



# 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

崔瑞廷 Tim Tsui

應用專家 Field Application Scientist

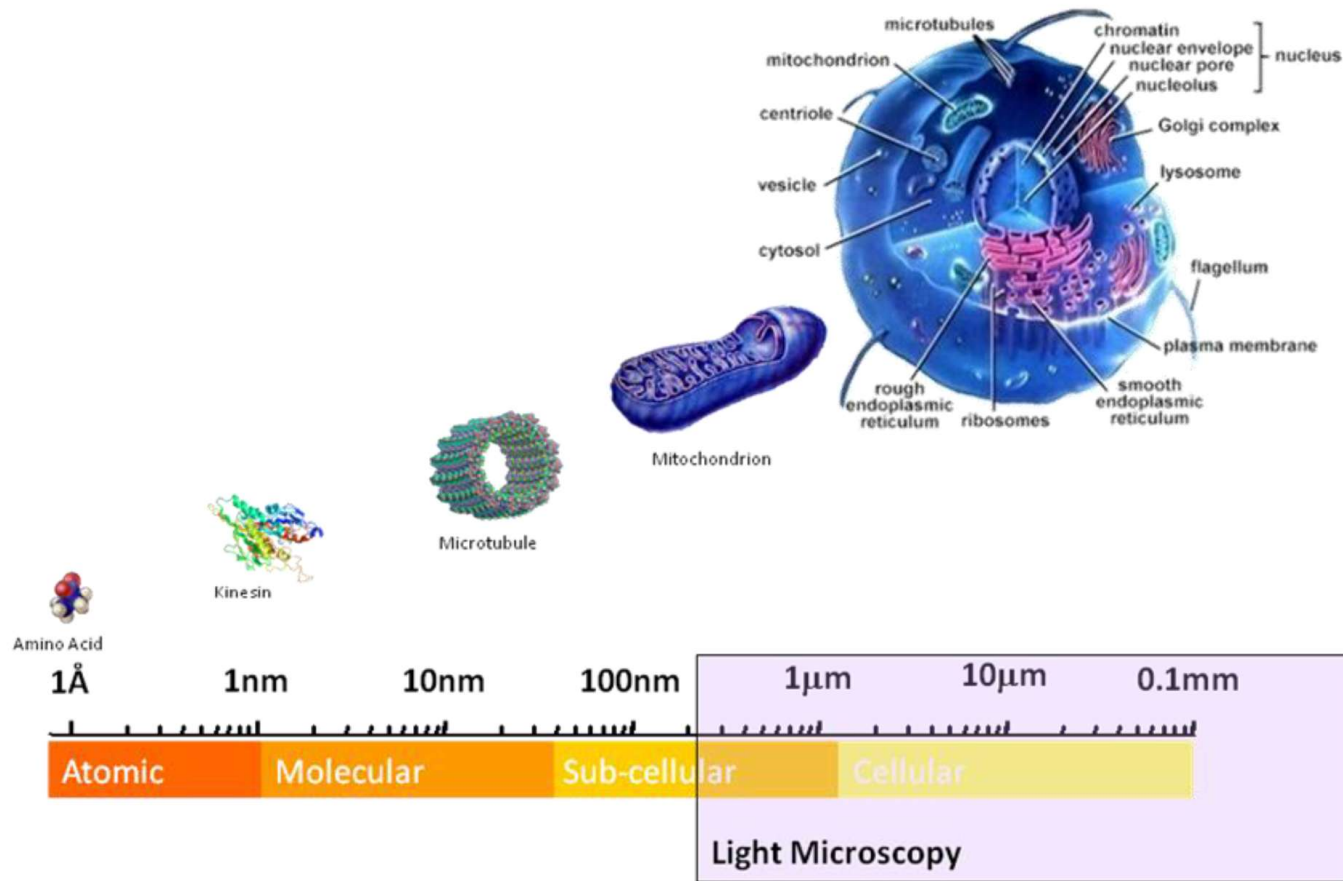
Molecular Devices (Hong Kong) Ltd.



# Outline

- Principle of High Content System
- Introduction of ImageXpress Micro Confocal System
  - Hardware features
  - Software features
- Trend of High Content System
- Case Study: 3D collagen high-throughput screen identifies drugs that induce epithelial polarity and enhance chemotherapy response in colorectal cancer

# Light Microscope for Micro-world



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

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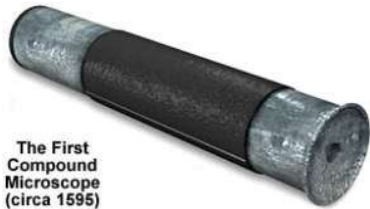
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# History of Microscope (before 20th)

## Improve Image Resolution

Zacharias Janssen



The First Compound Microscope (circa 1595)

Anthony Von Leeuwenhoek



Leeuwenhoek Microscope (circa late 1600s)

Time

late 16th

mid 17th

late 17th

late 19th

Magnification

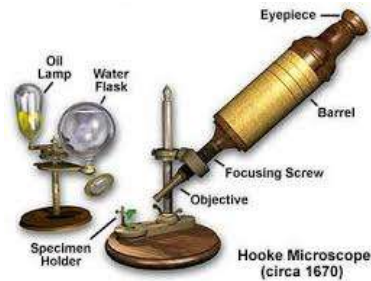
10X

100X

300X

1000X

Robert Hooke



Hooke Microscope (circa 1670)

Zeiss



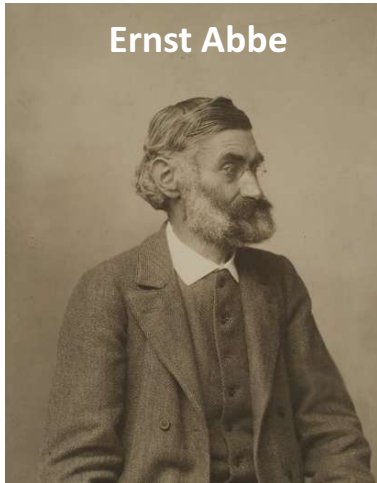
Pictures from :

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



Ernst Abbe

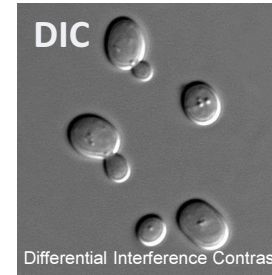
**Diffraction Limit**

$$d = \frac{\lambda}{2NA} \sim 200nm$$

(In late 19<sup>th</sup>)

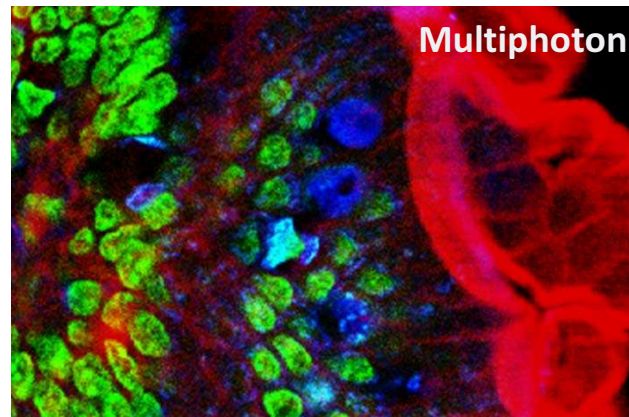


Phase Contrast

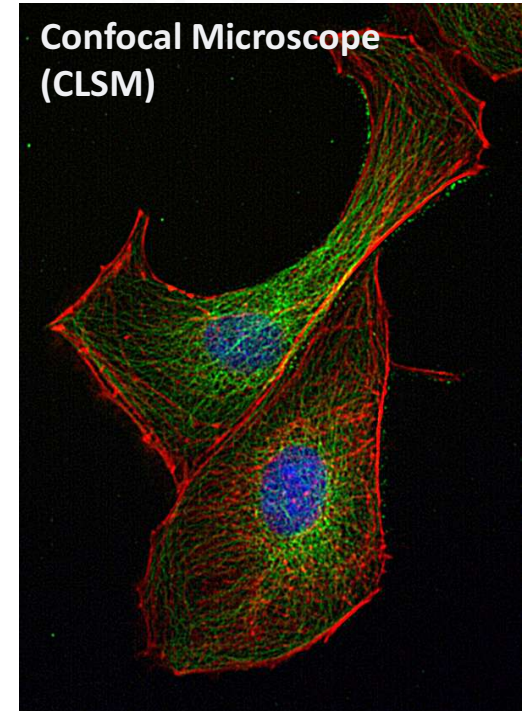


DIC

Differential Interference Contrast



Multiphoton



Confocal Microscope (CLSM)

**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.

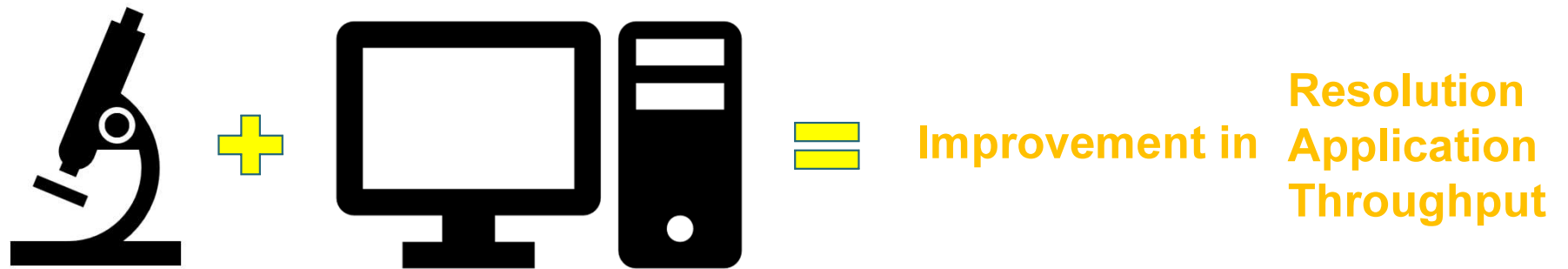
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# Progress of Microscope in 21st Century



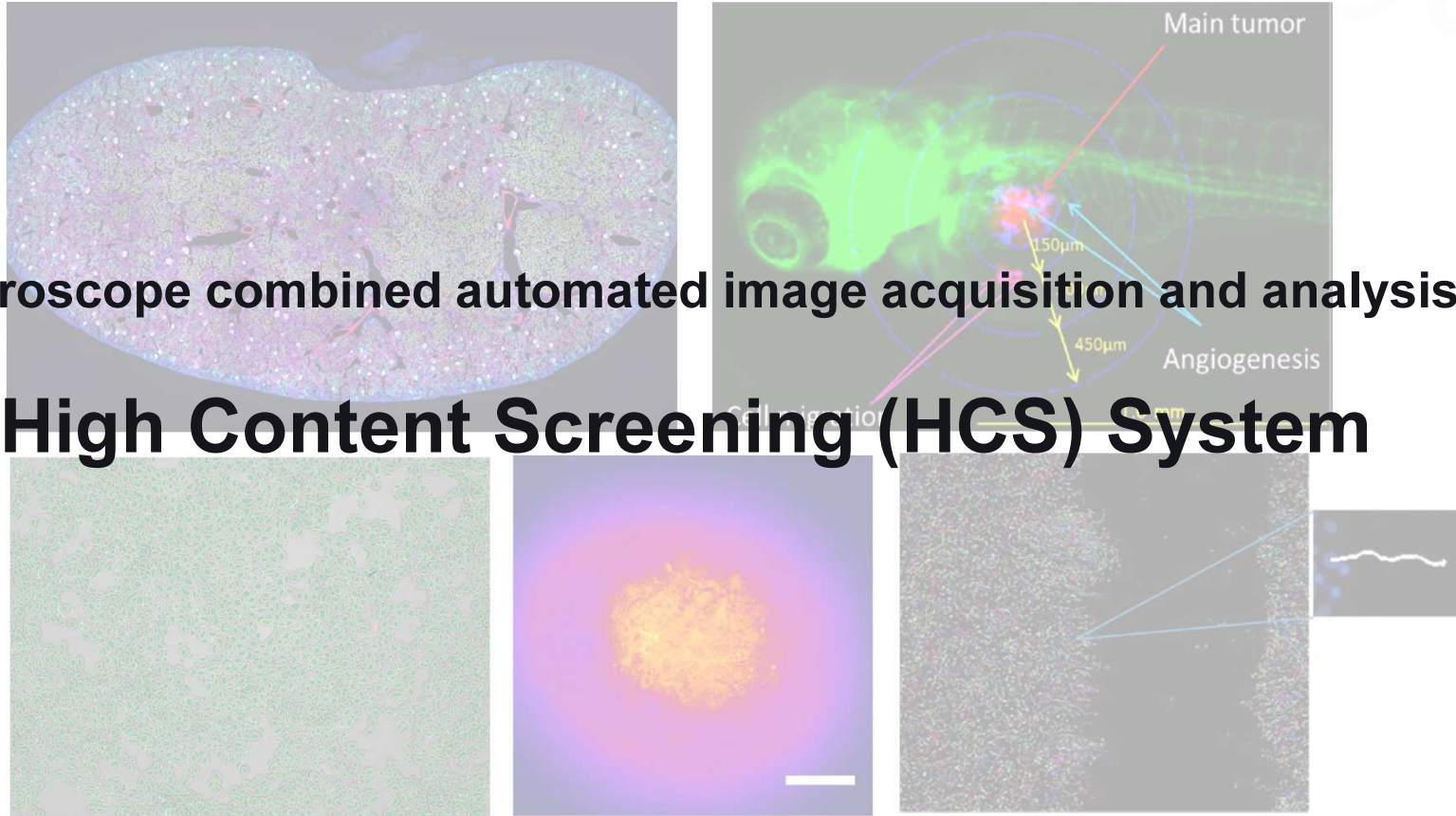
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# Progress of Microscope in 21st Century

Microscope combined automated image acquisition and analysis

## High Content Screening (HCS) System

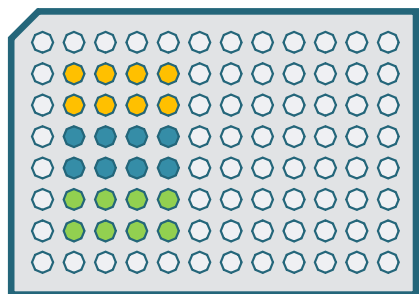


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# Live Cell Analysis for program death

Plating



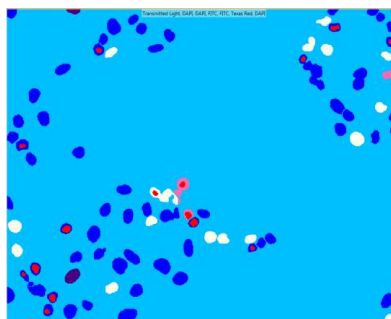
HeLa cell  
Hoechst (Nuclei )  
NucView (Apoptotic Cells)  
PI (Necrotic Cells)

