The relationship between optical volume & cell volume in biological samples

November 2016

phasefocus

- Ptychographic quantitative phase imaging can be used to determine the optical volume of each cell
- Optical volume can be used to increase cell tracking robustness
- Relative optical volume changes can be used to determine influence of drugs

1. Introduction

Changes in cell volume occur during a number of important physiological processes including the cell cycle, cell death and differentiation. The ability to report upon relative changes in cell volume is, therefore, of considerable importance to any system used to study these processes. Traditional techniques, such as cell impedance coulter counters and confocal laser scanning microscopy (CLSM) are time-intensive, especially when trying to effectively sample a population of cells. A faster, 2D, non-invasive alternative to assess the volume of adherent cells is offered through quantitative phase imaging, by outputting optical volume as a proxy.

2. Measuring the optical volume of a cell

The Phasefocus Livecyte uses ptychography in order to generate quantitative phase images of cells, which can be segmented using the Cell Analysis Toolbox (CAT). The optical volume of each individual cell measured; this value is calculated as the sum of phase values over the area of each cell (in μm^2) multiplied by $\lambda/2\pi$ (where λ is the wavelength of the illuminating light). The optical volume of each cell is related to the actual volume by the following equation:

$$\text{Actual Volume} = \frac{\text{Optical Volume}}{(n_{cell} - n_{media})}$$

Where n is the refractive index. Under the assumptions that the n components of the equation are constant, the actual volume of the cell will be directly related to the optical volume. Here we sought to determine the extent of correlation between actual and optical cell volume of a cell population under these assumptions.

3. Comparison of confocal cell volume and optical cell volume

Correlative phase images and fluorescence z-stacks of live dye-(CFSE)-loaded cells were acquired on the Phasefocus system and an LSM780 confocal microscope, respectively (Fig. 1). The same cells were imaged on both systems, equipped with environmental chambers maintained at 37 °C, 5% CO_2, within 30 min of one another. A total of 96 regions were acquired across three independent experiments yielding > 250 cells with correlative phase and fluorescence datasets for two cell lines (A549 and NIH-3T3). The optical volume was extracted from phase images and the confocal volume measurement for each cell (μm^3) was calculated using Volocity software from PerkinElmer.

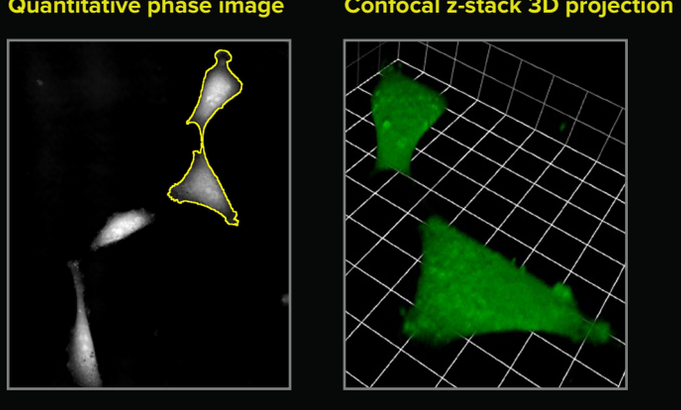

Fig. 1: Optical volume as a measure of cell volume. (a) Phase image of A549 cells overlaid with segmentation ROIs (yellow) and a 3D-projection of CFSE-labelled A549 cells acquired by CLSM.

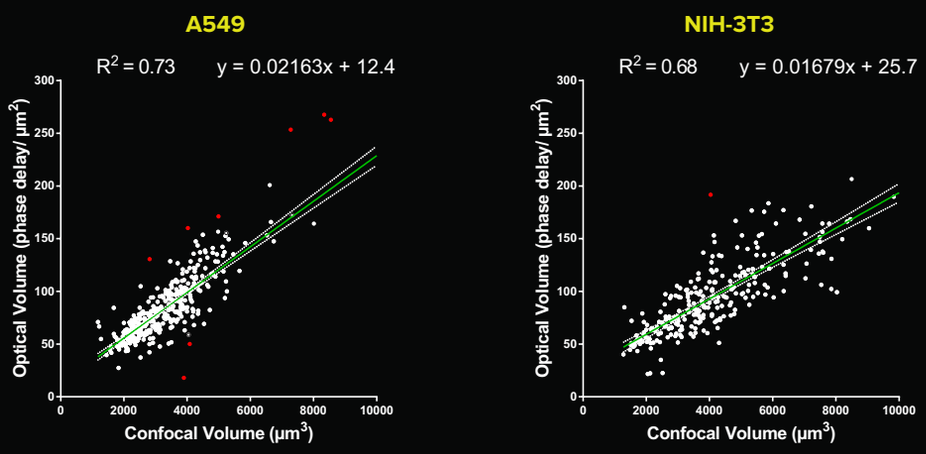

Fig. 2: Plots of optical and confocal volumes of A549 cells (n=381) and NIH-3T3 cells (n=261). The linear fit (-) and 95% confidence intervals (- -) are shown.

4. Conclusion

Plots of optical volume against confocal volume reveal a loose linear relationship between these two parameters for both cell types (Fig. 2). The general trend shows that cells of greater confocal volume were associated with a higher optical volume than those cells that exhibited a lower confocal volume. Thus, for a whole population, the average optical volume provides a good estimate of the average cell volume. With regards to individual cells, it is evident from the scatter around the linear fit, as well as the R2 value, that the optical and confocal volume are not directly proportional. This phenomenon could be explained by cell-to-cell variation in reflective index (n_{cell}) within the same cell type, which exemplifies the extent of cell heterogeneity within a given asynchronous cell population. Using the assumptions of a constant n_{cell} for each cell line, the optical volume can be used as an effective metric to assess the average cell volume within a population at a given time point.

livecyte

For more information on the benefits of the Livecyte system, to access application notes and for additional product information, please visit: www.phasefocus.com/livecyte

A sample of time-lapse videos can be found at: www.youtube.com/phasefocuslimited

T: +44 (0)114 286 6377
E: enquiries@phasefocus.com

Phase Focus Limited
Electric Works
Sheffield Digital Campus
Sheffield
S1 2BJ
UK

www.phasefocus.com

phasefocus