

常用细胞成像分析模块

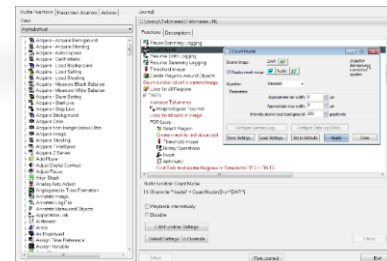
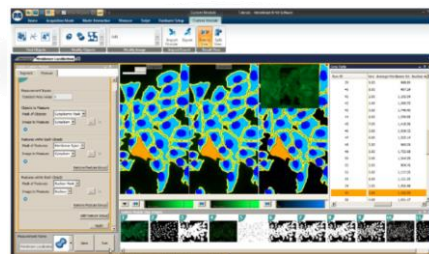
Hui Zhu

Hui.zhu@moldev.com

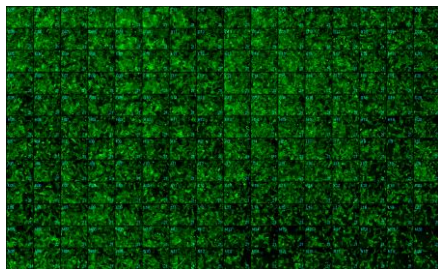
June 2024



MetaXpress Image Analysis Toolbox



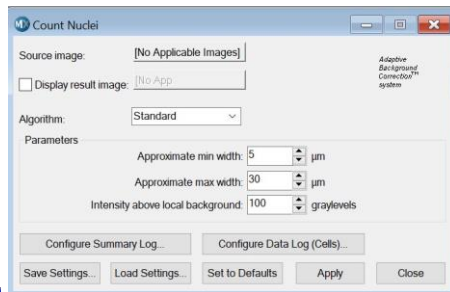
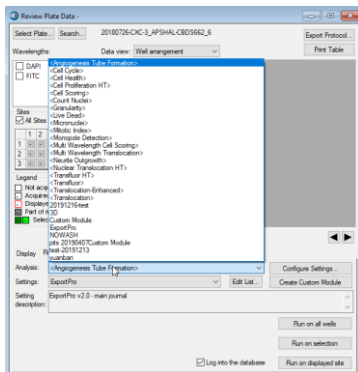
Analysis continuum with increasing flexibility and complexity



从批量拍摄到批量分析

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23
A																							
B																							
C																							
D																							
E																							
F																							
G																							
H																							
I																							
J																							
K																							
L																							
M																							
N																							
O																							
P																							

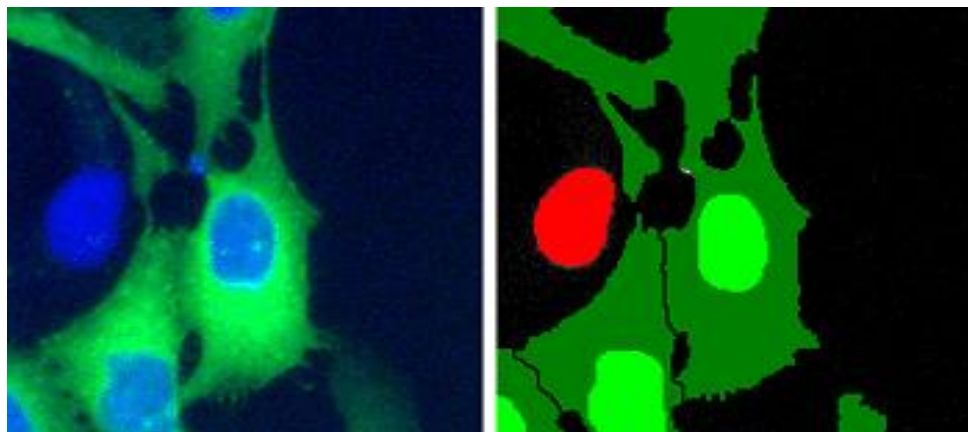
Cellular Results for Transfluor																							
Cell Assigned Label ID	Cell Pk Count	Cell Pk Total Area	Cell Pk Integrated Intensity	Cell Pk Average Intensity	Cell Vessicle Count	Cell Vessicle Total Area	Cell Vessicle Integrated Intensity	Cell Vessicle Average Intensity	Cell Nucleus Integrated Intensity	Cell Nucleus Total Area	Cell Nucleus Average Intensity	Cell Texture Index	Cell Gradient Index	Cell Laplacian Index									
116 116	101	54.4031	112957	556.436	18	31.2018	50492	279.36	325.171	243595	322.534	93.7137	34.6997	71.2611									
117 117	111	54.0032	106959	605.038	28	88.6113	243977	1144.36	322.878	332542	429.534	236.997	262.492	295.379									
118 118	22	11.2327	18447	683.222	3	9.96956	25795	1119.70	323.251	299734	385.790	77.3633	26.0367	20.0943									
119 119	91	44.9147	79688	708.167	17	34.5307	72797	389.12	325.744	36917	464.1	116.919	170.662	130.727									
120 120	123	56.9954	69695	656.241	28	41.9077	123404	1029.752	329.244	302762	381.716	172.157	153.517	111.135									
121 121	114	54.4993	89790	695.42	26	53.6472	107526	1023.535	329.36	348917	427.620	125.695	154.127	122.051									
122 122	124	57.4115	69629	421.149	16	76.1138	149340	179.795	329.902	294469	381.446	153.645	176.426	119.693									
123 123	114	39.9384	60726	715.096	29	71.1403	177690	1039.12	340.941	343084	403.443	246.329	230.342	187.267									
124 124	137	57.8121	107366	592.098	28	64.8999	144401	951.805	359.446	359462	415.26	232.959	189.15	135.180									
avg 136	176	43.7517	977362	676.093	19	47.4146	174481	1047.704	363.115	376983	426.736	176.704	117.424	88.4795									



成熟模块英文名称	中文名称
Angiogenesis Tube Formation	血管生成
Cell Cycle Application Module	细胞周期
Cell Proliferation HT	细胞增殖
Cell Health	细胞健康 (凋亡和坏死)
Cell Scoring	
Multi Wavelength Cell Scoring	细胞分类及多波长细胞分类
Count Nuclei	核计数 (细胞计数)
Granularity	颗粒度分析
Live/Dead	细胞死活
Micronuclei	微核
Mitotic Index	有丝分裂分析
Monopole Detection	单极纺锤体分析
Multi Wavelength Translocation	
Translocation	蛋白转位
Translocation-Enhanced	
Nuclear Translocation HT	核转位分析
Neurite Outgrowth	神经生长
Transfluor	受体内存

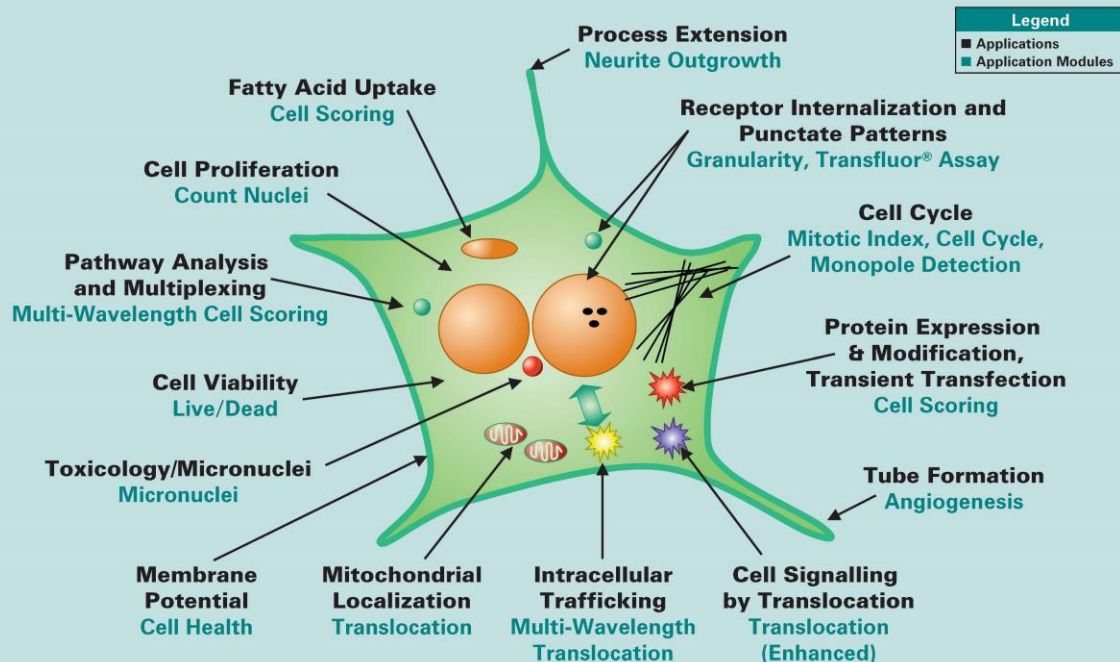
MetaXpress 分析模块

- 无人值守的自动化分析
- 图像分割、特征检测和计算测量
- Site-by-site and cell-by-cell data
- 可自定义分析方法



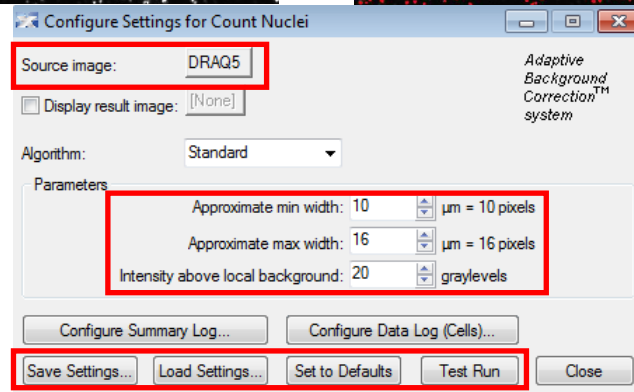
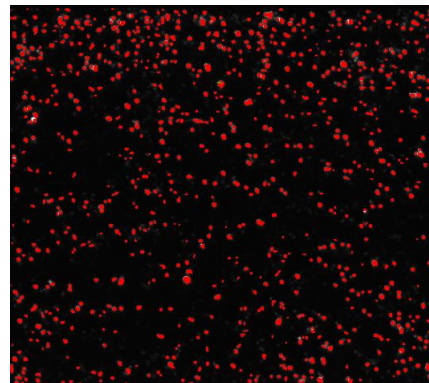
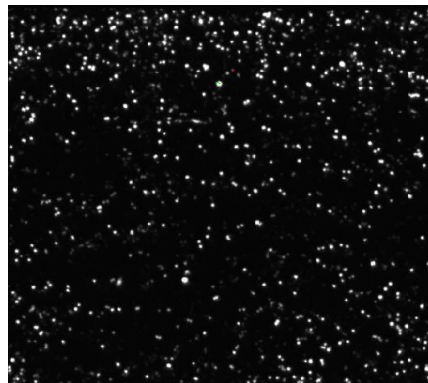
针对细胞生物学的分析模块

Application Modules for Hundreds of Assays Easy Custom Analysis with Journaling

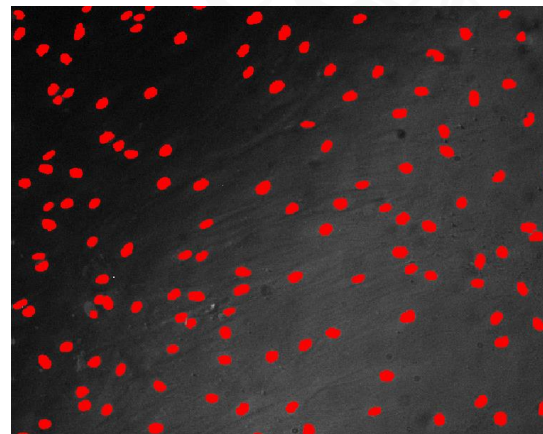
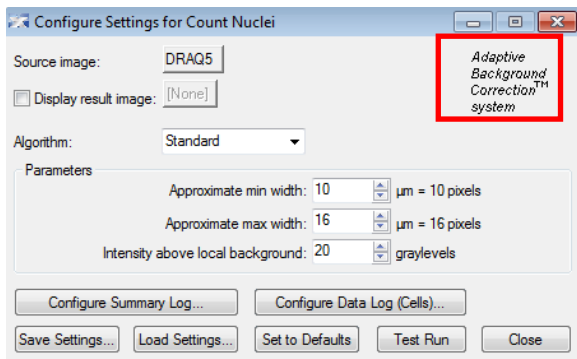


MetaXpress 分析模块

- 所有模块均具有相同的基本设置
- 图像识别参数设置简洁
 - 选择波长
 - 设置目标的大小范围
 - 设置目标高于周围背景的信号强度
 - 测试和保存设置
- 模块会自动分割接触的细胞



自适应背景校正 Adaptive Background Correction



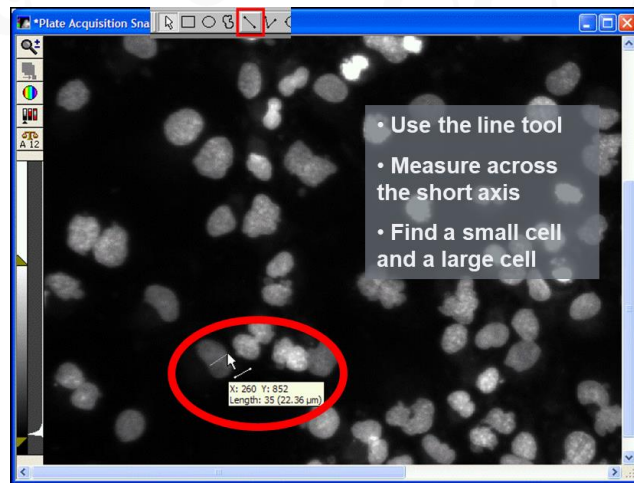
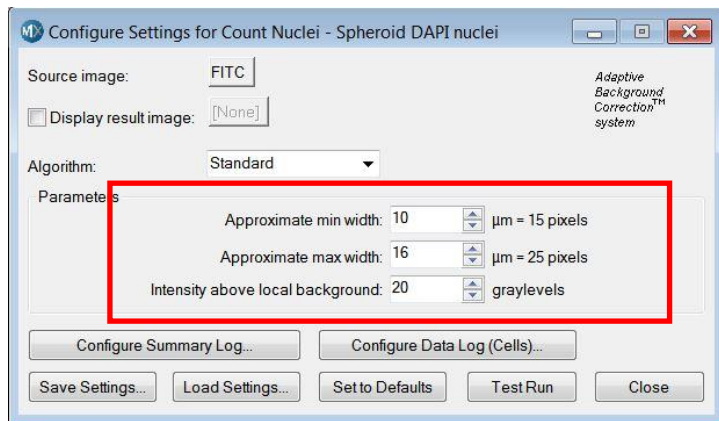
Built in background management

