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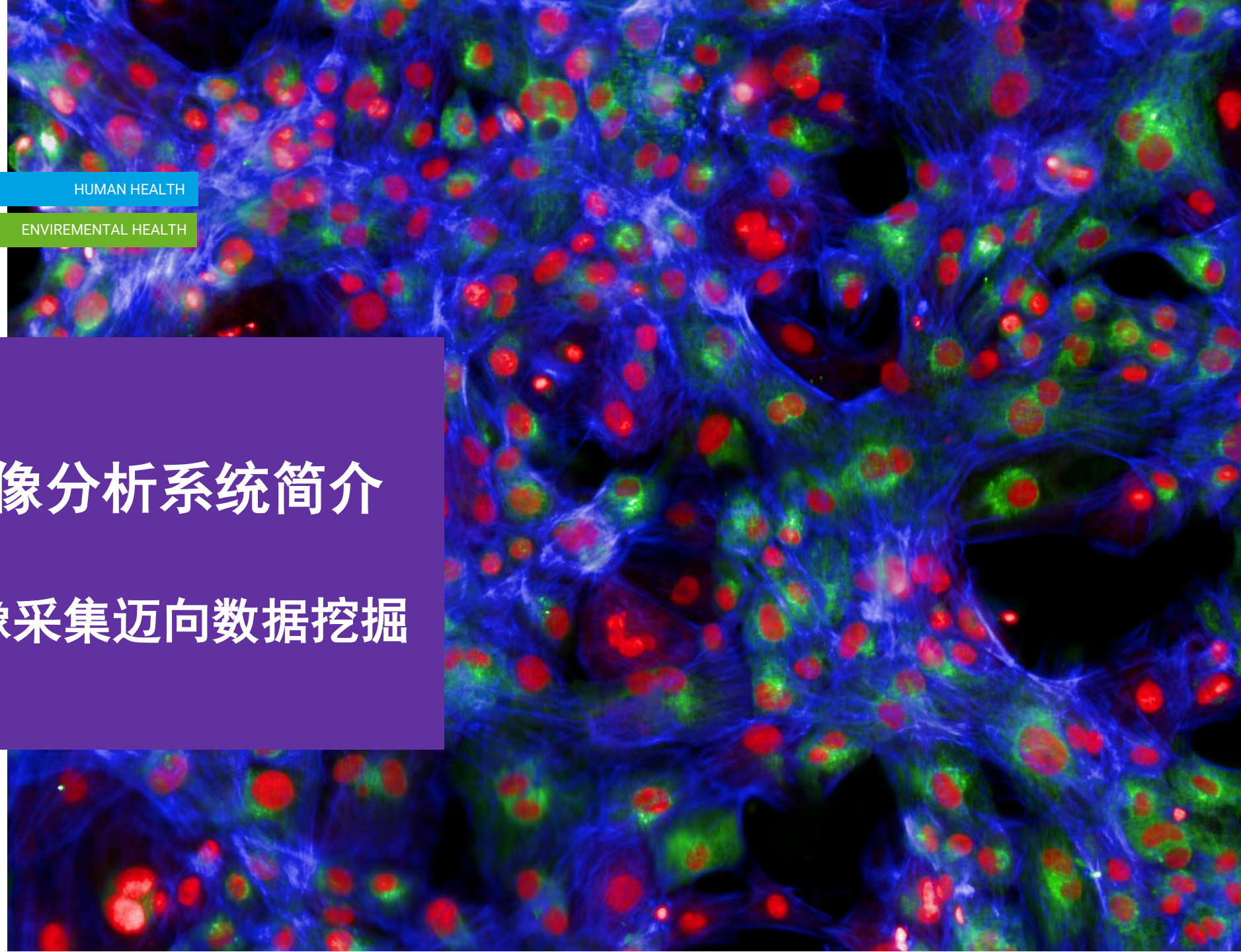
ENVIREMENTAL HEALTH

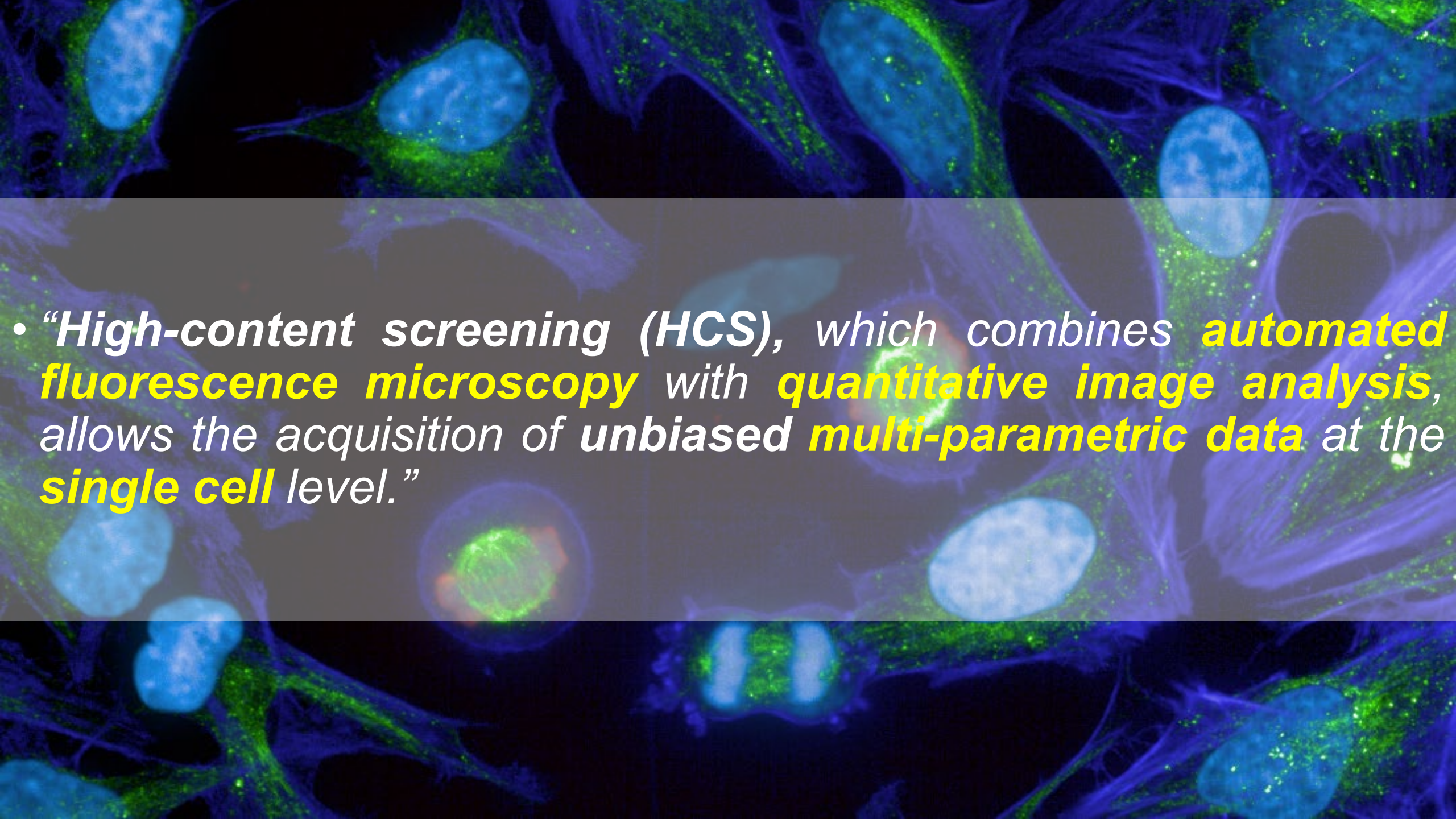


高内涵细胞成像分析系统简介

——由图像采集迈向数据挖掘

李妍

Yan.Li2@perkinelmer.com



- *"**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."*

What can HCS do?

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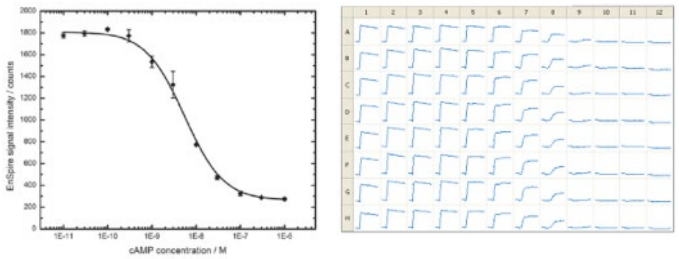


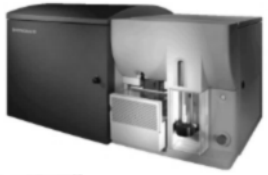
酶标仪

通量

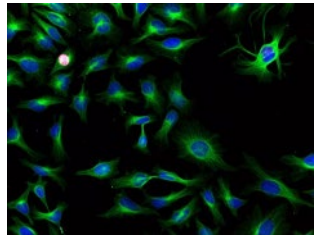
"深度"

图 像

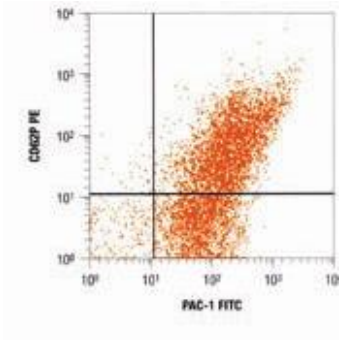
流式
细胞仪



荧光
显微镜

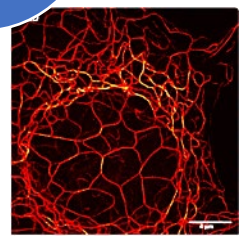


细胞学
分　析

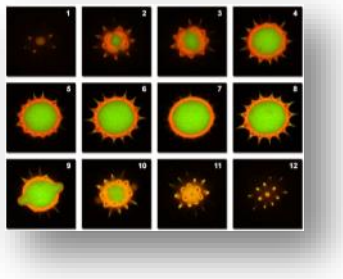


数 据

超高
分辨



共聚焦



高内涵增加了完整的实验设计，提供客观一致检测方式

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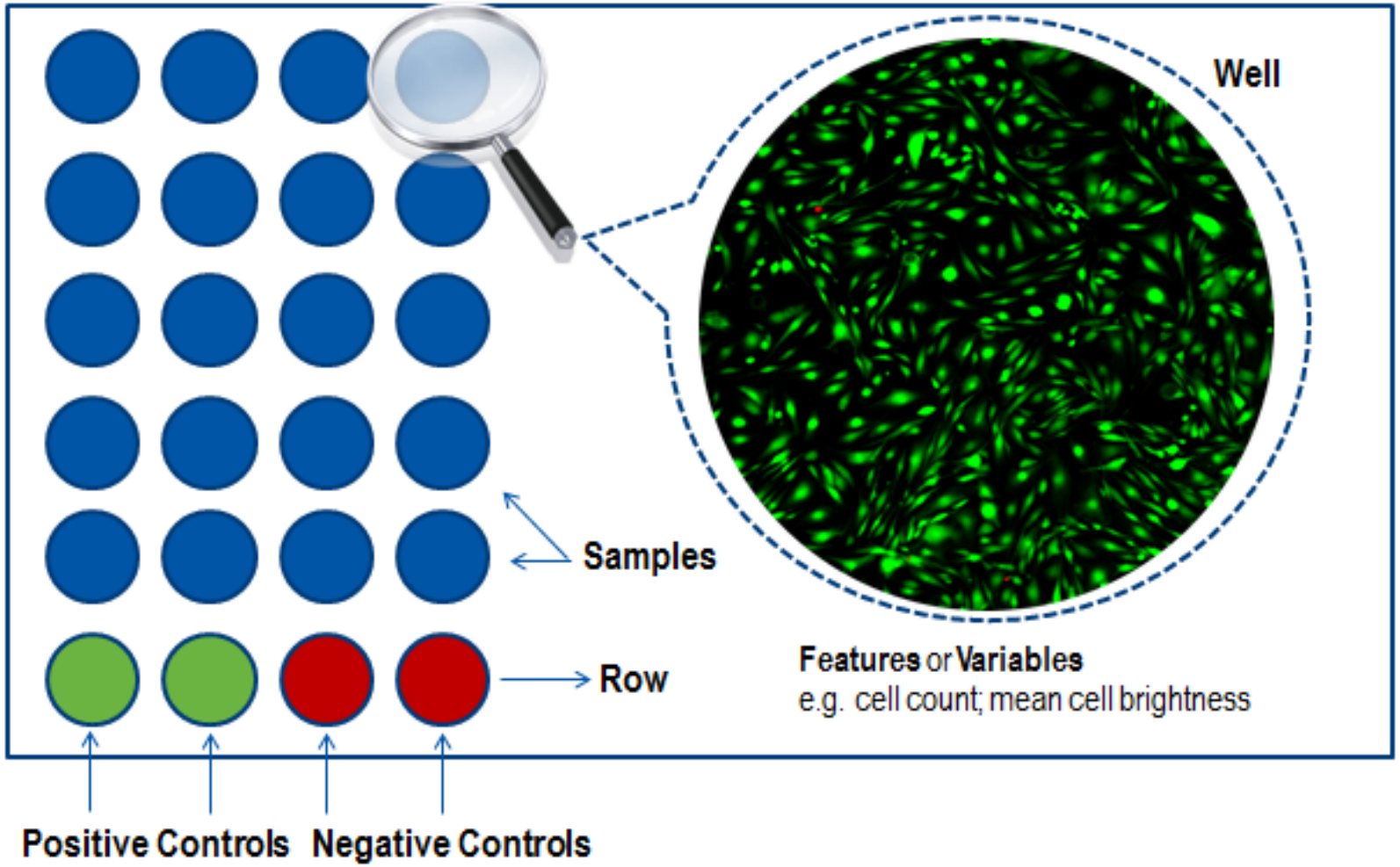
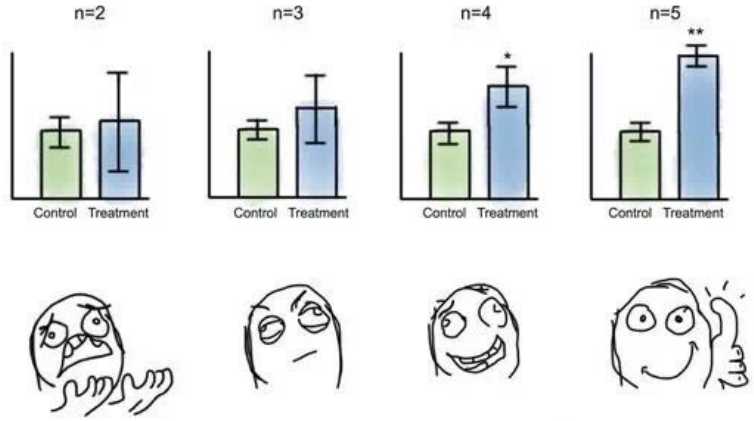
- ❖ 表型、亚细胞、动态
- ❖ 平行对照、重复统计



细胞模型！
对照试验！

- 多参数分析！
- 形态学数据！



PerkinElmer高内涵整体实验方案

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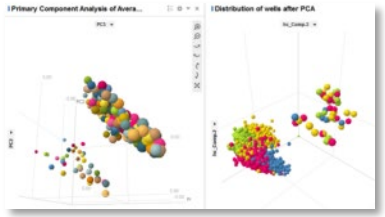
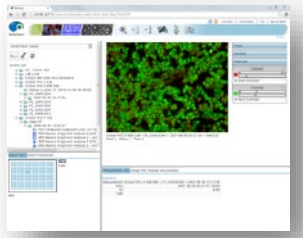
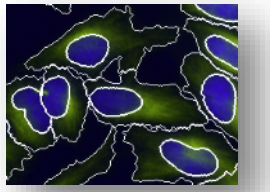


Spotfire
Easy. Fast. Smart.

