BOSTON UNIVERSITY

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RPC Report

Super-Resolved Spatial Light Interference Microscopy

by

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B.S., Bilkent University, 2012

Submitted in partial fulfillment of the requirements for the Ph.D. candidacy

April 26, 2013
ABSTRACT

In this report, structured illumination applications for spatial light interference microscopy (SLIM), based on the study titled “Super-Resolved Spatial Light Interference Microscopy” by Chu et al., is investigated. The study shows that the structured illumination technique can be used in spatial light interference microscopy to improve the lateral resolution by a factor of two. Both direct and modified applications of the structured illumination are presented in this study and it is concluded that even though the direct application of the structured illumination improves the resolution, it also results in low contrast and considerable amount of artifacts in the image, whereas with a modification in the SLIM setup and demodulation process, the image contrast can be increased and the artifacts can be lessened considerably. This report includes the following sections: Background, Spatial Light Interference Microscopy, SLIM with Structured Illumination, Results and Summary & Discussions.
1 Background

Most of the biological samples are transparent, which act as phase objects when they are incident to illumination light [1]. Although phase contrast microscopy introduced by Zernike [2] provides information of the biological samples non-invasively with nanoscale precision without using any agents, the information gathered is rather qualitative, and obtaining sample information quantitatively by making use of the phase difference induced by the samples would render the measurements of the biological structures with nanometer scales [1]. In the last decade, studies such as [3-7] have been able to collect sample information quantitatively through combining the phase contrast microscopy with various techniques. For instance, in [1], spatial light interference microscopy (SLIM), which combines the phase contrast microscopy with holography, is introduced. Due to the spectrally broad and spatially incoherent illumination applied in SLIM, it has an advantage of providing clean background over the other quantitative methods that involve phase contrast imaging. In addition, as the unscattered light from the illumination and the scattered light from the sample travel a common optical path before they interfere at the image plane, the system is not affected by vibrations. Although it provides an axial resolution with nanometer scale, SLIM lacks a high-lateral resolution due to Abbe’s diffraction limit.

Various fluorescence microscopy techniques such as two-photon microscopy, total internal reflection fluorescence microscopy (TIRF), stimulated emission depletion microscopy (STED), and stochastic optical reconstruction microscopy (STORM) [8-12] have been demonstrated to perform imaging beyond the diffraction limit, whereas the techniques used in those microscopy methods have not been proven to be useful for non-fluorescence microscopy techniques except for structured illumination microscopy, which can improve the lateral resolution by factor of two as explained in [13]. The fundamental principle behind is that by applying a sinusoidally patterned illumination to an object, we obtain a moiré pattern that combines the high spatial frequencies of the object with the spatial frequency of the sinusoidal illumination, which as a result, shifts the high spatial frequencies of the object into the passband of the imaging system [14]. In other words, structured illumination moves the imaging information from outside region into the physically observable region through moiré fringes, hence making that information observable [13]. In this study that we are investigating in this report, it is shown that by reconfiguring SLIM system such that when combined with structured illumination technique, high contrast images with increased lateral resolution can be achieved.

In the next section, we first provide a short introduction to SLIM and then formulate the Fourier analysis of its image formation.

2 Spatial Light Interference Microscopy

Quantitative phase imaging (QPI) is of great importance as it promises nanometer scale structure measurements in a non-invasive fashion [15]. SLIM, whose schematic is given in the Figure 1(a), offers a high sensitive QPI by combining conventional phase contrast microscopy with holography [1].

In phase contrast microscopy, $\pi/2$ phase shift is introduced between the scattered and unscattered light from the sample as explained in [2], and in addition to that, SLIM module shown in Figure 1(a) adds phase shifts by multiples of $\pi/2$ from liquid crystal phase modulator (LCPM) [1]. The patterns on LCMP is determined to match the phase ring image so that the additional phase shifts can be controlled between the scattered and unscattered parts of the image field, and as shown in the Figure 1(b), four different images corresponding to four different phase shifts ($0, \pi/2, \pi, 3\pi/2$) are obtained to produce a quantitative phase image, which is given in Figure 1(c) [1]. This quantitative phase image is proportional to $\phi(x, y)$, which is formulated in [1] as follows:

$$\phi(x, y) = \frac{2\pi}{\lambda} \int_{0}^{h(x,y)} [n(x, y, z) - n_0]dz$$

(1)

Note that in Equation 1, $n(x, y, z) - n_0$ denotes the local refractive index difference between the cell
and its surrounding medium, \( h \) denotes the local thickness of the cell and \( \lambda \) is the central wavelength of the illumination light, also the local phase shift, \( \phi \), which is determined very precisely, provides detection of local thickness changes, \( h \), with a scale that is much smaller than the wavelength of the light [1]. In the next section, Fourier analysis of the image formation of SLIM is presented.

