4D imaging with nanometer sensitivity with Digital Holographic Microscopy

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Abstract: *Digital Holographic Microscopy (DHM) appears as an innovative imaging modality offering nanometer accuracies and precision both in the space and time domain. Nanometer accuracies in the space domain can be allied to real time imaging by the use of high acquisition rate camera and stroboscopic modality. In some cases, resolution can also be scaled down to the nanometer range if some assumptions are made concerning the shape of the specimen. DHM is expected to take an increasing role in metrology and in biomedicine where it can be used in conjunction with other imaging modalities and manipulations such as optical trapping.*

1. General Considerations

Digital holographic microscopy (DHM) is an interferometric microscopy technique which has the advantage of providing real-time, full-field complex wavefront data. Because the phase of the complex field can be evaluated with accuracies around one degree, the axial accuracy with which the surface of an object can be measured is around one nanometer or even less, while lateral resolution remains diffraction-limited. The first idea of reconstructing the wavefront by processing digitally an hologram acquired with a camera dates back to the sixties[1]. The propagation of the wavefront was discovered to be precisely simulated by the numerical computation of the Huyghens Fresnel expression of a diffracted wave [2] . Further on, the basic approach developed for Digital holography has been applied to Microscopy [3 4 5 6]. The main interest of Digital Holographic Microscopy (DHM) resides in the ultrahigh accuracy (sub-nanometer) of the measurement on the optical axis. Compared to classical phase shifting interferometry or white light interferometry, used as standards in high precision metrology and quality control, DHM offers major advantages. Measurements are performed in a time interval (acquisition time) much shorter, as the complete description of the complex wavefront is obtained from a single hologram capture[6] (taken in a fewμs), while several acquisitions are required with phase shifting techniques and vertical scanning in white light interferomety. The acquisition rate can be also much higher, in the order of several thousands of holograms per second. A larger immunity to external perturbations (vibration and ambient light) can be achieved. Secondly the accuracy of the apparatus is not intrinsically limited by the precision of the control of moving parts such as adjustment knobs and other controls, as DHM is exempt of them. In DHM, most procedures can be achieved by processing numerically the holograms and reconstructed images. In particular, numerical procedures can be applied for automatic wavefront corrections, which brings a considerable simplification of the optical design [7,8,9,10,11,12]. DHM imaging can be carried out in transmission (DHMt) for transparent specimens (for example micro-optics or biological objects) and reflection configuration (DHMr) for opaque or reflecting specimens: metallic or dielectric surfaces.

DHM is a versatile tool for Micro- and Nano technology

DHM has proven its efficiency on numerous applications in metrology, going from micro-optics testing [13], MEMS and MOEMS characterization [14], microstructures investigation or roughness measurements. The applicability of DHM to metrology is facilitated thanks to the remarkably high measurement stability and robustness of DHM, which results from the off-axis configuration allowing all the necessary information to be recorded from a single hologram. Finally, DHM enables the so-called "digital focusing" by treating the complex wavefront as containing all the data to reconstruct the image at any depth, thus extending the depth-of-field by pure numerical methods. Long distance measurement can be achieved with synthetic wavelength resulting from the combination of holograms taken simultaneously with two or more wavelengths [15]. Full tomographic imaging results from the acquisition of holograms at different wavelengths [16]. Another advantage of DHM resides in the fact that the detection is based on coherence properties linking object and reference wave: coherent detection. The wavefront is reconstructed from the cross terms. The signal amplitude results from the product of the intensities of the object and reference wave and therefore can result in an increase of SNR. Limitations on accuracies of measurements by DHM have been estimated from the evaluation of the role of shot noise on reconstructed wavefront [17,18,19]. For material characterisation, two wave fronts orthogonally polarized permit the measurement of the specimen birefringence and the estimation of stress and strains [20,21].

Applications in biomedecine:

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. DHM in transmission configuration: DHMt allows for the precise determination of Optical Pathlength (OPL) which is directly results from the integration of the refractive index (RI) over the propagation of the light beam. Point to point OPL determinations yield an absolute phase contrast image in microscopy that can be directly interpreted by the biologists as proportional to the dry mass concentration. The high sensitivity and accuracy of the OPL determination make DHMt attractive in biomedicine to evaluate quantitatively protein content, hemoglobin in particular. The advantages of robustness and accuracy also hold for applications in biology and biomedicine.

Although methods to decouple thickness of the specimen and its refractive index exist, the measurement remains a value which is averaged over the pathlength, 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 and permit to recover full-3D refractive index map of intra-cellular structures. These techniques however use mechanical scanning, either by rotating the object or varying the illumination beam angle. However, a wavelength-scanned method can be used so that neither the object nor any part of the setup is mechanically moved. DHMr is used for these investigations in biology. 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.

The determination of exact morphology and volume of cells, the observation of dynamical phenomena are important fields of investigation in modern biology, where living tissues are continuously changing in shape and composition [22,23,24,25]. These combined features make DHM appear as a real innovative modality in absolute phase contrast microscopy. Accurate 3D investigations are also the result of DHM. Recently true 3D conformation of cells has revealed particularly attractive: measurements of the refractive index RI distribution within the cell has been made possible with DHM. Tomography a biological sample performed at multiple wavelength or at multiple incidences have been demonstrated [26,27.28].

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