

# LSM 880 Basic Operation



MultiChannel & TimeSeries & Z-Stack & TileScan



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2016-05-15

- 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

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# Startup and Shutdown of the System



## Startup of the System

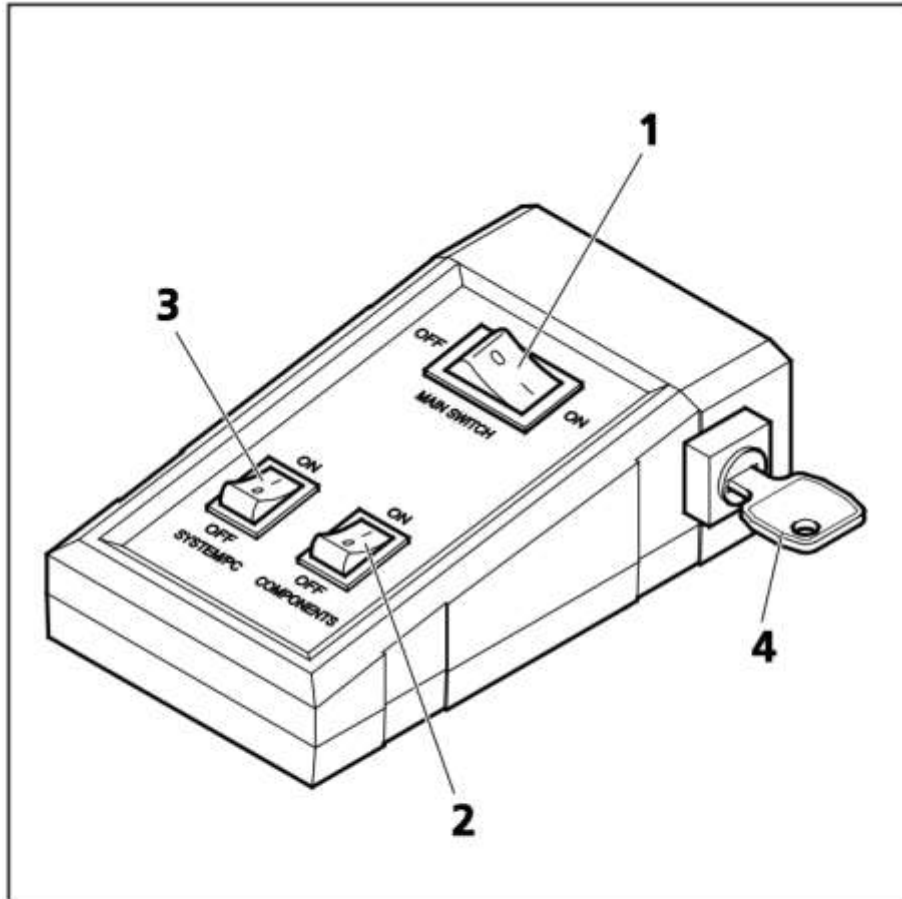


- 1、打开稳压电源：
  - a、打开稳压电源背后总开关；
  - b、打开稳压电源前面开关；
  - c、确定稳压电源显示220V；

# 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）；

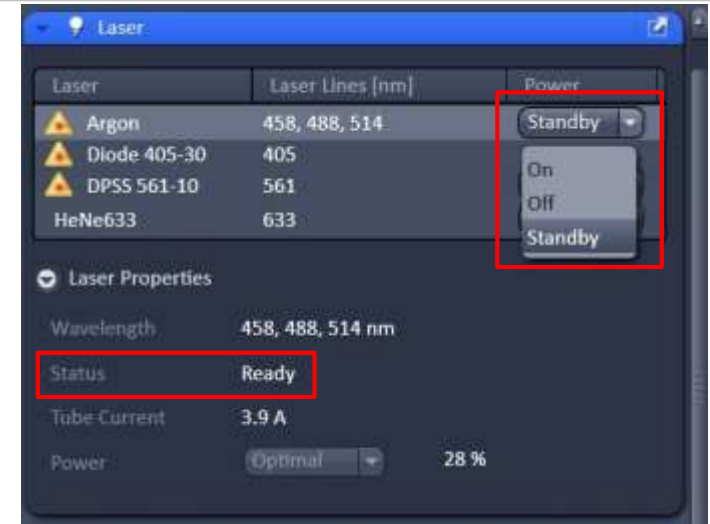
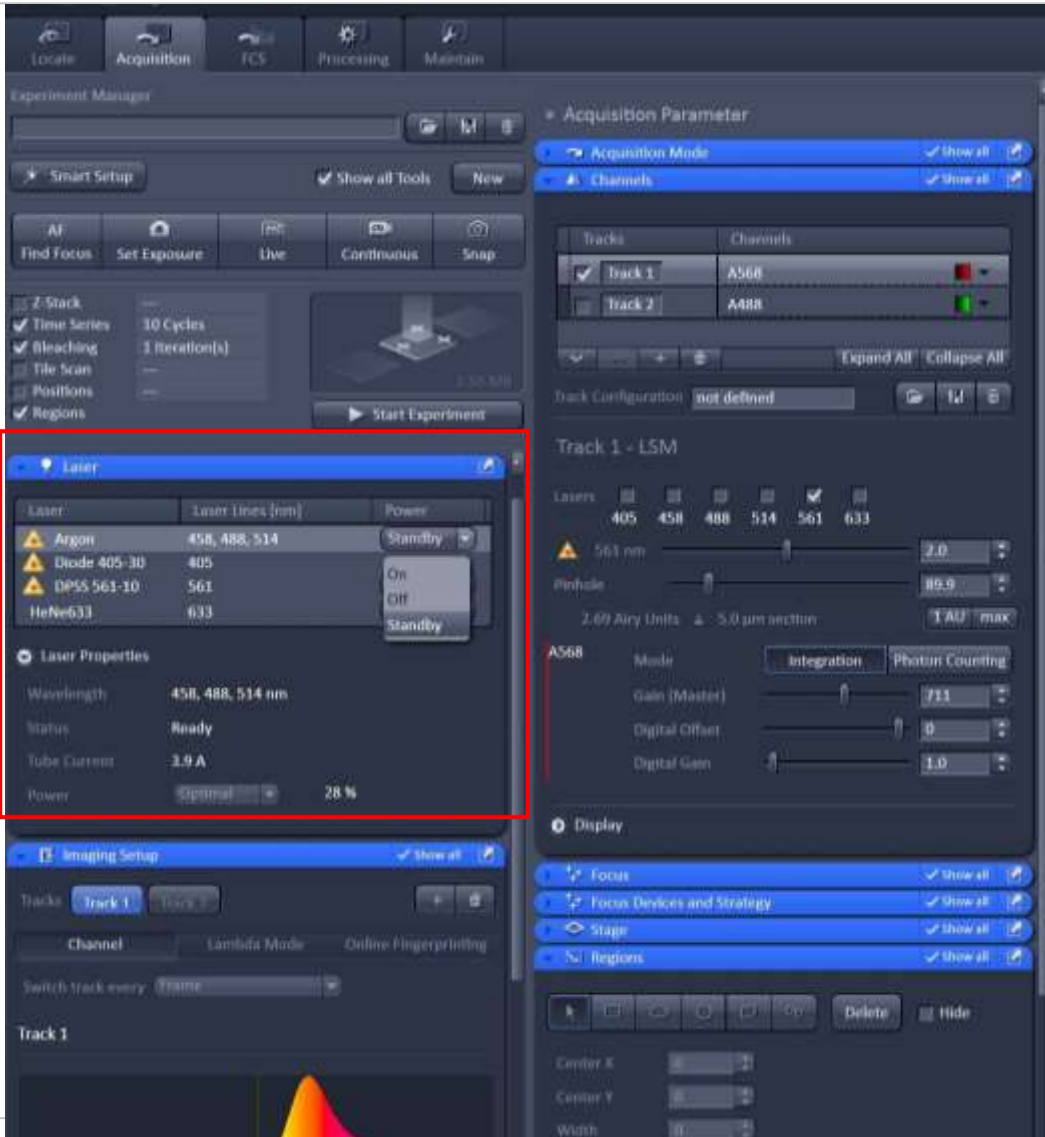


7、点击“Start System”

# 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；

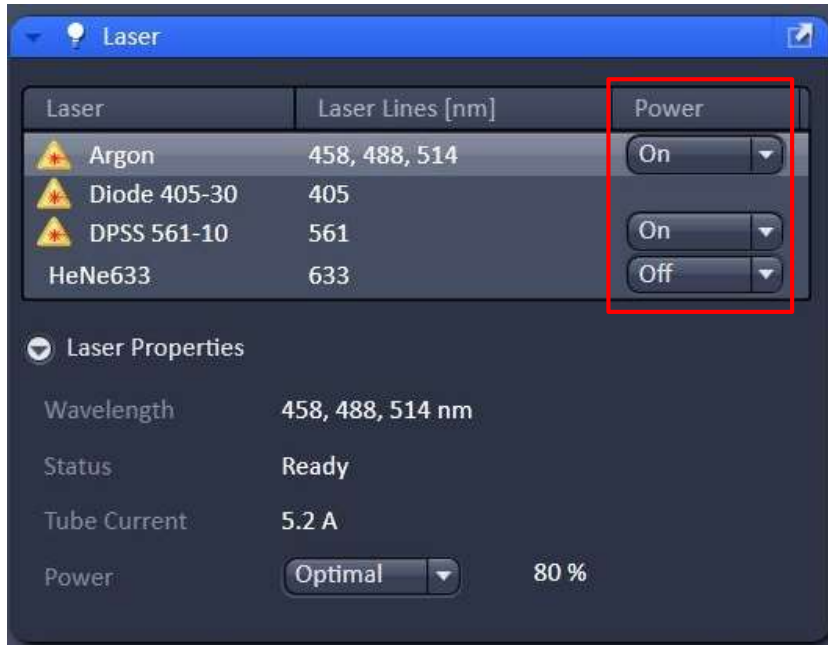
b、预热结束后“status”显示“Ready”后选择“On”；

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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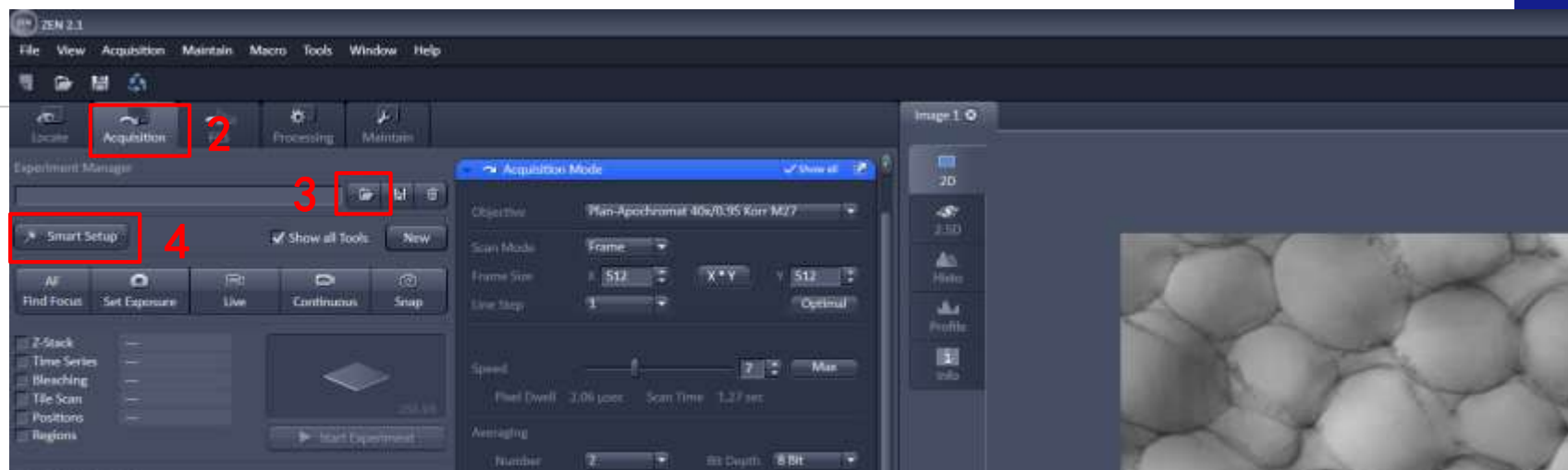
# Acquiring Multi-Channel images



