陽明交通大學
儀器資源中心

Carl Zeiss LSM 900 / ZEN Blue
Quick Guide

NYCU
儀器資源中心
YCIRC

目錄

ZEISS LSM 900 with Airyscan 2

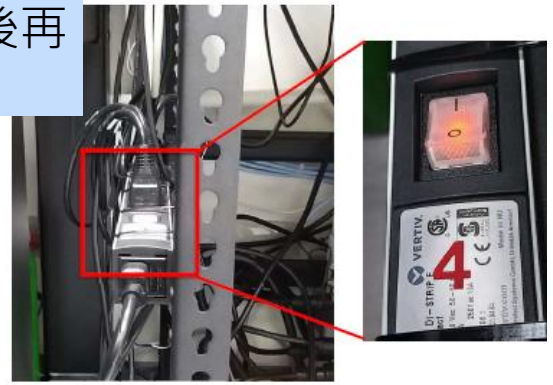
Start system

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

待白色燈光熄滅後再進入軟體

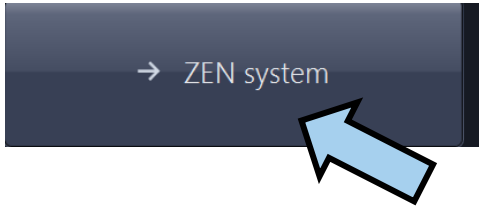


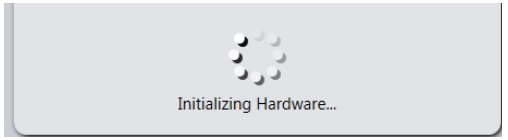
6

進入軟體 ZEN

1 進入ZEN BLUE

2

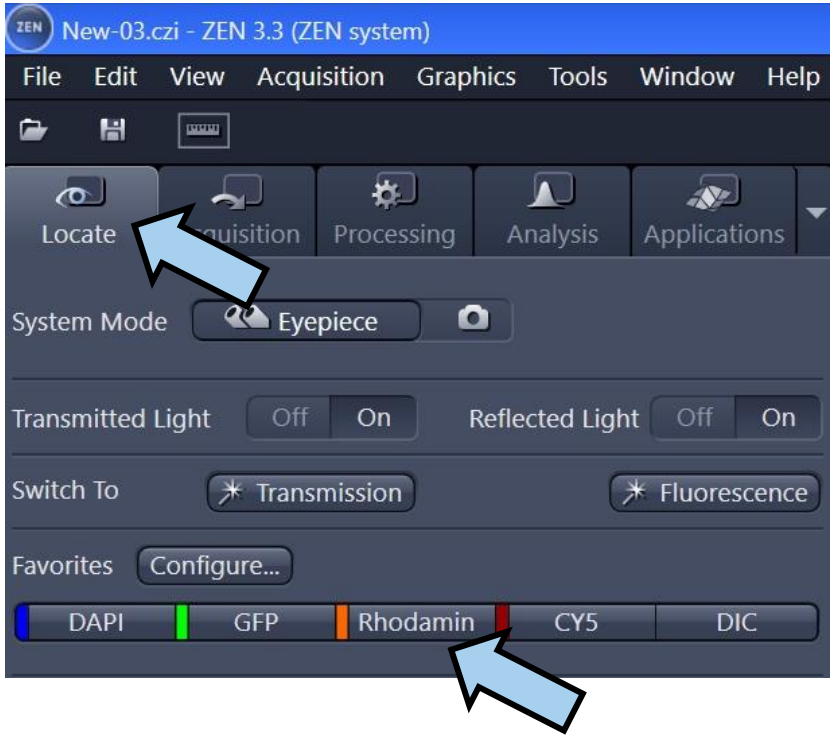




等待約一分鐘

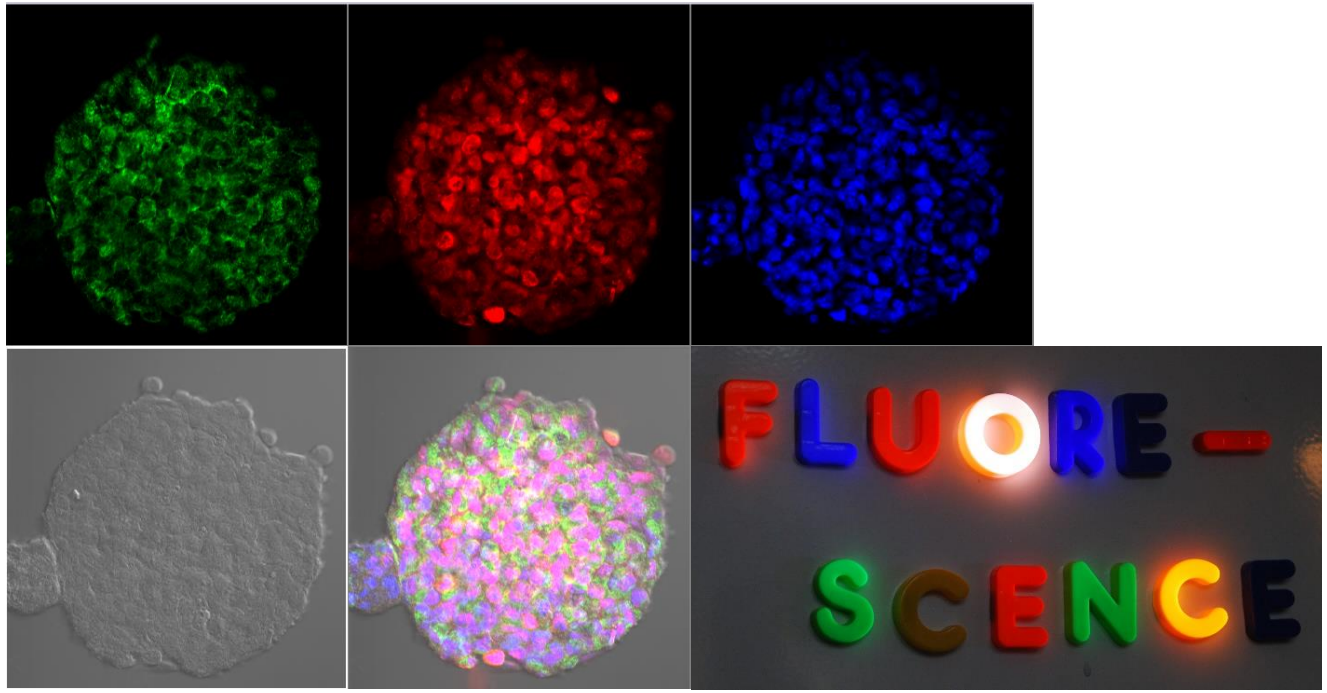
3

眼睛觀察

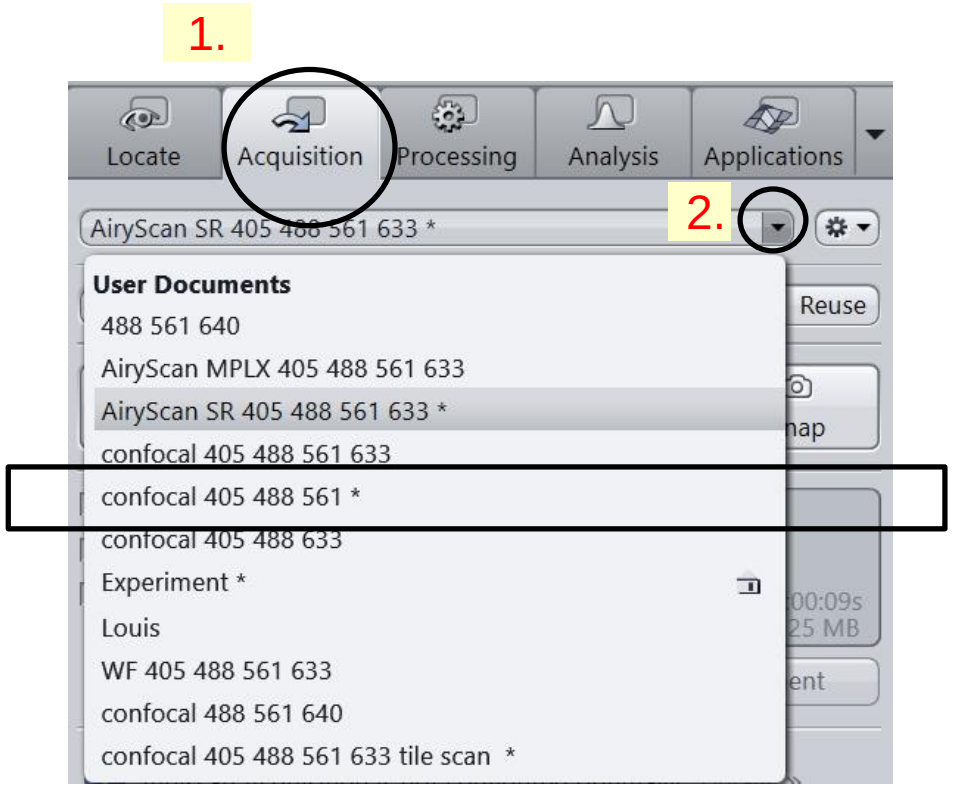


Choose the FL configuration you need

Multichannel Image Acquisition

