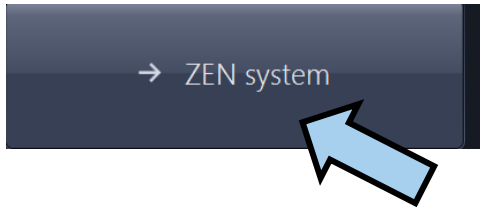
Carl Zeiss LSM 900 / ZEN Blue

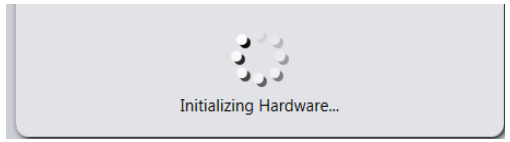
Quick Guide

ZEISS LSM 900 with Airyscan 2

目錄

Turn on the system





等待約一分鐘

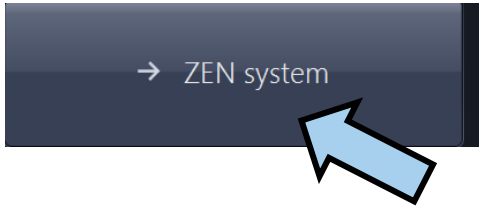
1. System
2. Components
3. Laser
4. Computer
5. ZEN blue
6. ZEN system

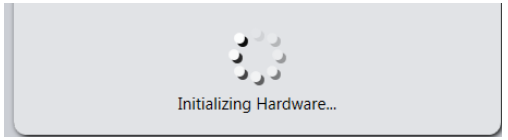
進入軟體 ZEN

1

進入ZEN BLUE

2

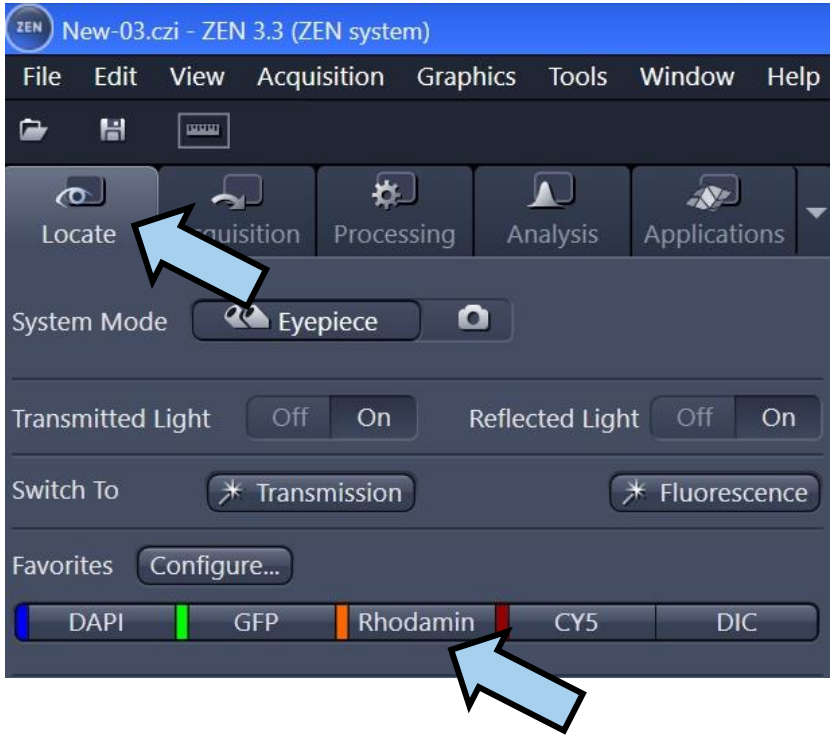




等待約一分鐘

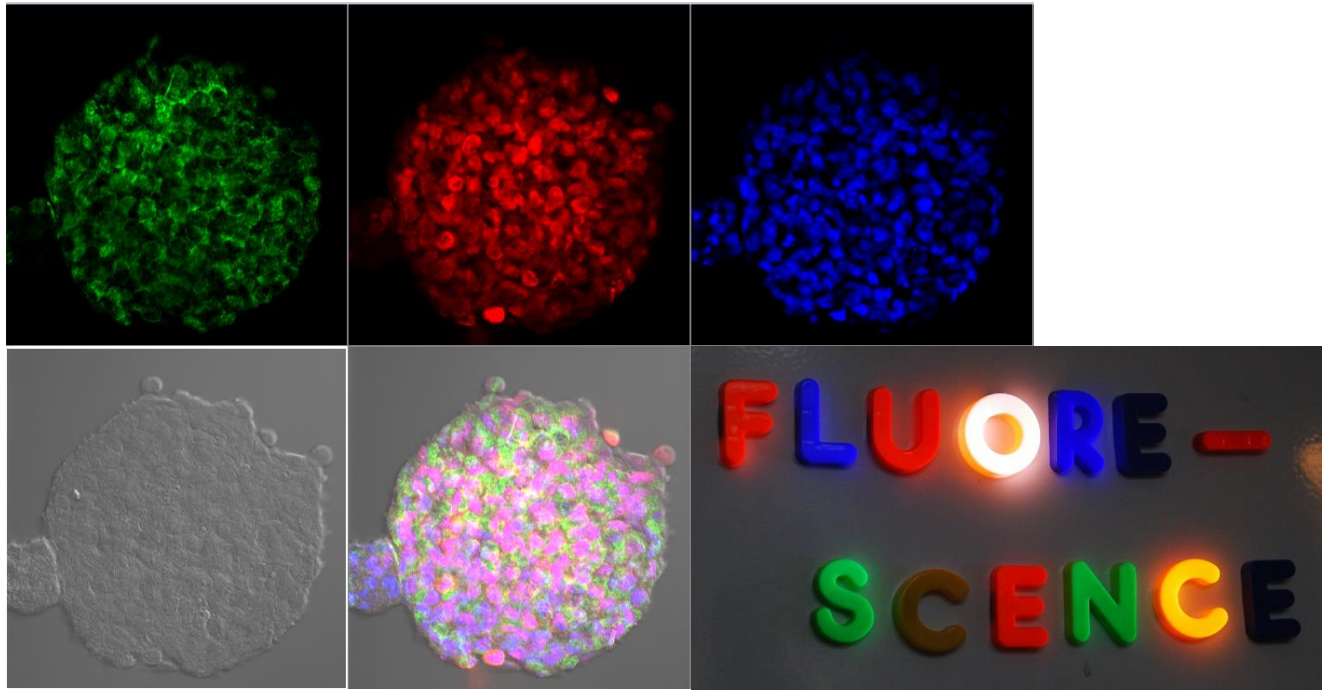
3

眼睛觀察

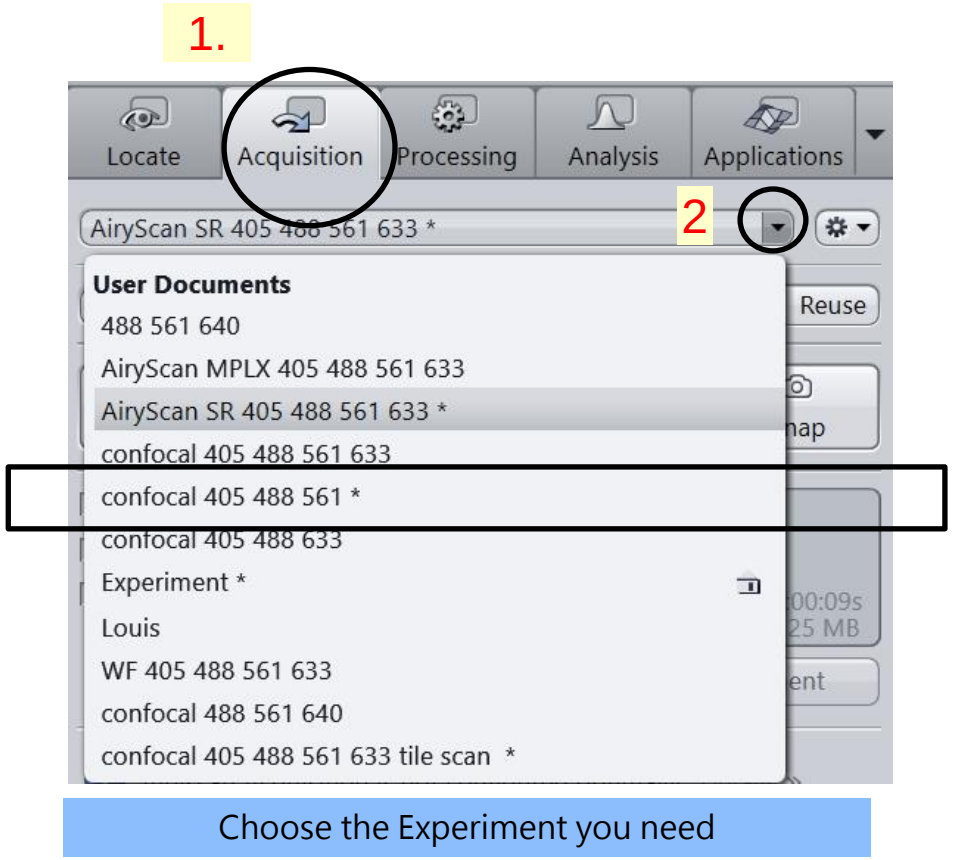


Choose the FL configuration you need

Multichannel Image Acquisition

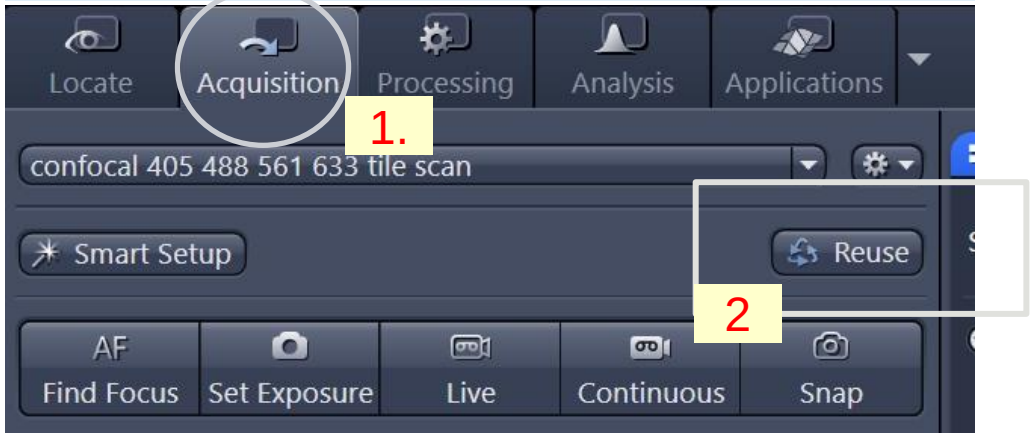


Multichannel Image Acquisition 1
Load Experiment methods form Experiment Setup



Choose the Experiment you need

OR……
Reuse

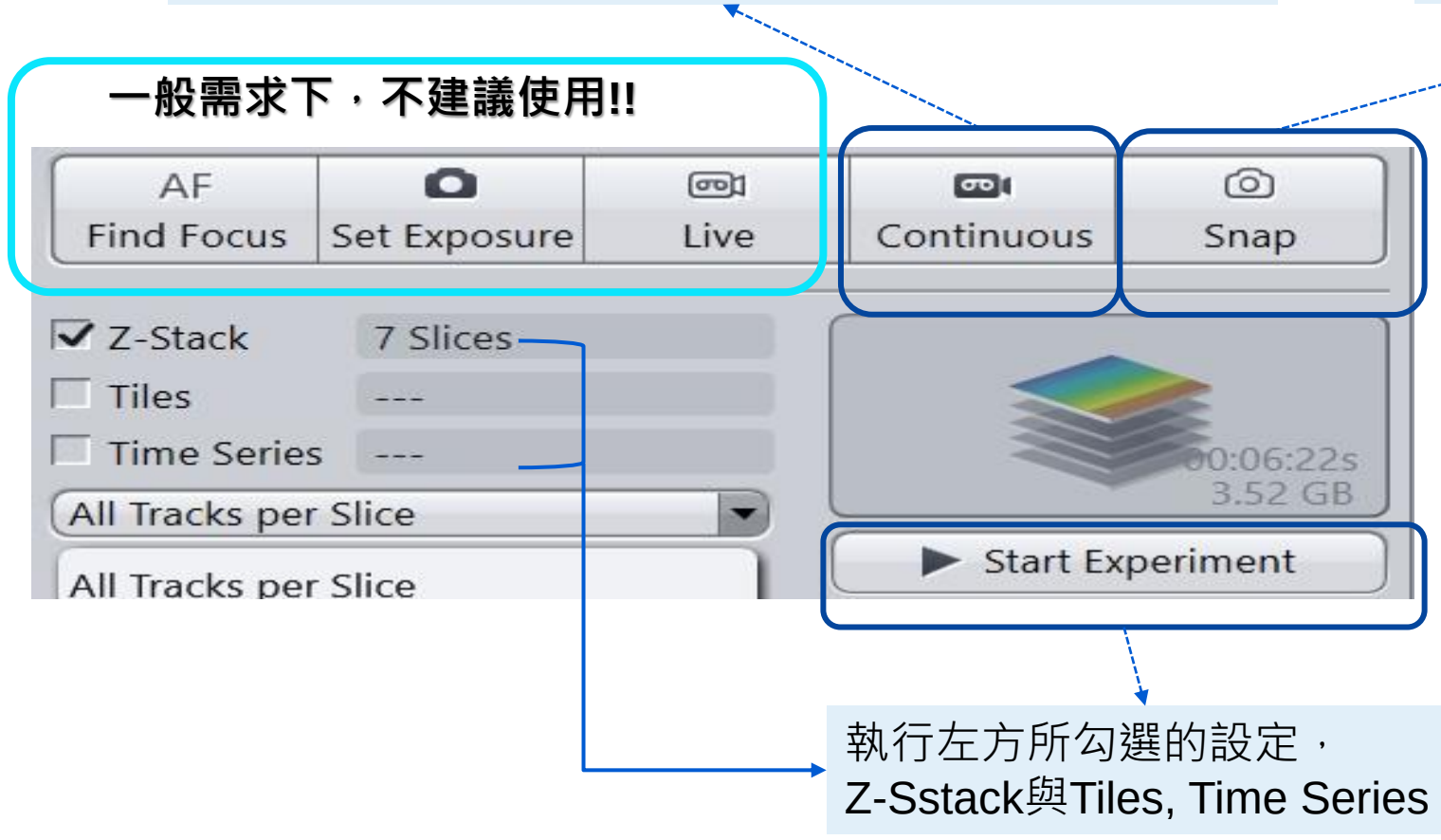


- 開啟欲套用的檔案後，按下Reuse系統會將舊檔案的設定apply至硬體中。
- 如果有Z 設定請取下樣品或先回到5x 物鏡
- 含有Tile等xyz設定請套用完畢後再刪除不需要的位置

Multichannel Image Acquisition 2
各種拍照function說明

連續掃描影像連續更新，可以看到即時影像，掃描不會自動停止，用於調整焦距與laser 與gain值看見及時變化。

在所停留的焦距位置，單拍一張，會執行以勾選的Channel，**不執行Z、T、Tile...等設定。**

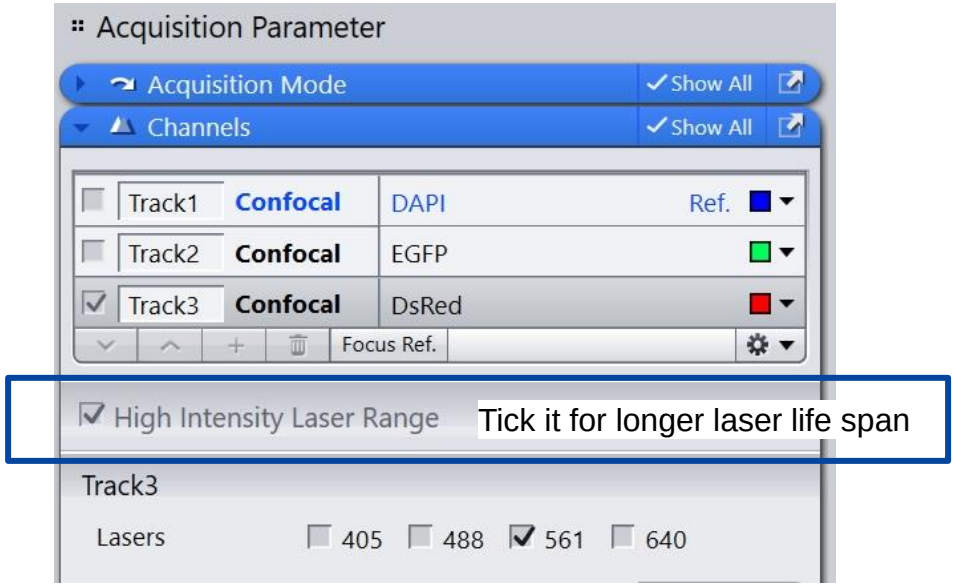


執行左方所勾選的設定，
Z-Sstack與Tiles, Time Series

Multichannel Image Acquisition 3
Acquisition Parameter

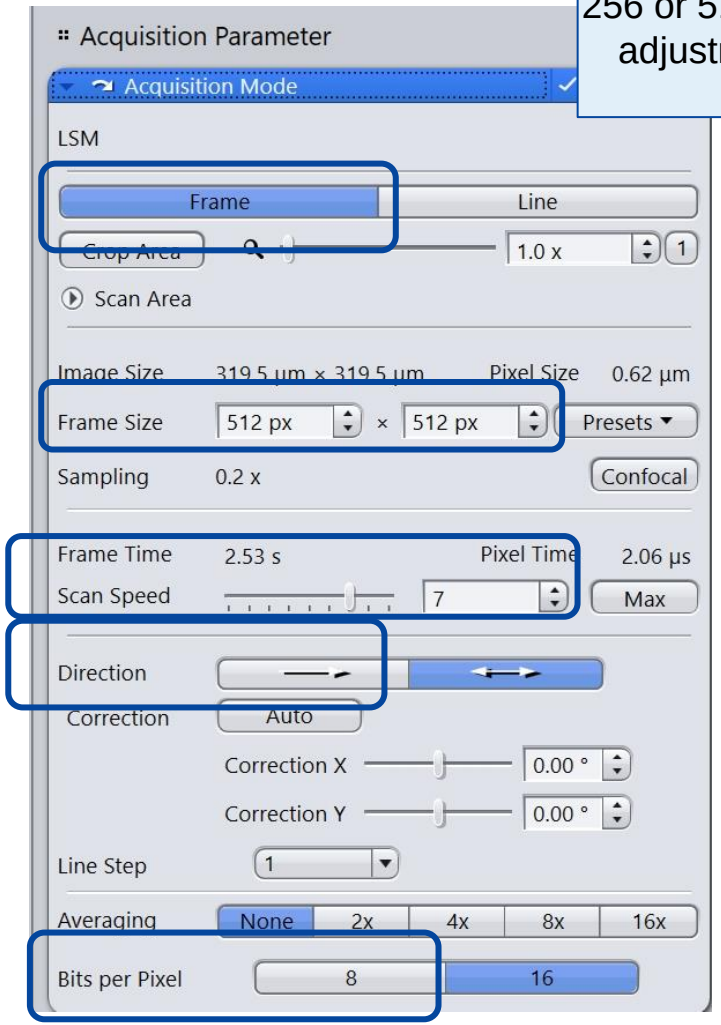
1

選擇需要的channel



2

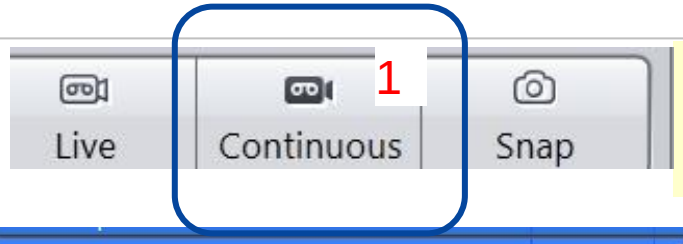
256 or 512 @ scan speed 7 for fast adjustment for focus, laser and detector gain



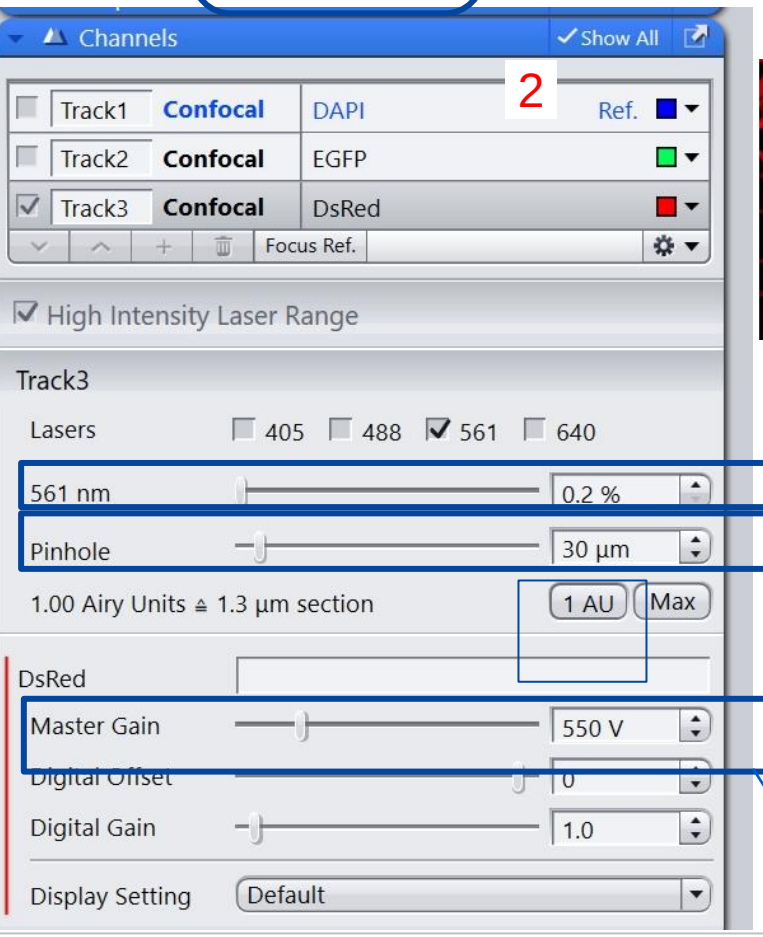
檔案小，大面拼圖時或不須定量時可選

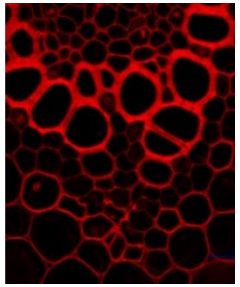
有定量需求可選16

Multichannel Image Acquisition 4
2D Image



- Adjust Laser Intensity and Master Gain for All Channels
- 一次調整一個channel， 依序調整所有channel的laser強度 與 Master Gain



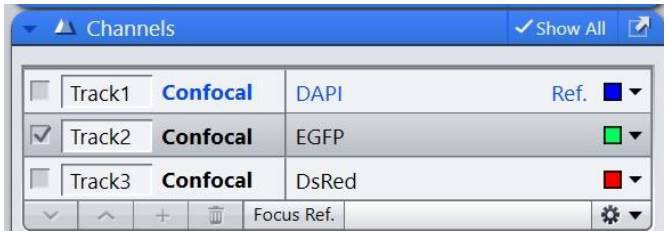


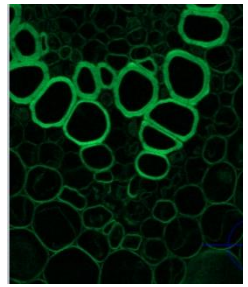
調整雷射強度
大部份情況下10%以內即足夠應付。

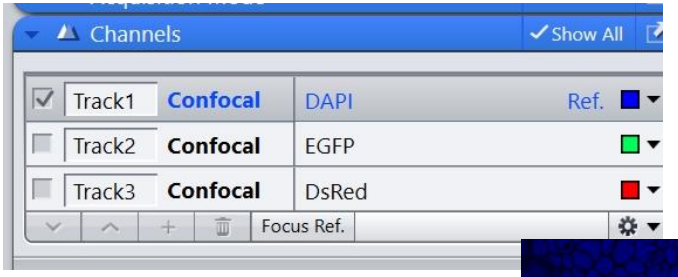
- Pinhole 大小，所有channel 設定相同(建議大約1AU)
- 若亮度足夠設在1AU左右即可
- 調整Pinhole大小 選一個channel 1AU， 其餘Channel對齊 (in this case, 30um)

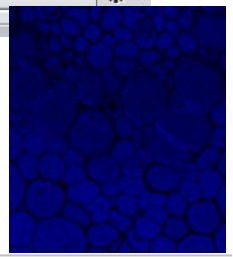
- 調整所有channel的laser 強度 與 Master Gain
- 一般亮度樣品不超過700， 切莫過曝損傷感測器

3.