- **Adaptive Background Correction** is automatically performed by each application module
- **Detection even in noisy** and poorly stained images
- **Splits touching cells**
- **Consistent** performance across multiple plates



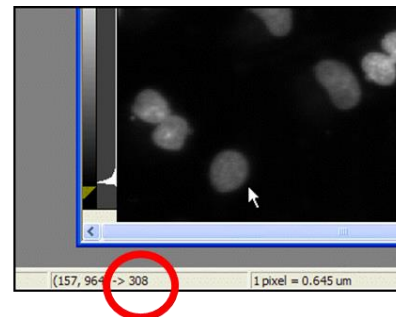
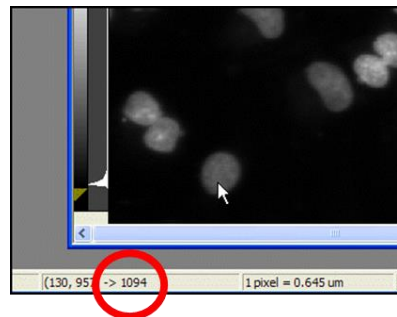
基本设置

➤ Measuring width

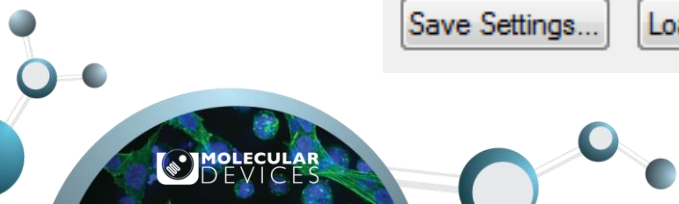
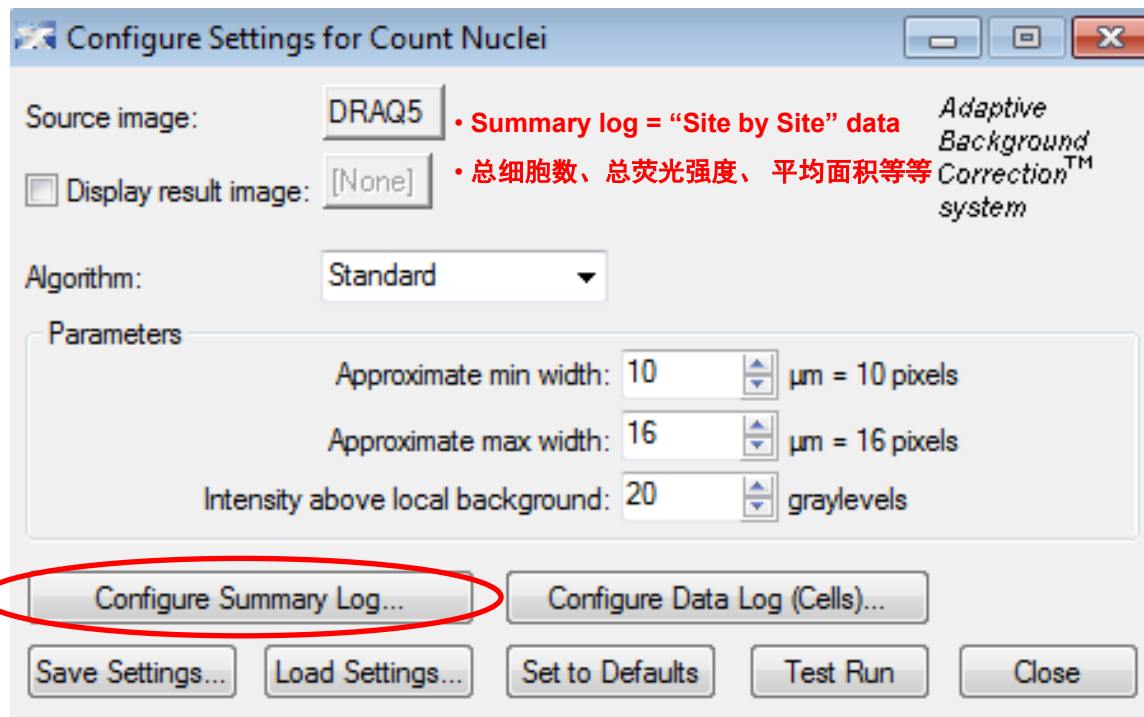


➤ Measuring Intensity above Local Background

1. Find a dim cell
2. Measure intensity just inside and outside the cell
3. Subtract to find the difference ($1094 - 308 = 786$)
4. "Pad" the value by about 100 gray values ($786 - 100 = 686$).



选择测量的数据



选择测量的数据

Configure Settings for Count Nuclei

Source image:

☐ Display result image:

Algorithm:

Parameters

Approximate min width: μm = 10 pixels

Approximate max width: μm = 16 pixels

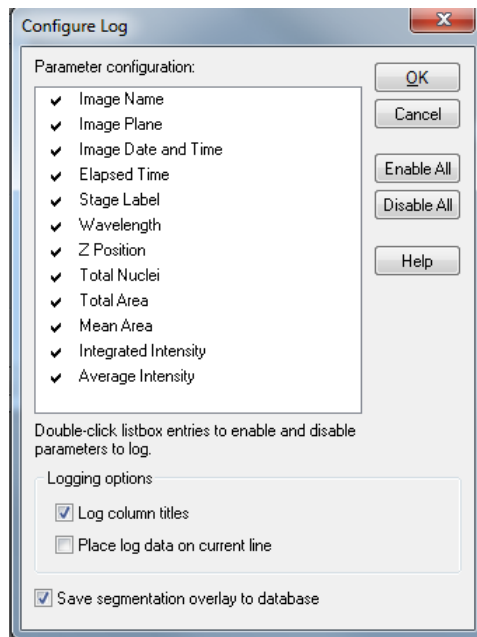
Intensity above local background: graylevels

Adaptive Background Correction™ system

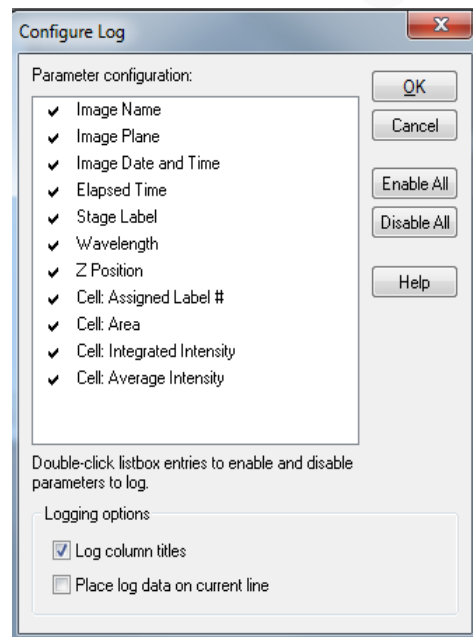
- Data log = "Cell by cell" data
- 每个细胞的面积、荧光强度等等



选择测量的数据



Configure Summary Log



Configure Data Log(Cells)

分析模块 – 交互式反馈

- 测试参数的分割效果

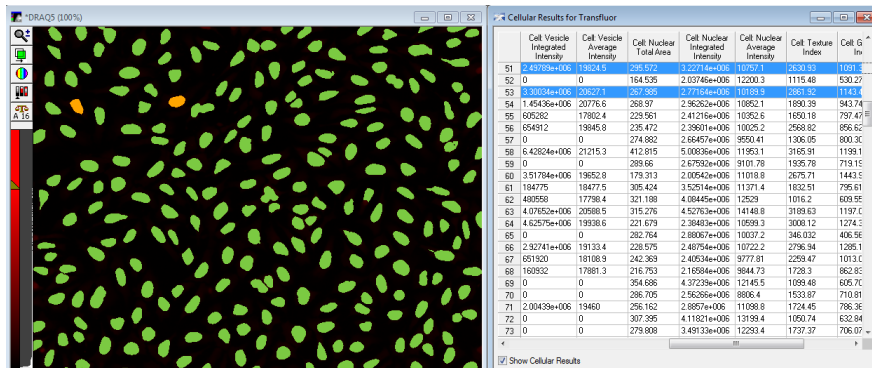
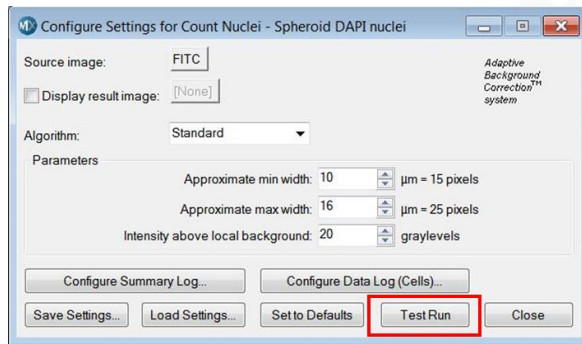
- Test Run

- Preview

- 优化图像分割参数

- 一次只更改一个参数

- 最小宽度对分割结果的影响最大



选择待查看或分析的图像

选择孔板

Review Plate Data -

Select Plate... Search...

Imc20170401_APSHAL-2C4KWT2_17

Export Protocol...

Print Table

Wavelengths:

☒ DAPI
☒ TRITC

Sites

☐ All Sites

Legend

- ☐ Not acquired
- ☐ Acquired, not measured
- ☐ Displayed well
- ☐ Part of montage
- ☒ Selected wells

Data view: Well arrangement

	01	02	03	04	05	06	07	08	09	10	11	12
A												
B		80.01	89.65	58.89								
C		64.98	74.04	144.18								
D		98.15	117.68	97.10								
E												
F												
G												
H												

Montage: 3 x 3



选择分析模块和已设置的方法

The screenshot shows the 'Run Analysis' window of a software application. The window has a tabbed interface with 'Run Analysis' selected. It contains several input fields and buttons. Red arrows point from Chinese labels to specific UI elements:

- 选择模块** (Select Module) points to the 'Analysis:' dropdown menu.
- 选择方法** (Select Method) points to the 'Settings:' dropdown menu.
- 新建方法** (New Method) points to the 'Configure Settings...' button.
- 分析所有孔** (Analyze All Wells) points to the 'Run on all wells' button.
- 分析已选孔** (Analyze Selected Wells) points to the 'Run on selection' button.

Other visible UI elements include:

- Buttons: 'Edit List...', 'Create Custom Module', 'Run on all wells', 'Run on selection', 'Run on displayed site', 'Load Selected Images', 'Reset Image Displays', 'Cellular Results...', 'Clear Selection', and 'Close'.
- Text: 'Display', 'Measurements', 'Graph' (tabs); '<Count Nuclei>' (Analysis); '1' (Settings); 'Counts nuclei using a single nuclear stain.' (Setting description); 'Log into the database' (checkbox).
- Decorative elements: A molecular structure graphic in the bottom left corner.

选择分析模块和已设置的方法

选择显示的数据

The screenshot displays a software window with the following components:

- Analysis:** A dropdown menu set to "Count Nuclei: 1: 4".
- Export Settings:** A button.
- Show Heat Map:** A checked checkbox.
- Heat Map...:** A button.
- Measurement:** A dropdown menu set to "Cell: Area (CountNuclei)".
- Display Format:** A dropdown menu set to "#.##".
- Select Wells Based On Variable Range:** A section containing:
 - Value is:** A dropdown menu set to "Between".
 - 0** and **100**: Two input fields with up/down arrows.
 - Select:** A button.
- Press "Open Log" button to start exporting the table**: A text instruction.
- Data Log Not Open**: A text status.
- Configure Log...:** A button.
- Open Log:** A button.
- Load Selected Images:** A button.
- Navigate Selections:** A section with left and right arrow buttons.
- Clear Selection:** A button.
- Reset Image Displays:** A button.
- Cellular Results...:** A button.
- ?**: A help icon button.
- Close:** A button.

选择显示热图

选择数据查看方式

Review Plate Data -

Select Plate... Search... Imc20170401_APSHAL-2C4KWT2_17

Wavelengths: ☒ DAPI ☒ TRITC

Sites ☐ All Sites

Legend

- ☐ Not acquired
- ☐ Acquired, not measured
- ☐ Displayed well
- ☐ Part of montage
- ☒ Selected wells

Data view: Well arrangement

Montage: 3 x 3

	01	02	03	04	05	06	07	08	09	10	11	12
A												
B	80.01	89.65	58.89									
C	64.98	74.04	144.18									
D	98.15	117.68	97.10									
E												
F												
G												
H												

单数据查看
全数据查看

Data view: Measurement vs Well

Print Table

	Cell Area (CountNuclei)	Cell Integrated Intensity (CountNuclei)	Cell Average Intensity (CountNuclei)	Laser focus score (CountNuclei)	Total Nuclei (CountNuclei)	Total Area (CountNuclei)	Mean Area (CountNuclei)	Integrated Intensity (CountNuclei)	Average Intensity (CountNuclei)
A12									
B01									
B02	80.01	610621.75	8428.57	30.00	2993.00	239478.05	80.01	1827590912.00	12857.61
B03	89.65	505112.77	7706.18	24.70	2611.00	234074.88	89.65	1318849408.00	9492.70
B04	58.89	299804.20	7483.20	18.57	4543.00	267536.78	58.89	1362010496.00	8577.22
B05									
B06									
B07									
B08									
B09									
B10									

数据导出

Analysis: Count Nuclei: 1: 4 Export Settings ☒ Show Heat Map Heat Map...