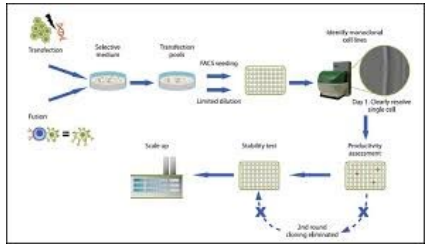


Columbus



Harmony

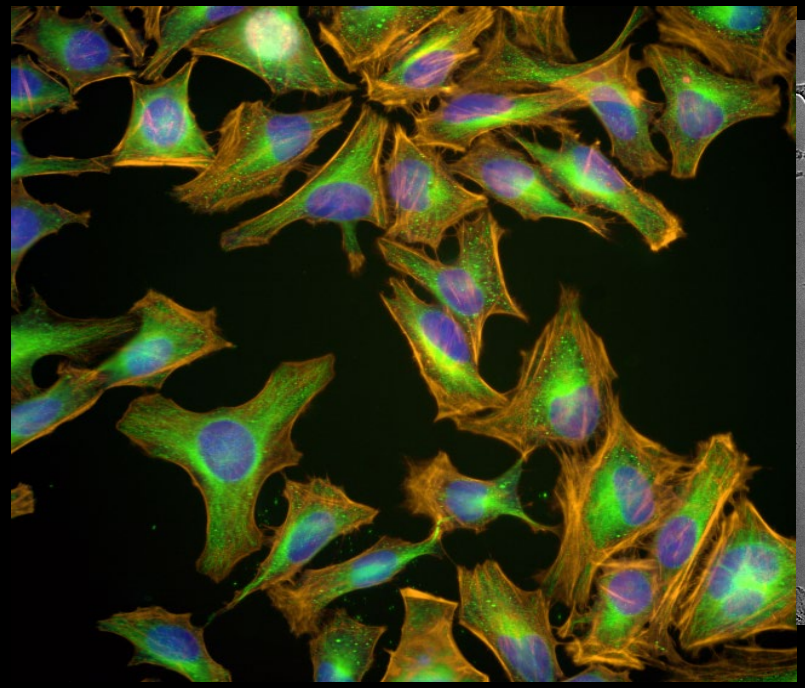
Software



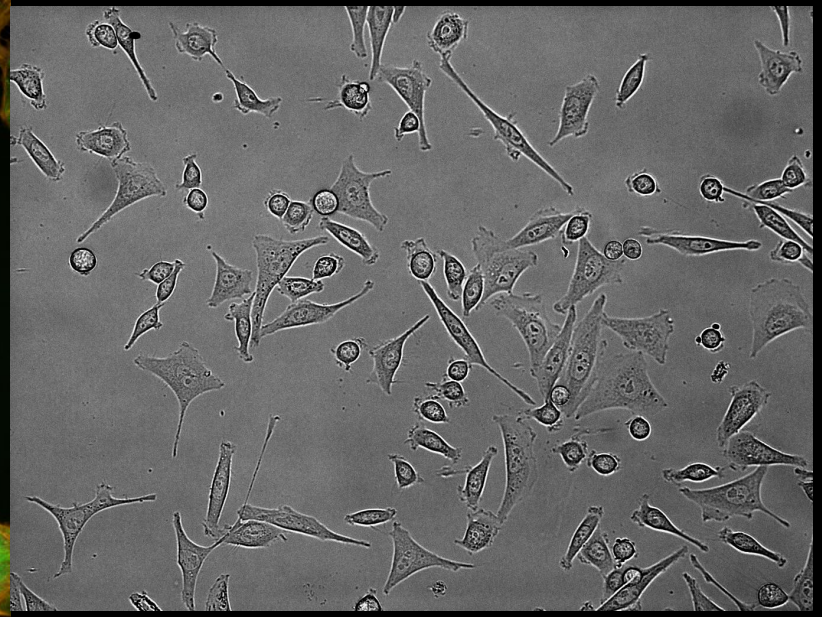
Wetware

Adapted from Korn & Krausz,
Current Opinion in Chemical Biology 2007, 11:503–510

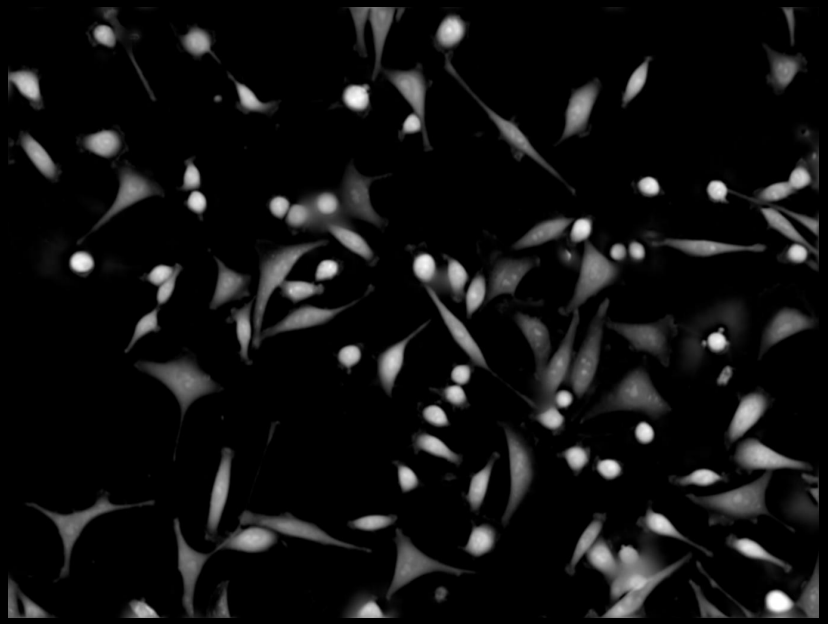
成像功能：荧光、明场、明场无标记（DPC）



荧光

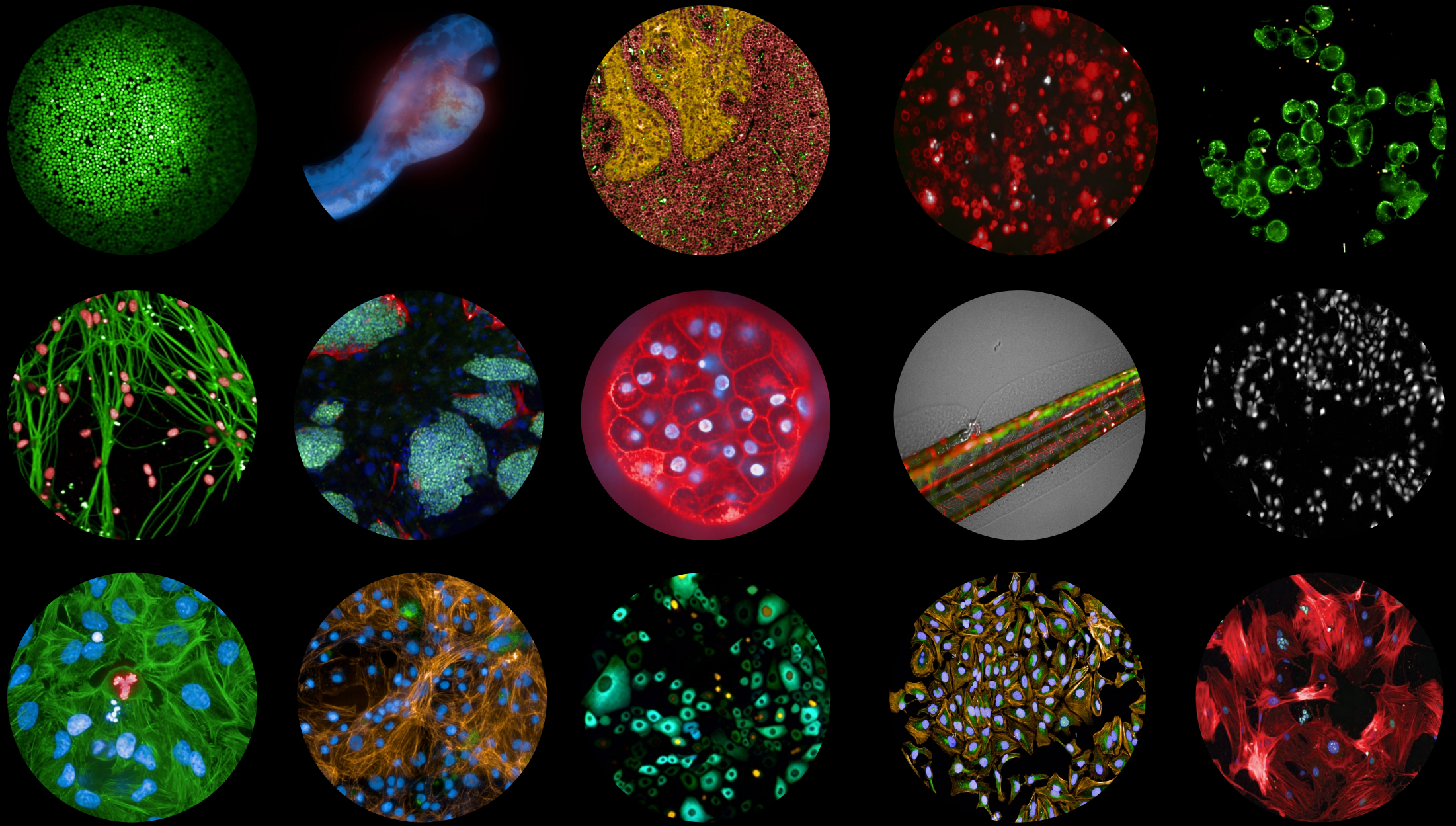


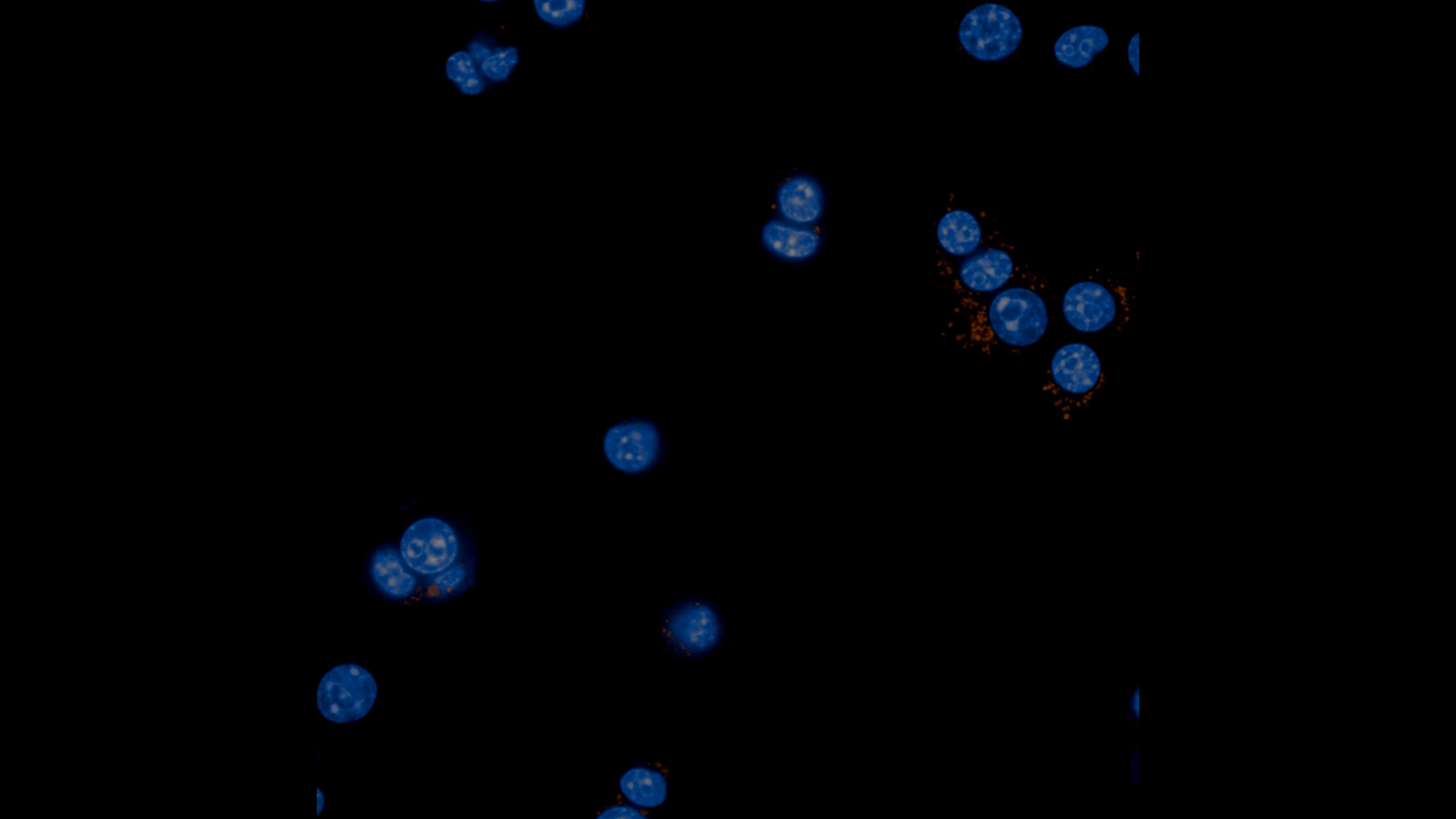
明 场

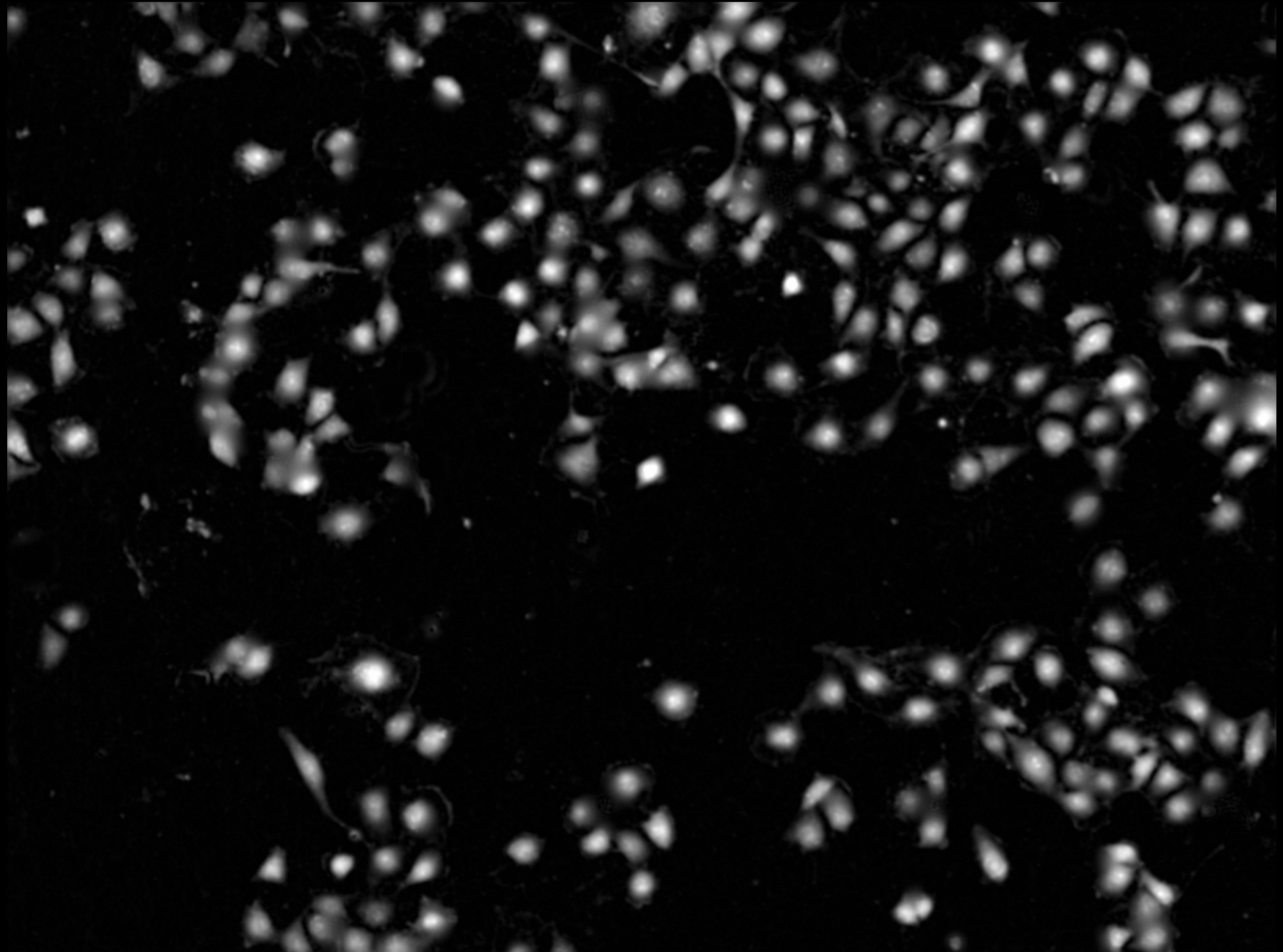


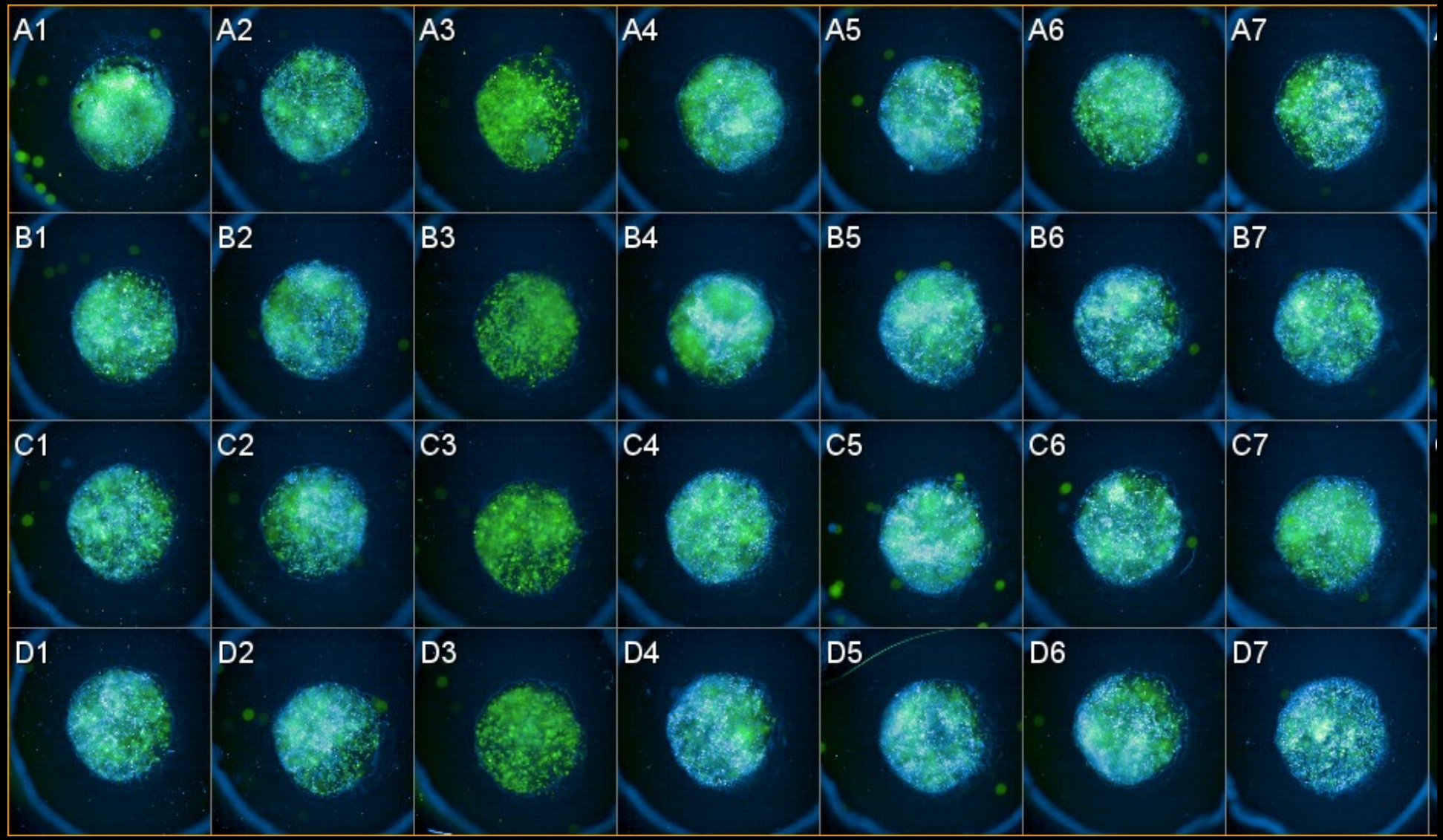
DPC

Better models….








A1
A2
A3
A4
A5
A6
A7
B1
B2
B3
B4
B5
B6
B7
C1
C2
C3
C4
C5
C6
C7
D1
D2
D3
D4
D5
D6
D7

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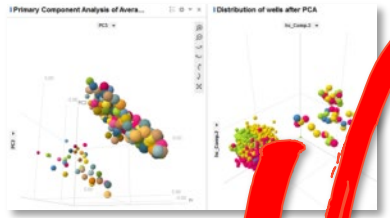
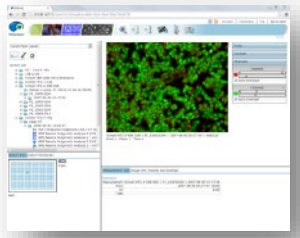
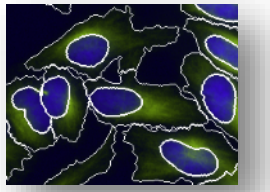


Spotfire
Easy. Fast. Smart.



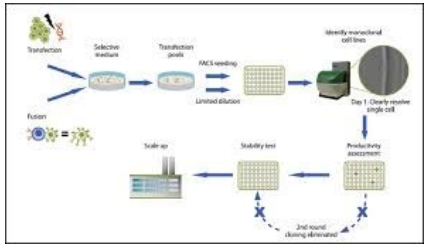
Columbus®



Harmony®

Software

Bioinformatics analysis
Chemistry
Cell biology
Wetware
Statistics, data analysis
Assay design
HCA
Data management
Fluorescent reagents
Image analysis
Robotics, liquid handling
Automated imaging systems
Hardware



Adapted from Korn & Krausz,
Current Opinion in Chemical Biology 2007, 11:503–510

细胞表型差异的高通量多参数量化

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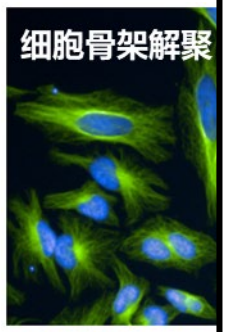
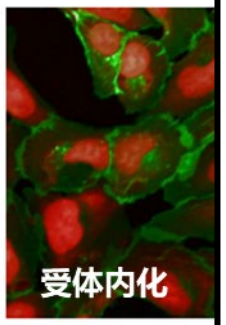
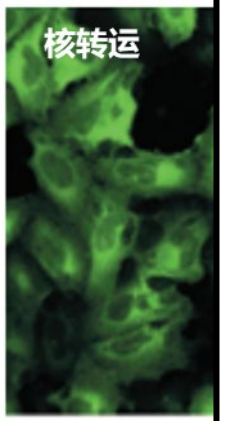
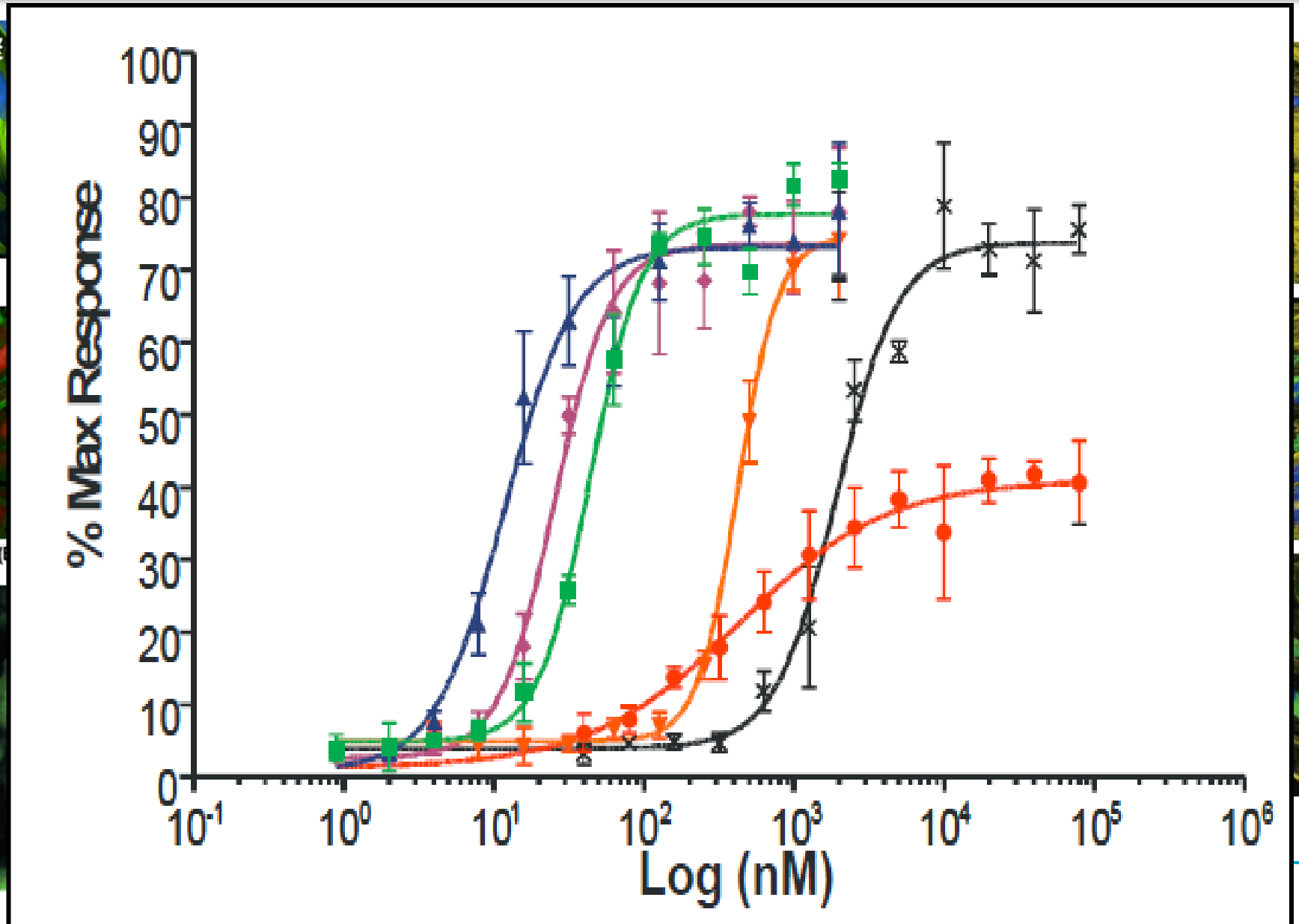


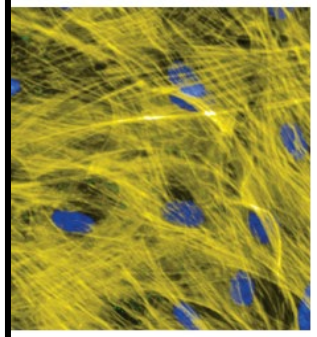
Untreated cells

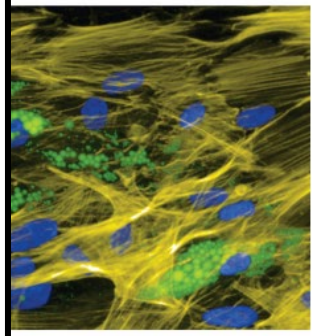


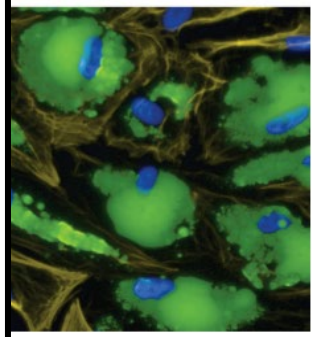
ET-1/ET_A receptor (











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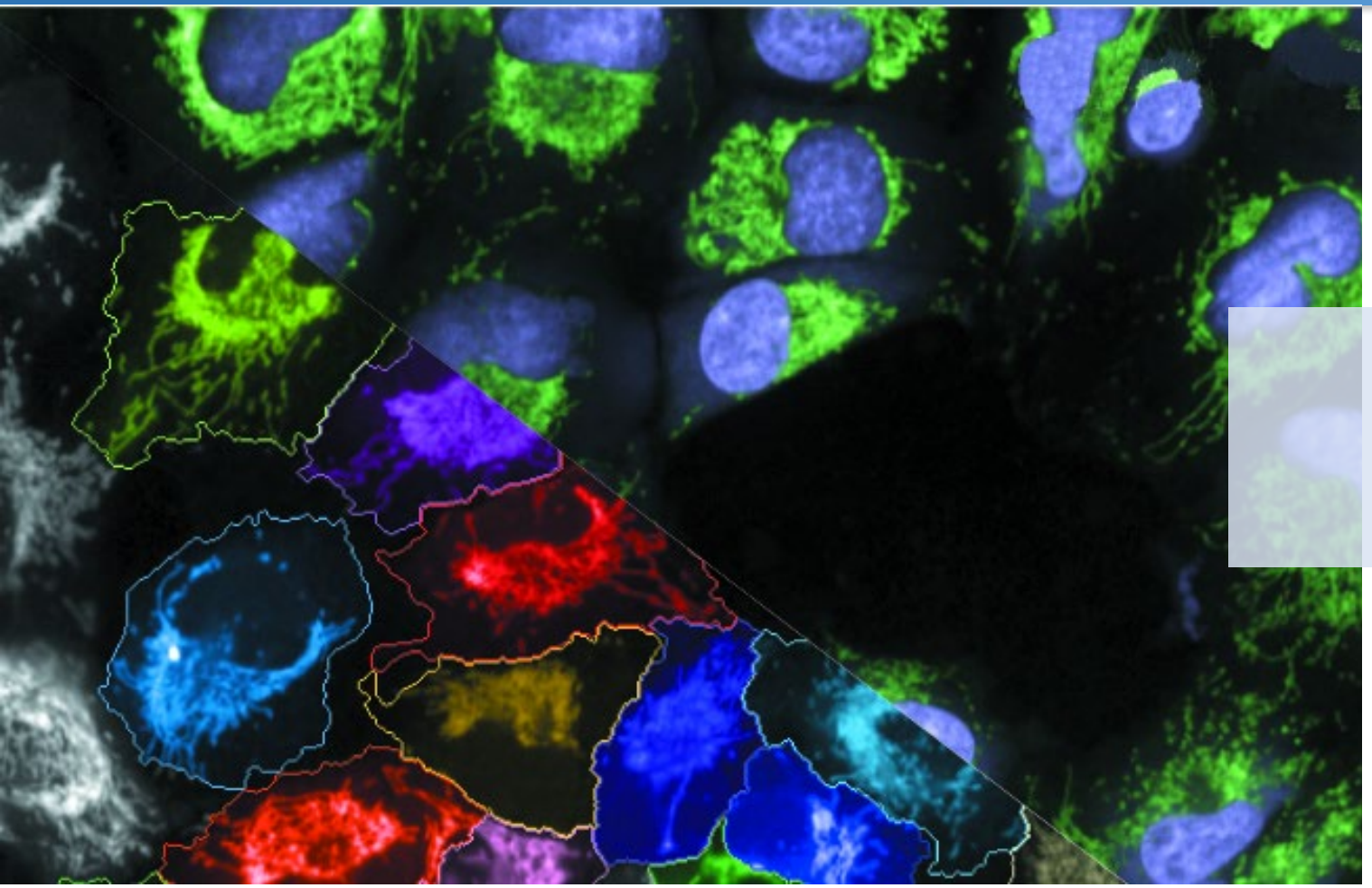

细胞表型分析

细胞表型分析-1

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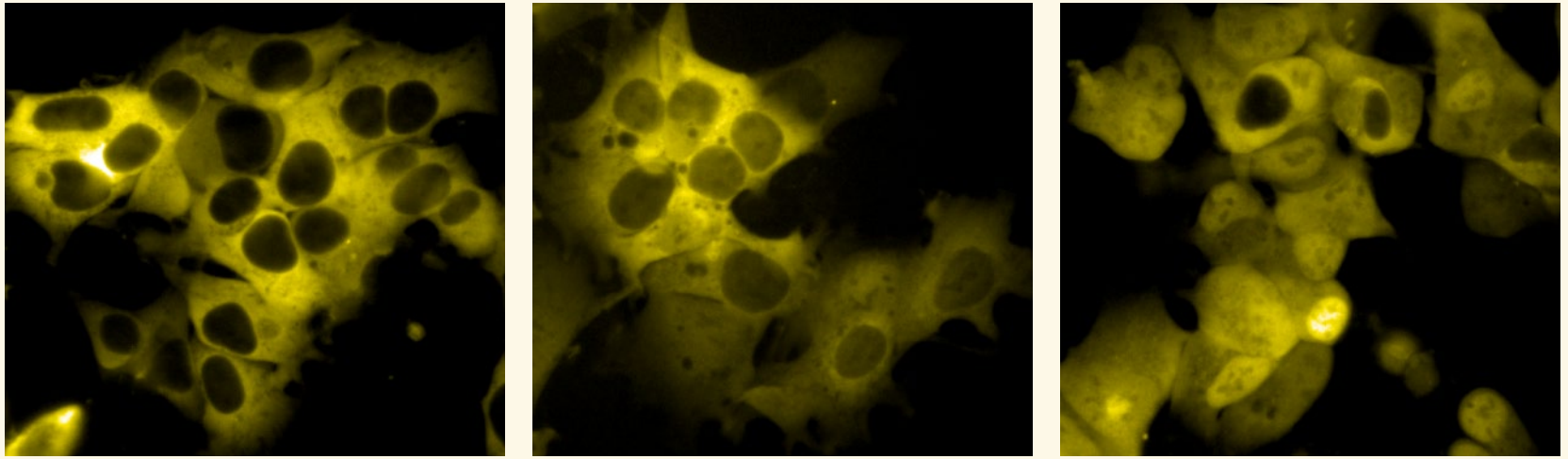
蛋白转位—活细胞内分析NF-κB 动态变化

- TNFα induces nuclear import of p65

HaloTag® TMR ligand (fused to p65 subunit of NF-κB); 30 min post-TNFα addition



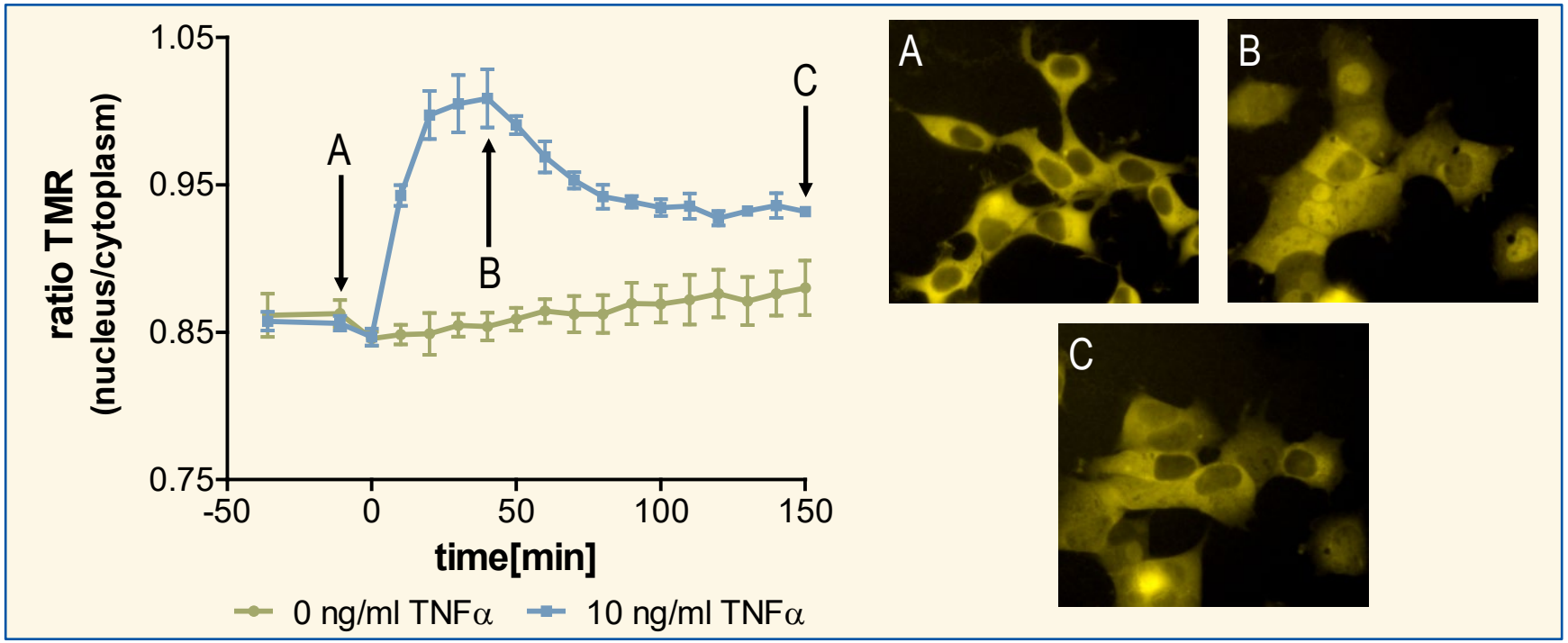
0 ng/ml TNFα　　0.5 ng/ml TNFα　　50 ng/ml TNFα

HaloTag® TMR ligand染活细胞

- HEK293细胞稳定表达NF-κB家族 带 HaloTag® 融合标签的p65 (p65-HT)， 通过 HaloTag® TMR 配体透过细胞膜染活细胞
- 用不同浓度TNFα 刺激NF-κB 信号

NF-κB转位具有时间效应

- 通过Harmony® 高内涵成像和分析软件计算
- 计算NF-κB荧光 信号的核质比

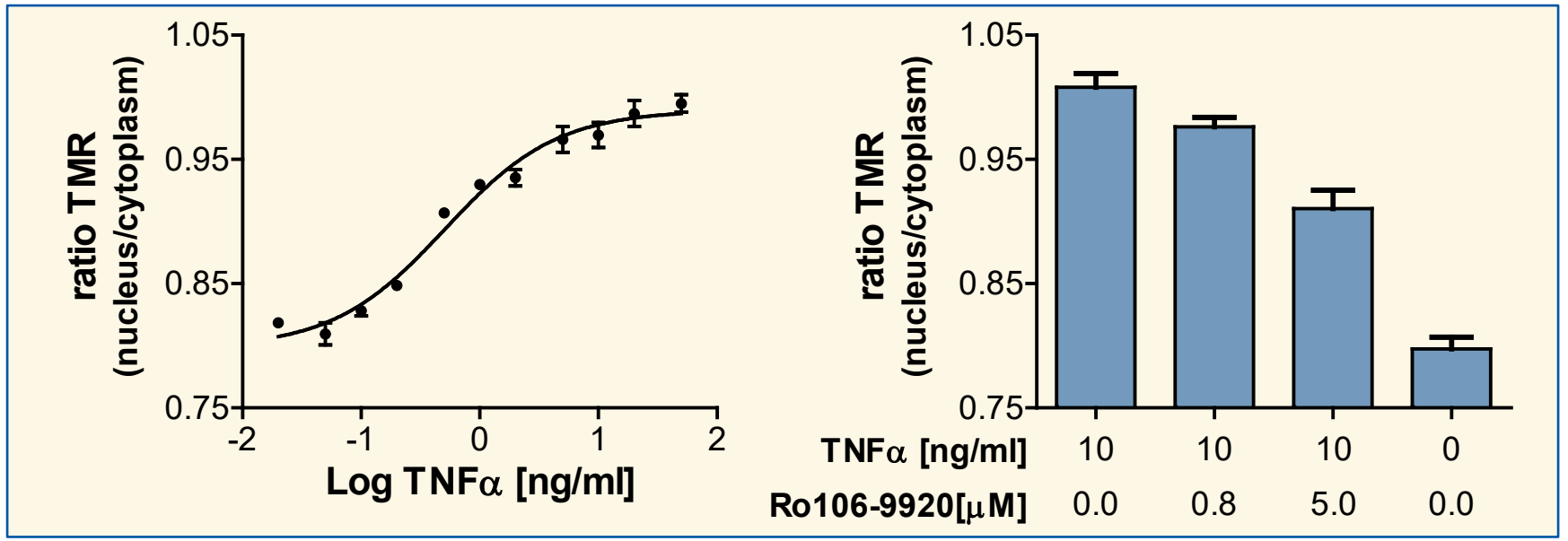


- 刺激30min后，核内p65-HT百分比 达到最高值
- 与固定终点分析相反，向实验中添加时间维度，允许鉴定抑制NF-κB易位的化合物以及影响细胞核中NF-κB分子驻留时间的化合物

