Fixing Dye Imaging

While changes in cell shape and volume have been linked to essential biological processes including proliferation and locomotion, studying cells in three dimensions can be difficult, requiring highly specialized methods such as scanning electron or atomic force microscopy. Although some imaging methods such as differential interference contrast microscopy or confocal sectioning can provide a sense of the third dimension, most widely available techniques only provide accurate quantitative data for two dimensions. Transmission-through dye (TTD) imaging, developed by Model and colleagues at Kent State University (Kent, OH), enables researchers to extract cell thickness information from a regular bright field image, generating data through which cell volume may be calculated. To use TTD imaging, living cells must first be covered with a non-toxic and strongly absorbing dye that cannot leak into cells. The cells are then imaged with near monochromatic illumination at the wavelength maximally absorbed by the dye. In regions where the cell body displaces the dye, more light will be transmitted, causing areas of increased cell thickness to appear brighter in the resulting images. Absolute values of cell thickness and volume can be calculated based on the amount of light transmitted through the sample at each point. TTD imaging is fast, has a high signal to noise ratio and can be performed using laser scanning or conventional microscopy with a bandpass filter. However, resolution is limited when using lower concentrations of dye, and increasing dye concentrations may interfere with cell function or block light penetration such that imaging thinner parts of a cell becomes difficult or impossible. Finding the right balance between the health of the cells during imaging, vertical resolution, and the ability to image at a maximum depth has been a challenge with this technique. Now, writing in this issue of BioTechniques, Model and colleagues describe an adaptation of TTD microscopy to enable imaging of fixed cells, thus increasing vertical resolution by allowing use of high dye concentrations while also eliminating cell changes from occurring during observation. The authors discuss multiple fixation and dye combinations that can be used for TTD imaging, and present an optimal protocol for increasing vertical resolution using higher concentrations of the dye while balancing against the onset of fixation-induced artifacts.

See “Thickness profiling of formaldehyde-fixed cells by transmission-through-dye microscopy” on page 389.

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