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Near-Common-Path Self-Reference Quantitative Phase Microscopy

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Abstract

We report a near-common-path self-reference quantitative phase microscope, wherein a quantitative phase image is formed through the off-axis interference of the sample wave with a 180-degree rotated version of itself. A pair of dove prisms, oriented in different directions, is used to effect the relative transformation between the beams. Our technique features a simple optical design that requires no maintenance, rendering it accessible to nonspecialists in the field of optics. Additionally, its variable magnification and low-noise phase measurement capabilities make it suitable for high-accuracy imaging of biological samples of different sizes.

Index Terms

Bioinstrumentation; interferometry; optical imaging; phase retrieval

I. Introduction

Quantitative phase microscopy (QPM), as implemented by digital holography [1], is a precise method for studying the internal structures of biological cells without requiring exogenous staining. Phase-shifting-interferometry-based QPM techniques require multiple interferograms to be recorded with precise $\lambda/4$ phase shifts of the reference wave, limiting their ability to perform real-time imaging and adding complexity to the system [2]. By comparison, off-axis-interferometry-based approaches provide the opportunity for dynamical quantitative phase measurements [3], [4].

To acquire accurate phase profiles of living cells, low measurement noise is highly desirable. Diffraction phase microscopy (DPM) achieves an accurate assessment of fast cellular dynamics by combining off-axis interferometry with a near-common-path geometry [4]. The single-shot fringe patterns are typically analyzed using Fourier or Hilbert transform methods in order to extract the sample phase information [5], [6]. Related QPM work includes the demonstration of an in-line point-diffraction-interferometry configuration with optimized lateral resolution [7]. More recent work by the same group demonstrates quantitative phase imaging using Zernike phase contrast microscopy [8] (as distinct from holographic interferometry), providing both high lateral resolution and low coherent noise. However,

both techniques use phase shifting to extract the two-dimensional (2D) phase information of the sample. A prior adaptation of DPM to a phase-contrast setup, known as instantaneous spatial light interference microscopy (iSLIM), was presented in Ref. [9]. This approach permits the high stability of DPM to be combined with white-light illumination, diminishing coherent speckle effects.

However, the requirement that the reference beam be passed through an optical pinhole [4], [7], [8] (or an annular aperture as in iSLIM [9]) creates an alignment and optimization constraint that would constitute a demanding problem for a non-specialist in microscopy. Likewise, it is not a trivial task to replace a system hardware element (such as the objective lens) with another, because the pinhole size must be optimized with respect to the system optics. The pinhole constraint becomes even more stringent if multiple illumination wavelengths are to be used.

Earlier, a self-reference quantitative phase microscope (SrQPM) was reported [10] along with the claim to eliminate the need for point-diffraction interferometry. The transmitted sample wave was propagated through a Michelson interferometer, where an additional objective lens in one of the arms performed the image inversion. The technique features an important advantage, its utilization of “extended-depth-of-field” optics. A simpler approach has been reported more recently that utilizes Michelson-interferometer mirror tilt to provide interference between two wavefronts subject to a relative lateral translation [11]. However, both approaches (the latter to a lesser degree) exhibit susceptibility to phase noise due to their separate sample and reference arms. In this letter, we propose a SrQPM design that preserves the two key advantages of DPM: its near-common-path geometry and single-shot imaging ability. In addition, the proposed setup offers variable magnification without any system modifications or adjustments. These features convey exceptional phase stability, fast imaging speed, and the ability to adjust the field of view (FOV) for imaging different-sized samples.

II. Experimental Setup

Fig. 1(a) presents the general schematic for the proposed near-common-path SrQPM. Collimated light from a 637-nm fiber-coupled laser diode source (color-coded red) is incident upon the sample, which occupies one-half or less of the objective lens FOV. The light collected by the microscope objective is magnified and incident upon a diffraction grating (1000 LPI), in a conjugate image plane (IP2). The 0th and +1st diffraction orders (color-coded green and blue, respectively) are both imaged onto a high-speed CMOS camera (Photron 1024PCI) via a 4 *f* imaging system. The qualifier “near” in “near-common-path” reflects the fact that the two beams traverse separate paths between IP2 and the CMOS camera, passing through different dove prisms (oriented in different directions). The ability of a dove prism to “flip” an image is illustrated in the ray-tracing diagram of Fig. 1(b). The two beams are therefore flipped in opposite directions prior to their recombination. We note that the dove prisms are not subject to a strict alignment constraint. A high-spatial-frequency carrier is imposed on the interference pattern generated by the two beams due to their angular separation at the camera plane. This permits single-shot (off-axis) holography to be performed. The necessity that the beams interfere after being flipped mandates that a

spatially coherent source be used for the approach. Also, the reason that one half of the field of view must be empty (optically flat) is so that the corresponding region of each beam can serve as the reference wave for the sample region of the other beam [Fig. 1(c)]. This might limit the achievable magnification for some samples.

At the detection plane, we denote the two wavefields corresponding to the +1st- and 0th-order beams as $E_{+1}(x, y)$ and $E_0(x, y)\exp(jk_x x)$, respectively, where k_x is the spatial carrier frequency. The measured intensity pattern, given by the squared modulus of their sum, contains multiple terms that are separable due to this carrier-frequency factor. In particular, the profile $T(x, y) = E_{+1}(x, y)E_0^*(x, y)$ can be recovered. Assuming the sample acts as a phase mask with profile $\phi(x, y)$, we can express the flipped wavefields as $E_{+1}(x, y) = \exp[j\phi(x, y)]$ and $E_0(x, y) = \exp[j\phi(-x, -y)]$, where the origin of the coordinate system corresponds to the intersection point between the dove prisms' axes of symmetry. The profile $\phi(x, y)$ is recovered from

$$\text{Arg}[T(x, y)] = \phi(x, y) - \phi(-x, -y), \quad (1)$$

provided that the two right-hand-side terms do not overlap.

