Tissue engineering and HCS (high content screening) for preclinical research

Is the future at our doorstep?

Julian Bursztyka, PhD

Adebiotech, 9th of December 2021

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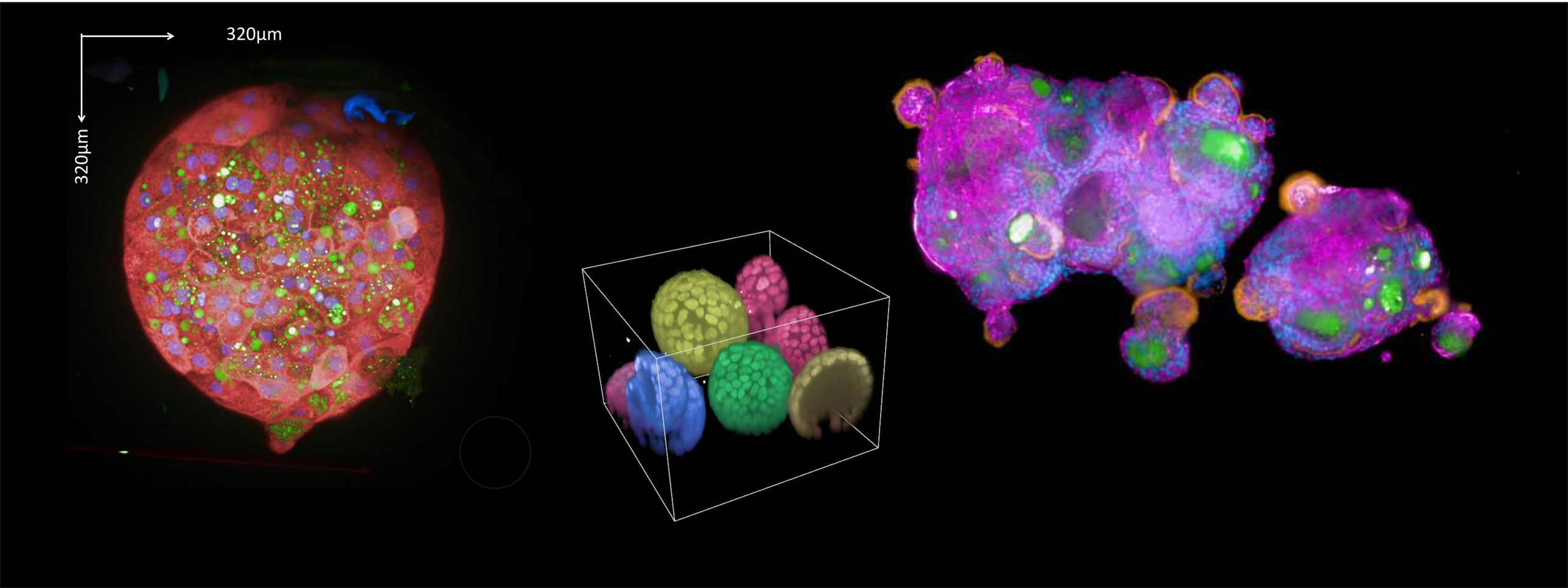
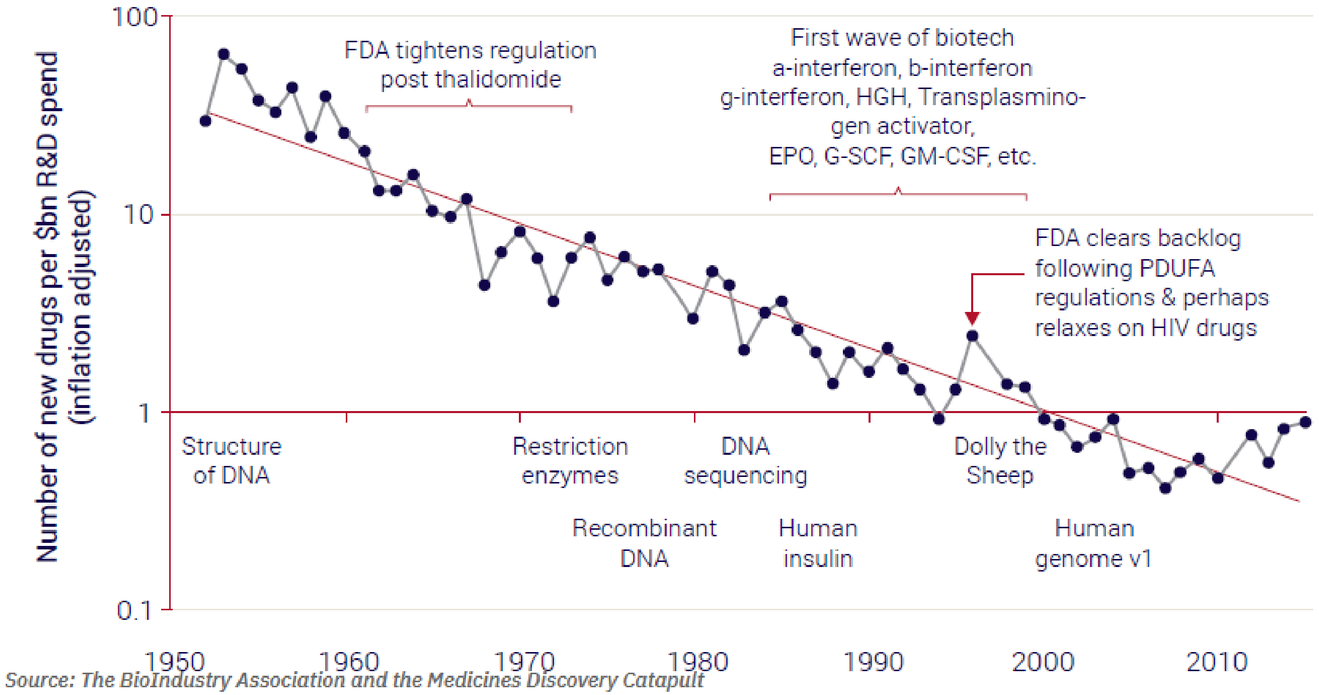
For the Better



R&D Productivity Decline: where are we ?

The life sciences productivity paradox — new drug launches are one-30th of what they were 40 years ago
18th January 2018

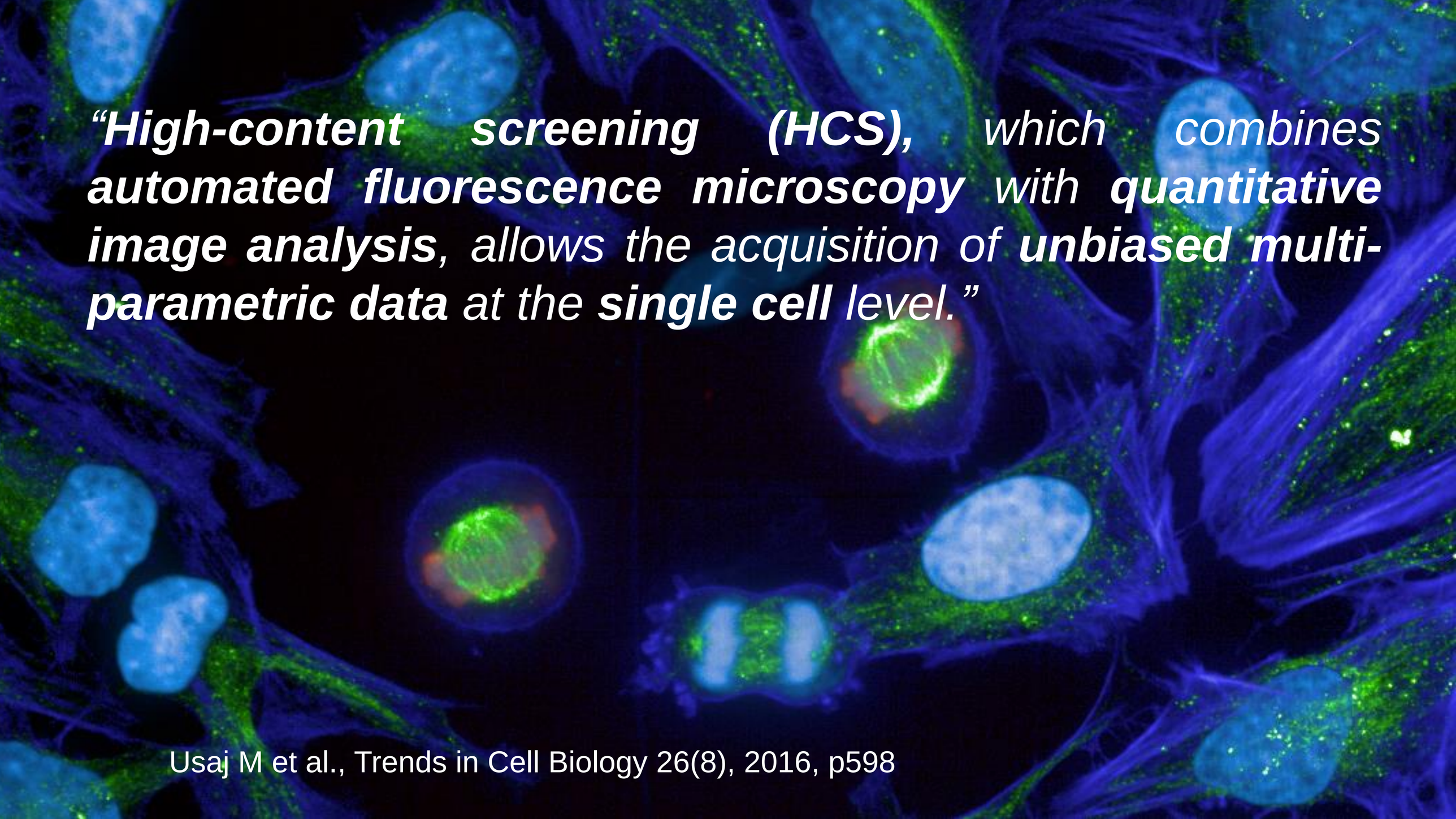
Figure 1: Long term productivity of drug discovery is in decline (courtesy Jack Scannell, UBS)



Source: The BioIndustry Association and the Medicines Discovery Catapult

How to overcome the challenge ?

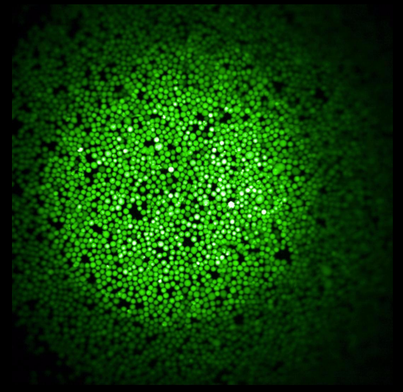
- More interest into orphan diseases
- Outsourcing R&D
- New R&D methods including:
  - **More efficient R&D tools** (hardware as well as software)
  - **More relevant in vitro models**

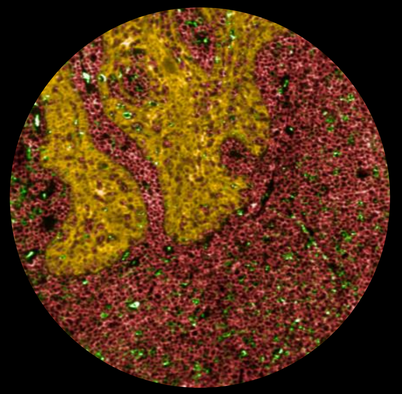


*“**High-content screening (HCS),** which combines **automated fluorescence microscopy** with **quantitative image analysis**, allows the acquisition of **unbiased multi-parametric data** at the **single cell** level.”*

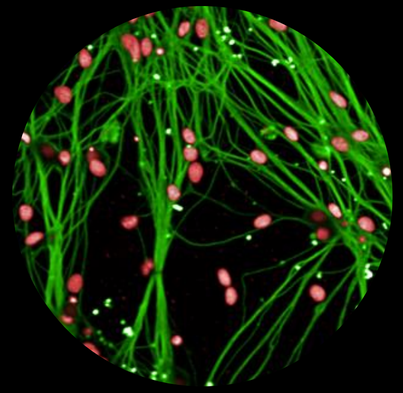
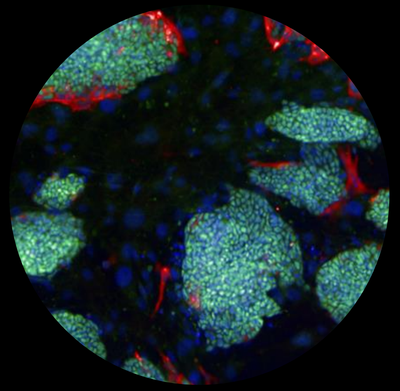
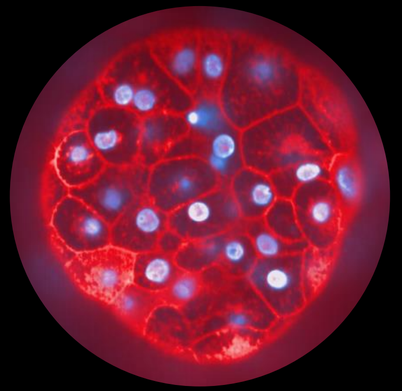
Usaj M et al., Trends in Cell Biology 26(8), 2016, p598

Better Models….

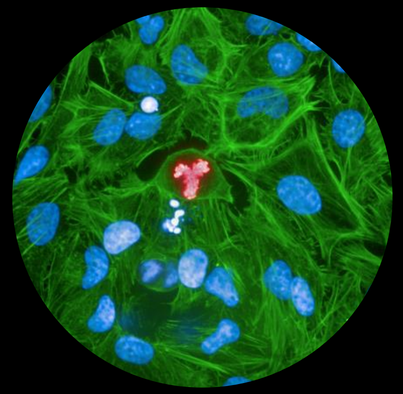
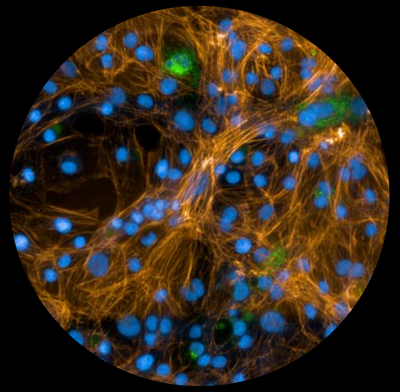
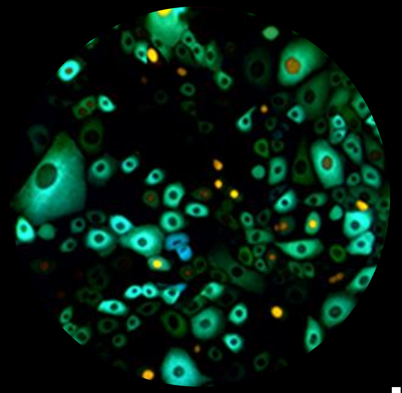




Live Cells
Zebrafish
Tissues

Spheroids

FRET

… More Images

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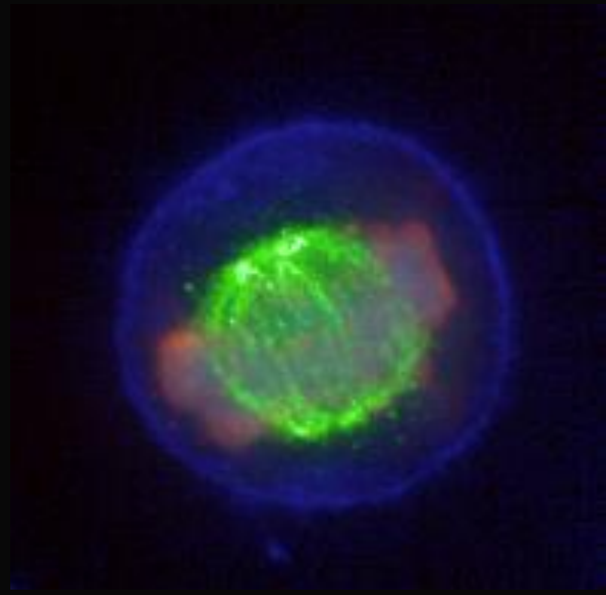
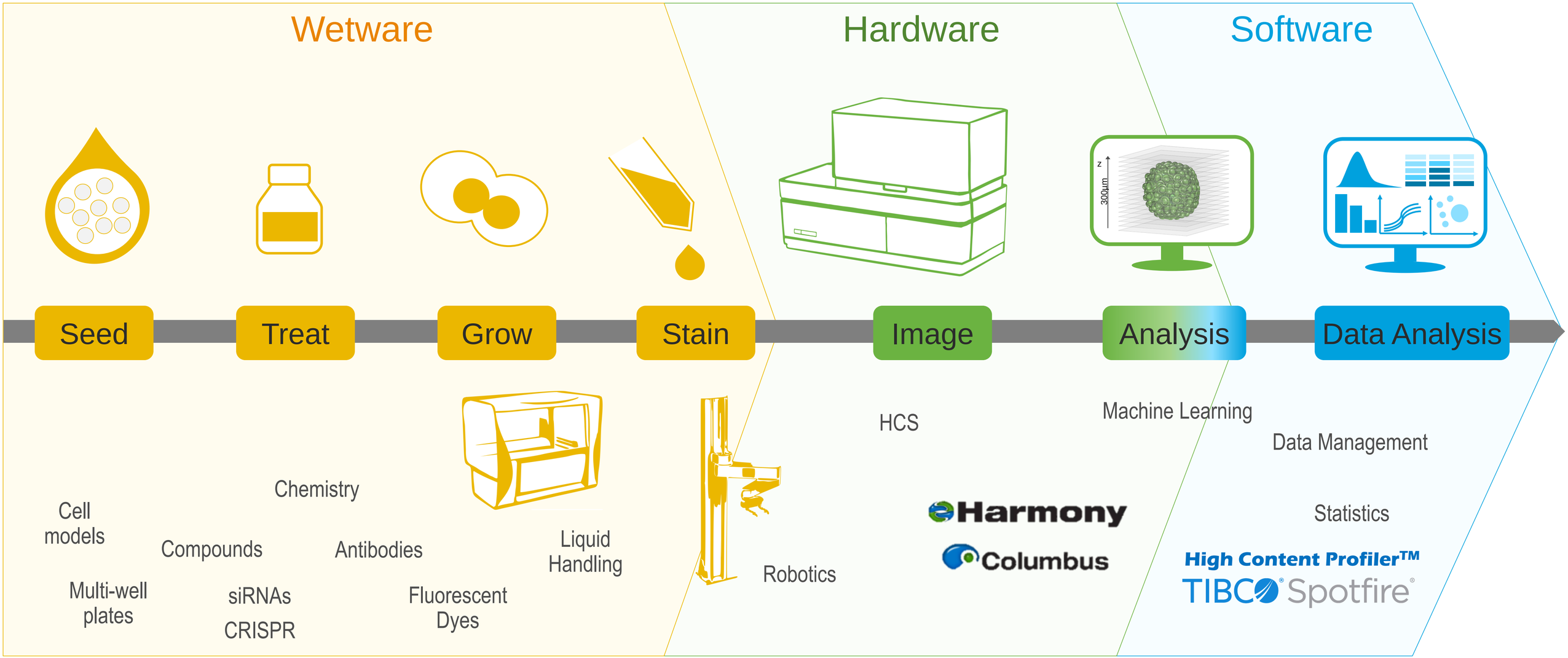
Biological Questions addressed with HCS