Acquisition

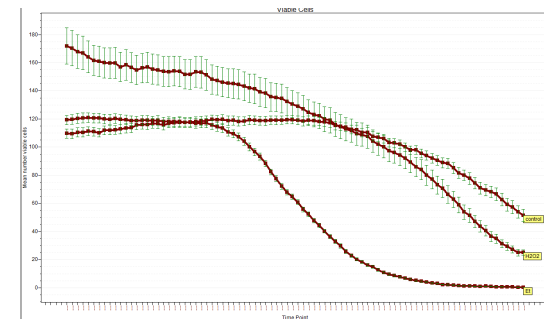


5% CO<sub>2</sub>, 37°C, 48hr

Analyze



Statistics

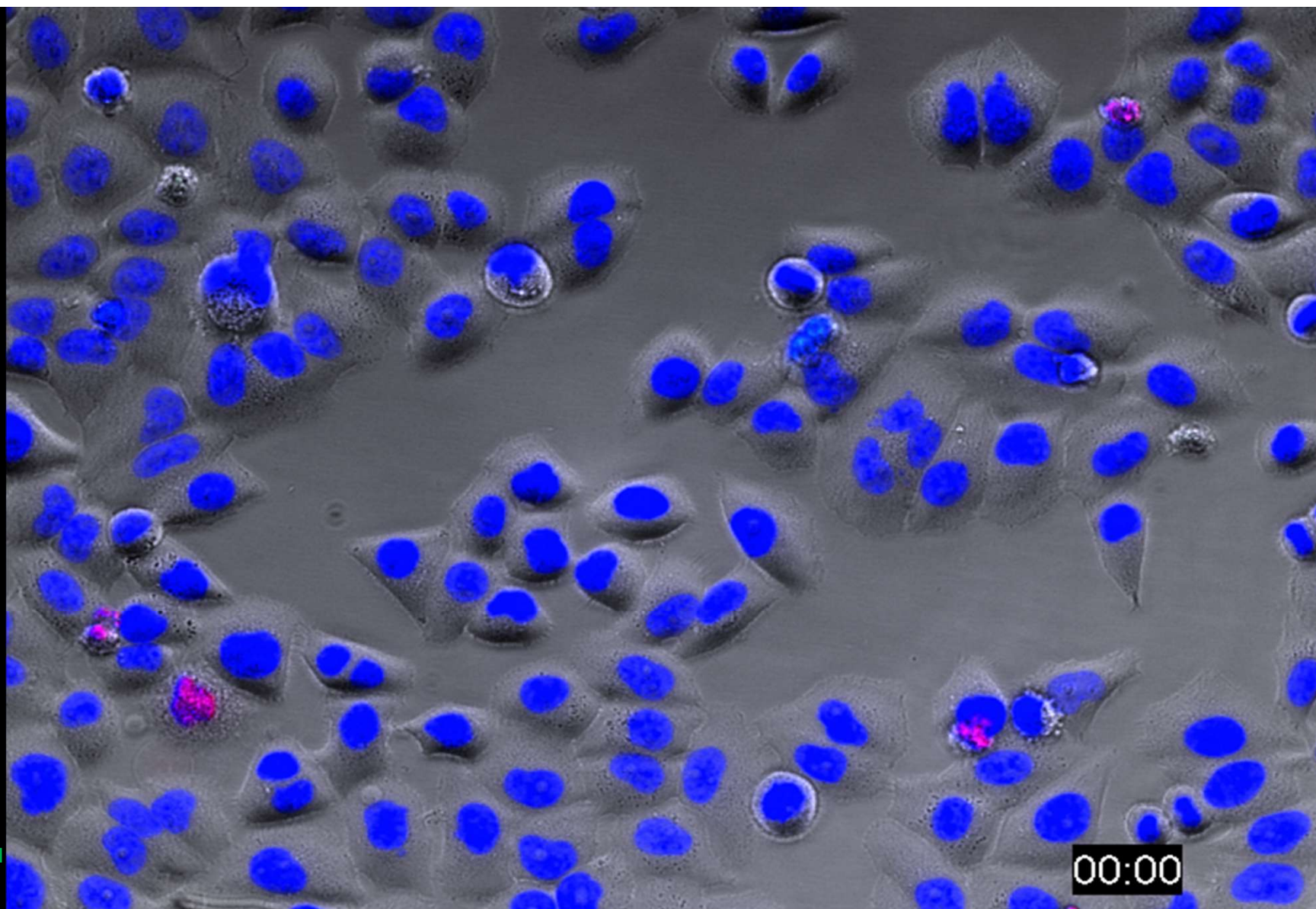


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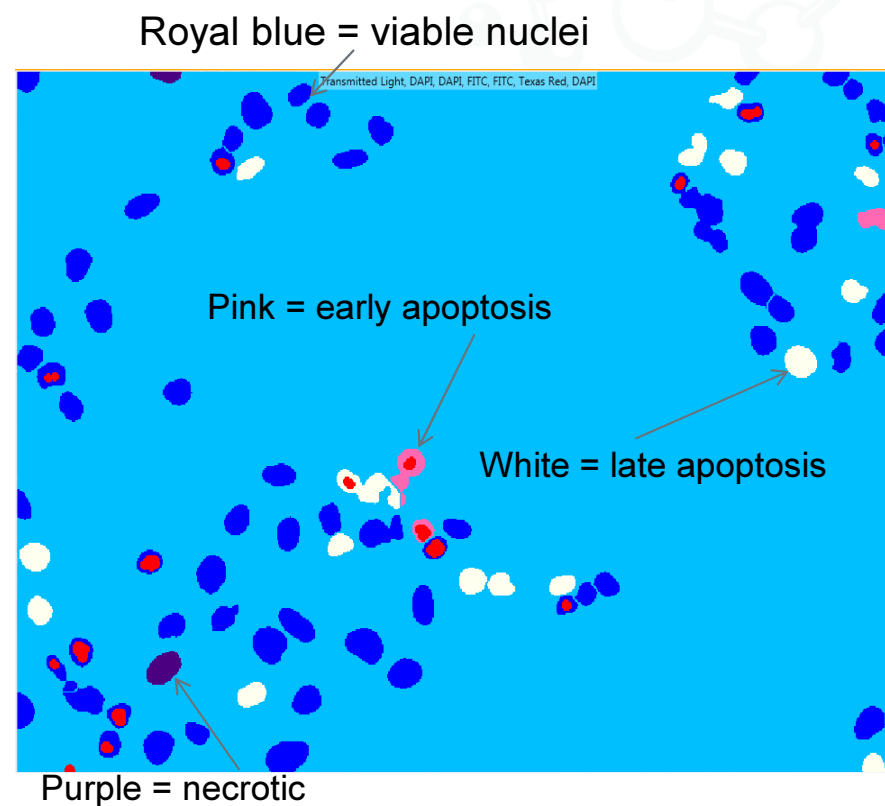
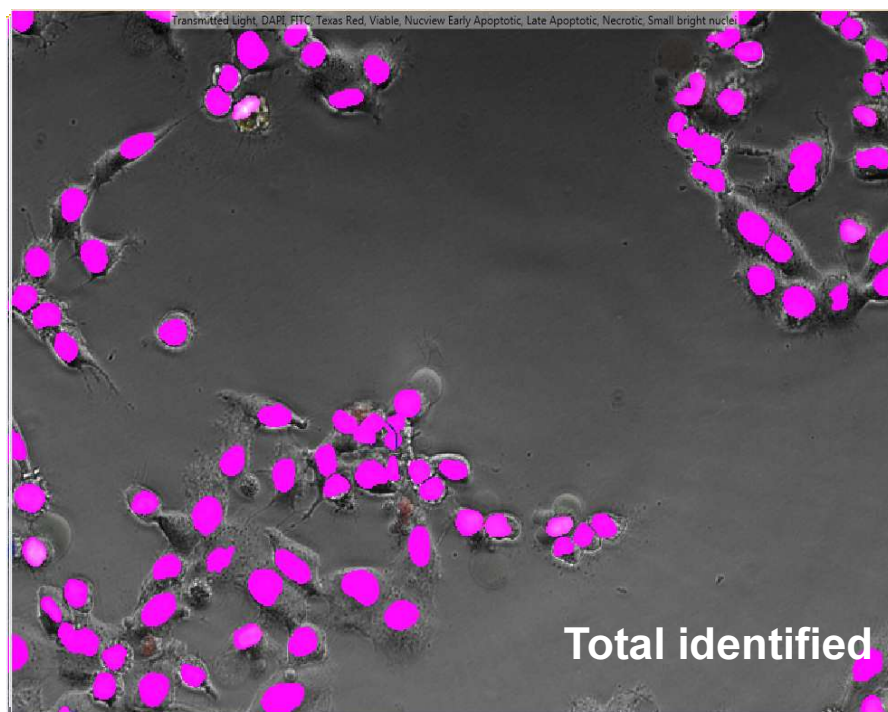


Nuclei  
Apoptotic Cell  
Necrotic Cell

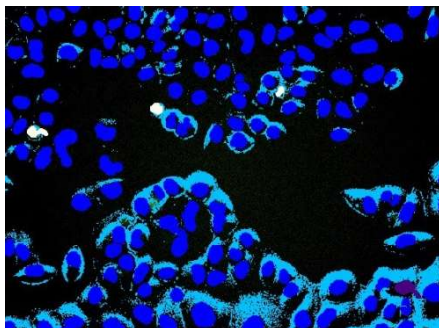


00:00

# Multi-parametric Image Analysis

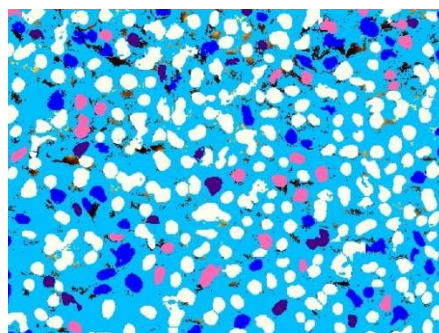
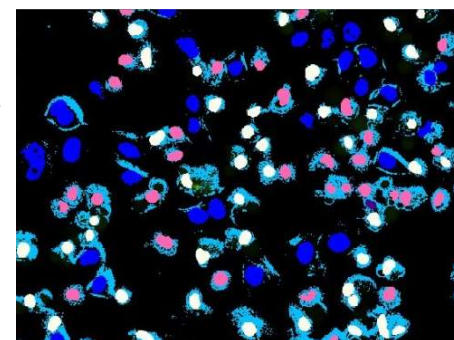


# Multi-parametric Image Analysis



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

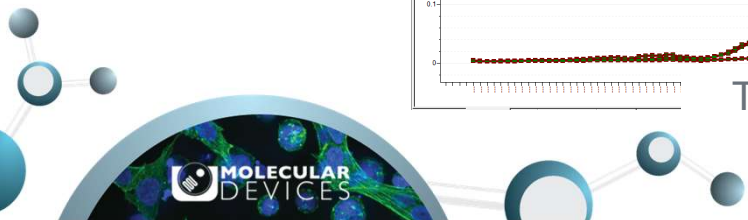
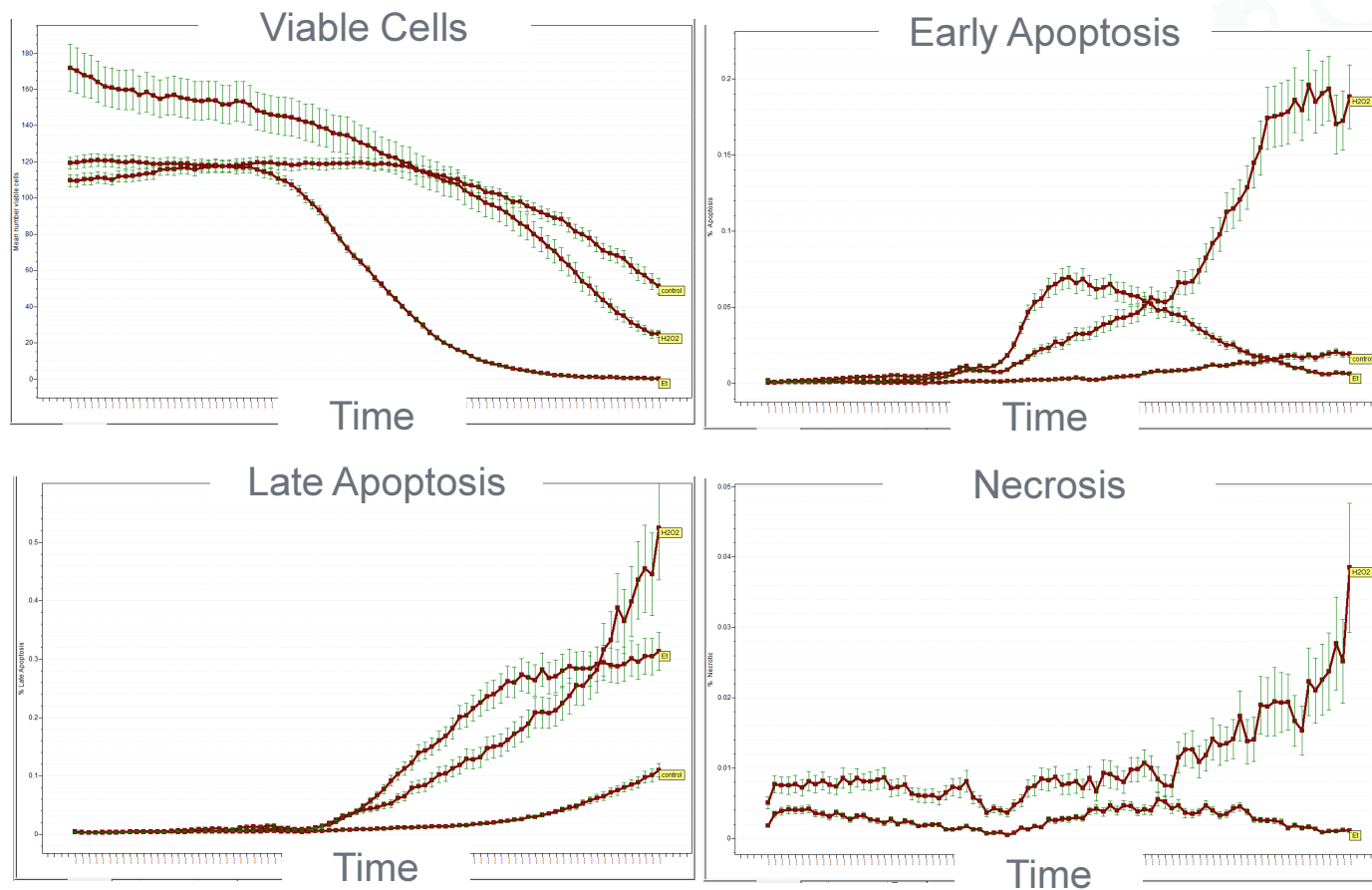
At time point 50, Etoposide treated cells show many cells in early apoptosis (pink mask) and late apoptosis (white mask)



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



# Multi-parametric Image Analysis



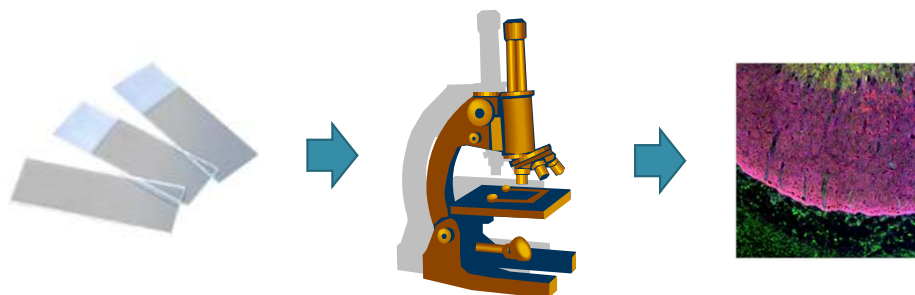
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# What's MD ImageXpress Imaging System?

