

IncuCyte® S3 – Live-Cell Imaging and Analysis around-the-clock

Real-time, live-cell analysis...

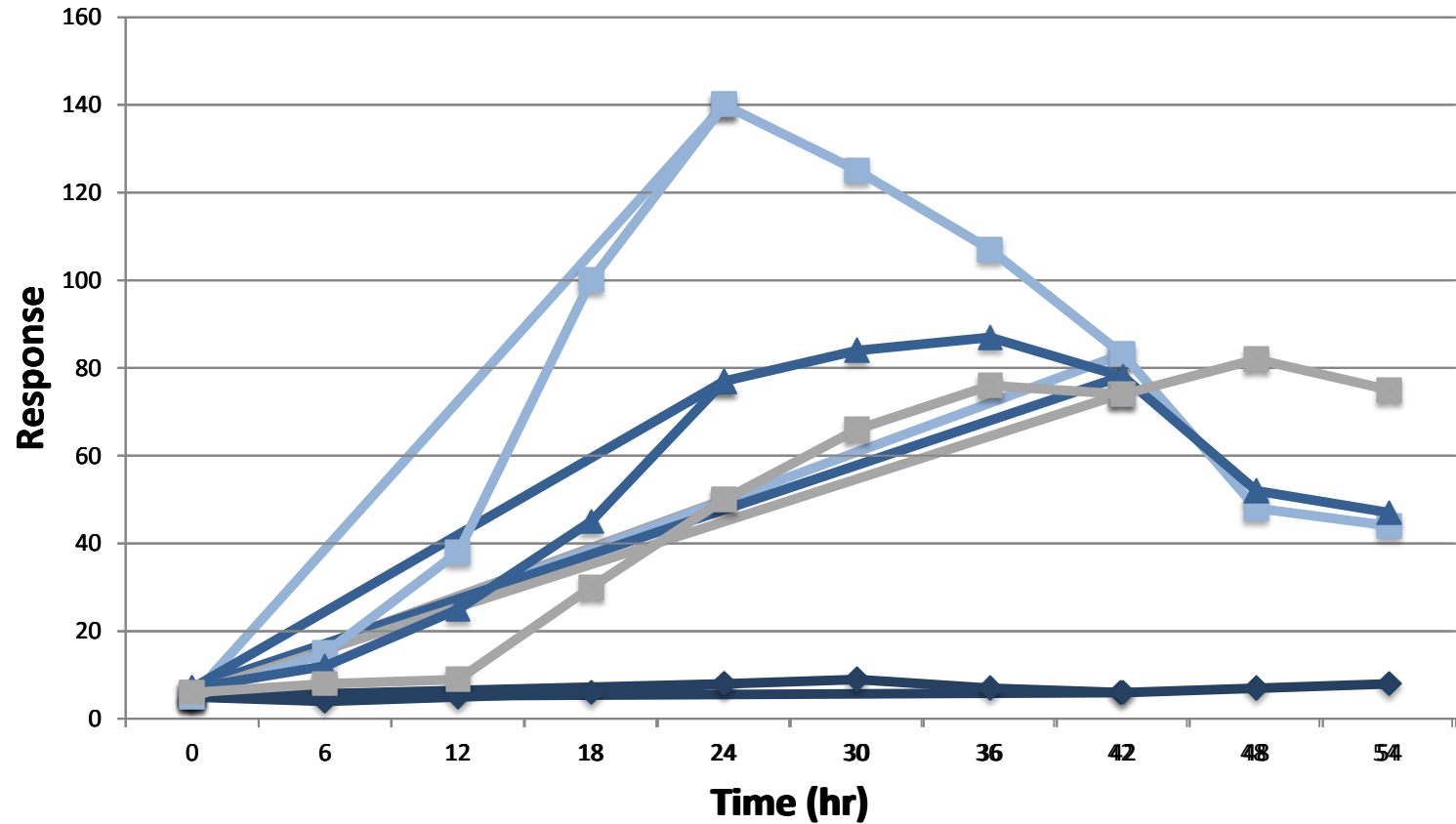
IncuCyte S3

IncuCyte®
by ESSEN BIOSCIENCE

<https://youtu.be/ugbPMrFrYWE>



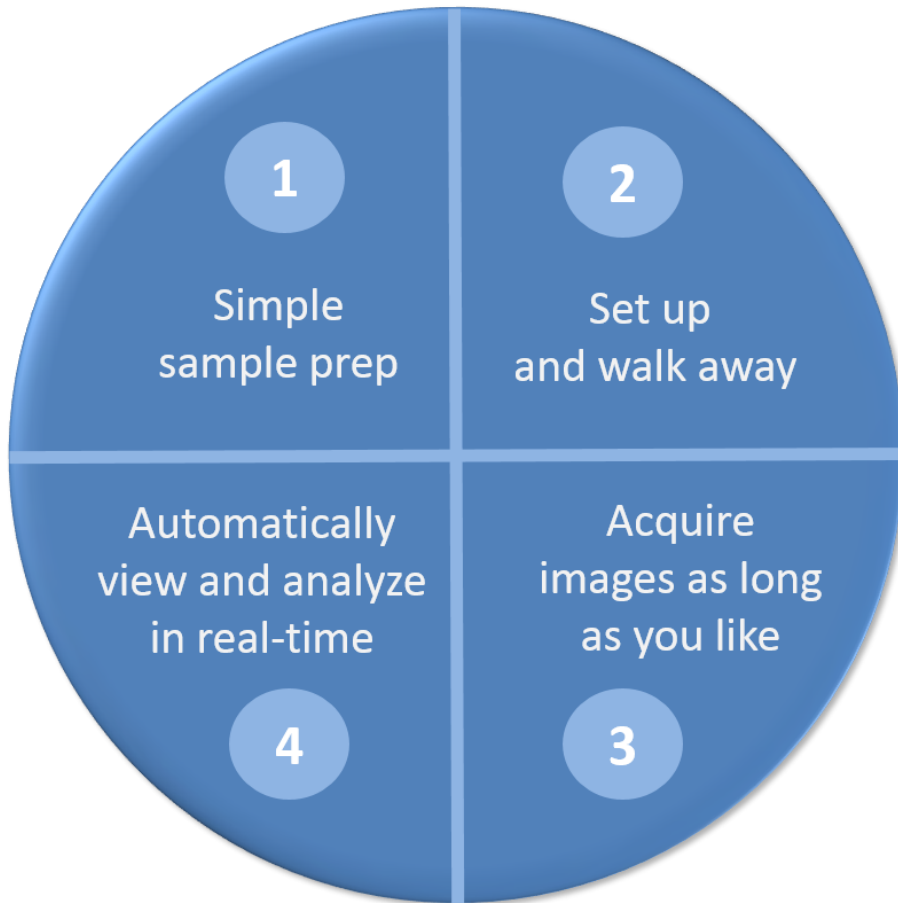
Biology happens in real-time, but what about your analysis?



See what your cells are doing and when!

How IncuCyte Transforms Research

Supports your entire research team day and night

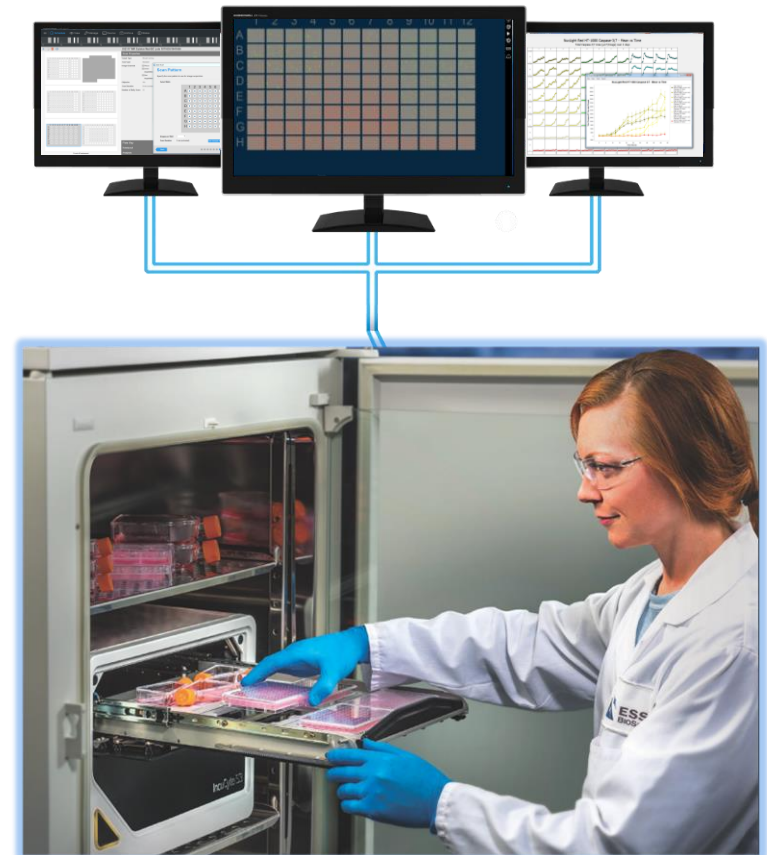


- ✓ Flexible assay platform
- ✓ Information-rich analysis
- ✓ Simple, powerful workflow
- ✓ Designed for multiple users and applications

How IncuCyte Transforms Research

Support for your entire research team

- ✓ Accommodate multiple users and applications in parallel
- ✓ Networked access and unlimited free licenses
- ✓ Fits in standard tissue culture incubators



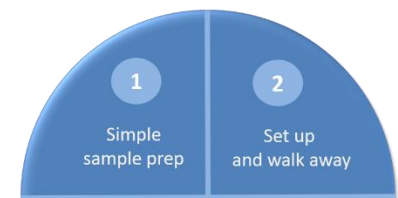
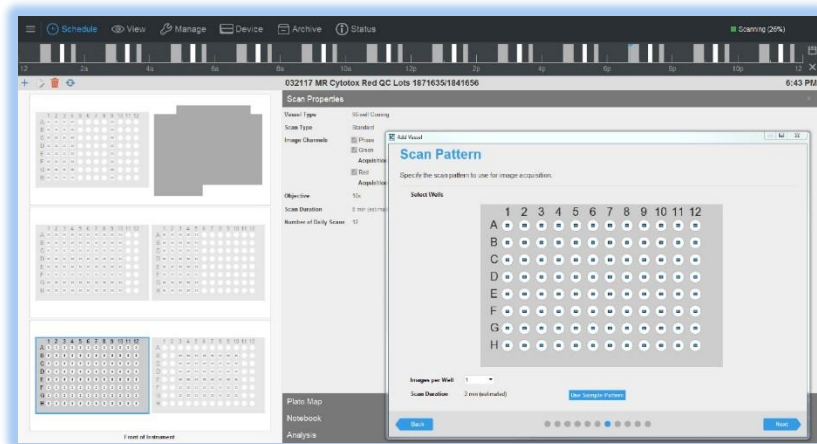
How IncuCyte Transforms Research

Simple, flexible sample prep then set up and walk away

http://www.essenbioscience.com/media/uploads/files/ncuCyte_S3_Vessel_Support_April_2017A_Release.xlsx

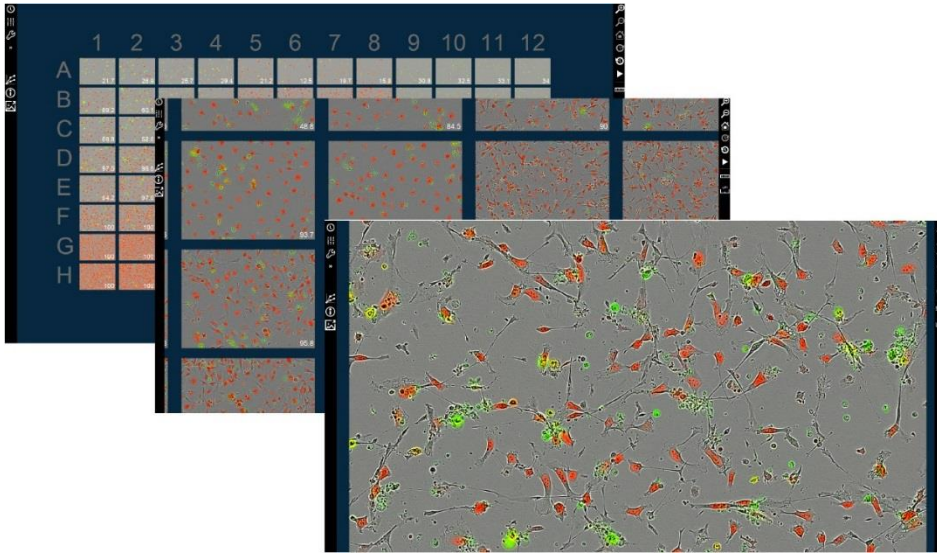


- ✓ Compatible with a wide range of culture vessels and applications
- ✓ Pair with IncuCyte reagents to maximize efficiency and preserve cell health
- ✓ Quickly set up experiments with easy-to-use guided interface

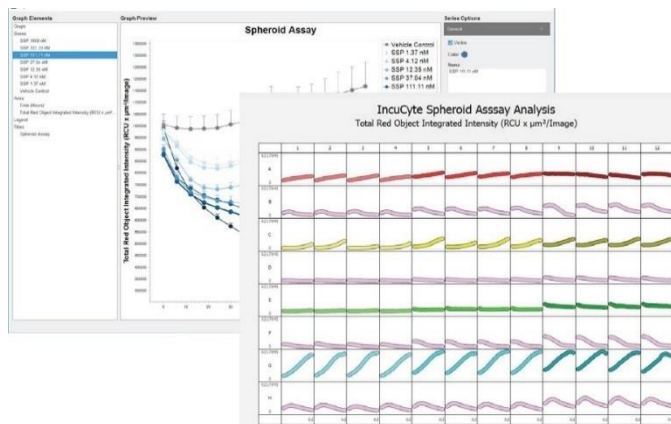


How IncuCyte Transforms Research

Automatically acquire images over days or weeks while viewing and analyzing in real-time



- ✓ Automatically acquire images in a stable, physiologically-relevant environment
- ✓ View all locations in a vessel at once to quickly identify trends and outliers
- ✓ Generate statistically relevant data using automated analysis
- ✓ Generate presentation-ready graphs



Published Worldwide

<http://www.essenbioscience.com/en/products/testimonials/>

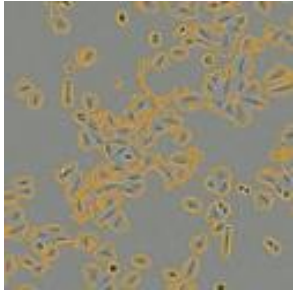


Over 1000 peer reviewed publications and growing!

