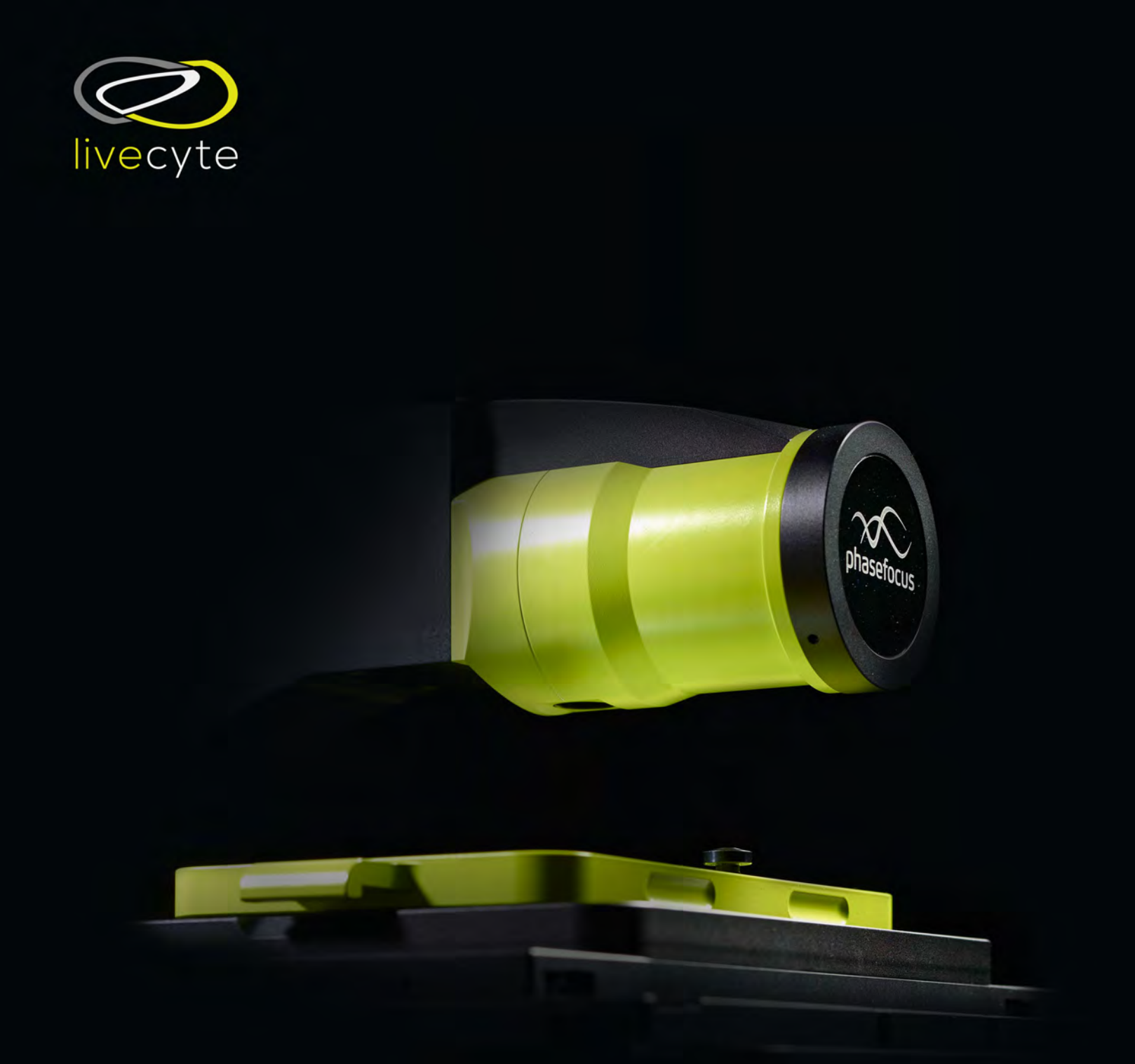
livecyte



A step forward in quantitative phase imaging

phasefocus

A new approach to live cell time-course imaging

Fluorescent cell labelling techniques can produce high contrast images, however, the introduction of fluorescent dyes and high intensity illumination to visualise the location of those dyes can potentially disturb normal cell functions. Quantitative Phase Imaging (QPI) generates quantitative data for analysis from unlabelled cells.

What is the Livecyte?

The Livecyte is a unique system for live cell analysis, which uses QPI technology to enable Kinetic Cytometry: the automatic tracking and analysis of phenotypic and kinetic behaviour of individual cells and cell populations over hours or days. Using an imaging technique called Ptychography (tie-cog-rafee), the system produces high contrast, high fidelity images which are artefact free and quantitative, without the need for cell labelling or high intensity light imaging.

How does Ptychography differ to traditional phase contrast imaging?

Phase contrast methods provide label free imaging techniques but often produce pseudo-3D images or halo artefacts and are not quantitative. However, the Livecyte delivers a QPI technology that :

- Exploits the intrinsic contrast of the cell itself to avoid the need for cell labelling.
- Is non-invasive, label and artefact-free.
- Can continuously monitor cells in culture, yielding information rich data from viable, unperturbed cells.
- Produces images that are always in focus and are not subject to imaging artefacts such as speckle.
- Delivers image quality that is never compromised by external vibrational interference.

Reducing phototoxicity:

Since the power of the illumination laser is thousands of times less than that used for traditional fluorescence light microscopy, cells suffer far less phototoxic shock-a particular advantage for fragile cells such as stem cells or primary cells.

