Live cell imaging by quantitative phase imaging with an add-on module for standard optical microscopes and with focal series reconstruction

Müller, J.[1], Eljarrat, A.[1], Wang, F.[1], Blessing, K.[2] and Koch, C.T.[1]

[1] Department of Physics, Humboldt University of Berlin, Germany, [2] Friedrich-Alexander-Universität Erlangen-Nürnberg, Interdisciplinary Center for Clinical Research, Germany

Introduction

Live cells are almost transparent and must therefore be imaged either by staining to increase their amplitude contrast or by imaging the induced phase shift of light that passes through them. Unlike standard phase imaging methods (e.g. Zernike phase contrast microscopy), quantitative phase imaging (QPI) can measure this phase shift precisely and determine cell characteristics without time consuming and often poisonous staining leading to only qualitative image contrast. Many QPI methods require special equipment, are expensive or introduce significant artefacts. We focus with our approach on low cost methods that only requires standard optical microscopes. We will present two approaches: our multi focus transport of intensity equation (MFTIE) algorithm that requires an automated sample stage for defocusing and our phase inline astigmatic holography (PIAH) method based on a camera module (patent pending) attached to a standard camera port that avoids having to change the objective lens focus and facilitates an even more quantitative phase reconstruction.

Objectives

Our goal is to offer low cost and quantitative methods for live cell imaging without staining that can be used with existing microscopes and their equipment. We will introduce MFTIE and PIAH and present their applications in life sciences (e.g. cell morphology, cell monitoring) highlighting their advantages over other cell imaging methods.

Materials & methods

The data acquisition for MFTIE consists in the acquisition of a special focal series and requires a microscope with a computer-controlled z-stage. The PIAH module can be attached to standard camera ports. Both methods work with halogen illumination with a colour filter or LED illumination. Both work with normal optical microscopes and are thus compatible with other imaging techniques, like fluorescence imaging.

Results

Figure 1 left shows the in-focus image of a HeLa cell without staining from a focal series acquired with our Zeiss Axiovert 200M optical microscope with a 40x objective lens (1.35 NA), a pco.edge 5.5 sCMOS camera and LED illumination with an average wavelength of 525 nm (figure 2). Figure 1 right shows the reconstructed phase shift by MFTIE of the HeLa cell, all the spatial frequencies are nicely recovered. Figure 2 is our new PIAH module, which can be attached to standard camera ports of optical microscopes.

Conclusion

Our QPI approaches can be used with existing optical microscopes with standard illumination and a PC equipped with a GPU to run the reconstruction. No staining and no special equipment, like gratings or dedicated microscopes is needed. The PIAH camera module can also be used for other imaging applications, like fluorescence imaging. Our approaches have the potential of being simple, fast, low-cost and versatile methods for quantitative phase imaging. We will present our techniques, compare them with other dedicated methods and discuss their viability.

We acknowledge financial support by the German Research Foundation (grant nr. 03EGSBE366).