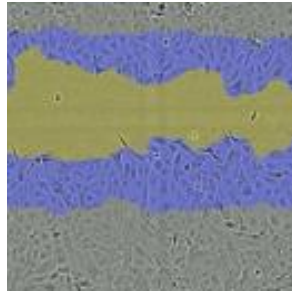
Explore the IncuCyte™ publications library - an up-to-date and fully searchable resource!

<http://www.essenbioscience.com/en/resources/publications/>

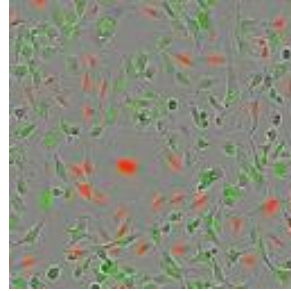
IncuCyte® Key Applications



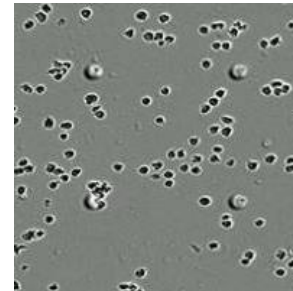
Confluence, QC & Cell Health



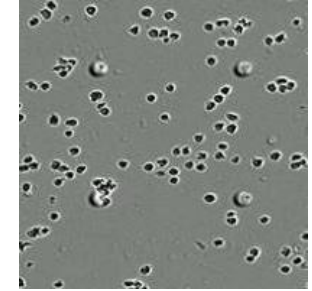
Cell Migration & Invasion



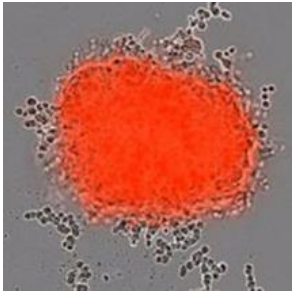
Proliferation & Cell Counting



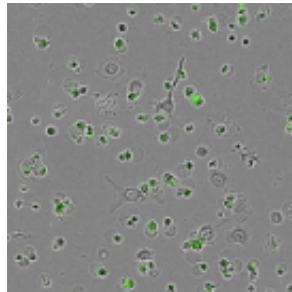
Chemotaxis Migration & Invasion Adherent



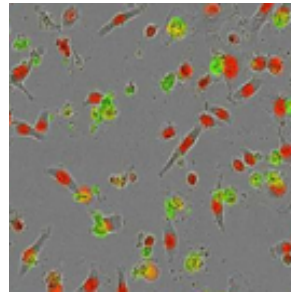
Chemotaxis Migration Suspension



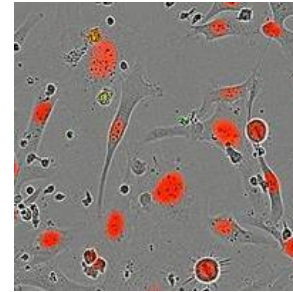
Spheroids



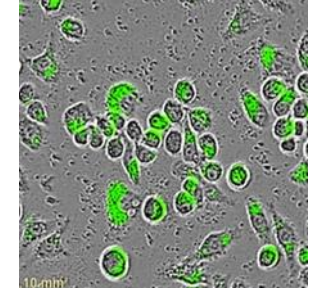
Apoptosis



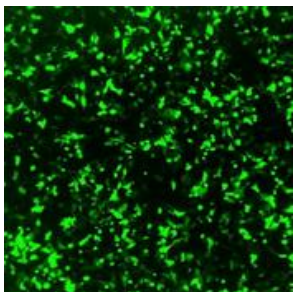
Cytotoxicity



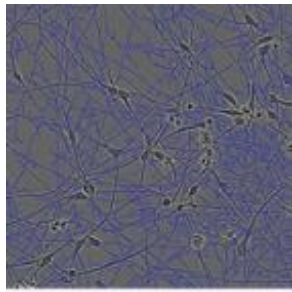
T-cell killing



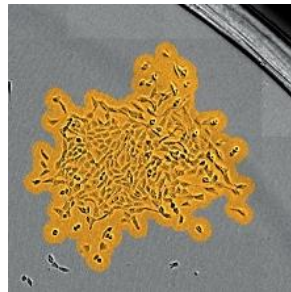
Phagocytosis



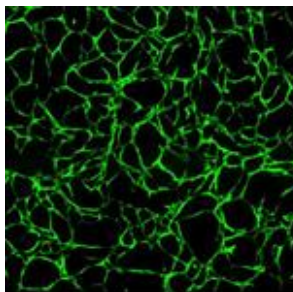
Gene Expression



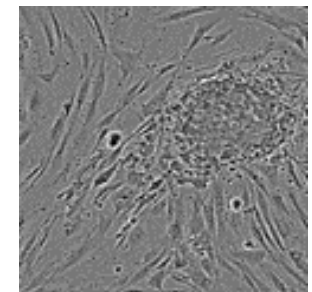
Neurite Analysis



Clonal Dilution



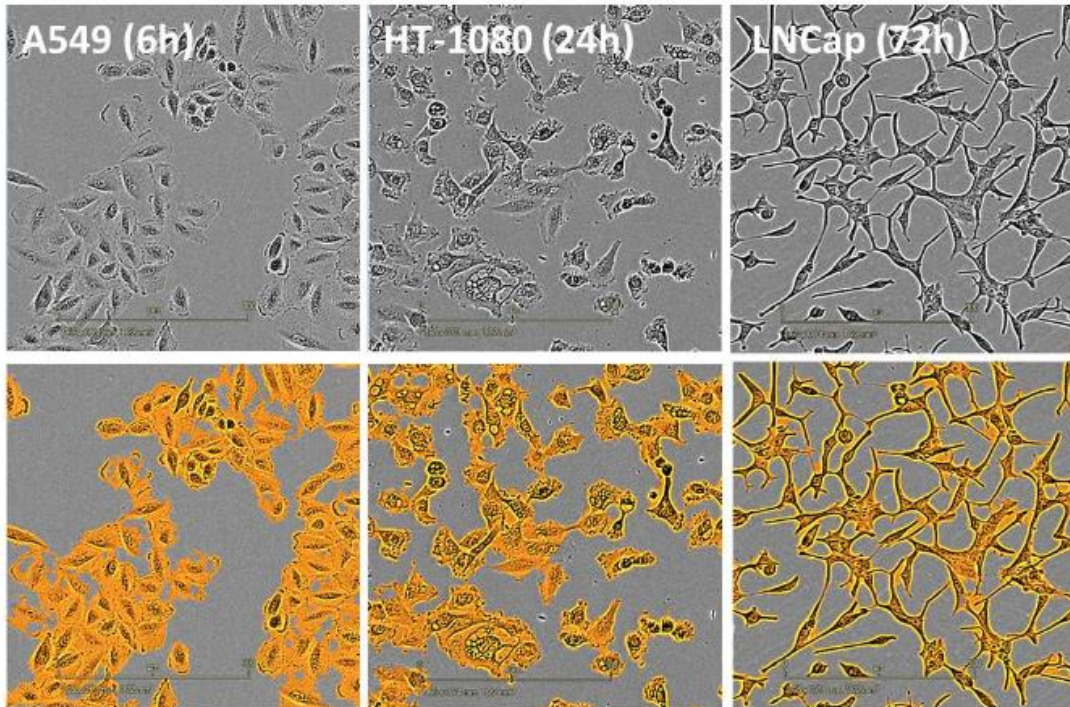
Angiogenesis



Stem Cell Monitoring & Reprogramming

Cell Confluence, QC & Health I

→ Optimize your cell growth conditions

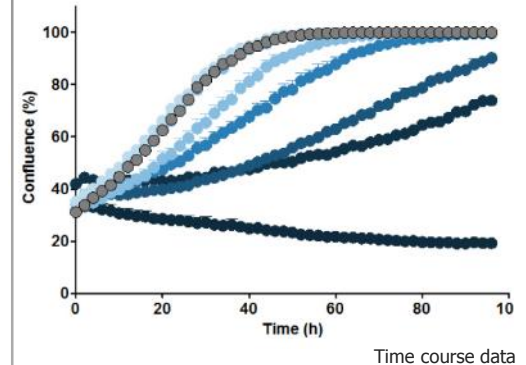


Movies

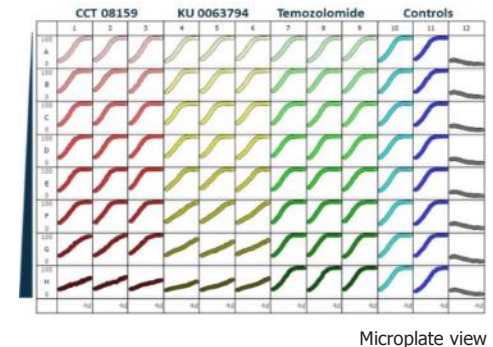


<https://youtu.be/N9vgnWbt6vQ>

Metrics

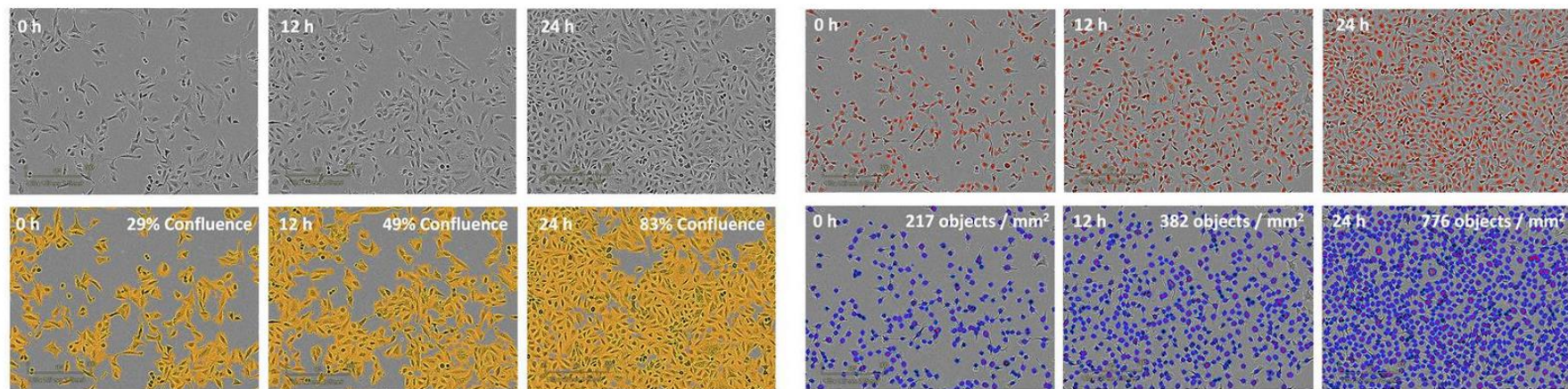


Throughput



- Measure cell proliferation over time & without labels
- Cells remain in your incubator throughout assay
- Applicable to a wide range of cell types
- Validate data and morphology with images & movies

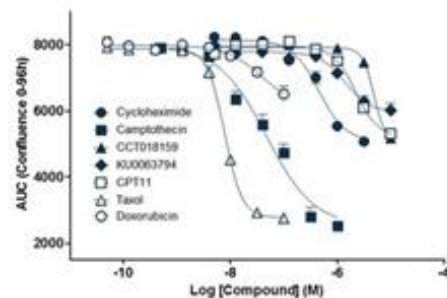
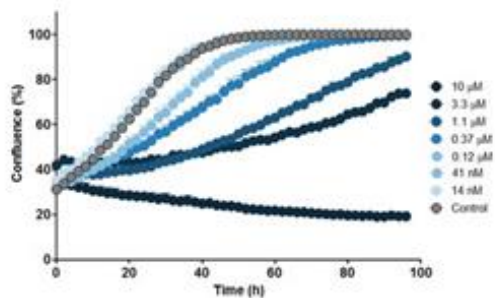
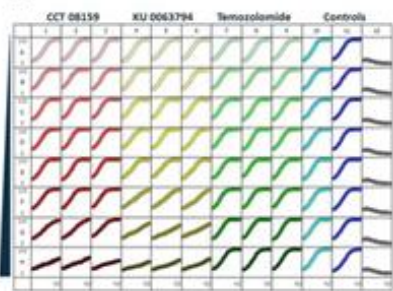
Cell Confluence, QC & Health II



Quantify cell proliferation in real-time using cell confluence (label free, left) and cell counting (fluorescence, right) in HT-1080 fibrosarcoma cells.

