



Carl Zeiss LSM 900 / ZEN Blue

Quick Guide



ZEISS LSM 900 with Airyscan 2

- 開機-----3
- 啟動軟體 -----4
- 螢光樣品拍攝-----5
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Turn on the system



等待約一分鐘



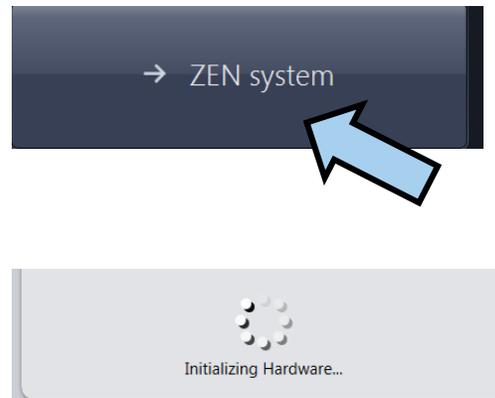
1. System
2. Components
3. Laser
4. Computer
5. ZEN blue
6. ZEN system

1

進入ZEN BLUE



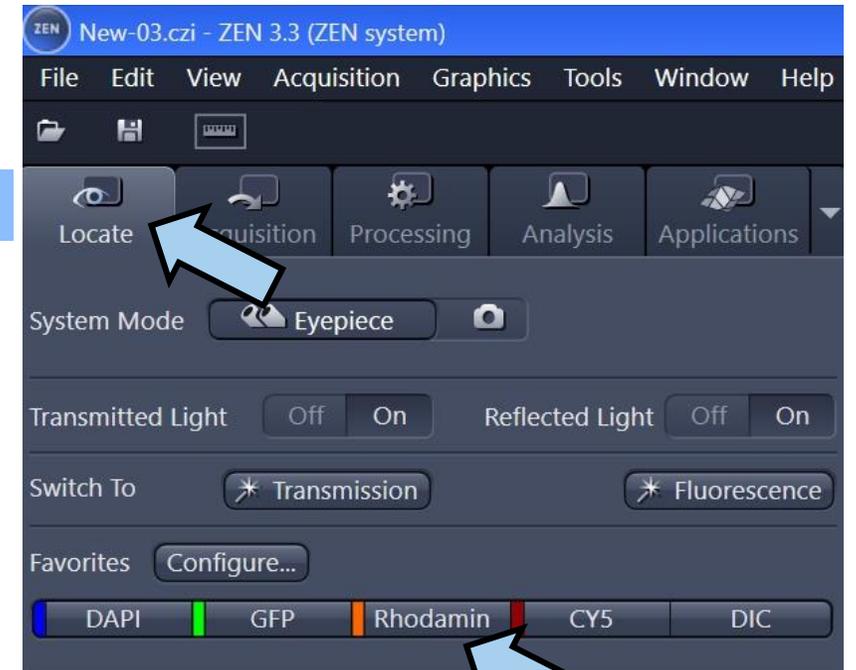
2



等待約一分鐘

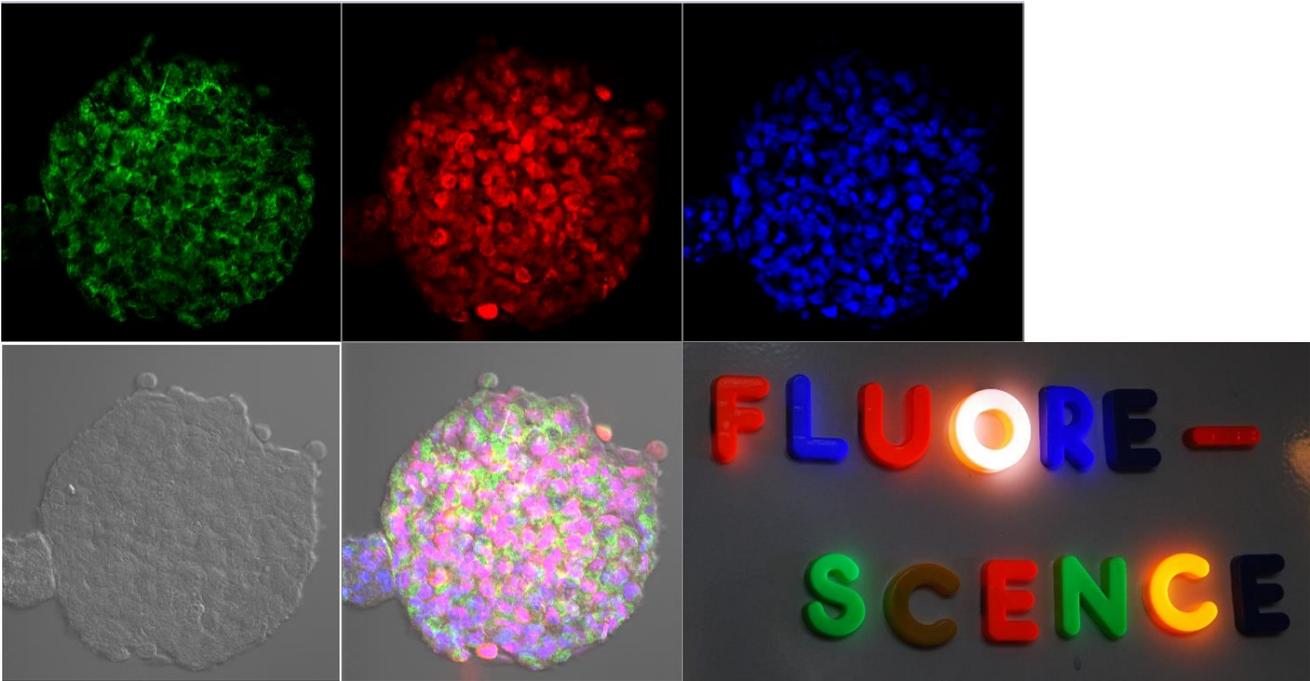
眼睛觀察

3



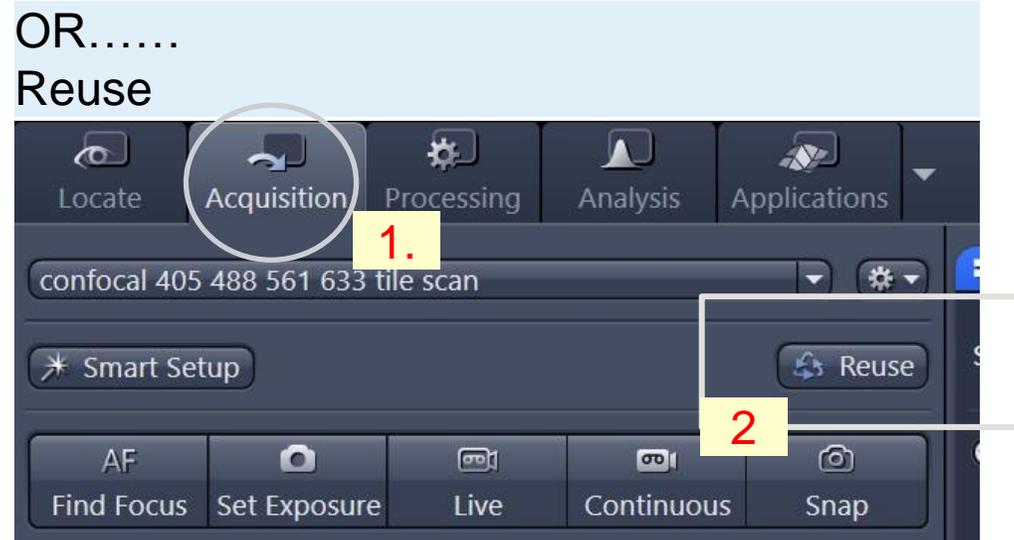
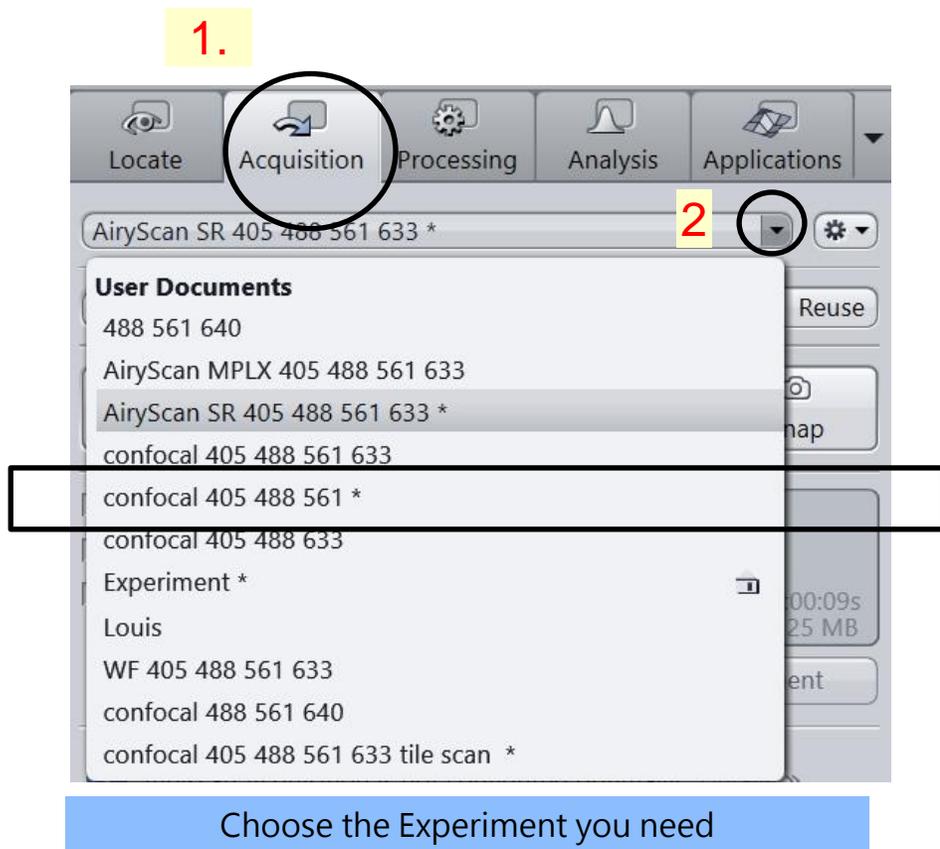
Choose the FL configuration you need

Multichannel Image Acquisition



Multichannel Image Acquisition 1

Load Experiment methods form 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。
- 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。

在所停留的焦距位置，單拍一張，會執行以勾選的Channel，不執行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-Sstack與Tiles, Time Series

