國立台灣師範大學生命科學系研究生貴儀教育訓練
高內涵顯微分析系統

ImageXpress Micro Confocal High Content Imaging System

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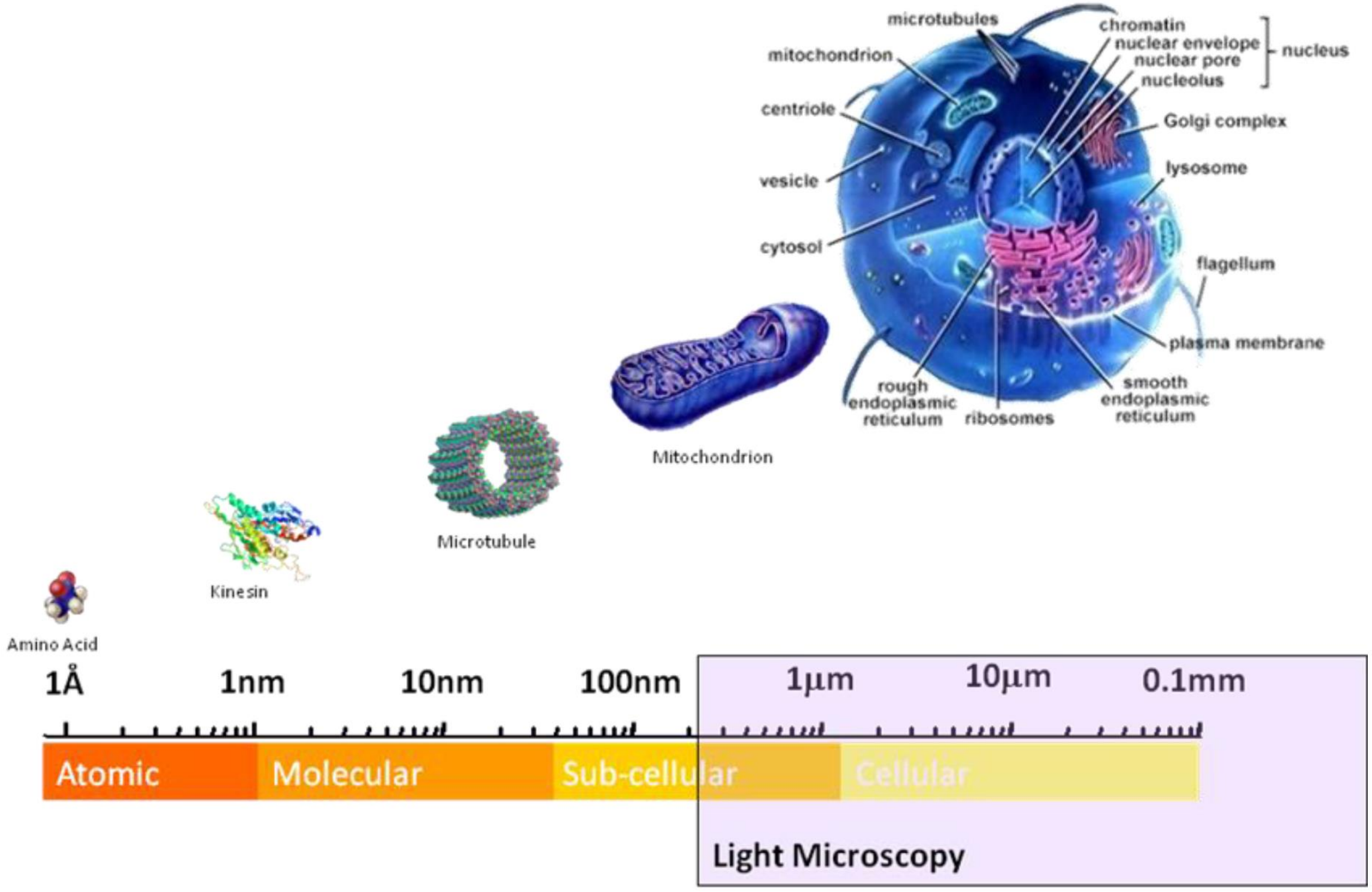
應用專家 Field Application Scientist

Molecular Devices (Hong Kong) Ltd.

Outline

- Principle of High Content System
- Introduction of ImageXpress Micro Confocal System
  - Hardware features
  - Software features
- Trend of High Content System Application

Light Microscope for Micro-world



www.3dchem.com; wikipedia.org/wiki/Kinesin; cvcweb.ices.utexas.edu; Fotin et al., *Nature* 2004; hrsbstaff.ednet.ns.ca; www.ebi.ac.uk

History of Microscope (before 20th)

Improve Image Resolution

Zacharias Janssen

Anthony Von Leeuwenhoek

Time	late 16th	mid 17th	late 17th	late 19th
Magnification	10X	100X	300X	1000X

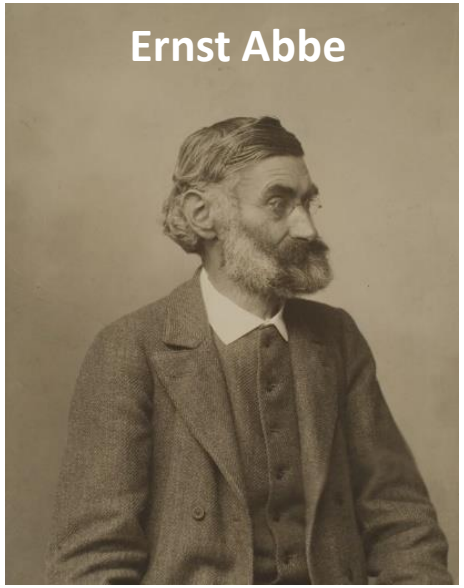
Robert Hooke

Zeiss

Pictures from :
By Chad Anderson, staff photographer for SFO Museum - SFO Museum, CC BY-SA 2.0, https://commons.wikimedia.org/w/index.php?curid=45625745

History of Microscope (after 20th)

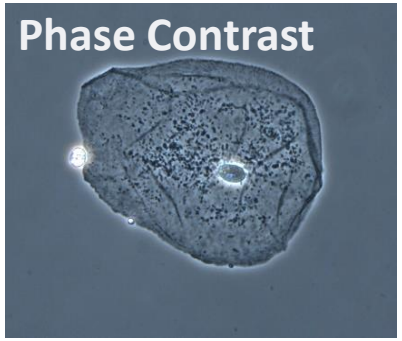
Improve microscope variation

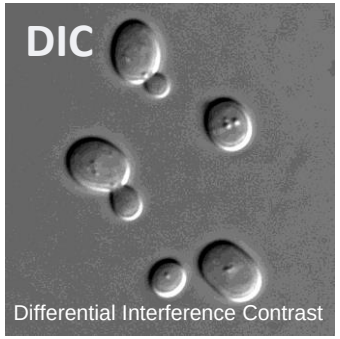


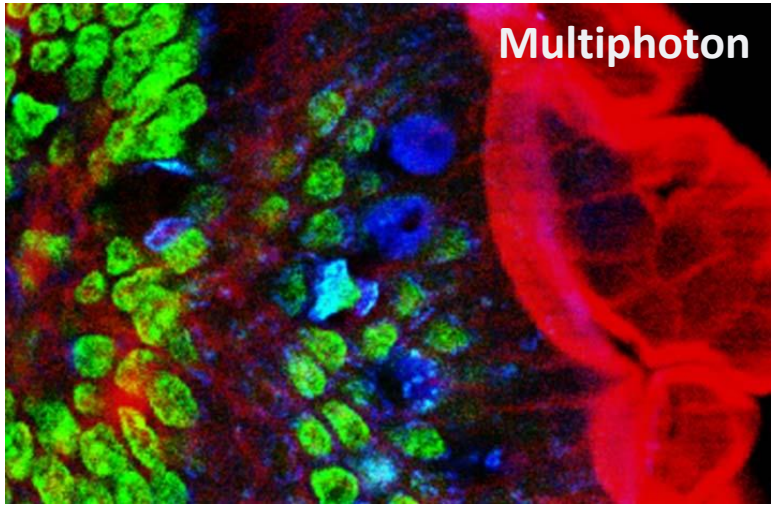
Diffraction Limit

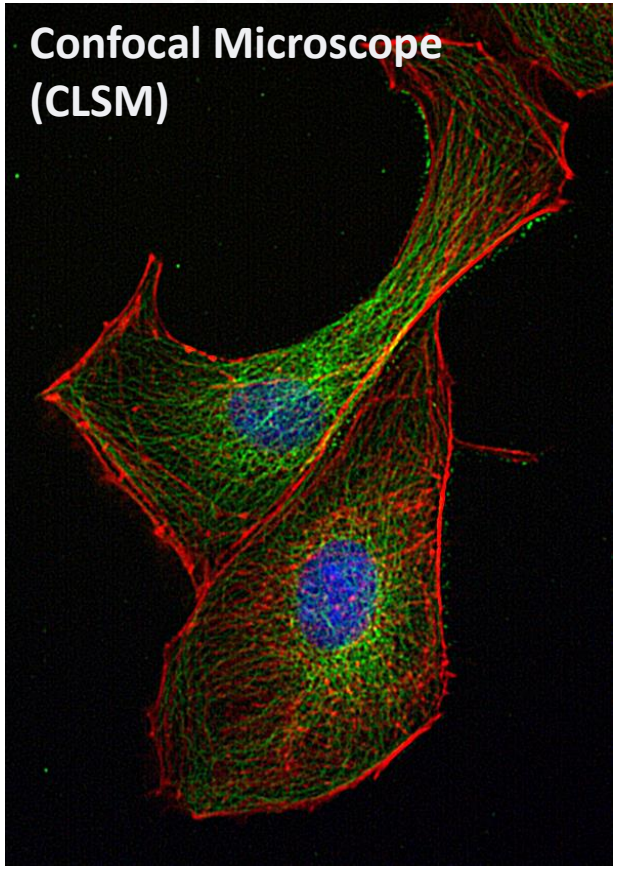
$$d = \frac{\lambda}{2\mathbb{N}A} \sim 200nm$$

(In late 19th)





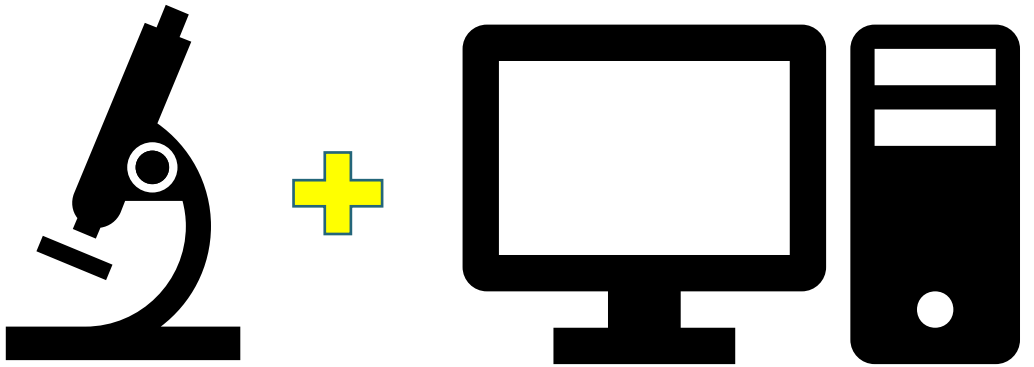




Other microscopy: polarization, TIRF, FRAP, FLIM……

Pictures from :
By Spencer Diamond at the Biological Imaging Facility in Koshland Hall on the campus of UC Berkeley
https://commons.wikimedia.org/w/index.php?curid=7903392
By Universitätsbibliothek Heidelberg, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=68788569
BioMedical Engineering OnLine, 2006, 5:36.DOI:10.1186/1475-925X-5-36.