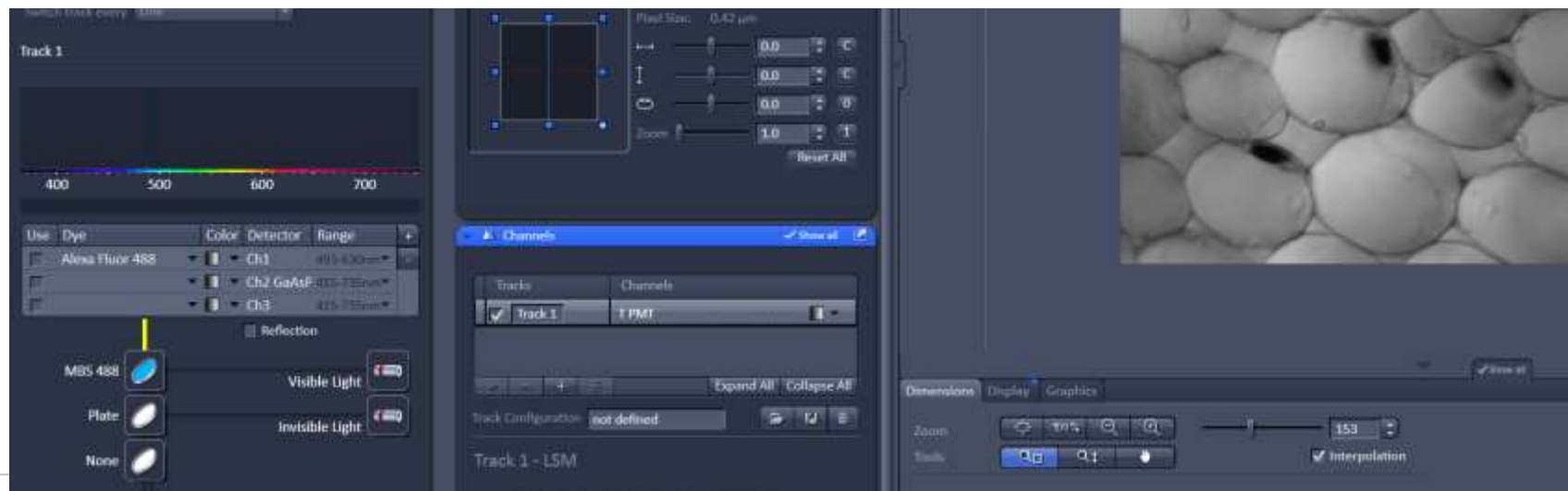
The screenshot displays the ZEISS software interface. The 'Locate' tab is selected and highlighted with a red box. Below it, the 'Configuration' section shows 'DIC II' selected, with buttons for 'BF', 'DAPI', 'GFP', 'DsRed', 'DIC II', and 'DIC III'. The 'Microscope Control' section shows a diagram of the microscope setup, including 'Aperture 0.55 DIC II', 'Plan-Apochromat 20x/0.8 M27', 'Analyzer module Pol ACR', and 'Lens 1x'. The main view shows a dark image of a sample with a small bright spot in the center.

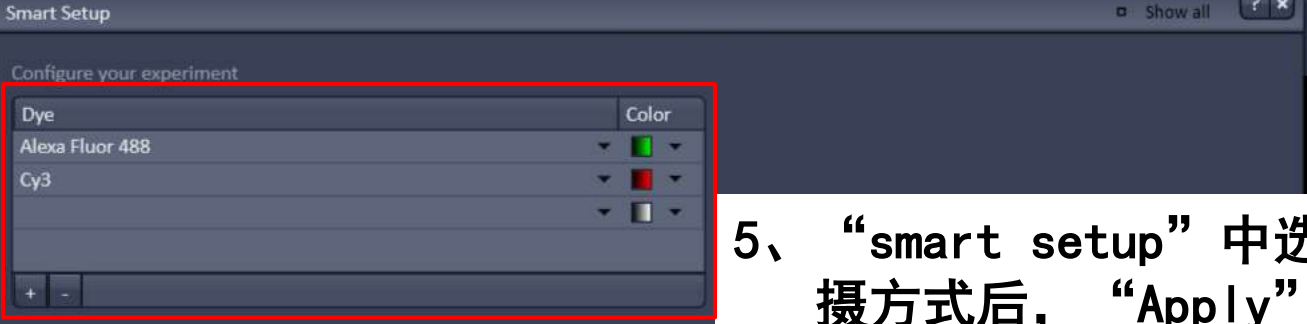
1、在“Locate”界面下选择快捷键，在镜下观察需要拍摄的样品区域，把需要拍摄的区域放在视野中央。

# Acquiring Multi-Channel images

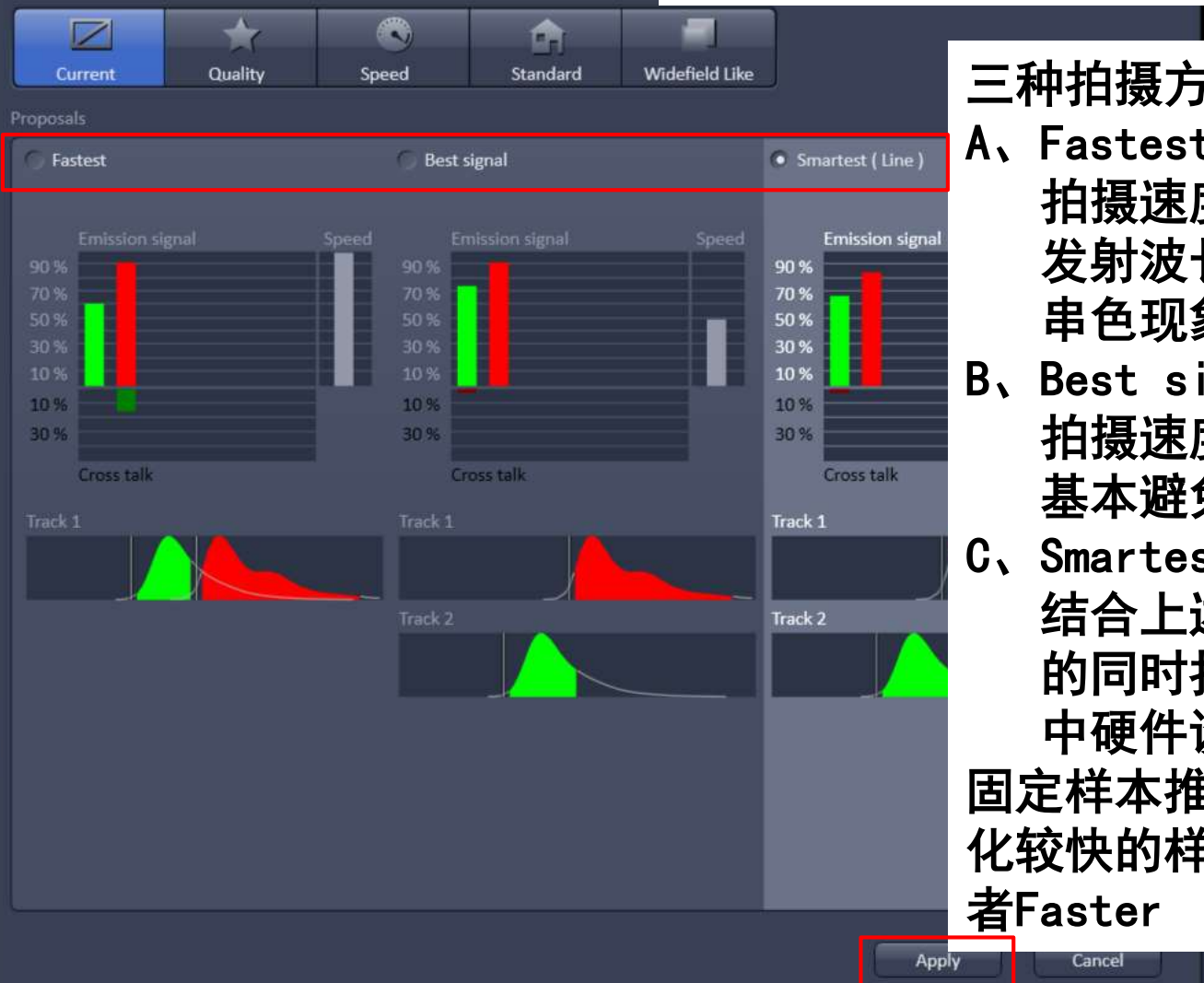


- 2、进入“Acquisition”界面；
- 3、选择之前保存好的光路设置→“channel”和“Acquisition mode”设置
- 4、或者新建光路设置→“Smart Setup”





