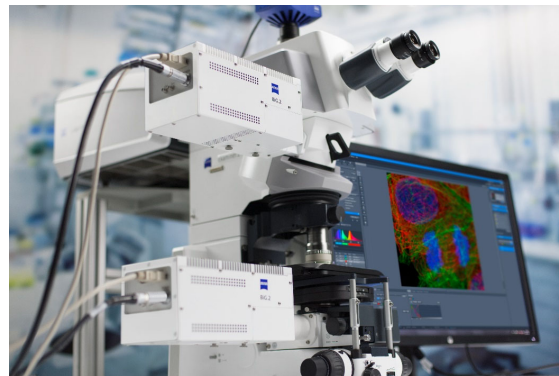
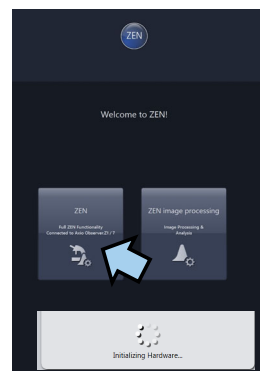
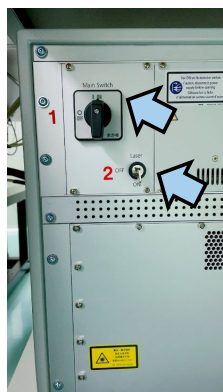




Carl Zeiss LSM 980 / ZEN Blue Quick Guide



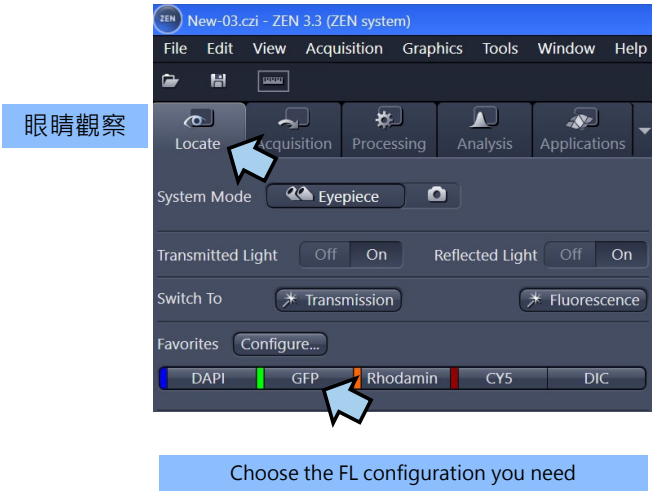
開機 System on



等待約三分鐘

1. Main Switch
2. Laser Key
3. Components
4. Computer
5. Multiphoton laser
key轉至on(水平)
6. ZEN Blue

目鏡下檢視樣品
Check sample under eyepiece



關機
System off



5. Multiphoton laser
key轉至stand by

4. Computer

3. Components

2. Laser Key

1. Main Switch

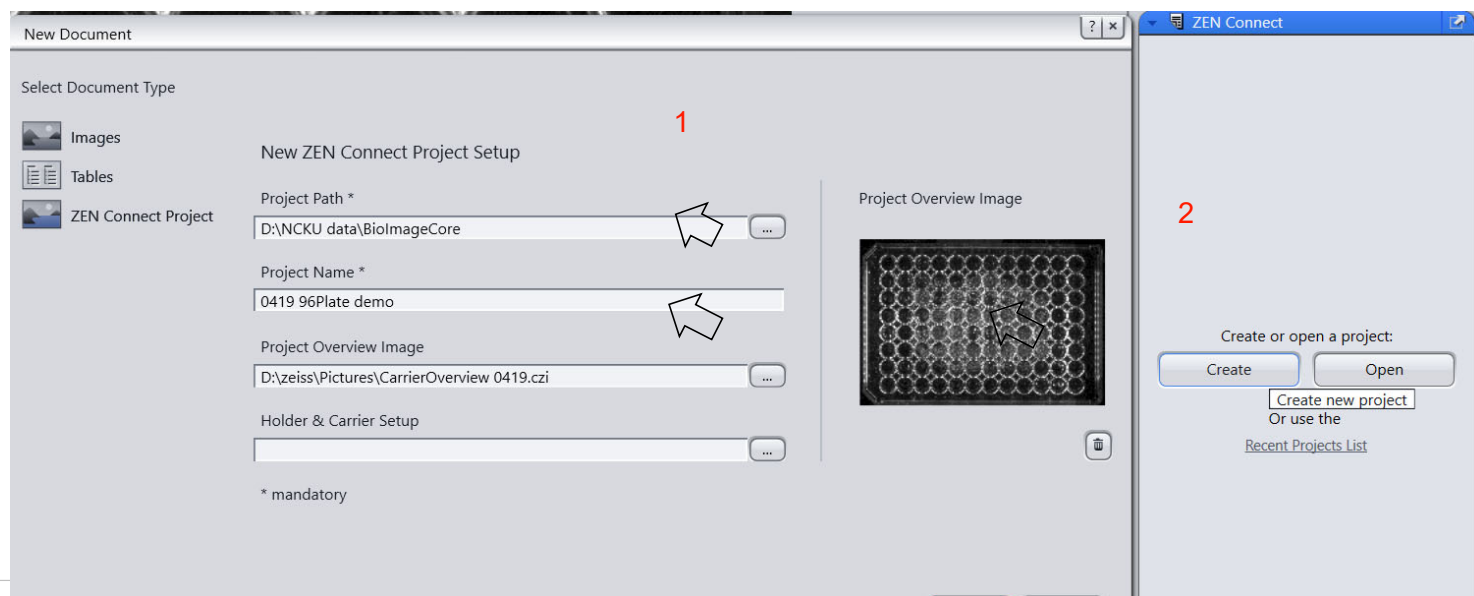


ZEN Connect (非必要) 同一Slide的檔案相互連結，自動儲存檔案座標位置



Create Project

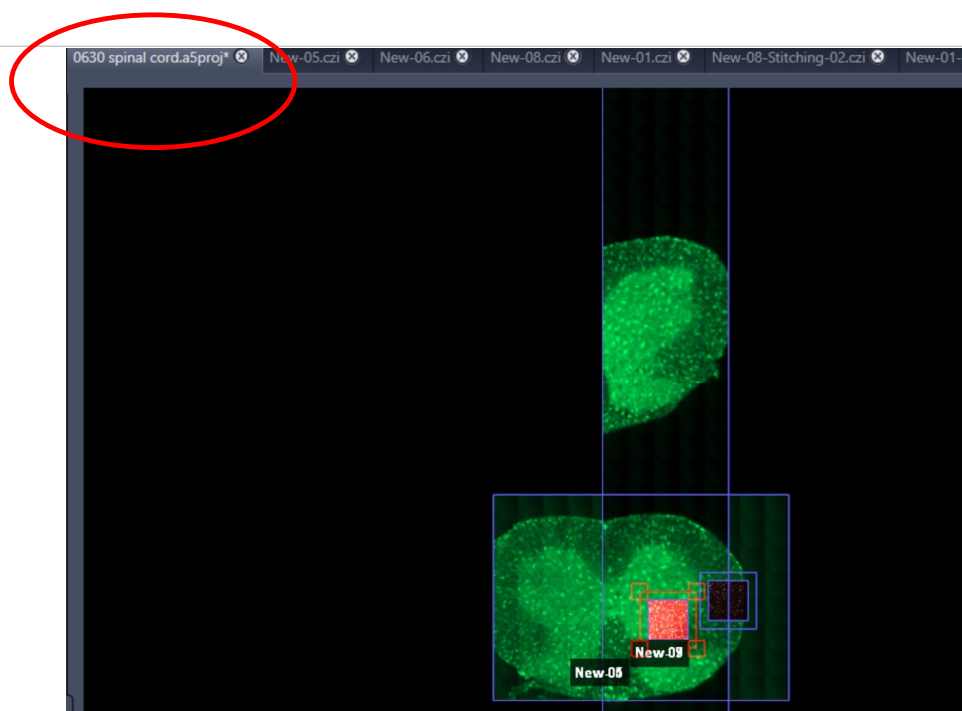
檔案管理工具，此為 非必要步驟



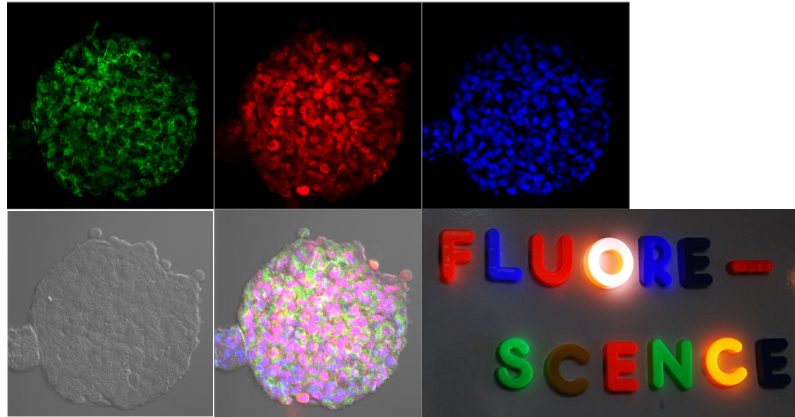
ZEN Connect 同一Slide的檔案相互連結，自動儲存檔案座標位置



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



Multichannel Image Acquisition

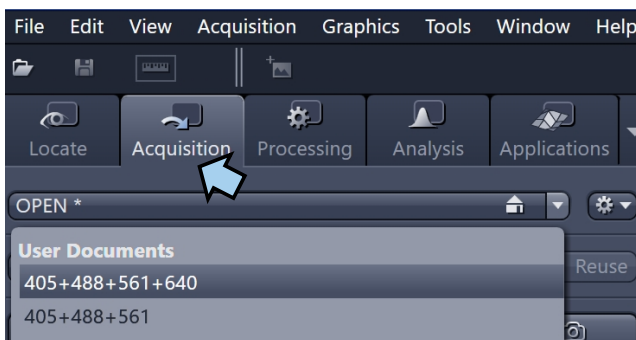


ZEISS Research Microscopy Solutions, HuaMan Hsu

Multichannel Image Acquisition 1 Load Experiment methods form Experiment Setup



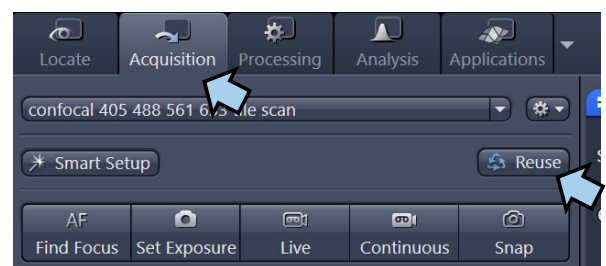
1.



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。

初學者不建議使用!!

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:

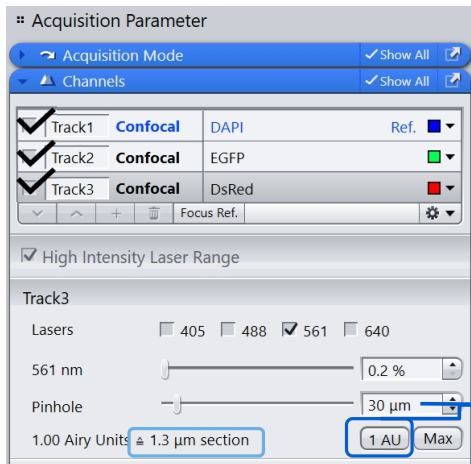
- Frame Size 256 or 512
- Scan Speed 8 or 7

Multichannel Image Acquisition 4 Acquisition Parameter Pinhole 設定



1.

2.



