LSM 880 Basic Operation

MultiChannel & TimeSeries & Z-Stack & TileScan

Lu Xi

2016-05-15

Agenda

1	Startup and Shutdown of the System
2	Acquiring Multi-Channel images
3	Z-stack image
4	Time Series image
5	Tile Scan
6	Airyscan Imaging
7	
8	

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Startup and Shutdown of the System

Startup of the System

1、打开稳压电源：

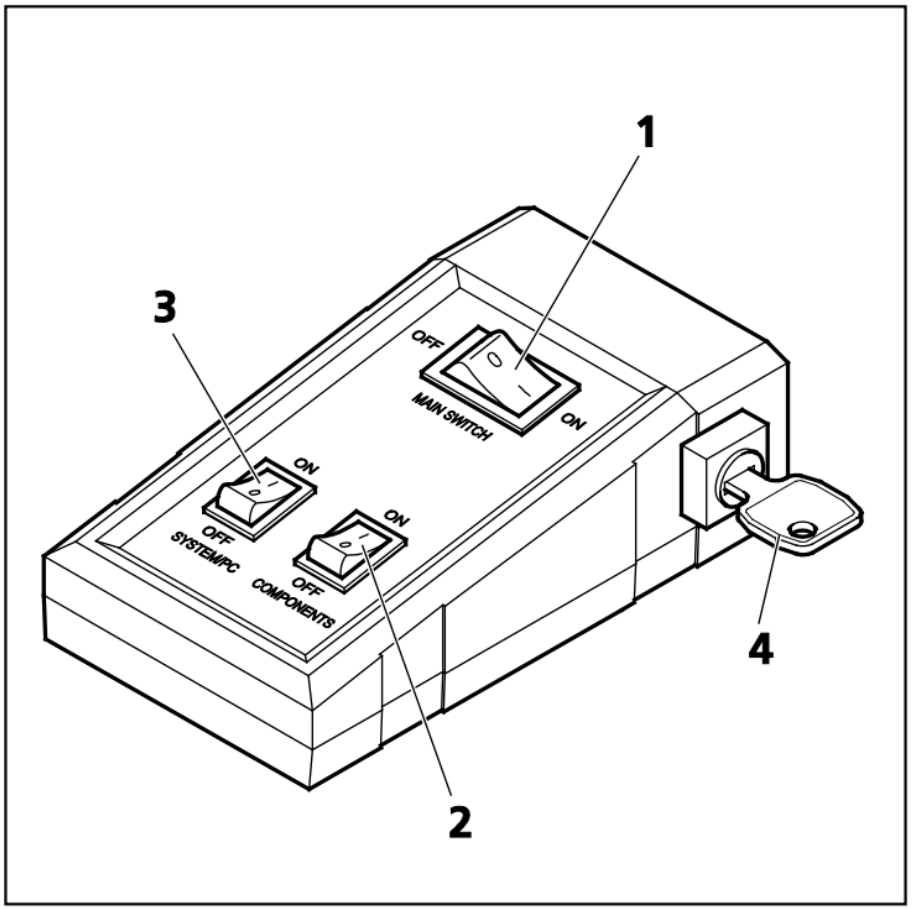
a、打开稳压电源背后总开关；

b、打开稳压电源前面开关；

c、确定稳压电源显示220V；

Startup and Shutdown of the System

Startup of the System



1 Main switch ON/OFF
2 COMPONENTS switch ON/OFF
3 SYSTEM PC switch ON/OFF
4 Key switch

*在打开整个系统电源之前，确保钥匙（图中4）处于“ON”；
2、打开“MAIN SWITCH”（图1）；
3、打开“SYSTEM/PC”（图3），随后打开电脑，进入系统；
4、打开“COMPONENTS”（图2）；
5、打开金属卤化物灯；

Startup and Shutdown of the System

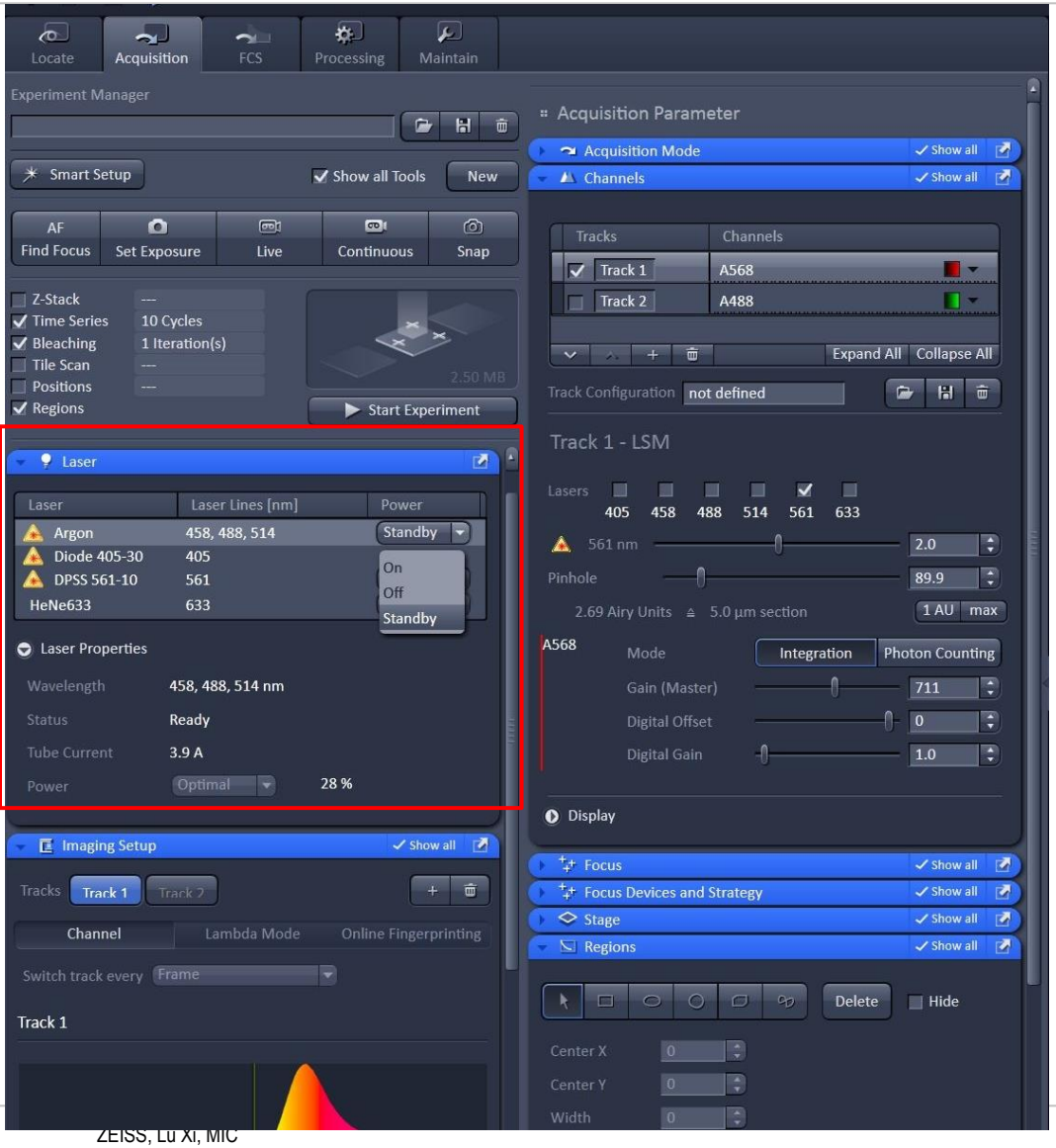
Startup of the System

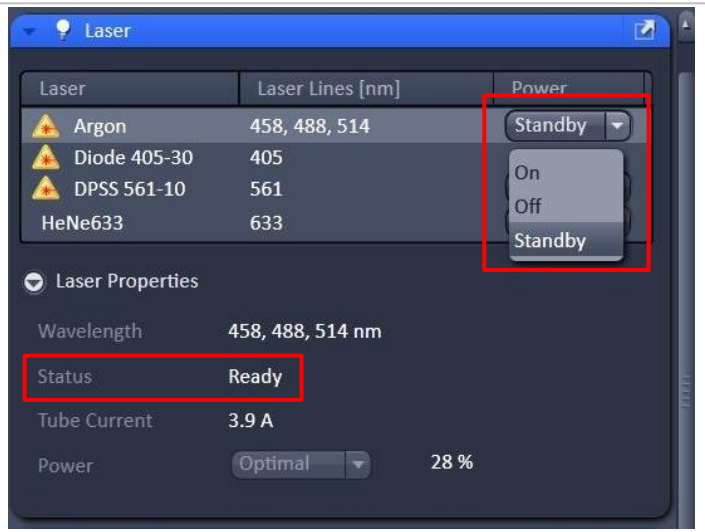
6、启动ZEN软件（black）；

7、点击“Start System”

Startup and Shutdown of the System

Startup of the System





8、如果需要使用458、488、514需要打开Argon激光器：

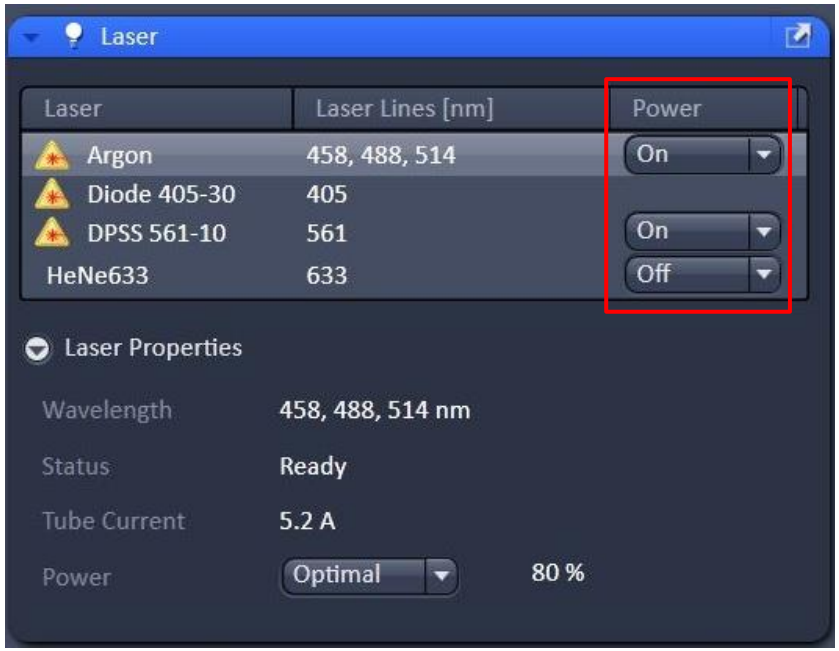
a、在“Laser”中“Argon”栏选择“Standby”，等待激光器预热；

b、预热结束后“status”显示“Ready”后选择“On”；

9、随后可以开始共聚焦成像

Startup and Shutdown of the System

Shutdown of the System



关机过程基本类似开机的倒序过程：

1、关闭金属卤化物灯；

2、关闭激光器，在“power”中选择“off”，关闭已打开的激光器；

3、关闭ZEN软件；

4、关闭电脑；

5、关闭电脑后等待激光器冷却约5min，此时Argon激光器的风扇停转；

6、按顺序关闭“COMPONENTS”→“SYSTEM/PC”→ “MAIN SWITCH”

