陕西师范大学
SHAANXI NORMAL UNIVERSITY

研究生教育教学改革研究项目
（研究生优质课程项目）

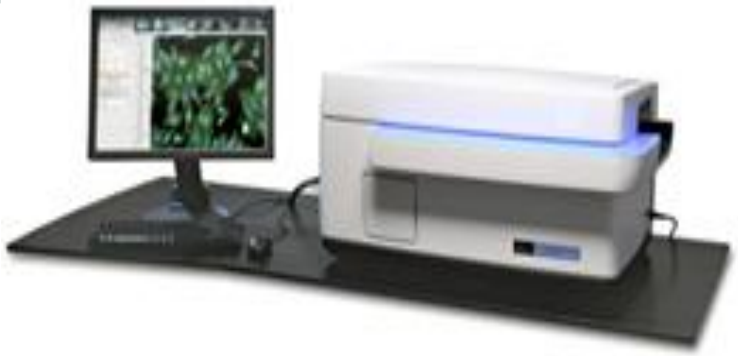
生物实验室安全及大型仪器应用

高内涵细胞成像系统

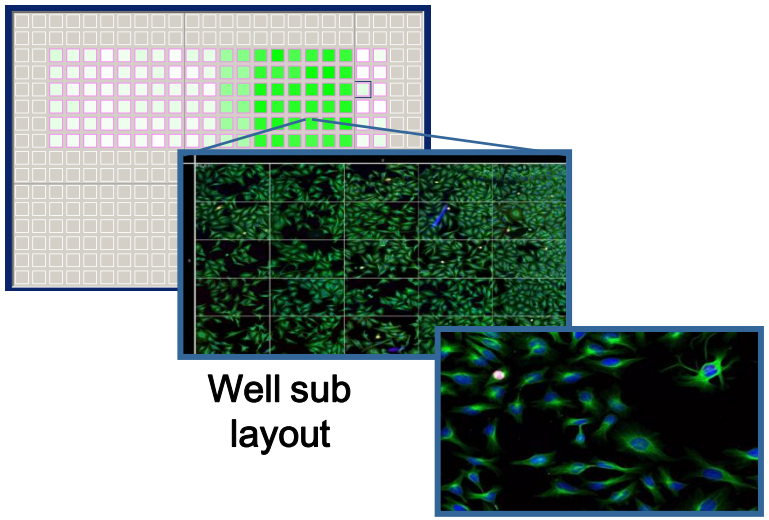
主讲教师 郑晓晶

生命科学学院实验教学中心

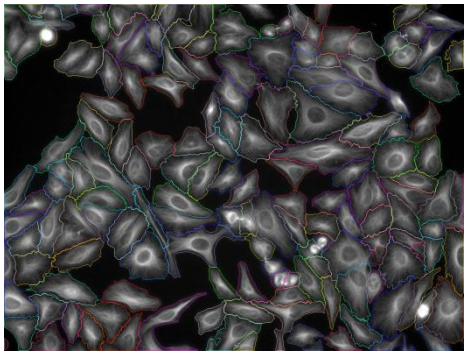
高内涵的工作流程

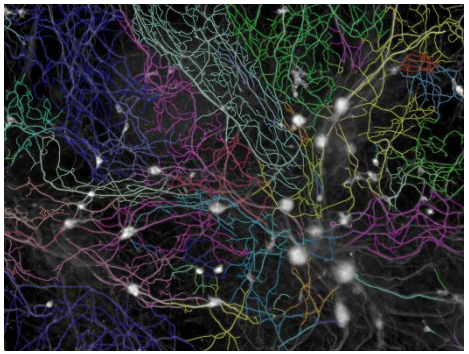


1. 自动成像

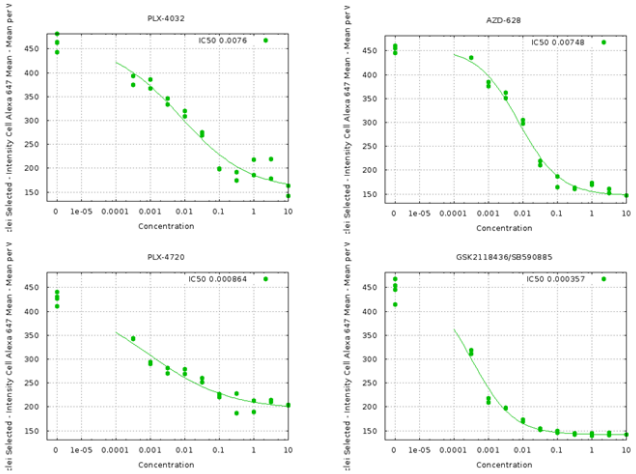


2. 图像分析



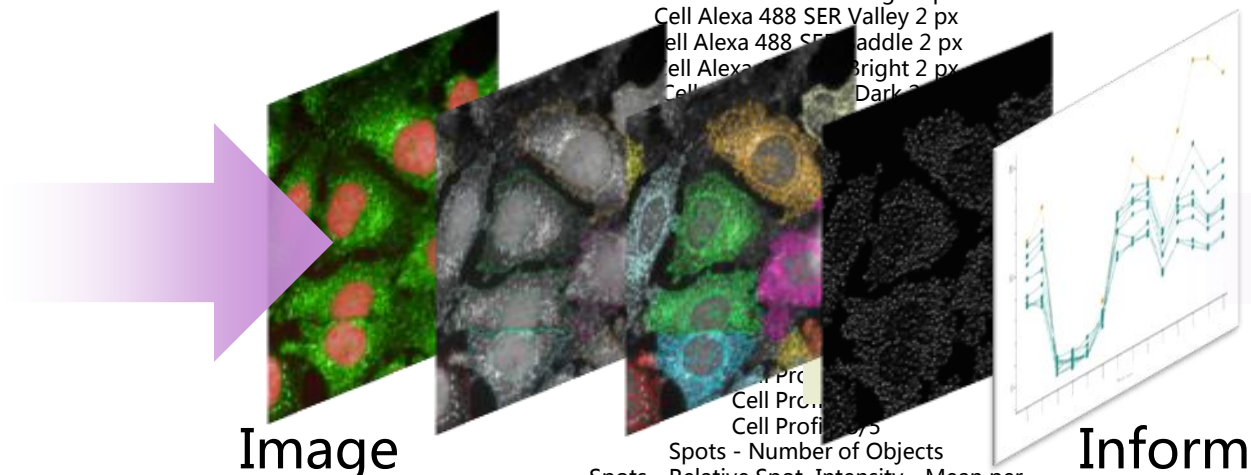


3. 数据质控/结果评估



The challenges of High Content Screening

Nuclei - Number of Objects Nuclei
Cells - Number of Objects
Cells - Total Spot Area - Mean per Well
Cells - Relative Spot Intensity - Mean per Well
Cells - Number of Spots - Mean per Well
Cell Alexa 488 SER Edge 2 px
Cell Alexa 488 SER Ridge 2 px
Cell Alexa 488 SER Valley 2 px
ell Alexa 488 SE addle 2 px
ell Alexa Bright 2 px
Cell Dark
Cell Pro
Cell Pro
Cell Profi /5
Spots - Number of Objects
Spots - Relative Spot Intensity - Mean per Well
Spots - Corrected Spot Intensity - Mean per Well
Spots - Uncorrected Spot Peak Intensity - Mean per Well
Spots - Spot Contrast - Mean per Well
Spots - Spot Background Intensity - Mean per Well
Spots - Spot Area [px²] - Mean per Well
Spots - Region Intensity - Mean per Well
Spots - Spot To Region Intensity - Mean per Well
Spots - Spot Area [µm²] - Mean per Well
Spots - Spot Ratio Width to Length - Mean per Well



Image

Information

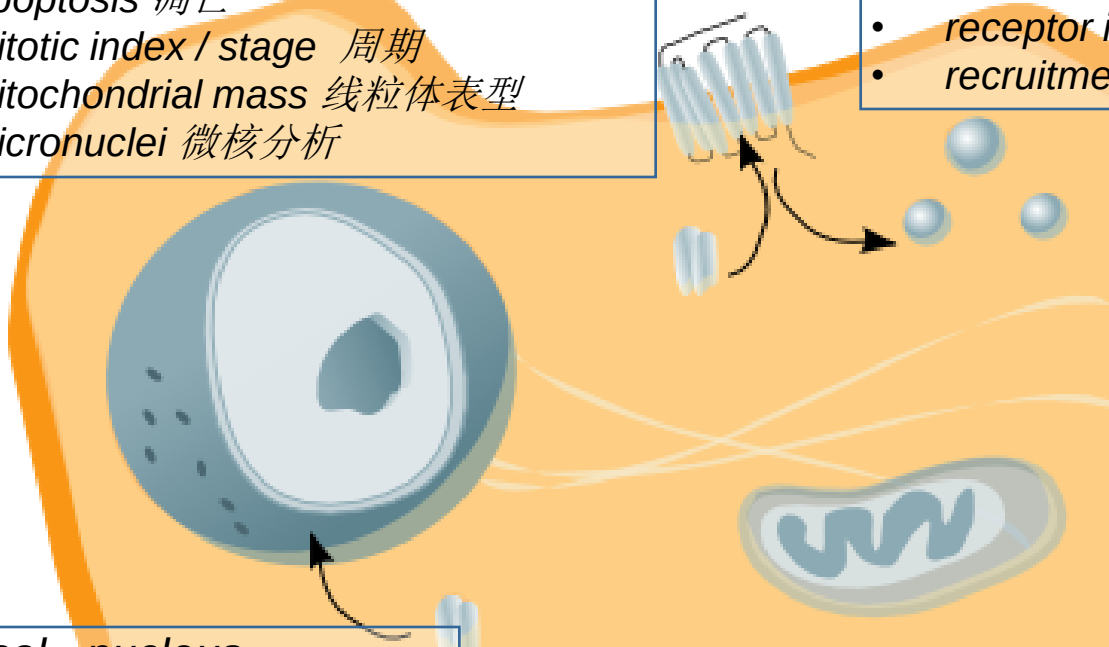
Knowledge

Cellular Images contain a lot of information!

HCS Applications Include

organelle morphology / staining
- *Apoptosis 凋亡*
- *mitotic index / stage 周期*
- *mitochondrial mass 线粒体表型*
- *Micronuclei 微核分析*

membrane – cytosol translocation
- *receptor internalization 受体内化*
- *recruitment of signaling proteins*



whole cell fluorescence
- *target phosphorylation*
- *transporter activity*
- *cytotoxicity*
- *membrane potential*
- *calcium flux*

cytosol - nucleus translocation
- *protein kinase activation*
- *transcription factor activation 转录因子激活*

others
- *Proliferation 增殖*
- *Migration 迁移*
- *Differentiation 分化*
- *Angiogenesis 血管生成*

membrane fluorescence
- *ligand binding 配体结合*
- *Apoptosis 凋亡*

cell morphology
- *neurite outgrowth*
- *apoptosis*
- *cytotox*

…and combinations

…enabling assays providing important information in

Marketing Diagram

A Add Your Text

B Add Your Text

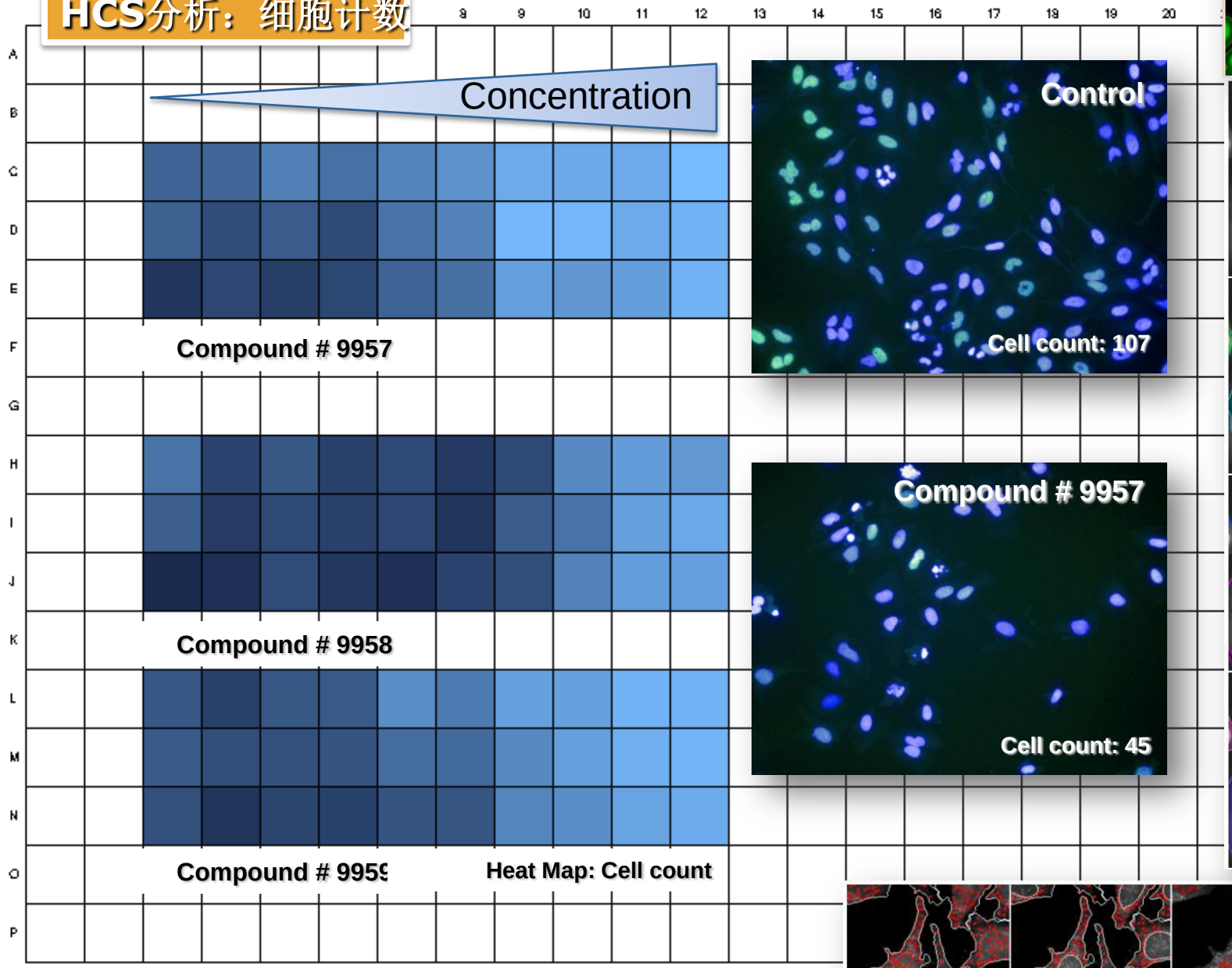
C Add Your Text

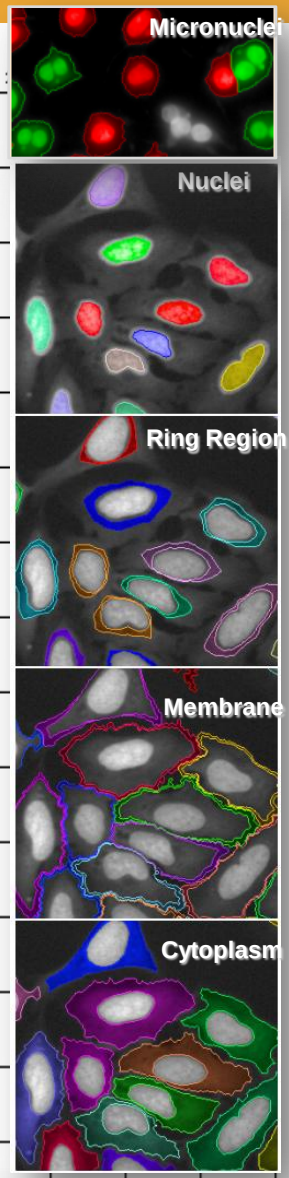
D Add Your Text

Add Your Text here

应用	预制方案
细胞周期	
G2 期向 M 期转变（Cyclin B1）	细胞质标记物定量（获取包括细胞核在内整个细胞的荧光）
细胞有丝分裂指数（组蛋白 H3）	细胞有丝分裂指数
S-期 (BrdU, EdU)	定量分析细胞核标志物
DNA 含量	细胞核分类- DNA 含量
组蛋白 H3、EdU、DNA 含量和细胞核形态的同步反应	细胞周期分类
细胞增殖	细胞计数或核计数
细胞毒性	
细胞计数	细胞计数或核计数
细胞活力：活/死细胞计数	活/死细胞计数
细胞核膨胀和皱缩	细胞核分析- 细胞核皱缩
线粒体膜损伤的趋势	定量分析细胞质标志物
线粒体数量	定量分析细胞质标志物
钙离子平衡	定量分析细胞质标志物
质膜的完整性	活/死细胞计数
微核分析	定量分析细胞核标记物
磷脂化	定量分析细胞质标志物
脂肪肝	定量分析细胞质标志物
过氧化物酶的定量	Spot 分析或定量分析细胞质标志物
细胞凋亡	
细胞核裂解	细胞核裂解
Caspase-3 活化	定量分析细胞核标志物
线粒体数量	定量分析细胞质标记物
线粒体膜损伤的趋势	定量分析细胞质标记物
蛋白表达	
细胞核内蛋白表达	定量分析细胞核标志物
细胞质内蛋白表达	定量分析细胞质标记物
质膜上蛋白表达	定量分析质膜标记物
受体激活	
GPCR 激活：吞噬作用	受体内化
GPCR 激活：抑制蛋白招募 (如 Transfluor®)	Spot 分析
标记配体的内化	受体内化

