Bulldog Bio

One New Hampshire Avenue

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What is the Livecyte system?

Why is the Livecyte system a new way of looking at my cells?

The Livecyte system calculates the phase delay induced in the illumination light to extract a suite of information about your cells' behaviour over time. The phase delay is represented as high contrast label free, quantitative images, which enables the behaviour of your cell population to be analysed right down to a single cell level. Combined with the time course ability, Livecyte enables a vast array of metrics to be calculated and combined to perform a number of applications such as true proliferation, advanced scratch wound, cell motility, chemotaxis and many more. The Livecyte can also perform correlative fluorescence and brightfield imaging.

Why is the Livecyte different to traditional light microscopes?

Traditional imaging is dependent on light scatter and/or absorption from the sample in order to produce contrast in the image. Cells are essentially transparent, so contrast is enhanced either by optical considerations (phase contrast, DIC) or by the addition of fluorescent labels. These techniques have inherent disadvantages when applied to live cells such as optical artefacts (individual cells are difficult to segment and track), risk of phototoxic damage and introduction of additional parameters into the experimental setup. These disadvantages will introduce a level of uncertainty into the final outcome of your experiment.

The Livecyte measurement is non-invasive (no labels required nor photo toxic effects on cells) and as such can be carried out over a long period of time on live sensitive cells. The ability to examine your data on the single cell level gives more refinement to your data. The non-invasive measurement makes Livecyte ideal for work on primary cells, neural cells and stem cells.

The nature of the technique also allows the areas of investigation larger than the field of view of the objective lens. Further to this it also allows for rectangular shaped regions to be measured (ideal for scratch wound assays) without the need for any image stitching and subsequently any image processing artefacts that may deteriorate the quality of your data.

In addition, due to the nature of the data collected, it is possible to automatically focus your sample post acquisition. This ensures that the microscope is not sensitive to focal drifts during a long term time lapse, or differing focus positions across an entire well plate.

Why is the Livecyte different to traditional flow cytometers?

The continuous single cell segmentation can be utilised to categorises heterogeneous cell populations at every time point. Population scatter graphs (similar to those produced by flow cytometers) can be produced for every time point on the exact same population of cells. Livecyte allows a far more efficient (cost, time) and refined representation of the cell population. Livecyte also produces a time-lapse video to compliment the data, which allows the user to interpret, mine and validate their data.

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How can the Livecyte system enhance my research

What advantages does the Livecyte offer over a phase contrast technique?

Phase contrast induces an additional phase delay to scattered light in order to increase the contrast between your cells and the background. Livecyte produces images, which due to their nature are inherently high contrast. In phase contrast, the contrast is far lower than the Livecyte images. In addition, phase contrast images are not optically quantitative (thickness measurements cannot be extracted). Phase contrast images also contain well known diffraction effects (such as halos around scattering objects) which limit the downstream analysis which can be applied to the data.

The high contrast Livecyte images enables single cell segmentation to be robustly applied and as such you are not limited to an estimate of cell behaviour based on assumptions from some form of population analysis. In combination with the quantitative nature, Livecyte can build a finger print for each individual cell and subsequently each individual cell can be tracked throughout a time course.

How does Livecyte support continuous non-invasive imaging and analysis?

Livecyte is a complete imaging and analysis time lapse system. Cells are supported in the custom designed Phasefocus POD (which ensures uniform CO_2 delivery to the entire plate) within an incubation environment which maintains a constant temperature of 37°C. The nature of the illumination required for phase imaging (very low power red laser (650 nm, with power output of 0.1 mW)) ensures that the risk of the measurement technique perturbing the natural behaviour of the cells is at an absolute minimum, even during a long term time lapse. Since the cells are not perturbed during the experiment they can be reused after measurement.

Why do you refer to the system as efficient?

