Expanding Live-cell Imaging Capability at Charnwood Discovery – Investigating 3D Tumour Spheroids and Immune Cell Models with the IncuCyte® SX5

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Introduction

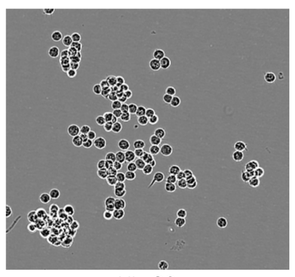
Cell-based assays are an essential component of many drug discovery programs and the ability to monitor how cells react to novel compounds in real time is invaluable.

At Charnwood Discovery we use the IncuCyte® SX5 for all our real-time live-cell imaging needs. This instrument allows us to investigate cell behaviour in a kinetic manner for extended time periods, from simple confluency monitoring to experiments including multiple cell dyes and labels. This capability is essential when developing cell-based assays and when screening compounds to fully understand how cells behave and add value to client projects.

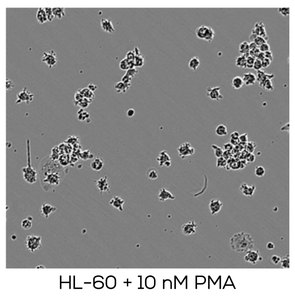
Often the first step in our assay optimization workflow, live-cell imaging with the SX5 can be used for highly accurate confluency monitoring to inform optimal assay conditions such as cell seeding density and timepoint selection. Advanced label-free cell counting using the Cell-by-Cell analysis algorithm takes quantification to the next level. These functions when combined with up to three colour fluorescence microscopy makes the IncuCyte® a powerful cell biology tool. Recently we have worked to expand our 3D cell culture and immune cell model capabilities and live-cell imaging was the ideal tool to capture these experiments.

Monocyte Differentiation and Phagocytosis

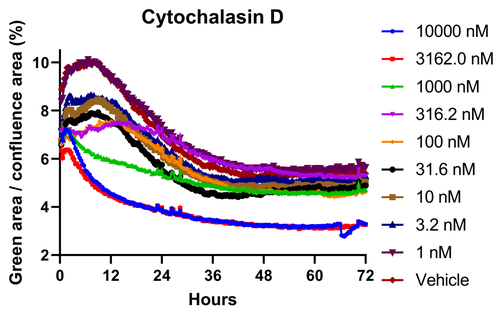
To monitor phagocytosis using the IncuCyte® SX5 we used HL-60 cells which can be differentiated to monocytes using PMA. This differentiation inhibits proliferation and induces a change in cell morphology which were also monitored using live-cell imaging. Flow cytometry confirmed that differentiated cells were CD11b positive. PMA-treated HL-60 cells were then incubated with IncuCyte® pHrodo® green *E. coli* Bioparticles® which fluoresce in an acidic environment, i.e. the phagosome and phagolysosome. Fluorescent signal was measured using the SX5 and normalized to cell confluency. The actin polymerization inhibitor cytochalasin D was employed as a control to prevent phagocytosis of the bioparticles.

