Compact and low-cost structured illumination microscopy using an optical fiber coupler

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In this paper, a compact and low-cost structured illumination microscope (SIM) based on a $2 \times 2$ fiber coupler is presented. Fringe illumination is achieved by placing two output fiber tips at a conjugate Fourier plane of the sample plane as the point sources. Raw structured illumination (SI) images in different pattern orientations are captured when rotating the fiber mount. Following this, high resolution images are reconstructed from no-phase-shift raw SI images by using a joint Richardson-Lucy (jRL) deconvolution algorithm. Compared with an SLM-based SIM system, our method provides a much shorter illumination path, high power efficiency, and low cost.

Fluorescence microscopy plays a vital role in modern biological research owing to its non-invasive visualization of biological samples. However, due to its optical diffraction limit, the lateral resolution of a conventional fluorescence microscope is restricted at $\lambda/(2NA)$, where $\lambda$ is the wavelength of the light source and $NA$ is the numerical aperture of the objective lens \cite{1}. In order to obtain fine structures at sub-diffraction sizes, several super-resolution (SR) imaging techniques have been developed to bypass resolution barriers \cite{2}. Among these SR techniques, structured illumination microscopy (SIM) stands out owing to its simple optical configuration and wide-field full-frame images at high acquisition speeds \cite{3}.

In a typical SIM setup, sinusoidal patterns are used to excite the sample fluorescence, which can shift the high-frequency component of the sample structure into the passband of the optical imaging system; resolution-enhanced images could be recovered from multiple SI image acquisitions with at least three phase shifts. Isotopic SR results can be obtained by repeating this process at other orientations. Thus, several devices have been proposed to generate those structured illuminations: gratings \cite{4}, programmable diffractive elements (DOEs) \cite{5}, spatial light modulators (SLMs) \cite{6}, silicon-on-insulator (SOI) chips \cite{7}, plasmonic nanostructured substrates \cite{8} and fiber devices \cite{9}. Among these devices, the SLM is the most widely used for building a high-performance SIM system that can provide a flexible and accurate way to achieve phase, frequency, and orientation modulation of illumination fringes. However, its prohibitive cost and low power efficiency owing to the diffraction of grating structures limited the penetration of SIM technique.

In recent years, it has been proposed that SR images can be recovered from three raw SI images by using a maximum likelihood approach \cite{10} with a joint Richardson-Lucy (RL) deconvolution algorithm \cite{11} \cite{12}, meaning the phase modulation of the illumination pattern is no longer required for SIM image processing.

In this paper, we propose a simple and low-cost method to achieve structured illumination super-resolution imaging using a $2 \times 2$ fiber coupler, wherein two output fiber tips were mounted in parallel on a nested rotation mount modified with a 3D-printed component. By placing this fiber mount at the conjugate Fourier plane of the illumination path, interference illumination can be obtained at the sample plane. High resolution images were recovered from no-phase-shift raw SI images (one SI image per orientation) by using a joint Richardson-Lucy deconvolution algorithm. Our method offers an inexpensive way to build a compact SIM system with high power efficiency.

In mathematics, an illumination field of two-beam interference can be expressed as:

$$i(r) = \cos(\omega_0 r + \phi), |\omega_0| \leq \omega_c$$  \hspace{1cm} (1)

where $\omega_0$ and $\phi$ represent the frequency vector and the phase of illumination and $\omega_c$ is the cut-off frequency of the imaging system; its Fourier form is expressed as:

$$i(\omega) = \delta(\omega - \omega_0)e^{-i\phi} + \delta(\omega + \omega_0)e^{i\phi}$$  \hspace{1cm} (2)

where $\delta(\omega)$ is a pulse function, which can be considered a point light source. The central idea of our pattern generation strategy is to place two point light sources at the conjugate Fourier plane of the illumination path in order to generate two-beam interference patterns at the sample plane.