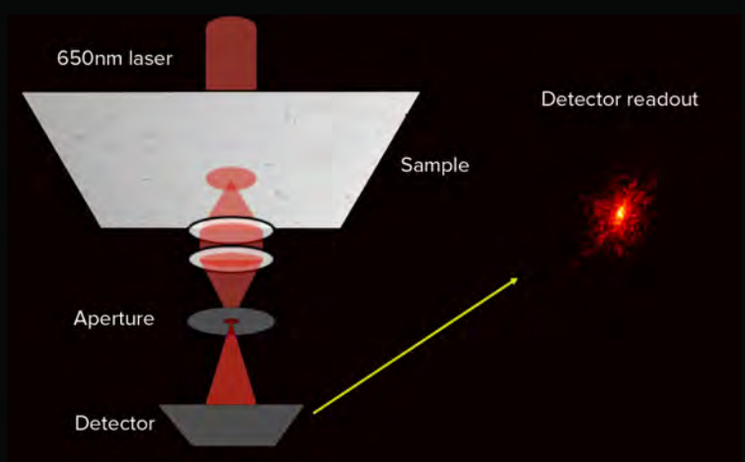


Fig 1. In Ptychography, the specimen and an illuminating laser are moved with respect to one another to create a sequential array of overlapping illuminated areas on the specimen. For each of these areas, the light scattered as it passes through the specimen is captured as a diffraction pattern on a sCMOS camera.
Diffraction patterns are then processed using a proprietary algorithm to reconstruct quantitative phase images.
The scanning technique enables generation of a very large field-of-view, invaluable in cell tracking assays with highly mobile cells.

A unique and versatile system for kinetic cytometry

The Livecyte has been specifically engineered, and employs unique technology, to overcome many of the limitations experienced when running live cell assays. It offers multiple benefits that together offer a practical and powerful solution for quantitative long term live cell imaging and analysis.

Easy to use

- No labelling, fixing or harvesting cells.
- Simple set up takes minutes.
- No complicated alignment required.
- Automated acquisition.
- Easy and effective segmentation and analysis software.
- Cell movies automatically generated.
- System immune to external vibrations-no av table required.

Highly versatile

- Monitor single cells and complex co-cultures simultaneously.
- Petri dishes and multi-well plates accommodated.
- 3 channel fluorescence capability as required.
- Multi area time lapse capability in single or multiple wells.
- Live cells available for additional experiments at conclusion of label free imaging.

Powerful cell tracking capability

- Patent-pending, robust tracking software ensures enhanced lineage tracking.
- Low laser power means cells can be imaged for long time periods.
- A large FOV (up to 2.5 x 2.5mm) ensures that highly motile cells are not “lost” during long time courses.
- Highly consistent and accurate results compared to manual analysis.

Information-rich data

- Multi-parametric quantitative data for **every** cell at each time-point.
- Multiplexed data outputs.
- Very large FOV means large numbers of cells can be studied at the same time.
- Csv files ensure easy transfer of data.

Complete imaging system

- All hardware, software, environmental and storage components included.
- Fully integrated phase and fluorescence workflows.
- Imaging recipes can be created and reused.
- System complements and enhances existing fluorescence analyses.

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A system optimally designed for long term, live cell assays

High resolution imaging with very large field-of-view

The Livecyte delivers the capability to combine data to produce a continuous field-of-view which is arbitrarily large-and can be up to a few millimetres in a single image, thus removing the need to stitch images. While the choice of objective lens will determine the resolution, the field-of-view is independent of this and is not limited by the objective lens being used. Conversely, as Fig 2 shows, large fields-of-view do not compromise the resolution obtained.

Accurately track even highly motile cells - Large fields-of-view means that no cells are “lost” during the imaging process, enabling individual, highly motile cells to be tracked over long time periods without the need for image stitching. The low power laser illumination ensures that cells remain healthy over extended imaging periods.

Multi-Area Time lapse – this is a powerful feature that enables imaging continuously over multiple regions, within a single well of a multi-well plate or multiple regions within each well of a multi-well plate.

The regions of interest may also be imaged at different magnifications, allowing for maximum flexibility within an imaging protocol.

Robust environment control

The LiveCyte system is housed within an environmental chamber, with the cells further protected within a “sample pod”. Even if the sample pod has to be removed during an experiment, it can be perfectly realigned, allowing continued monitoring and tracking of relevant cells.

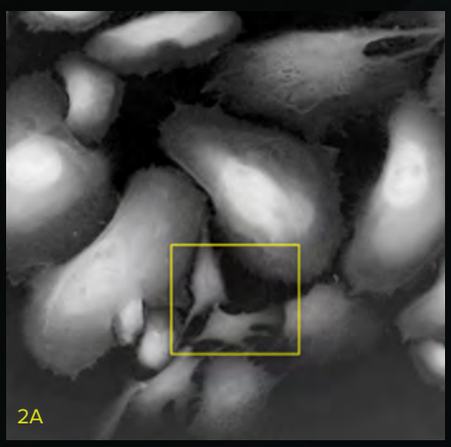
Always in focus with perpetual focus technology

The advanced technology used to engineer the Livecyte system means that during label free imaging, minimal focussing is required prior to acquisition. Due to the nature of the data collected during the imaging process, the perpetual focus feature results in images that are always in focus, thus the issue of focal drift in long term imaging is completely negated.