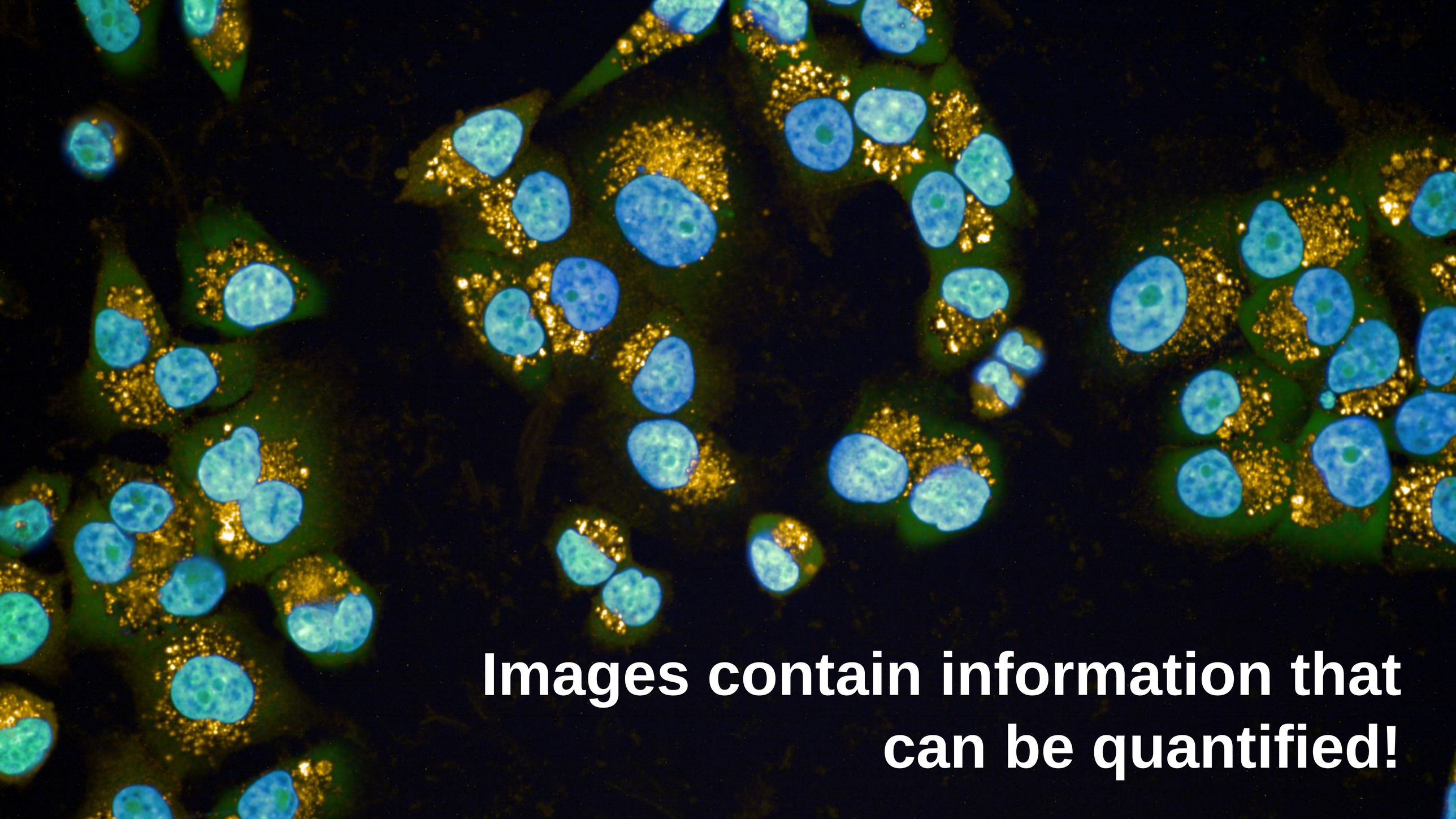
- ✓ Quantify changes in response to chemical or genetic perturbations
- ✓ Identify genes required for a specific process
- ✓ Identify genes required for differentiation and proliferation
- ✓ Explore models of disease
- ✓ Chemical and genetic profiling



Usaj et al, Trends in Cell Biology, August 2016, Vol. 26, No. 8

High Content Screening – experimental workflow

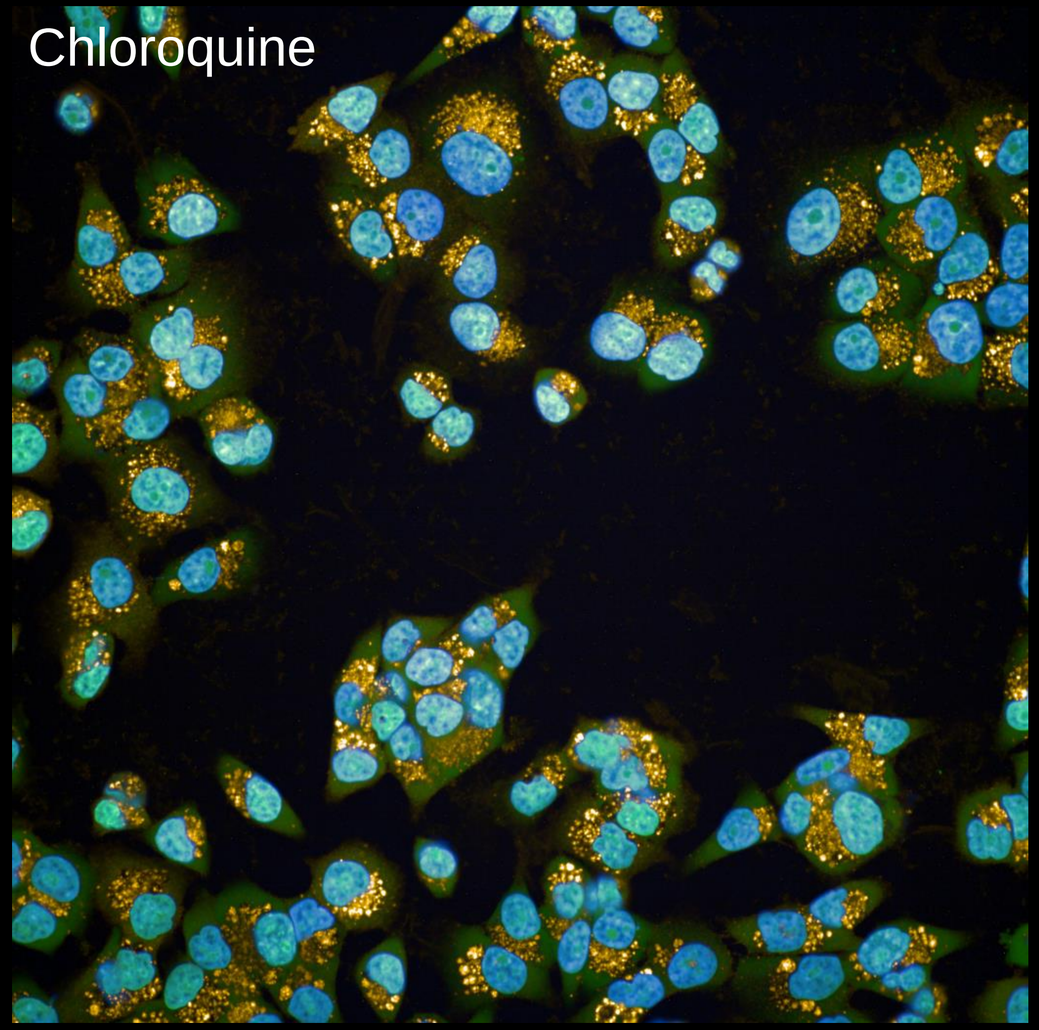




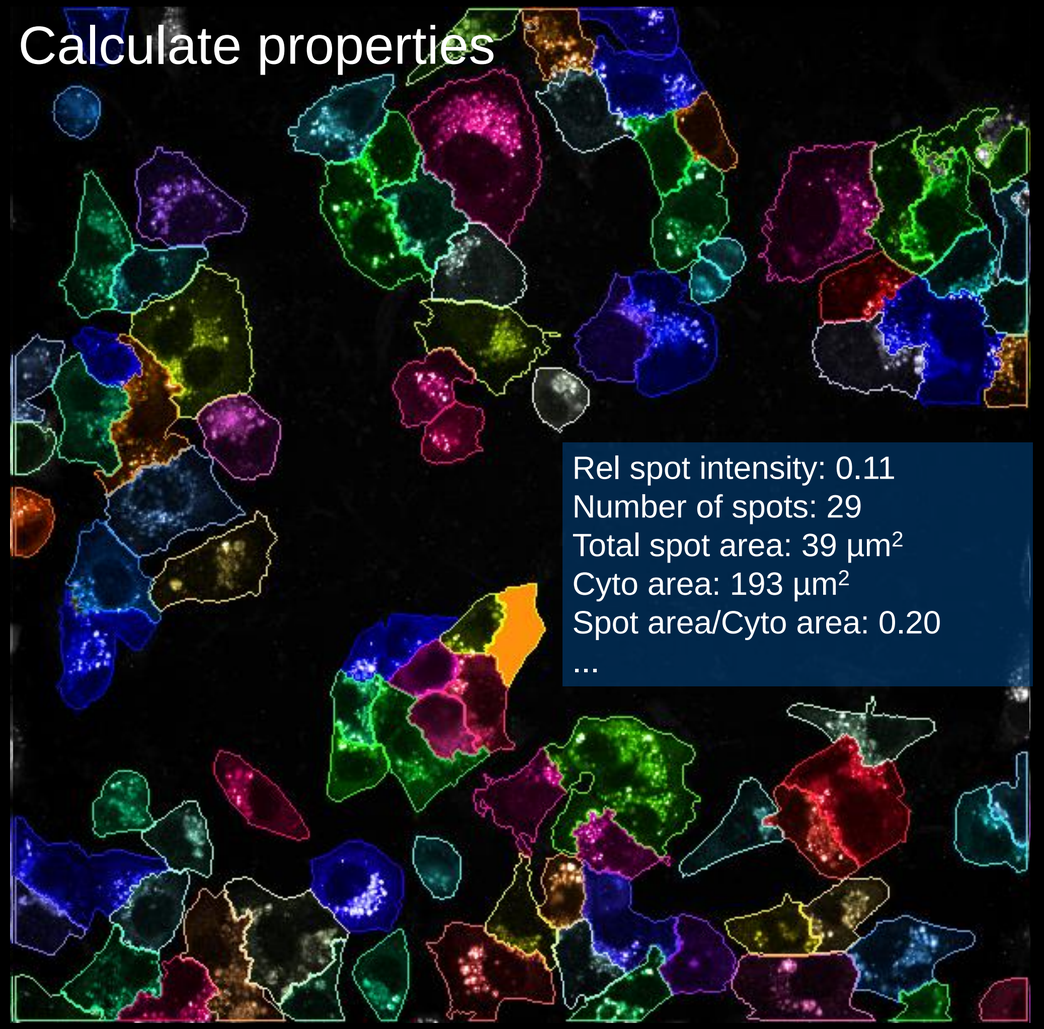
Images contain information that can be quantified!

From Images to Numbers

Chloroquine



Calculate properties



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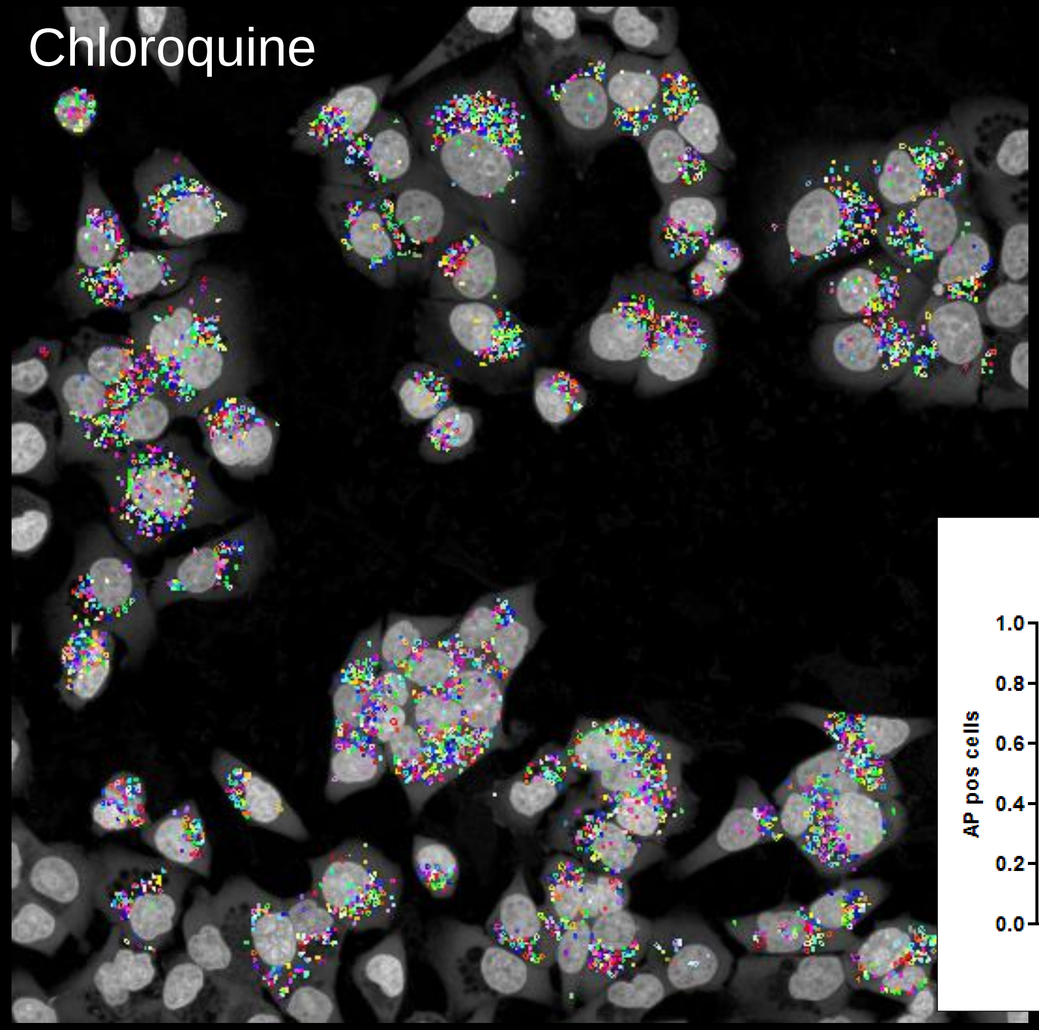
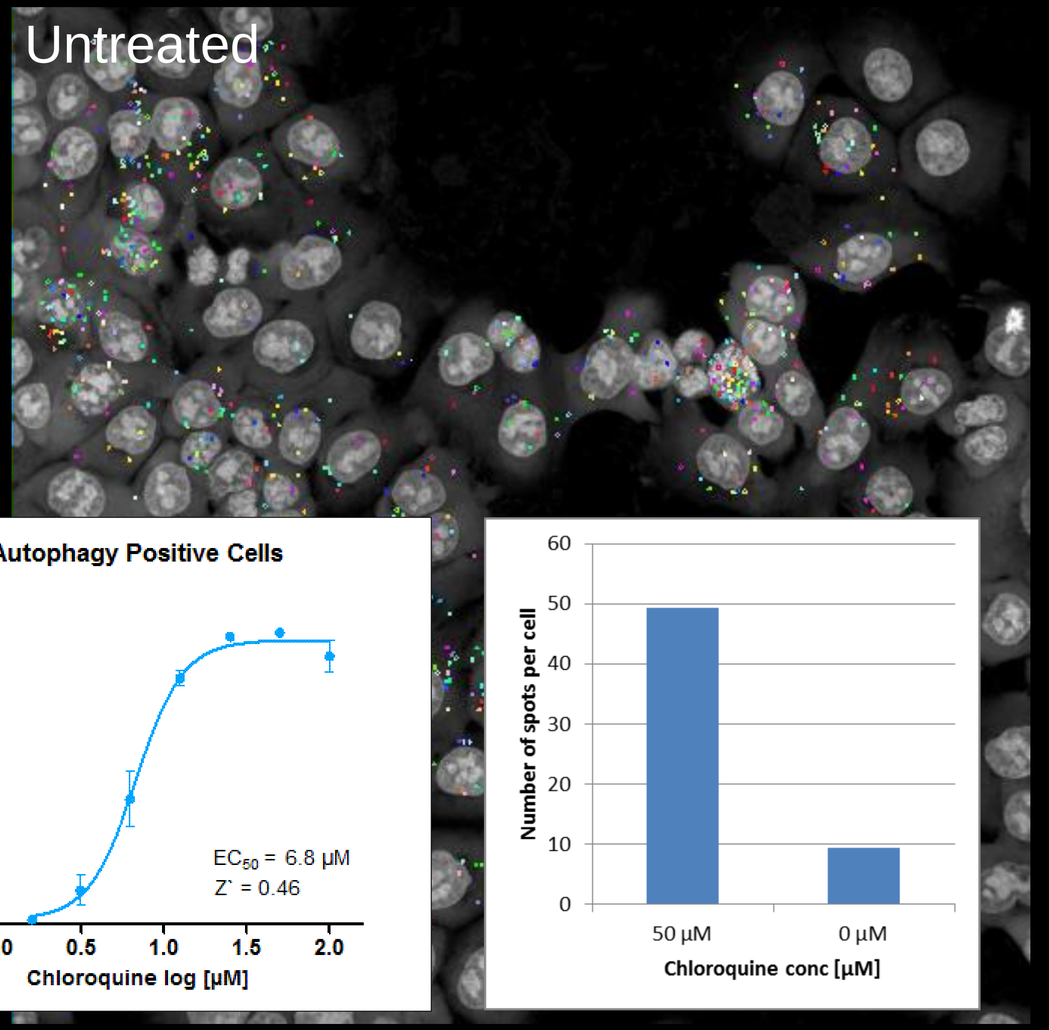
From Numbers to Knowledge