Live cell imaging allows the study of signaling dynamics

胞内p65 分布具有剂量依赖性

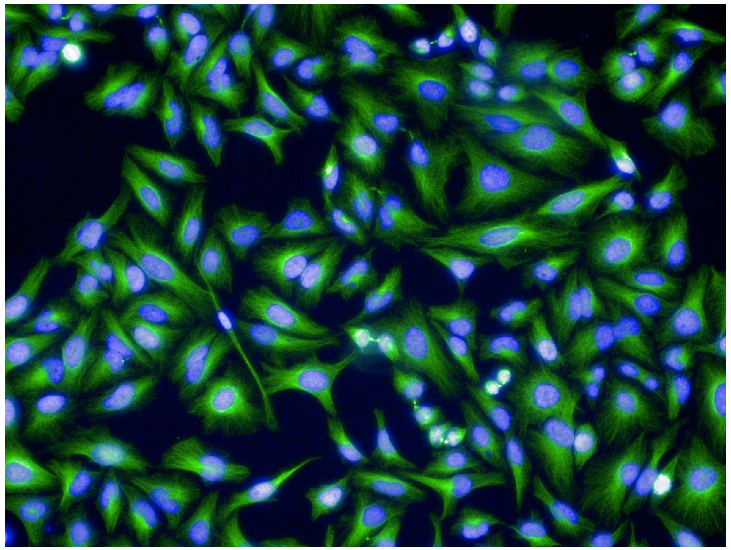
- 分析TNFα刺激30 min 后，p65-HT分布的剂量依赖性

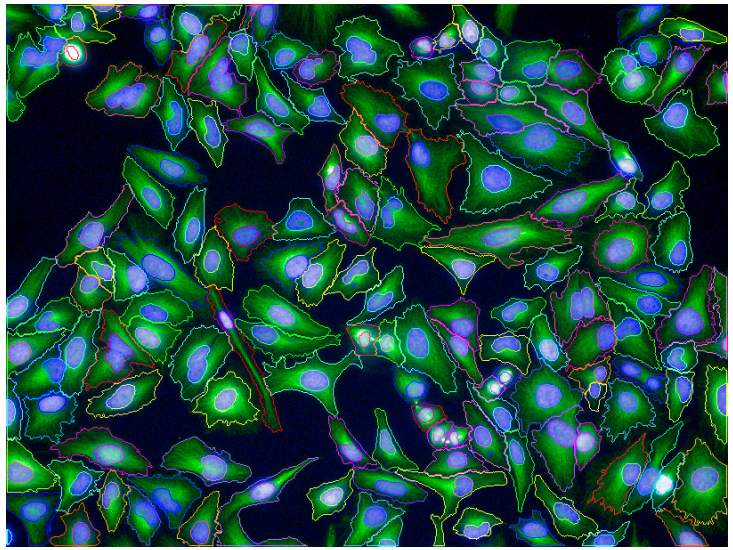


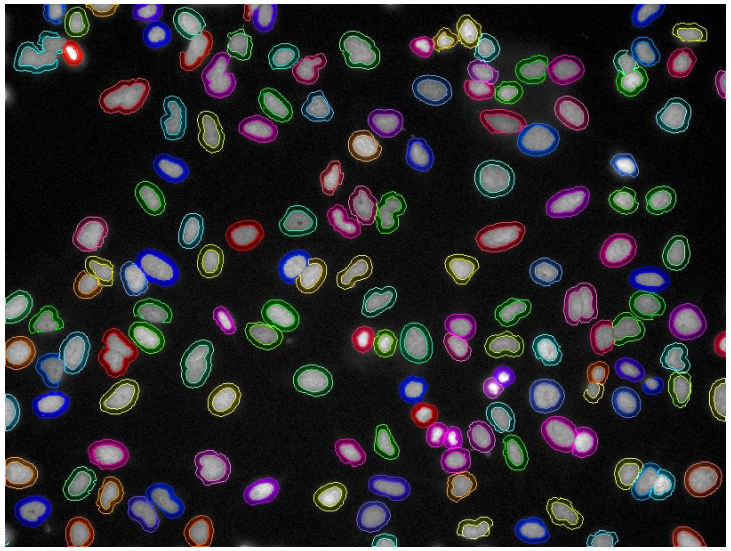
Quantitative analysis yields excellent Z' values ≥ 0.7

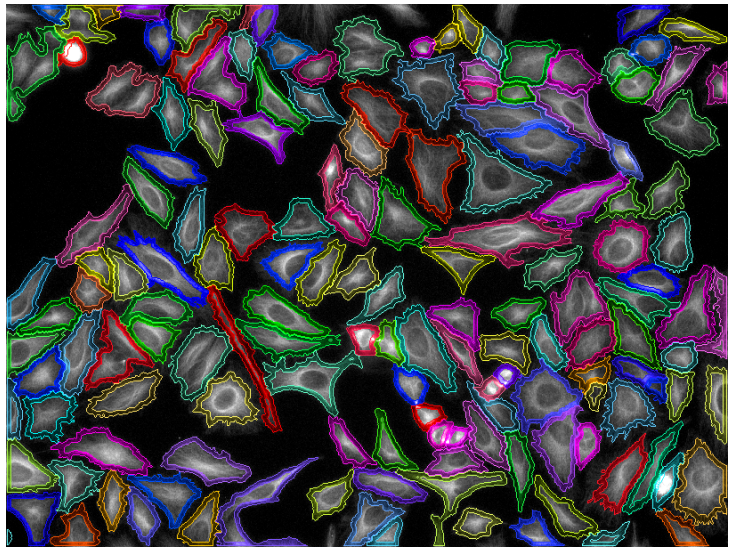
- 核内NF-κB信号随TNFα浓度的增加而增加 (EC_{50} = 0.5 ng/ml)
- 进入核内的NF-κB随Ro106-9920（稳定抑制NF-κB信号）浓度的增加而降低，具有剂量依赖性
- 通过活细胞的方法检测化合物对NFκB入核或出核的动态变化进行检测
- 高内涵扩展了蛋白转运的研究维度，让我们可以同时在时间维度上和处理方法维度上进行监测，所有这些因素都有可能为二次筛选或进一步分析过程节省时间和金钱。

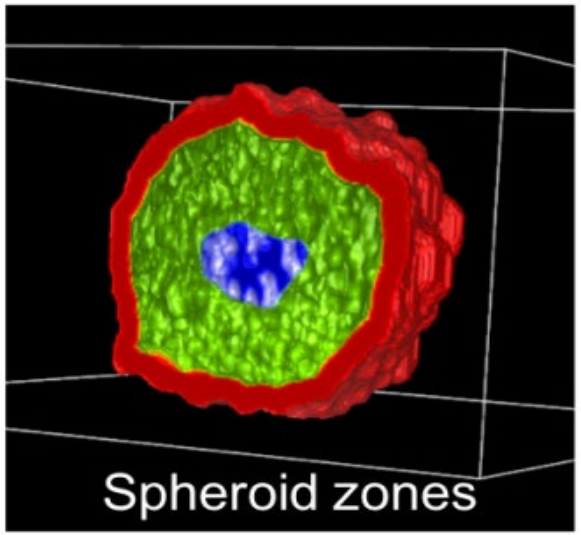
不同ROI定义

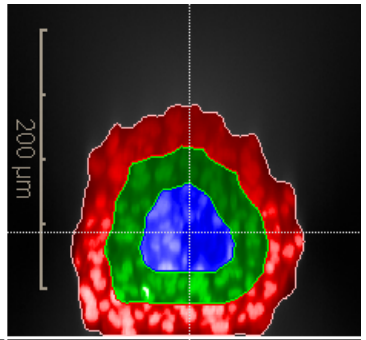


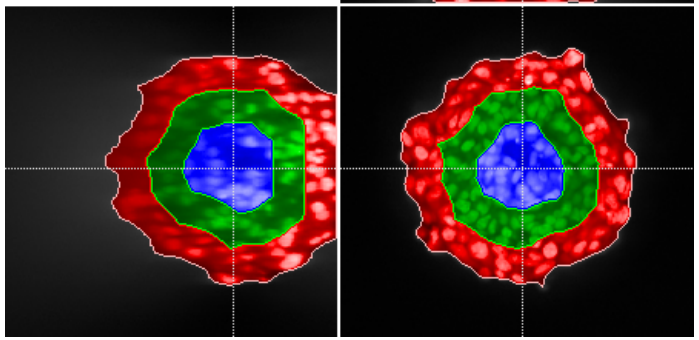




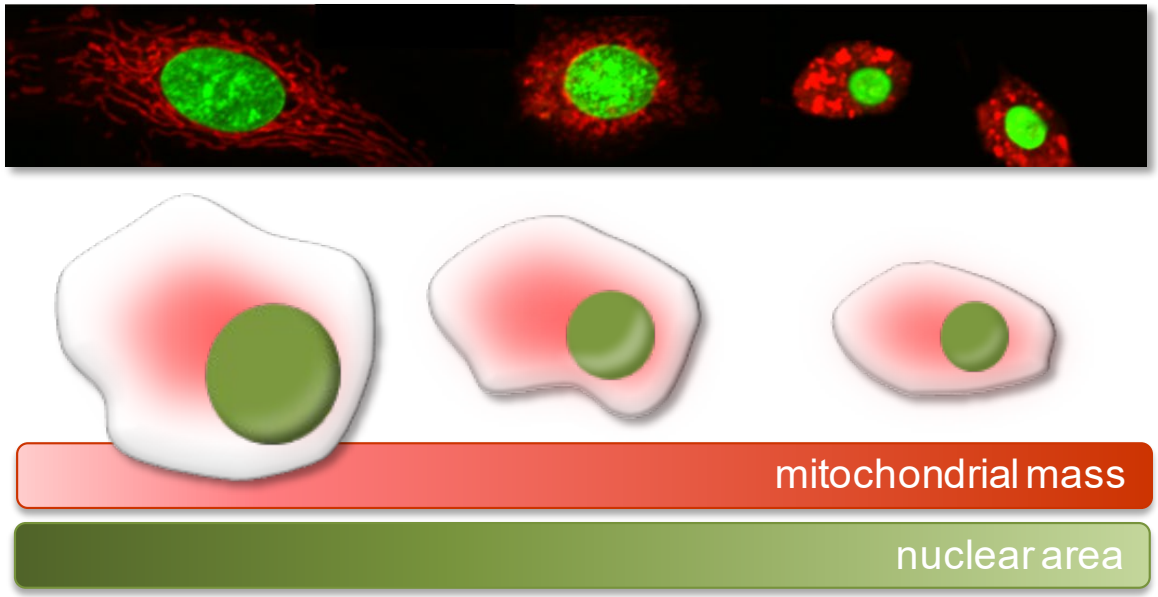


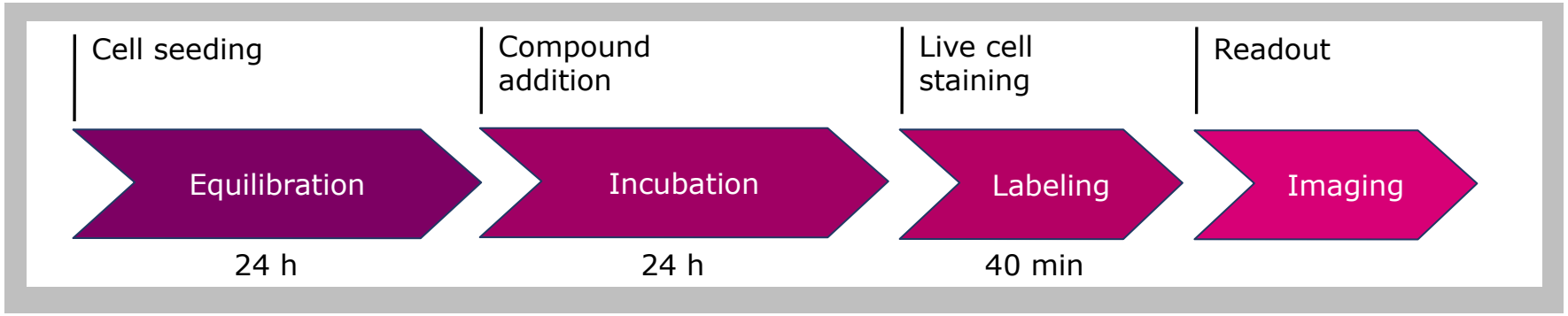






细胞表型分析-2


mitochondrial mass
nuclear area


Cell seeding
Compound addition
Live cell staining
Readout
Equilibration
Incubation
Labeling
Imaging
24 h
24 h
40 min

细胞毒性检测：成像

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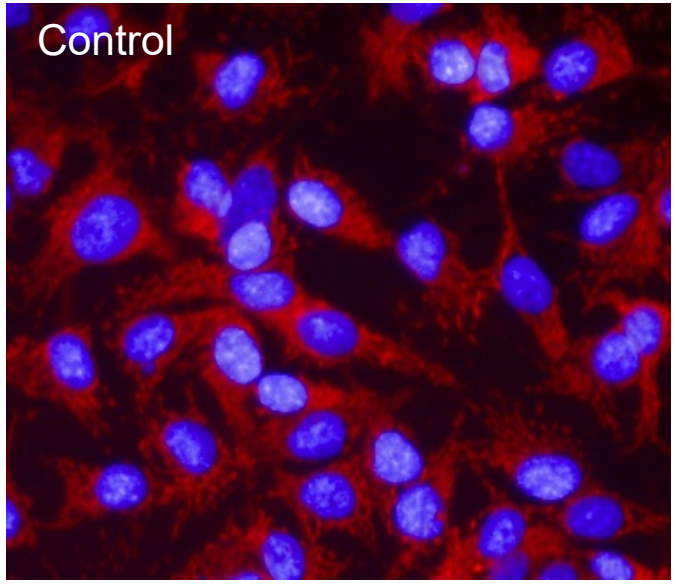
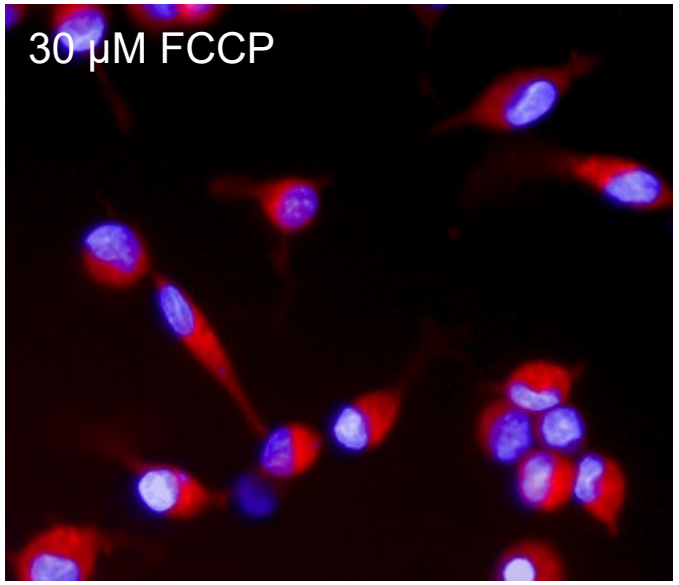
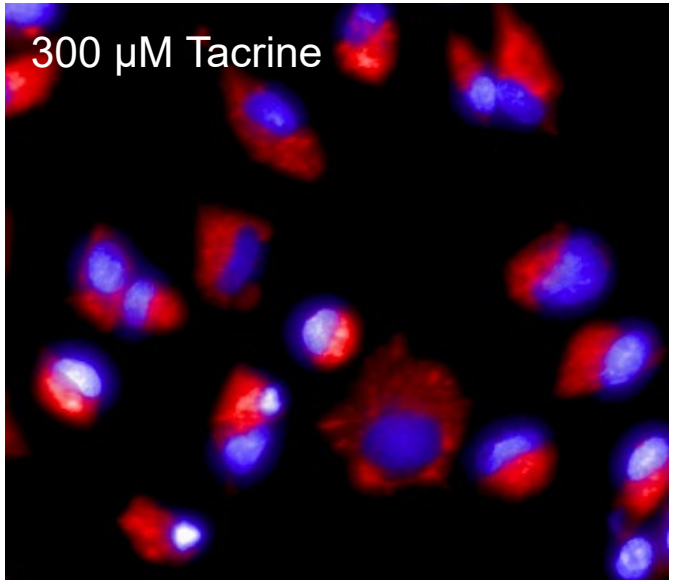
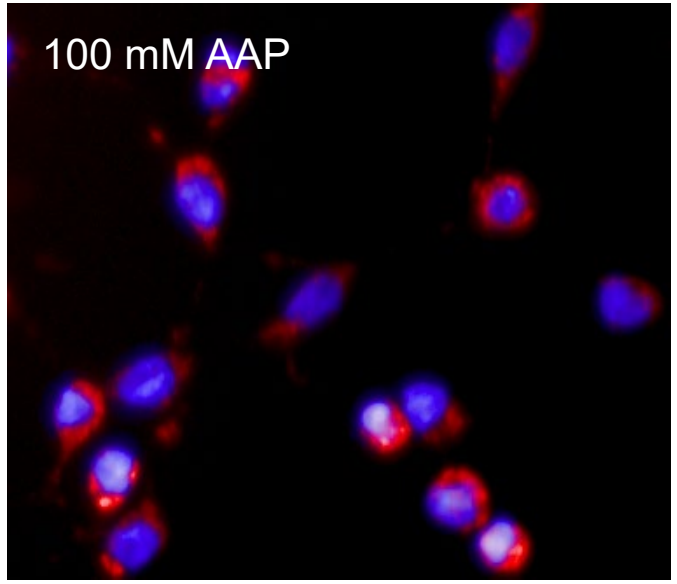




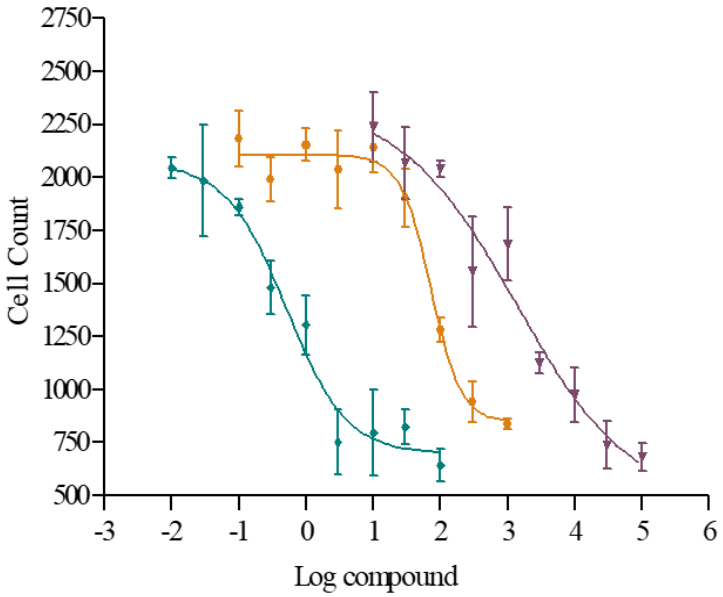


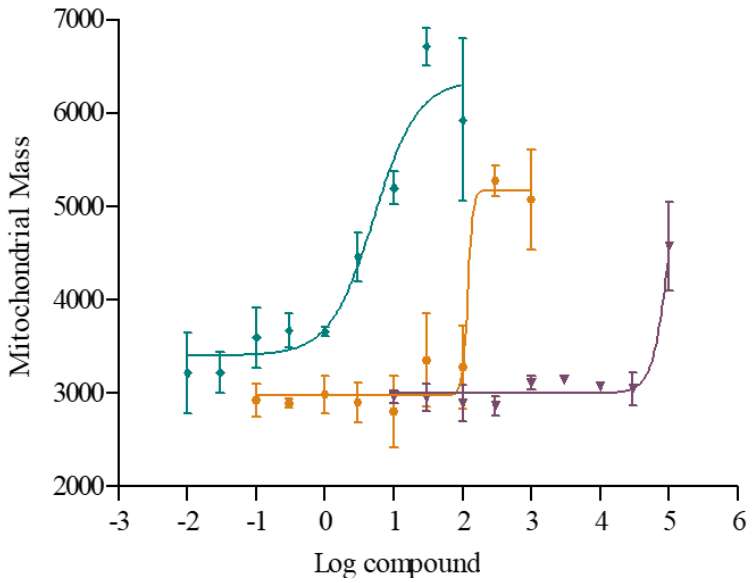


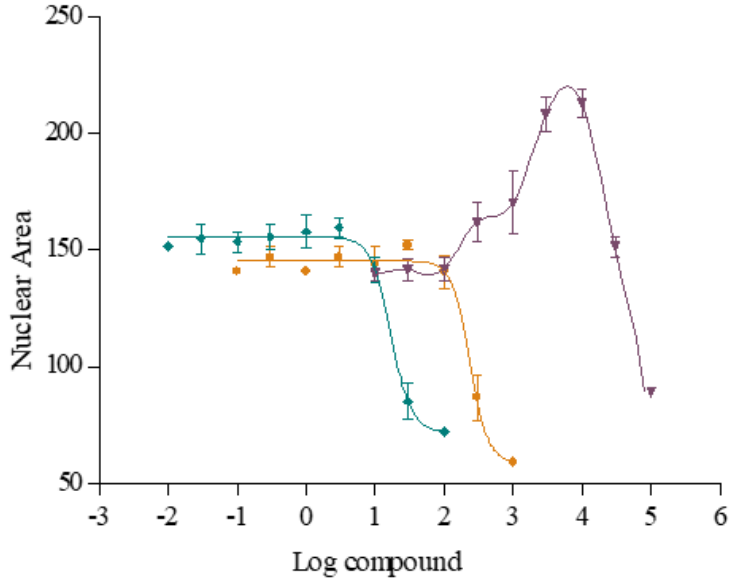
- 细胞计数　Hoechst 33342
- 细胞膜透性　BOBO™-3 (not displayed), Hoechst 33342
- 线粒体数量　MitoTracker® Deep Red
- 细胞核增大/皱缩　Hoechst 33342

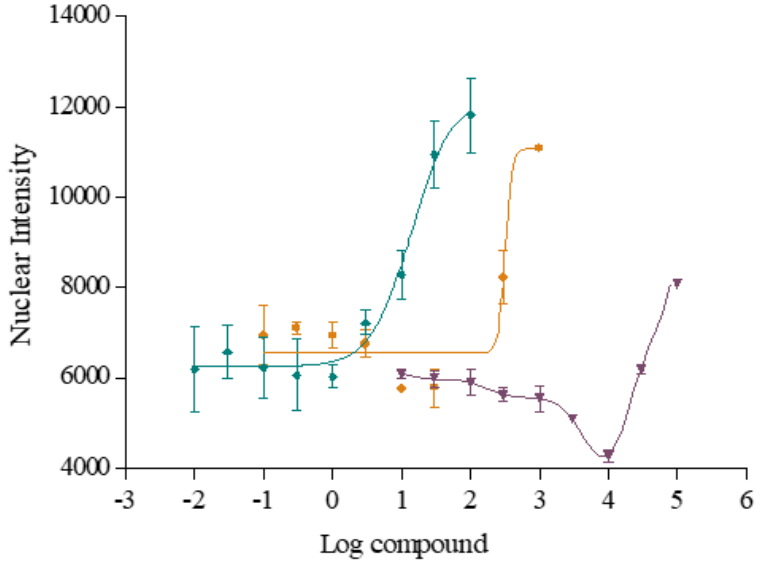
- 20x long WD 物镜成像
- 对HepG2细胞进行3种化合物处理（Carbonyl Cyanide P-(Trifluoromethoxy) Phenylhydrazone , Tacrine and Acetaminophen），观察细胞毒性
- 处理后细胞数量减少，质膜透性增加，线粒体数量增多，细胞核变小

细胞毒性


Cell Count
2750
2500
2250
2000
1750
1500
1250
1000
750
500
-3 -2 -1 0 1 2 3 4 5 6
Log compound


Mitochondrial Mass
7000
6000
5000
4000
3000
2000
-3 -2 -1 0 1 2 3 4 5 6
Log compound


Nuclear Area
250
200
150
100
50
-3 -2 -1 0 1 2 3 4 5 6
Log compound


Nuclear Intensity
14000
12000
10000
8000
6000
4000
-3 -2 -1 0 1 2 3 4 5 6
Log compound

细胞形态分布特征的分析复杂性

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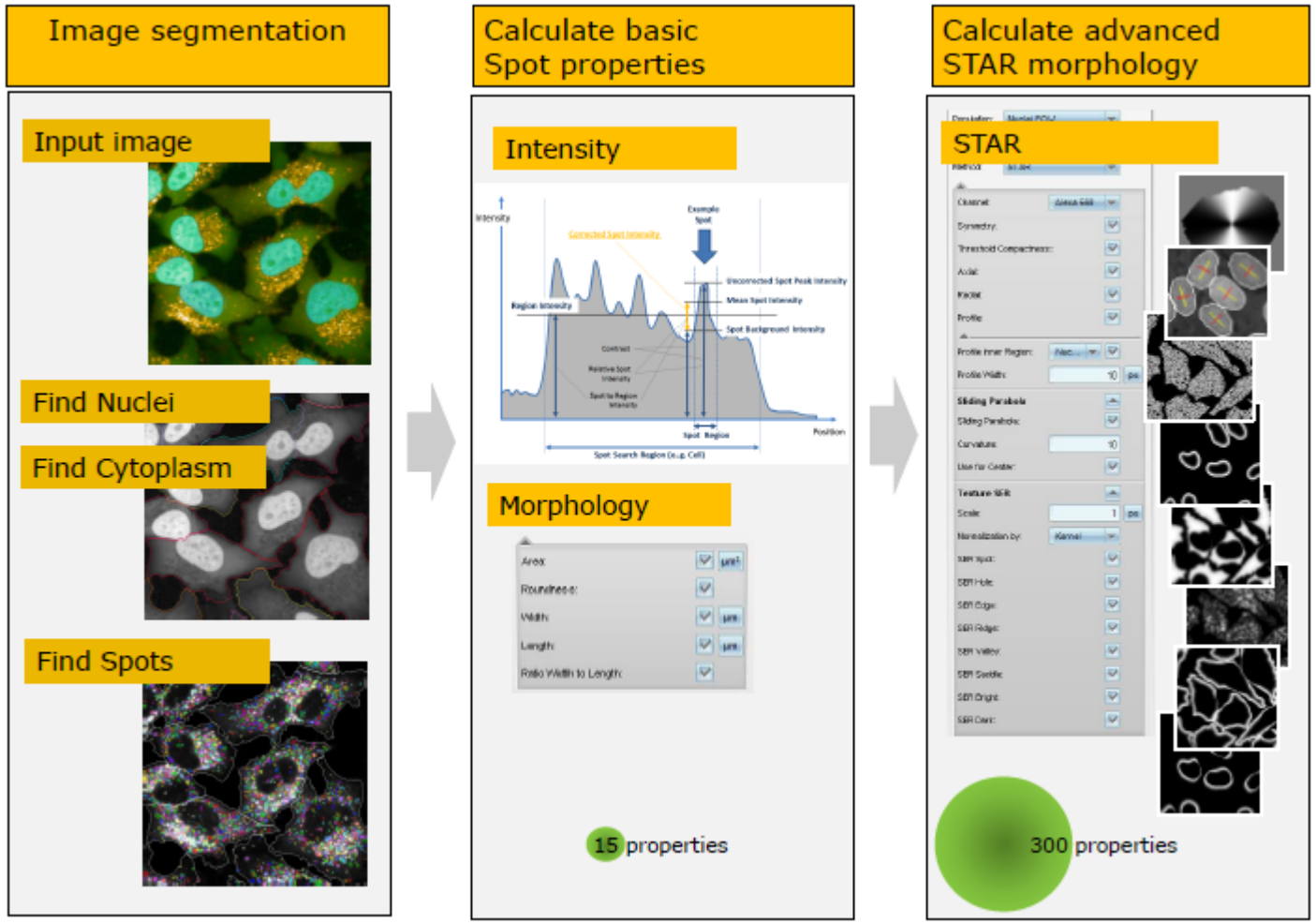

专为高内涵海量细胞分析设计的 STAR 全参数分析

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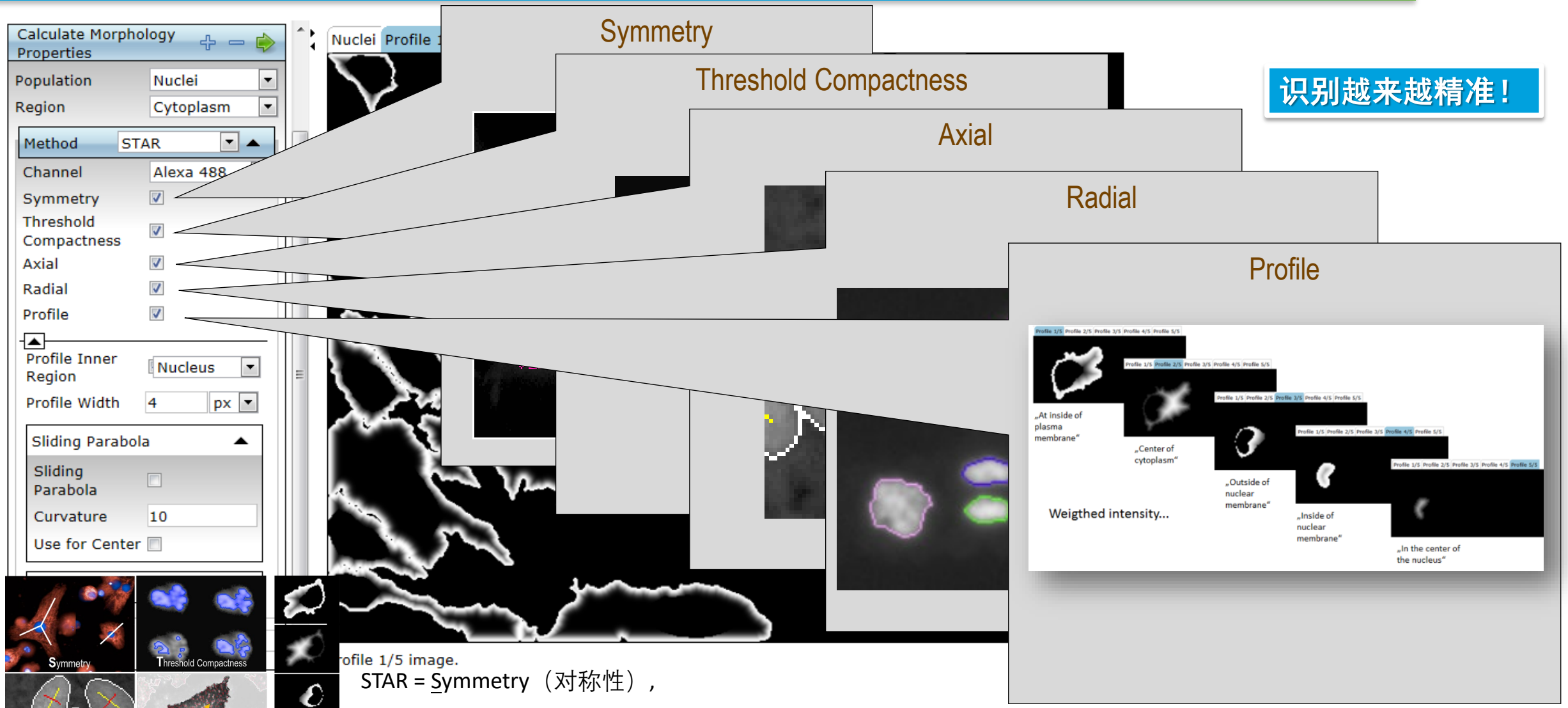