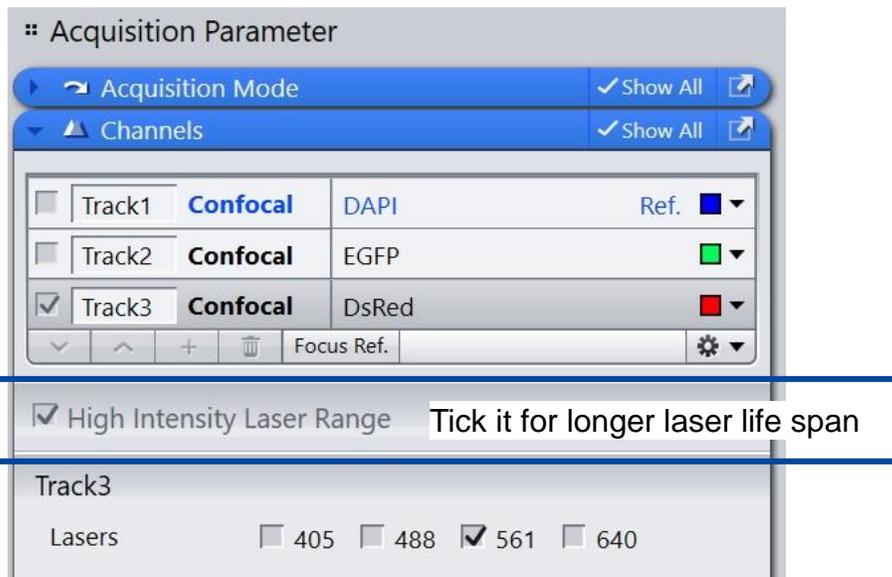
Multichannel Image Acquisition 3

Acquisition Parameter

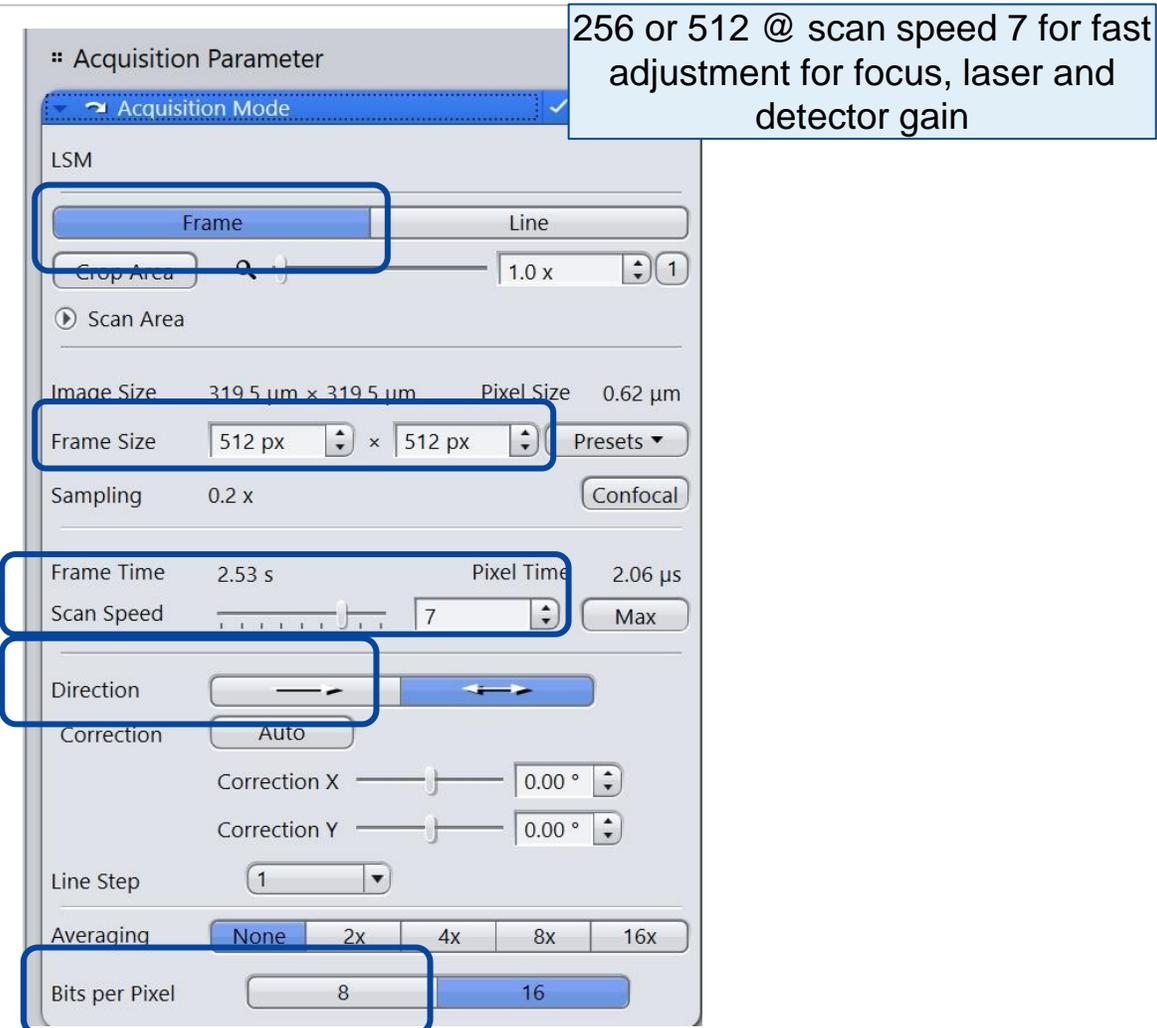


1

選擇需要的channel



