

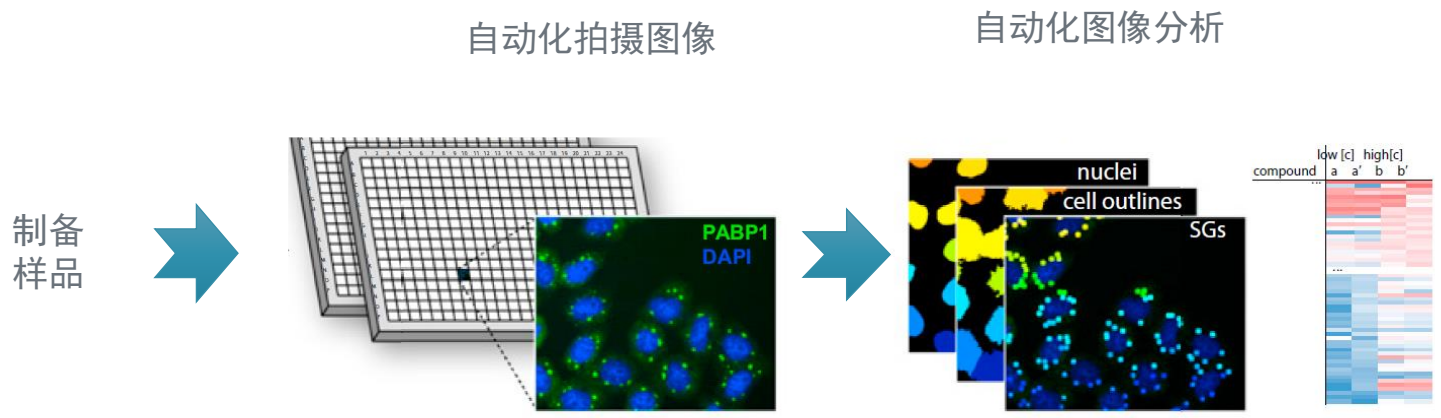


ImageXpress Micro高内涵系统操作培训

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高内涵实验基本流程



Frank Wippich, Bernd Bodenmiller. Dual Specificity Kinase DYRK3 Couples Stress Granule Condensation/Dissolution to mTORC1 Signaling. Cell 152, 791–805, February 14, 2013

主要内容

- MetaXpress软件拍摄设置流程

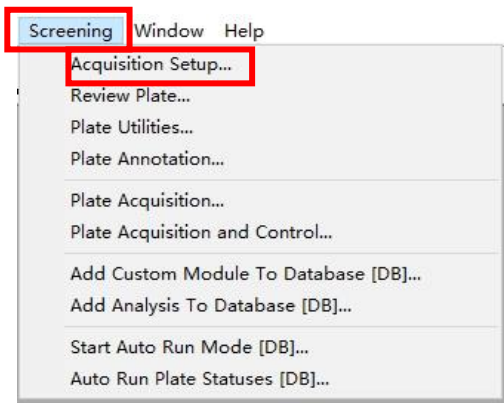
- 基本设置
- 长时间拍摄设置
- Z-Stack拍摄设置
- Protocol保存与应用
- 聚焦设置

- 高内涵图像常用处理方法与技巧

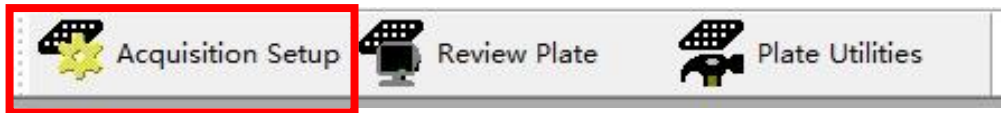


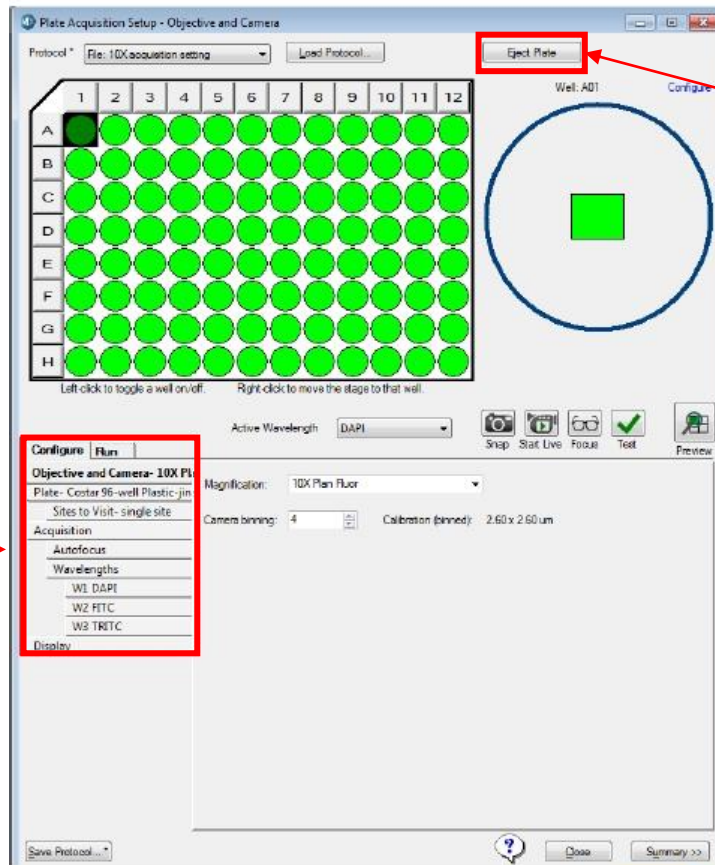
基本设置

进入软件后，在菜单栏Screening下拉菜单中，选择 Acquisition Setup



或者直接找到工具栏快捷键 Acquisition Setup

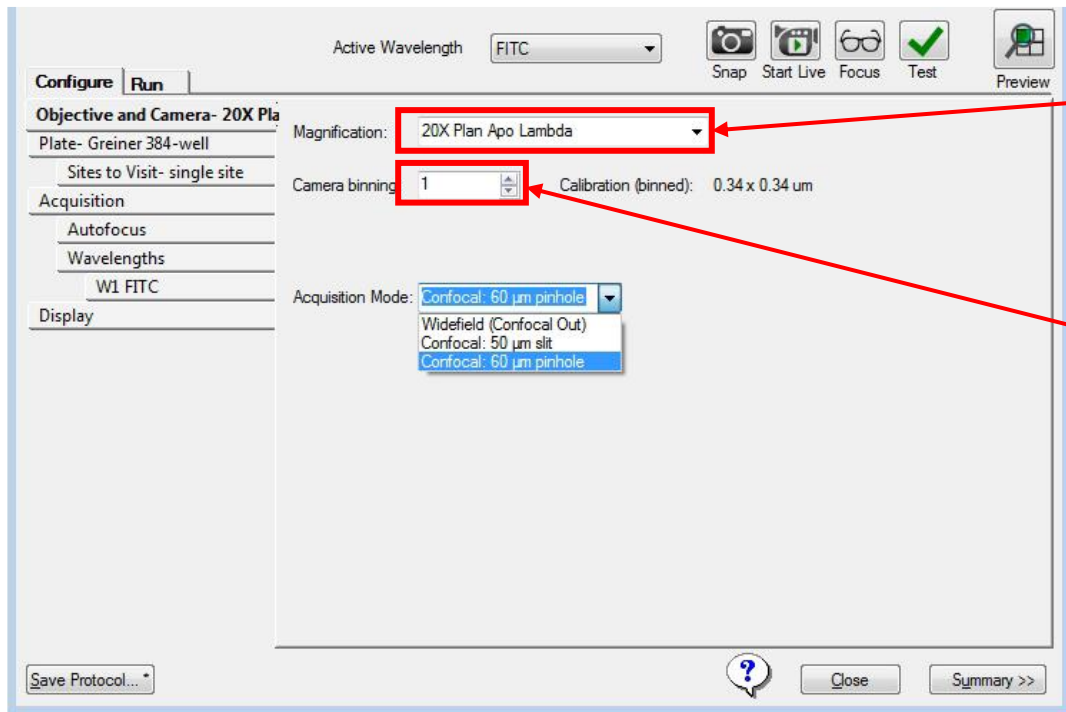




打开舱门，放样本进去

在弹出的Plate Acquisition Setup窗口，按照左侧标签顺序依次进行设置

物镜和图像大小设置

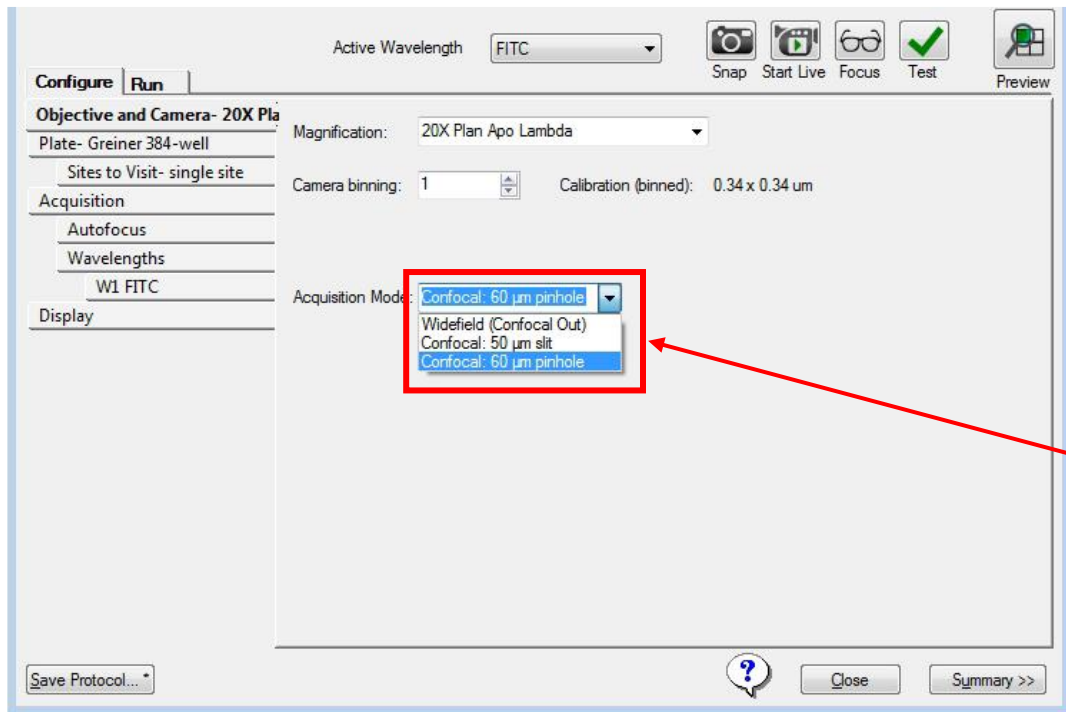


从下拉菜单中选择物镜类型

选择图像的大小，数值越大，图像越小，一般选择2的时候效率最高

IXM-Confocal

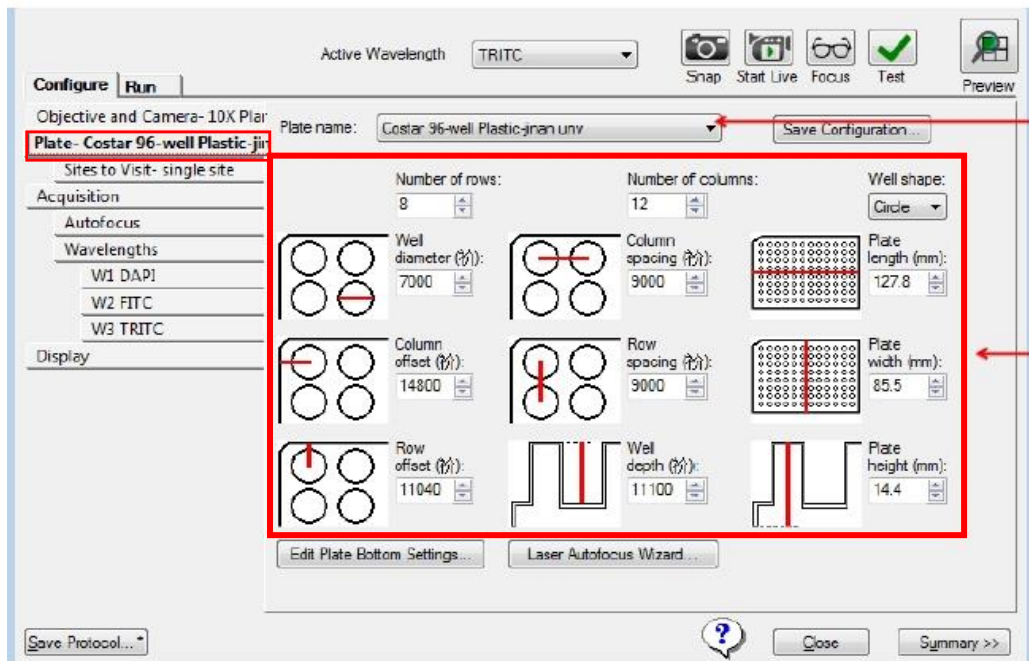
成像模式选择



成像模式：宽场或共聚焦
（根据系统配置可选择60
um pinhole, 42 um
pinhole, or 50 um slit）

IXM-Confocal

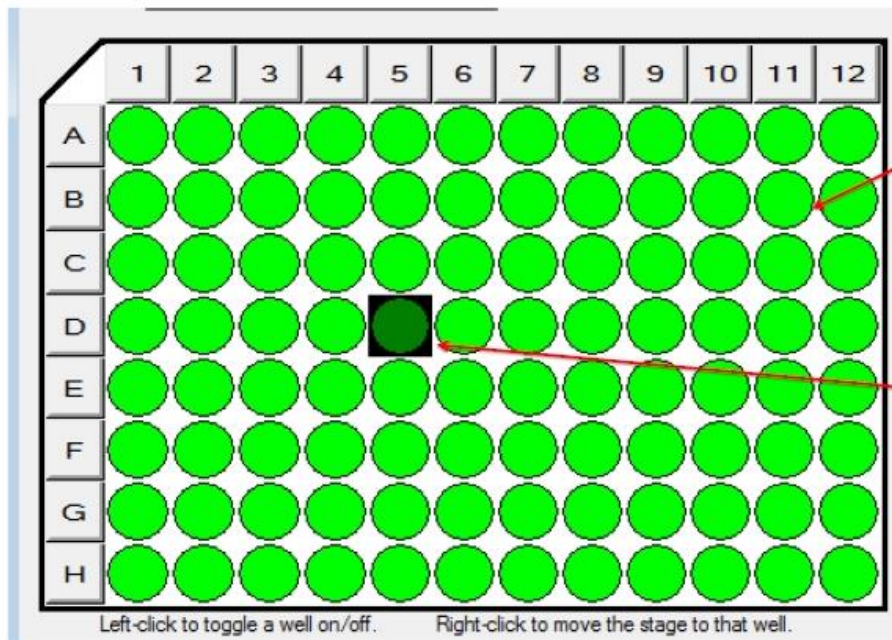
样本板的设置



选择放入仪器的多孔板类型

这些数据均是来自于多孔板厂商的数据，不能修改

多孔板内样品的选择



以鼠标**左键**选择有样品的样品孔，用以获取图像

以鼠标**右键**将镜头移至有样品的孔下方（相应孔周边会出现黑色边框）

选择孔内的拍摄视野

每个孔只拍一个视野

每个孔拍摄固定的视野数量，如3X3，九个视野

根据细胞数量自动决定拍摄的视野数，如每孔拍摄800个细胞

视野排布与排列的选择和每个视野之间的间隔距离

各个视野紧邻

各个视野分散于孔边缘

各个视野之间自动重叠10%（常用于图像拼接）

Active Wavelength: TRITC

Objective and Camera- 10X Plan

Plate- Costar 96-well Plastic-jin

Sites to Visit- multi-site

Acquisition

Autofocus

Wavelengths

W1 DAPI

W2 FITC

W3 TRITC

Display

Site Options

Single site

Fixed number of sites

Adaptive acquisition

Multi-well

Custom field of view (%)

Well size: 38 mm?

Number of sites: 4

24.28% Well Coverage

Site/image size: 1.66 x 1.40 mm

Acquires a fixed number of sites in each well

Columns: 2

Rows: 2

Spacing (µm)

0

0

Tile sites

Fit sites to well

Overlap sites 10%

Columns: 2

Rows: 2

Spacing (µm)

0

0

Columns: 4

Rows: 4

Spacing (µm)

455.445

631.445

Columns: 4

Rows: 4

Spacing (µm)

543.627

630.56

采集设置



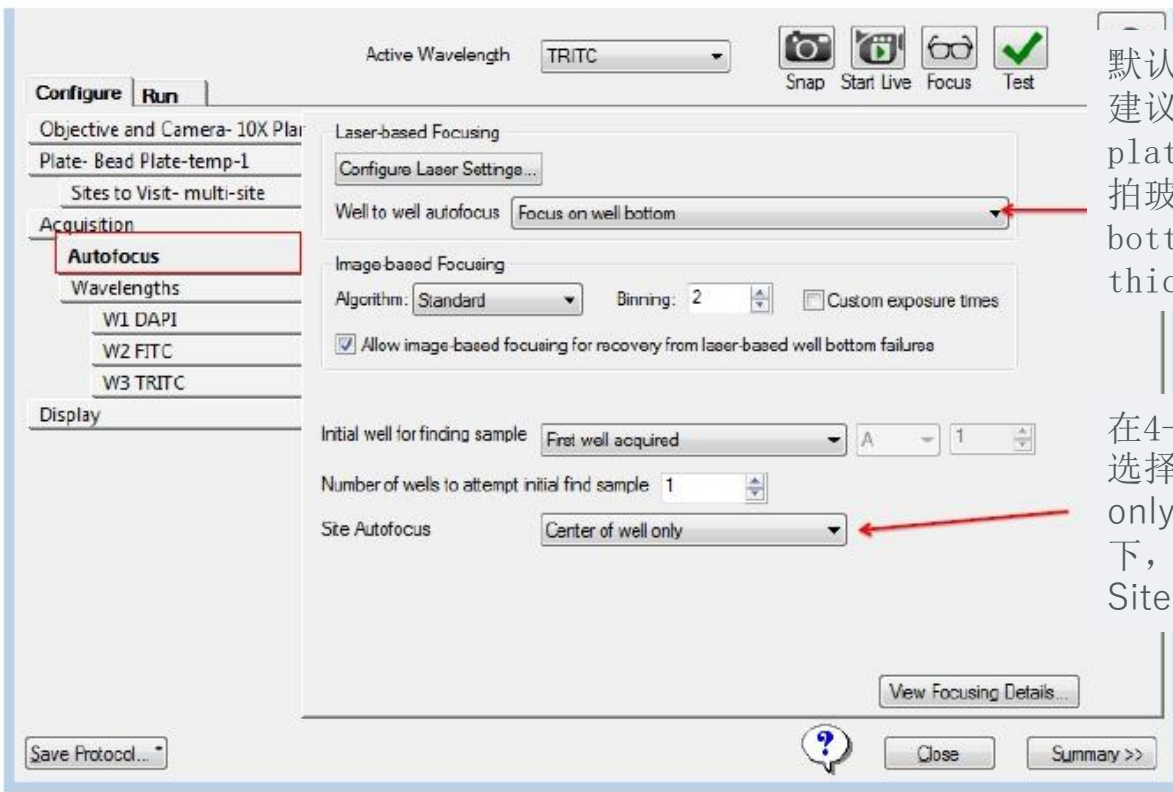
激活激光自动聚焦，
通常作为必选

激活图像自动聚焦，
图像自动聚焦能够
与激光自动聚焦同
时使用。

延时成像设置
多层成像设置

进行特殊实验（如：斑马鱼成像）和
大规模自动图像采集分析时需设置，
一般情况不做设置

激光自动聚焦选项



默认的聚焦方式为“聚焦于孔底”
 建议拍孔板时选择“Focus on plate and well bottom”
 拍玻片时选择“Focus on plate bottom, then offset by bottom thickness”

在4-10x物镜下，通常选择“Center of well only”，在>10x物镜下，通常选择“All Sites”

通道设置

Active Wavelength: TRITC

Configure | Run

Objective and Camera- 10X Plat

Plate- Bead Plate-temp-1

Sites to Visit- multi-site

Acquisition

Autofocus

Wavelengths

W1 DAPI

W2 FITC

W3 TRITC

Display

Number of wavelengths: 3

Snap Start Live Focus Test Preview

Save Protocol... ? Close Summary >>

荧光通道数量，如
 果需要采集透射光
 图像，透射光也作
 为一个通道

各个通道的设置



选择荧光
自动曝光

手动曝光时间

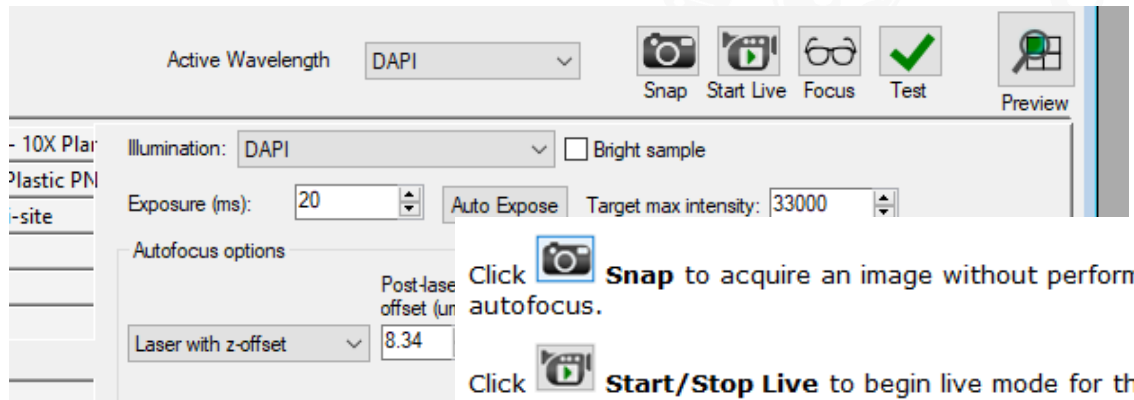
选择聚焦调整方式，
通常选择“Laser
with Z off-set”


自动计算
Offset值


数字共聚焦，
可提高一定分
辨率


设置步骤:


- (1) 选择一个通道，荧光或者透射光
- (2) 设置曝光时间，点击Focus或Test，自动聚焦一次
- (3) 点击Calculate Offset，获取z-offset值





Click  **Snap** to acquire an image without performing (or reperforming) an autofocus.