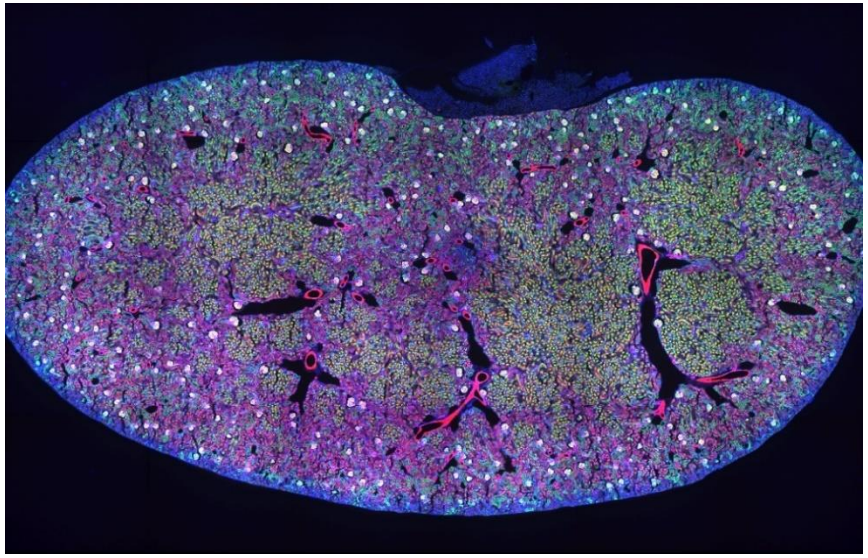
Progress of Microscope in 21st Century

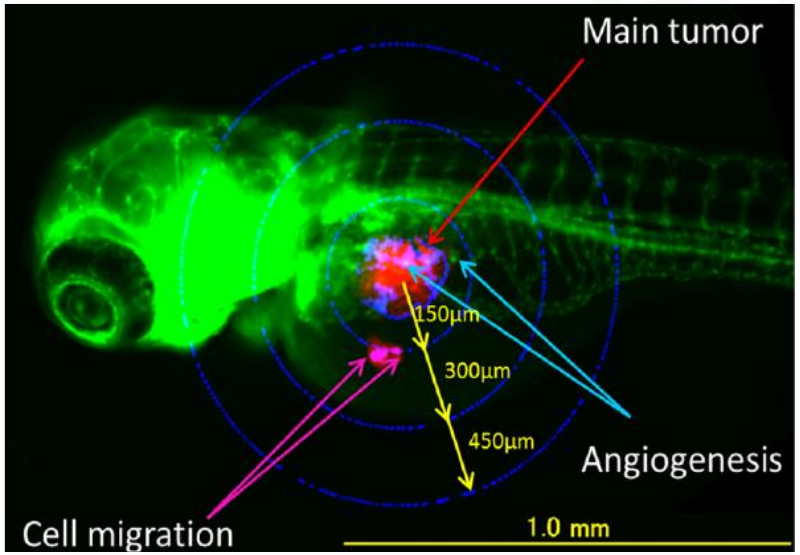


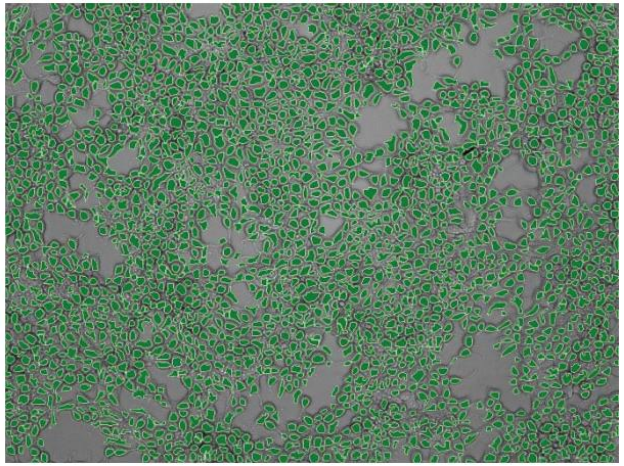
Improvement in

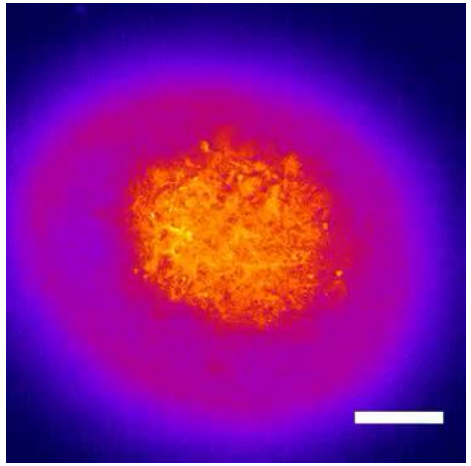
Resolution
Application
Throughput

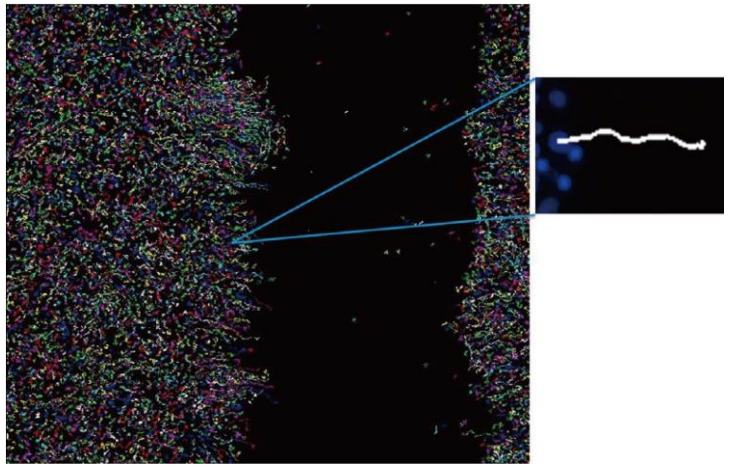
Progress of Microscope in 21st Century





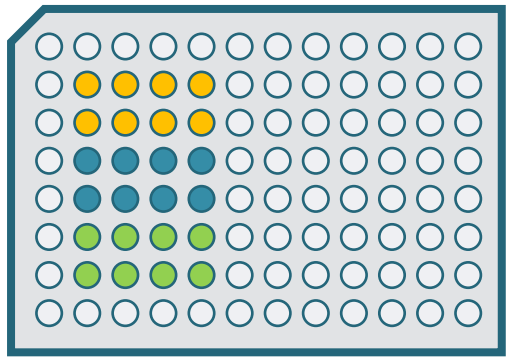






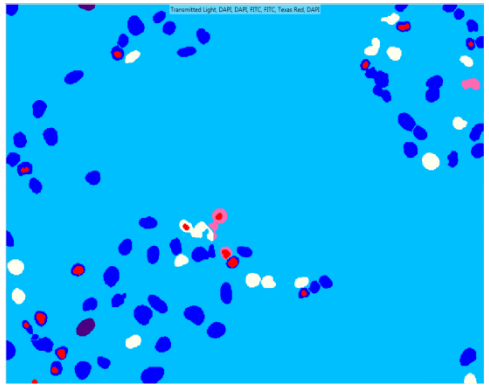
Live Cell Analysis for program death

Plating

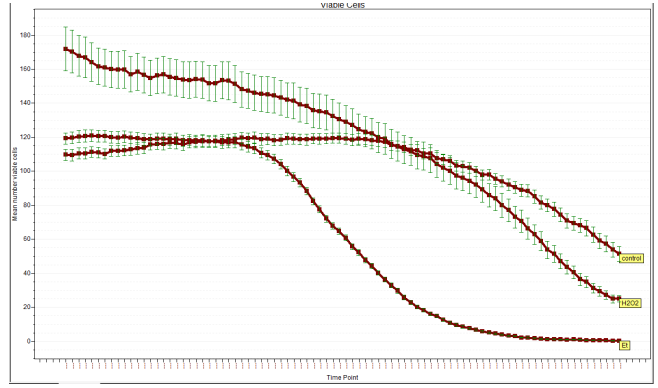


Acquisition

Analyze

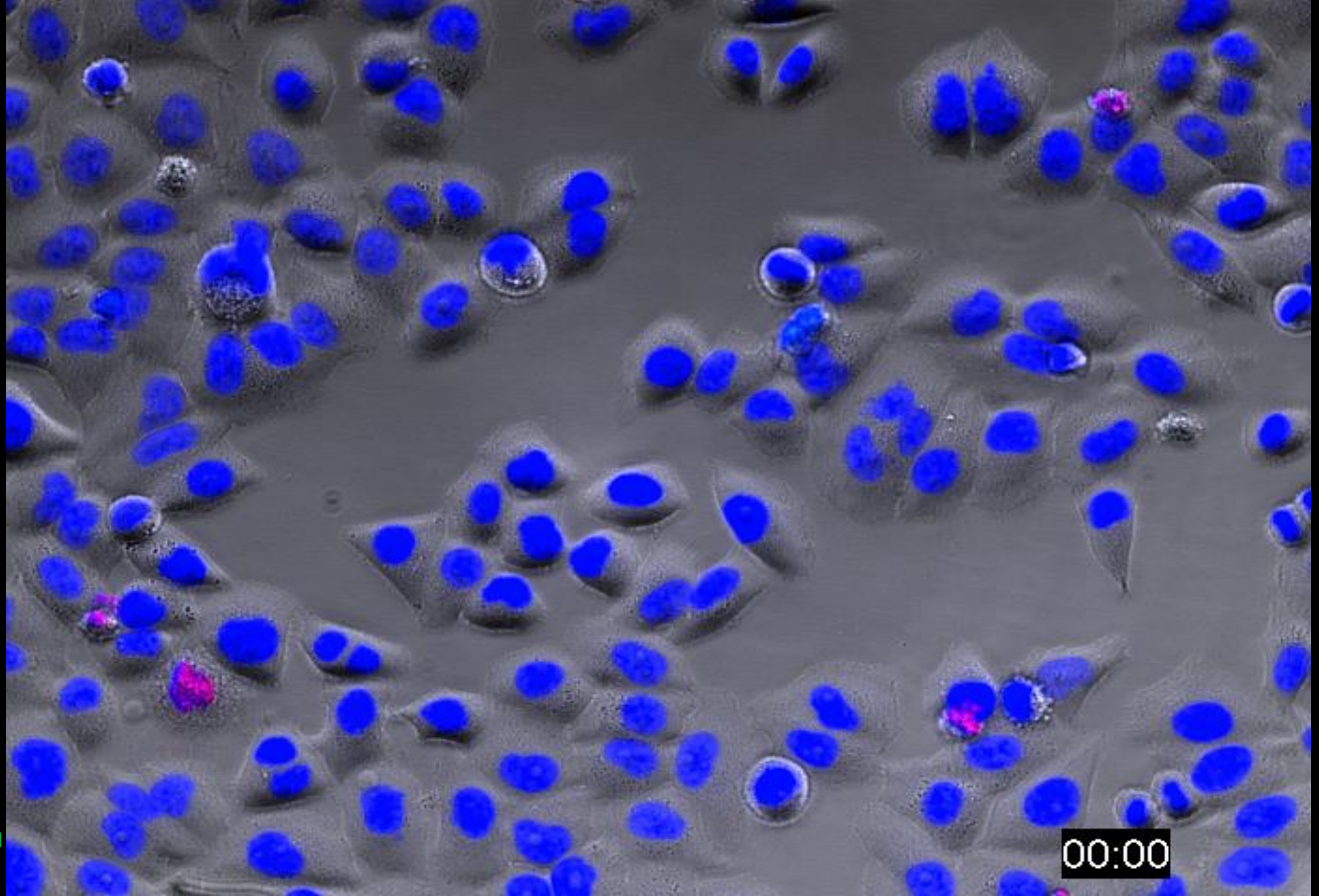


Statistics

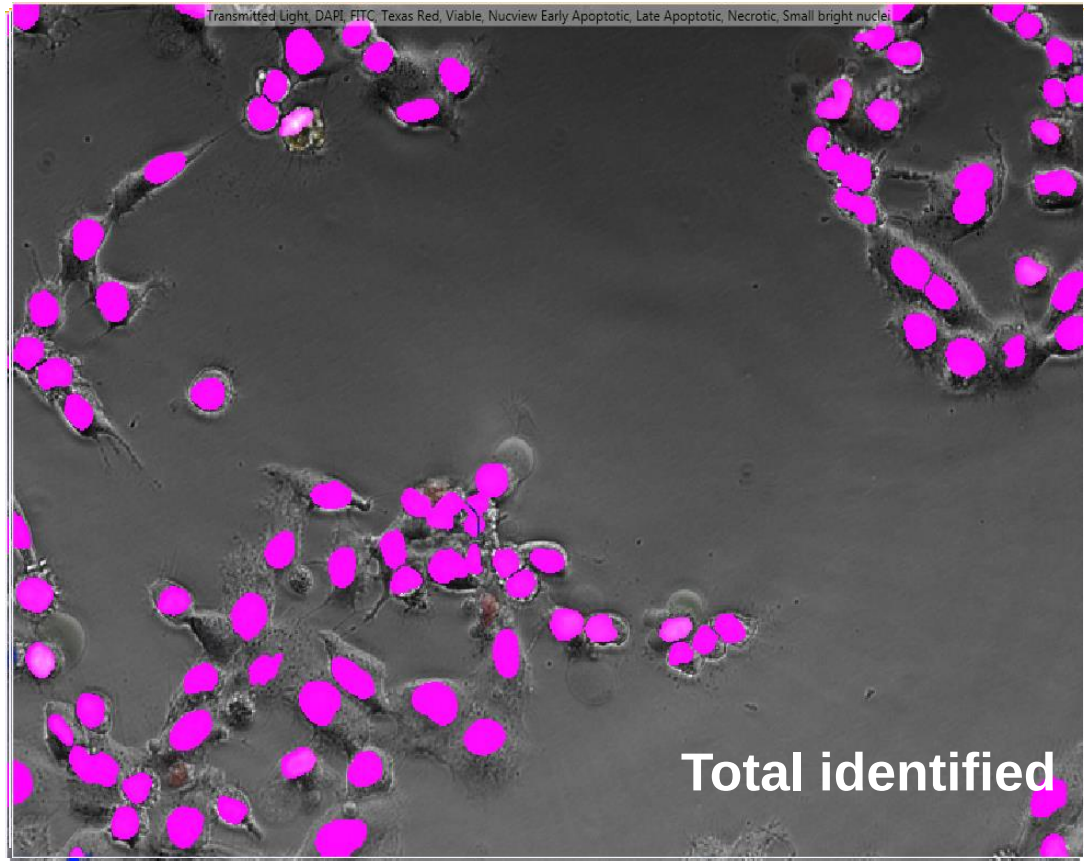


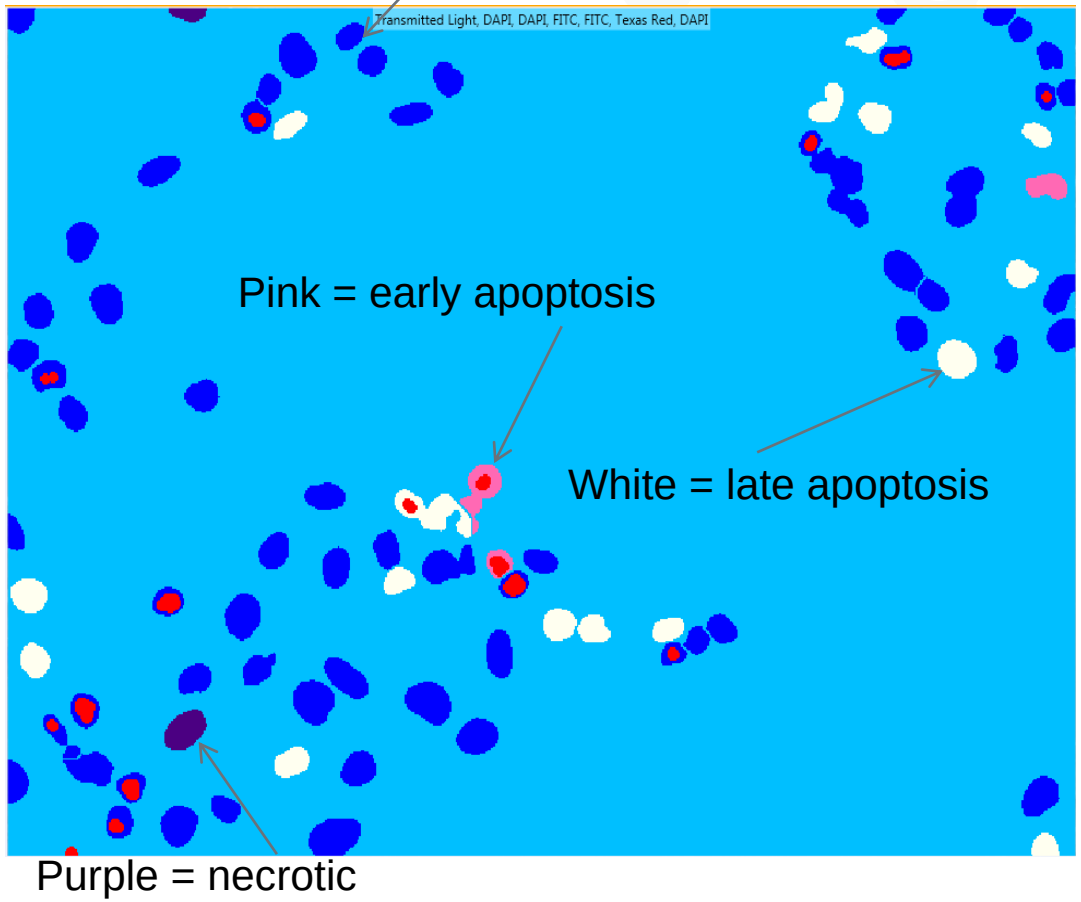
HeLa cell
Hoechst (Nuclei)
NucView (Apoptotic Cells)
PI (Necrotic Cells)

5% CO_2, 37℃, 48hr

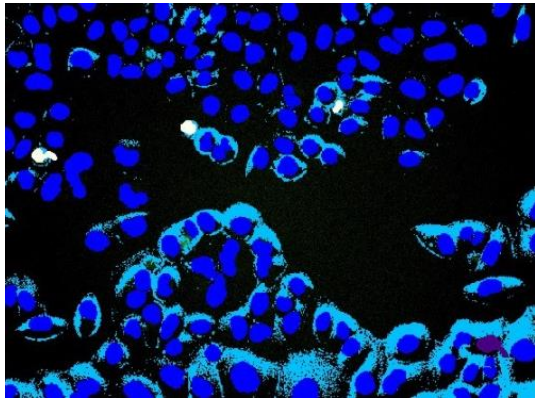

Nuclei
Apoptotic Cell
Necrotic Cell
00:00

Multi-parametric Image Analysis



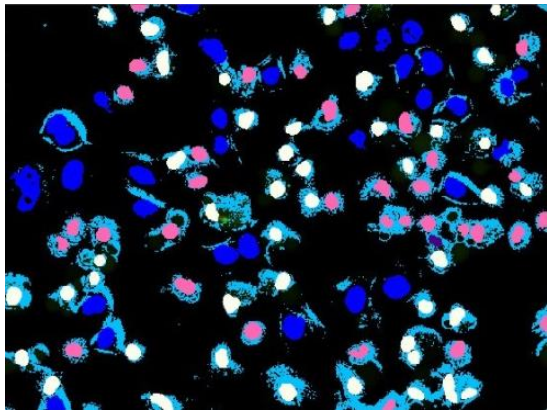


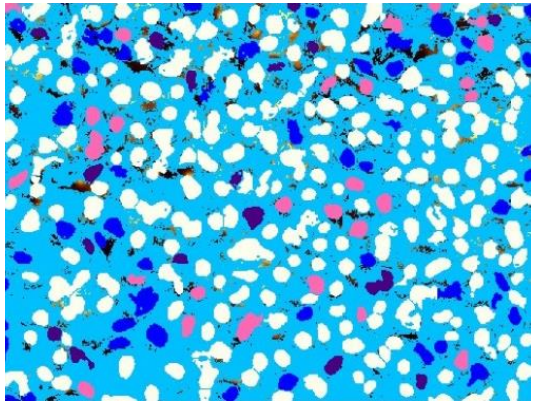
Multi-parametric Image Analysis



Early timepoint control shows mostly viable nuclei (royal blue mask)

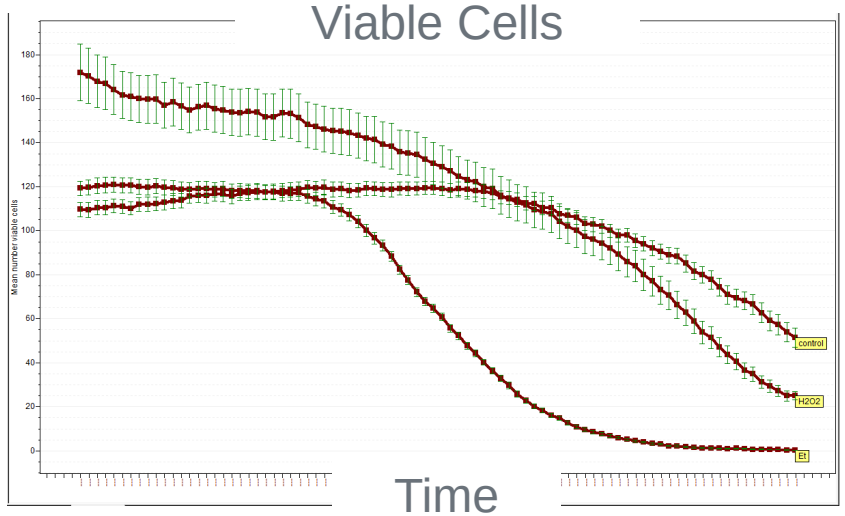
At time point 50, Etoposide treated cells show many cells in early apoptosis (pink mask) and late apoptosis (white mask)

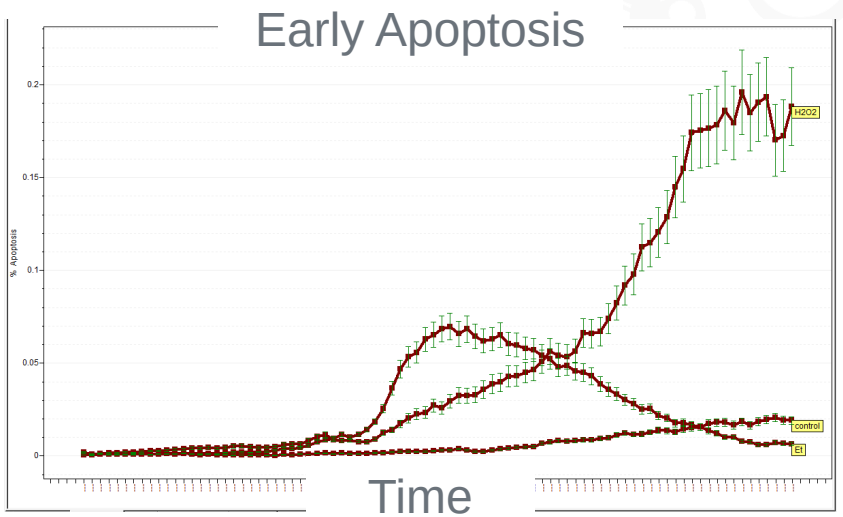


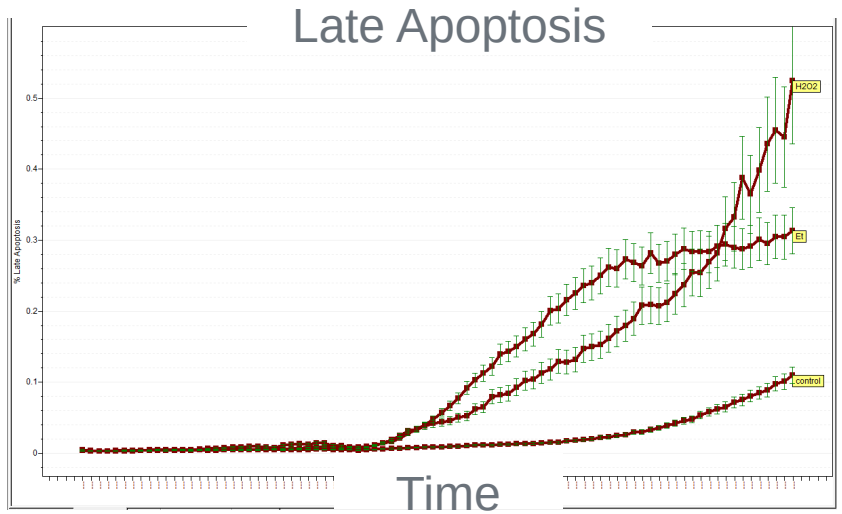


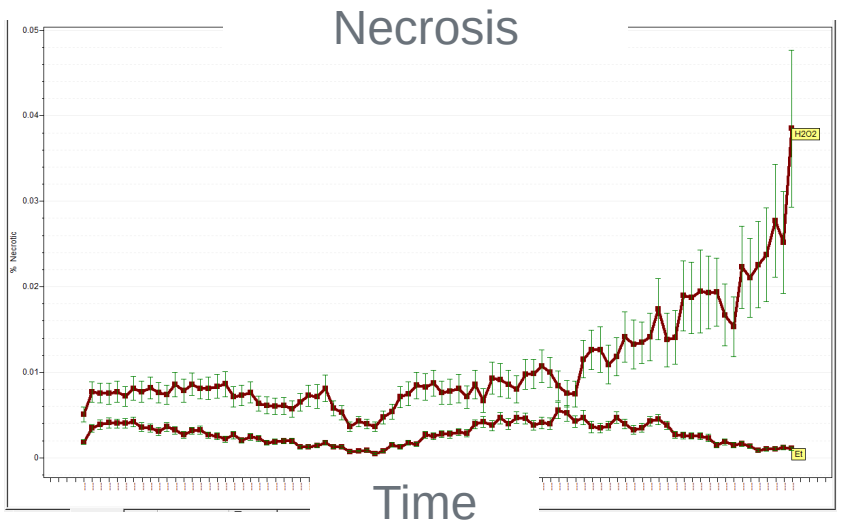
At time point 86, Peroxide treated well shows most in late apoptosis (white mask) and some suffered necrosis (purple mask)

Multi-parametric Image Analysis





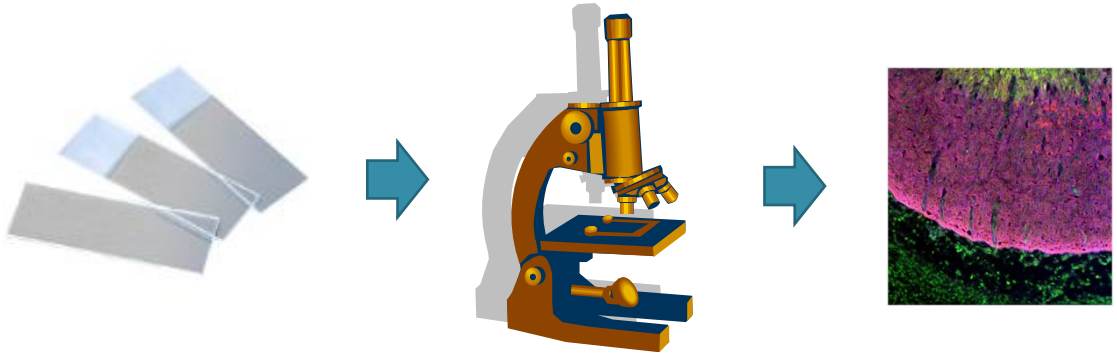




What's MD ImageXpress Imaging System?

Conventional Microscopy

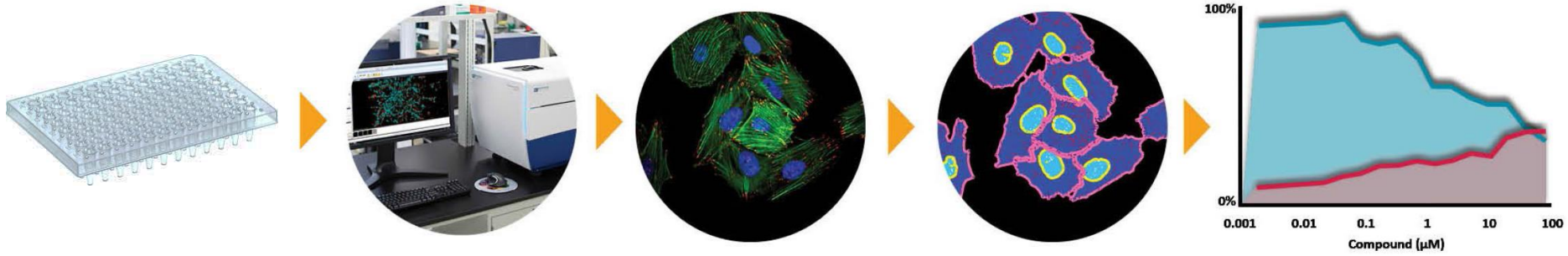
Limited throughput, qualitative results



- Weeks, months
- More subjective
- More labor work

ImageXpress Automated Imaging System

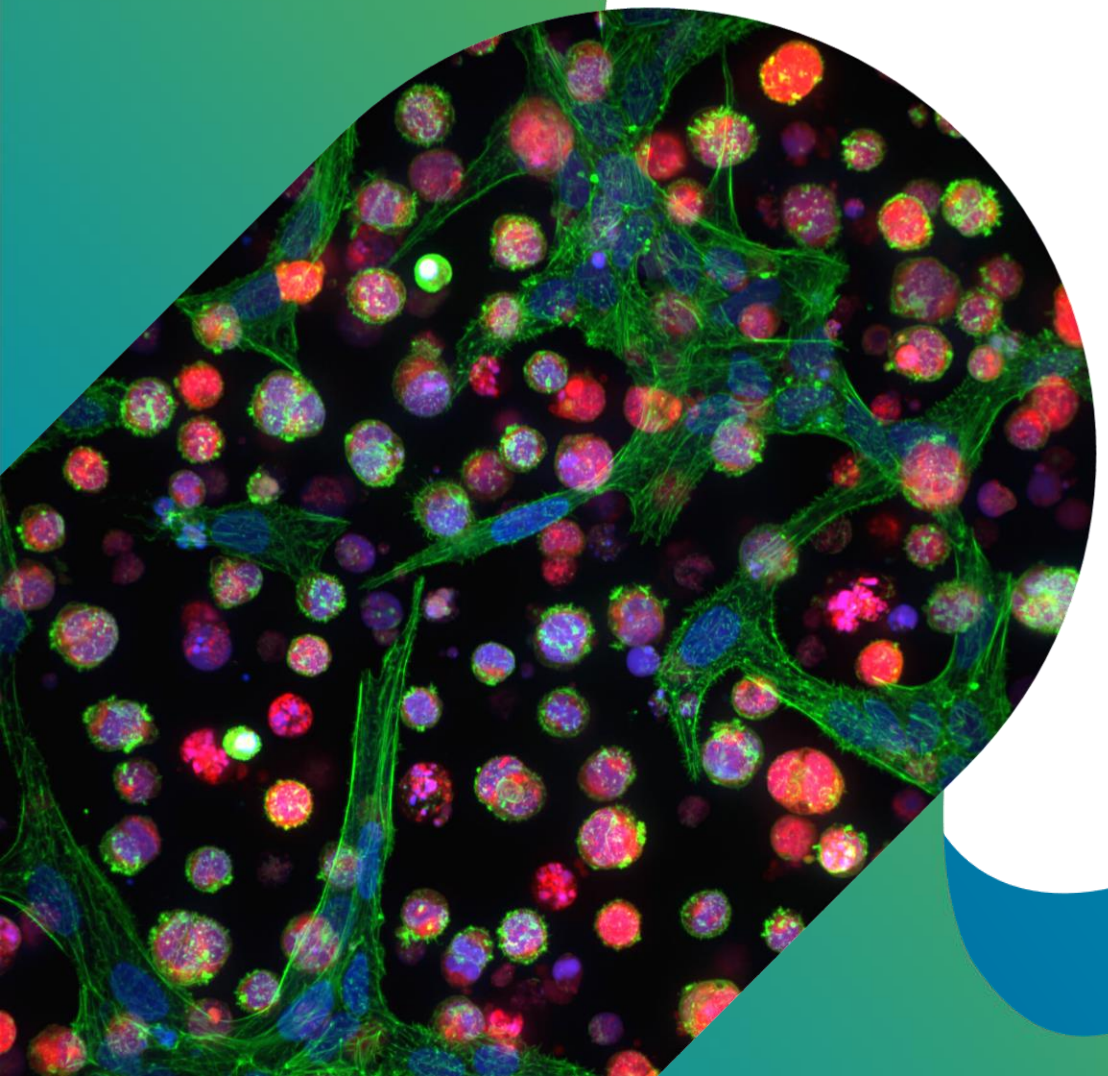
High throughput, qualitative + quantitative results



- **Hours, days**
- **More objective**
- **More automation**

ImageXpress Micro Confocal

High Content Imaging System



High Content System Overview

Features of Hardware

High reading speed

Automated stage
Autofocus system

High quality Image

Digital confocal module
Spinning disk confocal module
Automated water immersion objective module

Various modules

Transmitted light module
Environmental controller
Liquid handling module*
Robotic system*

