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

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TOOLS, REAGENTS, ASSAYS

- IncuCyte Zoom Live Cell Imaging Device resides within a standard cell culture incubator.
- Cell culture consumables (e.g. micro-titre plates, T-flasks, petri dishes) are placed within for *in situ* assays.
- Gathers time lapse images from cells – high definition **phase contrast, green and red fluorescence.**
- User Interchangeable objectives – 4x, 10x, 20x.
- Up to 6 x 384-well plates simultaneously.
- Simple to use but highly advanced phase and fluorescent image processing software tools.

cellplayer™ REAGENTS

Targeted GFP & RFP lentiviruses

- 3rd generation HIV-based, VSV-G pseudotyped lentiviral particles encoding cytolasmic (CytoLight) or nuclear restricted (NucLight) GFP or RFP, or a combination of the two (DuoLight).
- Expression driven off either an EF-1α or CMV promotor with antibiotic resistance cassette for stable cell line generation.
- Validated as non-perturbing to cell health across a range of MOIs.

Lentiviral targeted GFP & RPFs

Catalog	Name	Type	Localization	Promoter	Selection
4475	NucLight Green	Lentivirus	Nucleus	EF-1α	Puromycin
4476	NucLight Red	Lentivirus	Nucleus	EF-1α	Puromycin
4477	NucLight Green	Lentivirus	Nucleus	EF-1α	Bleomycin
4478	NucLight Red	Lentivirus	Nucleus	EF-1α	Bleomycin
4481	CytoLight Green	Lentivirus	Cytoplasm	EF-1α	Puromycin
4482	CytoLight Red	Lentivirus	Cytoplasm	EF-1α	Puromycin
4483	CytoLight Green	Lentivirus	Cytoplasm	EF-1α	Bleomycin
4484	CytoLight Red	Lentivirus	Cytoplasm	EF-1α	Bleomycin
4413	CytoLight Green	Lentivirus	Cytoplasm	CMV	None
TBD	DuoLight (Red/Green)	Lentivirus	Nuc + Cyto	CMV	Puromycin

Nuclear / Cytoplasmic 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. Validated as comparable to host cell lines (morphology, growth rates, migration rates).

Stable cell lines expressing targeted GFP & RPFs

Catalog No.	Cell Type	Fluorescent Marker	Selection
4485	HT-1080	NucLight Red	Puromycin
4486	HT-1080	NucLight Green	Puromycin
4487	MDA-MB-231	NucLight Red	Puromycin
4488	MDA-MB-231	NucLight Green	Puromycin
4489	HeLa	NucLight Red	Puromycin
4490	HeLa	NucLight Green	Puromycin
4491	A549	NucLight Red	Puromycin
4492	A549	NucLight Green	Puromycin
4506	HUVEC (Primary cells)	NucLight Green	None
4511	Neuro-2a	NucLight Green	Puromycin
4512	Neuro-2a	NucLight Red	Puromycin
TBD	HUVEC-DuoLight	NucLight Red/CytoLight Green	None

cellplayer™ ASSAYS

Cell Player Assays

Assay Type	Measurement	Markers	Cell types	Format
Proliferation	Number of nuclei (count)	Nuclear-restricted GFP/RFP: NucLight Red/NucLight Green	>20, including immortalised and primary cells	96, 384w
Apoptosis	Caspase 3/7 positive nuclei (count)	Bifunctional DEVD/DNA binding fluorescent substrate	>20, including immortalised and primary cells	96, 384w
Cytotoxicity	YoYo1 positive nuclei (count)	YoYo-1 cell intact cell impermeant DNA	>20, including immortalised and primary cells	96, 384w
Migration	Scratch wound migration	Wound confluence (Phase)	>20, including immortalised and primary cells	96w
Invasion	Scratch wound through substrate (e.g. Matrigel, Collagen-1)	Wound confluence (Phase)	>20, including immortalised and primary cells	96w
Angiogenesis	Vascular Tube Formation	GFP-labelled HUVECs/ECFCs	HUVECs & human dermal fibroblasts (Prime Kit), Adipocyte Derived Stem Cells &	96w
Neurotrack	Neurite Outgrowth	Phase (& Fluoresence)	Rat hippocampal neurones, neuronal IPSCs, neuroblastoma (Neuro-2a)	96w

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 end points 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 micro-titre plate-based kinetic assays for apoptosis, cell proliferation, cytotoxicity, angiogenesis, cell migration, cell invasion and neurite outgrowth. Simultaneous phase contrast and single colour fluorescence assays as well as multiplexed 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 types) 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-culture cell systems such as in studies on the tumour microenvironment.

