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Using live-cell imaging systems to transform perspectives in drug discovery

Live-cell imaging has transformed the study of cell behaviour and function. The technique allows researchers to monitor internal cell structures, interactions and processes in real-time, and over time. In drug discovery, understanding the dynamic processes of cells provides an enhanced picture of drug pharmacology, enabling more reliable predictions of clinical responses earlier in the process. In this article, we highlight some of the key commercial products helping to drive the adoption of this valuable approach and how it could help improve efficiencies and advance the development of medicines.

By Helen Stewart-Miller and Dr Kate Tointon

Traditional imaging experiments in drug discovery involve endpoint measurement approaches that typically use fixed cells requiring staining or artificial labelling (for example, using genetically-encoded fluorescent proteins). These artificial conditions mean that the cells do not sufficiently mimic the complex *in vivo* cellular responses to pharmacological or molecular perturbations that would be seen in the patient.

"To increase the chance that a drug's effects *in vitro* translate to the clinic, cell models must be as physiologically relevant as possible, comments Tim Dale, Head of BioAnalytics Applications, Sartorius Corporation. "We also need to implement new methods to gather information over time. As biology is incredibly dynamic and complex, single snapshots using endpoint measurements cannot provide the full picture."

Fortunately, a solution has emerged with the advent of novel time-lapse microscopy technologies. These enable powerful imaging analysis of live cells, fresh tissue or live *in vivo* models, performed in a time-resolved manner. Live-cell imaging enables the monitoring of internal cellular structures, processes and interactions to reliably determine the safety and efficacy of a drug, such as whether it selectively binds to the intended target or whether it has any off-target effects. These insights enable better predictions about which drugs will be successful in clinical development to reduce late-stage attrition.

A more complete picture of cellular processes

As well as offering a better understanding of cell behaviour and function, live-cell imaging offers drug discovery several other advantages over the traditional endpoint approach. This includes the ability to study dynamic cellular processes, behaviour and function from both spatial and tem-poral perspectives to get an enhanced picture of the pharmacological or molecular effect on the cell. Additionally, as live-cell imaging allows cellular structures to be studied in their native environ-ment, it is less prone to experimental artefacts. This means the technique typically provides more reliable and statistically-relevant information than fixed-cell microscopy.

Live-cell imaging can also reduce workload and streamline laboratory operations: "Live-cell imag-ing allows both controls and treated samples to be placed in the same plate, with the same sample fol-lowed over an extended period. This can dramati-cally reduce the experimental set-up time and com-plexity, as well as the number of samples required compared to endpoint experiments," comments Joffry Maltha, CEO, CytoSMART Technologies BV. "On top of that, live-cell imaging typically doesn't require the calibration or normalisation that endpoint assays need, which can save a lot of pre-experiment preparation time."

Live-cell imaging is already facilitating a wide range of drug discovery applications, including early target discovery and validation, information-al screens, secondary cell-based assays and ADME-Tox (Absorption, Distribution, Metabolism, Excretion and Toxicity) studies for prioritising pri-mary hits. Prominent examples where live-cell assays have been particularly valuable include the assessment of fast signalling responses, and exper-iments where multiple cell types interact to affect the disease model, such as in complex cellular models such as organoids and immune cell killing assays, which require both spatial and temporal insight.

Overcoming the challenges of live-cell imaging

To harness the power of live-cell imaging and the many benefits it offers drug discovery research, it is vital to overcome the challenges that commonly arise throughout the workflow, from image acqui-sition to analysis. One major issue is to sufficiently mimic the *in vivo* environment and avoid disrupt-ing the cells throughout the entire duration of the experiment, which can last days, weeks or even months.

"Controlling environmental parameters, such as temperature, humidity and CO_2 is critical for reducing the variability between observations and guaranteeing the relevance of the results in live-cell imaging assays," notes Karin Boettcher, Associate Product Manager for High-Content Screening and Applications, PerkinElmer. "The use of an onstage incubator can help manage the environmental con-ditions to keep the cells alive and functioning nor-mally for extended periods."

As cells often move during a time-lapse exper-iment, it is beneficial for live-cell imaging sys-tems to be capable of keeping the cells of interest within the image. Furthermore, because there tends to be inhomogeneous growth and response of biological wells in culture plates, modern live-cell imaging systems must be able to take a full scan of the entire plate to produce statistically-relevant data.

There are further issues associated with data storage and processing power. Time-lapse imaging at high resolution and high magnification gener-ates large numbers of images, particularly when data is collected over extended time periods, such as for long-term cultures like 3D spheroids. This means that modern live-cell imaging systems need enough data storage for potentially hundreds of thousands of large images, as well as a high levels of processing power for image stitching and run-ning image analysis software.

"It's also crucial to minimise photobleaching and phototoxicity caused by the fluorescent illumi-nation in live-cell imaging, especially in the UV range, says Chris Shumate, CEO, Etaluma. "Repetitive exposure to illumination and excita-tion sources can bleach the fluorescent labels and create reactive oxygen species that cause cell dam-age. Often, this effect isn't recognised until an extended time lapse has been performed."