STAR = Symmetry（对称性），

Threshold Compactness（信号密度），Axial（轴向）Radial（放射性）

Profile: 亚细胞分区

纹理分析——量化“看上去不一样”的细胞

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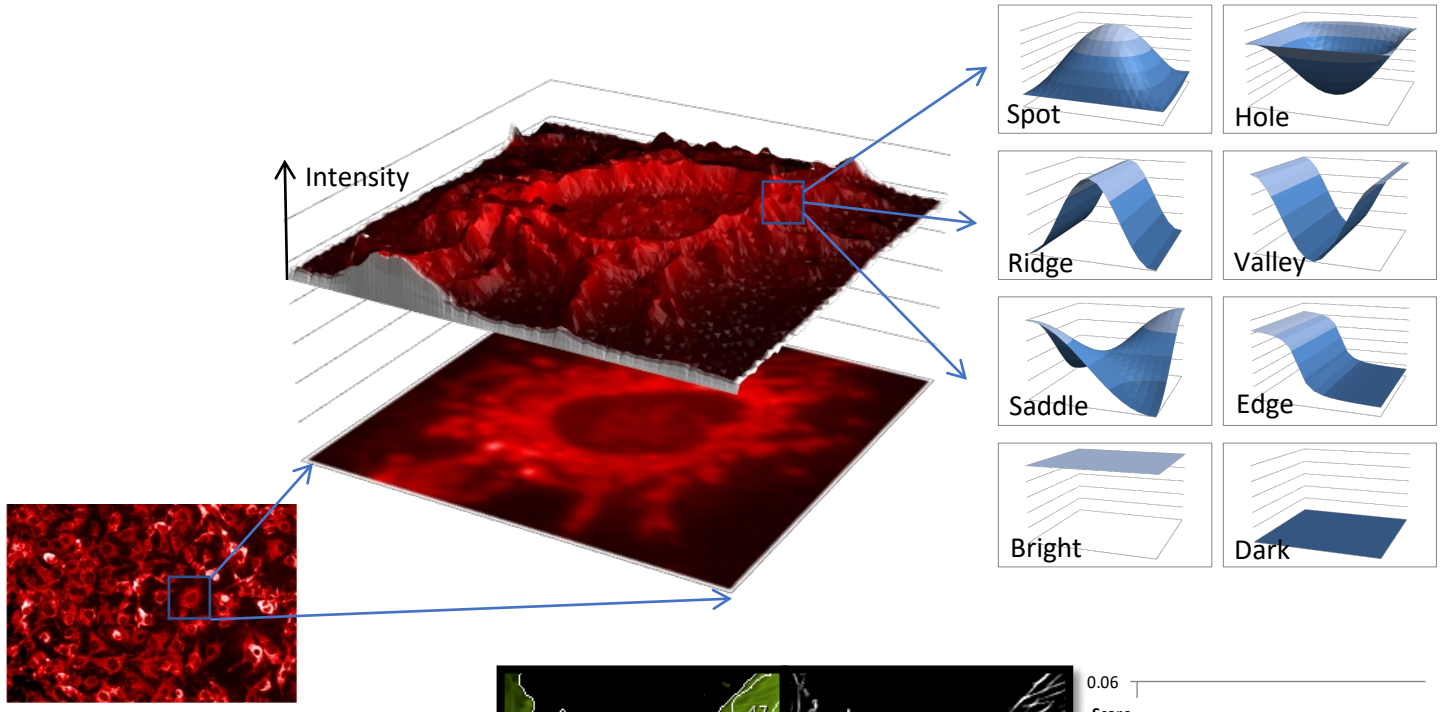
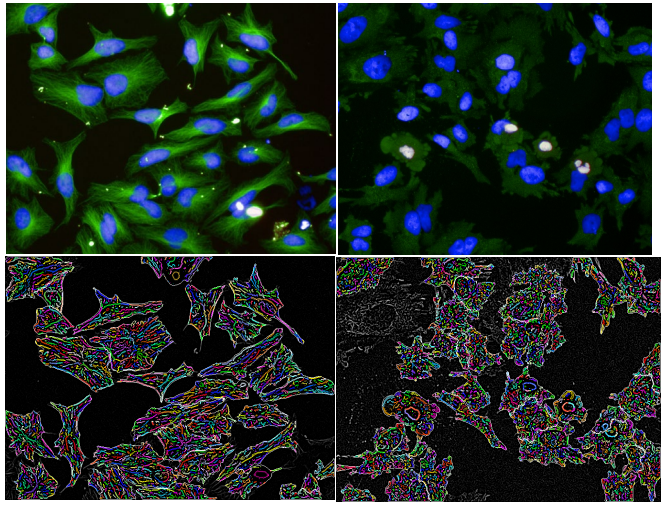
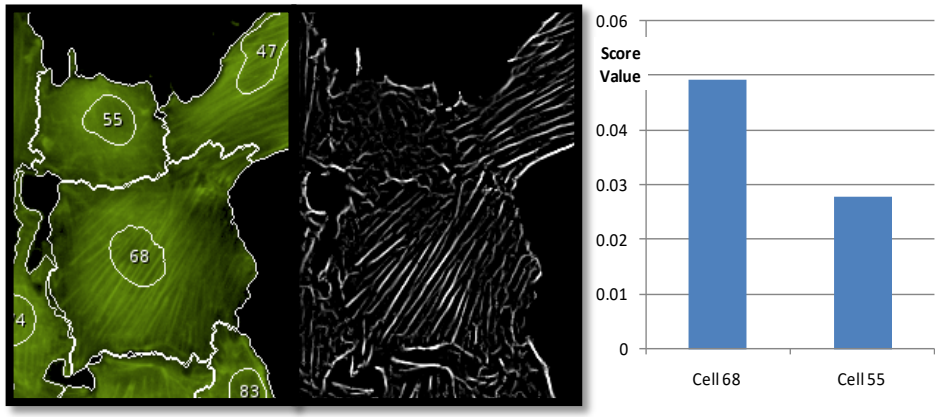
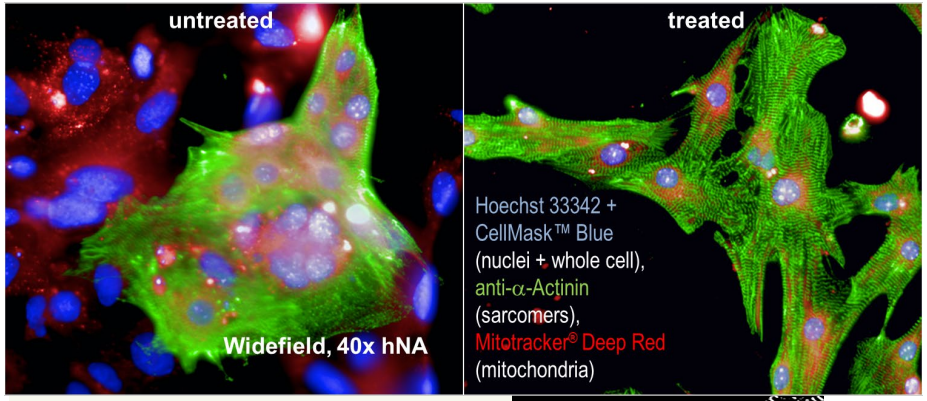
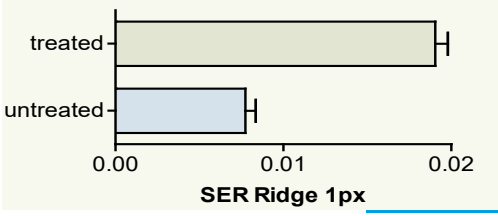


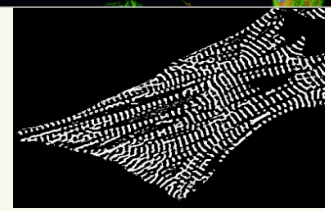




对比越来越显著！

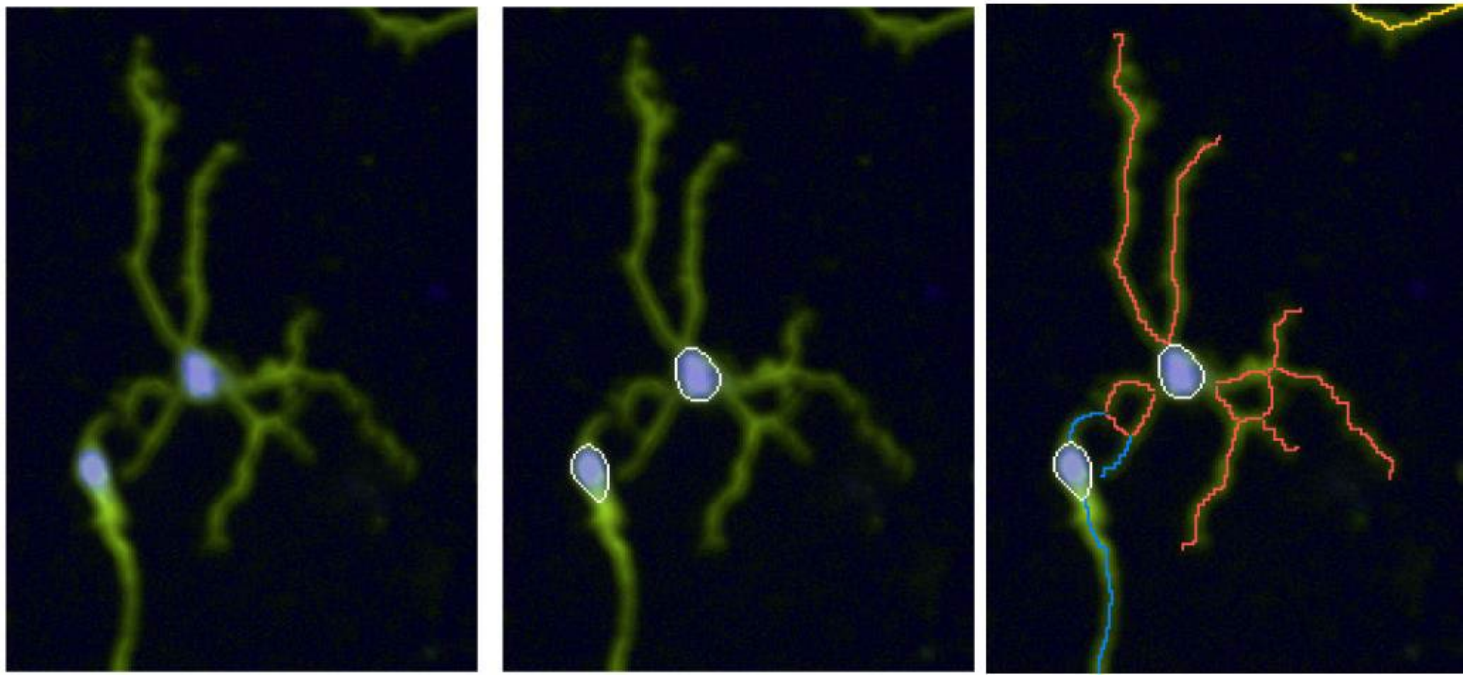






独有的神经突触分析模块 – 相关分析

- Powerful neurite analysis
  - Left: individual segments (different colors)
  - Right: Roots (circles), nodes (arrows), and ends (numbers)
- Unique colocalization analysis
  - Neurites can be used as search mask for signals colocalized on neurites ⇨ essential for axon analysis
  - Middle: mask created from neurites
  - Lower: Spot identification on neurite (arrow)



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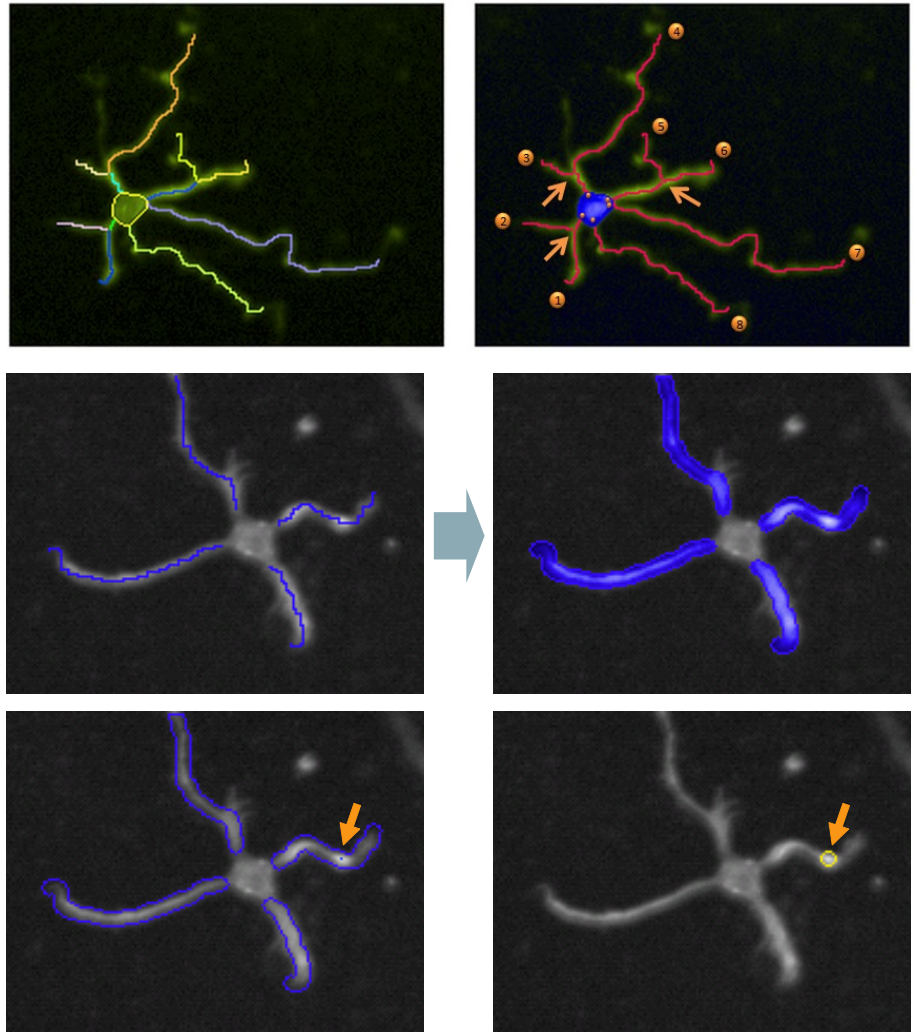
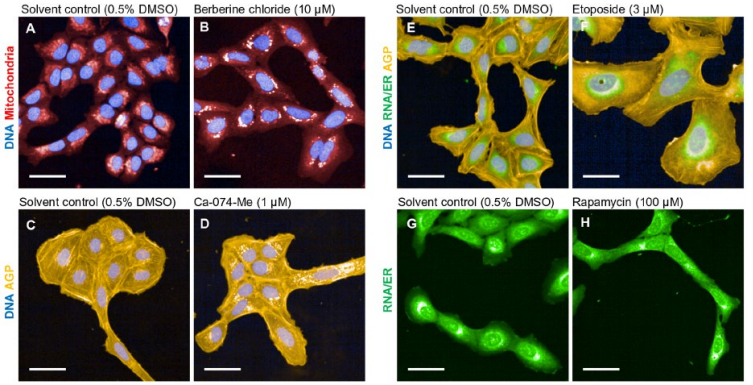


Toxicology and Applied Pharmacology
Volume 389, 15 January 2020, 114876

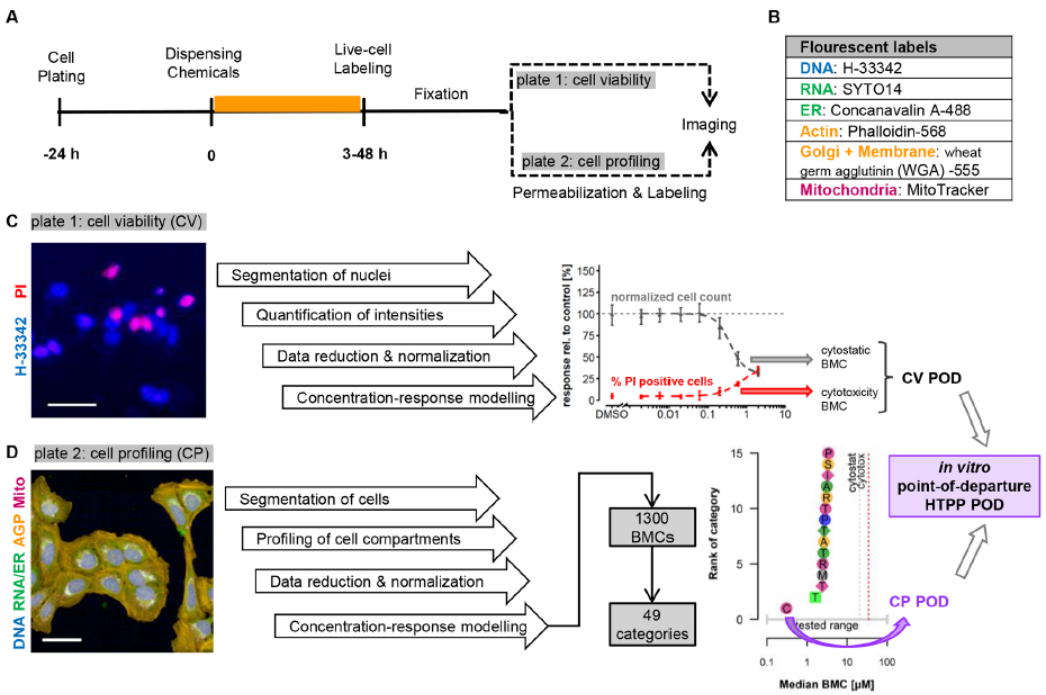
Bioactivity screening of environmental chemicals using imaging-based high-throughput phenotypic profiling

Johanna Nyffeler [a, b], Clinton Willis [a, c], Ryan Lougee [a, b], Ann Richard [a], Katie Paul-Friedman [a], Joshua A. Harrill [a]

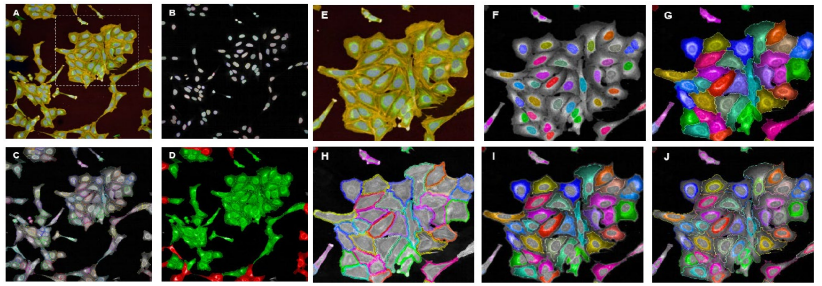
基于高通量成像的表型分析法筛选化学品的生物活性



细胞器染色



技术路线

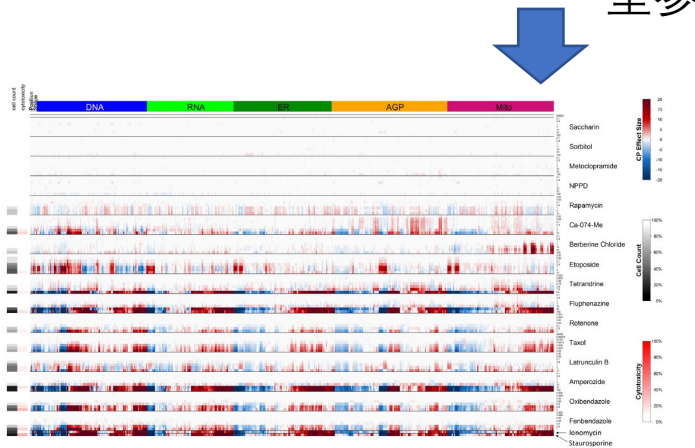


不同细胞分区：核、膜、环

		Module								
		Position [7]	Basic morph-ology [5]	SCARP morphology					Intensity [9]	Texture [14]
				Symmetry [80]	Compactness [40]	Axial [20]	Radial [28]	Profile [20-30]		
Channel	DNA			Nuclei	Nuclei	Nuclei	Nuclei Cell	Nuclei Cytoplasm	Nuclei	Nuclei
	RNA			Nuclei	Nuclei	Nuclei	Nuclei	Nuclei	Nuclei	Nuclei
	ER			Cell	Cell	Cell	Cell	Cytoplasm	Ring Cytoplasm	Ring Cytoplasm
	AGP			Cell	Cell	Cell	Cell	Nuclei Cytoplasm	Ring Cytoplasm Membrane	Ring Cytoplasm Membrane
	Mito			Cell	Cell	Cell	Cell	Nuclei Cytoplasm	Ring Cytoplasm	Ring Cytoplasm
	Not associated with a channel	Nuclei Cell	Nuclei Cell							

Supporting Information Fig. S7. CP assay: Overview of the modules used for analysis of each fluorescent channel.

每个细胞共计算~1300 个表型参数



不同通道、不同细胞区域的形态学变化与细胞毒性相关性

PhenoLOGIC机器自学习区分不同类型细胞

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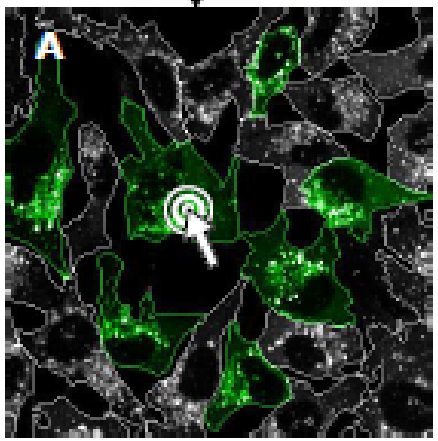
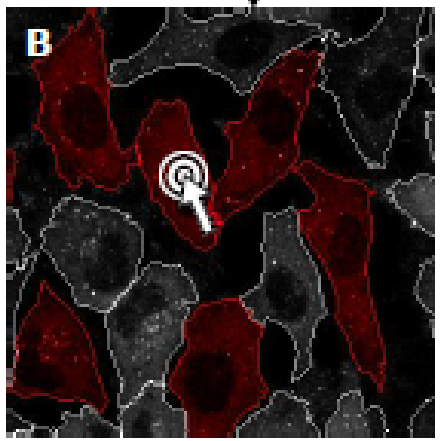
PhenoLOGIC™ - machine learning

Training

+ve

-ve

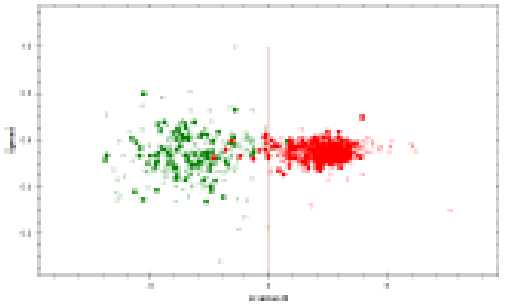


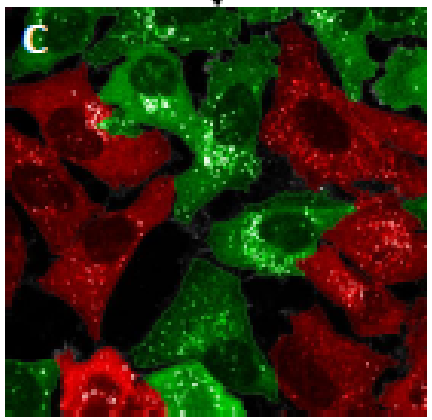


Click on autophagy positive cells

Click on autophagy negative cells

Apply Training to whole dataset





经典应用--干细胞分化、克隆分析

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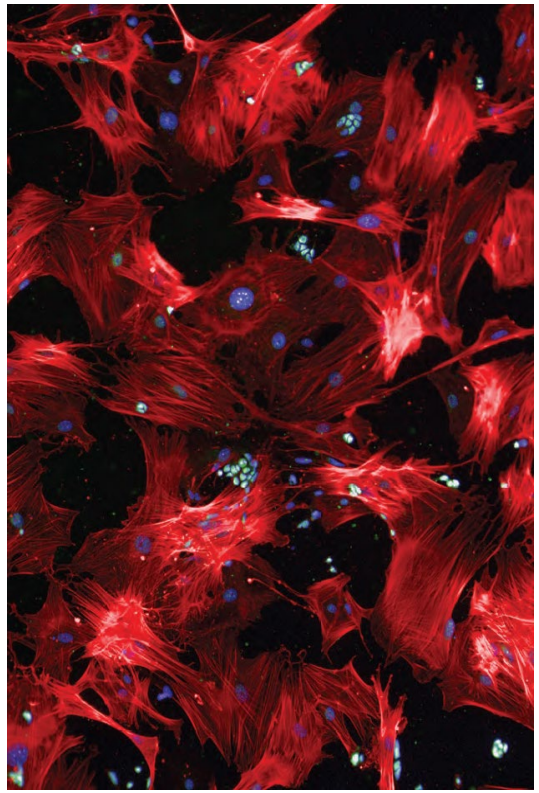
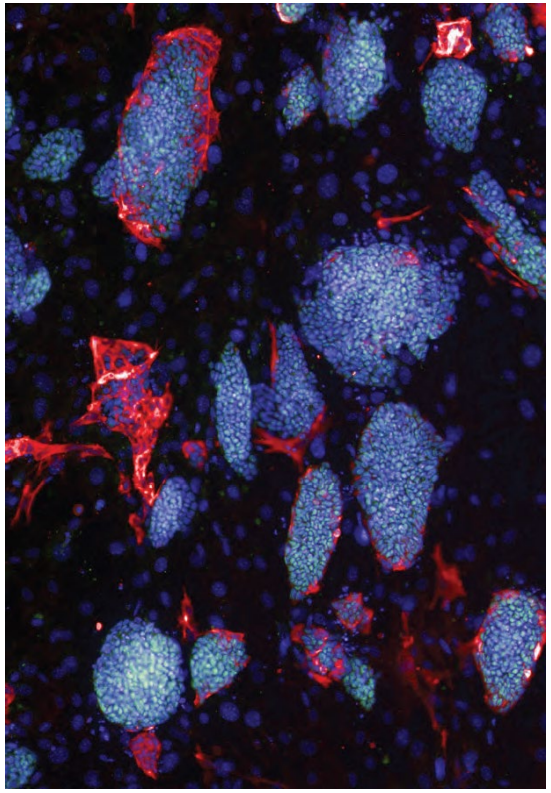
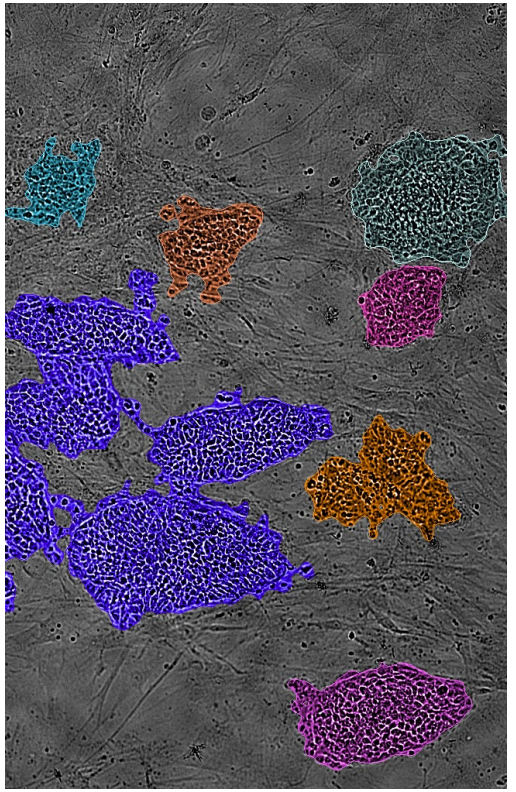




PhenoLOGIC

Texture

Machine Learning



克隆大小

克隆面积

克隆个数

Distinguishing Cell Types by Phenotypic Profiling of the Nucleus

PhenoLOGIC机器自学习区分不同类型细胞案例

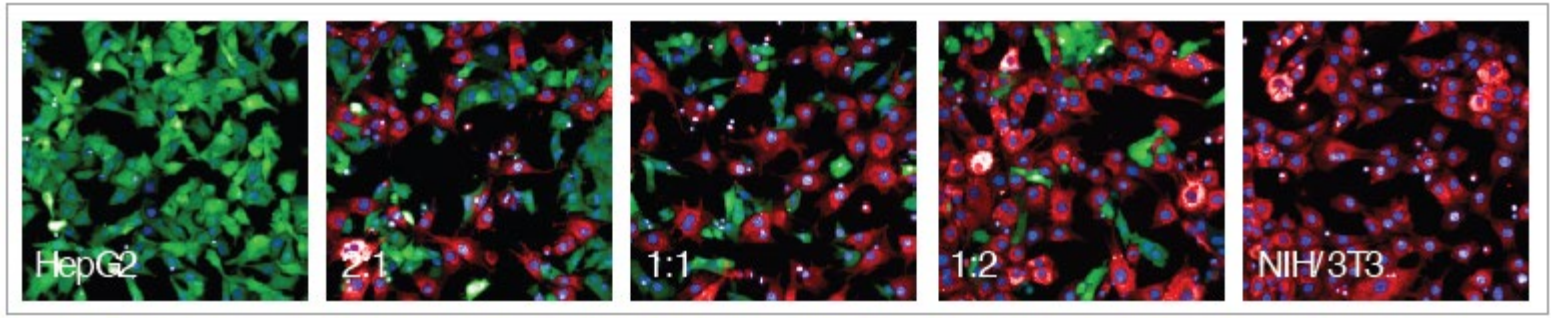


Figure 1. Representative images from wells containing either HepG2 or NIH/3T3 alone or co-cultures of HepG2 and NIH/3T3 cells mixed at ratios 2:1, 1:1 and 1:2 respectively. HepG2 cells are stained with Hoechst 33342 and CellTracker Green CMFDA. NIH/3T3 cells are stained with Hoechst 33342 and CellTracker Red CMTPX. Images were acquired on the Opera Phenix system in confocal mode using a 20x water immersion objective.