Automatically analyze growth curves and extrapolate doubling times

A

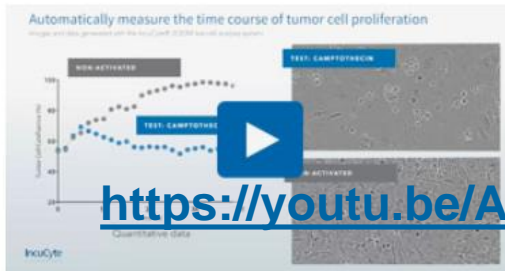


Measure treatment effects automatically and non-invasively. IncuCyte™ cell proliferation assays allow every well of a 96/384 well plate to be imaged and analyzed automatically to provide a microplate readout of cell proliferation over time (A). Proliferation time-courses reveal concentration-dependent treatment effects (B). Transform data into concentration-response curves to compare pharmacology (C).

Proliferation quantification / Cell Confluence I

(1) Label-free, Confluence

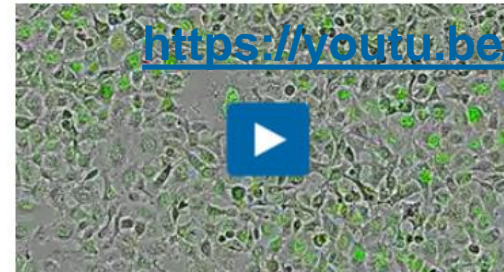
Cell proliferation is monitored by analyzing the occupied area (% confluence) of cell images over time. As cells proliferate, the confluence increases. Confluence is an excellent surrogate for proliferation, until the point that cells are densely packed or when large changes in morphology occur.



Monitor proliferation of A549 cells in real time with confluence image mask (Gold) using IncuCyte™ cell proliferation assays.

(2) Fluorescent Labeling, Direct Cell Count

Cell proliferation is quantified by counting the number of fluorescent nuclei over time to give true cell growth rates. Cells are labeled with nuclear-restricted non-perturbing fluorescent labels (e.g. IncuCyte® NuCLight Red). In co-culture, different labels can be combined to simultaneously measure proliferation of two cell types.

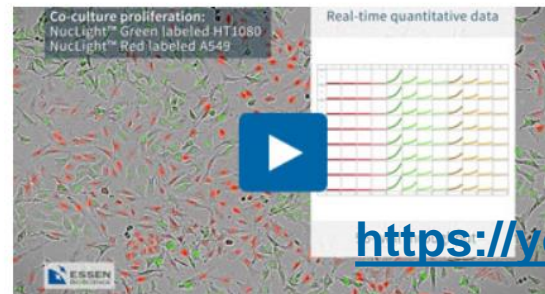


Monitor proliferation of HT1080 in real time using IncuCyte® NuCLight Green labeled cells and cell proliferation assays.

Proliferation measurement / Cell Confluence II

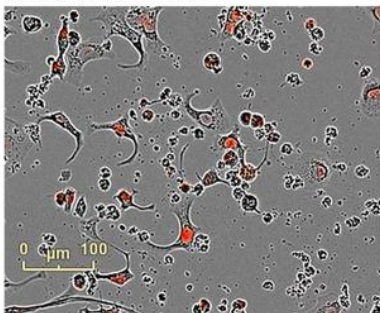
Explore co-cultures using IncuCyte® NuLight live cell labeling reagents

- Identify and quantify cells in co-culture by labeling with IncuCyte® NuLight fluorescent probes

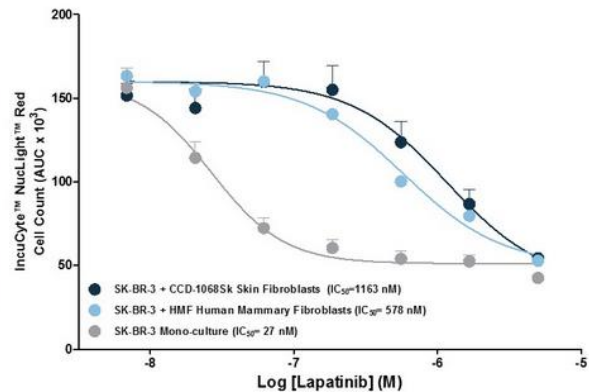
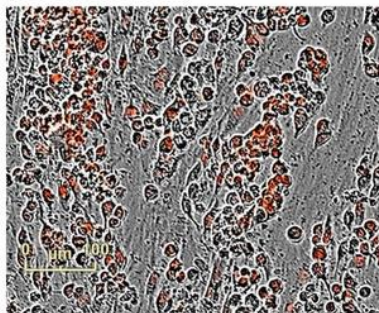


IncuCyte® Co-culture cell proliferation assays. Time-lapse movie of co-cultured nuclear-labeled HT1080 cells (green nuclei, NuLight Green) and A549 cells (red nuclei, NuLight Red).

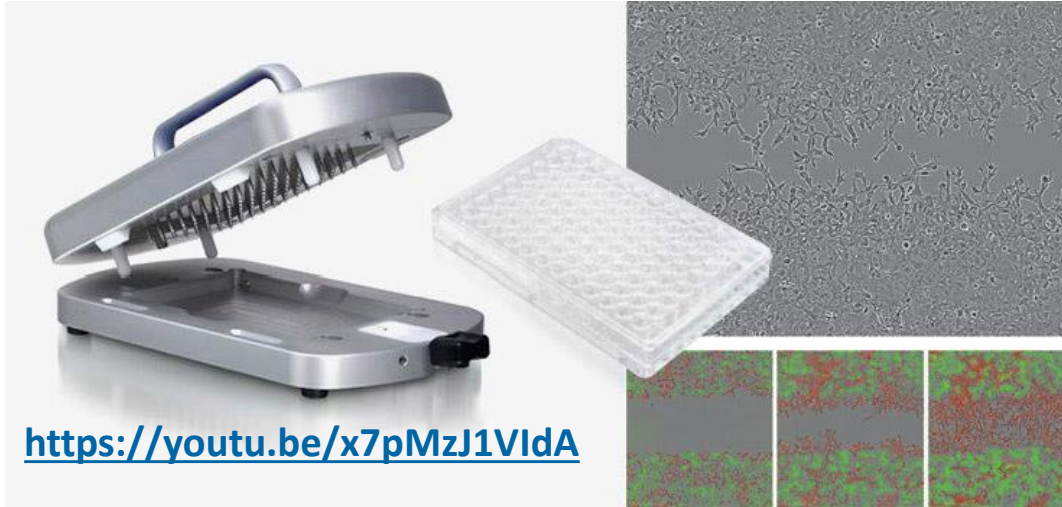
SK-BR-3 Monoculture
(550 nM Lapatinib)



SK-BR-3 Co-culture
(550 nM Lapatinib)



Cell Migration & Invasion



<https://youtu.be/x7pMzJ1VIdA>

ImageLock™ 96-well Plates

IncuCyte™ Cell Migration Software Module

- Create scratch wounds in seconds on 96-well plates
- Automatically analyse wound healing over time
- No labels required, amenable to wide range of cells
- Study migration or invasion through ECM

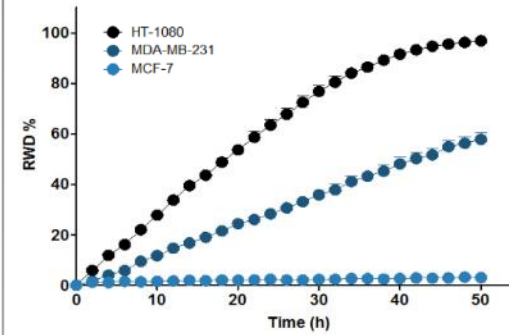
Movies



HT1080 cells, Matrigel Biomatrix

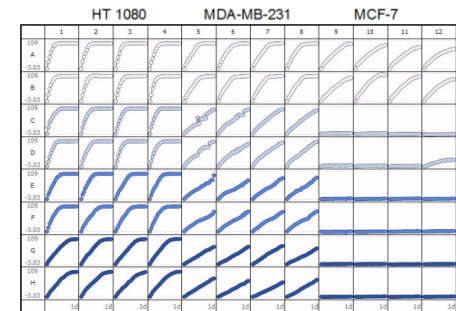
<https://youtu.be/qYfwcFDbyW4>
<https://youtu.be/Jrk3o9dMDWU>

Metrics



Time course data

Throughput



Microplate view

Directly compare 2D migration and 3D invasion within an assay

Investigate Migration & Invasion Behavior in Mixed Cultures
 Images and data generated with the IncuCyte® ZOOM live-cell analysis system.

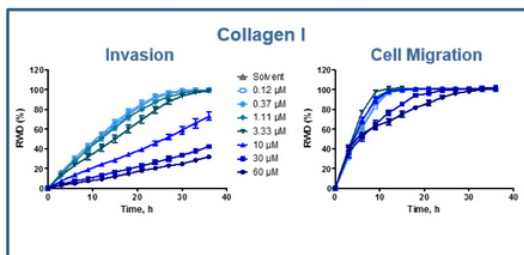
MCF-7 (Green) **non-invasive**
 +
 HT-1080 (Red) **invasive**



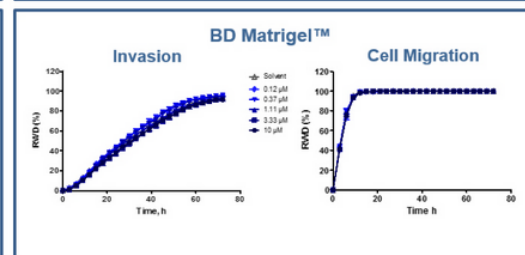
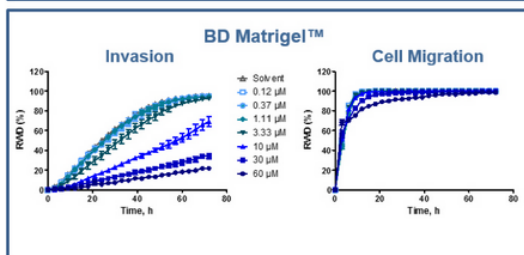
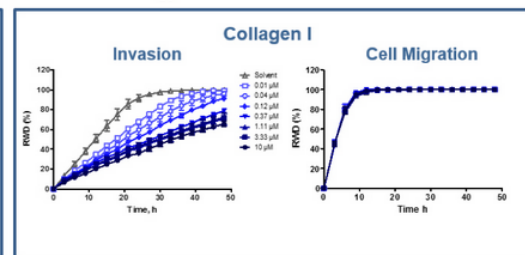
<https://youtu.be/ZxNXG3U2SyA>

ESSEN BIOSCIENCE

Pharmacology of blebbistatin: A myosin II inhibitor

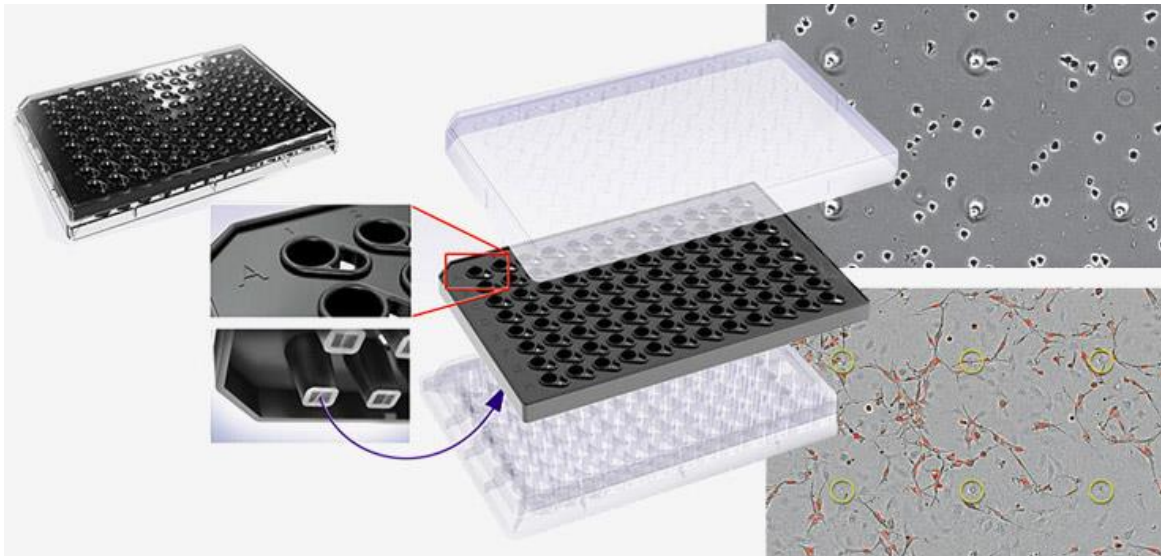
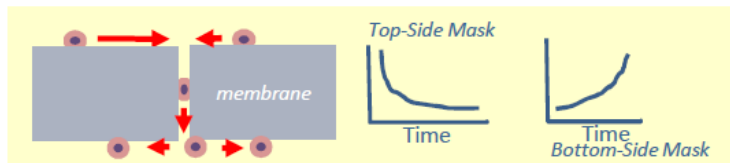


Pharmacology of GM6001: A broad MMP inhibitor



Pharmacological comparison of blebbistatin and GM6001 effect on the invasive phenotype of HT-1080 cells in Collagen I and Matrigel® matrix. Data show that blebbistatin inhibits invasion through both Collagen I and Matrigel® matrix (left), GM6001 inhibits invasion through Collagen I but not Matrigel® matrix (right).