Powerful tracking software

The Cell Analysis Toolbox contains powerful, automated cell tracking software, (patent pending), that can track individual cells over long time periods and identify changes in morphology and kinetic behaviour, removing the need for manual processing and saving many hours of tedious work. The system can accurately track cell lineage over multiple cell divisions.

Sub-cellular detail can be visualised label free and if required, additional fine detail can be identified using fluorescence.



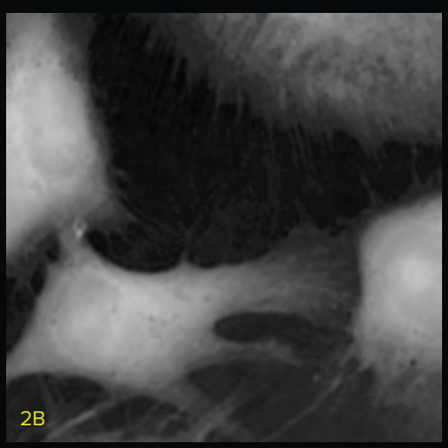


Figure 2A) Large field-of-view ptychography phase image. Note continuous high-resolution field. 2B) Zoom of highlighted region - the entire image in A) has this resolution and image quality.

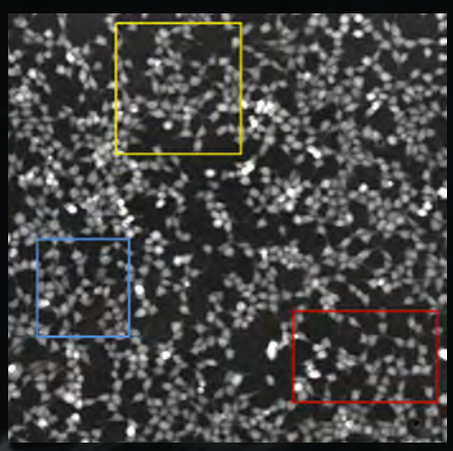


Fig 3. Multiple regions of varying sizes can be specified within a field-of-view.

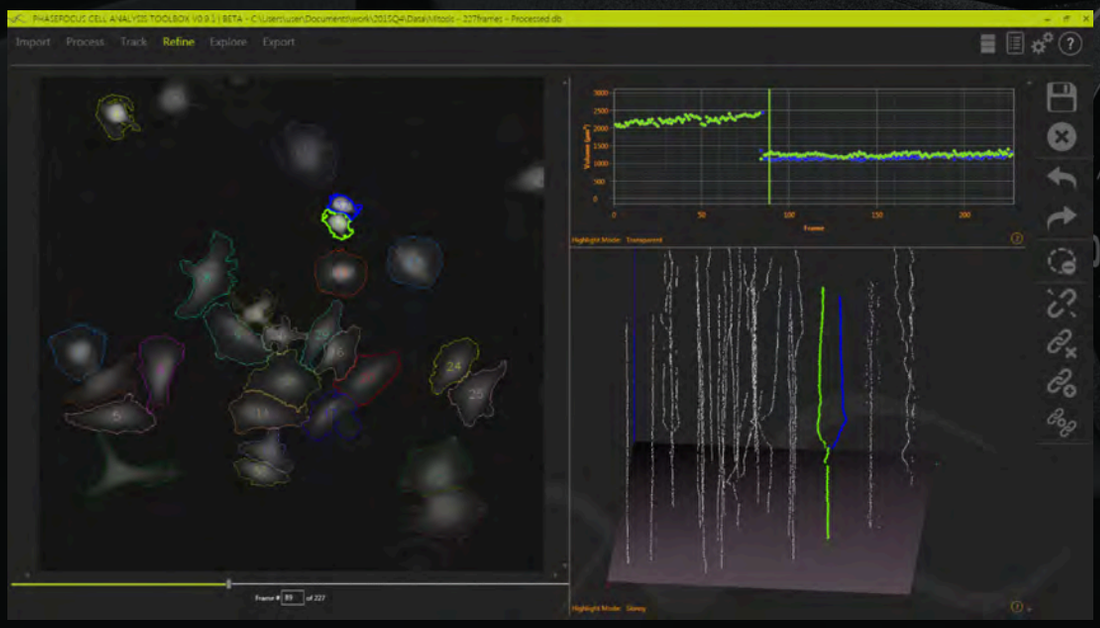


Fig 4. Typical outputs from cell tracking software.

Linking kinetic and morphological features

Multi-parametric kinetic and phenotypic data can be readily extracted and the behaviour of these, at a single cell level and within cell populations, recorded in response to different treatments or environmental conditions. Taken together, these make the Livecyte highly suitable for many different types of assay including:

• **Cell motility:** robustly measures cell migration, including scratch-wound assays. Automated software enables study of multiple cells within a mixed population and provides behavioural motility data while simultaneously revealing morphological information. The software can follow all cells for a complete time-course, even if those cells pass over each other.

• **Quantitative outputs relating to cell populations:** e.g. cell count, confluence, total dry mass, mitotic index and proliferation, at multiple time-points - for simple yet robust cell population growth monitoring.

• **Automated cell segmentation and tracking:** Software identifies and continuously monitors 10 different cellular characteristics for each cell, resulting in consistently accurate tracking of all cells within a population. Multiple cellular measurements are captured for the whole time-course and videos of fully segmented cell populations can be generated automatically.

