As dynamic as life

Cellular Assays Offer Key Insights into Cell Health and Behaviour

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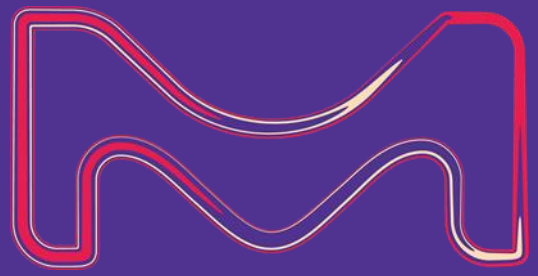
Solution Scientist



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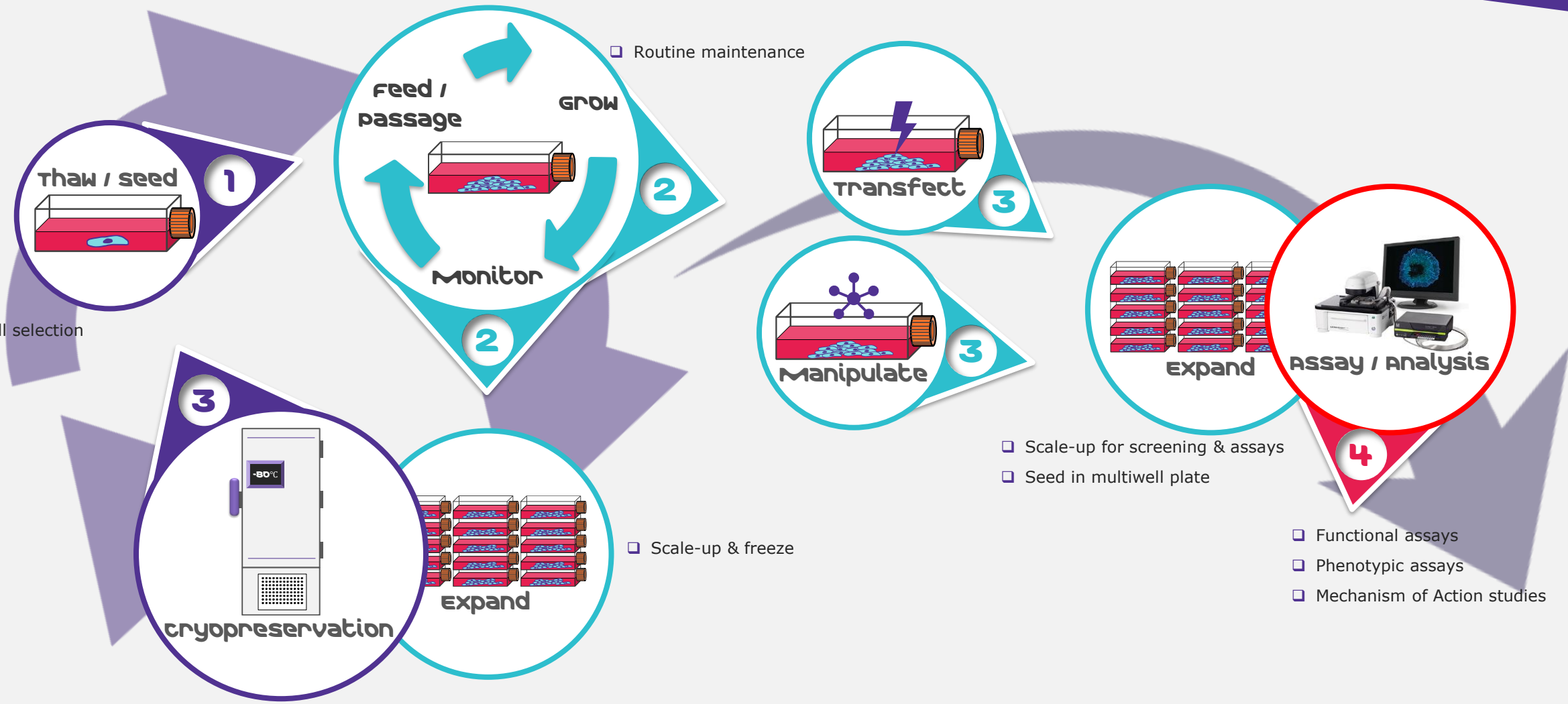
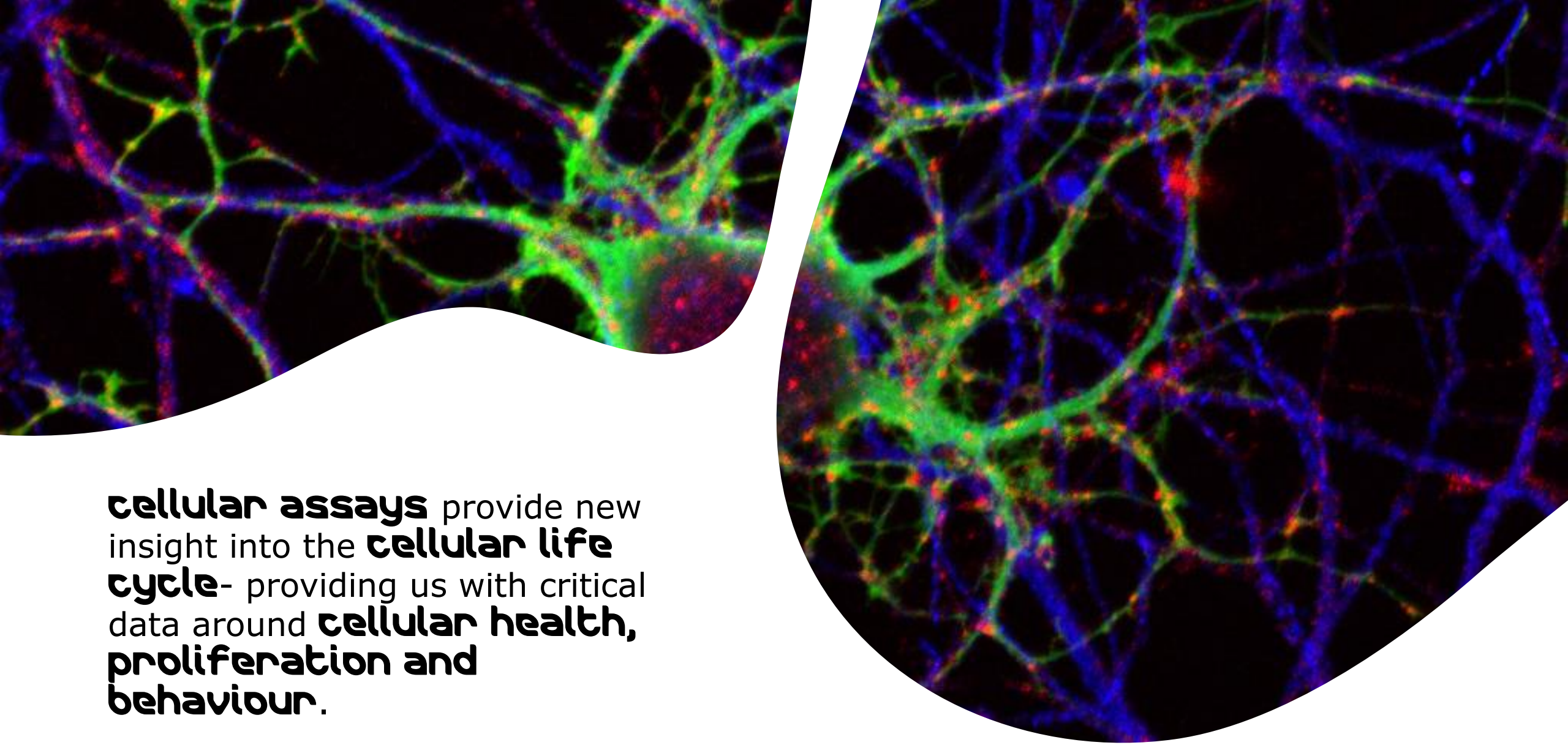
cell culture workflow





cellular assays provide new insight into the **cellular life cycle**- providing us with critical data around **cellular health, proliferation and behaviour**.

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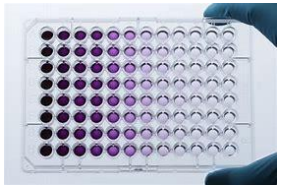
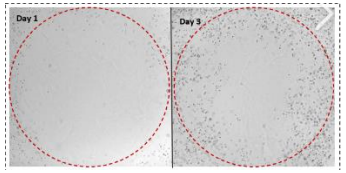
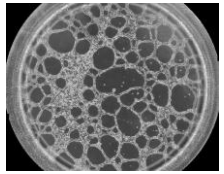
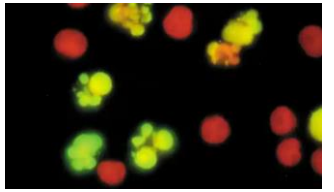
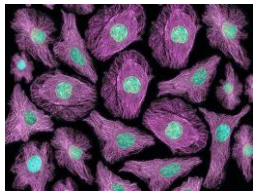
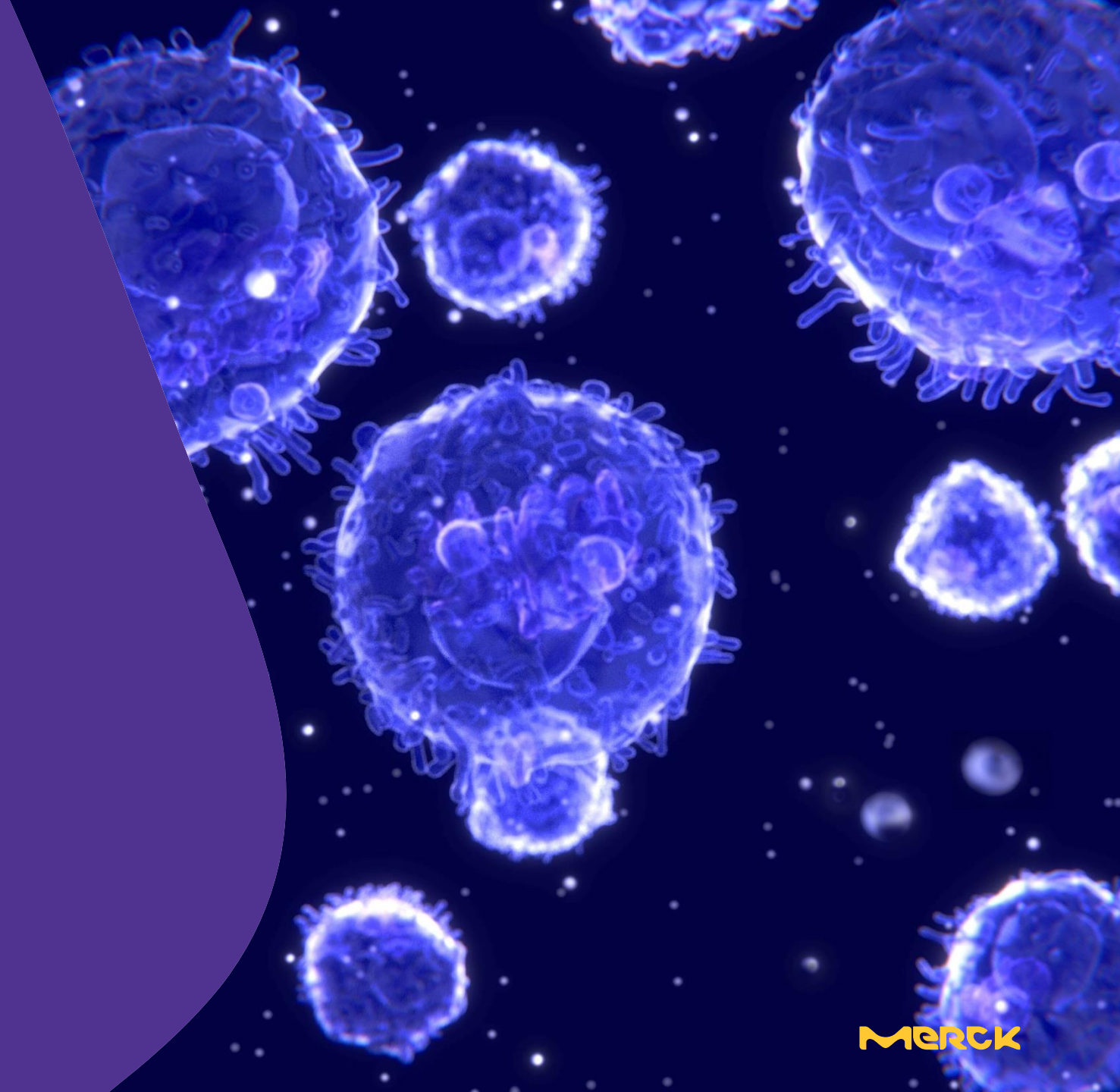
Types of
Cell Based Assays

Assay Type	Description	Common assay visuals
Cell Health	Evaluate how well cells grow	
Migration and Invasion	Evaluate if cells can invades/migrate	
Angiogenesis	Evaluate potential for blood supply	
Apoptosis/Autophagy	Cell death evaluations	
Live Cell Imaging	Dynamic evaluation of specific markers	

cell Health Assays



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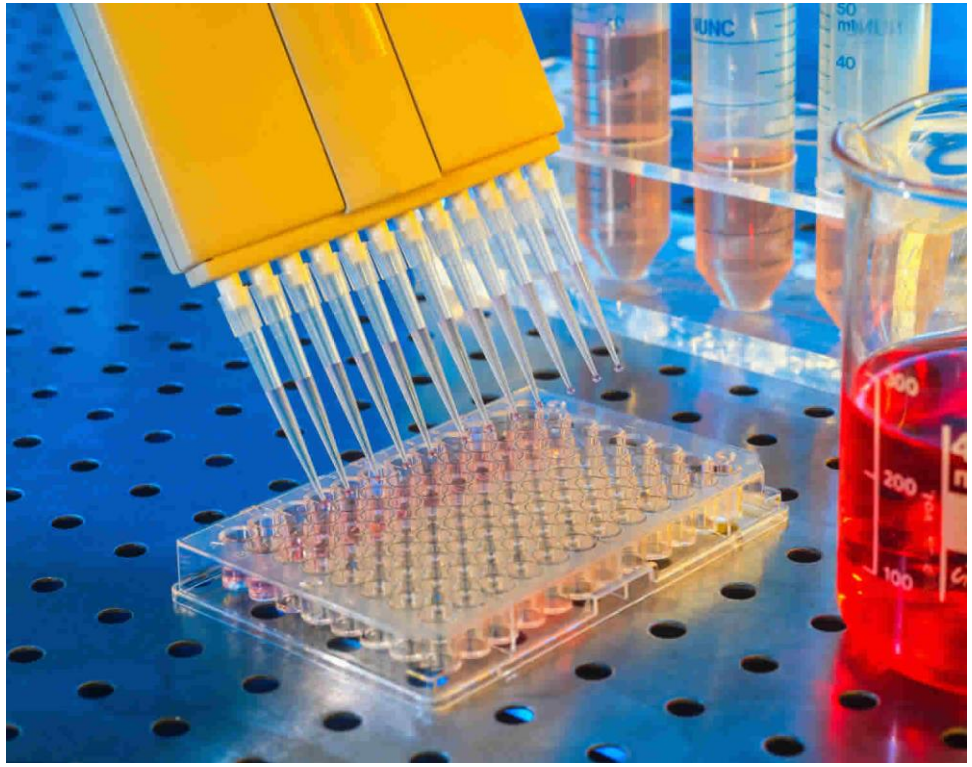
Cell Based Assays
Assessing Cell Health

- Cell health analysis reagents, kits, and tools measure general and specific indicators of cell culture vigor.
- **Viability assays** may be used as a general measure of cell health, or to assess the effects of treatment along with cytotoxicity assays.
- **Proliferation assays** often use DNA synthesis and cell division as a measure of metabolic activity of cells as the result of conditions or treatments.
- **Cytotoxicity kits** can employ bioreduction readouts to assess metabolic activity.
- Culture contamination is one of the most common barriers to efficient, reliable cell culture and should also be routinely monitored.