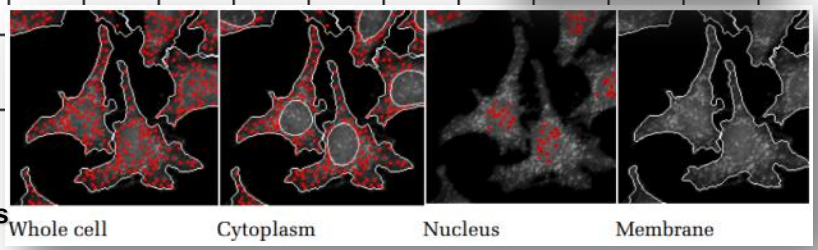
HCS分析：细胞计数





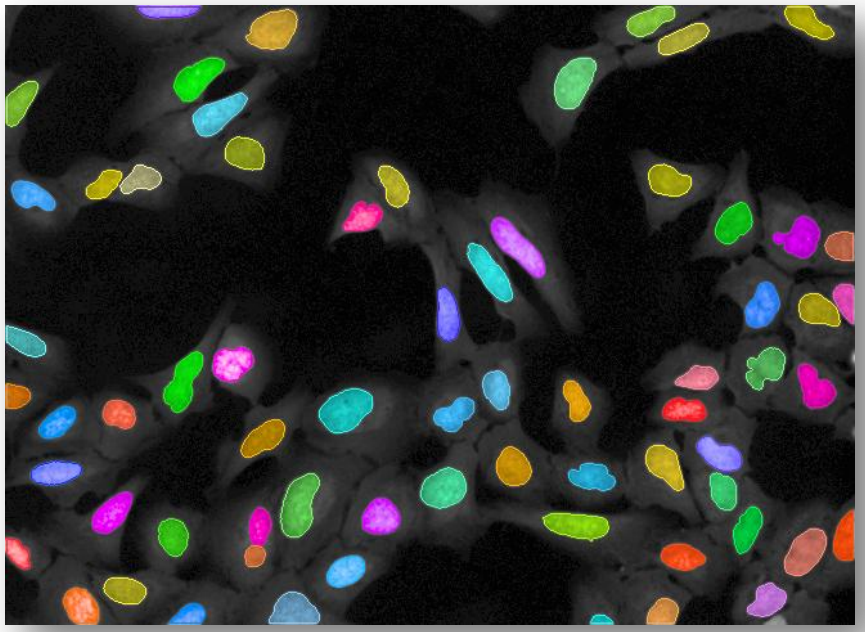
Hoechest 33342 / DAPI / DRAQ5
BOBO3
Calcein-AM/PI

Find Points



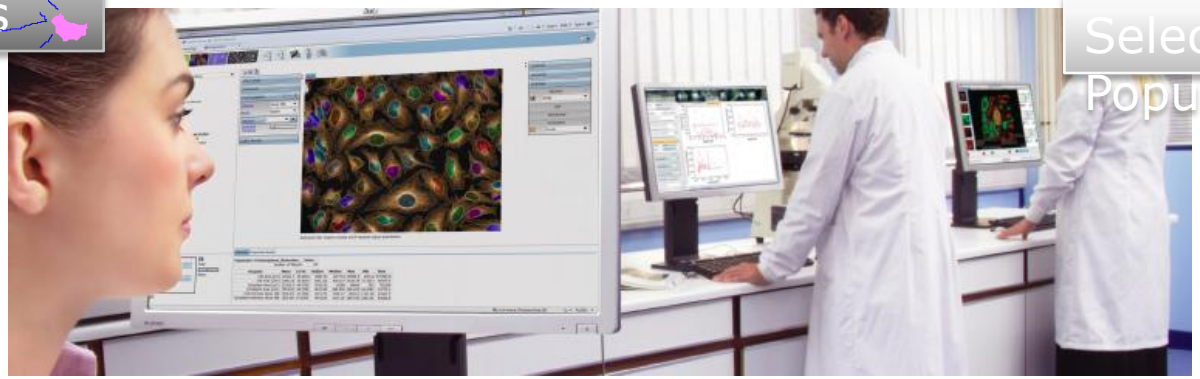
Building blocks for image analysis

- Find Nuclei
- Find Cells
- Find Cytoplasm
- Find Spots
- Find Micronuclei
- Find Texture Regions
- Find Image Regions
- Find Neurites



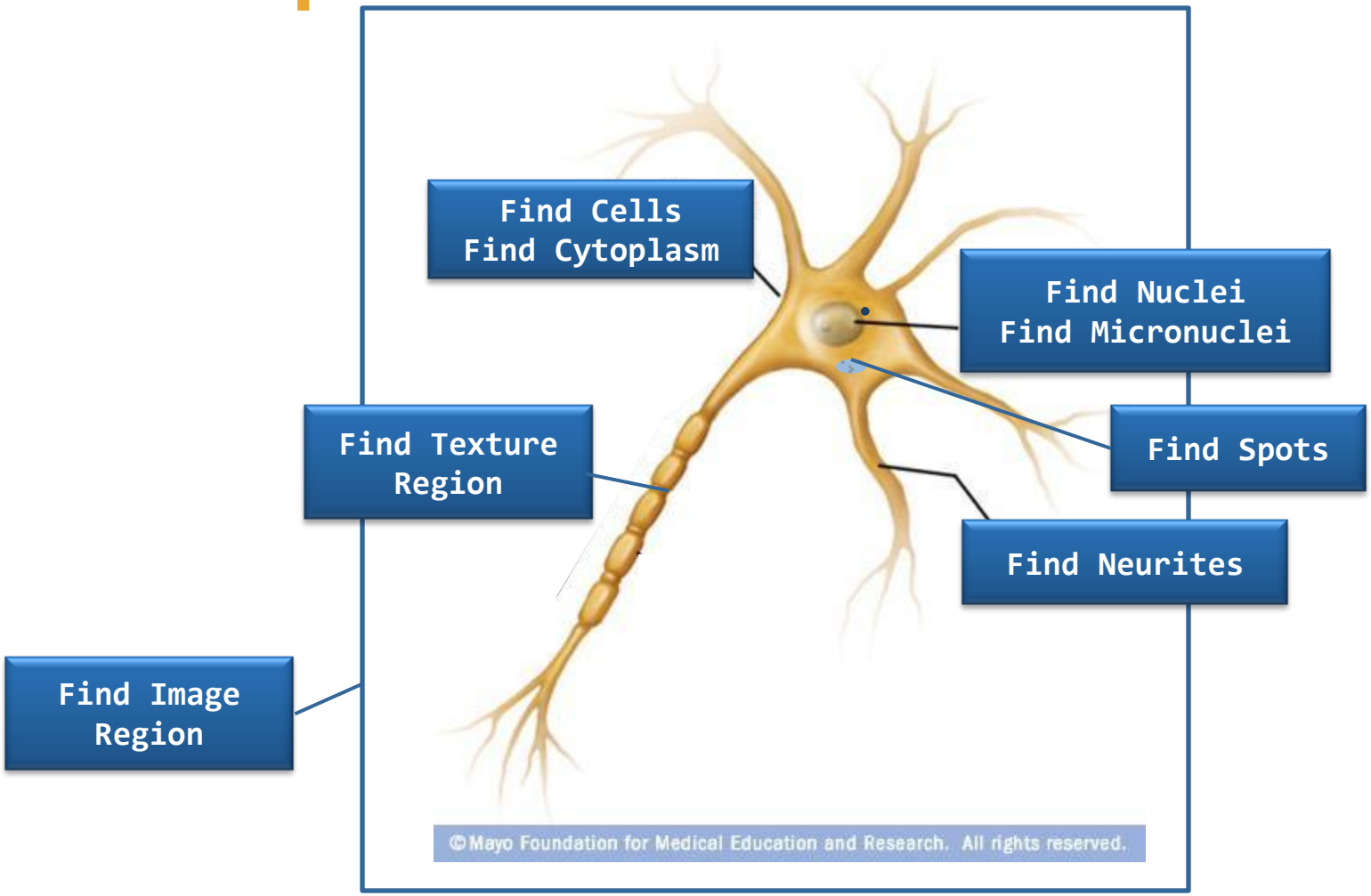
- Calc. Intensity
- New Calc. Morphology
- Calc. Texture
- Calc. Properties

- Select Cell Region
- Select Region
- Select Population



应用模块化组件进行图像分析

Example - Nervous Cell

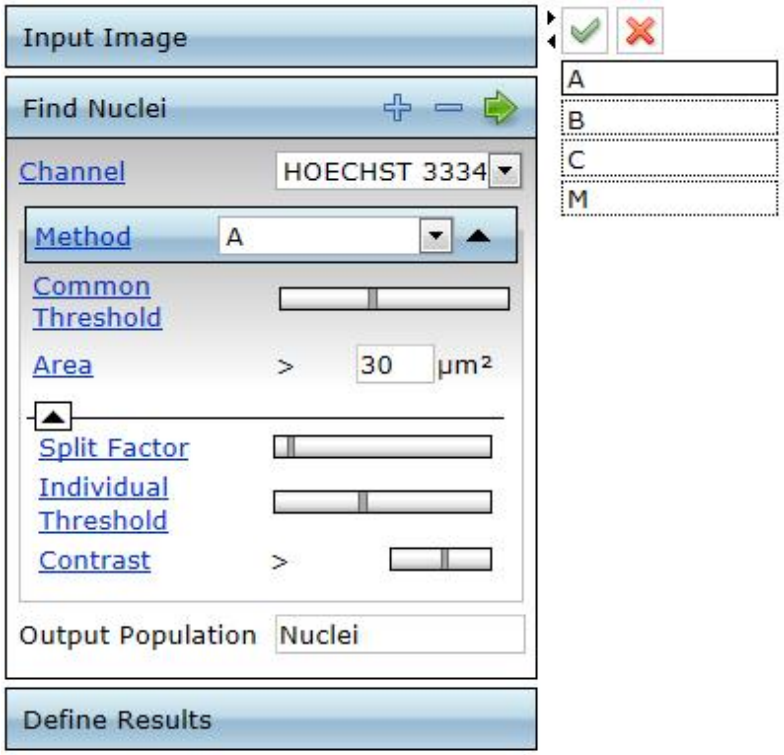


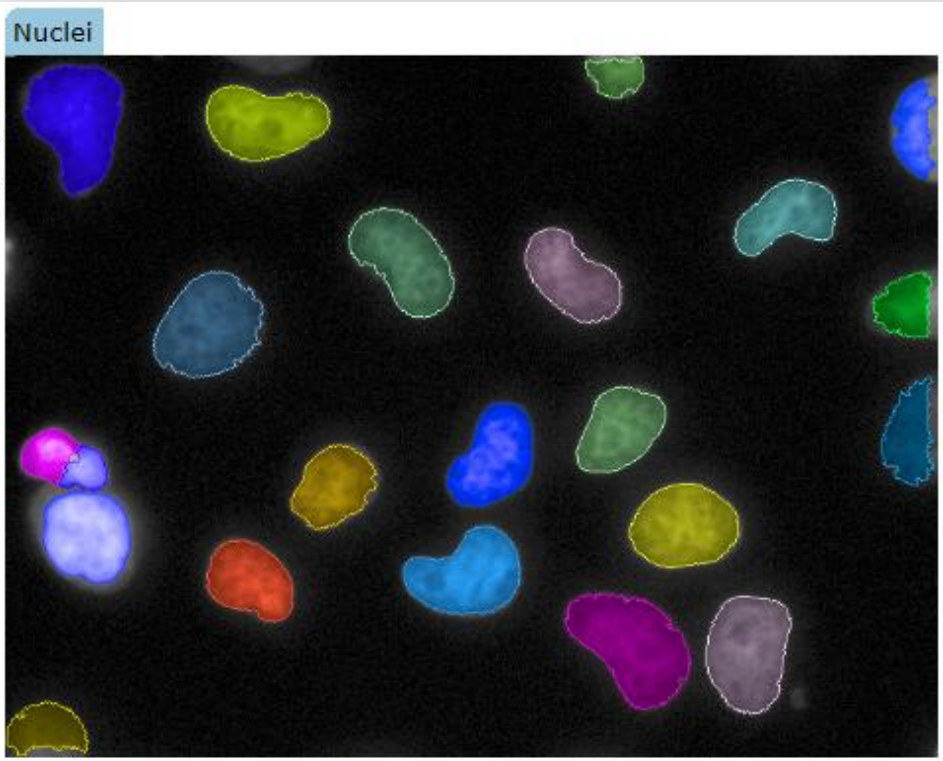
Building Block: Find Cells / Find Nuclei – Methods

Four different **methods** can be applied for the Cell / Nuclei detection:

A,B,C and M

	General robustness	Low background	Intensity variations	Stuck Cells / Nuclei	Calculation Speed
A				+	+
B	++				+
C		+	+	+	+
M	++		+	++	

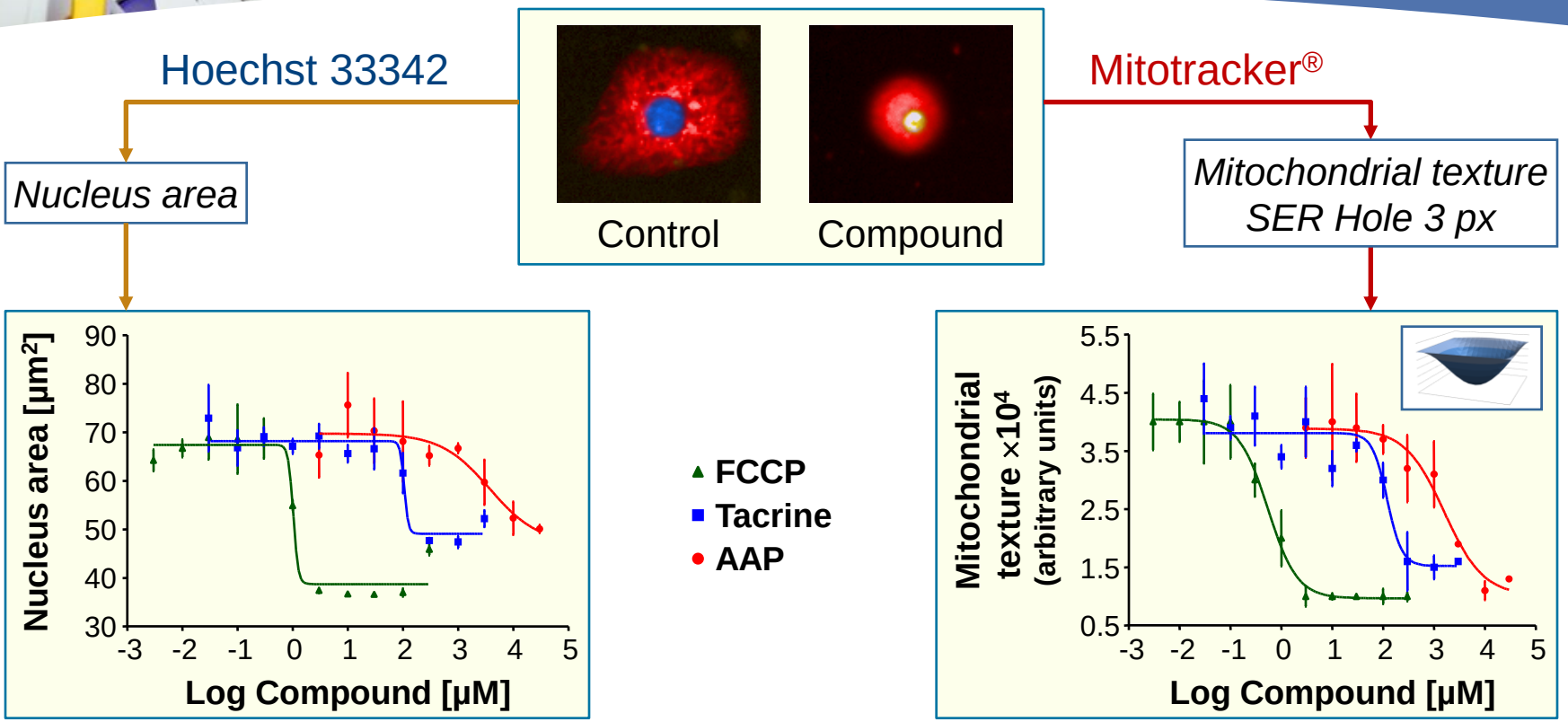




Tuning of parameter Method. Compare the detection results by the different methods and select the most appropriate one.

