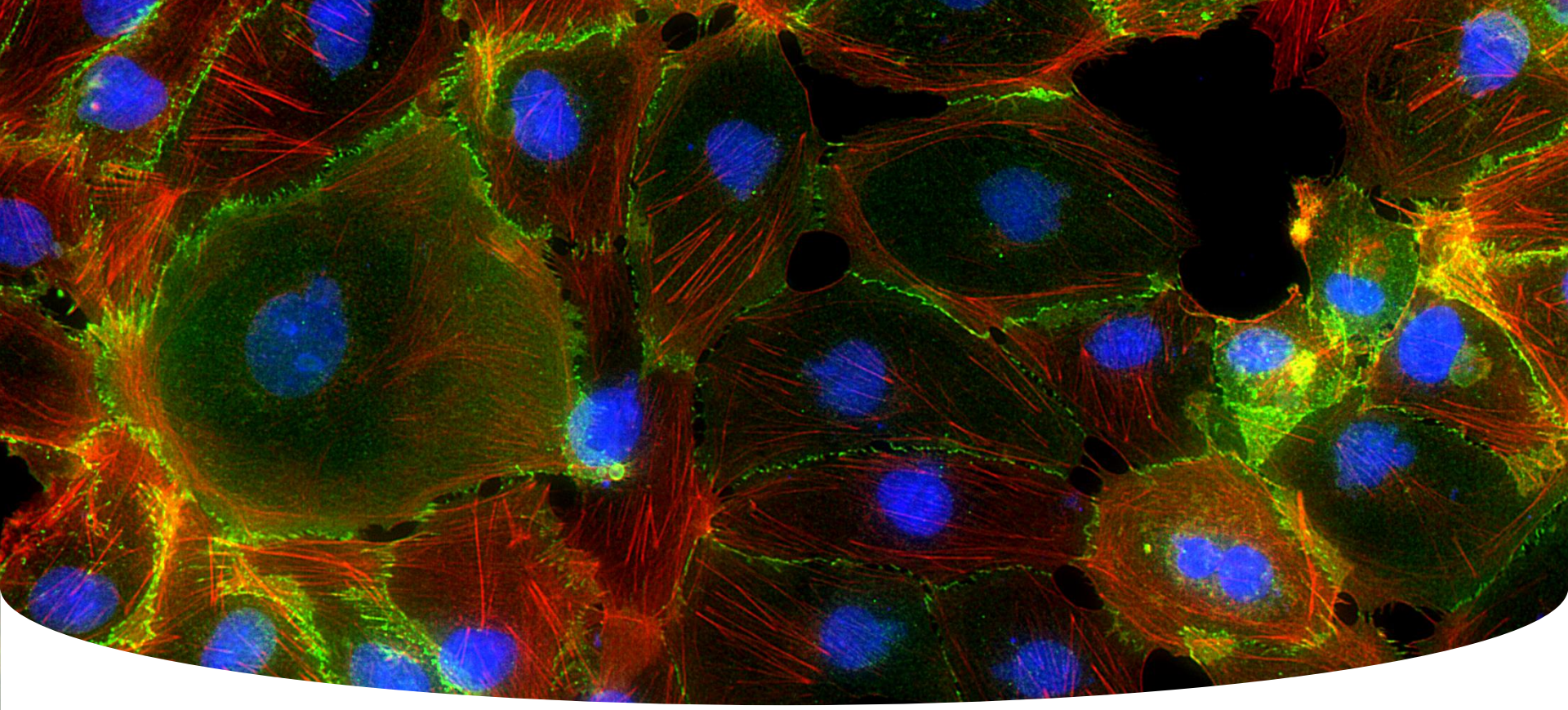
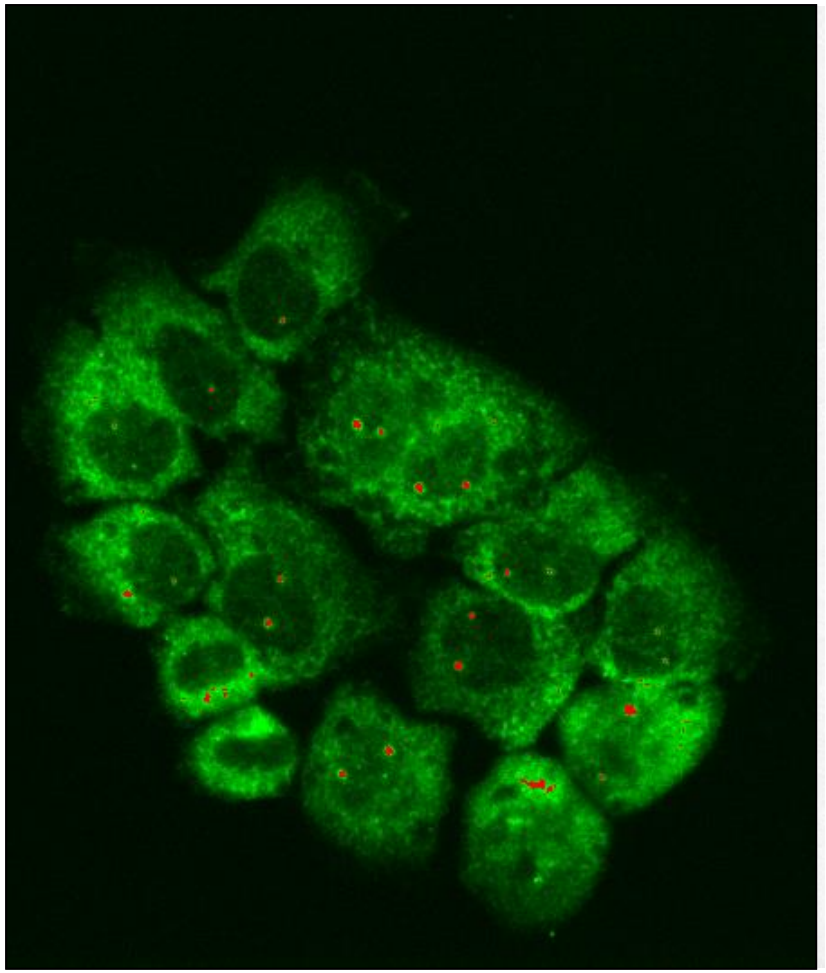


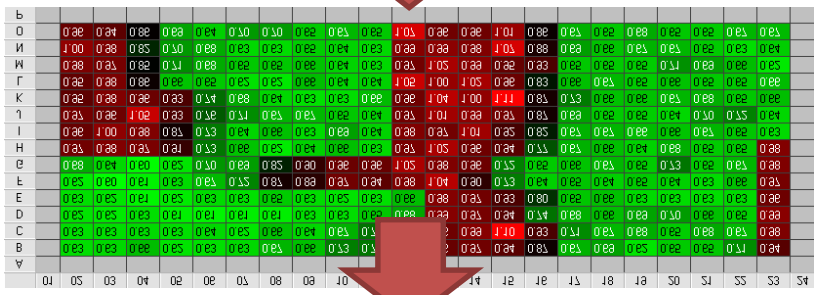
High Content Image System

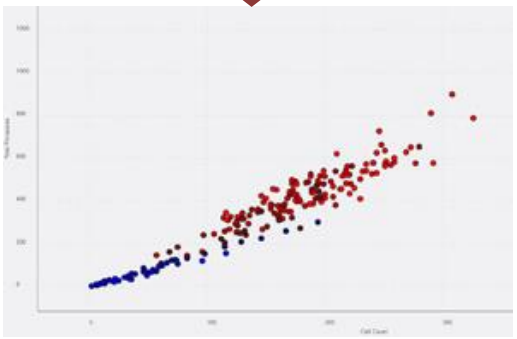
ImageXpress Micro XLS System

藥篩暨影像技術專員 崔瑞廷

High Content Image system



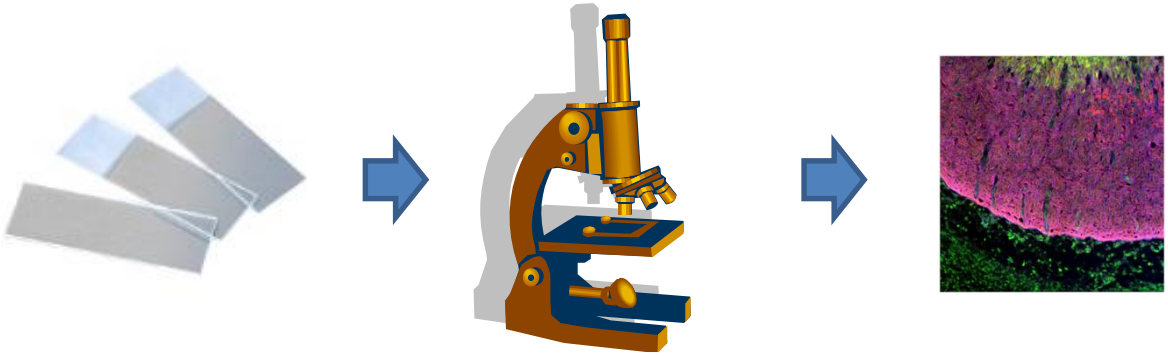




Why High Content System?

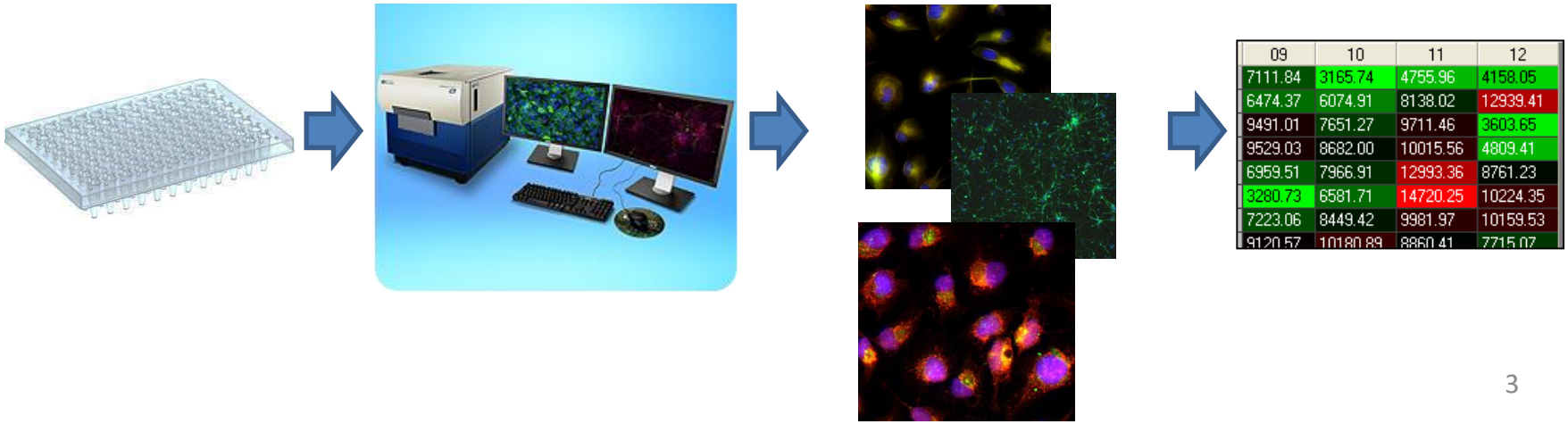
Conventional Microscopy

Limited throughput, qualitative results



High Content Imaging System

High throughput, qualitative+quantitative results

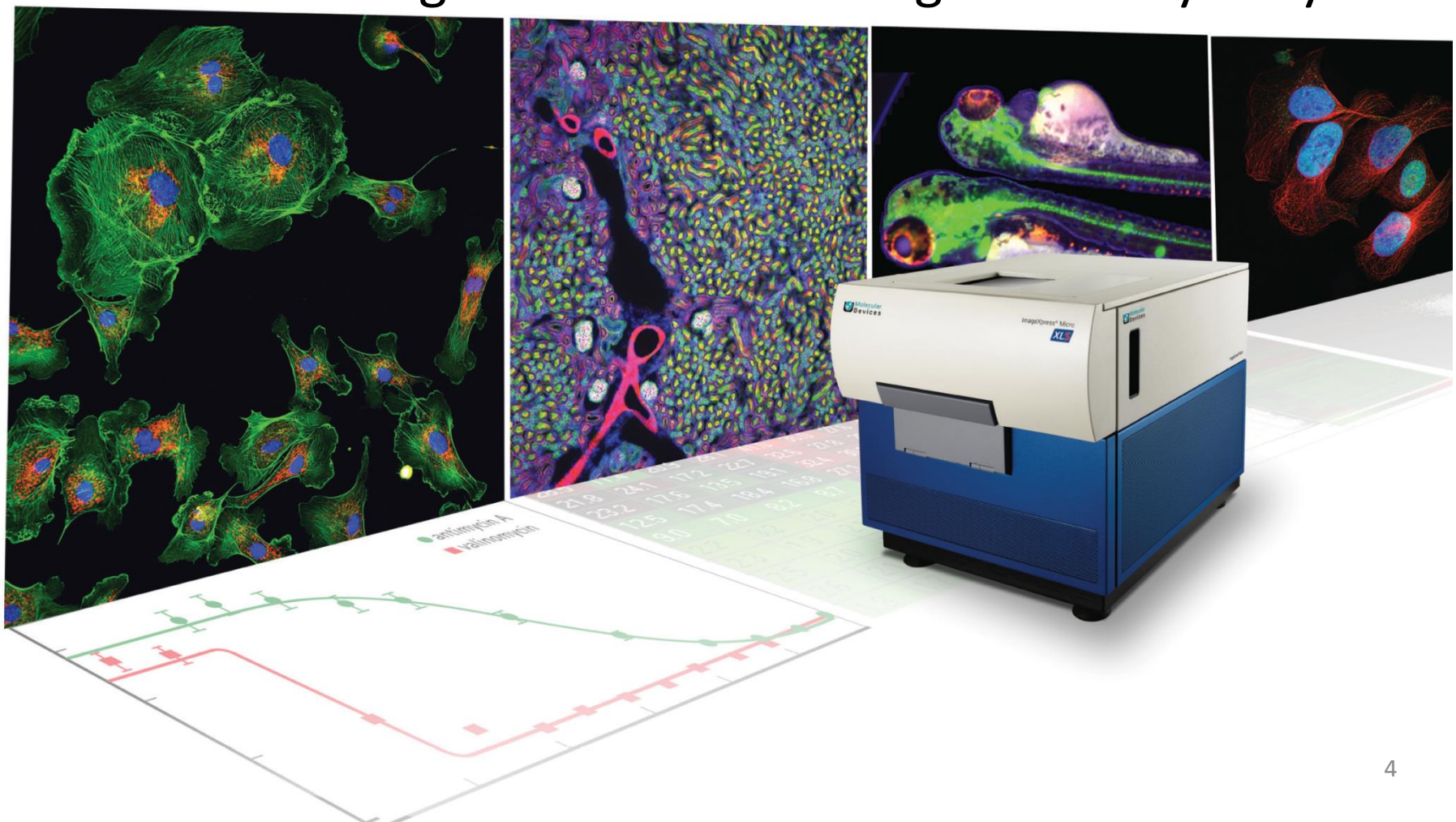


09	10	11	12
7111.84	3165.74	4755.96	4158.05
6474.37	6074.91	8138.02	12939.41
9491.01	7651.27	9711.46	3603.65
9529.03	8682.00	10015.56	4809.41
6959.51	7966.91	12993.36	8761.23
3280.73	6581.71	14720.25	10224.35
7223.06	8449.42	9981.97	10159.53
9120.57	10180.89	8860.41	7715.07

IXM system

- IXM system: ImageXpress Micro system

  Wide-field High Content Screening and Analysis system

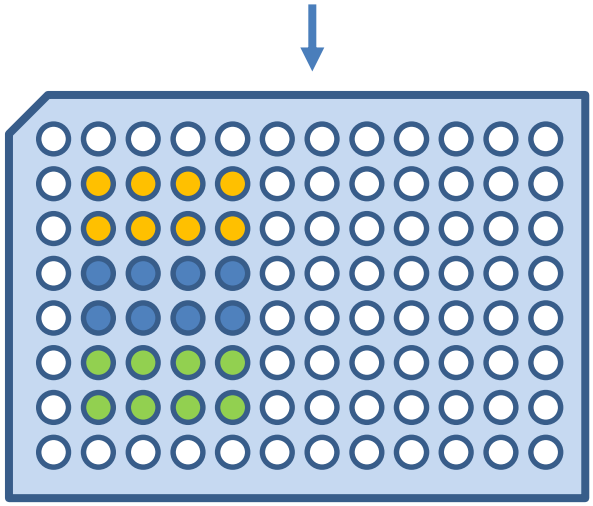


Live Cell Analysis for program death

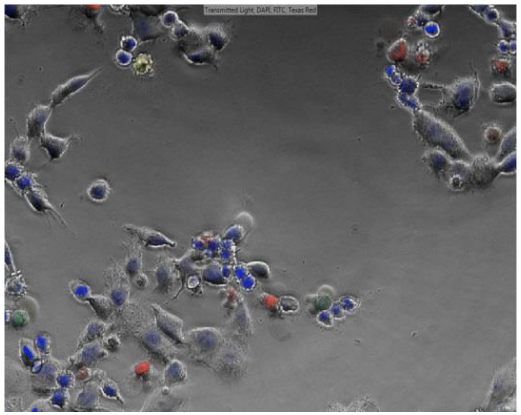
Nuclei (Hoechst)

Apoptotic Cells (NucView 488)

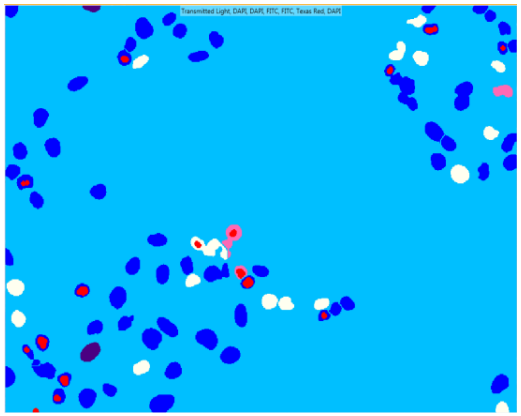
Necrotic Cells (Propidium Iodide)