Figure 1 (a) shows the optical setup of our homemade SIM system. Here, a $2 \times 2$ fiber coupler (TN532R5F2, THORLABS) with 50:50 coupling ratio was used to split an incident light source (532nm, 100mW) into two light beams with the same intensity. The fiber core of each output tip was 2.5μm, which could be considered a point light source. The A 3D printed fiber mount $M$, shown in Figure 1 (a), was used to mount the two output fiber tips in parallel, which were then placed at the conjugate Fourier plane of the illumination path. With two lenses ($L_1$ 150nm, $L_2$ 100nm), those two point light sources were projected onto the back aperture of the objective lens (OL, 60×, NA = 1.25, Olympus) while being interfered at the sample plane. The fluorescence signal was captured by a sCMOS camera (ORCA-Flash 4.0 V2,
HAMAMATSU) with a 200\(\mu\)m tube lens \((L_3)\). By rotating the fiber mount, \(M\), SI images with different pattern orientations were obtained. It should be noted that the spatial frequency of the illumination pattern was determined by both the distance between two output fiber tips and the combination of \(L_1\) and \(L_2\).

In the proposed SIM system, the fiber coupler was a passive device which could not modulate the phase of the output light. In other words, only one SI image per orientation was captured at one time, which means the sum of all raw images could not be considered a uniform illuminated sample image. Thus, neither the most common SIM image processing algorithms, which are based on separating high-frequency components in the Fourier domain \([2, 13]\) or blind reconstruction algorithms \([13, 16]\) were suitable in this case. As was recently reported in \([11]\), a joint RL deconvolution algorithm can combine multiple images collected under different excitation patterns; these could then be directly applied to our image recovery processing. In two-dimensional image reconstruction, the measurement matrix \(H\) mentioned in \([11]\) may require excessive memory for progressing. Thus, for each iteration of RL deconvolution, we used direct convolution to compute the blurring operations as follows:

\[
\hat{m} = h \otimes (e_i \times I) + b
\]

\[
r = m/\hat{m}
\]

\[
\hat{e}_{i+1} = e_i \times [h \otimes (r \times I)]/[h \otimes (ones \times I)]
\]

where \(e_i\) is an estimate of the real fluorescence distribution of the sample for iteration \(i\), \(h\) is the point spread function (PSF) of the imaging system, and \(b\) is the random noise. Furthermore \(m\) represents the raw SI data of our measurements, \(\hat{m}\) is the estimate SI data from \(e_i\); \(I\) represents the illumination profile of the raw SI data \(m\), and \(ones\) is a ones array with the same size as \(m\). Here, \(\otimes\) represents the convolution operation. Resolution-enhanced images could be reconstructed after several iterations.

In this experiment, we set the distance of two fiber tips to 6\(\mu\)m; the SI patterns were set to 53.3% of the maximal spatial frequency of the 532\(\text{nm}\) excitation light. Following this, we imaged some fluorescent microspheres with a diameter of 200\(\text{nm}\) (TetraSpeck, Thermo Fisher Scientific) on our SIM system. Five raw SI images were captured by the camera at a size of 2048\(\times\)2048; the field of view was 198.6\(\times\)198.6\(\mu\)m\(^2\). Figure 2 (a) shows part of one raw SI image. In order to determine the resolution enhancement, we blocked one of the fiber tips to produce a uniform illuminated sample image. Figure 2 (b1)-(b3) show the wide-field, deconvolved, and super-resolution images at the same area as those in Figure 2 (a); two beads were distinguished in the SR image but not in the wide-field and deconvolved images.

