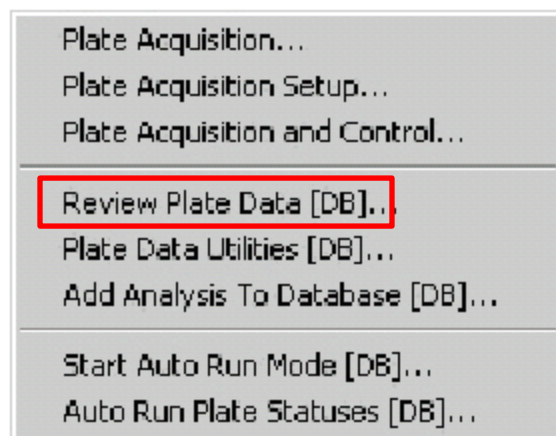


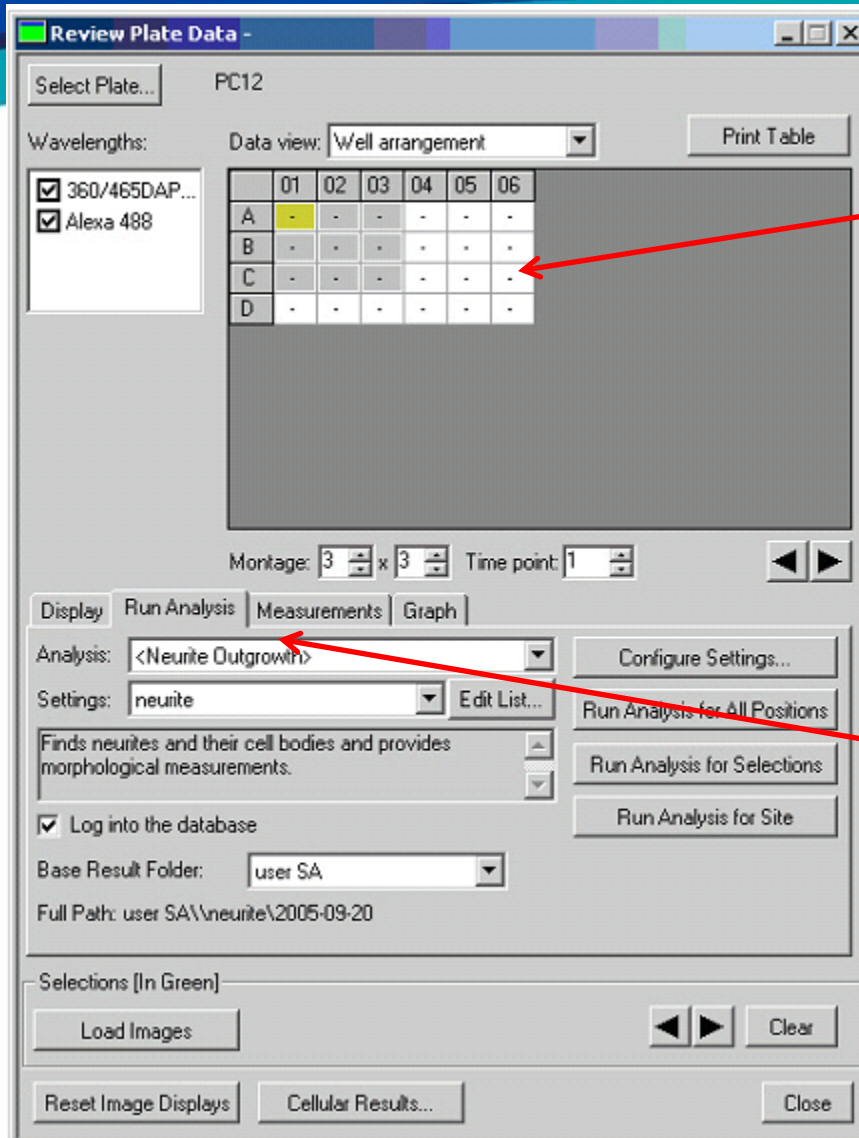


MD ImageXpress Micro高内涵筛选系统分析说明

- 进入软件后，从菜单栏中选择Screening菜单后，弹出以下选项



- 选择Review Plate Date，即可浏览采集过的图像，并进行图像批量分析

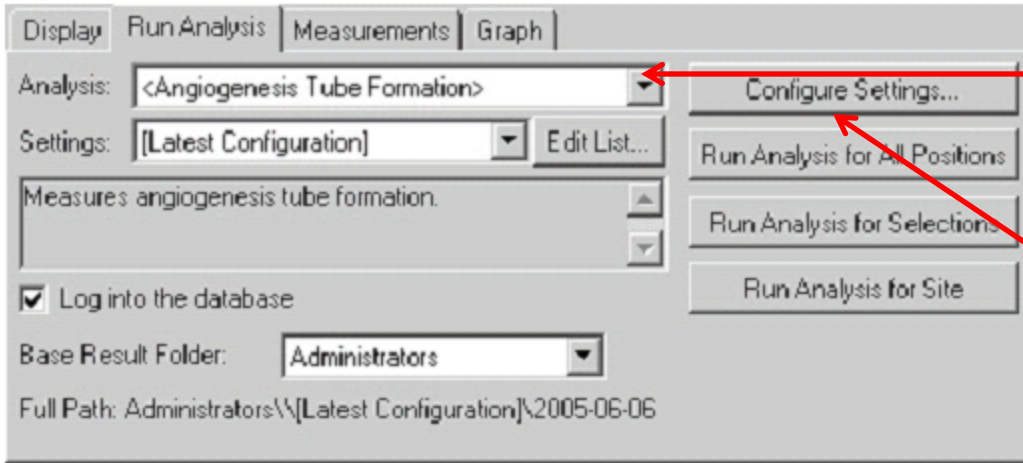


数据区，未进行图像分析的图像显示为“-”；图像进行过分析，则显示分析结果

标签区域，有Display、Run Analysis、Measurement和Graph共四个标签，完成相应功能。

进行图像分析时使用Run Analysis和Measurement标签

Run Analysis的设置

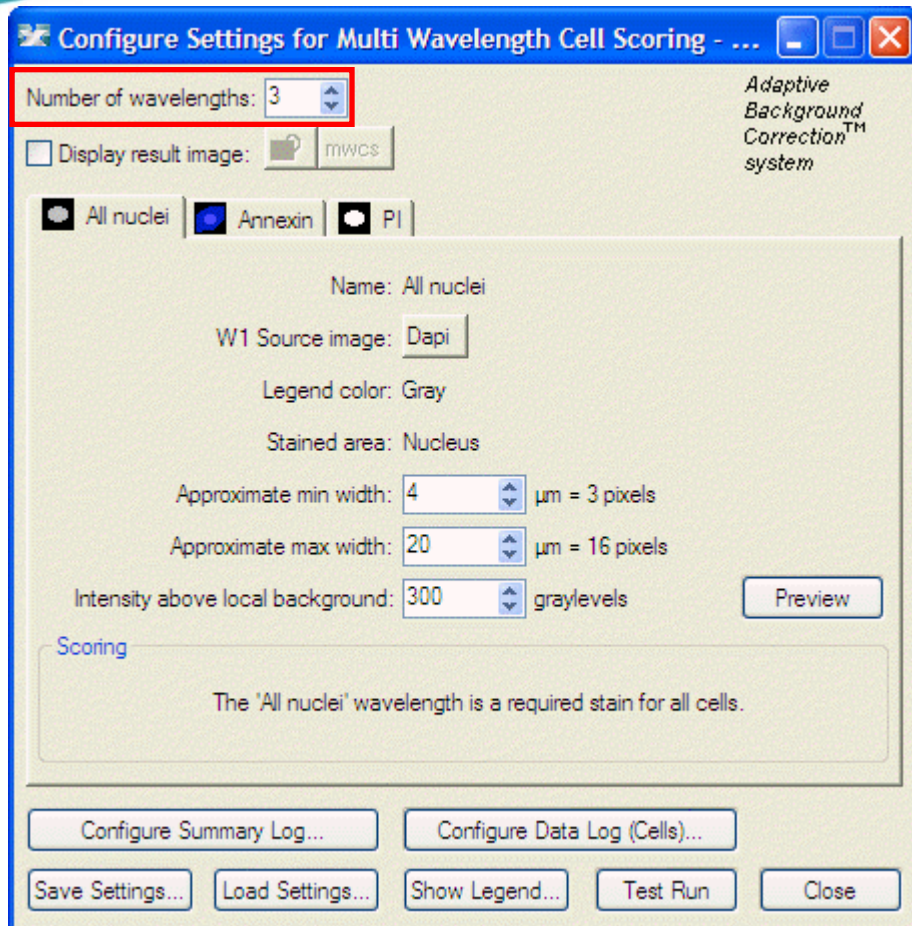


从下拉菜单中选择相应的分析方法（分析模块）

设置分析模块，点击后能够对该模块进行设置

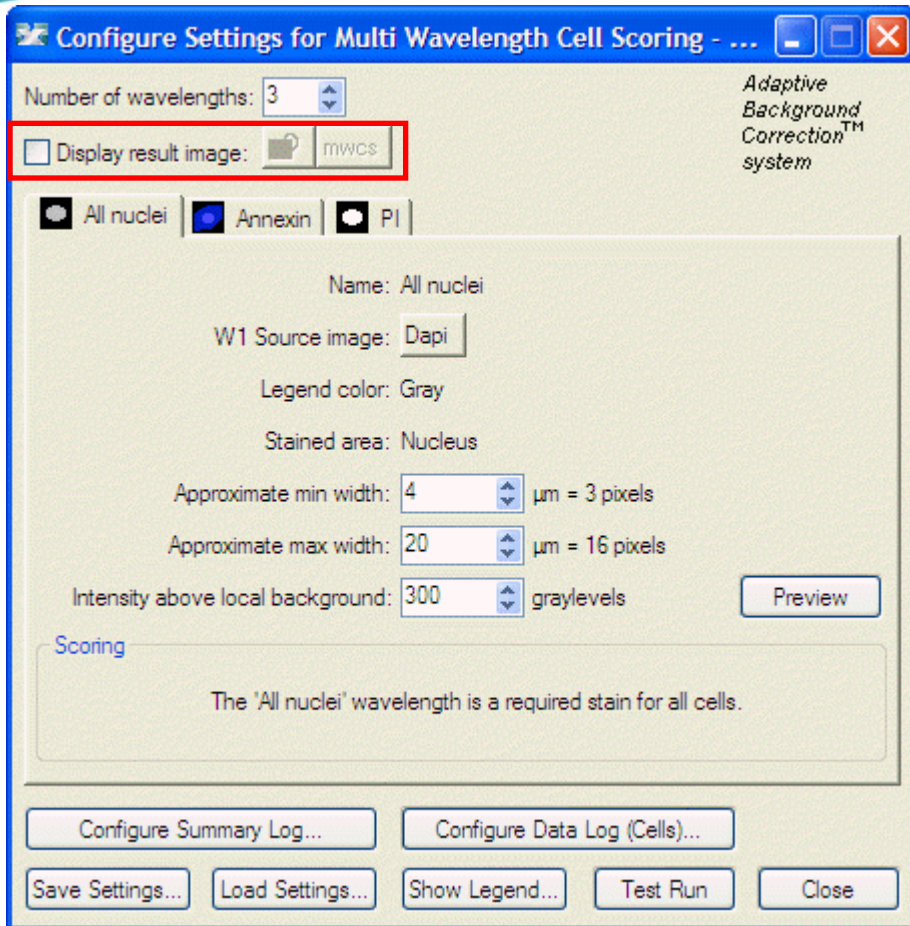
后文以多波长细胞分类计数模块（Multi-Weavelength Cell Sorting）为例，说明设置过程

模块设置



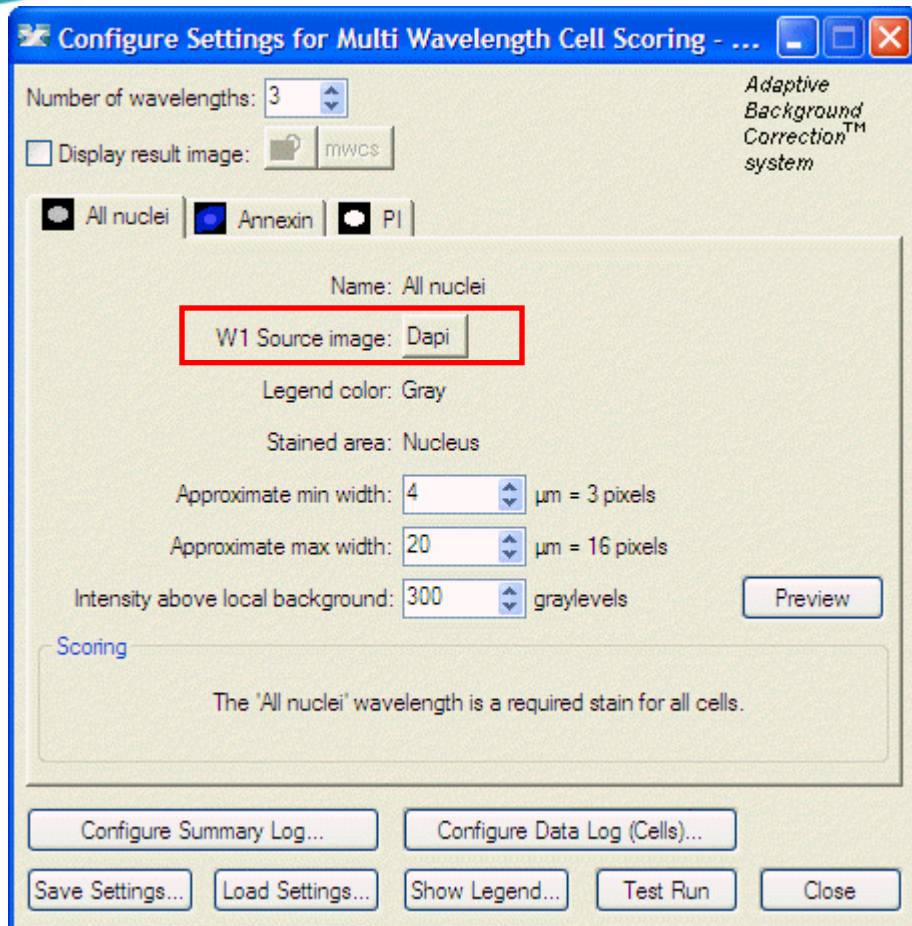
- 波长(荧光)数量
- 该处应设为需要进行分析的总的荧光波长数量(核荧光加感兴趣的探针数量)
- 能够同时分析7种荧光探针