Figure 1: (a) Schematic of the SLIM setup. (b) Phase rings and their corresponding images. (c) Quantitative phase image of a hippocampal neuron.

2.1 Fourier Analysis of SLIM Image Formation

As briefly introduced in the previous section and shown in the Figure 2 below, typically, SLIM setup is comprised of a circular source, a condenser lens, a sample plane, an objective lens, a pupil plane, an imaging lens and a sensor. Note that in Figure 2 it appears that the phase modulation element is integrated into the pupil plane, whereas in the real setup, it has been placed separately in a plane that is conjugate to the pupil plane. Therefore, in the pupil plane there are two masks overlapping; one is an amplitude attenuation mask, and the other one is a phase modulation mask. Note that both of the masks are only effective on the unscattered light.

Figure 2: Schematic of the SLIM setup.

Furthermore, Köhler illumination is used, i.e., illumination and the image paths are different, and the system is achromatic, i.e., phase modulations are uniform for all wavelengths, hence the image formation can be considered just for the center wavelength. The source and the back focal objective plane are Fourier planes, denoted by the coordinates in frequency domain: \( f = (f_x, f_y) \) with units of \( NA_o/\lambda \) where \( NA_o \) is the numerical aperture of the objective and \( \lambda \) is the center wavelength. The sample and the image planes are denoted by the spatial coordinates: \( r = (x, y) \). Furthermore, the
circular source can be expressed as follows:

\[ I_s(\vec{f}_s) = \begin{cases} 1, & |\vec{f}_s| \in f_s + [-\varepsilon, \varepsilon], \\ 0, & \text{elsewhere}; \end{cases} \]  

(2)

where \( f_s = NA_c/\lambda \) is the radius of the circular source, \( NA_c \) is the numerical aperture of the condenser lens, and \( 2\varepsilon \) is the width of the circle. Then the signal recorded by the sensor (either CCD or CMOS) can be expressed as given in the following equation:

\[ I(\vec{r}) = \int |T(\vec{r})e^{i2\pi(\vec{f}_s \cdot \vec{r})} \otimes p(\vec{r})|^2 d\vec{f}_s, \]  

(3)

where \( T(\vec{r}) \) is the complex transmission function of the sample, \( p \) is the point spread function (PSF), which is the Fourier transform of the pupil function \( (H) \), and \( \otimes \) is the convolution operator. Note that \( T \) can be decomposed into two parts: unscattered light and scattered light as follows:

\[ T = U_1 + U_2, \]  

(4)

where \( U_1 \) is the unscattered light and \( U_2 \) is the scattered light. Then the Fourier transform of the recorded signal can be expressed as follows:

\[ \tilde{I}(\vec{f}) = \int [\tilde{T}(\vec{f} + \vec{f}_s)H] \ast [\tilde{T}(\vec{f} + \vec{f}_s)H] d\vec{f}_s, \]  

(5)

where \( \tilde{T} \) is the Fourier transform of \( T \), the object field, and \( \ast \) is the correlation operator. Since the cutoff frequency of the pupil is \( f_o \), then frequencies lower than \( f_o + f_s \) can pass through the SLIM system, i.e., the cutoff frequency of the SLIM system is:

\[ f^{(SLIM)} = f_o + f_s. \]  

(6)

As explained earlier, there are two masks overlapping in the pupil plane: amplitude mask and phase mask, hence the pupil function, \( H \), can be expressed as the sum of the two mask functions as follows:

\[ H(\vec{f}, \phi) = ae^{i\phi}H_1(\vec{f}) + H_2(\vec{f}), \]  

(7)

where \( H_1 \) is comprised of the amplitude and the phase mask, where \( a \) denotes the attenuation coefficient, and the \( \phi \) denotes the phase shift. \( H_2 \) is the unmodulated part of the pupil. Thus, the recorded signal can be written as the interference of the light passing through the pupils, \( H_1 \) and \( H_2 \) as follows:

\[ \tilde{I}(\vec{f}; \phi) = a^2\tilde{I}_{11} + \tilde{I}_{22} + ae^{i\phi}\tilde{I}_{12} + ae^{-i\phi}\tilde{I}_{12}^*, \]  

