

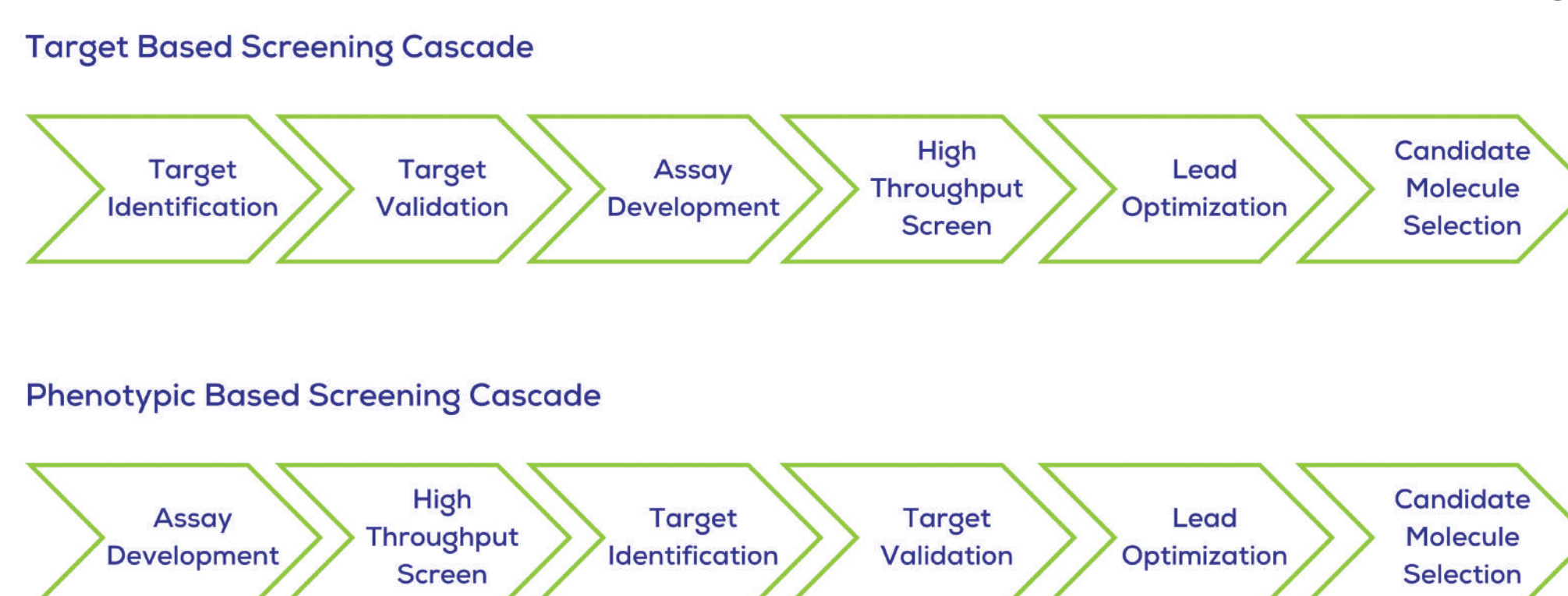
Phenotypic Assays – Diving into the Cellular Response to Expand the “Druggable” Target Space

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Introduction

Phenotypic screening assays provide a strategic approach to drug discovery to identify lead compounds based upon their ability to induce an effect - or phenotypic change - in a disease relevant cell scenario. Taking a target-agnostic approach is the underlying attribute that differentiates phenotypic drug discovery from hypothesis-driven target-based drug discovery. Over the past decade there has been more acceptance in directing efforts to modulate a cellular process rather than a specific molecular target.

This change has led to an expansion of the “druggable” target space and the concept of polypharmacology. Phenotypic changes can be monitored beyond a single cellular response and assays can often be multi-parametric to give a more informative profile of the compounds tested. The use of live-cell imaging in phenotypic approaches, rather than an end-point assay, also expands the opportunities to have a deeper understanding of the effect of test compounds. Ultimately screening compounds in a more physiologically relevant system helps to mitigate the risk of downstream failure as candidate compounds transition to the clinical phase of drug discovery.



