高内涵实验设计与优化

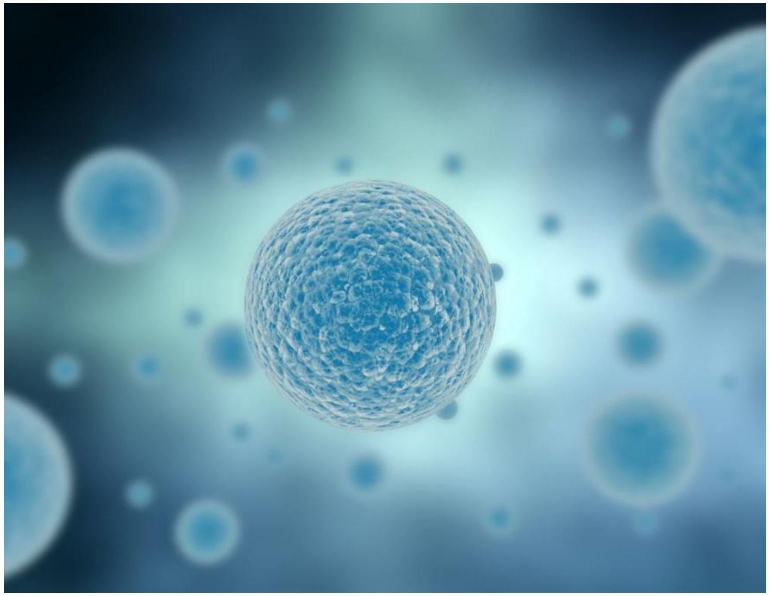
PerkinElmer®
For the Better

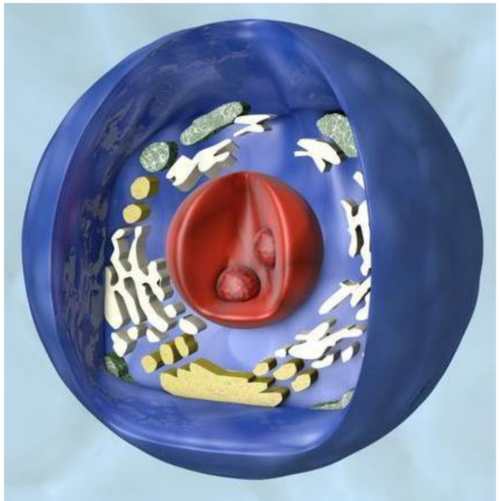
PerkinElmer生物影像学技术支持

孙兵　博士

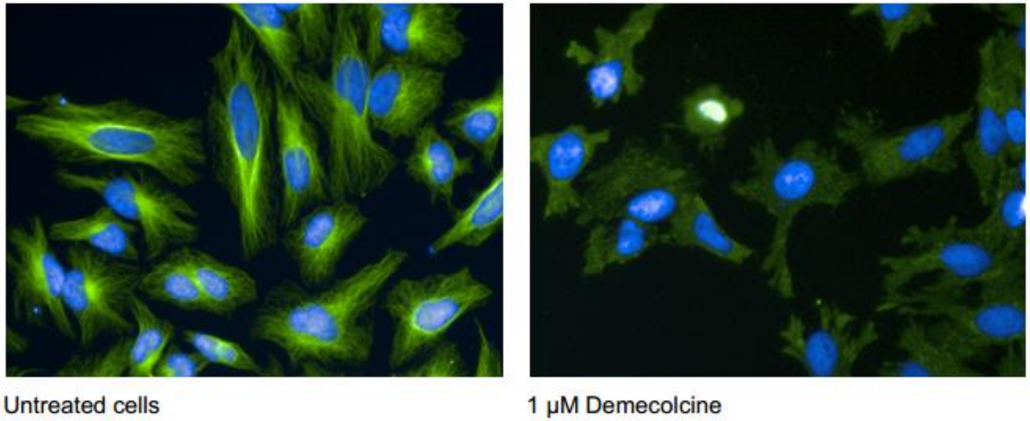
高内涵实验特点

针对细胞的光学成像分析





细胞结构与功能研究

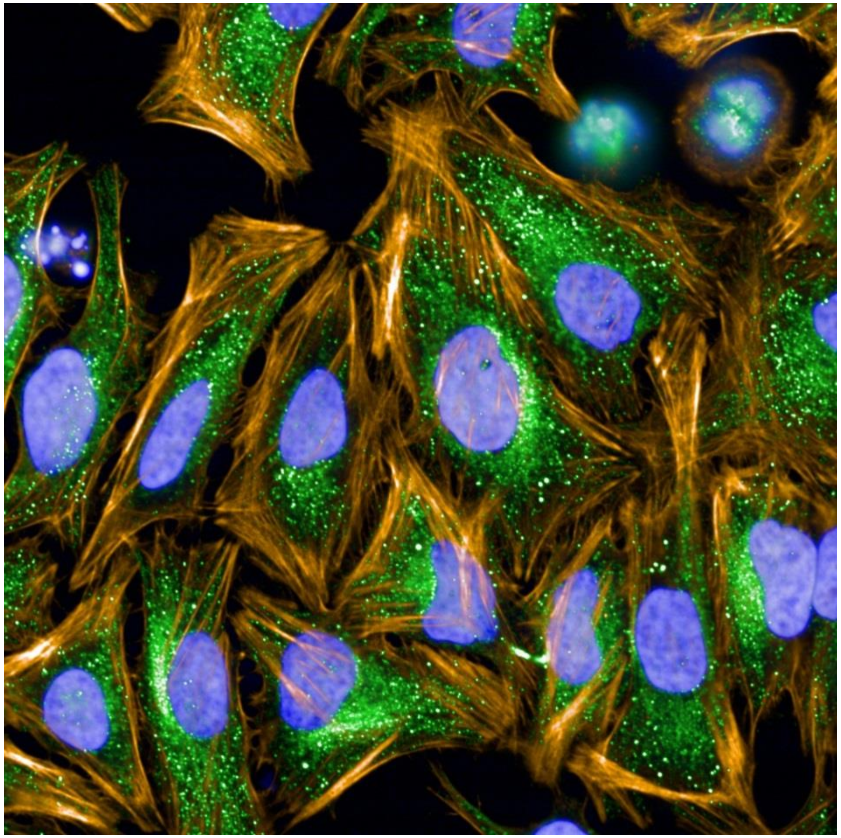

Untreated cells
1 µM Demecolcine

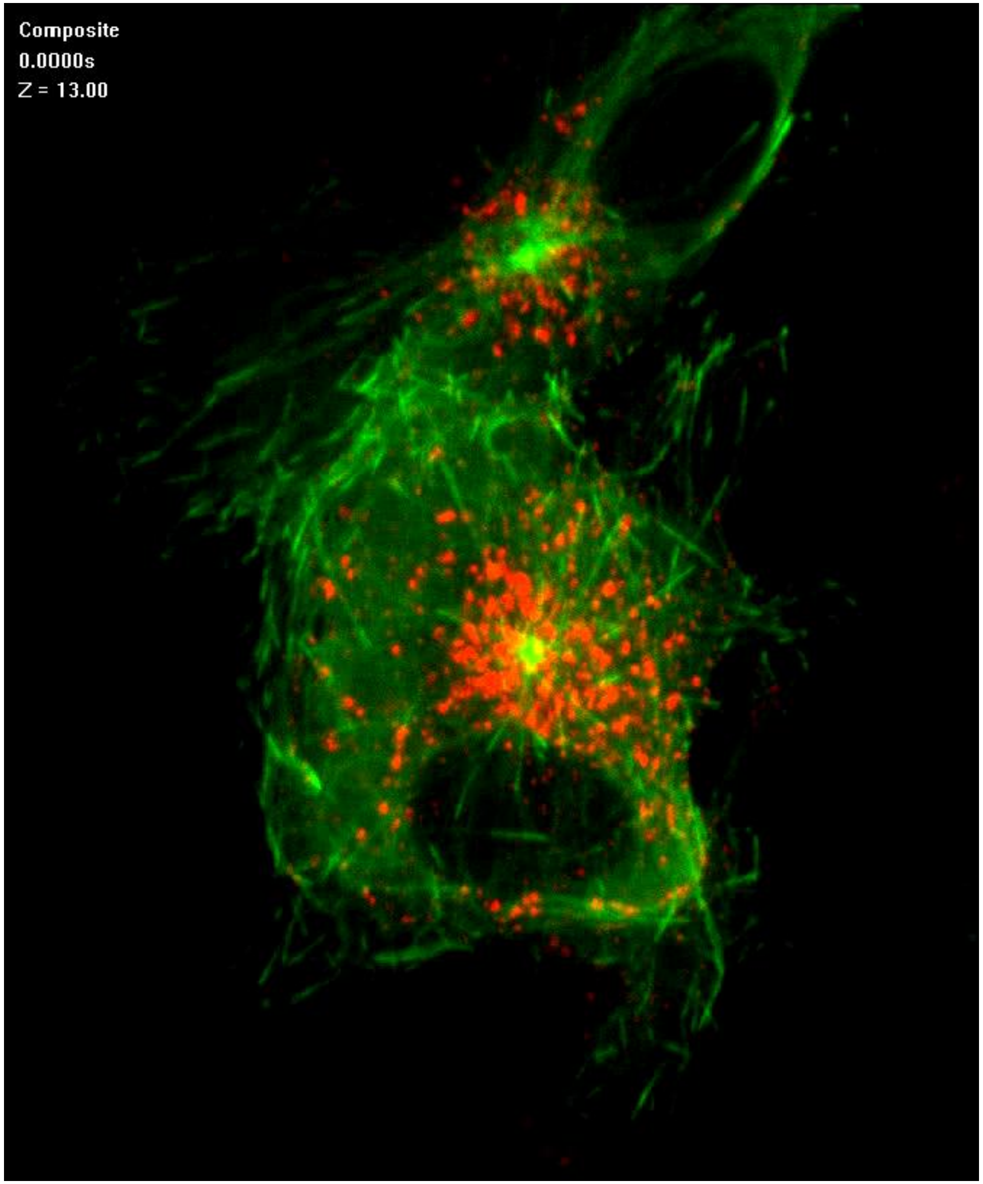
以细胞为评价模型的研究

高内涵实验特点

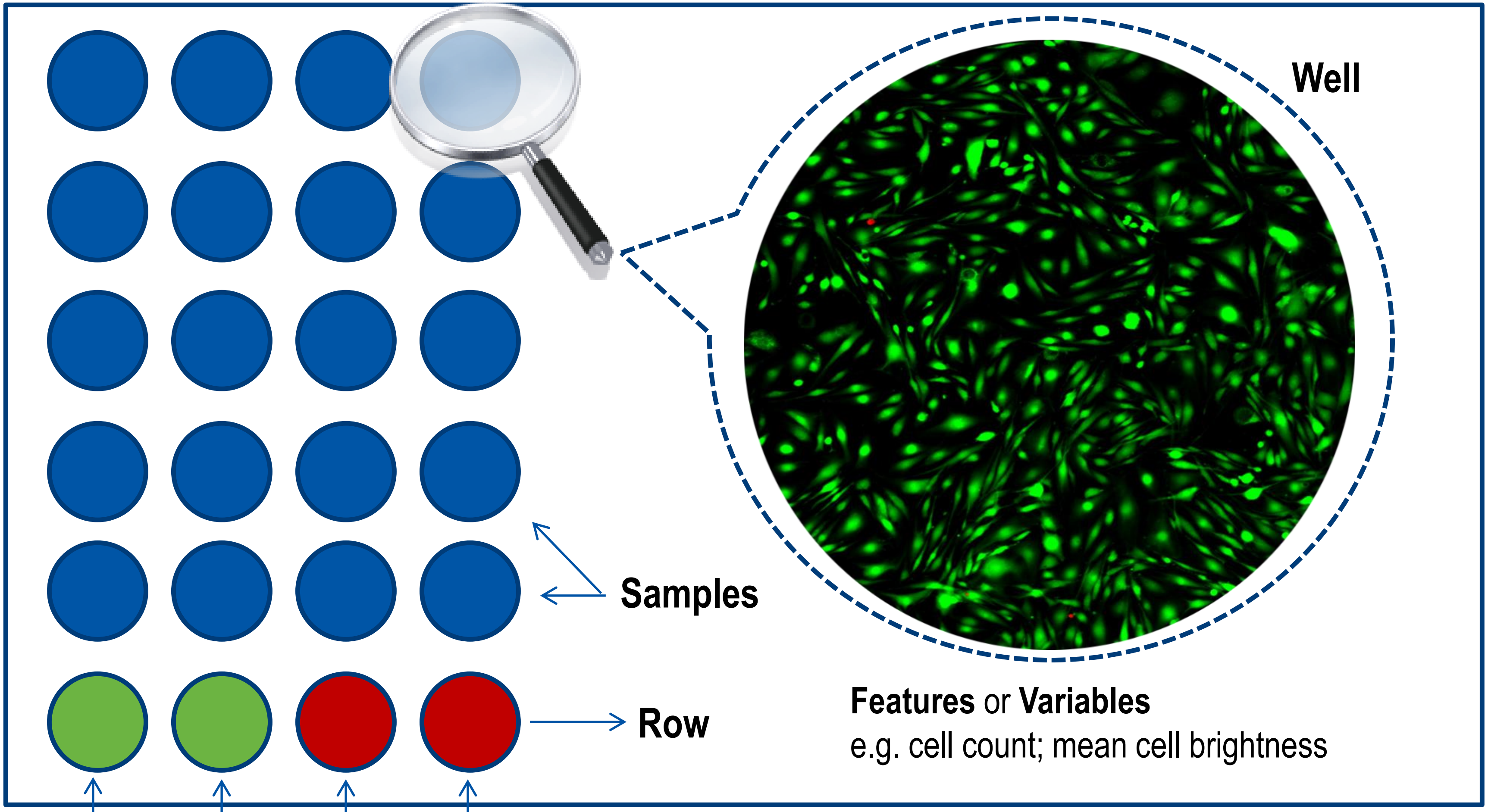
细胞结构与功能研究

清晰，快速




Composite
0.0000s
Z = 13.00

高内涵实验特点



Well

Samples

Row

Features or **Variables**
e.g. cell count; mean cell brightness