Click  **Start/Stop Live** to begin live mode for the site.

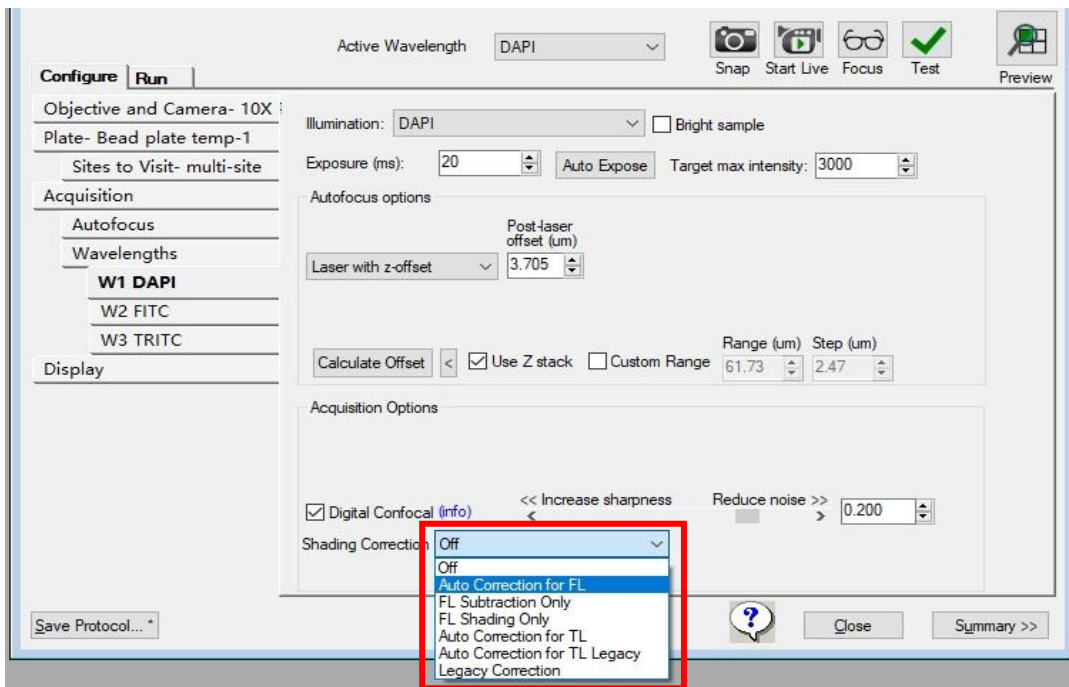
 **CAUTION!** You may photobleach the site if you acquire for too long.

Click  **Focus** to perform the autofocus routine and then acquire an image.

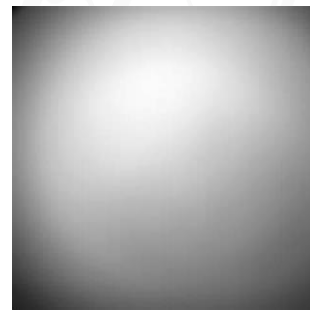
Click  **Test** to perform the autofocus routine and then acquire a z series and present the corresponding 2D projection when you set up a z acquisition.

Click  **Preview** to perform the autofocus routine and then acquire a z series at each of the wavelengths you set up in the protocol and present the corresponding 2D projection when you set up a z acquisition.

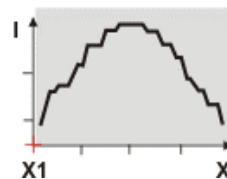
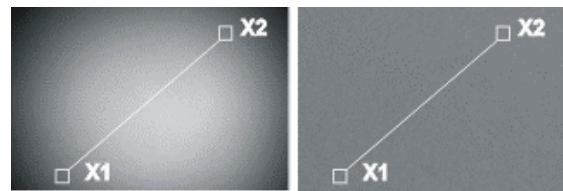
Shading Correction



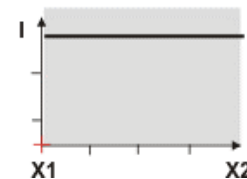
一定要选择与当前通道对应的shading模式



Shading image

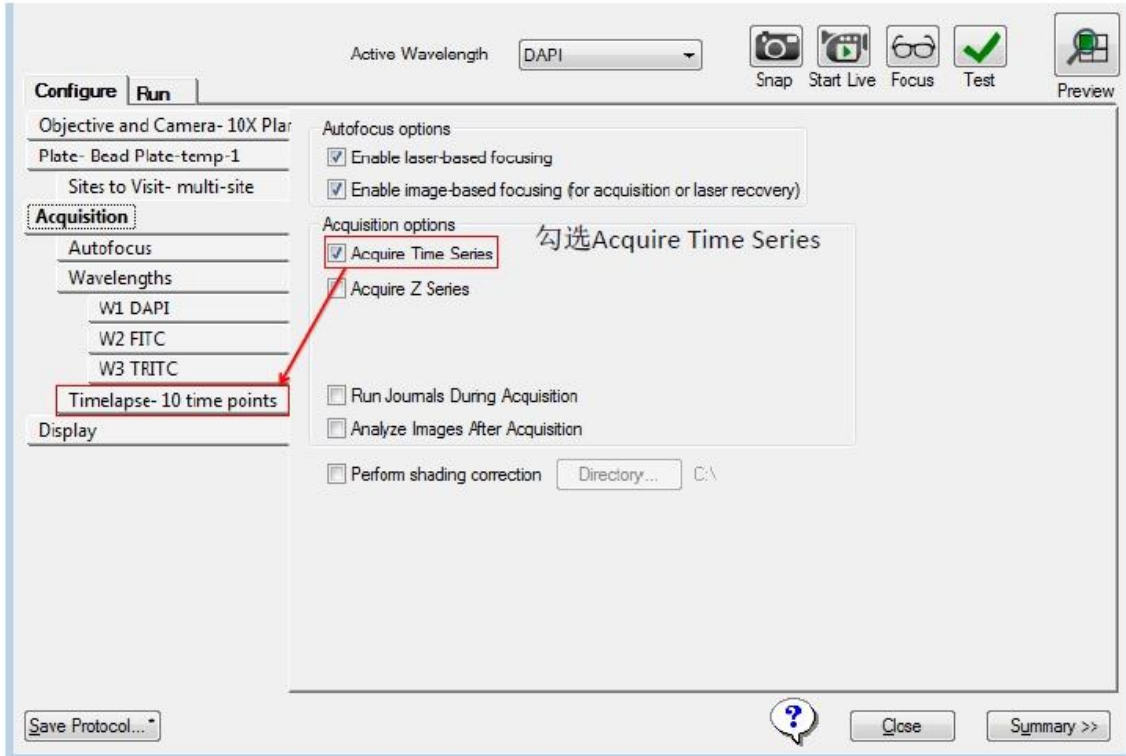


Before correction



After correction

延时成像设置



延时成像设置

Active Wavelength: DAPI

Configure | Run

Objective and Camera- 10X Plat
Plate- Bead Plate-temp-1

Sites to Visit- multi-site

Acquisition

Autofocus

Wavelengths

W1 DAPI

W2 FITC

W3 TRITC

Timelapse- 11 time points

Display

Number of timepoints: 11

Perform time series for: All selected wells

Approximate minimum time interval: 5.9 min

Interval: 1 min

Duration: 10 min

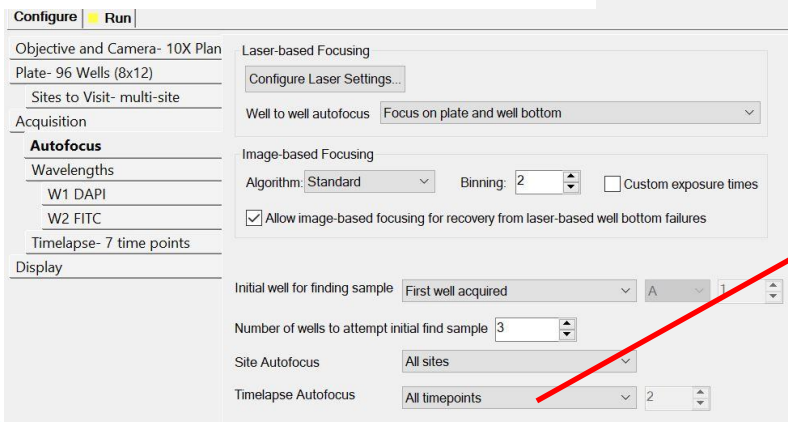
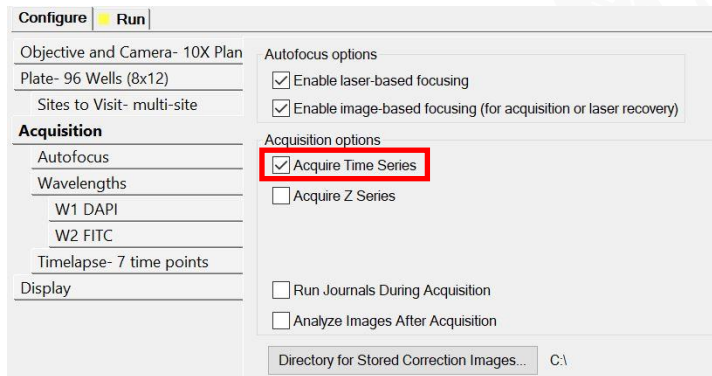
Annotations:

- 拍摄的时间点数量 (Number of timepoints)
- 各孔细胞的拍摄方式 (Perform time series for)
- 系统根据设置计算出的间隔时间的最小值 (Approximate minimum time interval)
- 每两个时间点的间隔时间 (不得小于系统给出的间隔时间最小值) (Interval)
- 整个实验的持续时间 (Duration)

Buttons: Snap, Start Live, Focus, Test, Preview, Save Protocol..., Done, Summary >>

进行长时间拍摄时，即拍摄多个时间点的实验，需要对此进行设定，如果只拍摄一个时间点则不需要设置这个选项。

Timelapse Autofocus



1. First timepoint only :

第一个时间点聚焦；

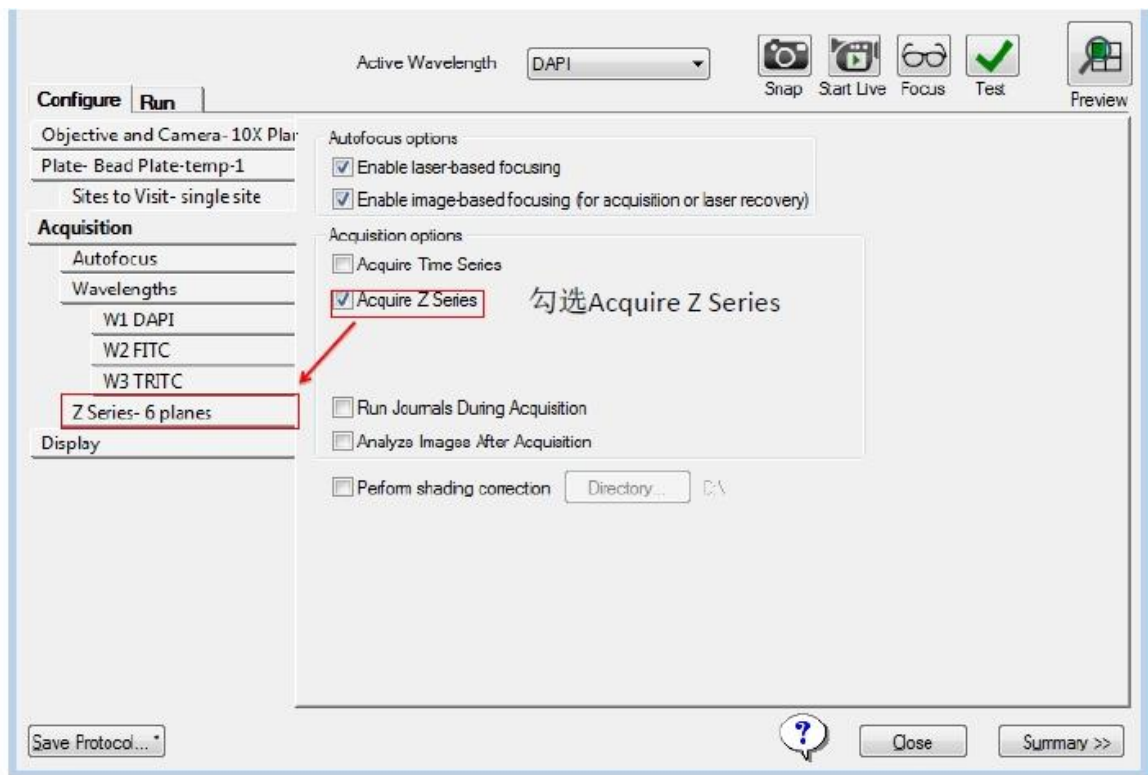
2. All timepoints :

所有时间点均聚焦；

3. Every Nth timepoint :

每隔N个时间点聚焦

多层扫描设置



多层扫描设置

在焦平面两侧对称拍摄

Active Wavelength: DAPI

Center Z Series Around Focus Result

of Steps: 6

Step Size: 1.4 μm

Recommended Step Size: 1.4 μm

Range: 7 μm

Units: μm

拍摄层数

间隔距离

FOCUS

TOP

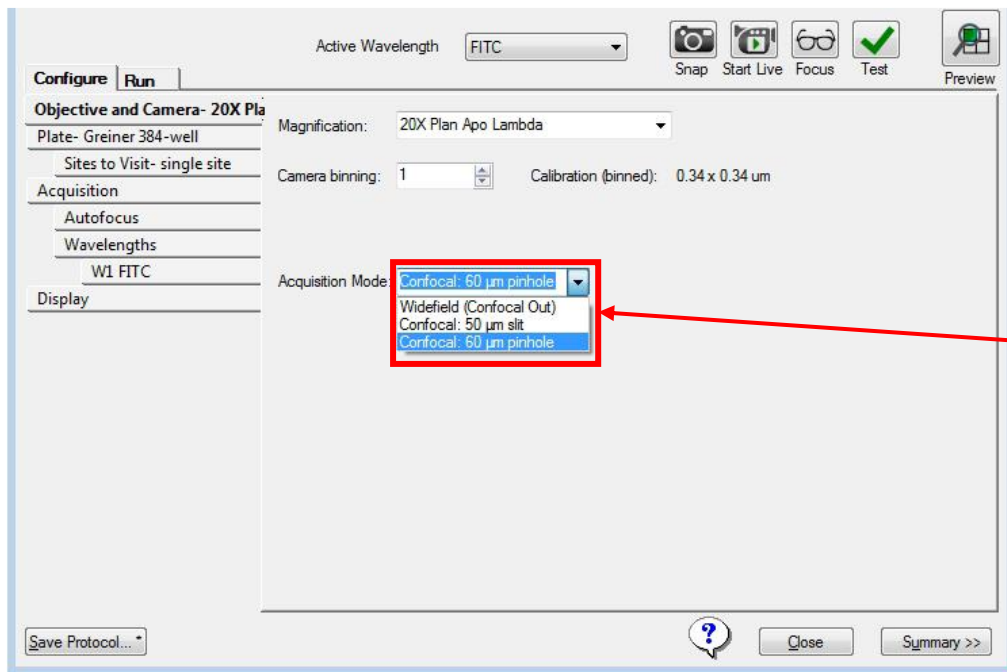
BOTTOM

Ruler Zoom: -

Save Protocol... ? Close Summary >>

拍摄多个层面的较大样本时，需要对此进行设定，通常需要配合使用共聚焦成像模式，如果只拍摄一个层面则不需要设置这个选项。

成像模式选择



成像模式：拍摄Z-Stack时
最好选择confocal模式

IXM-Confocal

实验名称和描述

输入实验的名称

输入实验组别的名称

输入的具体描述

Active Wavelength: DAPI

Buttons: Snap, Start Live, Focus, Test, Preview

Folder Name: 150612-1

Barcode: [Empty]

Plate Name: plate03 4X

Description: test

Storage Location: Database

	Exposure Time (ms)	Snap	Test	Focus Offset (μm)
DAPI	Auto Expose 120	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Calculate 1
FITC	Auto Expose 130	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Calculate 24.86
TRITC	Auto Expose 171	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Calculate 18.4

Buttons: Save Protocol..., Close, Summary >>



点击Acquire Plate开始采集图像

该窗口内包含了各通道的参数信息，用于采集图片之前进行核对

Plate Acquisition Setup - Wavelength 2

Protocol * DB: test Load Protocol... Eject Plate

Well: --, Site: -- Configure

Left-click to toggle a well on/off. Right-click to move the stage to that well.

Active Wavelength FITC Snap Start Live Focus Test Preview

Configure Run

Objective and Camera- 10X Plan Plate- NUNC 96-well Plastic PN

Sites to Visit- multi-site

Acquisition

Autofocus

Wavelengths

W1 DAPI

W2 FITC

Z Series- 6 planes

Display

Illumination: FITC Bright sample

Exposure (ms): 70 Auto Expose Target max intensity: 33000

Autofocus options

Offset (um)

Z-offset from W1 -68.460

Calculate Offset Use Z stack Custom Range Range (um) Step (um)

138.89 5.56

Acquisition Options

Z Series: Single Plane

Digital Confocal << Increase sharpness Reduce noise >> 0.200

AutoQuant 2D Real Time Deconvolution

Shading Correction Auto Correction for TL Legacy

Experiment1

Plate type- NUNC 96-well Plastic PN-165306

Well Information

Acquiring 77 Wells of 96
16 sites/well
140 µm between images in X direction
140 µm in Y direction

Objective

Magnification Setting: 10X Plan Fluor
Acquisition Mode: Widefield
Immersion Medium: Air

Wavelength Information

2 Wavelengths - Unbinned
DAPI W1 DAPI - 20ms, Auto Correction for TL Legacy, Refinement level = 2, Z series with Best Focus Projection
FITC W2 FITC - 70ms, Auto Correction for TL Legacy, Refinement level = 2, Single Z Plane

Storage Information

9856 Total Images, Requiring 4.81 GB
Folder Name: Moldev
Plate Name: Experiment1
Barcode:
Storage Location: Moldev

Digital Confocal Information

W1: Digital confocal enabled with filter K value: 0.2
W2: Digital confocal enabled with filter K value: 0.2

Z Series Information

Center Z Series: False
of Steps: 6
Step Size: 1.4
Range: 7

Focus Information

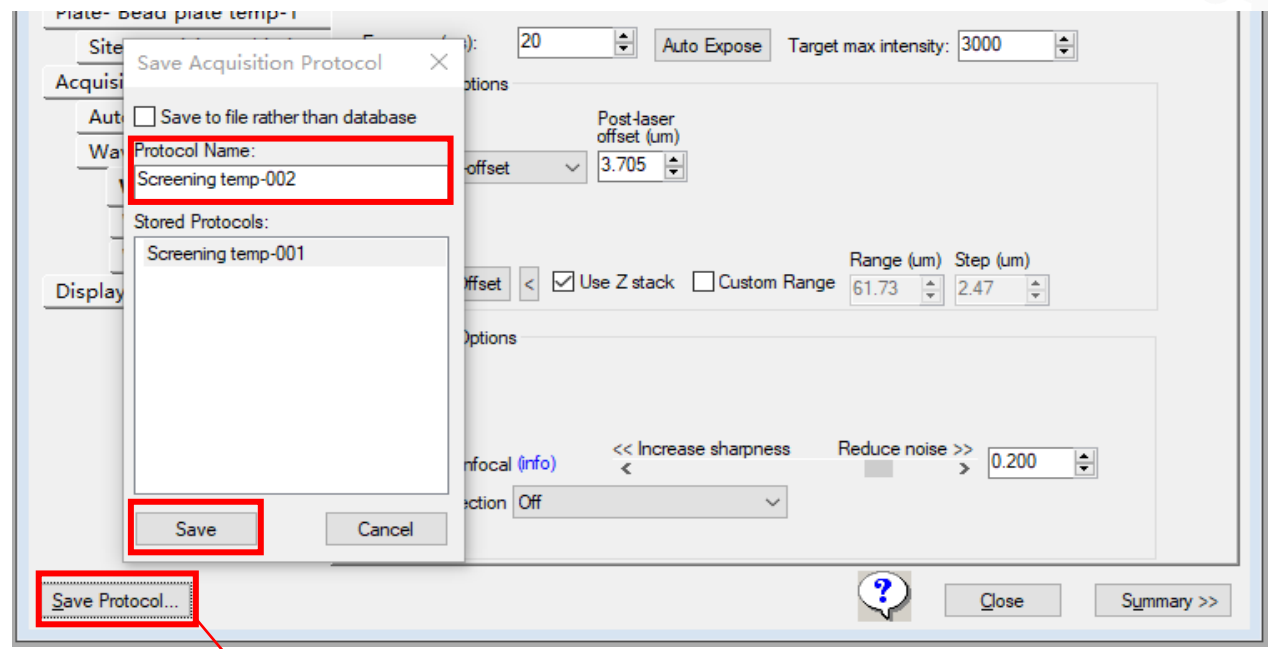
Laser focusing enabled
Image focusing enabled- focus binning = 2
Focusing each well- autofocus at first site in well
W1: Laser with z-offset 8.34 µm
W2: Z-offset from W1 -68.4609 µm

Instrument

Serial Number 0

Copy Print

Protocol 保存



点击Save Protocol可保存当前实验设置，以便下一次直接应用

Plate Acquisition Setup - Wavelength 1

Protocol: DB: Screening temp-002

Load Protocol... Eject Plate

Well: A01, Ste: 1

Configure

Left-click to toggle a well on/off. Right-click to move the stage to that well.