## Conventional Microscopy

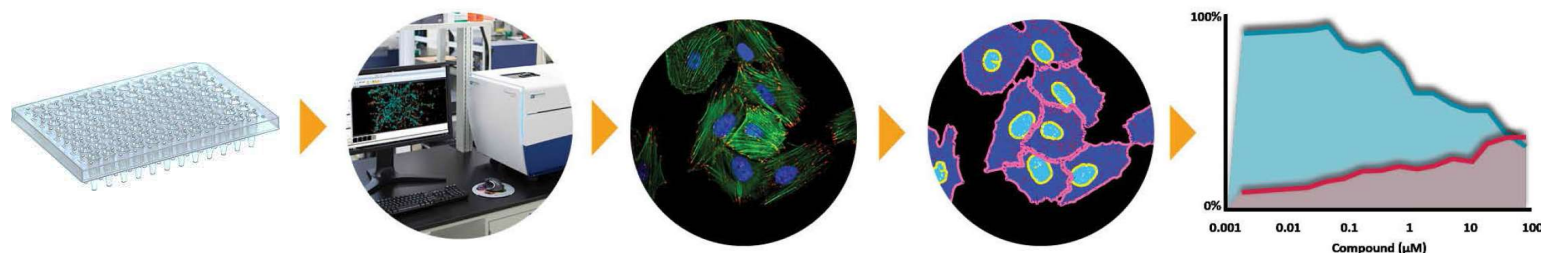
Limited throughput, qualitative results



- Weeks, months
- More subjective
- More labor work

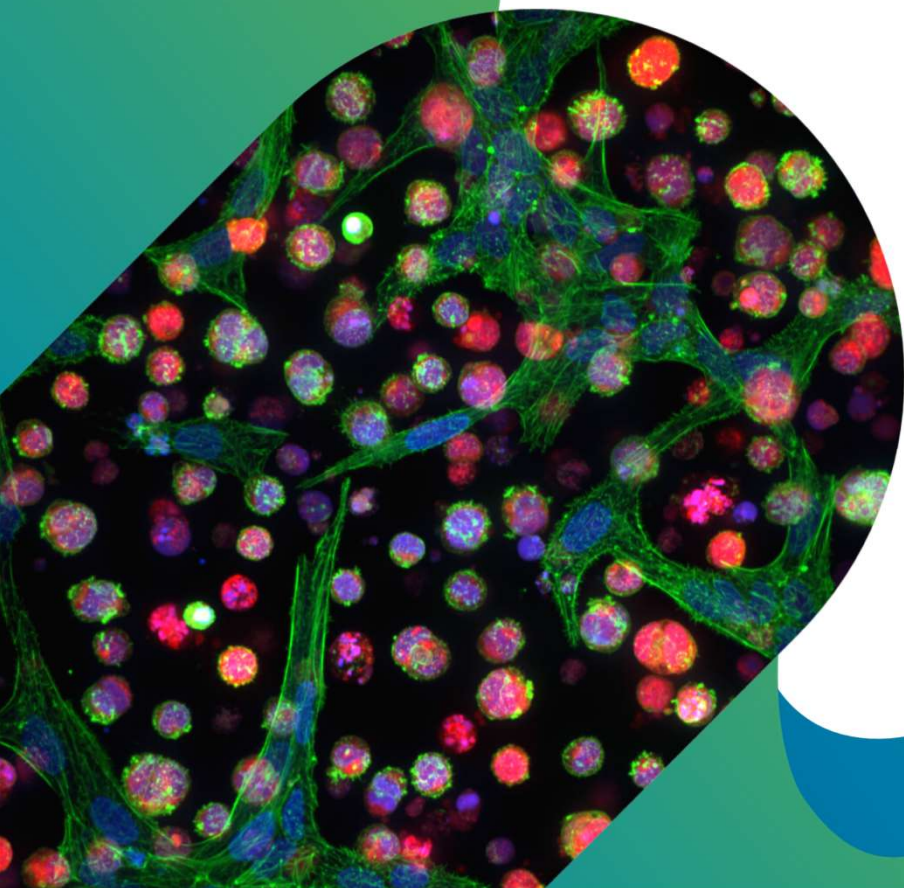
## ImageXpress Automated Imaging System

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

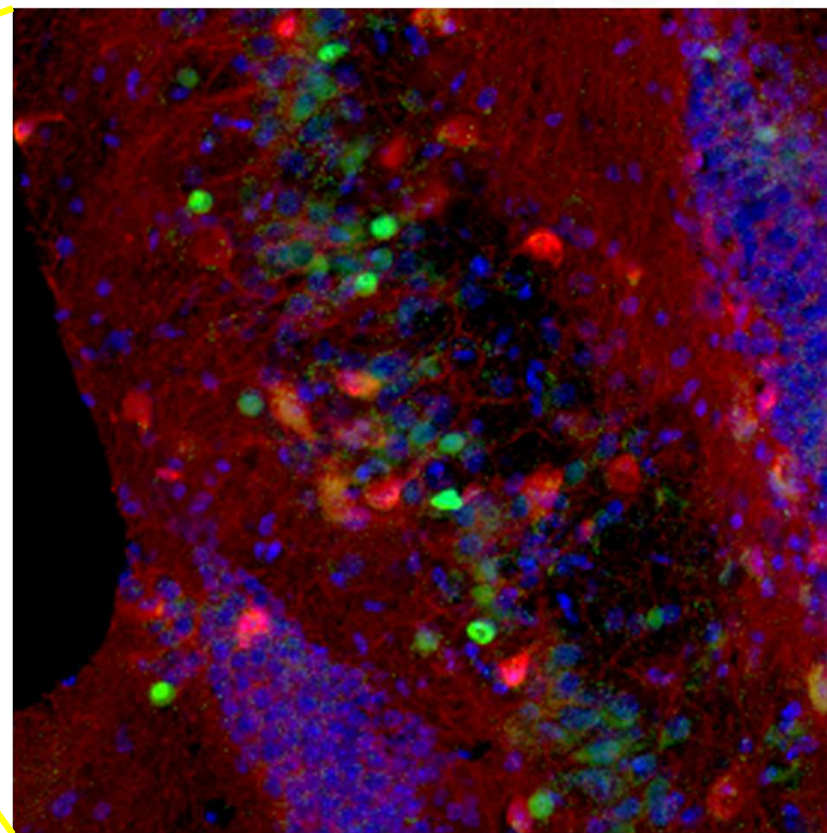
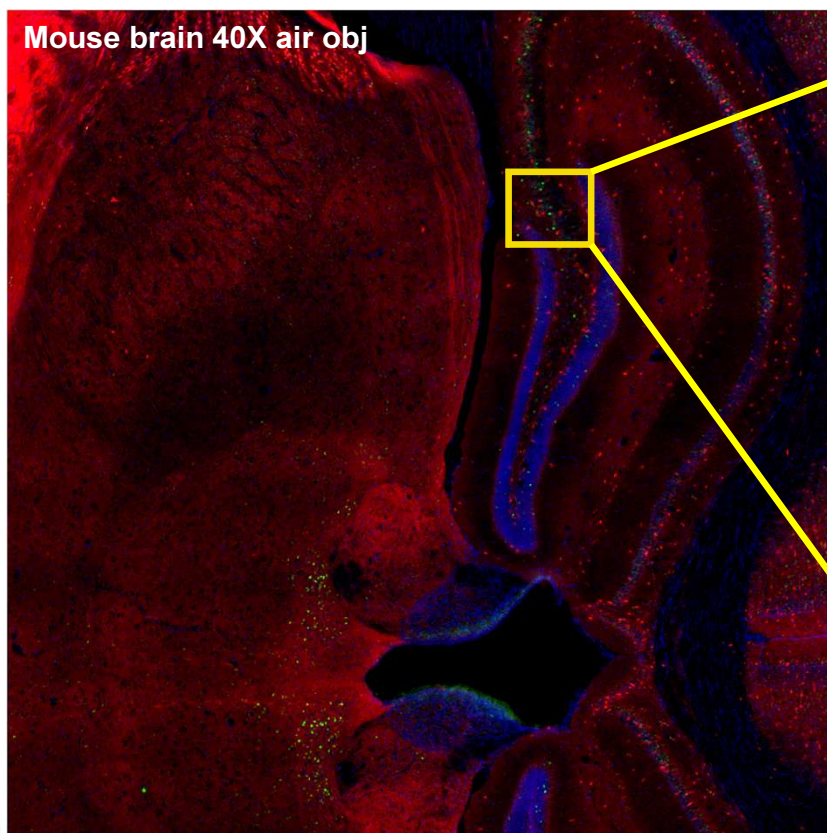
Slide/ chamber slide  
Multi-well plate  
Dish\*



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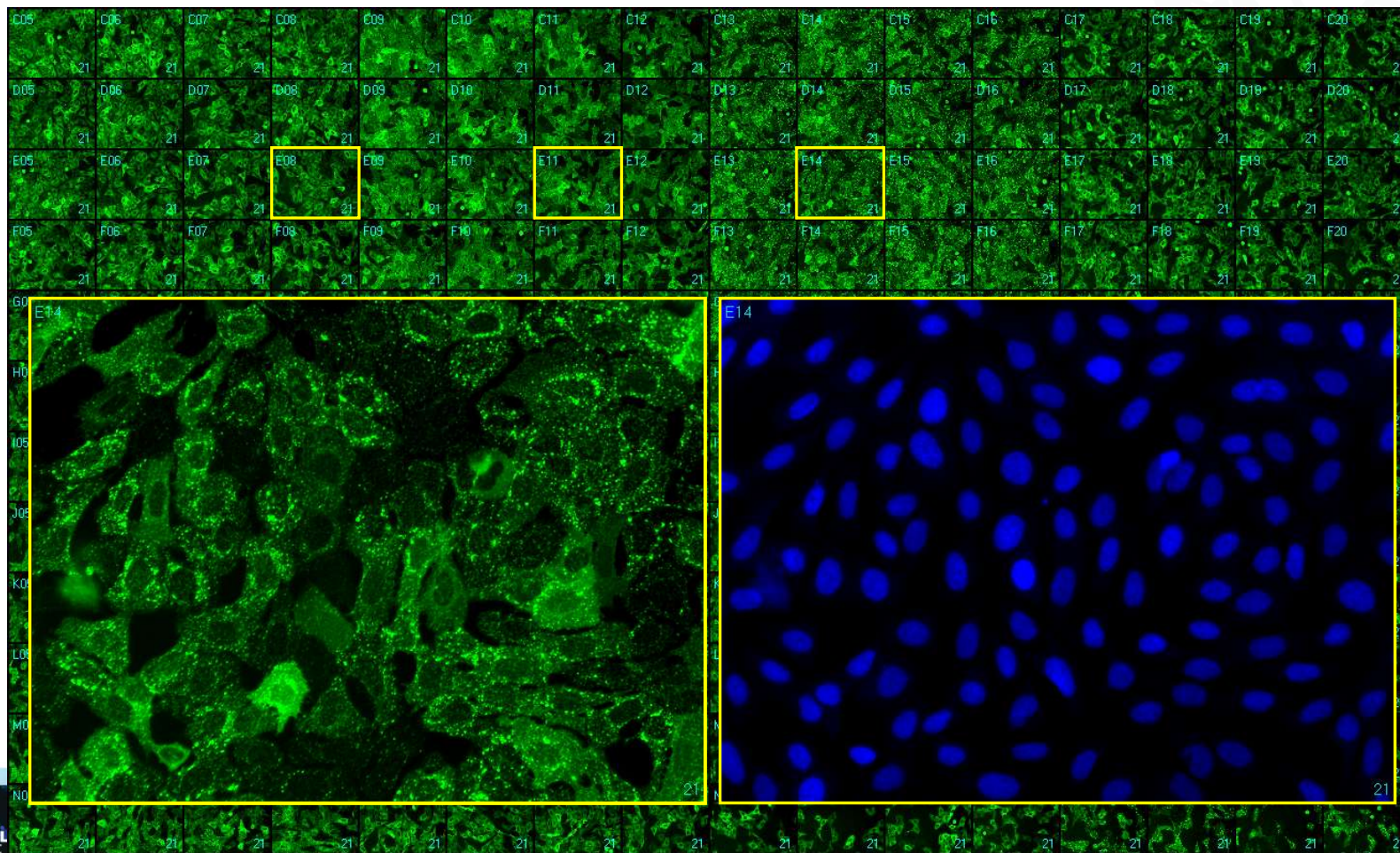
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# Slide Screening





# Multi-well plate acquisition

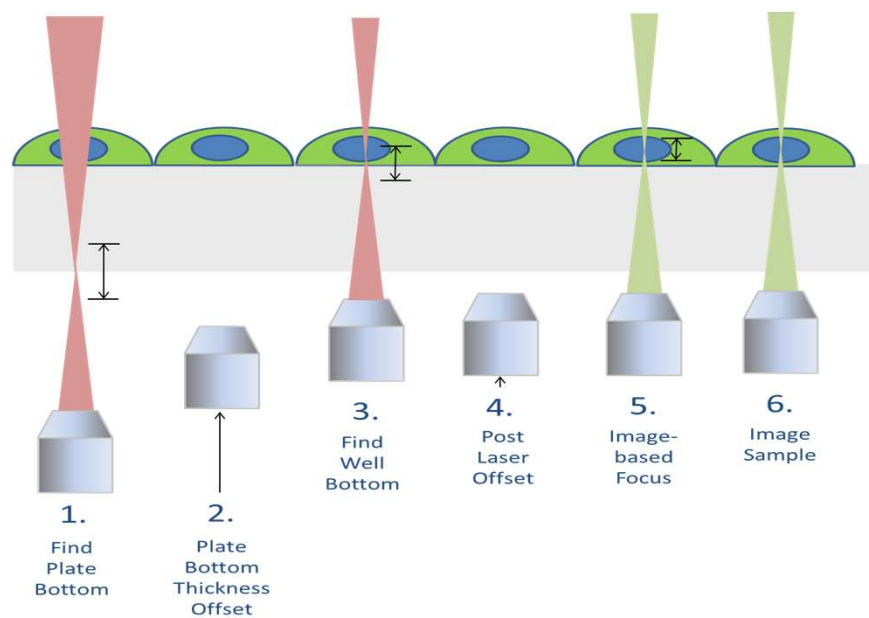


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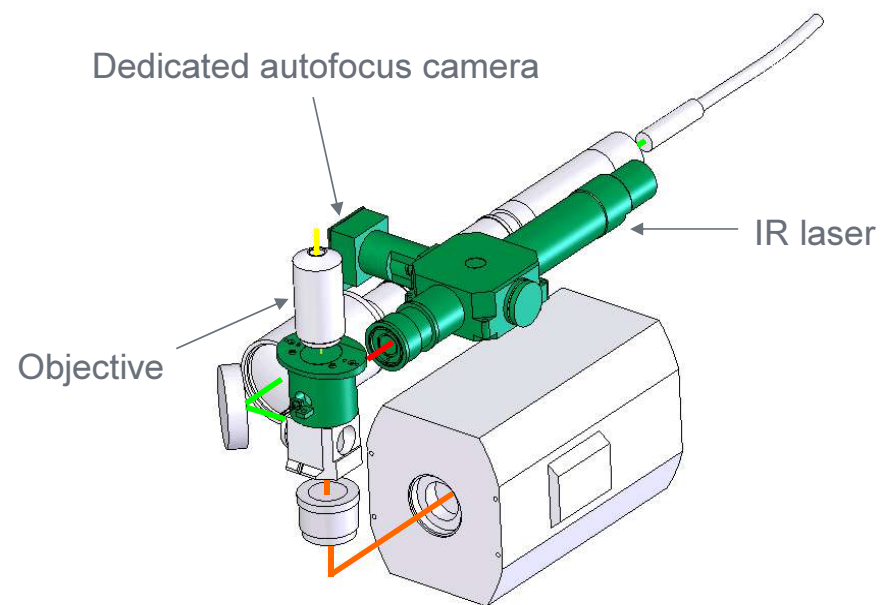
# Autofocus System

## Laser and image hybrid autofocus system



**Laser Autofocus**

**Image Autofocus**



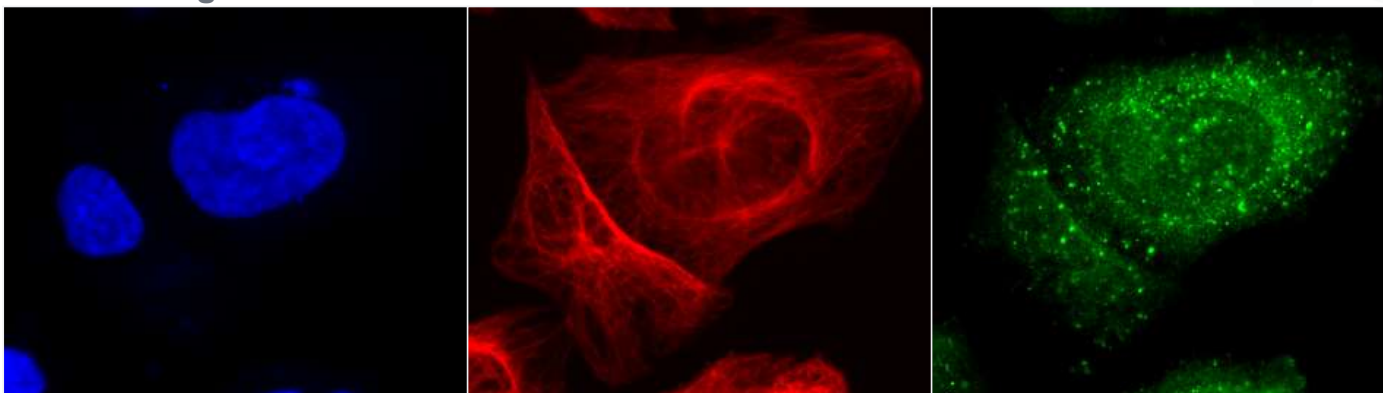
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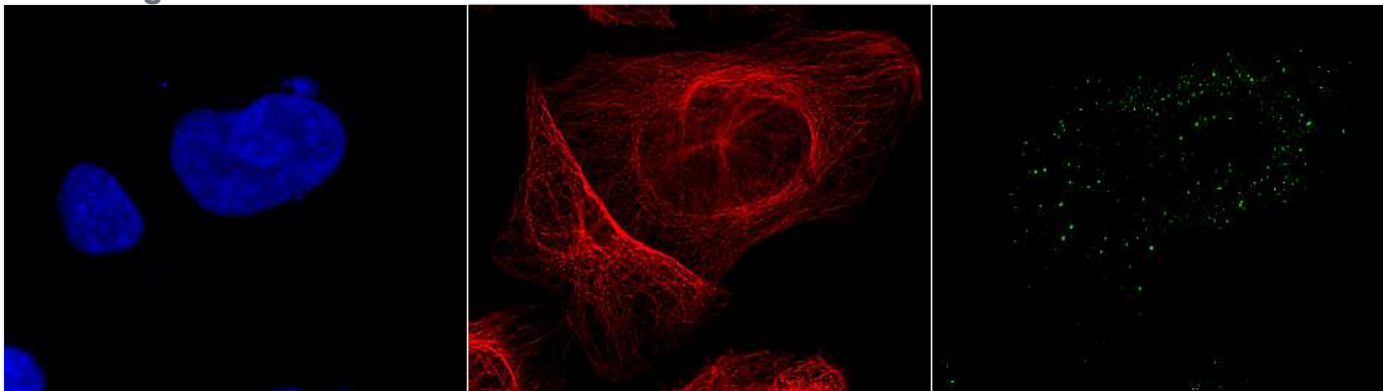


