



Carl Zeiss LSM 900 / ZEN Blue Quick Guide



• 開機	3
• 啟動軟體	4
• 螢光樣品拍攝	5
• Z-stack與疊圖	11
• 大面積拼圖	15
• 存/轉檔	20
• 長時間多位置拍攝	24
• 加入尺規...等標記	29
• 簡易自動量測與影像處理	31
• 穿透光影像拍攝	45
• DIC觀察設置	46
• 活細胞建議dish and plate	48
• 關機	49



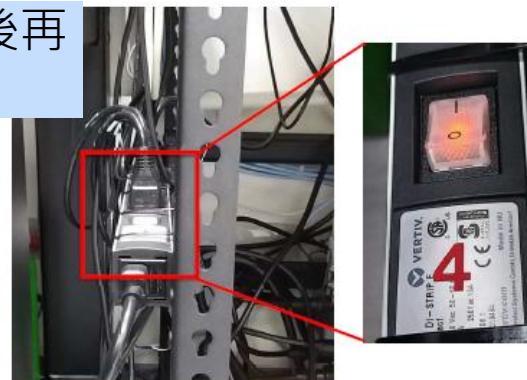
ZEISS LSM 900 with Airyscan 2

Start system



開機順序：1→6
關機順序：6→1

為維護雷射使用壽命，若兩小時內有下一位使用者，
請不要將系統關閉，只需清理物鏡與環境



6



1

進入ZEN BLUE



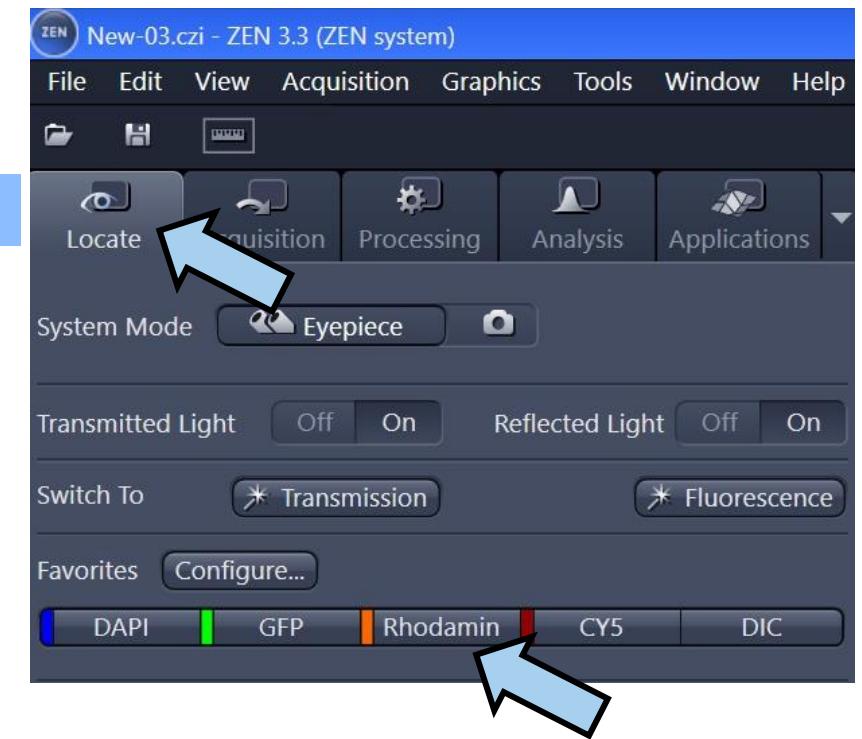
2



等待約一分鐘

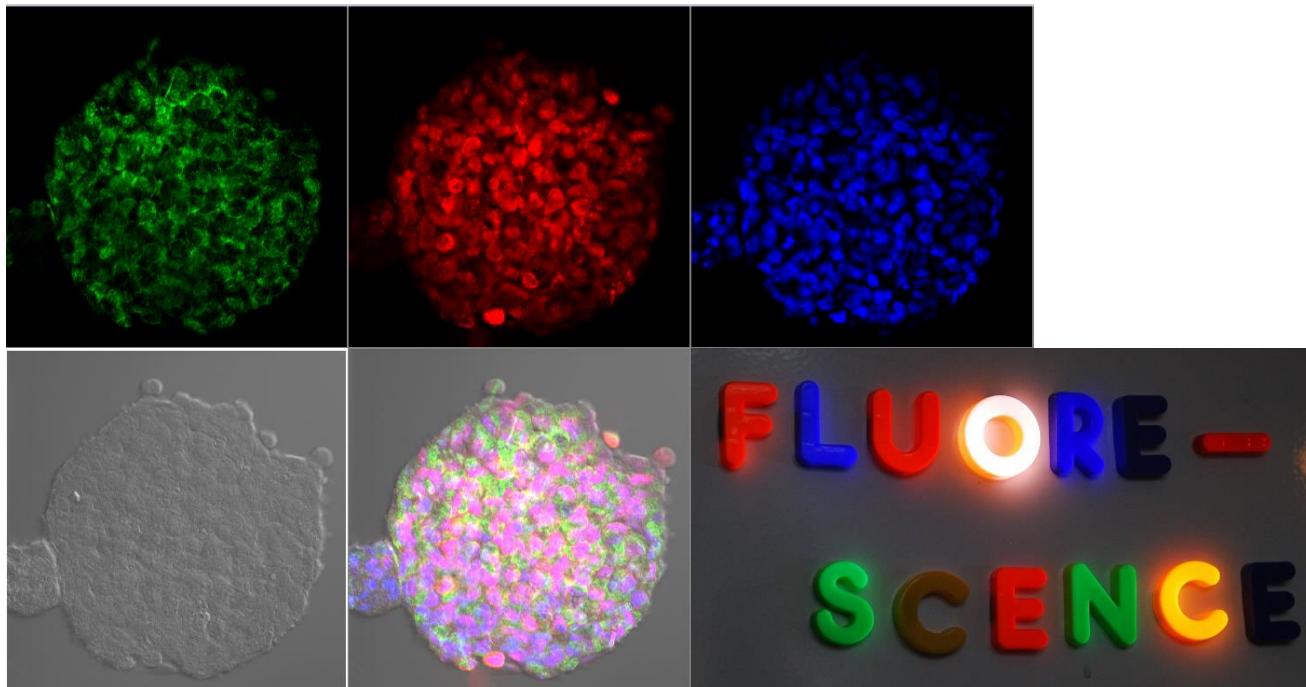
3

眼睛觀察



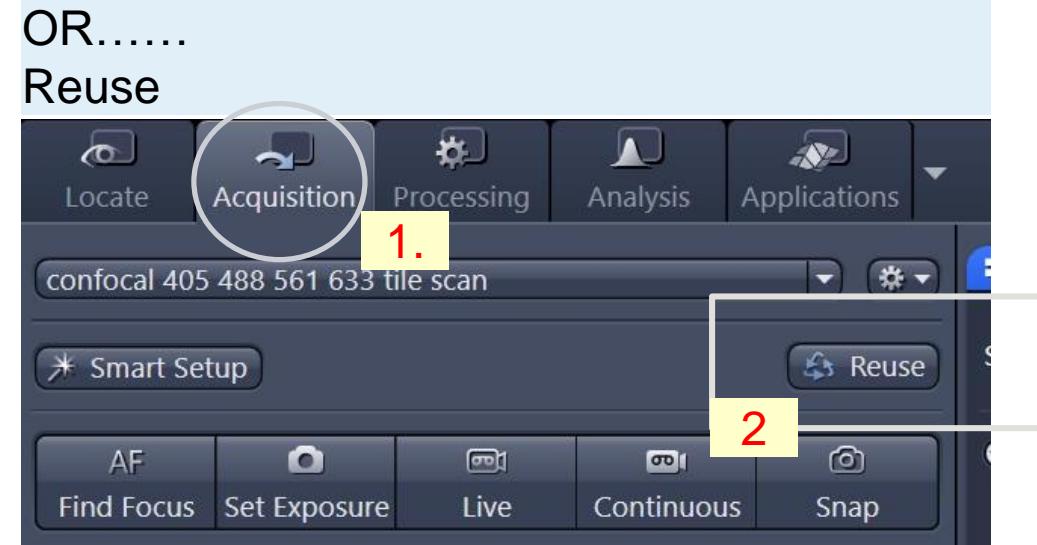
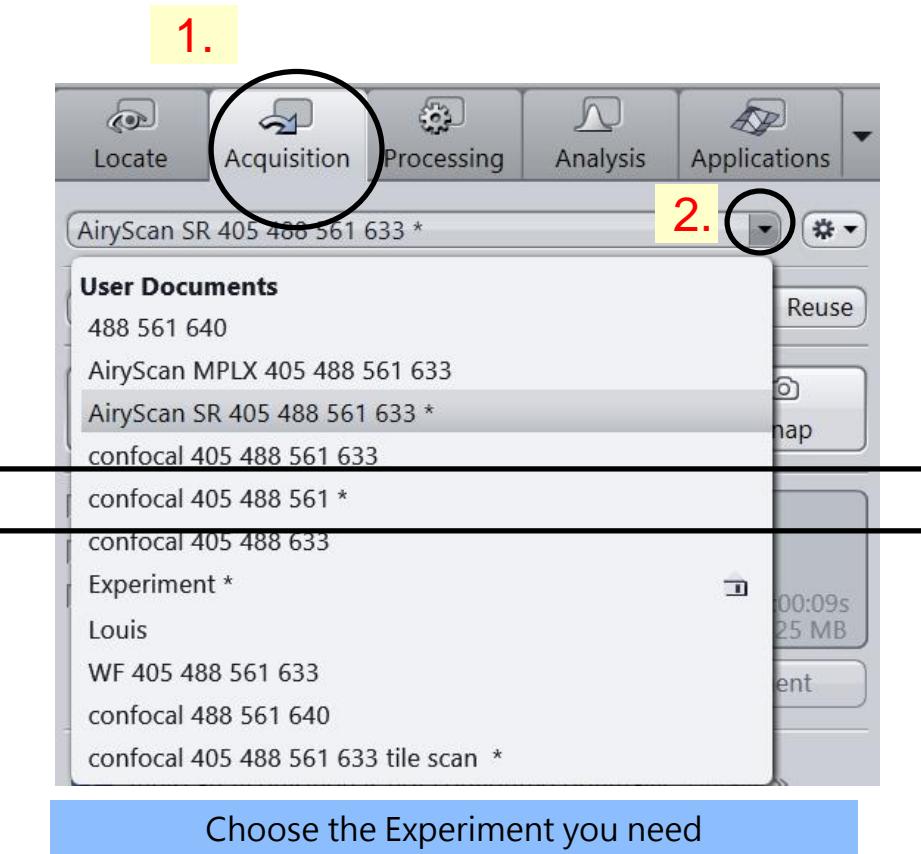
Choose the FL configuration you need

