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Visualizing transparent biology with sub-nanometer accuracy

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Interferometric phase microscopy measures transparent biological samples without labeling or physical contact with the sample, yielding a powerful and unique tool for research and medical diagnosis.

When coherent light interacts with an object, both its amplitude and phase are spatially modulated in the image of the object. However, conventional detectors, including digital cameras, are sensitive solely to intensity and record only the amplitude of the image. At the same time, many biological samples, including biological cells in vitro and some organs in vivo such as part of the human eye, are translucent 3D objects that have very little effect on the amplitude of light that interacts with them. This results in poor light intensity contrast in the sample image. Exogenous contrast agents, such as fluorescent dyes, are often used to solve this problem. However, these agents may be toxic, especially over extended periods. Fluorescent dyes also tend to photobleach, which limits imaging time.

Transparent or translucent objects can be much better analyzed by phase imaging. Phase is an inherent property of an object and is proportional to its optical thickness, which is a product of its refractive index and physical thickness. Therefore, phase imaging has the benefit of not requiring exogenous labeling. Phase contrast microscopy and differential interference contrast microscopy are widely used phase imaging techniques. However, these methods are not inherently quantitative and present distinct imaging artifacts that prevent straightforward determination of optical thickness. Interferometric phase microscopy (IPM), on the other hand, quantitatively records the phase of transparent and translucent samples.

Our group develops new IPM-based methods that enable imaging of biological samples in a label-free, non-contact manner, allowing sub-nanometer optical thickness changes to be tracked quantitatively at a rate of thousands of frames per second with minimal noise. This yields a powerful tool for biological research and medical diagnosis.

IPM-based techniques can be divided into wide-field and single-point methods. Wide-field IPM includes digital holographic microscopy, in which interference between the sample and reference beams creates a spatial interferogram or hologram on a digital camera. This interferogram can be processed into the quantitative amplitude and phase profiles of the sample. For cells that do not have a nucleus, such as mature red blood cells, the refractive index can be assumed to be constant, so that the quantitative phase measurement is proportional to the physical thickness of the cell (see Figure 1).

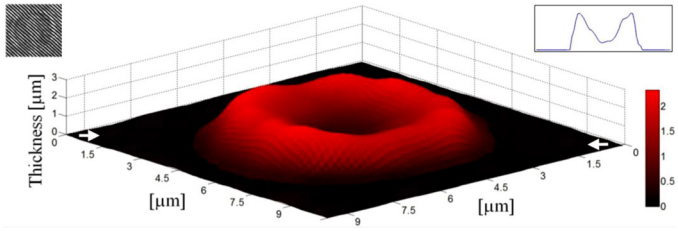

Figure 1. *Thickness profile of a red blood cell obtained by wide-field interferometric phase microscopy (IPM) under 40× magnification. This shows the valuable quantitative morphological data obtainable from a single exposure using this technique, without sample preparation or labeling, and with a low level of spatial noise. Top left: Spatial interferogram of the cell. Top right: Cross section across the cell diagonal (marked in the center by arrows). (Figure reproduced from Shaked, 2012,[1] courtesy of the Optical Society of America.)*

Recently, our group defined generalized parameters that can be calculated from wide-field IPM, which obviates prior decoupling of refractive index and physical thickness. This means that one can quantitatively analyze complex-refractive-index cells, such as cancer cells, which contain numerous organelles. Using these new parameters, we have been able to uniquely distinguish between the different stages of a cell life cycle and learn about cancer cell behaviors (see Figure 2).[2]

Wide-field IPM could be widely used for clinical applications owing to the unique advantages described above. Currently, however, there are not many options for commercial interferometric microscopes compared with other microscopy tech-