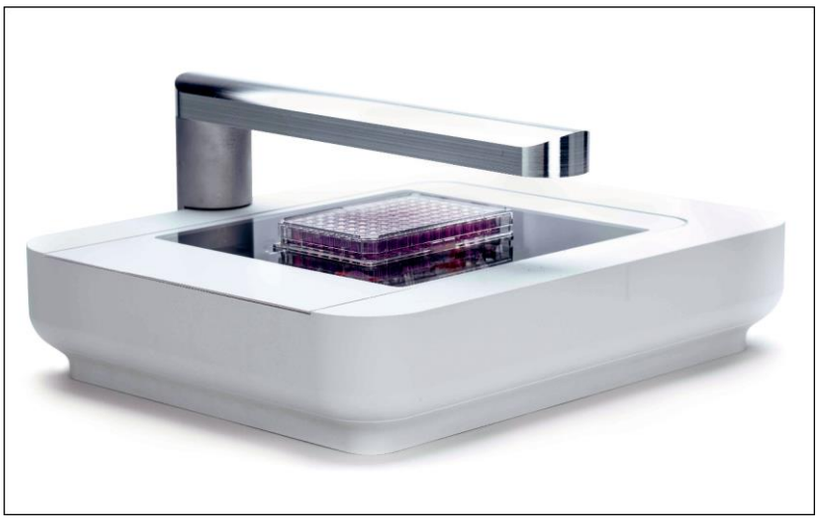
Commercially-available live-cell imaging products

Many commercially-available products overcome the challenges associated with live-cell imaging. Here we provide a summary of some of the key products available on the market:

CytoSMART™ – imaging with cloud-based storage and analysis

The CytoSMART™ Lux2 is a cell-culture monitor that operates at low-voltage and can be safely used in any regular CO_2 incubator. The system can run for extended periods (days or weeks) and will image cells in a wide range of cell-culture vessels, including slides, petri dishes, T-flasks and

Figure 1: The CytoSMART Lux2 live-cell monitor (above) and CytoSMART Omni automated live-cell imager (below)



microplates. The monitor can be used across a wide range of applications, including cell migra-tion, wound healing and scratch assays analysis, stem cell behaviour studies and chemotaxis stud-ies.

The CytoSMART™ Omni is an automated live-cell imager small enough to fit in any regular cell-culture incubator. The system is capable of imag-ing a complete T-flask or well plate by taking 6,500 high magnification digital phase contrast images at predefined time intervals. The system comes with a CytoSMART™ Connect Cloud, which is a suite of image analysis software for high volume confluency analysis, colony detection and scratch assays.

The CytoSMART™ Connect Cloud runs on Microsoft Azure, which provides cost-efficient, large storage capacity and high levels of processing power for analysing and storing images. The plat-form is accessible from any computer, tablet or mobile phone to enable remote, real-time monitor-ing. It can also be set up to send automatic notifi-cations to users once the cell-culture has reached the confluency threshold (**Figure 1**).

Etaluma Lumascopes – real microscopy simplified

Etaluma's technology centres on a compact and simple optics path allowing lower power fluores-cence excitation for the avoidance of photo dam-age. The solid state filter design eliminates pixel shift between channels and real phase contrast pro-vides high quality transmitted light images. An open design allows fluidics and micro injectors to be integrated. Magnifications from 2.5x to 100x are available for research quality images.

All four Lumascope models can be placed in environmental chambers, including common cell-culture incubators, as well as hypoxia chambers and refrigerators. Additionally, tiling and z-stack-ing capabilities help to keep the cells of interest within the image during time-lapse experiments (**Figure 2**).

PerkinElmer – application flexibility for all throughputs

The new PerkinElmer MuviCyte™ live-cell imag-ing system is designed to operate inside an incuba-tor to enable long-term observations under con-trolled conditions. The system can scan a broad range of vessel types and is compatible with label-free transmitted light readout as well as three-channel (red, green, blue) fluorescent imaging to enable a wide range of commonly-used live-cell assays (**Figure 3**).

The PerkinElmer Opera Phenix™ and Operetta

Figure 2
Etaluma offers manual as well as automated microscopes. Pictured are the LS720 (above) and LS560 (right) microscopes

CLS™ high-content screening systems can run a wide-variety of assays in a live-cell format. When used with automation, both systems enable high throughput live-cell assays and can be configured with the integrated environmental control chamber and spinning disk optical technology to minimise photobleaching and phototoxicity of samples. Additionally, these systems enable gentle live-cell analysis (ie, without using a fluorescent probe) by using the digital-phase contrast imaging mode and brightfield imaging function (**Figure 4**).