Multichannel Image Acquisition



Multichannel Image Acquisition 1

Load Experiment methods from Experiment Setup



- 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。
- 為避免撞傷鏡頭：
 - 如果有 Z 設定請取下樣品或先回到 5x 物鏡
 - 如果有 Tile 等 xyz 設定，套用完畢後記得先刪除不需要的位置

Multichannel Image Acquisition 2

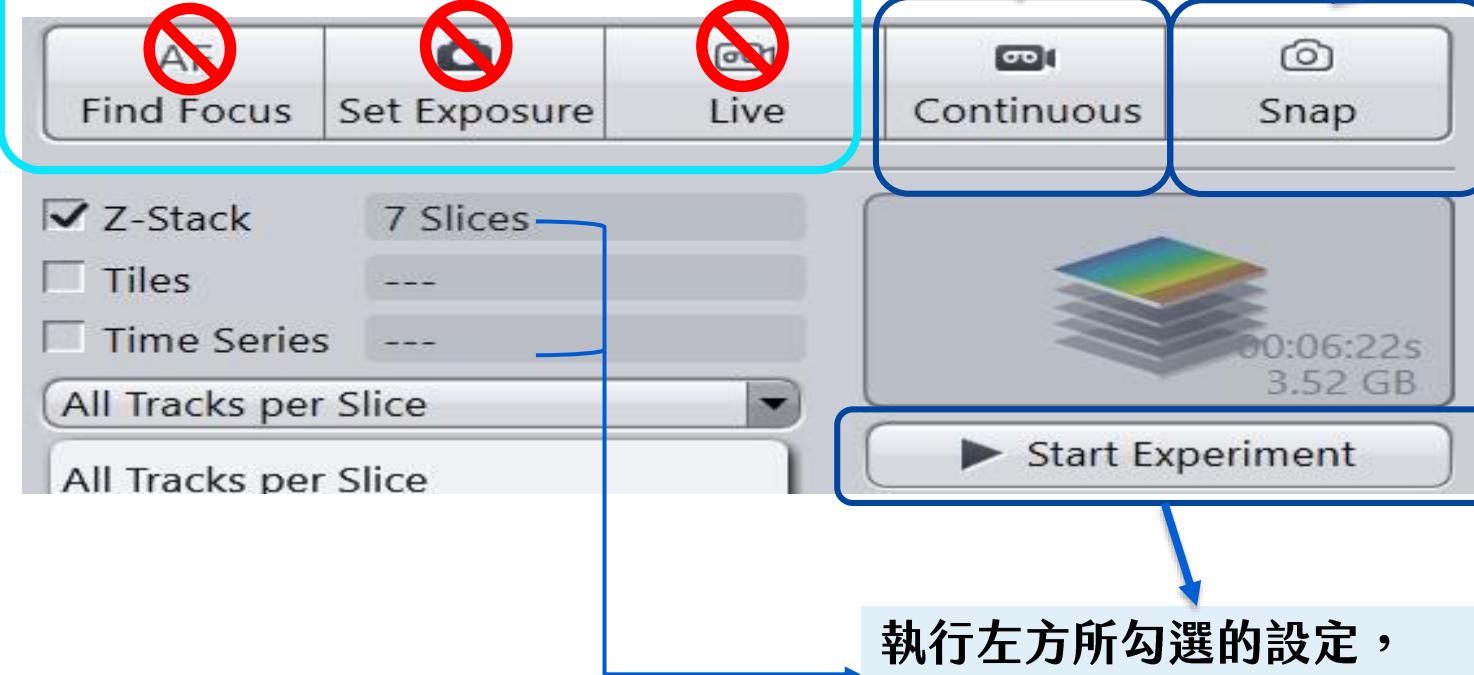
各種拍照function說明



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值時可看見即時變化。

在所停留的焦距位置，單拍一張，會執行已勾選的Channel，不會執行Z、T、Tile...等設定。

一般需求下，不建議使用!!