7、关闭稳压电源。

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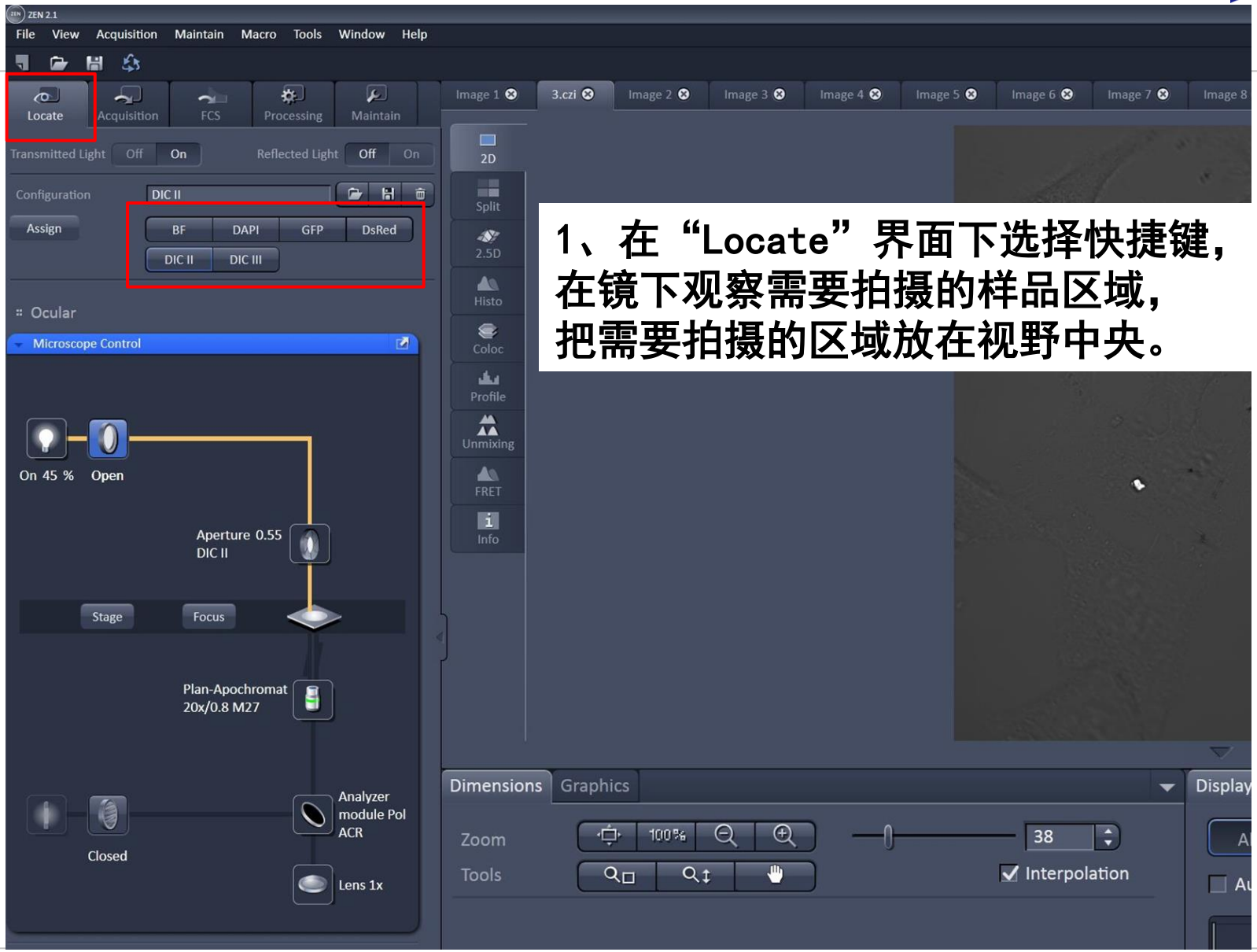
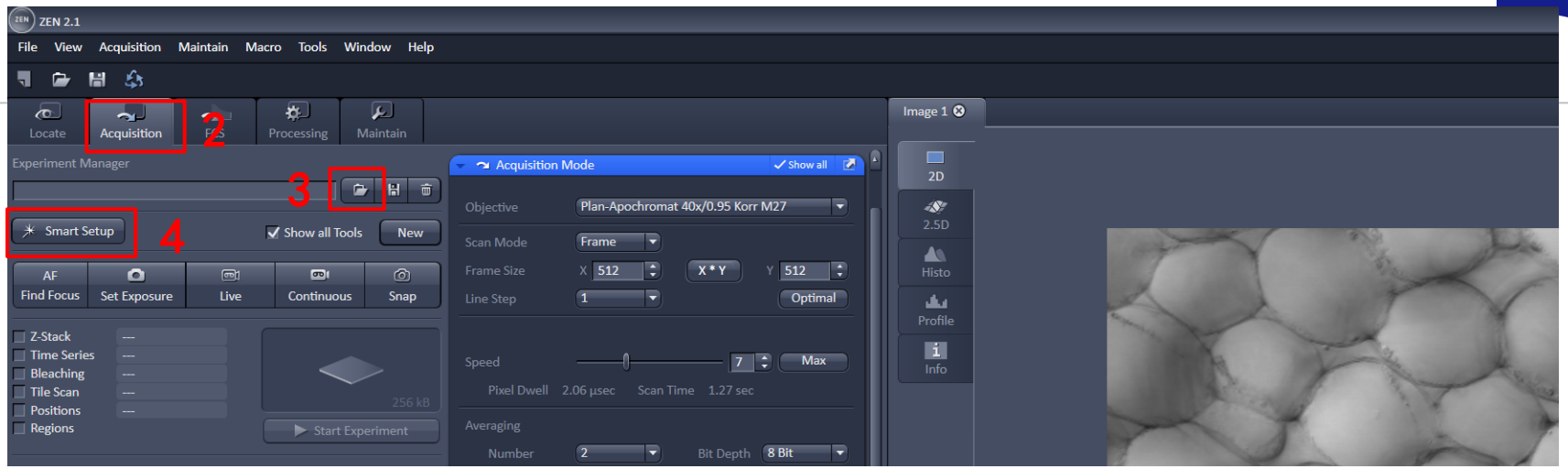
Acquiring Multi-Channel images



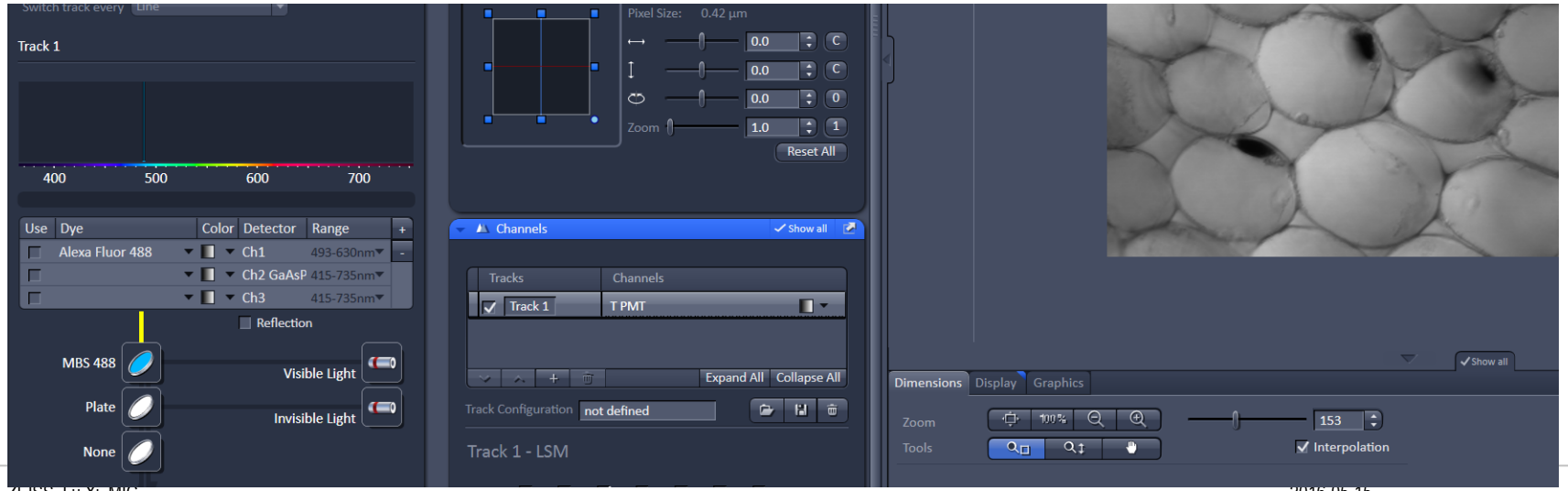
Acquiring Multi-Channel images



2、进入“Acquisition”界面；

3、选择之前保存好的光路设置→“channel”和“Acquisition mode”设置

4、或者新建光路设置→“Smart Setup”



5、“smart setup”中选择染料名称，并选择拍摄方式后，“Apply”