模块设置



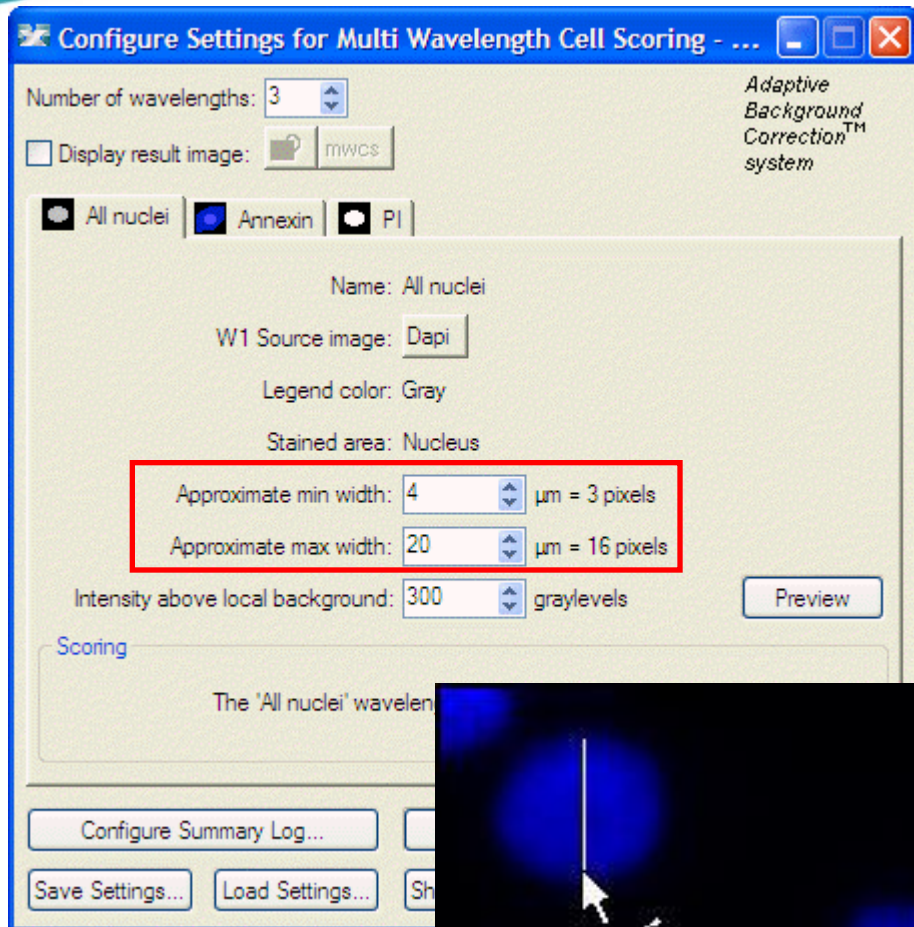
- 显示结果图像
- **Display result image**通常不打勾

模块设置

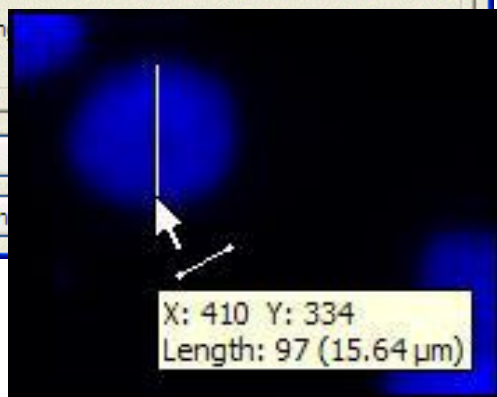


- 波长1 (W1)
- 选择细胞核图像

模块设置

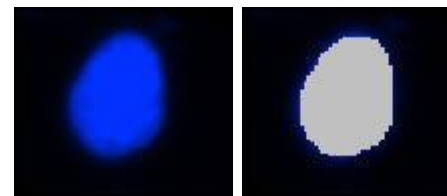


- 波长1(W1)
- 设置细胞核大概的最大宽度和最小宽度
- 宽度是指细胞核的短轴(μm)
- 可使用region 来测量



模块设置

设置不同宽度时的效果



最小宽度设置的太小：细胞核被分裂



最小宽度设置过大：会忽略小细胞核

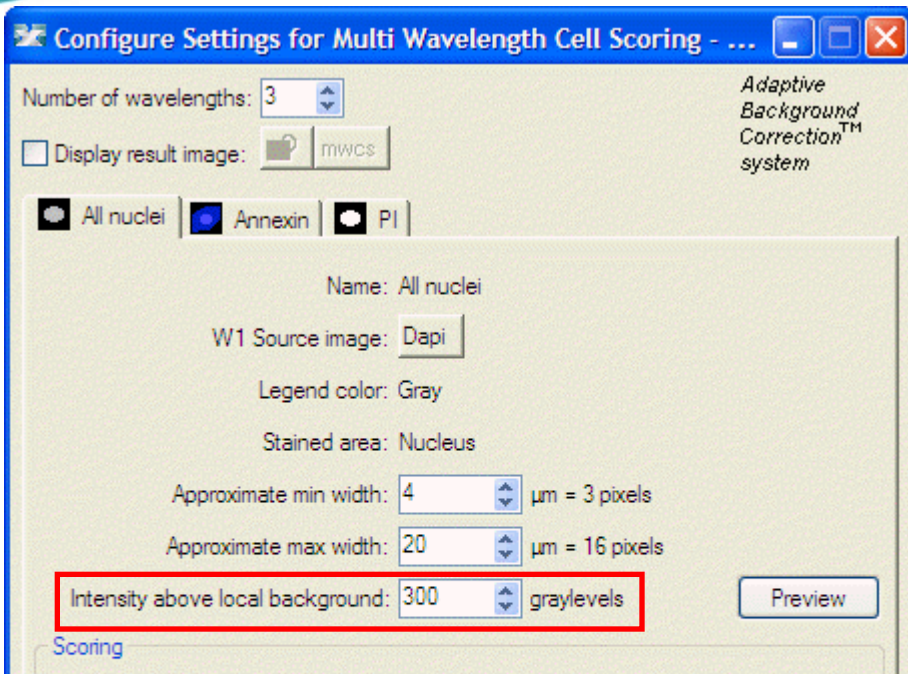
组大宽度设置过小：核边缘会收缩



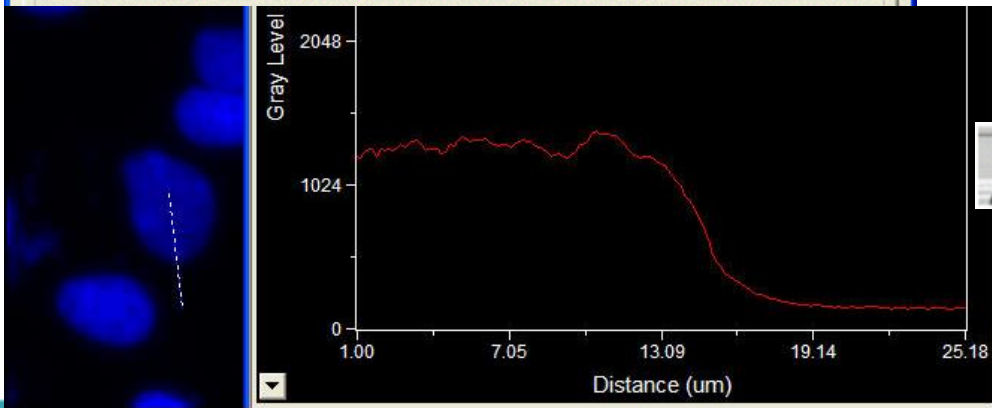
最大宽度设置过大：细胞核边缘或稍有扩大



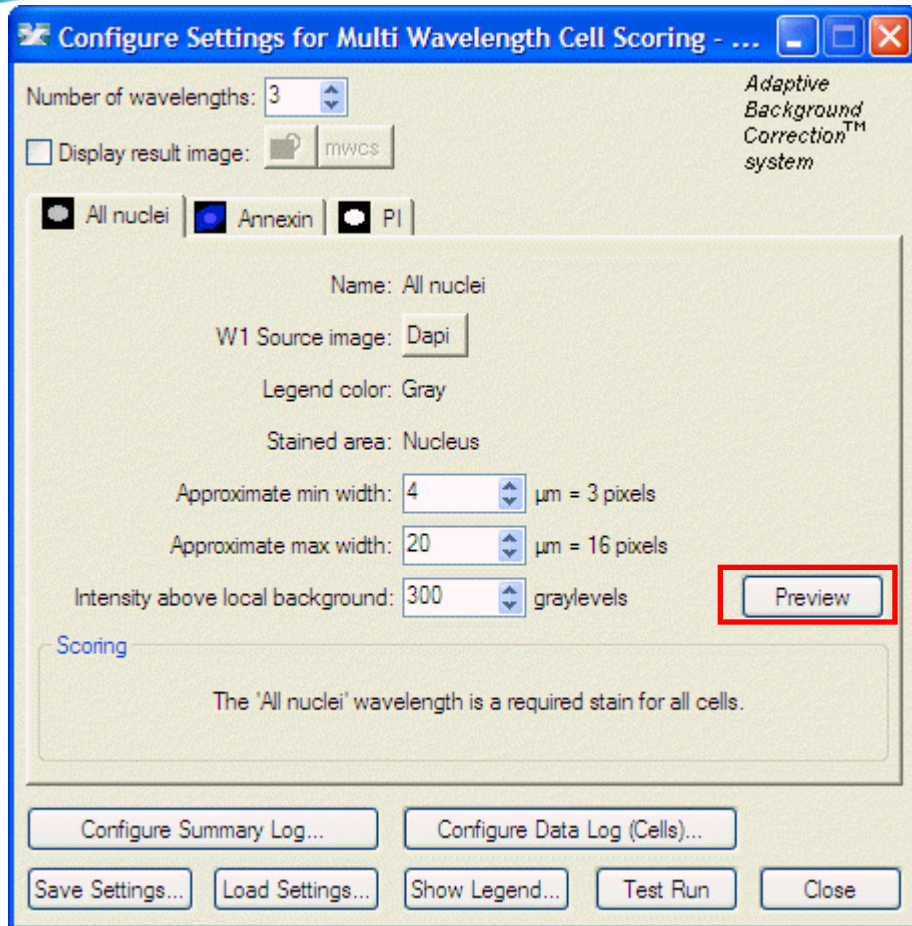
模块设置



- 波长1 (W1)
- **intensity above local background** 用于寻找细胞核
- 该值是最小值, 因此应该较图像中较弱的细胞获得的值低
- 画一条线同时穿过细胞核和背景, 选择菜单Measure 下的 Linescan 来决定该值;
- 或者将鼠标放在细胞核上和背景上, 在软件窗口下方会显示亮度值, 两者差值*0.6, 即为该值