Measurement: Cell: Area (CountNuclei) Display Format: ###

Select Wells Based On Variable Range

Value is: Between 0 and 100 Select

Press "Open Log" button to start exporting the table
Data Log Not Open

Configure Log... **Open Log**

Load Selected Images Navigate Selections ◀ ▶ Clear Selection

Reset Image Displays Cellular Results... ? Close

Open Data Log

Log Measurements to:

☒ Dynamic Data Exchange (DDE)

☐ A text file

OK Cancel

Export Log Data

Application: Microsoft Excel

Sheet Name: data OK Cancel

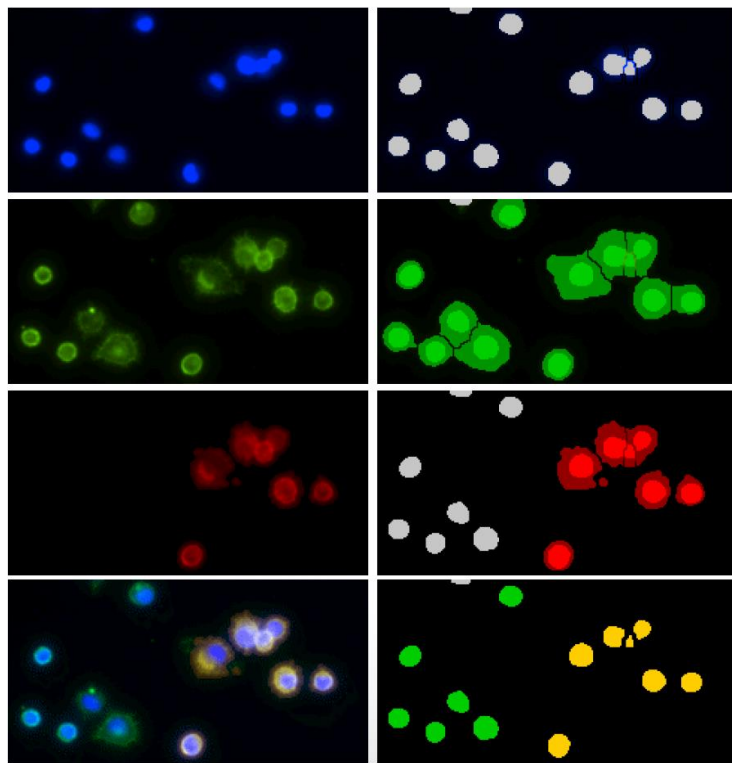
Starting Row: 1

Starting Column: 1 Default

Multi Wavelength Cell Scoring

多波长细胞分类

应用模块-多波长细胞分类



“对细胞图像中各个染料通道的信号进行识别和计算，通过不同通道信号组合来进行多参数的细胞分析”

- The MWCS module can be used to analyze cells imaged in 1-7 wavelengths
- The first wavelength should always be a stain for all nuclei (e.g. DAPI, Hoechst, or DRAQ5)
- With just 1 wavelength, it can be used for basic cell counting
- With 2 or more wavelengths, it will score cells as positive or negative for each wavelength and assign it a “profile” of its score in each wavelength

应用模块-多波长细胞分类

Number of wavelengths: 7 ———— 最多支持7个通道

Adaptive Background Correction™ system

Algorithm: Fast

All nuclei W2 W3 W4 W5 W6 W7

Name: All nuclei

W1 Source image: DAPI ———— 选择对应的图像

Legend color: Gray

Stained area: Nucleus

Approximate min width: 20 价 = 116 pixels

Approximate max width: 100 价 = 578 pixels ———— 图像识别的参数

Intensity above local background: 300 graylevels

Preview ———— 图像识别测试按钮

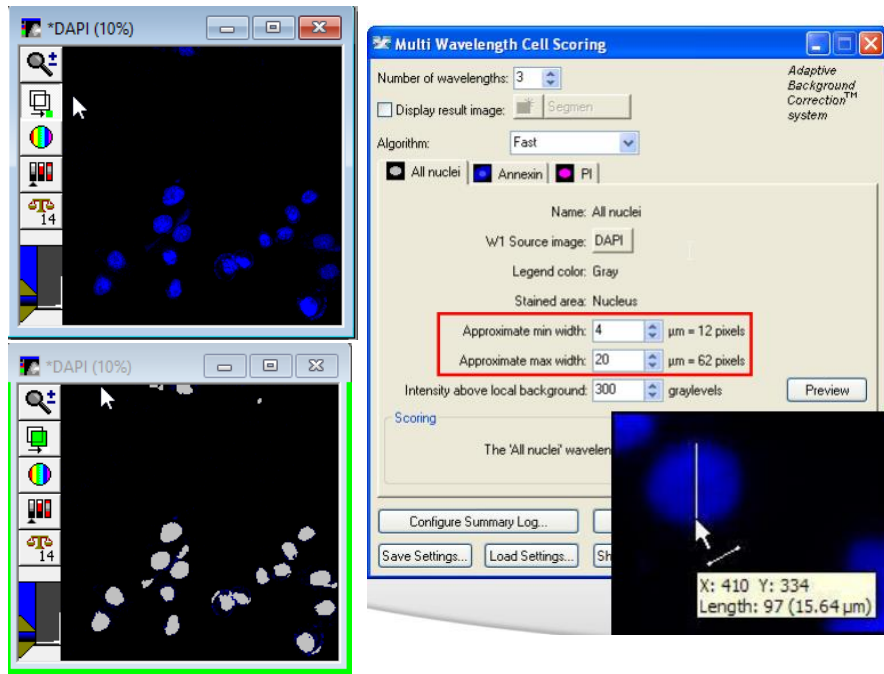
Scoring

The 'All nuclei' wavelength is a required stain for all cells.

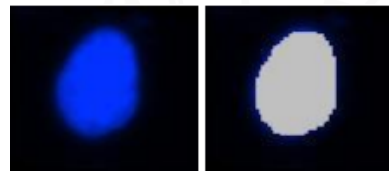
Configure Summary Log... Configure Data Log (Cells)... ———— 选择需要计算的数据

Save Settings... Load Settings... Show Legend... Test Run Close

应用模块-多波长细胞分类



参数设置适合



Min width 设置数值过小



分裂细胞核

Min width 设置数值过大

漏掉小细胞核

Max width 设置数值过小



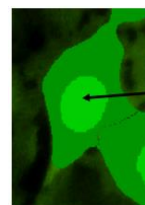
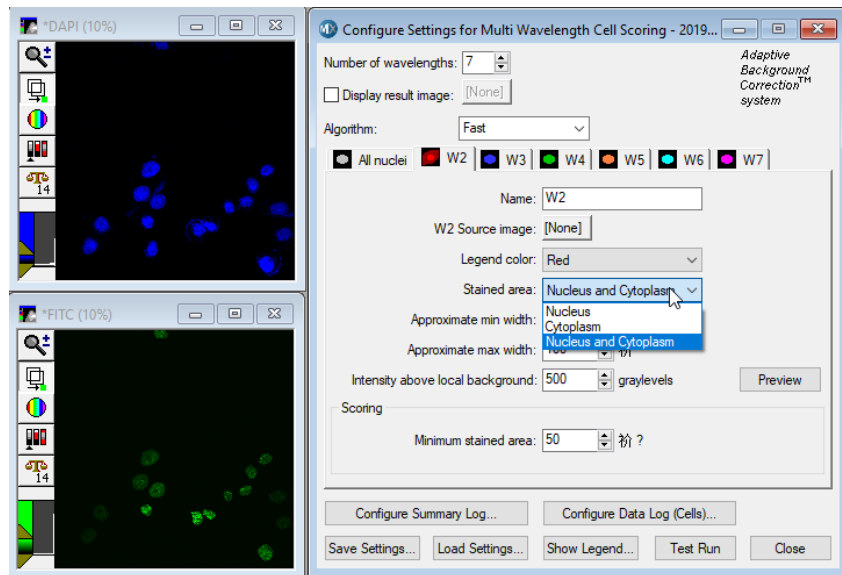
缩小核边界

Max width 设置数值过大



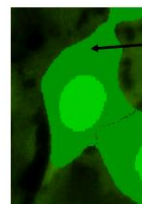
可能将多核合并

应用模块-多波长细胞分类



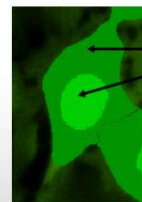
Nucleus

信号存在于细胞核



Cytoplasm

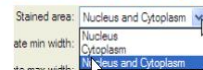
信号存在于细胞质



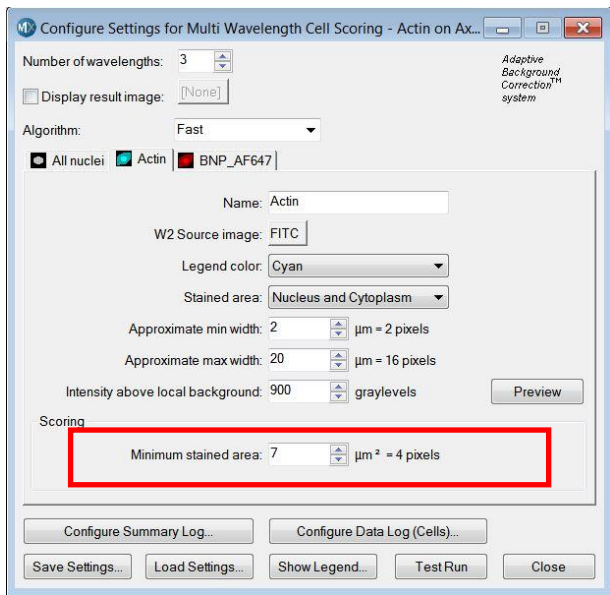
Cell (nucleus +

信号存在于细胞质
和细胞核

Stained Area (as
defined in settings)



应用模块-多波长细胞分类



Wavelength 2 (W2)

- Minimum stained area
- 最小染色面积是区分阳性细胞和阴性细胞的附加标准
- 若判定为阳性，则细胞的染色面积要达到或者比这个数值大
- 这有助于消除假阳性(具有一小块非常明亮的碎片的细胞)
- 可以使用圆形或其他区域选择工具，对图像中一个小的细胞面积进行测量



应用模块-多波长细胞分类

- ✓ Total Cells
- ✓ All Cells Mean Area
- ✓ All Nuclei Mean Area
- ✓ All Nuclei Mean Integr Intensity
- ✓ All Nuclei Mean Average Intensity
- ✓ All W2 Mean Stain Area
- ✓ All W2 Mean Stain Integr Intensity
- ✓ All W2 Mean Stain Aver Intensity
- ✓ All W2 Mean Nucl Integr Intensity
- ✓ All W2 Mean Nucl Aver Intensity
- ✓ All W2 Mean Cyto Integr Intensity
- ✓ All W2 Mean Cyto Aver Intensity
- ✓ All W2 Mean Cell Integr Intensity
- ✓ All W2 Mean Cell Aver Intensity
- ✓ Positive W2 Mean Stain Area
- ✓ Positive W2 Mean Stain Integr Intensity
- ✓ Positive W2 Mean Stain Aver Intensity
- ✓ Positive W2 Mean Nucl Integr Intensity
- ✓ Positive W2 Mean Nucl Aver Intensity
- ✓ Positive W2 Mean Cyto Integr Intensity
- ✓ Positive W2 Mean Cyto Aver Intensity
- ✓ Positive W2 Mean Cell Integr Intensity
- ✓ Positive W2 Mean Cell Aver Intensity
- ✓ Negative W2 Mean Stain Area
- ✓ Negative W2 Mean Stain Integr Intensity
- ✓ Negative W2 Mean Stain Aver Intensity
- ✓ Negative W2 Mean Nucl Integr Intensity
- ✓ Negative W2 Mean Nucl Aver Intensity
- ✓ Negative W2 Mean Cyto Integr Intensity
- ✓ Negative W2 Mean Cyto Aver Intensity
- ✓ Negative W2 Mean Cell Integr Intensity
- ✓ Negative W2 Mean Cell Aver Intensity
- ✓ Positive W3
- ✓ % Positive W3
- ✓ Scoring Profile 1--
- ✓ Scoring Profile 12-
- ✓ Scoring Profile 1-3
- ✓ Scoring Profile 123
- ✓ Percentage Scoring Profile 1--
- ✓ Percentage Scoring Profile 12-
- ✓ Percentage Scoring Profile 1-3
- ✓ Percentage Scoring Profile 123
- ✓ Subtotal Profile 1xx
- ✓ Subtotal Profile 12x
- ✓ Subtotal Profile 1x3
- ✓ Subtotal Profile 123
- ✓ Percentage Subtotal Profile 1xx
- ✓ Percentage Subtotal Profile 12x
- ✓ Percentage Subtotal Profile 1x3
- ✓ Percentage Subtotal Profile 123

细胞总数量和平均面积

W2 通道分别在核区、细胞质和全细胞的荧光强度

W2 通道阳性条件下的细胞数量百分比

各通道间的逻辑组合分析

- ✓ Cell: Assigned Label #
- ✓ Cell: Scoring Profile
- ✓ Cell: Custom Profile Name
- ✓ Cell: Total Area
- ✓ W1 Stained Area
- ✓ W1 Integrated Intensity
- ✓ W1 Average Intensity
- ✓ Cell: Positive W2
- ✓ Cell: W2 Stained Area
- ✓ Cell: W2 Stained Integr Intensity
- ✓ Cell: W2 Stained Average Intensity
- ✓ Cell: W2 Nucleus Integr Intensity
- ✓ Cell: W2 Nucleus Average Intensity
- ✓ Cell: W2 Cytoplasm Integr Intensity
- ✓ Cell: W2 Cytoplasm Average Intensity
- ✓ Cell: W2 Cell Integr Intensity
- ✓ Cell: W2 Cell Average Intensity
- ✓ Cell: Positive W3
- ✓ Cell: W3 Stained Area
- ✓ Cell: W3 Stained Integr Intensity
- ✓ Cell: W3 Stained Average Intensity
- ✓ Cell: W3 Nucleus Integr Intensity
- ✓ Cell: W3 Nucleus Average Intensity
- ✓ Cell: W3 Cytoplasm Integr Intensity
- ✓ Cell: W3 Cytoplasm Average Intensity
- ✓ Cell: W3 Cell Integr Intensity
- ✓ Cell: W3 Cell Average Intensity