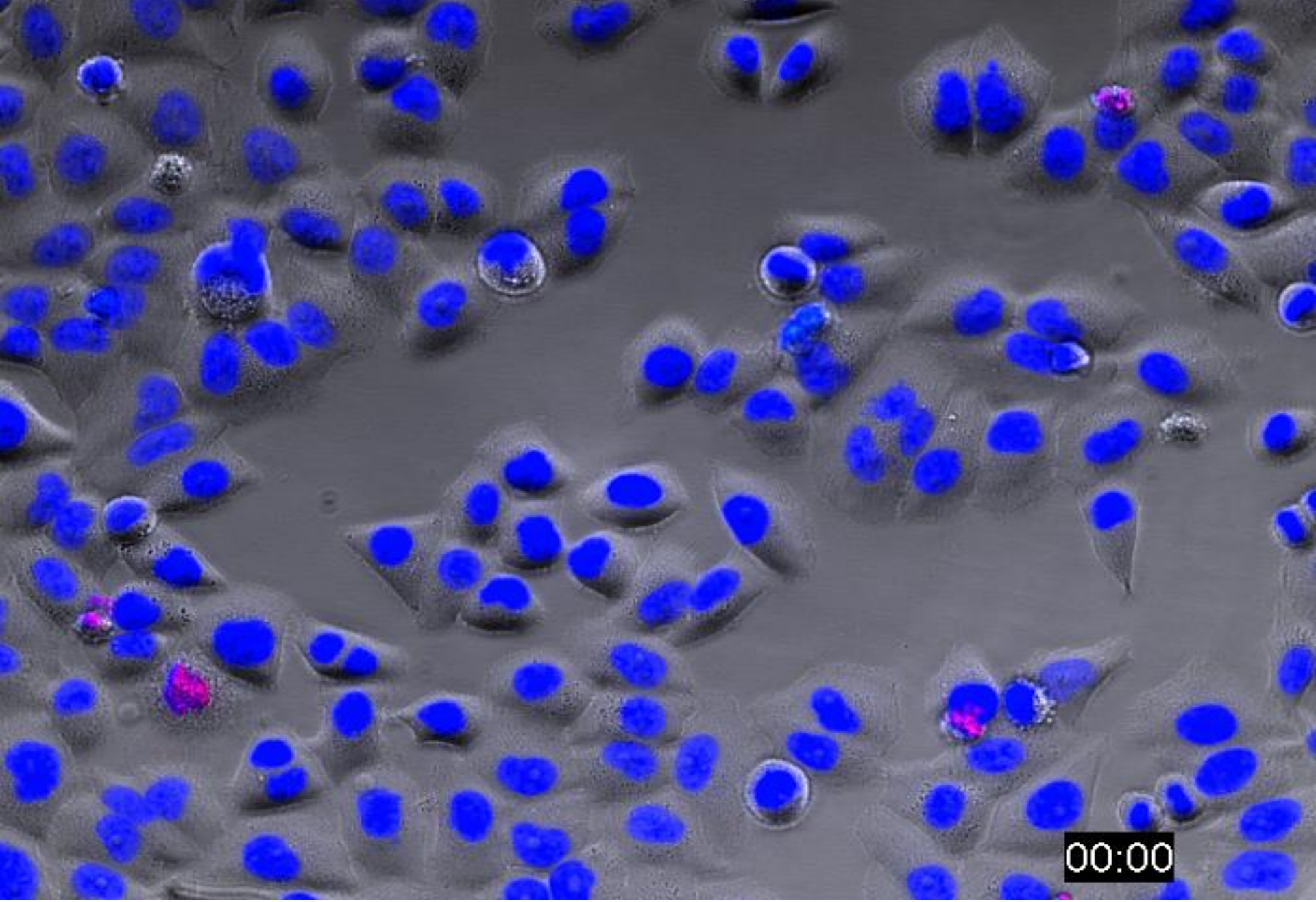
Acquisition



Analyze

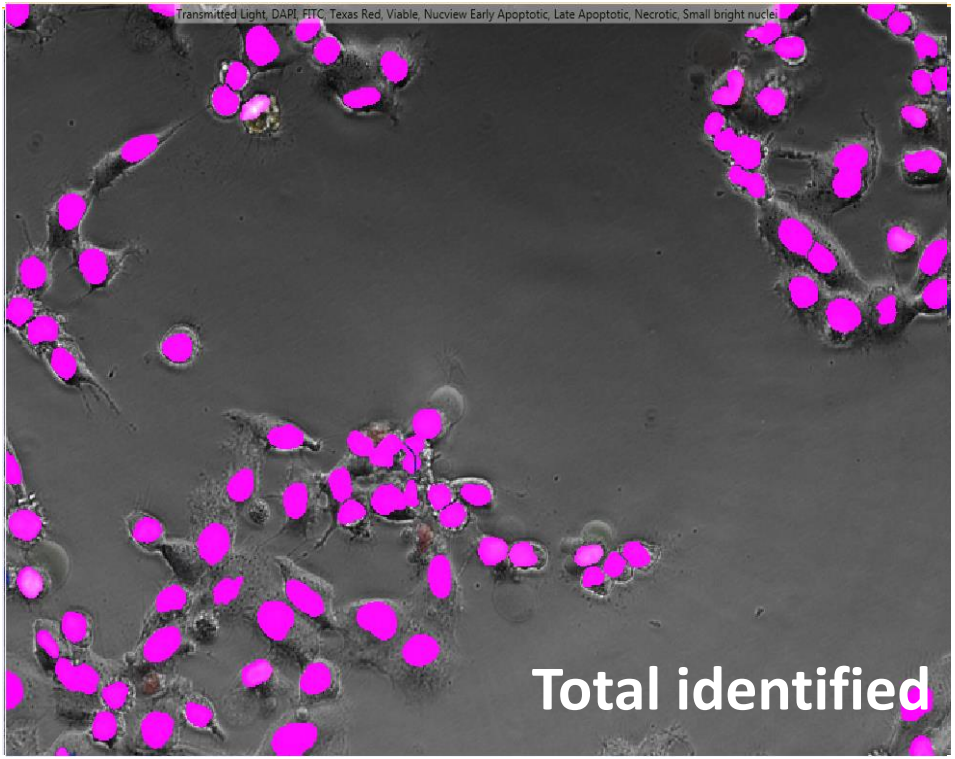


37° C, 5% CO_2, 43 hours

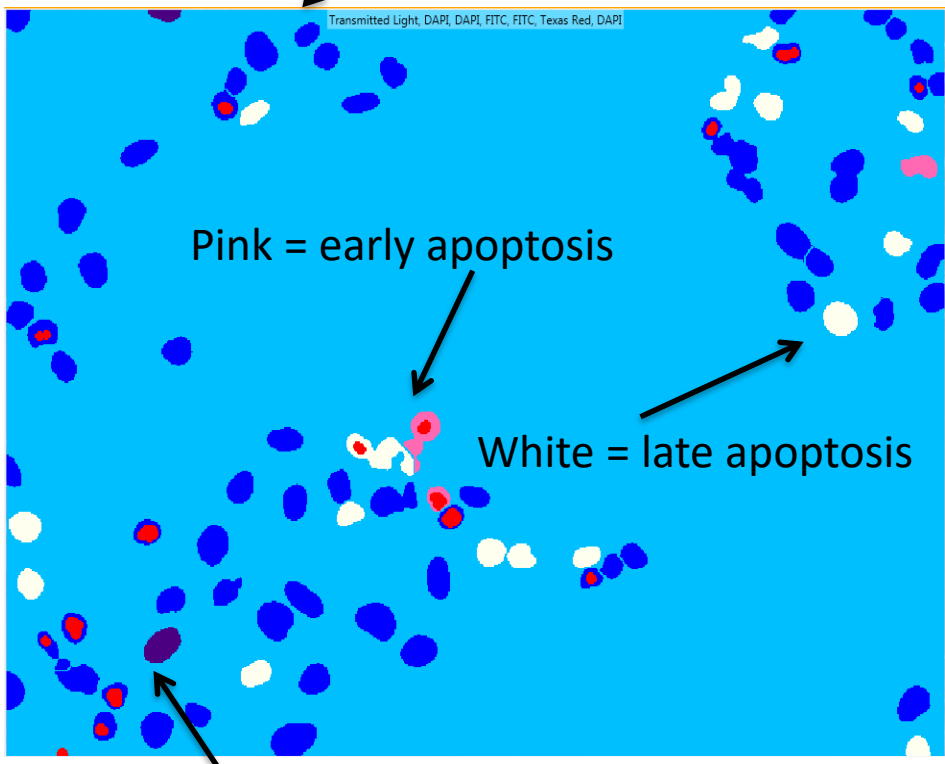


Nuclei **Apoptotic Cells** **Necrotic Cells**

Multi-parametric Image Analysis

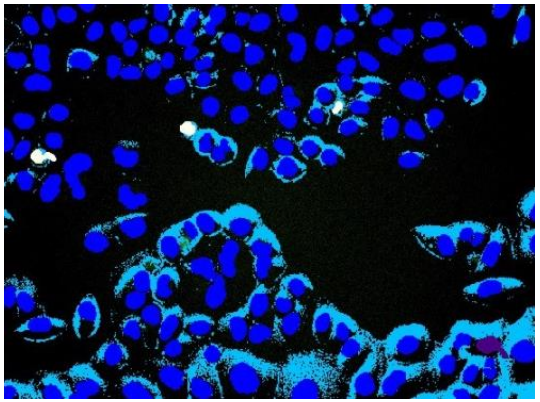


Royal blue = viable nuclei

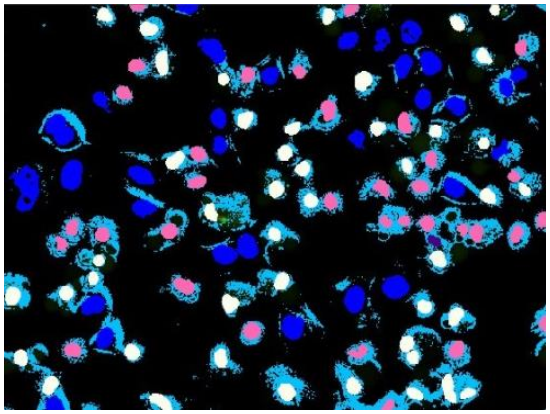


Purple = necrotic

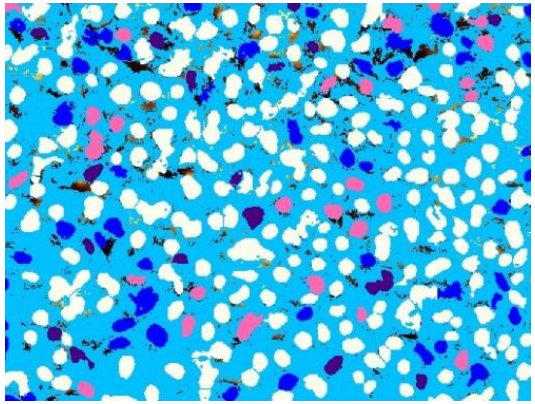
Multi-parametric Image Analysis



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

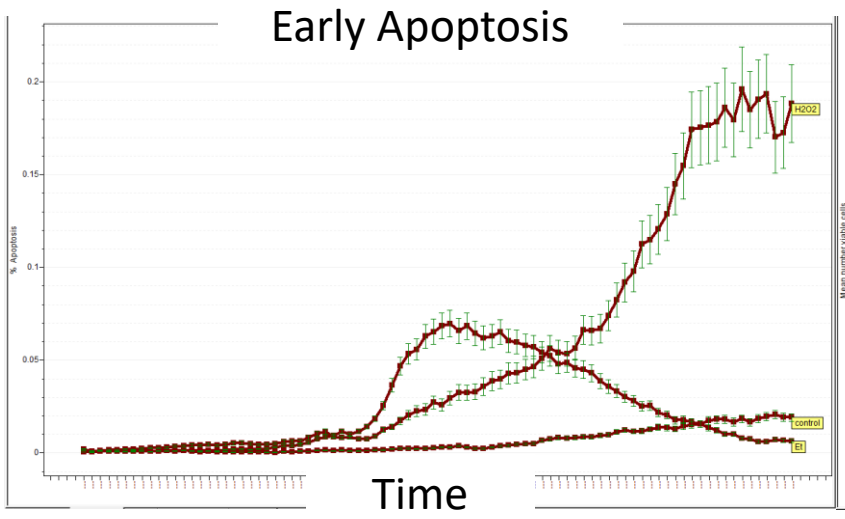


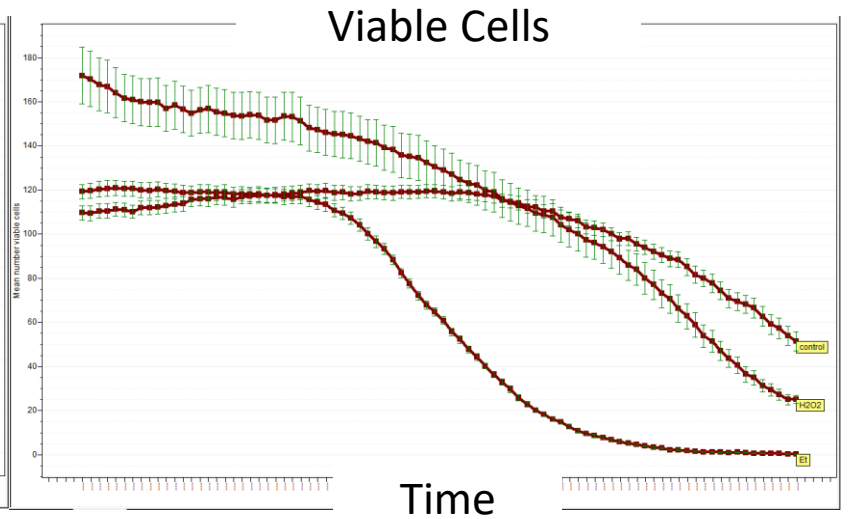
At time point 50, Etoposide treated cells show many cells in early apoptosis (pink mask) and late apoptosis (white mask)

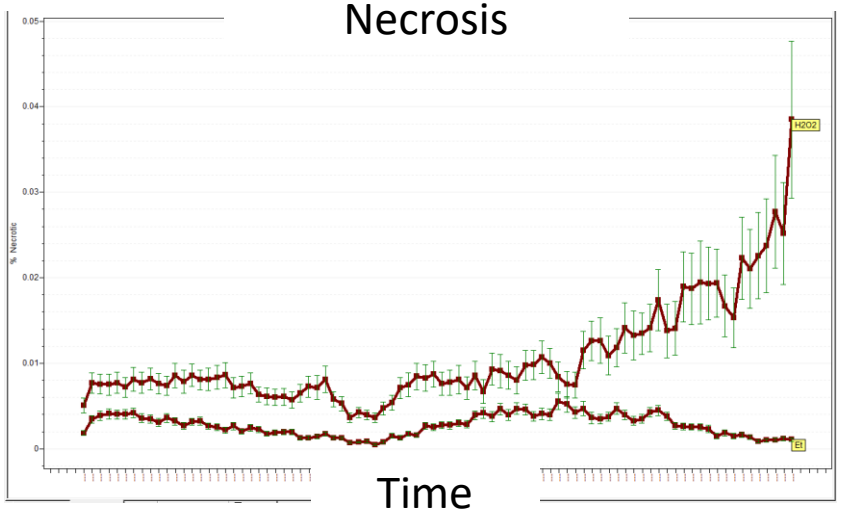


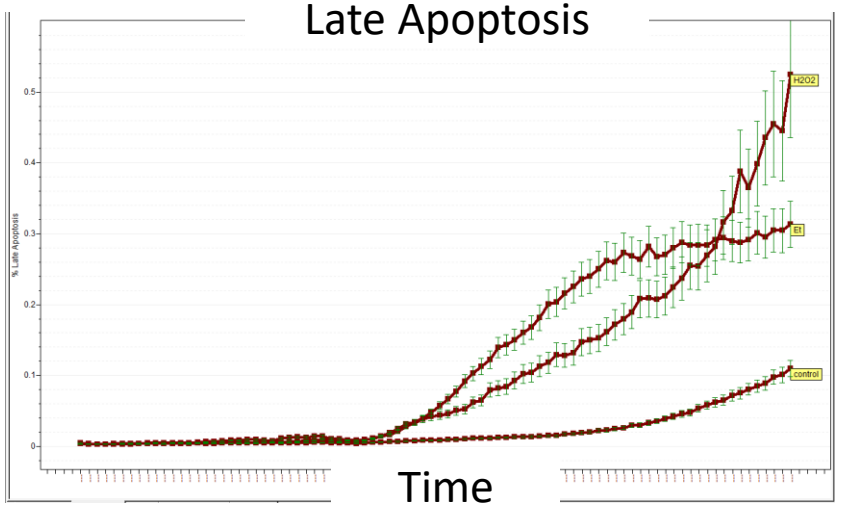
At time point 86, Peroxide treated well shows most in late apoptosis (white mask) and some suffered necrosis (purple mask)

Visualization of Cell Health









Complete Image Solution

IXM Features

High reading speed

3 color, 4 min/ 96 well

High quality Image

Hybrid autofocus system
Digital confocal module

User friendly interface

All in one system

Sample format flexibility

Slide, dish, multi-well plate

Live cell experiment

At least 3 day

Bench-top automation system

No darkroom requirement

High precision seamless splicing

– Auto splicing from 256 pictures of no-aperture slices

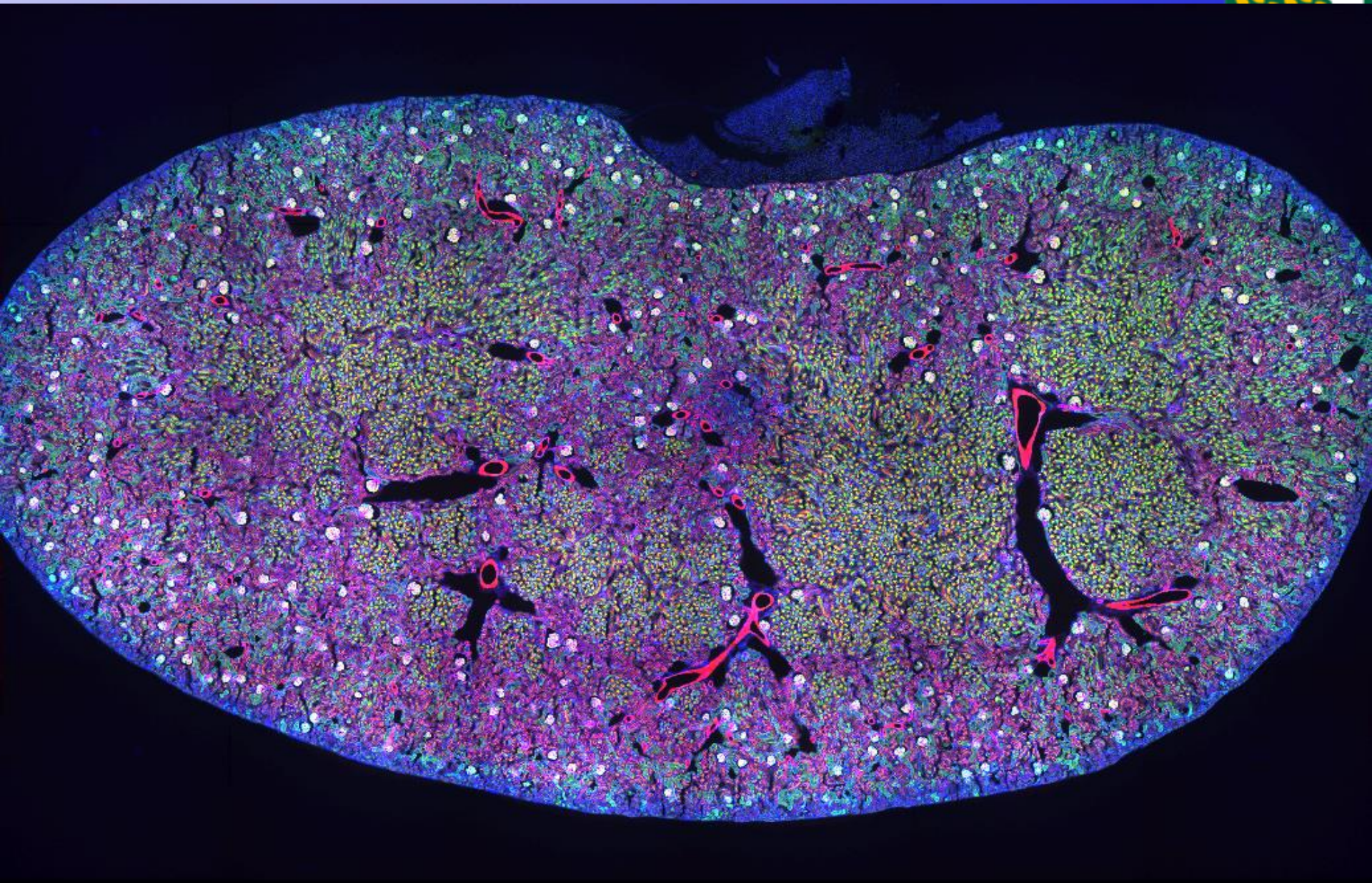
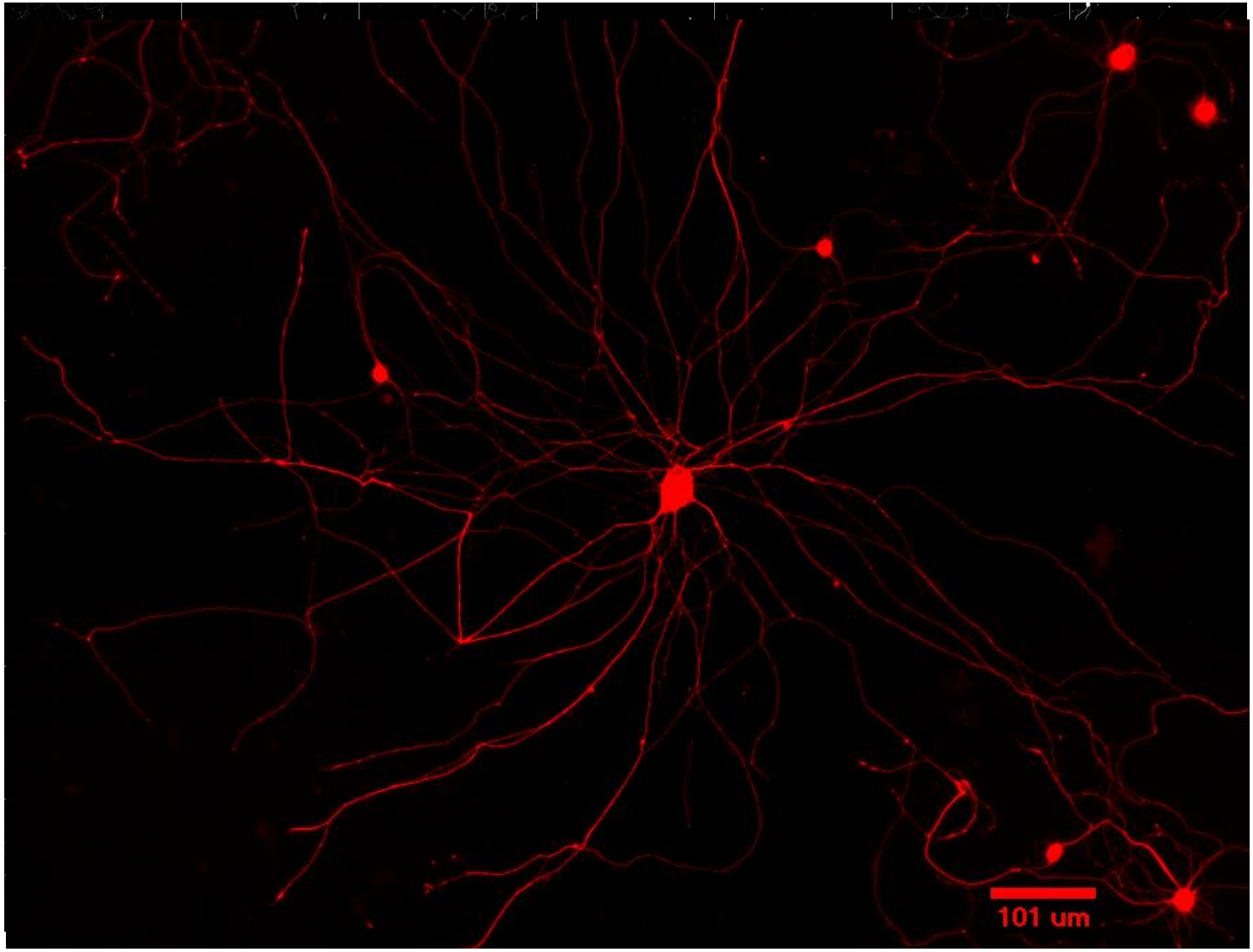
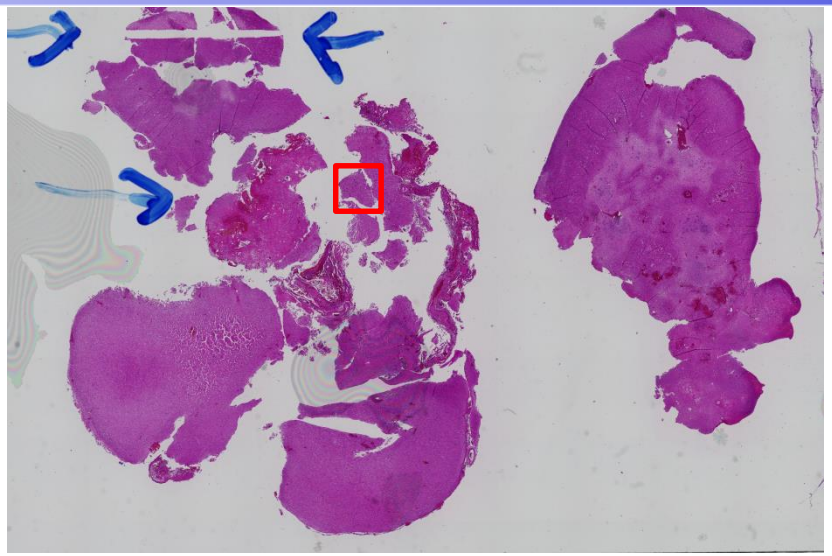