三种拍摄方式：

A、Fastest

拍摄速度最快，

发射波长接近的荧光染料间存在串色现象；

B、Best signal

拍摄速度最慢；

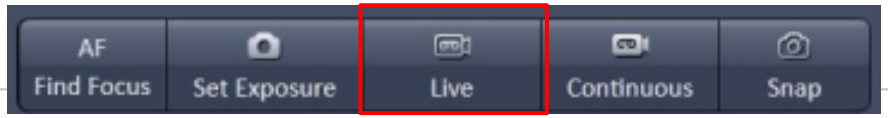
基本避免了发射荧光的串色；

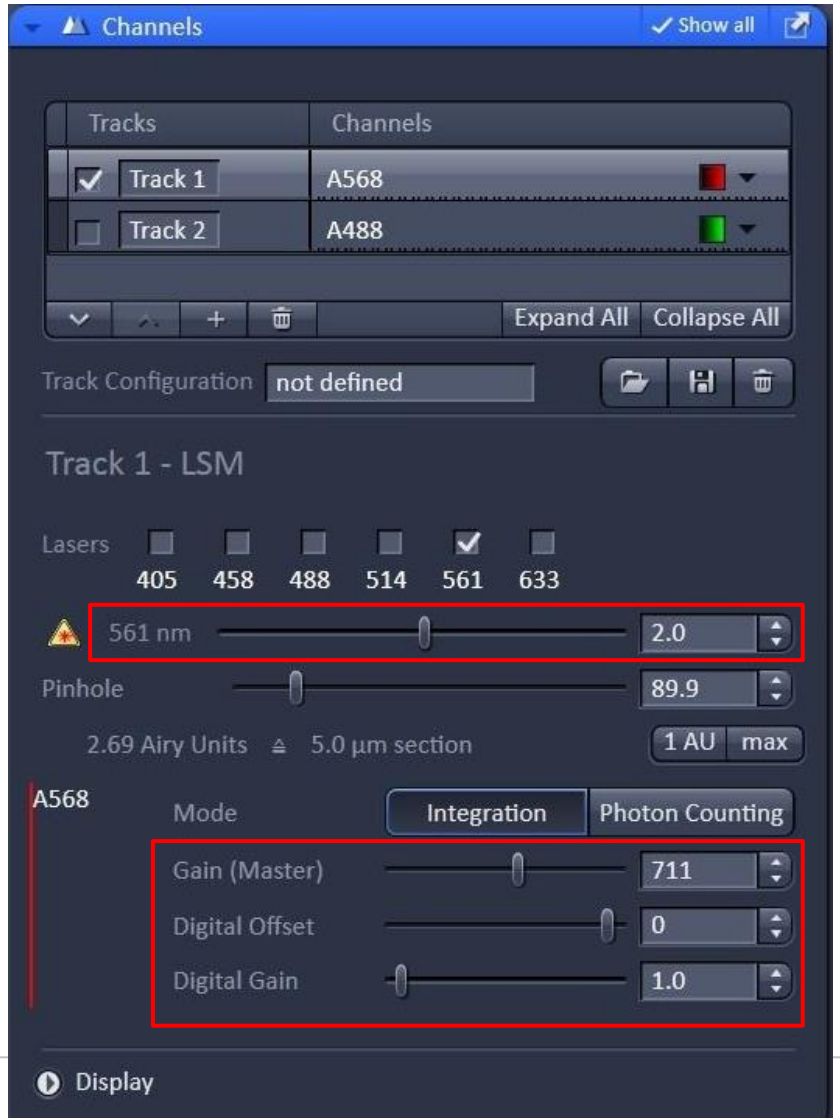
C、Smartest（Line）

结合上述两者优势，减少串色的同时拍摄速度较快，但是光路中硬件设置不能改变。

固定样本推荐使用Best signal、变化较快的样本推荐使用Smartest或者Faster

Channels

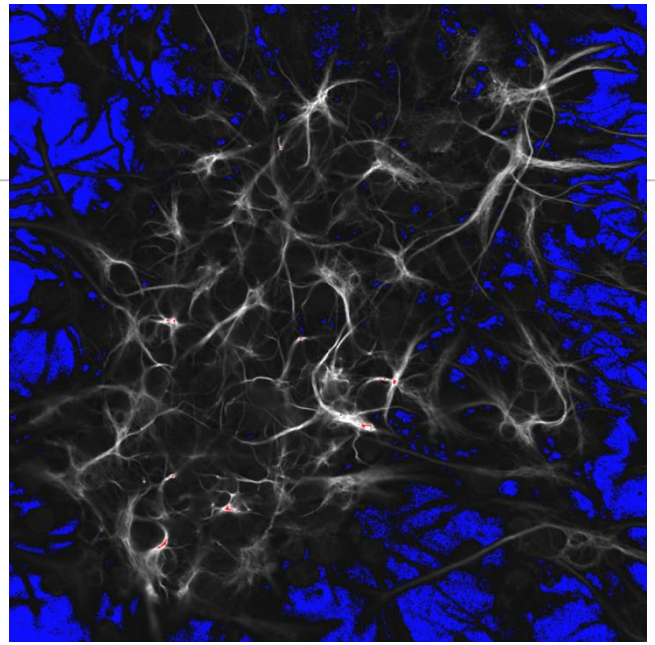


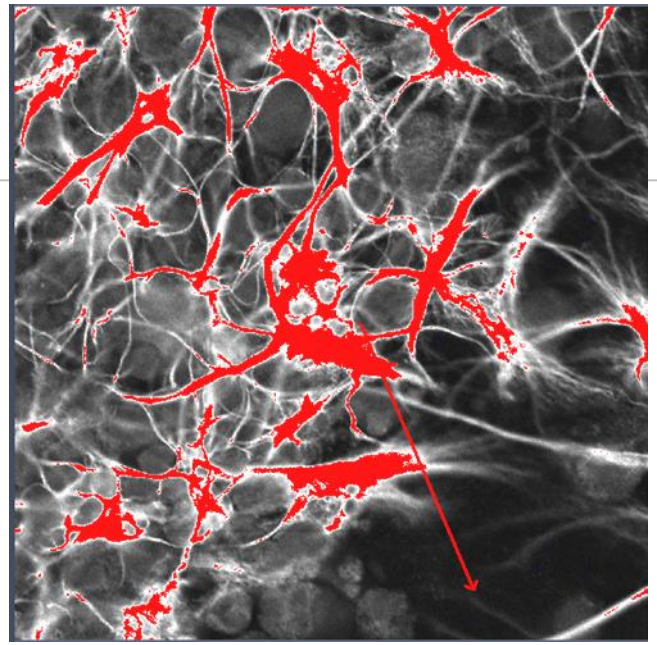


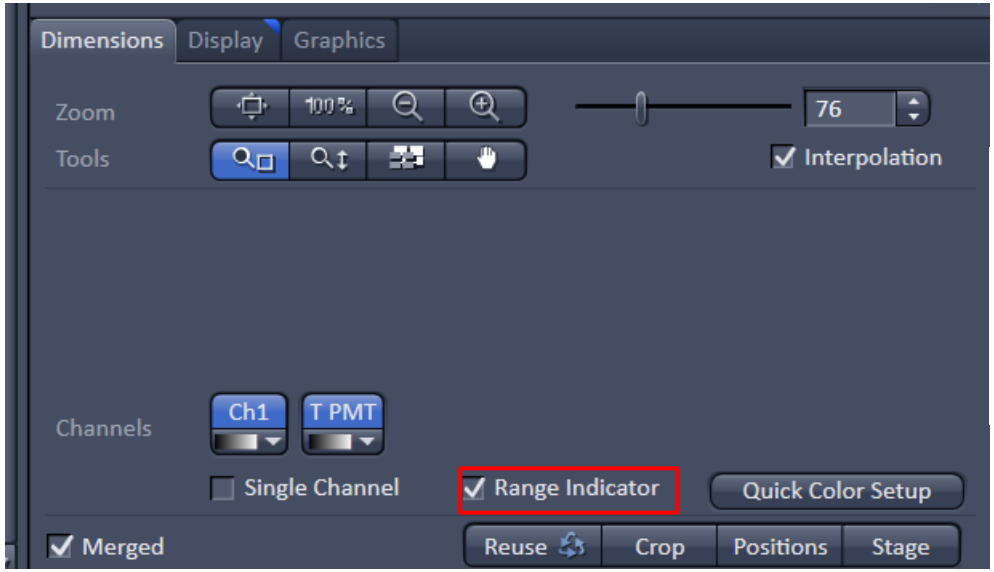
6、在“live”下设置Channels中的激光强度“Laser”，针孔大小“Pinhole”，检测器“Gain”值，以及“digital gain”或“digital offset”；每个track单独设置，所以每次只勾选一个track，并选中该track(选中track高亮)；

A、“Pinhole”一般设置为1 AU，增大Pinhole可以提高图像亮度，但会增加非焦面信息；减少Pinhole可以增加景深，但是会减少图像亮度；

B、“Gain”和“Digital Gain”增加可以增加图像亮度，但是也会提高背景噪音。



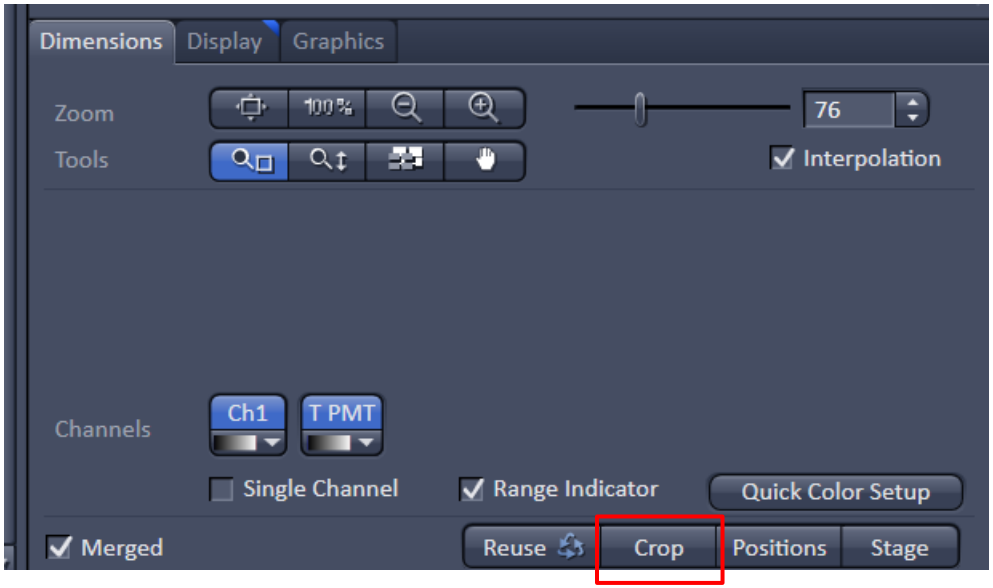




设置原则保证图像不要过曝，尽量减少背景噪音：
在live下选择“range indicator”可以显示出曝光程度；

Acquisition Mode

7、在Acquisition Mode下主要设置如下参数：
A、通过scan area选择扫描区域或通过图像窗口下的“crop”选择扫描区域；



Acquisition Mode

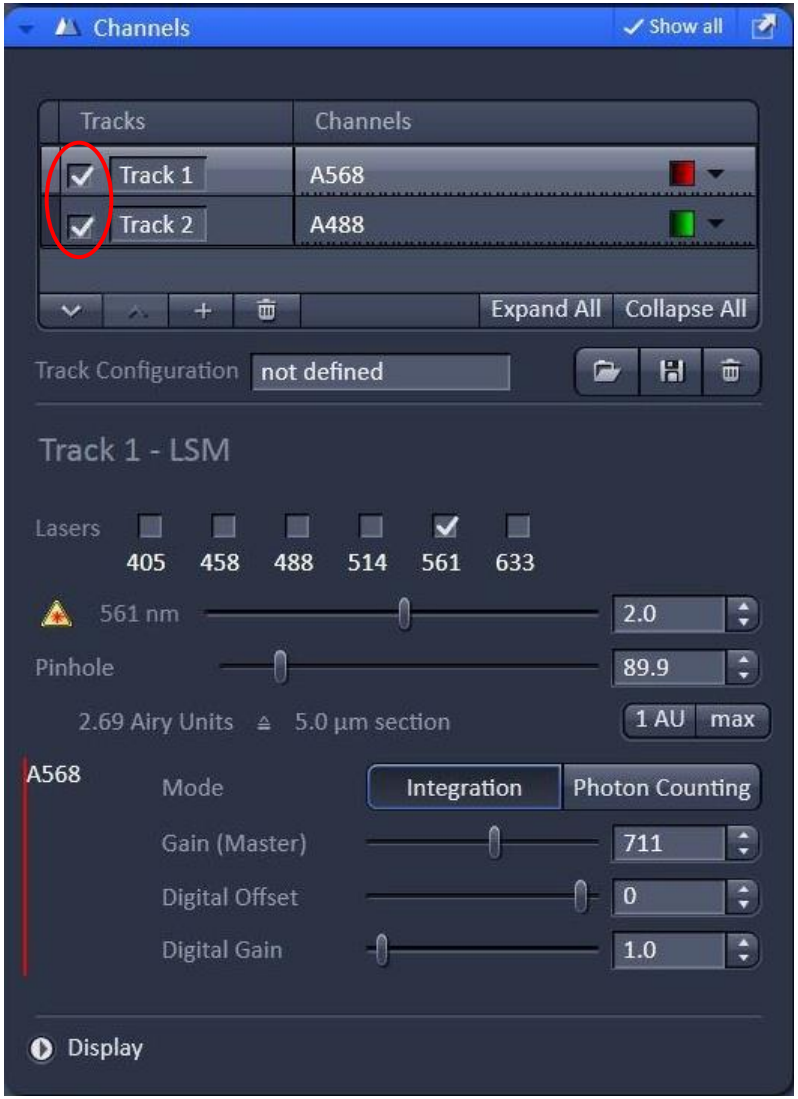
B、设置speed：扫描速度越慢，信噪比越好，但光漂白越多；

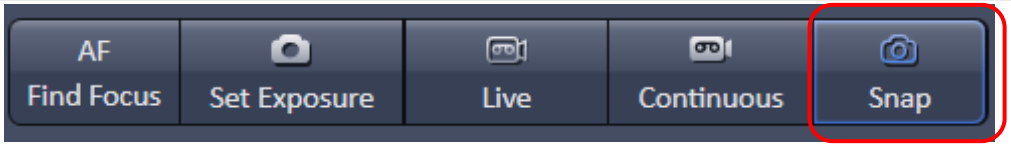
C、Averaging：增加averaging次数可以减少噪音，但会增加扫描时间；