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

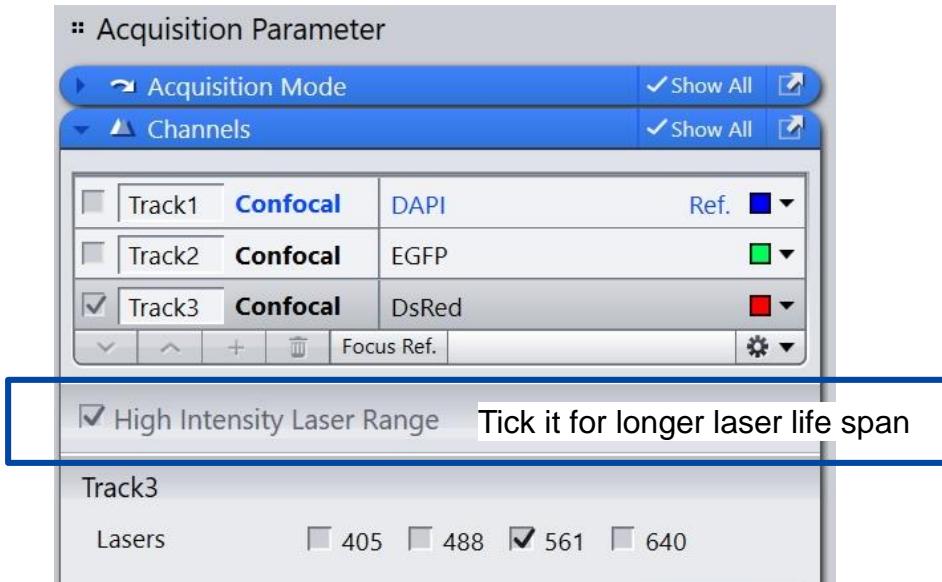
Multichannel Image Acquisition 3

Acquisition Parameters

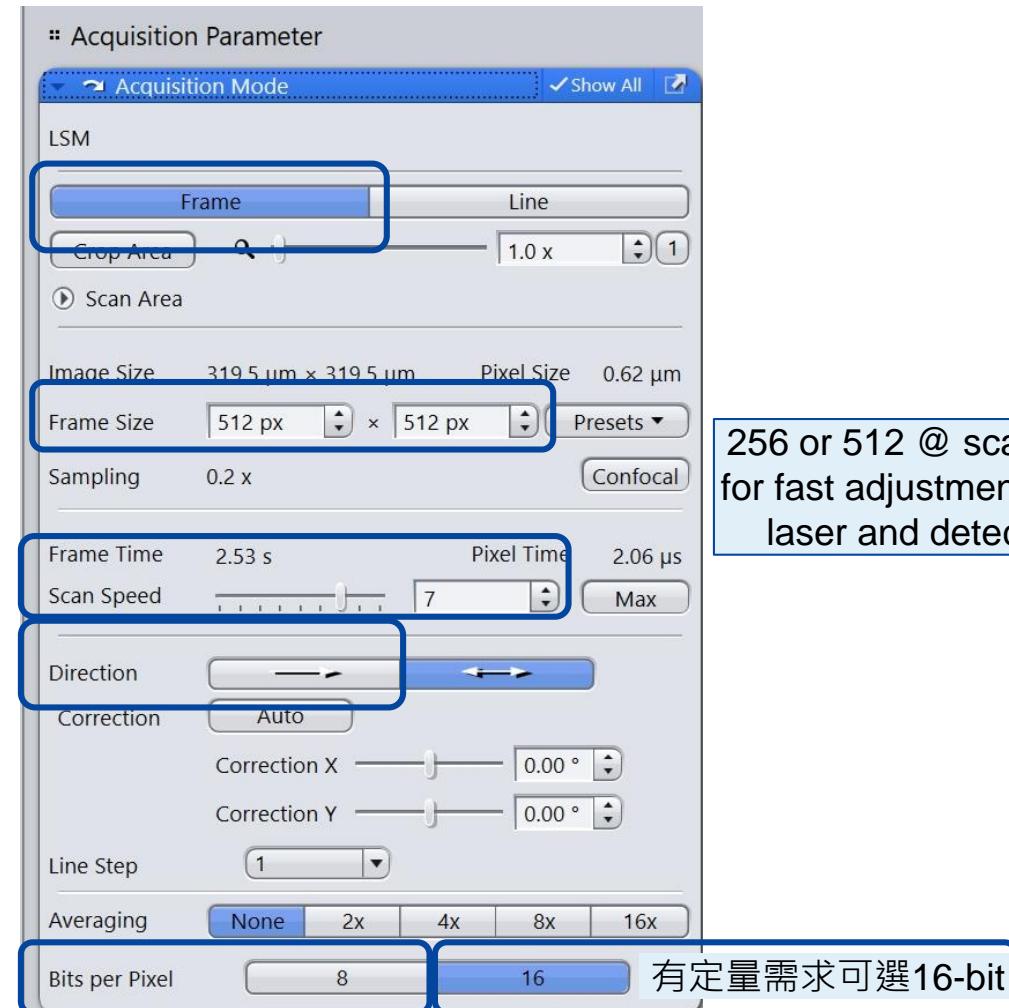


1

選擇需要的channel



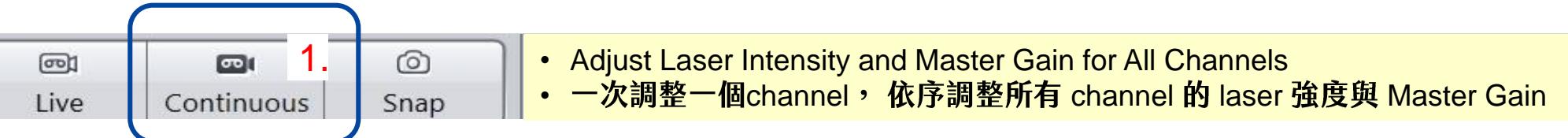
2



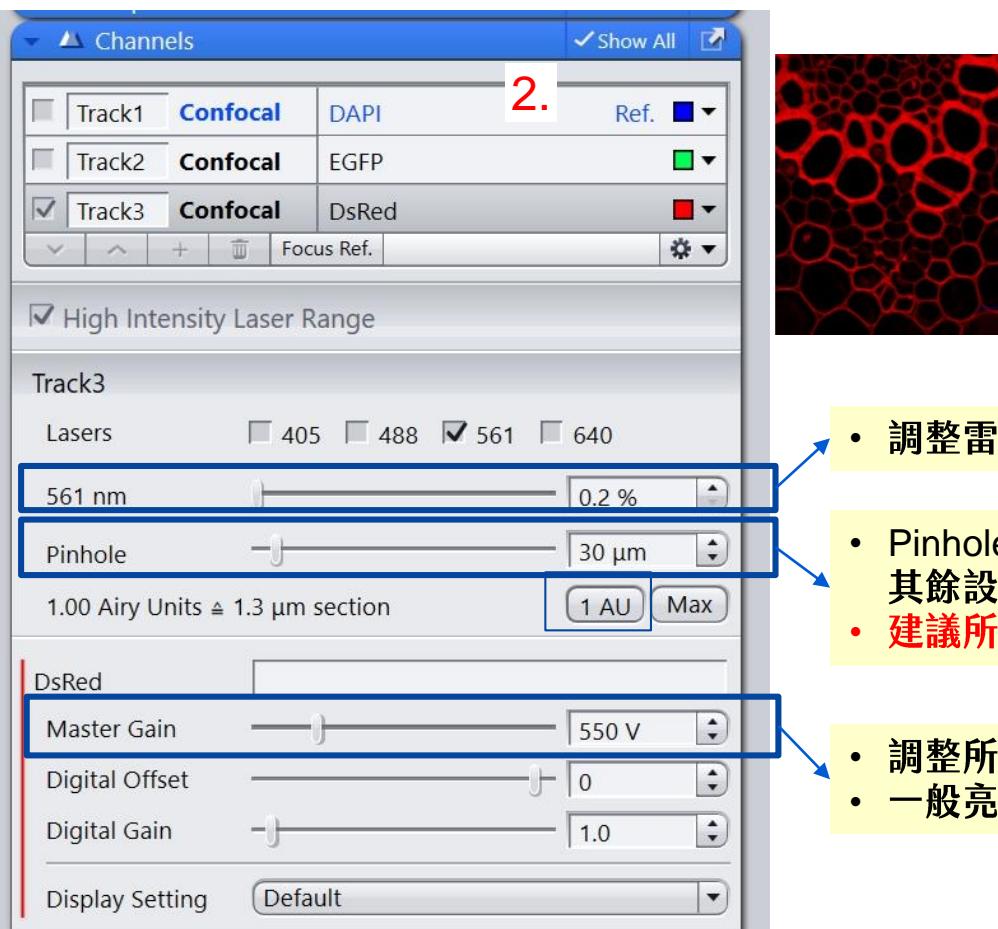
檔案小，大面拼圖時或不須定量時可選 8-bit

Multichannel Image Acquisition 4

2D Image

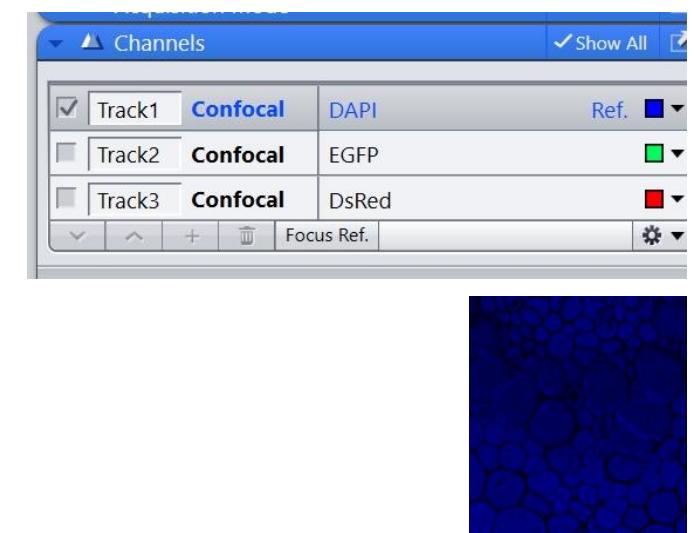


- Adjust Laser Intensity and Master Gain for All Channels
- 一次調整一個channel，依序調整所有 channel 的 laser 強度與 Master Gain



- 調整雷射強度：大部份情況下10%以內即足夠應付。
- Pinhole大小調整：選一個主要 channel 設定為1AU，其餘設定一樣數值 (in this case, 30um)
- 建議所有channel 設定相同(大約1AU)

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



Multichannel Image Acquisition 5

2D Image



1. 選取所有要準備要拍的track

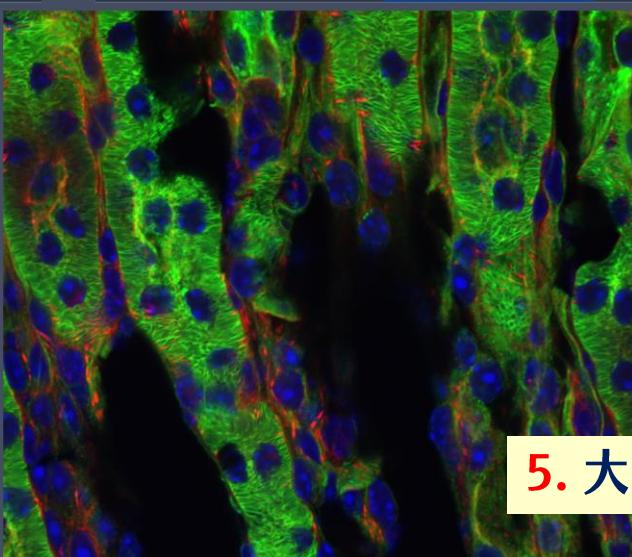
Channels

Track1	Confocal	DAPI	Ref.
Track2	Confocal	EGFP	Green
Track3	Confocal	DsRed	Red

Focus Ref.

High Intensity Laser Range

sure Live Continuous Snap 4.



5. 大功告成!!