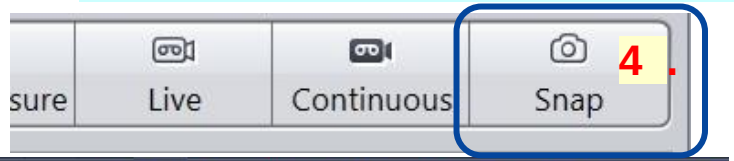


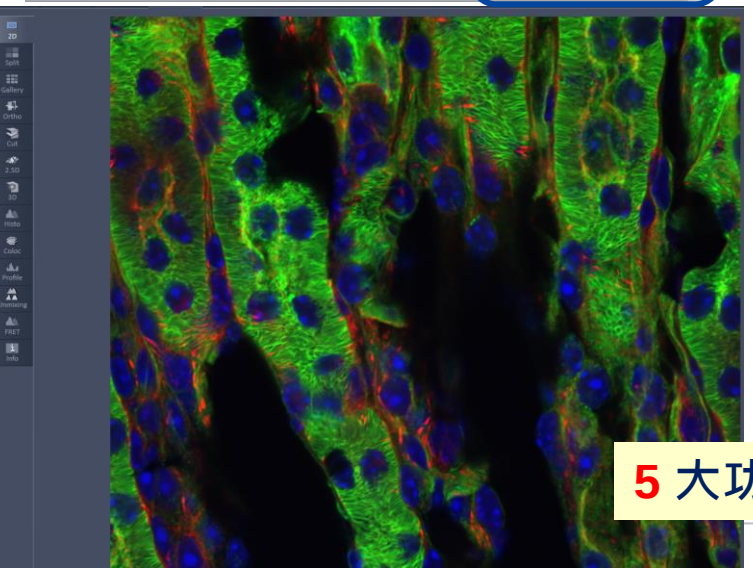


Multichannel Image Acquisition 5
2D Image

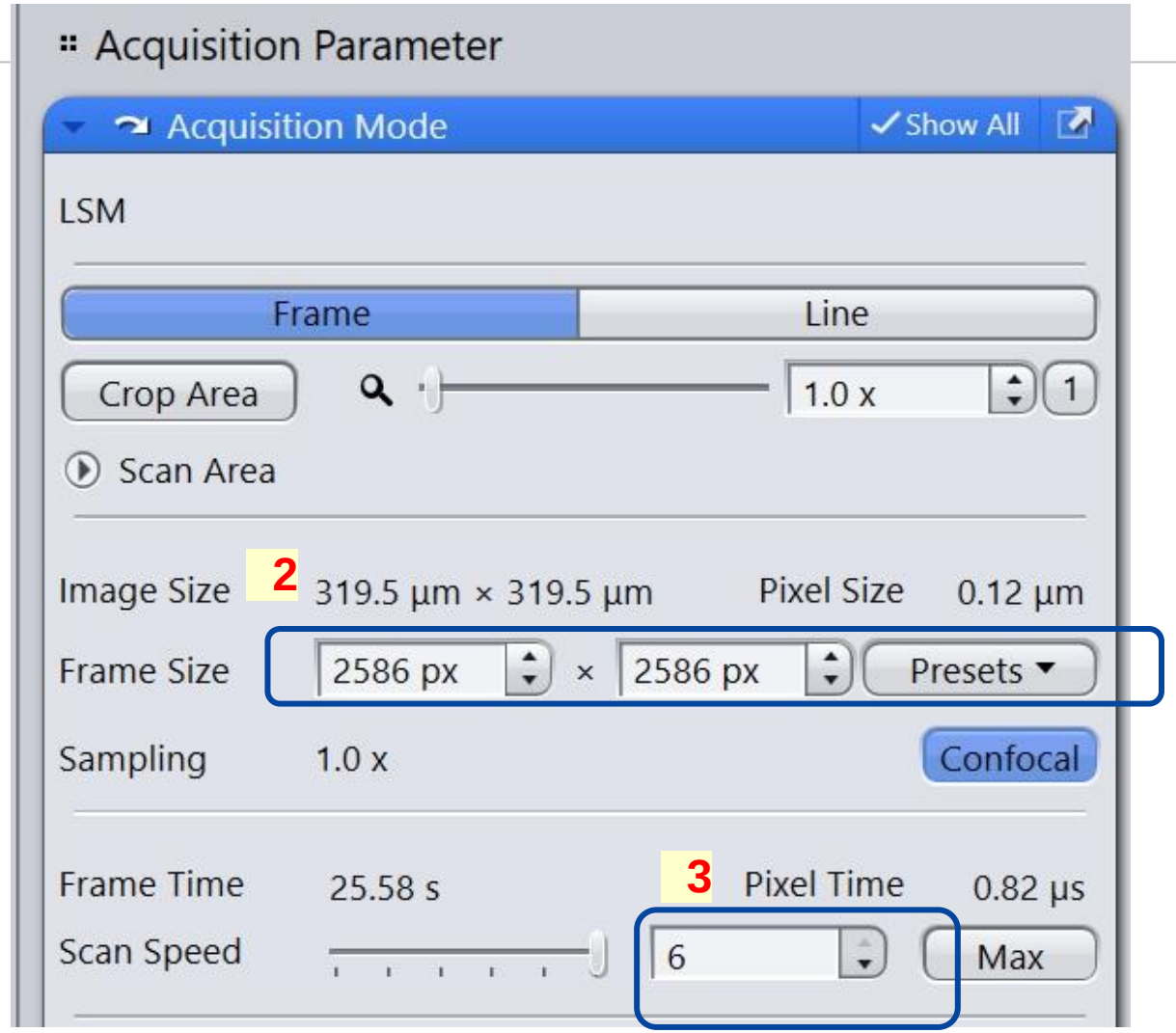
1

選取所有要準備要拍的track



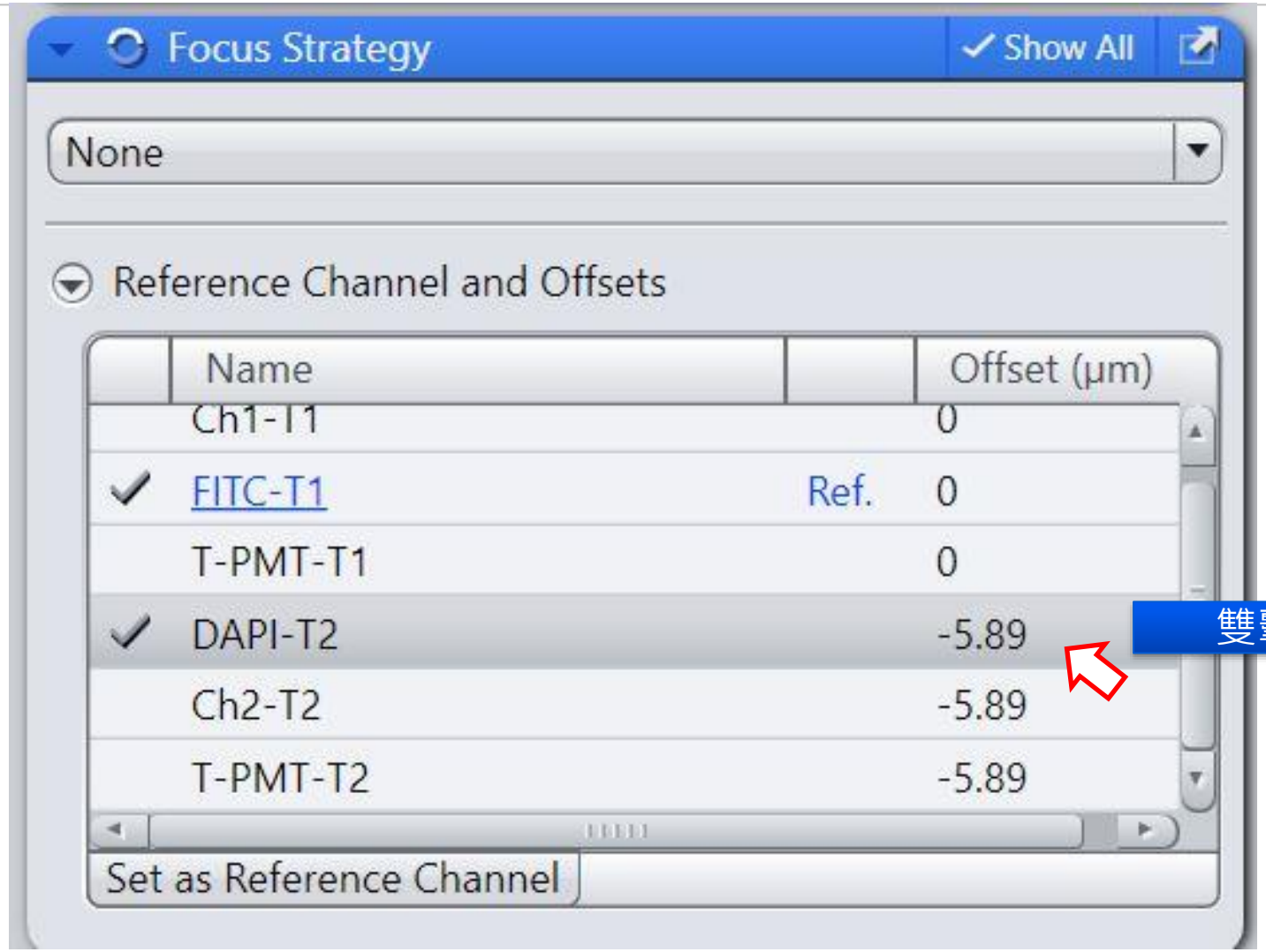


5 大功告成!!



•提高frame sisze 、降低掃瞄速度，是獲得高解析影像的最後祕技!
•1024, 2048，speed 5~7是很安全的設定值。

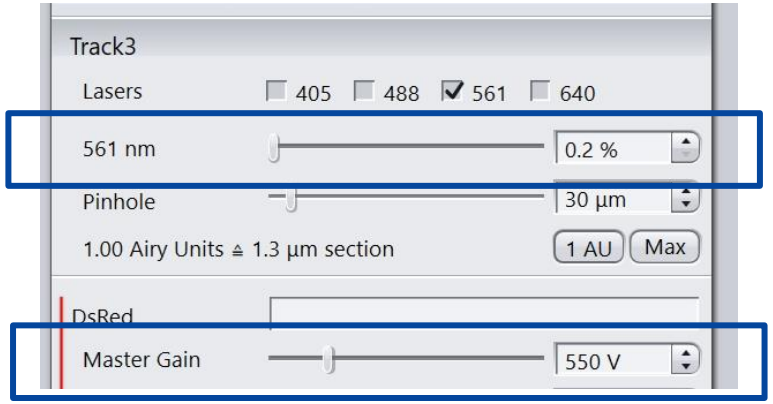
物鏡焦距誤差設定



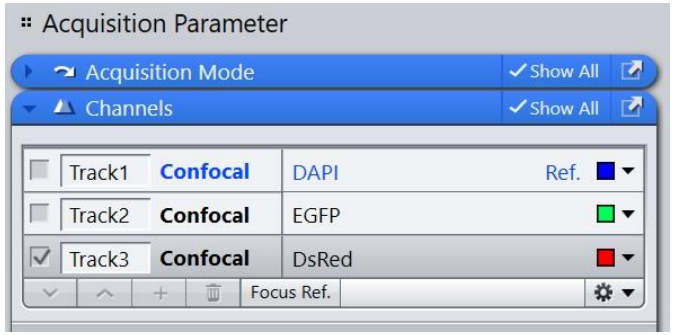
雙擊填入焦距誤差值

Multichannel Image Acquisition 6
3D Image - Z Stack Acquisition

1. 當所有channel的laser 強度 與Master Gain皆已設置完畢

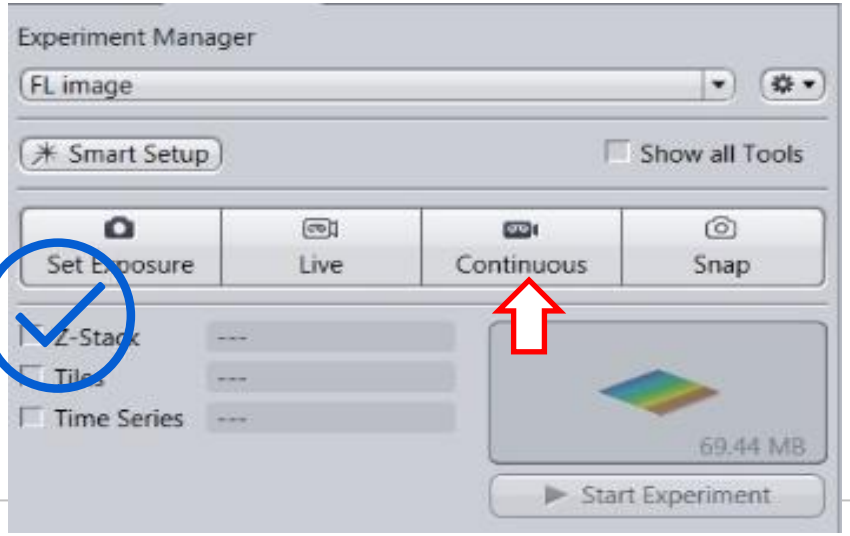


2 選擇一個Track



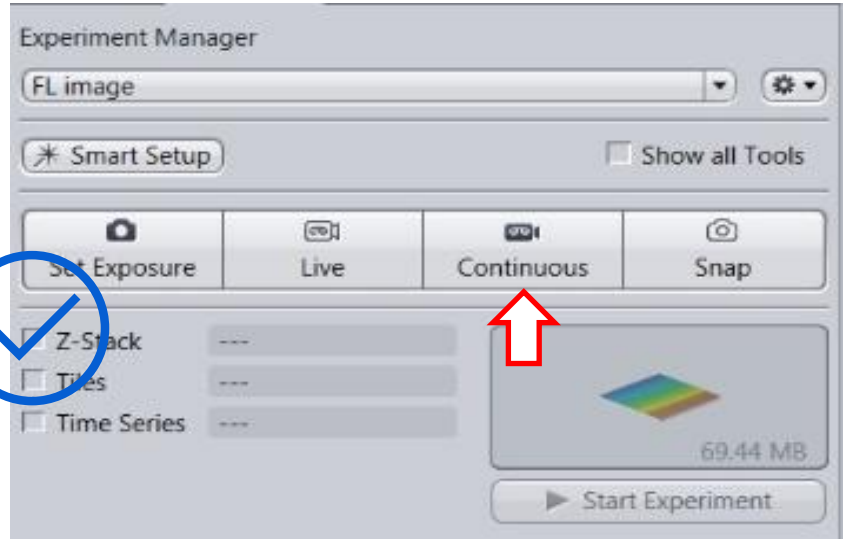
3. Put your hand on focus wheel and preparing for focusing

4 Continuous with higher frame rate (ex: 512^2 @ speed 7)

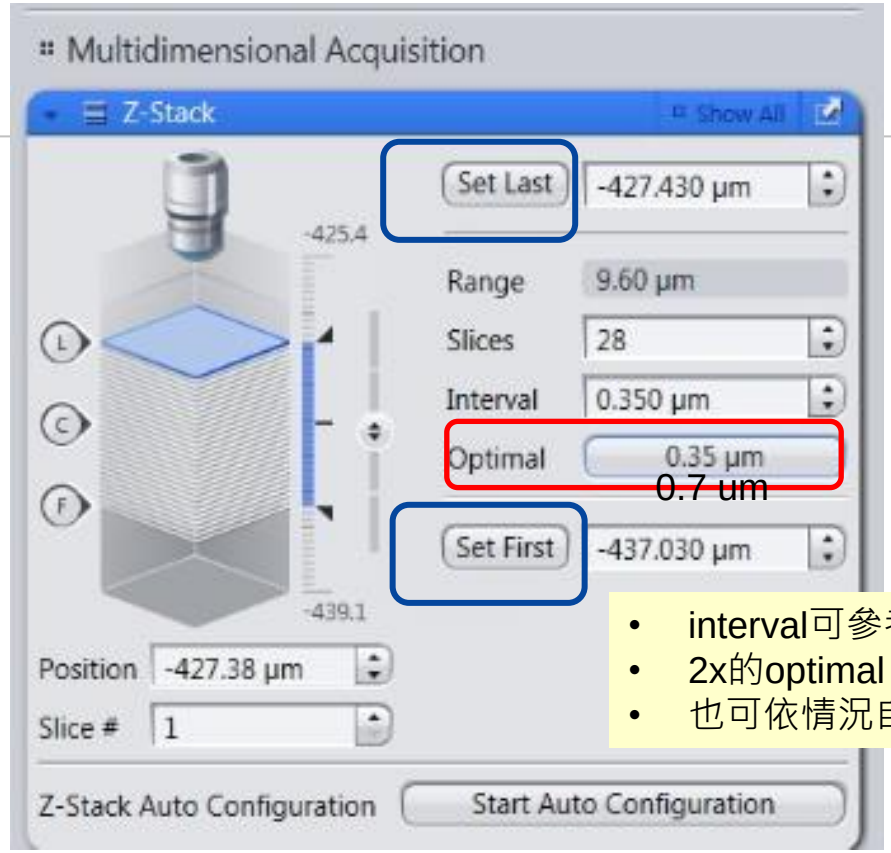


Multichannel Image Acquisition 7
3D Image - Z Stack Acquisition

1. Continuous with higher frame rate (ex: 512^2 @ speed 7)



- interval可參考optimal
- 2x的optimal 0.35*2 = 0.7也很好
- 自行決定間隔

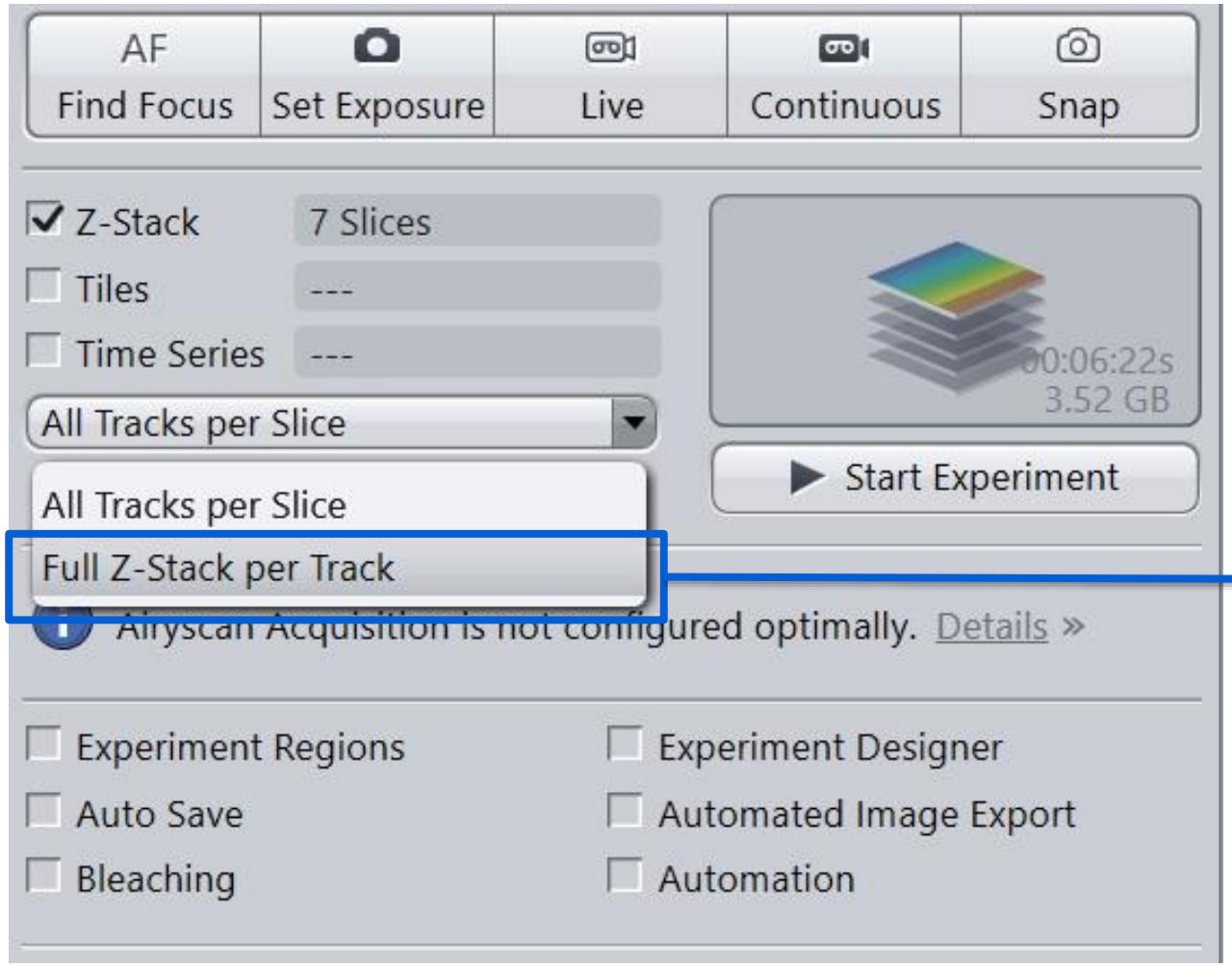


- interval可參考optimal
- 2x的optimal 0.35*2 = 0.7也很好
- 也可依情況自行決定間隔

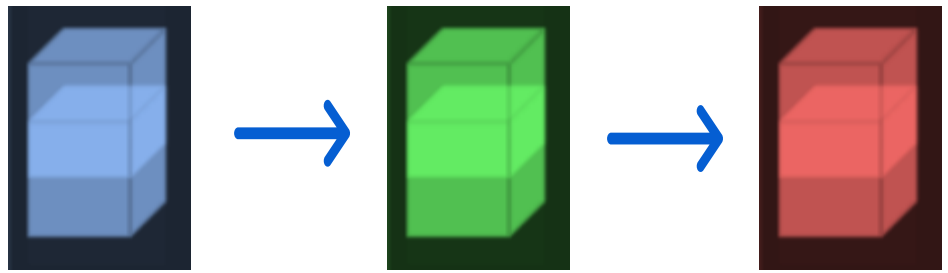
設訂Z stack的上下界限

- 選擇一個channel > Continuous
- 搭配Z stack視窗 > Z-stack
- 找到樣品焦距起點 >Set First
- 找到樣品焦距終點> Set Last

Multichannel Image Acquisition 8
3D Image - Z Stack Acquisition

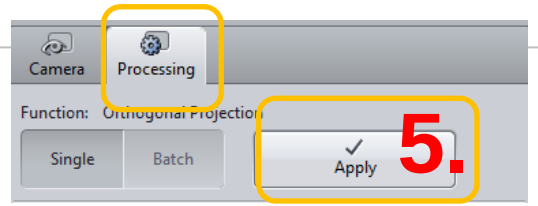


- 每次一個Channel的Z Stack 掃完才換下一個 Channel 的Z Stack
- 可再搭配line scan ， bi-direction scan， 為最省時間之掃描方式



Z stack: 把多張Z section疊成一張
製造全景深影像: Orthogonal Projection

1.



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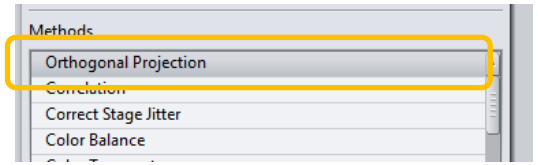

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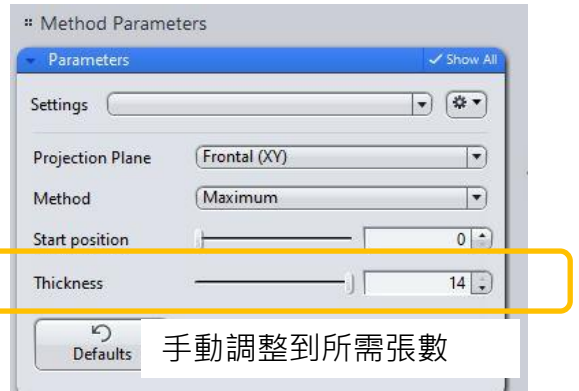
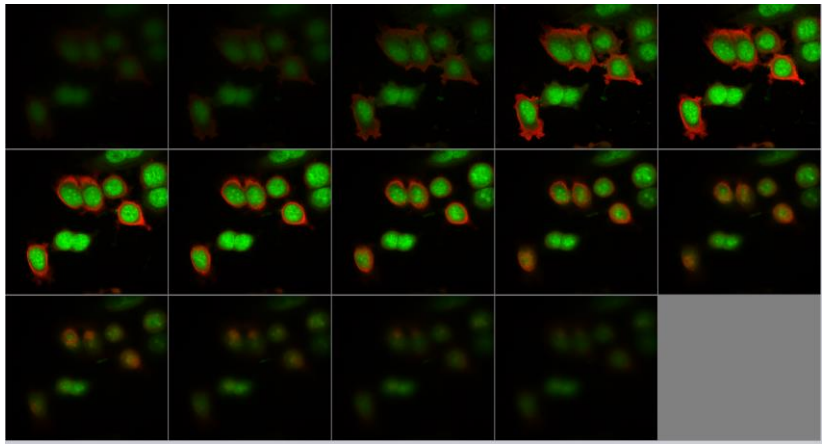
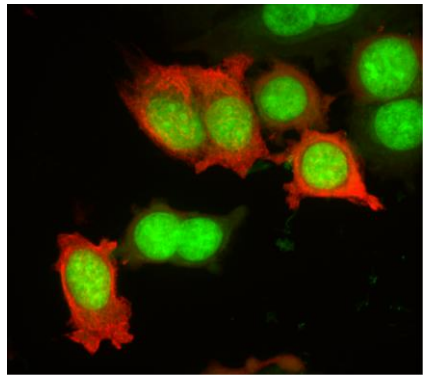


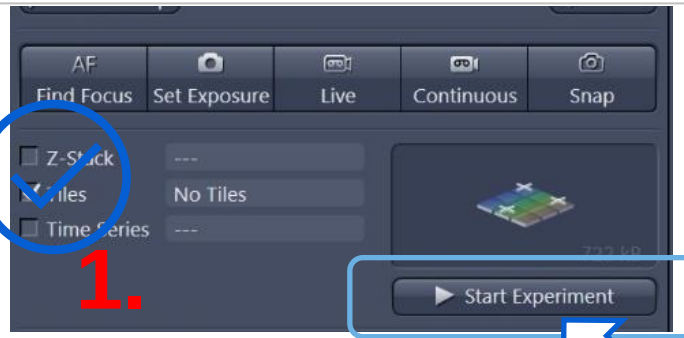
手動調整到所需張數

3.



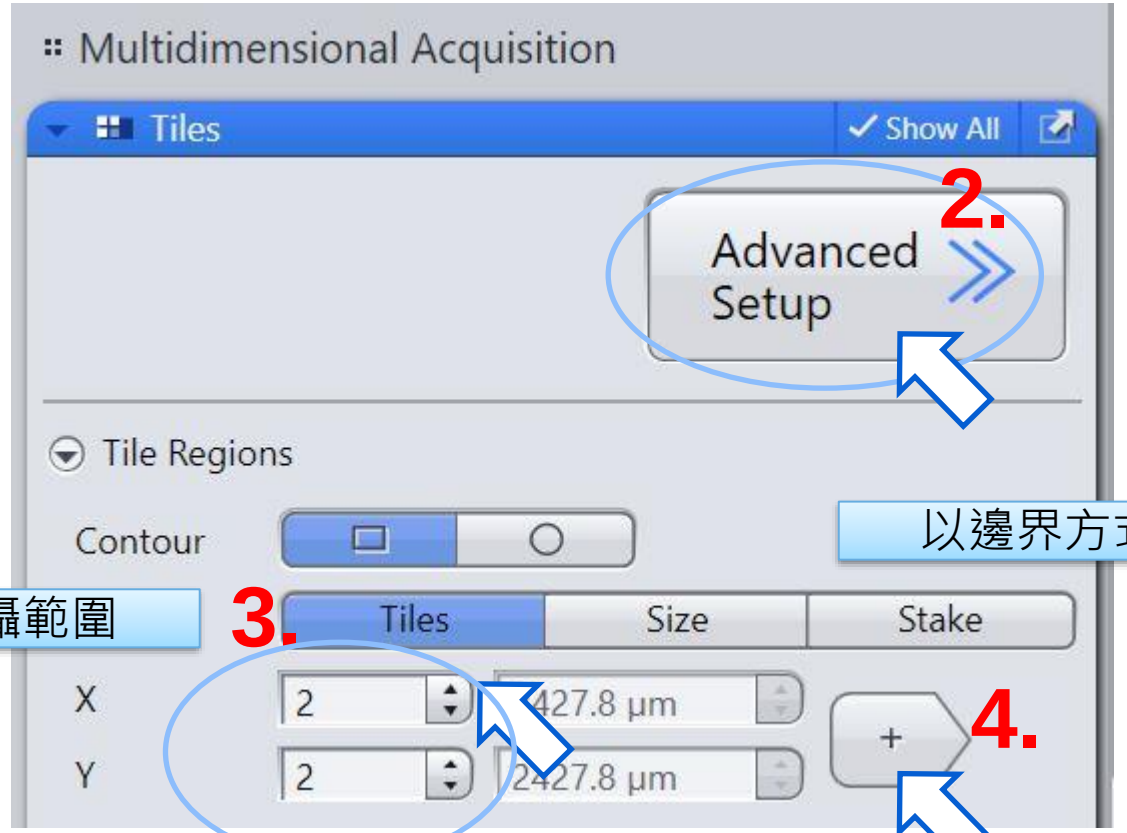


Tile Scan Imaging Setup
以目前視野為中心點做拼圖



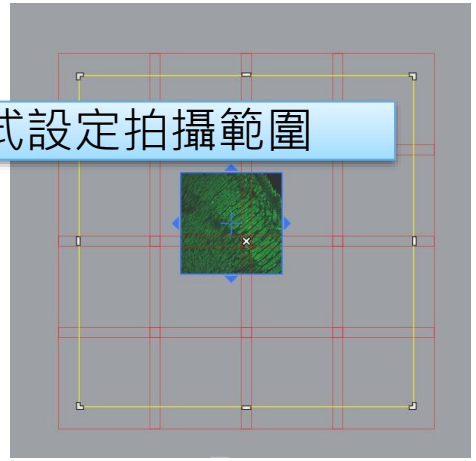
5.