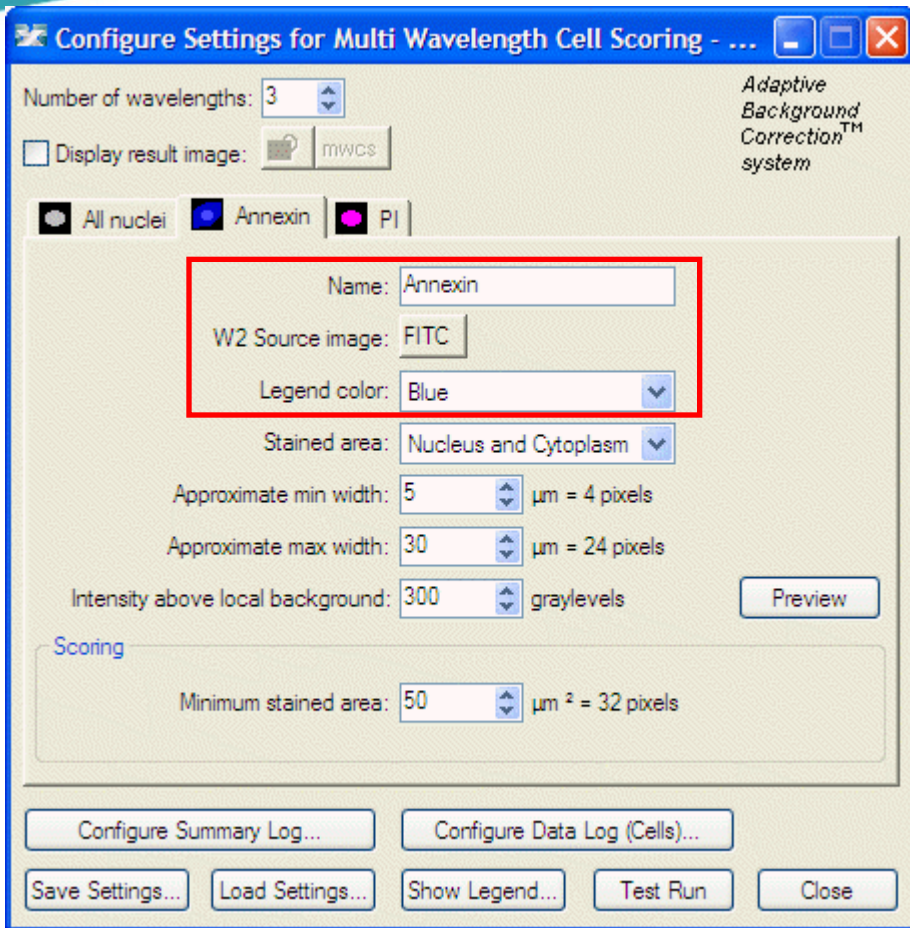


模块设置



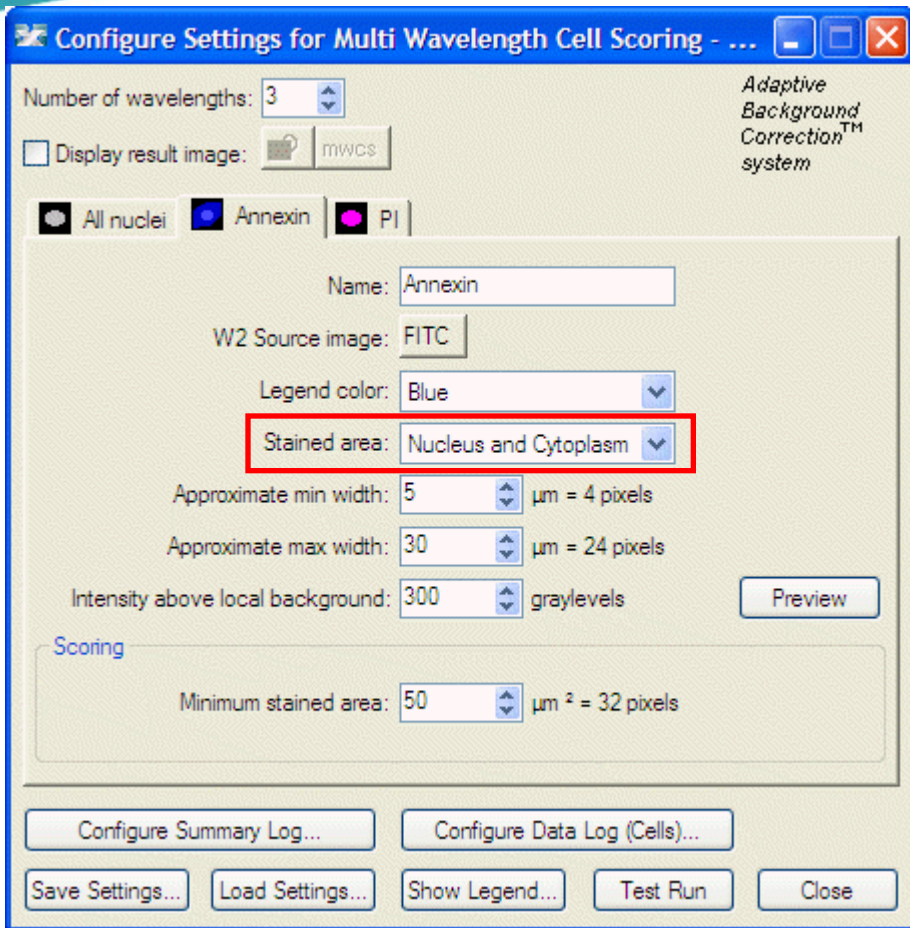
- 波长 1 (W1)
- 点击 **Preview** 来检验该波长的识别情况。

模块设置



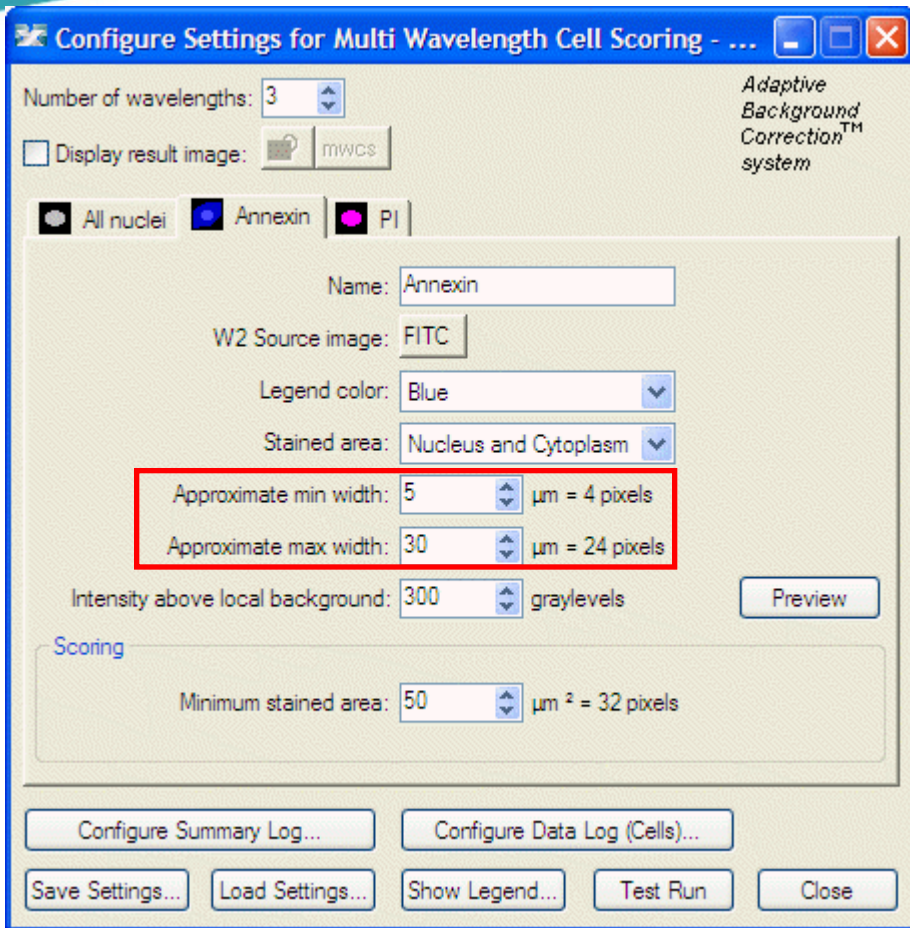
- 波长2 (W2)
- 输入该波长显示的结构名称, 相应的图像和识别后给予的示意颜色 (以Annexin为例)

模块设置



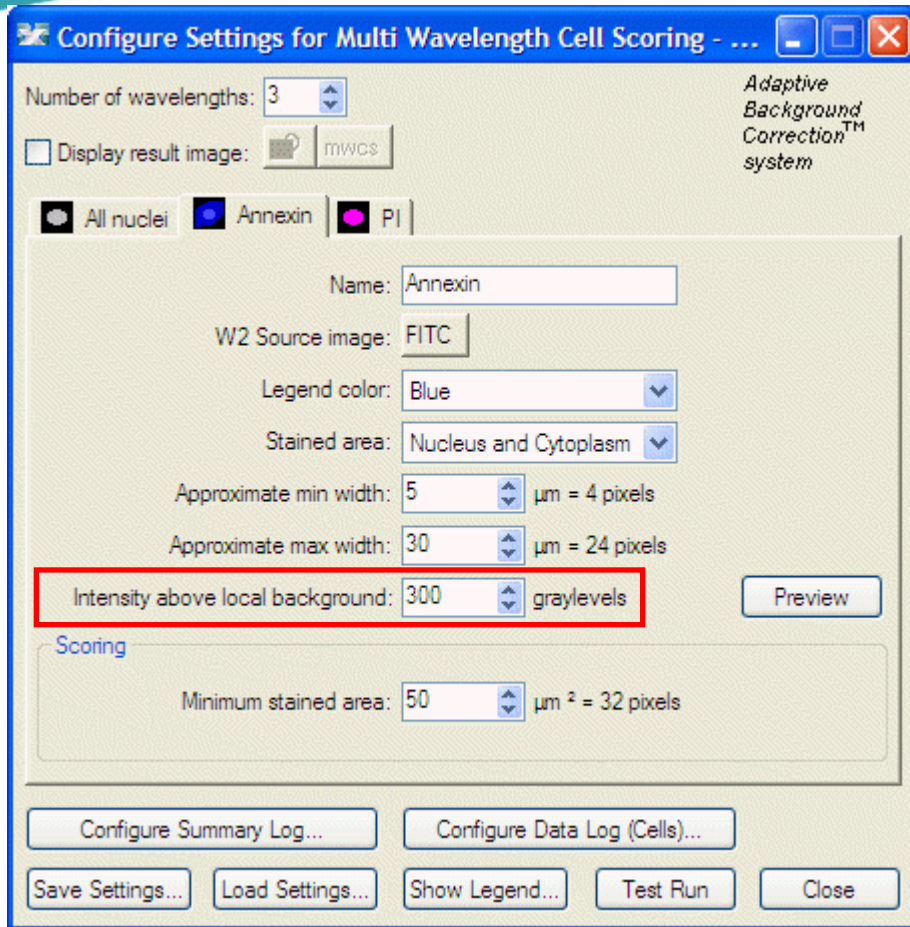
- 波长 2 (W2)
- 定义 **Stained area**:
 - Nucleus (细胞核区, 染色区域与W1相同)
 - Cytoplasm (胞浆区域, 染色区域环绕W1, 不与W1重合)
 - Nucleus and Cytoplasm (细胞核和胞浆, 染色区域包含W1区域, 和周围的环绕区域)

模块设置



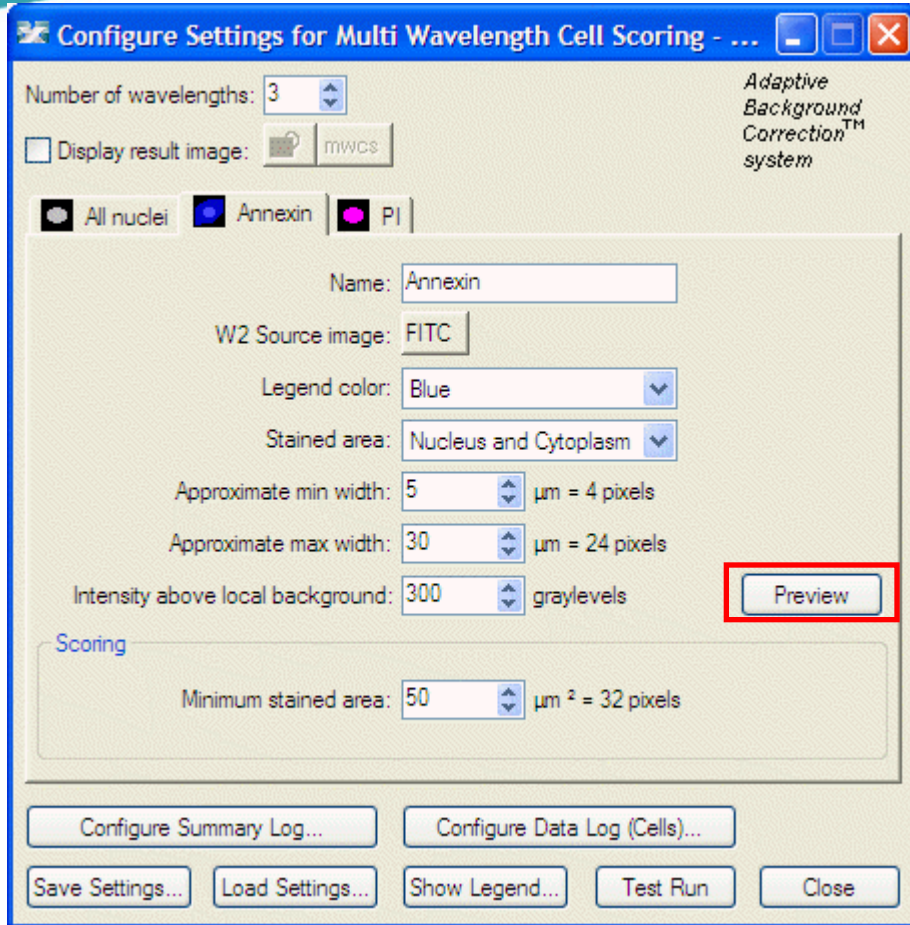
- 波长 2 (W2)
- 设置该波长 **Approximate min width** 和 **Approximate max width** 如果该波长染的是细胞核，使用与W1相同的设置
- 如果染 cytoplasmic 或 nuclear + cytoplasmic stain, 以设置W1的方法计算整个细胞的大小

模块设置



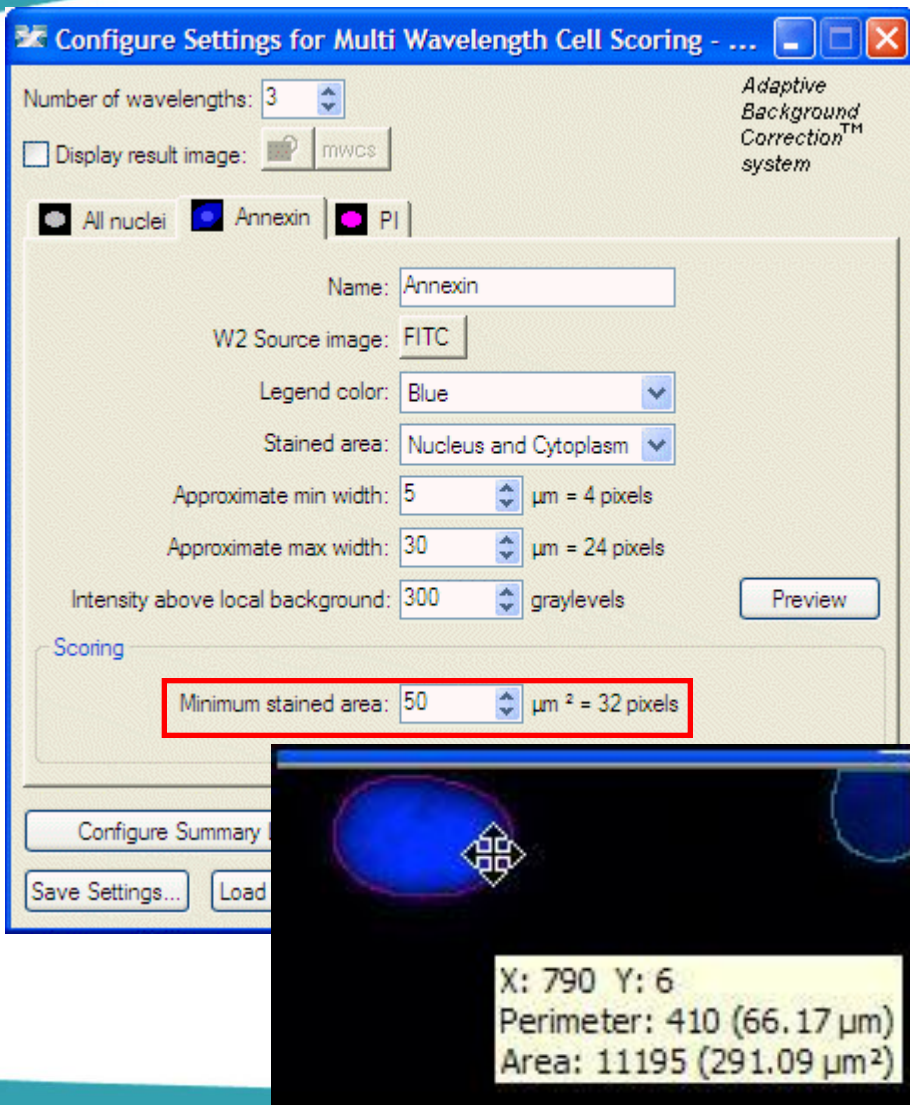
- **Wavelength 2 (W2)**
- **Intensity above local background** 的设置同W1

