Quantitative phase microscopy - a new way to interrogate the structure and function of unstained viable cells

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The optical transparency of unstained viable cell specimens limits the extent to which information can be recovered from bright field microscopic view as these specimens generally lack visible, amplitude modulating components. However, if the phase-shift component of the cellular material is utilised, an information-rich image can be obtained. Optical phase microscopy, and derivatives of this technique such as Differential Interference Contrast (DIC) and Hoffman Modulation Contrast (HMC) have been widely applied in the visualisation of cellular specimens to enhance contrast. Whilst providing significantly enhanced contrast, useful in viewing specimens, the capacity to extract quantitative information from the phase content available in these optical techniques has not previously been explored.

Quantitative Phase Microscopy (QPM) is a new method for visualising transparent objects. This recently developed computational approach extracts quantitative phase measurements from images captured using a bright-field microscope without phase or interference contrast optics. QPM works via an algorithm which is applied to an in-focus image and a pair of equidistant de-focus images (one positive and one negative de-focus). From these images a phase map is generated which can be used to quantitatively emulate other contrast image modes such as DIC, dark field or HMC. The generation of these analogue images using phase mapping obviates the inherent problems associated with optical phase imaging, including cell edge distortion and edge halo effects. As it is implemented on an optically simple bright field microscope, the QPM methodology is also an economical alternative for cellular imaging applications.

Of particular importance is the capacity to quantitatively analyse the recovered phase images using QPM. The phase map generated from the bright field images contains information about cell thickness and refractive index and can allow quantitation of cellular morphology under ‘real-time’ conditions. For instance, the proliferative properties of human airway smooth muscle cells have been evaluated using QPM techniques to track cell culture confluency and growth (Curl *et al.*, 2002). In addition, cell volume measurement techniques have been applied to investigate the responses of erythrocytes to different osmotic challenges using QPM (Curl *et al.*, 2003).

QPM is a valuable new imaging tool which extends the capacity to interrogate viable cells to obtain structural and functional information in a rapid and non-destructive manner.

Curl C.L., Harris T., Kabbara A.A., Allman B.E., Roberts A., Nugent K.A., Harris P.J., Stewart A.G. & Delbridge L.M.D. (2002) *Proceedings of Australian Health and Medical Research Congress*, 1: 1126.

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