VALIDATION OF TARGETTED GFP/RFP LENTIVIRAL REAGENTS & CELL LINES

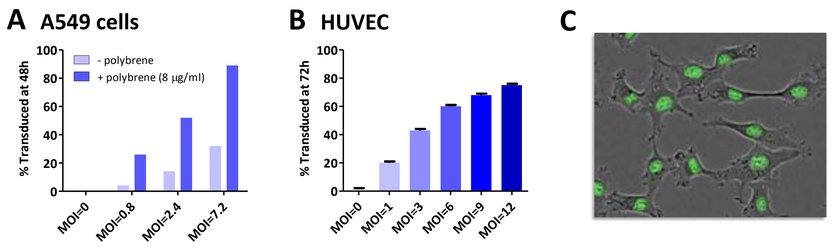


Figure 1. Lentiviral infection of immortalised and primary cells. Transduction efficiency of NucLight Green at different multiplicities of infection (MOI) ± polybrene in A549 (**A**) and HUVEC (**B**) cells following 24-48h transduction. Image of A549 cells expressing the NucLight Green (**C**). Note the homogeneous nuclear restricted GFP label and healthy appearance of the cells.

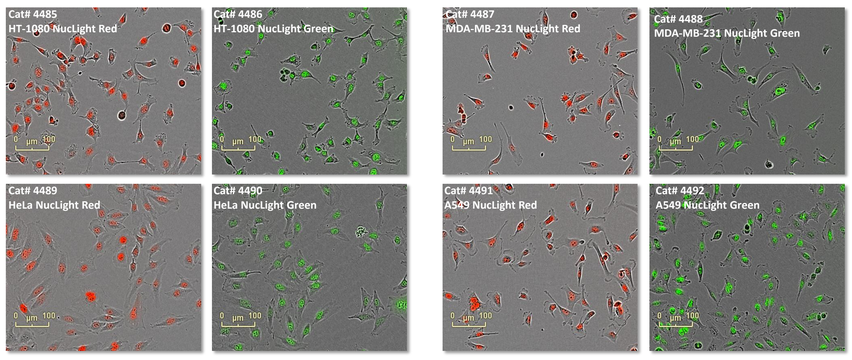
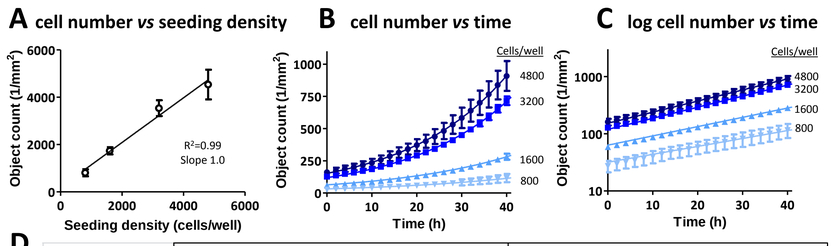


Figure 2. Panel of NucLight Green and NucLight Red stable cell lines in different host cell backgrounds. Note (1) the discrete nuclear localisation of the fluorescent protein (2) the homogeneous expression of almost all cells in the field of view and (3) the healthy appearance of the cells. In cell proliferation & migration experiments no differences were observed between the properties of the parental and transfected cells (data not shown).