Load Plate Acquisition Settings

Plates

- System Administrator [Creator Name - Plate Info]
 - 10/23/19 [Date Created - Plate Info]
 - 11/19/19 [Date Created - Plate Info]
 - 11/26/19 [Date Created - Plate Info]
 - 12/02/19 [Date Created - Plate Info]
 - 12/12/19 [Date Created - Plate Info]
 - 03/03/20 [Date Created - Plate Info]

Name [Plate Info]	Acquisition Name [Plate Info]	Barcode [..
20191127-Mito-Con-40x-2_APSHAL-6NP16Q2_23	20191127-Mito-Con-40x-2	<NULL>
20191127-Mito-Con_APSHAL-6NP16Q2_24	20191127-Mito-Con	<NULL>
20191127-Mito-Mid-40x-2_APSHAL-6NP16Q2_25	20191127-Mito-Mid-40x-2	<NULL>
20191127-Mito-Mid_APSHAL-6NP16Q2_26	20191127-Mito-Mid	<NULL>
20191127-Mito-Rig-40x-2_APSHAL-6NP16Q2_27	20191127-Mito-Rig-40x-2	<NULL>
20191127-Mito-Rig_APSHAL-6NP16Q2_28	20191127-Mito-Rig	<NULL>

Plate Statistics

Plate N... Site C... Well C... Series C... Compound Co... Controls C... Control Stat... Data... Measurement S...

Select Cancel

Plate Acquisition Setup - Wavelength 1

Protocol: DB: Screening temp-002

Load Protocol... Eject Plate

Well: --, Ste: --

Configure

Load Plate Acquisition Protocol

Protocols in Database

- Screening temp-001
- Screening temp-002

Load From File

Load From Plate

Load From DB

Delete From DB

Export to File

Cancel

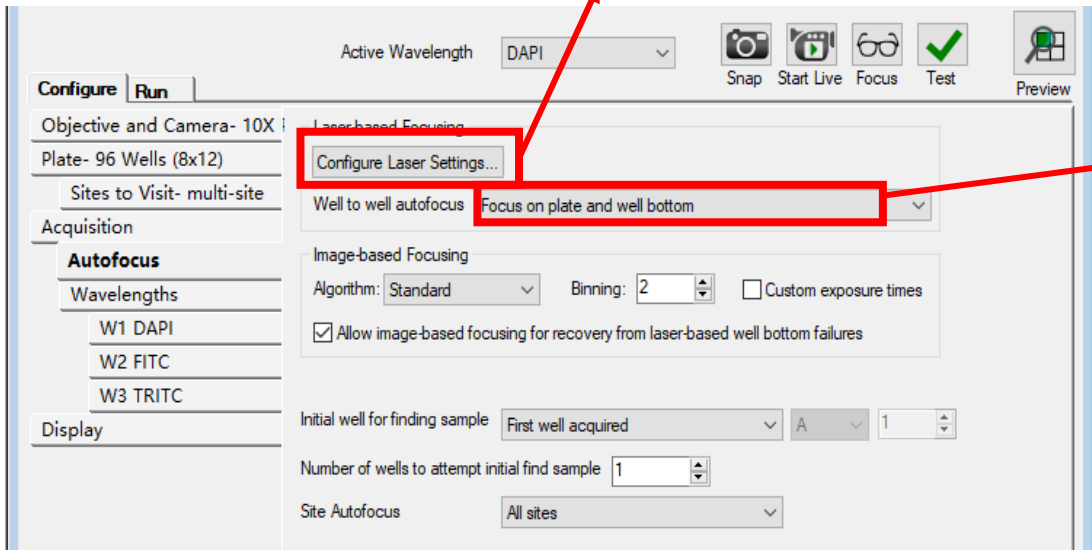
Left-click to toggle a well on/off. Right-click to move the stage to that well.

Active Wavelength: DAPI

Snap Start Live Focus Test Preview

激光自动聚焦选项

点击Configure Laser Settings进入自动聚焦设置



选择一种自动聚焦方式

自动聚焦方式:

- (1) Focus on well bottom
- (2) Focus on plate bottom, then offset by bottom thickness
- (3) Focus on plate and well bottom

玻片扫描时不可选第(3)种

确认孔板厚度及激光自动对焦的曝光时间

Configure Laser Autofocus Settings

Autofocus Settings
 Plate Name: 96 Wells (8x12)
 Mag setting:
 Settings file: C:\MX6\plates\96 Wells (8x12).plt

Save Settings Load Settings Laser Autofocus Wizard...

Plate Bottom Settings
 Bottom thickness: 800um [Edit Plate Bottom...]
 Bottom thickness max variation: 10um
 Adjacent well max variation: 34um
 Intra-well max variation: 35um
 Plate max variation: 600um

Step Size
 Coarse step (um): 10 [] Fine step (um): 2 []

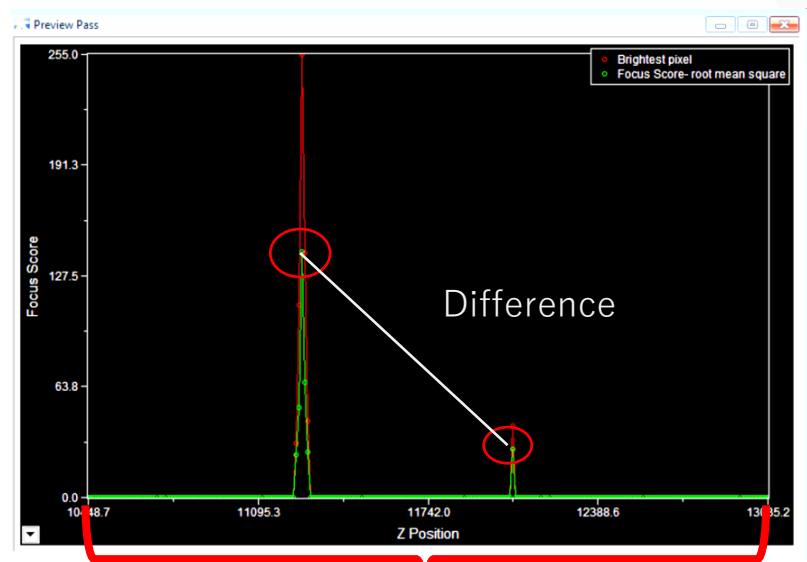
Plate Bottom Exposure		Well Bottom Exposure	
Attempt	Exposure (us)	Attempt	Exposure (us)
1	10.00	1	40.00
2	25.00	2	60.00
3	50.00	3	120.0

Preview Pass
 Note: Set the Preview Pass exposure by selecting an exposure time above or use "Override exposure" below

Start: 877.0um Start from current position
 Range: 2170um Override range (um) 1000 []
 Step size: 10.00um Override step (um) 10 []
 Exposure: 50.00us Override exposure (us) 10 []

Preview Pass

Find Sample Autofocus Focus Snap Close

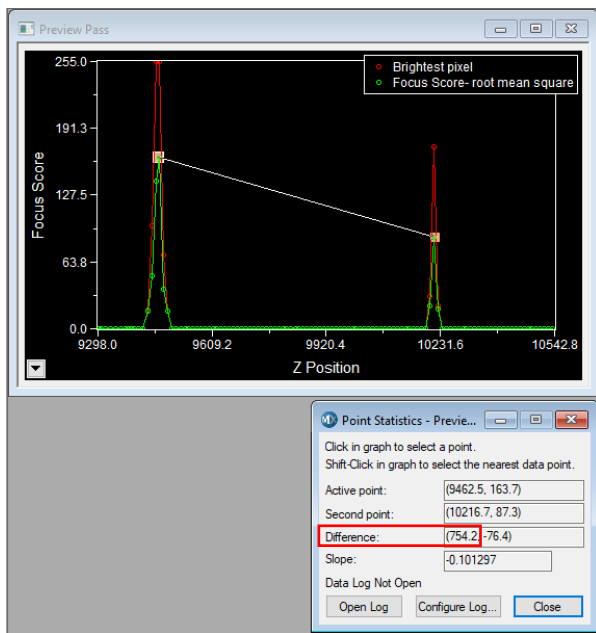


- Graph Settings...
- Print...
- Print Settings...
- Copy Graph to Clipboard
- Copy Graph to Image...
- Save Graph as Bitmap...
- Show Graph Data...
- Point Statistics...**
- Zoom In/Out (Ctrl+Click)

自动找焦范围

点击后拉取一条测量线，测量两个峰的间距即 difference

激光自动聚焦设置



Autofocus Settings

Plate Name: 96 Wells (8x12)
 Mag setting: 10X Plan Flour
 Settings file: C:\MX6\plates\96 Wells (8x12).plt

Save Settings Load Settings Laser Autofocus Wizard...

Plate Bottom Settings

Bottom thickness: 800 um Edit Plate Bottom...
 Bottom thickness max variation: 100 um
 Adjacent well max variation: 300 um
 Intra-well max variation: 200 um
 Plate max variation: 800 um

Step Size

Coarse step (um): 6 Fine step (um): 1.5

Attempt	Exposure (us)
1	5.000
2	10.00
3	20.00

Attempt	Exposure (us)
1	50.00
2	200.0
3	400.0

Preview Pass

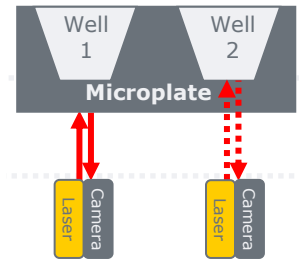
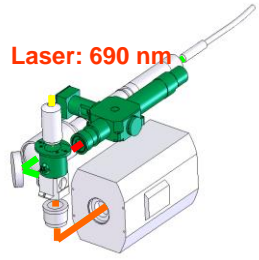
Note: Set the Preview Pass exposure by selecting an exposure time above or use "Override exposure" below

Start: 1332 um Start from current position
 Range: 1336 um Override range (um) 1000
 Step size: 6.000 um Override step (um) 10
 Exposure: 5.000 us Override exposure (us) 10

Preview Pass

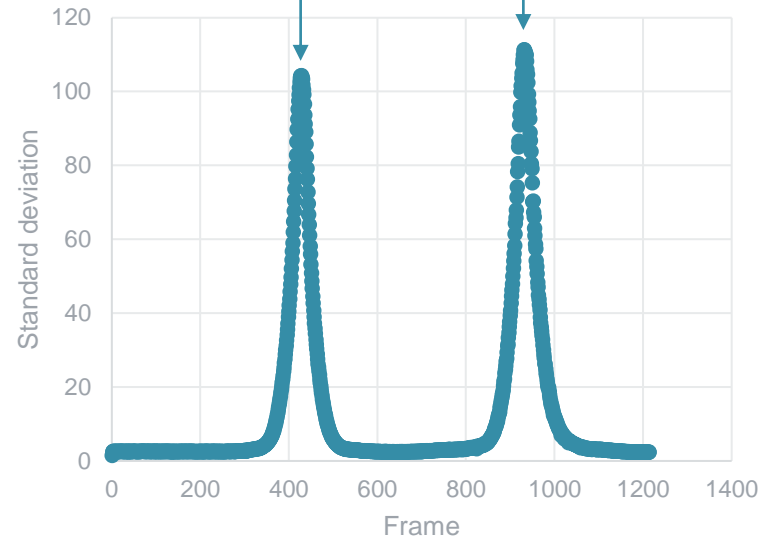
激光自动聚焦原理

激光自动聚焦

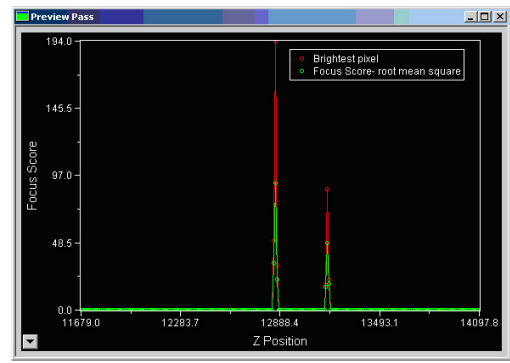


1st reflection

2nd reflection



Typical autofocus reflection "Spot"



激光照射耗材底部产生的反射光

激光自动聚焦设置

Autofocus Settings

Plate Name: 96-wells test 20200526
Mag setting: 10X Plan Fluor
Settings file: C:\MX6\plates\96-wells test 20200526.plt

Save Settings Load Settings Laser Autofocus Wizard...

Plate Bottom Settings

Bottom thickness 800 um
Bottom thickness max variation 100 um
Adjacent well max variation 300 um
Intra-well max variation 200 um
Plate max variation 800 um

Step Size

Coarse step (um) 6 Fine step (um) 1.5

Plate Bottom Exposure

Attempt	Exposure (us)
1	5.000
2	10.00
3	20.00

Well Bottom Exposure

Attempt	Exposure (us)
1	50.00
2	200.0
3	400.0

Preview Pass

Note: Set the Preview Pass exposure by selecting an exposure time above or use "Override exposure" below

Start: 632.0 um Start from current position
Range: 2636 um Override range (um) 1000
Step size: 6.000 um Override step (um) 10
Exposure: 5.000 us Override exposure (us) 10

板底厚度设置

步进设置

曝光时间设置

激光自动聚焦设置

Autofocus Settings

Plate Name: 96-wells test 20200526
Mag setting: 10X Plan Flour
Settings file: C:\MX6\plates\96-wells test 20200526.pt

Save Settings Load Settings Laser Autofocus Wizard...

Plate Bottom Settings

Bottom thickness	800 um	Edit Plate Bottom...
Bottom thickness max variation	100 um	
Adjacent well max variation	300 um	
Intra-well max variation	200 um	
Plate max variation	800 um	

Step Size

Coarse step (um) 6 Fine step (um) 1.5

Plate Bottom Exposure

Attempt	Exposure (us)
1	5.000
2	10.00
3	20.00

Well Bottom Exposure

Attempt	Exposure (us)
1	50.00
2	200.0
3	400.0

Preview Pass

Note: Set the Preview Pass exposure by selecting an exposure time above or use "Override exposure" below

Start: 632.0 um Start from current position

Range: 2636 um Override range (um) 1000

Step size: 6.000 um Override step (um) 10

Exposure: 5.000 us Override exposure (us) 10

Preview Pass

Bottom thickness:
板底厚度

Bottom thickness max variation:
整个孔板底部厚度的最大变化量

Adjacent well max variation:
相邻孔之间板底z轴位置的最大差异

Intra-well max variation:
每个孔内不同位置的板底z轴位置的最大差异

Plate max variation:
两个相距最远的孔之间板底z轴位置的最大差异



激光自动聚焦设置

Autofocus Settings

Plate Name: 96-wells test 20200526
 Mag setting: 10X Plan Flour
 Settings file: C:\MX6\plates\96-wells test 20200526.pit

Save Settings Load Settings Laser Autofocus Wizard...

Plate Bottom Settings

Bottom thickness 800 um [Edit Plate Bottom...](#)
 Bottom thickness max variation 100 um
 Adjacent well max variation 300 um
 Intra-well max variation 200 um
 Plate max variation 800 um

Step Size

Coarse step (um) Fine step (um)

Plate Bottom Exposure

Attempt	Exposure (us)
1	5.000
2	10.00
3	20.00

Well Bottom Exposure

Attempt	Exposure (us)
1	50.00
2	200.0
3	400.0

Preview Pass

Note: Set the Preview Pass exposure by selecting an exposure time above or use "Override exposure" below

Start: 632.0 um Start from current position
 Range: 2636 um Override range (um)
 Step size: 6.000 um Override step (um)
 Exposure: 5.000 us Override exposure (us)
[Preview Pass](#)

Coarse step:
粗调步进

Fine step:
细调步进

Meta Imaging Series Application Help

Hide Back Forward Home Print

Contents Index Search

Type in the word(s) to search for:

coarse

List Topics Display

Select topic: Found: 10

Title	Location	Rank
Configure Las...	Meta Im...	1
Screen Acquis...	Meta Im...	2
Plate Acquisit...	Meta Im...	3
Acquiring Scre...	Meta Im...	4
Screen Acquis...	Meta Im...	5
Configure Las...	Meta Im...	6

STEP SIZE

A step is an increment of an objective's vertical (Z-axis) movement. The laser autofocus system first moves the objective up the Z-axis to search for the plate bottom using the **coarse** (large) step size. Then, to refine its search, the laser autofocus system moves the objective down the Z-axis using the fine (small) step size. Click [here](#) to see an illustration of the **coarse** and fine step search process.

Step sizes vary depending on the objective that you are using. The larger the depth of field, the larger the **coarse** step. Typically, the fine step size is approximately 10-25% of the **coarse** step size. For example:

Table 29: Coarse and fine step sizes

Magnification of objective	Depth of field	If the coarse step size is...	Then the fine step size might be...
4X	Wide	20	5
10X	Narrower	6	1.5
40X	Narrowest	1.5	0.25

激光自动聚焦设置

Autofocus Settings

Plate Name: 96-wells test 20200526
 Mag setting: 10X Plan Flour
 Settings file: C:\MX6\plates\96-wells test 20200526.plt

Save Settings Load Settings Laser Autofocus Wizard...

Plate Bottom Settings

Bottom thickness 800 um Edit Plate Bottom...
 Bottom thickness max variation 100 um
 Adjacent well max variation 300 um
 Intra-well max variation 200 um
 Plate max variation 800 um

Step Size

Coarse step (um) 6 Fine step (um) 1.5

Plate Bottom Exposure

Attempt	Exposure (us)
1	5.000
2	10.00
3	20.00

Well Bottom Exposure

Attempt	Exposure (us)
1	50.00
2	200.0
3	400.0

Preview Pass

Note: Set the Preview Pass exposure by selecting an exposure time above or use "Override exposure" below

Start: 632.0um Start from current position
 Range: 2636um Override range (um) 1000
 Step size: 6.000um Override step (um) 10
 Exposure: 5.000 us Override exposure (us) 10

Preview Pass

Plate Bottom Exposure:
板底曝光时间

Well Bottom Exposure:
孔底曝光时间

1. 孔底曝光时间高于板底曝光时间
2. 高倍物镜曝光时间高于低倍物镜曝光时间

Laser Autofocus Wizard

Plate: 96 Wells (8x12) Save Configuration...

Number of rows: 8 Number of columns: 12 Well shape: Circle


Well diameter (µm): 7000 Column spacing (µm): 9000 Plate length (mm): 127.8

Column offset (µm): 14380 Row spacing (µm): 9000 Plate width (mm): 85.5

Row offset (µm): 11240 Well depth (µm): 12000 Plate height (mm): 14.3

Edit Plate Bottom Settings... Laser Autofocus Wizard...

Plate Acquire LAF Setup Wizard



LAF Setup Wizard for 96 Wells (8x12)

Creating optimized laser autofocus settings is a 2 step process:

1. Measure bottom variation across the entire plate.
2. Calculate a set of tiered exposure levels to use for successive LAF attempts during plate acquisition.

If necessary, you will be asked to load a 'dry' plate into the instrument for measuring bottom variation.

Next you will be asked to load a plate containing sample in the wells for calculating exposure times.

The opportunity to perform exposure measurements for multiple objectives will be available.

<< Back Next>> Cancel

Laser Autofocus Wizard

Plate Acquire LAF Setup Wizard

Measure Bottom Variation

Well selection
 All wells Selection pattern:
 Groups of wells Checkerboard including A1

Group size (NxN): 2 Gap between groups: 0

Selecting more wells takes longer to finish measurement but provides more robust autofocus settings, especially for plates with a large amount of variation.

Sites per well
 Multiple sites 2 Performing measurements on multiple sites/well is slower but provides intra-well variation data, which may result in faster site-to-site LAF.
 Single site

	1	2	3	4	5	6	7	8	9	10	11	12
A	●	●	●	●	●	●	●	●	●	●	●	●
B	●	●	●	●	●	●	●	●	●	●	●	●
C	●	●	●	●	●	●	●	●	●	●	●	●
D	●	●	●	●	●	●	●	●	●	●	●	●
E	●	●	●	●	●	●	●	●	●	●	●	●
F	●	●	●	●	●	●	●	●	●	●	●	●
G	●	●	●	●	●	●	●	●	●	●	●	●
H	●	●	●	●	●	●	●	●	●	●	●	●

Time estimate: 0:02:24

<< Back Next>> Cancel

Plate Acquire LAF Setup Wizard

Measure Bottom Variation

The following magnification setting will be used to measure plate bottom variation:
10X Plan Apo Lambda

Press **Next>>** to measure new bottom variation settings.

<< Back Next>> Cancel

Plate Acquire LAF Setup Wizard

Measure Bottom Variation

Load a DRY plate into the instrument.
Open door/clamp Close door

Select LAF step sizes (um)
 Existing values fine step = 2.0, coarse step = 10.0
 Calculated values fine step = 0.6, coarse step = 4.9
(based on objective N.A.)

