Livecyte: Creating a comprehensive cell profile

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SUMMARY

Live-cell time-lapse microscopy is an established and powerful technique for the study of mammalian cell biology *in vitro*. Common time-lapse applications use label-free and/or fluorescence approaches, with the latter requiring introduction of dyes or labels. For many label-free techniques, constraints caused by low levels of contrast preclude the automated segmentation of individual cells, and by association, the capability to differentiate them based on subtle differences in their phenotypic or kinetic characteristics. On the other hand, fluorescence microscopy overcomes such contrast limits, but labels have the potential to alter normal cell function and induce toxicity. Phasefocus Livecyte uses ptychography to generate high-contrast, label-free yet fluorescent-like images, using low powered illumination, allowing for robust individual cell segmentation during long-term time-lapse experiments.

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Introduction

With the advent of the -omics approaches and personalized medicine, the need for monitoring many parameters on many cells at the same time is becoming of paramount importance in the scientific community. Whether the goal is to test the effects of different drugs or to study the impact of gene therapy, scientists are exploiting microscopy more and more to observe cell behaviour from both morphological and kinetic perspectives.

The biggest challenge for many live cell researchers is to characterise individual cells without impacting their behaviour through the act of monitoring them. On one hand, when using most label-free microscopy techniques, the low level of contrast only allows for a population analysis where only major changes in morphology and confluency can be determined. On the other hand, whilst the use of fluorescent labels allows both single cells and cellular functions to be visualised, the levels of illumination needed to excite the fluorophores (and the labels themselves) can alter innate properties of cells, with the associated cytotoxicity limiting the scope, duration and integrity of any experiment.

Hence the emergence of label free techniques, which exploit the inherent contrast of cellular components to create images, allowing single cells to be tracked and monitored over longer periods of time.

Livecyte™ Cell Imaging and Analysis system

The Livecyte from Phasefocus exploits Ptychography, a form of Quantitative Phase Imaging (QPI) – an emerging imaging technique that retrieves phase-delay of light passing through a cell. Traditional techniques such as phase contrast (PC) and differential interference contrast (DIC) microscopy employ specific optical setups that translate differences in phase into changes in light wave amplitude, in order to be detected by the human eye or recorded as pixel intensity changes on a camera. However, the intensity in PC and DIC images is non-quantitative and low image contrast can make automated image segmentation difficult and sometimes impossible.

Ptychographic Quantitative Phase Imaging (QPI) leverages phase shift information to generate high contrast images in which cells appear as bright objects on a dark background (Figure 1). The sample is illuminated with a low power (<1 mW), 650 nm laser and a series of diffraction patterns are collected by the detector. A very sophisticated algorithm converts these patterns into phase shift information and recreates the high contrast image. The low levels of light intensity applied allow individual cells to be identified and tracked for prolonged periods without perturbation. Furthermore, the sample is accommodated in a dedicated pod, where temperature, CO_2 and humidity are controlled

