Recent Progress and Perspectives in Digital Holographic Microscopy

Christian Depeursinge
Ecole Polytechnique Fédérale de Lausanne, Institut d'Imagerie et d'Optique Appliquée, CH-1015 Lausanne, Switzerland

Abstract: Recent developments in Digital Holographic Microscopy (DHM) have permitted to achieve imaging accuracies and resolutions down to the nano-range. This result could be obtained by careful control of all parameters involved in the wavefront reconstruction.

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1. Introduction

The present proceeding paper will report mainly the results of the collaboration achieved in the Lausanne group and, intentionally, does not cover the litterature of other groups, since the limited place allotted to this communication could not offer the opportunity of a review of the DHM field.

Digital Holographic Microscopy (DHM) is an imaging technique offering both sub-wavelength resolution [1-3] and real time observation capabilities. Moreover, the possibility of reconstructing "automatically", i.e. without the "manual" intervention of the microscopist, the profile of the object or, more recently its true 3D conformation (refractive index distribution) from the digitized hologram is particularly attractive for end users. These combined features make DHM appear as a real innovative modality in phase contrast microscopy. From that point of view , it outsurpasses many of the previous interference microscopy methods.

2. Working principle:

DHM microscopy accomodates reflection as well as transmission geometries [3]. In DHM, The principles of hologram formation, acquisition and wavefront reconstruction from digital holograms, acquired in a non-scanned modality, have been described in details in several papers [1-5]. The reconstruction of the wavefront from the hologram provides the amplitude and the absolute phase of the wave diffracted by the microscopic objects [3]. If a carefull control of the experimental conditions allows maintaining the phase STD below one degree, the Absolute phase contrast yields longitudinal accuracies as low as four [6], down to less than one nanometer in air (depending on the statistical treatment of the holograms), or even less in dielectric media. In a transmission geometry, the accuracy can be kept below 15nm [6]. Thanks to the introduction of a Microscope Objective (M.O.) between the object and the camera in order to pick up the hologram (proposed by our group in 1997 [1]), the lateral accuracy, after appropriate image processing is also in the nanometer range and the corresponding resolution could be kept at a sub-micron level by the use of a high Numerical Aperture (N.A.) M.O. In the present state of the art, resolution can be kept commonly below 600nm [7]. The role of the M.O. consists in acquiring the high spatial frequency components of the beam diffracted by the object. Their high fidelity restitution in the image plane through the M.O. and an optional tube lens, has permitted to reduce the scale of the lateral wavevector k after the passage through the M.O. and tubelens by a factor equal to the magnification, providing the final advantage of adapting the spatial frequencies of the hologram to the sampling capacity of the camera.

The performances, in term of accuracy, resolution, of 3D imaging is limited by considerations which directly follow from the practical realization of the optical DHM setup as well as from the fundamental optics laws:

3. Correction of image aberrations :

Corrections of the imperfections of the optical DHM setup: Numerical methods [8-11] have been recently developed which enables to reconstruct correct and accurate phase distributions, even in the presence of strong and high-order aberrations. The developed procedure compensates for phase aberrations in digital holographic microscopy by computing a polynomial phase mask directly from the hologram. The so-called phase-mask parameters are computed automatically without knowledge of physical values such as wave vectors, focal lengths, or distances. The implementation of this technique allows palliating the imperfections of optical components and setups, opening the way to quasi perfect, numerically corrected, optical systems: the corrected images can be superimposed and combined for 3D imaging by true tomography.

Corrections to compensate the diffraction and aberrations induced by the M.O: a method to measure the complex 3D amplitude point spread function (APSF) of an optical imaging system has been developed [12] and possibly used to correct the aberrations caused by the M.O. The approach consists in measuring and interpreting the APSF by evaluating in amplitude and phase the image of a single emitting point. A single hologram gives access to the transverse APSF. The 3D APSF is obtained by performing an axial scan

4. Axial and lateral imaging accuracy and resolution down to the physical limits

Accuracies: Imaging objects along the optical axis of the DHM is directly limited in its accuracy by the fluctuations of the phase of the reconstructed wavefront . These fluctuations can occur both in time and space, due to the uncertainty in the acquisition of the hologram. These uncertainties arise basically from the time fluctuations of the signal: the quantum nature of light: shot noise, is the unavoidable tribute to pay to the physical nature of light, but fluctuations can also arise from thermodynamic fluctuations of the systems in presence: optical DHM setup, medium crossed by the optical beam and the observed object itself. The space variance of the hologram arises from stationary variations of light intensity in the hologram, due to various mechanisms, originating either from the camera or the optical setup: unavoidable interferences or components defects. Recent progresses [13] in the understanding of the origin of the noise have allowed to gain much in the accuracy of images, both in the axial and lateral domain. It was found that the experimental and theoretical (shot noise limitations) uncertainties were off only by a factor of two.