#: Acquisition Parameter

Acquisition Mode

LSM

Frame Line

Crop Area 1.0 x 1

Scan Area

Image Size 2. 319.5 μm x 319.5 μm Pixel Size 0.12 μm

Frame Size 2586 px x 2586 px Presets

Sampling 1.0 x Confocal

Frame Time 25.58 s 3. Pixel Time 0.82 μs

Scan Speed 6 Max

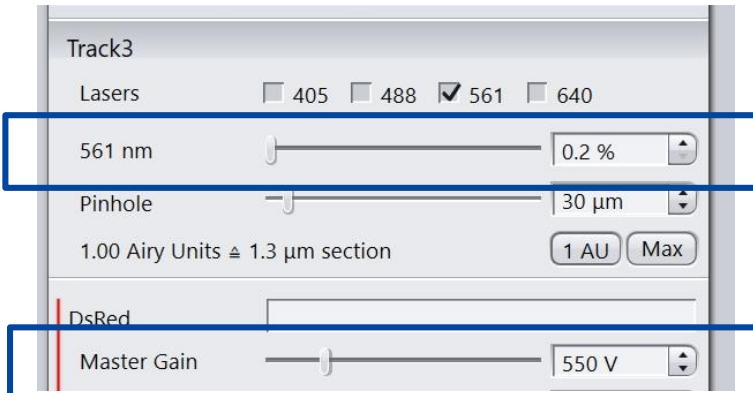
- 提高frame size、降低掃描速度，是獲得高解析影像的最後祕技！
- 1024 or 2048，speed 5~7 是很安全的設定值。

Multichannel Image Acquisition 6

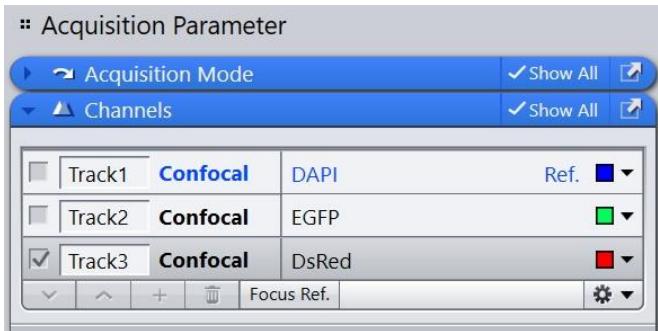
3D Image - Z Stack Acquisition



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 be preparing for focusing



4. Check Z-stack

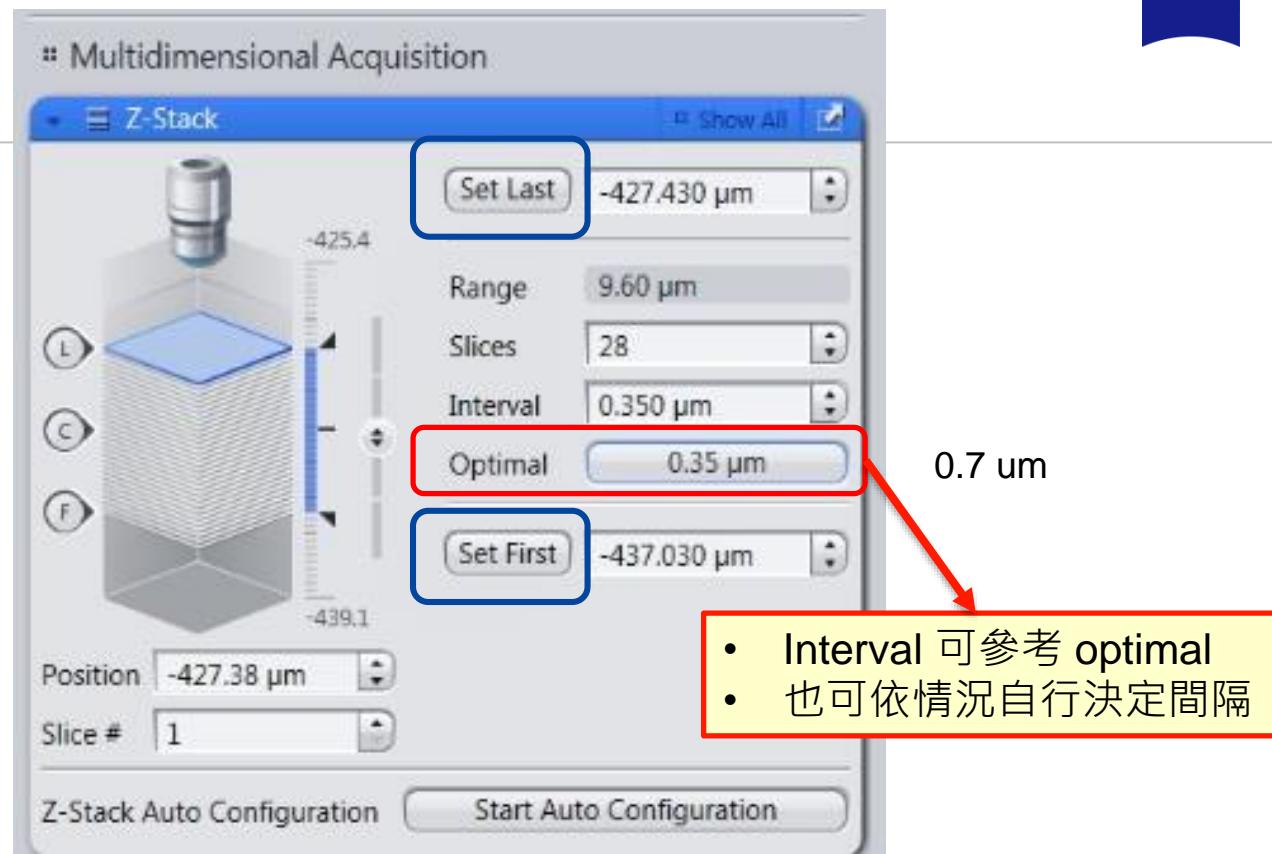
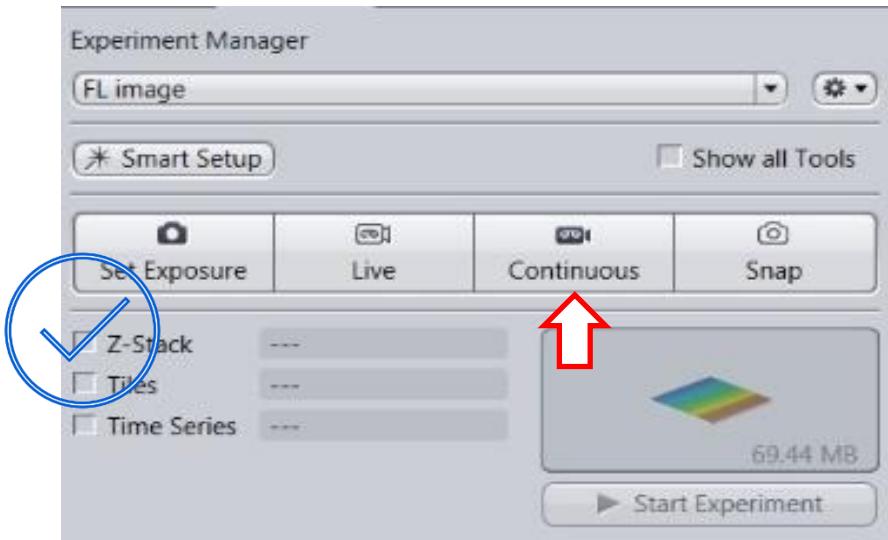


Multichannel Image Acquisition 7

3D Image - Z Stack Acquisition



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



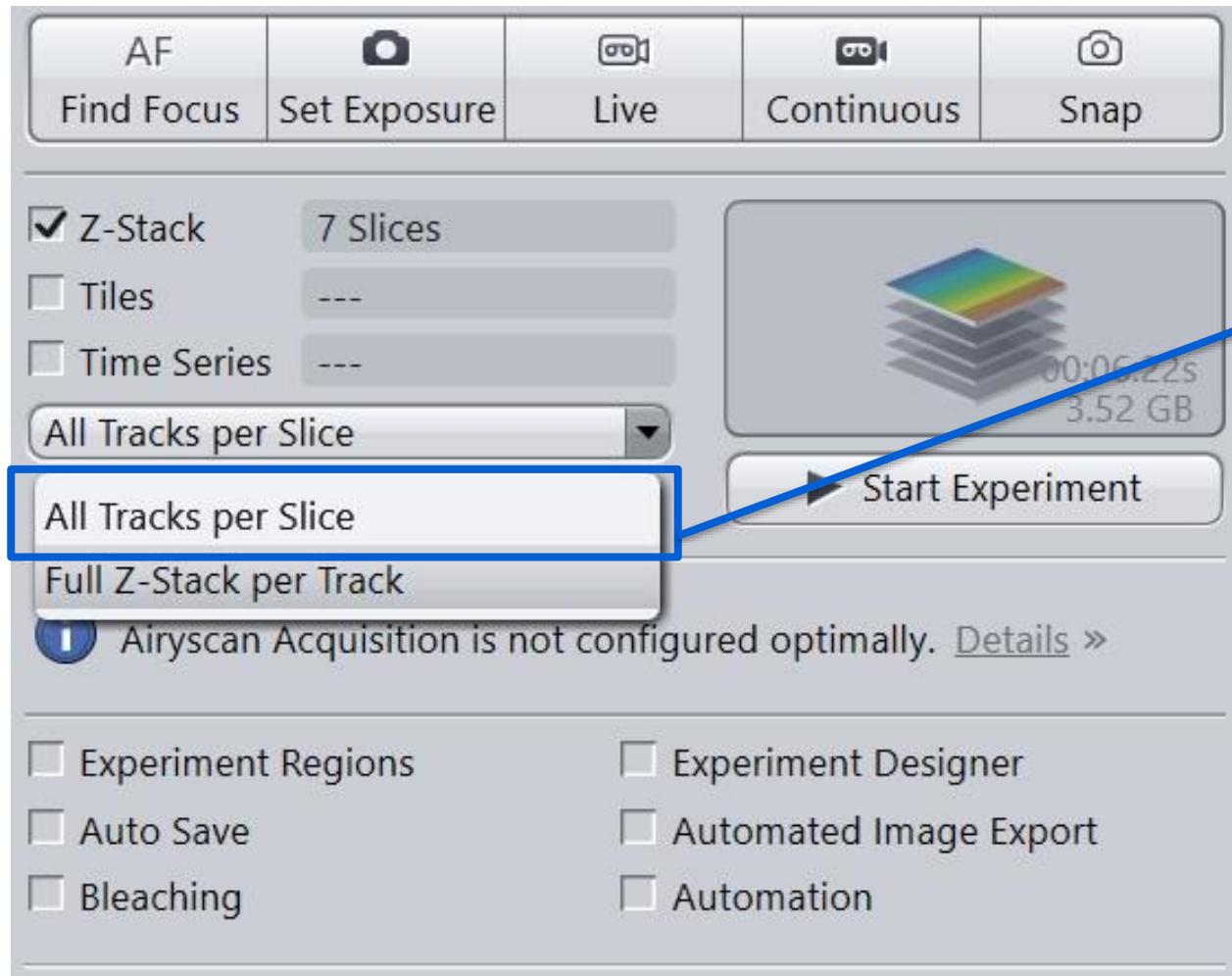
6. 確認拍攝要用的frame size, speed等設定後，
Start Experiment 開始拍攝Z-stack