D、Direction：双向扫描可以减少扫描时间；

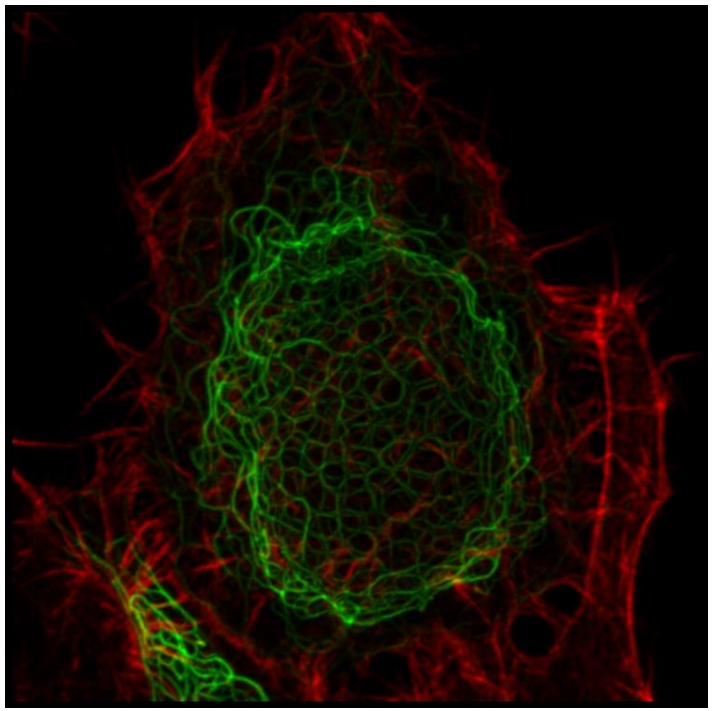
E、Frame Size：一般选择512×512或1024×1024，图像越大，扫描时间越长

Acquiring Multi-Channel images

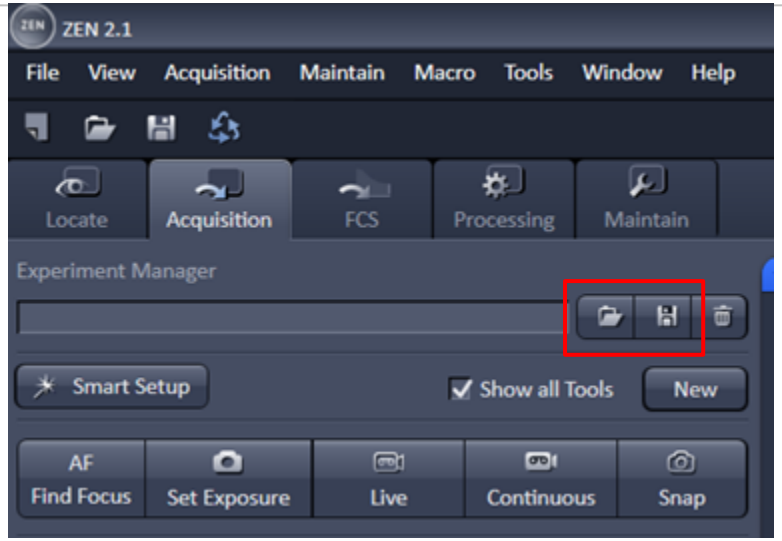




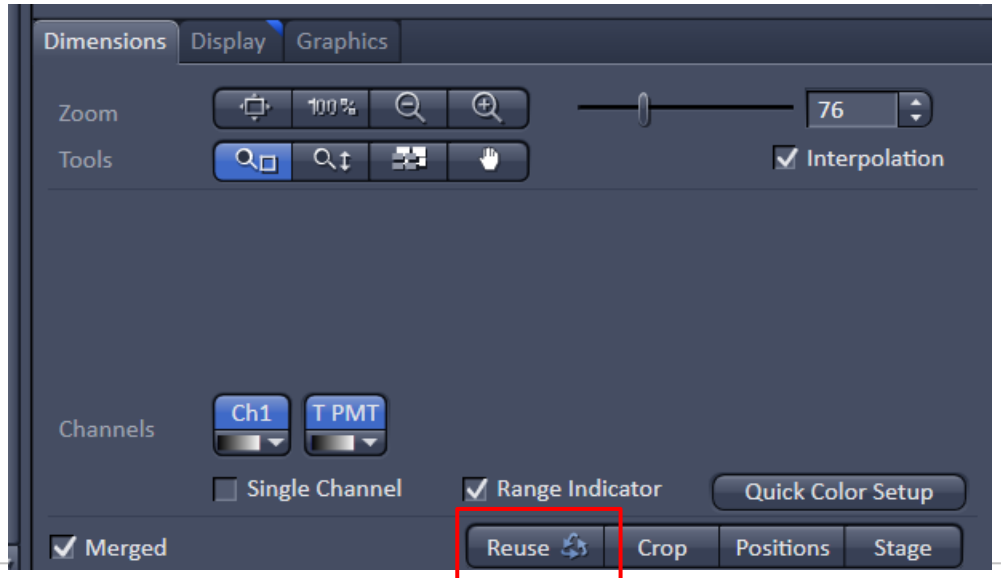
8、选择需要成像的track，单击“Snap”；获得一张多通道图像。



Experiment Manager and Reuse



可以通过Experiment Manager来保存拍摄的参数，或打开已经保存的图片（czi格式），通过“Reuse”来调用上次拍摄的参数设置



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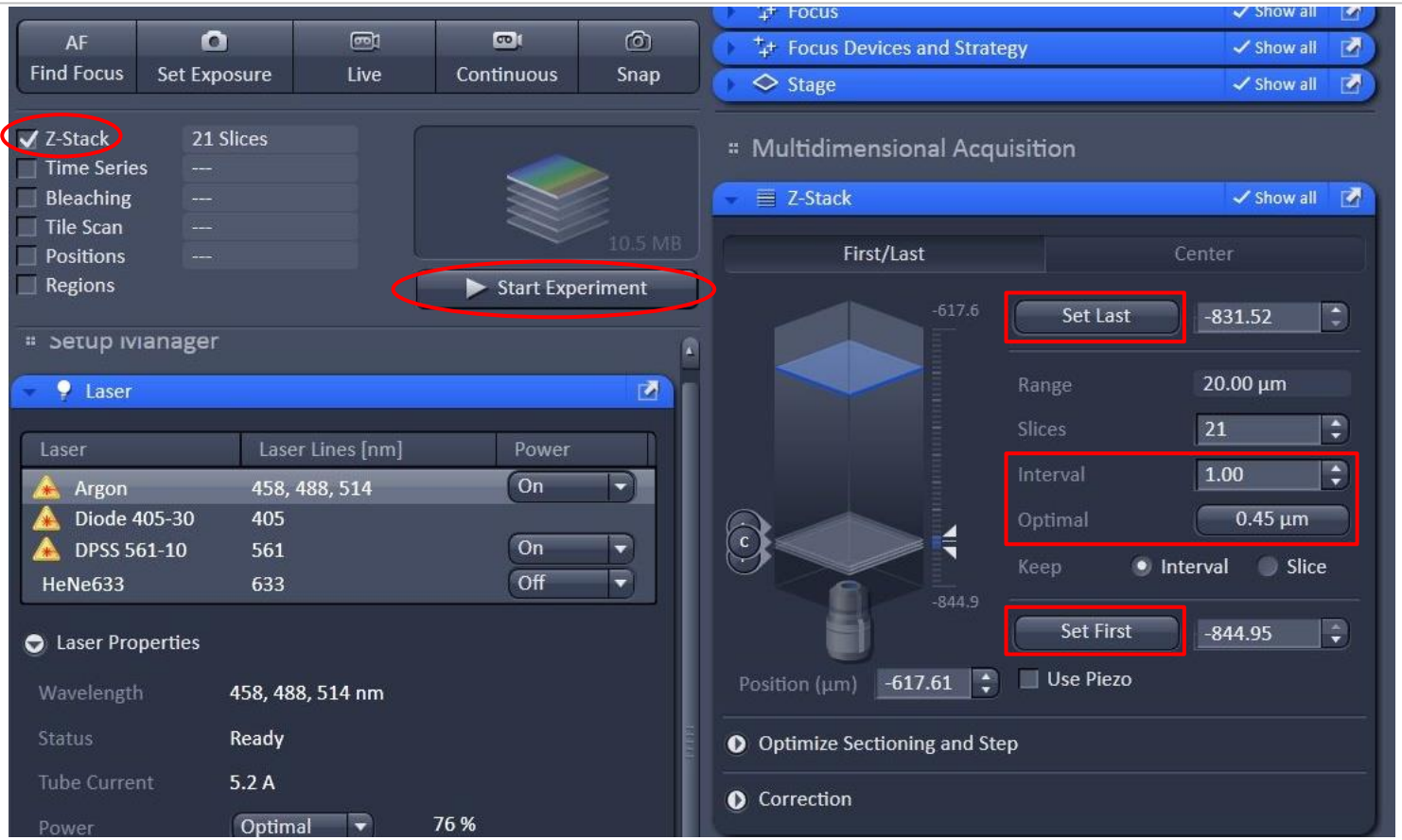
Z-stack image

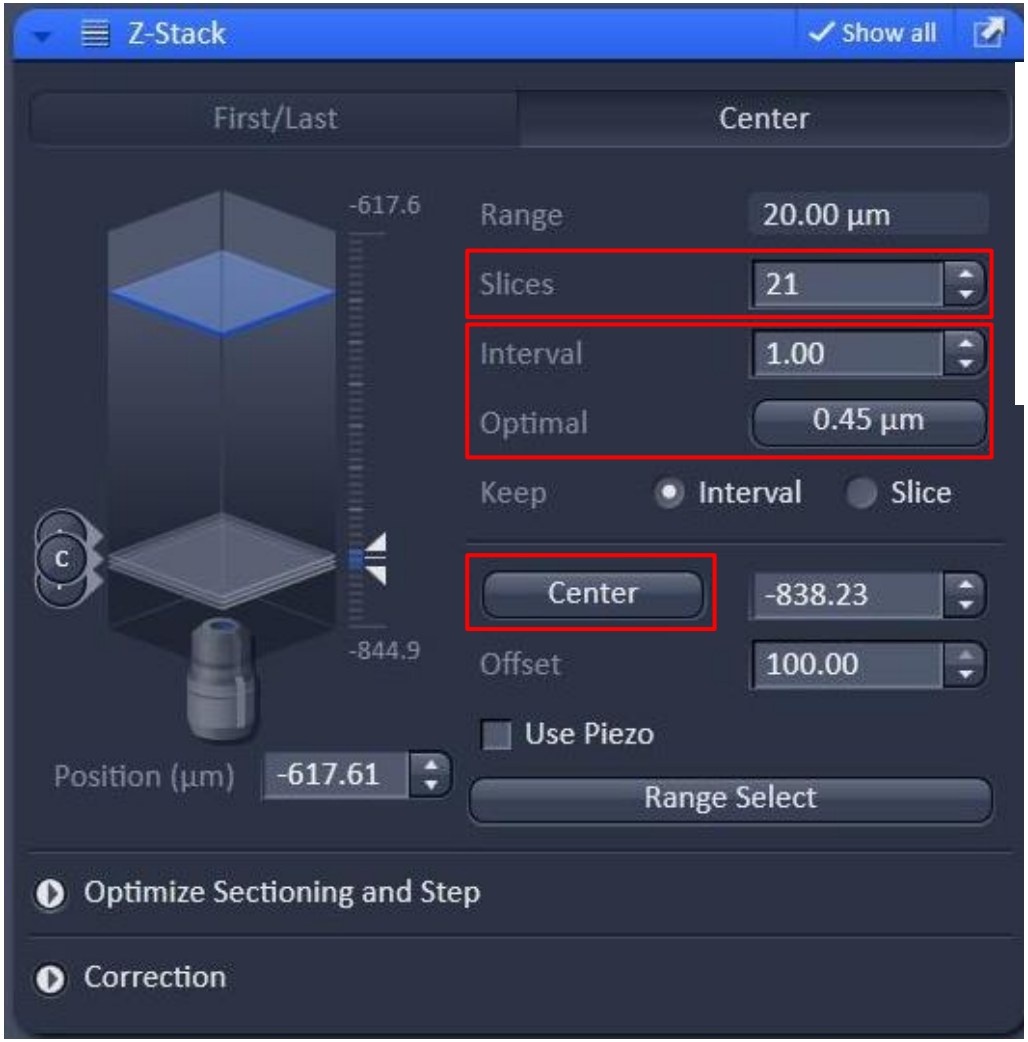
First/Last



1、选择Z-stack；
2、在live下调节焦距选择层扫图像的上下范围：“set first”和“set last”；
3、单击“optimal”让间距在最适合范围；
4、单击“Start Experiment”

