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Epi-mode tomographic quantitative phase imaging in thick scattering samples

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ABSTRACT

Quantitative phase imaging (QPI) is an important tool in biomedicine that yields label-free access to cellular and subcellular structures with nanometer-scale sensitivity. However, implementation of QPI involves a transmission-based geometry and requires thin samples, severely hindering its overall utility in biomedicine. Here we describe our recently developed method, quantitative oblique back illumination microscopy (qOBM), which overcomes this significant limitation and achieves epi-mode, tomographic, quantitative phase imaging in thick samples, including intact thick tissues. Here we describe the method in detail, show validation experiments and results from thick scattering samples.

1. INTRODUCTION

Quantitative phase imaging (QPI) is an important tool in biomedicine that yields label-free access to cellular and subcellular structures with nanometer-scale sensitivity[1]. However, implementation of QPI involves a transmission-based geometry and requires thin samples, severely hindering its overall utility in biomedicine, particularly for clinical application. Here we describe our recently develop method, quantitative oblique back illumination microscopy (qOBM), which overcomes this significant limitation and achieves epi-mode, tomographic, quantitative phase imaging in thick samples, including intact thick tissues[2].

qOBM combines two recent advances in phase imaging to achieve this feat. First, as suggested by its name, we leverage the infrastructure of OBM[3] where light is deployed in a thick scattering medium in epi-mode which, through multiple scattering, produces a virtual oblique light source within the sample that mimics a transmission geometry. Qualitative differential phase is produces by subtracting images taken with two diametrically opposed sources. Out of focus contributions are removed thought the subtraction process. Next, we use advances in differential phase contrast (DPC) where a well-defined asymmetric illumination is used in combination with a deconvolution algorithm to produce quantitative phase[4,5]. In qOBM, instead of using a well-define illumination pattern, we derive the illumination produced through the multi-scattering process using a Monte Carlo photon transport program.

Here we describe the method in detail, show validation experiments, and show results from thick samples. qOBM overcomes a significant limitation of QPI and opens the door to many exciting new applications in biomedicine.

Keywords: quantitative phase imaging, microscopy, label-free imaging, 3D imaging

2. SYSTEM AND METHODS

qOBM yields quantitative phase in 3D with high-resolution using a traditional brightfield microscope geometry with epi-illumination emanating from two pairs of optical fibers positioned around the objective housing (Fig. 1)[2,6,7]. Each fiber pair is placed diametrically opposite from one another. When light from an LED light source is deployed through one of the fibers, light undergoes multiple scattering, causing some of the photons to turn around and effectively produce a virtual light source from the opposite side of the imaging plane with a slight offset to the optical axis (Fig. 1a). This is known as oblique back-illumination. Under these circumstances, lateral variations in index of refraction at the focal plane redirect light toward or away from the acceptance angles of the micro-scope objective (given by its numerical aperture), producing phase contrast in observed intensity. Images acquired with a pair of diametrically opposed fibers are subtracted to generate differential phase contrast[3]. Further, because out-of-focus contributions from

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either illumination image are the same, the subtraction process rejects out-of-focus content, allowing for tomographic sectioning[3,8].

The procedure described above, first proposed by Mertz et al.[3], produces qualitative differential phase contrast in 3D. To translate these measurements into quantitative phase, we take advantage of recent developments in phase microscopy which do not relay on interferometry to derive phase[4,5], unlike conventional QPI methods[1]. The key requirement to achieve this feat is that the angular distribution of light at the focal plane has to be nonsymmetric and known, which ultimately leads to the transfer function of the system. Then, phase can be computed via deconvolution. In previous intensity-based QPI work, illumination was in transmission mode and the samples were thin[5,9]; thus, it was trivial to fulfill the aforementioned requirements. Recently, we extended this framework to thick samples by considering the multiple scattering process within the sample to generate an estimate of the ensemble average angular distribution of light at the focal plane from oblique back-illumination[2]. Because tissue is weakly scattering at any one point, tissue inhomogeneity, including its complex microscopic structure, does not significantly affect the light's ensemble angular distribution at the focal plane, and thus bulk scattering properties (which are tabulated) are sufficient to arrive at an accurate estimate of the transfer function to compute the phase. With this novel in-sight, qOBM overcomes a significant limitation of traditional QPI, yielding access to the same rich level of quantitative cellular detail but in thick scattering samples, with epi-mode illumination, in 3D. Plus, compared to 3D qualitative differential phase contrast, qOBM is not only quantitative, but also offers improved image quality and higher conspicuity to small structures.

