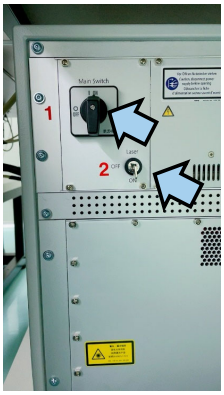
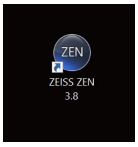
Carl Zeiss LSM 980 / ZEN Blue
Quick Guide

開機
System on





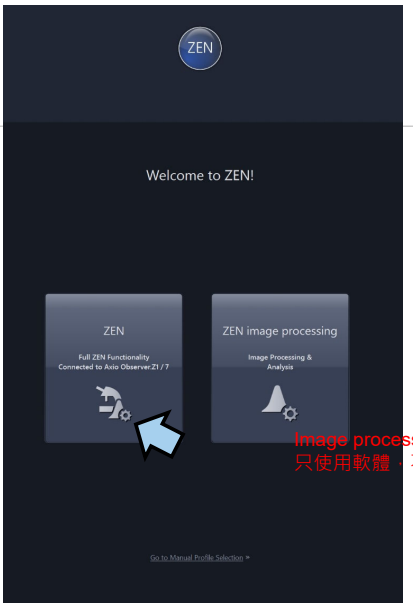


Image processing
只使用軟體，不連接硬體

1. Main Switch
2. Laser Key
3. Components
4. Computer
5. ZEN Blue

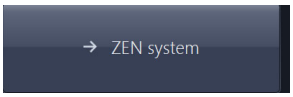
等待約三分鐘

進入軟體 ZEN

1

進入ZEN BLUE

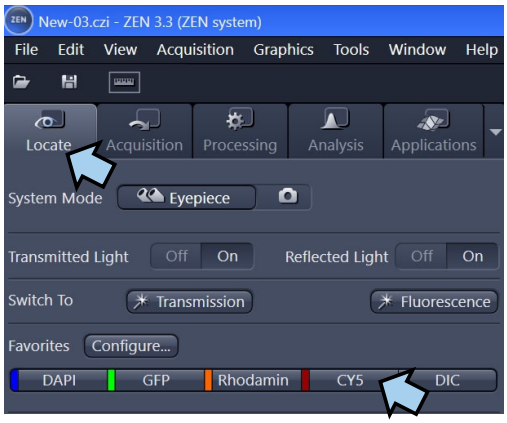
2



等待約一分鐘

眼睛觀察

3

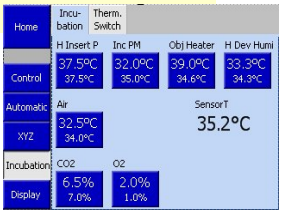


Choose the FL configuration you need

關機
System off

4. Computer

- 如果有使用活細胞培養裝置
- 關閉C02 (鋼瓶與TFT控制面板 Incubation)
- **Incubator XL 降溫至30度內(降溫完成, 控制面板incubation → off)**
- 加濕水瓶倒除



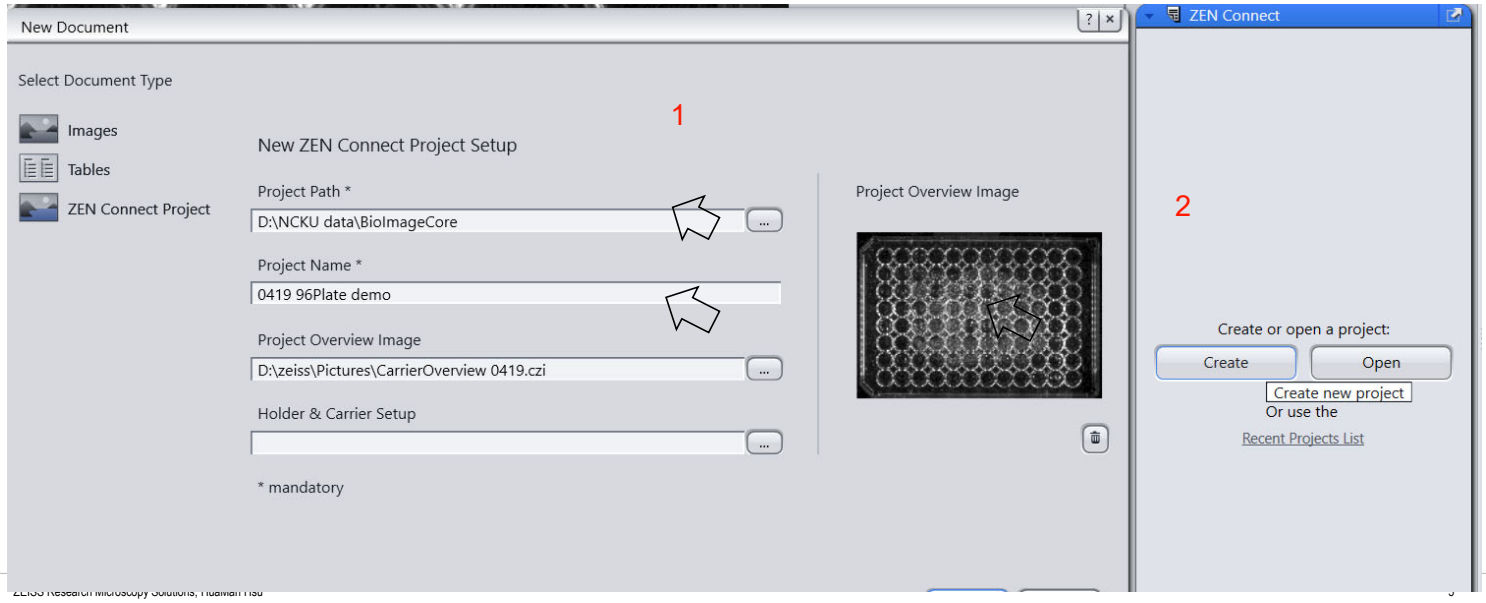
3. Components

2. Laser Key

1. Main Switch

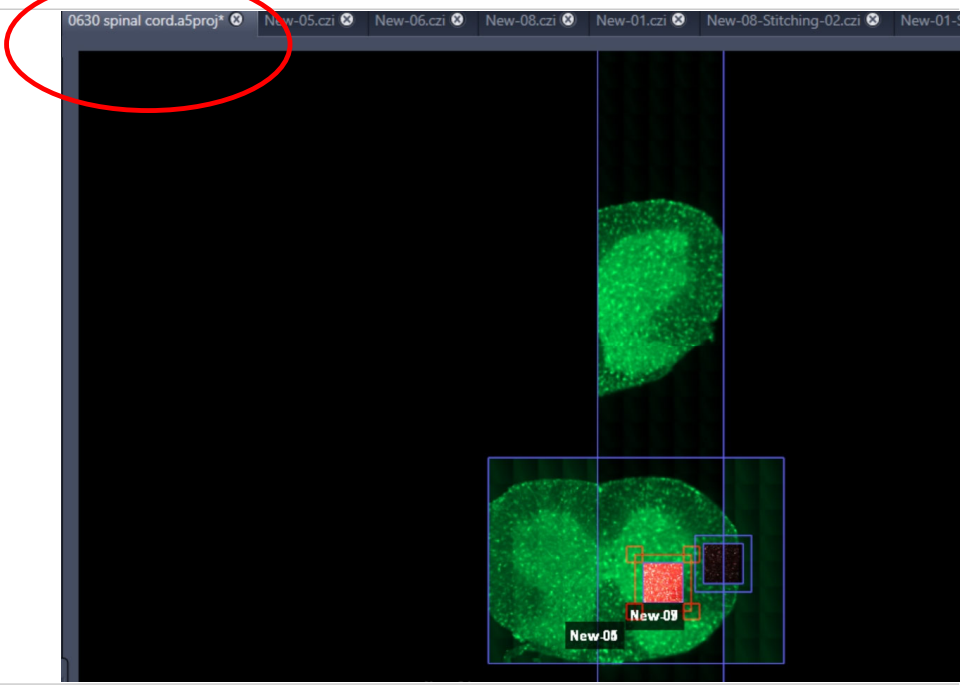
ZEN Connect (非必要)
同一Slide的檔案相互連結，自動儲存檔案座標位置

Create Project
檔案管理工具 ，此為 非必要步驟

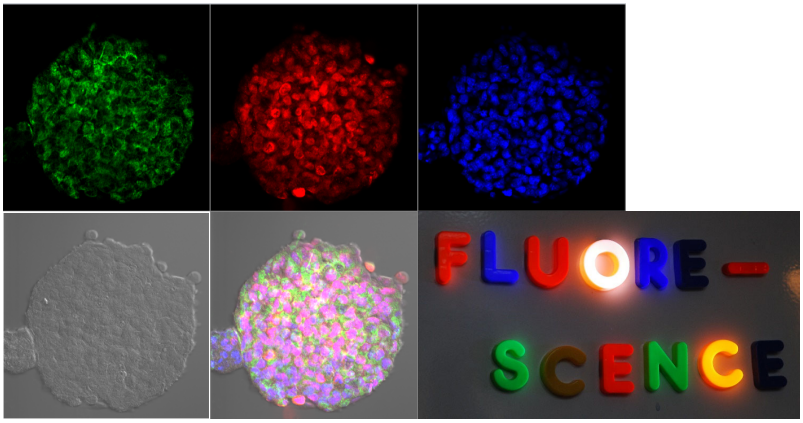


ZEN Connect
同一Slide的檔案相互連結，自動儲存檔案座標位置

- ***. a5proj**
- 所有該Project的檔案皆會以關聯地圖方式示顯示於其中，並且Autosave
- 每次換一片slide可以新增一個project

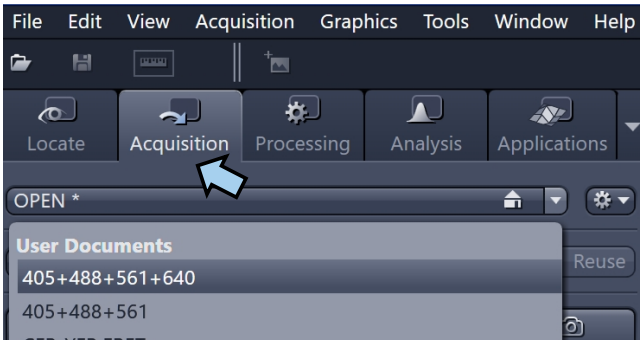


Multichannel Image Acquisition



Multichannel Image Acquisition 1
Load Experiment methods form Experiment Setup

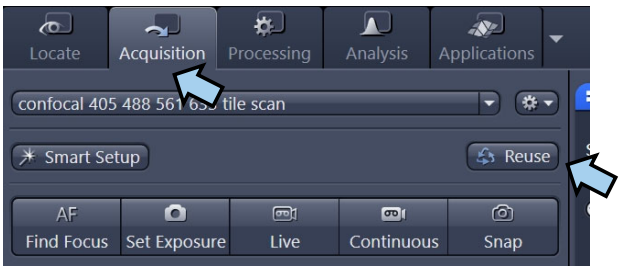
1.



Choose the Experiment you suits your need

OR

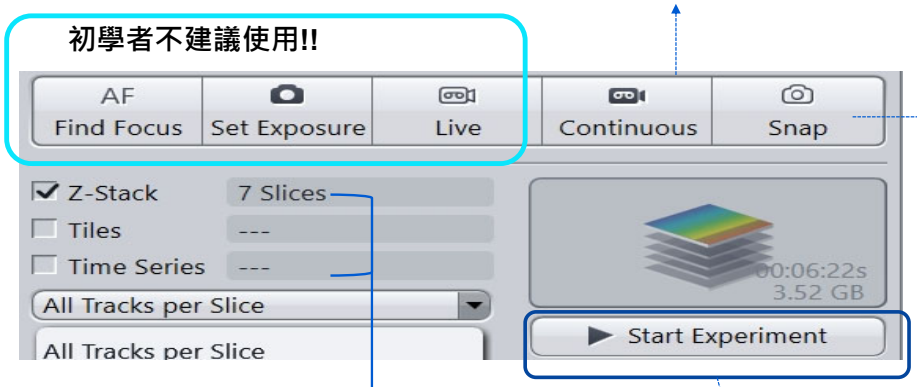
Reuse



- 開啟欲套用的檔案後，或按下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

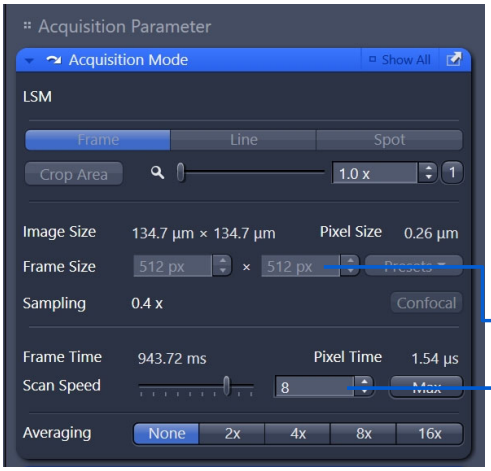
連續掃描影像連續更新，可以看到即時影像，掃描不會自動停止，用於調整焦距與laser 與gain值看見及時變化。



在所停留的焦距位置，單拍一張，會執行已勾選的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
設定預覽速度(快)與畫素(小)



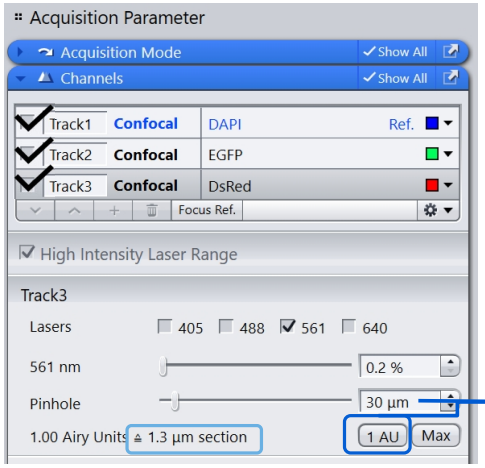
Fast adjustment for focusing, laser and detector gain:

- Frame Size 256 or 512
- Scan Speed 8 or 7

Multichannel Image Acquisition 4
Acquisition Parameter
Pinhole 設定

1.

2.

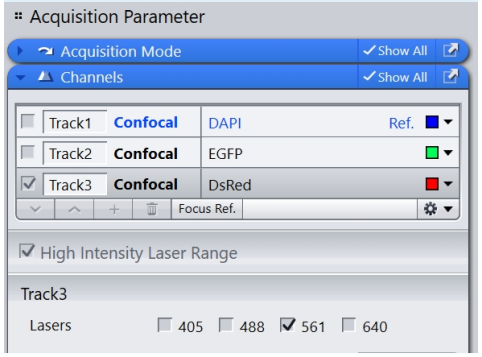


Optical Section Thickness

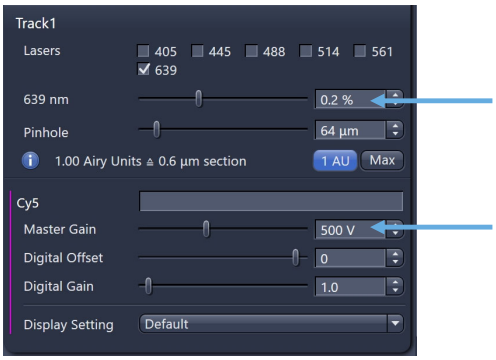
1. 所有Tracks打勾，Pinhole 所有channel 設定相同
2. 擇一Track作為運算依據，按1AU，或者依需求調整合適大小。

Multichannel Image Acquisition 5
Acquisition Parameter
為每個Track 設定Laser強度 與 感測器Master Gain

1 調整時，一次僅打勾一個Track

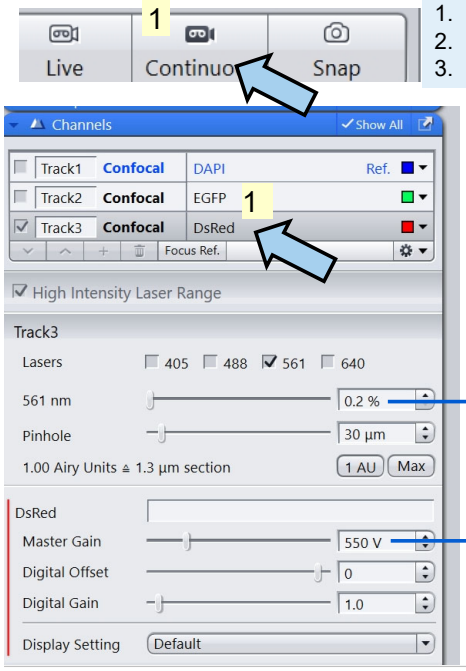


2 將各Track內的Laser 與Master Gain 設定完成

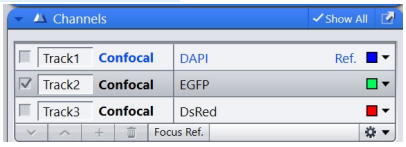


Multichannel Image Acquisition 6
Acquisition Parameter
為每個Track 設定Laser強度 與 感測器Master Gain

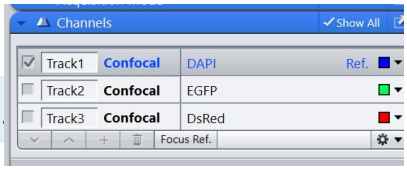
1. 對焦，Continous，一次調整一個channel， Master Gain
2. Adjust Laser Intensity and Master Gain for All Channels
3. 依序調整所有Tracks(重複上步驟)的laser強度 與 Gain



3.



3.

