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A Lens Assisted Phase Microscope Based on Ptychography *

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Based on the recently developed ptychographical iterative engine (PIE), we suggest a lens assisted microscopy to realize quantitative phase imaging without using interferometry. The sample is imaged with a lens system; a pinhole on the image plane scans the image at a proper step interval; the diffraction pattern is recorded simultaneously by a CCD at Fresnel area. With a slightly changed PIE algorithm, the phase image of the sample can be accurately reconstructed from the recorded diffraction pattern. The main advantage of this suggested method lies in its capability to retrieve the phase information from the recorded intensity directly, and thus it has more flexibility over conventional interferometric techniques. The feasibility of the suggested method is verified by reconstructing the modulus and phase image of a biological sample from a set of 10 by 10 diffraction patterns, and the result matches the analysis well.

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Many biological specimens, such as cells and cornea, are almost invisible using conventional bright field microscopes, because their absorption at visible light is so tiny that the intensity image contrast is almost zero. Their structural information is mainly indicated by the phase changed inside the transmitted light wave. Two kinds of optical microscope are commonly used to observe these kinds of roughly pure phase specimens, differential interference contrast and Zernike phase contrast, but these techniques do not offer quantitative measurement of phase.[1,2] Accurate phase measurement is important to extract the specimen properties such as the variations in thickness, surface topology and refractive index changes. Digital holographic microscopy and other techniques based on interferometry can measure the complex valued wave front and correspondingly generate a three-dimensional image by altering the focus of the image. However, the requirement of a smooth reference wave makes the optical system complex, at the same time, since only one third of the CCD's band width can be utilized by the reconstructed real image; the spatial resolution of digital holographic microscopes commonly is not ideal.

Andrew presented a lensless approach based on a ptychographical iterative engine (PIE) to realize the full-valued complex wave front measurement.[4] In this method, the specimen is illuminated by a plane light wave and the diffraction pattern of its transmitted field is recorded by a CCD camera after passing through a diffusing pinhole. In the data recording process, the specimen is laterally shifted relative to the specimen and a set of diffractions are recorded at different specimen positions. In the reconstruction process, an extend PIE (ePIE) algorithm is used to calculate the complex transmission function of the diffuser and the specimen's transmit wave function simultaneously, and then the phase and modulus images are generated by refocusing the transmit wave to the specimen plane.[5] This technique is quite simple in setup compared with digital holography, but there are still some problems limiting its application. Firstly, the requirement on the translation stage is quite high, because the scanning pinhole is placed in the Fresnel area of the sample and the influence of the shifting error of the pinhole can be amplified by the diffuser. Secondly, though the diffuser on the pinhole can eliminate a strong un-diffracted wave to lower the dynamic range, it is not suitable for the measurement of very week specimens. An empty glass slide with ideally uniform inner structures is a typical example, since all recorded diffractions will be the same, and reconstructing the diffuser structure with an ePIE algorithm becomes impossible. In this Letter we suggest another approach to realize PIE[6−10] measurement by placing an empty pin hole on the image plane and shifting it laterally to record the diffraction patterns.

We use a slightly changed PIE algorithm to reconstruct quantitative phase images from Fresnel diffraction patterns. The setup of our microscope is schematically shown in Fig. 1. The specimen is imaged by $4f$ system. A pinhole (with a diameter of 1.5 mm) fixed on a translation stage is located on the image plane and can be shifted laterally relative to the specimen; a 16 bit 782×582 pixel CCD camera with a pixel spacing of 8.3 µm at distance of 147.8 mm behind the pinhole records the diffraction patterns at a series of pinhole positions. The inset in Fig. 1 shows one of the diffraction patterns recorded.