• **Changes in cell or sub-celluar volume:** a highly versatile measurement that can offer new insights into cell behaviour and responses, relating not only to cell growth, but also enabling deeper understanding in areas such as metabolic studies.

• **Toxicity, cell viability and apoptosis rate:** providing information on the kinetics of the cytotoxic response rather than the snapshot generated by many colorimetric endpoint assays.

• **Mitotic index vs time:** Measurement at multiple time-points allows identification of changes in proliferation as a population evolves.

• **Morphology:** Measures such as sphericity, volume, length: width ratio and surface profile/texture can identify many different types of changes in cell behaviour, e.g. such as formation of neuronal processes.

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Example Applications

Cell Migration

Assay parameters can be combined to generate deeper understanding of cell behaviour. Gap closure assays are just one example in which such combinatorial phenotypic metrics can be used in order to correctly interpret potential drug-induced changes in cell motility. Does faster gap closure relate to a drug induced increase in cell motility, proliferation, or both?

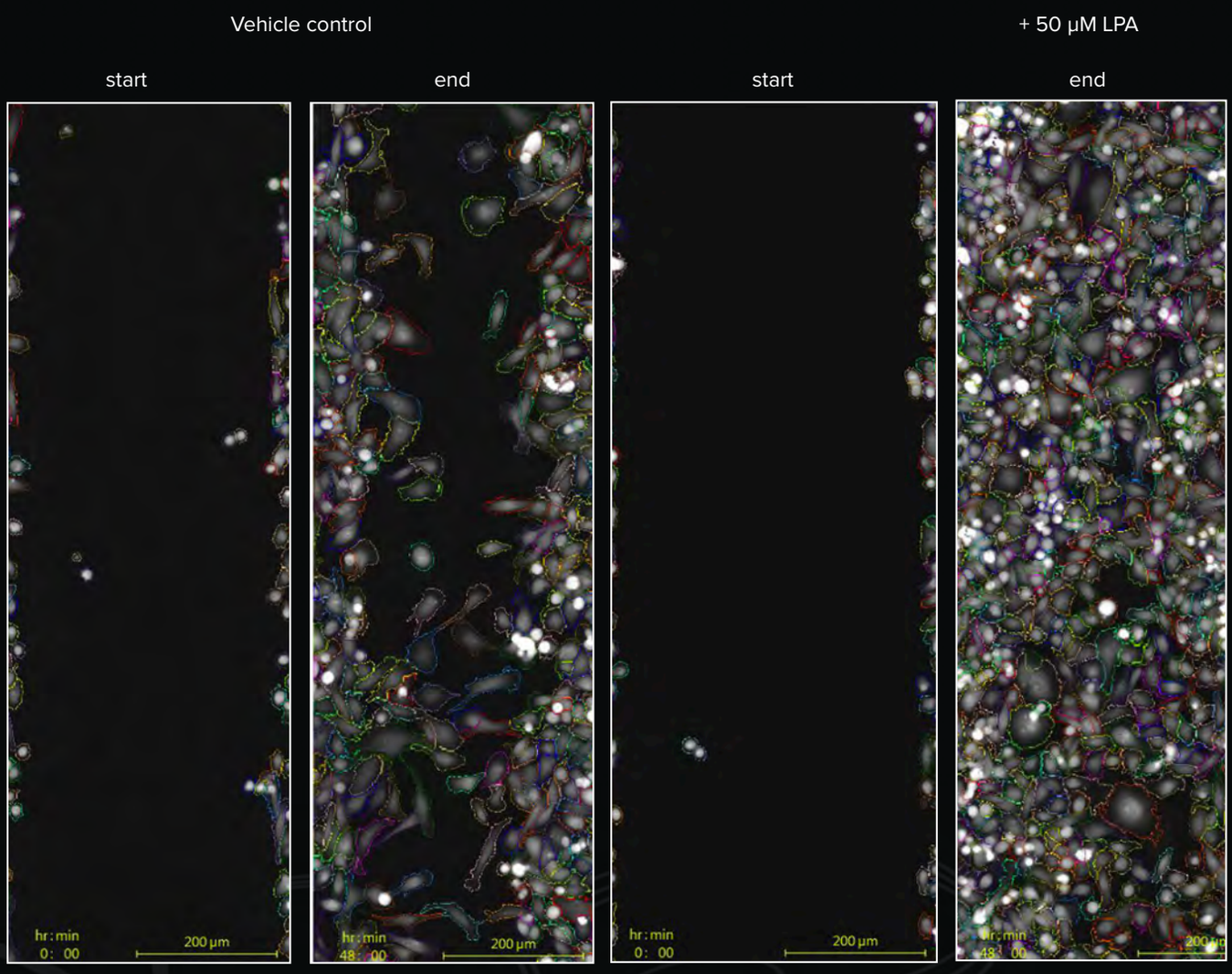

Fig 5. Images showing start and end frames of a 48h time-lapse experiment in which MDA-MB-231 cells were treated with 50 µM lysophosphatidic acid (LPA) or a vehicle control. Unlike the vehicle control, cells treated with LPA show complete closure of the gap within the duration of the experiment. Yet, judging by the images alone, it is unclear whether this closure is due to direct effects of the drug on cell motility and/or proliferation.