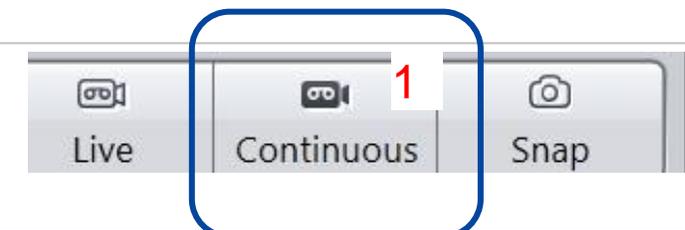
2



檔案小，大面拼圖時或不須定量時可選

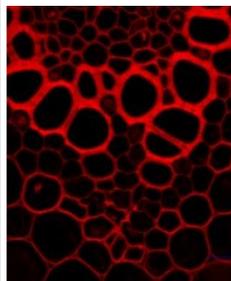
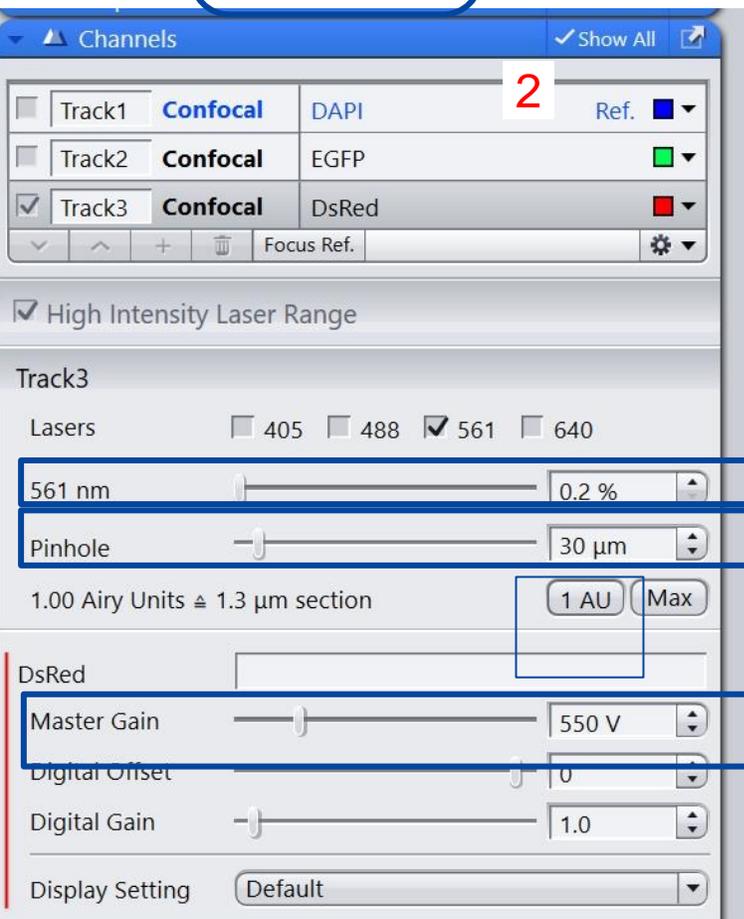
有定量需求可選16

