



ZEISS LSM 900 System Tutorial

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開機順序:123456
關機順序:654321
(***為維護雷射使用壽命,如果不是當
天最後一個使用者,請不要將系統關
閉,只需清理物鏡和環境)





Start System - Software



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Light

path

40x

Oil

Load

position

TL Illumination

RL Illumination

Definite Focus

On

On

On

3.5

Off

Off

Off

De-

flector

20x

Objec- Colibri

tives

5x

63x

Oil

FL

Home

Control

Automatic

XYZ

Incubation

Display

5x/0.16

LED

10x

100×

Oil

Pos. 1: Pin Apo 5x/0.16

BF

Pos. -6-

Contrast Manager



點選"Objectives"選擇物鏡

從非油鏡切換至油鏡前會出現如下方提示畫面, 務必注意鏡油是否已點上; 反之,請先擦除油鏡上及玻片上的油再切換至非油鏡

按"Done"完成切換 若誤切換物鏡,"Back"可返回前物鏡







1. 放置樣品前,先將樣品清潔乾淨

2. 點選Load position將物鏡降低 (避免撞到物鏡)









Snap

Locate - Microscope Control



Locate Acquisition Processing Analysis Applications	—— 點選Locate
System Mode System Mode	Eyepiece:切換至目鏡,眼睛觀察樣品
Transmitted Light Off On Reflected Light Off On	—— 螢光燈開關
Switch To * Fluorescence * Transmission	
Favorites Configure	
BF DIC DAPI GFP Cy3	—— 光路檢視樣本
CY5	選擇濾片:
	穿透光:BF-明視野、DIC-干涉相位差、
	螢光:DAPI-藍螢光 、 GFP-綠螢光、Cy3-紅螢光、

Cy5-遠紅光

Acquisition - Experiment Manager



Acquisition - Channels



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Acquisition - Acquisition Mode



Acquisition - Channels



14

✓ Lhannels ✓ Show All	先前在Experiment Manager選擇拍照雷射後 光路組態(Tracks)已自動產生,並自動開啟所需雷射
 ✓ Track1 Confocal AF488 Track2 Confocal Rhoda Track3 Confocal DAPI Ref. ✓ ▲ + Focus Ref. 	— 8. 設定拍照需要的波長種類(Track) 一次單獨勾選一個Track,取消勾選其他Track
High Intensity Laser Range	
Track1 Lasers 405 488 561 640	— 9. 軟體自動啟動對應的雷射
488 nm 0 0.2% 640 nm 0.2% Pinhole 0.2% $1.00 \text{ Airy Units} \triangleq 4.0 \ \mu\text{m} section 1 \text{ AU} Max$	 10. 點選"1 AU",將Pinhole調整成1 Airy Unit Pinhole設定: 一般選擇1AU 亦可以設定成所有channel 為相同的um厚度
	11. 調整 雷射強度 及 Gain (建議值)
Alexa Fluor 488	405 0.2~0.5% 600
Master Gain 710 V 🗘	488 0.2~0.5% 600
Digital Offset	561 0.2~0.5% 600
Digital Gain - 1.0	640 0.2~0.5% 600
	(雷射切勿過強,長時間過曝將造成樣本bleach及感測器燒燬)
Display Setting Default	1

Acquisition - Continuous and Snap

	- 🛆 Channels		- Show All
Locate Acquisition Processing Analysis Applications	Track1 Confocal	AF488	
2-405 561+488 BE 640 *		Cy5	 _ •
		T-PMT	
★ Smart Setup ★ Reuse	Track2 Confocal	DAPI	Ref. 🗖 🔻
	Fo	AF546 cus Ref.	■ ▼ *
Find Focus Set Exposure Live Continuous Snap	High Intensity Laser F	Range	
Tiles	Track1		
Time Series	Lasers 📃 40	5 🗹 488 🔲 561 🦻	√ 640
2.5 MB	488 nm	•	- 0.3 %
	640 nm		- 0.3 %
12. "Continuous"預覽掃圖畫面	Pinhole		- 24 μm 🗘
*切勿點選左邊"Live"按鈕·Live速度過快將導致scanner毀損	 1.13 Airy Units ≙ 4. 	3 µm section	1 AU Max
13. 適富調整對焦、雷射強度及Gain ————————————————————————————————————			
-14. "Stop" Continuous 停止預寬		0	
	Master Gain		665 V -
▶ 若還需拍攝其他波長,改選其他track,	Digital Offset	U	- 0 🗘
調整Gain值&雷射強度至適當亮度。	Digital Gain – () –		1.0
	Display Setting Defa	ault	•

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Acquisition - Continuous and Snap



若在預覽圖下方"Dimensions"頁面裡勾 選 "Range Indicator" 將有助於將intensity調 到最佳分布,

- 紅色表示飽和,建議降低亮度
- 藍表示背景無訊號



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Acquisition - Acquisition Mode



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Acquisition - Continuous and Snap

C Locate	Acquisition	Processing	Analysis	Applications	
2-405_561+4	88_BF_640 *			• * •	
(⊁ Smart Set	up			🍰 Reuse	
AF		et	ല്വ	0	
Find Focus	Set Exposure	e Live	Continuous	Snap	— 16. 按"Snap"元成汨撮
Z-Stack					
Tiles					
Time Series	5				
				2.5 MB	
			Start E	xperiment	

REVIEW SNAP Steps

- . Load Experiment setting 選擇掃圖需要的波長
- . 調整預覽解析度≦512x512, 並設定掃圖速度≦7
- . 單獨調整預覽Track的雷射強度、Pinhole、Gain
- . Continuous開始預覽、對焦,再調整雷射、Gain
- . Stop Continuous · Snap!

