ImageXpress Micro高内涵系统操作培训

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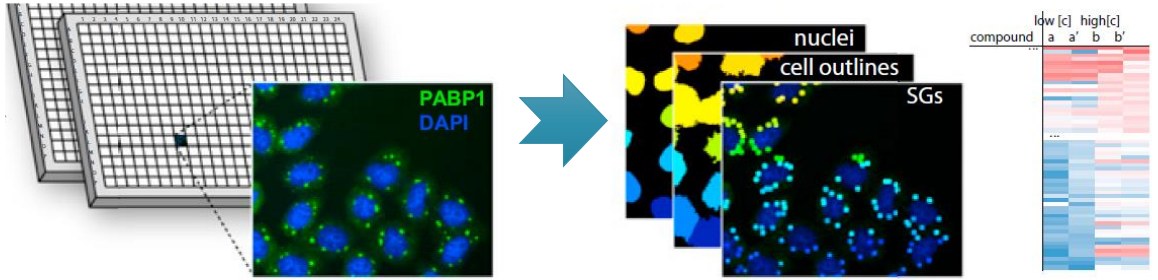
MOLECULAR DEVICES

高内涵实验基本流程

自动化拍摄图像

自动化图像分析

制备
样品



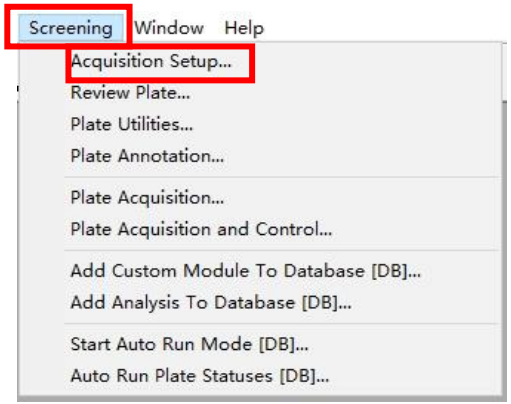
Frank Wippich,Bernd Bodenmiller.Dual Specificity Kinase DYRK3 Couples Stress Granule Condensation/Dissolution to mTORC1 Signaling. Cell 152, 791–805, February 14, 2013

主要内容

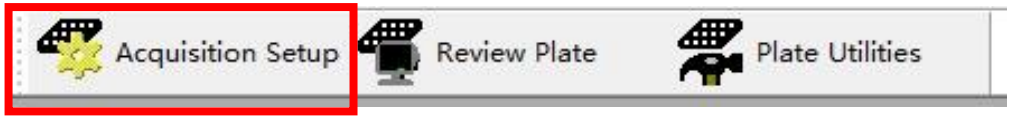
- MetaXpress软件拍摄设置流程
  - 基本设置
  - 长时间拍摄设置
  - Z-Stack拍摄设置
  - Protocol保存与应用
  - 聚焦设置
- 高内涵图像常用处理方法与技巧

基本设置

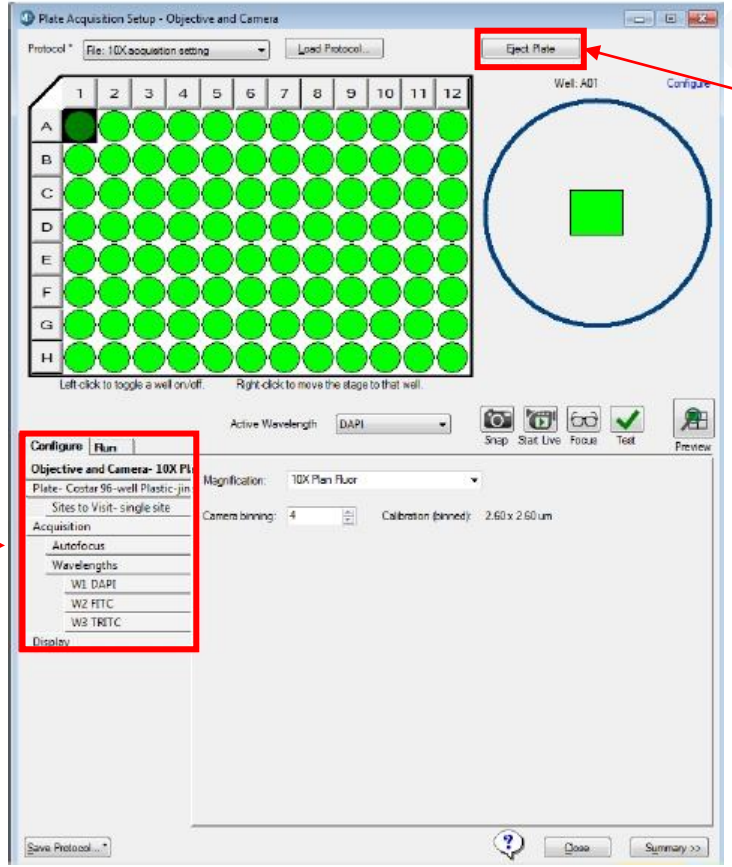
进入软件后，在菜单栏Screening下拉菜单中，选择 Acquisition Setup



或者直接找到工具栏快捷键 Acquisition Setup

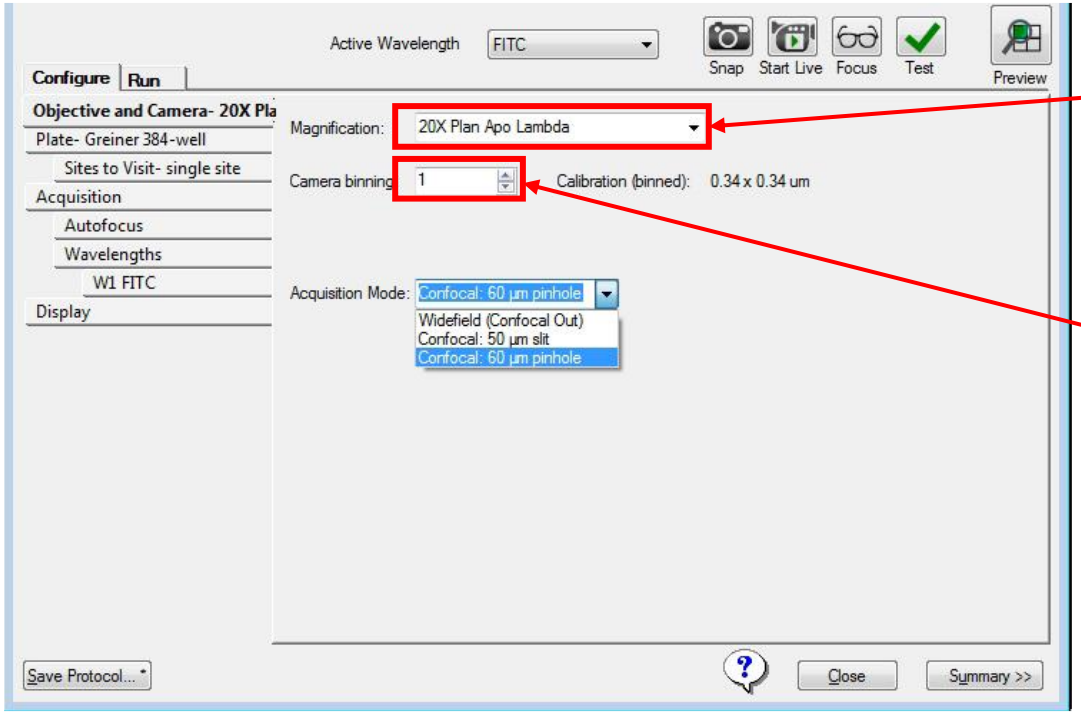


在弹出的Plate Acquisition Setup窗口，按照左侧标签顺序依次进行设置



打开舱门，放样本进去

物镜和图像大小设置

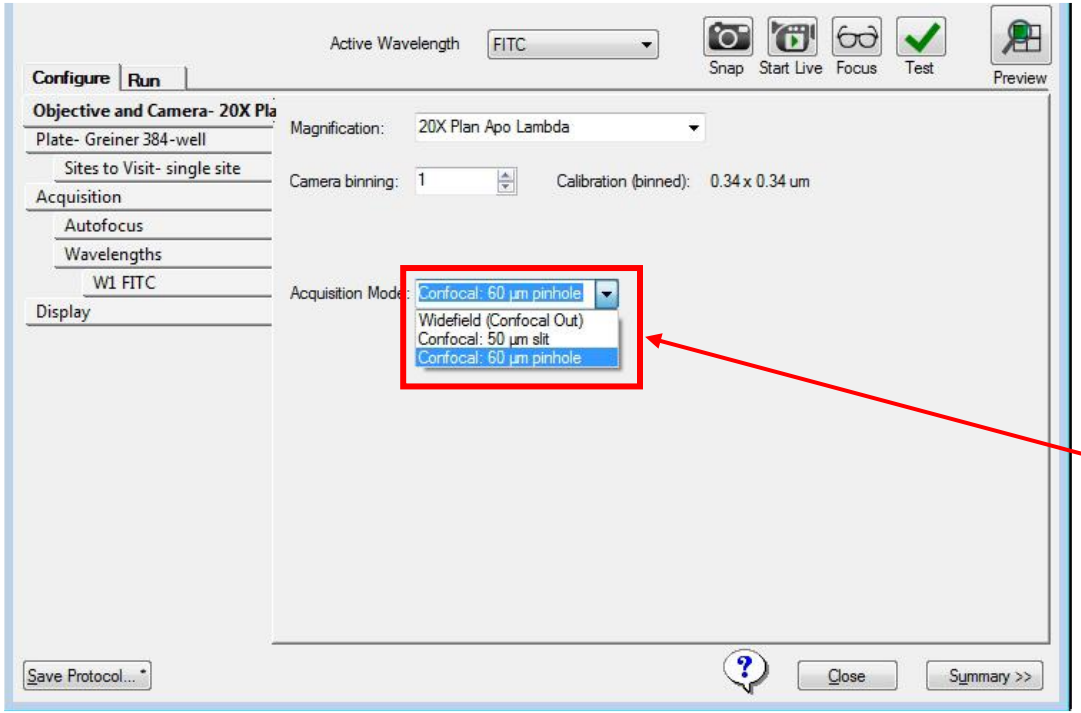


从下拉菜单中选择物镜类型

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

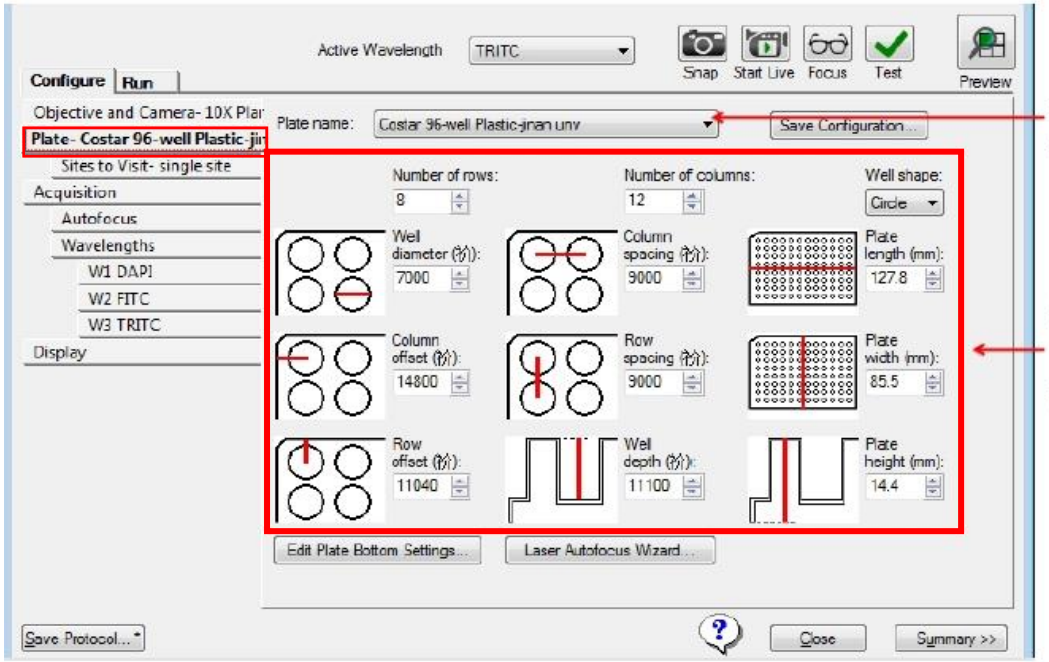
成像模式选择



IXM-Confocal

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 ）

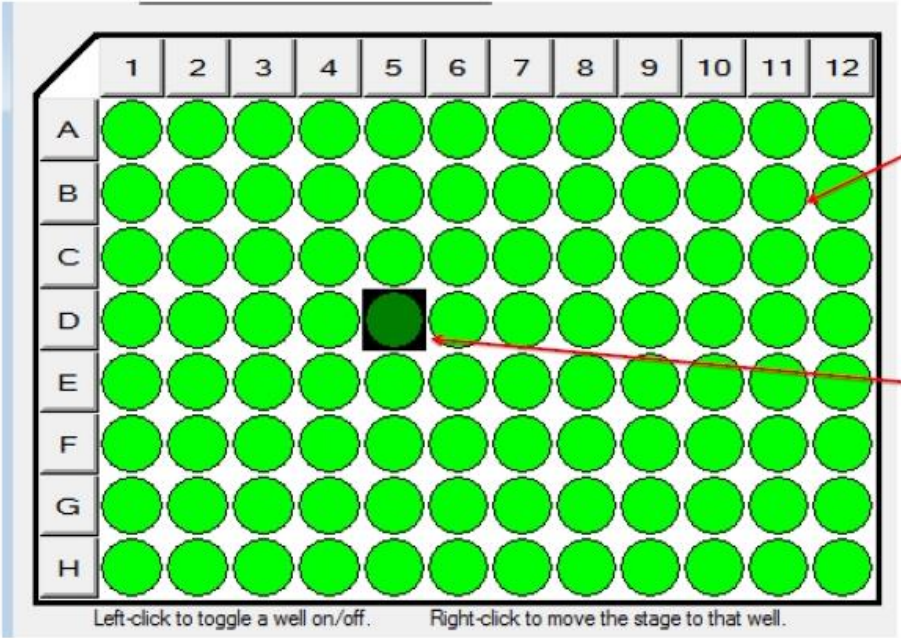
样本板的设置



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



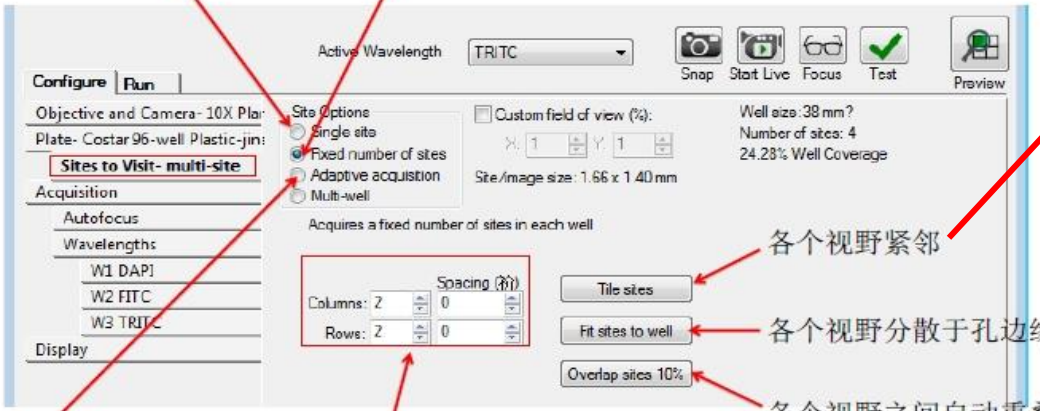
以鼠标左键选择有样品的样品孔，用以获取图像

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

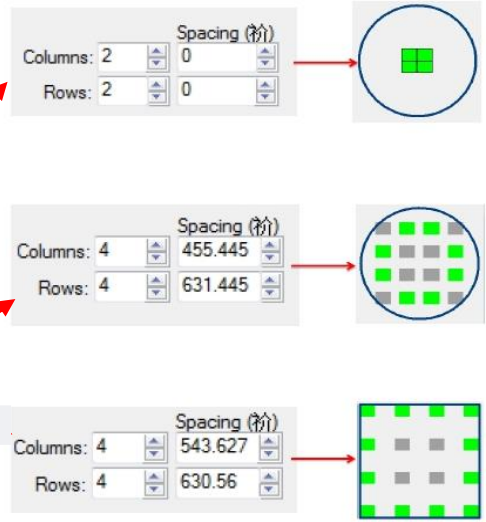
每个孔拍摄固定的视野数量，如3X3，九个视野



各个视野紧邻

各个视野分散于孔边缘

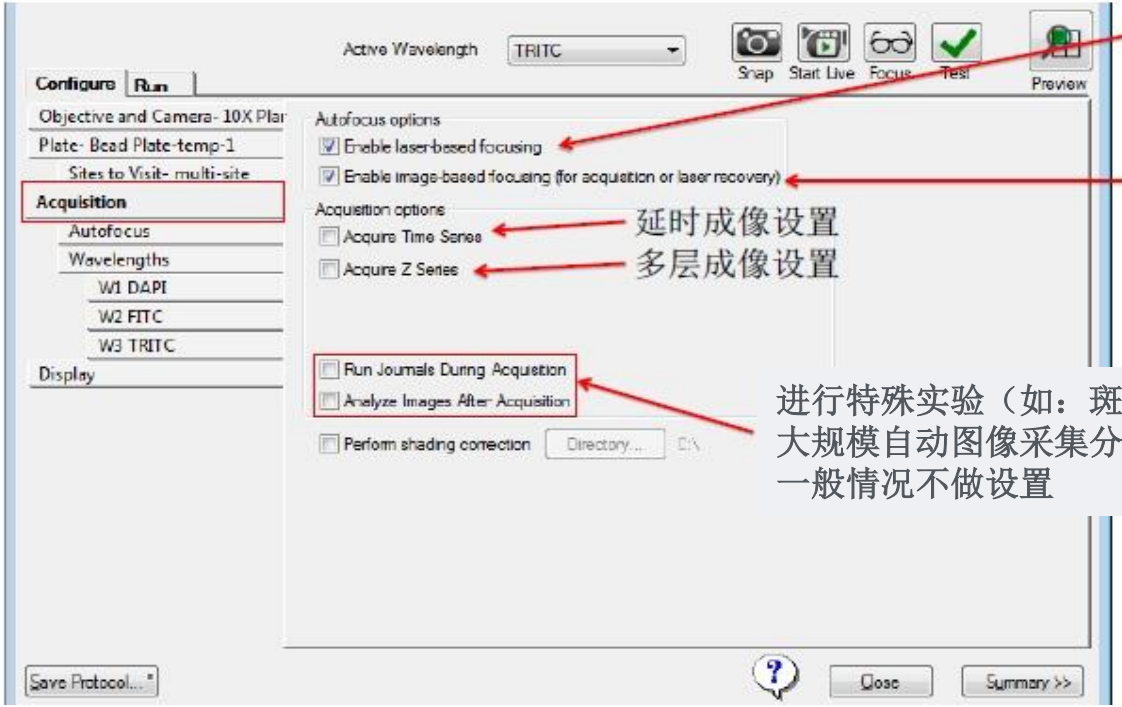
各个视野之间自动重叠10%（常用于图像拼接）



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

采集设置



激活激光自动聚焦，通常作为必选

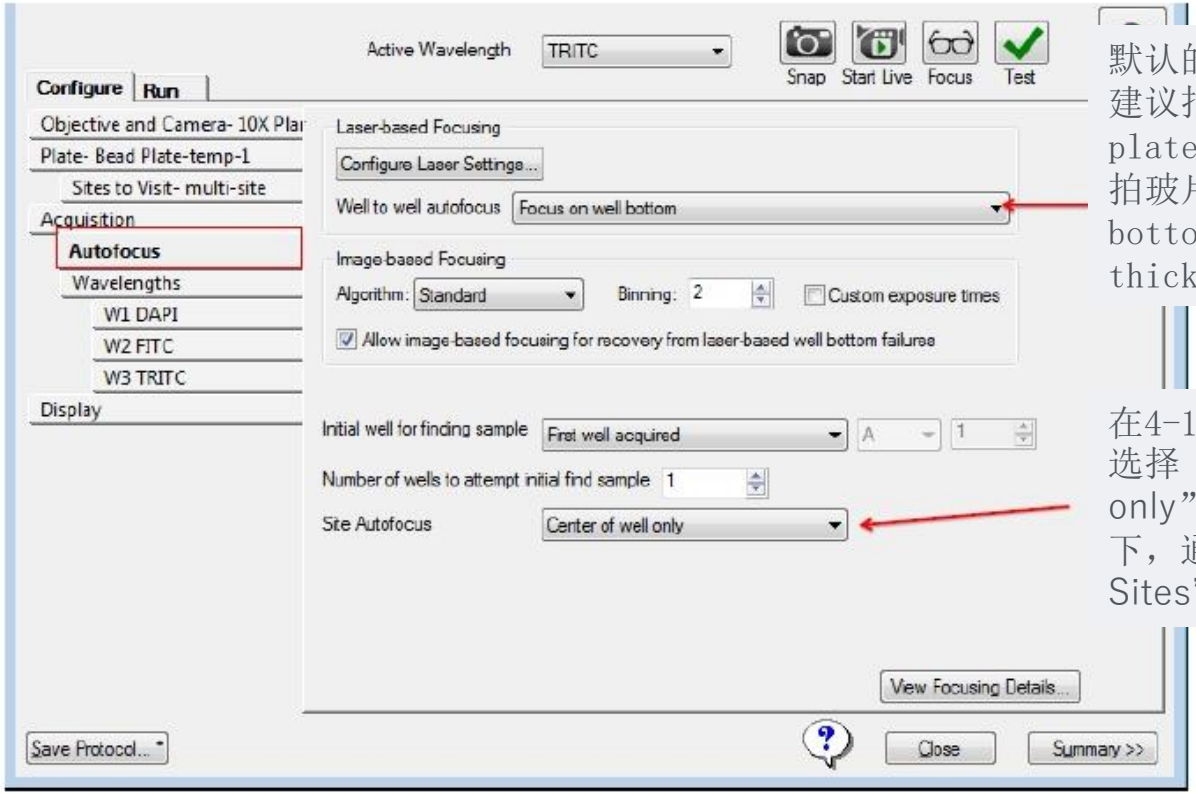
激活图像自动聚焦，图像自动聚焦能够与激光自动聚焦同时使用。

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

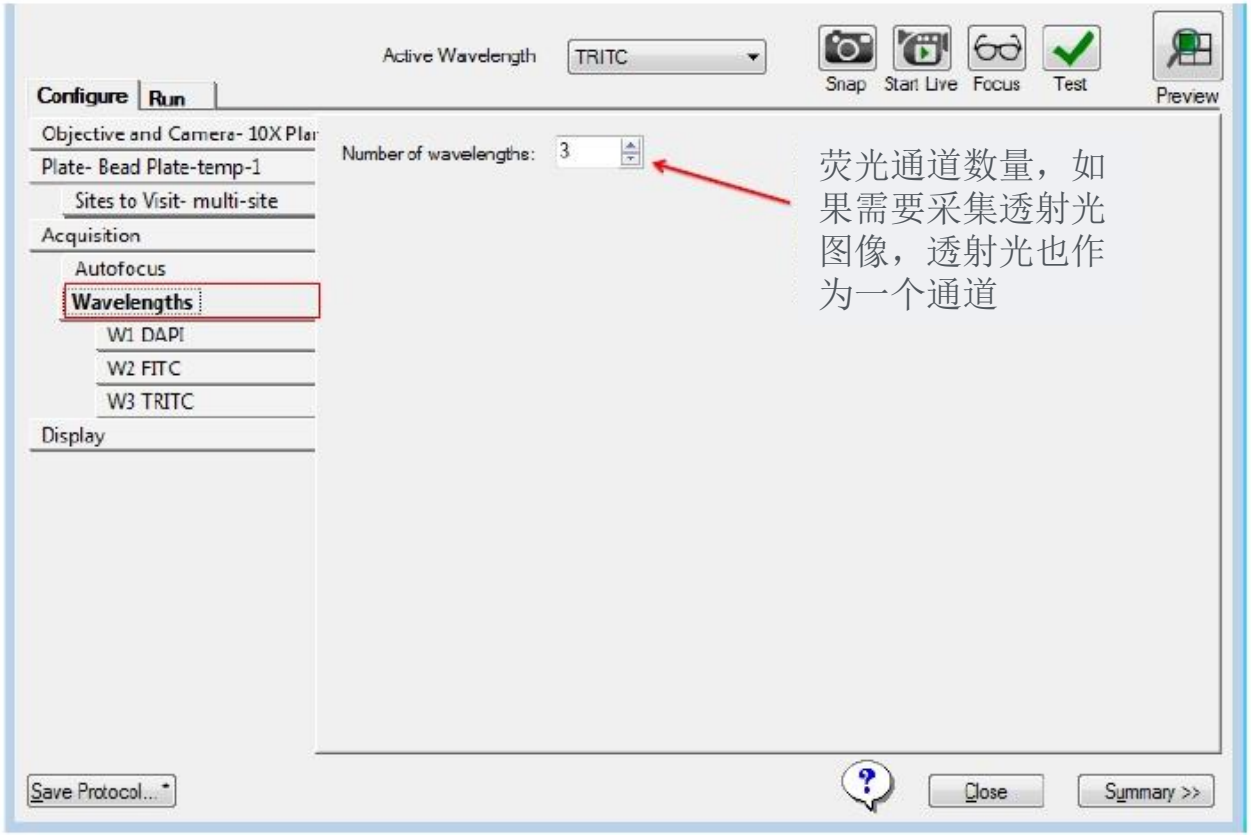
激光自动聚焦选项



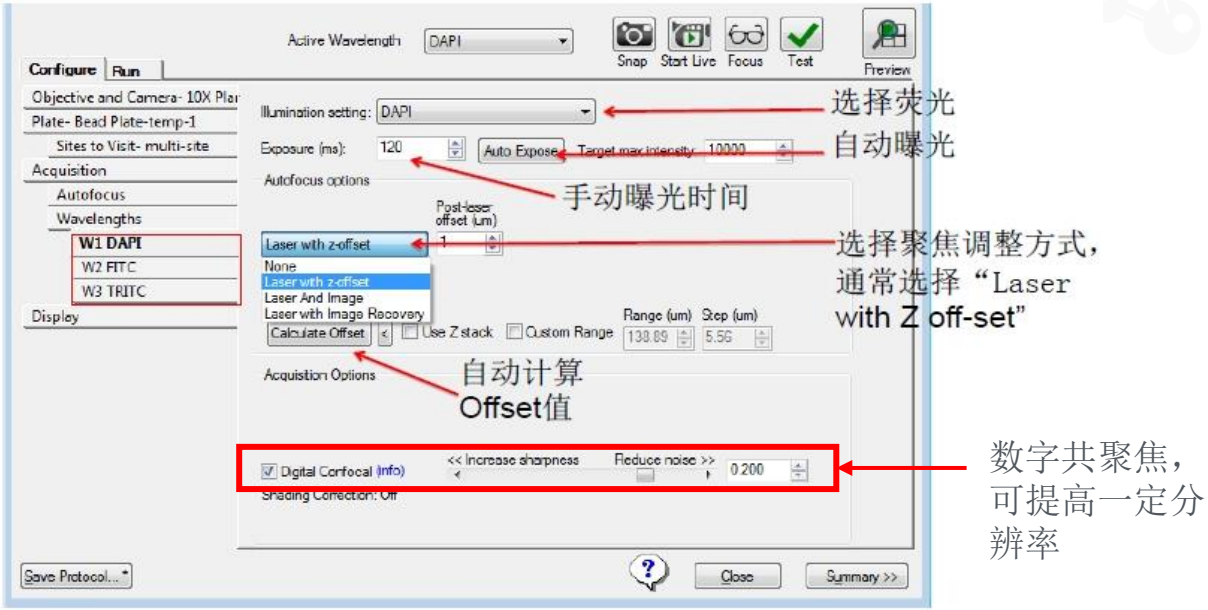
默认的聚焦方式为“聚焦于孔底”
建议拍孔板时选择“Focus on plate and well bottom”
拍玻片时选择“Focus on plate bottom, then offset by bottom thickness”

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



各个通道的设置

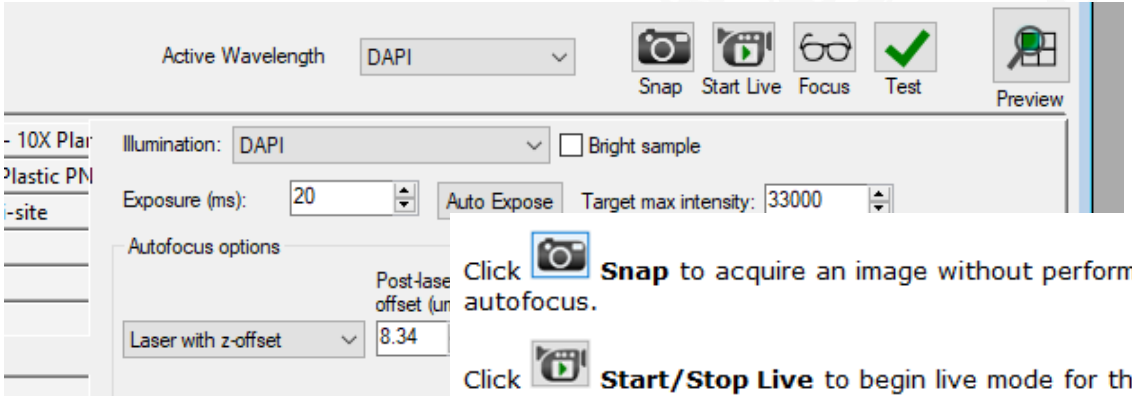


设置步骤：

（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.