# Digital Confocal Module

Without Digital Confocal



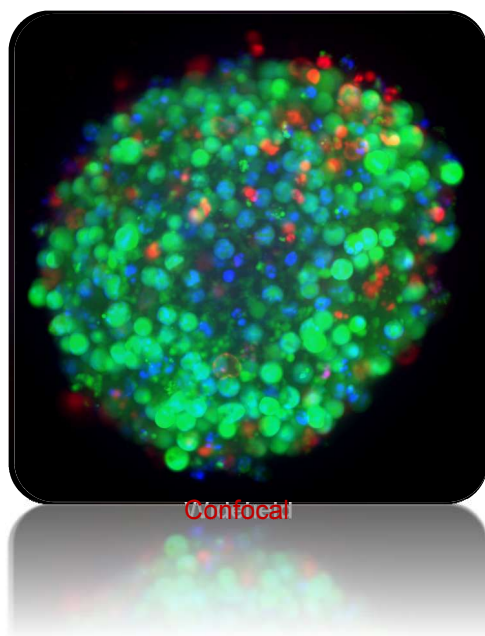
With Digital Confocal



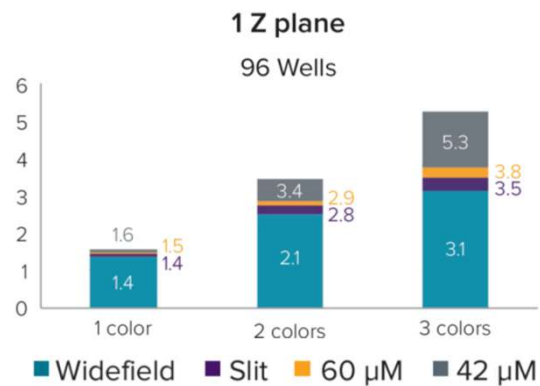
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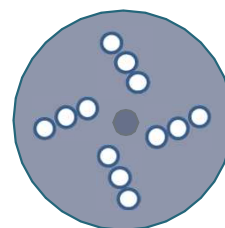
# Spinning Disk Confocal



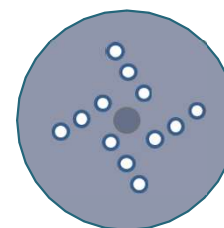
- Multi-type configuration
- Compatible with live cell assay
- Widefield switchable
- High-resolution and high-throughput



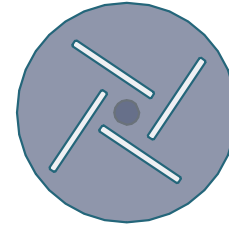
60 μm pinhole



42 μm pinhole



50 μm slit



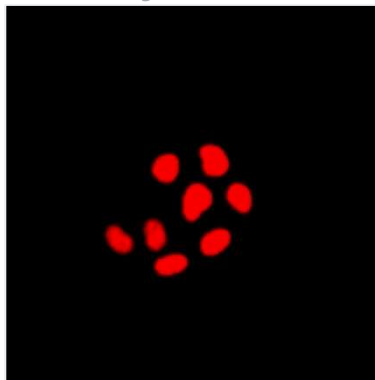
# Environmental Control Module

IXM can monitor and control:

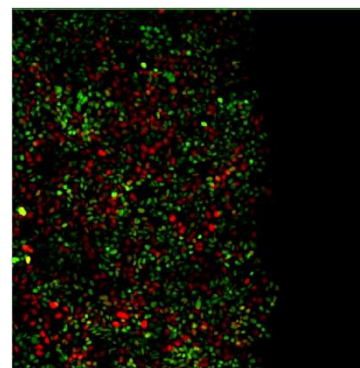
- Temperature
- Humidity
- Gas

Continuous time course  
from millisecond to multi-  
days

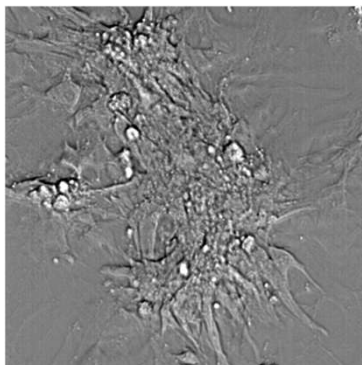
Colony formation



Migration assay

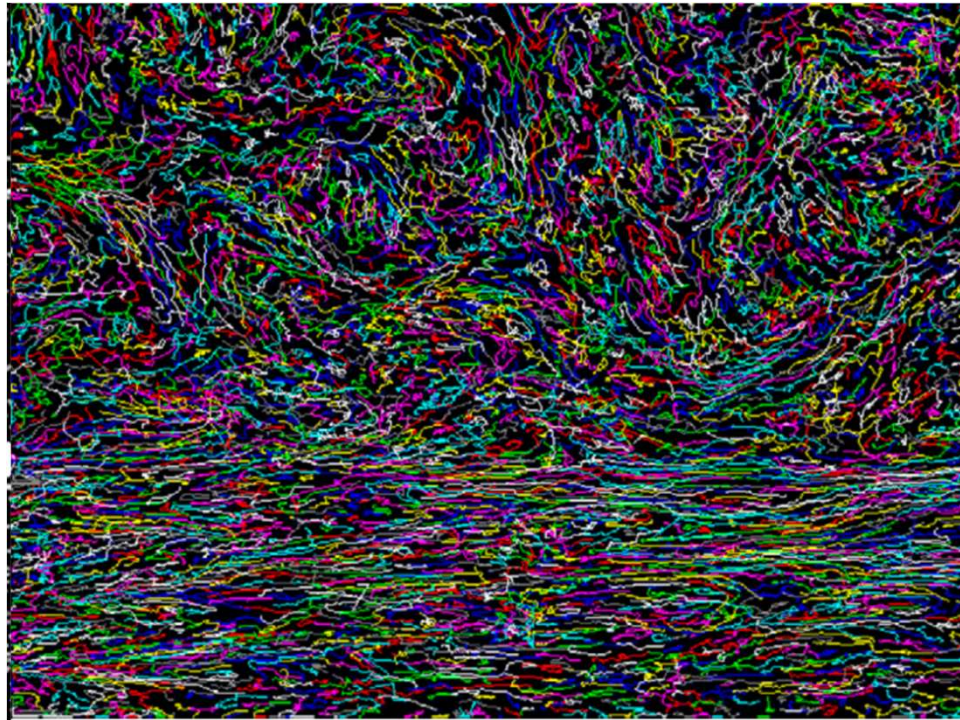


Cardiomyocyte assay



# 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



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# High Content System Overview



# 3-Stage Image Analysis Tools



MetaXpress® Software



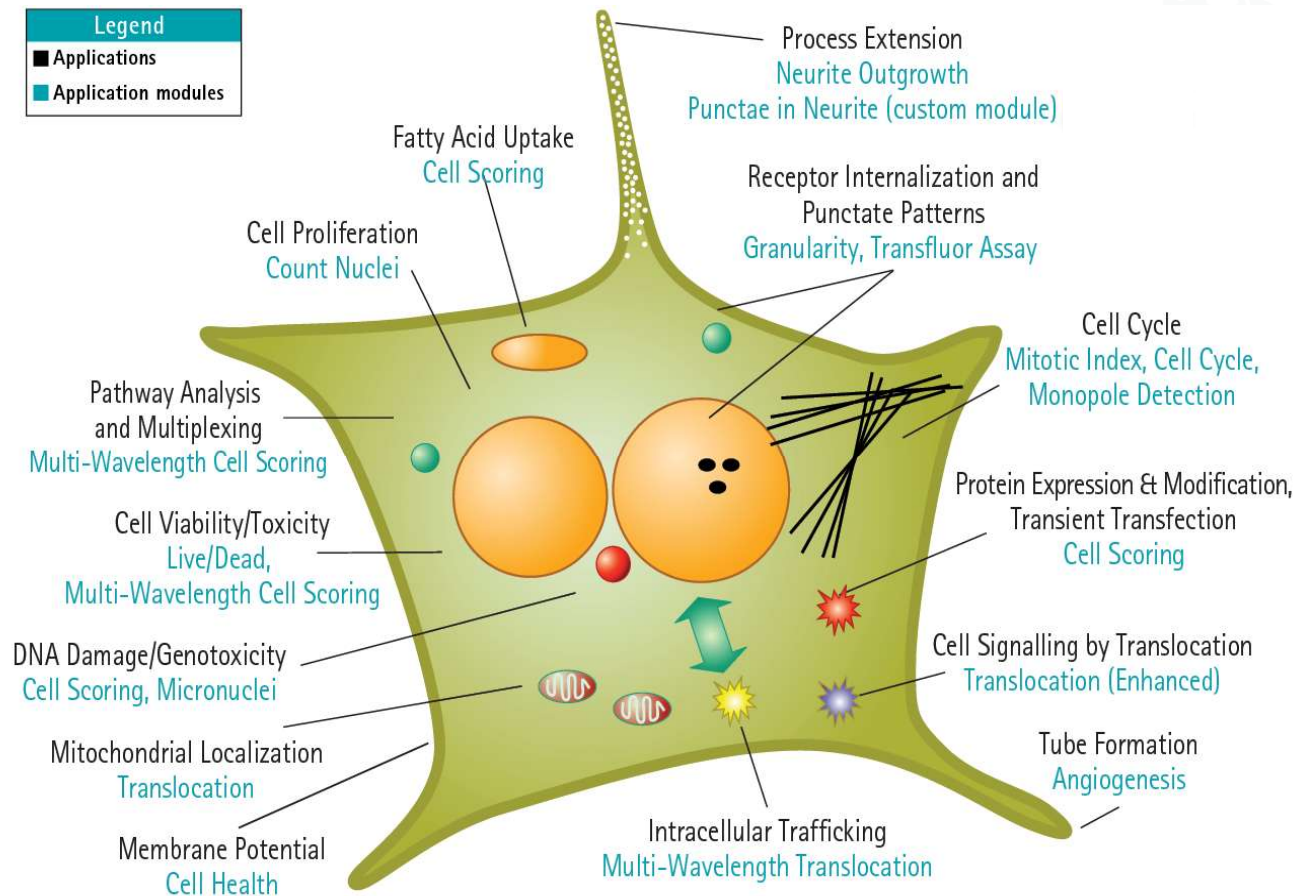
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# Application Modules

| Legend                |  |
|-----------------------|--|
| ■ Applications        |  |
| ■ Application modules |  |

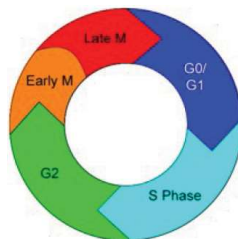
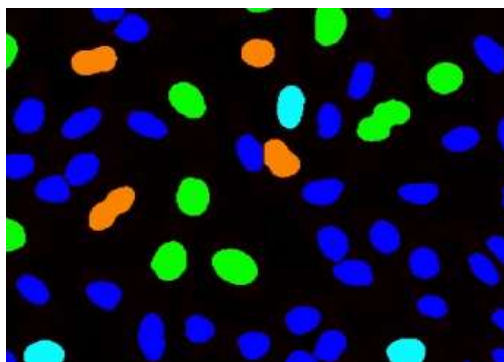
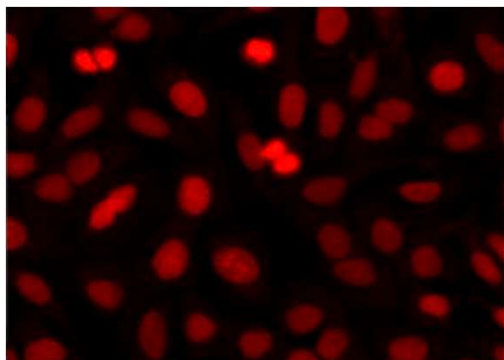


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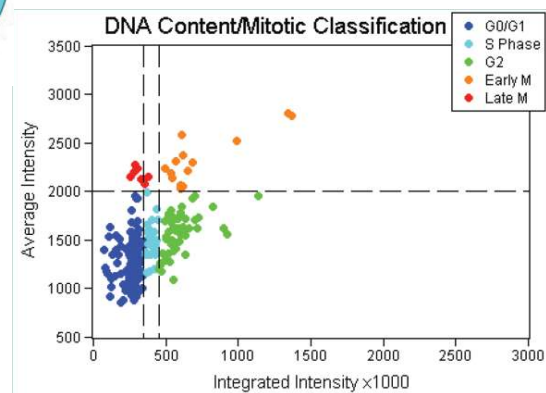
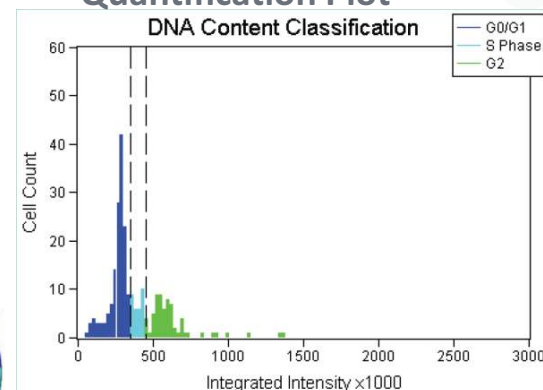
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# Application Module: Cell Cycle

DAPI



Quantification Plot

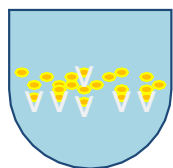


## Measurements:

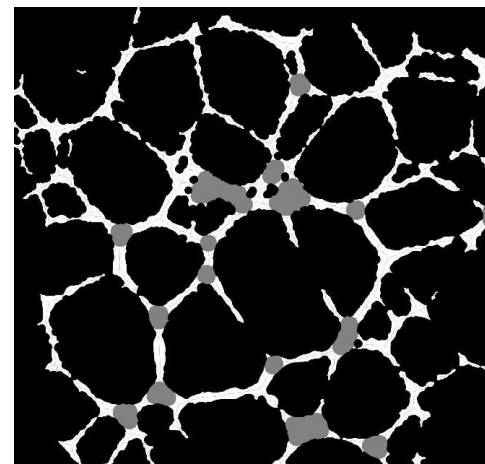
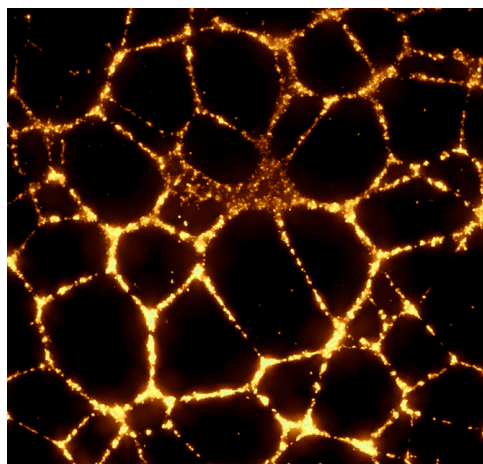
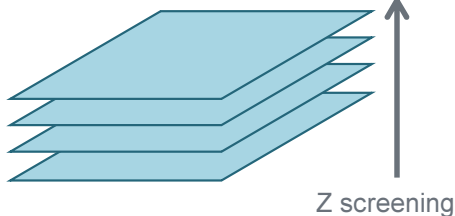
Count and percentage of G0/G1, S, G2, early M, late M and apoptotic cells, area and intensity of DNA.....