Z-stack image

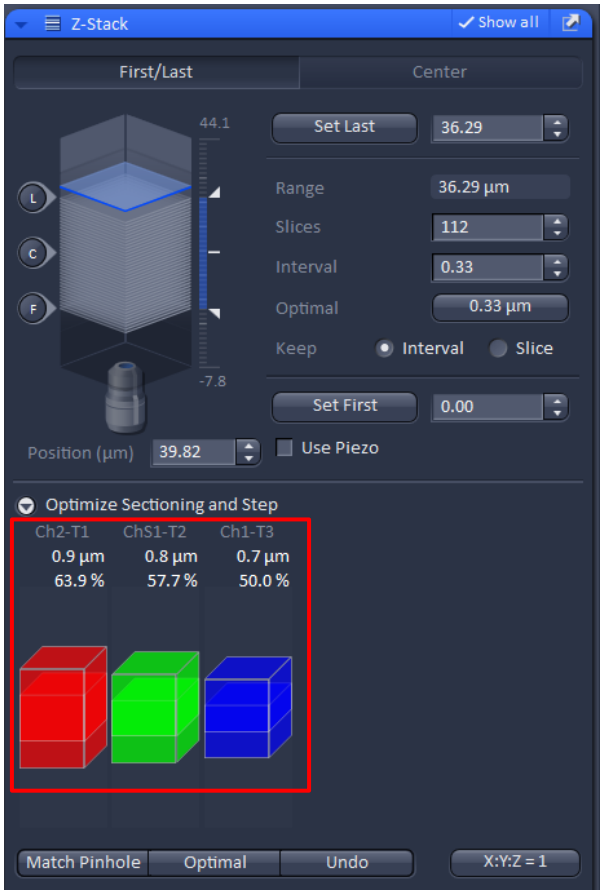
Center



1、center模式下，live下选择成像的中间位置，单击“center”，然后设置需要层扫的层数Slices，并单击“optimal”；
2、单击“Start Experiment”

Z-stack image

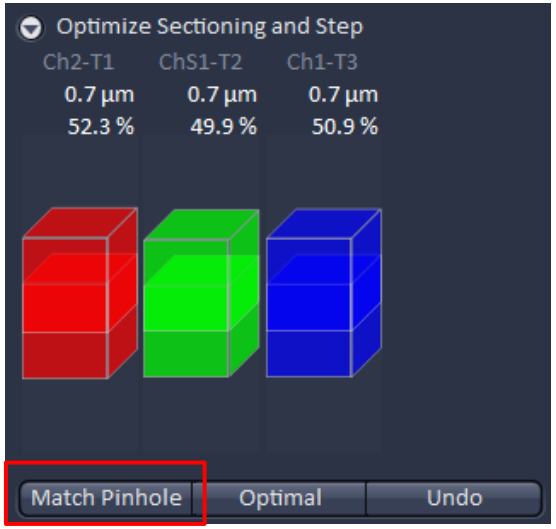
Match Pinhole



多通道荧光拍摄Z-stack需要考虑光切厚度不一致的问题：

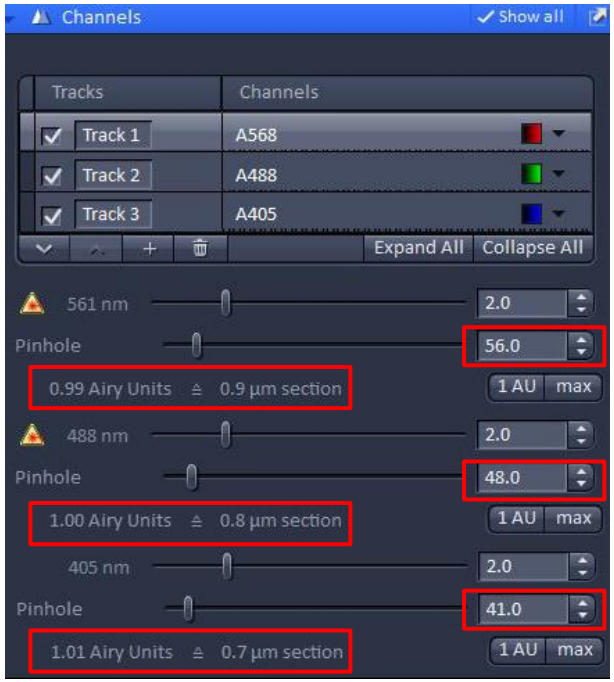
1、可以通过点击“Match Pinhole”自动调节不同track的针孔使光切厚度相似；

*这种方法的缺点在于可能会使长波长的针孔过于小，不利于弱荧光成像。

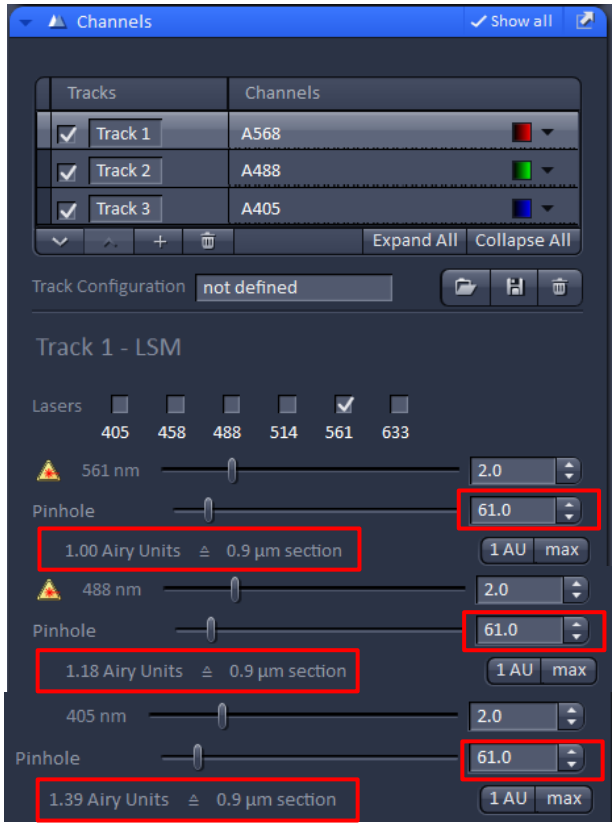


Z-stack image

Match Pinhole



2、通过手动调节针孔到一致，可以保证荧光强度的同时，保证光切厚度一致。



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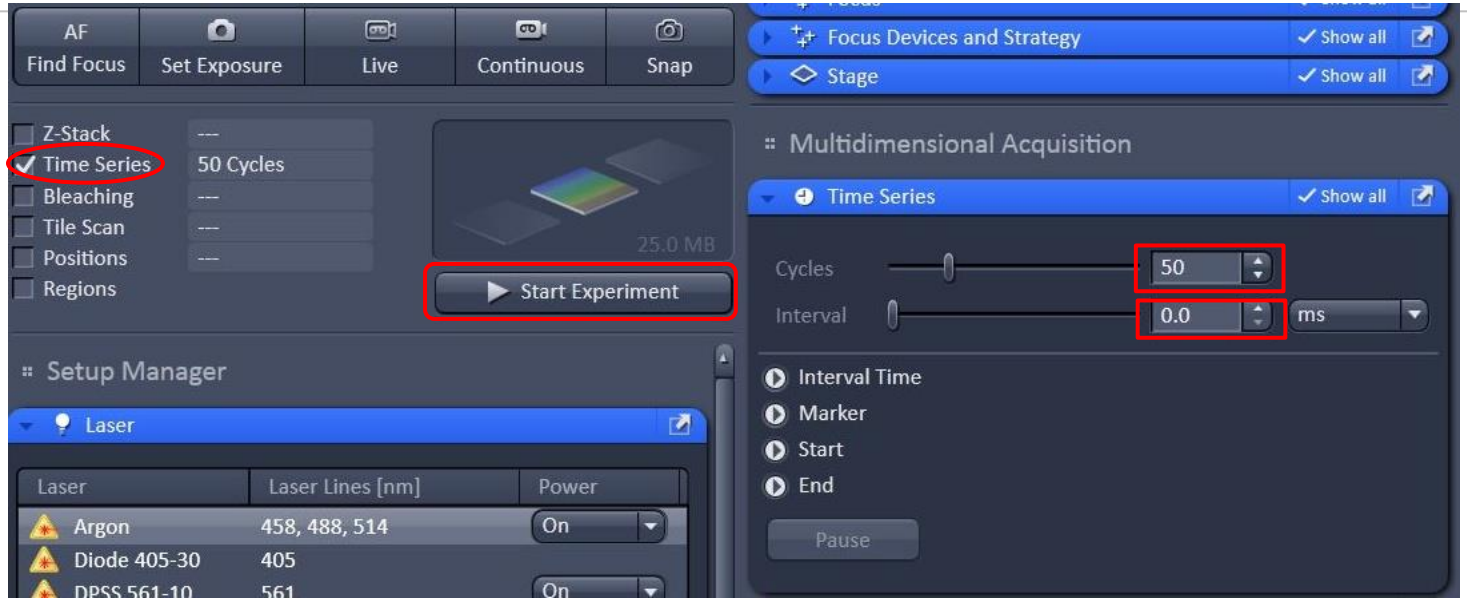
Time Series image



1、选择“Time Series”；
2、拍摄要进行的循环数cycles；
3、循环之间的间隔Interval；
interval：两次循环开始时间的间隔，因此interval包含了上一个循环的拍摄时间；
4、“Start Experiment”

