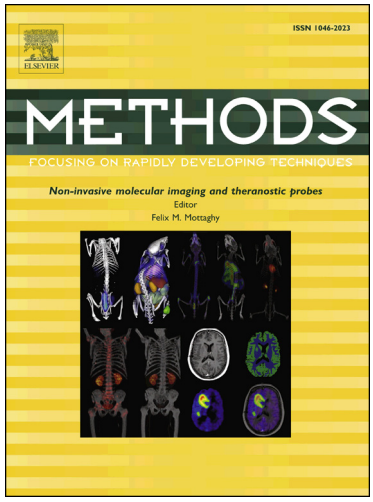

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Single-Shot Dual-Wavelength Interferometric Microscopy

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Interferometric microscopy (IM) can provide complex field information of the biological samples with high spatial and temporal resolution with virtually no photodamage. Measuring wavelength-dependent information in particular has a wide range of applications from cell and tissue refractometry to the cellular biophysical measurements. IM measurements at multiple wavelengths are typically associated with a loss in temporal resolution, field of view, stability, sensitivity, and may involve using expensive equipment such as tunable filters or spatial light modulators. Here, we present a novel and simple design for an interferometric microscope that provides single-shot off-axis interferometric measurements at two wavelengths by encoding the two spectral images at two orthogonal spatial frequencies that allows clean separation of information in the Fourier space with no resolution loss. We demonstrated accurate simultaneous quantification of polystyrene bead refractive indices at two wavelengths.

Keywords: Interferometric Microscopy, Dispersion, Cellular Imaging, Label-free Imaging, Quantitative Phase

1. Introduction

Interferometric microscopy (IM) provides complex field information for a variety of samples at the diffraction limit [1,2]. The label-free nature of these techniques makes them particularly suitable for studying biological samples in a minimally invasive way for an extended period of time. IM enables measurement of the quantitative phase over the field of view with high resolution in space and time. The measured phase represents the cumulative effect of the morphology and the refractive index (RI) distribution of the specimen in distorting the transmitted wavefront. This phase delay, in the case of eukaryotic cells, has been shown to be linked to the cellular dry mass [3], which provides a powerful tool for studying cellular processes such as cell division and growth over days or weeks [4]. When either of RI or morphology of the sample is known, one can measure the other without ambiguity. For instance, in the case of red blood cells (RBCs) in healthy individuals where cellular RI can be approximated with good precision, IM provides an accurate measurement of the morphology with nanometer precision [5]. Monitoring this morphology with sufficient temporal resolution enables measurement of the membrane fluctuations of RBCs that in turn provides a window to the cellular biomechanics [6,7]. Optical measurements of the biomechanical properties of RBCs have been used in studying the pathology of the diseases such as malaria [7] and sickle cell disease [8]. Similarly, a priori information about the sample morphology enables direct measurement of the refractive index from the quantitative phase information.

Early during the development of the interferometric techniques, it was realized that measurement of the phase delay at multiple wavelength can be a useful tool in studying cell and tissue refractometry or measuring concentrations of cellular proteins [9–11]. Additionally, IM measurements at a continuum spectrum in the visible range have been shown to provide tomographic measurement of the subcellular structures within the hematopoietic stem cells, while enabling an increase in the speed of the tomographic interferometric microscopy by orders of magnitude [12]. The sequential IM measurements of multiple wavelengths is either done through filter wheels [11], spatial light modulators [13], or tunable filters in combination with supercontinuum sources [14,15]. In the first case, the mechanical switching of the wavelength, sacrifices the temporal resolution, necessary for measurement of fast dynamics such as RBC fluctuation. In the case of acousto-optic tunable filters (AOTFs) or spatial light modulators, while they have faster switching time, the instrument cost is significantly higher. Color cameras have been suggested as an alternate way of simultaneous measurement of the phase information for multiple colors [16,17]. Because of the spectral ranges of the filters in the camera sensor are fixed by the manufacturer, color cameras compromise sensitivity of the IM measurements and reduce the flexibility in design for various applications. Stability of the interference is another factor that determines the sensitivity of the measurements and is maximized when reference and sample arms travel side by side [5,18]. Several designs for simultaneous measurements of two wavelengths has been suggested in non-common-path designs where the two colors travel different paths before interfering at the image plane limiting stability and sensitivity [9,19]. Two additional designs have been suggested to enable single-shot common-path measurements of multiple wavelengths by creating