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Imaging in focus

Characterising live cell behaviour: Traditional label-free and quantitative phase imaging approaches

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ABSTRACT

Label-free imaging uses inherent contrast mechanisms within cells to create image contrast without introducing dyes/labels, which may confound results. Quantitative phase imaging is label-free and offers higher content and contrast compared to traditional techniques. High-contrast images facilitate generation of individual cell metrics *via* more robust segmentation and tracking, enabling formation of a label-free dynamic phenotype describing cell-to-cell heterogeneity and temporal changes. Compared to population-level averages, individual cell-level dynamic phenotypes have greater power to differentiate between cellular responses to treatments, which has clinical relevance *e.g.* in the treatment of cancer. Furthermore, as the data is obtained label-free, the same cells can be used for further assays or expansion, of potential benefit for the fields of regenerative and personalised medicine.

1. Introduction: the need for label-free imaging

Label-free imaging ensures that native cell behaviour remains uninfluenced by the recording process. In this mini-review, we focus on differences between quantitative phase imaging (QPI) and traditional label-free imaging techniques regarding: (i) the importance of image contrast for enabling robust, automated extraction of metrics describing individual cell behaviour; (ii) the power of a label-free dynamic phenotype over global population-level measurements in identifying changes in cell behaviour.

1.1. Visualising cells and contrast-enhancing agents

Cells are phase objects, *i.e.* absorb little light, resulting in only minor changes in the amplitude of transmitted light through the cell. Since the human eye relies on changes in amplitude of a light wave, cells can be difficult to visualise using a light microscope without a system to enhance cell contrast. One widespread solution is to introduce dyes/labels; these provide molecular specificity but can involve procedures (*e.g.* fixation) incompatible with live cell imaging. Even labels designed for use with live cell imaging can cause perturbation to normal cellular function and concentration-dependent toxicity effects (Alford et al., 2009; Coutu and Schroeder, 2013).

1.2. Phototoxicity

Phototoxicity poses additional barriers to imaging native cell behaviour, as the light intensity required to excite a fluorophore can cause cells to behave abnormally or die (Mov. 1). Phototoxicity is primarily attributed to generation of reactive oxygen species, which adversely affect cell physiology, health, behaviour, movement and shape by various mechanisms (Magidson and Khodjakov, 2013). Subtler phototoxic effects can easily be overlooked, confounding experimental results (Saetzler et al., 1997; Tinevez et al., 2012), and are further exacerbated when imaging over extended periods, *e.g.* causing impairment of cell doubling time (Carlton et al., 2010). The impact of phototoxic damage can be assessed and limited but not negated (Magidson and Khodjakov, 2013; Tinevez et al., 2012). Thus, imaging under very low light intensity without labels is an attractive solution to enhance cell contrast whilst minimising uncertainty in the recording of native cell behaviour. Furthermore, label-free techniques enable researchers to avoid the cost of time-intensive dye/label optimisation or stable fluorescent-reporter cell line generation.

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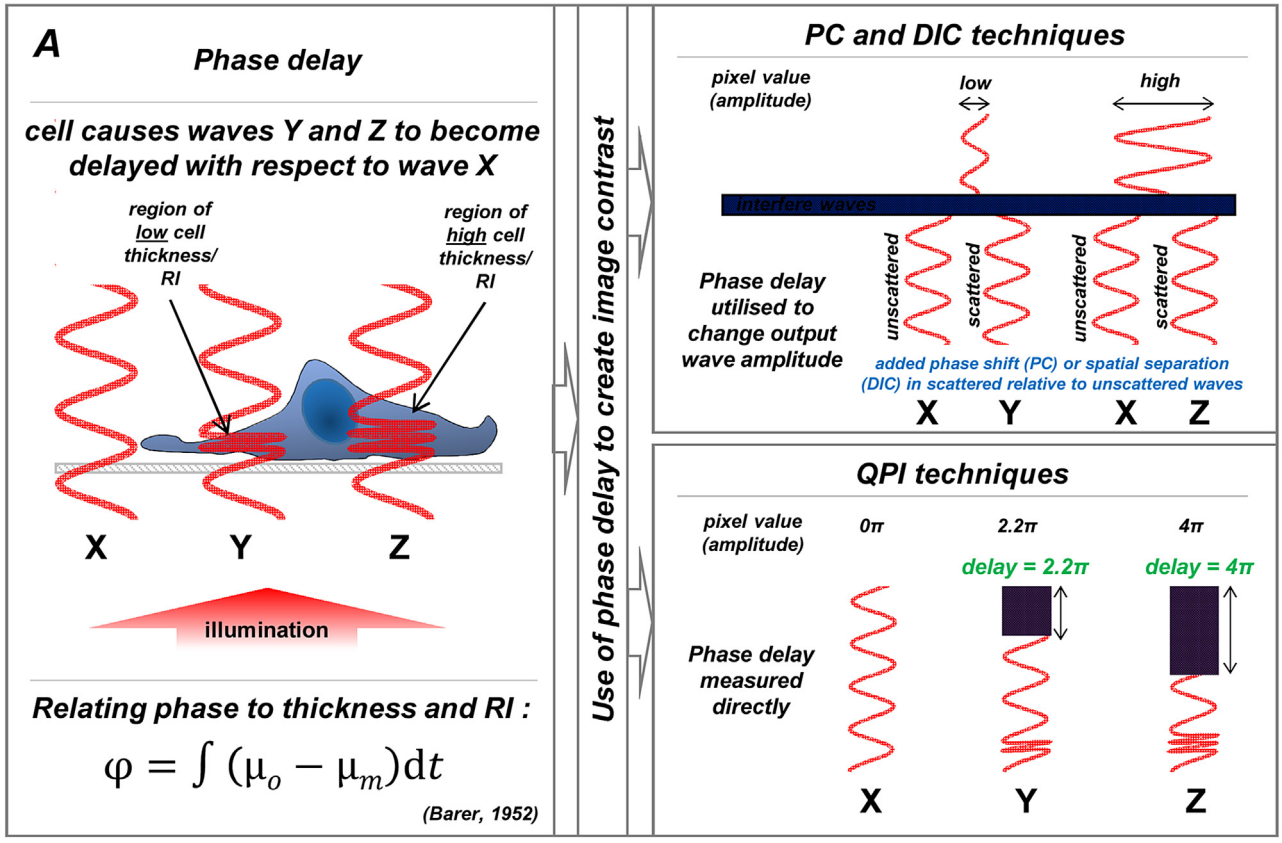

