Cell Life Cycle Characterization Based on Generalized Morphological Parameters for Interferometric Phase Microscopy

Pinhas Girshovitz[*] and Natan T. Shaked

Department of Biomedical Engineering, Faculty of Engineering, Tel Aviv University, Tel Aviv 69978, Israel
[*]pinhasgi@post.tau.ac.il

Abstract: We developed biological cell analysis tools for interferometric phase microscopy based directly on the quantitative phase profile and used them to characterize cancer-cell life cycle, and uniquely distinguish and predict the cell life cycle phases.

OCIS codes:(090.2880) Holographic interferometry; (170.1530) Cell analysis.

1. Introduction

Interferometric phase microscopy (IPM) is a powerful quantitative tool that can provide information on biological cell structure and dynamics [1,2]. The sensitivity of IPM setups for small changes in the refractive index provides label-free contrast and enables us to see cells and inner cell organelles without the use of exogenous contrast agents. Various methods have been suggested for decoupling cell thickness and the differential refractive index [1,3-8], all of which are generally inadequate for measuring heterogeneous and highly dynamic cells.

For heterogeneous refractive-index-structure cells, studies have shown that some cell parameters can be calculated directly from the phase profile of the cell. These include dry mass, cell area, and relative volume in certain cases [1-4,9]. Other studies used differential-time analysis on the cell phase profile to record dynamic properties of highly-heterogeneous cells [10].

We propose to quantify cells with heterogeneous refractive-index structure without losing the dynamic properties of the cells [11]. To obtain this goal, we used generalized parameters providing new analysis tools that extend the ability of IPM systems to measure biologically-relevant parameters to the general case of highly-dynamic cells with heterogeneous refractive-index structure, without decoupling cell thickness and refractive index.

Using these tools, we investigated the dynamics of HeLa (human epithelial carcinoma) cells, and showed various biological phenomena occurring during the cell life cycle [11]. The suggested tools managed to distinguish cell life-cycle phases and sub-phases.

2. Methods

In order to acquire the dynamic phase profile of the cells, we constructed a wide-filed digital interferometer (WFDI) microscope, which can obtain an optical path delay spatial stability of 1.1 nm and a temporal stability of 0.62nm [11].

The optical path delay of the cell (OPD_c) is defined as follows:

$$OPD_c(x,y) = [\bar{n}_c(x,y) - n_m] \times h_c(x,y), \tag{1}$$

where h_c is the thickness profile of the cell, n_m is the refractive index of the medium, and $\bar{n}_c$ is the cell integral refractive index [1-4]. OPD_c can be used to directly calculate the cells dry mass, which is used to describe cell behavior and defined as follows, where α is the refractive increment constant and approximated as 0.18-0.21ml/g:

$$M = \frac{1}{\alpha}\int_{Sc} OPD_c(x,y)ds, \tag{2}$$

We defined new generalized parameters based directly on the cell phase for describing cells such as the statistical parameter, cell phase skewness. Full list of the parameters can be found in Ref. 11.

In order to use statistical parameters, such as skewness, the phase values over the projected cell area were written as a single vector containing *n* values. Then, the cell skewness was calculated using the following formula:

$$Skewness = \sum_{i=1}^{n} \frac{E(OPD_c(n) - \mu_{OPDc})^3}{\sigma^3}, \tag{3}$$

where $OPD_c(n)$ is the cell phase values and μ_{OPDc} is the mean of these values and σ is the variance.

3. Results

Using IPM, we acquired two off-axis interferograms of HeLa cells per minute for 50 hours, and processed them into the time-dependent quantitative phase profile of the sample.

Figure 1 presents results measured during a part of the life cycle of a HeLa cell starting right after the mother cell divided into two new cells and includes cell transition from G1 phase to S phase. G1 phase is defined as the part of the life cycle where the cell grows, and S phase is defined as the part of the life cycle where the cell is metabolically inactive and DNA synthesis occurs.

Figure 1(a) presents the OPD profile of the measured cell. Figure 1(b) presents the cell dry mass which is calculated according to Eq. (2). This graph shows the growth of the cell during G1 phase and an approximately constant value of the dry mass during S phase, taking into account the dry mass values at the beginning of G1 and S phase. The growth rate during G1 phase was calculated as 10.31 pgr per hour. The time dependency of the phase skewness parameter [Eq. (3)] is presented in Fig.1(c). This graph shows a change occurring at hour 6:18, after which the value of OPD_c stops changing, meaning that the cell stops growing, which agrees with the definition of S phase.