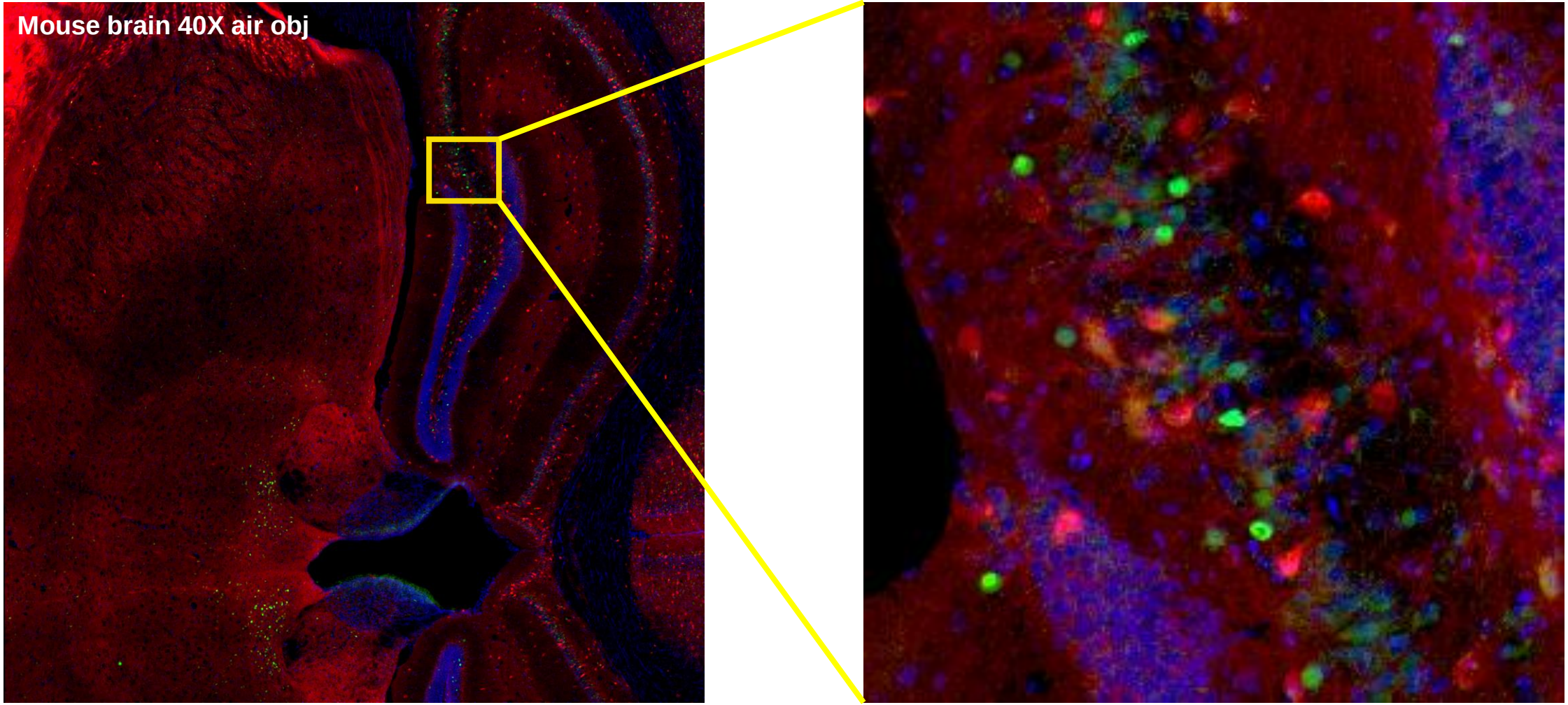
Server system

Offline PC
Network server system*
Cloud (VM) system*

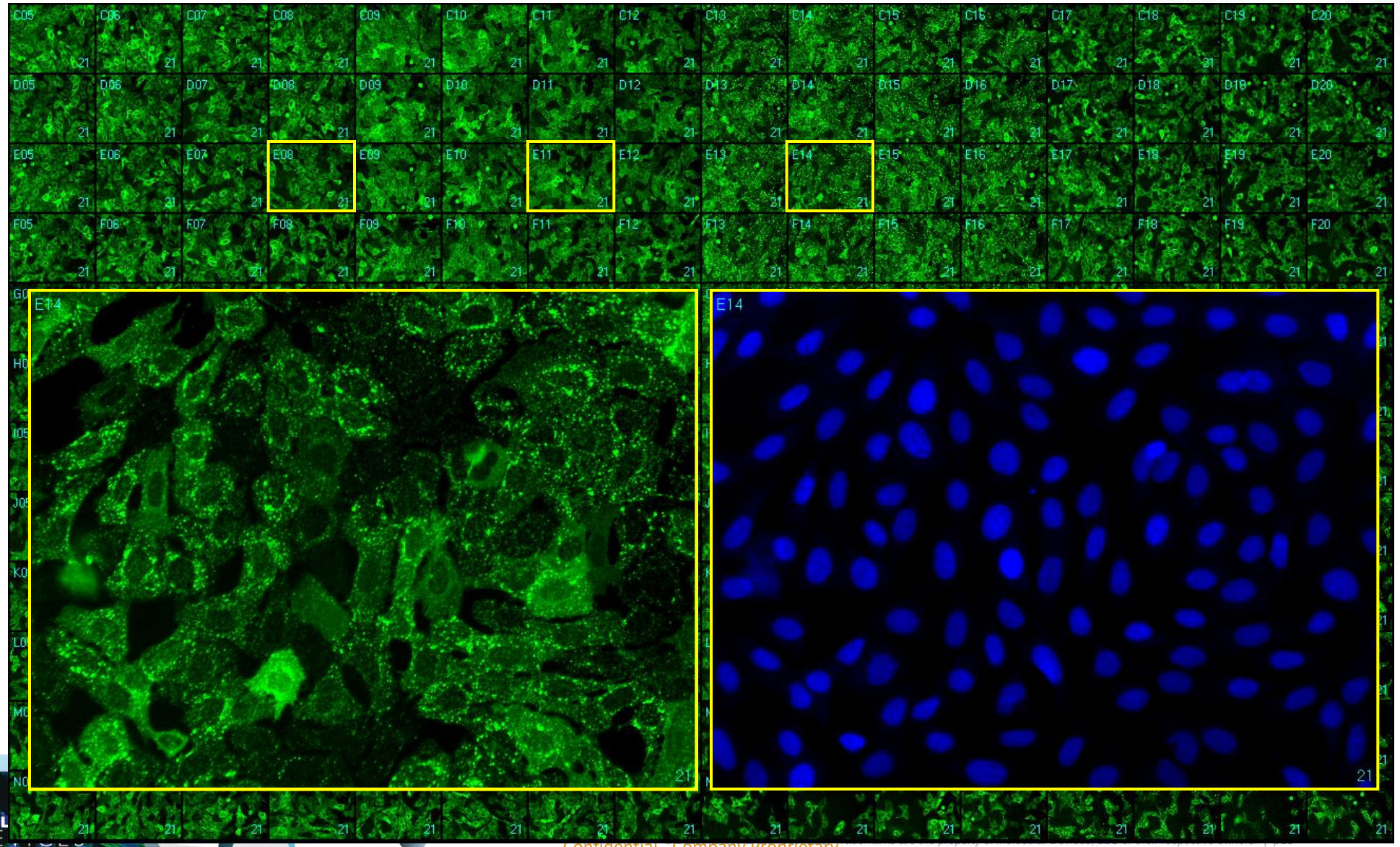
Vessel applicability

Slide/ chamber slide
Multi-well plate
Dish*

Slide Screening

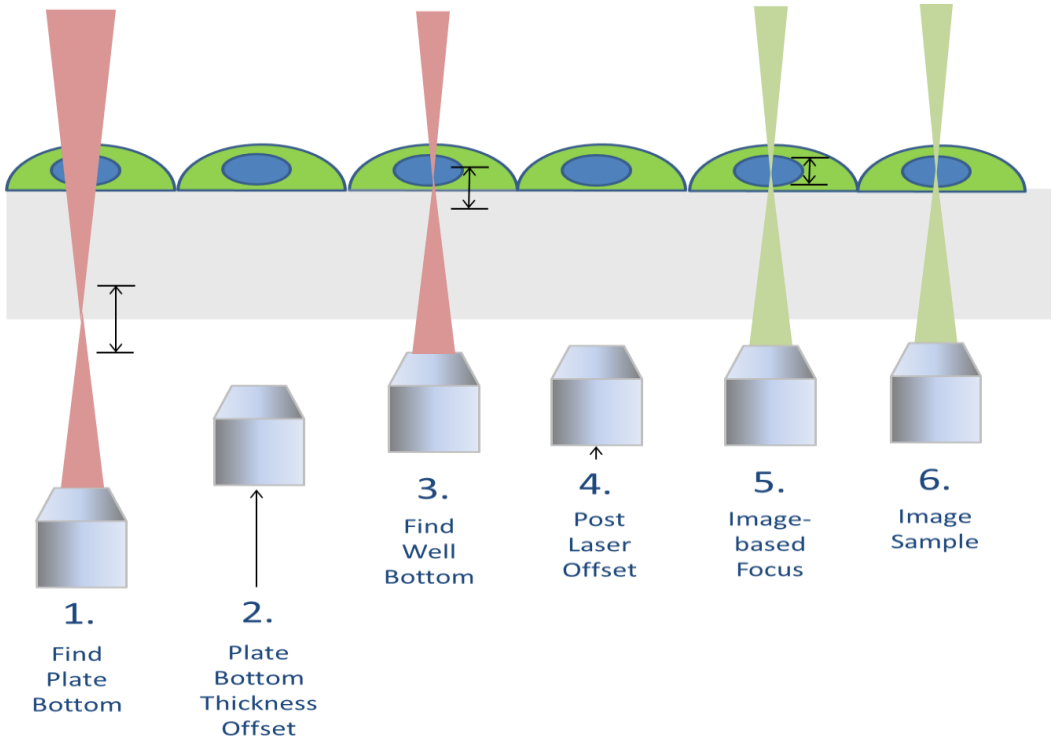


Multi-well plate acquisition



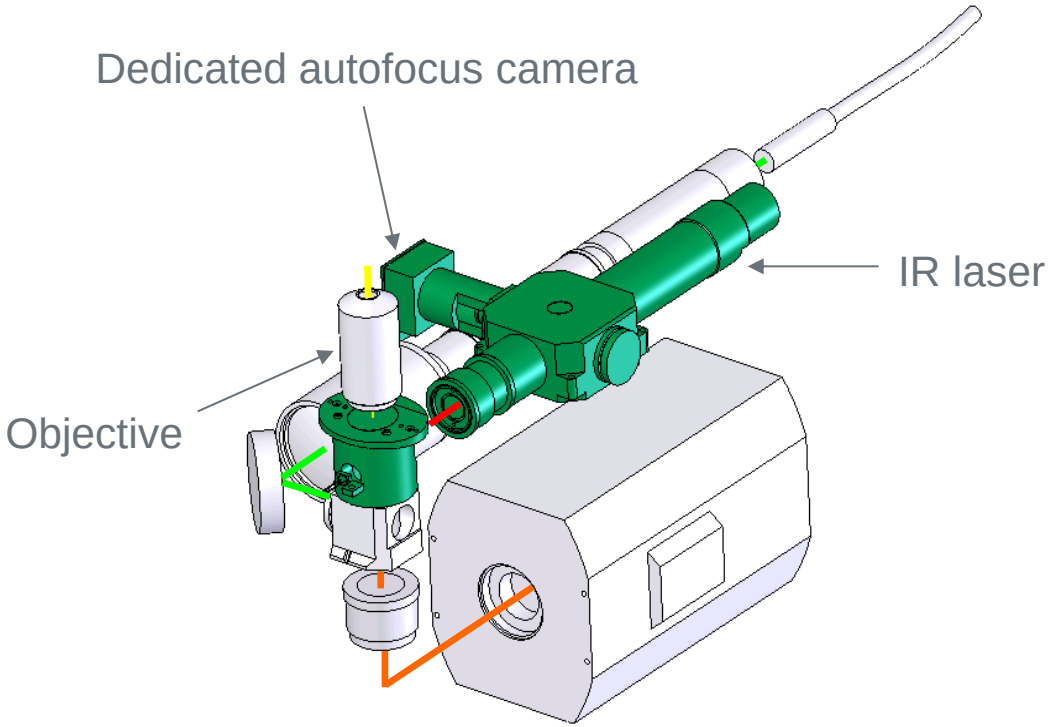
Autofocus System

Laser and image hybrid autofocus system



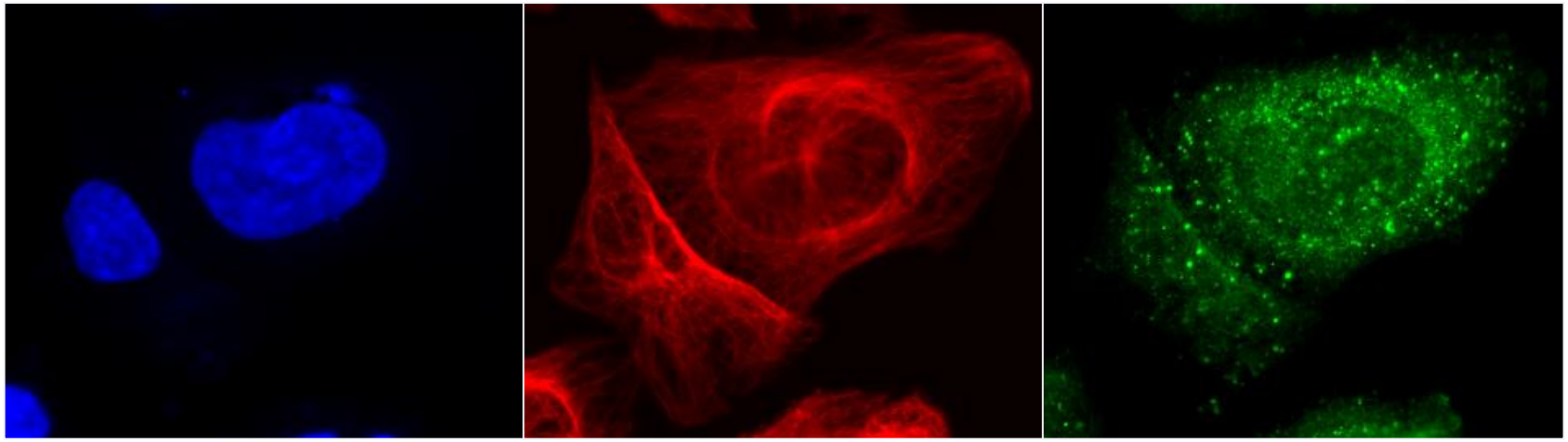
Laser Autofocus

Image Autofocus

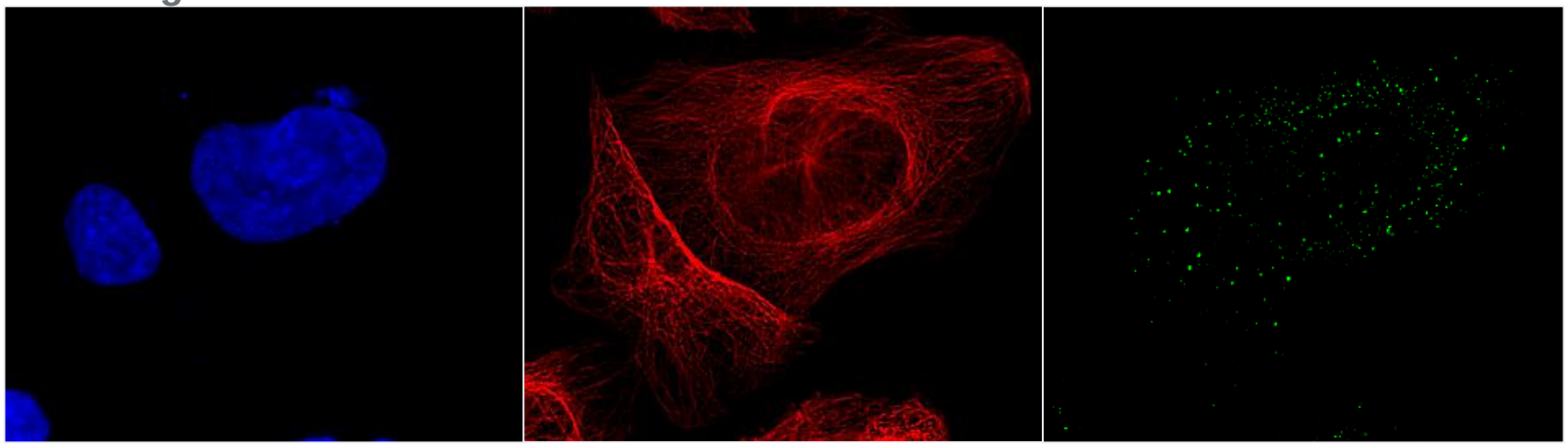


Digital Confocal Module

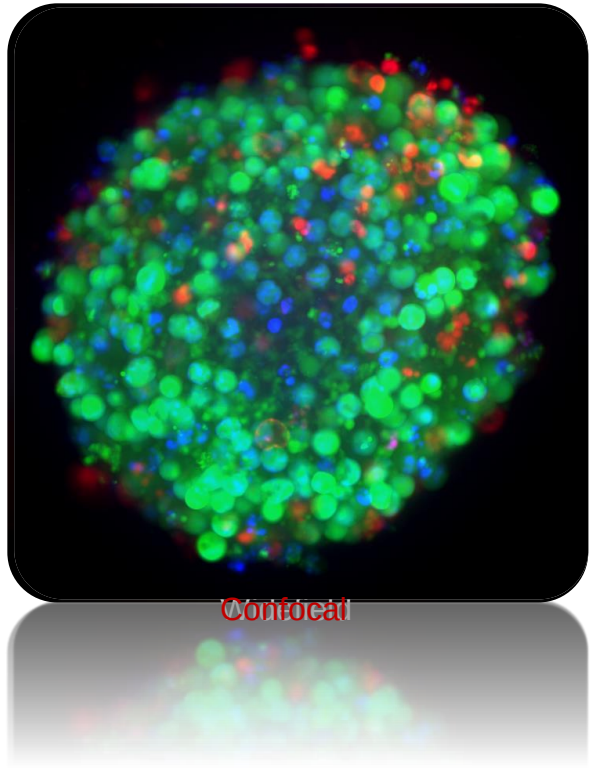
Without Digital Confocal



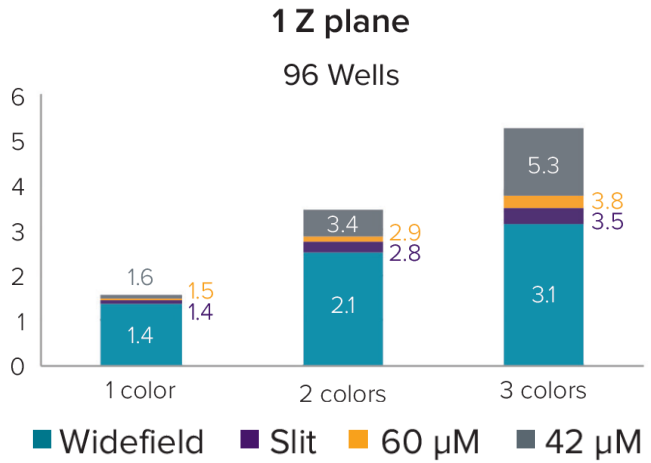
With Digital Confocal

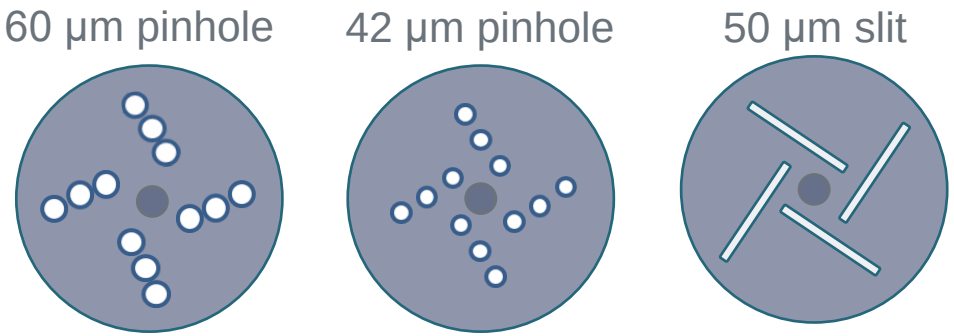


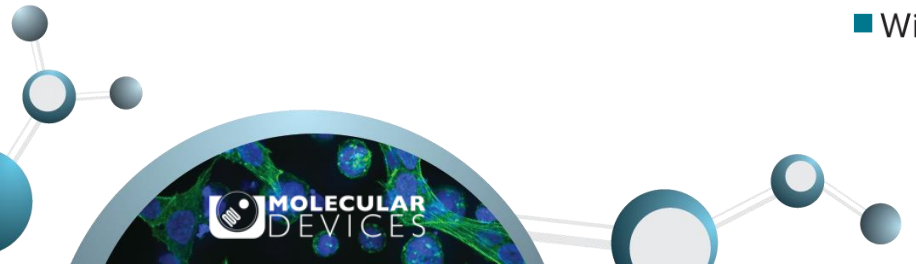
Spinning Disk Confocal



- Multi-type configuration
- Compatible with live cell assay
- Widefield switchable
- High-resolution and high-throughput







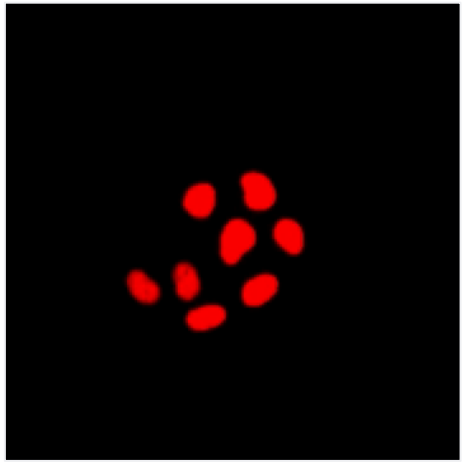
Environmental Control Module

IXM can monitor and control:

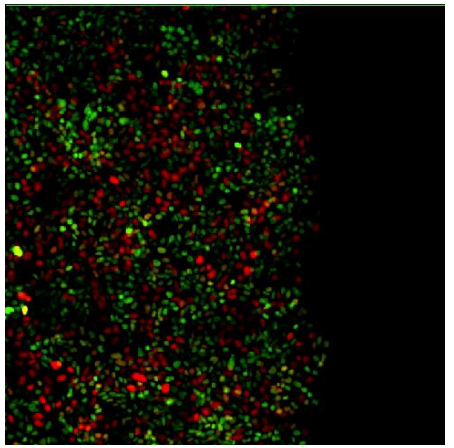
- Temperature
- Humidity
- Gas

Continuous time course from millisecond to multi-days

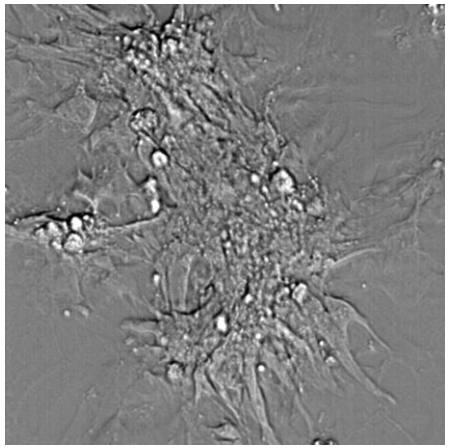
Colony formation



Migration assay

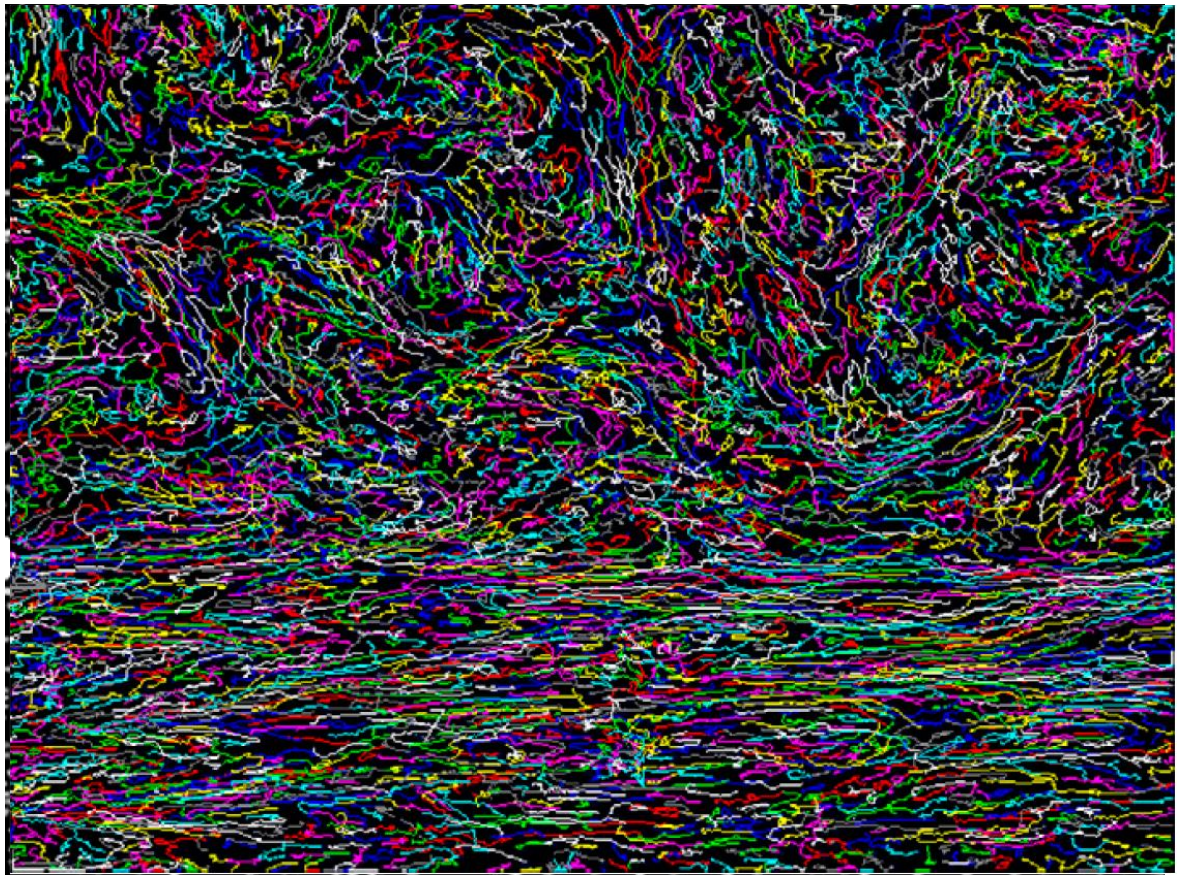


Cardiomyocyte assay



Example: Single Cell Migration Assay

Cell migration assay



Nonautonomous contact guidance signaling during collective cell migration
Proc Natl Acad Sci U S A. 2014 Feb

Transmitted Light Module

Transmitted Light Module

- Bright field/ Phase contrast
- Köhler illumination
- Compatible with

  Environmental control system

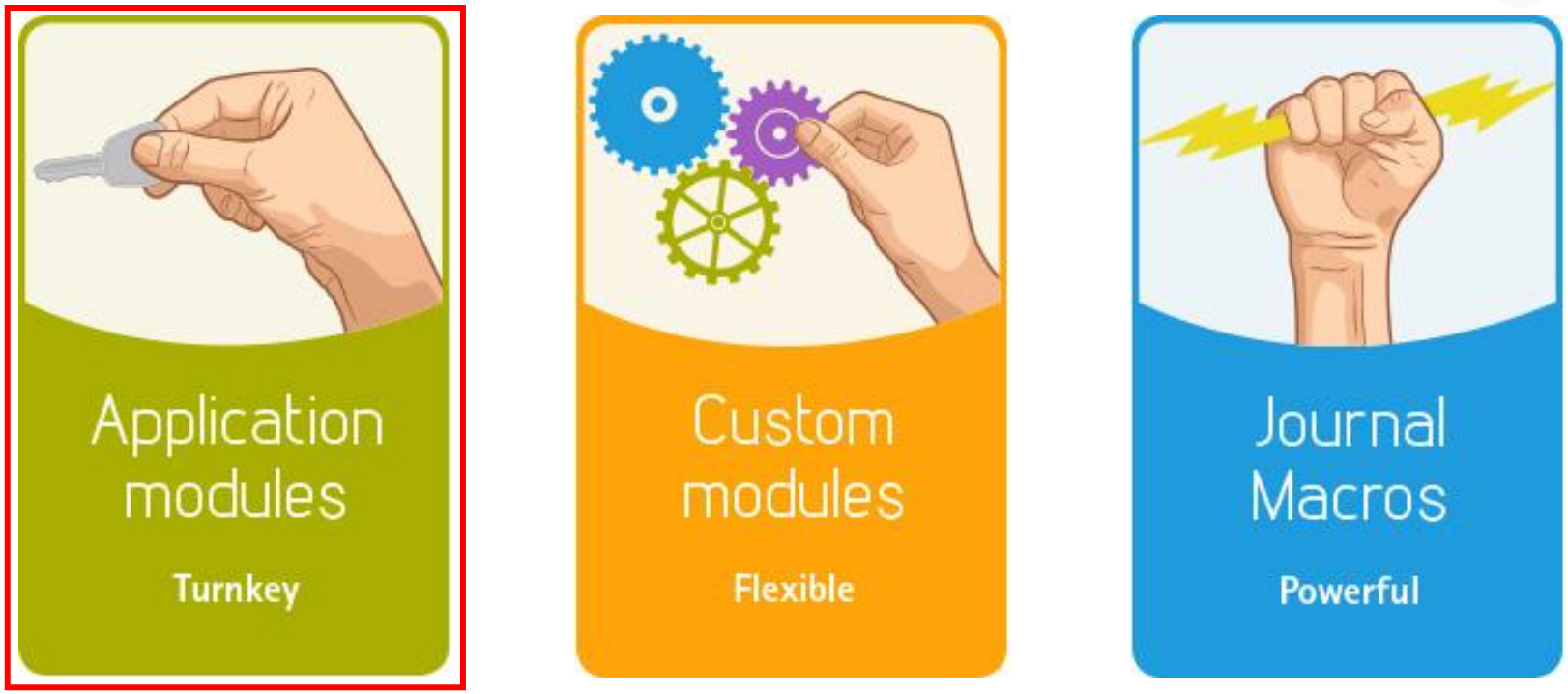
Liquid Handling & Robotic System (Optional)

Liquid Handling Module

Robotic System

High Content System Overview

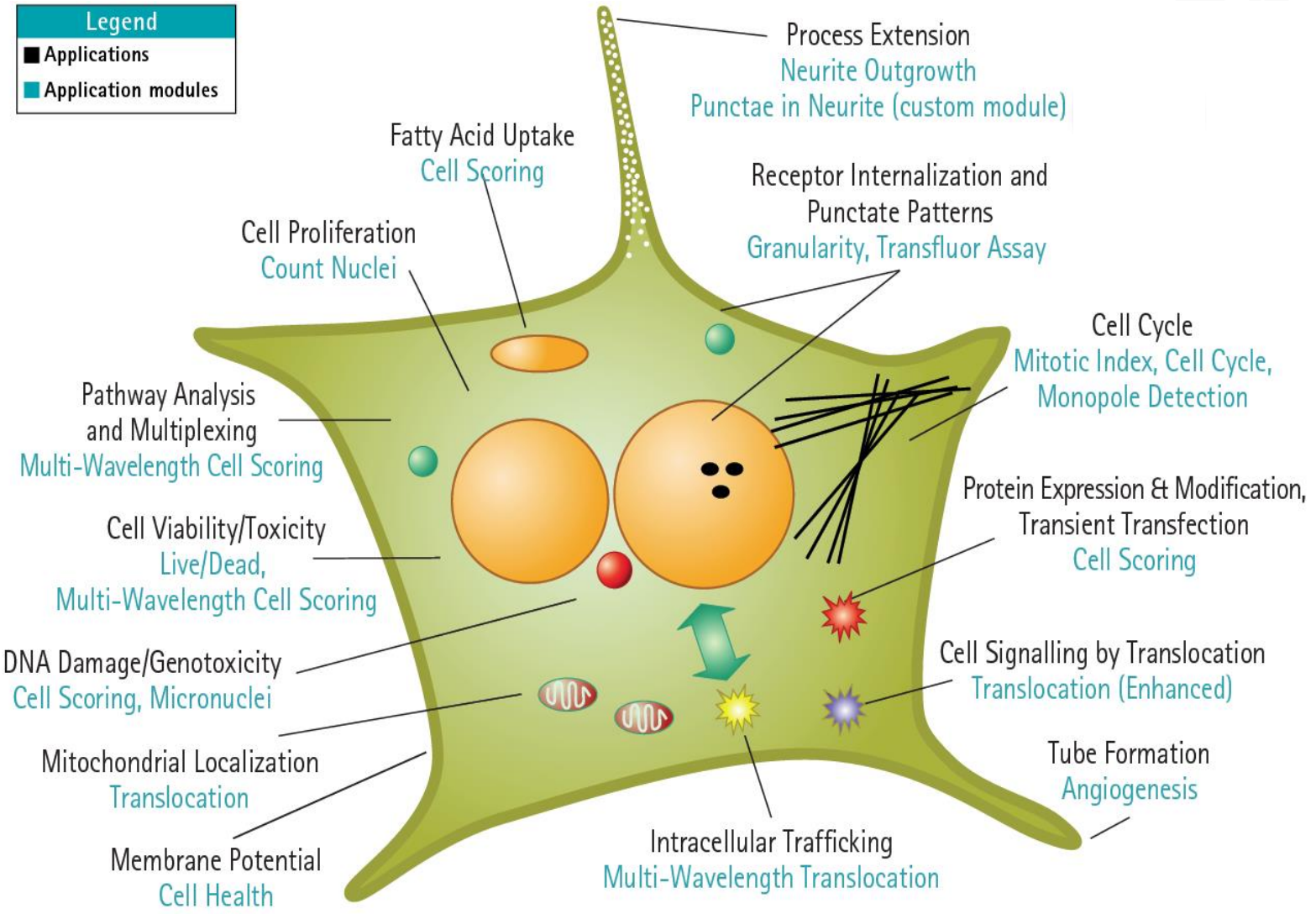
3-Stage Image Analysis Tools



Analysis continuum with increasing flexibility and complexity

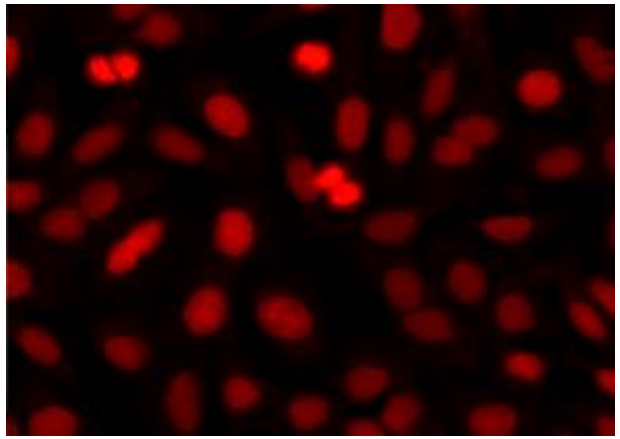
MetaXpress® Software

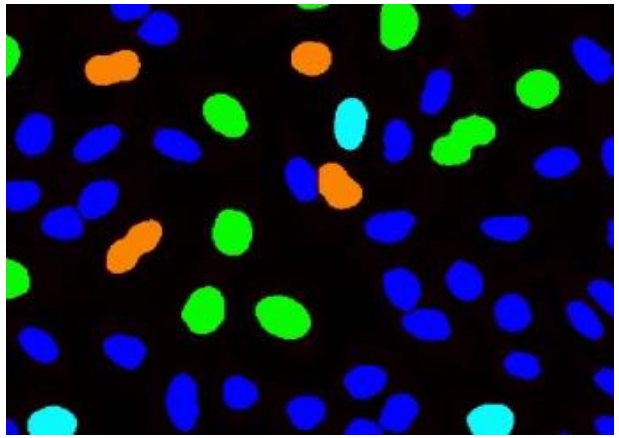
Application Modules

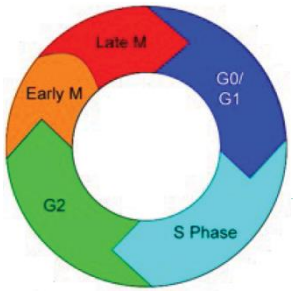


Application Module: Cell Cycle

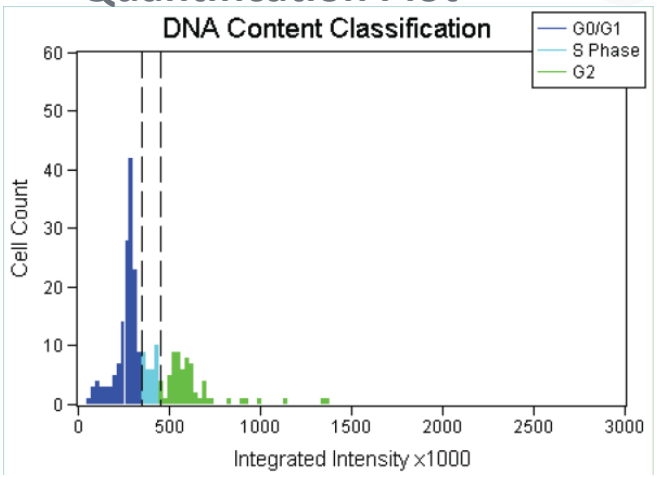
DAPI

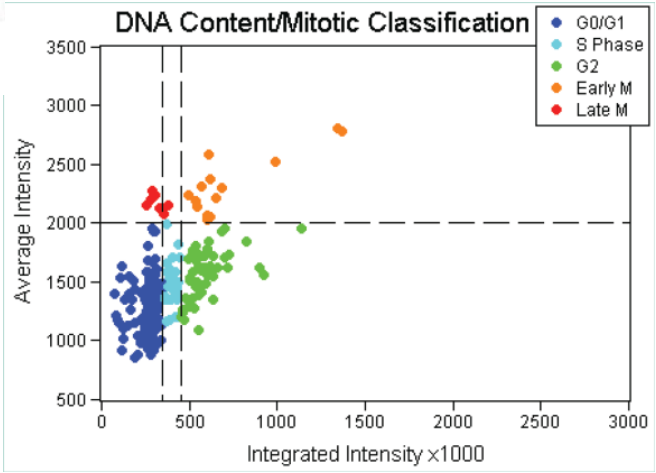






Quantification Plot





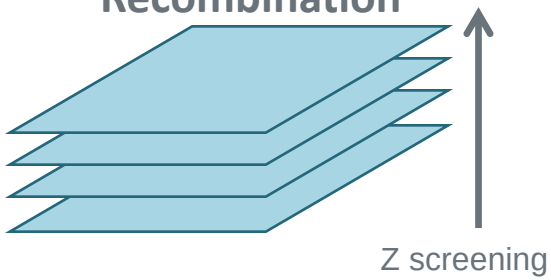
Measurements:

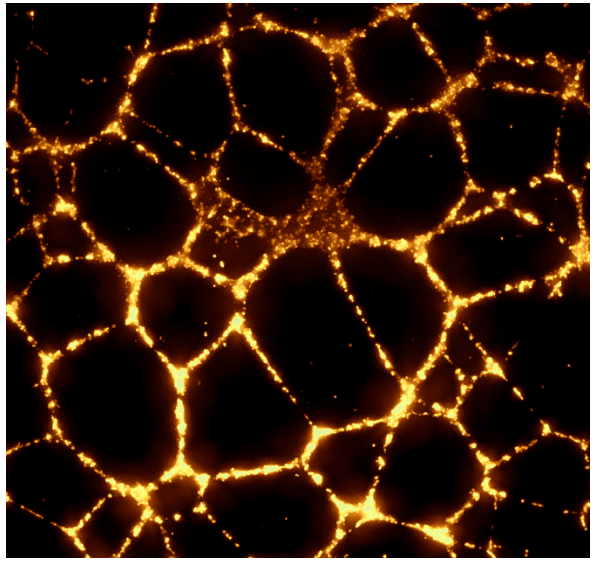
Count and percentage of G0/G1, S, G2, early M, late M and apoptotic cells, area and intensity of DNA……

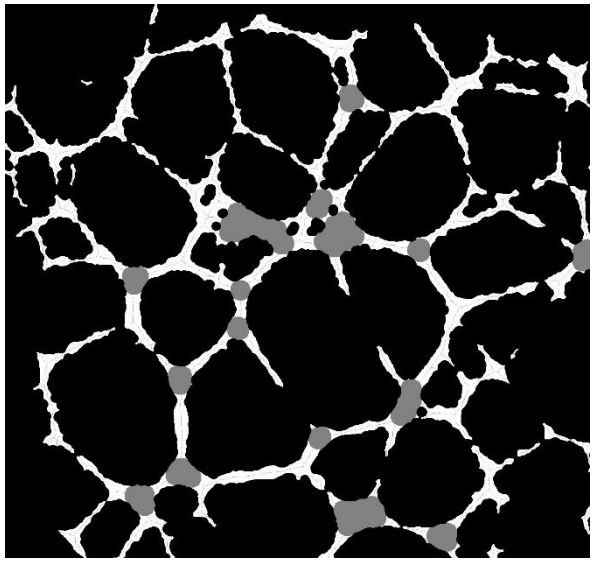
Application Module: Angiogenesis

Locating and Scanning

Best Focus Recombination



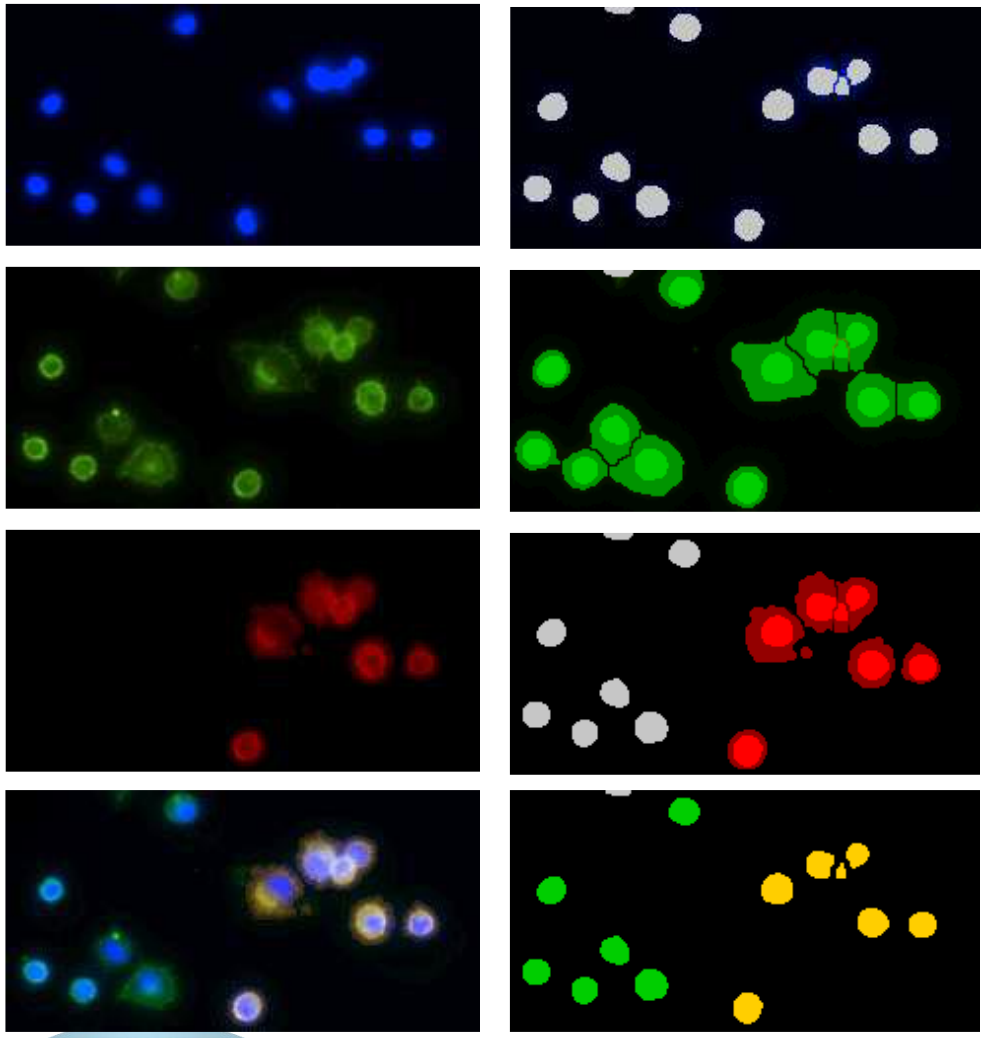


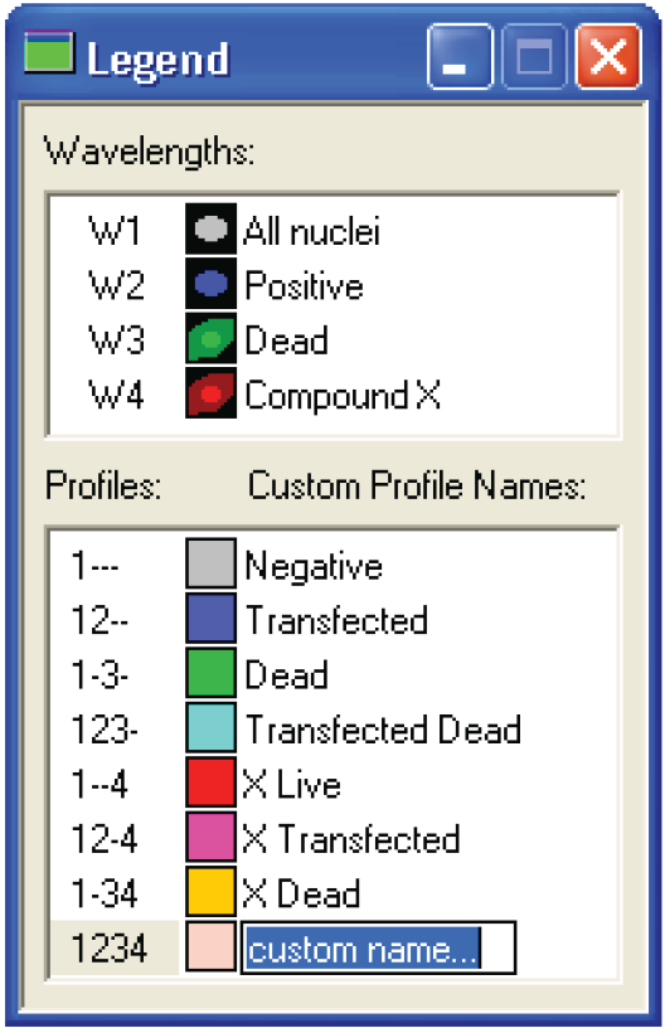


Measurements:

Tube length, tube area, branch, segment, nodes……

Application Module: Multi-wavelength Cell Scoring





Measurements:

Cell count

Percentage of each cell type

Wavelength intensity of each channel in cell

Wavelength intensity of each channel in nucleus

Nucleus area

Cell area

Application Module: Granularity

Mito Tracker

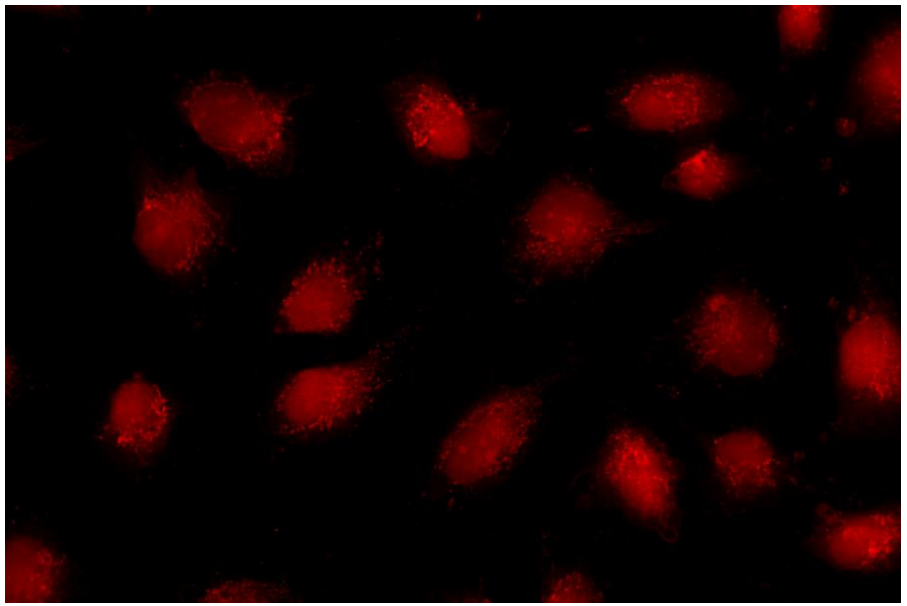
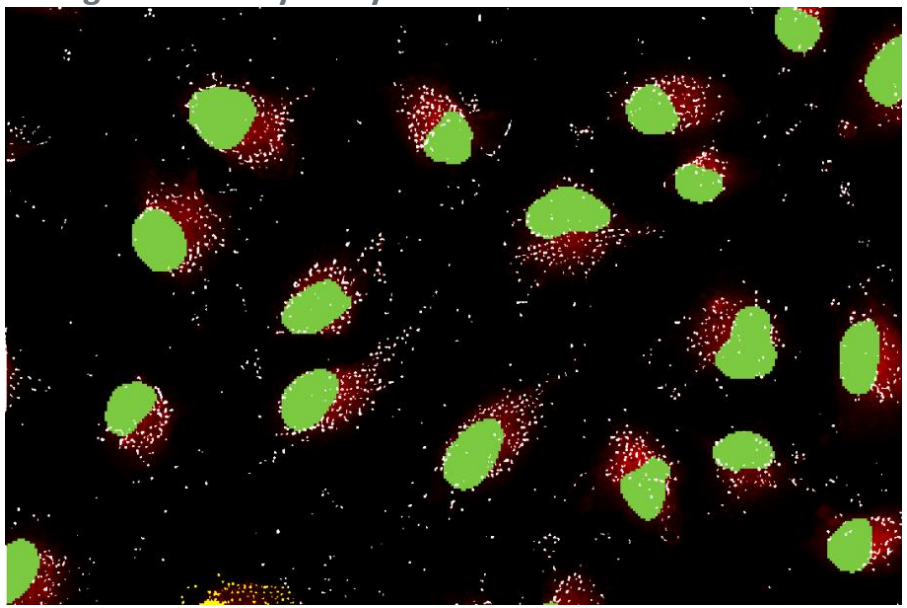


Image masked by analysis result

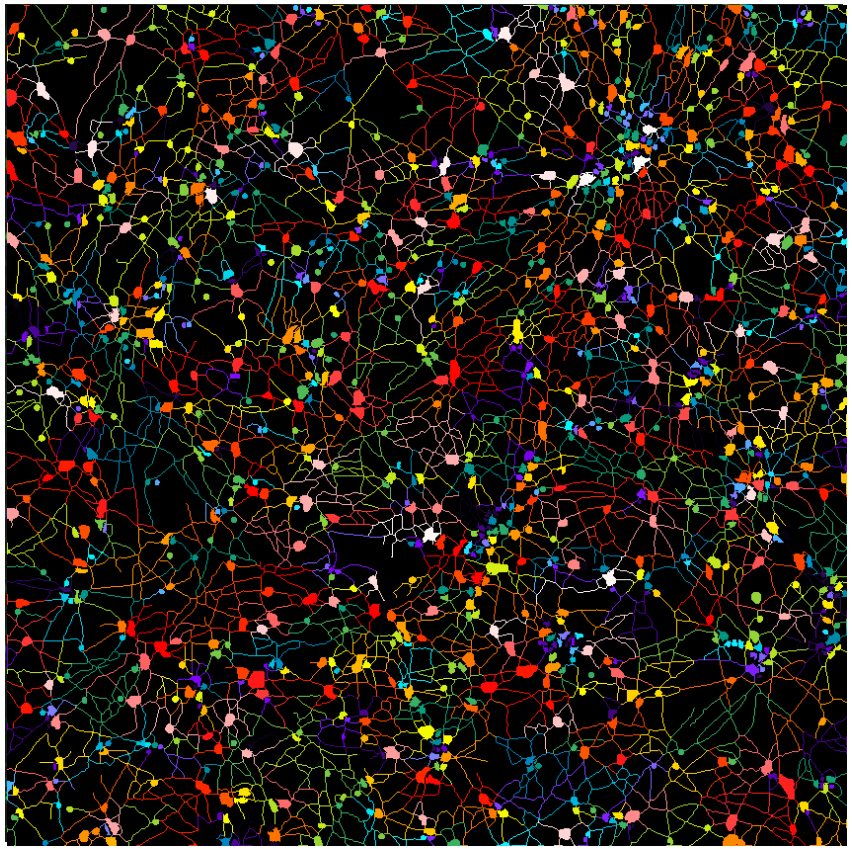


Measurements:

Granule count per cell, total granule area per cell, granule integrated intensity per cell, nuclear count/ intensity/ area.

Application Module: Neurite Outgrowth

Nuclear β-tubulin



Quantification

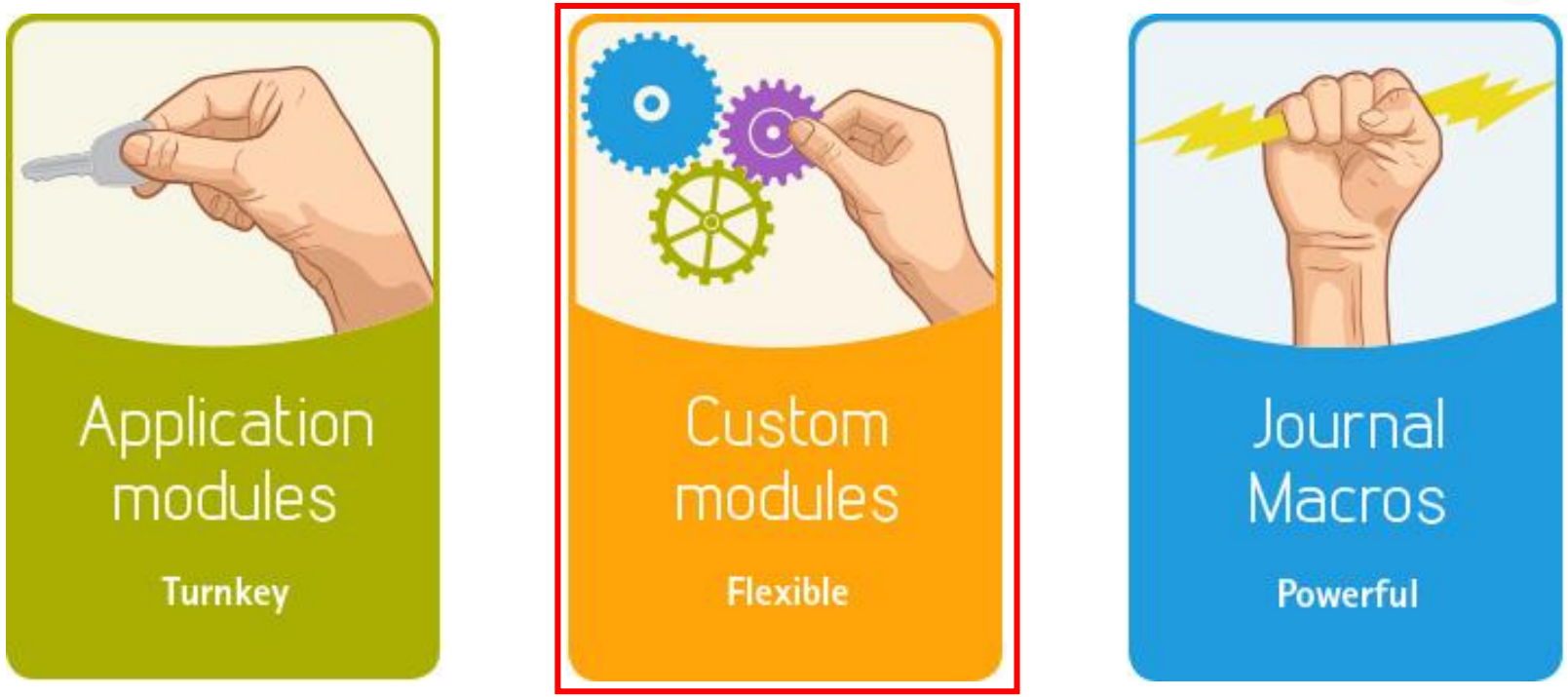
Cellular Results for Neurite Outgrowth

	Cell: Assigned Label #	Cell: Total Outgrowth	Cell: Processes	Cell: Mean Process Length	Cell: Median Process Length	Cell: Max Process Length	Cell: Branches	Cell: Straightness	Cell: Cell Body Area	Cell: Mean Outgrowth Intensity
1	1	35.124	2	17.562	17.562	27.9832	2	0.908596	39.9384	574.945
2	2	0	0	0	0	0	0	0	15.3929	0
3	3	0.645	1	0.645	0.645	0.645	0	1	27.4577	0
4	4	37.7957	2	18.8978	18.8978	37.1507	1	0.927003	19.9692	607.4
5	5	32.6088	2	16.3044	16.3044	31.9638	1	0.939445	22.0493	502.553
6	6	0	0	0	0	0	0	0	24.1295	0
7	7	15.2587	4	3.81467	1.29	12.0337	0	0.935226	27.4577	731.757
8	8	97.9909	2	48.9955	48.9955	97.3459	4	0.932935	44.5147	611.191
9	9	55.0542	2	27.5271	27.5271	41.9977	0	0.885411	51.1711	719.173
10	10	25.449	2	12.7245	12.7245	24.804	0	0.913902	57.4115	711.3
11	11	46.3372	4	11.5843	12.0242	21.6438	1	0.94364	40.7705	617.612
12	12	34.4142	3	11.4714	5.69434	27.1627	3	0.957466	67.8121	662.172
13	13	60.241	2	30.1205	30.1205	59.596	0	0.932452	84.4531	632.494
14	14	17.2395	2	8.61975	8.61975	9.72084	2	0.927697	32.0339	878.871
15	15	101.327	3	33.7755	10.32	84.0222	5	0.931383	30.3698	686.784
16	16	20.4645	2	10.2323	10.2323	19.8195	3	0.912585	40.3544	869.62
17	17	4.13717	1	4.13717	4.13717	4.13717	0	0.95086	34.5301	782.167
18	18	59.3479	2	29.6739	29.6739	30.9869	5	0.895381	32.45	684.953

Measurements:

Outgrowth, Processes, Process length, Branches, Cell body area……

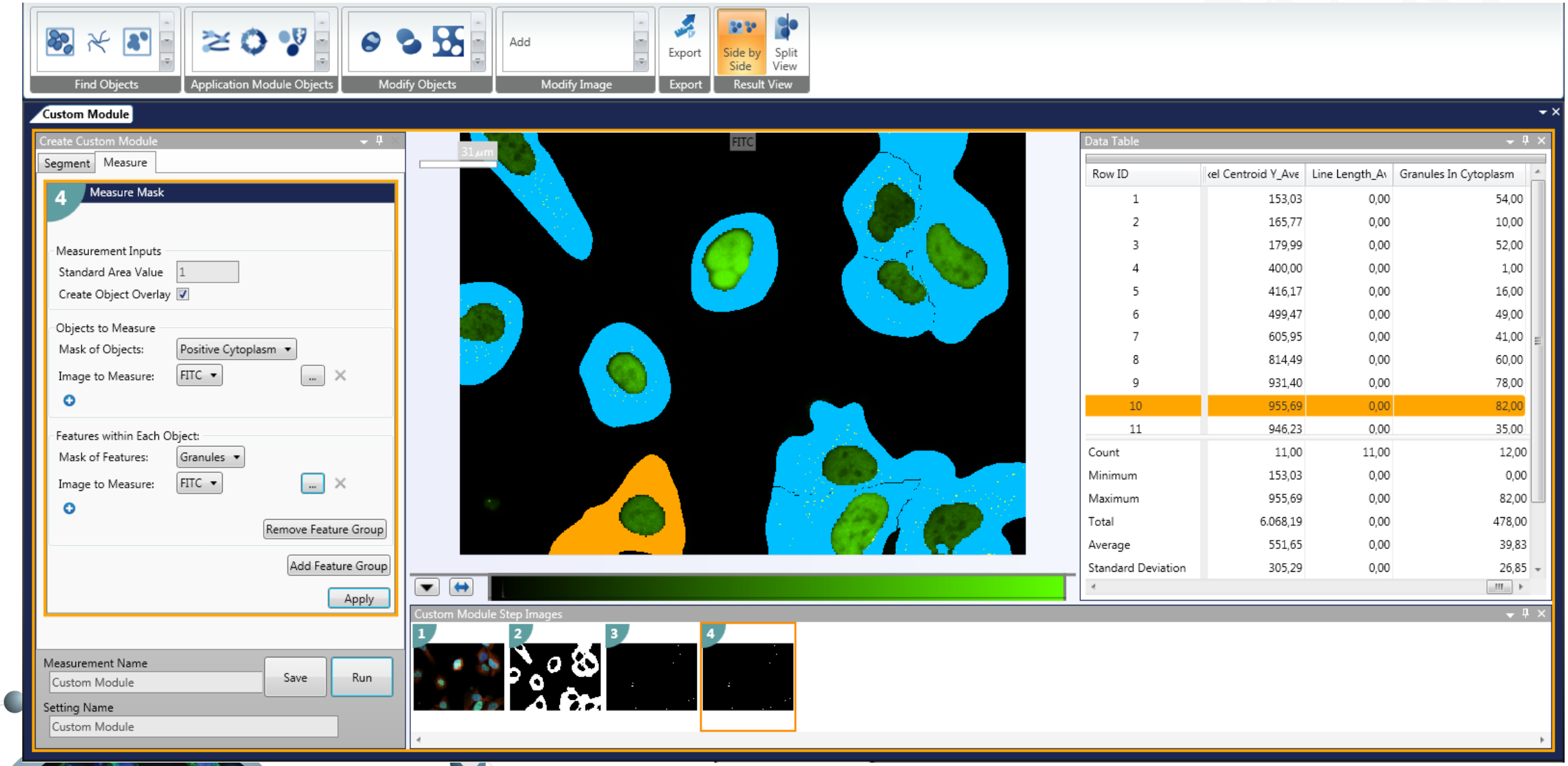
3-Stage Image Analysis Tools



Analysis continuum with increasing flexibility and complexity

MetaXpress® Software

Customized Analysis Workflow

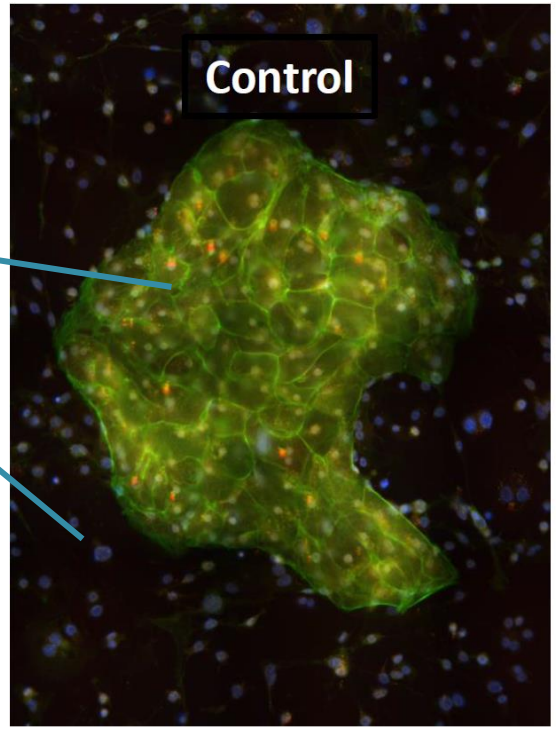


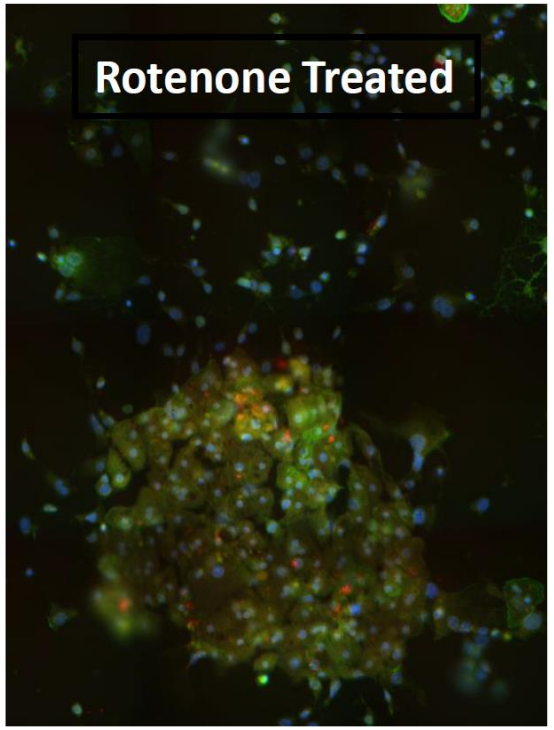
Example: Toxicity Assay by Hepatocyte Co-Culture Model

