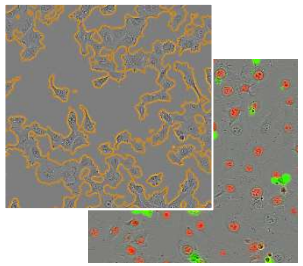
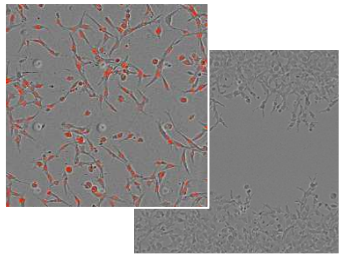
IncuCyte® Key Applications

Cell Health & Viability



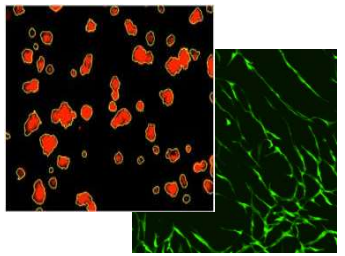
- Proliferation
- Cytotoxicity
- Apoptosis
- Cell Cycle

Cell Function & Interactions



- Cell Migration & Invasion
- Immune Cell Activation
- Immunocytochemistry
- Immune Cell Killing
- Antibody Internalization
- Phagocytosis
- NETosis

Long Term Cell Monitoring



- Spheroid
- Angiogenesis
- Dilution Cloning

Continuously monitor & analyze cells inside your incubator using simple mix-and-read protocols!

INCUCYTE® KEY APPLICATIONS

Proliferation

Monitor cell proliferation inside your incubator in real-time

LABEL-FREE CONFLUENCE

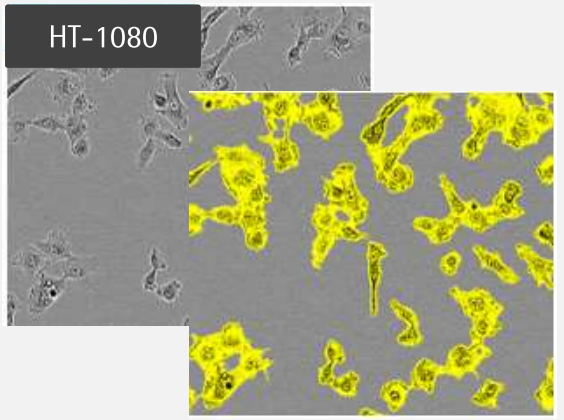
✓ Generate kinetic growth curves and track trends using **label-free confluence** measurements

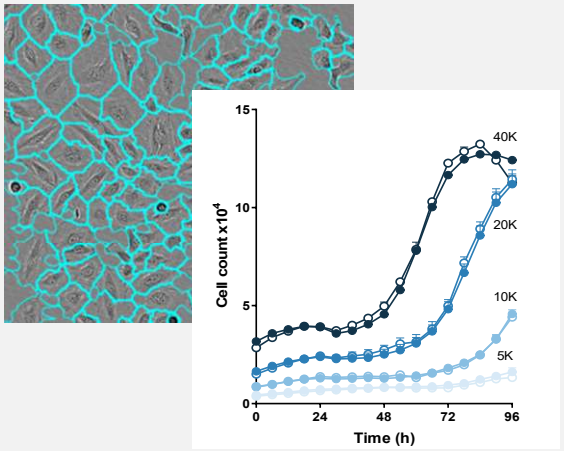
LABEL-FREE COUNTING

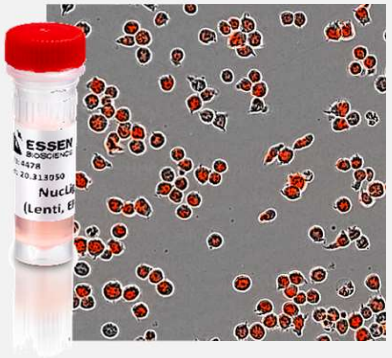
✓ Calculate specific cell counts and analyze sub-populations using label-free **Cell-by-Cell Analysis**

FLUORESCENT LABELING FOR CELL COUNT

✓ Use NucLight reagents to **fluorescently label** nuclei of cells to better track proliferation and cell count





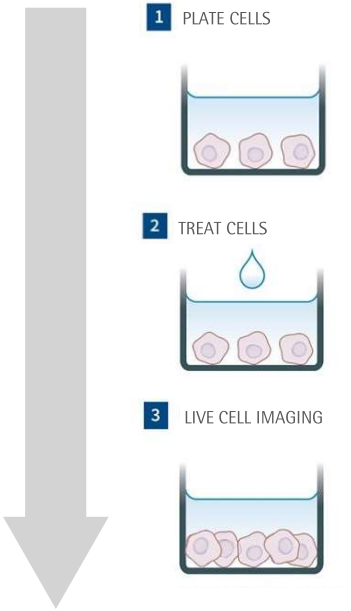


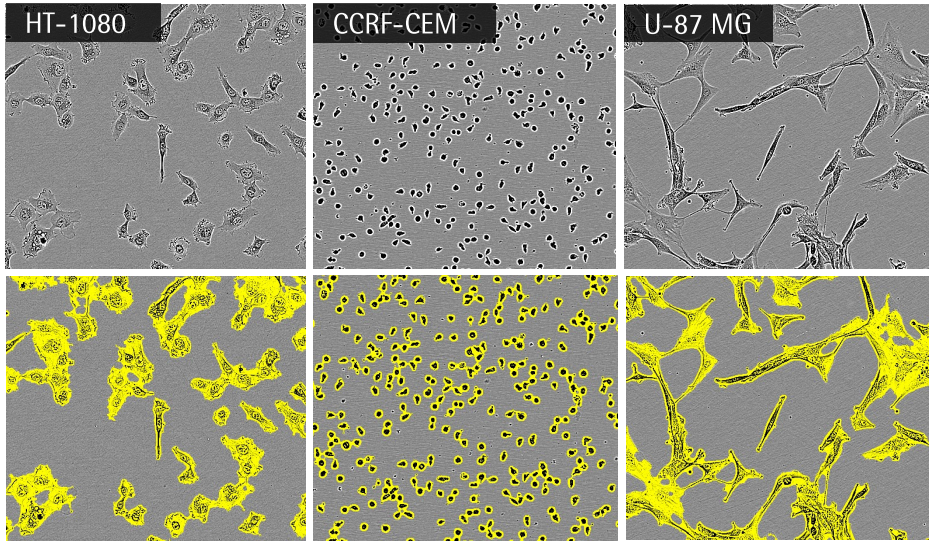
Cell Health & Viability

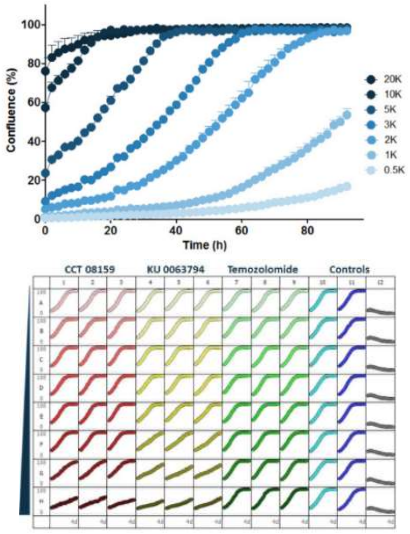
Proliferation

Quantify cell growth with label-free confluence metrics

- ✓ Monitor multiple cell lines with different morphologies using HD phase optics
- ✓ Establish assay quality with accurate, automated confluence metrics





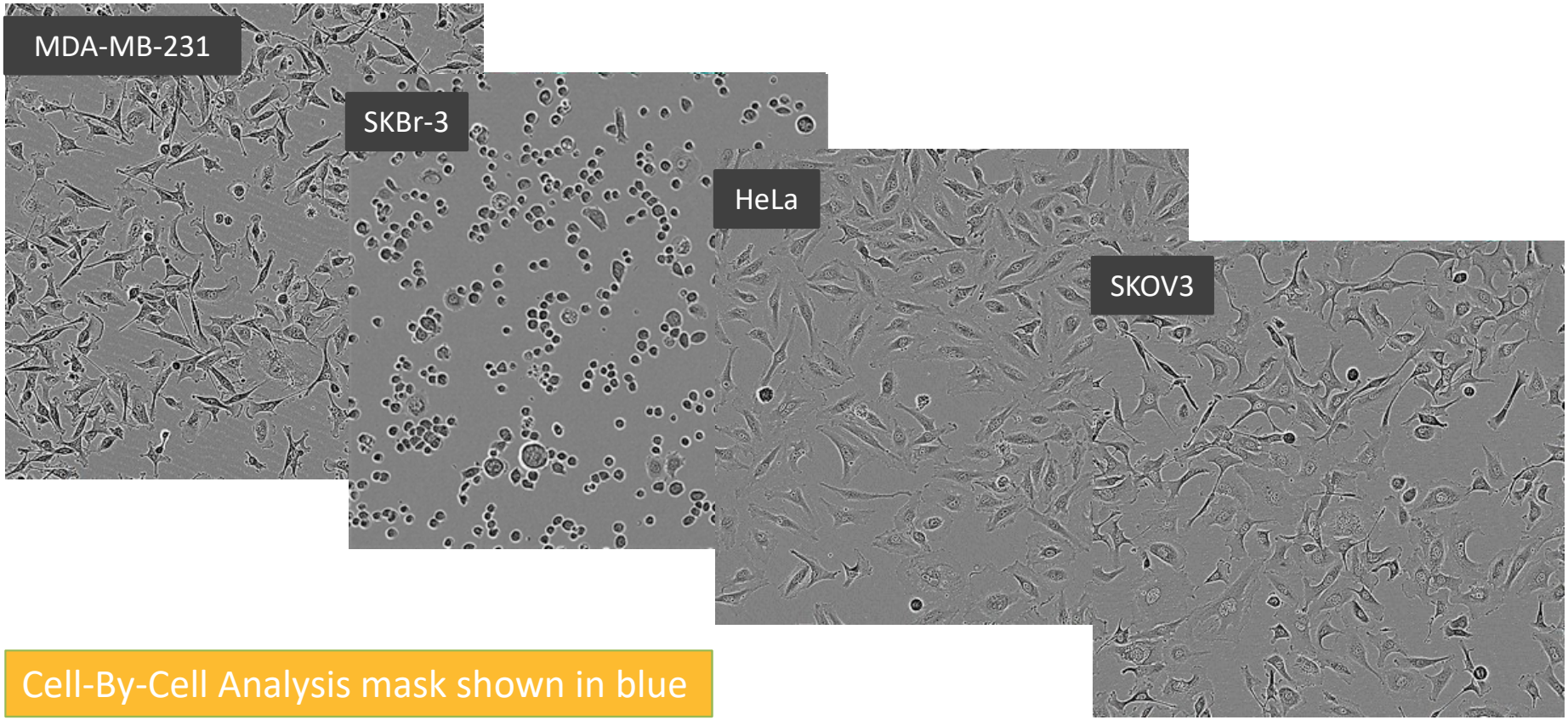


Cell Health & Viability

Proliferation

Adherent label-free cell counts with Cell-by-Cell Analysis

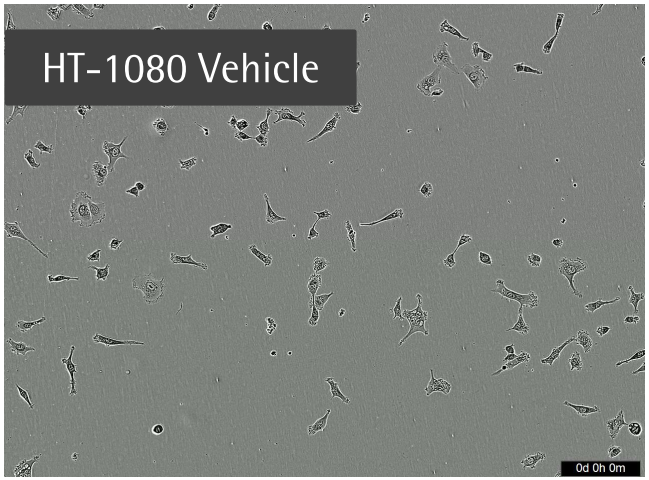
- ✓ Perform label-free cell count through individual cell segmentation
- ✓ Analyze sub-populations of cells with different morphologies

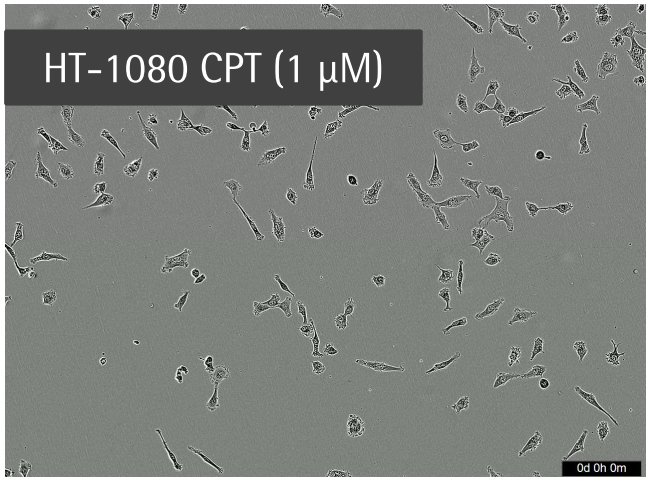


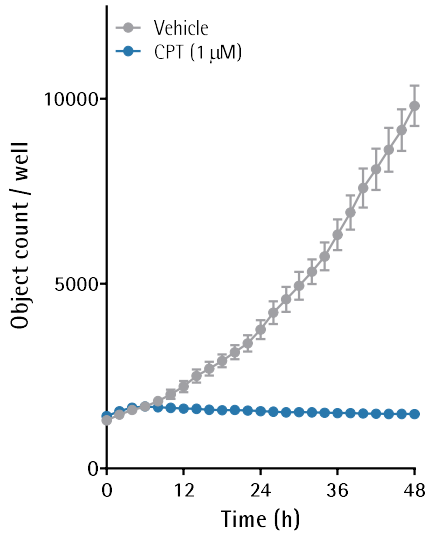
Cell-By-Cell Analysis mask shown in blue

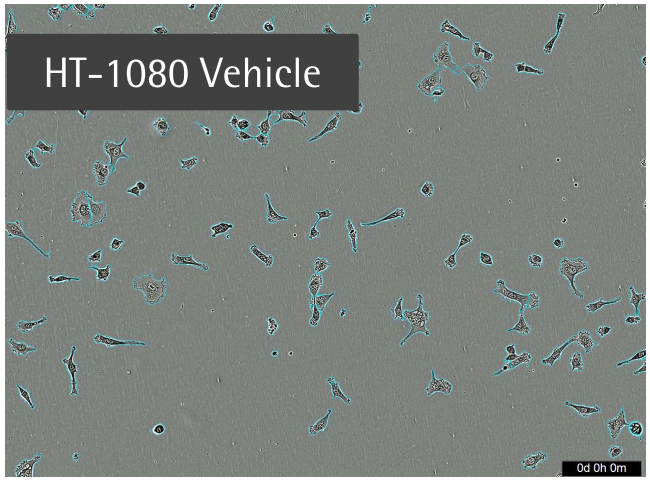
Proliferation

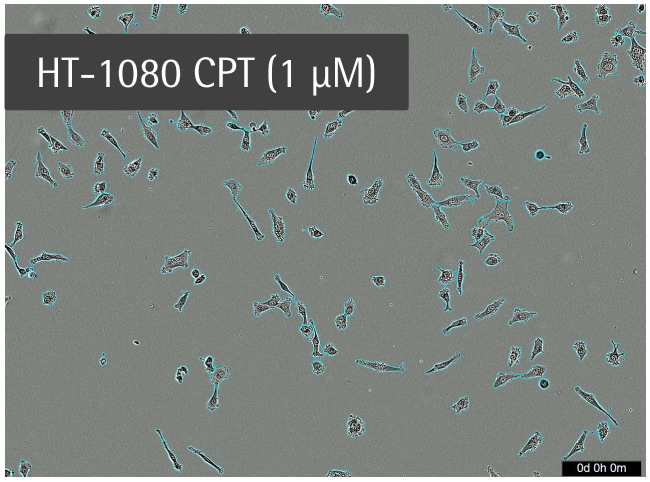
Label-free quantification of proliferation with Cell-By-Cell Analysis shows concentration dependent response to camptothecin

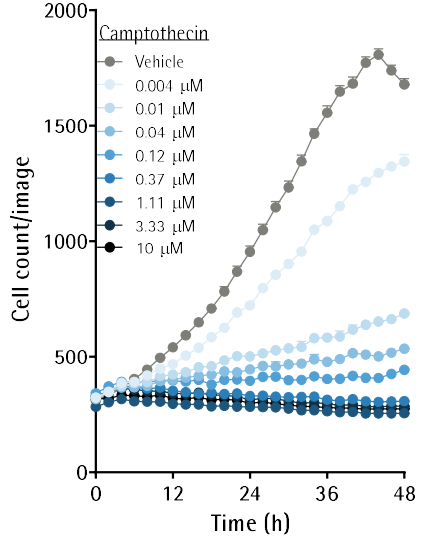










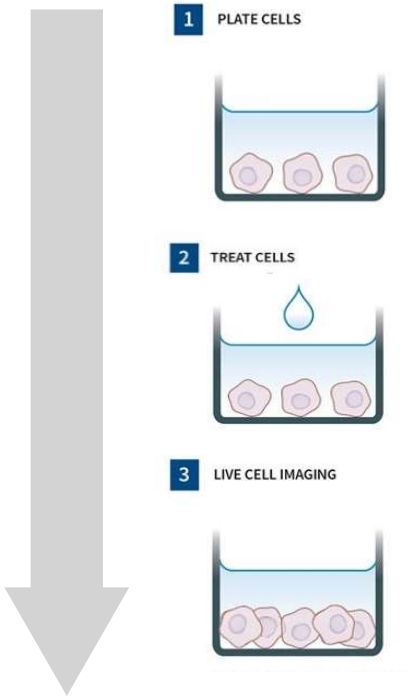


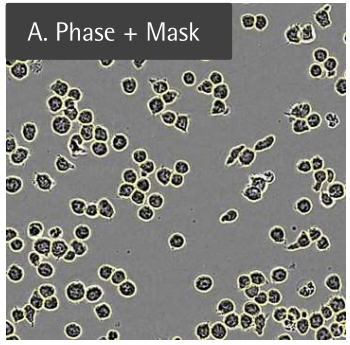
Cell Health & Viability

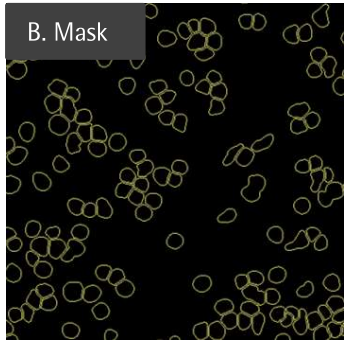
Proliferation

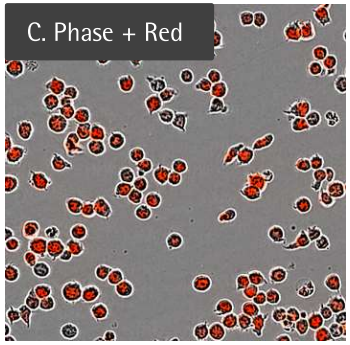
Measure non-adherent cell counts using label-free Cell-by-Cell Analysis

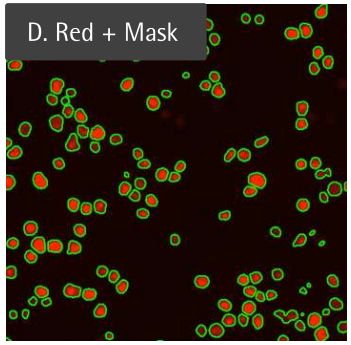
- ✓ Individual cell segmentation using the Cell-by-Cell Analysis Software Module
- ✓ Label-free counting of non-adherent phase objects
- ✓ Can be multiplexed with cell health reagents and FabFluor antibody reagents

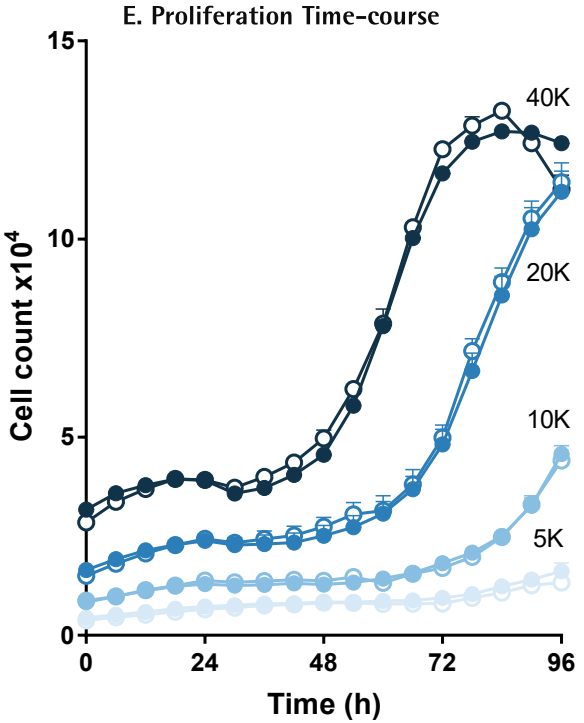










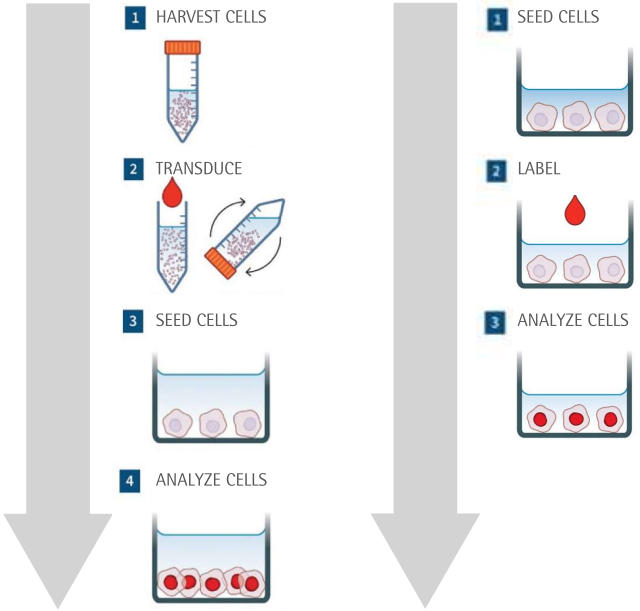


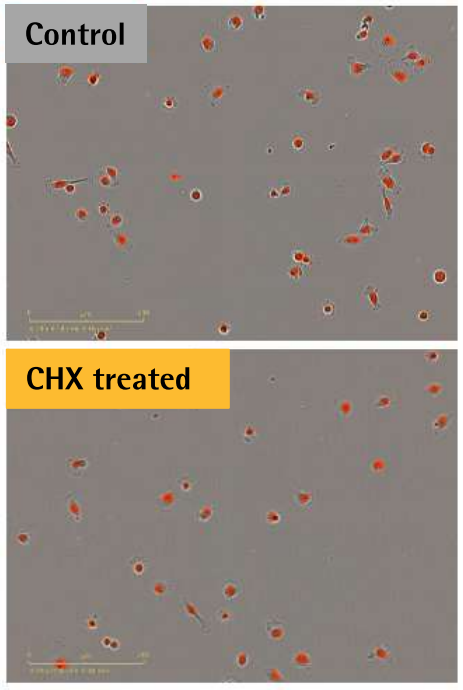
Cell Health & Viability

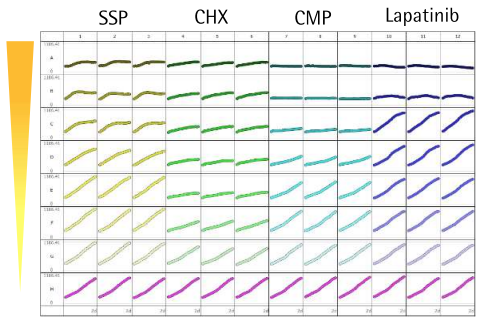
Proliferation

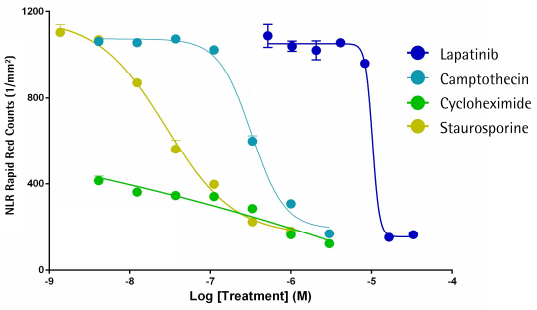
Measure viable cell counts with IncuCyte® NucLight Reagents

- ✓ Label cells using non-perturbing reagents for real-time cell counts
- ✓ Evaluate dynamic co-culture models such as immune cell killing assays
- ✓ Multiplex with cell health reagents to characterize test compounds