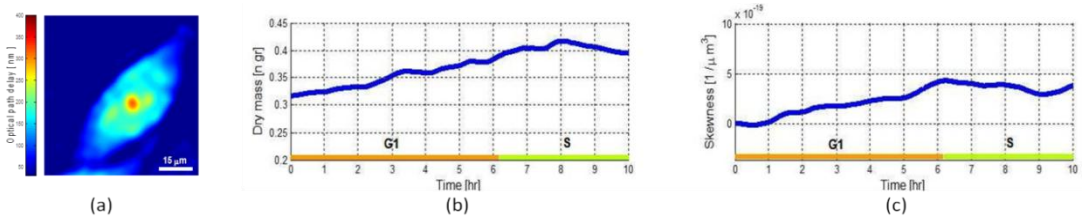

Fig. 1. Measured cell parameters during cell growth: (a) OPD image of the cell; (b) Dry mass; (c) Phase skewness.

The presented results were confirmed by additional measurements done on nine more cells showing similar behaviors and values [11]. In the case of dry mass the results showed values of 241.1±24.7 pgr at the beginning of G1 phase, 355±19.21 pgr during S phase and 546±45.75 pgr in the end of G2 phase, with significant statistical difference between the lifecycle phases (p<1e-8) [11].

4. Conclusion

Using generalized parameters, we uniquely describe cell growth and distinguish between cell life-cycle phases for heterogeneous cells, without the need to decouple the refractive index and thickness from the OPD measurement. The results showed good agreement with measurements done using other label-based and cell-invasive techniques, but in our case, it was done in a label-free, non-contact manner for live cells. The new generalized parameters, obtained from a single interferogram, can be used to better understand cell behaviors including cell-growth regulation.

5. References

[1]N. T. Shaked, L. .L.Satterwhite, M. T. Rinehart, and A. Wax, "Quantitative analysis of biological cells using digital holographic microscopy," Holography Research and Technologies, Joseph Rosen (Ed.), InTech,219-236 (2011).

[2] B. Rappaz, P. Marquet, E. Cuche, Y. Emery, C. Depeursinge, and P. J. Magistretti, "Measurement of the integral refractive index and dynamic cell morphometry of living cells with digital holographic microscopy," Opt. Express 13,9361-9373 (2005).

[3] G. Popescu, T. Ikeda, C.A. Best, K. Badizadegan, R.R. Dasari, and M.S. Feld, "Erythrocyte structure and dynamics quantified by Hilbert phase microscopy," J. Biomed. Opt. Lett. 10 (2005).

[4] B. Rappaz, A. Barbul, Y. Emery, R. Korenstein, C. Depeursinge, P.J. Magistretti, and P.Marquet , "Comparative study of human erythrocytes by digital holographic microscopy, confocal microscopy, and impedance volume analyzer," Cytometry A 73, 895-903 (2008).

[5] W. Choi, C. Fang-Yen, K. Badizadegan, S. Oh, N. Lue, R. R. Dasari, and M. S. Feld, "Tomographic phase microscopy," Nature Methods 4, 717-719 (2007).

[6] F. Charrière, A. Marian, F. Montfort, J. Kuehn, T. Colomb, E. Cuche, P. Marquet, and C. Depeursinge, "Cell refractive index tomography by digital holographic microscopy," Opt. Lett. 31,178-180 (2006).

[7]B. Kemper, D. Carl, J. Schnekenburger, I. Bredebusch, M. Schäfer, W. Domschke, and G.von Bally, "Investigation of living pancreas tumor cells by digital holographic microscopy," J. Biomed. Opt. 11, 034005 (2006).

[8] N. Lue, G. Popescu, T. Ikeda, R.R. Dasari, K. Badizadegan, and M. S. Feld, "Live cell refractometry using microfluidic devices," Opt. Lett. 31, 2759-2761(2006).

[9] G. Popescu, Y. K. Park, N. Lue, C. B. Popescu, L. Deflores, R.R. Dasari, M.S. Feld, and K.Badizadegan, "Optical imaging of cell mass and growth dynamics,"Am. J. Physiol. Cell.Physiol. 295538-544 (2008).

[10] N.T. Shaked, L. L. Satterwhite, N. Bursac, and A. Wax, "Whole cell imaging based on wide-field interferometric phase microscopy and its application to cardiomyocytes," Proc. SPIE 7904, 790405 (2011).

[11] P. Girshovitz and N.T. Shaked, "Generalized cell morphological parameters based on interferometric phase microscopy and their application to cell life cycle characterization," Accepted to Biomedical Optics Express (2012).