Allow Extreme Variation (Example: Thin bottom plastic plate)

<< Back Start>> Cancel

Laser Autofocus Wizard

Plate Acquire LAF Setup Wizard

Measure Bottom Variation Status

96 Wells (8x12)

Elapsed time: 0:00:00
Estimated time remaining: 0:00:01

■ success
 ■ some failures
 ■ all failed

Magnification setting: 10X Plan Apo Lambda
Current well/site: C3 site 1 of 2
Status: Running

Thickness and average bottom variation
 Typical thickness (um): 800.0
 Adjacent wells (um): 0.0
 Intra-well (um): 0.0

<< Back Next >> Cancel

Plate Acquire LAF Setup Wizard

Measure Bottom Variation Summary

Plate bottom thickness
 Typical thickness (um): 800.0 Variation (+/-): 0.0

Plate bottom variation

	Minimum	Maximum	Average	Std. Dev.
Adjacent wells (um):	0.0	0.0	0.0	0.0
Intra-well (um):	0.0	0.0	0.0	0.0
Plate maximum variation (um):	94.0			

All LAF searches succeeded!

<< Back Next >> Cancel

Laser Autofocus Wizard

Plate Acquire LAF Setup Wizard

Calculate Exposure Parameters

Select the medium that will be used when running this plate! magnification setting to use for calculating exposure times.

Medium: Aqueous media Refractive index: 1.33

Magnification setting for calculating exposures:

- 10X Plan Apo Lambda
- 4X S Fluor
- 10X Plan Apo Lambda
- 20X Plan Apo Lambda
- 40X S Plan Fluor ELWD

Select LAF:

- Existing values (fine step = 2.0, coarse step = 10.0)
- Calculated values (based on objective N.A.)

Press **Next>>** to measure new exposure

<< Back **Next>>** Cancel

换一个物镜做校准

用已有参数校准还是重新计算校准参数

只有已做过校准的物镜会有这个选项，没做过的都默认第二种

Plate Acquire LAF Setup Wizard

Exposure Measurement Summary

Exposure variation	Minimum	Maximum	Average	Std. Dev.
Plate bottom exposure (us):	20.0	20.0	20.0	0.0
Well bottom exposure (us):	30.0	30.0	30.0	0.0

LAF search step sizes (um)

Fine step = 1.0 Coarse step = 5.0

Plate bottom exposures (us)

1st pass-	20.0
2nd pass-	20.0
3rd pass-	24.0

Well bottom exposures (us)

1st pass-	30.0
2nd pass-	32.0
3rd pass-	38.0

All LAF searches succeeded!

Save settings and exit wizard

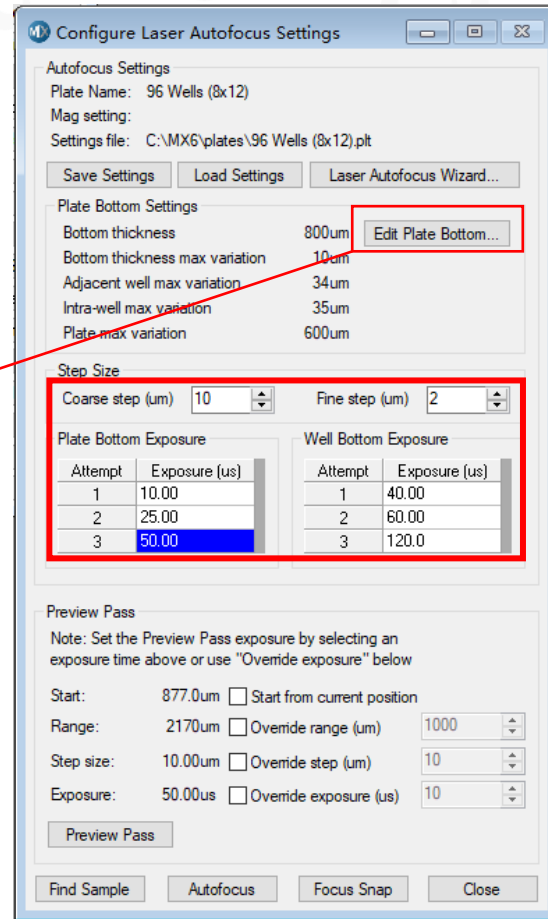
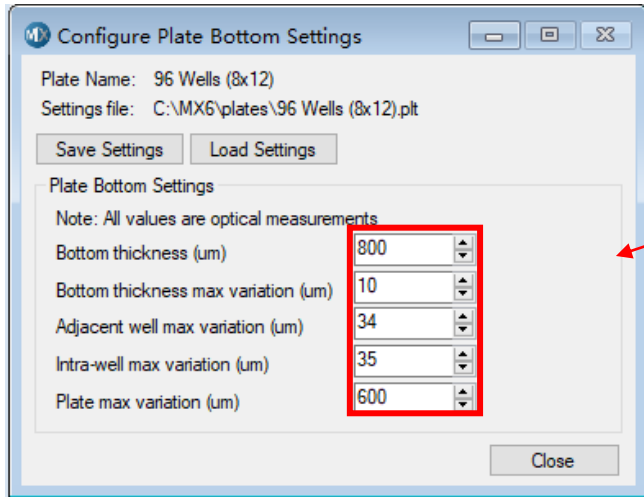
Save settings and measure with another objective

Discard settings and measure with another objective

<< Back **Next>>** Cancel

保存退出或进行下一个物镜校准

Laser Autofocus Wizard



做完 laser autofocus wizard 之后，框内的数字会自动填入

不用Laser Autofocus Wizard, 如何设置激光自动聚焦?

激光自动聚焦设置流程-1

新孔板规格参数设置

Configure Run
Preview

Objective and Camera- 10X Plan

Plate- 96 Wells (8x12)

Sites to Visit- multi-site

Acquisition

Autofocus

Wavelengths

W1 DAPI

W2 FITC

Display

Plate Name: 96 Wells (8x12) Save Configuration...

<p>Number of rows:</p> <input style="width: 100%;" type="text" value="8"/>	<p>Number of columns:</p> <input style="width: 100%;" type="text" value="12"/>	<p>Well shape:</p> <input style="width: 100%;" type="text" value="Circle"/>	
<p>Well diameter (μm):</p> <input style="width: 100%;" type="text" value="7000"/>	<p>Column spacing (μm):</p> <input style="width: 100%;" type="text" value="9000"/>	<p>Plate length (mm):</p> <input style="width: 100%;" type="text" value="127.8"/>	
<p>Column offset (μm):</p> <input style="width: 100%;" type="text" value="14380"/>	<p>Row spacing (μm):</p> <input style="width: 100%;" type="text" value="9000"/>	<p>Plate width (mm):</p> <input style="width: 100%;" type="text" value="85.5"/>	
<p>Row offset (μm):</p> <input style="width: 100%;" type="text" value="11240"/>	<p>Well depth (μm):</p> <input style="width: 100%;" type="text" value="12000"/>	<p>Plate height (mm):</p> <input style="width: 100%;" type="text" value="14.3"/>	

Edit Plate Bottom Settings...
Laser Autofocus Wizard...

激光自动聚焦设置流程-2

Autofocus Settings

Plate Name: 96-wells test 20200526
 Mag setting: 10X Plan Flour
 Settings file: C:\IMX6\plates\96-wells test 20200526.pit

Save Settings Load Settings Laser Autofocus Wizard...

Plate Bottom Settings

Bottom thickness 800 um Edit Plate Bottom...
 Bottom thickness max variation 100 um
 Adjacent well max variation 300 um
 Intra-well max variation 200 um
 Plate max variation 800 um

Step Size

Coarse step (um) 6 Fine step (um) 1.5

Plate Bottom Exposure

Attempt	Exposure (us)
1	5.000
2	10.00
3	20.00

Well Bottom Exposure

Attempt	Exposure (us)
1	50.00
2	200.0
3	400.0

Preview Pass

Note: Set the Preview Pass exposure by selecting an exposure time above or use "Override exposure" below

Start: 632.0 um Start from current position
 Range: 2636 um Override range (um) 1000
 Step size: 6.000 um Override step (um) 10
 Exposure: 5.000 us Override exposure (us) 10

Preview Pass

分别设置“Step Size ”和“Bottom Exposure”

激光自动聚焦设置流程-3

Autofocus Settings

Plate Name: 96-wells test 20200526
 Mag setting: 10X Plan Flour
 Settings file: C:\MX6\plates\96-wells test 20200526.plt

Save Settings Load Settings Laser Autofocus Wizard...

Plate Bottom Settings

Bottom thickness: 800 um Edit Plate Bottom...
 Bottom thickness max variation: 100 um
 Adjacent well max variation: 300 um
 Intra-well max variation: 200 um
 Plate max variation: 800 um

Step Size

Coarse step (um): 6 Fine step (um): 1.5

Plate Bottom Exposure

Attempt	Exposure (us)
1	5.000
2	10.00
3	20.00

Well Bottom Exposure

Attempt	Exposure (us)
1	50.00
2	200.0
3	400.0

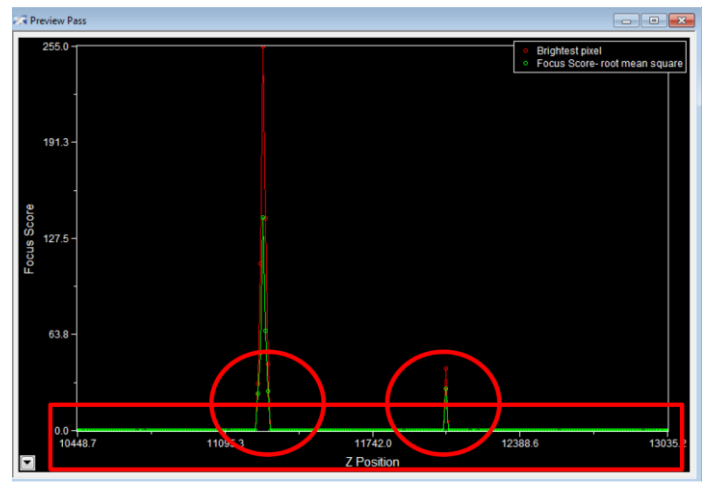
Preview Pass

Note: Set the Preview Pass exposure by selecting an exposure time above or use "Override exposure" below

Start: 632.0 um Start from current position
 Range: 2636 um Override range (um) 1000
 Step size: 6.000 um Override step (um) 10
 Exposure: 5.000 us Override exposure (us) 10

Preview Pass

“Preview Pass”检查双反射峰



激光自动聚焦设置流程-4

Plate Name: 96-wells test 20200526
 Mag setting: 10X Plan Flour
 Settings file: C:\MX6\plates\96-wells test 20200526.plt

Save Settings Load Settings Laser Autofocus Wizard...

Plate Bottom Settings

Bottom thickness 800 um
 Bottom thickness max variation 100 um
 Adjacent well max variation 300 um
 Intra-well max variation 200 um
 Plate max variation 800 um

Step Size

Coarse step (um) 6 Fine step (um) 1.5

Plate Bottom Exposure

Attempt	Exposure (us)
1	5.000
2	10.00
3	20.00

Well Bottom Exposure

Attempt	Exposure (us)
1	50.00
2	200.0
3	400.0

Preview Pass

Note: Set the Preview Pass exposure by selecting an exposure time above or use "Override exposure" below

Start: Current Start from current position

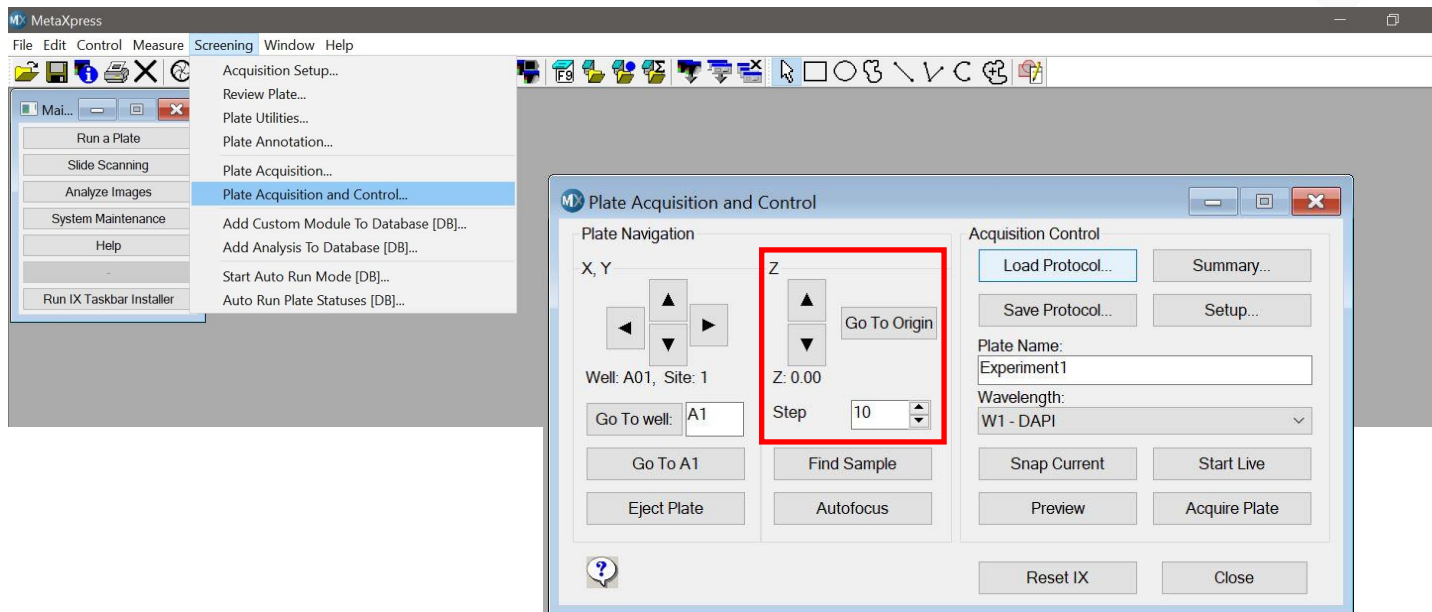
Range: 1000 um Override range (um) 3000

Step size: 6.000 um Override step (um) 10

Exposure: 5.000 us Override exposure (us) 10

- 如果无法观察到双反射峰，先勾选“Start Current”和“Range”，并设置“Override range”
- 手动调焦直到找到双反射峰(未完待续)

手动调焦方法



激光自动聚焦设置流程-5

- 找到双反射峰后，记录下双反射峰横坐标中点值A
- 取消“Start Current”和“Range”选项，重新“Preview Pass”
- 记录下整个横坐标中点值B
- 计算A-B值，假设“Well depth”为C，将当前C值设为C-(A-B)

Configure | Run | Preview

Objective and Camera- 10X Plan
Plate- 96 Wells (8x12) Plate Name: 96 Wells (8x12) Save Configuration...

Acquisition

Autofocus

Wavelengths
 W1 DAPI
 W2 FITC

Display

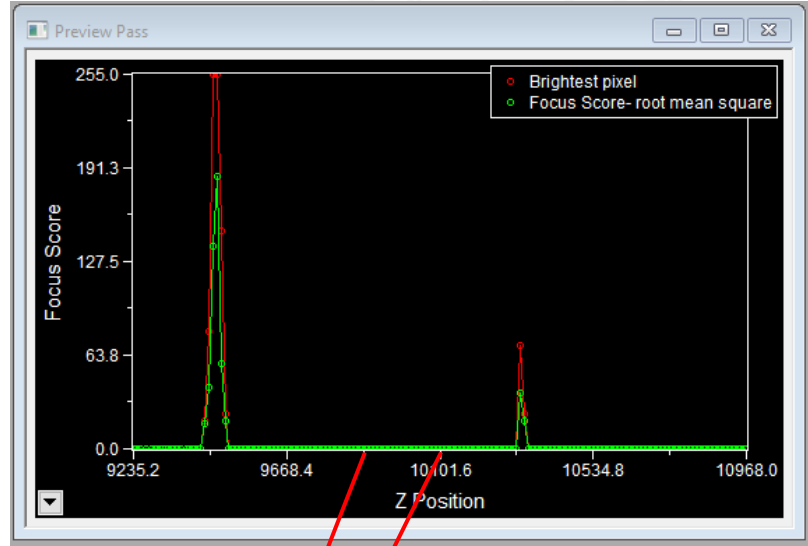
Number of rows: 8
 Number of columns: 12
 Well shape: Circle

Well diameter (µm): 7000
 Column spacing (µm): 9000
 Plate length (mm): 127.8

Column offset (µm): 14380
 Row spacing (µm): 9000
 Plate width (mm): 85.5

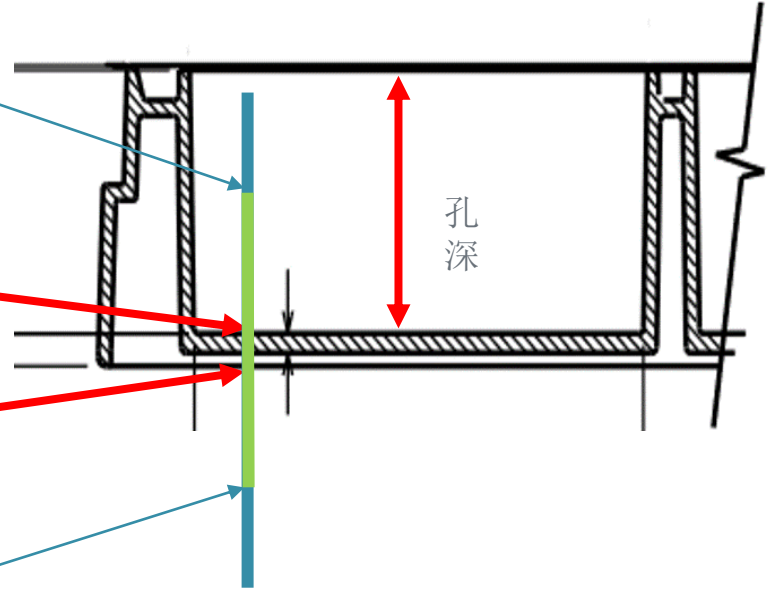
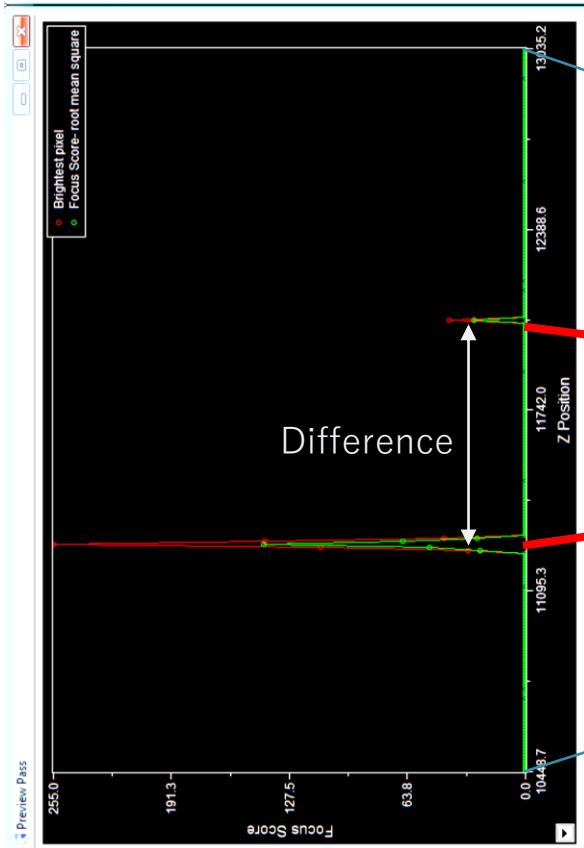
Row offset (µm): 11240
Well depth (µm): 12000
 Plate height (mm): 14.3

Edit Plate Bottom Settings... Laser Autofocus Wizard...



A

B



激光自动聚焦设置流程-6

- 测量双反射峰之间的横坐标距离并将其设置为板底厚度，进一步设置其他板底厚度变量

Autofocus Settings

Plate Name: 96-wells test 20200526
 Mag setting: 10X Plan Fluor
 Settings file: C:\MX6\plates\96-wells test 20200526.plt

Save Settings Load Settings Laser Autofocus Wizard...

Plate Bottom Settings

Bottom thickness 800 um
 Bottom thickness max variation 100 um
 Adjacent well max variation 300 um
 Intra-well max variation 200 um
 Plate max variation 800 um

Step Size
 Coarse step (um) 6 Fine step (um) 1.5

Plate Bottom Exposure

Attempt	Exposure (us)
1	5.000
2	10.00
3	20.00

Well Bottom Exposure

Attempt	Exposure (us)
1	50.00
2	200.0
3	400.0

Preview Pass

Note: Set the Preview Pass exposure by selecting an exposure time above or use "Override exposure" below

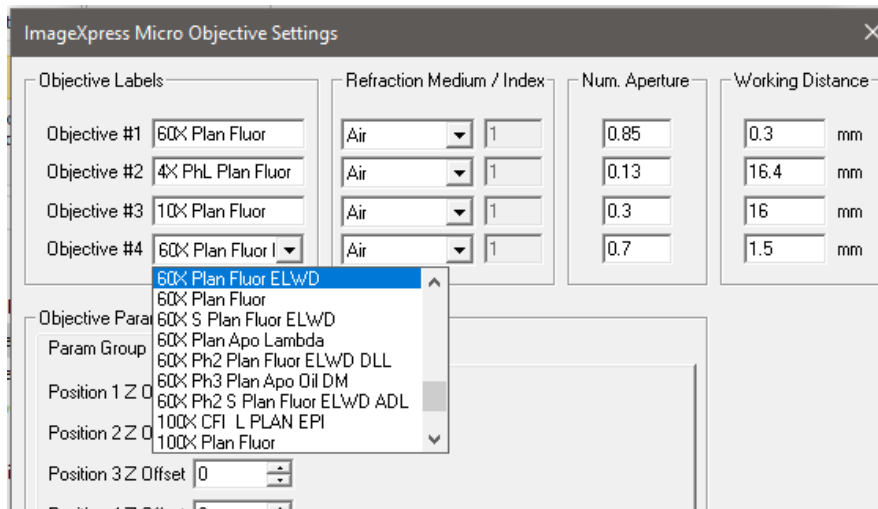
Start: 632.0 um Start from current position
 Range: 2636 um Override range (um) 1000
 Step size: 6.000 um Override step (um) 10
 Exposure: 5.000 us Override exposure (us) 10

The screenshot shows the software interface during a focus scan. A graph titled 'Preview Pass' displays 'Focus Score' on the y-axis (ranging from 0.0 to 255.0) and 'Z Position' on the x-axis (ranging from 9229.2 to 10542.8). Two distinct peaks are visible, with the 'Brightest pixel' (red dot) and 'Focus Score - root mean square' (green line) overlaid. A context menu is open over the graph, with 'Point Statistics...' selected. The 'Point Statistics - Preview...' dialog box is also open, showing the following data:

Active point:	(9462.5, 163.7)
Second point:	(10216.7, 87.3)
Difference:	(754.2, -76.4)
Slope:	-0.101297

The 'Difference' row is highlighted with a red box in the dialog, indicating the measured distance between the two peaks.

