Nanometer fluctuations of erythrocytes imaged by Hilbert phase microscopy

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Abstract: Using Hilbert phase microscopy, a technique recently developed in our laboratory, the nanometer level structure and dynamics associated with live red blood cells have been quantified on the 10 millisecond time scale.

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1. Introduction

Red blood cells play a crucial role in health and disease, and structural/dynamical abnormalities of these cells have been associated with important diseases such as sickle cell disease and thalassemias. Phase contrast (PC) and differential interference contrast (DIC) microscopy [1] have been used extensively to infer morphometric features of normal and abnormal red cells. Erythrocytes lack nuclei and major organelles and thus can be modeled as optically homogeneous objects, i.e. they produce optical phase shifts that are approximately proportional to their thickness profile. However, PC and DIC are qualitative in terms of the phase shift produced by the cell, and thus cannot directly infer 3D cellular shape information. More recently, atomic force microscopy (AFM) has emerged as a high resolution technique that can be used for structural analysis of minimally fixed erythrocytes [2]. In addition, AFM has been successfully used for analysis of cell wall fluctuations in yeast cells [3]. Although quantitative, the AFM approach samples a single point and requires contact, and it is technically limited when applied to softer structures such as membranes of mammalian cells.

Fourier phase microscopy (FPM) has been developed in our laboratory as an extremely low-noise phase imaging method [4]. Due to the sub-nanometer phase stability over extended periods of time, FPM is suitable for investigating dynamics in biological systems on time scales from seconds to a cell lifetime. However, each image requires collection of four phase-shifted frames, thus limiting the frame rate.

Many processes that take place at the cellular level, including cytoskeletal dynamics, cell membrane fluctuations, and neural activity, occur at short time scales, down to the millisecond range. Therefore, a microscope that allows acquisition of full-field quantitative phase images at kHz frame rates may open the door to quantifying unexplored biological phenomena, such as full-field nanometer erythrocyte membrane fluctuations.

2. Hilbert phase microscopy (HPM)

Recently, Hilbert phase microscopy (HPM) has been developed in our laboratory as a novel optical technique for measuring high transverse resolution quantitative phase images associated with optically transparent objects [5]. Because each frame is collected in a single shot, HPM is suitable for investigating rapid phenomena that take place in biological cells.

The HPM principle is presented in detail in reference [5]. This technique extends the concept of analytic signals to spatially varying fields, in complete analogy with temporal measurements. With HPM, the frame acquisition rate is limited only by the recording device (CCD). This contrasts with phase-shifting techniques, in which multiple frames are required to construct a single phase image. In addition, HPM

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inherently allows for robust phase unwrapping, which enables the study of phase objects much larger than the wavelength of light.

In order to conveniently investigate live cells in their physiological conditions, the HPM method was combined with a standard inverted microscope. The experimental setup is depicted in Fig. 1.

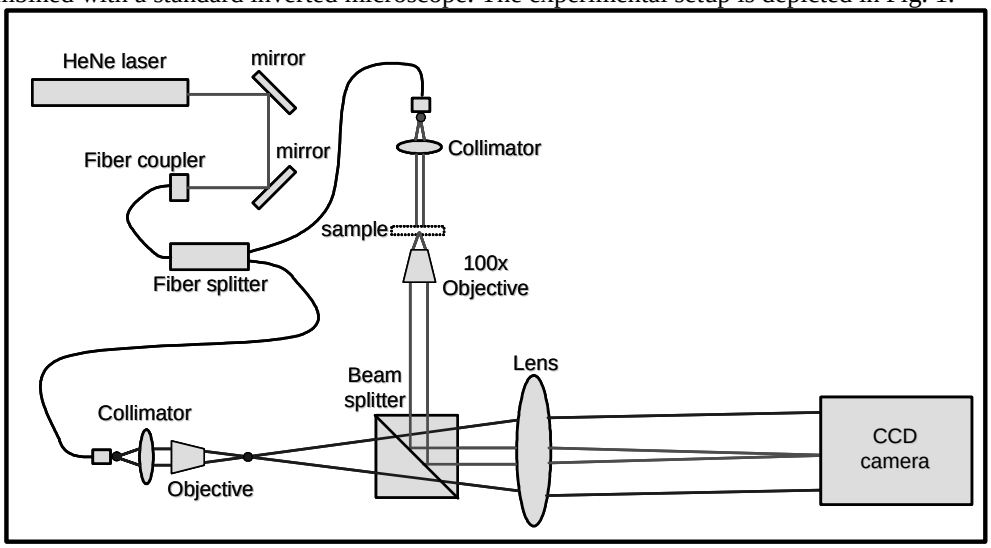

Figure 1. Experimental setup.