完成設定，正式開始掃圖



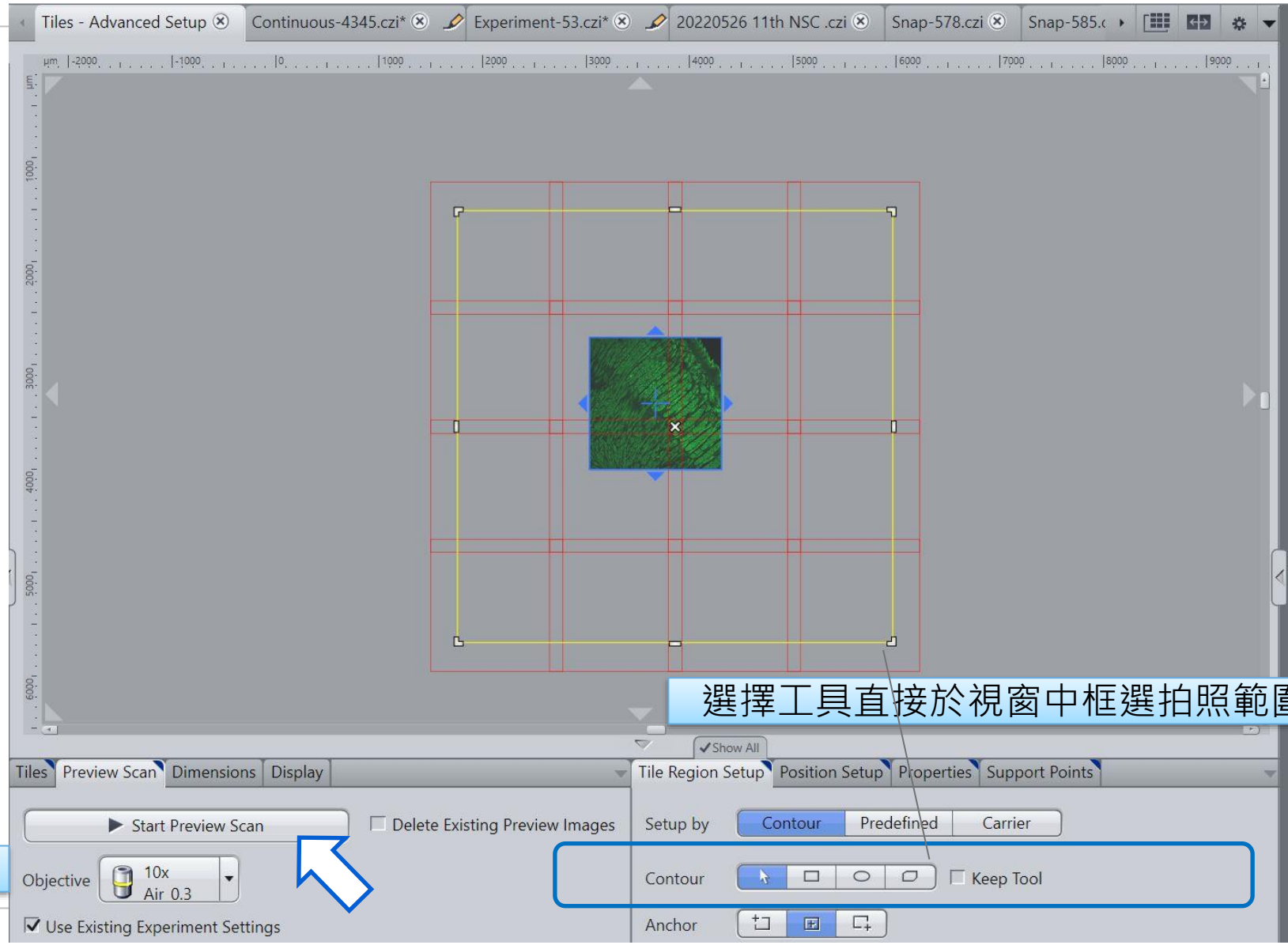
中心點模式設定拍攝範圍

以邊界方式設定拍攝範圍



按下之後右方視窗會出現大視野拼圖，如下頁所示

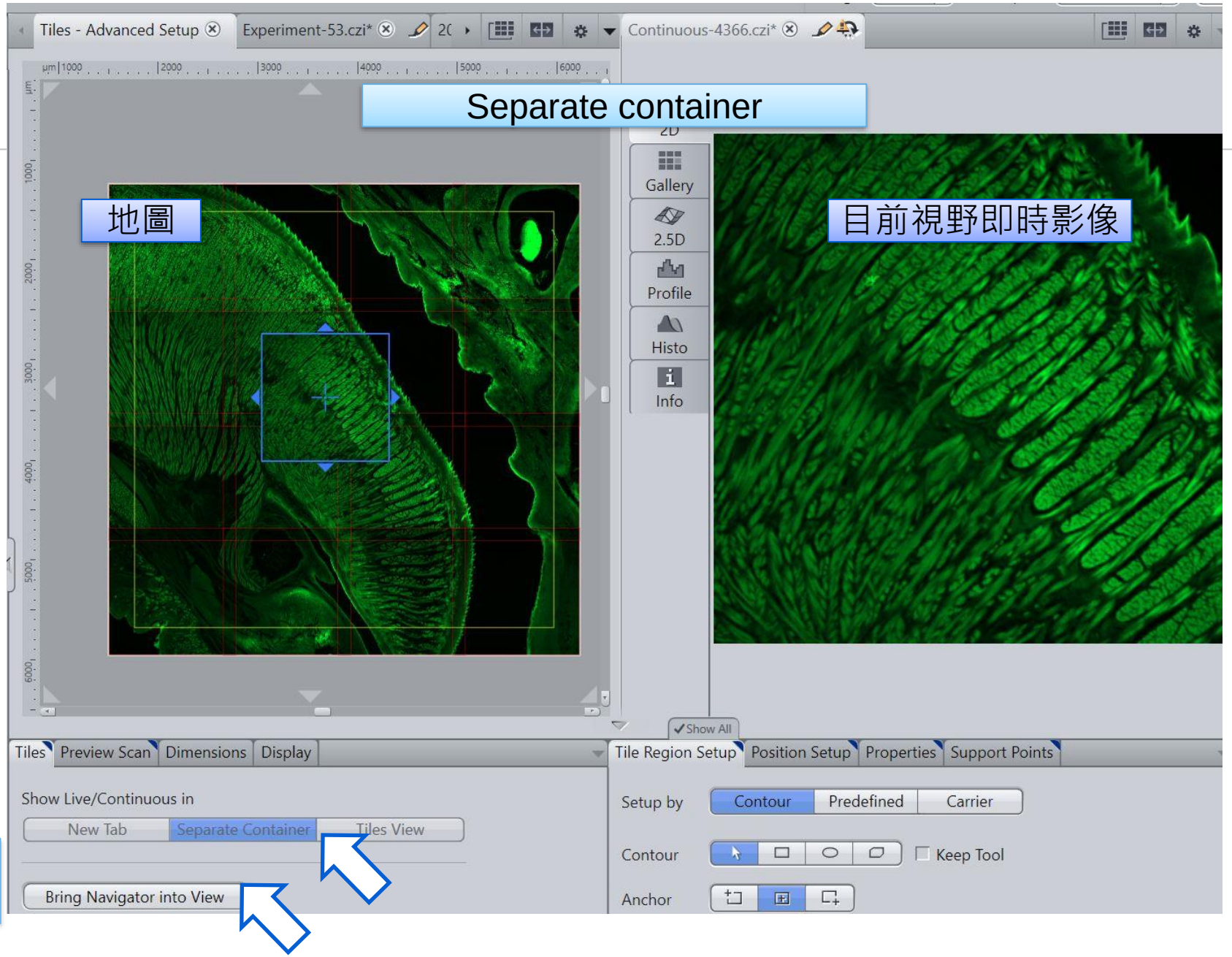
Tile Scan Imaging Setup
拼圖範圍設定 補充1



選擇工具直接於視窗中框選拍照範圍

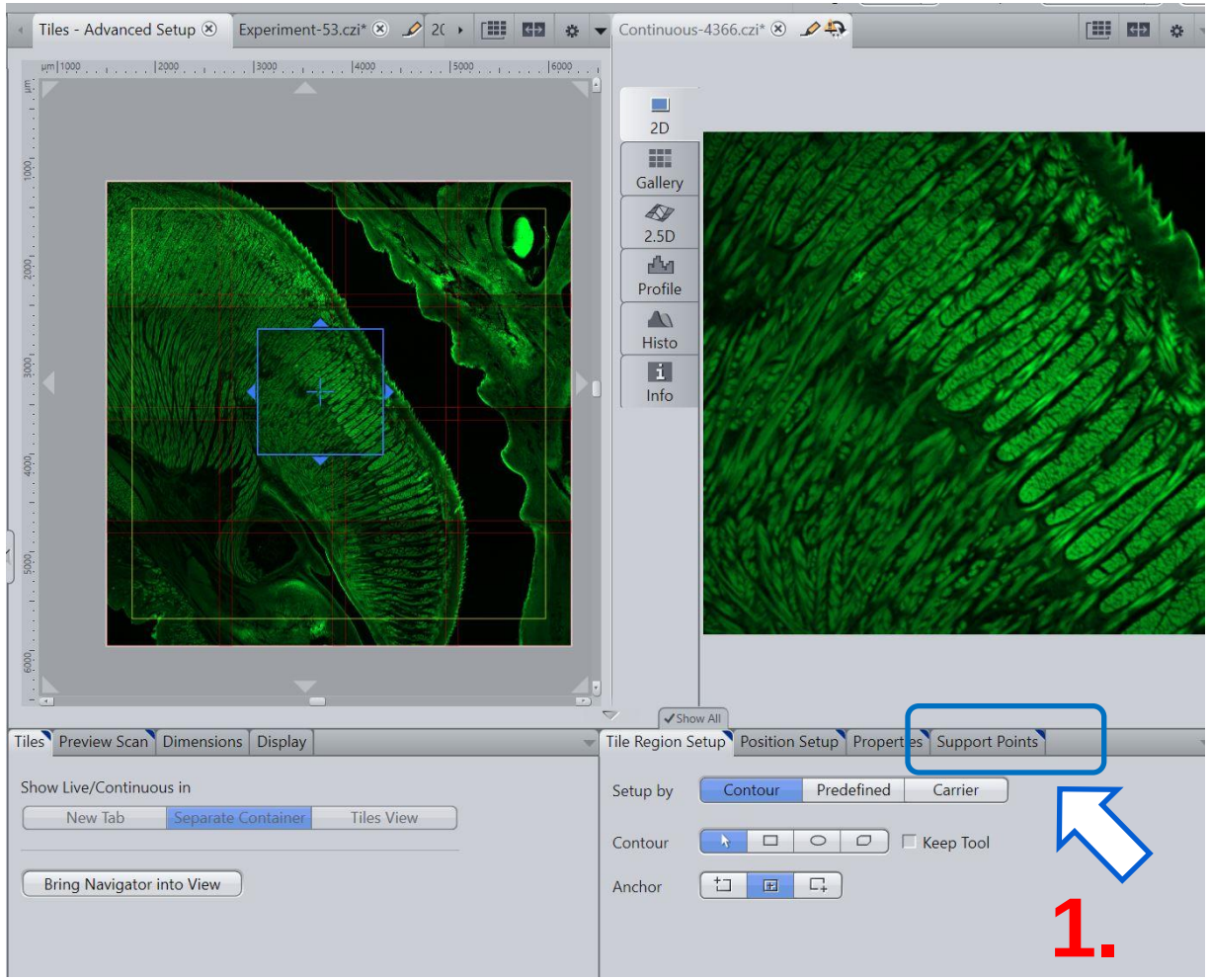
於視窗中預覽，非正式掃描

Tile Scan Imaging Setup
拼圖視窗設定 補充2

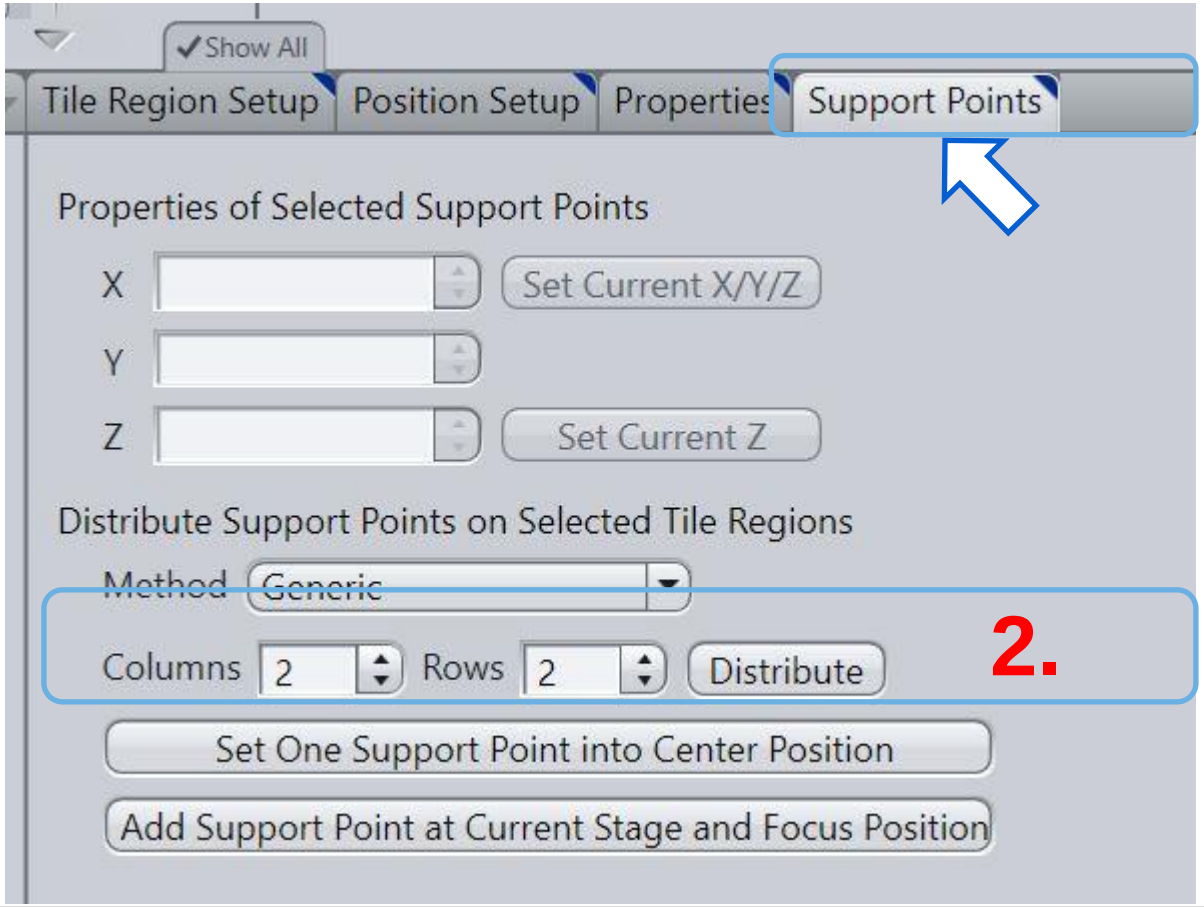


Bring Navigator into View
將視目前視野置中於視窗

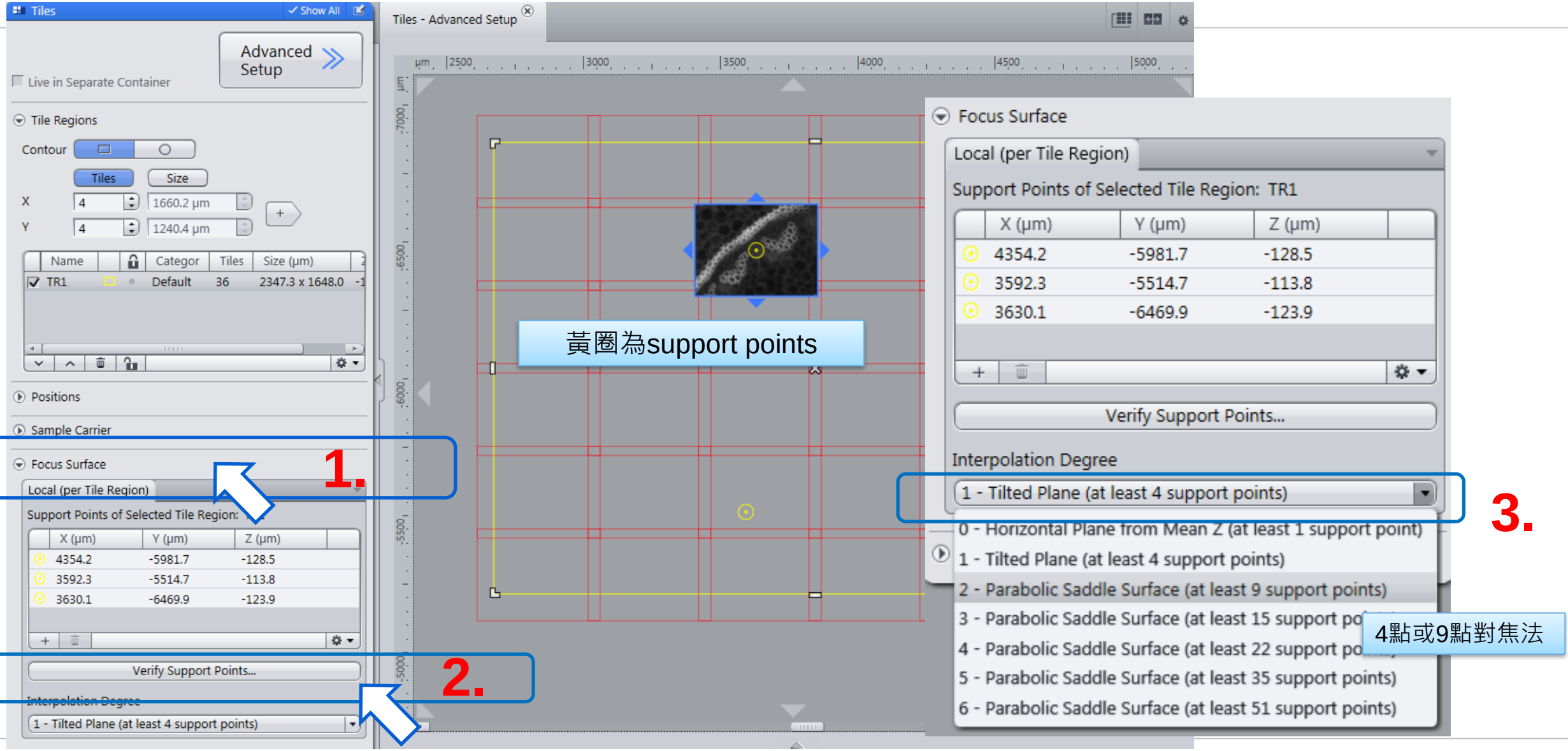
Tile Scan Imaging Setup
拼圖設定對焦輔助點 補充3



1.

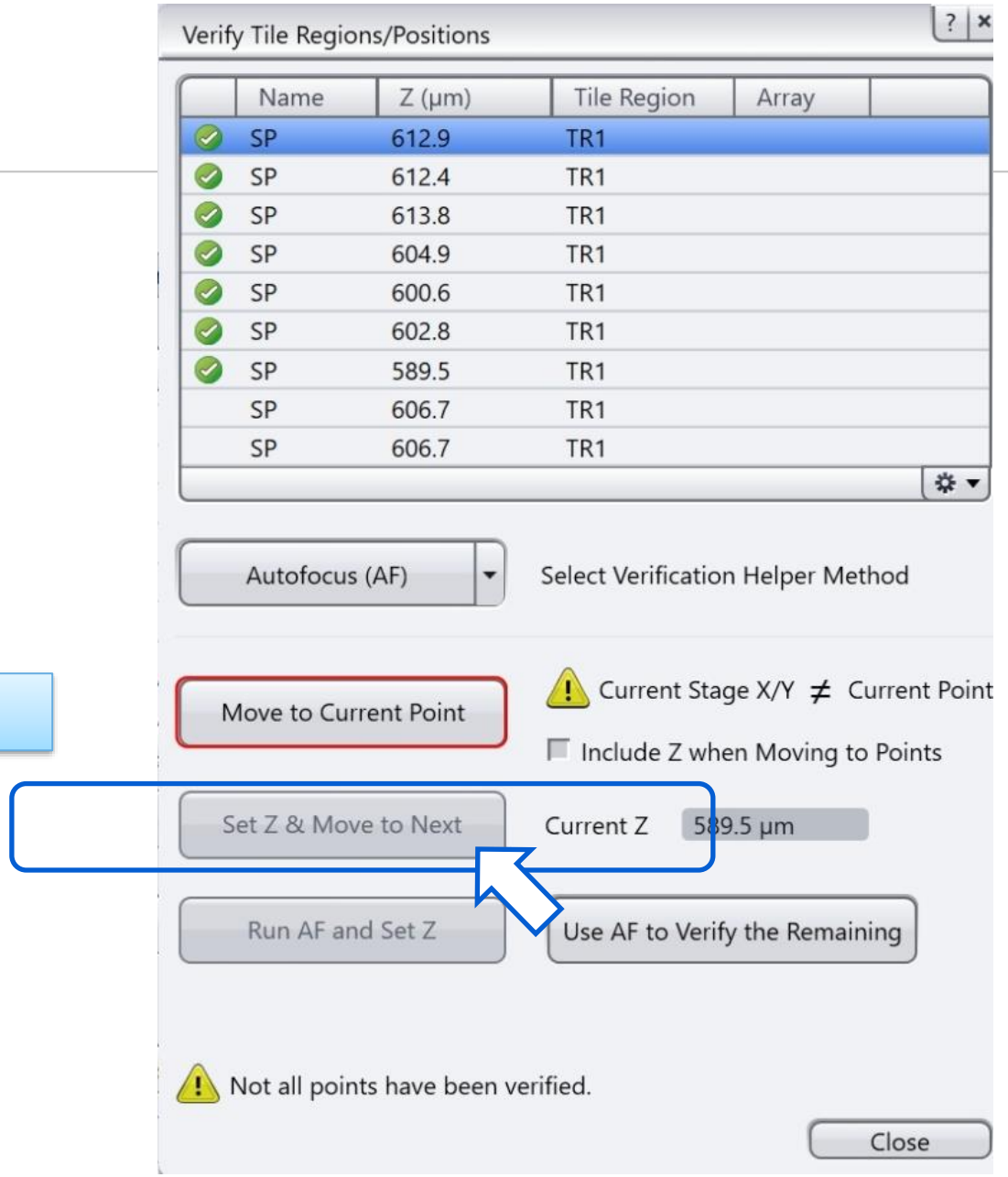


Tile Scan Imaging Setup
拼圖設定對焦輔助點 補充4

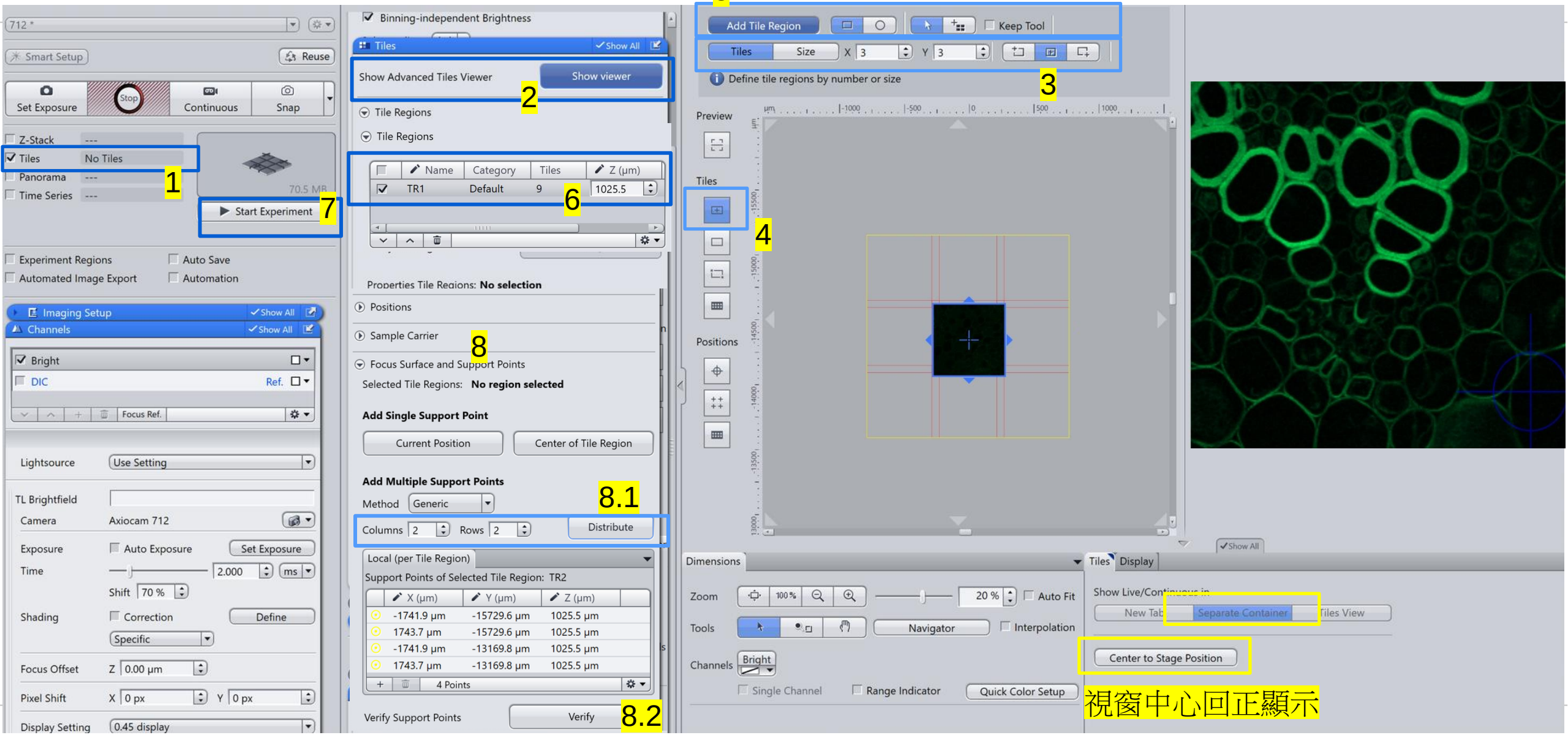


Tile Scan Imaging Setup
拼圖設定對焦輔助點 補充5

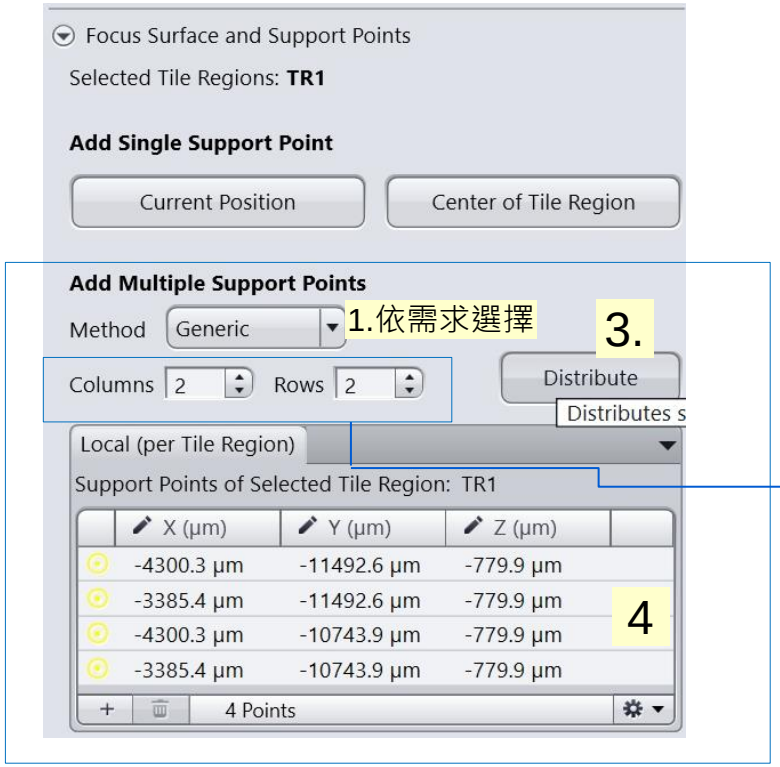
為每個Support point手動調整焦距



Tile Scan Setup
ZEN 3.3補充設定 (多位置焦距support points)



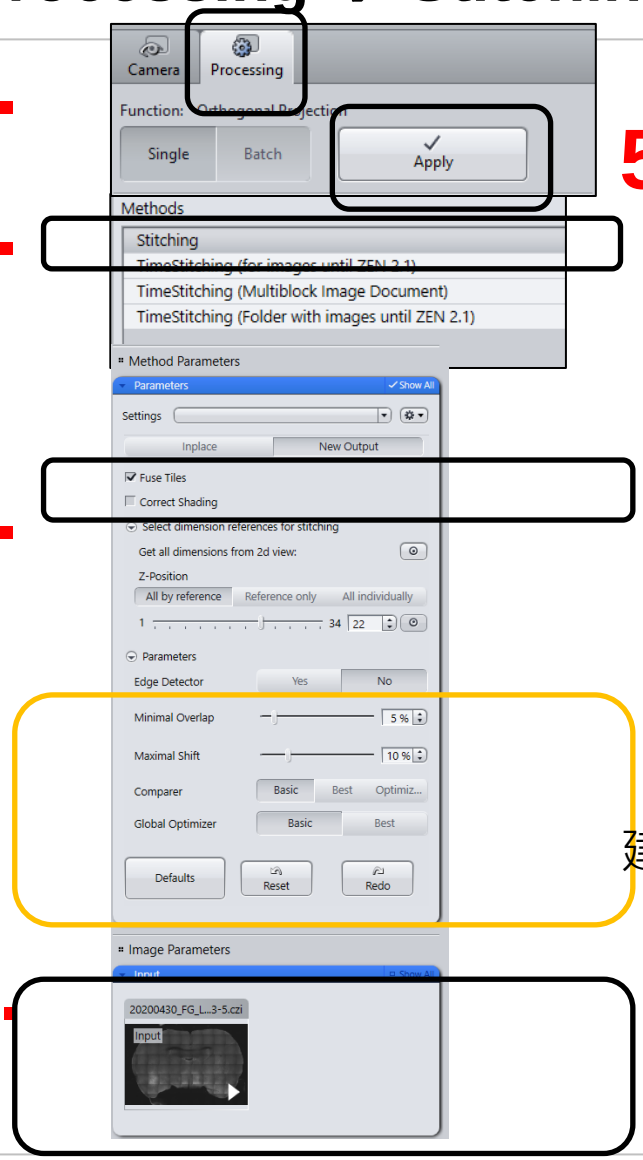
Tile Scan Setup
ZEN 3.3補充設定 (多位置焦距support points)



2.

