

共軛焦顯微鏡簡易操作手冊 LSM 800 Takeoff Guide



Taiwan Instrument Company, 余雅倩

LSM 800/Axio Observer 開關機步驟



1. On/ Off

2. On/ Off

3. Turn Key

On: 水平
Off: 垂直

開機1→5順序開啟
關機5→1順序關閉

4. 電腦

5. ZEN 2.6 軟體啟動

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1. 進入軟體ZEN




2. 開啟軟體與硬體連結



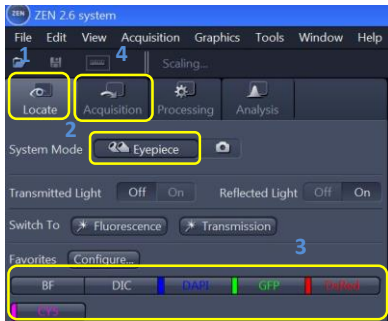
啟動機器掃圖

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3. 控制顯微鏡找到樣品焦距



- 1. 切到**Locate**的頁面
- 2. **Eyepiece**，從目鏡觀察樣品，找到焦距。
- 3. 點選切換各螢光濾片組與透光設定。
- 4. 確認樣品位置後切換至**Acquisition**，即可進入LSM影像擷取模式。
- (Option: 5.如需要記憶多點位置，可使用Stage的視窗，”+”將位置記錄下來)



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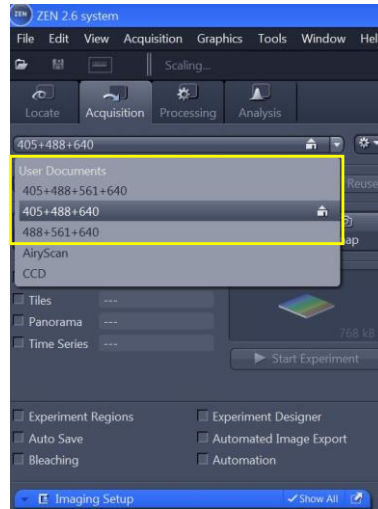
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4. 套用適合的掃圖條件

點選樣品有表現的
螢光組合掃圖條件，
例如：405+488+640

*****請勿任意更改、
儲存、或刪除設定



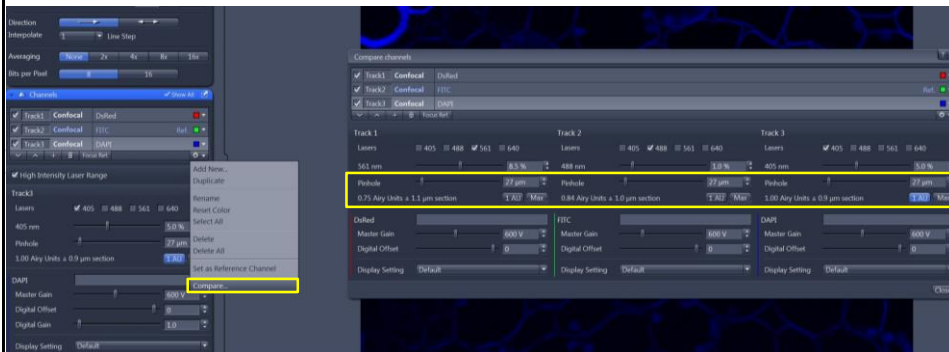
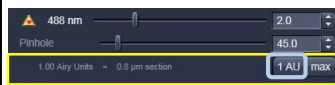
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5. Acquisition setting