Chemotactic Migration and Invasion

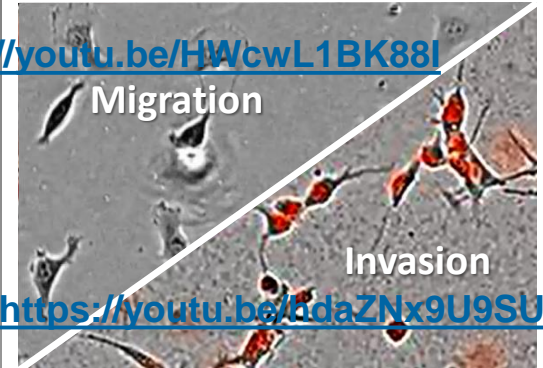


CellPlayer™ 96-well ClearView™ Cell Migration Plate (Cat No 4582)

- Visualize cells migrating toward chemo-attractants
- No labels required, low cell usage, surface interactions
- Monitor full time course of chemotaxis
- Set it & forget it – fully automated analysis

Movies

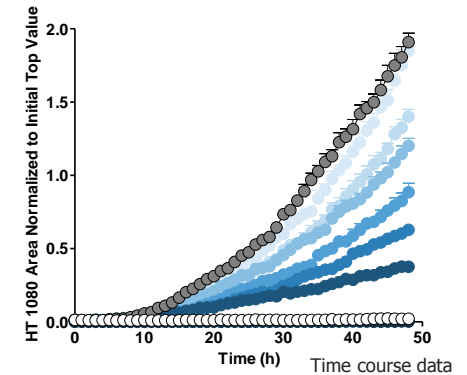
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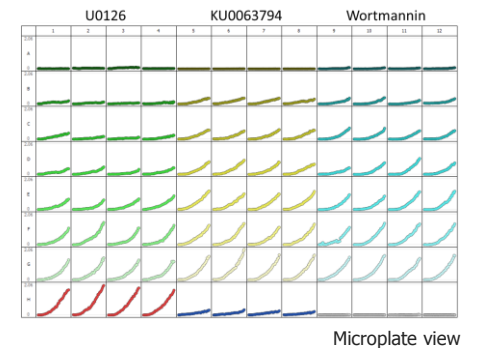
<https://youtu.be/tdaZNx9U9SU>

HT 1080 fibrosarcoma cells

Metrics

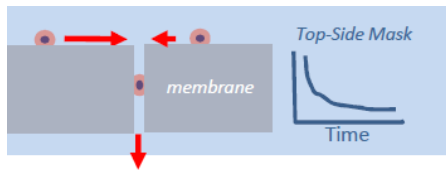


Throughput

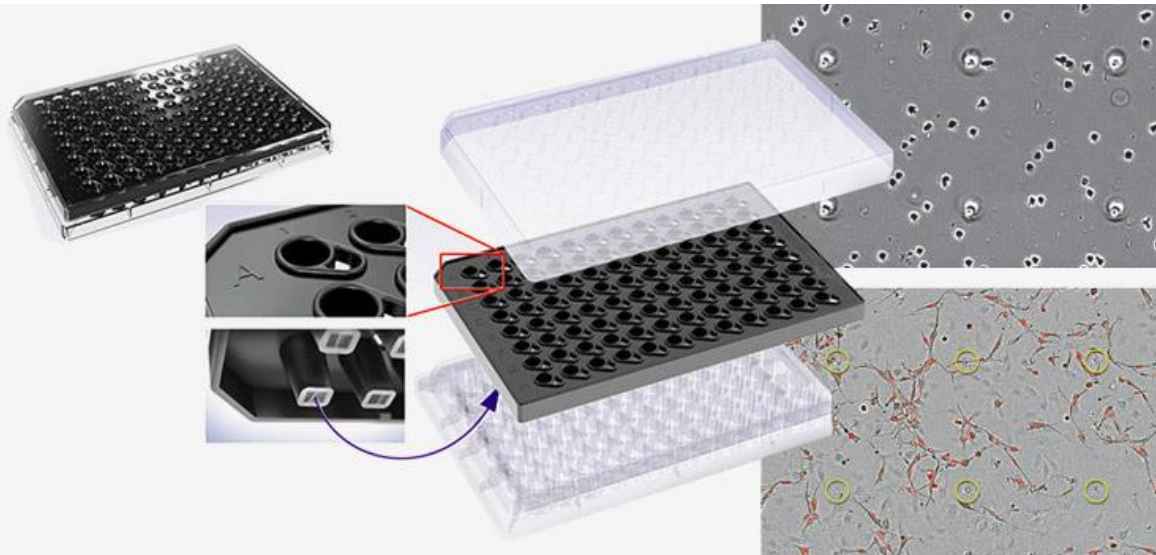


Microplate view

Chemotaxis & Directional Migration



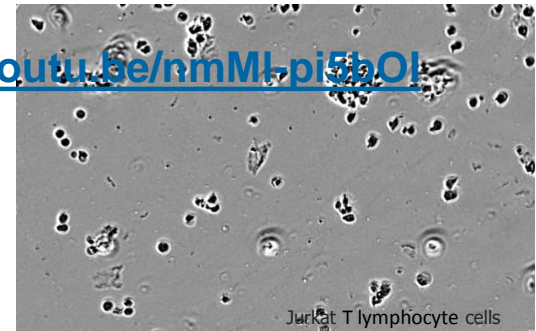
<https://youtu.be/nmMl-pi5bOl>



CellPlayer™ 96-well ClearView™ Cell Migration Plate (Cat No 4582)

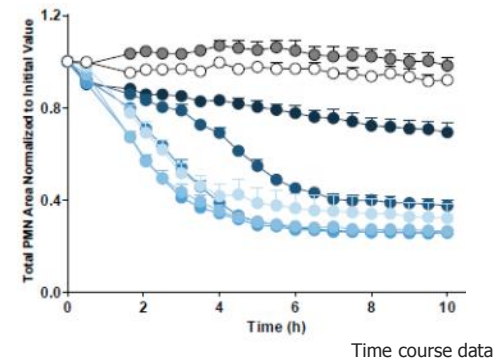
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Movies

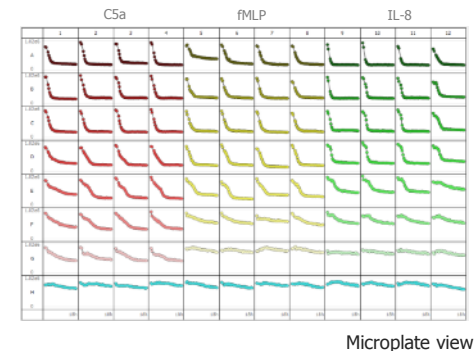


Jurkat T lymphocyte cells

Metrics

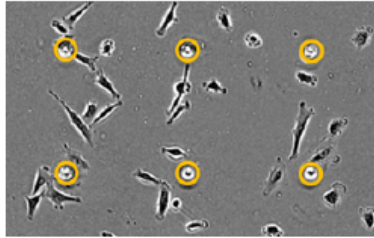


Throughput



Measure Chemotactic Migration, Invasion and Transendothelial Migration in Real Time

<http://www.essenbioscience.com/en/applications/live-cell-assays/cell-migration-chemotaxis/>

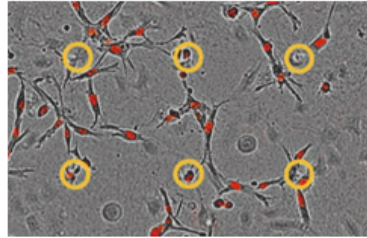


Chemotactic migration across substrate surfaces

- Monitor and quantify directional cell migration across plastic or biomatrix coatings in real time
- Validated with a wide range of immune, tumor and vascular cell types
- Study the effect of treatments on full time course chemotaxis profiles

<https://youtu.be/oElaY8QWe-g>

https://youtu.be/VIJp5G_PhAQ

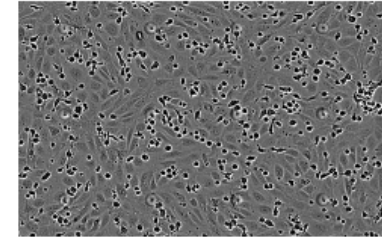


http://www.essenbioscience.com/media/uploads/files/Untitled-1_4.jpg

Chemotactic invasion through 3D biomatrix gels

- Real time imaging and quantification of cell invasion through 3D biomatrix gels
- Assess metastatic potential and define the effect of treatments on invasive phenotype.
- Explore differential biology of chemotactic cell migration and invasion in the same plate.

<https://youtu.be/W2Gg26FVYBo>



Chemotactic transendothelial migration of leukocytes

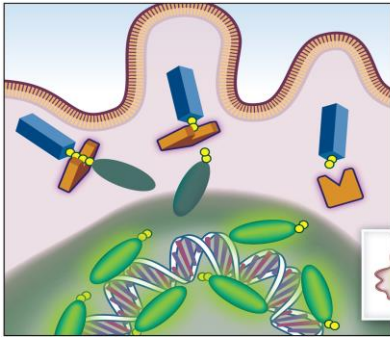
- Monitor and quantify directional leukocyte migration across an endothelial monolayer in real time
- Visualize endothelial monolayer integrity and leukocyte diapedesis
- Investigate the biological effects of inhibitors and neutralizing antibodies

<https://youtu.be/JHnDkKeeBfg>

Apoptosis Assays

<http://www.essenbioscience.com/en/applications/cell-health-viability/apoptosis/>

Caspase-3/7

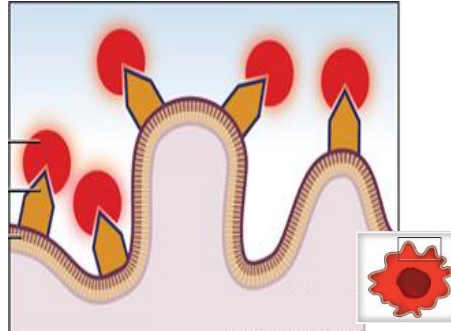


IncuCyte™ Caspase-3/7 Reagent

Detect activation of caspase 3 or 7

Activated caspase-3/7 cleaves the reagent releasing a DNA binding dye

Annexin V



IncuCyte™ Annexin V Reagents

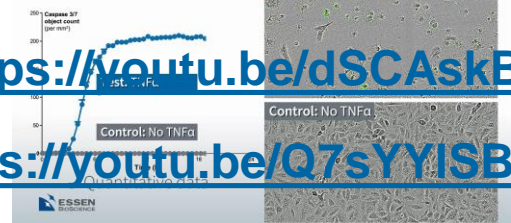
Detect PS externalisation

Exposure of PS on the extracellular surface enables reagent binding

- Quantify apoptosis automatically and in real time
- Simple mix-and-read 96/384-well assays – no washing
- Detect & confirm apoptosis through 2 different pathways
- Multiplex with proliferation and cytotoxicity measures

Movies

Automatically measure the time course of caspase-3/7 mediated apoptosis
Images and data generated with the IncuCyte™ ZOOM live-cell analysis system.