Live-cell Imaging Assays

Phenotypic assays, facilitated by live-cell imaging can add value in any drug discovery cascade by developing a deeper understanding of cellular responses to therapeutic treatment (small molecules, biologics etc.) that may otherwise be overlooked in more traditional end-point assays. In phenotypic screening, a wide array of cellular functions can be studied, that can reveal insight into the growth and morphology changes of cells over several days.

We utilize the **Sartorius Incucyte® SX5**, for our live-cell imaging needs together with specialized tools, assay plates and analysis algorithms.

Scratch Wound Migration and Cell Health

Here, we use a scratch wound model to investigate phenotypic responses to PROTAC treatment. Migration of MCF7 cells is impaired due to the degradation of BRD4. Cytochalasin D, an actin polymerisation inhibitor, was used as a positive control to compare to the BRD4 PROTAC dBET6 and the BRD4 inhibitor JQ1(+).

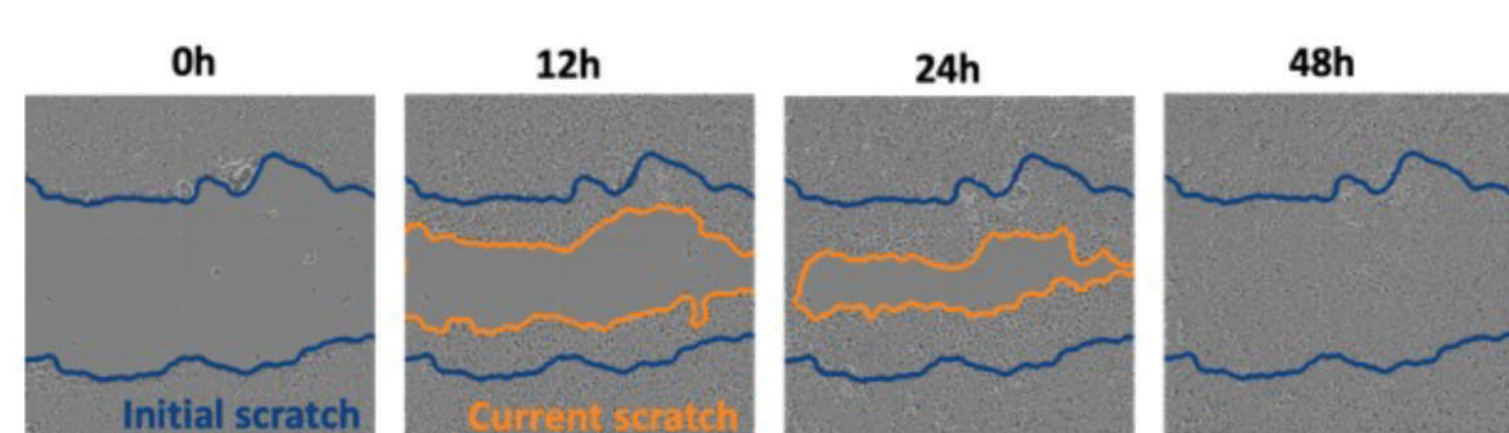


Figure 1: Scratch wound assay. Image analysis was handled by trainable algorithms that identified the initial scratch wound and tracked wound closure over the course of the experiment to calculate a “relative wound density”.

Chemotaxis through a porous membrane in a 96-well plate format gives a detailed view of the migration process and its regulation. It also helps separate the effects of compound treatment on chemotaxis from cytotoxicity artifacts.