细胞、细胞核分析组件：分析方法

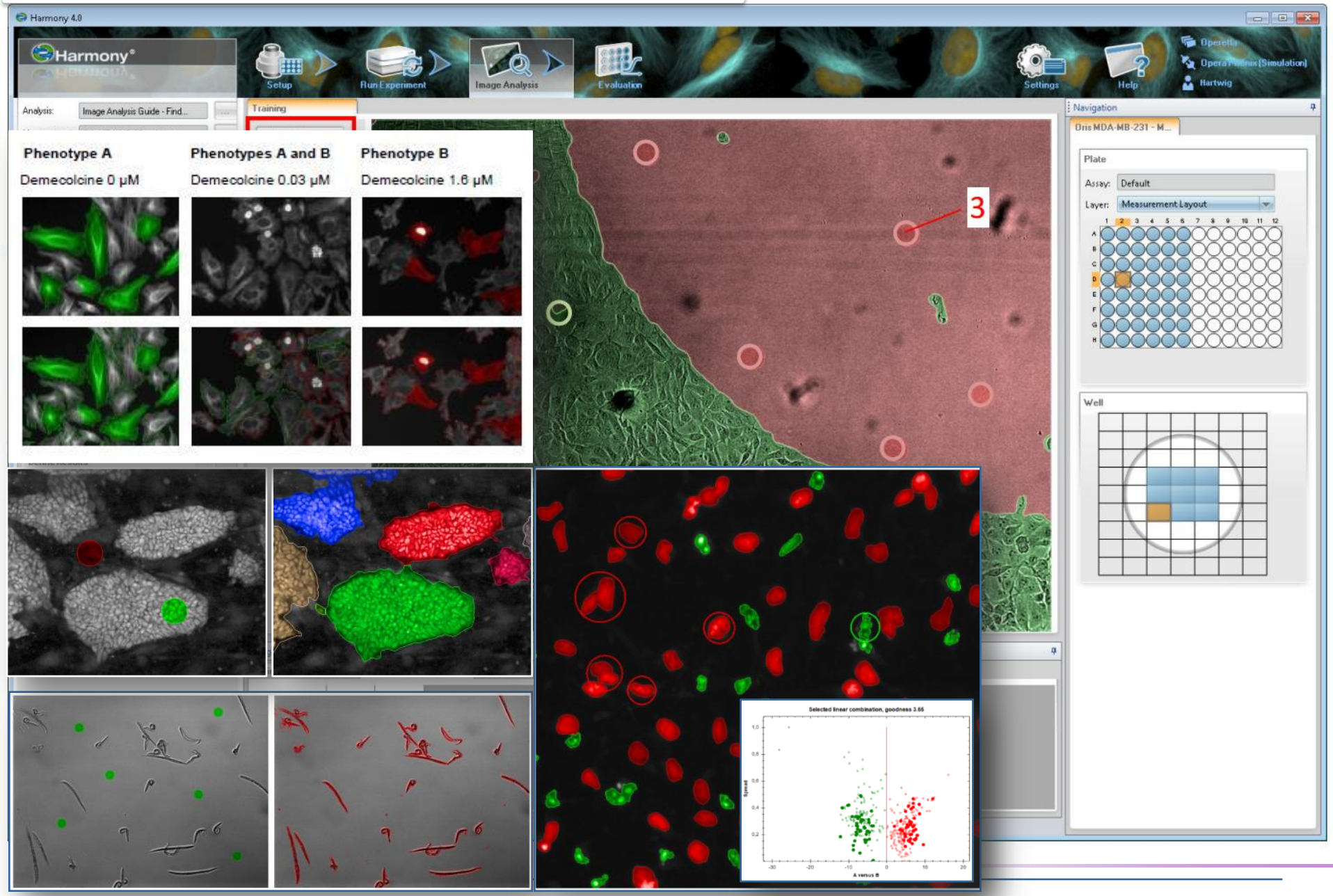
Multiparametric analysis of cytotoxicity



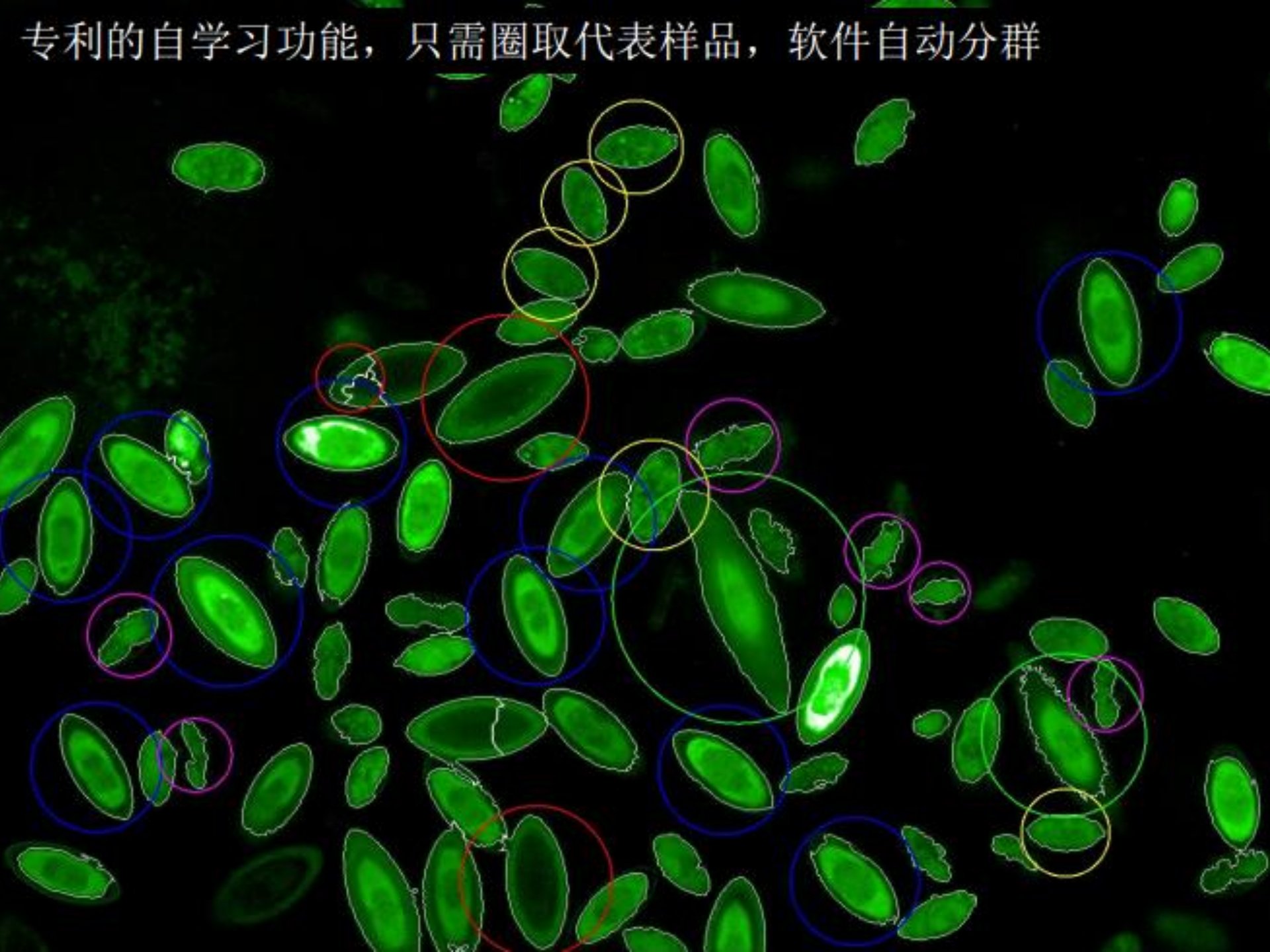
- **Both readouts deliver similar results, but examine different aspects of cytotoxicity**
- **Further possible readouts: nucleus intensity, cell size, Mitotracker® intensity, BOBO™-3 intensity, additional mitochondrial texture parameters**

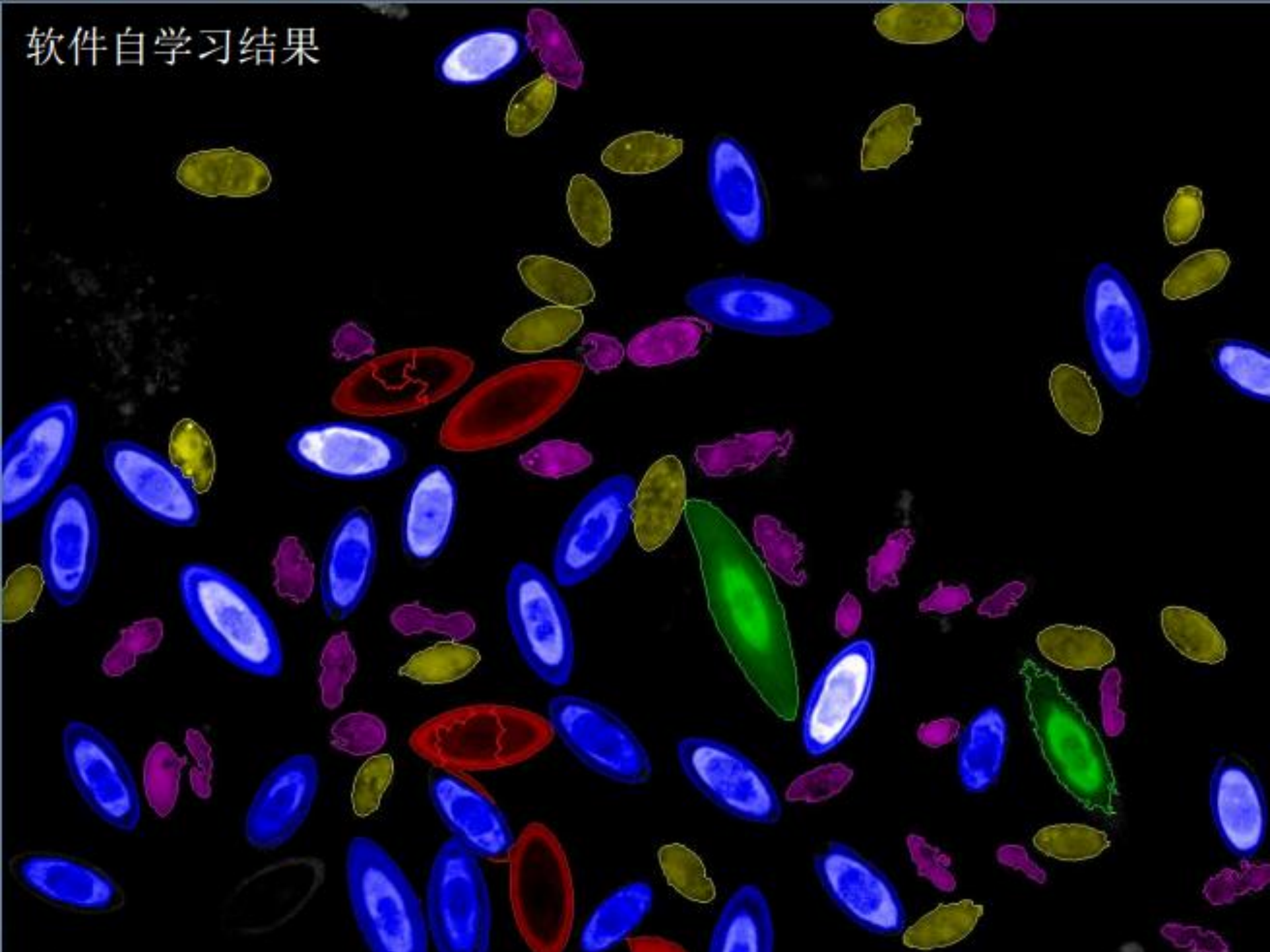
Different readouts provide robustness in toxicity studies

软件自学习，轻松分组样品



专利的自学习功能，只需圈取代表样品，软件自动分群




软件自学习结果

细胞凋亡（Apoptosis RMS Apoptosis-1）

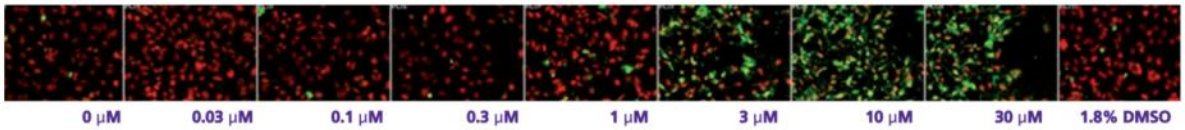
Nuclear fragmentation

Caspase-3 activation

Nuclear DRAQ5

Caspase-3 Alexa Fluor 488

20X

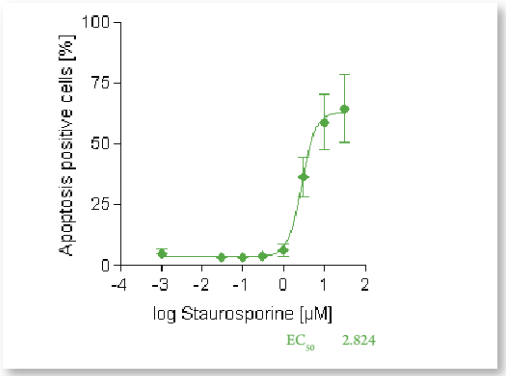


Layout

6 x 10^3 cells / well - 4 h compound treatment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G			0 µM	0.03 µM	0.1 µM	0.3 µM	1 µM	3 µM	10 µM	30 µM	DMSO													
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

十字孢碱



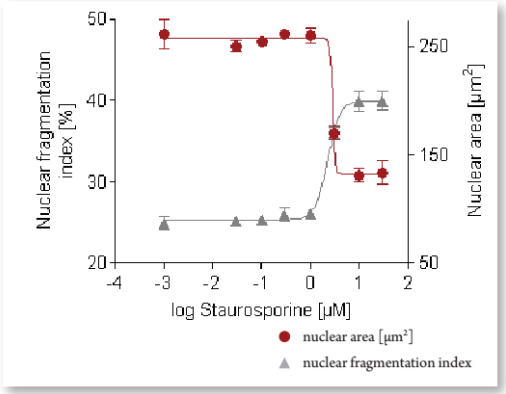


Figure 3. The graphs display the staurosporine-generated dose-response curves after 4 h.
Top | In order to quantify cells according their apoptotic state a threshold with respect to caspase-3-related fluorescence intensity was introduced to the data (the threshold was variable and was adapted to individual experimental intensity results, here 980). In this way the subpopulation of apoptotic cells became classified, and the resulting numerical readout was called "percentage of apoptosis positive cells". Taking these percentages as a basis, an EC_{50} of 2.8 µM for staurosporine was calculated using the Prism-software to fit the curve.
Bottom | The graph shows the fluctuation of the nuclear intensity on one axis (fragmentation index) and the nuclear area on an other axis. It clearly shows the rapid decrease in nuclear area and the increase in fragmentation, upon treatment with 3 µM staurosporine.

参数指标：
- 细胞核数目
- 细胞核碎片：DRAQ5信号强度的波动（CV）
- 细胞核面积：识别DRAQ5标示的细胞核，计算面积
- Caspase-3强度：核区Alex Fluor 488信号强度

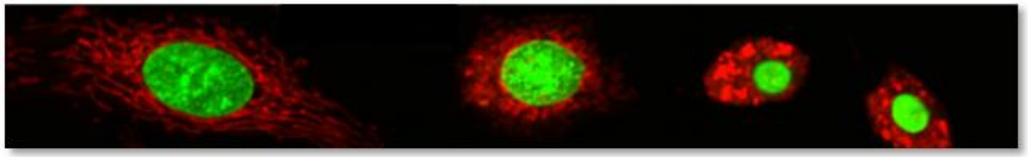
统计：100细胞/视野 x 10个视野 x （8个浓度梯度+1个对照）x 3组重复
~27000细胞，EC50=2.824，Z=0.96
细胞类型：Hela cell

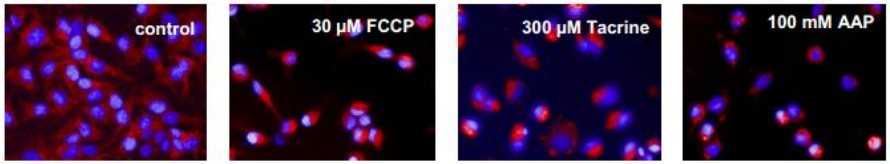
Digram

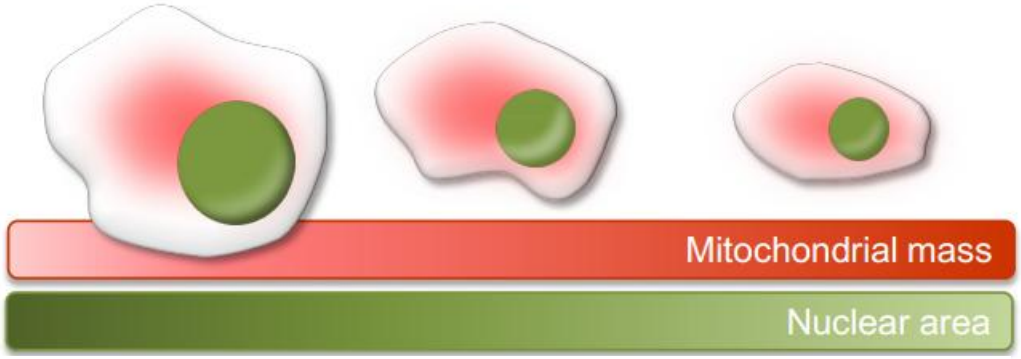
Dye	Ex./ Em. Max. [nm]	Channel name in Harmony	Readouts
DRAQ5™ (nucleus/ cytoplasm, specific stain for nucleic acids)	647 / 670	DRAQ5™	Loss of cells Nuclear shrinking Nuclear fragmentation
Cleaved-caspase-3 antibody (AlexaFluor® 488 conjugate)	494 / 520	AlexaFluor® 488	Apoptosis activation

Fluorescence characteristics of each dye used, and the corresponding readouts.