設定 Z-stack的上下界限

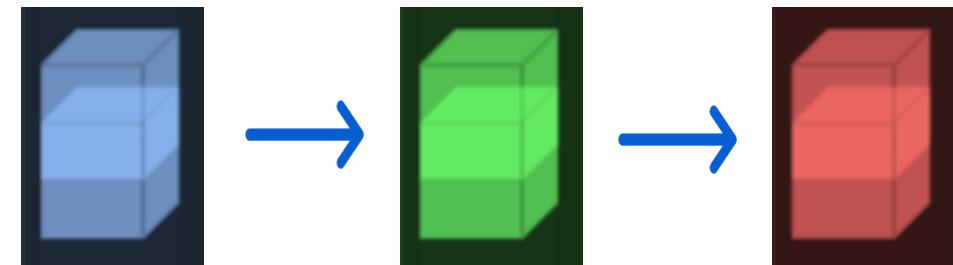
- 選擇一個channel → Continuous
- 搭配Z stack視窗 → Z-stack
- 找到樣品焦距起點 → Set First
- 找到樣品焦距終點 → Set Last

Multichannel Image Acquisition 8

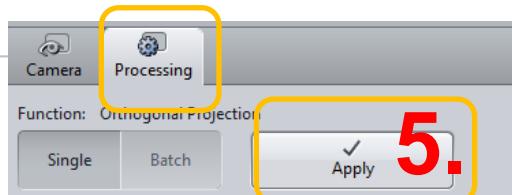
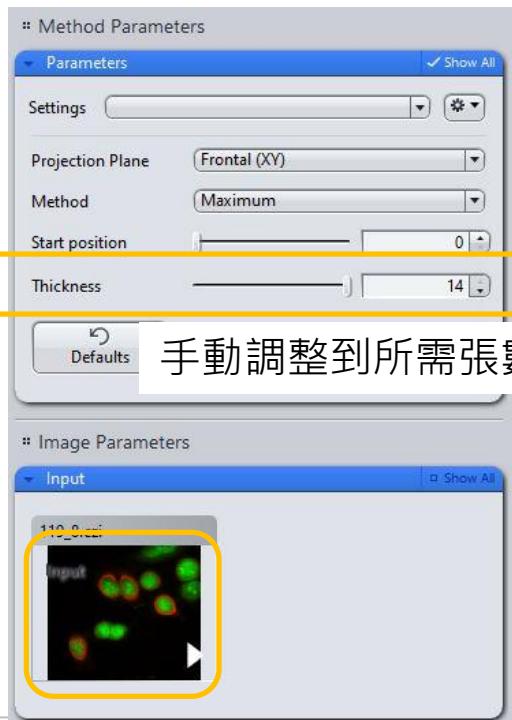
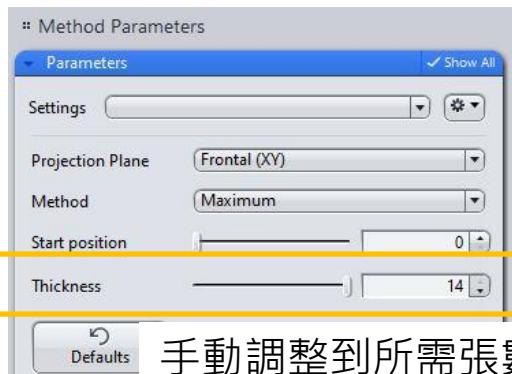
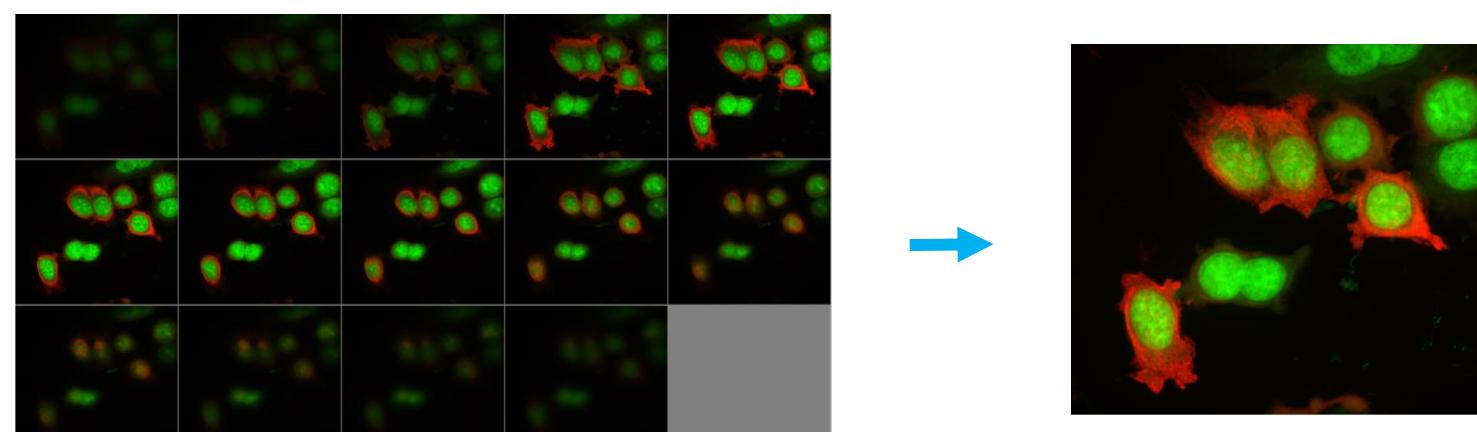
3D Image - Z Stack Acquisition



- 一般建議使用 **All Tracks per Slice** 以避免不同顏色Z錯位
- 若因實驗需求，以Full Z-stack per Track方式掃描，可得到最快速度
 - 每個 Channel 的 Z Stack 掃完才換下一個 Channel 的掃 Z Stack
 - 若搭配 line scan, bi-direction scan，為最省時間之掃描方式

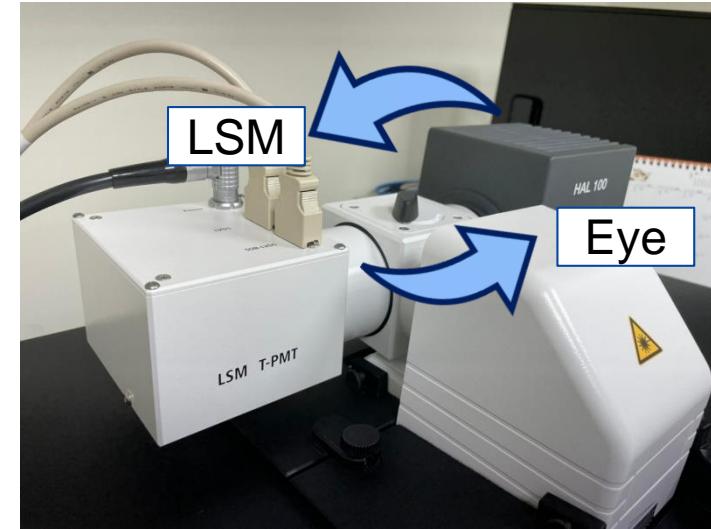
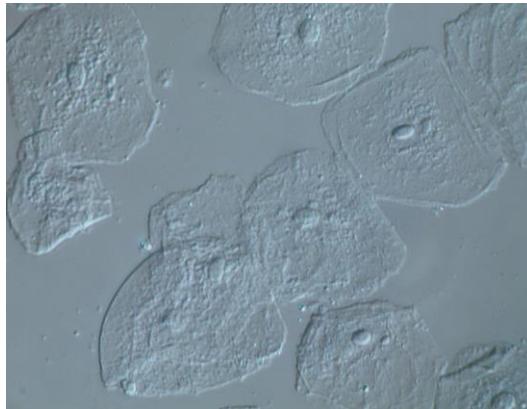


Z stack: 把多張Z section疊成一張 製造全景深影像: Orthogonal Projection

1. 
2. 
3. 
4. 
5. 

The image shows a 5x5 grid of smaller microscopy images on the left, each showing a different Z-section of a sample. To the right of the grid is a large blue arrow pointing to a single, larger processed image on the right. This processed image is a panoramic reconstruction of the sample, showing both green and red fluorescence across the entire depth of the stack.

