi_n} \\ + \frac{m}{2}\mathcal{H}(f_x, f_y)\mathcal{H}(-f_o, 0)\mathcal{G}_g(f_x + f_o, f_y)e^{-i\phi_n} \end{aligned} \quad (1)$$

where  $\mathcal{G}_g$  is the Fourier transform of the actual object intensity and  $n = 1, 2, \dots, N$ . Each image has three copies of the object spectrum. The shifted spectra carry higher spatial frequencies that enhance resolution. Precise knowledge of the phase shifts enables these spectra to be extracted and combined to produce enhanced-resolution images.

## 3. Estimation of phase shift in each of $N$ image frames

We assume no prior knowledge of the randomly spaced phase shifts,  $\phi_n$ , of the sinusoidal illumination in each of the  $N$  images. One way of estimating the phase shift in each image is to fit a sinusoid to the existing pattern in the image. The method we propose uses the image spectrum at  $(f_o, 0)$ . As long as  $f_o$  is a medium spatial frequency, the

magnitude of the second term,  $\mathcal{H}^2(f_o, 0)\mathcal{G}_s(0, 0)$ , dominates over  $\mathcal{H}(f_o, 0)\mathcal{H}(0, 0)\mathcal{G}_s(f_o, 0)$  and  $\mathcal{H}(f_o, 0)\mathcal{H}(-f_o, 0)\mathcal{G}_s(2f_o, 0)$ . Since  $\mathcal{G}_s(0, 0)$  has zero phase and  $\mathcal{H}^2(f_o, 0)$  has zero phase when there are no aberrations,  $e^{i\phi_n}$  contributes the dominant phase component to Eq. (1) at  $(f_o, 0)$ . Therefore, the phase of  $\mathcal{G}_{sn}(f_o, 0)$  is approximately the desired phase,  $\phi_n$ .

Residual aberrations in the image should be compensated before estimating the phase. Further optimization routines and iterative algorithms could be used to achieve greater precision with this initial phase estimate [7].

We simulated a structured illumination setup with three orientations of the sinusoid (to achieve superresolution in all directions) with completely incoherent light. The errors vary with the orientation of the sinusoidal illumination because when a significant component of the object spectrum overlaps with the spectrum of the sinusoid, we see a decrease in accuracy of the estimated phase.

The accuracy in our estimate depends on the spatial frequency of the sinusoidal illumination (Fig. 1). We achieved accuracy better than  $6 \times 10^{-3}$  radians for frequencies,  $f_o$ , between 15 – 85% of the cutoff frequency,  $f_c$ . At higher spatial frequencies of the sinusoid, error in the estimate rises due to the reduction in the contrast of the incoherent grating illumination proportional to  $\mathcal{H}(f_o, 0)$ . At lower spatial frequencies of the sinusoid, error in the estimate may rise if the value of  $|\mathcal{G}_s(f_o, 0)|$  is large.

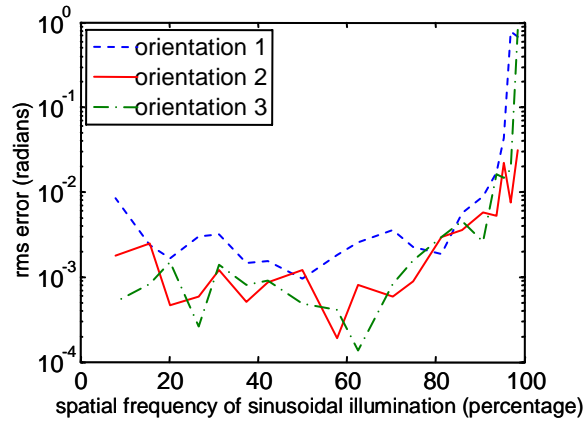


Fig. 1. Rms error in phase shift estimate versus spatial frequency of sinusoidal illumination,  $f_o/f_c$  with no noise

As shown in Fig. 2, for  $f_o = 0.5f_c$  and moderate SNR, the accuracy was better than  $1.5 \times 10^{-3}$  radians. For lower SNR values in the range 2 – 7, our error increased to  $4 \times 10^{-3}$  radians.

As shown in Fig. 3, for  $f_o = 0.97f_c$  and moderate to high SNR the accuracy was better than  $3 \times 10^{-2}$  radians. For lower SNR values in the range 2 – 13, our error increased up to 0.95 radians.

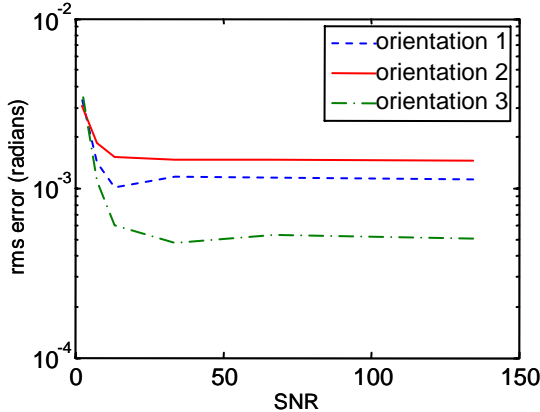


Fig. 2. Rms error in phase shift estimate versus SNR for sinusoidal illumination with  $f_o = 0.5f_c$ .