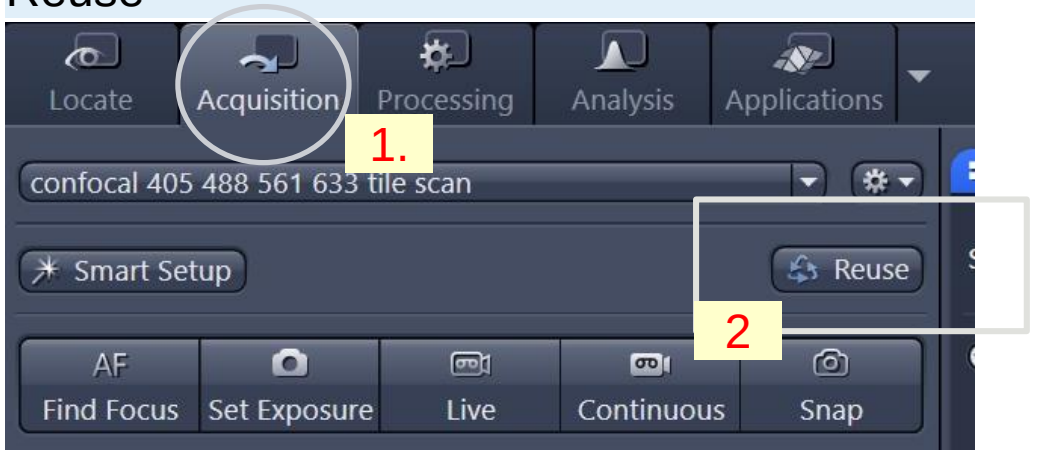


Multichannel Image Acquisition 1
Load Experiment methods from Experiment Setup



Choose the Experiment you need

OR……
Reuse

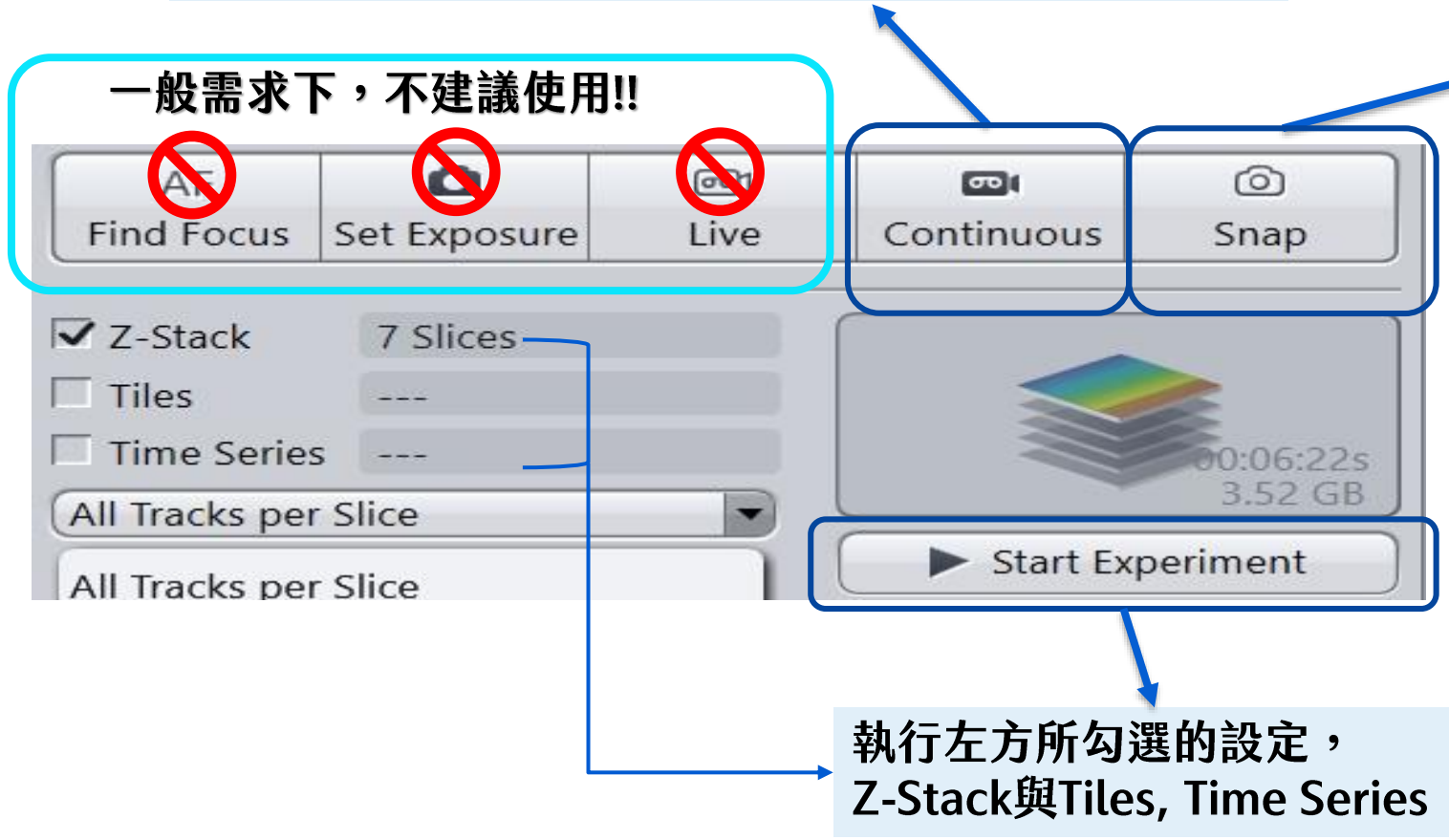


- 開啟欲套用的檔案後，按下Reuse，系統會將舊檔案的設定apply至硬體中。
- 為避免撞傷鏡頭：
  - 如果有 Z 設定請取下樣品或先回到 5x 物鏡
  - 如果有 Tile 等 xyz 設定，套用完畢後記得先刪除不需要的位置

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。

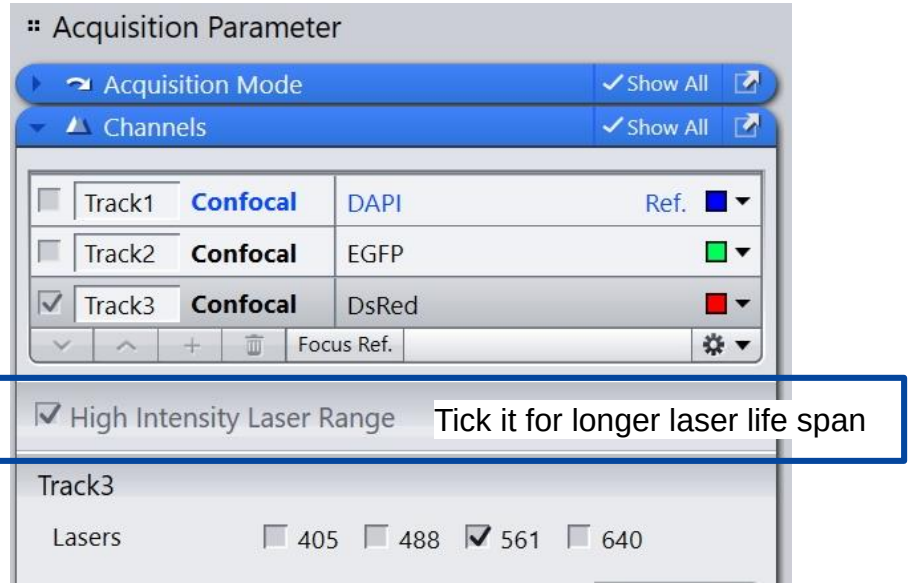


執行左方所勾選的設定，
Z-Stack與Tiles, Time Series

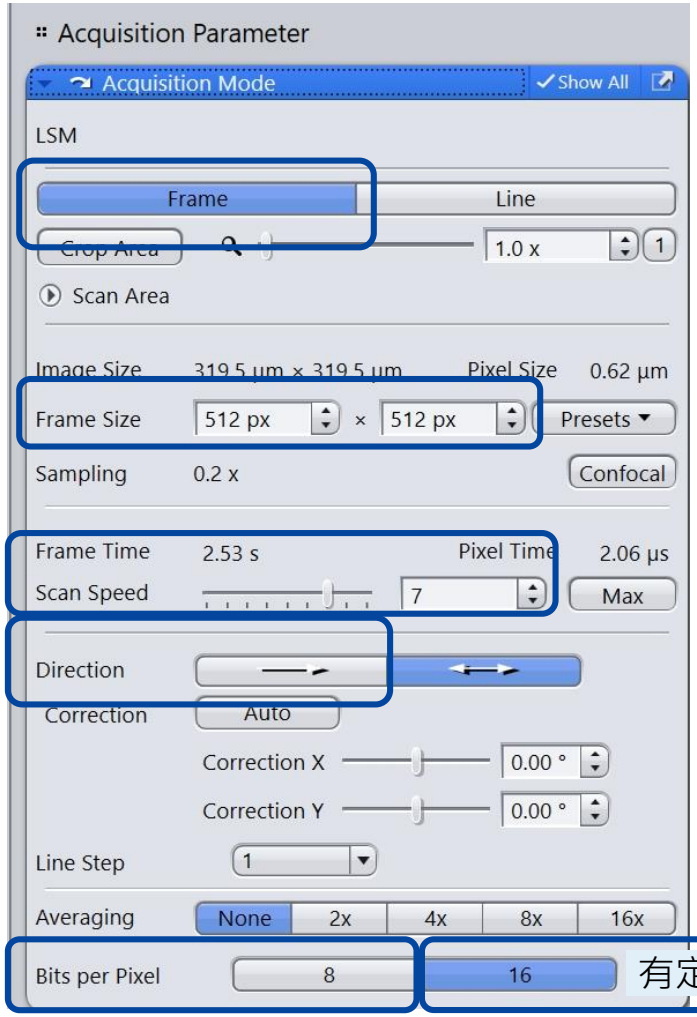
Multichannel Image Acquisition 3
Acquisition Parameters