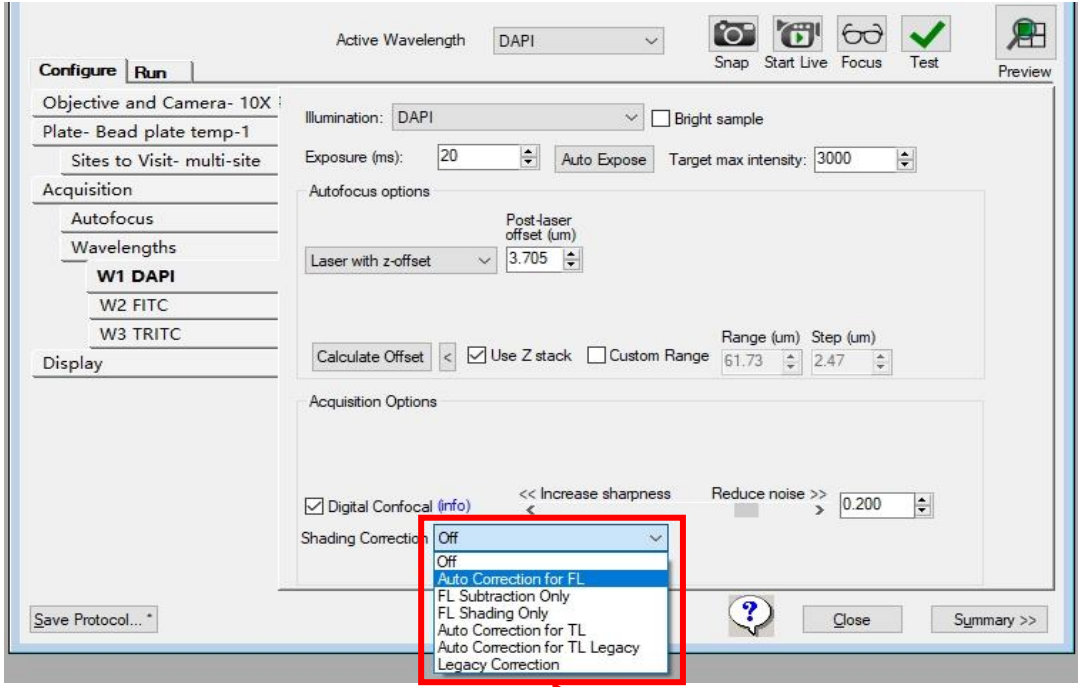
CAUTION! You may photobleach the site if you acquire for too long.

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

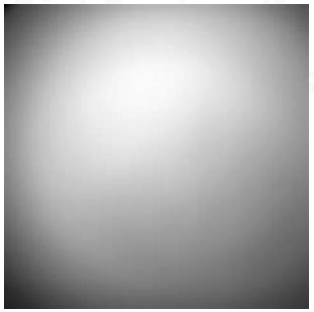
Click **Test** to perform the autofocus routine and then acquire a z series and present the corresponding 2D projection when you set up a z acquisition.

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.