Cell Based Assays

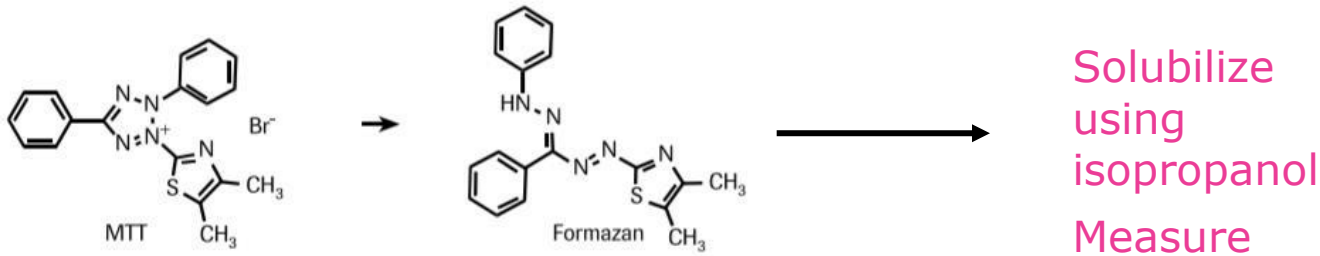
Cell Viability And Proliferation Assays

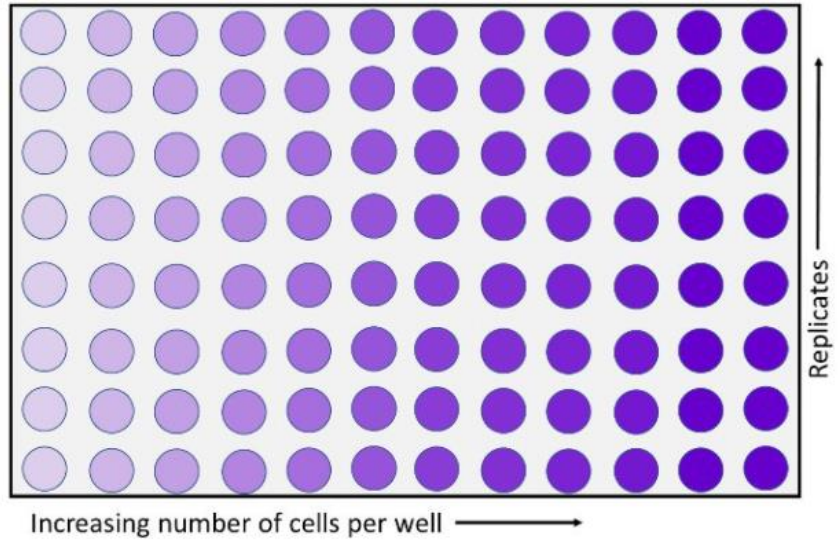
- Assays for measuring **cell proliferation and viability** are used to monitor the response and health of cells in culture after treatment with various stimuli.
- Our comprehensive tools and solutions for measuring cell viability and proliferation employ various methods, and include:
  - DNA Synthesis Proliferation Assays, e.g 5-bromo-2’-deoxy-uridine (BrdU assays)
  - Metabolic Proliferation Assays
  - Live/Dead/Total Cell Triple Staining for 3D Cultures
  - Trypan Blue Dye Exclusion Viability Counting
  - Luminescent Cell Viability Assays
  - Fluorescent Dye Proliferation Assays

Comparison of Cell Proliferation Assays

Name	Overview	Detection Method	Advantage	Disadvantage
BrdU Assay	BrdU incorporates into newly synthesized DNA and detected using anti-Brdu Antibody	ICC, IHC, FACS, ELISA	Cell Cycle Kinetics Single Cell Resolution	Lengthy Protocol Potential DNA Damage Low throughput
EdU Assay	Similar to BrdU technique but uses Click-Chemistry detection without antibodies	ICC, IHC, FACS, ELISA	Less Toxic than BrdU, Faster Protocol, No DNA Denaturation	Expensive Reagents Low throughput
MTT Assay	MTT, a yellow tetrazole, is reduced to purple formazan in living cells	Spectrophotometer	Fast Protocol High Throughput	Endpoint Assay Overestimation of Viability Final Solubilization Step
XTT Assay	Actively respiring cells convert the XTT to a water-soluble, orange colored formazan product	Spectrophotometer	High Sensitivity Large Dynamic Range, Water Soluble High Throughput	Endpoint Assay Overestimation of Viability
WST-1 Assay	WST-1 is cleaved to a soluble formazan by a complex cellular mechanism that occurs primarily at the cell surface.	Spectrophotometer	Highest Sensitivity Faster Protocol High-throughput Not an endpoint assay	Overestimation of Viability

MTT Cell Proliferation Assay



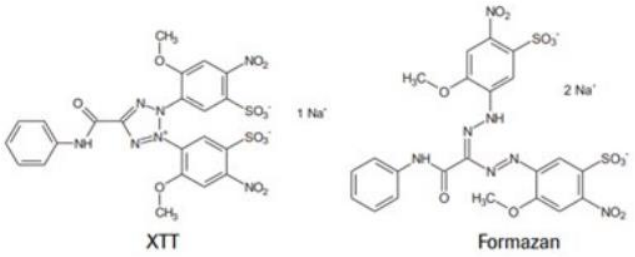


- Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole salt) is reduced to blue/purple formazan by intracellular NAD(P)H-dependent cellular oxidoreductase enzymes which present in the mitochondria of living cells.
- Formazan crystals are dissolved using a solubilization solution
- Measuring absorbance at 500-600 nanometers using a multi-well spectrophotometer.
- The darker the solution, the greater the number of viable, metabolically active cells.

Key Applications:

1. Measurement of cytotoxicity
2. Quantification of cell growth and viability
3. Measurement of cell proliferation in response to growth factors, cytokines and nutrients.

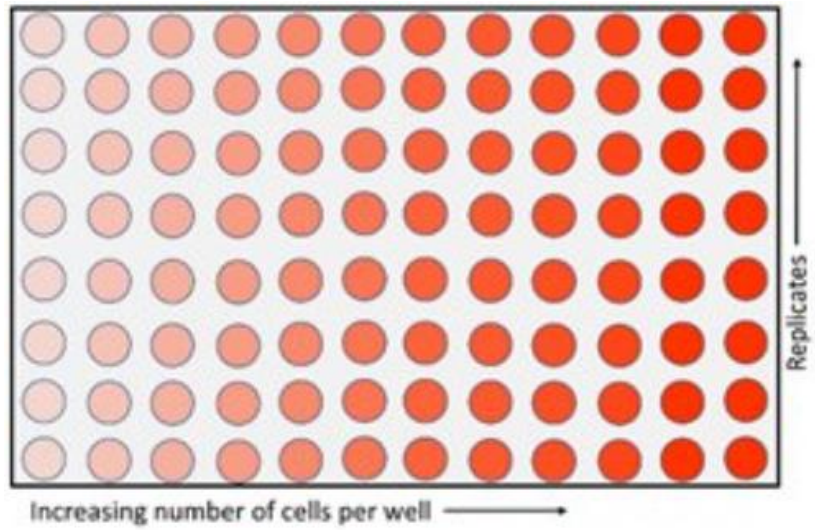
XTT Cell Proliferation Assay



Yellow, Water soluble

Orange, Water soluble

ELISA reader for microplates

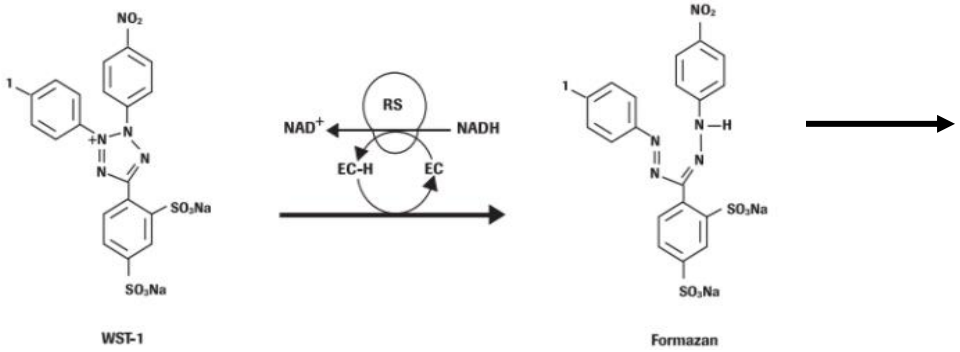


- Sample material is either **adherent or suspension cells** cultured in 96-well microplate
- Cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye by metabolically active cells
- The formazan dye formed is soluble in aqueous solutions
- Reading through scanning multiwell spectrophotometer (ELISA reader)
- An increase in the number of living cells results in an increase in the overall activity of mitochondrial dehydrogenases in the sample

Key Applications:

1. Measurement of cytotoxicity
2. Quantification of cell growth and viability
3. Measurement of cell proliferation in response to growth factors, cytokines and nutrients.

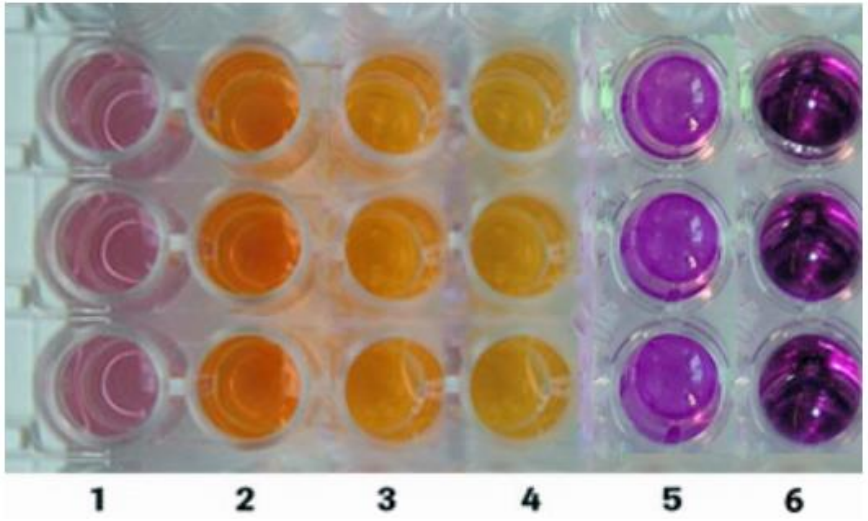
WST/ CCK8 Cell Proliferation Assay



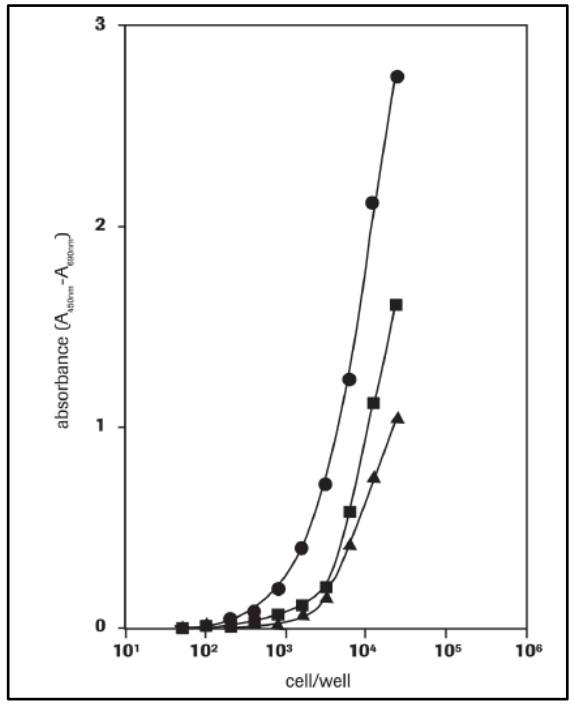
Slightly Red, Water soluble

Dark Red, Water soluble

ELISA reader for microplates



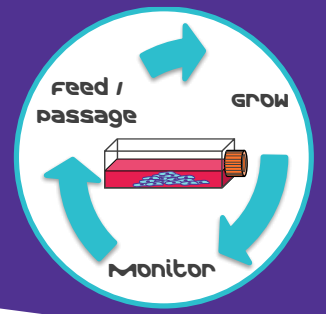
- More stable, sensitve and wider wavelength absorbance of XTT
- WST-1 can be used as a ready-to-use solution and can be stored at +2 to +8°C for several weeks without significant degradation.
- Sample material is either **adherent or suspension cells** cultured in 96-well microplate
- WST-1 is cleaved to a soluble formazan by a complex cellular mechanism that occurs primarily at the cell surface
- Wavelength measurement is between 420 and 480 nm; the reference wavelength should be >600 nm



Comparison of MTT (▲), XTT (■), and Cell Proliferation Reagent WST-1

P815 cells at cell concentrations indicated in the figure were preincubated for 20 hours before the addition of the various tetrazolium salts. After 4 hours substrate reaction, the absorbance was determined at the respective wavelength with an ELISA reader.

passaging cells



Digital Cell Imager For Routine Cell Monitoring

Cells | Grow | Passage | Transfection | Cryopreservation | Assays

What is it?

- Affordable easy to use digital cell imager designed to add convenience, consistency & reliability to routine cell monitoring & maintenance
- Compact footprint minimizes benchtop or hood space
- Additional software package that addresses advanced image analysis calculations off device

Wifi Enabled

Compatible with most vessels

Hemocytometer Counting

Small Footprint
11”x12”x6”

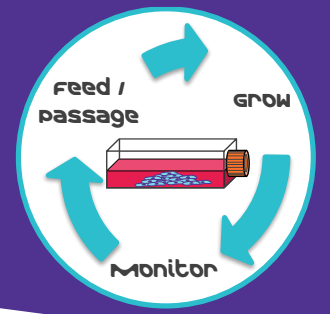
10x Objective with 20x Digital Zoom

Masking for Confluency & Estimated Cell Density

7” Touch Screen

Ethernet & USB Ports

Adjustable Light Source



viability assay

Scepter Handheld Automated Cell Counter

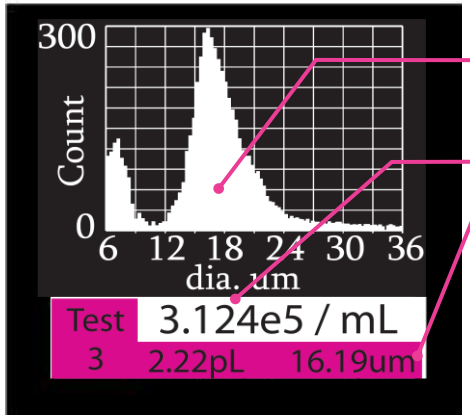
Cells | Grow | Passage | Transfection | Cryopreservation | Assays

Handheld Pipette Form

- Compact, easy to use
- Ergonomic action feels like pipetting
- On-screen instructions
- USB port for downloading data and charging
- Stores 72 histograms

Plastic Consumable Sensor

- 2 sensor types available
- Integrated sensing electrodes
- Precision-molded sampling chamber
- Precision-manufactured electronic sensing zone
- Discriminates cell sizes with sub-micron resolution
- Discriminates cell volumes with sub-picoliter resolution



Integrated Display

- Histogram data on cell populations
- Cell concentration
- Mean cell volume and size
- Can apply custom gating
- Gain insight into cell health

Scepter 3.0 Handheld Automated Cell Counter

Scepter 3.0 Handheld Automated Cell Counter
Product Enhancements

Increased Screen Size

Software updates

- Easier on-instrument visualization & manipulation of cell counts
- Storage for 999 histograms (2.0 only stores 72)
- No software to download

Mountable Charger

- Error proof charging
- Mounts anywhere
- More efficient charging mechanism

Bluetooth®/WiFi Capability

USB Port

- Easier/more flexible data export

Sensor Insertion/Ejection

- Reduce risk of damage
- Ensures proper usage
- Improved ergonomics

Redesigned Sensor

- Enables easy insertion & ejection
- 2.0 sensors not compatible

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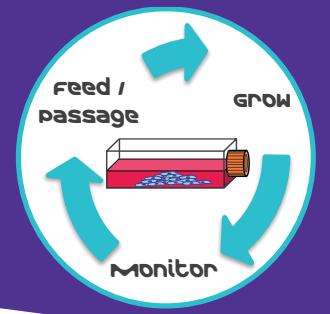
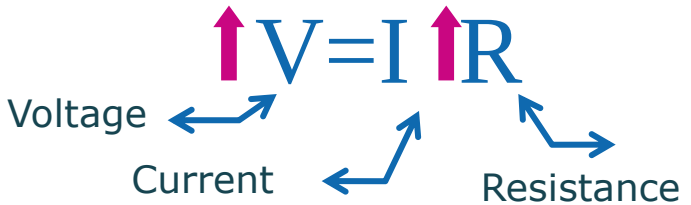
viability assay

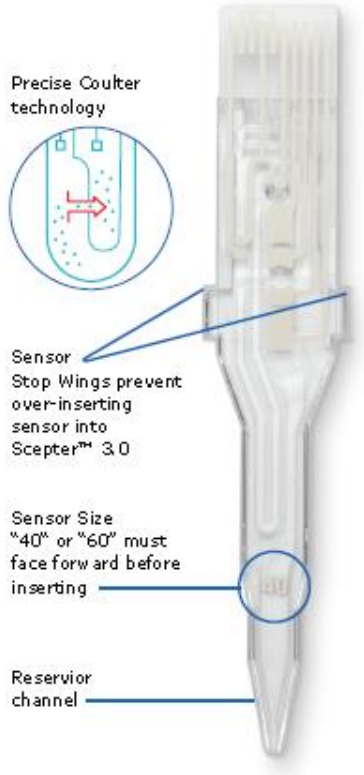
Scepter 3.0 Handheld Automated Cell Counter



Scepter Sensors use Coulter Counting Principle

- Precise sample volumes are drawn into the Scepter sensor
- As cells flow through the aperture in the sensor, resistance increases. This increase in resistance causes a subsequent increase in voltage.
- Voltage changes are recorded as spikes with each passing cell.
- Spikes of the same size are bucketed into a histogram and counted. This histogram gives you quantitative data on cell morphology that can be used to examine the quality and health of your cell culture.





viability assay

Scepter 3.0 Handheld Automated Cell Counter

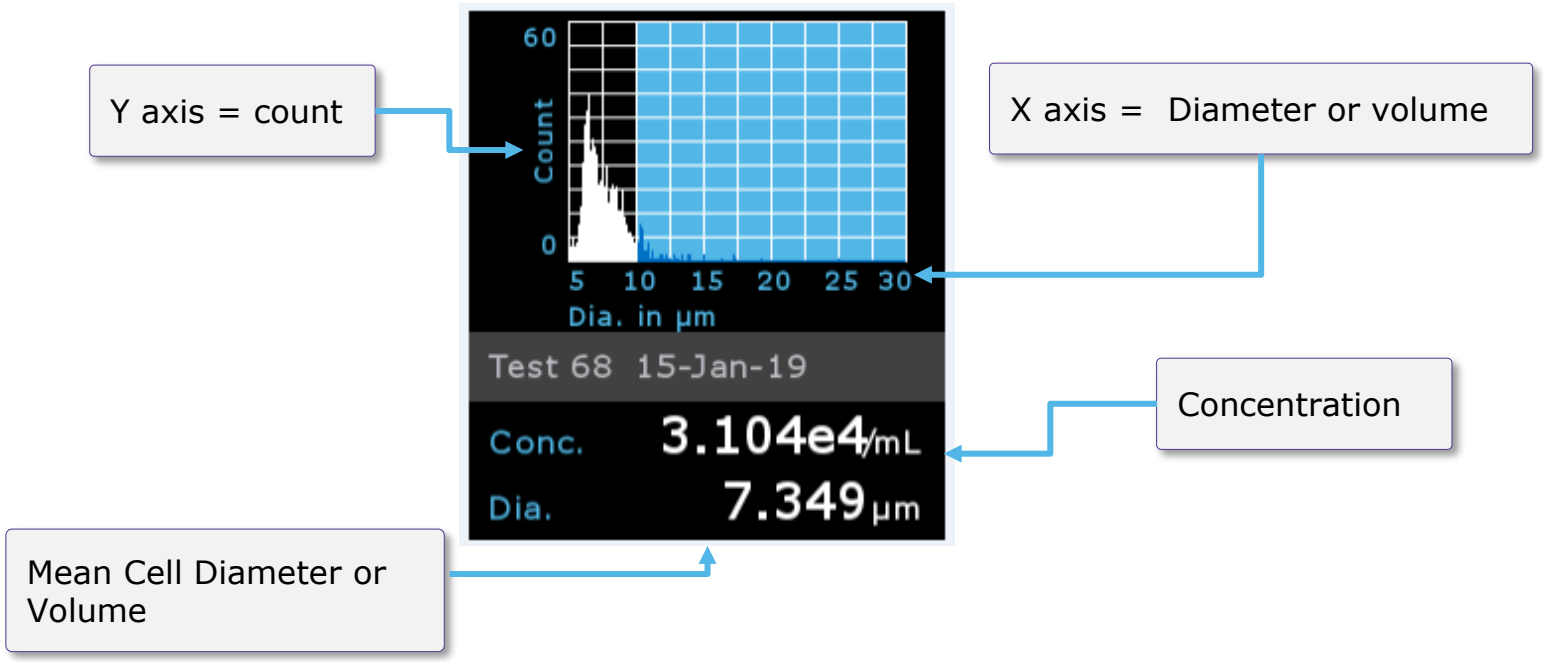
Scepter Histograms provide more information than other systems

