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Self-referenced Diffraction Phase Microscopy

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ABSTRACT

Self-referenced diffraction phase microscopy (SrDPM) is reported, wherein quantitative phase imaging is achieved through the interference of the sample wave with a reflected version of itself. The off-axis interference between the two beams generates a spatially modulated hologram that is analyzed to quantify the sample's amplitude and phase profile. SrDPM requires approximately one-half of the object field of view to be empty and optically flat, which serves as a reference for the other half of the field of view containing the sample.

Keywords: Cellular imaging, digital holography, quantitative phase microscopy, self-referencing

1. INTRODUCTION

Contrast-agent-free microscopy is highly desirable for studying the dynamics and physiological activity of various structures in living cells. Unlike spectroscopic imaging modalities such as fluorescence [1] or Raman [2-3] techniques that offer molecular/biochemical specificity, coherent field-based microscopy techniques use optical phase as the contrast mechanism. The use of optical phase in biological imaging of cells has a rich and long history dating back to Zernike's phase-contrast microscopy [4-5]. Later, differential interference contrast (DIC) microscopy was introduced, which directly converts sample phase gradients into intensity changes [6]. DIC's ability to provide improved sensitivity, outstanding contrast, high spatial resolution, and optical sectioning has enabled researchers to study live transparent biological samples without staining. However, one of the major limitations of DIC microscopy is its qualitative nature, due to the nonlinear relationship between intensity and phase gradient. Moreover, the sample's amplitude profile also affects the image intensity distribution, which sometimes makes data interpretation difficult. Recent developments in phase microscopy have focused on obtaining the quantitative phase information through digital holography. The quantitative phase $\Delta\phi$ measured at specific lateral location (x, y) of the cell is a function of both the refractive index of the cell along the optical axis and the height of the cell: $\Delta\phi(x, y) = 2\pi h(x, y)\{n_c - n_m\}/\lambda$, where n_c and n_m are the average refractive indices of the cell and the culture medium, respectively, $h(x, y)$ is the cell height, and λ is the illumination wavelength.

Techniques based on low-coherence interferometry, or optical coherence tomography (OCT) [7], are capable of quantifying phase shifts in the signal measured from different locations in a three-dimensional sample. For example, depth-resolved phase-sensitive measurements have been performed for studying static cells [8], monitoring electric activity in nerve cells [9-10], and spontaneous beating in cardiomyocytes [11]. Such techniques are capable of accessing one lateral sample position at a time; they require raster scanning for imaging purposes, limiting their applicability for the analysis of whole-cell or wide-area dynamics.

Full-field quantitative phase imaging techniques include: digitally recorded interference microscopy with automatic phase-shifting (DRIMAPS) [12-13]; quantitative phase microscopy using the transport-of-irradiance equation [14-15]; and the aforementioned digital holography [16], whose implementation can utilize phase shifting interferometry [17]. Digital holography has been adapted for the quantitative phase imaging of cells [18-20], and our laboratory has been responsible for several significant technological advances [21-25]. The Hilbert phase microscope (HPM) [22-23] was

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based on a Mach-Zehnder design and an off-axis interferometer; it allowed full-field quantitative phase images from single interferograms. Later, our research group developed diffraction phase microscopy (DPM)[24-25] with a common-path configuration to meet the need for both high-speed quantitative phase imaging and high phase stability over broad temporal scales. The technique has been successfully used for nanometer-scale membrane fluctuations in red blood cells, in healthy or diseased states [26-30].

Importantly, DPM requires the reference beam be passed through an optical pinhole to generate a plane wave required for off-axis interference in the image plane. Such a requirement creates an alignment constraint particularly for non-specialists in the field of optics. In this paper, we propose self-referenced diffraction phase microscopy (SrDPM), an adaptation of DPM that overcomes this constraint.

An alternative "self-reference" phase microscope was recently reported [31]. For the setup in this reference, the illumination beam is transmitted through the sample, and subsequently enters a Michelson interferometer. The two beams generated by the beam splitter (of the interferometer) are subjected to different optics in their respective arms, such that they are inverted with respect to each other prior to their recombination. If the sample only occupies one-half of the object-plane field of view, and the other half is left empty (optically flat), then at the recombination stage, the "sample" region of each beam will overlap with the empty ("reference") region of the other beam. Thus, the interference pattern between the two beams will incorporate two representations of the sample; it can be processed to determine sample amplitude and phase information. This configuration, however, is susceptible to phase noise due to the separate arms of the Michelson interferometer. (It has a separate, important advantage, its utilization of "extended-depth-of-field" optics.) In our proposed technique, SrDPM, the sample also occupies one-half of the object-plane field of view. However, SrDPM features a common-path design similar to DPM. This design offers high phase stability when acquiring single-shot wide-field quantitative phase images of biological samples over long durations.