Optical Section
Thickness

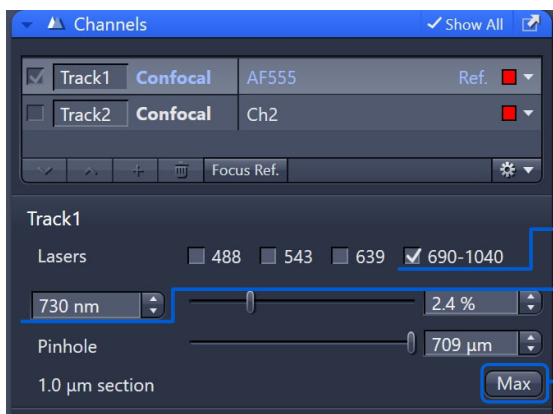
1. 所有Tracks打勾，Pinhole 所有channel 設定相同
2. 擇一Track作為運算依據，按1AU，或者依需求調整合適大小。

NLO專屬 Multichannel Image Acquisition 5 Acquisition Parameter multiphoton 作為激發光源 Pinhole 設定， use internal detector



1.

2.
確認



1. Select multiphoton laser as a light source

2. Assign the wavelength

3. Pinhole 全開



Beam path中MP laser 使用MBS 690 or MBS 760

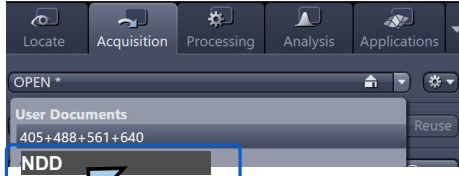
NLO專屬 Multichannel Image Acquisition 5

Acquisition Parameter

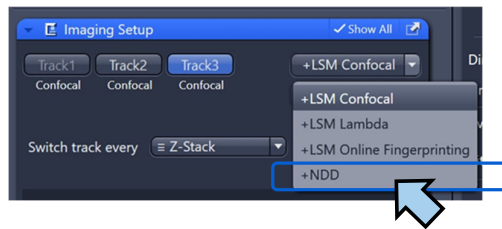
multiphoton 作為激發光源 NDD as detector



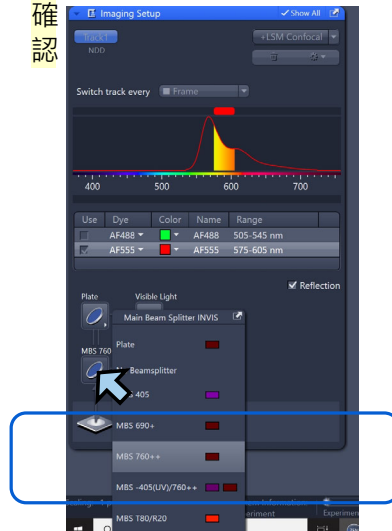
1.



OR



2.
確認



Beam path中MP laser 使用MBS 690 or MBS 760

3

正立顯微鏡 AxioExaminer



Multichannel Image Acquisition 5

Acquisition Parameter

為每個Track 設定Laser強度 與 感測器Master Gain



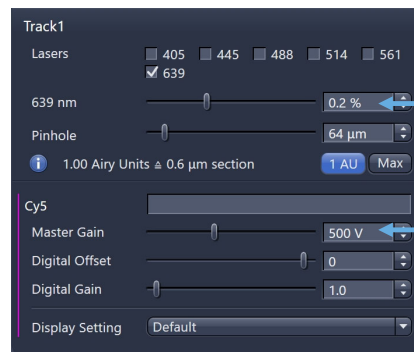
1

調整時，一次僅打勾一個Track



2

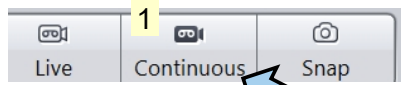
將各Track內的Laser 與Master Gain 設定完成



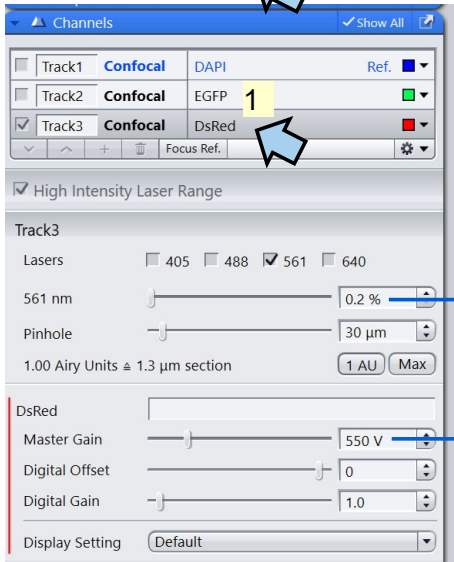
Multichannel Image Acquisition 6

Acquisition Parameter

為每個Track 設定Laser強度 與 感測器Master Gain



1. 對焦 · Continuous · 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



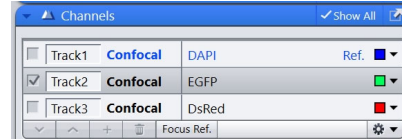
2

- 調整雷射強度
- 大部份情況下20%以內即足夠應付

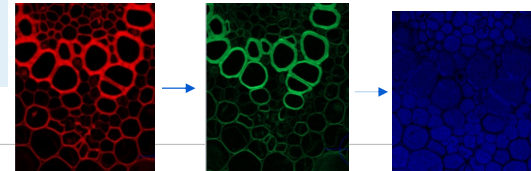
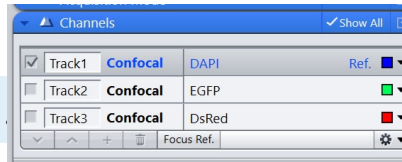
2

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

3.



3.



Multichannel Image Acquisition 7

2D Image

正式拍照: 提高Frame Size 降低Scan Speed

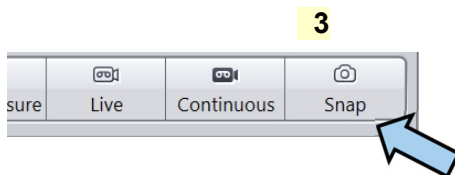


選取所有要準備要拍的track

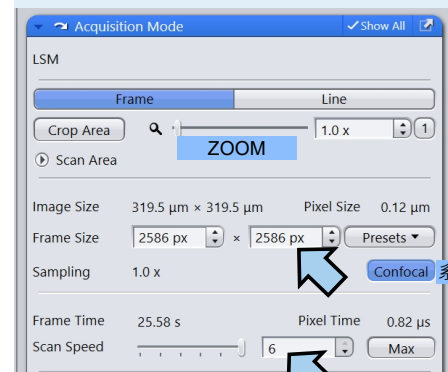
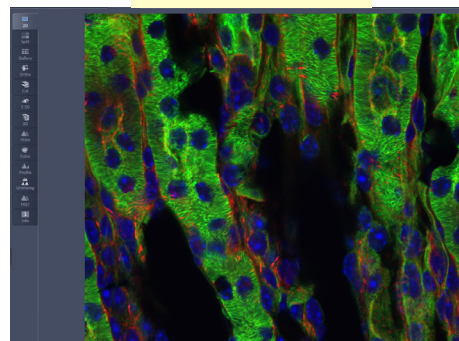
2

- 提高Frame Size (pixel number)、降低掃描速度，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

4 大功告成!!



3

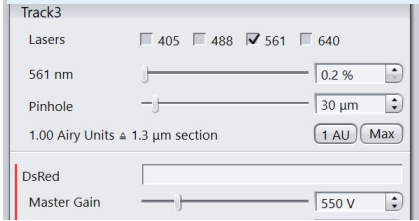


系統建議畫素

Multichannel Image Acquisition 8 3D Image - Z Stack Acquisition



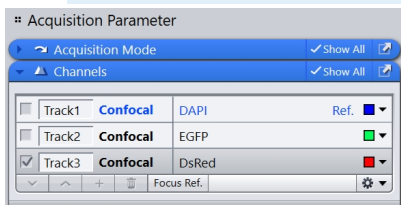
1. 當所有channel的laser 強度 與Master Gain皆已設置完畢



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

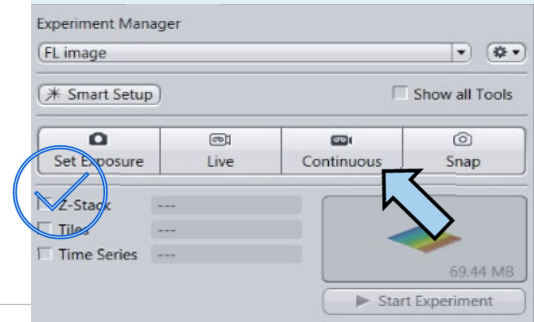


- 2 選擇一個Track



- 4

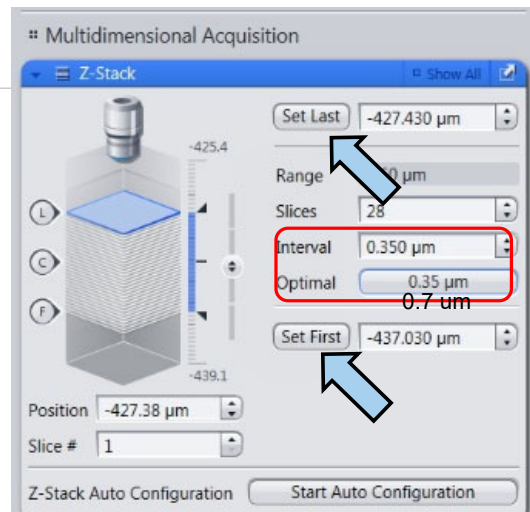
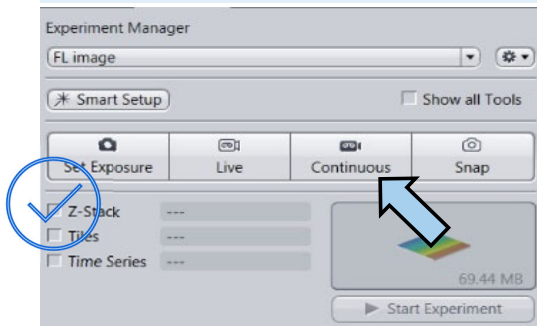
“Continuous” scanning under higher frame rate (ex: 512² @ speed 7)



Multichannel Image Acquisition 9 3D Image - Z Stack Acquisition



1. Continuous scanning under higher frame rate (ex: 512² @ speed 7)

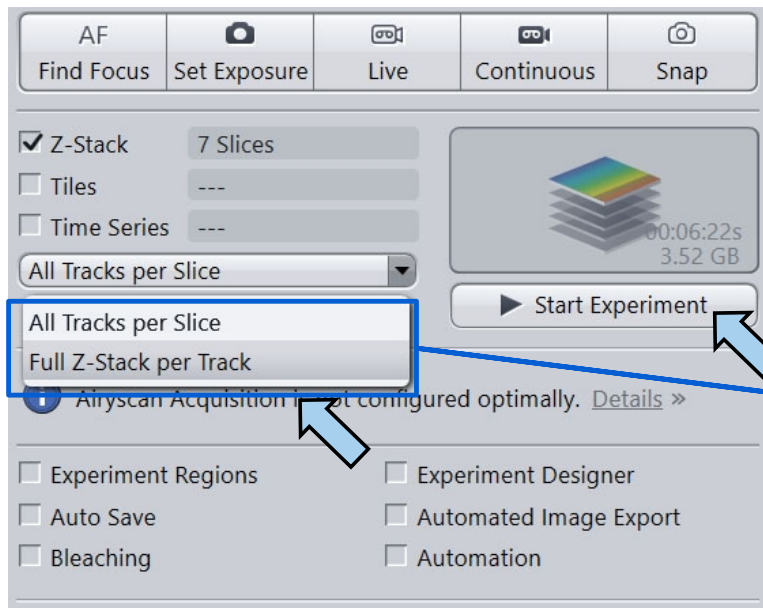


- interval可參考optimal
- 2x的optimal 0.35*2 = 0.7也很好，張數少，較省時
- 也可自訂定間隔

設訂 Z stack的上下界限

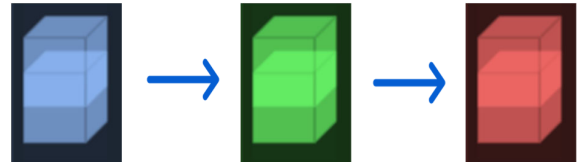
- 選擇一個Track02. > Continuous
- 搭配 Z stack視窗 > Z-stack
- 找到樣品焦距起點 > Set First
- 找到樣品焦距終點 > Set Last

