Development of Microscopy Tools to Inform Drug Discovery

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Introduction

- Microscopy is a key tool in drug discovery, enabling visualisation of disease phenotypes and therapeutic intervention
- It can be utilised at all stages of the drug discovery process to provide novel biological insights
- High content imaging is capable of visualising large numbers of samples at high resolution, becoming a vital approach in industry for evaluating new medicines and disease biology
- Here, we present a series of case studies from work at Medicines Discovery Catapult showcasing the impact of high-content imaging on the Opera Phenix spinning disk confocal

1. High Content Imaging of Complex Cell Models

- Cell models with improved translational relevance are expected to improve the success rate of delivery of new medicines to patients
- Exploitation of patient derived iPSC stem cells assembled into multicellular and 3D structures may yield drug screening approaches more representative of patient biology than traditional 2D approaches
- High content imaging can be used to screen and evaluate the effect of new novel drugs in more physiological 3D models

Live antibody binding assay established in CNS iPSC-derived tricultures

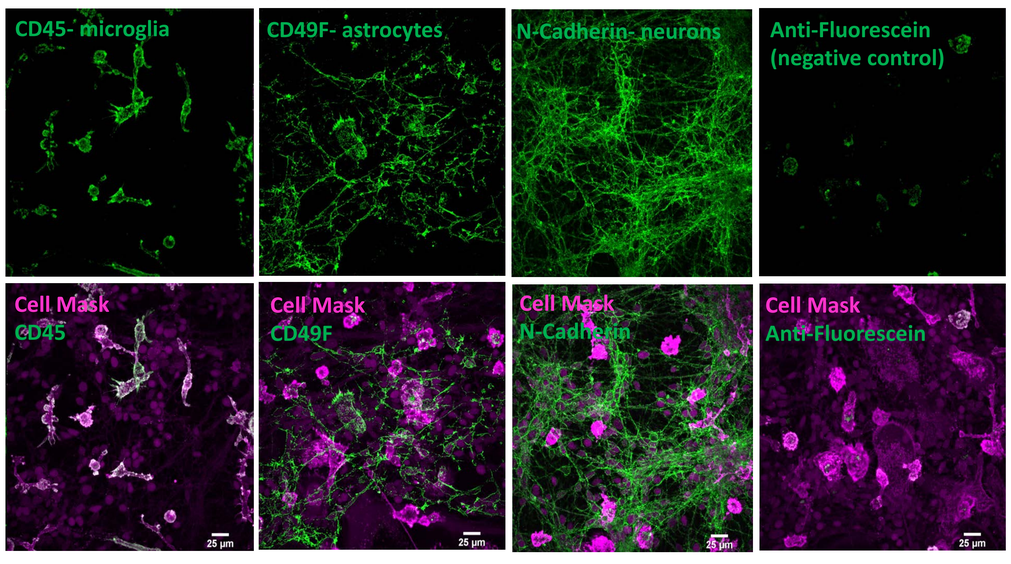


Figure 1. CNS tricultures were imaged using an Opera Phenix at 40x magnification. Shown are control antibodies binding the extracellular portion of membrane proteins expressed in each of the 3 cell types (microglia, astrocytes and neurons). Anti-fluorescein antibody labelling is shown as a negative control. Harmony analysis software was used to build up imaging analysis pipelines, assessing the fluorescence intensity of the antibodies.