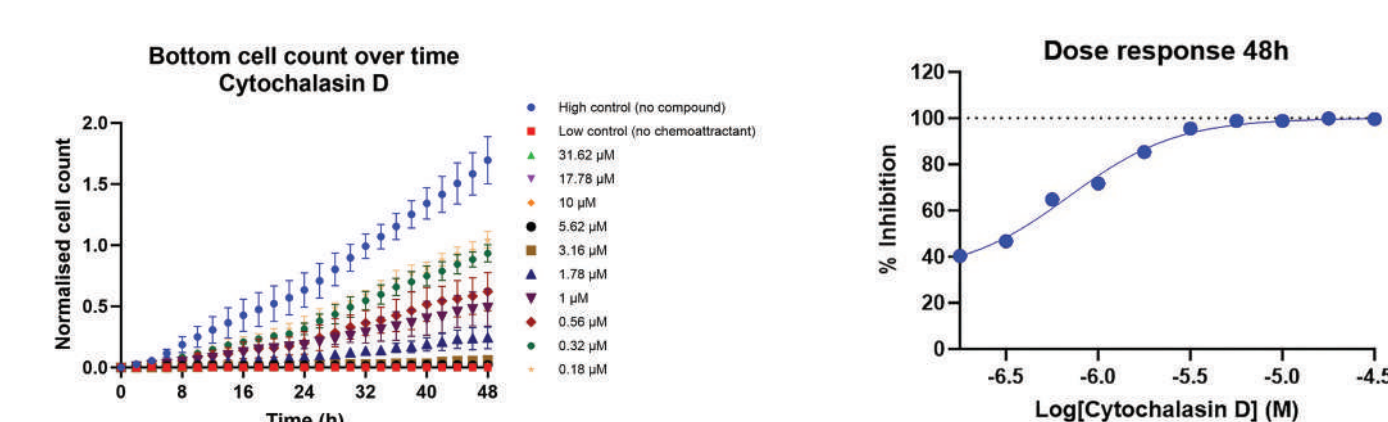


Figure 2: Cytochalasin D inhibits chemotactic migration of HT-1080 cells in a concentration-dependent manner. Left – normalized cell count on the bottom of the membrane over time. Right – inhibition of chemotaxis by cytochalasin D after 48 hours.

Tracking Neuronal Differentiation

The ability to investigate neuronal differentiation and development is key to developing in vitro models of neurological disease and degeneration. At Charnwood Discovery, we have monitored the differentiation process in real time using the Incucyte SX5, tracking metrics such as neurite length, branch points and cell body area.

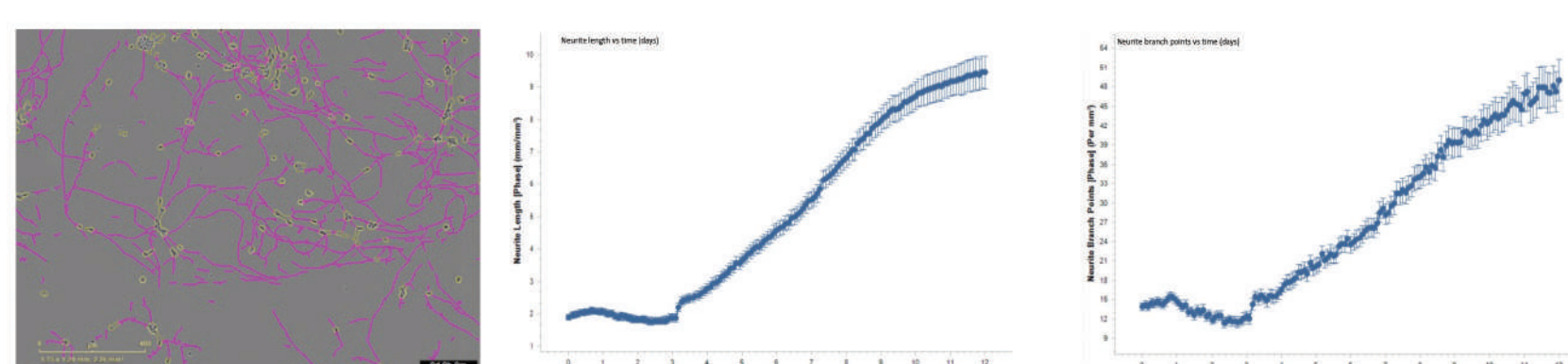


Figure 3: Images and associated graphs of neurons captured on the Incucyte SX5, displayed with overlaid analysis mask – neurites in pink and cell bodies in yellow.

High Content Imaging

3D cellular models have increased in significance as a tool of drug discovery with the introduction of systems to allow the culture of 3D spheroids e.g. ultra-low attachment (ULA) plates, hanging drop and organ-on-a chip. At Charnwood Discovery, we have utilized several 3D spheroid assay approaches including simple culture in ULA plates with a CellTiter Glo® 3D readout to a multi-stained high content image analysis which can be applied to a specific cellular phenotype.