Chloroquine treatment leads to autophagosome accumulation

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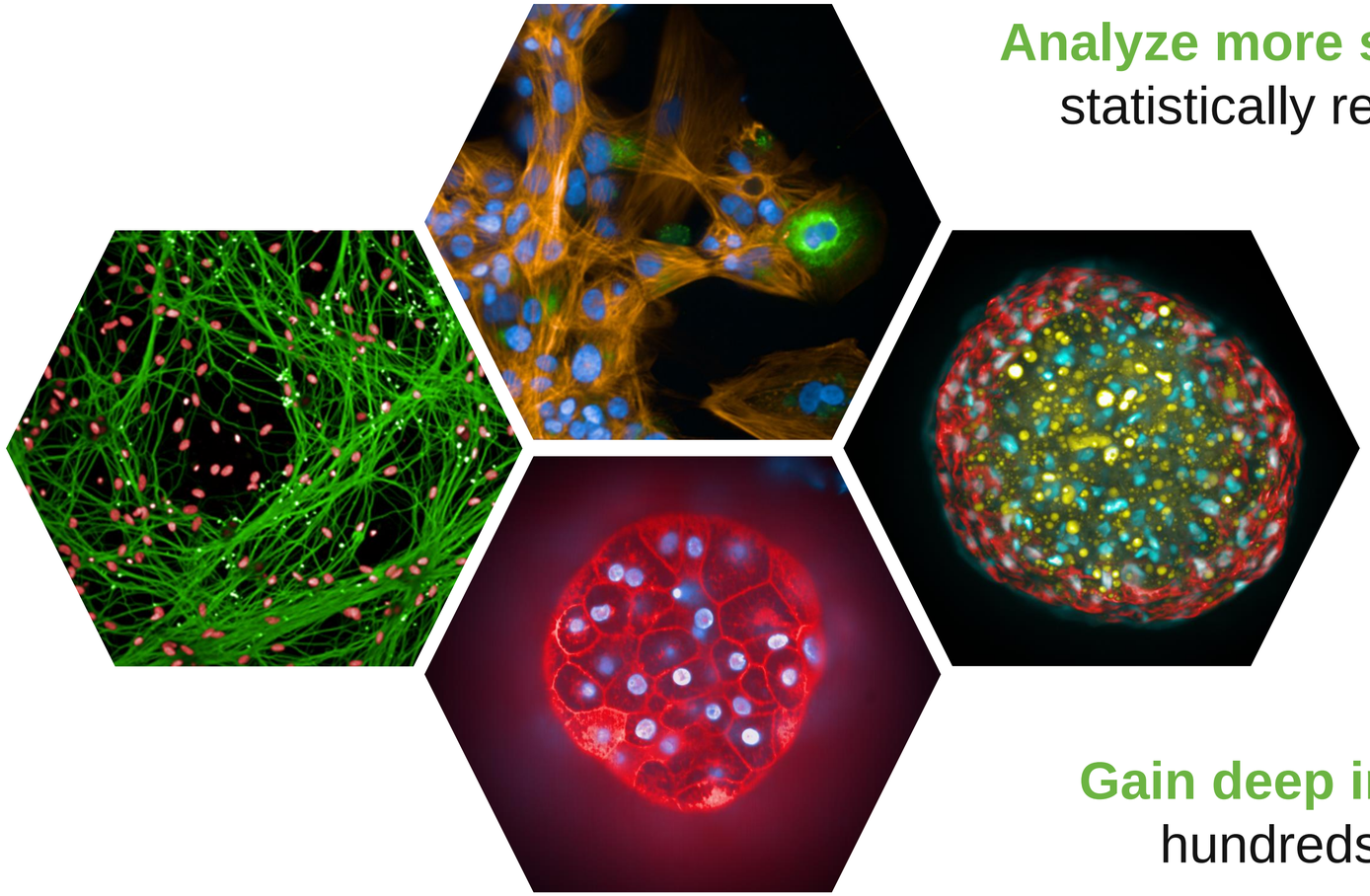
What does High Content Analysis bring to you?



Analyze more samples

statistically relevant readouts, reduce bias

Achieve higher physiological relevance

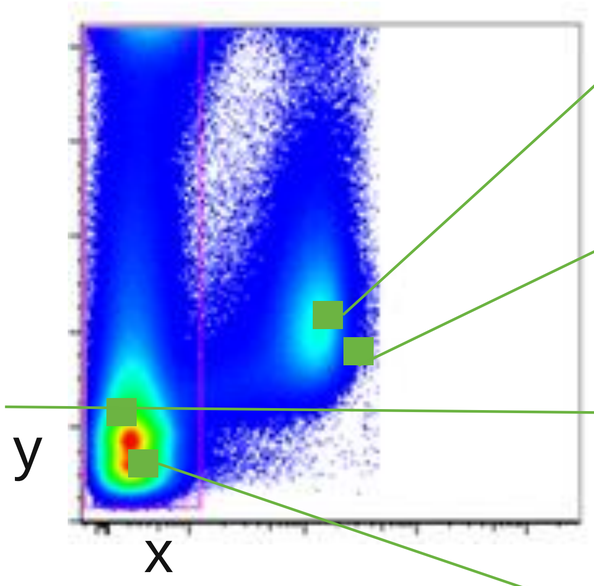
disease-relevant cell models

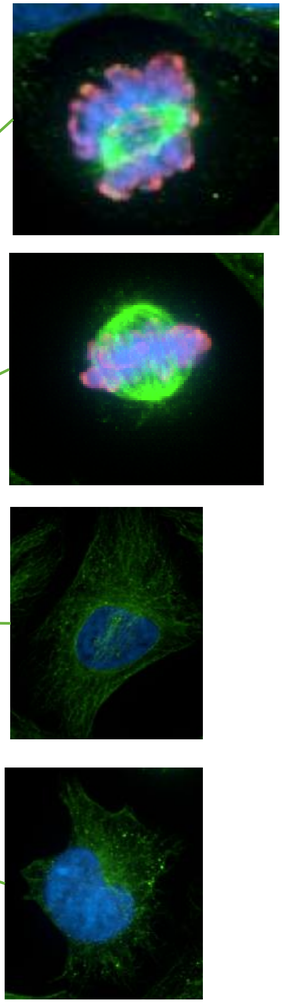
Gain deep insights into your biology

hundreds of readouts per cell, phenotypic analysis

Gain More Information From Your Cellular Samples

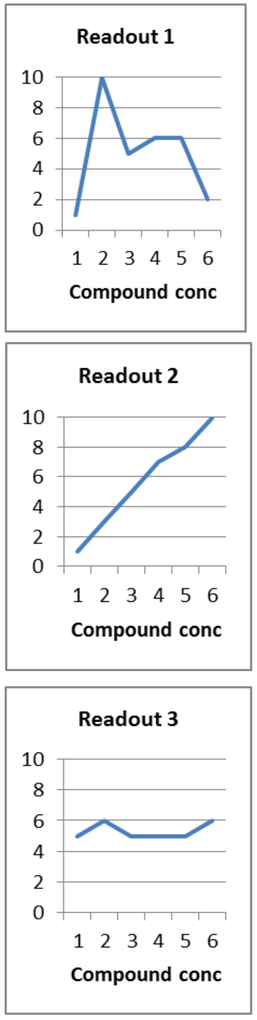
Flow Cytometry





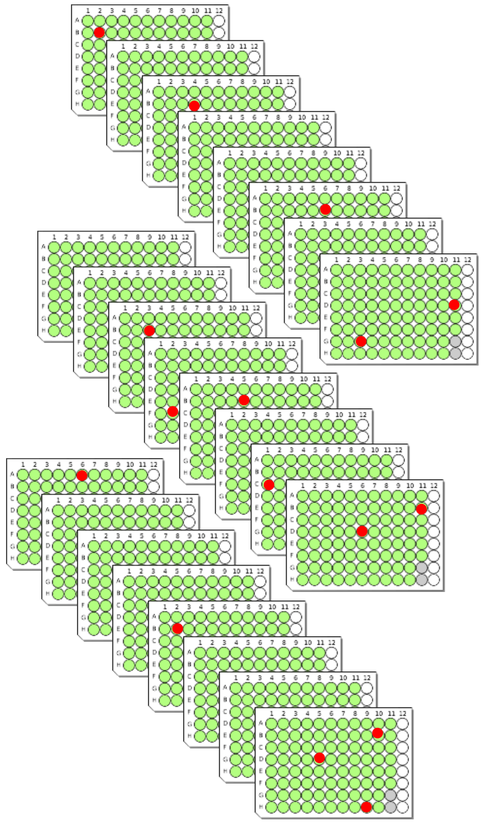
High-Content Screening

Nucleus area
Nucleus intensity
Nucleus roundness
Cell area
Cell roundness
Cytoplasm intensity
Cytoplasm texture
...



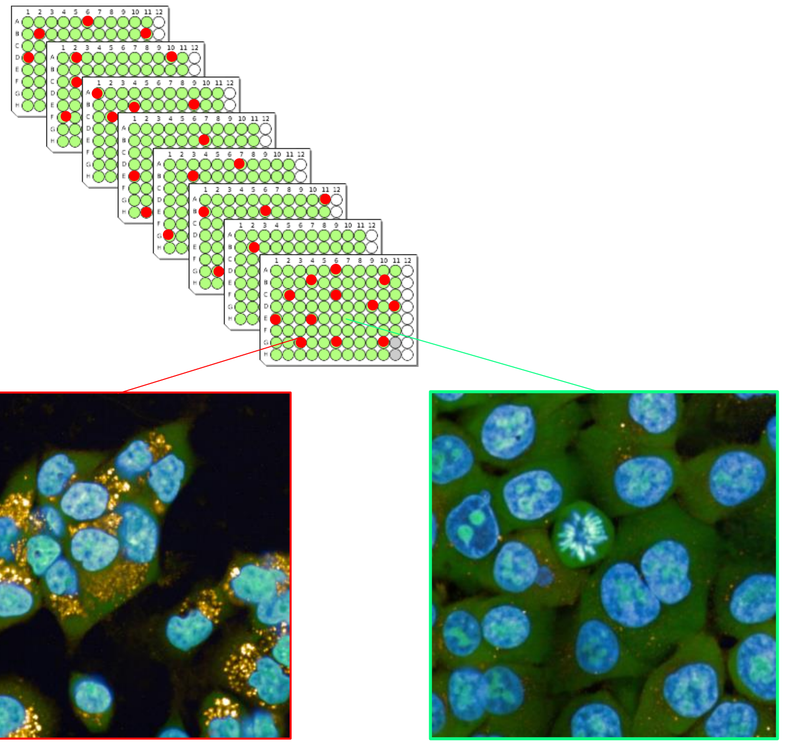
Gain More Information From Your Cellular Samples

High Throughput Screening



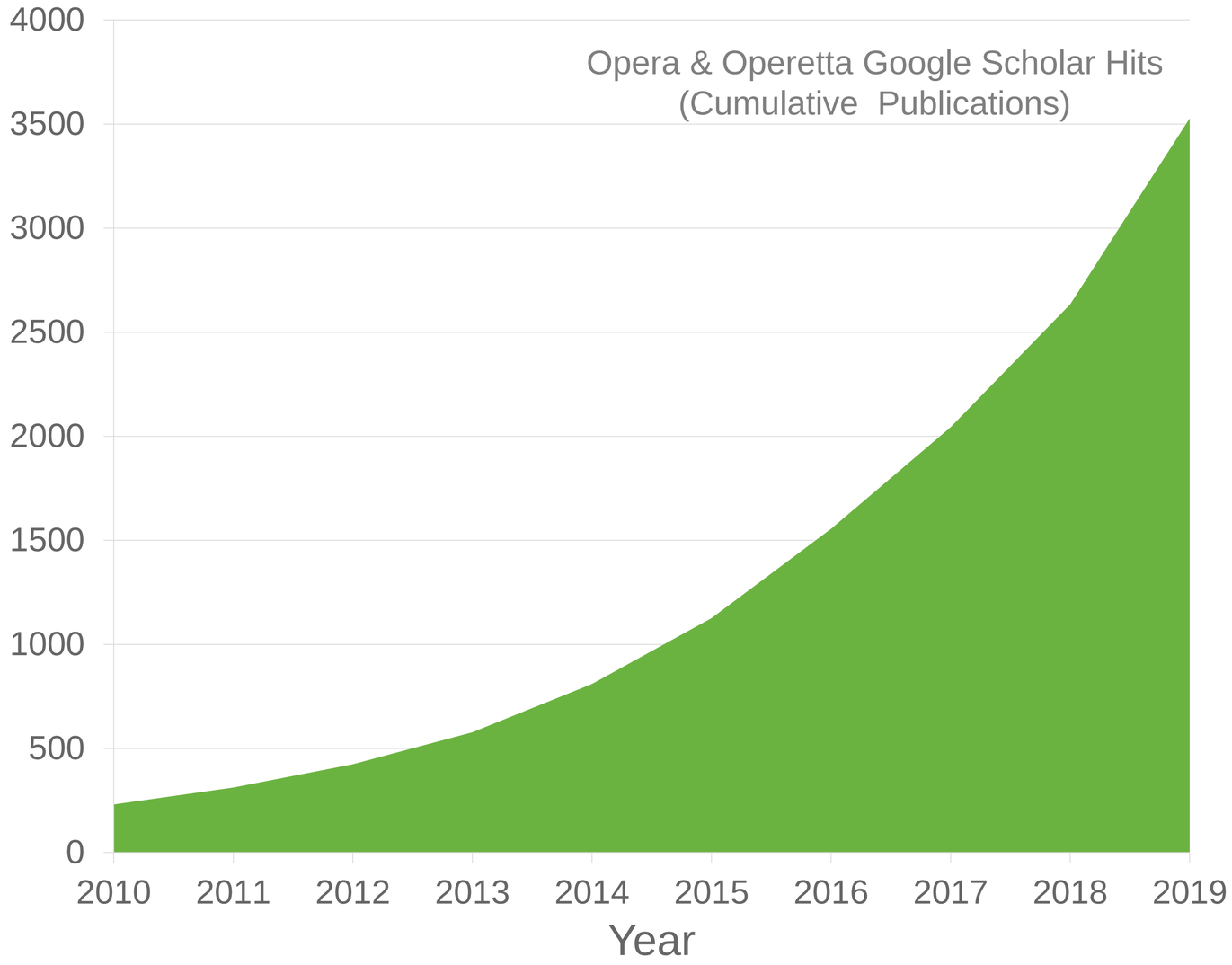
1 well – one readout

High-Content Screening



1 well – many readouts, phenotypic information

PerkinElmer HCS Publications

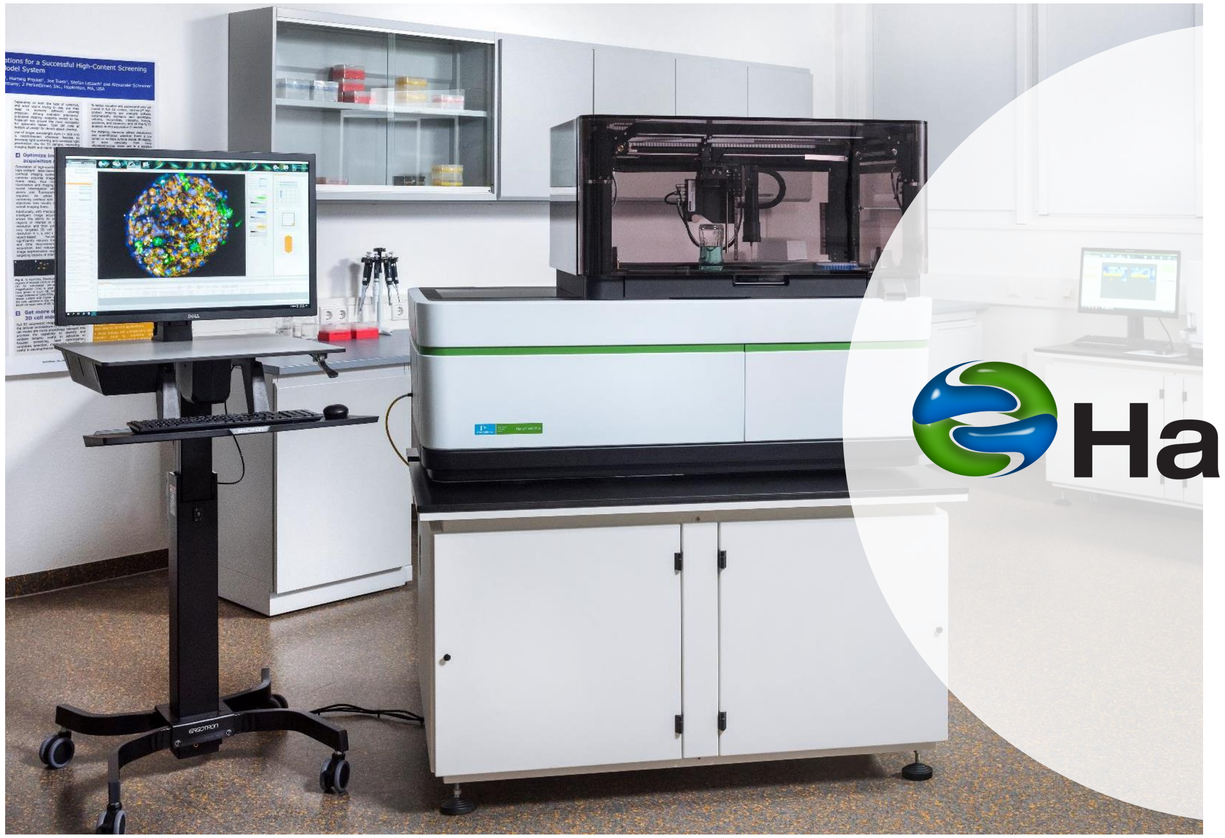


Scientific Productivity with:

- Unbiased, statistically significant data
- Image quality
- Powerful yet easy to use software

Annual Google Scholar hits: Operetta and Opera PerkinElmer

PerkinElmer Instruments for HCS



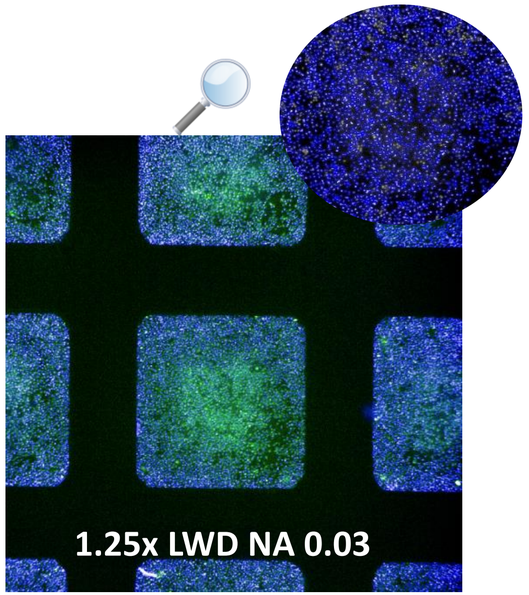
Harmony®

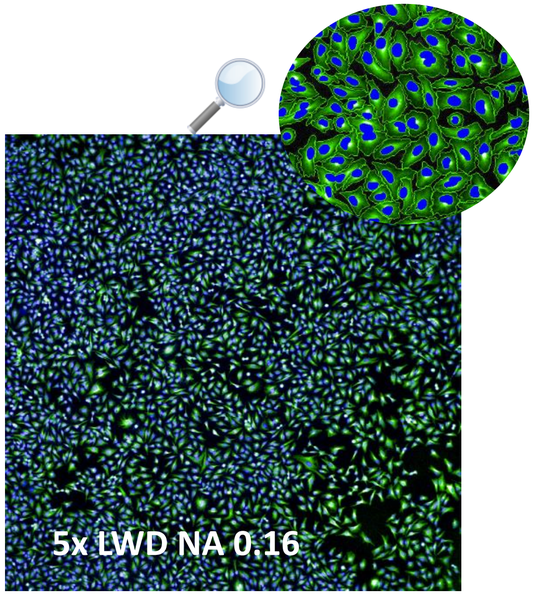
Opera Phenix Plus

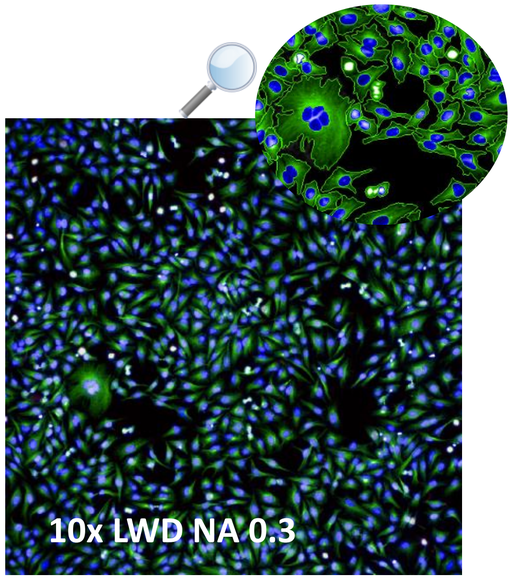
Operetta CLS

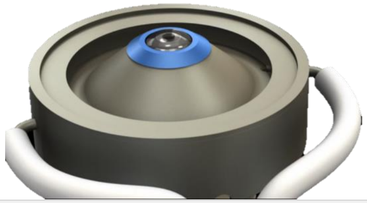
PerkinElmer

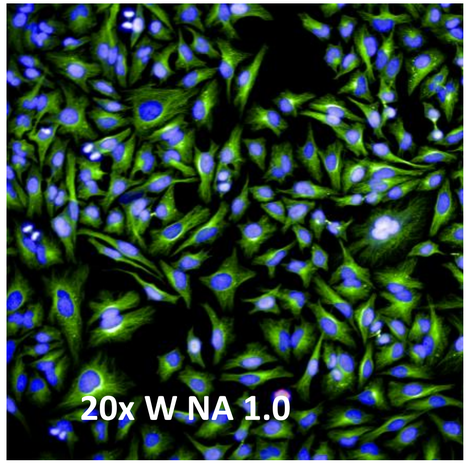
Choice of Magnification

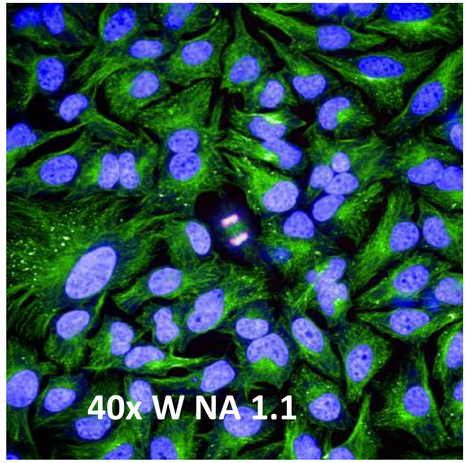


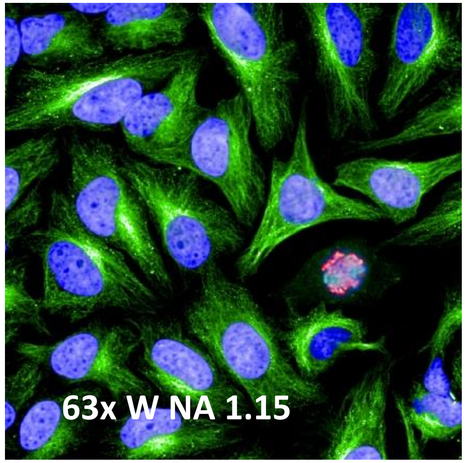












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PerkinElmer Instruments for HCS

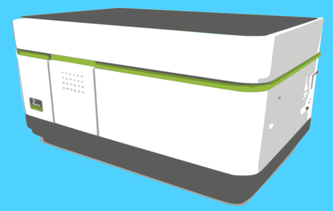
1 Camera

4 or 8 high power LED

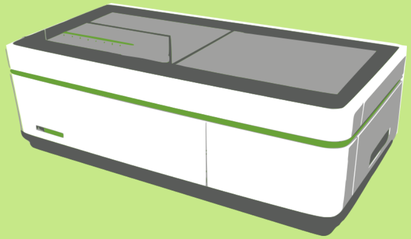
Spinning disc confocal

Accessible 8 position emission filter wheel

Temperature and CO_2 control



Shared features



1 – 4 Cameras

4 – 5 Lasers

Synchrony Optics™ crosstalk suppression

On-Board Liquid Handling

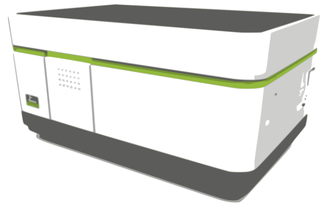
Temperature, CO_2 and humidity control

- 4.7MPx sCMOS camera
- Air and Water immersion objectives
- Widefield and confocal imaging
- Brightfield and digital phase imaging

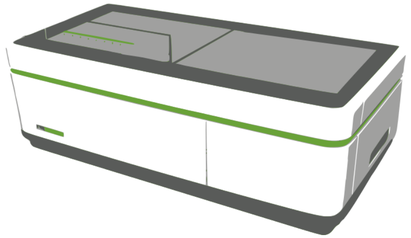
- Fast, high resolution x,y-table and single lens z-drive
- 6 position objective turret
- Sample based flatfield correction
- Easy to use Harmony software
- Automation ready

PerkinElmer

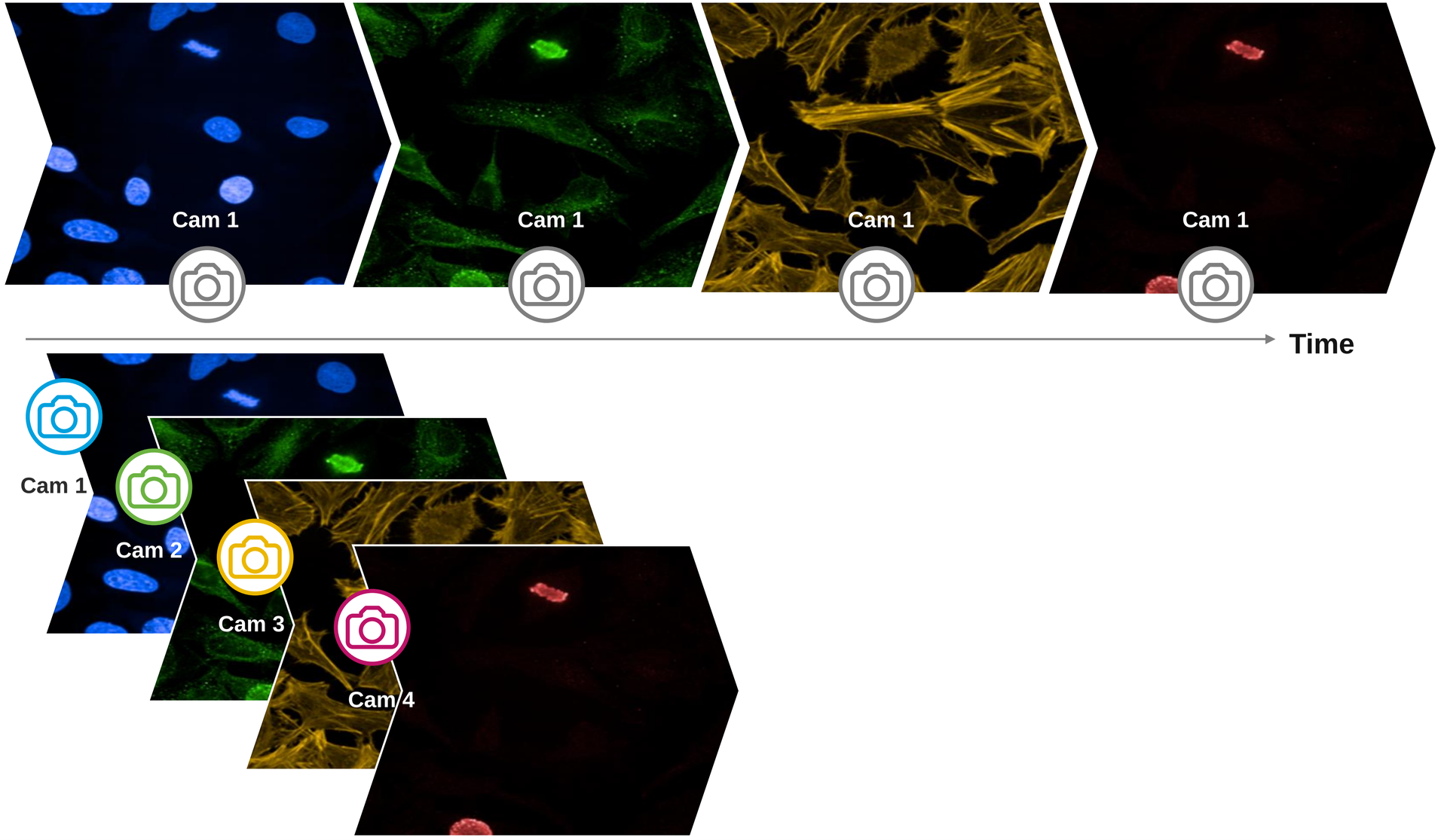
Add More Cameras for Increased Speed



Operetta CLS
Sequential acquisition
(1 camera)

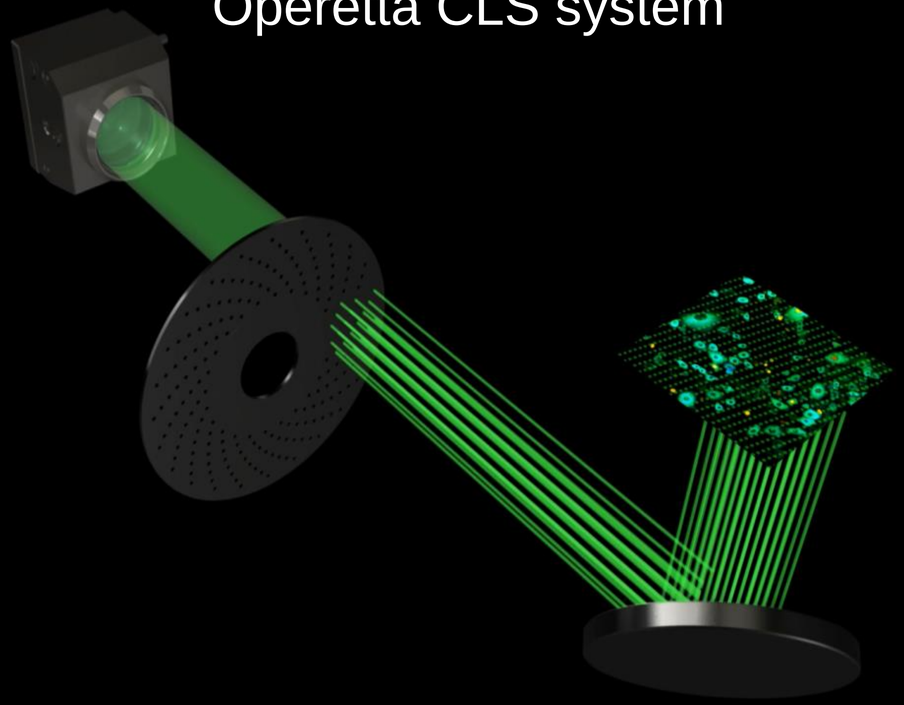


Opera Phenix Plus
Simultaneous acquisition
(2 or 4 cameras)