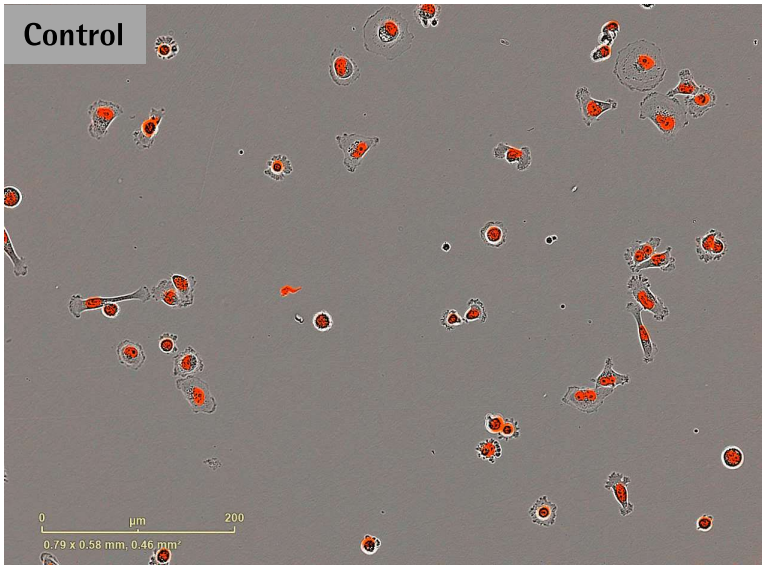


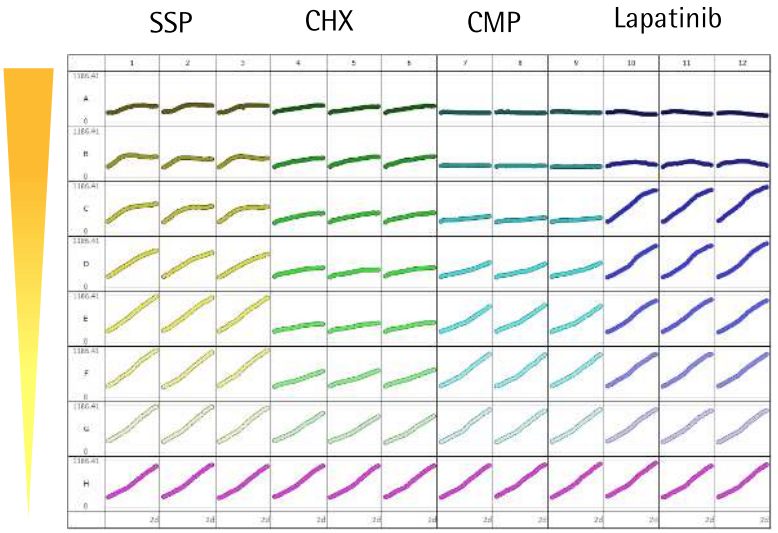


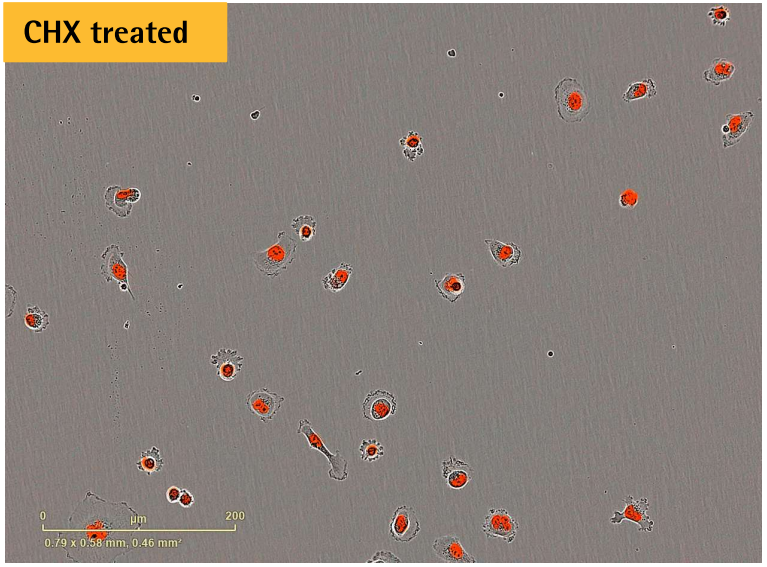


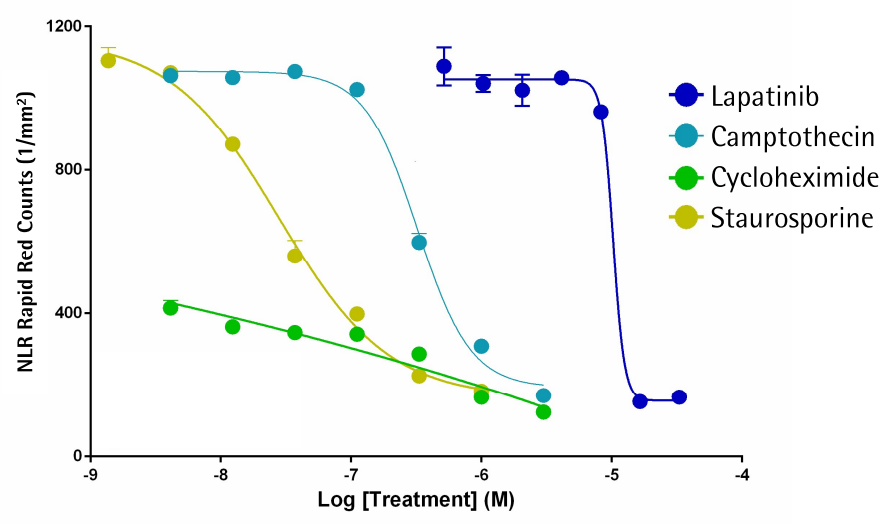
Proliferation

MDA-MB-231 cells + NucLight Red Lentivirus in presence of different compounds







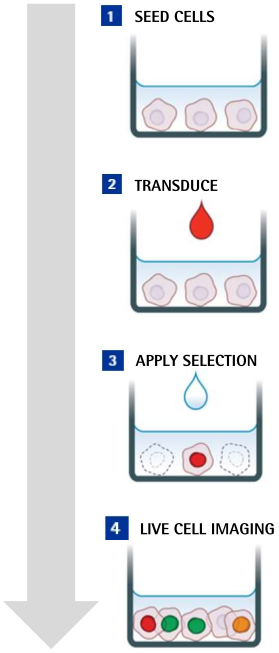


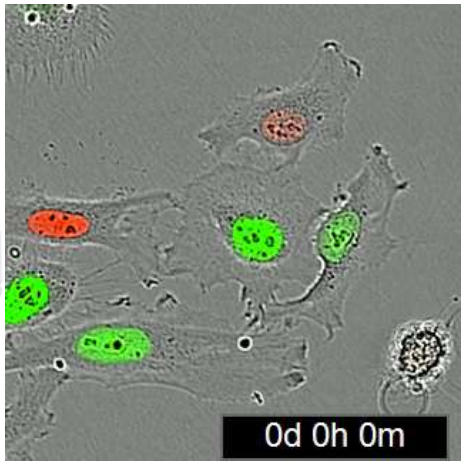
Cell Health & Viability

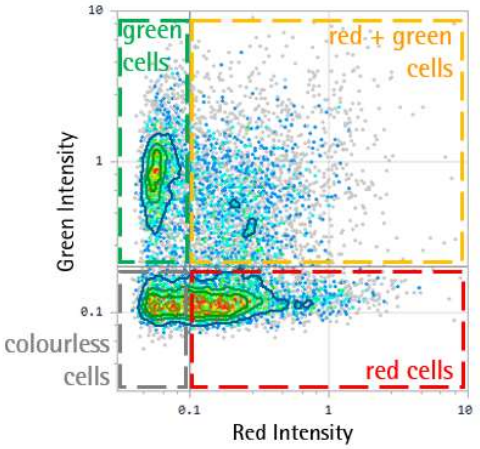
Cell Cycle

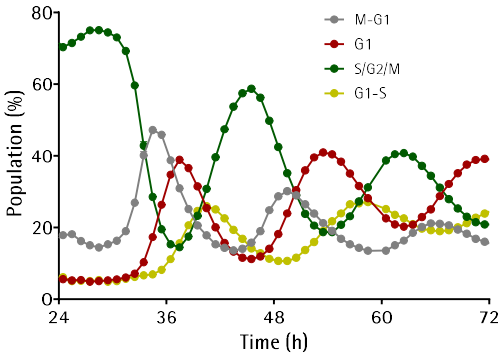
Follow cell cycle progression over time in living cells

- ✓ Track cell cycle populations over multiple cell divisions
- ✓ Achieve microplate throughput with a turnkey solution
- ✓ Gain dynamic insight into drug-induced treatment effects on proliferation







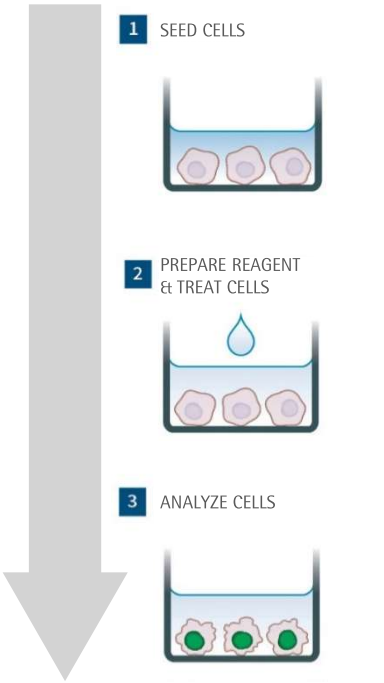


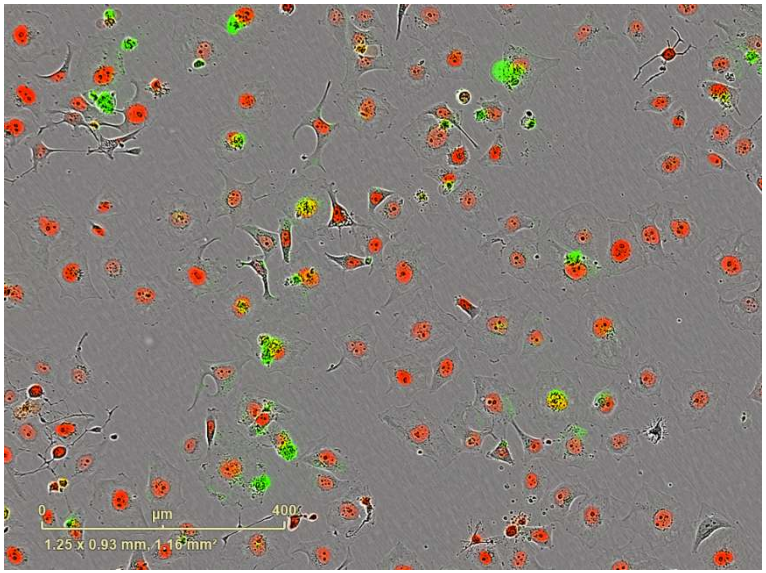
Cell Health & Viability

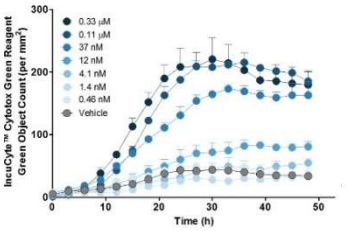
Cytotoxicity

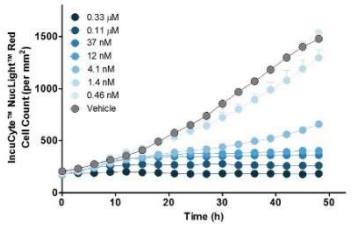
Quantify cytotoxicity using IncuCyte® CytoTox Reagents

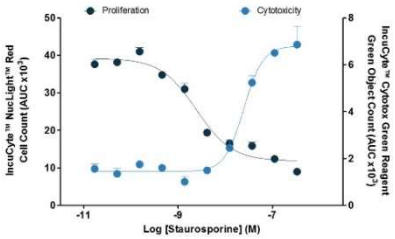
- ✓ Automatic analysis of time course of cell death inside your incubator
- ✓ Multiplex with proliferation and apoptosis measurements





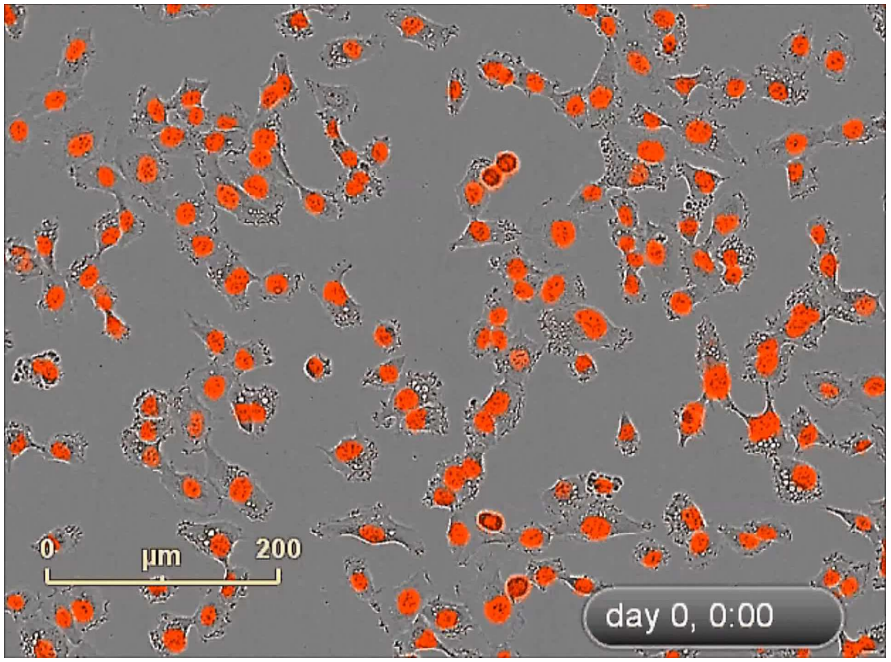


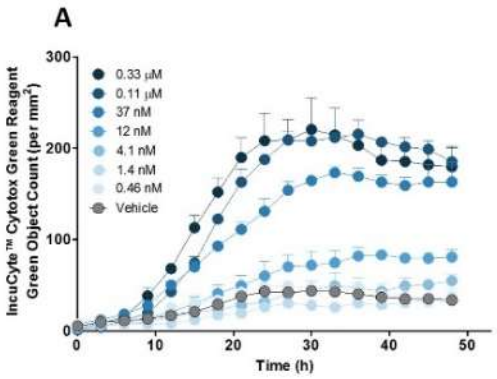


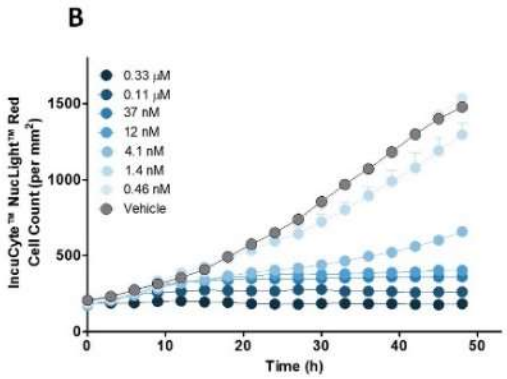


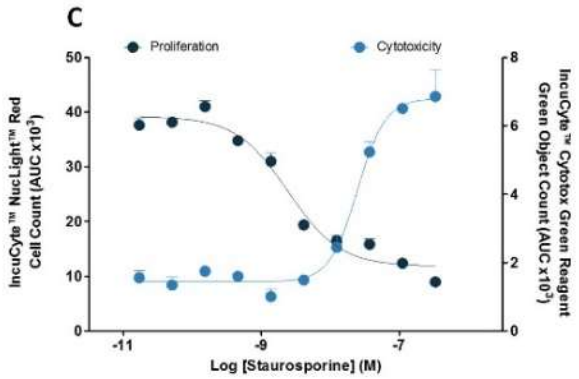
Cytotoxicity

MDA-MB-231 NucLight Red Cells treated with different concentrations of Camptothecin in presence of Cytotox Green







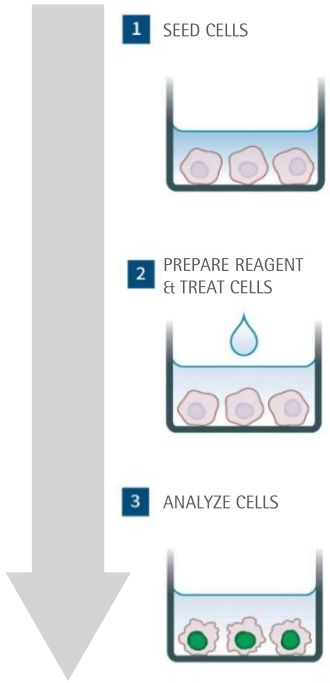


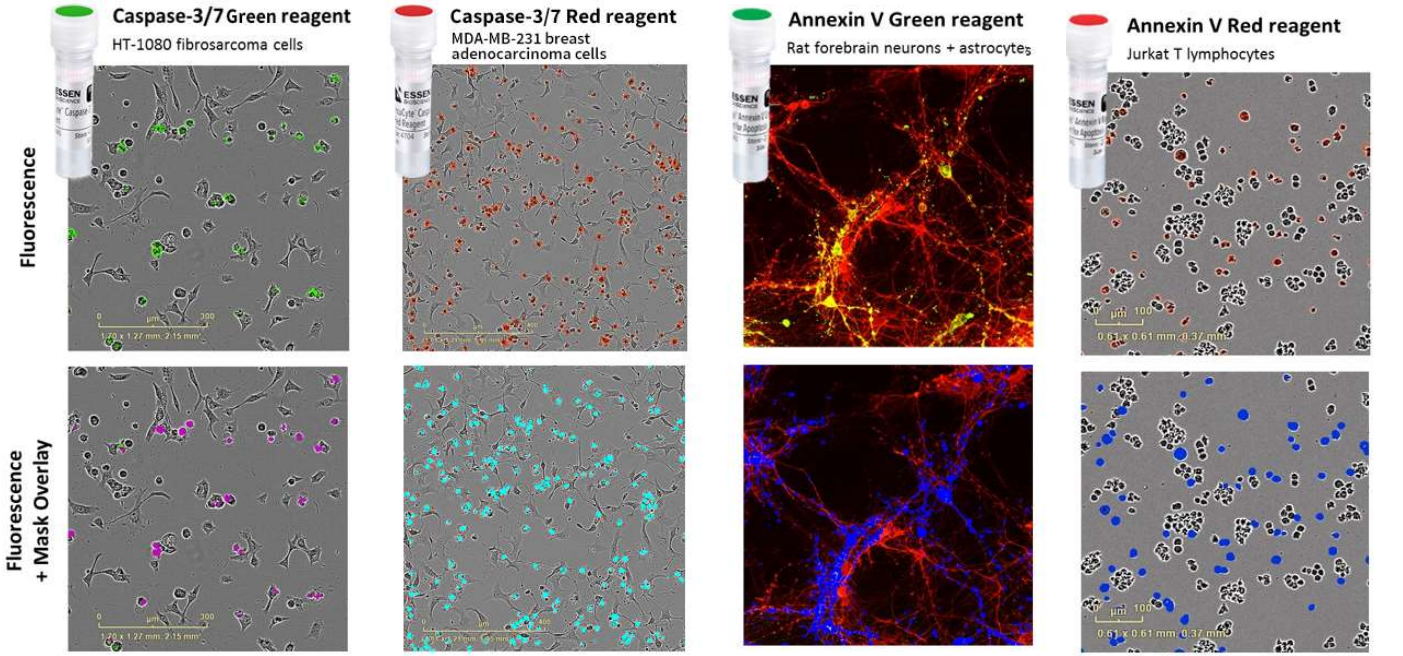
Cell Health & Viability

Apoptosis

Measure apoptotic cell death using IncuCyte® Caspase-3/7 & Annexin V Reagents

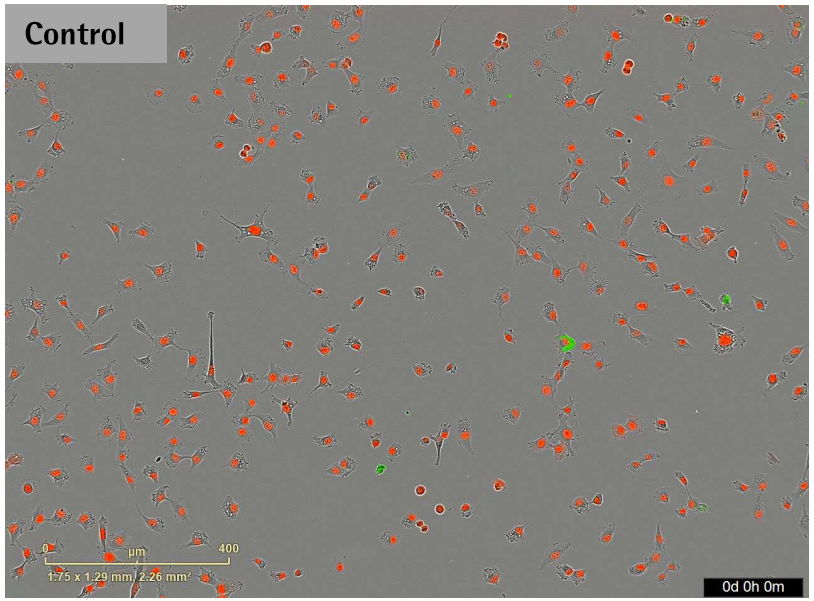
- ✓ Non-perturbing, mix-and-read reagents
- ✓ Caspase-3/7 detects both short-term and long term treatment effects by fluorescing in presence of activated caspases.
- ✓ Annexin V provides early detection of apoptosis upon binding to phosphatidylserine on the outer membrane

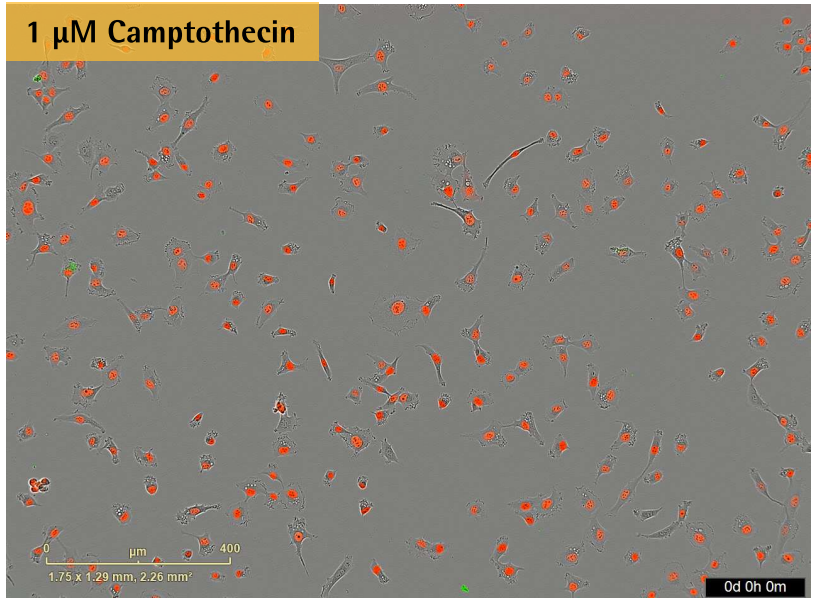


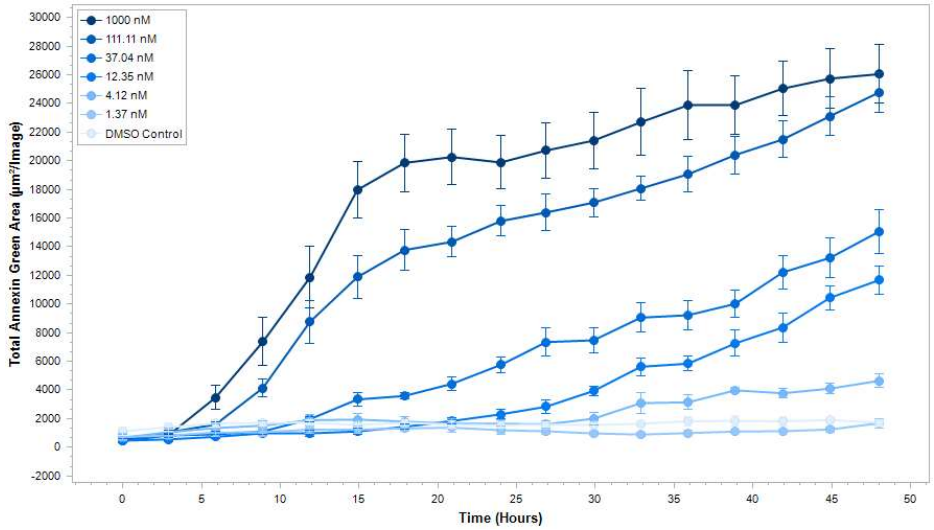


Apoptosis: Adherent Cells

HT-1080 NucLight Red Cells treated with Camptothecin in presence of Annexin V Green

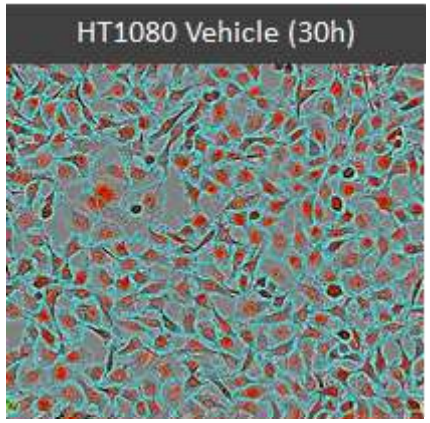


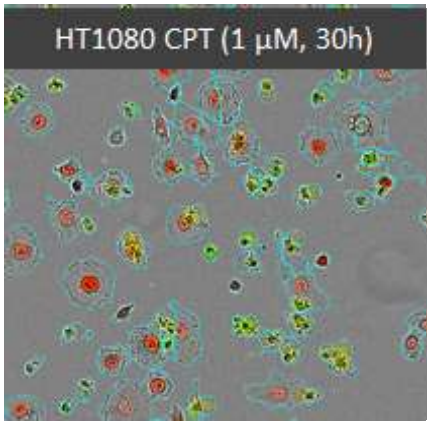




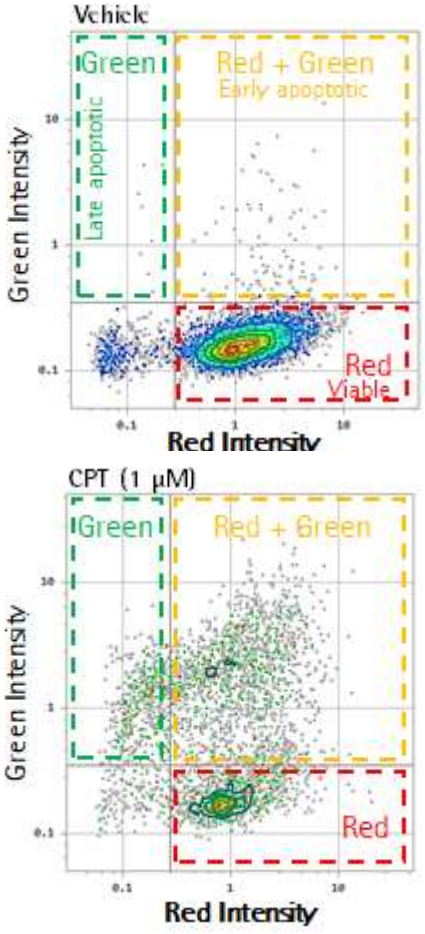
Apoptosis: Adherent Cells

Multiplex apoptosis measurements with label-free cell count and quantify % of live / dead cells with Cell-by-Cell Analysis