孔板与物镜选择



- 板底的厚度应与所要使用的物镜的工作距离相匹配。
- 一般来说，数值孔径(NA)较大的物镜往往需要薄底板。
- 超长工作距离(ELWD)目标兼容较大的板厚范围，但往往有较小的NA。
- 板的底部厚度相当于一个标准的盖玻片(0.17毫米)与大多数物镜都可以匹配。

Correction Collar

- ✓ If you are using an objective with a correction collar, ensure that the correction collar is set appropriately for the plate you are using
- ✓ high numerical aperture dry objectives (numerical aperture of 0.8 or greater)



→ 矫正环

调节矫正环系数值

塑料: Difference x 1.59
 玻璃: Difference x 1.52
 170um厚度的玻璃底也可直接调到0.17

Adjustment of Objective Correction Collars

Microscope Image

Choose a Specimen: Rat Neuron (FL)

Microscope Focus: [Slider]

Correction Collar: [Slider with scale .1, .15, .2, .25]

Adjustment of Objective Correction Collars

Microscope Image

Choose a Specimen: Rat Neuron (FL)

Microscope Focus: [Slider]

Correction Collar: [Slider with scale .1, .15, .2, .25]

主要内容

- MetaXpress软件拍摄设置流程

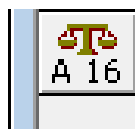
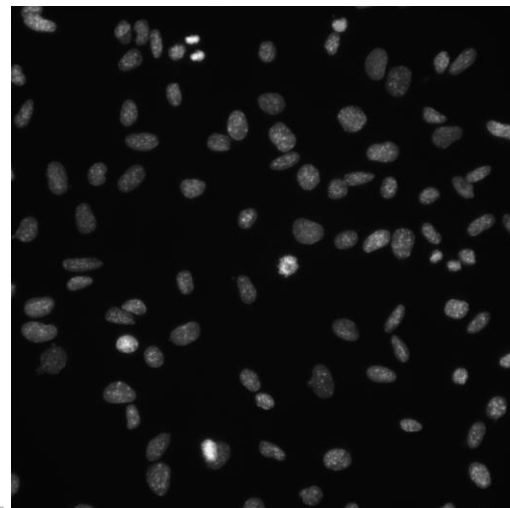
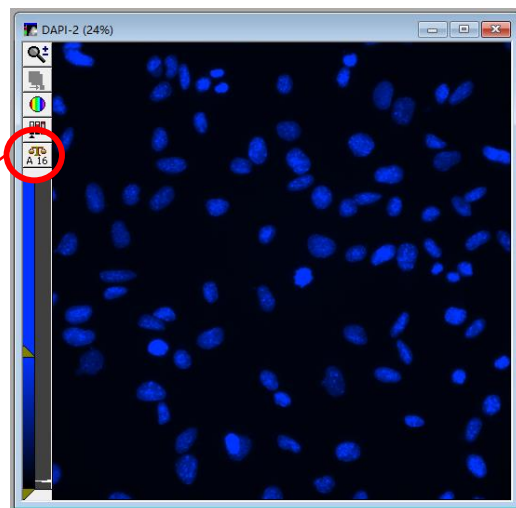
- 基本设置
- 长时间拍摄设置
- Z-Stack拍摄设置
- Protocol保存与应用
- 聚焦设置

- 高内涵图像常用处理方法与技巧



A screenshot of a software menu for selecting bit ranges. The menu is titled "A 8" and contains the following options:

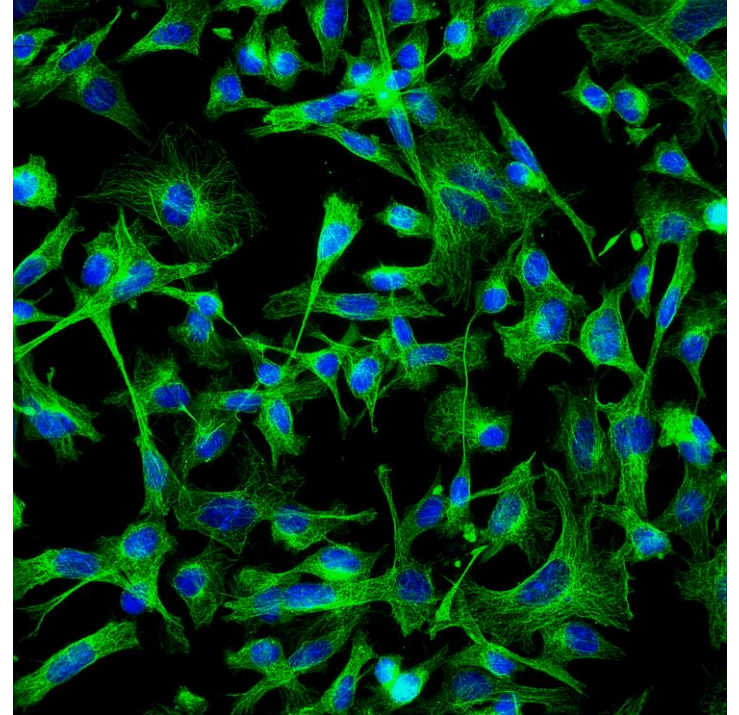
- Best Fit Range
- 8-Bit Range (0-255)
- 10-Bit Range (0-1023)
- 12-Bit Range (0-4095)
- 14-Bit Range (0-16383)
- 16-Bit Range (0-65535)
- Auto Scale
- Scale Within Active Region
- Scale Image...

