

Laser-based dual-space microscopy



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ABSTRACT

We show using two-dimensional simulations that the dual-space microscopy (DSM) phase-recovery algorithm converges to the correct result for arbitrary samples. We present experimental results obtained by implementing the DSM technique using a laser. We demonstrate that DSM produces synthetic images with a large field of view when a laser is used as the illumination source. However, speckles affect the quality of the obtained images.

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1. Introduction

Fourier ptychographic microscopy (FPM) is a phase-recovery imaging technique [1–9] that permits to obtain high-resolution images with a large field of view (FOV) from a set of low-resolution images obtained by illuminating the sample from a different direction each time [10–15]. FPM was initially demonstrated using planar [10–12] and hemispherical [13,14] arrays of light emitting diodes (LED) as white-light illumination sources and it was subsequently extended to the infrared spectral range [16]. FPM was also demonstrated using a laser as the illumination source, and mirrors to illuminate the sample from different directions using the same laser beam [16–18]. The low-resolution images of the sample used in FPM only record the intensity of the electric field in the microscope's real plane (RP). Nevertheless, in general, the FPM phase-recovery algorithm is successful in extracting the unmeasured phase of the electric field in the sample's plane because each low-resolution RP image carries partial information about the phase. The resolution limit of FPM is $\sim \lambda/NA_s$, where λ is the wavelength of the light used for imaging, $NA_s = NA_o + NA_c$, and NA_o and NA_c are the numerical apertures of the objective lens and condenser, respectively [12,14,15]. Therefore, when $NA_c > NA_o$, FPM permits to obtain images with better resolution than the Rayleigh resolution limit $\sim \lambda/(2NA_o)$ [10]. DSM is another recently demonstrated phase-recovery imaging technique which is based on the simultaneous observation of the sample in the position and momentum spaces; i.e., the DSM phase-recovery algorithm can be considered an extension of the FPM algorithm where intensity images obtained in the microscope's Fourier plane (FP) are used in addition to the low-resolution RP images used

in FPM [18,19]. When DSM was demonstrated using a hemispherical array of LEDs, it was found that the obtained high-resolution RP images have a reduced FOV due to the small divergence of the light emitted from each LED [18]. The solution of this problem is the main focus of this work. Here we demonstrate for the first time that synthetic images with a large FOV are obtained by implementing the DSM technique using a laser beam as the illumination source. In order to facilitate the comparison between the FPM and DSM techniques based on both laser and LEDs, we present results obtained with both phase-recovery techniques using a hemispherical digital condenser (HDC) [14,18], and using a hemispherical laser guider (HLG) fabricated with similar geometry than the HDC. The rest of this paper is organized in the following manner: In Section 2 we describe the experimental arrangements used in this work. In Section 3, the DSM algorithm is discussed and simulation results are presented. For the first time, we demonstrate using realistic two-dimensional simulations that the DSM phase-recovery algorithm works well for arbitrary samples. Experimental results are presented and discussed in Section 4. Finally, the conclusions of this work are presented in Section 5.

2. Experimental set up

We imaged a 600 lines/mm Ronchi Ruling ($p = 1.67 \mu\text{m}$) using a Nikon Elipse Ti inverted microscope with an objective lens with $NA_o = 0.8$ and 50X magnification. As sketched in Fig. 1(a), the microscope set up includes two charge coupled device (CCD) cameras for obtaining RP and FP images of the sample.

The microscope's built in illumination source was substituted either by a computer controlled HDC formed by 64 white-light LEDs

