Applications of digital holographic microscopes with partially spatial coherence sources

Frank Dubois, Catherine Yourassowsky, Natacha Callens, Christophe Minetti, and Patrick Queeckers

Université Libre de Bruxelles
Microgravity Research Center
50 Av. F. Roosevelt, CP 165/62
B-1050 Brussels (Belgium)

e-mail: frdubois@ulb.ac.be

Abstract. We implemented partially spatial coherent illuminations in digital holographic microscopes (DHM) working in transmission. The benefits gained with those sources are outlined. A major advantage is the drastic reduction of the speckle noise making it possible high image quality comparable to the best classical transmission microscopes. Several implementations of biomedical applications, where digital holography provides significant information, are described. With a rapid DHM permitting the analysis of dynamical phenomena, applications in microfluidics are also provided.

1. Introduction
Optical microscopy is limited by the small depths of focus due to the high numerical apertures of the microscope lenses and the high magnification ratios. The extension of the depth of focus is thus an important goal in optical microscopy. In this way, digital holography microscopy (DHM), where the hologram is recorded with a CCD camera, yields the reconstruction in depth ¹.

As the holographic information involves both optical phase and intensity, the complex amplitude is computed to refocus slice-by-slice, by implementing the Kirchhoff-Fresnel (KF) equation, the depth images of a thick sample. In addition, the optical phase is the significant information to quantitatively measure the optical thicknesses of the sample which are not available from the measurements with classical optical methods ². DHM has been applied in numerous applications of interest as observation of biological samples ³-⁶, living cells culture analysis ⁷-⁹ and accurate measurements inside of cells as refractive indexes and even 3D tomography ¹⁰-¹¹.

DHM is very flexible to implement powerful processing of the holographic information or of the processed images. For examples, there exist methods to correct phase aberration ¹²-¹⁴ to perform 3D pattern recognition ¹⁵-¹⁶, to process the border artefacts ¹⁷, to emulate classical phase contrast imaging ⁶,⁹, to implement autofocus algorithms ¹⁸,¹⁹ and to perform object segmentation ²⁰.
Usually, DHM is implemented with coherent laser beam. However, laser beams are very sensitive to any defect in the optical paths in such a way that the results can be badly corrupted by the coherent artefact noise. For that reason, we developed two types of DHM with a partially spatial coherent illumination in Mach Zehnder configurations in order to reduce this noise\textsuperscript{5,21,22}. The first type is using a LED which is spatially filtered to achieve the spatial coherence. As the spectral bandwidth is about 20nm, the source is also of partially temporal coherence. This feature improves further the noise reduction but imposes to properly align reference and object beams. Therefore, the computation of the optical phase results from the implementation of a phase shifting method. For applications where the phenomena are too rapidly varying for the phase shifting, we implemented a DHM where the complex amplitude is computed with the Fourier transform method\textsuperscript{23}. In this case, we realized the partially spatial coherent source from a laser beam focused close to a rotating ground glass. The two configurations are described in the next section. The benefits in using the partially spatial coherence in a DHM are given in section 3 and some applications in section 4.

2. Optical set-ups and digital holographic reconstructions

2.1. DHM working with a LED

The DHM that benefits from a partially spatial coherent illumination created from a LED is described by the figure 1. A LED beam is injected in a liquid optical fiber to homogenize the beam in the front focal plane of a lens L1 which is imaged in the plane of the sample So. An aperture between the mirror M1 and M2 increases the spatial coherence. A plane of the sample is imaged on the CCD camera by the couple of lenses ML2-L6. The sample (So) is placed inside a Mach Zehnder interferometer to record the interference patterns between the sample beam and the reference beam.

The mirror M4 is placed on a piezoelectric transducer to implement a 4 images phase stepping method.

![Figure 1. DHM working with a LED: Light-emitting diode (LED), optical fiber (OF), lenses (L1-L6), beam splitters (BS1-BS2), mirrors (M1-M5), microscope lenses 10x (ML1-ML2), optical flat (Sr), sample (So), camera (CCD).](image)

The typical acquisition time of the four frames is about ¼ second. The resulting complex optical field is used to refocus the optical intensity and phase fields. The lateral resolution computed according to the Rayleigh criteria is 1.3 µm, and the Z resolution, computed with the formula $\delta = \frac{\lambda}{2NA}$, is 1.4 µm, where $\lambda$ is the average wavelength (660nm) and NA the numerical aperture (0.30). The depth of investigation by means of digital holographic reconstruction is about 100$\delta$. The resolution of the optical thickness computed on the phase map is about 2nm.
As DHM records the complex amplitude, and thanks to the low noise level, it is possible to emulate usual microscopy modes such as the differential interference contrast (DIC); this latter is particularly useful in the observation of living cells by providing the scientists an usual visualization mode.