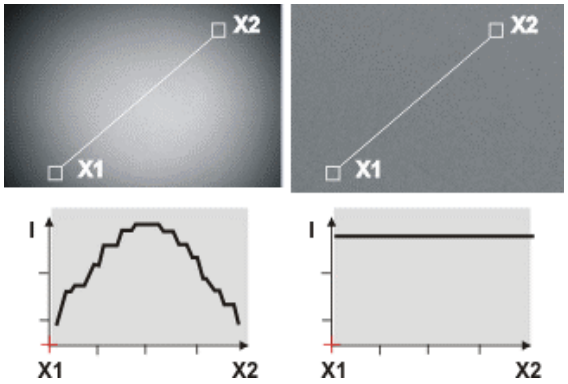
Shading Correction



一定要选择与当前通道对应的shading模式



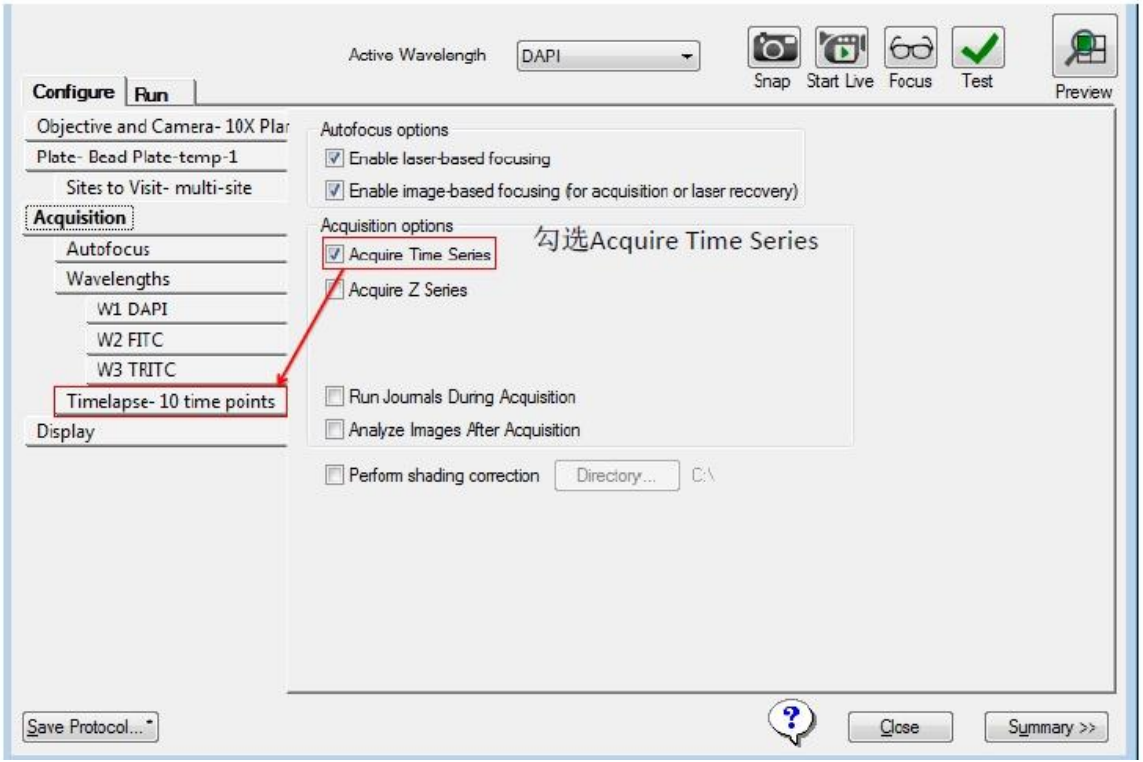
Shading image



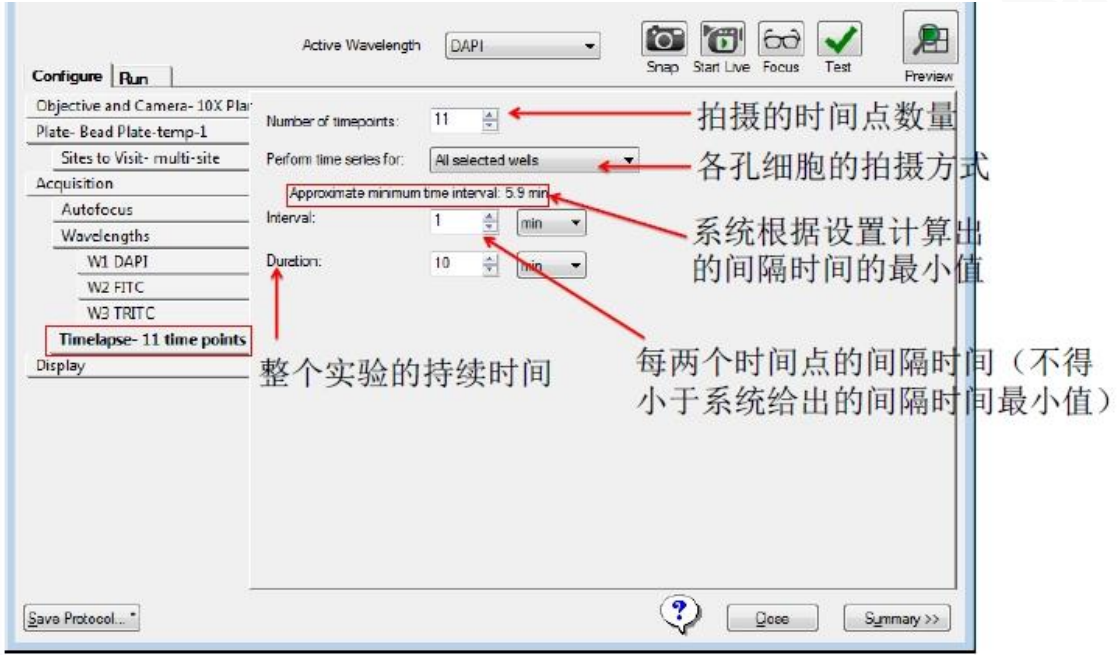
Before correction

After correction

延时成像设置

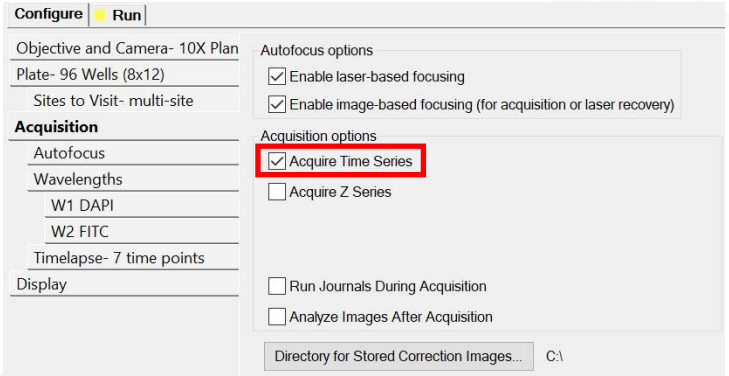


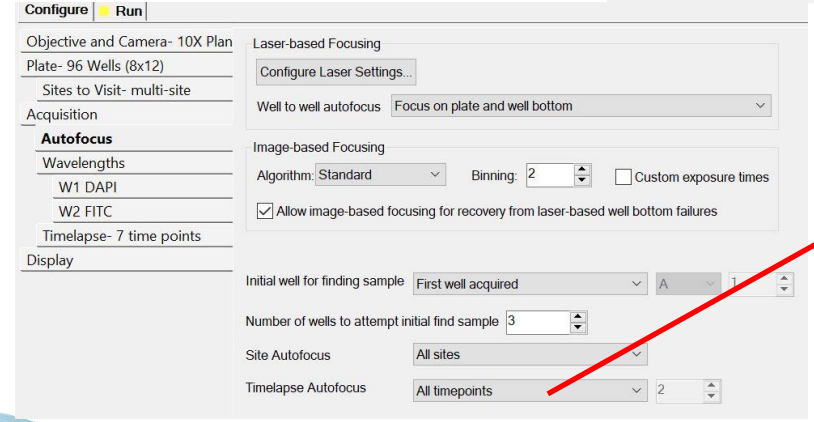
延时成像设置



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



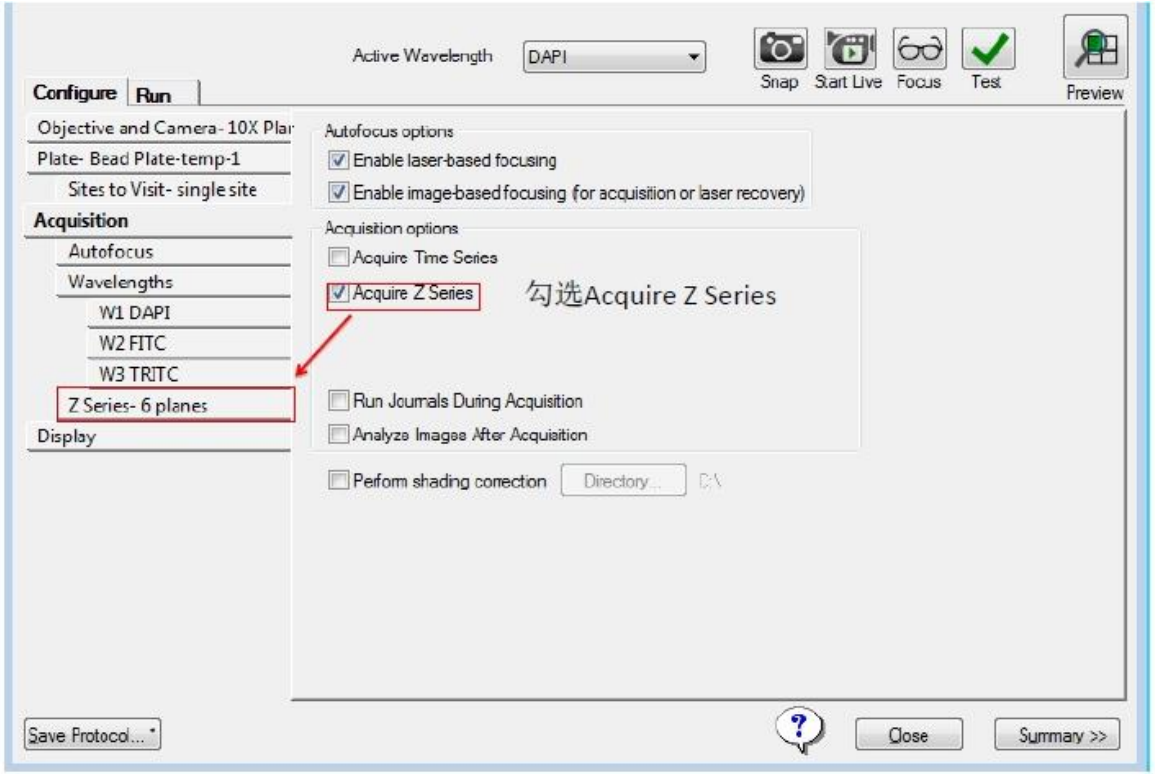


1. First timepoint only：
第一个时间点聚焦；

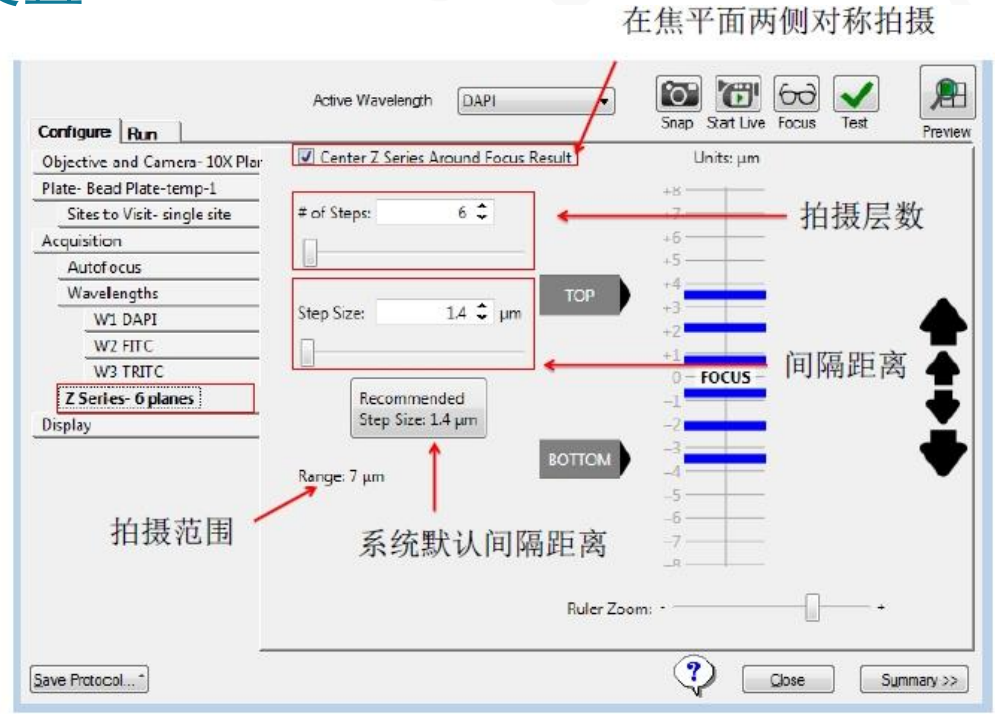
2. All timepoints：
所有时间点均聚焦；

3. Every Nth timepoint：
每隔N个时间点聚焦

多层扫描设置

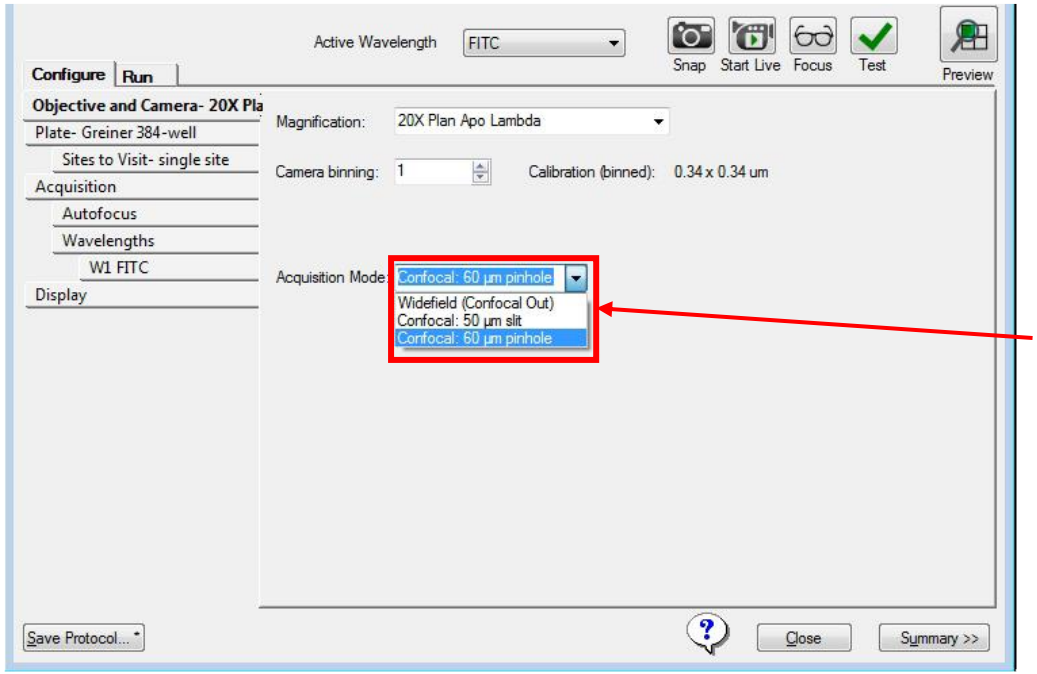


多层扫描设置



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。

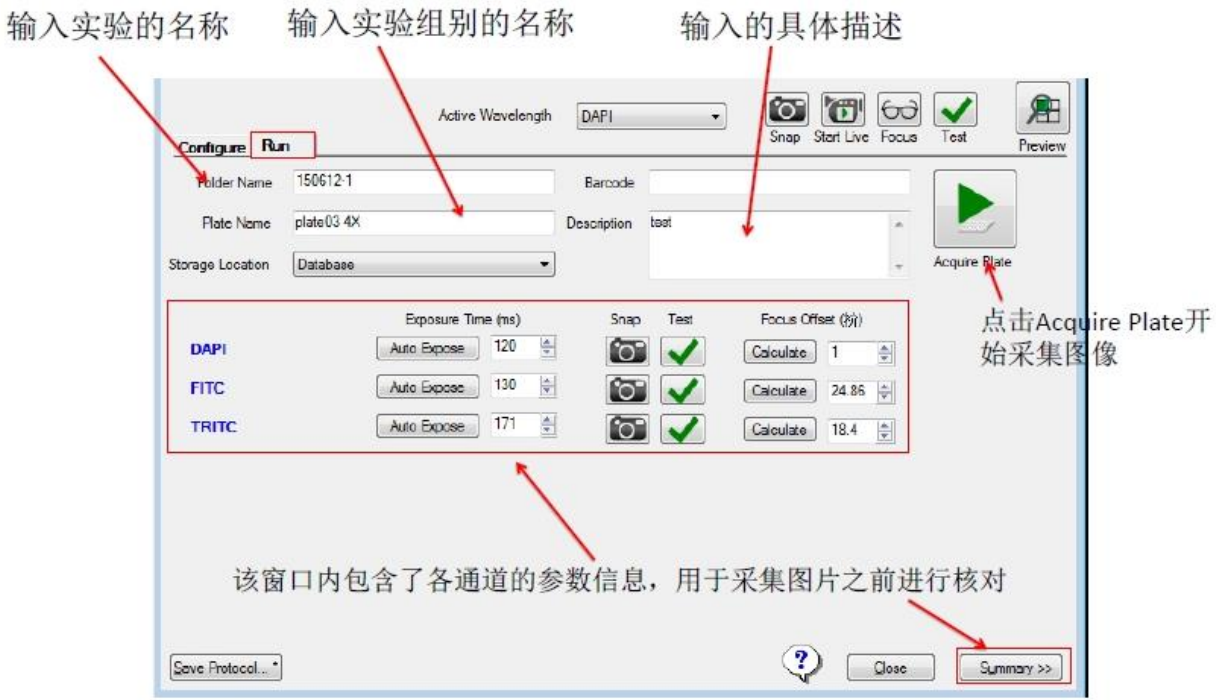
成像模式选择

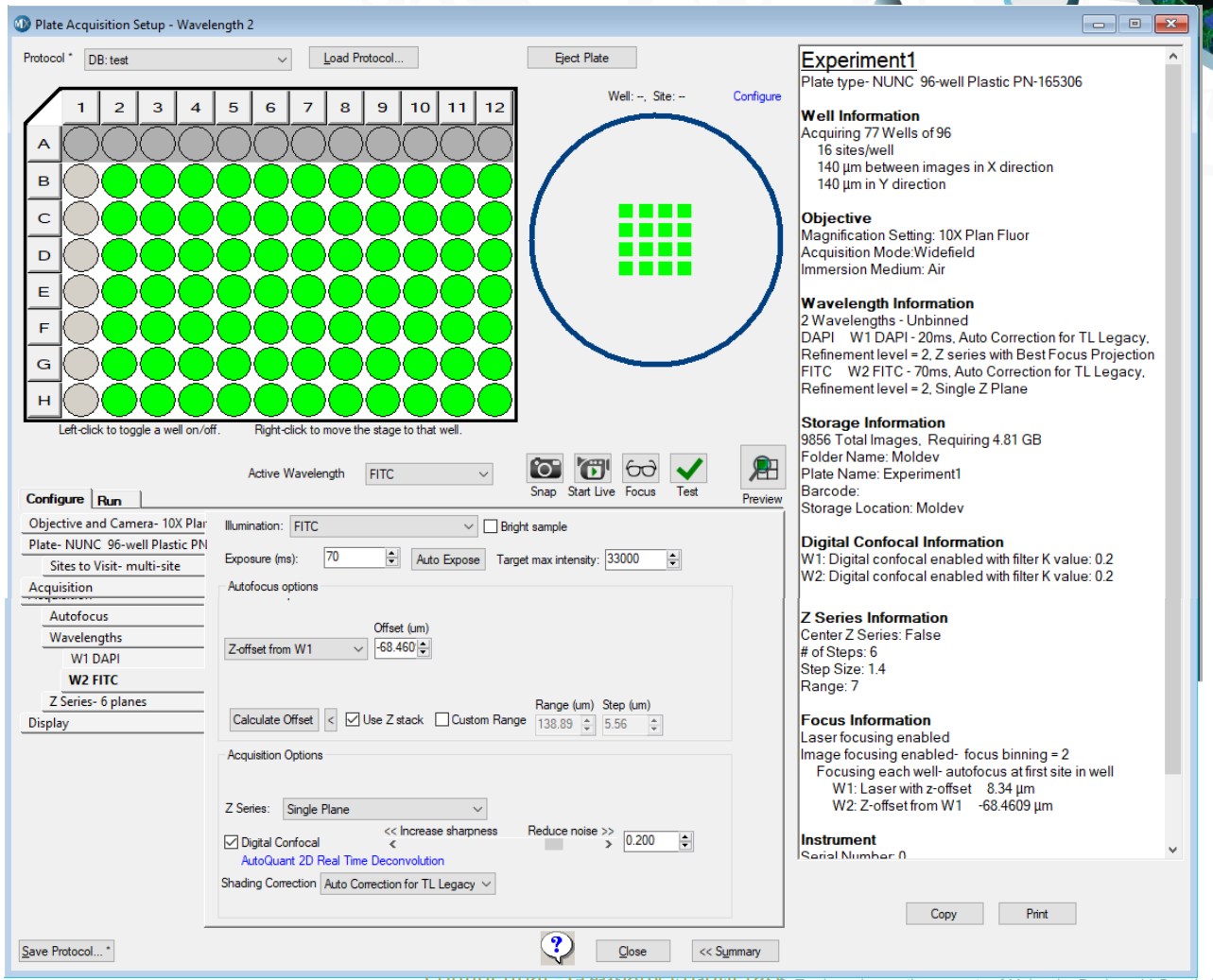


IXM-Confocal

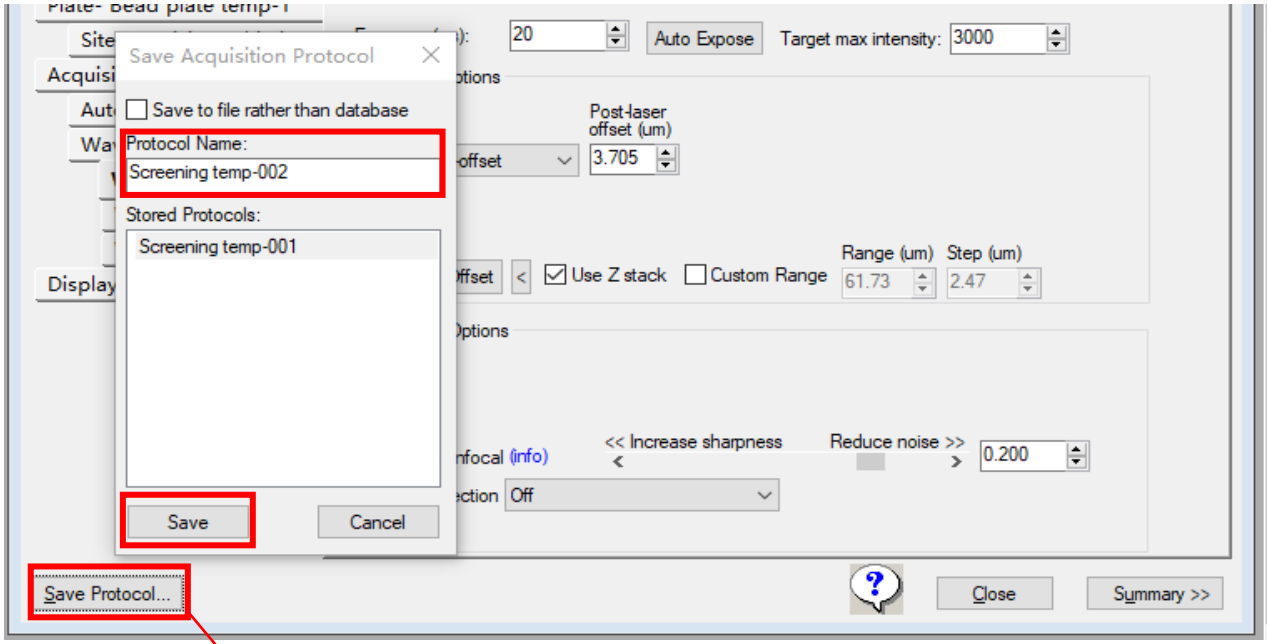
成像模式：拍摄Z-Stack时最好选择confocal模式

实验名称和描述

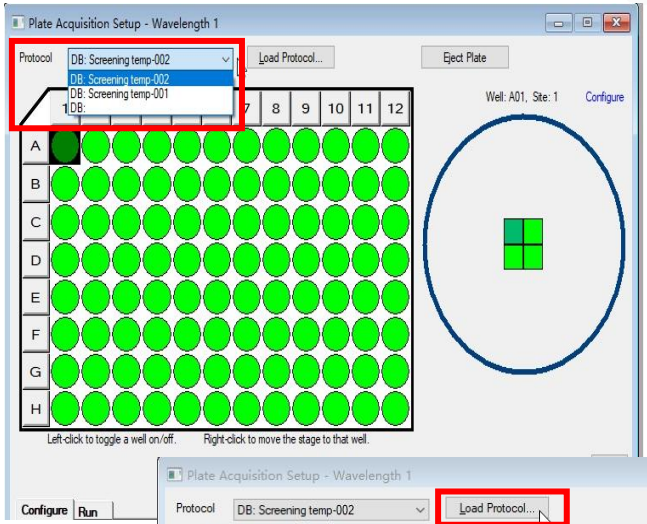


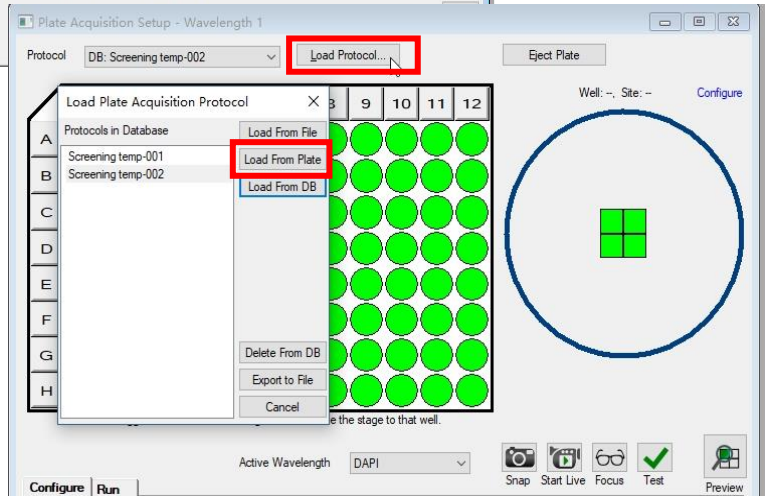


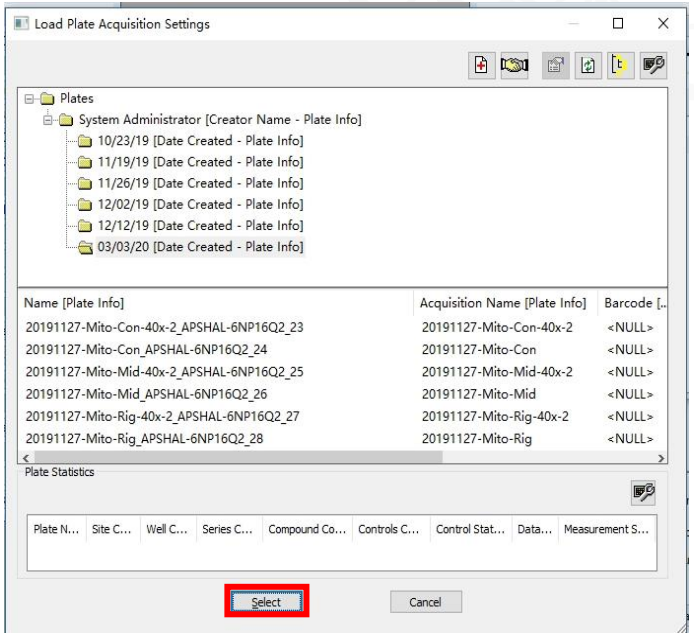
Protocol 保存



点击Save Protocol可保存当前
实验设置，以便下一次直接应用

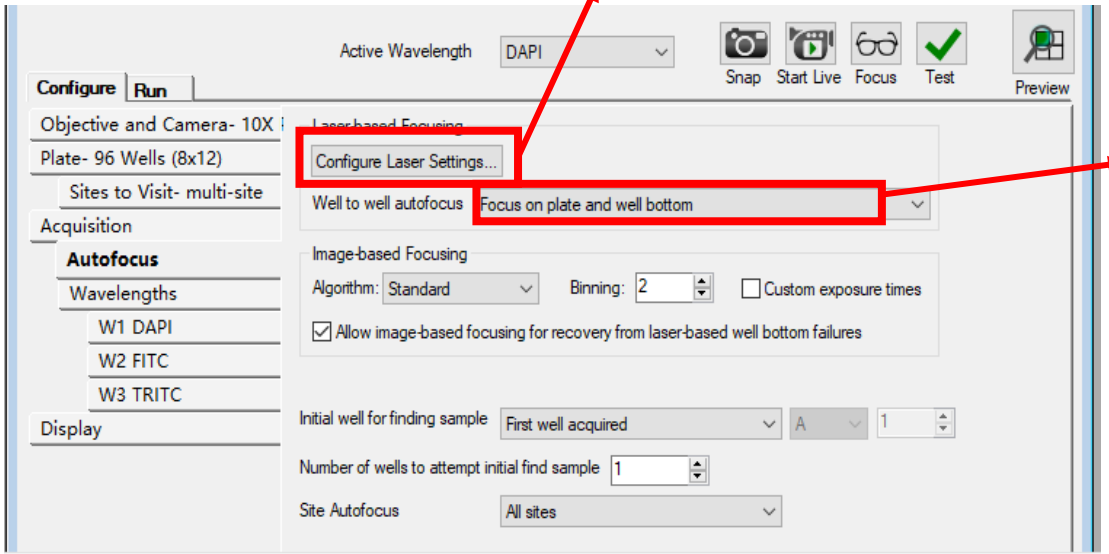






激光自动聚焦选项

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：

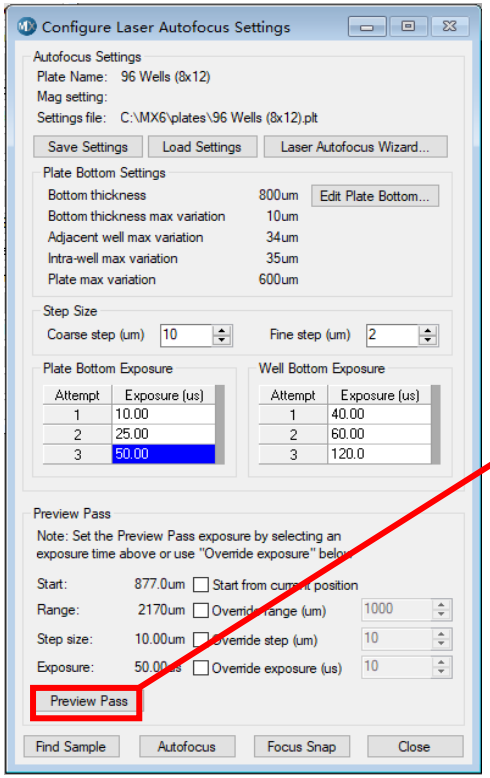
（1）Focus on well bottom

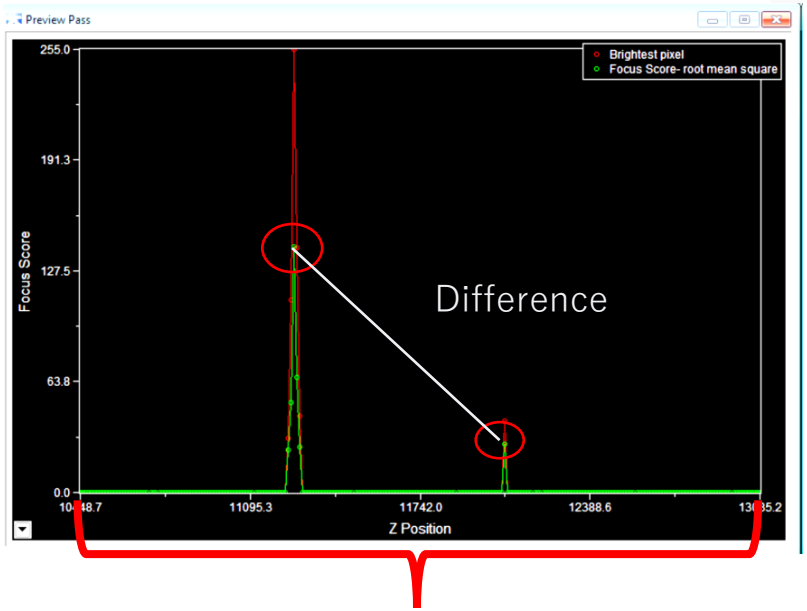
（2）Focus on plate bottom, then offset by bottom thickness

（3）Focus on plate and well bottom

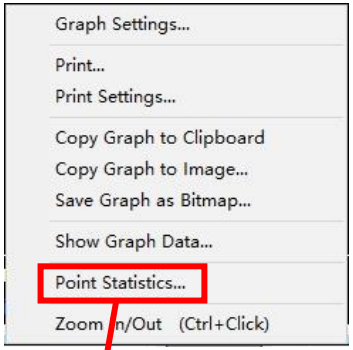
玻片扫描时不可选第（3）种

确认孔板厚度及激光自动对焦的曝光时间



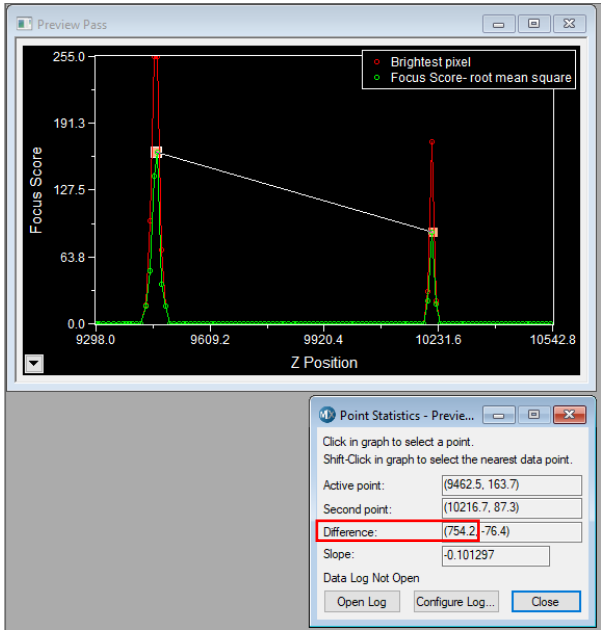


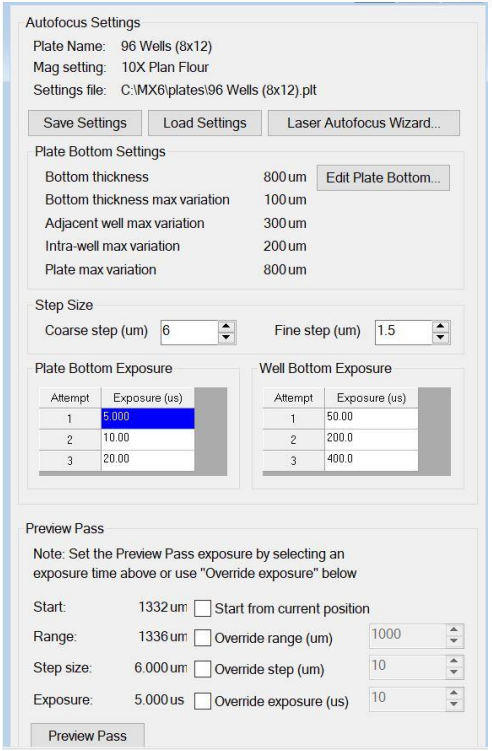
自动找焦范围



点击后拉取一条测量线，测量两个峰的间距即difference

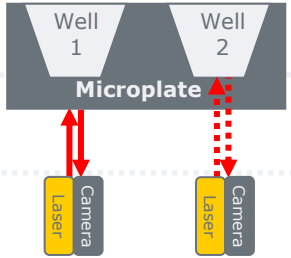
激光自动聚焦设置

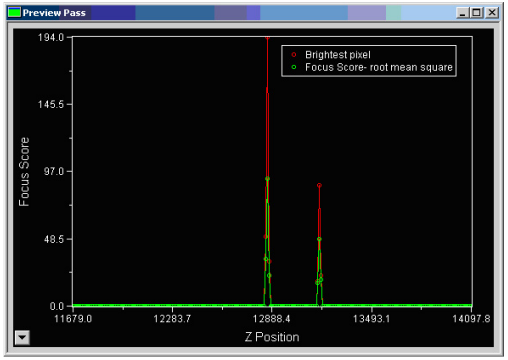




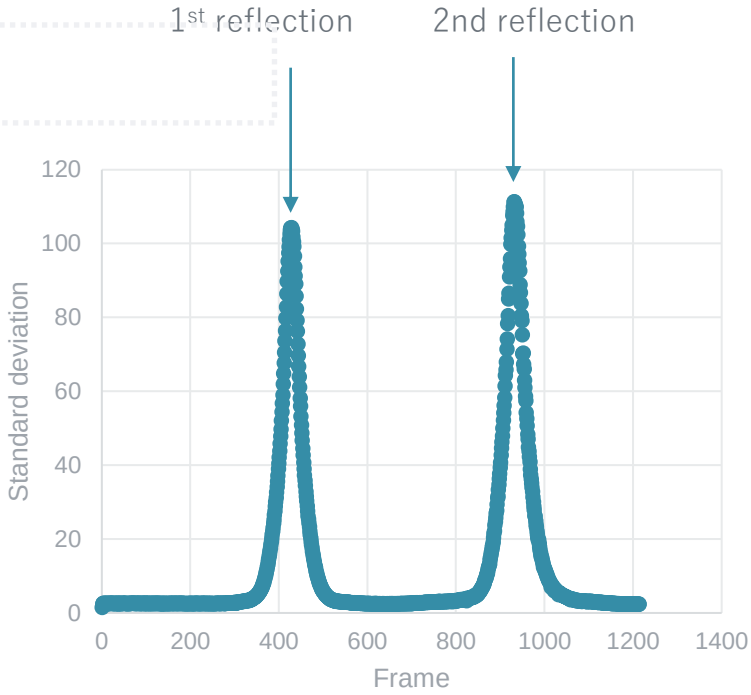
激光自动聚焦原理

激光自动聚焦



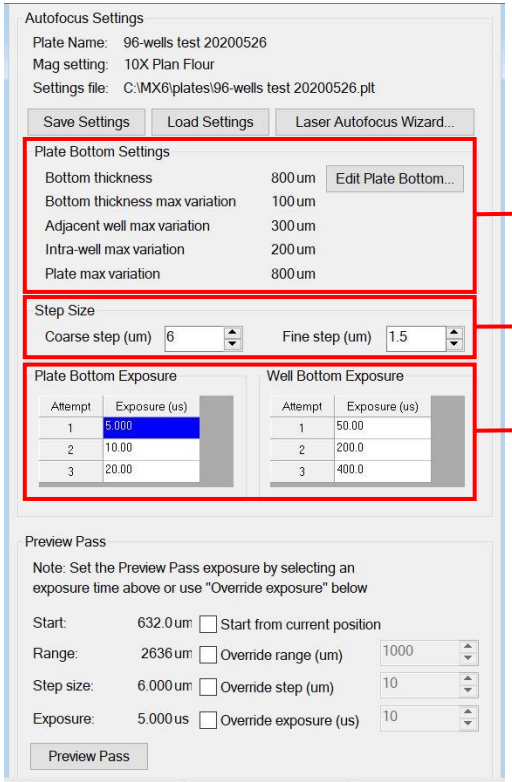


Typical autofocus reflection“Spot”



激光照射耗材底部产生的反射光

激光自动聚焦设置

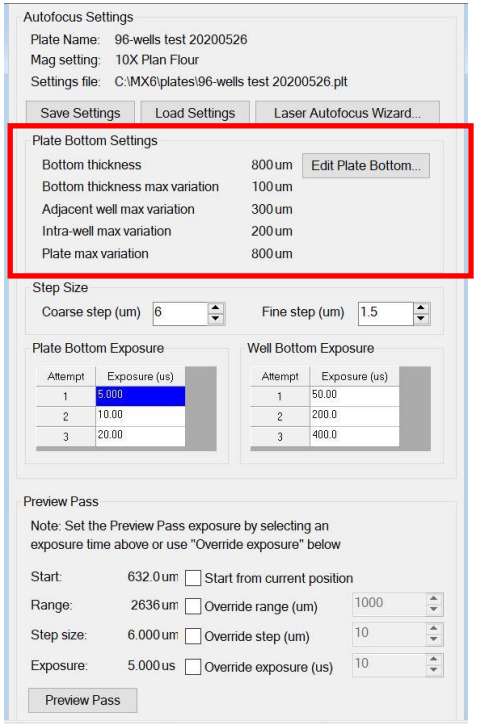


板底厚度设置

步进设置

曝光时间设置

激光自动聚焦设置



Bottom thickness:
板底厚度

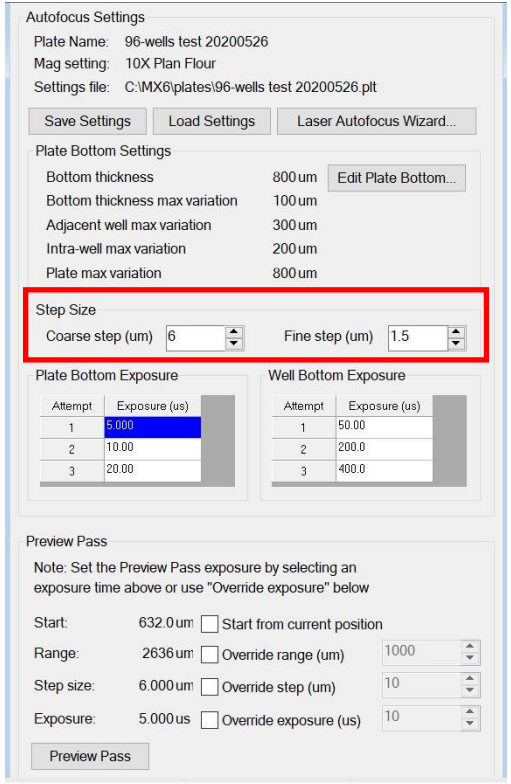
Bottom thickness max variation:
整个孔板底部厚度的最大变化量

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

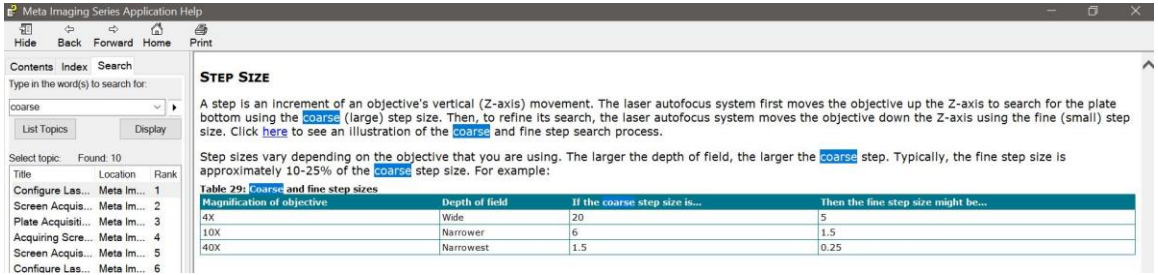
Plate max variation:
两个相距最远的孔之间板底z轴位置的最大差异

激光自动聚焦设置



Coarse step:
粗调步进

Fine step:
细调步进



激光自动聚焦设置

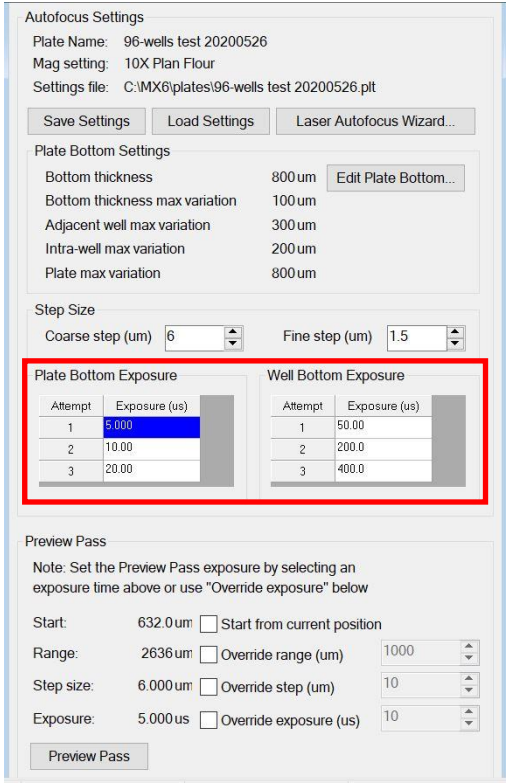
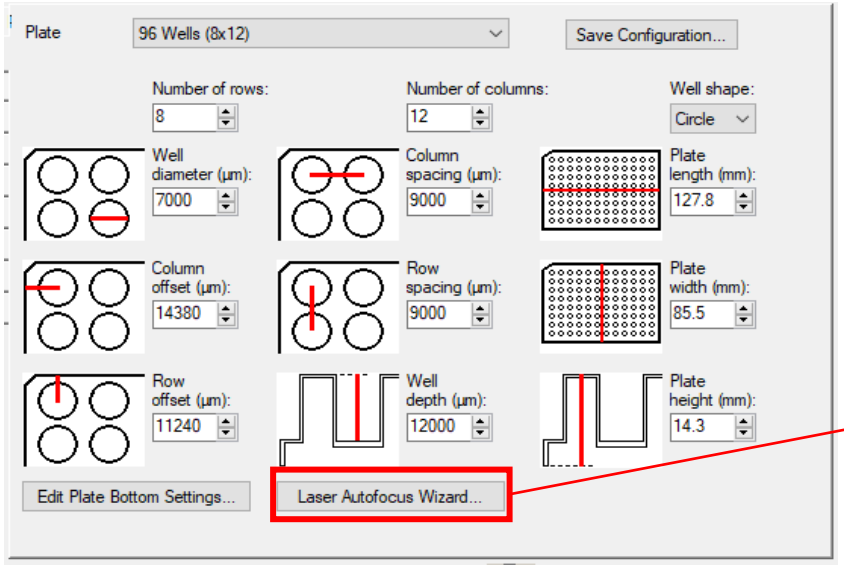