Multichannel Image Acquisition 10 3D Image - Z Stack Acquisition

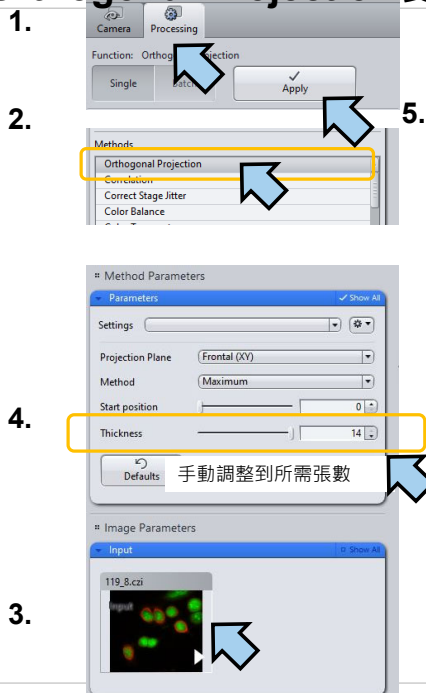


Full Z-Stack per Track:可避免硬體移動過於頻繁而降速

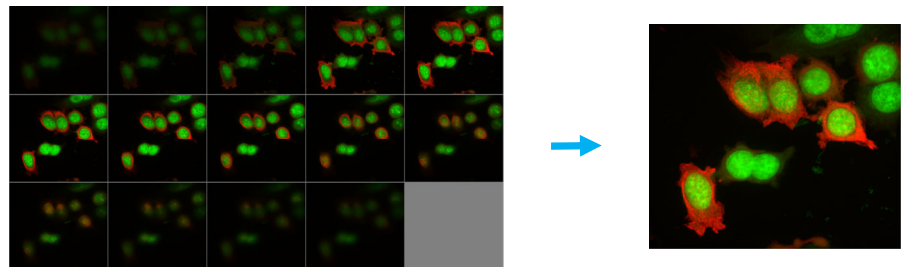
- 第一個Track的Z-Stack
- 第二個Track的Z Stack
- 第三個Track的Z Stack



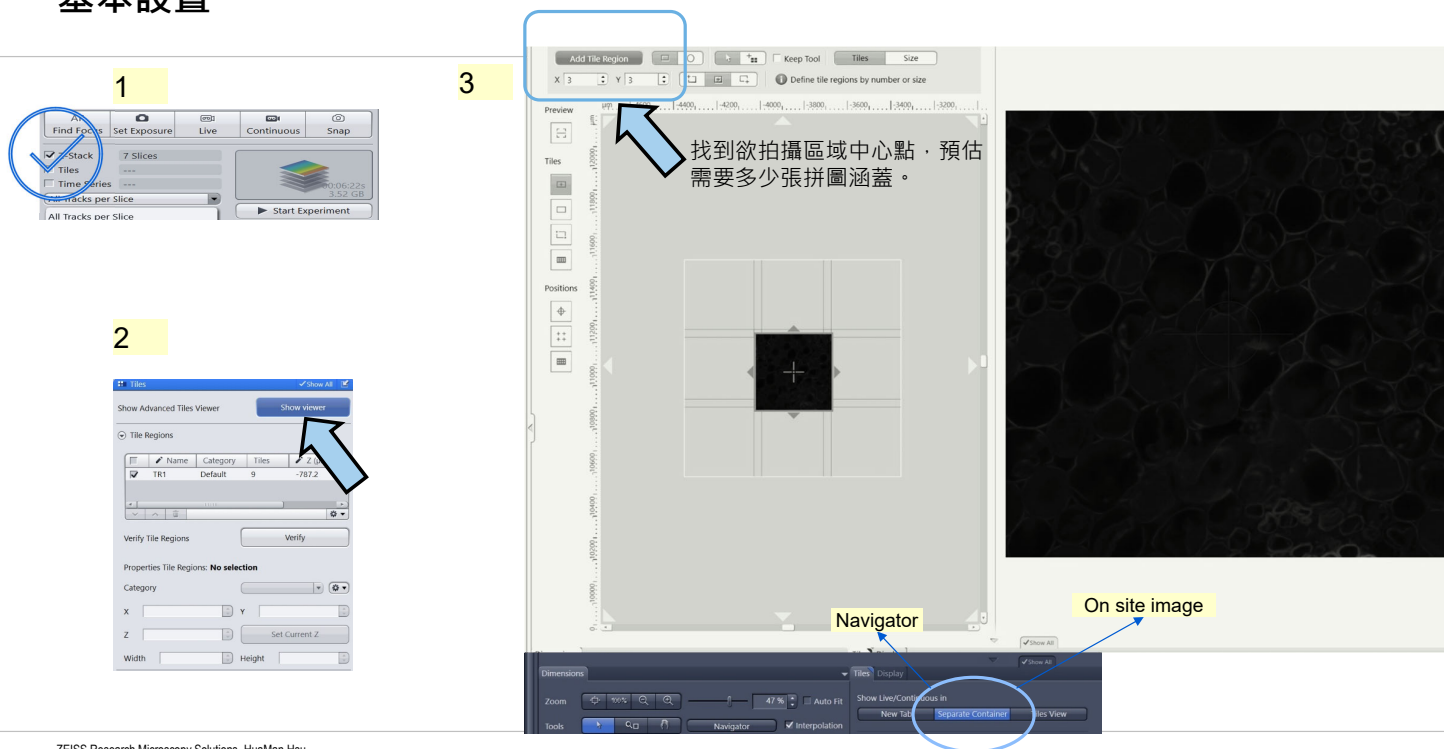
Multichannel Image Acquisition Z Stack Series Image Orthogonal Projection製造全景深影像:



Z stack: 把多張Z section疊成一張



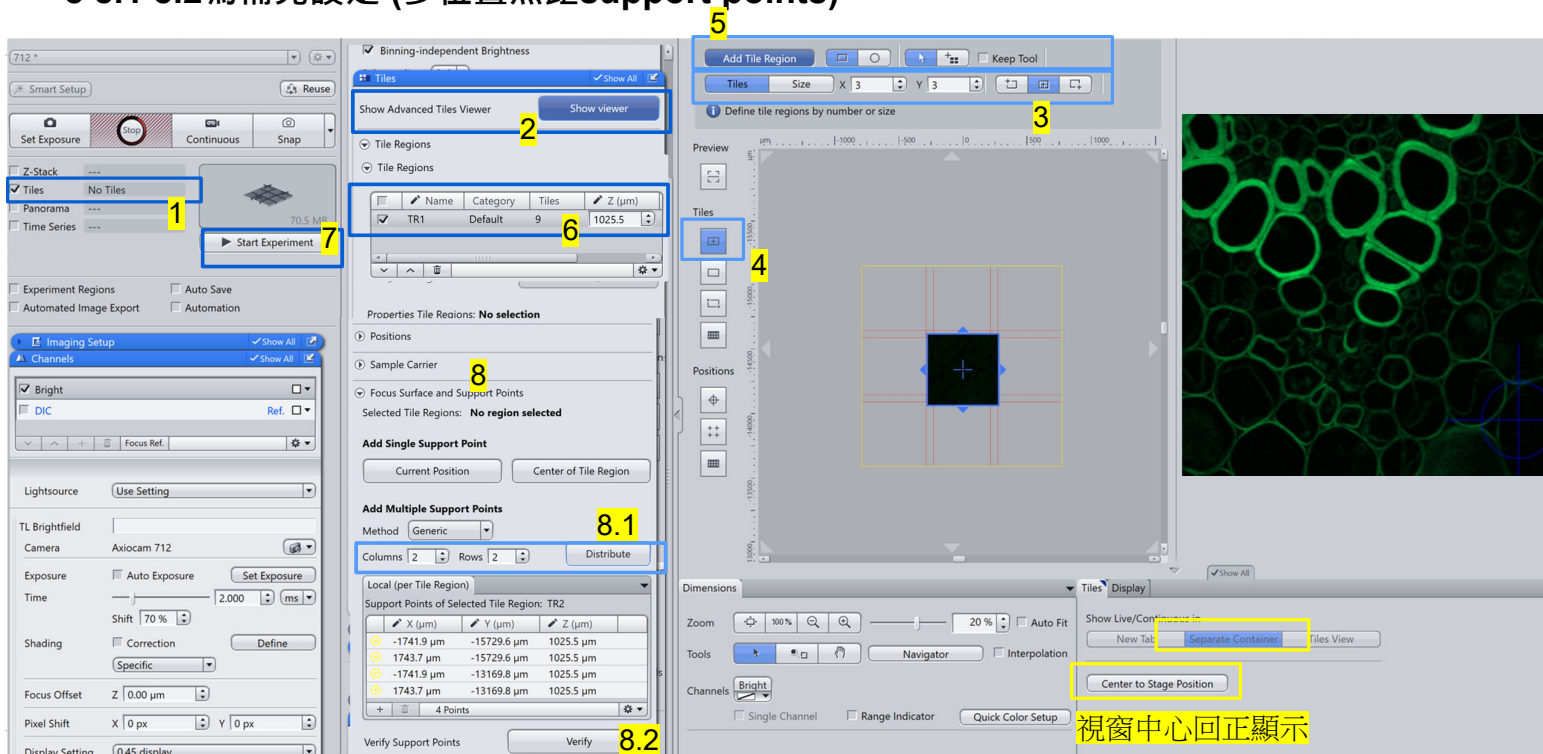
Tile Scan Imaging Setup 基本設置



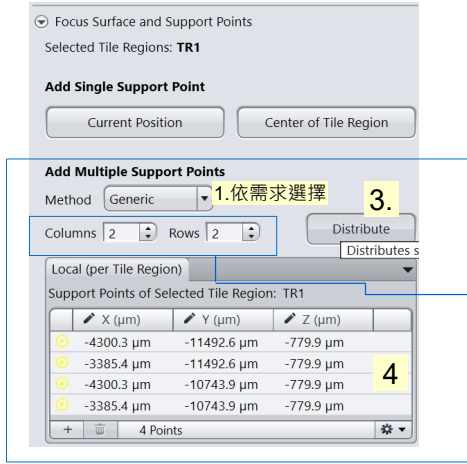
ZEISS Research Microscopy Solutions, HuaMan Hsu