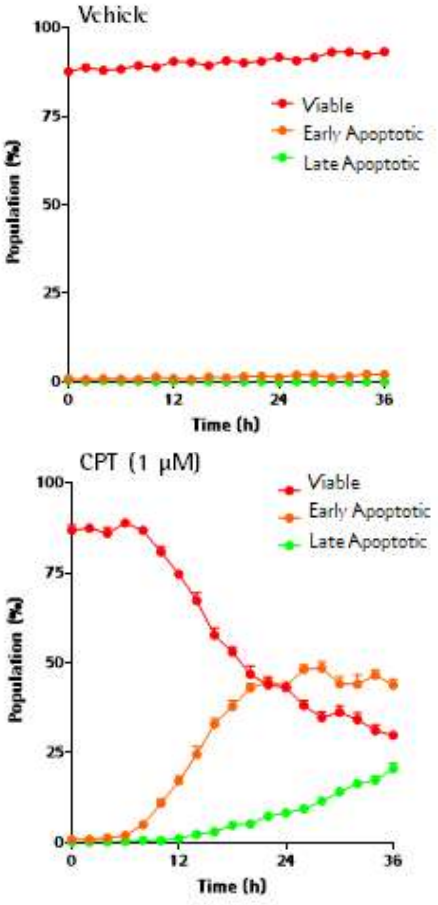




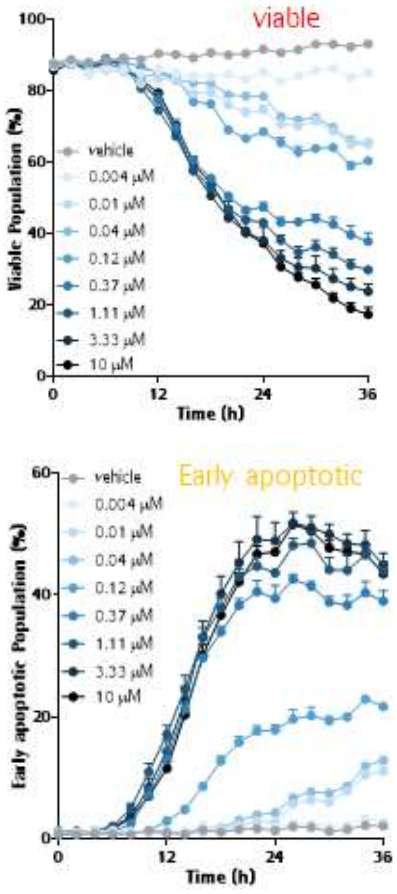
Identification



Classification



Population tracking



Concentration response

Apoptosis: Non-Adherent Cells

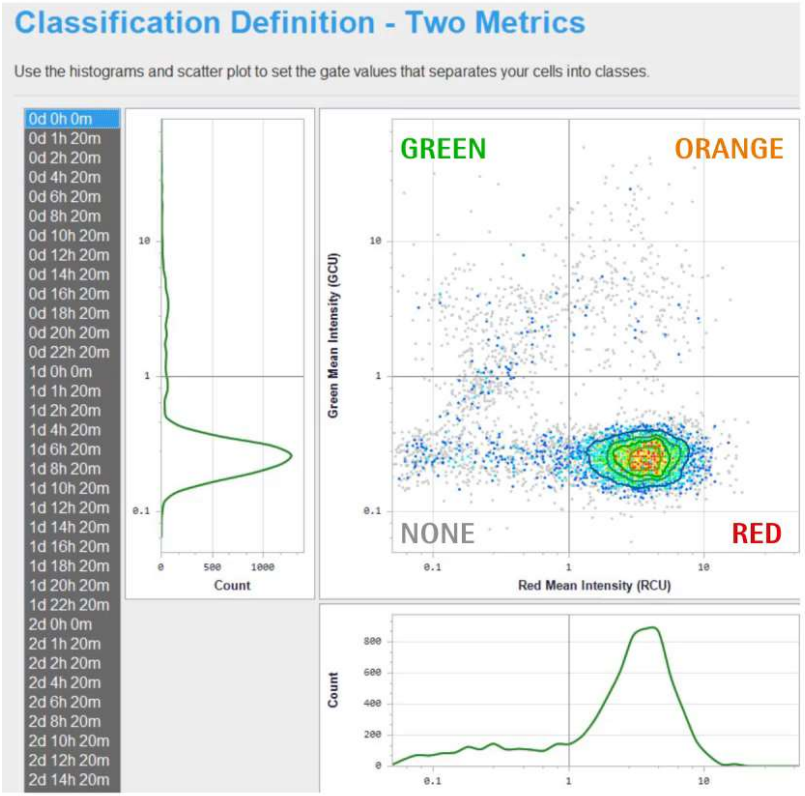
Jurkat NucLight Red Cells treated with Camptothecin in presence of Caspase-3/7 Green

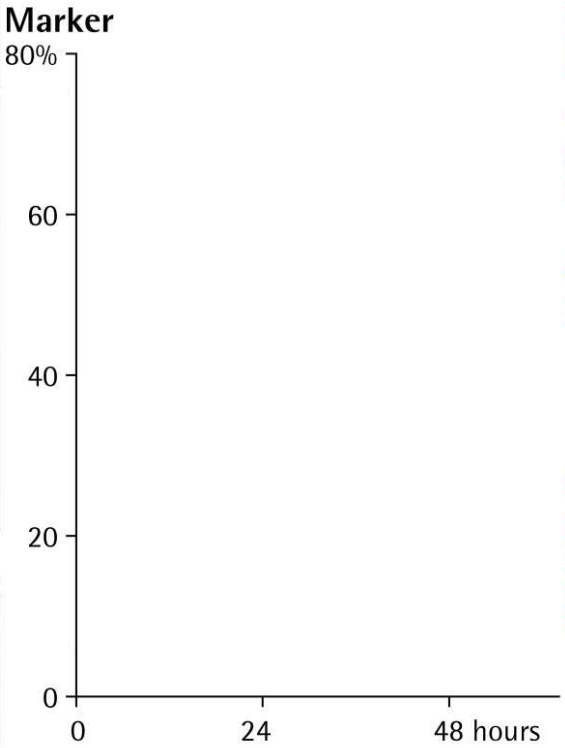
sartorius

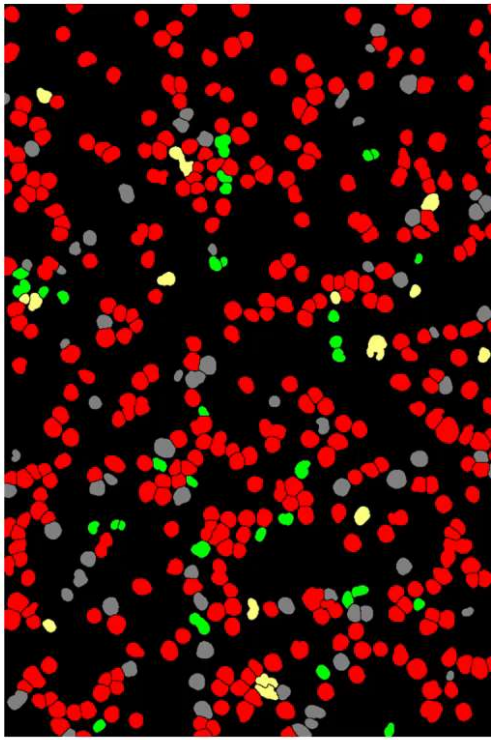
IncuCyte® S3 Cell-by-Cell Classification and Analysis

Images and data generated with the IncuCyte® S3 Live-Cell Analysis System.

sartorius





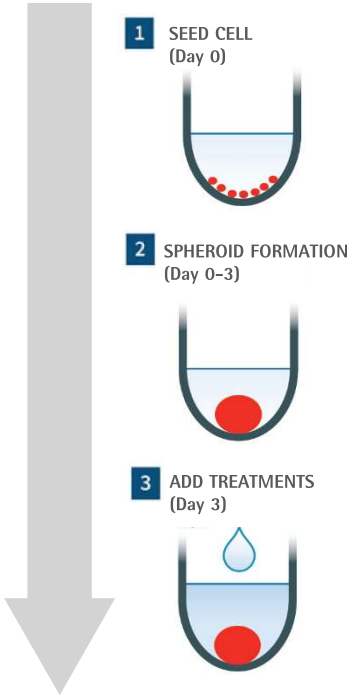


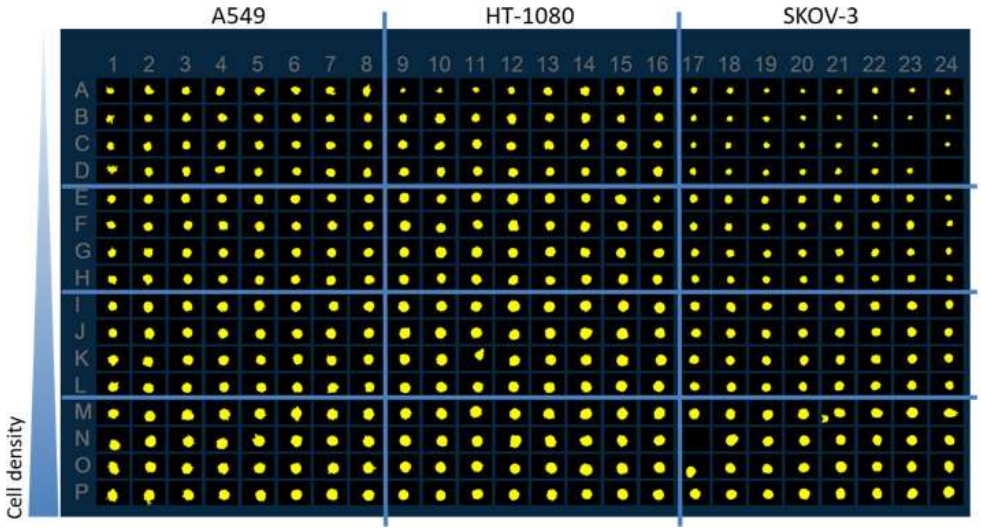
Cell Health & Viability

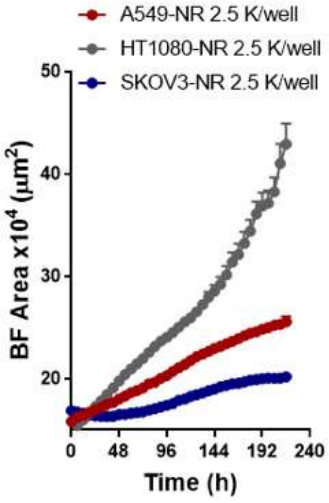
Spheroid

Quantify spheroid cell growth, shrinkage, and death in U-bottom plates

- ✓ Continuously measure spheroid growth and shrinkage in 96- & 384- well format
- ✓ Duplex brightfield analysis with cell health readouts



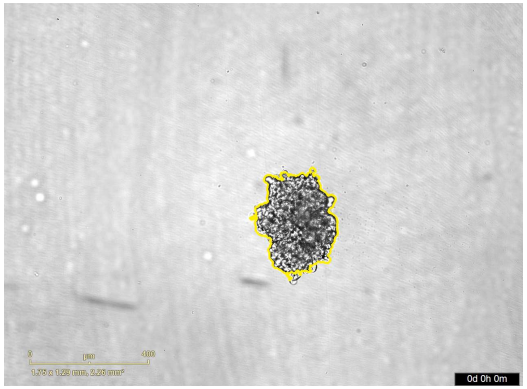
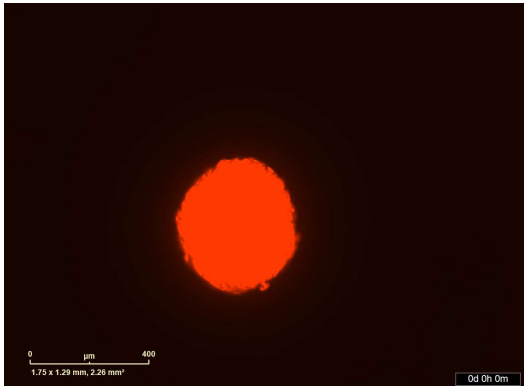
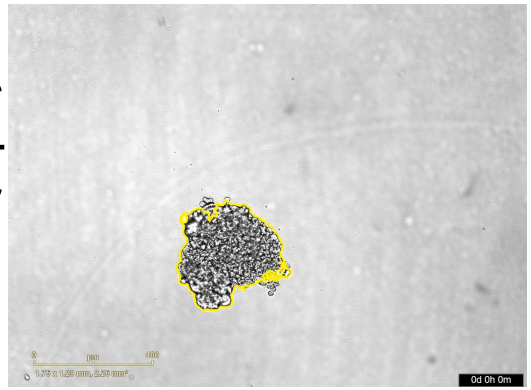
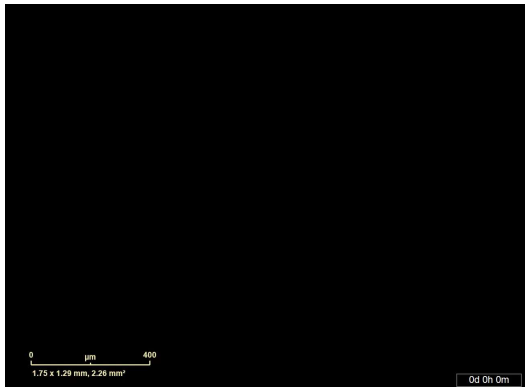
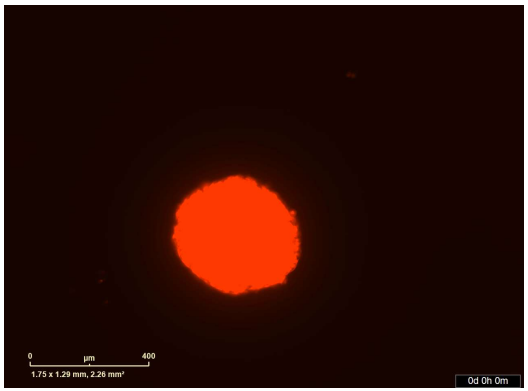


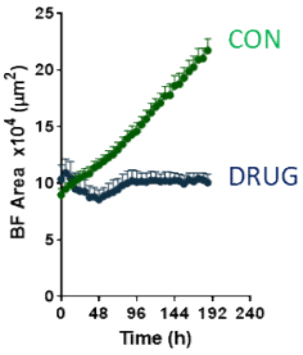


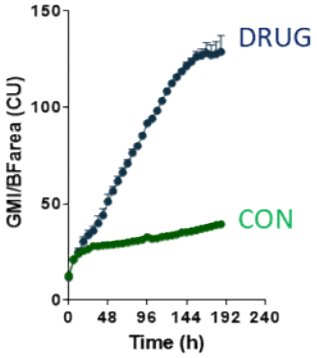
Spheroid: U-Bottom Plates

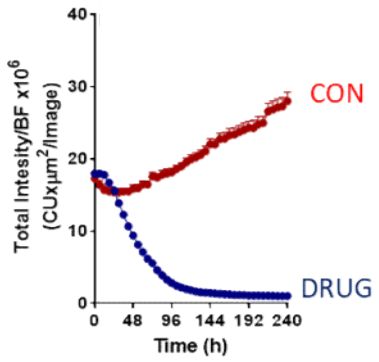
SKOV-3 cells treated with Camptothecin

sartorius

	Brightfield	CytoTox Green	NucLight Red
Untreated			
+ CMP (1 µM)			





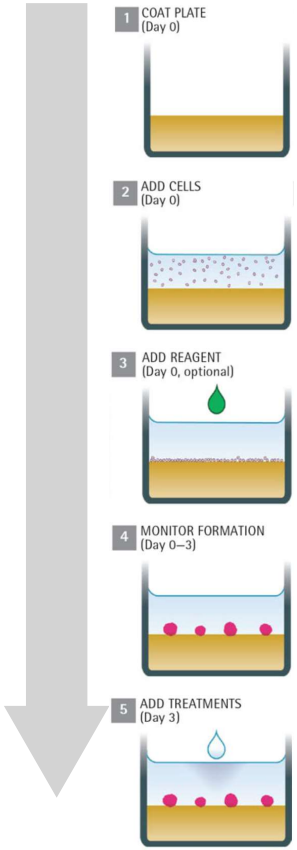


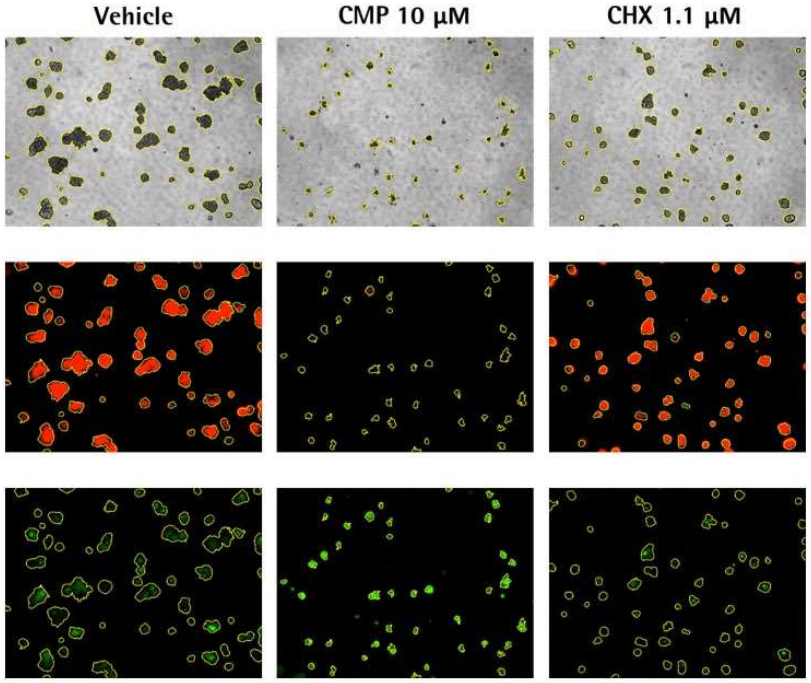
Cell Health & Viability

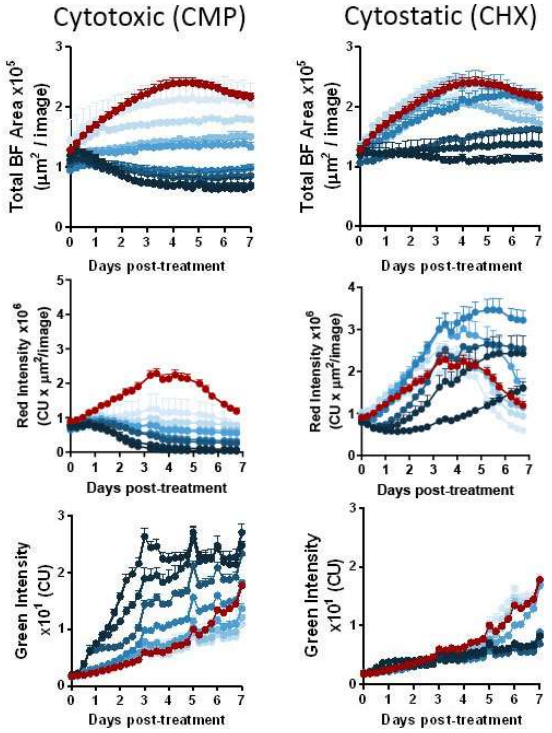
Spheroid

Quantify growth, shrinkage, and death of 3D Scaffold-Based Multi-tumor Models

- ✓ Quantify label-free growth and investigate morphology of 3D multi-tumor spheroid cultures on Matrigel®
- ✓ Multipex with cell health reagents to investigate mechanism of action

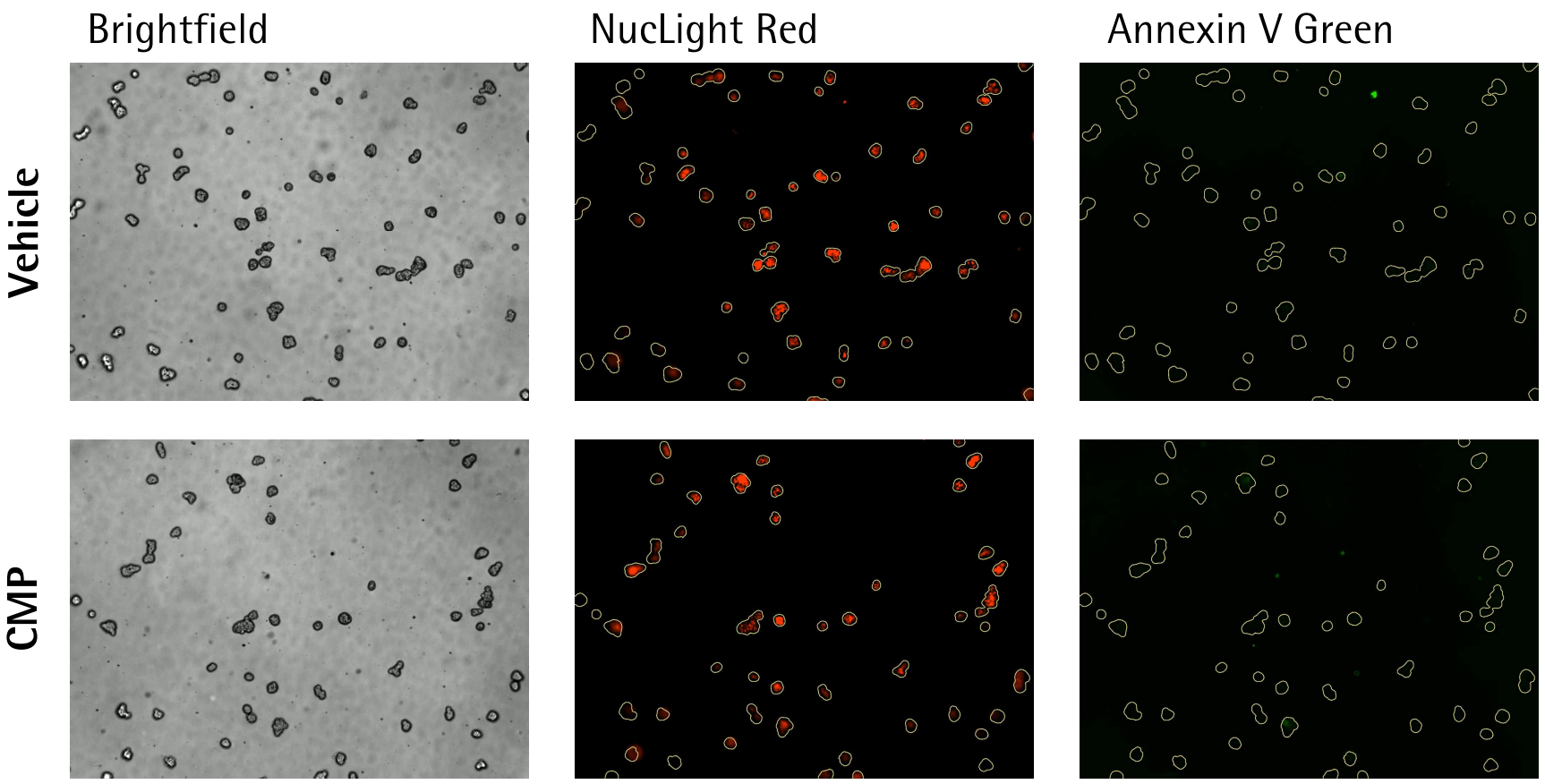






Spheroid: 3D Scaffold Based Multi-Tumor Models

A549 NucLight Red cells treated with Camptothecin in presence of Annexin V Green

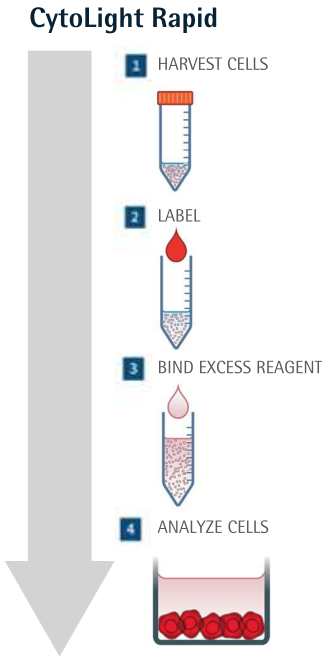


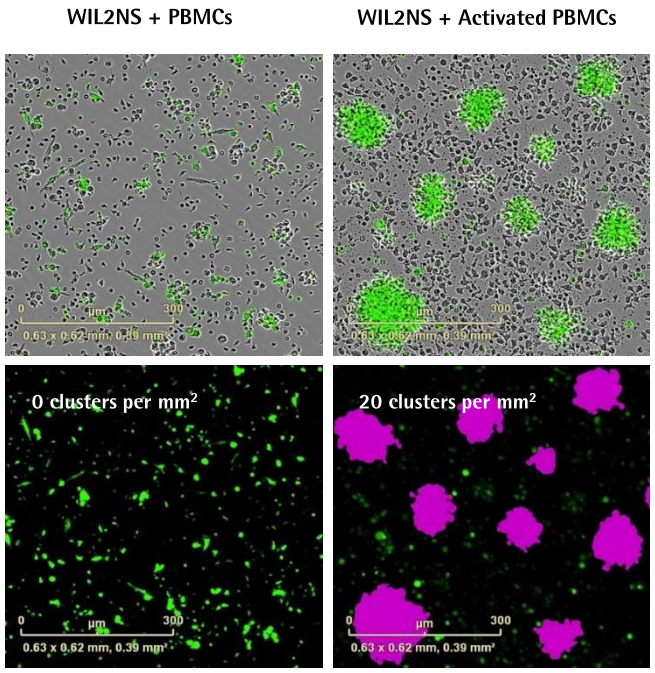
Cell Function & Interactions

Immune Cell Activation

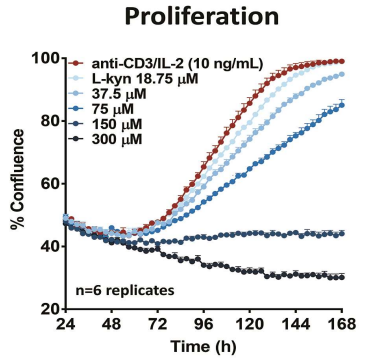
Classify activated immune cell populations using IncuCyte® reagents and Cell-by-Cell Analysis

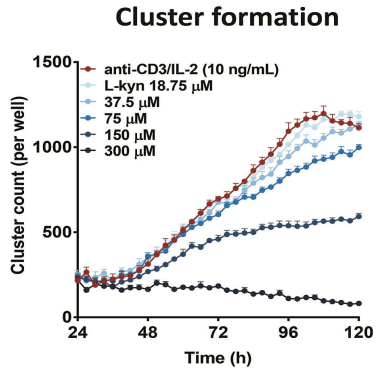
- ✓ Label-free or labelled real-time measurements of morphology changes associated with immune cell activation
- ✓ Individual cell segmentation and classification using Cell-by-Cell Software Module
- ✓ Monitor clustering of activated T-cells using CytoLight Rapid Reagents