模块设置



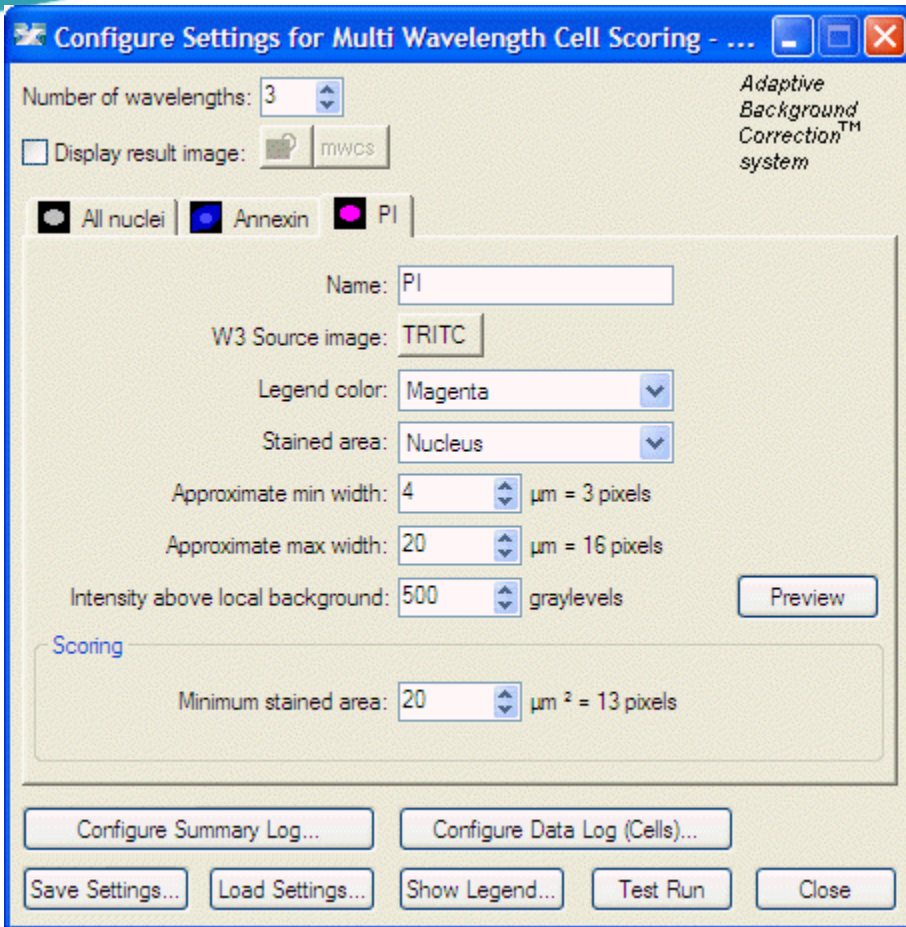
- **Wavelength 2 (W2)**
- 点击**Preview** 来测试设置是否合适
- 可以参考Preview的结果进行 Intensity above local background 设置的调整

模块设置



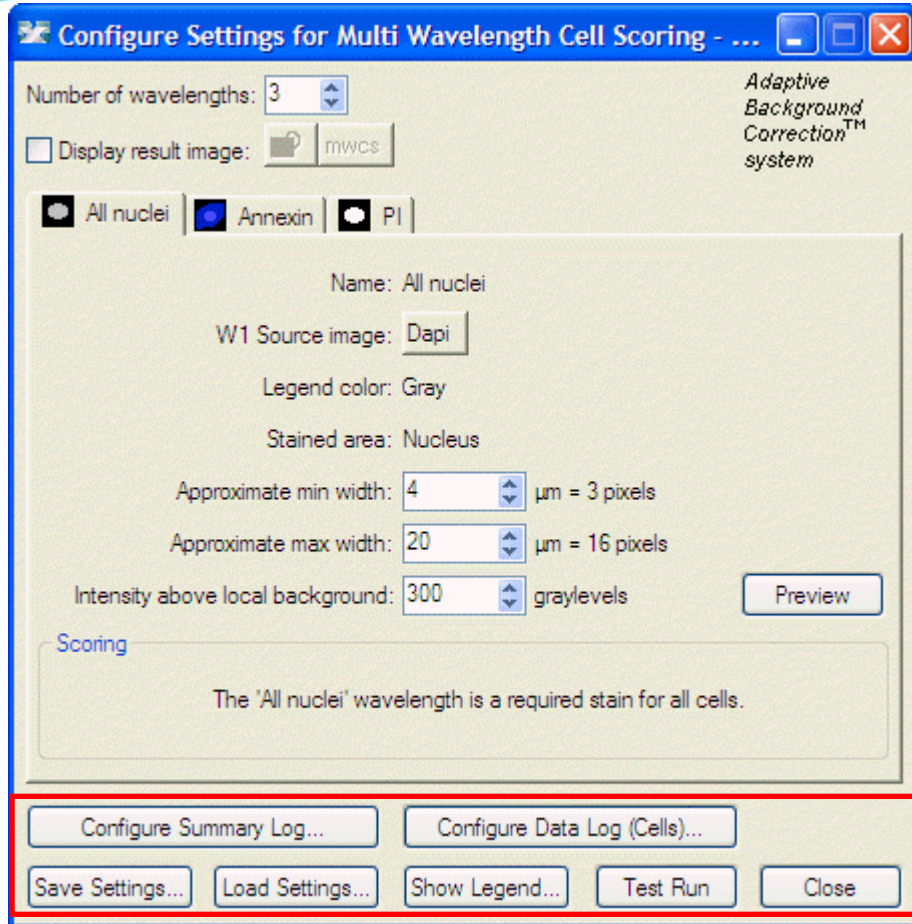
- **Wavelength 2 (W2)**
- **Minimum stained area** 是区别阳性和阴性细胞的一个区别标准
- 可以使用圆形或其他区域选择工具来进行测量

模块设置



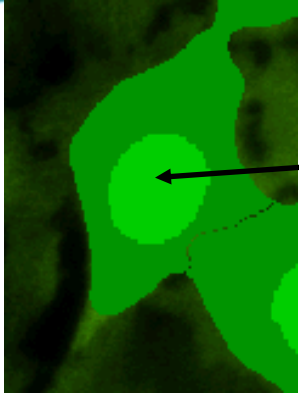
- **Wavelength 3 (W3) 和其他的波长**
- 以之前的方法进行设置(以PI 为例)

模块设置

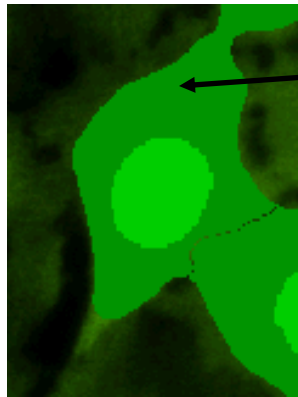


- **Configure Summary Log** – 选择每个视野图像的测量结果
- **Configure Data Log** – 选择每个细胞的测量数据
- **Save Settings** – 保存分析参数
- **Test Run** – 测试所有的设置, 同时显示细胞的分析结果

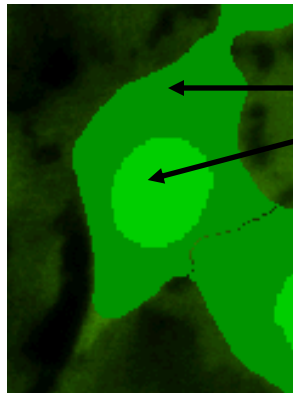
测量区域说明



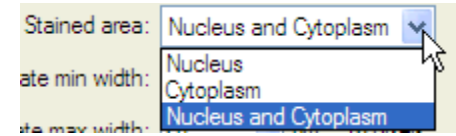
Nucleus



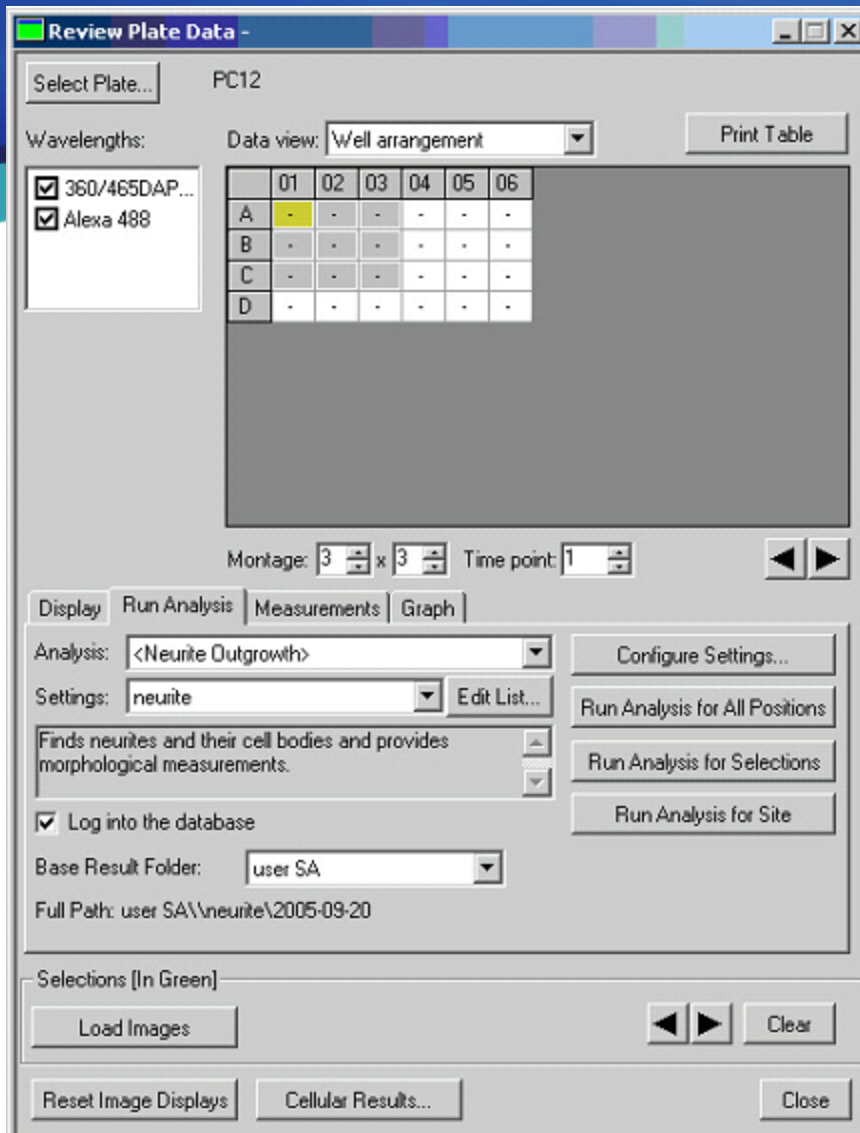
Cytoplasm



Stained Area (as defined in settings)



Cell (nucleus + cytoplasm)



分析模块设置完成后，Setting内
可以选择该分析设置

点击Run Analysis for all
Position对所有图像进行分析

Review Plate Data - PC12

Select Plate... PC12

Wavelengths: 360/465DAP... Alexa 488

Data view: Well arrangement Print Table

	01	02	03	04	05	06
A	25.00	5.88	46.67	5.22	7.53	1.79
B	41.94	0.00	4.17	6.67	11.83	4.09
C	33.12	10.00	7.38	13.79	10.00	4.76
D	39.70	9.38	5.02	3.60	5.87	14.60

Montage: 4 x 3 Time point: 1

相应分析参数的分析结果显示于此

Display | Run Analysis | Measurements | Graph

Analysis: Count Nuclei: CountNucleiDAI Show Heat Map Heat Map...

Measurement: Laser focus score (CountNuclei) Display Format: #.###

Select Wells Based On Variable Range

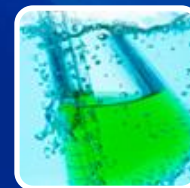
Value is: Between 0 and 100 Select

Data Log Not Open Configure Log... Open Log

分析完毕后，在 Measurement 标签中选择分析过的模块及设置

选择需要比较的分析参数





数据导出

部分数据导出

Select Plate... Transflur Fixed Demo Plate 36_APBIJL-L3CFX5X_1

Wavelengths: Data view: Well arrangement Print Table

	07	08	09	10	11	12
A						
B						
C	1322.00	1318.00	7795.00	8031.00	6130.00	5424.00
D	963.00	1202.00	6639.00	6569.00	6158.00	5305.00
E	905.00	1001.00	6032.00	6348.00	6284.00	5643.00
F	1077.00	1099.00	6273.00	6892.00	5290.00	4135.00
G	1089.00	1018.00	5090.00	4548.00	4611.00	5524.00
H	2129.00	2210.00	3037.00	3565.00	2589.00	2613.00
I	3964.00	5178.00	1567.00	1165.00	1170.00	1206.00
J	4543.00	5281.00	853.00	780.00	660.00	1097.00

Montage: 4 x 3 Time point 1 of 1

Display | Run Analysis | Measurements | Graph

Analysis: Transflur HT: Demo Plate Pits Show Heat Map Heat Map...