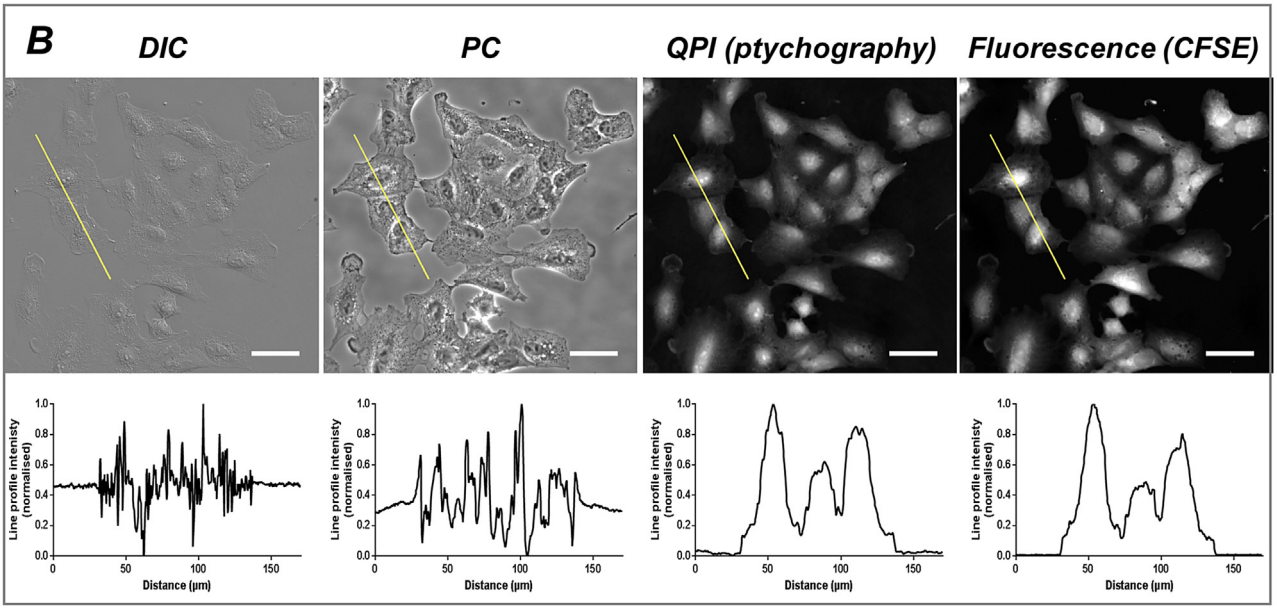

Fig. 1. High-contrast images are obtained by QPI techniques. (A) Diagram of phase delay caused by a cell and the basis by which the phase delay is used to create contrast in the image. The equation describes how phase delay (φ) is calculated from thickness (t) and the difference in refractive index (RI) of the object (μ_o) and media (μ_m). Whilst traditional techniques (PC, DIC) use the phase delay to alter the amplitude of the exit wave resulting in changes in pixel intensity, in quantitative techniques (QPI) the phase delay is measured directly and is enumerated as a pixel intensity. (B) Line profiles across three adjacent A549 cells in an identical field of view imaged by DIC, PC, ptychographic QPI and whole-cell fluorescence. A549 cells were labelled with CFSE and fixed. Scale bar, 50 μm.

2. What are the label-free options?

Rather than requiring contrast-enhancing dyes/labels, label-free solutions rely on components of the optical setup that exploit cells' inherent contrast mechanisms (thickness and refractive index (RI)) to create image contrast.

2.1. Traditional techniques

Phase contrast (PC) and differential interference contrast (DIC) microscopy remain the most prevalent label-free imaging techniques in biological research. Both techniques employ specific optical setups that translate differences in phase caused by cells and intracellular features into changes in light wave amplitude.