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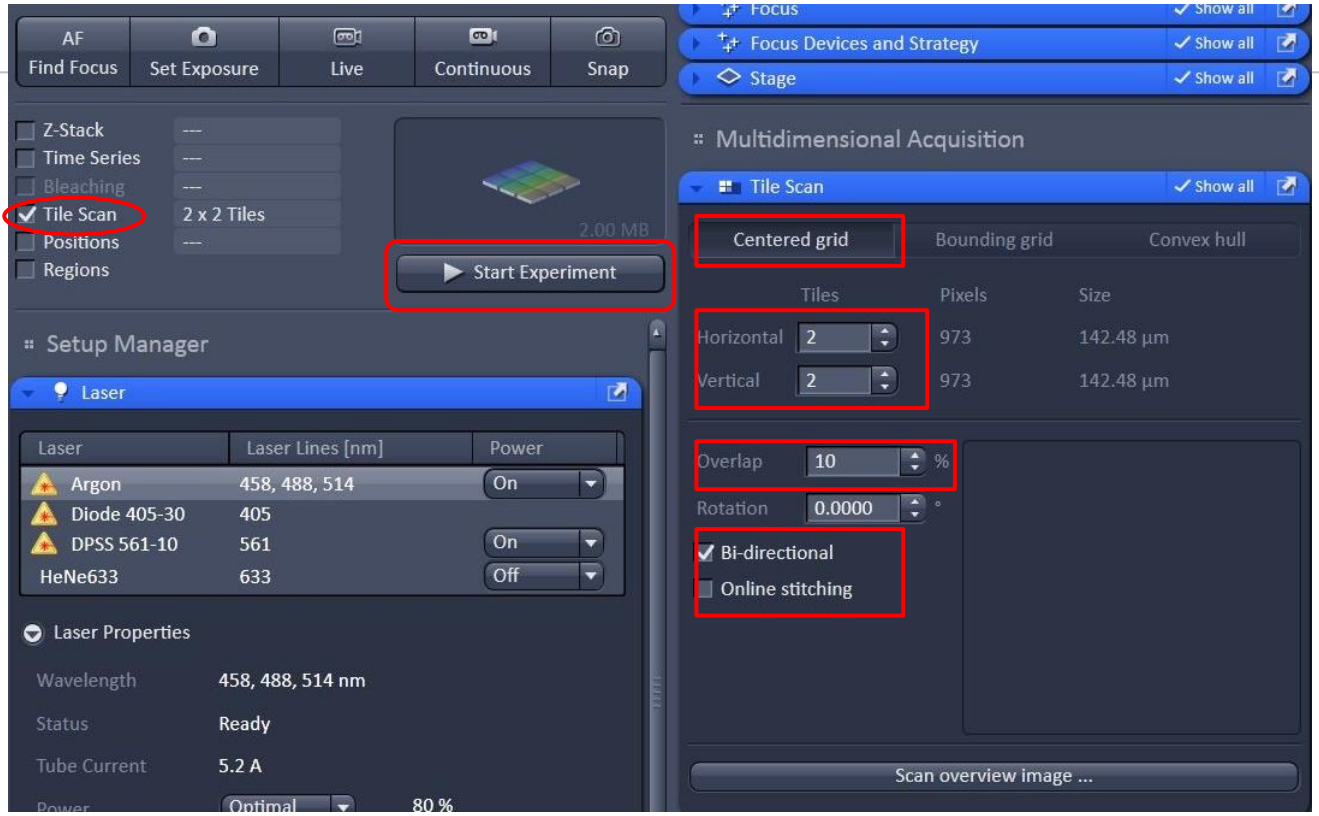
Tile Scan



1、选择“Tile Scan”；

2、Centered grid下以现在视野为中心，拍摄拼图：

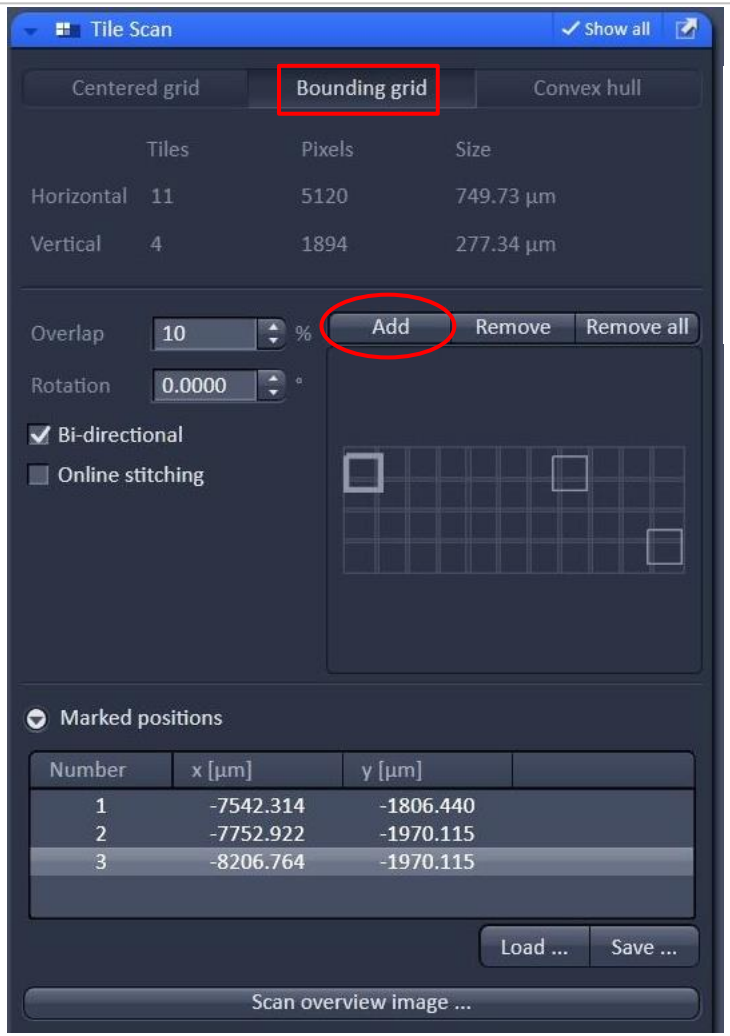
A、Horizontal & Vertical 水平和垂直拍摄范围；

B、拼图之间的重叠overlap、是否双向拼图Bi-directional以及拍摄后拼接Online stitching；

3、“Start Experiment”

Tile Scan

Bounding grid

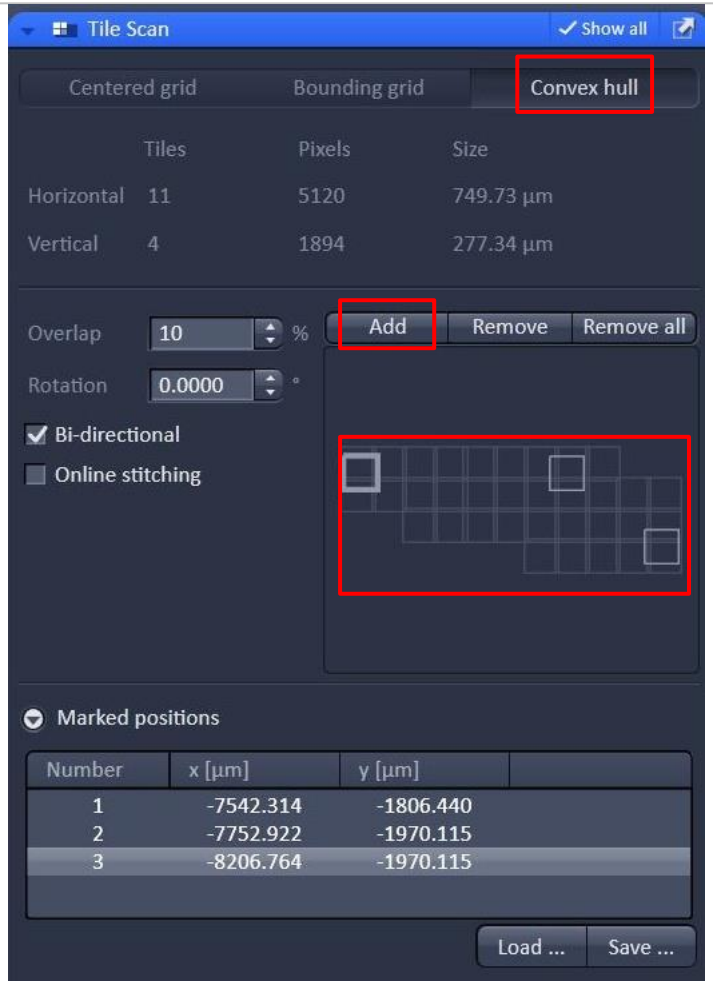


1、Bounding grid下通过移动载物台到想拼接的图像边缘，单击“Add”，添加边缘范围。软件自动定义拼图范围。

2、“Start Experiment”。

Tile Scan

Convex hull



1、Convex hull类似Bounding grid，通过移动载物台到想拼接的图像边缘，单击Add，添加边缘范围点。软件自动定义拼图范围。不同于Bounding grid软件定义拼图范围会更接近选择的位置点。