2.2. Rapid DHM working with a Laser beam incident on a rotating ground glass

This section describes the rapid DHM working with partial spatial coherent source created from a laser beam. The optical set up is shown by the Figure 2.

![Figure 2. Rapid DHM with a partially spatial coherent source from a laser beam.](image)

A mono-mode laser diode beam (\(\lambda\)=635nm) is transformed into a partially spatial coherent source by focusing the beam, by to lens L1, close to the plane a Rotating Ground Glass (RGG). The spatial partial coherence is adjusted by changing the position of the focused spot with respect to the RGG plane. The lens L2 collimates the beam that is divided by a beam splitter BS1. The transmitted part, the object beam, illuminates the sample S in transmission. A plane of S is imaged by the lenses L3-L5 on the CCD camera sensor. The reference beam, reflected by BS1, interferes with the object beam on the CCD sensor (1280 x 1024 pixels). The reference beam is slanted with respect to the object beam in order to record a grating-like thin interference pattern and to implement the Fourier method to compute the complex amplitude for every recorded frame.

3. Benefits of the partially spatial coherence for the DHM in transmission

Consider a transparency \(t(x,y)\) in a plane separated to the imaged plane on the CCD by a distance \(d\). It can be shown that the partial spatial coherence nature of the source results in a low pass spatial filtering process that can be expressed by:

\[
V(v_x, v_y, d) = T(v_x, v_y) S\left(\frac{v_x d}{f}, \frac{v_y d}{f}\right)
\]  

(1)

Where \(T\) and \(S\) are the Fourier transformations of \(t\) and of the source aperture \(s, f\) the focal length of the collimating lens and \(v_x , v_y\) the spatial frequencies. The resulting signal \(V\) is filtered by a scaled Fourier transformation of the source. The factor \(d/f\) means that the filtering process increases with \(d\). Eq.(1) allows us to set accurately the partial coherence state of the source with respect to the requested resolution, refocusing distance and the location of the optical defects to be rejected. As in Biomedical applications, it happens often that the selection of the experimental container is not made for the alone optical quality but has to take into account the biocompatibility. Then, the experimental containers are often of poor optical quality. In this situation, the partial coherence can be matched to refocus the sample while keeping the container defects at distances to be efficiently filtered.

The resolution loss is controlled by adjusting the spatial partial coherence of the source and can be kept smaller than a limit defined by the user. The spatial coherence reduction also increases the
visibility of the refocused plane by reducing the influence of what is out of focus. It is useful when the sample is highly scattering the light. Indeed the speckle noise in coherent illumination results from the coherent superposition of random contributions that are originated from out of focus locations. The benefit of the noise reduction by the partial spatial coherent illumination is illustrated by the figure 3 where images of 5µm particles in distilled water are recorded in partially and full coherent illumination.

**Figure 3-a.** Part of an interferometric image of 5µm particle in distilled water – partially spatial coherent illumination.  

**Figure 3-b.** Part of interferometric image of 5µm particle in distilled water – spatial coherent illumination.

Partial coherent illumination also removes the multiple reflections occurring with coherent illumination. It is obvious with the LED illumination due to the small temporal coherence. However, it is also true with the microscope working with the RGG and the laser source. In this case, a reflection introduces an increase of the optical path $d$. When the distance $d$ introduces a significant decorrelation depending on the spatial coherence, the contrast of the interference fringe pattern between the reflected and the direct beam is largely reduced.

4. Applications

4.1. Biomedical applications – study of cell cultures  
The biomedical applications here below have been performed with the DHM illumination with a LED, as described by figure 1. The DHM is a powerful tool to study cell cultures and their evolution during the time. The holographic microscope provide a bright field image quality equivalent to a classical microscope, even with sample container of poor quality like plastic dishes (figure 4-a).

With the complex amplitude, it is possible to emulate classical optical microscopy modes as the differential interference contrast (DIC) to improve the visibility of unstained living cells $^{6,9}$ (figure 4-b). The quantitative phase computation is used to analyze dynamically the cell morphology with nanometric accuracies (figure 4-c, 4-d and 4-e).

In collaboration with several biomedical departments of the Université Libre de Bruxelles, different experiments were performed with the DHM. With the RUBIO department, we performed the observation and morphology analysis of cell fusions and hybrids formation. In the scope of the anti-tumor vaccination strategy, hybrids between tumor and dendritic cells combine the expression of specific tumor antigens and the machinery for their optimal presentation for the induction of an efficient immune response. As we implemented the fluorescence mode in DHM $^6$, this multimode diagnostic allows to study fusion processes of different cell lines in details.

The capability to observe living unstained cells in turbid media is shown by results obtained on the migration of cancerous cells embedded in a collagen gel $^9$. With the Laboratories of Toxicology and of SLN, we performed time laps experiments with DHM on unstained HT-1080 fibrosarcoma cells embedded in a thick 3D collagen matrix (thickness around 1,500 µm) to study the cell motility.
4.2. Microfluidic experiments

The rapid DHM of figure 2 is currently used to perform two types of microfluidic experiments.