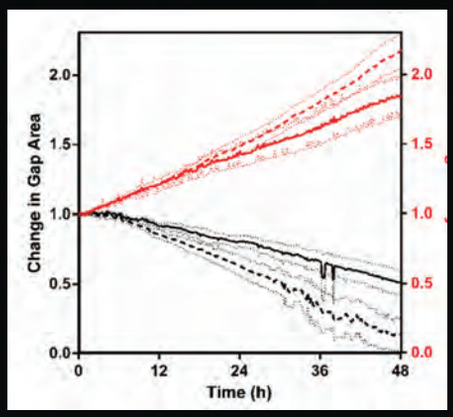

Fig 6A. The change in gap area (black lines) was quantified using the Cell Analysis Toolbox, confirming that cells treated with 50 µM LPA (dashed lines) demonstrate a faster gap closure compared to control cells (solid lines). However, after ~20 h a divergence in dry mass (red lines) between control and LPA-treated cells is evident, which indicates that there has been a drug-induced change in the rate of cell growth. Together, global dry mass and gap closure metrics show that the gap area metric is unaffected by proliferation up until ~20 h, but after that becomes unreliable as a measurement of motility.

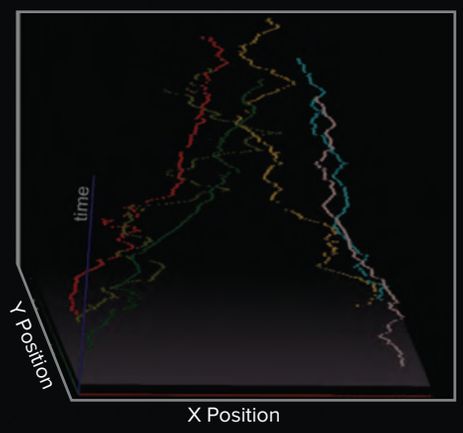

Fig 6B. A cell tree (3D graph of the [x,y] position of cells plotted against time), from the sample treated with LPA. This highlights the tracks of three selected cells on either side of the gap and shows that individual cells can be followed over the course of the gap closure assay, enabling direct measurement of their motility.

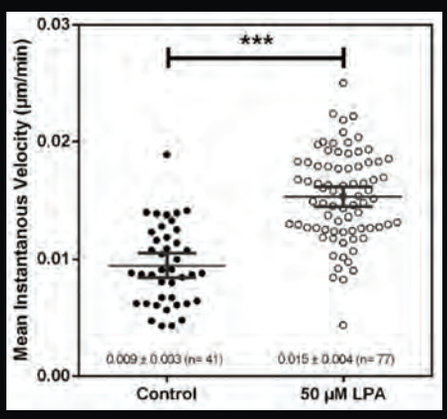

Fig 6C. The plot shows how the direct motility measurement, in the form of mean instantaneous velocity of cells with tracks lasting > 33 h, can be used to assess the effects of LPA on the motility in a manner that is completely independent of proliferation. When used in combination with the global gap closure and dry mass metrics, this confirms that LPA causes an increase in cell motility.

A Mann-Whitney U-test was performed; ***, P<0.001; bars show mean ± 95% confidence interval.

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Example Applications

Identifying mitosis & cell division

Mitosis is very easy to identify, even in near confluent cell layers. Dividing and non-dividing populations can be readily segmented and tracked.

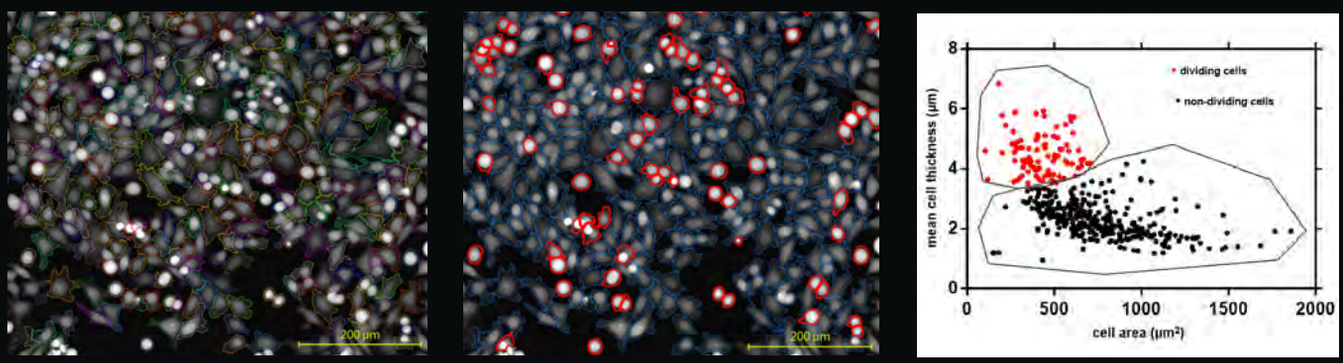


Fig 7. Segmented Hela cells growing at high density - cells undergoing mitosis are marked. The plot demonstrates that cell thickness and area parameters can be used to identify cells that are undergoing cell division. Points highlighted in red relate to the cells outlined in red in the image.