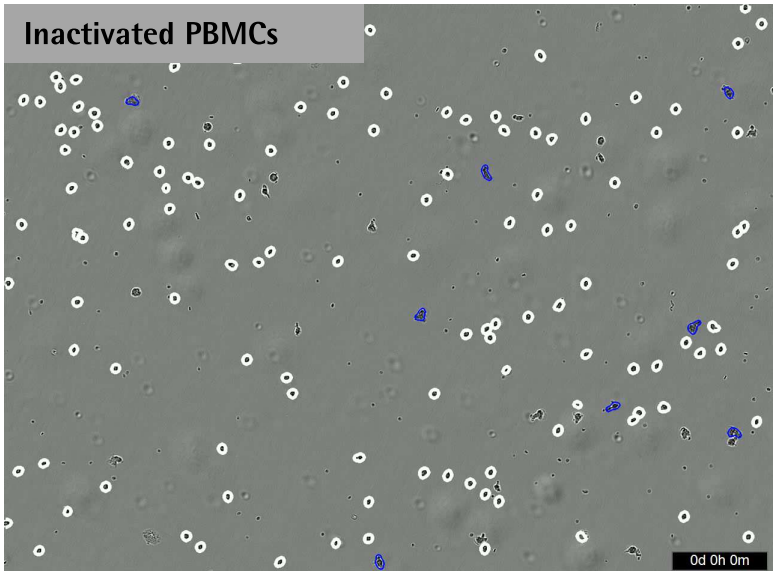
PBMCs labelled with IncuCyte® CytoLight Rapid Green

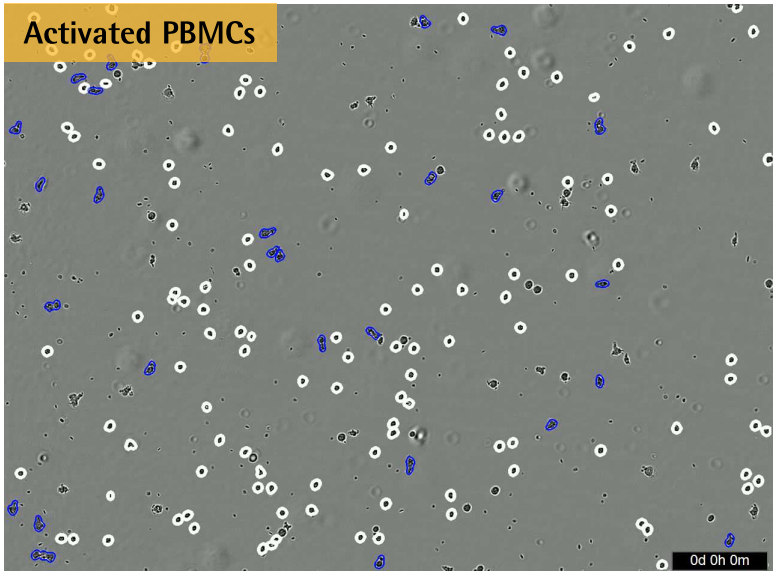


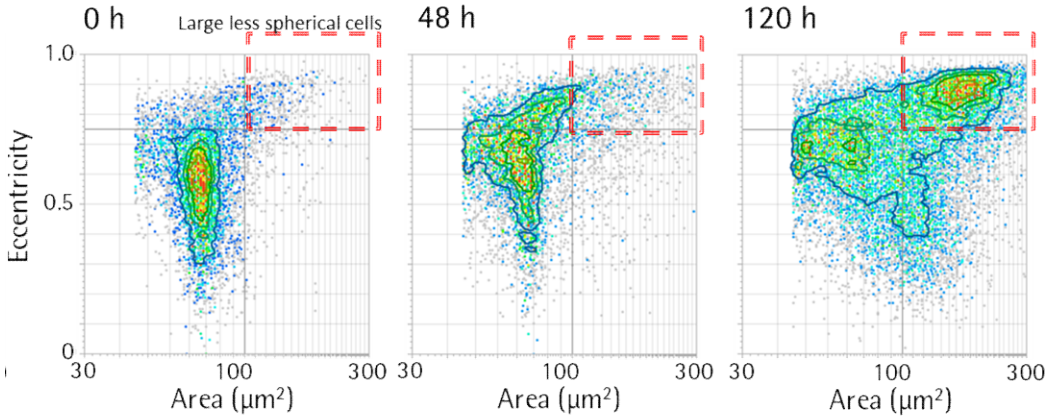


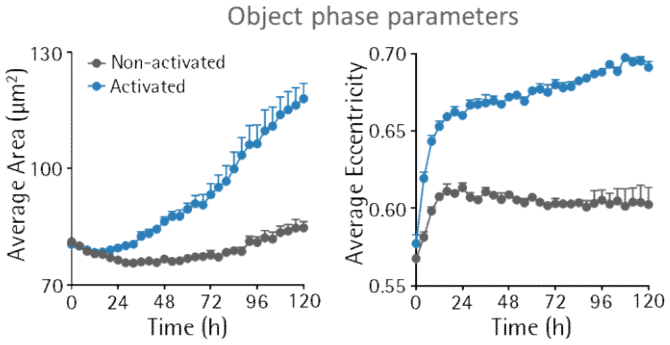
Immune Cell Activation

PBMC + CD3/IL-2 Measuring Roundness and Size of Cells









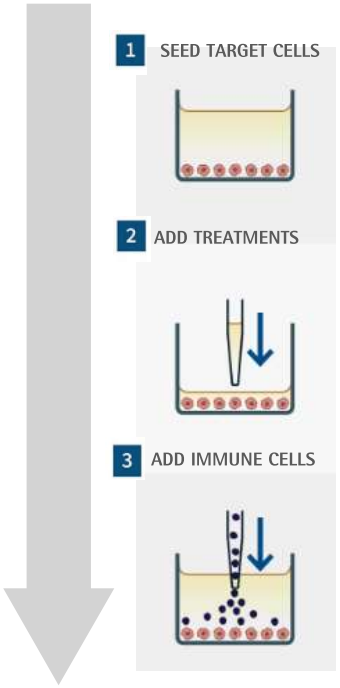
Live Cell Immunocytochemistry

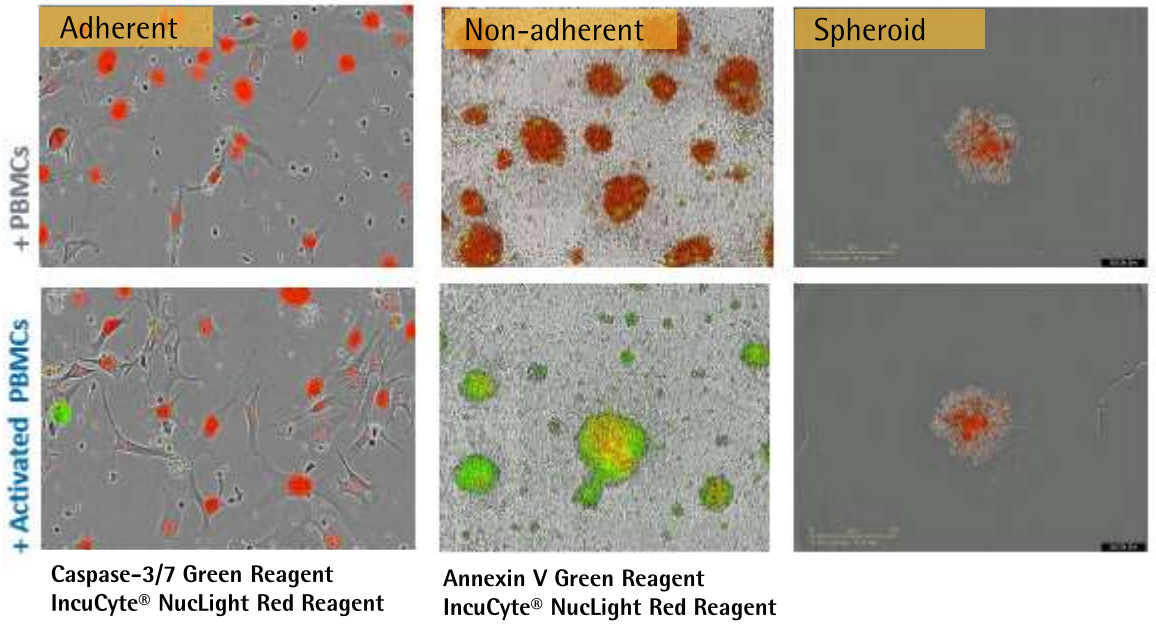
Cell Function & Interactions

Immune Cell Killing

Visualize and quantify immune cell killing using Cell Health reagents

- ✓ Direct & robust measurement – true cell counting and cell death
- ✓ No cell lifting, no isotopes or labelling Abs, no wash
- ✓ Flexible application – cell pairings and models (ADCC and cytotoxic)



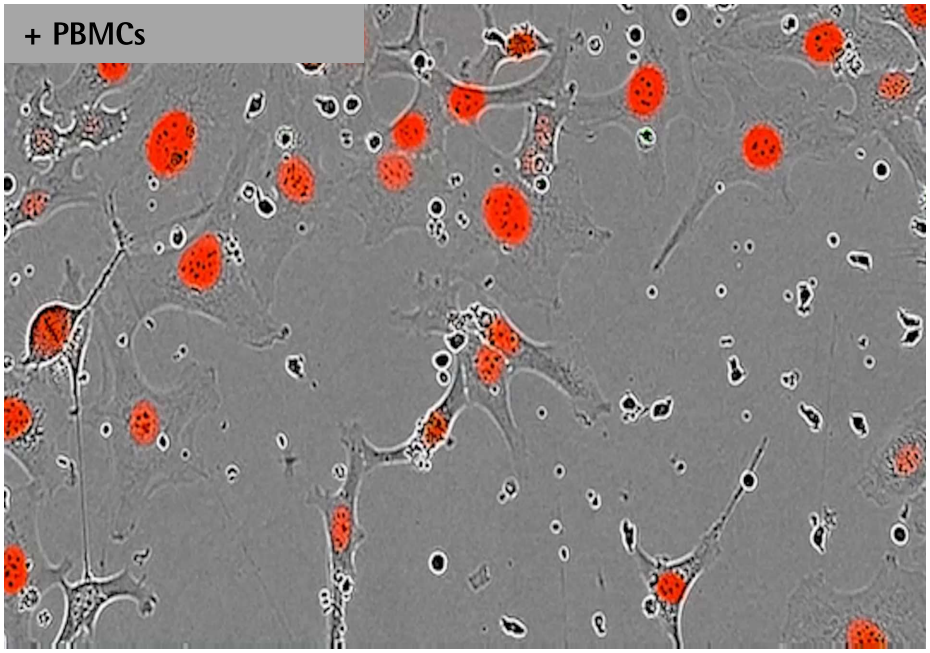


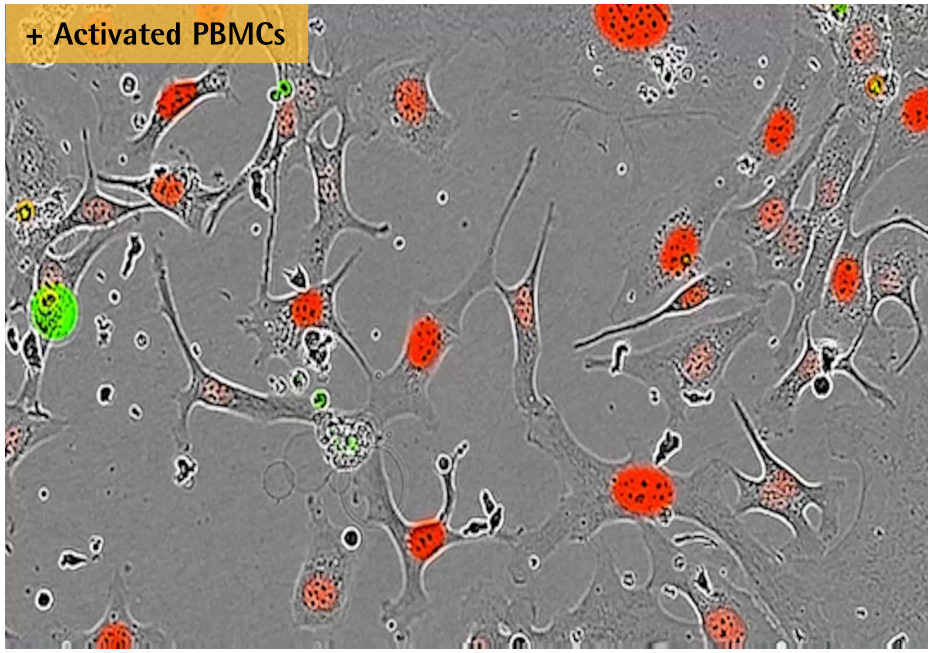
Caspase-3/7 Green Reagent
IncuCyte® NucLight Red Reagent

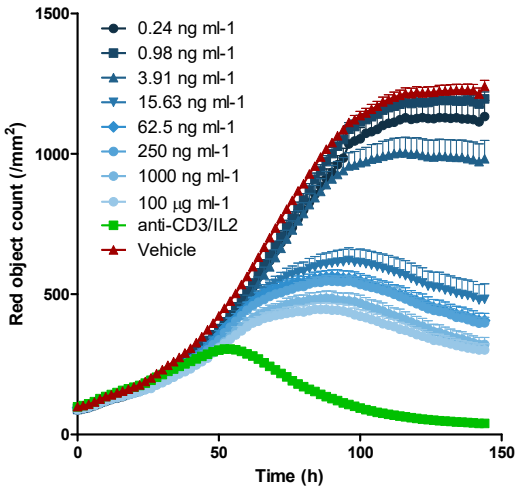
Annexin V Green Reagent
IncuCyte® NucLight Red Reagent

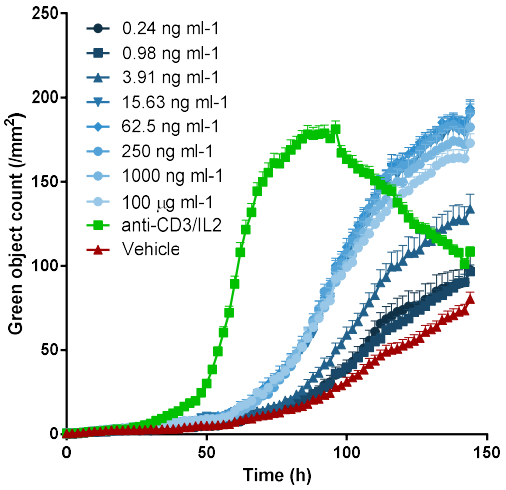
Immune Cell Killing: Adherent Cells

NucLight Red labelled SK-OV-3 cells + human PBMCs in presence of IncuCyte® Caspase-3/7 Green Reagent



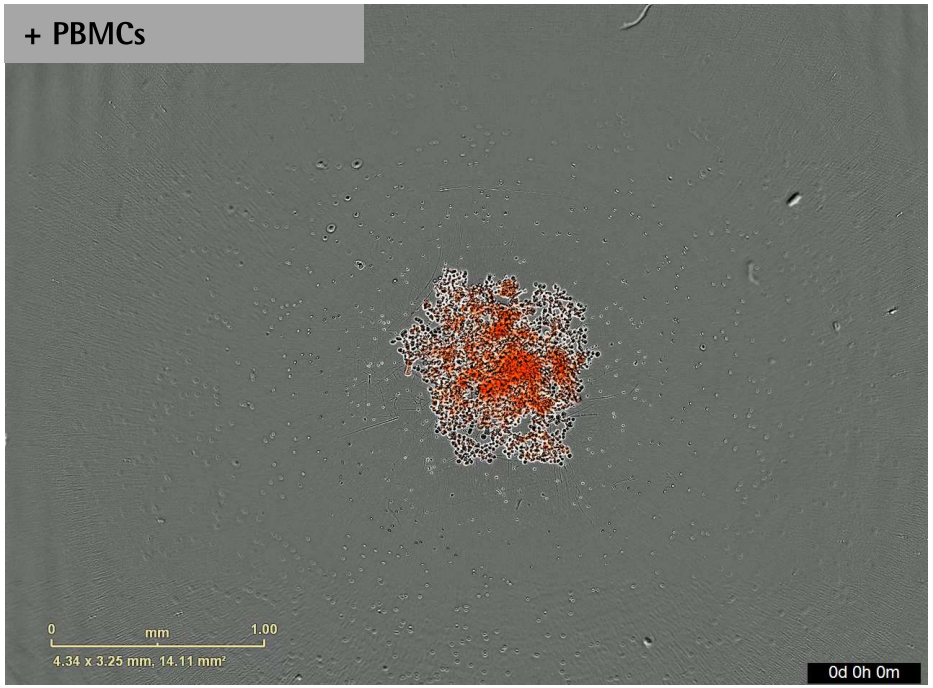


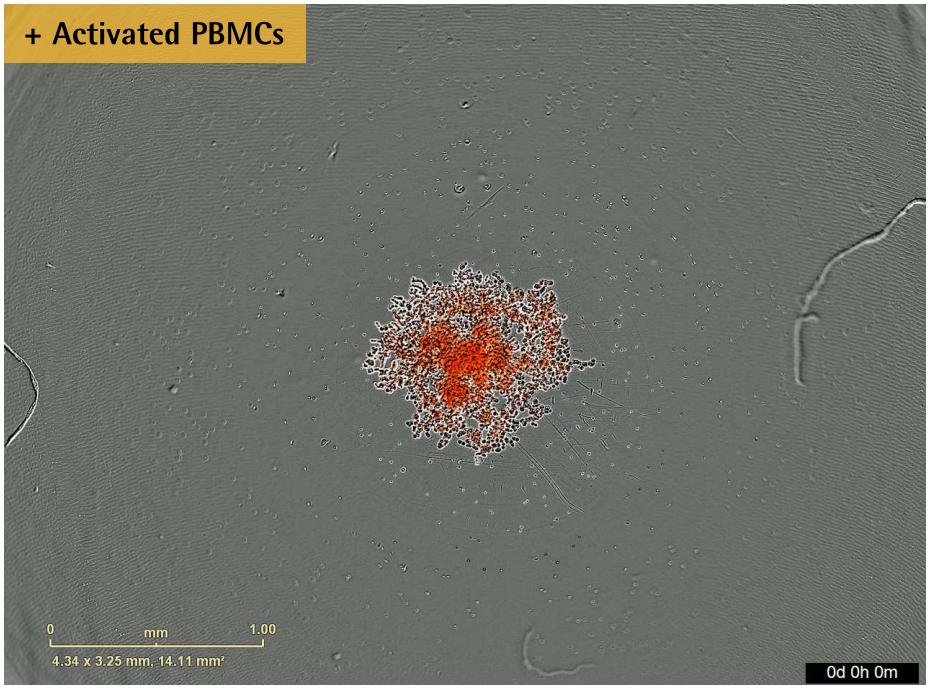


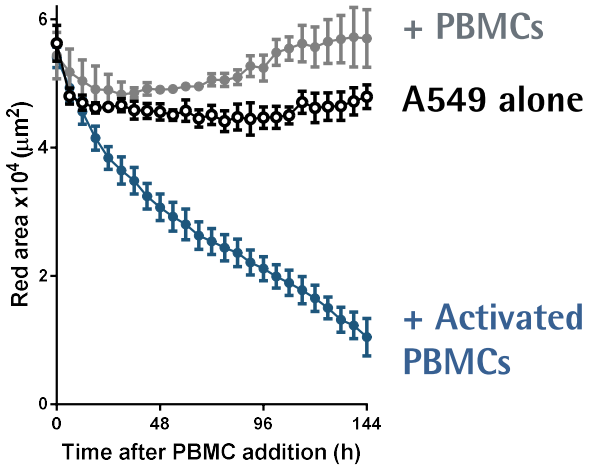


Immune Cell Killing: Tumor Spheroids

NucLight Red labelled WIL2A549+ PBMCs





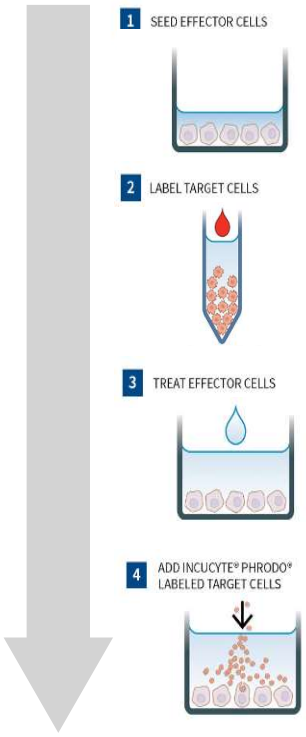


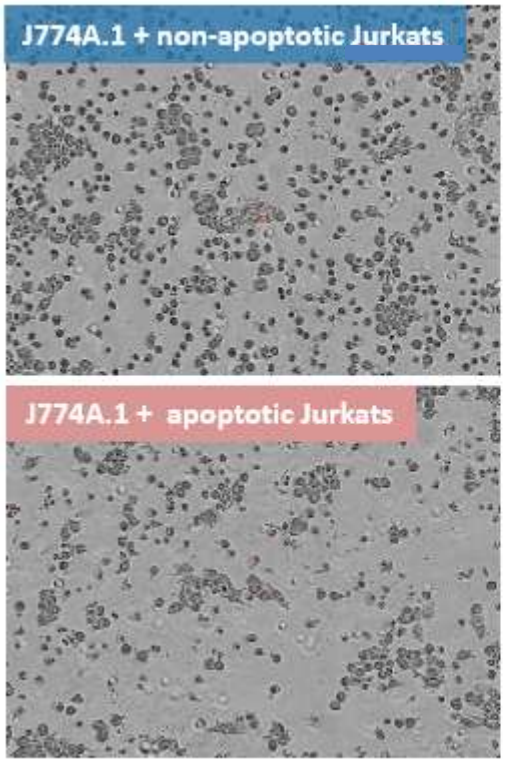
Cell Function & Interactions

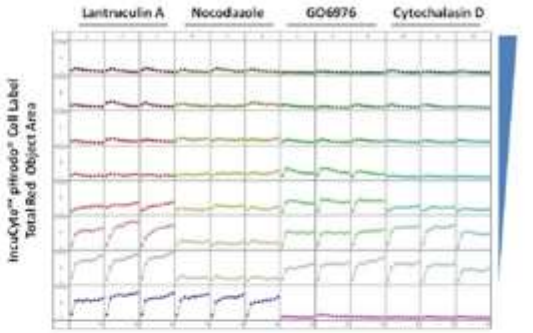
Phagocytosis

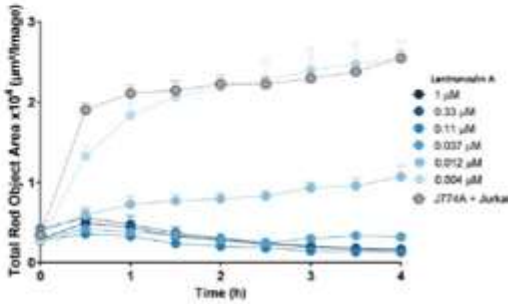
Quantify phagocytosis of dying cells using IncuCyte® pHrodo® Red Cell Labelling Reagent

- ✓ Real-time measurements of cell internalization using your choice of target cells labelled with IncuCyte® pHrodo® Red Cell Labelling Reagent.
- ✓ Confirm phagocytosis using high definition phase contrast images to directly visualize cell engulfment.