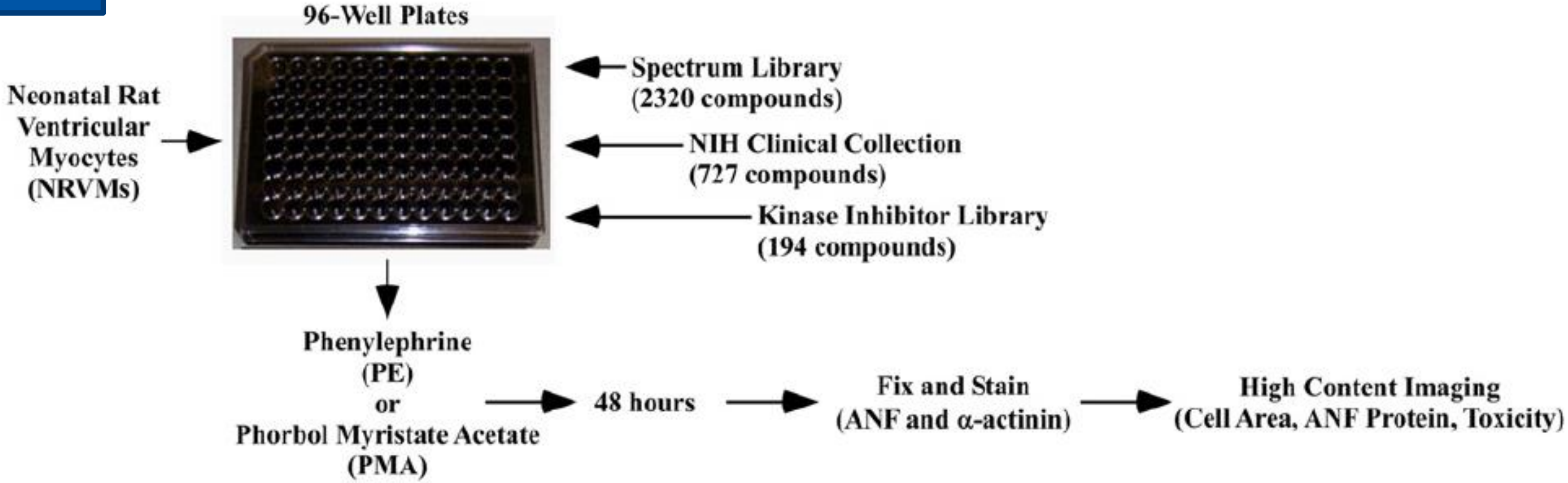
Positive Controls **Negative Controls**

以细胞为评价模型的研究

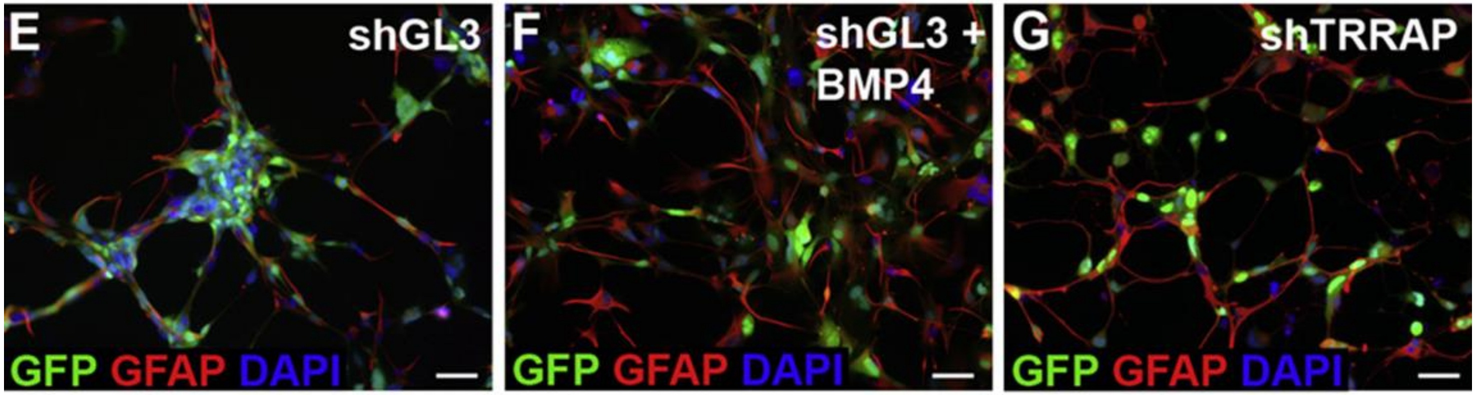
高内涵实验细胞模型

药物

以细胞为评价模型的研究

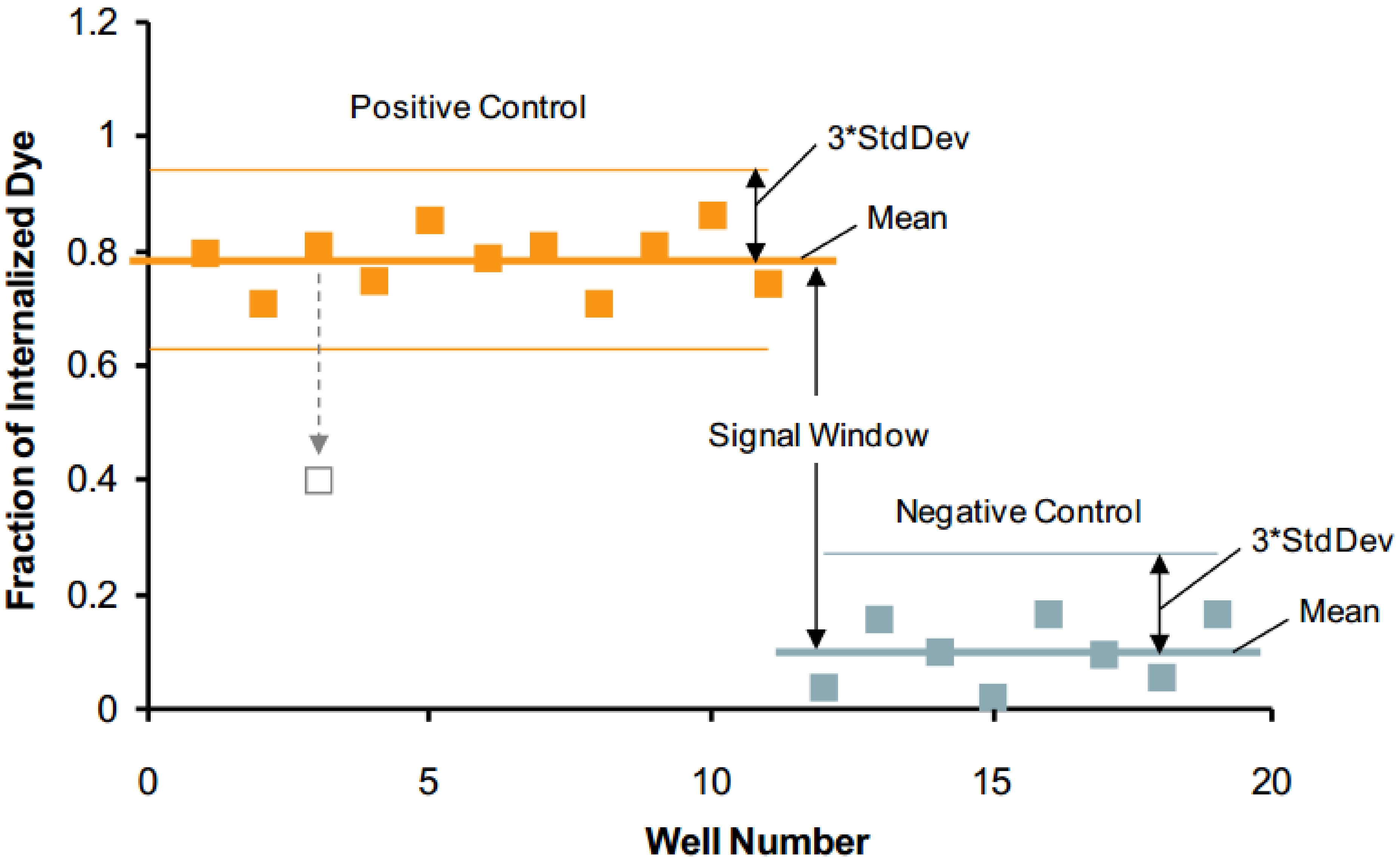

96-Well Plates
Neonatal Rat Ventricular Myocytes (NRVMs)
Spectrum Library (2320 compounds)
NIH Clinical Collection (727 compounds)
Kinase Inhibitor Library (194 compounds)
Phenylephrine (PE) or Phorbol Myristate Acetate (PMA)
48 hours
Fix and Stain (ANF and α-actinin)
High Content Imaging (Cell Area, ANF Protein, Toxicity)