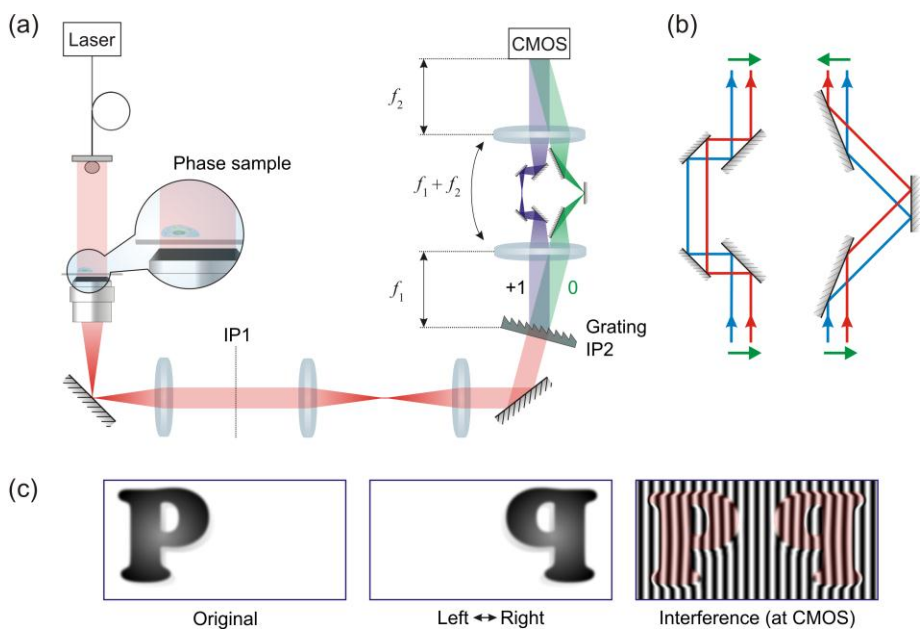

Figure 1. (a) Schematic of the near-common-path self-referenced diffraction phase microscope. (b) Beam propagation through the two mirror assemblies indicating left ↔ right inversion in one beam path and path-length delay adjustment in the other. (c) Cartoons of: a phase object located in the left half of the field of view (left); the left ↔ right-inverted copy of the same phase object located in the right half of the field of view (middle); and the interference pattern due to off-axis interference between the two beams (right).

2. APPROACH

Figure 1(a) shows the general SrDPM schematic. Collimated light is incident upon the sample, which occupies only one-half or less of the objective-lens field of view. The light collected is magnified and incident upon a diffraction grating,

placed in a conjugate image plane, which generates multiple copies of the incident beam. Only two diffraction orders, the 0th and $+1^{st}$ (color-coded green and blue, respectively) are used. The diffraction grating and digital camera are related via a $4f$ imaging system. Note that each beam passes through a different mirror assembly. The 0th-order beam encounters three mirrors; this odd number results in image inversion (from left to right). However, the $+1^{st}$-diffraction-order beam is reflected an even number of times, so that the beam/image orientations are the same. The inverted and non-inverted beams meet in the image plane at a suitable angle for off-axis interferometry.

The proposed SrDPM setup was built around an inverted microscope (Olympus, IX71). A 637-nm fiber-coupled laser diode was used as the light source. The transmitted light was collected using a 40× / 0.65 NA objective lens (Olympus). A 1000 lines/inch diffraction grating was used in the setup. Instead of the mirror assemblies mentioned above, we used a pair of dove prisms (placed side by side but oriented in different directions) to achieve inversion of the incident beams. More precisely, one prism flipped the incident beam from left to right whereas the other inverted the incoming beam upside down. For this modification, the two beams were not reflected copies of each other, but instead, rotated by 180° with respect to each other about the center of the field of view. For imaging, the setup used a high-speed CMOS camera (Photron 1024PCI).

The intensity profile of the recorded hologram can be written as:

$$\begin{aligned} H(x,y) &= \left|E_{+1}(x,y) + E_0(x,y)\exp(jk_x x)\right|^2 \\ &= \left|E_{+1}\right|^2 + \left|E_0\right|^2 + E_{+1}E_0^* \exp(-jk_x x) + E_{+1}^* E_0 \exp(jk_x x), \end{aligned} \tag{1}$$

where k_x is the carrier frequency, and $E_{+1}(x,y)$ and $E_0(x,y)\exp(jk_x x)$ represent the two wavefields, corresponding to $+1^{st}$ and 0th order beams, respectively, arriving at the image plane.