3D Liver Organ on a Chip Model

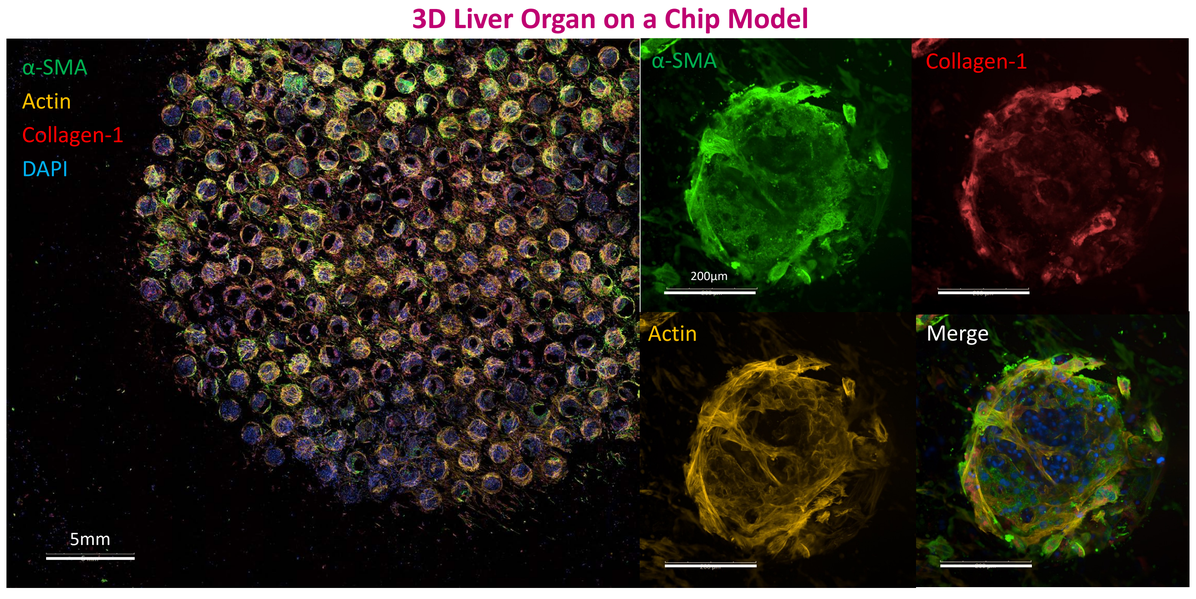


Figure 2. Primary hepatocytes, hepatic stellate cells and Kupffer cells were added to scaffolds within a PhysioMimix® platform, stimulated to induce fibrosis and analysed at 14 days post induction. Scaffolds were stained with markers for fibrosis and imaged on an Opera Phenix at 10x (left) and 20x (right). Intensity of each channel could be calculated and normalised to DAPI. Inhibitors could be screened in this system.

2. Morphological Profiling using Cell Painting

- Cell painting is a high-content image-based technique used to generate complex, information-rich, and interdependent measurements of cellular activity *in vitro*
- Cell painting is a powerful tool to analyse morphological profiles of cells and when applied to drug discovery, can provide invaluable information on new drugs
- Cell painting can also be applied in complex cell systems and primary cells to enable translation of findings in drug and target evaluation studies