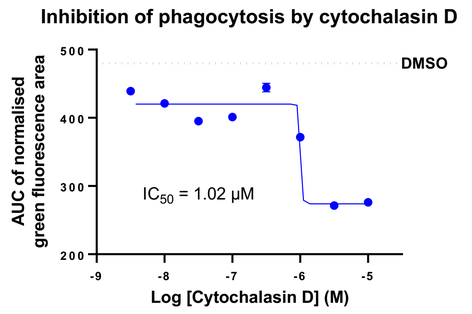


HL-60



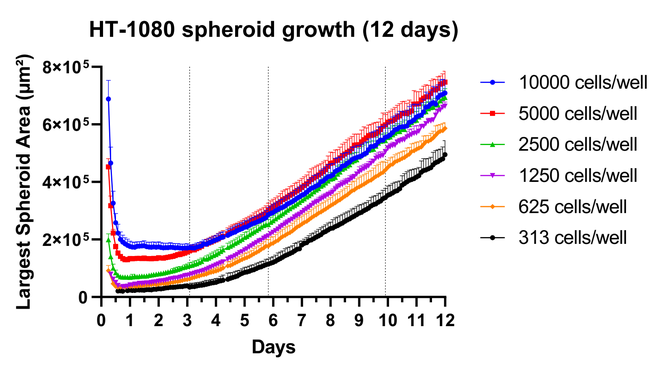
HL-60 + 10 nM PMA

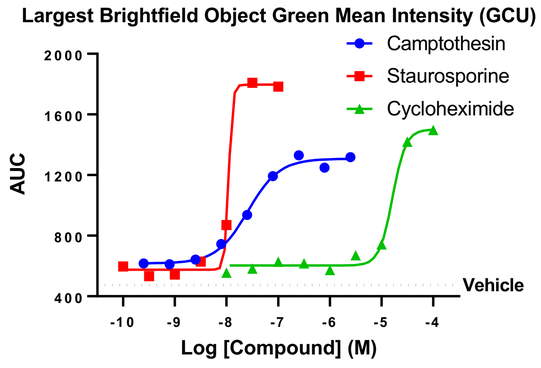


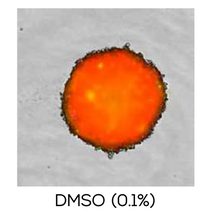


Tumour Spheroid Culture and Health Screening

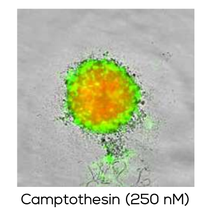
3D cell culture is an expanding field and is gaining interest as a more physiological model for screening assays. The SX5 is set up for 3D cell culture monitoring with a dedicated set of spheroid analysis tools. To test this functionality we cultured Incucyte® Nuclight red HT-1080 cells in 96-well ultra-low attachment plates at a range of densities over an extended period to inform the optimal seeding density. Once selected as $5x10^3$ cells/well, spheroids were incubated with three cytostatic/toxic compounds and the IncuCyte® Cytotox Dye (green) to monitor cell permeability. Spheroids were monitored over 6 days and area under curve (AUC) analysis used to plot dose-response curves. As expected, the two cytotoxic compounds (staurosporine and camptothesin) induced an increase in green fluorescent signal at lower concentrations that the cytostatic compound cycloheximide.



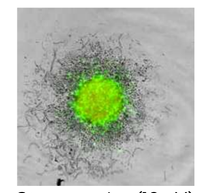




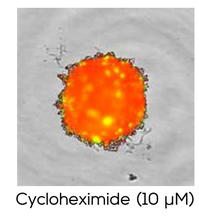
DMSO (0.1%)



Camptothesin (250 nM)



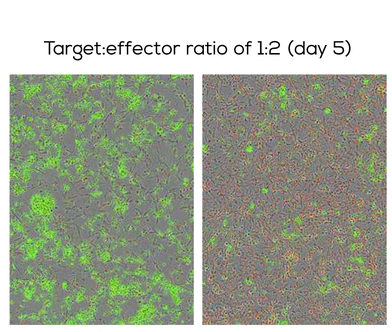
Staurosporine (10 nM)

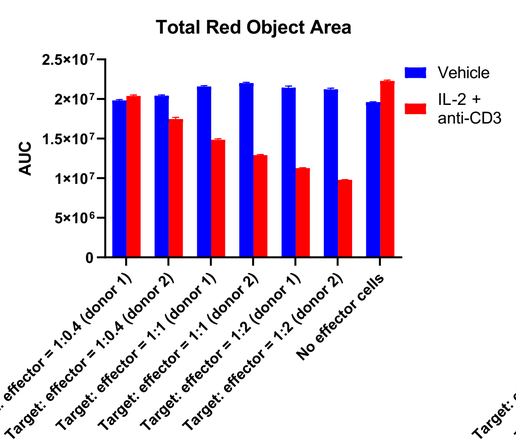


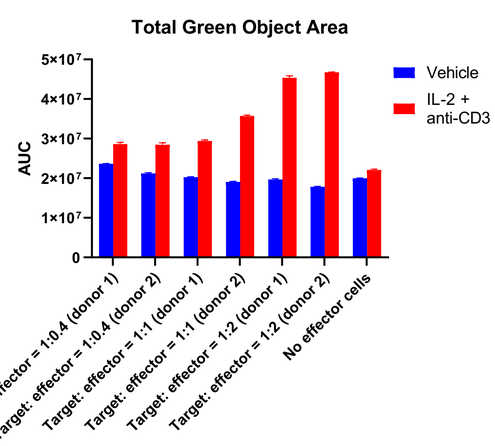
Cycloheximide (10 µM)