21

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

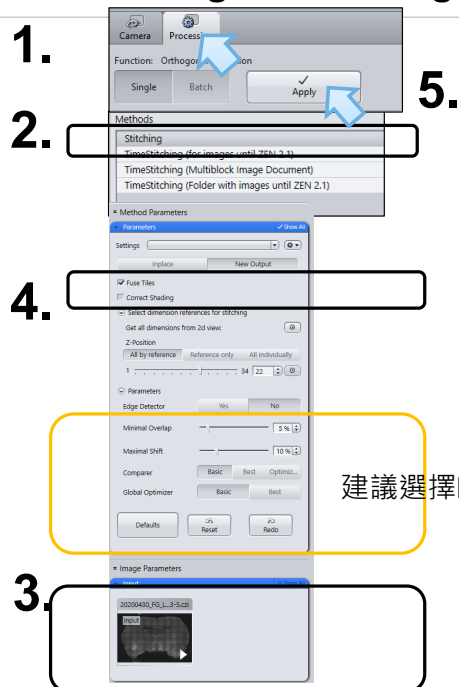


Tile Scan. 設置多位置焦點 Focus Map 多位置焦距Add support points

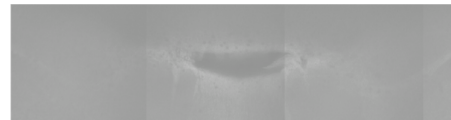


- 2.
- 依樣品狀況決定support points數量
 - 一般單純傾斜不必太多點，系統會自行計算焦點

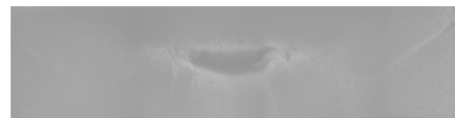
拼圖結果融合處理 Processing → Stitching / Fuse



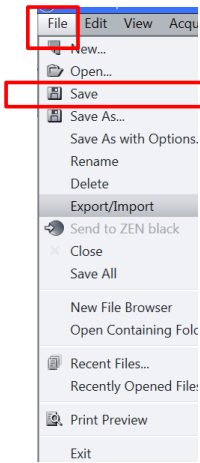
Before



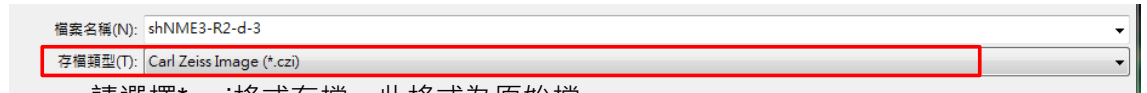
After



5



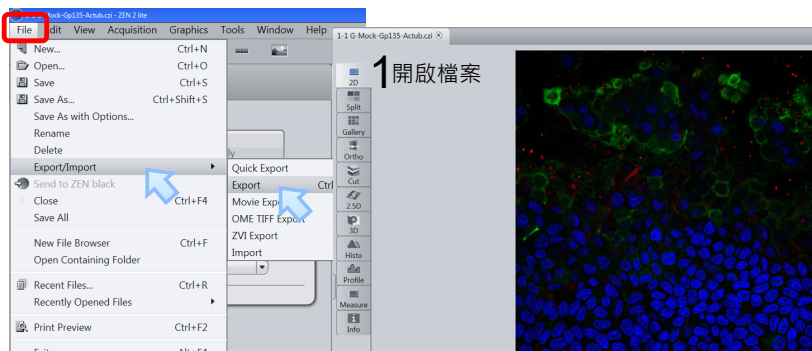
存檔



- 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

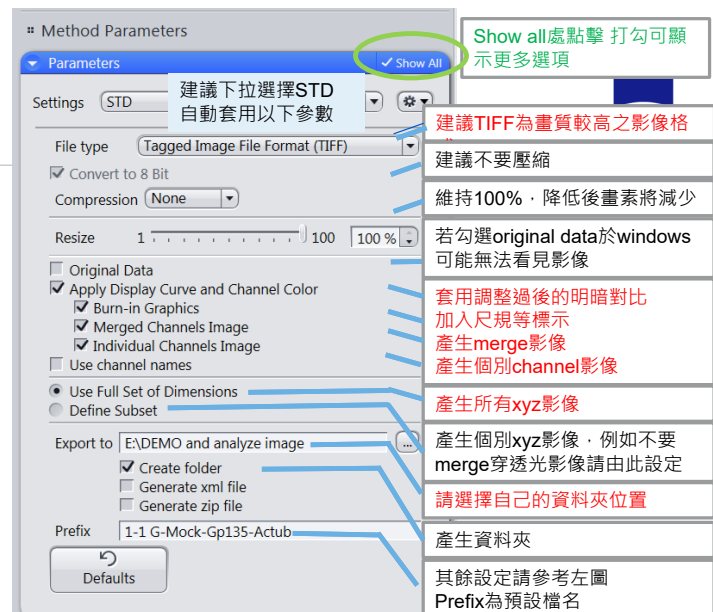
圖檔輸出export (單一檔案)

2



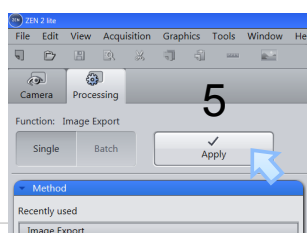
1開啟檔案

4



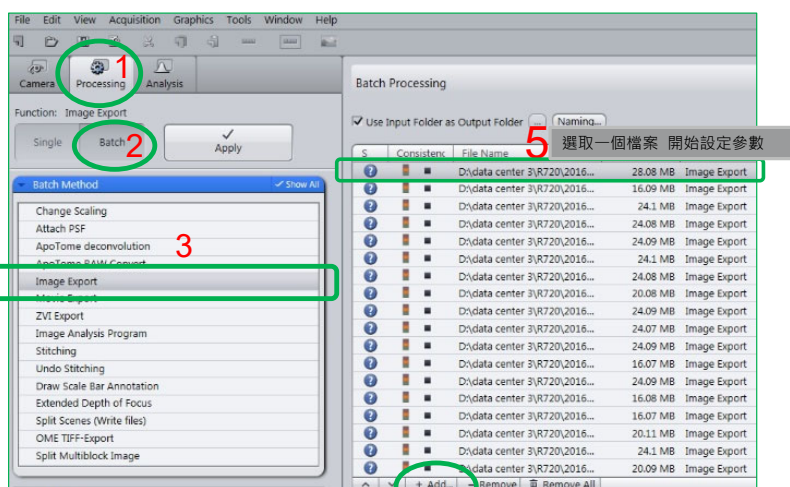
3

確認input 檔名是否為您所要輸出
的檔案

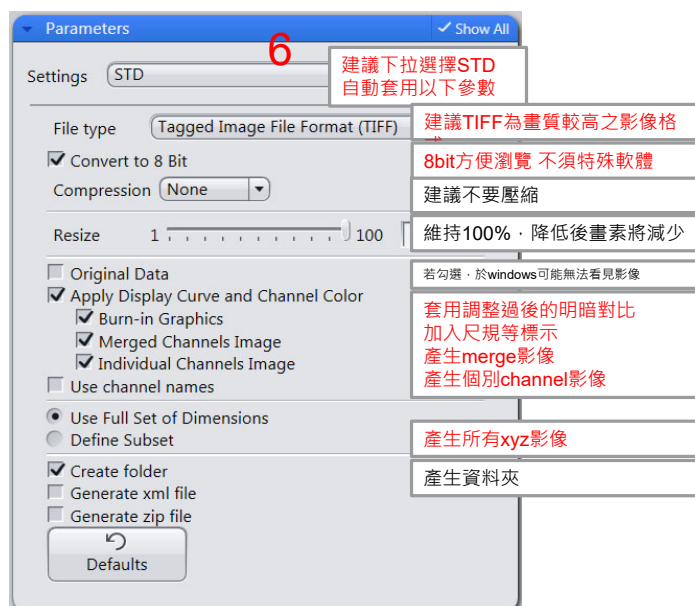


5

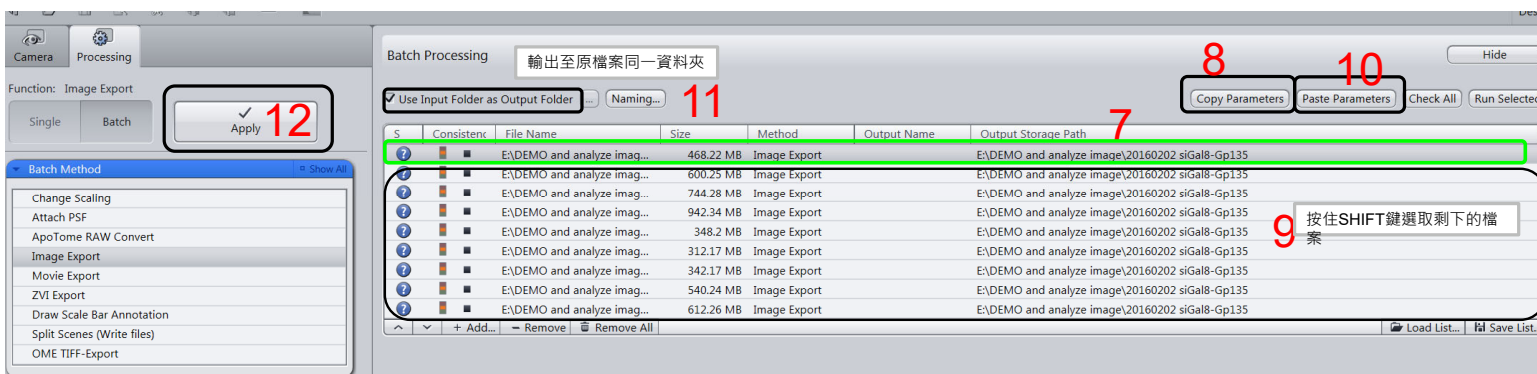
大量批次轉檔batch export 1



加入你所想要轉檔的項目



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.

3.

4.

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

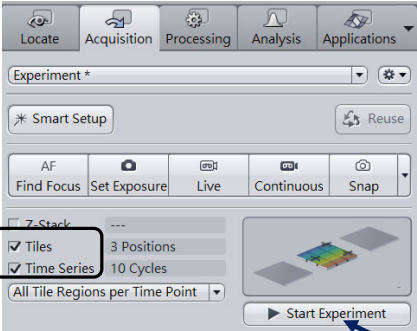
2.

3.

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

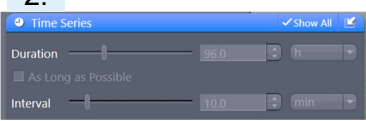
多位置長時間自動追焦 Multi-position Time Lapse Imaging 2. 適用於微小焦距差異設定



1. 

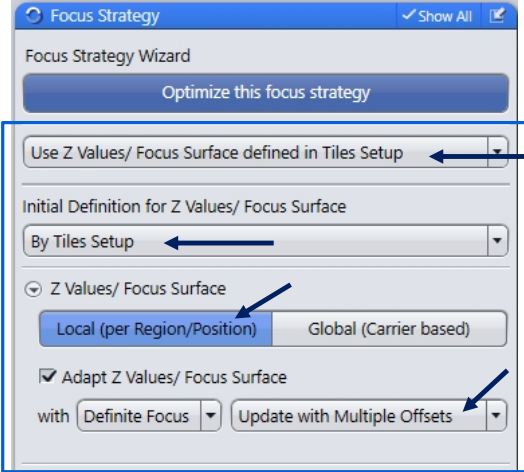
4. 

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

2. 

5. 

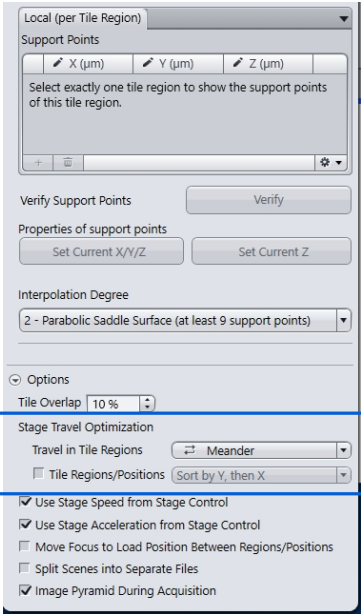
6. 

3. 