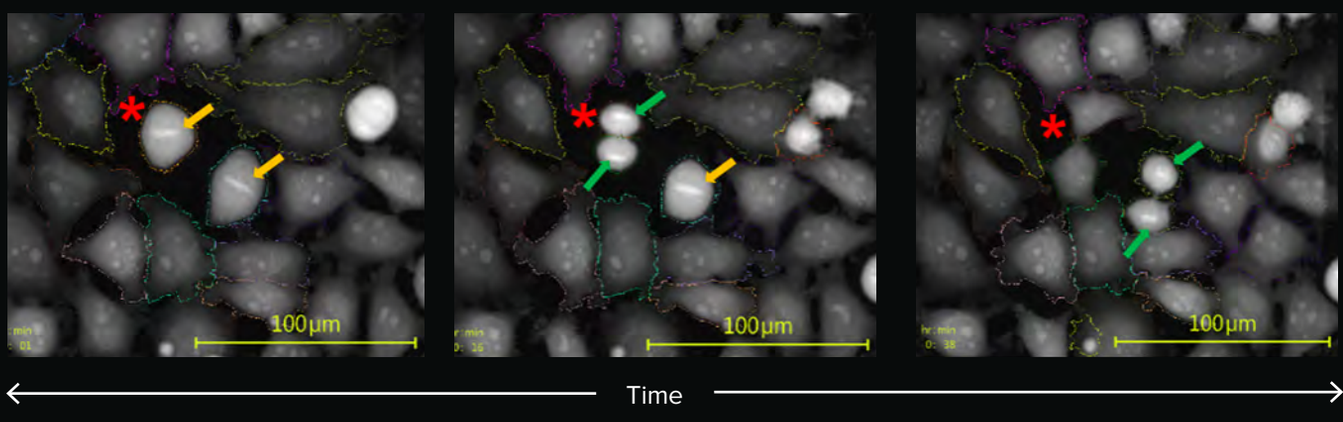


Fig 8. Yellow arrows highlight where chromosomes can be seen to align on the metaphase plate. Green arrows indicate the presence of aligned chromosomes that are evident in each daughter cell immediately after cytokinesis.

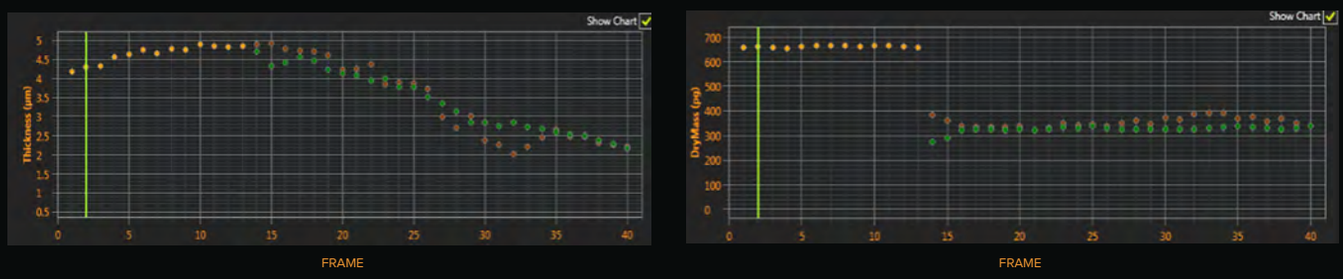
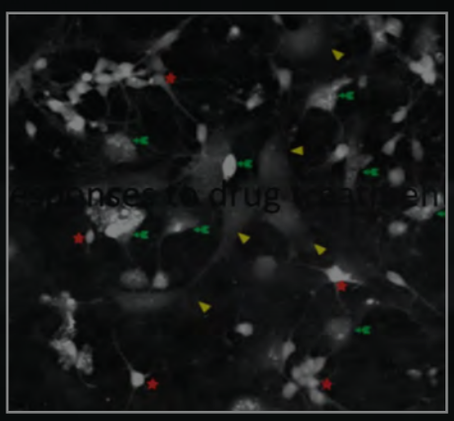
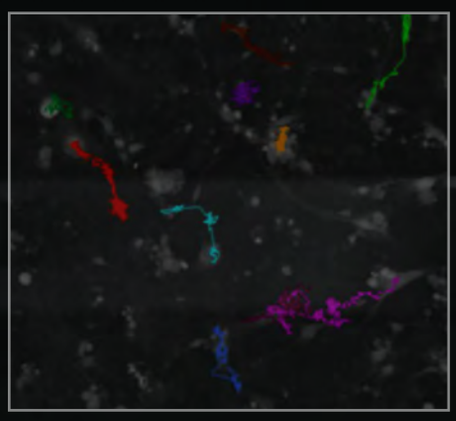


Fig 9. Graphs show temporal changes in metrics of cell thickness and dry mass that demonstrate the feasibility of single cell analysis of mitosis using the Cell Analysis Toolbox. Red asterisk (Fig 8) indicates the selected cells shown on the plot.

Primary cells and stem cells

Primary cells and stem cells can be difficult to study using fluorescence imaging, as they are particularly sensitive to phototoxic shock. However, behaviour and effects of treatments can be studied readily over long periods of time without perturbing function and growth using the Livecyte system. Additionally, samples may be used for subsequent analyses such as end point fluorescent assays following label free analysis.





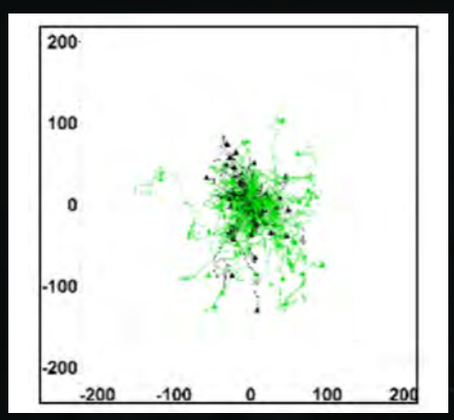


Fig 10. In this example, microglia, astrocytes and neurons were identified and behaviour subsequently studied. Time lapse video identified two distinct behaviours by the microglia, one being a highly active sweeping behaviour, while the other was relatively static. This behaviour was further characterised by studying the velocity of individual cells over time, quantitative data generated for each, and populations identified. These can then be further studied for, for example, differences in response to drug treatments.