Immune Cell Killing of 2D and 3D Cultures

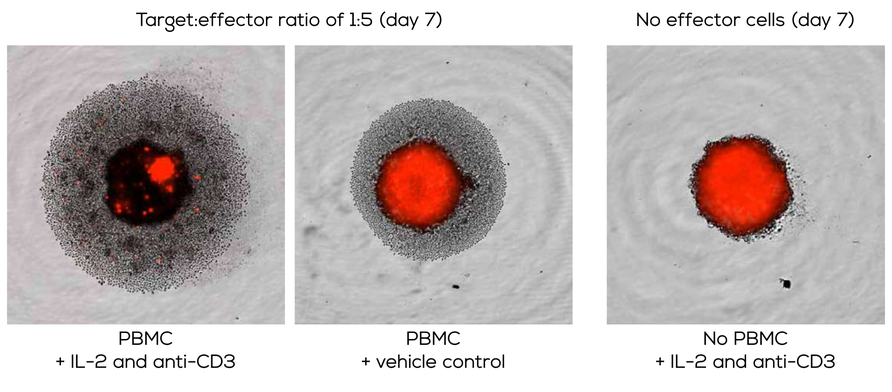
In vitro assays investigating immune cell mediated killing of cancer cells aim to replicate the complex interactions between the immune system and their targets. To capture this using live-cell imaging peripheral blood mononuclear cells (PBMCs) were isolated and activated using IL-2 and anti-CD3 then incubated with a monolayer of Nuclight red HT-1080 cells at different target:effector cell ratios. IncuCyte® Cytotox green dye was included to monitor cell permeability as a measure of cell death. Using red or green fluorescent signal area as a readout it is evident that incubating HT-1080 cells with activated PBMCs results in cancer cell killing, with more cell death occurring when a greater ratio of PBMCs to target cells are included. IL-2 and anti-CD3 did not impact HT-1080 cell health in the absence of PBMCs.

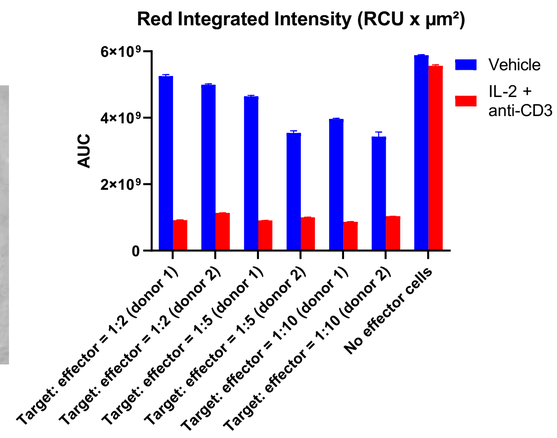






Similar results were obtained using IncuCyte® Nuclight red HT-1080 spheroid cultures. Spheroids were formed for 48-hours prior to addition of PBMCs then spheroid health monitored over 7 days. Those incubated with activated PBMCs display reduced red integrated intensity over this period compared to those incubated without IL-2 and anti-CD3.





Summary and Conclusion

Our work here illustrates the insights that live-cell imaging can bring to cell-based assays and outlines a few of the extensive range of applications this technique offers. Kinetic measurements produce additional data, that is not captured with end-point assays, which can provide detail into cell behaviour that would otherwise be missed. In this work we demonstrate several of our live-cell imaging capabilities here at Charnwood Discovery:

- **3D cell culture and viability monitoring** – spheroids present a more physiologically relevant model for cell-based assays and can be manipulated and interrogated in a similar manner to traditional 2D cell culture.
- **Investigating complex interactions between immune cells and their targets** – live-cell imaging provides the tools to capture and analyze these interactions across different immune cell models.
- **Immune cell activation and co-culture** – techniques that can be applied to a range of assays and monitored using the IncuCyte® SX5.

IncuCyte® SX5

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