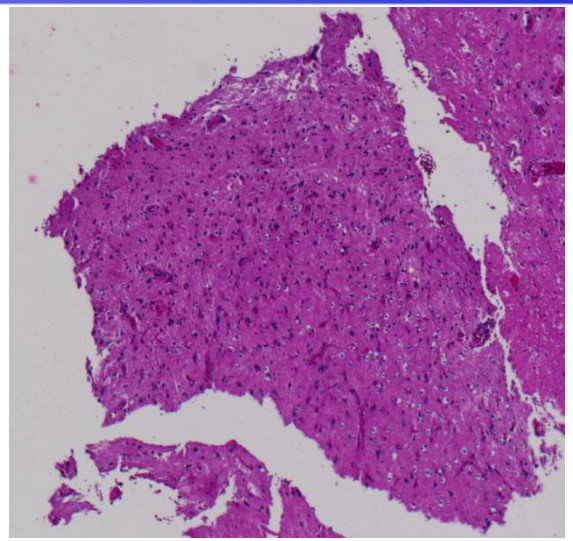
Image stitching



IHC Tissue Scanning

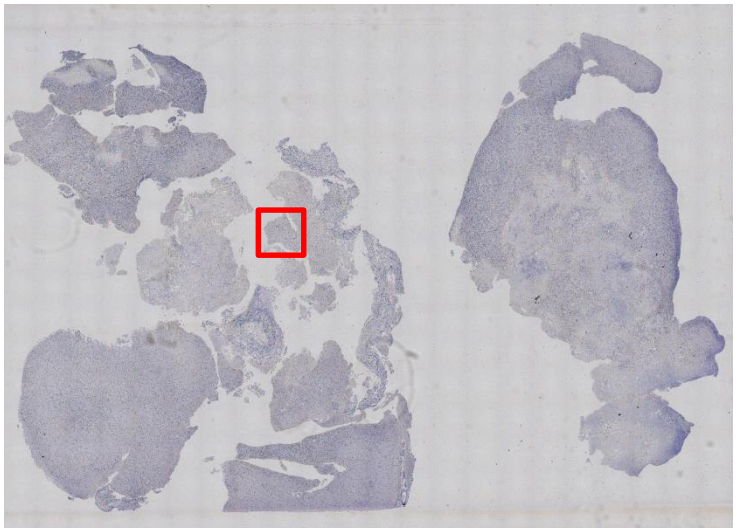
H & E staining

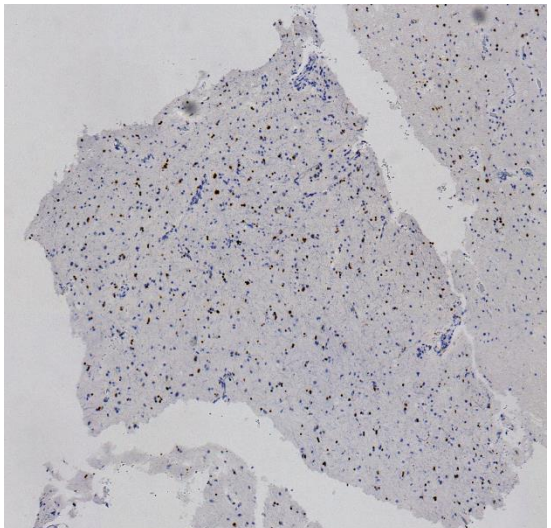




Acquired by 10X objective

HRP color reaction





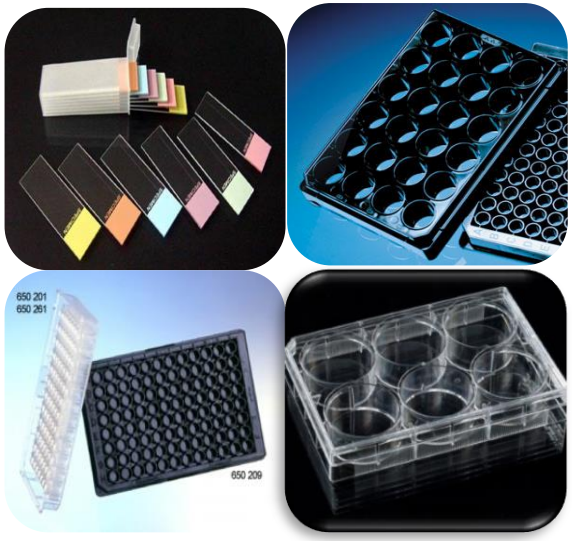
Various Objective & Plate Choice

- From 1X to 100X
- From Air to Oil

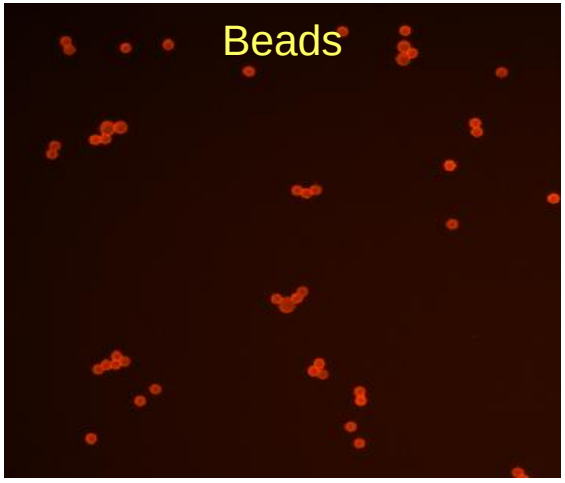
(4/10/20/40X in KMU)

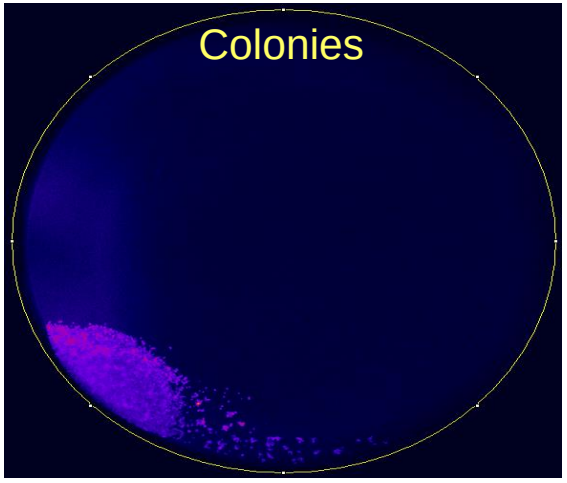
- From slide, plate to 1536 well

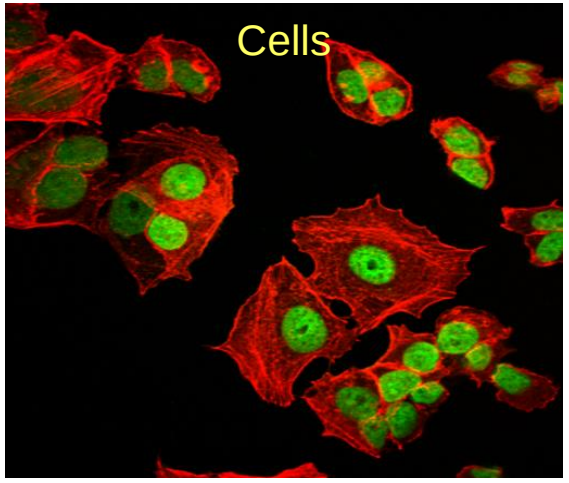
(Glass/plastic are both acceptable)

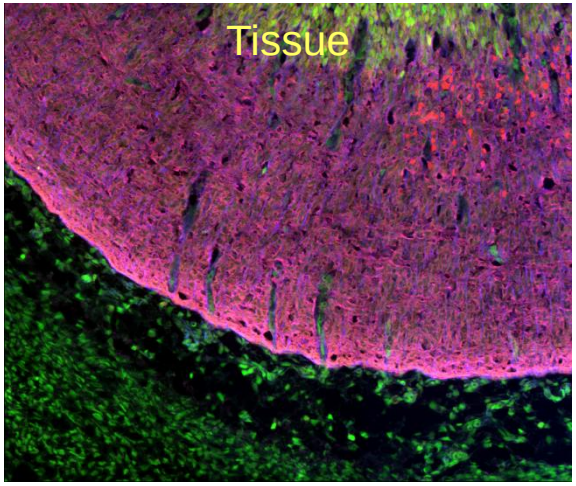


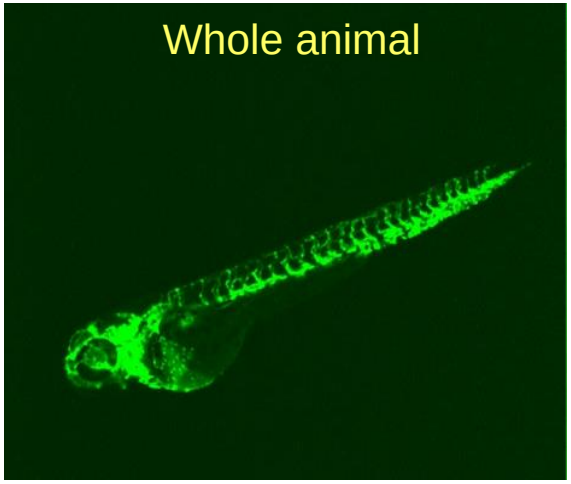
Sample Flexibility

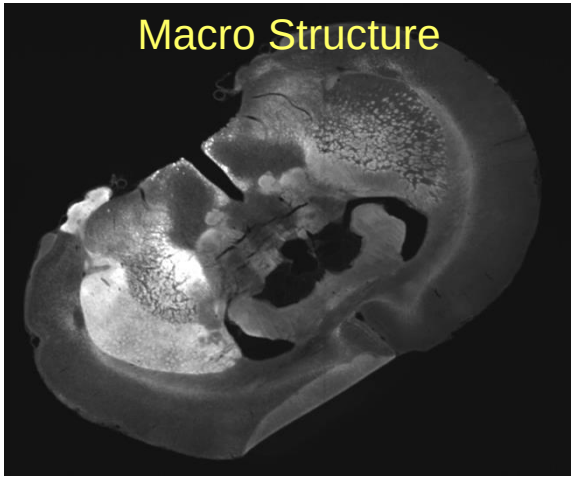






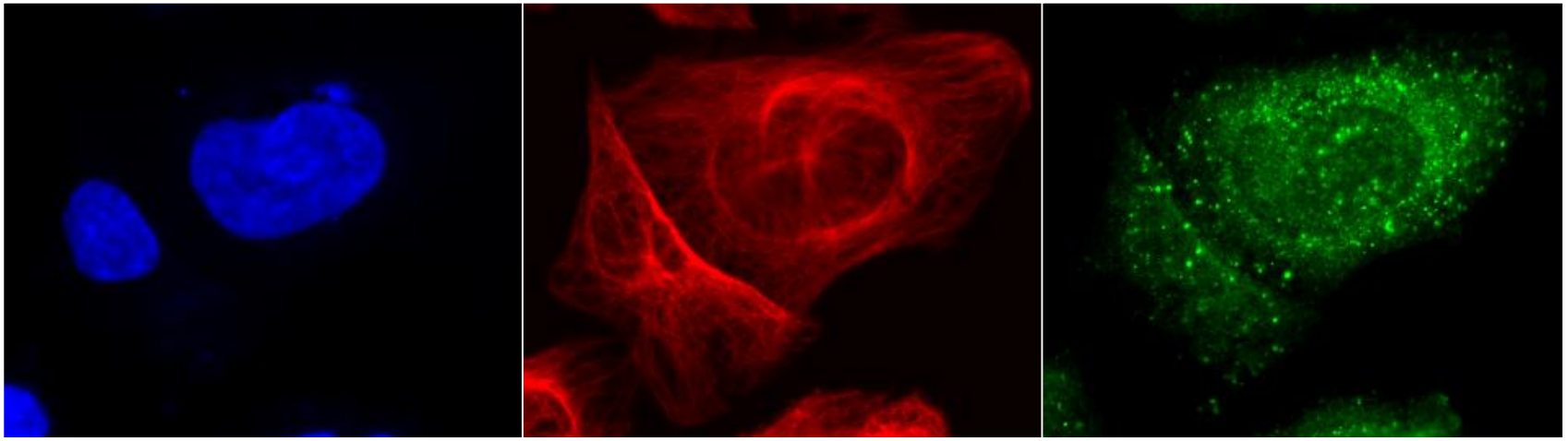




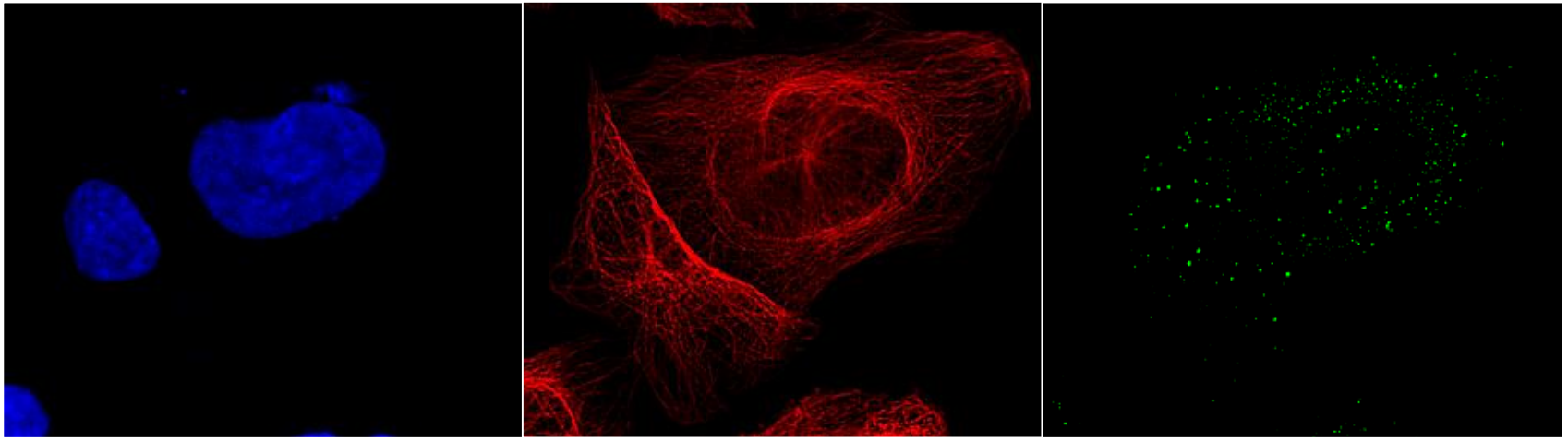


Digital Confocal

No Digital Confocal



With Digital Confocal



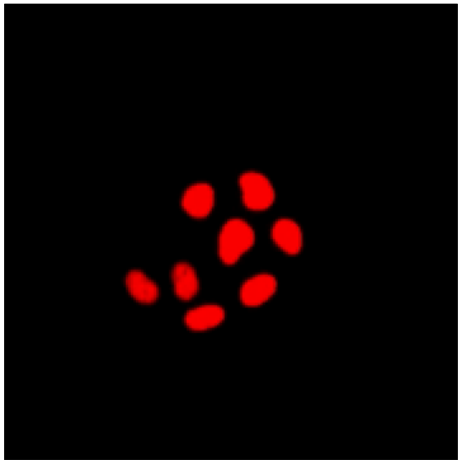
Environmental Control Module

IXM can monitor and control:

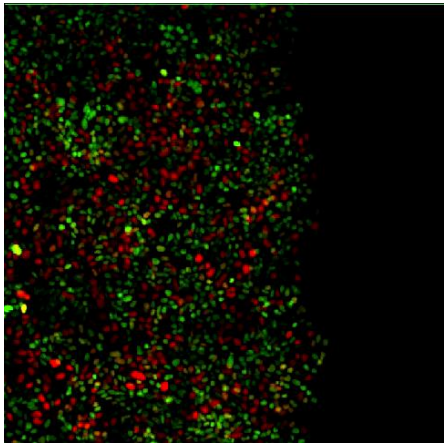
- Temperature
- Humidity
- Gas

Continuous time course for at least 3 days

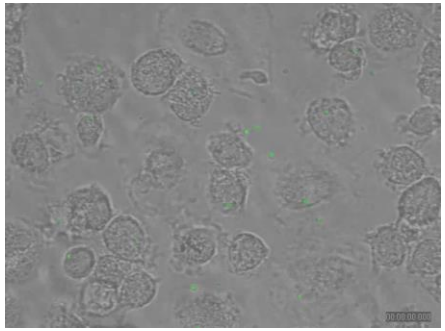
Colony formation



Migration assay



Bacteria invasion assay



Transmitted Light Module

Transmitted Light Module

- Bright field/ Phase contrast
- Köhler illumination
- Compatible with Environmental control system

Other Available Modules

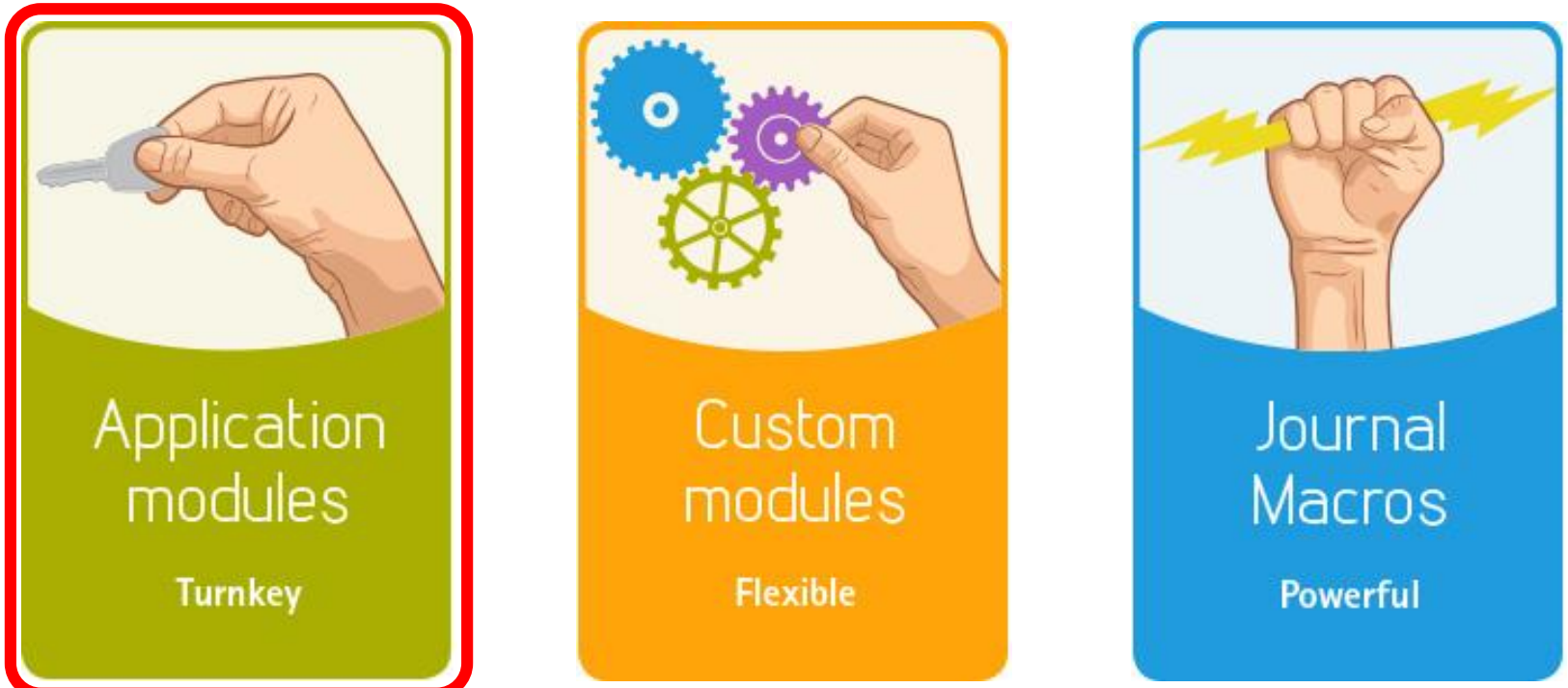
Liquid handling

- 96- or 384-well
- 2 compound plates available
- Plate heating

Robotic System

- Adequate to most robotic systems
- Compatible with Environmental control and Transmitted light systems

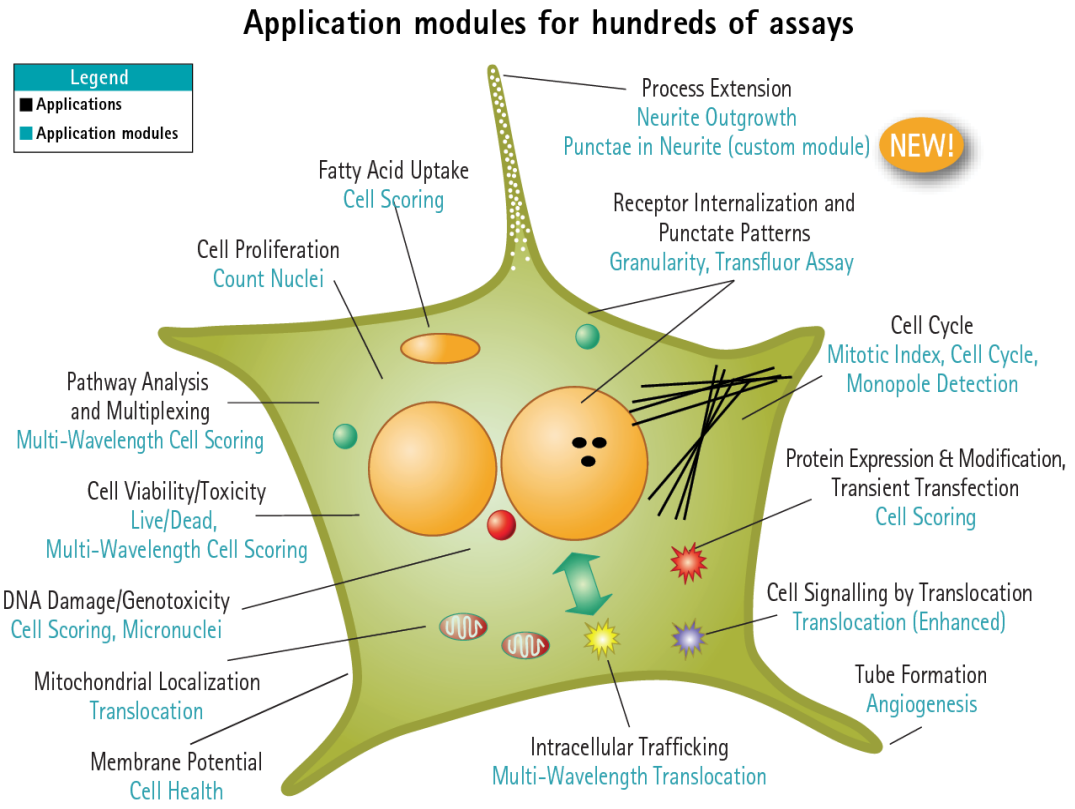
Comprehensive Image analysis tools



MetaXpress® Software

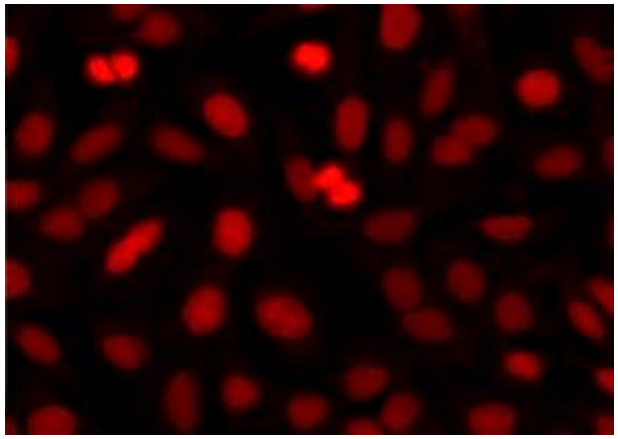
Application Modules

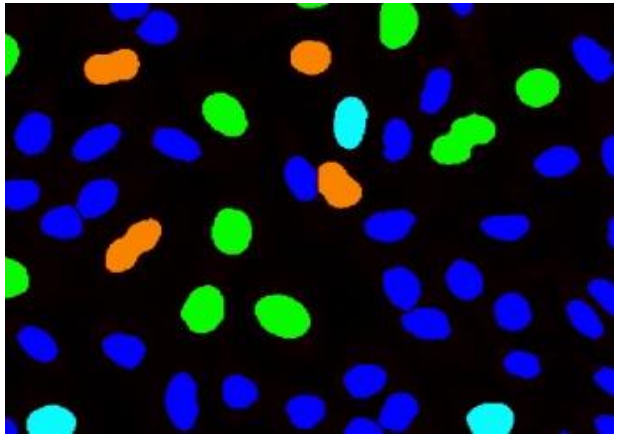
1. Micronuclei
2. Neurite Outgrowth
3. Angiogenesis Tube Formation
4. Mitotic
5. Cell Cycle
6. Monopole Detection
7. Cell Scoring
8. Multi-Wavelength Cell Scoring
9. Count Nuclei
10. Cell Proliferation HT
11. Cell Health
12. Live/Dead
13. Granularity
14. Transfluor
15. Transfluor HT
16. Translocation
17. Translocation-Enhanced
18. Multi-Wavelength Translocation
19. Nuclear Translocation HTs