1

選擇需要的channel



2

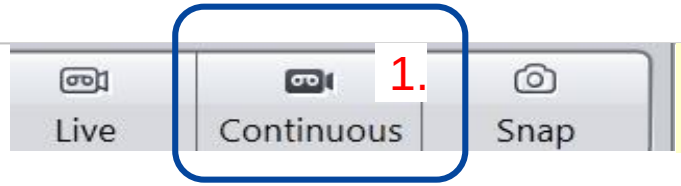


256 or 512 @ scan speed 7 for fast adjustment for focus, laser and detector gain

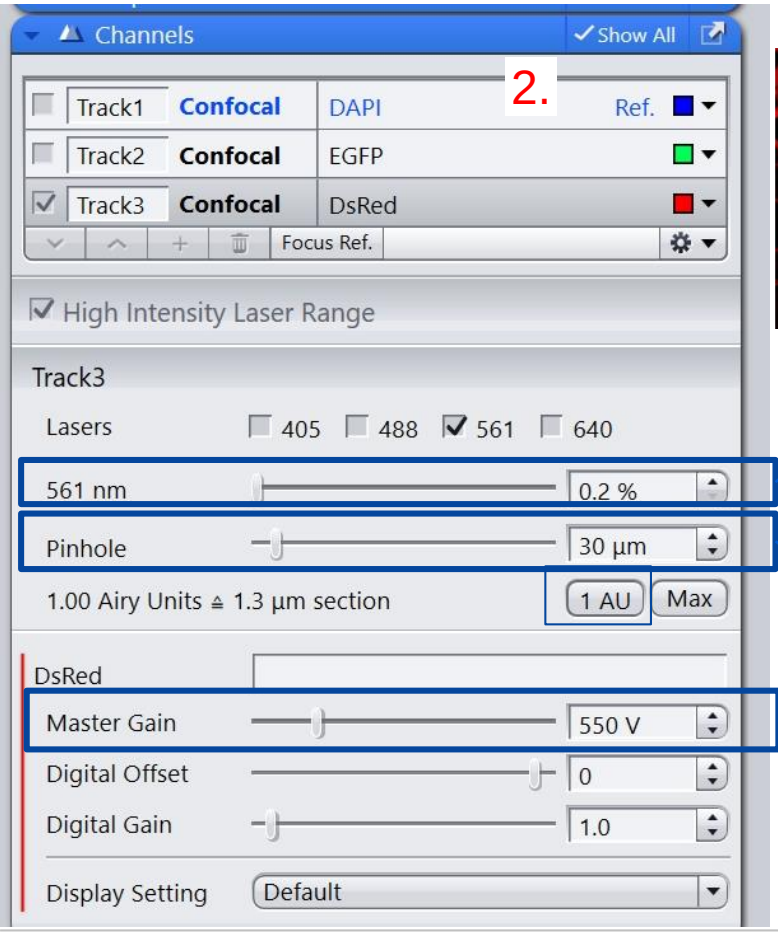
有定量需求可選16-bit

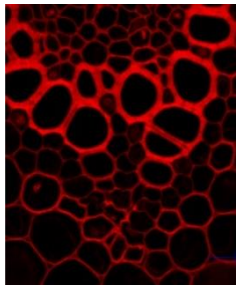
檔案小，大面拼圖時或不須定量時可選 8-bit

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



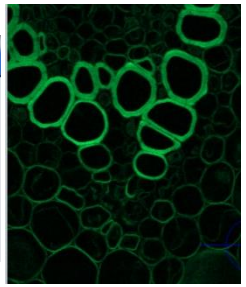


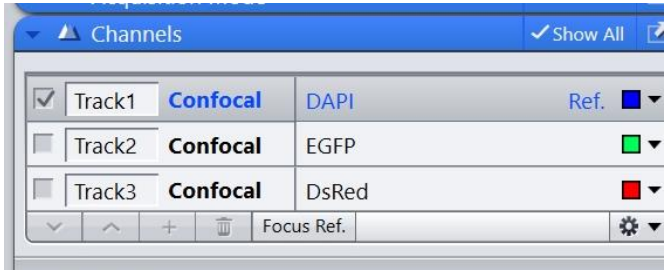
- 調整雷射強度：大部份情況下10%以內即足夠應付。

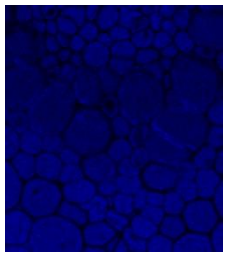
- Pinhole大小調整：選一個主要 channel 設定為1AU，其餘設定一樣數值 (in this case, 30um)
- 建議所有channel 設定相同(大約1AU)

- 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

3.