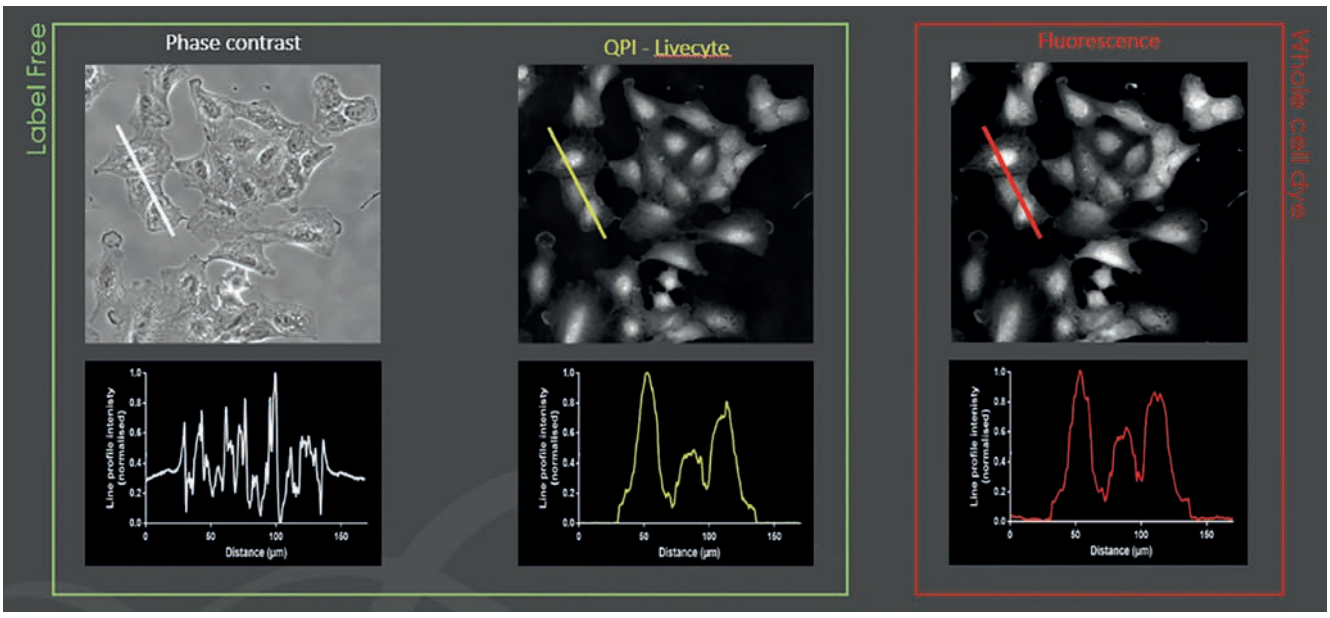

Figure 1. Line profiles across three adjacent A549 cells in an identical field of view imaged by Phase Contrast, ptychographic QPI and whole-cell fluorescence. A549 cells were labelled with CFSE and fixed.

and monitored over the entire duration of the experiment. This ability to image under a more natural environment with reduced risk of phototoxicity not only supports the use of sensitive cell types such as primary and stem cells, but also enables viable cells to be recovered for subsequent experimentation or downstream analysis, giving it broad spectrum appeal for clinical applications in particular. The combination of cell segmentation and QPI information provides a suite of morphological metrics that include cell area, perimeter, thickness, dry mass and sphericity (Marrison *et al.*, 2013). Researchers not only gain a true measure of cell count for the population as a whole but have the added capability to define and quantify distinct sub-populations within complex heterogeneous cultures, achieving a more realistic narrative of cell behaviour. Furthermore, the technology employed in Livecyte delivers a continuous field of view with no loss of resolution, or stitching errors, permitting even highly motile cells to be tracked during time lapse