细胞毒性（RMS Cytotoxicity-1）







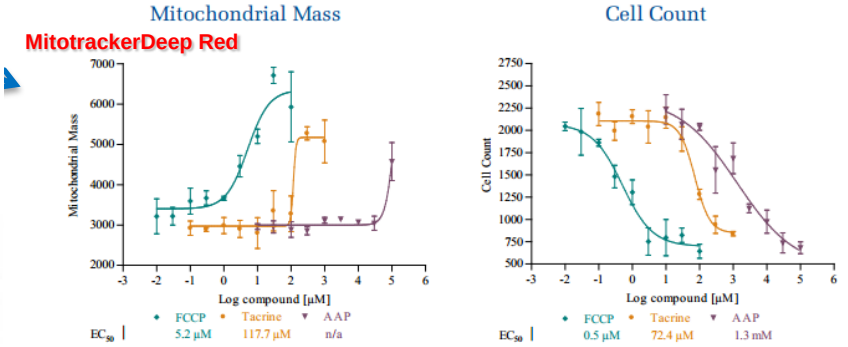


Figure 3–10: FCCP, Tacrine and Acetaminophen dose-response curves, determined from the mitochondrial mass (left) and live cell count (right). N = 4 wells.

Layout

4 x 10^3 cells / well - 24 h cpd treatment - 45` staining

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B			0	10 nM	30 nM	100 nM	300 nM	1 µM	3 µM	10 µM	30 µM	100 µM			0.2 % DMSO									
C																								
D																								
E																								
F																								
G			0	100 nM	300 nM	1 µM	3 µM	10 µM	30 µM	100 µM	300 µM	1 mM			10 % ddH_2O									
H																								
I																								
J																								
K																								
L			0	10 µM	30 µM	100 µM	300 µM	1 mM	3 mM	10 mM	30 mM	100 mM			2% DMSO									
M																								
N																								
O																								
P																								

FCCP Tacrine AAP 1µM Hoechst / 0.75µM BOBO-3 / 0.3µM Mitotracker

HepG2 肝癌细胞
(Human hepatocellular carcinoma) cells

参数指标：
- **细胞数目**
- **细胞核面积：Hoechst**
- **线粒体团聚：MitoTracker信号强度**
- **胞膜渗透性：BOBO-3阳性细胞核比例**

统计：100细胞/视野 x 5个视野 x （4个浓度梯度+1个对照）x 4组重复 x 3种化合物，~30000细胞；HepG2 cell

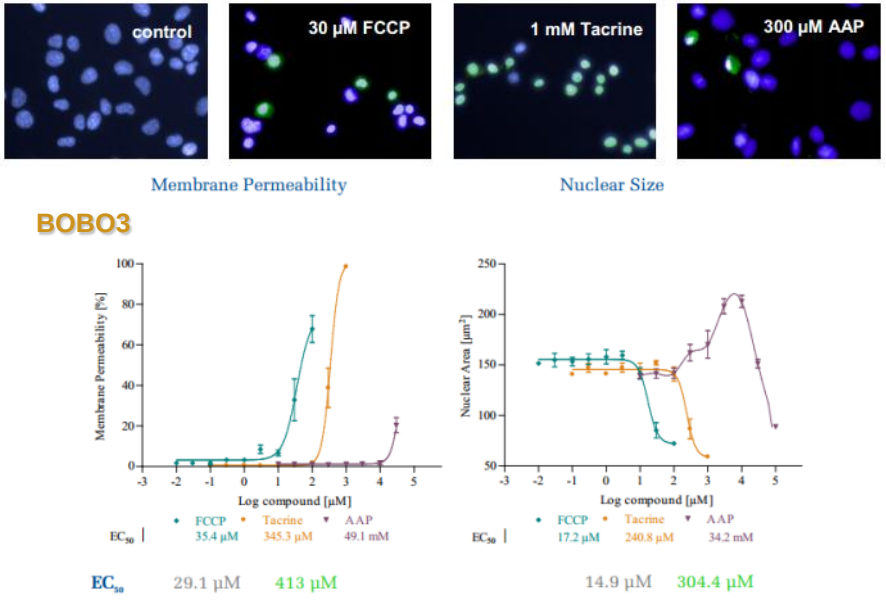


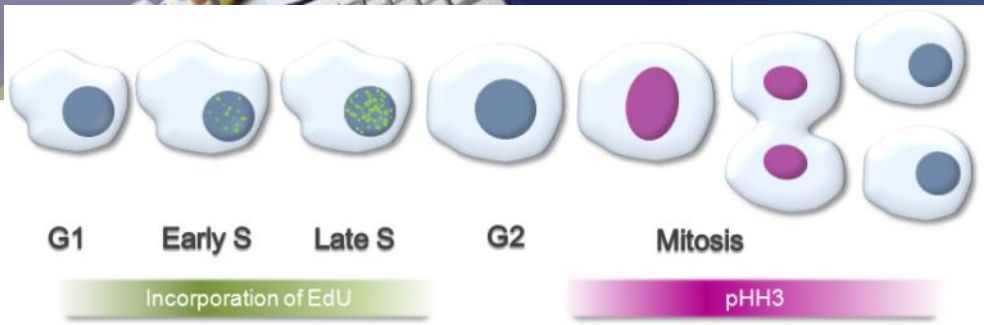
Figure 3–12: FCCP, Tacrine and Acetaminophen-generated dose-response curves deduced from membrane permeability (left) and nuclear size (right). N = 3 wells.

- **Hoechst 33342** 是一个广受欢迎的细胞膜可透过的染料，可用于活细胞核的染色。该染料我们用于细胞数计算，细胞核强度计算，细胞核皱缩程度计算。
- **BOBOTM-3**是一个细胞膜不可透过的细胞染料。它特异性的与DNA双链结合。该染料用于评估细胞膜的通透性。该染料只能对细胞膜被损失的细胞进行着色。
- **MitoTracker® Deep Red** 用于检测线粒体质量，这个活细胞可透过的细胞器染料会富集在代谢活跃的线粒体区域。

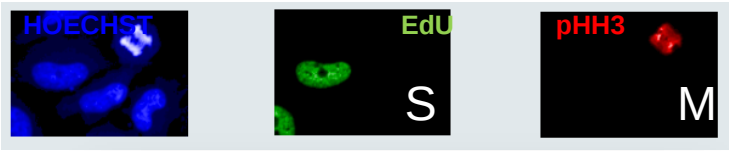
Dye	Ex./ Em. Max. [nm]	Channel name in Harmony	Readouts
Hoechst 33342 (nucleus / DNA)	350 / 461	HOECHST 33342	Loss of cells Nuclear shrinkage
BOBO™-3 iodide (nucleus / DNA)	570 / 602	BOBO-3™	Cell membrane disruption
MitoTracker® Deep Red FM (mitochondria)	635 / 690	MitoTracker® Deep Red	Changes in mitochondrial mass

Fluorescence characteristics of each dye used, and the corresponding readouts.

细胞周期（RMS Cell Cycle-1）



Mitotic index	Anti phosphohistone H3 antibody, labeled with Alexa Fluor® 647
S-phase	Anti EdU antibody, labeled with Alexa Fluor® 488
DNA content	HOECHST 33342, combined with CellMask Blue



DNA Content

Layout

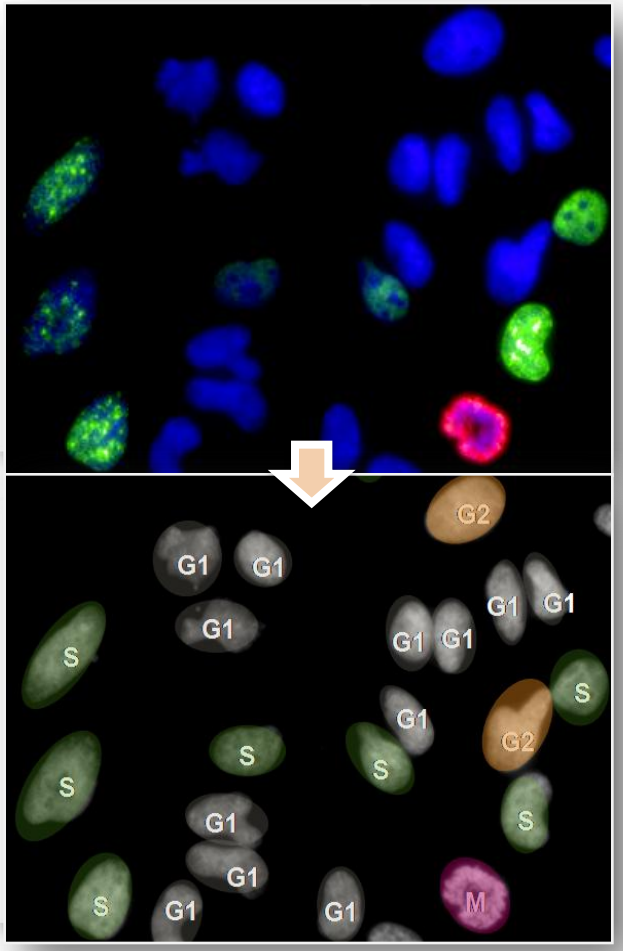
2.5 x 10^3, 5 x 10^3 cells / well - 6 / 18 /24 / 30 h cpd treatment on 4 separate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B		0	1	5	10	50	70	100	500	1000	5000			0	1	5	10	30	50	100	500	1000	5000	
C																								
D																								
E																								
F																								
G		control												control (2 % DMSO)										
H																								
I																								
J																								
K																								
L																								
M																								

参数指标：
- 细胞数目、面积：Hoechst 33342染色细胞核
- 高DNA含量的细胞核数目和比例：信号强度的Sum和mean
- S期细胞比例：EdU阳性细胞数/细胞总数
- M期细胞比例：pHH3阳性细胞数/细胞总数

统计：100细胞/视野 x 10个视野 x （10个浓度梯度+1个对照）x 3组重复 x 2种处理，~66000细胞；Hela Cell

Thymidine（胸腺嘧啶）EdU EC50=37.57，Nocodazole（诺考达唑）pHH3 EC50=0.11

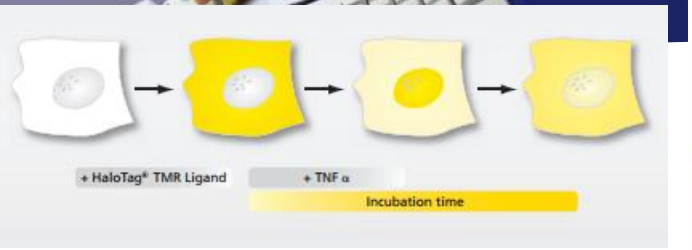


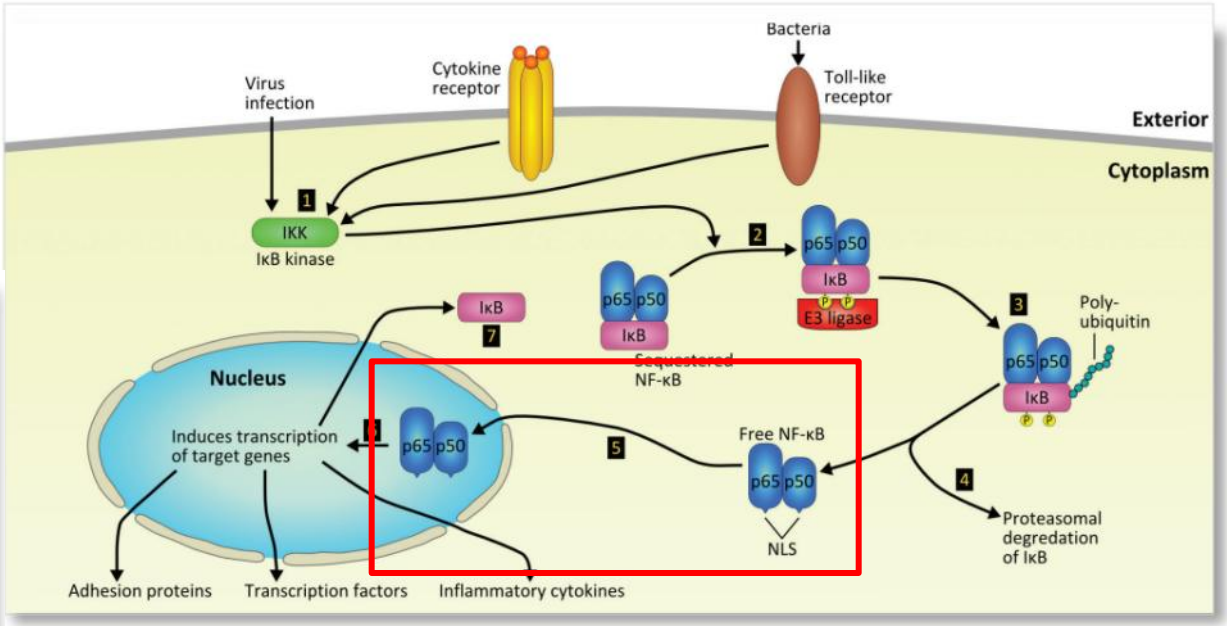
EdU检测S期细胞，借助将Uridine引入新合成的DNA链，使用时在细胞固定之前加入EdU孵育30min

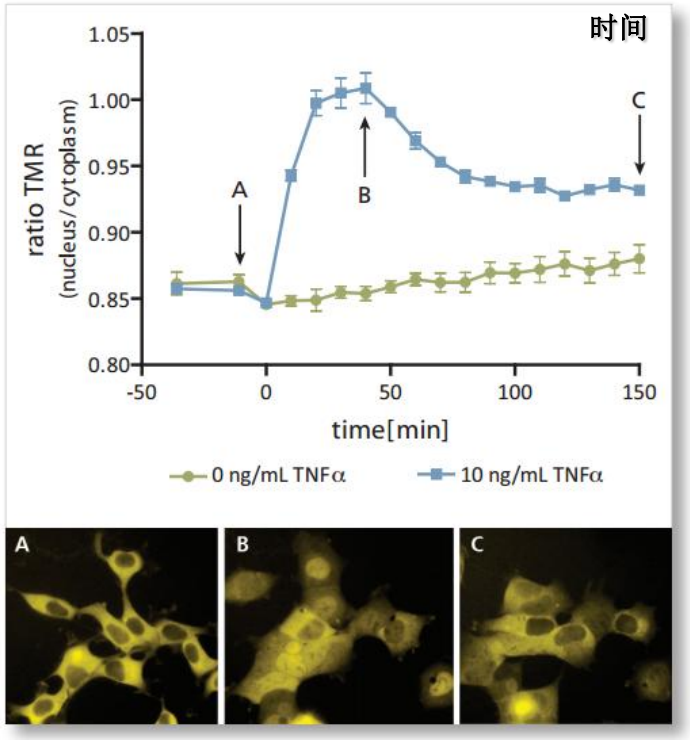
pHH3检测的是细胞内源的磷酸化组蛋白，直接用抗体染色即可；磷酸化组蛋白H3（PHH3）抗体，能够特异地检测到10位或28位的丝氨酸被磷酸化的组蛋白。这个磷酸化过程在细胞间期几乎不发生，而仅发生在有丝分裂期，而且细胞凋亡时组蛋白不发生磷酸化。因此，PHH3可作为一个有效的在免疫组织化学中应用的细胞有丝分裂标记抗体。

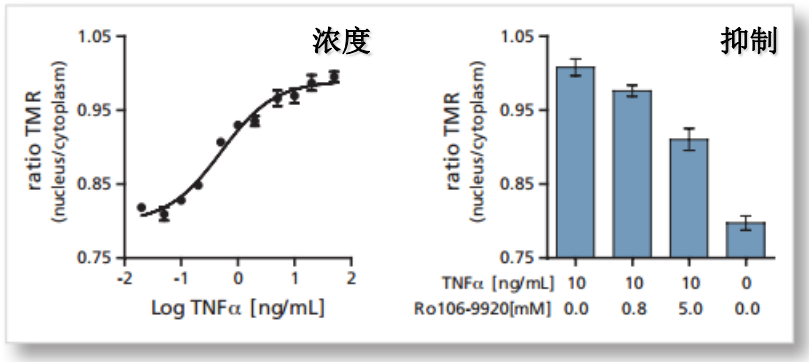
Dye	Ex./ Em. Max. [nm]	Channel name in Harmony	Readouts
Hoechst 33342 (nucleus / DNA)	350 / 461	HOECHST 33342	Loss of cells Nuclear shrinkage
Alexa Fluor® 488 (EdU)	490 / 519	AlexaFlour® 488	S-phase cells
Alexa Fluor® 647 (pHH3)	653 / 669	AlexaFlour® 633	M-phase cells

质核转运（NF-kB Cytosol to Nucleus Translocation）









Promega HaloTag

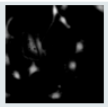
HEK293 cell line

Figure 3. NF-κB translocation at the time point of maximum response (30 min post stimulation) (A) TNFα dose dependent nuclear translocation of p65-HT in HEK293 cells. The ratio between nuclear and cytoplasmic TMR fluorescence increases with increasing TNFα concentrations. N=3 wells, Z'=0.76. (B) Inhibition of NF-κB signaling by Ro106-9920. The fraction of nuclear p65-HT decreases dose-dependently with increasing Ro106-9920 concentrations. Concentrations of Ro106-9920 > 5 µM were cytotoxic in these experiments. N=3 wells.