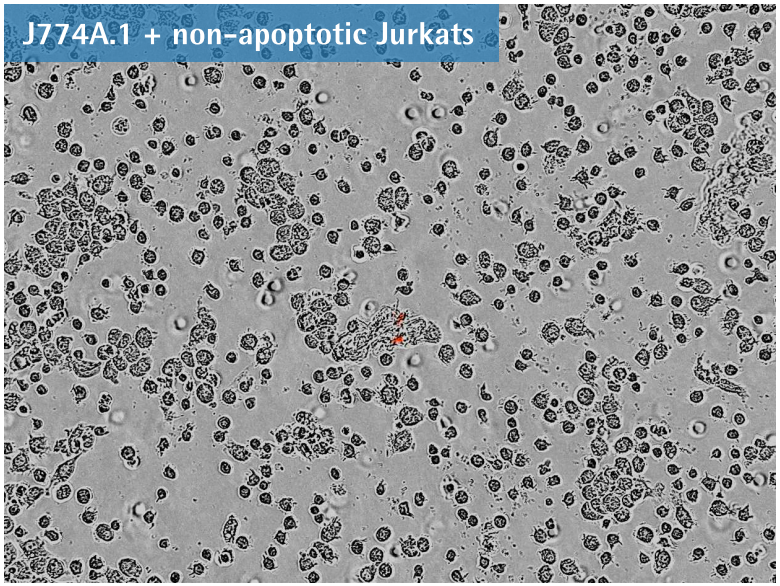


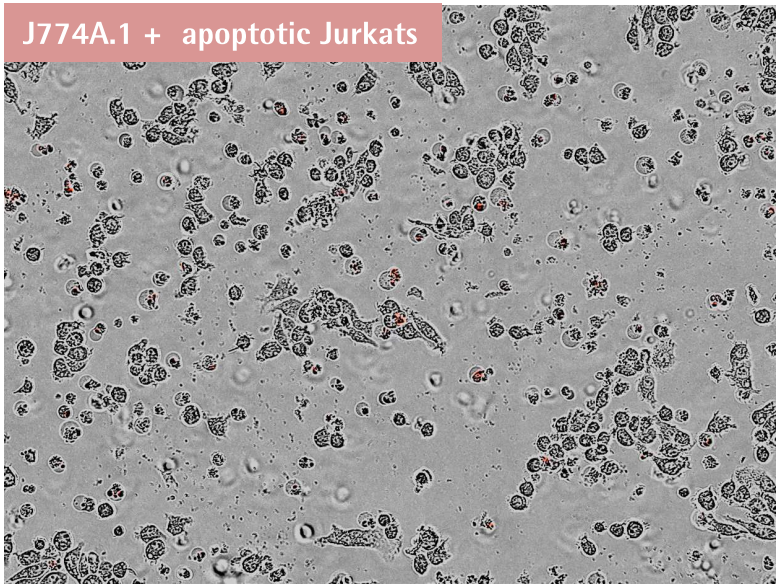


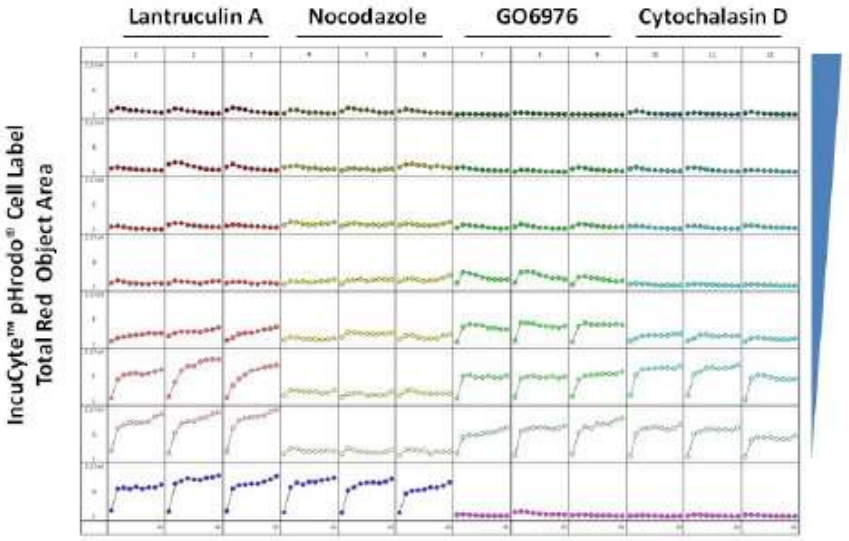


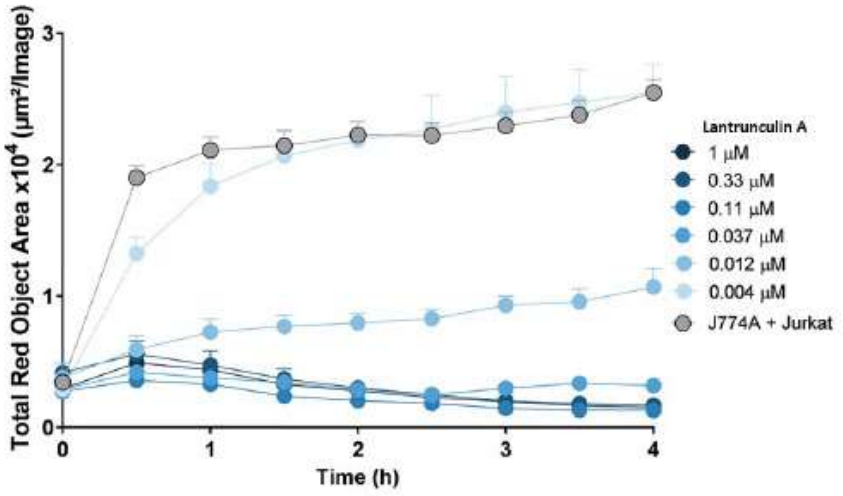
Phagocytosis: Cells

J774A.1 macrophages + pHrodo Red labelled Jurkats









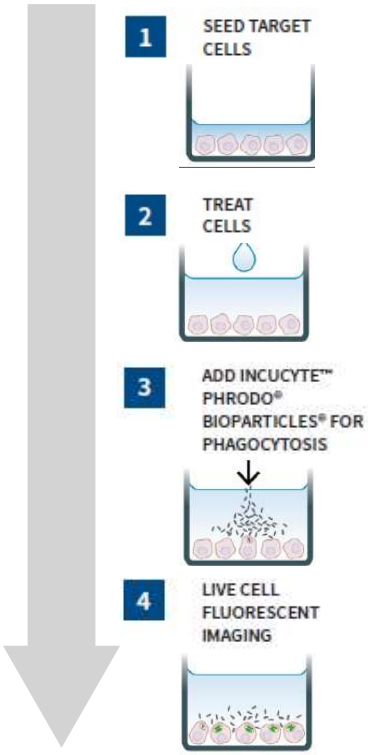
Phagocytosis of Bioparticles

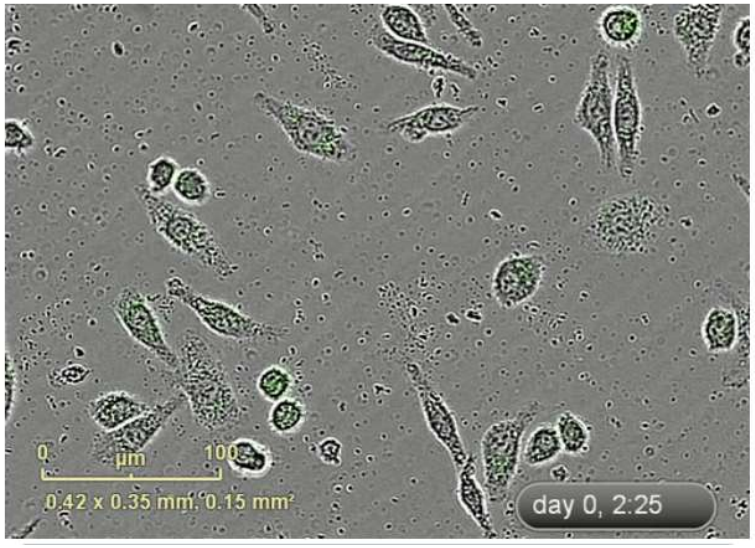
Cell Function & Interactions

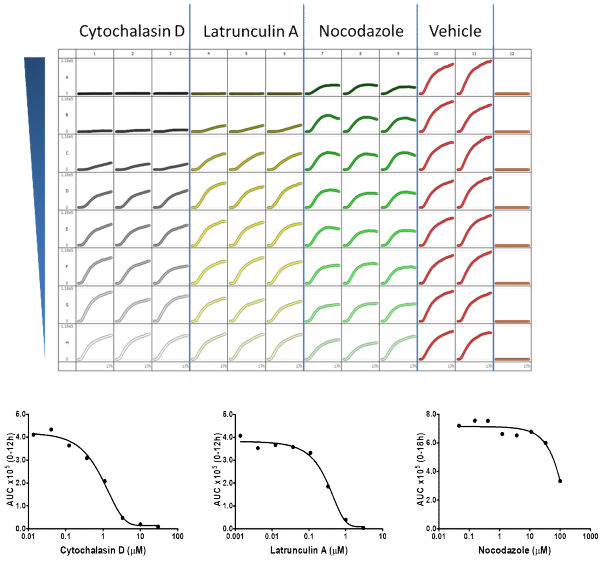
Phagocytosis

Quantify phagocytosis of bacteria or yeast pHrodo® Bioparticles®

- ✓ Simple mix-and-read protocols - no washing, no fixing, no lifting
- ✓ Validate phagocytosis with images and movies

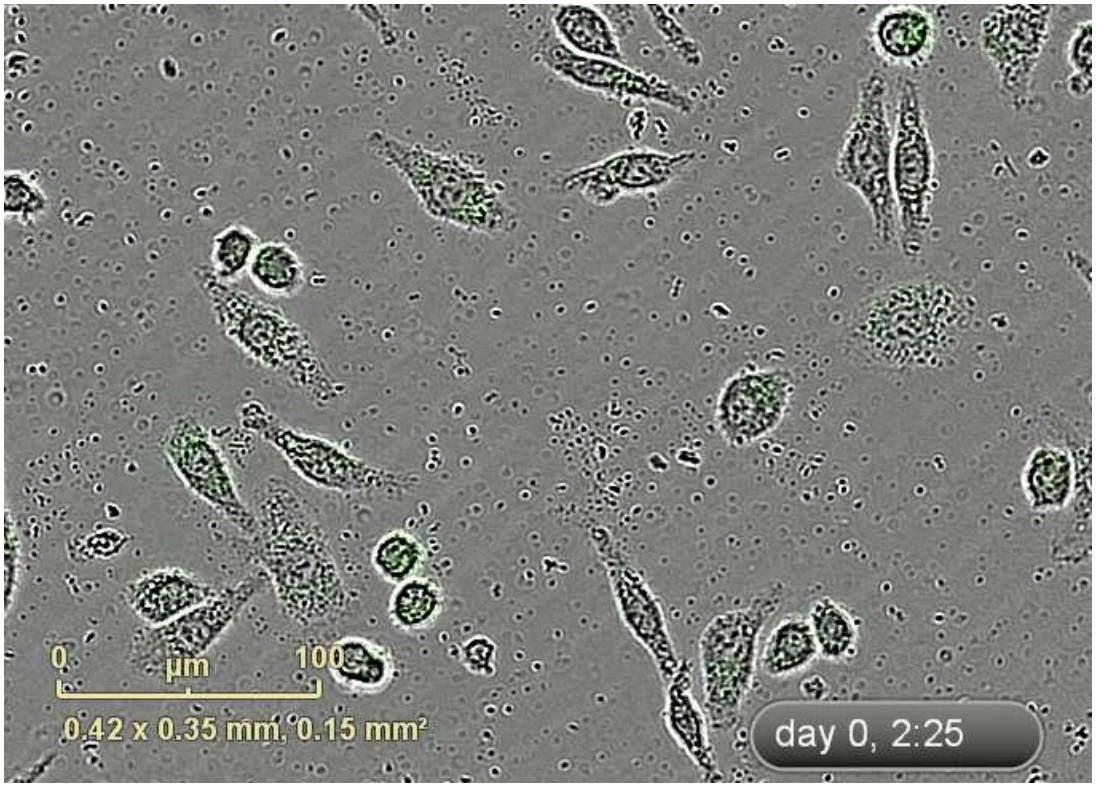


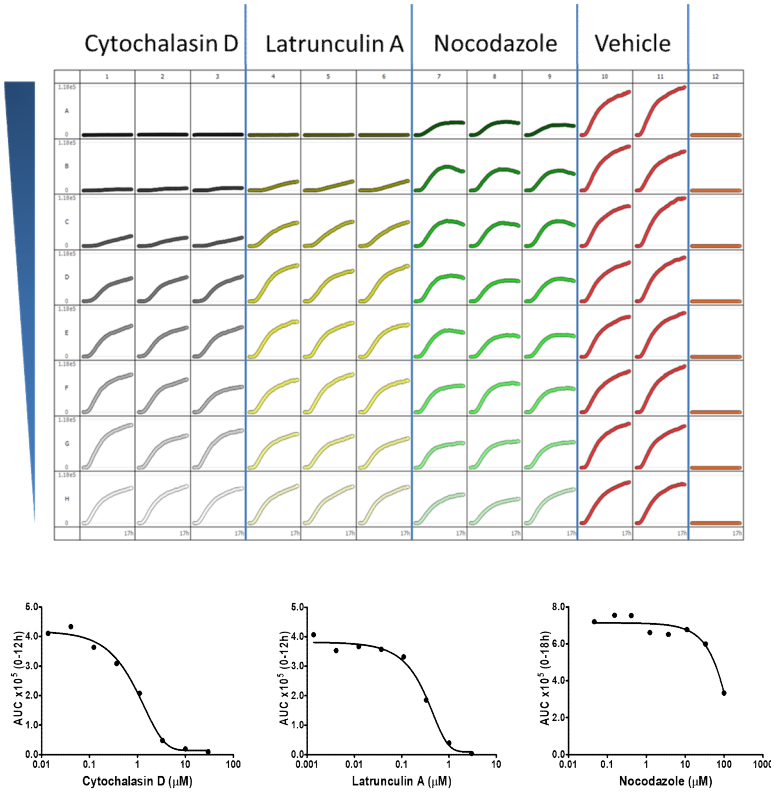




Phagocytosis: Bioparticles

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





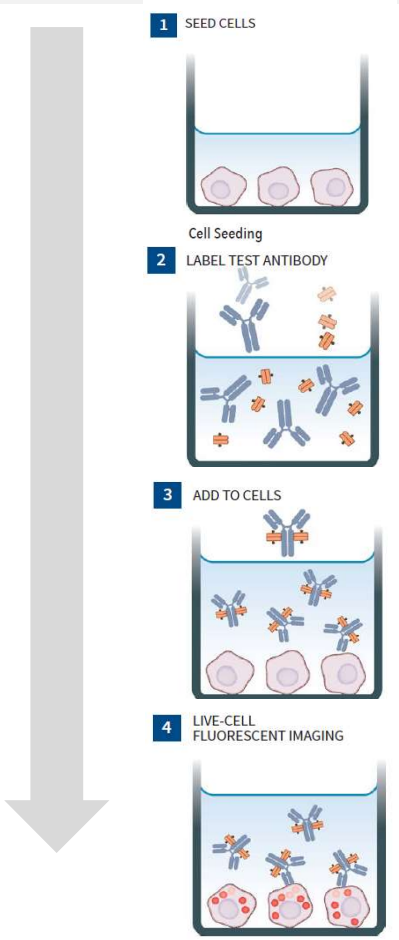
Phagocytosis of Cells

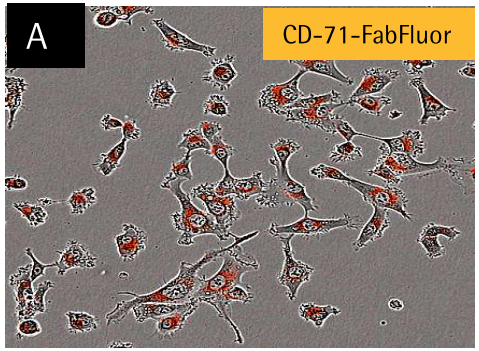
Cell Function & Interactions

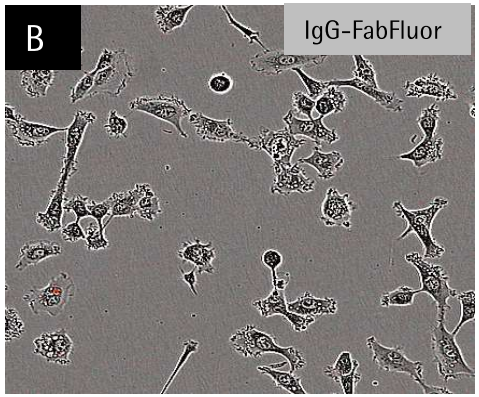
Antibody Internalization

Sensitive, kinetic measurements of internalization rates

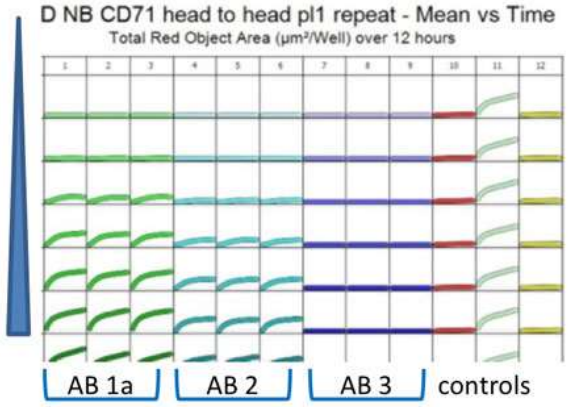
- ✓ Rapid, single step antibody labeling – label in 15 minutes, no optimization required
- ✓ Amenable to both adherent and non-adherent cell types
- ✓ Rapid internalization signal appears in as little as an hour





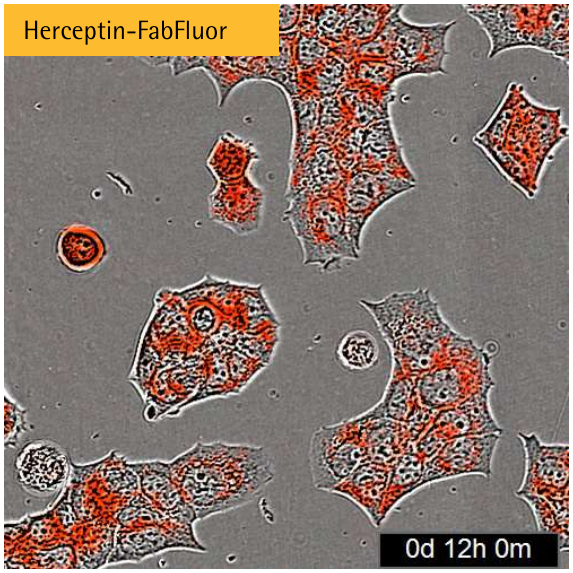


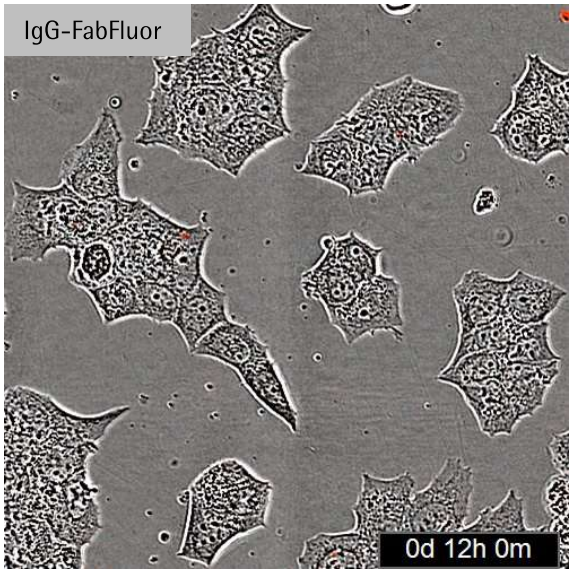
IncuCyte® FabFluor pH-sensitive Antibody Labeling Reagent

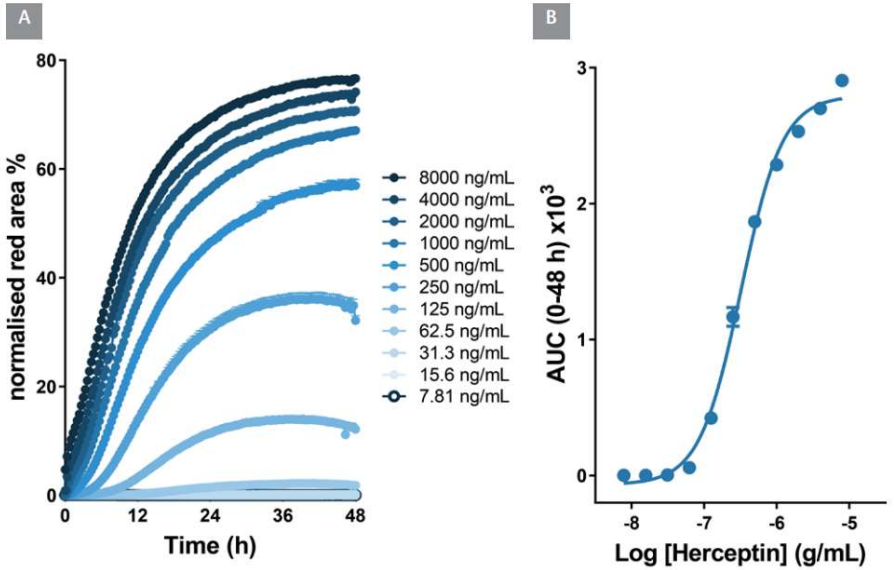


Antibody Internalization

BT-474 Her2 positive cells + FabFluor labelled Herceptin





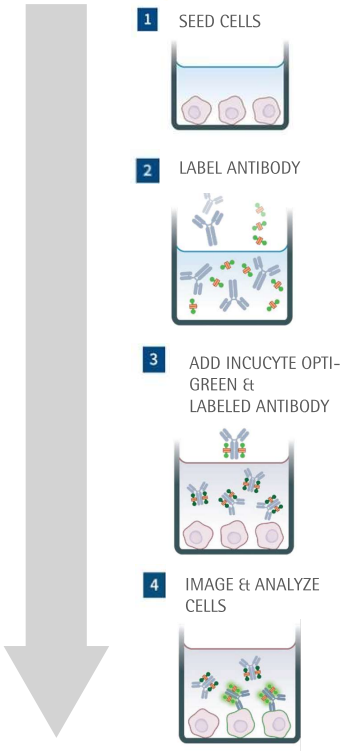


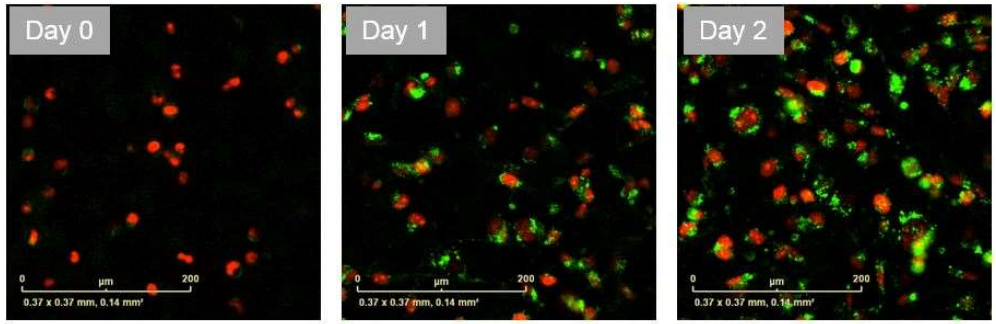
Cell Function & Interactions

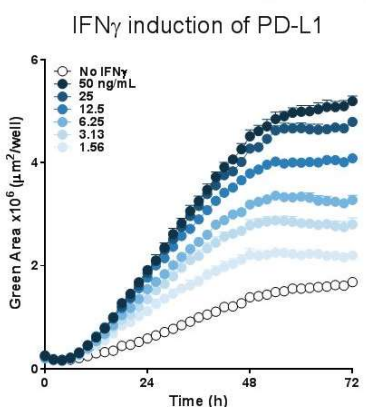
Live-Cell Immunocytochemistry

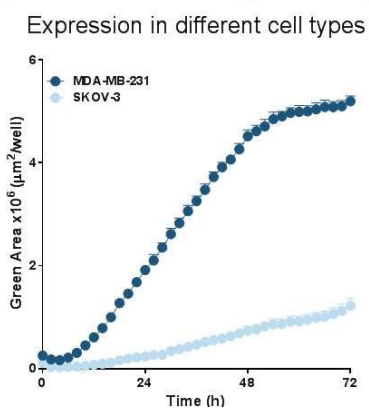
Long-term tracking and quantification of cell surface protein markers

- ✓ Rapid, single-step labelling with automated acquisition and analysis
- ✓ Couple protein expression dynamics to morphology and function
- ✓ Visualize and quantify cell-cell interactions overtime









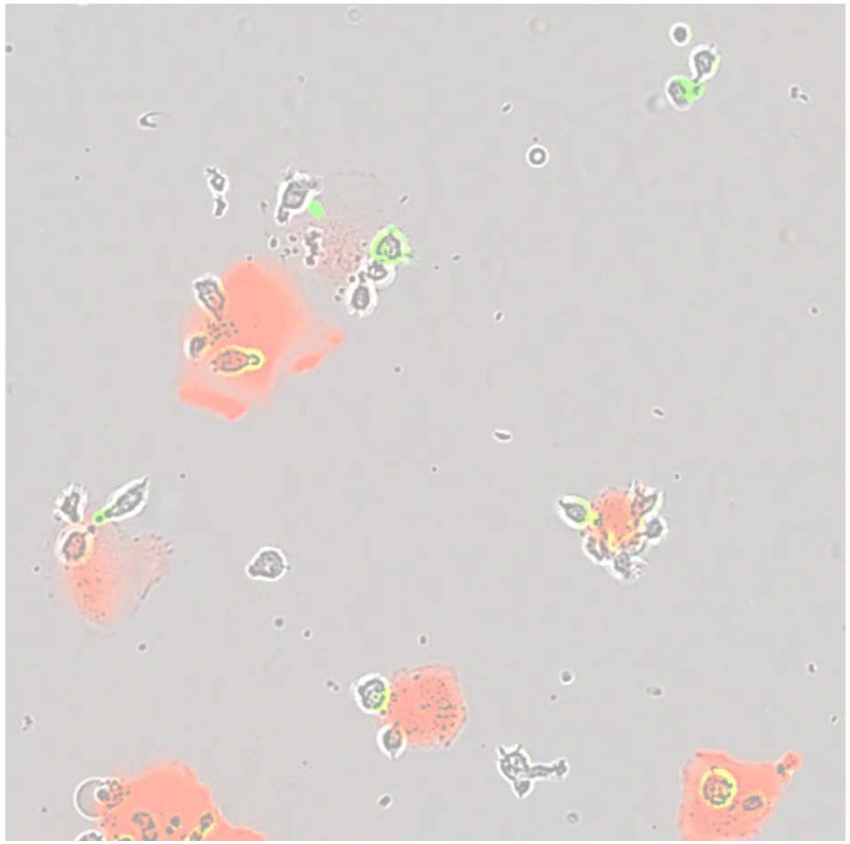
Live-Cell Immunocytochemistry

Visualize and quantify cell-cell interactions

sartorius

IncuCyte® Live-Cell Immunocytochemistry

Images and data generated with the IncuCyte® S3 Live-Cell Analysis System.



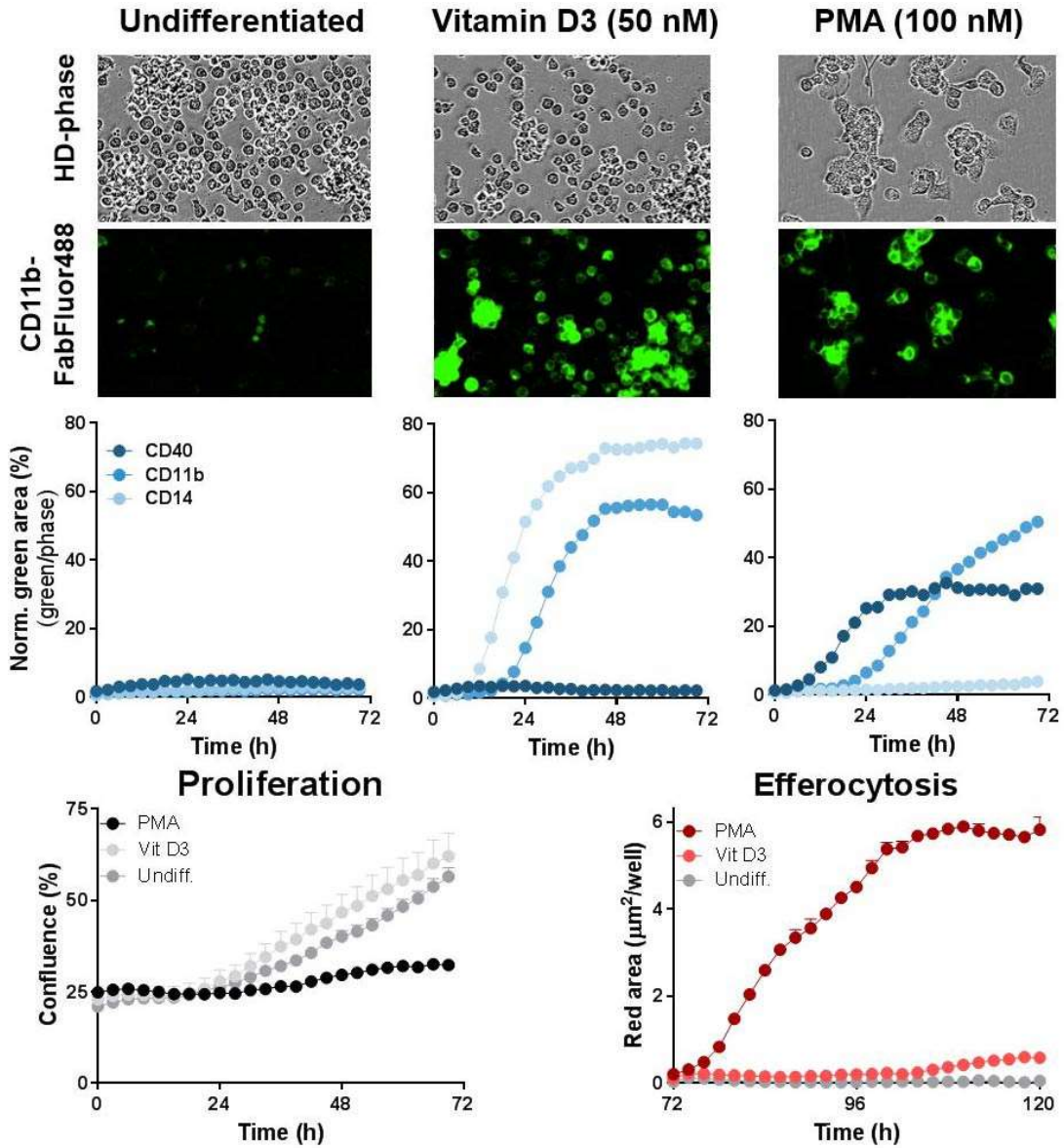
– PBMC labeled with IncuCyte® FabFluor-488 tagged CD8 antibody.