(8)

where

\[ \tilde{I}_{ij}(\vec{f}) = \int [\tilde{T}(\vec{f} + \vec{f}_s)H_i(\vec{f})] \ast [\tilde{T}(\vec{f} + \vec{f}_s)H_j(\vec{f})] d\vec{f}_s, \quad i, j = 1, 2. \]  

(9)

and in spatial domain, it can be expressed as follows:

\[ I(\vec{r}; \phi) = a^2I_{11} + I_{22} + ae^{i\phi}I_{12} + ae^{-i\phi}I_{12}^*. \]  

(10)

Note that \( H_1 \), the part of the pupil that has the amplitude and the phase mask, is chosen to match the circular source, so the unscattered light from the source will pass through \( H_1 \), whereas only the scattered light from the sample will pass through \( H_2 \). Therefore, the recorded signal can be considered as the interference between the scattered and the unscattered light as given in the following equation:

\[ I(\vec{r}; \phi) = |aU_1e^{i\phi} + U_2|^2 = |U_1||ae^{i\phi} + \beta e^{\phi_2}|^2, \]  

(11)
where $\beta$ is the amplitude ratio and $\phi_{12}$ is the phase difference between scattered and unscattered light. The added phase, $\phi$, is modulated by a spatial light modulator (SLM). Equation 10 can be decomposed into four terms, each of which can be considered as an image. Among those four terms, the term that has $\phi_{12}$ in it is the modulated term, which can be rewritten as follows: $I_{12} = |I_{12}|e^{i\phi_{12}}$, where $I_{12}$ and $\phi_{12}$ are given in Equation 12 and Equation 13 respectively.

$$\phi_{12} = \arctan \left( \frac{I(\vec{r}; -\pi/2) - I(\vec{r}; \pi/2)}{I(\vec{r}; 0) - I(\vec{r}; \pi)} \right), \quad (12)$$

$$|I_{12}| = \sqrt{\left( I(\vec{r}; -\pi/2) - I(\vec{r}; \pi/2) \right)^2 + \left( I(\vec{r}; 0) - I(\vec{r}; \pi) \right)^2} / 4a. \quad (13)$$

The rest of the unmodulated terms in Equation 11 are also given in the following equation:

$$\langle I \rangle = a^2 I_{11} + I_{22} = \frac{I(\vec{r}; -\pi/2) + I(\vec{r}; \pi/2) + I(\vec{r}; 0) + I(\vec{r}; \pi) }{4}. \quad (14)$$

Furthermore, $U_2/U_1$, the ratio between scattered light and unscattered light can be calculated as follows:

$$\beta(\vec{r}) = \left| \frac{U_2}{U_1} \right| = \frac{\langle I \rangle - \sqrt{\langle I \rangle^2 - 4a^2 |I_{12}|^2} }{2|I_{12}|}. \quad (15)$$

Hence the estimated object field in the sample plane can be expressed as given below:

$$U^{(SLIM)}(\vec{r}) = \sqrt{I_{11}(1 + \beta e^{i\phi_{12}})}, \quad (16)$$

where $I_{11} = \langle I \rangle / (a_2 + \beta_2)$, then the object’s phase information can be found as follows:

$$\phi^{(SLIM)}(\vec{r}) = \arctan \left( \frac{\text{imag}(U^{(SLIM)})}{\text{real}(U^{(SLIM)})} \right). \quad (17)$$

Note that the estimated field and the phase information, given in Equation 16 and Equation 17 respectively, are approximations of the original field and phase information, as the SLIM is bandlimited with cutoff frequency, $f_c = f_o + f_s$. The lateral resolution of the system can be calculated as follows:

$$d^{(SLIM)} = 1.22 \frac{\lambda}{NA_o + NA_c} = \frac{1.22}{f_c^{(SLIM)}}. \quad (18)$$