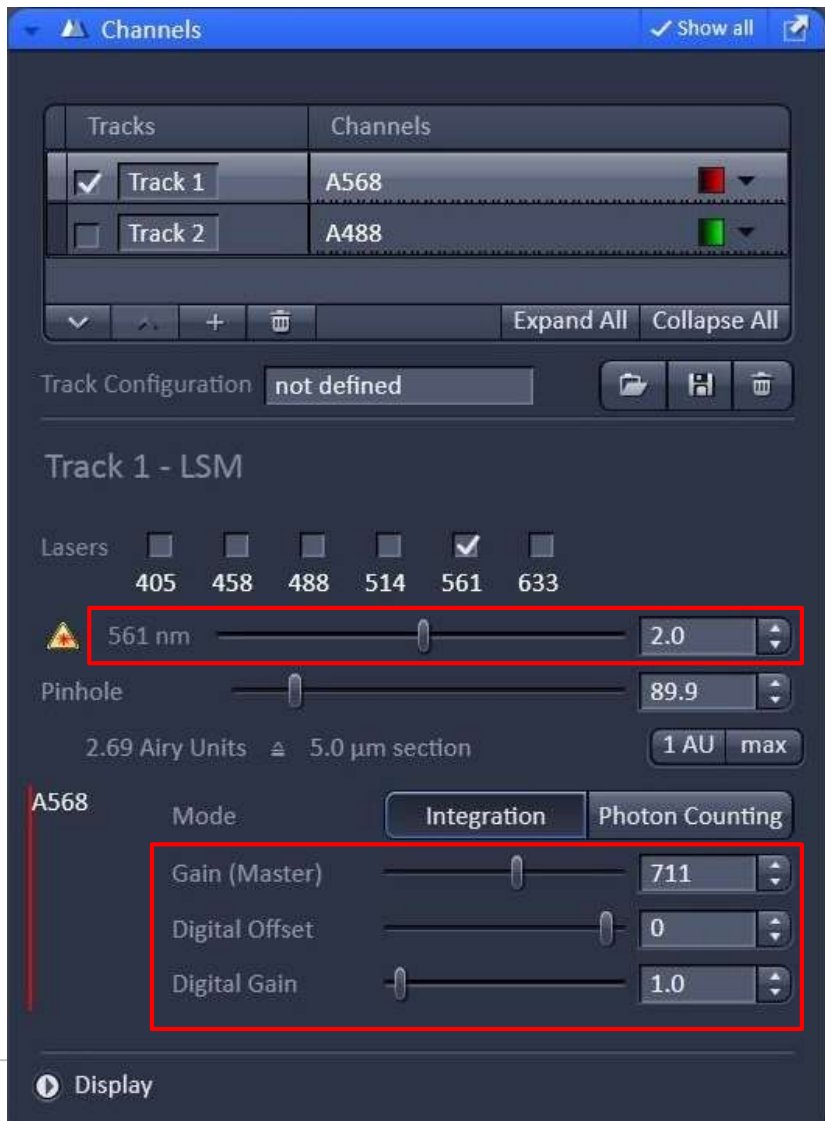
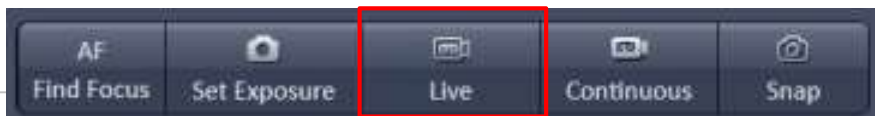
5、“smart setup”中选择染料名称，并选择拍摄方式后，“Apply”



### 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

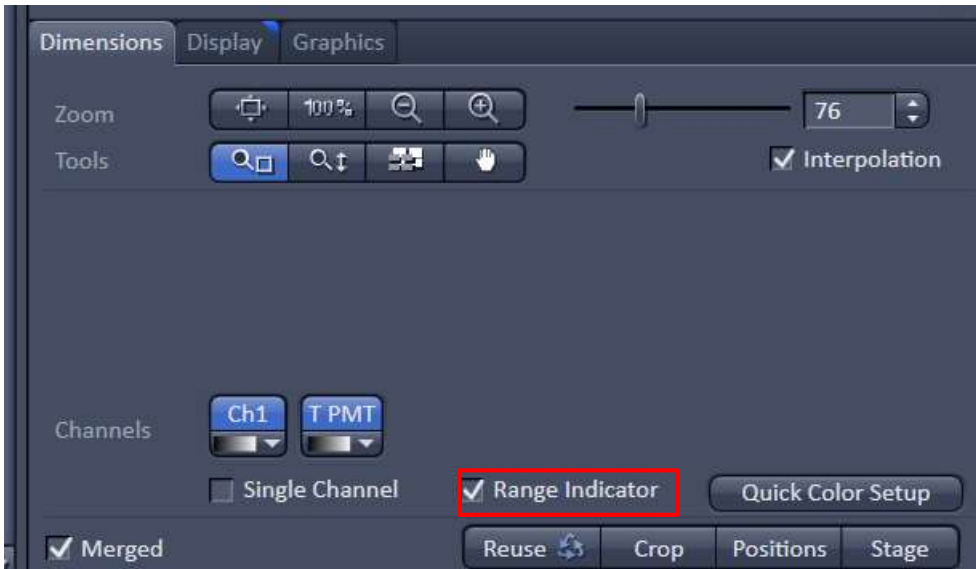
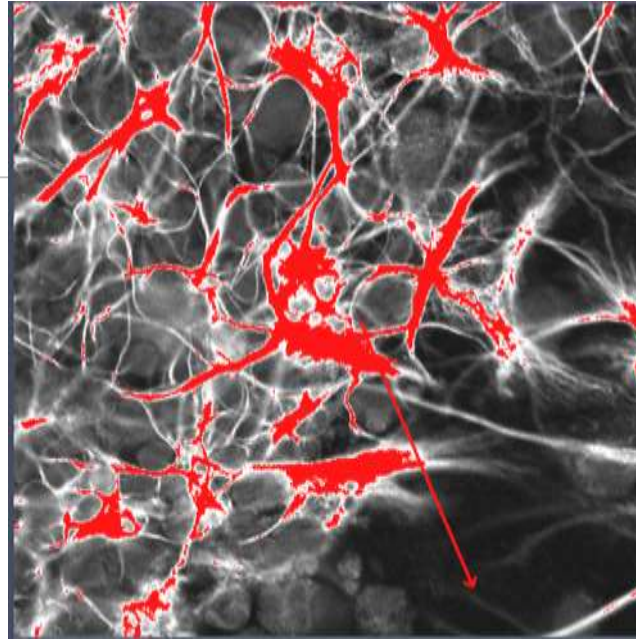
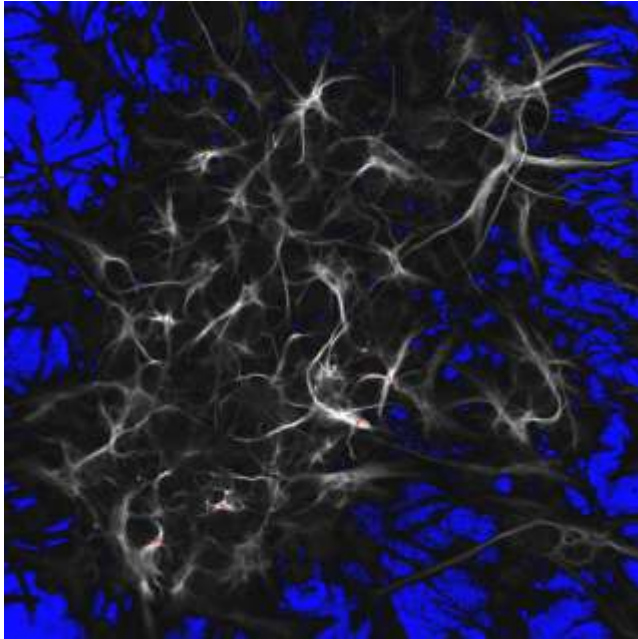
# Channels



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。

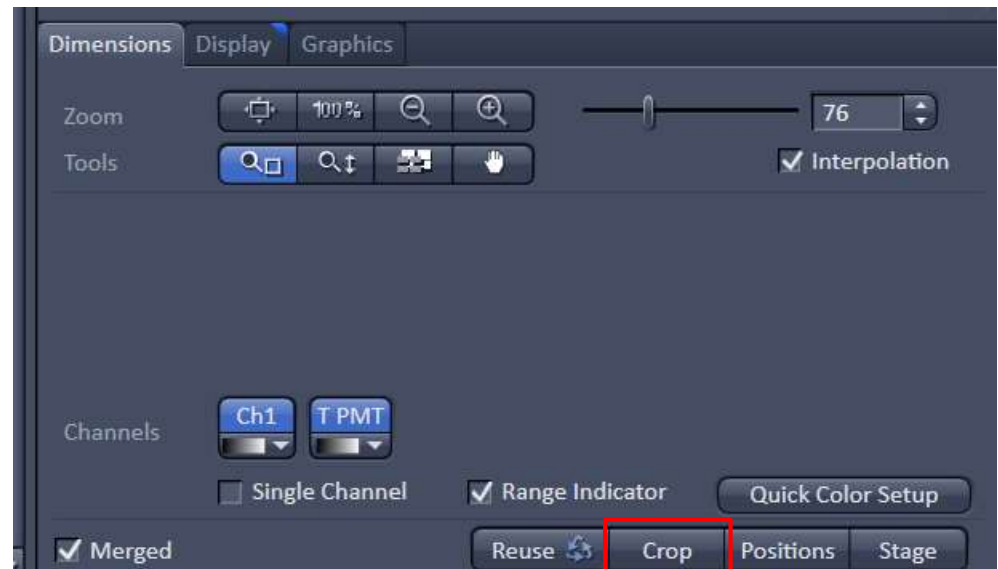


设置原则保证图像不要过曝，尽量  
减少背景噪音：  
在live下选择“range indicator”  
可以显示出曝光程度；

# 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；



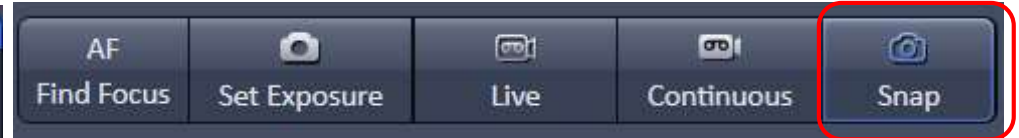
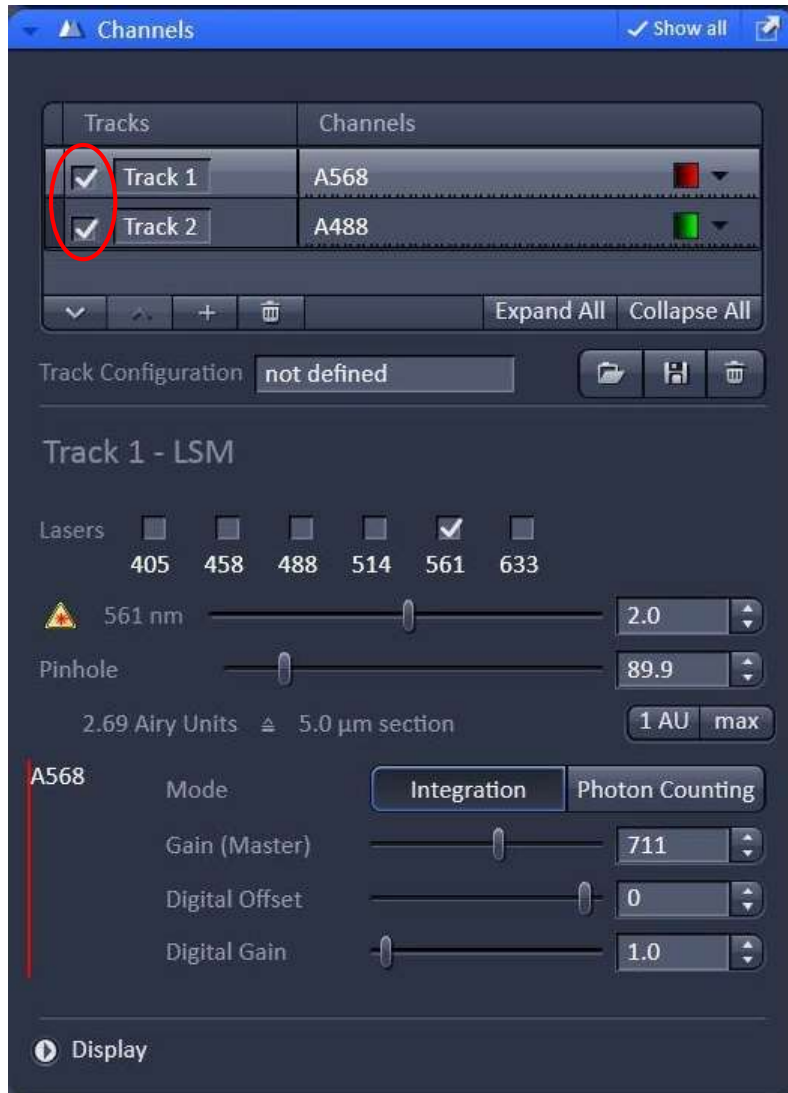
# Acquisition Mode