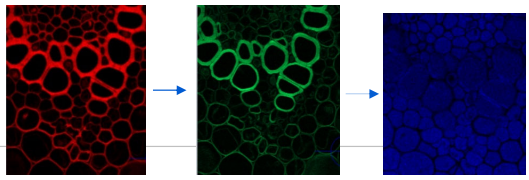


2

- 調整雷射強度
- 大部份情況下10%以內即足夠應付

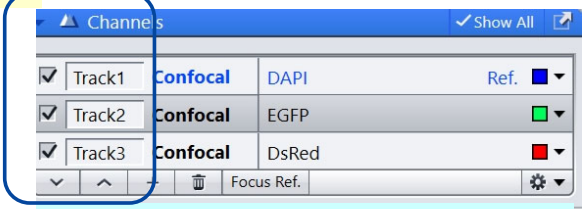
2

- 調整Master Gain
- 一般Master Gain不超過900， 避免過曝損傷感測器



Multichannel Image Acquisition 7
2D Image
正式拍照: 提高Frame Size 降低Scan Speed

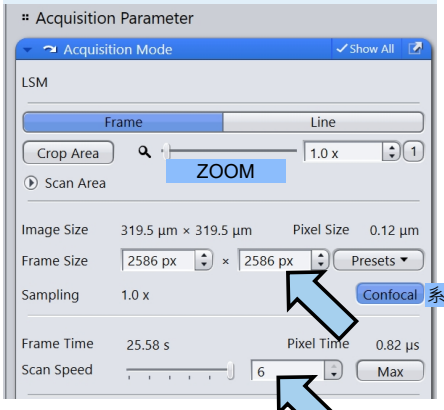
1



選取所有要準備要拍的track

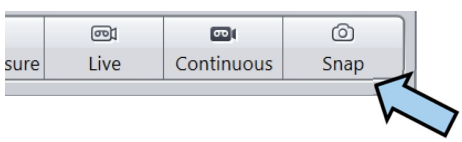
2

- 提高Frame Size 、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

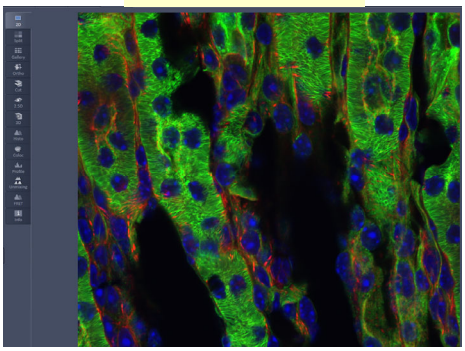


系統建議畫素

3

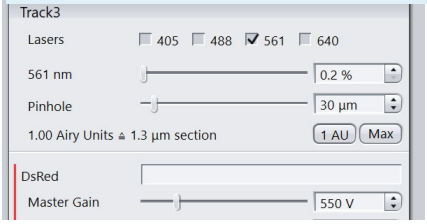


4 大功告成!!

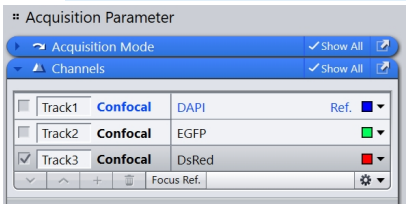


Multichannel Image Acquisition 8
3D Image - Z Stack Acquisition

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

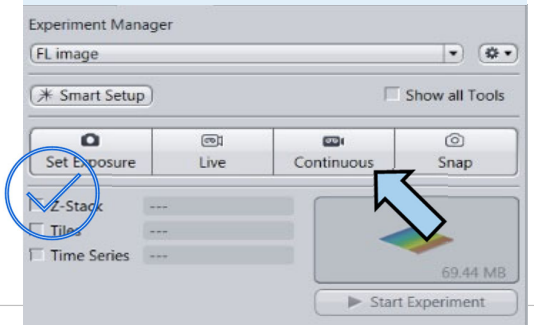


2 選擇一個Track



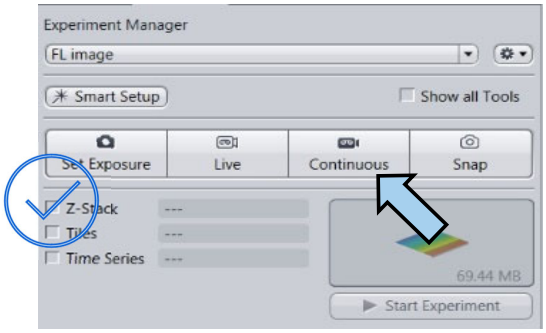
3. Put your hand on focus wheel and preparing for focusing

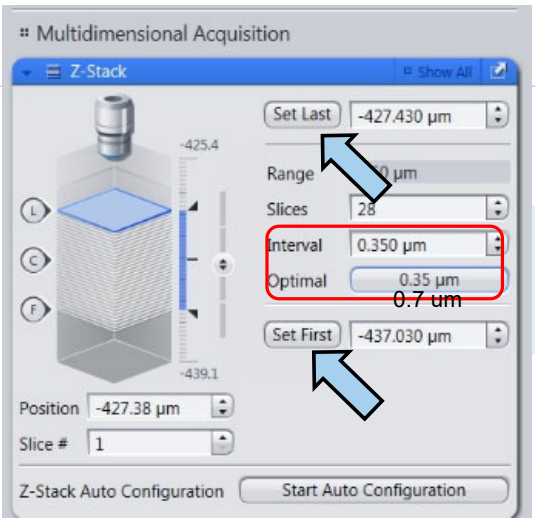
4 “Continuous” scanning under higher frame rate (ex: 512^2 @ speed 7)



Multichannel Image Acquisition 9
3D Image - Z Stack Acquisition

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



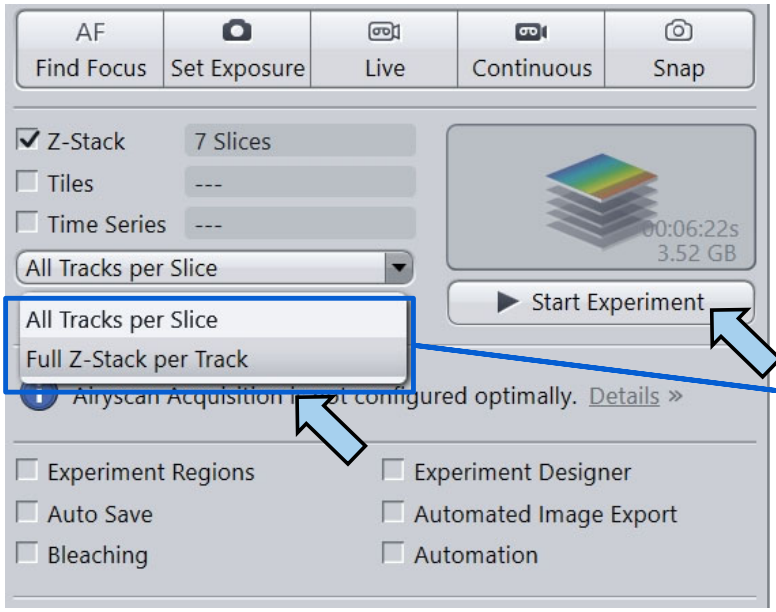


- interval可參考optimal
- 2x的optimal 0.35*2 = 0.7也很好 ，張數少，較省時
- 也可自訂定間隔

設訂Z stack的上下界限

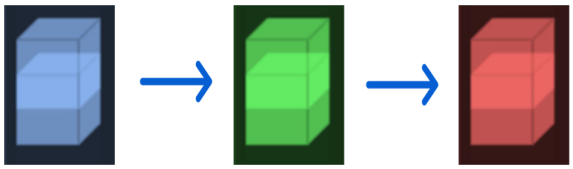
- 選擇一個Track02. > Continuous
- 搭配Z stack視窗 > Z-stack
- 找到樣品焦距起點 >Set First
- 找到樣品焦距終點> Set Last

Multichannel Image Acquisition 10
3D Image - Z Stack Acquisition



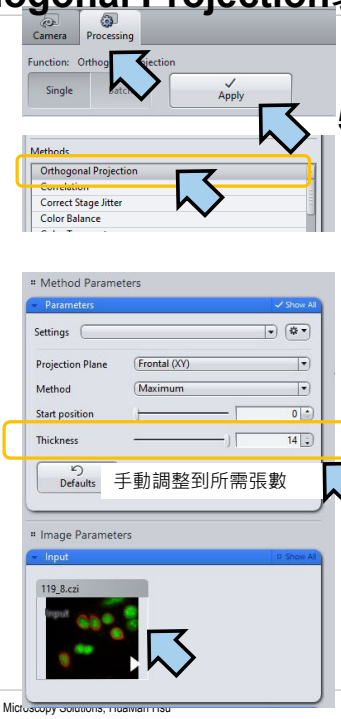
Full Z-Stack per Track:可避免硬體移動過於頻繁而降速

- 第一個Track的Z -Stack
- 第二個Track 的Z Stack
- 第三個Track 的Z Stack

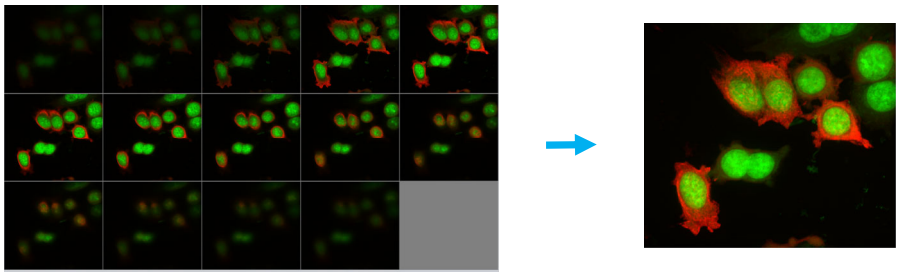


Multichannel Image Acquisition
Z Stack Series Image
Orthogonal Projection製造全景深影像:

1.
2.
3.
4.
5.

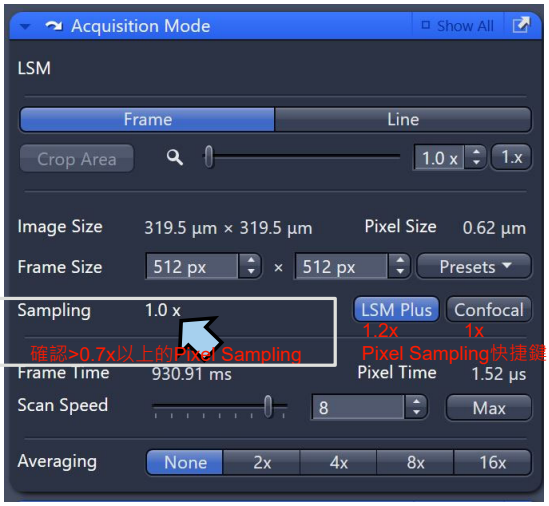


Z stack: 把多張Z section疊成一張

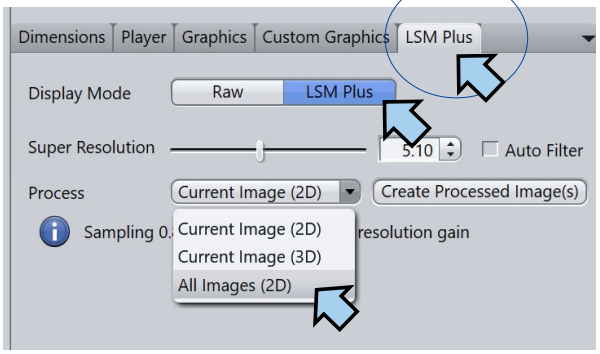


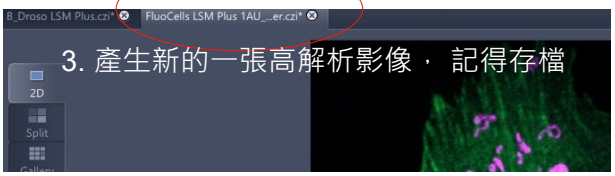
LSM Plus

1.掃圖設置，畫素要夠

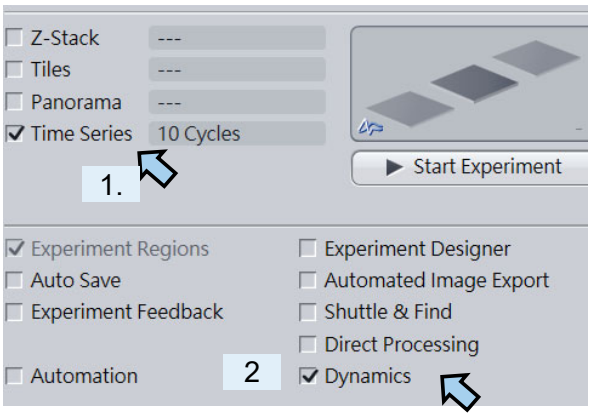


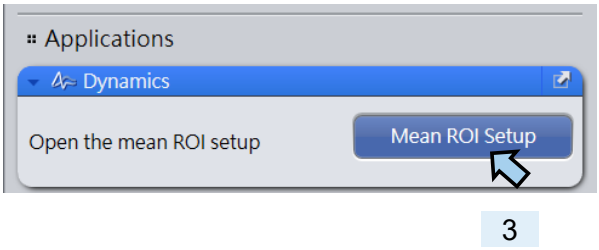
2.執行LSM Plus運算，
拍完圖，產生新的一張高解析影像



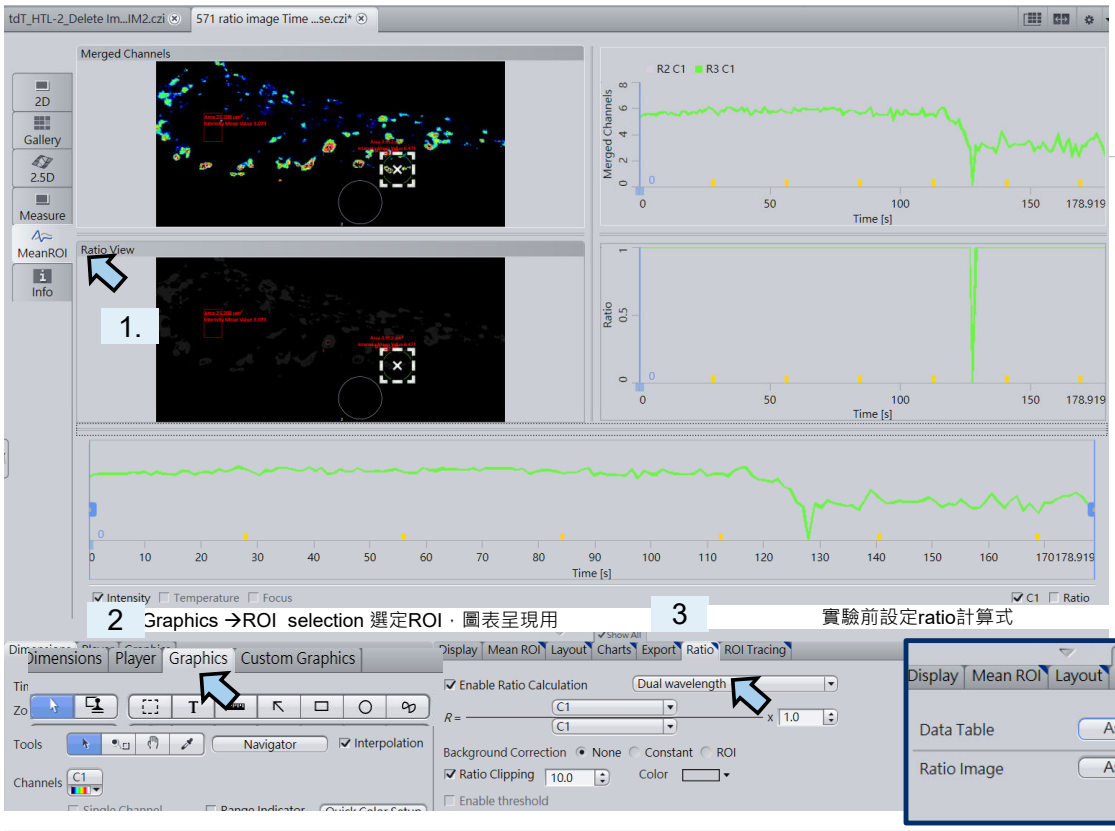


Ratio image with timelapse
(離子濃度監控)(intensity change on-line ratio plot display)