Measurement: Granules (Transflur HT) Display Format: ###

Select Wells Based On Variable Range

Value is: Between 0 and 100 Select

Data Log Not Open Configure Log... Open Log

Selections [In Green]

Load Images Clear

Reset Image Displays Cellular Results... Close

1. 点击Measurements标签
2. 选择相应的分析方法和设置
(之前进行过的分析)
3. 选择所需要的参数
4. 点击Open Log
5. 弹出以下窗口

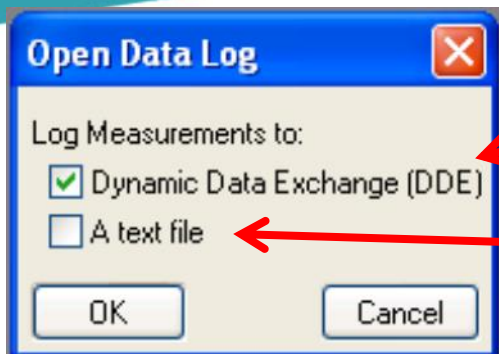
Open Data Log

Log Measurements to:

Dynamic Data Exchange (DDE)

A text file

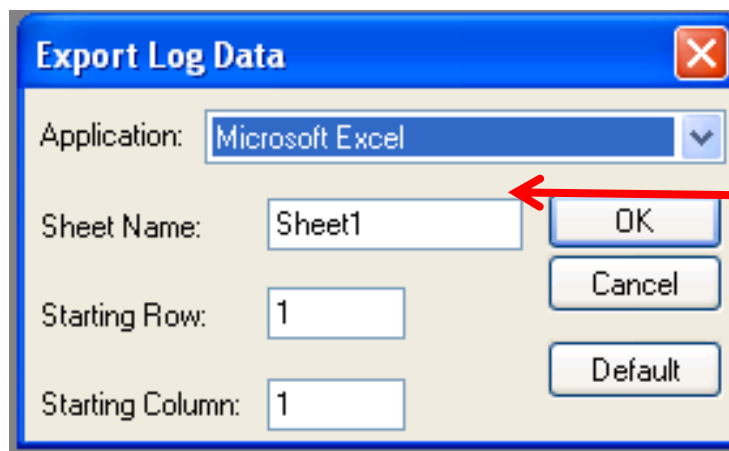
OK Cancel



导出到Excel

导出到文本文件

点击OK后弹出以下窗口



可以修改Excel表单的名称，点击OK，系统会自动打开一个Excel表格

Review Plate Data -

Select Plate... Transflur Fixed Demo Plate 36_APBIJL-L3CFX5X_1

Wavelengths: DAPI GFP

Data view: Well arrangement

	07	08	09	10	11	12
A						
B						
C	1322.00	1318.00	7795.00	8031.00	6130.00	5424.00
D	963.00	1202.00	6639.00	6569.00	6108.00	5305.00
E	905.00	1001.00	6032.00	6348.00	6284.00	5643.00
F	1077.00	1099.00	6273.00	6892.00	5290.00	4155.00
G	1089.00	1018.00	5090.00	4548.00	4611.00	5524.00
H	2129.00	2210.00	3037.00	3565.00	2589.00	2613.00
I	3964.00	5178.00	1567.00	1165.00	1170.00	1206.00
J	4543.00	5281.00	853.00	780.00	660.00	1095.00

Montage: 4 x 3 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

Analysis: Transflur HT: Demo Plate Pit: Show Heat Map

Measurement: Granules (Transflur HT) Display Format: ###

Select Wells Based On Variable Range

Value is: Between 0 and 100

Data Log: DDE App

Selections [In Green]

此时，原来的Open Log会变成Log Data

点击Log Data，即可将各个孔相应数据导入到Excel中。



Review Plate Data -

Select Plate... Transfluor Fixed Demo Plate 36_APBIJL-L3CFX5X_1

Wavelengths: DAPI GFP

Data view: Well arrangement Print Table

	07	08	09	10	11	12
A						
B						
C	1322.00	1318.00	7795.00	8031.00	6130.00	5424.00
D	963.00	1202.00	6639.00	6569.00	6108.00	5305.00
E	905.00	1001.00	6032.00	6348.00	6284.00	5643.00
F	1077.00	1099.00	6273.00	6892.00	5290.00	4155.00
G	1089.00	1018.00	5090.00	4548.00	4611.00	5524.00
H	2129.00	2210.00	3037.00	3565.00	2589.00	2613.00
I	3964.00	5178.00	1567.00	1165.00	1170.00	1206.00
J	4543.00	5281.00	853.00	780.00	660.00	1095.00

Montage: 4 x 3 Time point: 1 of 1

Display Run Analysis Measurements Graph

Analysis: Transfluor HT: Demo Plate Pit: Show Heat Map Heat Map...

Measurement: Granules (Transfluor HT) Display Format: #.##

各个孔相应指标的结果会自动导入到Excel中。

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Time point	1														
Measurement	Granules (Transfluor HT)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
					1407	1503	1322	1318	7795	8031	6130	5424	6921	8274	6656
					1121	1227	963	1202	6639	6569	6108	5305	6875	7049	7237
					1300	1088	905	1001	6032	6348	6284	5643	7574	7014	7372
					1067	1131	1077	1099	6273	6892	5290	4155	7449	7032	7301
					900	949	1089	1018	5090	4548	4611	5524	7041	7214	6213
					1745	2078	2129	2210	3037	3565	2589	2613	4243	4262	4461
					4584	3032	3964	5178	1567	1165	1170	1206	2112	2262	2419
					4036	4927	4543	5281	853	780	660	1095	1354	1227	1308
					4635	4105	4834	5631	801	789	875	817	828	819	836
					3961	4131	5153	5987	1068	834	695	840	735	643	669
					3874	4991	6317	5704	892	948	846	735	537	566	482
					4644	5443	4517	5426	974	760	867	679	548	410	637

全部数据导出

Review Plate Data -

Select Plate... Transflur Fixed Demo Plate 36_APBIIJL-L3CFX5X_1

Wavelengths: DAPI GFP

Data view: Well arrangement Print Table

	10	11	12
A			
B			
C	1322.00	1318.00	7795.00
D	963.00	1202.00	6639.00
E	905.00	1001.00	6032.00
F	1077.00	1099.00	6273.00
G	1089.00	1018.00	5090.00
H	2129.00	2210.00	3037.00
I	3964.00	5178.00	1567.00
J	4543.00	5281.00	853.00

Montage: 4 x 3 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

Analysis: Transflur HT: Demo Plate Pits Show Heat Map Heat Map...

Measurement: Granules (Transflur HT) Display Format: #.##

Select Wells Based On Variable Range

Value is: Between 0 and 100 Select

Data Log: DDE App Configure Log... Log Data

选择Measurement vs Well

Review Plate Data -

Select Plate... Transflur Fixed Demo Plate 36_APB1JL-L3CFX5X_1

Wavelengths: DAPI GFP

Data view: Measurement vs Well Print Table

	Granules	Granule	Total	Mean	Integrated	Average	Nuclei	Total
C05	1407.00	10.91	5434.12	3.86	11243685.00	860.79	125.88	33611.49
C06	1503.00	14.74	6150.93	4.09	13081814.00	884.80	102.00	27457.65
C07	1322.00	13.09	5261.05	3.98	11200956.00	885.73	101.00	27422.70
C08	1318.00	11.87	4641.17	3.52	9118496.00	817.36	111.00	29248.22
C09	7795.00	73.54	10535.00	1.35	20436056.00	807.02	106.00	28489.39
C10	8031.00	71.71	11080.83	1.38	21074132.00	791.22	112.00	30170.13
C11	6130.00	60.10	8025.12	1.31	14443160.00	748.74	102.00	25818.51
C12	5424.00	71.37	7567.08	1.40	13712077.00	753.87	76.00	19496.60
C13	6921.00	60.18	13833.25	2.00	27870740.00	838.19	115.00	30704.31
C14	8274.00	61.75	16521.19	2.00	34820964.00	876.84	134.00	35525.21

Montage: 1 x 3 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

Analysis: Transflur HT: Demo Plate Pit:

Measurement: Granules (Transflur HT) Display Format: #.###

Select Wells Based On Variable Range

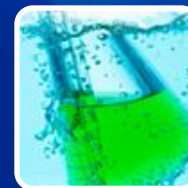
Value is: Between 0 and 100 Select

Data Log Not Open Configure Log... Open Log

软件会展示所有的分析指标和结果

点击Open Log, 可将所有结果导出到Excel或txt文件中。

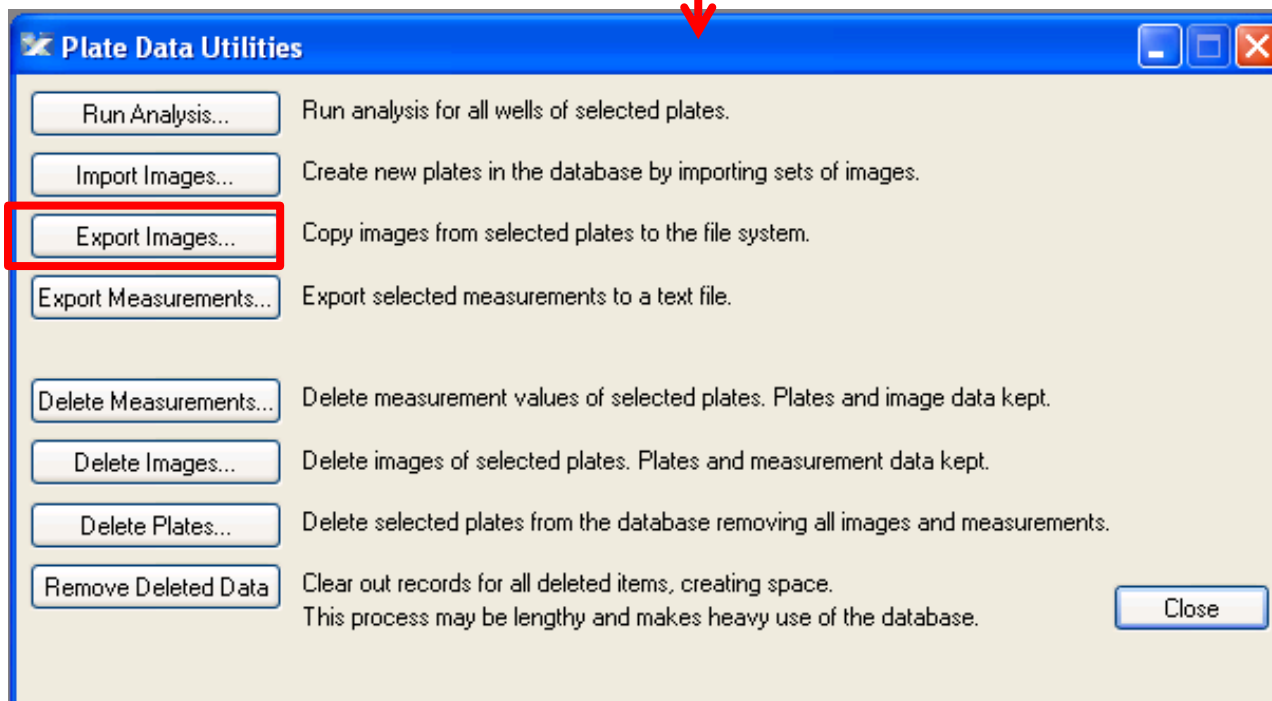
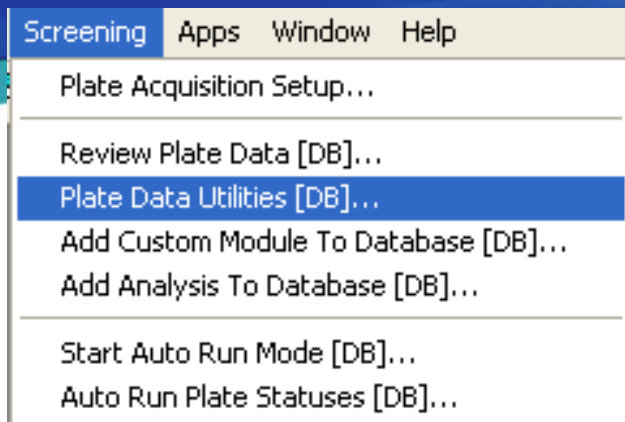