应用模块-多波长细胞分类

✓ Total Cells	✓ All W3 Mean Cyto Integr Intens	→ 细胞总数量和平均面积
✓ All Cells Mean Area	✓ All W3 Mean Cyto Aver Intens	
✓ All Nuclei Mean Area	✓ All W3 Mean Cell Integr Intens	→ W2 通道分别在核区、细胞质和全细胞的荧光强度
✓ All Nuclei Mean Integr Intens	✓ All W3 Mean Cell Aver Intens	
✓ All Nuclei Mean Average Intens	✓ Positive W3 Mean Stain Area	→ W2 通道阳性条件下的细胞数量百分比
✓ All W2 Mean Stain Area	✓ Positive W3 Mean Stain Integr Intens	
✓ All W2 Mean Stain Integr Intens	✓ Positive W3 Mean Stain Aver Intens	→ 各通道间的逻辑组合分析
✓ All W2 Mean Stain Aver Intens	✓ Positive W3 Mean Nucl Integr Intens	
✓ All W2 Mean Nucl Integr Intens	✓ Positive W3 Mean Nucl Aver Intens	
✓ All W2 Mean Nucl Aver Intens	✓ Positive W3 Mean Cyto Integr Intens	
✓ All W2 Mean Cyto Integr Intens	✓ Positive W3 Mean Cyto Aver Intens	
✓ All W2 Mean Cyto Aver Intens	✓ Positive W3 Mean Cell Integr Intens	
✓ All W2 Mean Cell Integr Intens	✓ Positive W3 Mean Cell Aver Intens	
✓ All W2 Mean Cell Aver Intens	✓ Negative W3 Mean Stain Area	
✓ Positive W2 Mean Stain Area	✓ Negative W3 Mean Stain Integr Intens	
✓ Positive W2 Mean Stain Integr Intens	✓ Negative W3 Mean Stain Aver Intens	
✓ Positive W2 Mean Stain Aver Intens	✓ Negative W3 Mean Nucl Integr Intens	
✓ Positive W2 Mean Nucl Integr Intens	✓ Negative W3 Mean Nucl Aver Intens	
✓ Positive W2 Mean Nucl Aver Intens	✓ Negative W3 Mean Cyto Integr Intens	
✓ Positive W2 Mean Cyto Integr Intens	✓ Negative W3 Mean Cyto Aver Intens	
✓ Positive W2 Mean Cyto Aver Intens	✓ Negative W3 Mean Cell Integr Intens	
✓ Positive W2 Mean Cell Integr Intens	✓ Negative W3 Mean Cell Aver Intens	
✓ Positive W2 Mean Cell Aver Intens	✓ Positive W3	
✓ Negative W2 Mean Stain Area	✓ % Positive W3	
✓ Negative W2 Mean Stain Integr Intens	✓ Scoring Profile 1--	
✓ Negative W2 Mean Stain Aver Intens	✓ Scoring Profile 12-	
✓ Negative W2 Mean Nucl Integr Intens	✓ Scoring Profile 1-3	
✓ Negative W2 Mean Nucl Aver Intens	✓ Scoring Profile 123	
✓ Negative W2 Mean Cyto Integr Intens	✓ Percentage Scoring Profile 1--	
✓ Negative W2 Mean Cyto Aver Intens	✓ Percentage Scoring Profile 12-	
✓ Negative W2 Mean Cell Integr Intens	✓ Percentage Scoring Profile 1-3	
✓ Negative W2 Mean Cell Aver Intens	✓ Percentage Scoring Profile 123	
✓ Positive W2	✓ Subtotal Profile 1xx	
✓ % Positive W2	✓ Subtotal Profile 12x	
✓ All W3 Mean Stain Area	✓ Subtotal Profile 1x3	
✓ All W3 Mean Stain Integr Intens	✓ Subtotal Profile 123	
✓ All W3 Mean Stain Aver Intens	✓ Percentage Subtotal Profile 1xx	
✓ All W3 Mean Nucl Integr Intens	✓ Percentage Subtotal Profile 12x	
✓ All W3 Mean Nucl Aver Intens	✓ Percentage Subtotal Profile 1x3	
✓ All W3 Mean Cyto Integr Intens	✓ Percentage Subtotal Profile 123	
✓ All W3 Mean Cyto Aver Intens		
✓ All W3 Mean Cell Integr Intens		
✓ All W3 Mean Cell Aver Intens		

名词解释:

Average: 平均每个像素点

Integr: 总体

Mean: 平均每个细胞

nuci: 核区的

Cyto: 细胞质的

Cell: 全细胞的

1-3: 1和3通道阳性且2通道阴性的细胞数或百分比

1x3: 1和3通道阳性且2通道既可为阳性又可为阴性的细胞数或百分比



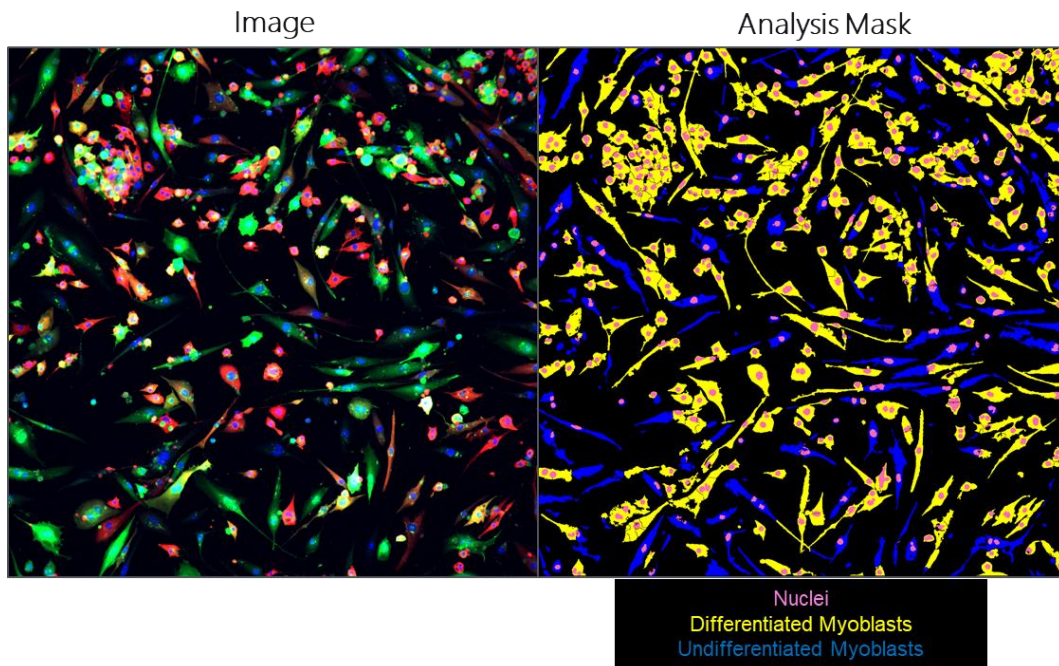
Multi Wavelength Cell Scoring 多波长细胞分类

应用实例



应用模块-多波长细胞分类

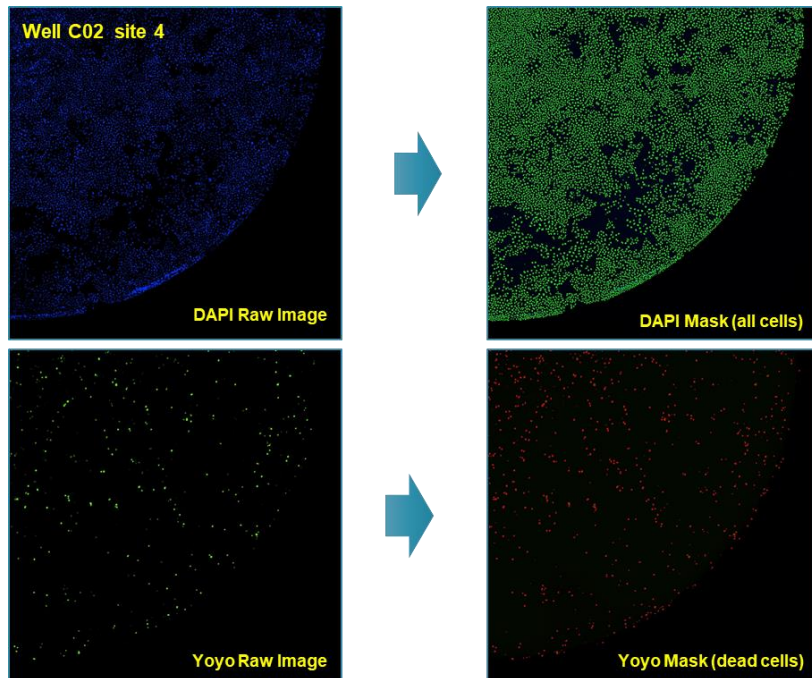
成肌细胞分化



成肌细胞 C2C12 是在成人骨骼肌组织中发现的，是创伤重建肌肉组织的前体细胞，具有很好的分化能力，C2C12 成肌细胞经常被用来在体外系统研究肌肉的发育和分化

应用模块-多波长细胞分类

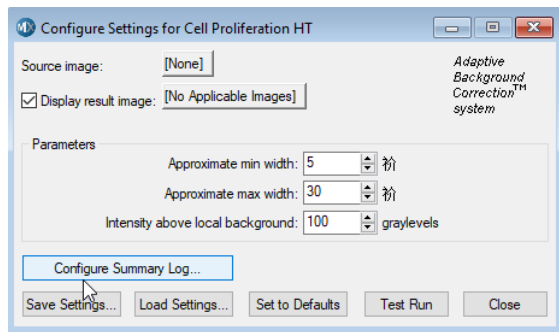
细胞死活



- 乳腺癌细胞
- 4X Plan Apo
- 96 well plate
- 染料:
 - Hoechst (nuclei)
 - Yoyo-1 (dead cells)

细胞增殖、细胞计数、细胞 死活、细胞健康分析模块

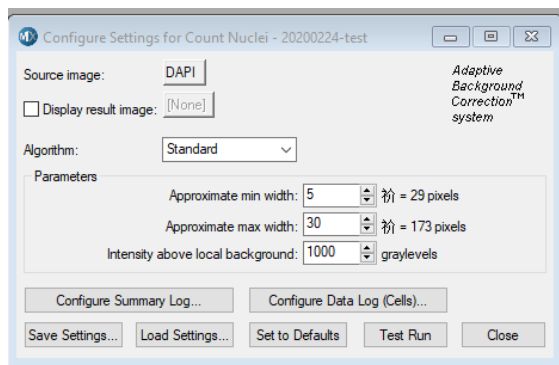
细胞增殖分析模块



Configure Summary Log

Total Nuclei

细胞计数分析模块



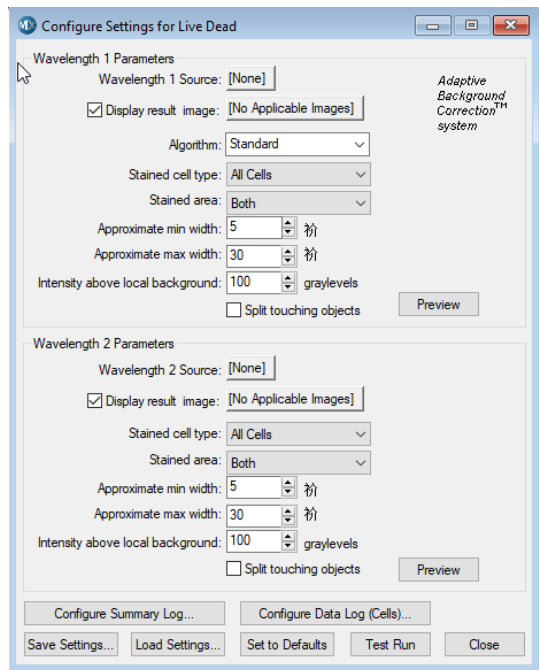
Configure Summary Log

Total Nuclei
Total Area
Mean Area
Integrated Intensity
Average Intensity

Configure Data Log(Cells)

Cell: Area
Cell: Integrated Intensity
Cell: Average Intensity

细胞死活分析模块



Configure Summary Log

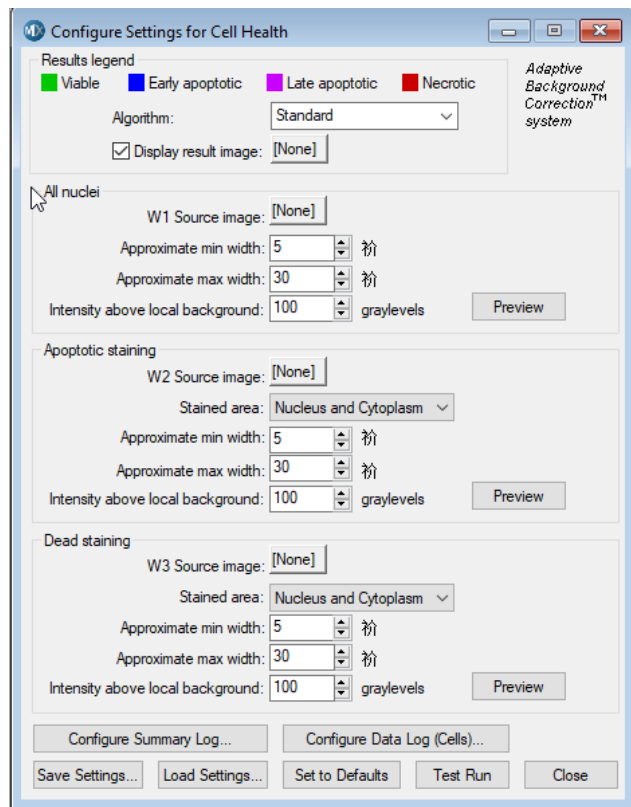
Total Cells
Live Cells
Dead Cells
% Live Cells
% Dead Cells

W1 Total Area
W1 Area Per Cell
W1 Integrated Intensity
W1 Average Intensity
W2 Total Area
W2 Area Per Cell
W2 Integrated Intensity
W2 Average Intensity

Configure Data Log(Cells)

Cell: Live Classification
Cell: W1 Total Area
Cell: W1 Integrated Intensity
Cell: W1 Average Intensity
Cell: W2 Total Area
Cell: W2 Integrated Intensity
Cell: W2 Average Intensity

细胞健康分析模块



Configure Summary Log

Total Cells
Viable Cells
Early Apoptotic Cells
Late Apoptotic Cells
Necrotic Cells
% Viable Cells
% Early Apoptotic Cells
% Late Apoptotic Cells
% Necrotic Cells

All Cells Total Area
All Cells Mean Area
All Cells W1 Integrated Intensity
All Cells W1 Average Intensity
All Cells W2 Integrated Intensity
All Cells W2 Average Intensity
All Cells W3 Integrated Intensity
All Cells W3 Average Intensity

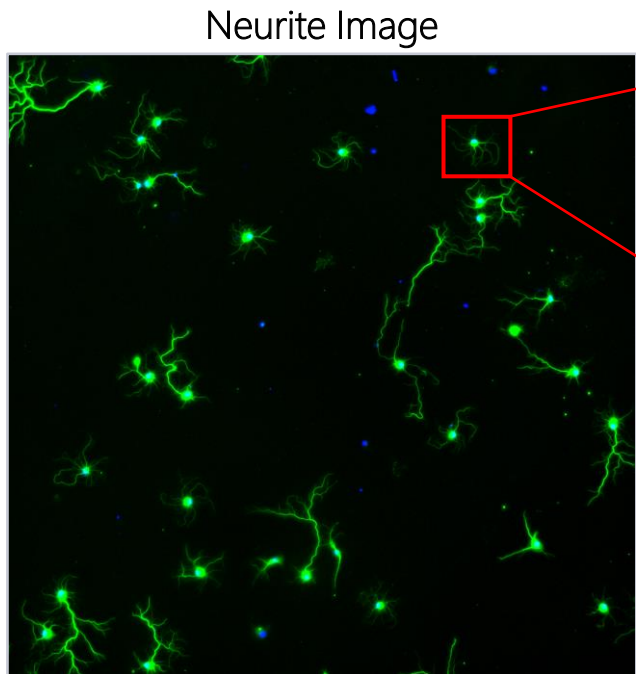
Configure Data Log(Cells)

Cell: Health Classification
Cell: Nuclear Area
Cell: W1 Integrated Intensity
Cell: W1 Average Intensity
Cell: W2 Integrated Intensity
Cell: W2 Average Intensity
Cell: W3 Integrated Intensity
Cell: W3 Average Intensity

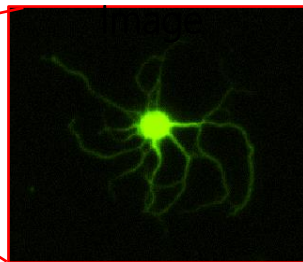
Neurite Outgrowth 神经生长分析模块

应用模块-神经生长模块

“通过对胞体数量、突出长度和分支多少等参数的综合分析，评估神经生长的状态”