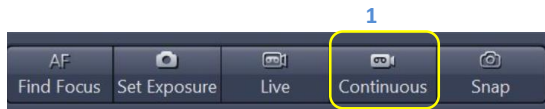
- 1 AU或調整piphole至相同的光學切片厚度



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6. 預覽掃圖



2 fine focus drive
細調節輪



1. Continuous

- 按下Continuous 進入預覽模式，同時使用2. focus drive精調螢幕中影像焦距與掃圖條件

3. STOP

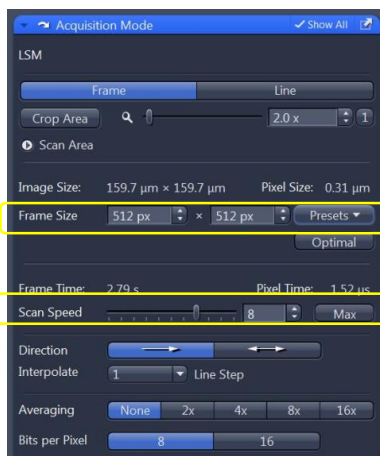
- 找到焦距與設定後停止掃描



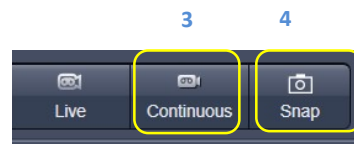
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6.1 掃描參數值設定



- 1.&2 於frame size 512*512畫素、speed 8下進行影像快速掃描，以方便即時預覽更改參數後的結果 (speed 數值不要超過8)
- 3. Continuous: 持續掃描，要按停止才會停止掃描
- 4. Snap: 拍一張影像



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6.2 掃描參數值設定



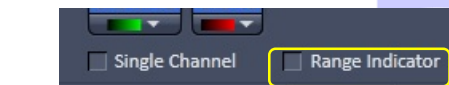
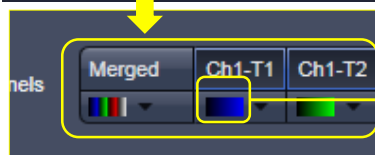
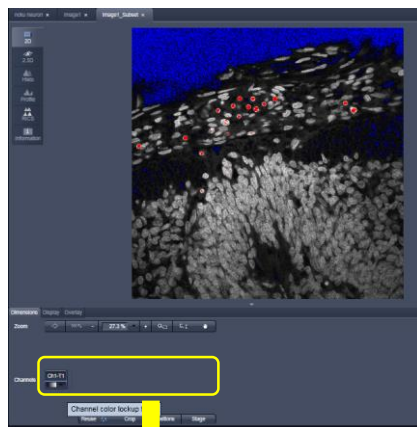
- 1. 依序設定每個track的掃圖參數
- 2. 調整PMT gain值，數值越大訊號被放大得越多，影像越亮，可使用滑鼠中鍵滾輪滑動調整。(建議數值'650~800')
- 3. 調整 laser強度，數值越大，影像越亮。
- 4. 調整好後進行下一個track的設定，重複1~3步驟直到每個track都設定完畢。

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6.3 掃描參數值設定



- 拍圖調整掃描條件時建議勾選 **Range indicator** 以套色方式表現色彩，將有助於將顏色之intensity調到最佳分布，。紅色表示飽和(調整 detector gain和laser量)，**background 藍**表示背景乾淨(調整 digital offset)
- 建議調整到全畫面當中訊號少部分飽和，background 部分為藍色。

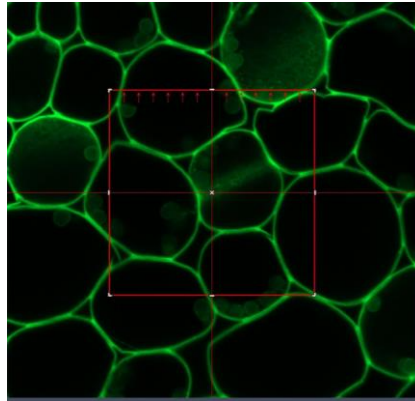
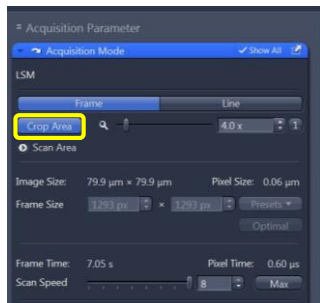
彩色方型內點下可做套色