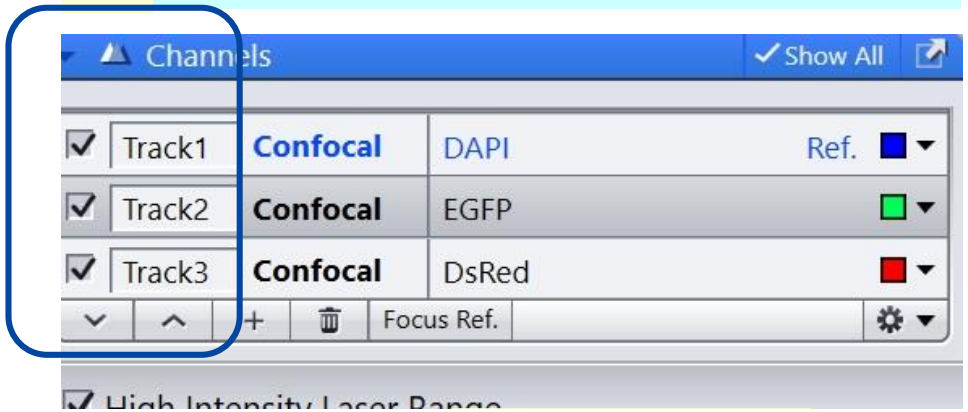


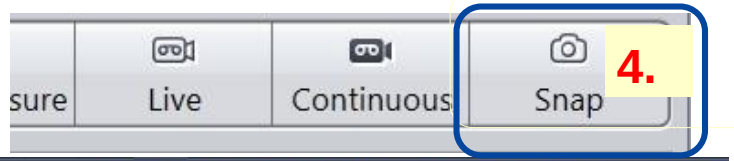


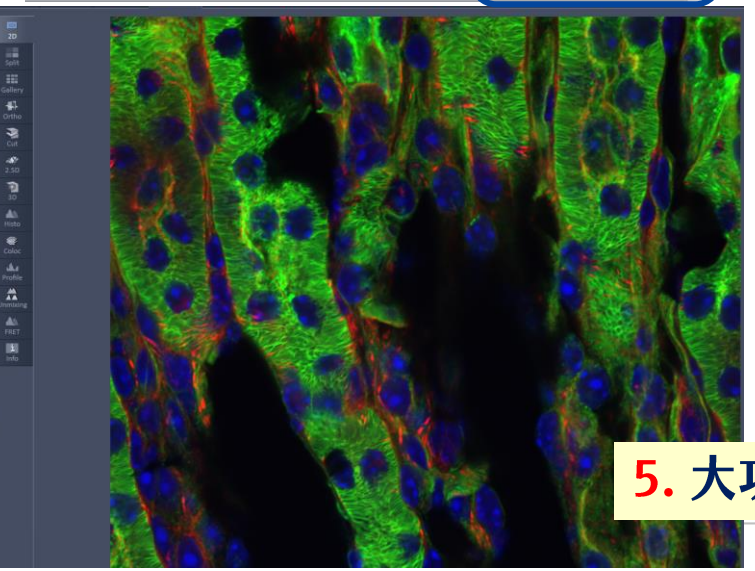


Multichannel Image Acquisition 5
2D Image

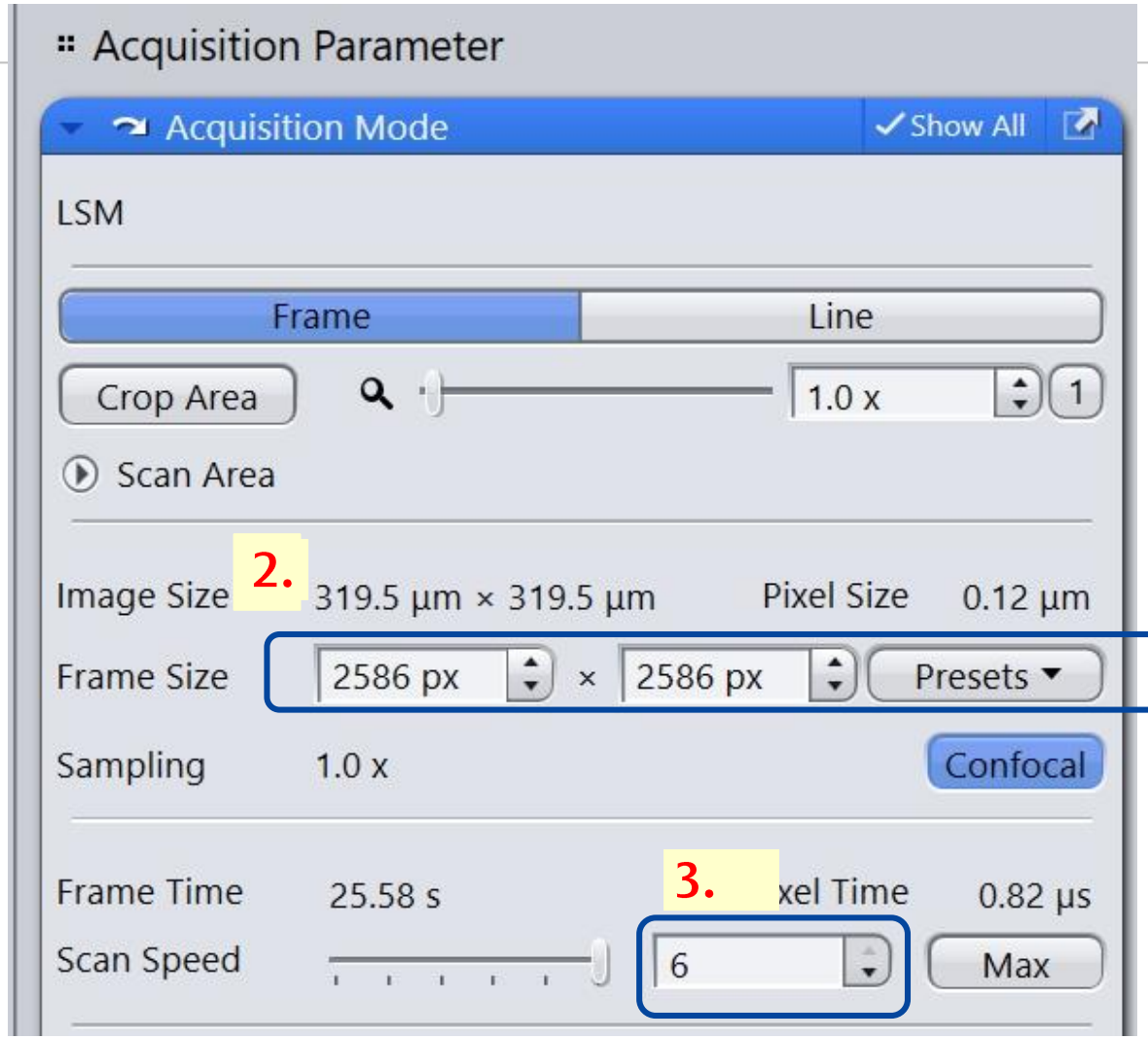
1. 選取所有要準備要拍的track







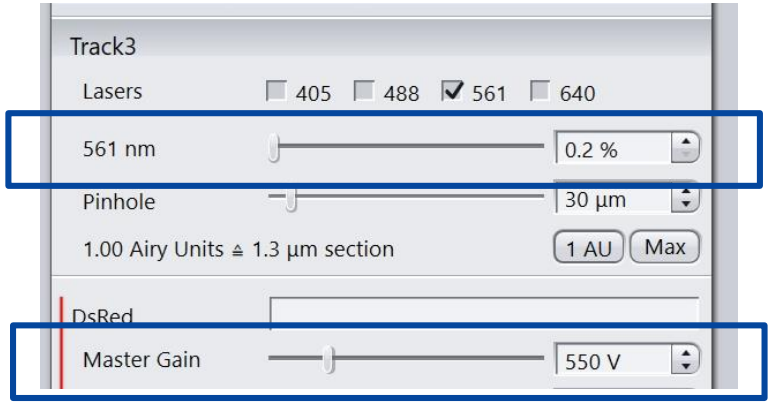
5. 大功告成!!



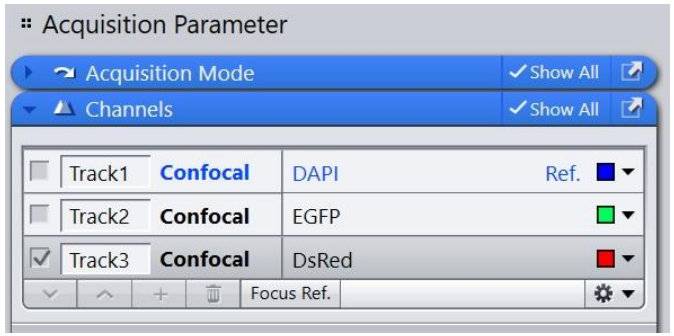
- 提高frame size 、降低掃描速度，是獲得高解析影像的最後祕技！
- 1024 or 2048，speed 5~7 是很安全的設定值。

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

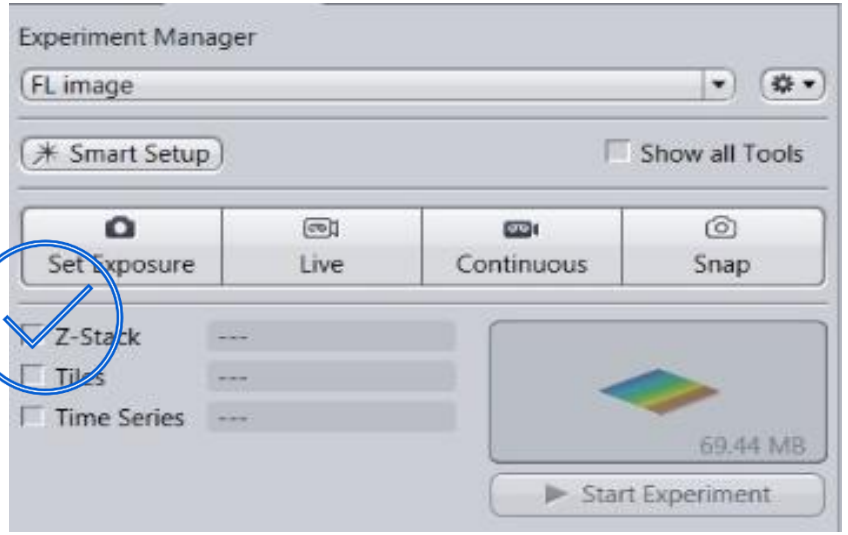


2. 選擇一個Track



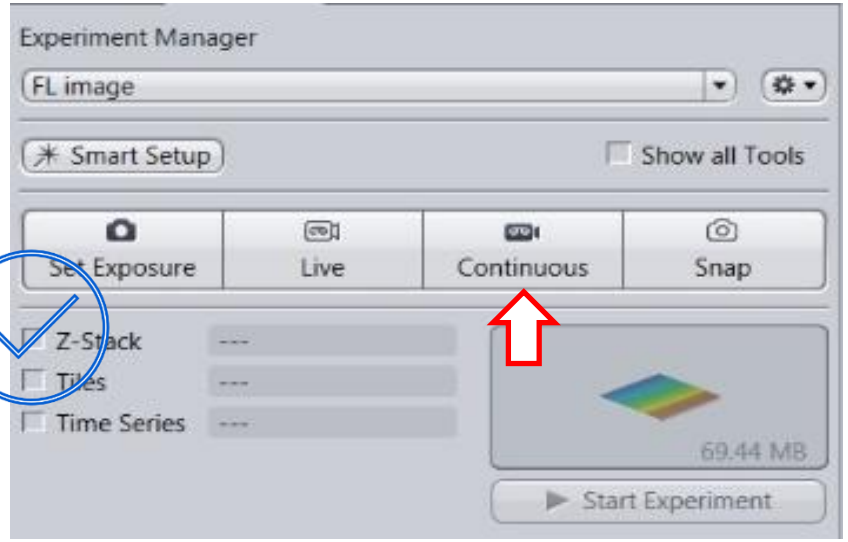
3. Put your hand on focus wheel and be preparing for focusing