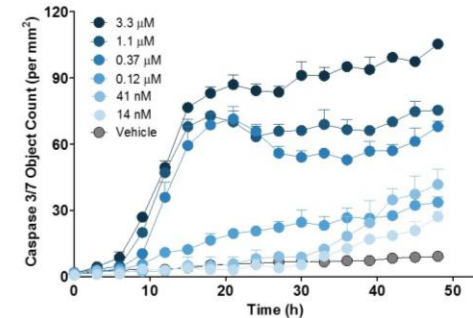


<https://youtu.be/dSCAskBH6Pk>

<https://youtu.be/Q7sYYISBKcA>

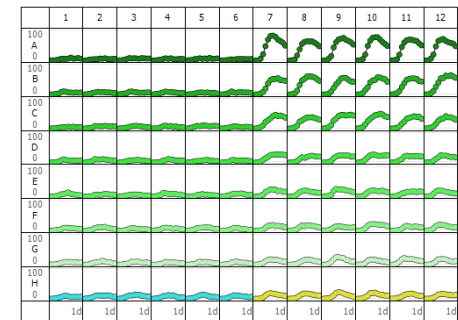
Real-time detection and analysis

Metrics



Time course data

Throughput

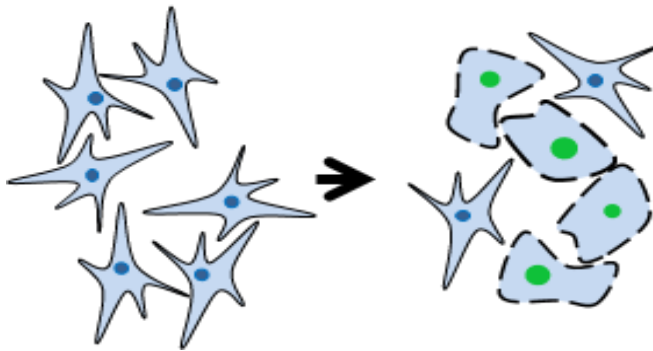


Microplate view

Cytotoxicity Assays

<http://www.essenbioscience.com/en/applications/cell-health-viability/cytotoxicity/>

Kinetic Cytotoxicity using IncuCyte™ Cytotox Red and IncuCyte™ Cytotox Green Reagents



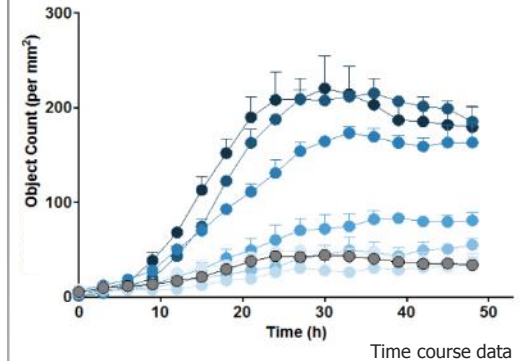
- Quantify real time cell death
- No wash, mix and read assays in 96- and 384-well format
- Easily duplex with NuLight Red Proliferation Assay
- Validate data with images and movies

Movies

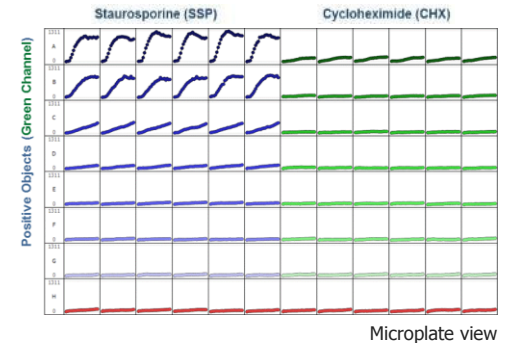


<http://youtu.be/46-4whHx6nl>

Metrics



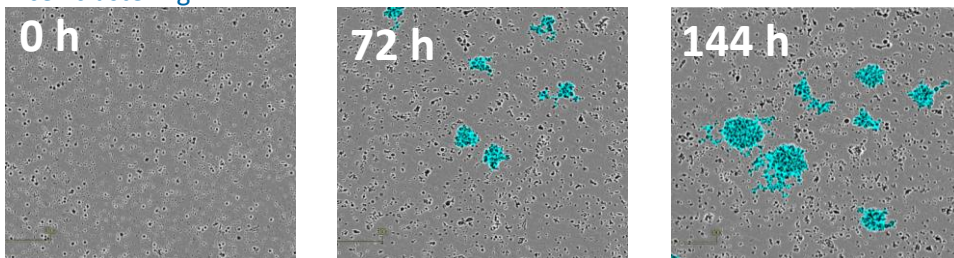
Throughput



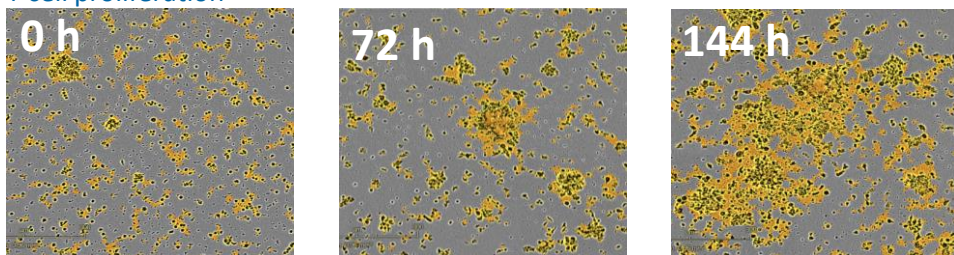
Immune Cell Clustering & Proliferation

http://www.essenbioscience.com/media/uploads/files/Suspension_cell_poster_ELRIg.pdf

T cell clustering



T cell proliferation



- Monitor proliferation and clustering in real-time
- Automated imaging and time-course analysis
- Label free, kinetic measurements
- No wash, no cell lifting, no antibody labelling

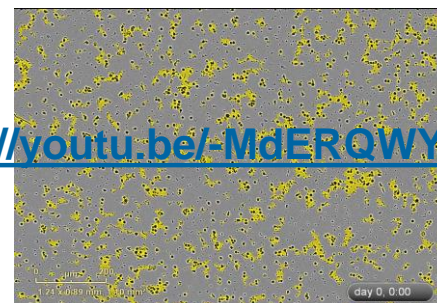
Movies

Immune cell Clustering



PBMCs activated with anti-CD3 antibody and IL-2

Immune cell Proliferation

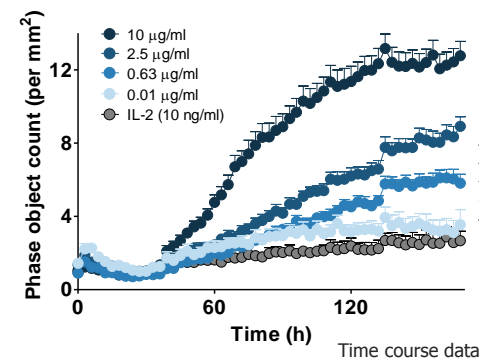


PBMCs activated with anti-CD3 antibody and IL-2

<https://youtu.be/0QOfmMeDMYU>

<http://youtu.be/-MdERQWYzV8>

Metrics



Immune Cell Killing (T cell, ADCC)

http://www.essenbioscience.com/media/uploads/files/Essen_Poster_ITOC_2016_final_1.pdf

Immune cell

Target tumor cell

Tumor cell apoptosis (green)

Tumor cell count (red)

IncuCyte™ Caspase-3/7 Apoptosis Assay Reagent

NuLight™ Red

- Direct, kinetic measures of tumor cell death
- Mix-and-read protocols - no radioactivity or cell lifting
- Images/movies validate target/effector cell interactions
- T cell killing, ADCC - your choice of effector/target cells

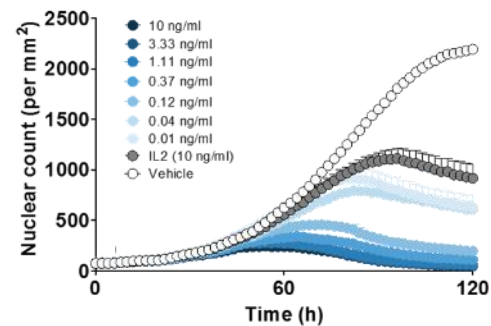
Movies



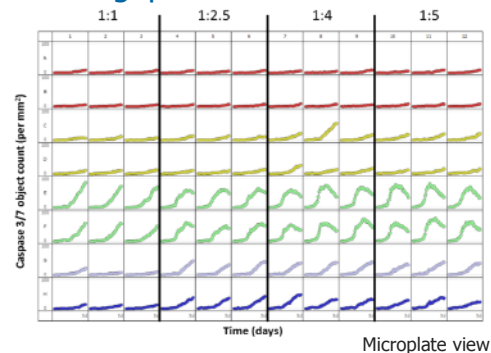
<https://youtu.be/fJLot4RIMAQ>
<https://youtu.be/j0HT7i-Dilo>

NuLight Red SK-OV-3 cells + PBMCs + Caspase 3/7 reagent

Metrics

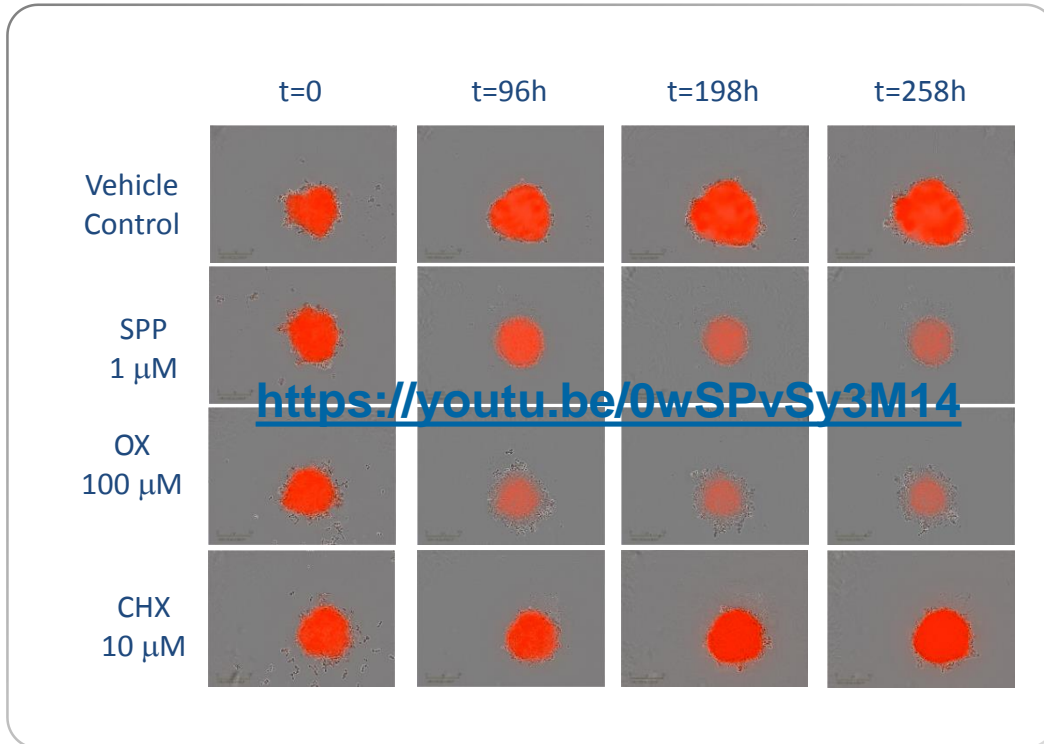


Throughput



Tumor Spheroid

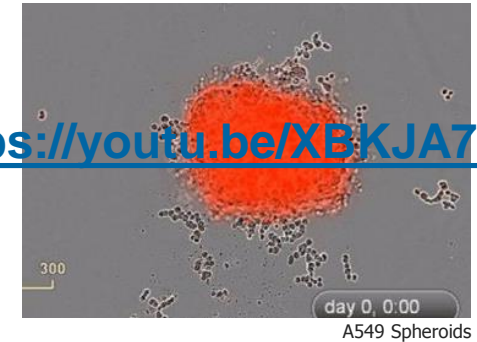
http://www.essenbioscience.com/media/uploads/files/Simplifying_high_throughput_3D_spheroid_growth_and_shrinkage_assays_using_live_content_imaging_1.pdf



<https://youtu.be/0wSPvSy3M14>

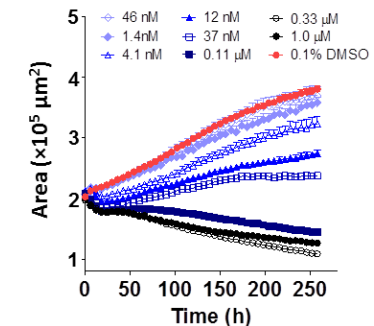
- Simple protocol for generating spheroids in Ultra Low Attachment Round Bottom Plates
- Label-free and fluorescence metrics used to measure growth, shrinkage and spheroid health
- Validate findings with images and movies