In Figure 3(a), a sample image of resolution chart obtained by SLIM is shown. In this particular example, following parameters are considered: NA of the objective ($NA_o$) is 0.6, NA of the condenser ($NA_c$) is 0.54, the width of the source is 1% of its radius, the attenuation of the unscattered light is 0.1 and the center wavelength is 0.5 $\mu$m. Thus, from Equation 18, we find $f_c = (NA_o + NA_c)/\lambda = 2.28 \mu m^{-1}$, hence the lateral resolution is found as 535 nm. Due to the limited bandwidth, as shown in Figure 3(a), the system is unable to resolve the elements smaller than element 5 of group 1. As can be seen from the Equation 18, if the cutoff frequency ($f_c$) can be increased, the lateral resolution can be improved, hence the system can resolve finer details. In the next section, it is explained that by using a structured illumination scheme, the cutoff frequency can be increased, which in turn increases the lateral resolution.
3 SLIM with Structured Illumination

In this study, two different methods are explained for integrating structured illumination into SLIM: A direct application with an only addition of grating while the rest of the technique remains the same, and a modified application with the changes in the SLIM setup and the demodulation process, which improves the contrast and lessens the artifacts. First we analyze the direct application scheme, which is explained in the following section.

3.1 Direct Application of Structured Illumination in SLIM (SLIM+SI-direct)

In this technique, the grating is added in front of the sample plane providing structured illumination of the sample. With the grating added, the object field, $T$, is now as follows:

$$T'(\vec{r}) = T(\vec{r})(1 + m \cos(2\pi \vec{f}_g \cdot \vec{r} + \theta)),$$

where $\vec{f}_g$ is the spatial frequency, $\theta$ is the phase of the grating and $m$ is the contrast of the sinusoidal pattern on the phase grating. Fourier transform of the object field is given as follows:

$$\tilde{T}'(f) = \tilde{T}(f) + \frac{m}{2} e^{i\theta} \tilde{T}(f + f_g) + \frac{m}{2} e^{-i\theta} \tilde{T}(f - f_g).$$

By using Equation 5, we can obtain the recorded signal as follows:

$$\tilde{I}'(\vec{f}; \theta) = \int [\tilde{T}'(\vec{f} + \vec{f}_s) H] \ast [\tilde{T}'(\vec{f} + \vec{f}_s) H] d\vec{f}_s$$

$$= \tilde{I}_0(f) + \frac{m}{2} e^{i\theta} \tilde{I}_{+1} + \frac{m}{2} e^{-i\theta} \tilde{I}_{-1} + \frac{m^2}{4} e^{i2\theta} \tilde{I}_{+2} + \frac{m^2}{4} e^{-i2\theta} \tilde{I}_{-2}.$$
where

\[ \tilde{I}_0 = \int \left\{ \sum_{i=-1}^{1} [\mathcal{T}(\tilde{f} + i\tilde{f}_g + \tilde{f}_s)H] * [\mathcal{T}(\tilde{f} + i\tilde{f}_g + \tilde{f}_s)H] \right\} d\tilde{f}_s, \]  

(22)

\[ \tilde{I}_{+1} = \int \{ [\tilde{T}(\tilde{f} + \tilde{f}_g + \tilde{f}_s)H] * [\tilde{T}(\tilde{f} + \tilde{f}_g + \tilde{f}_s)H] + [\tilde{T}(\tilde{f} + \tilde{f}_g + \tilde{f}_s)H] * [\tilde{T}(\tilde{f} - \tilde{f}_g + \tilde{f}_s)H] \} d\tilde{f}_s, \]  

(23)

\[ \tilde{I}_{-1} = \int \{ [\tilde{T}(\tilde{f} - \tilde{f}_g + \tilde{f}_s)H] * [\tilde{T}(\tilde{f} + \tilde{f}_g + \tilde{f}_s)H] + [\tilde{T}(\tilde{f} + \tilde{f}_g + \tilde{f}_s)H] * [\tilde{T}(\tilde{f} - \tilde{f}_g + \tilde{f}_s)H] \} d\tilde{f}_s, \]  

(24)

\[ \tilde{I}_{+2} = \int [\tilde{T}(\tilde{f} + \tilde{f}_g + \tilde{f}_s)H] * [\tilde{T}(\tilde{f} - \tilde{f}_g + \tilde{f}_s)H] d\tilde{f}_s, \]  

(25)

\[ \tilde{I}_{-2} = \int [\tilde{T}(\tilde{f} - \tilde{f}_g + \tilde{f}_s)H] * [\tilde{T}(\tilde{f} + \tilde{f}_g + \tilde{f}_s)H] d\tilde{f}_s. \]  