基因


E
shGL3
F
shGL3 + BMP4
G
shTRRAP
GFP GFAP DAPI
GFP GFAP DAPI
GFP GFAP DAPI

高内涵实验细胞模型

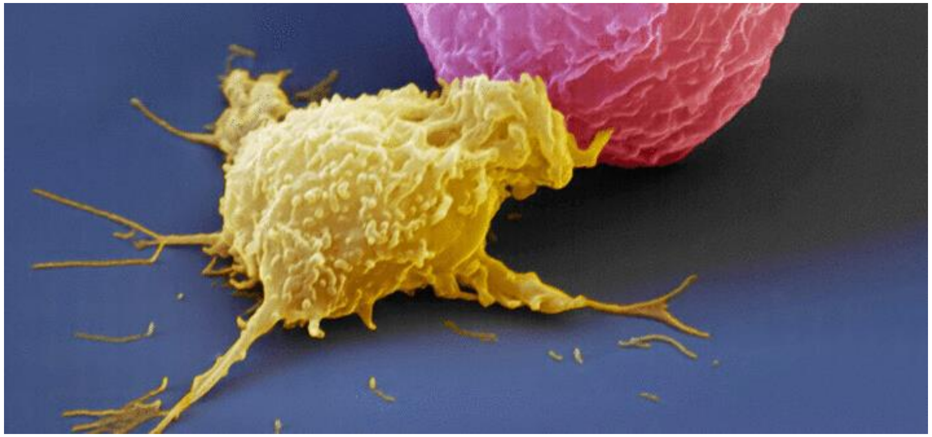
细胞模型—稳定



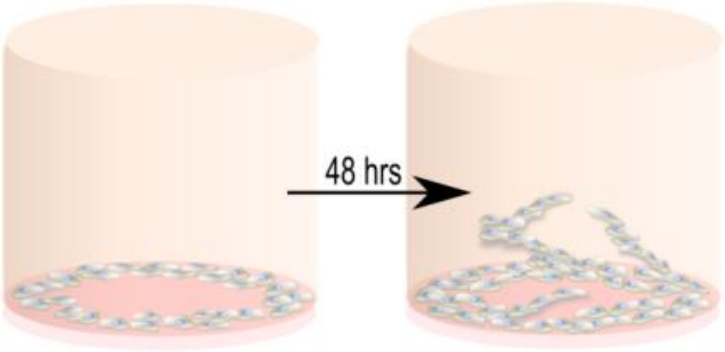
高内涵实验细胞模型

评价指标选择

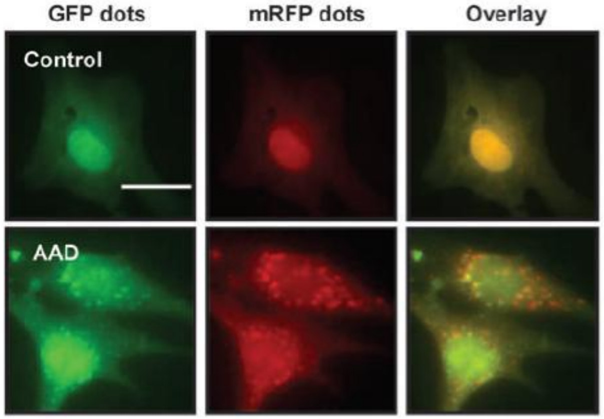
研究对象
细胞模型



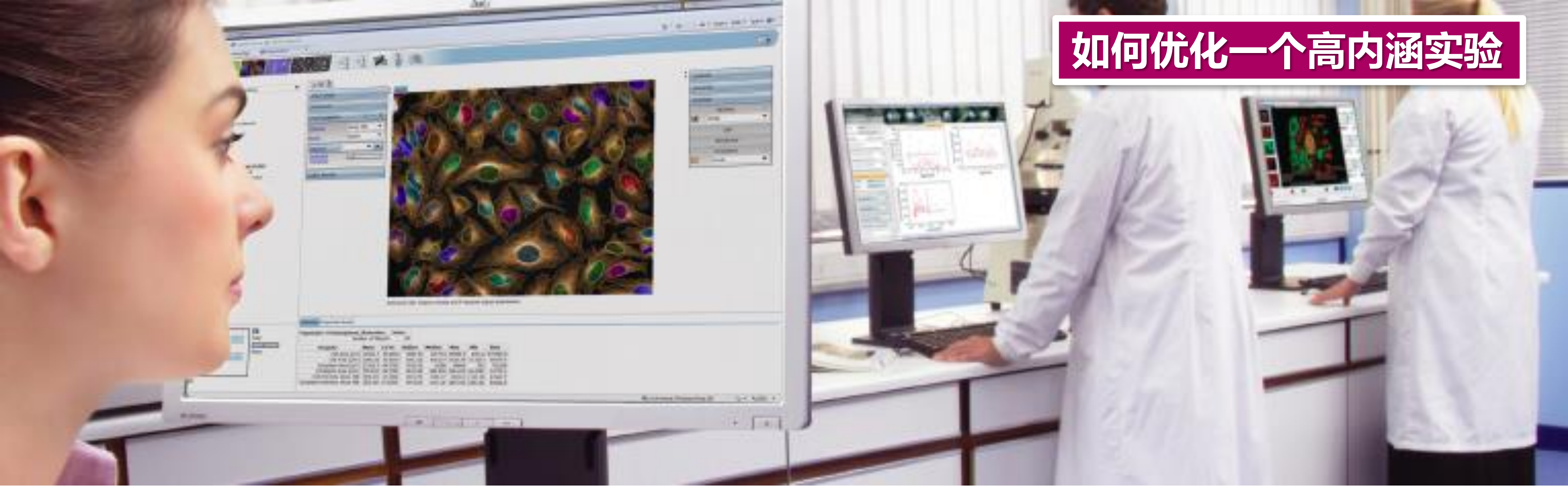
现象（表型）



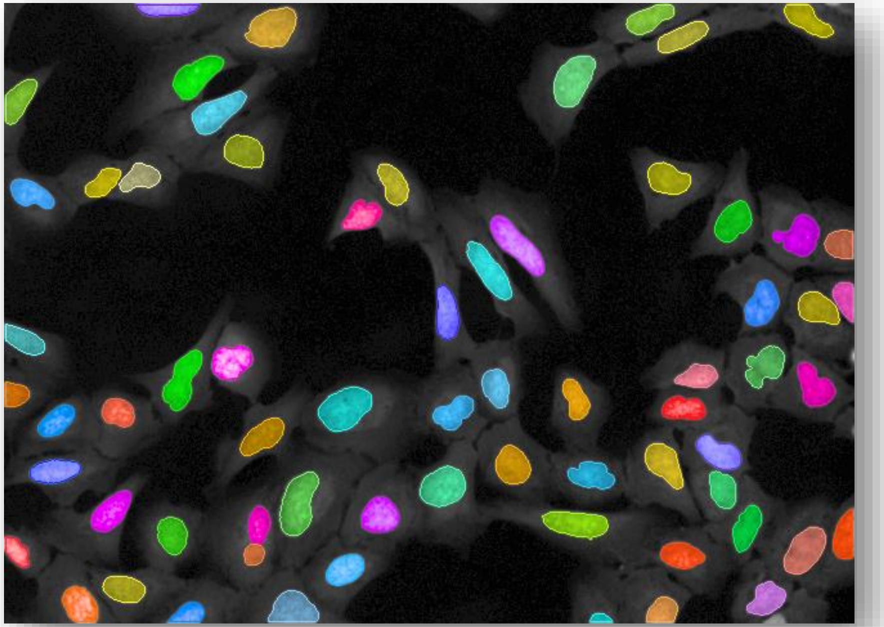
机制（靶标）


GFP dots
mRFP dots
Overlay
Control
AAD

如何优化一个高内涵实验



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



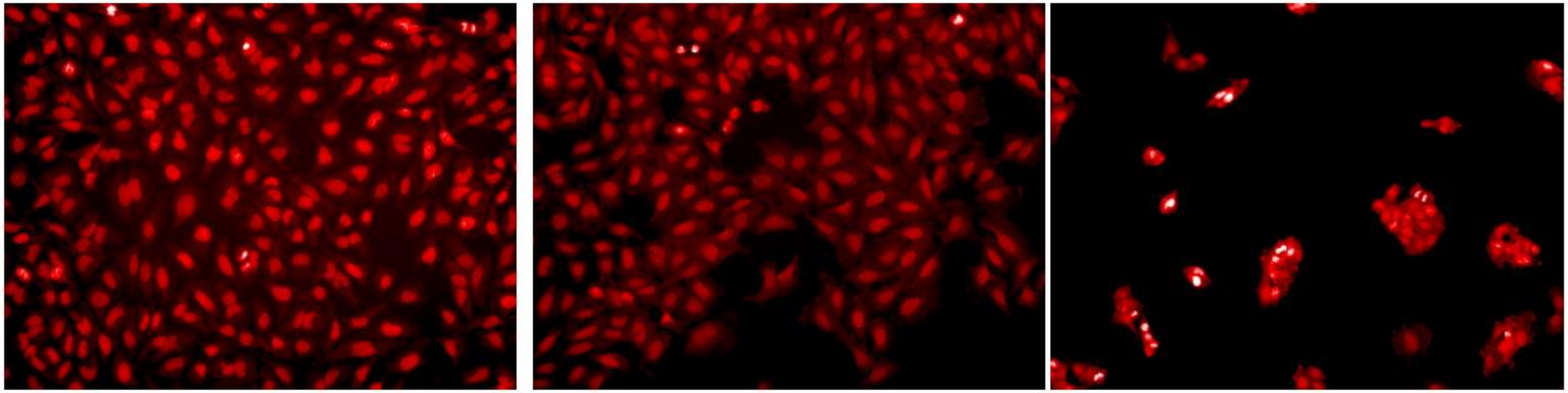
利于数据分析

- Calc. Intensity
- Calc. Morphology
- Calc. Texture
- Calc. Properties a^2+b

- Select Cell Region
- Select Region
- Select Population

1.选择适合高内涵细胞系

细胞的贴壁性以及**生长特性**是影响后续成像和分析的重要因素。



A – HeLa

B – U2OS

C – HepG2

充分重悬细胞，孔板包被

2.选择高内涵微孔板

选择合适的微孔板是成功高内涵实验的重要因素。

如何优化一个高内涵实验
Find Nuclei
Find Cells
Find Cytoplasm
Find Spots
Find Micronuclei
Find Texture Regions
Find Image Regions
Find Neurites
Calc. Intensity
Calc. Morphology
Calc. Texture
Calc. Properties
a²+b
Select Cell Region
Select Region
Select Population
利于数据分析

微孔板影响实验：

- ➢ 提供合适的细胞生长环境
- ➢ 透光度良好
- ➢ 与镜头匹配

2.选择高内涵微孔板

微孔板底材质：常规微孔板底材质为玻璃，聚丙乙烯PE，烯烃CO

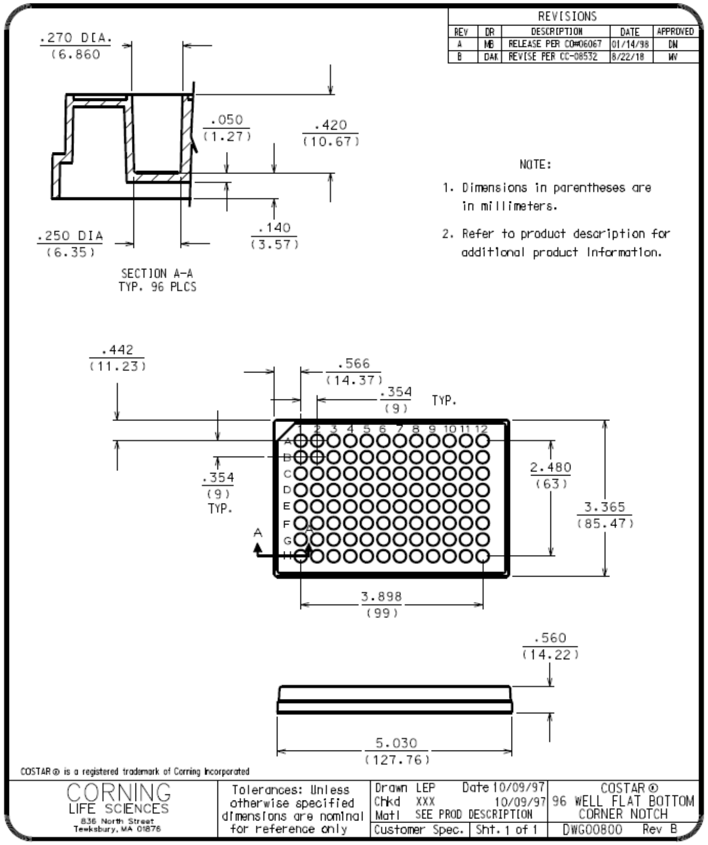
- TC-Treat PS板以及CO板都能够粘附蛋白，从而促进细胞的贴壁。
- 有些PS板在UV的激发下会产生较强的自发荧光，而CO板中的自发荧光会更少。
- 玻璃底板的透光度更好，但是不适合细胞培养，因此，如使用一般需要进行包被。

Tips:对于绝大部分实验来说，建议使用TC-Treat PS或CO板，透明底，黑色板

2.选择高内涵微孔板

微孔板的厚度是影响透光性以及成像质量的重要因素。

- 为保证共聚焦图像效果，建议使用板底厚度为110μm-200μm的微孔板，可使用高数值孔径镜头以及水镜。
- 板底厚度在250μm以上的，需要长工作距离镜头，并且需要调整矫正环。
- 不推荐板底厚度大于1mm的微孔板。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B | DAK | REVISE PER CC-08532 | 8/22/18 | MV
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(6.860
.050
(1.27)
.420
(10.67)
.250 DIA
(6.35)
.140
(3.57)
SECTION A-A
TYP. 96 PLCS
NOTE:
1. Dimensions in parentheses are in millimeters.
2. Refer to product description for additional product information.
.442
(11.23)
.566
(14.37)
.354
(9)
TYP.
1 2 3 4 5 6 7 8 9 10 11 12
A B C D E F G H
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TYP.
A
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3.898
(99)
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5.030
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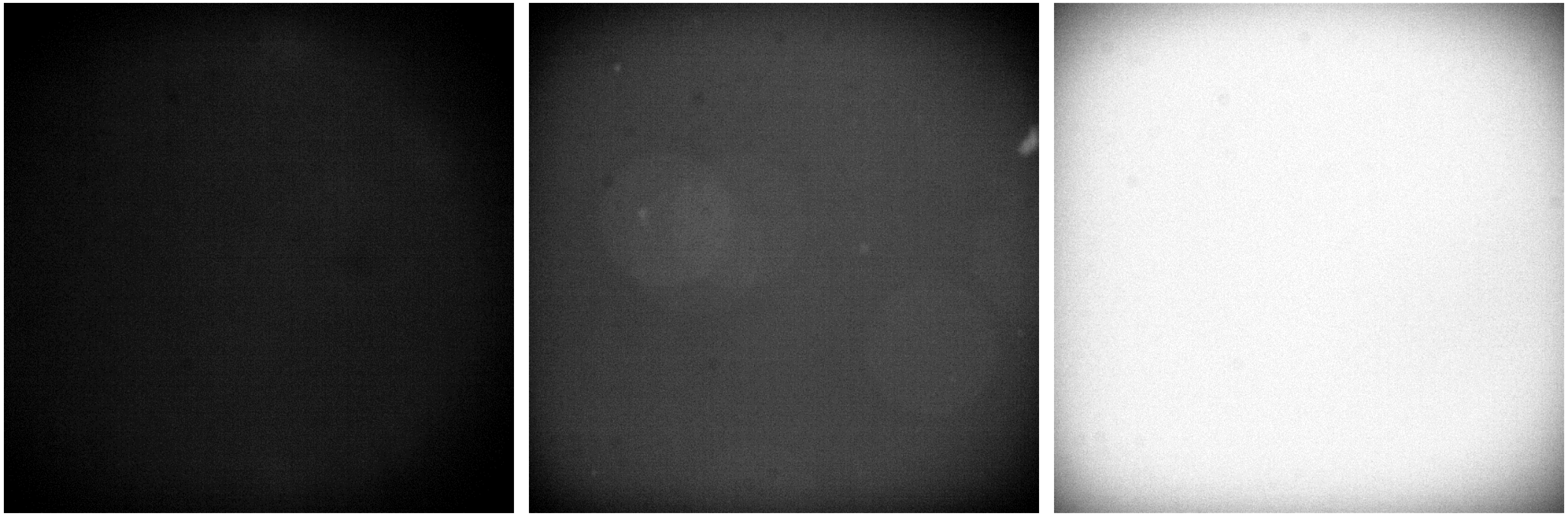
Chkd XXX
10/09/97
Mat'l SEE PROD DESCRIPTION
Customer Spec.
Sht. 1 of 1
COSTAR®
96 WELL FLAT BOTTOM
CORNER NOTCH
DWG00800
Rev B