First, we analyze the micro-sized particles distributions in hydrodynamic flows passing through a Split-flow Lateral-Transport Thin (SPLITT) separation cell. This class of separation devices makes possible the continuous and rapid fractionation of macromolecular, particulate materials and particles according to their sizes. The cell is a thin ribbon-like channel of 234 µm thickness, having at its extremities two inlets and two outlets separated by splitters. The inlet splitter allows to separately inject the sample through and the carrier liquid. The outlet splitter divides the flow into two sub-streams in the outlets. DHM provides measures of the particle distributions to assess the influence of the hydrodynamic effects on the separation efficiency, as well as the particle-particle and particle-wall interaction. It permits to obtain the particle diameters, their 3D positions and to estimate their mean velocities.
The second application concerns the study of the dynamics of vesicles (lipid membranes) enclosing a fluid and suspended in an aqueous solution. Vesicles can be viewed as a simple model to represent the basic mechanical properties of cells such as red blood cell with which they share several common features and behaviours under flow. Although this is a simplified model, they are an interesting tool for physicists. Parameters such as diameter, volume-to-surface ratio and viscosity of the filling fluid can be easily varied over significant ranges. As their behavior under shear flow is not completely understood. Digital holographic microscopy gives the opportunity to investigate the 3D distribution of vesicles flowing inside a shear-flow chamber and to determine their size and shape. As the vesicles are completely transparent (figure 5), the quantitative phase contrast imaging capability of the DHM is used.

5. Conclusions
We show two configurations of DHM working with spatial partial coherent sources. The benefits of this type of sources are given. Biomedical and fluid physics applications illustrate those advantages.

References
[1] Zhang T and Yamaguchi I 1998 Opt. Lett. 23 1221
[2] Cuche E Bevilacqua F and Depeursinge C 1999 Opt. Lett. 24, 291
[3] Ikeda T Popescu G Dasari RR and Feld MS 2005 Opt. Lett. 30 1165
[4] Popescu G T. Ikeda T Best CA Badizadegan K Dasari RR and Feld MS 2005 J. Biomed. Opt. 10, 060503
[5] Dubois F Joannes L Legros JC 1999 Appl. Opt. 38 7085
[6] Dubois F Yourassowsky C Monnom O 2004 Ed. Faupel M Smigielski P and Grzymala R Fontis Media, Formartis 287
[7] Marquet P Rappaz B Magistretti PJ Cuche E Emery Y Colomb T and Depeursinge C 2005 Opt. Lett. 30, 468
[8] Carl D, Kemper B Wernicke G von Bally G 2004 Appl. Opt. 43 6536
[9] Dubois F Yourassowsky C Monnom O Legros JC Debeir O Van Ham P Kiss R Decaestecker C 2006 J. Biomed. Opt. 11 054032
[10] Lue N G Popescu G Ikeda T Dasari RR Badizadegan K, and Feld MS 2006 Opt. Lett. 31 2759
[11] Charrière F Marian A Montfort F Kühn J Colomb T Cuche E Marquet P and C. Depeursinge 2006 Opt. Lett. 31 178
[12] Ferraro PS De Nicola S Finizio A Coppola G Grilli S Magro C and Pierattini G 2003 Appl. Opt. 42 1938
[13] Colomb T Kühn J Charrière F Depeursinge C Marquet P and Aspert N 2006 Opt. Exp. 14, 4300
[14] Miccio L Alfieri D Grilli S Ferraro P Finizio A De Petrocellis L and De Nicola S 2007 Appl. Phys. Lett. 90 041104
[15] Kim D and Javidi B 2004 Opt. Exp. 12, 5539
[16] Dubois F Minetti C Monnom O Yourassowsky C Legros JC 2002 Appl. Opt. 41, 4108
[17] Dubois F Monnom O Yourassowsky C and Legros JC 2002 Appl. Opt. 41, 2621
[18] Dubois F Schockaert C Callens N and Yourassowsky C 2006 Opt. Exp. 14, 5895
[19] Li W Loomis NC Hu Q and Davis CS 2007 J. Opt. Soc. Am. A 24, 3054
[20] McElhinney CP McDonald JB Castro A Frauyl Javidi B Naughton TJ 2007 Opt. Lett. 32, 1229
[21] Dubois F Novella Requena ML Minetti C Monnom O and I stasse E 2004 Appl. Opt. 43, 1131
[22] Dubois F Callens C Yourassowsky C Hoyos M Kurowski P and Monnom O 2006 Appl. Opt. 45, 864
[23] Kreis T 1986 J. Opt. Soc. Am. A 3, 847
[24] Giddings JC 1985 Sep. Sci. Technol. 20, 749
[25] P.S. Williams 1994 Sep. Sci. Technol. 29, 11