(26)

Using the same phase shifting method as explained in [14], an image can be constructed from Equation 21 as follows:

\[ \tilde{I}(\tilde{f}) = \tilde{I}_0(\tilde{f}) + \tilde{I}_{+1}(\tilde{f} - \tilde{f}_g) + \tilde{I}_{-1}(\tilde{f} + \tilde{f}_g) + \tilde{I}_{+2}(\tilde{f} - 2\tilde{f}_g) + \tilde{I}_{-2}(\tilde{f} + 2\tilde{f}_g). \]  

(27)

As Equation 21 has five terms, five images are to be extracted from \( \tilde{I} \). Also note that the cutoff frequency is now increased by \( \tilde{f}_g \). To achieve superresolution in all directions, the rotation of the sinusoidal fringe illumination by 0°, 120°, and 240° is carried out. Each image requires 15 frames, and changing the phase of the pupil function, \( H_1 \), four times; one super-resolved phase image can be obtained with 60 frames in total. Earlier in this report, it is assumed that the unscattered light passes through \( H_1 \) and only the scattered light passes through \( H_2 \). However, in reality, as the phase grating leads to bending of the unscattered light such that a portion of it actually passes through the \( H_2 \), which results in reduced image contrast. The performance of the SLIM with the directly applied structured illumination technique is given in Figure 3(b). The same SLIM setup is used with an addition of a phase grating placed in front of the sample plane with frequency of 1.8 \( \mu m^{-1} \), and all the other parameters are kept same as in the previous case whose result is shown in Figure 3(a). As is clear from Figure 3(b), there is an improvement in the resolution compared to the previous case. Yet, the contrast is low and there are significant amount of artifacts in the image. The reason for that is based on the fact that in phase microscopy, there is no linear relation between object and the image intensity, and in contrast to the structured illumination microscopy where \( \tilde{I}_0 \) only denotes the unshifted spectrum while \( \tilde{I}_{\pm1,\pm2} \) denote down and up shifted parts of the spectrum, \( \tilde{I}_{0,\pm1,\pm2} \) denote mixture of shifted and unshifted spectrum as can be seen from the Equations 22-26. As a result, the true object spectrum cannot be reconstructed by the demodulation process. As there is a linear relation between the object and the field, a method for performing demodulation with field, which results in better contrast and improved lateral resolution is explained in the next section.

### 3.2 Modified SLIM and the Demodulation Process for Structured Illumination (SLIM+SI-adapted)

As explained in the previous section, the unscattered light has three components with center frequencies at 0, ±\( \tilde{f}_g \), which can also be deduced from the Equation 20. To modulate the entire unscattered light, the pupil function is modified as follows:

\[ H' = ae^{i\phi}H'_1 + H'_2, \]  

(28)

where
The composite image in the spatial domain can be obtained by taking the inverse Fourier transform of $U$.

Note that $H'_2$ is the unmasked area of the pupil, hence, now we can assume that the unscattered light will pass through $H'_1$, whereas the scattered light will pass through $H'_2$. With the added phase grating, the estimated object field can be considered as a product of the grating function and the original object field, so the estimated object field is as follows:

$$U'(\vec{r}; \theta) = U(1 + m \cos(2\pi \vec{f}_g \cdot \vec{r} + \theta)),$$

(30)

Also the Fourier transform of the object field is given below:

$$\tilde{U}'(\vec{f}; \theta) = \tilde{U}(\vec{f}) + \frac{m}{2} e^{i\theta} \tilde{U}(\vec{f} + \vec{f}_g) + \frac{m}{2} e^{-i\theta} \tilde{U}(\vec{f} - \vec{f}_g).$$

(31)

As stated in the previous section, there is a linear relation between the object and the field; the Fourier transform of the object field is composed of three terms: two shifted fields and one unshifted field as can be seen in Equation 31. For three different values of phase grating ($\theta$), there are three object fields, and their Fourier transforms are given in a matrix form as follows:

$$\begin{bmatrix} \tilde{U}'(\vec{f}; \theta_1) \\ \tilde{U}'(\vec{f}; \theta_2) \\ \tilde{U}'(\vec{f}; \theta_3) \end{bmatrix} = \begin{bmatrix} e^{-i\theta_1} & 1 & e^{i\theta_1} \\ e^{-i\theta_2} & 1 & e^{i\theta_2} \\ e^{-i\theta_3} & 1 & e^{i\theta_3} \end{bmatrix} \begin{bmatrix} \frac{m}{2} \tilde{U}(\vec{f} - \vec{f}_g) \\ \tilde{U}(\vec{f}) \\ \frac{m}{2} \tilde{U}(\vec{f} + \vec{f}_g) \end{bmatrix}.$$  