Tips:如需要观察共聚焦亚细胞结构或纹理分布，必须用推荐薄底板

2.选择高内涵微孔板

不同材质，不同厚度的细胞培养板的背景光不同

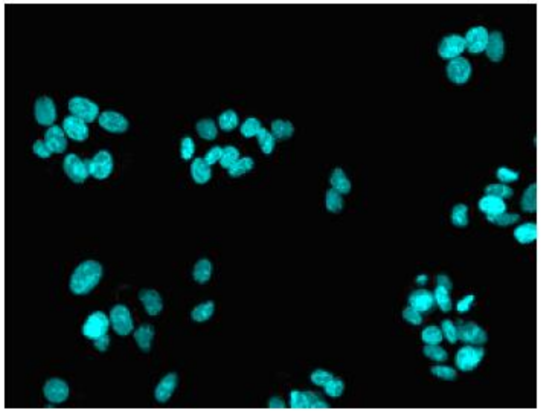
100 µl of PBS in the well.
Channel: Alexa488,
Exposure time: 50 ms



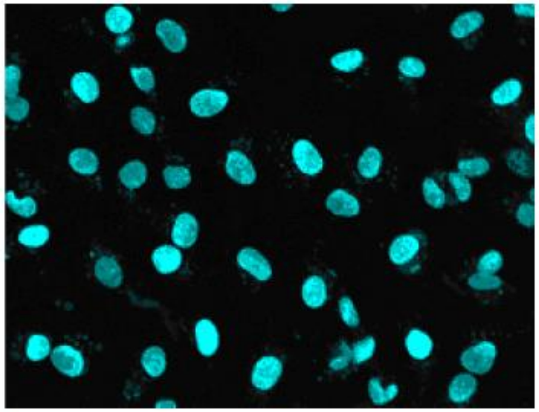
	CellCarrier Ultra	ViewPlate	Coster 3599 (Culture Plate)
背景信号值	**595.6**	**752.2**	**1455.9**

3.孔板包被

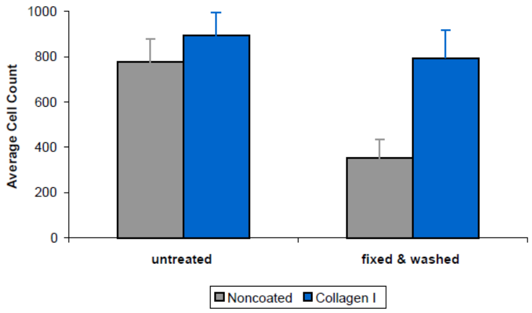
玻璃或塑料底孔板都可以通过包被不同的生物分子来提高细胞的贴壁以及改善细胞的生长特性。



Non-Coated



Collagen I Coated

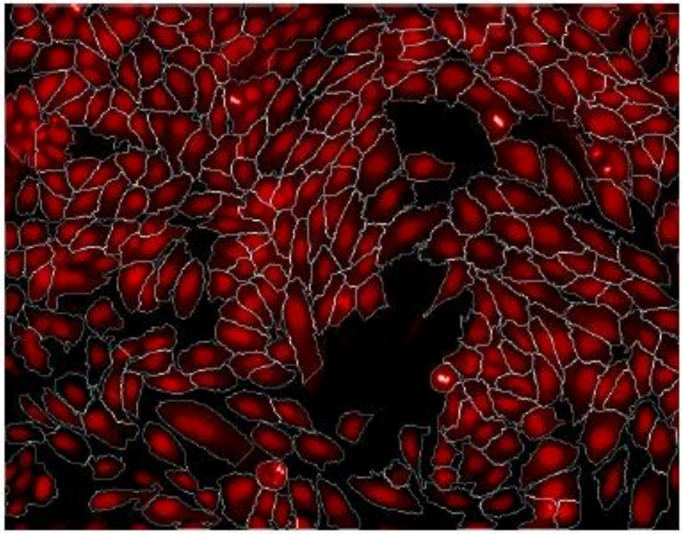


包被液	细胞类型
多聚赖（鸟）氨酸	适合绝大部分细胞
胶原蛋白I&IV	适合内皮，上皮，肌肉，肝细胞
层黏蛋白	适合上皮细胞，白细胞，成肌细胞，神经细胞
纤连蛋白	适合内皮细胞，成纤维细胞

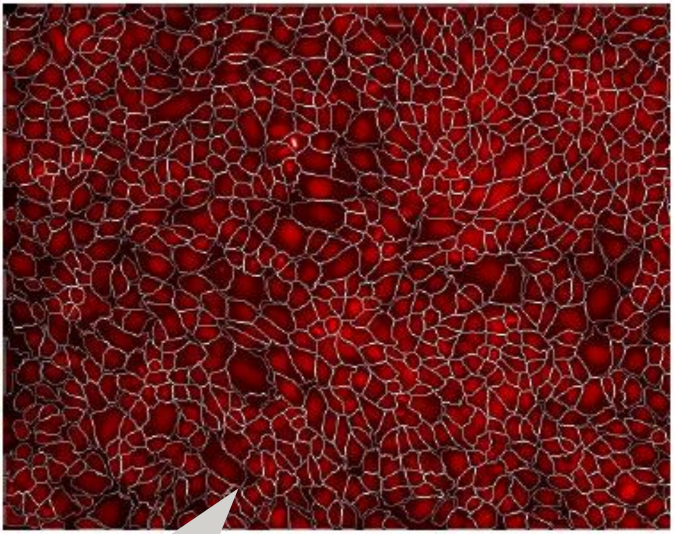
4.实验铺板细胞量

合适的细胞密度

- 高细胞密度，能提高统计学分析的准确性
- 低细胞密度，能提高细胞软件识别分析的准确性



80%



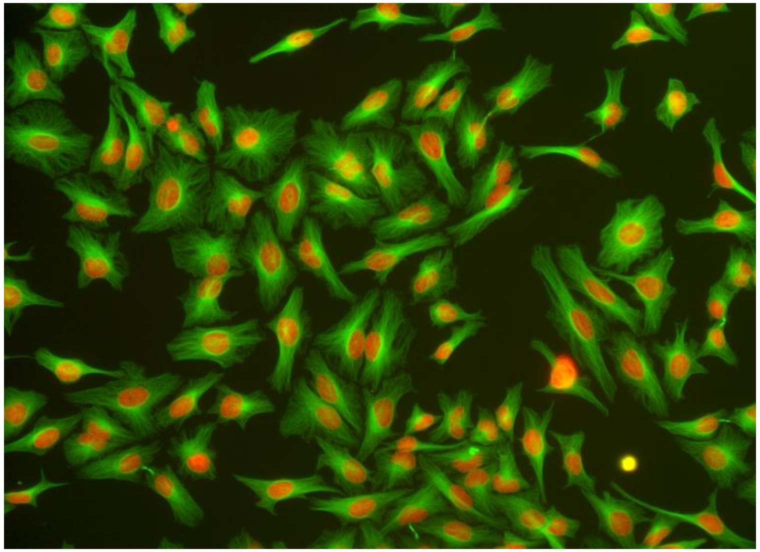
100%

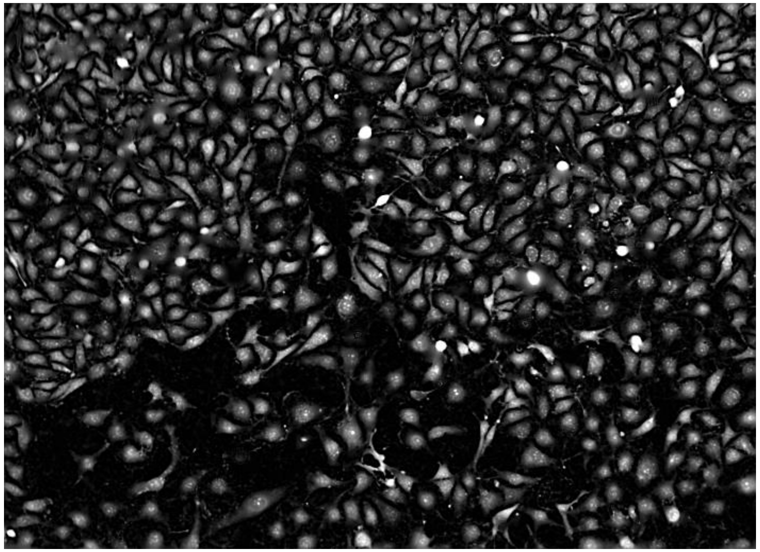
- 细胞核染色有重叠
- 影响正常细胞形态
- 细胞状态不一致

4.实验铺板细胞量

合适的细胞密度

- 高细胞密度，能提高统计学分析的准确性
- 低细胞密度，能提高细胞软件识别分析的准确性

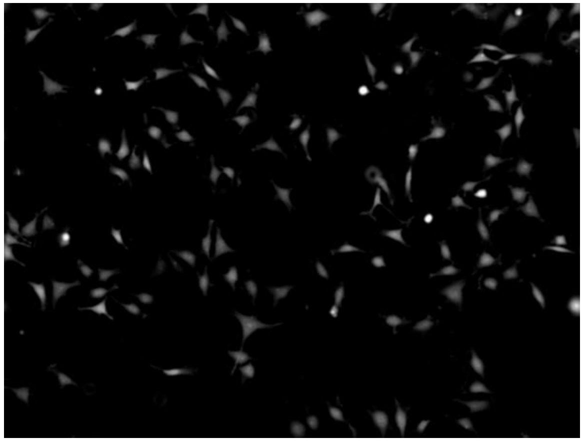


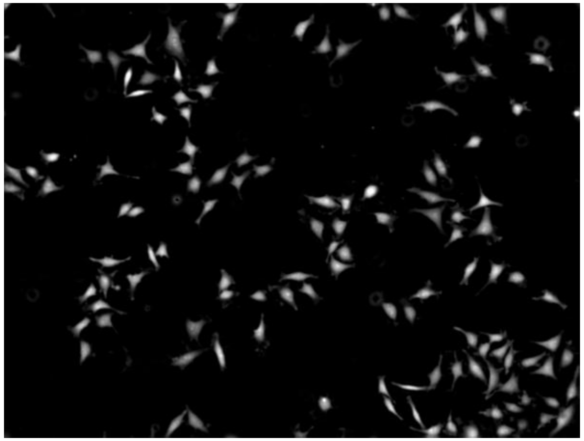


60%-80%可满足较好的统计学分析以及软件识别要求

如无染核的分析方案，需降低细胞量至40%-50%

4.实验铺板细胞量



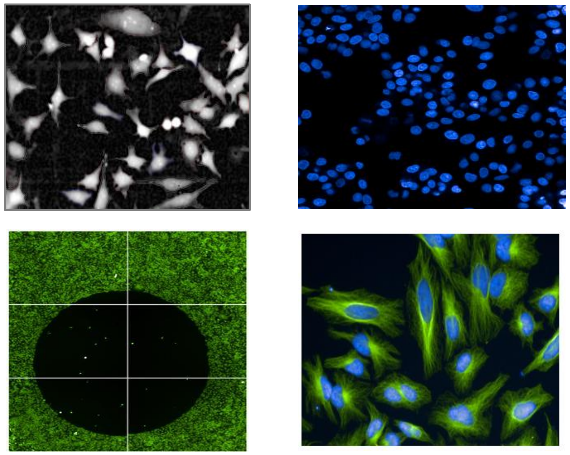


铺板的细胞量需考量因素：

- 细胞生长速度
- 距离高内涵拍摄时间
- 细胞系类型
- 孔板类型

When cells grow in multiple layers (confluency > 100 %) it is difficult to achieve robust and reliable cell detection. Make sure that you use cultures of less than 100 % confluency.