The scepter's screen displays:

- Cell concentration
- Mean cell size/diameter
- Mean cell volume
- Histogram of size or volume distribution

Fun facts:

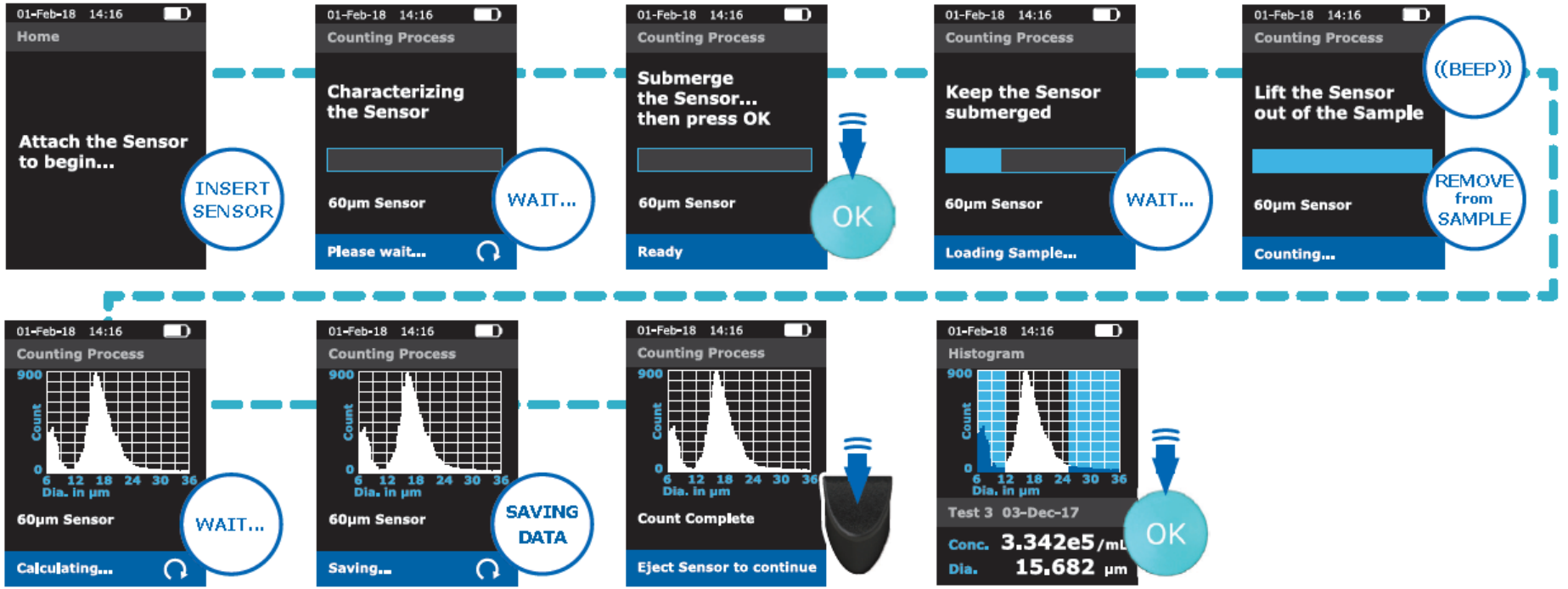
Over 1,200 histograms have been generated for the Scepter 3.0 validation testing



viability assay

Scepter 3.0 Handheld Automated Cell Counter

Work Procedure

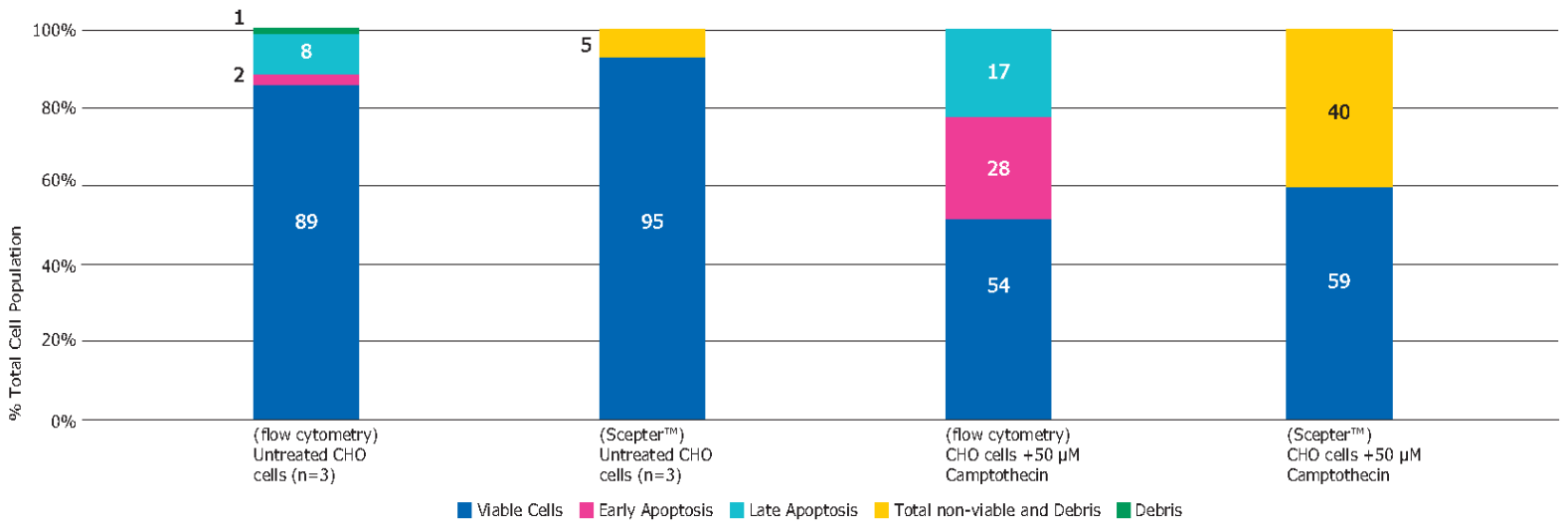


viability assay

Scepter 3.0 Handheld Automated Cell Counter

Application

predicting cell death by rapidly assessing size changes with the scepter™ cell counter



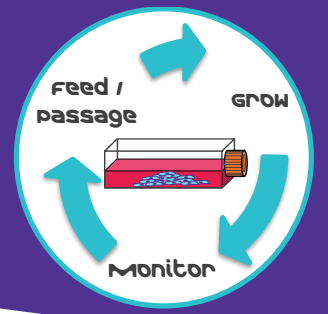
Comparison of the Scepter™ cell counter with a flow cytometer in measuring apoptotic and non-apoptotic cell populations. Percentages of viable, early, and late apoptotic CHO cells determined using flow cytometry, and compared with viable and non-viable/debris populations determined using a Scepter™ cell counter. Cells were enzymatically dissociated, washed and resuspended in PBS.

The Scepter™ cell counter was used to qualitatively monitor apoptosis events in CHO. The cell line was incubated with camptothecin, an inhibitor of nuclear topoisomerase and known inducer of apoptosis.

Cell line exhibited an increased percentage of smaller cells, as seen by the shift in the histogram population to the left after a 24 hour exposure to camptothecin.

viability assay

Scepter 3.0 Handheld Automated Cell Counter



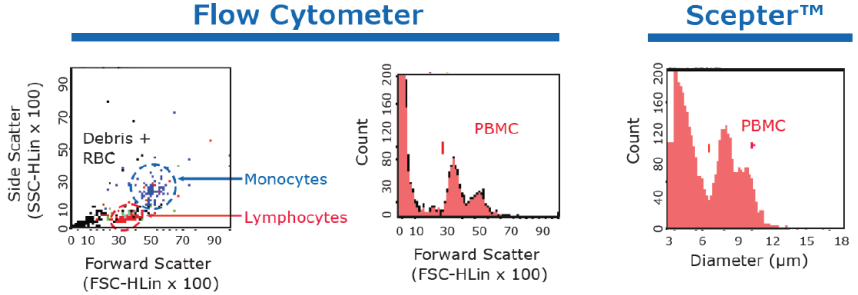
Cells | Grow | Passage | Transfection | Cryopreservation | Assays

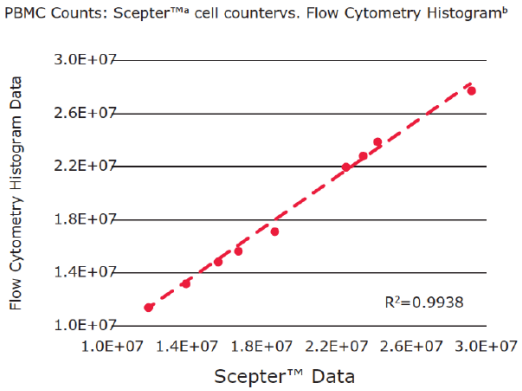
Application

Rapid counting for PBMC

Test	Cell Fraction	Scepter™ cell countera	Forward Scatterb	Stainingc
1	Lymphocyte	58	65	63
	Monocyte	42	35	37
2	Lymphocyte	68	72	71
	Monocyte	32	28	29
3	Lymphocyte	66	69	71
	Monocyte	34	31	29
4	Lymphocyte	62	67	64
	Monocyte	38	33	36
5	Lymphocyte	64	66	67
	Monocyte	36	34	33
6	Lymphocyte	62	58	60
	Monocyte	38	42	40
7	Lymphocyte	65	72	72
	Monocyte	35	28	28
8	Lymphocyte	59	61	61
	Monocyte	41	39	39
9	Lymphocyte	64	72	72
	Monocyte	36	28	28

PBMC Sample 1





Lymphocyte and monocyte subset frequencies from nine individual PBMC samples. Aliquots from each sample were analyzed using the Guava easyCyte™ and Scepter™ instruments.

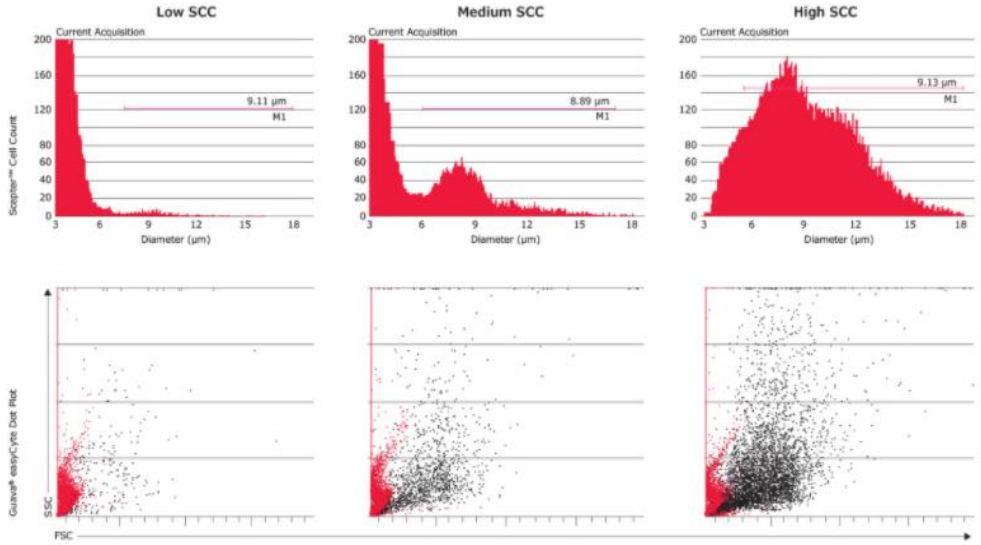
viability assay

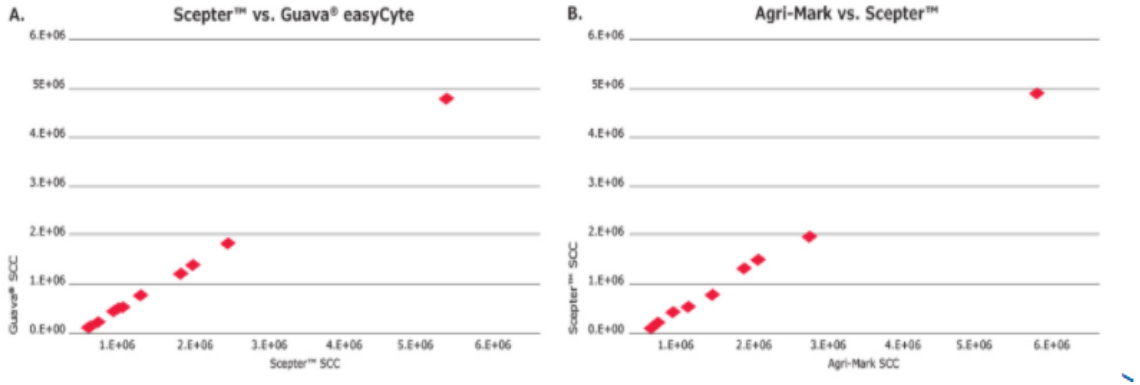
Scepter 3.0 Handheld Automated Cell Counter

Cells | Grow | Passage | Transfection | Cryopreservation | Assays

Application

Rapid counting of somatic cells in dairy milk





Scepter™ cell counting and Guava® easyCyte flow cytometry provide interpretable SCC data for dairy milk samples containing low, medium, and high numbers of somatic cells. For the Guava® data, events were gated to separate smaller (low FSC) fat globules (red dots) from larger (higher FSC) somatic cells.

High correlation of SCC data between three cell counting platforms number of fluorescently labeled cells following ViaCount staining. Agri-Mark is an external SCC testing facility serving the dairy industry.

Live/Dead® Cell Viability Assay Kit

- *Simultaneous fluorescence staining of live & dead cells*
- *Calcein-AM stains live (green)*
- *Propidium Iodide stains dead (red)*
- *Hoechst 33342 stains all cells blue for total cell*

Advantages of the kit include:

- 3D and 2D cell culture and flow cytometry
- Easy-to-use assay protocol
- Intensity of Calcein-AM and Hoechst 33342 stains are stronger compared to other assay kits
- Identification of spatial and temporal patterns of cell death occurring in complex tissues

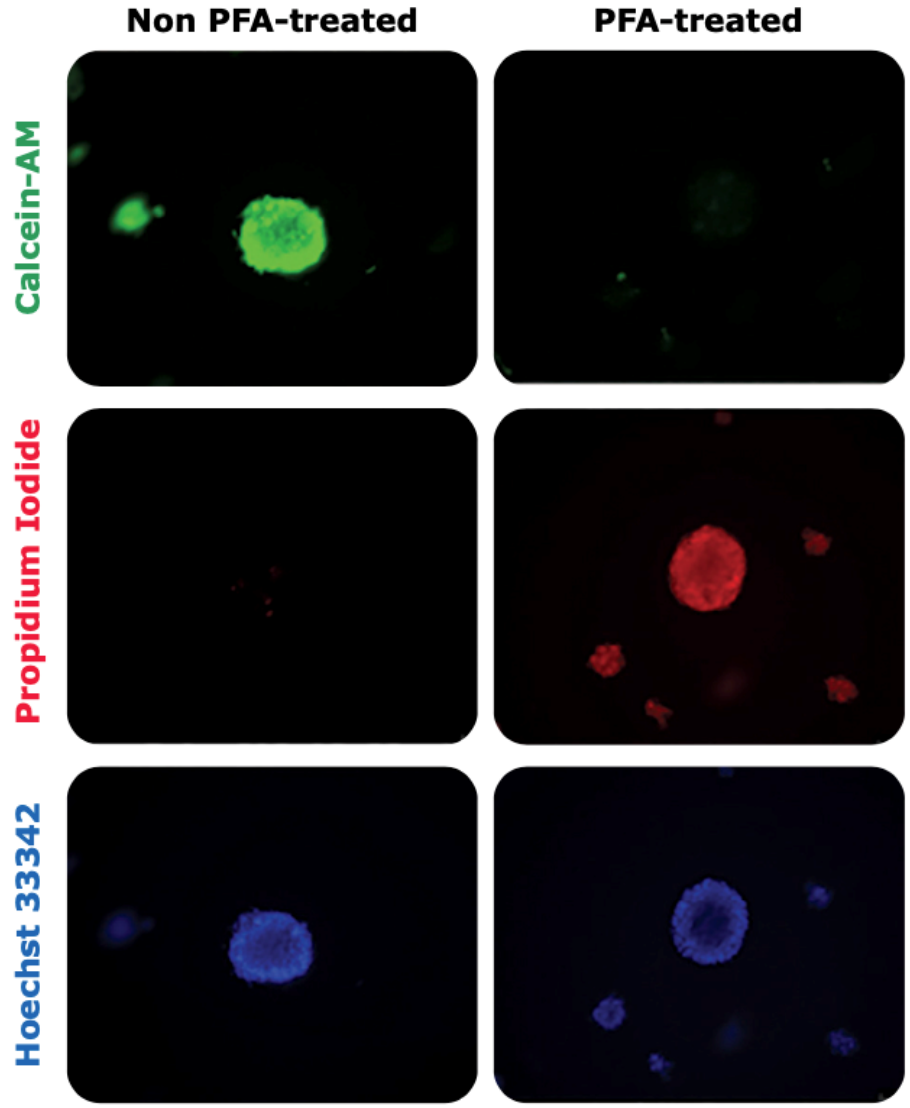


Figure 16. Live/Dead staining of liver spheroids. Day 7 HepG2 spheroids embedded in Matrigel® substrate and stained with the Live/Dead assay before and after selective paraformaldehyde (PFA) fixation/killing.

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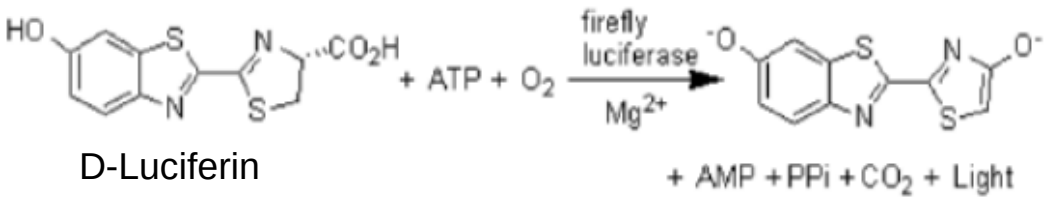
Viability Assays
ATP Cell Viability Luciferase Assay

A highly sensitive firefly luciferase cell-based assay for quantifying ATP in cell cultures, used to measure cell viability.

