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COMMENTARY

Cytometry PART A
Journal of the International Society for Advancement of Cytometry

ISAC
International Society for Advancement of Cytometry

Quantitative Phase-Contrast Imaging—A Potential Tool for Future Cancer Diagnostics

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• **Key terms**
angiogenesis; cancer; digital holographic microscopy; imaging; non-invasive; phase contrast

DIGITAL holographic microscopy is a technique that utilizes quantitative phase-contrast imaging with high resolution (1). Living cells and tissues are transparent phase objects, giving different signals in refractive index (RI) generated by the cellular content of organic molecules in the specimen, such as the nuclei, other organelles, lipids, proteins, and DNA (2). The images generated by the technique contain quantitative information about both the local thickness and refractive index of the structure (3).

In digital holographic microscopy, a sample beam will pass through the object and merge together with a reference beam and create an interference pattern. An image sensor, for example, a CCD-sensor, will capture the interference pattern, and computer algorithms convert the signal into a holographic image based on the light phase shifting properties of the cells. Several cellular parameters can be calculated from the hologram, including the area, thickness, volume, confluence and the number of cells (4,5). The area of an imaged cell is obtained from the total number of pixels covering the cell. The cellular thickness can be translated into the phase of light that occurs when the light passes through the cell. The phase shift is related to optical path length and the wavelength of the light. As a consequence, the optical path length is directly correlated to the thickness and the RI of the cell. In addition, the area and the thickness together will determine the volume of the cell.

Label-free imaging of living cells was published for the first time in 2005 by Marquet et al. (6). Since then, a significant increase in biological applications targeted by the methods of quantitative phase-contrast imaging has been witnessed. A wide range of different cell types, for example, protozoa, bacteria and plant cells, mammalian cells including tumor cells, bacterial–cell interactions, red blood cells (RBC), sperm cells, nerve cells and stem cells have been analyzed (2,7–10). Interestingly, the number of clinical specimen undergoing analysis by quantitative phase-contrast imaging is now increasing. The method has been extensively used to quantify RBC morphological parameters, but screening for white blood cells has also been performed (11). Indeed, development of the technique has emerged in widely different areas of medicine such as sperm motility, blood cell analysis, inflammation, migration and cancer screening. Using quantitative phase-contrast imaging for cell cycle arrest analysis in cancer cells could be another future goal in medical diagnostics. Cell cycle control plays an essential role in cell differentiation and proliferation of both normal cells and cancer cells. Quantitative phase-contrast imaging can be a helpful tool when identifying specific changes in cell phase volumes that correlate to either G1 or G2/M arrest. Average cell phase volume changes in response to treatment of cancer cells with cell cycle arresting compounds can be successfully used as markers for monitoring cycle arrest in cultured cells. Previous results revealed the usefulness of the method in analyzing pancreatic cancer cells (7) and endothelial cells (12), as well as in measuring cancer cell growth and drug testing (13,14). The field of digital holographic microscopy is now emerging due to the novel capabilities of nanoscale digital phase-contrast imaging that can be performed instead of invasive time consuming photochemical procedures.

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Received 3 March 2017; Accepted 8 March 2017

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Published online 6 April 2017 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cyto.a.23104

In the study by Guo et al. (15), cellular behavior of angiogenic and nonangiogenic tumor cell phenotypes are examined by using quantitative phase-contrast holographic microscopy and imaging. The authors express the lack of reproducible and reliable quantification systems for evaluating these cell types. The tumor cell phenotypes are described as dormant cells with the possibility to switch to actively proliferating cells, if the proper angiogenic signals are present (16). Rogers et al. emphasize that rather than an obligate property of only the tumorigenically transformed cells in a tumor, the angiogenic switch may be an ensemble property comprised of contributions from all the cells in the tumor. The authors have previously published numerous papers about tumor angiogenesis related to the field, but this is the first time they demonstrate the feasibility of using the quantitative phase-contrast imaging methodology.

The nonangiogenic (KHOS-N) and angiogenic (KHOS-A) human osteosarcoma cells were used in the study. Except from quantitative phase-contrast imaging, the authors use proliferation and migration assays for comparison. To evaluate a more cost-effective and simple procedure to monitor fundamental cellular behaviors of KHOS cells, the authors state that quantitative phase-contrast imaging can be used. For these two cell types, the motility pattern, but not the proliferative pattern, was different. This is in line with the study performed by Naumov et al. who elegantly showed similar proliferation rates of angiogenic and nonangiogenic tumor cells inoculated in SCID mice (17). Instead, major differences were demonstrated before the developed tumors could be palpable. The angiogenic human osteosarcoma cells in the present study showed a higher motility speed compared to the nonangiogenic counterpart. This results in a longer migration distance and motility distance, which can be used as a parameter between angiogenic and nonangiogenic cell types and being exploited as a potential mechanism to isolate angiogenic and dormant phenotypes from a heterogeneous tumor population. The results of the traditional cell migration assay further confirmed that angiogenic cells exhibited increased migratory activity when comparing with the nonangiogenic cells.

Quantitative phase-contrast imaging is a very useful method for the determination of cellular area, thickness and volume as a measure of cellular morphological parameters that can be monitored in cell populations or in individual cells (7). Indeed, the authors show that KHOS-A cells have a significantly smaller cell area and increased cell thickness, in comparison with the nonangiogenic KHOS-N cells, although no difference in their average cell volume was observed. Volume measurement is an area of outmost interest in field of quantitative phase-contrast imaging and drug response. As already mentioned, measuring volumes is useful for determining cell division and cell cycle phases. In this study, cell cycle stages were clarified, especially for the interphases G1, G2, and S. Interestingly, volume changes are now utilized for the monitoring of cell death. Apoptosis is a process of various morphological changes that can be monitored over time with quantitative phase-contrast imaging (7,18). The first visible indication of a cell undergoing an early stage of apoptosis is a decrease of the cell phase shift. Pavillion et al. recognized early apoptotic cells within minutes after incubation by measuring the phase signal, whereas identification of dead cells using trypan blue staining could only be done after several hours.

Altogether, quantitative phase-contrast microscopy is a novel and efficient imaging technique that allows label-free and continuous monitoring of cell morphology and behaviors. Indeed, it may provide tools for being a promising tool in near future studies. The technique is cheap, fast and simple and has the potential of imaging cells noninvasively over time while the cells are kept in their normal culture environment.

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