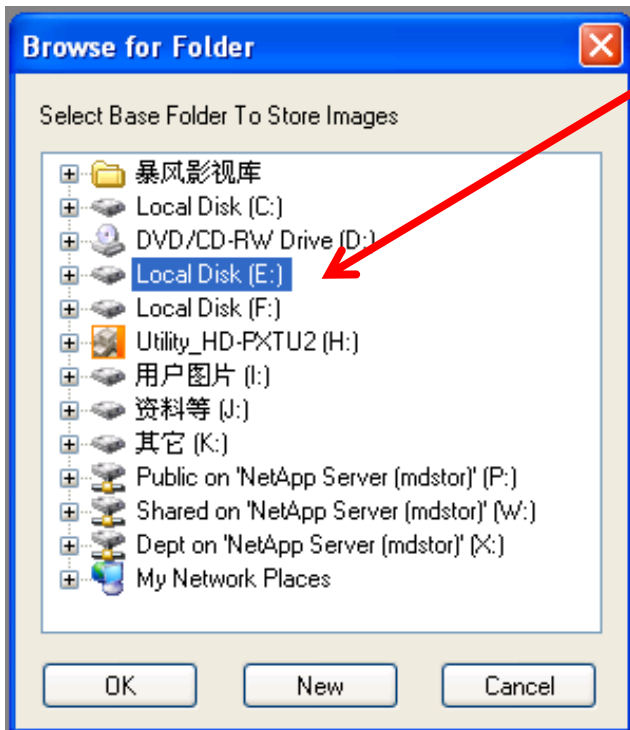


导出图像有两种方式

- 1. 导出原始图像(黑白)
推荐采用该方式导出图像
- 2. 导出彩色图像

导出原始图像

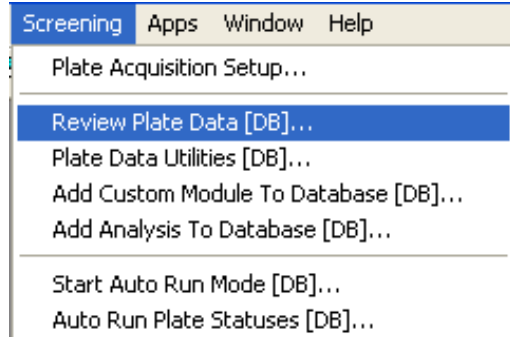




选择图像导出路径

原始图像即导出到相应文件夹

导出彩色图像



Review Plate Data -

Select Plate... Transflur Fixed Demo Plate 36_APBIJL-L3CFX5X_1

Wavelengths: DAPI GFP

Data view: Well arrangement Print Table

	04	05	06	07	08	09	10	11	12
B									
C		5434.12	6150.93	5261.05	4641.17	10535.00	11080.83	8025.12	7567.0
D		3749.63	4473.52	3072.76	5252.32	9289.42	9450.01	7669.00	6468.3
E		5304.32	4151.51	3824.10	3189.25	8642.92	9197.48	8608.39	7515.4
F		3983.02	4519.70	4090.36	4180.22	9486.62	9304.40	7147.73	6360.6
G		3267.04	3217.54	3225.44	3309.48	7996.42	6930.14	7355.32	7408.5
H		3686.40	3896.91	4761.41	4688.19	5678.74	5679.99	4729.37	4757.2
I		6876.48	5449.51	5655.44	8297.20	4680.70	2896.37	3213.38	3396.4
J		6359.36	7015.43	7007.94	7718.10	3248.32	3148.89	2160.00	4486.8
K		6675.54	5904.23	7134.00	8498.97	2914.26	2932.98	3941.42	3212.9

Montage: 1 x 3 Time point: 1 of 1

Display Run Analysis Measurements Graph

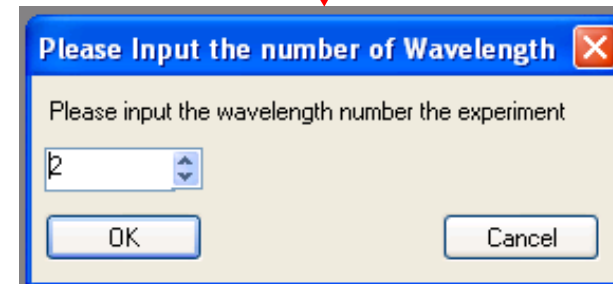
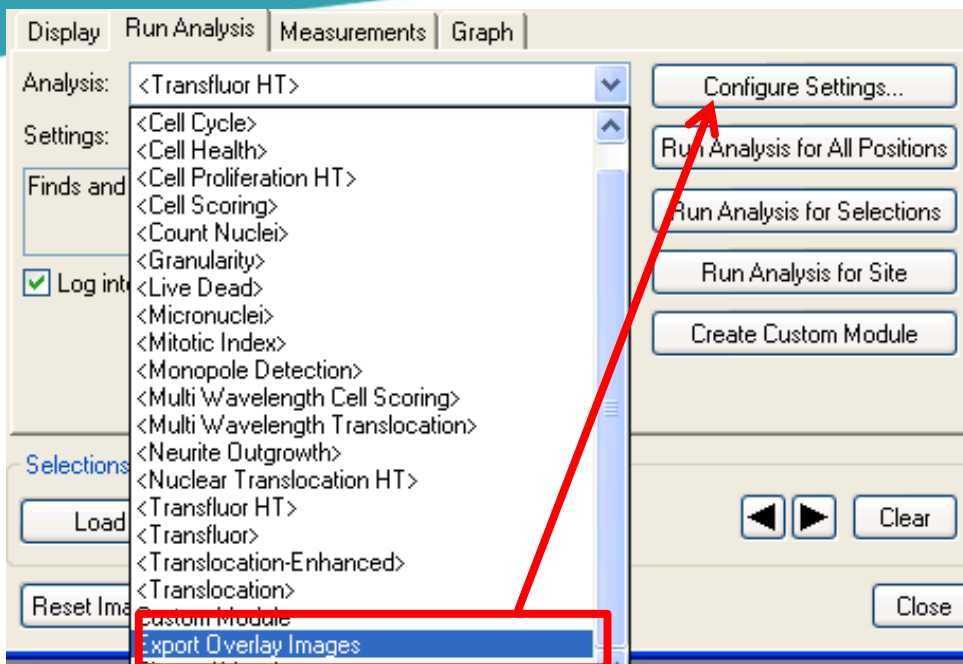
Analysis: Transflur HT: Demo Plate Pit: Show Heat Map Heat Map...

Measurement: Total Granule Area (Transflur) Display Format: ###

Select Wells Based On Variable Range

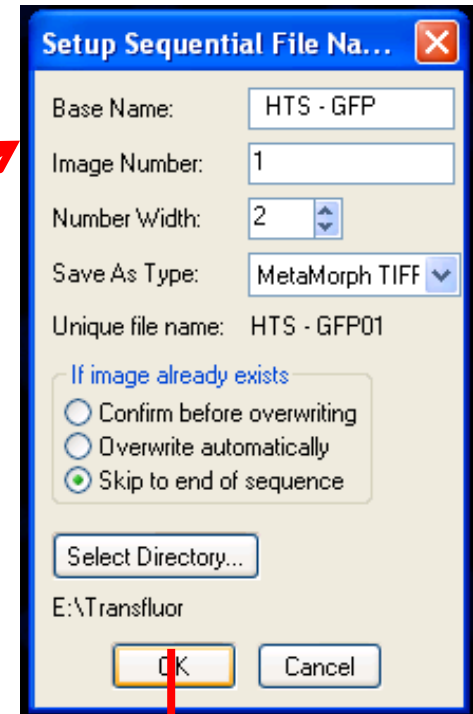
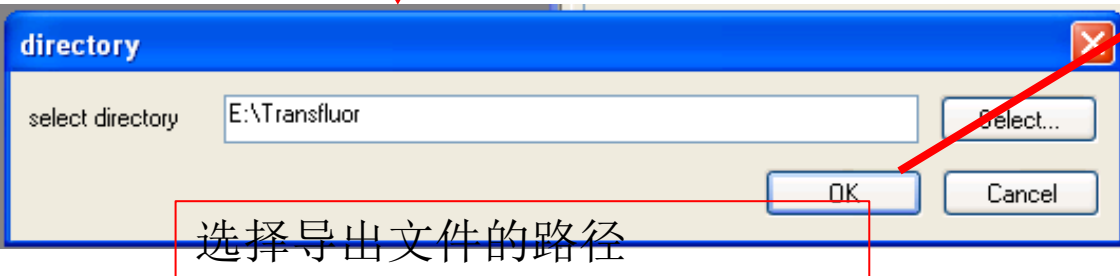
Value is: Between 0 and 100 Select

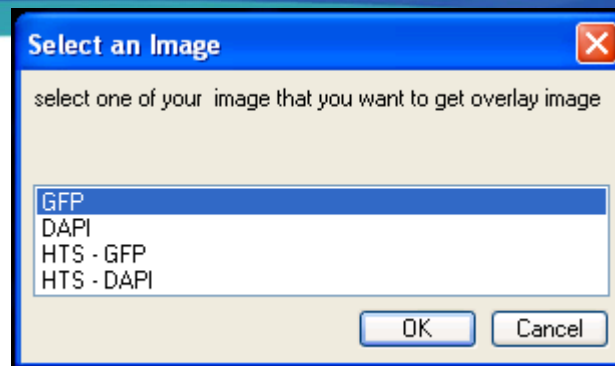
Data Log Not Open Configure Log... Open Log



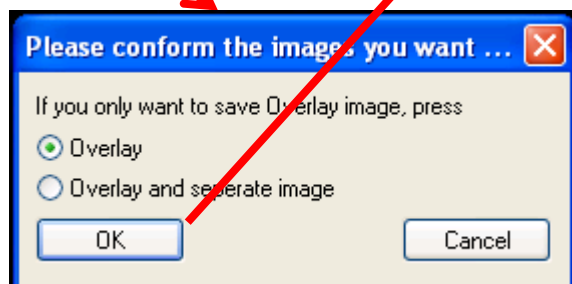
输入荧光波长数，
如同时标记了2中
荧光，就选择2



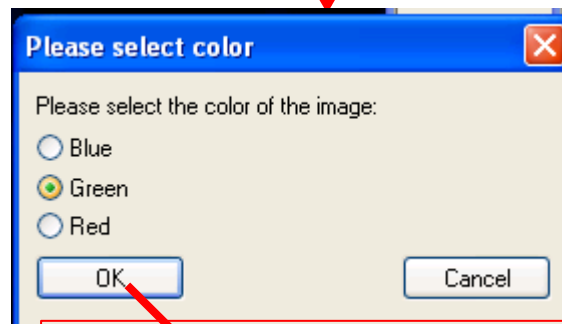




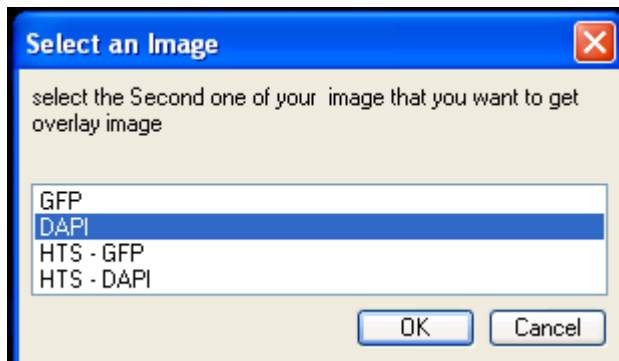
选择某个荧光通道的一个图像（不要选择HTS开头的图像）



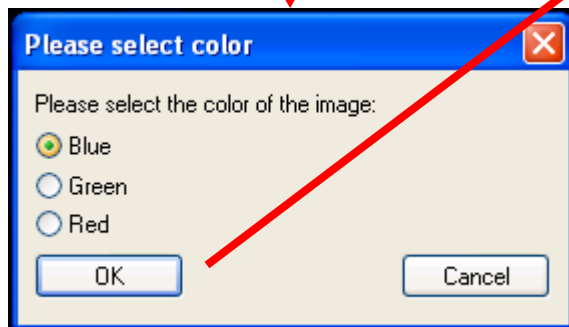
如果仅需要彩色叠加图像，就选**Overlay**；如果彩色叠加图像和各个通道单独的彩色图像，选后者