手動或自動確認焦距

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

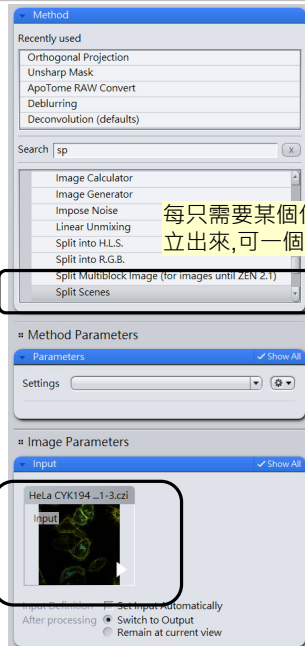




- 按照positions 順序拍攝請取消Meander!
- 否則位置拍攝順序會重新排序

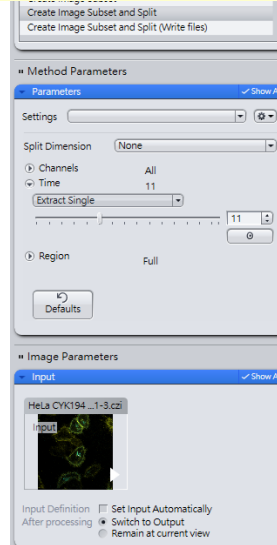
多位置拍攝長時間 依位置切割檔案

Processing → Split Scenes/ Create Subsets



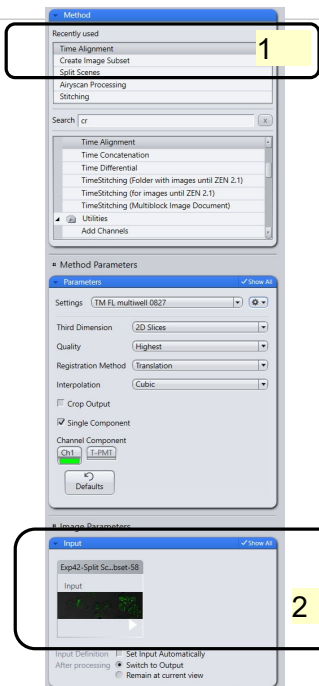
每只需要某個位置的結果獨立出來,可一個個分別存檔

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

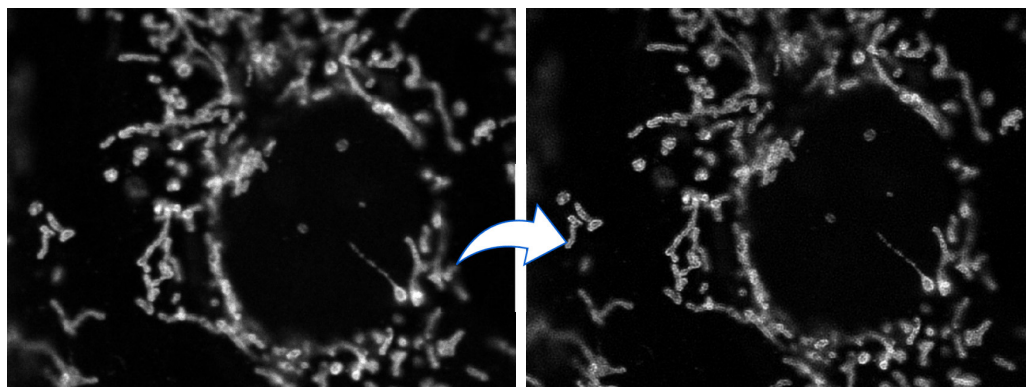
- 請放好樣品後對焦完畢，以黏土或工具固定樣品以免因載物台長時間多點移動造成樣品位移。
- 若合併多點(位置)拍攝此步驟請split scene切割完畢再執行

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



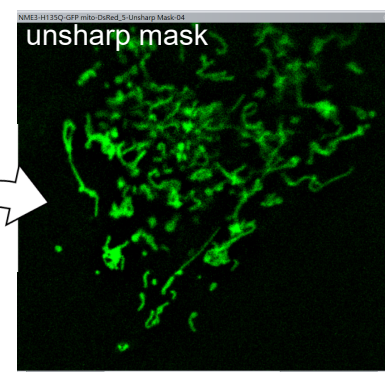
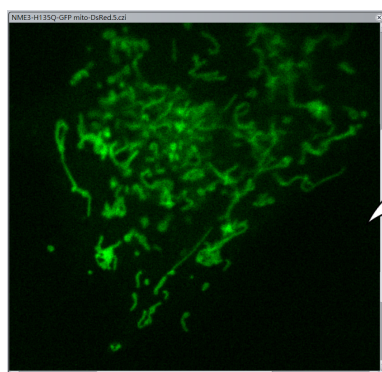
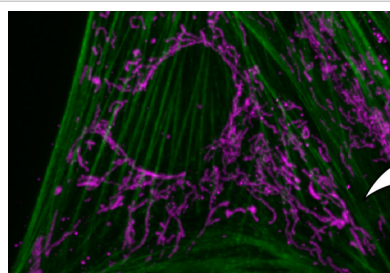
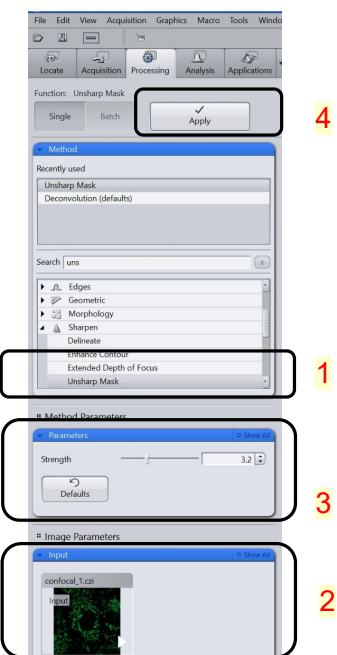
Before

After



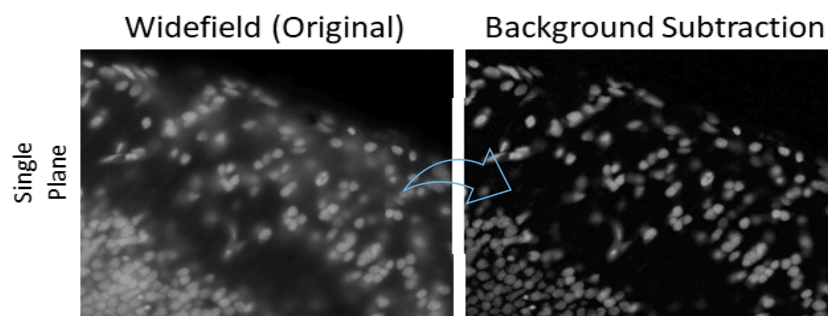
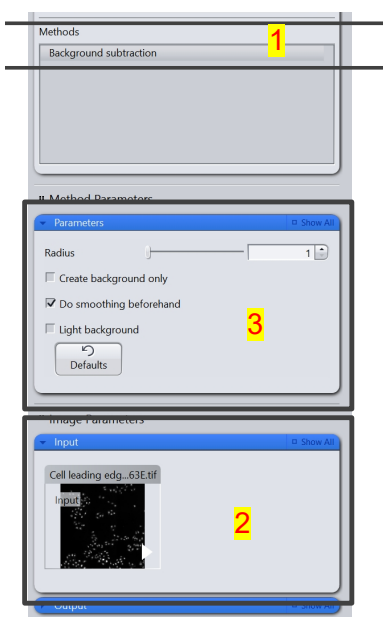
35

ZEN Image Processing Image Clearing Option 2: Unsharp masking

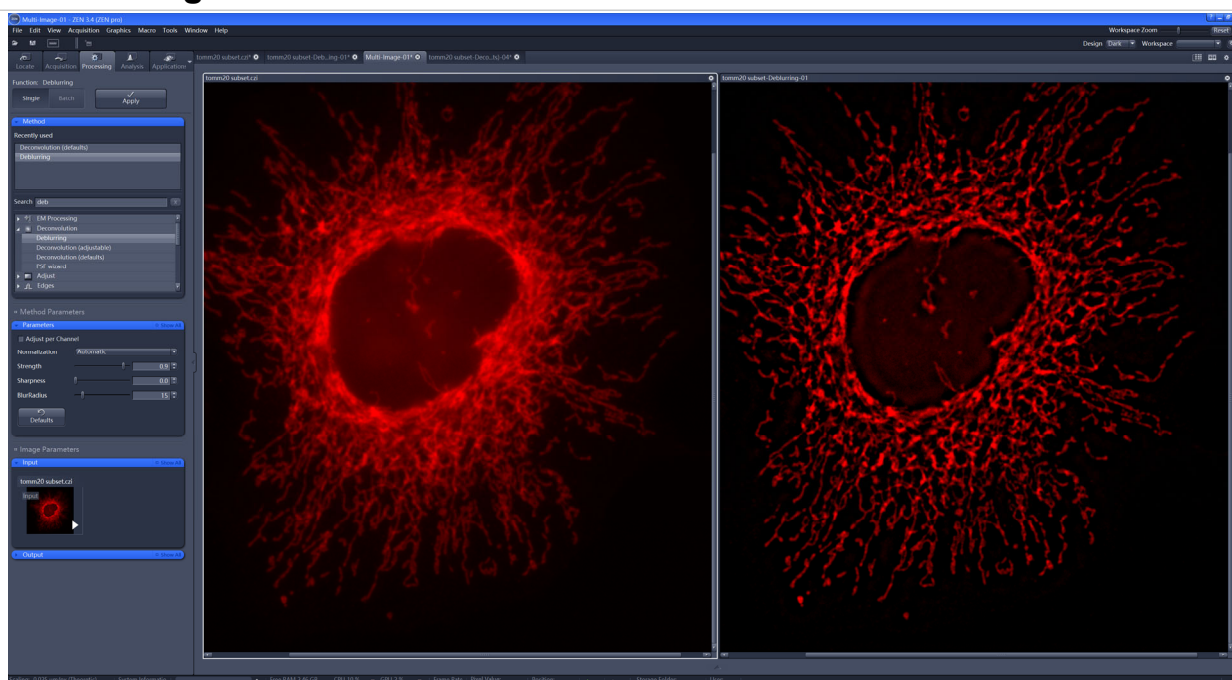


36

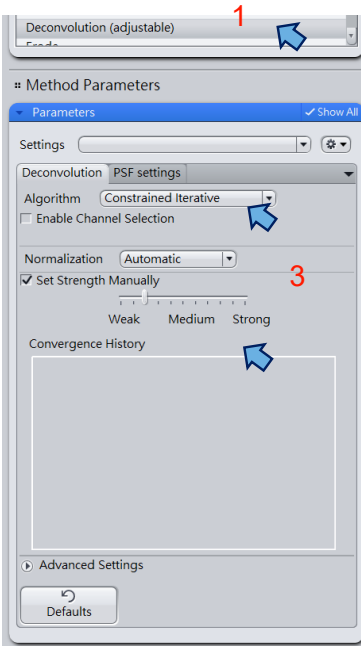
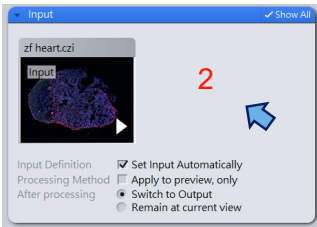
ZEN 3.3 Image Processing Image Clearing Option 3: Background subtraction



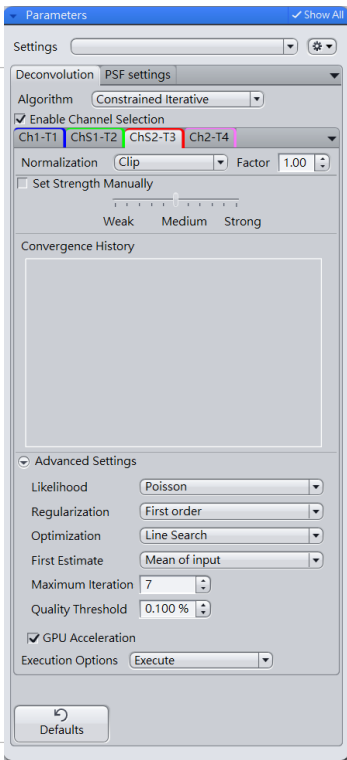
Option 4: ZEN 3.4 WF vs Deblurring/2D DCV



Deconvolution (adjustable)



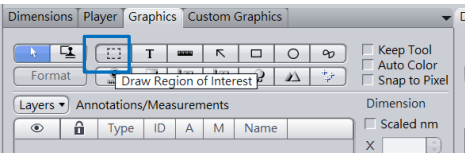
進階選項



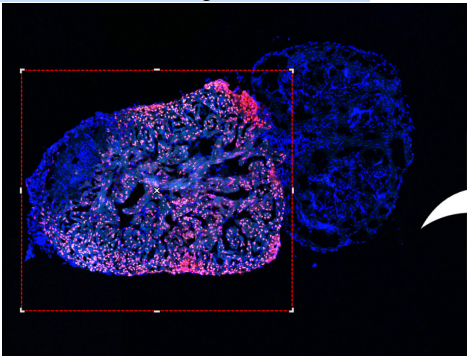
裁圖，複製選取範圍影像



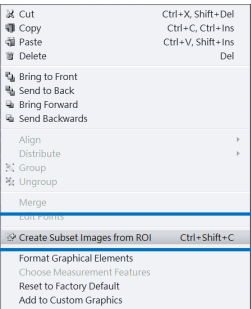
1.