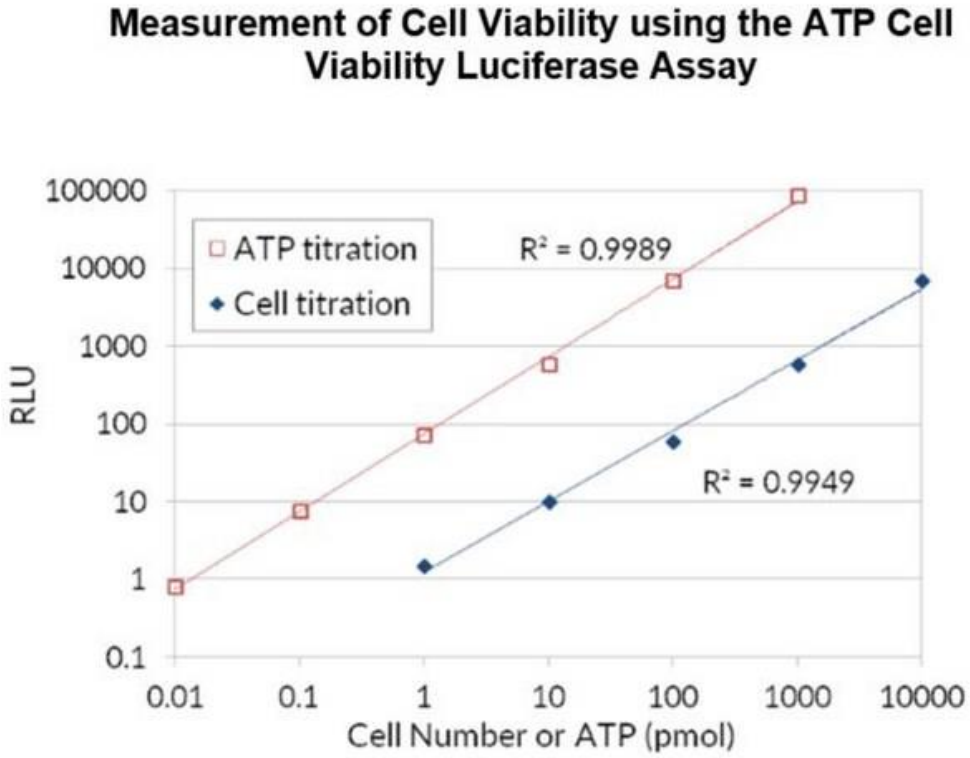
This kit can be used to detect as little as a single cell or 0.01 picomoles of ATP.

Flash luciferase assay



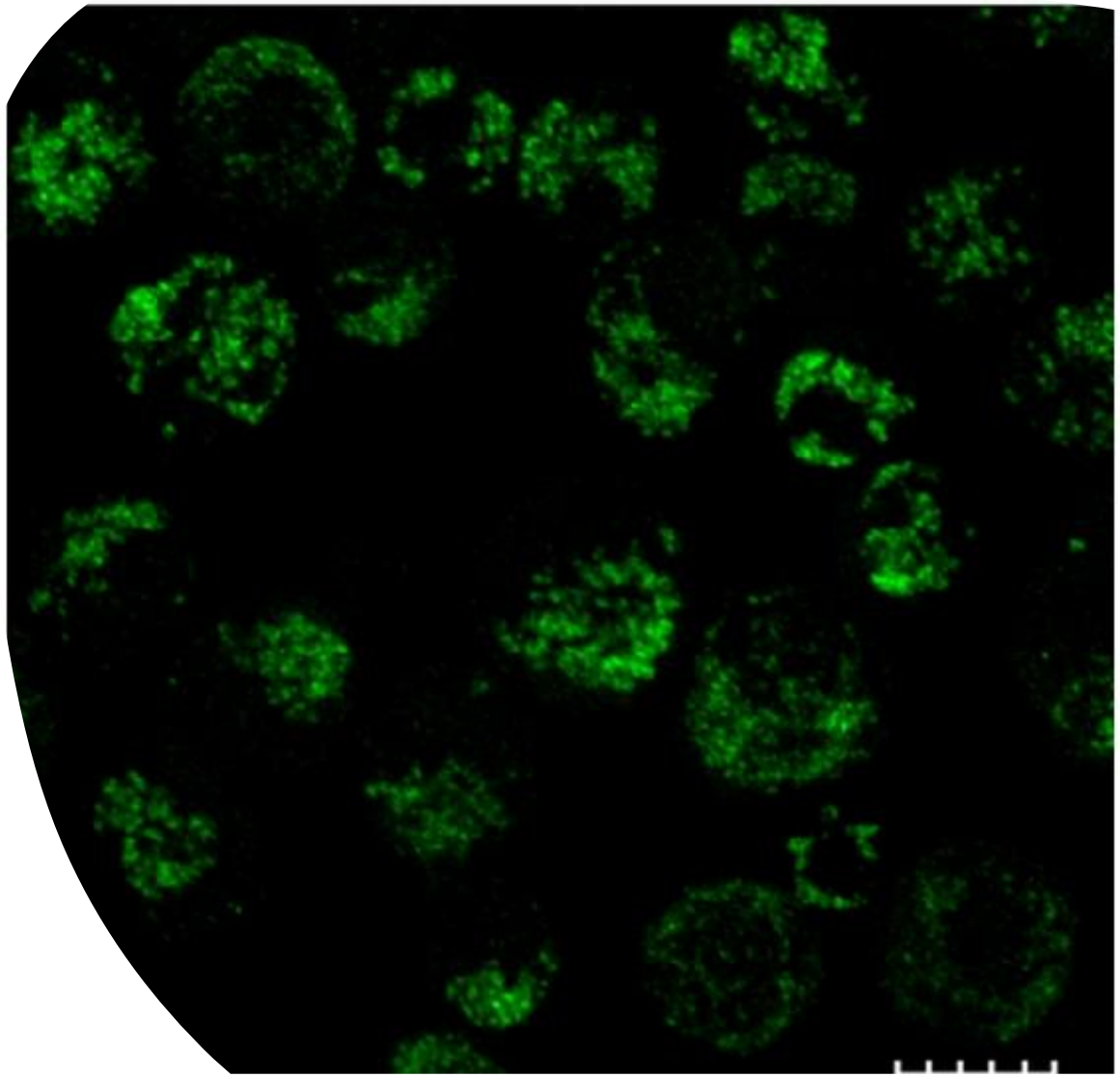
D-Luciferin

Firefly luciferase uses ATP molecules to oxidize D-Luciferin and produces light, proportional to the amount of ATP available



Cell Based Assays
Apoptosis Assays

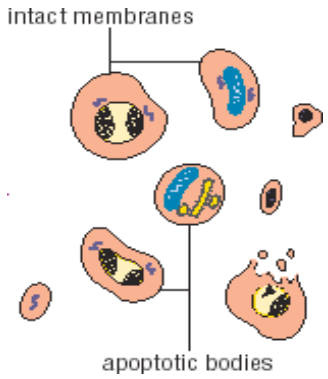
- Apoptosis, or programmed cell death, is a growth-limiting regulatory mechanism by which cells can trigger their own death in response to extracellular signals because of irreparable cellular or DNA damage.
- The ability of tumor cells to elude **apoptosis is a hallmark of most types of cancer.**
- Apoptosis plays an important role in various developmental mechanisms, such as preventing the overgrowth of neuronal cell lineages in the developing brain and regulating interdigital spacing in limb development.
- Apoptosis is a multistep process including early, mid and late-stage cellular events which can be detected using various cellular assays including Annexin V, Caspase and TUNEL detection.



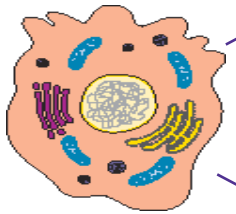
Life cycle of cells

What happens & how can it be measured?

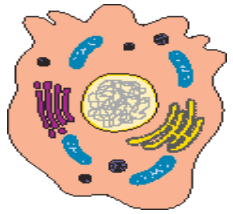
Dying cells ⟷ **Viable cells**



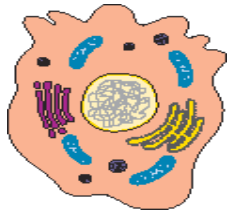
Cytotoxicity



Cell division



Cell proliferation



Apoptosis is a physiological process
Necrosis is a pathological process

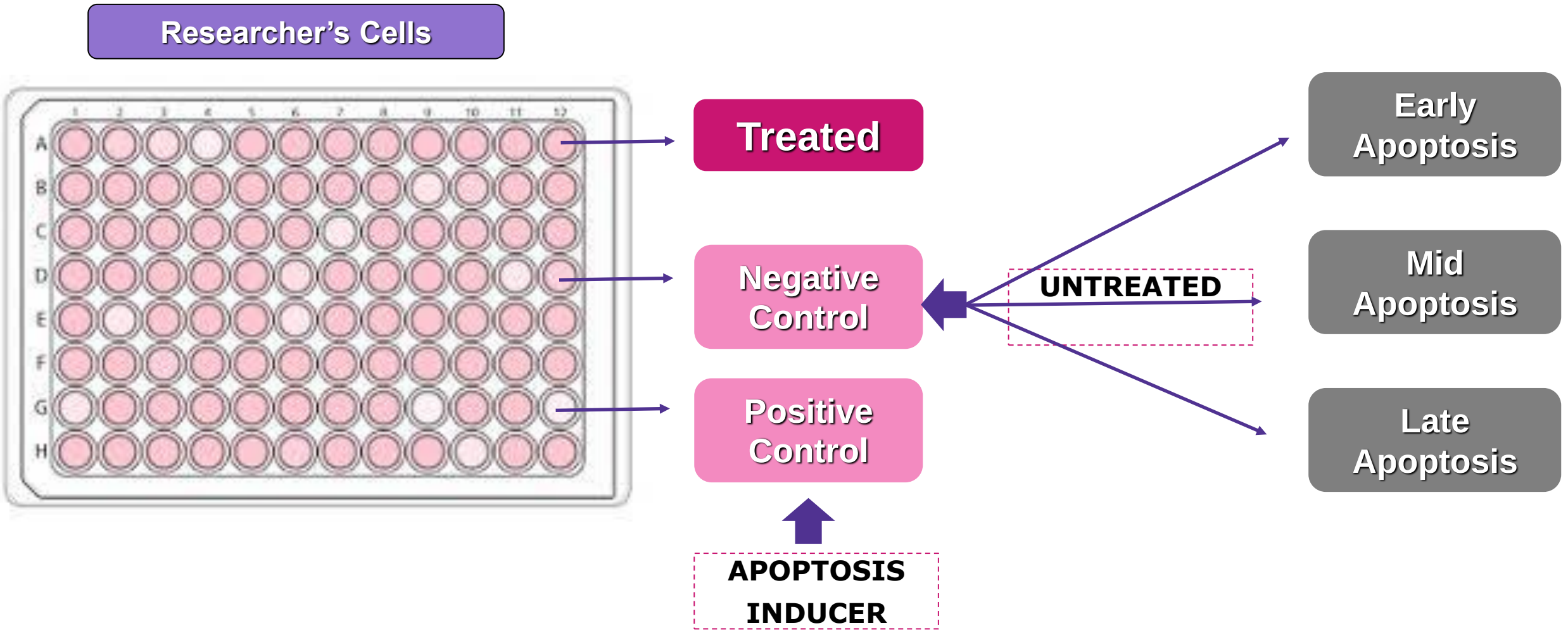
Apoptosis:

- From Greek word: Falling off (leaves)
- Normal process in development and morphogenesis
- Programmed cell death and does not induce inflammatory response

Necrosis:

Response to injury or toxic damage which induces an inflammatory response

Classical Approach to Study Apoptosis



Apoptosis Inducer

Apoptosis inducer can be used to induce DNA fragmentation in apoptosis event for used for positive control

No.	Agent	Dose	Solvent For Stock Solution	Cat. No.
1	Actinomycin D	500 ng/ml	Methanol	114666
2	Aphidocolin	2 mg/ml	DMSO	178273
3	A23187	10 mg	DMSO	100105
4	Caffeine	16 mM	Boiling H_2O	205548
5	Camptothecin	4 mg/ml	DMSO	208925
6	Cycloheximide	100 mg/ml	H_2O	239764
7	Dexamethasone	1 mM	Ethanol	265005
8	Doxorubicin (Adriamycin)	0.2 mg/ml	H_2O	324380
9	5-Fluorouracil	25 mg/ml	DMSO, Hot H_2O	343922
10	Hydroxyurea	500 nM	H_2O	400046
11	Paclitaxel (TAXOL)	100 - 580 nM	DMSO	580555
12	Staurosporine	500 nM	DMSO	569397
13	Thymidine	2 mM	PBS	6060
14	Vinblastine	60 nM	Methanol	677175

Not every agent will induce apoptosis in every cell type; indeed, dexamethasone will actually stimulate growth of some cells. Depending on the agent selected and the concentrations used, maximal induction of a particular protein may occur within 8 to 72 hours post-treatment.

APOPTOSIS STAGES

Early Apoptosis

1. Translocation of phosphatidylserine to the outer leaf of plasma membrane
2. loss of mitochondrial membrane potential
3. Cytochrome C & ATP release activation of caspase-8 and 9

ASSAY:

1. DETECTION OF PHOPHOTIDYL SERINE (PS) using **ANNEXIN V ASSAY**
2. MITOCHONDRIAL DETECTION

Mid Apoptosis

1. Activation of caspase-3, 6 and 7
2. Poly-ADP-Ribosepolymerase (PARP) cleavage
3. Cell shrinkage
4. Activation of nucleases

ASSAY:

1. DETECTION OF CASPASE

Late Apoptosis

1. DNA fragmentation
2. Nuclear collapse
3. Formation of apoptotic bodies phagocytosis by macrophages

ASSAY:

1. DNA FRAGMENTATION DETECTION using **TUNEL ASSAY**
2. H2Ax PHOSPHORYLATION ASSAY

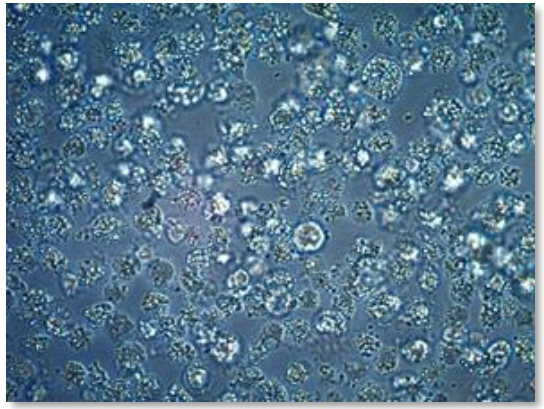
Apoptosis Assays

Product Description	Sample Type	Application
Early stage Apoptosis		
ApopNexin™ Kits	Adherent Cells/ Suspension Cells	Immunofluorescence
Annexin-V Kits	Adherent Cells/ Suspension Cells	Immunofluorescence Flow Cytometry
MitoLight® Mitochondrial	Adherent Cells/ Suspension Cells	Immunofluorescence
Apoptosis Detection Kits (EMD)		
BioTracker Mitochondria Dyes (EMD)	Adherent Cells/ Suspension Cells	Live Cell Imaging
Mitochondrial Membrane Potential Kit (Sigma)	Adherent Cells/ Suspension Cells	Immunofluorescence/ Flow Cytometry
Cytochrome C Assay Kits (Sigma)	Adherent Cells/ Suspension Cells	Plate Reader
Mitochondria Isolation Kits (Sigma)	Adherent Cells/ Suspension Cells	Isolation
JC-1 Dye (Sigma)	Adherent Cells/ Suspension Cells	Live Cell Imaging
Mid-stage Apoptosis		
BioTracker NucView® Caspase-3 Dyes (EMD)	Adherent Cells/ Suspension Cells	Live Cell Imaging
BioTracker NucView® Caspase-3 Dyes (EMD)	Adherent Cells/ Suspension Cells	Flow Cytometry
CaspaTag™ Pan-Caspase	Adherent Cells/ Suspension Cells/Tissue	Immunofluorescence/ Cytometry/Plate Reader
Caspase-3,7	Adherent Cells/ Suspension Cells/Tissue	Immunofluorescence/ Cytometry/Plate Reader
Caspase-8	Adherent Cells/ Suspension Cells/Tissue	Immunofluorescence/ Cytometry/Plate Reader
Caspase-9 (EMD)	Adherent Cells/ Suspension Cells/Tissue	Immunofluorescence/ Cytometry/Plate Reader
CaspSCREEN™ Kit (EMD)	Suspension Cells	Cytometry
Caspase Activity Kits (EMD)	Cell Lysates	Plate Reader
Caspase 3/8 Assay Kit (Sigma)	Adherent Cells/ Suspension Cells	Immunofluorescence/Cytometry
Late Apoptosis		
ApopTag® TUNEL Kits (EMD)	Adherent Cells/ Suspension Cells/Tissue	Immunohistochemistry/ Immunofluorescence/ Cytometry
ApopTag® ISOL Kits (EMD)	Adherent Cells/ Suspension Cells/Tissue	Immunohistochemistry/ Immunofluorescence
FragEL™ DNA Fragmentation Detection Kits (EMD)	Adherent Cells/ Suspension Cells/Tissue	Immunohistochemistry/ Immunofluorescence
Flow Cytometry Kit for Apoptosis (Sigma)	Suspension cells	Flow Cytometry
Cell Death Detection Kits (Roche)	Adherent Cells/ Suspension Cells/ Tissue	Immunohistochemistry/ Immunofluorescence/ ELISA

Mycoplasma contamination

It's me- I'm the problem

Most cell culture contamination can be detected by a visual check



preventative measures

- Always work in biosafety cabinet
- Disinfection: Bleach, Alcohol (ie. ethanol, isopropanol)
- Sterile filter reagents before use
- Do not work or pass over open vessel containers or open bottles of reagents
- Reduce clutter in biosafety cabinet

Mycoplasma contamination is cell culturists' worst nightmare…

what is mycoplasma?

- Small bacteria (~100nm) and are not visible to the naked eye
- Lack cell wall therefore not affected by antibiotics
- Feed off of host cell to survive
- May alter cell morphology
- May alter cell growth
- May cause chromosomal aberrations

→ **Invalidate Results**

test early and often

- Culture isolation
- PCR
- Indirect DNA stain (Hoechst)

❑ **Testing requirements (ICH, FDA, EMA)**

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Contamination Detection
Mycoplasma Detection/Elimination Kits

The LookOut® Mycoplasma PCR Detection Kit

PCR based method for highest sensitivity in the detection of Mycoplasma, Acholeplasma, and Ureaplasma contamination in cell cultures and other cell culture derived biologicals.