Hepatocyte island

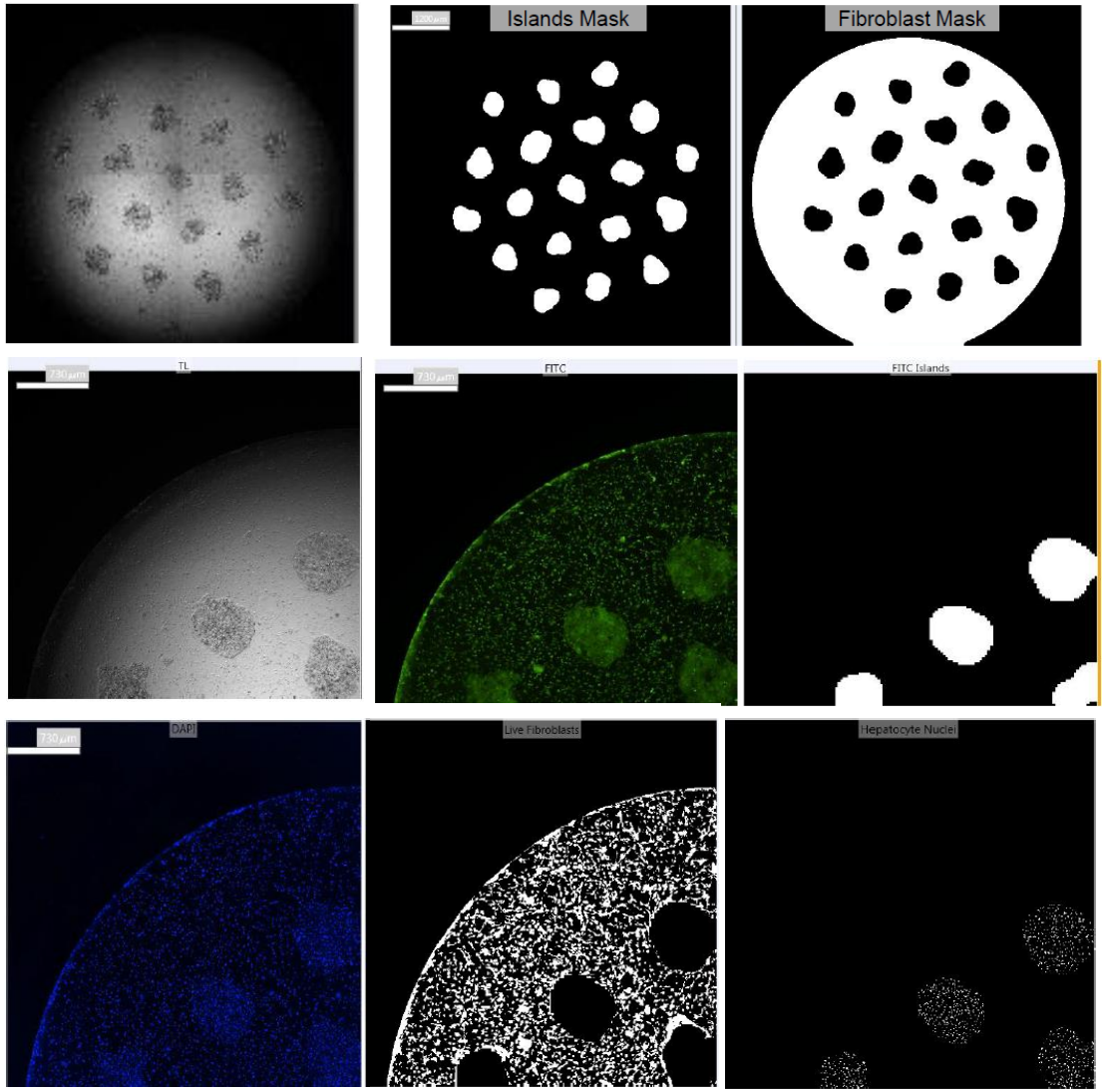
Fibroblast

Nuclei
Actin
Mitochondria





Example: Toxicity Assay by Hepatocyte Co-Culture Model



Identify well margin and hepatocyte islands

- **Island number/ area**
- **Fibroblast area**

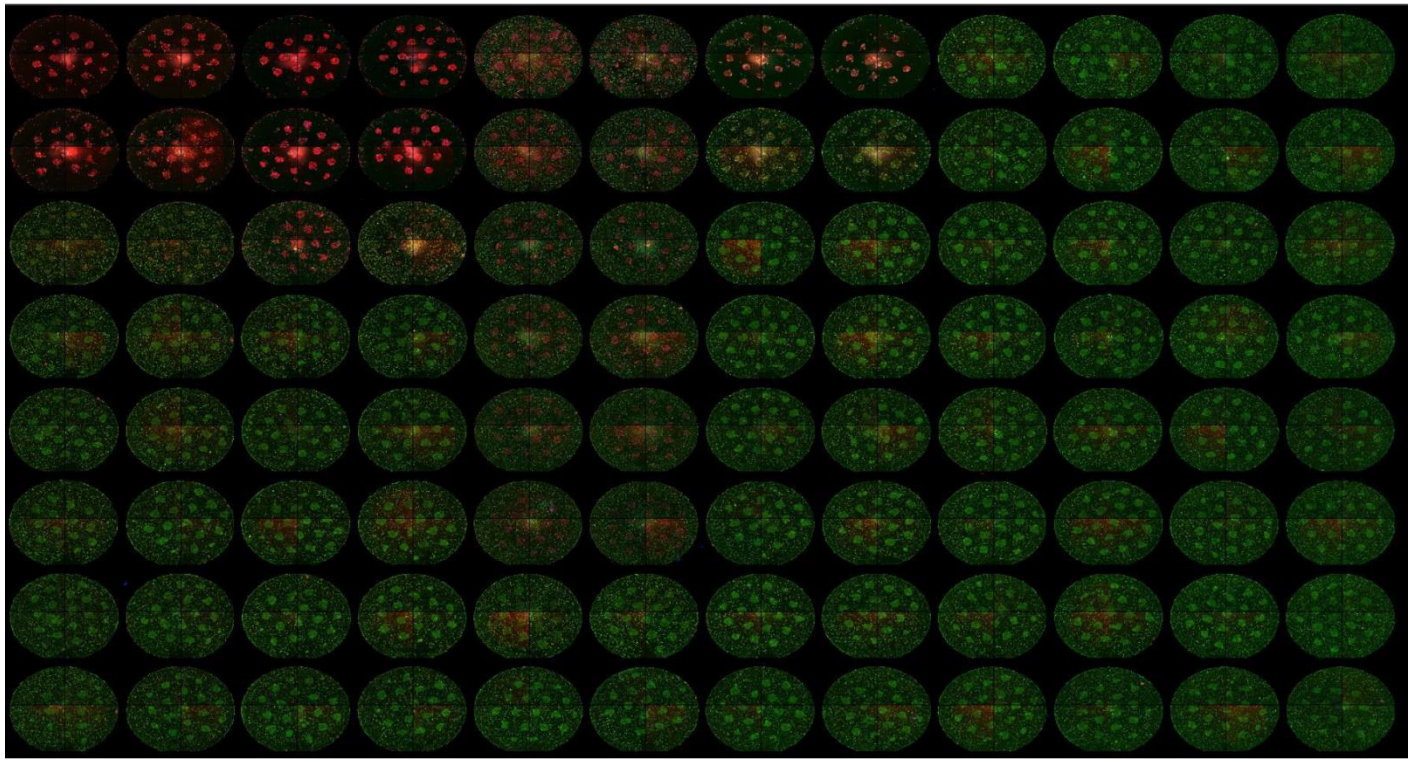
Determine parameters of hepatocyte islands

- **DAPI/ Mitochondria intensity of island**

Determine parameters of cells

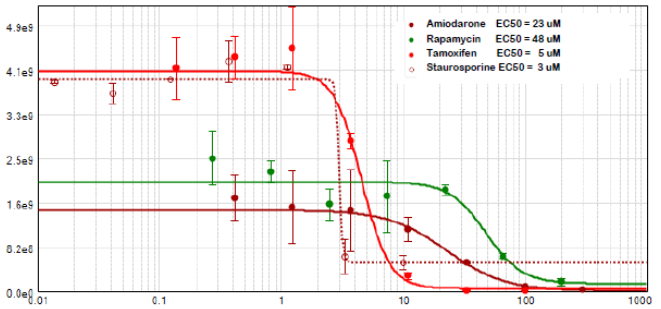
- **Live cell number of hepatocytes and fibroblast**
- **Mitochondria intensity of each cell**

Example: Toxicity Assay by Hepatocyte Co-Culture Model

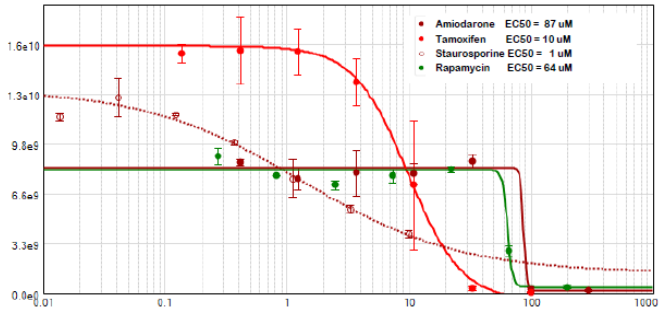


Compound concentration

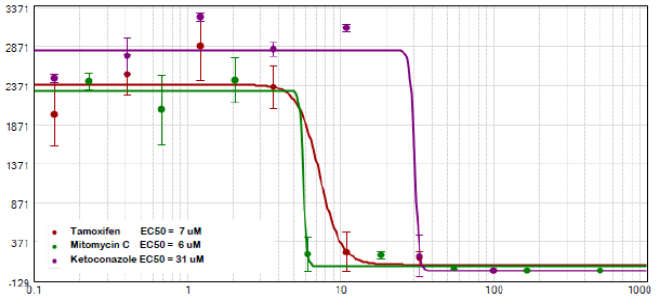
Live hepatocyte number



Live fibroblast number

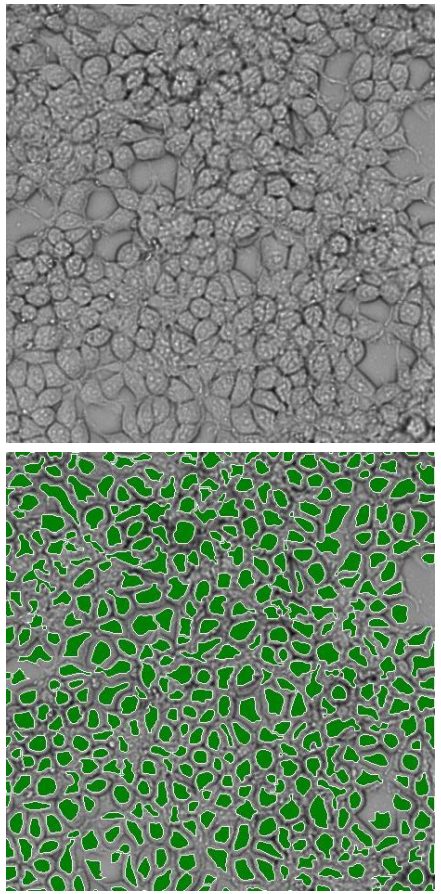


Mitochondria intensity

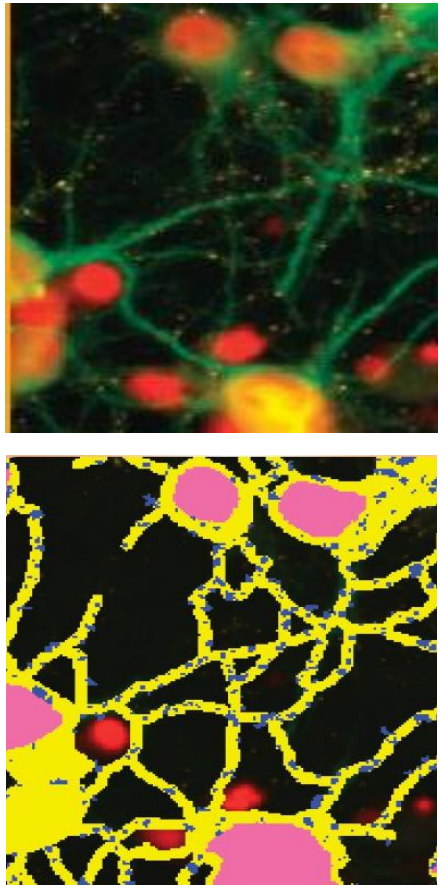


Versatile Analysis Applications

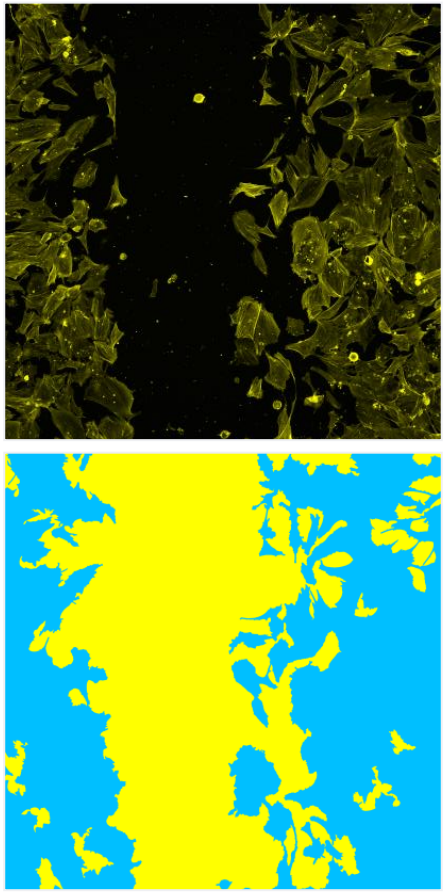
Proliferation with Transmitted Light



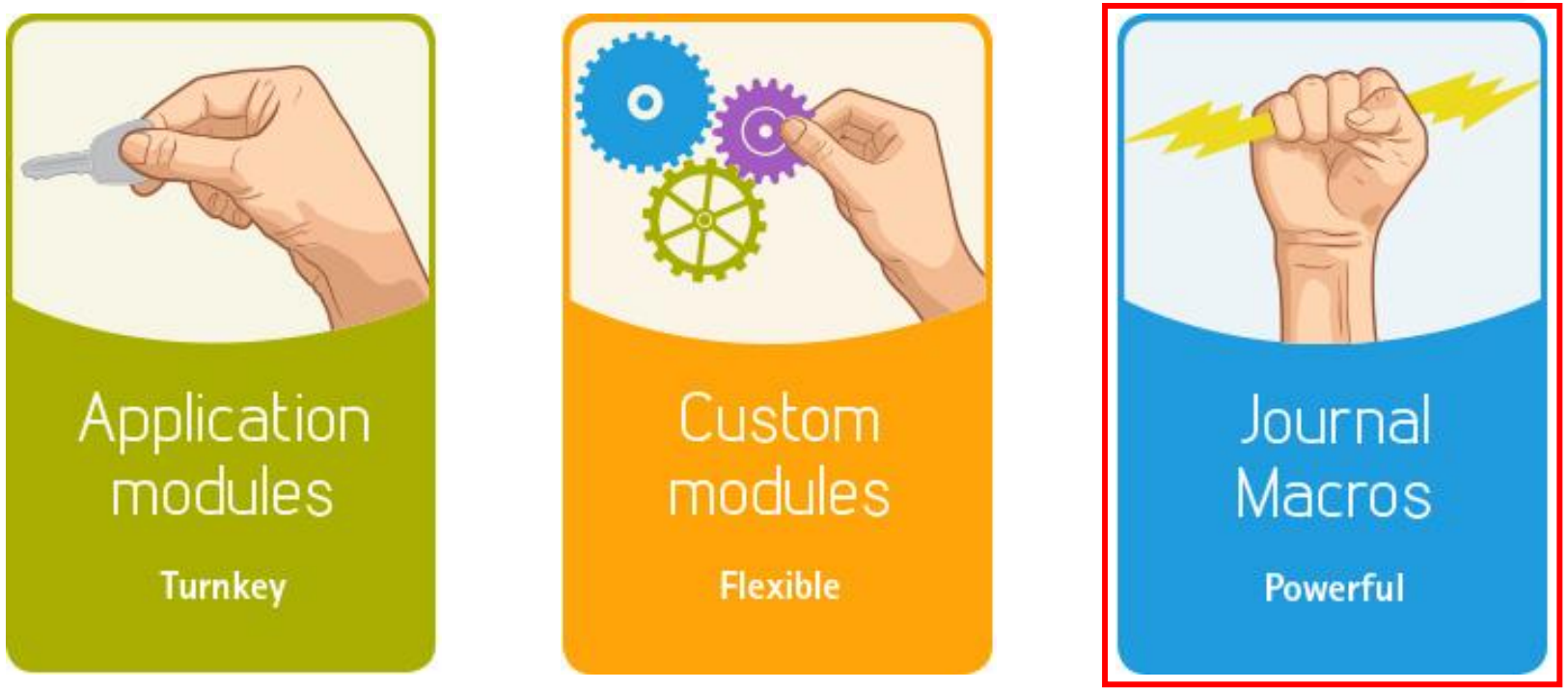
Neurite Outgrowth and Puncta



Wound Healing



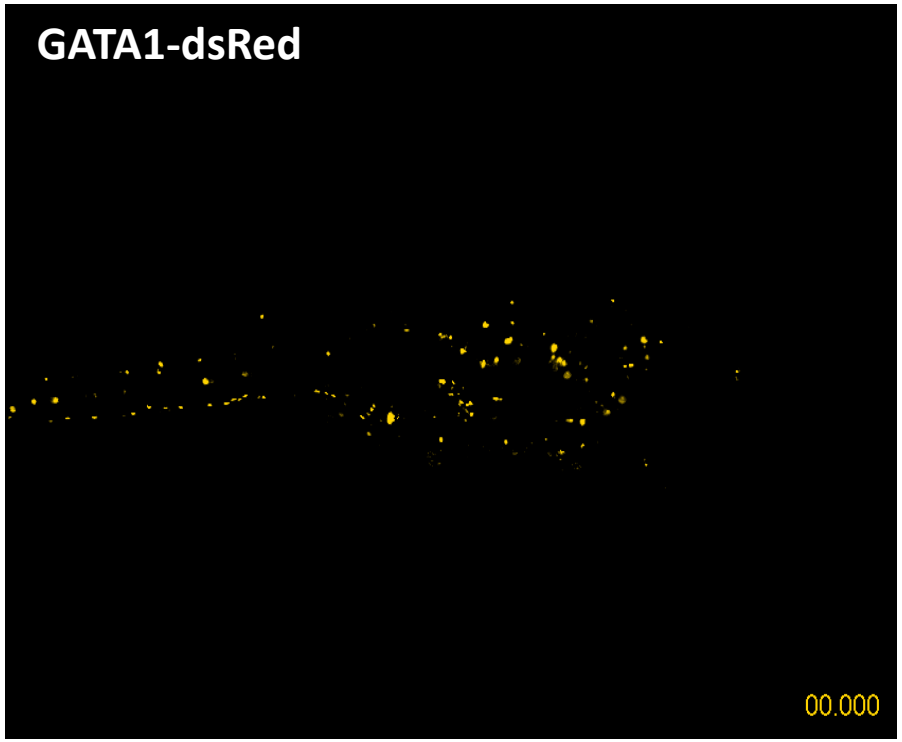
3-Stage Image Analysis Tools

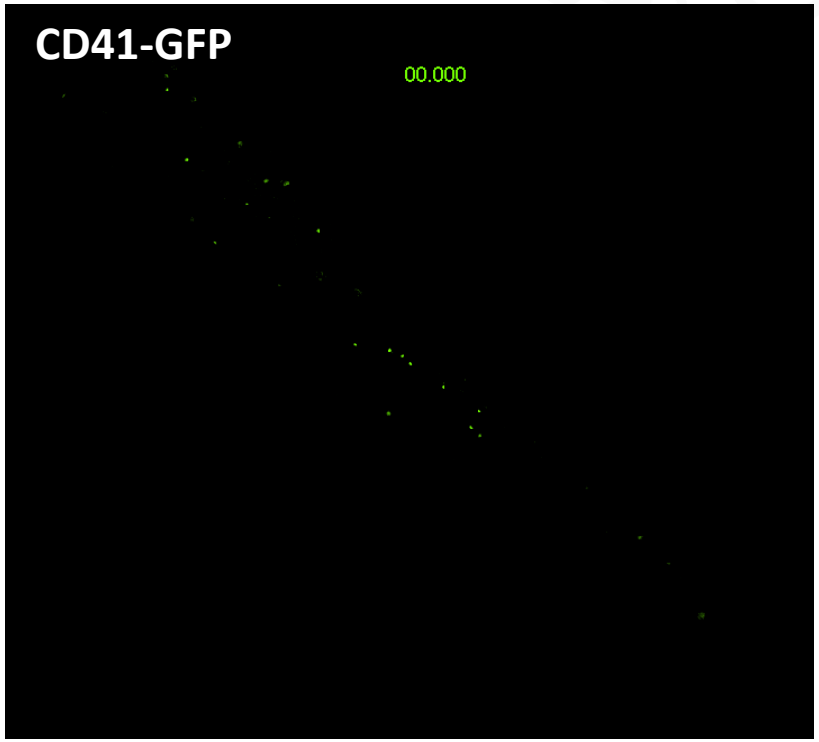


Analysis continuum with increasing flexibility and complexity

MetaXpress® Software

Analyzing Circulating Cells in Zebrafish



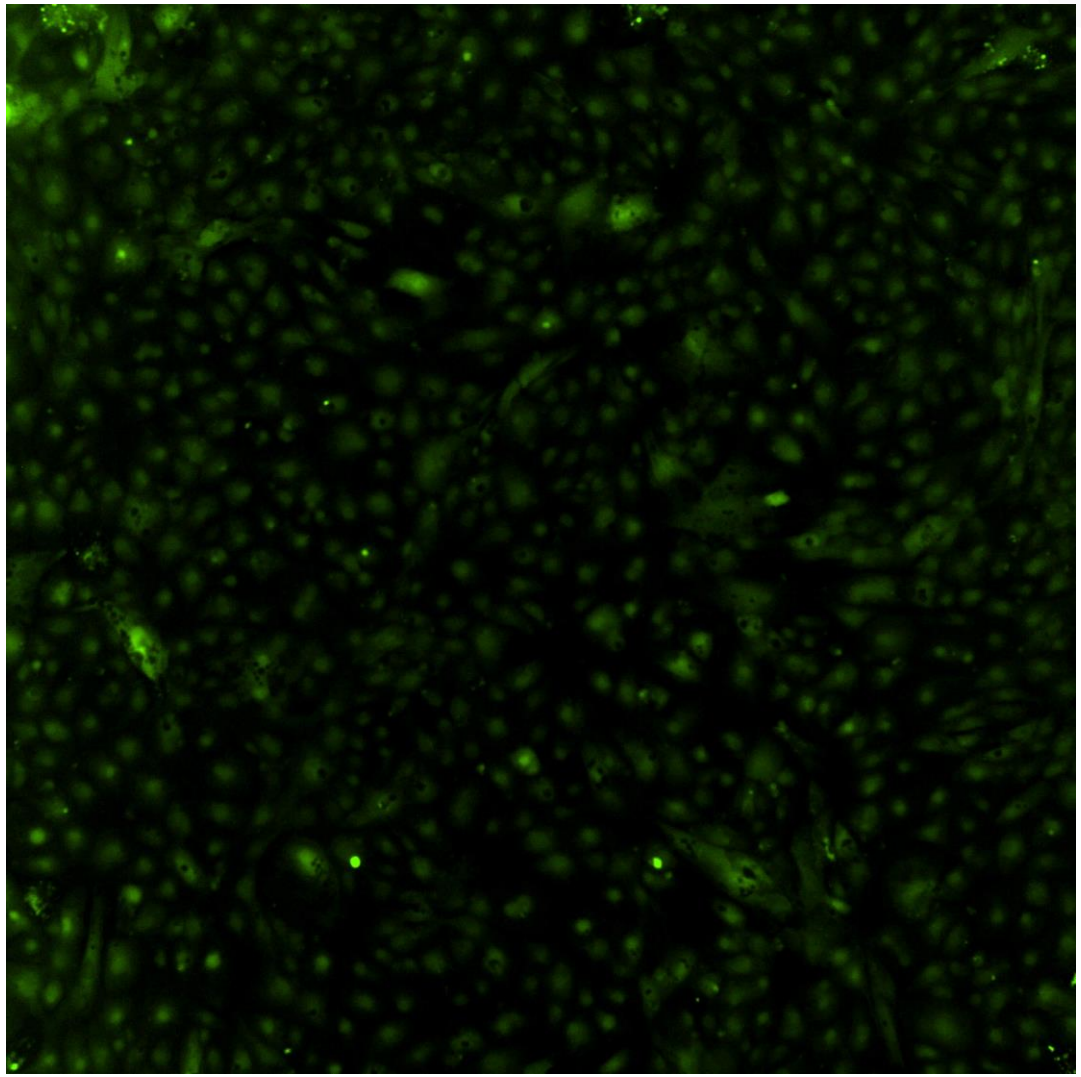


- Remove static signal from the image to be able to measure the cells that are actually circulating

Number of Circulating Cells	
GATA1	CD41
92	25

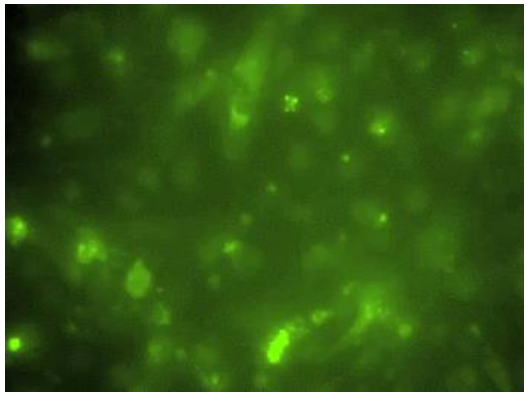
In vitro Cardiomyocyte Toxicity Assay

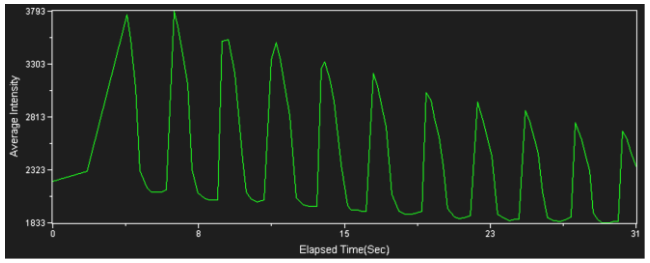
- iPSC derived cardiomyocytes
- Stained with FLIPR Calcium 6 assay kit
- 10X Plan Apo objective
- Image acquired with 100 FPS