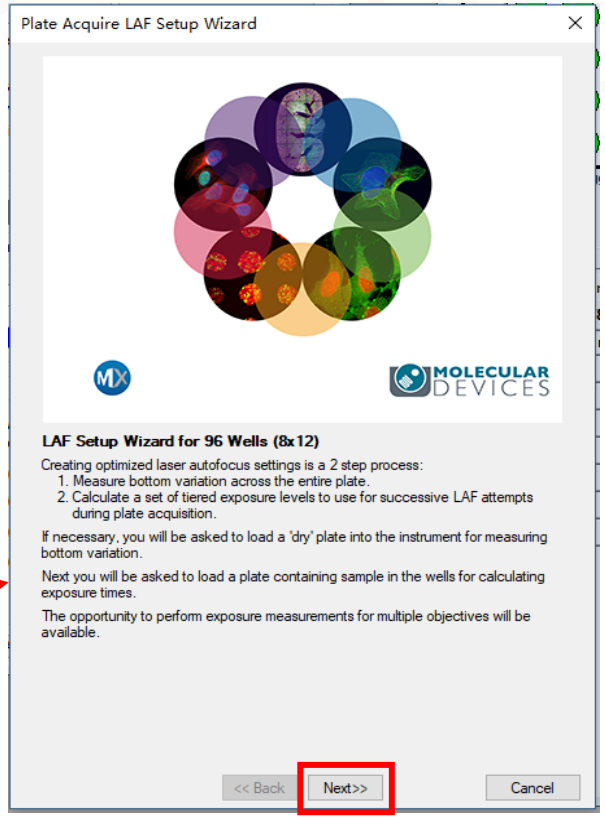
Plate Bottom Exposure:
板底曝光时间

Well Bottom Exposure:
孔底曝光时间

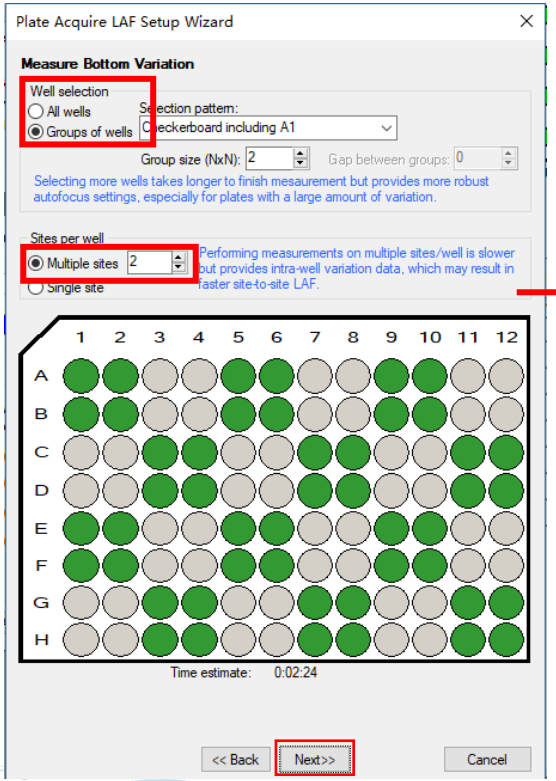
1. 孔底曝光时间高于板底曝光时间
2. 高倍物镜曝光时间高于低倍物镜曝光时间

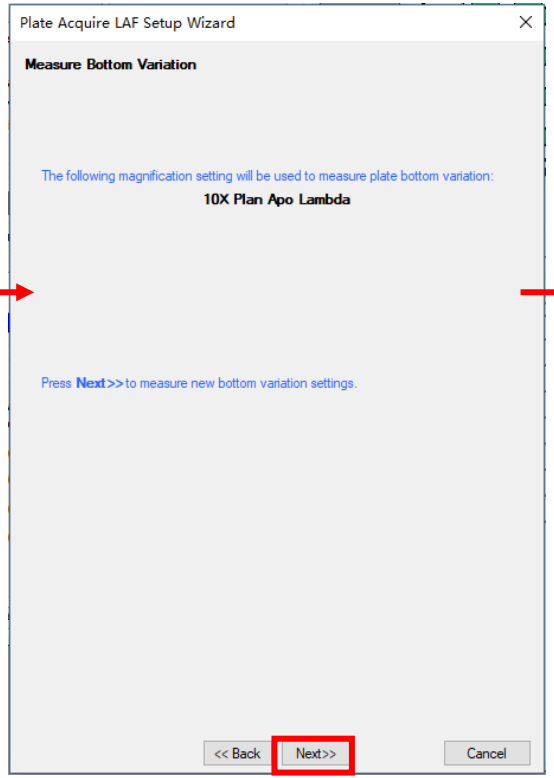
Laser Autofocus Wizard

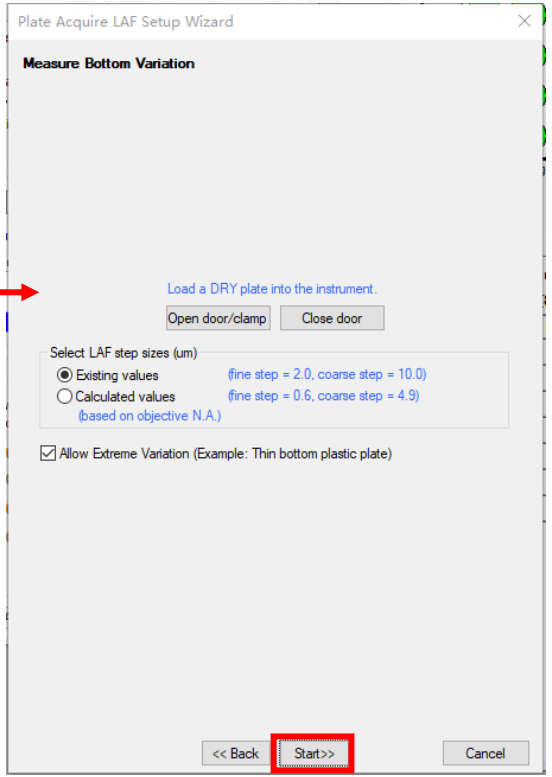




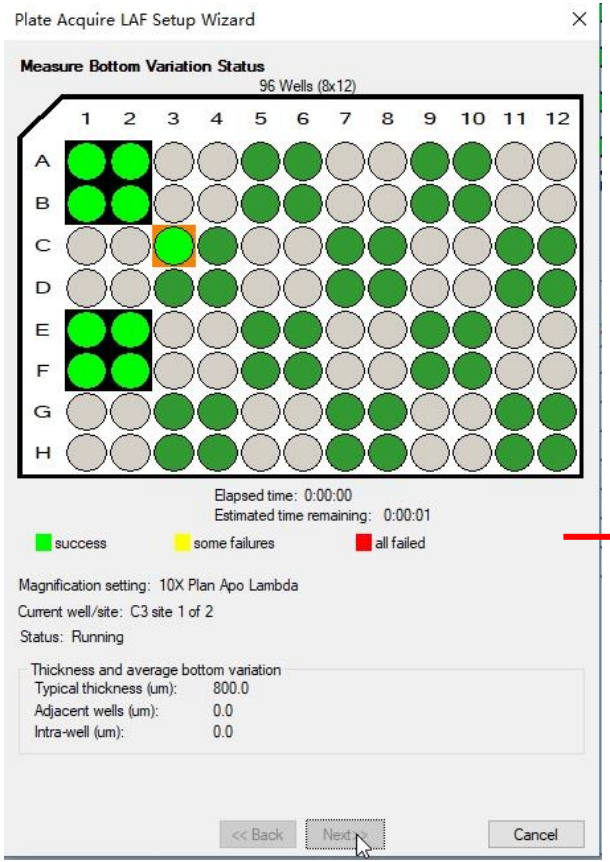
Laser Autofocus Wizard

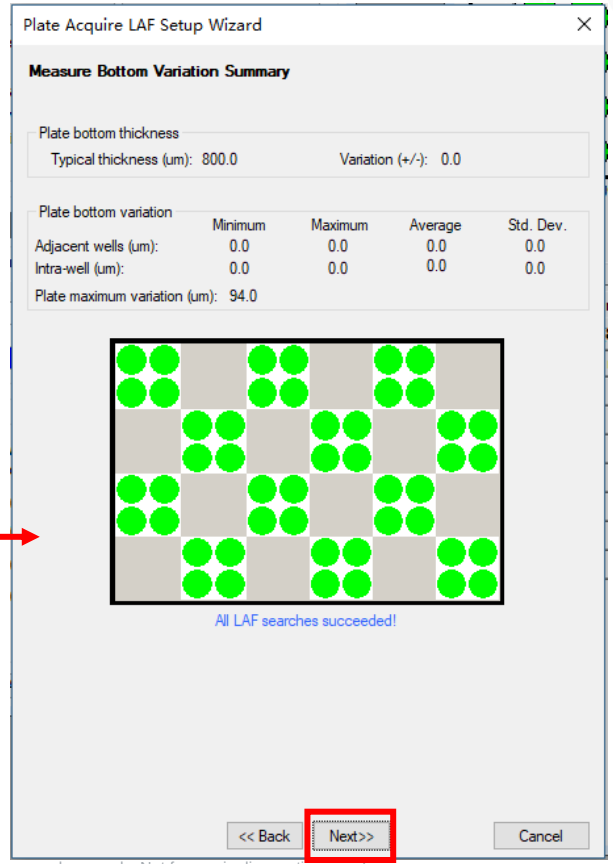




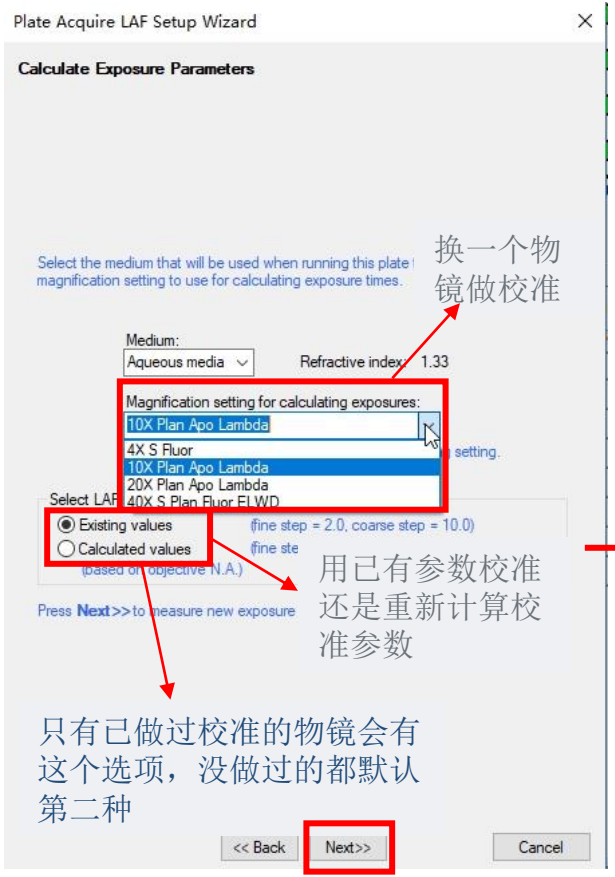


Laser Autofocus Wizard





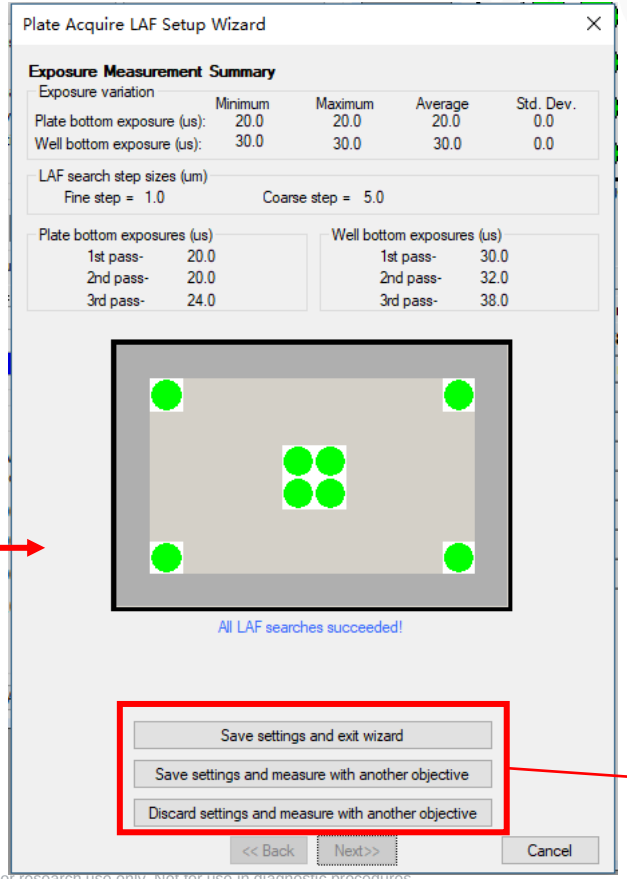
Laser Autofocus Wizard



换一个物镜做校准

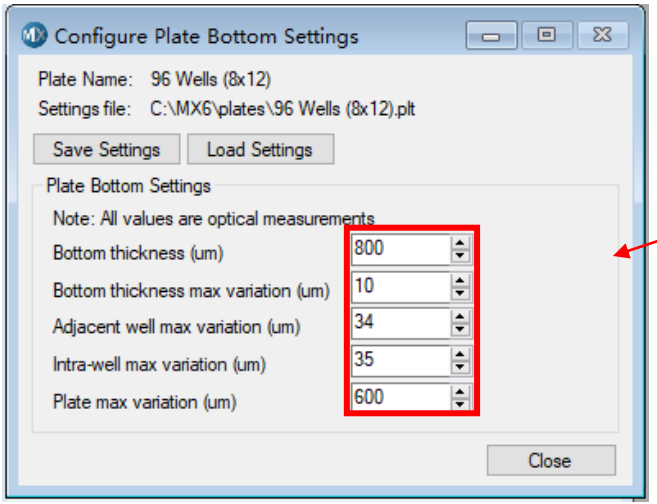
用已有参数校准还是重新计算校准参数

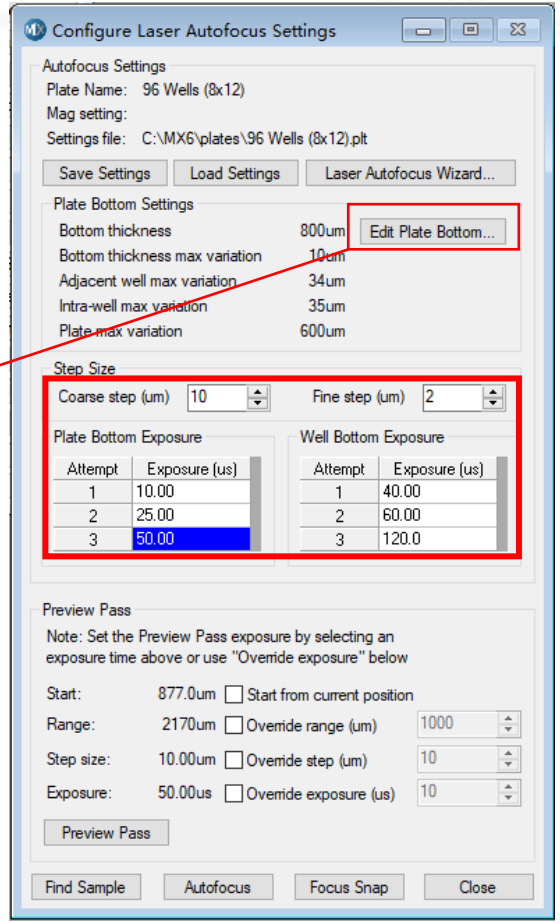
只有已做过校准的物镜会有这个选项，没做过的都默认第二种



保存退出或进行下一个物镜校准

Laser Autofocus Wizard





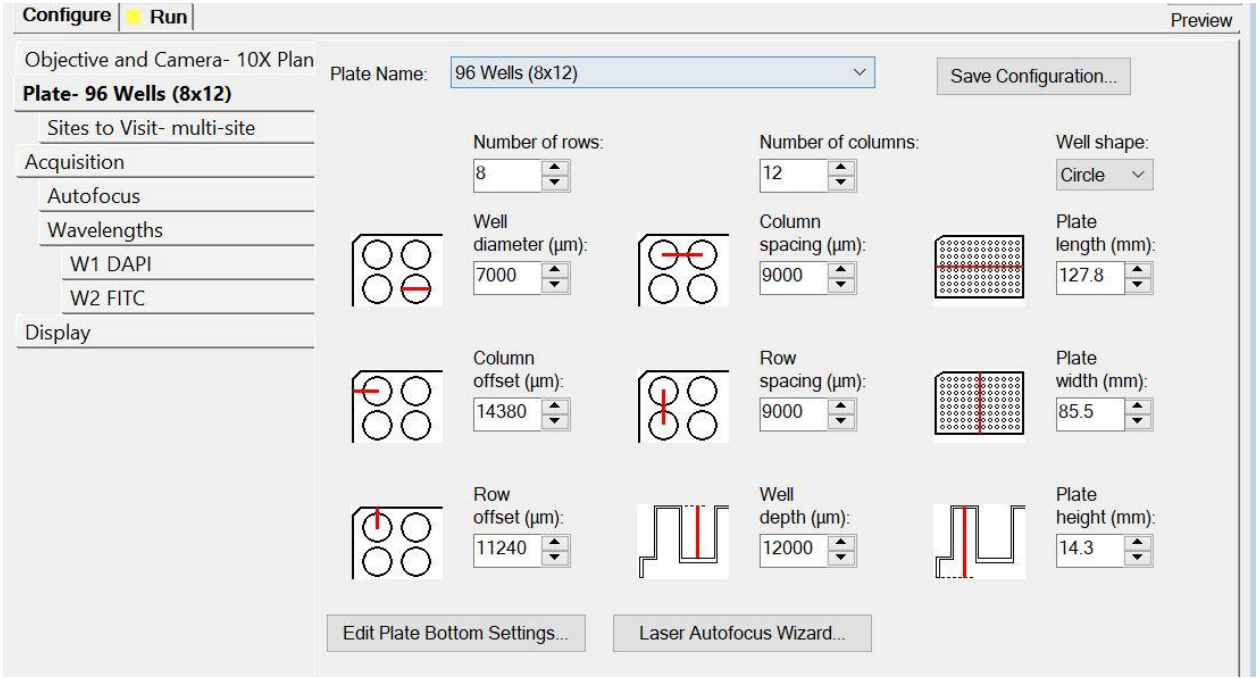
做完 laser autofocus wizard 之后，框内的数字会自动填入

不用Laser Autofocus Wizard，

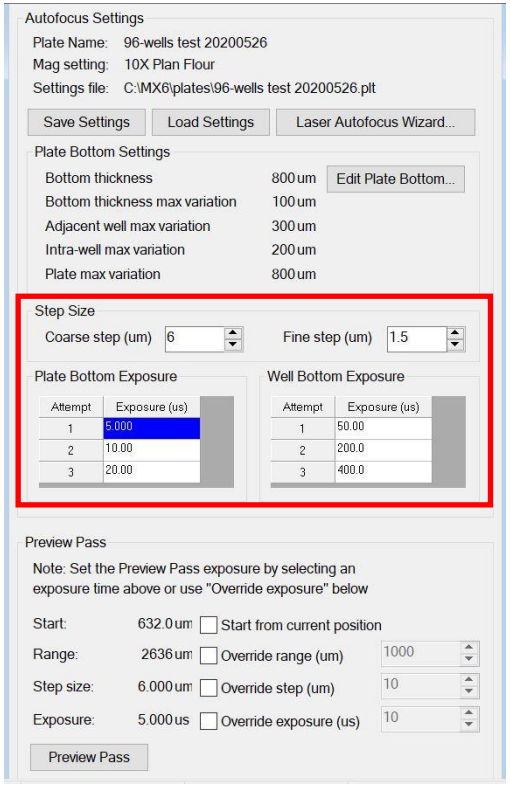
如何设置激光自动聚焦？

激光自动聚焦设置流程-1

新孔板规格参数设置

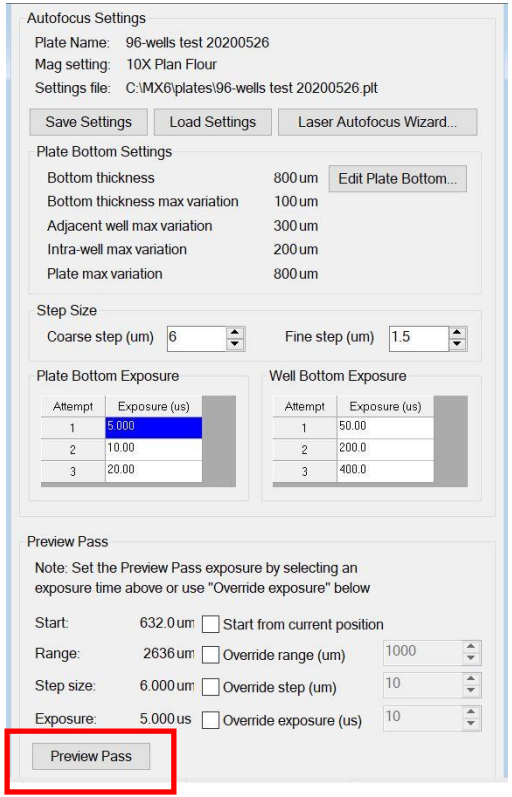


激光自动聚焦设置流程-2

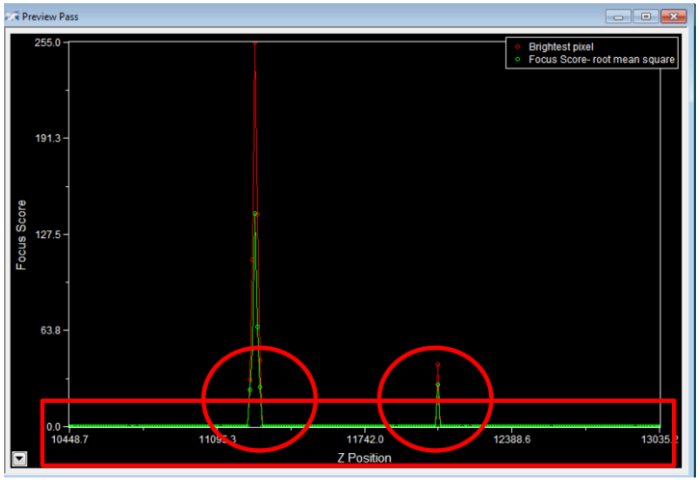


分别设置“Step Size ”和“Bottom Exposure”

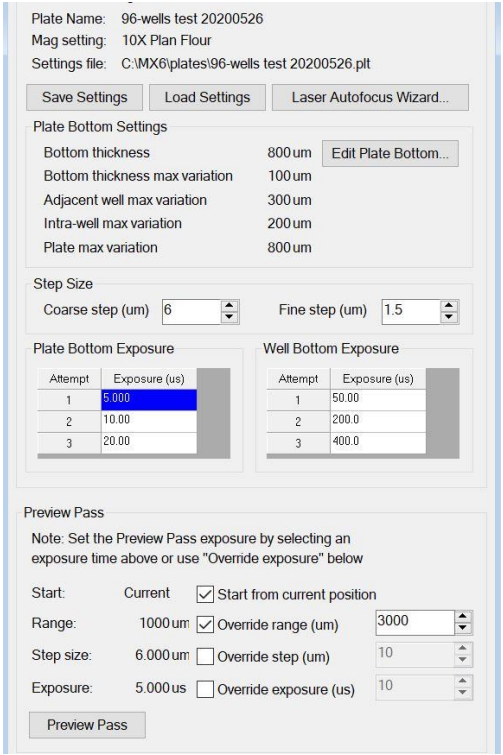
激光自动聚焦设置流程-3



“Preview Pass”检查双反射峰

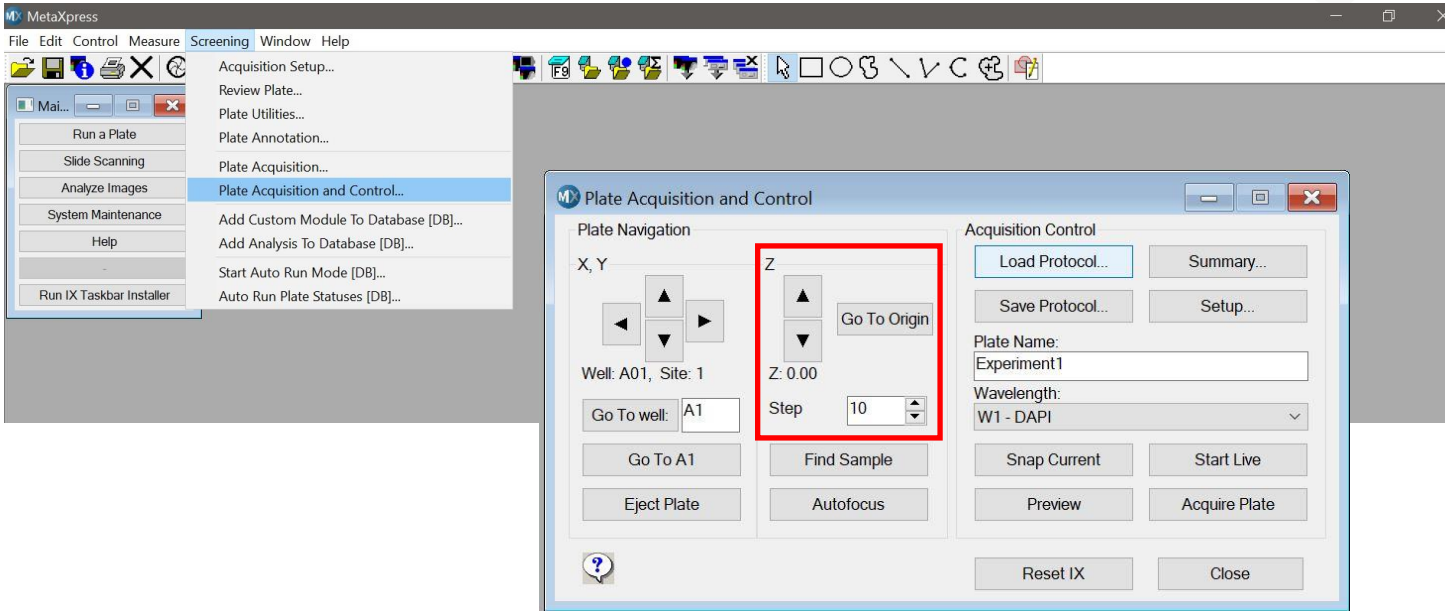


激光自动聚焦设置流程-4



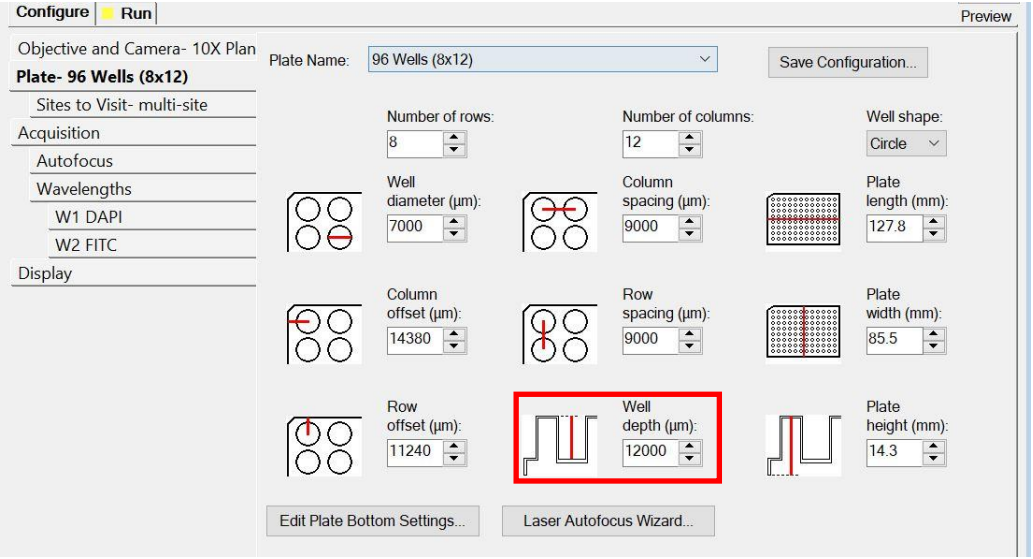
- 如果无法观察到双反射峰，先勾选“Start Current”和“Range”，并设置“Override range”
- 手动调焦直到找到双反射峰(未完待续)