D

	HT1080				Hela			
Seeding density (Cells mm^{-2})	Y_0	K	Doubling Time (h)	R^2	Y_0	K	Doubling Time (h)	R^2
4.8	152	**0.045**	**15.5**	1.00	294	**0.021**	**33.6**	0.98
3.2	119	**0.045**	**15.5**	1.00	222	**0.023**	**30.8**	0.99
1.6	63	**0.037**	**18.6**	1.00	93	**0.025**	**27.2**	1.00
0.8	32	**0.032**	**21.7**	1.00	42	**0.024**	**28.5**	1.00

Figure 3. **(A)** Correlation between cell seeding density and nuclear count (HT1080 NucLight-Green stable cell line). **(B)** Time-course of cell proliferation at different initial cell densities. **(C)** Log_{10} of the nuclear count time-course, illustrating exponential cell growth. **(D)** Kinetic data were fitted to $y = y_0 . e^{kt}$ to yield comparative growth rate constants (K values) and doubling times.

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

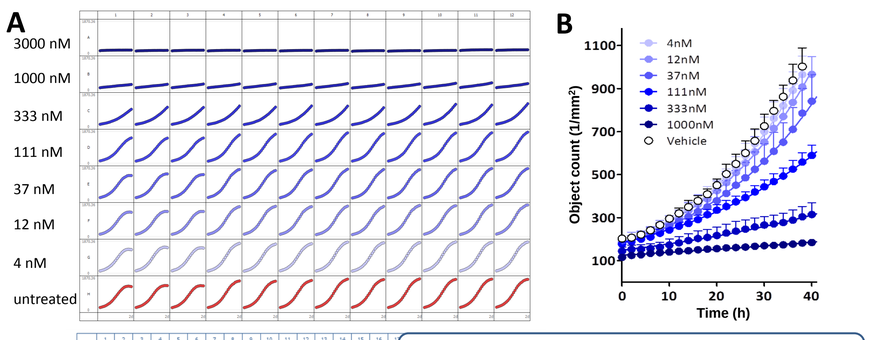


Figure 4: (**A**) 96-well plate view of kinetic cell proliferation assay in HT1080-NucLight Green cells in the presence of different concentrations of cyclohexamide (CHA). (**B**) Overlaid timecourses. (**C**) 384-well assay plate view and IC_{50} curves.

PHASE/2-COLOUR ASSAY APPLICATIONS

Co-Culture Kinetic Proliferation

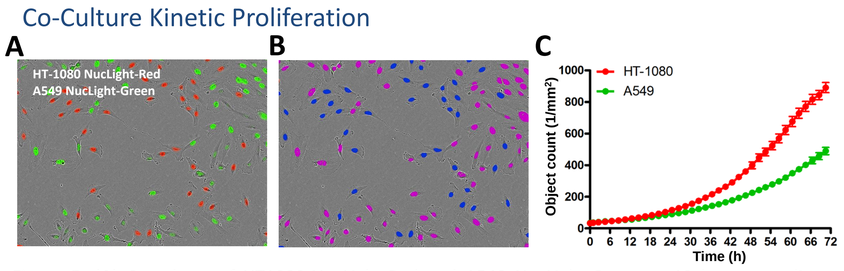


Figure 5: (**A**) Co-culture of HT1080-NucLight-Red and A549 NucLight-Green at 12 h post-seeding. (**B**) IncuCyte software image mask independently identifying red and green nuclei. (**C**) Time-course of cell count.

Duplex Cell Proliferation & Apoptosis/Cytotoxicity

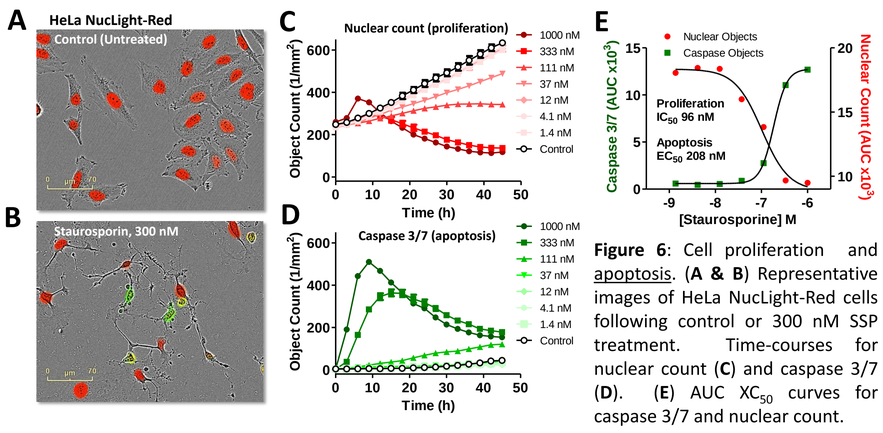


Figure 6: Cell proliferation and apoptosis. (**A & B**) Representative images of HeLa NucLight-Red cells following control or 300 nM SSP treatment. Time-courses for nuclear count (**C**) and caspase 3/7 (**D**). (**E**) AUC XC_{50} curves for caspase 3/7 and nuclear count.