细胞追踪（RMS2.29 Tracking ）

Image Analysis Sequence: Cell Tracking – Migration Analysis

Input Image
Channel 1:
Digital Phase Contrast (DPC)


Untreated

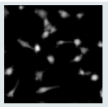
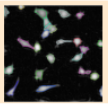

Cytochalasin D treated

Image Segmentation

Find Cells
Finds cellular outlines (Method P for DPC images)



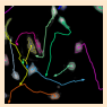
Track Objects

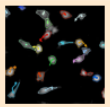
Use as time window
Selects a subset of time points as time window

Images from these time points will be loaded for tracking

Track Objects
Uses the overlap of cells between adjacent time points for tracking

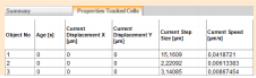
Cell masks overlayed with object tracks


Cell migration


Cell migration inhibited

Quantify Properties

Calculate Kinetic Properties
Calculates Current Speed and Current Step Size

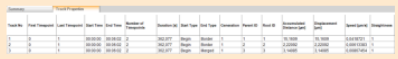


Calculate Properties
Calculates the Current Square Displacement

Uses a formula to calculate a derived property on a per object basis:
Current Square Displacement = (Current Displacement X [µm])² + (Current Displacement Y [µm])²

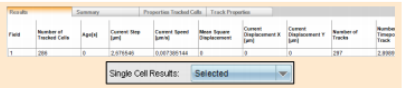
Quantify Track Properties

Calculate Track Properties
Calculate accumulated distance, displacement and speed

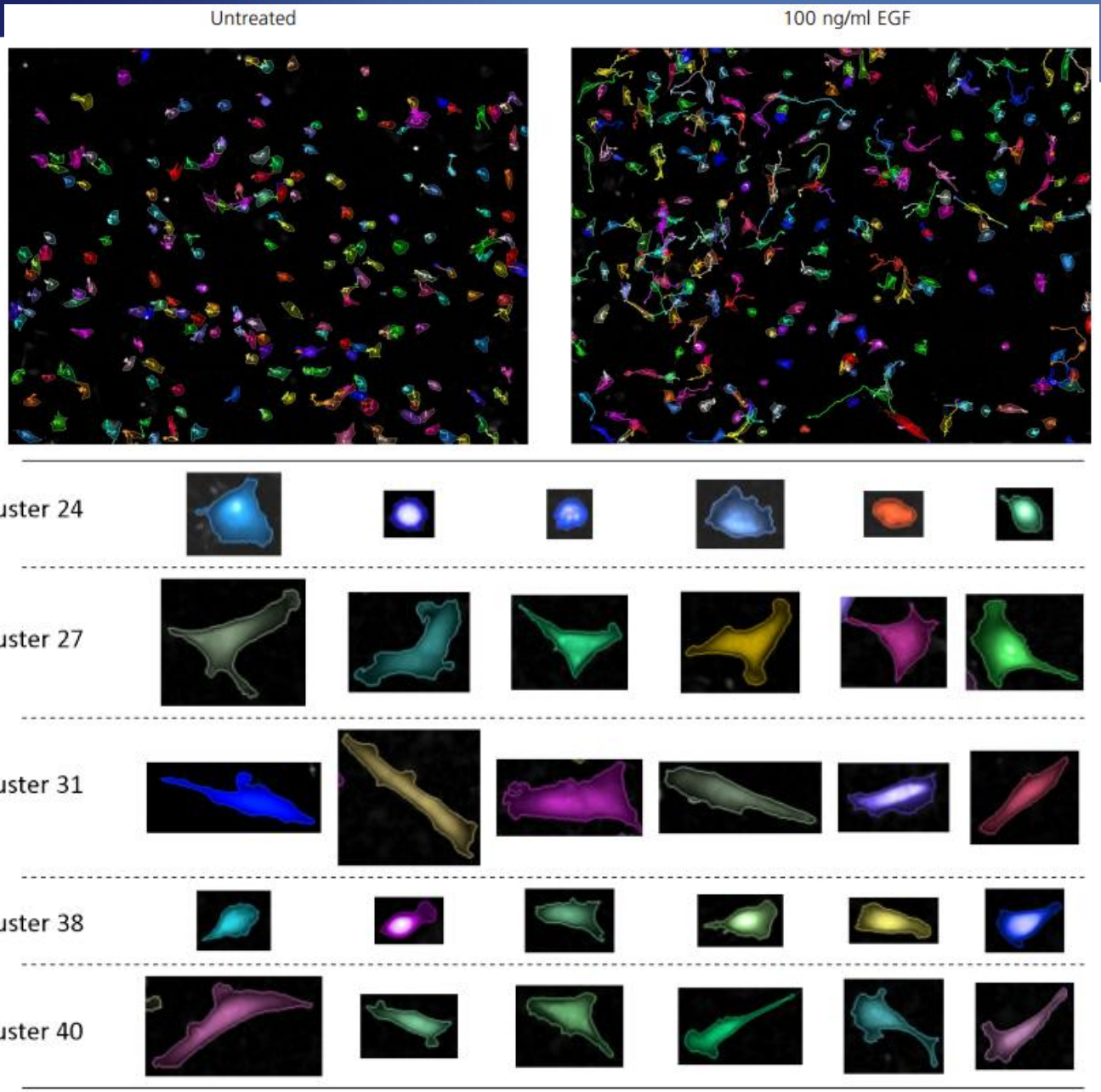


Calculate Readout Values

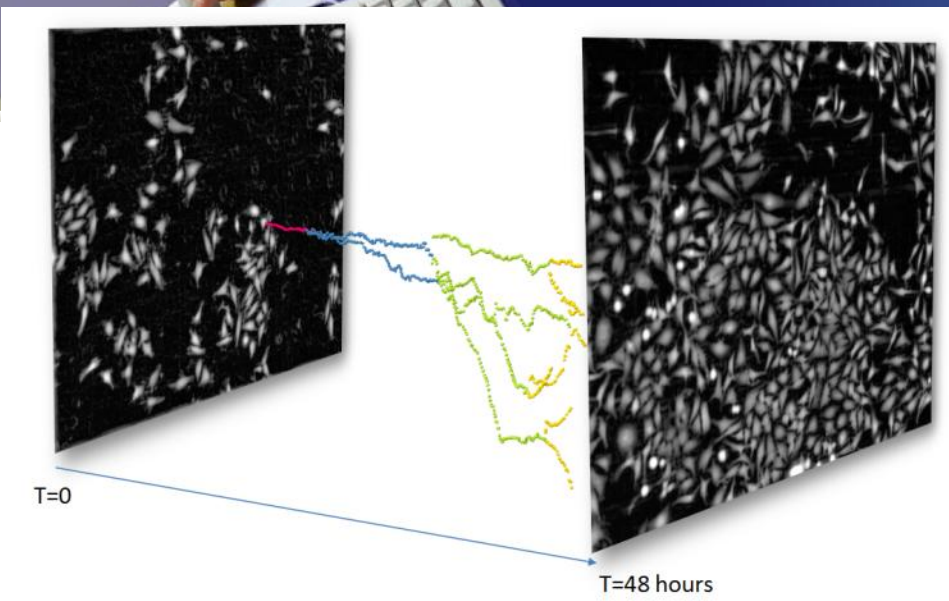
Define Results
Select meaningful names for your readouts and save single cell data



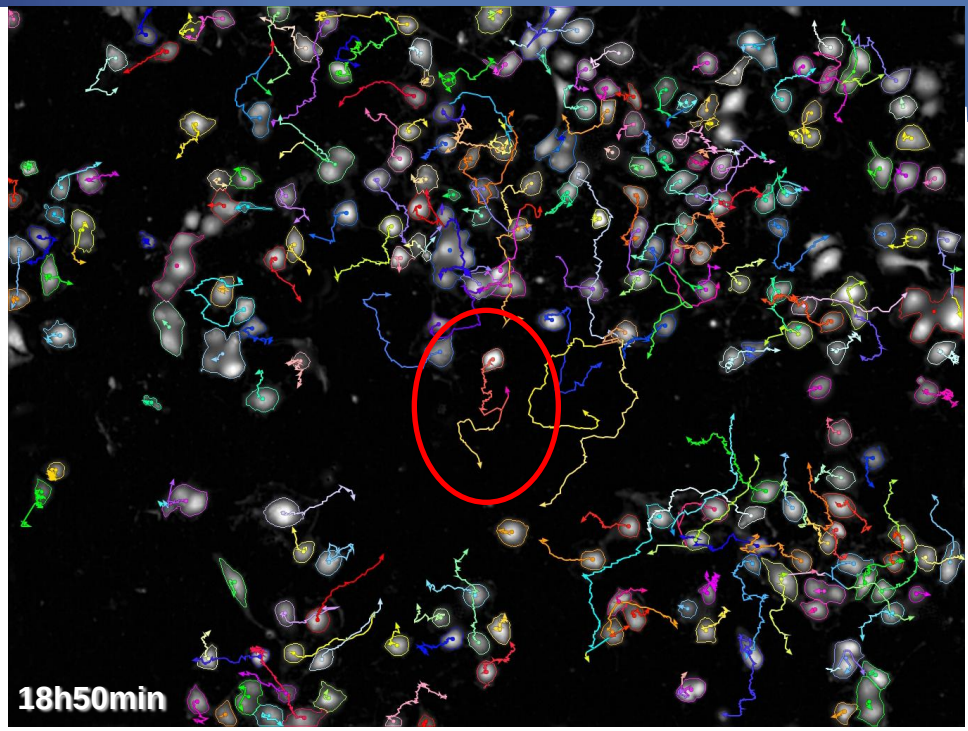
Readout Values calculated per time point	
Number of Tracked Cells	Number of cells per well
Age [s]	Passed time since the cell was first observed in seconds; averaged over all cells per well
Current Speed [µm/s]	Distance (in µm) a cell traveled per second; averaged over all cells per well
Current Step [µm/s]	Distance (in µm) a cell traveled between two time points; averaged over all cells per well
Mean Square Displacement	Squared norm of a vector from the point of the first to the point of the current observation; averaged over all cells per well
Current Displacement X [µm]	X component of a vector from the point of the first to the point of the current observation; averaged over all cells per well
Current Displacement Y [µm]	Y component of a vector from the point of the first to the point of the current observation; averaged over all cells per well
Readout Values calculated per track	
Number of Tracks	Number of detected cell tracks per well
Number of Timepoints per Track	Number of time points that a cell track was observed; averaged over all tracks per well
Accumulated Distance [µm] per Track	Total length of all segments of an object track in µm; averaged over all tracks per well
Displacement [µm] per Track	Distance from the first to last observation of a cell track in µm; averaged over all tracks per well
Average Speed [µm/s] per Track	Accumulated distance in µm divided by the track duration in seconds; calculated per track and then averaged over all tracks per well



细胞追踪（RMS2.29 Tracking，世代分析Family tree ）



Tracking of HeLa cells on digital phase image. The track of one cell starting at T=0 (red line) is shown over 48 hours. Several cell devisions occur, each color represents a new generation of daughter cells.



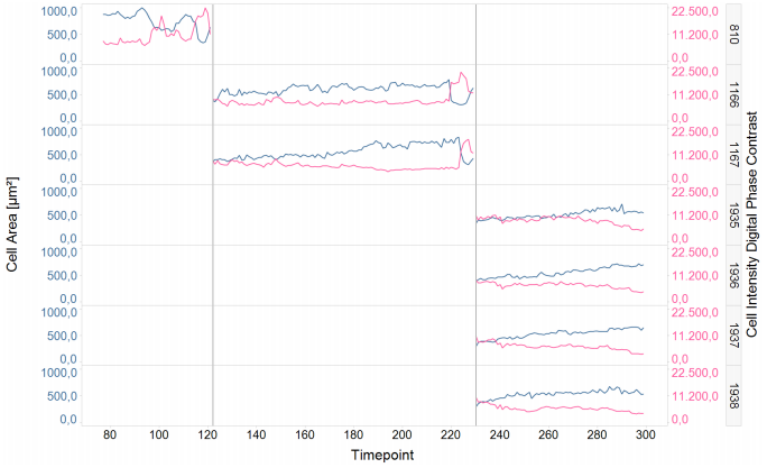
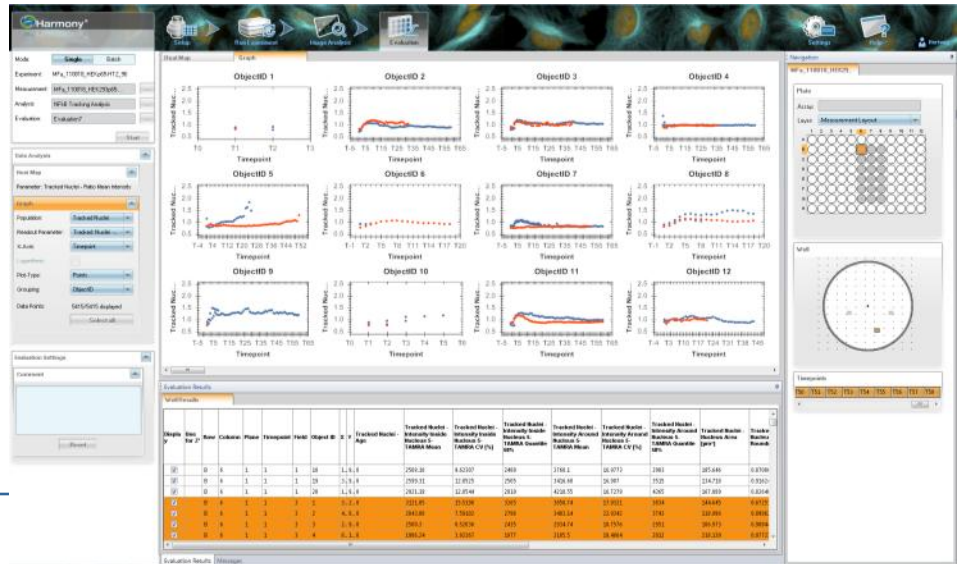


Figure 1–125: Analysis of three cell generations using TIBCO Spotfire® software. The vertical grey lines indicate cell division. Plotted are the cell area (blue) and the cell intensity in a digital phase contrast image (red). Note how the cell area decreases and the phase contrast signal increases prior to mitosis. The two daughter cells of the 2nd generation divide synchronously. Object numbers are depicted in the grey rectangles to the right. The plot combines information from the single object results table (area and intensity versus time) with information from the track results table (object number, generation, root ID).

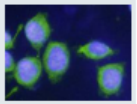


神经细胞分析（RMS2.21 Neurite Outgrowth Analysis）

Image Analysis Sequence: Neurite Outgrowth Analysis

Input Image
Channel 1:
Nuclear stained image
Channel 2:
Neurite stained image

Untreated control



NGF- treated cells

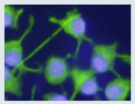
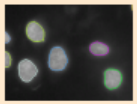


Image Segmentation

Find Nuclei
Finds nuclear outlines using the nuclear stain image

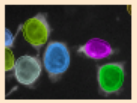
Nuclear region overlaid on the nuclear stain image (untreated cells)



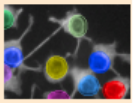
Resulting detection for treated cells

Select Region
Estimate the cell body position by enlarging the nuclear mask

Estimated cell body position

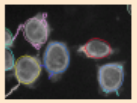


Estimated cell body position (treated cells)

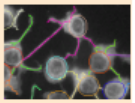


Find Neurites
Detect neurites starting from the estimated cell body positions

Detected neurites



Detected neurites (treated cells)



Quantify Neurite Properties

The **Find Neurites** building block automatically calculates a set of neurite properties.