2、“Start Experiment”。

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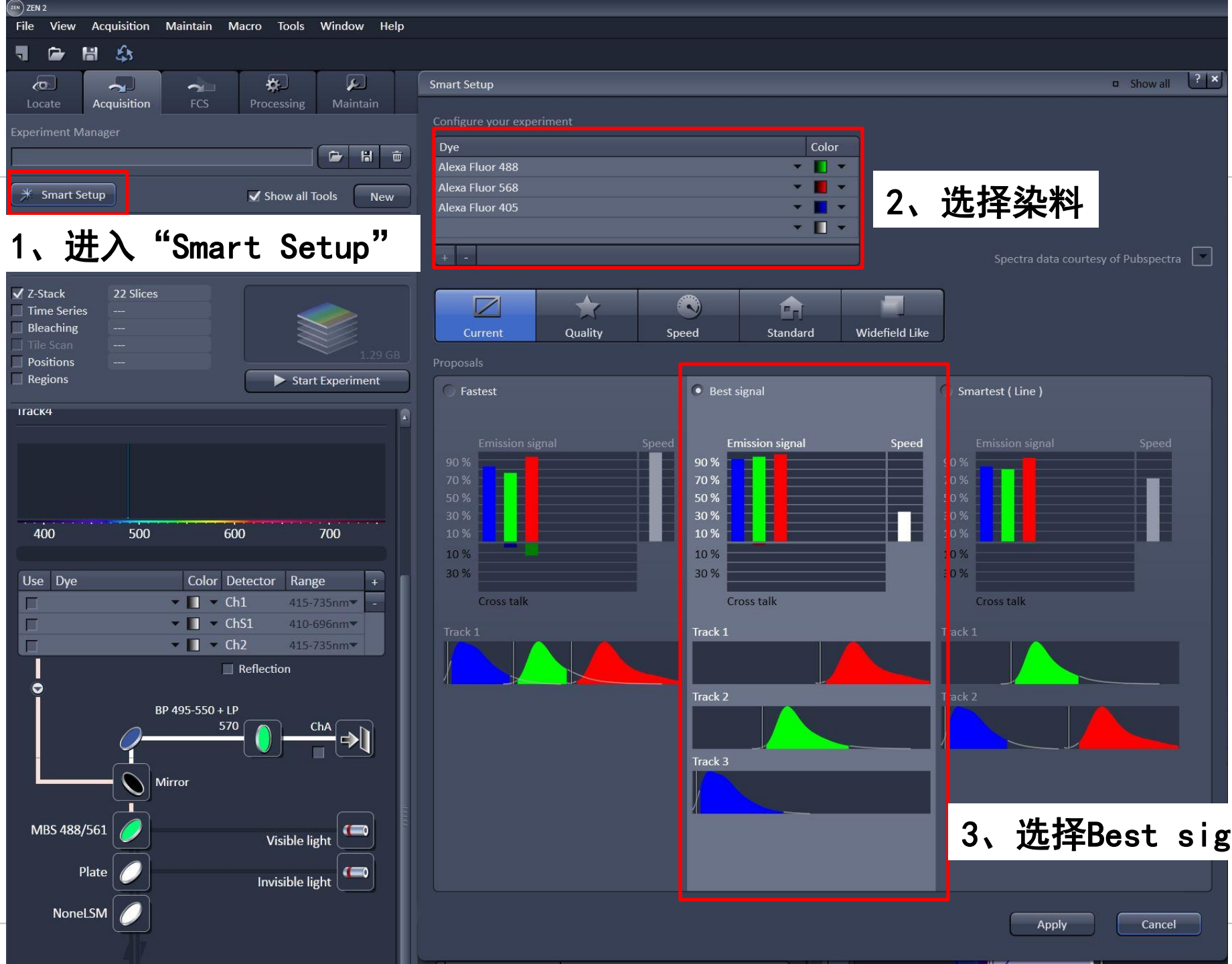
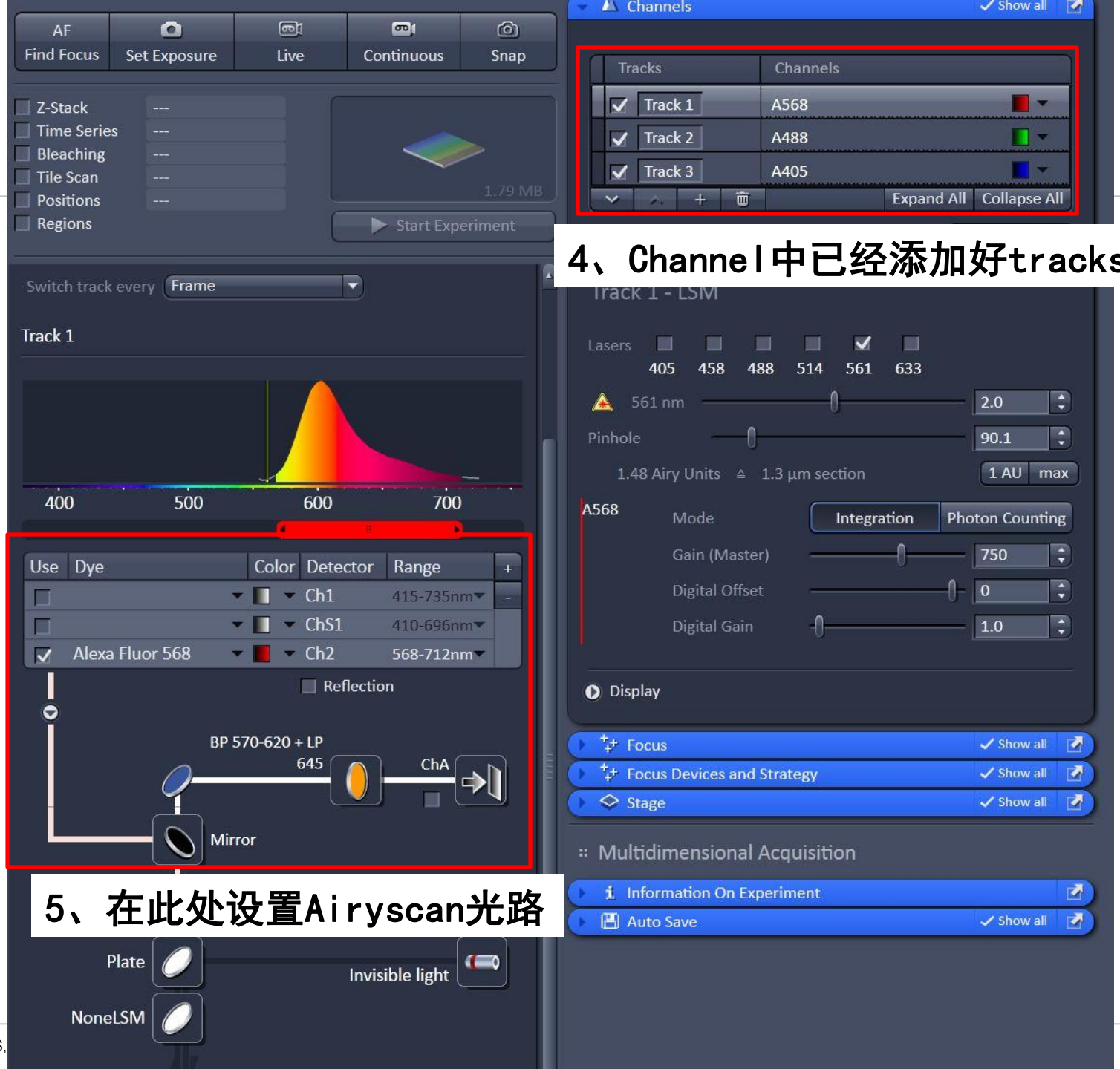
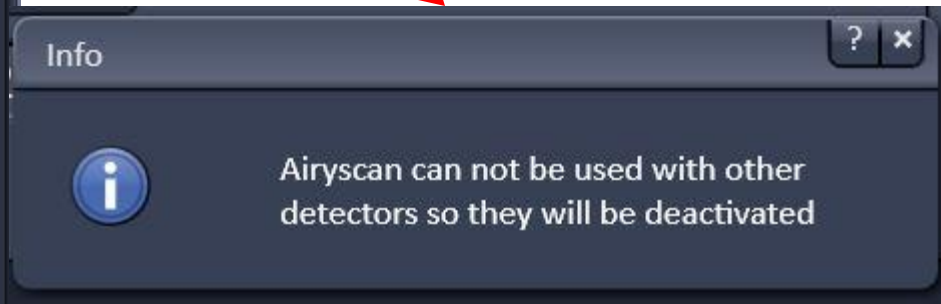
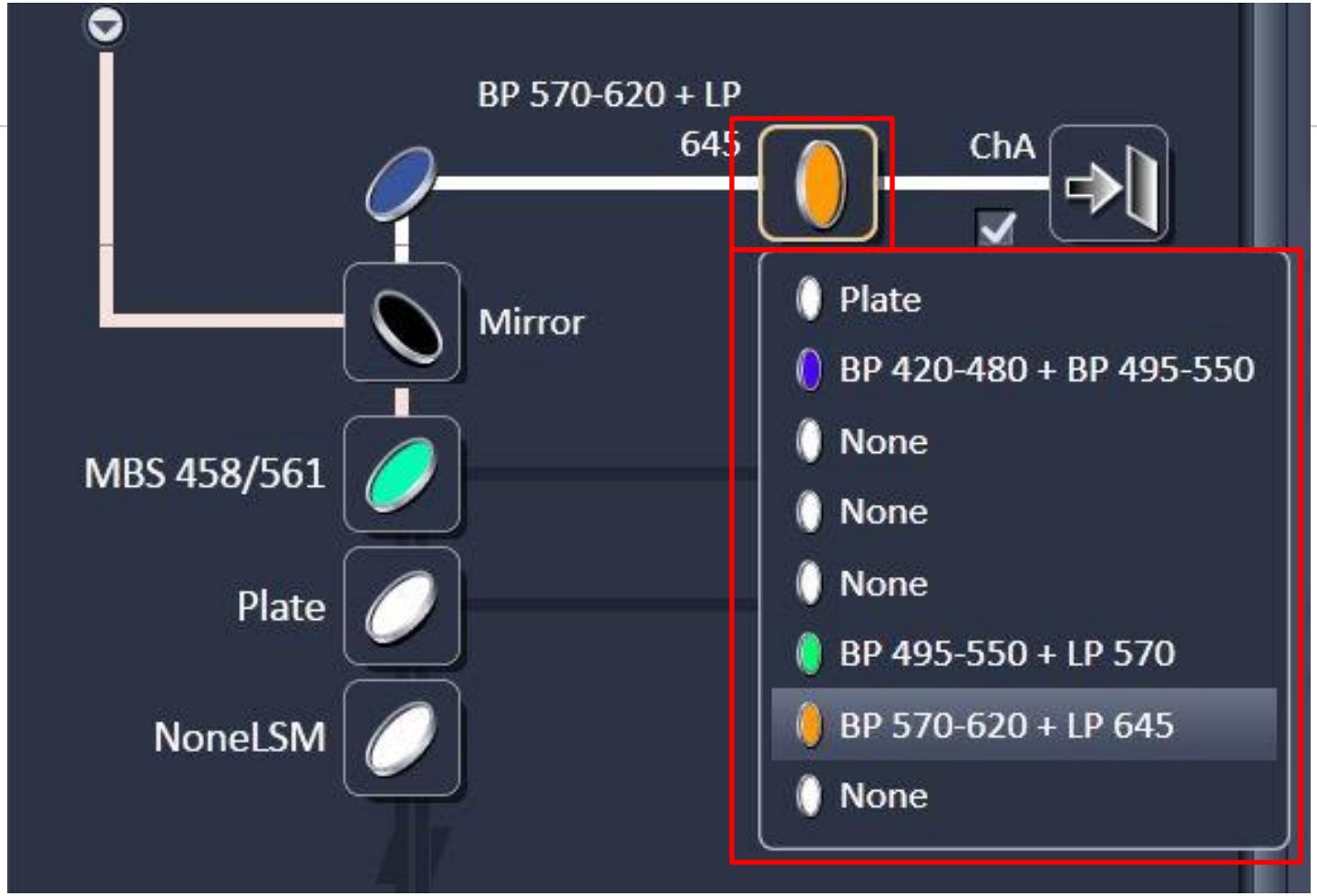

ZEN 2
File View Acquisition Maintain Macro Tools Window Help
Locate Acquisition FCS Processing Maintain
Experiment Manager
Smart Setup
Show all Tools
New
1、进入“Smart Setup”
Z-Stack 22 Slices
Time Series ---
Bleaching ---
Tile Scan ---
Positions ---
Regions ---
1.29 GB
Start Experiment
Track4
400 500 600 700
Use Dye Color Detector Range
Ch1 415-735nm
ChS1 410-696nm
Ch2 415-735nm
Reflection
BP 495-550 + LP 570
ChA
Mirror
MBS 488/561
Visible light
Plate
Invisible light
NoneLSM
Smart Setup
Show all
Configure your experiment
Dye Color
Alexa Fluor 488
Alexa Fluor 568
Alexa Fluor 405
2、选择染料
Spectra data courtesy of Pubspectra
Current Quality Speed Standard Widefield Like
Proposals
Fastest
Best signal
Smartest (Line)
Emission signal Speed
90 % 70 % 50 % 30 % 10 % 10 % 30 %
Cross talk
Track 1
Track 2
Track 3
3、选择Best signa
Apply
Cancel


AF
Find Focus
Set Exposure
Live
Continuous
Snap
Z-Stack
Time Series
Bleaching
Tile Scan
Positions
Regions
1.79 MB
Start Experiment
Channels
Show all
Tracks
Channels
Track 1
A568
Track 2
A488
Track 3
A405
Expand All
Collapse All
4、Channel中已经添加好tracks
Switch track every
Frame
Track 1
400
500
600
700
Use
Dye
Color
Detector
Range
Ch1
415-735nm
ChS1
410-696nm
Alexa Fluor 568
Ch2
568-712nm
Reflection
BP 570-620 + LP
645
ChA
Mirror
5、在此处设置Airyscan光路
Plate
NoneLSM
Invisible light
Track 1 - LSM
Lasers
405
458
488
514
561
633
561 nm
2.0
Pinhole
90.1
1.48 Airy Units
1.3 µm section
1 AU
max
A568
Mode
Integration
Photon Counting
Gain (Master)
750
Digital Offset
0
Digital Gain
1.0
Display
Focus
Show all
Focus Devices and Strategy
Show all
Stage
Show all
Multidimensional Acquisition
Information On Experiment
Auto Save
Show all