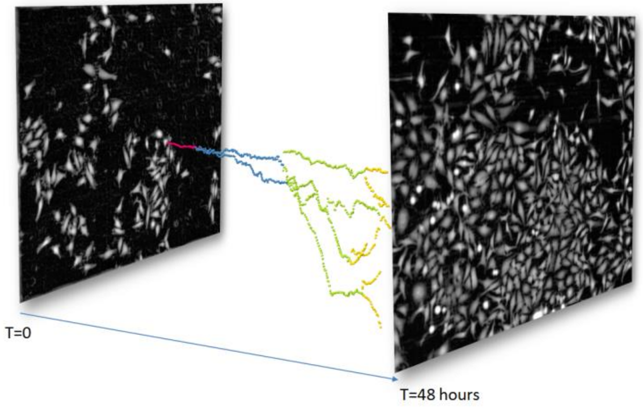
5.荧光类型选择

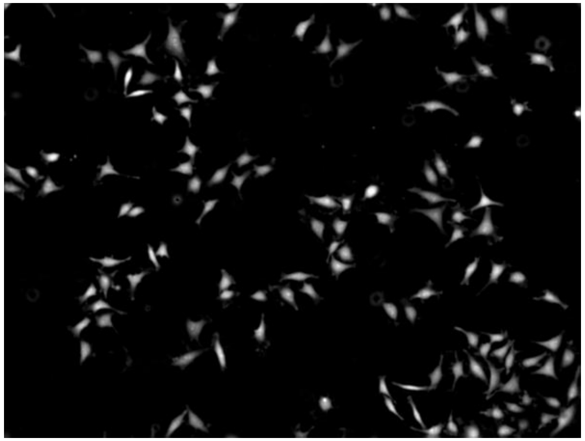


- 是否需要做活细胞实验?
  - 活细胞
    是否需要荧光?
    - 无荧光标记检测
    - 转染荧光蛋白
    - 活细胞染料
    - 活细胞摄入荧光材料或颗粒
  - 固定细胞
    荧光处理?
    - 已有转染蛋白
    - 细胞染料
    - 免疫荧光

5.荧光类型选择

无荧光标记检测



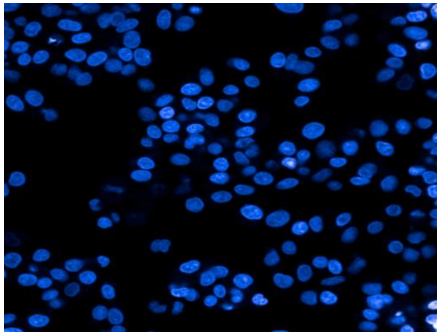


无标记检测模式特点

- □ 对细胞无干预，能最大限度保证细胞状态
- □ 近红外波长检测，最大限度降低光毒性和光损伤
- □ 特别适合长时间动态观察。
- □ 成本最小

无标记模式无亚细胞结构信息

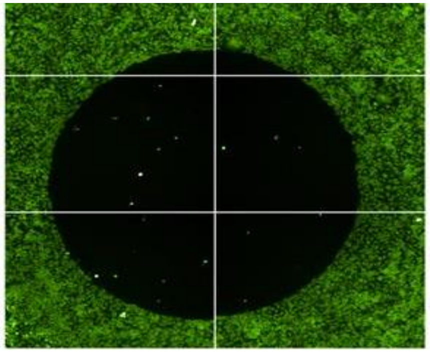
5.荧光类型选择



荧光染料

优点： 有活细胞和固定细胞染料选择，荧光强度高，染色方式简单

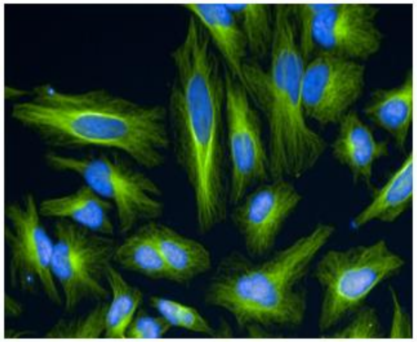
缺点： 活细胞长时间观察随着细胞分裂强度降低



荧光蛋白

优点： 适合活细胞和固定细胞方案检测，特别适合活细胞长时间观测

缺点： 荧光强度相对较低，且荧光强度受细胞状态影响，需要对细胞进行转基因干预



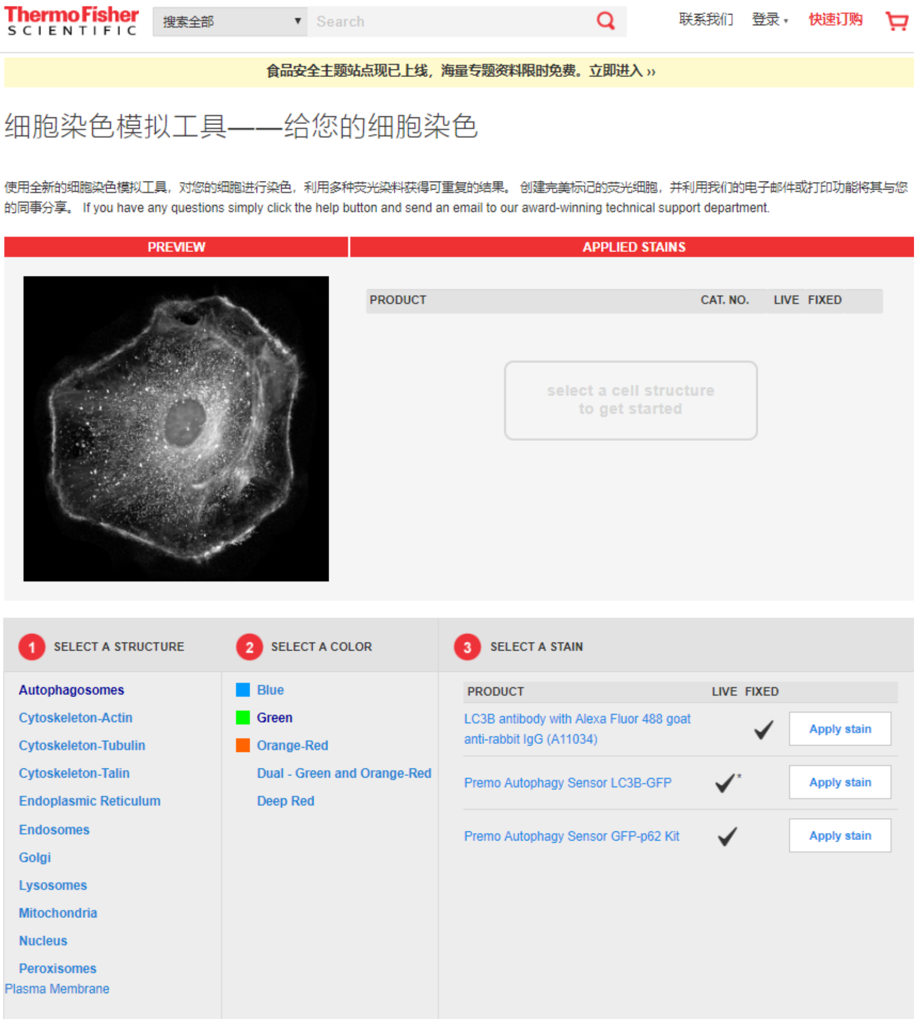
免疫荧光

优点： 荧光强度高，抗体选择多能够满足绝大部分标志蛋白以及信号分子检测

缺点： 免疫荧光步骤相对麻烦，标记效果受抗体质量影响，不适合活细胞检测

5.荧光类型选择

荧光染料，免疫荧光抗体选择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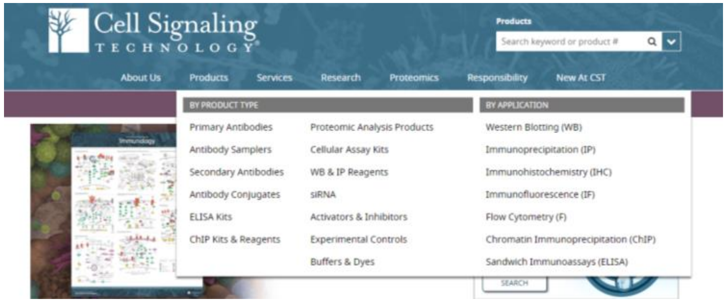
使用全新的细胞染色模拟工具，对您的细胞进行染色，利用多种荧光染料获得可重复的结果。 创建完美标记的荧光细胞，并利用我们的电子邮件或打印功能将其与您的同事分享。 If you have any questions simply click the help button and send an email to our award-winning technical support department.

PREVIEW
APPLIED STAINS
PRODUCT
CAT. NO.
LIVE
FIXED
select a cell structure to get started
1 SELECT A STRUCTURE
Autophagosomes
Cytoskeleton-Actin
Cytoskeleton-Tubulin
Cytoskeleton-Talin
Endoplasmic Reticulum
Endosomes
Golgi
Lysosomes
Mitochondria
Nucleus
Peroxisomes
Plasma Membrane
2 SELECT A COLOR
Blue
Green
Orange-Red
Dual - Green and Orange-Red
Deep Red
3 SELECT A STAIN

PRODUCT	LIVE	FIXED	
LC3B antibody with Alexa Fluor 488 goat anti-rabbit IgG (A11034)		✓	Apply stain
Premo Autophagy Sensor LC3B-GFP	✓*		Apply stain
Premo Autophagy Sensor GFP-p62 Kit	✓		Apply stain


Cell Signaling TECHNOLOGY
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Responsibility
New At CST
BY PRODUCT TYPE
Primary Antibodies
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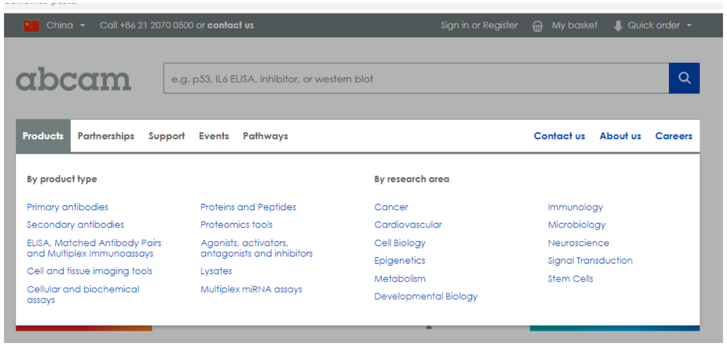
Secondary Antibodies
Antibody Conjugates
ELISA Kits
ChIP Kits & Reagents
Proteomic Analysis Products
Cellular Assay Kits
WB & IP Reagents
siRNA
Activators & Inhibitors
Experimental Controls
Buffers & Dyes
BY APPLICATION
Western Blotting (WB)
Immunoprecipitation (IP)
Immunohistochemistry (IHC)
Immunofluorescence (IF)
Flow Cytometry (F)
Chromatin Immunoprecipitation (ChIP)
Sandwich Immunoassays (ELISA)
SEARCH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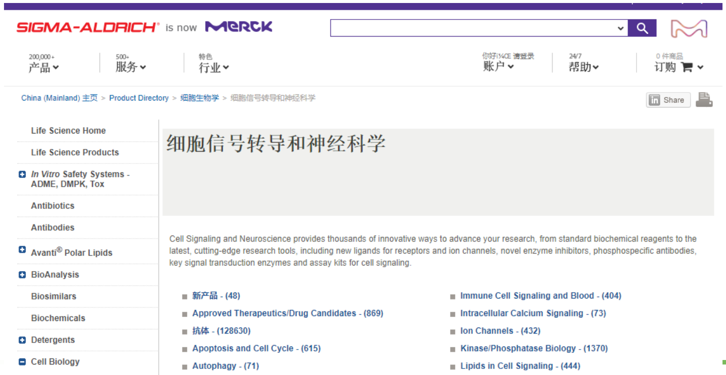
Proteomics tools
Agonists, activators, antagonists and inhibitors
Lysates
Multiplex miRNA assays
By research area
Cancer
Cardiovascular
Cell Biology
Epigenetics
Metabolism
Developmental Biology
Immunology
Microbiology
Neuroscience
Signal Transduction
Stem Cells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细胞信号转导和神经科学

Cell Signaling and Neuroscience provides thousands of innovative ways to advance your research, from standard biochemical reagents to the latest, cutting-edge research tools, including new ligands for receptors and ion channels, novel enzyme inhibitors, phosphospecific antibodies, key signal transduction enzymes and assay kits for cell signaling.