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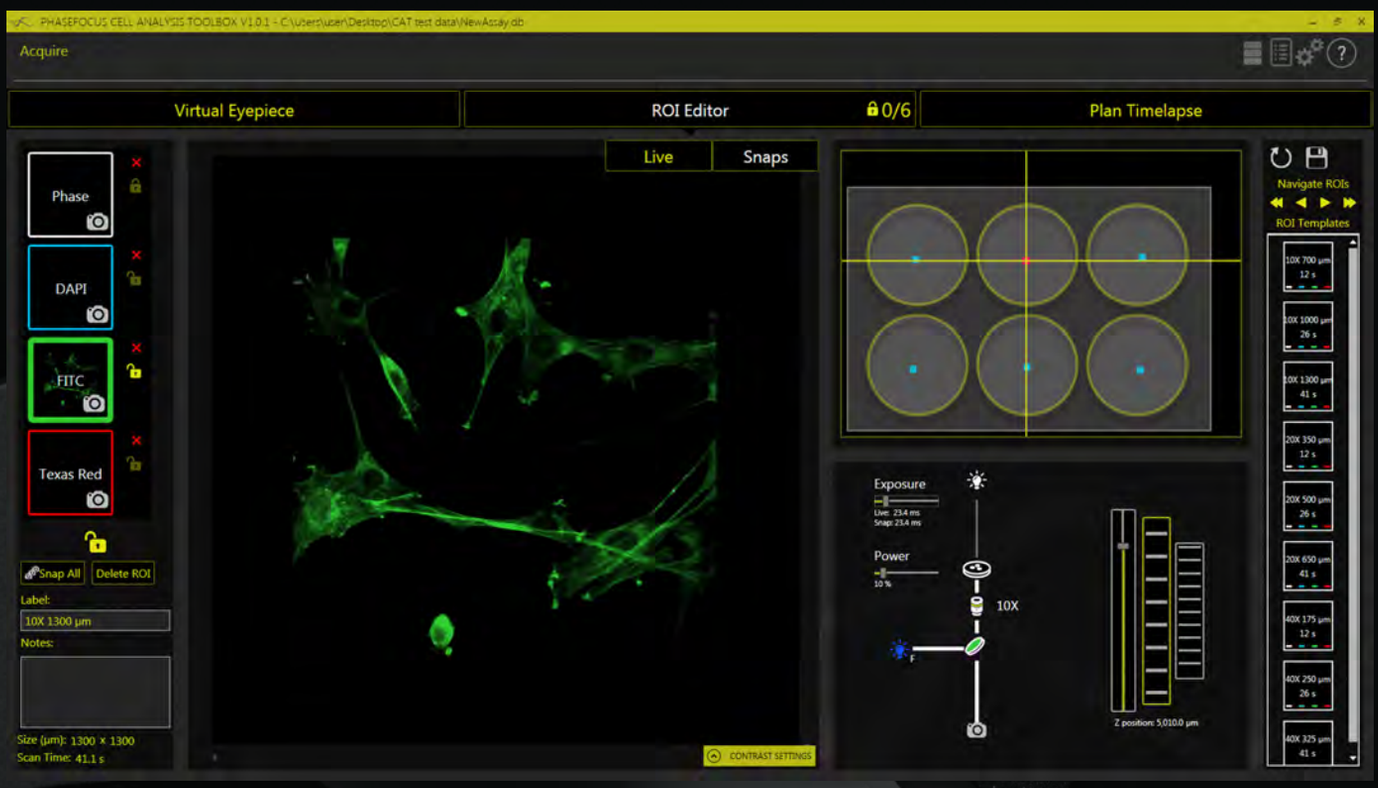
An easy transition from set up to images to data

Simple acquisition set-up

The LiveCyte system can be fully controlled using acquisition software designed to be easy and intuitive for both new and advanced users.

Interactive graphical tools such as a “mini-map” and a mouse-controlled focus wheel aid navigation around a sample. Interesting regions can be quickly identified using a 4X objective lens; and a simple click changes to any one of up to 5 other magnifications. Alternatively, there is an option for the software to collect a low magnification, well-preview scan automatically, on start-up.

Switching between fluorescence channels and a bright-field preview is simple, with a light path diagram that makes it clear which setup is being viewed at any time.



Easy time lapse workflows

For time lapse assays, the workflow is also simple: an interactive view of the graphical timeline makes set- up and visualisation of even the most complex assays stress-free. While a time lapse assay is running, progress can be monitored by browsing the images as soon as they are generated and the microscope can be paused at any time.