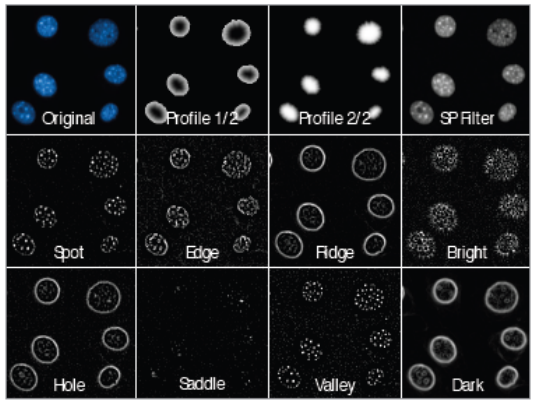
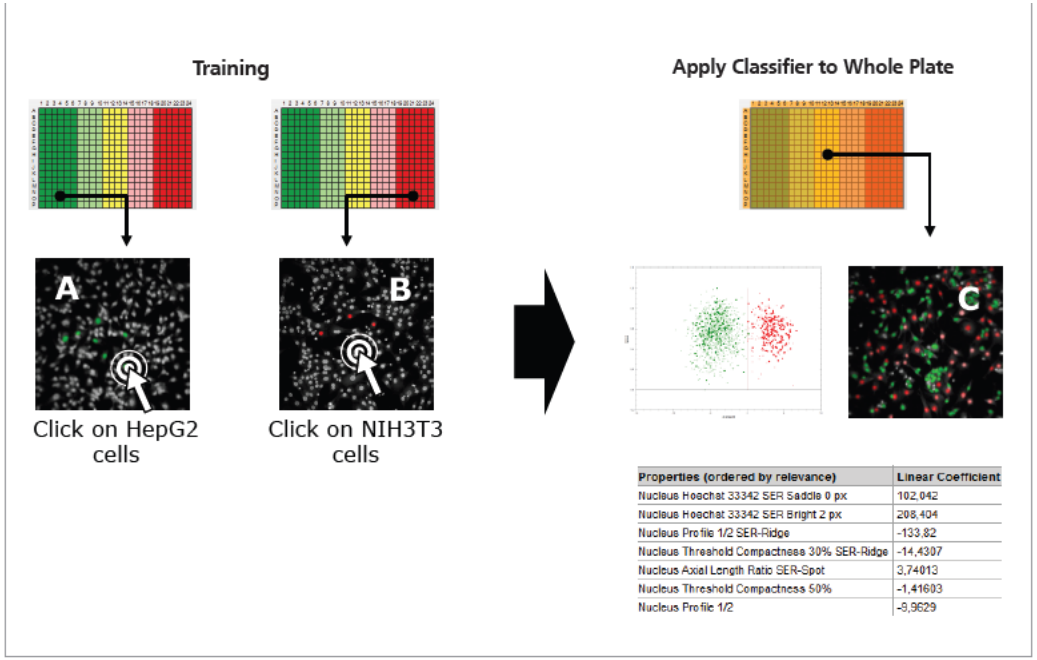
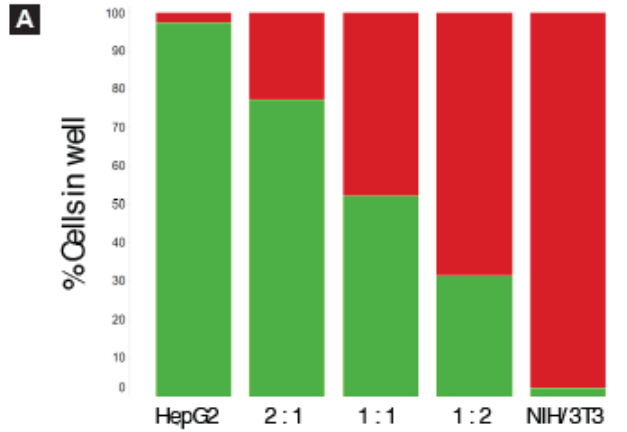


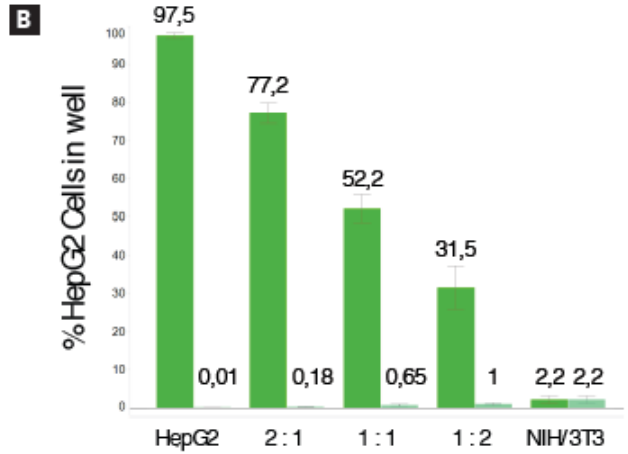
Figure 2. SER texture and STAR morphology properties are key parameters for phenotypic profiling within Harmony software. Original input image, profile images (Profile 1/2 and Profile 2/2), sliding parabola filtered (SP Filter) image and SER texture filtered (Spot, Edge, Ridge, Bright, Hole, Saddle, Valley and Dark) images of the same NIH/3T3 nuclei.

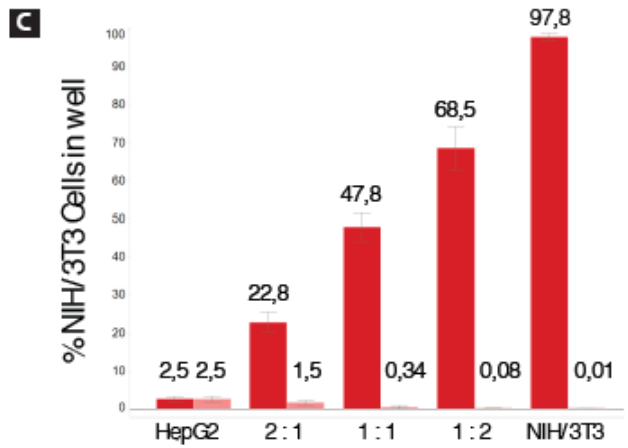


Properties (ordered by relevance)	Linear Coefficient
Nucleus Hoechst 33342 SER Saddle 0 px	102,042
Nucleus Hoechst 33342 SER Bright 2 px	208,404
Nucleus Profile 1/2 SER-Ridge	-133,82
Nucleus Threshold Compactness 30% SER-Ridge	-14,4307
Nucleus Axial Length Ratio SER-Spot	3,74013
Nucleus Threshold Compactness 50%	-1,41603
Nucleus Profile 1/2	-9,9629

Figure 3. Identifying cellular phenotypes using PhenoLOGIC machine learning. In "Training" mode, about 100 single cells within different wells were selected to teach the software to identify the different cell types in "mono cultures" (A and B). Once cells for each class were marked, the resulting classifier was applied to the whole data set. PhenoLOGIC combines the most meaningful parameters, to achieve accurate classification of the two cell types (panel C). In this case, seven properties were chosen to distinguish HepG2 from NIH/3T3 (properties shown in table below the scatter plot). Note how advanced SER (first 2) and STAR (position 3 to 7) properties dominate the selection.







软件自学习，轻松分组样品，不仅仅是细胞分类

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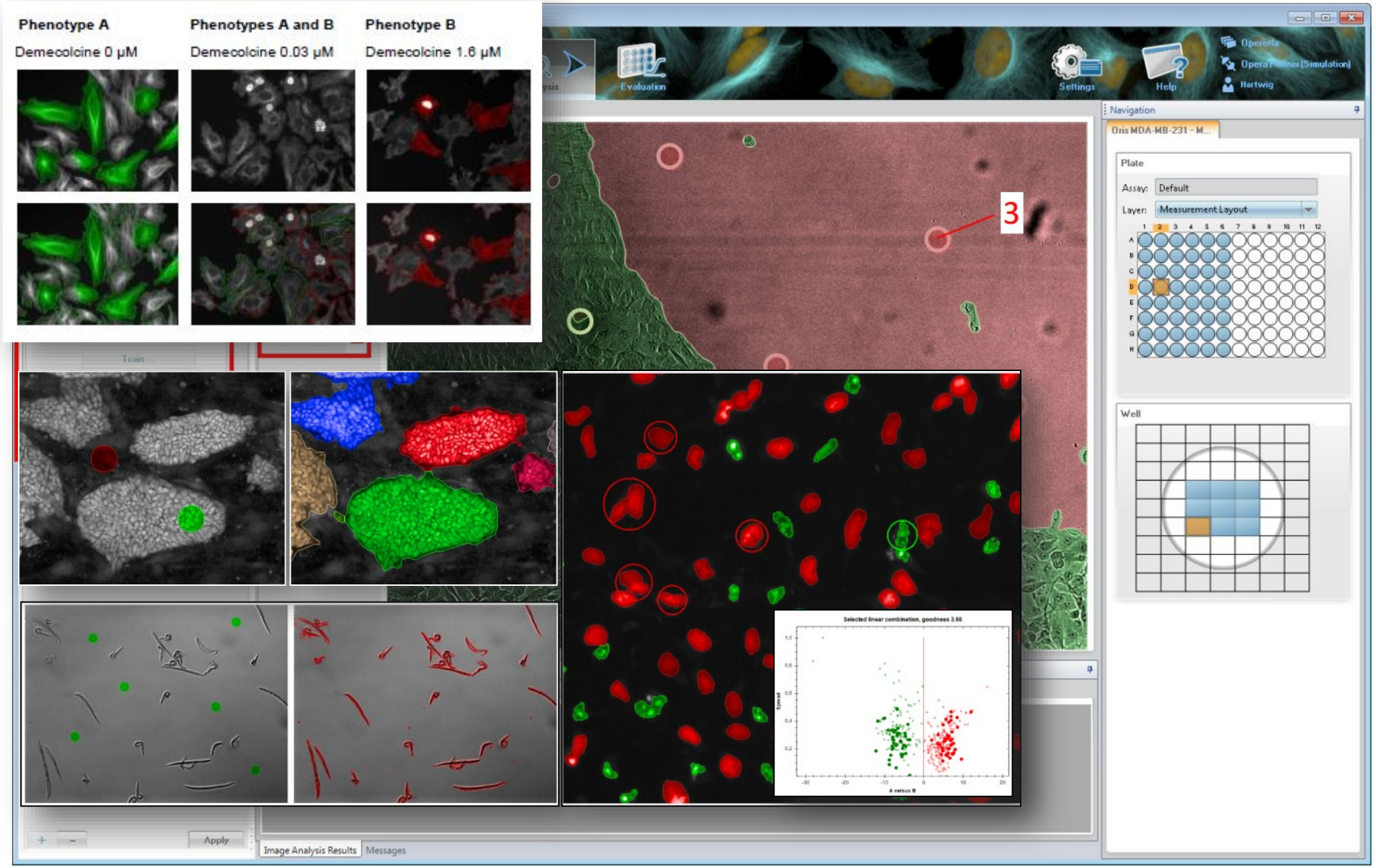

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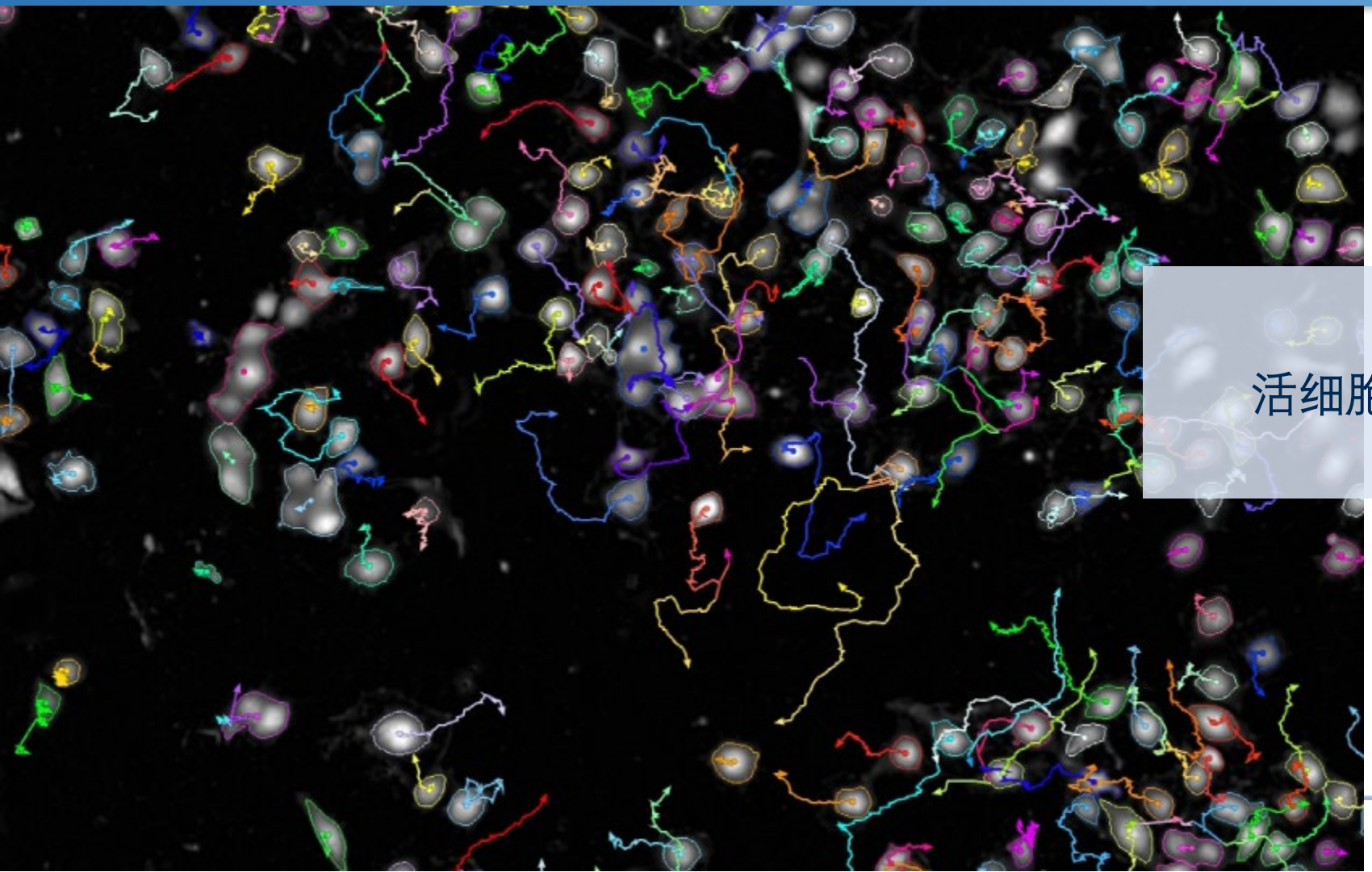

活细胞的增殖和迁移

活细胞的增殖和迁移

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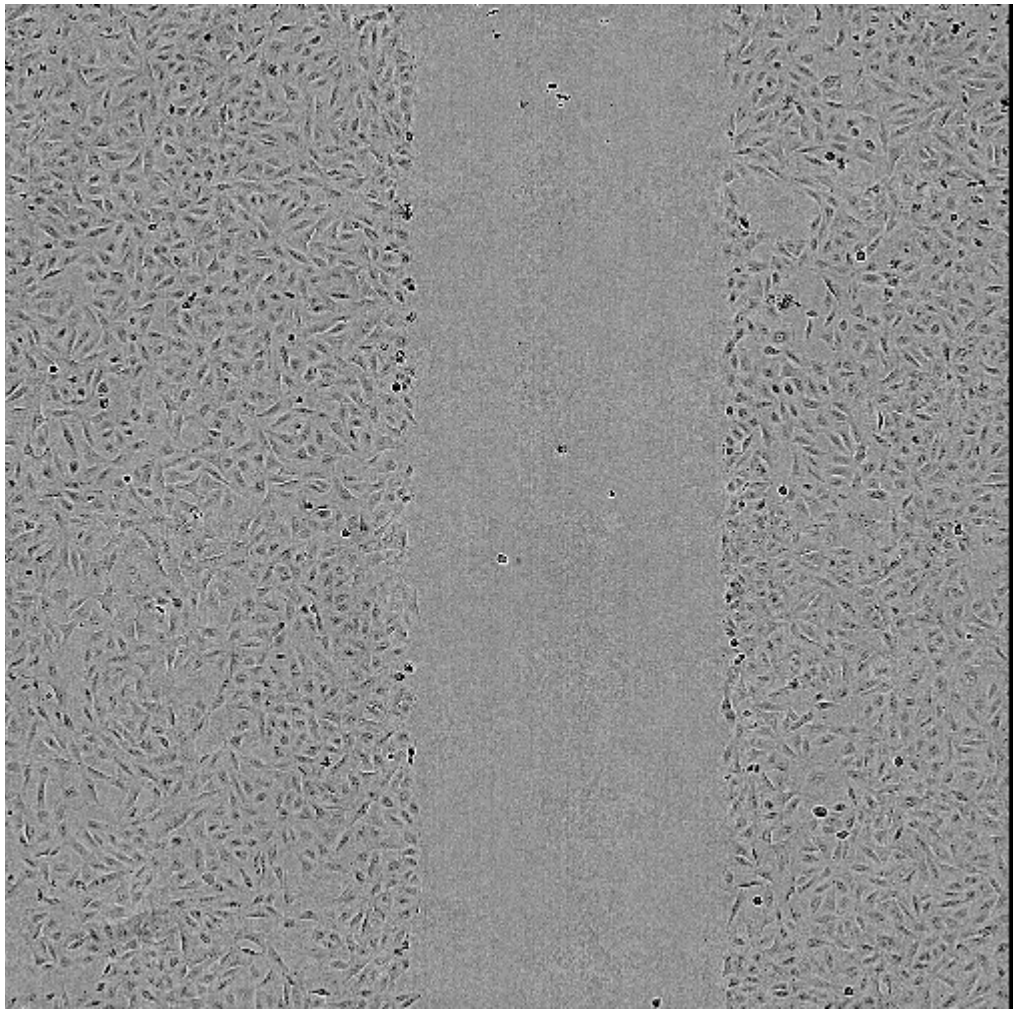
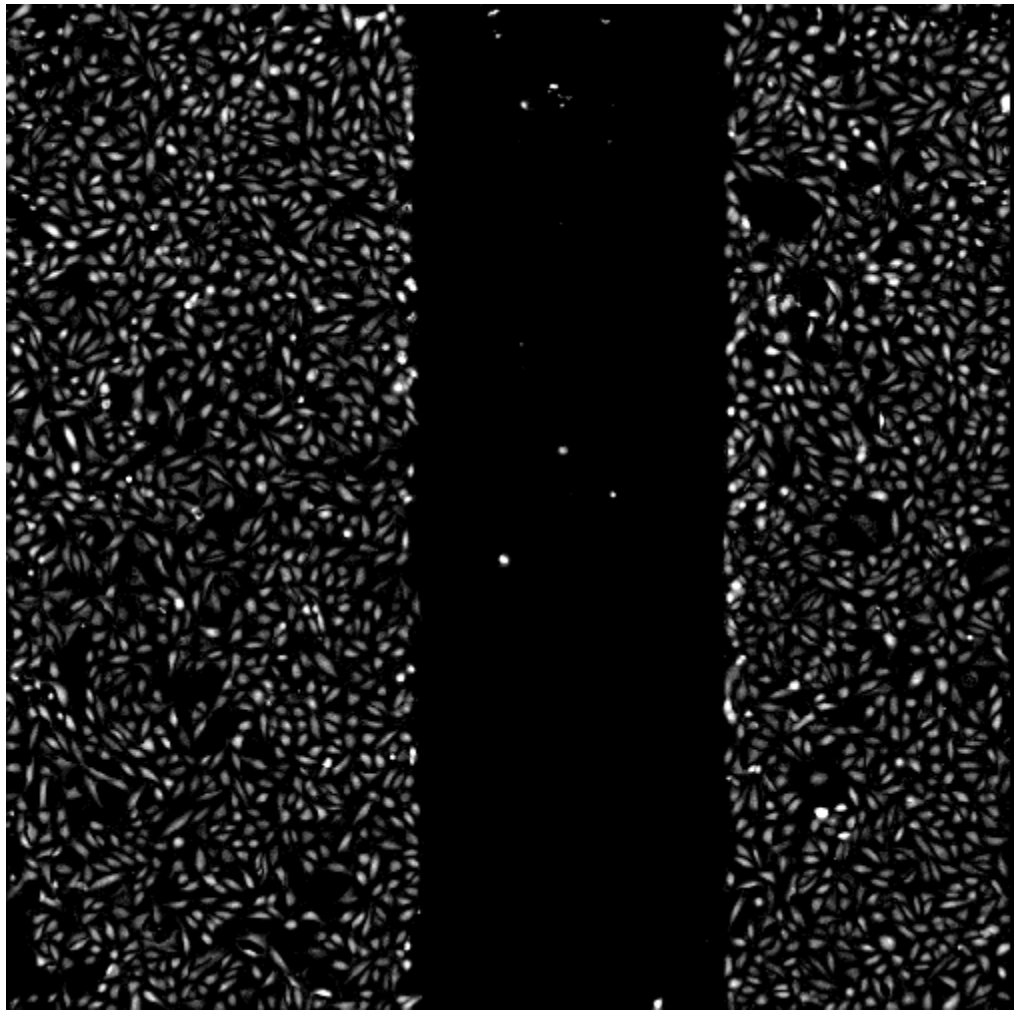
划痕实验



明场



DPC无标记模式

划痕实验

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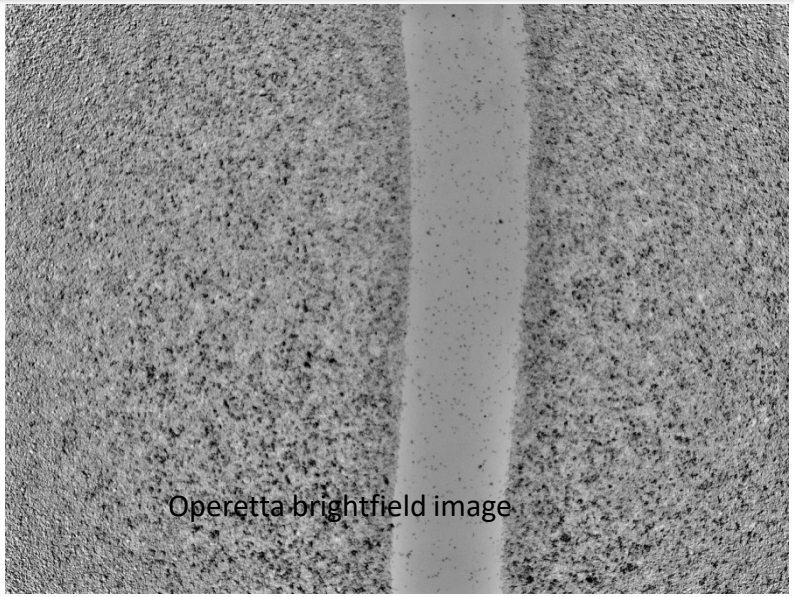