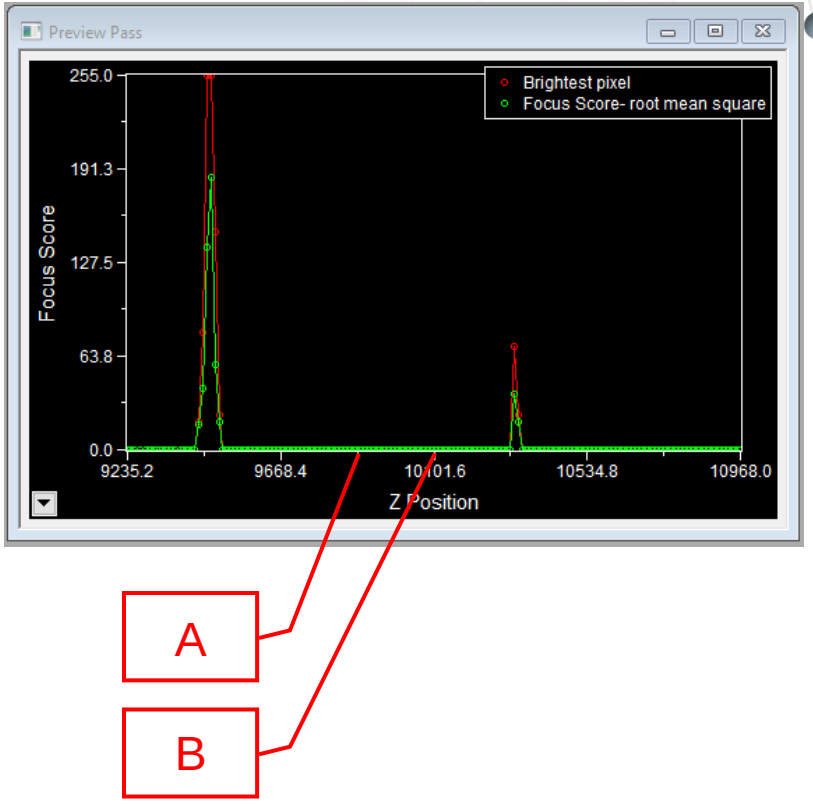
手动调焦方法

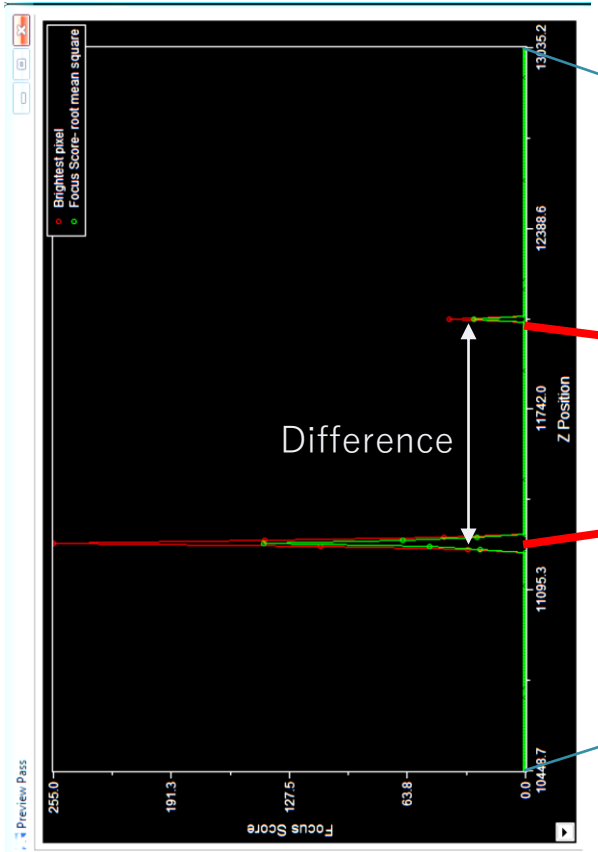


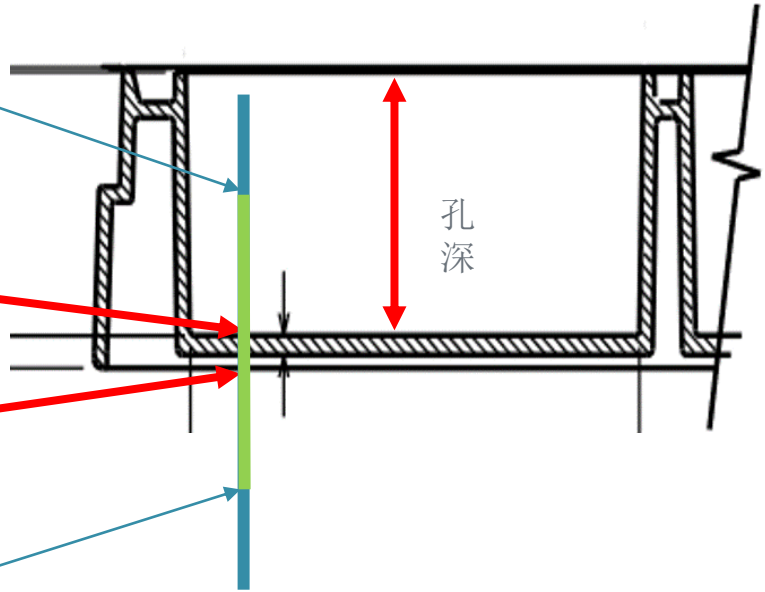
激光自动聚焦设置流程-5

- 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)

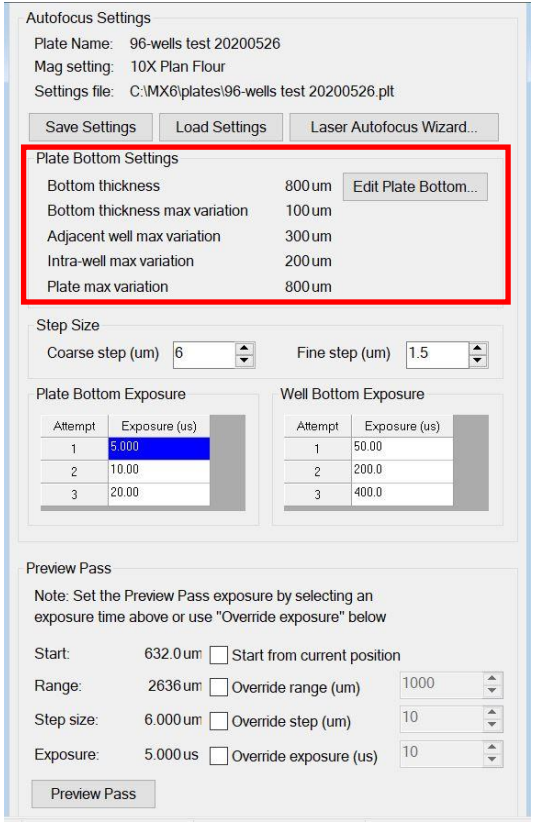




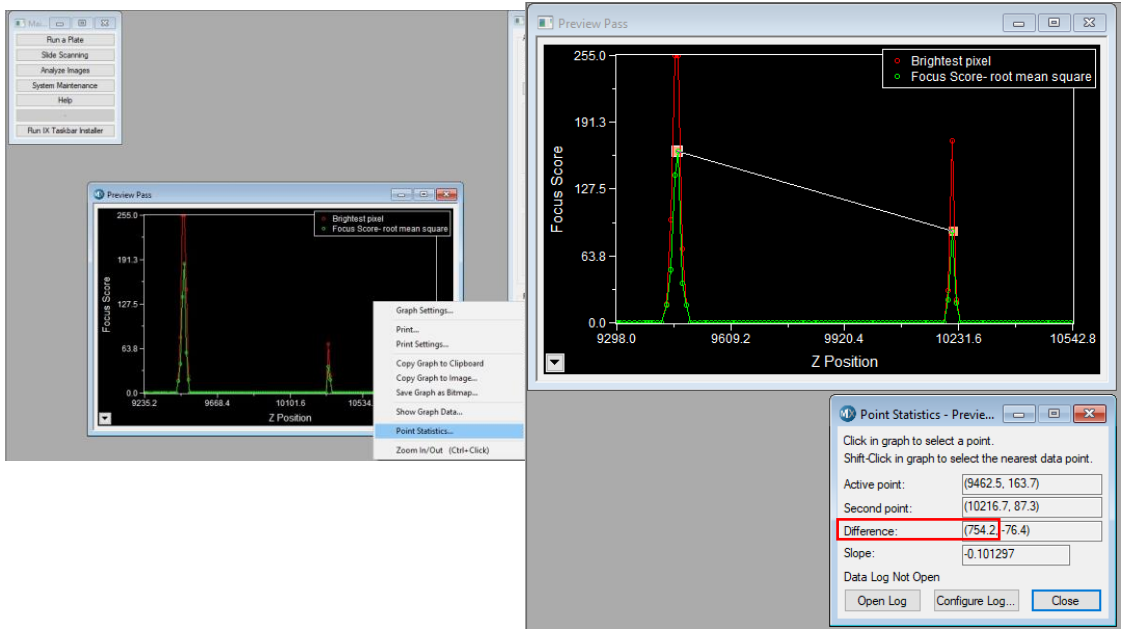




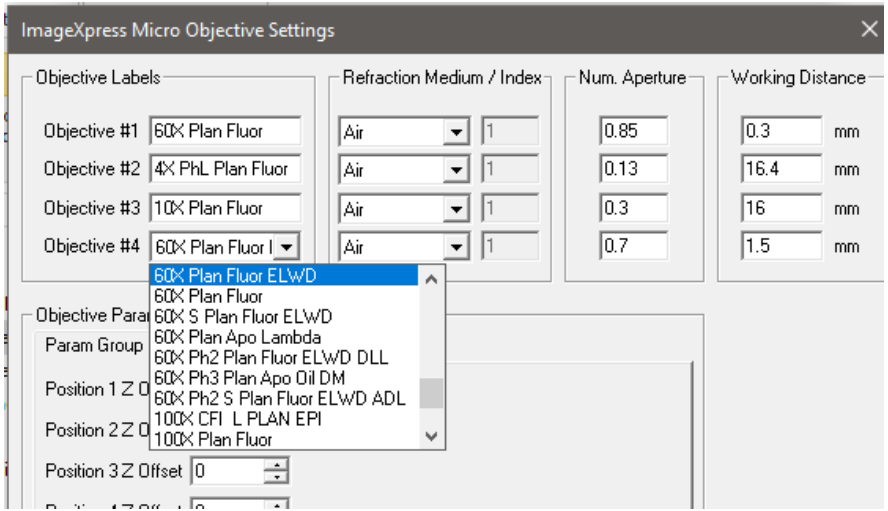
激光自动聚焦设置流程-6



- 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



孔板与物镜选择



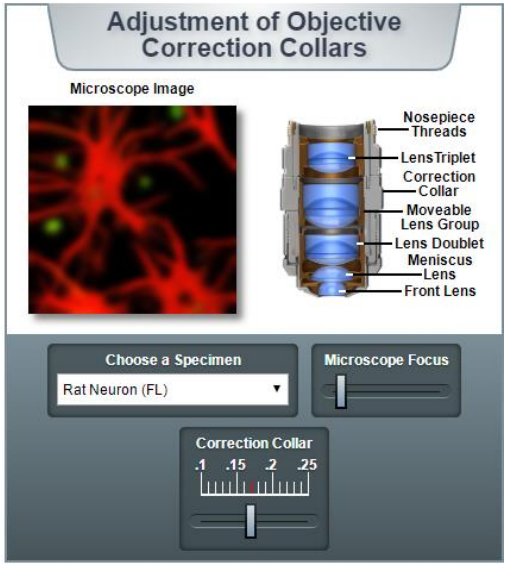
- 板底的厚度应与所要使用的物镜的工作距离相匹配。
- 一般来说，数值孔径(NA)较大的物镜往往需要薄底板。
- 超长工作距离(ELWD)目标兼容较大的板厚范围，但往往有较小的NA。
- 板的底部厚度相当于一个标准的盖玻片(0.17毫米)与大多数物镜都可以匹配。

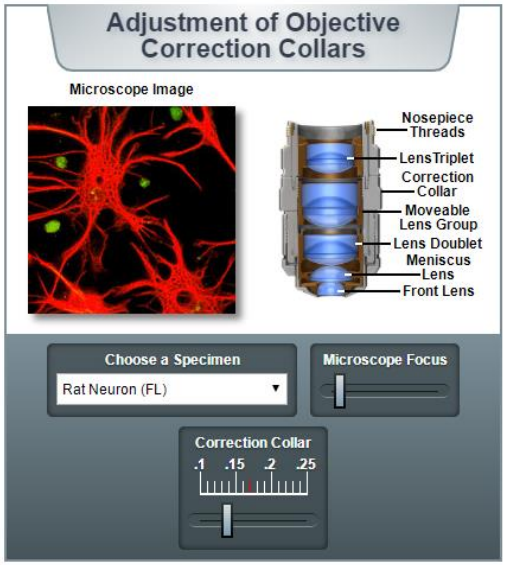
Correction Collar

- ✓ 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)

调节矫正环系数值

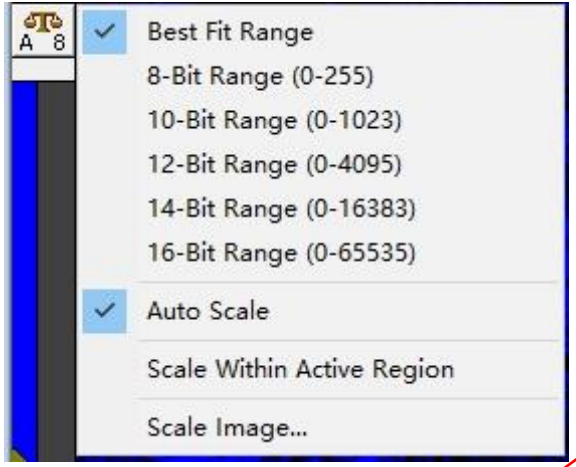
塑料：Difference x 1.59
玻璃：Difference x 1.52
170um厚度的玻璃底也可直接调到0.17