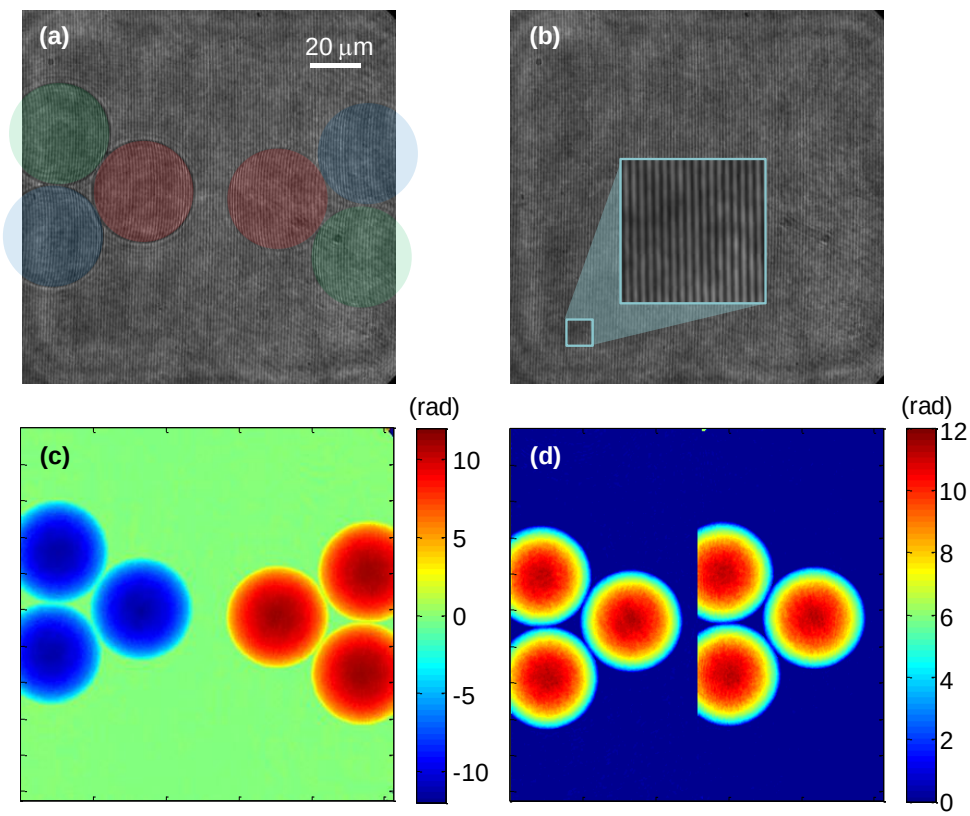

Figure 2. (a) Single-shot hologram of 40-μm latex beads (in oil) recorded using SrDPM implemented with a pair of dove prisms. The locations of the three beads are highlighted using different-colored discs. (b) "Background" hologram recorded with no sample in the field of view. (c) Quantitative phase profile reconstructed from the measured hologram after background removal and 2-D phase unwrapping. (d) Final phase image with duplicate profiles of the latex beads.

The measured hologram is processed using Hilbert transform, where after taking Fourier-transform of the hologram, all but the third term are suppressed using an amplitude mask. Furthermore, the effect of the carrier frequency is removed by translating this term (in Fourier space) to the origin. The resulting reconstruction, $T(x,y) = E_{+1}(x,y)E_0^*(x,y)$. Now, assuming the sample acts as a phase mask with profile $\varphi(x,y)$, then $E_{+1}(x,y) = \exp[j\varphi(x,y)]$, and moreover, $E_0(x,y) = \exp[j\varphi(-x,-y)]$, where the origin of the (x,y) coordinate system corresponds to the center of the 180° rotation mentioned in the previous paragraph, specifically, the intersection point between the dove prisms' axes of symmetry. The phase profile of the sample, $\varphi(x,y)$, is recovered according to: $\mathrm{Arg}[T(x,y)] = \varphi(x,y) - \varphi(-x,-y)$; we require that the $(+x,+y)$ and $(-x,-y)$ terms do not overlap in the image plane. A hologram is also recorded in the absence of the sample to remove any system-dependent phase variations over the field of view. Two-dimensional (2D) phase unwrapping is then applied to determine sample's optical height map. The existence of two distinct reconstructions of the phase profile in SrQPM implies that they can be combined [31], so as to further reduce the still-present phase noise in the reconstruction. This may be achieved, of course, by simply averaging the two phase profiles $\varphi(x,y)$ generated in the reconstruction.

