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Quantitative Phase Imaging with Epi-mode Illumination for Fiber-Optic Endoscopy

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ABSTRACT

Quantitative phase imaging (QPI) provides unique access to cellular and subcellular structures with nanometer-scale sensitivity, making it a valuable tool for non-destructive, label-free imaging of biological samples. However, implementation of QPI typically involves a transmission-based geometry and requires thin samples, preventing use of QPI in many important clinical settings, including endoscopy. In this work we demonstrate a fiber-optic device, with epi-illumination, capable of providing quantitative phase information that is well suited for clinical endoscopy, among other biomedical applications.

KEYWORDS: Microscopy, Phase measurement, Phase retrieval, Endoscopy, Computational imaging, Image reconstruction techniques

INTRODUCTION

Quantitative phase imaging (QPI) reveals refractive index or sample thickness information of biological samples which enables us to study cellular and sub-cellular structures and their dynamics with great detail^{1,2}. The capability of QPI to retrieve quantitative phase, as compared with conventional Zernike phase contrast or differential interference contrast (DIC) microscopy which only give qualitative phase information, has enabled numerous applications in biology, immunology, hematology, and pathophysiology. For example, morphological structures of cells or tissues can be monitored in a typical life cycle, which can be extremely useful to understand drug exposure and cell necrosis and apoptosis^{3,4}. Moreover, QPI can provide sample information without staining or labeling making it possible to observe biological behaviors in an unaltered and non-destructive manner^{1,2,5-8}.

Although QPI can provide label-free, non-toxic analysis of biological samples, the approach involves a transmission optical geometry and requires thin samples to work, preventing the use of QPI in many important clinical situations, such as in-vivo imaging and real-time endoscopic diagnosis. This significantly limits the use of QPI in many clinical and medical settings.

In this work, we demonstrate a fiber-optic device, with epi-illumination, capable of providing quantitative phase information that is well suited for clinical endoscopy among other biomedical applications. Our approach, termed quantitative oblique back-illumination microscopy (qOBM), makes use of multiple scattering within a thick sample as a source of transmissive illumination from within⁹⁻¹¹. Coupled with a deconvolution algorithm that accounts for the angular distribution of scattered light within the sample and transfer function of the optical system, qOBM is able to recover the same rich level of quantitative detail as QPI, even in thick samples which was previously not possible for conventional QPI¹². Here we leverage this qOBM infrastructure to enable QPI-based contrast using a flexible fiber-bundle probe coupled to a gradient refractive index (GRIN) lens to provide cellular resolution, which could be readily applied for endoscopy.

In this paper, we will detail the theory and design of the qOBM fiber-based system. First, we review the working principles of QPI and qOBM; then we introduce the fiber-bundle-based qOBM system, illustrating the capabilities of the fiber-optic approach to retrieve quantitative phase. Lastly, we will present preliminary data from an amplitude-and-phase microscopic

resolution target, tissue phantoms consisting of polystyrene beads and a (ex-vivo) healthy mouse brain. Finally, future work will be discussed.

THEORY

Conventional microscopy techniques, such as wide-field microscopy, perform well in capturing intensity information of the transmitted light from biological samples. The captured information provides a good picture of biological structures in terms of attenuation (due to absorption and scattering), especially for samples with characteristic absorption profiles. However, most biological samples are nearly transparent in their natural state and, as a result, techniques to reveal sample phase information are highly desired.

In biological samples, under transmission illumination, a typical differential interference contrast (DIC) microscope will record the sample phase information by introducing a phase difference between the signal field and the reference field (which is a spatially shifted replica of the signal field), which can convert the spatial phase differences $\Delta\phi(x, y)$ into observable intensity variations.

Another technique to recover phase information is differential phase-contrast (DPC) microscopy which does not rely on interferometry¹³. Using an opposite illumination configuration, the DPC image $I_{DPC}(x, y)$, can be computed as a normalized difference between the two components¹⁴.

$$I_{DPC}(x, y) = \frac{I_+ - I_-}{I_+ + I_-} \quad (1)$$

Where I_+ and I_- are the intensity images with oblique illumination with opposed directions. This simple difference produces images similar to DIC. However, DIC and DPC only contains phase variation along one direction and do not contain any phase-contrast in the orientation perpendicular to the shear direction or opposed illumination direction^{9,15}.