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

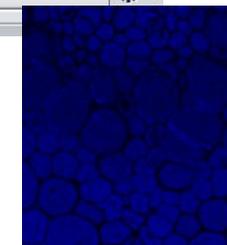
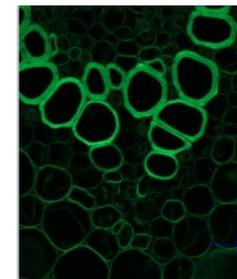
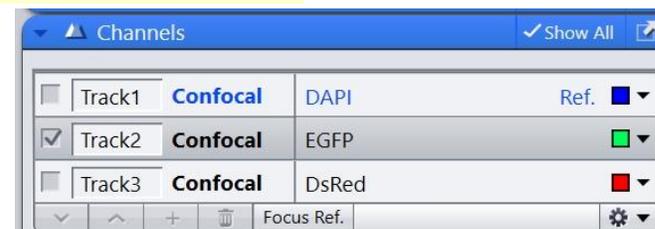
3.



調整雷射強度
大部份情況下10%以內即足夠應付。

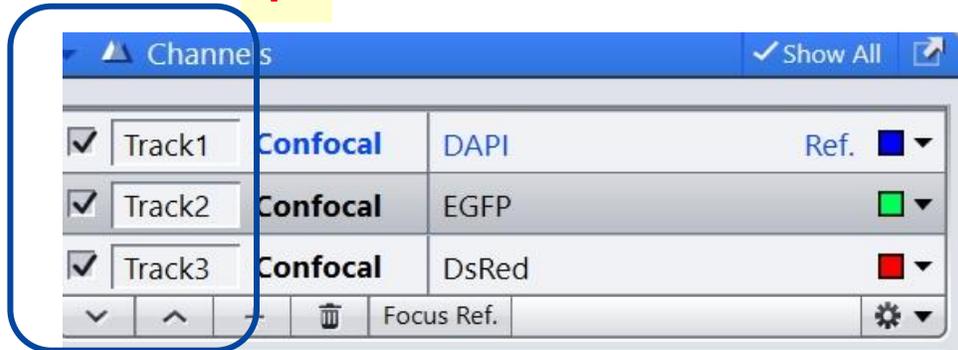
- Pinhole 大小，所有channel 設定相同(建議大約1AU)
- 若亮度足夠設在1AU左右即可
- 調整Pinhole大小 選一個channel 1AU，其餘Channel對齊 (in this case, 30um)

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



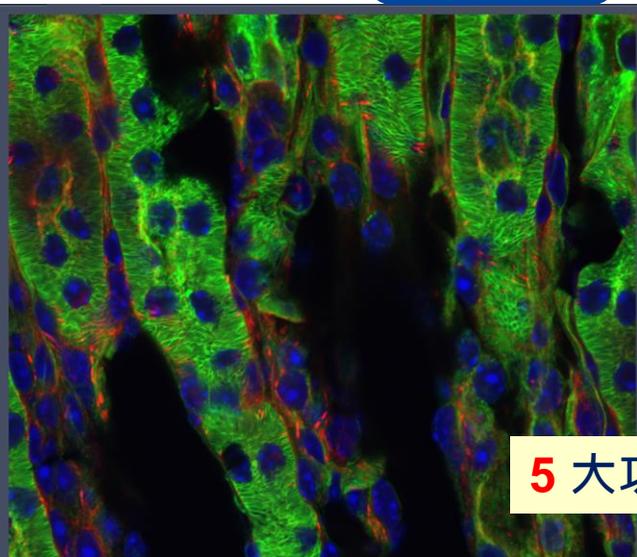
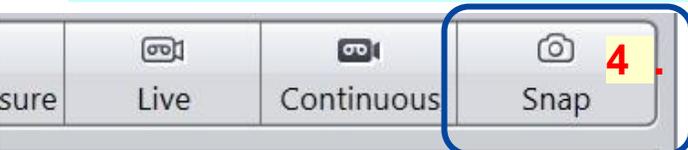
Multichannel Image Acquisition 5 2D Image