Since an empty pinhole is used in our setup to select the exit wave and form the diffraction pattern, the reconstruction process is very similar to Fienup's algo-

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rithm. That is, at the beginning of the computation, the image of the specimen $A(x, y)$ is assumed to be a random complex-valued metric, and then its portion is selected by multiplying with the binary transmission function of the pinhole $P(x + M\Delta, y + N\Delta)$, where M and N are the position index and Δ is the step interval. The selected wave indicated by the production of $A(x, y)P(x + M\Delta, y + N\Delta)$ is propagated to the recording plane with the Fresnel formula to form a computed diffraction pattern $D(u, v)$, where the u and v are the coordinates of the recording plane. Then the computed diffraction pattern is updated to $D'(u, v)$ by replacing its modulus with the square root of the recorded intensity $I(u, v)$ while keeping its phase unchanged. Then the updated diffraction is backward propagated to the image plane to get a new guess for the image. After updating the image with the following formulas, the above computation is repeated at the next pinhole position until an accurate image is generated. The updated functions used are

$$\begin{aligned} A_{n+1}(x, y) = & \frac{1}{2}P(x + M\Delta, y + N\Delta)[A_n(x, y) \\ & + A'_n(x, y)] + [1 - P(x + M\Delta, y \\ & + N\Delta)]A_n(x, y), \\ A'_n(x, y) = & \text{Fres}^{-1}[D'(u, v)], \\ D'(u, v) = & \sqrt{I_n(u, v)}\exp(-i\text{Ang}[D(u, v)]), \\ D(u, v) = & \text{Fres}[A_n(x, y)], \end{aligned} \quad (1)$$

where $\text{Fres}^{-1}[D'(u, v)]$ means propagating the wave field $D'(u, v)$ from the recording plane to the image plane, $\text{Fres}[A_n(x, y)]$ means propagating the wave field $A_n(x, y)$ from the image plane to the recording plane, and $\text{Ang}[D(u, v)]$ means extracting the phase of $D(u, v)$.

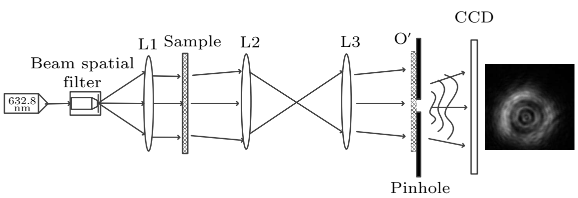

Fig. 1. Schematic of the setup for our microscope.

We verified the feasibility of the above-described method with a prepared microscope slide of a plant section. The pinhole is stepped to a grid of 10 × 10 positions with an interval of 415 µm, to ensure the exit wave selected by the pinhole overlaps with its neighbours at a portion around 50%.[11] The wavelength of the laser used is 632.8 nm. The focal length of the lens used is 100 mm, and the magnification of the system is about 1.0. Figures 2(a) and 2(b) are the reconstructed modulus and phase images, respectively, which were obtained with 240 iterations or 240 × 10 × 10 times of iterative computations at all of the positions. For clarity, Figs. 2(a) and 2(b) show the zoom-in amplitude and the phase images, respectively, and the whole field images are shown as the insets. Figure 2(c) is the phase profile of the digitized phase image along the broken line in Fig. 2(b). Figure 3(a) is the common bright field image of the sample used, and by comparing it to Fig. 2, we can find that almost all of the fine structures of the sample are faithfully reconstructed in both the modulus and phase images. This result demonstrates the feasibility of our suggested method well.