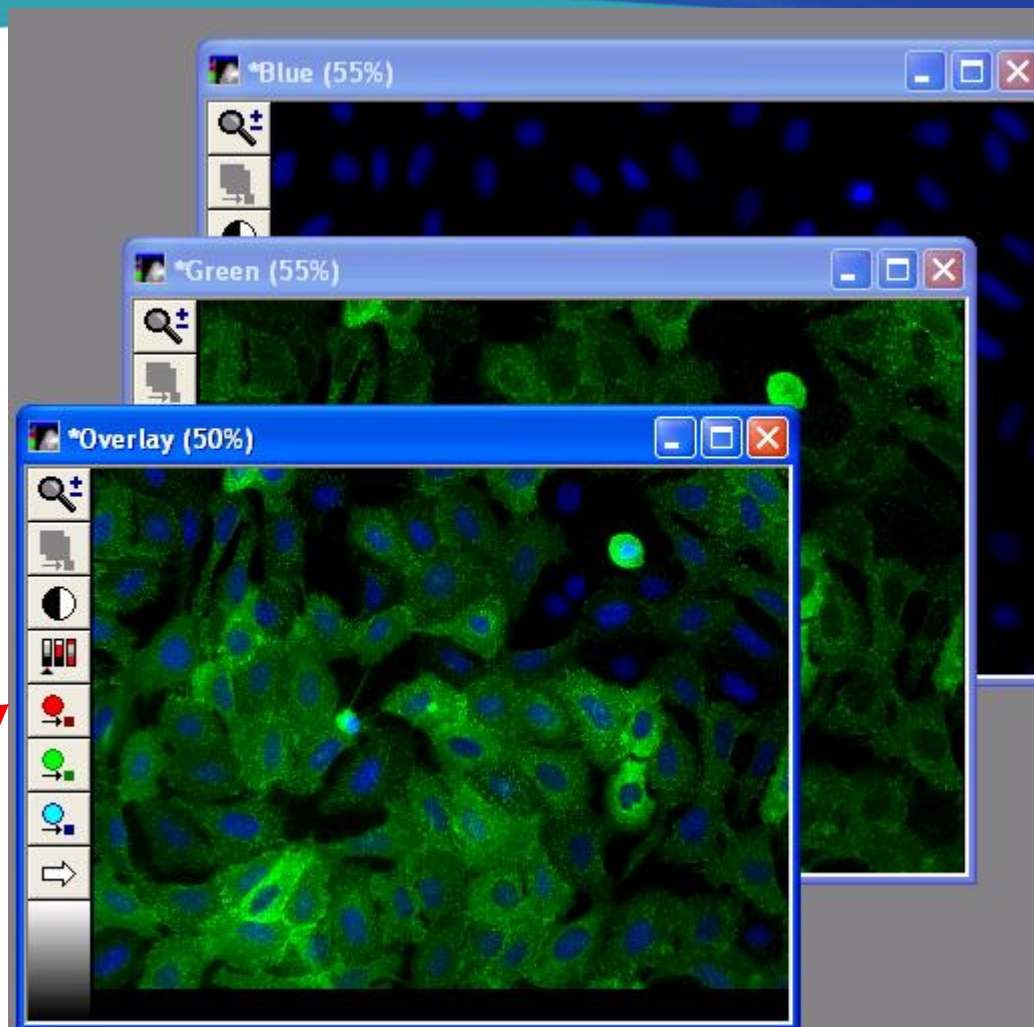
选择该通道图像的颜色



选择另外一个荧光通道的图像（不要选择HTS开头的图像）

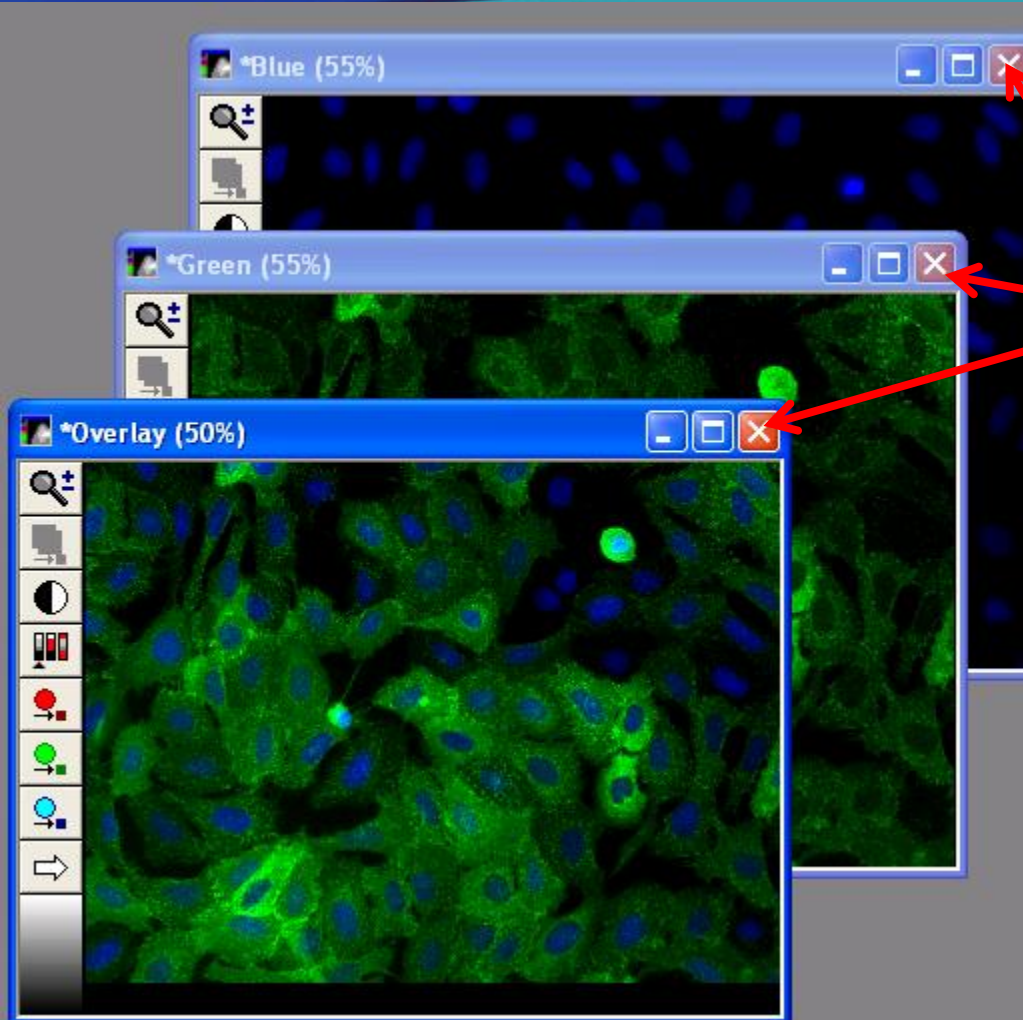


选择该通道图像的颜色

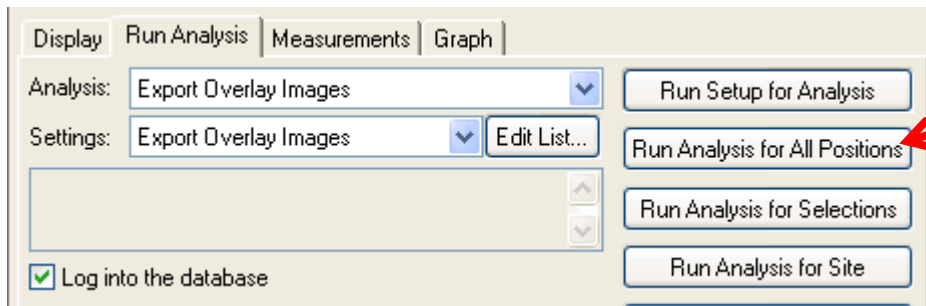


软件会根据之前的选择自动生成彩色图像



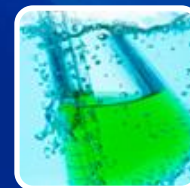


关闭生成的图像

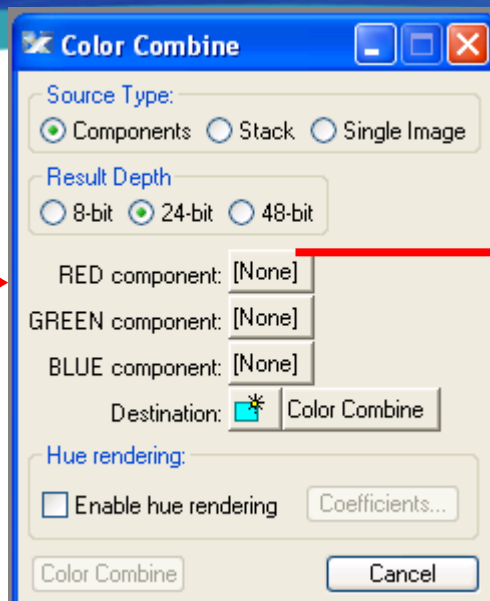
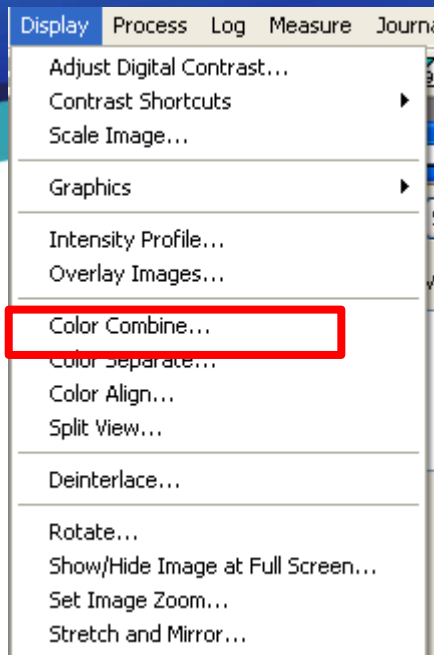