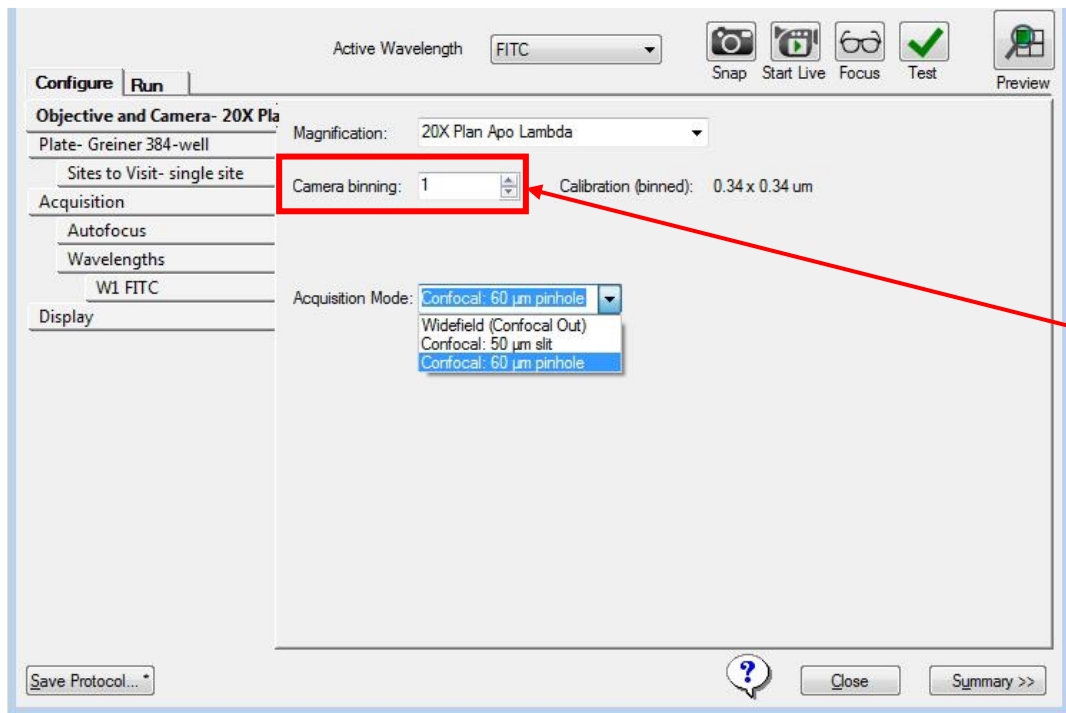


Bit depth : **16 bit**
Resolution : **2048 x 2048**



sCMOS





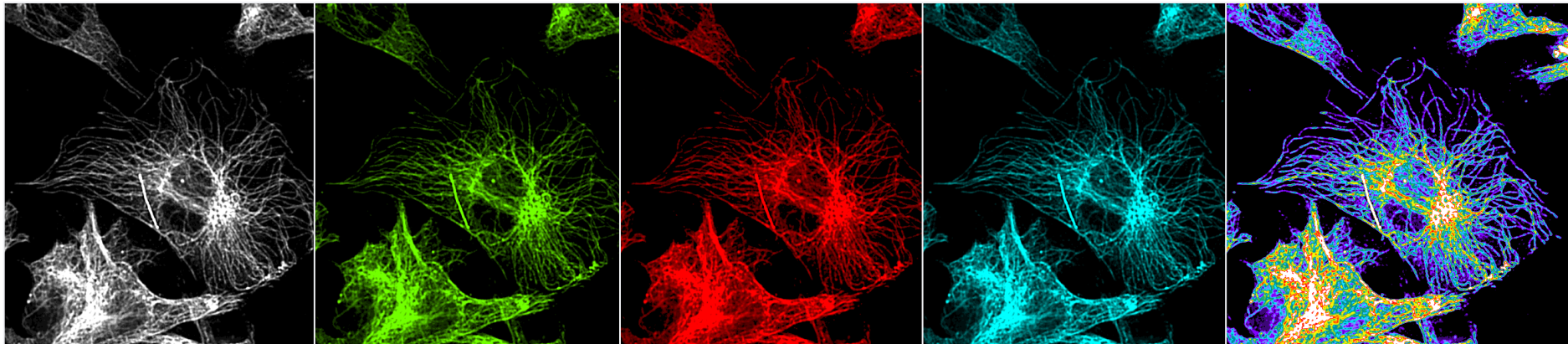
选择图像的大小，数值越大，图像越小，一般选择2的时候效率最高

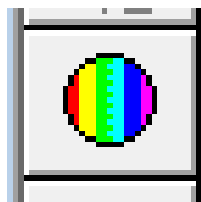


Binning

5	9	18	15
8	15	19	16
6	12	15	12
11	14	22	14

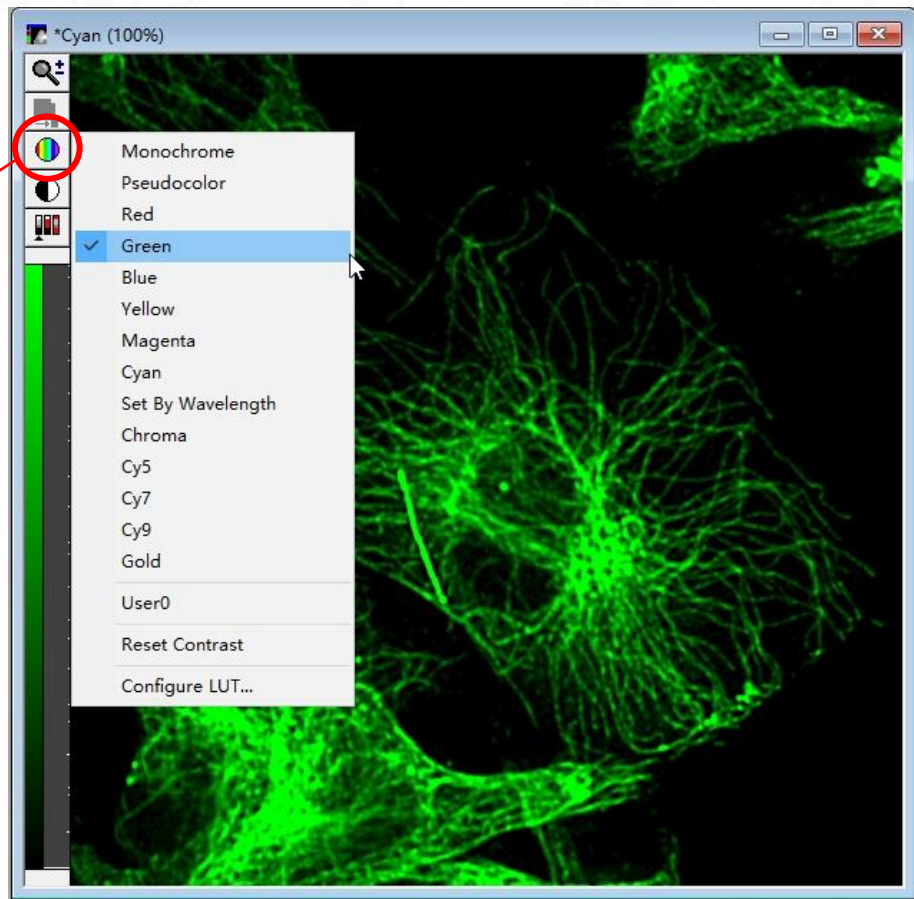
Camera Binning

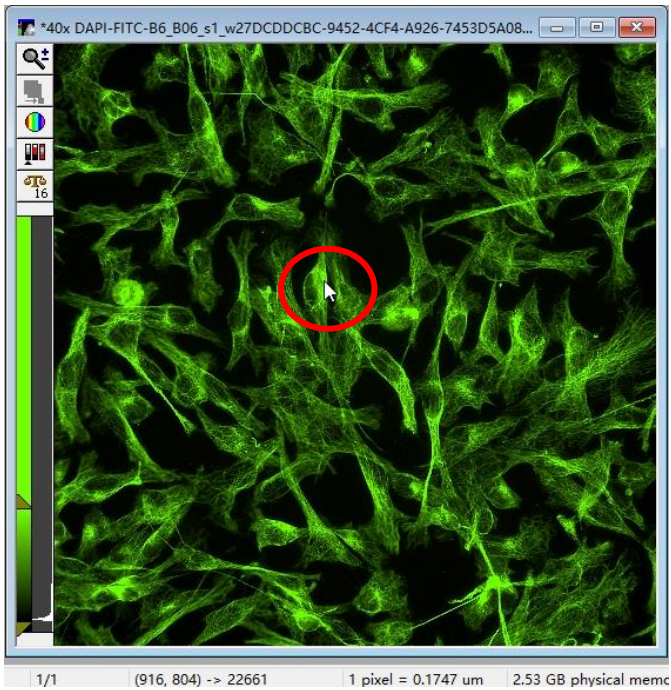




自定义像素的颜色

通常习惯用波长本身的色彩

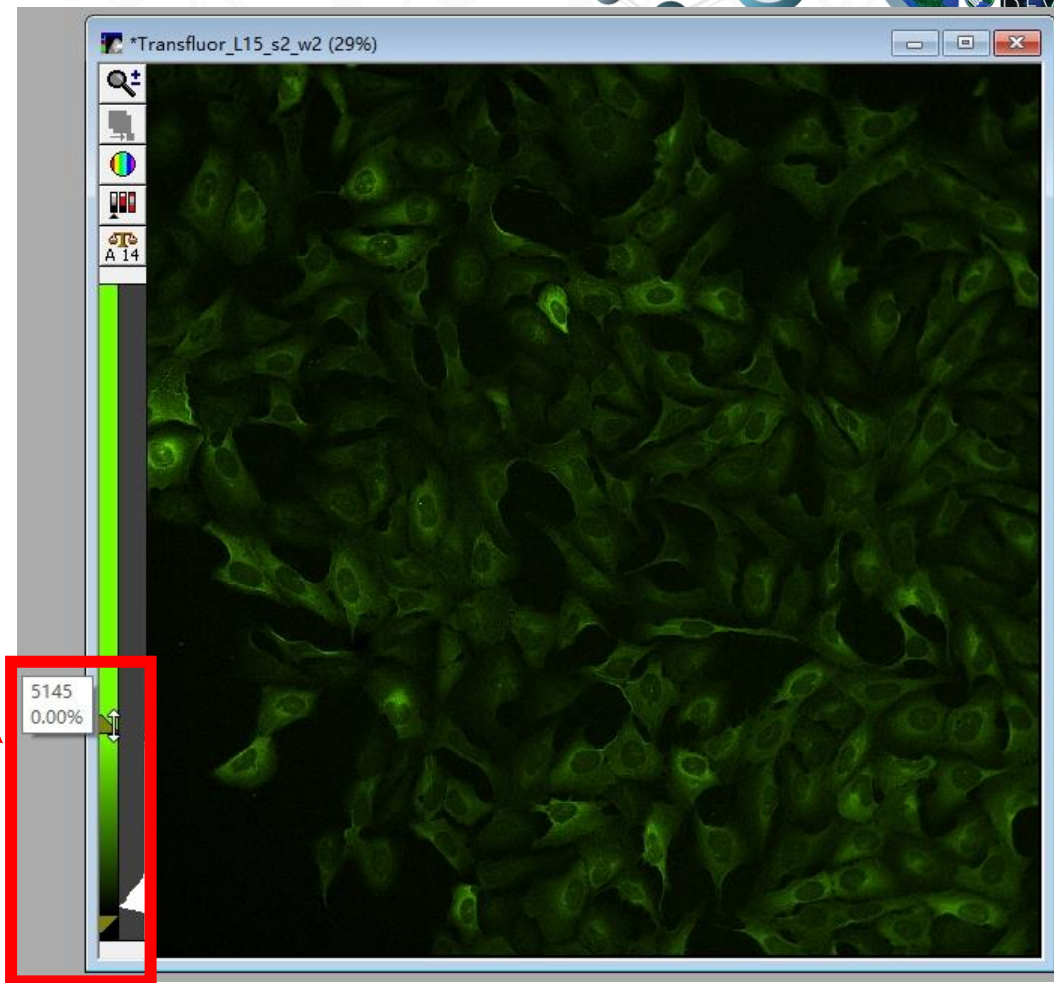


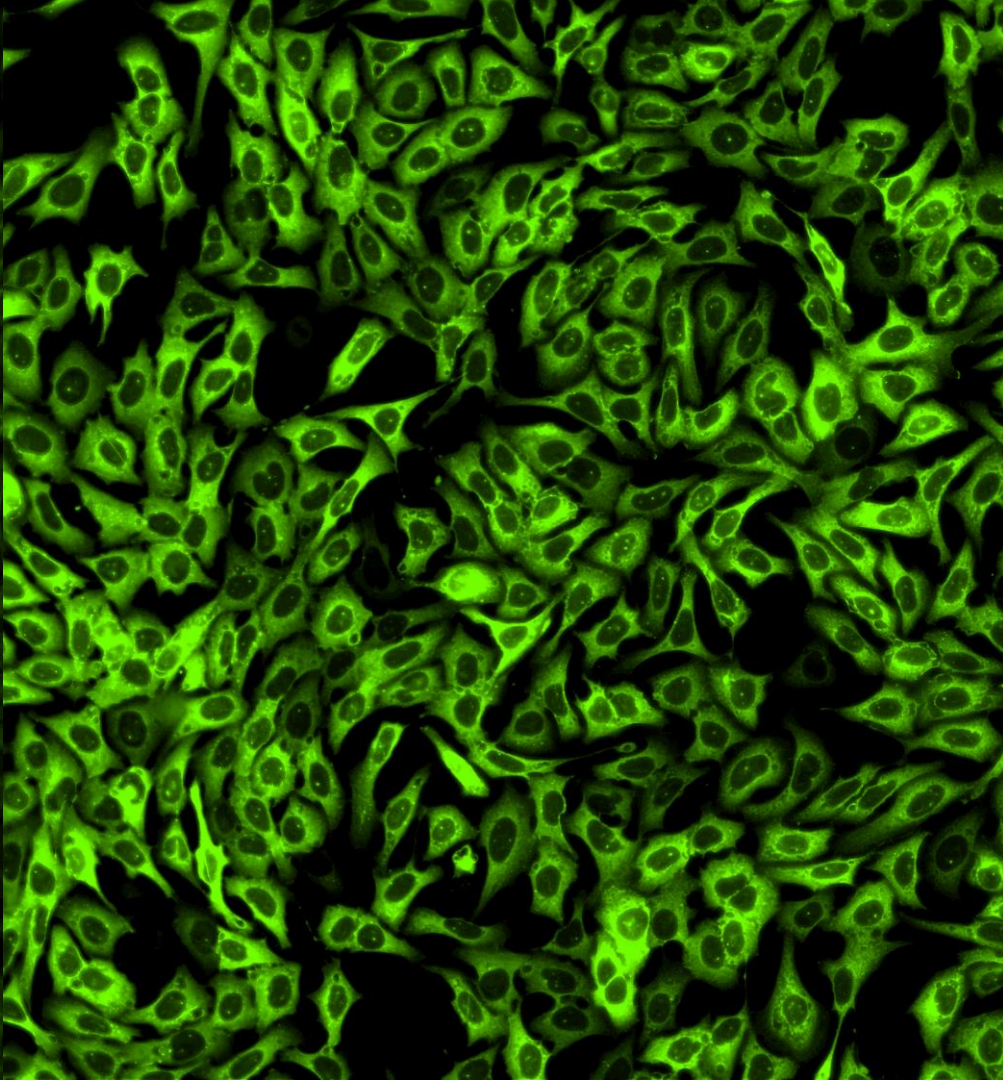
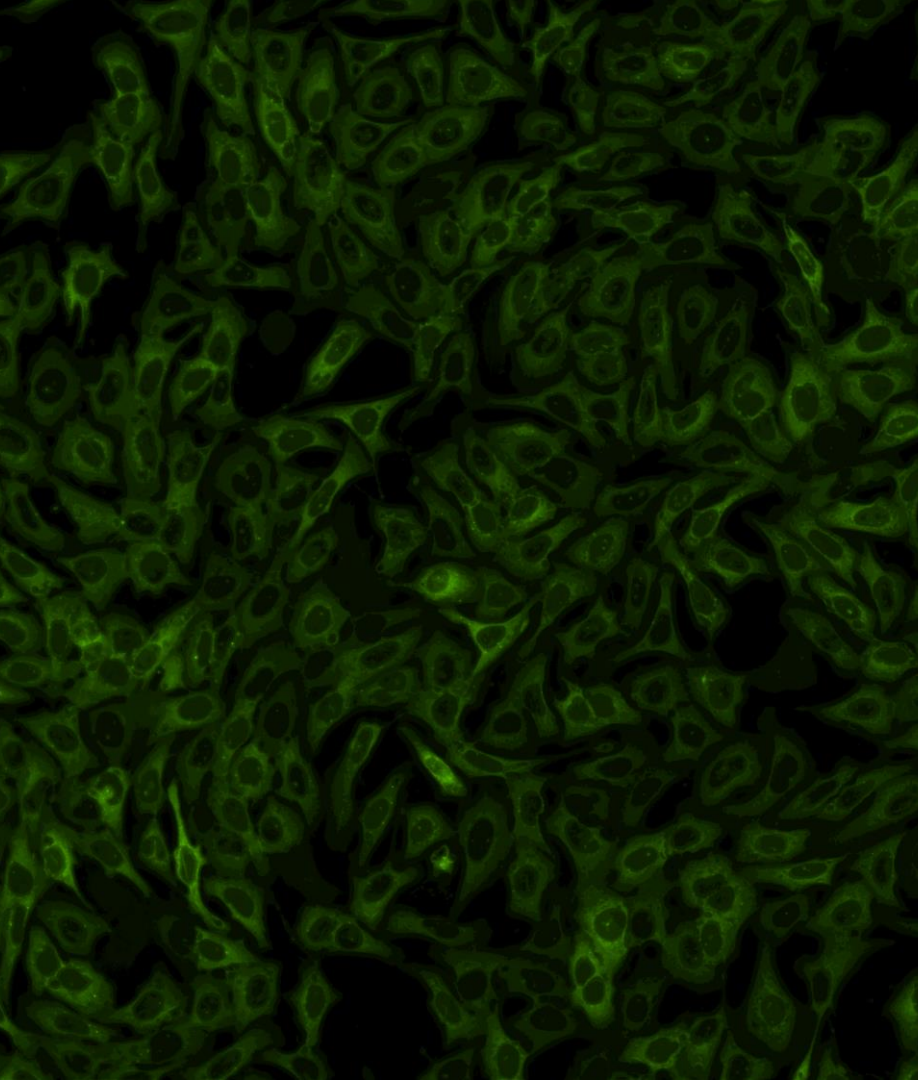


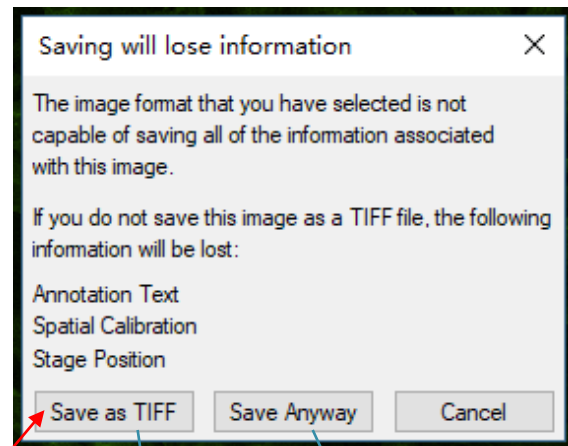
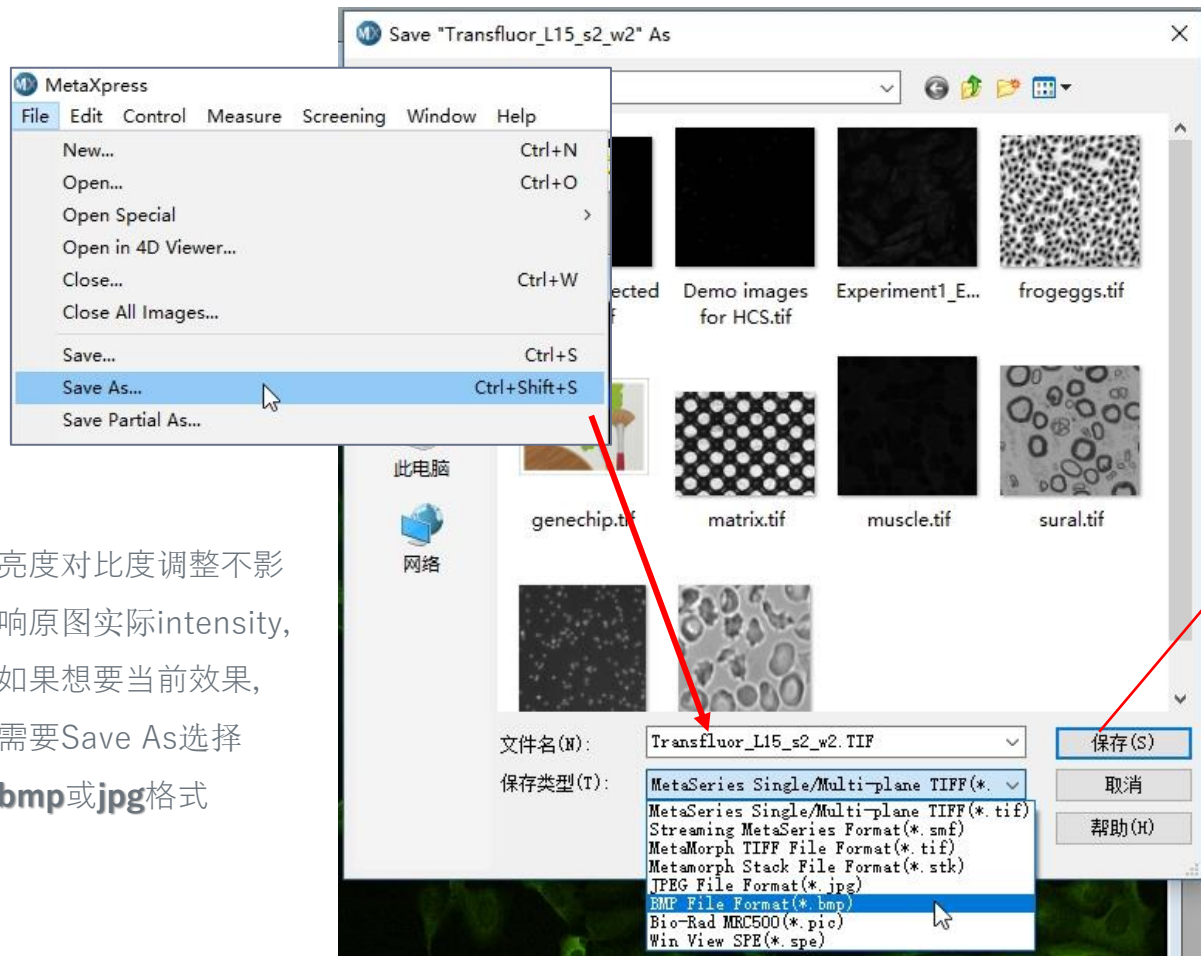
5	9	18	15
8	15	19	16
6	12	15	12
11	14	22	14

40x DAPI-FITC-B6_B06_s1_w27DCDDCBC 25% 1/1 (916, 804) -> 22661 1 pixel = 0.1747 um 2.66 GB physical memory 6.12 GB virtual memory

图像后期调整
亮度对比度





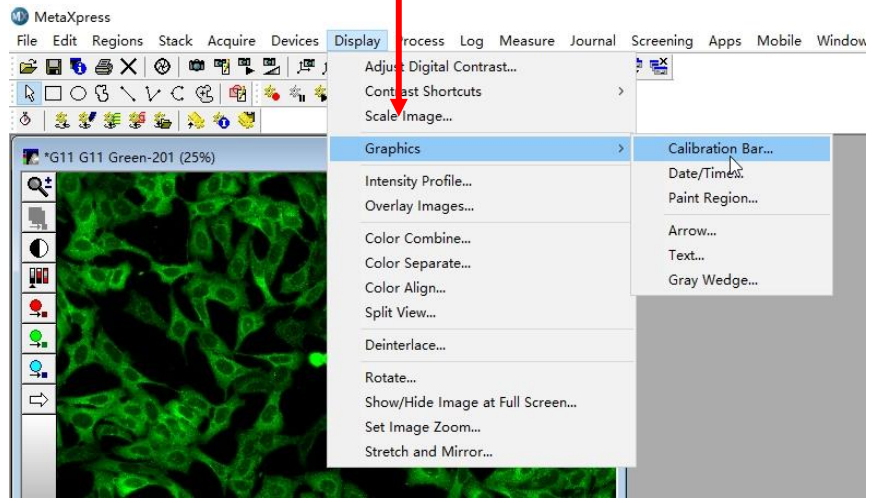
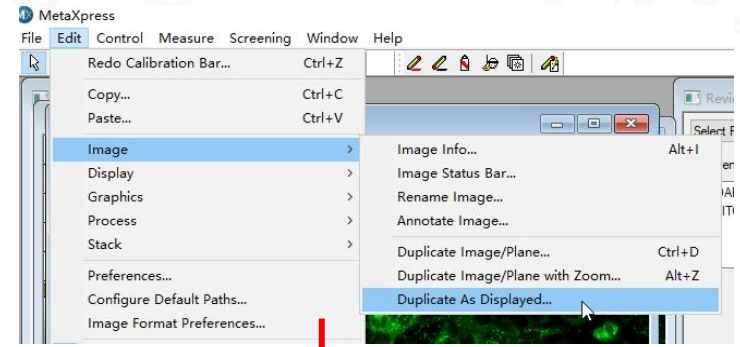
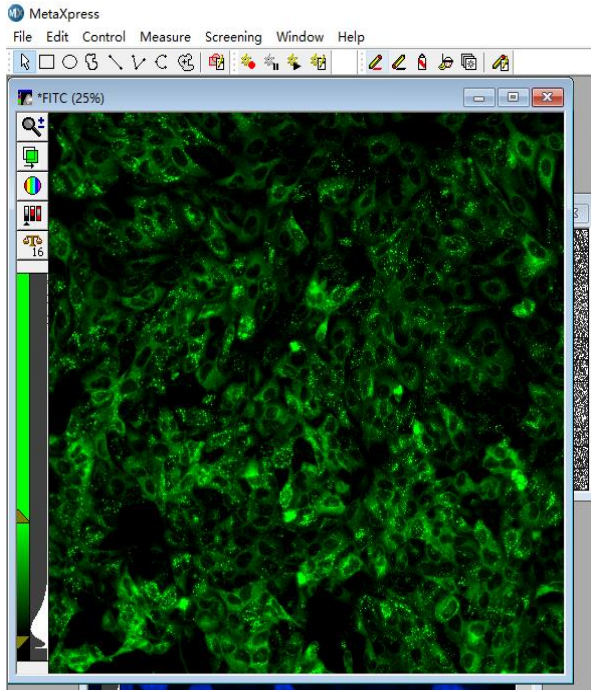


亮度对比度调整不影响原图实际intensity, 如果想要当前效果, 需要Save As选择 **bmp**或**jpg**格式

最终格式仍为tif

最终格式为所选格式

添加scale bar



*G11 G11 Green-201 (25%)

Calibration Bar

Image: G11 G11 Green-201

Label: 180 um

Bar size (um): 180

Thickness (pixels): 78

Bar Orientation

Horizontal

Vertical

X: 1458

Y: 1892

Stamp Location

Image

Measurement Overlay

Measurement Color: Red

Color:

Bar border color: Color...

Fill interior area of bar Color...

Draw label onto image Color...

Erase image behind label Color...

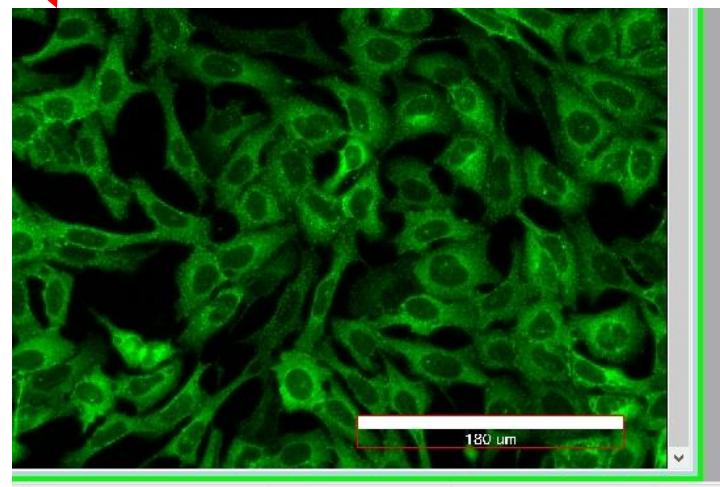
Font... MS Sans Serif Regular 23

X: 1458 Y: 1892

Width: 508 (179.83 μm)

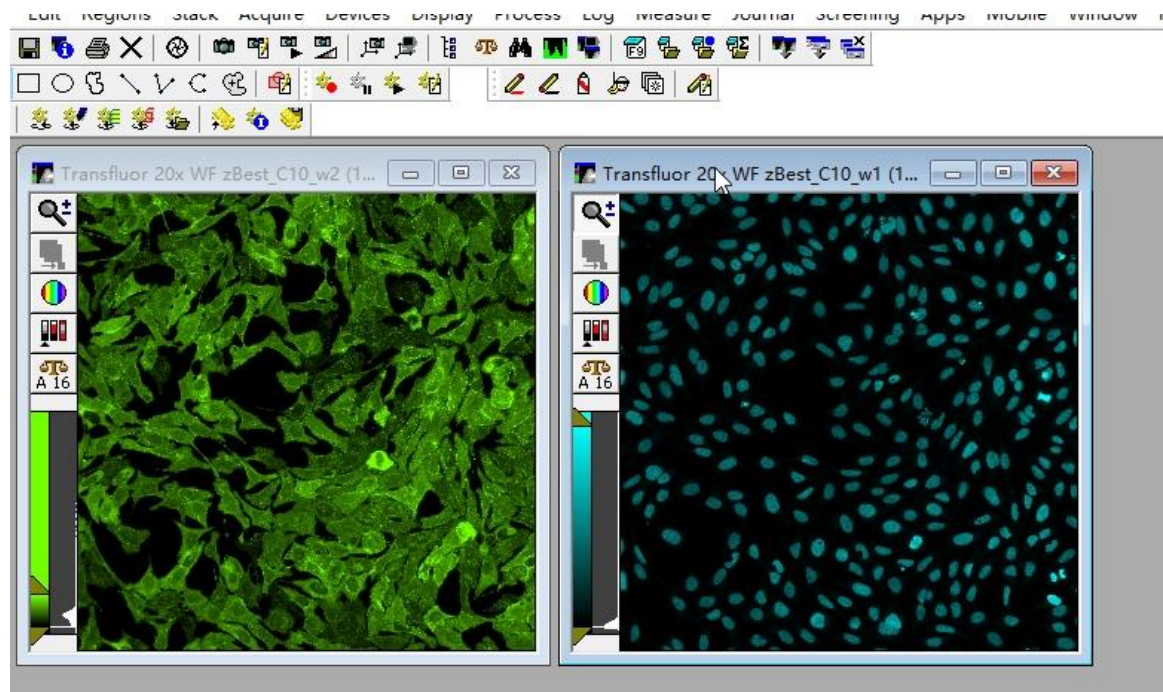
Height: 114 (40.36 μm)

Area: 57912 (7257.30 μm^2)

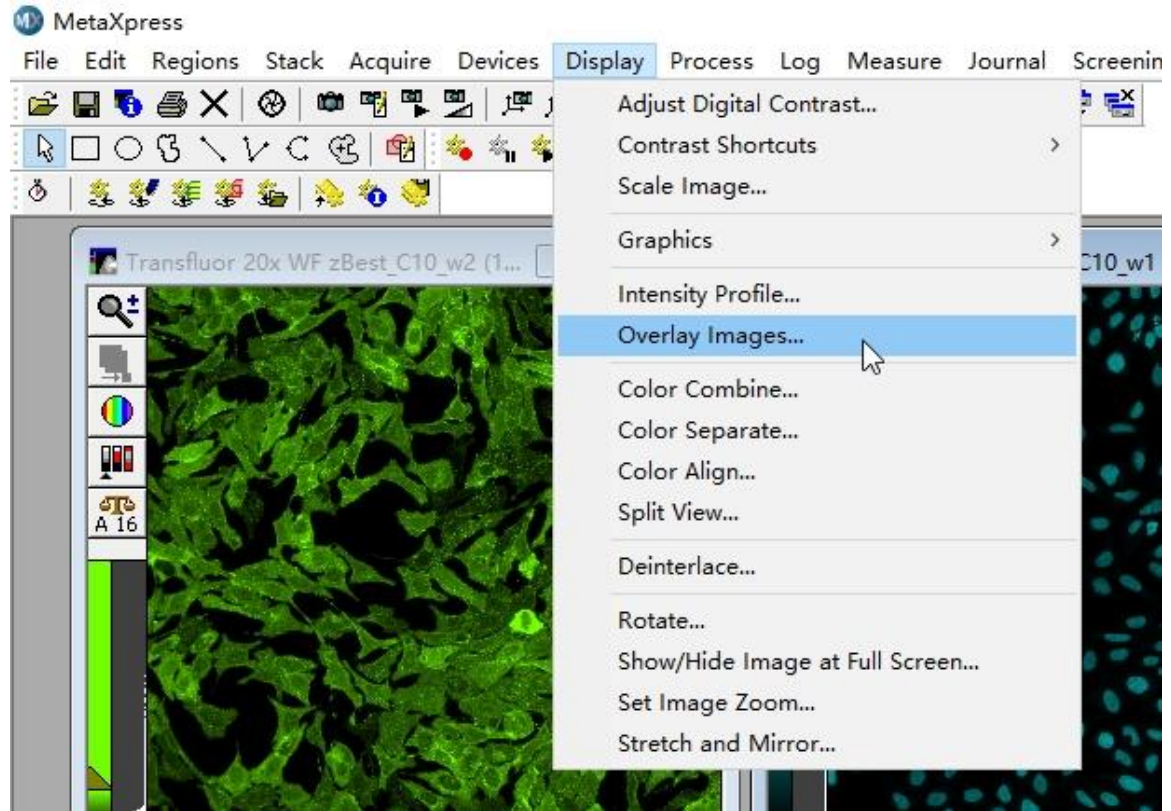


彩色多通道图像叠加

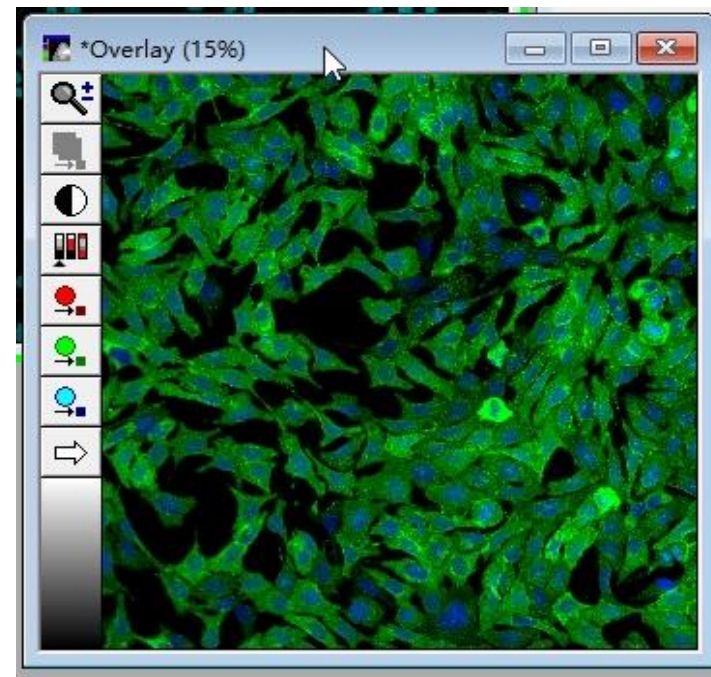
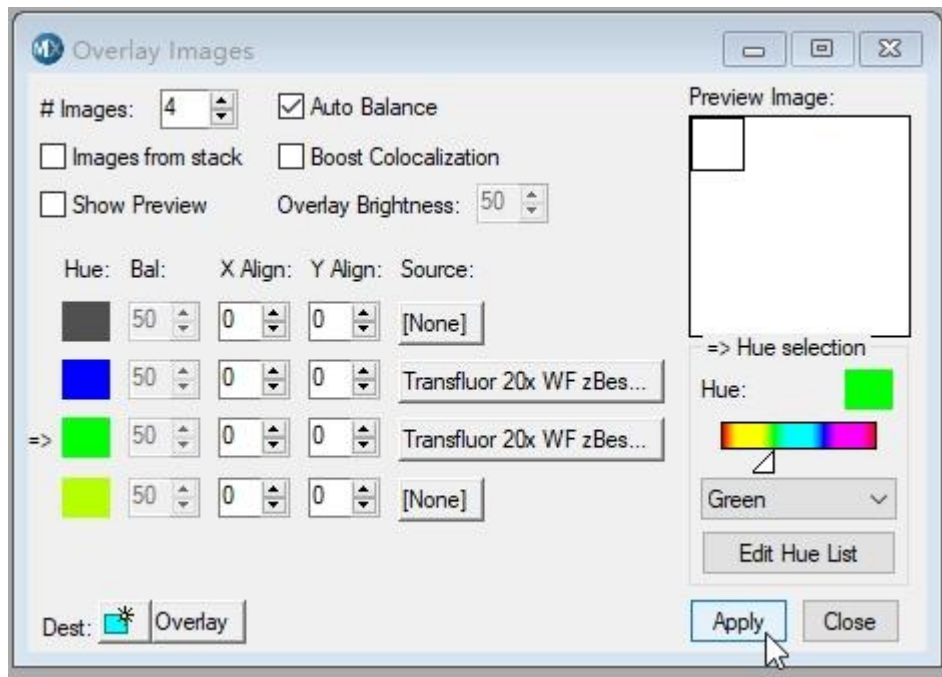
1. 打开需要被叠加在一起的多张图片；



找到Display→Overlay images功能（或在Edit→Display→Overlay images）



在不同通道处分别选择需要叠加的图像名称，调好单色图像的对比度，点击apply



Move the square to preview zoomed areas

The screenshot shows the 'Overlay Images' software interface. On the left, the 'Show Preview' checkbox is checked and highlighted with a red box. A red arrow points from a text box above to a small square in the 'Preview Image' window. The main preview window shows a multi-color fluorescence microscopy image of cells. The interface includes various controls for image processing, such as 'Auto Balance', 'Boost Colocalization', and 'Overlay Brightness'.