Of course, with QPI, one does not recover phase gradient information but in fact the actual phase profile of the sample. Typically, a QPI setup uses an interferometric geometry (such as Mach-Zehnder interferometer) to record the sample phase information. For example, in an off-axis interferometry configuration, the sample field interferes with a reference field that is incident on the camera with an angle α , which yields an intensity:

$$I_{QPI}(x, y) = I_0 + I_1(x, y) + 2\sqrt{I_0 I_1(x, y)} \cos[kx \sin(\alpha) + \phi(x, y)] \quad (2)$$

where the oscillating cross term contains the complete spatial phase information.

However, for QPI to reveal detailed (sub-)cellular structures, the sample must fulfill certain requirements. For example, the biological sample needs to be nearly transparent or thinly sliced. As the transmitted light travels through the biological sample, phase information accumulates along its complete trajectory. Thus, if the sample itself is thick, the information will be corrupted by the multiple “layers” of the sample, and no meaningful results can be obtained. Therefore, an approach to investigate the quantitative phase from thick, multiple scattering media is needed.

Oblique back-illumination microscopy (OBM) is one such technique which uses the back-scattered light as an illumination light source from inside multiply-scattering samples. When the illumination from the epi-mode light source (for example, low-cost LED) gets into the sample, multiple scattering occurs which converts the forward-propagation photons into back illumination that mimics a transmission illumination system. The multiple scattering process of photons will depend on the scattering medium and the illumination configuration. Multiple approaches had been proposed in the past to understand the scattering process¹⁶, among which the Monte Carlo simulation is a powerful and computationally effective way to find the system photon response. The illumination field $E(\mathbf{x})$ (and its Fourier transform pair $E(\mathbf{u})$) and the measured intensity on the camera $I(\mathbf{r})$ are related by⁹:

$$I(\mathbf{r}) = |\mathcal{F}^{-1}\{P(\mathbf{f})\mathcal{F}\{o(\mathbf{x})\mathcal{F}^{-1}\{E(\mathbf{u})\}\}\}|^2 \quad (3)$$

where $P(\mathbf{f})$ is the pupil function of the imaging system, and $o(\mathbf{x})$ is the object transmittance function in the object spatial domain. $\mathcal{F}$ represents a 2D Fourier transform from spatial coordinates into spatial-frequency coordinates, $\mathbf{x}$ are the spatial coordinates at the focal plane, $P(\mathbf{f})$ is the pupil function, either 0 or 1, in spatial frequency coordinates $\mathbf{f}$ that correspond to physical coordinates at the back focal plane, and $\mathbf{r}$ represents spatial coordinates at the camera. The coordinates $\mathbf{u}$ and $\mathbf{f}$ are both spatial frequency units, which map to units of propagation angle when scaled by a factor of λ . The coordinates $\mathbf{x}$ and $\mathbf{r}$ are in units of distance and correspond when scaled appropriately for magnification.

It can be shown that due to the relation between the light source intensity and angular spectrum distribution in the scattering medium, the DPC image can be expressed as:

$$I_{DPC}(\mathbf{r}) = \frac{-\text{Im}\{c_\delta(\mathbf{r})\} * \phi(\mathbf{r})}{C(0,0)} \quad (4)$$

where $C(0,0)$ is a real-valued constant background term, $c_\delta(\mathbf{r})$ is the Fourier transform of an equivalent 2D optical phase transfer function of the DPC system, and $\phi(\mathbf{r})$ is the quantitative phase under investigation.

From the distribution obtained from the photon transport simulation of a single LED source, an optical transfer function can be produced for the DPC image formed by the microscope which can then be applied to recover the object function with a deconvolution. The quantitative phase image can be determined by combining two pairs of illumination sources (and their DPC images), with the Tikhonov regularized deconvolution¹⁷:

$$\phi = \mathcal{F}^{-1}\left\{\frac{\sum_k \tilde{I}_{DPC}^k C_{DPC}^*}{\sum_k |C_{DPC}|^2 + \alpha}\right\} \quad (5)$$

where C_{DPC} is the net 2D optical transfer function of the system, $k = 2$ corresponds to the two orthogonal DPC images, and α is a regularization parameter that could be determined by generalized cross validation¹⁸.