Immune Cell Function

Immune Cell Activation

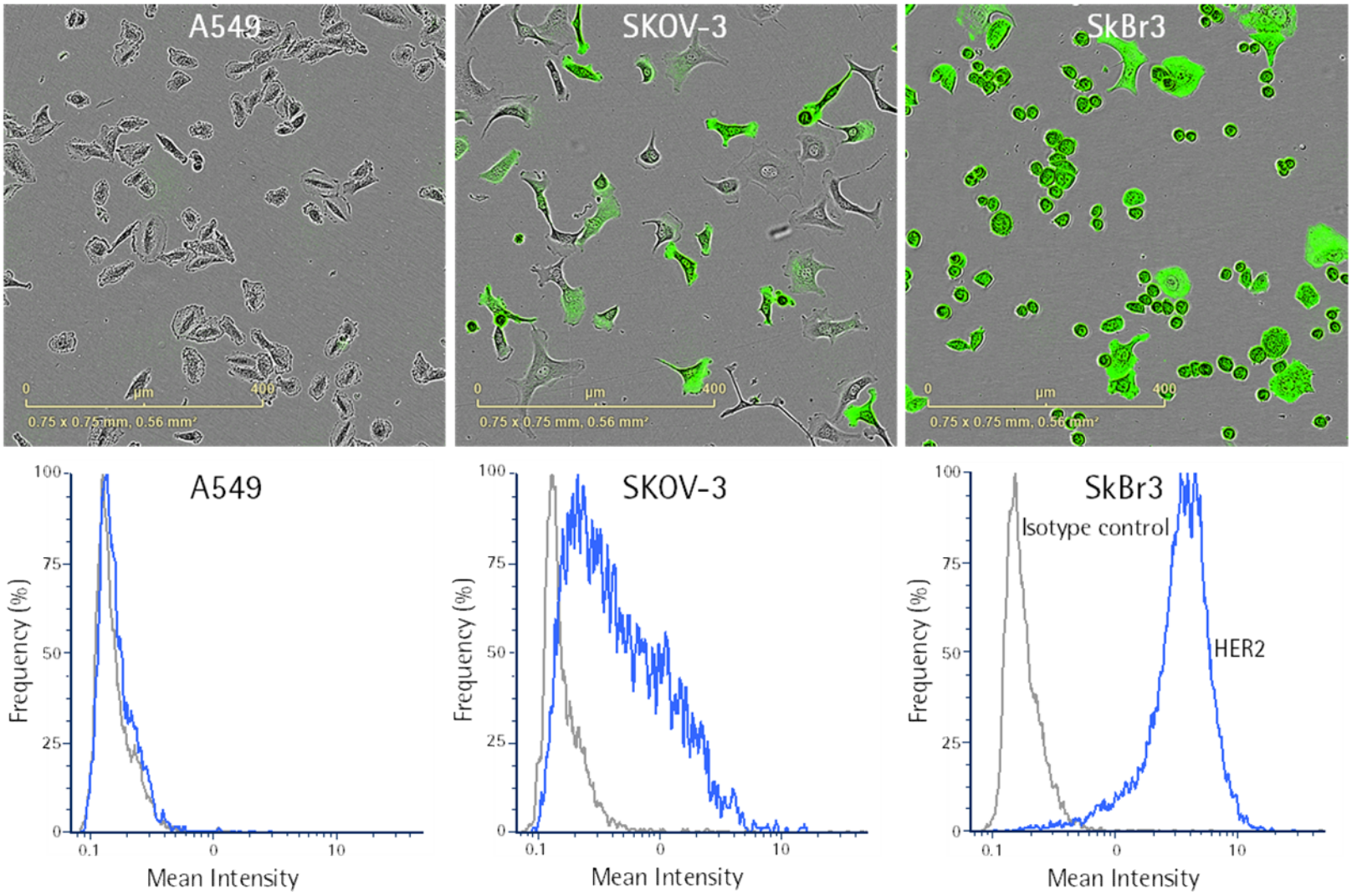
Live-Cell Immunocytochemistry

THP-1 cells + FabFluor conjugated to different antibodies coupled to cell functions



Live-Cell Immunocytochemistry

Kinetic phenotyping of cell sub-populations using fluorescent labelling with Cell-by-Cell Analysis



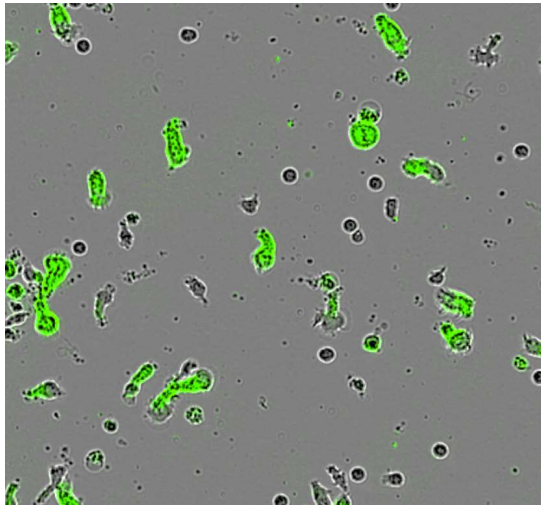
IncuCyte
by ESSEN BIOSCIENCE

IncuCyte.com

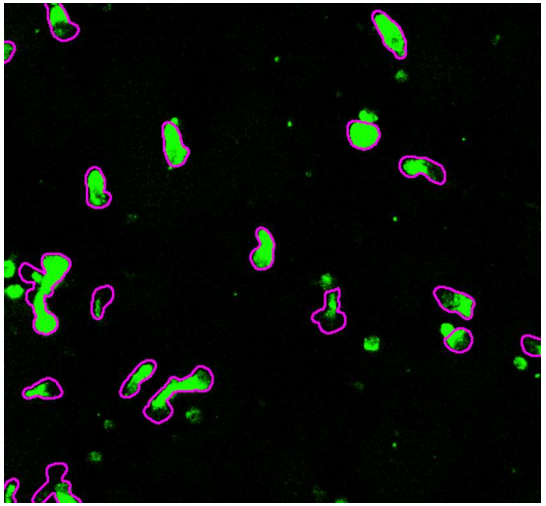
Live-Cell Immunocytochemistry

PBMC + CD3/IL-2 + FabFluor-488-CD71 Measuring Size and CD71+ Activation of Immune Cells

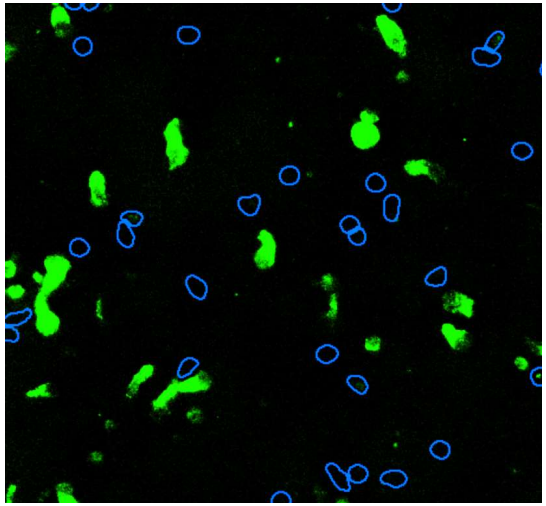
Total Population

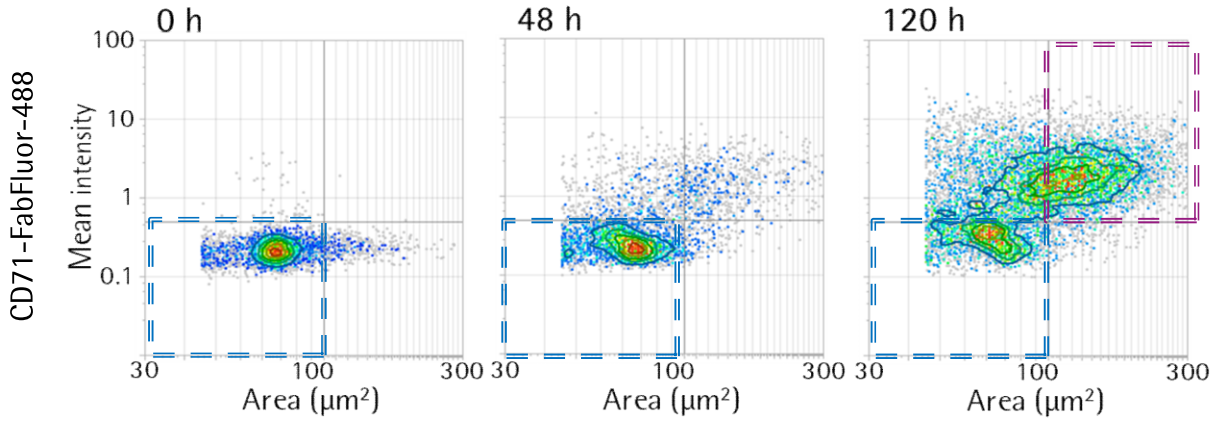


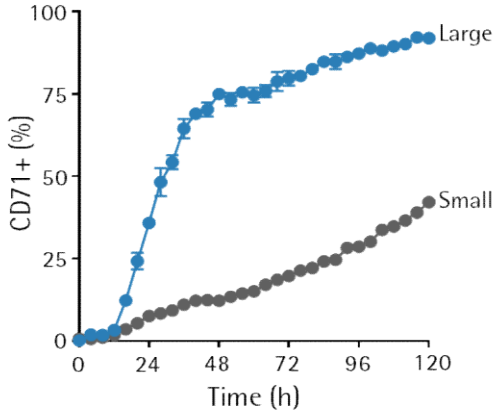
Large and Bright (CD71+)



Small and Dim (CD71-)







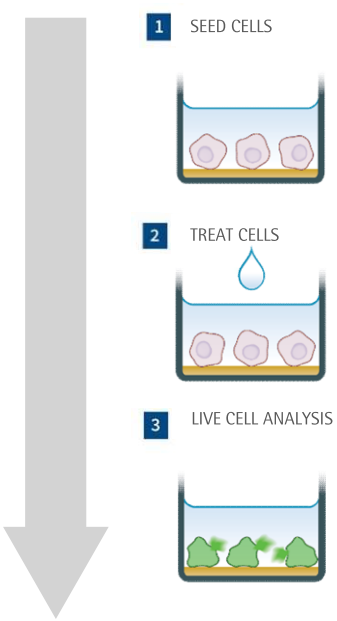
CD71 expression (bright cells) is higher in activated cells (larger cells)

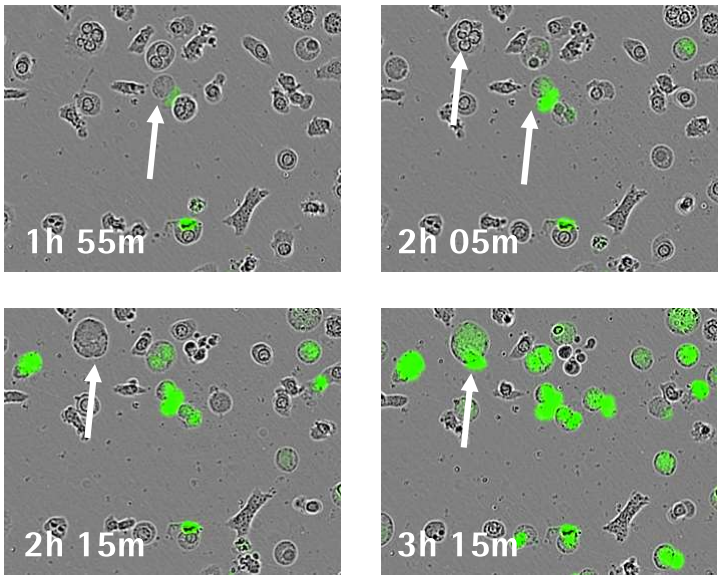
Cell Function & Interactions

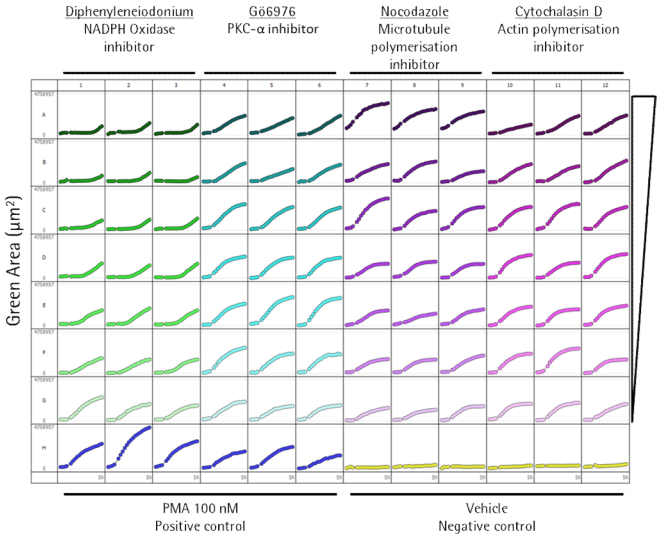
NETosis Assay

Quantification of neutrophils undergoing NETosis in real time

- ✓ Automated analysis using IncuCyte Cytotox Green Reagent
- ✓ Simple mix-and-read 96 well protocols suitable for pharmacological screening
- ✓ Validate stages of NETosis with images and movies

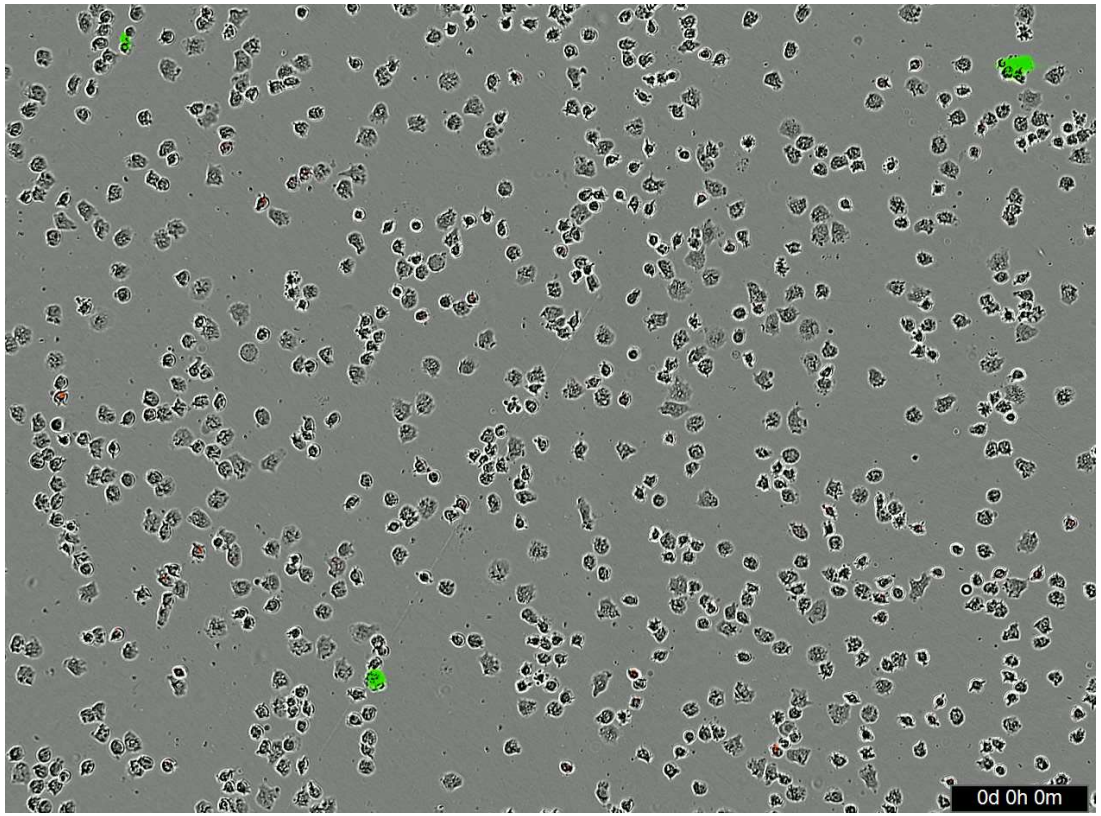


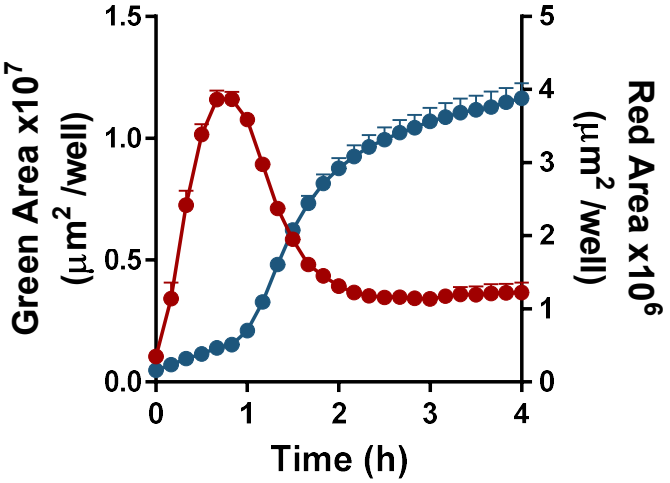




NETosis Assay

Human neutrophils stimulated by PMA to induce NET release



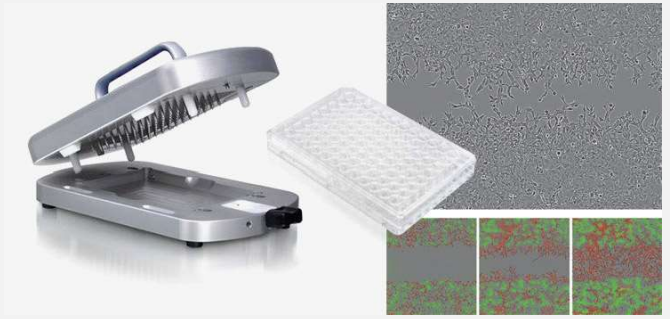


- ROS Detection: CellROX Deep Red (red line)
- NET release: IncuCyte® Cytotox green NETs (ble line)

INCUCYTE® KEY APPLICATIONS

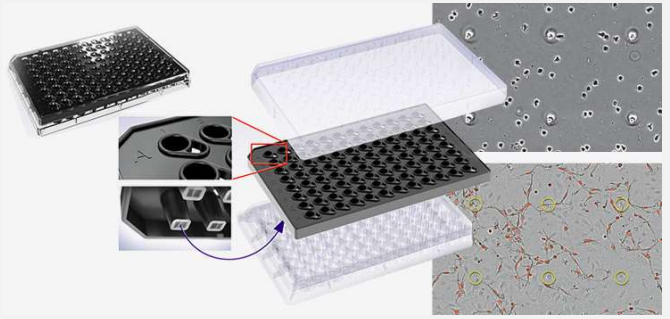
Cell Migration & Invasion

Analyze cell motility inside your incubator in real-time



SCRATCH WOUND

✓ **Label-free** or **fluorescent** analyses of migration and invasion into a wounded region

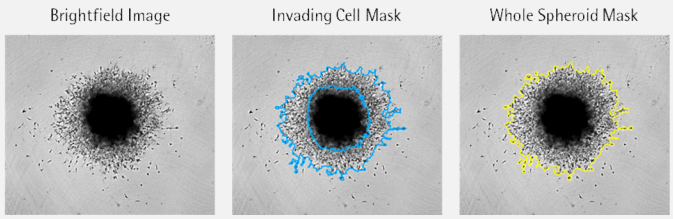


CHEMOTAXIS

✓ Measure **adherent** or **non-adherent** cell movement towards a chemoattractant across a cell membrane

SPHEROID INVASION

✓ Measure **adherent** or **non-adherent** cell movement towards a chemoattractant across a cell membrane

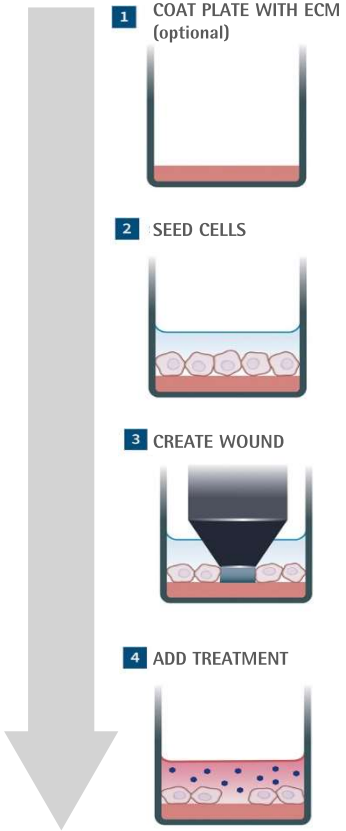


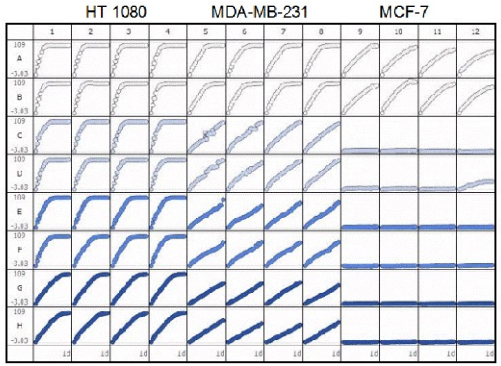
Cell Migration & Invasion

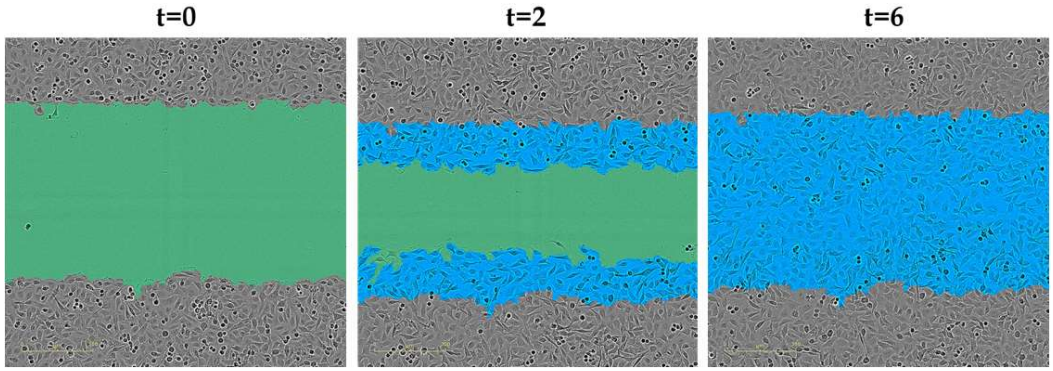
Scratch Wound

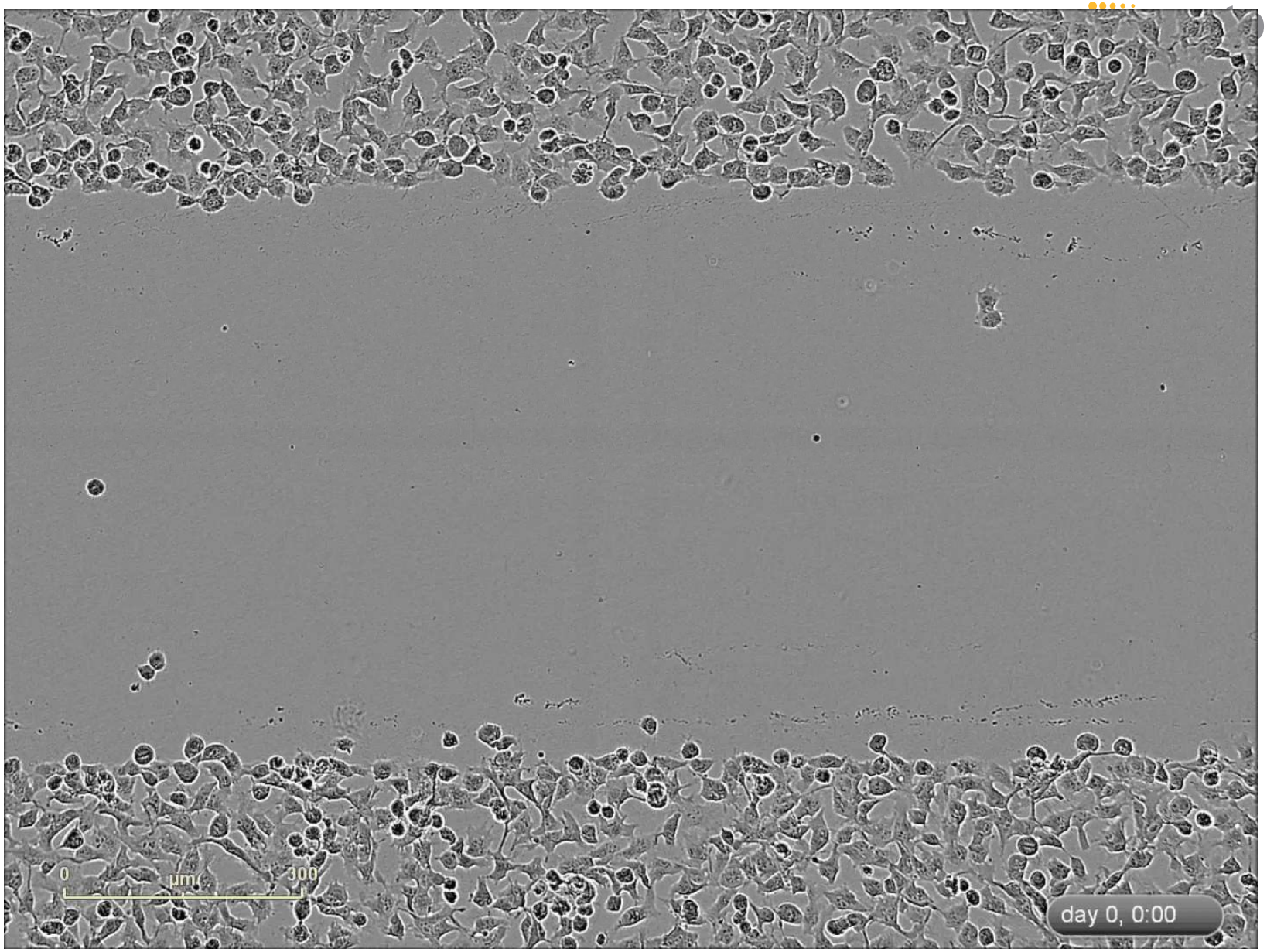
Measure cell density-dependent migration and invasion into wound region