4. Check Z-stack

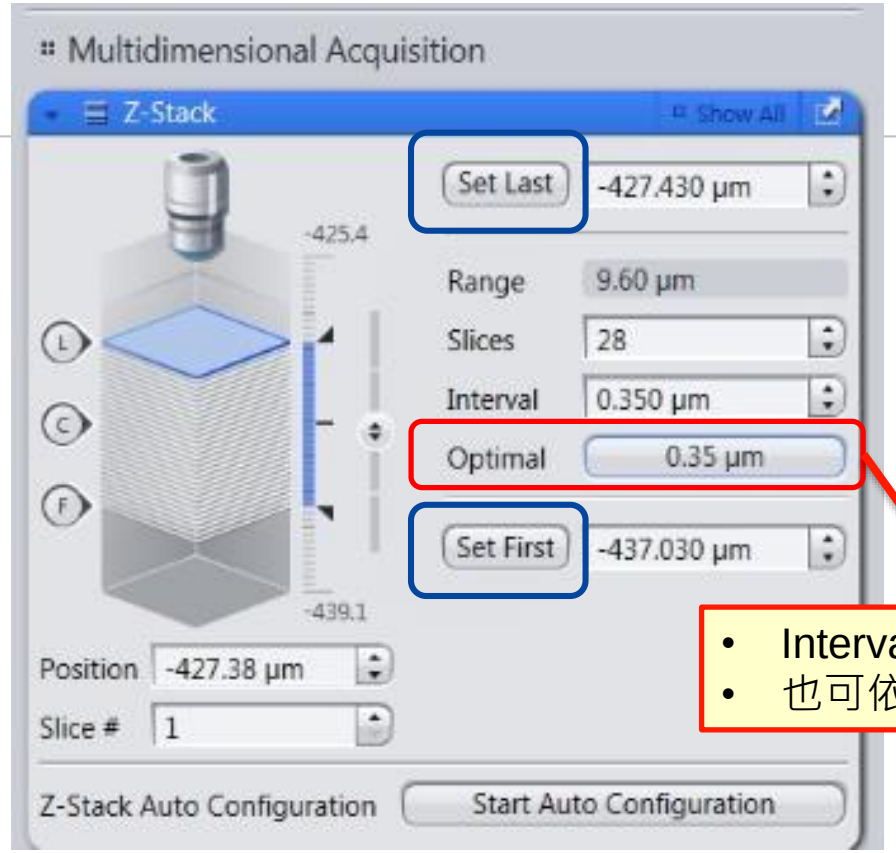


Multichannel Image Acquisition 7
3D Image - Z Stack Acquisition

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



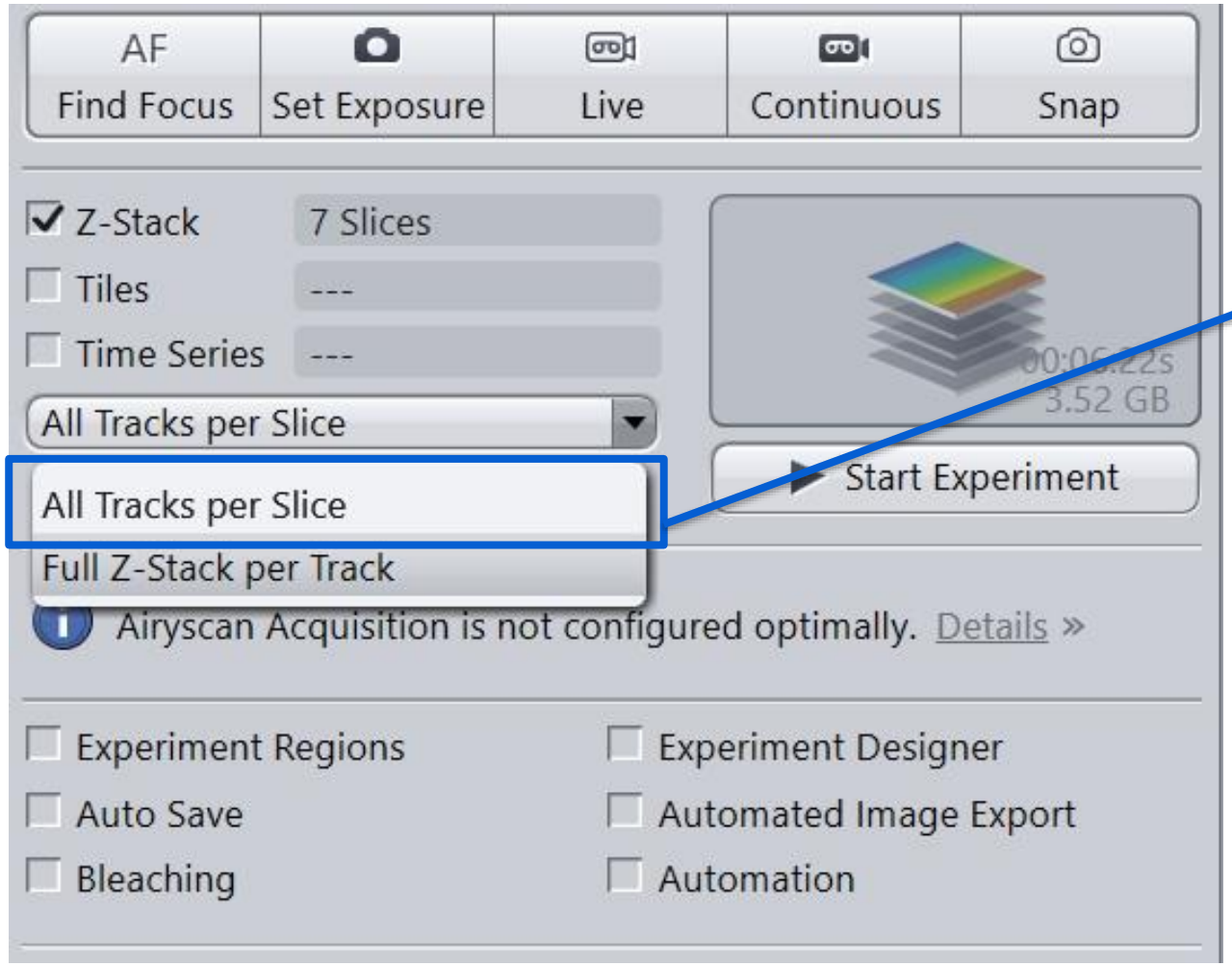
0.7 um

- Interval 可參考 optimal
- 也可依情況自行決定間隔

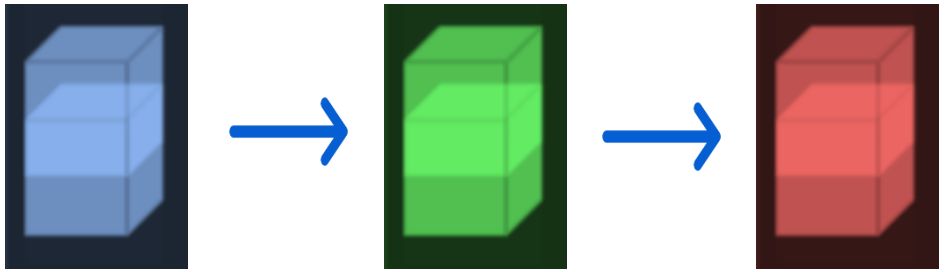
設定 Z-stack的上下界限

- 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

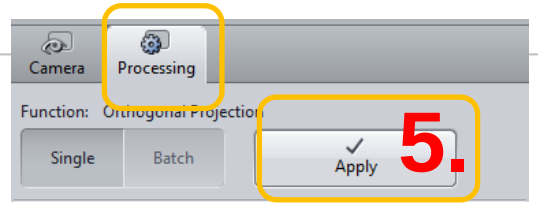


- **一般建議使用 All Tracks per Slice 以避免不同顏色Z錯位**
- 若因實驗需求，以Full Z-stack per Track方式掃描，可得到最快速度
  - 每個 Channel 的 Z Stack 掃完才換下一個 Channel 的掃 Z Stack
  - 若搭配 line scan, bi-direction scan，為最省時間之掃描方式



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

1.



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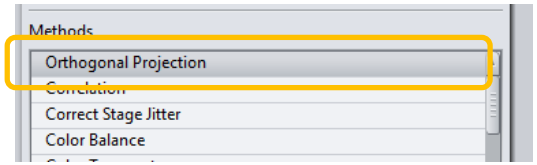
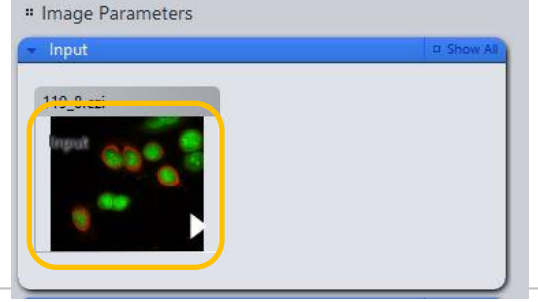
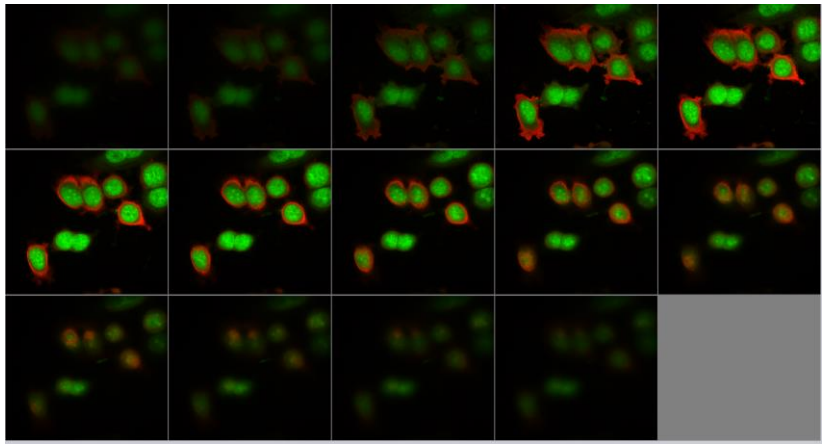


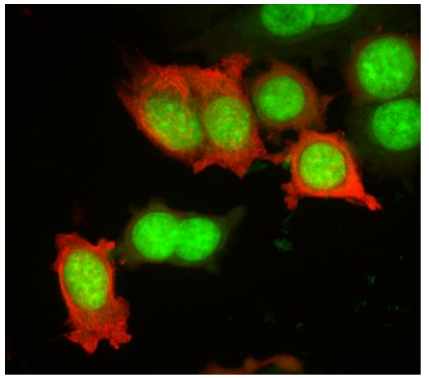
4.

手動調整到所需張數

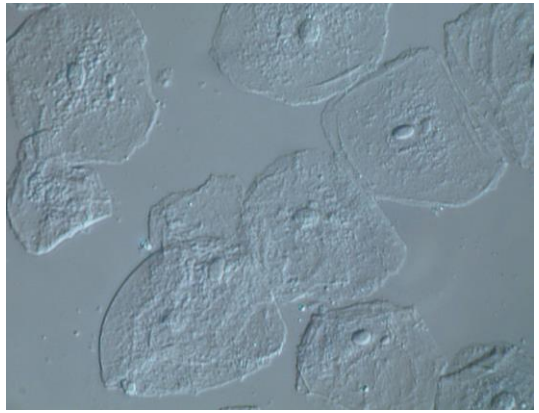
3.