Venor™ GeM Mycoplasma Detection Kit, PCR-based
Kit employs PCR technology for rapid and reliable detection of mycoplasma DNA in cell cultures and virus stocks

The LookOut Mycoplasma Elimination Kit

Suitable for the elimination of Mollicutes and related organisms (Mycoplasma, Acholeplasma, Spiroplasma, and Entomoplasma) in cell and virus cultures.

The LookOut® Mycoplasma qPCR Detection Kit:

qPCR Probe Kit for sensitive, reliable quantitative detection of mycoplasma DNA in research, industrial application and product testing.

NEW!

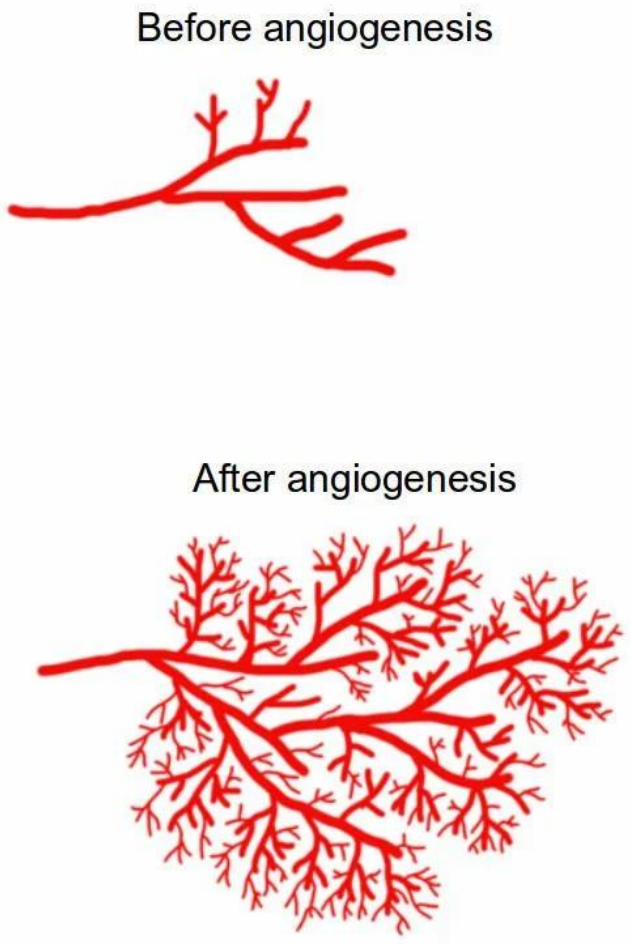
LookOut® One-step Mycoplasma qPCR Detection Kit is a PCR detection kit with all components (including polymerase) lyophilized in one tube

LookOut® Mycoplasma DNA Erase Wipes are ready-to-use wet wipes for the elimination of DNA, RNA, DNases, and RNases from lab surfaces and PCR workstations, for enhanced PCR accuracy for mycoplasma detection or any PCR applications

Cell Health
Angiogenesis Assays

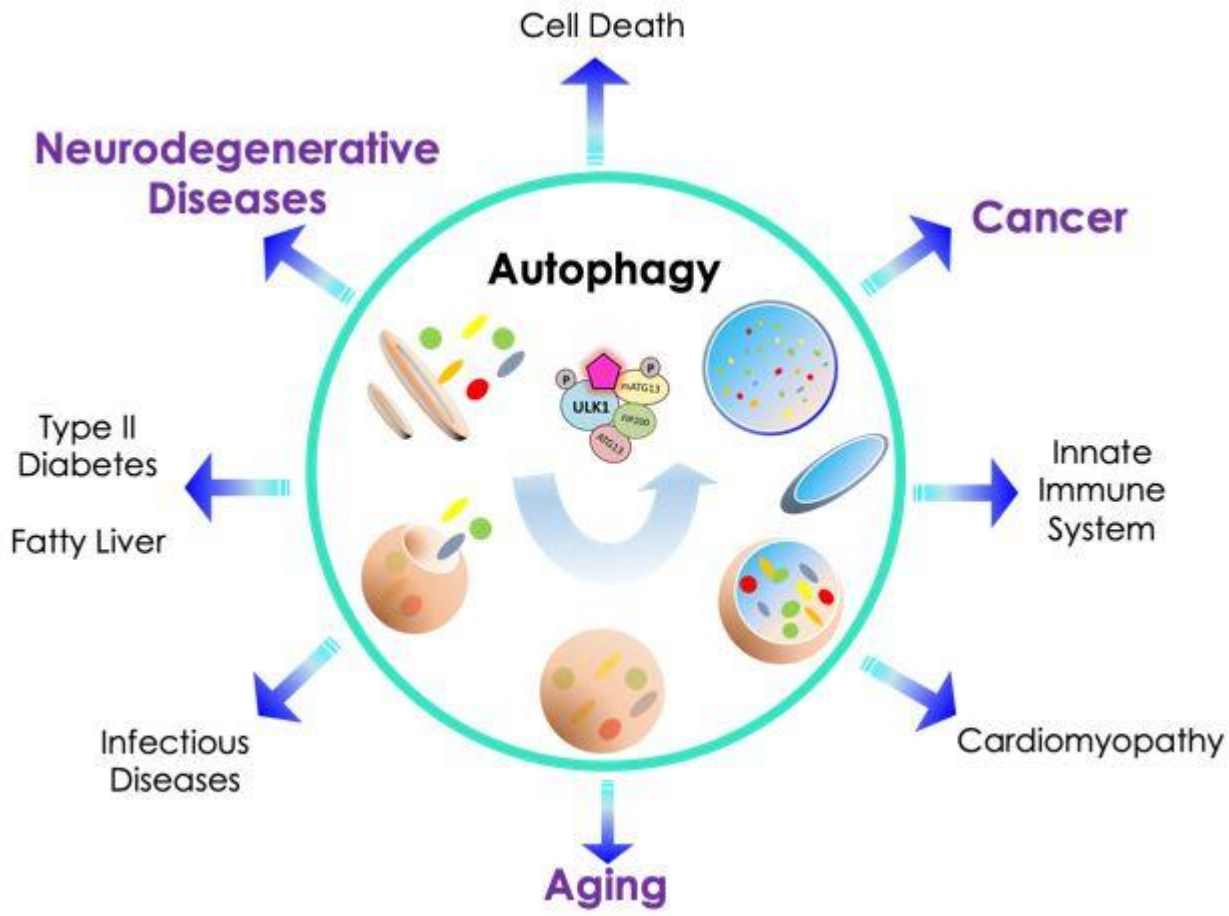
- Angiogenesis is the process of generating new capillary blood vessels and is a fundamental component of a number of normal (reproduction and wound healing) and pathological processes including tumor growth and metastasis.

- Types of Angiogenesis Assay
  - Angiogenesis Tube Formation Assays
  - Microfluidic Angiogenesis Assays
  - Endothelial Adhesion, Invasion, And Migration Assays
  - Scratch Wound Healing Assays

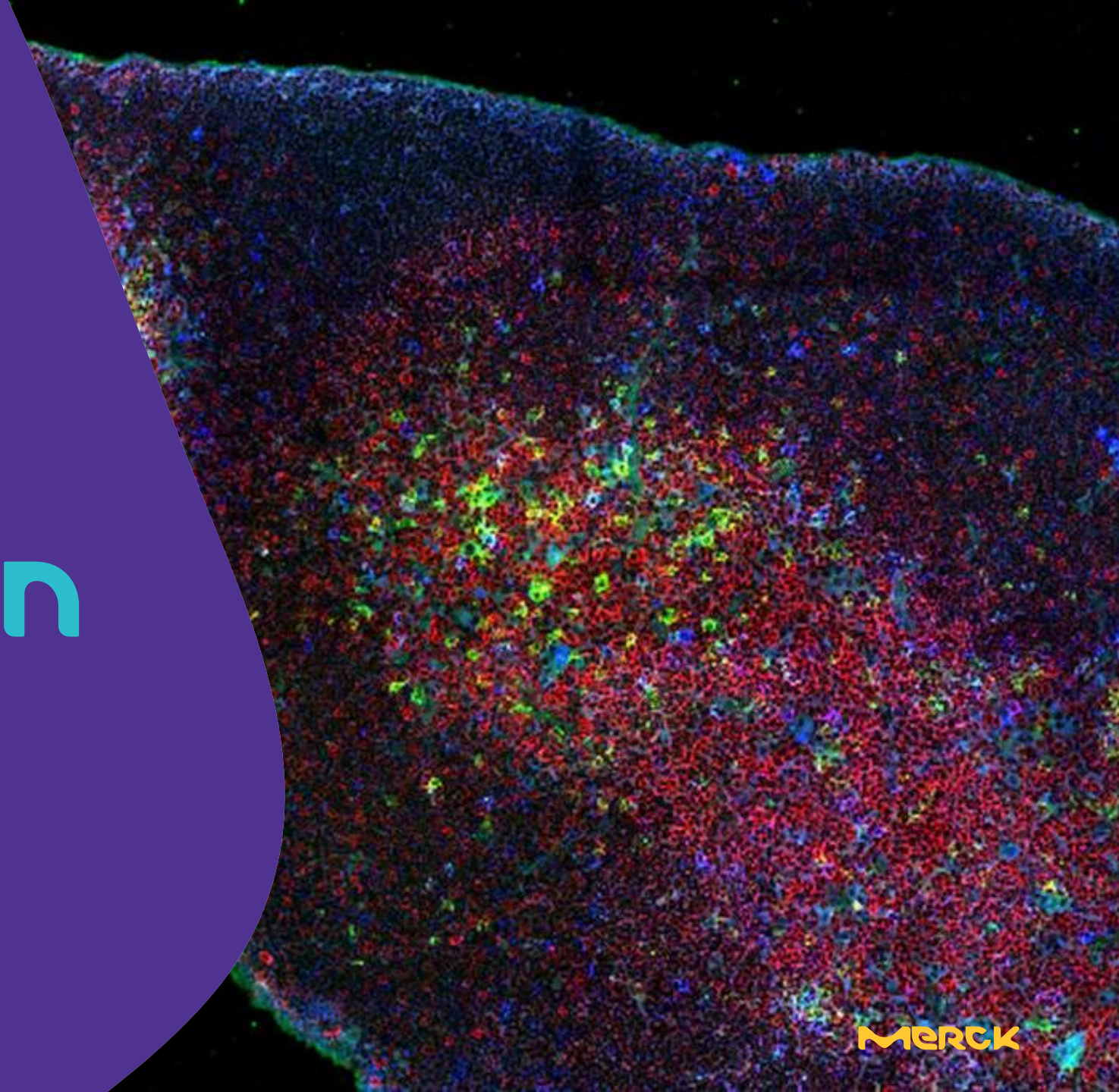


Cell Health
Autophagy Assays

- Autophagy is a highly regulated homeostatic degradative process where cells destroy their own components via the lysosomal machinery and recycle them.
  - This process is associated with diverse diseases including Alzheimer's disease, aging, cancer and Crohn's disease.
- Autophagy Assays:
  - Live Cell Lc3 Lentiviral Fluorescent Biosensors
  - Lc3-ii Autophagy Enrichment Kits
  - Flow Cytometry Autophagy Detection



Cell Migration and Invasion Assays



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Migration and Invasion Assays
The Boyden Chamber Assay

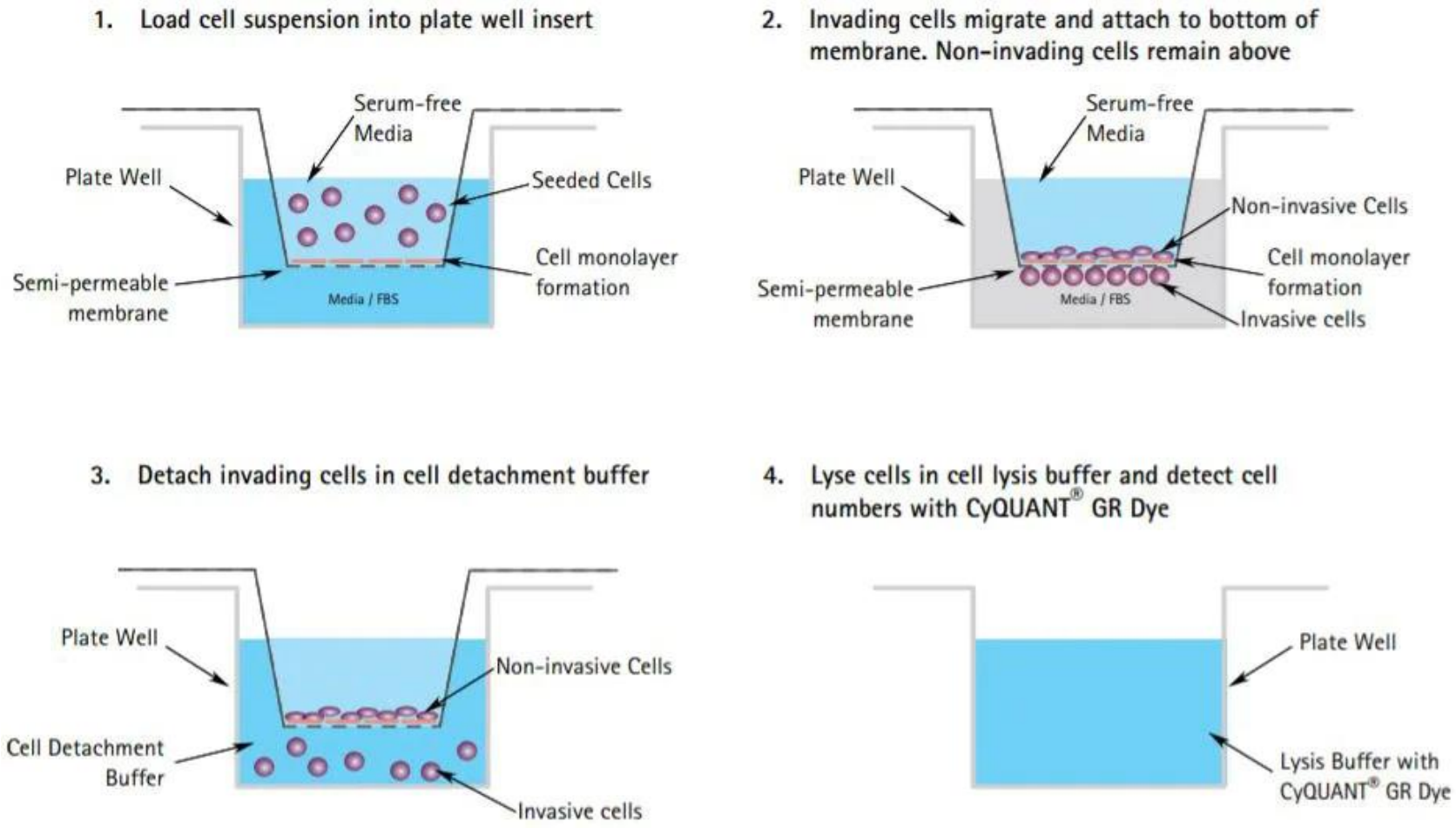
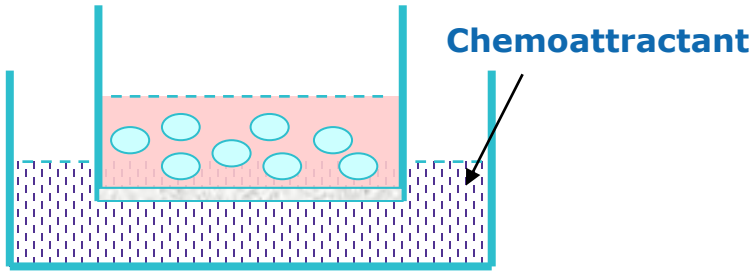


Figure 2. The Boyden Chamber Assay Protocol. Cells are allowed to migrate through a cell monolayer or ECM protein mixture which have been seeded onto a semi-permeable membrane cell culture insert with chemoattractants added below the membrane. Migrated cells can then be quantified by staining cells with DNA dyes such as Calcein-AM or CyQUANT GR Dyes

Types of Migration Assays

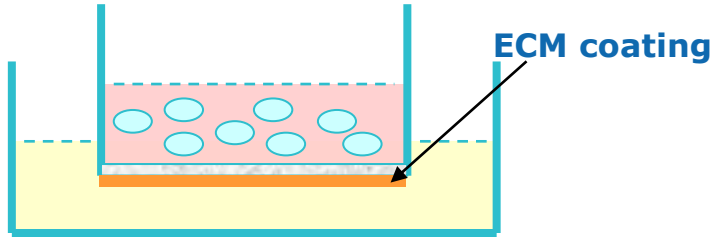
Chemotaxis

- Directed cell movement toward chemical gradient
- Microporous membrane - uncoated



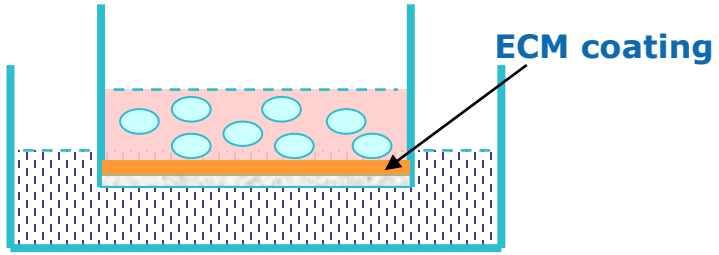
Haptotaxis

- Directed cell movement toward ECM protein
- Microporous membrane – outer side coated with ECM protein