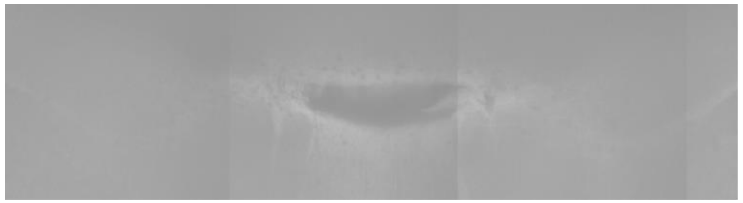
- 依樣品狀況決定support points數量
- 一般單純傾斜不必太多點，系統會自行計算焦聚

拼圖結果融合處理
Processing → Stitching / Fuse

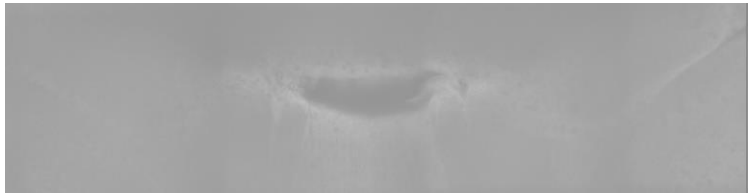


建議選擇Basic即可

Before

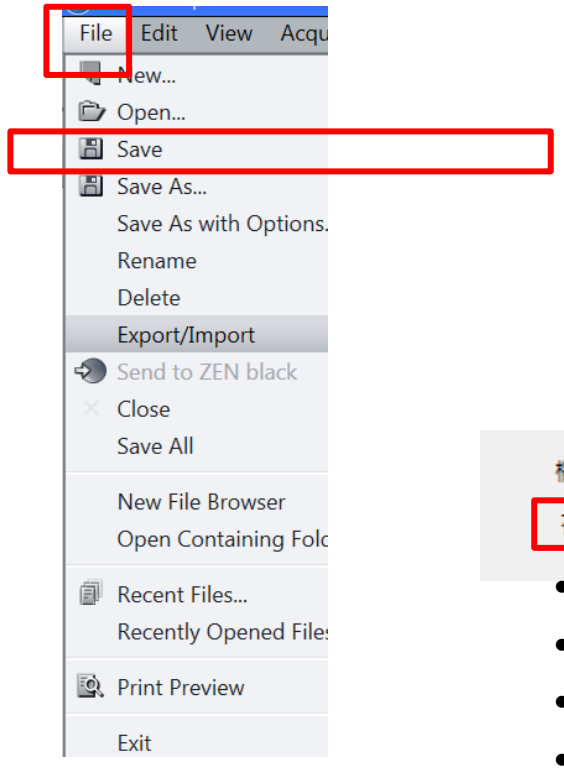


After

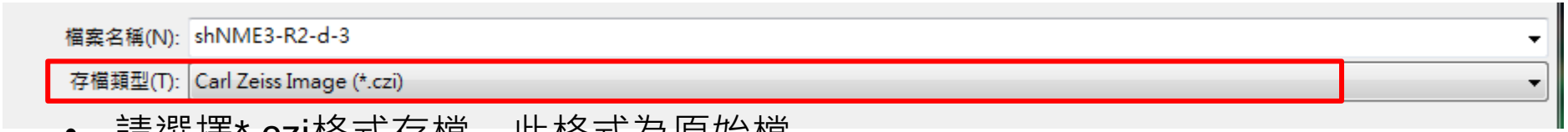


存檔

5



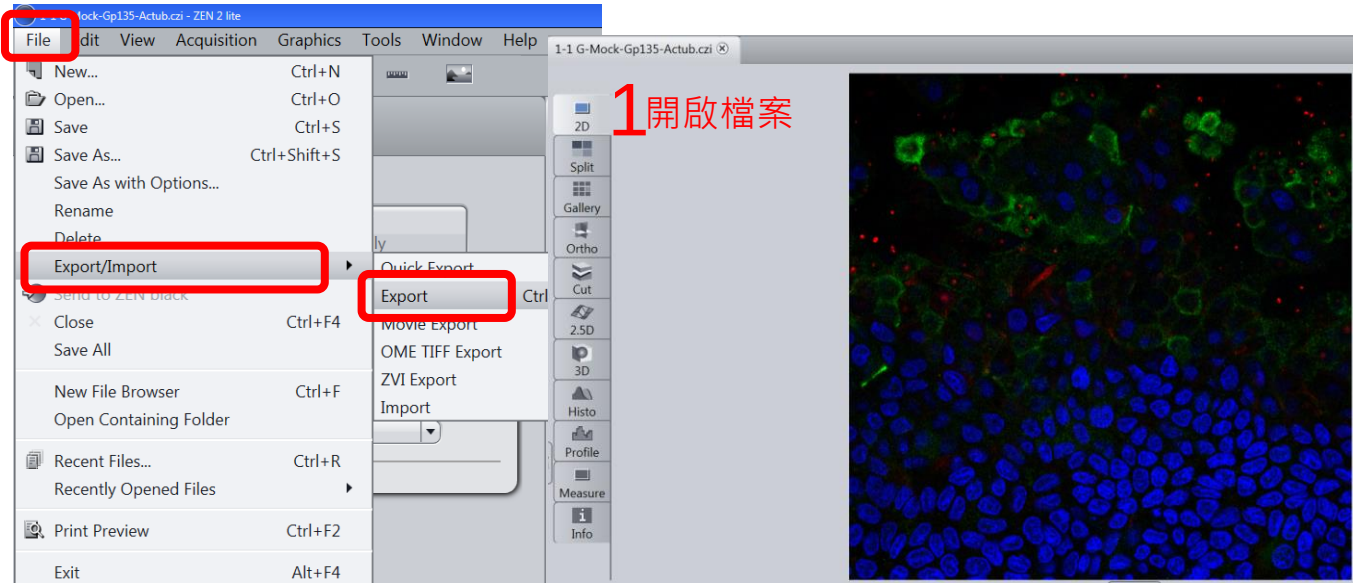
存檔



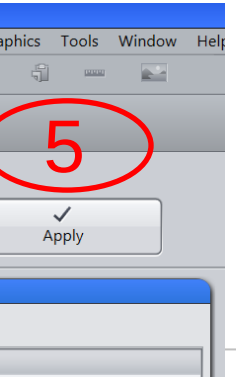
- 請選擇*.czi格式存檔，此格式為原始檔
- *.czi 日後可reuse
- 請勿直接存成tif/jpg檔，以免日後無法定量及添加尺規
- 需要純圖檔請進行圖檔輸出export或大量批次轉檔batch export，詳見下頁

圖檔輸出export (單一檔案)

2



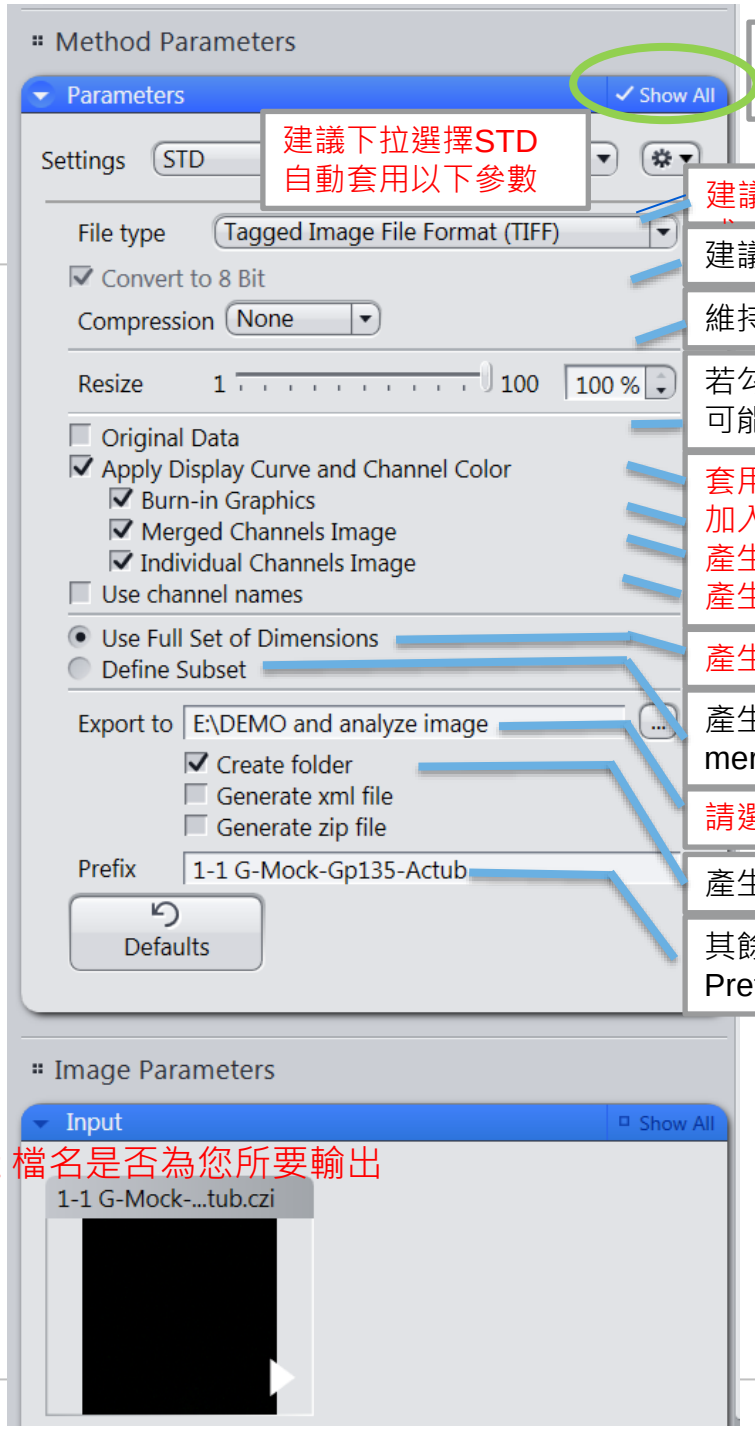
1開啟檔案



3

確認input 檔名是否為您所要輸出的檔案

4



Show all處點擊 打勾可顯示更多選項

建議TIFF為畫質較高之影像格

建議不要壓縮

維持100%，降低後畫素將減少

若勾選original data於windows可能無法看見影像

套用調整過後的明暗對比
加入尺規等標示
產生merge影像
產生個別channel影像

產生所有xyz影像

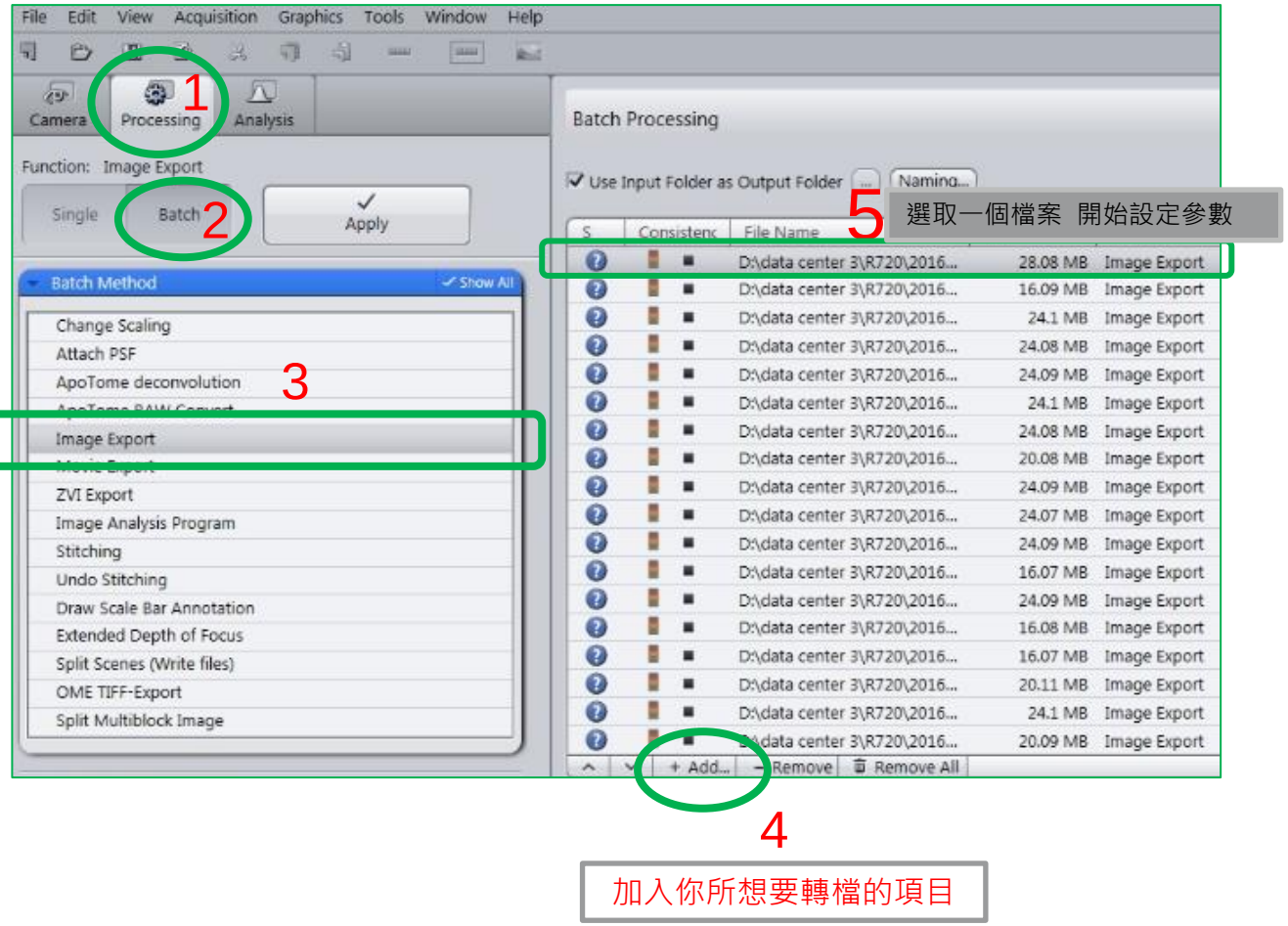
產生個別xyz影像，例如不要merge穿透光影像請由此設定

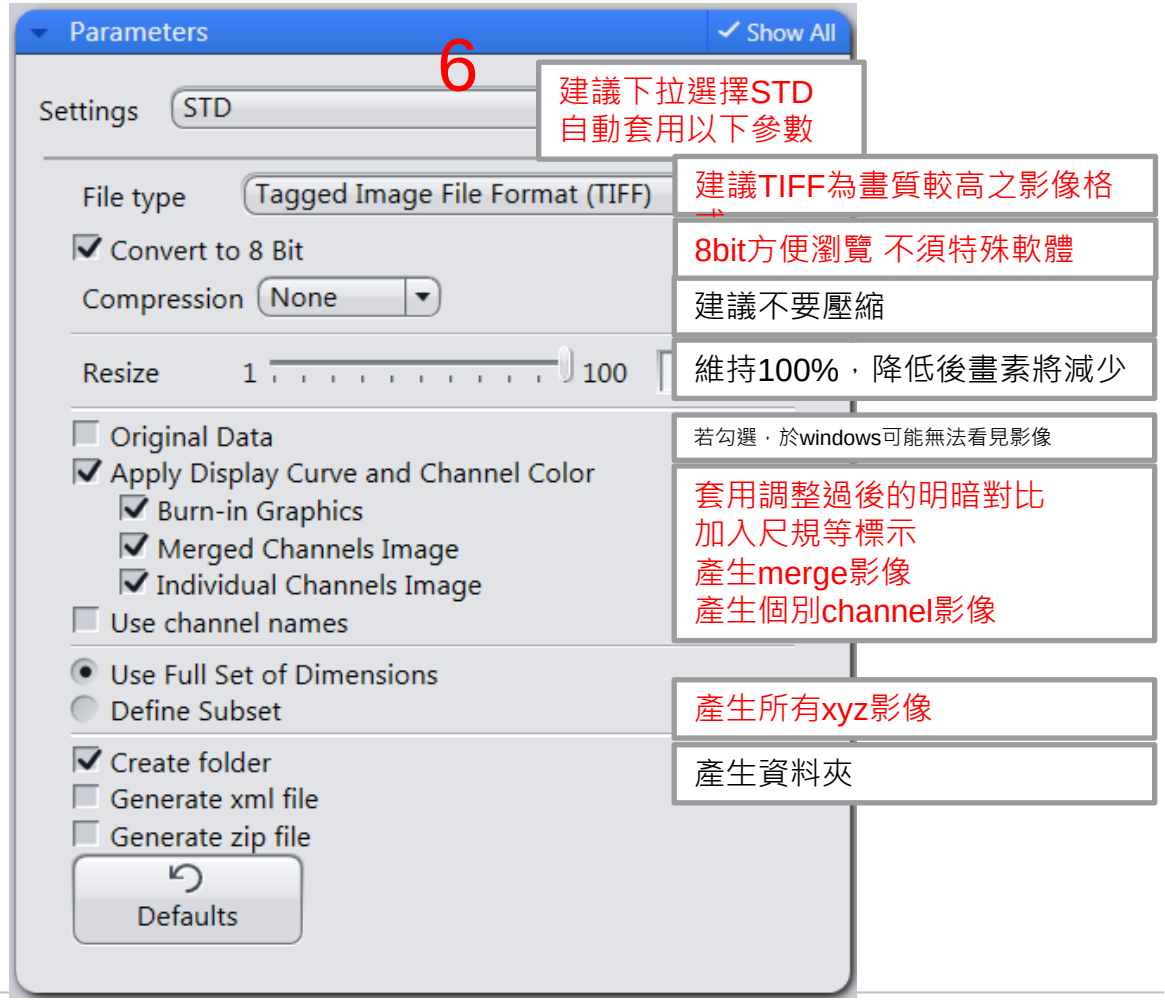
請選擇自己的資料夾位置

產生資料夾

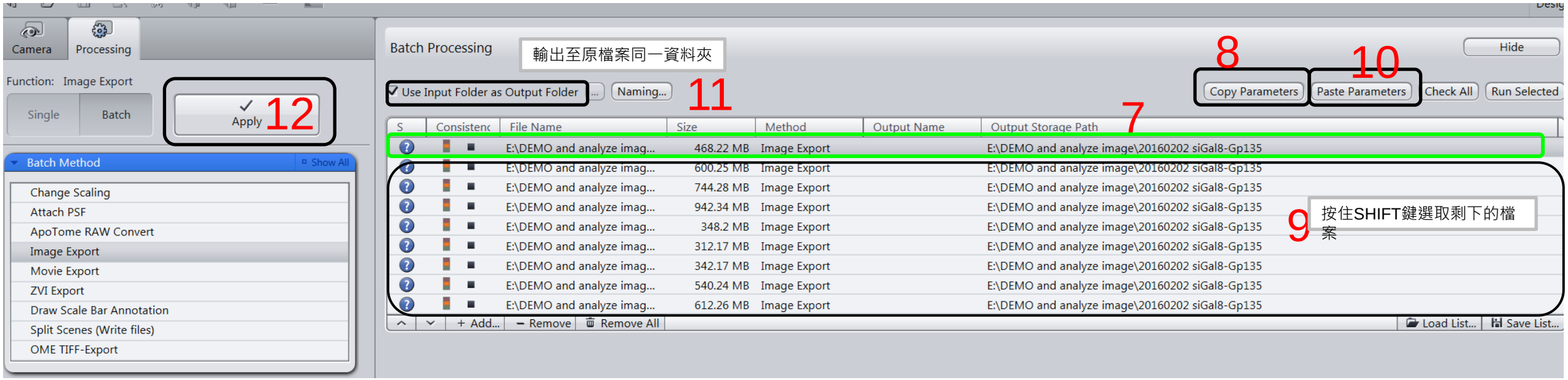
其餘設定請參考左圖
Prefix為預設檔名

大量批次轉檔batch export 1





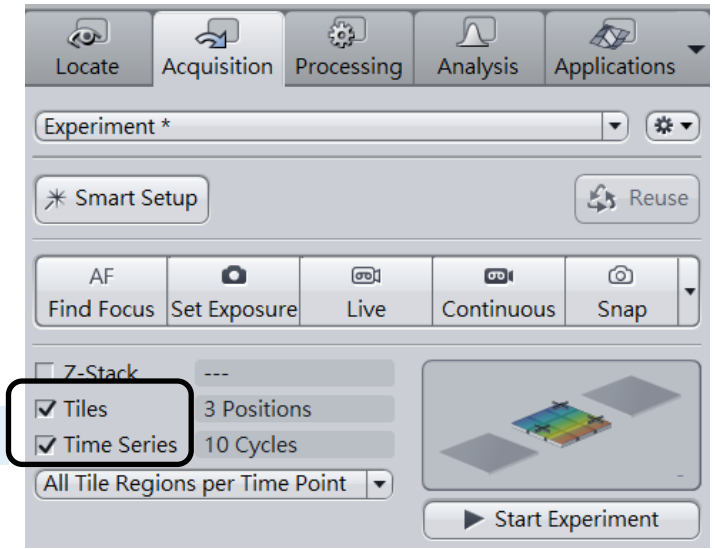
大量批次轉檔batch export 2



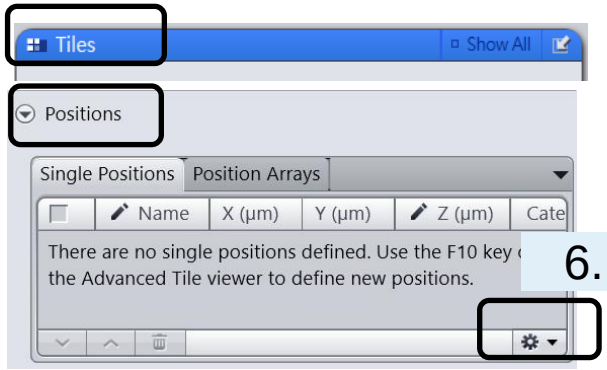
7~10 將設訂好的參數貼至其餘檔案當中，若沒有做paste parameter的動作，可能會失敗!

Multi-position Time Lapse Imaging 1.
Mark the positions of interests
Import stage marks

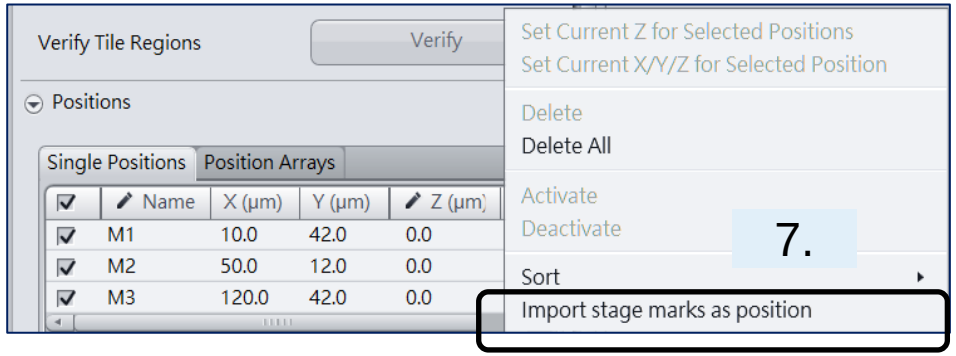
1.



5.

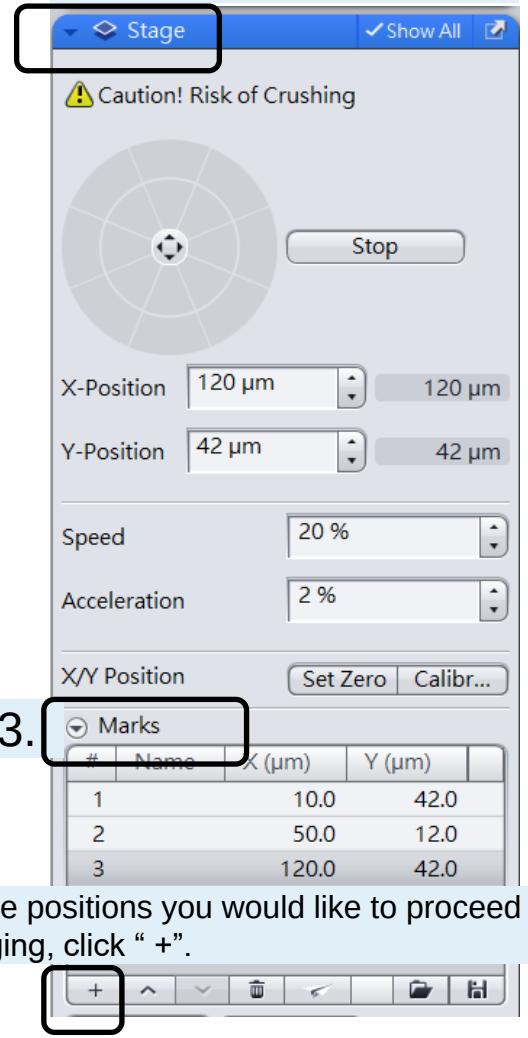


6.



7.

2. Stage → Marks

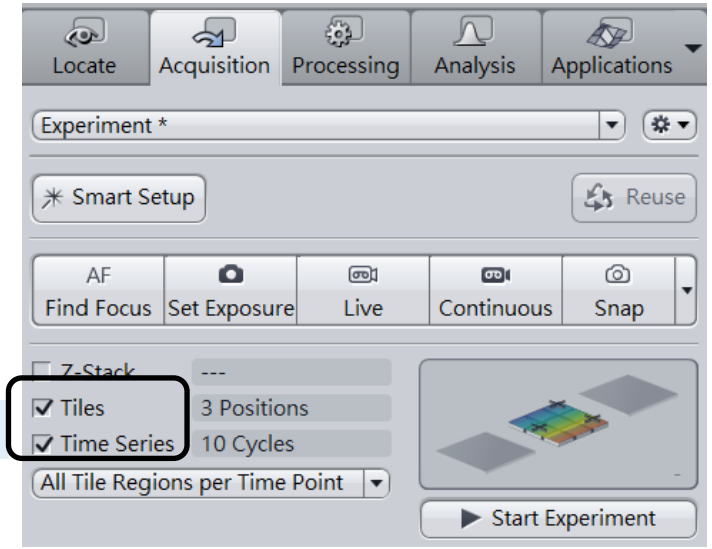


3.

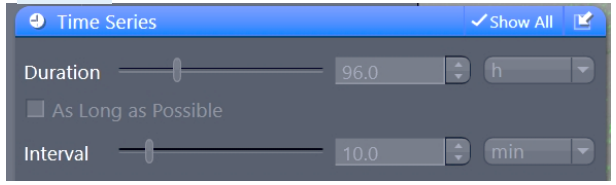
4. At the positions you would like to proceed imaging, click " +".

Multi-position Time Lapse Imaging 2.
Definite Focus
Focus Strategy 長時間自動追焦

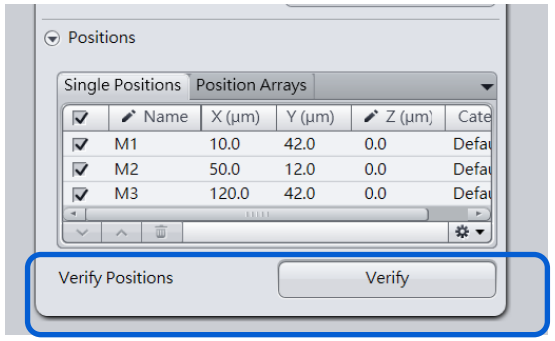
1.



