Optical-Mechanical Signatures of Cancer Cells Measured by Interferometry

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Abstract: We used interferometric microscopy to uniquely measure live cancer cell fluctuations, in a noncontact, label-free manner, and showed that cancer cells fluctuate more than similar but healthy cells, demonstrating the method potential for cancer diagnosis.

OCIS codes: (090.2880) Holographic interferometry, (170.1530) Cell analysis, (170.4580) Optical diagnostics for medicine.

1. Introduction

Changes in viscosity-related properties of cancer cells compared to healthy cells have been reported [1]. Lately, it has been shown that cancer cells are nearly four times softer than similar but healthy cells [2]. However, the contribution of the mechanical properties of cells in invasion is not completely clear [3]. A possible explanation is that greater elasticity of cancer cells helps them metastasize, and more easily squeeze through the body tissues [4,6]. In Ref. [4], it has been shown that stiffness can be used to accurately distinguish between normal and cancerous cells that have similar appearances. This is important for preventing false diagnosis using the conventional and subjective cancer identification that is currently performed under a regular optical microscope. This study used atomic force microscopy (AFM), which is expensive, hard to implement, and applies forces on the cell measured. In addition, stiffness alone can yield a wrong diagnosis, since cells might be less rigid due to other reasons than cancer. Reference [5] reviews various mechanical biomarkers for cancer, as well as invasive methods that are currently used to measure these biomarkers [6]. These methods, however, rely on mechanical biomarkers measured by direct contact or force application on the cells.

In the current research, we used a wide-field optical interferometric technique to uniquely and quantitatively measure morphological and mechanical properties of live cancer cells *in-vitro* in a noncontact, label-free manner. Interferometry is a noncontact technique that is able to record the entire complex wavefront (amplitude and phase) of the light which has interacted with the sample by using a low-power coherent or partially-coherent light, where no exogenous labeling or special sample preparation are involved [7,8]. It was lately shown that wide-field interferometry is useful to characterize the stiffness changes of live red blood cells in sickle cell disease [9].

2. Experimental Setup

Figure 1(a) presents an interferometric microscopy system [10], for measuring the quantitative phase fluctuation maps of cancer cells. A beam from a He-Ne laser passes through the sample, magnified by a microscope objective (40×, infinity corrected, 0.67 numerical aperture), and projected onto the digital camera by a 15 cm tube lens, while being split at the beam splitter BS into two beams. One beam goes to a perpendicular mirror and returns to BS, while the second beam goes to the right and reflected by another mirror which is tilted at a slight angle (~3º). The reflected beams are combines at BS and projected onto the camera, where the interference pattern is captured.

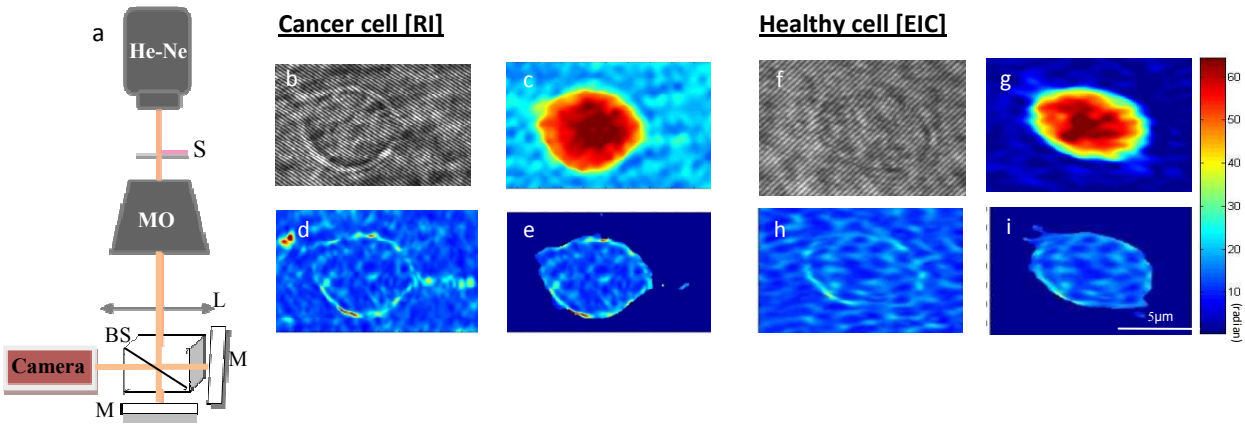

Fig. 1: (a) Scheme of the system. S: sample, MO: microscope objective, L: lens, BS: cube beam splitter, M: mirror. (b-e) cancer cell (RI). (f-i) healthy cell (EIC). (b,f) Off-axis interferograms of the cells. (c,g) Quantitative phase profile of the cells, each obtained from one off-axis interferogram of the sample. (d,h) Fluctuation STD map. (e,i) Fluctuation STD map of the cells with no background.

The advantages of this interferometric system are as follows: (a) The interferometric system is positioned only in the output of an inverted microscope and thus it is more stable due to cancellation of noise between the reference and sample arms in most of the beam path. Due to the low noise level, the system can measure smaller thickness fluctuations in time; (b) Only one microscope objective is used; (b) Off-axis interferograms can be acquired while only single shot is required (not like in on-axis), which is suitable for measuring fast fluctuations.