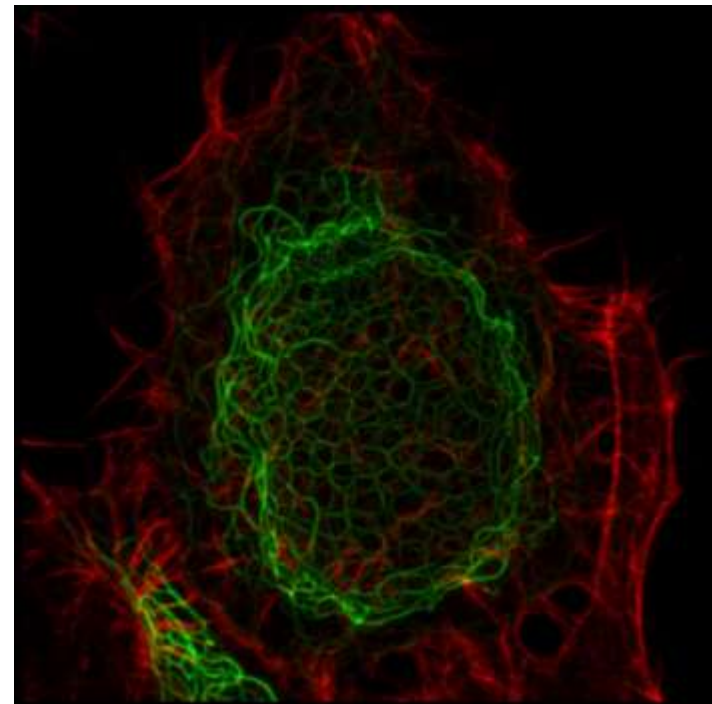
- B、设置speed: 扫描速度越慢, 信噪比越好, 但光漂白越多;
- C、Averaging: 增加averaging次数可以减少噪音, 但会增加扫描时间;
- D、Direction: 双向扫描可以减少扫描时间;
- E、Frame Size: 一般选择512×512或1024×1024, 图像越大, 扫描时间越长



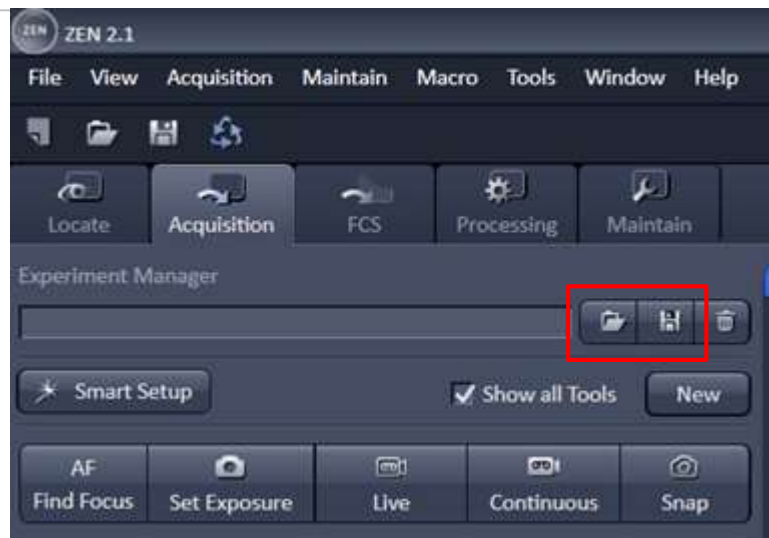
# Acquiring Multi-Channel images



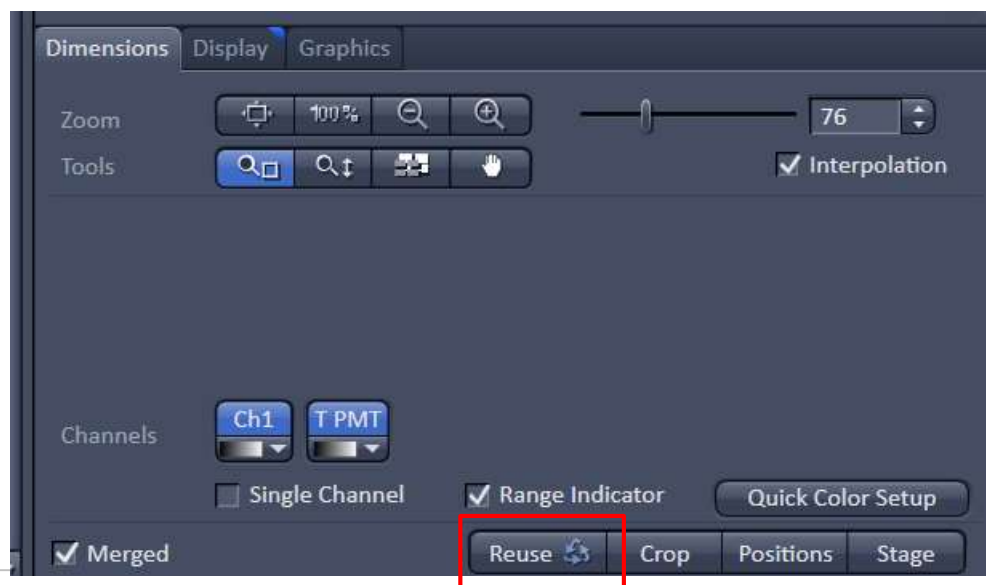
8、选择需要成像的track，单击“Snap”；  
获得一张多通道图像。



# 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

The screenshot shows the Zeiss software interface for Z-stack acquisition. The 'Z-Stack' checkbox is checked. The 'Start Experiment' button is highlighted. The 'Set Last' and 'Set First' buttons are highlighted. The 'Interval' dropdown is set to 1.00. The 'Optimal' button is highlighted. The 'Keep' radio buttons are set to 'Interval'.

Laser	Laser Lines [nm]	Power
Argon	458, 488, 514	On
Diode 405-30	405	On
DPSS 561-10	561	On
HeNe633	633	Off

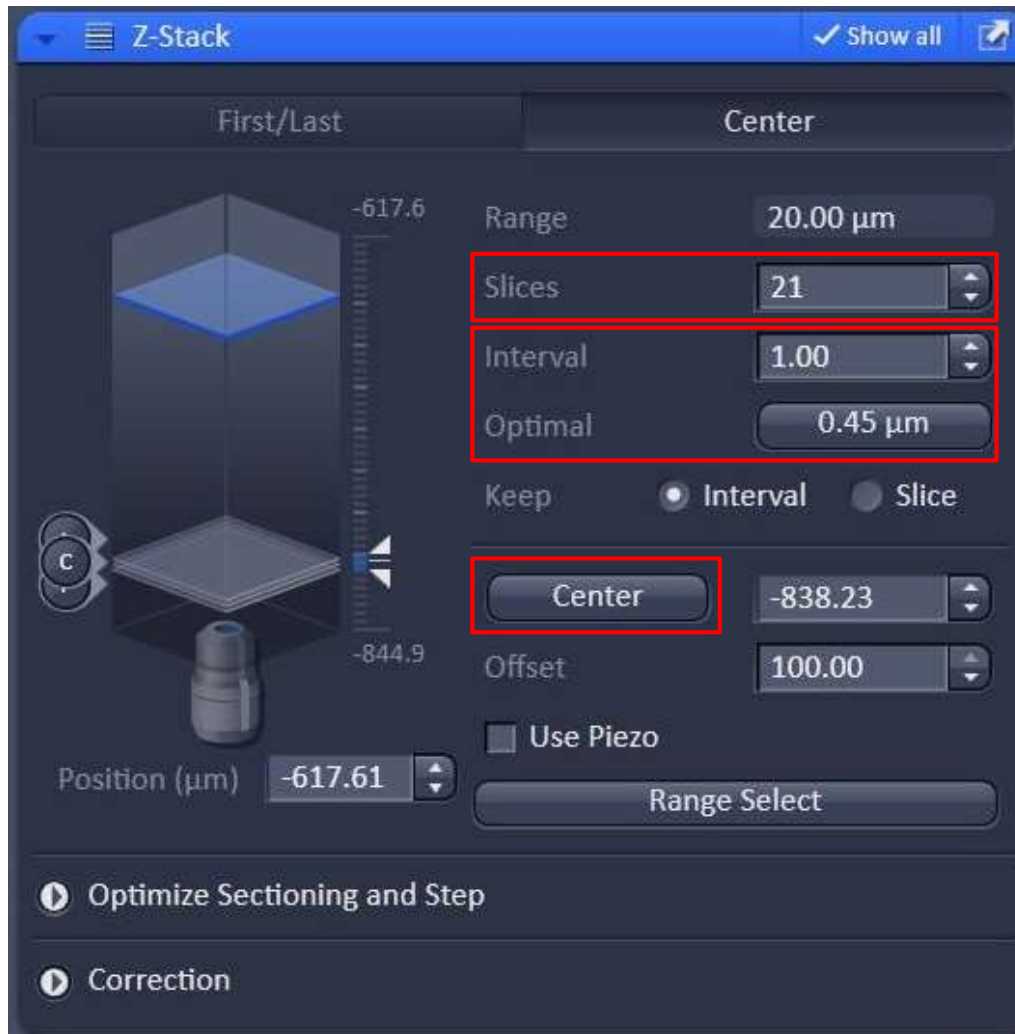
Property	Value
Wavelength	458, 488, 514 nm
Status	Ready
Tube Current	5.2 A
Power	Optimal 76 %