- ✓ WoundMaker creates 96 precise, uniform cell-free zones every time
- ✓ Wound closure is visualized and analyzed in real-time inside your incubator







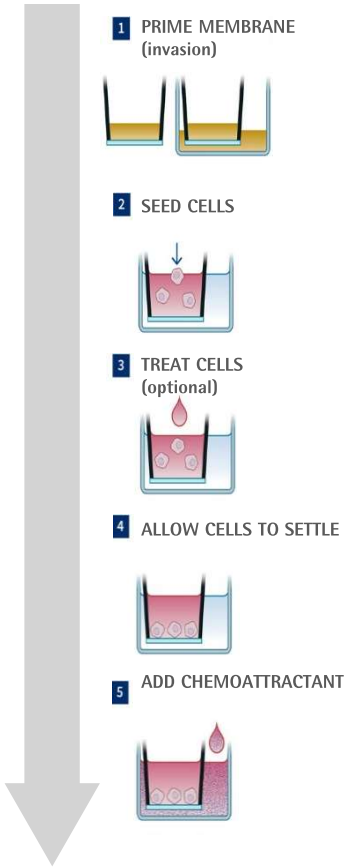

0
µm
300
day 0, 0:00

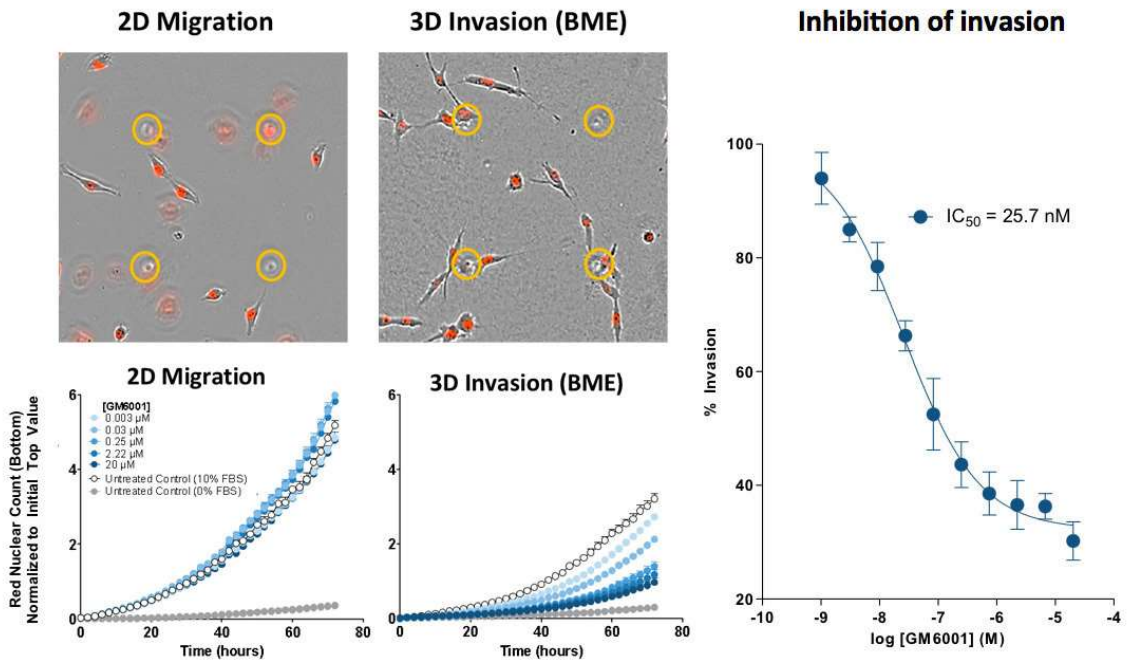
Cell Migration & Invasion

Chemotaxis: Adherent

Measure cell migration or invasion toward a stable chemoattractant gradient

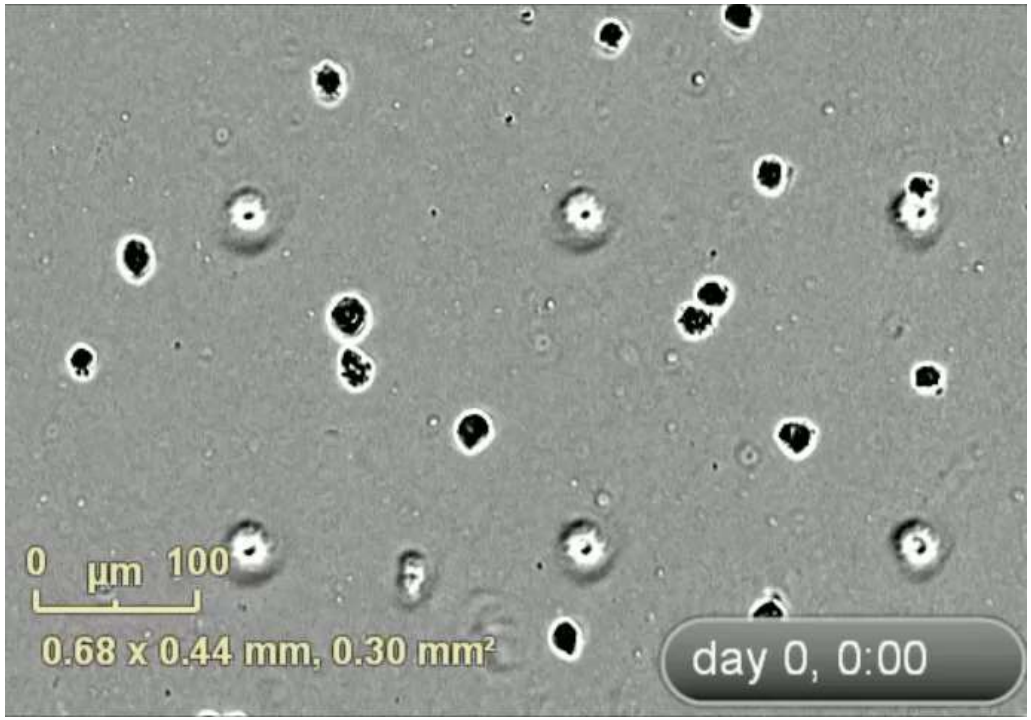
- ✓ Requires fewer cells compared to traditional Boyden chamber approaches
- ✓ Validate data and visualize invasive phenotype with images and movies

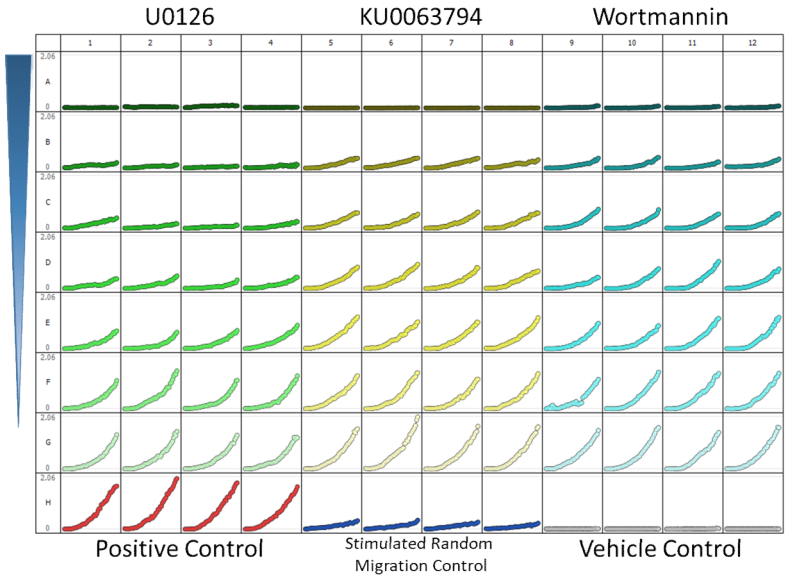




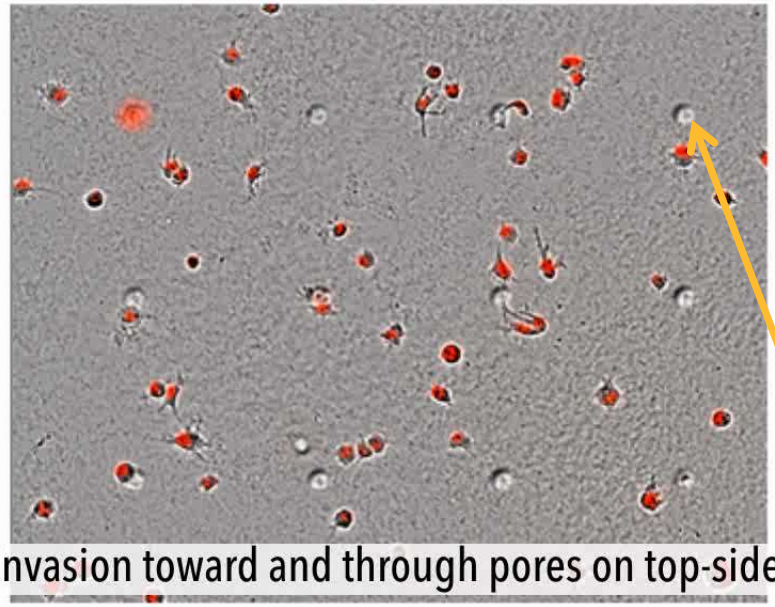
Chemotaxis: Adherent Migration

HT-1080 chemotaxis toward FBS

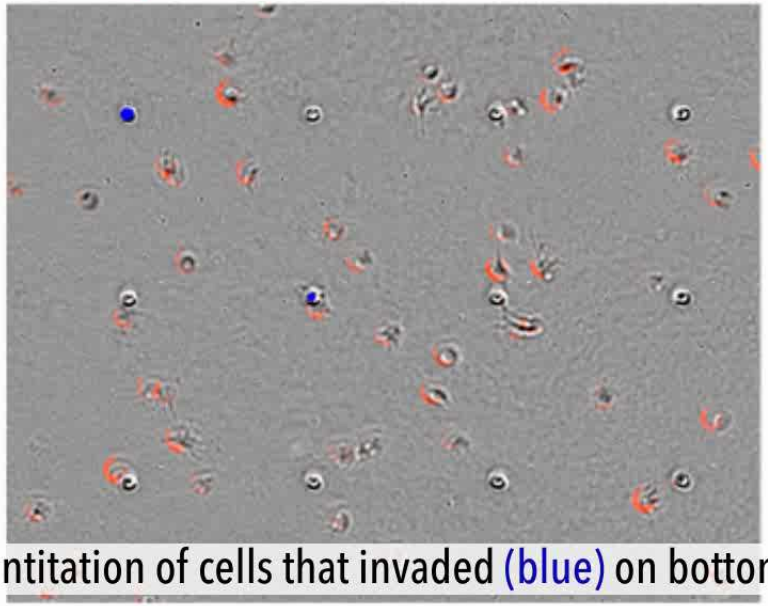




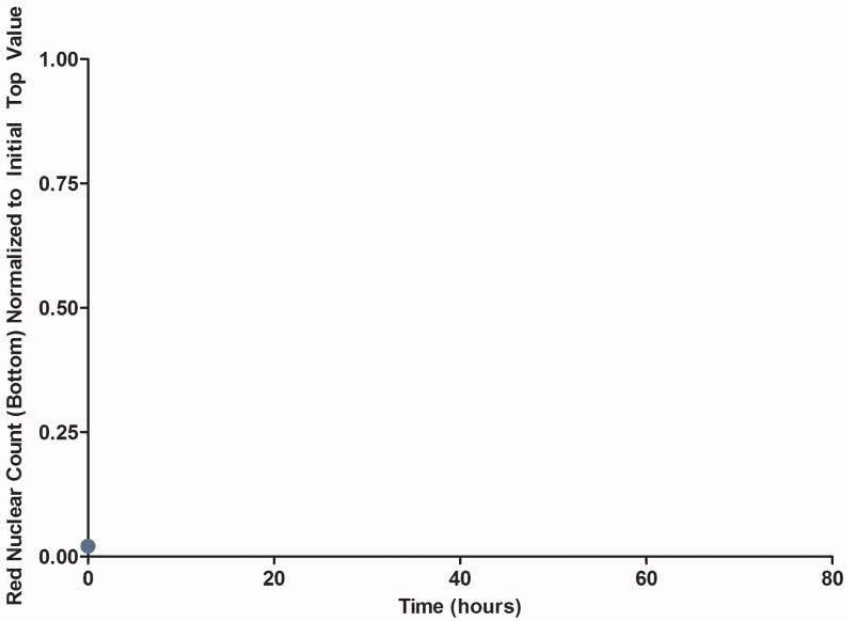
Chemotaxis: Directional Invasion



Invasion toward and through pores on top-side



Quantitation of cells that invaded (blue) on bottom-side



* Invasive phenotype seen as cells invade through ECM matrix toward pores on top-side of ClearView Chemotaxis plate

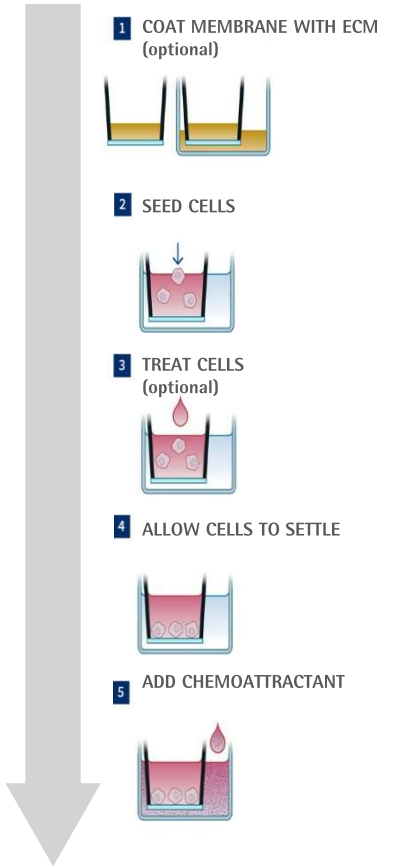
* Cells that invade to the bottom-side of ClearView Chemotaxis plate are masked and counted as shown in the graph above

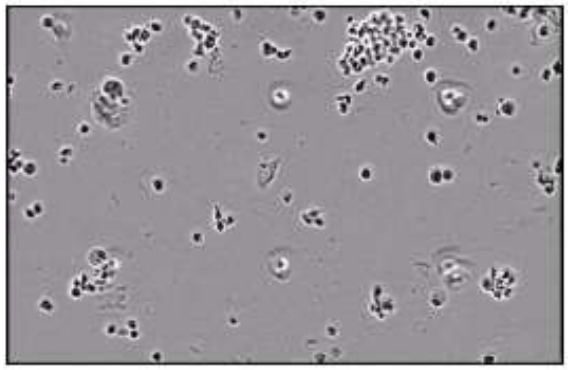
Cell Migration & Invasion

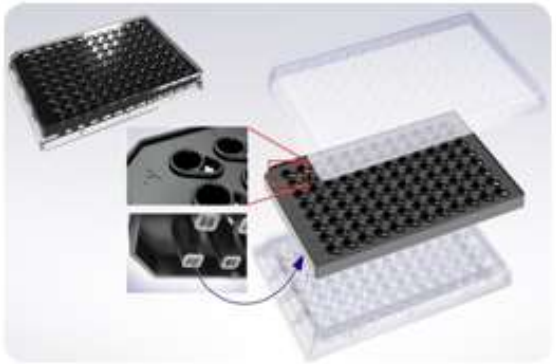
Chemotaxis: Non-Adherent

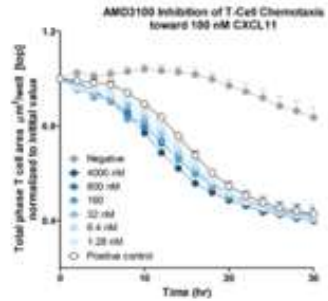
Measure cell migration toward a stable chemoattractant gradient

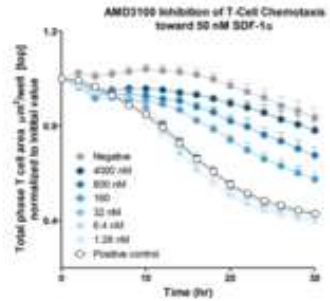
- ✓ Requires fewer cells compared to traditional Boyden chamber approaches
- ✓ Ideal for rare, expensive, and primary cells

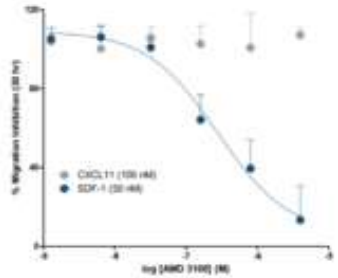






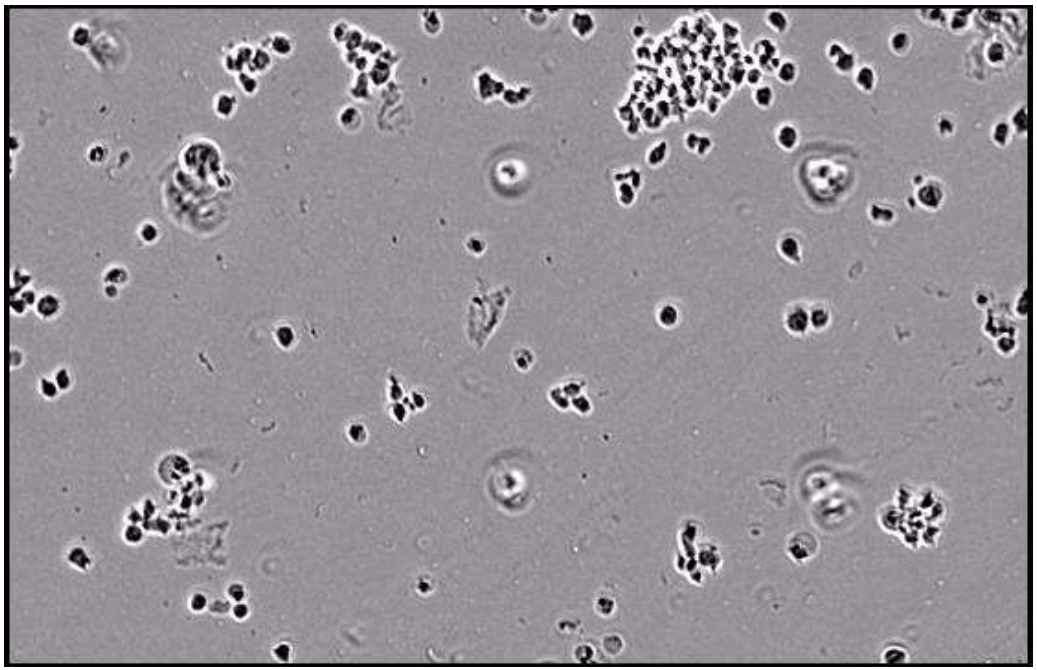


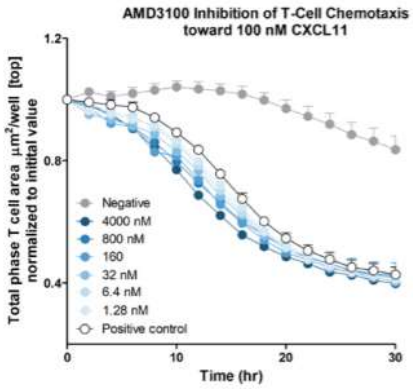


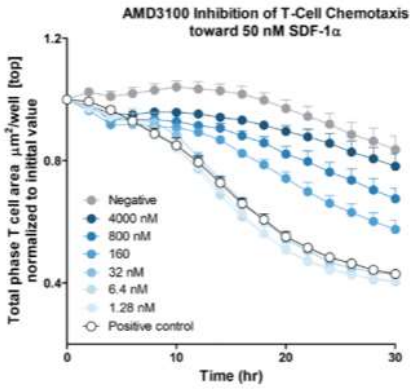


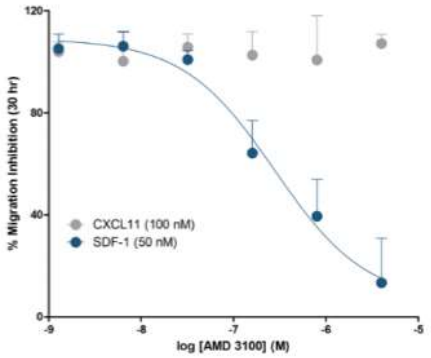
Chemotaxis: Non-Adherent Migration

T-cell chemotaxis towards CXCL11 or SDF-1α







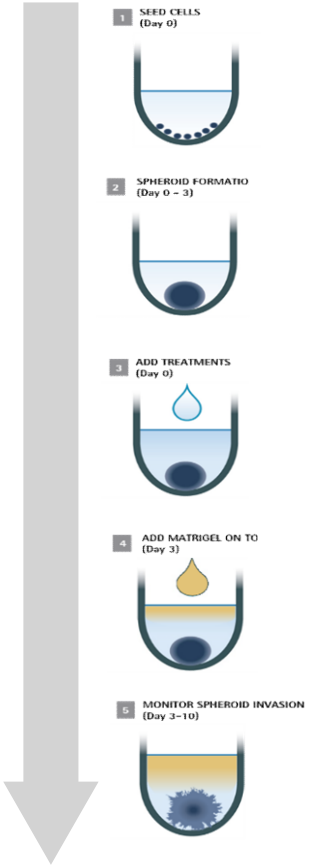


Cell Migration & Invasion

Single Spheroid Invasion

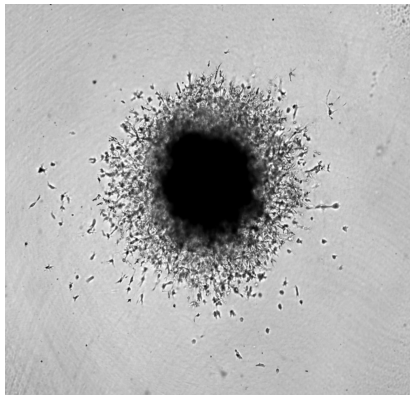
Quantify label-free invasion and investigate inhibitors of metastasis

- ✓ Create uniform spheroids suitable for highly reproducible invasion assays
- ✓ Investigate cell-type-specific differences in metastatic potential of tumor cells
- ✓ Acquire, analyze and graph thousands of images from up to six 96-well plates in parallel