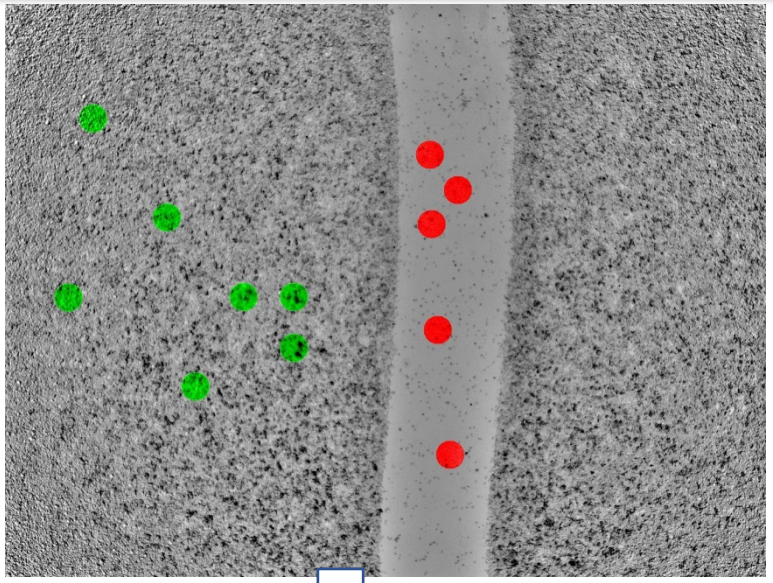

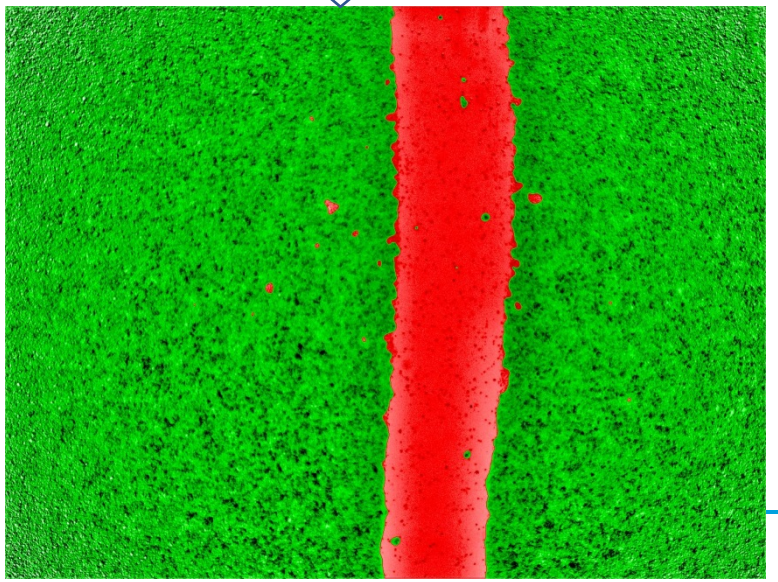

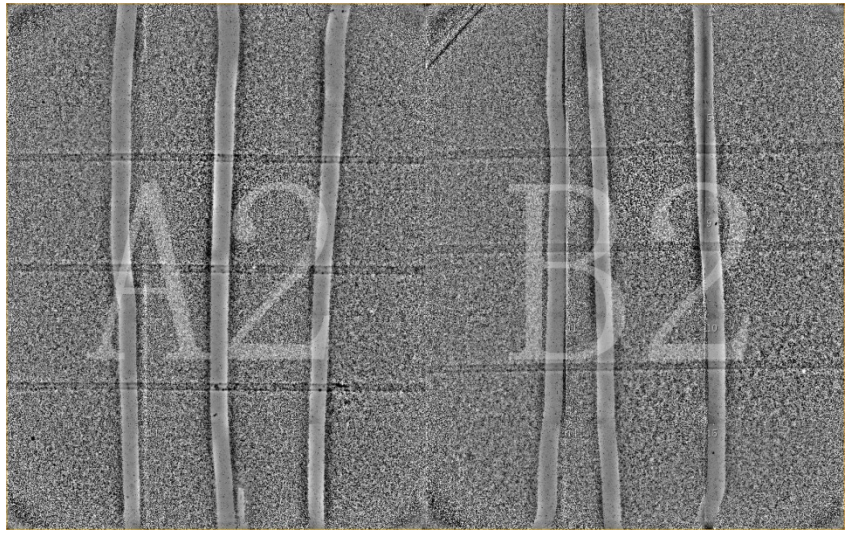

划痕实验

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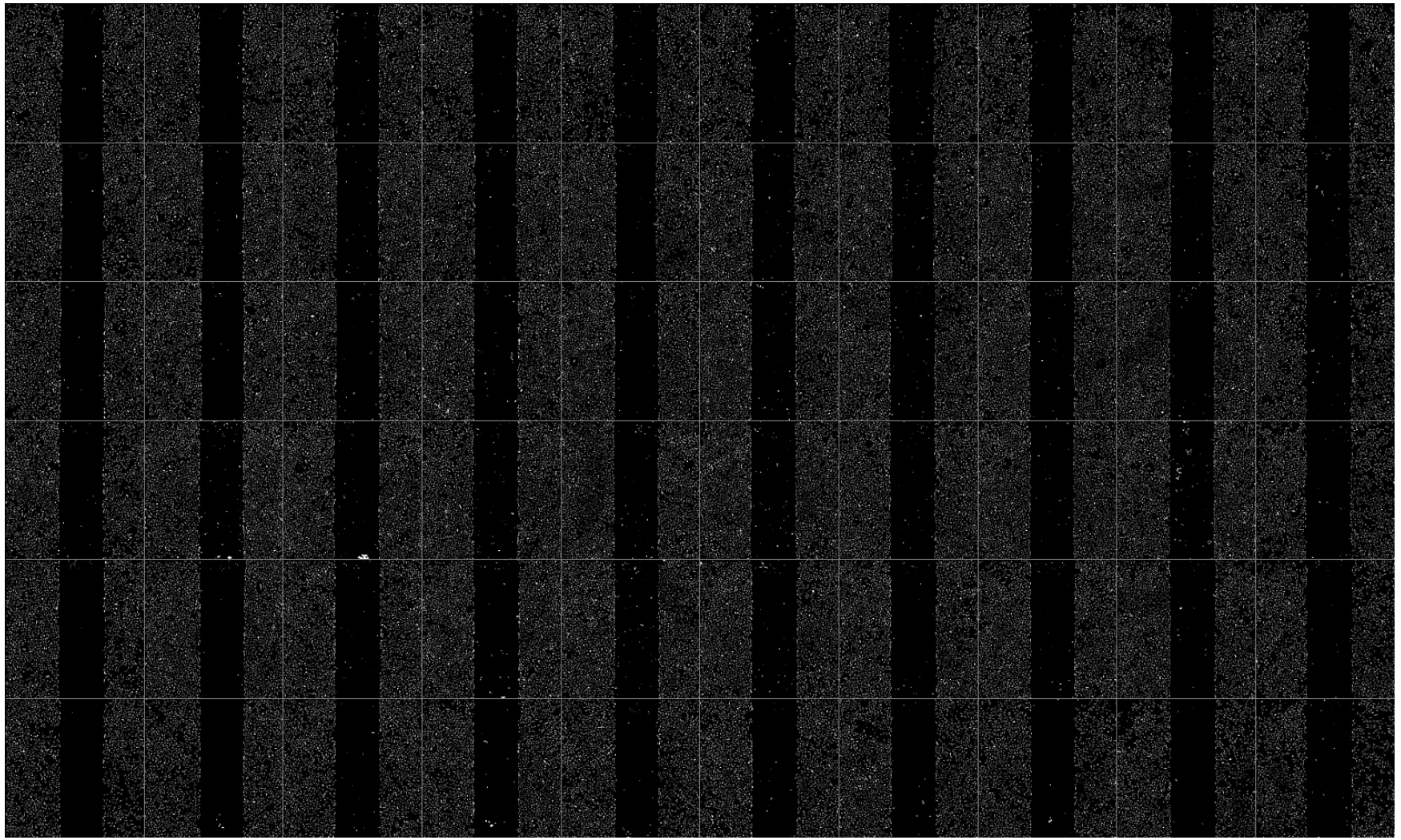
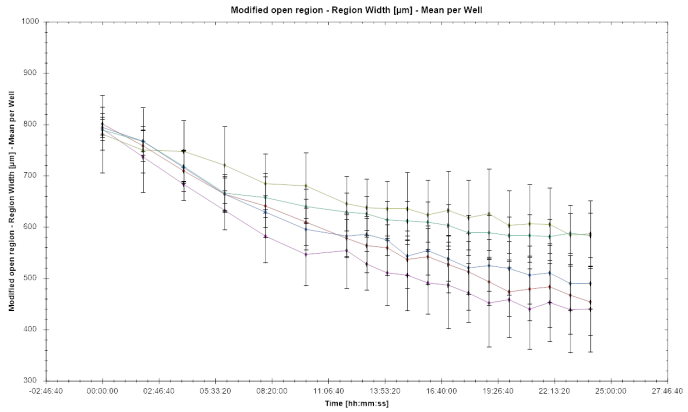
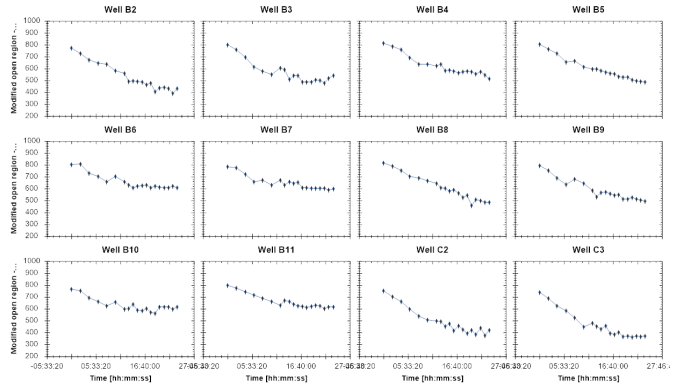
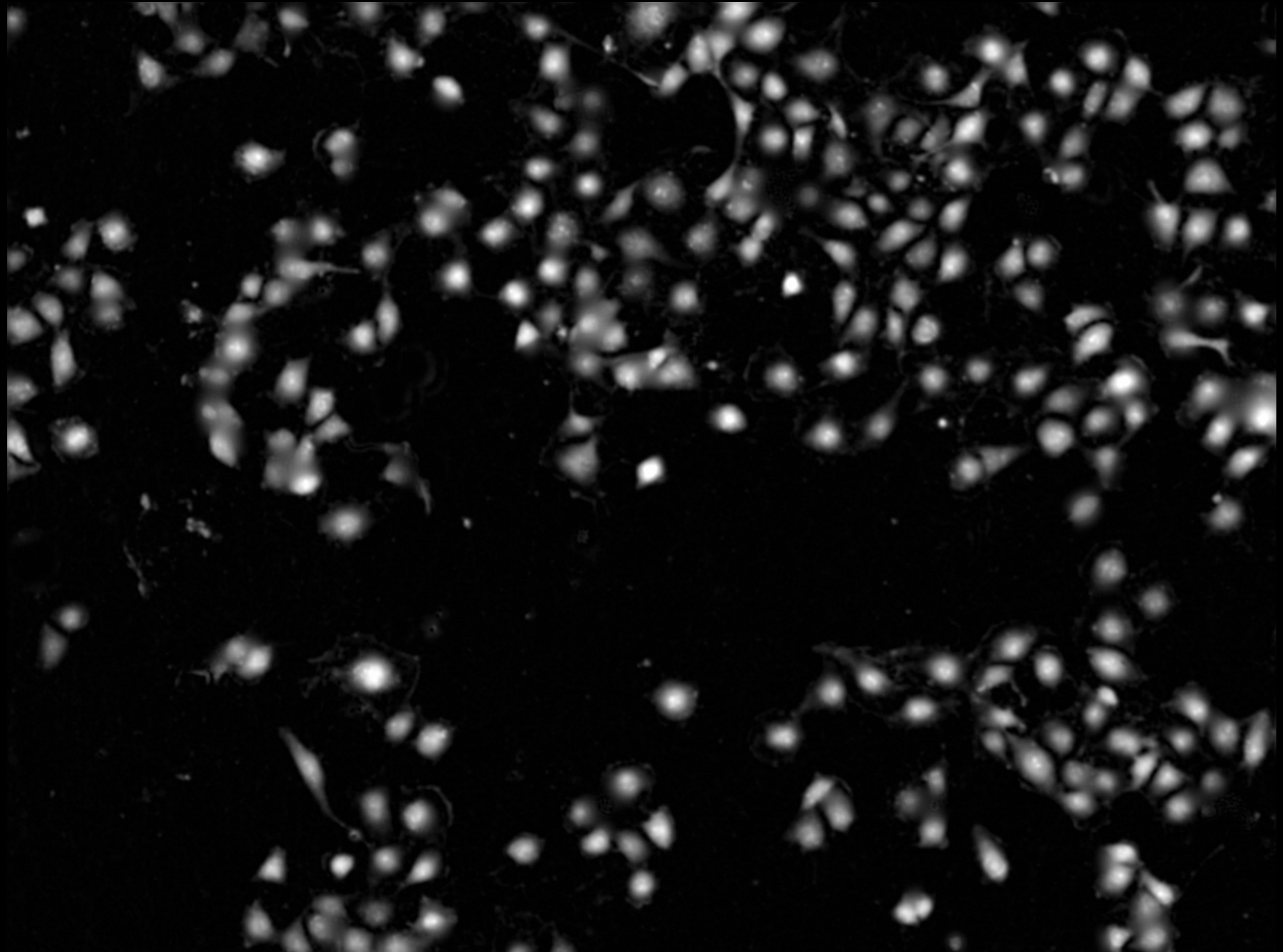






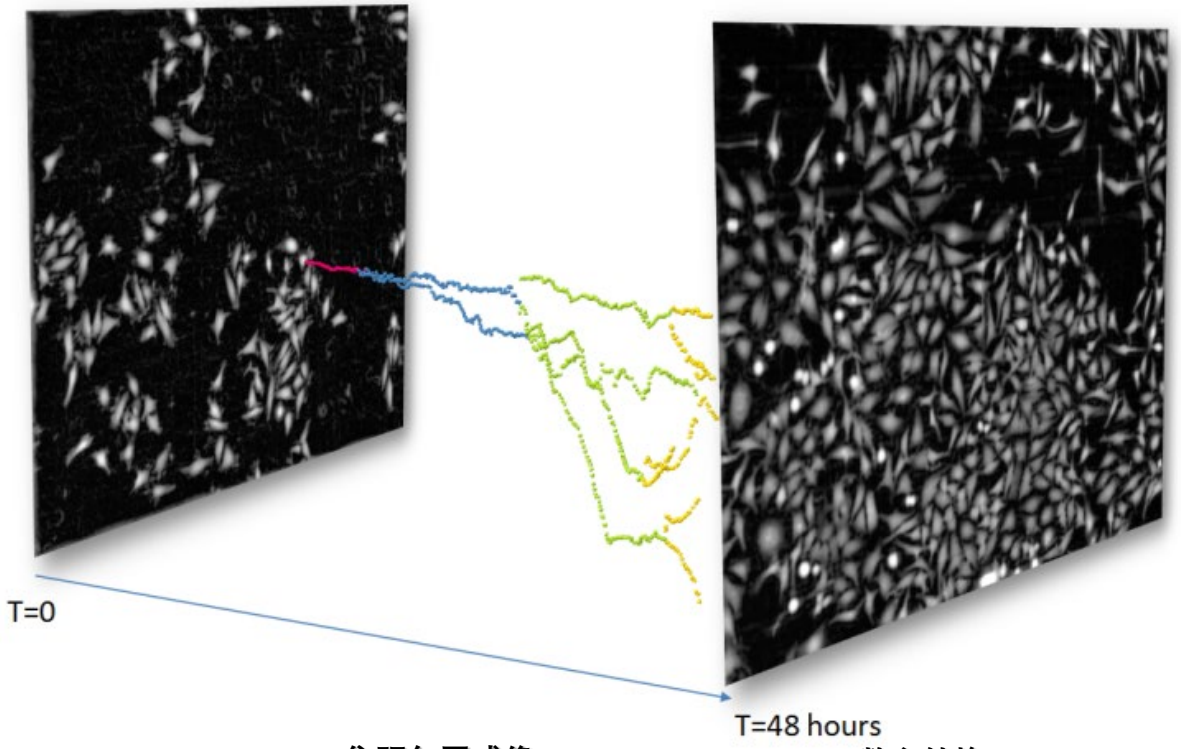
成像图片（60 wells，4 field，10X Air，DPC通道）

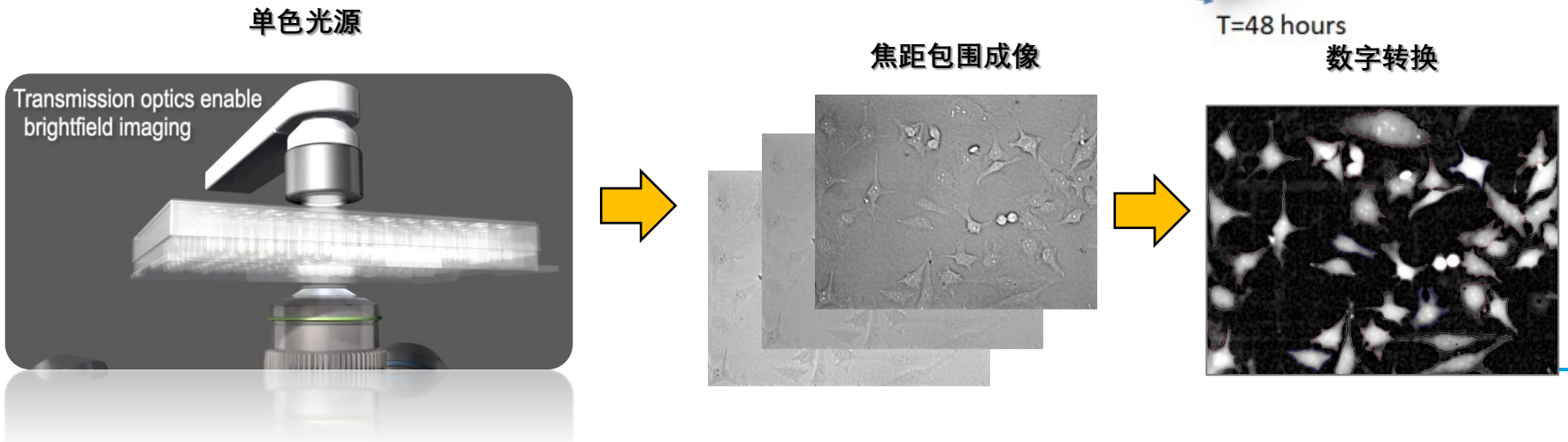


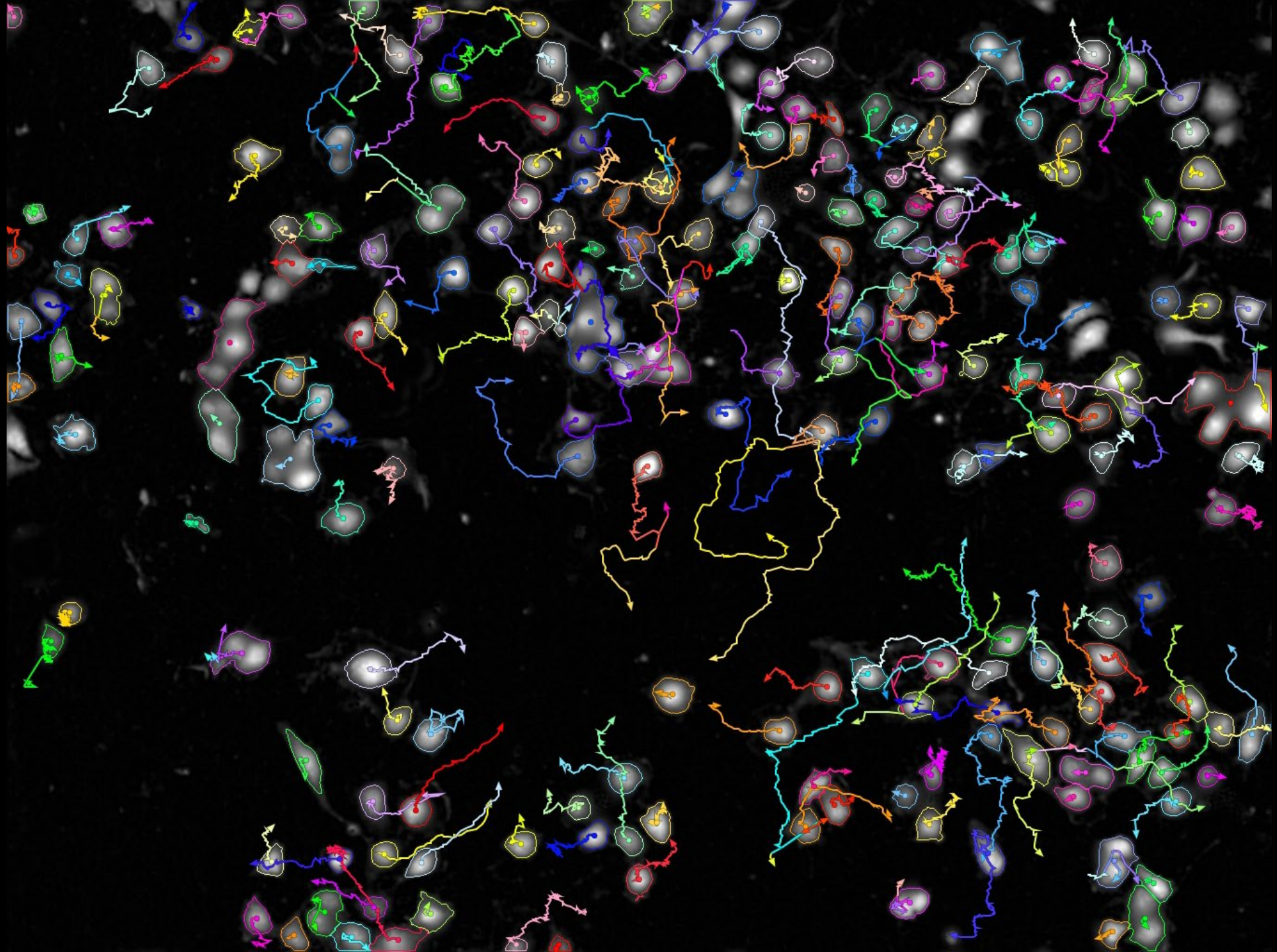
无标记细胞检测

景深包围式无标记成像模式

（Digital phase contrast , DPC）







DPC细胞运动追踪结果

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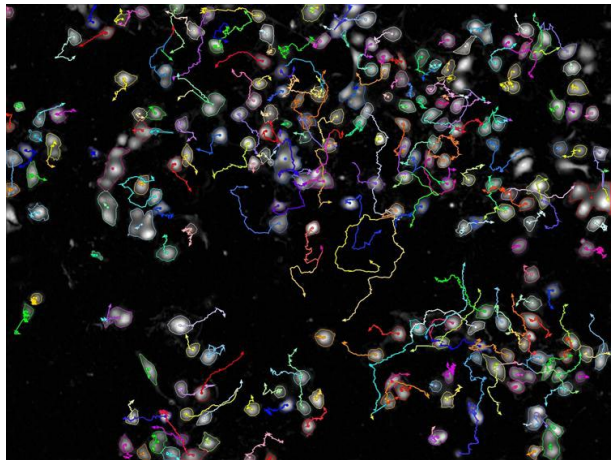
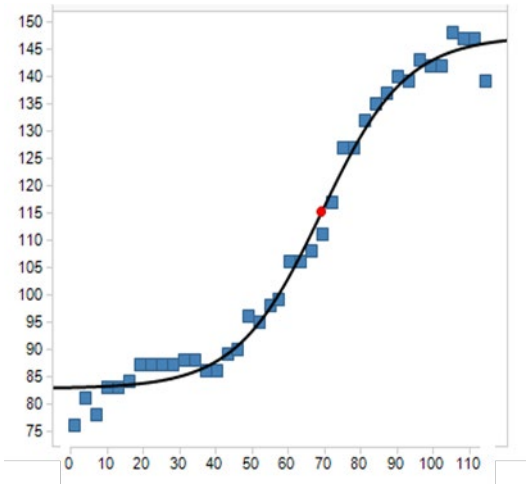
运动轨迹





生长曲线

Row	Column	Plane	Timepoint	Number of Tracked Cells	Age [s]	Current Displacement X [??m]	Current Displacement Y [??m]	Current Speed [??m/s]	Intensity Cell Mean - Digital Phase Contrast	Intensity Cell Contrast - Digital Phase Contrast	Cell Area [??m??]	Cell Roundness	Number of Analyzed Fields	Number of Timepoints per Track	Track Duration [s]	Tracked Cells - Generation	Accumulated Distance [??m] per Track	Displacement [??m] per Track	Average Speed [??m/s] per Track	Number of Tracks	Height [??m]	Time [s]	Temture
7	7	1	3	312	1649.786	-4.83242	-0.63987	0.003764	588.6884	0.888622	728.3874	0.902662	1	9.627933	15403.56	1.334078	57.23537	34.17703	0.003863	1790	0	1799.766	
7	8	1	3	112	1608.188	-4.98386	0.088681	0.003622	579.8935	0.883073	795.476	0.918525	1	9.836237	15773.51	1.529617	53.84848	31.32942	0.003697	574	0	1801.17	
7	9	1	3	265	1653.185	-3.34323	0.161165	0.003607	589.1942	0.892303	724.132	0.920082	1	10.51684	16987.59	1.446359	64.1123	38.8705	0.003983	1277	0	1802.856	
7	10	1	3	226	1557.196	-5.22981	-0.27248	0.00373	601.4256	0.881812	823.505	0.899123	1	9.473144	15128.47	1.424171	51.45512	31.32123	0.00362	1266	0	1804.75	
7	11	1	3	200	1651.874	-2.08577	1.027987	0.002832	599.5664	0.835778	908.2412	0.862136	1	8.226003	12898.77	1.452007	42.72207	26.58144	0.003446	1146	0	1805.327	
7	12	1	3	178	1621.532	-5.09446	-0.07254	0.004052	570.6434	0.877574	780.2122	0.907618	1	10.486	16941.09	1.450168	61.28012	35.10516	0.003952	893	0	1803.954	
7	13	1	3	147	1705.868	1.067547	3.128319	0.003234	578.8935	0.883047	843.3525	0.91603	1	10.061	16180.07	1.464115	55.96933	33.79595	0.003632	836	0	1804.047	
7	14	1	3	262	1680.424	-4.02976	-0.31465	0.003673	593.9718	0.887071	754.4879	0.911571	1	9.583685	15316.88	1.40647	55.11333	32.76789	0.003768	1422	0	1804.39	
7	15	1	3	181	1565.934	-0.70788	0.444482	0.003174	573.368	0.870023	828.7648	0.904248	1	9.08596	14441.15	1.434575	53.68213	33.62974	0.003841	1047	0	1805.313	

Operetta高内涵成像系统DPC明场功能和Harmony软件强大的cell tracking分析功能完美结合，只需简单操作，即可获得细胞分裂或运动的详细数据，包括**分裂次数、生长曲线、运动轨迹、位移、方向、速度**等多种参数结果。

DPC明场观察细胞迁移—运动速度变化

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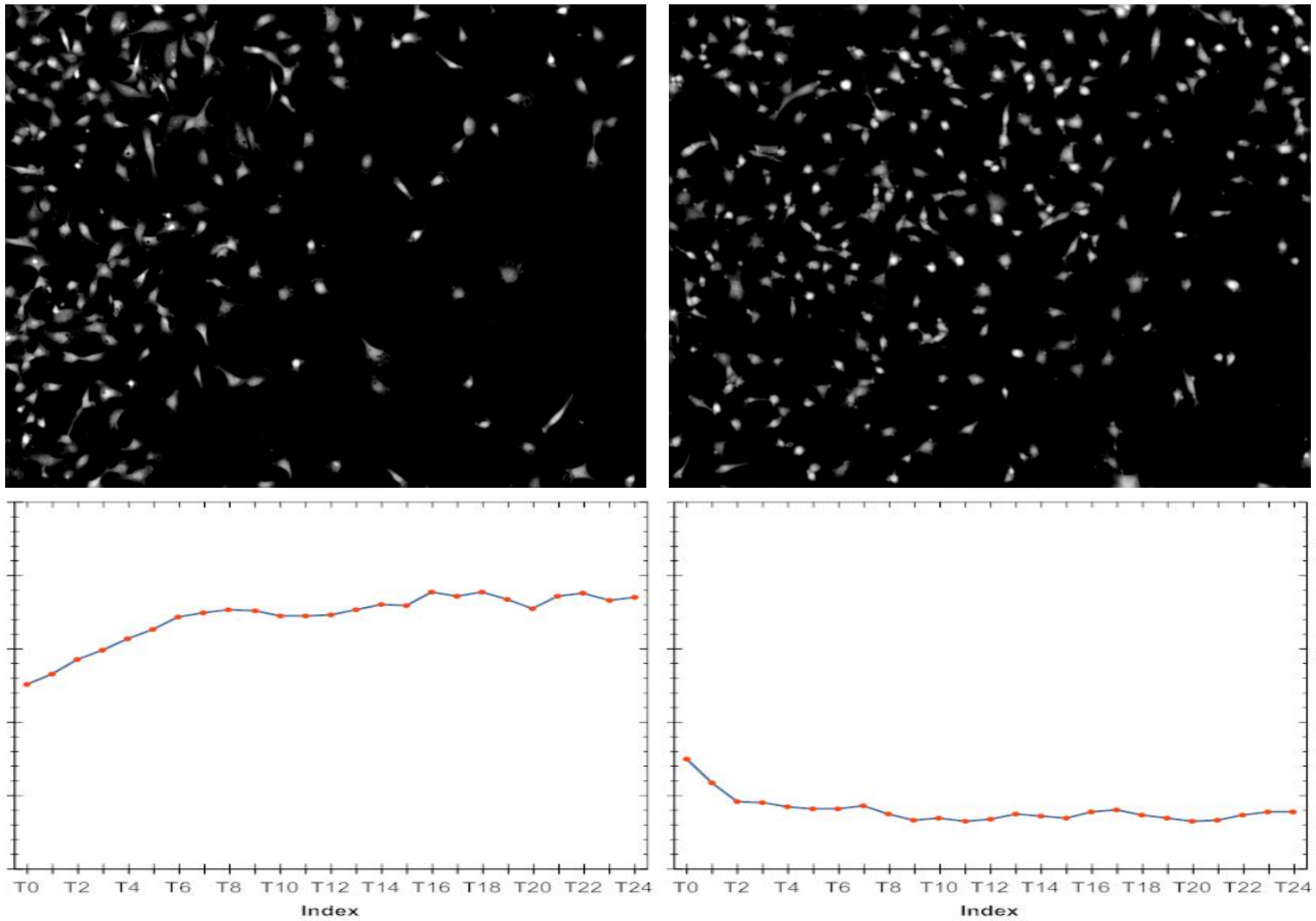
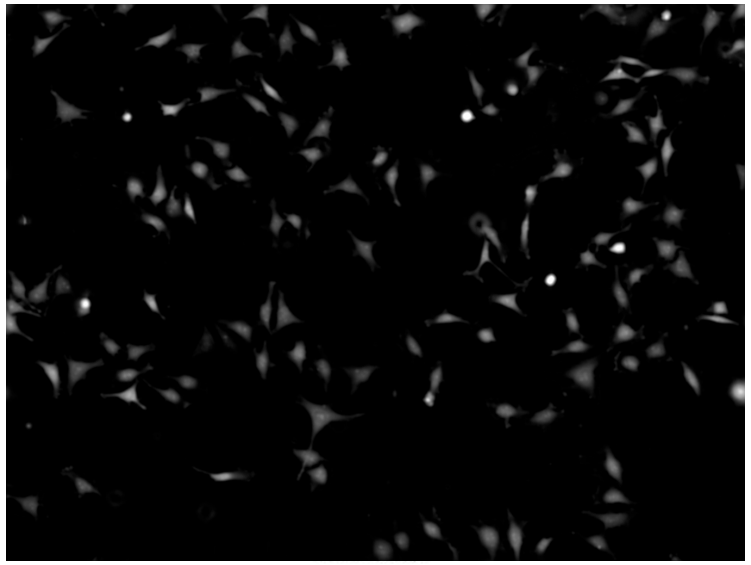
对照组