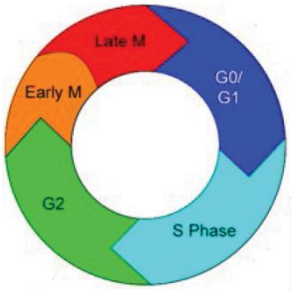


Applications: Cell cycle

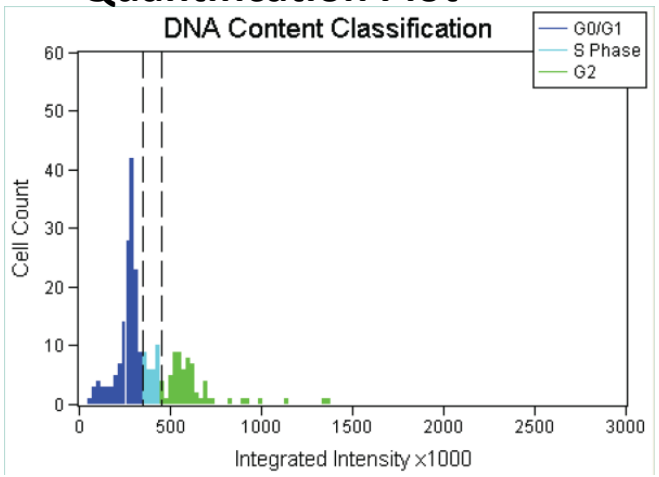
DAPI

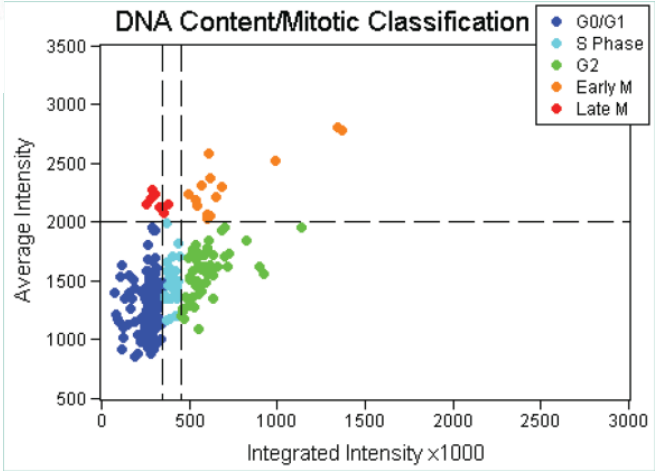






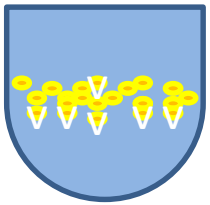
Quantification Plot



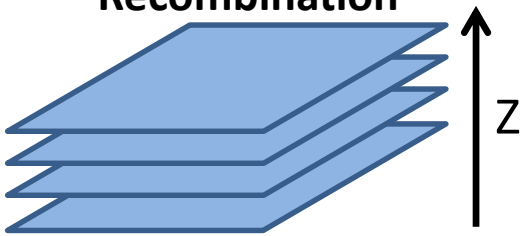


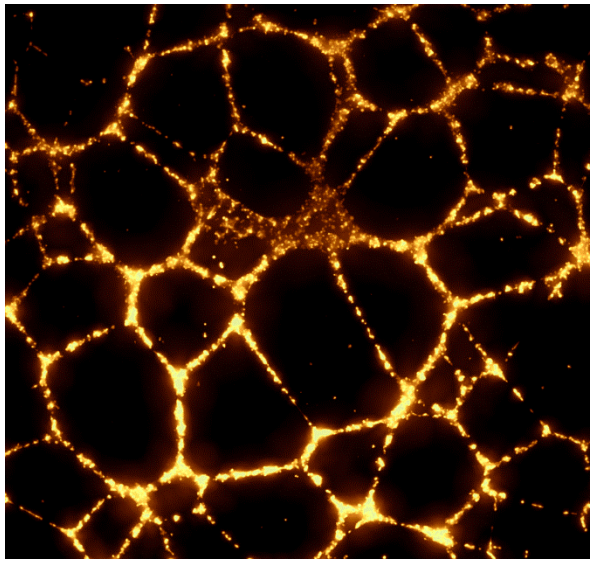
Applications: Angiogenesis

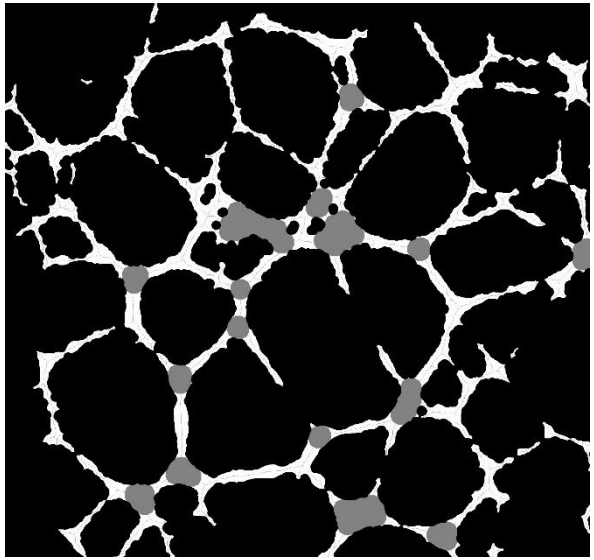
Locating and Scanning



Best Focus Recombination





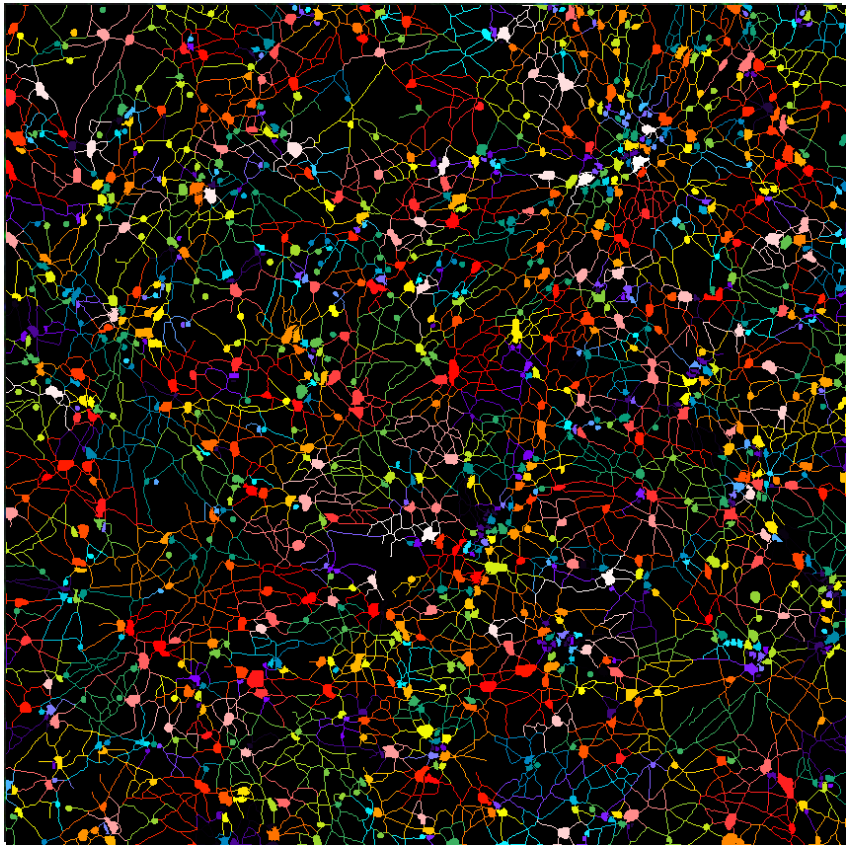


Measurements:

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

Applications: Neurite outgrowth

Nuclear β-tubulin



Quantification

Cellular Results for Neurite Outgrowth

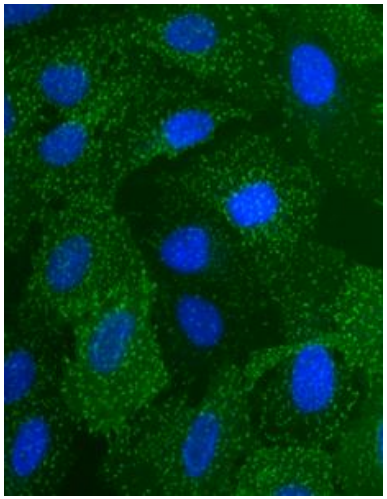
	Cell: Assigned Label #	Cell: Total Outgrowth	Cell: Processes	Cell: Mean Process Length	Cell: Median Process Length	Cell: Max Process Length	Cell: Branches	Cell: Straightness	Cell: Cell Body Area	Cell: Mean Outgrowth Intensity
1	1	35.124	2	17.562	17.562	27.9832	2	0.908596	39.9384	574.945
2	2	0	0	0	0	0	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

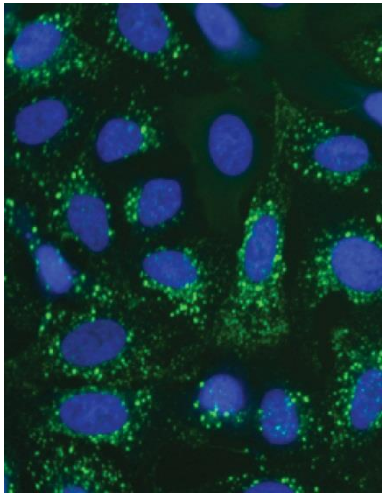
Measurements:

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

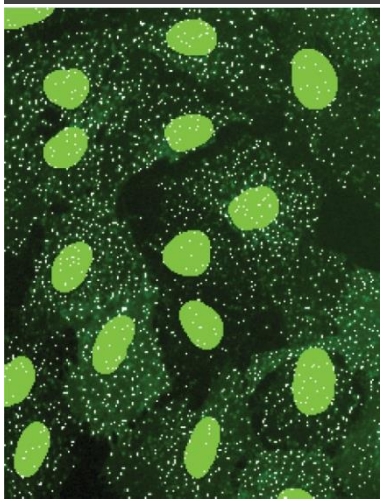
Applications: Transfluor

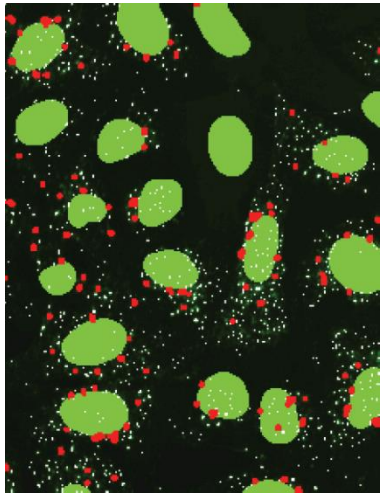
Nuclear β-arrestin



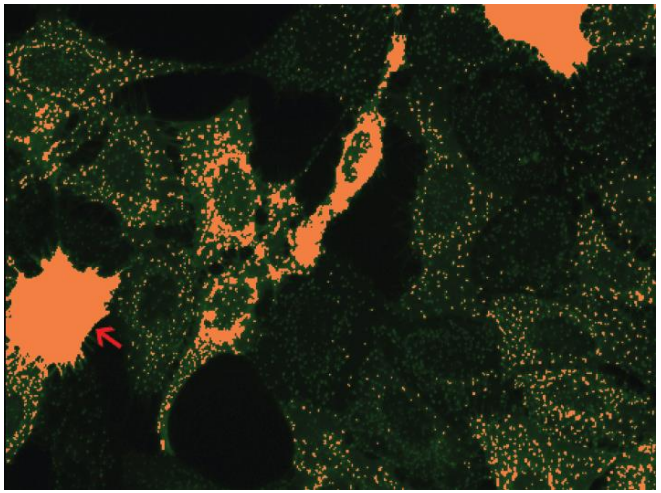


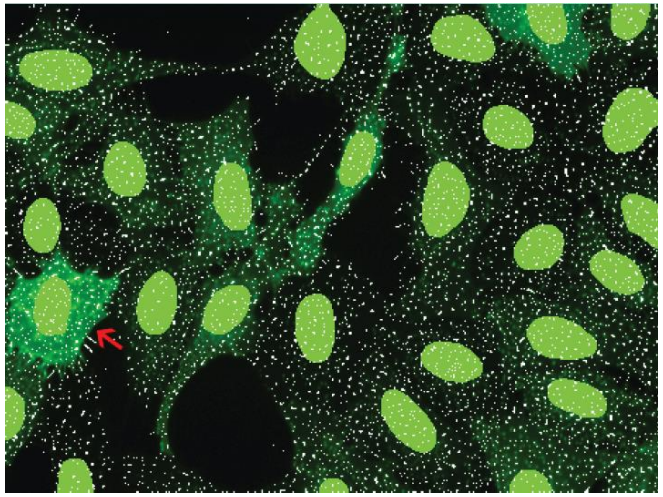
Nuclear Pit Vesicle





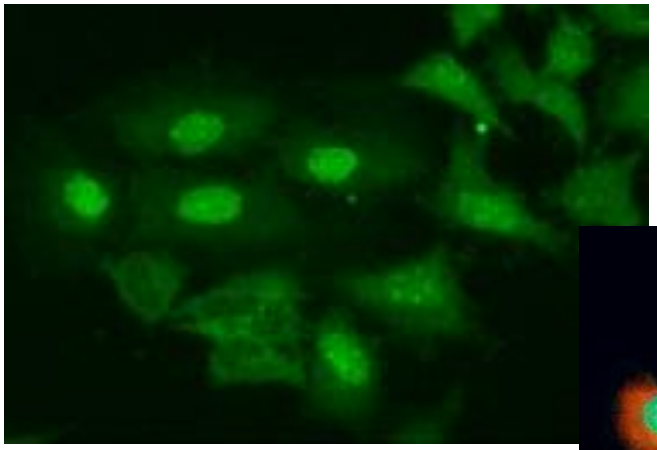
Adaptive Background Correction





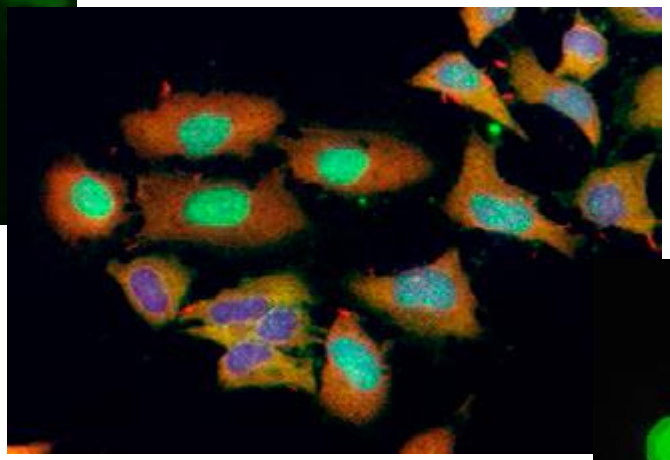
Applications: Translocation

Image of Translocation Protein

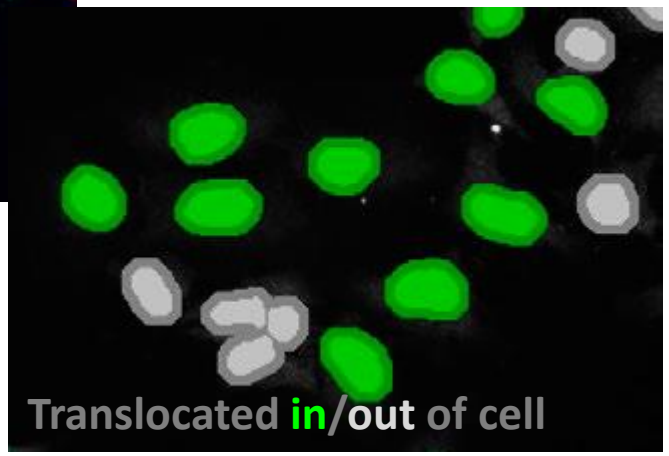


Translocation protein
Nuclear
Cytoplasm

Merged Image



Analysis Image



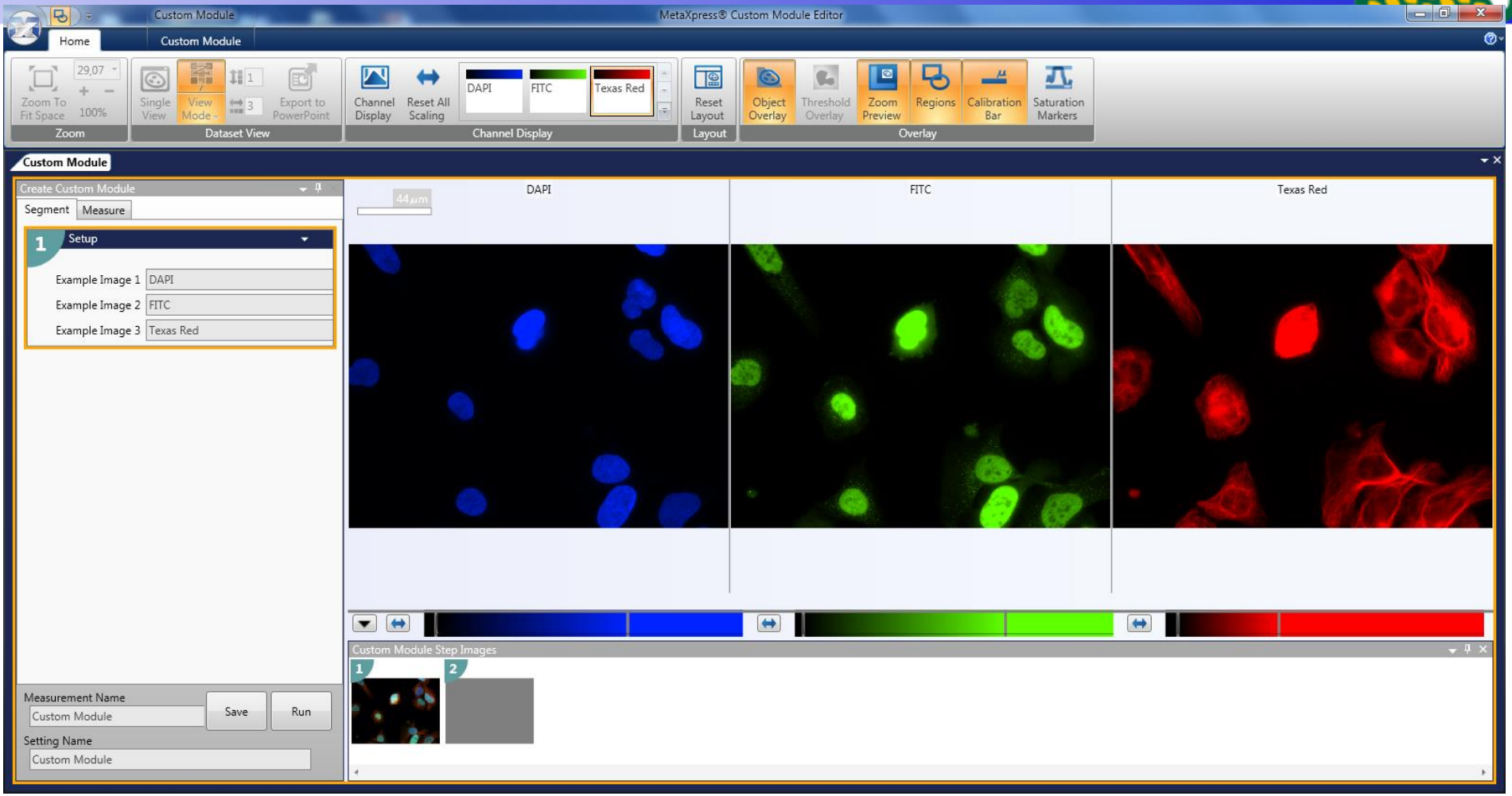
Measurements:
Count and percentage of positive/negative cells, scoring profile account, wavelength-specific intensity statistics…

Comprehensive Image analysis tools

Analysis continuum with increasing flexibility and complexity

MetaXpress® Software

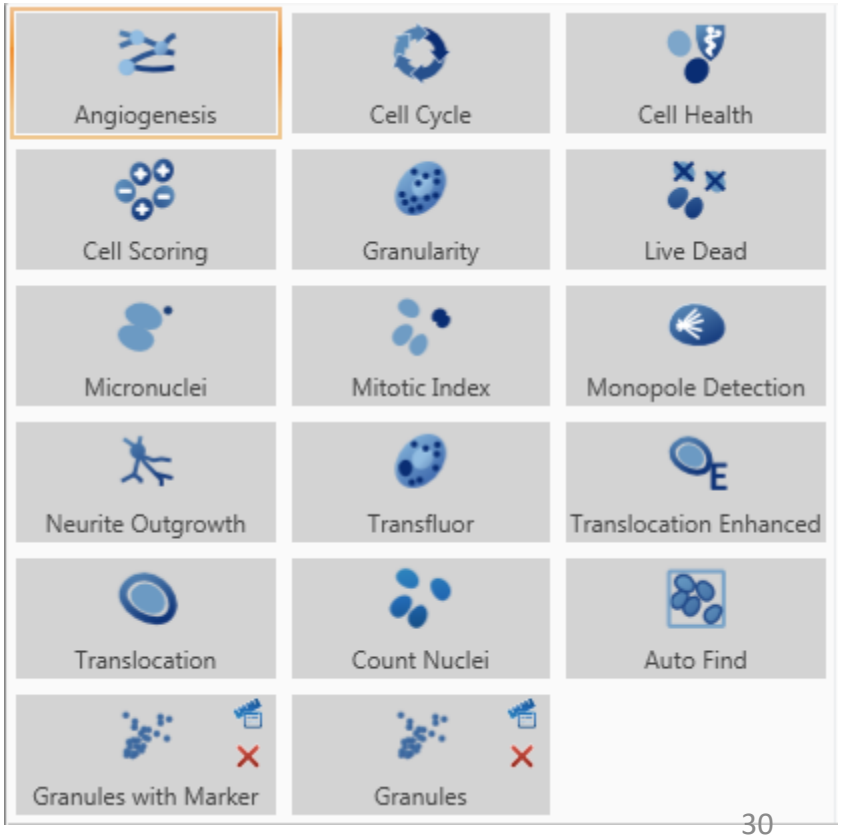
Intuitive Interface



Assign image names to wavelengths

Including all Application Modules and analysis components

- **Custom Modules**
  - Use the Analysis Builder to make custom modules
  - Open the Analysis Builder using the Create Module on the Modules palette