Movies



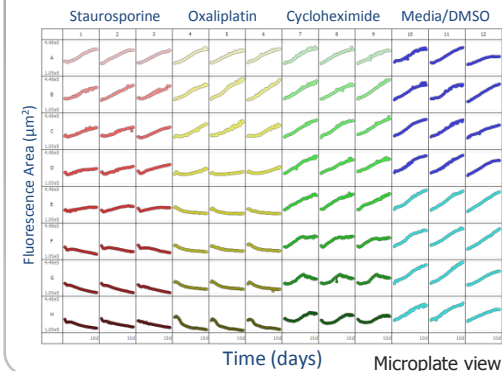
<https://youtu.be/XBKJA7fatKs>

Metrics

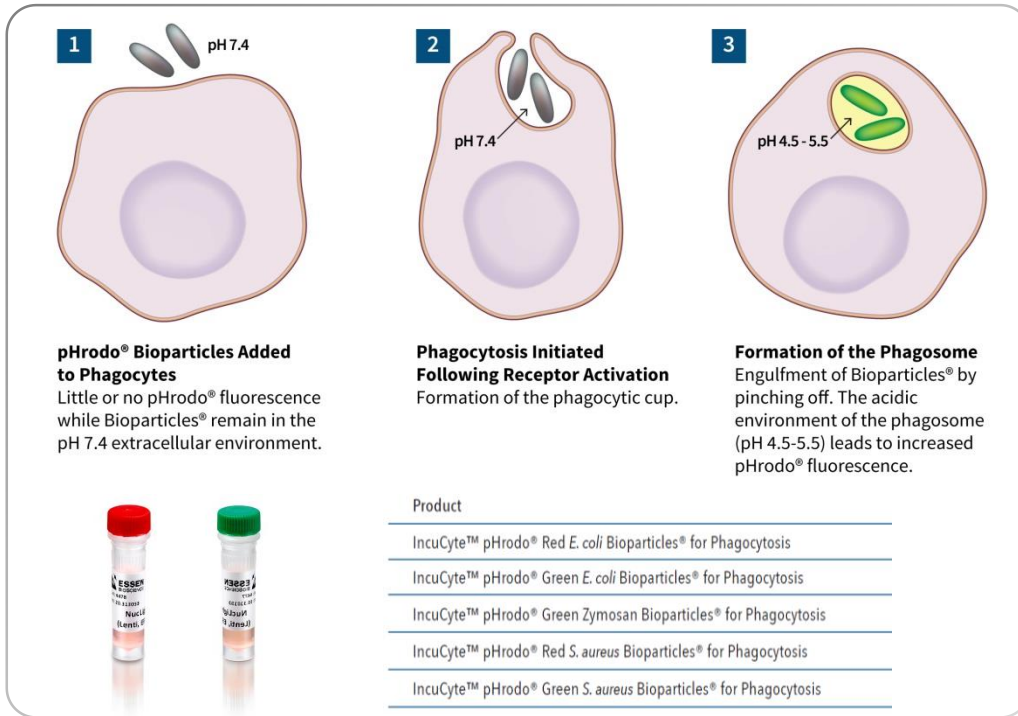


Time course data – Dose response to Staurosporine

Throughput

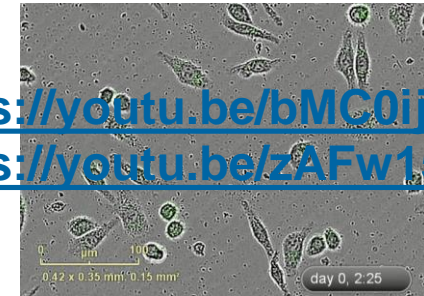


Phagocytosis & IncuCyte pHrodo® Bioparticles



- Validation of phagocytosis using time-lapse imaging
- Automated analysis and quantification
- Mix-and-read; no fixing, no quenching, no lifting
- Highly sensitive, low background, low cell numbers

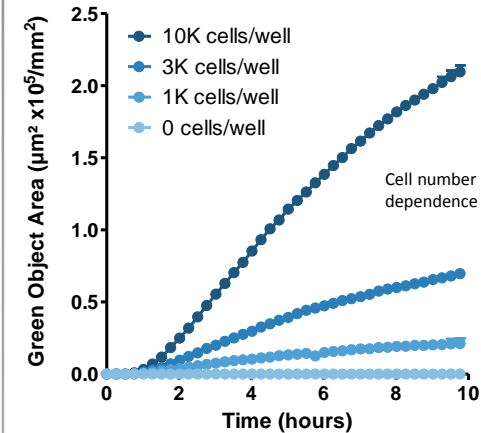
Movies



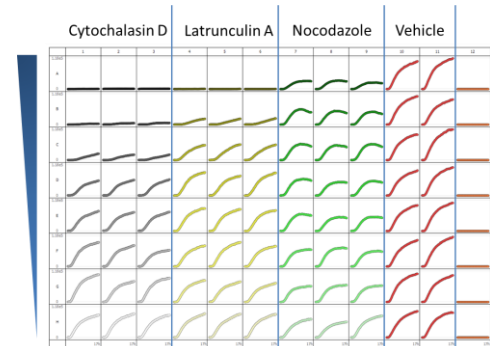
<https://youtu.be/bMC0ijUdtzA>
<https://youtu.be/zAFw15Kr6q8>

J774A.1 macrophages + IncuCyte® pHrodo® Green E. coli bioparticles®

Metrics

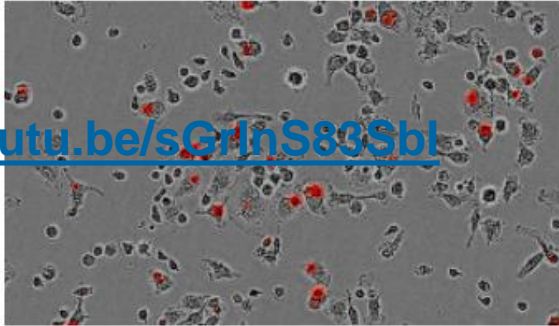


Throughput

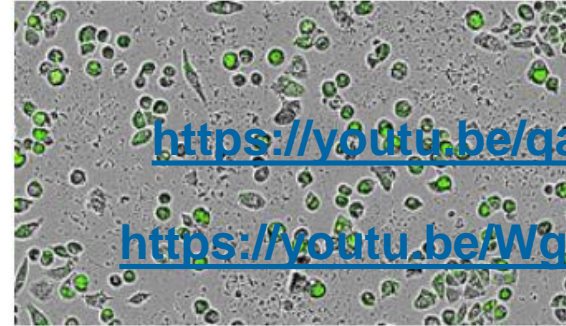


Measure Phagocytosis in Real Time

http://www.essenbioscience.com/media/uploads/files/CIMT_Poster_2016.pdf



<https://youtu.be/sGrInS83Sbl>



https://youtu.be/ga_LKKJ3ok

<https://youtu.be/WgdsuJgMh4w>

Measuring Phagocytosis of Cells with IncuCyte® Phagocytosis Assay

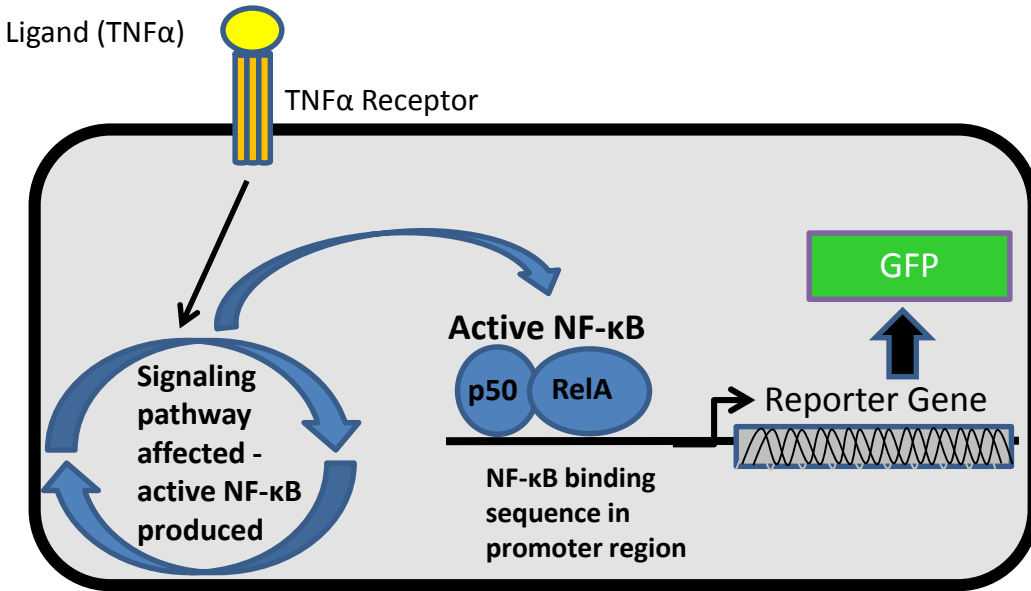
- Automatically quantify the phagocytosis of cells in real time
- Investigate efferocytosis and antibody mediated cellular phagocytosis (e.g., anti-CD47 / ADCP)
- Compatible with your choice of target cells labelled with the IncuCyte® pHrodo® Red Cell Labeling Kit

Measuring Phagocytosis of Bioparticles® with IncuCyte Phagocytosis Assay

- Monitor and measure uptake of bacteria or yeast pHrodo® Bioparticles®
- Validate phagocytosis with images and movies
- Study the effect of treatments on full time course phagocytosis profiles

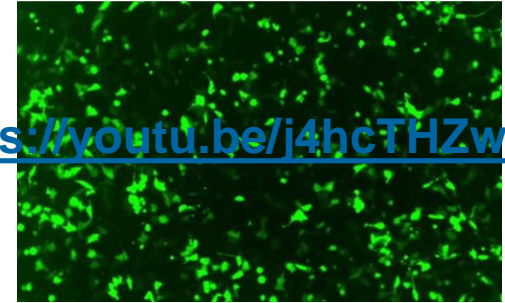
Reporter Gene Example

TNF α Activation of NF- κ B



- Dose response of TNF α added to cells transfected with NF κ B-GFP reporter genes is shown in this example.
- The activation of the TNF receptor leads to the activation of NF- κ B, a transcription factor that, in turn, stimulates gene expression.
- After the addition of the TNF α , the cells are placed into the IncuCyte ZOOM which kinetically measures GFP expression.

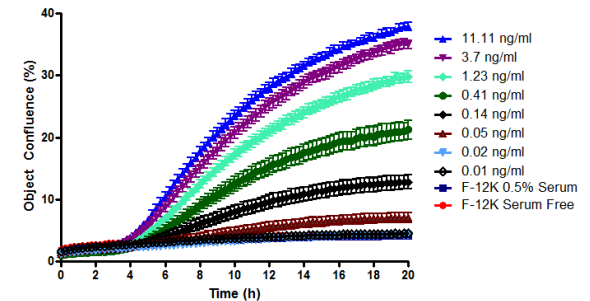
Movies



<https://youtu.be/j4hcTHZwUhg>

Cells transfected with a reporter gene for NF- κ B treated with TNF α

Metrics

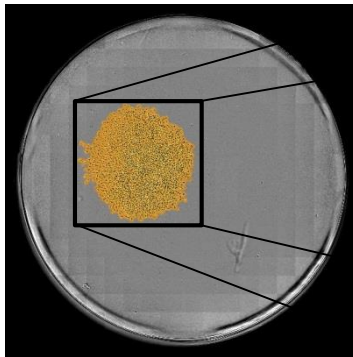


Time course dose response to TNF α addition

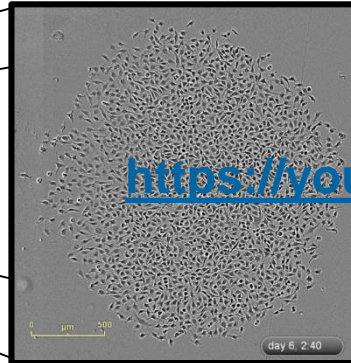
Research Area	Reporter Constructs (Transcription Factor)
Cancer	NF- κ B, p53, MAPKs, Wnt, HIF-1 α , PI3K
Inflammation	NF- κ B, TGF- β , Interferon Response
Cardiovascular Research	cAMP/PKA, Notch, MEF-2
Stem Cells	KLF4, Oct4, Nanog
Nuclear Receptors	PPAR γ
Toxicity	AhR (CYPs), Nrf1, Nrf2

Clonal Dilution Monitoring

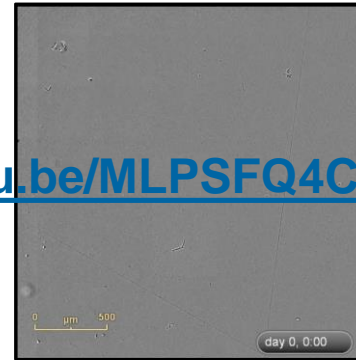
Step 1: Acquire whole well image and identify wells with single colonies



Step 2: Zoom in and Inspect colony morphology



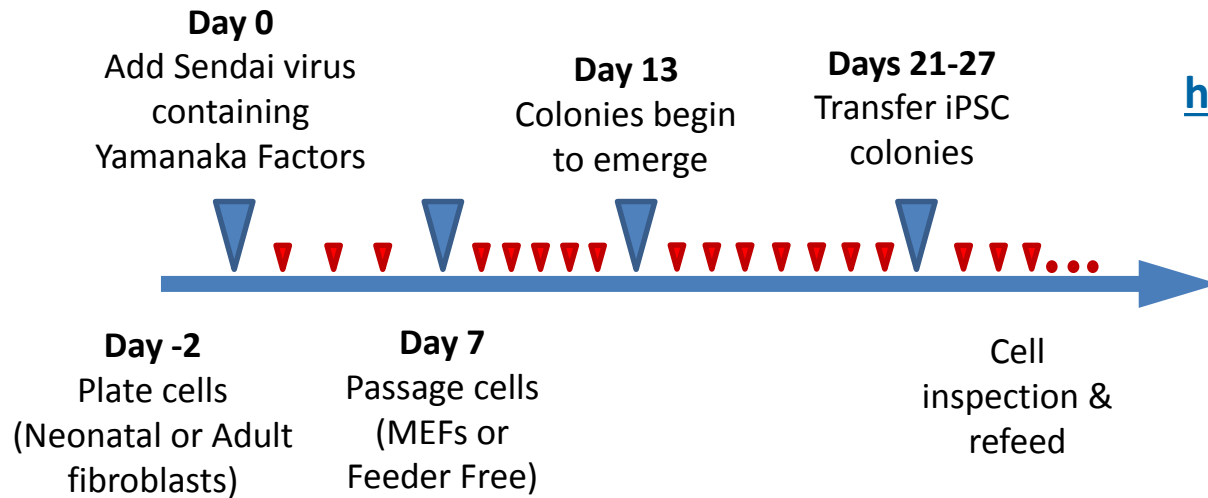
Step 3: Confirm clonality by navigating to earlier time points