- 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



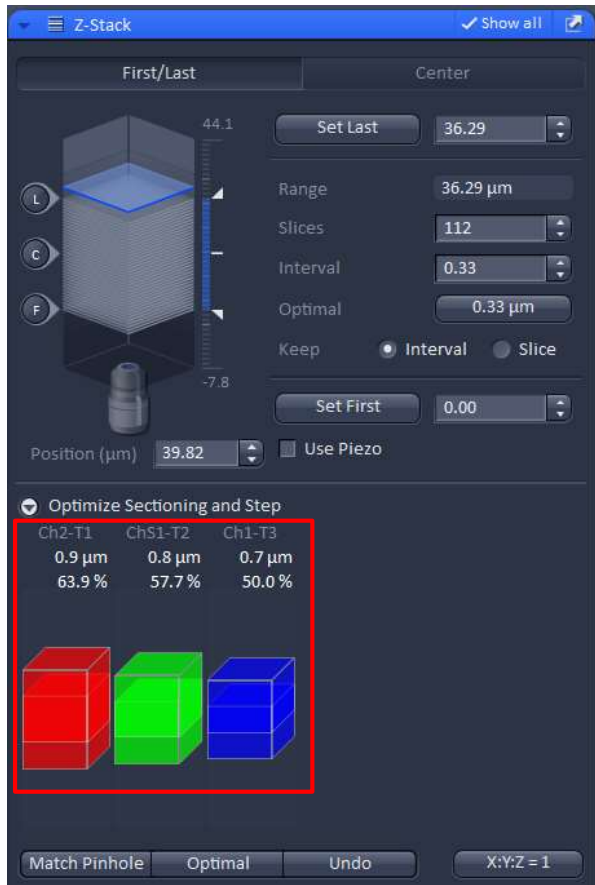
## Center



- 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

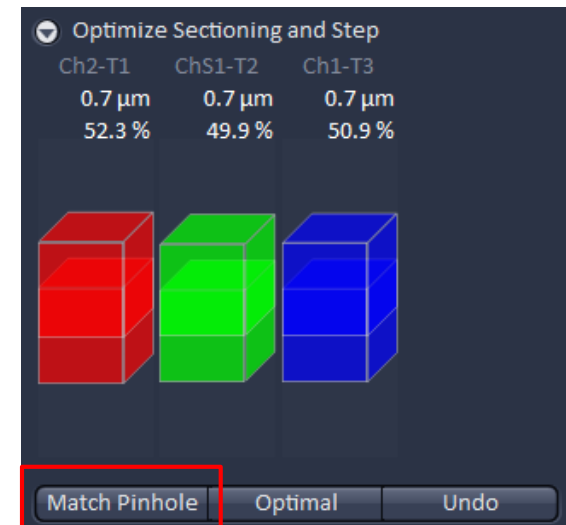
## Match Pinhole



多通道荧光拍摄Z-stack需要考虑光切厚度不一致的问题：

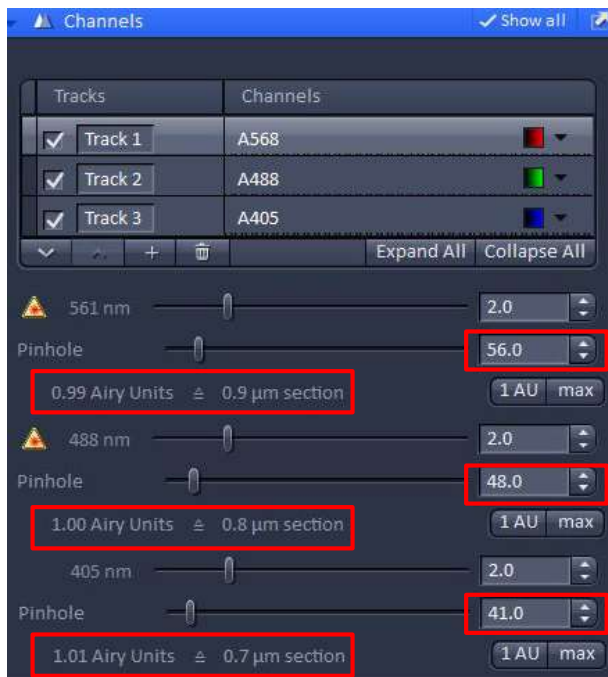
1、可以通过点击“Match Pinhole”自动调节不同track的针孔使光切厚度相似；

\*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。

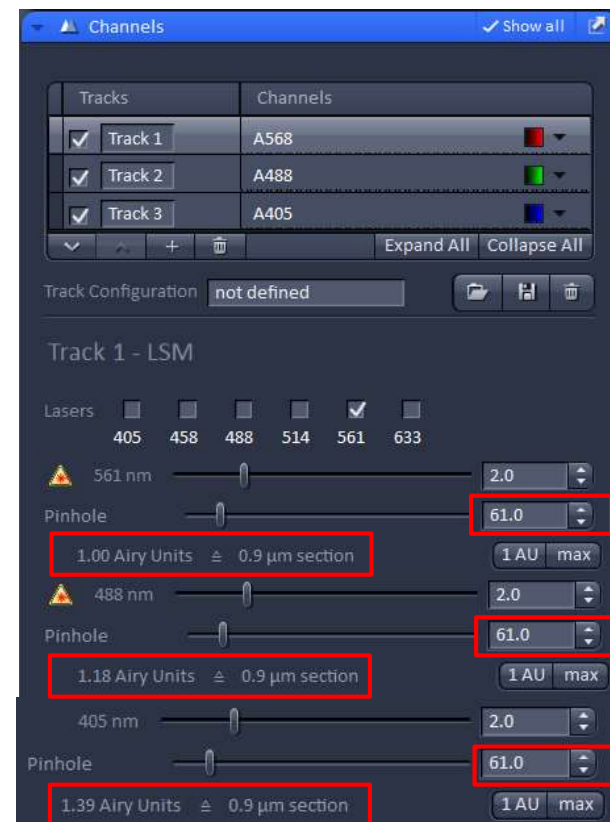


# Z-stack image

## Match Pinhole



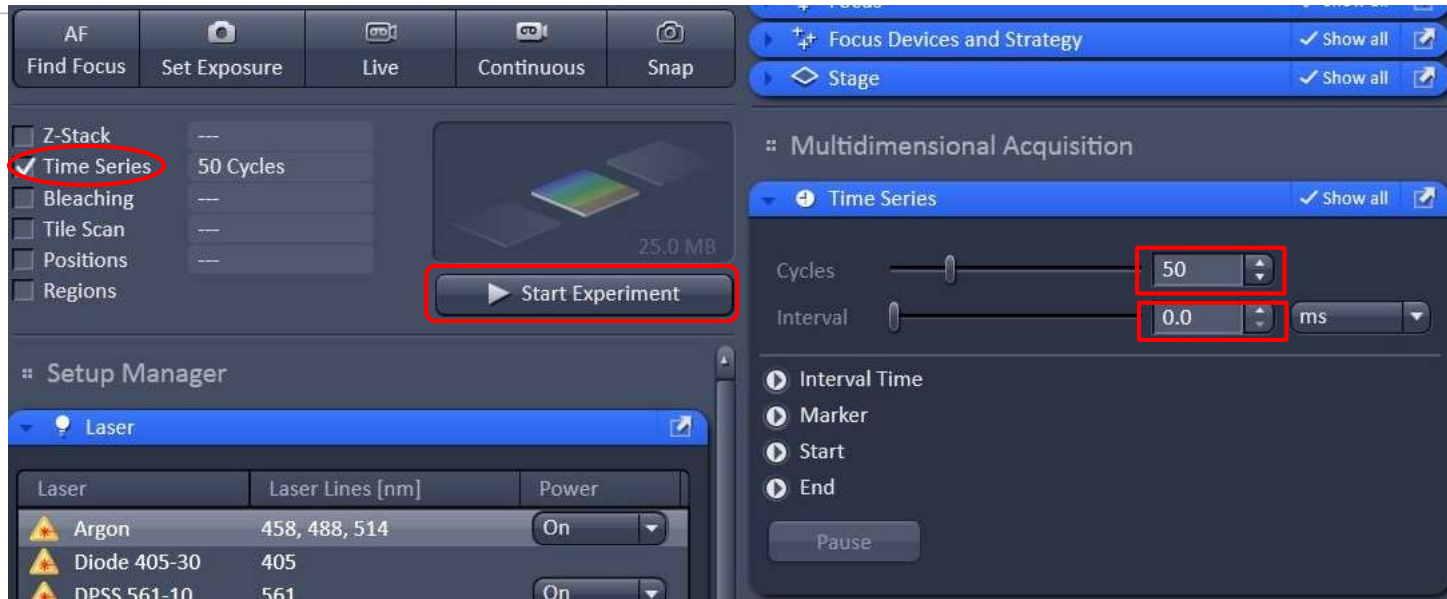
2、通过手动调节针孔到一致，可以保证荧光强度的同时，保证光切厚度一致。



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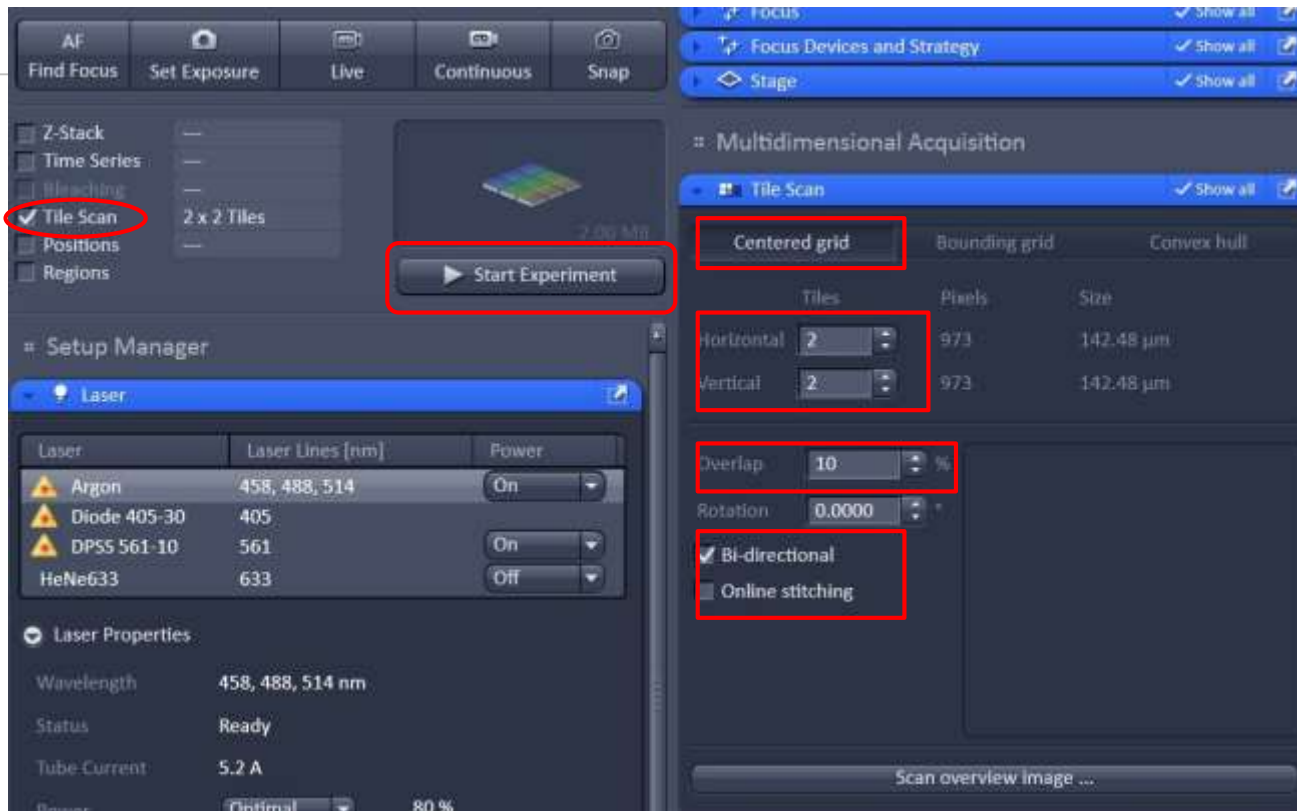
# Time Series image