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6.4 掃描參數值設定 ZOOM、掃描區域選擇



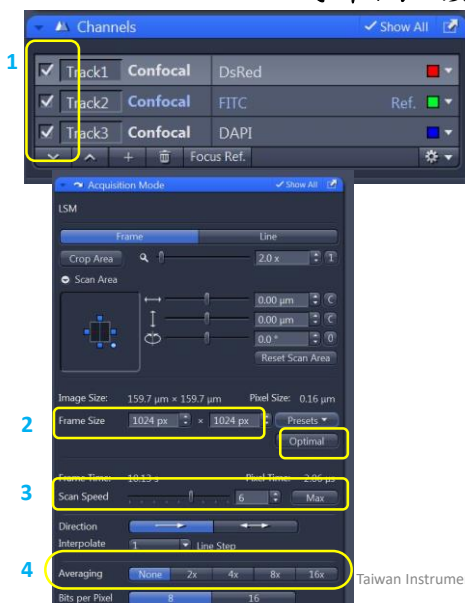
- Crop功能包含zoom in/ zoom out, rotation
- Zoom 勿過度使用,一般不會超過3 否則將造成bleach樣品的效果

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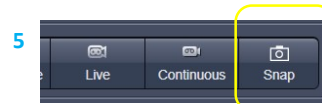
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7. 正式掃圖:獲得高解析度影像

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1. 將所有已經調整好參數的track 都打勾
2. 選擇需要的畫素,一般需要發表可固定1024*1024.或是選擇Optical
3. 調整掃圖至慢速度,高品質影像建議scan speed為5~7
4. 一般均設定1,若影像品質不佳可採用平均數次可以使影像品質提升,降低雜訊
5. SNAP拍一張,獲得漂亮的数据!!




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ZEN blue

Z-stack



Acquisition of image series from different focus positions

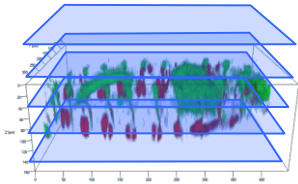
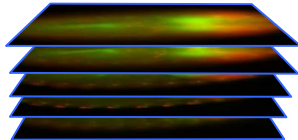

- Requires motorized Z-drive

Flexible Z-stack configuration


- 2 modes
 - Start/Stop and Center
- Optimal z-interval adjustment for 3D DCV
 - According to Nyquist criterion
- Z-stack navigation control
 - Start, Center, Stop, Stepwise
- Mixed acquisition mode
 - Z-stack and single plane channels

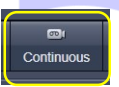

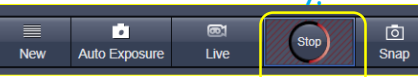
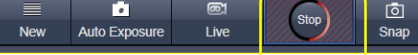
Optimum precision and reproducibility

- Automatic adjustment of minimum step size according to microscope type
- Integrated Z-drive backlash compensation
- Automatically determine z-stack range and slices via software autofocus

8. Z stack



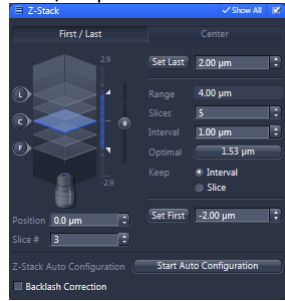
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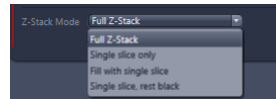
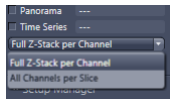
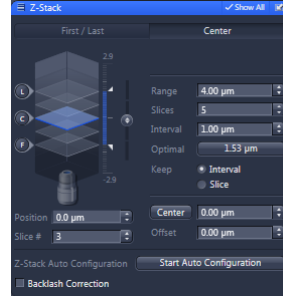
8.1 Z stack