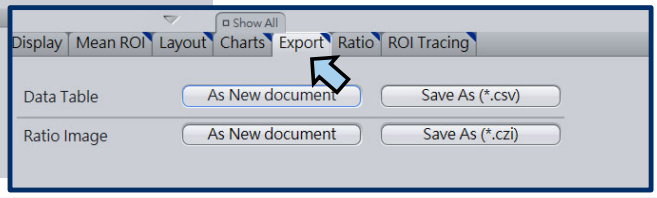


先滿足Channel 與 Time lapse 控制，Dynamics選項方會出現



2 Graphics →ROI selection 選定ROI，圖表呈現用

3 實驗前設定ratio計算式

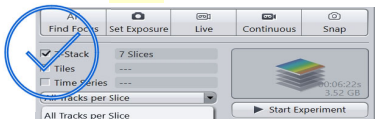


4

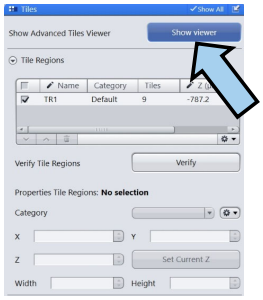
實驗結束後的影像/數據輸出

Tile Scan Imaging Setup
基本設置

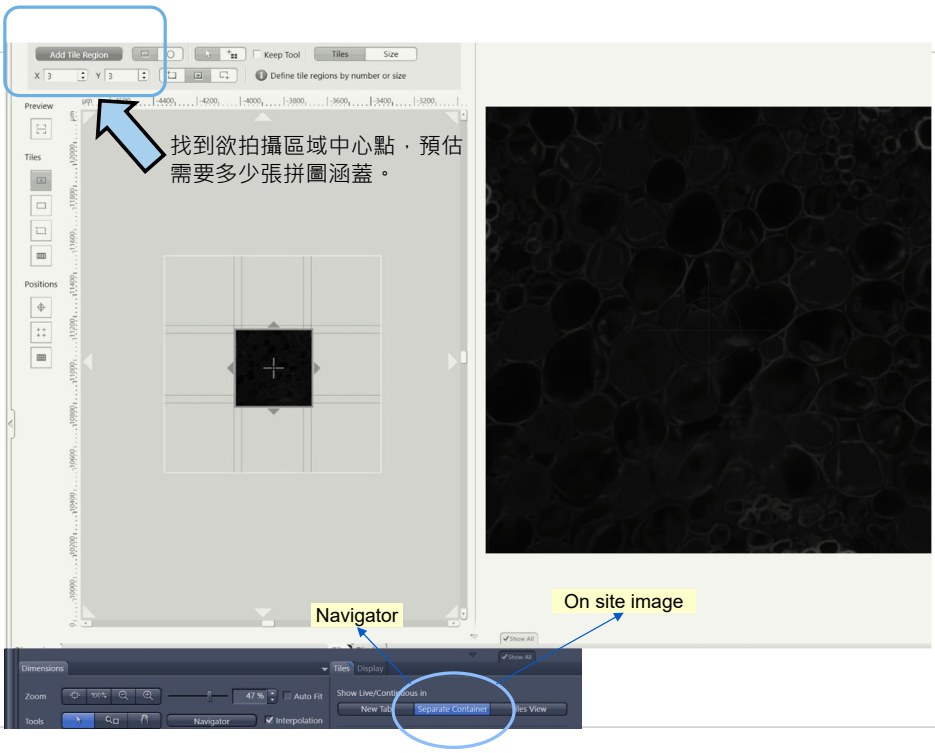
1



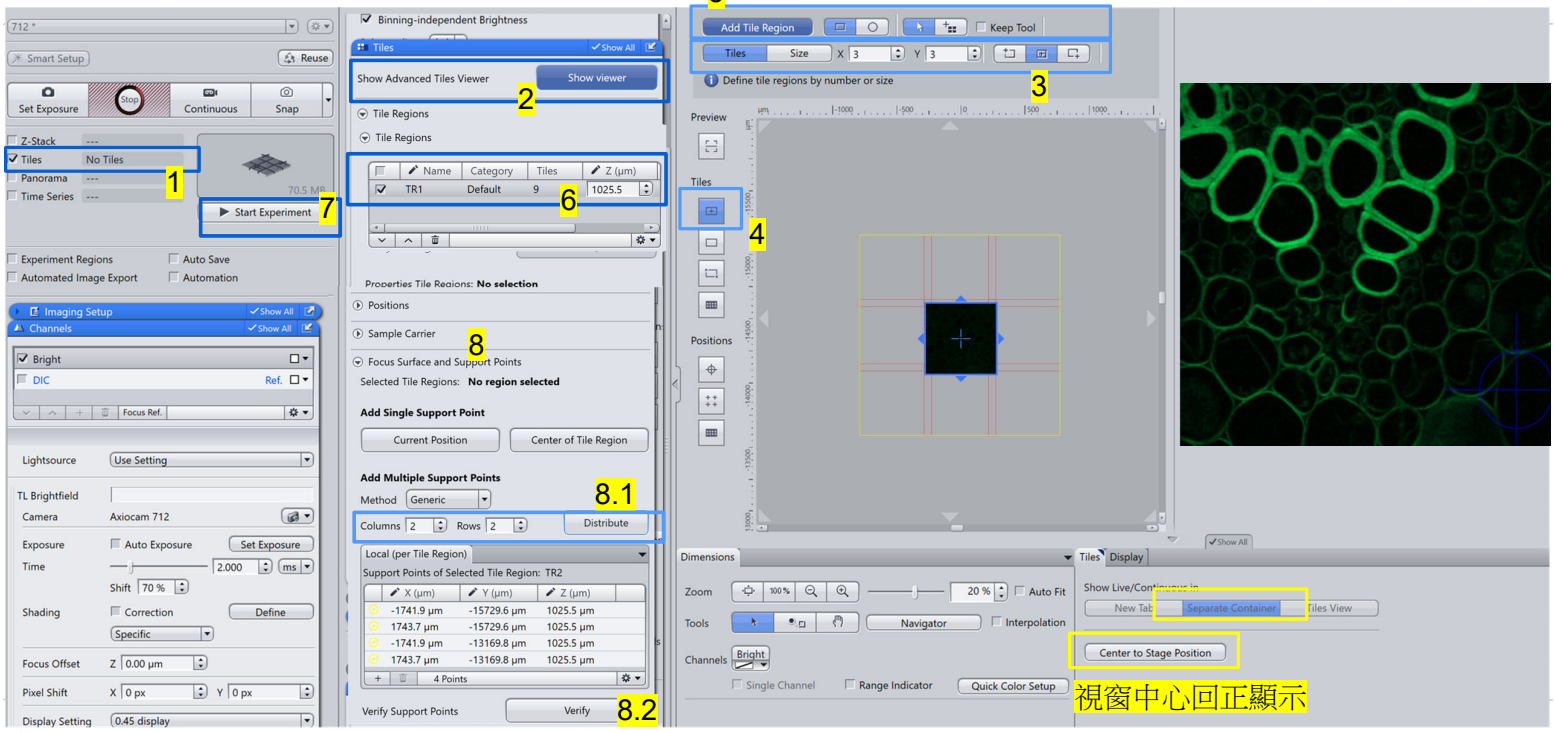
2



3



Tile Scan Setup
8 8.1 8.2為補充設定 (多位置焦距support points)



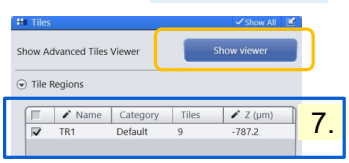
Tile Scan __ 1.
以WF方式(AxioCam702)輔助
快速設定拍照區域

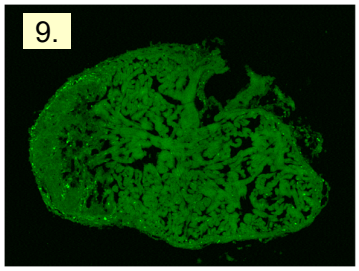
2.

- 先以WF快速設定拍照區域，
- 決定拼圖範圍後，取消WF Tracks 選擇
- 勾選其餘Confocal tracks 進行後續設定

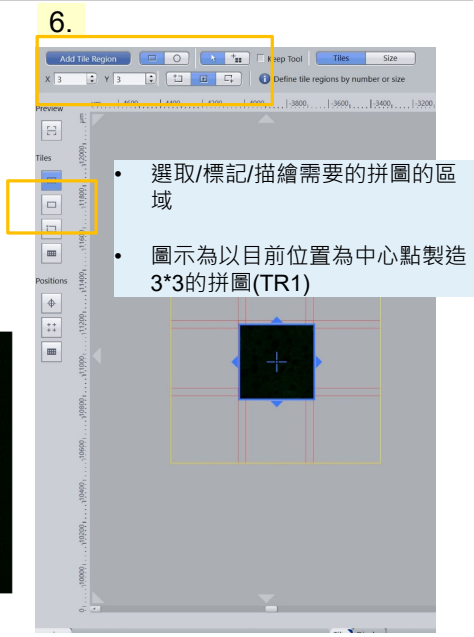
- 9.預覽TR區域WF試拍結果
- 10.取消WF，勾選confocal Tracks

5.地圖模式



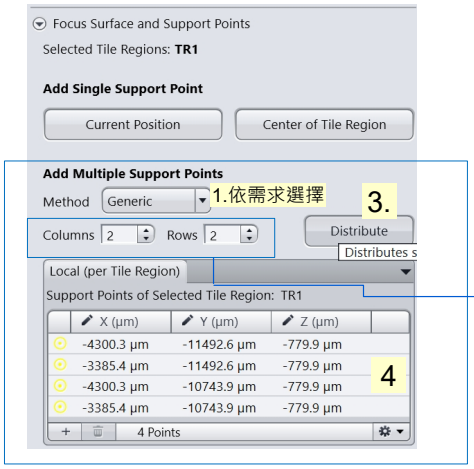


6.



- 選取/標記/描繪需要的拼圖的區域
- 圖示為以目前位置為中心點製造3*3的拼圖(TR1)

Tile Scan.
設置多位置焦點 Focus Map
多位置焦距Add support points

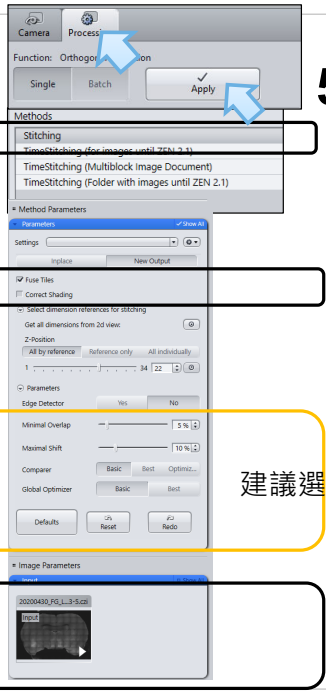


2.
- 依樣品狀況決定support points數量
- 一般單純傾斜不必太多點，系統會自行計算焦聚

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

1.

2.

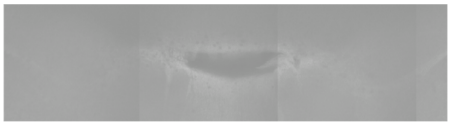


4.

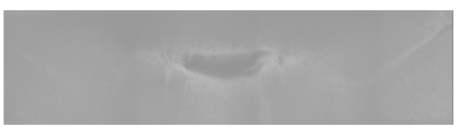
3.

建議選擇Basic即可

Before

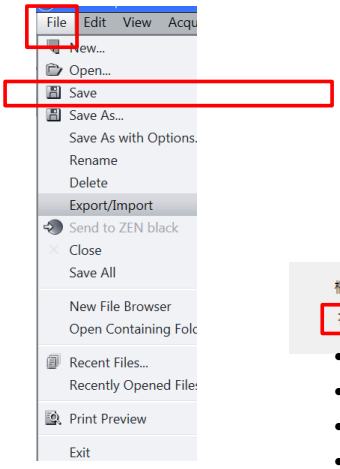


After

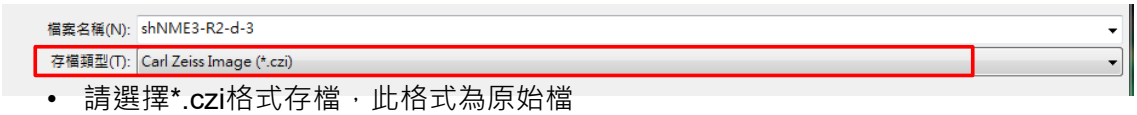


存檔

5



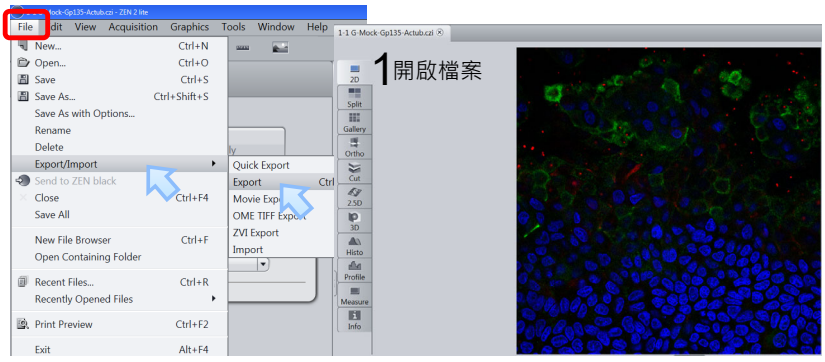
存檔

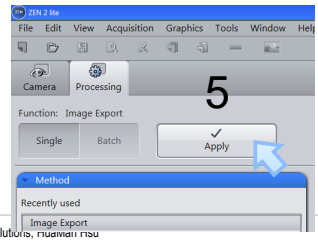


- 請選擇*.czi格式存檔，此格式為原始檔
- *.czi 日後可reuse
- 請勿直接存成tif/jpg檔，以免日後無法定量及添加尺規
- 需要純圖檔請進行圖檔輸出export或大量批次轉檔batch export，詳見下頁

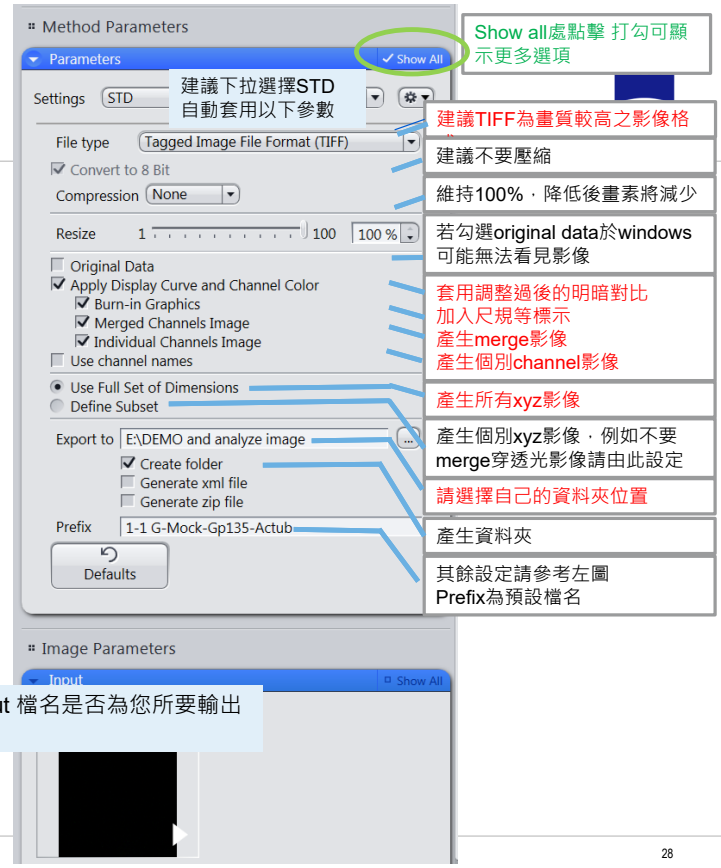
圖檔輸出export (單一檔案)

2





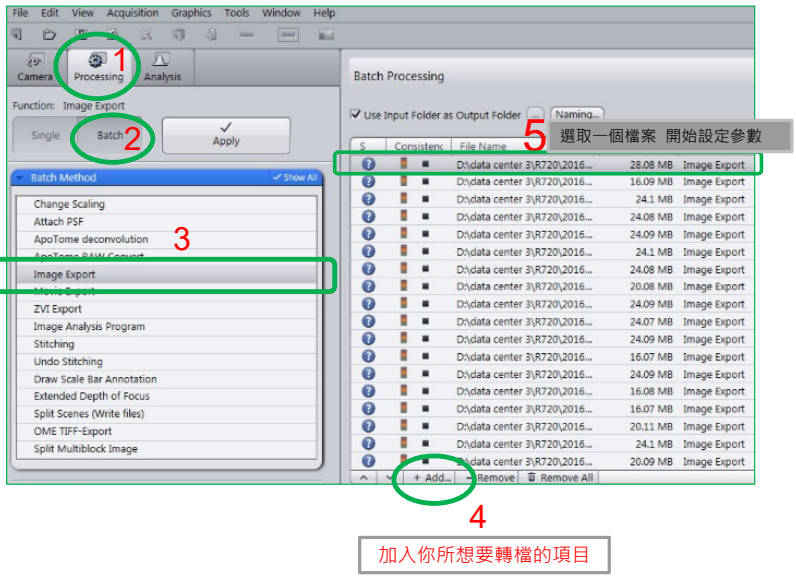
4

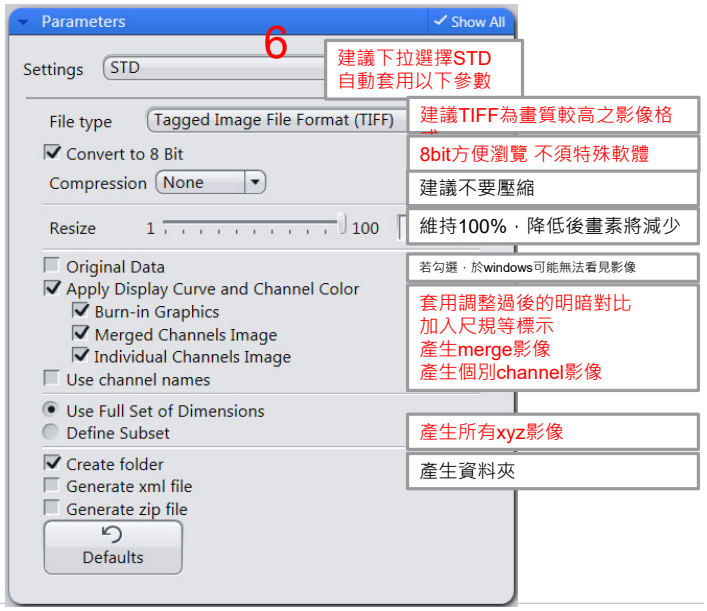


3

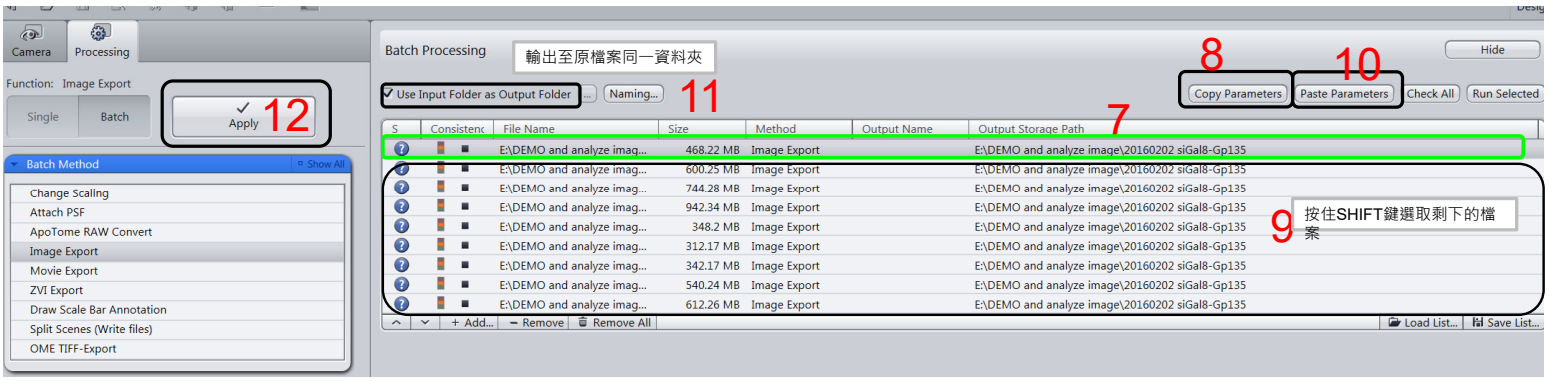
確認input 檔名是否為您所要輸出的檔案

大量批次轉檔batch export 1



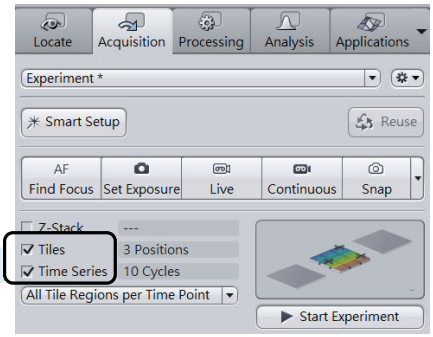


大量批次轉檔batch export 2

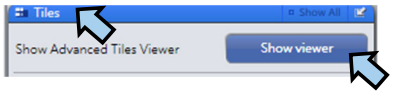


7~10 將設訂好的參數貼至其餘檔案當中，若沒有做paste parameter的動作，可能會失敗!