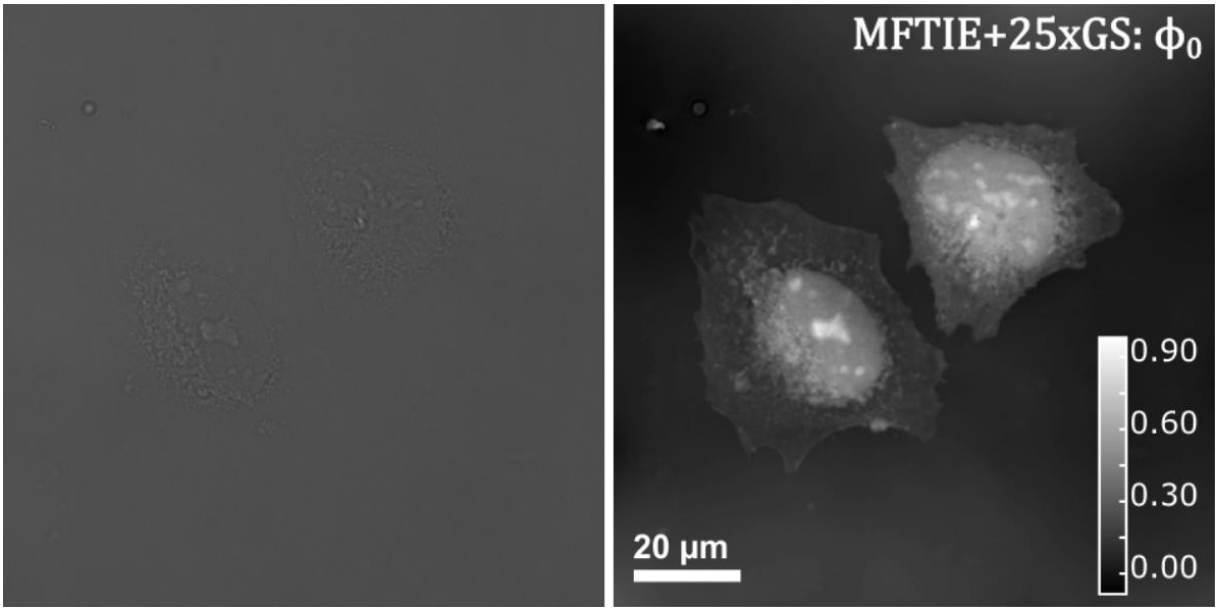

Image 1: In-focus image of a focal series from unstained hela cells (left), quantitative phase image reconstructed from the focal series (right).

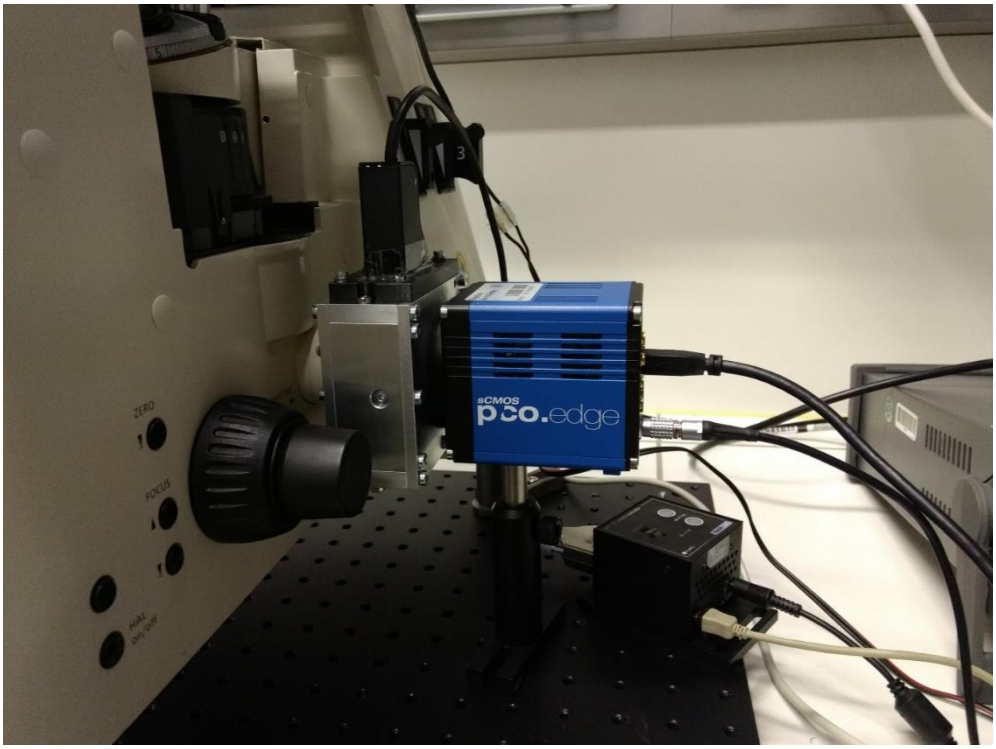

Image 2: The add-on camera module PIAH attached to a standard optical microscope. It allows to image cell quantitatively without staining.