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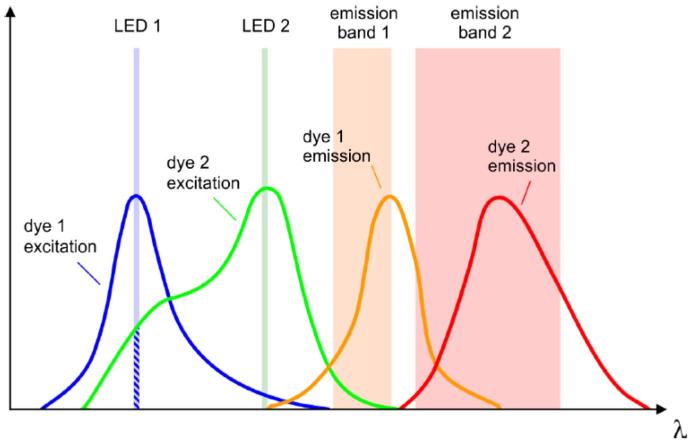
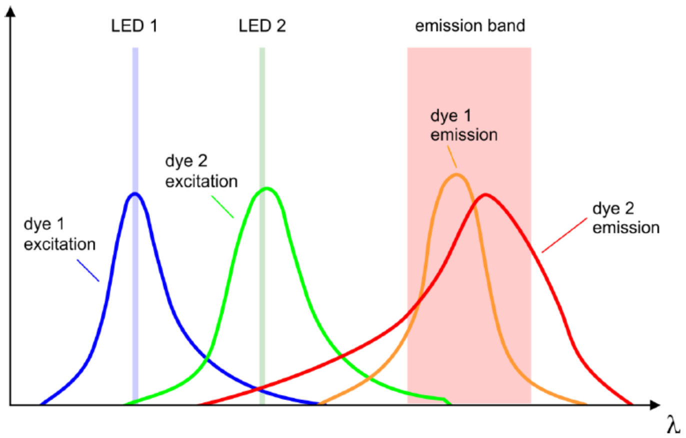
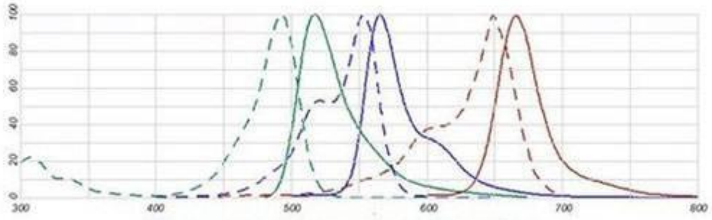
5.荧光类型选择

多荧光检测需要考虑荧光搭配问题，从而防止荧光串扰问题。





选择合适的荧光搭配，需要了解激发和发射波谱

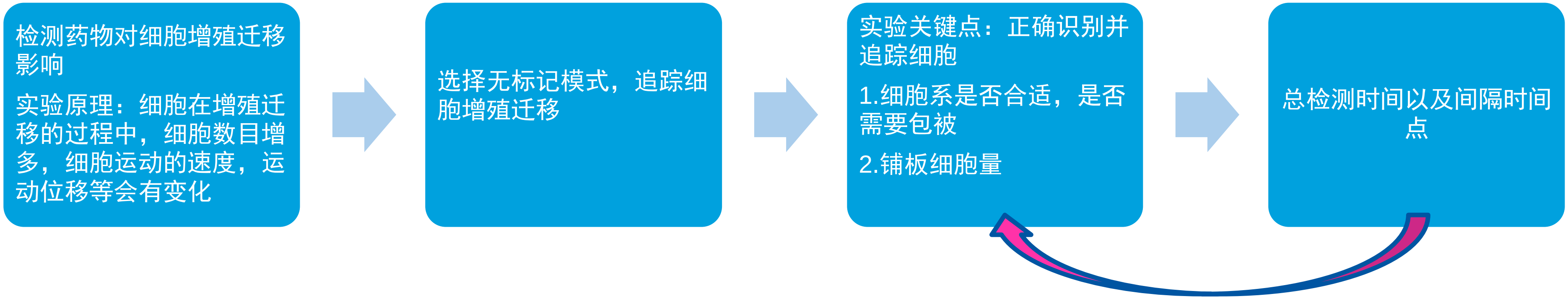


绿色：Apollo®488；蓝色：Apollo®567；红色：Apollo®643

Tips：高内涵检测多荧光不宜超过四个，建议搭配DAPI，ALEX488，ALEX594，ALEX647

6.高内涵实验设计案例

案例一：检测药物对细胞增殖迁移影响



检测药物对细胞增殖迁移影响

实验原理：细胞在增殖迁移的过程中，细胞数目增多，细胞运动的速度，运动位移等会有变化

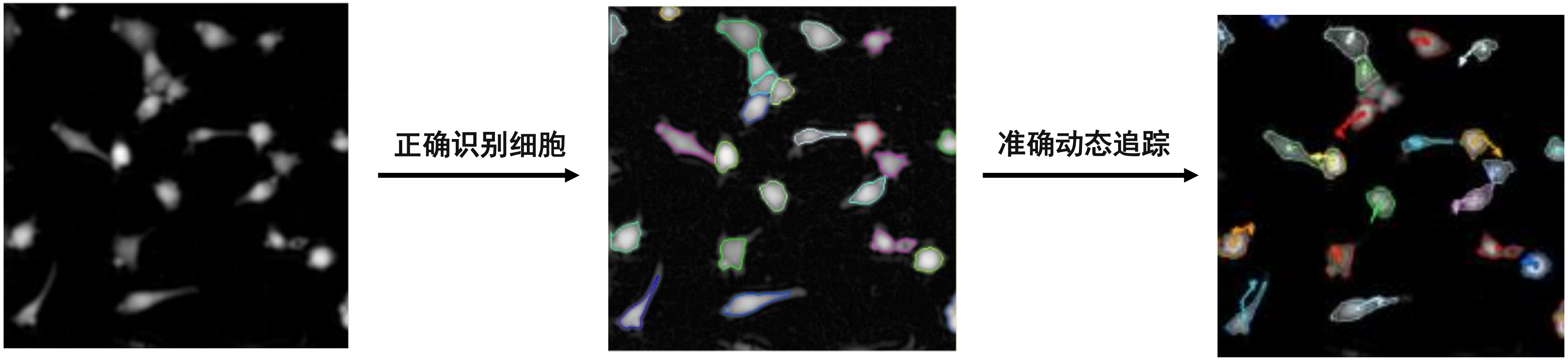
选择无标记模式，追踪细胞增殖迁移

实验关键点：正确识别并追踪细胞

1.细胞系是否合适，是否需要包被

2.铺板细胞量

总检测时间以及间隔时间点



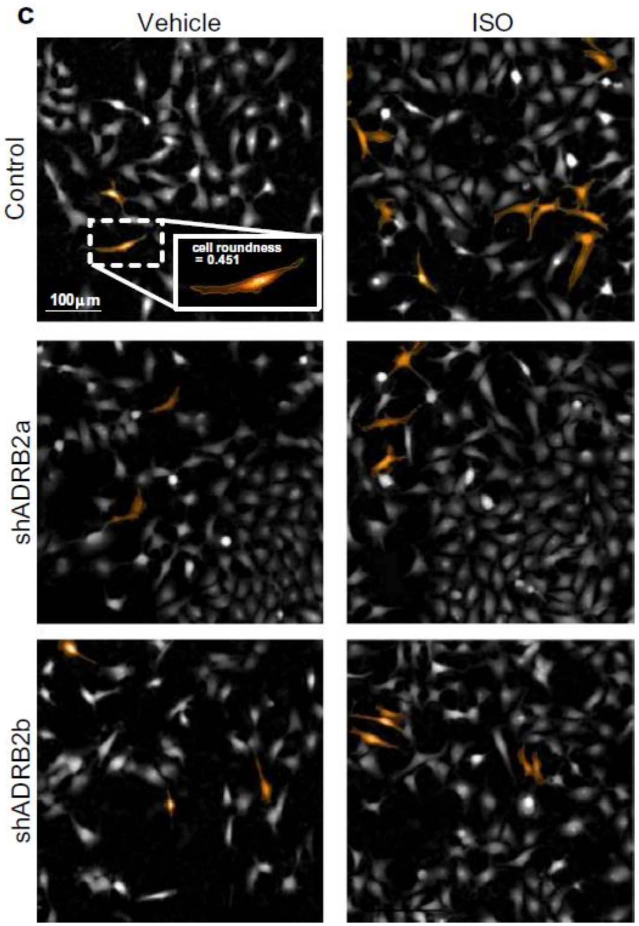
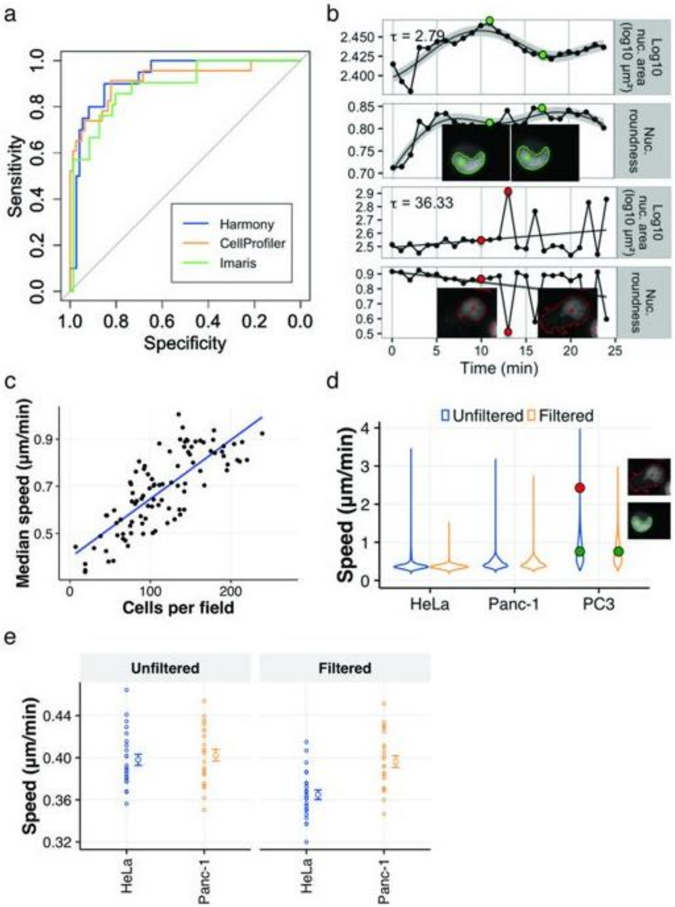

c
Vehicle
ISO
Control
shADRB2a
shADRB2b
cell roundness = 0.451
100µm

Figure 2: Tracking individual cells in heterogeneous populations.