Livecyte is an automated easy to use system. Therefore, minimum training is required. The system is also cell friendly so minimum perturbation is experienced by the cells. The nature of the data also ensures that an entire suite of information can be extracted at every time point. Therefore, it is highly suitable for limited cell lines and /or sensitive cell lines as the information rich data produced ensures that a vast suite of metrics can be extracted to accurately describe the behaviour of your cells over time. Therefore, Livecyte is efficient in terms of cost and time.

Why does the system also have fluorescence capabilities?

Livecyte has the ability to acquire fluorescent images of your sample and automatically overlay these on your phase images. The Livecyte comes equipped with 3 filter cubes as standard (optimised for DAPI, FitC and TxRED) but also has the scope for an additional four to be added.

Fluorescence is envisaged as a validation step, to verify specific features/processes visualised during the phase imaging. All precautions are in place to minimise the risk of phototoxic damage while utilising the fluorescence modality. For

example, phase imaging can be used to extract the kinetic behaviour of the individual cell, while less frequent fluorescence data can be acquired to validate any specific process.

What is meant by the dry mass measurement?

Biological context: What cell parameters does dry mass measure?

Dry mass is the summed mass of all cellular components (e.g. biomolecules such as proteins, lipids, carbohydrates, DNA, etc.) excluding water. As such, the dry mass measurement is an accurate measure of cell size; accounting for the extent of biosynthetic and degradative processes in addition to uptake and expulsion material by the cell. Thus, the growth of an individual cell, defined as a change in cell size over time, can be monitored by measuring changes in a cell's dry mass over time. For a population of cells, the sum of the dry mass is a useful measure to enumerate the combined growth and proliferation rate of the population.

How dry mass is measured by quantitative phase imaging:

The pixel intensity of the quantitative phase image relates to the extent of phase delay. The phase delay information captured within our images can be directly converted to dry mass using the equation below. This equation makes use of a constant refractive increment ($\alpha = 1.8 \times 10^{-4}$ m^3/kg), which is a mean of the tight range of specific refractive increments measured for biomolecules that predominate cell composition. Typically, however, the specific value of α is not critical as normalisation is performed to compare the rate of change of dry mass between treatments.

$$m = \frac{1}{\alpha} \int \varphi \lambda dA$$

$\boldsymbol{m}$ = mass
$\boldsymbol{\alpha}$ = refractive increment constant
$\boldsymbol{\varphi}$ = phase delay
$\boldsymbol{\lambda}$ = illumination wavelength

What QPI technology is utilised

What technique does Livecyte use to obtain the phase information?

The Livecyte utilises the technique of Ptychography to solve the phase problem in optics. Images representative of the phase delay (differences in relative optical path differences) can be constructed to produce high contrast quantitative images. The phase data is extracted from the reconstructed wavefront. The reconstructed wavefront can be propagated

mathematically to post acquisition focus your cells, therefore the technique is not sensitive to focal drift or any differences in focal positions across a well plate.

What do you mean when you say the images are quantitative?

The quantitative nature of the images refers to the fact that the intensity values in the image contain information relating the phase delay experienced by the light passing through the sample. This allows information representative of the thickness of your cell to be extracted. This enables relative changes in volume of cells to be investigated over time and/or compared across your experiment. It also provides Livecyte with an additional parameter to 'finger print' each individual cell, which aids in the robust tracking of individual cells throughout an entire time-lapse.

What is the resolution of the images produced?

The spatial resolution of the images produced is ultimately determined by the resolution of the objective used in the measurement. Due to the increased contrast in the images produced by Livecyte, smaller sample features are more discernible than in the intensity/bright-field image. For a 40X measurement the spatial resolution is approximately 0.6μm.

The depth resolution (z resolution) of the measurement is ultimately limited by the difference in refractive index between the cell and media. This can range from tens of nanometres to 1 μm (typically 40nm).

What is the nature of the data produced?

The data produced from the Livecyte is a native format to the CAT: Analysis software (containing both intensity and phase images and a database file). This native data is used to reconstruct and post focus the images. The data can now be analysied within CAT but is also fully supported to export to a variety of image analysis packages.