Object No	Maximum Neurite Length	Number of Extremities	Number of Roots	Number of Segments	Number of Nodes type 1	Number of Nodes type 2	Total Neurite Length
1	20.2441	1	1	1	0	0	20
2	0	0	0	0	0	0	0
3	34.9957	1	1	1	0	0	35

A set of per-cell neurite properties is added to the "All Cells" population

Select Cells

Select Population
To avoid artifacts, remove cells touching the image border as a large part of their neurite tree is cut off

Calculate Readout Values

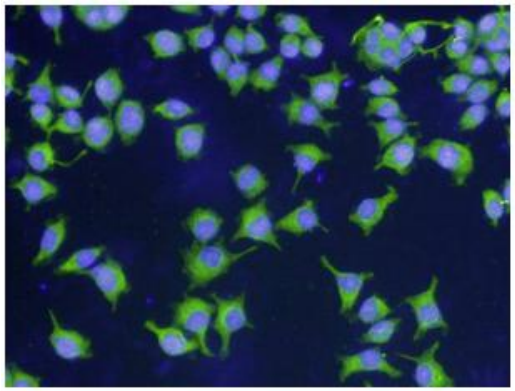
Define Results
Selected average values of all the cells in the well

Results | Summary | Properties Completely ...

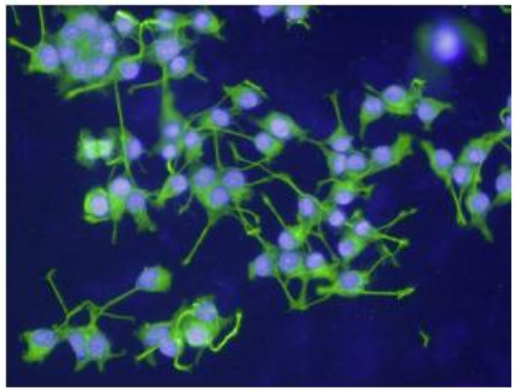
Field	Number of Cells	Mean Total Neurite Length	Mean Length of longest Neurite	Mean Number of Segments	Mean Number of Extremities	Mean Number of Roots	Mean Number of Nodes 1	Mean Number of Nodes 2
1	225	98.84	49.8348	3.49333	2.40444	1.83556	1.08889	0.795556

Readout Values

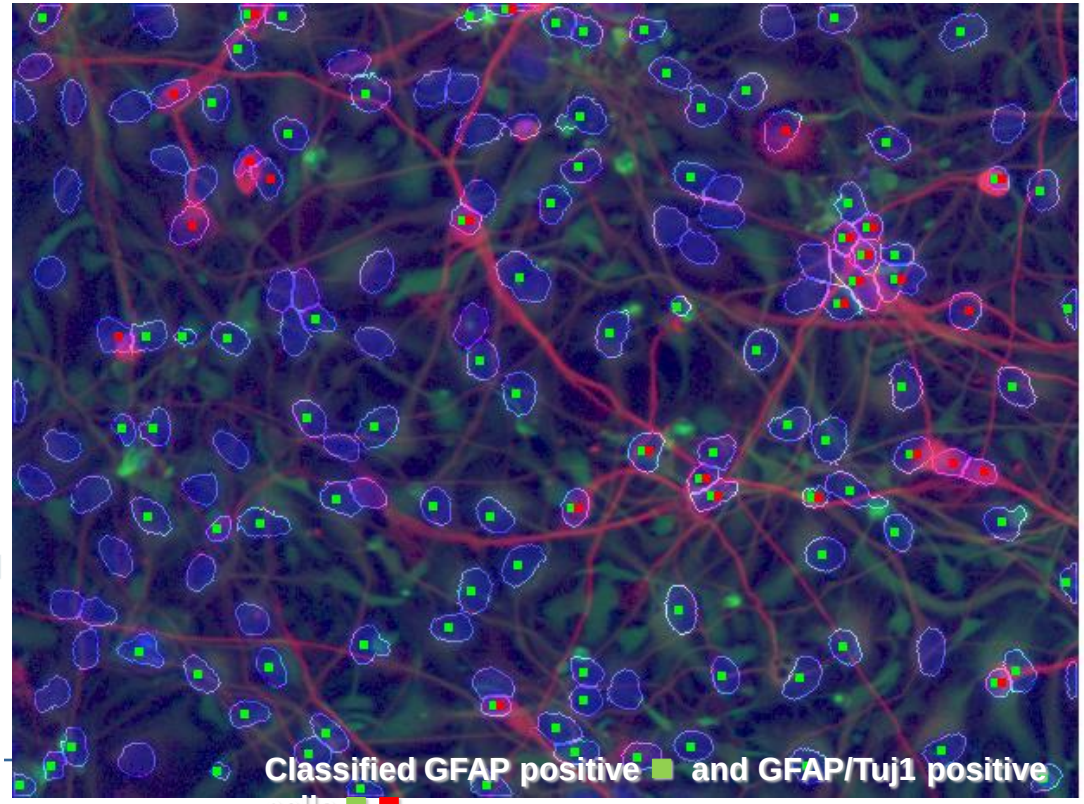
Number of Cells	Number of analyzed cells (Border cells are excluded)
Mean Total Neurite Length	Total length of all neurites of the neurite tree for each cell; averaged over all cells
Mean Length of longest Neurite	Length of the longest neurite for each cell; averaged over all cells
Mean Number of Segments	Number of segments of each neurite tree; averaged over all neurite trees
Mean Number of Extremities	Number of endpoints of each neurite tree; averaged over all neurite trees
Mean Number of Roots	Number of root points of each neurite tree; averaged over all neurite trees
Mean Number of Nodes	Number of branching points (type 1 and type 2) of each neurite tree; averaged over all neurite trees



3 ng/ml NGF



60 ng/ml NGF

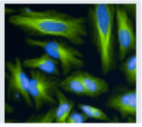


细胞骨架分析（RMS2.23 Cytoskeleton）

Image Analysis Sequence: Phenotype classification

Input Image
Channel 1:
Nuclear stain image
Channel 2:
Tubulin stain image

Untreated control



Demecolcine treated cells

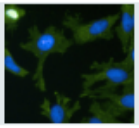
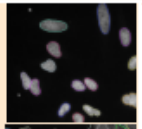


Image Segmentation

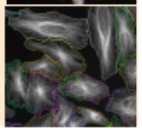
Find Nuclei
Finds nuclear outlines using the nuclear stain image

Find Cytoplasm
Finds cytoplasmic outlines around each of the previously detected nuclei

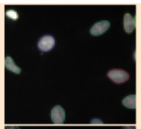
Nuclear region overlaid on the nuclear stain image (untreated cells)

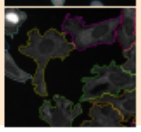


Detected cytoplasmic outlines



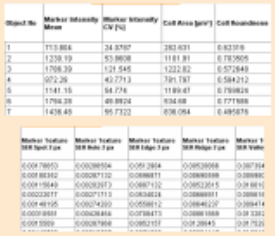
Resulting detection for treated cells

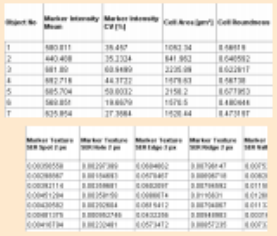




Quantify Properties in Regions

Calculate Morphology Properties
Calculate Intensity Properties
Calculate Texture Properties
Calculate a set of properties for each individual cell including texture features to measure cytoskeletal re-arrangement

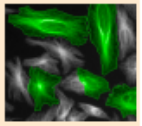




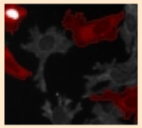
Select Cells

Select Population
Linear classifier
Classification using pre-calculated object properties
Requires a training phase of selecting cells

Training mode:
Select cells for Phenotype A (green)



Training mode:
Select cells for Phenotype B (red)



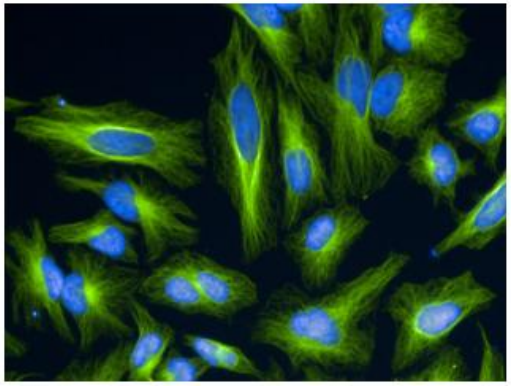
Calculate Readout Values

Define Results
Calculate the percentage of cells of phenotype A and B

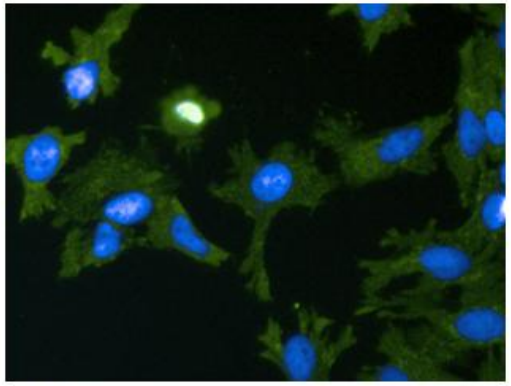
Number of Cells	Percentage of Phenotype A	Percentage of Phenotype B
41	37.2727	62.7273

Readout Values

Number of Cells	Number of analyzed cells in the well
Percentage of Phenotype A	Percentage of "Phenotype A" classified cells in the well
Percentage of Phenotype B	Percentage of "Phenotype B" classified cells in the well



Untreated cells



1 µM Demecolcine

Train ...

Click on cells in the image to select examples for "Class A" (green) and "Cass B" (red)

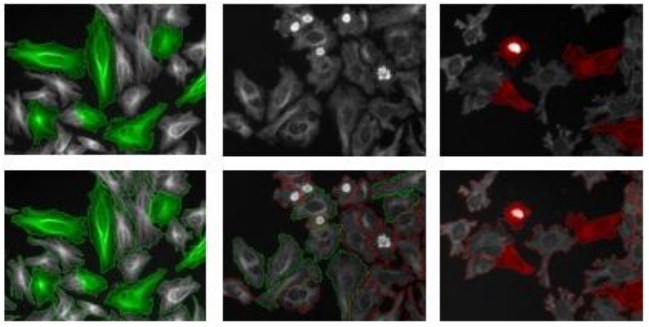
Apply Changes

The classifier is calculated and applied to all cells in the image

Phenotype A
Demecolcine 0 µM

Phenotypes A and B
Demecolcine 0.03 µM

Phenotype B
Demecolcine 1.6 µM



A versus B

The best linear combination of properties for separation of phenotypes A (green) and B (red) is determined and plotted on the x-axis. The y-axis is selected to give the best visualization (spread of populations).

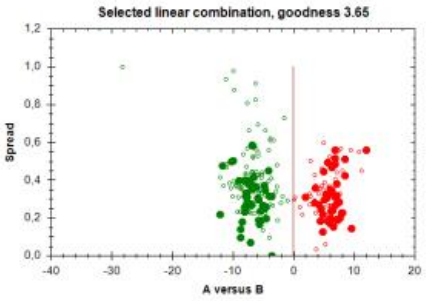


Figure 2-48: Illustration of the process of training the building block. Top row: Example cells, selected during the training, are marked red and green. Middle row: Classification results are added as red and green rims to each cell. Bottom row: scatter plots of property pairs and classifiers. Red and green dots represent the trained cells and black circles represent all other cells.

质核转运（RMS 2.9 Cytosol to Nucleus Translocation）

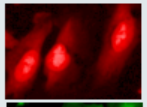
Image Analysis Sequence: Cytosol to Nucleus Translocation

Input Image

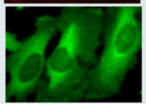
Channel 1: Combined nuclear and cytoplasmic stain image

Channel 2: Signal image

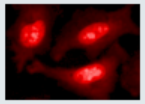
Untreated control: Nuclei + cytosol



Labeled NF_KB antibody



Treated cells



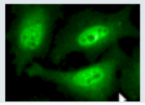


Image Segmentation

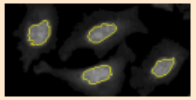
Find Nuclei

Finds nuclear outlines using the nuclear stain image

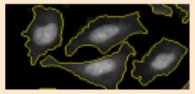
Find Cytoplasm

Finds cytoplasmic outlines around each of the previously detected nuclei

Nuclear regions overlaid on nuclear stain image



Detected cytoplasmic outlines



Define Region of Interest

Select Cell Region

Select the region to measure the cytoplasmic fluorescence based on the detected nuclear and cytoplasmic outlines.

Search the region overlaid on the signal stain image (control)

Quantify Properties in Regions

Calculate Intensity Properties

in the nuclear region

Calculate Intensity Properties

in the cytoplasmic region

Object No	Intensity Nucleus Mean
10	346.552
11	405.603

Object No	Intensity Cytoplasm Mean
10	446.409
11	547.204

Object No	Intensity Nucleus Mean
22	850.549
23	720.187

Object No	Intensity Cytoplasm Mean
22	280.082
23	264.559

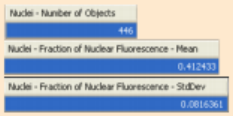
Calculate Ratios of Properties

Calculate Properties

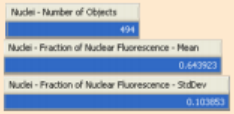
Determine the fraction of fluorescence that is located in the nucleus

Define Results

Select readout values to report



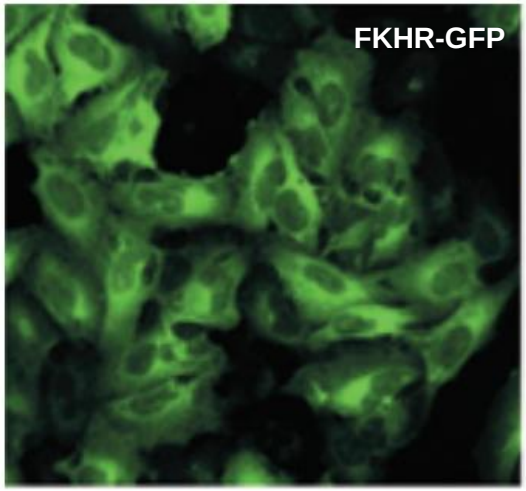
➢ Marker is mainly located in the cytoplasm

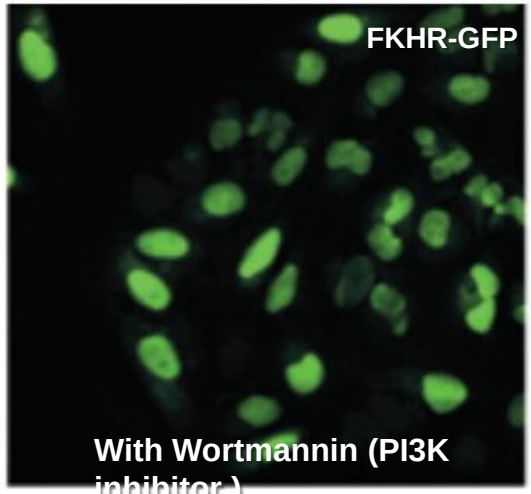


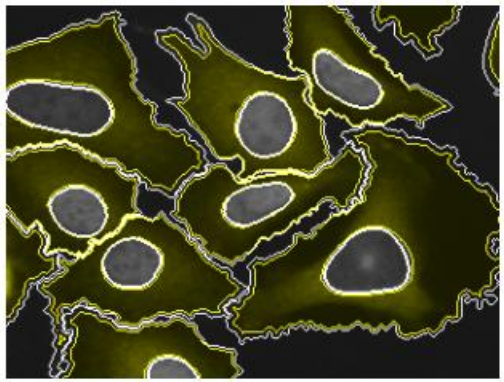
➢ Marker is mainly located in the nucleus

Readout Values

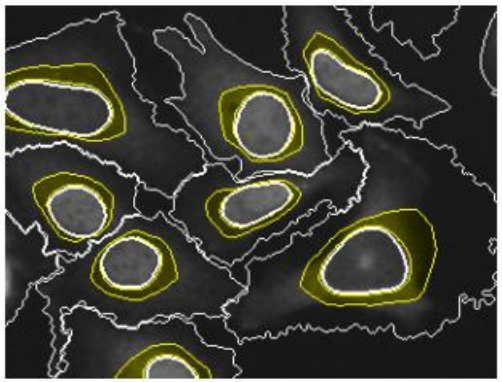
Total Number of Cells	For information and quality control
Mean Fraction of Nuclear Fluorescence	Indicates the location of the marker: 0.0 – marker is only located in the cytoplasm; 1.0 – marker is only located in the nucleus
StdDev Fraction of Nuclear Fluorescence	Homogeneity of the marker distribution in the cell population. A low value indicates that all cells show a similar translocation





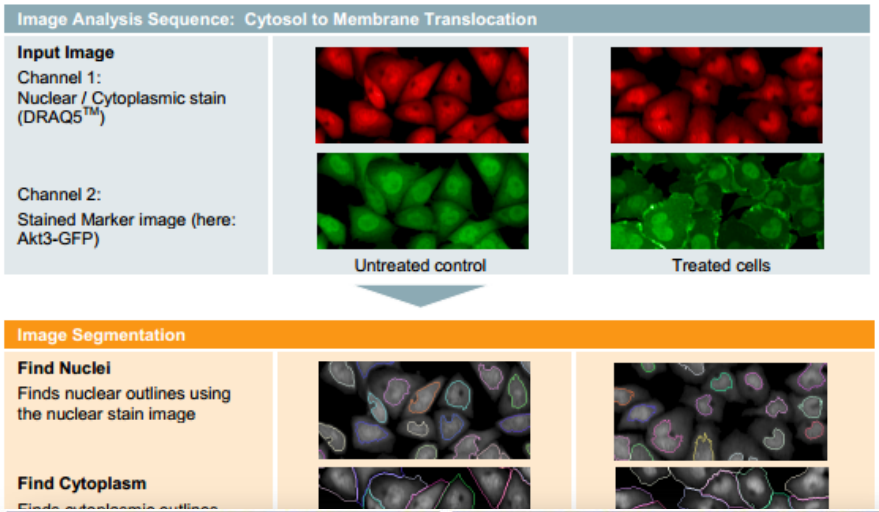


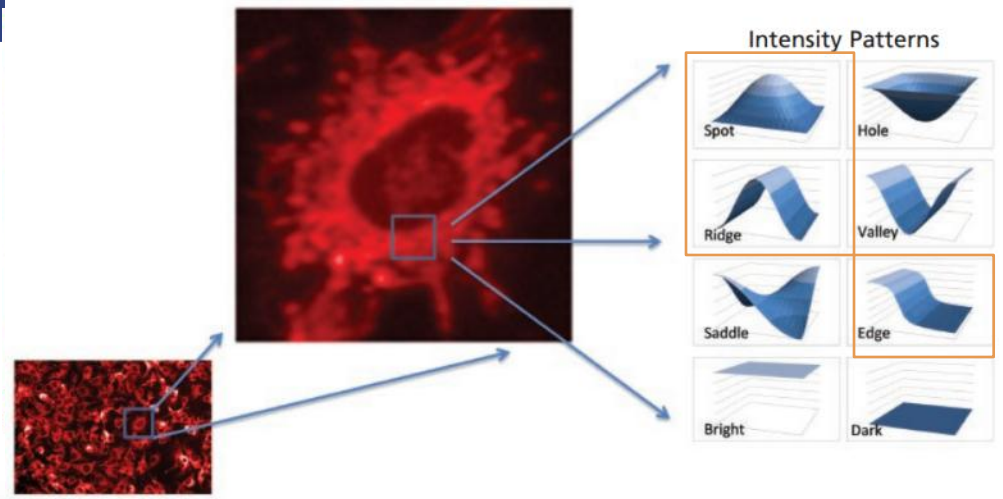
Standard choice: Membrane and nuclear border excluded to avoid noise.