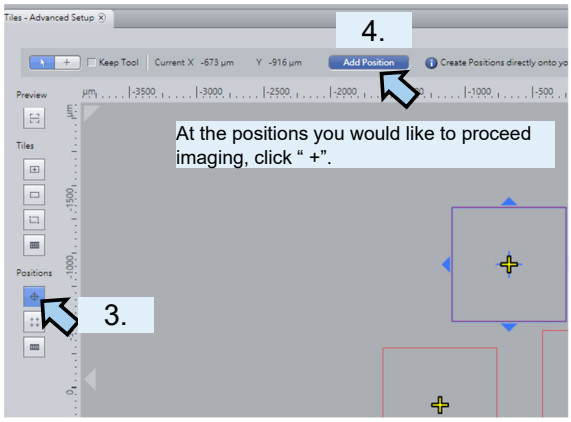
Multi-position Time Lapse Imaging 1.
Mark the positions of interests
Focus Strategy_ Definite focus (適用於焦距差不多的樣品，通用法)



1.



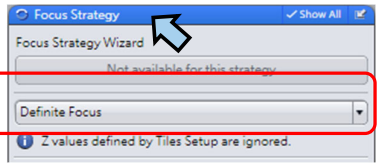
2.



4.

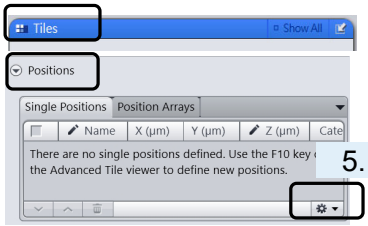
3.

5. Focus Strategy → Definite Focus

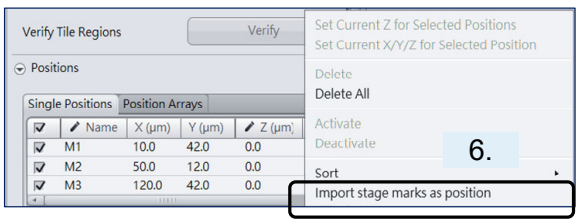


(補充)Multi-position Time Lapse Imaging 1.
Mark the positions of interests
Import stage marks as position

4.

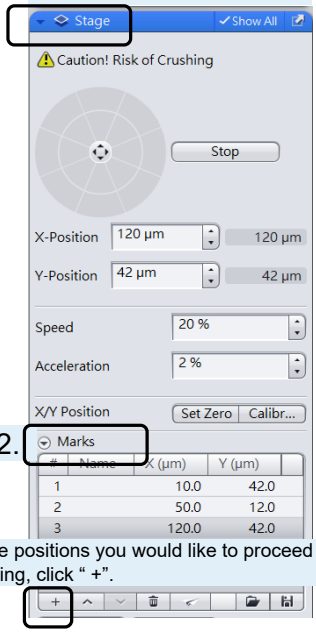


5.



6.

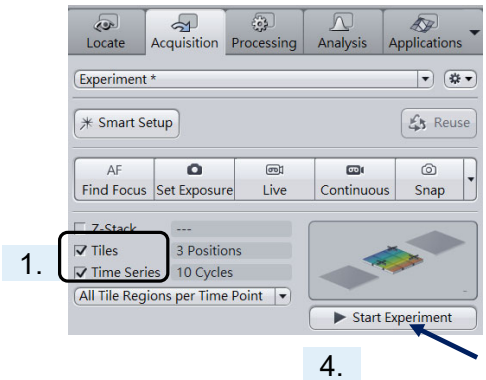
1. Stage → Marks

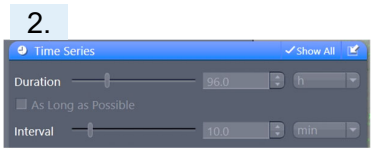


2.

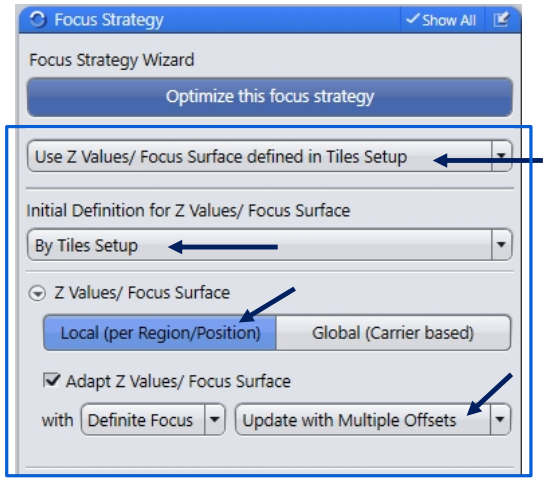
3. At the positions you would like to proceed imaging, click " +".

多位置長時間自動追焦
Multi-position Time Lapse Imaging 2.
適用於微小焦距差異設定



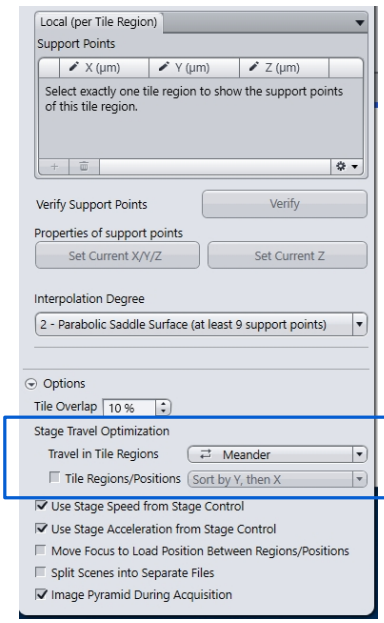


手動或自動確認焦距



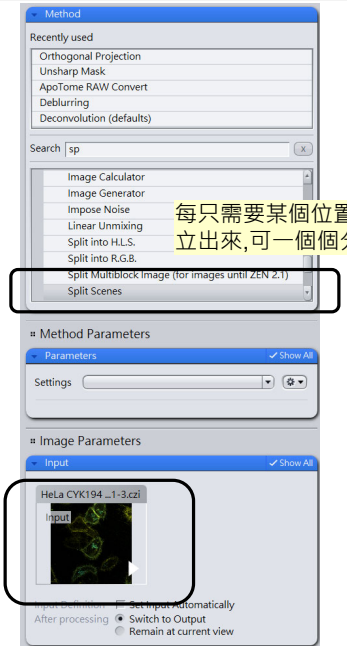
- 所有位置焦距設定完畢
- 按下Start Experiment後軟體會自動設Multiple Offsets

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



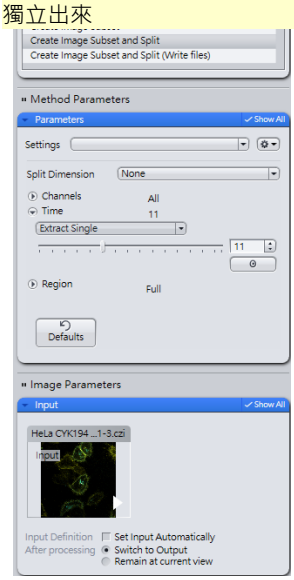
- 按照positions 順序拍攝請取消Meander!
- 否則位置拍攝順序會重新排序

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

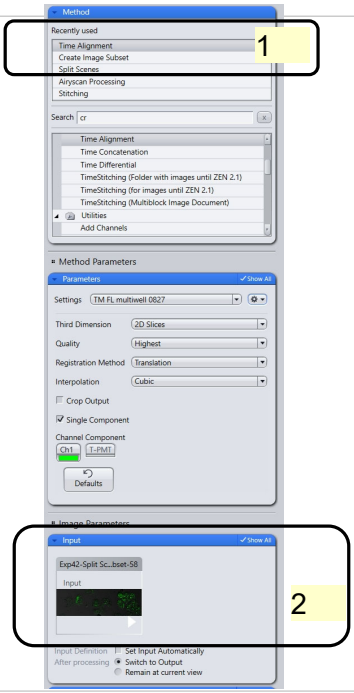


每只需要某個位置的結果獨立出來,可一個個分別存檔

只需要某個位置或時間的結果獨立出來



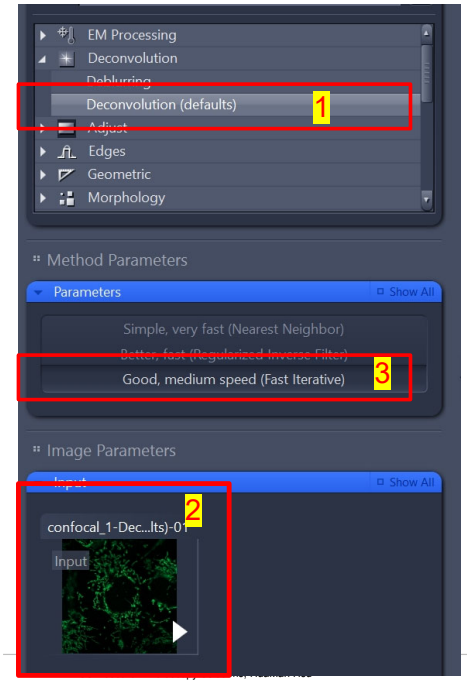
多位置拍攝長時間晃動問題
Processing → Time lapse Alignment



關於連續撥放影片晃動

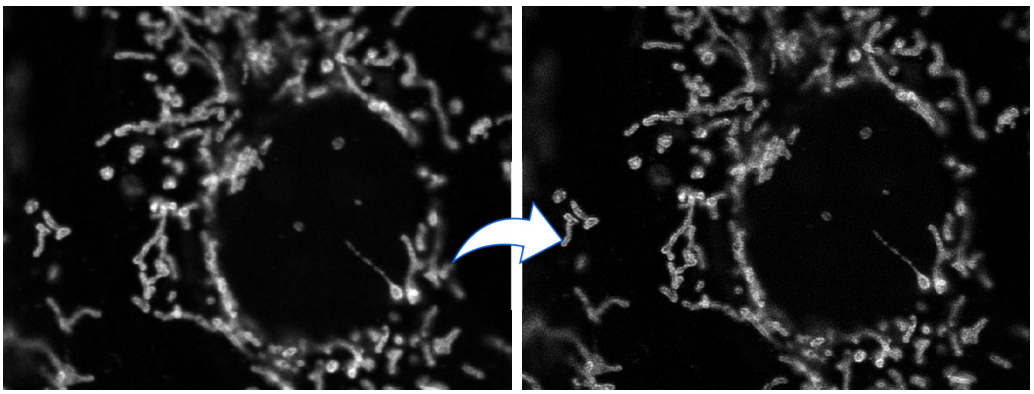
- 請放好樣品後對焦完畢，**以黏土或工具固定樣品**以免因載物台長時間多點移動造成樣品位移。
- 若合併多點(位置)拍攝此步驟請split scene切割完畢再執行

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

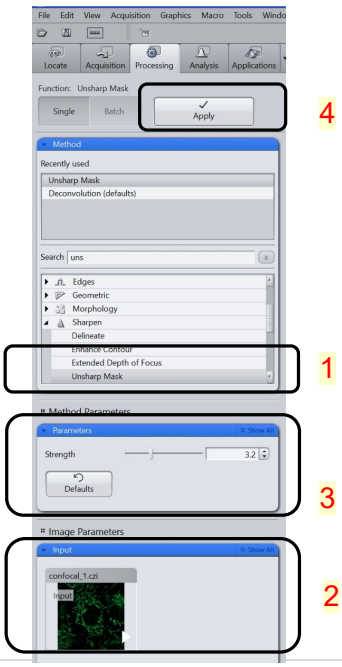


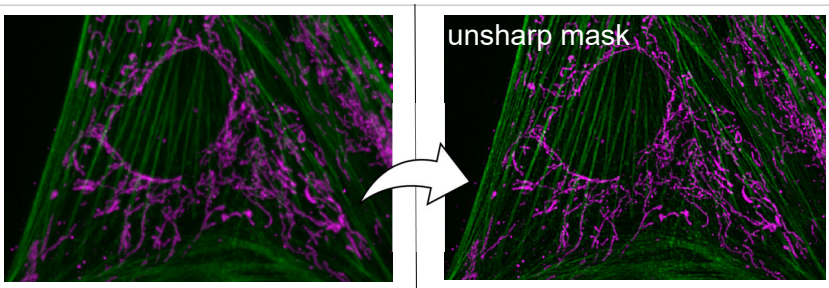
Before

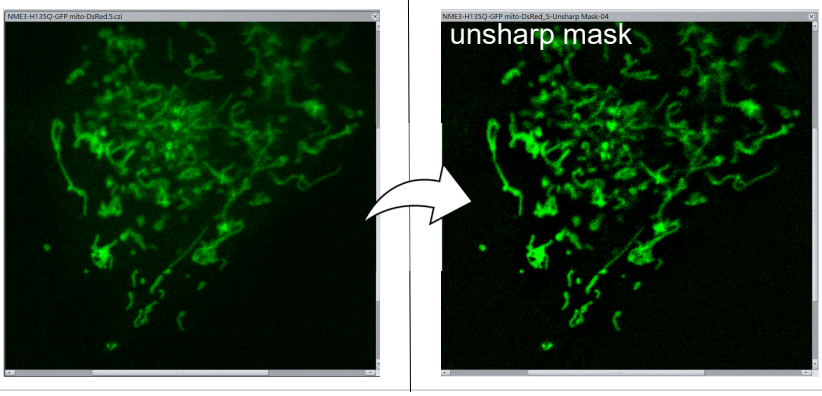
After



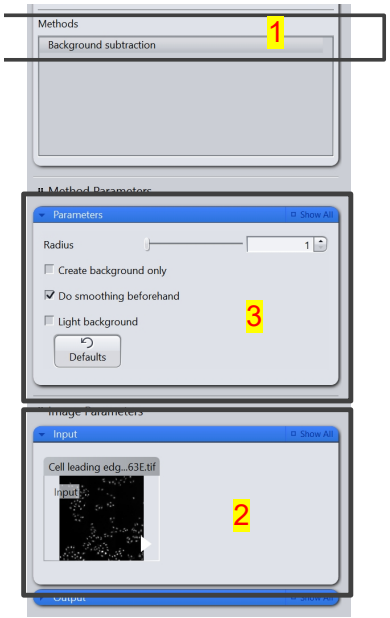
ZEN Image Processing
Image Clearing Option 2:
Unsharp masking