- 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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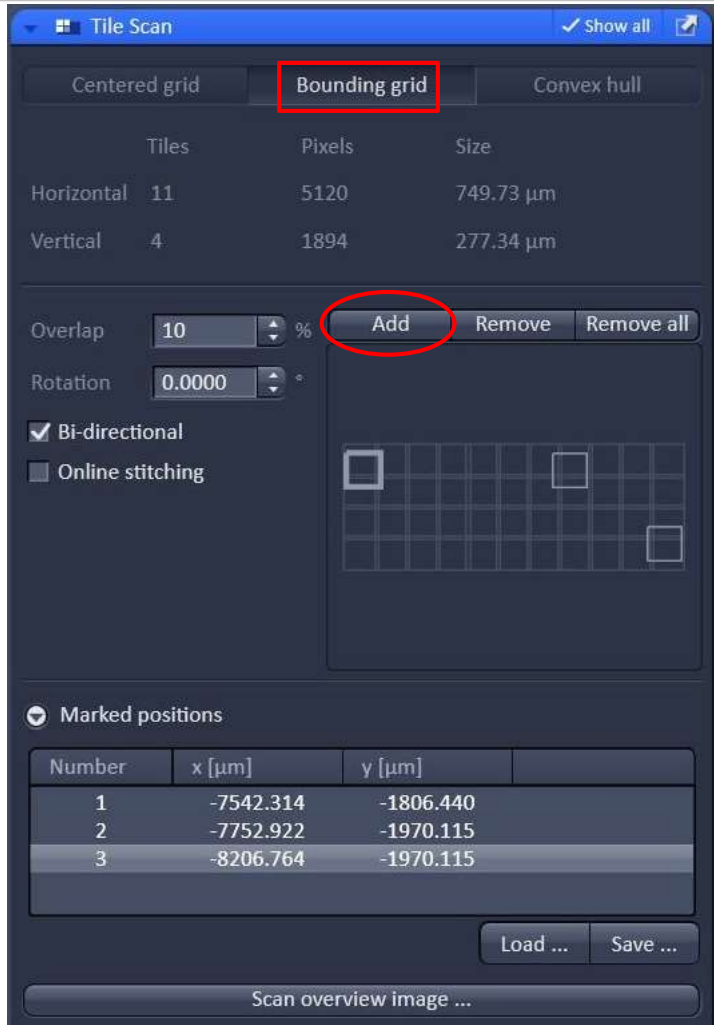
# Tile Scan



- 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

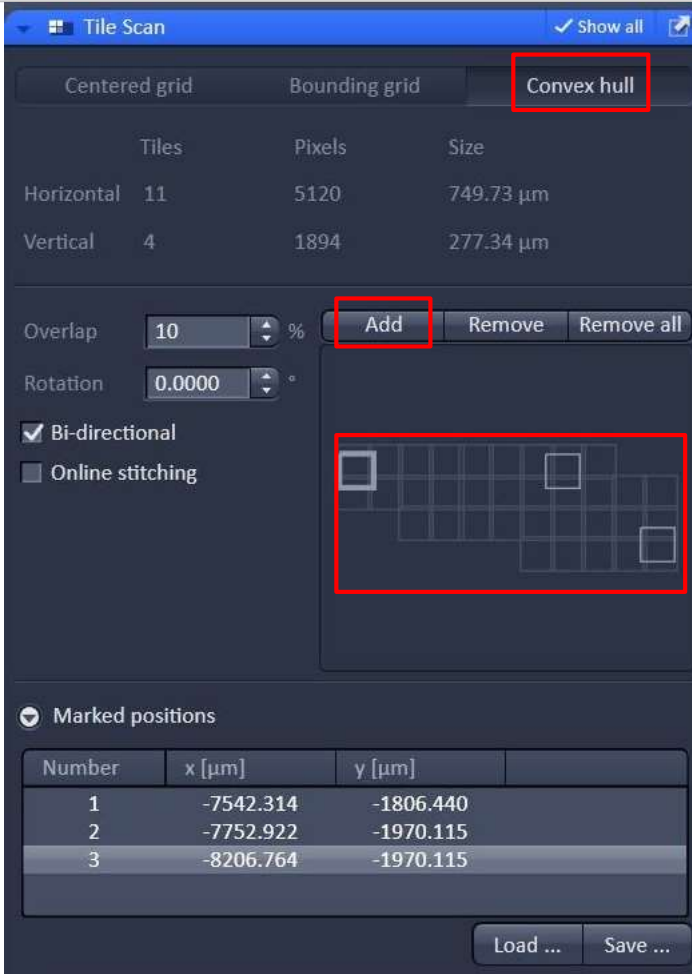
## Bounding grid



- 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



Centered grid    Bounding grid    **Convex hull**

	Tiles	Pixels	Size
Horizontal	11	5120	749.73 $\mu\text{m}$
Vertical	4	1894	277.34 $\mu\text{m}$

Overlap: 10 %    **Add**    Remove    Remove all

Rotation: 0.0000 °

Bi-directional  
 Online stitching

Marked positions

Number	x [ $\mu\text{m}$ ]	y [ $\mu\text{m}$ ]
1	-7542.314	-1806.440
2	-7752.922	-1970.115
3	-8206.764	-1970.115

Load ...    Save ...

- 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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Locate Acquisition FCS Processing Maintain

Experiment Manager

Smart Setup  Show all Tools New

# 1、进入“Smart Setup”

Smart Setup Show All

Configure your experiment

Dye	Color
Alexa Fluor 488	
Alexa Fluor 568	
Alexa Fluor 405	

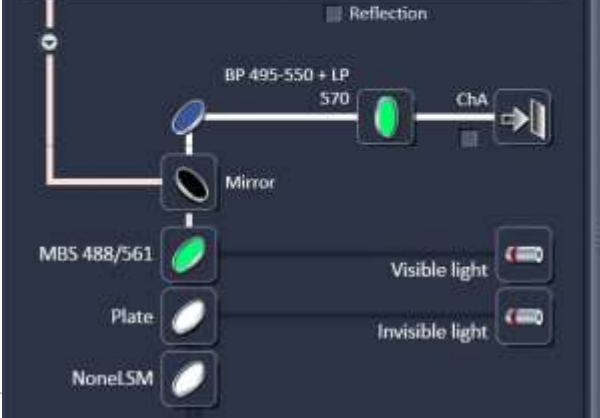
# 2、选择染料

Z-Stack 22 Slices  
 Time Series  
 Bleaching  
 Tile Scan  
 Positions  
 Regions

Start Experiment



Use	Dye	Color	Detector	Range
<input type="checkbox"/>			Ch1	415-735nm
<input type="checkbox"/>			ChS1	410-696nm
<input type="checkbox"/>			Ch2	415-735nm



Current  Quality  Speed  Standard  Widefield Like

Proposals

Fastest  Best signal  Smartest (Line)

# 3、选择Best signal

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

Tracks	Channels
<input checked="" type="checkbox"/> Track 1	A568
<input checked="" type="checkbox"/> Track 2	A488
<input checked="" type="checkbox"/> Track 3	A405

Expand All   Collapse All

### 4、Channel 中已经添加好tracks

Switch track every: Frame

Track 1

Use	Dye	Color	Detector	Range
<input type="checkbox"/>			Ch1	415-735nm
<input type="checkbox"/>			ChS1	410-696nm
<input checked="" type="checkbox"/>	Alexa Fluor 568		Ch2	568-712nm

Reflection

BP 570-620 + LP 645

Mirror

ChA

### 5、在此处设置Airyscan光路

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   Show all
- Auto Save   Show all





Use	Dye	Color	Detector	Range	
<input type="checkbox"/>			Ch1	415-735nm	+
<input type="checkbox"/>			ChS1	410-696nm	-
<input checked="" type="checkbox"/>	Alexa Fluor 568		Ch2	568-712nm	