Zoom



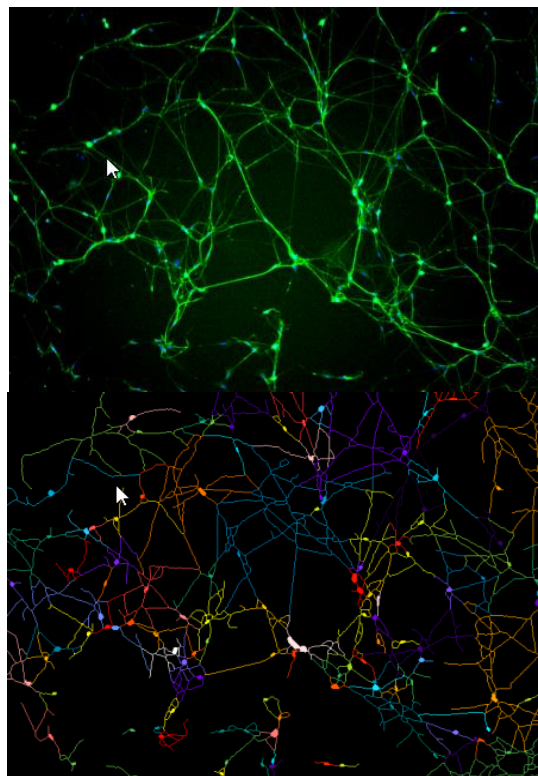
Raw



Mask

- ✓ 识别和测量连接到细胞体的 processes
- ✓ 可选择使用核染料协助细胞体的识别
- ✓ 细胞体/突起可以是荧光，也可以是透射光图像

应用模块-神经生长模块



Configure Settings for Neurite Outgrowth

Neurite image: Adaptive Background Correction™ system

☐ Display result image:

Illumination

☒ Fluorescence ☐ Transmission

Cell bodies

Approximate max width: 袞 = 173 pixels

Intensity above local background: graylevels

Minimum area: 袞 ? = 1671 pixels

☒ Nuclear stain (optional)

Nuclear image:

☐ Display result:

Approximate min width: 袞 = 29 pixels

Approximate max width: 袞 = 173 pixels

Intensity above local background: graylevels

Outgrowths

Maximum width: 袞 = 58 pixels

Intensity above local background: graylevels

Minimum cell growth to log as significant: 袞 = 0 pixels

识别细胞胞体

- 如果设置太大，则可能会将细胞体簇连接在一起
- 如果设置太小，较厚的突起或分支点可能会被识别为细胞体

识别突起

应用模块-神经生长模块

Outgrowths

Maximum width: 10 μm

Intensity above local background: 50 graylevels

Minimum cell growth to log as significant: 0 μm

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Set to Defaults Apply Close

区分突起起始部分和胞体、分支点，故选择生长较粗的部位设置参数

用于评价细胞阳性或阴性生长，不会影响神经突起的识别，可用测量工具测量长度，此参数可按自己的实验判断标准设置

应用模块-神经生长模块

Configure Summary Log

Number of Cells

Total Outgrowth

Mean Outgrowth Per Cell

Total Processes

Mean Processes Per Cell

Total Branches

Mean Branches Per Cell

Total Cell Body Area

Mean Cell Body Area

Straightness

Cells Significant Growth

%Cells Significant Growth

Mean Outgrowth Average Intensity

生长总长度和平均长度，单位 mm

Process 数量

Branch 数量

直线率，点与点直线距离除以实际曲线长度，0到1之间

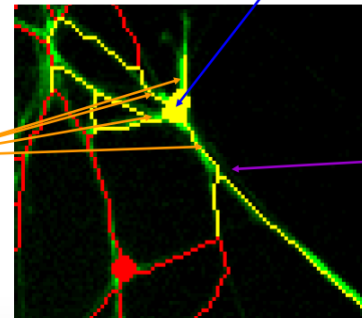
阳性生长的细胞数量，由参数设置决定

所有神经生长的平均像素强度

Processes (4)
connected to
cell body

Cell Body

Branch



Configure Data Log(Cells)

Cell: Total Outgrowth

Cell: Processes

Cell: Mean Process Length

Cell: Median Process Length

Cell: Max Process Length

Cell: Branches

Cell: Straightness

Cell: Cell Body Area

Cell: Mean Outgrowth Intensity

单位 um

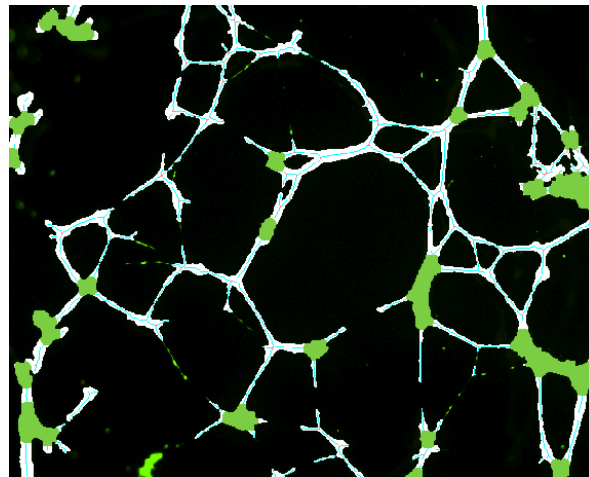
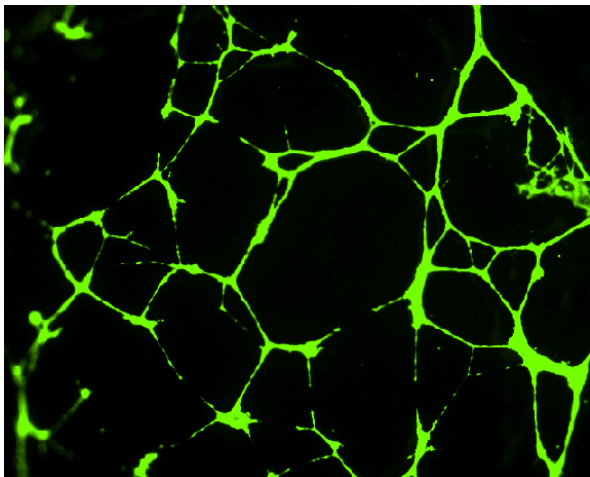
Process 总长度除以其数量

神经生长的平均像素强度

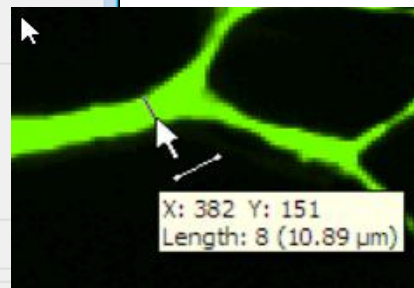
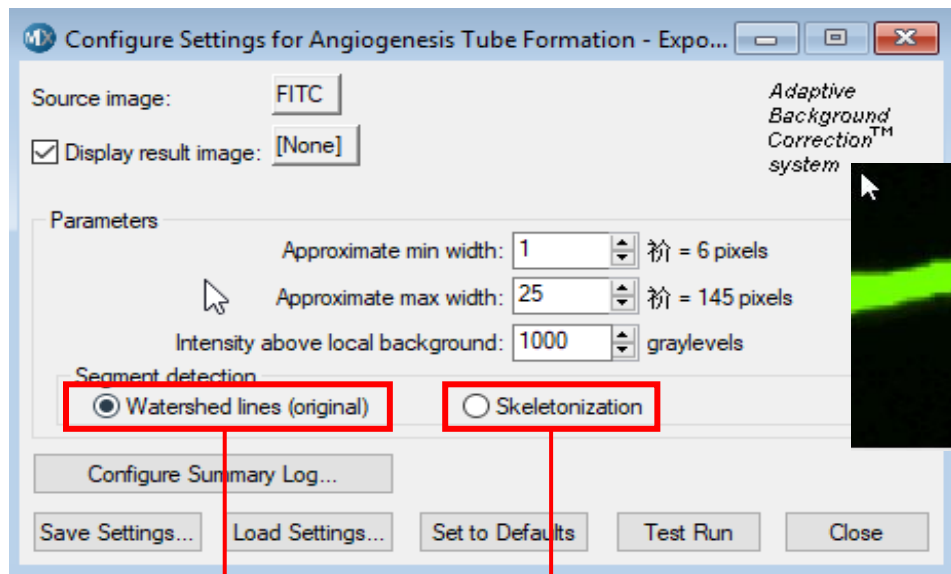
Angiogenesis Tube Formation 血管生成分析模块

应用模块-血管生成模块

- 在单个波长下，识别和测量管（细长物体）和节点（管之间的连接点）
- 除了进行管形成测定外，还可用于测量神经突生长（如胞体模糊或在FOV以外的情况）



应用模块-血管生成模块



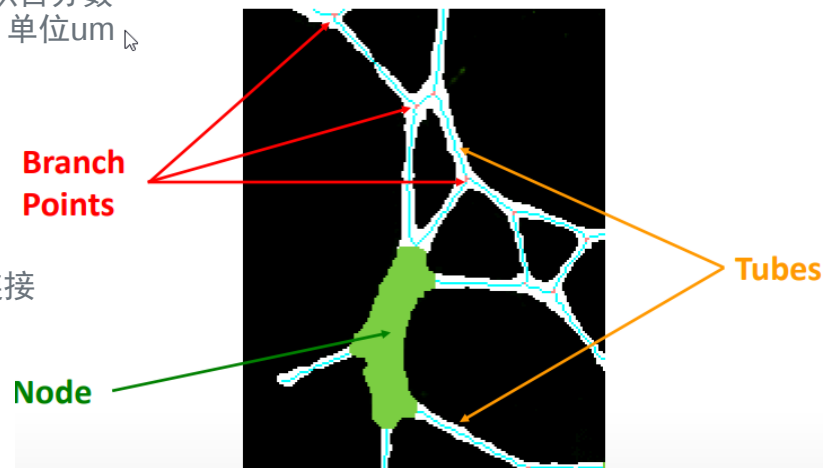
更早期的传统分割方法

使用一种新的分割方法，可以为一些具有较厚和较稀疏的血管提供更精确的分割识别

应用模块-血管生成模块

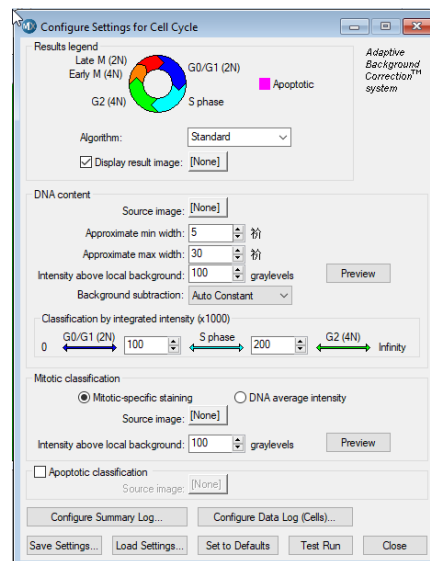
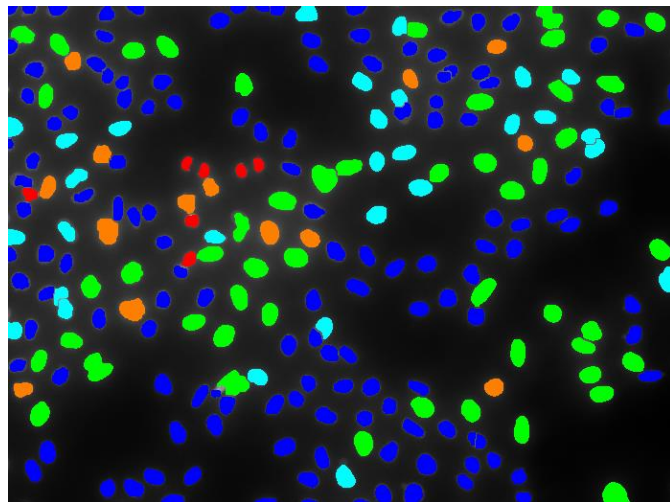
Configure Summary Log

Total Tube Length	→	血管总长度(不包括nodes)，单位um
Mean Tube Length	→	血管总长度除以segments数量
Total Tube Area		
Mean Tube Area		
Tube % Area Covered	→	血管总面积(不包括nodes)除以图像总面积百分数
Average Tube Thickness	→	血管总面积除以总长度(不包括nodes)，单位um
Segments	→	血管分段数量
Branch Points		
Nodes		
Total Node Area		
Mean Node Area	→	Node的总面积除以node的数量
Node % Area Covered		
Connected Sets	→	独立血管的数量，血管与血管之间没有连接
Tube Length Per Set		



Cell Cycle 细胞周期分析模块

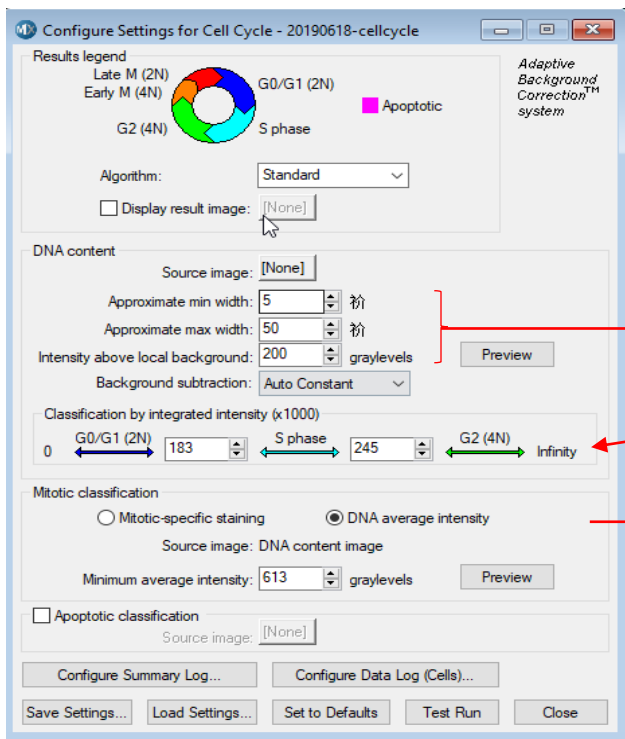
应用模块-细胞周期



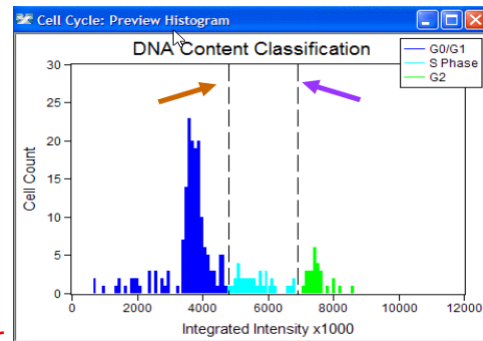
- ✓ G0 / G1
- ✓ S
- ✓ G2
- ✓ Early M
- ✓ Late M