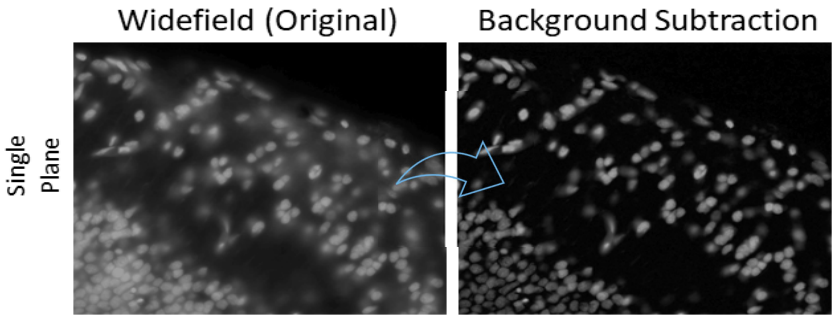




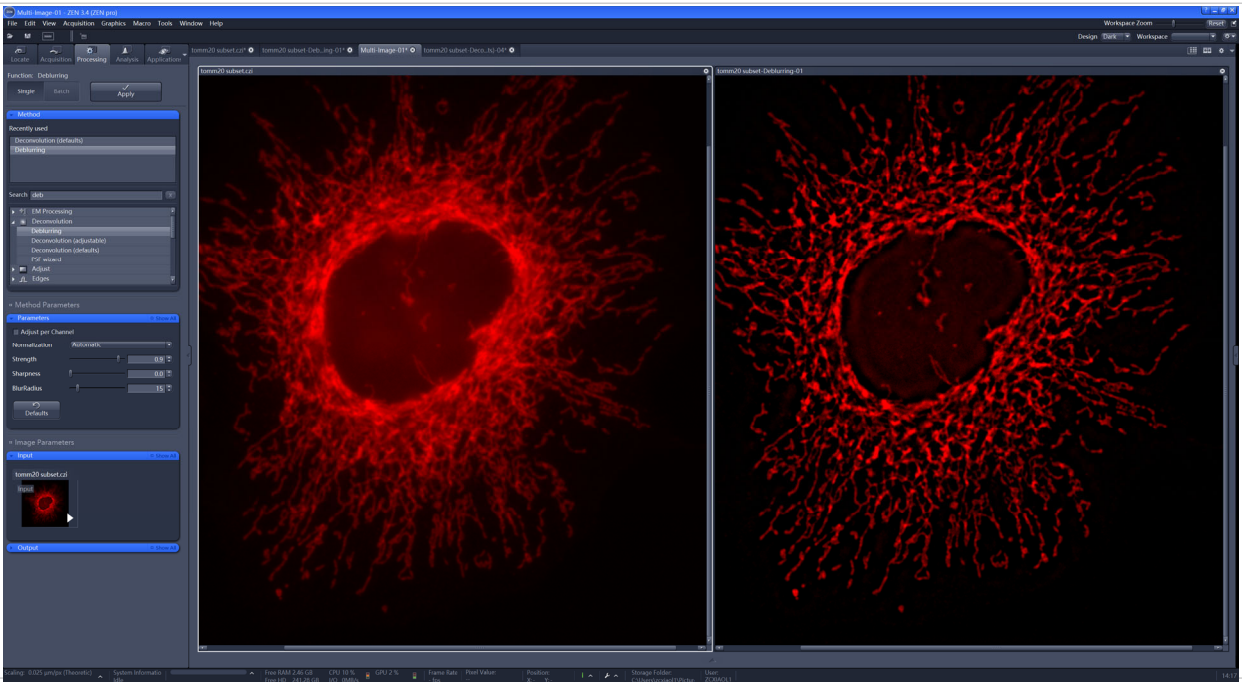


ZEN 3.3 Image Processing
Image Clearing Option 3:
Background subtraction



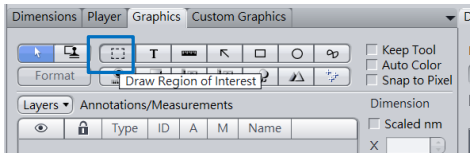


Option 4:
ZEN 3.4
WF vs Deblurring/2D DCV

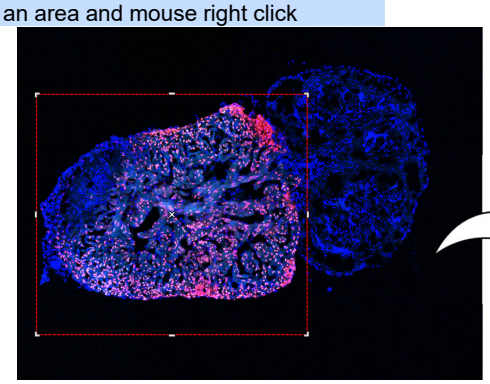


裁圖，複製選取範圍影像

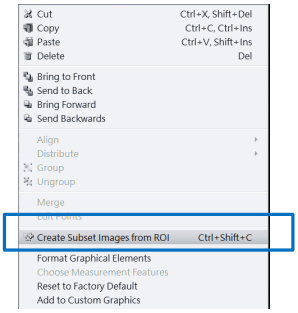
1.



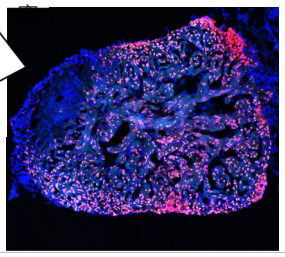
2. Draw an area and mouse right click



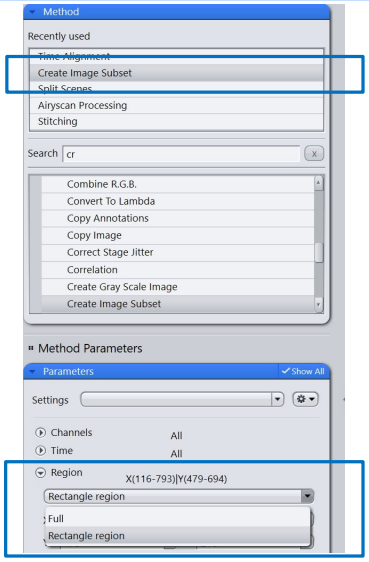
3. Create Subset Images from ROI



4. 獲得裁切範圍於新視

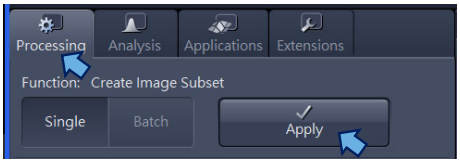


3. 或者
Processing → Create Image Subset

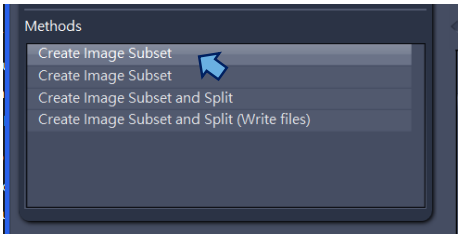


裁切，分割 channel , time , region, scene片段資料
create image subset

1



2

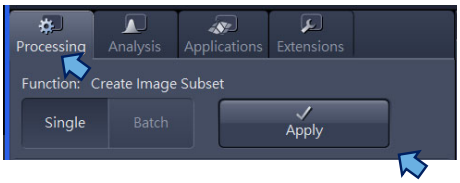


3

4

裁切結果自動存檔，
分割 channel , time , region, scene片段資料
create image subset

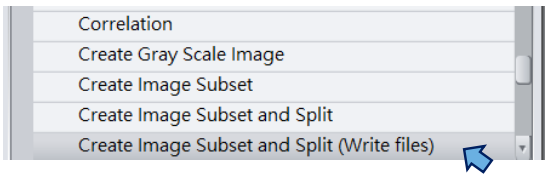
1



5

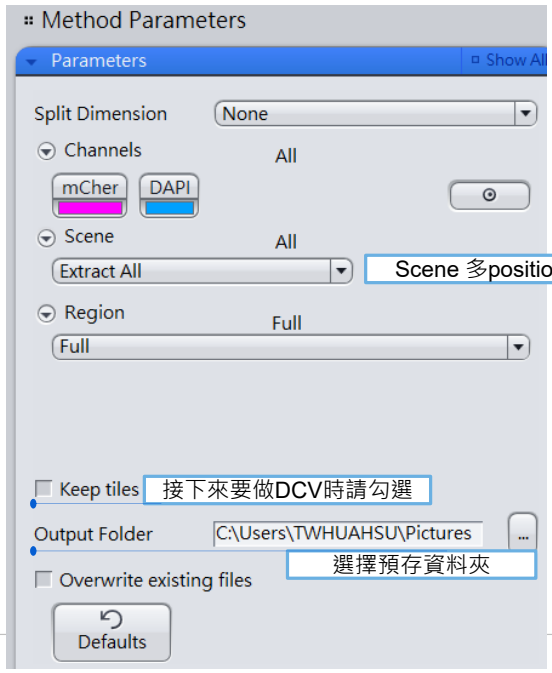
3

2



4

選擇欲分割參數



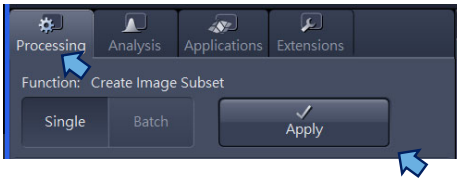
Scene 多positions切割

接下來要做DCV時請勾選

選擇預存資料夾

Scene分離，每個位置一個單獨檔案
Split Scenes (Write files)

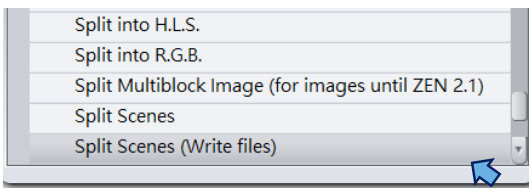
1



5

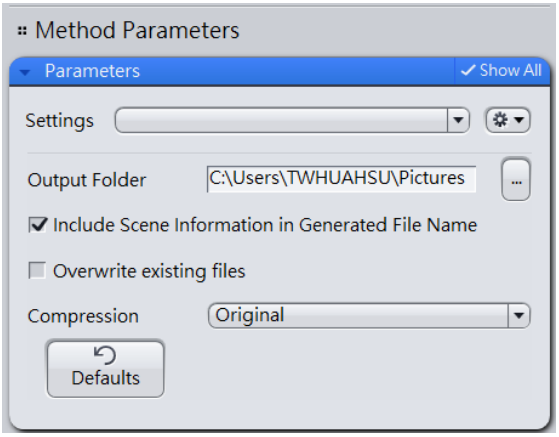
3

2

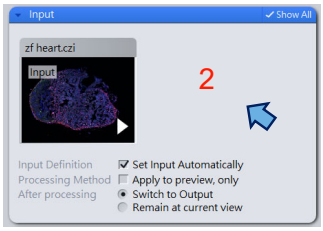


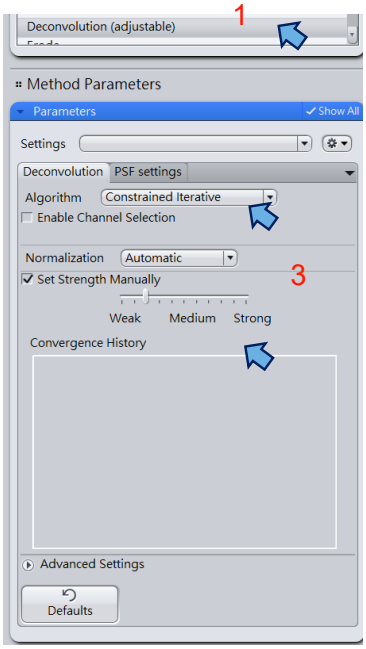
4

選擇目的資料夾

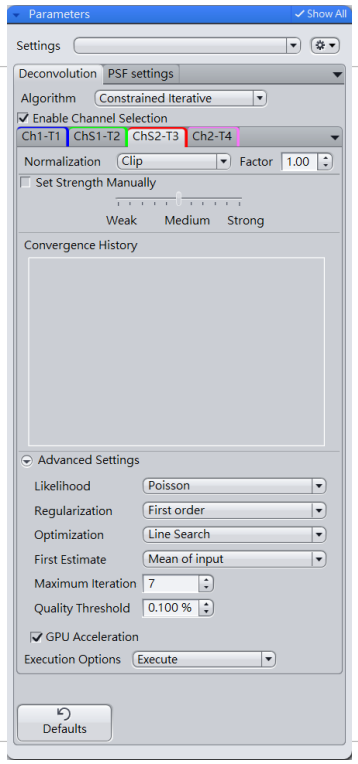


Deconvolution (adjustable)

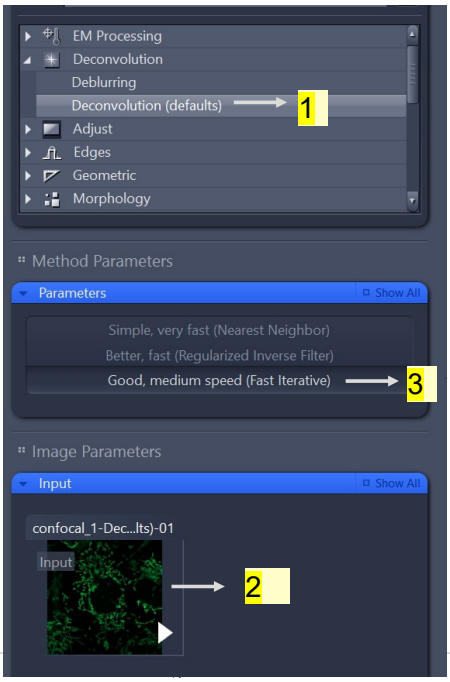




進階選項

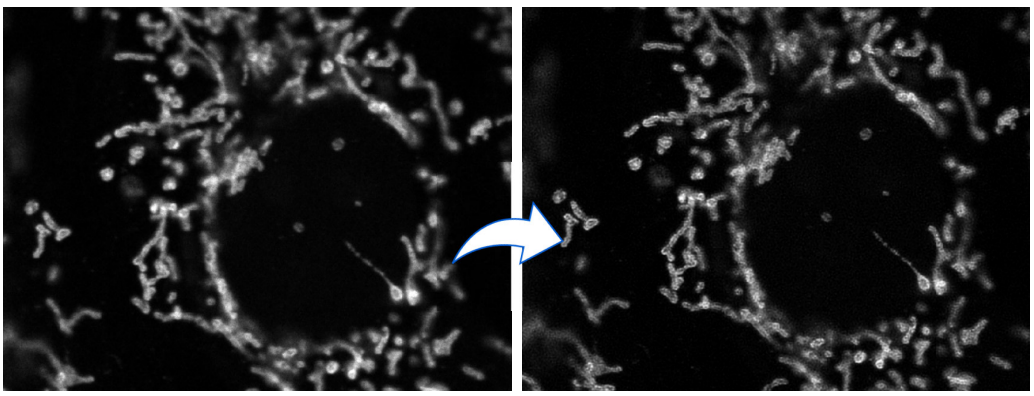


ZEN 3.3 Image Processing Image Clearing Option 1: 2D DCV (defaults)