# Application Module: Angiogenesis

Locating and  
Scanning



Best Focus  
Recombination



**Measurements:**

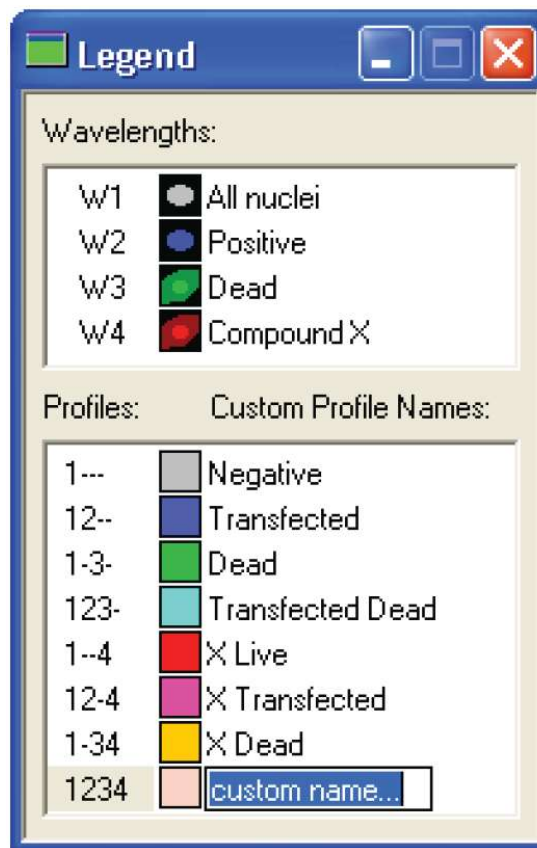
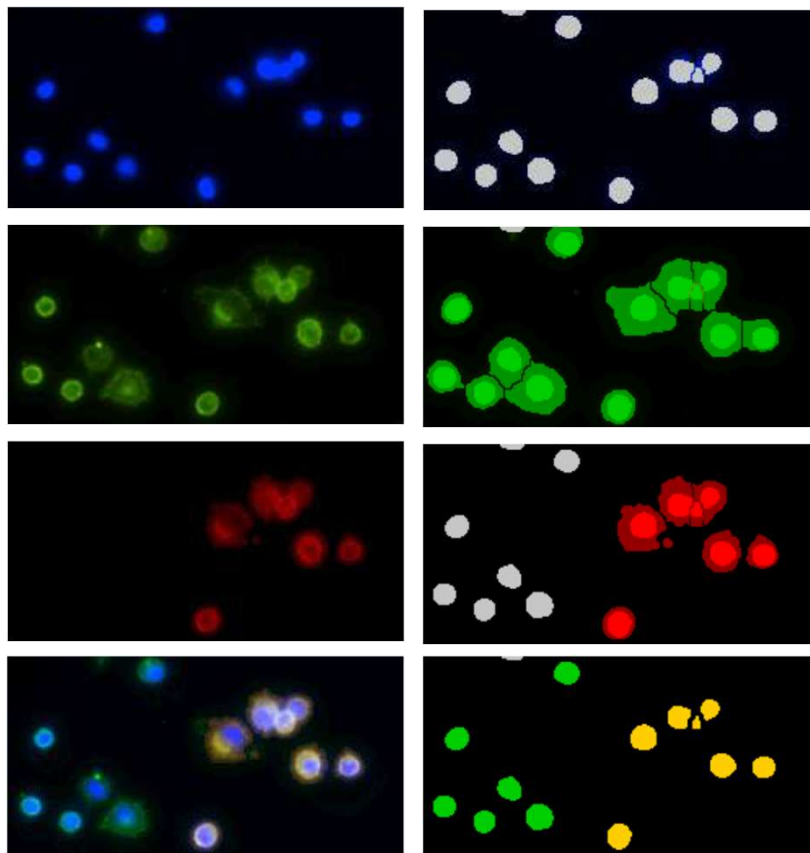
Tube length, tube area, branch, segment, nodes.....



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# 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

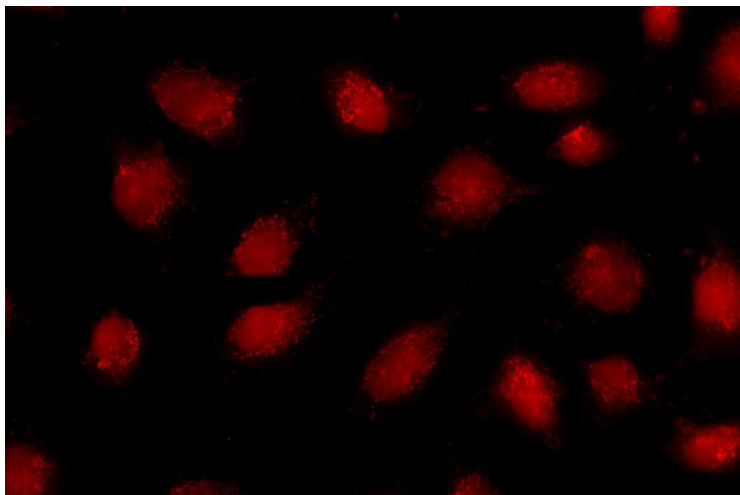
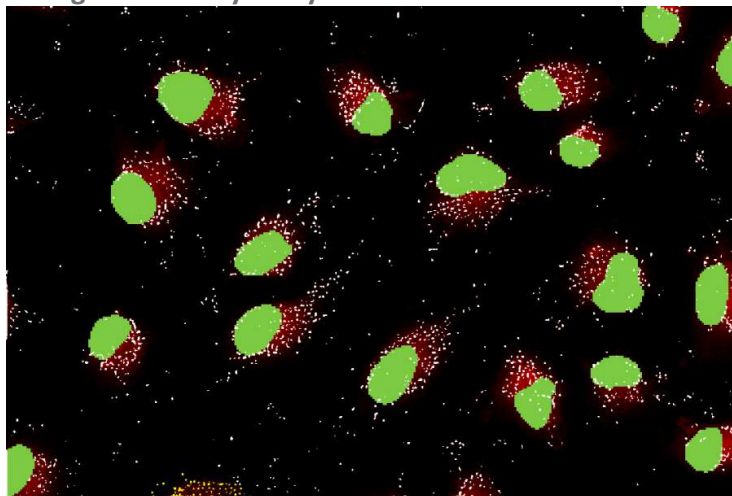


Image masked by analysis result



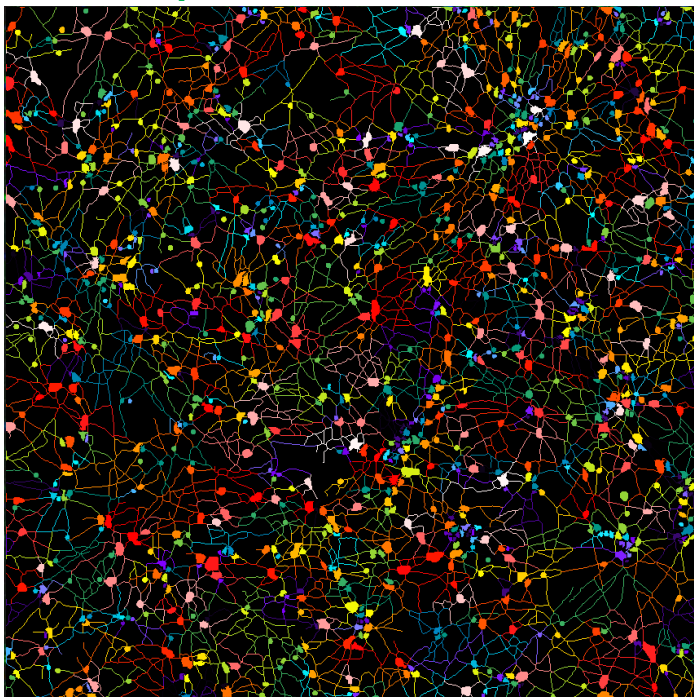
## Measurements:

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



# Application Module: Neurite Outgrowth

## Nuclear $\beta$ -tubulin



## Quantification

|    | 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



MetaXpress® Software



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# Customized Analysis Workflow

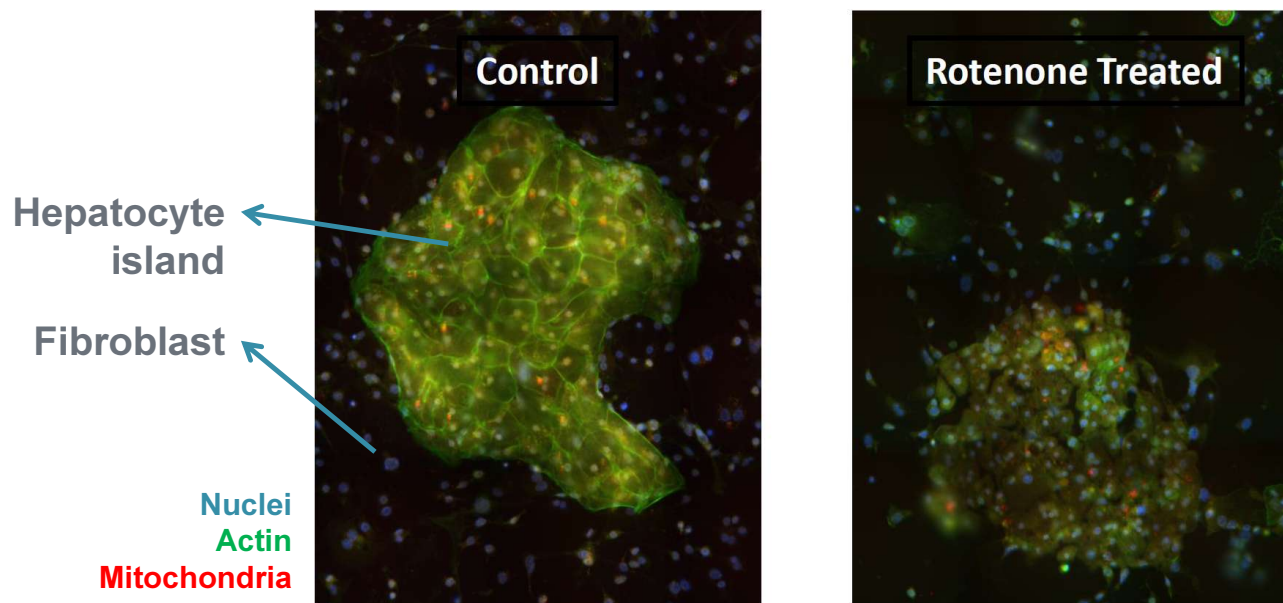
The screenshot displays the Molecular Devices software interface, specifically the 'Custom Module' window. The interface is divided into several sections:

- Top Toolbar:** Contains icons for 'Find Objects', 'Application Module Objects', 'Modify Objects', 'Modify Image', 'Export', 'Side by Side', and 'Split View'.
- Custom Module Panel (Left):**
  - Create Custom Module:** Includes tabs for 'Segment' and 'Measure'.
  - Measure Mask:**
    - Measurement Inputs:** 'Standard Area Value' is set to 1, and 'Create Object Overlay' is checked.
    - Objects to Measure:** 'Mask of Objects' is set to 'Positive Cytoplasm', and 'Image to Measure' is set to 'FITC'.
    - Features within Each Object:** 'Mask of Features' is set to 'Granules', and 'Image to Measure' is set to 'FITC'.
    - Buttons: 'Remove Feature Group', 'Add Feature Group', and 'Apply'.
  - Measurement Name:** 'Custom Module'.
  - Setting Name:** 'Custom Module'.
  - Buttons: 'Save' and 'Run'.
- Main Image View:** Displays a fluorescence image with cell outlines in blue and granules in green. A scale bar indicates 31.4 μm.
- Data Table (Right):**

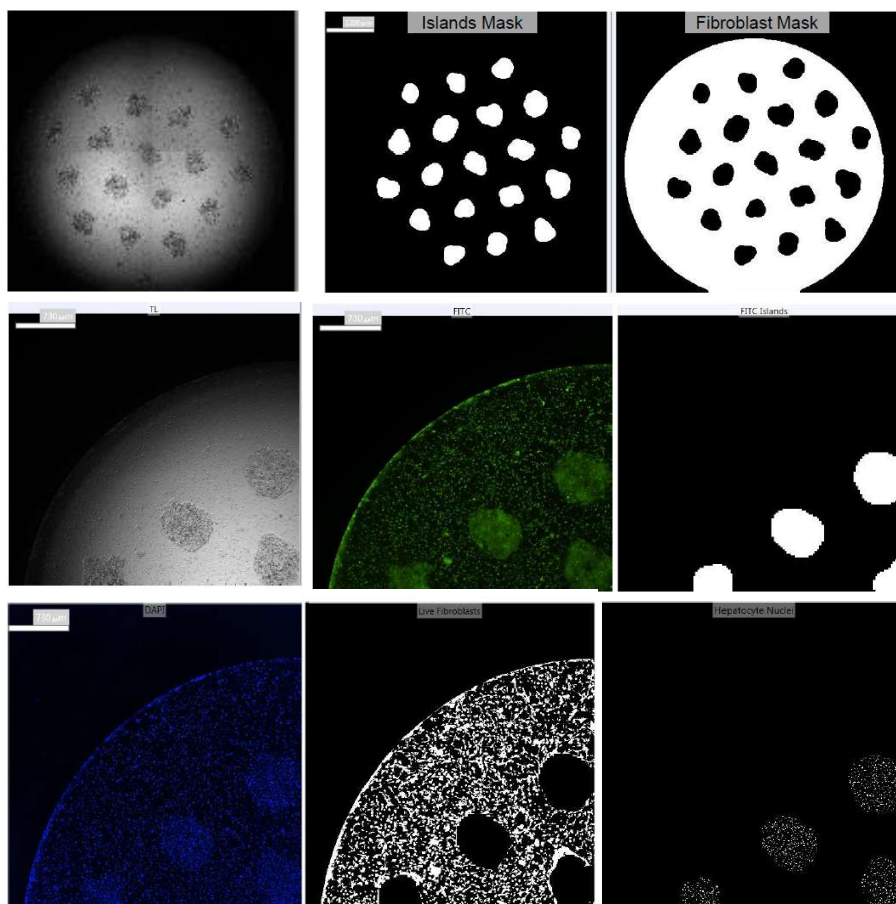
| Row ID             | Cell Centroid Y_Ave | Line Length_A | Granules In Cytoplasm |
|--------------------|---------------------|---------------|-----------------------|
| 1                  | 153,03              | 0,00          | 54,00                 |
| 2                  | 165,77              | 0,00          | 10,00                 |
| 3                  | 179,99              | 0,00          | 52,00                 |
| 4                  | 400,00              | 0,00          | 1,00                  |
| 5                  | 416,17              | 0,00          | 16,00                 |
| 6                  | 499,47              | 0,00          | 49,00                 |
| 7                  | 605,95              | 0,00          | 41,00                 |
| 8                  | 814,49              | 0,00          | 60,00                 |
| 9                  | 931,40              | 0,00          | 78,00                 |
| 10                 | 955,69              | 0,00          | 82,00                 |
| 11                 | 946,23              | 0,00          | 35,00                 |
| Count              | 11,00               | 11,00         | 12,00                 |
| Minimum            | 153,03              | 0,00          | 0,00                  |
| Maximum            | 955,69              | 0,00          | 82,00                 |
| Total              | 6.068,19            | 0,00          | 478,00                |
| Average            | 551,65              | 0,00          | 39,83                 |
| Standard Deviation | 305,29              | 0,00          | 26,85                 |
- Custom Module Step Images (Bottom):** A sequence of four images showing the analysis steps, with the fourth image highlighted.