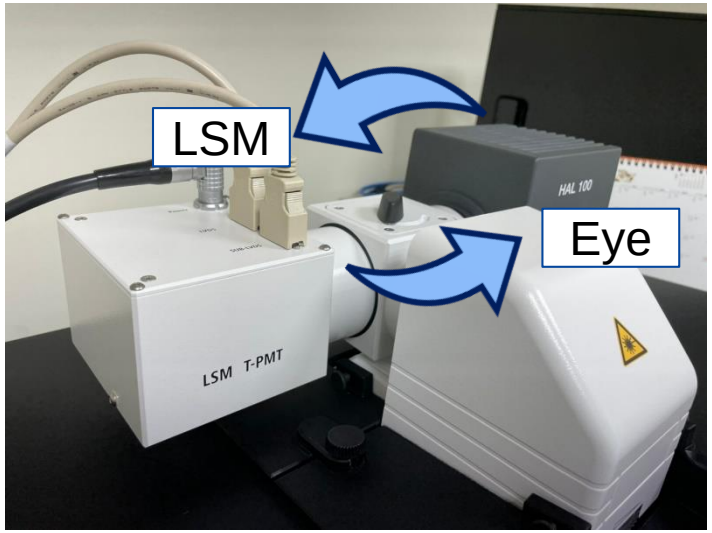






加拍穿透光
Bright Field / DIC Observation

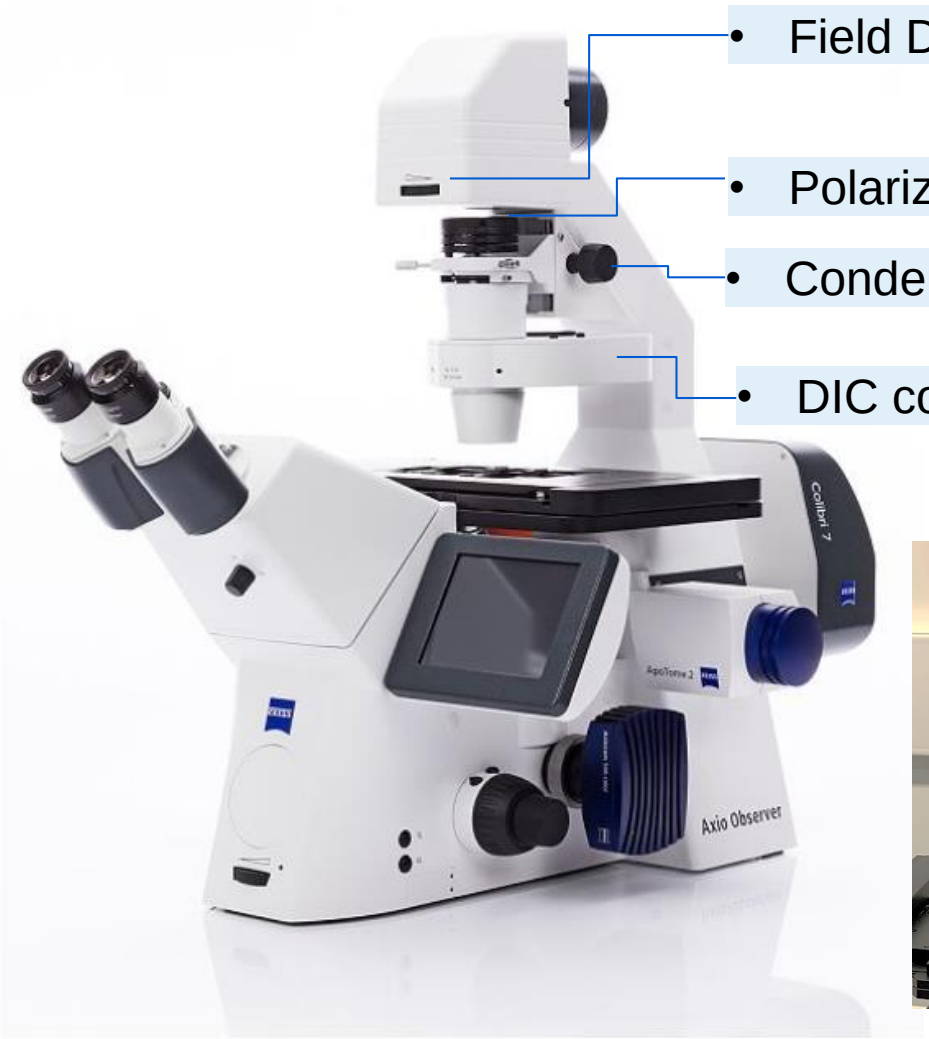




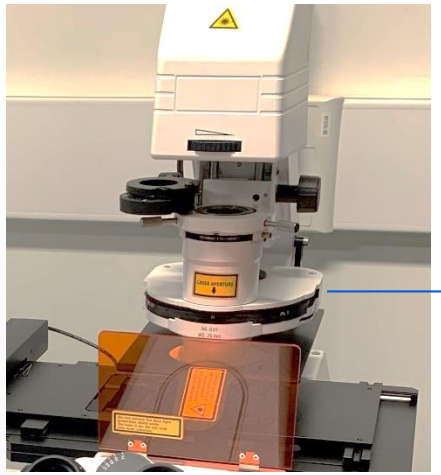
Turn the T-PMT Rotary Switches to

- Right for eye observation
- Left for confocal imaging

Bright Field / DIC Observation
Microscope setting for DIC



- Field Diaphragm
- Polarizer
- Condenser focusing
- DIC contrast

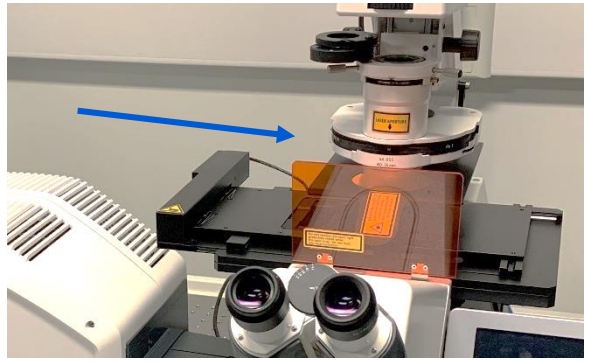


1. Focus the sample with objective
2. Adjust the condenser height by condenser focus knob.
3. Check the condenser center position by closing the field diaphragm and reopen it after focusing the condenser.
4. Choose the DIC filter position (as last page)
5. Swing the polarizer holder in
6. Choose condenser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