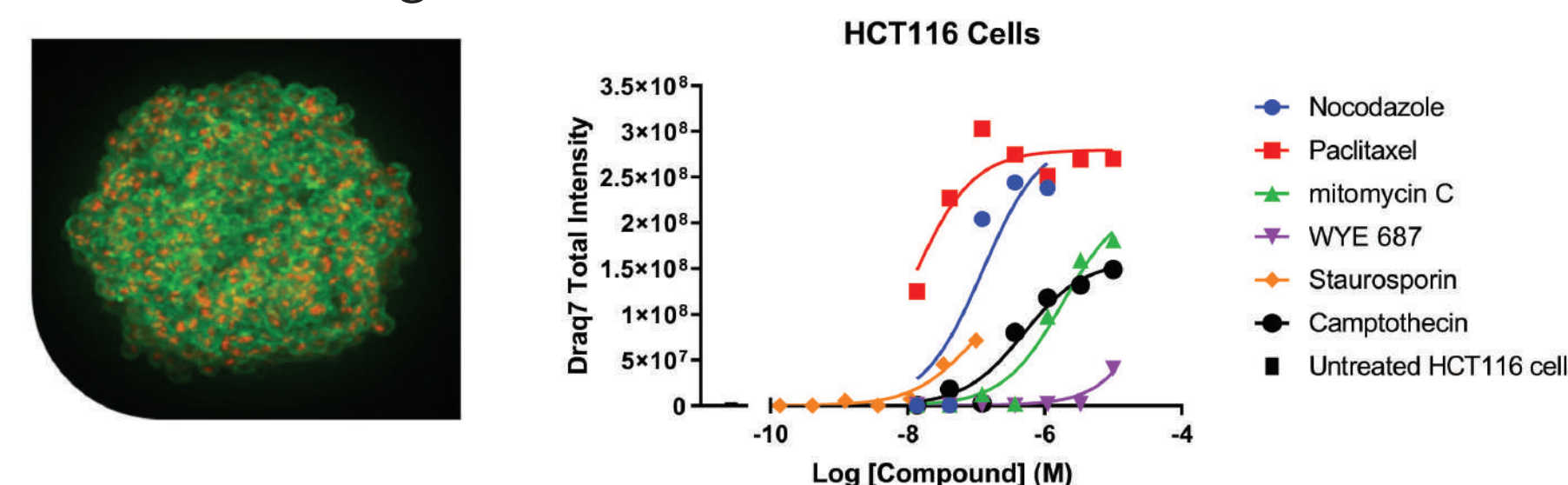


Figure 4: High content imaging of HT1080 cells expressing a nuclear RFP stain grown within the CelVivo for 72-hours and co-stained with Phalloidin 488 (left-hand image). DRAQ7TM intensity plotted against compound concentration using HCT116 cells (right-hand image).

DNA damage is one of the central mechanisms of cancer, indeed most common cancer treatments are designed to induce cell death by direct or indirect DNA damage. There are multiple technologies that can be applied to study DNA damage and the DNA damage repair pathways. One such assay measures the formation of γH2AX foci in cell nuclei. These foci are formed in response to DNA damaging agents, in a dose-dependent manner. This phenomenon enables the quantification of DNA damage, facilitating the screening of genotoxic compounds.