3. Results and Conclusions

Using the system described above, we carried out measurements for gastrointestinal healthy (EIC) and cancerous (RI) cells lines (cells were generously provided by Prof. Nadir from Ichilov Hospital). Note that both cell lines (normal and cancer) were taken from the exact same source (rat's colon), so all their attributes are identical, except of cancer. Since we record the interference of the sample beam with itself, half of the sample needs to be empty. To achieve this requirement, we plated the cells in a low concentration.

Using the system described above, we captured interferograms of 13 cancer cells and 11 healthy cells [example are shown in Figs. 1 (b,f)]. The off axis interferograms were processed by digital spatial filtering (using two digital Fourier Transforms), followed by phase unwrapping algorithm to solve 2π ambiguities [11], which yielded the final phase profile of the cell [see examples in Figs. 1(c,g)]. 100 images were acquired per each cell at 120 frames per second and the standard deviation (STD) fluctuation map was calculated, indicating on the cell stiffness.

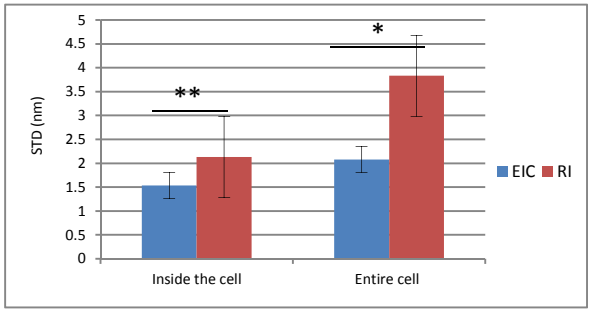

Fig. 2: Graph of maximum STD value of the flucations [nm]. n the left: maximum STD without cell edges (without the membrane). On the right: maximum STD of the entire cell (including edges/membrane). (*, $P < 0.01$; **, $P < 0.025$).

We created a database of 13 cancer cells and 11 healthy cells and compared the maximum STD of the fluctuations in the cells (see Fig. 2). For this purpose, two assessments were checked: whole-cell fluctuations (including membrane on the edges) and fluctuations inside the cell (without membrane on the edges).

For comparison, we performed stiffness testing experiments with healthy and cancer cells using AFM, and obtained similar results to the ones previously published [2] (not shown here).

In conclusion, we found that cancer cell fluctuations are significantly higher than these of similar but healthy cells, especially in the membrane area [12,13]. This evidence implies on decrease stiffness of cancer cells and their membrane fluidity. According to these promising preliminary results, our system has a potential to be used as a new tool for diagnosis and monitoring of cancer.

[1] J. Farrell, "The origin of cancer and the role of nutrient supply: a new perspective," Med. Hypotheses **2** 119-23 (1988).
[2] S. E. Cross, Y. S. Jin, J. Rao, and J. K. Gimzewski, "Nanomechanical analysis of cells from cancer patients," Nature Nanotechnology **2** 780-783 (2007).
[3] V. Swaminathan, K. Mythreye, E. T. O'Brien, A. Berchuck, G. C. Blobe and R. Superfine,"Mechanical Stiffness Grades Metastatic Potential in Patient Tumor Cells and in Cancer Cell Lines," Cancer Res. **71**, 1-6 (2011).
[4] K. Bhadriraju and L. K. Hansen, "Extracellular matrix- and cytoskeleton-dependent changes in cell shape and stiffness," Exp. Cell Res. 278, 92–100 (2002).
[5] S. Suresh, "Biomechanics and biophysics of cancer cells," Acta Materialia **55**, 3989-4014 (2007).
[6] G. Bao and S. Suresh, "Cell and molecular mechanics of biological materials," Nature Mater. **2**, 715–25 (2003).
[7] N. T. Shaked, M. T. Rinehart, and A. Wax, "Dual-interference-channel quantitative-phase microscopy of live cell dynamics," Optics Letters **34**, 767-769 (2009).
[8] N. T. Shaked, J. D. Finan, F. Guilak, and A. Wax, "Quantitative phase microscopy of articular chondrocyte dynamics by wide-field digital interferometry," Journal of Biomedical Optics Letters **15**, 010505 (2010).
[9] N. T. Shaked, L. L. Satterwhite, G. A. Truskey, M. J. Telen, and A. Wax, "Quantitative microscopy and nanoscopy of sickle red blood cells performed by wide field digital interferometry," Journal of Biomedical Optics Letters **16**, 030506 (2011).
[10] B. Kemper, D. Carl, J. Schnekenburger, I. Bredebusch, M. Schafer, W. Domschke, and G. von Bally, "Investigation of living pancreas tumor cells by digital holographic Microscopy," Journal Biomedical Optics **11**, 34005 (2006).
[11] N. T. Shaked, and A. Wax, "Quantitative analysis of biological cells using digital holographic microscopy," In *Holography, research and technologies* (Intech, 2011), Chapter 10.
[12] M. Shinitzky and M. Inbar, "Difference in microviscosity induced by different cholesterol levels in the surface membrane lipid layer of normal lymphocytes and malignant lymphoma cells," Journal of molecular biology 85, 603-615 (1973).
[13] A.Vasanji, P. K. Ghosh, L. M. Graham, S. J. Eppell, and P. L. Fox, "Polarization of plasma membrane microviscosity during endothelial cell migration," Developmental Cell **6**, 29-41 (2004).