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+

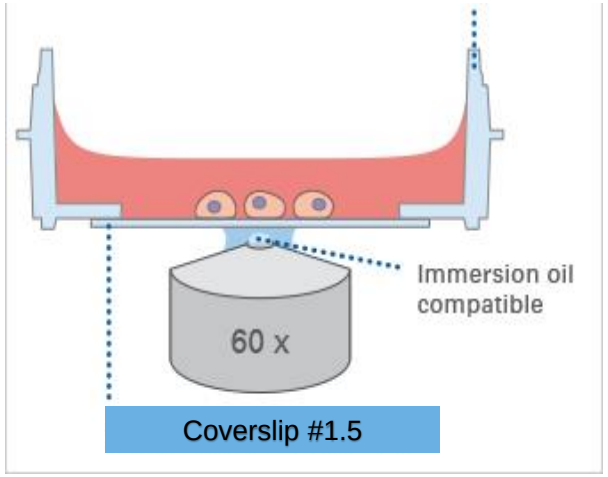
POC-R

µ-Dish 35 mm, high

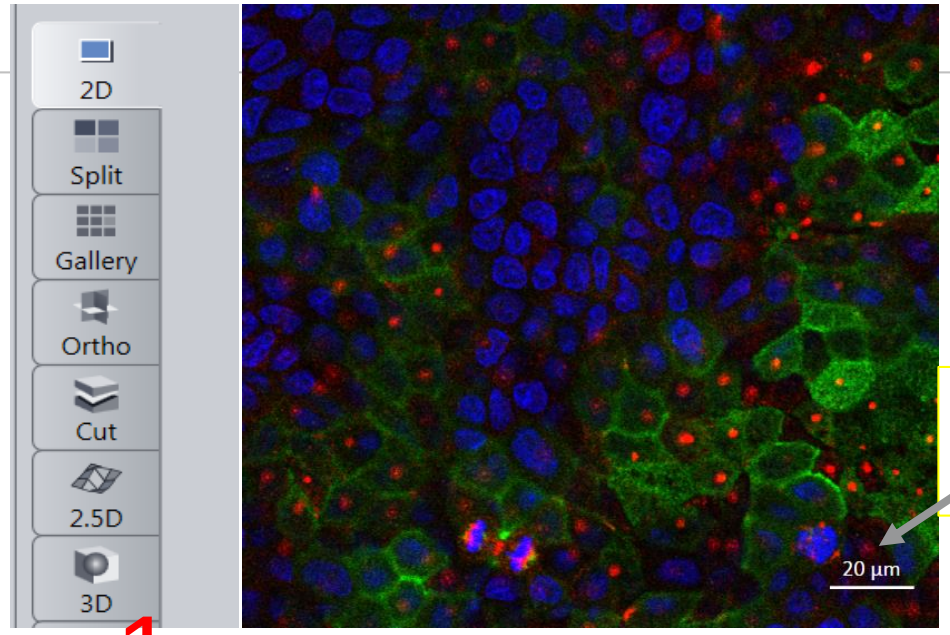
POC-R mini

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

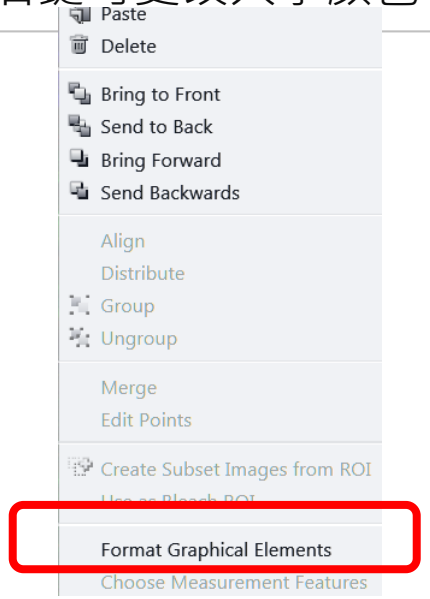


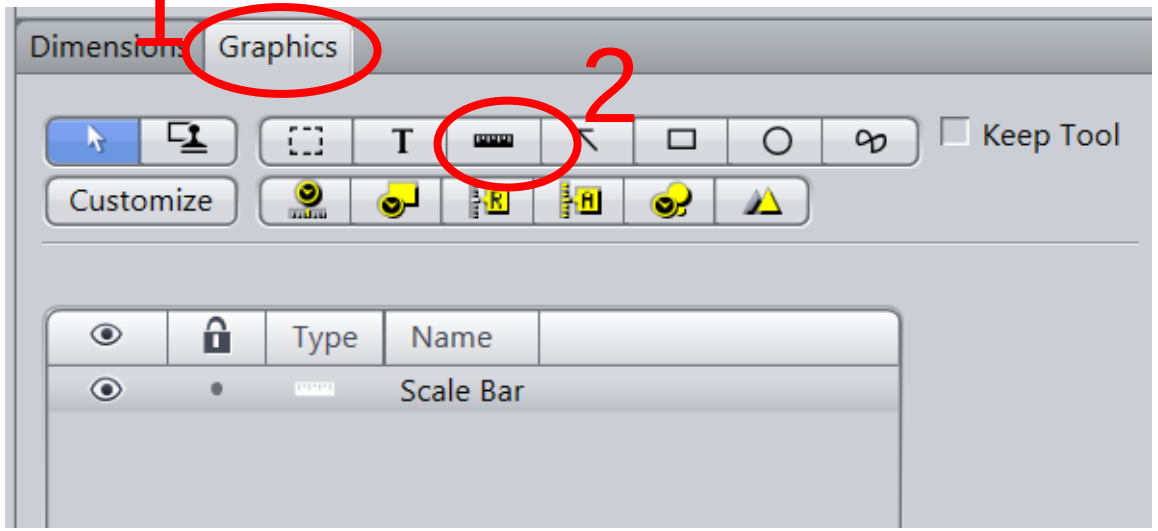
Add Scale Bar加入尺規



3 scale bar會自動出現

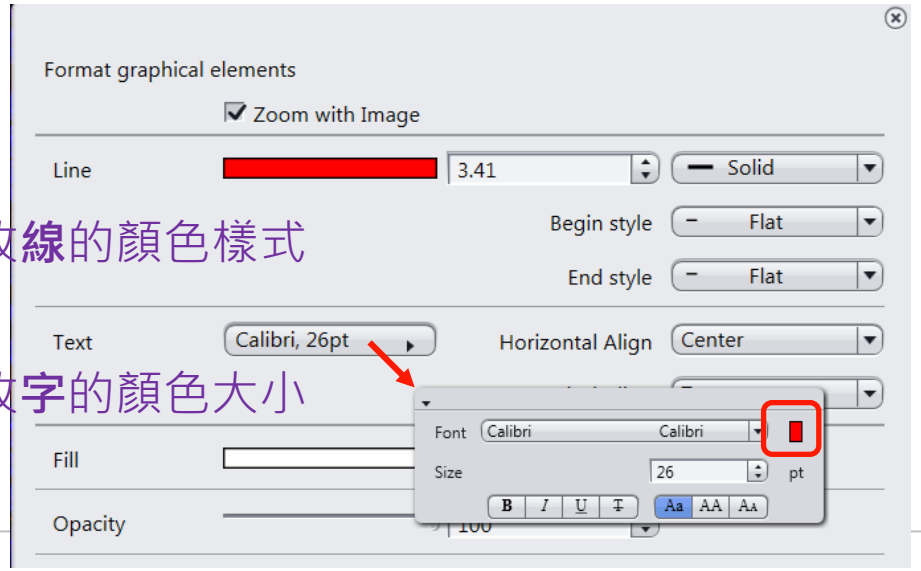
4 於scale bar按右鍵可更改大小顏色粗細等格式





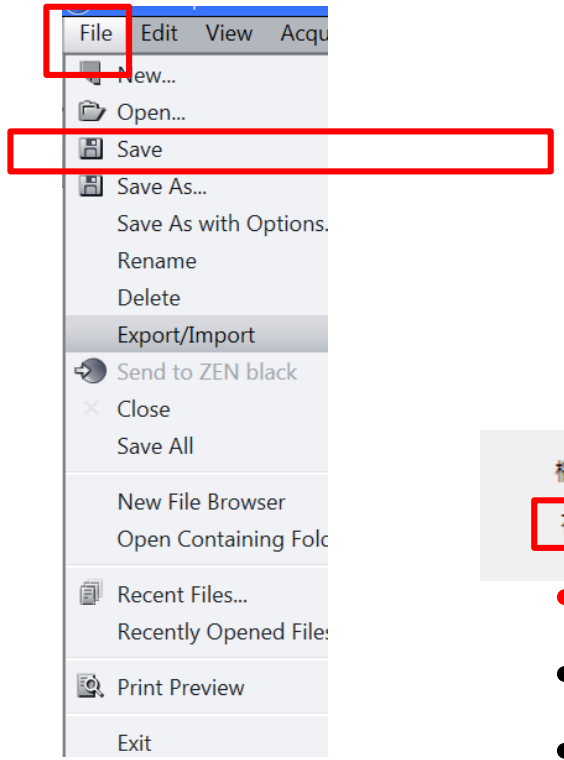
更改線的顏色樣式

更改字的顏色大小

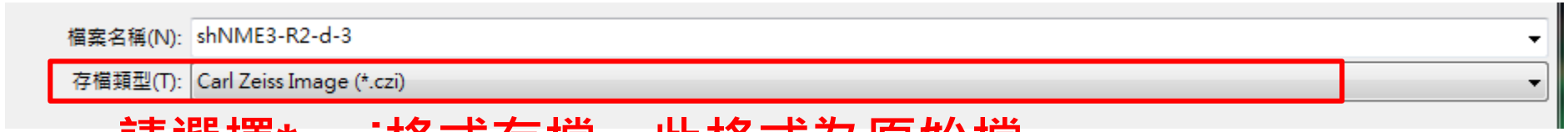


存檔

5



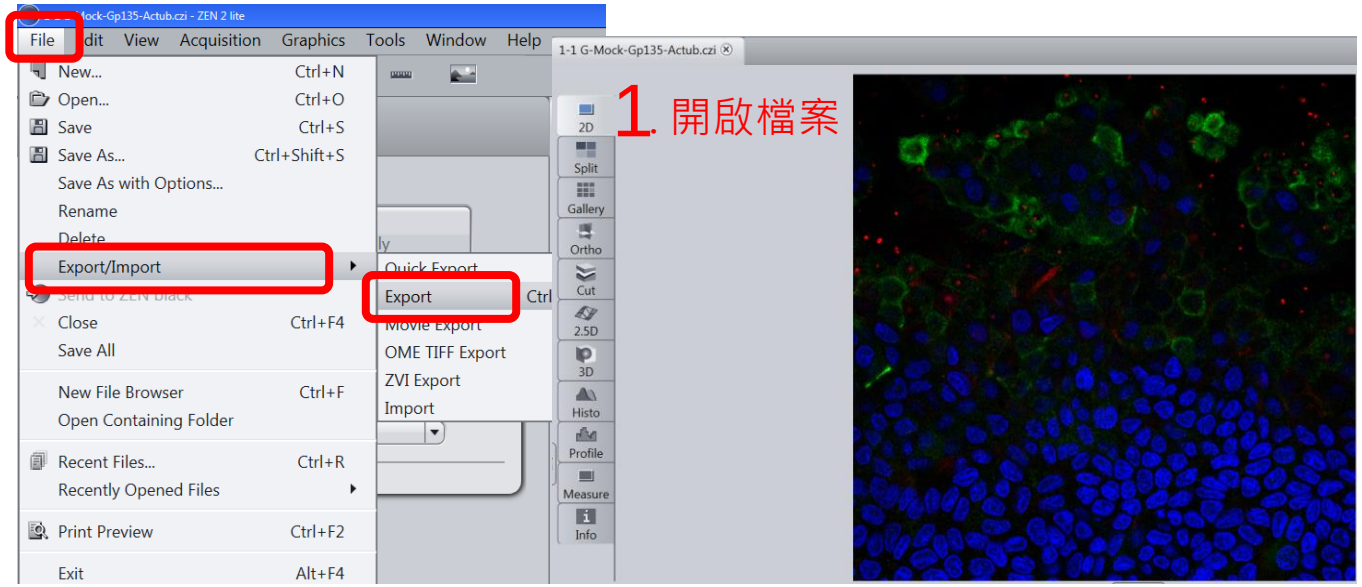
存檔

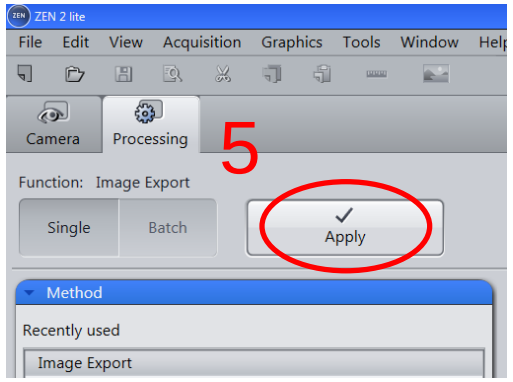


- **請選擇*.czi格式存檔，此格式為原始檔**
- *.czi 日後可reuse
- 請勿直接存成 tif/jpg檔，以免日後無法定量及添加尺規
- 需要純圖檔請進行圖檔輸出export或大量批次轉檔batch export，詳見下頁

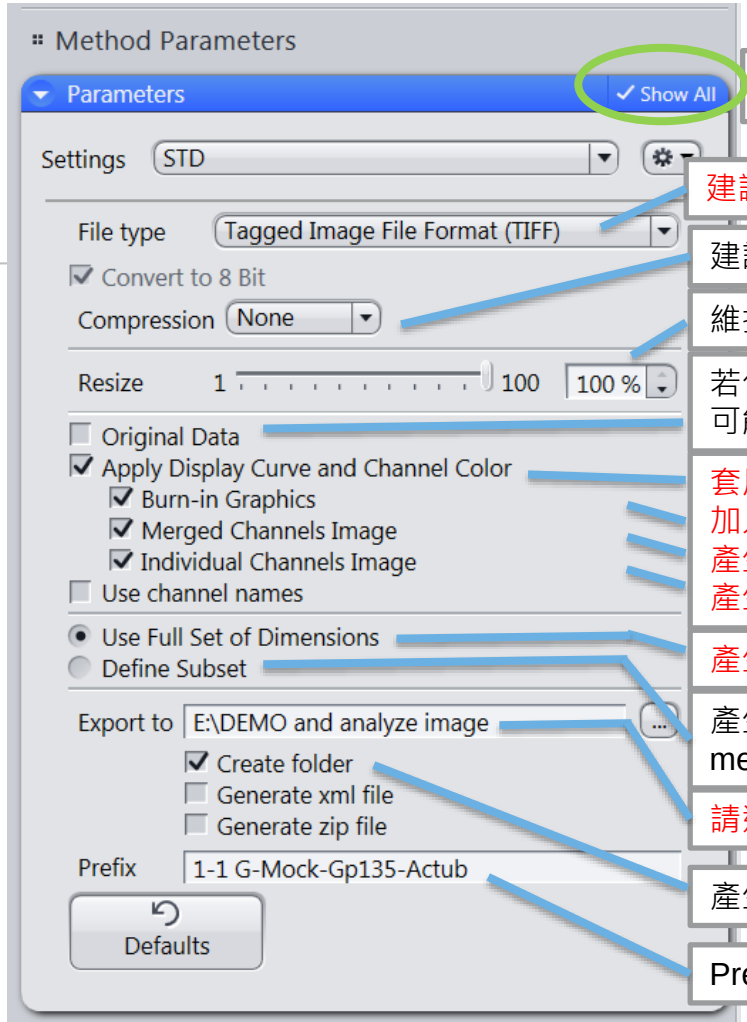
圖檔輸出export (單一檔案)