点击Run Analysis for All
Position

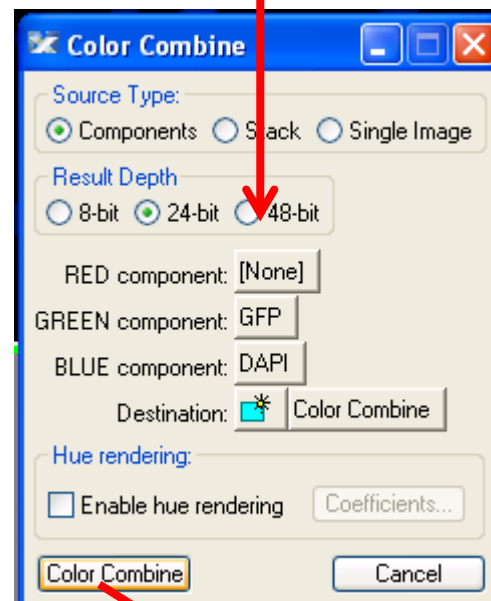
所有通道生成的叠加彩色图
像自动保存在设置好的路径
内。



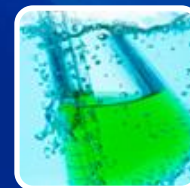
彩色多通道图像手动叠加



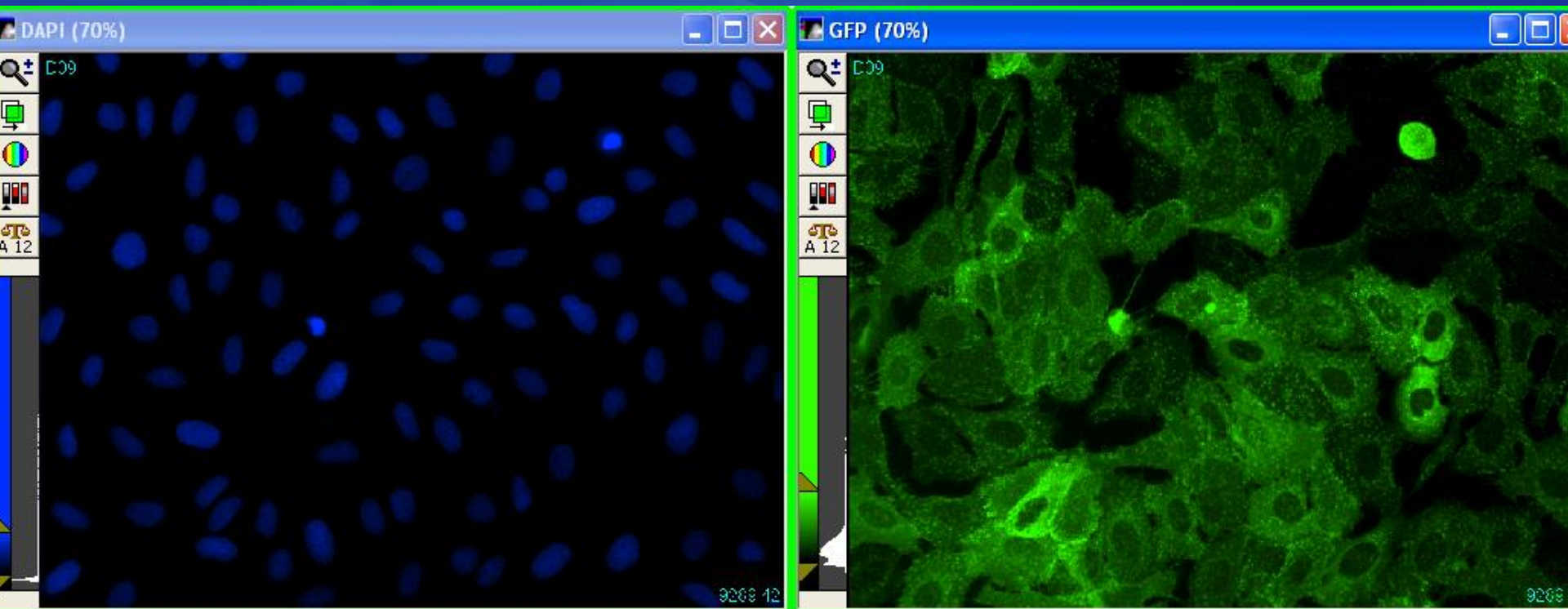
在不同的颜色中选择相应的图片



点击Color Combine，就可获得多通道叠加图像。



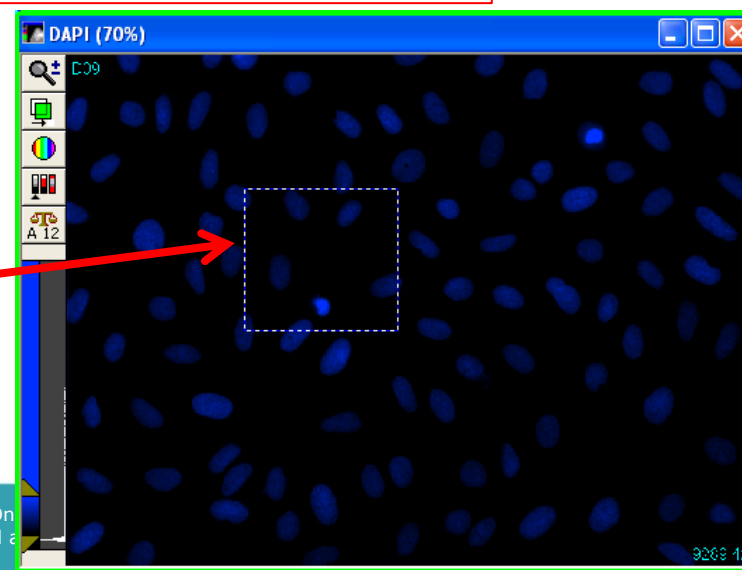
相同部位的图像剪切

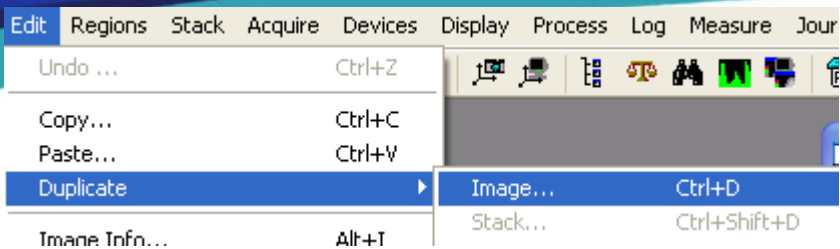


打开同一位置不同通道的图像

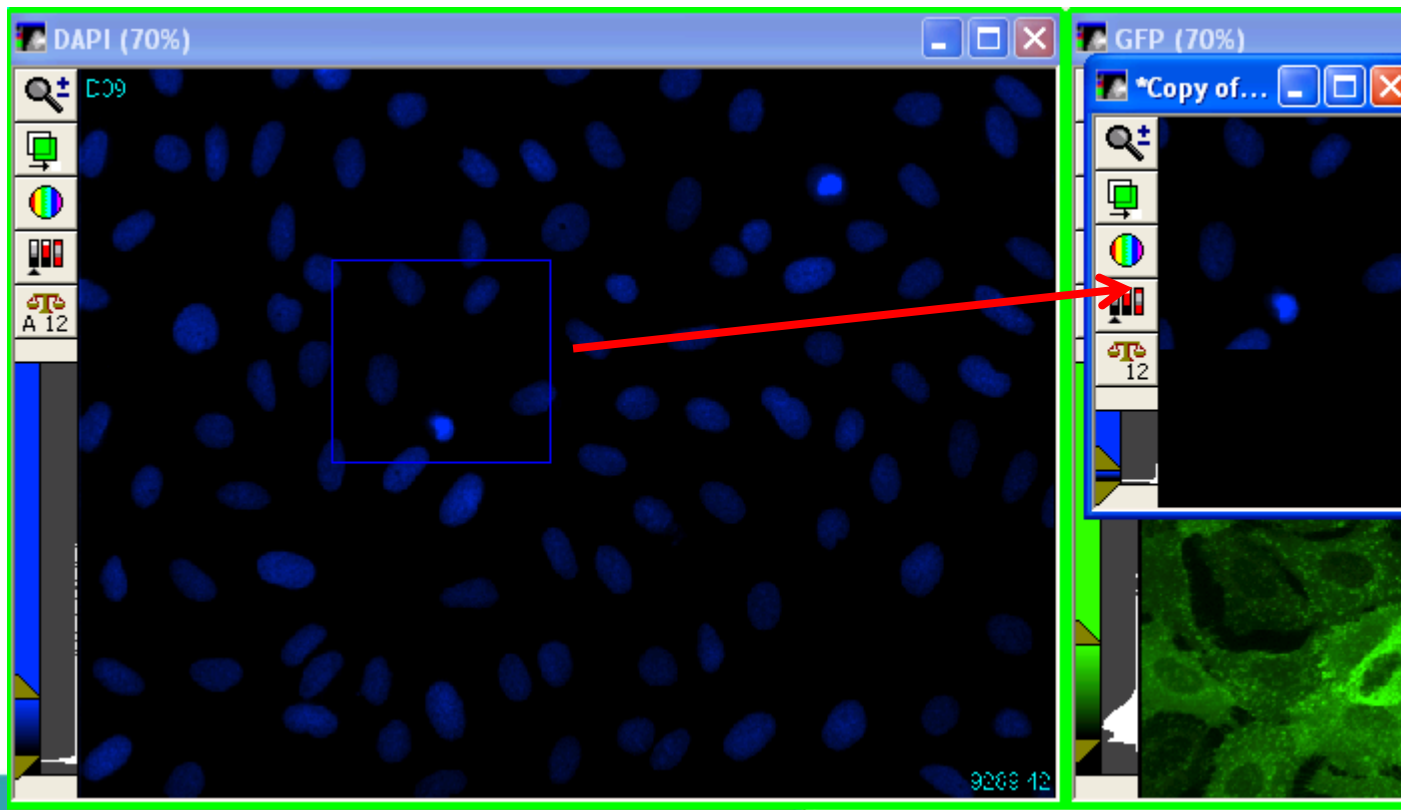


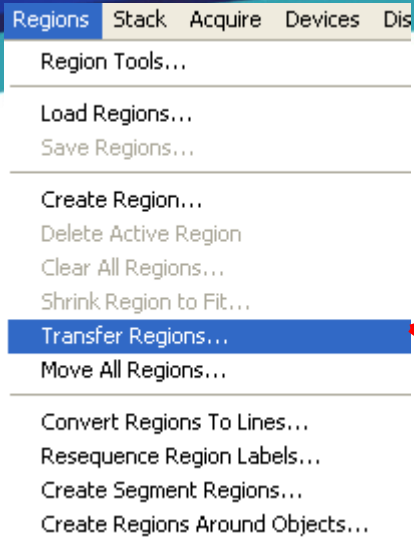
使用Region工具在图像上画出剪切区域



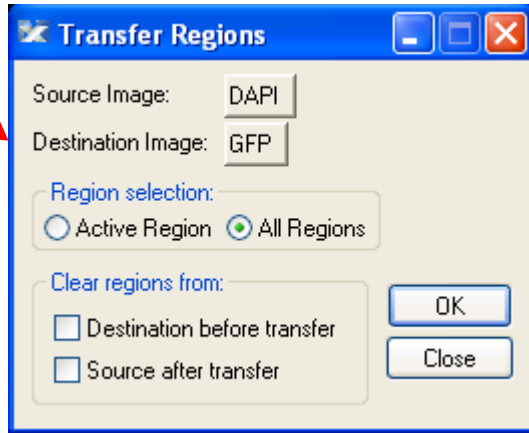


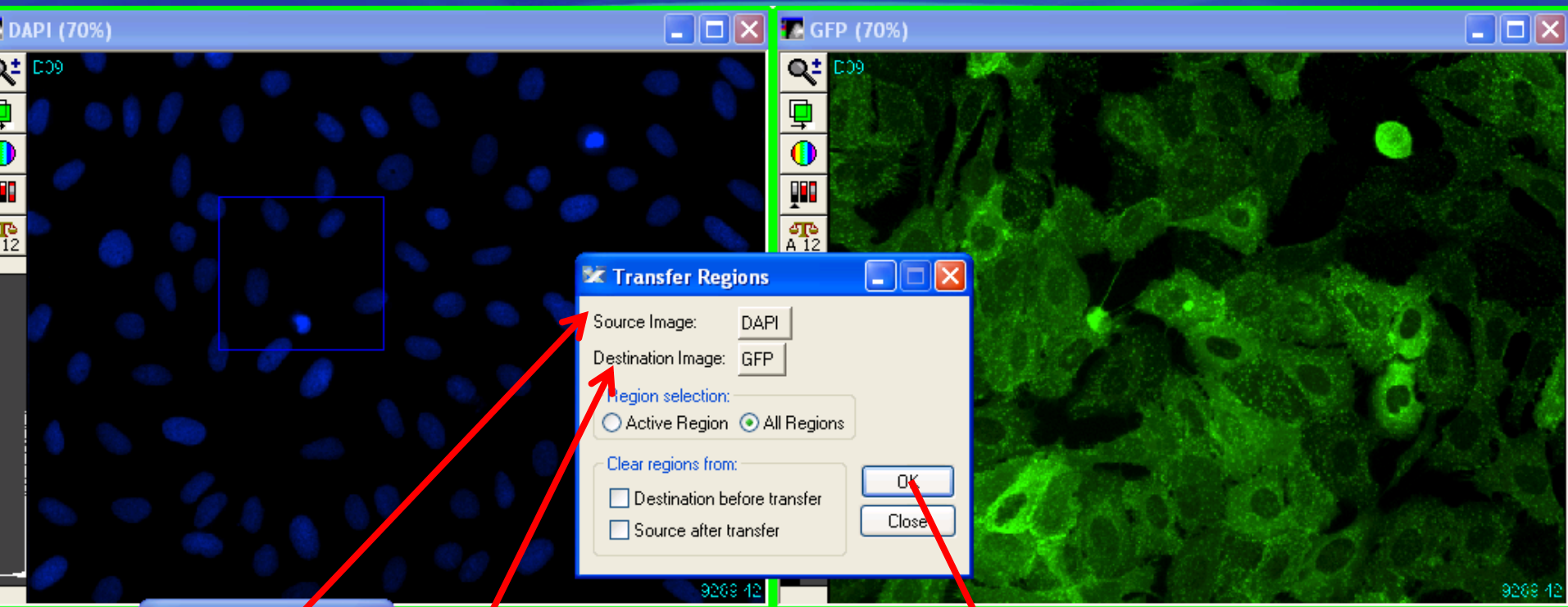
选择Edit-Duplicate—Image,即可
将图像相应区域剪切下来





可将一个图片中的区域直接转移到另一张图上

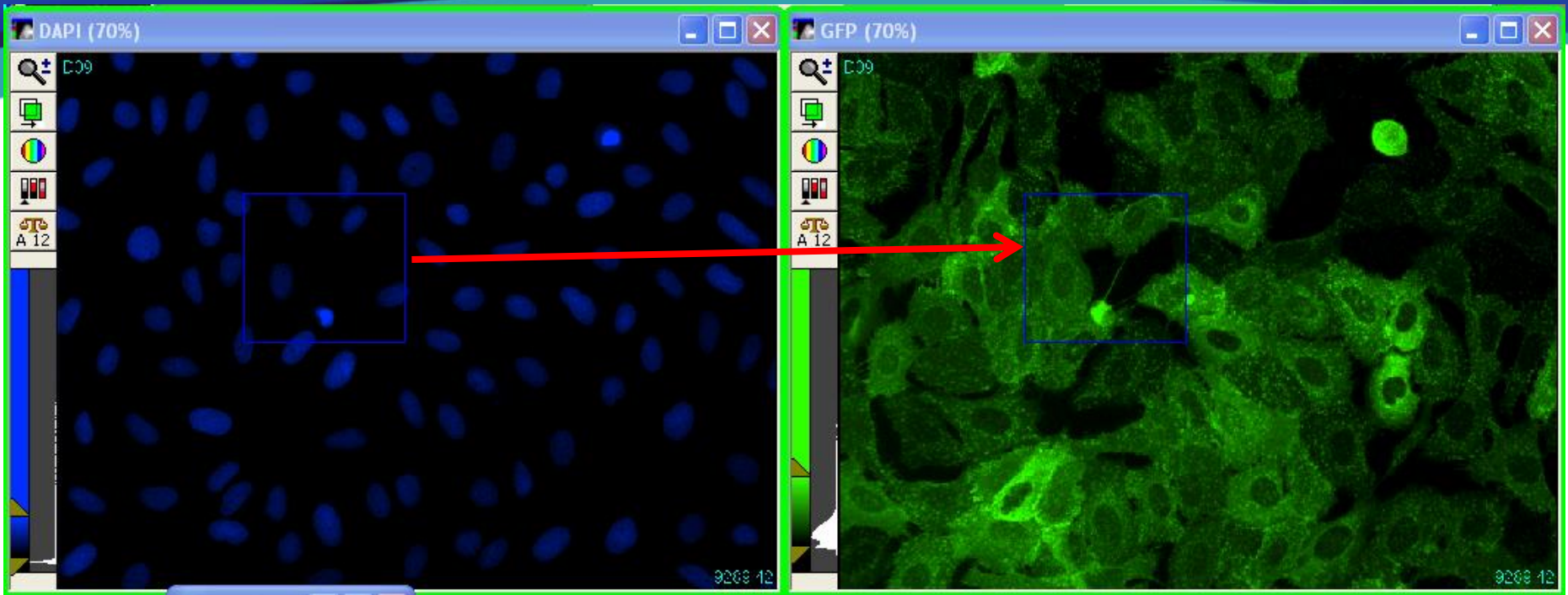




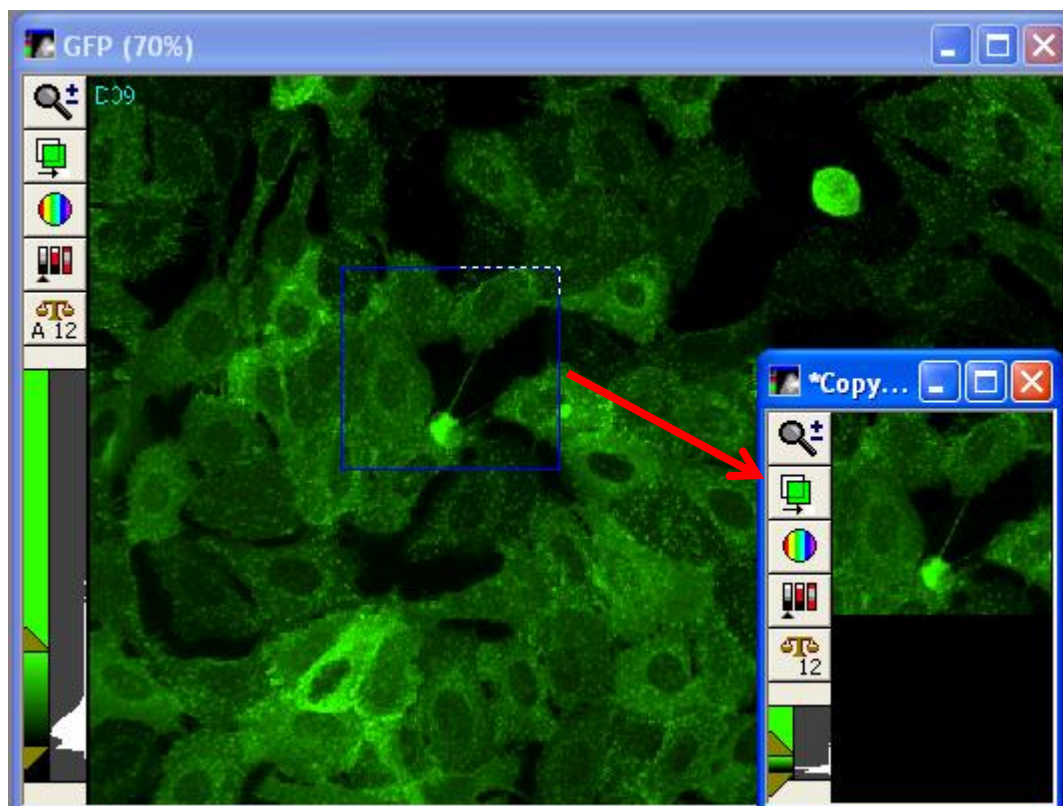
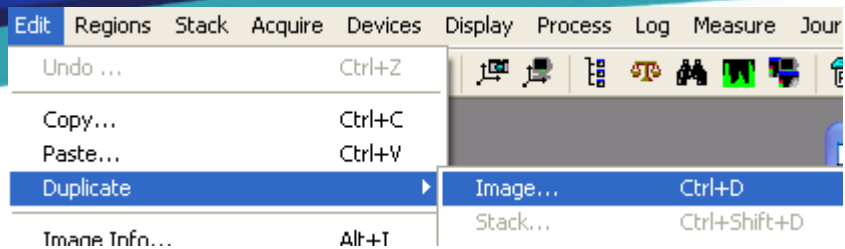
选择源图像（即已经划定区域的图像，如上图中的DAPI）

选择目标图像（即需要将DAPI中的方形区域转移到哪张图像上，如上图GFP）

点击OK



方形区域从DAPI复制到GFP上



- 可对生成的方形区域大小的图像进行颜色叠加等操作