3. RESULTS AND DISCUSSION

To demonstrate quantitative phase imaging using SrQPM, we first utilized a phase sample comprising 40-µm polymer microspheres (Duke Scientific Corp.) immersed in 1.55 refractive index oil. Figures 2(a) and (b) show the measured interference patterns both with and without the sample (three microsphere beads) in the microscope's field-of-view. Figure 2(c) shows quantitative phase reconstructions of the beads after background removal and 2D phase unwrapping. The final quantitative phase image (Fig. 2(d)) presents duplicate profiles of the microspheres, with matching phase signs (+/-) and orientations. It illustrates the robustness and working principle of the proposed near-common path self-reference quantitative phase microscope.

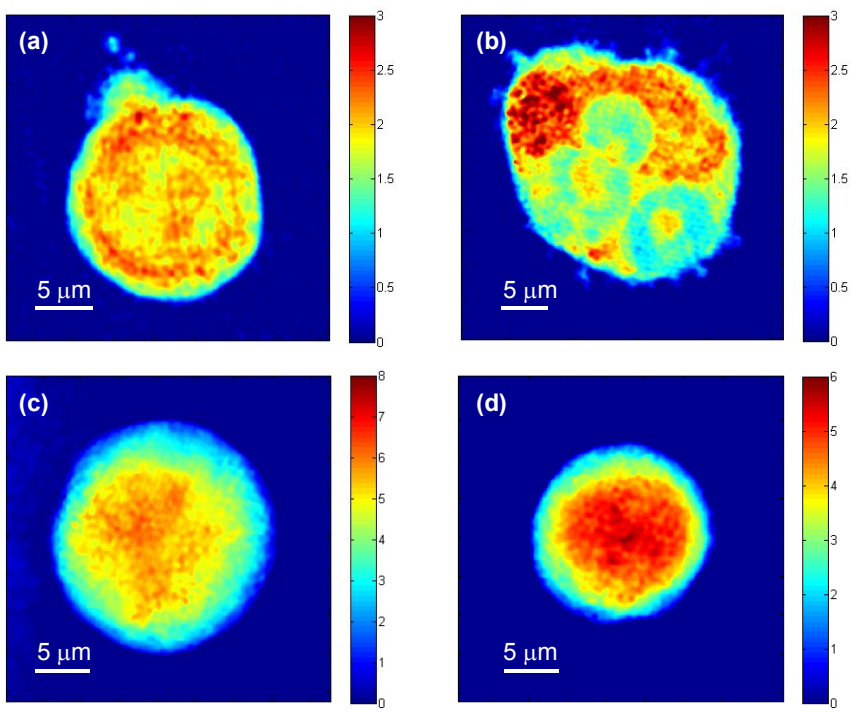

Figure 3. Quantitative phase images of live RKO human colon cancer cells obtained using SrQPM. For imaging, the cells were placed between #1 coverslips; the lateral position of each cell was adjusted so that the duplicate images of the cell did not overlap in the SrQPM field-of-view.

We also employed SrQPM for quantitative phase imaging of live RKO human colon cancer cells. The cells from the culture dish were placed between #1 glass coverslips for imaging. The position of the sample was adjusted so that the

cells under observation were entirely in either in the left or the right half of the field-of-view. The holograms were recorded both with and without the sample present, again for the purpose of background removal. Figure 3 shows quantitative phase images of four different RKO cells, demonstrating the capability of SrDPM to deduce optical pathlength and reveal intricate cellular features.

4. CONCLUSION

In summary, a method for quantitative phase microscopy is proposed based on self-referenced interferometry, which is especially suitable for monitoring cells in a microfluidic setting. The proposed SrDPM technique maintains the two key advantages of DPM: its near-common-path geometry and its single-shot, wide-field imaging ability. These advantages translate to dynamic phase measurements with sub-nanometer measurement sensitivity and millisecond imaging speed. SrDPM also eliminates DPM's requirement to pass the reference-wave illumination through a pinhole spatial filter. This fact relaxes the key alignment constraint associated with DPM, rendering it more accessible to non-specialists and more amenable to long-term measurements over several cell cycles. A dove-prism-pair-based version of the technique was implemented and employed to demonstrate phase imaging of latex microspheres and live human colon cancer cells.

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