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）

MBS 458/561

Plate

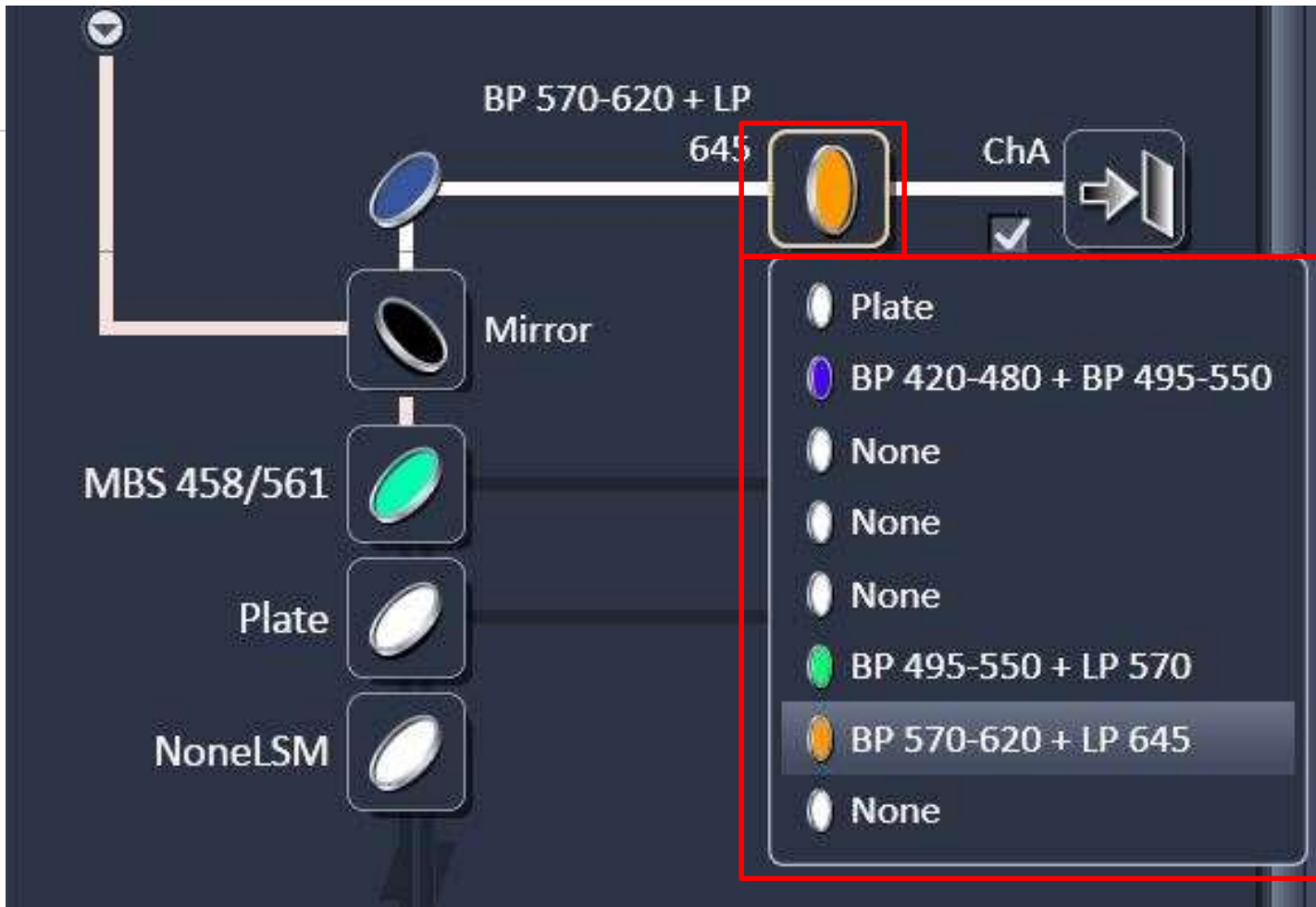
NoneLSM

Visible light

Invisible light

Info

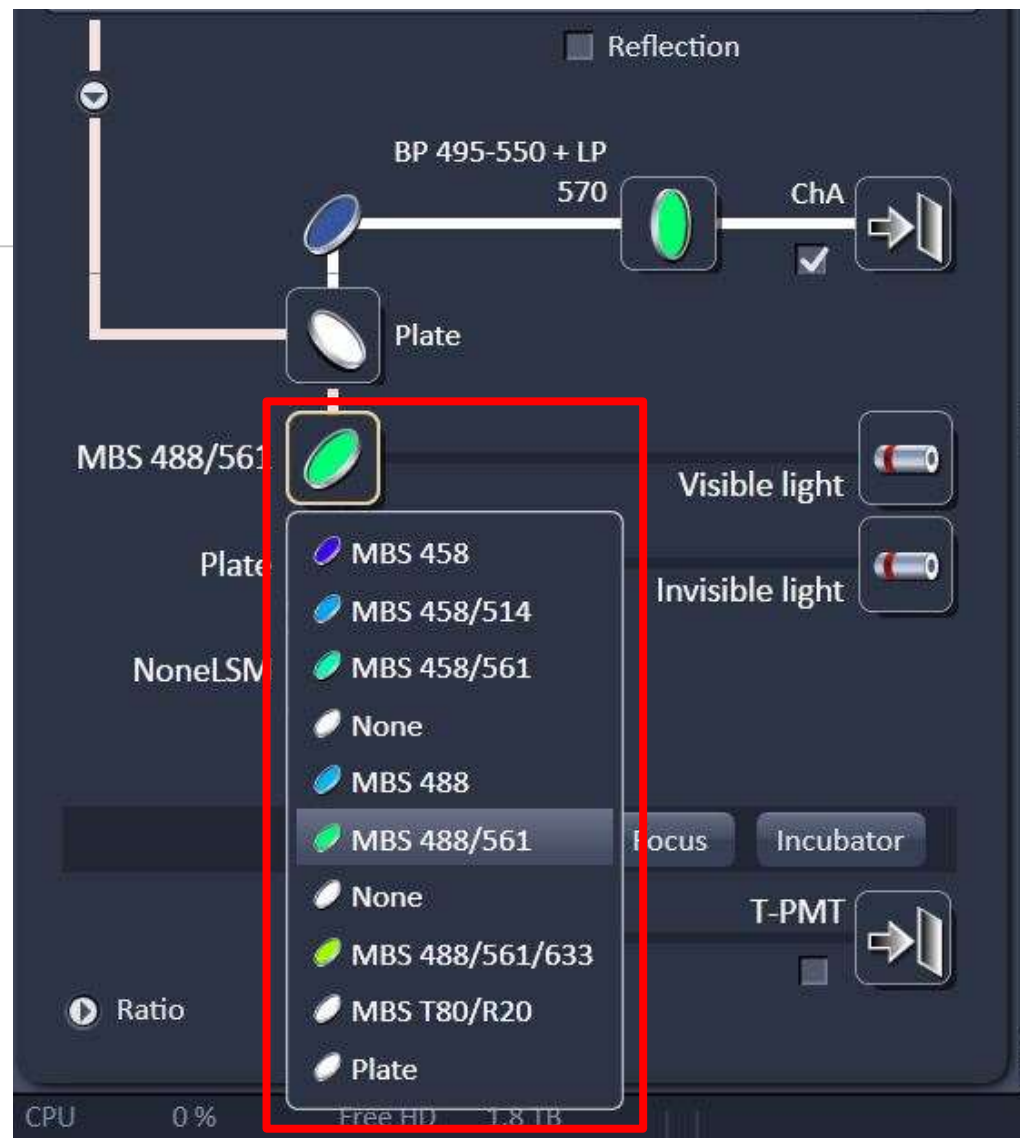
Airyscan can not be used with other detectors so they will be deactivated



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

**Channels**

Tracks	Channels
<input checked="" type="checkbox"/> Track 1	ChA
<input checked="" type="checkbox"/> Track 2	ChA
<input checked="" type="checkbox"/> Track 3	ChA

Track Configuration: not defined

**Track 1 - LSM**

Lasers: 405, 458, 488, 514, 561, 633

561 nm

**Pinhole**: 142.4 (2.51 Airy Units, 0.4 μm section)

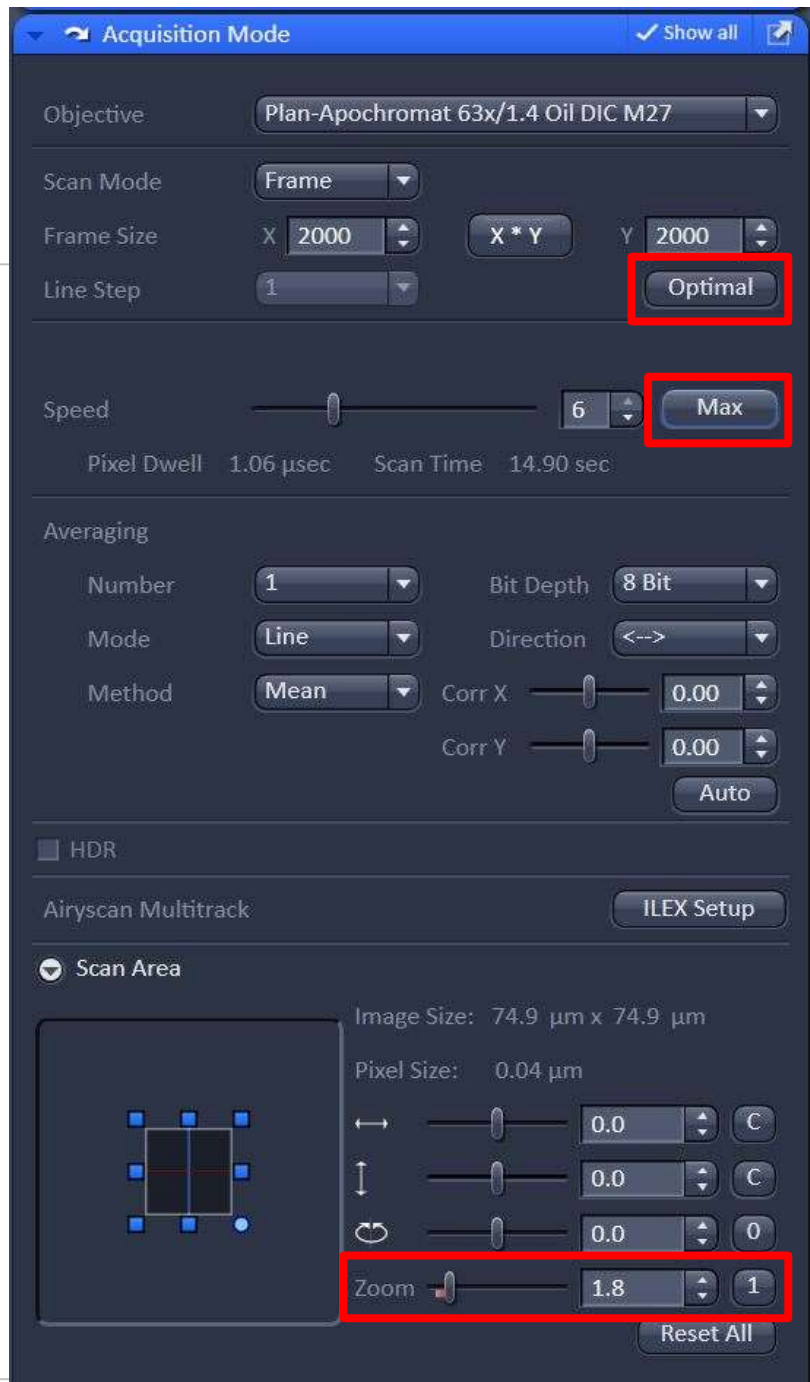
ChA Airyscan Mode: **SR**, VP, CO

Gain (Master): 0

Digital Gain: 1.0

Display: Auto copy from last acquired, Range indicator

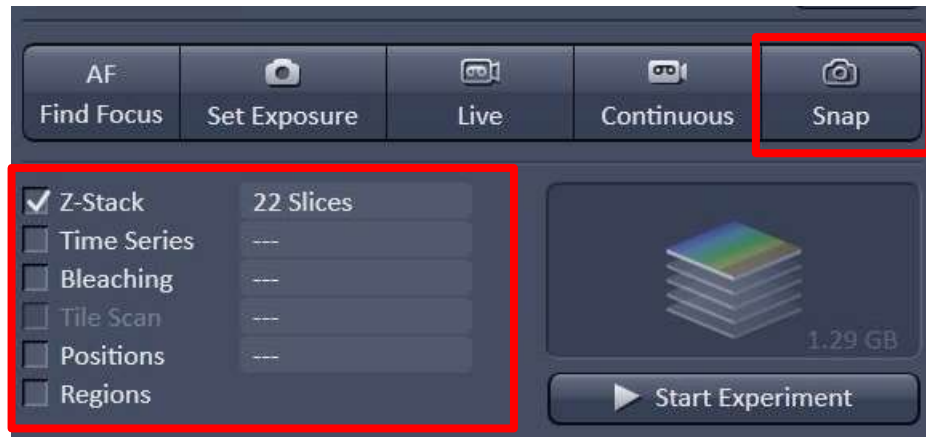
11、不要更改针孔 (Pinhole) 大小, Airyscan Mode 确认是 “SR” 模式



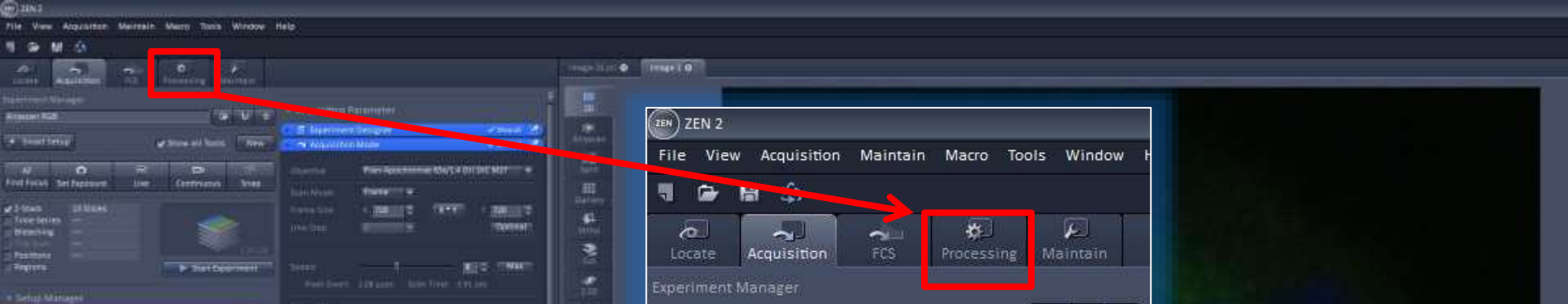
13、Frame Size选择“optimal”

14、Speed选择“Max”