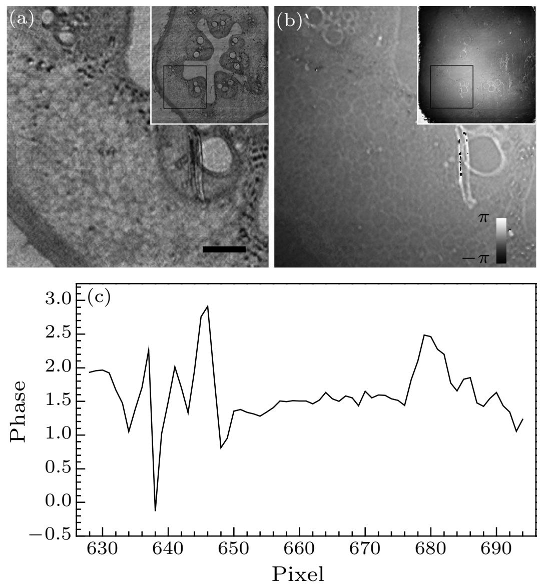

Fig. 2. (a) The reconstructed modulus image, (b) the phase image and (c) the phase profile along the broken line in (b). The full modulus and phase images are shown as the insets in (a) and (b) respectively, and the main images are the zoomed region, indicted by the black box in (a) and (b). The scale bar is 332 µm.

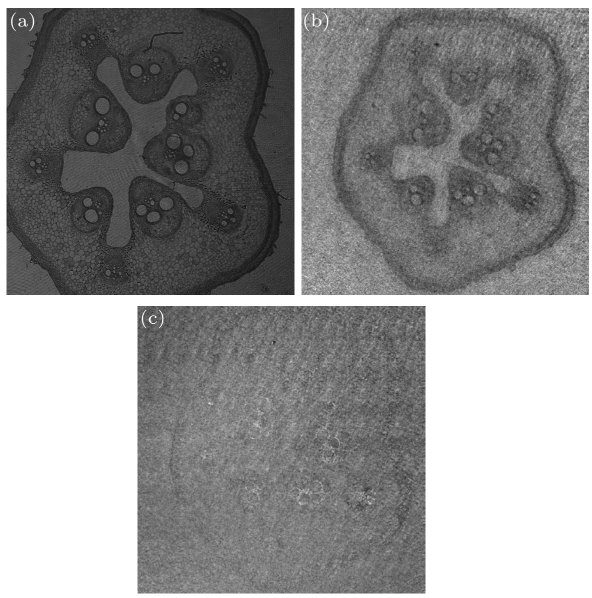

Fig. 3. The common bright field image of the sample (a), the reconstructed modulus image (b) and the phase image (c) obtained by scanning the pinhole after the sample.

To get good reconstructions with PIE, much attention should be paid to the alignments of the experimental setup. We can know from Ref. [11] that, to realize fast and accurate reconstructions, the overlapping ratio between two neighbouring scanning probes should be about 50%, for our method this decides a scanning step roughly equal to the radius of the pinhole. At the same time, a large enough sample-CCD distance is necessary to obtain fast convergence in computation, and generally speaking a shorter wavelength means a longer sample-CCD distance required. If the quality of the lens in Fig. 1 is not good, it can induce remarkable aberrations to the sample image O' especially in the phase distribution. However, this aberration can be removed by measuring the phase distribution of an empty sample first and then subtracting it from the reconstruction of a real sample.

The setup in Ref. [4] has higher requirement on the accuracy of the scanning mechanism, because the influence of the positioning error of the pinhole can be amplified by the diffuser. For comparison, we also repeated the above experiment by positioning the CCD at the plane of 96.5 mm behind the pinhole; the modulus and phase images are shown in Figs. 3(b) and 3(c), respectively. A great deal of noise is contained in these two images, compared with that in Fig. 2.

In conclusion, we have suggested a lens assisted ptychographical microscope for phase imaging with visible light. The specimen is firstly imaged with a common lens system and a pinhole then selects a portion of the image to form Fresnel diffraction patterns on the CCD target. In the data recording process, the pinhole is laterally shifted to a series of positions and the diffraction patterns are recorded simultaneously. Since the pinhole overlaps its neighbours at a portion around 50%, the standard PIE algorithm is used for the images reconstruction. This method can provide quantitative phase information; it also has obvious advantages over digital holography or other techniques based on interferometry in system design and operation, since no reference wave is used. The feasibility of this suggested method is also demonstrated experimentally.

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