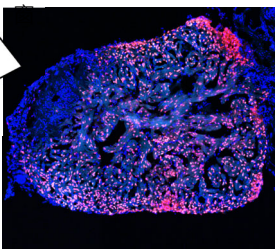
2. Draw an area and mouse right click



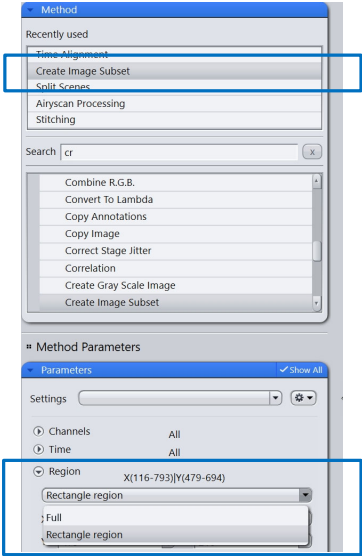
3. Create Subset Images from ROI



4. 獲得裁切範圍於新視



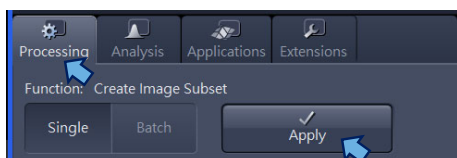
3. 或者
Processing → Create Image Subset



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



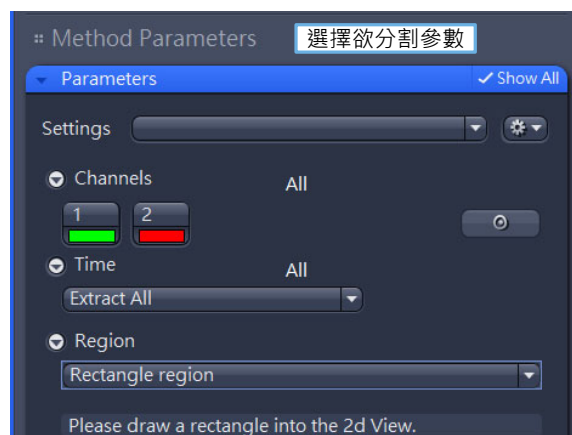
1



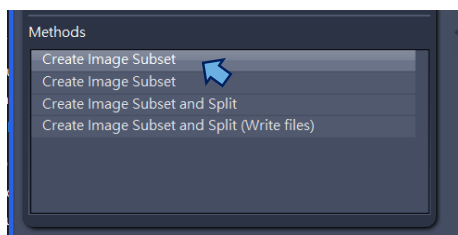
3



4



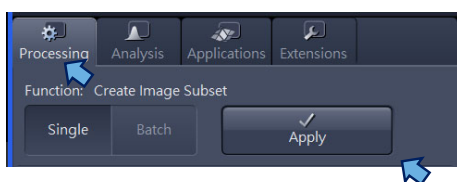
2



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



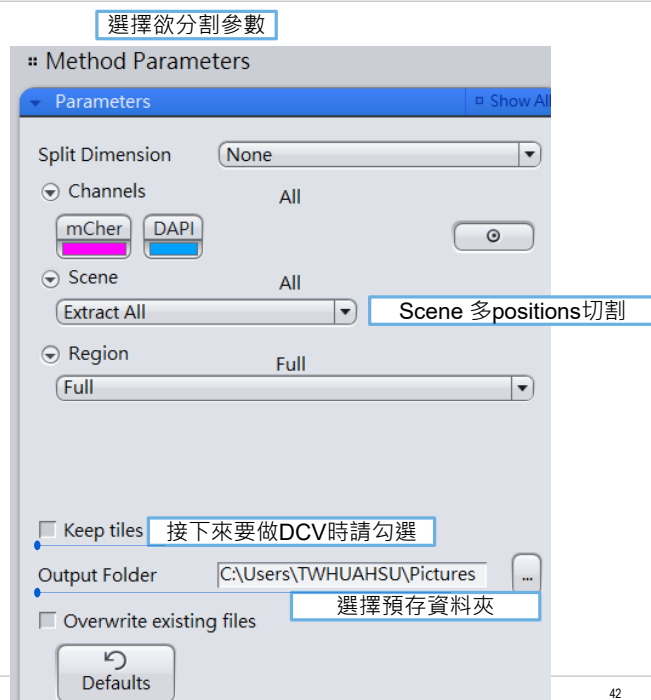
1



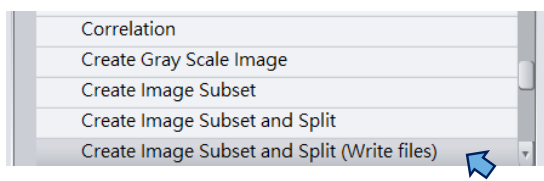
3



4



2



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



1

Processing Analysis Applications Extensions

Function: Create Image Subset

Single Batch Apply

2

Split into H.L.S.
Split into R.G.B.
Split Multiblock Image (for images until ZEN 2.1)
Split Scenes
Split Scenes (Write files)

3

Input

CFP-YFP-ble...-02.czi

Input

選擇欲分析的圖檔

4

選擇目的資料夾

Method Parameters

Parameters Show All

Settings

Output Folder C:\Users\TWHUAHSU\Pictures

☒ Include Scene Information in Generated File Name

☐ Overwrite existing files

Compression Original

Defaults

5

Add Scale Bar加入尺規



1

2D
Split
Gallery
Ortho
Cut
2.5D
3D

2

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

更改線的颜色樣式

更改字的颜色大小

Dimension Graphics

Customize

Scale Bar

Format graphical elements

Zoom with Image

Line 3.41 Solid

Begin style Flat

End style Flat

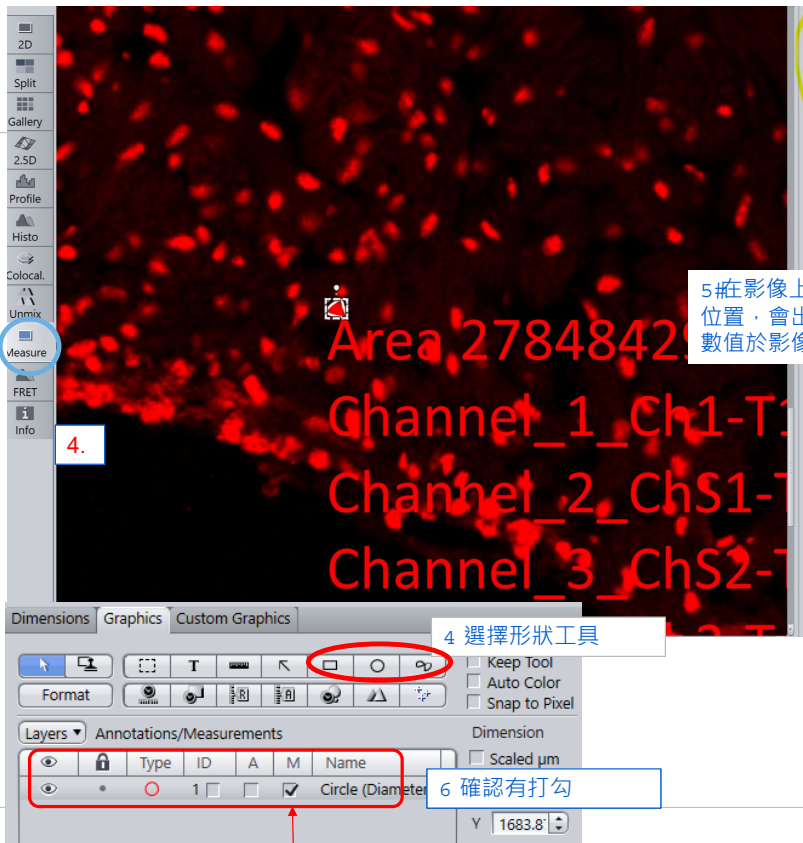
Text Calibri, 26pt Horizontal Align Center

Font Calibri

Size 26 pt

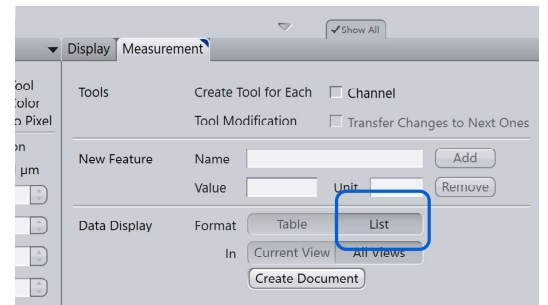
Opacity

量測area & intensity

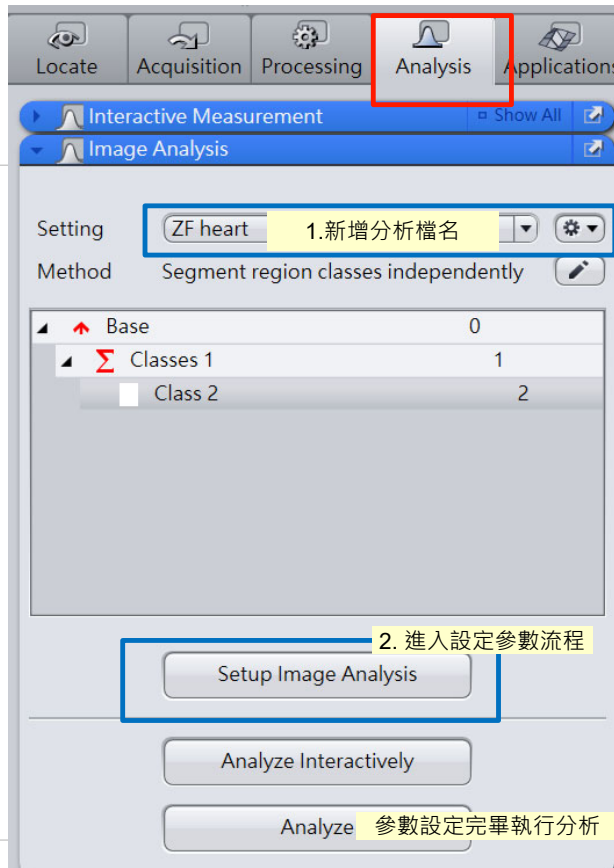


7. 圖表位於右方視窗

	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	

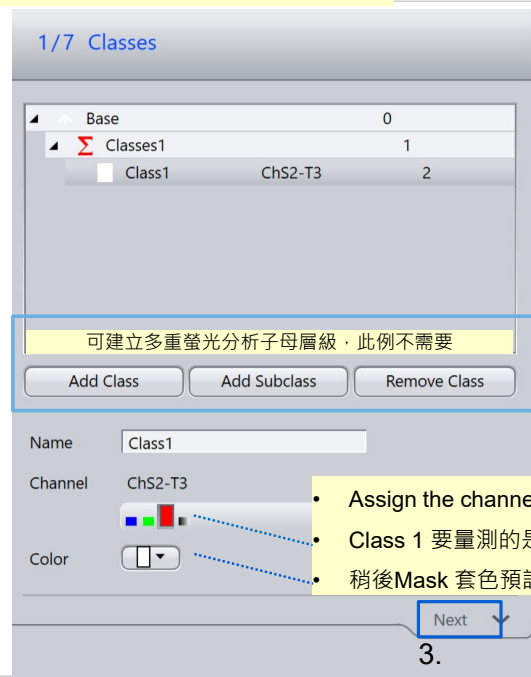


45



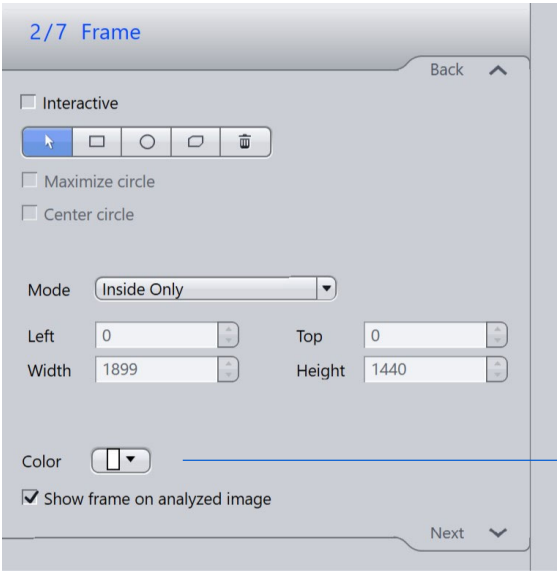
自動量測 Image Analysis Module 1.