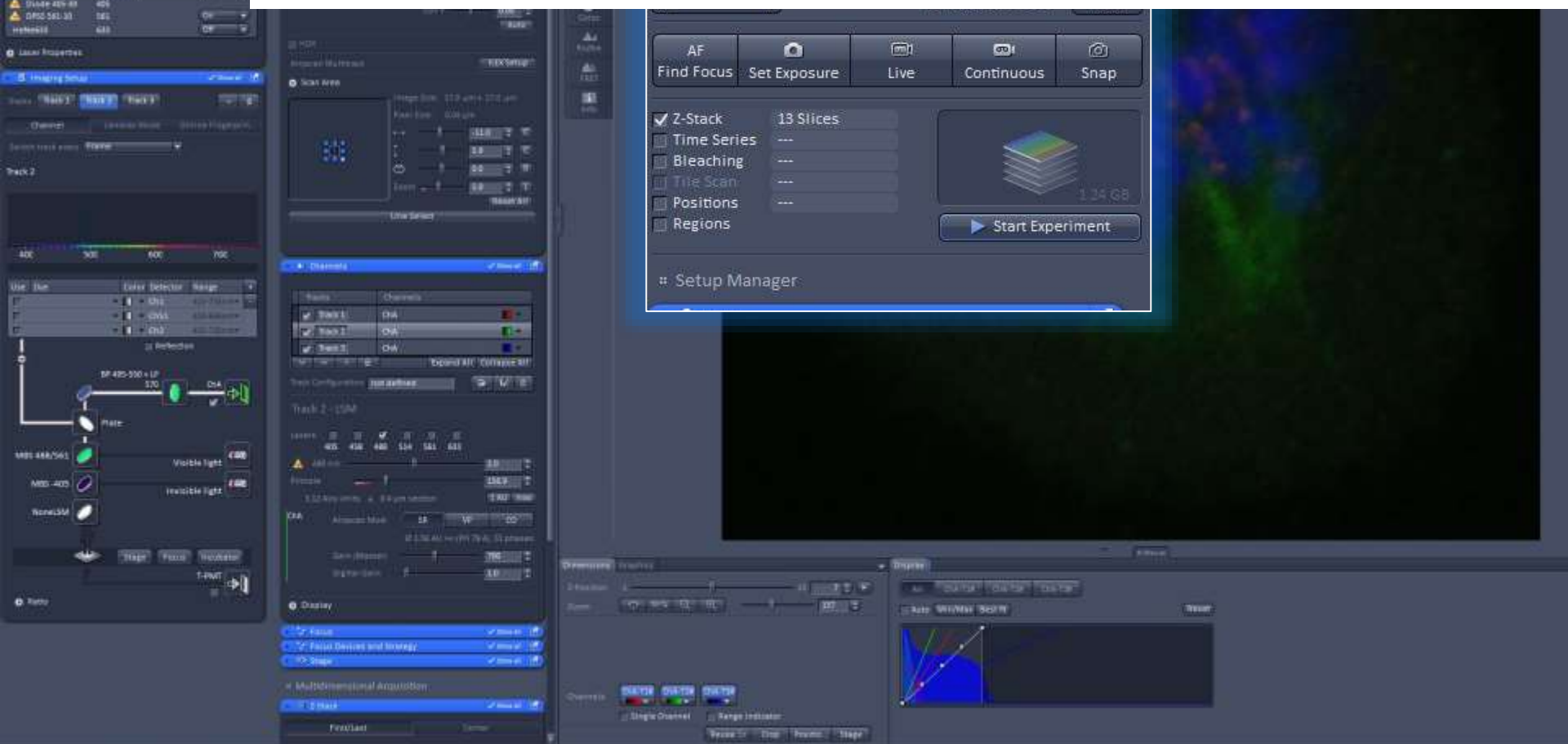
12、Zoom不能小于1.8



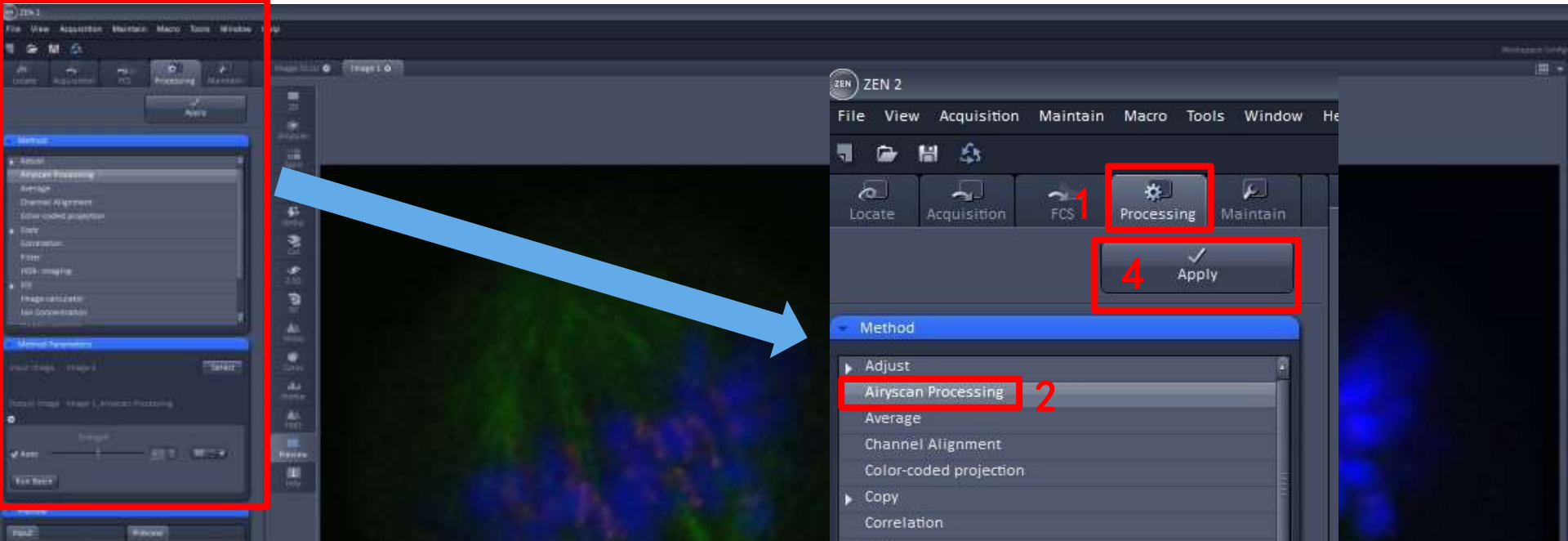
15、随后可以进行二维图像拍摄（Snap）或者多维图像拍摄



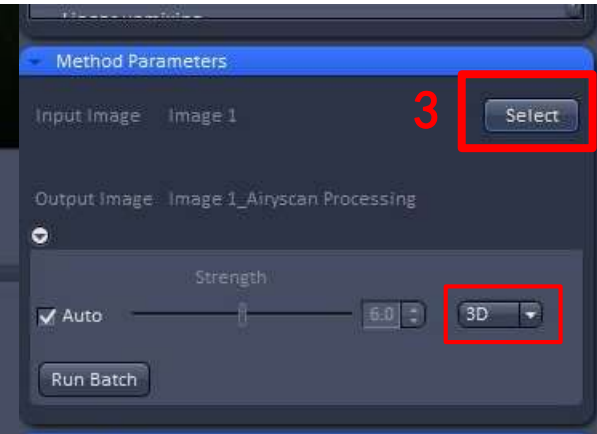
## 16、拍摄后的airyscan图像需要在“Processing”中进行后续处理

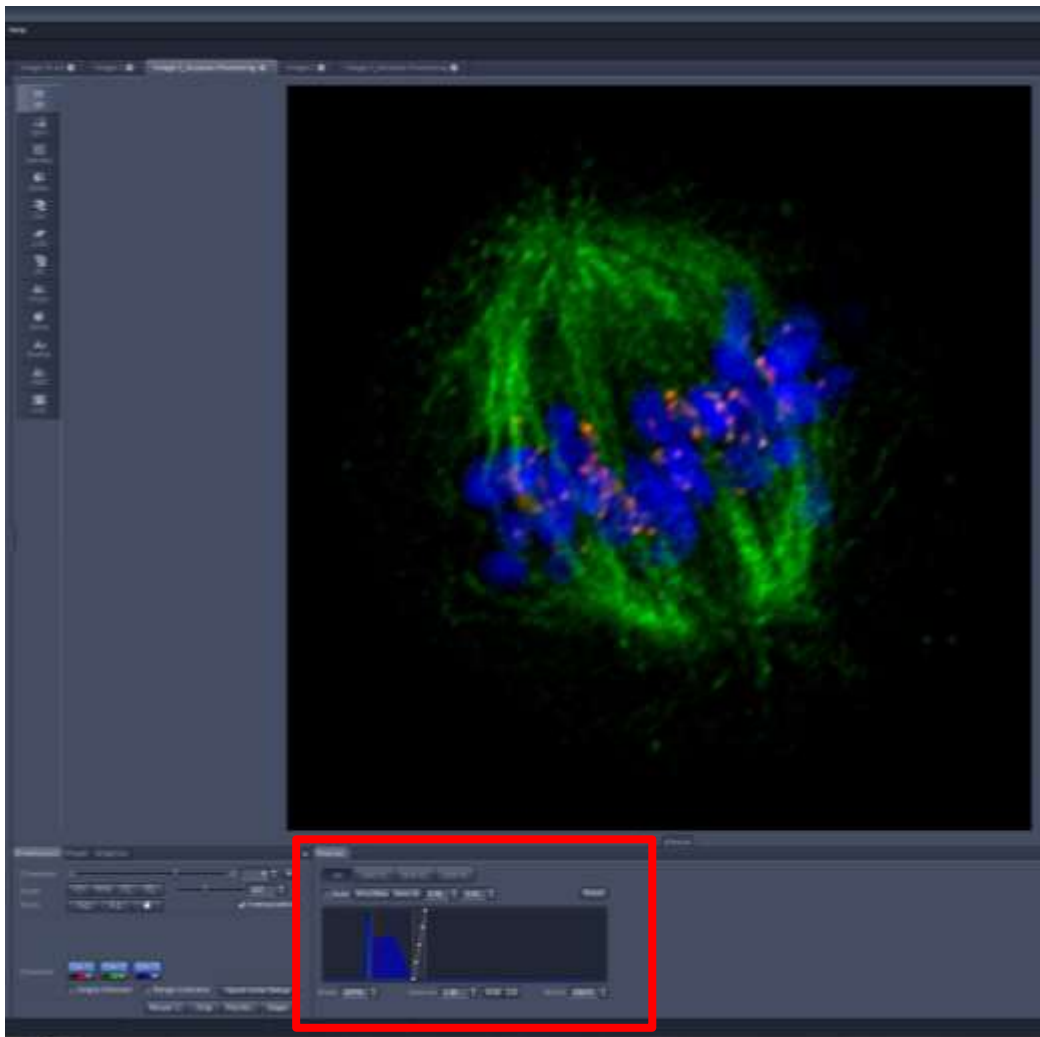






17、按照“Processing → Airyscan Processing → Select → Apply”进行图像处理。如果是z-stack图像，请选择“3D”





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