Spinning Disk Confocality

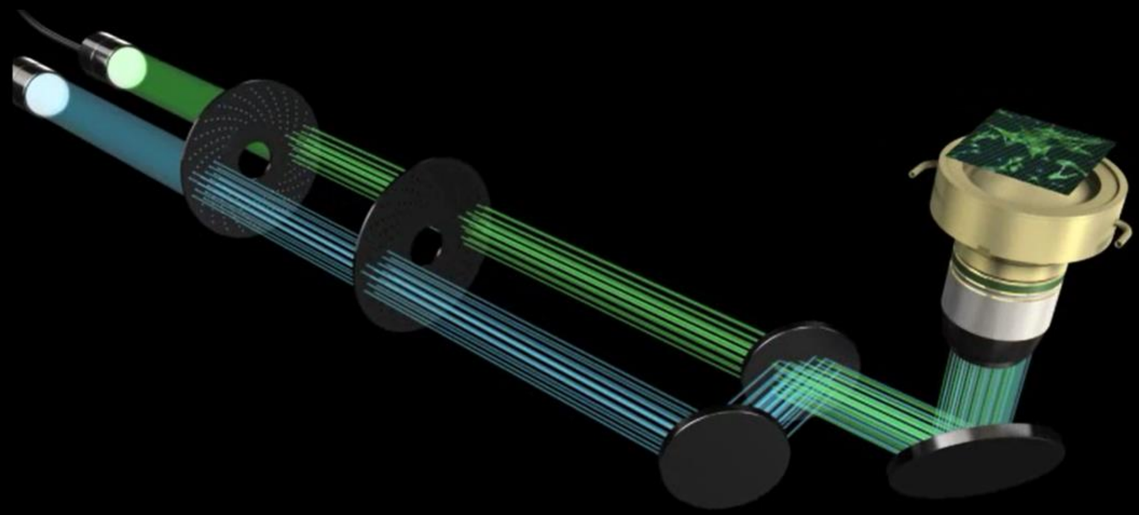
Operetta CLS system



Excitation light path schematic

- Single spinning pinhole disk

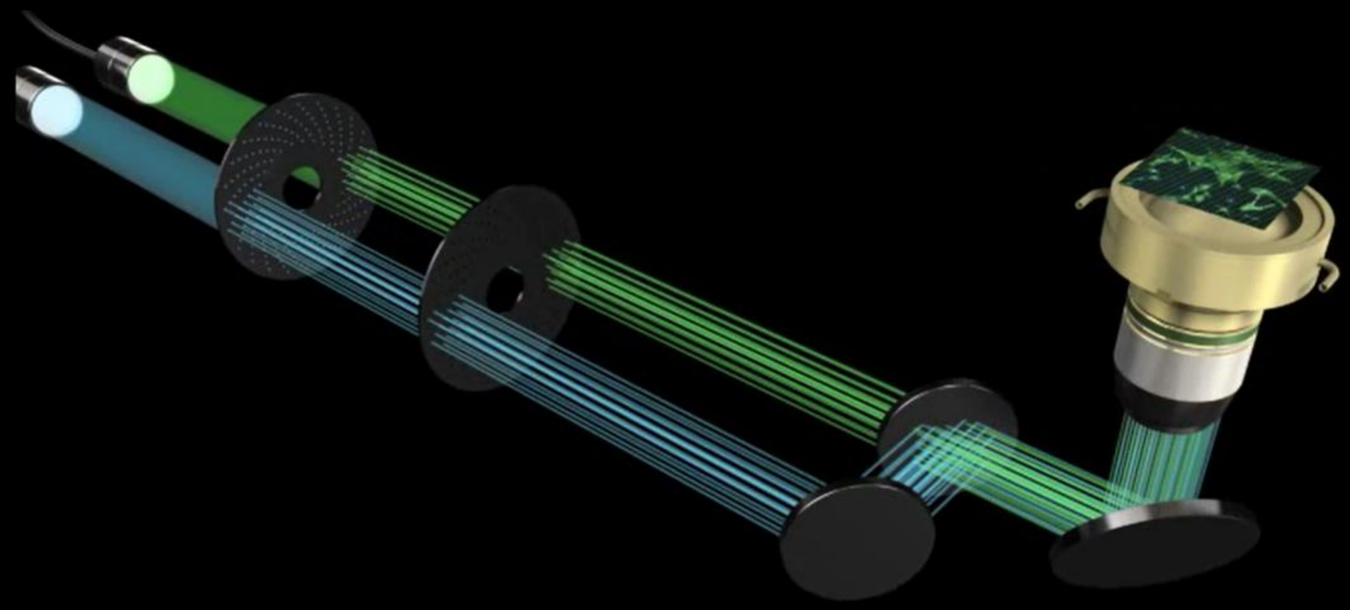
Opera Phenix system



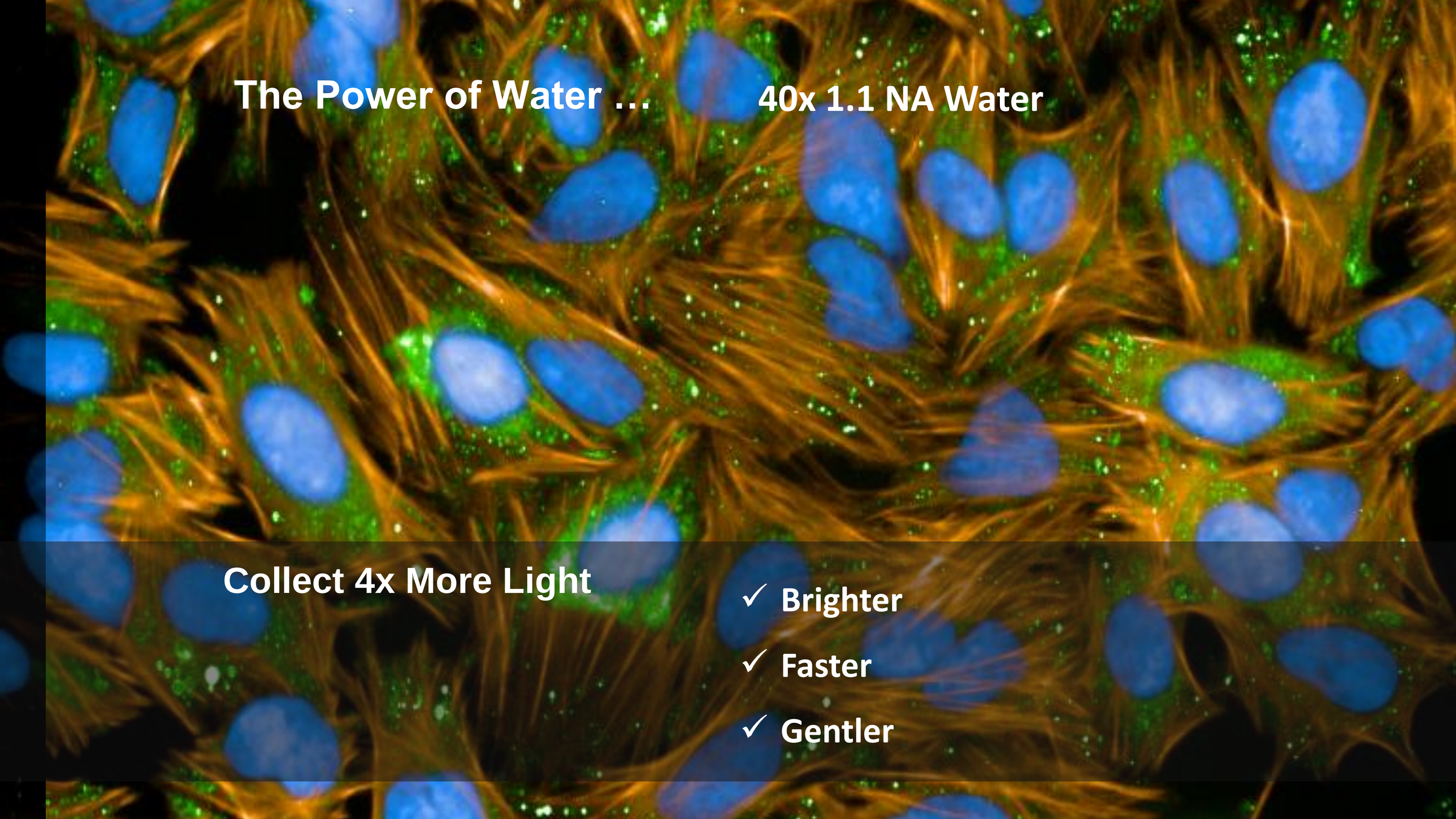
Synchrony Optics excitation schematic

- Microlens-enhanced dual spinning disc
- Proprietary confocal light path
- Simultaneous acquisition with minimal crosstalk

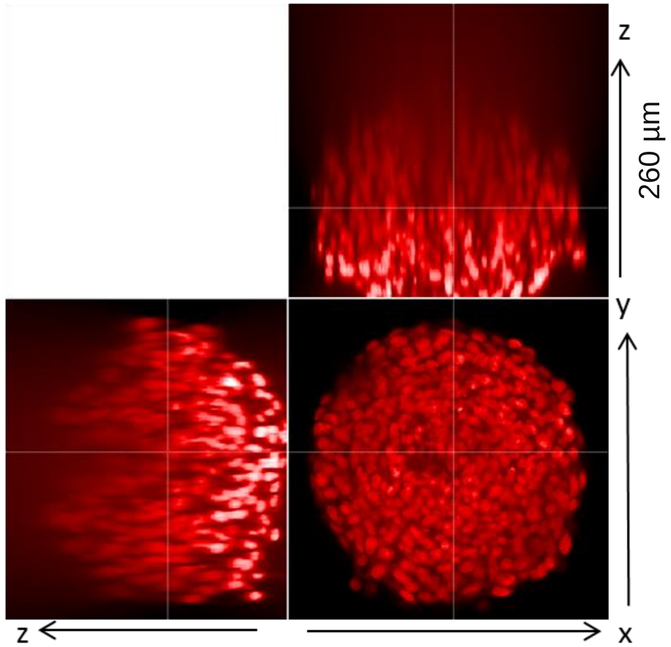
Opera Phenix - Synchrony Optics



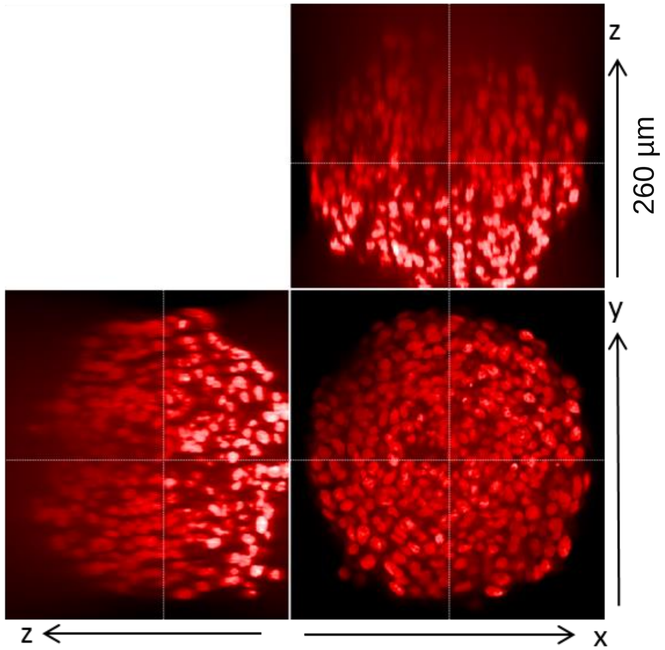
- Microlens-enhanced dual spinning disc for faster confocal imaging
- Dual excitation path for simultaneous acquisition with minimal crosstalk
- Large pinhole distance for improved image quality from 3D samples


The Power of Water …
40x 1.1 NA Water
Collect 4x More Light
✓ Brighter
✓ Faster
✓ Gentler

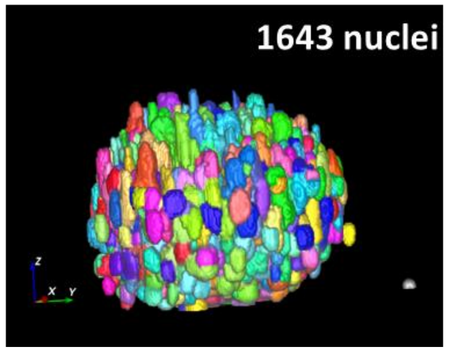
Use Water Immersion Objectives to Improve 3D Image Quality



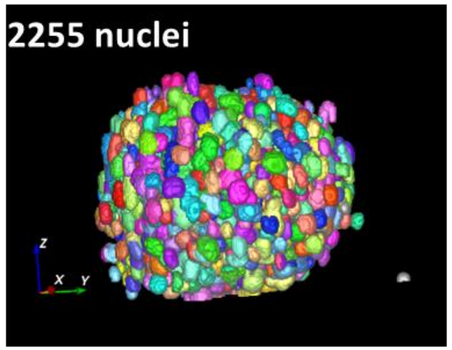
20x Air NA 0.75



20x Water NA 1.1



Exposure time 400ms



Exposure time 300ms

Water immersion objectives improve:

- z resolution
- imaging depth
- nuclei segmentation
- in 3D cell models

~1.5x
more nuclei detected

Solid cancer spheroids, cleared with Scale A2
DRAQ5™

Aquired on Opera Phenix system

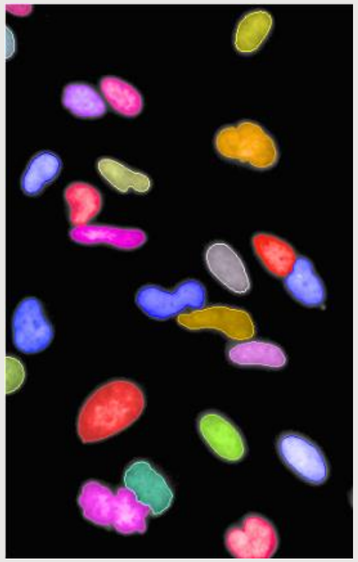
PerkinElmer

Harmony Image Analysis

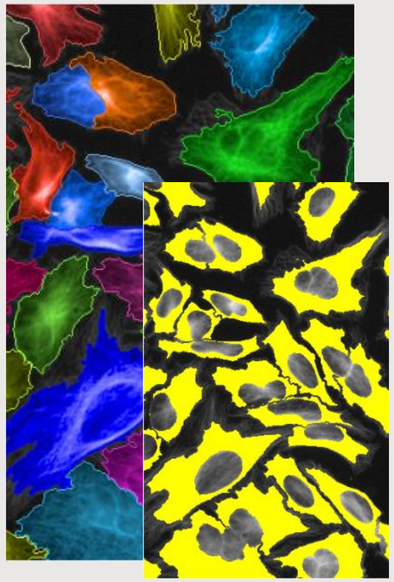
Harmony Interface for Image Analysis

Image Analysis Workflow- Constructed From Building Blocks

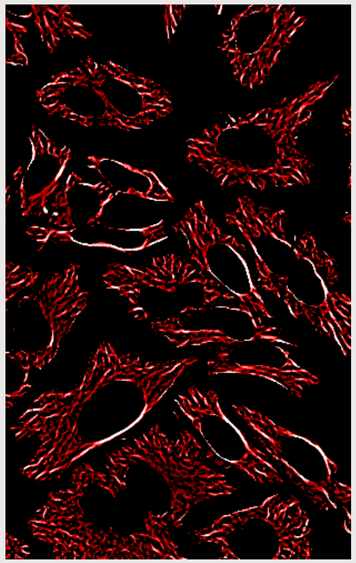
Segment
image to identify objects



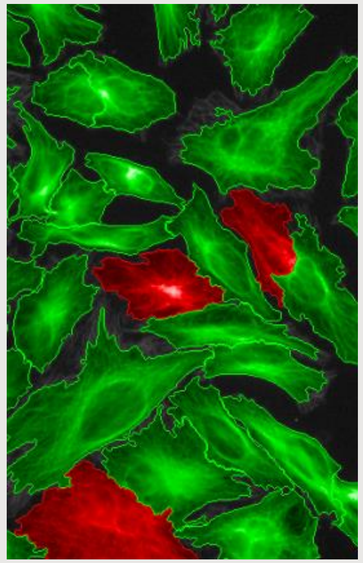
Refine
regions of interest



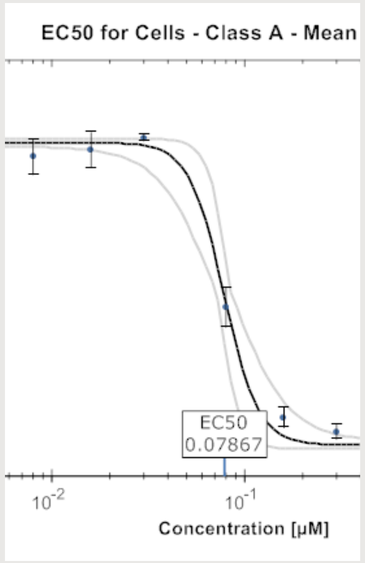
Quantify
relevant features



Classify
define and select specific cellular populations



Calculate
population measurements and statistics



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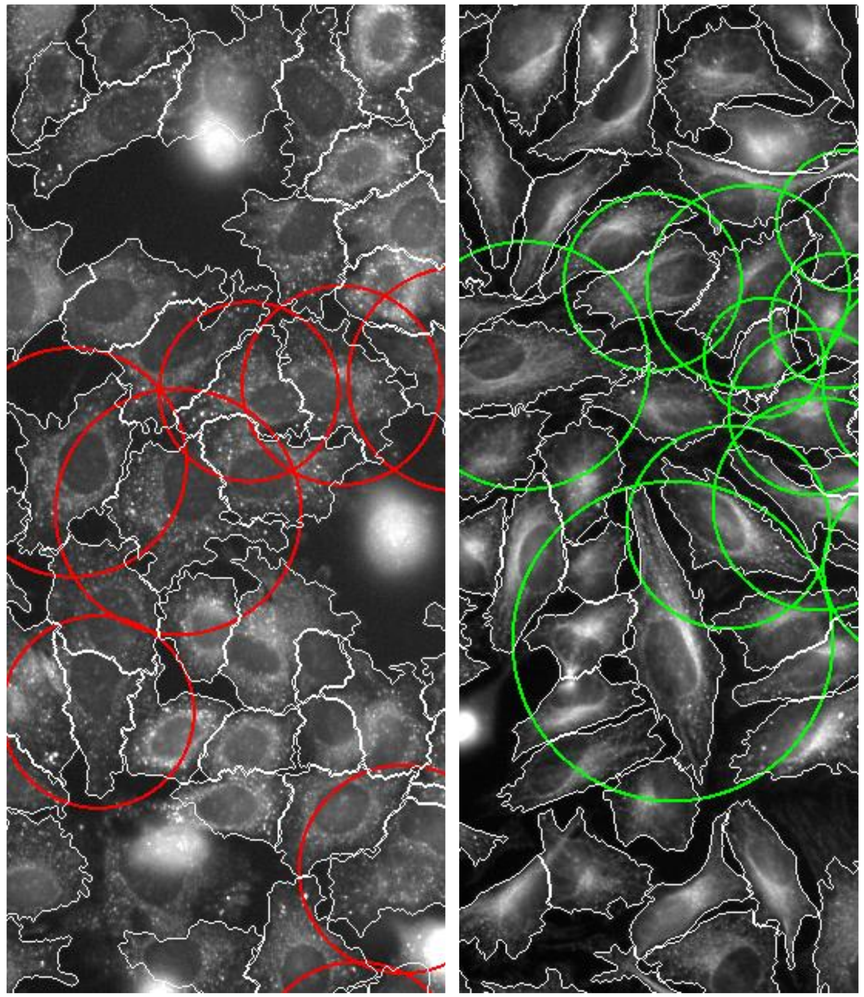
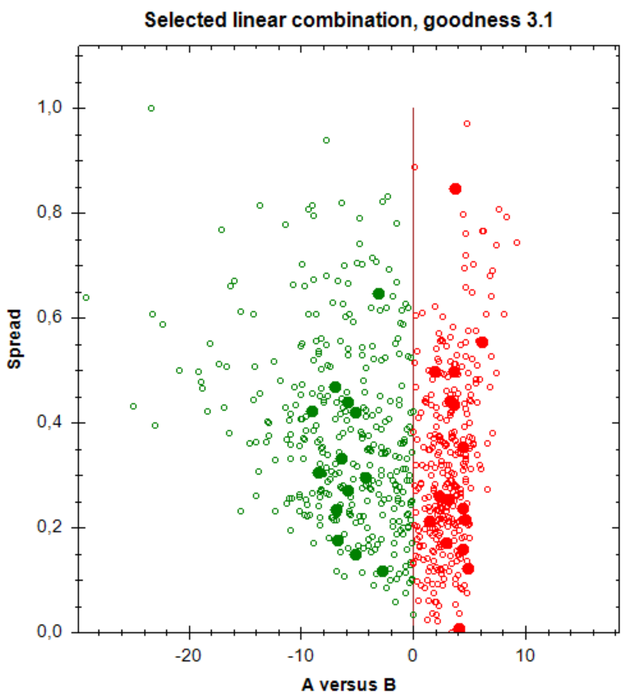
Advanced Image Analysis Made Easy – PhenoLOGIC™: Supervised Machine Learning