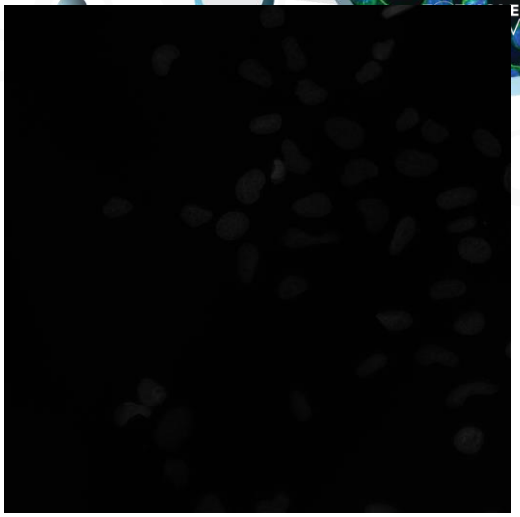


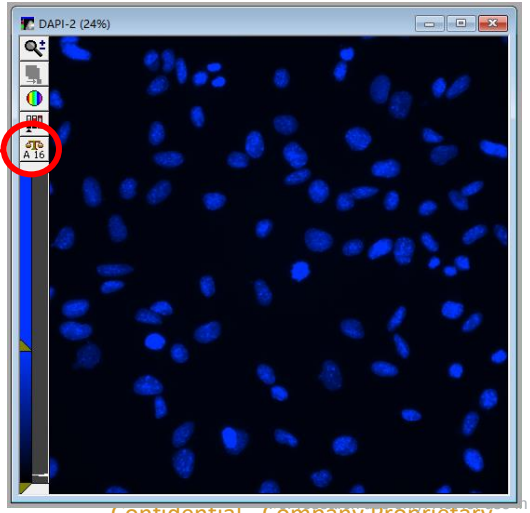


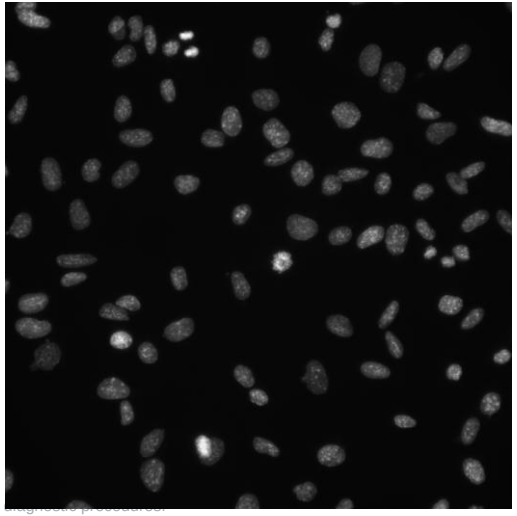
主要内容

- 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





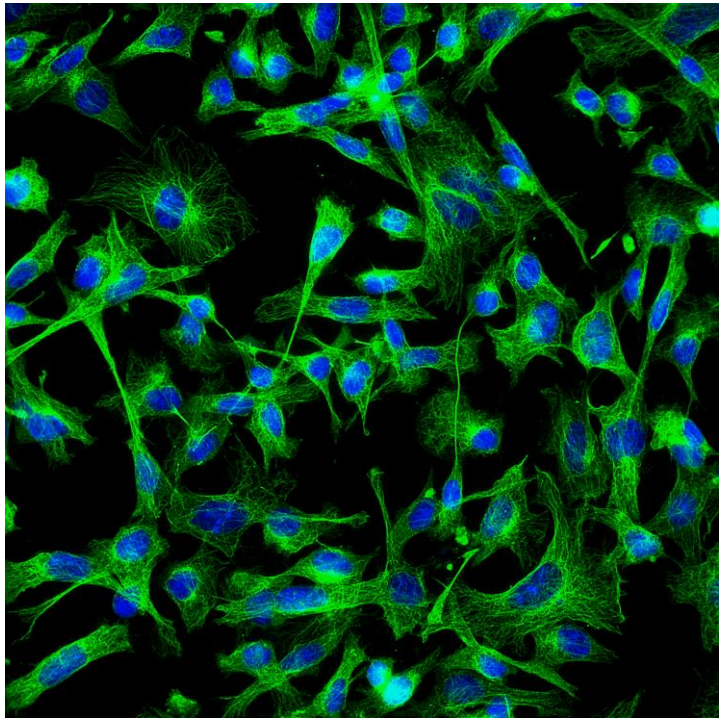


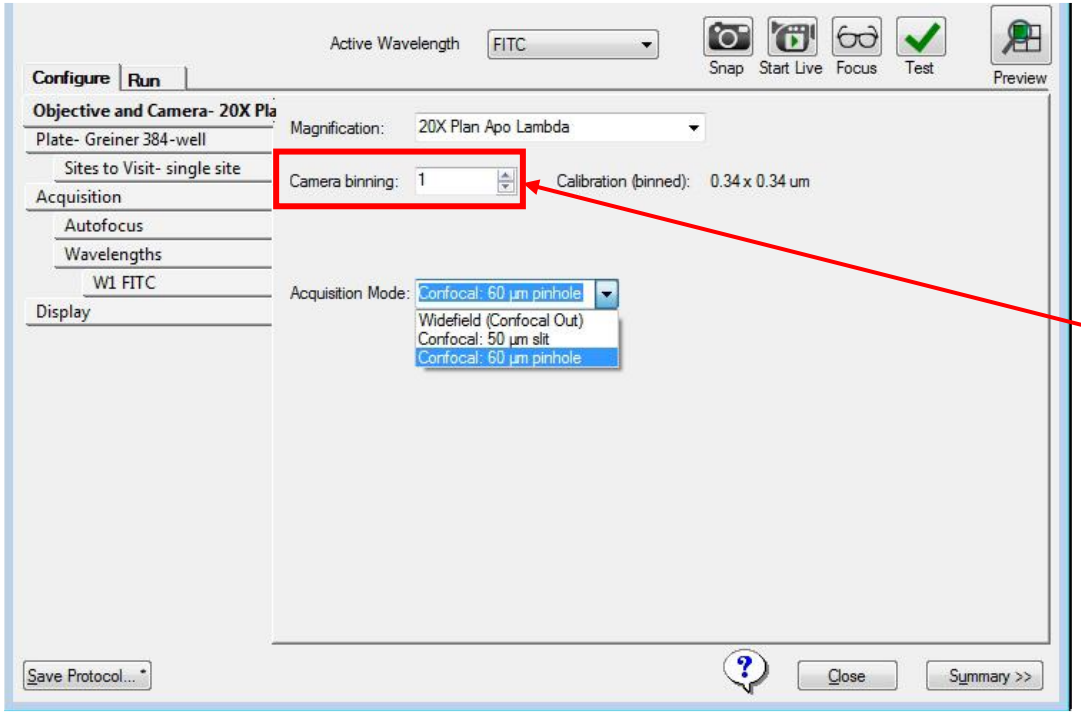


Bit depth：**16 bit**

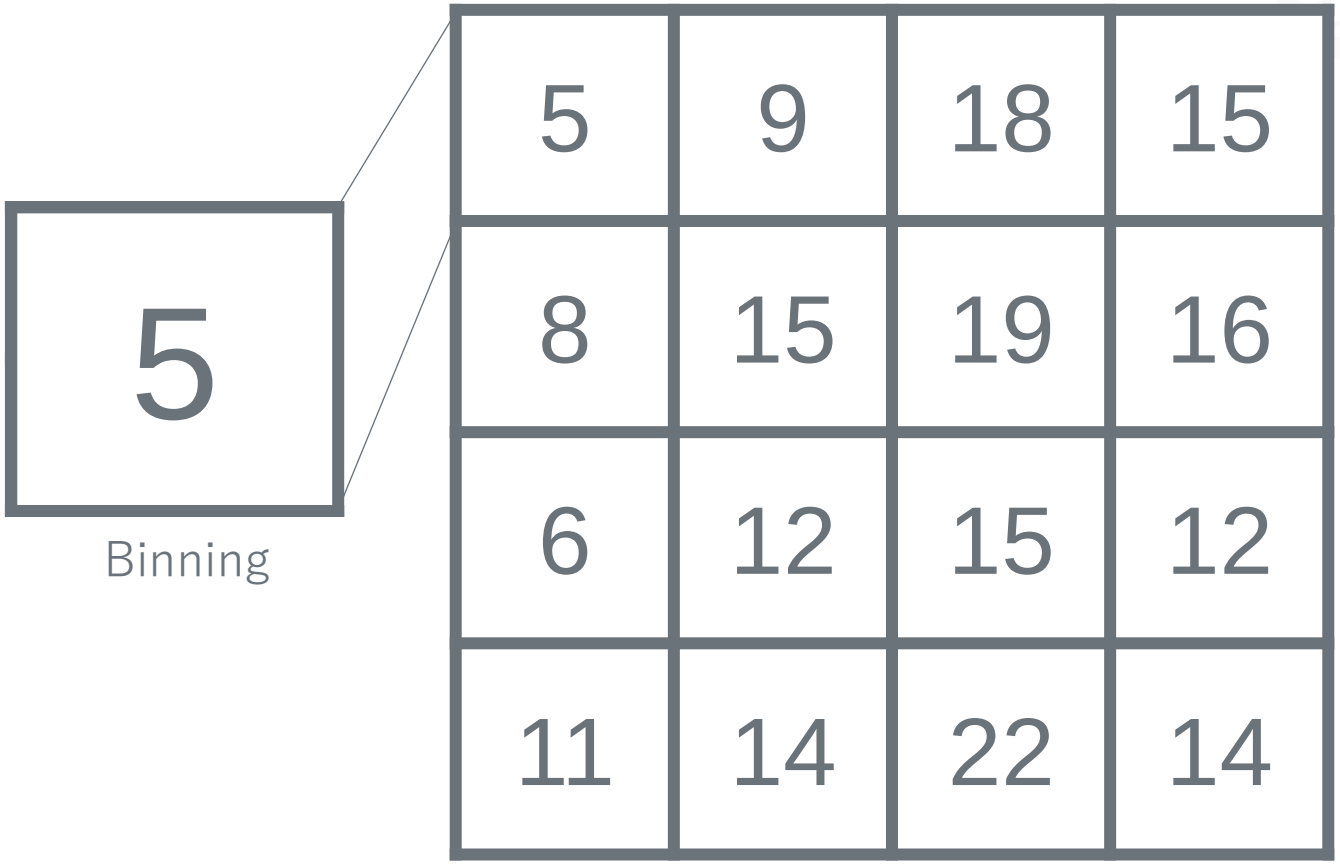
Resolution：**2048 x 2048**

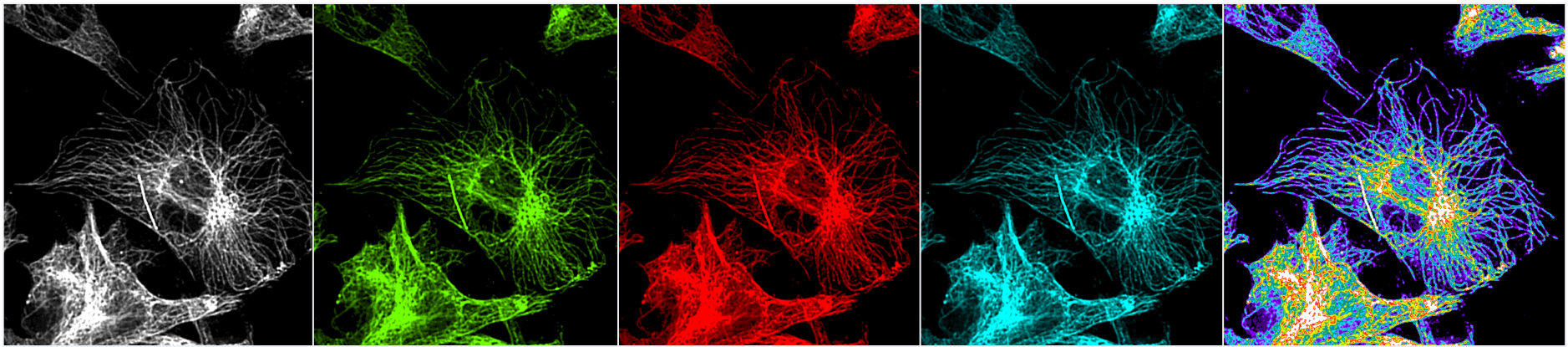
sCMOS

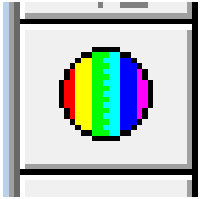


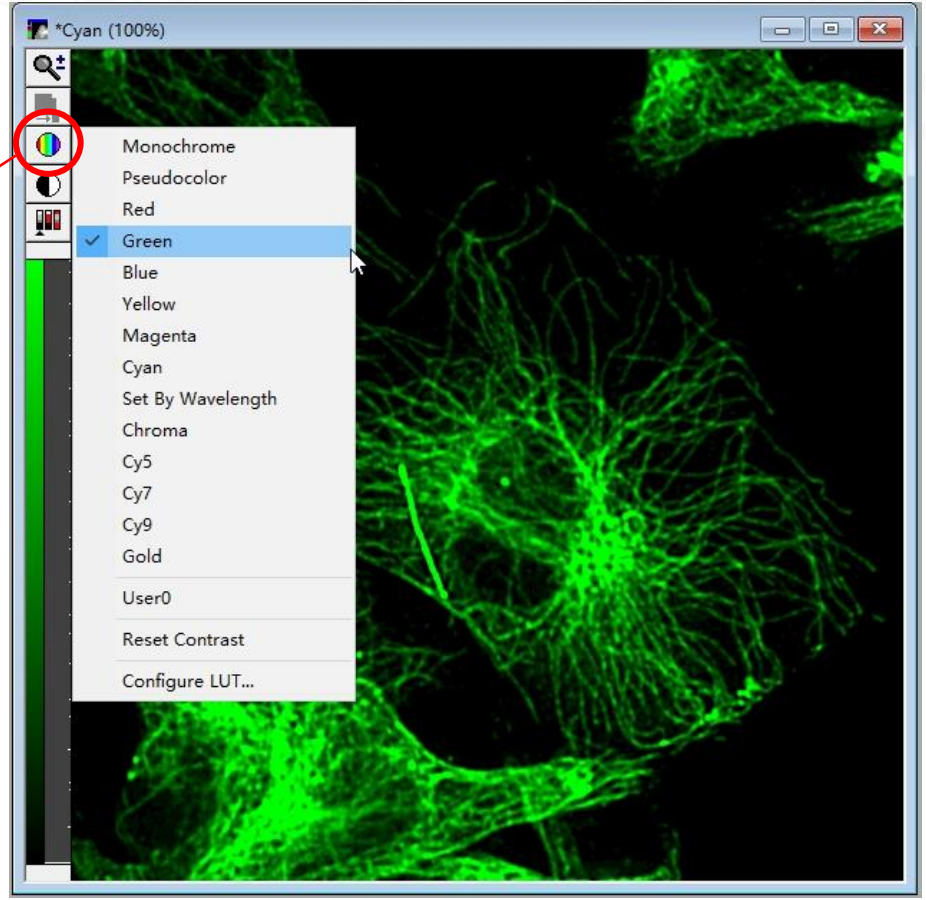


选择图像的大小，数值越大，图像越小，一般选择2的时候效率最高



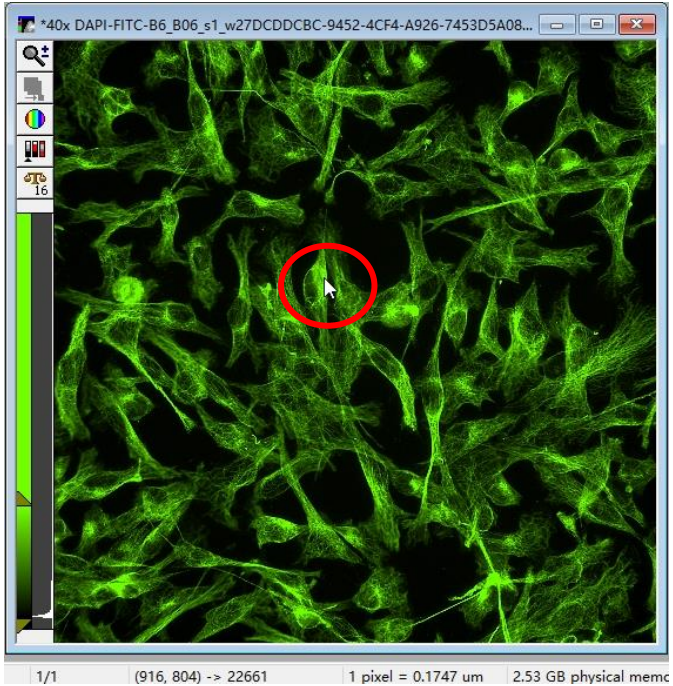






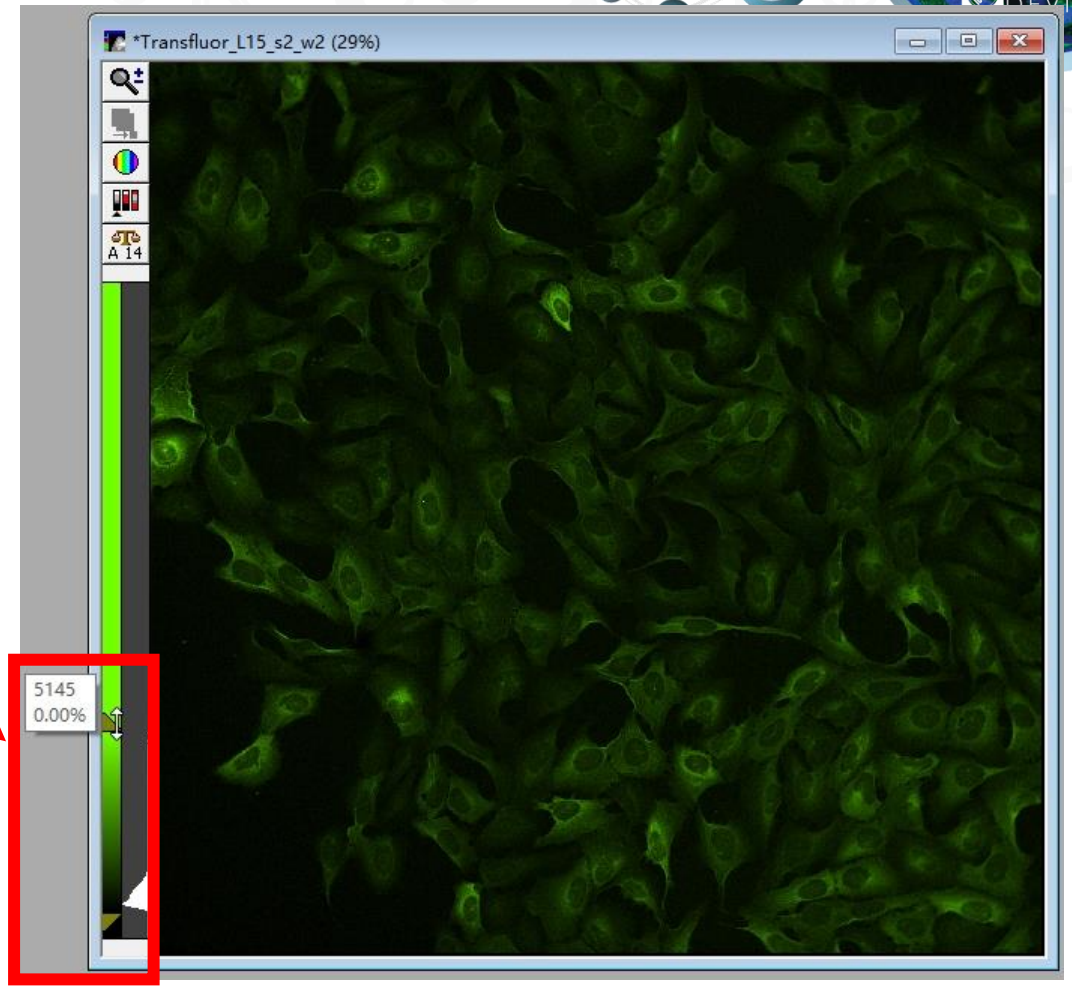
自定义像素的颜色

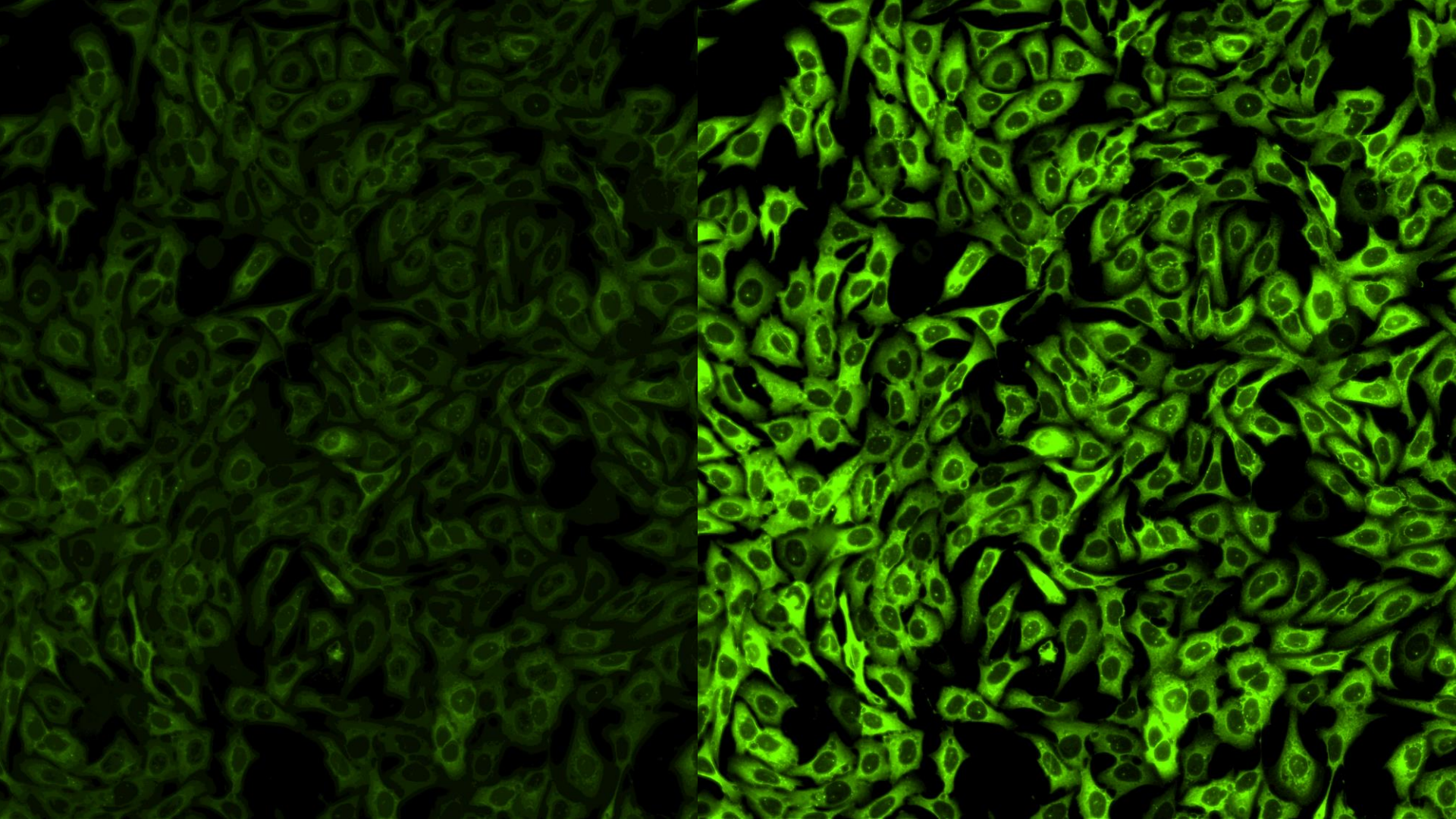
通常习惯用波长本身的色彩

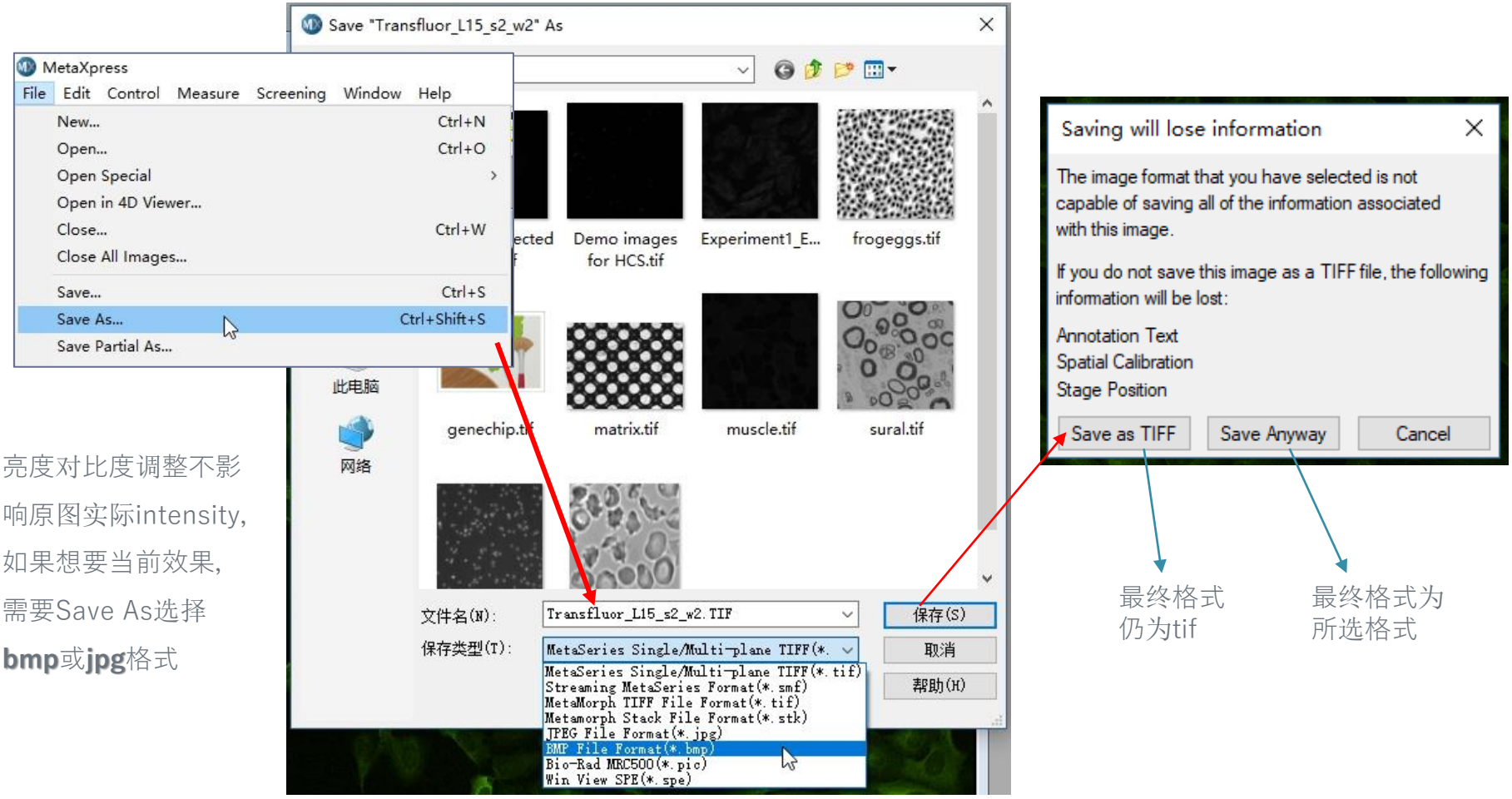


5	9	18	15
8	15	19	16
6	12	15	12
11	14	22	14

图像后期调整
亮度对比度





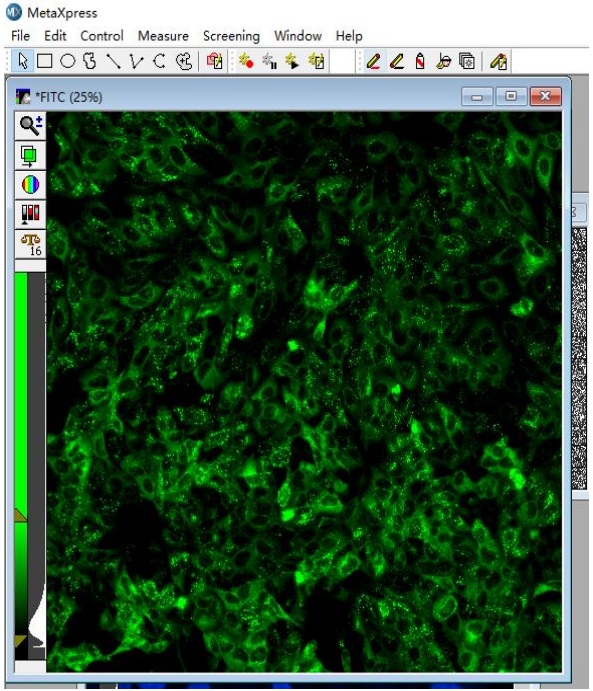


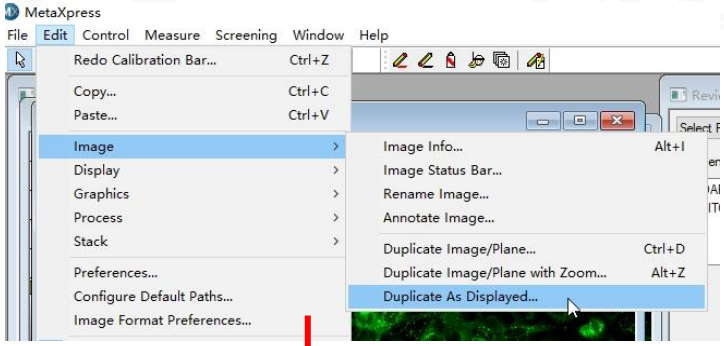
亮度对比度调整不影响原图实际intensity，如果想要当前效果，需要Save As选择**bmp**或**jpg**格式