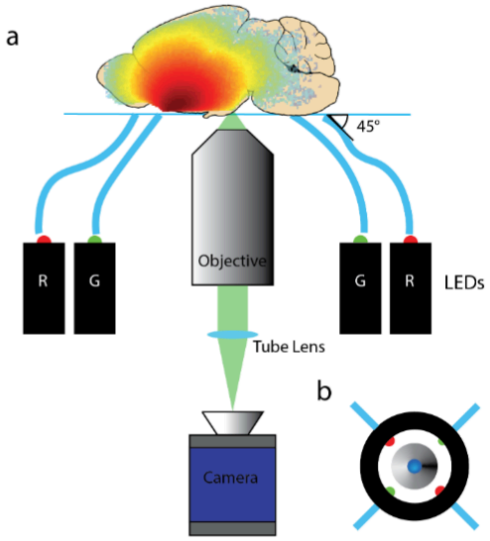

Figure 1. (a) Diagram of qOBM assembly. Four LEDs, two red (630 nm) and two green (530 nm), sequentially illuminate the target from some distance off axis and at some angle (45◦ in this work), and an image is produced with an inverted microscope. (b) Top-down view of imaging apparatus indicating the entry positions of the four LEDs. Taken from Ref[2].

3. RESULTS

Figure 2 shows the quantitative capabilities of qOBM using a lithography relief (Fig. 2a-b) with 300, 200, and 100 nm height structures, and a 2 μm polystyrene bead phantom (Fig. 2c-d), both imbedded in an intralipid agar scattering medium as illustrated in Fig. 2e. The reconstructed quantitative images show remarkable agreement with the expected values of each sample, with a sensitivity of 1.75 nm in height**,** which is within range of transmission-based, interferometric QPI using thin samples. The lateral resolution is diffraction limited (0.6 μm), and axial resolution ~1.5 μm, similar to confocal microscopy. The total time to acquire 1 qOBM image is 100 msec. (10Hz), limited by the frame rate of the camera, which is 40Hz, with <30 mW optical power on the sample from inexpensive LEDs. Image processing and display are done in real-time.