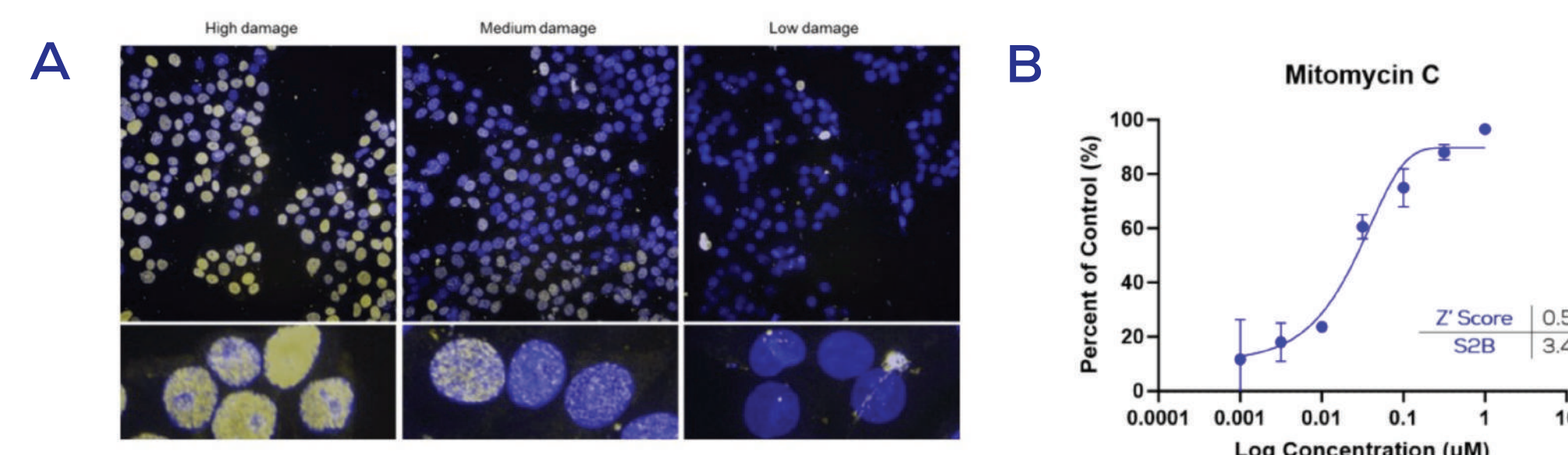


Figure 5: HT115 cells were treated with a serial dilution of mitomycin C. γH2AX foci were detected with a labelled secondary antibody. A) Images were captured using a ImageXpress® Confocal HT.ai B) Graph showing the dose-dependent effect of Mitomycin C on the number of γH2AX foci.

Autophagy is a central process that functions to remove aggregated proteins and damaged organelles from the cell via the autophagosome targeting them for elimination. High content imaging assays can be applied to study the effects of compounds which may be beneficial in studying cellular ageing.

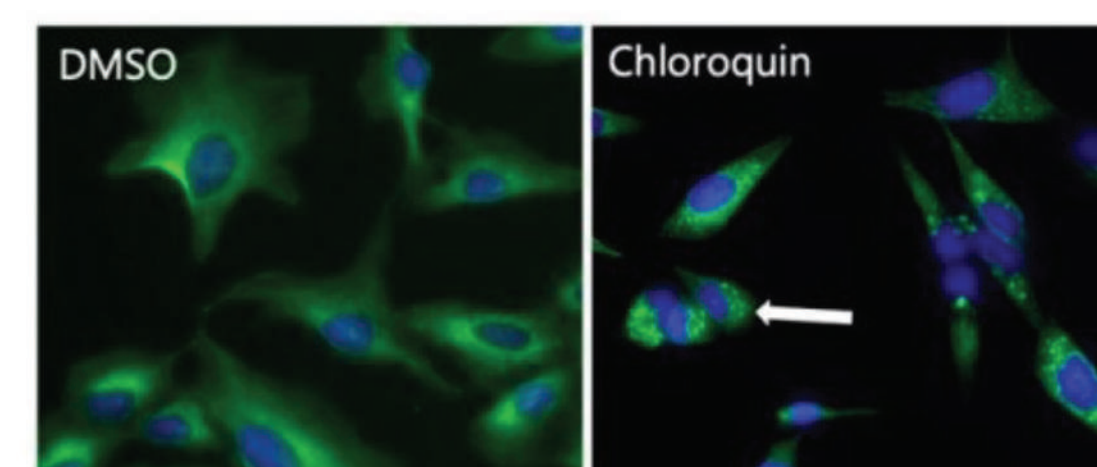


Figure 6: LC3B antibody staining (Alexa 647) in HEK-293 cells imaged on Cellinsight CX5 (Hoescht stained nuclei in blue). Cells were treated (left to right) with DMSO showing diffuse cytoplasmic LC3B staining, chloroquine (25 µM) (right), in which the LC3B forms perinuclear punctate staining of autolysosomes in the cytoplasm.

Summary and Conclusion

Phenotypic screening assays provide a strategic approach to drug discovery, identifying lead compounds based on their ability to induce an effect, or phenotypic change, in a disease relevant cell scenario. Charnwood Discovery's experienced bioscientists are skilled at working with a wide range of cell lines from immortalized cell lines and iPSC cell-based models to patient derived stem cells.

This ensures the generation of information-rich data within an appropriate, disease-applicable cellular background. In addition, we can work within a 2D, and 3D format allowing for a more biological relevant scenario. From simple monitoring of cell confluency to investigating differentiation of neuronal iPSC cells, live-cell imaging unlocks more in-depth analysis of a wide array of cellular processes.

We can advise and guide you through this process to achieve candidate drug selection.