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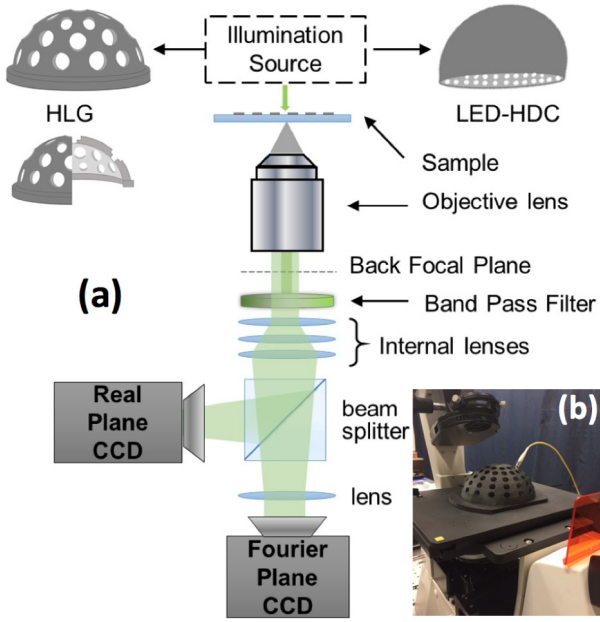


Fig. 1. (a) Schematic illustration of the experimental set up. The illumination source can be either a HDC (top-right) or a laser-HLG combination (top-left). (b) Photograph of the HLG-collimator combination.

distributed uniformly in four circular-rows having numerical apertures $NA_c = 0.58, 0.73, 0.89, 0.97$ [14,18], or by a HLG, which was fabricated by a 3D-printer with geometry similar to the HDC. As shown in Fig. 1(b), the HLG has 76 circular-holes for manually inserting a collimator connected by a single mode optical fiber to a $\lambda = 532$ nm wavelength laser beam produced by a solid-state laser. Sixty four of the 76 HLG holes permit to illuminate the sample in the same directions as the LEDs in the HDC can illuminate the sample. This allows a direct comparison of the images obtained using LED and laser illumination. Eleven of the other 12 holes in the HLG are distributed uniformly in a circular row with $NA_c = 0.37$. The last hole is located directly at the center and permits to illuminate the sample perpendicularly ($NA_c = 0$) with the laser beam. As sketched in Fig. 1(a), the HDC and the HLG were alternatively placed directly on top of the sample. When the HDC was used, a band-pass spectral filter centered at $\lambda = 570$ nm with a 10 nm bandwidth was inserted after the objective lens.

3. Dual-space microscopy

The DSM algorithm has been described in detail before [18,19]; nonetheless, here we include a fast description of it for consistency. As sketched in block (1) in Fig. 2, the DSM algorithm starts by assuming an initial approximation of the complex function describing the high-resolution optical disturbance (OD) at the microscope's RP [20]. For instance, the DSM algorithm could start by assuming that both the amplitude ($a_{m=0}(\vec{r})$) and the phase ($p_{m=0}(\vec{r})$) of the OD are equal to zero.

As shown in block (2) of Fig. 2, the input approximation is then Fourier-transformed to obtain the actual approximation of the OD in the microscope's FP:

$$A_{m,j}^{act}(\vec{k}) e^{iP_{m,j}^{act}(\vec{k})} = F \left[a_{m=0}(\vec{r}) e^{iP_{m=0}(\vec{r})} \right]. \quad (1)$$

As shown in block (3) in Fig. 2, Eq. (2) is used to calculate the first approximation to the amplitude ($A_{m,j}(\vec{k})$) and phase ($P_{m,j}(\vec{k})$) corresponding to the intensity ($A_{m,j}^2(\vec{k})$) of the FP image with small NA_o that would be observed by illuminating the sample from the direction j .

$$A_{m,j}(\vec{k}) e^{iP_{m,j}(\vec{k})} = A_{m,j}^{act}(\vec{k} - \vec{k}_j) e^{iP_{m,j}^{act}(\vec{k} - \vec{k}_j)} \cdot W_o. \quad (2)$$

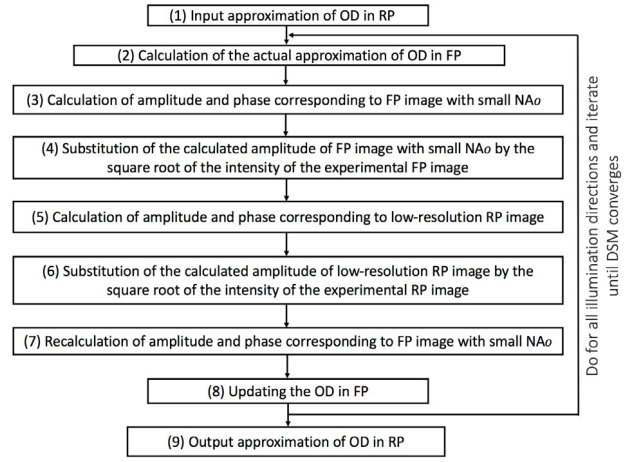


Fig. 2. Block diagram of DSM algorithm.