检测细胞周期和细胞凋亡状态是肿瘤学研究和肿瘤药物筛选中的重要方法。传统方法如流式细胞仪，存在需要大量细胞，且分辨率低，仅为5um左右，信息量小，无法数据回溯等问题。ImageXpress系统使用灵活，能够提供每个细胞的多参数分析结果，并能报告不同亚细胞群的分布情况，并能同时分析细胞的凋亡状态，综合分析细胞周期相关蛋白，获得更多信息。

应用模块-细胞周期

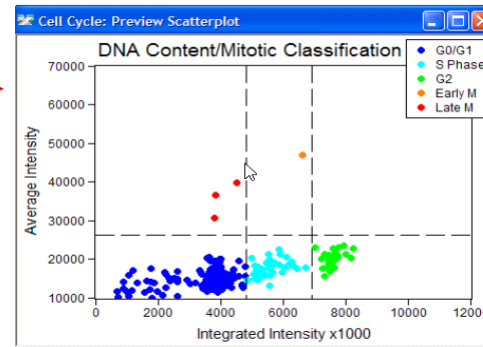
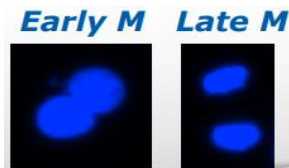


识别所有核染信号



通过拉动双条竖线来分出G0及G2期区间

通过平均像素点荧光强度区分分裂早期和晚期



通过拉动单条横线来分出EM及LM期区间

应用模块-细胞周期

Configure summary log

- ✓ DNA Structures
- ✓ DNA Background Value
- ✓ G0/G1 Cells
- ✓ % G0/G1
- ✓ S Phase Cells
- ✓ % S Phase
- ✓ G2 Cells
- ✓ % G2
- ✓ Early M Cells
- ✓ % Early M
- ✓ Late M Cells
- ✓ % Late M
- ✓ Apoptotic Cells
- ✓ % Apoptotic

细胞核总数

每个时期细胞总数和占比

Configure data log (Cells)

- ✓ Cell: Assigned Label #
- ✓ Cell: Classification
- ✓ Cell: G0/G1
- ✓ Cell: S Phase
- ✓ Cell: G2
- ✓ Cell: Early M
- ✓ Cell: Late M
- ✓ Cell: Apoptotic
- ✓ Cell: DNA Area
- ✓ Cell: DNA Integrated Intensity
- ✓ Cell: DNA Average Intensity
- ✓ Cell: Mitotic Integrated Intensity
- ✓ Cell: Mitotic Average Intensity
- ✓ Cell: Apoptotic Integrated Intensity
- ✓ Cell: Apoptotic Average Intensity

细胞周期分类

有丝分裂特异染色的总强度
只在此选项使用时出现
凋亡特异染色的总强度
只在此选项使用时出现

单个细胞数据

Granularity

颗粒度分析

应用模块-颗粒度分析

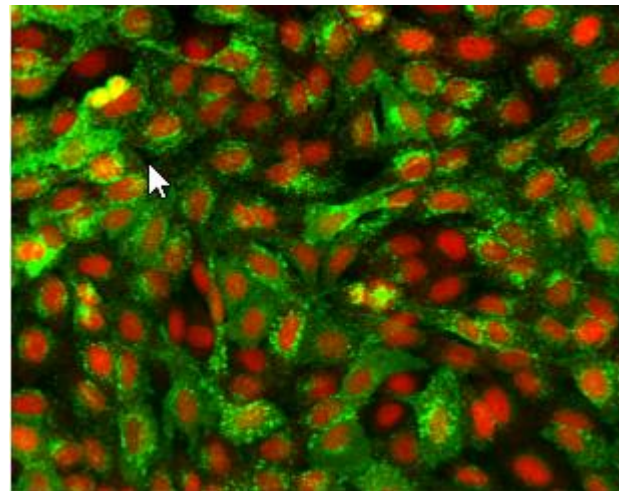
› [Methods Enzymol](#), 587, 71-86 2017

High-Throughput Quantification of GFP-LC3⁺ Dots by Automated Fluorescence Microscopy

J M Bravo-San Pedro¹, F Pietrocola², V Sica³, V Izzo², A Sauvat⁴, O Kepp⁴, M C Maiuri², G Kroemer⁵, L Galluzzi⁶

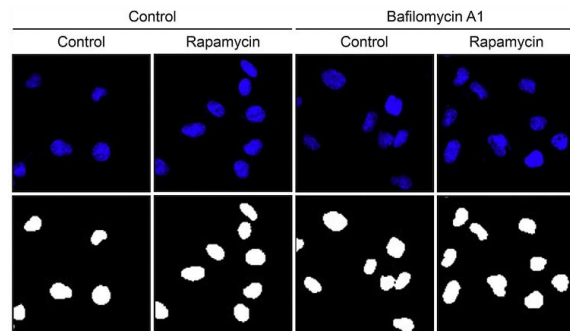
Affiliations + expand

PMID: 28253977 DOI: [10.1016/bs.mie.2016.10.022](https://doi.org/10.1016/bs.mie.2016.10.022)

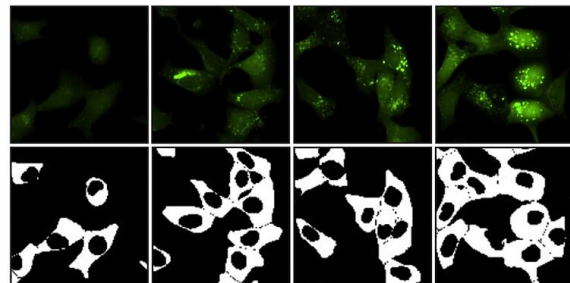


“对细胞图像中染料信号的集聚体进行识别，分析数量和强度，指示细胞内微结构和蛋白集聚等”

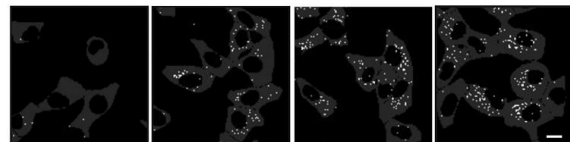
应用模块-颗粒度分析



Nuclear segmentation



Cytoplasmic segmentation



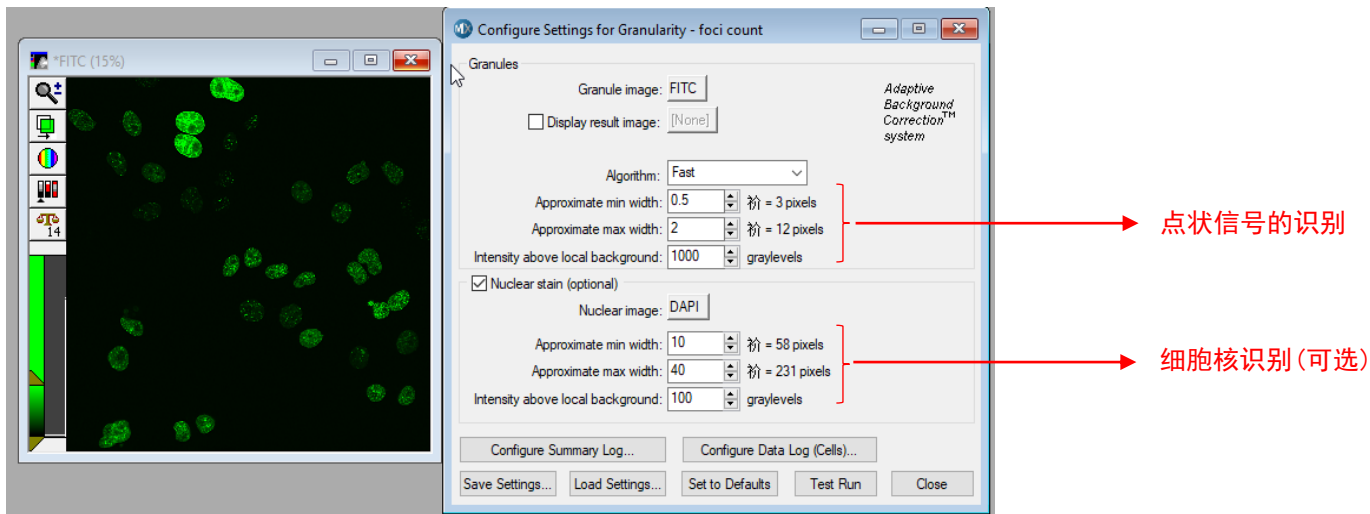
Dots identification

自噬诱导剂或抑制剂的筛选

巨自噬是自噬的一种特殊过程，其涉及一个双膜微结构-自噬体。通过使用 GFP 标记的 LC3 蛋白来监测细胞内的自噬体。在自然条件下，GFP-LC3 在细胞质和细胞核上表现出弥散的绿色荧光。当响应于促进巨自噬的刺激时，GFP-LC3 信号会以点状的形式主要集中在细胞质中

使用 ImageXpress Micro 成像系统，并使用 Granularity 模块对转染 GFP-LC3 的人脑神经胶质瘤 H4 细胞进行建模

应用模块-颗粒度分析



应用模块-颗粒度分析

Configure summary log

- ✓ Granules
- ✓ Granules Per Cell
- ✓ Total Granule Area
- ✓ Mean Granule Area → 平均每个细胞的颗粒面积(所有细胞总颗粒面积除以总细胞核数量)
- ✓ Integrated Granule Intensity
- ✓ Average Granule Intensity → 平均每个细胞的颗粒强度(所有细胞总颗粒强度除以总细胞核数量)
- ✓ Nuclei
- ✓ Total Nuclear Area
- ✓ Mean Nuclear Area
- ✓ Integrated Nuclear Intensity
- ✓ Average Nuclear Intensity → 平均每个细胞的细胞核强度(所有细胞核染色区域的强度除以总细胞核数量)

Configure data log

- ✓ Cell: Assigned Label #
- ✓ Cell: Granule Count
- ✓ Cell: Granule Total Area
- ✓ Cell: Granule Integrated Intensity
- ✓ Cell: Granule Average Intensity → 平均每个颗粒的强度(指定细胞的总颗粒强度除以这个细胞的颗粒数量)
- ✓ Cell: Nuclear Total Area
- ✓ Cell: Nuclear Integrated Intensity
- ✓ Cell: Nuclear Average Intensity → 细胞核染色区域的平均像素强度

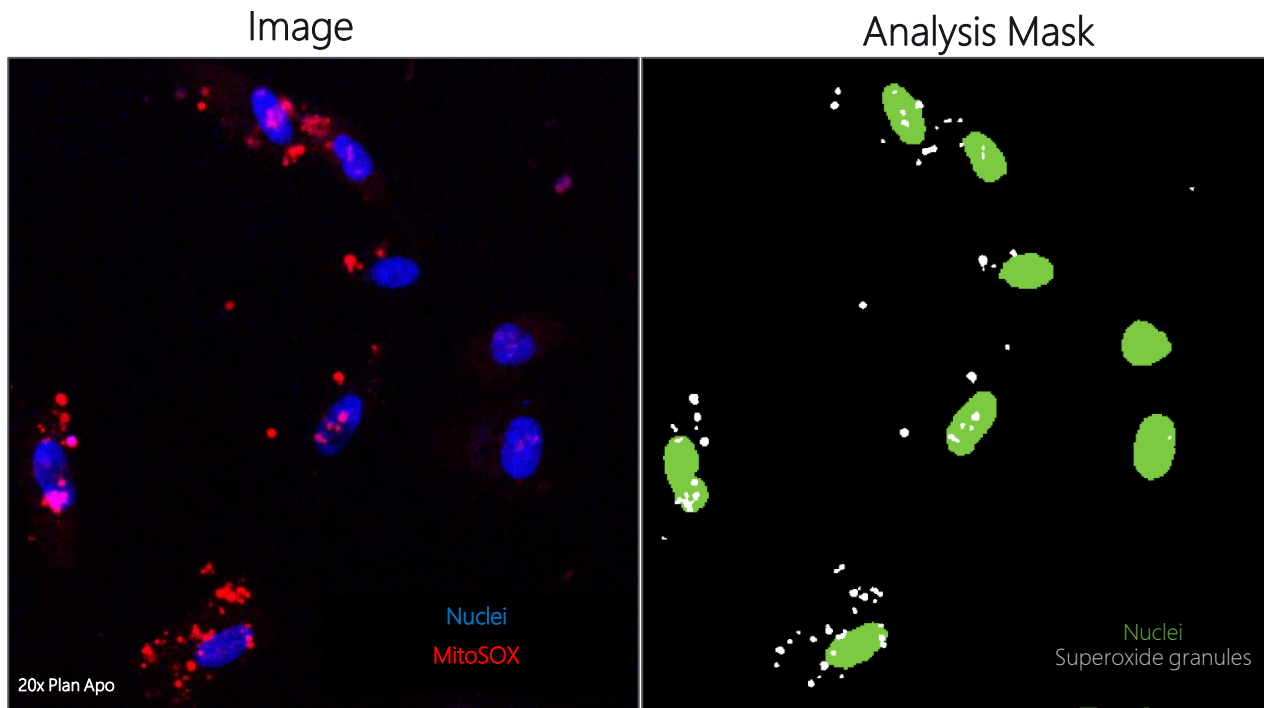
Granularity 颗粒度分析

应用实例



应用模块-颗粒度分析

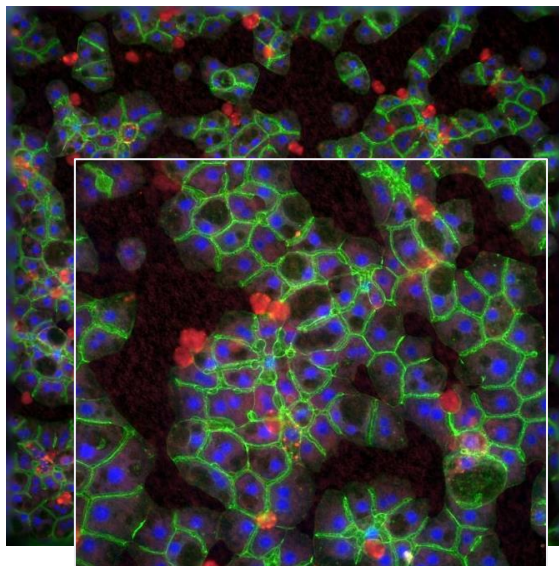
线粒体损伤



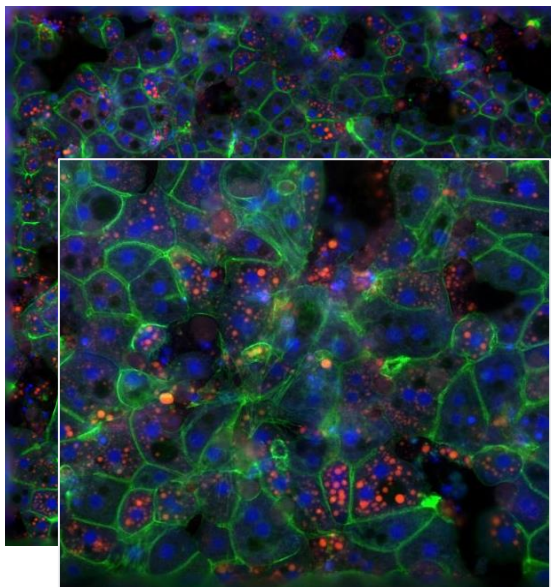
MitoSOX 染料可标记线粒体并能被超氧化物氧化产生红色荧光

应用模块-颗粒度分析

测量脂滴



Control



Drug Treated

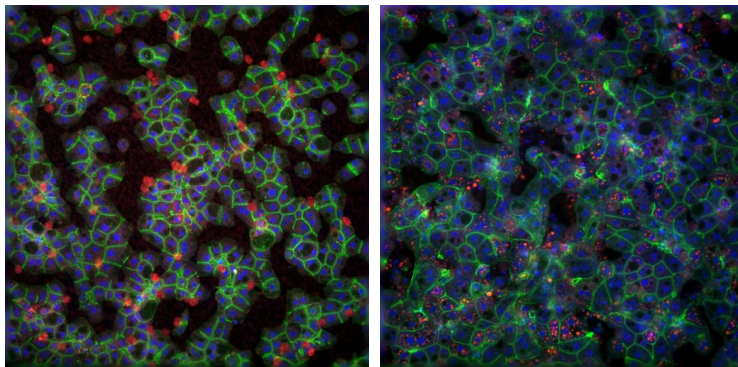
Blue = topro (false color)
Green = E-cadherin
Red = lipid staining

