

Exploring cell dynamics with Digital Holographic Microscopy

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Abstract:

Quantitative phase signal (QPS) of digital holography microscopy has permitted to investigate Cellular Membrane nano-Fluctuations (CMnF), of red blood cells as well as to non-invasively provide an optical signature of the electrical activity of cells.

Digital Holographic Microscopy

DHM allows to quantitatively measure the wavefront transmitted through a specimen and magnified by a microscope objective. A hologram, which contains the whole information of the transmitted wavefront, is formed by the interference of the wave coming from the object with a reference wave, and recorded with a camera. (cf. Figure 1) Holograms are recorded in an off-axis geometry i.e. the reference wave reaches the camera with a small incidence angle ($\sim 1^\circ$) with respect to the propagation direction of the object wave. The hologram reconstruction process, based on the scalar wave propagation theory, permits to numerically process the hologram in order to extract both amplitude and phase information of the transmitted wavefront. A detailed description of the algorithm used for hologram reconstruction in such an off-axis configuration and aberration compensation has been previously described in [1, 2].

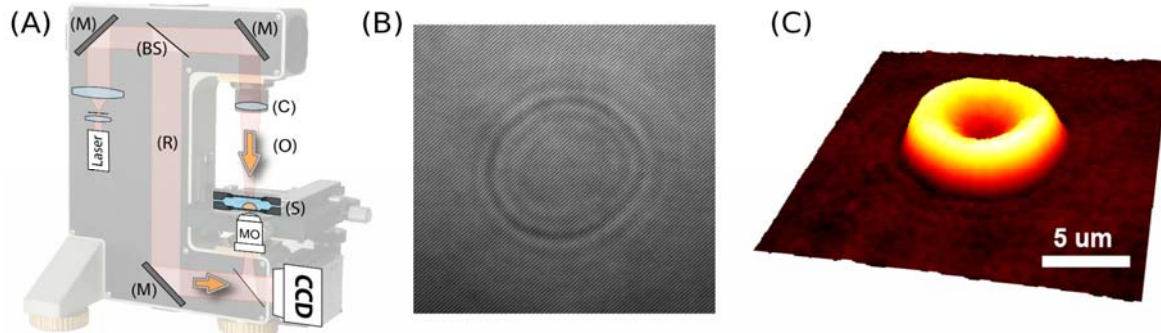


Figure 1: (A) Basic configuration for digital holographic microscopy (DHM). A laser diode produces the coherent light ($\lambda = 683$ nm) which is divided by a beam splitter (BS). The specimen (S) is illuminated by one beam through a condenser. The microscope objective (MO) collects the transmitted light and forms the object wave (O) which interferes with a reference beam (R) to produce the hologram recorded by the CMOS camera. The sample is mounted in a chamber used for recording. (B) Typical example of a hologram acquired by the camera (256×256 pixels). C. 3D representation of a quantitative phase image of an erythrocyte. Color scale in degree.

The quantitative phase signal Ψ is a local measure that holds information about the integral refractive index n and the thickness d of the observed cell.

$$\Psi = \frac{2\pi}{\lambda} (n_{cell} - n_{solvent}) d \quad (1)$$

Therefore DHM can be used to investigate cellular dynamics of morphological shape alterations, as well as refractive index related changes such as protein flux or diffusion of water through the membrane triggered by ionic permeability changes.

Red Blood Cell Membrane Fluctuations

Erythrocyte cells were prepared according to the procedure described in [3]. During 10 s phase signals were recorded with the DHM setup. Membrane fluctuation is measured in term of temporal phase variation. The measured standard deviations of the pixel inside and outside the cell indicate membrane fluctuation amplitudes significantly larger than the background noise level. (cf. Figure 2).

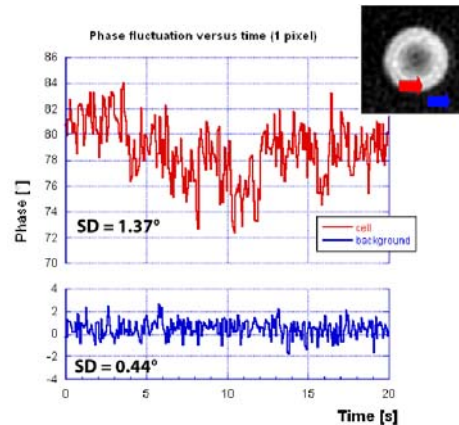


Figure 2: Phase signals of two different regions (1 pixel region outside the cell (blue) and within the cell (red)) recorded at 25 Hz during a 10 s period. The standard deviations of the signal are 0.44° and 1.37°, respectively. Inset: phase image of the monitored erythrocyte with the corresponding regions indicated by color arrows.

A decoupling procedure of the phase signal as described in [3] allows to measure the integral index of refraction, which is assumed to be constant over the surface of the cell. The measurements of the refractive index of 13 erythrocytes showed a very stable value amongst different cells of $n = 1.394 \pm 0.008$ (STD). This measured mean refractive index lies within the range of 1.37 to 1.42 reported by [3-7]. Regions within the RBC show a rather constant fluctuation amplitude of about 35.9 ± 8.9 nm (mean \pm STD, $n = 9$ cells), within the range of previously measured values of 52.6 nm [8-10]. As a control we employed 90% ethanol-fixed cells. As expected we obtained negligible CMF of 4.7 ± 0.5 ($n = 6$) close to the sensitivity of the DHM

Optical recording of chloride current by Digital Holographic Microscopy

A second example of the Digital Holographic Microscopy (DHM) application is in the pharmacological field. Especially, DHM could be a relevant tool to study non-invasively the properties of membrane ionic conductances.

As described above, DHM is a non invasive optical imaging technique able to provide quantitative phase images of living cells. Specifically, the phase signal depends on both the cell morphology (cell volume and shape) and the intracellular refractive index. Initial studies with DHM have shown that the impact of the refractive index is a stronger determinant of the value of the phase than the volume changes [11]. We have determined that the intracellular refractive index is mostly dependent on the protein content of the cell. Thus, an entry of water (accompanying the transmembrane movement of ions) will dilute the intracellular protein content resulting in a decrease in the phase while an exit of water will lead to a phase increase.

Against this background, we have analyzed the optical signal detected by DHM associated to the chloride flux triggered by GABA application to a neuronal GABA_A receptor-expressing Human Embryonic Kidney (HEK) cells. We have combined patch clamp recordings and DMH in the same cells following GABA application. We have observed a phase shift in the optical signal detected by DHM after application of GABA (3 μ M, 30s) for a holding potential to -100mV. Furthermore, the value for the reversal potential for chloride obtained by establishing phase/voltage relations (ϕ/V) is superimposable to that obtained by voltage/current relations (I/V); this experimentally-determined reversal potential is dependent on the intracellular concentration of chloride. Finally, there is a mathematical correlation between the phase signal and the recorded current triggered by GABA, allowing a prediction of the actual current by a simple analysis of phase shift. These data indicate that the quantitative monitoring of the DHM phase signal affords the possibility to analyze at the single-cell level with a simple and non-invasive optical method the effect of neurotransmitter-receptor activations known to result in excitability changes.

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