A hologram recorded in the absence of the sample is used to remove systematic system-dependent phase variations over the FOV. This is a step that need only be performed once for any system configuration. After two-dimensional phase unwrapping, any residual phase ramp occurring in the reconstruction can be removed by fitting such a ramp (or, indeed, a higher-order polynomial) to the still-present background.

The absence of the spatial filter in the SrQPM allows nearly equal strengths of the interfering beams as well as increased total optical power delivery to the detector, which generally improves SNR. However, deviation of the unfiltered reference beam from a precise plane wave will introduce (static) phase-measurement error. To quantify the (temporal) stability of our setup, we acquired no-sample phase images at 125 Hz for 10 seconds using a 40 $\times$ objective lens. After applying median filtering and spatial averaging (the latter over the diffraction-limited spot size in the recording plane), the phase stability was measured to be ~ 1 mrad. The two distinct (twin, phase-conjugate) phase-profile reconstructions represented by the right-hand-side terms of Eq. (1) can be combined, reducing the phase noise by a factor of up to $\sqrt{2}$. Slightly different defocus or higher-order relative aberrations between the pair of complex-amplitude distributions can be corrected through the application of low-order-polynomial phase correction to their respective, individual Fourier transforms.

III. Results and Discussion

To demonstrate quantitative phase imaging using our near-common-path SrQPM, we utilized a 40- μm polymer microsphere (Duke Scientific Corp.) immersed in oil with refractive index 1.55. A 40 $\times$ /0.65-NA objective lens was used for imaging. Based on holograms recorded both with and without the sample present (Figs. 2(a), (b), respectively), the phase difference between the +1st- and 0th-order wavefields was generated; it is

displayed (after background phase ramp subtraction) in Fig. 2(c). The twin, phase-conjugate reconstructions of the near-transparent bead are evident. Figs. 2(d), (e) display the reconstructions with matching orientations and phase signs. They have been digitally corrected up to second-order relative aberrations (including defocus). The mean phase profile of the 40- μm polymer bead is presented in Fig. 2(f). The radial phase profiles for the reconstructions in Figs. 2(d), (e), plotted in Fig. 2(g), are in good agreement with each other.

Fig. 3 demonstrates the robustness of the proposed SrQPM to modifications in the optical system. Three exposures of a 40- μm polymer microsphere, immersed in 1.57 refractive index oil, were captured in rapid succession using 10 $\times$, 20 $\times$, and 40 $\times$ microscope objectives, respectively. No additional system adjustments were required. This ability of the near-common-path SrQPM to provide variable magnification can help mitigate the sacrificed-FOV limitation.

To demonstrate further, we performed quantitative phase imaging of live RKO human colon-cancer cells and drosophila embryos using 40 $\times$ and 4 $\times$ microscope objectives, respectively. The RKO cells were dissociated from the culture flask and placed between #1 glass coverslips. The drosophila embryos were treated with bleach to remove chorion (the outer egg shell), and later fixed with 5% formaldehyde before being transferred to 50% glycerol solution on a glass slide. When imaging, the lateral position of each sample was adjusted so that the twin sample images did not overlap. Holograms were recorded both with and without the sample present, and were processed in the manner described earlier. Figs. 4(a), (b) depict the respective phase reconstructions, $\phi(x, y)$. For the RKO cell, structures such as the cytoplasmic-nuclear boundary and nucleolus are clearly visible. Similarly, the progress of gastrulation in the middle section as well as the dorsal appendages can be observed in the quantitative phase image of the drosophila embryo.

IV. Conclusion

In summary, we have described a near-common-path SrQPM, which provides a straightforward approach for generating highly sensitive phase profiles of biological samples. Although the SrQPM restricts the sample FOV, the proposed setup offers several advantages including: a common-path configuration for low-noise dynamic phase measurements; phase-noise reduction via simultaneous measurement and combination of dual phase profiles; the ability to easily modify the optical magnification by simply interchanging the microscope objectives; and a simple modular design requiring no maintenance, rendering it accessible to the non-specialists in optics. Microfluidic devices, for which narrow microchannels are surrounded by optically flat background regions, are ideal targets for a self-reference approach [10]. We note that a multiple-wavelength source may also be incorporated for implementing near-common-path single-shot multi-color quantitative phase microscopy without any alignment modifications [12], [13].

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Fig. 1.

(a) SrQPM schematic (IP: image plane. L1, L2: lenses, with respective focal lengths f_1 , f_2).

(b) Inversion of an image pattern (green arrow) on propagation through dove prism. (c) Original image, Up ↔ Down and Left ↔ Right inverted images due to respective dove prisms, and the interference pattern (incorporating a high-frequency carrier).

Fig. 2.

Near-common-path SrQPM reconstruction of a 40- μm polymer bead sample. (a) and (b) Single-shot holograms with and without sample, respectively. (c) Phase reconstruction showing twin, phase-conjugate images. (d) and (e) Cropped left/right bead images from (c), after low-order aberration correction and image registration. (f) Combined final phase profile. (g) Radial profiles in red and blue for (d) and (e), respectively. Color bars are in radians. [Scale bars in (a) and (d), 10 μm .]

Fig. 3. SrQPM (twin, phase-conjugate) reconstructions of a 40- μm polymer bead utilizing (a) 10 $\times$, (b) 20 $\times$, and (c) 40 $\times$ microscope objectives. Color bar is in radians.

Fig. 4. Quantitative phase images of (a) RKO human colon cancer cell and (b) drosophila embryo obtained using 40× and 4× microscope objectives, respectively. Color bars are in radians.