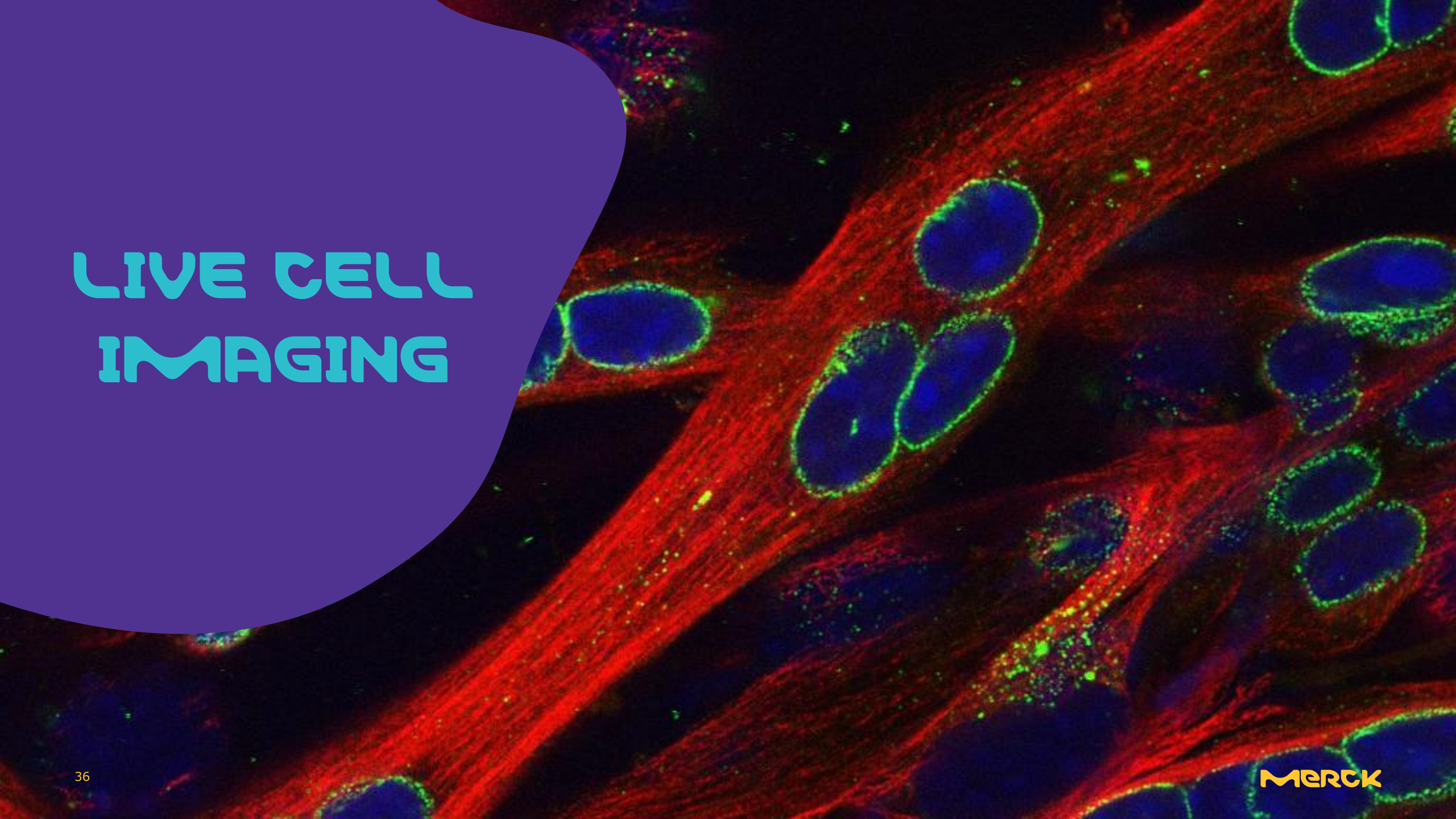
Invasion

- Cell invasion through an ECM protein &/or another cell layer
- Microporous membrane – inner side coated with ECM protein



35

LIVE CELL IMAGING



Bind and Shine

What are Live Cell Probes? How are they used?

Probes track and analyze cellular events in real time in living cells, instead of fixed cells or tissues, or extracts

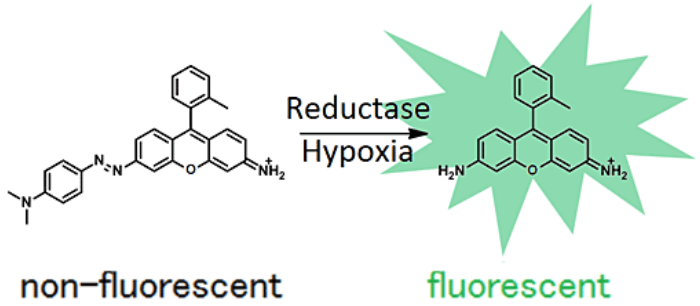
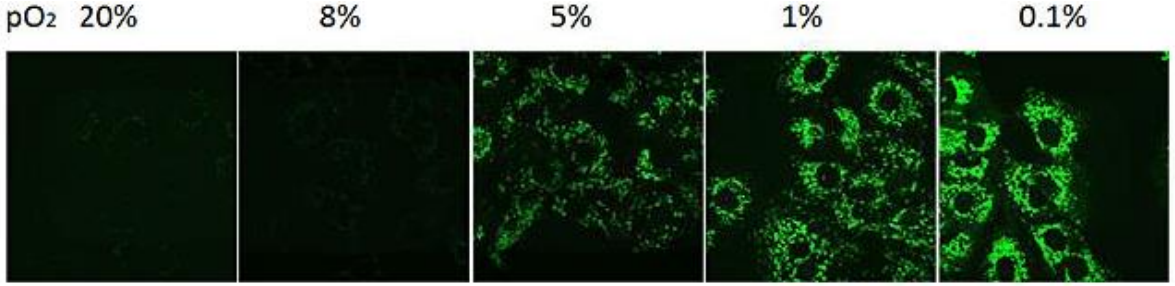


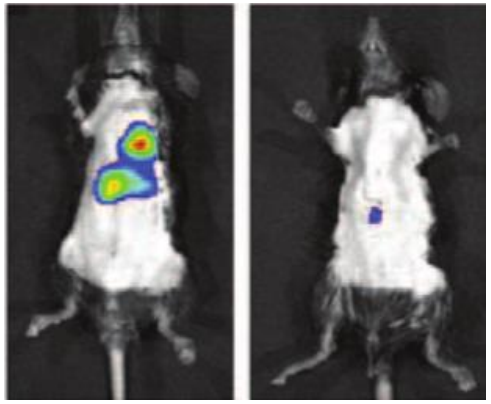
Fig. 2. Reduced reaction of MAR under hypoxia



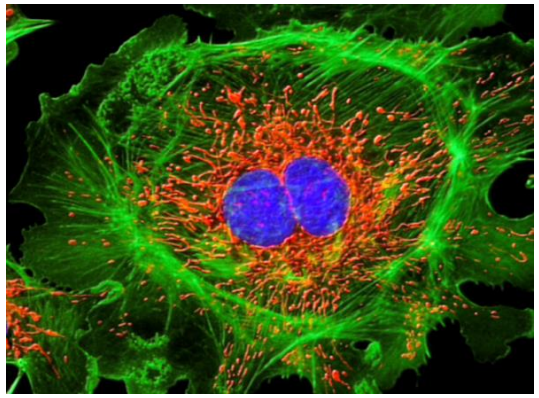
Fluorescent imaging of A549 cells under decreasing oxygen concentration conditions.

Biological Applications

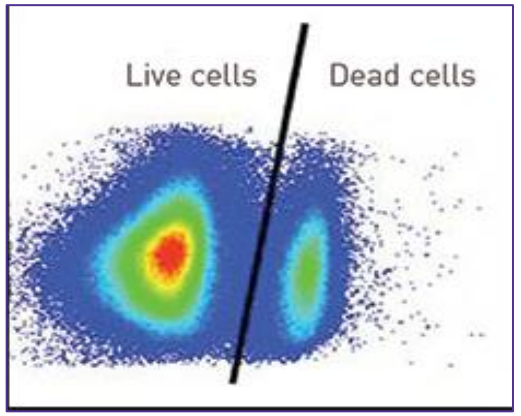
Cell Tracking



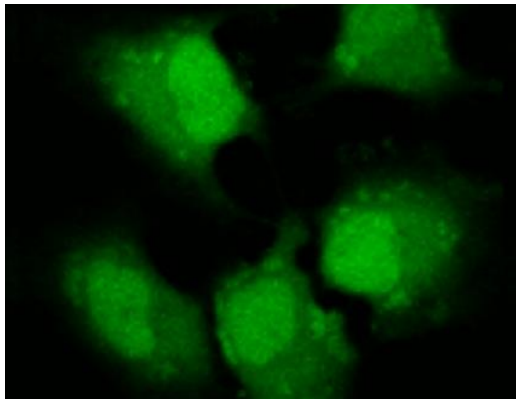
Organelles



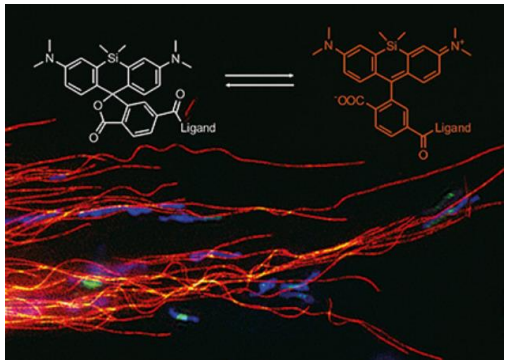
Cell Health



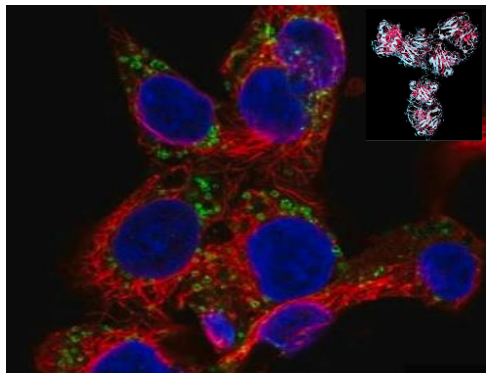
Ions, pH, and more



Live Cell Probes



or



Antibodies?

	Live Cell Probes	Antibodies	Why it matters
Size	Small molecule	Large molecule	Intracellular targets
Target diversity	Moiety (+)	Protein-specific (+++)	Manage customer expectations
Spectral diversity	✓	✓	• Autofluorescence • Cytotoxicity • Multiplexing
Real-time analysis	✓	✗	Biorelevance
Ease of use	+++	+	Convenience, time
HT-friendly	+++	+	Screening
Low cytotoxicity	+++	varies	Biorelevance, time studies
Reliability/consistency	+++	− to +++	Reproducibility

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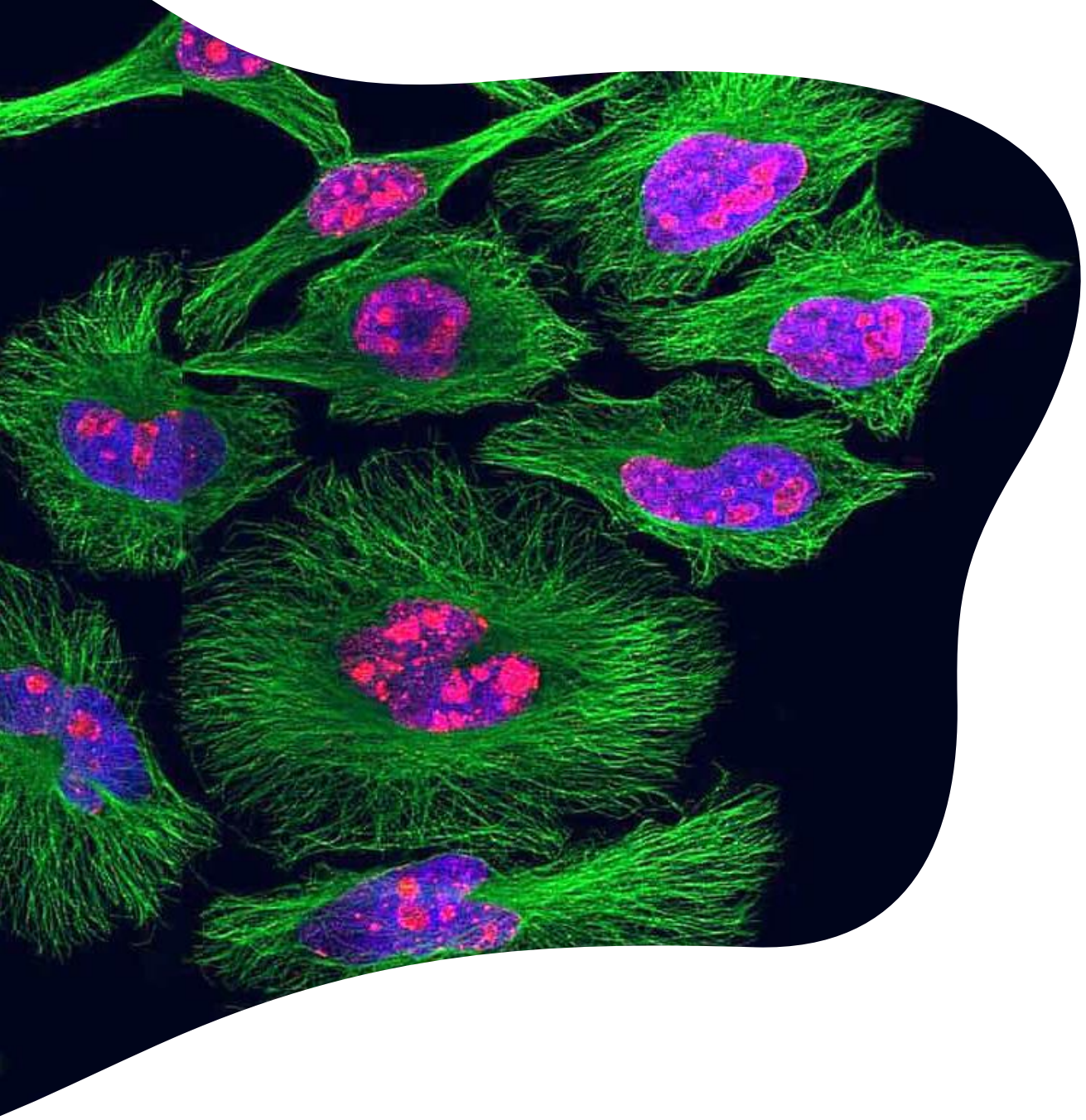


using live cell imaging to improve our understanding of hypoxia and apoptosis

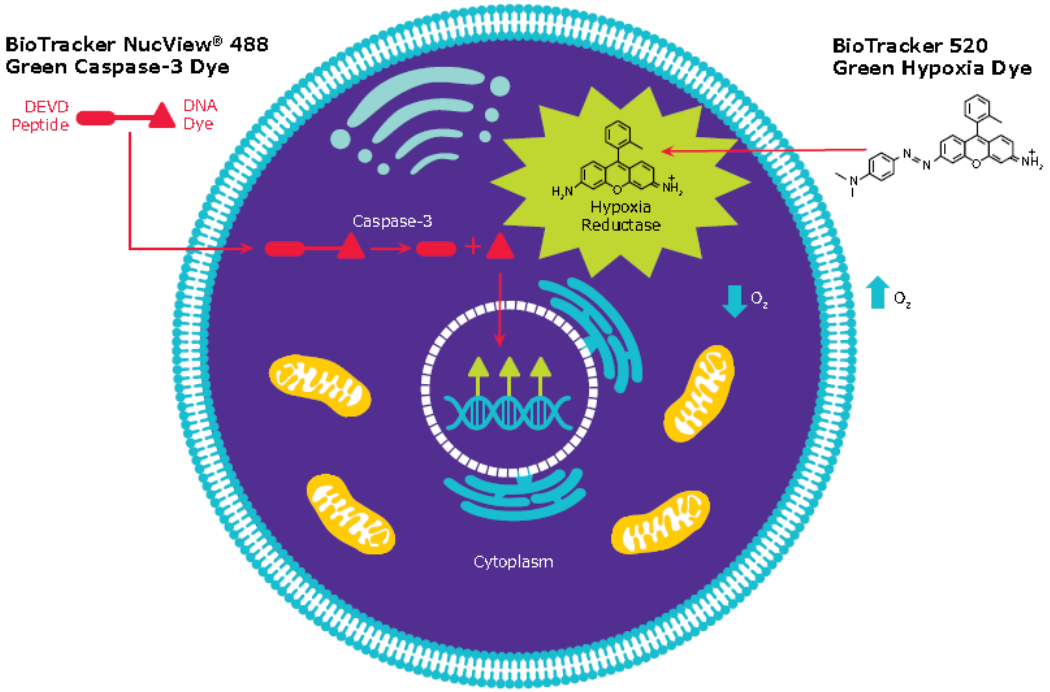
Live Cell Imaging

Dynamic visualisation of hypoxia and apoptosis

- Resistance to Apoptosis (programmed cell death) and Hypoxia (low O2) are hallmarks of cancer
- Development of these attributes are correlated to increasing aggression and resistance to treatment
- Making them important parameters in understanding cancer
- Traditional assays to measure apoptosis (Annexin-V, Caspase and TUNEL assays) and hypoxia (HIF1α expression and Hypoxyprobe/EF5) are end-point assays which require cell fixation or lysis and do not detect real-time cellular events

Live Cell Imaging
Dynamic visualisation of hypoxia and apoptosis

- Here, we used the CellASIC ONIX live cell imaging system and new BioTracker live cell dyes for caspase-3 and hypoxia to measure apoptosis and hypoxia in living cells
- The Caspas-3 BioTracker consists of a fluorogenic DNA dye coupled to the Caspase-3/7 DEVD recognition sequence
- The hypoxia dye relies on 2meRG production under low oxygen levels



Mechanism of BioTracker live cell fluorescent apoptosis and hypoxia dyes

Assay

CellAsic® ONIX2

Cells | Grow | Passage | Transfection | Cryopreservation | Assays

Traditional biology	Microfluidics
Static cultures	Continuous perfusion
Snapshots, endpoint analysis	Dynamic assays over time
Difficult to image while incubating	Micro-incubator fits microscopes
Expensive (e.g. reagents, rare cells)	Micro- and nano- volumes
Difficult to create stable gradients	Constant, reproducible gradients

Assay

CellAsic® ONIX2

Cells | Grow | Passage | Transfection | Cryopreservation | Assays

What it is:

- Microfluidics instrument that pairs with inverted microscope
- Rapid real time control of fluidics due to small volume control
- Precise control of temp, media, gas
- 96 well format with 4 chambers for long term experiments

Application:

- Applicable for 3D cell culture, primary cells, and stem cells

Why it's better :