Hue	Bal	X Align	Y Align	Source
[None]	50	0	0	[None]
Blue	50	0	0	DAPI
Green	50	0	0	FITC
Red	50	0	0	Cy5
Yellow	50	0	0	Cy3
Cyan	50	0	0	[None]
Magenta	50	0	0	[None]

Review Plate Data -

Select Plate... Search... HCT in MG CF60 20xPA 24Z-240-65_APSHAL-24QLJR2_8

Wavelengths: DAPI FITC TRITC

Data view: Z Step vs Well

	01				11	12	13	14
C10								
C11								
C12								
D01								
D02								
D03								
D04								
D05								
D06								
D07	-	-	-	-	-	-	-	-
D08								
D09								

Legend

- Not
- Acc
- Dis
- Par
- Z

Load Selected Images

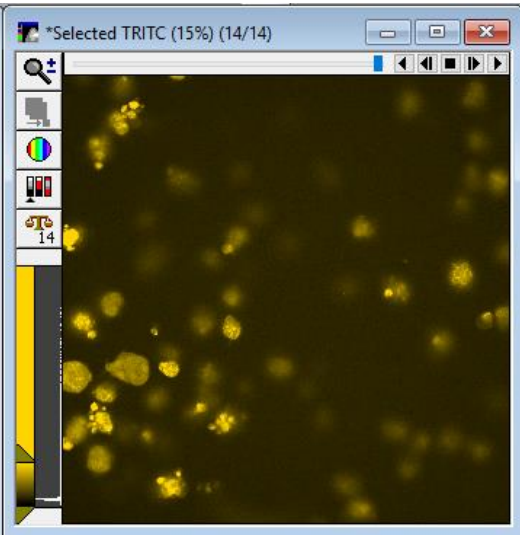
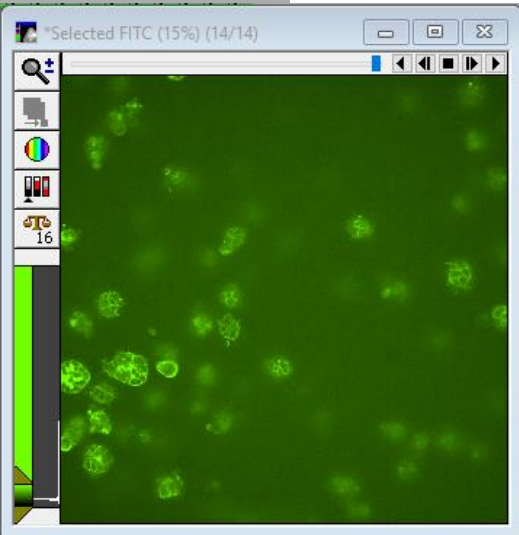
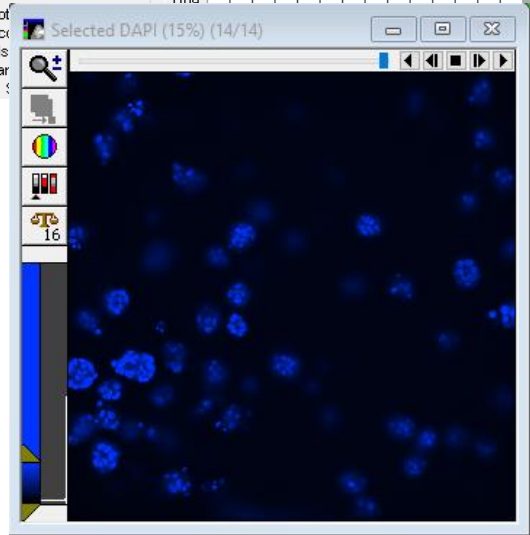
Reset Image Displays

Cellular Results...

Navigate Selections

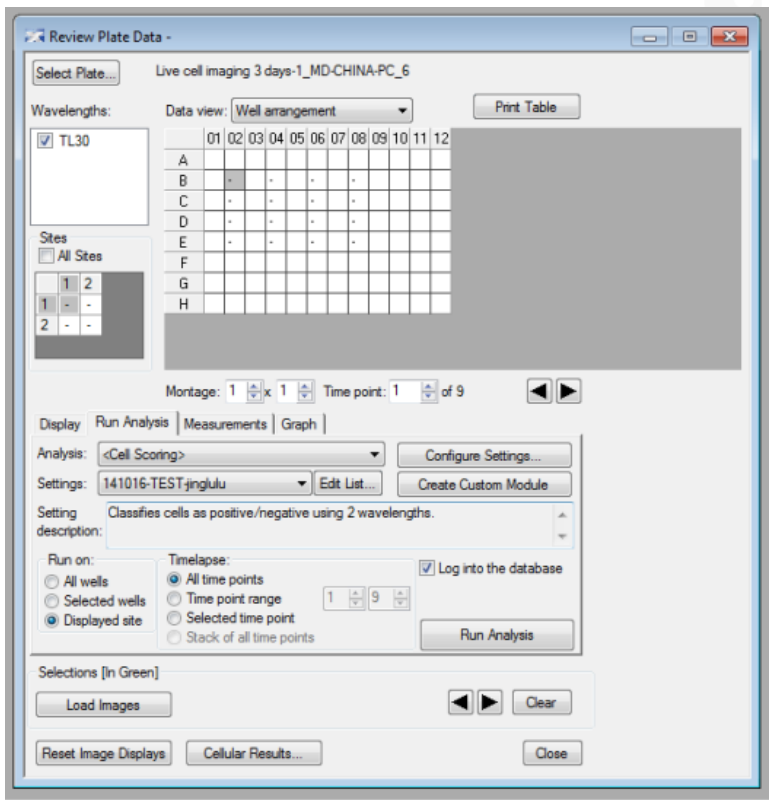
Clear Selection

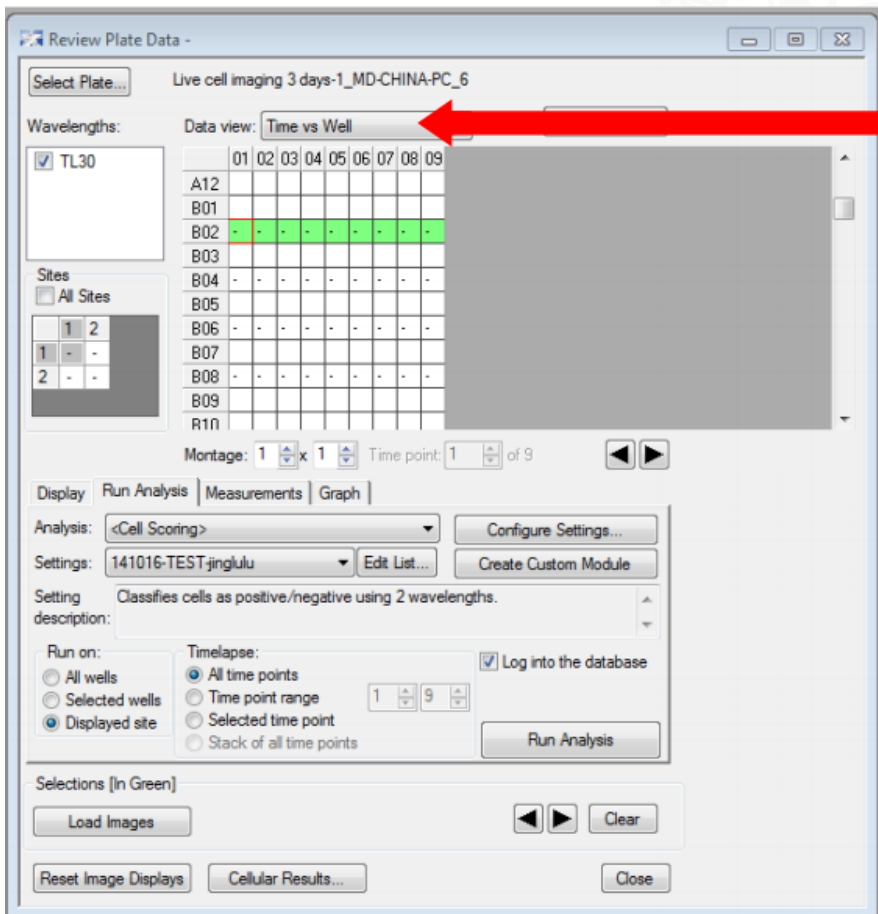
Close



制作movie

在 Screening---Review plate date 里打开要做 movie 的数据





在Data view 选项（红色箭头）里选择“Time vs Well”，鼠标右击要做 movie 的孔（图中举例为 B02），选中后显示为绿色。矩阵图上方的 01-09 数字代表拍了几个时间点。左方的 wavelength 要选中需要的荧光通道

Review Plate Data -

Select Plate... Live cell imaging 3 days-1_MD-CHINA-PC_6

Wavelengths: TL30

Sites All Sites

	01	02	03	04	05	06	07	08	09
A12									
B01									
B02	-	-	-	-	-	-	-	-	-
B03									
B04	-	-	-	-	-	-	-	-	-
B05									
B06	-	-	-	-	-	-	-	-	-
B07									
B08	-	-	-	-	-	-	-	-	-
B09									
R10									

Montage: 1 x 1 Time point: 1 of 9

Display Run Analysis | Measurements | Graph

Analysis: <Cell Scoring> Configure Settings...

Settings: 141016-TEST:jnglulu Edit List... Create Custom Module

Setting description: Classifies cells as positive/negative using 2 wavelengths.

Run on: All wells Selected wells Displayed site

Timelapse: All time points Time point range Selected time point Stack of all time points

Log into the database

Run Analysis

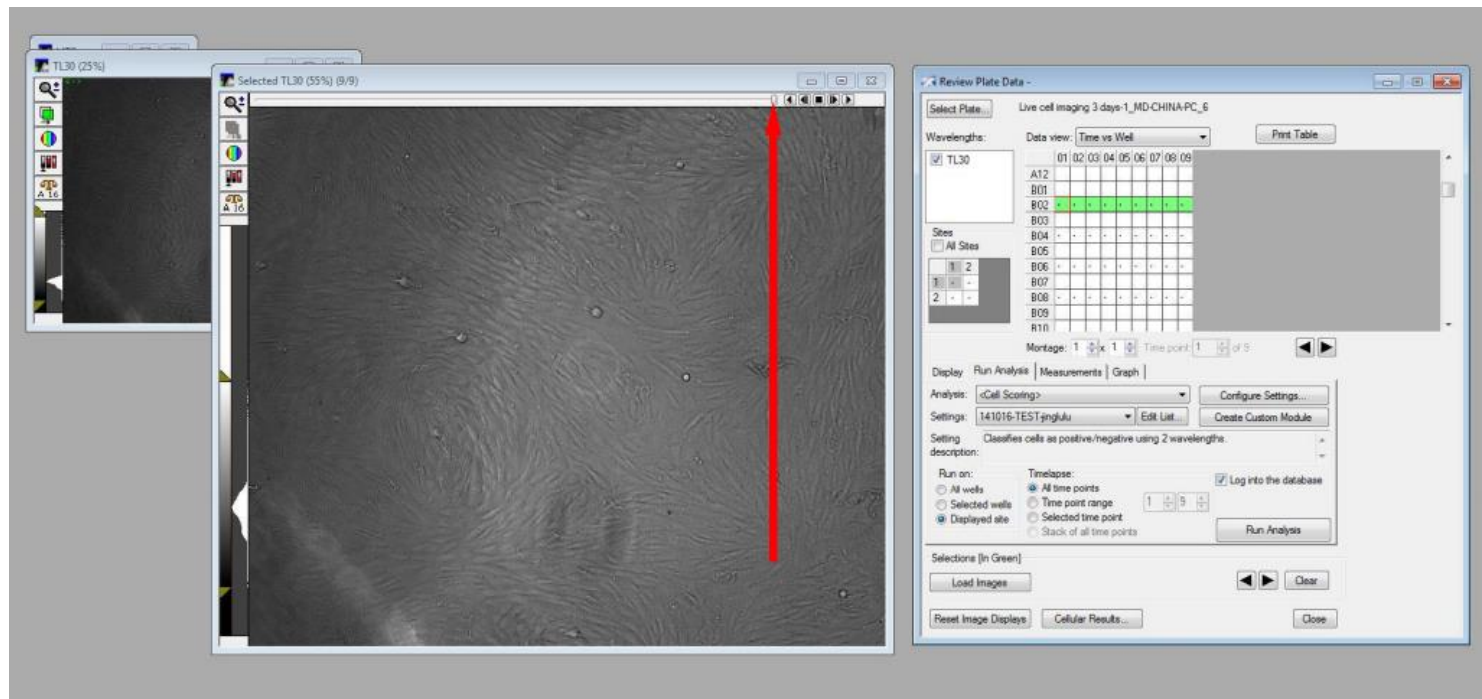
Selections [In Green]

Load Images

Reset Image Displays Cellular Results... Close

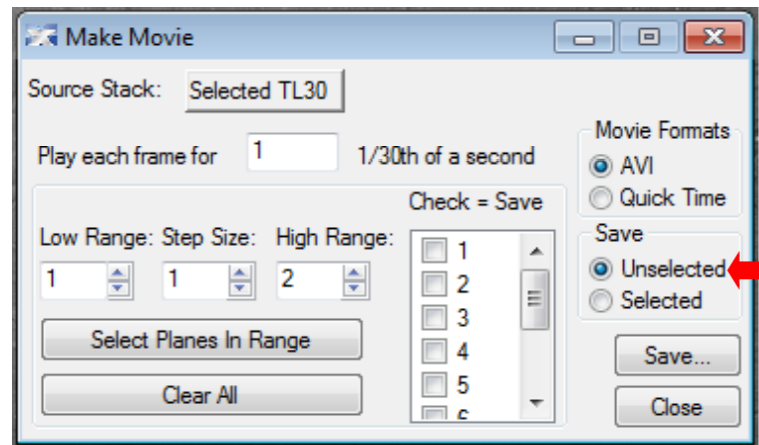
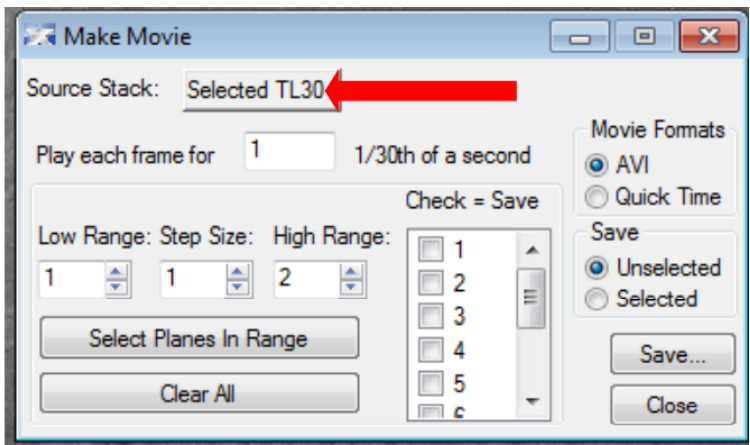
点击” load Images”

出现如图所示的 image stack，其中包含同一位置下不同时间照片的集合

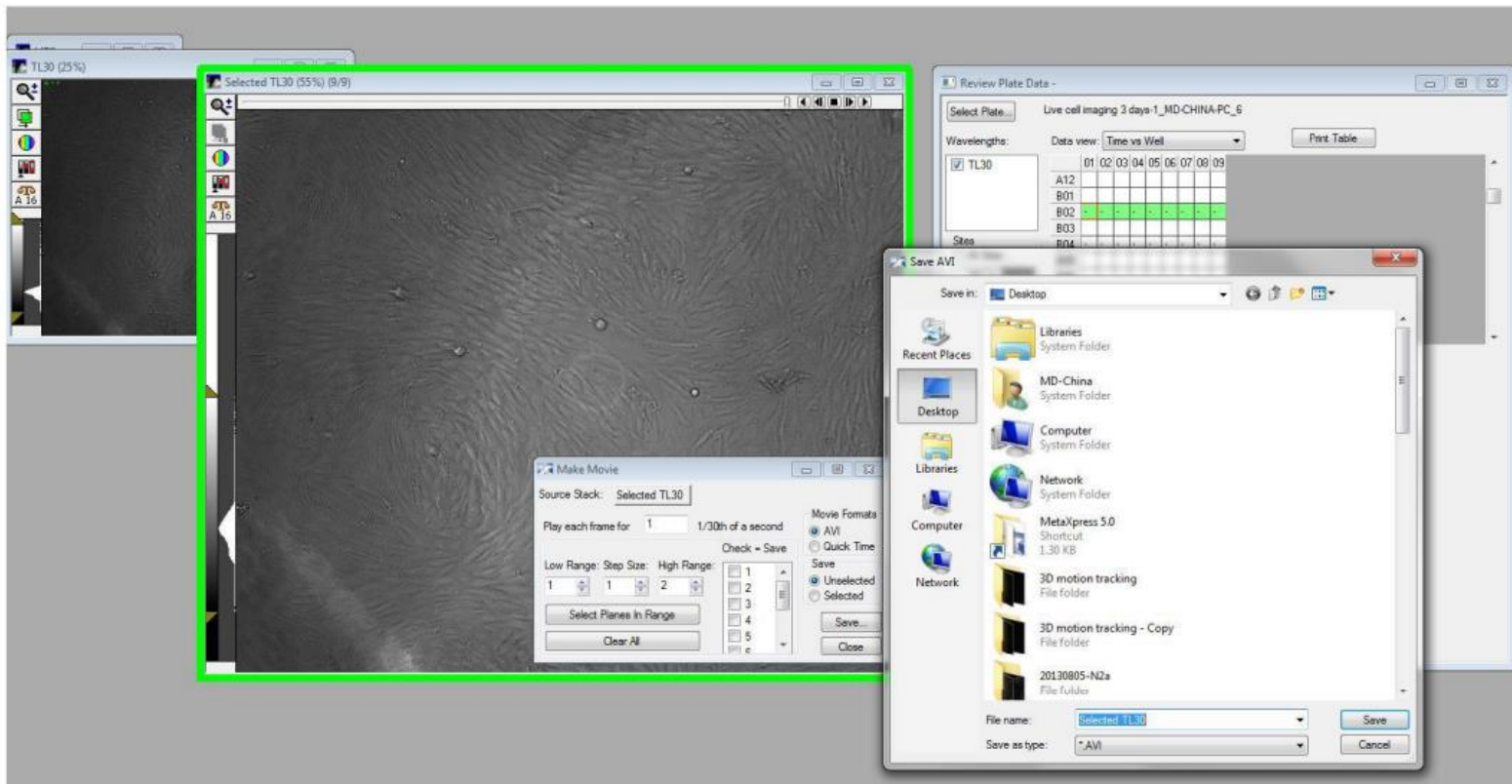


如果前述 Image stack 建立成功，则下图中红色箭头所指的位置会自动导入建好的 image stack（本例中名为 Selected TL30）

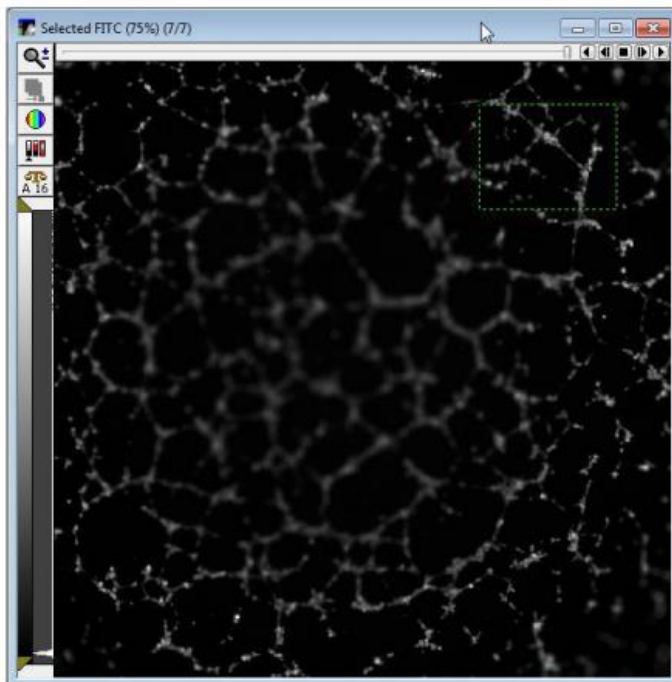
直接点击红色箭头所指的“Unselected”选项，将所有图片包含入 movie 中。