2





4



☑ 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

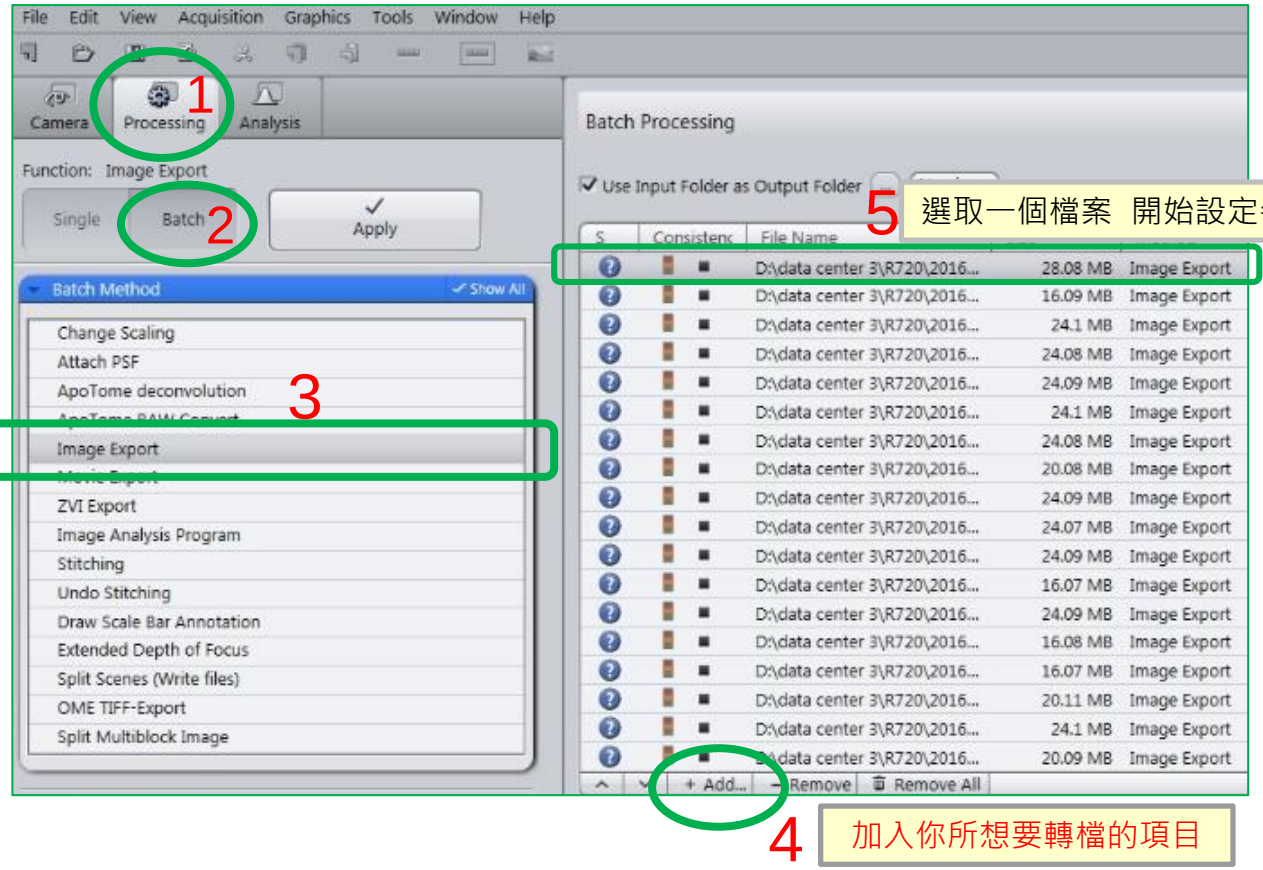
產生資料夾

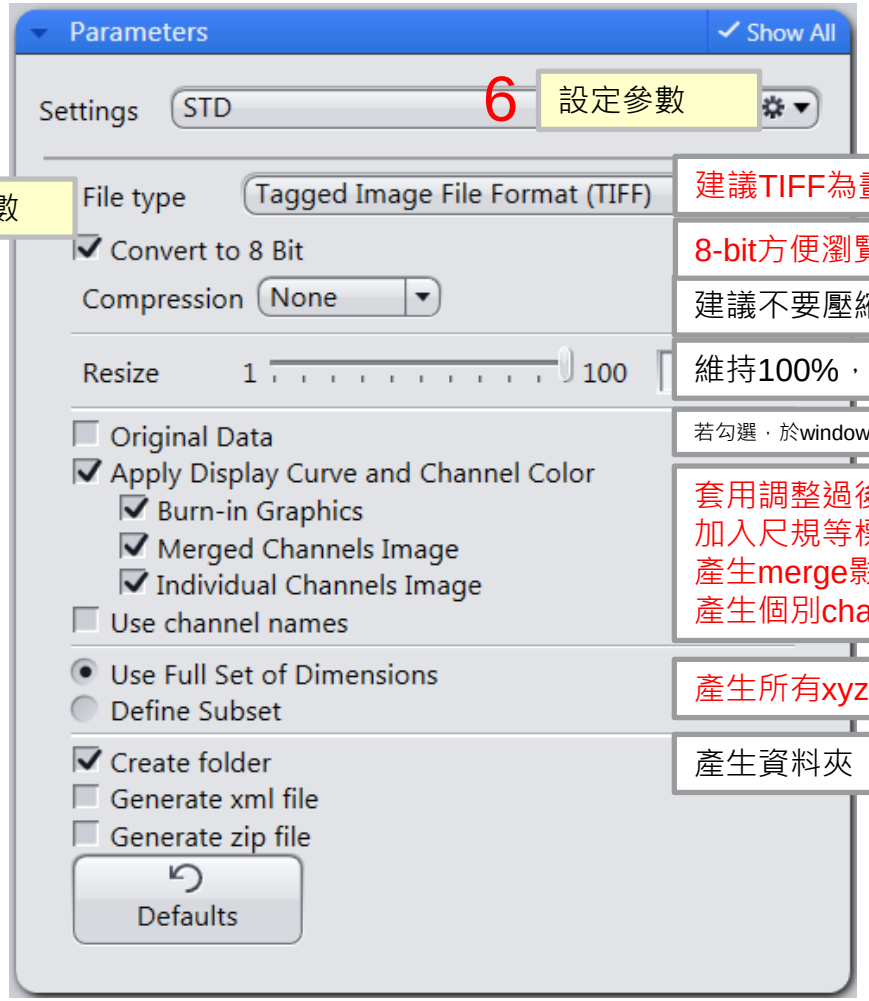
Prefix為預設檔名

3

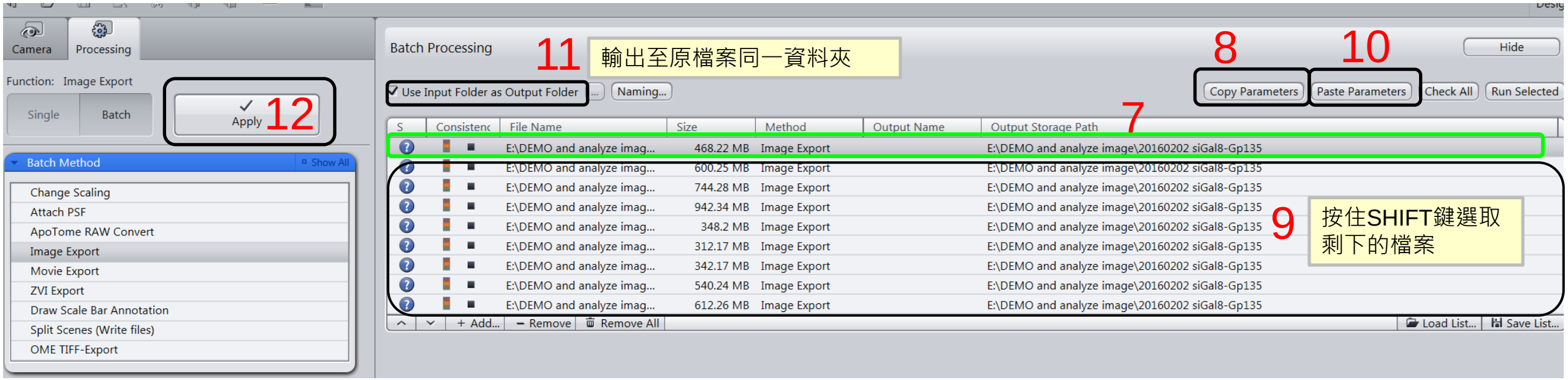
確認input 檔名是否為您所要輸出的檔案

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！


ZEISS