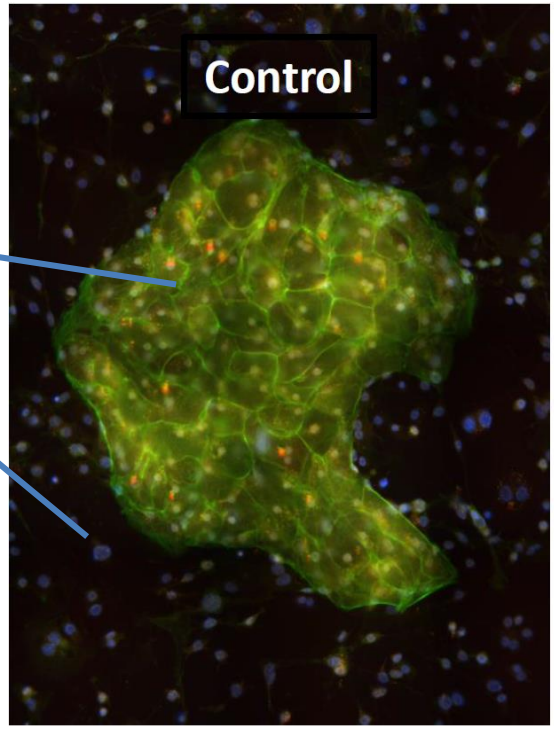


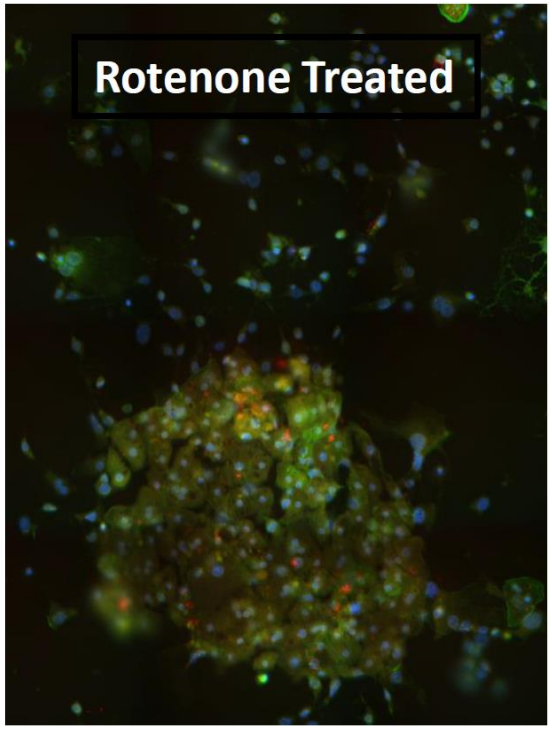
Hepatocyte Co-Culture Model

Hepatocyte island

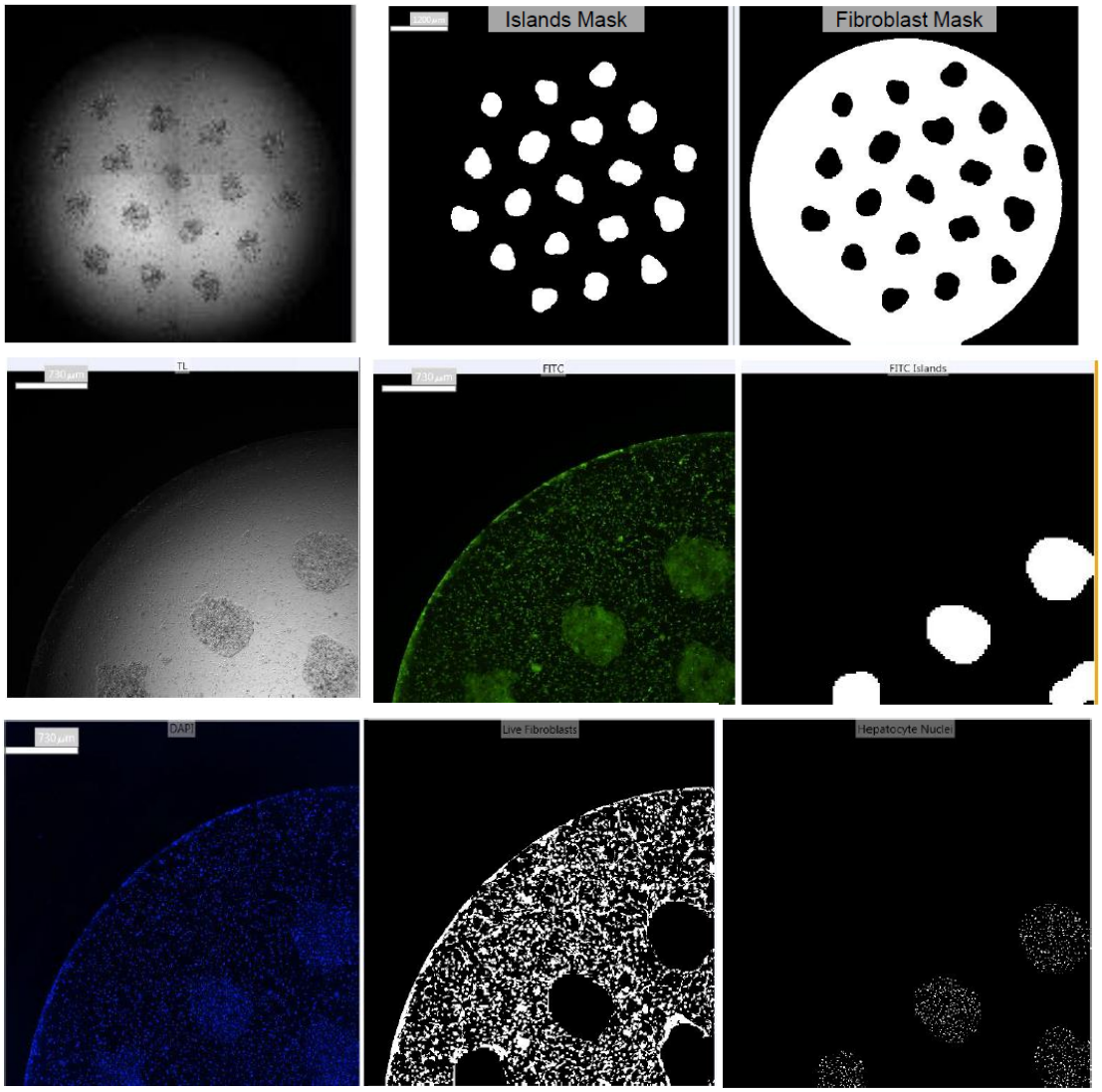
Fibroblast

Nucleus
Actin
Mitochondria





Customized analysis workflow



Identify Islands and Fibroblasts

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

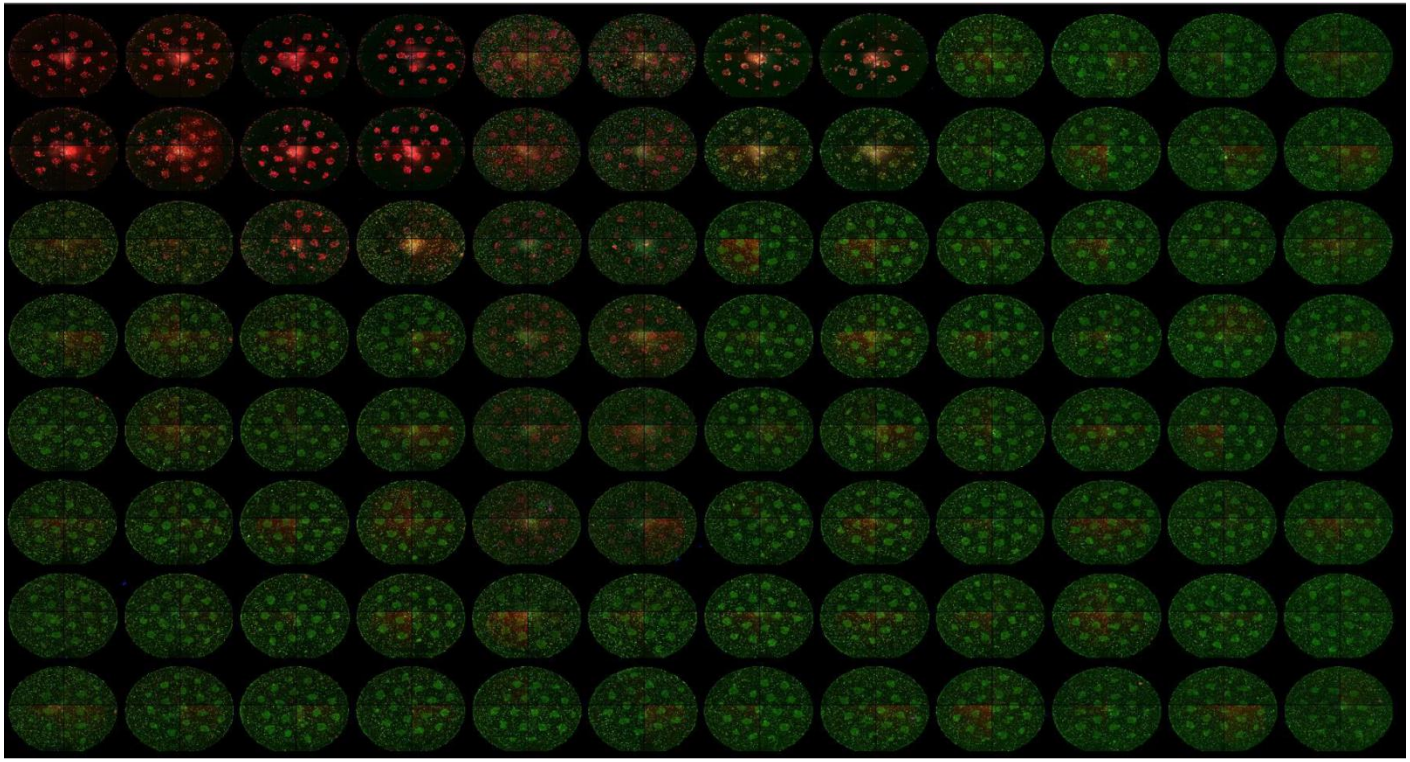
Combine TL and Fluorescent Images

- **DAPI/Mito intensity**

Determine parameters inside each group

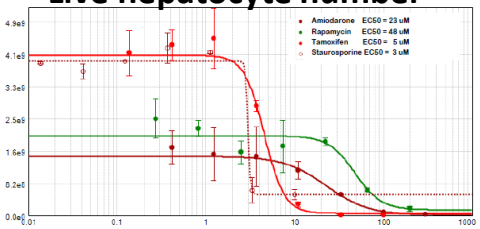
- **Live cell number of island/ fibroblast**
- **Mitochondria intensity of each cell**