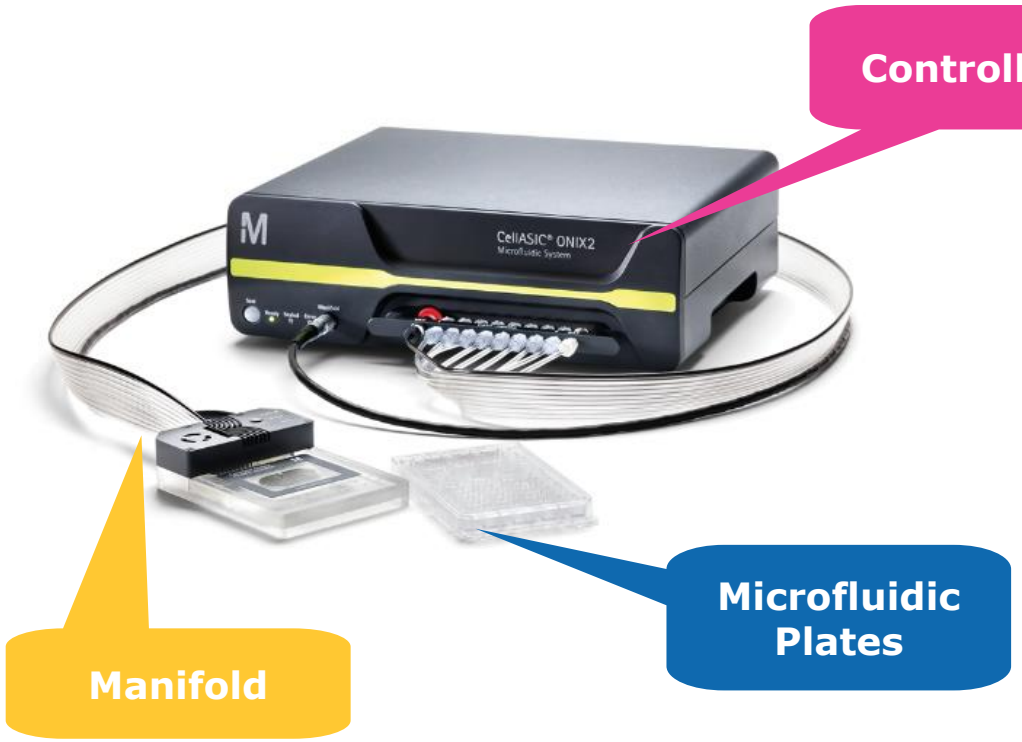
- Only device with:
  - Microfluidics cell culture
  - Perfuse up to 6 reagents in any combination
  - Run long term experiments with flexible perfusion protocol
- Price competitive and more features than competitors

Assay

CellAsic® ONIX2

Cells | Grow | Passage | Transfection | Cryopreservation | Assays

The CellASIC® ONIX2 Microfluidic System

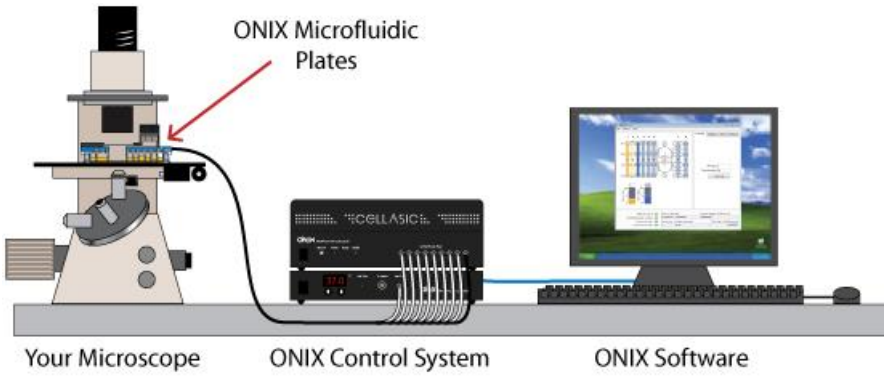


Assay

CellASIC ONIX

Cells | Grow | Passage | Transfection | Cryopreservation | Assays

The CellASIC® ONIX2 Microfluidic System



Manifold Benefits

Localizes environmental control to within the plate

Pressurized air from instrument used to move media through plate

Filters prevent any decontamination from entering system

Peltier heater to control temperature

Rubber gasket helps create vacuum seal to create closed controlled system

Closed environment for media

Stays within plate

Only air in this region is heated & recirculated

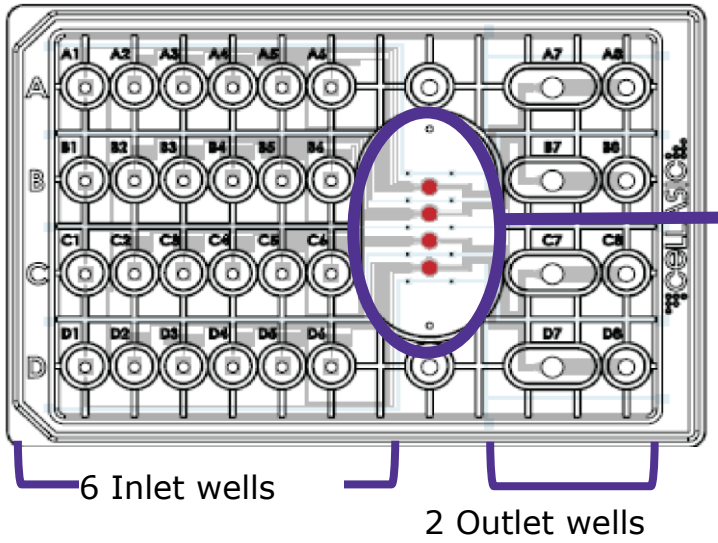
Faster changes to temperature & gas composition

Without worrying about evaporation

Assay

CellASIC ONIX

Versatile microfluidic plates

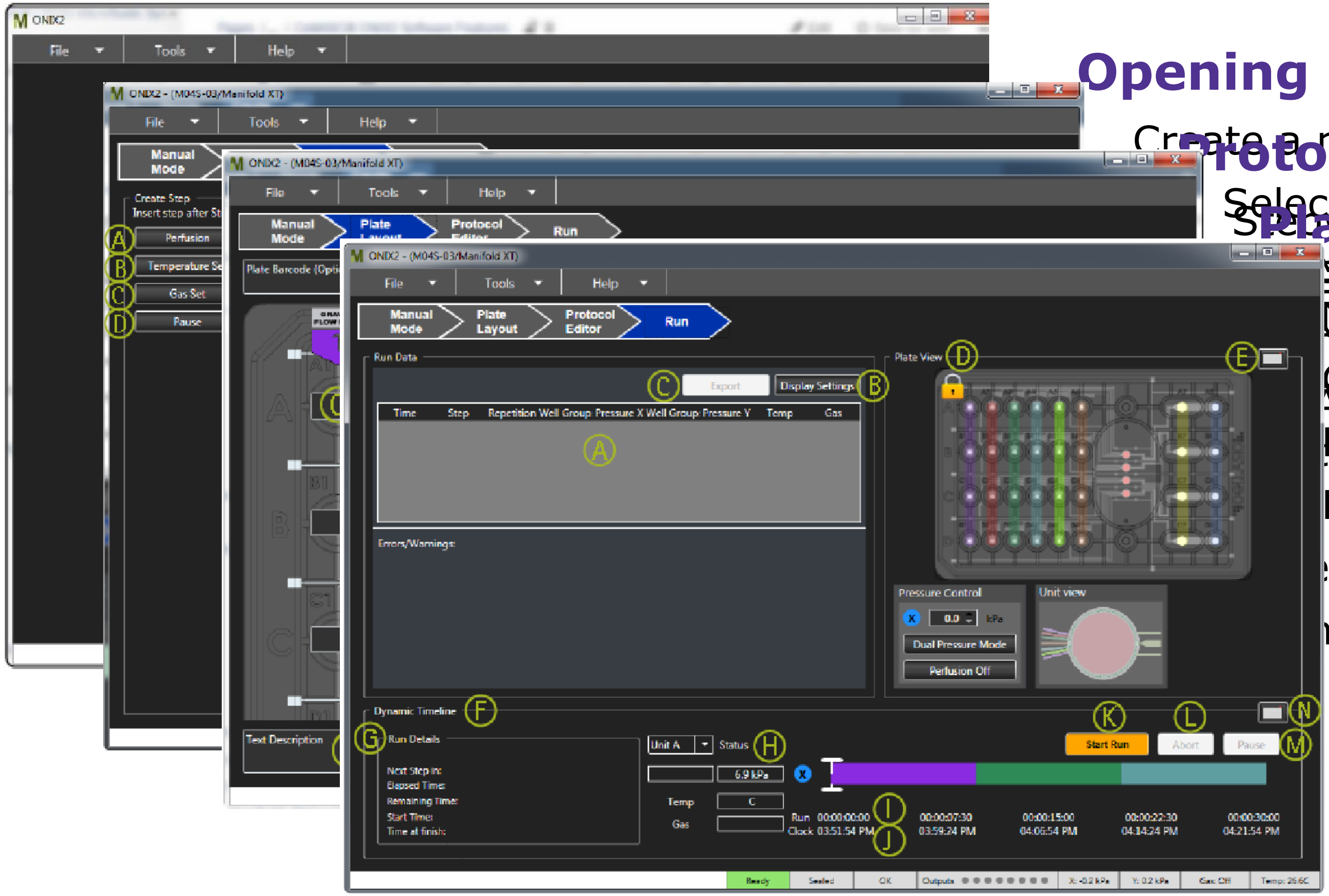


4 cell chambers

- Chamber volume = 1µL vs.
  - 4 mL for 6-well plate
  - 200 µL for 96-well plate
- Single viewing window - minimize travel of phase objectives

- Inlet and outlet wells for media and reagent flow
- Standard microplate dimensions - compatible with microscope stages
- Composed of gas/heat-permeable materials
- High optical glass base – enable imaging

ONIX2 Software



Opening Screen

Create a new experiment

Protocol Editor Tab

Select the plate format

Plate Layout Tab

Select the plate format
M04S Mammalian
Temperature, Gas Source (2 options),
Enter well information (cell type, media) or general notes
Choice of input Well(s)
All information will be saved in the Run file
Flow Rates
Duration
-Map mirrors plate Layout
mber Diagram demonstrates flow

Run Tab

Previously saved experiment execution of a protocol

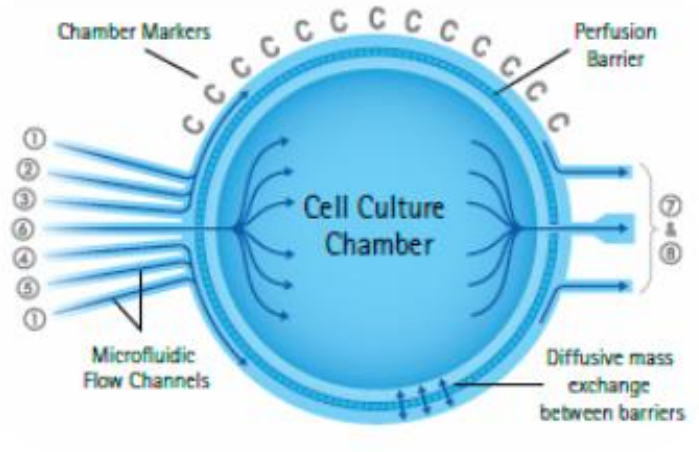
- Dynamic Timeline – graphical display of protocol in progress
- Status Bar
  - Plate sealed/ready
  - Wells in use (Outputs)
  - Flow rates
  - Temp and Gas status
  - Error alert messaging

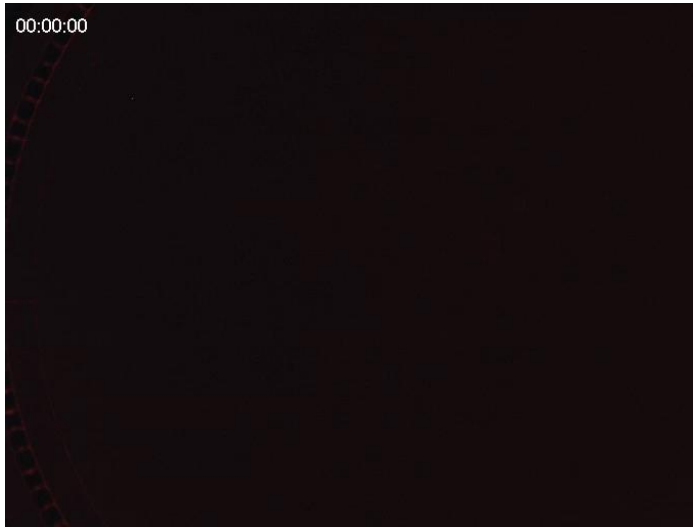
MERCK

Assay

CellASIC ONIX

Versatile microfluidic plates





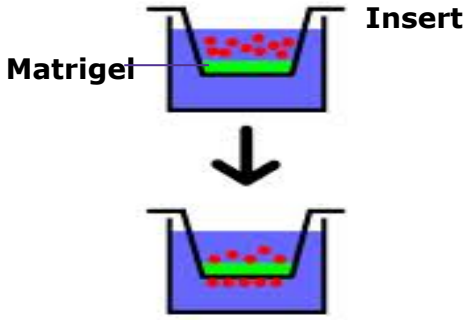
- Microfluidic channels and perfusion barriers mimic fluid dynamics of in vivo diffusion conditions
- Deliver media changes without shear stress
- New media is continuously perfused in and waste out for healthy, long-term cell culture conditions

Assay

CellASIC ONIX Application

Limitations of Traditional Invasion Assays

Modified Boyden Chamber

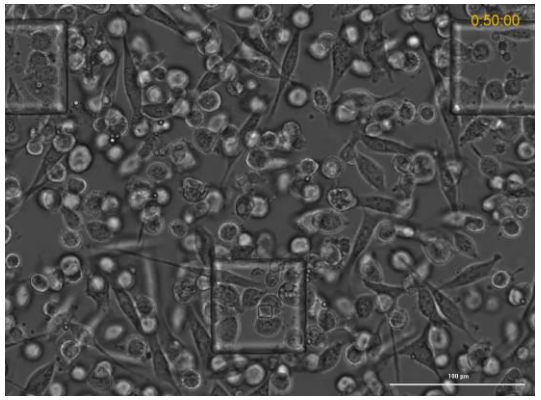


Limitations

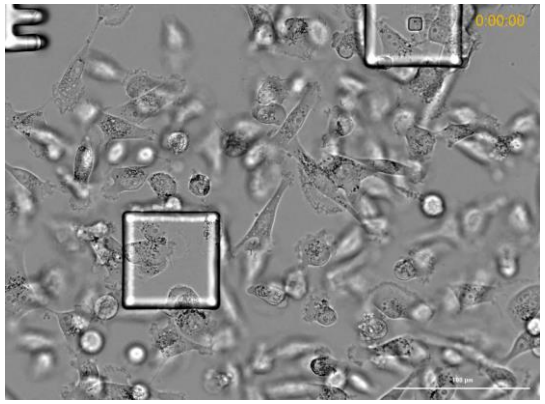
- Establishing true gradient
- Maintaining any gradient
- Incompatible with scope

MDA-MB-231: Invasive breast cancer cell line

Static Culture



Perfusion Culture



Healthy, happy cells will respond

Assay

CellASIC ONIX Application

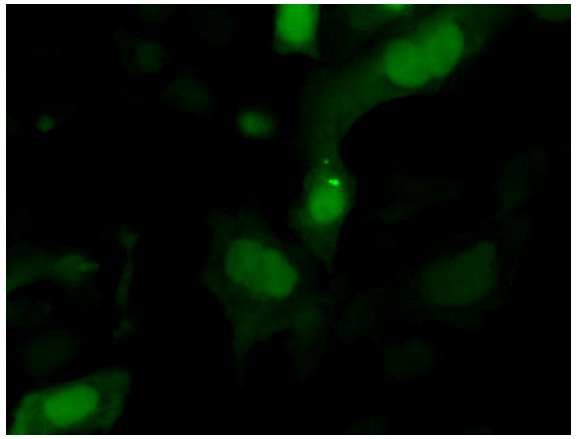
Cells | Grow | Passage | Transfection | Cryopreservation | Assays

Autophagy (Media Switching)

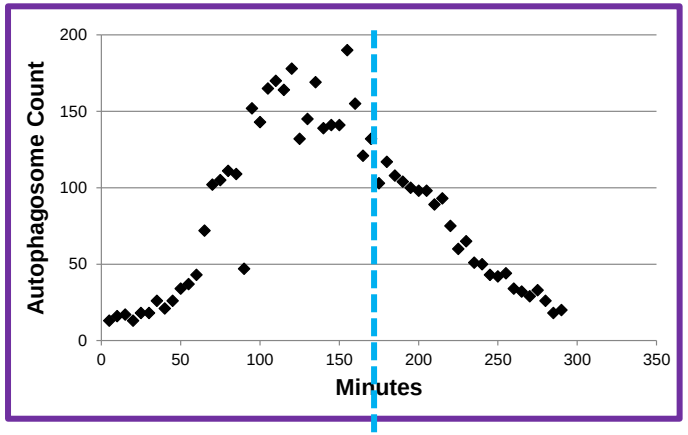
- Tumor cells use the autophagy pathway to promote survival under stressed conditions, such as nutrient deprivation or hypoxia.
- Static end-point assays do not permit understanding of this dynamic process

LC3+ Autophagosomes

Cells with GFP-LC3 Reporter



Starve (stress) Lyso. Inhibition → **Normal Culture**



Programmable, precise changes in culture conditions permit investigation of dynamic cellular processes

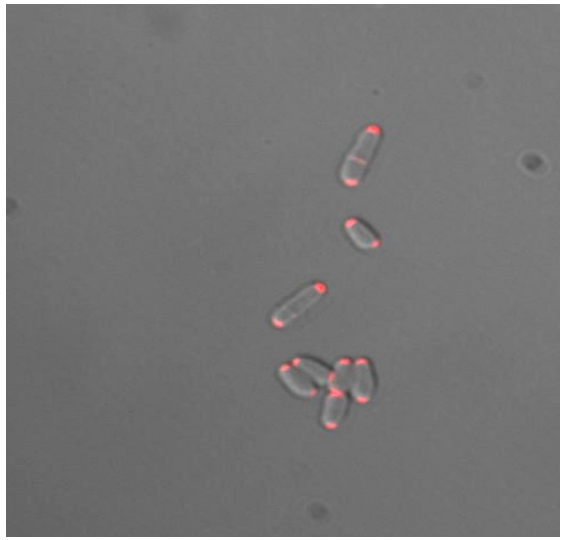
Assay

CellASIC ONIX Application

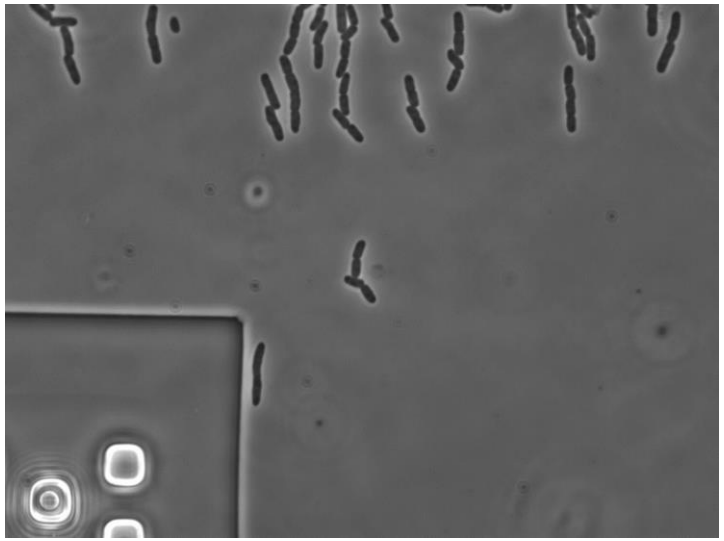
Cells | Grow | Passage | Transfection | Cryopreservation | Assays