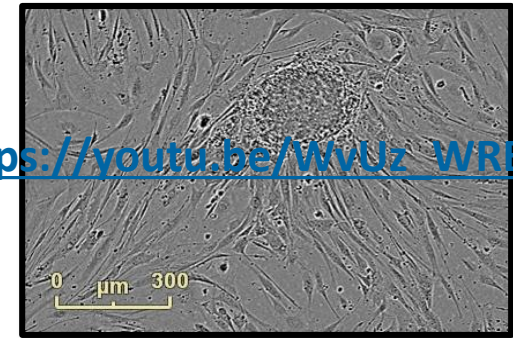
<https://youtu.be/MLPSFQ4CK9k>

- Automated image processing to Identify and track clones, confirm clonality
- Automated image panning of large vessels such as 35mm dishes

Stem Cell Reprogramming



Movies

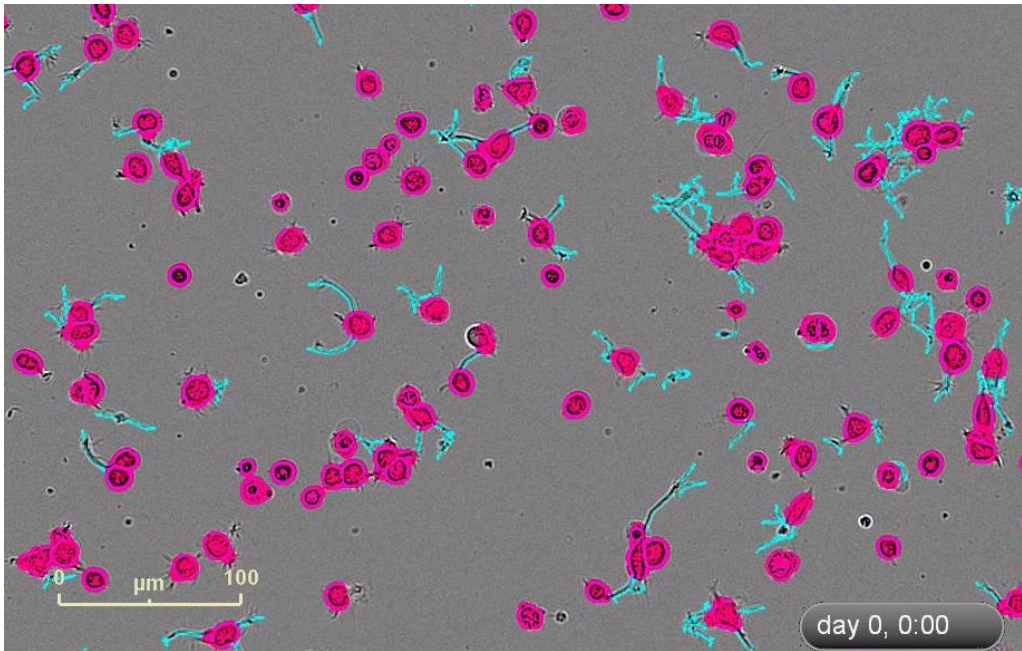


Stem cell colony 12 days post passage

https://youtu.be/WvUz_WRE3Zo

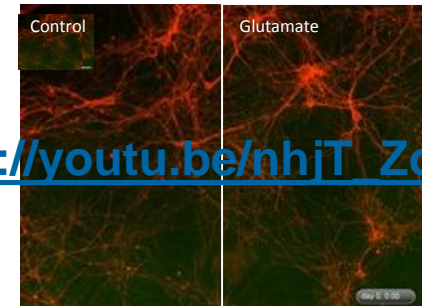
- **Days 0 - 7**
 - Evaluate toxicity of reprogramming reagents
- **Days 8 – 21**
 - Track colony emergence, growth, and morphology
 - Standardize calculation of reprogramming efficiencies
- **Days 21+**
 - Post-Reprogramming - monitor clone transfer and survival

Neurite Analysis



- Measure neurite dynamics automatically and in real-time
- Label-free analysis in monoculture
- Specialized neuronal fluorescent labels for co-culture
- Multiplex with cell health reagents for additional insight

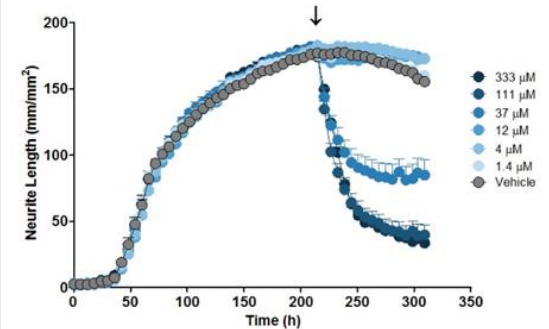
Movies



Neurite analysis multiplexed with Annexin V

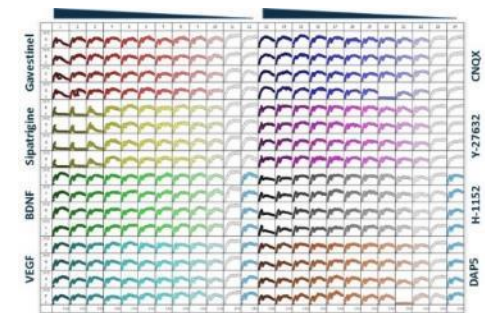
http://youtu.be/nhjT_ZoYA5w

Metrics



Time course data

Throughput



Microplate view

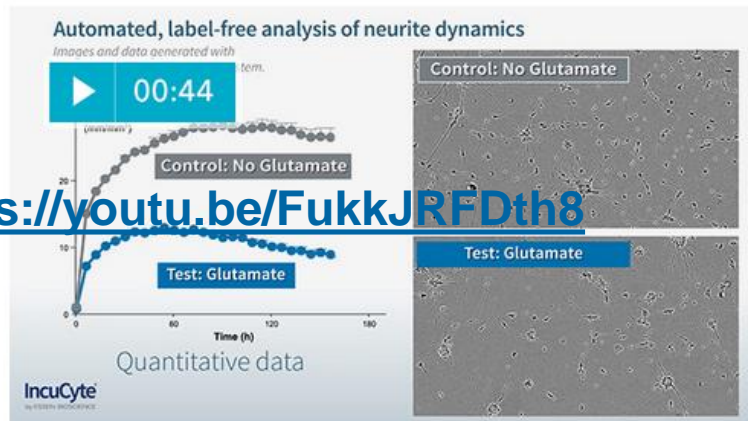
Real-time neurite analysis inside your incubator

IncuCyte® Neurite Analysis Assays enable automated, continuous analysis of neurite outgrowth and neural network stability - all inside your cell culture incubator. Quantify neurite length and branch points in your choice of neurons (e.g. primary, iPSC-derived, immortalized neuronal cell lines) in monoculture or in co-culture with astrocytes.

- Screen for neurotoxicity
- Investigate neuroprotective treatments
- Study pathways involved in neurodegeneration
- Measure neuronal differentiation in real time

1. Label-free analysis of neurons in monoculture

Monitor neurite outgrowth in live neuronal monocultures using IncuCyte NeuroTrack™ analysis software. Fully automated, long-term label-free analysis.

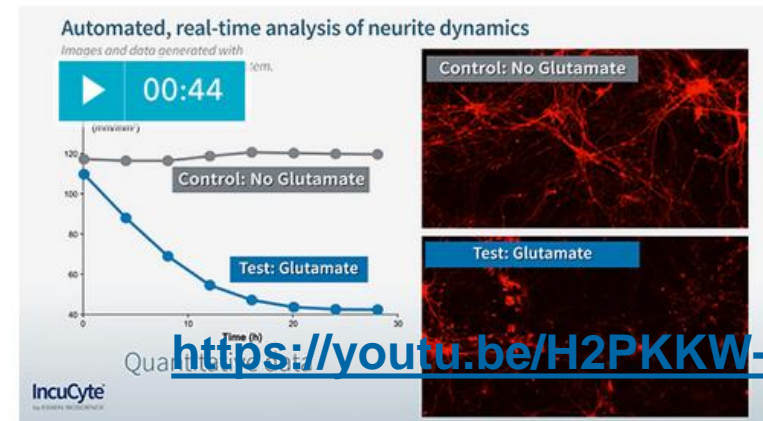


<https://youtu.be/FukkJRFDth8>

Human iPSC-derived neurons (iCell Neurons, CDI) in monoculture.

2. Fluorescence analysis of neurons in co-culture with astrocytes

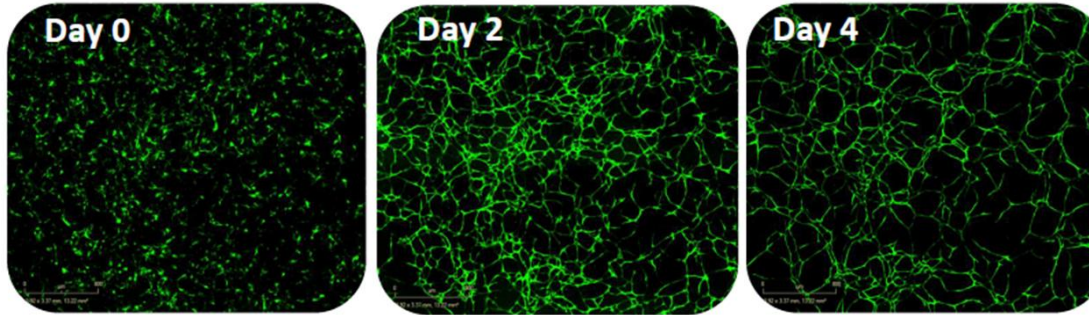
Measure changes in neurite length and branch points in mixed cultures of neurons and astrocytes using fluorescent neuronal labelling reagents and IncuCyte analysis software.



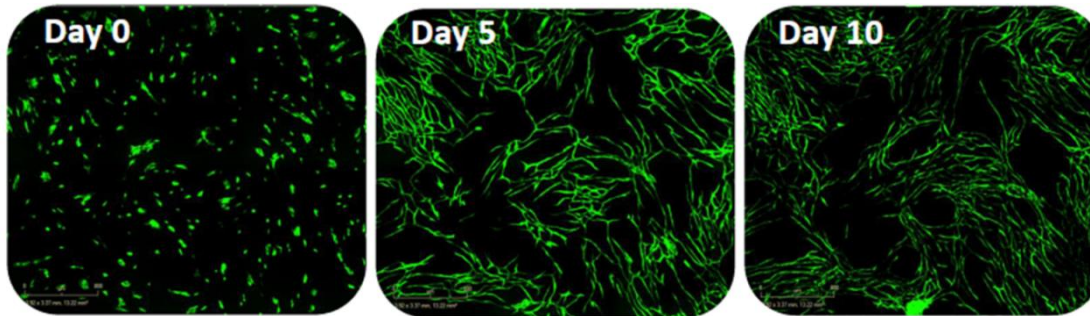
<https://youtu.be/H2PKKW-bh2A>

Primary rat forebrain neurons labeled with the IncuCyte® NeuroLight Red reagent in co-culture with rat astrocytes.