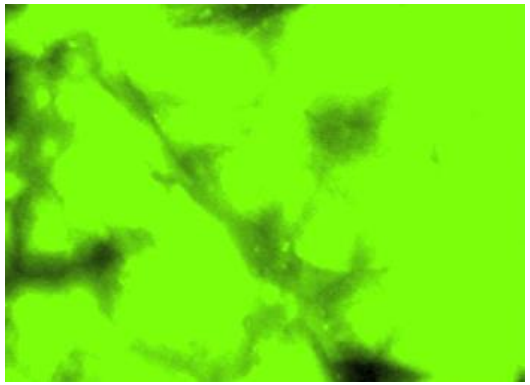
Spontaneous Contracting Cardiomyocytes Monitoring

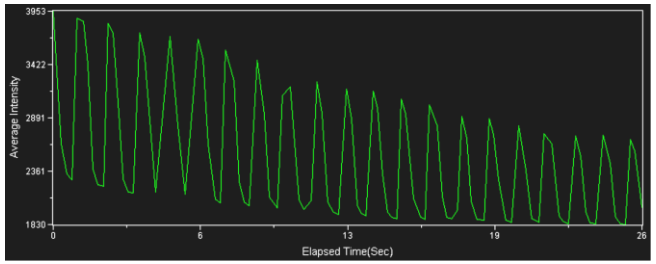
Control



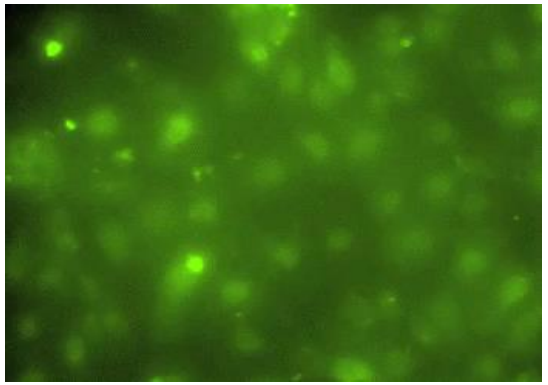


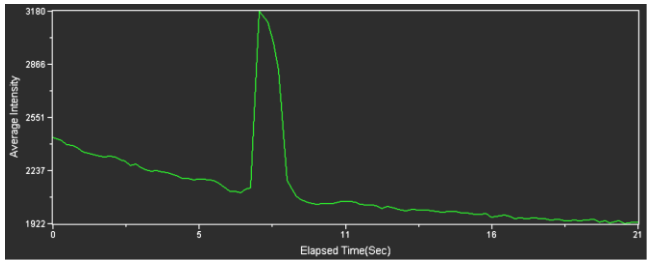
Epinephrine





Verapamil

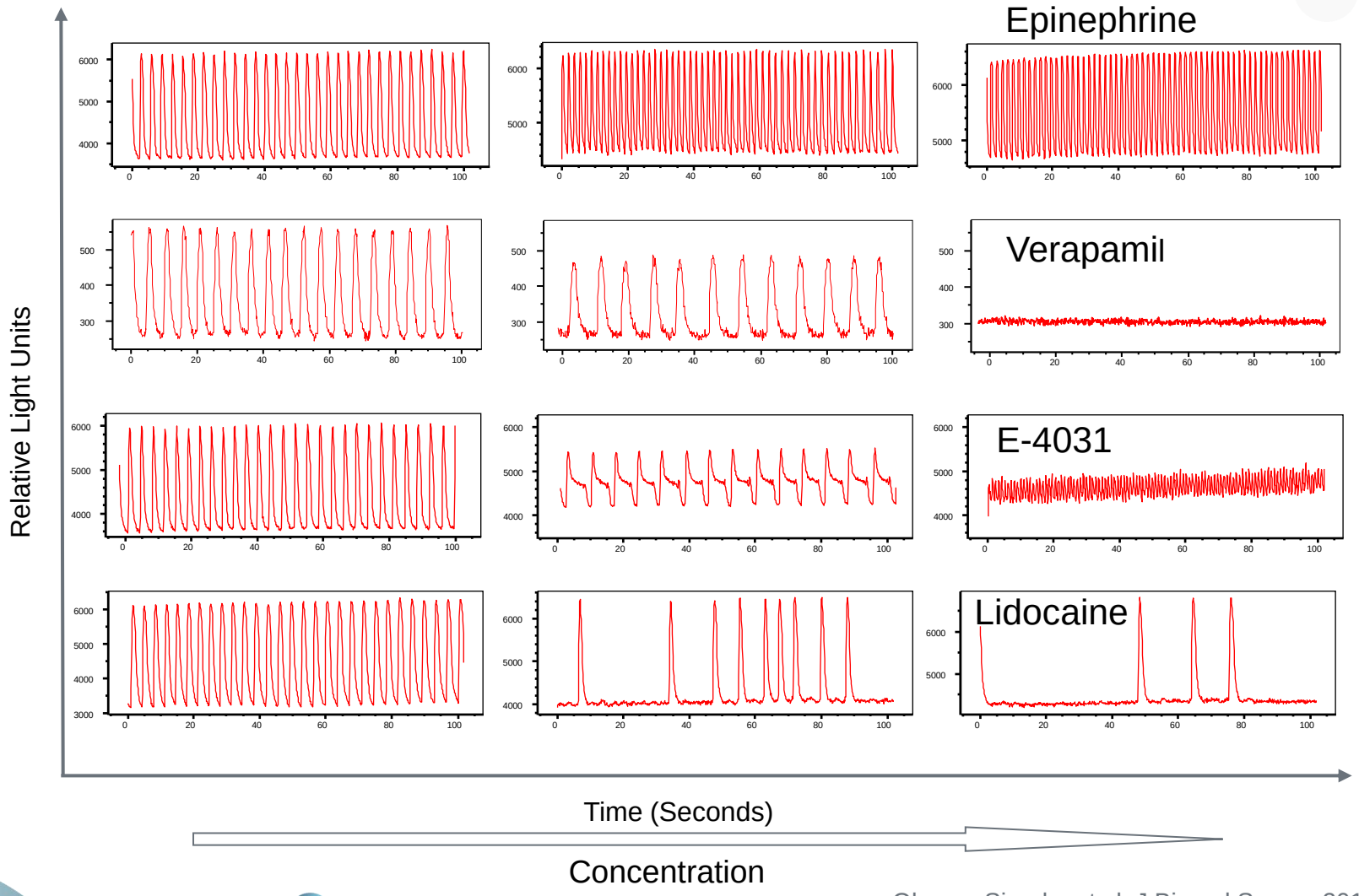




- Cell contractions visualized with Ca2+ sensitive dye
- Ca2+ levels fluctuate with contraction events
- Provide surrogate assessment of beat rate and sarcolemmal activity

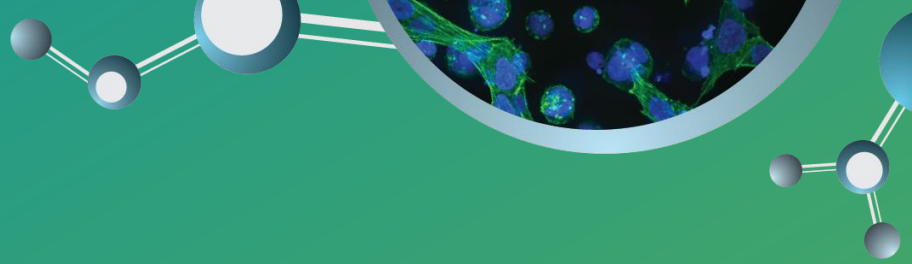
Oksana Sirenko et al. J Biomol Screen 2013 Jan;18(1):39-53.

Spontaneous Contracting Cardiomyocytes Monitoring



Oksana Sirenko et al. J Biomol Screen 2013 Jan;18(1):39-53.

High Content System Overview



Trend of High Content System Application

MOLECULAR DEVICES

12–18 YEARS + $13B = 1 DRUG

90% FAIL

DRUG DISCOVERY — 3-5 years
- Target identification/validation
- Assay development
- Lead generation

PRE-CLINICAL — 1-2 years
- In vitro and in vivo toxicity
- ADMET
- PK/PD

CLINICAL TRIALS — 6-7 years
- Phase I
- Phase II
- Phase III

REGULATORY APPROVAL — 1-2 years

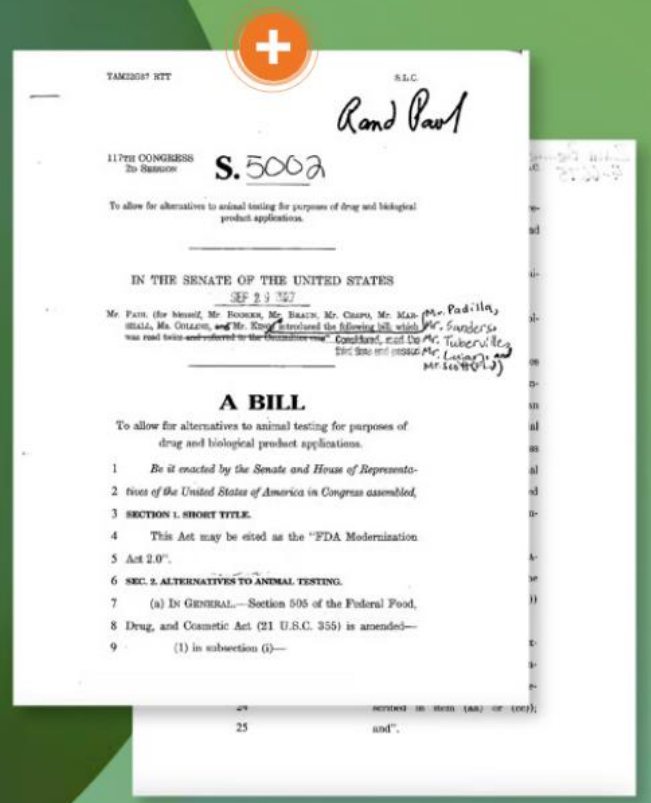
1 DRUG

90 percent of drug candidates **fail** during the first of three phases **in clinical trials**

This alarming failure rate can be traced in part to reliance on 2D cell cultures or animal models that don't closely mimic complex human biology. The results are inaccurate predictions of a drug's potential and extended drug development timelines.

View in fullscreen

FDA Modernization Act 2.0



TAM22037 NTT S.L.C.

Rand Paul

117TH CONGRESS 2D SESSION **S. 5002**

To allow for alternatives to animal testing for purposes of drug and biological product applications.

IN THE SENATE OF THE UNITED STATES

SEP 29 2022

Mr. PAUL (for himself, Mr. BOOKER, Mr. BRAUN, Mr. CRAPO, Mr. MARSHALL, Ms. COLLINS, and Mr. KING) introduced the following bill; which was read twice and referred to the Committee on [illegible]

Mr. Padilla, Mr. Sanders, Mr. Tuberville, Mr. Lujan, and Mr. Scott of Florida

A BILL

To allow for alternatives to animal testing for purposes of drug and biological product applications.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SECTION 1. SHORT TITLE.

This Act may be cited as the "FDA Modernization Act 2.0".

SEC. 2. ALTERNATIVES TO ANIMAL TESTING.

(a) IN GENERAL.—Section 505 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355) is amended—

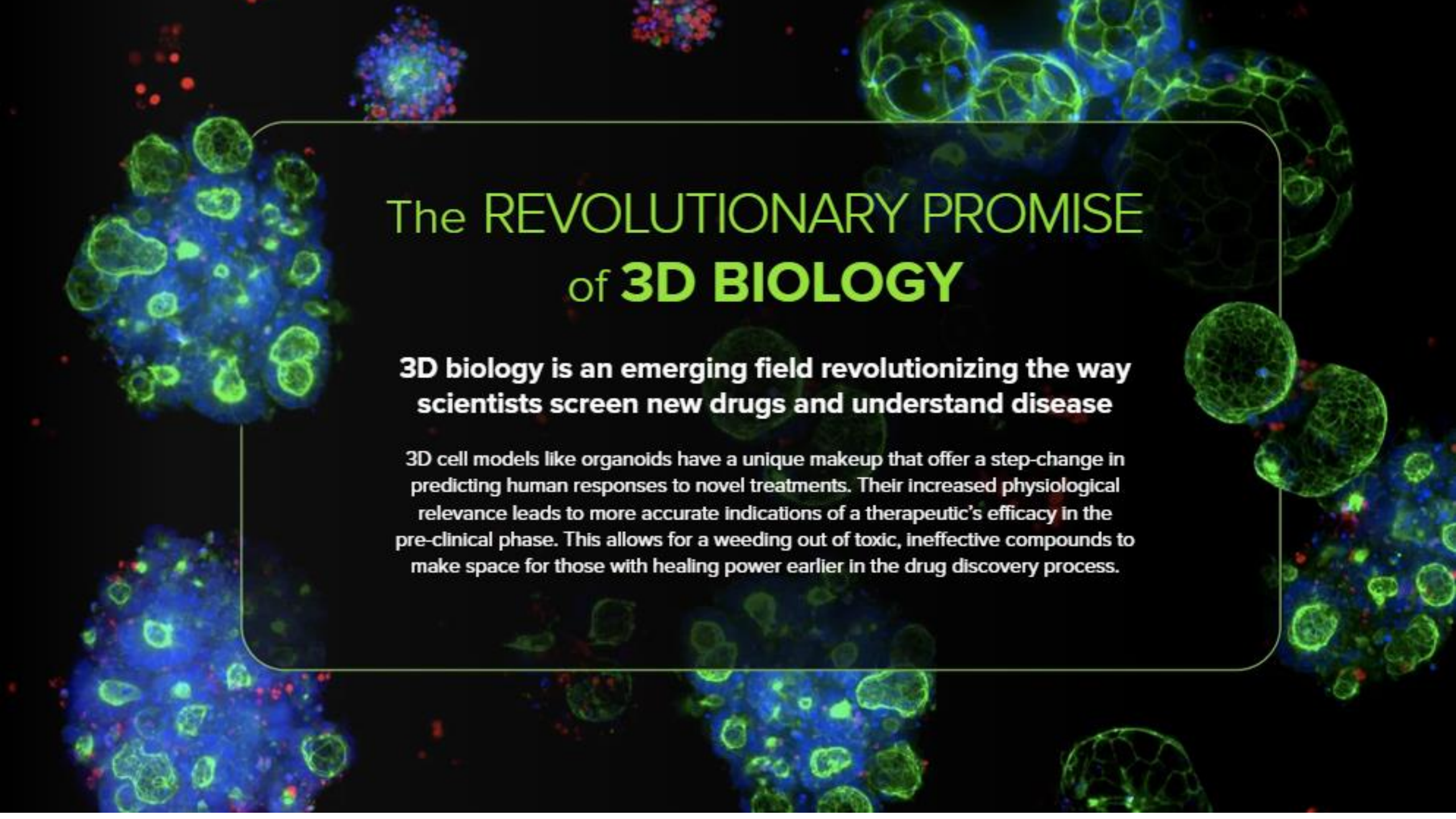
(1) in subsection (i)—

and".

Bipartisan bill signed into law allowing alternatives to animal testing for drug and biological product applications.

This historic move toward animal-free testing refutes the Federal Food, Drug and Cosmetics Act (FFDCA) of 1938 mandating that all new drugs be tested in animals to protect patients from unknown toxicity.

The U.S. Food and Drug Administration (FDA) can now consider alternatives fordrug testing methods, like those rooted in 3D biology which offer higher predictive power and could limit the need for up to 156 million animals used in clinical testing today.

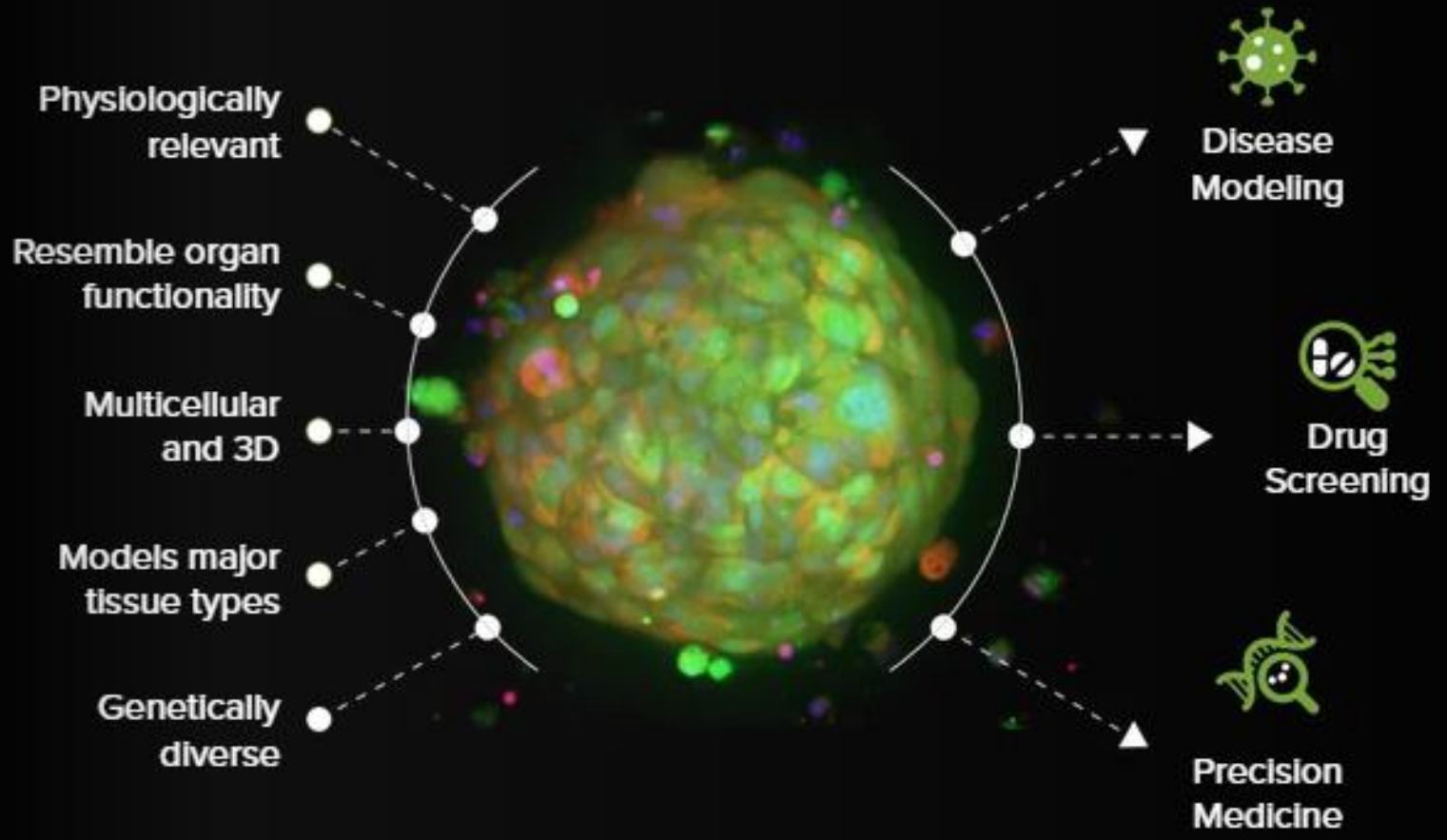


The REVOLUTIONARY PROMISE of **3D BIOLOGY**

3D biology is an emerging field revolutionizing the way scientists screen new drugs and understand disease

3D cell models like organoids have a unique makeup that offer a step-change in predicting human responses to novel treatments. Their increased physiological relevance leads to more accurate indications of a therapeutic's efficacy in the pre-clinical phase. This allows for a weeding out of toxic, ineffective compounds to make space for those with healing power earlier in the drug discovery process.

Organoids in action



Case studies and expert insight

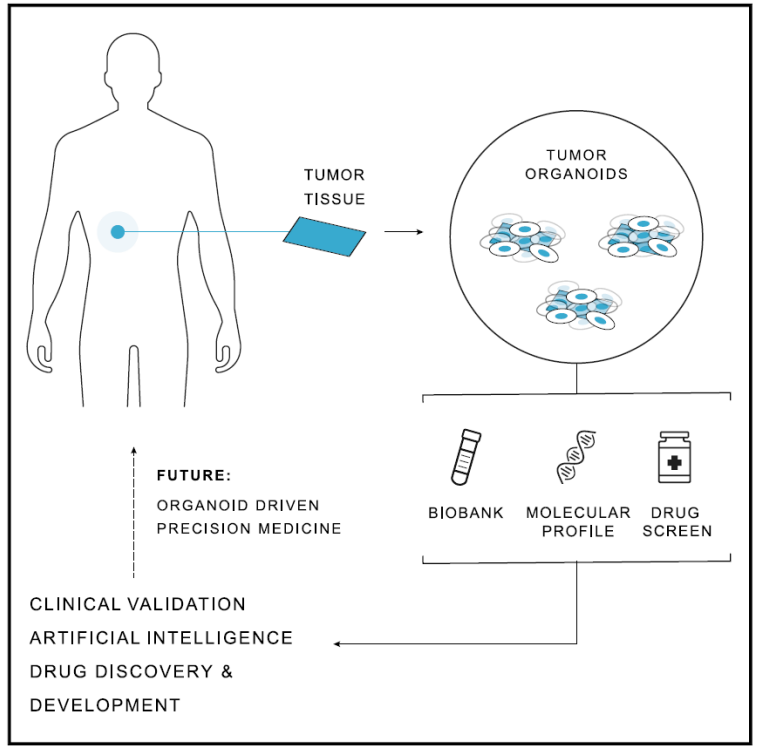
Because they so closely resemble their internal organ counterparts, organoids have proven to be an accurate model for studying human disease, screening drugs, and testing potential therapeutics.

They're also an especially useful tool for precision medicine. For example, patient derived organoids can be generated from individuals with specific ailments and used to better understand disease, develop drugs, and customize personalized therapies.