加拍穿透光 Bright Field / DIC Observation

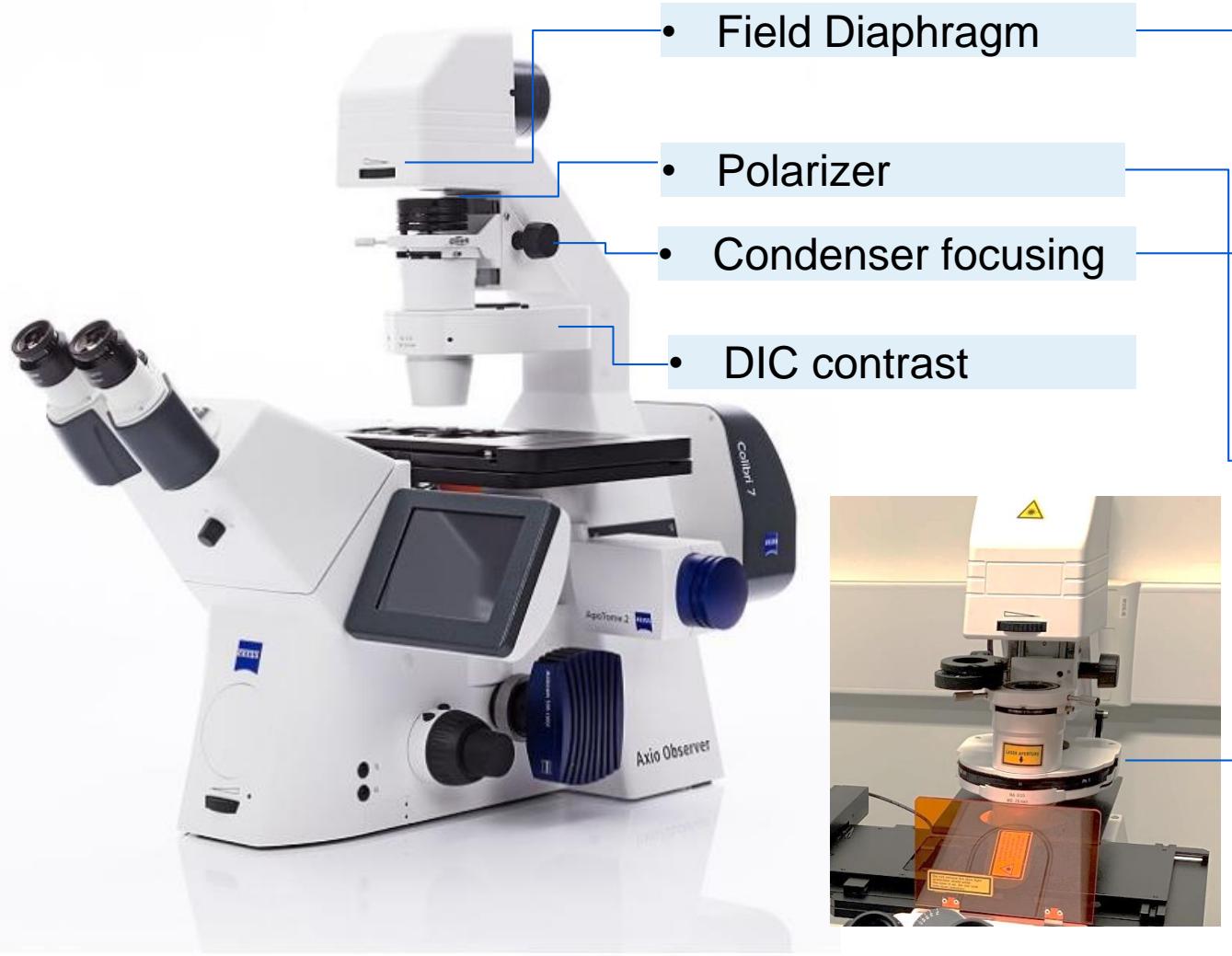


Turn the T-PMT Rotary Switches to

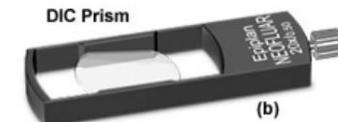
- Right for eye observation
- Left for confocal imaging

Bright Field / DIC Observation

Microscope setting for DIC

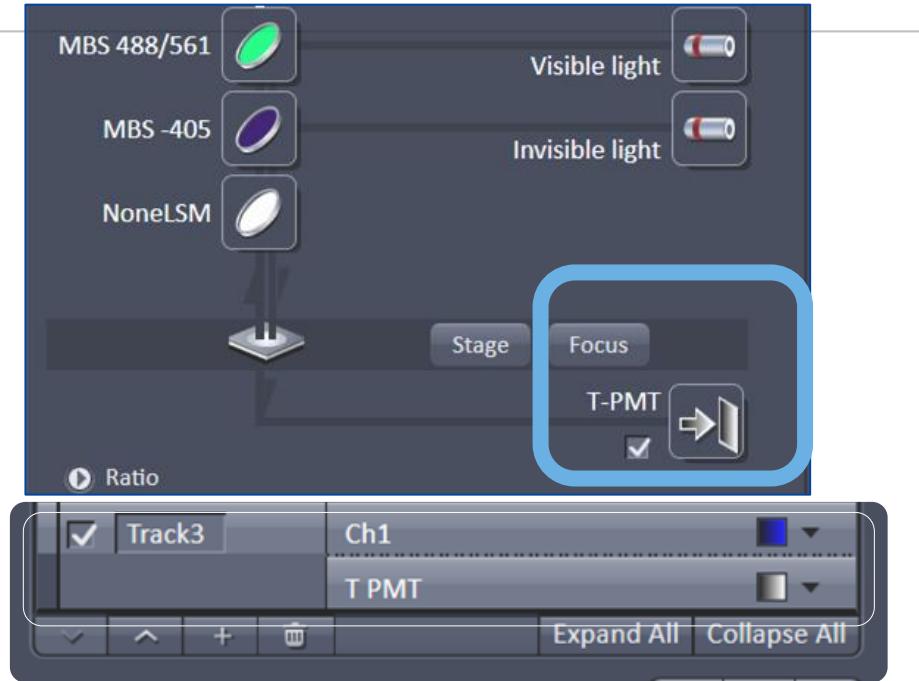
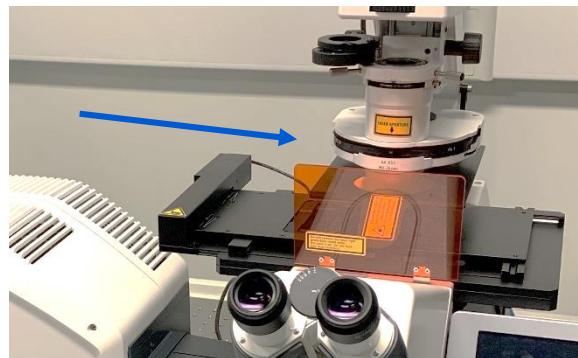


1. Focus the sample with objective
2. Adjust the condenser height by condenser focus knob.
3. Check the condenser center position by closing the field diaphragm and reopen it after focusing the condenser.
4. Choose the DIC filter position (as last page)
5. Swing the polarizer holder in
6. Choose condenser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



拍攝DIC影像

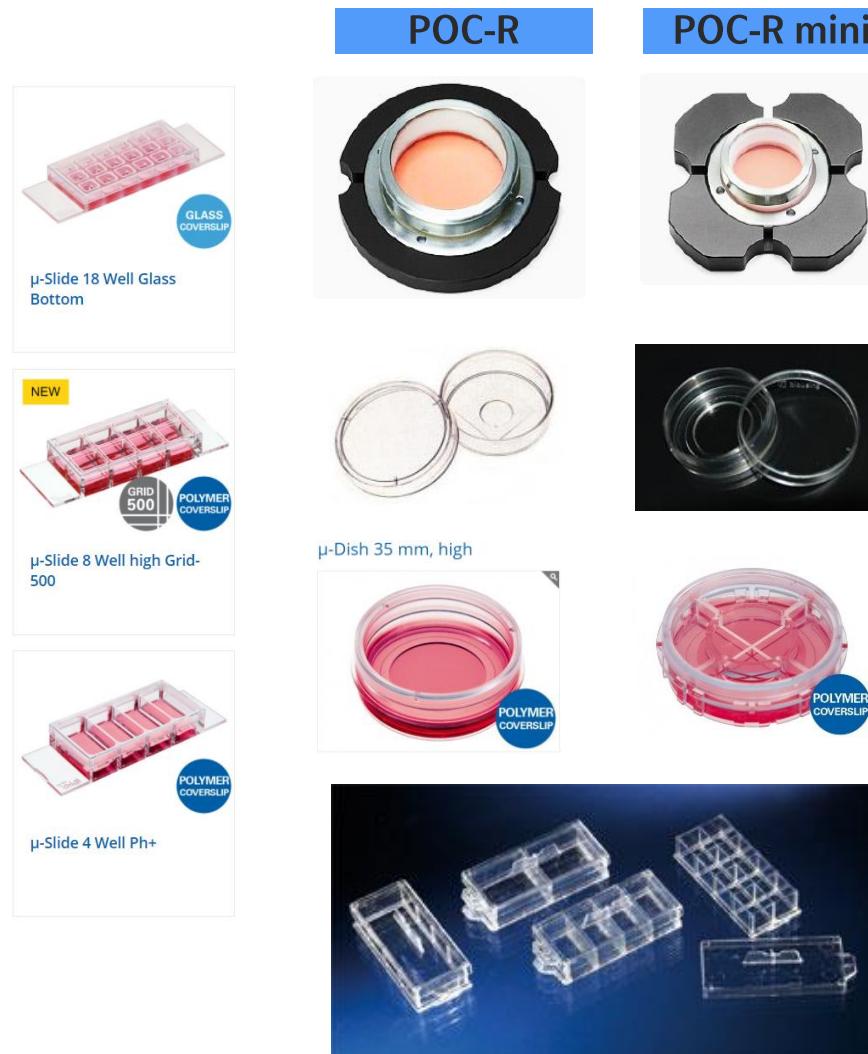
1. 如果講求完美效果聚光鏡校正要先做好! (設置請參考前頁)
2. 先將螢光設定好，最後再開啟T-PMT
3. 可選取任一個Track合併拍攝穿透光，或者增加track單獨拍攝
4. 確認一下聚光鏡轉盤位置是否在DICII (10x& 20x) 或DICIII (40x以上) (見下圖)。