2.



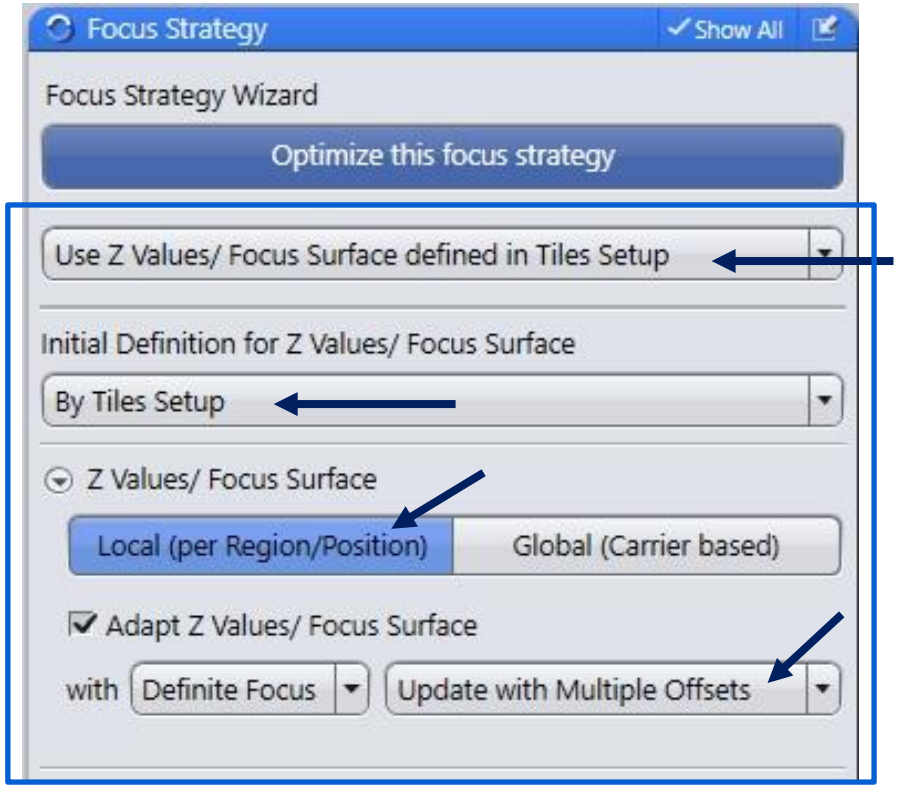
4.



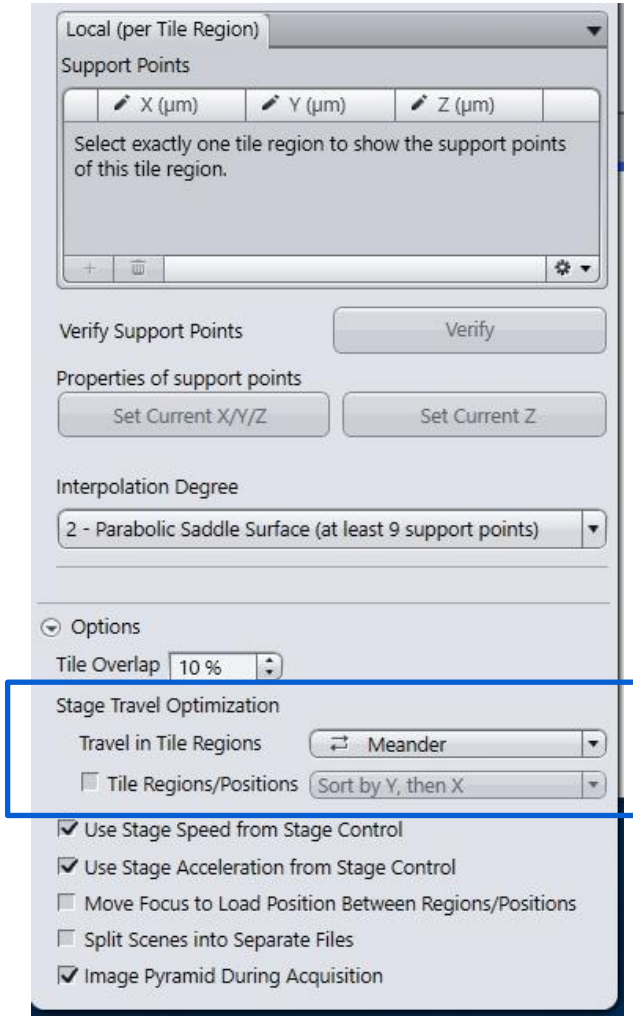
5.

- 所有位置焦距設定完畢
- 按下Start Experiment後軟體會自動設Multiple Offsets

3.

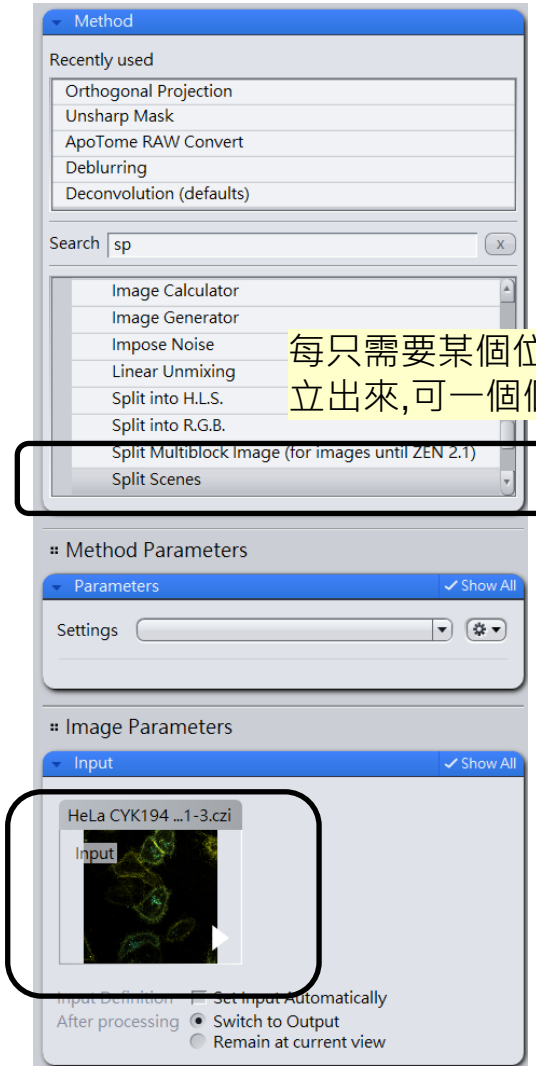


Multi-position Time Lapse Imaging 3. Options
多孔盤注意: 避免位置拍攝順序混亂



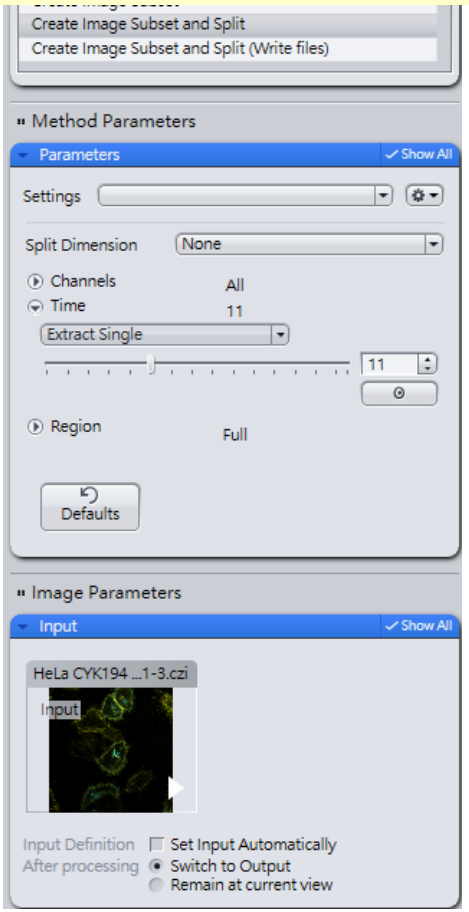
- 按照positions 順序拍攝請取消Meander!
- 否則位置拍攝順序會重新排序

多位置拍攝長時間
依位置切割檔案
Processing → Split Scenes/ Create Subsets

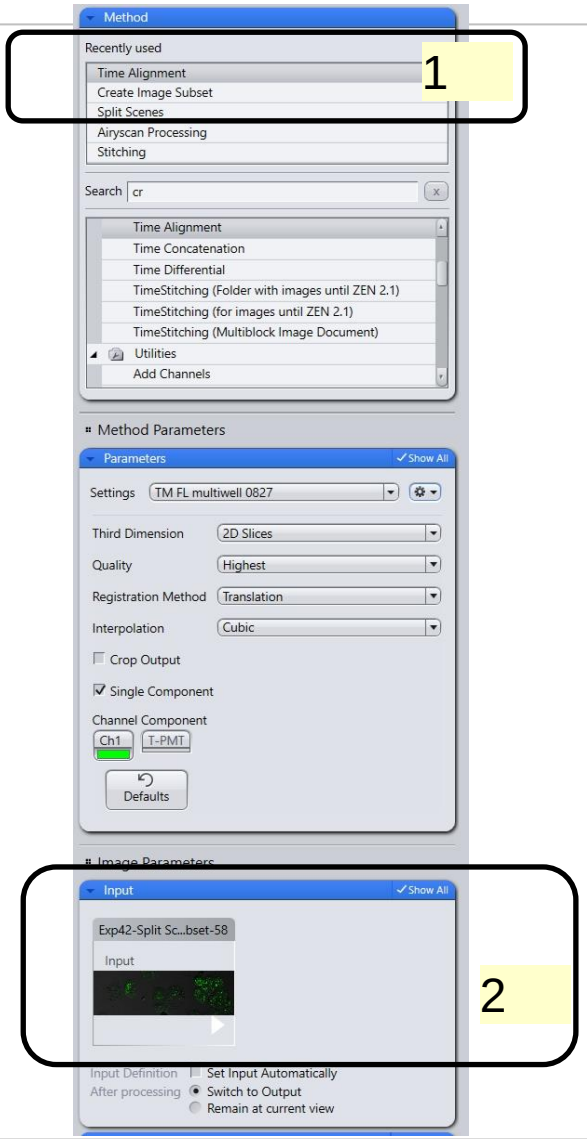


每只需要某個位置的結果獨立出來,可一個個分別存檔

只需要某個位置或時間的結果獨立出來



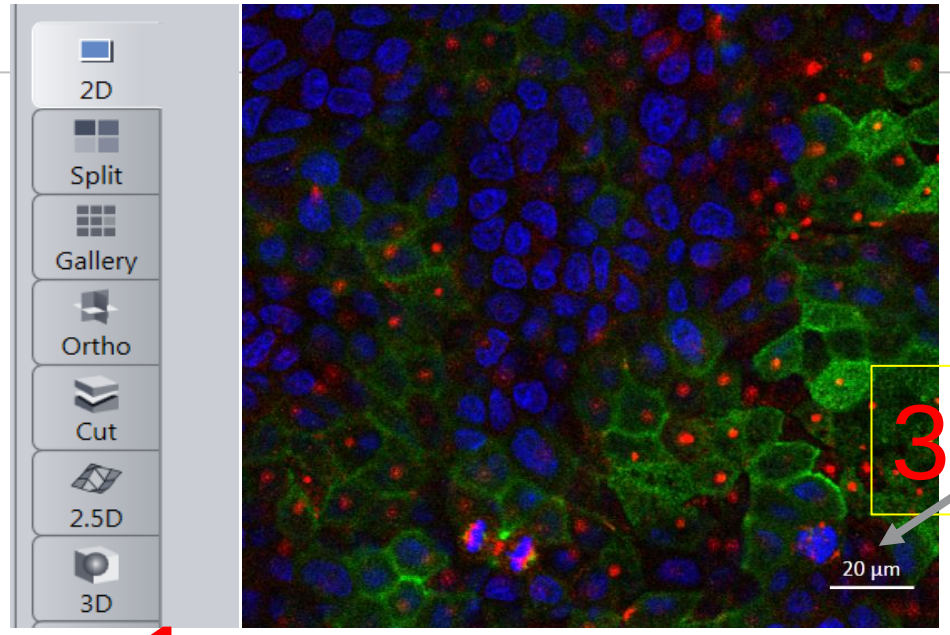
多位置拍攝長時間晃動問題
Processing → Time lapse Alignment



關於連續撥放影片晃動

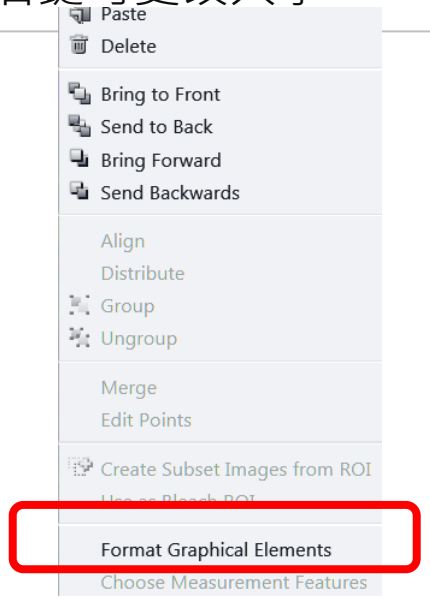
- 請放好樣品後對焦完畢，**以黏土或工具固定樣品**以免因載物台長時間多點移動造成樣品位移。
- 若合併多點(位置)拍攝此步驟請split scene切割完畢再執行

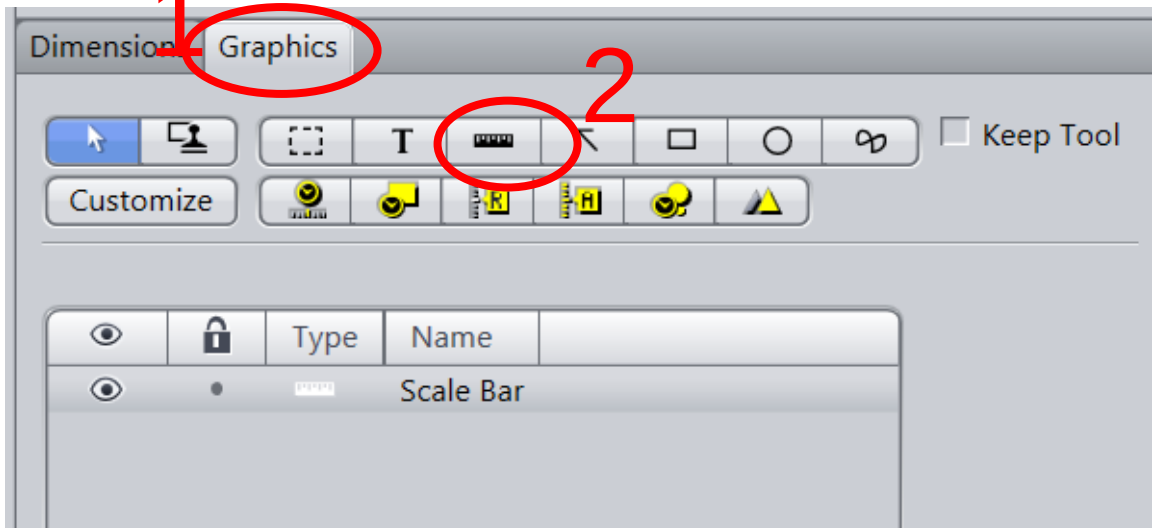
Add Scale Bar加入尺規

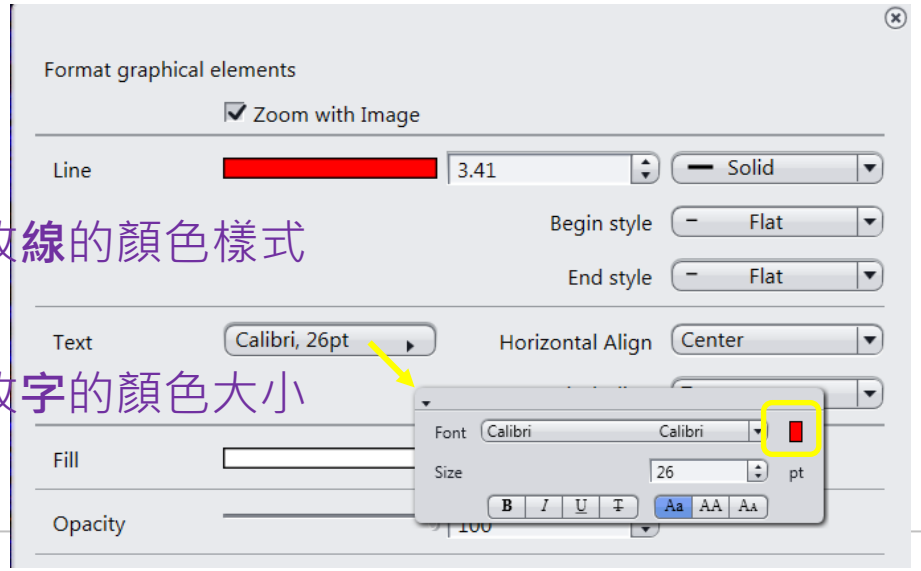


3 scale bar會自動出現

4 於scale bar按右鍵可更改大小顏色粗細等格式



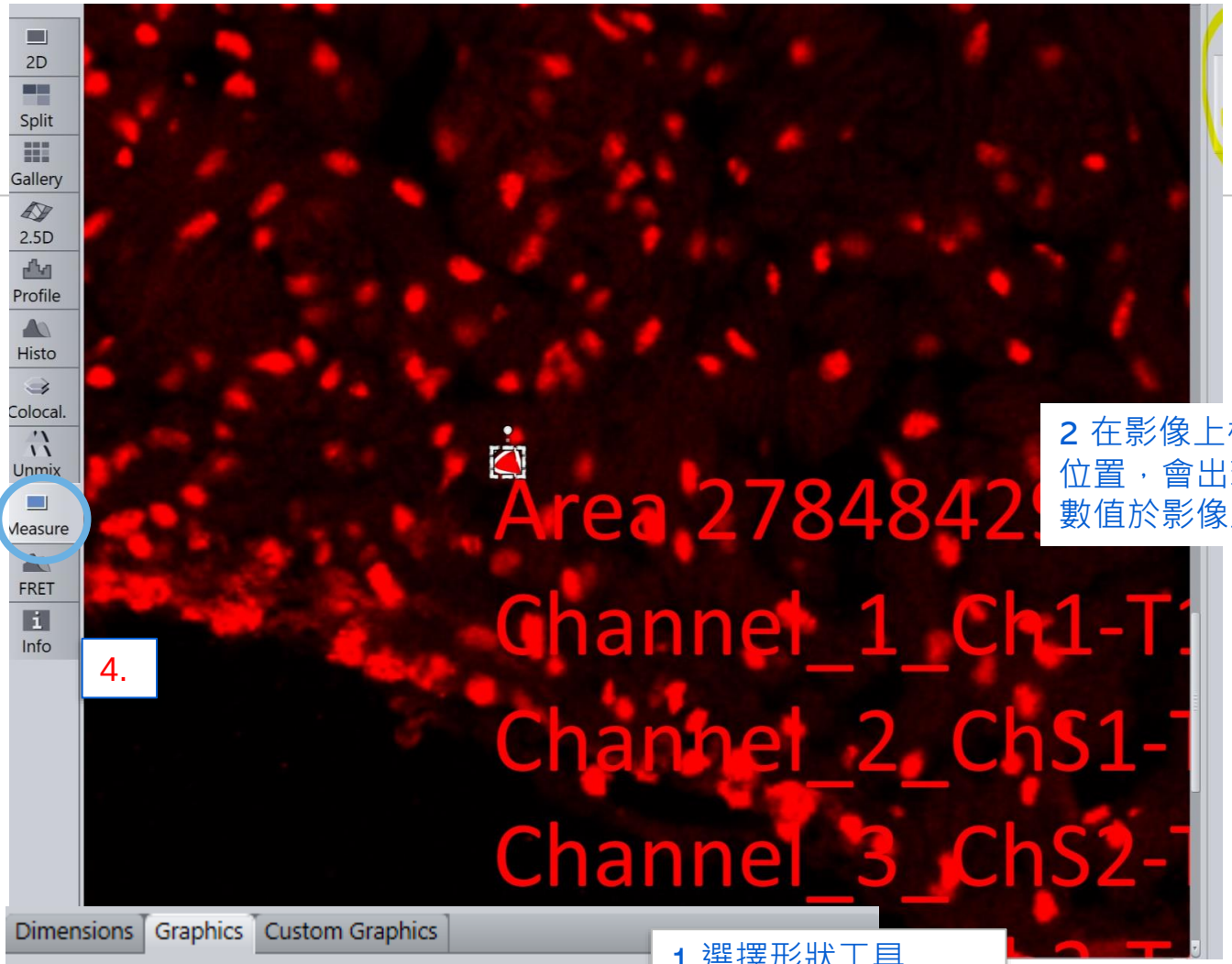




更改線的顏色樣式

更改字的顏色大小

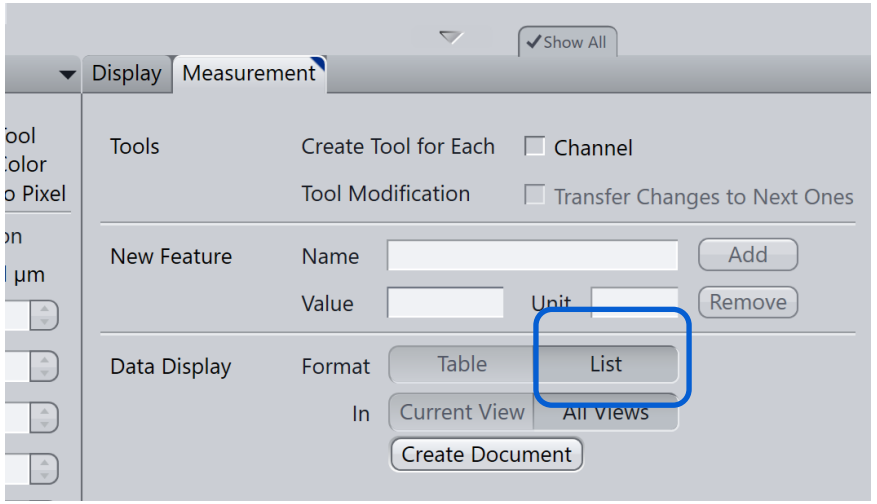
量測area & intensity

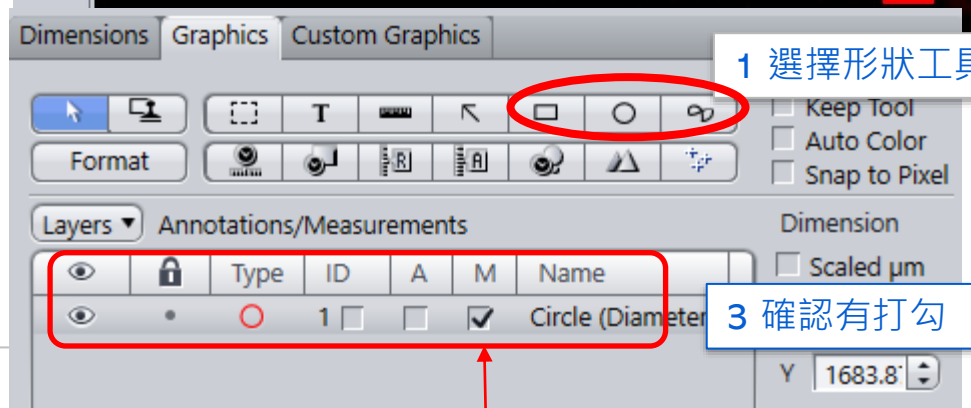


2 在影像上框選有興趣的位置，會出現面積及亮度數值於影像上

4.圖表位於右方視窗

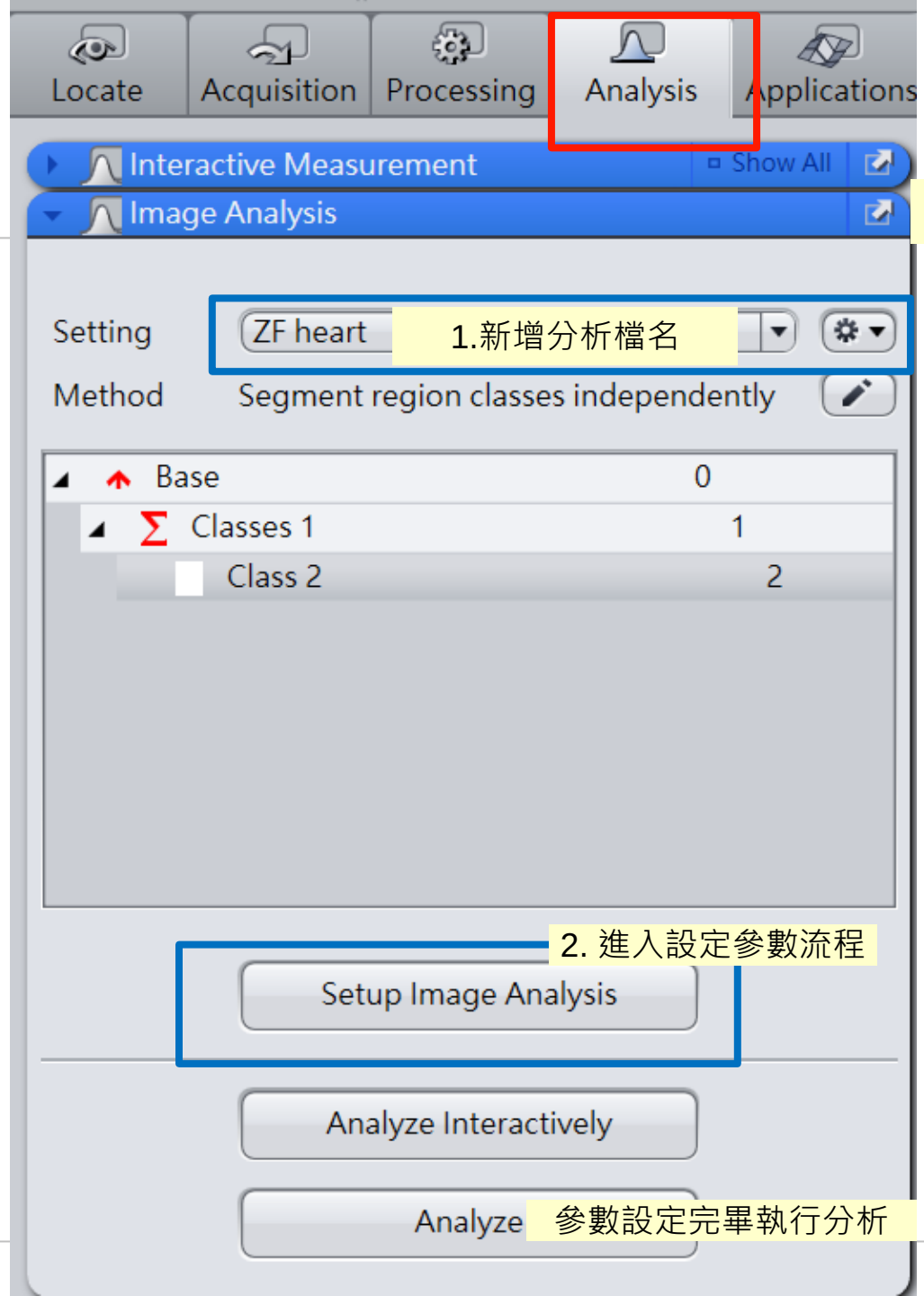
	Name	Feature	Value	Unit
	A	B	C	D
1	Circle (Diameter)	Area	1,146,259.648	µm²
2	Circle (Diameter)	Channel_3_mPlum.Intensity Mean Value	8,855.551	
3	Circle (Diameter)	Channel_2_EGFP.Intensity Mean Value	5,622.191	
4	Circle (Diameter)	Channel_1_DAPI.Intensity Mean Value	3,224.520	
5	Circle (Diameter)	Diameter	1,208.082	µm
6	Circle (Diameter)	Channel_4_Cy5.Intensity Mean Value	808.316	





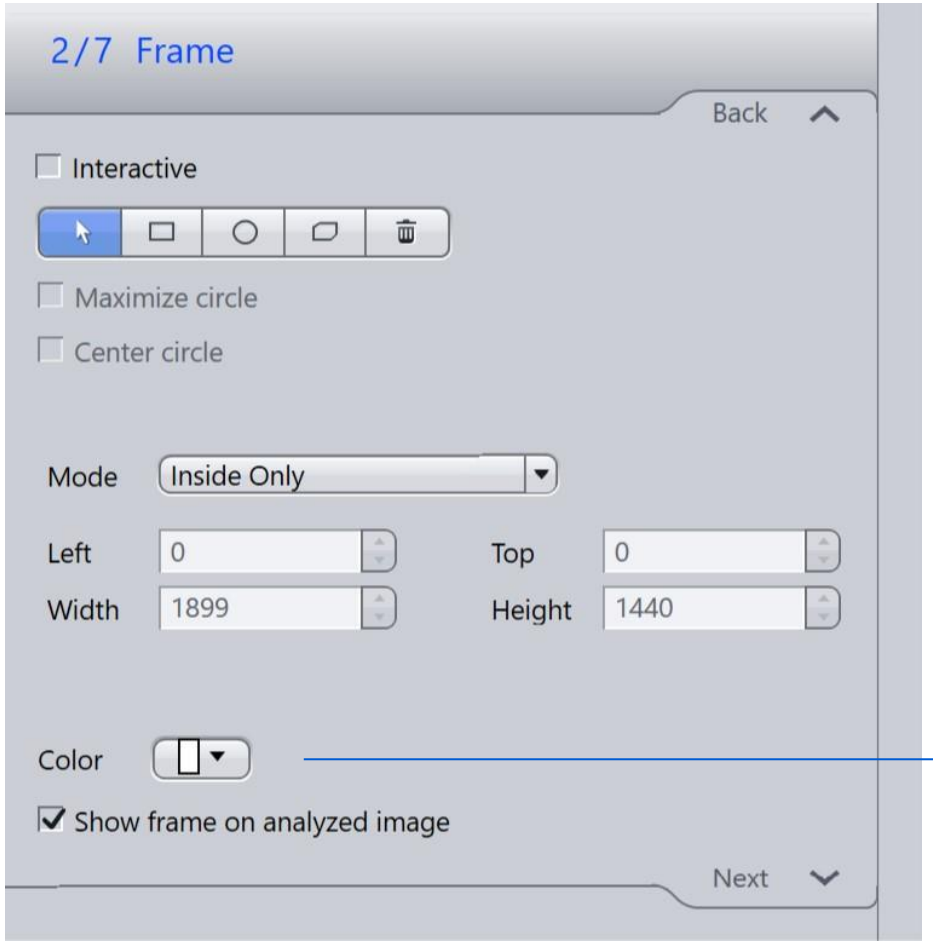
自動量測 Image Analysis Module 1.