Start/Stop mode



Center mode



可以選擇
Full Z-stack per Channel:同色(track)
 掃完Z再切到下一個顏色。
All Channels per Slice: 每一層所有顏色掃完後，再換下一層

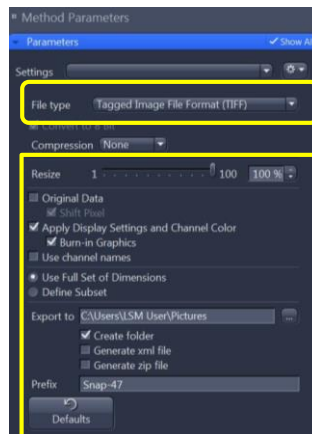
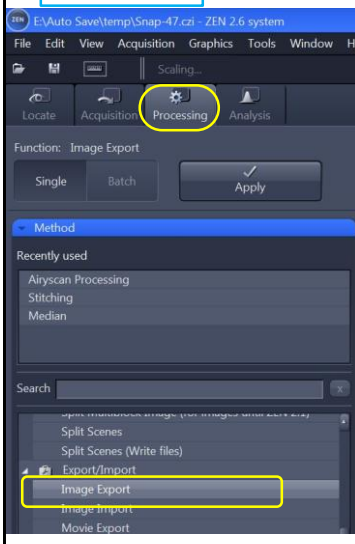
每一個顏色(track)可以選擇單張，或是掃Z堆疊

9.存檔—

Carl Zeiss Image (*. Czi) 完整檔案, 可以reuse!



File → Export



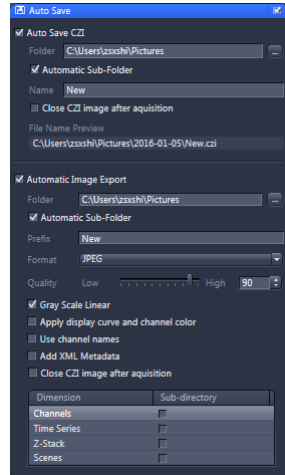
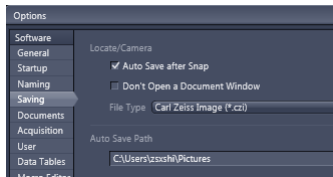
Format : 下拉選擇欲存檔之類型 (tif、jpg...), 建議以tif檔儲存, 減少壓縮損失, 亦可儲存serial section 成動畫影片檔(.avi)



9.1 Auto Save and Automatic Export

Under "Acquisition" tab, you can option for Auto Save or Automatic Export

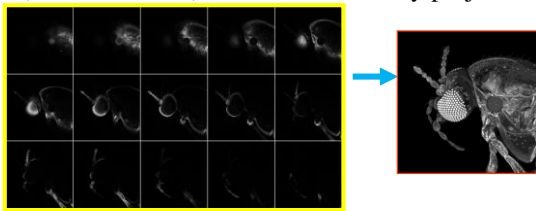
You can also auto save snap images under "Locate" tab. To do so, go to Tools → Options, under the "Saving" tab, check "Auto Save after Snap", and choose file type and Save Path:



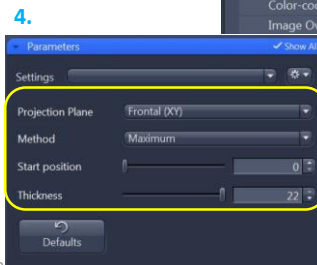
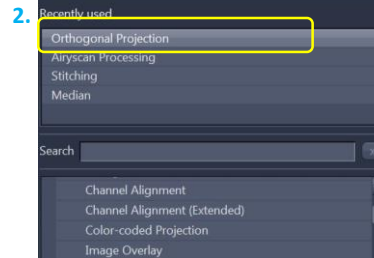
10. 把多張Z section 疊成一張



製造全景深影像: maximum intensity projection



1.



5.



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