T-PMT Gain (Master) 建議250左右開始嘗試
調整，注意勿過曝以保護感測器

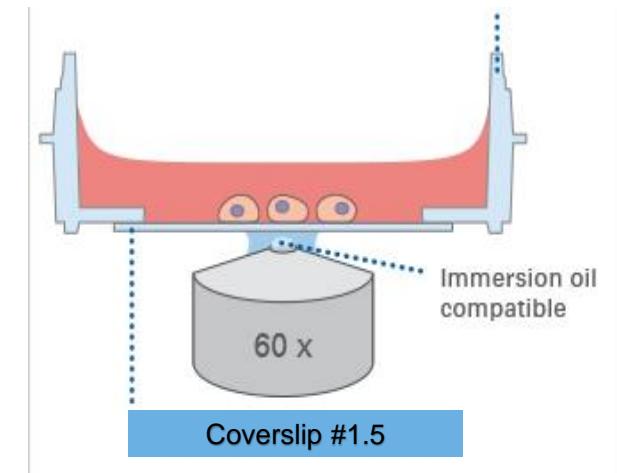


Recommend Single/Multi-well Chamber Types for Living Cell Application

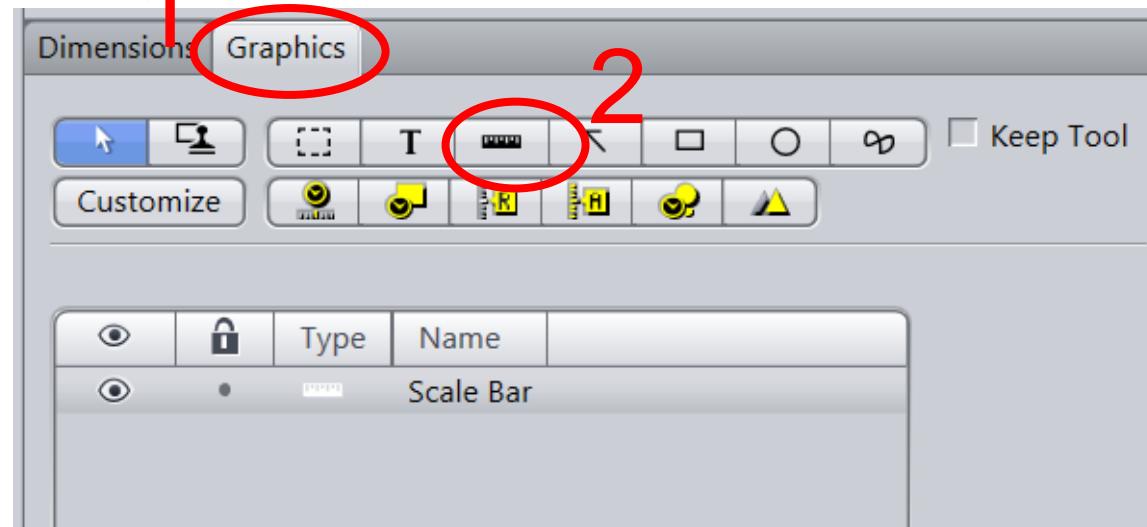
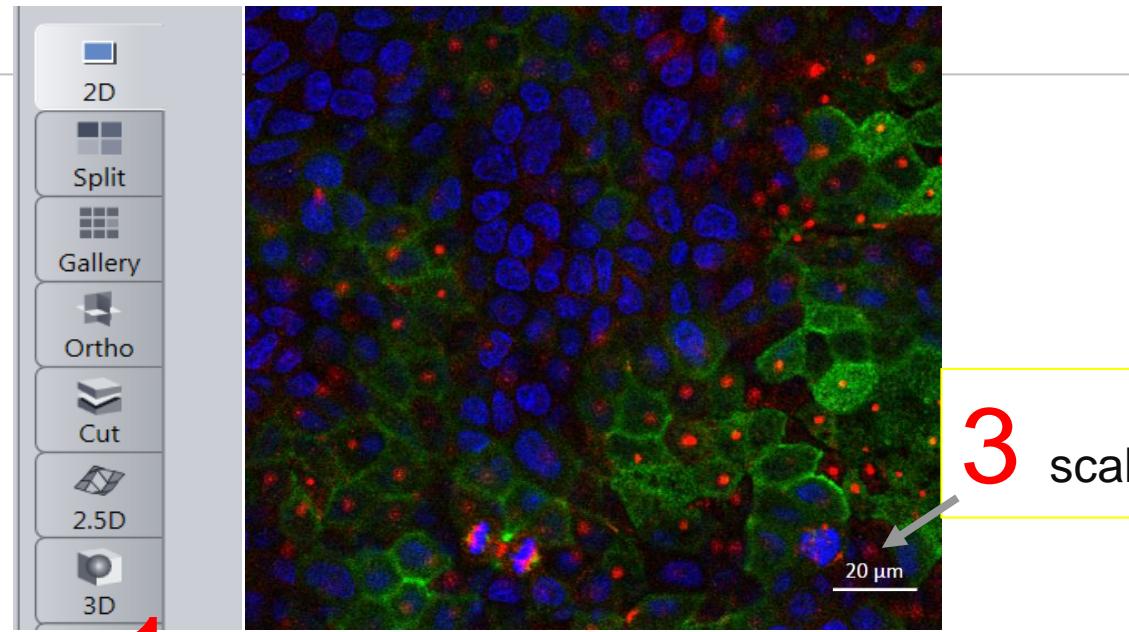


Cell Imaging Plate

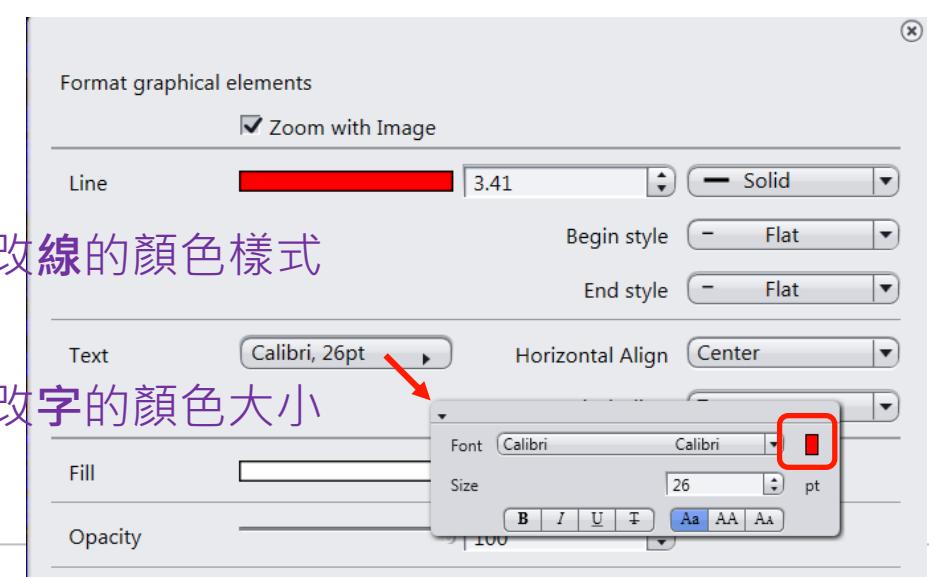
- #1.5 cover glass/ polymer bottom dish/plate/slide for inverted microscope with high N.A objectives.
- Thickness: no 1 ½ 0.17mm ±0.005mm



