

Exploring red blood cell membrane dynamics with Digital Holographic Microscopy

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ABSTRACT

Digital Holographic Microscopy (DHM) has been used to investigate the spontaneous cell membrane fluctuations (CMF) of the Red Blood Cell. DHM as an interferometric technique is able to accurately provide the wavefront deformation induced by a transparent specimen, including living cells in a transmission configuration. From a numerical reconstruction of a single hologram, quantitative phase contrast images are obtained. The local phase shift is proportional to the specimen thickness with accuracy of 5-10 nm. As a non invasive full field technique DHM is particularly well suited to assess and study membrane fluctuations of a large number of cells simultaneously. In our analysis we show that CMF amplitudes are unhomogenously distributed on the cellular surface and seem to correlate with the biconcave equilibrium shape of erythrocytes. A mean fluctuation amplitude of 47 nm is measured in a group of 198 erythrocytes.

Keywords: Red Blood Cell Membrane Fluctuations, Digital Holographic Microscopy

1. INTRODUCTION

The remarkable mechanical properties of normal red blood cells (RBCs) allow the cell to undergo enormous reversible deformations when passing for instance through small capillaries. On the other hand the cell must be rather robust and show high resistance to stretching forces that could rupture the membrane. A consequence of these mechanical properties is that red blood cells show spontaneous membrane fluctuations (CMF) with deformation amplitudes in the order of 50 nm. These fluctuations are observed under a microscope as so called flickering motions of the membrane and have been investigated in the past with various optical techniques, including phase contrast microscopy,^{1,2} reflection interference microscopy,³ dark field microscopy⁴ or bright field microscopy.^{5,6} A drawback of these techniques is however that CMF can be assessed either only pointwise on the cellular surface, or at the rim of the cell based on some edge detection criteria. Recently however quantitative phase contrast microscopy allowed measuring cellular thickness with a nanometric sensitivity along the optical axis. This provides the means to assess CMF on the whole fluctuating membrane.⁷⁻⁹ We use Digital Holographic Microscopy (DHM) to measure the phase shift induced by living cells in a single shot acquisition. This phase signal can be converted to cellular thickness under the assumption of a spatially homogeneous refractive index of the RBC. Spatial maps of fluctuation amplitudes are evaluated on the cell surface. These amplitudes give clues on the physical origin that causes CMF. Up to day this is an open question: Studies reported a CMF decrease upon ATP depletion, suggesting an ATP consuming metabolic mechanism that increases CMF.^{4,9,10} Other studies could not observe such a dependency.⁵

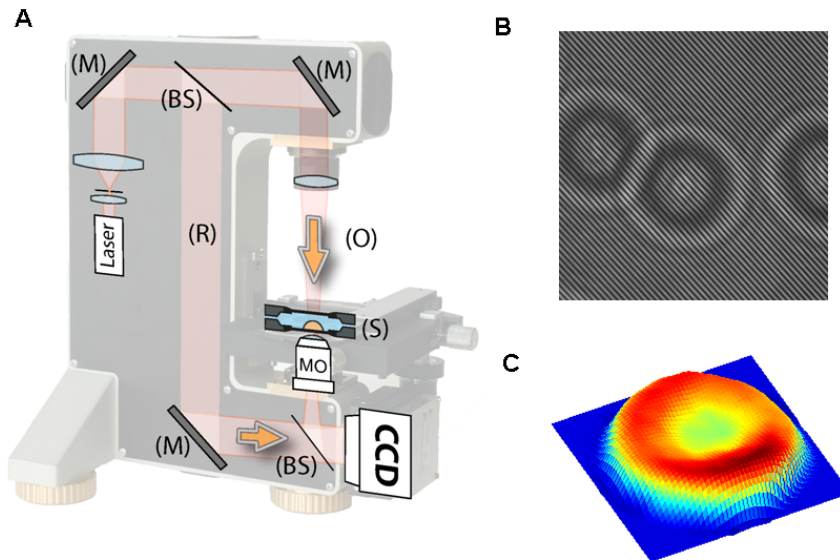


Figure 1. (A) Basic setup for digital holographic microscopy (DHM). A laser diode produces the coherent light which is divided by a beam splitter (BS). The microscope objective (MO) collects the light that is transmitted through the specimen (S). This object wave (O) interferes with a reference beam (R) to produce the hologram recorded by the CCD camera. The sample is mounted in a chamber used for recording. (B) Typical example of an out-of-focus hologram acquired by the camera. (C) 3D representation of a quantitative phase image of an erythrocyte.

2. MATERIAL AND METHODS

2.1 Cell Preparation

20-30 μl of blood was drawn from a healthy laboratory personnel by fingerpick, collected and diluted in 200 μl cold HEP buffer (15mM HEPES pH 7.4, NaCl 130 mM, KCl 5.4 mM and 10 mM glucose). Blood cells were sedimented at 200 g, 4 $^{\circ}\text{C}$ for 10 min and buffy coat was gently removed. Red blood cells were washed twice in HEP buffer (1000 g \times)2 min at 4 $^{\circ}\text{C}$). Finally, erythrocytes were suspended in HEPA buffer (15mM HEPES pH 7.4, 130 mM NaCl, 5.4 mM KCl, 10 mM glucose, 1 mM CaCl₂, 0.5 mM MgCl₂ and 1 mg/ml bovine serum albumin) at 0.2% hematocrit. 4 μl of the erythrocyte suspension were diluted to 150 μl of HEPA buffer and introduced into the experimental chamber, consisting of two cover slips separated by spacers 1.2 mm thick. The bottom coverslip was coated with polyornithine to ensure that cells adhere to the coverslip surface and to suppress translational movements of the cells during acquisition. Cells were incubated for 30 min before mounting the chamber on the DHM stage. All experiments were conducted at room temperature (22 $^{\circ}\text{C}$) and only cells displaying a normal biconcave shape were selected for analysis.