In Eq. (2), the magnitude of the wavevector \vec{k}_j is $k_j = (2\pi/\lambda)NA_c$, where NA_c takes the value corresponding to the illumination direction j , and W_o is a circular window with radius NA_o , amplitude equal to one, and is centered at $\vec{k}_j = 0$. The step indicated in the block (4) in Fig. 2 is absent in FPM, where only RP images are used. In DSM the calculated amplitude of the FP image with small NA_o ($A_{m,j}(\vec{k})$) is substituted by the square root of the measured intensity of the corresponding experimental FP image with small NA_o ($A_j(\vec{k})$). The rest of the steps of the DSM algorithm coincide with the well-known steps of the FPM algorithm [10–15]. In short, as indicated in block (5) in Fig. 2, an inverse Fourier transform (F^{-1}) operation is performed to calculate the amplitude and phase associated with the low-resolution RP image using Eq. (3):

$$a_{m,j}(\vec{r}) e^{iP_{m,j}(\vec{r})} = F^{-1} \left[A_j(\vec{k}) e^{iP_{m,j}(\vec{k})} \right]. \quad (3)$$

As sketched in block (6) in Fig. 2, in the next algorithm step the calculated amplitude of the low-resolution RP image ($a_{m,j}(\vec{r})$) is substituted by the square root of the measured intensity of the corresponding experimental low-resolution RP image ($a_j(\vec{r})$) and, as shown in block (7) in Fig. 2, Eq. (4) is used to recalculate the amplitude and phase corresponding to the FP image with small NA_o :

$$A_{m,j}^{rec}(\vec{k}) e^{iP_{m,j}^{rec}(\vec{k})} = F \left[a_j(\vec{r}) e^{iP_{m,j}(\vec{r})} \right] \cdot W_o. \quad (4)$$

Then, as indicated in block (8) in Fig. 2, the updated approximation of the optical disturbance in the FP is calculated using Eq. (5):

$$A_{m,j}^{upd}(\vec{k}) e^{iP_{m,j}^{upd}(\vec{k})} = A_{m,j}^{act}(\vec{k}) e^{iP_{m,j}^{act}(\vec{k})} + \alpha \left[\gamma A_{m,j}^{rec}(\vec{k} + \vec{k}_j) e^{iP_{m,j}^{rec}(\vec{k} + \vec{k}_j)} - \beta A_{m,j}(\vec{k} + \vec{k}_j) e^{iP_{m,j}(\vec{k} + \vec{k}_j)} \right] \quad (5)$$

where α , β , and γ are coefficients that can be conveniently adjusted. In this work, we used the set of values $\alpha = 1, \beta = 1, \gamma = 1$ in simulations and $\alpha = 1, \beta = 0, \gamma = 1$ while processing experimental images. An iteration of the DSM algorithm occurs when the steps indicates in blocks (2)–(8) in Fig. 2 are successively done for each RP–FP image pair. Generally, several iterations are needed in order that the DSM algorithm convergence is established. Finally, as shown in block (9) in Fig. 2, a final Fourier transform operation provides the output high-resolution RP image and its corresponding phase. Fig. 3 shows DSM simulation results corresponding to an OD at the sample's plane with arbitrary intensity (Fig. 3(a)) and phase (Fig. 3(b)).

The simulation was conducted supposing that the sample was imaged with the experimental set up illustrated in Fig. 1 and illuminated successively from 44 different directions with the laser beam using

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