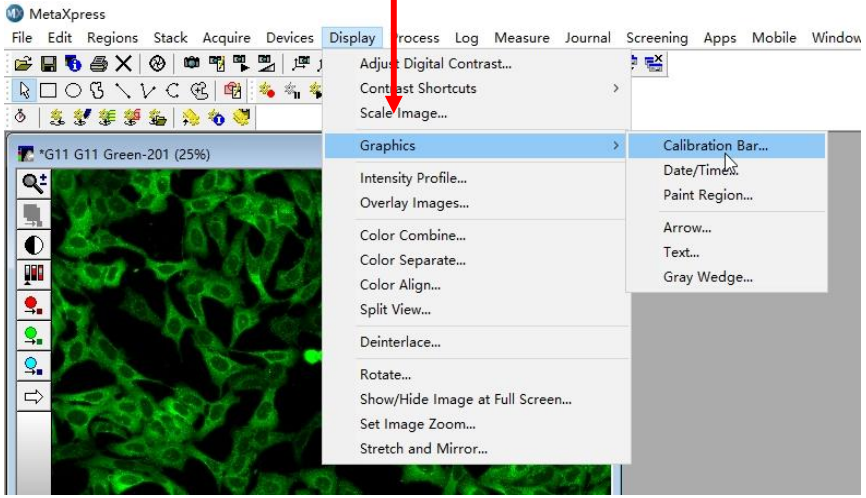
最终格式仍为tif

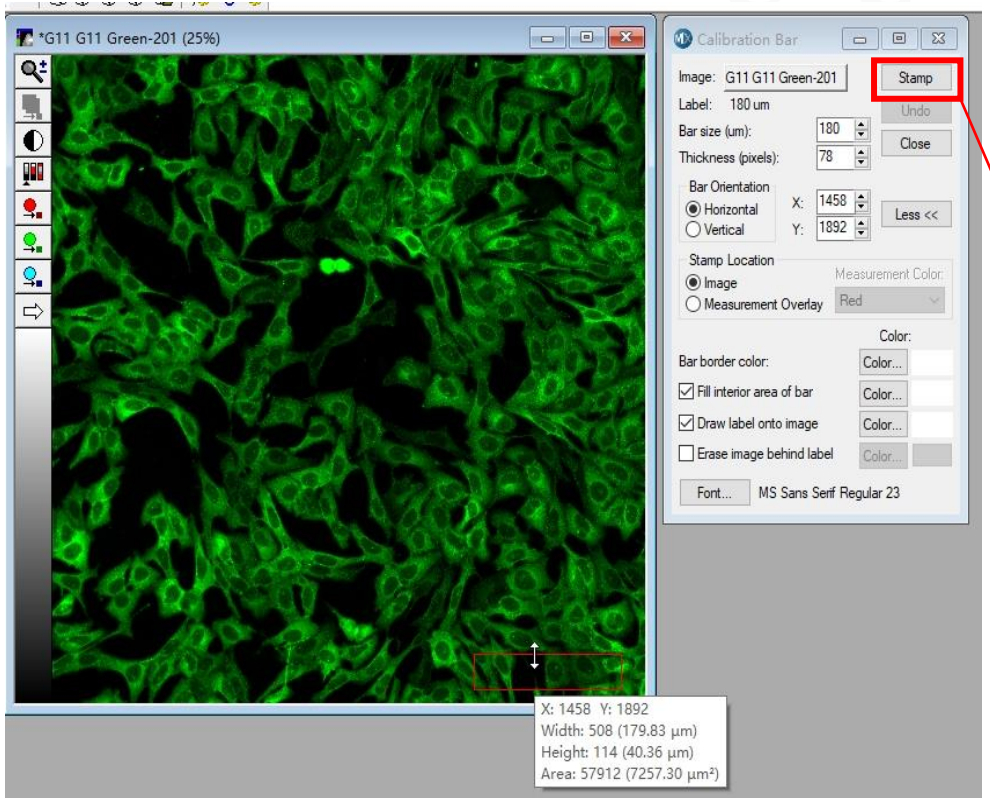
最终格式为所选格式

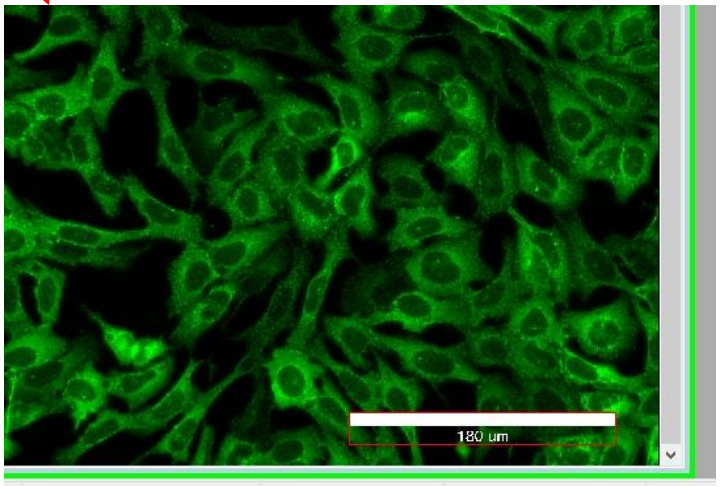
添加scale bar





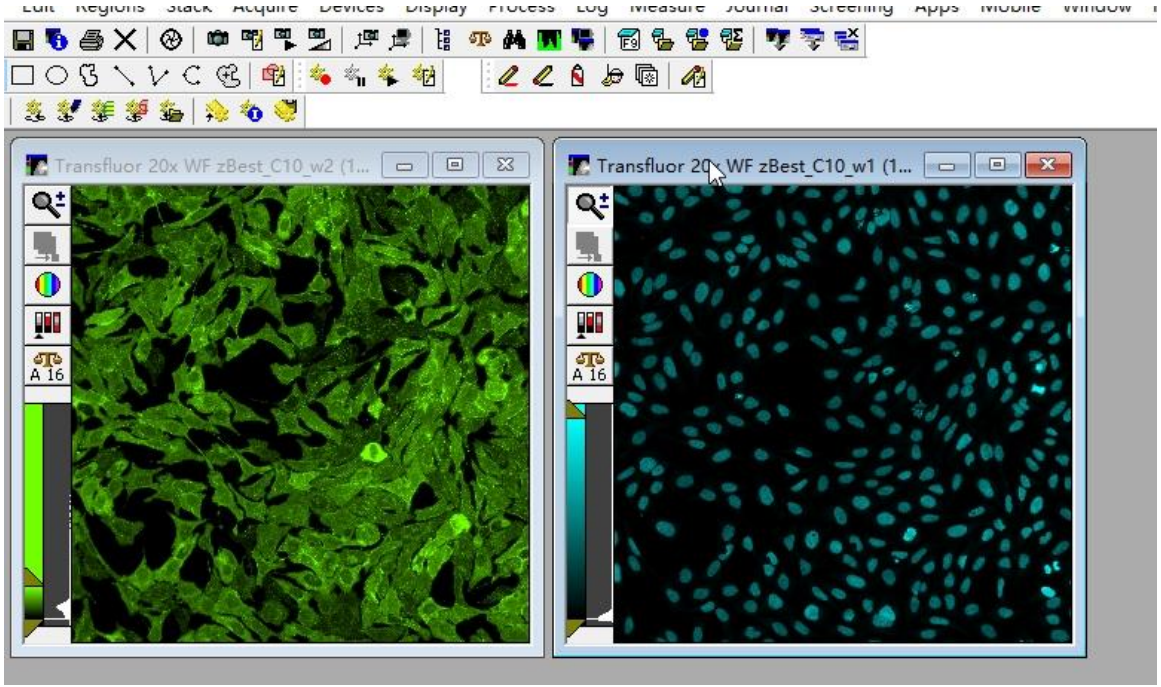




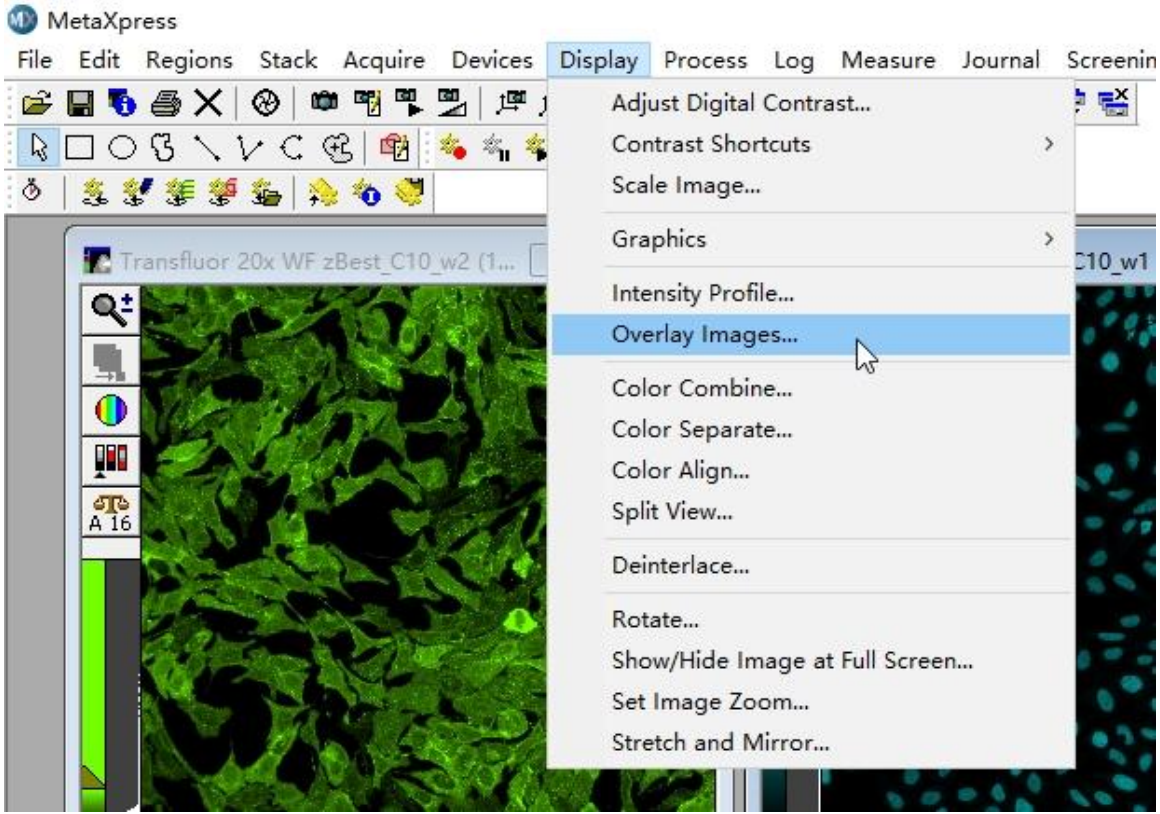


彩色多通道图像叠加

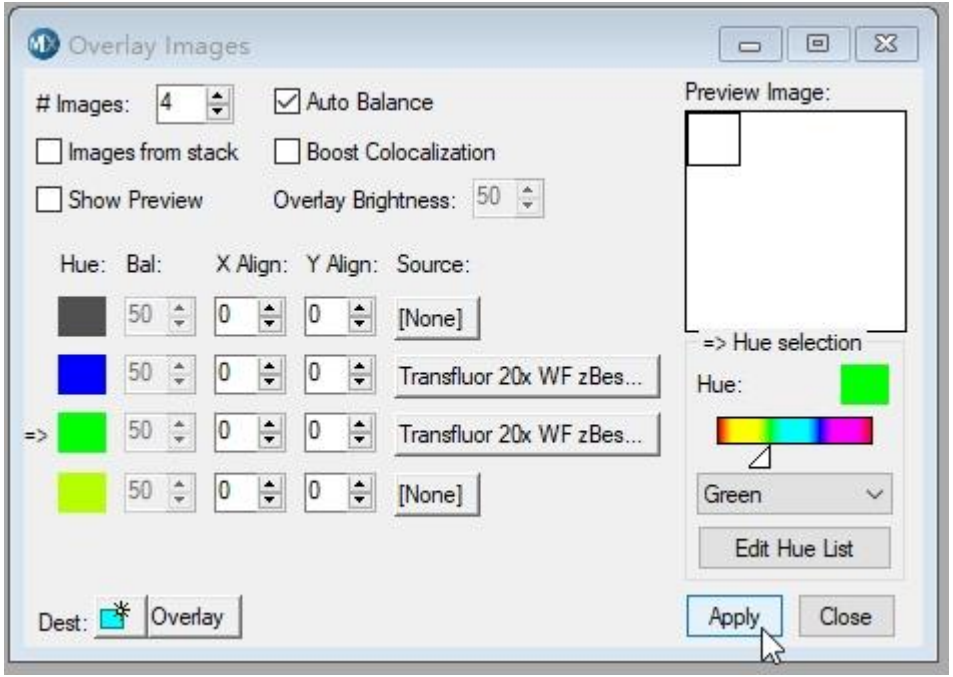
1. 打开需要被叠加在一起的多张图片；

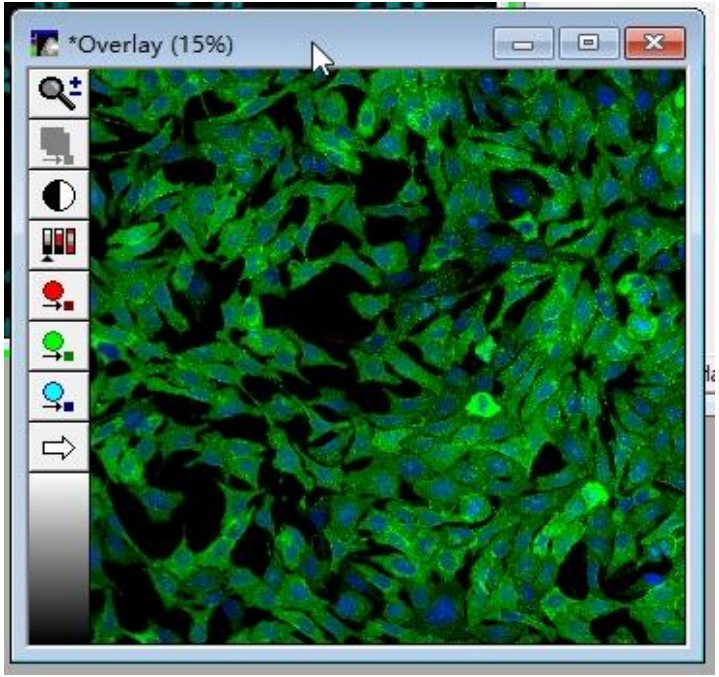


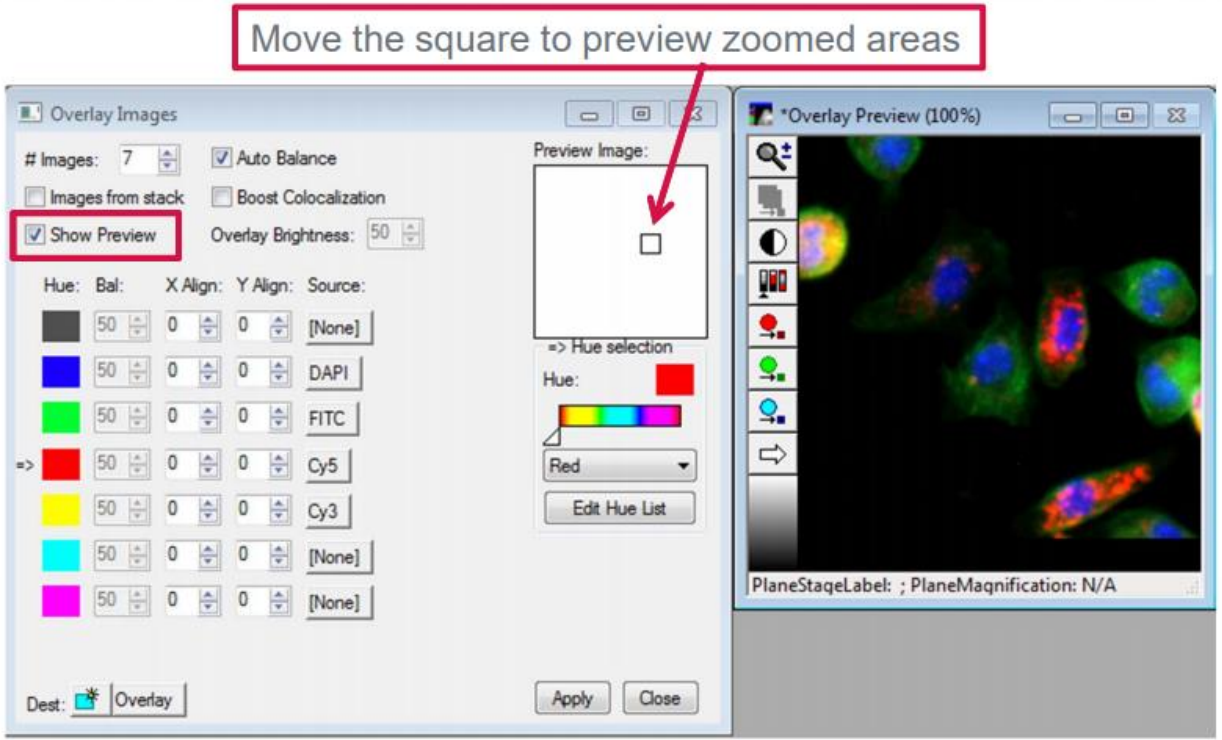
找到Display→Overlay images功能（或在Edit→Display→Overlay images）

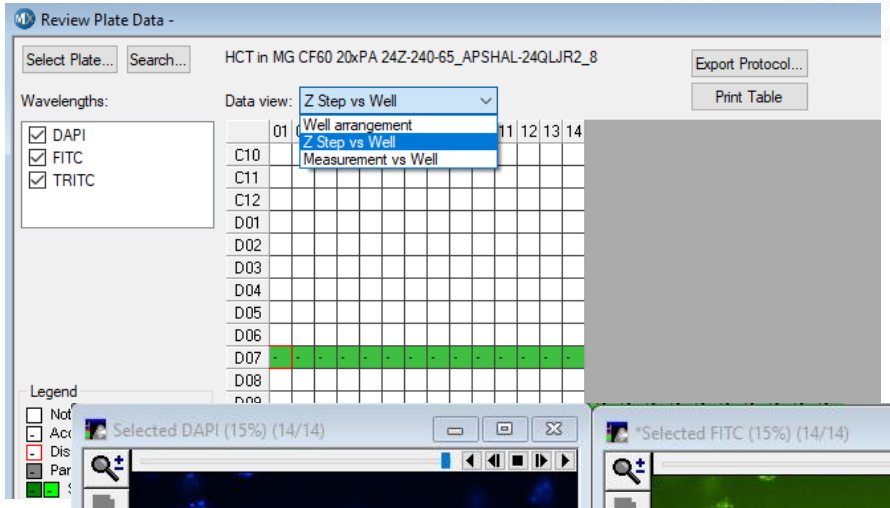


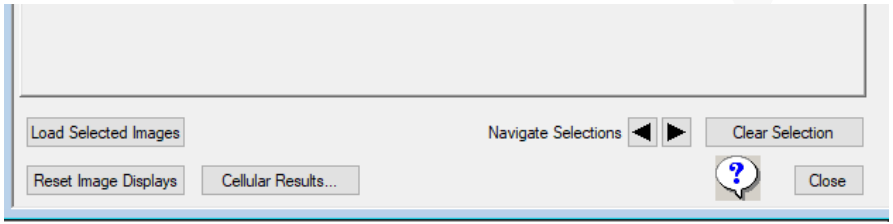
在不同通道处分别选择需要叠加的图像名称，调好单色图像的对比度，点击apply

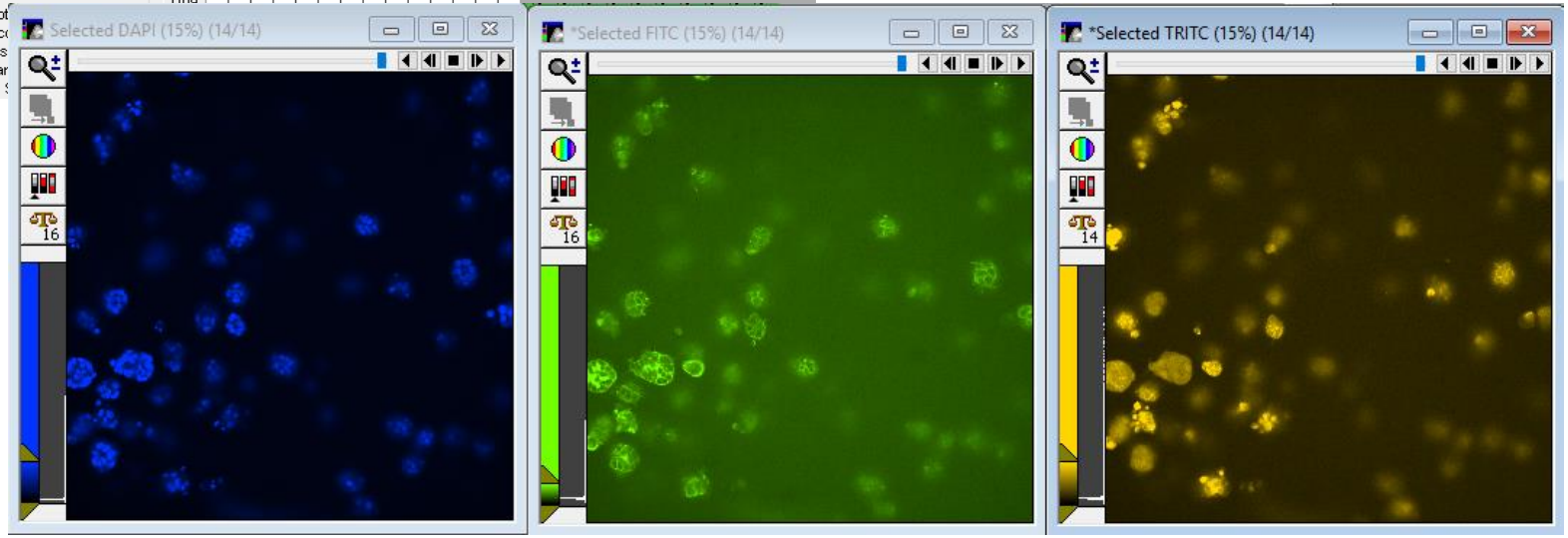






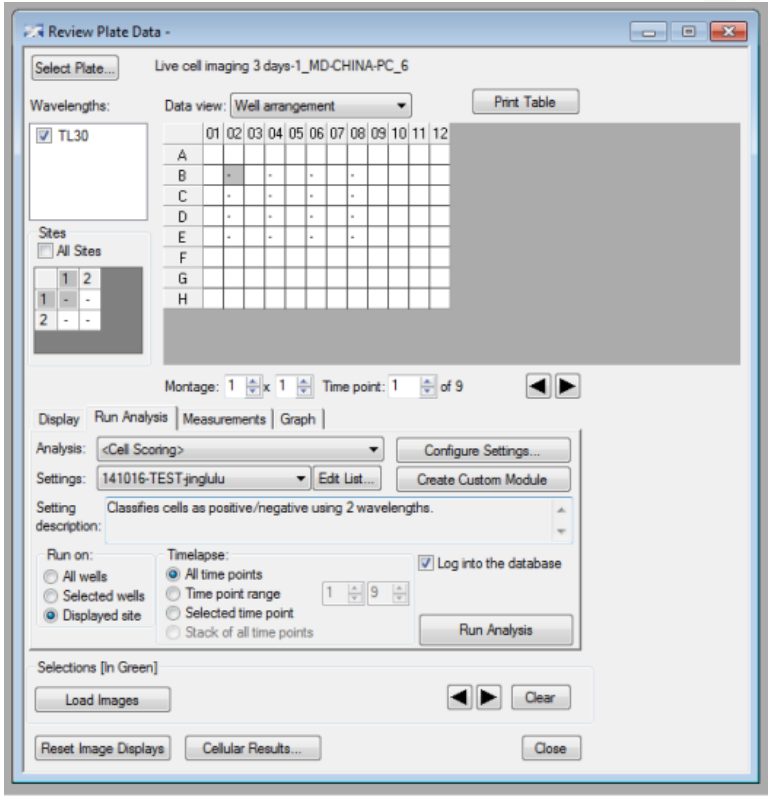


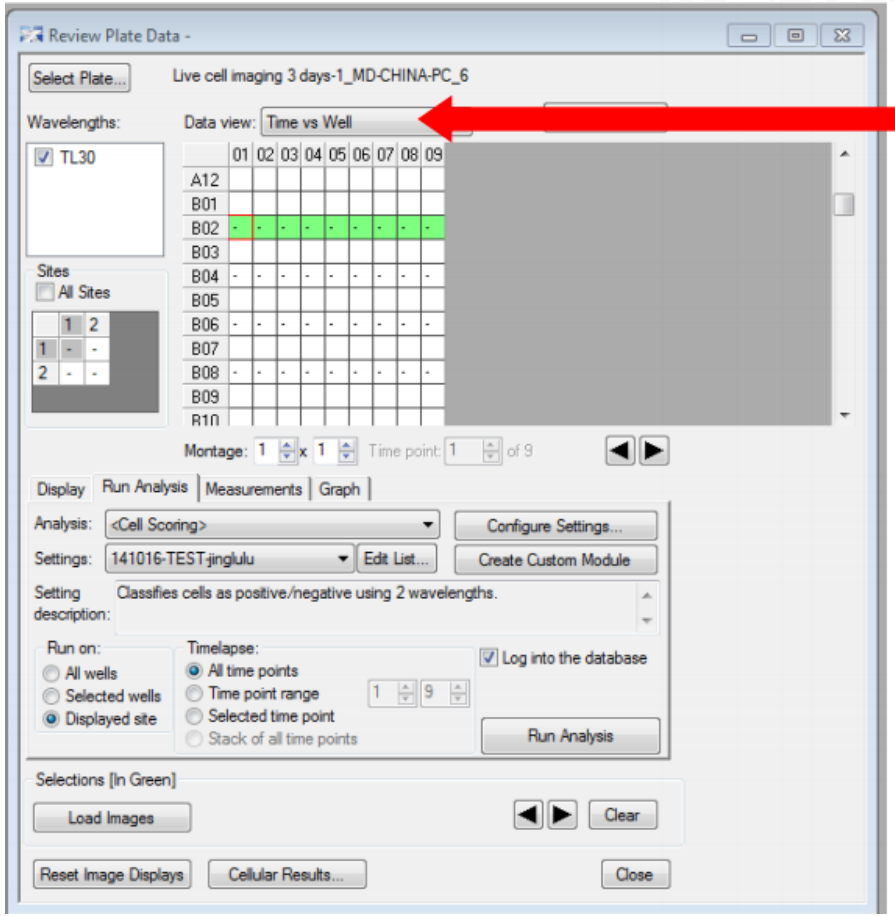




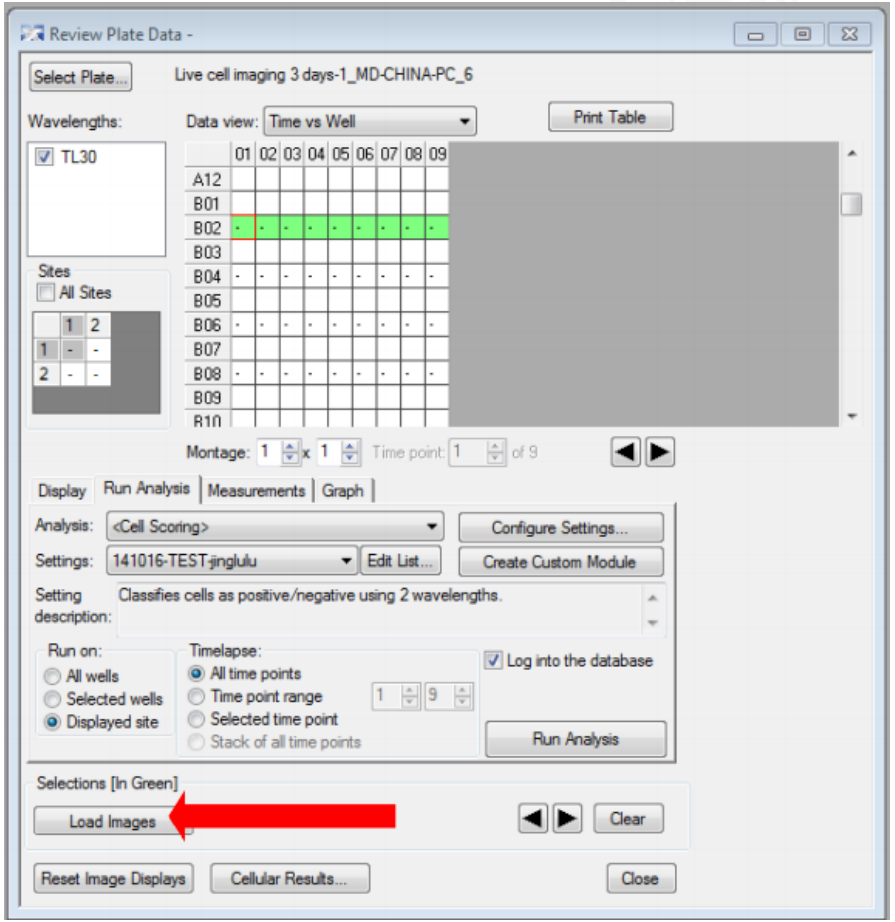
制作movie

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



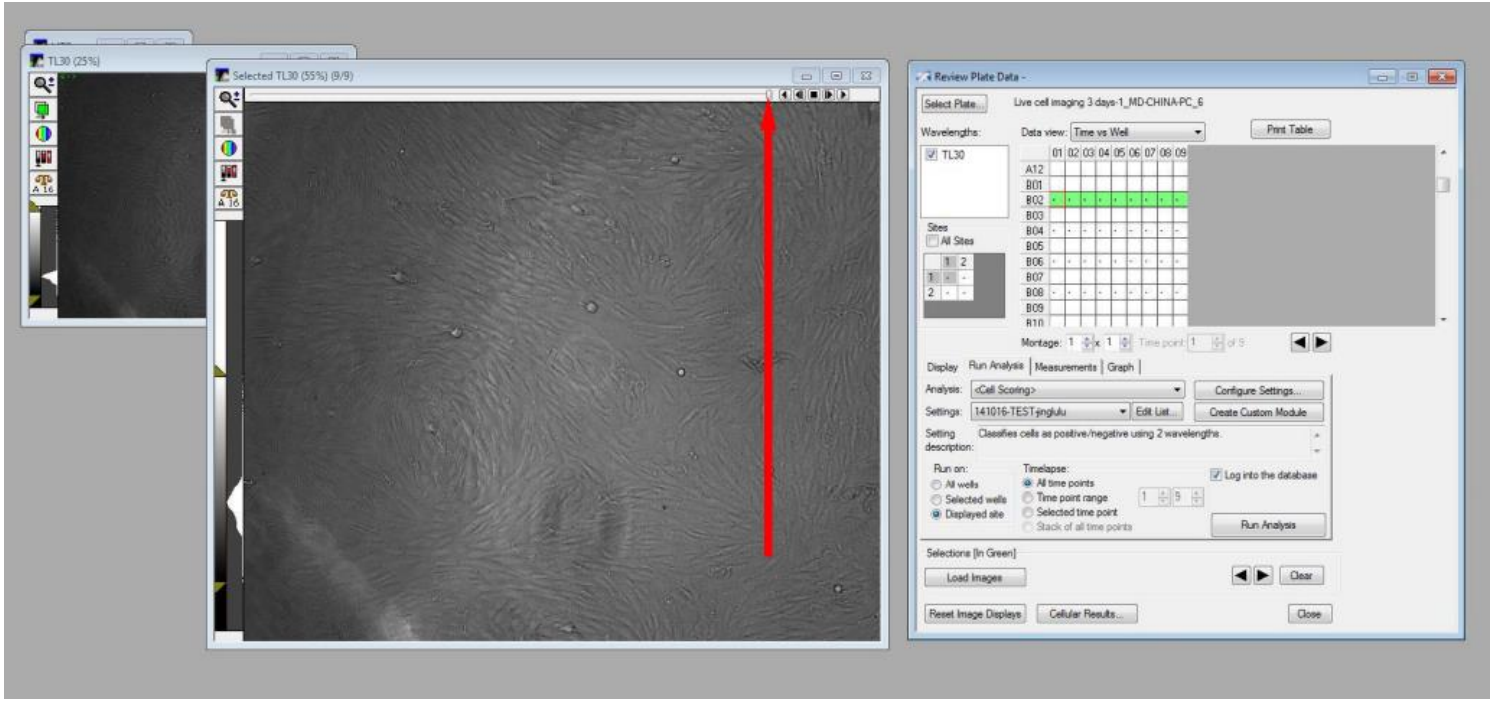


在Data view 选项（红色箭头）里选择“Time vs Well”，鼠标右击要做 movie 的孔（图中举例为 B02），选中后显示为绿色。矩阵图上方的 01-09 数字代表拍了几个时间点。 左方的 wavelength 要选中需要的荧光通道

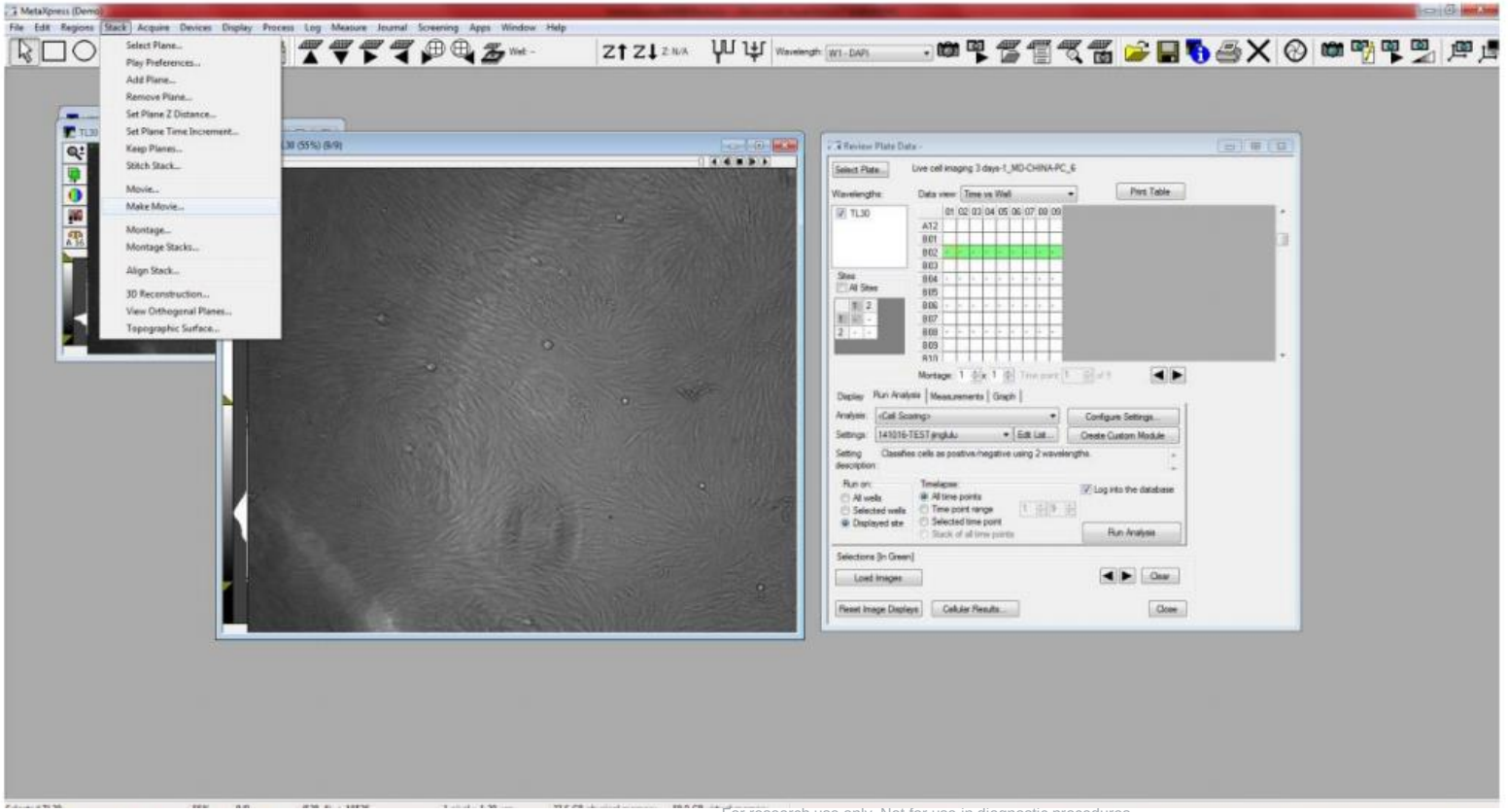


点击”load Images”

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

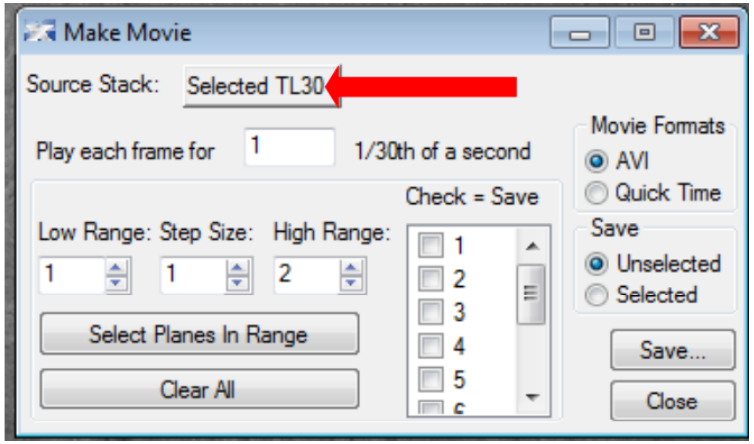


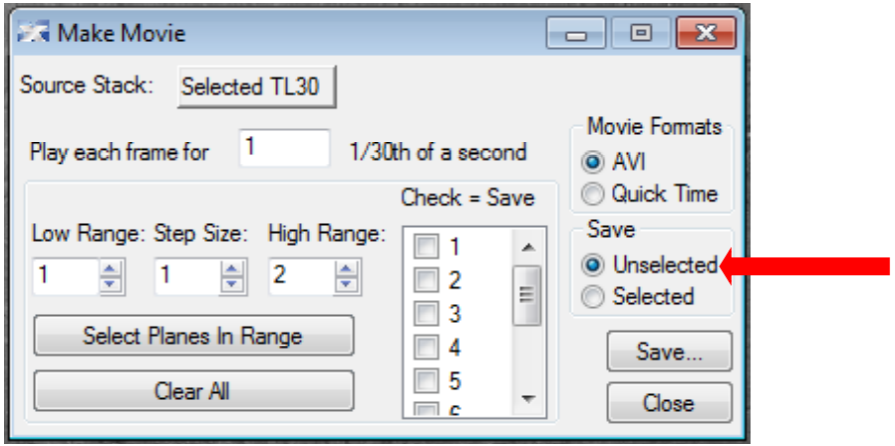
点击 Stack---Make movie



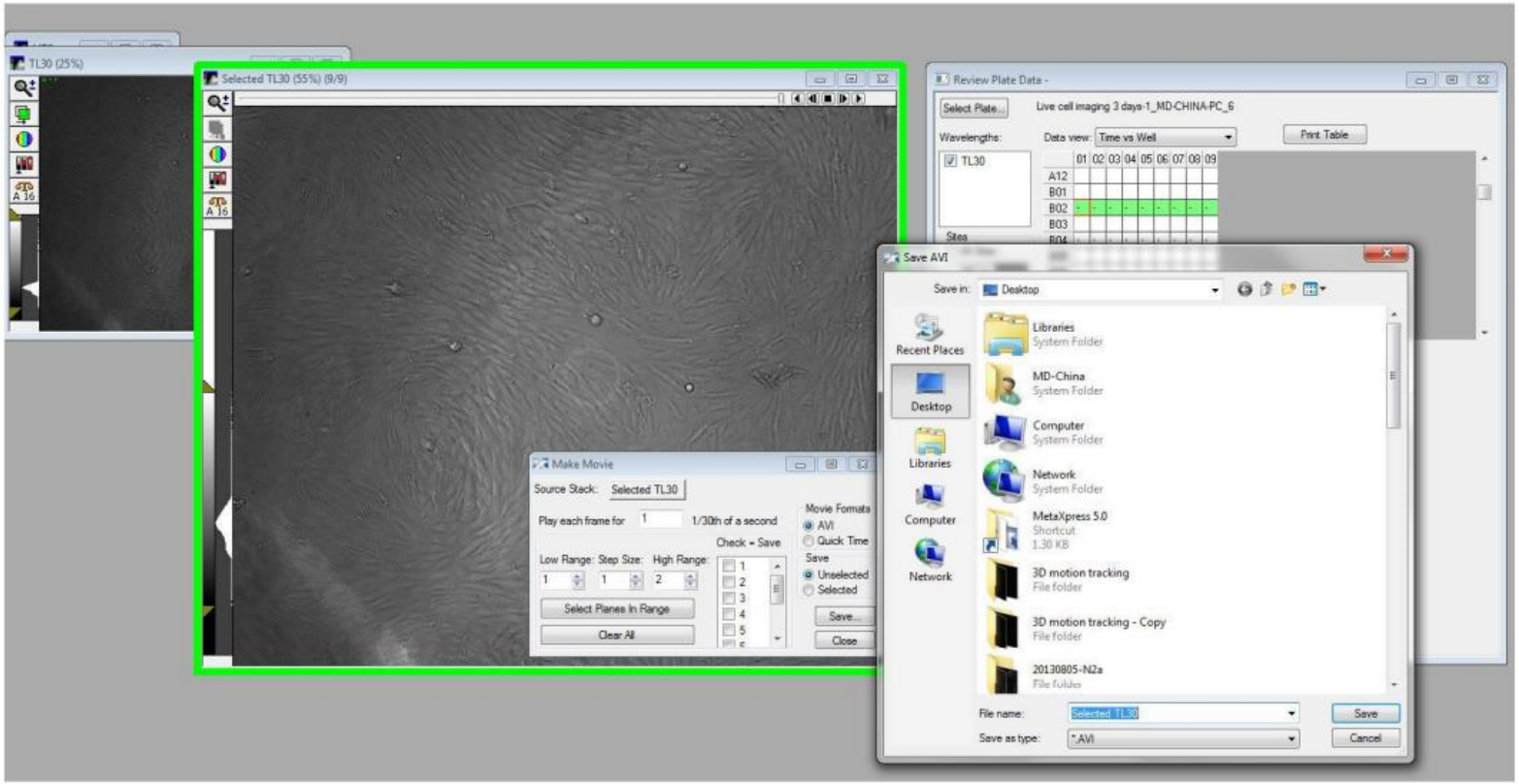
如果前述 Image stack 建立成功，则下图中红色箭头所指的位置会自动导入建好的 image stack（本例中名为 Selected TL30）