METHODOLOGY

The experimental fiber based qOBM setup is shown in Figure 1. The sample under investigation can be a thick, scattering sample, such as intact whole mouse brain. The fiber-based system is made of a fiber optic bundle (FIGH-30-850N by Fujikura, made of 30,000 single-mode cores) and a gradient refractive index (GRIN) lens (NEM-100-06-08-520 by GrinTech). LED sources (Luxeon sink-PAD II) were used to provide epi-illumination, through four guiding fibers (Thorlabs FP1000ERT), forming a configuration as shown in the bottom-right inset—four LED fibers surrounded the GRIN lens with 90 degrees azimuthal angle in between.

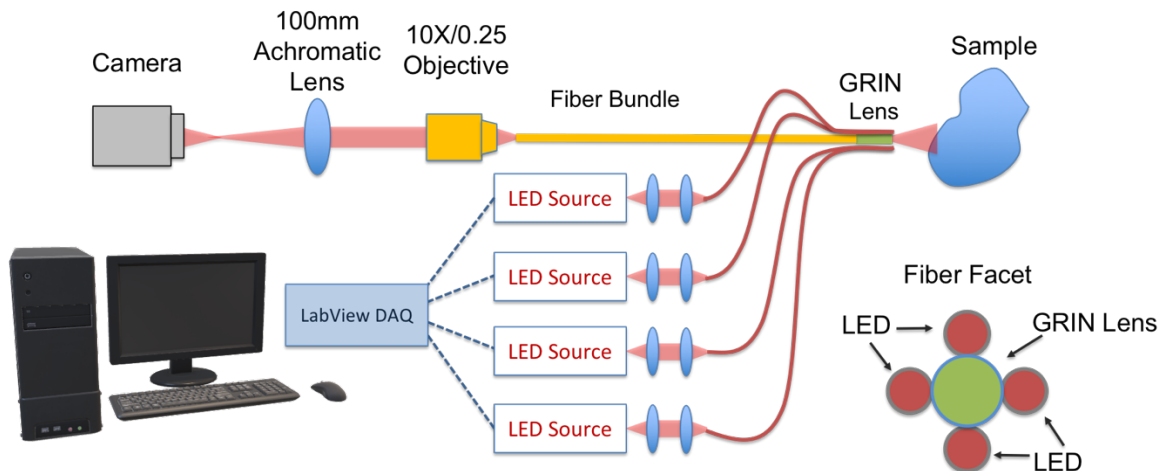


Figure 1. The experimental setup of the fiber-optic endoscope based on quantitative oblique back-illumination microscopy (qOBM). The sample was under epi-illumination from four opponent LED fibers, and the back-scattered light was collected by a fiber-bundle-GRIN-lens assembly (shown in the bottom-right inset). The image was captured by a microscopic imaging system formed using an objective lens, achromatic tube lens and a camera.

The GRIN lens provided 2.6~2.7 magnification from the object space (0.5 NA, working distance 60 μ m in water) to the image space (working distance 80 μ m in air). The flexible fiber bundle, enabling the system for endoscopy, transmits forward-scattered light from the sample back to a microscopic imaging system formed by a 10x objective (Olympus Plan N-10x-0.25), an achromatic lens (100mm) and a 10-bit camera (Thorlabs DCC3240M). Both the illumination and image capture are controlled by LabView software on a conventional computer.

RESULTS

Using the qOBM fiber system, we were able to look at a series of interesting targets and biological samples. Here we include data for the analysis of the system's capability and characteristics.