以量測前頁影像內的紅色細胞核數目為例



- Assign the channel to the Class
- Class 1 要量測的是Chs2-T3
- 稍後Mask 套色預設白色

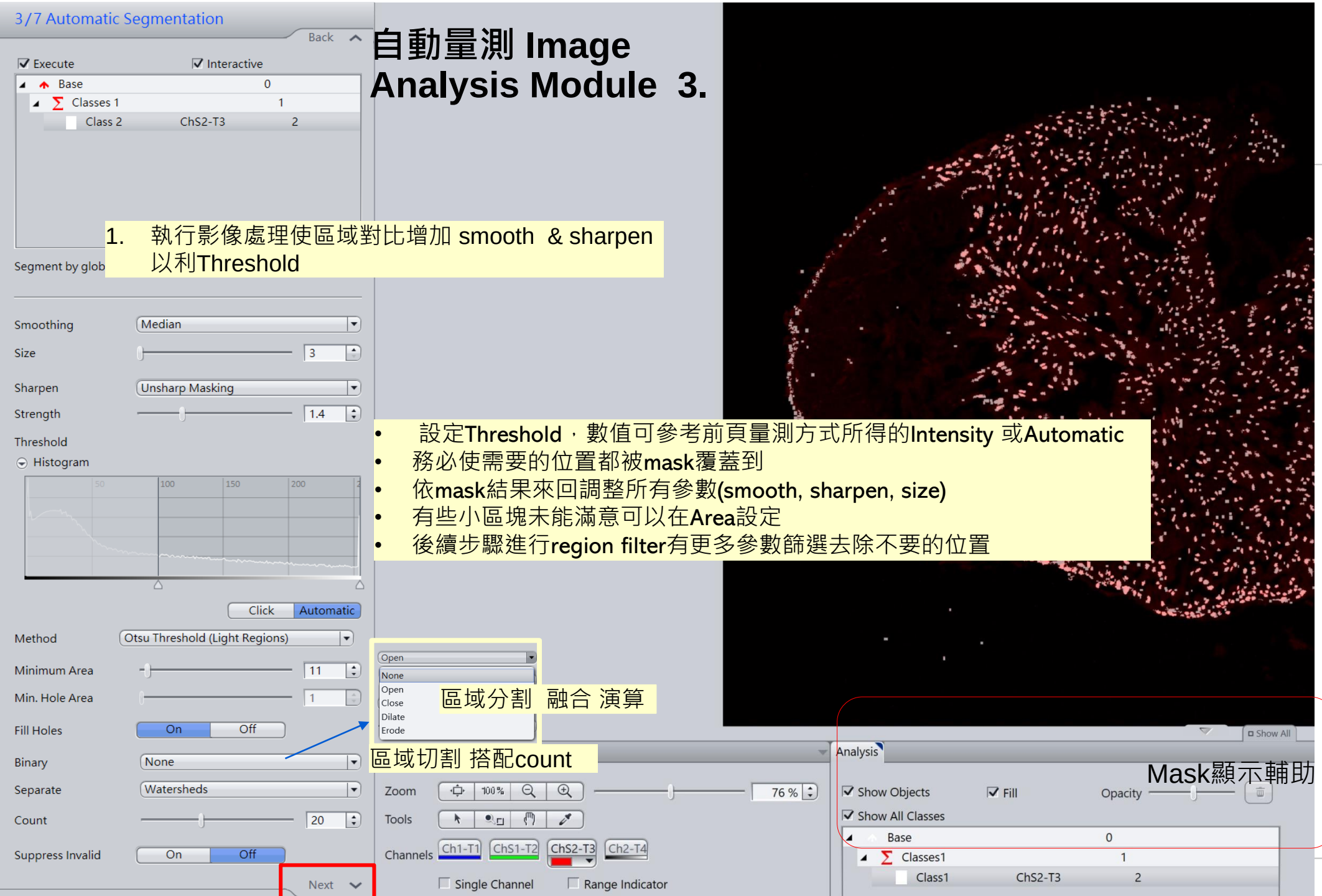
自動量測 Image Analysis Module 2.
圈選分析範圍，可略過



- 圖檔不大請略過此頁步驟
- 可針對要分析的位置框選位置
- 整面要量測請略過此步驟

- 量測區域外框顏色

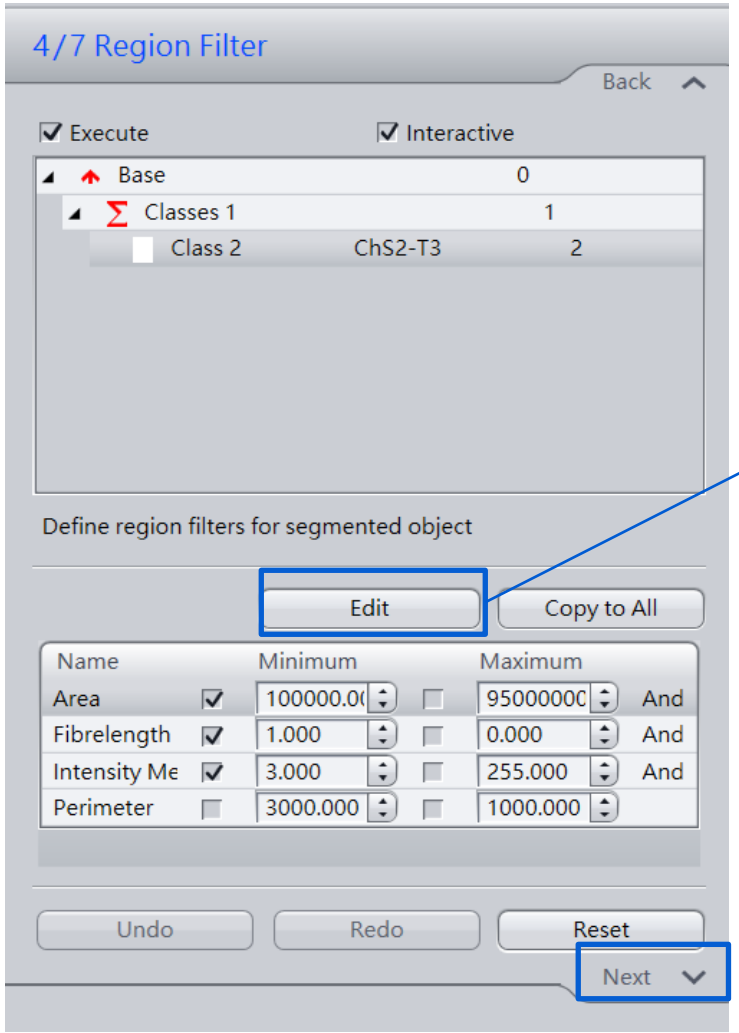
自動量測 Image Analysis Module 3.

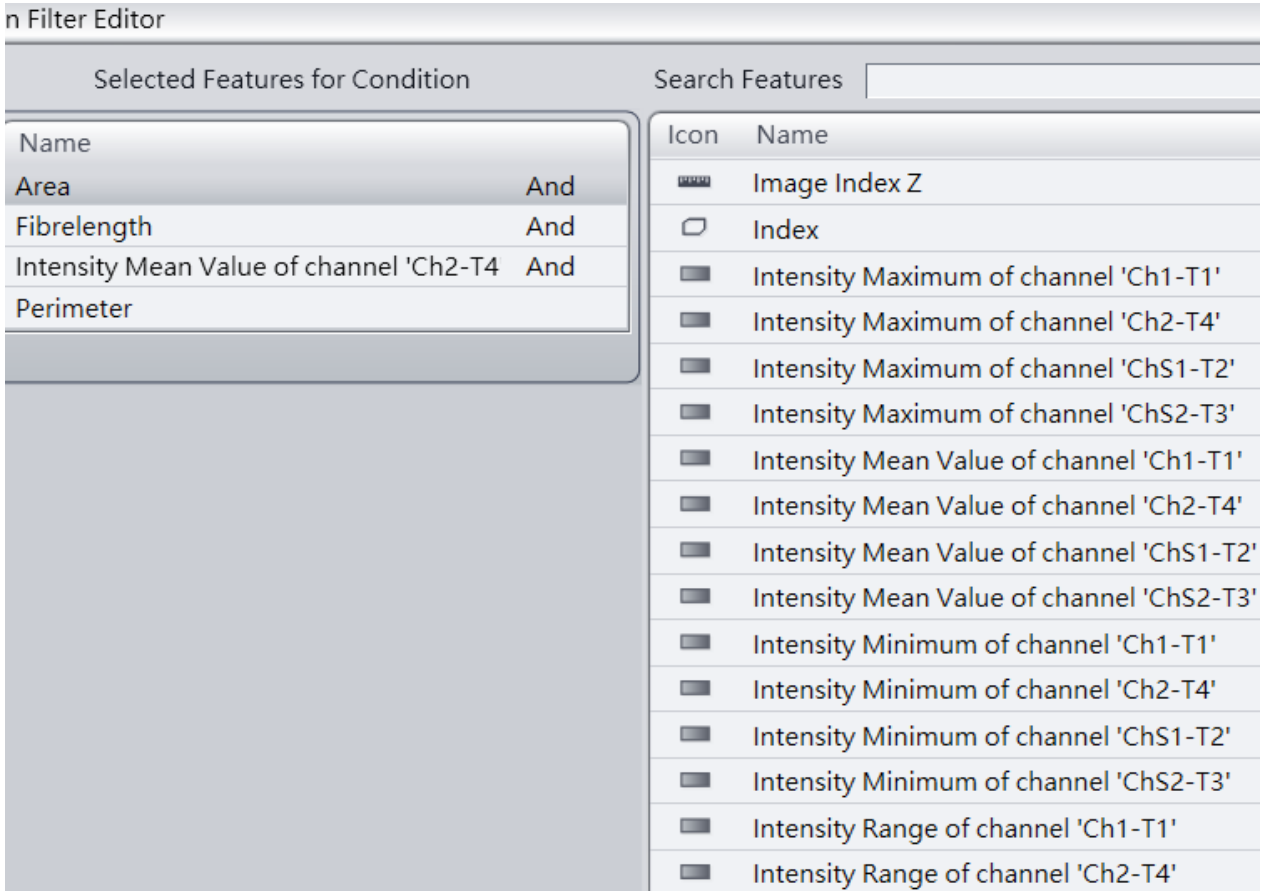


1. 執行影像處理使區域對比增加 smooth & sharpen 以利Threshold

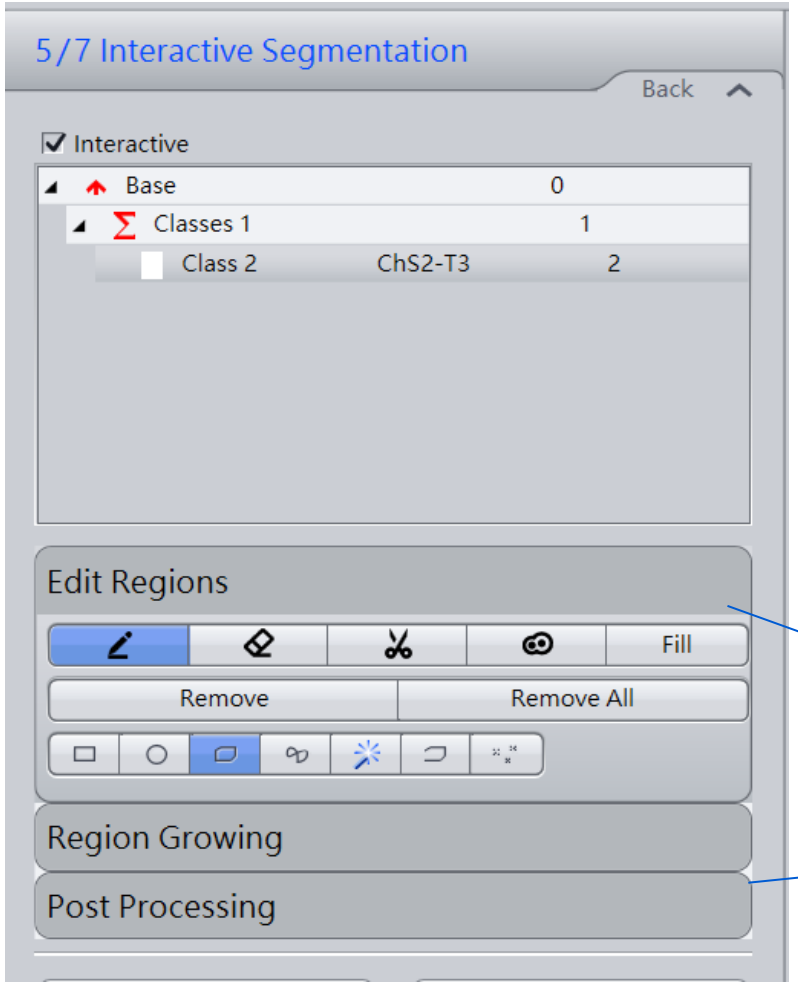
- 設定Threshold，數值可參考前頁量測方式所得的Intensity 或Automatic
- 務必使需要的位置都被mask覆蓋到
- 依mask結果來回調整所有參數(smooth, sharpen, size)
- 有些小區塊未能滿意可以在Area設定
- 後續步驟進行region filter有更多參數篩選去除不要的位置

自動量測 Image Analysis Module 4.
若仍有不需計算的位置，此步驟可進一步篩出需要的位置，依需求加入各式range filter





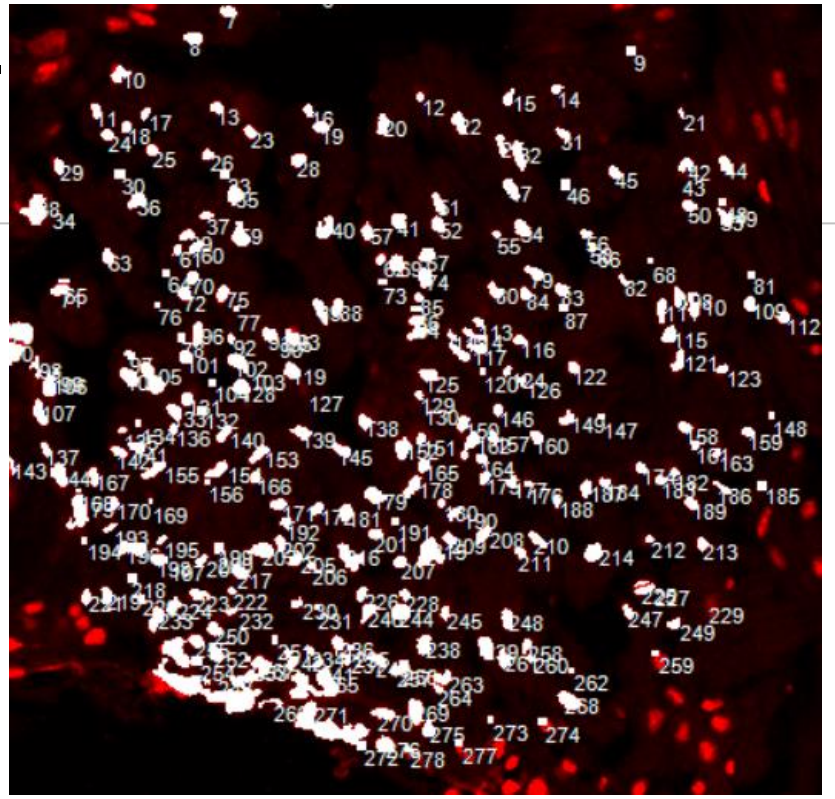
自動量測 Image Analysis Module 5.
互動式調整量測區域 增加 減少 或 切割 縫合
批次處理需求請略過此步

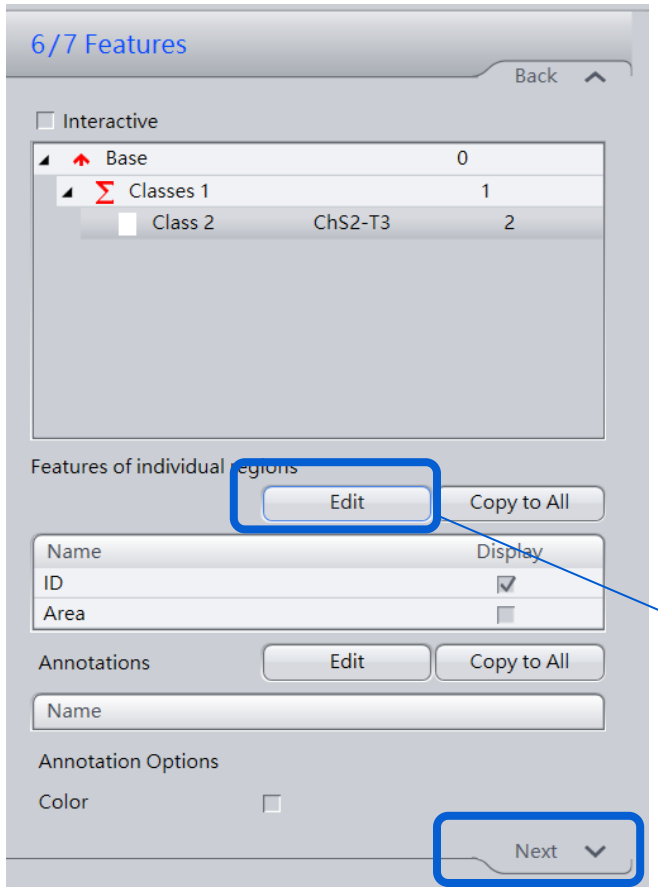


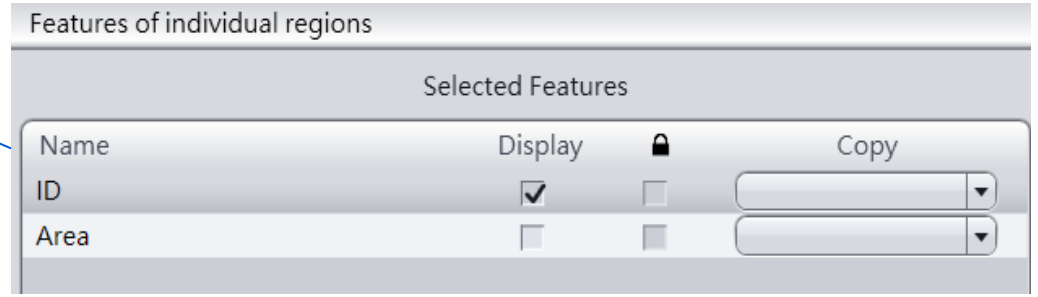
- 手動增加/刪除區域
- 手動切割/接合區域
- 若要進行自動大量圖檔批次分析，請避免使用此步驟

- 此例上述步驟已滿足需求，略過不再進行調整

自動量測 Image Analysis Module 6. Features 顯示設定

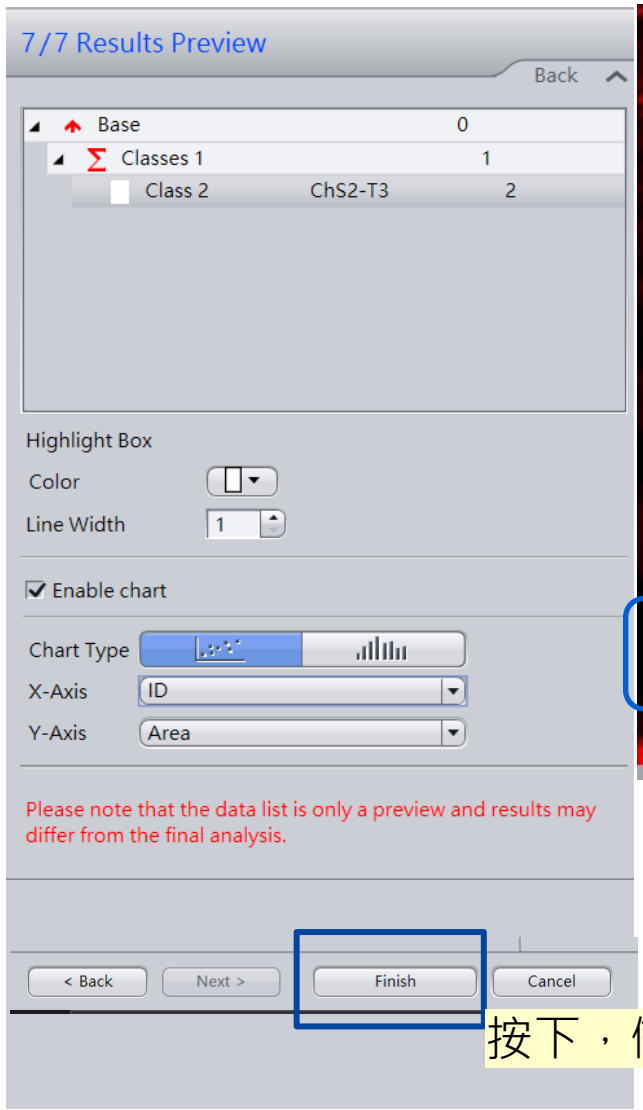


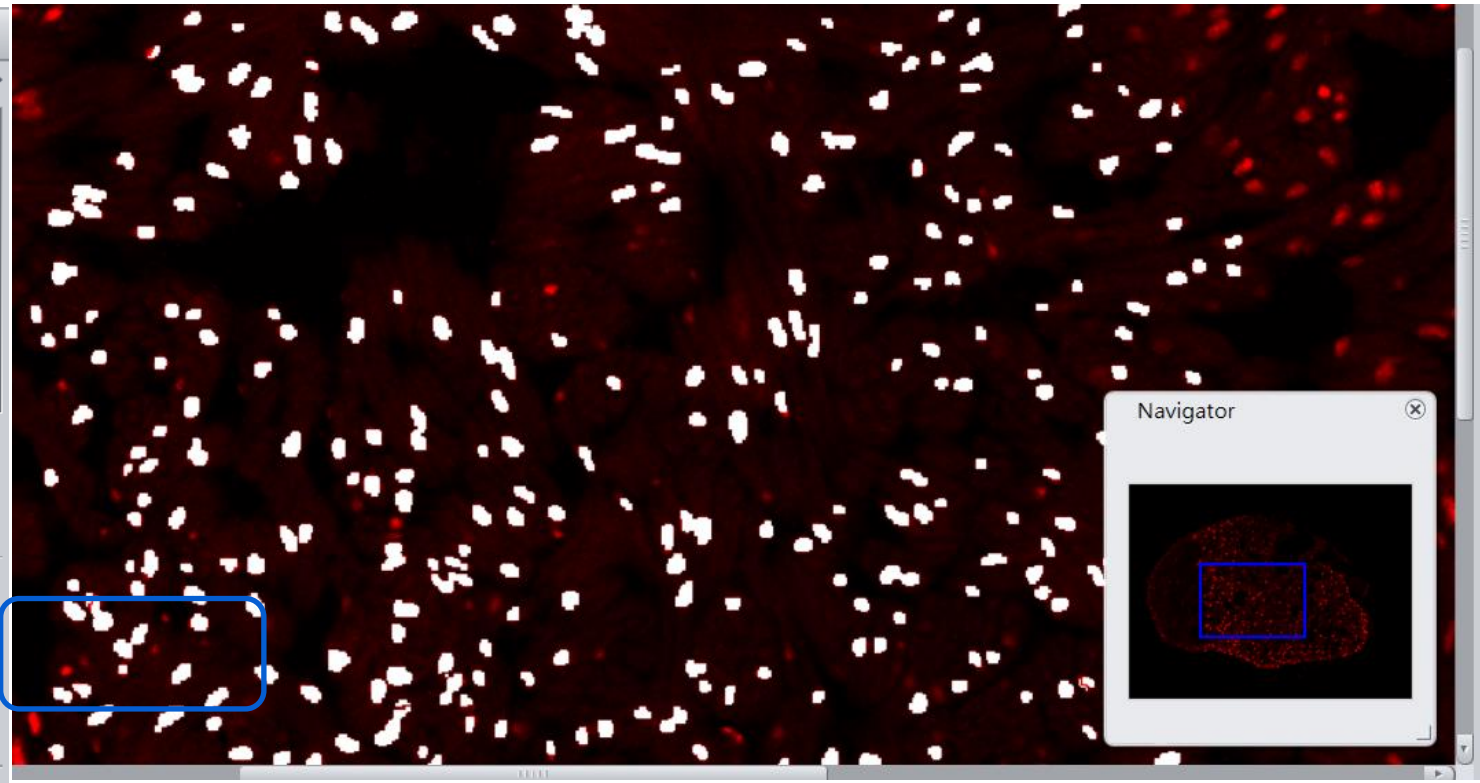




- 圖上若不需要顯示量測結果不要勾Display
- 量測結果顯示設定 ， 如果密密麻麻的mask 請避免使用，以免影響觀察量測區域

自動量測 Image Analysis Module 7.
參數設定完畢，結果預覽



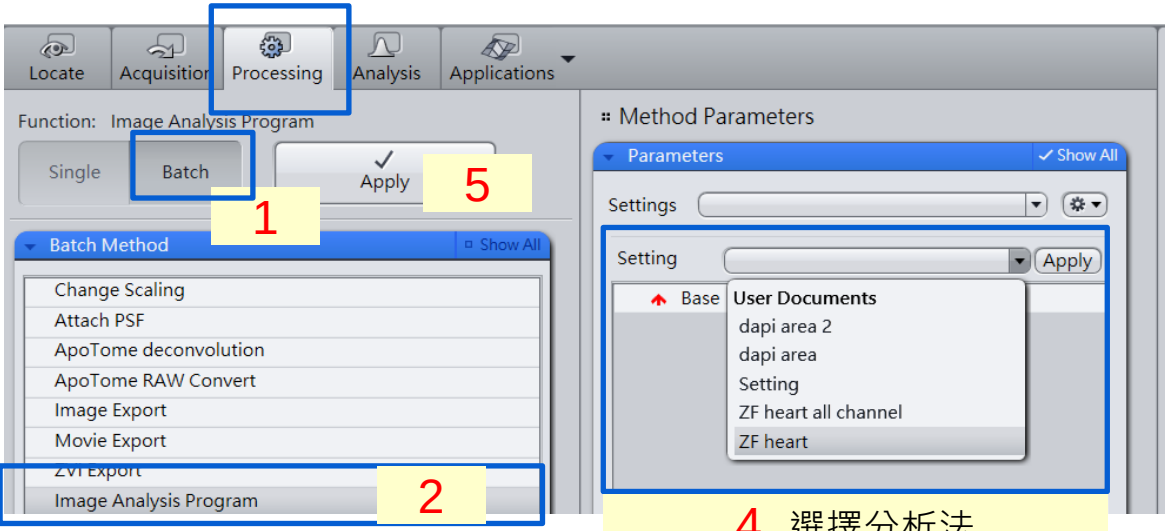


10	11	12,408,144.002
11	12	16,544,192.003
12	13	19,990,898.670
13	14	15,854,850.670
14	15	18,612,216.003
15	16	15,854,850.670
16	17	39,981,797.341
17	18	51,700,600.010
18	19	23,437,605.338
19	20	31,709,701.339
20	21	15,854,850.670
21	22	26,194,970.672
22	23	22,748,264.004
23	24	37,913,773.340
24	25	17,922,874.670
25	26	13,097,485.336
26	27	32,399,042.673
27	28	24,816,288.005
28	29	22,748,264.004
29	30	113,051,978.688
30	31	26,194,970.672
31	32	12,408,144.002