药物处理组

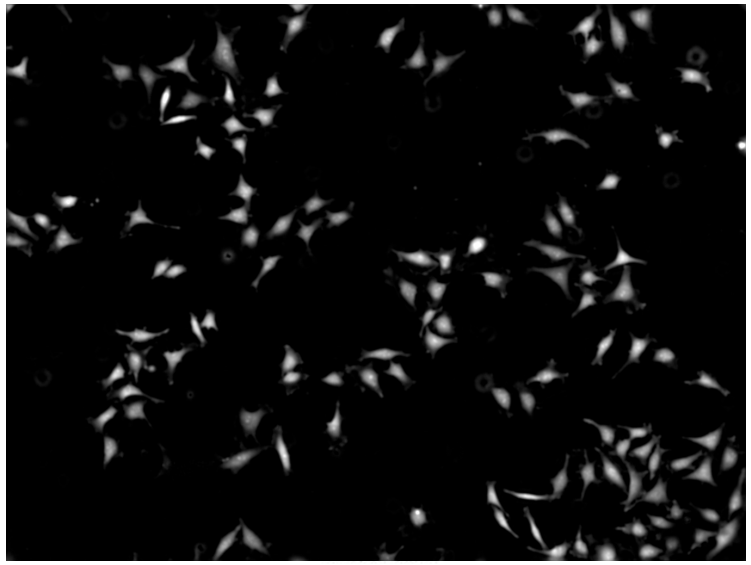


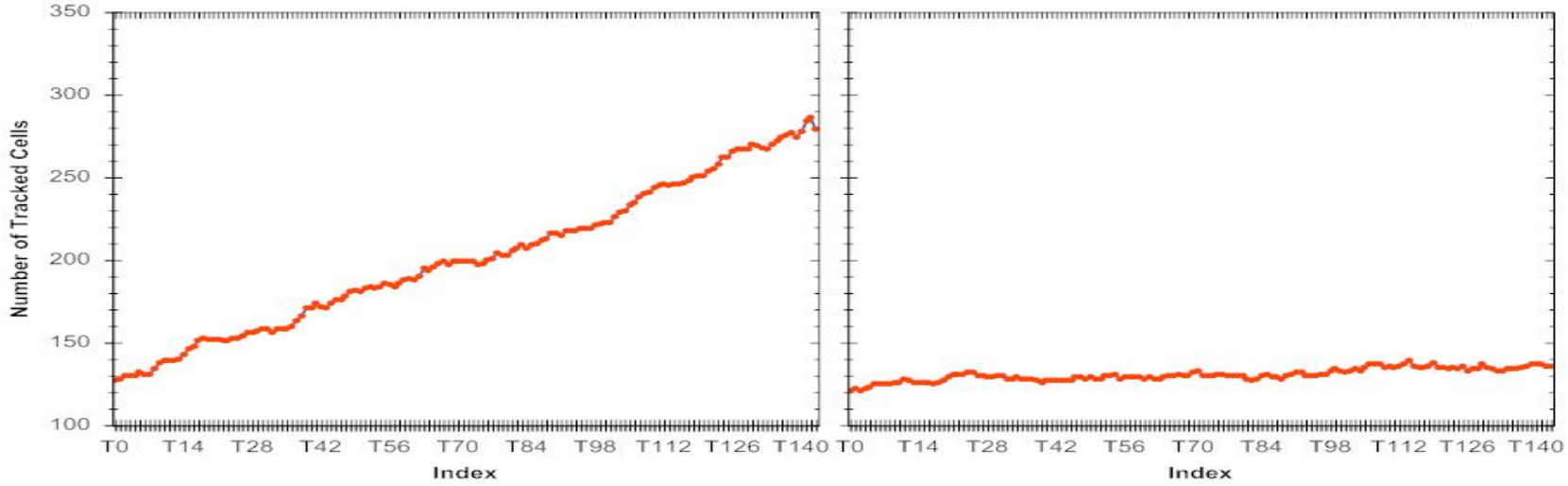
DPC明场观察细胞增殖—细胞数变化

对照组



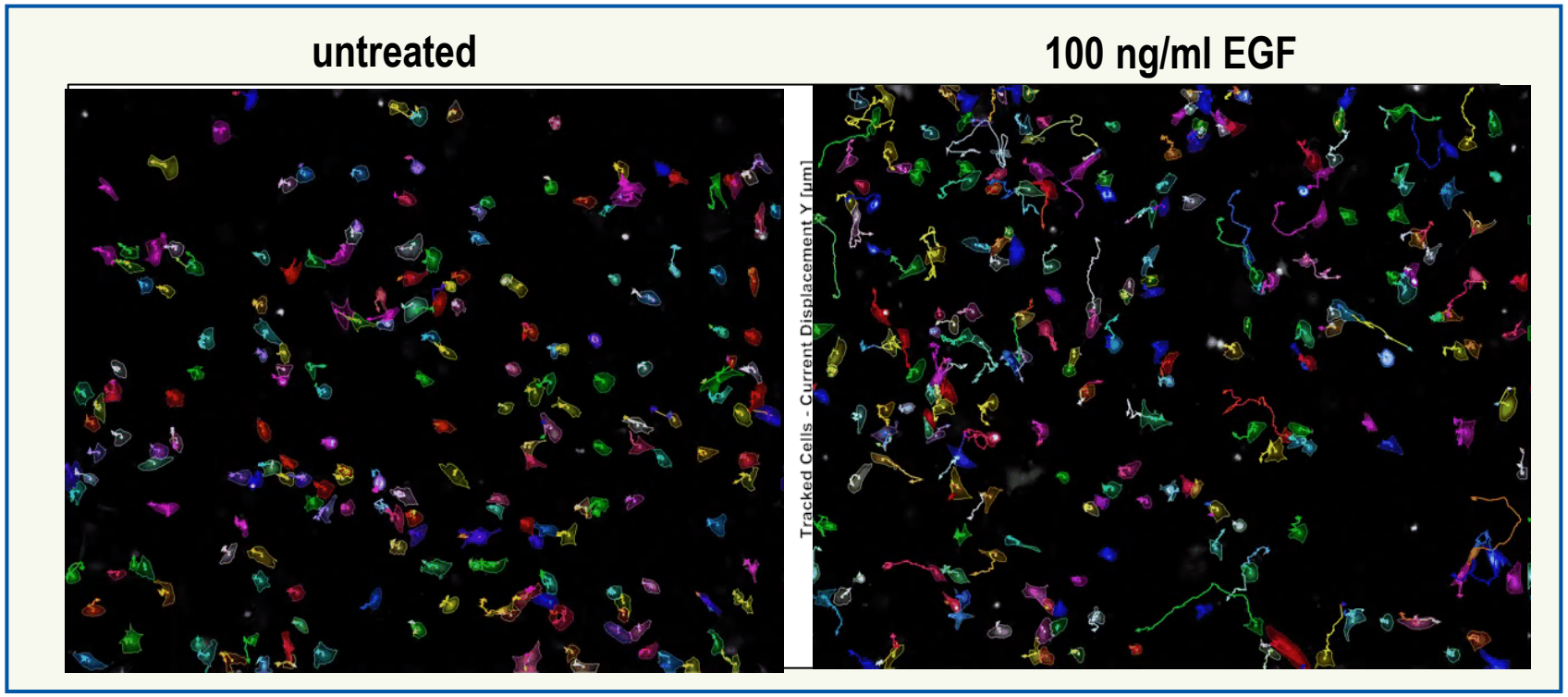
药物处理组





化学运动性研究

- A549 非小细胞癌细胞化学运动性模型(Caino *et al.*, 2012)
- Operetta® 活细胞无标记成像
- 每间隔5-10 min 连续成像6 hrs， 10x 物镜



- EGF stimulates cell migration resulting in longer cell tracks

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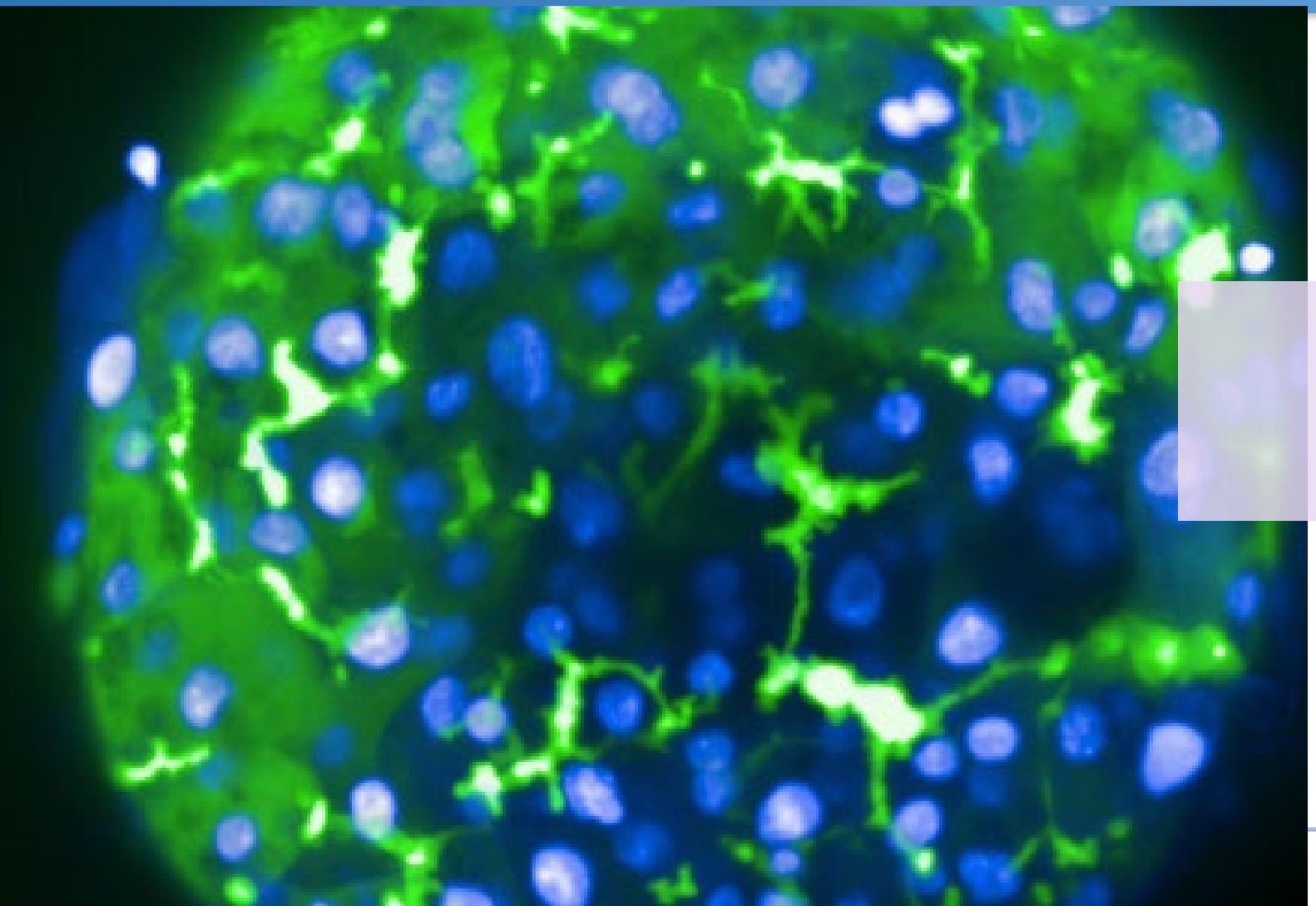

3D微组织

3D微组织 – 应用方向

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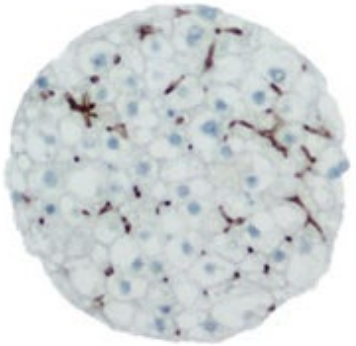
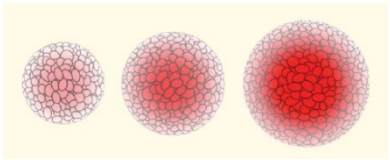


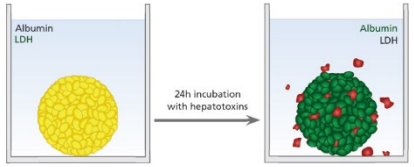
HST drug screening, Car-T, Nano materials, Neuron growth …

Cancer research



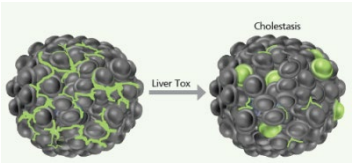
- 3D Tumor model using HT-29 cancer microtissues
- Cancer biomarker expression using in vivo NIR agents

Toxicity testing



- 3D Hepatotoxicity model using primary human liver microtissues
- Mitomembrane potential; Apoptosis; Cell death

Disease modelling



- 3D Cholestasis model using primary human liver microtissues
- Bile efflux transporter activity

variety of apps in preclinical research

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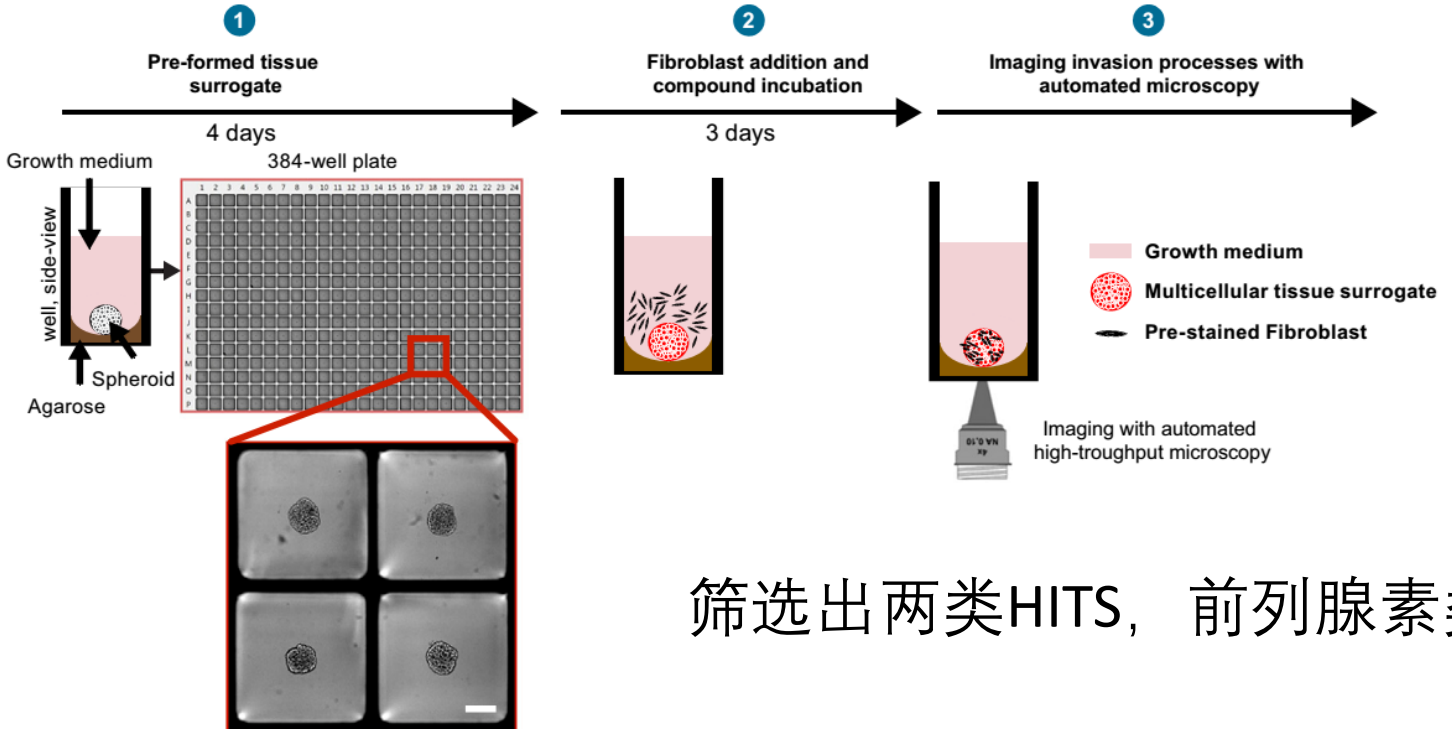
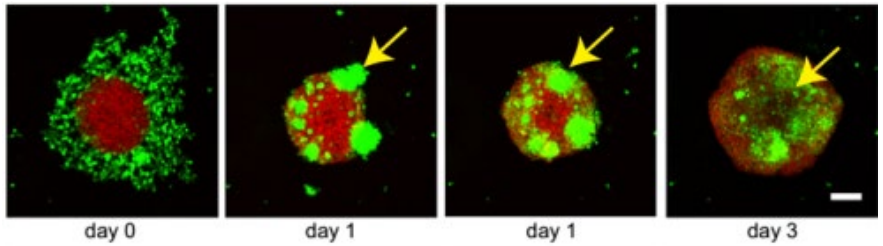
Research Article

A novel 3D high-content assay identifies compounds that prevent fibroblast invasion into tissue surrogates

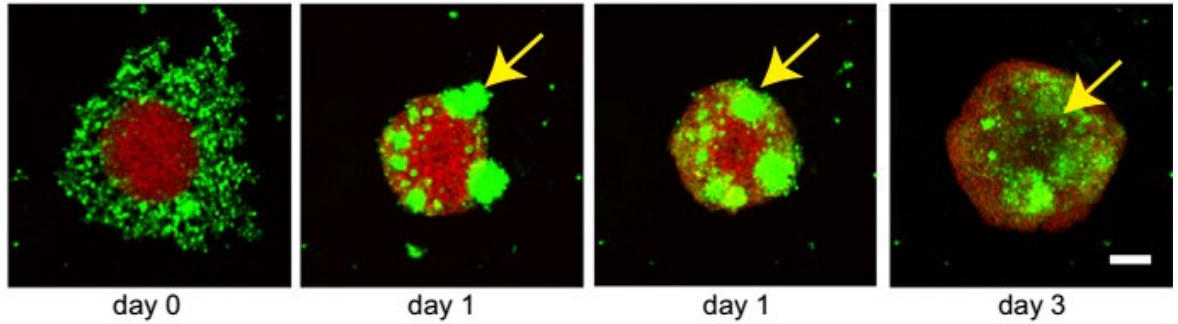
Carsten Wenzel, Saskia Otto, Stefan Prechtl, Karsten Parczyk, Patrick Steigemann *

Bayer Pharma AG, Global Drug Discovery, Berlin, Germany

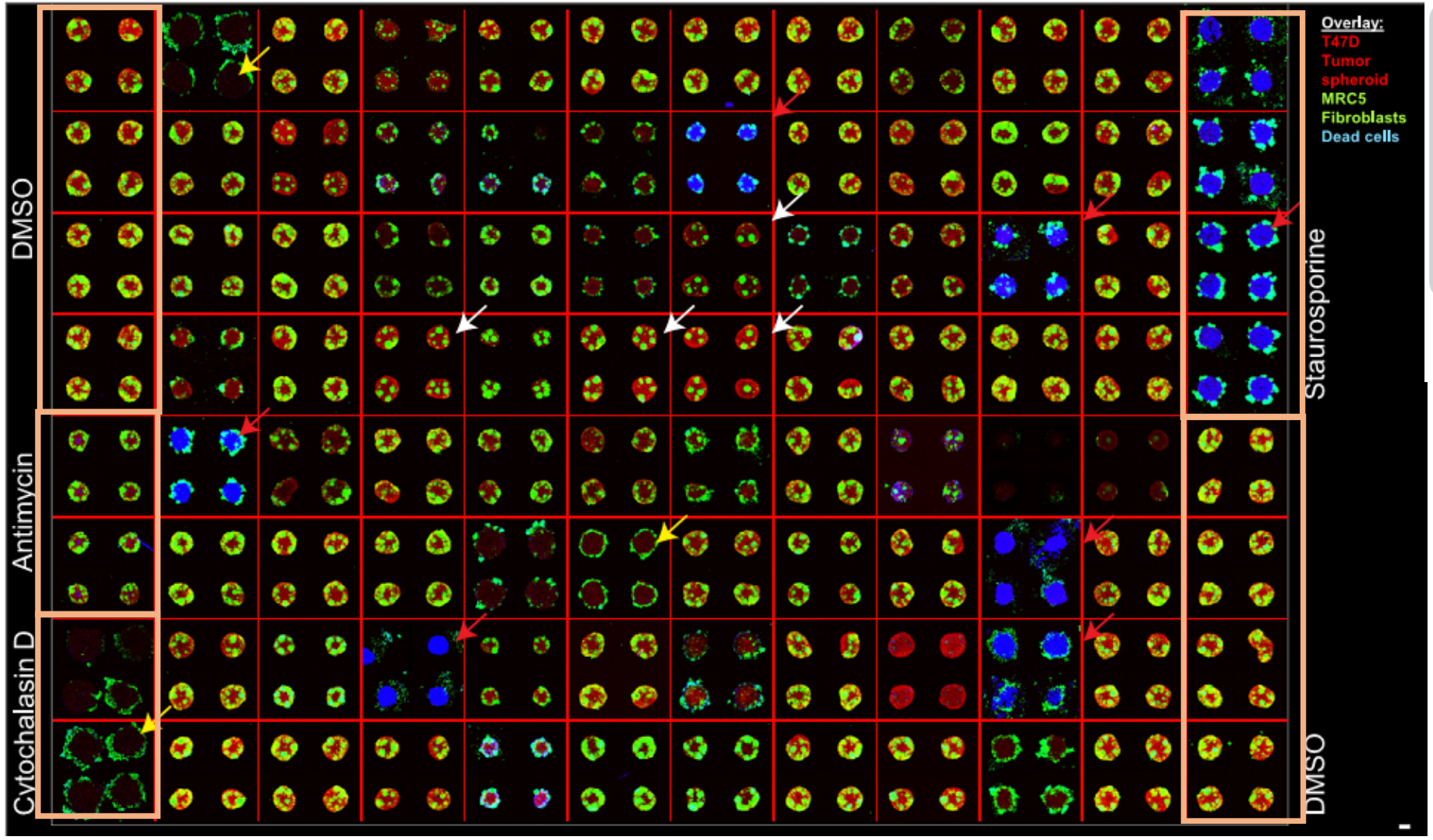


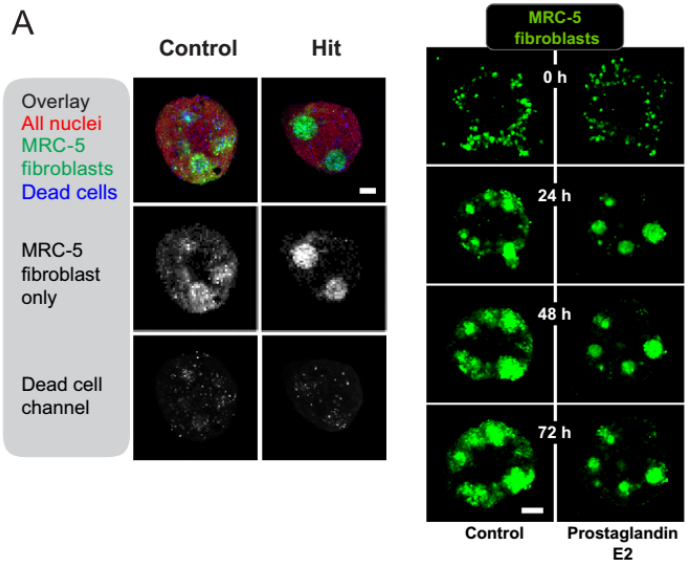


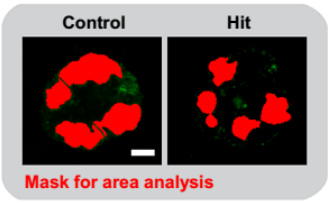
筛选出两类HITS，前列腺素类、ROCK抑制剂

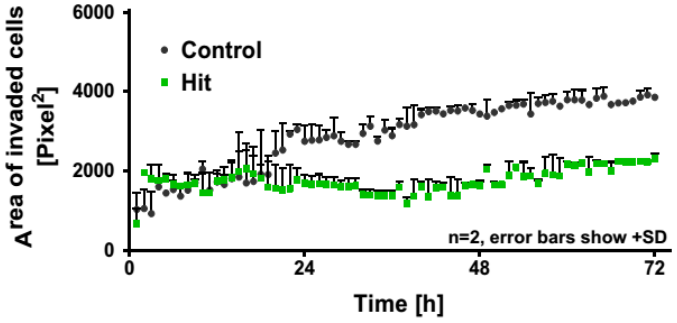


黄色箭头显示纤维细胞侵入肿瘤微球









临床样本3D微组织

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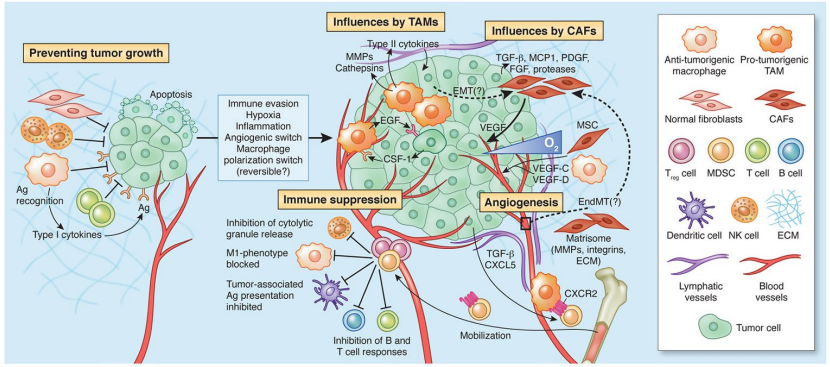
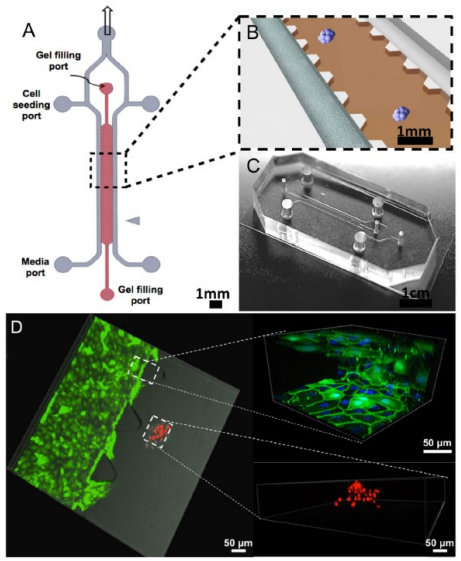
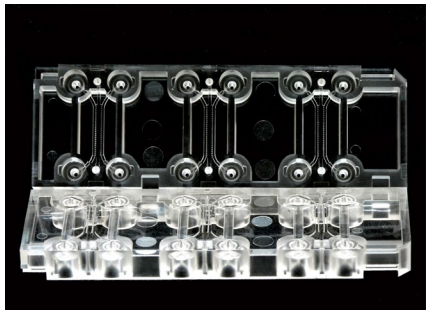