以量測前頁影像內的紅色細胞核數目為例



46

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.



3/7 Automatic Segmentation

☒ Execute ☒ Interactive

Base 0

Classes 1 1

Class 2 ChS2-T3 2

Segment by glob

Smoothing Median

Size 3

Sharpen Unsharp Masking

Strength 1.4

Threshold

Histogram

Click Automatic

Method Otsu Threshold (Light Regions)

Minimum Area 11

Min. Hole Area 1

Fill Holes On Off

Binary None

Separate Watersheds

Count 20

Suppress Invalid On Off

Next

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

設定閾值，數值可參考前頁量測方式所得的Iqwhqv1w 或Dxwvp dwif

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+vp rrvk/#kcdushq/#ljh,

有些小區塊未能滿意可以在Dund設定

後續步驟進行uhj lrg#lbn有更多參數篩選去除不要的位置

區域分割 融合 演算

區域切割 搭配count

Mask顯示輔助

Analysis

Show Objects ☒ Fill ☒ Opacity

Show All Classes

Base 0

Classes1 1

Class1 ChS2-T3 2

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



4/7 Region Filter

Back

☒ Execute ☒ Interactive

Base 0

Classes 1 1

Class 2 ChS2-T3 2

Define region filters for segmented object

Edit Copy to All

Name	Minimum	Maximum	And
Area	100000.0	95000000	And
Fibrelength	1.000	0.000	And
Intensity Me	3.000	255.000	And
Perimeter	3000.000	1000.000	

Undo Redo Reset

Next

n Filter Editor

Selected Features for Condition

Name	And
Area	And
Fibrelength	And
Intensity Mean Value of channel 'Ch2-T4	And
Perimeter	

Search Features

Icon	Name
	Image Index Z
	Index
	Intensity Maximum of channel 'Ch1-T1'
	Intensity Maximum of channel 'Ch2-T4'
	Intensity Maximum of channel 'ChS1-T2'
	Intensity Maximum of channel 'ChS2-T3'
	Intensity Mean Value of channel 'Ch1-T1'
	Intensity Mean Value of channel 'Ch2-T4'
	Intensity Mean Value of channel 'ChS1-T2'
	Intensity Mean Value of channel 'ChS2-T3'
	Intensity Minimum of channel 'Ch1-T1'
	Intensity Minimum of channel 'Ch2-T4'
	Intensity Minimum of channel 'ChS1-T2'
	Intensity Minimum of channel 'ChS2-T3'
	Intensity Range of channel 'Ch1-T1'
	Intensity Range of channel 'Ch2-T4'

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



5/7 Interactive Segmentation

Back

☒ Interactive

Base 0

Classes 1 1

Class 2 ChS2-T3 2

Edit Regions

Remove Remove All

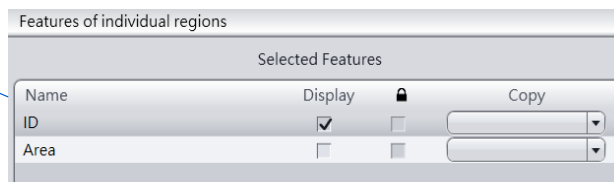
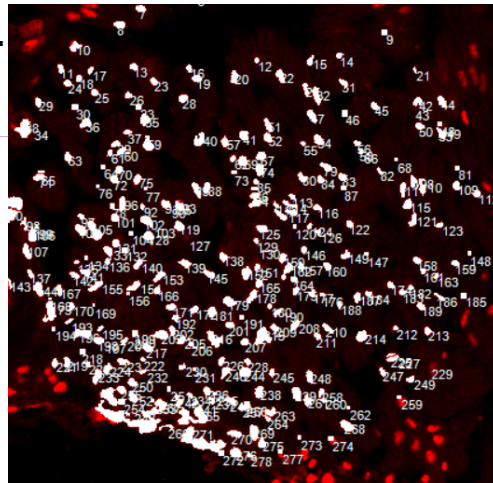
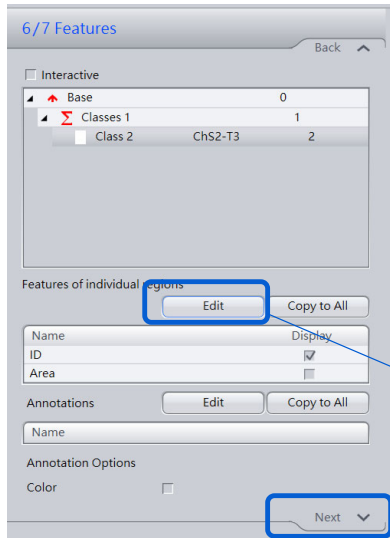
Region Growing

Post Processing

- 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

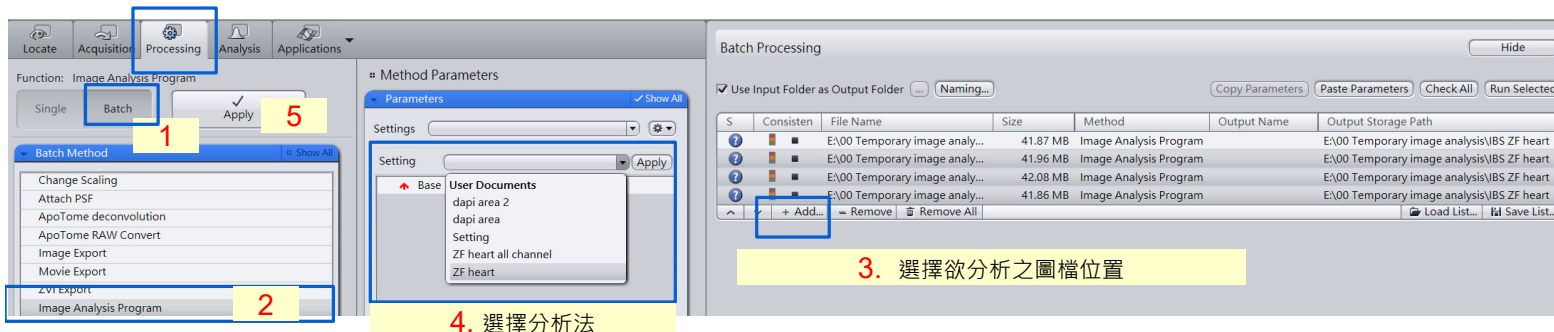


10	11	12,408,144.002
11	12	16,544,192.003
12	13	19,990,898.670
13	14	15,854,850.670
14	15	18,612,216.003
15	16	15,854,850.670
16	17	39,981,797.341
17	18	51,700,600.010
18	19	23,437,605.338
19	20	31,709,701.339
20	21	15,854,850.670
21	22	26,194,970.672
22	23	22,748,264.004
23	24	37,913,773.340
24	25	17,922,874.670
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