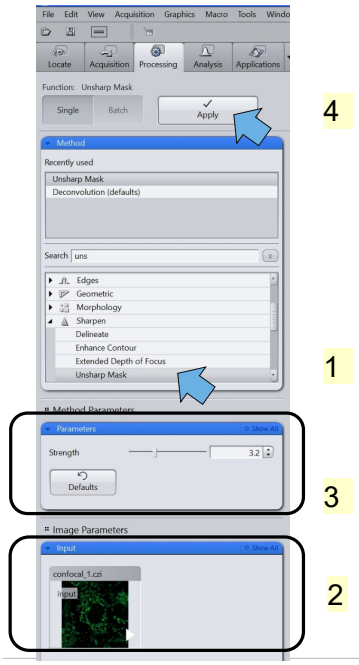


Before

After



ZEN Image Processing
Image Clearing Option 2:
Unsharp masking

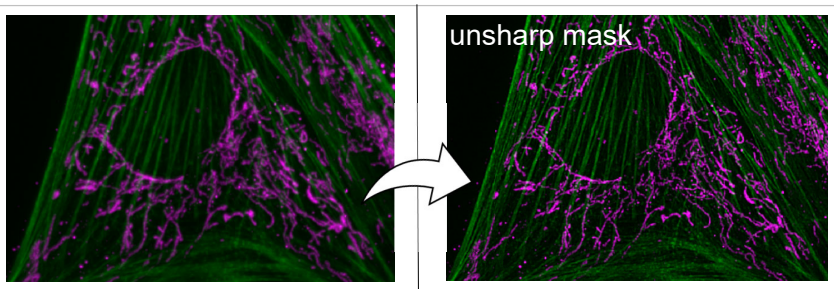


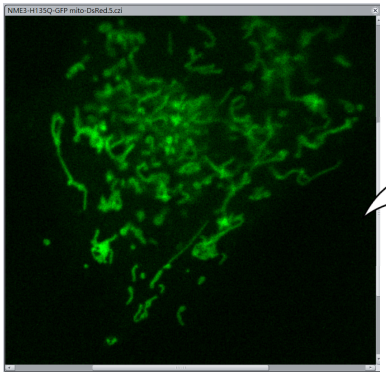
4

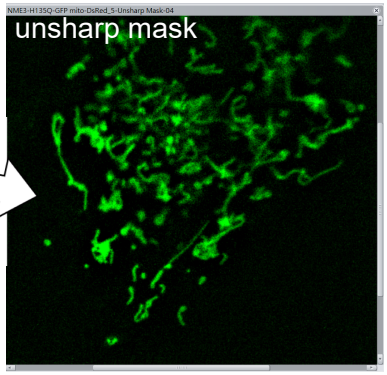
1

3

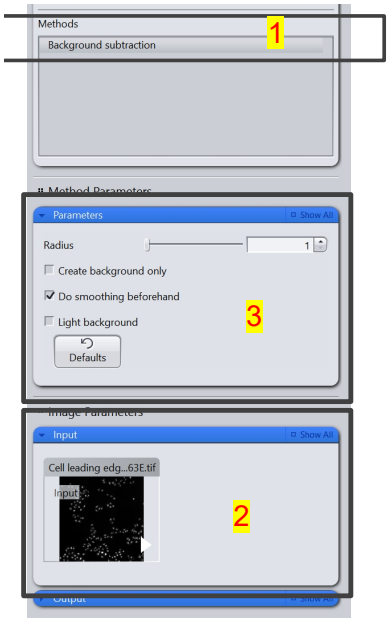
2

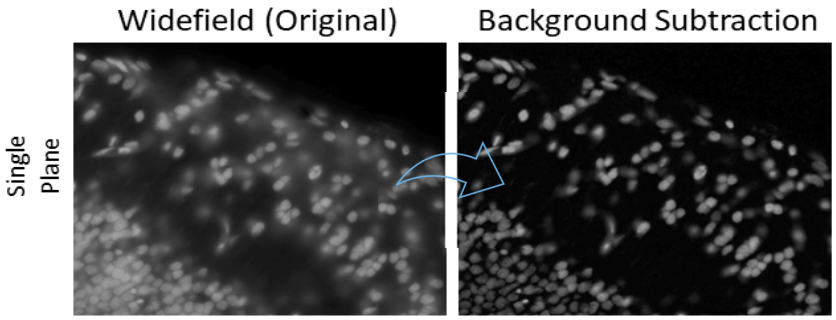




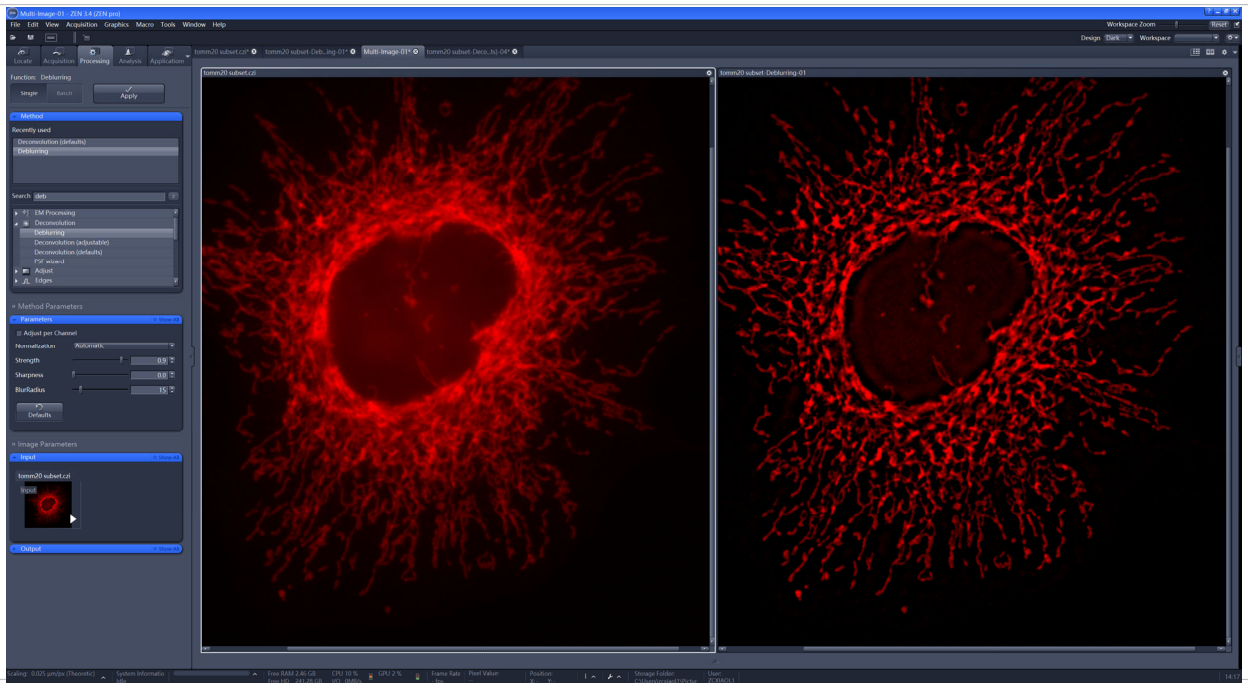


ZEN 3.3 Image Processing
Image Clearing Option 3:
Background subtraction



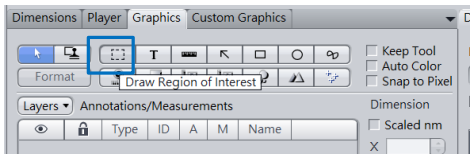


Option 4:
ZEN 3.4
WF vs Deblurring/2D DCV

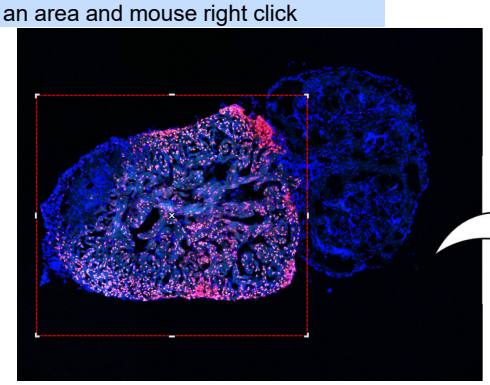


裁圖，複製選取範圍影像

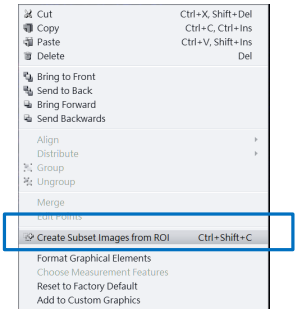
1.



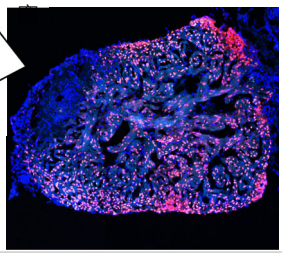
2. Draw an area and mouse right click



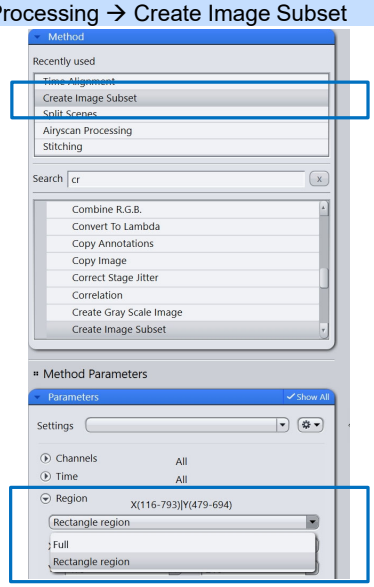
3. Create Subset Images from ROI



4. 獲得裁切範圍於新視

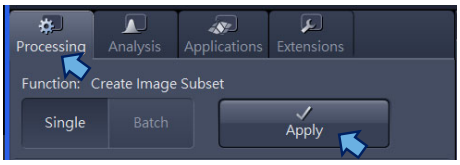


3. 或者
Processing → Create Image Subset

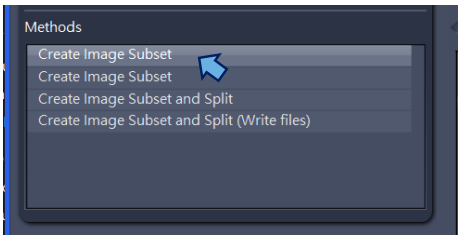


裁切，分割 channel , time , region, scene片段資料
create image subset

1



2

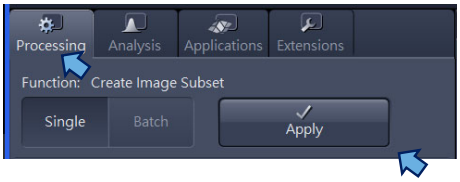


3

4

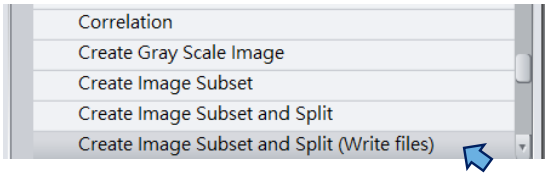
裁切結果自動存檔，
分割 channel , time , region, scene片段資料
create image subset

1



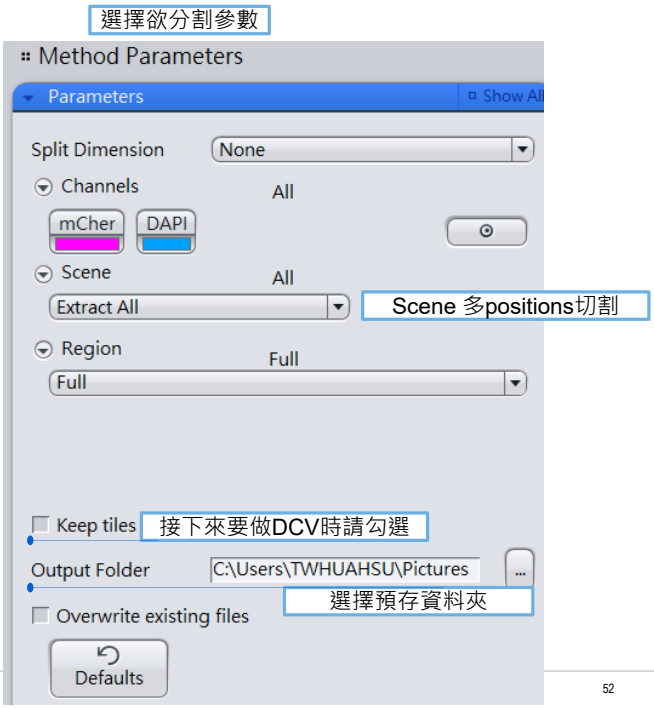
5

2



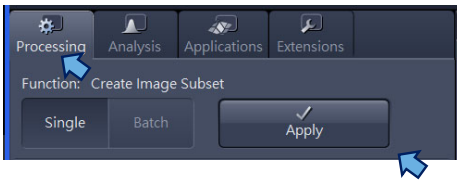
3

4

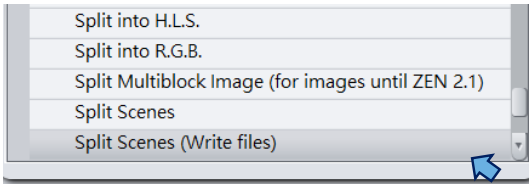


Scene分離，每個位置一個單獨檔案
Split Scenes (Write files)

1

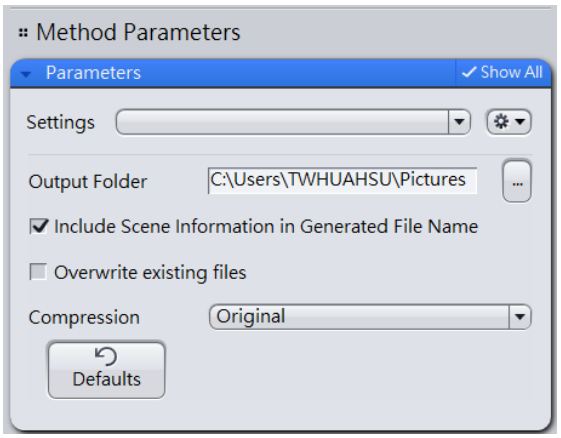


2



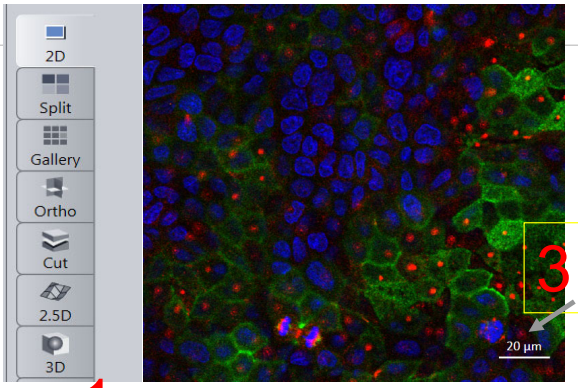
3

4 選擇目的資料夾



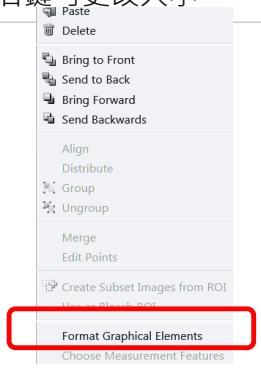
5

Add Scale Bar加入尺規



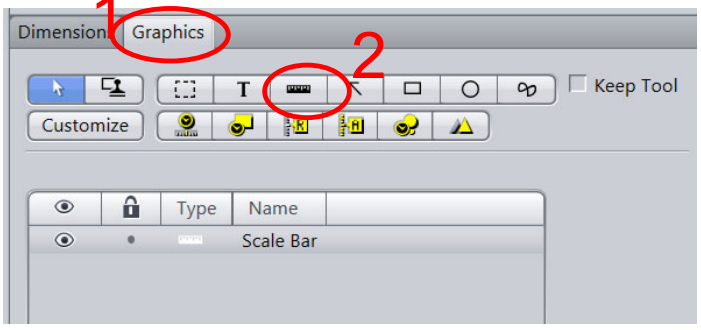
3 scale bar會自動出現

4 於scale bar按右鍵可更改大小顏色粗細等格式



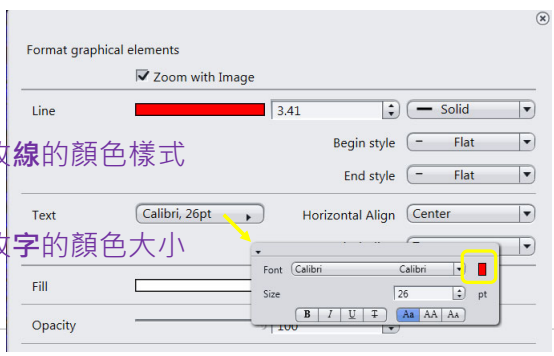
1

2

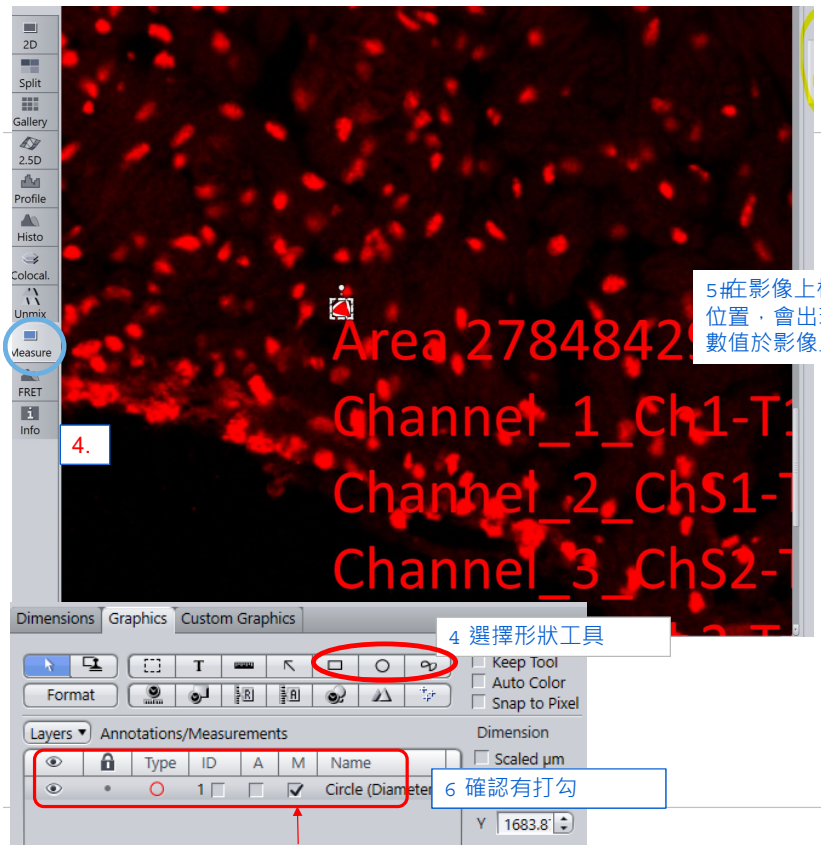


更改線的顏色樣式

更改字的顏色大小



量測area & intensity



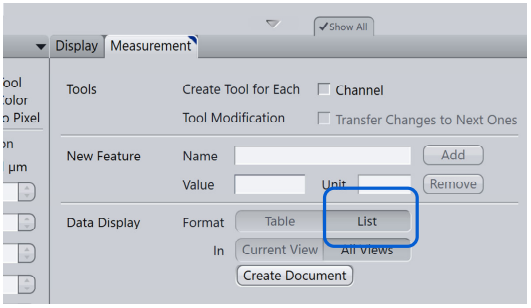
5#在影像上框選有興趣的位置，會出現面積及亮度數值於影像上

4 選擇形狀工具

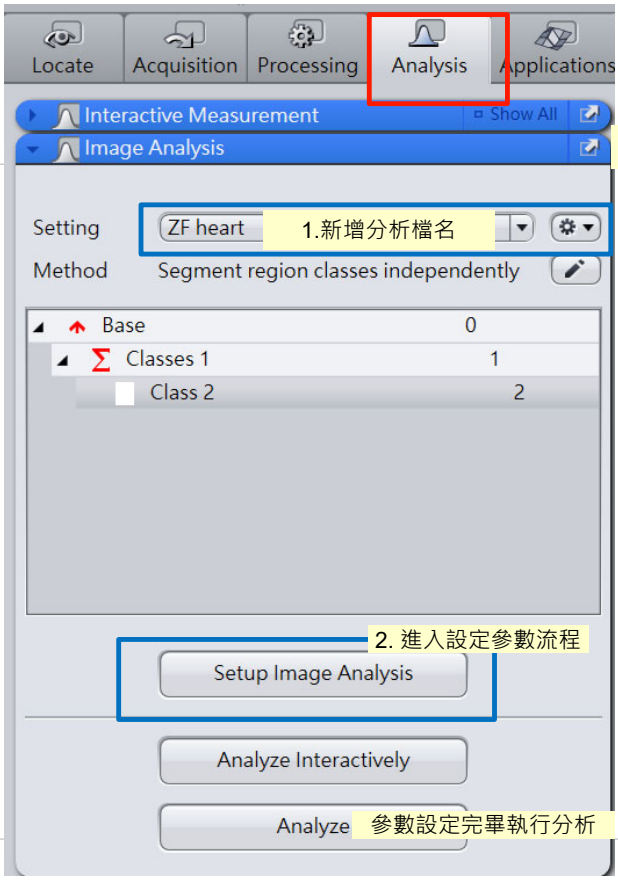
6 確認有打勾

7圖表位於右方視窗

	A	B	C	D
	Name	Feature	Value	Unit
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	



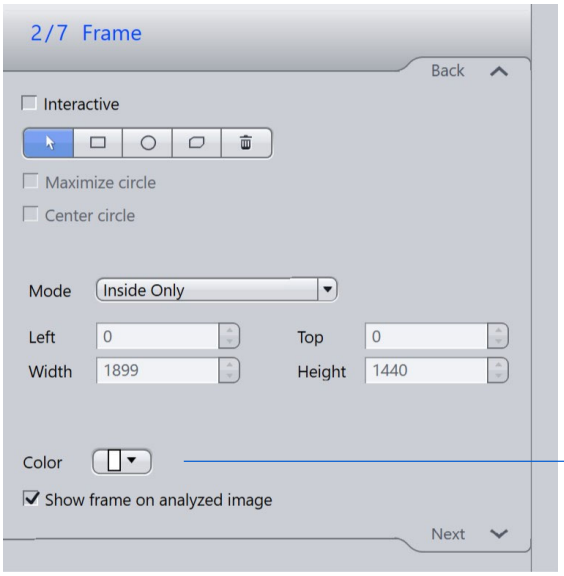
自動量測 Image Analysis Module 1.