(32)

By inverting the matrix given above, we can obtain $\tilde{U}(\vec{f})$, $\tilde{U}(\vec{f} + \vec{f}_g)$ and $\tilde{U}(\vec{f} - \vec{f}_g)$, and by shifting $\tilde{U}(\vec{f} + \vec{f}_g)$ and $\tilde{U}(\vec{f} - \vec{f}_g)$ components to $(f_x, f_y)$ coordinates, we get $\tilde{U}_\pm(\vec{f})$, and combining them with $U(\vec{f})$, the composite object spectrum is obtained as follows:

$$\tilde{U}^{(xSLIM)} = \tilde{U}_-(\vec{f}) + \tilde{U}(\vec{f}) + \tilde{U}_+(\vec{f}).$$

(33)

Now the cutoff frequency of the system becomes $f_c + f_g$ where $f_g \leq f_o + f_s$ hence the maximum cutoff frequency is extended by a factor of two:

$$\max(f_c^{(xSLIM)}) = 2(f_o + f_s) = 2f_c^{(SLIM)}.$$  

(34)

As a result of doubling the cutoff frequency, the lateral resolution is improved by a factor of two. The composite image in the spatial domain can be obtained by taking the inverse Fourier transform of $\tilde{U}^{(xSLIM)}$ as follows:

$$U^{(xSLIM)}(\vec{r}) = \mathcal{F}^{-1}\{\tilde{U}^{(xSLIM)}\}. $$  

(35)

Also, the phase information regarding the object can be found as follows:

$$\phi^{(xSLIM)}(\vec{r}) = \text{arctan} \left( \frac{\text{imag}(U^{(xSLIM)})}{\text{real}(U^{(xSLIM)})} \right).$$

(36)

The result of this method is shown in Figure 3(c). The same setup with the same parameters is used as in the previous case with the exception of the modifications in the pupil plane and the demodulation process. As can be seen from Figure 3(c), higher contrast image with fewer artifacts, compared to the previous cases, is obtained with this technique. Furthermore, in Figure 3(d), a comparison is made across the three methods discussed so far, plotting the cross-sectional image of an element obtained using the three methods (SLIM, SLIM+SI-direct and SLIM+SI-adapted). As can be seen from the comparison, the adapted scheme of structured illumination with SLIM procedure provides more accurate results.
compared to the direct application of structured illumination with SLIM. Moreover, the number of frames required to reconstruct the images is also reduced with adapted scheme; in the direct application of structured illumination with SLIM, there are four phase steps, each requiring three different directions of grating and each direction requires 5 phase shifts, thus adding to 60 frames in total to reconstruct a phase image, whereas in the adapted application of the structural illumination with SLIM, each direction requires only three phase shifts, resulting in 36 frames in total to reconstruct the phase image.

In Figure 4, the modified SLIM setup (xSLIM) is shown. In principle, as the grating is added in front of the sample plane, this can be translated into an experimental setup with add-on module consisting of an objective, mirror, lens and a grating as shown in Figure 4(a). Note that the NA of the objective inside of the add-on module should be same or higher than the NA of the objective used to collect scattered light. In Figure 4(b), the reflection mode setup where the back-scattered light from the sample is focused on an objective to obtain an image is shown. In addition, it may be necessary to add antireflection coating to the glass substrates surface to reduce the amount of unscattered light.

Figure 4: Schematics for xSLIM setup for (a) Transmission mode and (b) Reflection mode.

4 Results

In this section, first the results of imaging simulation of randomly positioned beads are presented. Then, the noise effects on the image quality is investigated, and lastly the the effects of grating contrast on the image is explored through the simulations of an original fluorescence image of rat hippocampal neurons.