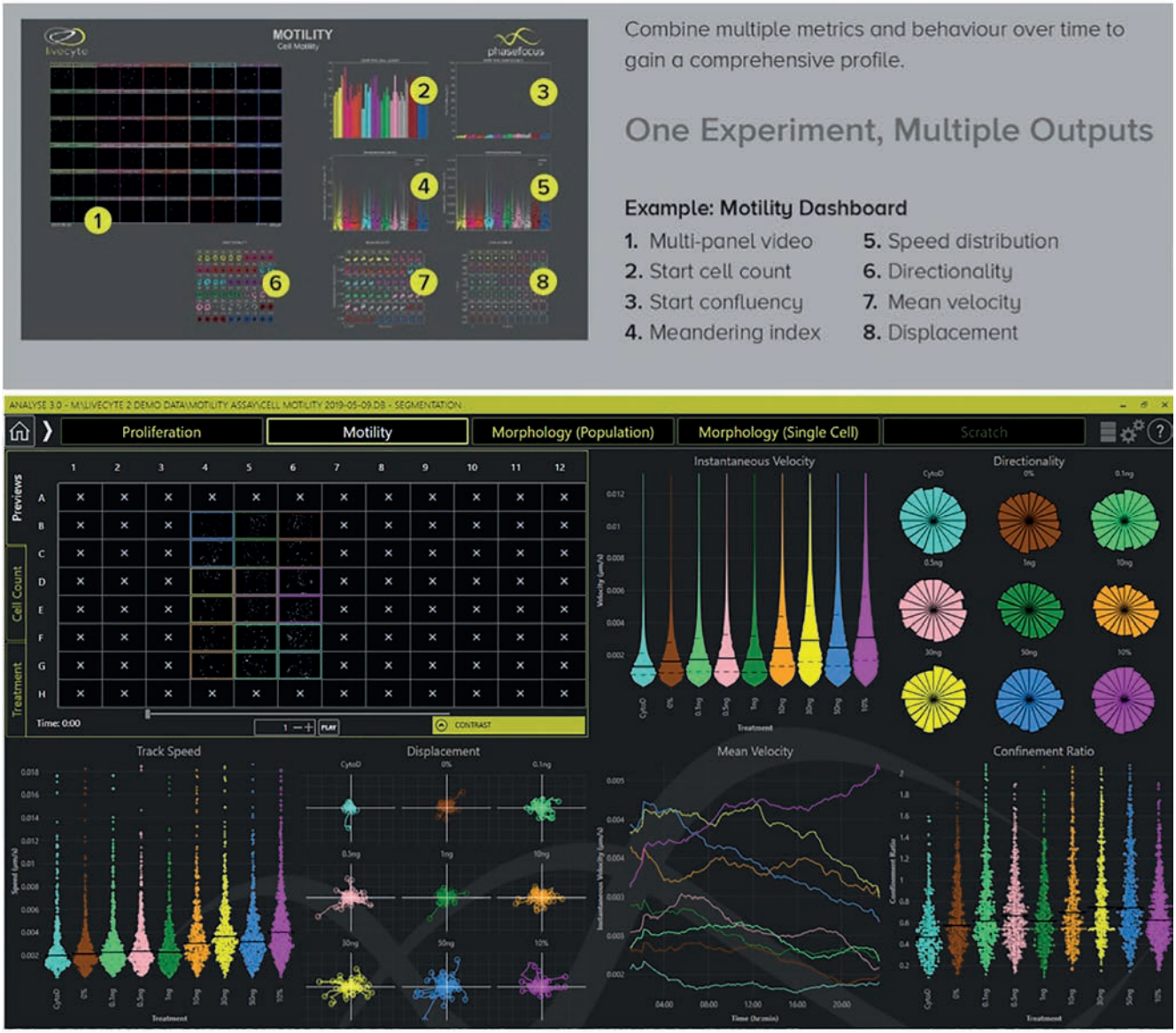

Figure 2. Example Livecyte dashboard (in this case motility) showing multi-well interactive videos and various cell movement metrics. Within the same experiment, cell proliferation and morphology metrics are also generated (accessible *via* the tabs across the top of the screen).

imaging, ensuring no cells are "lost".

Livecyte's Cell Analysis Toolbox™ (CAT) contains automated tracking software that monitors changes in individual cells through multiple cell divisions, eliminating the need for manual tracking and achieving a seamless integration of image acquisition and analysis.

Each experiment automatically yields a plethora of phenotypic metrics, all derived from the phase shift information and the subsequent segmentation and tracking of each individual cell, providing information both on the previously mentioned morphological parameters and on kinetic behaviour, determined by factors including cell speed, displacement and meandering index. Whether the initial biological question was on proliferation or motility, each assay performed with the Livecyte generates a complete dataset that can be reanalysed at a later time from a different perspective, helping creating a comprehensive profile of the cell behaviour (Figure 2).

In addition, the system has the ability to identify cells entering mitosis, as they appear brighter and with a more circular shape (Figure 3A). This allows for a deep investigation of the cell cycle in different conditions or in response to different drug treatments, giving the ability to identify sub-populations of cells with different mitotic times or rare single-cell mitosis or fusion events. Since each cell is segmented and numbered, it is also possible to perform lineage studies, keeping track of sub-populations generated by a single cell and analysing them separately (Figure 3B).