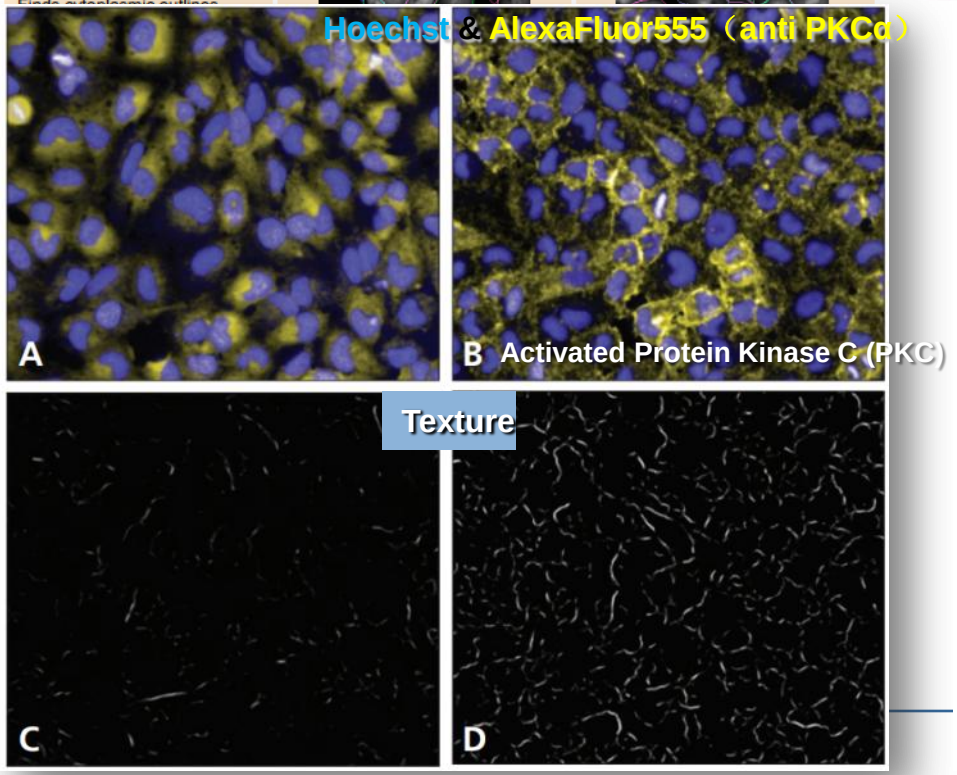


Special adjustment: Only the inner part of the cytoplasm is covered since the intensity fluctuations increase when getting closer to the membrane.

质膜转运（RMS 2.10 Cytosol to Membrane Translocation）







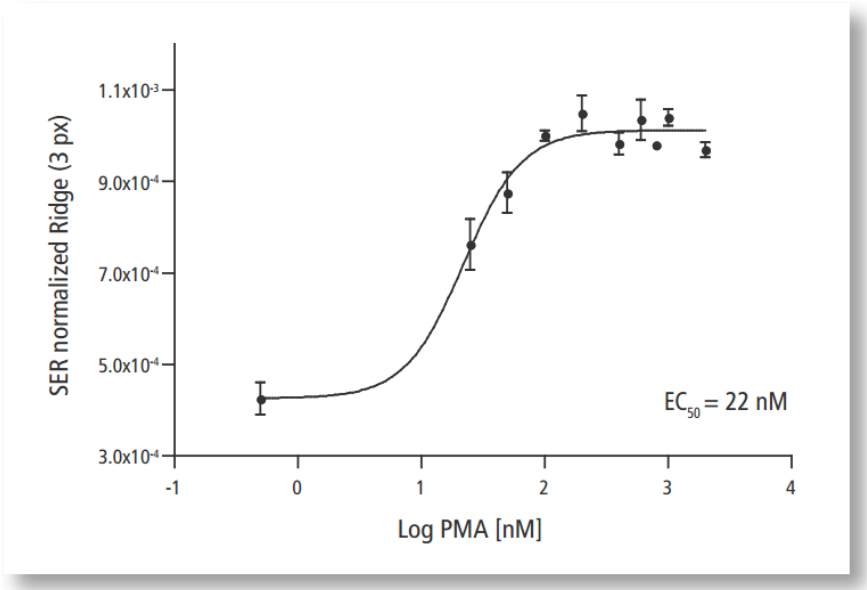


Figure 3. Dose dependent PKCα activation in HeLa cells stimulated with PMA. The SER 'ridge' signal, normalized by cell number and width parameter set to 3 pixels, increases as the PMA concentration increases. This signal represents PKCα association with cell membranes. N = 3 wells, z′ = 0.55.

受体内化（RMS 2.11Receptor Internalization）

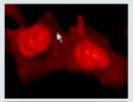
Image Analysis Sequence: Receptor Internalization

Input Image

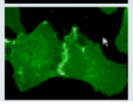
Channel 1: Combined nuclear and cytoplasmic stain image

Channel 2: Signal image

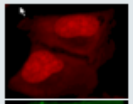
Untreated control: Nuclei and cytosol



Labeled ET_AR Receptor



Treated cells



Internalized ET_AR Receptor

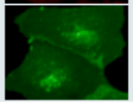


Image Segmentation

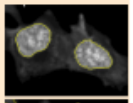
Find Nuclei

Finds nuclear outlines using the nuclear stain image

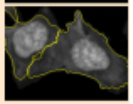
Find Cytoplasm

Finds cytoplasmic outlines around each of the previously detected nuclei

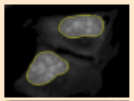
Nuclear region is overlaid on nuclear stain image (untreated cells)

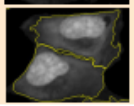


Detected cytoplasmic outlines



Results of detection on treated cells



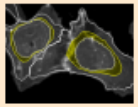


Define Region of Interest

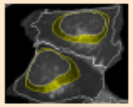
Select Cell Region

Select the region to search for the internalized receptor. The region is based on the detected nuclear and cytoplasmic outlines.

Search region is overlaid on the signal stain image (control)



Search region is overlaid on the positive control image



Quantify Properties in Regions and Calculate Ratios of Properties

Calculate Intensity Properties

Sum signal intensity in the selected region and in the whole cell

Calculate Properties

Calculate "Internalized Intensity" = ratio of intensity in the selected region and in the whole cell

Select Population

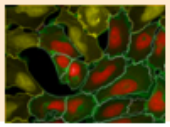
Only keep the cells that are completely visible in the image

Object No	Intensity Cell Sum	Intensity ROI Sum
14	3995635	890291
15	3948934	793078

Object No	Internalized Intensity
14	0.222816
15	0.200833

Control → low values of "Internalized Intensity"

Untreated cells

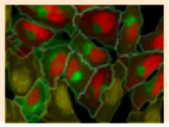


Object No	Intensity Cell Sum	Intensity ROI Sum
5	5407775	1467707
6	5210943	1422400

Object No	Internalized Intensity
5	0.271407
6	0.272964

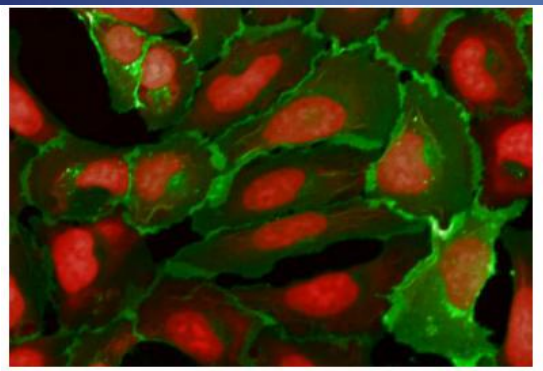
Activated cells → high values of "Internalized Intensity"

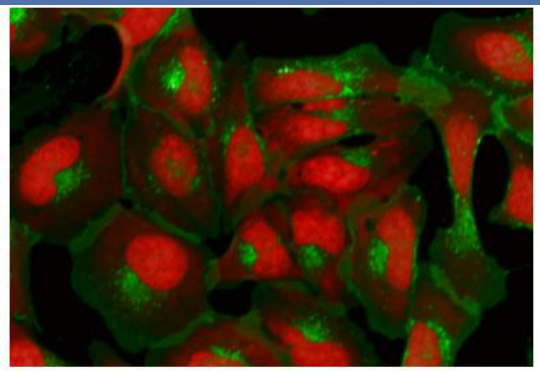
Treated cells with internalized ET_A Receptor



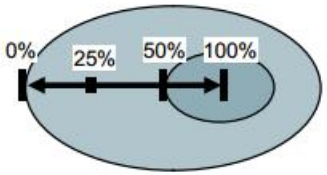
Readout Values

Total Number of Cells	For information and quality control
Mean Internalized Intensity	Measure of the mean degree of receptor internalization in the cell population
StdDev of Internalized Intensity	Homogeneity of receptor internalization within the cell population

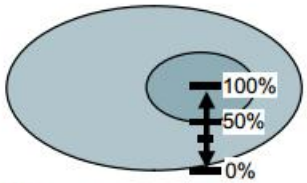




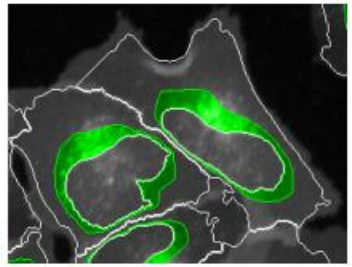
ET-1/ET_A receptor (ET_AR) Path way



A

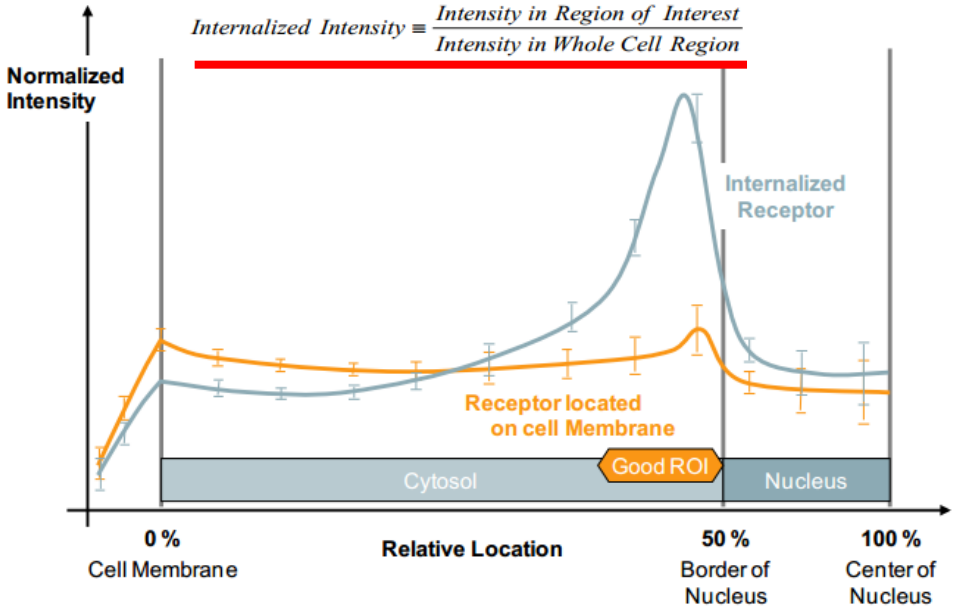


B

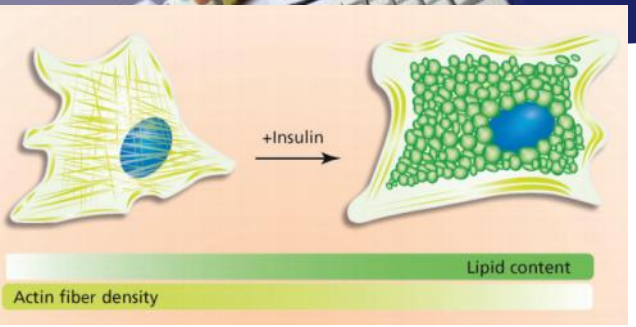


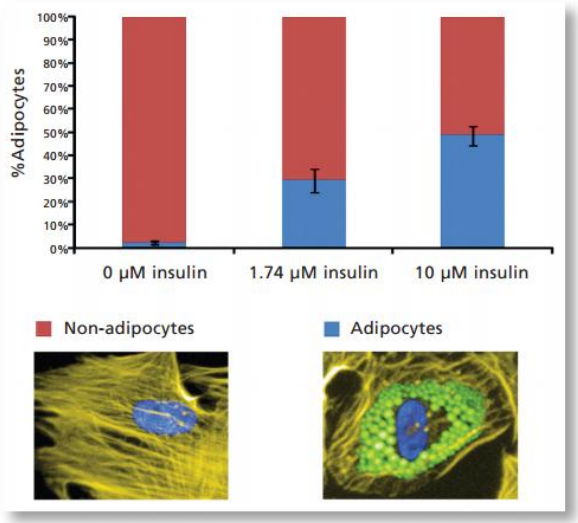
C

$$Internalized\ Intensity \equiv \frac{Intensity\ in\ Region\ of\ Interest}{Intensity\ in\ Whole\ Cell\ Region}$$