人肝细胞中的脂滴测量

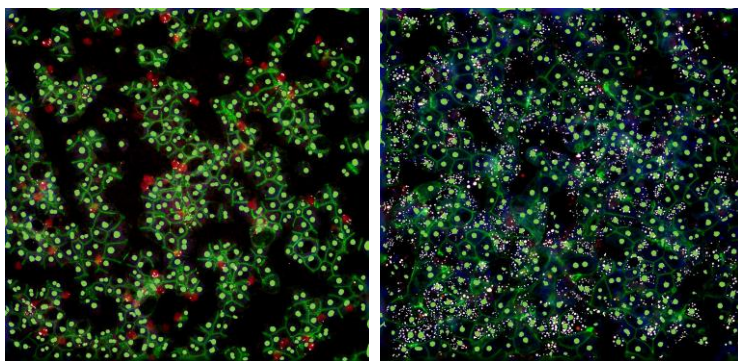
应用模块-颗粒度分析

测量脂滴

Image



Mask



Control
Granules = 252

Drug Treated
Granules = 3659

Analysis using Granularity application module

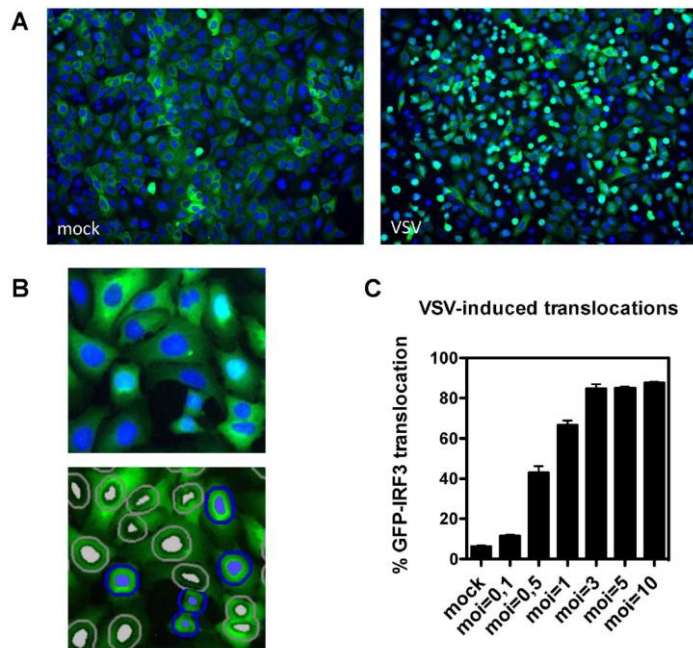
Translocation

转位分析(蛋白转位、核转位)

应用模块-转位分析

A method for the detection of virus infectivity in single cells and real time:
Towards an automated fluorescence neutralization test

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A: U2OS_GFP-IRF3 细胞感染水疱性口炎病毒8h, 在96孔板中使用高内涵进行 GFP 荧光核转位成像, 细胞核用 Hoechst 33342 标记, 统计发生核转位的细胞比例; B: 采用多波长核转位分析模块识别分析发生核转位的细胞; C: 在不同病毒感染浓度下的核转位率

应用模块-转位分析

Translocation-Enhanced

Compartment: [No Applicable Images]

Translocation probe image: [No Applicable Images]

☐ Display result image: [No App]

Adaptive Background Correction™ system

Compartments

Algorithm: Standard

Approximate width: 22 μm

Intensity above local background: 15 to 65535 graylevels

Minimum area: 200 μm^2

Maximum area: 1500 μm^2

☒ Auto separate touching compartments

Define regions for measurement

Inner region distance in from edge: 2 μm

Outer region distance out from edge: 2 μm

Outer region width: 6 μm

Translocation probe

Background estimation method: Auto Constant

Classify positive if: Correlation Coefficient $\geq$ 0.3

Configure Summary Log... Configure Data Log (Cells)...

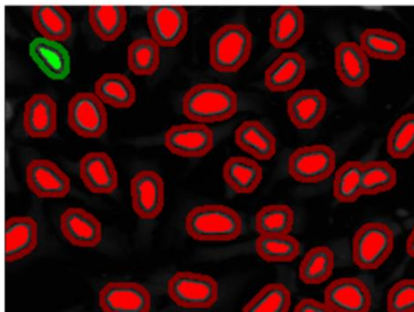
Save Settings... Load Settings... Set to Defaults Apply Close

识别细胞核区域

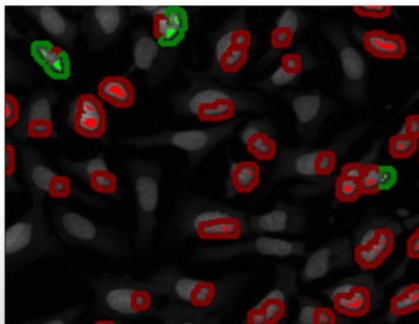
识别测量区域：
转位区域识别

阳性细胞判定条件

应用模块-转位分析



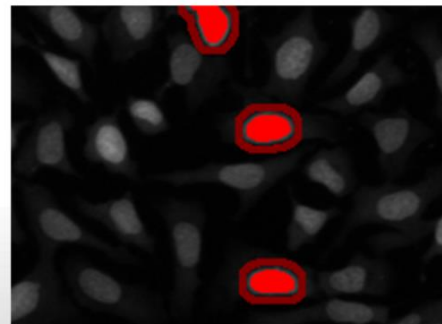
Set correctly



Set too low



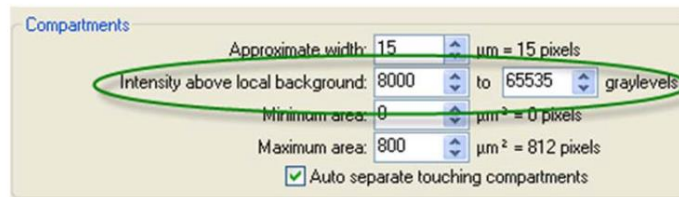
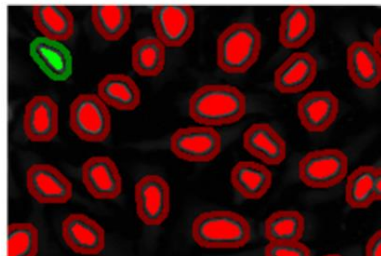
Note: The Minimum and Maximum area will have an impact as well!



Set too large

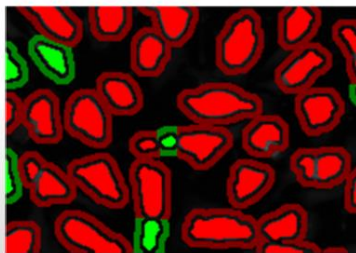
应用模块-转位分析

- Set correctly

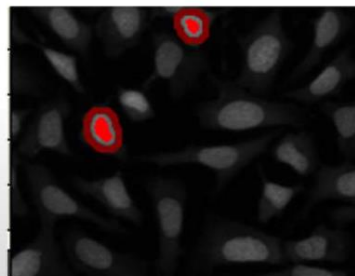


最低亮度值的注意事项

- Set too low → Compartments are too large

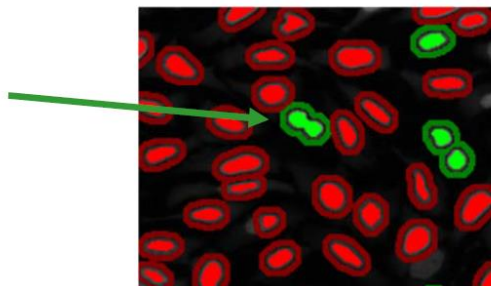


- Set too high → Compartments are not being detected

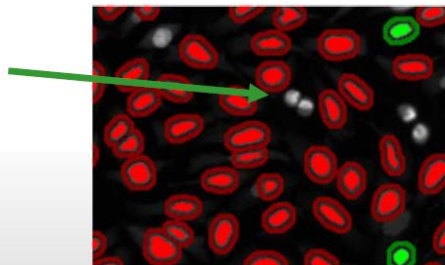


应用模块-转位分析

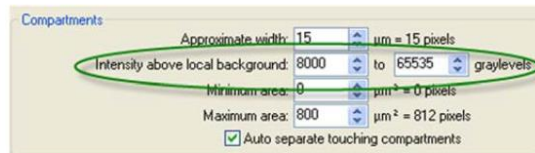
- All cells/ compartment detected



- Bright cells are being ignored

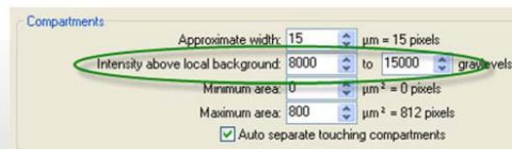


- Max intensity set to 65535 (bit dept of image)
- All nucleus detected



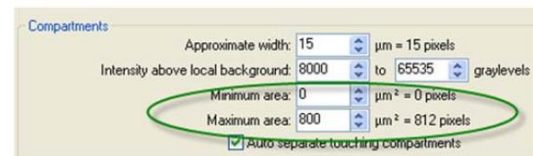
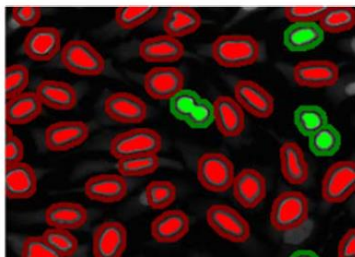
最高亮度值的注意事项

- Max intensity set lower then the bright (mitotic) cells
- Only “normal” cells detected

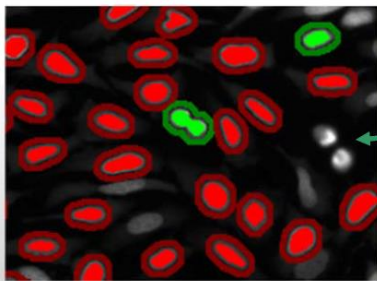


应用模块-转位分析

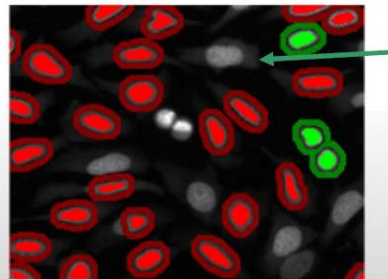
- Min= 0, Max=800
- Detect all compartment/ cells



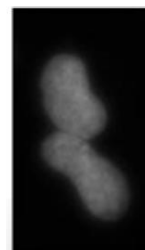
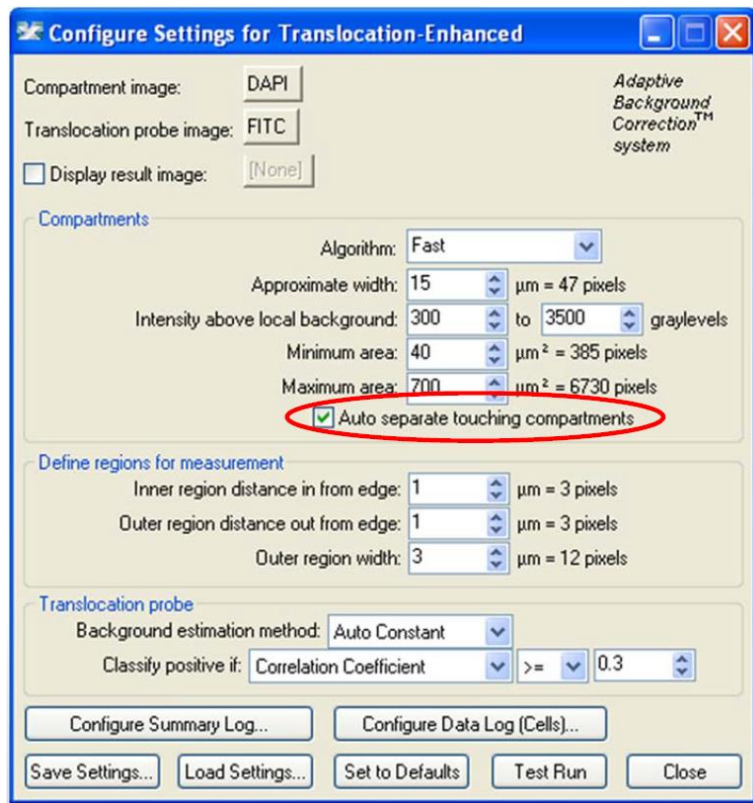
- Min= 150, Max=800
- Ignores small compartments/ cells



- Min= 0, Max=250
- Ignores large compartments/ cells



应用模块-转位分析



Auto
separate
option:



On



Off

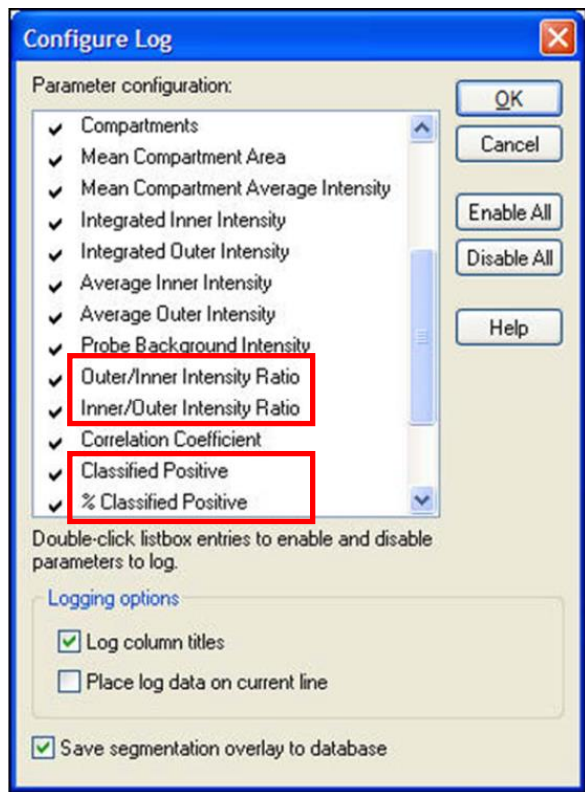
应用模块-转位分析

部分参数的常用设置

1. Intensity above local background.
 - Set max to max bit dept of the image
 - 4095 for 12 bit image (ImageXpress Micro, Discovery-1)
 - 63535 for 16 bit image (ImageXpress Ultra, ImageXpress Micro XL, mageXpress 5000A)
2. Area
 - Minimum: play with the value to get the same number of compartments/ cells
3. Auto separate toughing compartments
 - Selected
4. Inner and outer distance from Edge
 - Enter values that represent 1 pixel
5. Outer region width
 - Enter a value that represent: $1/3$ of the appropriate width for the compartment and subtract 1 pixel.
 - For instance is the width is 15 pixels, then enter 4 ($(15/3) - 1$)
6. Background estimation method:
 - Select Auto Constant
7. Classify positives
 - Select Correlation Coefficient
 - Select $\geq$

应用模块-转位分析

Configure summary log



Outer/Inner Intensity Ratio: 所有外部区域探针的平均像素强度(减去背景)除以所有内部区域探针的平均像素强度(减去背景)

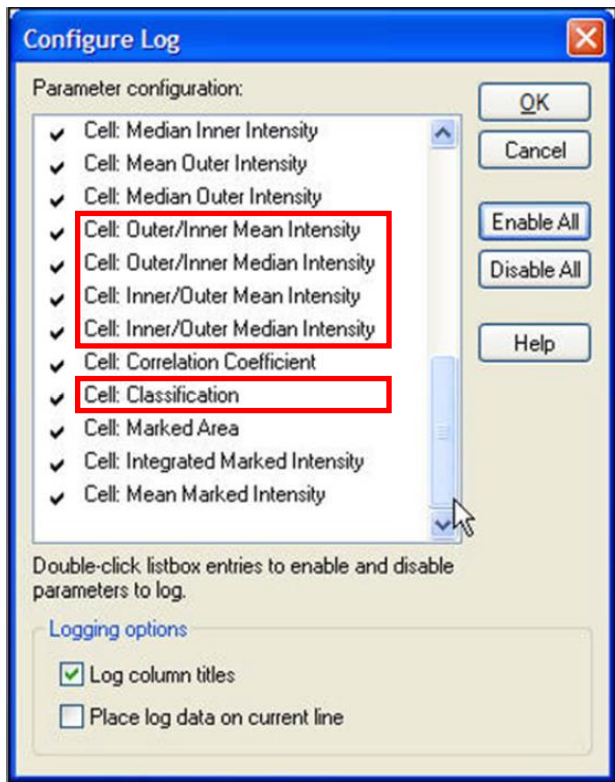
Inner/Outer Intensity Ratio: 所有内部区域探针的平均像素强度(减去背景)除以所有外部区域探针的平均像素强度(减去背景)

Classified Positive: 转位阳性分类的细胞总数

% Classified Positive: 转位阳性分类的细胞总数占所有细胞总数的百分比

应用模块-转位分析

Configure data log (Cells)



Cell: Outer/Inner Mean Intensity: 外部区域探针的平均像素强度(减去背景)除以内部区域探针的平均像素强度(减去背景)

Cell: Outer/Inner Median Intensity: 外部区域的探针像素强度中值(减去背景)除以内部区域的探针像素强度中值(减去背景)

Cell: Inner/Outer Mean Intensity: 内部区域探针的平均像素强度(减去背景)除以外部区域探针的平均像素强度(减去背景)

Cell: Inner/Outer Median Intensity: 内部区域的探针像素强度中值(减去背景)除以外部区域的探针像素强度中值(减去背景)

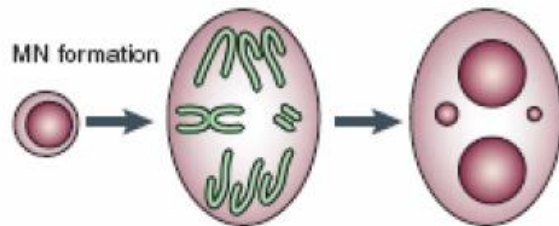
Cell: Classification: 1为阳性转位分类(核染色), 0为阴性转位分类(胞浆染色)

Micronuclei 微核分析模块

应用模块-微核分析

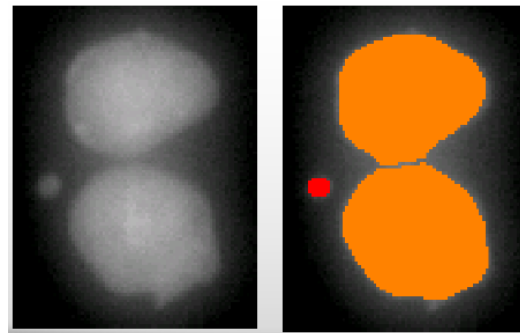
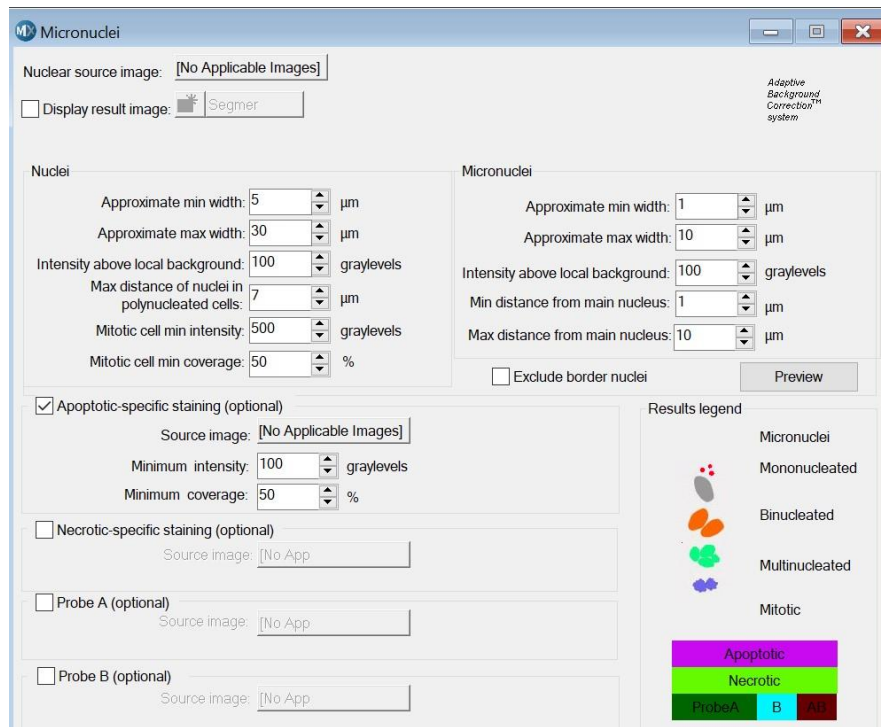
荧光微核

微核是细胞分裂过程中分裂过程落后的染色体或整个染色体形成的小细胞核。在细胞毒性物质，尤其是染色体毒性物质作用下，细胞分裂过程中通常会产生微核，因此，细胞微核能够反映出化合物的染色体毒性，检测细胞内微核能够定量检测化合物的染色体毒性。



Micronuclei是一个功能强大的软件功能，其样品准备简单，只需使用核荧光标记物（DAPI、Hoechst、吖啶橙等）标记细胞，即可进行细胞荧光微核的检测。同时结合其他特异荧光标记物（如凋亡、坏死等，可使用4种），该分析方法完全符合CBMN的要求，在荧光微核检测同时，检测出单核细胞、双核细胞、多核细胞、有丝分裂细胞、凋亡细胞、坏死细胞、特异性荧光标记细胞等多种细

应用模块-微核分析



应用模块-微核分析

Micronuclei

Nuclear source image: [No Applicable Images]

☐ Display result image:  Segmer

Nuclei

Approximate min width: 5 μm

Approximate max width: 30 μm

Intensity above local background: 100 graylevels

Max distance of nuclei in polynucleated cells: 7 μm

Mitotic cell min intensity: 500 graylevels

Mitotic cell min coverage: 50 %

☒ Apoptotic-specific staining (optional)

Source image: [No Applicable Images]

Minimum intensity: 100 graylevels

Minimum coverage: 50 %

☐ Necrotic-specific staining (optional)

Source image: [No App]

☐ Probe A (optional)

Source image: [No App]

☐ Probe B (optional)

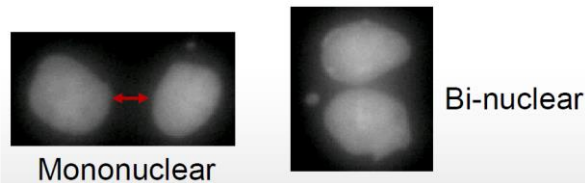
Source image: [No App]

这一部分参数是对细胞核的识别

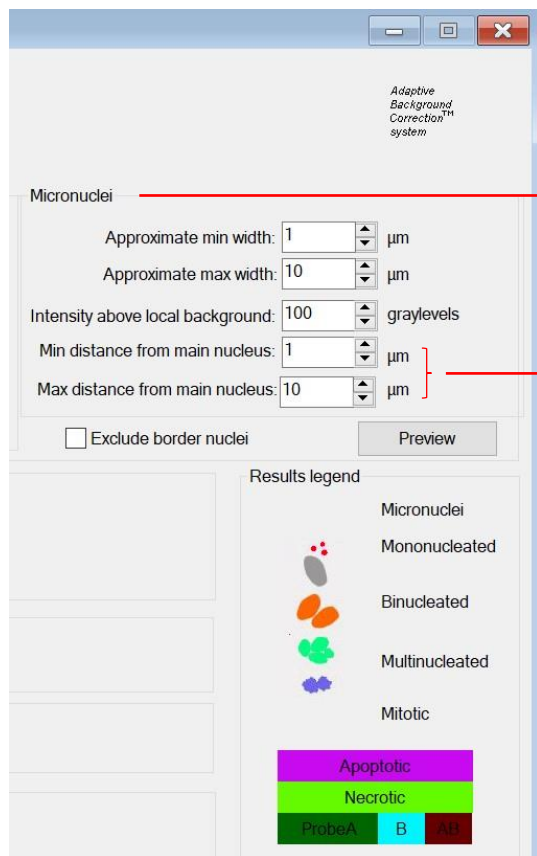
定义多核细胞中核与核之间的最大边界距离，在这个距离内的所有核将被认为是同一个细胞的

视野内最暗淡的有丝分裂细胞核的强度

细胞核的强度值等于或大于指定的有丝分裂细胞的最小强度值，有助于确保细胞核中有小亮点的细胞不属于有丝分裂



应用模块-微核分析



这一部分参数是对微核的识别

微核与细胞核的最小和最大距离(任何距离主核小于最小距离或大于最大距离的核结构都被排除在分析之外)

Exclusion of Nuclei at the border of an image

Identification of 2 close bi-nucleated cells

A "minimum distance from main nuclei" option allows identification of "Blebs"

Mitotic Cells



应用模块-微核分析

Configure summary log

Total Micronuclei	Total number of micronuclei for interphase cells
Micro-nucleated Cells	Total number of interphase cells with micronuclei
Cells with one micronucleus	Total number of interphase cells with one micronucleus
Cells with multi micronuclei	Total number of interphase cells with multi micronuclei
Micro and Mono-nucleated Cells	Total number of mono-nucleated cells with one or more micronuclei
Micro and Bi-nucleated Cells	Total number of bi-nucleated cells with one or more micronuclei
Micro and Multi-nucleated Cells	Total number of multi-nucleated cells with one or more micronuclei
Micro and ProbeA-positive Cells	Total number of ProbeA-positive cells with one or more micronuclei
Micro and ProbeB-positive Cells	Total number of ProbeB-positive cells with one or more micronuclei
Micro and ProbeAB-positive Cells	Total number of ProbeAB-positive cells with one or more micronuclei
% Healthy Cells	Healthy cells divided by total cells
% Mono-nucleated Cells	Mono-nucleated cells divided by total cells
% Bi-nucleated Cells	Bi-nucleated cells divided by total cells
% Multi-nucleated Cells	Multi-nucleated cells divided by total cells
% Micro-nucleated Cells	Micro-nucleated cells divided by total cells
% Cells with one micronucleus	Micro-nucleated cells with one micronucleus divided by total cells
% Cells with multi micronuclei	Micro-nucleated cells with multi micronuclei divided by total cells

总共68个计算数据

应用模块-微核分析

Configure data log (Cells)

Cell: Necrotic	1 if necrotic, 0 if not
Cell: Mononucleated	1 if mononucleated, 0 if not
Cell: Binucleated	1 if binucleated, 0 if not
Cell: Multinucleated	1 if multinucleated (more than 2 nuclei), 0 if not
Cell: ProbeA Positive	1 if ProbeA positive, 0 if not
Cell: ProbeB Positive	1 if ProbeB positive, 0 if not
Cell: Number of Micronuclei	Number of micronuclei in this cell
Cell: DNA Area	Area (in calibrated units of measure) identified as nuclear or micronuclear
Cell: DNA Integrated Intensity	Integrated intensity over all pixels identified as nuclear or micronuclear
Cell: DNA Average Intensity	Integrated intensity over all pixels identified as nuclear or micronuclear divided by number of corresponding pixels
Cell: Nuclear Area	Area (in calibrated units of measure) identified as main nuclear (includes bi- and multinucleated)
Cell: Nuclear Integrated Intensity	Integrated intensity over all pixels identified as main nuclear
Cell: Nuclear Average Intensity	Integrated intensity over all pixels identified as main nuclear divided by number of cells
Cell: Apoptotic Integrated Intensity	Integrated intensity over all pixels identified as main nuclear in an apoptotic cell

总共30个计算数据

Science, for me, gives a
partial explanation for life.
In so far as it goes, it is
based on fact, experience
and experiment.

— Rosalind Franklin

Q&A



ADVANCING DISCOVERY

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