With the capability to compare response to different treatments or environmental conditions at both population

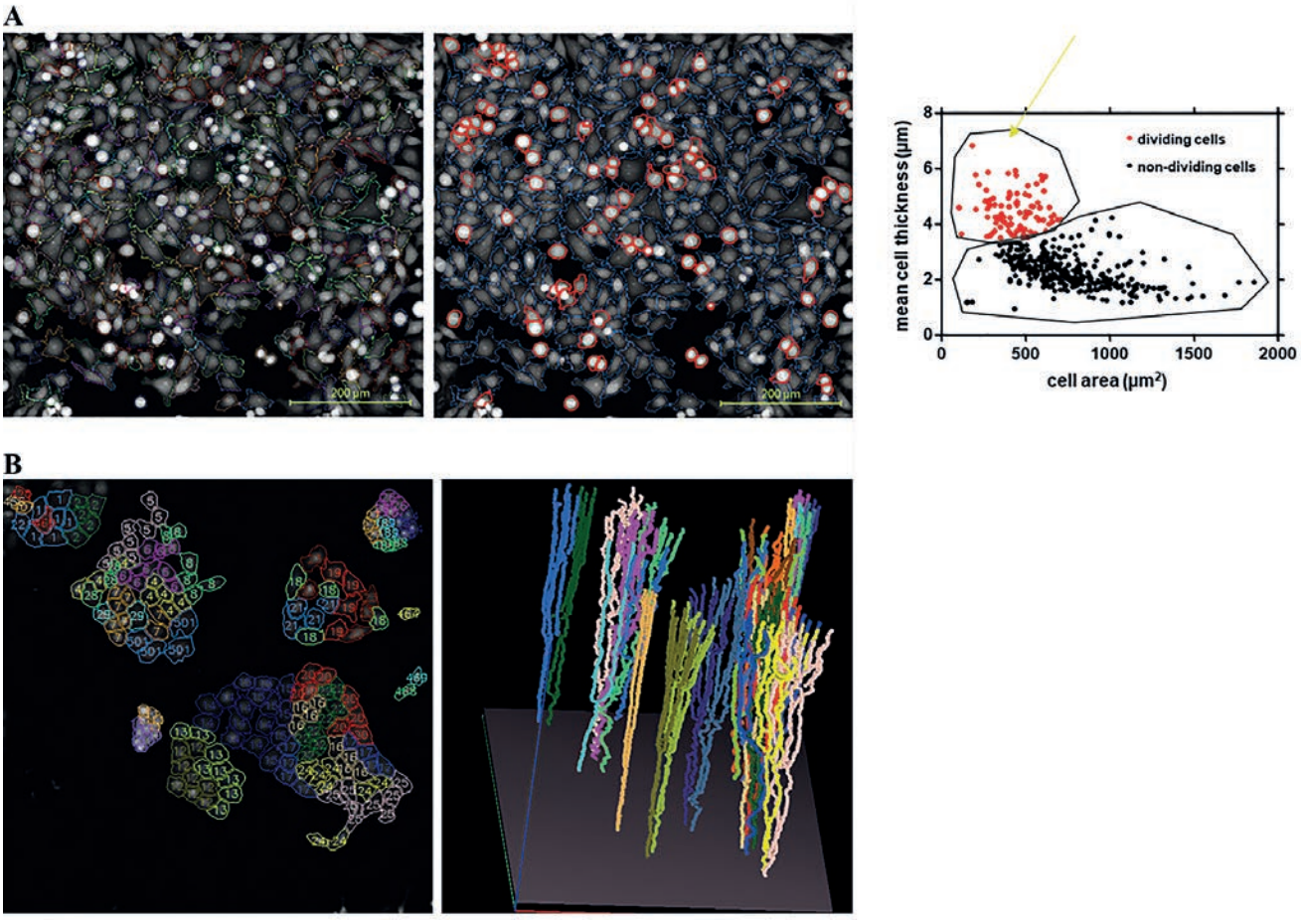

Figure 3. A) Detection of events undergoing mitosis in single time-point images; the gating tool in CAT software gives the percentage of mitosis events at one particular time; samples of the Translational Research Institute, Brisbane, Australia. B) Lineage study with 3D family tree output.

and single-cell level within a single experiment, laboratory workflows can be effectively streamlined, making best use of limited resources.

In the current economic climate, as researchers face ongoing cost pressures and demands for productivity improvements, Livecyte represents a rapid and cost-effective means of gaining deeper insights into biological processes, associated with a wide range of disease conditions with positive implications for drug discovery and development of personalised medicine.

Alfatest is pleased to be the new local distributor for the Livecyte™ Cell Imaging and Analysis system developed by UK-based company Phasefocus™.

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