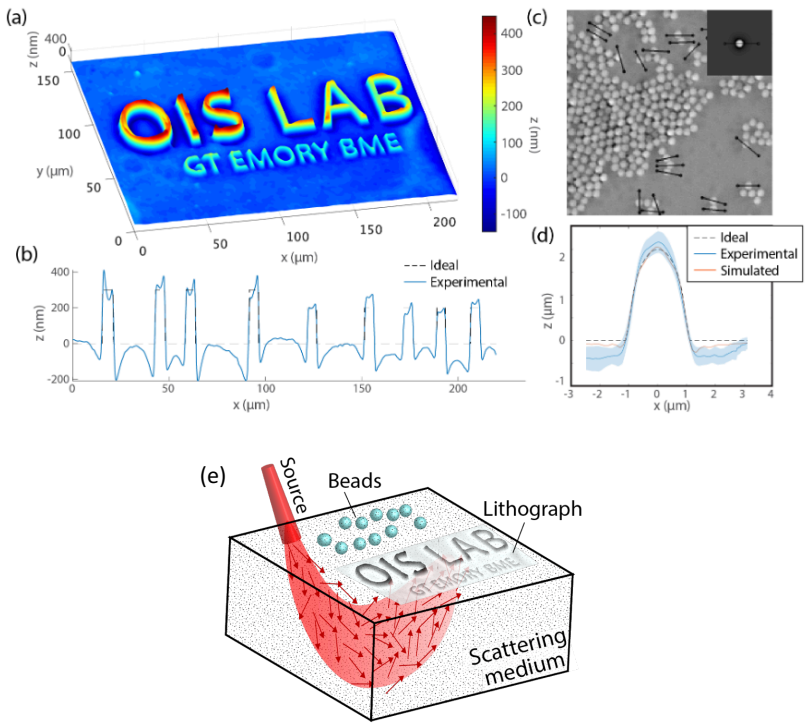

Fig. 2 (a) Relief depiction of qOBM image of lithography target. (b) Cross-section of phase image through the top 6 letters (blue) with actual height overlaid (black dashed). (c) qOBM image of several 2μm polystyrene beads immersed in a well of oil beneath an intralipid scattering phantom. (c inset) Phase reconstruction from simulated image of a 2μm polystyrene bead in oil. (d) The average height from cross-sections of 20 imaged beads (blue, standard deviation shaded), overlaid with the simulated phase recovery (orange), and ideal height (black dashed). (e) Cartoon depiction of the sample and illumination light source. Taken from Ref [2].

Figure 3 shows qOBM images from a freshly excised, but otherwise unaltered, whole mouse brain. Fig. 3a shows a 3D volumetric rendering of the brain using maximum intensity projection, while Figs 3b-f highlight various (sub-) cellular structures visible with qOBM. Here cellular and subcellular structures are clearly observed, such as neural cell soma with resolvable internal cell contents, smooth muscle cells, blood vessels and nearby glial cells. Axons and cell bodies of neurons and glia are also clearly resolvable. Blood vessels also clearly show red blood cells still inside. The figure also shows a rendition of a 3D volume (using maximum intensity projections) which highlight qOBM's 3D (i.e., tomographic) imaging capabilities. These images were taken 50-100 μm into the cortex. Currently, the maximum penetration depth is ~200 μm.