Train PhenoLOGIC

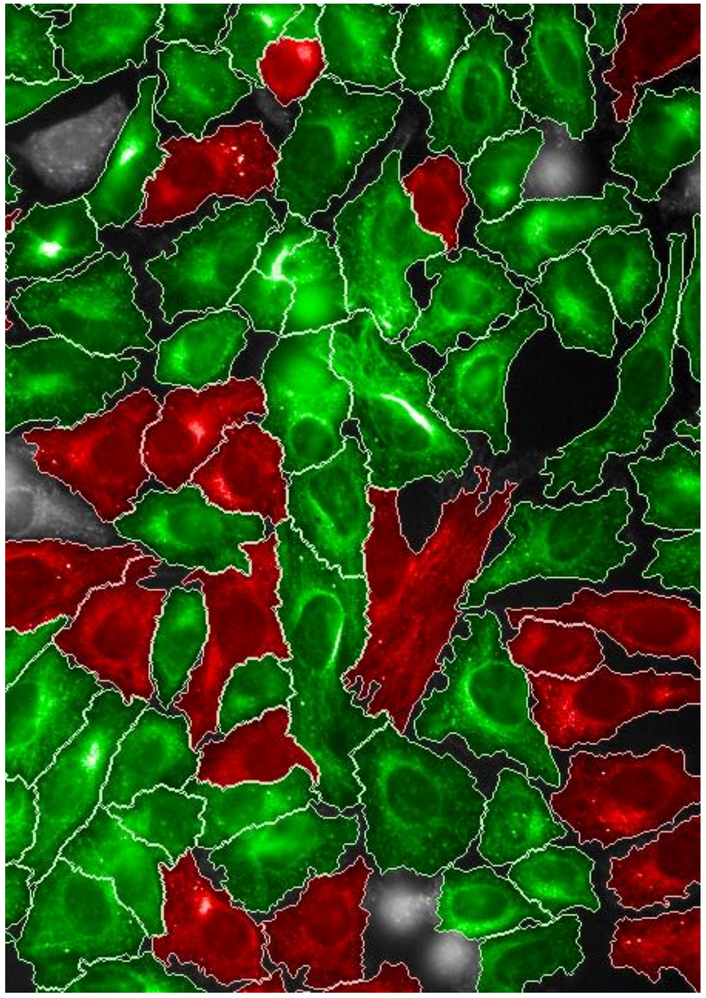




Training Set | A versus B

Goodness	Offset
3,1	-10,1384
Properties (ordered by relevance)	**Linear Coefficien**
Cell Profile 5/5 SER-Edge	-317,599
Cell Profile 4/5 SER-Bright	-396,434
Cell Symmetry 03	13,4113

Apply classification



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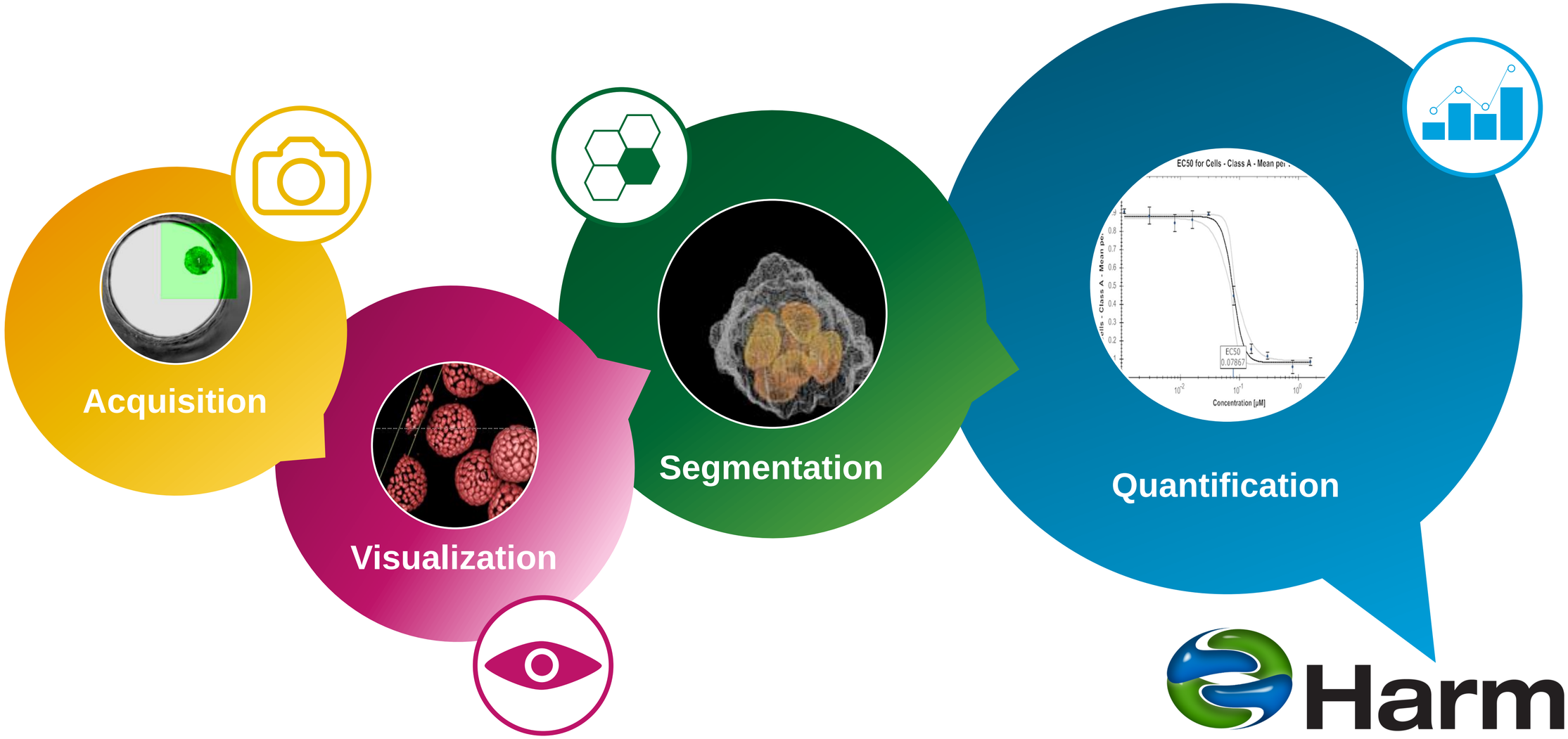
3D High Content Analysis Workflow



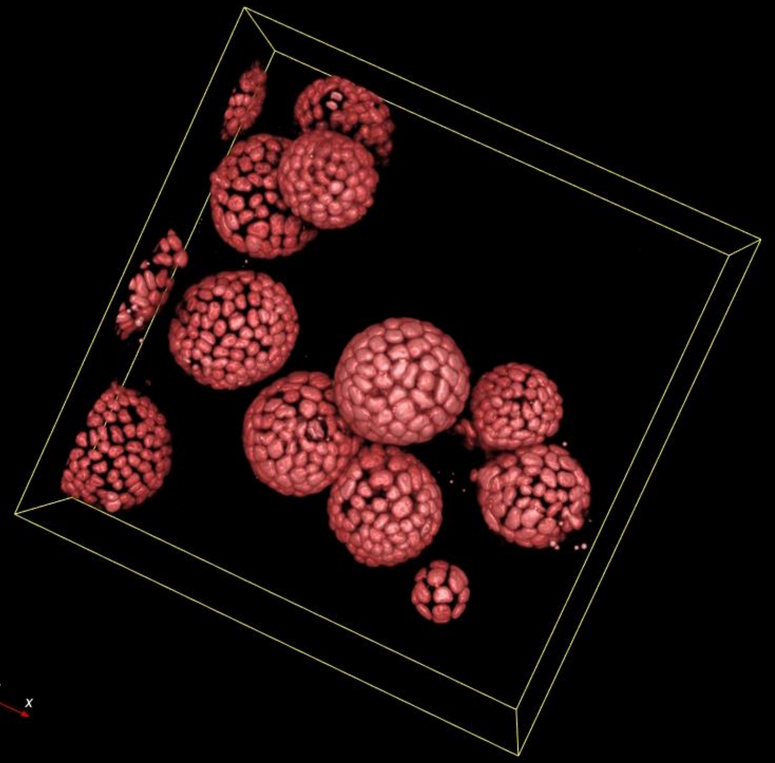
Harmony®

PerkinElmer

Multiple Ways for 3D visualization

Plane movie

3D movie



XYZ View
sectional planes in x-, y- and z- direction

3D View
Rotate, zoom, shift objects

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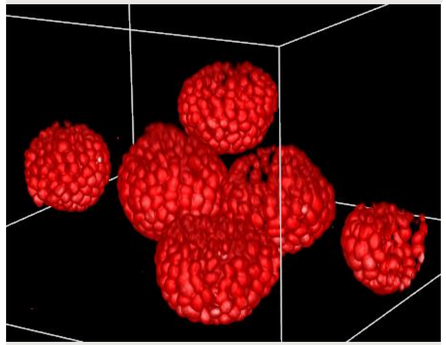
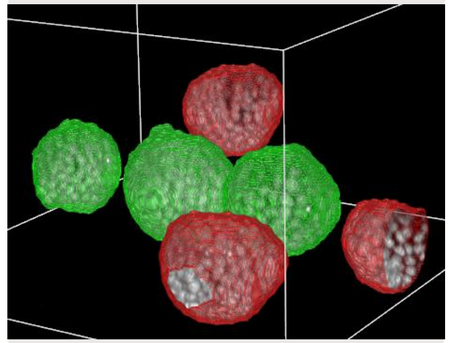
3D Volumetric Analysis of Luminal Spaces Inside Cysts or Organoids

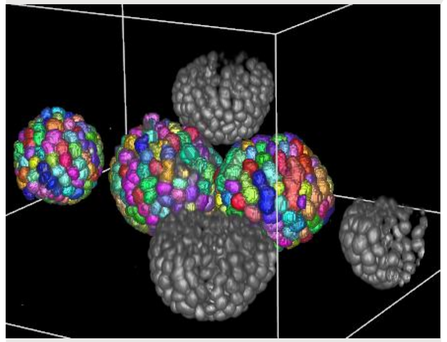
Input
image stack



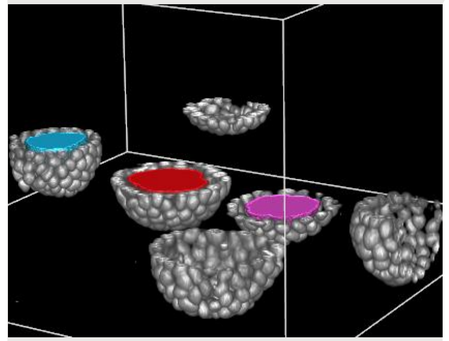
Find
Cysts and remove border objects



Segmentation
of single cells



Define
hollow space



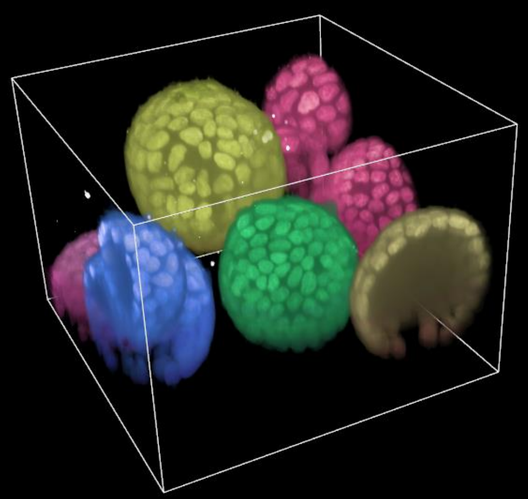
Calculate
specific properties for evaluation

Cyst Centroid X in Image [µm]	Cyst Centroid Y in Image [µm]	Cyst Centroid Z in Image [µm]
-117.0	13.1	47.0
86.5	-38.5	66.6
-1.7	-22.5	58.0

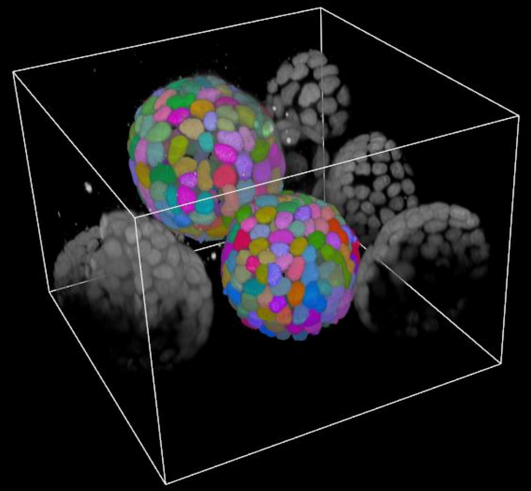
Cyst Volume [µm³]	Cyst Surface Area [µm²]	Cyst Sphericity
168879	17877	0.83
217712	21292	0.82
254947	23239	0.84

Lumen Volume [µm³]	Number of Nuclei- per Cyst	Cell Volume [µm³] - Mean per Cyst
33415	139	813
63350	130	1080
59776	179	922

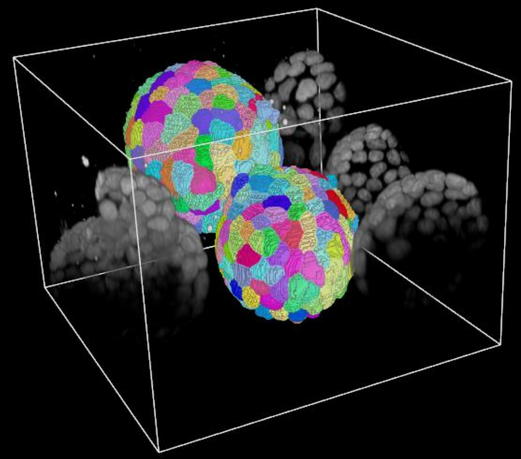
Harmony 3D Analysis - Segmentation Building Blocks



Cysts



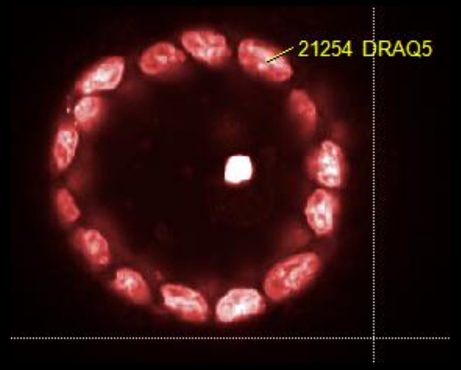
Nucleus



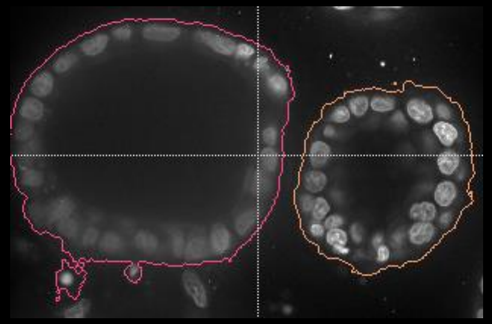
Cytoplasm

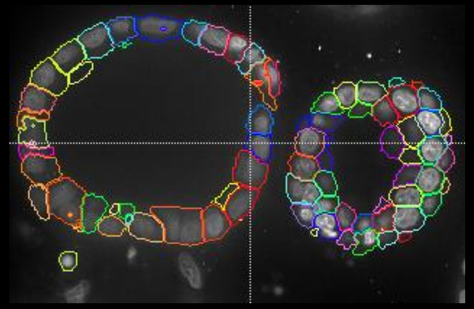
Find Image Region
Find Nuclei
Find Cytoplasm

Harmony 3D Analysis - Quantification Building Blocks

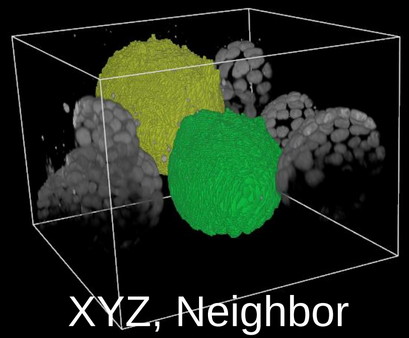


Intensity

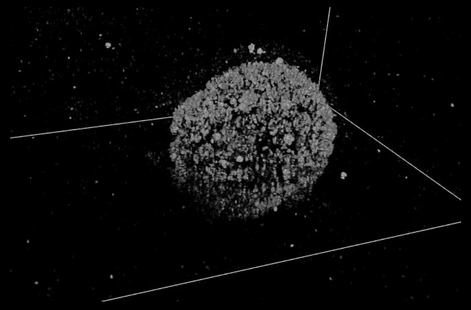




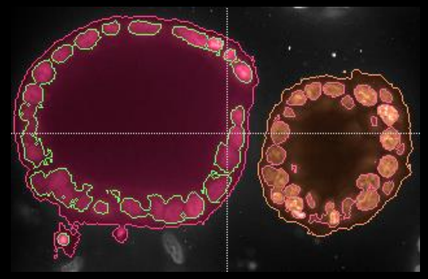
Volume, Sphericity…



Position



PLS Texture



Nuclei/Cyst

Calculate Intensity Properties
Calculate Position Properties
Calculate Morphology Properties
Calculate Texture Properties
Calculate Properties