# Example: Toxicity Assay by Hepatocyte Co-Culture Model



# Example: Toxicity Assay by Hepatocyte Co-Culture Model



Identify well margin and hepatocyte islands

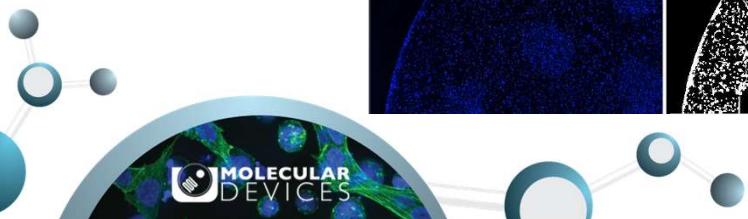
- Island number/ area
- Fibroblast area

Determine parameters of hepatocyte islands

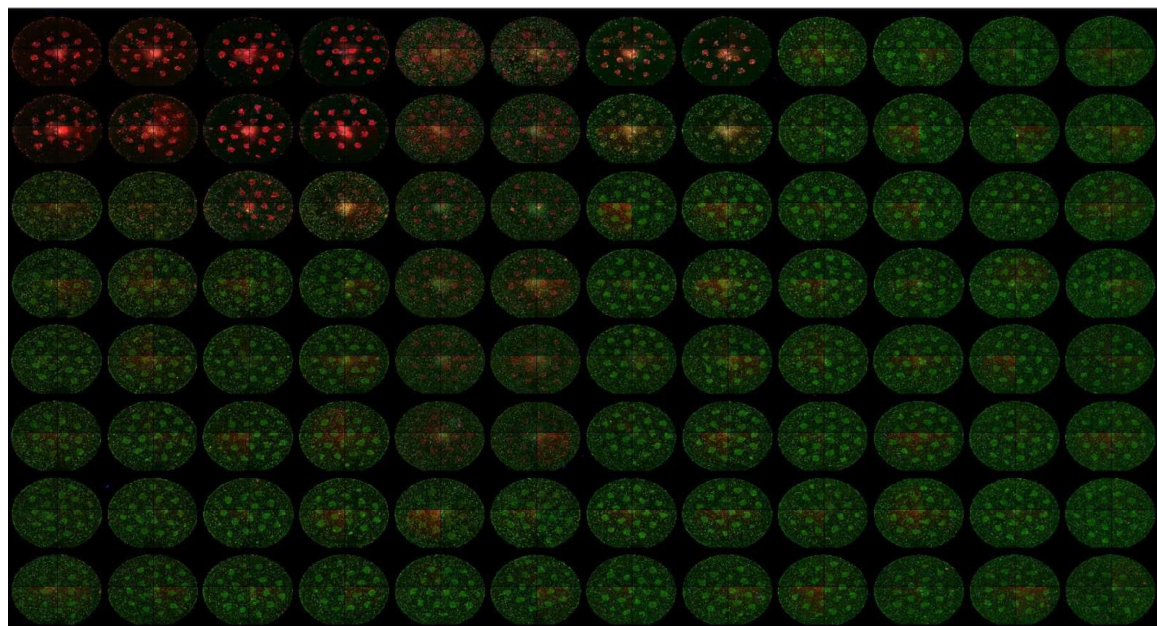
- DAPI/ Mitochondria intensity of island

Determine parameters of cells

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

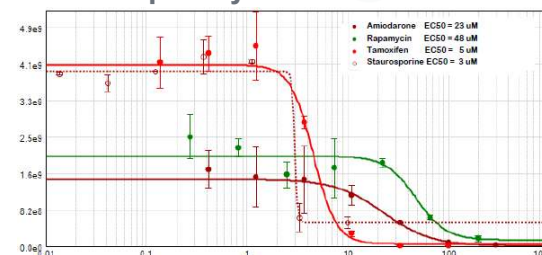


# Example: Toxicity Assay by Hepatocyte Co-Culture Model

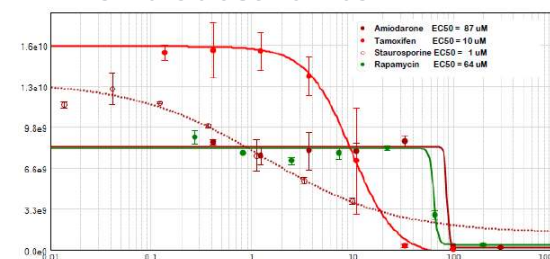


Compound concentration

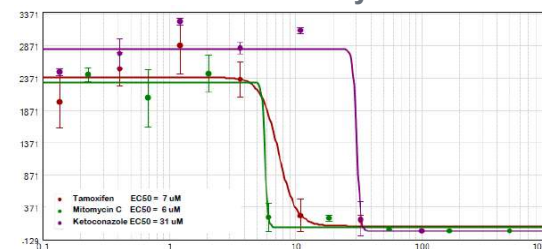
Live hepatocyte number



Live fibroblast number



Mitochondria intensity



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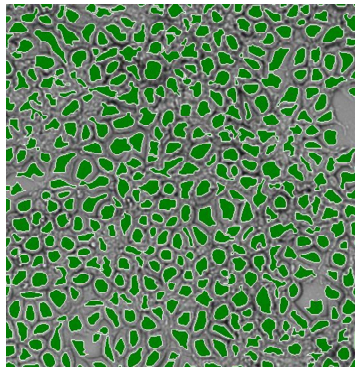
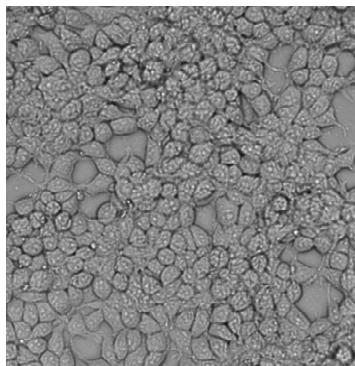
Confidential - Company Proprietary



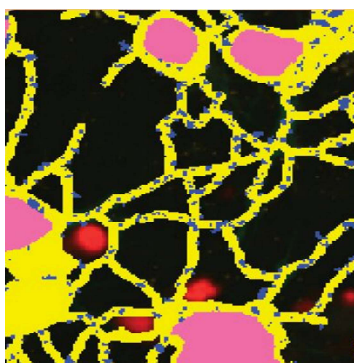
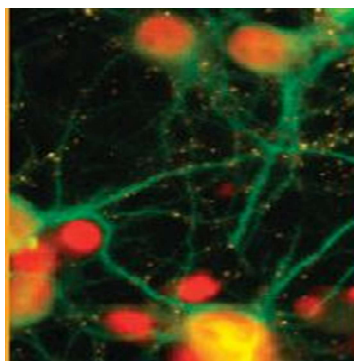


# Versatile Analysis Applications

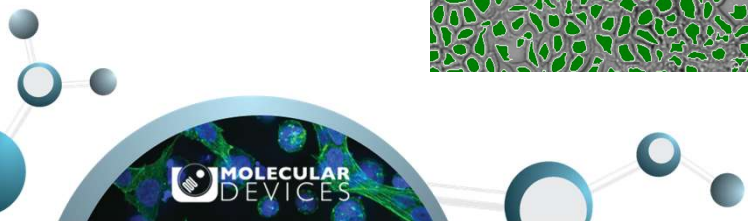
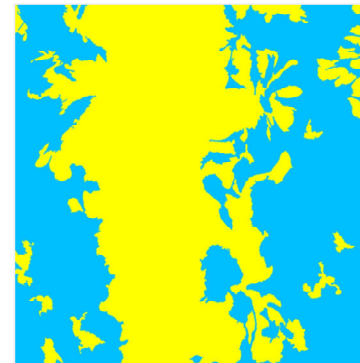
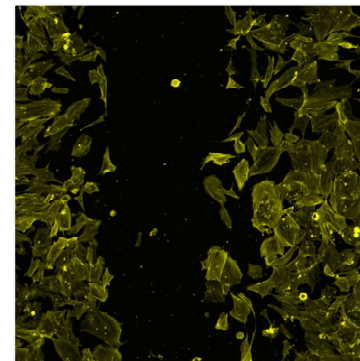
Proliferation with  
Transmitted Light



Neurite Outgrowth  
and Puncta



Wound Healing





# 3-Stage Image Analysis Tools



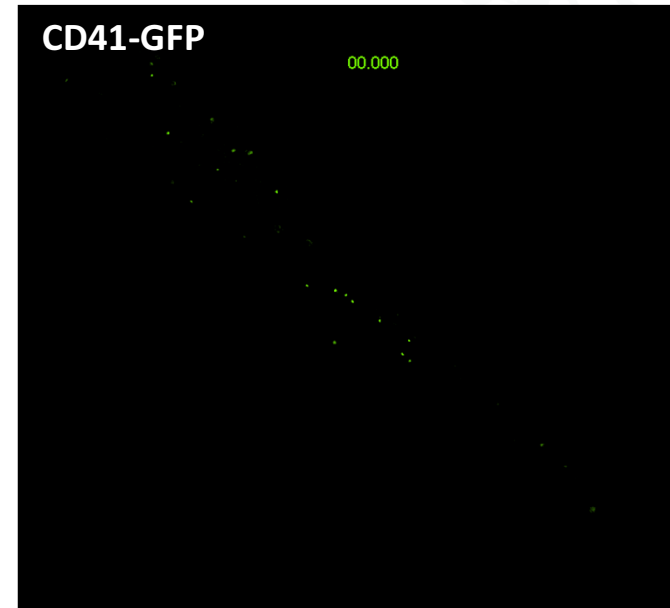
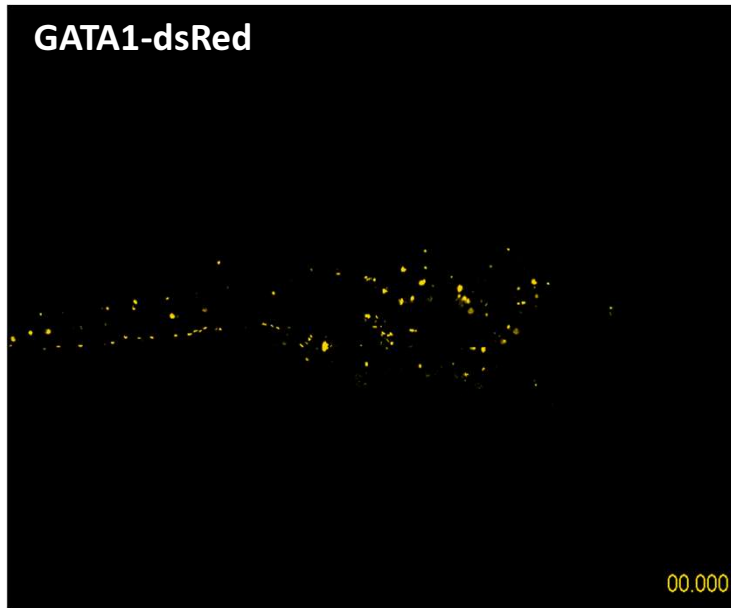
MetaXpress® Software



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# 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**

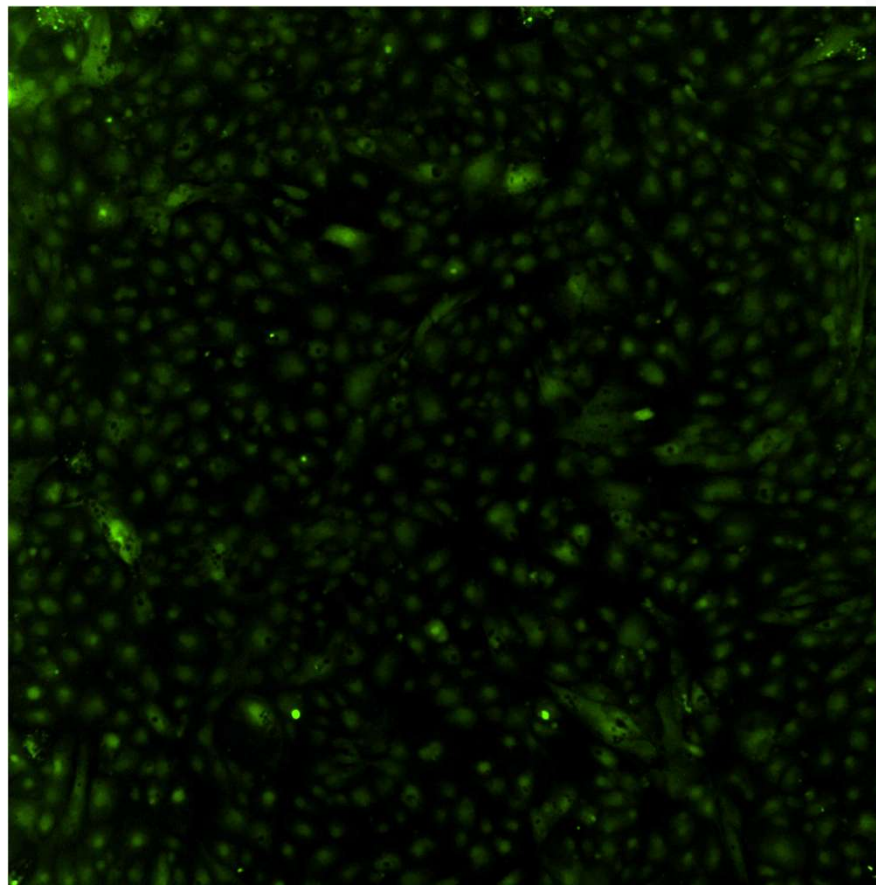
92

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

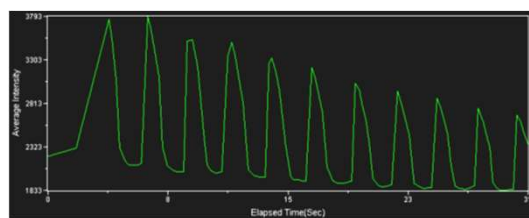
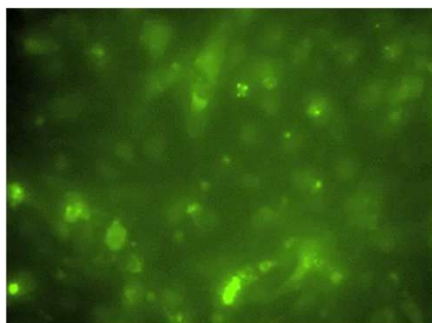


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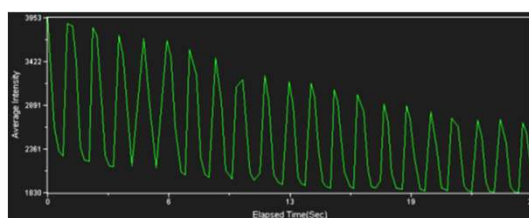
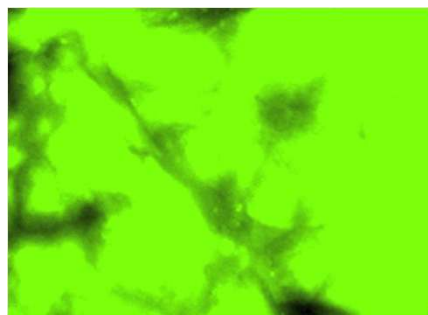
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# Spontaneous Contracting Cardiomyocytes Monitoring

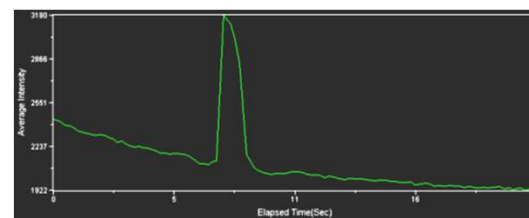
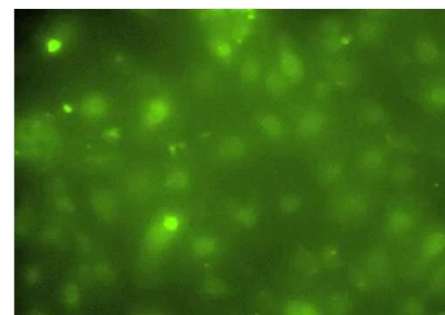
Control



Epinephrine



Verapamil



- Cell contractions visualized with  $\text{Ca}^{2+}$  sensitive dye
- $\text{Ca}^{2+}$  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.