2.2 DHM setup

The optical microscope is a transmission DHM setup (DHM T1000TM) from LyncéeTec SA. The setup is a Mach-Zehnder configuration with a laser diode source ($\lambda = 683 \text{ nm}$) (cf. Fig. 1a). Holograms are formed in an off-axis configuration by the interference of a reference wave R and an object wave O. The latter is transmitted

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through the specimen and magnified by a microscope objective (MO). The complex field of the object wave is encoded in the interference pattern of the hologram and can be reconstructed by re-illuminating numerically the hologram with a reference field. Fresnel transform allows then to backpropagate the field to the focus plane. More information on the method and the reconstruction algorithm with details on wave front aberration compensation can be found in references 11,12. For the conducted experiment, holograms of 256*256 pixels are acquired at a rate of 25 Hz from which phase images are reconstructed with custom made Software from LyncéeTec SA.

3. RESULTS AND DISCUSSION

The measured phase shift is converted into cellular thickness by the relation

$$d(x, t) = \frac{\lambda}{2\pi} (n_{cell} - n_{sol}) \phi(x, t) \quad (1)$$

A refractive index of the HEPA solution of $n_{sol} = 1.3334$ measured with a refractometer and a mean cellular refractive index of $n_{cell} = 1.394$, evaluated by the same microscopic technique,⁸ are therefore used. Further, individual RBC images are aligned with an image registration algorithm.¹³ The local fluctuation amplitude at an instance t for a pixel x is obtained by subtracting the temporal mean thickness from the current thickness.

$$u(x, t) = d(x, t) - \langle d(x, t) \rangle_t \quad (2)$$

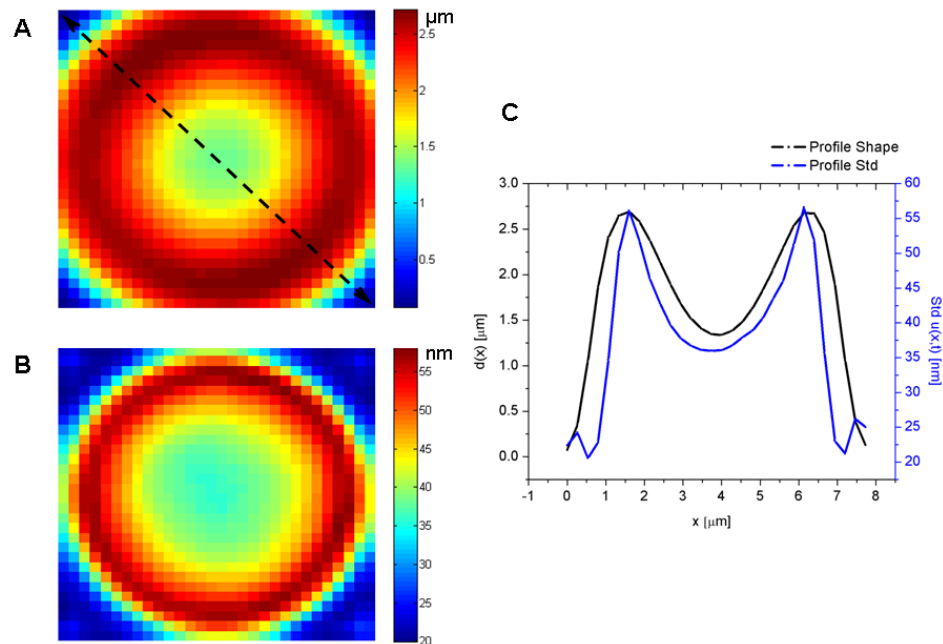


Figure 2. (A): Color coded average thickness profile of a group of $n=198$ cells. (B) Color coded standard deviation of CMF averaged over the group. (C) Profile lines extracted from Figures A and B. The profile line corresponds to the black arrow in Figure A

The evaluated mean fluctuation amplitude $\langle u(x) \rangle$ is then simply the temporal standard deviation of $u(x,t)$. As a simple correction, the mean fluctuation amplitudes are projected onto the z -axis (optical axis). This is done because in regions that show high inclinations with respect to the x - y plane, a small translational movement

would translate into a high displacement along the z-axis. In order to obtain a representative spatial map of CMF amplitudes of a population of RBCs, cells that differ in shape were reinterpolated to match the contour of a circular model cell. Figure 2 shows the result of the evaluation of the CMF amplitudes of a group of n=198 cells. Figure 2a is a color coded average thickness profile of all the cells in the group. A spatial map of CMF amplitudes is depicted in Figure 2c. Corresponding profile line extractions of Figures 2a and b are shown in Figure 2c. It can be seen that CMF amplitudes of RBCs are not homogeneously distributed on the cellular surface, but tend to increase from the center to the position of maximal height, where also CMF amplitudes are maximal. Similar observations have already been stated in references 9,14. However their interpretation of this result tended to favor a view where active ATP mediated contributions would increase CMF in the outer regions of the erythrocyte. They argued that in these regions where we have an increased curvature of the membrane, thermal CMF should be diminished. However in reference 15 it has been shown that such a distribution of CMF amplitudes can be expected for a purely thermal excitation of a freely undulating bilayer.

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