肿瘤微环境



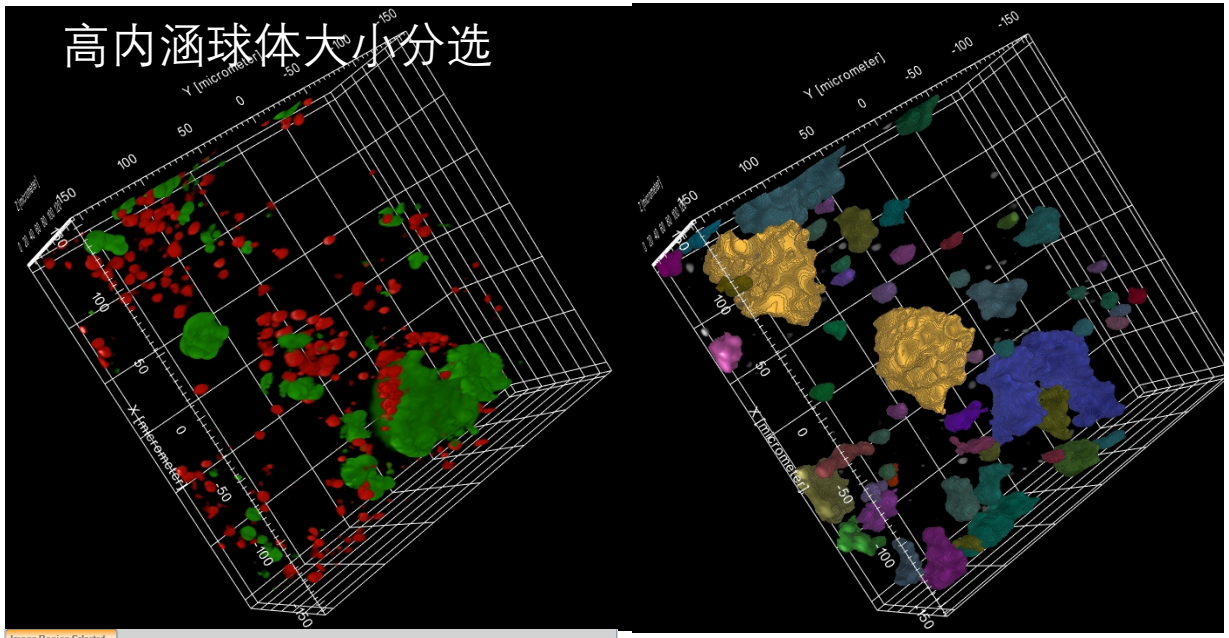


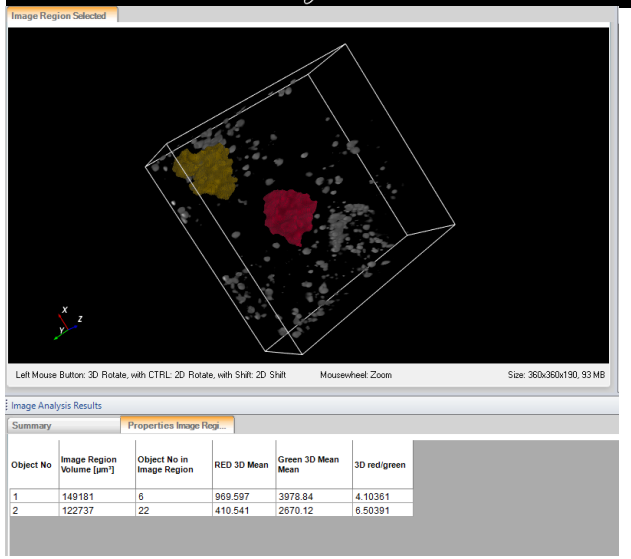
肿瘤球大小依赖的药物作用

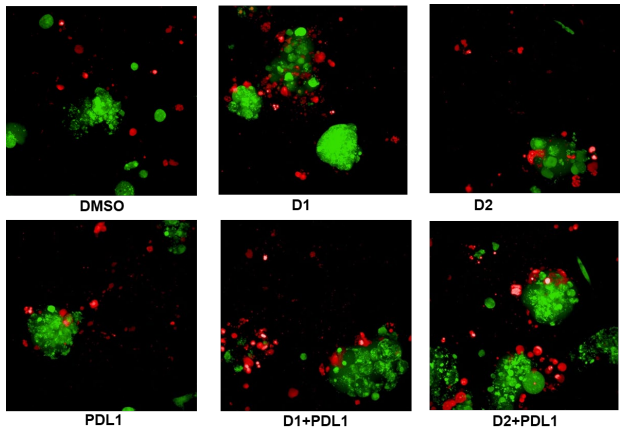
单个肿瘤微球体积

荧光强度

药物杀伤率







计算体积并且通过设置阈值确定特定范围的微球

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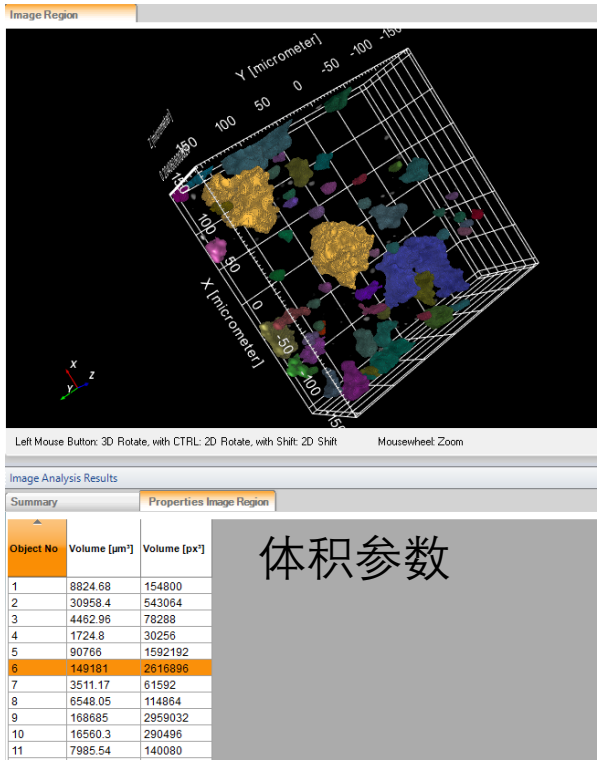
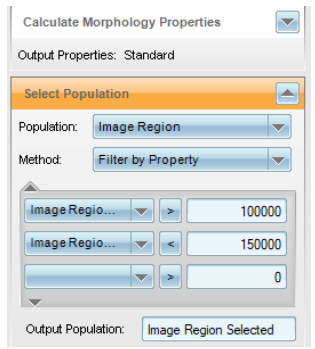


Image Analysis Results

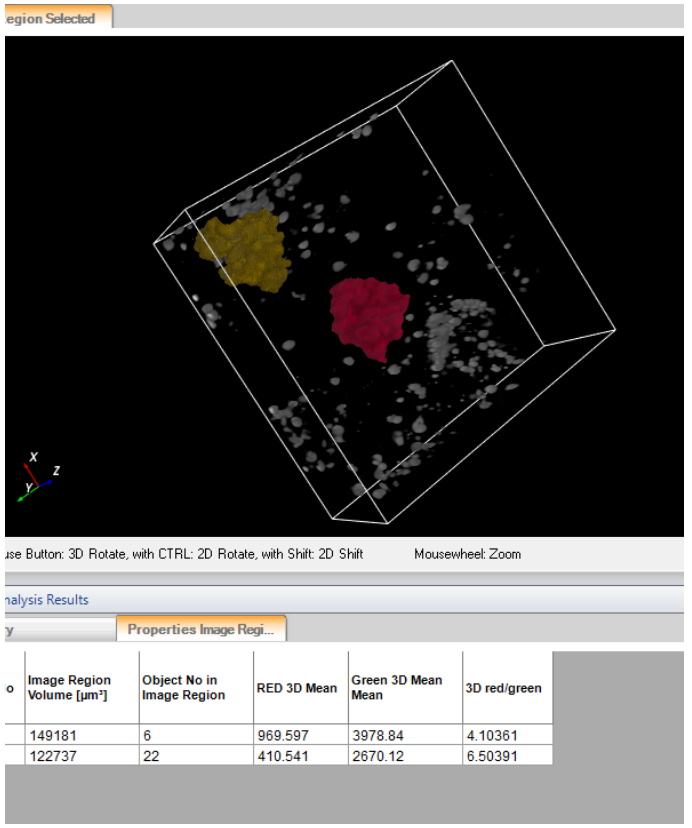
Summary | Properties Image Region

Object No	Volume [µm³]	Volume [px³]
1	8824.68	154800
2	30958.4	543064
3	4462.96	78288
4	1724.8	30256
5	90766	1592192
6	149181	2616896
7	3511.17	61592
8	6548.05	114864
9	168685	2959032
10	16560.3	290496
11	7985.54	140080

体积参数



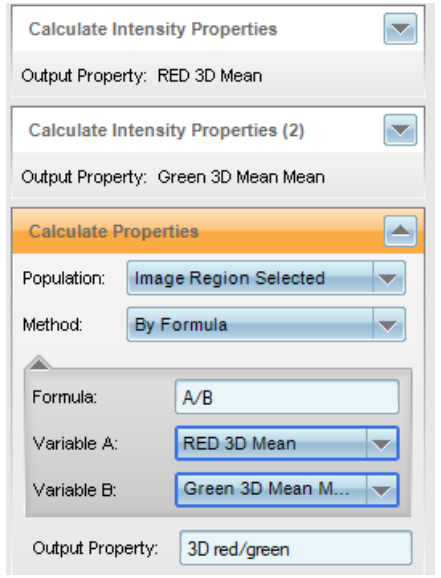
范围筛选



Properties Image Regi...

Image Region Volume [µm³]	Object No in Image Region	RED 3D Mean	Green 3D Mean Mean	3D red/green
149181	6	969.597	3978.84	4.10361
122737	22	410.541	2670.12	6.50391

结果计算



临床样本3D微组织

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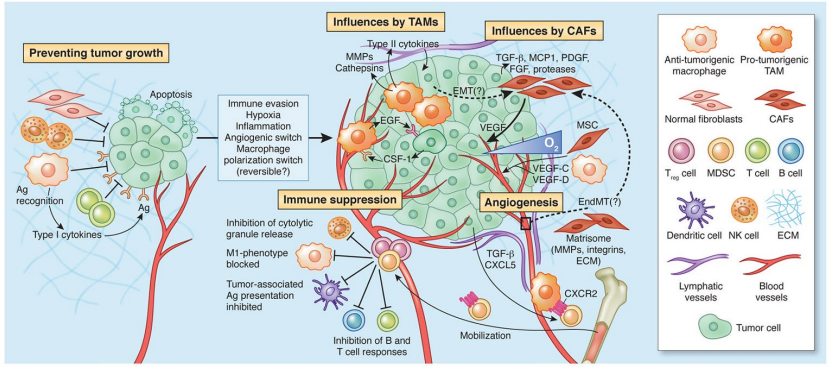
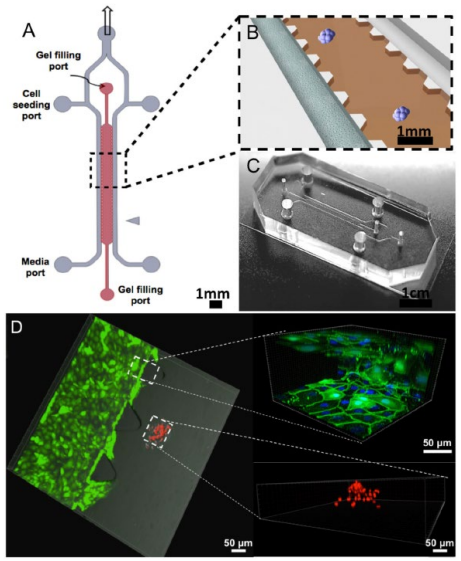
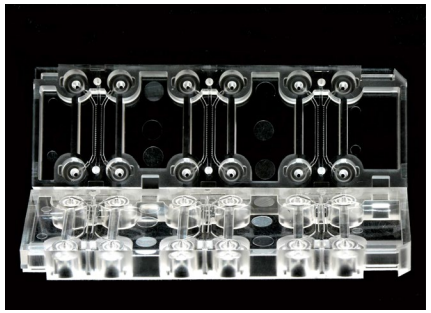


肿瘤微环境



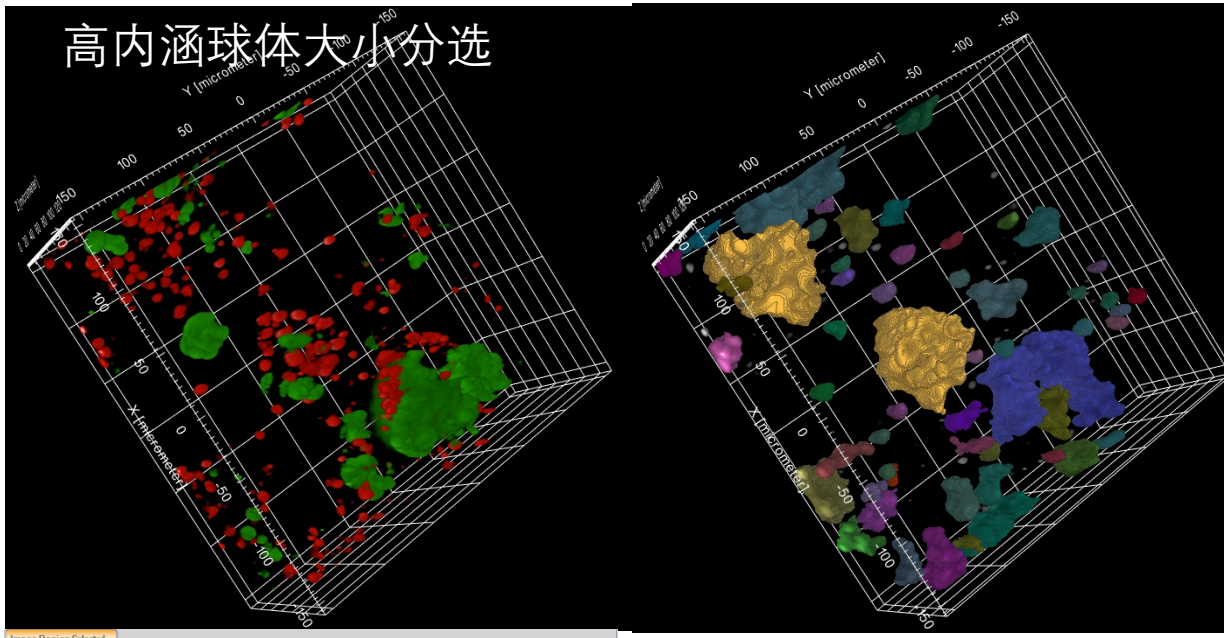


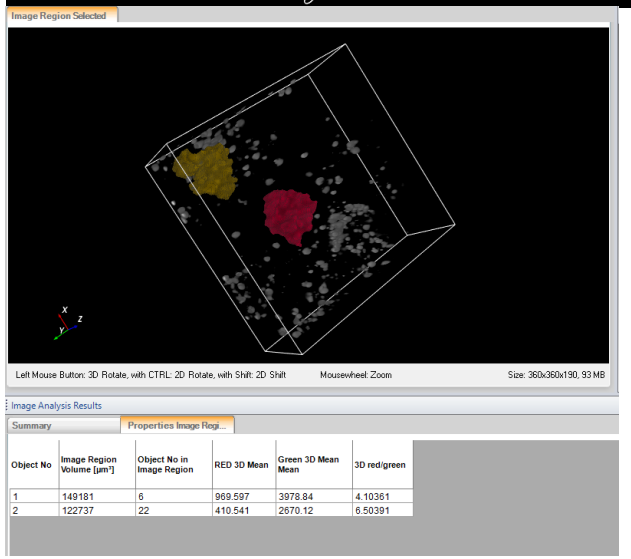
肿瘤球大小依赖的药物作用

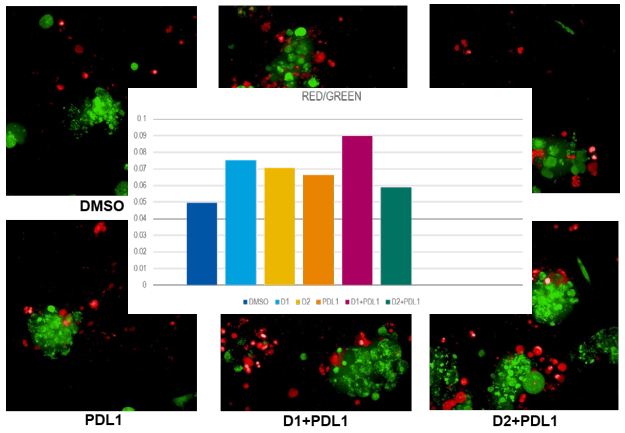
单个肿瘤微球体积

荧光强度

药物杀伤率







高内涵系统可进行丰富的细胞实验

细胞自噬分析	细胞凋亡分析	细胞迁移分析	细胞周期分析	细胞健康度分析	细胞毒性分析	神经细胞生长分析
神经突分析	细胞核形态分析	细胞核碎片化分析	细胞膜蛋白分析	DNA DAMAGE 分析	微核分析	线粒体分析
细胞骨架分析	细胞信号转导	转录因子激活	胞内蛋白表达分析	受体内化分析	脂滴分析	干细胞分化分析
酵母筛选	3D 细胞团分析	细胞共培养实验	共定位分析	胚胎分化	彗星分析	有丝分裂
转染效率	斑马鱼实验					

PerkinElmer高内涵整体实验方案

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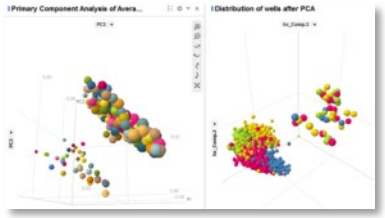
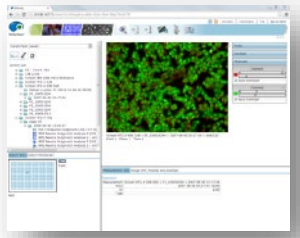
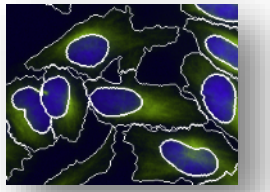


Spotfire
Easy. Fast. Smart.



Columbus

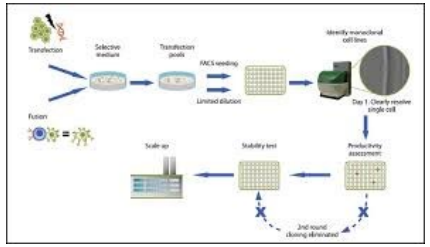


Harmony

Software

Chemistry
Cell biology
Assay design
Fluorescent reagents
Robotics, liquid handling
Automated imaging systems
Image analysis
Data management
Statistics, data analysis
Bioinformatics analysis
HCA

Wetware



Hardware

Adapted from Korn & Krausz,
Current Opinion in Chemical Biology 2007, 11:503–510

多维度数据管理

- 数据多维度管理：数据类型、用户名、项目名称、板型、放大倍数、日期、荧光通道、分析方法、细胞类型、化合物、关键词等等.....

完整的实验设计和排板，便于数据管理和回溯

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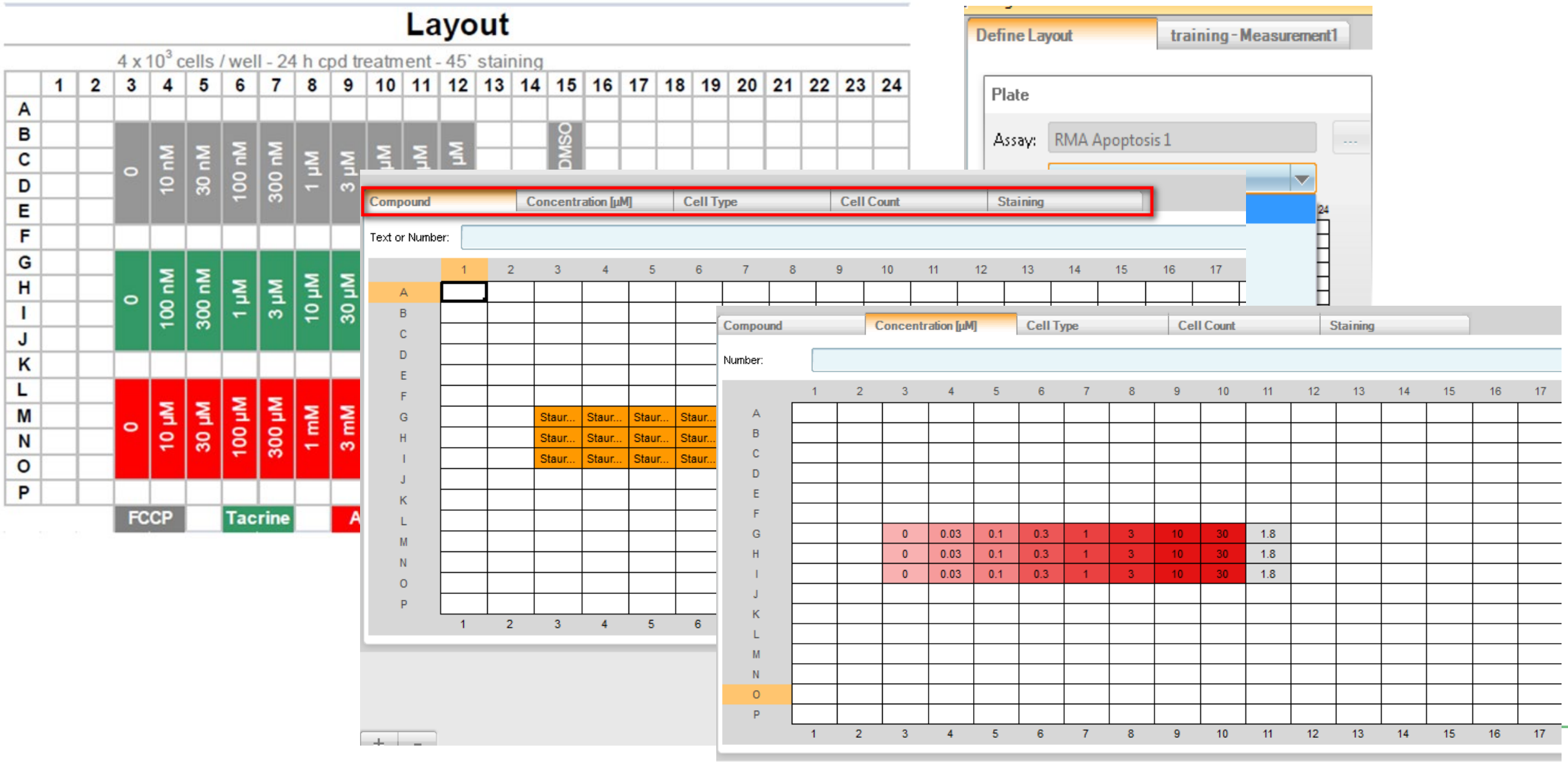
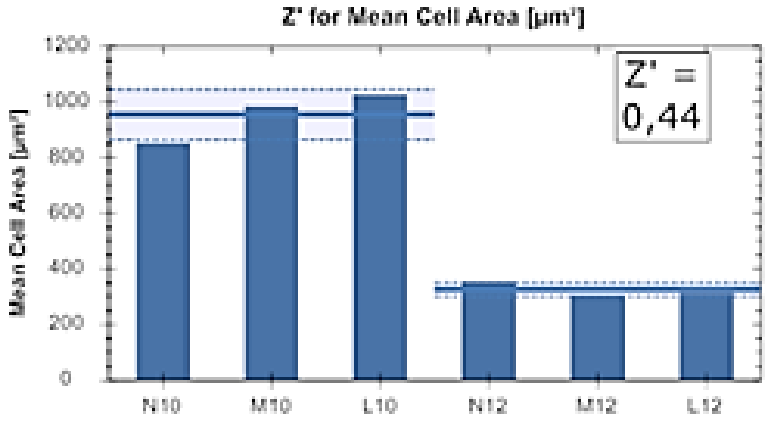
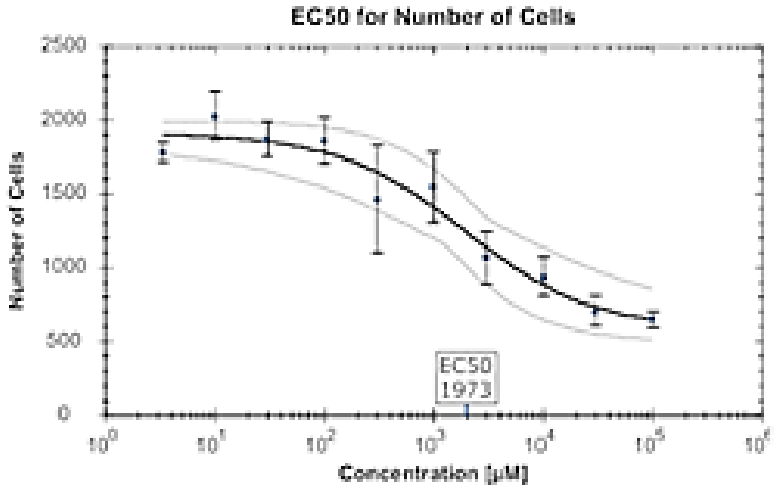
HUMAN HEALTH | ENVIRONMENTAL HEALTH

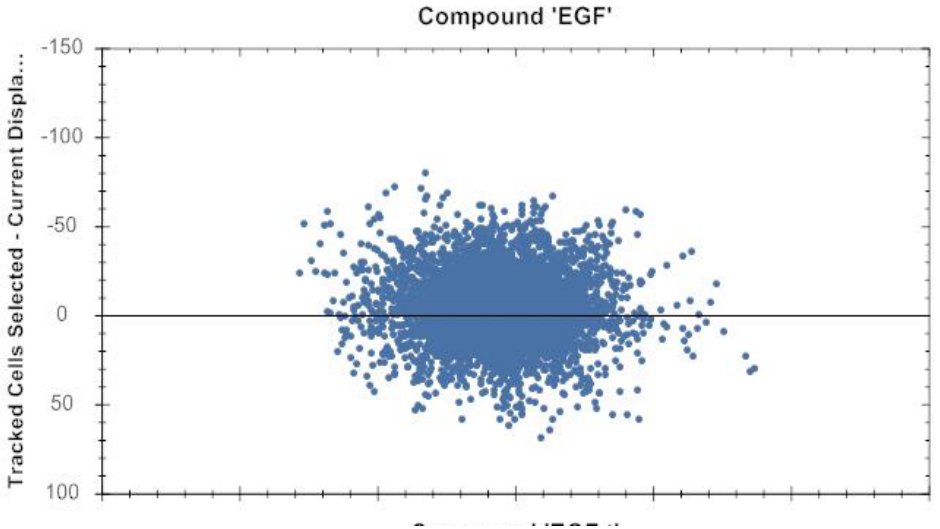


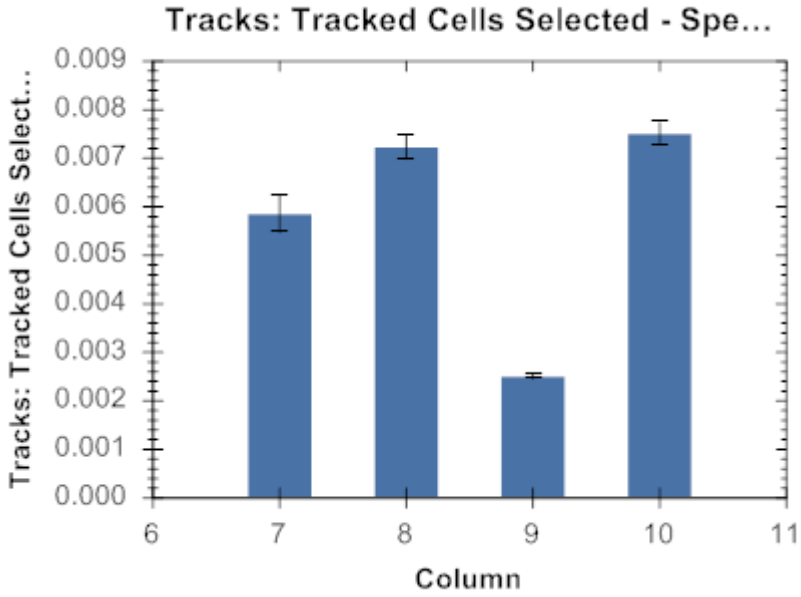
完善的数据计算，EC50、Z 值计算



- **EC50:** Displays a dose-response curve to determine the half maximal effective concentration.





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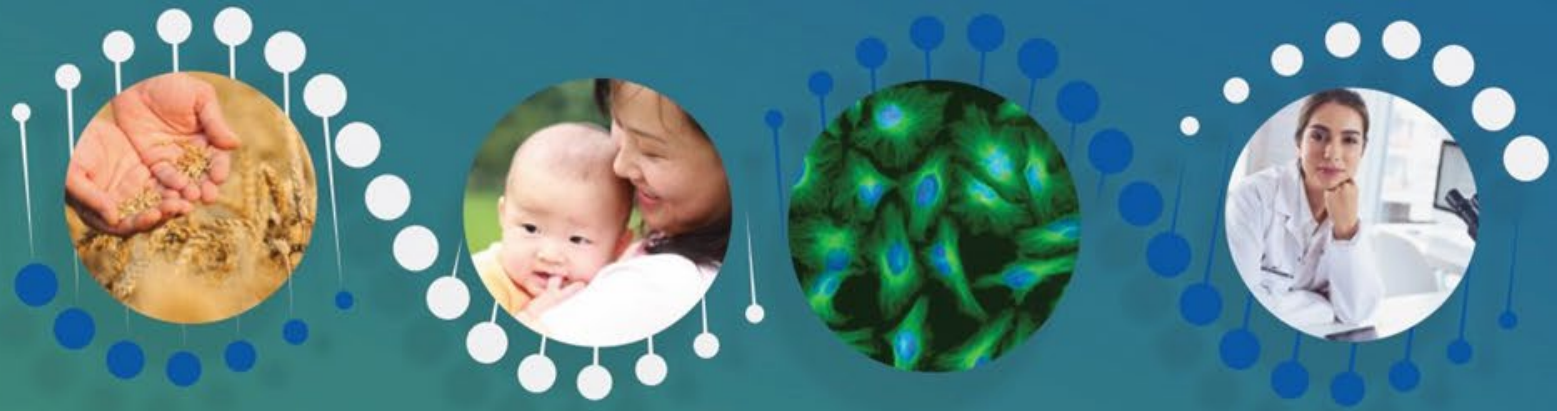
Detection Imaging Informatics Services



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