Image Overview & Toxicity Evaluation

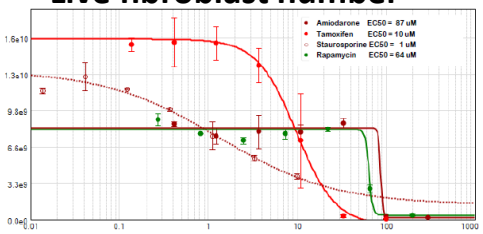


Compound concentration

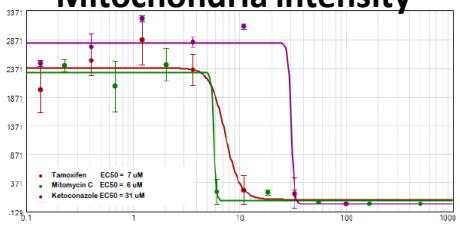
Live hepatocyte number



Live fibroblast number

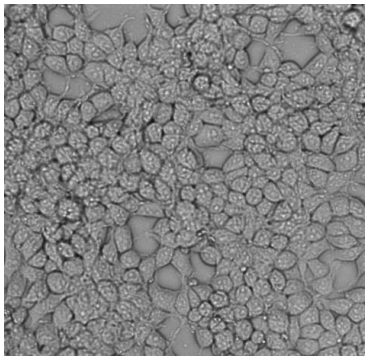


Mitochondria intensity

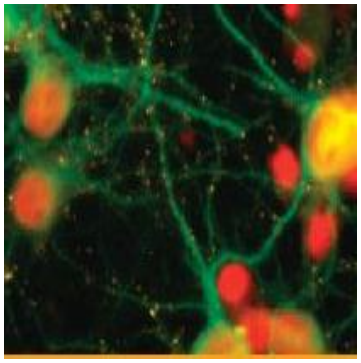


MetaXpress- Custom Module Editor

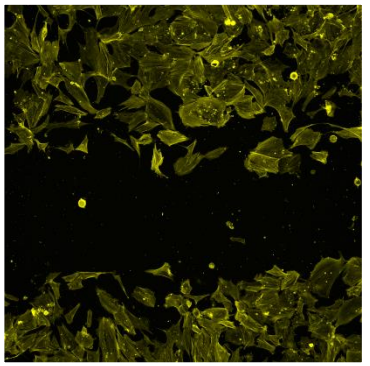
Proliferation with Transmitted Light



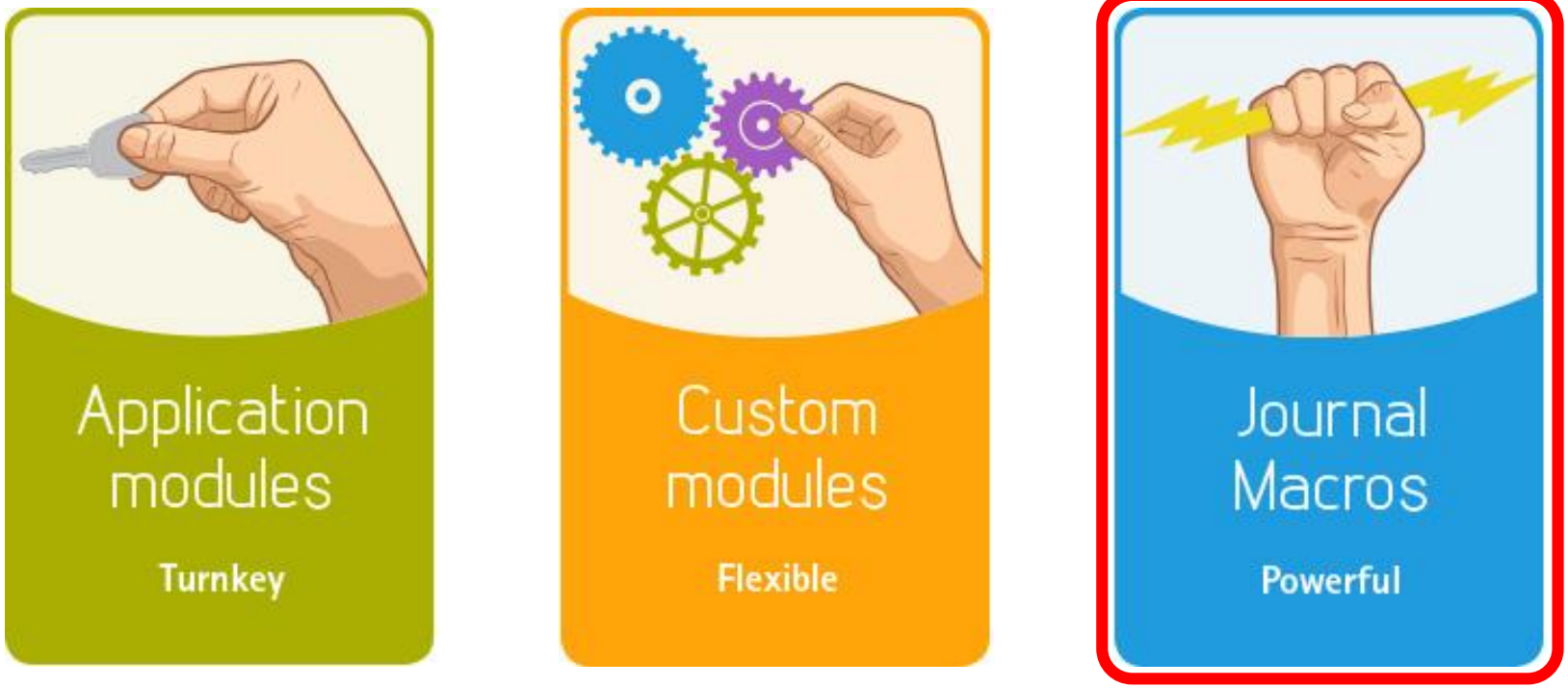
Neurite Outgrowth and Puncta



Wound Healing



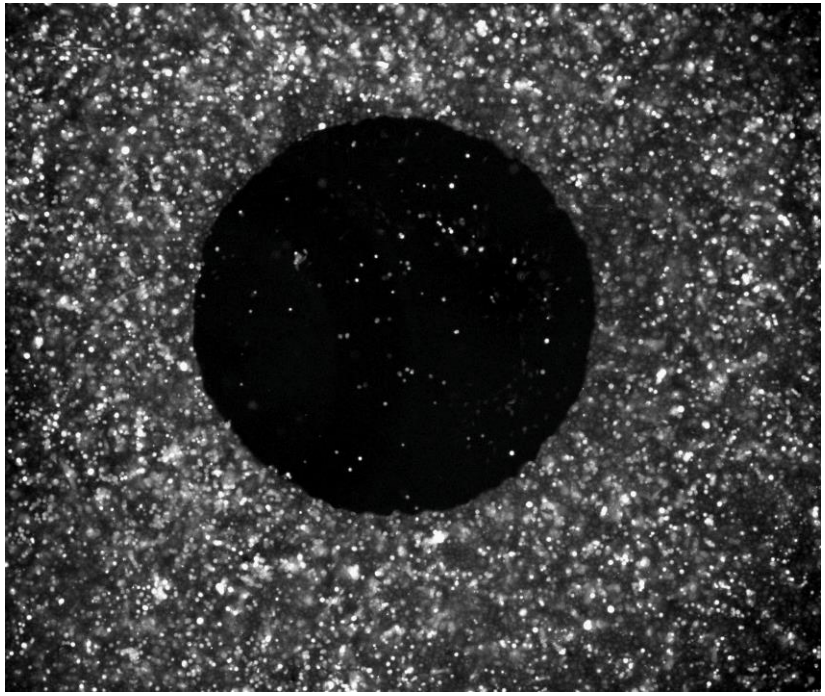
Comprehensive Image analysis tools



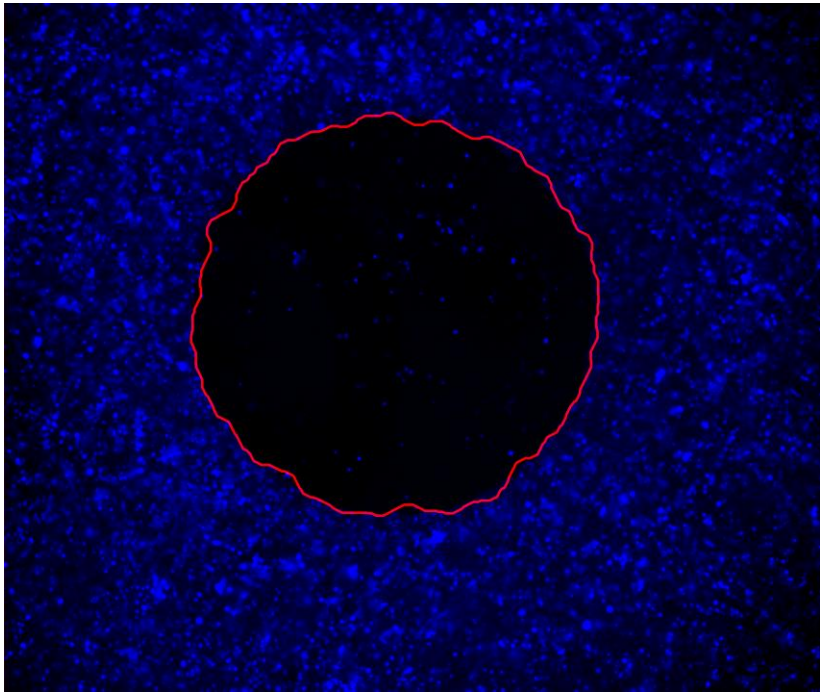
MetaXpress® Software

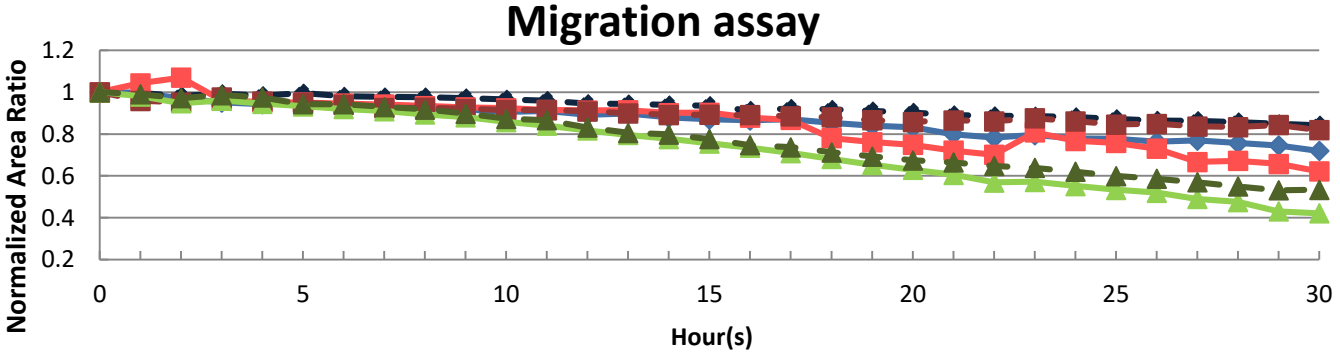
Migration/Wound Healing Assay

Original Image

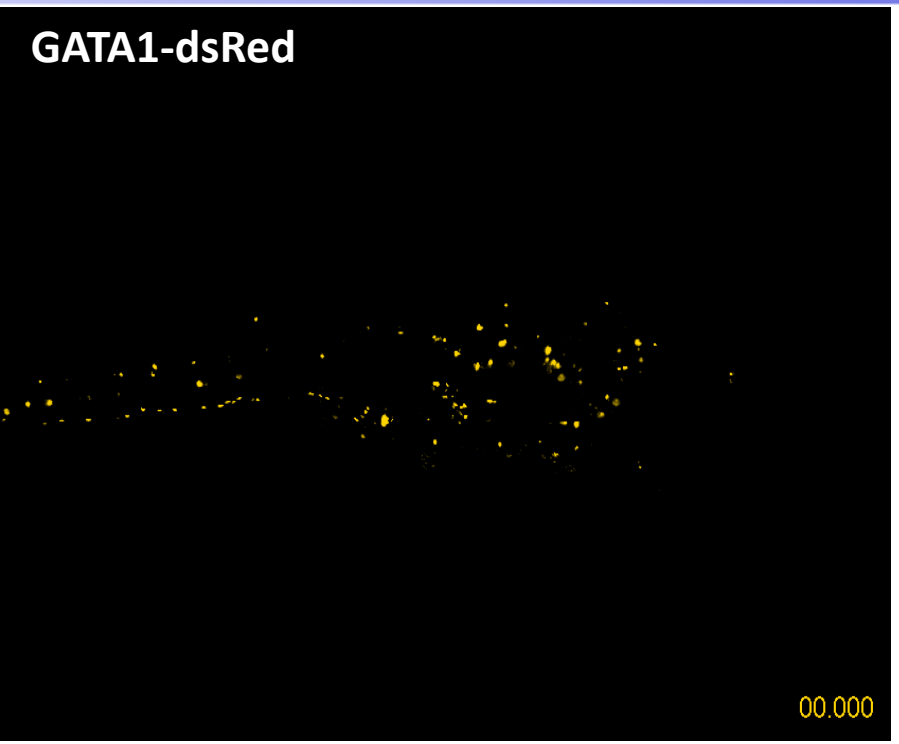


Analysis Image

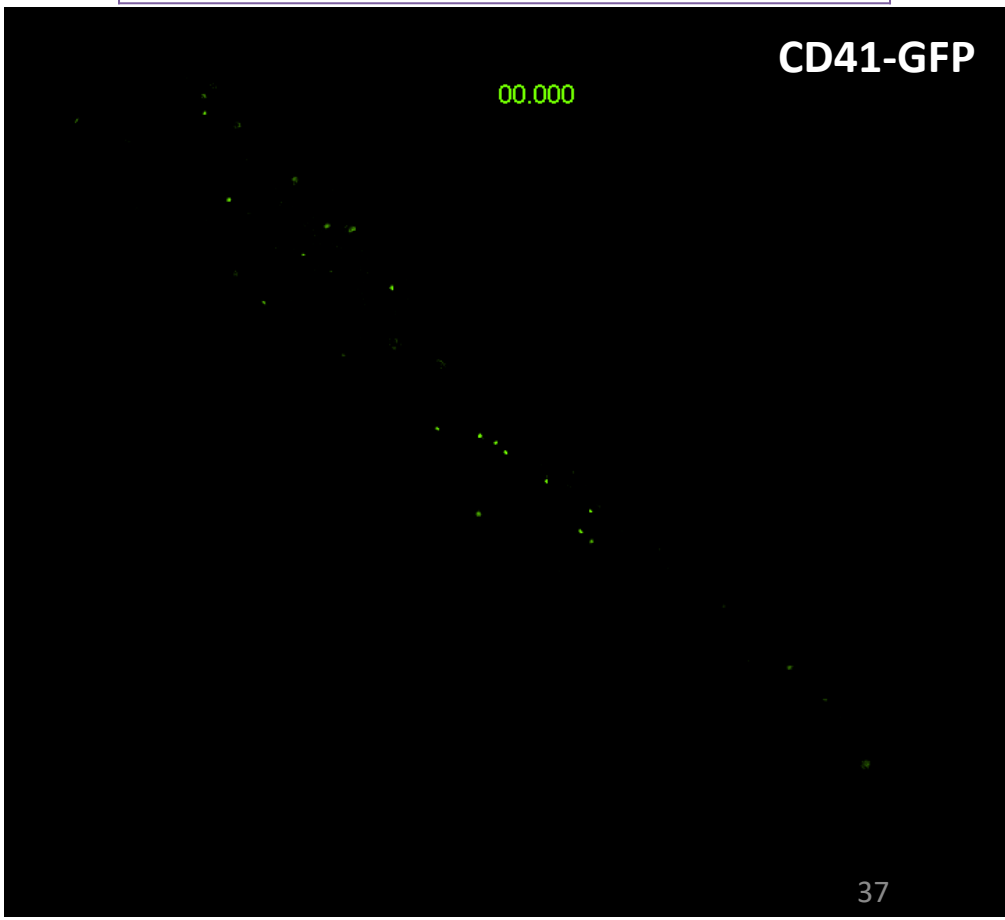




Analyzing Circulating Cells in Zebrafish

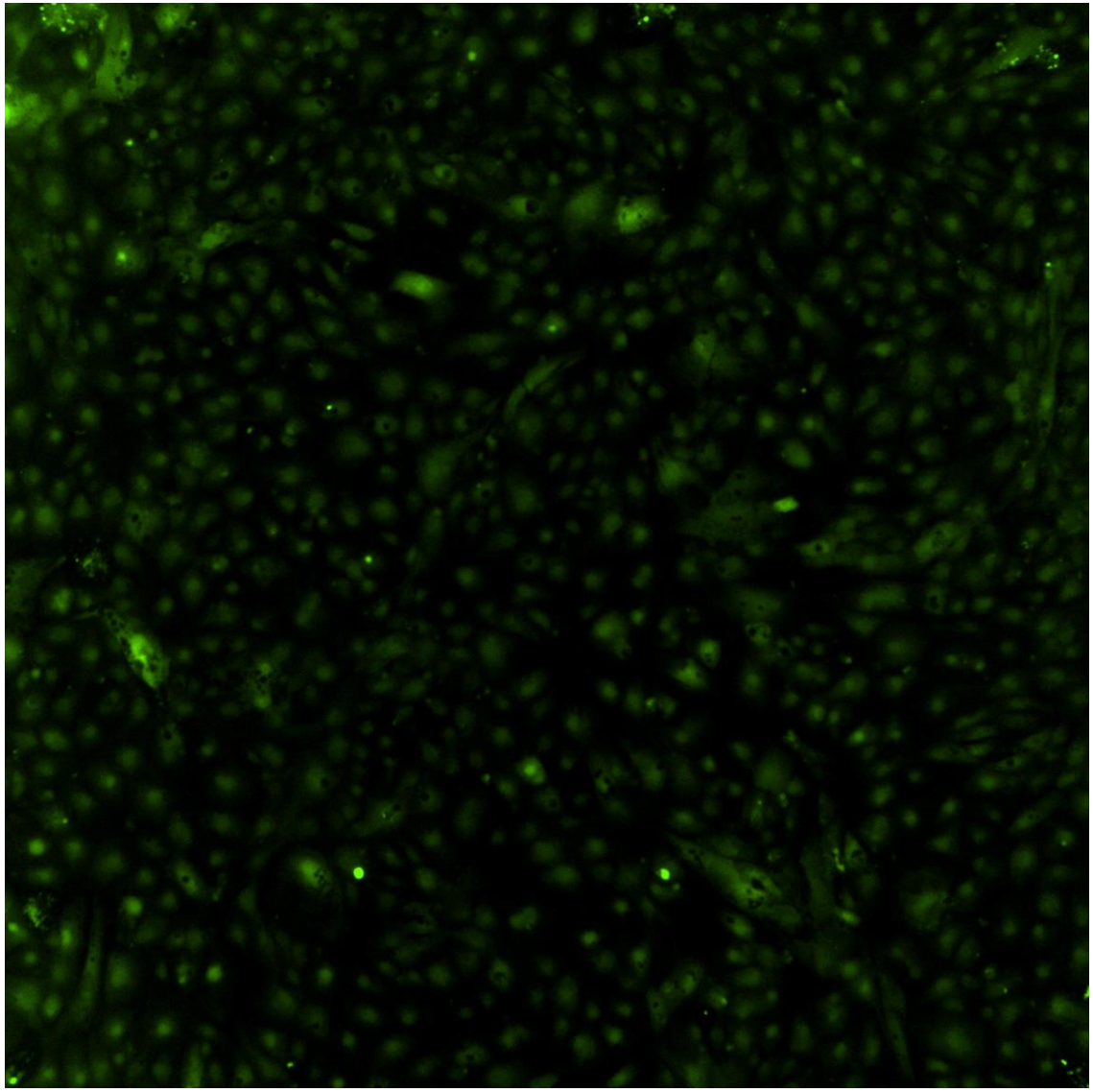


Number of Circulating Cells	
GATA1	CD41
92	25



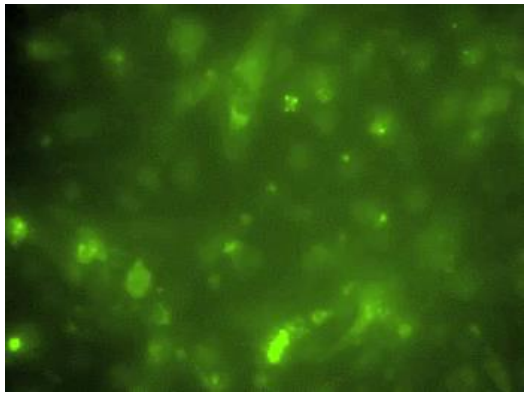
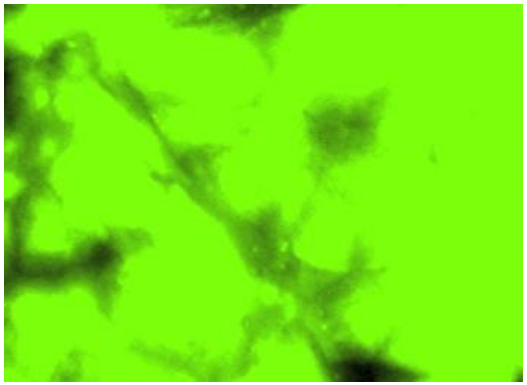
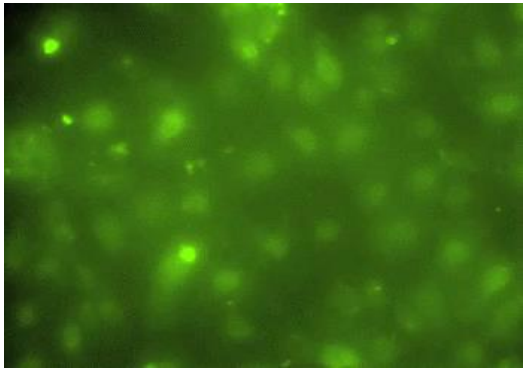
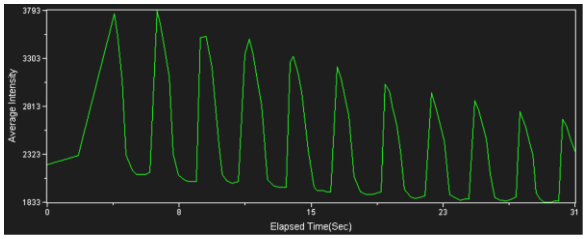
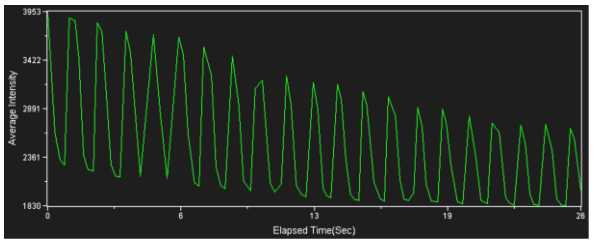
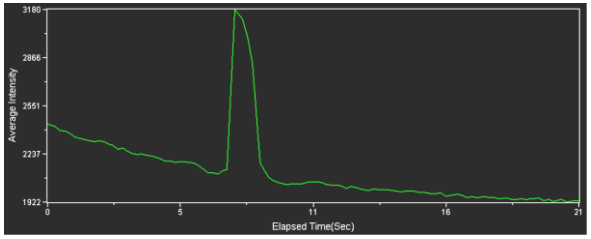
- Remove anything static from the image to be able to measure the cells that are actually circulating

Cardiomyocyte assay for toxicology studies



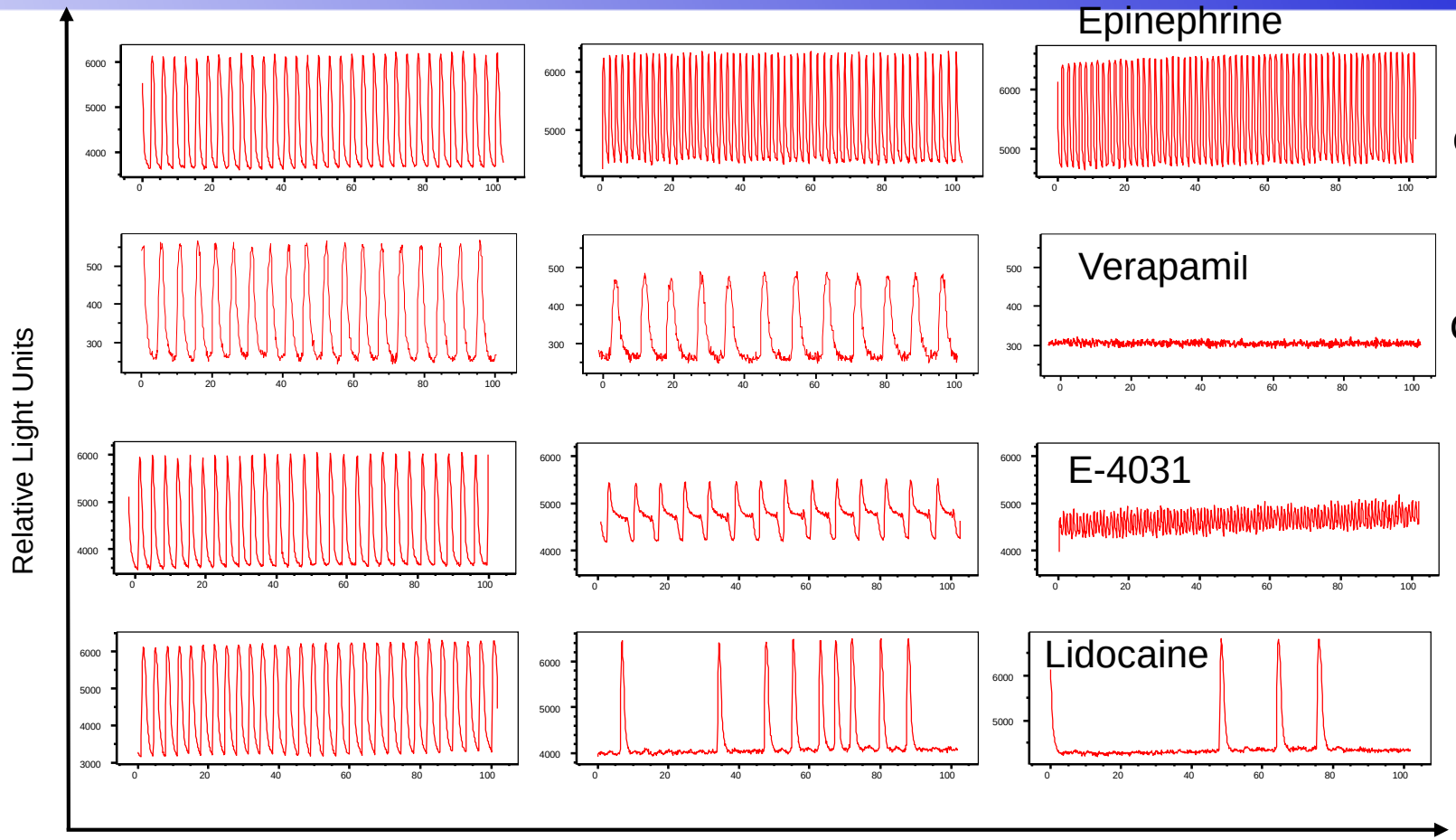
- FLIPR Calcium 6 Assay Kit
- 10X Plan Apo
- 100 FPS

Analysis of beat rate in cardiomyocytes from Ca^{2+} flux

Control	Epinephrine	Verapamil
		
		

- Cell contractions visualized with Ca^{2+} sensitive dye
- Ca^{2+} levels fluctuate with contraction events
- Provide surrogate assessment of beat rate and sarcolemmal activity

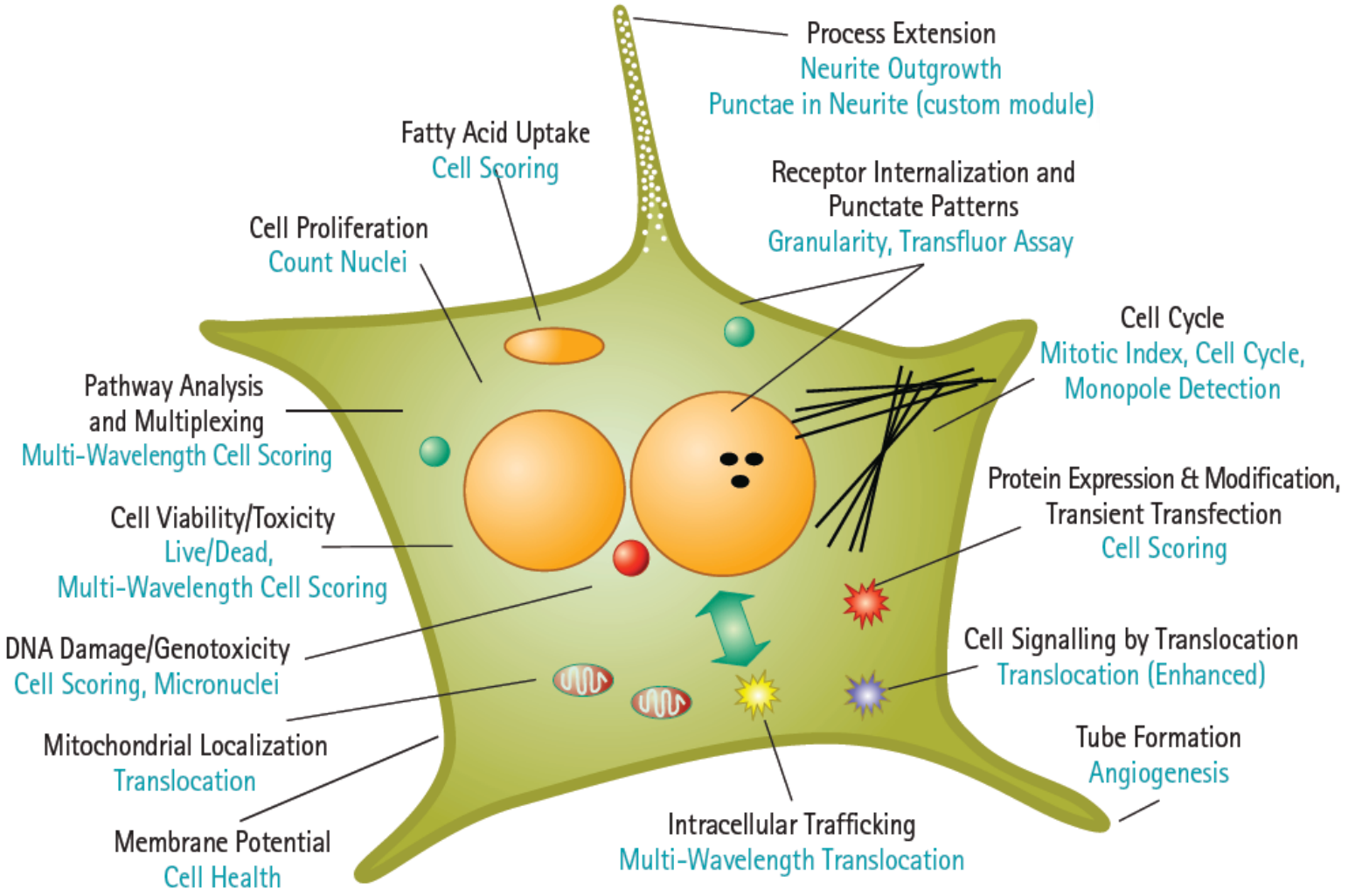
Characteristic Beating Profiles



Sirenko et al, J Biomol Scr, 2013

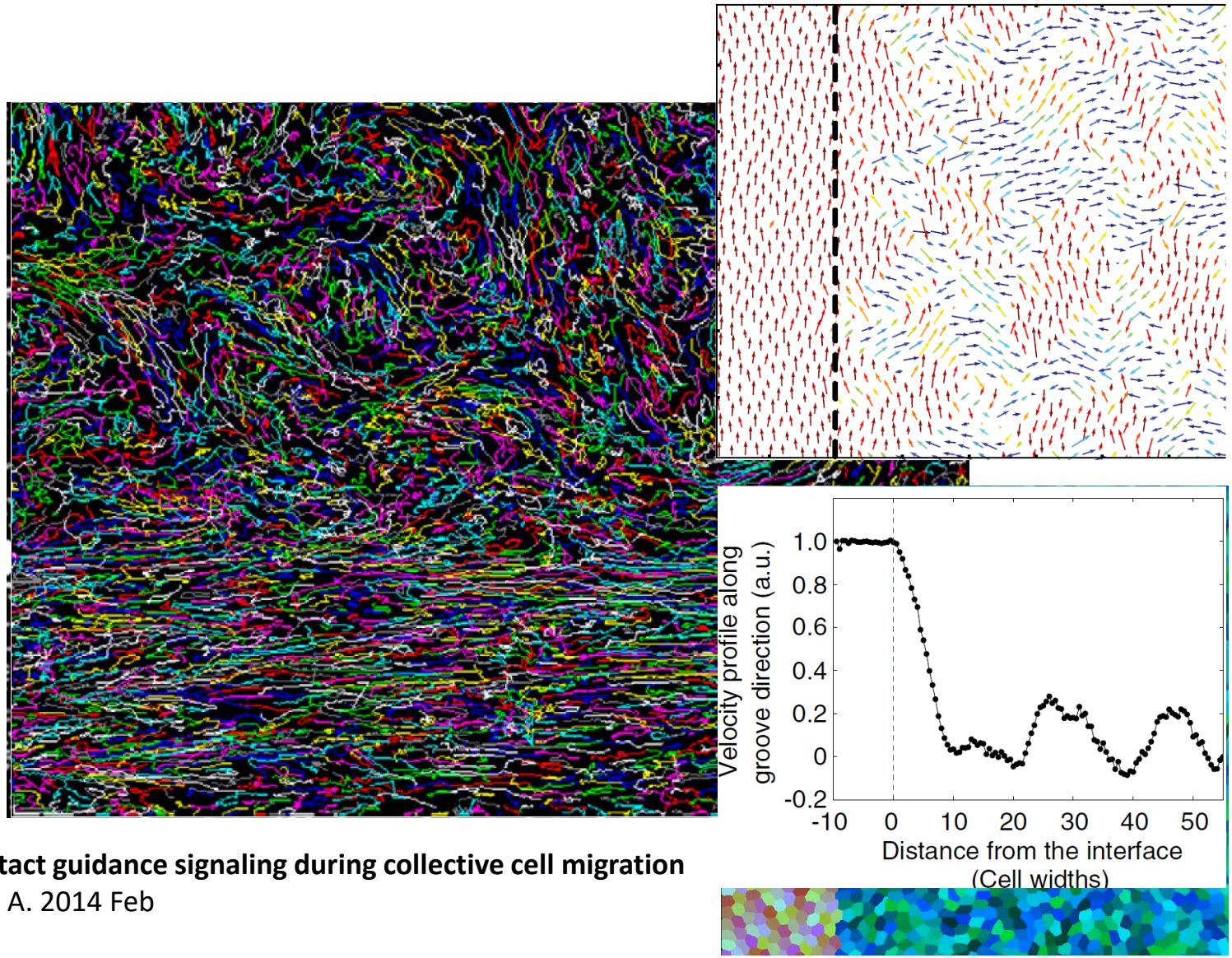
Sky is the limit!