The Cell Analysis Toolbox

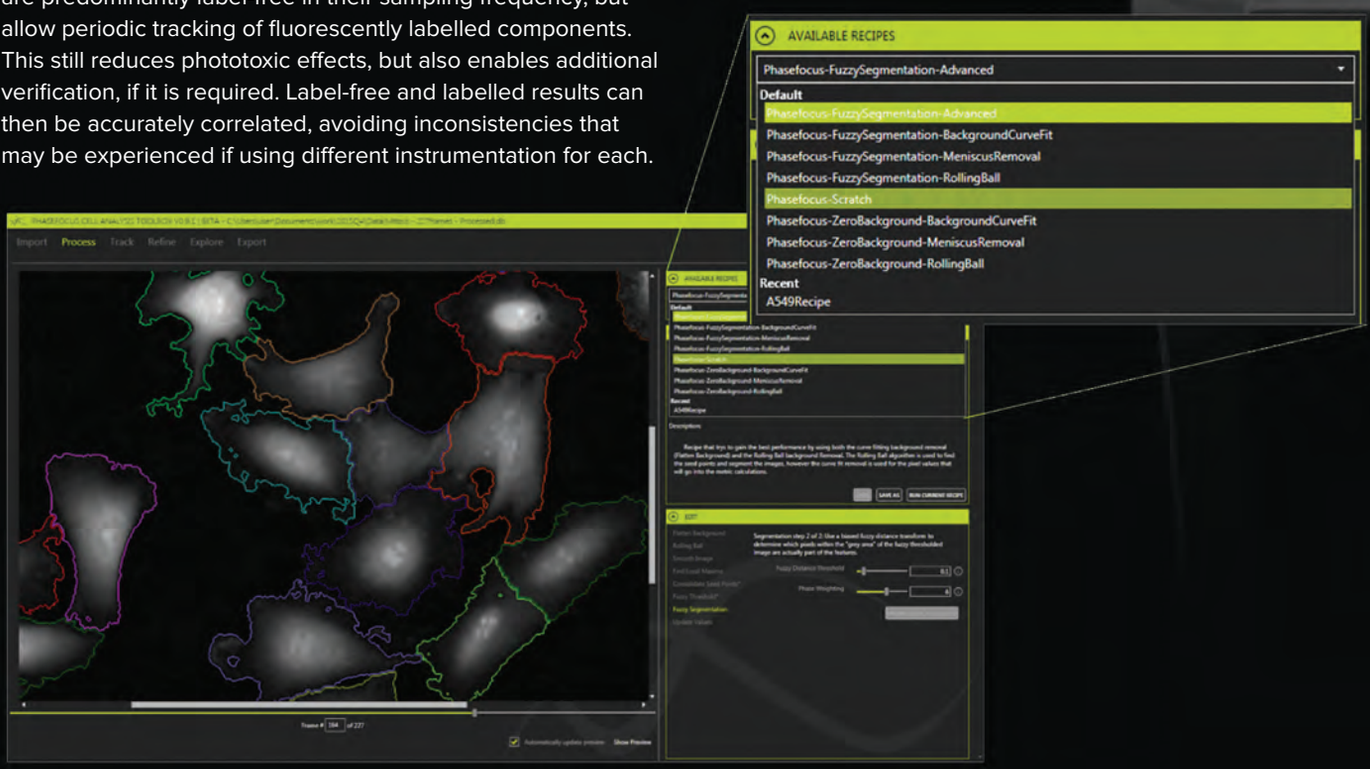
Once LiveCyte has collected high contrast, high fidelity images, detailed morphological and kinetic data about each cell can be generated using the Cell Analysis Toolbox, which provides a powerful array of image analysis tools. Default recipes for the most common analyses are provided, but these can be adjusted with real time feedback to be tailored to specific data. Customised recipes can be saved and shared.

Interrogation of results is very flexible: single cells, multiple cells or multiple populations of cells can be studied, always with the option to link images to cell tracking and data by moving the mouse over particular cells.

To produce outputs for presentation or publication, or for further analysis, data can be exported as graphs, data tables, images or videos. Data tables are saved in a csv file format.

Fluorescence enabled

A powerful and useful application of the LiveCyte system is the ability to combine label-free and fluorescence protocols automatically and seamlessly. Protocols can be used which are predominantly label-free in their sampling frequency, but allow periodic tracking of fluorescently labelled components. This still reduces phototoxic effects, but also enables additional verification, if it is required. Label-free and labelled results can then be accurately correlated, avoiding inconsistencies that may be experienced if using different instrumentation for each.



Uncomplicated Data handling

Long term live cell imaging assays often generate large datasets. With Livecyte, data handling is straight forward: data is saved directly to a dedicated database and can be accessed from any networked computer.

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A complete system solution

The Livecyte is a full, ready to go system providing all the hardware and software you need to get started quickly and simply. The system consists of the following high quality components:

Transmission Inverted Microscope

- Fully automated Brightfield, Ptychographic phase and Fluorescence modalities
- 120mm x75mm travel, high precision stage
- 4X to 40X objectives
- 6 position turret
- LED illumination for brightfield/fluorescence imaging
- 650nm diode laser for Ptychographic imaging
- sCMOS camera for high sensitivity phase and fluorescence image capture

Environmentally controlled incubator

- Full heat, $C0_2$ and humidity control plus Sample Pod

Acquisition PC and software

- 12TB data storage system (upgradable with options up to 92TB)
- Dedicated Cell Analysis Toolbox data processing unit

Fluorescence capability

- Fully integrated Cool LED illumination
- Filter sets optimised for DAPI, FITC, Texas Red fluorescence
- Fluorescence acquisition software fully integrated with Phase acquisition software
- Fluorescence analysis software fully integrated with Phase analysis software

Optional extras

- Customised filter sets are available
- Additional objectives are available
- Additional data storage is available
- Additional Cell Analysis Toolbox analysis software site licences available

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A powerful tool to bring the power of quantitative phase imaging into your research.

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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

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