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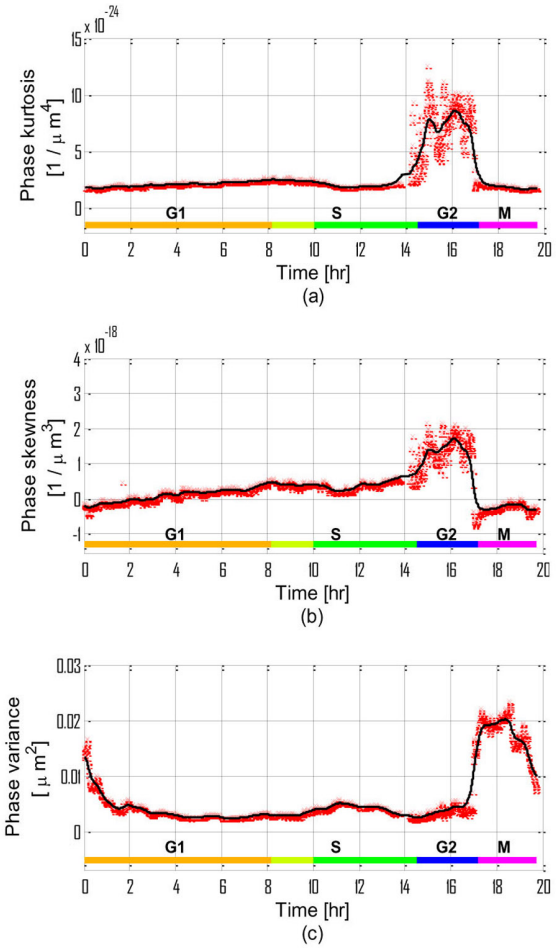

Figure 2. *Wide-field IPM-based statistical parameters change during the life cycle of cancer cells. It is therefore possible to predict the cell life cycle stages (marked as G1, S, G2, and M). Parameters measured: (a) phase kurtosis (peak distribution among the cell phase values); (b) phase skewness; and (c) phase variance. (Figure reproduced from Girshovitz and Shaked, 2012,[2] courtesy of the Optical Society of America.)*

niques. Hence, wide-field IPM is mostly used by optical and biomedical engineers for research purposes. The main reason is the difficulty in obtaining high-quality and stable interference patterns with modest and portable equipment and without an expert user.

To solve this problem, we have designed the τ interferometer,[1] an inexpensive portable device that can be attached to the output of any inverted microscope to obtain spatial interferograms of microscopic biological samples. It can do so without the strict stability and highly coherent illumination usually required for interferometric microscopy setups. Built using off-the-shelf optical elements and operable with low-coherence illumination, the τ interferometer is able measure the optical thickness profile with a temporal stability of 0.18nm and spatial stability of 0.42nm. We believe that the simplicity of connecting the device to a microscope and easily operating it will make wide-field IPM more accessible and affordable for biologists and clinicians, significantly broadening its range of applications.

Single-point IPM techniques, meanwhile, have an inherently higher signal-to-noise ratio than wide-field IPM. We recently proposed using a low-coherence spectral-domain phase microscopy system,[3] which relies on a single-point IPM technique based on low-coherence spectroscopy and optical coherence microscopy. This fiber-optic system is driven by a compact low-coherence source. The spectrum measurements are recorded by a portable spectrometer and processed to determine, with high accuracy, the optical thickness of the sample at a given point. This technique has a good potential for clinical, endoscopic, and in vivo applications.

In summary, IPM techniques for measuring transparent biological samples present unique advantages. They can measure remarkably small changes in the optical thickness of live samples even without labeling. They are also non-invasive, non-destructive, inexpensive, and relatively easy to implement and use. We expect that the IPM field will continue to grow rapidly in the near future, and many more biological researchers and medical doctors will start using it for new and exciting applications. We are currently using IPM to measure temperature-induced changes in red blood cells, cell fluctuations as an indicator for cancer, and neuronal activity. We are also adding molecular specificity to IPM by using laser heating of plasmonic metal nanoparticles to label targets of interest inside cells and tissues.

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