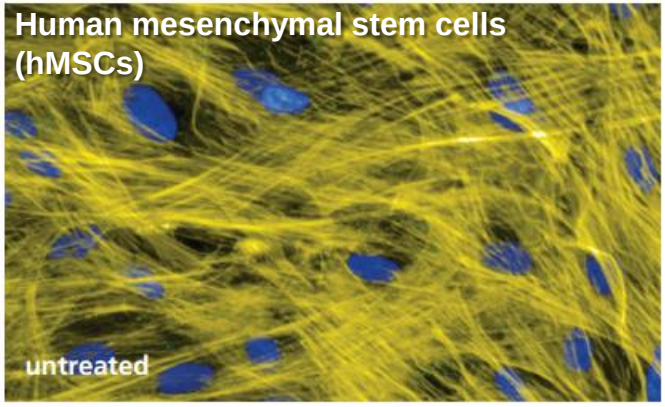


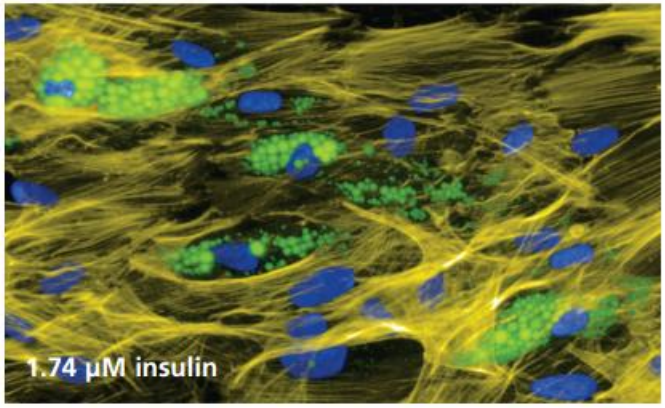
干细胞分化

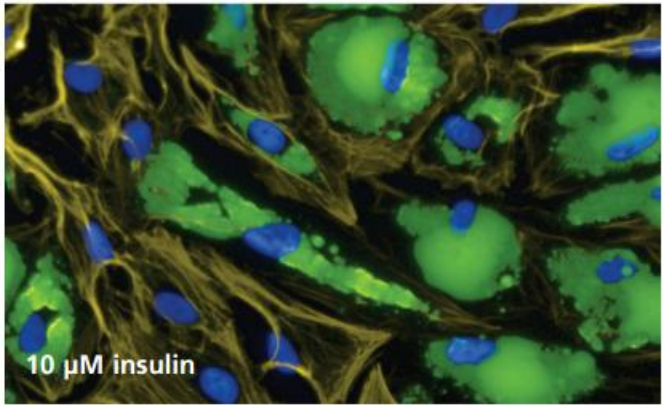


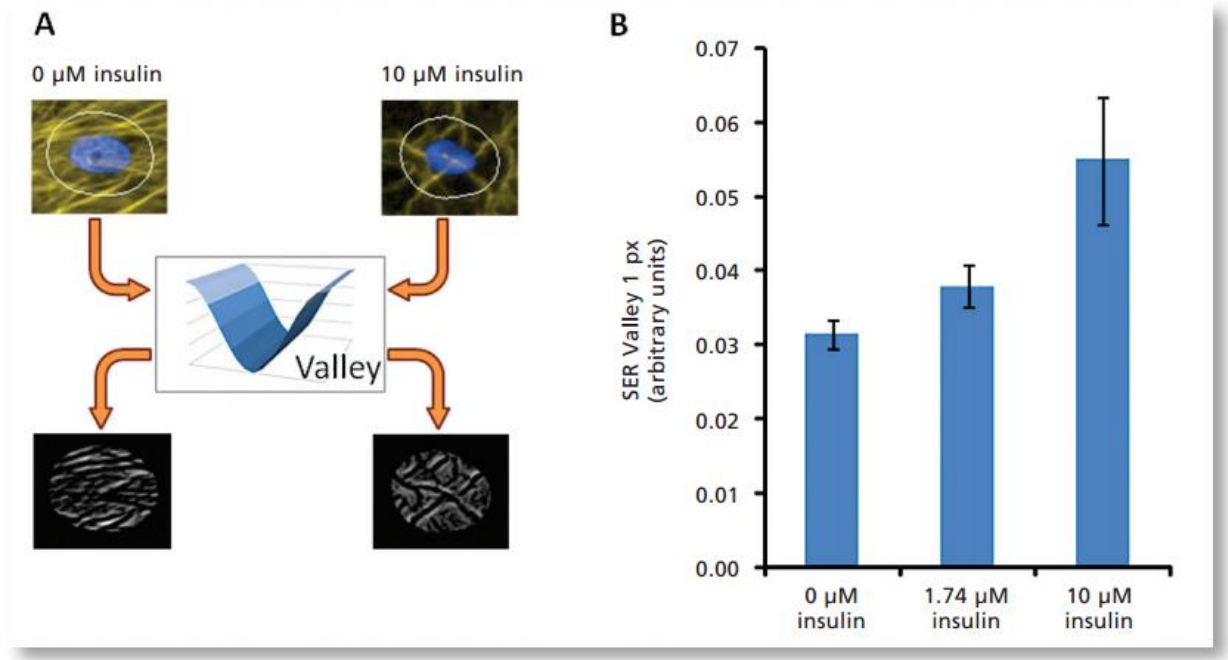


DAPI
PhalloidinTRI TC
LipidTOX Green





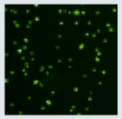




克隆计数

Image Analysis Sequence: Colony Formation

Input Image
Cytoplasmic stain image (CMFDA)


Cells on day 1

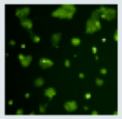
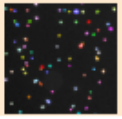
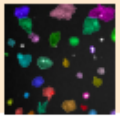

Colonies on day 5

Image Segmentation

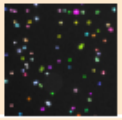
Find Cells
Detects single cells, cell clusters or colonies

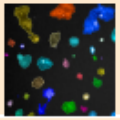




Refine Colony Detection

Modify Population
Merging of fragmented colonies using the **Cluster by Distance** method

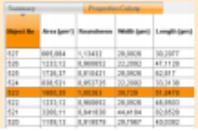


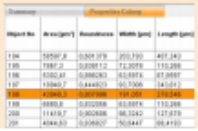


Distance set to 1 px: objects closer than 1 px are merged to one object

Quantify Properties of the Colonies

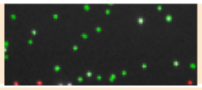
Calculate Morphology and Intensity Properties
Calculate area, roundness, width, length and intensity properties of each colony

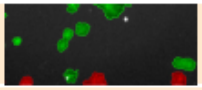




Select Population

The common filter **Remove Border Objects** excludes objects crossing the image border.





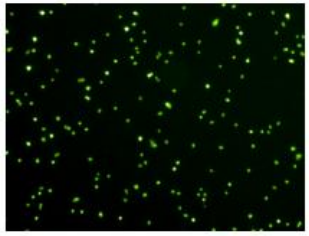
Complete colonies are shown in green and discarded border colonies in red

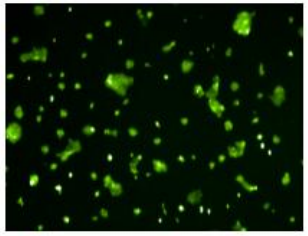
Calculate Readout Values

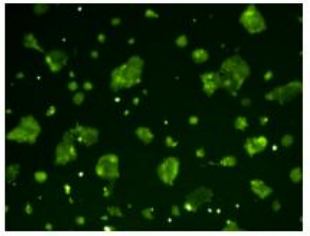
Define Results
Calculate averaged population results per well.

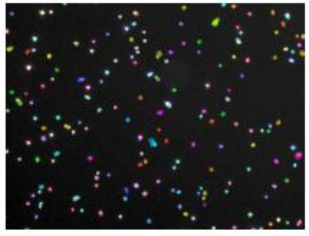
Readout Values

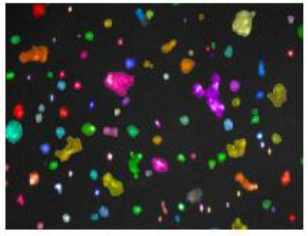
Number of Colonies	Equivalent to the number of cells at the beginning of the time series and the number of colonies as time proceeds
Mean Area [µm²]	Average size of cells and / or colonies per well
Sum Area [µm²]	Total area covered by cells and / or colonies per well
Mean Contrast	A measure of staining intensity against background

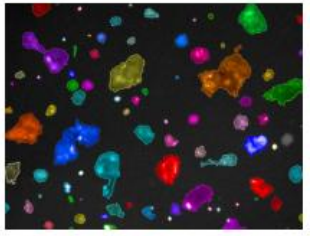




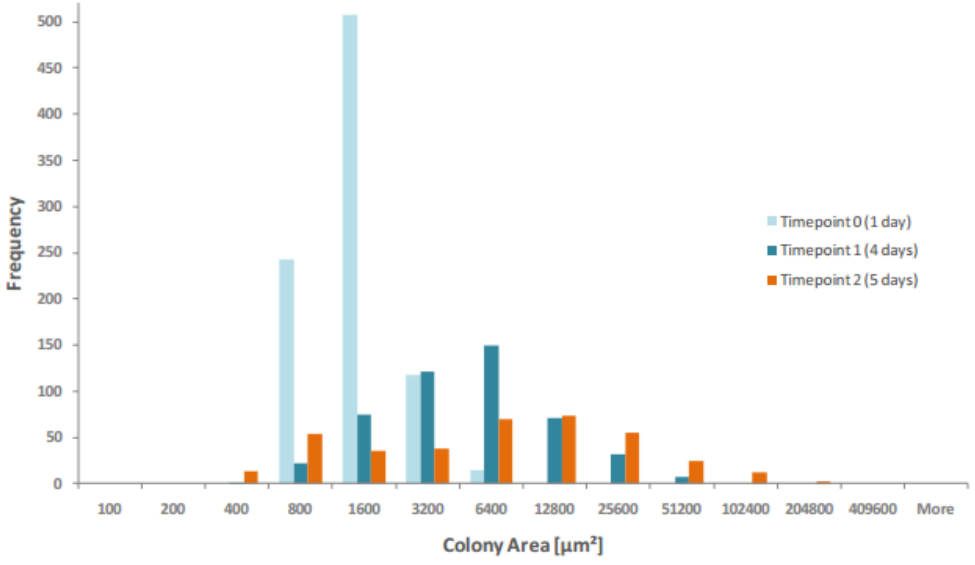








Time point 1 / day 1 (T0) | Time point 2 / day 4 (T1) | Time point 3 / day 5 (T2)



挑选克隆（RMS 2.28 Stem Cells Colonies）

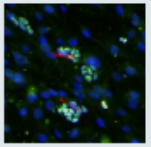
Image Analysis Sequence: ESC Colony Analysis

Input Image

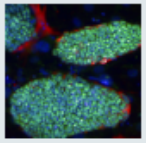
Channel 1: Nuclear stained image (blue)

Channel 2: Oct-4 stained image (green)

Channel3: Cytokeratin stained image (red)



36 h after cell seeding



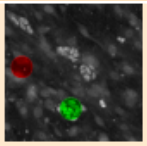
3 d after cell seeding

Image Segmentation

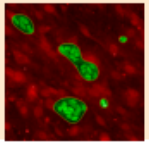
Find Texture Regions

Allows you to separate regions of an image with different texture properties.

Use "Train…" mode to select example regions of two texture classes, A and B



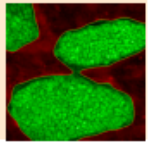
Training results:



Detected texture regions on the "36 h" image

Click on different texture regions to set training points for class A (stem cell colonies, green) and class B (feeder layer, red)

Detected texture regions on the "3 d" image

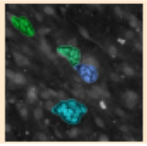


Modification of detected regions

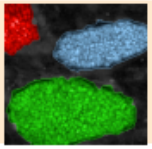
Modify Population

Split detected region into objects, adjust the minimum area and splitting distance for touching regions.

Valid colonies of the "36 h" image



Valid colonies of the "3 d" image



Quantify properties in regions

Calculate Morphology and Intensity Properties

Area and roundness, and marker intensities for Oct4 and Cytokeratin in each colony.

Object No.	Colony Area [µm²]	Colony Roundness
1	1855.8	0.92498
2	1407.73	0.909095
3	1485.66	0.951386

Oct-4 Intensity Mean	Oct-4 Intensity Sum	Cytokeratin Intensity Mean	Cytokeratin Intensity Sum
1371.94	2680628	1267.62	2384391
1392.07	1986478	7024.08	1.116498E+07
1377.08	2073876	470.515	708585

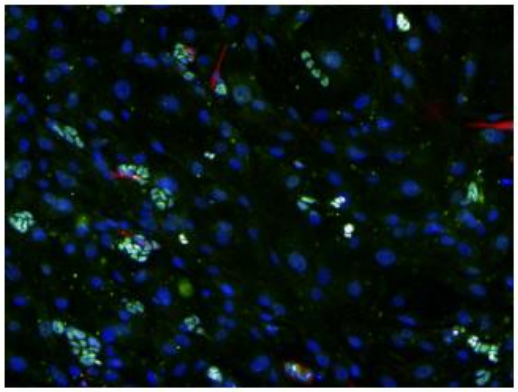
Object No.	Colony Area [µm²]	Colony Roundness
1	7477.83	0.925182
2	42439	0.782413
3	37256	0.823627

Oct-4 Intensity Mean	Oct-4 Intensity Sum	Cytokeratin Intensity Mean	Cytokeratin Intensity Sum
1985.91	1.585323E+07	728.873	5524854
2273.48	9.780506E+07	1781.18	7.662877E+07
2729.5	1.830823E+08	1291.93	4.879396E+07

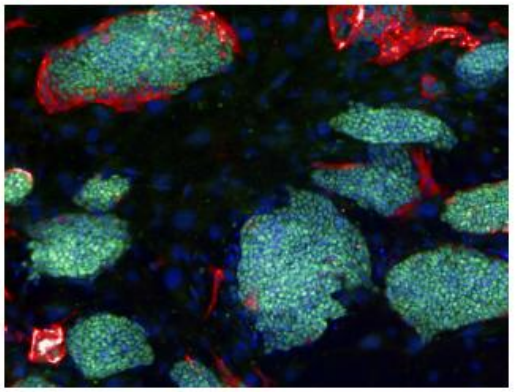
Readout Values

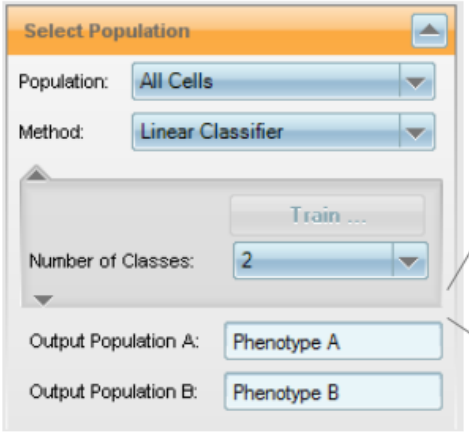
Number of Colonies	Number of all analyzed valid colonies in the well
Mean Colony Area	Area of the whole colony in µm²; averaged over all colonies in the well
Mean Oct4 Intensity per colony	Intensity of the Oct4 stem cell marker stain; first averaged over all pixels within each colony and then averaged over all colonies in the well
Mean Cytokeratin Intensity per colony	Intensity of the Cytokeratin marker stain; first averaged over all pixels within each colony and then averaged over all colonies in the well

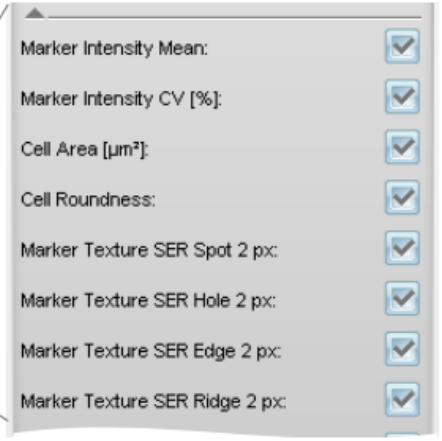
Colonies after 36 h incubation



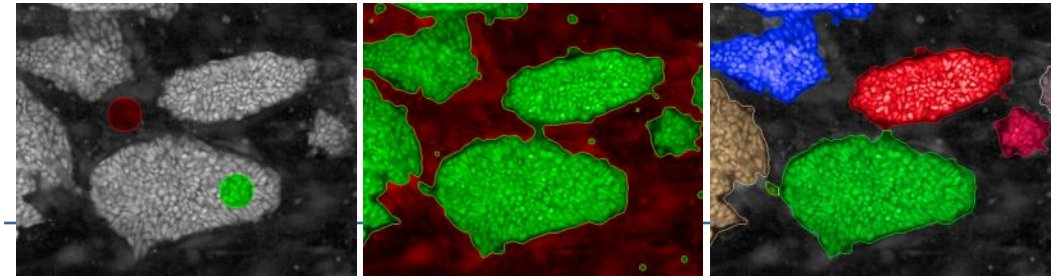
Colonies after 3 d incubation







PhenoLOGIC machine learning



一、细胞培养和实验操作技巧

1.普通多孔板厚度约为1um，适合于20倍及以下镜头。为达到更好的成像效果，当需要使用40倍、60倍镜时推荐用薄底板，板底厚度只有0.19um。

2.细胞均匀铺板的“秘密”：细胞加入微孔板后应立即振匀，振匀方法推荐“前后-左右”法。即把培养板方在桌面上，两手扶住板，先快速前后振动8-10次，振幅在15cm-20cm。然后将板水平转动90度，重复刚才的动作。振动完后平拿板，轻轻放入培养箱内。2小时内不要碰触。

二、药物筛选细胞排板建议

每次实验需包括阴性对照、阳性药对照、待测试剂做8倍梯度稀释，8个浓度以保证量效关系曲线的完整性。每个条件都需要2个或以上重复。

三、实验信噪比要求，常规提高信噪比的方法

信号与背景，细胞信号与背景之间的比值需大于3倍。在提高信噪比上面有些工作可以做，饥饿处理可以降低背景，例如做高糖诱导实验检测蛋白表达时可以先做低糖饥饿处理，可以降低阴性对照值，从而提高信号窗口。在免疫相关实验时需考虑血清对实验的影响，建议使用热灭活胎牛血清，检测刺激物影响前尝试血清饥饿。

Thank You !