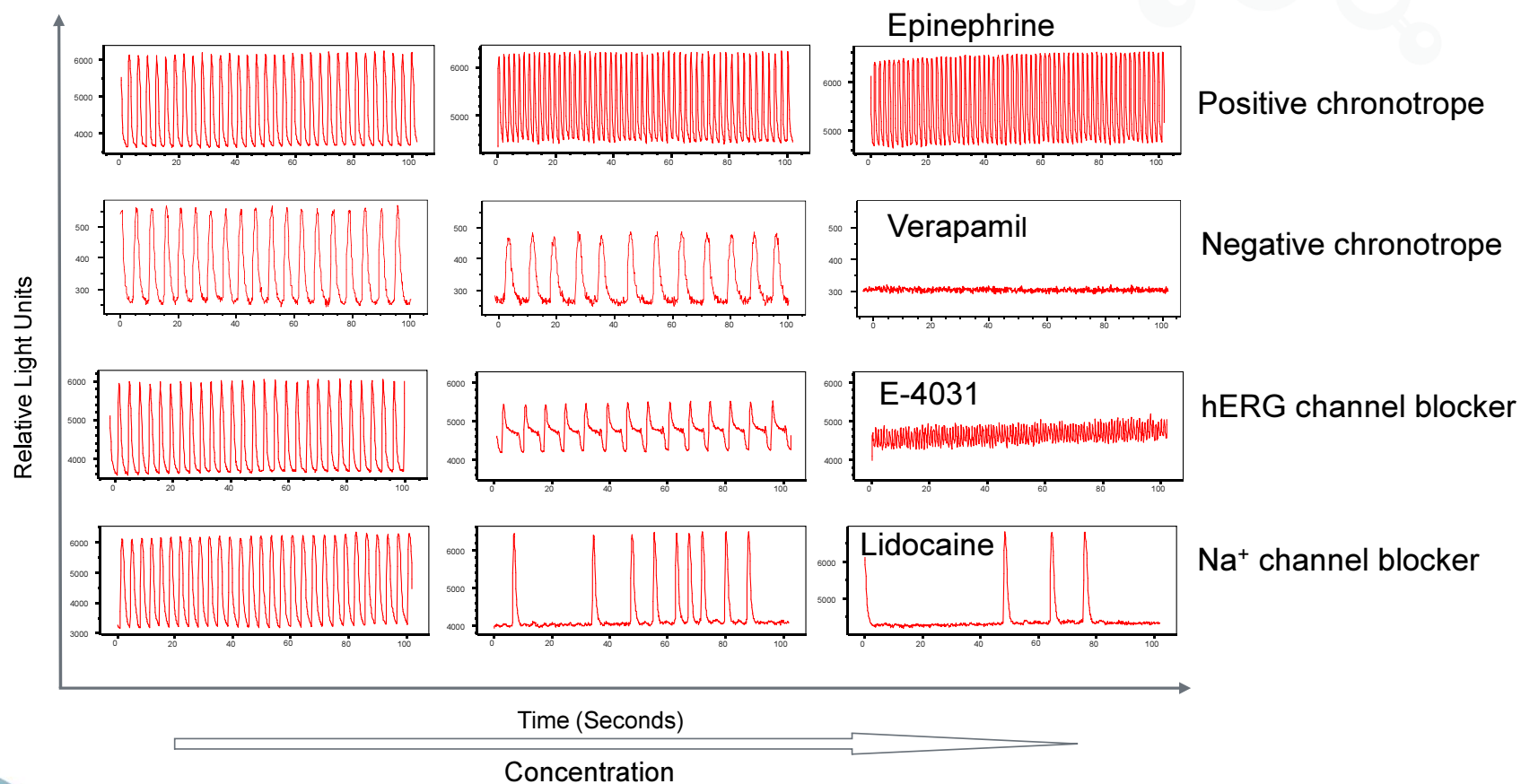
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# Spontaneous Contracting Cardiomyocytes Monitoring



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

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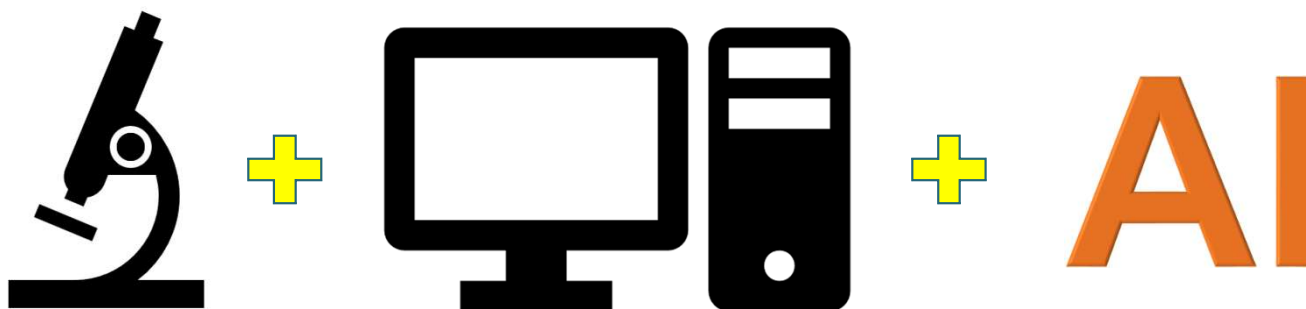


# High Content System Overview



# Trend of High Content System

# Progress of High Content System in 21st Century



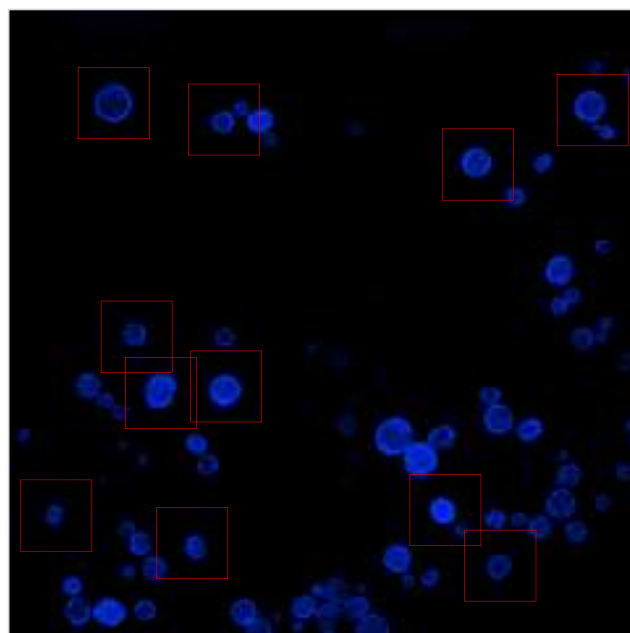
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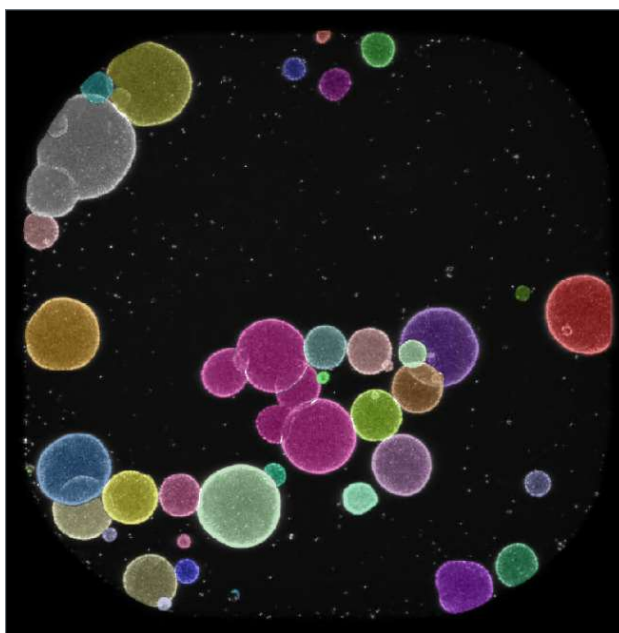


# AI-associated Breakthrough on HCS

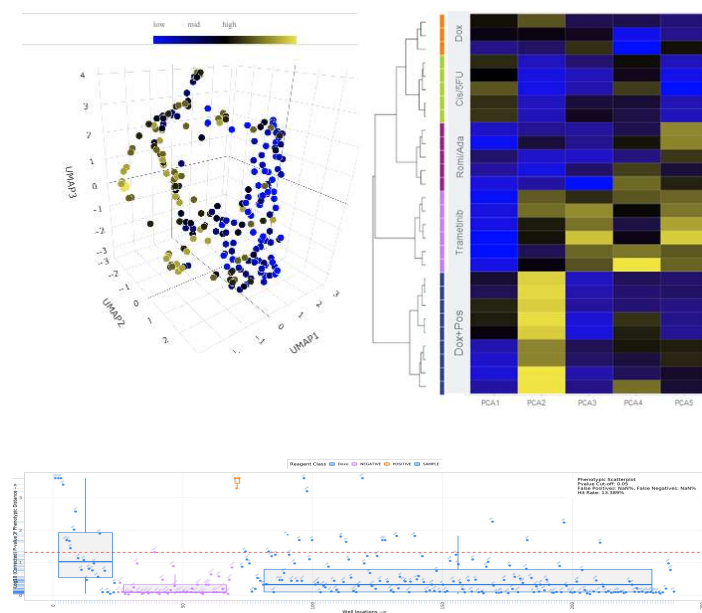
AI-assisted target acquisition

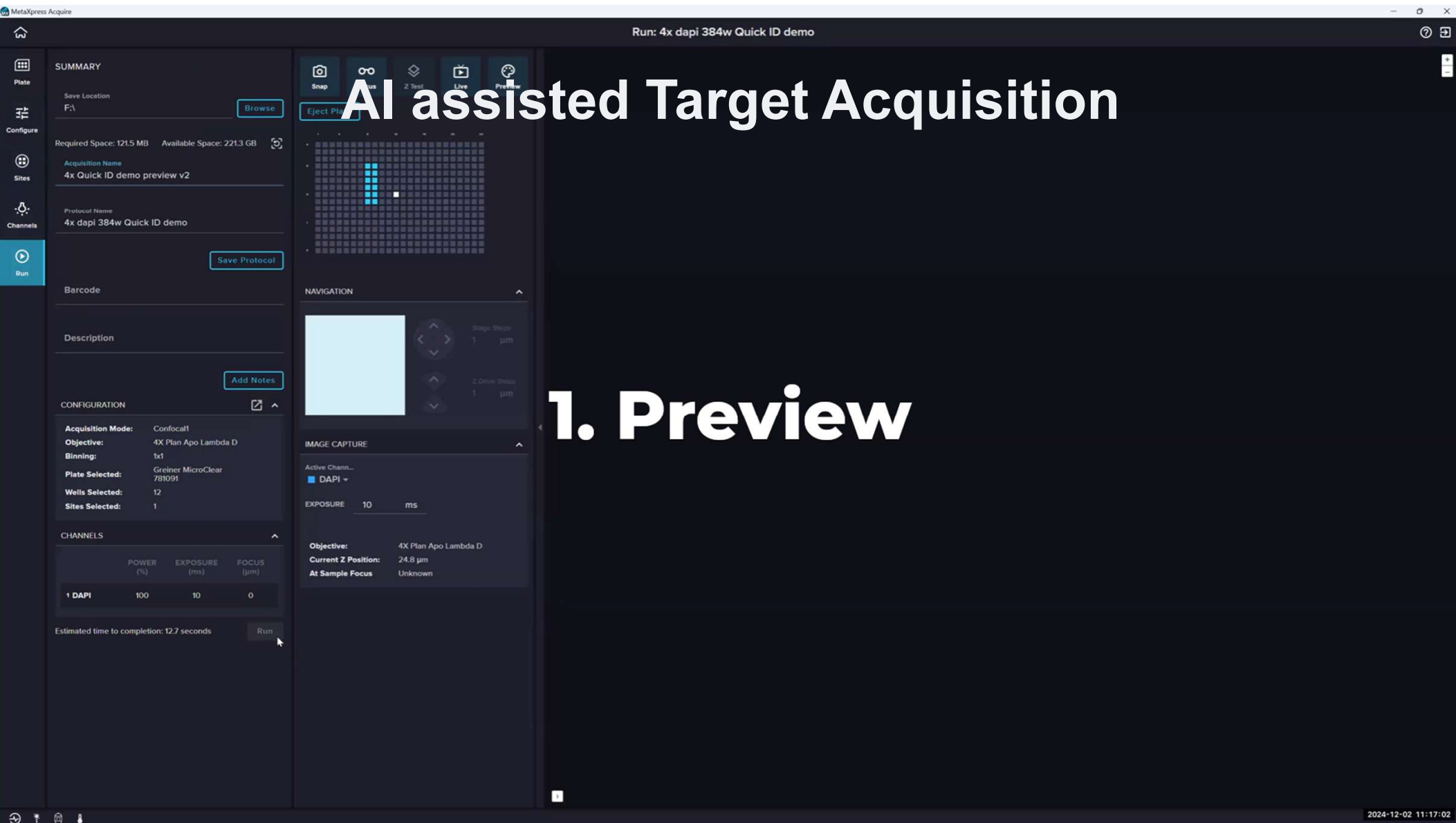


AI-based object identification



AI-embedded data analysis





# AI-based object identification

SINAP ⓘ Neurons\_b3tub\_1FOV\_1

**Image Selection**

Channel: 2 : Cy5-Cy5  
Row: All Column: All  
Time: All Z-Slice: All  
Random FOV ↻ Well: A - 1 | Field: 1  
Channel: 2 | Z: 1 | T: 1