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

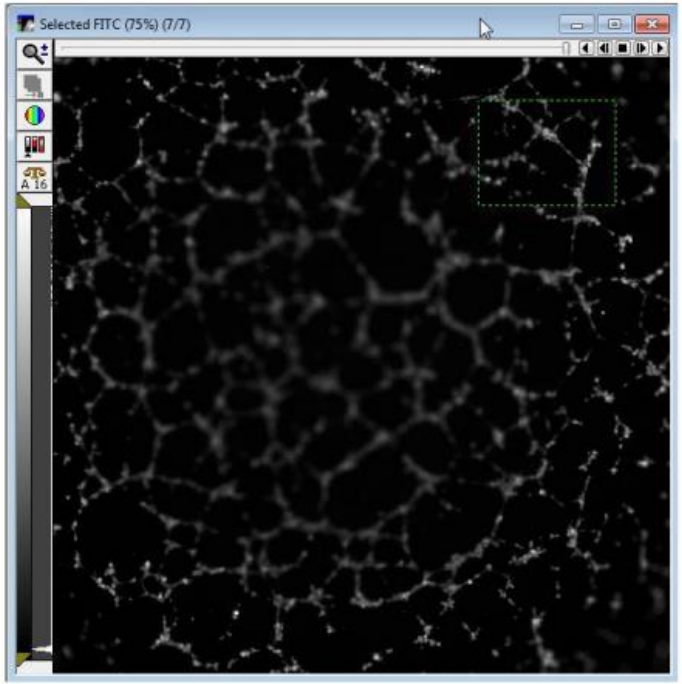




点击 save 后，保存为 Avi 格式或 Quicktime 格式即可



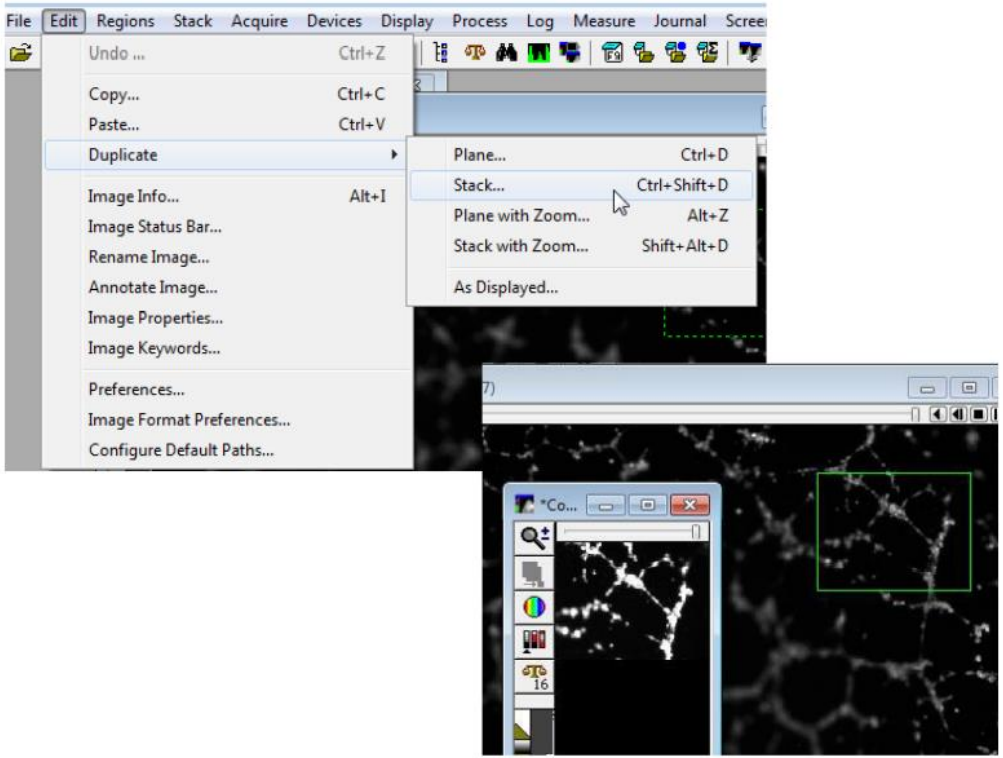
Stack 截取小区域生成视频



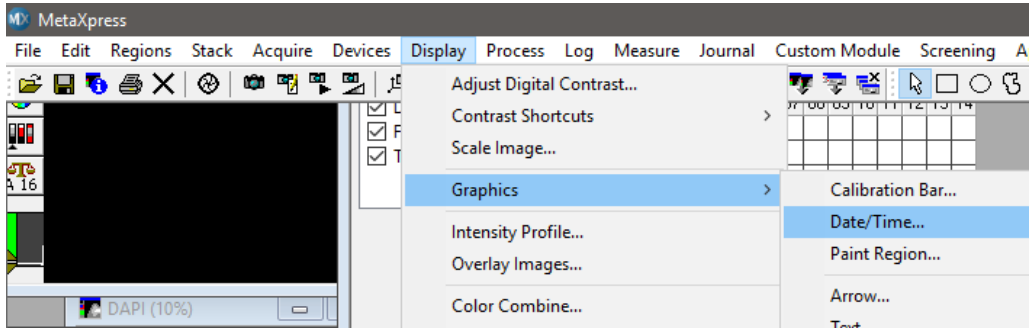
工具

在生成的 stack 文件上用选区工具框选中目标位

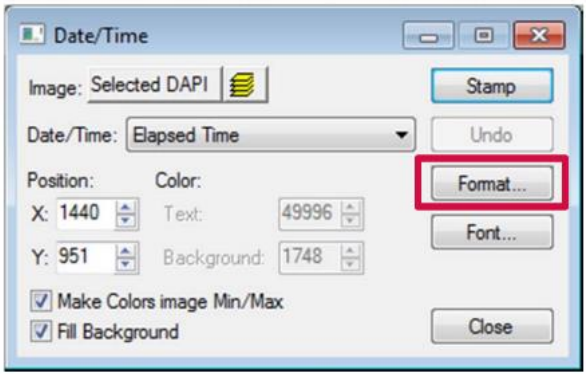
在 Edit 中选择 Duplicate—Stack，生成一个小区域的 stack 文件

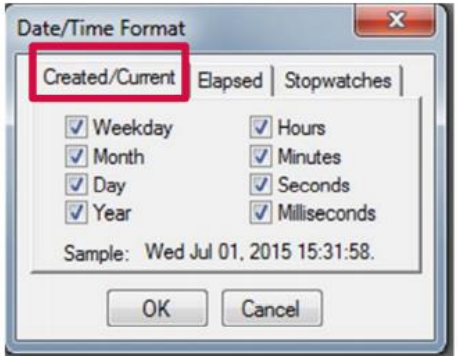


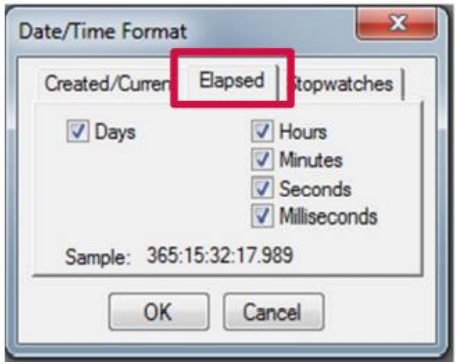
给movie加上日期/时间

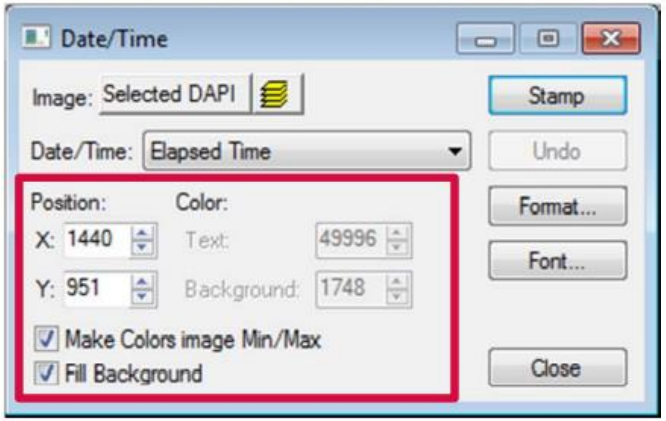


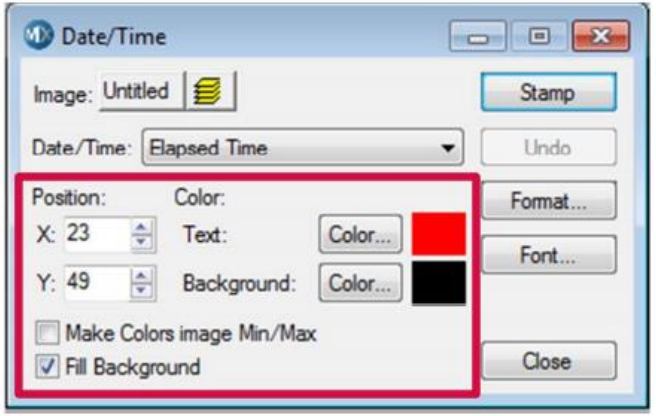
或Edit > Graphics > Date/Time

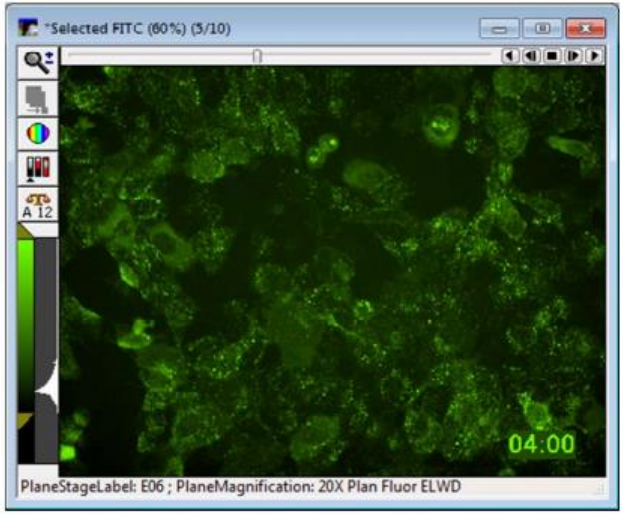


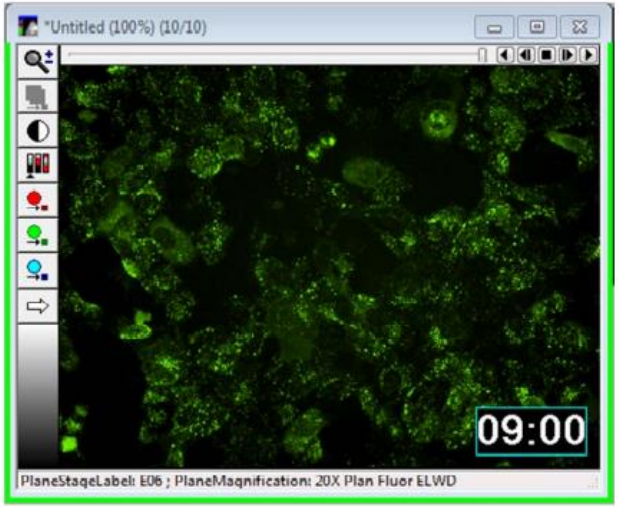










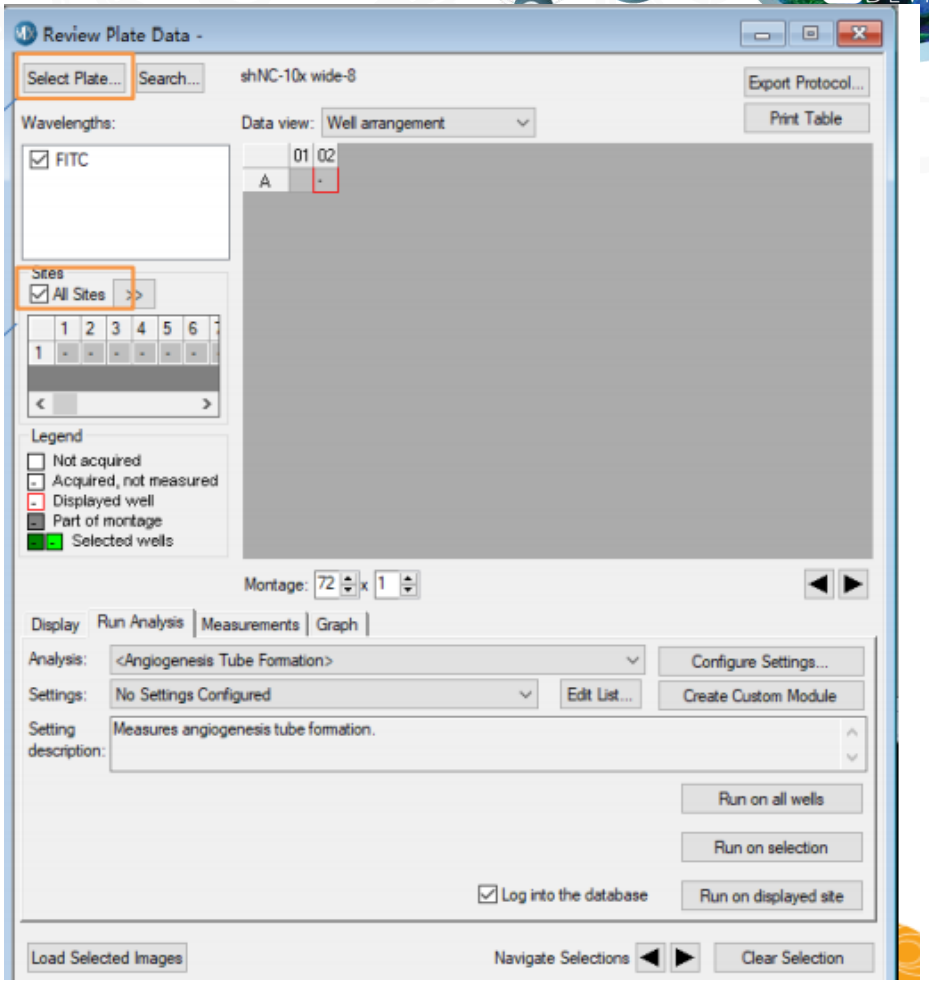


图像拼接

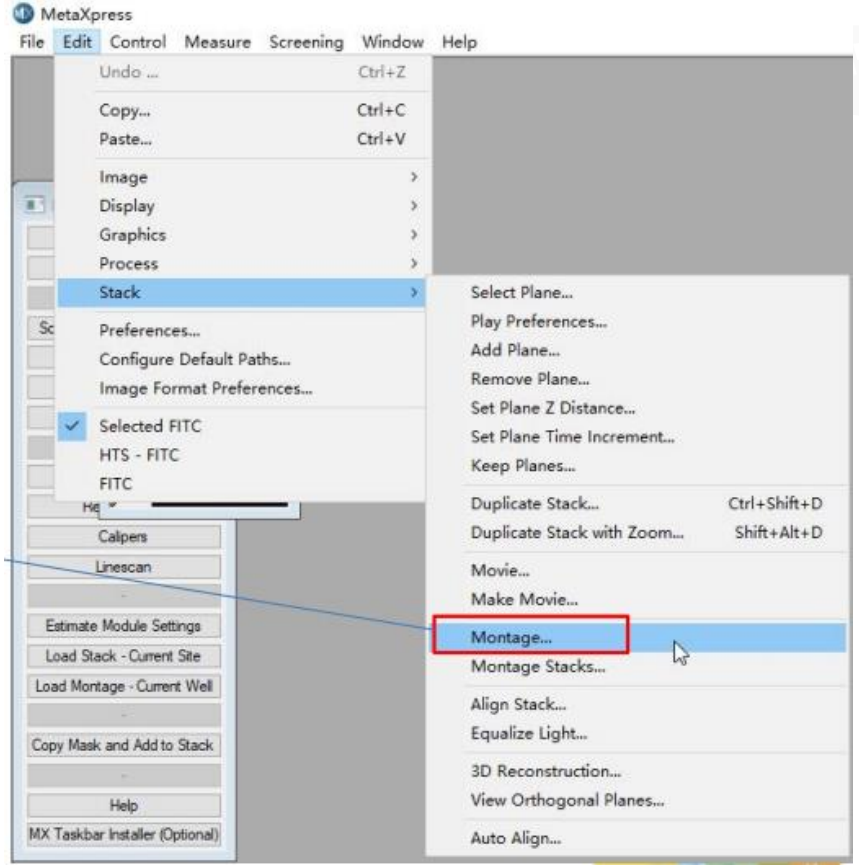
选好板子和通道以后，选择 all sites

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

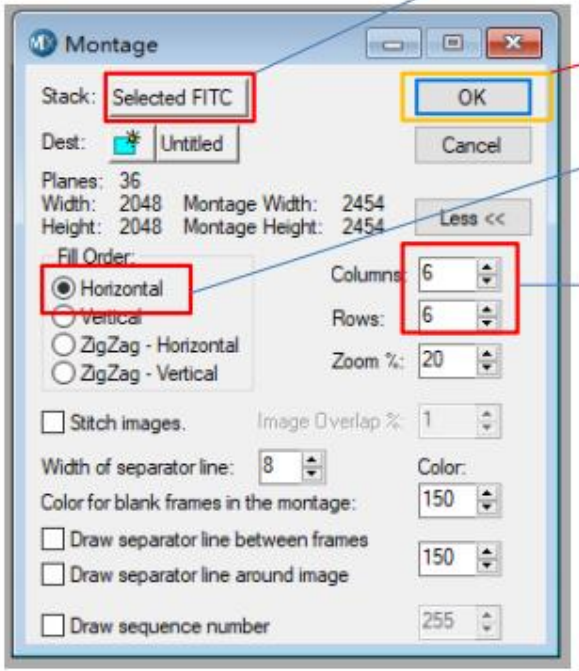
先调整好对比度，然后去掉 auto scale



Edit-Stack-Montage



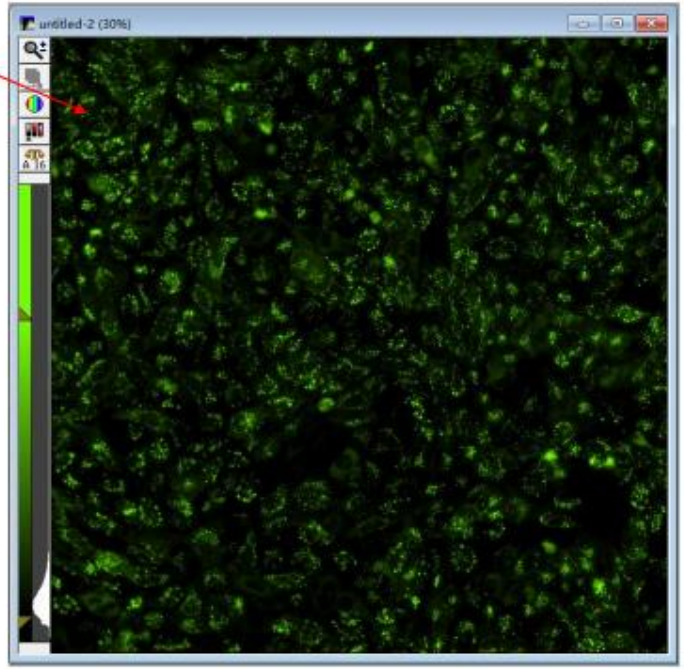
1 选择刚刚生成的stack文件



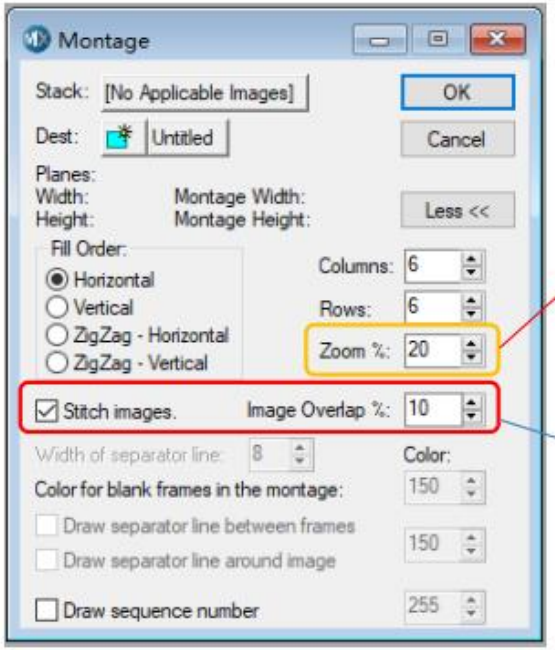
2 选择Horizontal模式

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）

4 全部设置完成后点OK生成大图

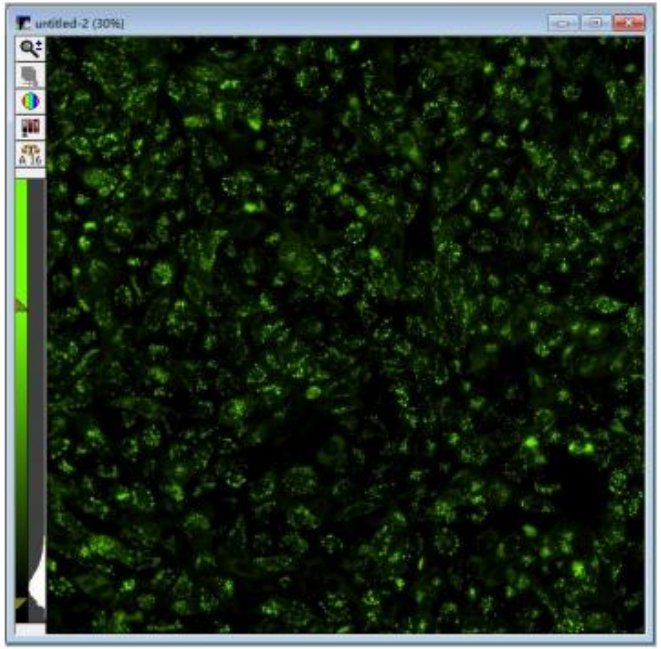


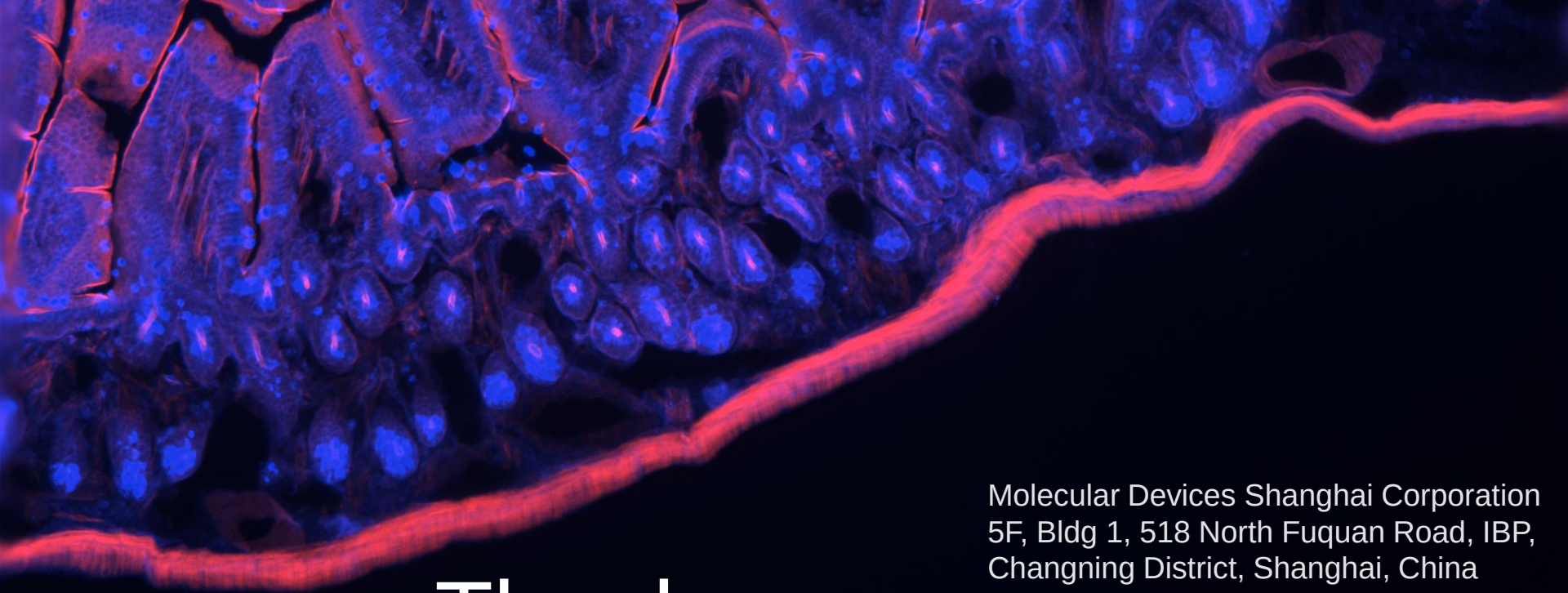
拼接后的大图



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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