Sartorius Corporation – accessible automated image acquisition and analysis

The Sartorius IncuCyte® is a live-cell analysis system that sits inside standard tissue culture incuba-tors and automatically analyses cells for extended periods. The cells stay stationary during image acquisition and are not shuttled between instru-ment and incubator, minimising disruption. The system has also been designed to make it accessible for even first-time users by automating as many of the decision points as possible. The images are organised alongside experimental details and real-time data analysis is provided by a guided user interface tailored for different experimental paradigms (**Figure 5**).

Picturing the future of live-cell imaging

The role of live-cell imaging is likely to grow across drug discovery and development. Enhanced data capture and analysis features will improve the accessibility of the technique, allowing researchers to keep pace with the ever-growing need for more physiologically-relevant and complex models of disease. This will likely lead to the generation of better statistically-relevant and reproducible results compared to traditional endpoint measurements.

Moreover, as a quicker and easier alternative to endpoint experiments, kinetic (time-based) data such as that generated from live-cell imaging is pre-dicted to become increasingly vital in drug discov-ery. This type of data enables a better understand-ing of cell behaviour as a result of different stress factors, meaning that a wider variation of cell types can be studied, including minor and less frequent effects as well as long-term effects.

Time-lapse microscopy such as that used in live-cell imaging could also help improve the efficiency and speed in which hit compounds can be identi-fied, by applying the technique to large compound screens in primary high-throughput screening.

As technologies such as CRISPR, CAR-T and other immuno-oncology techniques continue to expand the biology that can be measured in cells, it

Figure 4: The Perkin Elmer Opera Phenix™ (above) and Operetta CLS™ (below) high content screening systems

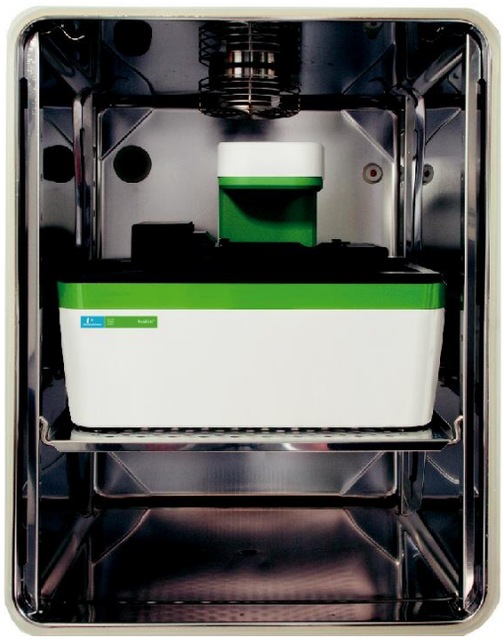


Figure 3: The PerkinElmer MuviCyte™ live-cell imaging system inside an incubator

is predicted that the scope of applications for live-cell screening will keep growing. As 3D biology and mixed cultures yield more physiologically-rel-evant measurements, live-cell imaging of these cul-tures will complement the replication of the *in vivo* environment.

Figure 5
The Sartorius IncuCyte® S3 live-cell analysis system

Conclusion

Live-cell imaging provides greater insights into cellular behaviour and function to enhance drug discovery. Modern technologies offer numerous benefits, including improved cost-efficiency and streamlined laboratory operations, as well as the genera-tion of more reliable and relevant data that could potentially expedite the successful progression of drugs through discovery. As live-cell imaging becomes progressively important in the develop-ment of new medicines, it is vital that technologies continue to adapt to the needs of researchers to fully unlock the benefits of this ground-breaking technique. **DDW**

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Unleashing subject matter expertise to drive results for your business

By Dr Paul Avery and Dr Clare Russell,
Managing Directors at BioStrata

For many life science companies, positioning themselves as thought lead-ers in their market will be a key tenet of their marketing and communi-cations strategy – after all, people buy from brands they know, trust and respect.

As scientific audiences tend to be sceptical of sales pitches and instead value 'peer-to-peer' level interactions, one of the most effective ways for life science companies to build trust and boost engagement is by unleashing the bank of knowledge amassed by their in-house subject matter experts. By converting this insight into valuable content and sharing it at a scale to reach your target audi-ence, you will be able to attract prospects, generate new leads and ultimately nurture them into customers.

However, translating subject matter expertise into engaging content that res-onates with prospects can be challenging and requires substantial time, energy and internal resources. Additionally, companies may not have the requisite infrastructure, such as prospect email lists, to promote customer-facing con-tent, or the relevant trade media contacts needed to secure interviews and con-tributed editorial articles.

Specialist marketing communications agencies can help businesses make the best use of their in-house knowledge by working with their subject matter experts to extract relevant insights and distil these into engaging content. In this way, effective life science marketing materials such as whitepapers, eBooks and blog posts can deliver real insight to your target audience via your website, while articles can be pitched to the media for publication. Combined, this con-tent can form an impactful part of a successful marketing and communications plan.

If you would like to learn more, our comprehensive guide to content market-ing has everything you need to create a content marketing plan, including how best to harness subject matter expertise. Download the guide here: http://bit.ly/GuideToContentMarketing.

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