4.1 Simulation Results

To validate the methods explained in the previous sections, imaging simulations of randomly positioned beads are carried out. Bead pairs, separated from each other by 0.25 µm, are generated and randomly positioned. Note that this separation is smaller than the resolution limit, which is 0.5 µm. The beads are Gaussian functions with 0.125 µm width. For the simulation of the SLIM setup, the following parameters are considered: The numerical aperture of the objective (NA₀) is 0.8, NA of the condenser (NAₖ) is 0.55, the width of the source is 1% of its radius, and the center wavelength is 0.5 µm. Thus, from Equation 18, we find \( f_c = (NA_0 + NA_k) / \lambda = 2.48 \, \mu m^{-1} \) hence the lateral resolution is 492 nm. For the simulation of the modified SLIM (xSLIM) setup, same parameters are used with an addition of a phase grating with \( f_g = 2.464 \, \mu m^{-1} \). The results are shown in the Figure 5.
As can be seen from Figure 5, the close bead pairs are well resolved by xSLIM, whereas, SLIM was not able to resolve them. Also, note that beads appear smaller in xSLIM image due to the fact that the PSF for xSLIM is narrower than that for SLIM. Figure 5(c) compares the cross-sectional images of the bead pairs obtained by SLIM and xSLIM to the original. As is clear from that figure, xSLIM provides twice the resolution, which is in this case 0.25 µm, compared to that of SLIM.

4.2 The Effects of Noise on Image Quality

In this section, the effects of noise in the image sensor is investigated through imaging simulations for various signal to noise ratios (SNR). Again, using the same parameters as in the previous section, and assuming the grating contrast to be 0.01, the bead pair images are simulated for $10^4$, $10^3$ and $10^2$ SNR values, and the results are shown in the Figure 6 below:

From Figure 6, we can conclude that even with relatively low value of SNR, $10^2$ in this case, the 0.25 µm bead separation is resolved. In Figure 7, we see results of another simulation where the effect of grating contrast on the image quality is investigated with an original fluorescence image of rat hippocampal neurons. Note that the intensity of the image is converted to height to turn the image into a phase-type object for this simulation. In the case of Figure 7(a) and Figure 7(c), the SNR is 1000 and in the case of Figure 7(b) and Figure 7(d), the SNR is 100. As can be seen when comparing Figure
7(a) to Figure 7(c) and Figure 7(b) to Figure 7(d), lower grating contrast leads to an image with more noise and lower contrast.

![Figure 7: Imaging results of xSLIM with real sample for the following grating contrast and SNR: (a) $m = 0.01$, SNR=1000, (b) $m = 0.01$, SNR=100, (c) $m = 0.001$, SNR=1000 and (d) $m = 0.001$, SNR=100.](image)

Moreover, the authors of this study also denote that with an addition of filtering procedure or optical transfer function (OTF) compensation, the resolution of xSLIM can be further improved.

## 5 Summary & Discussions

In this report, we investigated an optical method introduced by Chu et al., which renders a resolution beyond the diffraction limit for SLIM by employing structured illumination. The key point of this study is to utilize the imaging information beyond cutoff frequency for improving resolution. It is first demonstrated that by directly applying structured illumination to SLIM, the resolution can be improved, however with considerable amount of artifacts and low contrast in the images. Then, it is also shown that by applying structured illumination to a modified SLIM system, the resolution improvement by a factor of 2 with fewer artifacts and better contrast can be achieved, and the results comparing SLIM to xSLIM are presented in Section 4 as well. Furthermore, noise and contrast effects on the resulting image quality are also investigated through simulations.

As described in previous sections of this report, in super-resolved spatial light interference microscopy, phase grating is used to provide structural illumination to achieve super-resolution. However, in a recent study [16] by Hussain et al., an alternative super-resolution microscopy technique with a simple optical setup (shown in Figure 8), which uses a computer controlled spatial light modulator (SLM) to illuminate the object, is introduced.
Figure 8: Experimental setup: BEC (Beam expander and collimator), QWP+P (quarter wave plate and polarizer), BS (beam splitter), SLM (spatial light modulator), L (lens), IS (Imaging system).

Instead of using phase grating to increase the resolution, in this technique described in [16], the lateral resolution is increased by increasing the number of shifted beams produced by SLM. Furthermore, using SLM for both illumination and controlling the phase variations minimizes the errors, as it is controlled by a computer [16].

In conclusion, the study by Chu et al., which is investigated in this report, overall demonstrates a promising non-invasive and label-free super-resolution microscopy technique, which can further be developed to improve its lateral resolution and contrast through advancing its optical setup and additional processing.
6 References


