Using an inexpensive module for Quantitative Phase Imaging

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Abstract. Common-path and nearly-common-path interferometry are techniques whereby both the object and reference co-propagate for a large part of their optical paths before being split towards the end of the system. Common-path systems use a grating to split the object field into two parts, one of which can be spatially filtered to generate a reference field while nearly-common path systems are typically based on using a Michelson interferometer applied to the object wavefield; one arm of which is filtered to produce the reference wavefield. Recently we proposed a nearly-common-path phase imaging module based on the Mach-Zender interferometer which we report here.

1 Introduction

Quantitative phase imaging (QPI) is a set of optical techniques that allow for real-time measurement of the phase-delay introduced by a specimen. QPI provides a powerful means to study cellular dynamics related to nanometric changes in cell morphology. QPI methods include coherent interferometry known as digital holographic microscopy (DHM), as well as partially coherent white-light methods. DHM uses a spatio-temporal coherent source to produce an interference pattern that encodes the sample's complex transmittance.

Common-path and nearly-common-path interferometry are techniques whereby both the object and reference co-propagate for a large part of their optical paths before being split towards the end of the system. Common-path systems use a grating to split the object field into two parts, one of which can be spatially filtered to generate a reference field while nearly-common path systems are typically based on using a Michelson interferometer applied to the object wavefield; one arm of which is filtered to produce the reference wavefield. Recently we proposed a nearly-common-path phase imaging module based on the Mach-Zender interferometer which we report here.

2. The module

The proposed setup is shown in Fig. 1 and employs an inexpensive fiber-coupled laser diode which is collimated using a plano-convex lens. This lens replaces the condenser lens in the microscope or can be integrated into the existing condenser lens system of a brightfield microscope. The module is positioned at the output camera port of the microscope and splits the object wavefield outside the microscope and filters one of the two paths to obtain a plane reference wavefield. The first element is a bi-convex lens located at a focal length distance from the image plane of the microscope and is the first of two lenses in a 4-f imaging system that maps the image plane to the camera plane. A polarizing beamsplitter splits the wavefield into the object and reference wavefields.
A pinhole is positioned at the back focal plane in the reference path to spatially filter the object wavefield and produce a clean reference wavefield. Two wedge prisms are located after the pinhole to correct the tilt of the refracted wavefront. A second beam-splitter recombines the two wavefields before the second lens, and a polarizer is used to enhance the diffraction efficiency of the recorded hologram. The recorded intensity is given by

\[ I(x) = |O(x)|^2 + |R(x)|^2 + O(x)R^*(x) + R(x)O^*(x) \]

\[ = |O(x)|^2 + 1 + O(x)\exp\left(\frac{-j2\pi x^2\delta}{f\lambda}\right) + O^*(x)\exp\left(\frac{j2\pi x^2\delta}{f\lambda}\right) \]

Where \( O \) is the object field and \( R \) is the reference obtained by filtering a copy of \( O \). Note the off-axis terms, produced by the wedge prisms; the tilt angle (and therefore the separation of the twin in the DFT domain) is a function of the focal length \( f \) as well as the wedge prism separation. The system can be optimized for any camera pixel size by varying the distance between the prism pairs using cage optics. The separation between the twin image terms in the discrete Fourier domain is proportional to the distance between the prism pairs, and for a laser source with low temporal coherence, it is necessary to carefully match the separation between the prism pairs in both paths to ensure a common path length. More details on the design and implementation of this module can be found at Ref. 6.

3. Results

The modular implementation has the significant advantage of extending the functionality of an existing life-science microscope. This permits DHM recording of samples with different magnifications, movement of the sample on the microscope stage as well as concurrent recording of fluorescence images. We employed a 20x and a 50x microscope objective to record two holograms. The holograms were recorded on a digital sensor, which and the raw image was filtered in the frequency domain making use of a discrete Fourier transform. Results are provided in Fig. 1 for (a) a micro lens and (b) a buccal epithelial cell. Phase unwrapping was applied before rendering the phase image in three-dimensions.

4. Conclusion

We report on a new self-reference interferometer that is portable, inexpensive, and robust to vibration and differential noise. The proposed system has advantages over recently proposed Mach-Zender modules for phase imaging, including variable tilt control and path-length matching for low-coherence sources. The system uses prism-pairs to shift Fourier transforms and allows for simple control of tilt that can match the bandwidth of the camera used for recording holograms. The optical elements used in the module were purchased for less than EUR 2000 and could be mass-produced for as little as EUR 100. Although not as robust to vibration as true common-path modules, the module was surprisingly insensitive to vibration due to its small size and integrated optical elements.