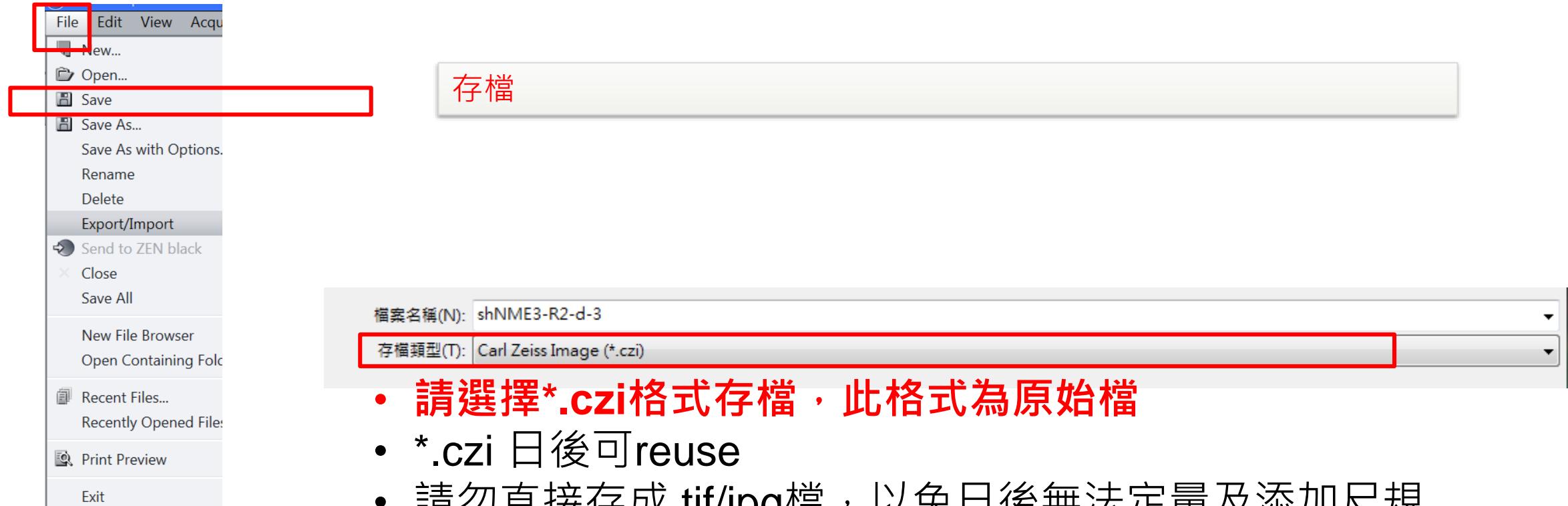
Add Scale Bar加入尺規



4 於scale bar按右鍵可更改大小顏色
粗細等格式



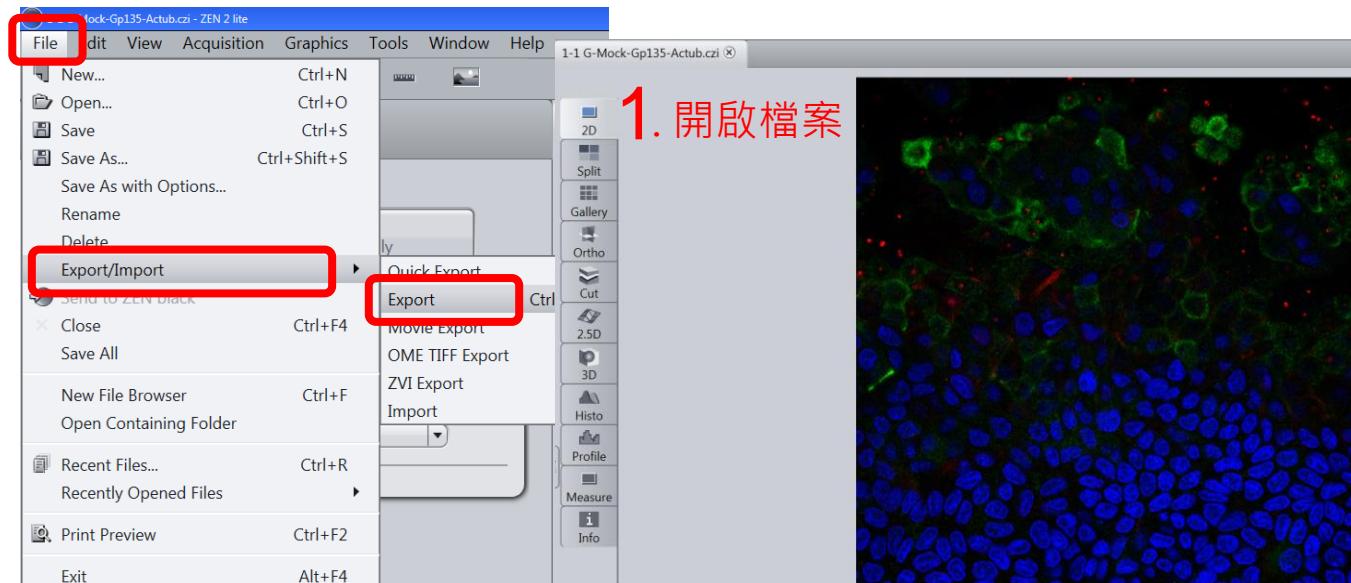
5



- 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

Method Parameters

Parameters

Show All

File type: Tagged Image File Format (TIFF)

Convert to 8 Bit

Compression: None

Resize: 100%

Original Data

Apply Display Curve and Channel Color

- Burn-in Graphics
- Merged Channels Image
- Individual Channels Image

Use channel names

Use Full Set of Dimensions

Define Subset

Export to: E:\DEMO and analyze image

- Create folder
- Generate xml file
- Generate zip file

Prefix: 1-1 G-Mock-Gp135-Actub

Defaults

Image Parameters

Input

1-1 G-Mock-...tub.czi

Show All

✓ Show all 可顯示更多選項

建議TIFF為畫質較高之影像格式

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
產生各別 channel 影像

產生所有xyz影像

產生各別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

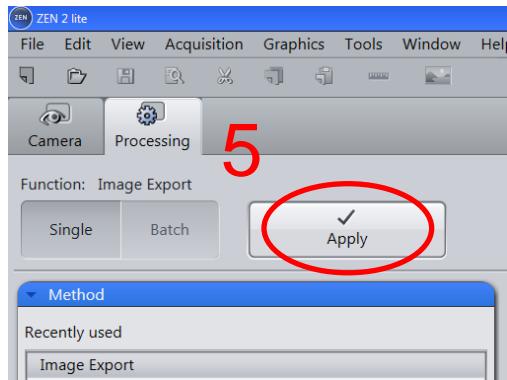
產生資料夾

Prefix為預設檔名

確認input 檔名是否為您所要輸出的檔案

3

5



大量批次轉檔 batch export 1

The screenshot shows the ZEISS Research Microscopy Solutions software interface. On the left, the 'Batch Processing' dialog is open, displaying a list of files to be processed. On the right, the 'Parameters' dialog is open, showing various settings for the batch export.

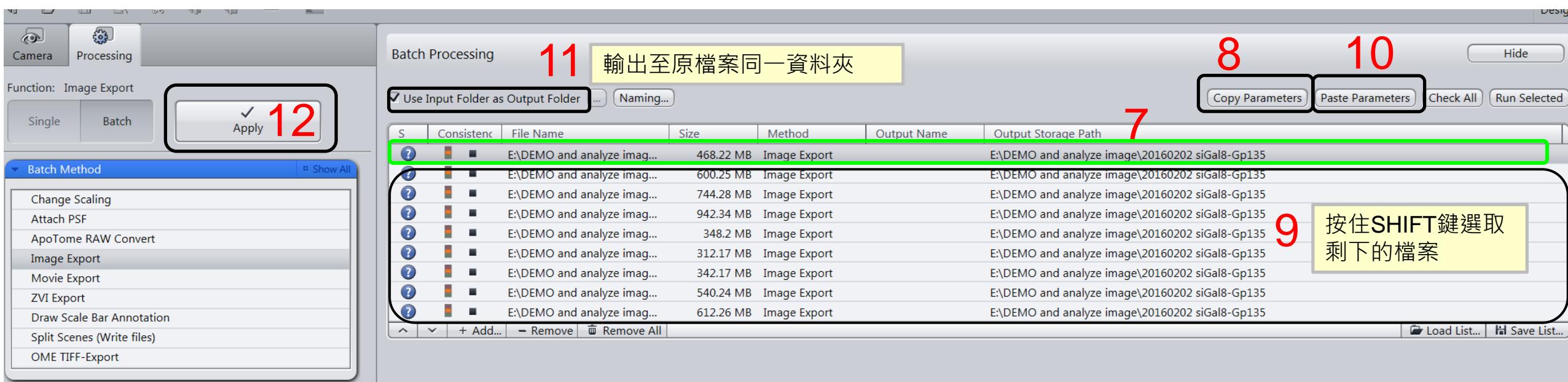
Callouts and Annotations:

- 1 Processing tab in the top menu bar.
- 2 Batch button in the 'Batch Processing' dialog.
- 3 'Image Export' option selected in the 'Batch Method' list.
- 4 'Add...' button in the 'Batch Processing' dialog to add files.
- 5 'Batch Processing' dialog showing a list of files to be exported. A yellow box highlights the first file entry.
- 6 Parameters dialog showing settings for the export. A yellow box highlights the 'File type' section.

Parameters Dialog Settings and Descriptions:

- File type:** Tagged Image File Format (TIFF) 建議TIFF為畫質較高之影像格式
- Convert to 8 Bit** 8-bit方便瀏覽 不須特殊軟體
- Compression:** None 建議不要壓縮
- Resize:** 100% 維持100% · 降低後畫素將減少
- Original Data:** 若勾選 · 於windows可能無法看見影像
- Apply Display Curve and Channel Color:** 套用調整過後的明暗對比
加入尺規等標示
- Burn-in Graphics**
- Merged Channels Image**
- Individual Channels Image** 產生merge影像
產生個別channel影像
- Use channel names**
- Use Full Set of Dimensions** 產生所有xyz影像
- Define Subset**
- Create folder** 產生資料夾
- Generate xml file**
- Generate zip file**

大量批次轉檔 batch export 2



7~10 將設定好的參數貼至其餘檔案當中。
若沒有做paste parameters的動作，batch export可能會失敗！