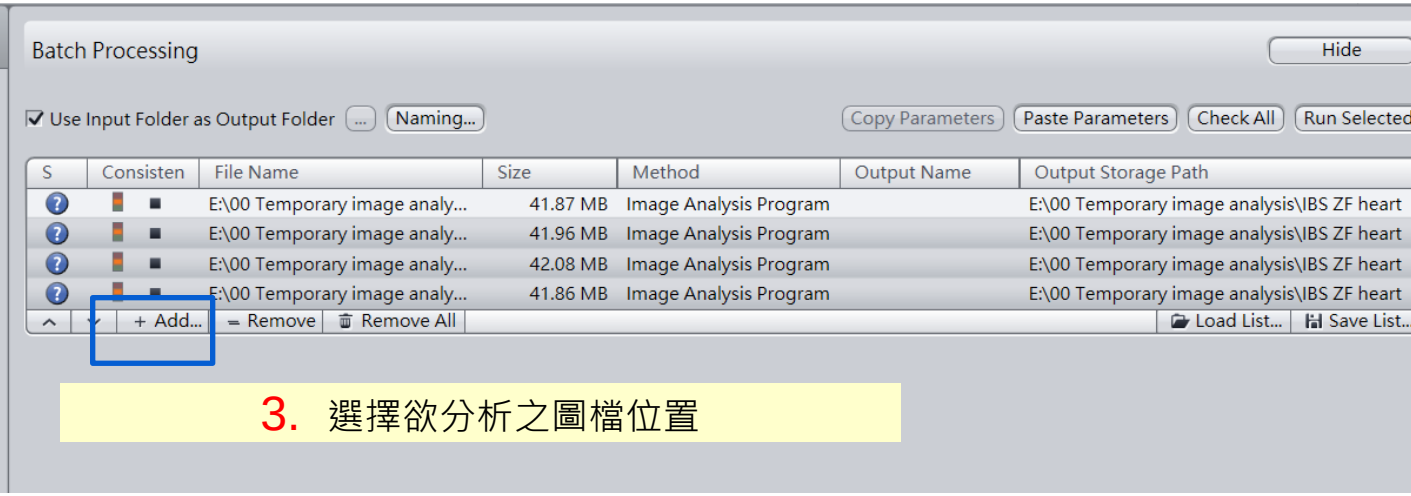
- 結果**預覽**
- 此時尚無法輸出結果

按下，儲存以上各步驟分析設定

自動量測 Image Analysis Module 8.
大量分析Batch Analyze



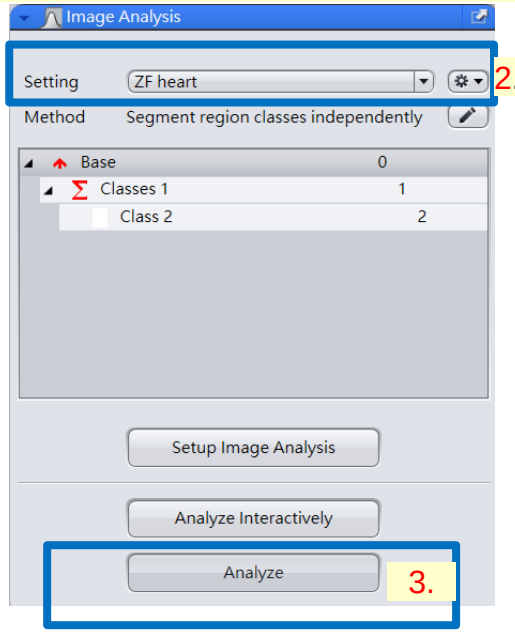
4. 選擇分析法



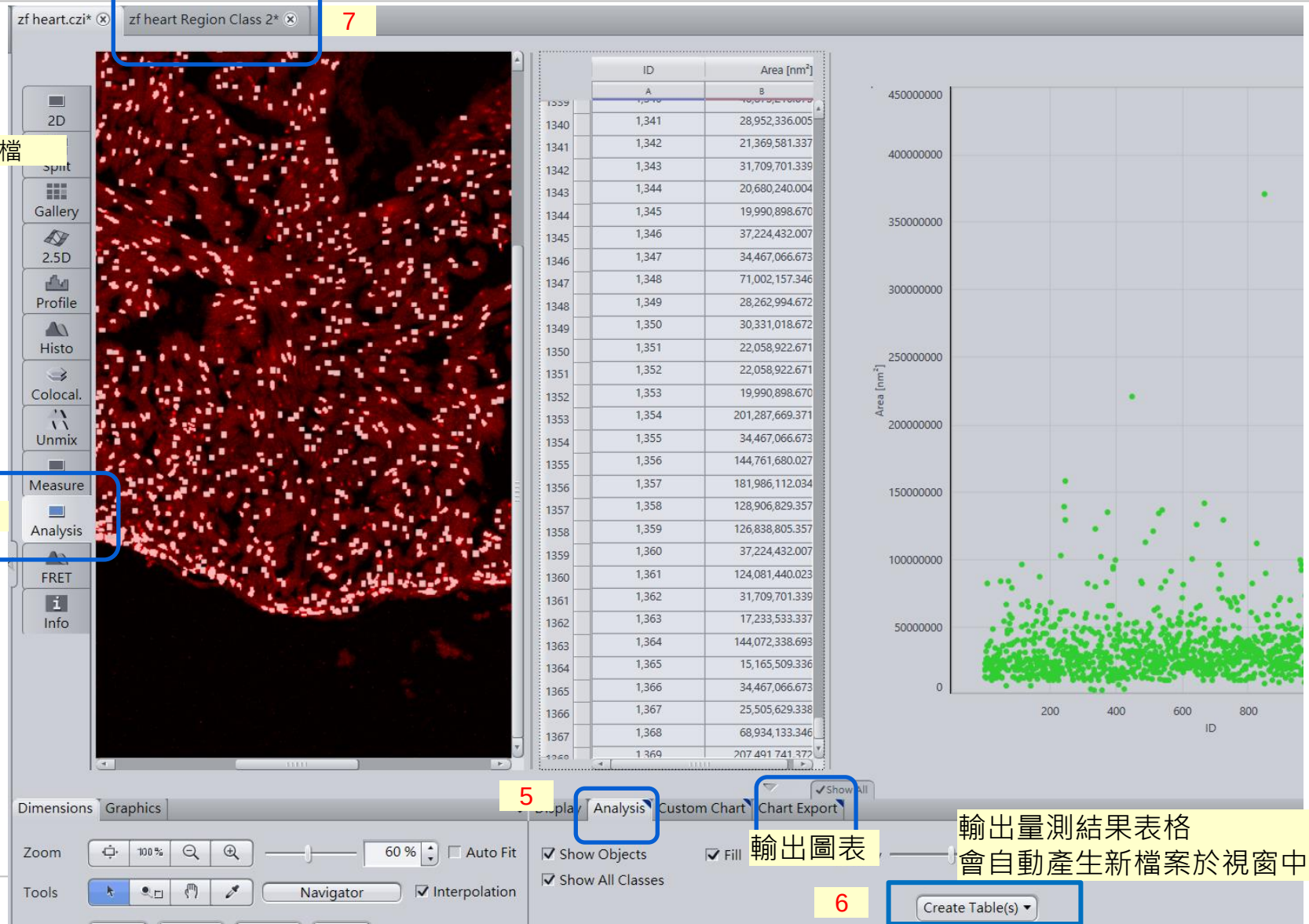
3. 選擇欲分析之圖檔位置

自動量測 Image Analysis Module 9.
Analyze the Image and Export the Data

1 打開原圖 或其他同系列要分析的圖



2. 選擇設定檔

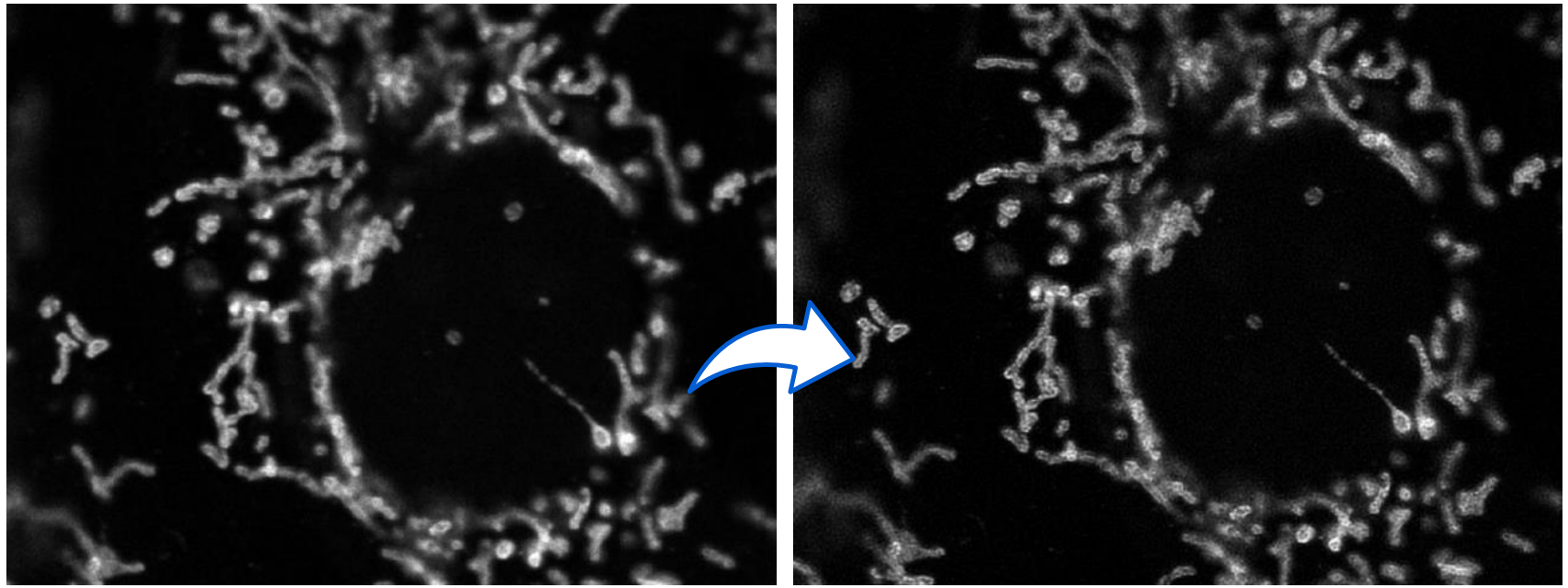


輸出量測結果表格
會自動產生新檔案於視窗中

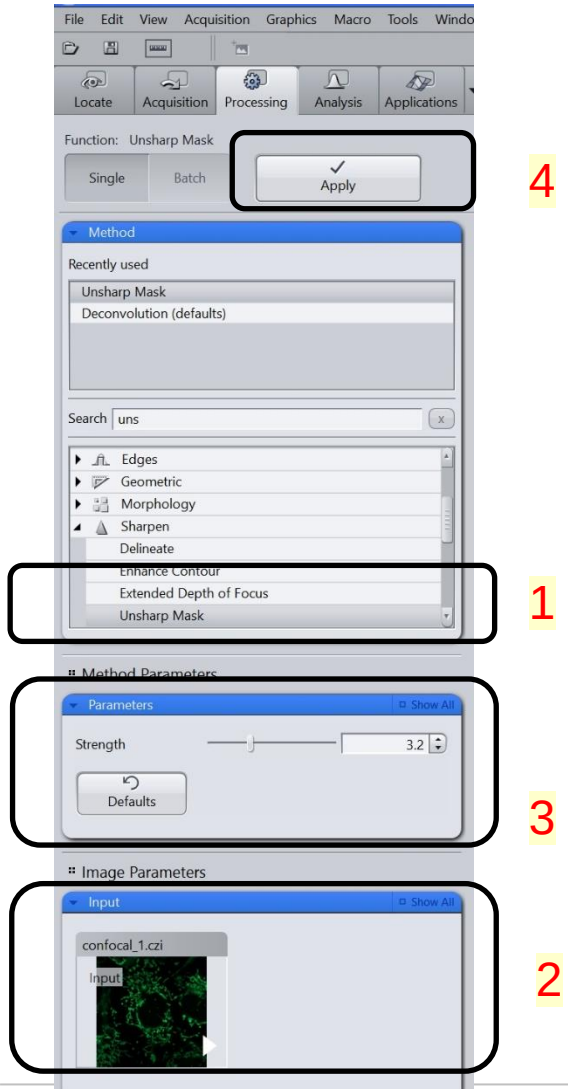
ZEN 3.3 Image Processing
Image Clearing Option 1:
2D DCV (defaults)

Before

After



ZEN Image Processing
Image Clearing Option 2:
Unsharp masking

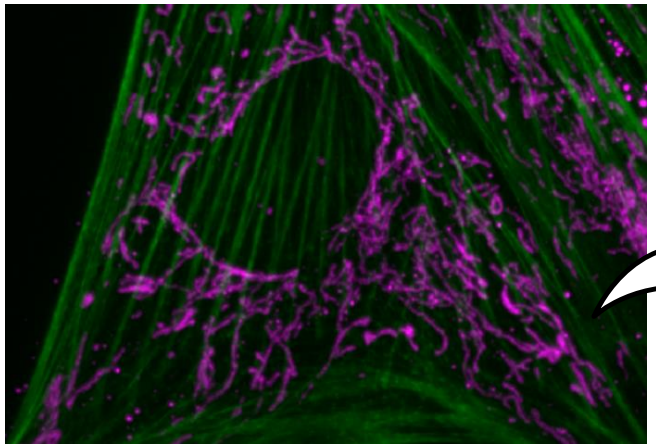


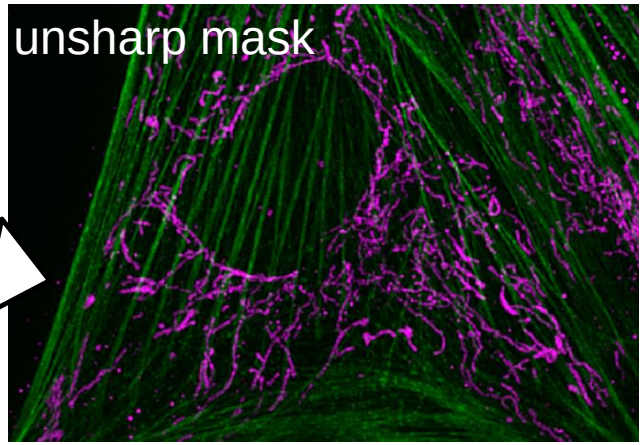
4

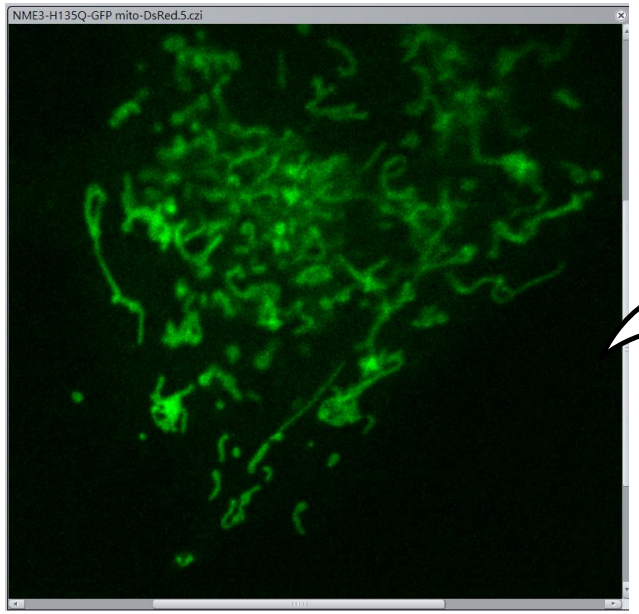
1

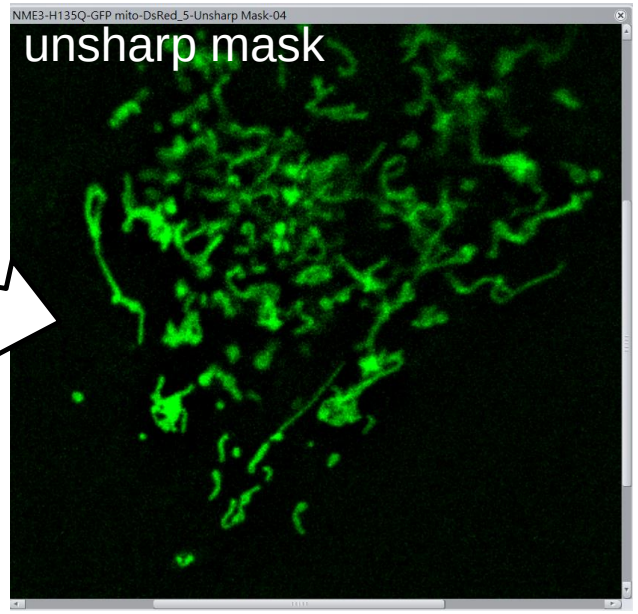
3

2

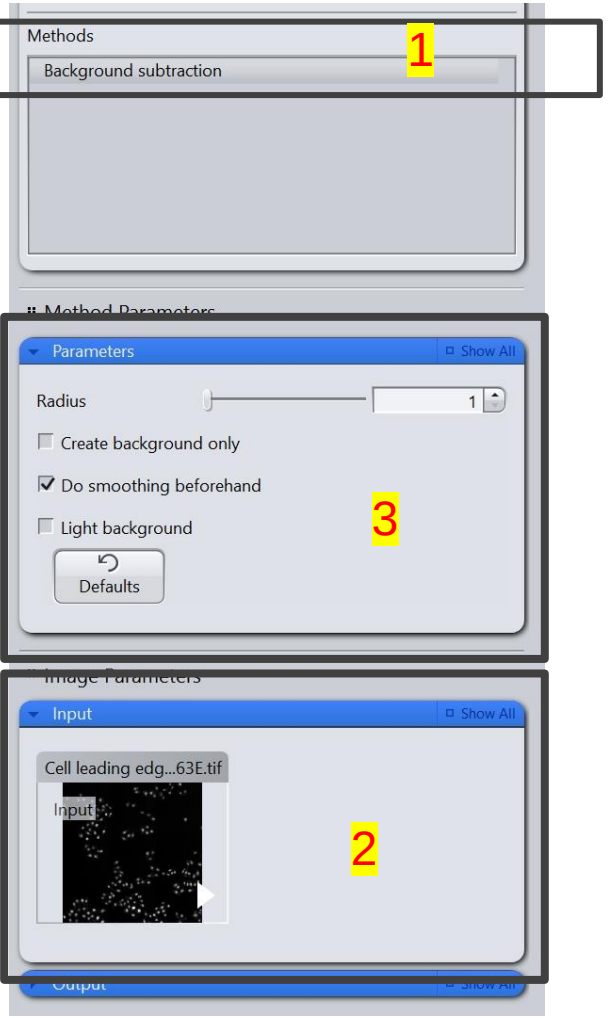


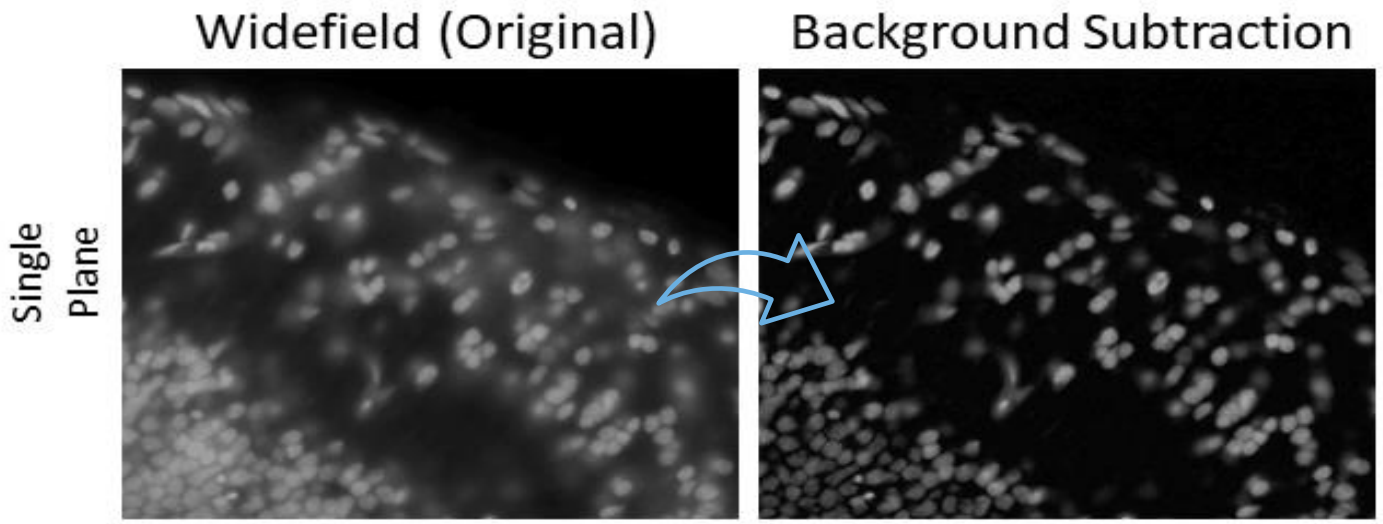




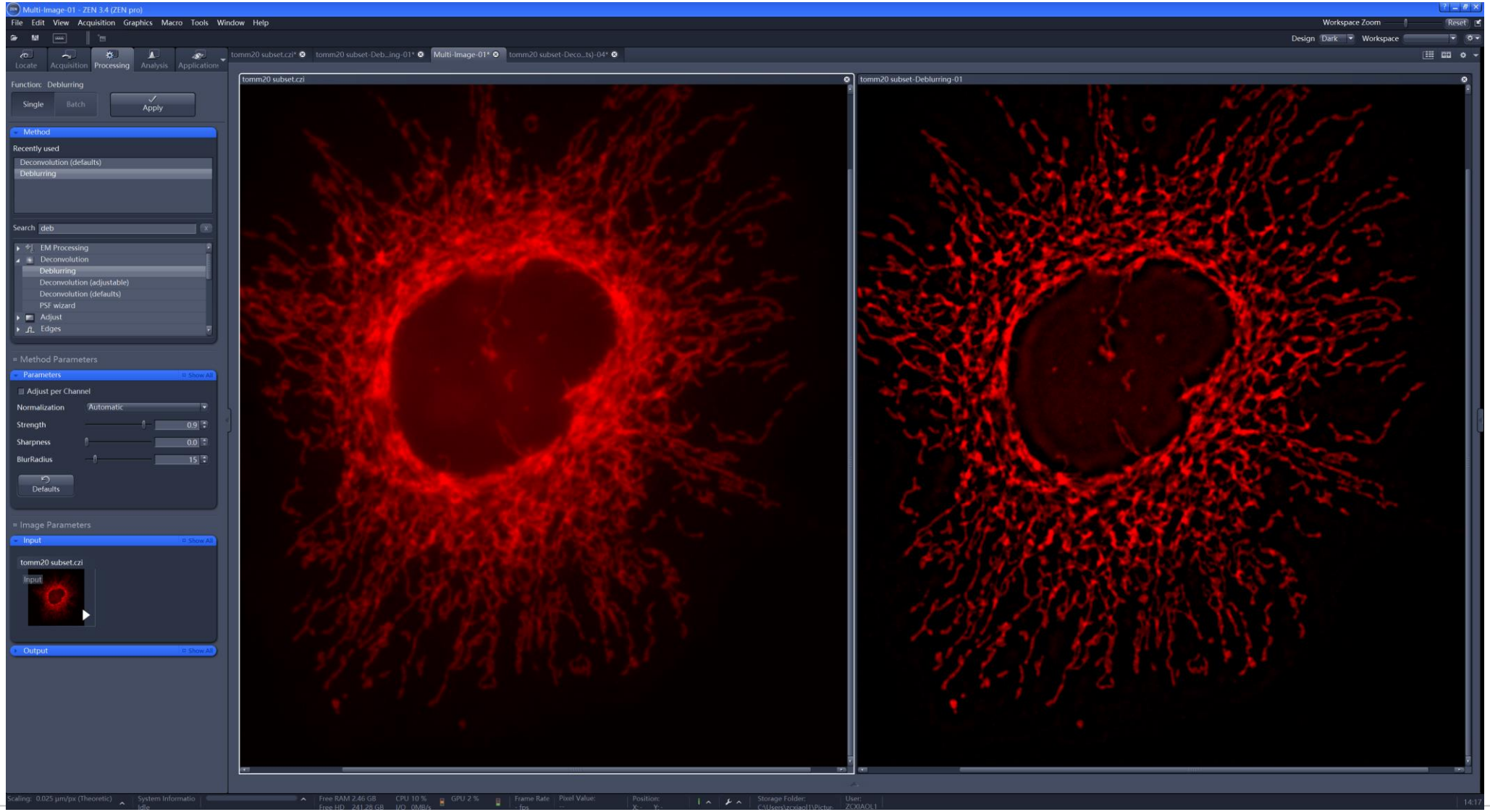


ZEN 3.3 Image Processing
Image Clearing Option 3:
Background subtraction



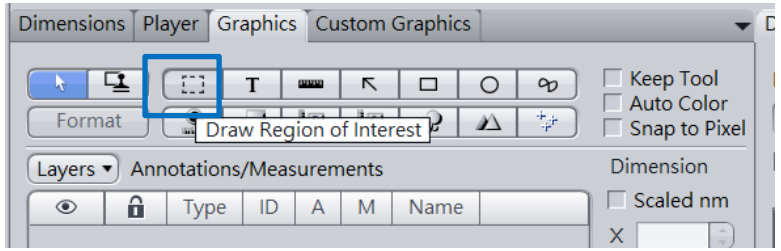


Option 4:
ZEN 3.4
WF vs Deblurring/2D DCV

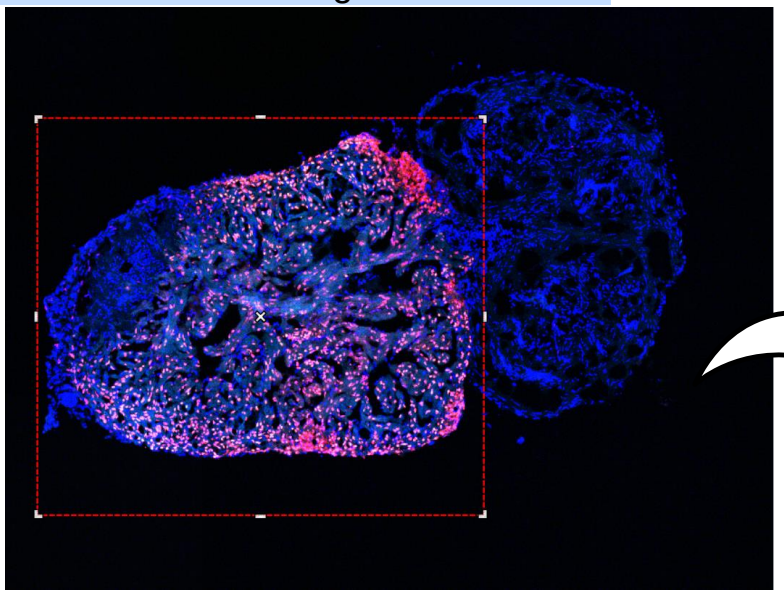


裁圖，複製選取範圍影像

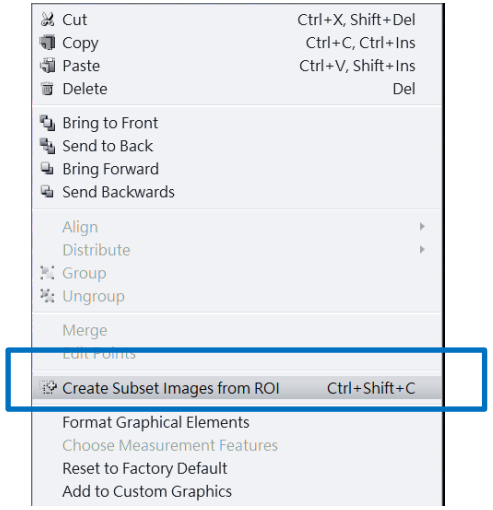
1.