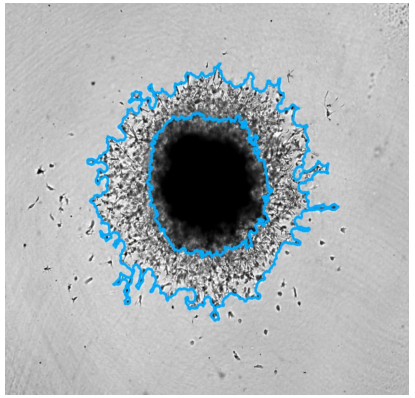


U87MG

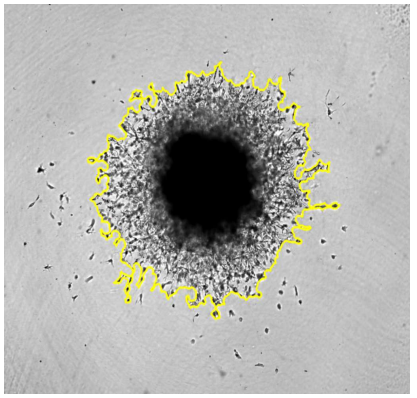
Brightfield Image



Invading Cell Mask



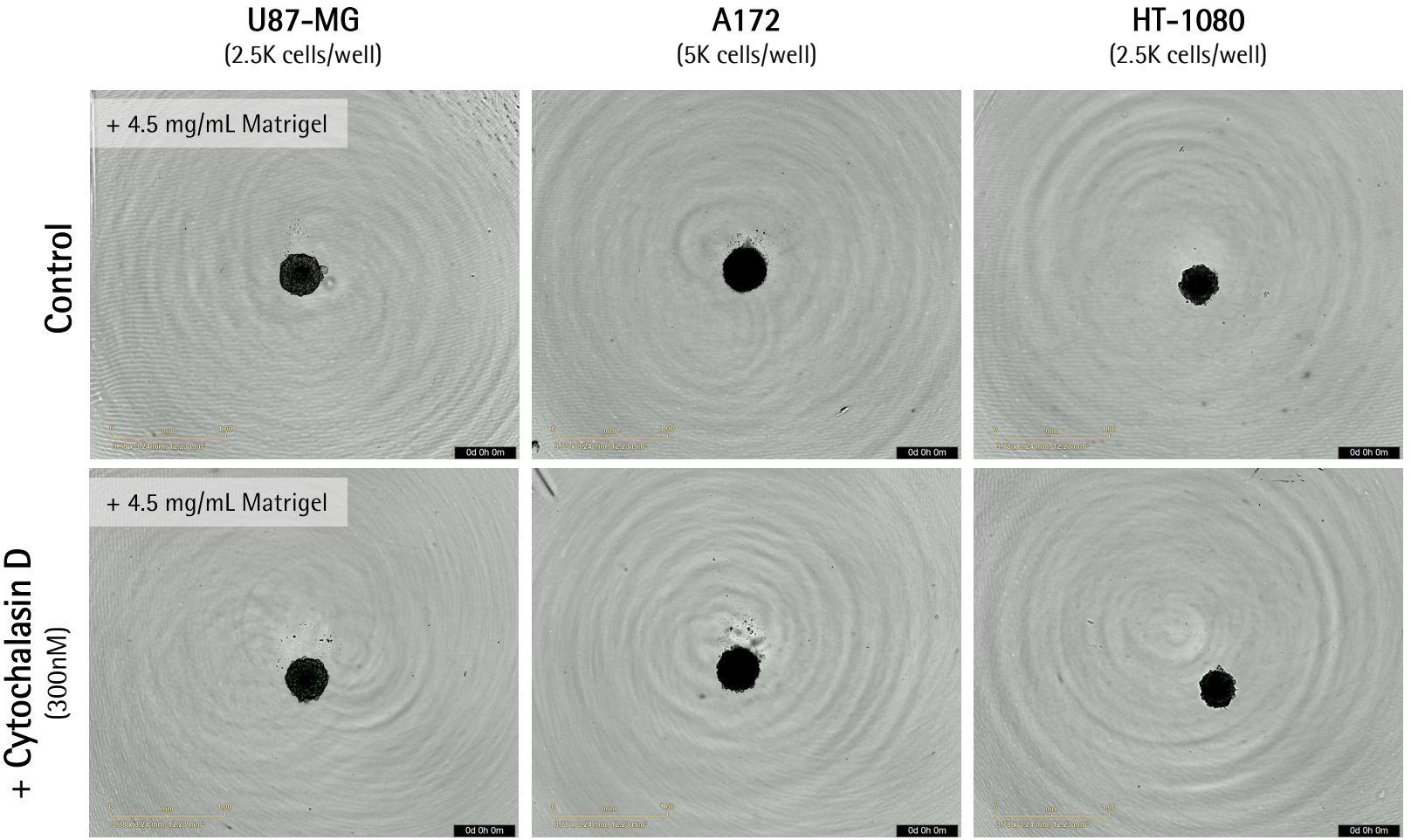
Whole Spheroid Mask



Single Spheroid Invasion

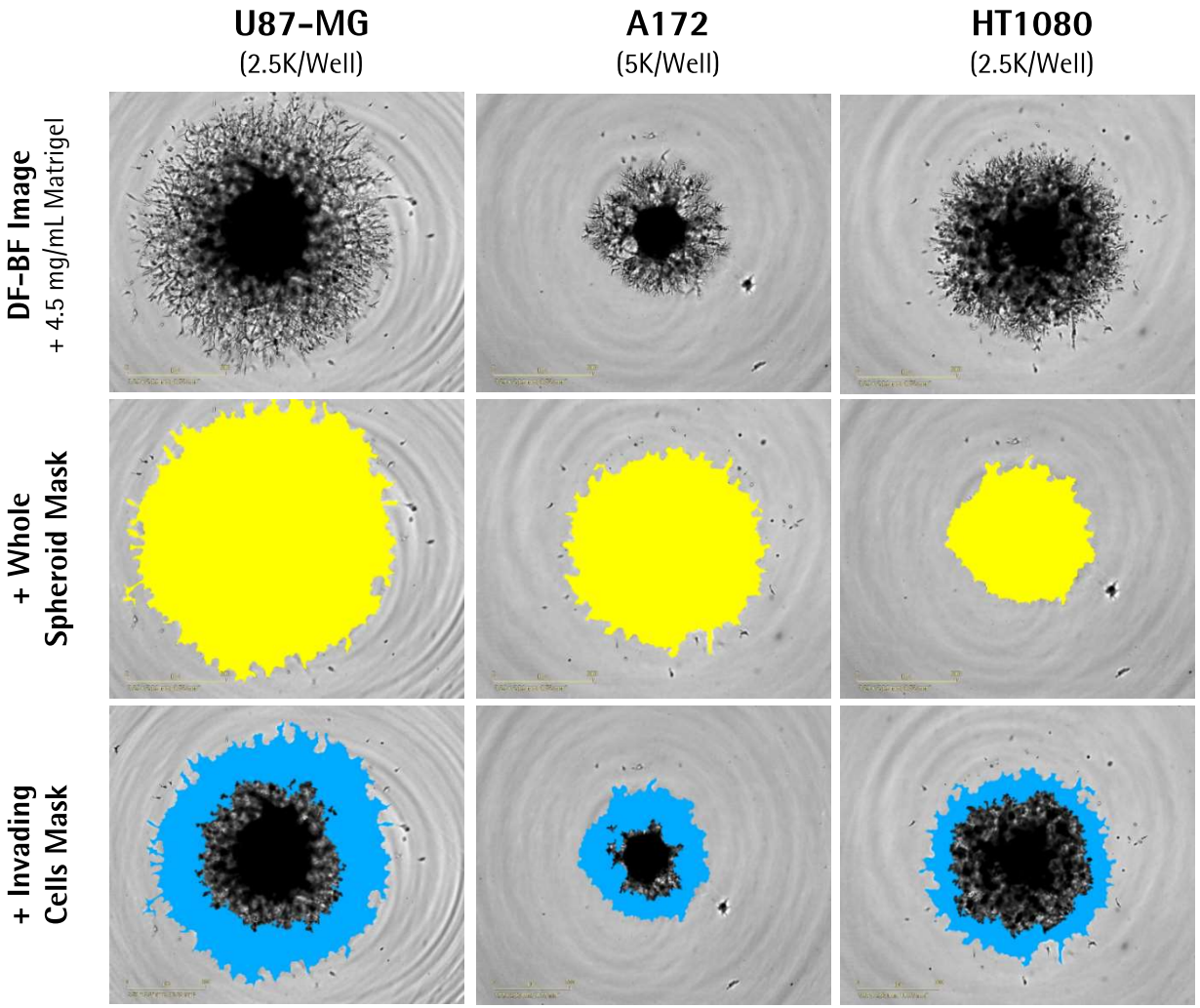
Quantifying inhibition of spheroid invasion

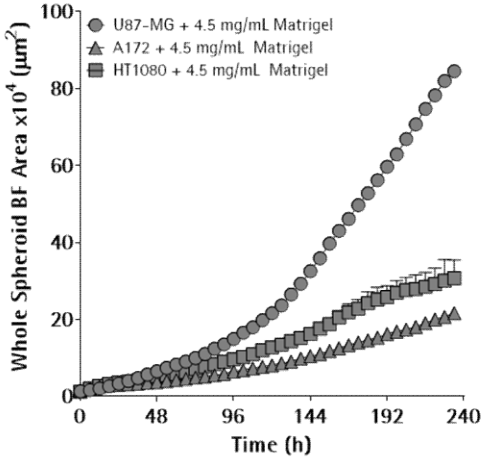
sartorius

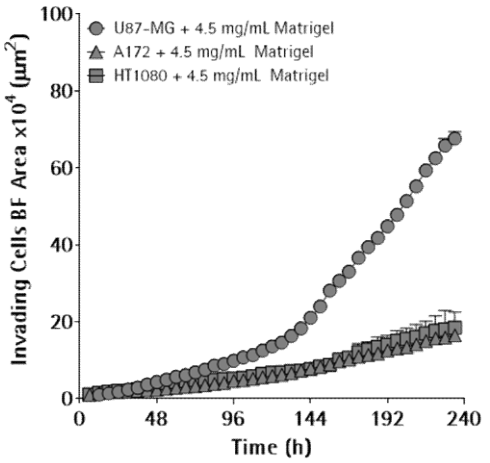


Single Spheroid Invasion

Quantifying whole spheroid and invasive phenotype





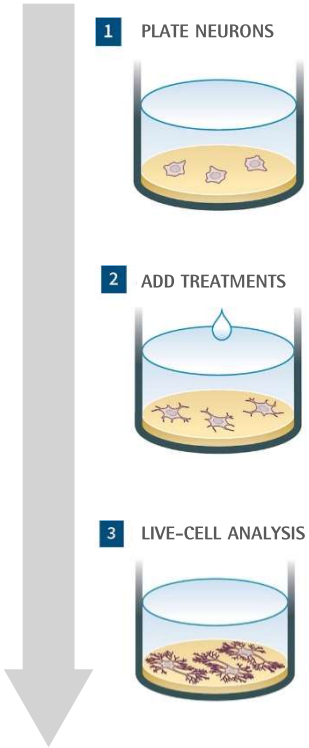


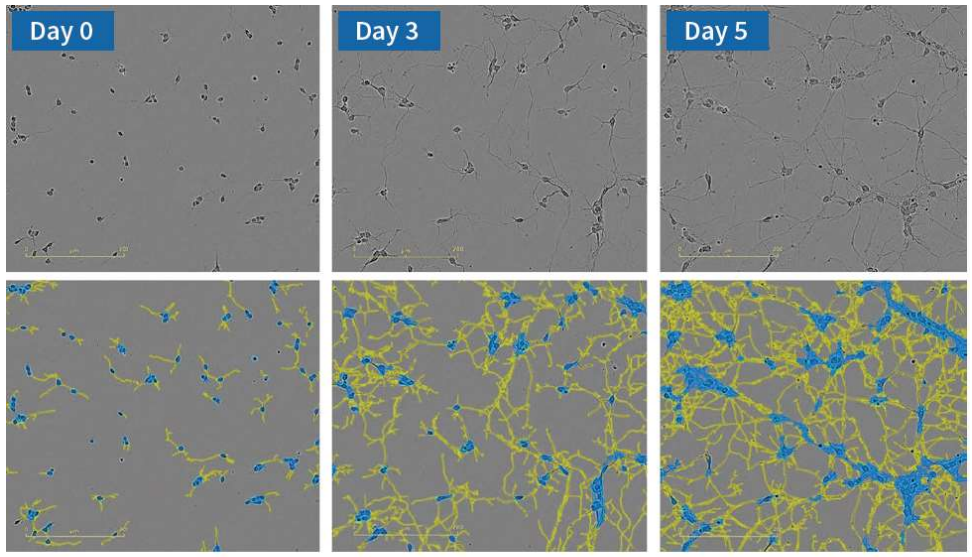
Continuous Live Cell Assays

Neurite Dynamics

Measure neurite dynamics in label-free mono-culture or fluorescent co-cultures

- ✓ IncuCyte® NeuroLight reagents or NeuroPrime cell kits for co-culture analysis
- ✓ Multiplex with IncuCyte® Apoptosis or Cytotoxicity Reagents to quantify cell viability and neurite dynamics in real time
- ✓ Assays compatible with primary neurons, stem-cell derived neurons, and neuronal-like cells

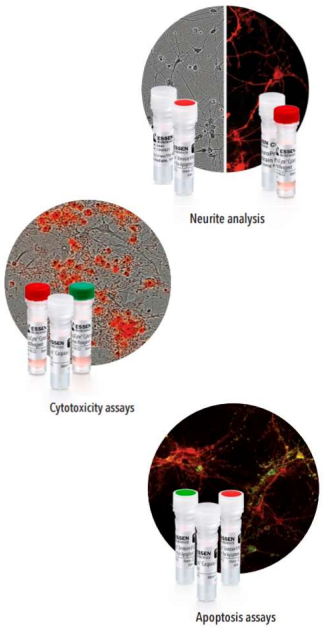




Neurite length: 11.2 mm/mm²
Cell body cluster: 134/mm²
Branch points: 259.9/mm²

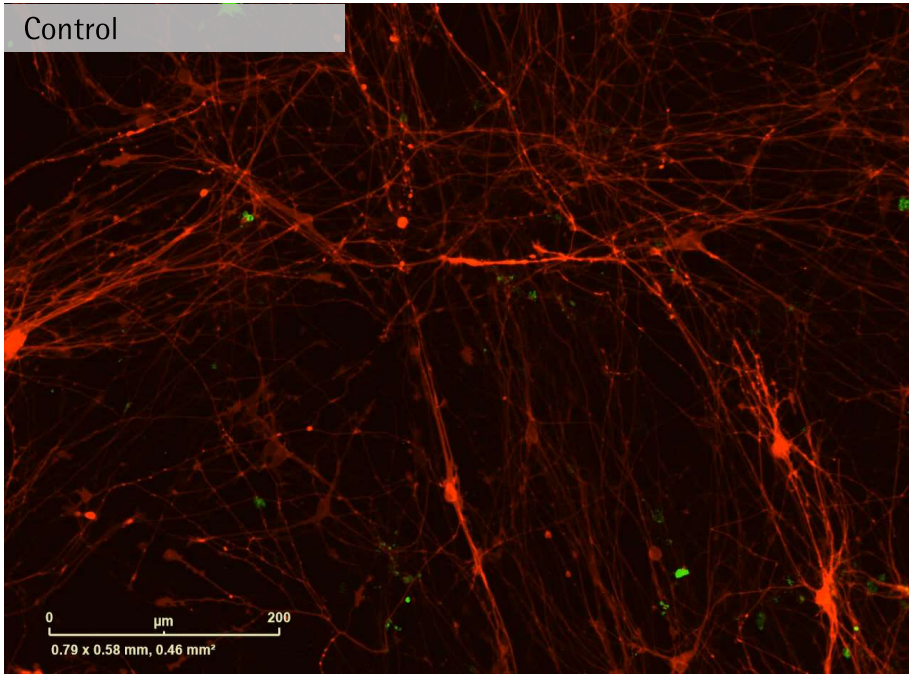
Neurite length: 55.1 mm/mm²
Cell body cluster: 130/mm²
Branch points: 1368/mm²

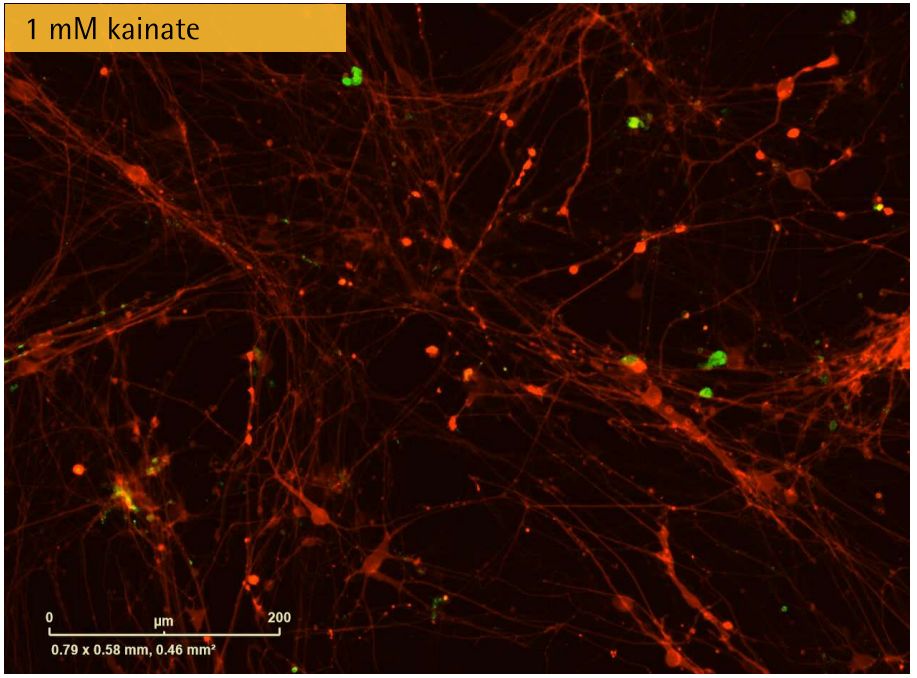
Neurite length: 75.1 mm/mm²
Cell body cluster: 139/mm²
Branch points: 2048/mm²

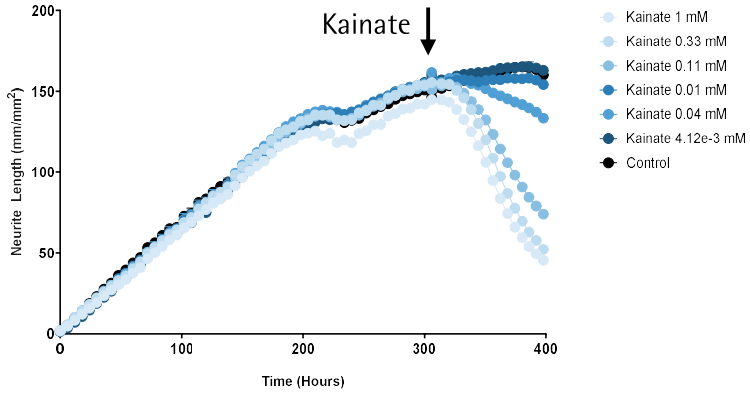


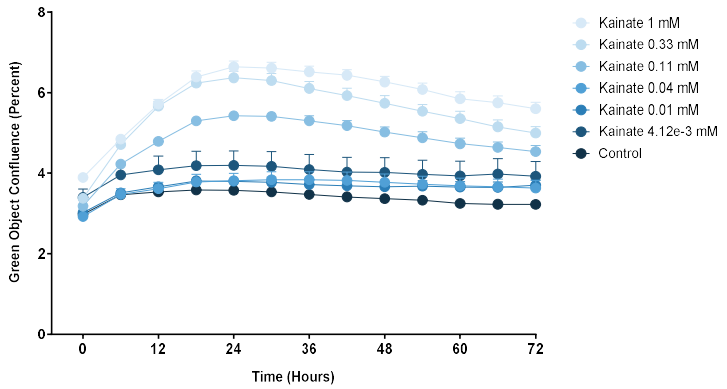
Neurite Dynamics: Fluorescent Co-Culture

Neurotoxicity in iGluta Neurons







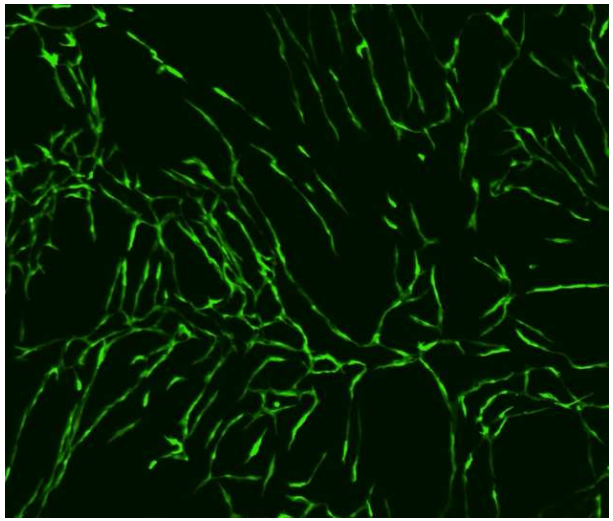


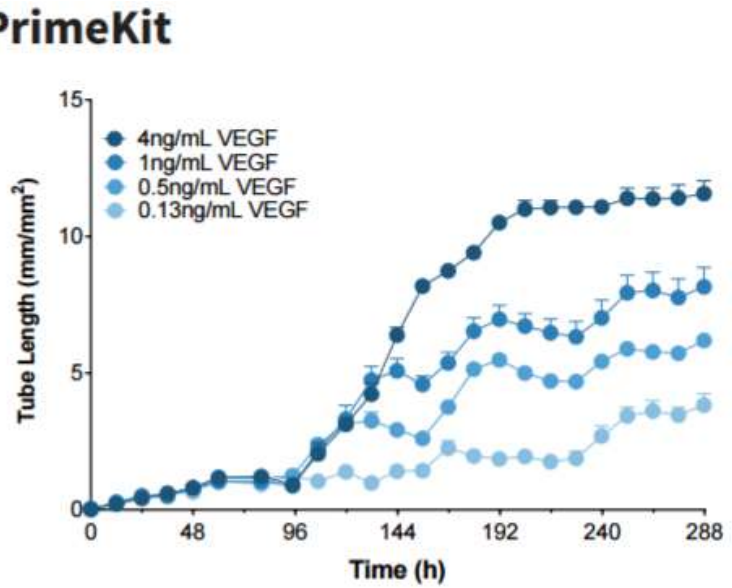
Continuous Live Cell Assays

Angiogenesis

Co-culture cell model for kinetic measurements of vascular tube formation

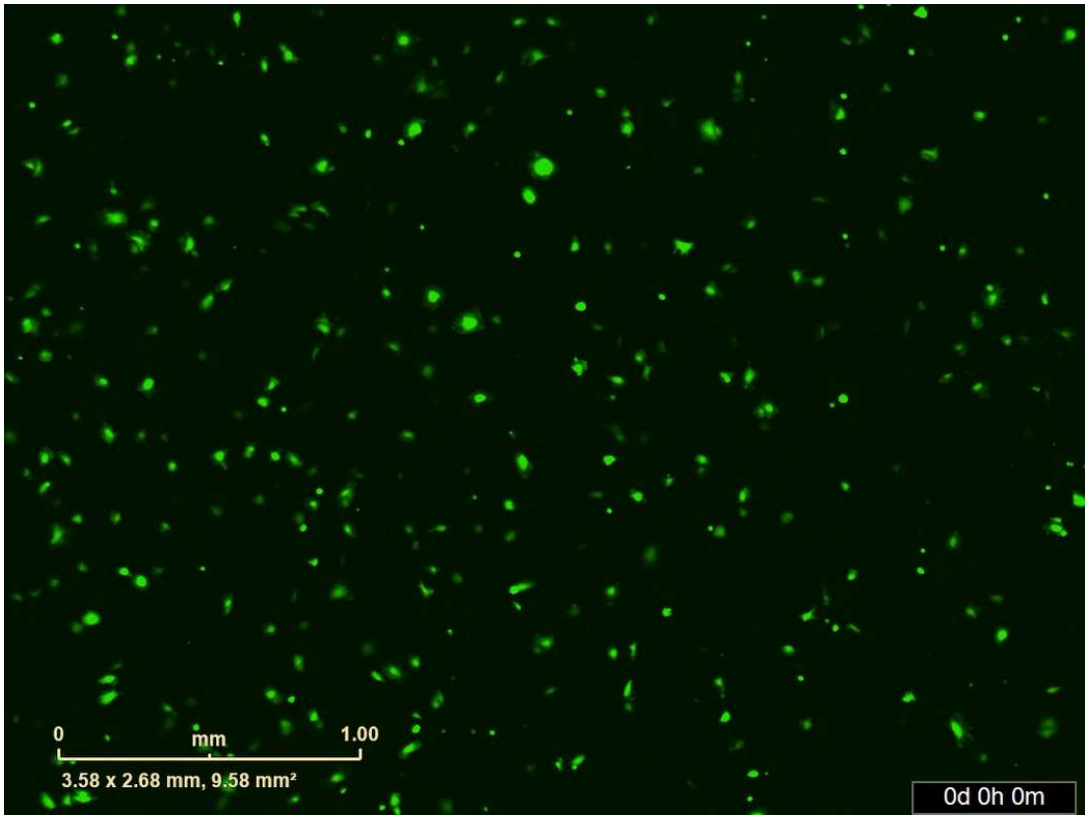
- ✓ Follow tube formation in real time for up to 2 weeks via fluorescent quantification of GFP-labelled endothelial cells
- ✓ Quantify pro- and anti-angiogenic treatments
- ✓ Recapitulates all phases of in vivo angiogenesis process (proliferation, migration, and differentiation)

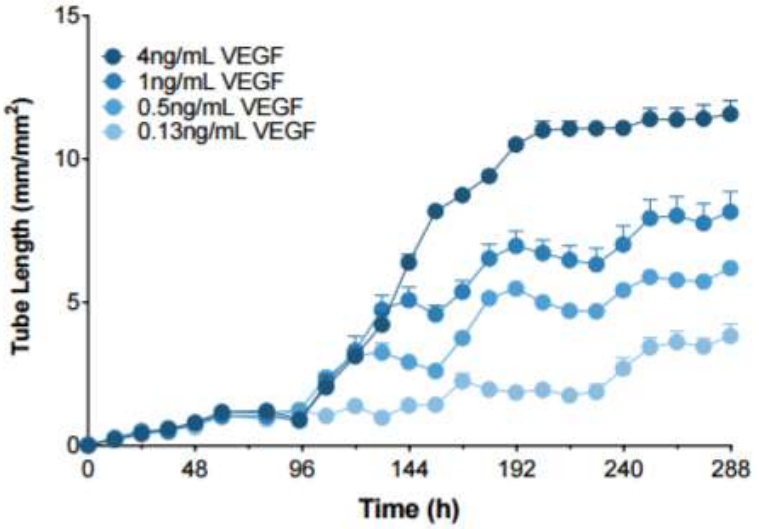




Angiogenesis: PrimeKit

VEGF dose dependent vascular tube formation



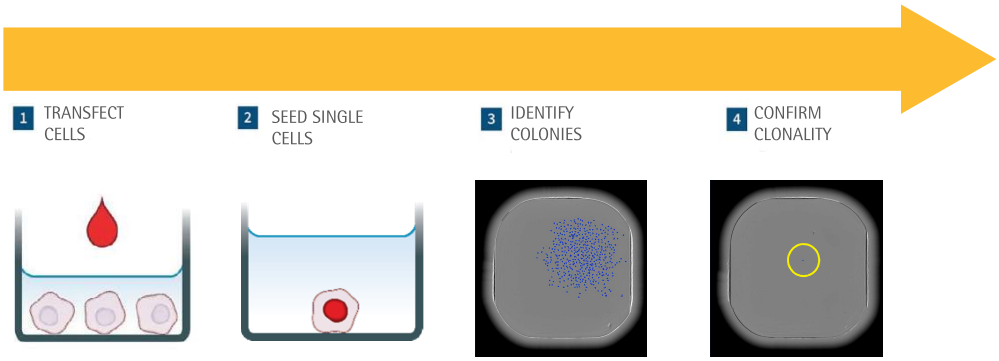


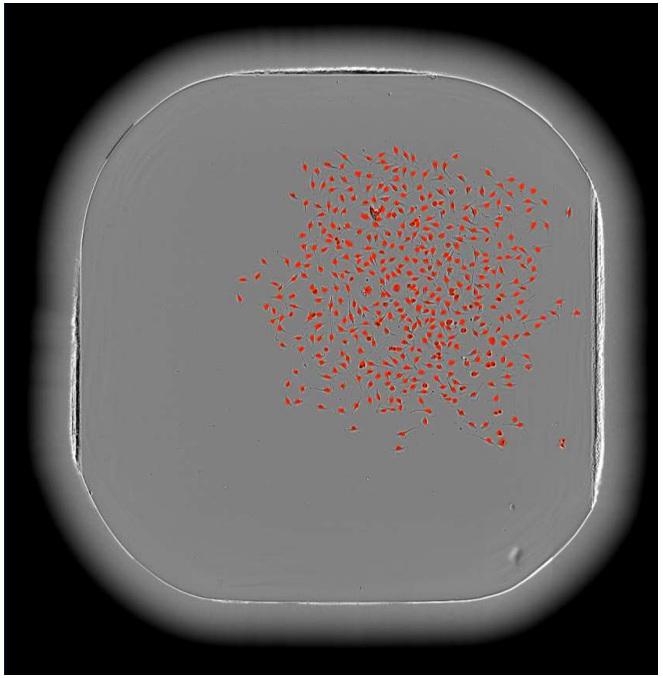
Continuous Live Cell Assays

Dilution Cloning

Simplify your dilution cloning workflow with whole-well imaging

- ✓ Automatically scan wells for rapid identification of clones of interest
- ✓ Quickly and easily validate clonality with the ability to move forward and backward in time
- ✓ Inspect colony morphology and monitor growth characteristics using HD-phase and 2-color fluorescence





Dilution Cloning: Whole-well 384-well plate