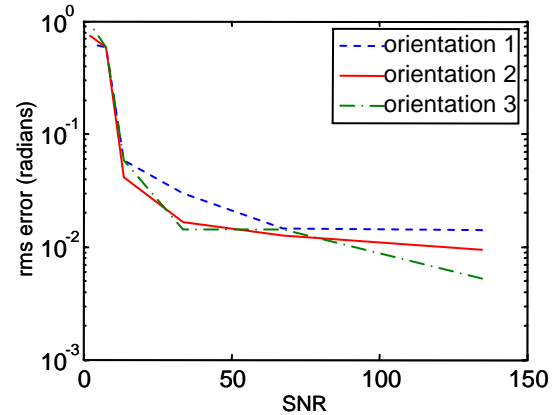


Fig. 3. Rms error in phase shift estimate versus SNR for sinusoidal illumination with  $f_o = 0.97f_c$ .

#### 4. Reconstruction of enhanced-resolution images

Using estimated values of phase shifts, we segregate the three copies of the object spectrum from these  $N$  equations (one per image) with singular value decomposition and pseudoinverse. A composite reconstruction is formed by appropriate weighting and superposition of the retrieved components. The results shown use OTF-compensated images with no aberrations. Large errors in the estimate usually cause artifacts in the reconstructed image. Further images taken with the sinusoidal illumination rotated by  $2\pi/3$  and  $4\pi/3$  radians are used to retrieve the complete object spectrum in all directions.

Figure 4 shows a simulated conventional image taken with uniform illumination. Images recovered from structured illumination images taken on the same system are shown in Fig. 5 and Fig. 6. The results shown in Fig. 5 used a sinusoidal illumination with  $f_o = 0.5f_c$  and SNR of 100. The maximum possible superresolution is 1.5x in this case. The results shown in Fig. 6 used a sinusoidal illumination with  $f_o = 0.97f_c$  and no noise which has maximum superresolution of almost 2x; for this case however, image quality degrades rapidly as noise is introduced. The images reconstructed used estimated phase shifts. We saw no significant artifacts such as sinusoidal patterns in the image [7].



Fig. 4. Conventional image taken with a uniform illumination.



Fig. 5. Reconstructed composite image with  $f_o = 0.5f_c$ , SNR of 100, using estimates of phase shift acquired by proposed method.



Fig. 6. Reconstructed composite image with  $f_o = 0.97f_c$ , no noise, using estimates of phase shift acquired by proposed method

#### 5. Conclusions

We have proposed a method of estimating randomly introduced, unknown phase shifts in the sinusoidal illumination used for incoherent structured illumination imaging. We also reconstruct images with enhanced lateral resolution using these estimated phase shifts. We have observed no visible sinusoidal patterned artifacts in these images.

We would like to acknowledge funding for this work from NSF Science & Technology Center for Adaptive Optics - AST-9876783 and NIH Core Grant in Vision Science - EY 001319.

#### 6. References

- [1] M. G. L. Gustafsson, "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy," *J. Microsc.*, 198: 2, 82- 87 (2000)
- [2] R. Heintzmann, C. Cremer, "Laterally Modulated Excitation Microscopy: Improvement of resolution by using a diffraction grating," in *Optical Biopsies and Microscopic Techniques III*, I. J. Biglo *et al.*, , Editors, Proc.SPIE 3568, 185 - 196 (1999)
- [3] M. Gustafsson, "Extended-Resolution Reconstruction of Structured Illumination Microscopy Data," in *Adaptive Optics: Analysis and Methods/Computational Optical Sensing and Imaging/Information Photonics/Signal Recovery and Synthesis Topical Meetings on CD-ROM*, Technical Digest (Optical Society of America, 2005), paper JMA2.
- [4] M. G. L. Gustafsson, L. Shao, D.A. Agard, J.W. Sedat, "Fluorescence microscopy without resolution limit," in *Biophotonics/Optical Interconnects and VLSI Photonics/WBM Microcavities*, 2004 Digest of the LEOS Summer Topical Meetings (2004)
- [5] M. G. L. Gustafsson, "Extended resolution fluorescence microscopy", *Curr. Opin. Struct. Biol.*, 9, 627-634, (1999)
- [6] J. W. Goodman, *Introduction to Fourier Optics*, Third Edition, (Roberts & Co., Greenwood Village, CO, 2005)
- [7] L. H. Schaefer, D. Schuster, J. Schaffer, "Structured illumination microscopy: artefact analysis and reduction utilizing a parameter optimization approach," *J. Microsc.*, 216:2, 165-174 (2004)