点击 save 后，保存为 Avi 格式或 Quicktime 格式即可



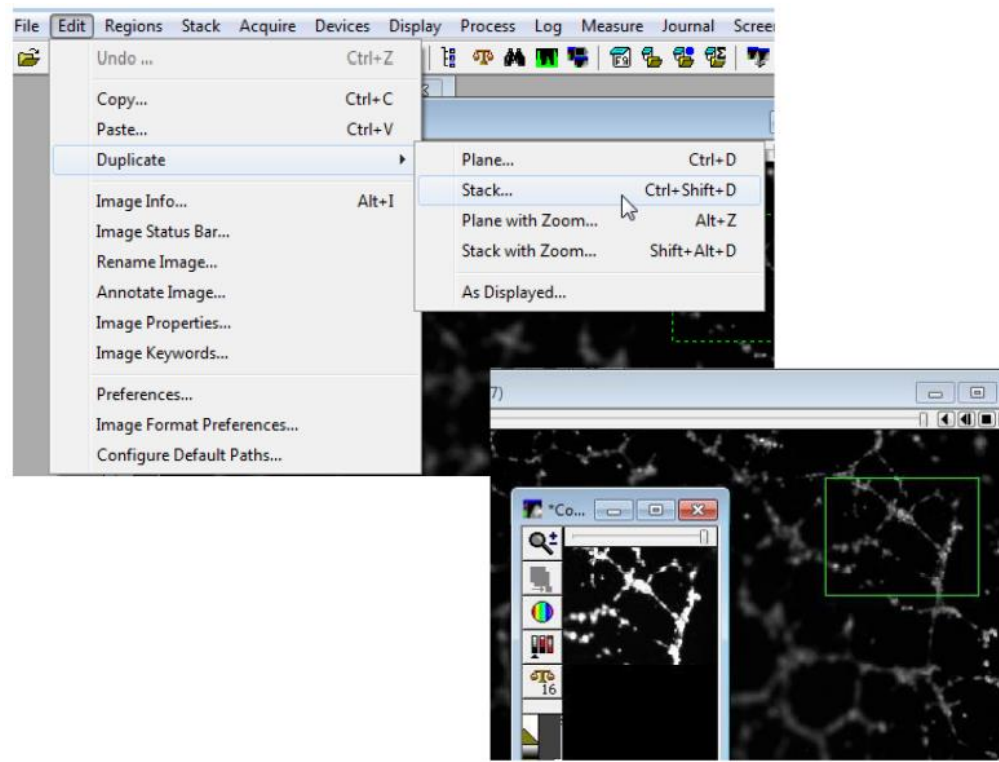
Stack 截取小区域生成视频



工具

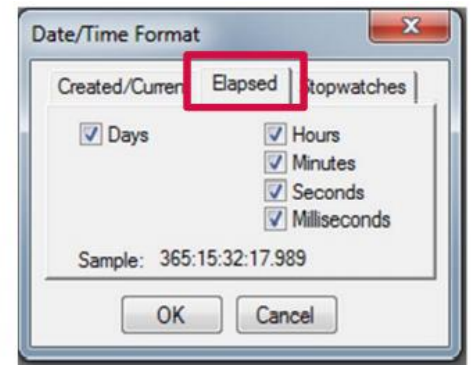
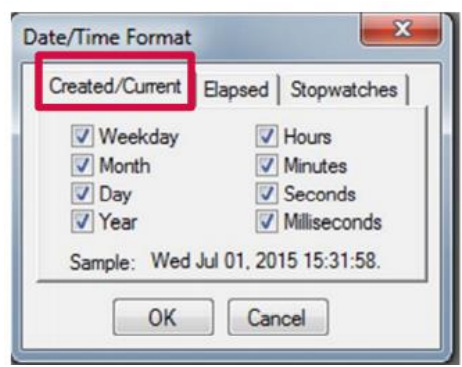
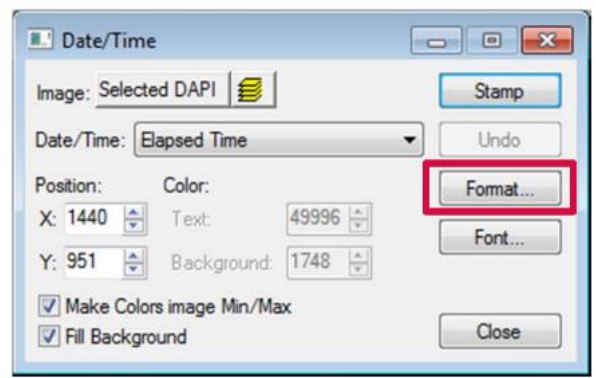
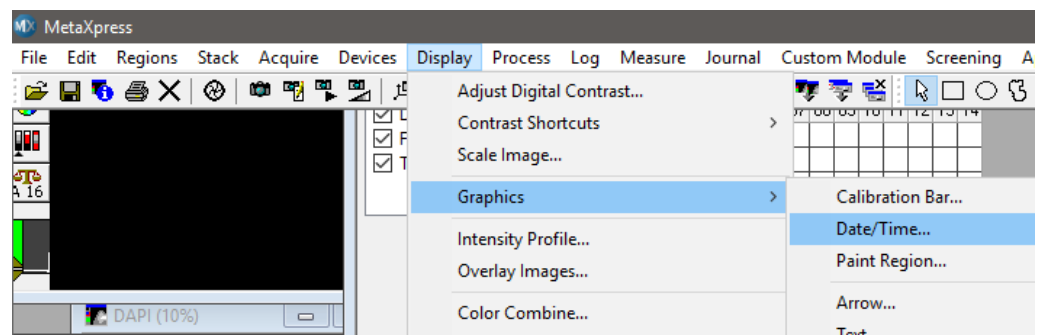
在生成的 stack 文件上用选区工
具框选中目标位

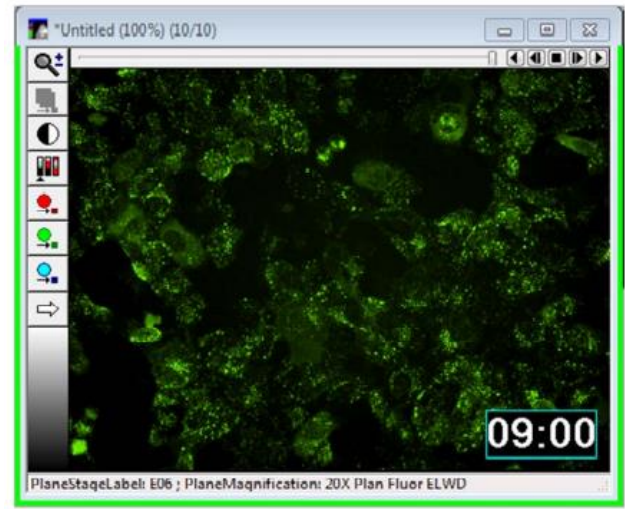
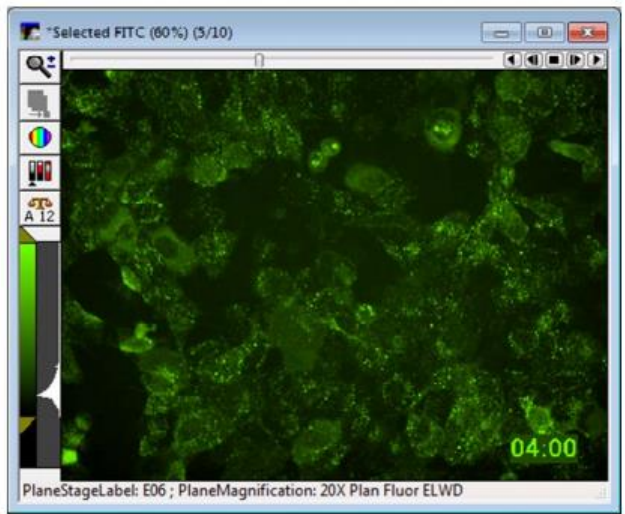
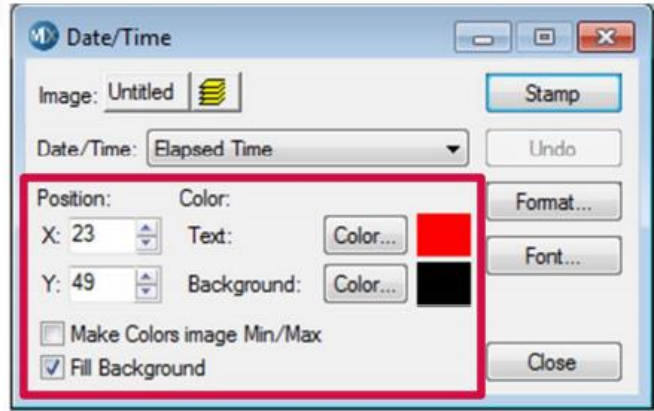
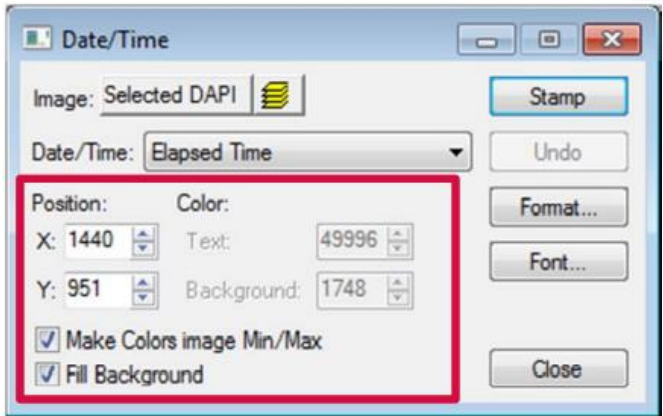
在 Edit 中选择 Duplicate—Stack，生成一个小区域的 stack 文件



给movie加上日期/时间

或Edit > Graphics > Date/Time



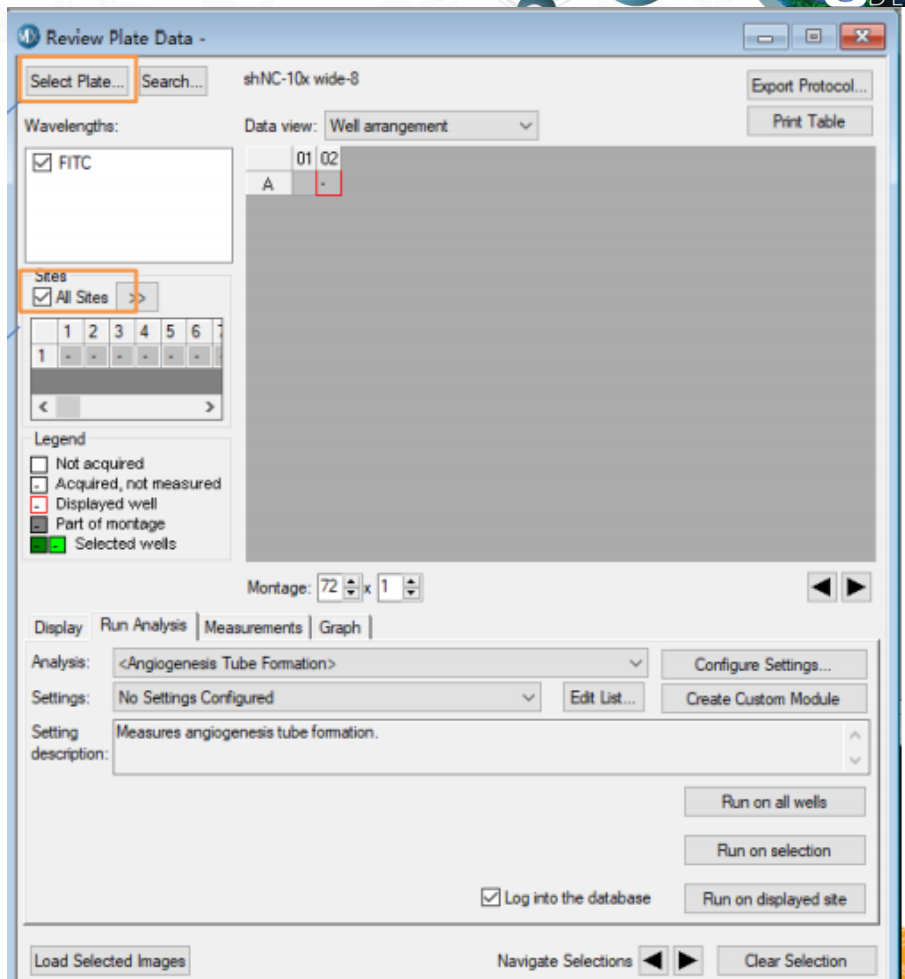


图像拼接

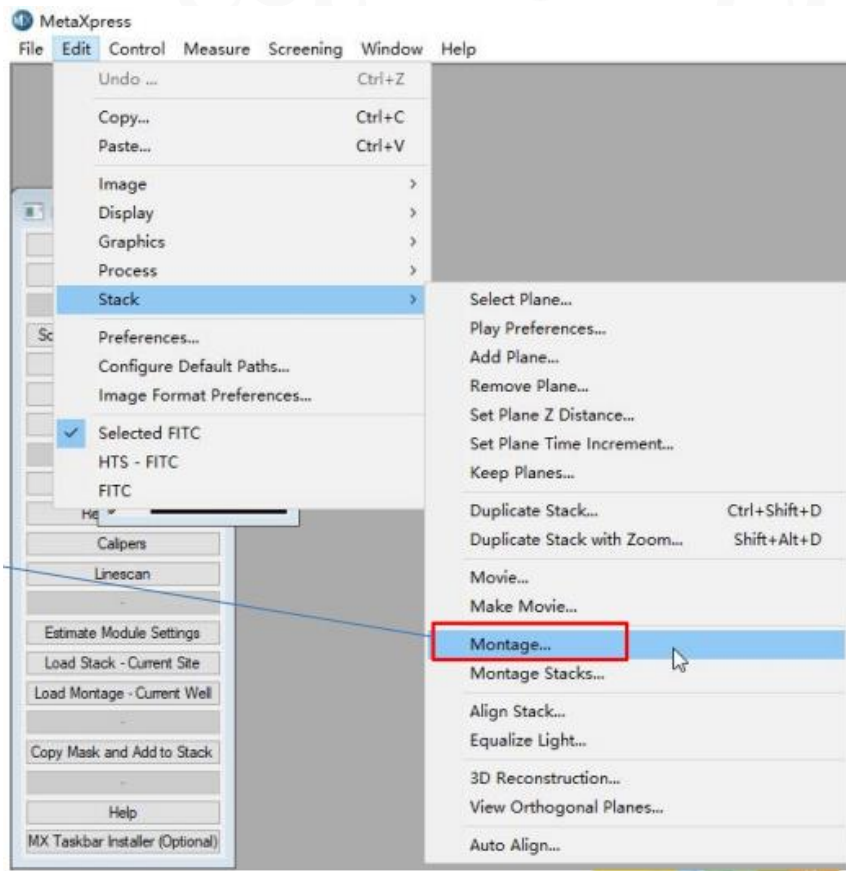
选好板子和通道以后，选择 all sites

右键选中待拼接的孔，然后点击下方 load selected images，生成一个stack文件

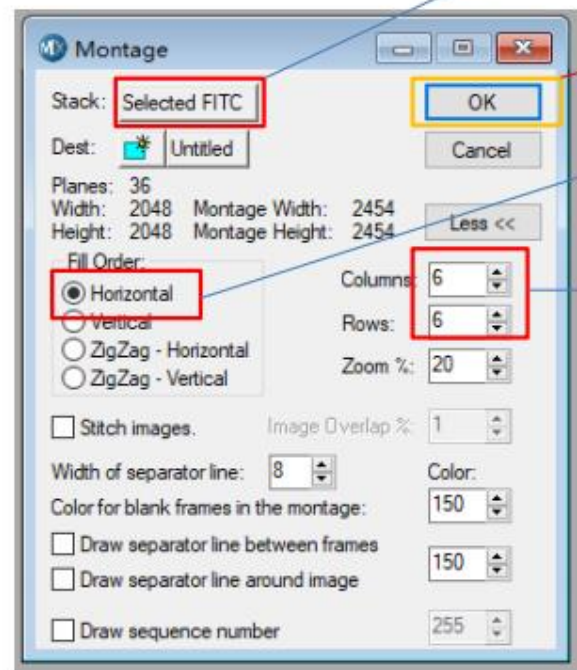
先调整好对比度，然后去掉 auto scale



Edit-Stack-Montage



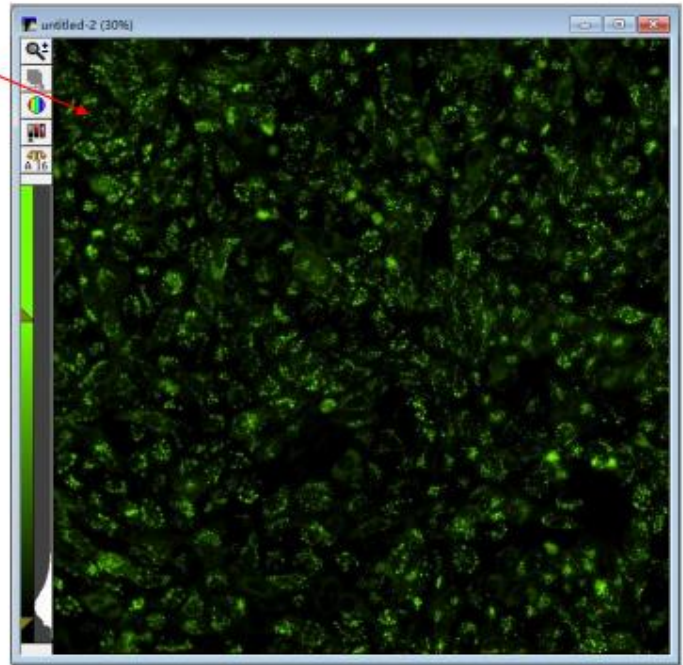
1 选择刚刚生成的stack文件



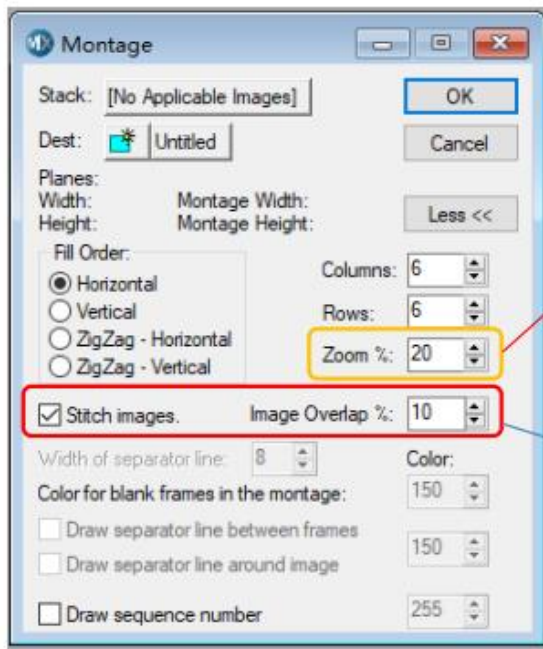
4 全部设置完成后点OK生成大图

2 选择Horizontal模式

3 设定拍摄时的排列方式（通常在Review Plate Data中的All Sites选项下可以看出排列模式）

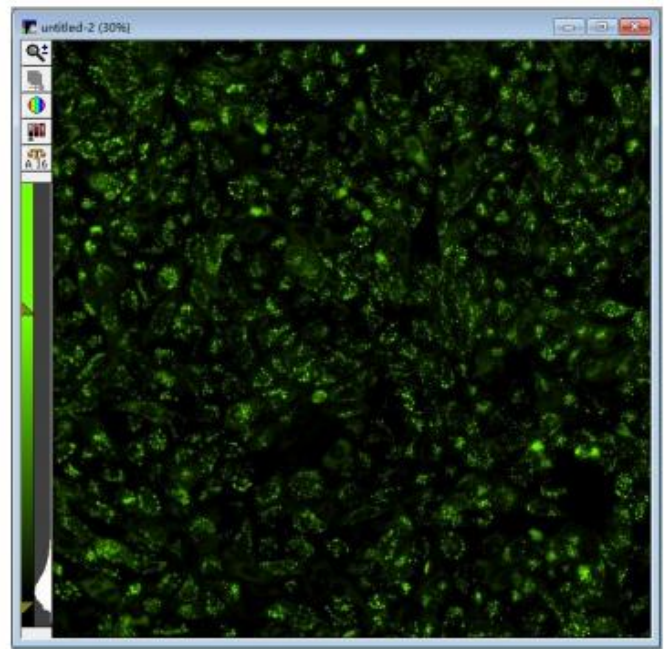


拼接后的大图



如果原图非常大，可能导致电脑无法直接打开，可以选择一定比例缩小拼接的大图，以便方便打开

如果拍摄设置里，sites 排布方式里选择了 **Overlay sites 10%**，则需要勾选 **Stitch images**，并将 **overlay** 比例设为 10%







Thanks




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