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

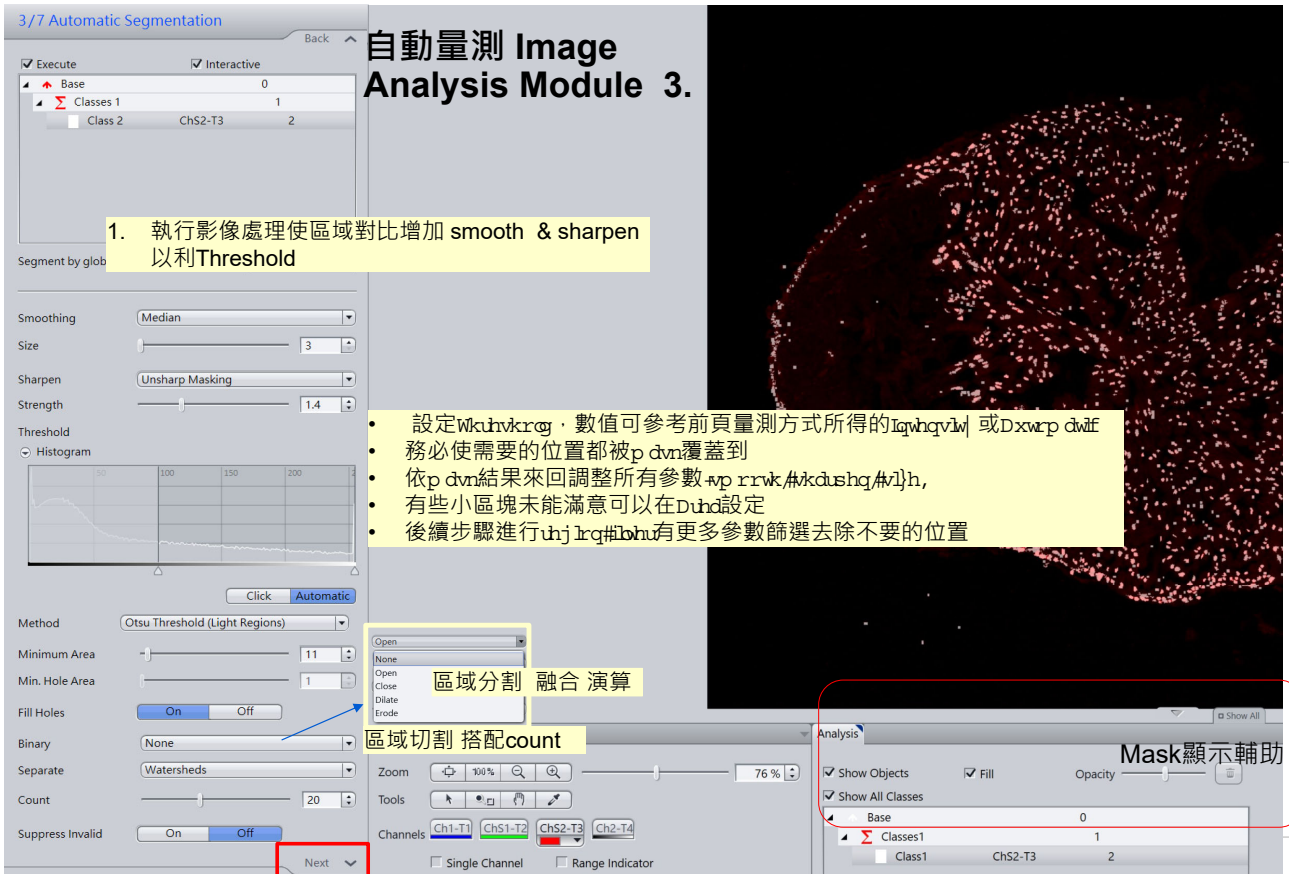
自動量測 Image Analysis Module 2.
圈選分析範圍，可略過



- 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

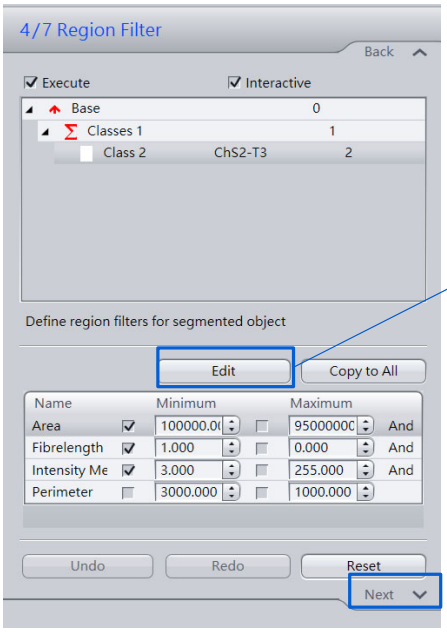
自動量測 Image Analysis Module 3.

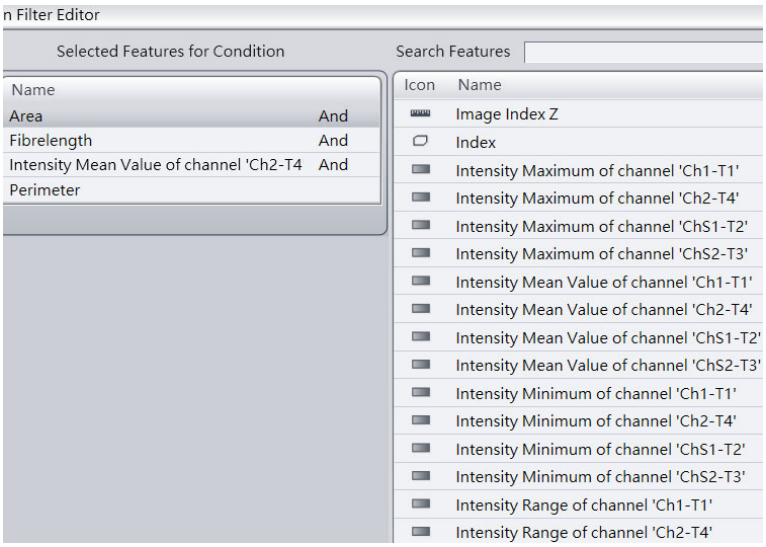


1. 執行影像處理使區域對比增加 smooth & sharpen 以利Threshold

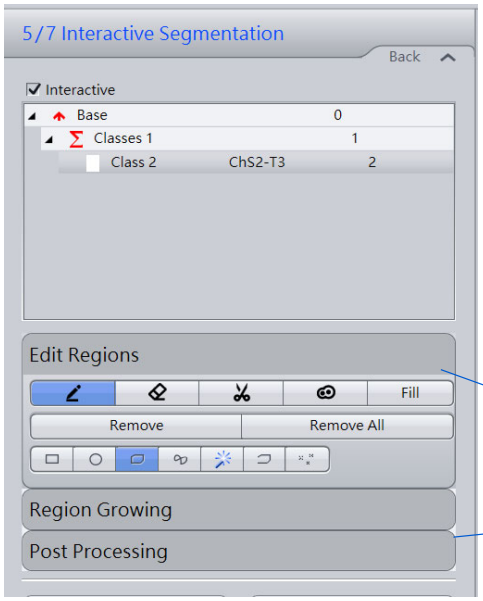
- 設定Wkuhvkrog，數值可參考前頁量測方式所得的Iqwhqvlw| 或Dxwrp dwlf
- 務必使需要的位置都被p dvn覆蓋到
- 依p dvn結果來回調整所有參數+wp rrwk/#vkdushq/#vl}h,
- 有些小區塊未能滿意可以在Duhd設定
- 後續步驟進行uhj lrq#lohu有更多參數篩選去除不要的位置

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





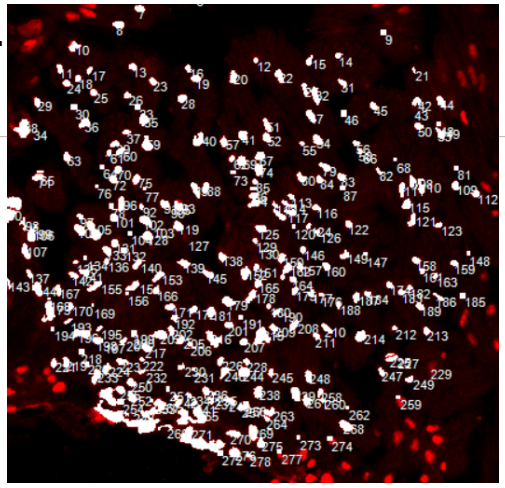
自動量測 Image Analysis Module 5.
互動式調整量測區域 增加 減少 或 切割 縫合
批次處理需求請略過此步

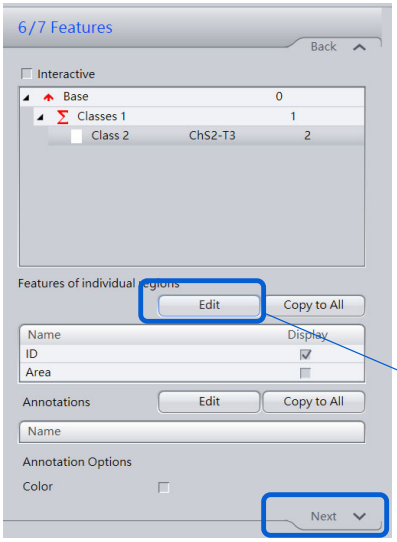


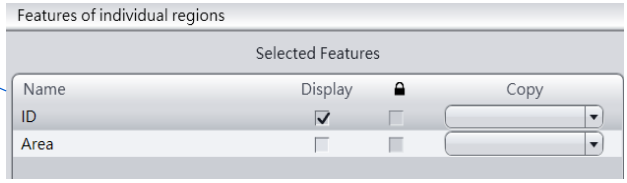
- 手動增加/刪除區域
- 手動切割/接合區域
- 若要進行自動大量圖檔批次分析，請避免使用此步驟

- 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

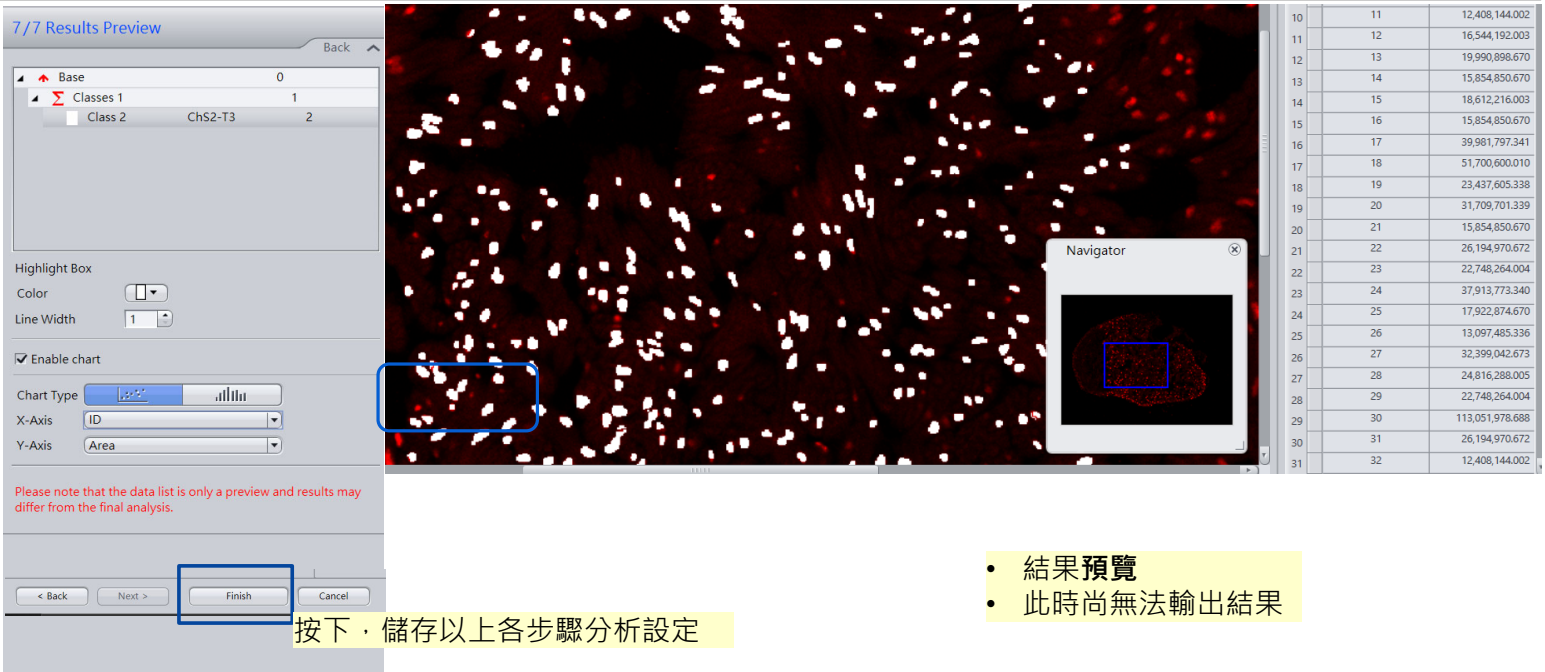






- 圖上若不需要顯示量測結果不要勾Display
- 量測結果顯示設定 ， 如果密密麻麻的mask 請避免使用，以免影響觀察量測區域

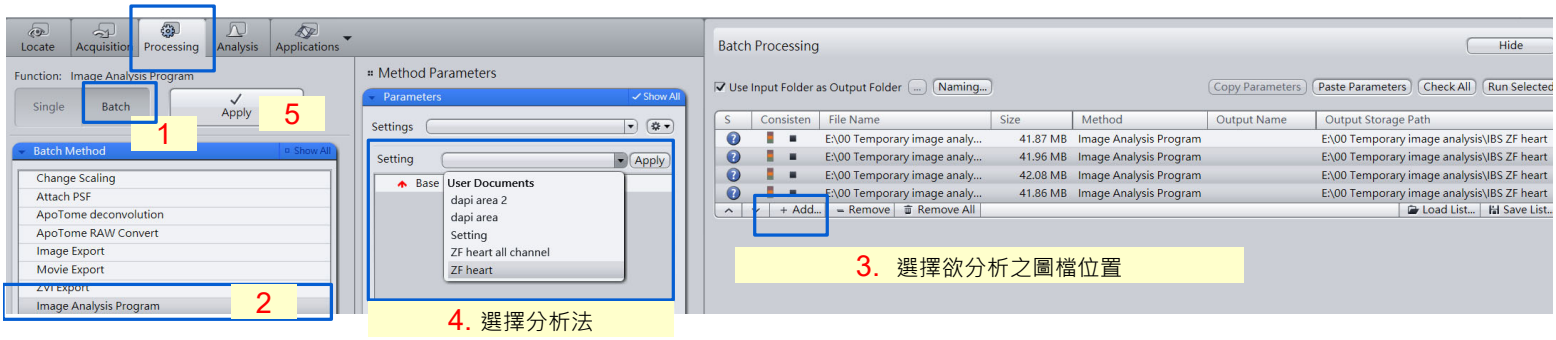
自動量測 Image Analysis Module 7. 參數設定完畢，結果預覽