Applications for HUNDREDS of assay



Case Study

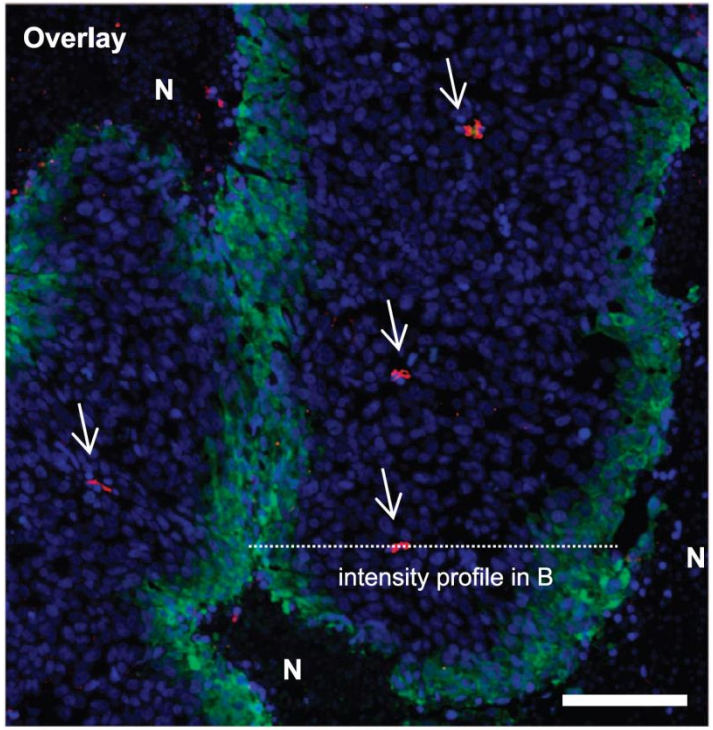
Cell Migration Assay



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

Hypoxia Study in Spheroid Model

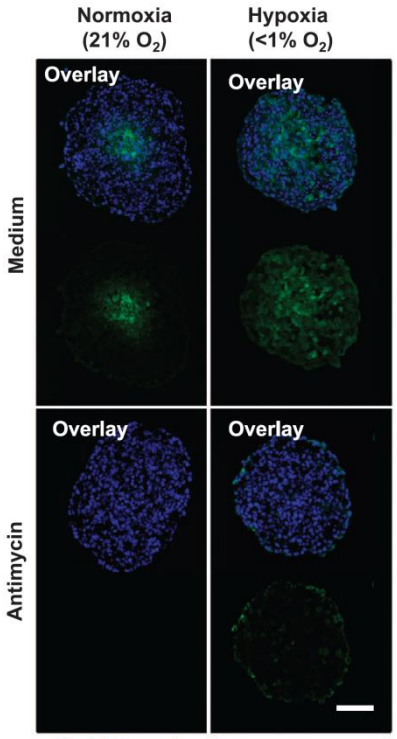
Tumor tissue section



Nuclei Hypoxic cells Vasculature

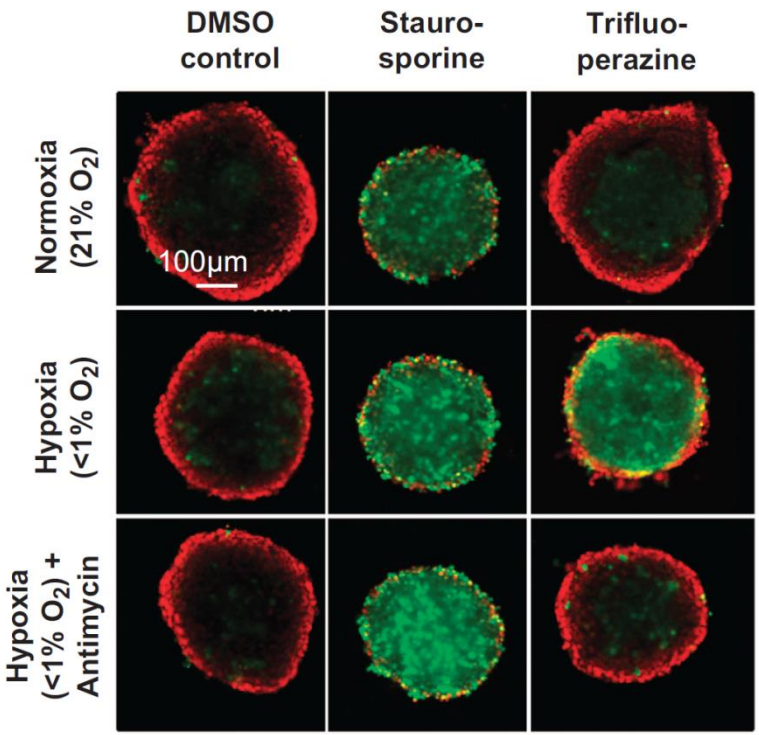
Hoechst Pimonidazole CD31

Spheroids



Nuclei Hypoxic cells

Hypoxia sensing drug treatment



Nuclei Dead cells

Cell Death Dis. 2017 Mar 30;8(3)

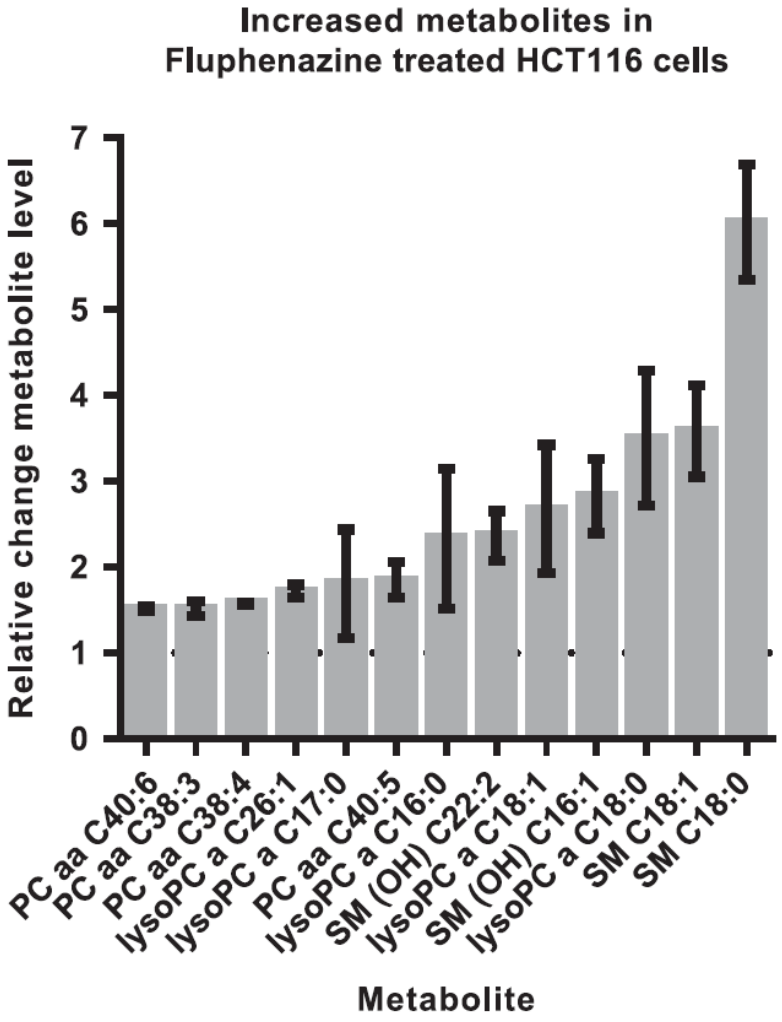
Hypoxia Study in Spheroid Model

EC50 generation of hypoxia specific hits in hypoxic and normoxic 3D tumor spheroids

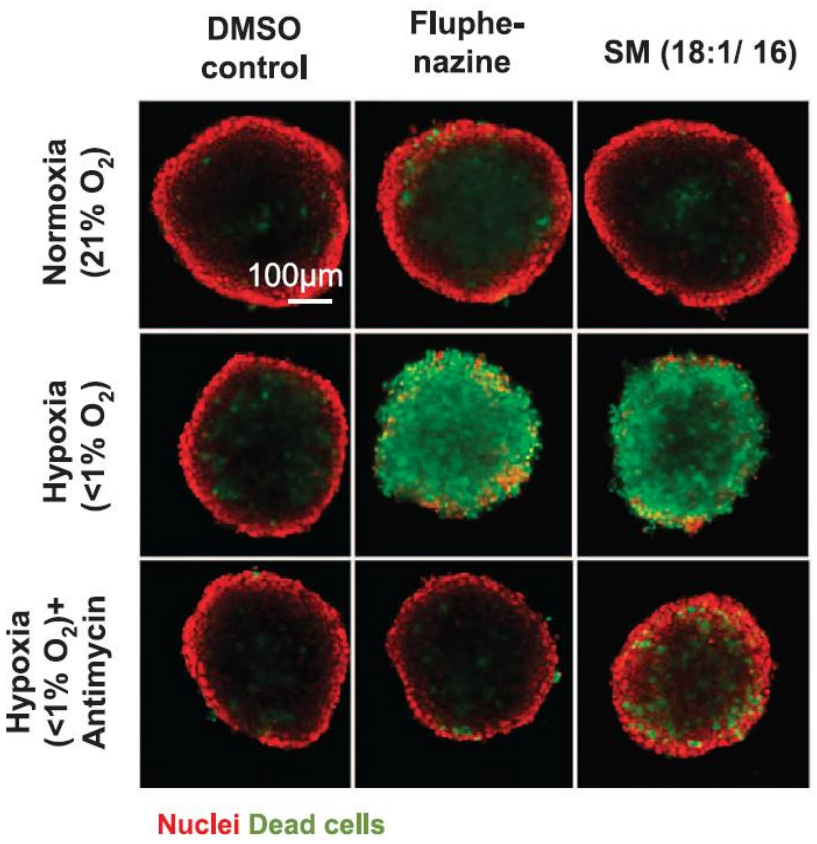
Compound	EC50 (cell death) in tumor spheroids (M)				EC50 (cell count) in 2D in (M)	
	Hypoxia <1% O_2	Nor-moxia 21% O_2	Respira-tory chain inh. at 21% O_2	Respira-tory chain inh. at <1% O_2	Hypoxia <1% O_2	Nor-moxia 21% O_2
Hypoxia-sensitizing compounds with novel mode of action						
Trifluoperazine	1.35E-06 (SD 7.2E-07)	>1E-05	>1E-05	>1E-05	> 1E-05	>1E-05
*Fluphenazine	1.63E-06 (SD 1.2E-06)	>1E-05	>1E-05	>1E-05	> 1E-05	>1E-05
ML9	2.79E-06 (SD 2.0E-06)	>1E-05	>1E-05	>1E-05	> 1E-05	>1E-05
*Chlorpromazine	3.31E-06 (SD 6.2E-07)	>1E-05	>1E-05	>1E-05	> 1E-05	>1E-05
Tamoxifen	3.44E-06 (SD 2.5E-06)	>1E-05	>1E-05	>1E-05	> 1E-05	>1E-05
ML7	3.92E-06 (SD 1.6E-06)	>1E-05	>1E-05	>1E-05	> 1E-05	>1E-05
*Thioridazine	4.09E-06 (SD 3.2E-06)	>1E-05	>1E-05	>1E-05	> 1E-05	>1E-05

Positive control: 10 μ M Staurosporine

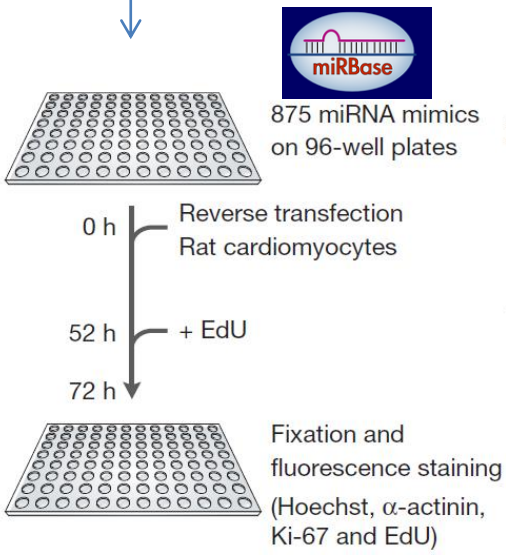
Hypoxia Study in Spheroid Model

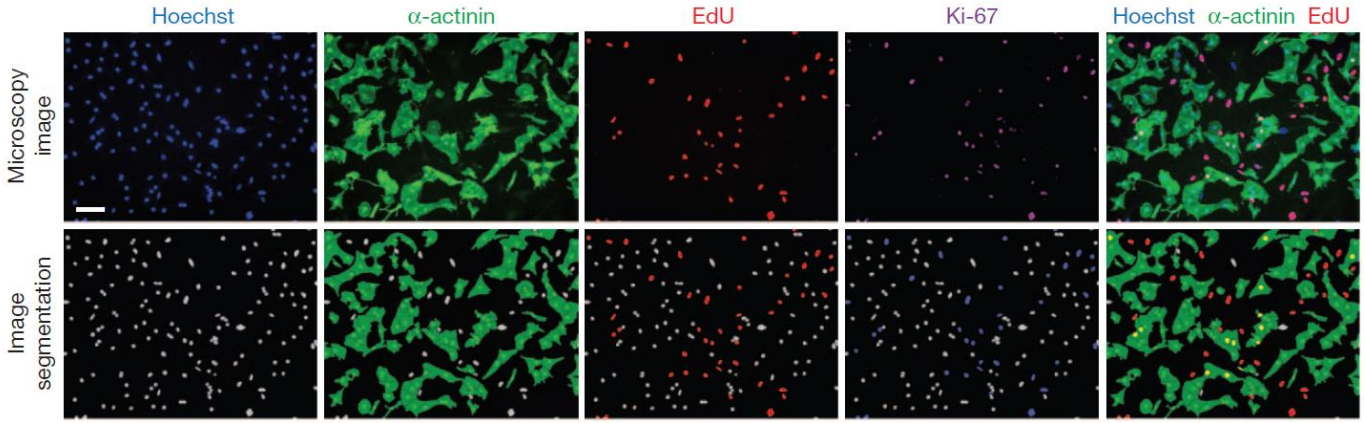


Identify the cause of cell death



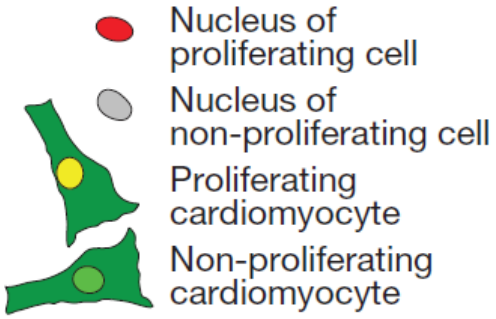
miRNA Functional Assay

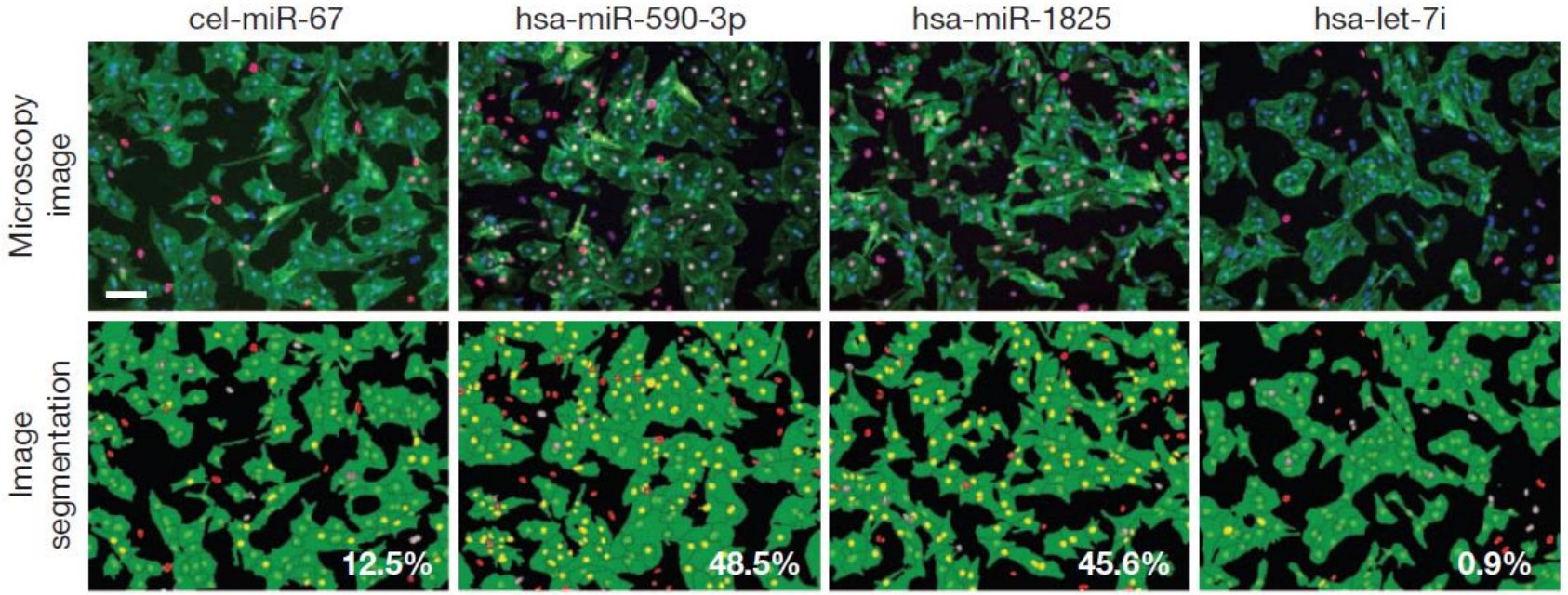




Nature. 2012 Dec 20, Eulalio A. et al.