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Experiment Z-Stack

Acquisition - Z-stack





- 1. 先完成Snap的設定步驟1~14
- 2. 勾選Z-Stack啟動Z軸拍攝功能

Acquisition - Z-stack





Acquisition - Z-stack





5. Stop "Continuous", 並確認掃圖解析度

6. "Start Experiment"開始拍攝Z-Stack ➤ 若拍攝時需終止拍攝可按"Stop"停止拍攝



- ➤ Stop:停止拍攝(當下的那張拍完即停止拍攝)
- ▶ Pause:暫停拍攝 (直接停在當下拍到的位置)

Processing - Orthogonal Projection



Z-stack可透過疊圖整合為一張全景深影像

* 6 1. "Processing" Acquisition Processing Analysis Applications Locate Function: Orthogonal Projection \checkmark Single Batch Apply **Orthogonal Projection** 或下方搜尋 Image Export Stitching Split Multiblock Image (for images until ZEN 2.1) Split Scenes Search **Z-Stack Alignment** Sharpen Smooth Time Series н. Utilities F Export/Import F APEER F h Image Analysis

2. 在Method中點選 "Orthogonal Projection" 或下方搜尋

Processing - Orthogonal Projection

	Locate Acquisition Processing Analysis Applications	Z-stack可透過疊圖整合為一張全景深影像
	Function: Orthogonal Projection	
	Single Batch Apply	
	" Method Parameters	
	Parameters Show All	
	Settings 💽 🔹 🔹	
	Projection Plane Frontal (XY)	
	Method Maximum 🔻	
4	Start position	
-	Thickness (Start position : Start
	ン Defaults	Thickness : Number of slice
		例如:
		Thickness: 5
	 Inage Parameters Input Show All 	就只有#2、#3、#4、#5、#6會疊圖
3	Experiment-680.czi	
		— 3. Input 欲處理的Z-Stack 檔条
	Input Definition Set Input Automatically	— 5. 回到視窗上方 · 點"Apply"開始處理影像
	After processing • Switch to Output • Remain at current view	





Image Processing

Image Processing - Graphics





在Image的下方可找到"Graphics"工具列, 使用此工具列可在Image上加入註記(包含 Scale bar及文字)及執行基礎的測量功能, 例如:



т 加入文字

□ 加入方框或設定方框後做測量

Image Processing - Graphics





線的粗細、顏色、 樣式

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字的大小與顏色

Image Processing - Basic Measurement



測量值會顯示在圖片上, 若要改變測量的參數(例如:不想 測量面積,想測量螢光強度等), 則在圖片上選取物件後按下滑鼠 右鍵,選擇 "Choose Measurement Features",及可修改參數。

Feature Selection						? ×
Available Elements Selected Features		Search	Features	All		
lcon Name	Name	Display		Name		
Region 2D	Area	\checkmark	0	Area		
🗆 Rectangle2 🕨	Intensity Mean Value	\checkmark	0	Diameter		
				ID		
				Intensity Mean Value		
				Intensity Mean Value.Blue		
				Intensity Mean Value.Green		
				Intensity Mean Value.Red		
				Perimeter		
			0	Radius		
					OK	Cancel

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Image Processing - Basic Measurement



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Save and Export Images

Save Raw Image





▶ 建議將影像先儲存為原始格式,以利日後的 影像處理及分析

1. 在軟體視窗右上角找到已拍攝完成的影像清單 選擇欲儲存的檔案

2. 點選 "Save" (儲存檔案)





➢ 若需將原始檔轉換為Tiff或JPG等一般影像格式

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4. 選擇轉檔格式 (Tiff, JPFG)

Original Data: 輸出螢光照片 Single Search Burn-in Graphics: 顯示圖片 上的註記及比例尺等繪圖物件 Smooth F Utilities 6. 設定儲存位置及檔名 F

* 0 Acquisition Processing Function: Image Export 1 Apply Geometric Sharpen **Time Series** Export/Import Convert TXM Files to CZI Image Export 7. "Apply" 存檔

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Locate A 1. Processing Analysis Report	Batch Processing					
Function: Single File Export					Conv Daramatora	Dasta Daramatara Chack All
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Z. Batch Apply	S Consistenc	File Name	Size	Method	Output Name	Output Storage Path
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Batch Method Show All	?	C:\Users\COLIBRI\Pictures\	11.39 MB	Single File Export		C:\Users\COLIBRI\Pictures
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ch PSF		C:\Users\COLIBRI\Pictures\	3.09 MB	Single File Export		C:\Users\COLIBRI\Pictures
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- ✓ Apply Display Curve and Channel Color
 ✓ Burn-in Graphics
- Use Full Set of Dimensions
- Define Subset
- ✓ Create folder
- Generate xml file
- Generate zip file
 - ා Defaults

							-	
Locate Acquisition Processing Analysis Reporting	Batch F	Processing				7.	8.	9.
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Apply Display Curve and Channel Color								

- Burn-in Graphics
- Use Full Set of Dimensions
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- Generate zip file
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Congratulations !!