RED BLOOD CELL TOMOGRAPHY IN REFLECTION BY MULTIPLE-WAVELENGTH DIGITAL HOLOGRAPHIC MICROSCOPY

Jonas Kühn,[1] Frédéric Montfort,[2] Tristan Colomb,[2] Benjamin Rappaz,[3] Corinne Moratal,[3] Nicolas Pavillon,[1] Pierre Marquet[3] and Christian Depeursinge[1]

[1]Institute of imaging and applied optics, EPFL, CH-1015 Lausanne, Switzerland
[2]Lyncée Tec SA, PSE-A, CH-1015 Lausanne, Switzerland
[3]Brain Mind Institute, EPFL, CH-1015 Lausanne, Switzerland

KEY WORDS: Digital holography, tomography, cell imaging, phase microscopy

1. INTRODUCTION

Digital holographic microscopy (DHM) is an interferometric microscopy technique providing real-time full-field complex wavefront retrieval: the axial resolution reaches nanometer-range, while lateral resolution remains diffraction-limited. In all biological-applications in transmission, like cell imaging, the DHM phase is proportional to the integrated optical path length (OPL) through the specimen, dependant on both topology and mean intracellular refractive index. Although methods to decouple both variables exist, the measurement remains an integrated value, thus no interface or sub-cellular component localization is possible in the z-direction. Recently, multiple angles DHM tomography and tomographic phase microscopy have rapidly evolved in order to recover full-3D refractive index map of intra-cellular structures. Nevertheless, all these techniques use mechanical scanning, either by rotating the object or varying the illumination beam angle. We are here using a wavelength-scanned method [1], thus neither the object nor any part of the setup is mechanically moved. We present a new configuration enabling to retrieve for the first time the reflection phase signal of fixed red blood cells (RBC), and reconstruction of the membrane geometry in 3D.

2. RESULTS

Our method, detailed in [2], relies on the reconstruction of 20 sequentially-acquired holograms between 485 and 675 nm of fixed RBC investigated in a reflection geometry. The sample coverslip is custom-treated with an anti-reflection coating to detect the on-cell reflection signal and the tomographic algorithm of [1] is improved.

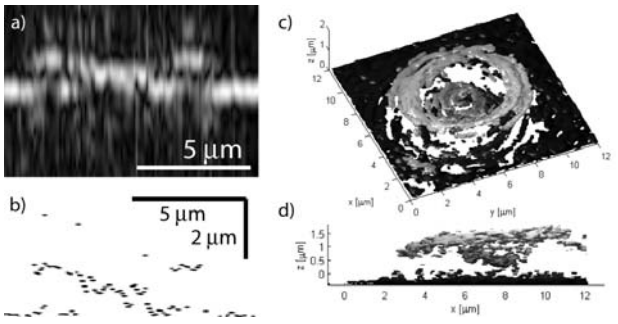

Figure: (a) Raw X-Z cut of the RBC
(b) Pseudo-deconvolution of (a)
(c-d) 3-D plots from all cuts like (b)

[1] F. Montfort *et al.*, "Submicrometer optical tomography by multiple-wavelength digital holographic microscopy," Applied Optics **45**, 8209{8217 (2006)
[2] J. Kühn *et al.*, "Sub-micrometer tomography of cells by multiple-wavelength digital holographic microscopy in reflection," Optics Letters (2009) (accepted)