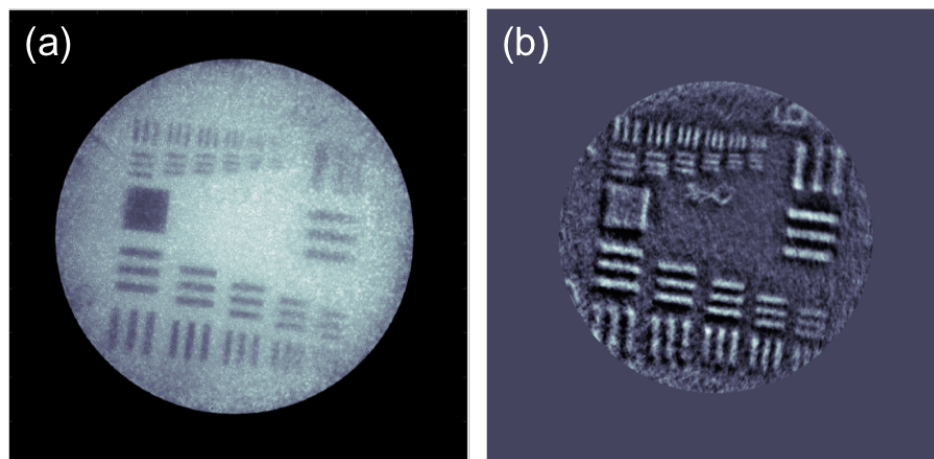


Figure 2. Quantitative phase retrieval of the 1951-USAF target. (a) Raw intensity image from epi illumination from one of the LED fibers. (b) Quantitative phase retrieval of the resolution target. The smallest feature of this target piece (Group 7, Element 6, 228 line pairs/mm) can be clearly seen.

First, we took a look at a target that presents both amplitude and phase modulations to the forward-scattered light. The 1951-USAF target has been widely used as a calibration target for microscope resolution and magnification. The qOBM retrieval results are shown in Figure 2. Aside from the intensity image (Figure 3(a)), the qOBM endoscope also retrieves the spatial phase profile as in Figure 2(b). The qOBM images show good contrast of the features on the target up to the smallest one available (Group 7, Element 6, 228 line pairs/mm). Both intensity and phase modulations (due to manufacturing of the opaque metallic structure and diffraction) can be clearly visualized. The intensity image shows granular pattern from the gap between individual fiber core inside the bundle, which ultimately determines the spatial resolution of the device.

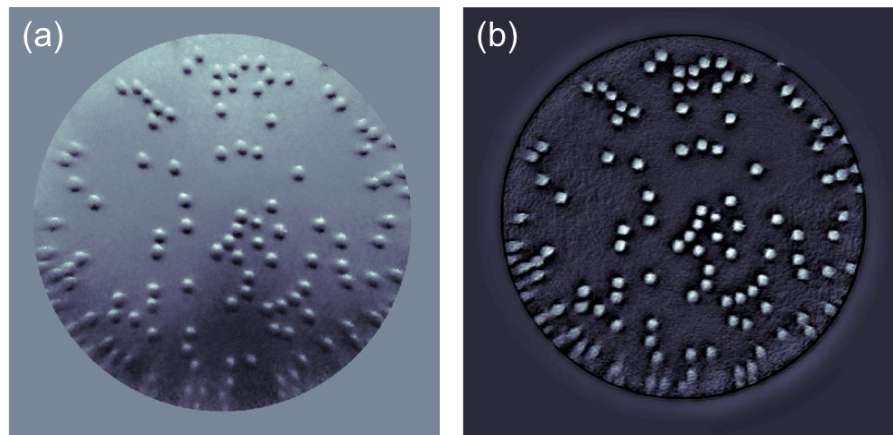


Figure 3. Quantitative phase retrieval of the 10µm polystyrene beads in water suspension. (a) DPC image of the beads, obtained from diametrically opposed LED illuminations. Granular patterns were removed from the raw image by Fourier filtering. (b) Quantitative phase retrieval of the polystyrene beads.

Next, we look at polystyrene beads in a water suspension. The beads are 10µm in diameter and were placed on top of a 100µm depth well on a slide. We use paper as the back-scattering medium. In this case, it was noted that there were aberrations (mostly field curvature) in the edge area of the fiber bundle, which is common in such fiber-bundle assemblies, and can largely be avoided by taking only data at the equivalent clear aperture around the center.