6.高内涵实验设计案例

案例二：检测细胞自噬情况

检测药物,细菌等对细胞自噬的影响

实验原理：细胞自噬发生过程中，如LC3，P62等标志性蛋白表达情况改变

选择标记LC3或者P62，并检测自噬小体个数以及相关蛋白荧光强度

实验关键点：如何标记自噬小体，如何识别

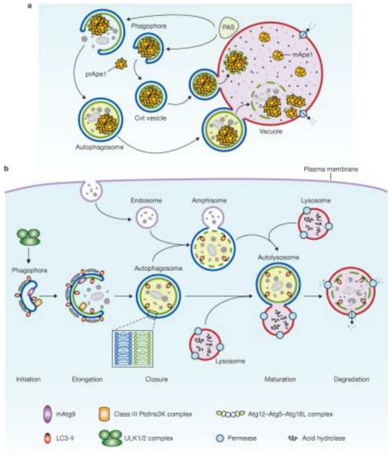
1.是否需要活细胞

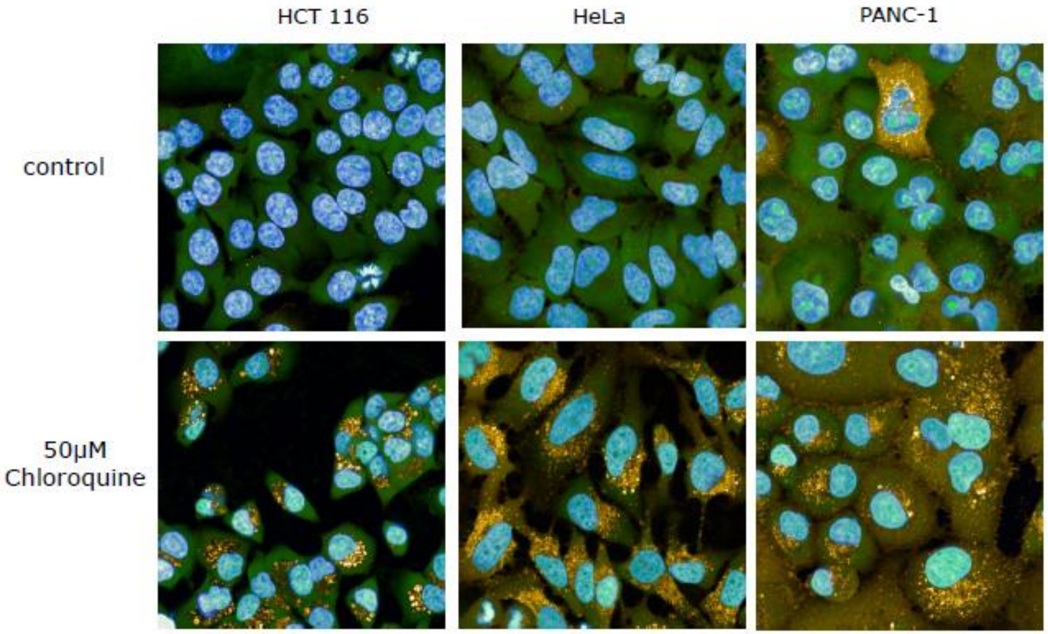
2.LC3-GFP-RFP活细胞，LC3,p62免疫荧光（固定细胞）

3.放大倍数，细胞密度

1.如何选择评价指标

2.如何保持实验体系稳定（自噬影响因素）

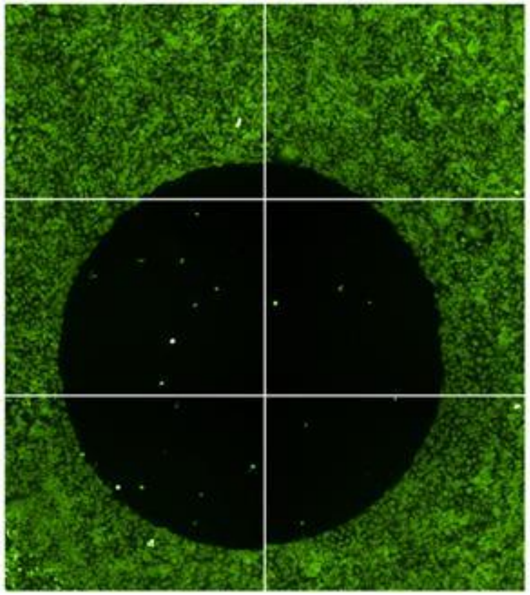

a
Phagophore
PAS
mApe1
prApe1
Cvt vesicle
Autophagosome
Vacuole
b
Plasma membrane
Endosome
Amphisome
Lysosome
Phagophore
Autophagosome
Autolysosome
Lysosome
Initiation
Elongation
Closure
Maturation
Degradation
mAtg9
Class III PtdIns3K complex
Atg12–Atg5–Atg16L complex
LC3-II
ULK1/2 complex
Permease
Acid hydrolase


HCT 116
HeLa
PANC-1
control
50μM
Chloroquine

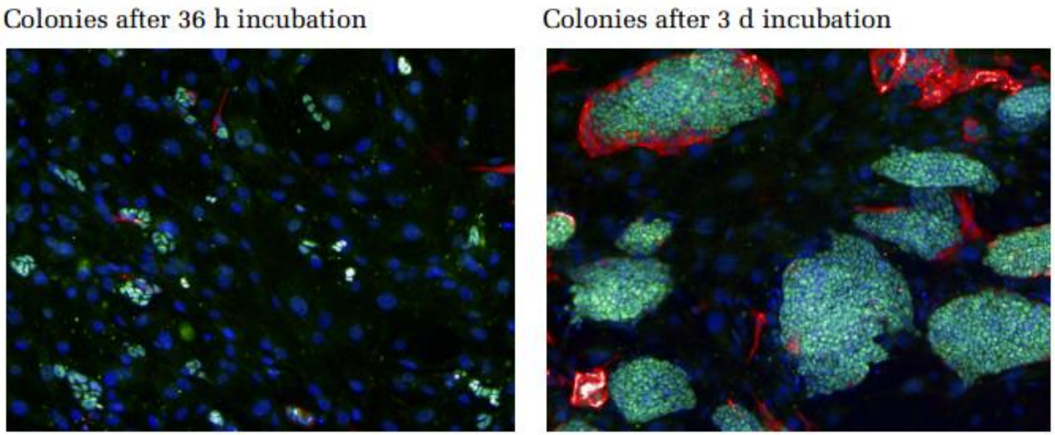
高内涵检测指标

1.细胞群体

细胞群体面积



细胞群体数量

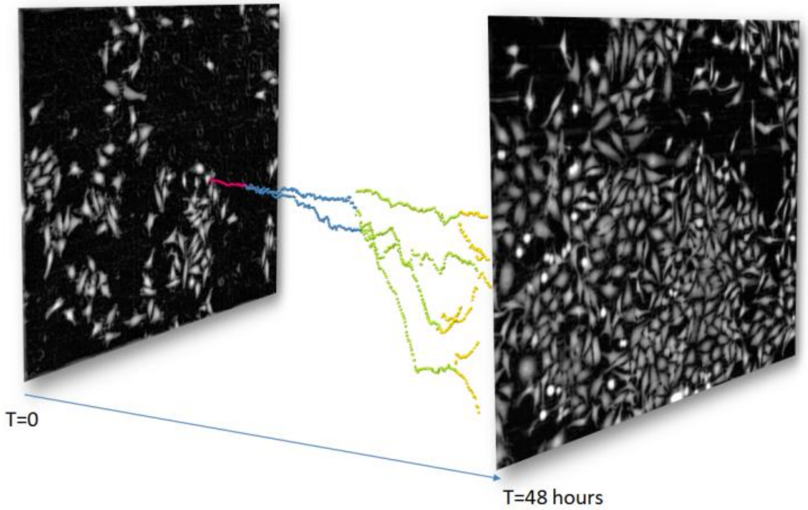


Colonies after 36 h incubation

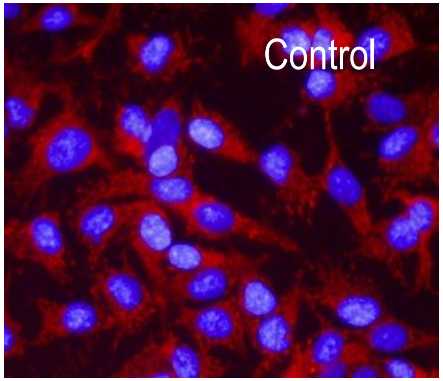
Colonies after 3 d incubation

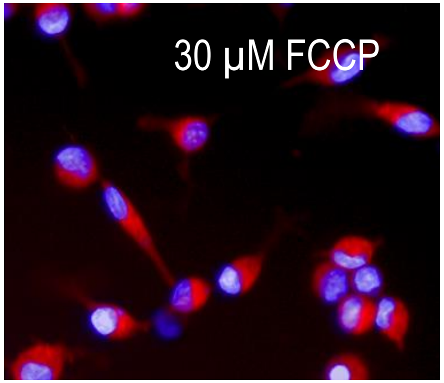
高内涵检测指标

2.1 细胞数量


T=0
T=48 hours

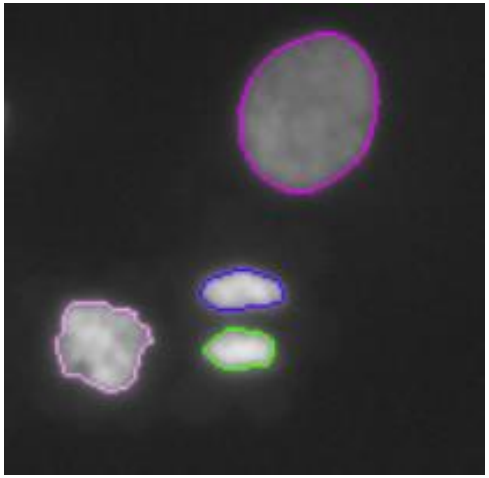
Tracking of HeLa cells on digital phase image. The track of one cell starting at T=0 (red line) is shown over 48 hours. Several cell devisions occur, each color represents a new generation of daughter cells.