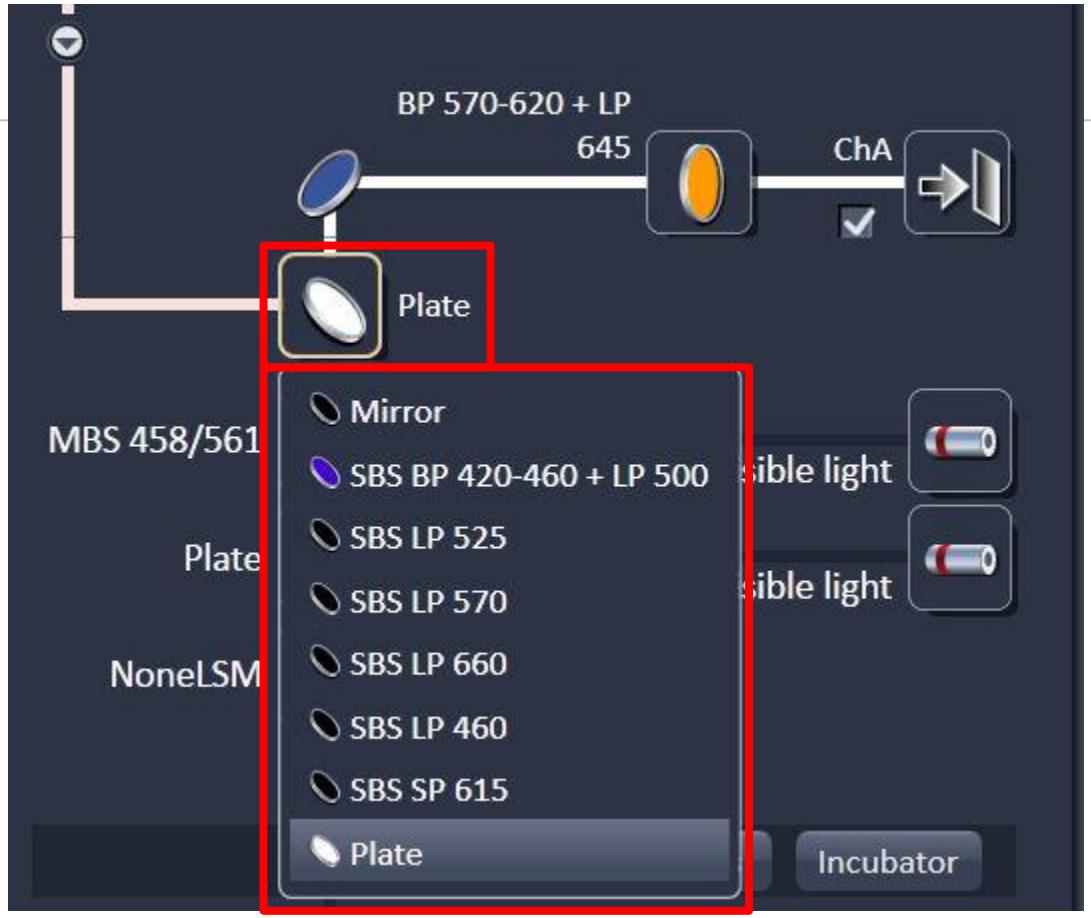
7、软件会自动关闭其他检测器，并在右下角提示“airyscan不能与其他检测器共用”

6、选择ChA检测器（即Airyscan检测器）

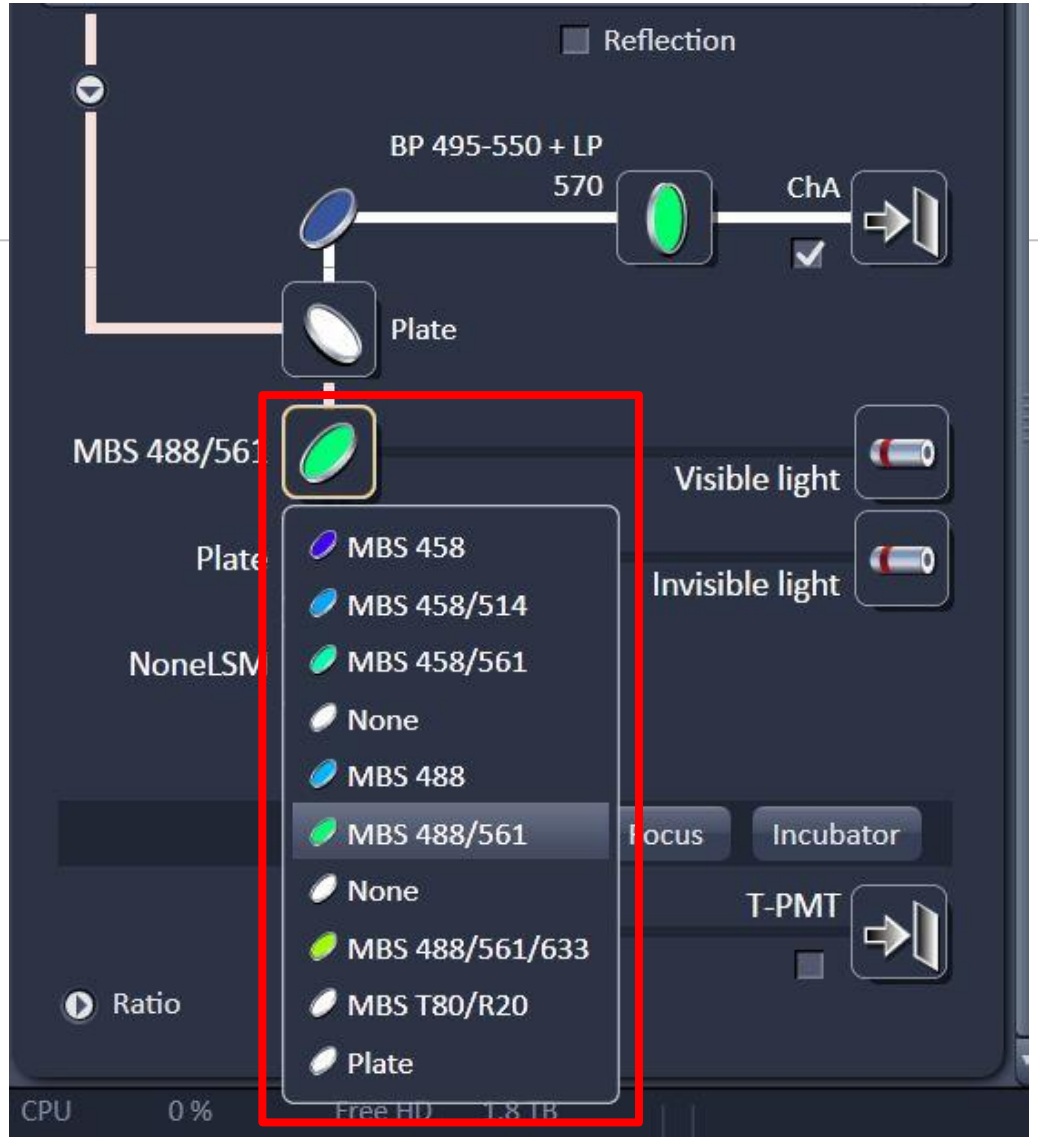




8、选择适合的滤片，如BP420-480代表蓝色波段，BP495-550代表绿色波段，BP 570-620 代表红色波段



9、次级分色镜（SBS）选择“Plate”



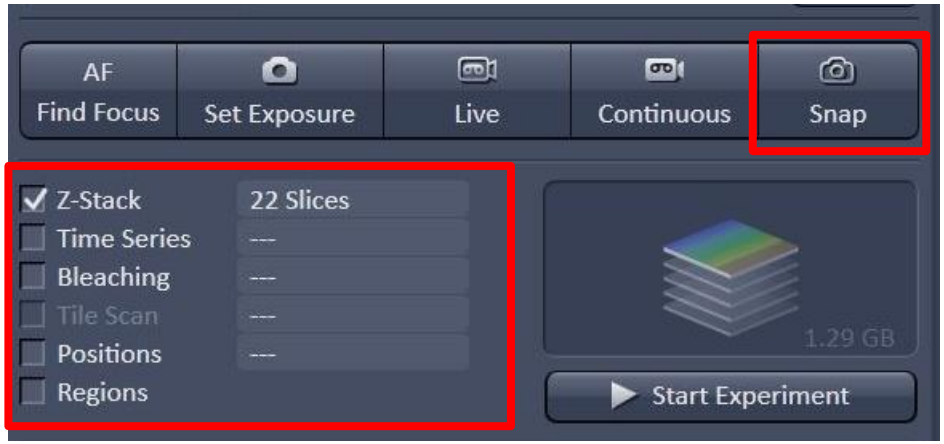
10、如果是多色荧光成像，可以选择多激光反射主分色镜（MBS），如图中MBS可以同时反射488和561，减少MBS转换

11、不要更改针孔（Pinhole）大小，Airyscan Mode确认是“SR”模式

13、Frame Size选择“optimal”

14、Speed选择“Max”

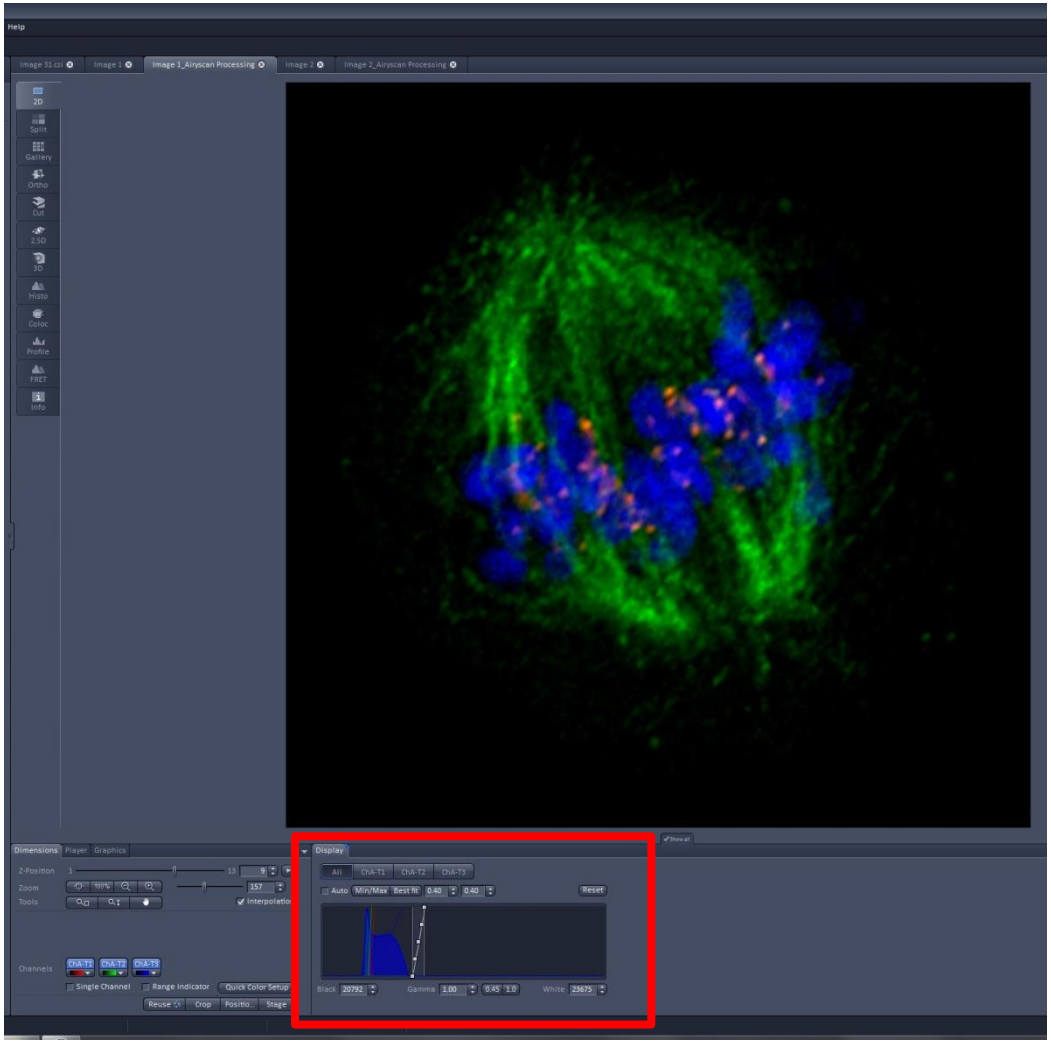
12、Zoom不能小于1.8



15、随后可以进行二维图像拍摄（Snap）或者多维图像拍摄


ZEN 2
File View Acquisition Maintain Macro Tools Window
Locate Acquisition FCS Processing Maintain
Experiment Manager
AF Find Focus Set Exposure Live Continuous Snap
Z-Stack 13 Slices
Time Series
Bleaching
Tile Scan
Positions
Regions
1.24 GB
Start Experiment
Setup Manager
16、拍摄后的airyscan图像需要在“Processing”中进行后续处理

17、按照“Processing → Airyscan Processing → Select → Apply”进行图像处理。如果是z-stack图像，请选择“3D”



18、处理后的图像，在Display处选择Min/Max或者手动调节显示效果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