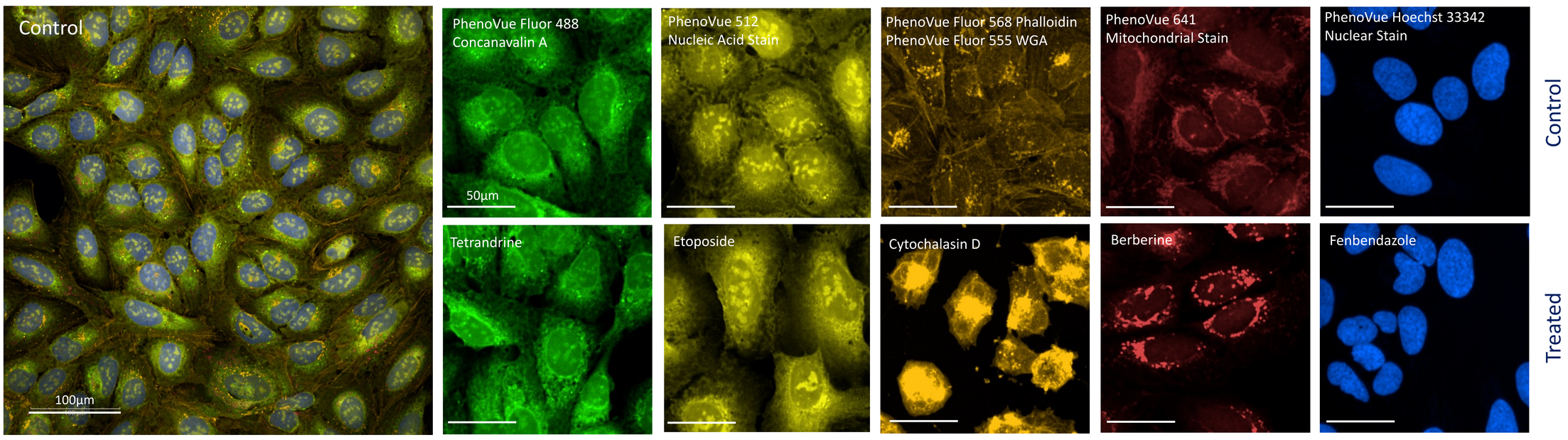
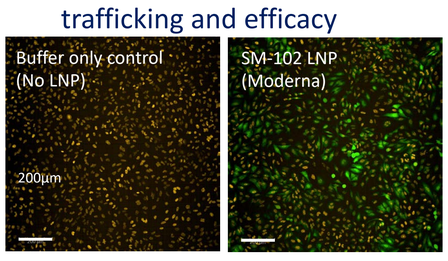
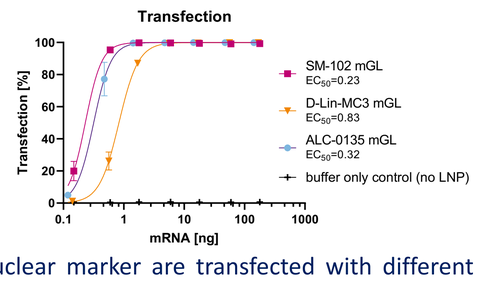


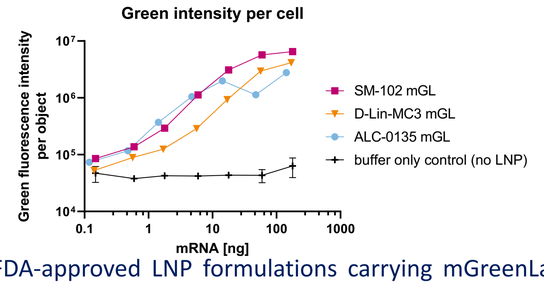
Figure 3. U2OS cells were seeded into a 384 well plate and treated with positive controls (see image annotations) known to result in diverse phenotypes. Here, cells were seeded, left to recover for 24 hours and subsequently stained with the PhenoVue-JUMP cell painting kit. Plates were imaged on the Opera Phenix high content system at 40x. Morphological changes can be detected after drug treatment. More than 1000 cell features can be analysed using Harmony 'Cell Painting' building block and further downstream analysis with Cell profiler software. In addition, MDC are building informatic tools to evaluate and automate analysis.

3. Screening of Complex Medicines

- MDC is a partner in the Intracellular Drug Delivery Centre (IDDC), a CPI led programme with the University of Strathclyde, University of Liverpool and Imperial College London providing a UK centre of excellence to support the development of nano-delivery systems
- High content imaging is employed for screening and characterisation of LNPs and other complex medicines to understand intracellular delivery, trafficking and efficacy







Read out	Negative control – CV (%)	Positive control – CV (%)	Z'
Cell count	3.4	3.7	N/A
Transfection	29	0.05	0.99
Green intensity / cell	16.3	6.3	0.58

Figure 4. HeLa cells transduced with a nuclear marker are transfected with different FDA-approved LNP formulations carrying mGreenLantern mRNA, identified by their ionisable lipid SM-102, D-Lin-MC3 and ALC-0135. The cells are screened on the Opera Phenix, with the transfection efficiency (%) and fluorescence intensity per cell calculated for each formulation. Evaluation of cell count is also performed to monitor potential toxicity. Assay development statistics demonstrate the robustness of the screening assay.

Conclusion

- High content imaging is transforming drug discovery with increased speed, resolution and throughput
- At MDC we use high content imaging to address a range of drug discovery questions

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