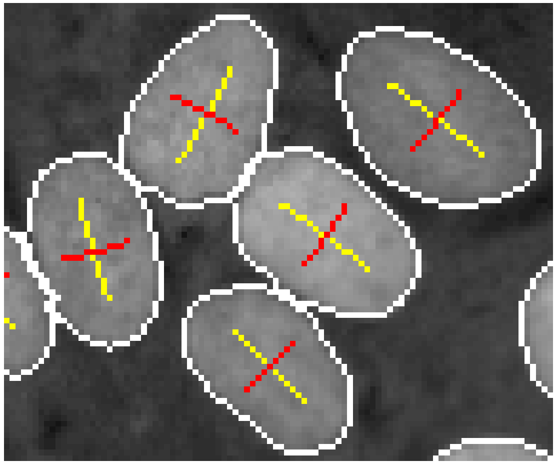


高内涵检测指标

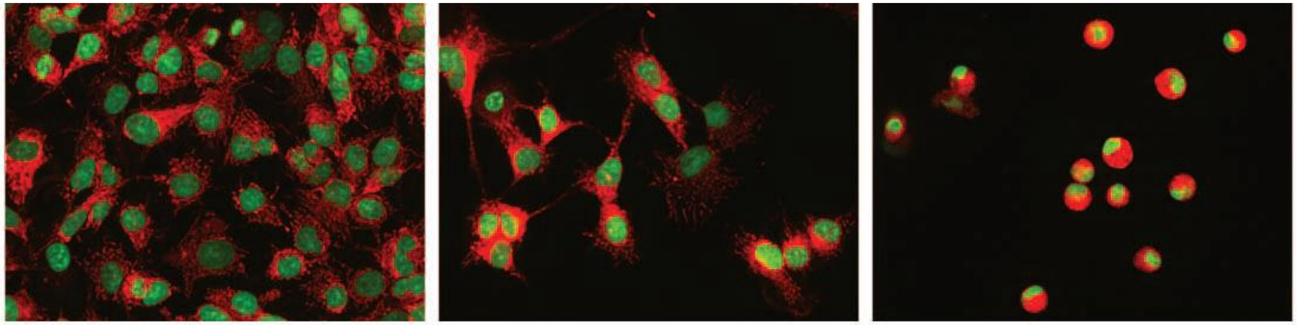
2.2 细胞及荧光形态



细胞及荧光面积

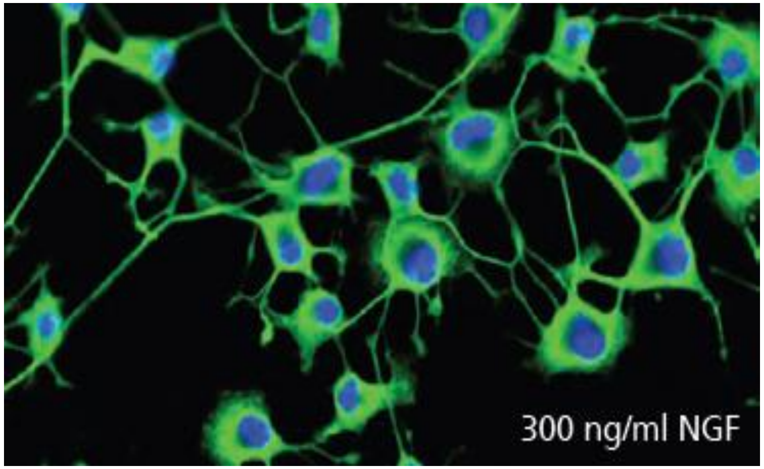


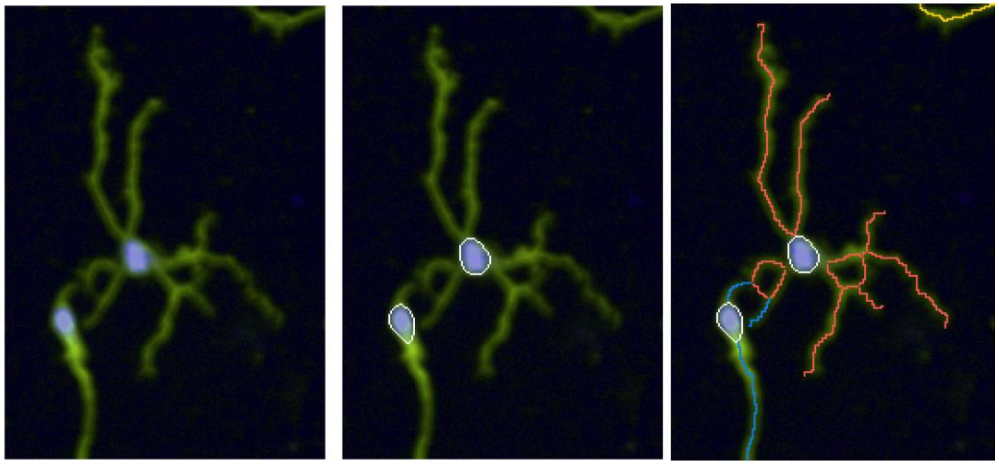
细胞及荧光形状：
圆度，轴向



高内涵检测对象

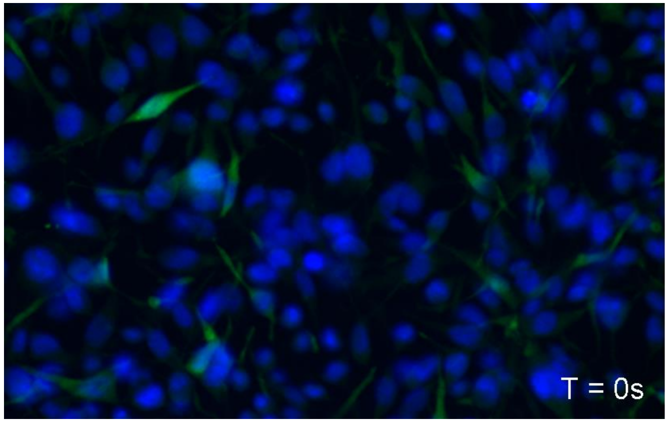
2.3 特殊细胞

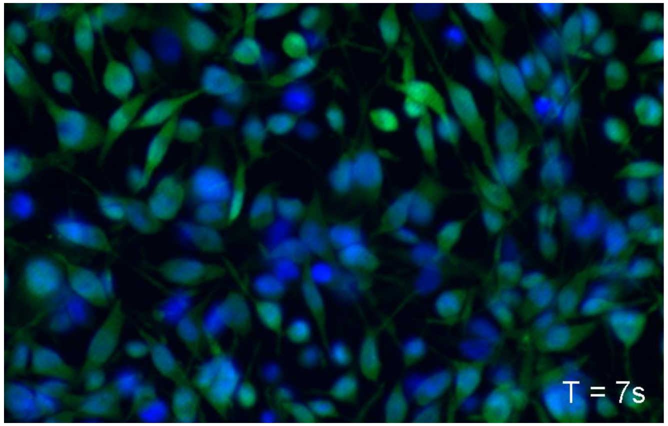

300 ng/ml NGF

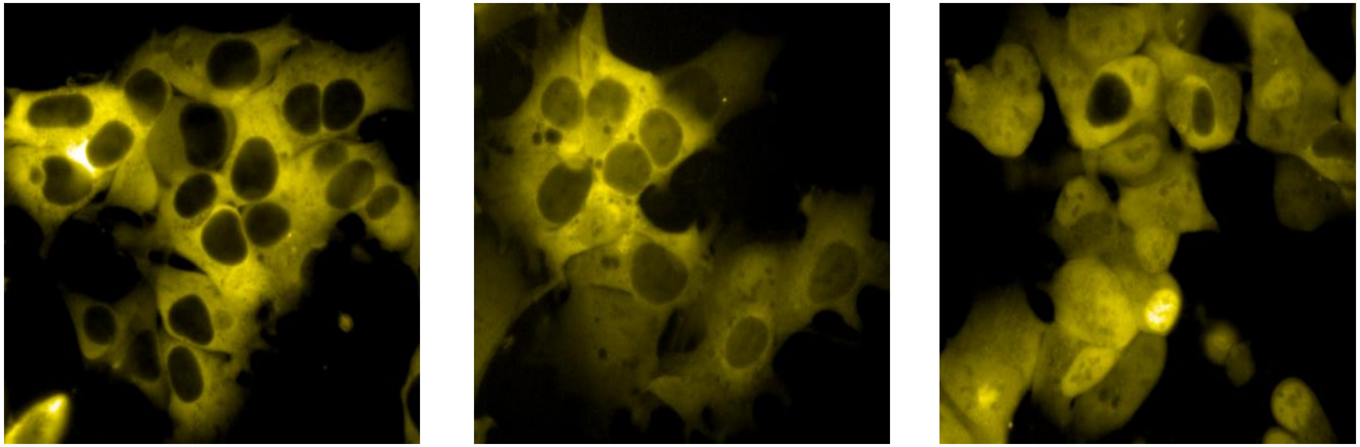


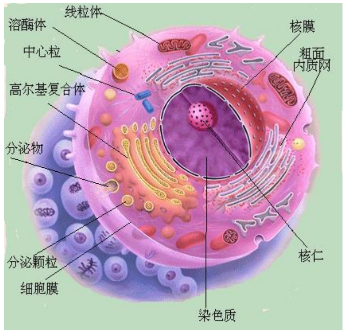
高内涵检测指标

2.4细胞荧光信号强度变化，位置变化



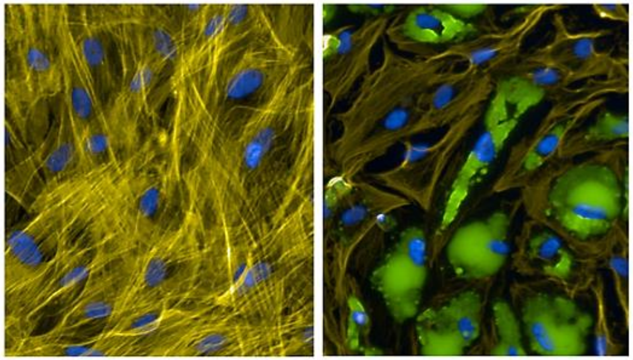


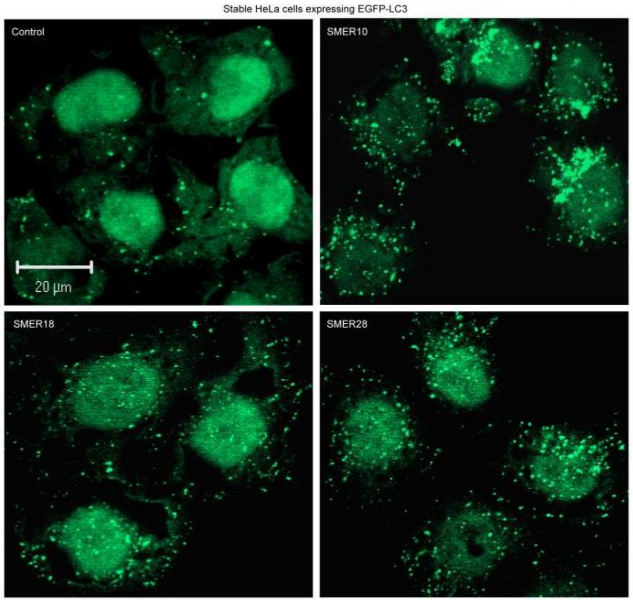


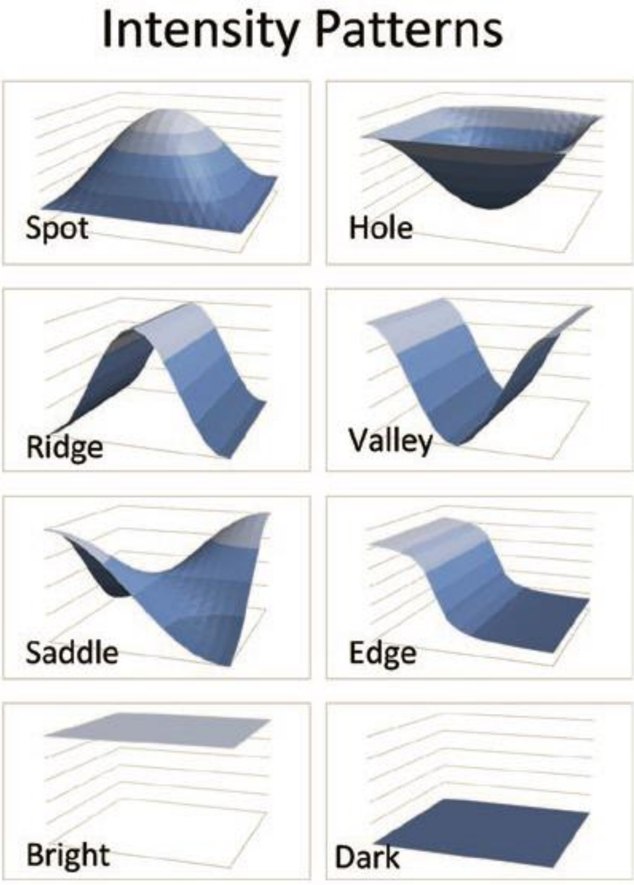

线粒体
溶酶体
中心粒
高尔基复合体
分泌物
分泌颗粒
细胞膜
核膜
粗面内质网
核仁
染色质

高内涵检测对象

2.5 细胞及荧光的纹理

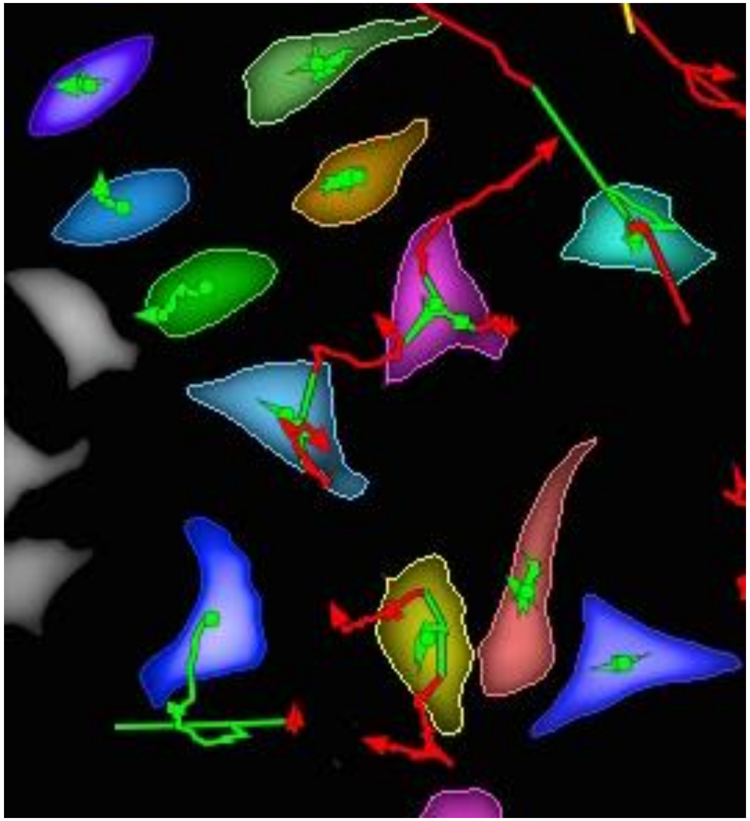


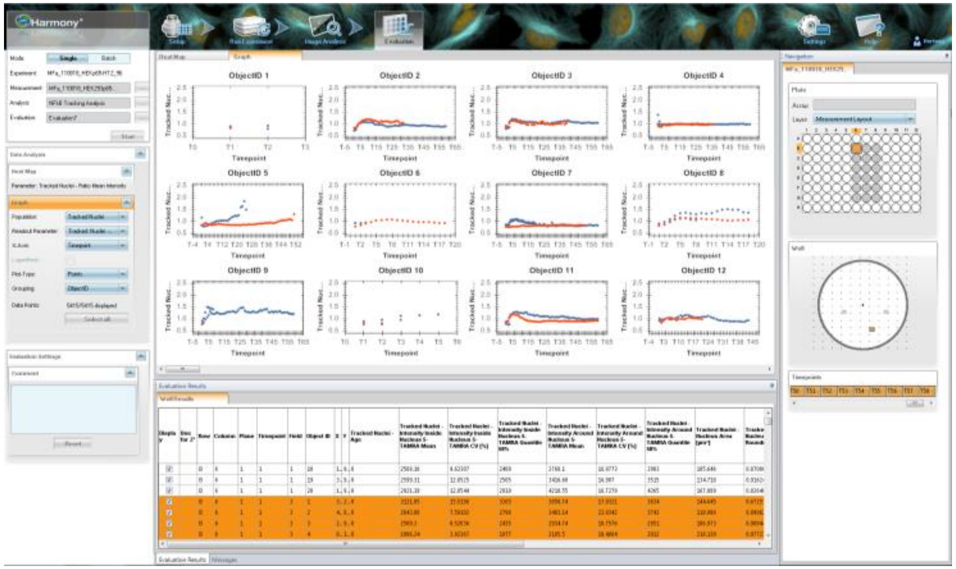

Stable HeLa cells expressing EGFP-LC3
Control
SMER10
20 µm
SMER18
SMER28


Intensity Patterns
Spot
Hole
Ridge
Valley
Saddle
Edge
Bright
Dark

高内涵检测对象

2.6 细胞运动参数




Harmony
Setup
Run Experiment
Image Analysis
Evaluation
Heat Map
Graph
ObjectID 1
ObjectID 2
ObjectID 3
ObjectID 4
ObjectID 5
ObjectID 6
ObjectID 7
ObjectID 8
ObjectID 9
ObjectID 10
ObjectID 11
ObjectID 12
Tracked Nuc...
Timepoint
Evaluation Results
Well Results
Evaluation Results
Messages

Thank You!