Angiogenesis



ADSC/ECFC Cord Formation

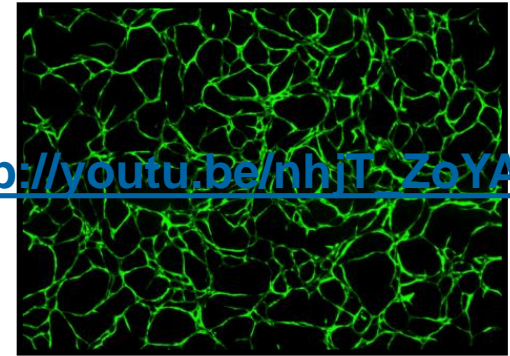


NHDF/HUVEC Tube Formation

Two well published Angiogenesis co-culture models consisting of cryopreserved cells and supplements available from Essen BioScience:

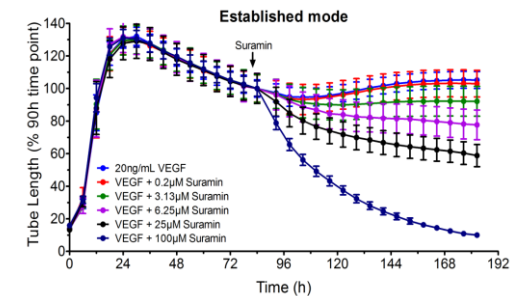
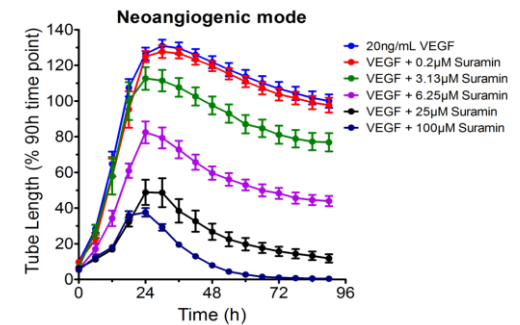
- 1) Adipose Derived Stem Cells/Endothelial Colony Forming Cells (model yields rapidly forming (<48h) endothelial cell 'cord' structures)
- 2) Normal Human Dermal Fibroblasts/HUVEC (slowly forming 'tube-like' structures appear, grow and branch for 10 days)
- 3) Different morphological, temporal, and pharmacological profiles

Movies



http://youtu.be/nhjT_ZoYA5w

Essen BioScience Stem Kit for Angiogenesis



IncuCyte[®] – Key Features

Multiple Imaging Modes

- HD phase-contrast, green & red fluorescence, 4x 10x 20x
- Sample area to whole well image capture, up to 6 plates at once
- 24h scheduling and 'scan on demand'

Multi-Vessel, Multi-User

- Compatible with over 600 standard plates, dishes & flasks
- Mix & match vessels & assays
 - (slides, dishes, flasks, and 6 to 384-well plates)
- Guided interface enables even first-time users

Remote Viewing & Analysis

- Quickly assess trends and outliers with visualization tools
- Fully automated, fast on the fly analysis
- Shared access via any networked Windows device

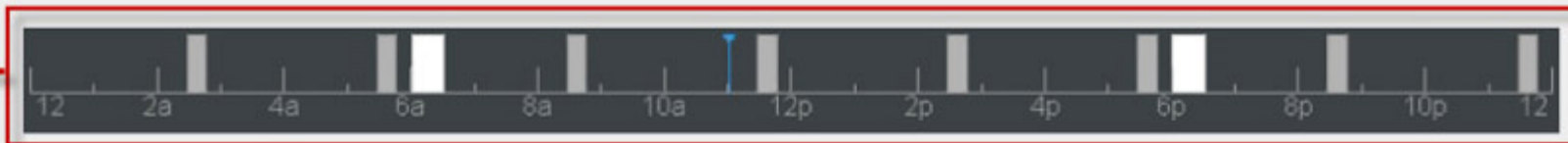
Software insights

The screenshot displays the IncuCyte software interface. At the top, a navigation bar includes 'Schedule', 'View', 'Manage', 'Device', 'Archive', and 'Status'. A progress bar shows 'Scanning (26%)' and a timeline from 12 to 12. The main window title is '032117 MR Cytotox Red QC Lots 1871635/1841656' with a timestamp of '6:43 PM'. On the left, there are three 'Plate Map' sections, each showing an 8x12 grid of wells (A-H, 1-12). The bottom-most plate map is highlighted with a blue border and labeled 'Front of Instrument'. The central 'Scan Properties' panel lists: 'Vessel Type: 96-well Corning', 'Scan Type: Standard', 'Image Channels: Phase, Green, Red', 'Objective: 10x', 'Scan Duration: 8 min (estimated)', and 'Number of Daily Scans: 12'. A 'Scan Pattern' dialog box is open, titled 'Add Vessel', with the instruction 'Specify the scan pattern to use for image acquisition.' It features an 8x12 grid of wells with blue squares indicating the scan pattern. Below the grid, 'Images per Well' is set to 1 and 'Scan Duration' is 3 min (estimated). A 'Use Sample Pattern' button is present. At the bottom of the dialog, there are 'Back' and 'Next' navigation buttons and a progress indicator.

Scan Schedule

Review and modify the daily scan times here.

1



+ ↑ ↓ ↻

3

2

Vessel ID	Name	Owner	Location	Scan duration h:mm
- 8 Scans Per Day - 0h 18m per Scan				
1027	1/19/17 MR Polymer Tox Ass...	LabUser	Rear Tray, Left Position	0:09
1028	1/19/17 MR Polymer Tox Ass...	LabUser	Rear Tray, Right Position	0:09
- 2 Scans Per Day - 0h 30m per Scan				
1029	384-well Corning 3764 MDA-...	LindyO	Front Tray, Left Position	0:16
	Test		Front Tray, Right Position	0:14

2a

2a

Add and modify scan times for the current scan group in the timeline above.
Right click rows in the grid for vessel grouping and ordering options.

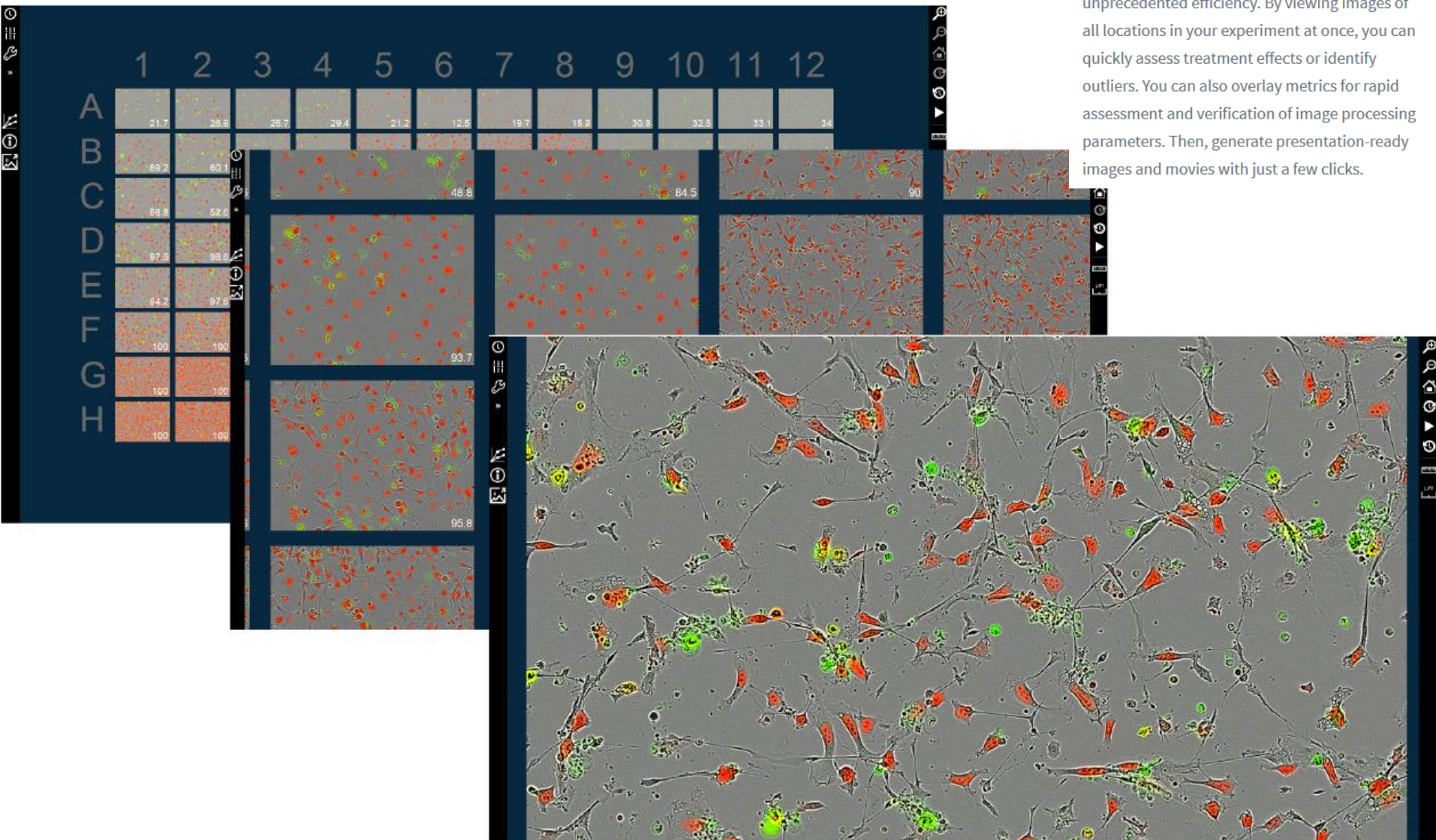
Back



Next

Viewing

IncuCyte® VesselView enables users to navigate and explore thousands of timelapse images with unprecedented efficiency. By viewing images of all locations in your experiment at once, you can quickly assess treatment effects or identify outliers. You can also overlay metrics for rapid assessment and verification of image processing parameters. Then, generate presentation-ready images and movies with just a few clicks.



Analysis Definition

Refine the Analysis Definition settings using Preview to analyze the Image Set.

2

3

1

Analysis Definition

Phase

Object Name

Parameters

Segmentation Adjustment: 1

Background Cells

Cleanup

Hole Fill (μm^2)

Adjust Size (pixels)

Filters

Area (μm^2):

min max

Eccentricity:

min max

Green

Red

Whole Well

Layers

Image Channels

Phase

Green

Red

Spectral Unmixing

% removed from

% removed from

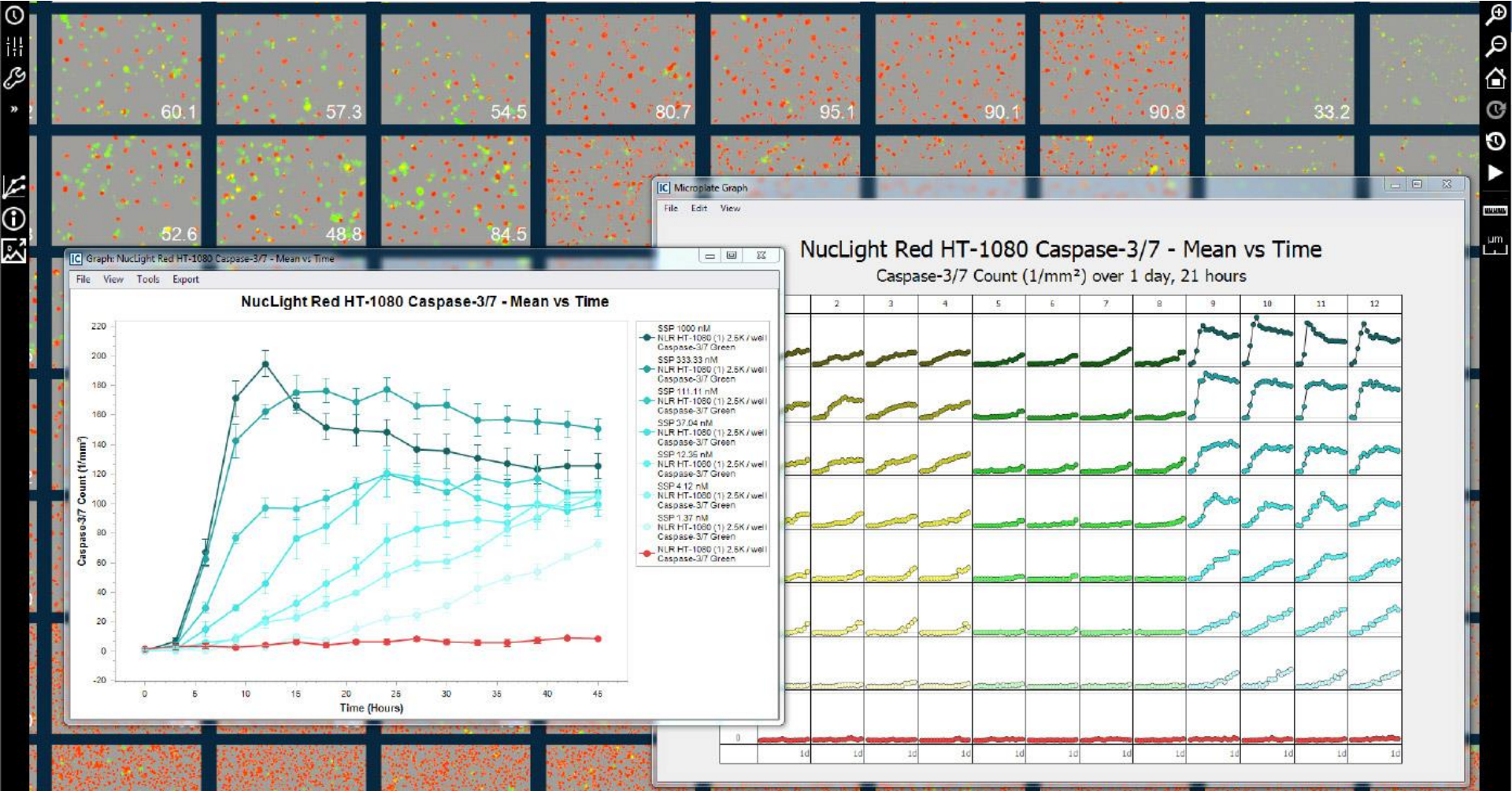
1 of 4

Press Preview Current or Preview All to Update

Back Next

Analysis

IncuCyte® Analysis interface enables even first-time users to convert images into insight. By providing purpose-built tools to answer your scientific questions, image processing and analysis is uncomplicated yet extremely powerful. Create analysis definitions once, and then reapply to subsequent experiments to generate real-time metrics that enable decision-making. Easily view all trends in an experiment at once with IncuCyte®'s PlateGraph, and create customized response curves that are ready for publication and presentation.



IMMUNO-ONCOLOGY



<https://www.youtube.com/watch?v=OyOLT73dUf8>

CELL-BASED ASSAYS

▶ ⏪ 🔊 0:16 / 4:16