The 633nm radiation from a HeNe laser is coupled into a single-mode 1x2 fiber optics coupler and subsequently collimated at both outputs, such that the fields are characterized by full spatial coherence. One of the beams (vertical in Fig. 1) is used as an illumination source for an inverted microscope, which uses a 100X, oil immersion microscope (NA=1.3). The sample is thus illuminated by a plane wave in transmission geometry. A microscope objective is also used on the reference (horizontal) arm of the interferometer and both beams are combined by a beam splitter. A 250 mm focal length lens is used to create telescoping systems with both arms of the interferometer. The CCD is placed in the focal plane of this lens, i.e. in the imaging plane of the inverted microscope that constitutes the sample arm. The reference field is slightly tilted with respect to the sample beam in order to create a uniform fringe structure oriented at 45^{o} with respect to the x and y axes of the CCD. The CCD used (C7770, Hamamatsu Photonics) has an acquisition rate of up to 291 frames/s at the full resolution of 480x640 pixels. As described in [5], using the analytic continuation method and the Hilbert transform operation, the spatially varying irradiance at the image plane allows retrieval of the full-field phase information from the sample.

3. Structure and dynamics of erythrocytes imaged by HPM

We have employed HPM to retrieve the phase information associated with red blood cells. Samples of whole blood smears were prepared by sandwiching a small blood droplet between cover slips. Series of up to 1,000 phase images were acquired at repetition rates of 10.3 ms/frame. An example of dynamic changes in live red blood cells is shown in Fig. 2. Figs 2(a) and 2(c) represent, respectively, phase images of a red blood cell at t=0 and t=10.3 seconds, i.e. the first and the last frames in a 1,000 frame data set. Due to the lack of major structures within the red blood cells, the phase information from the HPM images can be easily transformed into thickness information, which provides direct access to parameters such as cell shape and volume. Figs. 2(b) and 2(d) show both the horizontal and vertical profiles of the cells, with nanometer precision.

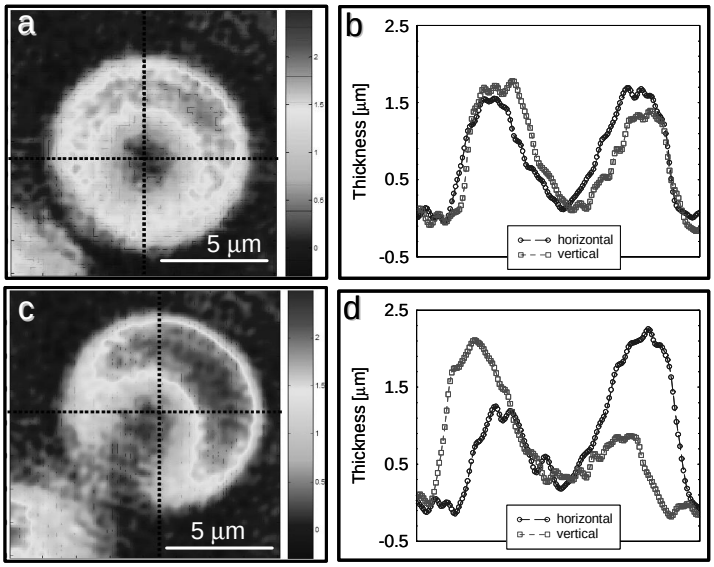

Figure 2. HPM images of a live red blood cell (a) and 10.3 seconds later (c); b and d indicate the thickness profile of the cells.

Interestingly, the significant change in the cell shape is due to a rapid interaction with a neighboring neutrophil [6]. This results in an asymmetric shape change that is easily quantified by the HPM technique.

Subsequent experiments have been performed on live erythrocytes under various chemical conditions. The results suggest that HPM is capable of measuring the cell fluctuations at the nanometer and millisecond level and thus may become a powerful tool for extracting quantitative information about the mechanical properties of the cell membrane. This type of information is crucial for describing the functioning and pathology of red blood cells.

4. References

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