Figure 3 shows the illumination path of the proposed SIM system. \(L_1\) was used to collimate the light beams put out by the fiber tips. In addition, with the help of \(L_2\), two point light sources were projected at the back aperture of the objective lens. The distance between two point light sources was determined by the focal length of \(L_1, L_2\), and \(D_1/D_4 = f_1/f_2\). \(D_4\) corresponded directly to the spatial frequency of the interference pattern at the sample plane. Typically, in a SIM system with a certain objective lens, the spatial frequency of the SI pattern must be close to the cut-off frequency of the imaging system to produce the best resolution enhancement. In other words, the pattern’s spatial frequency is not required to change frequently in daily use. We can use different fiber mounts with different \(D_1\) to modulate the spatial frequency of the SI pattern for a particular optical setup.
FIG. 2. Experiment result. (a) part of one raw SI image with 512×512 pixels; the full-size raw SI image is 2048×2048 pixels. (b1)-(b3) show the same area in (a); (b1) shows the wide-field image, (b2) shows the Richardson-Lucy deconvolution result of the wide-field image, and (b3) shows the SR reconstruction result. It is clear that the reconstructed image can distinguish two beads while the wide-field and deconvolved images cannot. All images in this figure were normalized to 1. The scale bars were 5μm and 200nm in the enlarged panels.

FIG. 3. The illumination path of the SIM setup. Two fiber tips were placed at the focal plane of $L_1$. $D_1$ is the distance between two optical fiber tips; $D_2$ is the diameter of the light field in front of $L_1$; $D_3$ is the diameter of optical lenses; $D_4$ is the distance between two point light sources at the back aperture of the objective lens. $f_1$ and $f_2$ are the focal lengths of $L_1$ and $L_2$.

Moreover, as shown in Figure 3, the fiber tips were placed off-axis, while the light beams collimated by $L_1$ had a certain angle in relation to the optical axis. If we set $L_1$ and $L_2$ to be as close as possible so that most of the energy could be used. In the optical setup, the diameter of the $D_3$ lenses was 25mm and the NA of the fiber tips was 0.13. When the focal length of the $L_1$ was set to 150mm, approximately 41% of the light energy crossed the illumination path. Moreover, power efficiency was improved by using lenses with different diameters and focal lengths. In this case, if we changed the diameter of the lenses to 50mm, 100% of the energy was projected to the back aperture of the objective lens. Here, we ignored the insertion loss of the optical fiber, which was about 3dB.

In a typical SLM-based SIM system, a relatively high-powered light source is needed owing to low power efficiency caused by the diffraction of the grating structure. However, due to the high power efficiency of our illumination strategy, the power of a single light source can be much lower. Furthermore, when compared with the high cost of an SLM, the fiber coupler and 3D-printing mount used in our setup was much cheaper and the illumination path was extremely short. Thus, this method could be easily applied to a commercial microscope.

In this experiment, the orientation modulation of the SI pattern was achieved using mechanical rotation of the 3D printed fiber mount, which caused the deformation of the fibers. This led to an uncertain phase shift and polarization change of the SI pattern, which could cause the appearance of artifacts in image reconstruction. In addition, the acquisition speed of SI images was limited by mechanical movement. These issues can be mitigated by using more fiber tips as a programmable point-source array, thereby avoiding mechanical movement.

In summary, we have proposed a compact and low-cost SIM system using a $2\times2$ fiber coupler. Two output fiber tips, mounted on a rotatable 3D-printed fiber mount, were placed at the conjugate Fourier plane of the illumination path, which generated two-beam interference excitation patterns at the sample plane. Resolution-enhanced images were recovered from the no-phase-shift raw SI images with a joint Richardson-Lucy deconvolution algorithm. When compared with the most widely used SLM-based SIM setups, our proposed method offers a cheaper and more compact way to build a SIM system with high power efficiency.

In future work, more point light sources could be added as an array placed at the conjugate Fourier plane, thereby replacing mechanical movement with a programmable operation of the point light source array. Thus, the reconstruction artifacts in the SR images could be reduced and the acquisition speed of the system could be improved. It should be noted that those point light sources could be provided using low-cost laser diodes (LDs) with relatively low power requirements. Furthermore, the modulation speeds of these LDs could easily reach 100MHz, which means the SI pattern generation speed could be much higher than with other SLM-based SIM systems. Moreover, a uniform illuminated sample image could be captured using single-source illumination. Hence, several reconstruction algorithms [17][20], based on 4 raw images.
(one wide-field image and three SI images), could be applied to recover SR images in future work.

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