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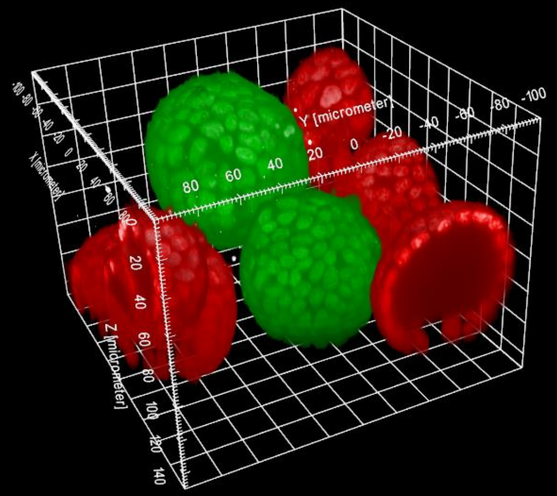
Harmony 3D Analysis - Building Blocks continued

Invert



Select Population

Filter Image
Calculate Image
Select Region
Select Population

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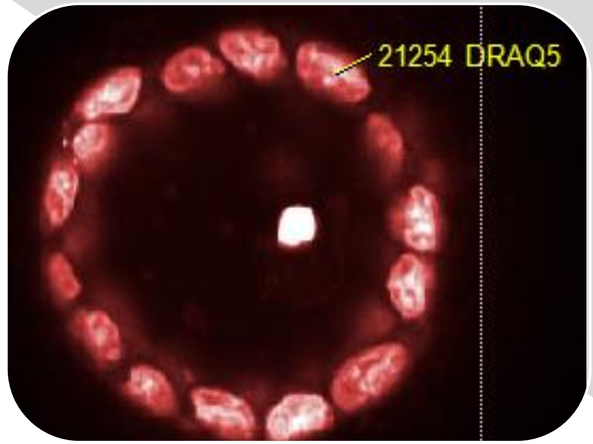
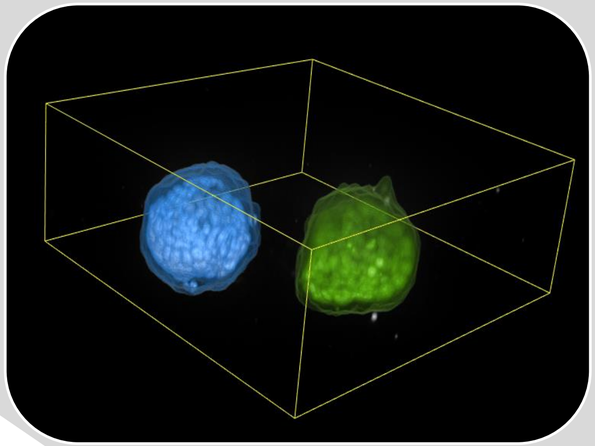
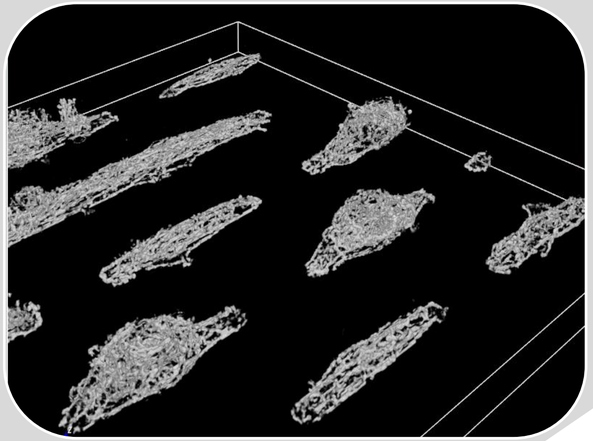
3D Image Analysis – made easy



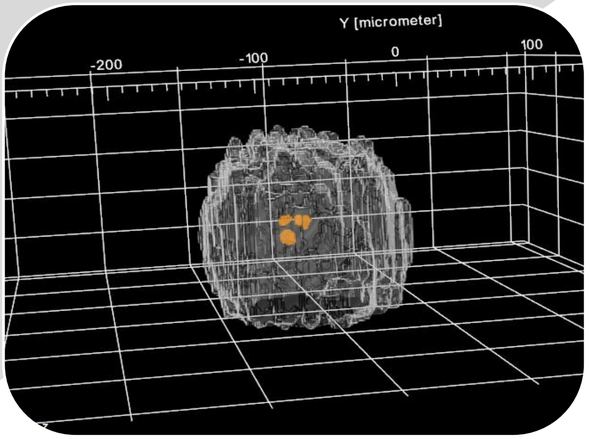
Intensity



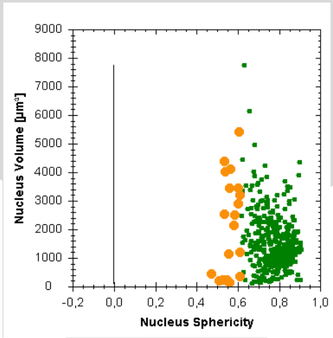
Morphology



Texture



XYZ position



Select Population

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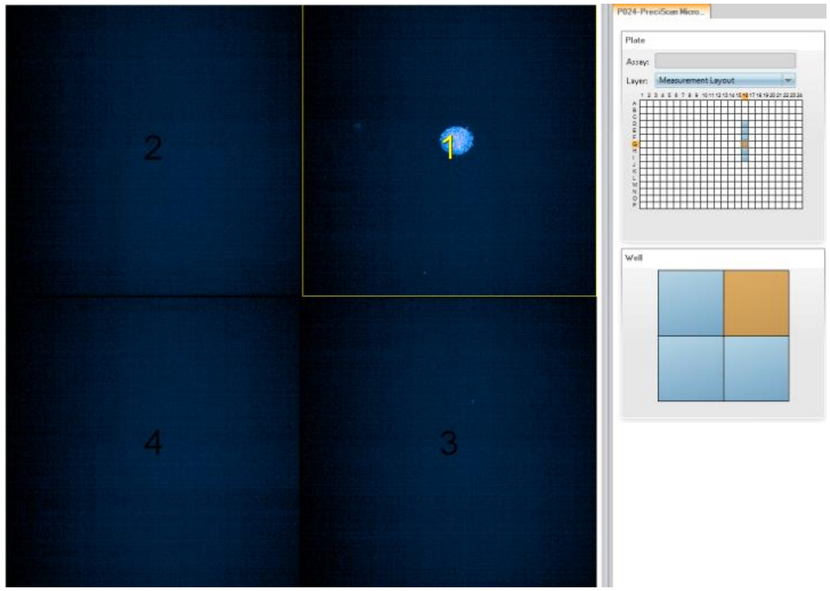
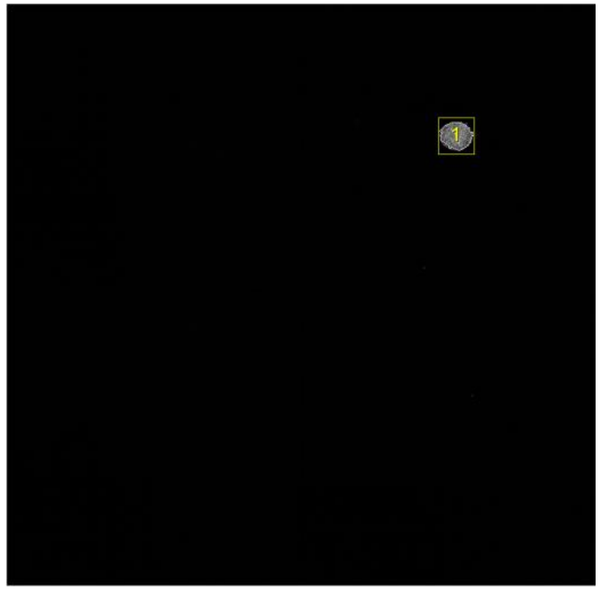
Intelligent Aquisition – PreciScan

PreciScan

PreScan – Measurement (5x)

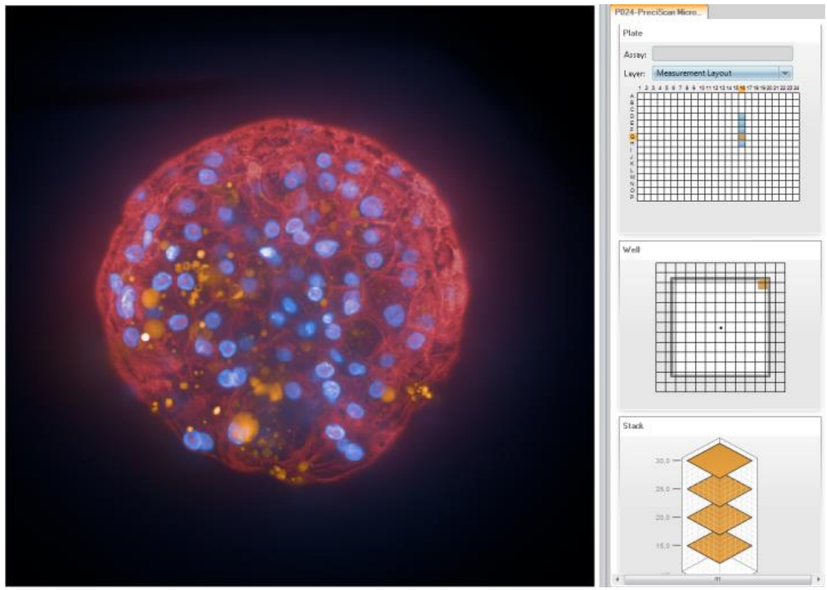


„Find Microtissue“ Analysis



Identify x/y positions of tissues for ReScan measurement

ReScan – Measurement (40x)

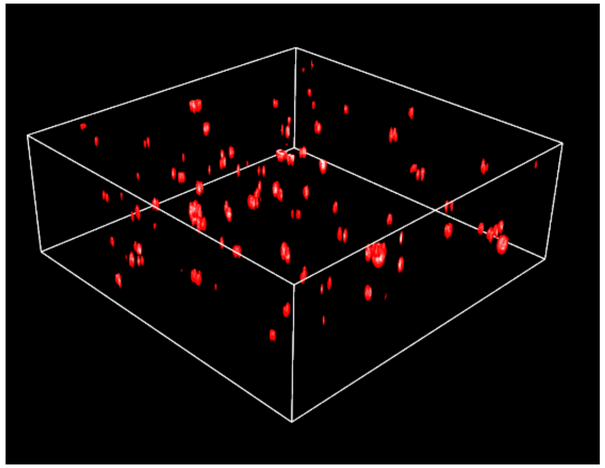


ReScan only wells that contain tissues

Intelligent Aquisition – Spheroids in Matrigel

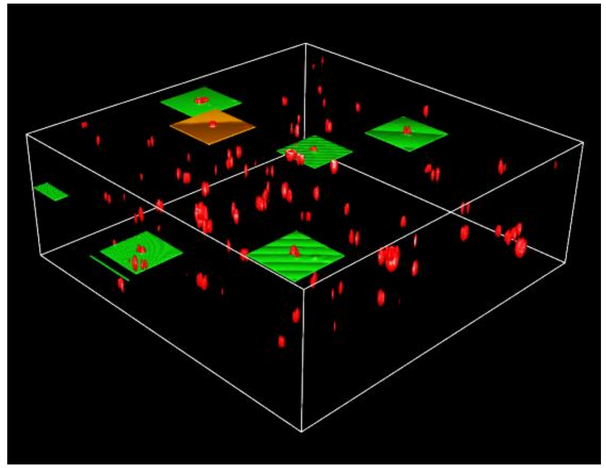
PreciScan (XY & Z)

Pre-scan – Measurement (10x)



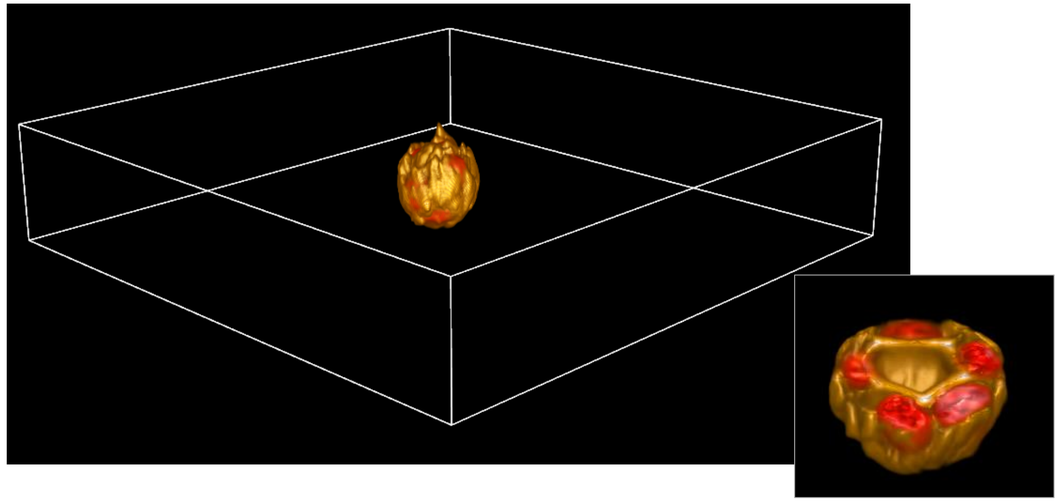
Pre-scan organoids with low magnification z-stack

Analysis to Find Organoids



Identify xy and z positions of organoids in matrigel

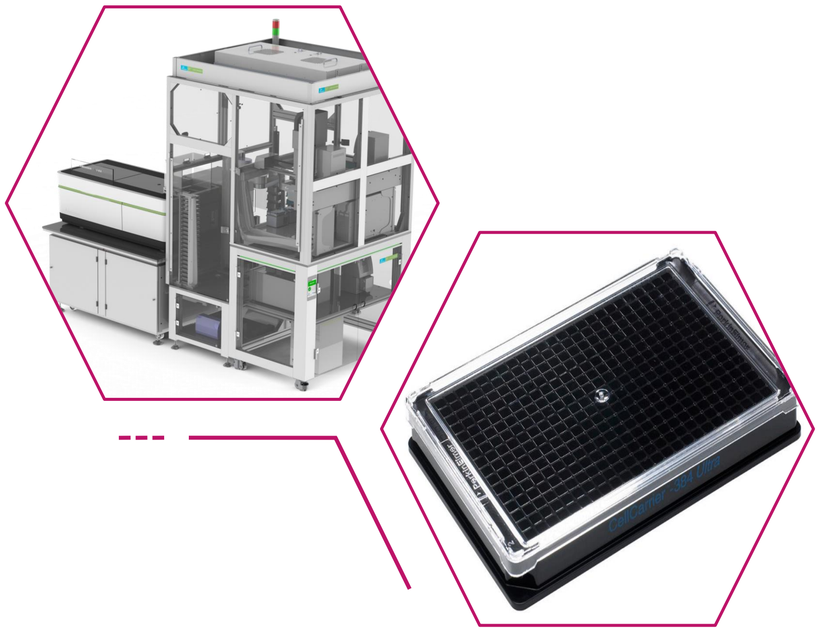
Re-scan – Measurement (63x)



Re-scan individual organoids with high magnification z-stack

WHAT WORKFLOW ?
Integrated High Content Screening Solutions - *from images to insights*

Sample Handling



explorer™ G3 workstation
Automate custom workflows and increase productivity and reproducibility

CellCarrier™ imaging plates
Optimal clarity and fast autofocusing from excellent flatness of the plate bottom

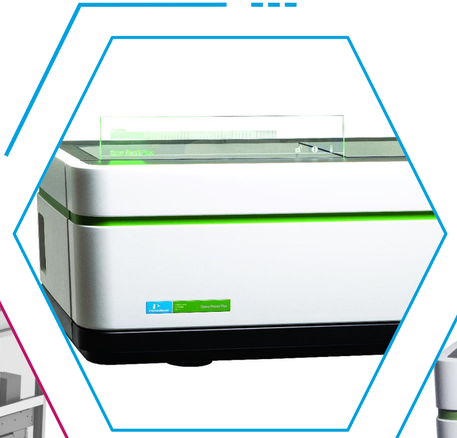
WHAT WORKFLOW ?
Integrated High Content Screening Solutions - *from images to insights*

Opera Phenix Plus
Maximal speed and sensitivity for 3D assays

Operetta CLS
Sensitivity and flexibility for all your biology

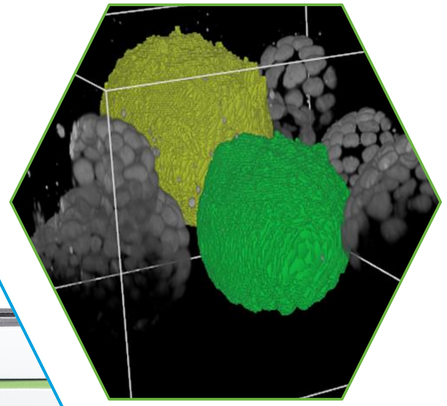
WHAT WORKFLOW ?
Integrated High Content Screening Solutions - *from images to insights*

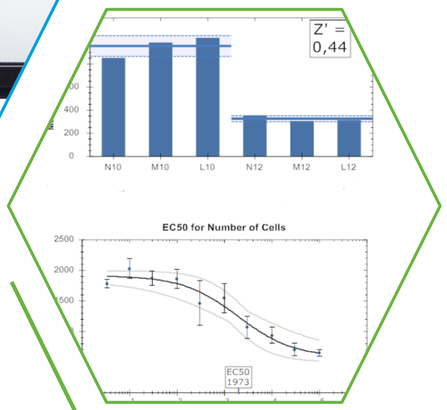
Sample Handling



Detection

Image Analysis





Harmony Software
for robust description of cellular phenotypes with texture and STAR morphology and showing results