**Labels**

Target Background

**Labeling Tools**

3 2 Fill Holes  
Segment Correct Add to Training Set

**Overlays**

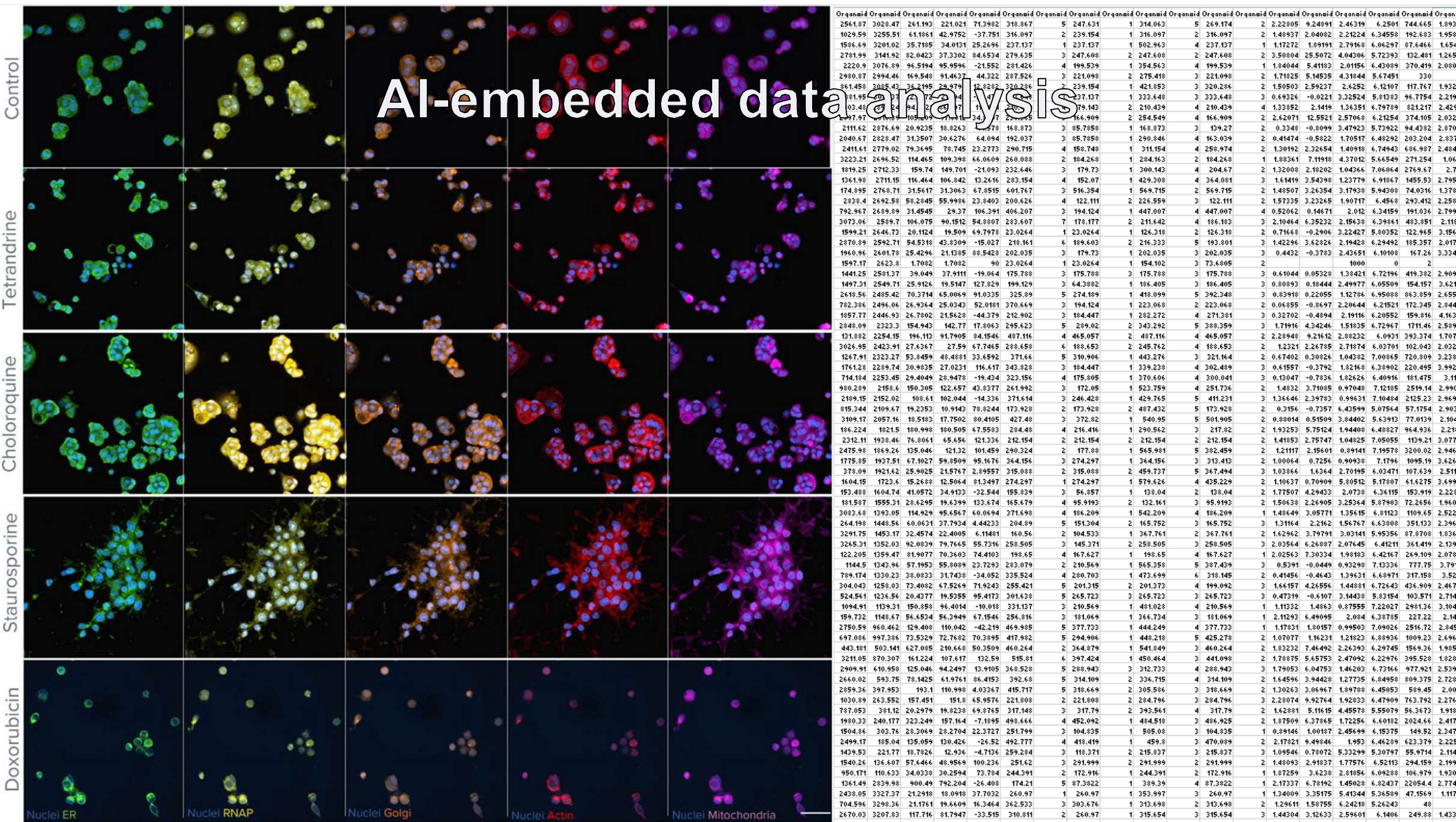
Annotations 0.3 Segmentation 0.2

**Neural Network Segmentation**

Default Custom  
Model  
Train Apply Advanced Save

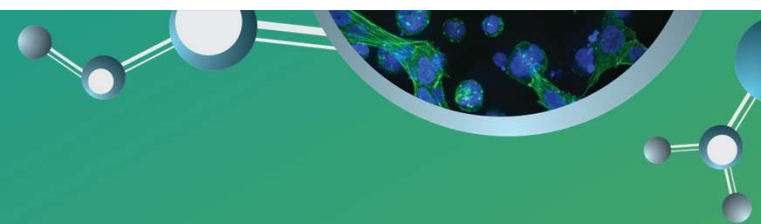
New field of view has been loaded







# Case Sharing



# AI-associated Breakthrough on HCS

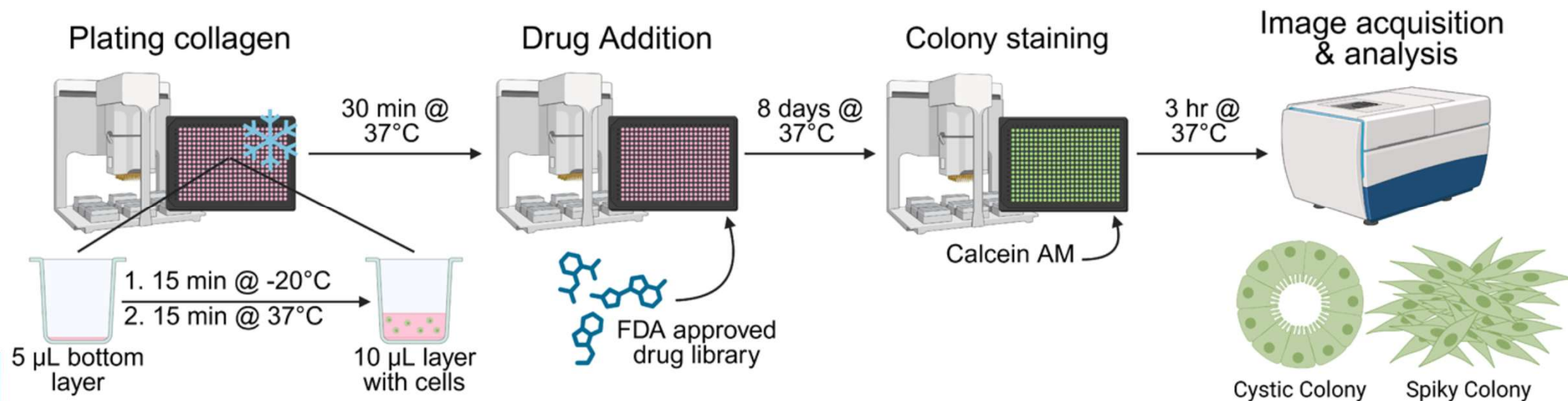
communications biology

A Nature Portfolio journal

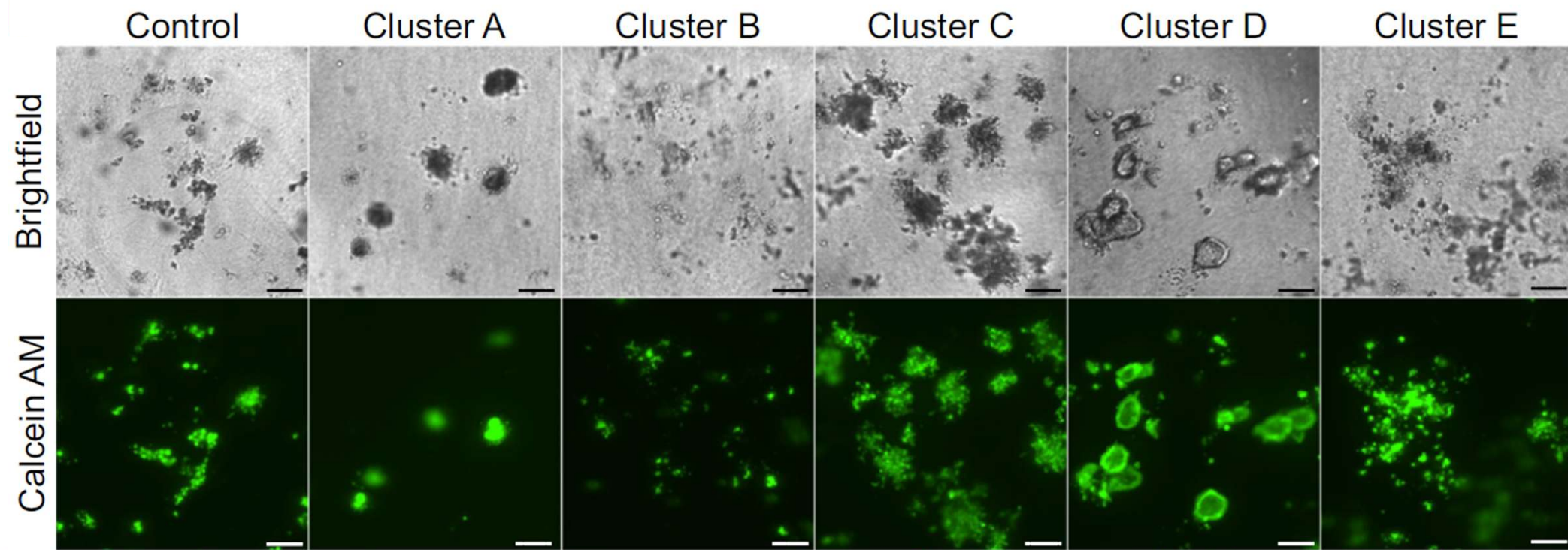
Article



## 3D collagen high-throughput screen identifies drugs that induce epithelial polarity and enhance chemotherapy response in colorectal cancer



# AI-associated Breakthrough on HCS

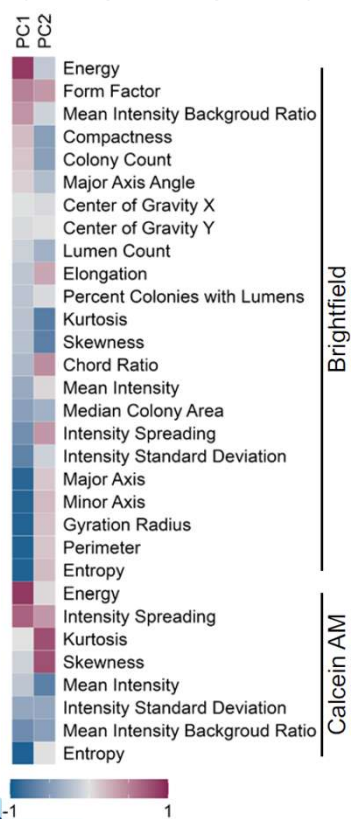


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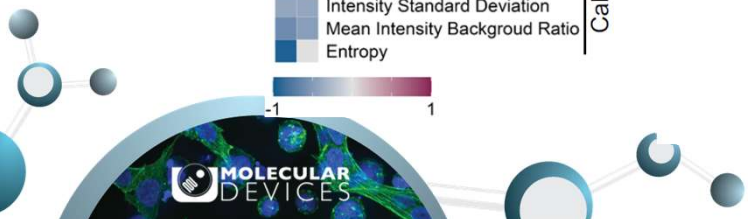
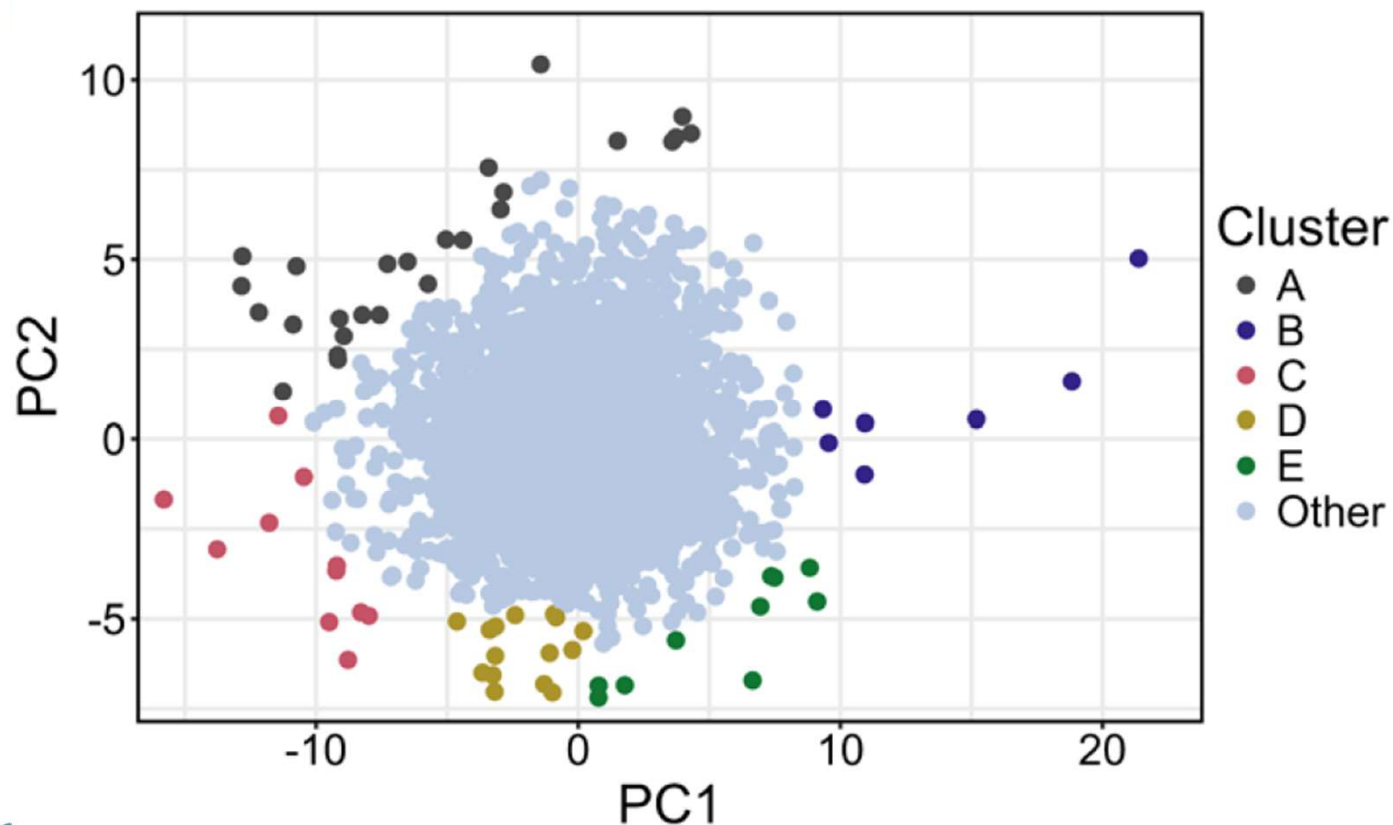
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# AI-associated Breakthrough on HCS

Heatmap of PC  
(Principal component)



PCA plot of morphological characteristics of colonies



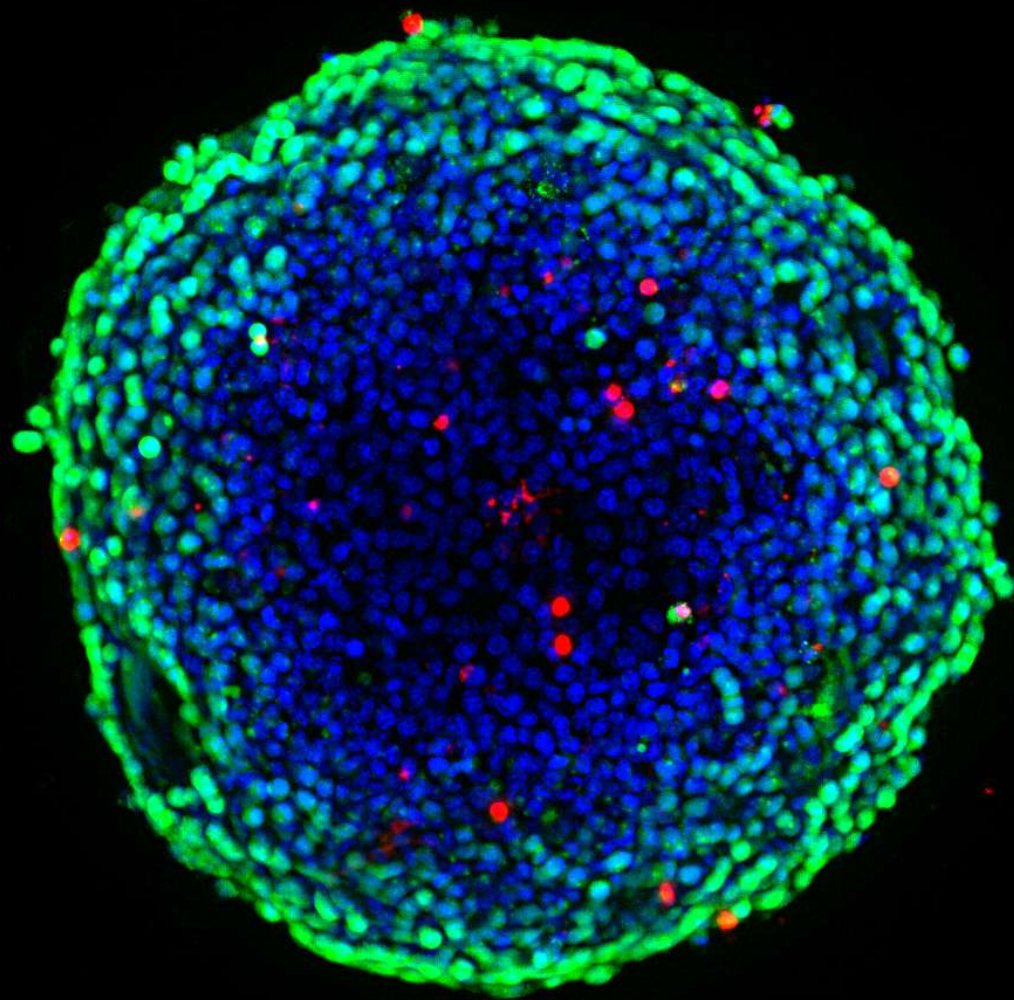


# AI-associated Breakthrough on HCS



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# Capture Deeper Inside

High Content Imaging System

**MIMETAS**  
the organ-on-a-chip company

Vascularized liver spheroid  
in the OrganoPlate® Graft.  
Blue: DNA, Green: Actin,  
Red: RFP-HUVEC,  
Magenta: VE-Cadherin.



**ImageXpress® Pico**  
Automated Cell Imaging System



**ImageXpress Nano**  
Automated Cell Imaging System



**ImageXpress Micro 4**  
High-Content Imaging System



**ImageXpress Micro Confocal**  
High-Content Imaging System



**ImageXpress Confocal HT.ai**  
High-Content Imaging System

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# Q & A







*Thanks to Your Attention!*

Tim Tsui

Field Application Scientist



Molecular Devices, LLC

114063 4F., No. 631, Ruiguang

Rd., Neihu Dist., Taipei City, ROC

☎ +886 2 2656 7584

🔧 support@moldev.com

🌐 moleculardevices.com