The variety of export workflows readily supported include exporting to ImageJ and Microsoft Excel compatible formats (tiff stacks, segmentation masks, .csv files), as well as a variety of other common image and video formats.

What plates can I use on the Livecyte?

What well plates and coatings can I use on the Livecyte?

The following plates have been routinely tested on the Livecyte system:

- 35 mm dishes (WPI, glass-bottomed Fluorodish [Item#: FD35-100]; ibidi 35 mm dishes (glass or ibiTreat))
- Ibidi μ-slides and flow chambers (e.g. ibidi μ-slide I; ibidi 4 well μ-slide Ph+; ibidi μ-slide VI $^{0.4}$)
- 6 well plates (Corning Costar plastic 6 well, individually wrapped [Item#: 3516]; or [Item#: 3506];
- Cellvis 6 well glass bottomed plates [Item#: P06-1.5H-N])
- 12 well plates (Corning Costar plastic 12 well, individually wrapped [Item#: 3513])
- 24 well plates (Cellvis 24 well glass bottomed plates [Item#: P24-1.5H-N])
- 96 well plates (Corning Costar plastic 96 well, individually wrapped [Item#: 3603]; Greiner Screenstar
- 96 well plate [Item#: 655 866]; Cellvis 96 well glass bottomed plates [Item#: P96-1.5H-N])
- However you can also use other suppliers for glass-bottom plates and most individually wrapped

- HCS (optical quality) plastic types.
- Measurements can be performed on a number of coatings included matrigel, agarose, cell derived matrix and fibronectin.

What applications could I run on Livecyte

Example applications:

Timelapse scratch wound assays *(recent example: 12 well plastic plate containing breast cancer cells (wt and point mutation) scratched with P200 tip, media added containing one of two different drugs)*

Label-free cytotoxicity assays *(recent example: 96 well HCS plate containing prostate cancer cells treated with a range of concentrations of an inhibitor – cytostatic effects of drug and cell death measured over 24 h)*

Random motility assays *(recent example: renal cell carcinoma line plated in a 6 well plate and treated with a positive control and test candidate drug, individual cells tracked, speed measured, positive control gave change in cell speed whilst test candidate did not – assay only needed 5 h imaging duration)*

Phenotyping heterogeneous responses in primary cells *(recent example: primary prostate cancer cells examined in random motility assay, able to discriminate between different cell sub-populations using unique QPI metrics (e.g. dry mass), morphometrics and dynamic behaviour)*

Assays measuring effects on mitosis *(recent example: effect of a drug on mitotic time in a cervical carcinoma cell line; effect of a drug upon the percentage of cervical carcinoma cells that stall in mitosis and those that fail during cytokinesis)*

Phagocytosis assays *(recent example: correlating the uptake of fluorescent bacteria by individual cells to their speed and roaming)*

Angiogenesis assays *(recent example: measuring the extent of tube formation for HUVEC cells plated on Matrigel in response to different ligands)*

Lineage determination *(recent example: measuring the differences in motility, cell cycle time and lineage of wt and cripsr stem cells)*

Chemotaxis assays *(currently using ibidi chemotaxis slides to address chemotactic response of macrophages towards novel chemoattarctants)*

General questions

What do I receive as standard?

The Livecyte system is a complete automated imaging and analysis system. The Livecyte system includes all you need to support your cells long term (incubation, C02 POD and all inserts to support various plate types, objectives (4X, 10X, 20X, 40X)), and produce information rich images. The system also includes CAT (Cell Analysis Toolbox) to provide the necessary tools for you to perform the required analysis in an easy to use and highly intuitive suite of software. The system also supports fluorescent imaging and you will be provided with 3 fluorescent cubes (RGB), as well as LED illumination, to

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perform fluorescence imaging. The system comes with 12TB of storage as standard (sufficient for 3 months of support). There are no hidden or additional costs. Full training and support is included.