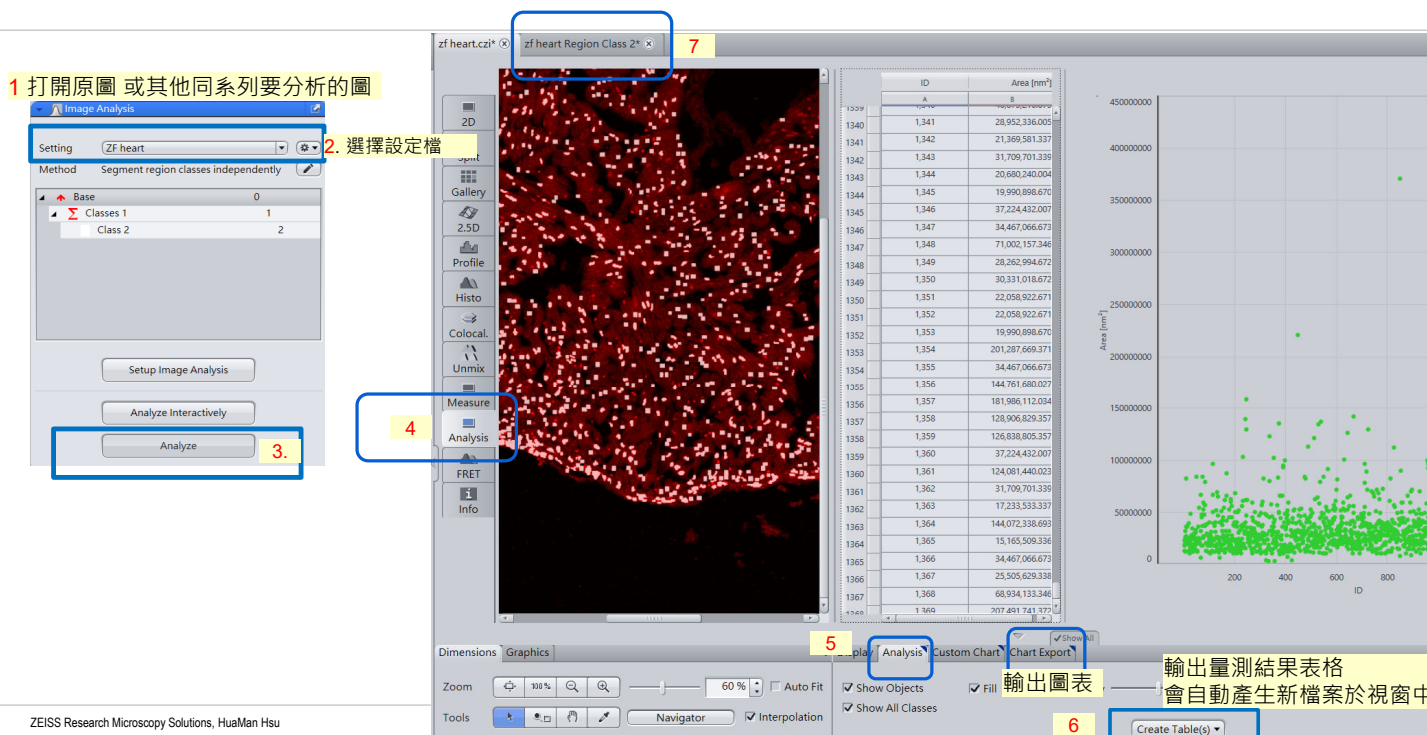
按下，儲存以上各步驟分析設定

- 結果預覽
- 此時尚無法輸出結果

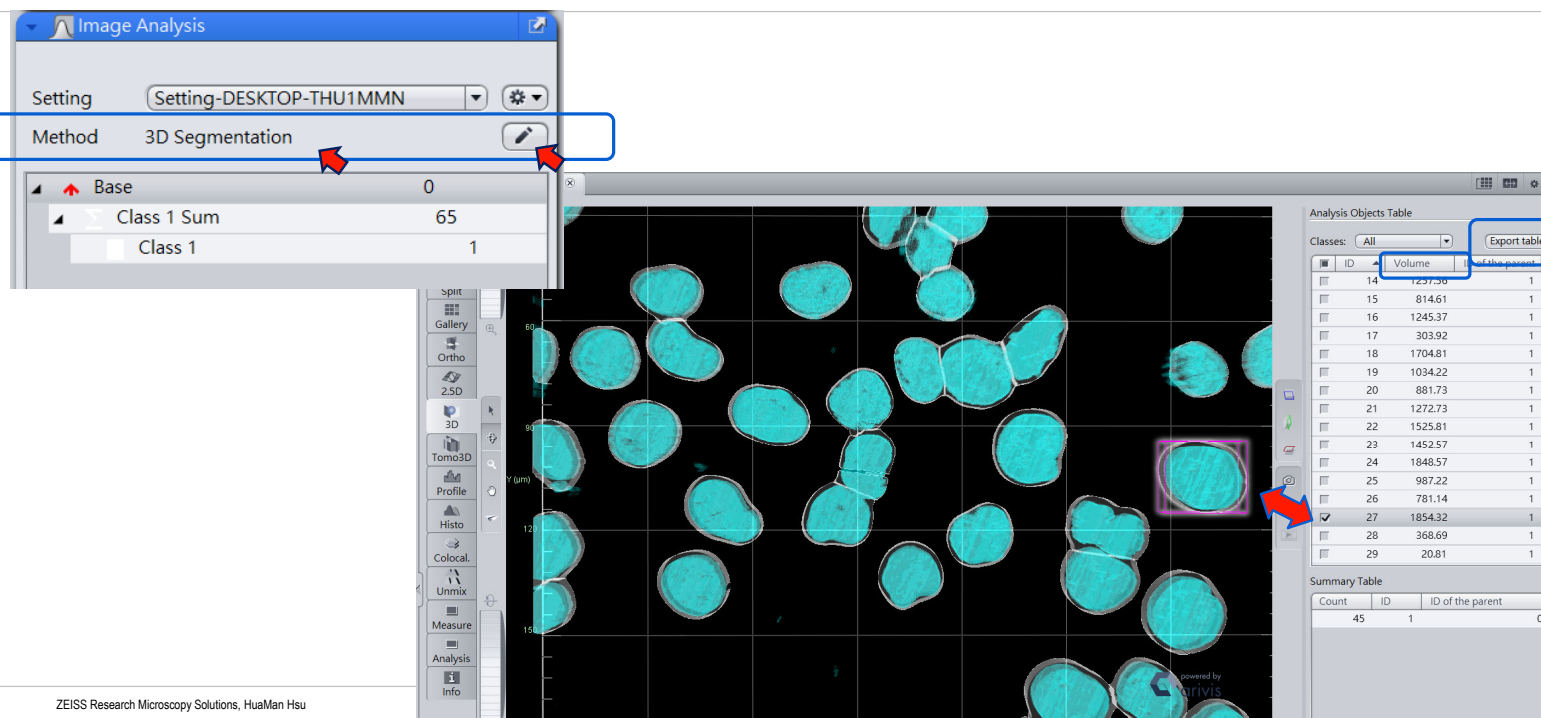
自動量測 Image Analysis Module 8. 大量分析Batch Analyze



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



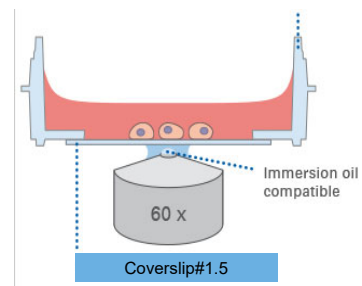
ZEN 3.5 3D Image Analysis



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



- **#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





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

Home	Incubation	Therm. Switch		
	H Insert P	Inc PM	Obj Heater	H Dev Humi
	37.5°C	32.0°C	39.0°C	33.3°C
Control	37.5°C	35.0°C	34.6°C	34.3°C
Automatic	Air			SensorT
	32.5°C			35.2°C
XYZ	34.0°C			
Incubation				
	C02	O2		
Display	6.5%	2.0%		
	7.0%	1.0%		



3. Components

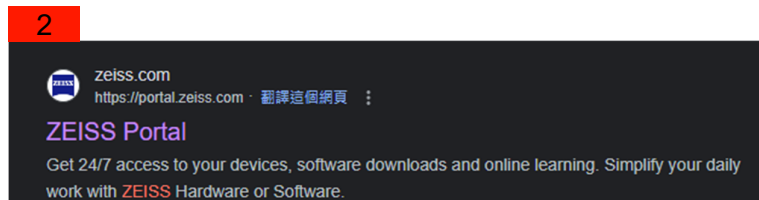


2. Laser Key

1. Main Switch

ZEN blue/lite Download

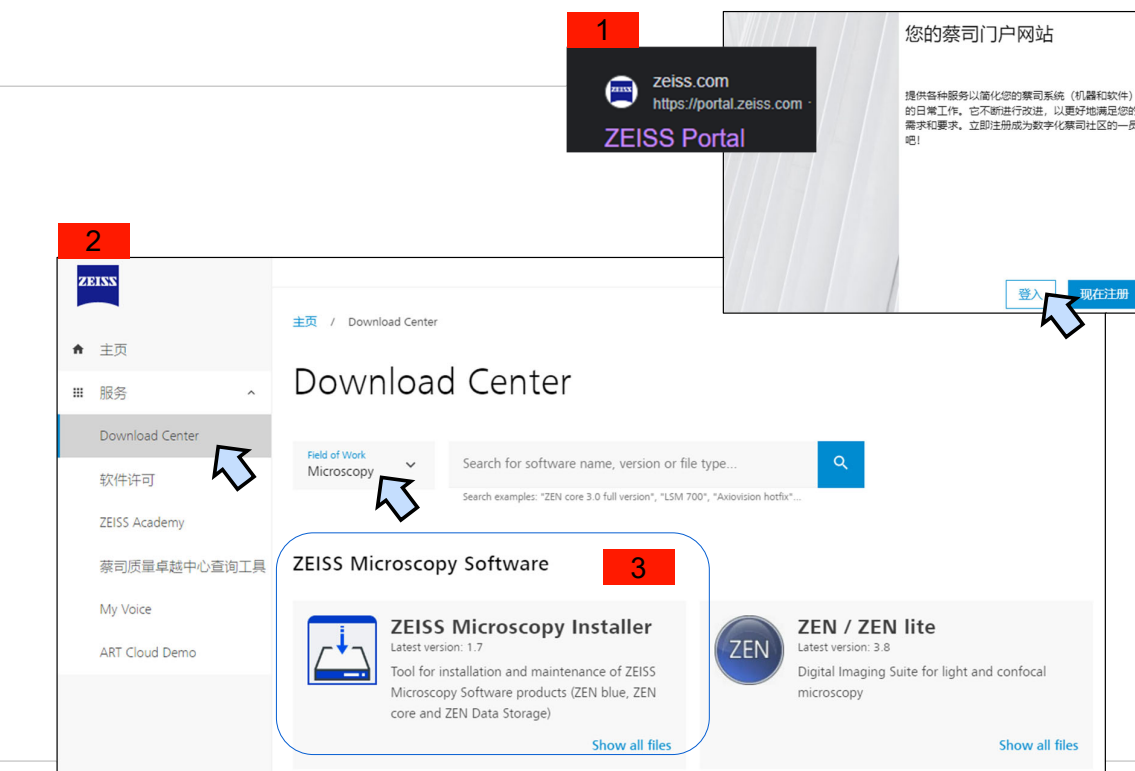
下載離線軟體 ZEN lite 1



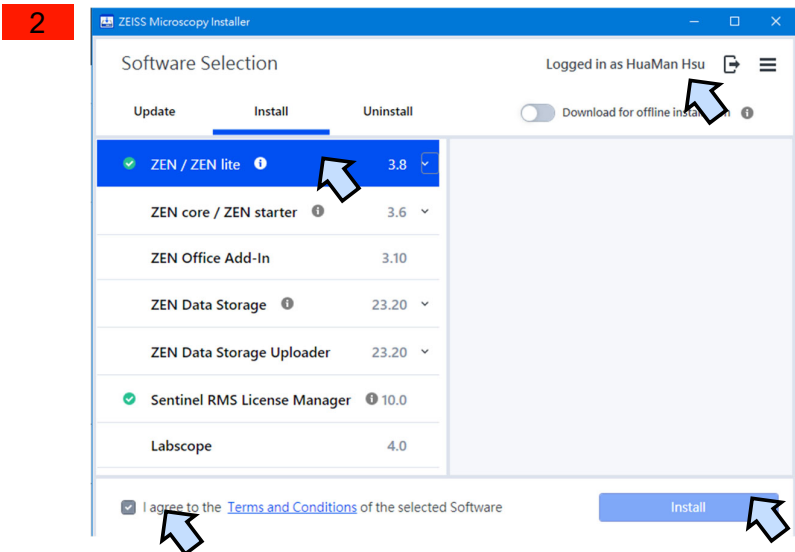
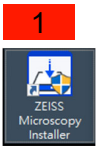
4

信箱收註冊確認信，沒有收到請製垃圾桶中找尋

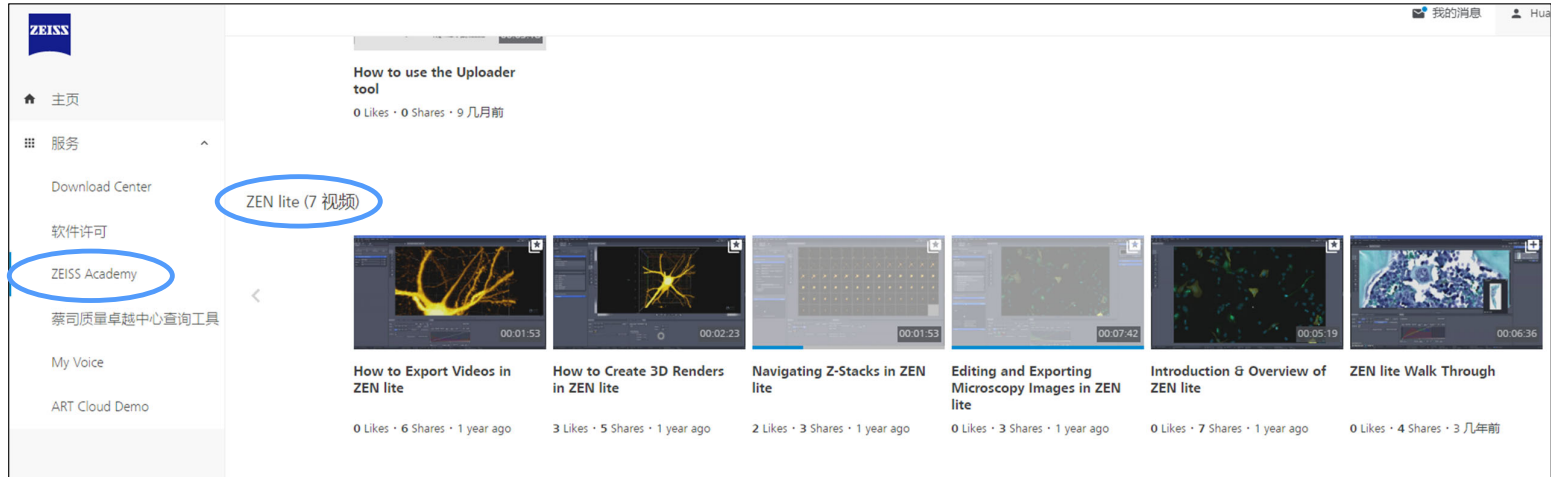
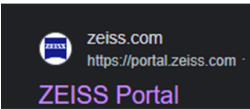
下載離線軟體 ZEN lite 2



下載離線軟體 ZEN lite 3



ZEN lite使用教學視頻





徐華蔓

Mobile/LINE : 0963 725 501

huaman.hsu@zeiss.com