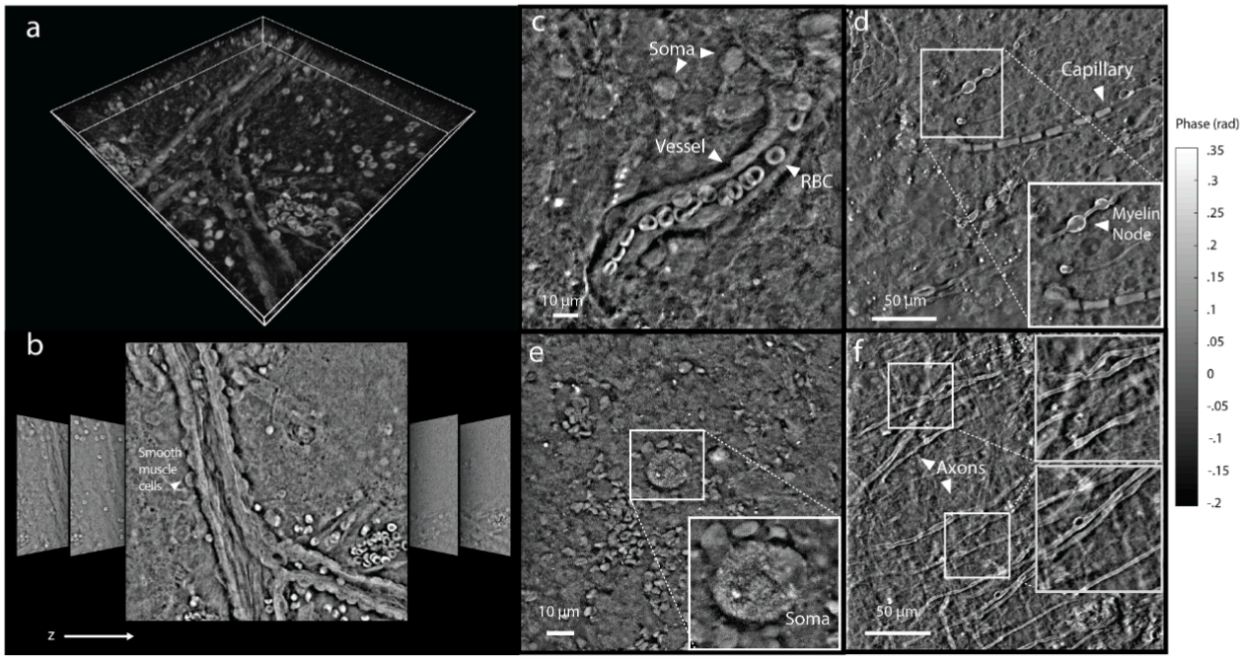

Fig. 3: qOBM images of whole mouse brains. (a) Maximum-intensity projection of a 60 μm vertical stack of qOBM images of blood vessel in unsectioned brain. (b) Representative images from the stack. (c) Blood vessel, with red blood cells and nearby brain cells in unsectioned cortex. (d) Descending capillary, axons, and myelin nodes from coronal section. (e) Neuron soma with internal structures visible from unsectioned cortex. (f) Axonal projections from white matter. Taken from Ref[2].

4. CONCLUSION

QOBM offers unique opportunities to leverage the clear subcellular detail and rich biophysical information provided by quantitative phase but now in a configuration that enables 3D imaging in thick samples[2]. The approach is easy to implement and use, it does not require any scanning optics (i.e., it is wide field), it is inherently tomographic, fast, and low-cost. Because qOBM relies on forward scattering, as opposed to forward scattering, it has higher sensitivity to low frequency structures; in turn, this yields unprecedented clarity cells' and tissues' structural composition which can only be rivaled by expensive nonlinear microscopy systems. Thus, qOBM is an exciting new tool that can greatly expand the utility of quantitative phase in many clinical and biomedical settings that were previously out of reach.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

1. Park, Y., Depeursinge, C. & Popescu, G. Quantitative phase imaging in biomedicine. *Nat Photonics* **12,** 1 12 (2018).

2. Ledwig, P. & Robles, F. E. Epi-mode tomographic quantitative phase imaging in thick scattering samples. *Biomed Opt Express* **10,** 3605 (2019).

3. Ford, T. N., Chu, K. K. & Mertz, J. Phase-gradient microscopy in thick tissue with oblique back-illumination. *Nat Methods* **9,** 1195 1197 (2012).

4. Mehta, S. B. & Sheppard, C. J. Quantitative phase-gradient imaging at high resolution with asymmetric illumination-based differential phase contrast. *Optics Letters* **34,** 1924 1926 (2009).

5. Tian, L. & Waller, L. Quantitative differential phase contrast imaging in an LED array microscope. *Opt Express* **23,** 11394 10 (2015).

6. Ledwig, P., Sghayyer, M., Kurtzberg, J. & Robles, F. E. Dual-wavelength oblique back-illumination microscopy for the non-invasive imaging and quantification of blood in collection and storage bags. *Biomed Opt Express* **9,** 2743 (2018).

7. Casteleiro Costa, P., Ledwig, P., Bergquist, A., Kurtzberg, J. and Robles, F. E., "Noninvasive white blood cell quantification in umbilical cord blood collection bags with quantitative oblique back-illumination microscopy," *Transfusion*, Accepted. Article DOI: 10.1111/trf.15704 (2020).

8. Streibl, N. Three-dimensional imaging by a microscope. *J. Opt. Soc. Am. A* **2,** (1985).

9. Yang, C., Zheng, G., Chung, J., Horstmeyer, R. & Ou, X. Diffraction tomography with Fourier ptychography. *Optica* **3,** 827–835 (2016).