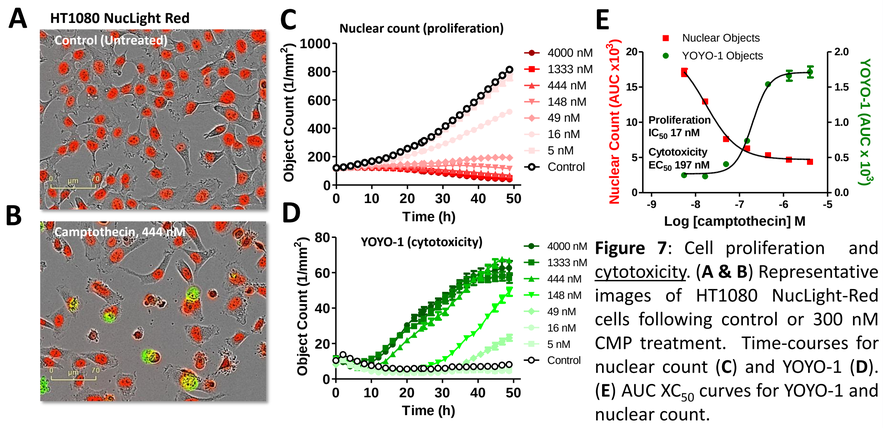


Figure 7: Cell proliferation and cytotoxicity. (**A & B**) Representative images of HT1080 NucLight-Red cells following control or 300 nM CMP treatment. Time-courses for nuclear count (**C**) and YOYO-1 (**D**). (**E**) AUC XC_{50} curves for YOYO-1 and nuclear count.

NeuroTrack™: Kinetic Neurite Outgrowth

Figure 8: (**A**) Representative image of Neuro-2A cells. (**B**) Neuro-2A cells with the quantification mask applied, identifying neurite outgrowth. (**C**) Time-course of neurite outgrowth and attenuation by the PKC inhibitor Ro-31-8220.

2-COLOUR ADVANCED BIOLOGY MODELS

Angiogenesis: Endothelial and stromal cell co-cultures

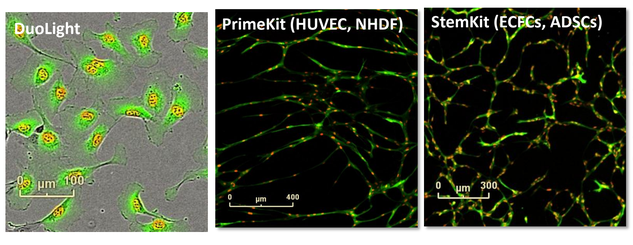


Figure 9: Use of the DuoLight construct for combined nuclear (Red) and cytoplasmic (Green) labelling. This approach allows the use of quantitative algorithms to measure the vascular tube network and HUVEC cell number simultaneously.

Cell invasion: HT1080 and MCF-7 cells

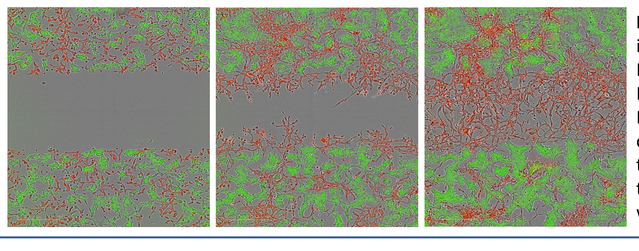


Figure 10: Co-culture cell invasion assay in matrigel. Representative images of HT1080 CytoLight-Red and MCF-7 CytoLight-Green cells. Note the invasion of the HT1080 cells, but not the MCF-7 cells into the wounded area.

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

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