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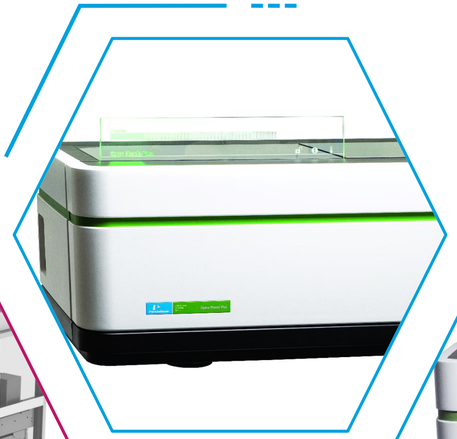
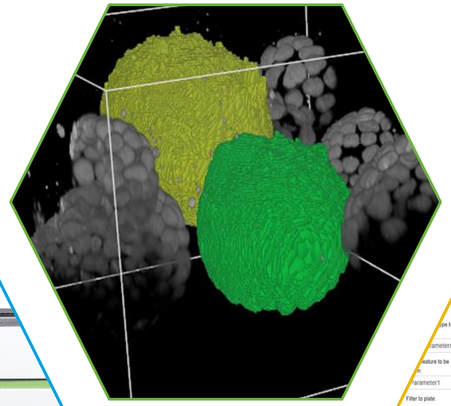
WHAT WORKFLOW ?
Integrated High Content Screening Solutions - *from images to insights*

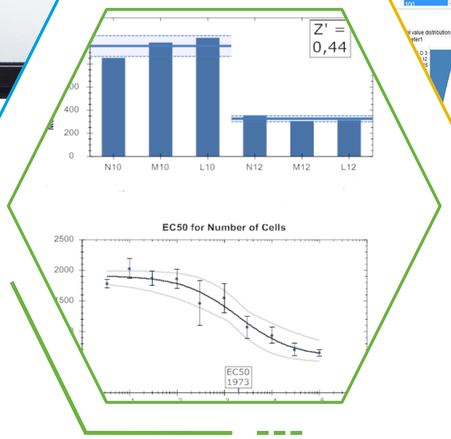
Sample Handling



Detection

Image Analysis

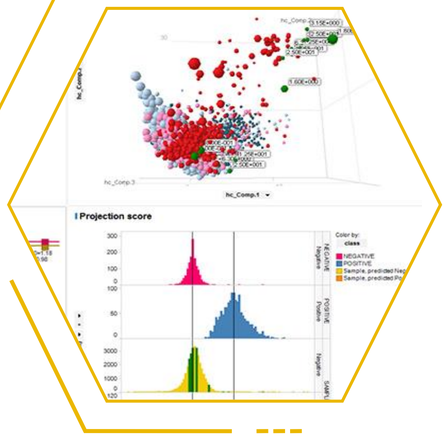




Signals VitroVvo powered by TIBCO Spotfire

Screen QC, data normalization, feature reduction, multiparametric hit selection

Profiling & Hit Selection



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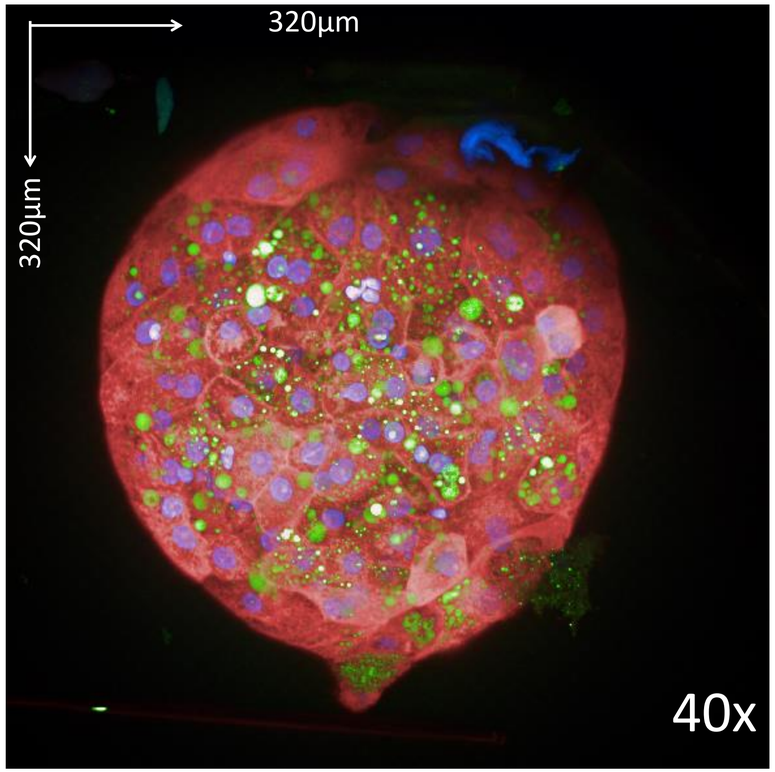
What 3D cell models ?



Spheroid: cells aggregate +/- cells growth

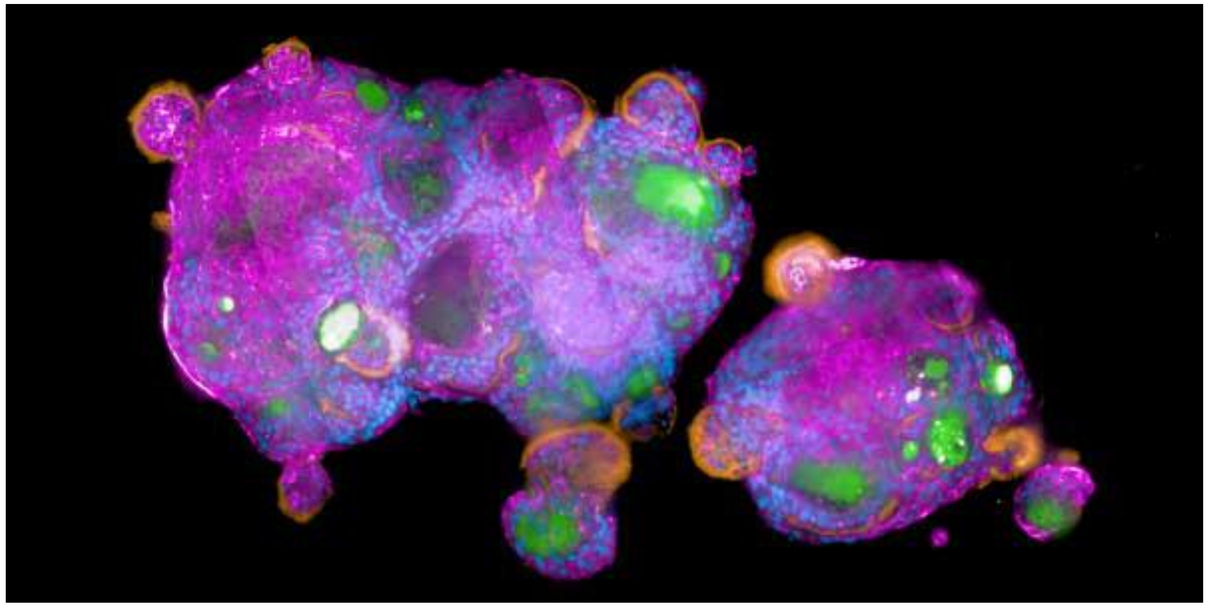
+/- Coculture

Without extracellular matrix : mostly in U shaped bottom plates

With extracellular matrix (matrigel, geltrex, growdex…)

What 3D cell models ?

Organoid: derived from one or a few cells from a tissue, embryonic stem cells or induced pluripotent stem cells, which can self-organize in three-dimensional culture



SARS-CoV-2-infected apical-out polarized lung organoids.
Höechst for nuclei (blue), MUC5AC for mucus (orange), phalloidin for tight junctions (pink), and an anti-SARS-CoV-2 antibody (green). Images were taken using the Operetta CLS system.
©D.Wilflingseder, A.Noureen

What 3D cell models ?

Going further ?

Automated Patient cells seeding into matrigel to grow cancerous organoids for **personalized medicine** and **translational research**

Embedded cells into matrigel dispensed at 4°C + medium addition: 15 x 384-well plates in 20mn

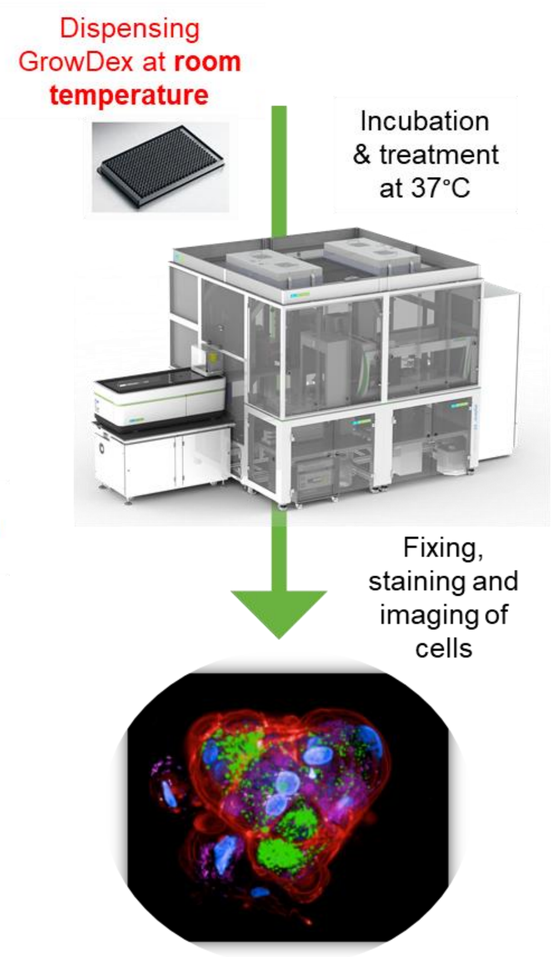
Mont Sinai Hospital, NY (USA)
Dr Ben Hopkins

What 3D cell models ?

Going further ?

What about 3D printing cells ?

Positioning matrices side by side or on top of one another



GrowDex®

GrowDex®-T

Exceptional imaging properties
Ideal for HCS platforms

GrowDex®-A

Protein functionalization
capability via avidin-biotin

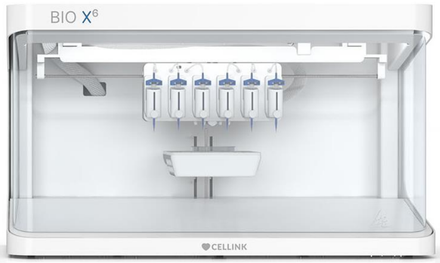
GrowDase™

What 3D cell models ?

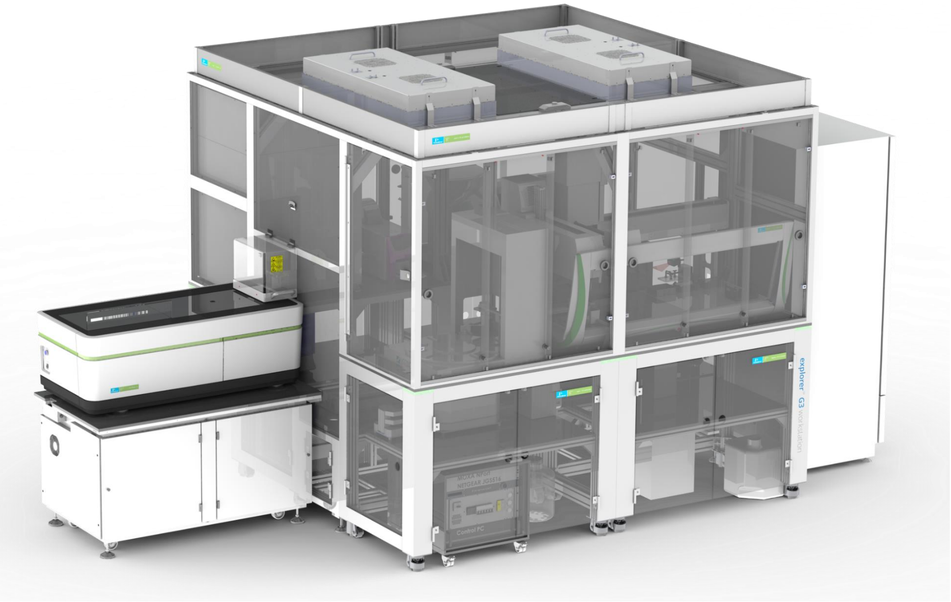
Going further ?

What about 3D printing cells ?

CellInk's printers



?



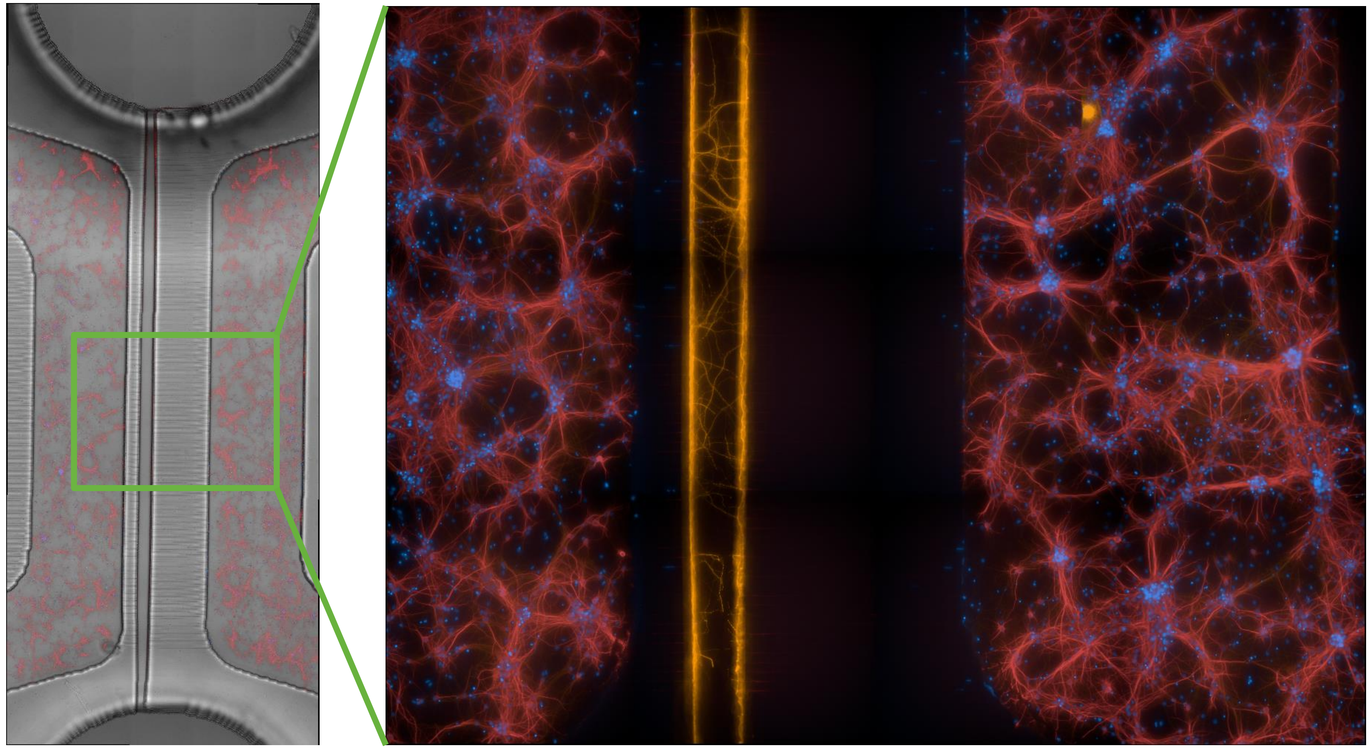
Inventia: Rastrum printer

What 3D cell model ?

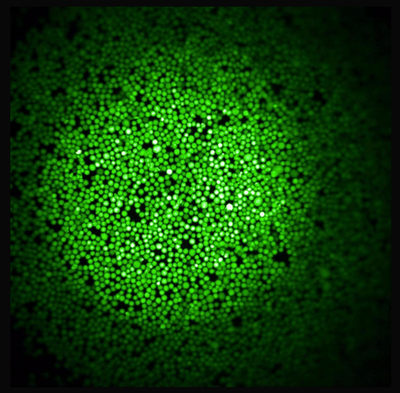
Going further ?

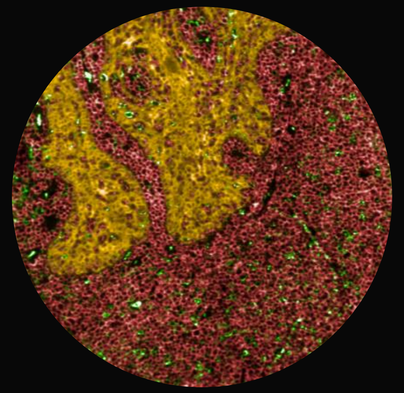
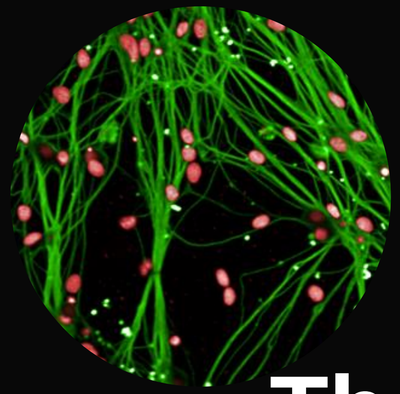
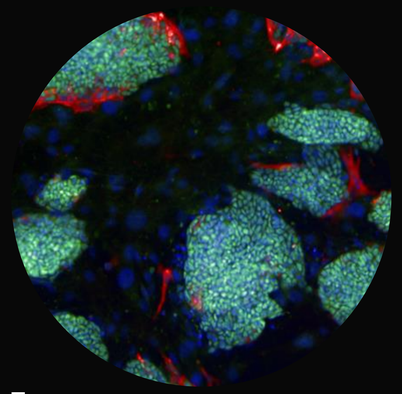
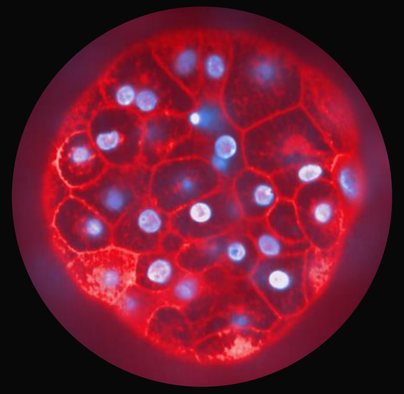
What about growing microtissues into microfluidic devices ?

96 well plate format Neurobento from NETRI



Operetta CLS 20x



Thank You