Multiplexed, live content cellular imaging enabled:
Cell Player™ reagents, assays and IncuCyte Zoom™

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TOOLS, REAGENTS, ASSAYS

- Measurement
- Puromycin
- Lentivirus
- HeLa
- 0.025 Puromycin
- Caspase 3/7 positive nuclei
- Lentivirus
- Bleomycin
- 96, 384w
- Lentivirus
- EF-1
- Cell Type
- EF-1
- CMV
- Doubling Time (h)

VALIDATION OF TARGETTED GFP/RFP LENTIVIRAL REAGENTS & CELL LINES

- A549 cells
- HUVECs
- NucLight Red

PHASE/2-COLOUR ASSAY APPLICATIONS

- A: Co-Culture Kinetic Proliferation
- B: Duplex Cell Proliferation & Apoptosis/Cytotoxicity
- C: Cell proliferation and apoptosis: NucLight

SUMMARY

- We define Live Content Imaging as the acquisition, analysis and quantification of images (phase and fluorescence) from living cells that remain unperturbed by the detection method, allowing for repeated measures over long periods of time (days to weeks).
- We differentiate Live Content from High Content Imaging which typically measures assay endpoints using fixed cells, or employs conditions (e.g. Ab labelling) under which cells are viable for only short periods of time (minutes to hours). Live Content Imaging offers clear advantages for measuring long term biological processes, providing full temporal resolution of the events of interest from viable healthy cells. The images and time-lapse movies are information rich and yield valuable confirmation of the experimental outcomes.
- Here we describe a novel suite of tools – reader technology, cellular reagents, assay protocols, software modules – that together enable true live content imaging assays. The building blocks of these assays are (1) the IncuCyte Zoom live cell imaging device (2) novel, highly validated targeted GFP/RFP lentiviral and stable cell lines (3) sophisticated algorithms for fluorescent object analysis and for quantifying phase structures. Using these tools we have configured microtitre plate-based kinetic assays for apoptosis, cell proliferation, cytotoxicity, angiogenesis, cell migration, cell invasion and neurite outgrowth. Simultaneous phase contrast and single colour fluorescent assays as well as multiplex two colour (red/green) and phase reads are exemplified in both homogeneous cell systems (e.g. 1 cell type) as well as co-culture (2 cell type) models.
- The introduction of targeted GFP and RFP cellular reagents suitable for long term live cell imaging along with the 2 colour IncuCyte zoom system, provide a powerful integrated solution for fully kinetic, multiplexed live cell assays. We foresee particular utility in co- and multi-cell culture systems such as in studies on the tumour microenvironment.

Nuclear / Cyttoplasmic GFP / RFP stable cell lines

- Created by transduction of the host cell with the targeted lentiviruses (above).
- Typically >95% of cells express the fluorescent protein.
- Characterized as comparable to host cell lines (morphology, growth rates, migration rates).

Scalable cell lines expressing targeted GFP & RFP

<table>
<thead>
<tr>
<th>Stable cell line expressing targeted GFP &amp; RFP</th>
<th>Time (h)</th>
<th>Measurement Format</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td></td>
<td>Number of nuclei (count)</td>
</tr>
<tr>
<td>HUVECs</td>
<td></td>
<td>Caspase 3/7 positive nuclei</td>
</tr>
<tr>
<td>Neuro-2a</td>
<td></td>
<td>Dual kinetic assay (polarization, proliferation, apoptosis)</td>
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</tbody>
</table>

96- & 384-WELL KINETIC PROLIFERATION ASSAYS BASED ON CELL COUNT

- A: NucLight Red
- B: NucLight Green
- C: NucLight Red

2-COLOUR ADVANCED BIOLOGY MODELS

- Angiogenesis: Endothelial and stromal cell co-cultures
- NeuroTrack™ Kinetic Neurite Outgrowth
- 2-Colour Advanced Biology Models

The data contained in this poster represents work designed and conducted by the entire Essen BioScience R&D team.

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