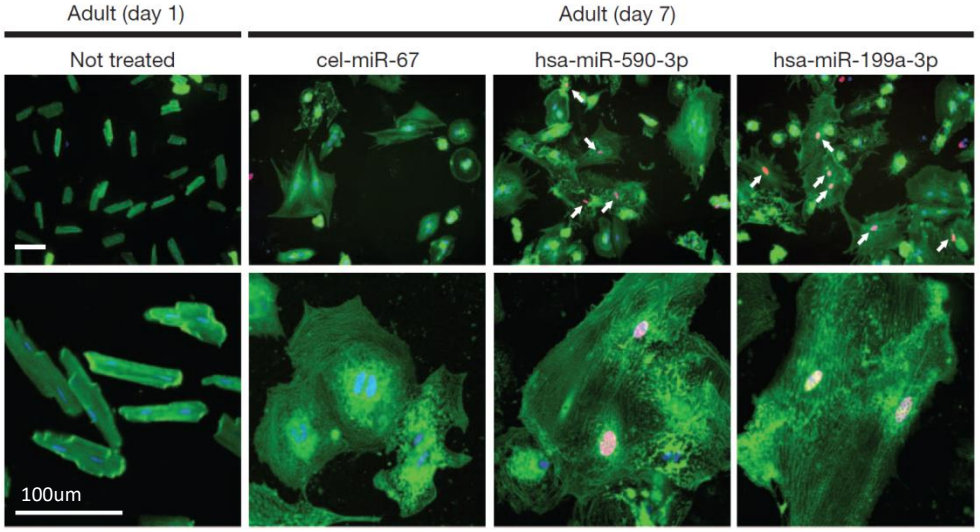
miRNA Functional Assay

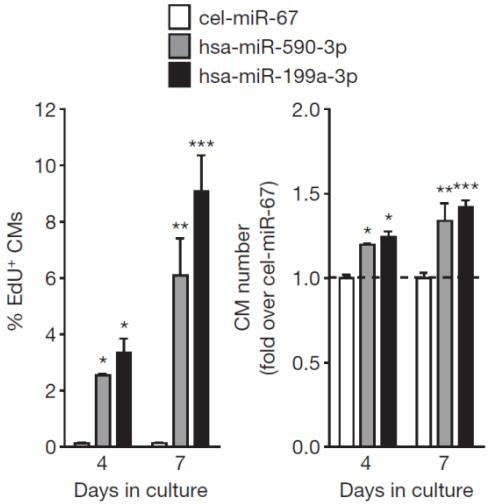


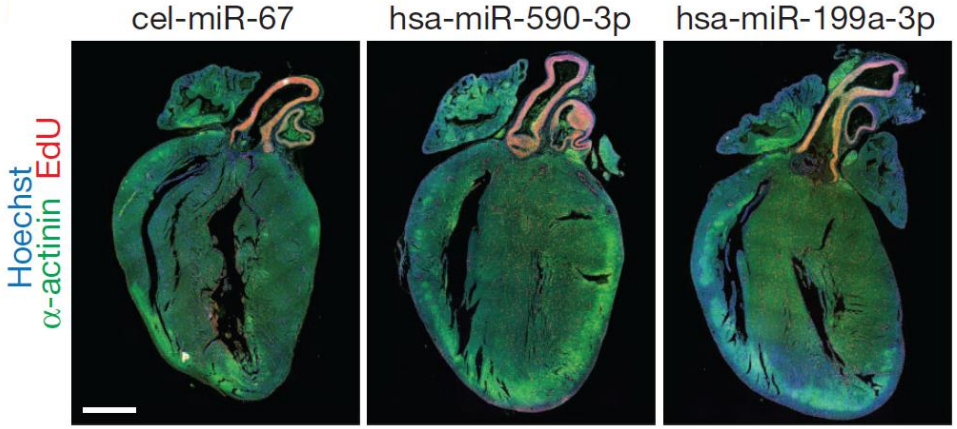


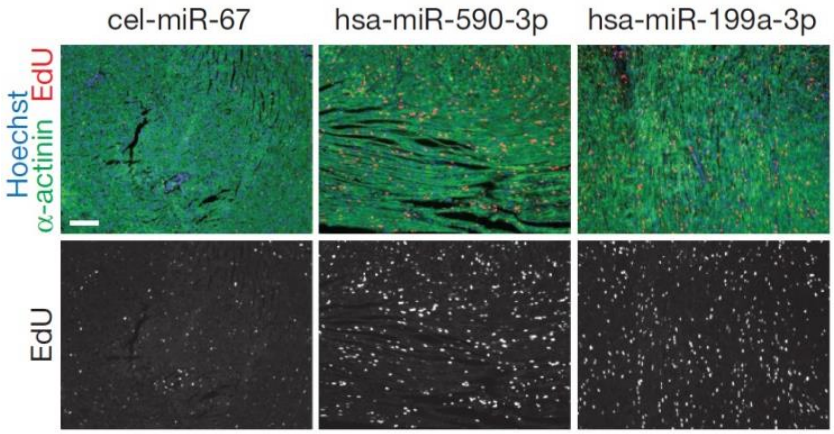
Hoechst β-actinin EdU

miRNA Functional Assay

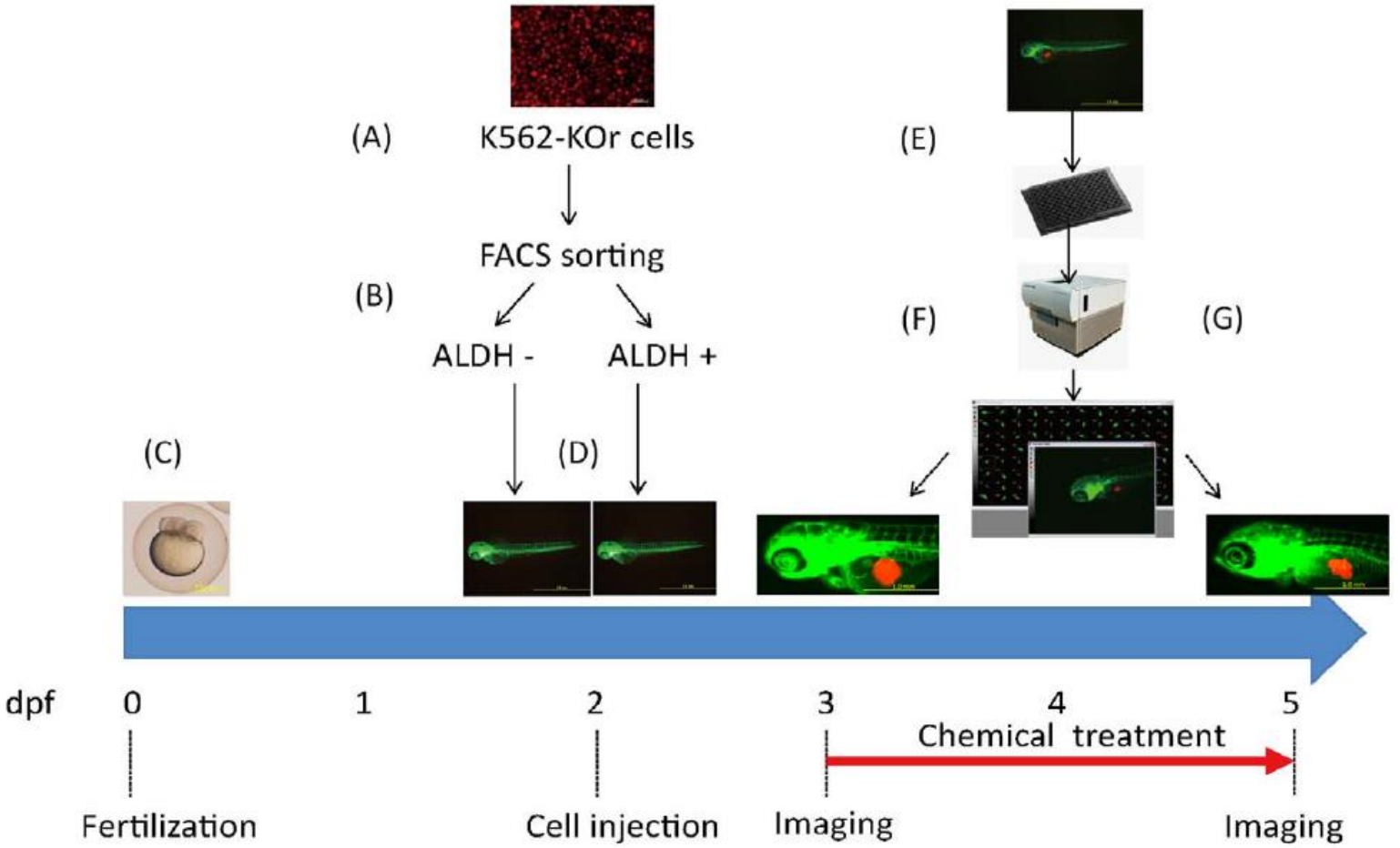




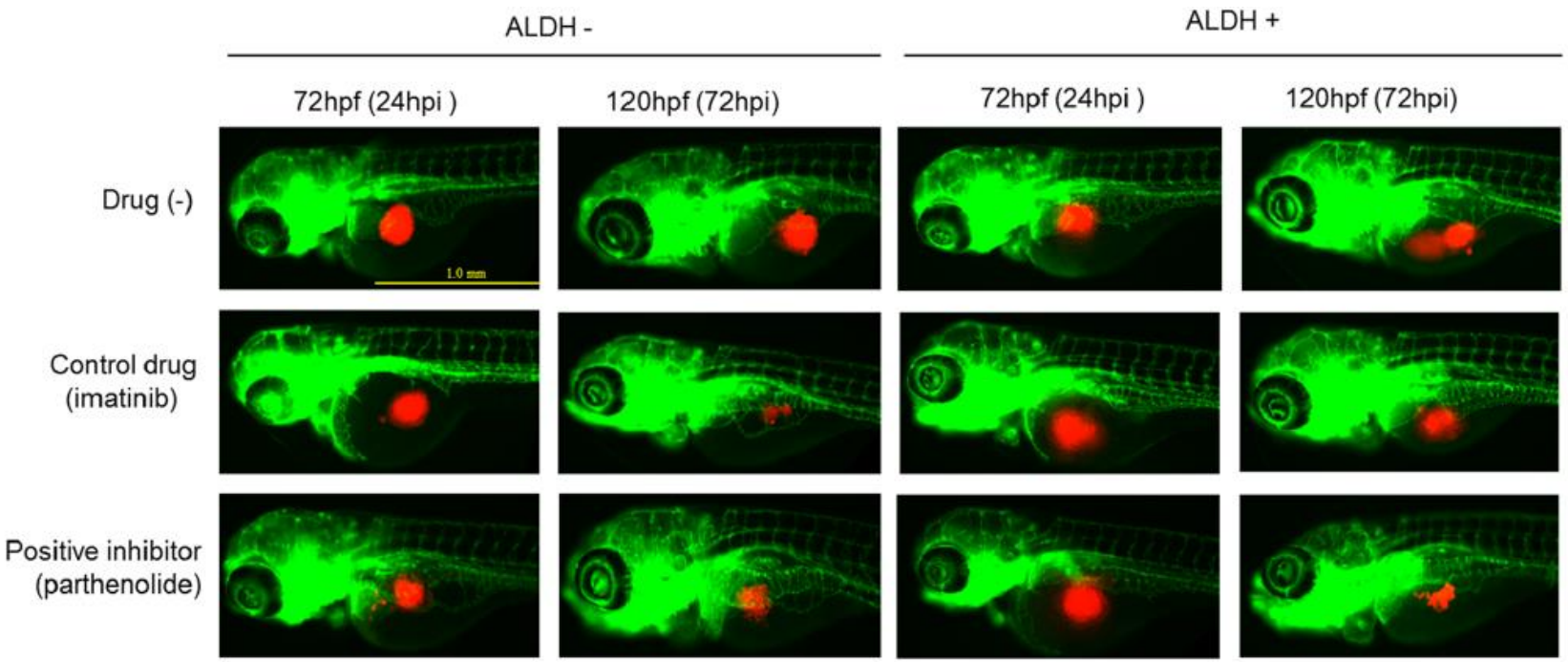




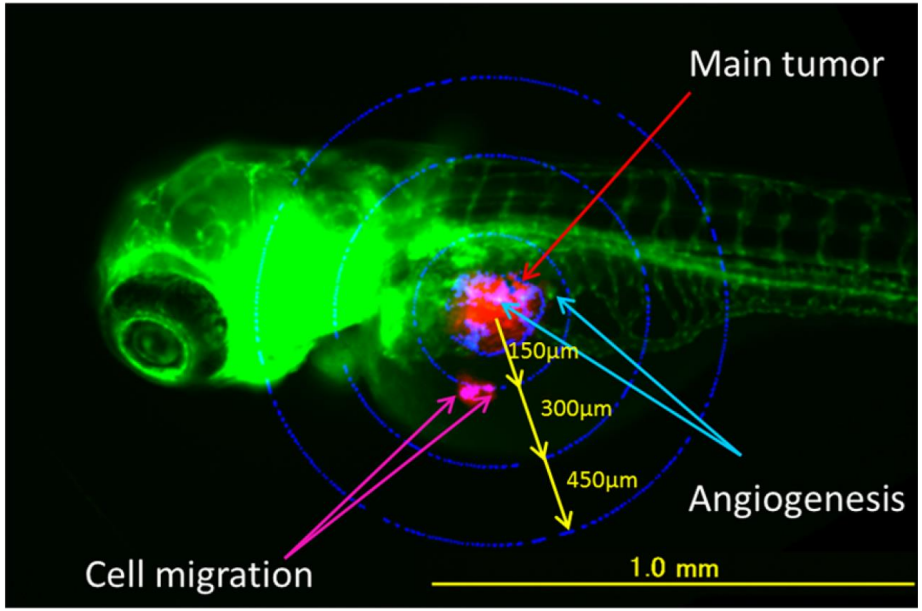
Tumor Xenotransplantation in Zebrafish



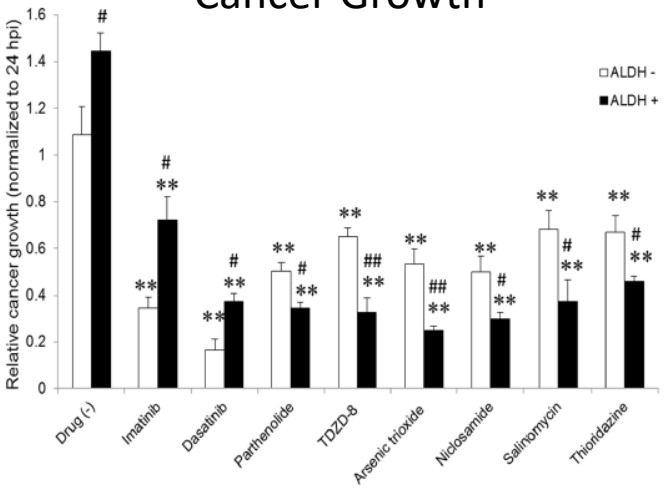
Tumor Xenotransplantation in Zebrafish



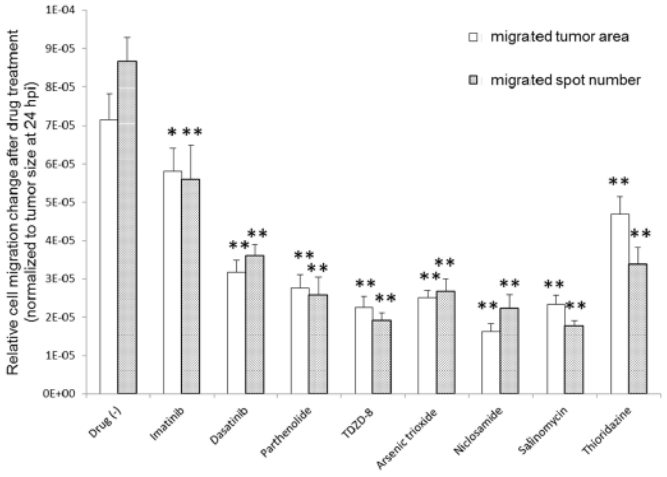
Tumor Xenotransplantation in Zebrafish



Cancer Growth



Migration change



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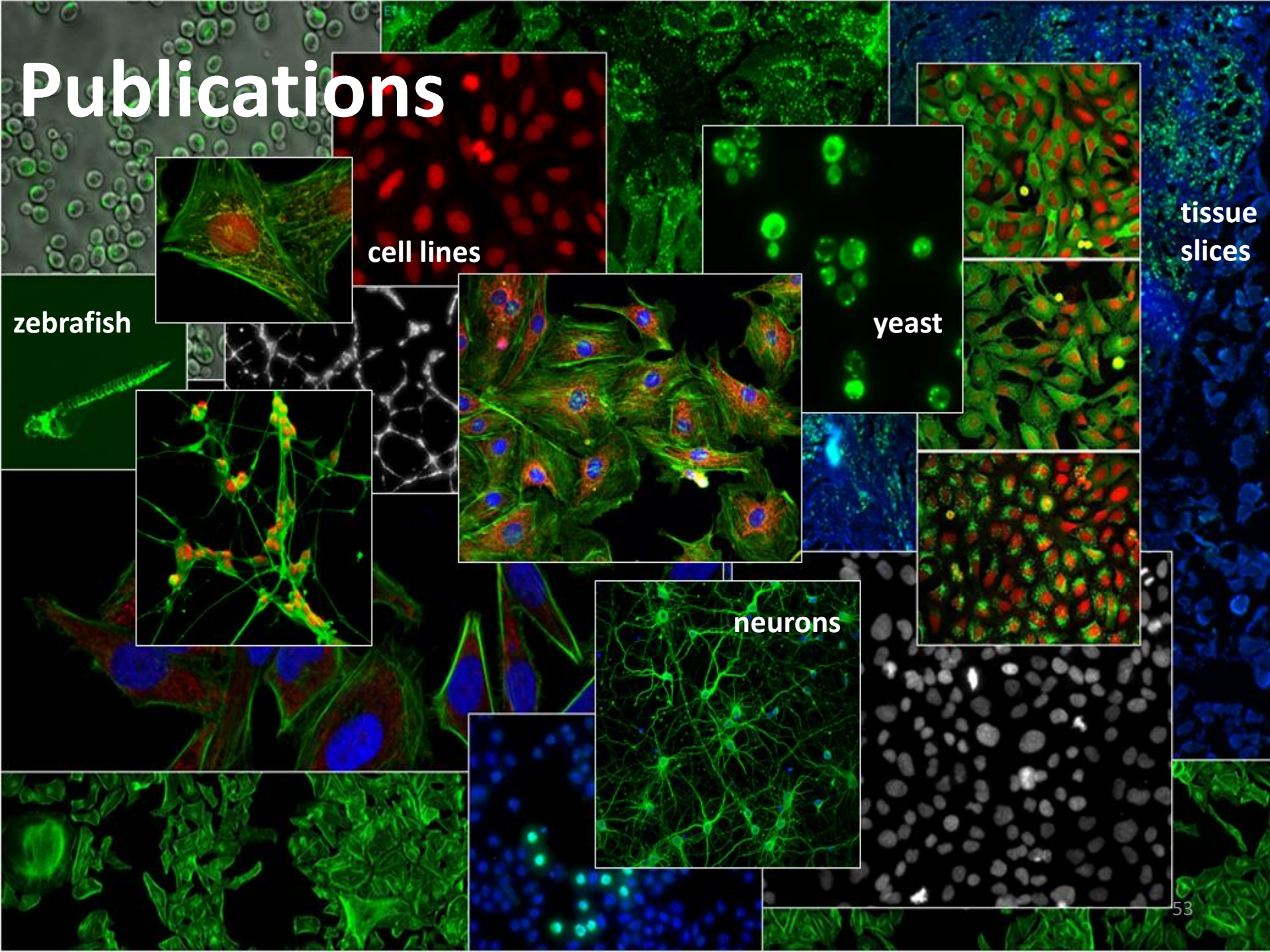

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Application Notes

APPLICATION NOTE

Assessment of drug effects on cardiomyocyte physiology using human iPSC-derived cardiac spheroids

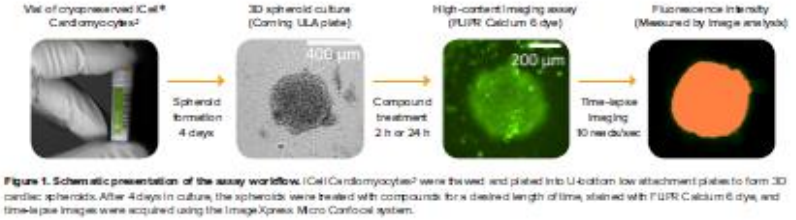
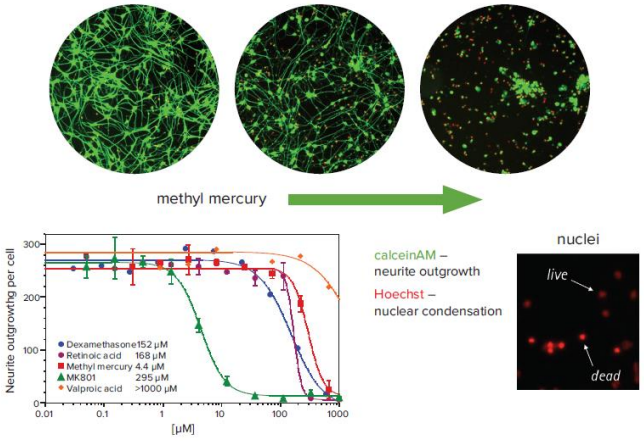


Figure 1. Schematic presentation of the assay workflow. iCell Cardiomyocytes² were thawed and plated into U-bottom low attachment plates to form 3D cardiac spheroids. After 4 days in culture, the spheroids were treated with compounds for a desired length of time, stained with FLIPR Calcium 6 dye, and time-lapse images were acquired using the ImageXpress Micro Confocal system.

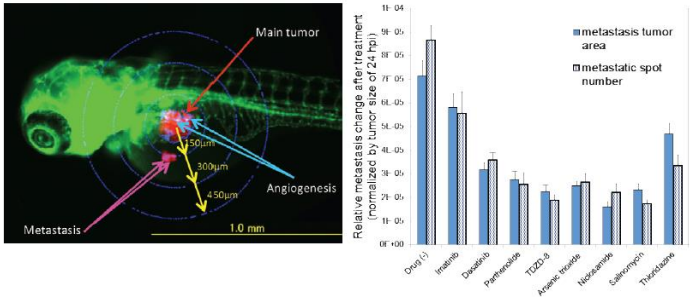
APPLICATION NOTE

High-content screening of neuronal toxicity using iPSC-derived human neurons



APPLICATION NOTE

High-throughput imaging assays using zebrafish, a model organism for human disease



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Customer Breakthroughs

HCS Pharma uses ImageXpress Micro Confocal Systems for phenotypic screening of physiologically relevant 3D cell models

Details

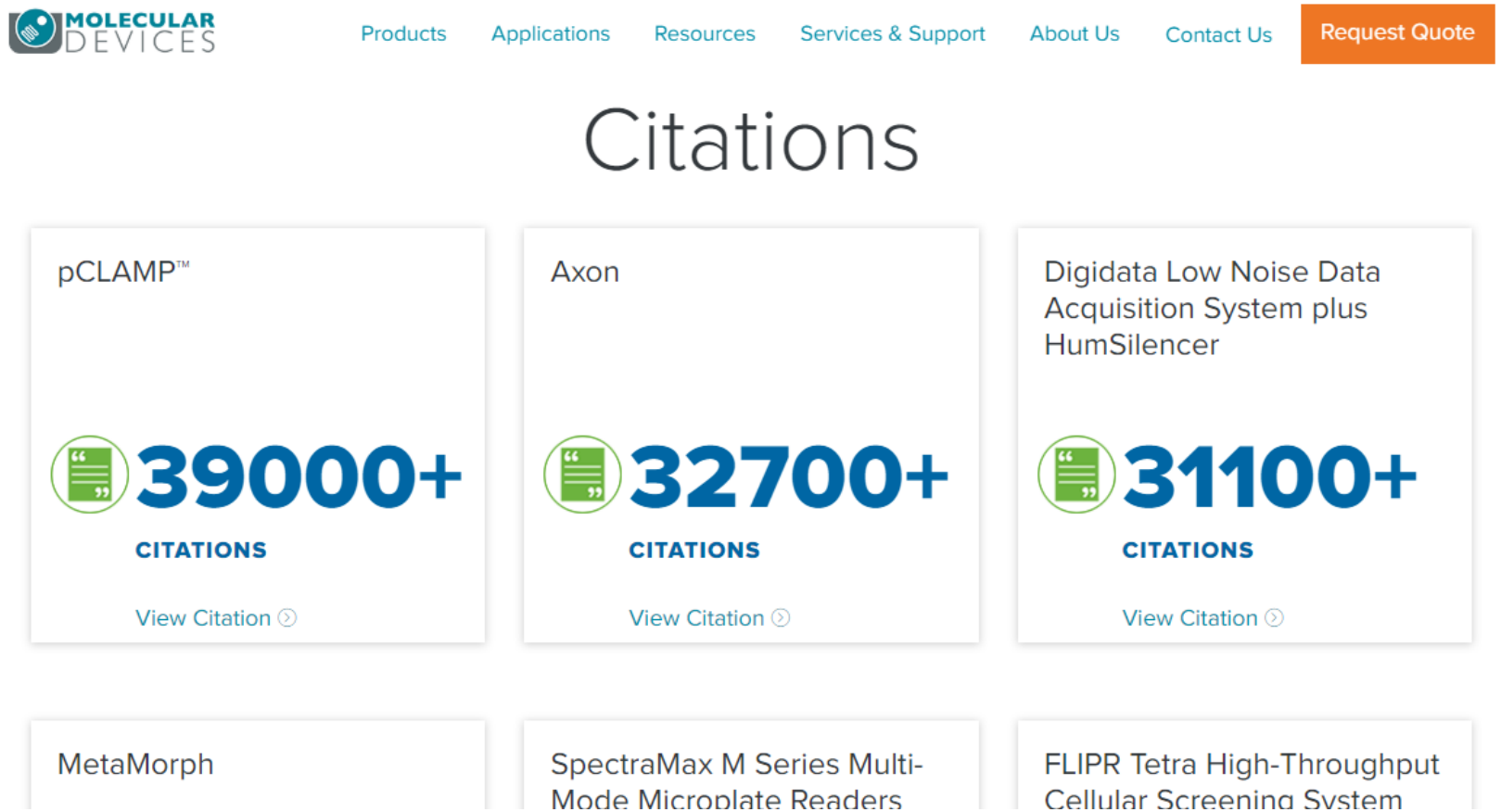
Gustave Roussy Cancer Center uses the ImageXpress Widefield Systems to assess mechanisms of cell stress and death in cancer treatment

Details

University College London uses the SpectraMax i3x reader and FLIPR Tetra System to develop personalized medicines

Details

Citations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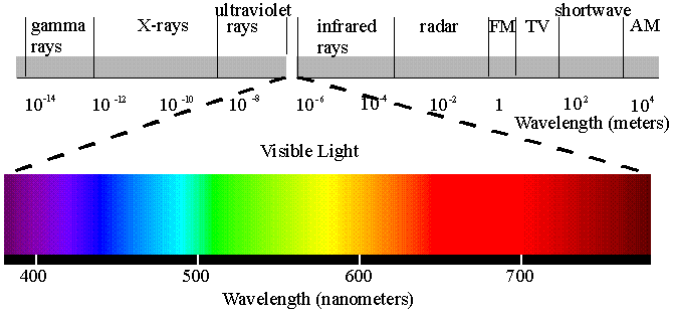
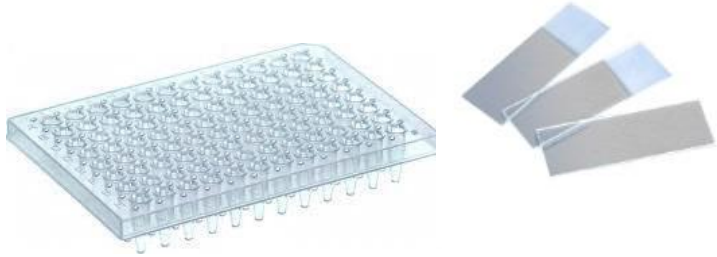
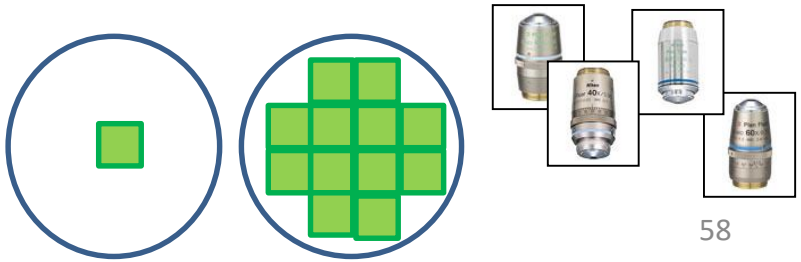
FLIPR Tetra High-Throughput Cellular Screening System

Notes before doing HCS

- **Wavelength: DAPI, FITC, TRITC, Texas Red, Cy5**
- **Devices format: 6~1536 well plate, regular or chamber slide, or others**
- **Sample type: Fixed cell or living cell, cell or non-cell sample(ex: E. coli, yeast, zebrafish ……)**
- **Field of view: single image per well to total well, 4~40X objective**







Thanks for your attention!

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