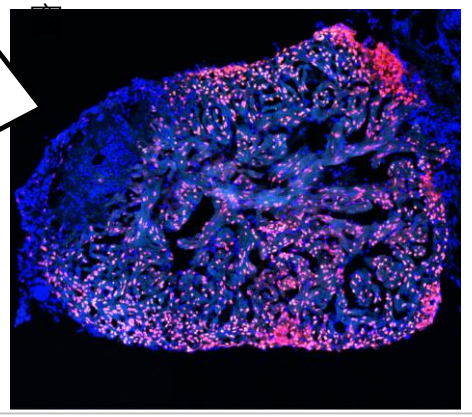
2. Draw an area and mouse right click



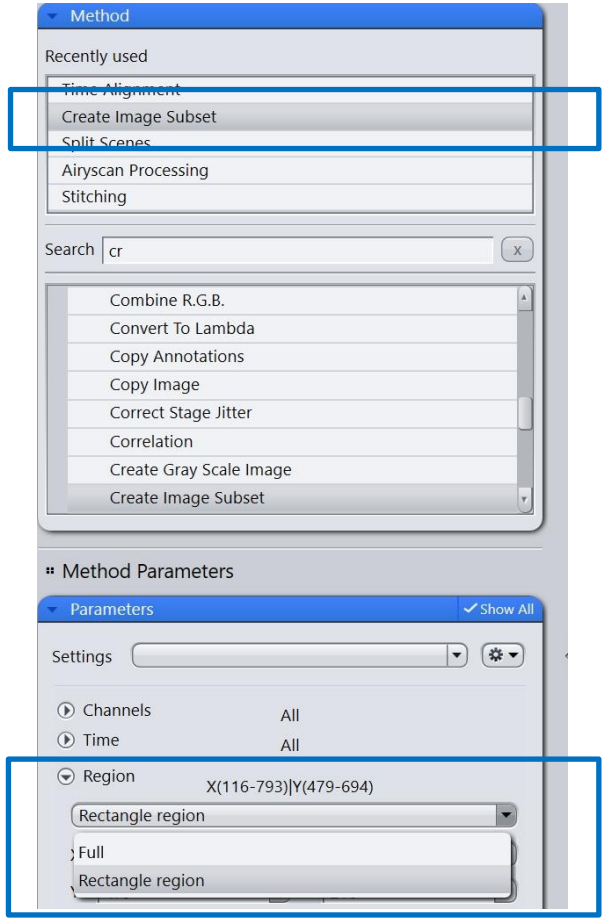
3. Create Subset Images from ROI



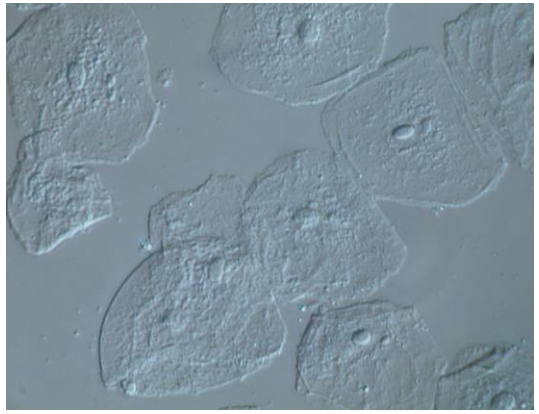
4. 獲得裁切範圍於新視



3. 或者
Processing → Create Image Subset

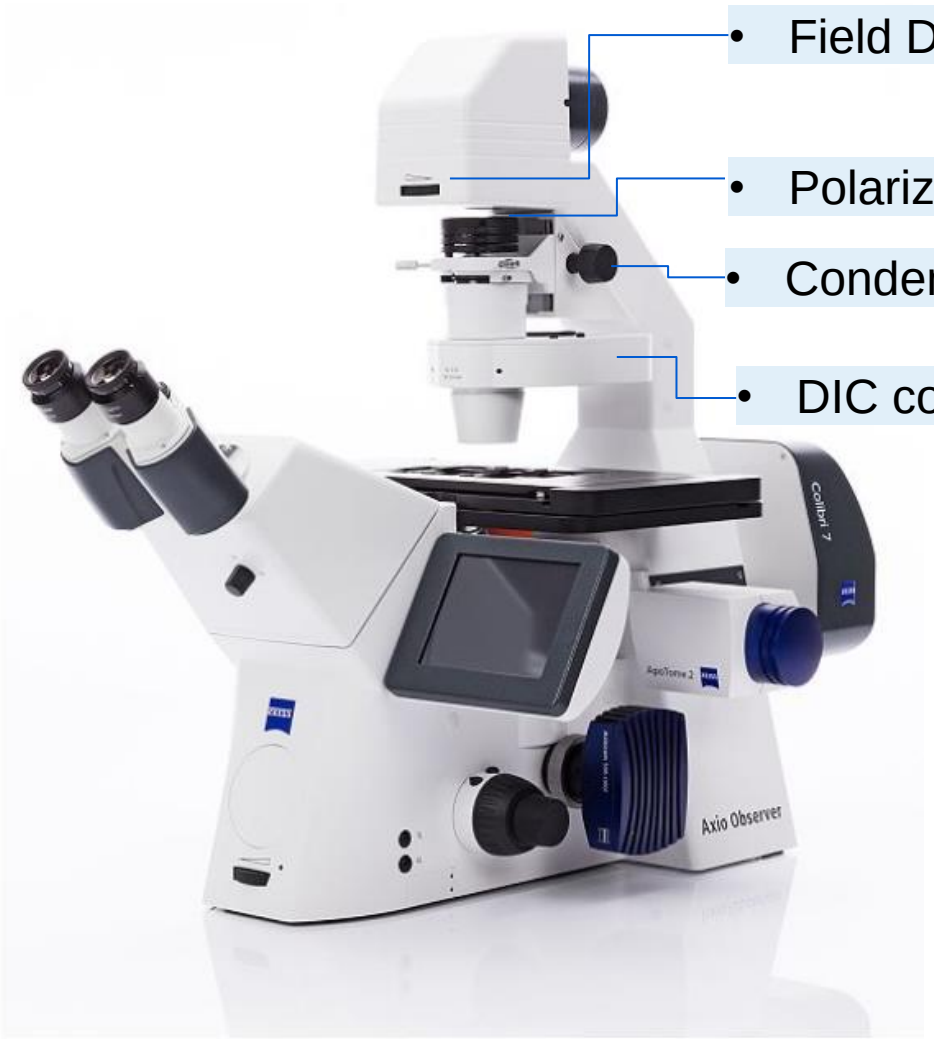


補充:穿透光
Bright Field / DIC Observervation

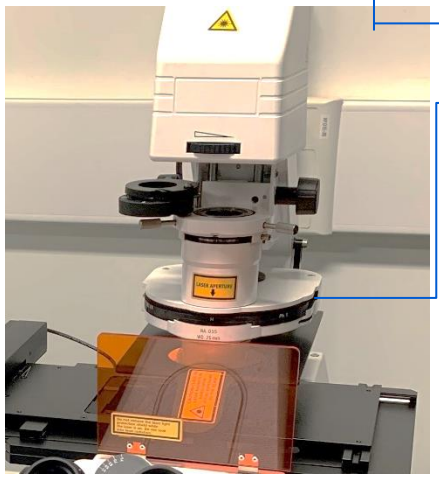


先於Ocular(目鏡)下調整最佳DIC 對比

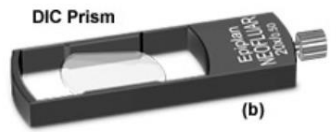
Bright Field / DIC Observation
Microscope setting for DIC



- Field Diaphragm
- Polarizer
- Condenser focusing
- DIC contrast



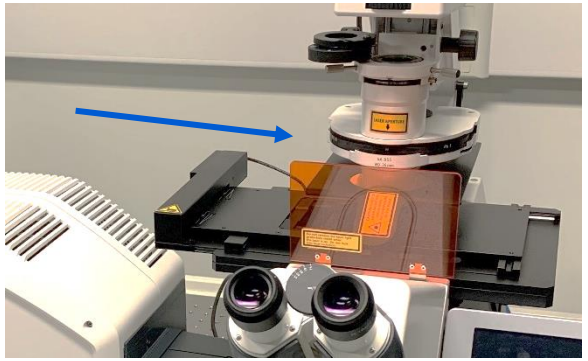
1. Focus the sample with objective
2. Adjust the condenser height by condenser focus knob .
3. Check the condenser center position by closing the field diaphragm and reopen it after focusing the condenser.
4. Choose the DIC filter position
5. Swing the polarizer holder in
6. Choose condenser turret position for DIC
7. Insert the objective DIC prism and adjust the knob



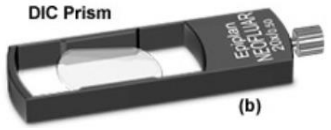
拍圖補充:
DIC 影像

拍攝DIC影像

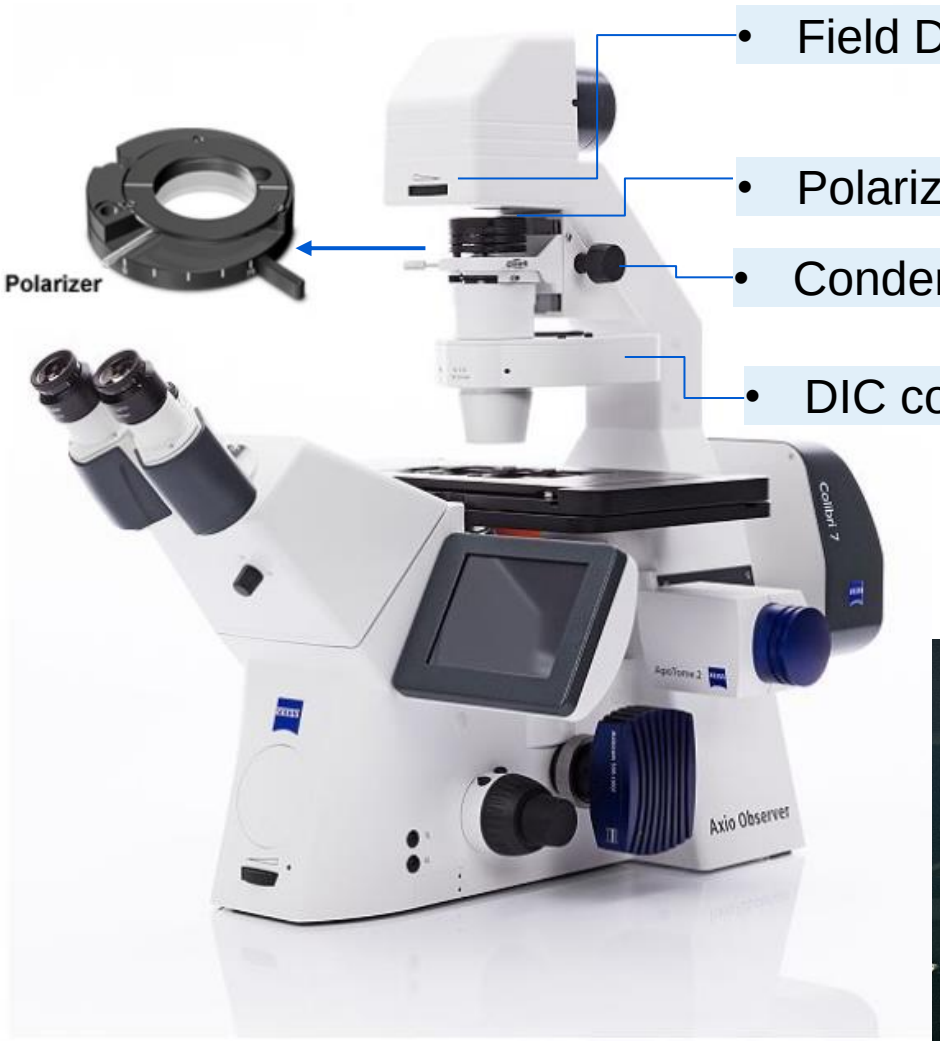
1. 如果講求完美效果聚光鏡校正要先做好! (設置請參考前頁)
2. 將螢光設定好最後再開啟T-PMT
3. 可選取任一個Track**合併**拍攝穿透光或者增加track單獨拍攝
4. 確認一下聚光鏡轉盤位置是否在DICII（10x& 20x）或DICIII（40x以上）（見下圖）。



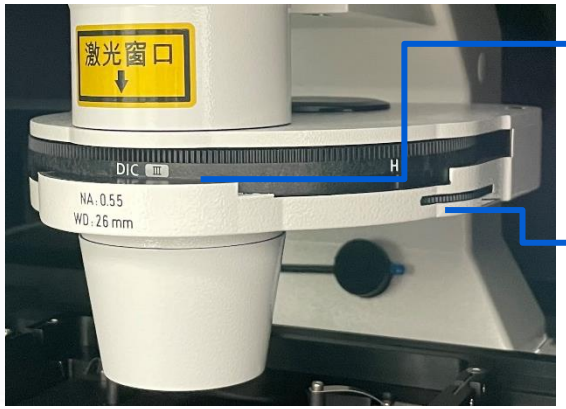
Bright Field / DIC Observation
Microscope setting for DIC



DIC prism可能會造成訊號誤差， AiryScan時建議拔出



- Field Diaphragm
- Polarizer
- Condenser focusing
- DIC contrast

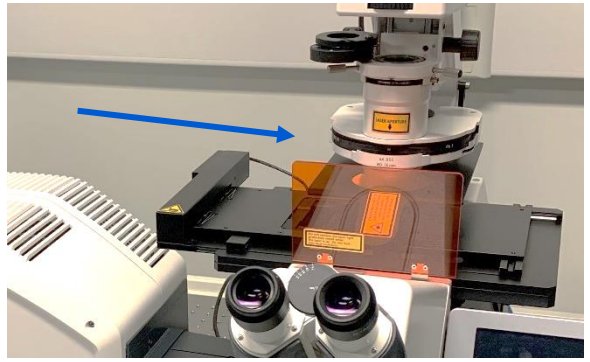


1. Focus the sample with objective
2. Adjust the condenser height by condenser focus knob (標記線對齊).
3. Check the condenser center position by closing the field diaphragm and reopen it after focusing the condenser.
4. Choose the DIC filter position (螢光濾片轉盤)
5. Swing the polarizer holder in
6. Choose condenser turret position for DIC(油鏡:轉到DICIII)
7. Adjust aperture diaphragm (油鏡:全開)
8. Insert the objective DIC prism and adjust the knob

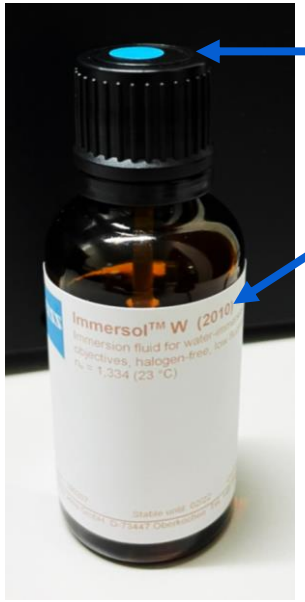
拍圖補充:
DIC 影像

拍攝DIC影像

1. 如果講求完美效果聚光鏡校正要先做好! (設置請參考前頁)
2. 將螢光設定好最後再開啟T-PMT(或ESID)
3. 可選取任一個Track**合併**拍攝穿透光或者增加track單獨拍攝
4. 確認一下聚光鏡轉盤位置是否在DICII（10x& 20x）或DICIII（40x以上）（見下圖）。



40x/ 1.1水鏡
請用專用鏡油



認明瓶蓋與瓶身標示

勿與其他鏡油混合使用或取代
(可以dd H2O 取代 ，需注意蒸發問題)

- 室溫請參考黑線位置
- 37度加熱時參考紅線

Recommend Single/Multi-well Chamber Types for Living Cell Application

µ-Slide 18 Well Glass Bottom

µ-Slide 8 Well high Grid-500

µ-Slide 4 Well Ph+

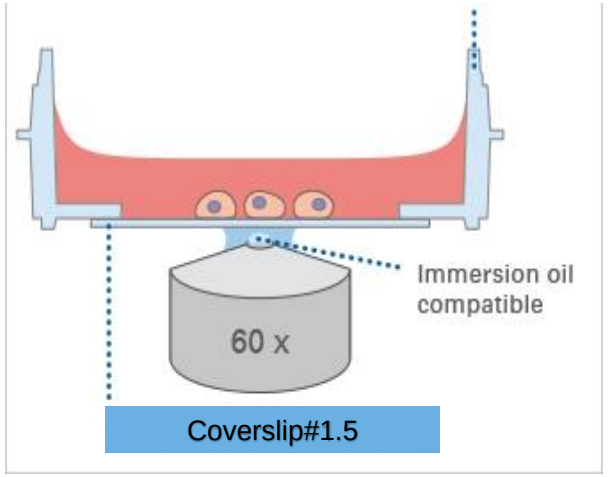
POC-R

µ-Dish 35 mm, high

POC-R mini

Cell Imaging Plate

- **#1.5 cover glass/ polymer bottom dish/plate/slide for inverted microscope with high N.A objectives.**
- Thickness **no 1** ½ 0.17mm ±0.005mm



Turn off the system

- Exit ZEN
- 如果有使用活細胞培養裝置，TFT面板內Incubation 頁面OFF 所有(Incubator 及Heating insert)溫控
- 關閉C02，鋼瓶鎖緊
- 加濕水瓶倒除

4

3

2

1

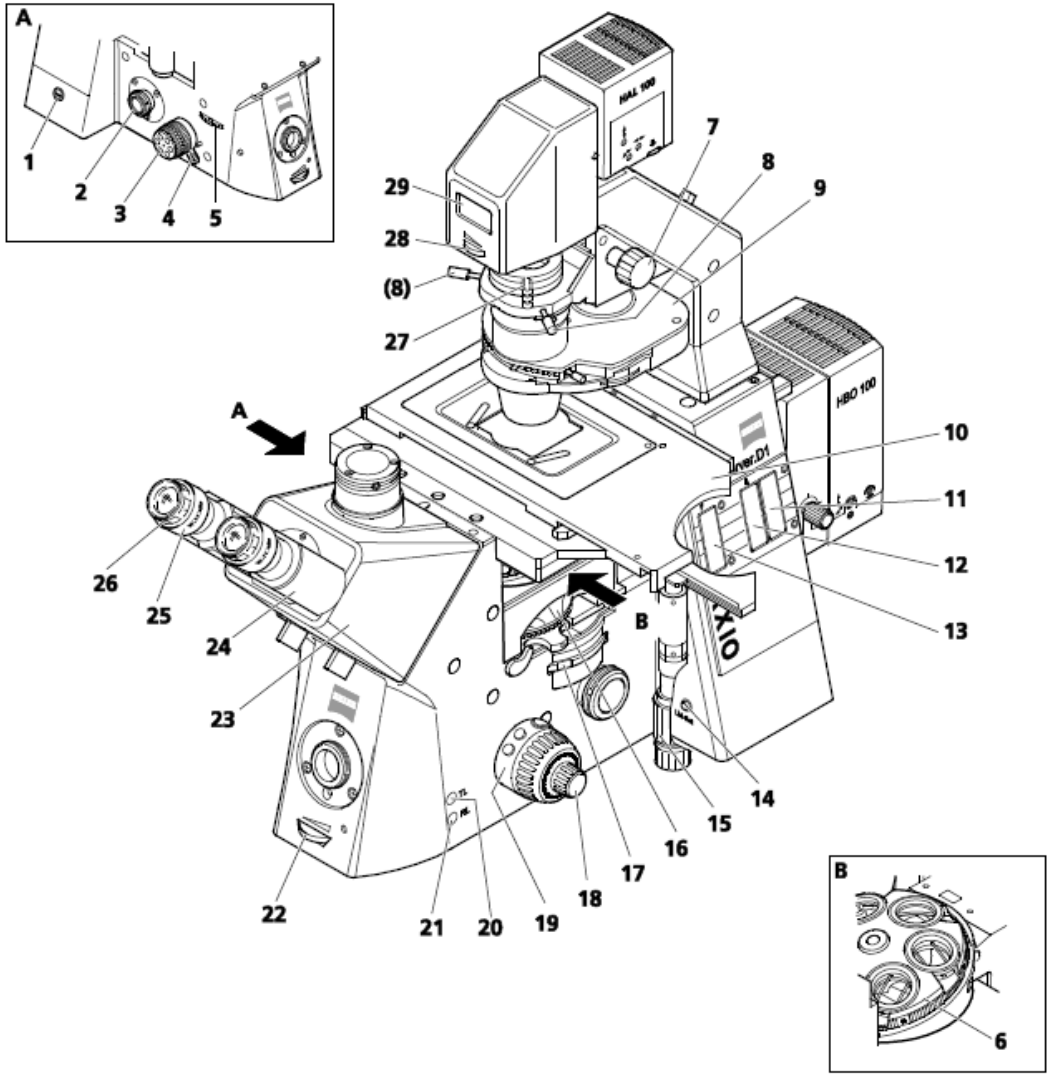
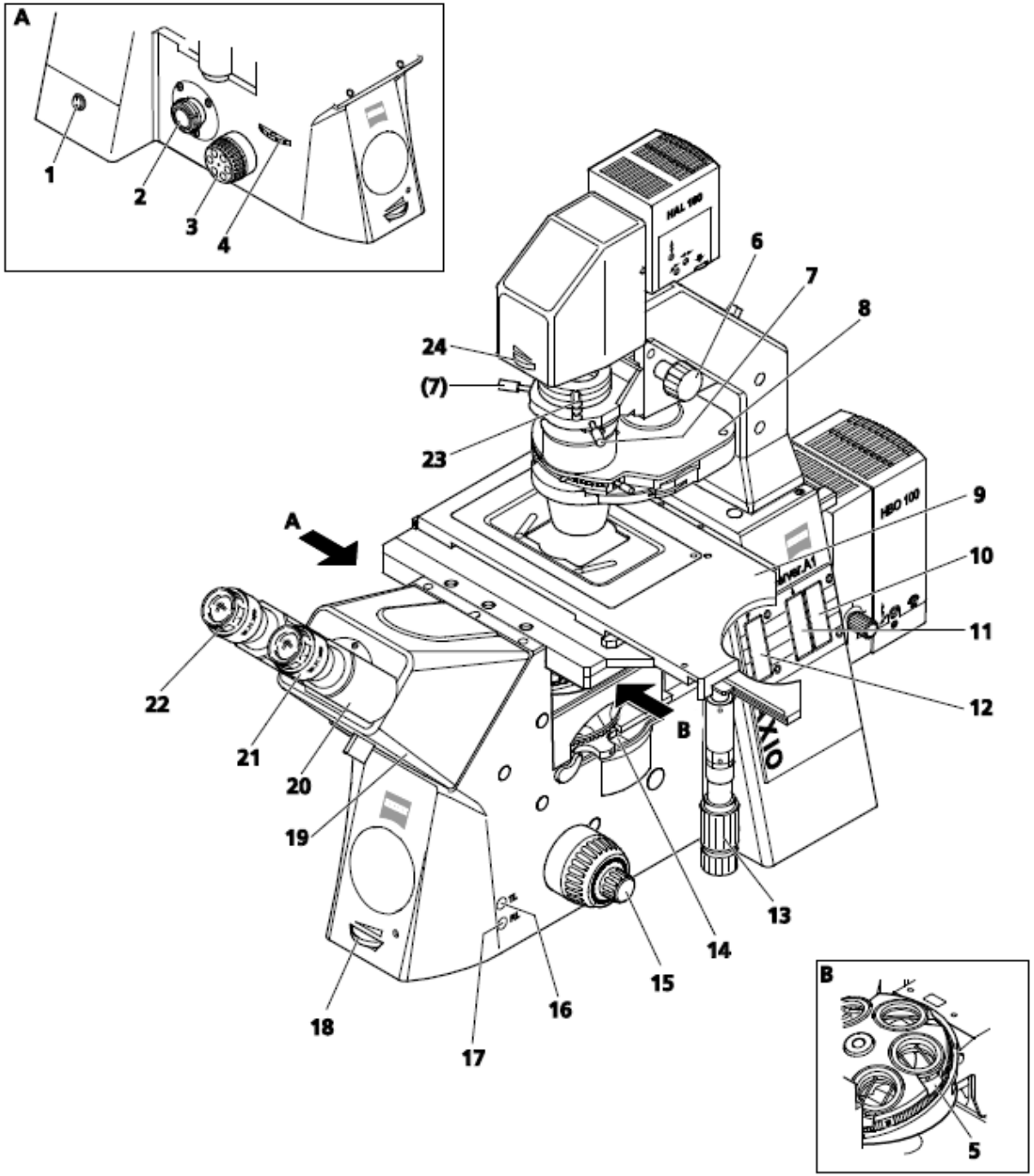


Fig. 4-2 Axio Observer.D1 components and controls (coded, semi-motorized)

1 On / off button 2 Left Sideport
3 Coarse / fine focus drive with fine drive, flat
4 Vertical stop for focus drive
5 Light path switching control
6 Objective nosepiece
7 Vertical adjustment knob for condenser
8 Condenser centering screw
9 Condenser
10 Microscope stage 11 3-position filter slider slot
12 Slot for FL attenuator
13 Slot for iris stop slider as reflected light luminous-field stop
14 LM set button
15 Drive knobs for controlling XY positioning of the mechanical stage
16 Reflector turret 17 Optovar turret control wheel
19 Control ring, right
20 TL button for transmitted light shutter
21 RL button for reflected light shutter on and off
22 Halogen illumination intensity control
23 Binocular tube
24 Binocular section of the binocular tube
25 Eyepiece 26 Eyepiece adjustment ring
27 Polarizer D with 2-position filter changer or 3-position filter changer
28 Luminous-field stop control
29 LCD display



1 On / Off switch
2 Left Sideport
3 Coarse / fine focus drive (left side) with fine drive, flat
4 Light path switching control (leftSideport / vis)
5 Objective nosepiece
6 Vertical adjustment knob for condenser
7 Condenser centering screw
8 Condenser
9 Microscope stage
10 3-position filter slider slot
11 Slot for iris stop slider as reflected light aperture stop or FL attenuator
12 Slot for iris stop slider as reflected light luminous-field stop
13 Drive knobs for controlling XY positioning of the mechanical stage
14 Reflector turret
15 Focus drive coarse / fine
16 TL button for switching the transmitted light halogen illuminator on and off or for opening and closing the transmitted light shutter
17 RL button for switching fluorescence shutter on and off
18 Halogen illumination intensity control
19 Binocular tube 20 Binocular section of the binocular tube
21 Eyepiece 22 Eyepiece adjustment ring
23 Polarizer D with filter changer
24 Luminous-field stop control

Fig. 4-1 Axio Observer.A1 components and controls (manual)


ZEISS