Bacterial responses to environmental change

Growth of RFP tagged Bacteria



Bacteria growth then treatment with antibiotic (ampicillin)



- Setup and Walkaway – simplified experimental workflow
- Generational view of growth and response to drug treatment

Assay

CellASIC ONIX Application

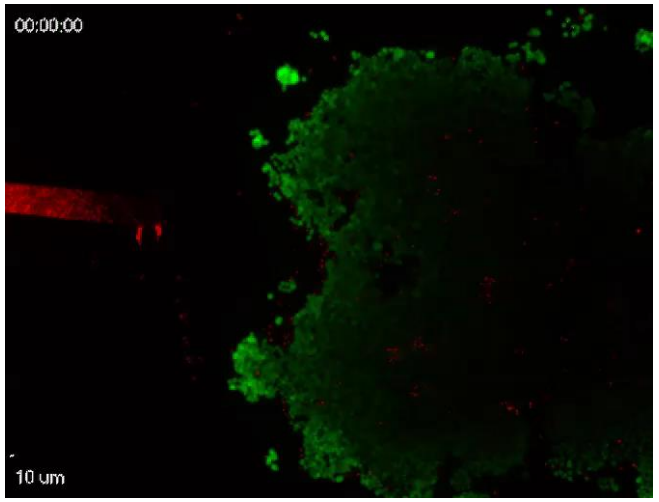
Cells | Grow | Passage | Transfection | Cryopreservation | Assays

Host-Pathogen interactions

***In vitro* model needs**

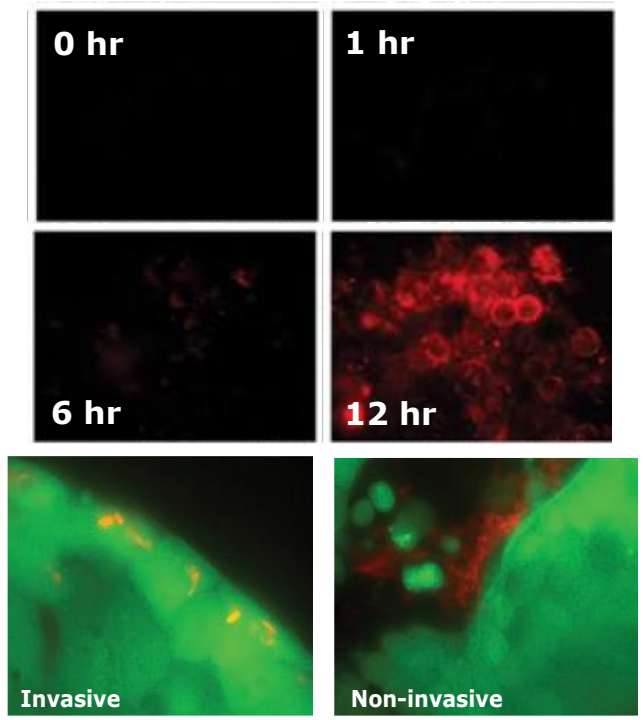
- Precise perfusion flow rates
- Definable exposure times
- Differential delivery of infectious agents and therapeutics
- Maintain longer term cell culture
- Real-time imaging
- Temporal and spatially-defined loading and perfusion capacity enable study of unique interactions and responses

Loading bacteria



- Establish Mammalian Cell culture (**GREEN**)
- Load infectious Bacteria (**RED**)
- Monitor Response

Infection by invasive *E. coli*



E. Coli (mCherry), HT29 (Calcein)

Assay

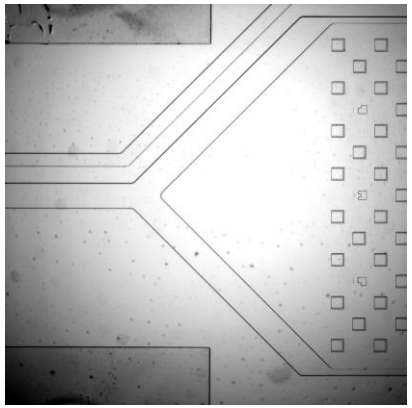
CellASIC ONIX Application

Cells | Grow | Passage | Transfection | Cryopreservation | Assays

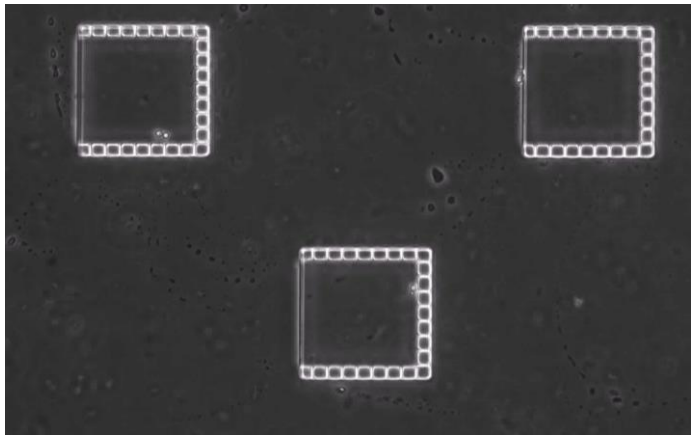
New Trap Array: Multi-generation yeast cell analysis

Y04T trap array for physically independent cell colonies with continuous media supply; removal of excess cells

Yeast cell loading (video)



Yeast growth (24h time-lapse)



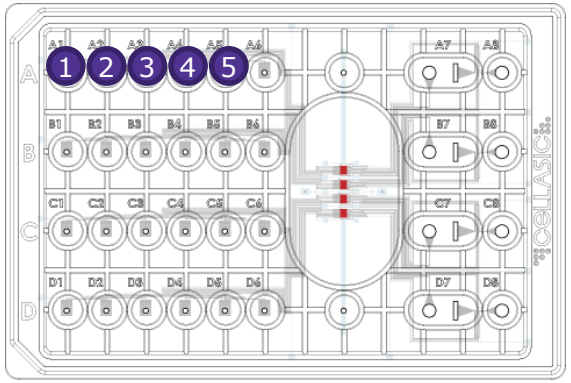
Study of generation dynamics and single cell tracking

Assay

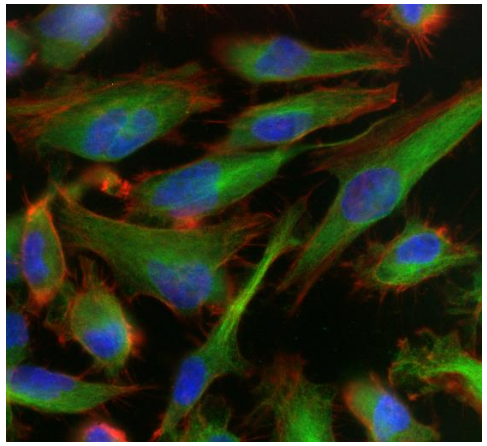
CellASIC ONIX Application

Cells | Grow | Passage | Transfection | Cryopreservation | Assays

In-Plate Fluorescent Labeling (Flexibility)



1. PBS
2. Fix
3. Perm
4. 1 Ab
5. 2 Ab



Actin

Tubulin

DAPI

- Use perfusion to stain live or fix (and permeabilize) cells
- Automate multi-solution washing and exposure programs
  - *Microfluidics is not just for media perfusion*

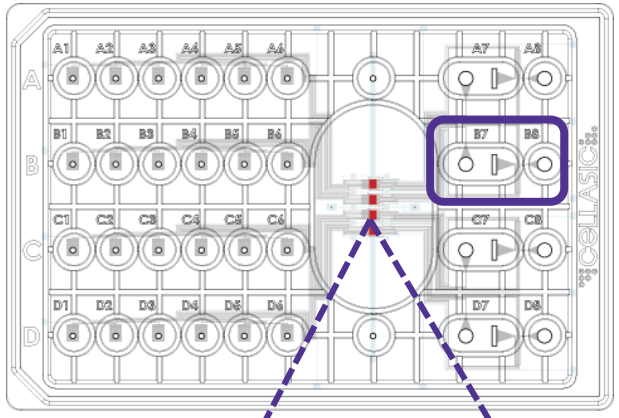
Assay

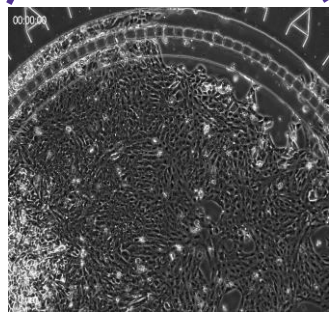
CellASIC ONIX Application

Downstream analyses (Workflow Linkage)

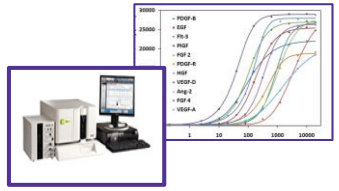
Sampling of supernatant from waste wells

Harvest cells by trypsinization

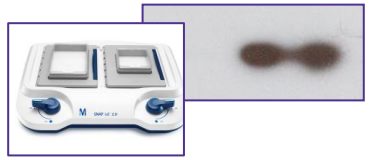




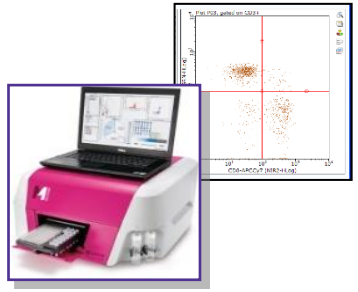
Bead-based Analyte/RNA detection

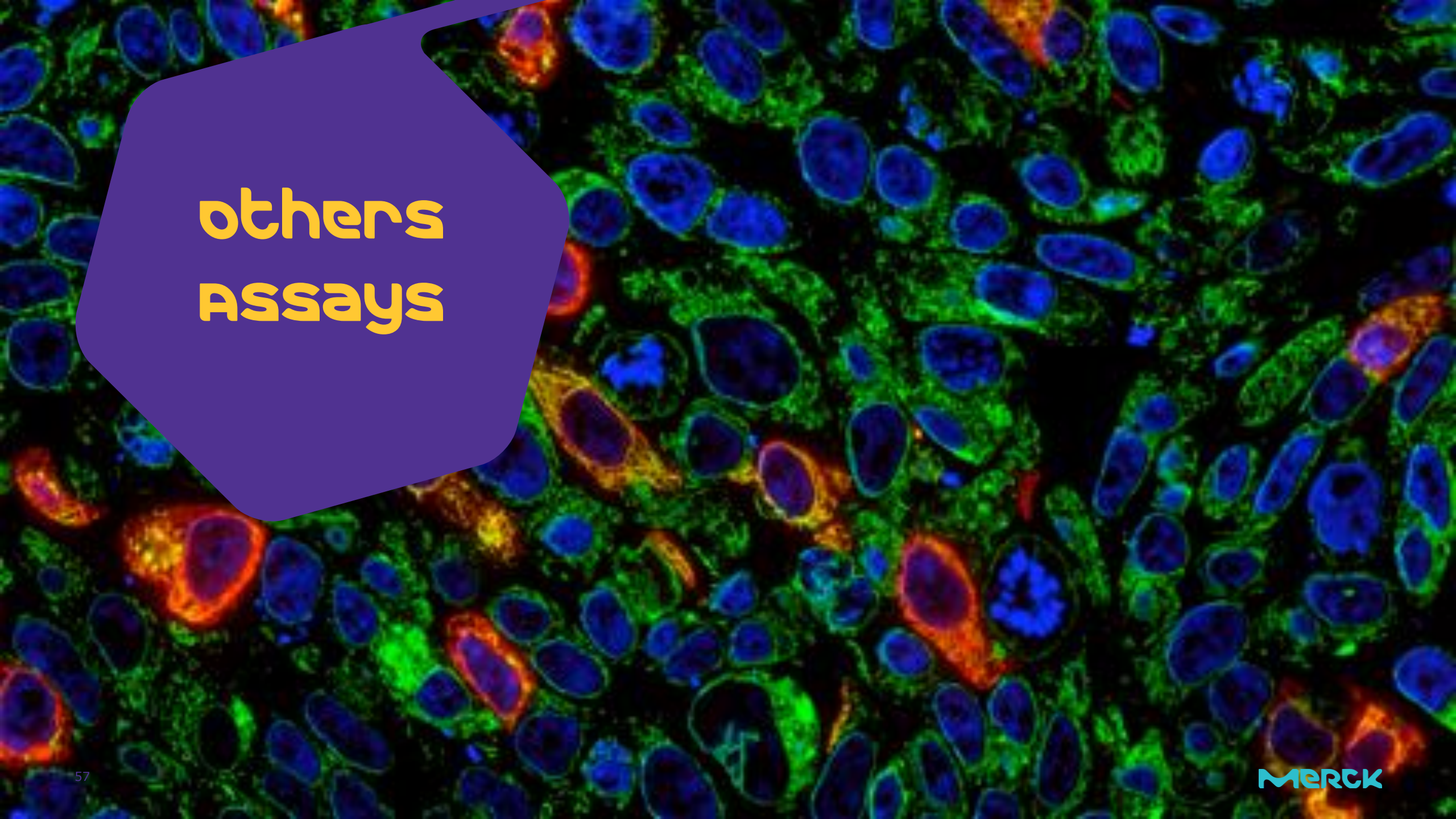


Western Blotting



Flow Cytometry




others
assays

Convenient Assay kits to analyze Metabolites and Enzymes

Cardiology
Cardiovascular
52 kits

Neurology
68 kits

Pulmonology
10 kits

Orthopedics
10 kits

Rheumatology
10 kits

Dermatology
2 kits

Endocrinology-
Diabetes
47 kits

Hematology
39 kits

Gastroenterology
37 kits

Obesity
6 kits

Genitourinary
15 kits

Oncology (continued)

Kit	Product
MAK131	Formaldehyde Assay
MAK044	Free fatty acid quantitation kit
MAK020	Fructose-6-phosphate assay kit
MAK060	Fumarate assay kit
MAK098	G1p colorimetric assay kit
MAK015	G6pdh activity assay kit
MAK277	Gapdh activity assay kit
MAK099	Gdh activity assay kit
MAK348	Gelatinase (Gelatin Degradation/Zymography) Assay Kit (Fluorometric)
MAK083	Glucose uptake colorimetric assay kit

Obesity

Kit	Product
MAK313	Adipolysis Assay Kit
MAK001	Alanine Assay Kit
MAK325	Cholesterol Uptake Assay Kit
MAK265	Fructose Colorimetric/Fluorometric Assay Kit
MAK180	High Sensitivity Fructose Assay Kit

Cell based Assays by Category

Cancer

Angiogenesis

Proliferation/Viability

Transmigration

Migration/Invasion

Apoptosis

Stem Cells

Proliferation/Viability

Apoptosis

Hypoxia

Cell Structure

Migration/Invasion

Adhesion

Cytoskeleton

Organelle (Mitochondria/lysosome/lipids)

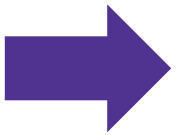
Extracellular Matrix

Neuroscience

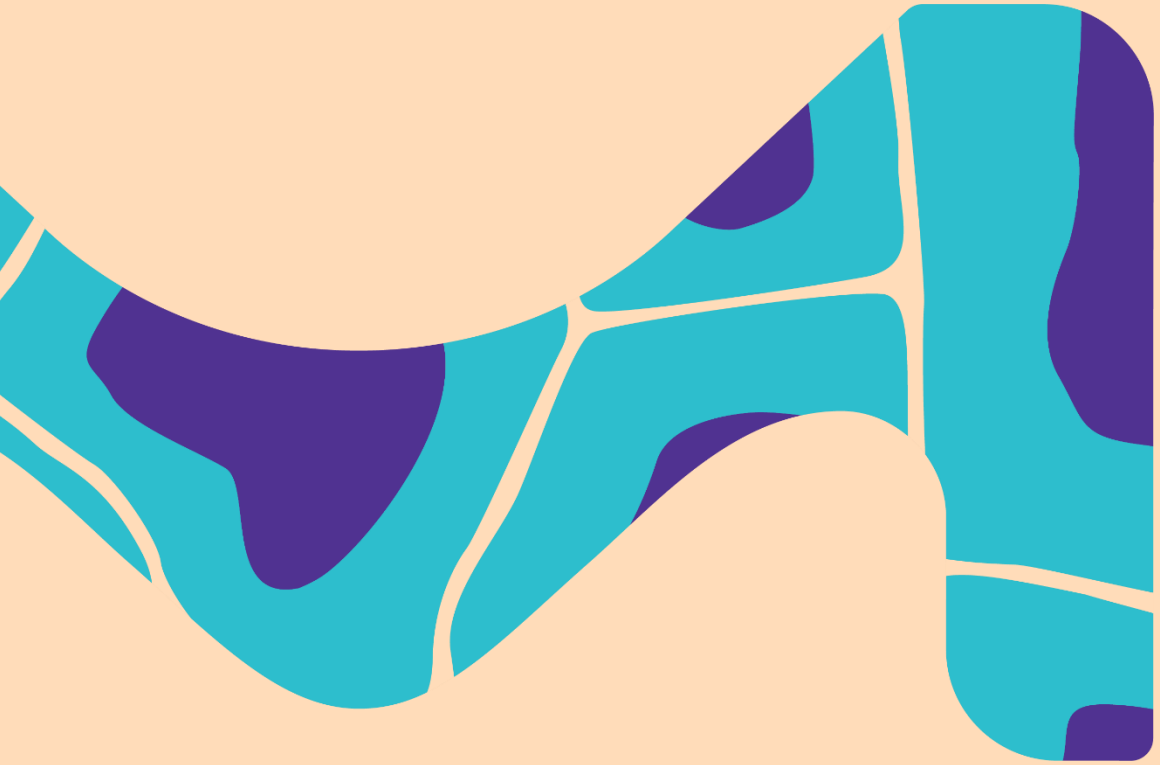
Proliferation/Viability

Apoptosis

Neurite Outgrowth



Mycoplasma Detection



THANK YOU

MERCK