Resolutions: The problem of resolution in DHM, must be addressed in the frame of a more general concept which must take into account the characteristics of the object. Some guess concerning the nature, the texture and constitution of the observed object must be made. A first distinction must be made between two or three dimensional objects: an object may be two dimensional or, in other terms, in the case of a 3D object, we could be interested only in its surface (2D object). In this case, one can shrink resolutions “in height” down to the nano level, thanks to a high strength of the signal. For 3D isolated punctual objects, resolution can be confounded with accuracy, making feasible localization at the nano level of isolated punctual objects like nano- particles. However, the signal is usually weak and the uncertainties reduce the accuracy and resolution. More generally , for true 3D objects: sparsity may play a key role, by permitting localization of individual parts of the object with ultra-high resolution

True 3D tomography: For denser physical objects, multi-wavelength acquisition of holograms can allow slicing of the object by superposition of reconstructed wavefronts. Out of focus images are thereby rubbed out and true 3D configuration of the object, in general characterized by its 3D distribution of the refractive index can be imaged in 3D. High resolutions or even so-called “ultrahigh resolutions” can be achieved in this case: typically 750 nm as established in [14]. The distribution of the refractive index can be also established by applying Radon theorem to projected phase images in a plurality of directions, each image being obtained by reconstruction of wavefront scattered by a rotated beam. Tomographic images have been obtained for pollen grains [15] and amoebas [16], with subwavelength resolution.

5. Applications: A variety of applications of this new type of optical microscopy are possible: material research: stress evaluation by imaging birefringence [17-21], surface and interface sciences, microtechnologies, micro-optics and MOEMS [22] are field where DHM brings innovative solutions. Applications to cell dynamics studies: nano-movements and cyto-architectures deformations constitute another field of investigations. [7,23]. Finally, tomographic images of tissues by short coherence gating have also been obtained: they show in depth and “en face” images of the anterior chamber of the eye [24].