Last, we present our results on a thick, biological sample: a whole mouse brain. The brain was excised and fixed with the 10% neutral buffered Formalin solution. The field of view is roughly ~170µm in diameter. Figure 4(a) to 4(c) show different parts of the imaged brain area.

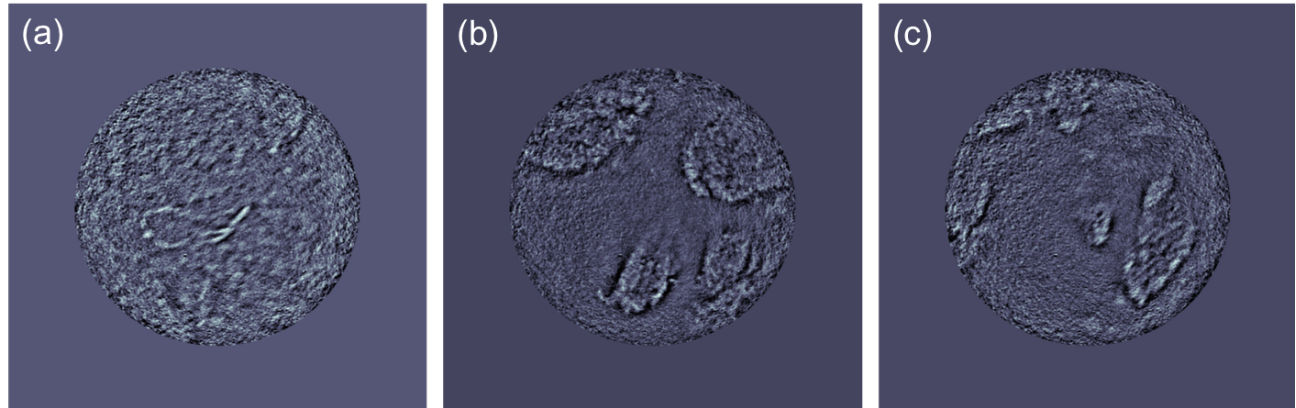


Figure 4. Quantitative phase retrieval of the mouse brain tissues. Different morphological features of the imaged areas can be clearly seen from the retrieved phase. The intensity plots (not shown), however, offered no comparable contrast.

DISCUSSION

We have presented a fiber-optic-based qOBM system to retrieve quantitative phase using epi-illumination from LEDs. Currently, the system retrieves features that are $<4\text{ }\mu\text{m}$ in separation (the smallest feature in the USAF-1951 target as shown in Figure 2; 228 line pairs/mm), and with a spatial ratio of $0.35\mu\text{m}/\text{camera pixel}$. Each core in the fiber bundle, from our calculation, represented $\sim 1\mu\text{m}$ in the object space, which ultimately determines the spatial resolution. Field of view (FOV) of the device was calibrated to be a circular area of around $195\mu\text{m}$ in diameter, which is mainly limited by the fiber bundle size that is available. Larger fiber bundles are commercially available¹⁹. Optical aberrations of the device are mostly from filed curvature (which is not a significant concern in thick tissues) but may also came from fabrication errors in making the fiber-bundle/GRIN-lens assembly. Aberrations had been noted in our system, and as a result, the equivalent clear aperture of our FOV was $\sim 170\mu\text{m}$ in diameter instead of the expected $190\text{ }\mu\text{m}$. Further, a careful analysis of the phase sensitivity with qOBM, measure as the standard deviation of a background region, reveals a sensitivity of $\sim 5\text{nm}$.

Our future work will involve improving the performance of the fiber-optic qOBM system. We will continue applying the technique to other biomedical samples and applications.

CONCLUSION

In conclusion, quantitative phase imaging offers a powerful way to investigate biological samples but it is restricted to thin samples, which significantly hinders its use in many clinical applications. QOBM, on the other hand, yields the same level of quantitative detail as QPI, but in thick scattering tissues. The fiber-based qOBM system presented here will have significant implications for leveraging quantitative phase contrast in many previously inaccessible scenarios/environments, including endoscopy.

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