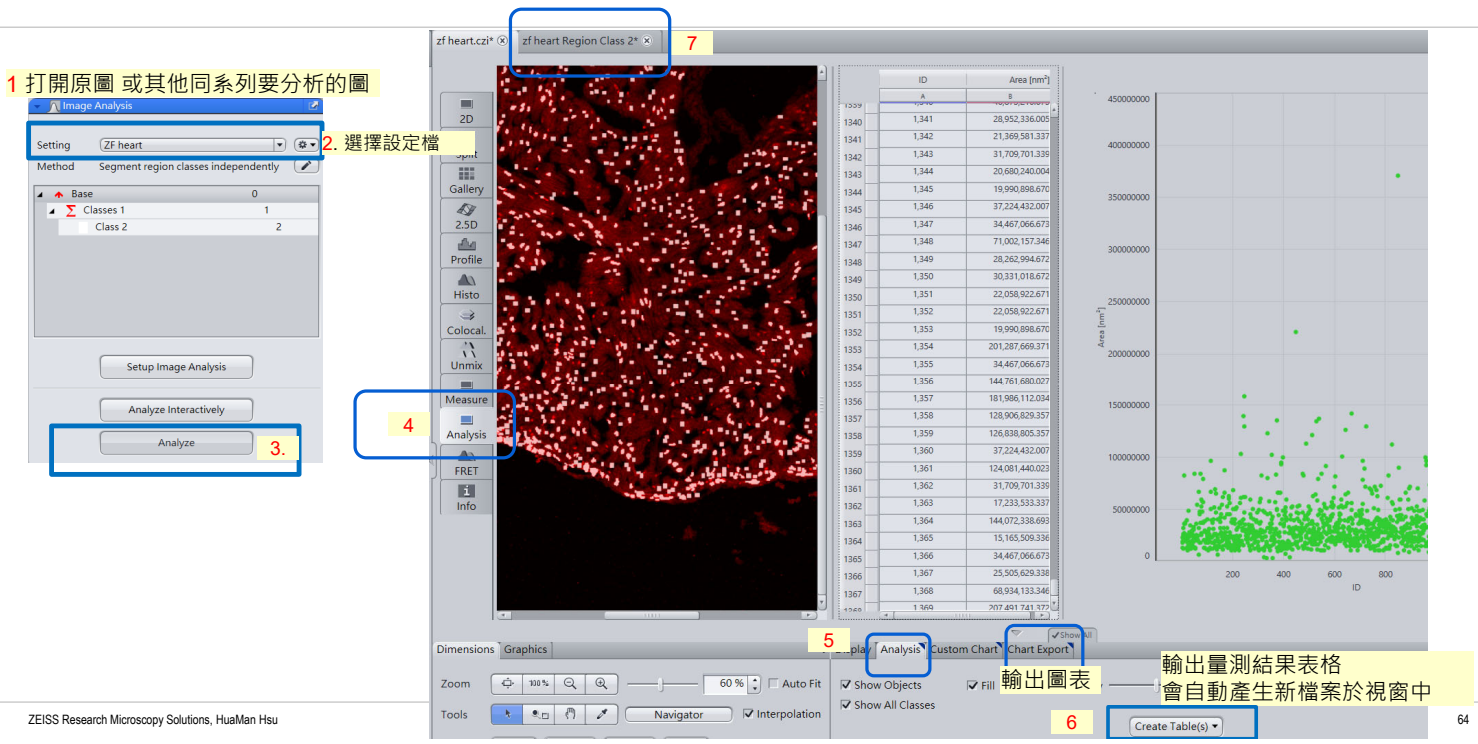
按下，儲存以上各步驟分析設定

- 結果**預覽**
- 此時尚無法輸出結果

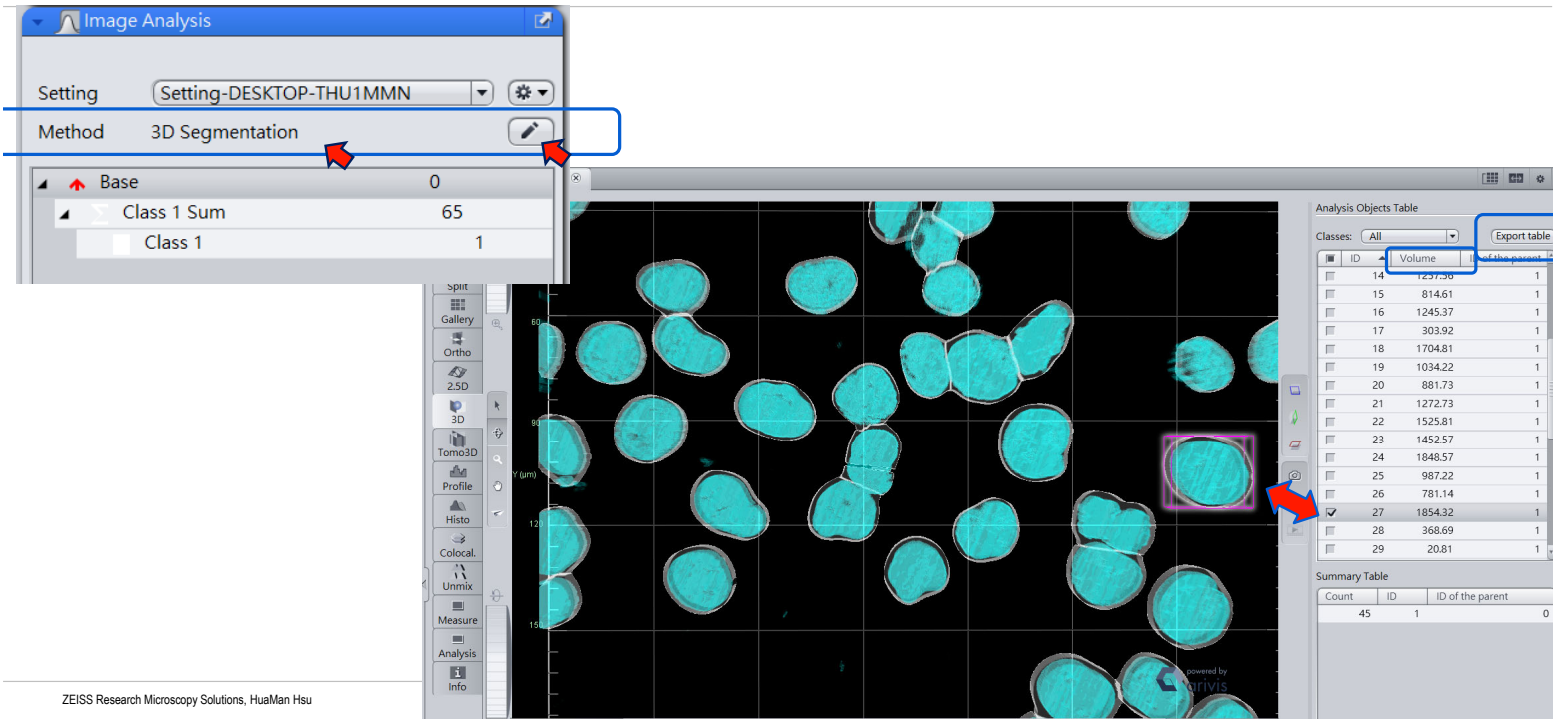
自動量測 Image Analysis Module 8.
大量分析Batch Analyze



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



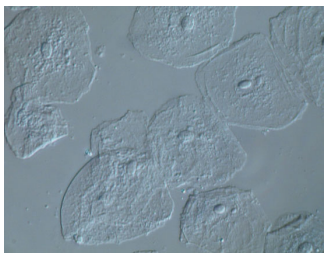
ZEN 3.5
3D Image Analysis

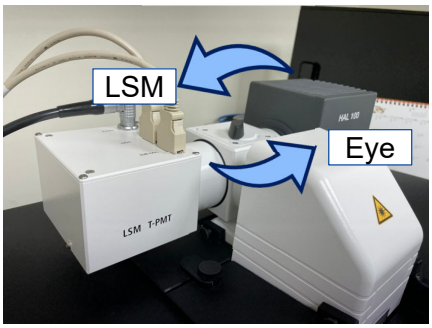


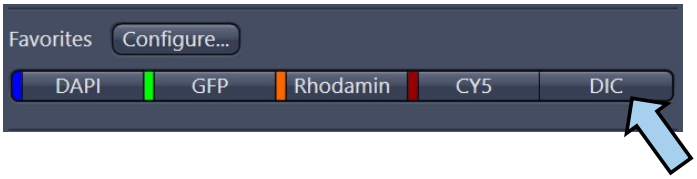
ZEISS Research Microscopy Solutions, HuaMan Hsu

Bright Field / DIC Observation

加拍穿透光
Bright Field / DIC Observation



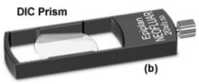




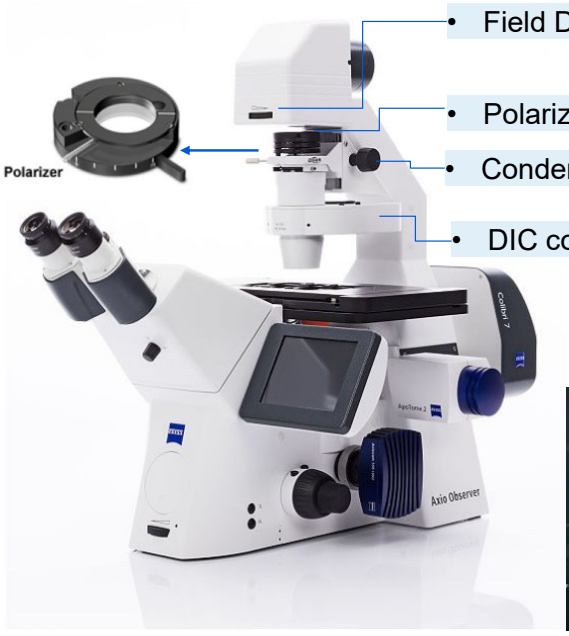
Turn the T-PMT Rotary Switches to

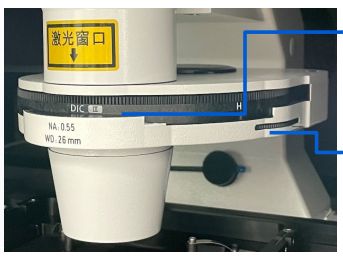
- Right for eye observation
- Left for confocal imaging

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



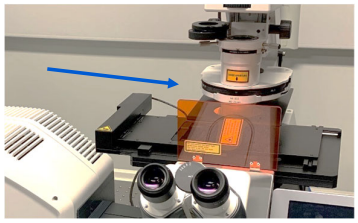


1. Focus the sample with objective
2. Adjust the condenser height by condenser focus knob (標記線對齊).
3. Check the condenser center position by closing the field diaphragm and reopen it after focusing the condenser.
4. Choose the DIC filter position (螢光濾片轉盤)
5. Swing the polarizer holder in
6. Choose condenser 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

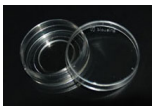
1. 如果講求完美效果聚光鏡校正要先做好! (設置請參考前頁)
2. 將螢光設定好最後再開啟T-PMT(或ESID)
3. 可選取任一個Track**合併**拍攝穿透光或者增加track單獨拍攝
4. 確認一下聚光鏡轉盤位置是否在DICII（10x& 20x）或DICIII（40x以上）（見下圖）。

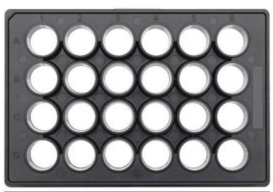


Recommend Single/Multi-well Chamber Types for Living Cell Application

POC-R

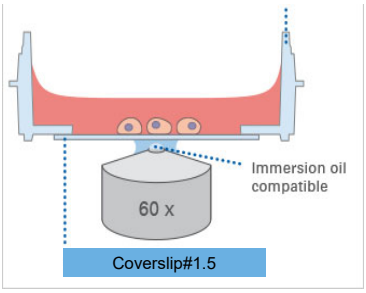
POC-R mini





Cell Imaging Plate

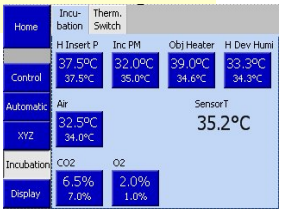
- **#1.5 cover glass/ polymer bottom dish/plate/slide for inverted microscope with high N.A objectives.**
- Thickness **no 1** ½ 0.17mm ±0.005mm
- 需設定多孔盤Templet 請找徐小姐



關機

4. Computer

- 如果有使用活細胞培養裝置
- 關閉C02 (鋼瓶與TFT控制面板 Incubation)
- **Incubator XL 降溫至30度內(降溫完成, 控制面板incubation → off)**
- 加濕水瓶倒除



3. Components

2. Laser Key

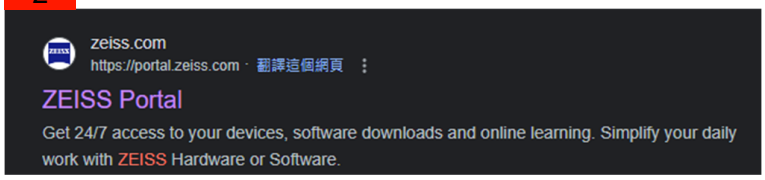
1. Main Switch

ZEN blue/lite Download

下載離線軟體 ZEN lite 1

1

2



3

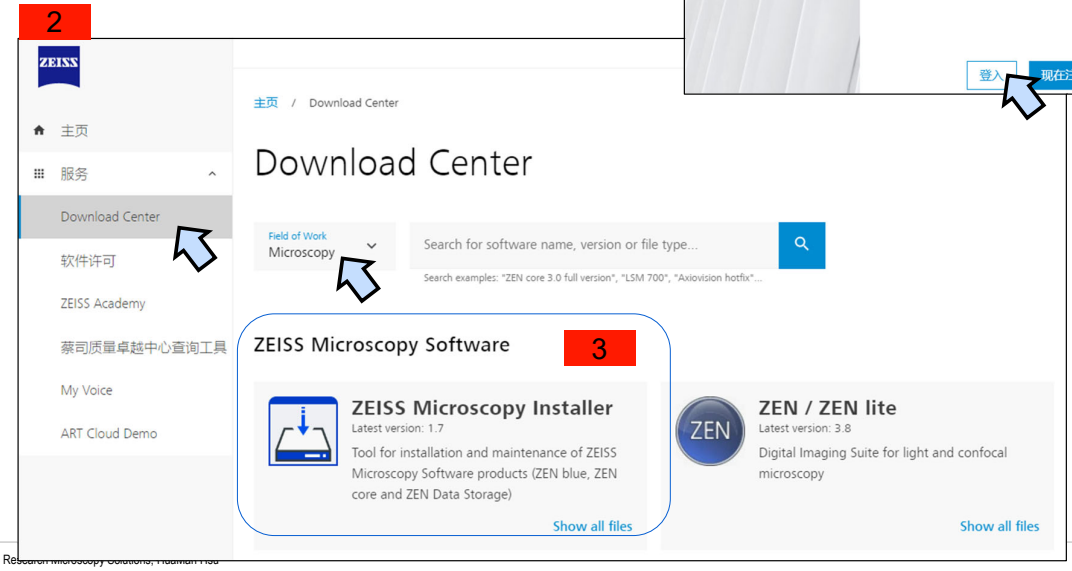
4

信箱收註冊確認信，沒有收到請製垃圾桶中找尋

下載離線軟體 ZEN lite 2

1

2



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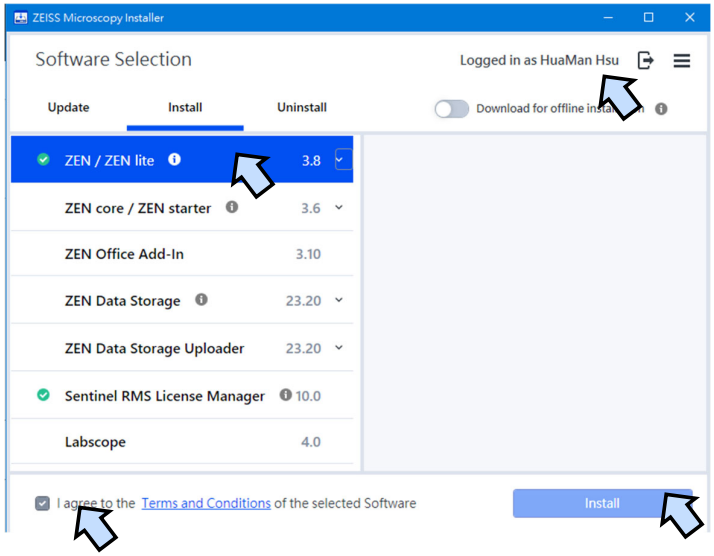
1

2



ZEN lite使用教學視頻

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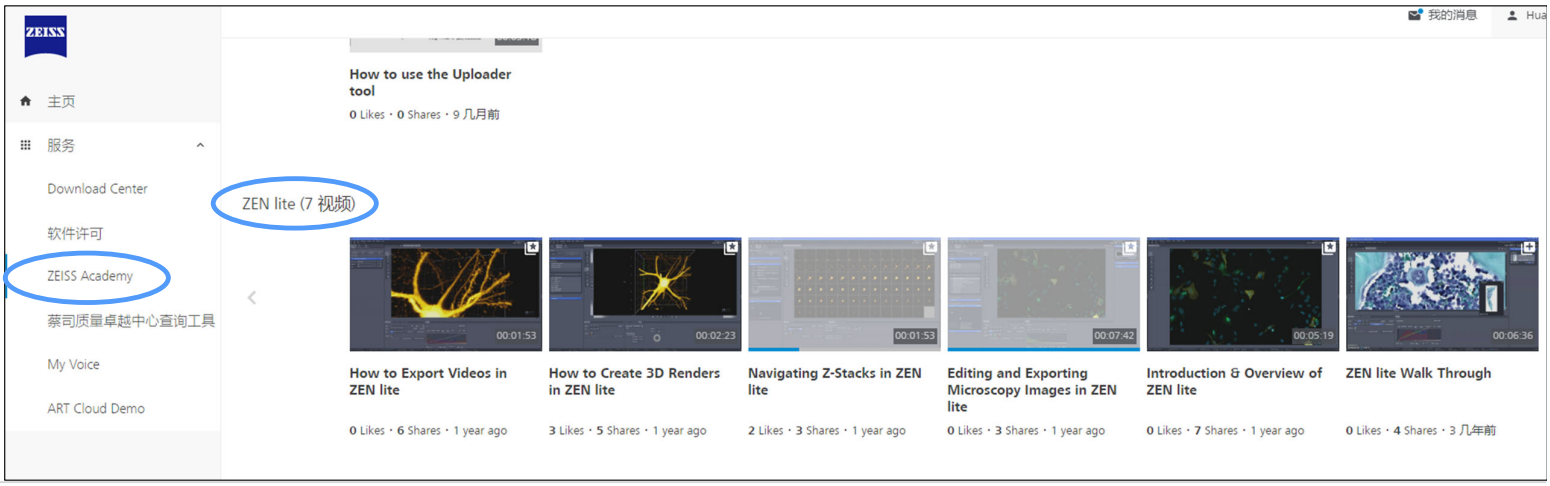

徐華蔓
Mobile/LINE : 0963 725 501
huaman.hsu@zeiss.com