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7. References

[1]. E. Cuche, P. Poscio, and C. Depeursinge, "Optical tomography by means of a numerical low-coherence holographic technique," Journal of Optics-Nouvelle Revue D Optique **28**, 260-264 (1997).
[2]. E. Cuche, F. Bevilacqua, and C. Depeursinge, "Digital holography for quantitative phase-contrast imaging," Opt. Lett. **24**, 291-293 (1999).
[3]. E. Cuche, P. Marquet, and C. Depeursinge, "Simultaneous amplitude-contrast and quantitative phase-contrast microscopy by numerical reconstruction of Fresnel off-axis holograms," Appl. Opt. **38**, 6994-7001 (1999).
[4]. E. Cuche, P. Marquet, and C. Depeursinge, "Spatial filtering for zero-order and twin-image elimination in digital off-axis holography," Appl. Opt. **39**, 4070-4075 (2000).
[5]. E. Cuche, P. Marquet, and C. Depeursinge, "Aperture apodization using cubic spline interpolation: application in digital holographic microscopy," Opt. Comm. **182**, 59-69 (2000).
[6]. F. Charriere, J. Kuhn, T. Colomb, F. Montfort, E. Cuche, Y. Emery, K. Weible, P. Marquet, and C. Depeursinge, "Characterization of microlenses by digital holographic microscopy," Appl. Opt. **45**, 829-835 (2006).
[7]. P. Marquet, B. Rappaz, P. J. Magistretti, E. Cuche, Y. Emery, T. Colomb, and C. Depeursinge, "Digital holographic microscopy: a noninvasive contrast imaging technique allowing quantitative visualization of living cells with subwavelength axial accuracy," Opt. Lett. **30**, 468-470 (2005).
[8]. T. Colomb, E. Cuche, F. Charriere, J. Kuhn, N. Aspert, F. Montfort, P. Marquet, and C. Depeursinge, "Automatic procedure for aberration compensation in digital holographic microscopy and applications to specimen shape compensation," Appl. Opt. **45**, 851-863 (2006).
[9]. T. Colomb, J. K. Kuhn, F. Charriere, C. Depeursinge, P. Marquet, and N. Aspert, "Total aberrations compensation in digital holographic microscopy with a reference conjugated hologram," Opt. Express **14**, 4300-4306 (2006).
[10]. T. Colomb, F. Montfort, J. Kuhn, N. Aspert, E. Cuche, A. Marian, F. Charriere, S. Bourquin, P. Marquet, and C. Depeursinge, "Numerical parametric lens for shifting, magnification, and complete aberration compensation in digital holographic microscopy,"JOSA A **23**, 3177-3190 (2006).
[11]. F. Montfort, F. Charriere, T. Colomb, E. Cuche, P. Marquet, and C. Depeursinge, "Purely numerical compensation for microscope objective phase curvature in digital holographic microscopy: influence of digital phase mask position," JOSA A **23**, 2944-2953 (2006).
[12]. A. Marian, F. Charriere, T. Colomb, F. Montfort, J. Kühn, P. Marquet, and C. Depeursinge, "On the complex three-dimensional amplitude point spread function of lenses and microscope objectives: theoretical aspects, simulations and measurements by digital holography," J. Microsc. **225**, 156-169 (2007).
[13]. F. Charriere, T. Colomb, F. Montfort, E. Cuche, P. Marquet, and C. Depeursinge, "Shot-noise influence on the reconstructed phase image signal-to-noise ratio in digital holographic microscopy," Appl. Opt. **45**, 7667-7673 (2006).
[14]. F. Montfort, T. Colomb, F. Charriere, J. Kuhn, P. Marquet, E. Cuche, S. Herminjard, and C. Depeursinge, "Submicrometer optical tomography by multiple-wavelength digital holographic microscopy," Appl. Opt. **45**, 8209-8217 (2006).
[15]. F. Charriere, A. Marian, F. Montfort, J. Kuehn, T. Colomb, E. Cuche, P. Marquet, and C. Depeursinge, "Cell refractive index tomography by digital holographic microscopy," Opt. Lett. **31**, 178-180 (2006).
[16]. F. Charriere, N. Pavillon, T. Colomb, C. Depeursinge, T. J. Heger, E. A. D. Mitchell, P. Marquet, and B. Rappaz, "Living specimen tomography by digital holographic microscopy: morphometry of testate amoeba," Opt. Express **14**, 7005-7013 (2006).
[17]. D. Beghuin, E. Cuche, P. Dahlgren, C. Depeursinge, G. Delacretaz, and R. P. Salathe, "Single acquisition polarisation imaging with digital holography," Electron. Lett. **35**, 2053-2055 (1999).
[18]. T. Colomb, P. Dahlgren, D. Beghuin, E. Cuche, P. Marquet, and C. Depeursinge, "Polarization imaging by use of digital holography," Appl. Opt. **41**, 27-37 (2002).
[19]. T. Colomb, E. Cuche, F. Montfort, P. Marquet, and C. Depeursinge, "Jones vector imaging by use of digital holography: simulation and experimentation," Opt. Comm. **231**, 137-147 (2004).
[20]. T. Colomb, F. Durr, E. Cuche, P. Marquet, H. G. Limberger, R. P. Salathe, and C. Depeursinge, "Polarization microscopy by use of digital holography: application to optical-fiber birefringence measurements," Appl. Opt. **44**, 4461-4469 (2005).
[21]. Y. Bellouard, T. Colomb, C. Depeursinge, M. Dugan, A. A. Said, and P. Bado, "Nanoindentation and birefringence measurements on fused silica specimen exposed to low-energy femtosecond pulses," Opt. Express **14**, 8360-8366 (2006).
[22]. C. Depeursinge, F. Charriere, and F. Montfort, "Digital holographic microscopy for comparative metrology in mirco-optics and MOEMs," Tm-Technisches Messen **73**, 172-179 (2006).
[23]. B. Rappaz, P. Marquet, E. Cuche, Y. Emery, C. Depeursinge, and P. J. Magistretti, "Measurement of the integral refractive index and dynamic cell morphometry of living cells with digital holographic microscopy," Opt. Express **13**, 9361-9373 (2005).
[24]. P. Massatsch, F. Charriere, E. Cuche, P. Marquet, and C. D. Depeursinge, "Time-domain optical coherence tomography with digital holographic microscopy," Appl. Opt. **44**, 1806-1812 (2005).

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