Pan-Cancer Organoid Platform for Precision Medicine

Cell Reports

A pan-cancer organoid platform for precision medicine

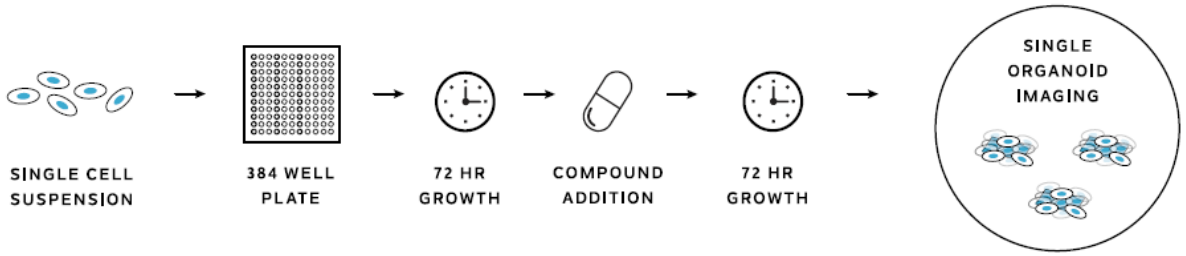


In brief

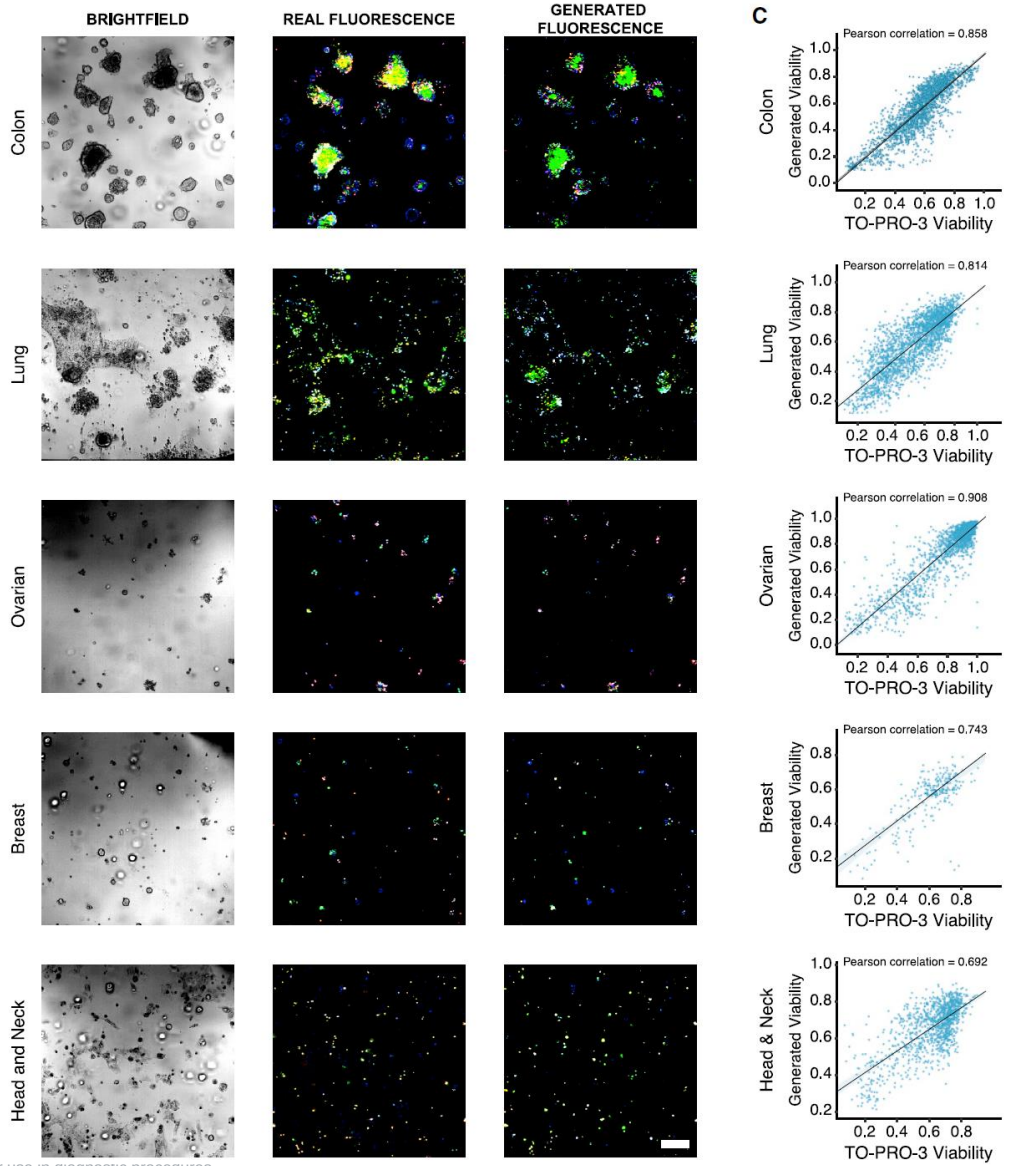
Patient-derived tumor organoids offer promise as tools for precision medicine but require scalable and reproducible methods for wide use. Larsen et al. optimize a pan-cancer platform with tumor organoids from over 1,000 patients. Standards are established for culture conditions, molecular characterization, and therapeutic profiling using deep learning coupled with light microscopy.

Cell Rep. 2021 Jul 27;36(4):109429.

Pan-Cancer Organoid Platform for Precision Medicine



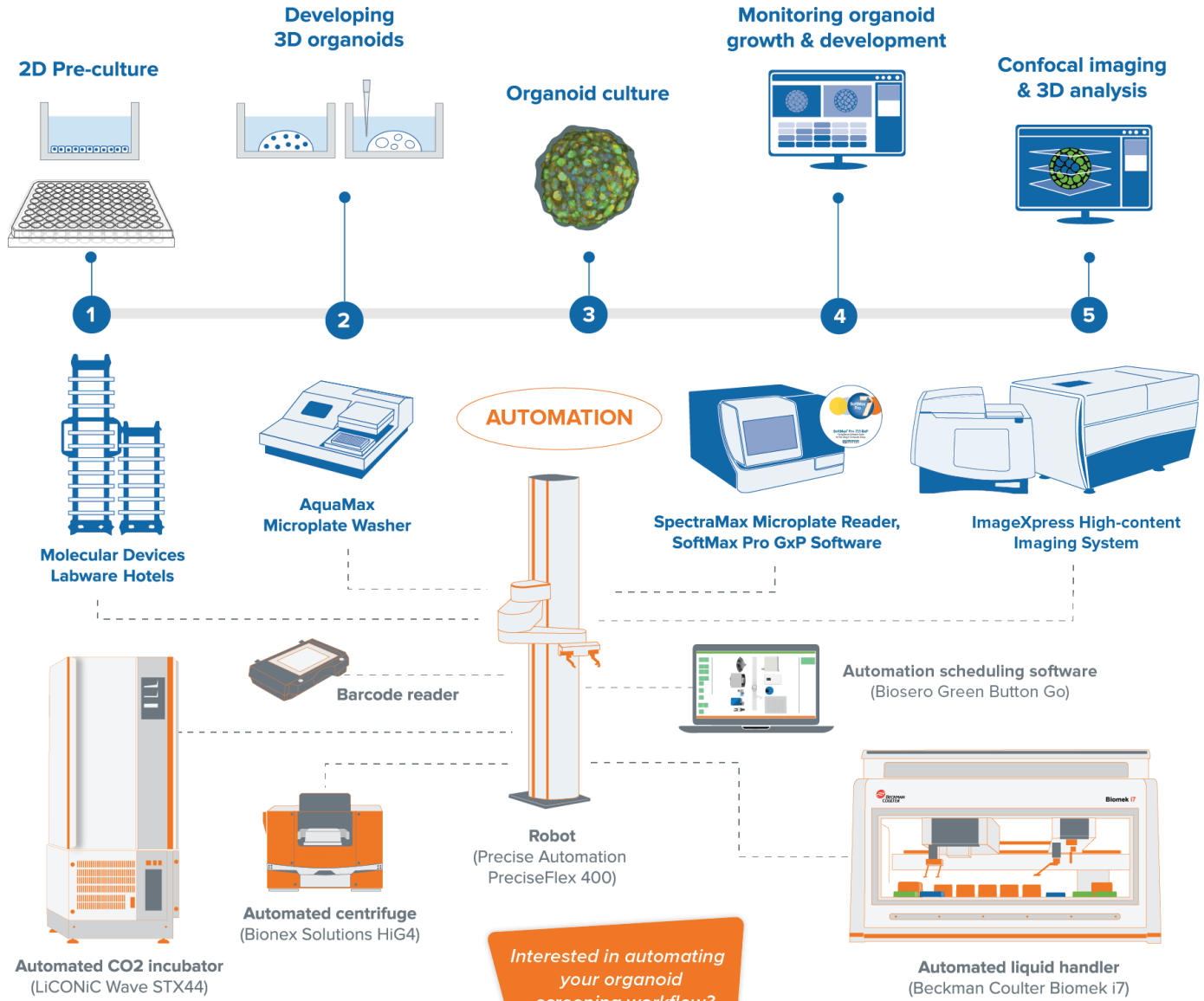
Prior to imaging, TOs were incubated with 4 μM Hoechst 33342 (Fisher Scientific) and 300 nM TO-PRO-3 Iodide (642/661) (Invitrogen) for 1.5-2 hours. Assay plates were imaged using an ImageXpress Micro Confocal (Molecular Devices) at 10x magnification so that ~100-200 TOs were imaged per well. Images were acquired as 4 × 15 μm Z stacks and the 2D projections were analyzed to assess cell viability. Confocal images were analyzed using the MetaXpress software (Molecular Devices) custom module editor feature to design an analysis module that identified TOs by clusters of Hoechst 33342 staining, individual cells by Hoechst 33342 staining, and dead/dying cells by either TO-PRO-3 or Caspase-3/7 staining. The result of this analysis module is a spreadsheet detailing the number of live and dead cells for every individual organoid.



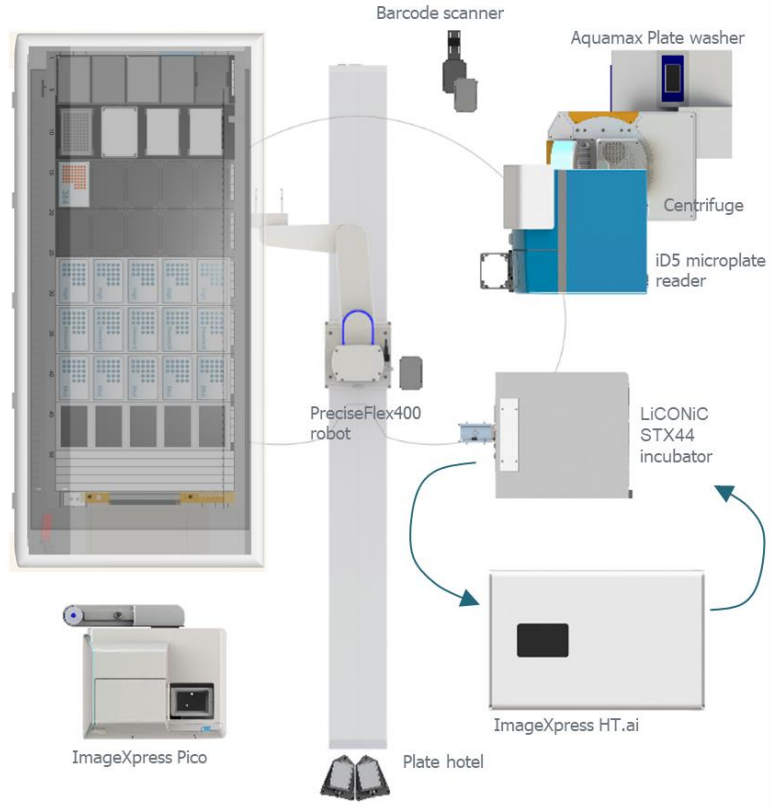
Cell Rep. 2021 Jul 27;36(4):109429.

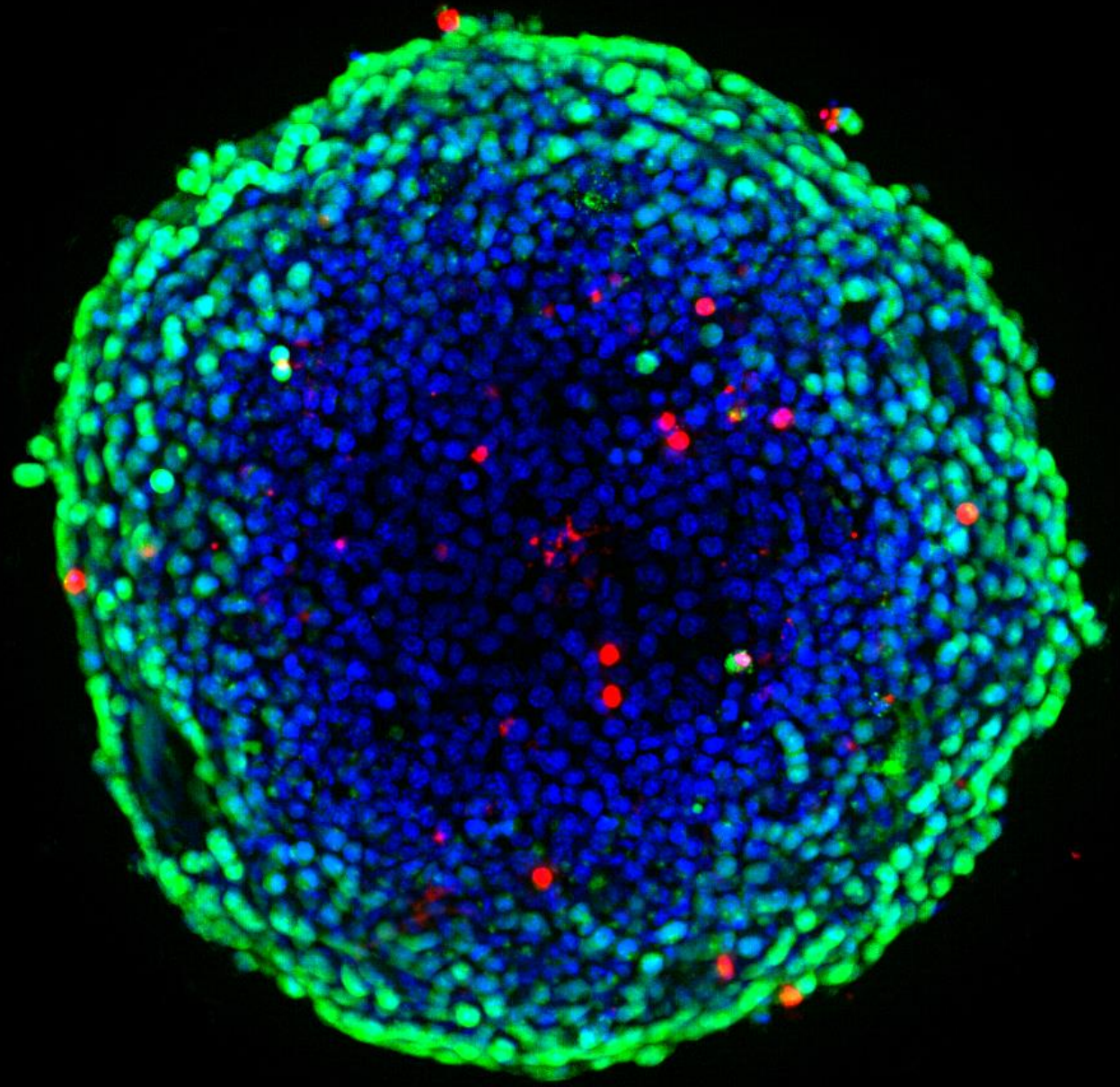
MOLECULAR DEVICES

Fully Automation HCS Workstation



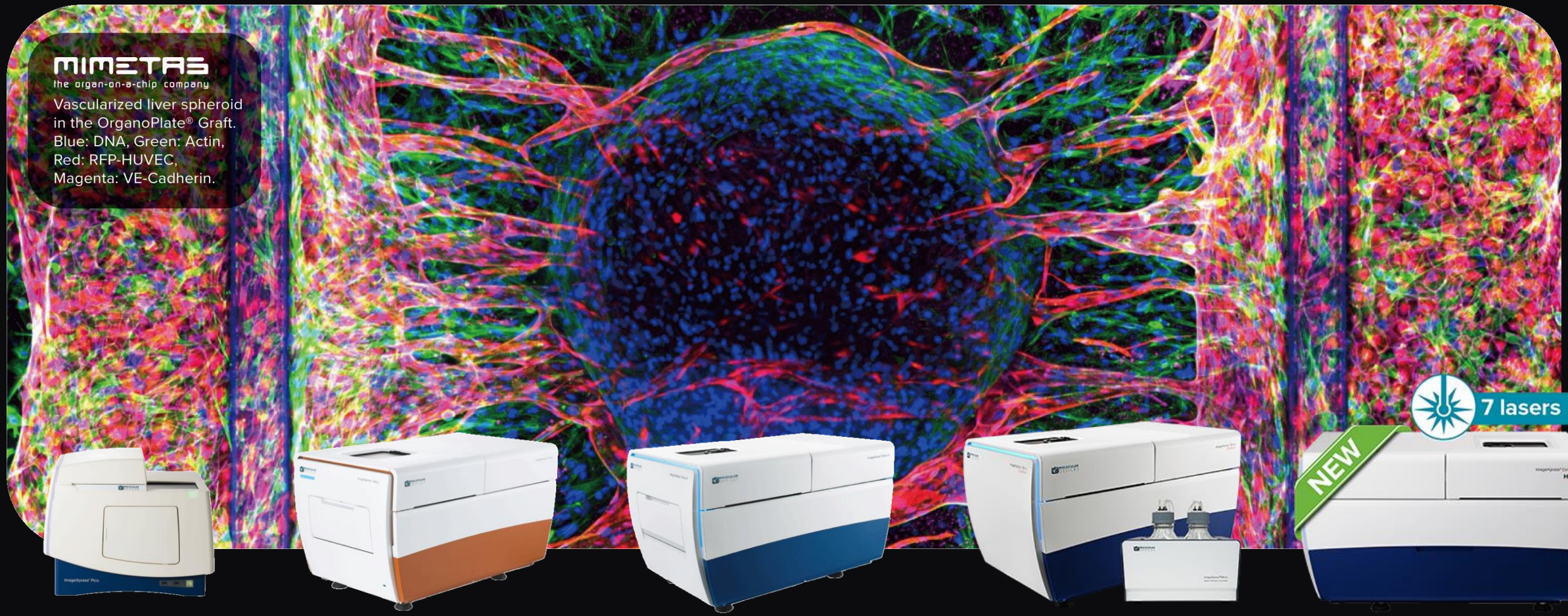
Automated Cell Culture, Dispensing, and Reading





Capture Deeper Inside

High Content Imaging System



ImageXpress® Pico
AutomatedCell Imaging System

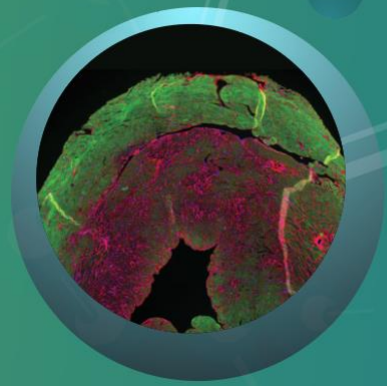
ImageXpress Nano
AutomatedCell Imaging System

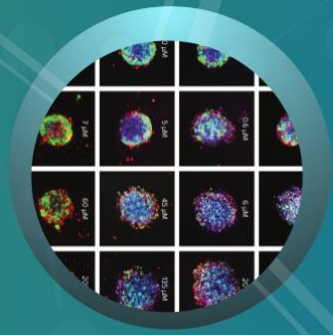
ImageXpress Micro 4
High-Content Imaging System

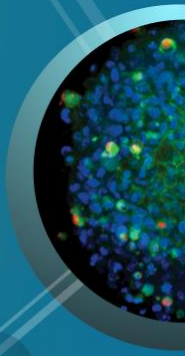
ImageXpress Micro Confocal
High-Content Imaging System

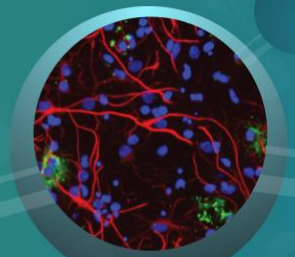
ImageXpress Confocal HT.ai
High-Content Imaging System

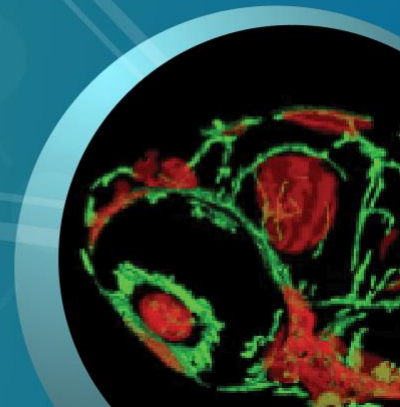
Q & A



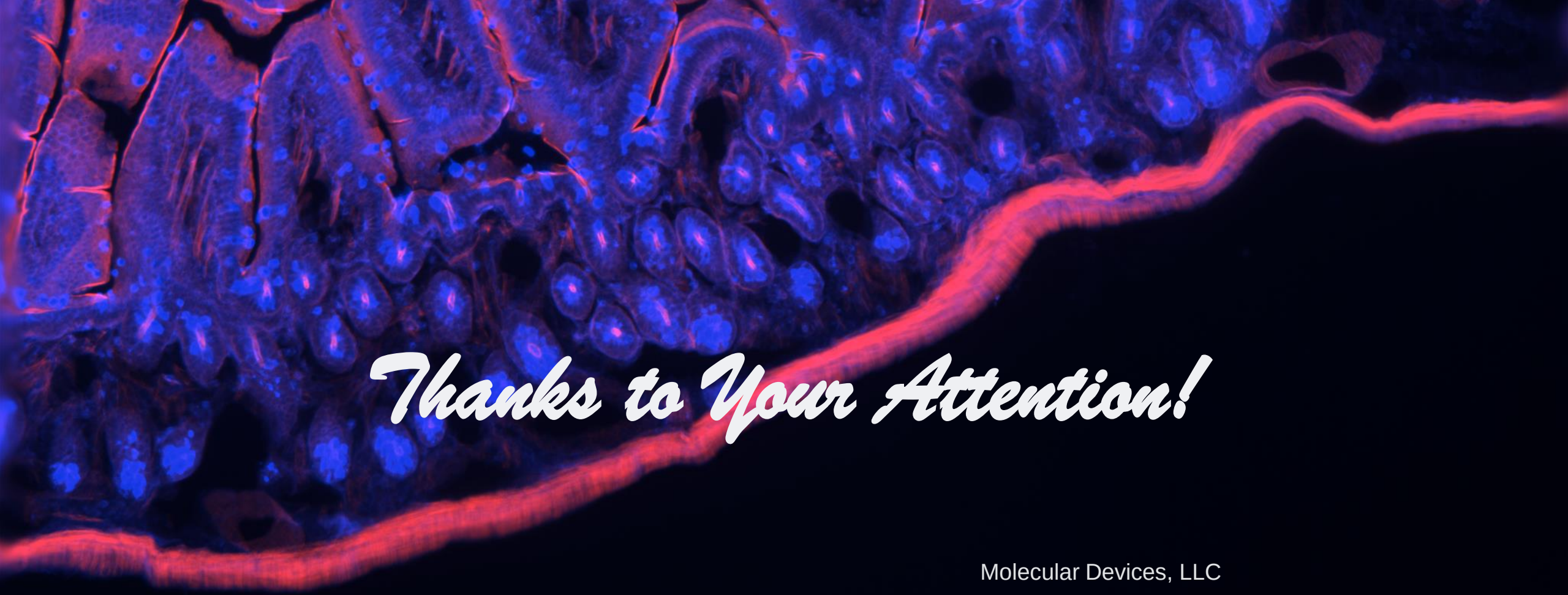








MOLECULAR DEVICES



Thanks to Your Attention!

Tim Tsui

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