1

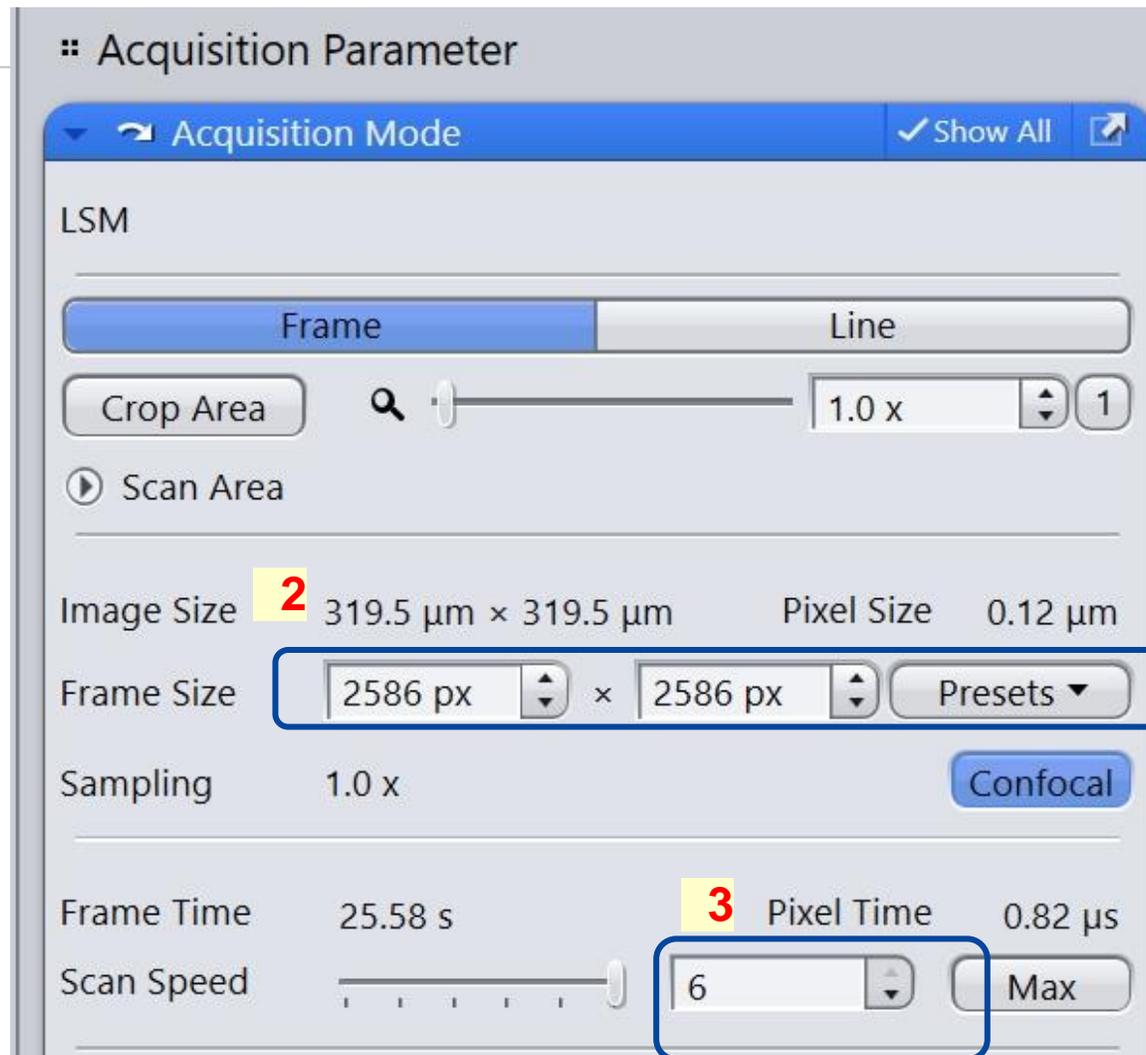


選取所有要準備要拍的track

4



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, 2048, speed 5~7是很安全的設定值。

Focus Strategy ✓ Show All

None

Reference Channel and Offsets

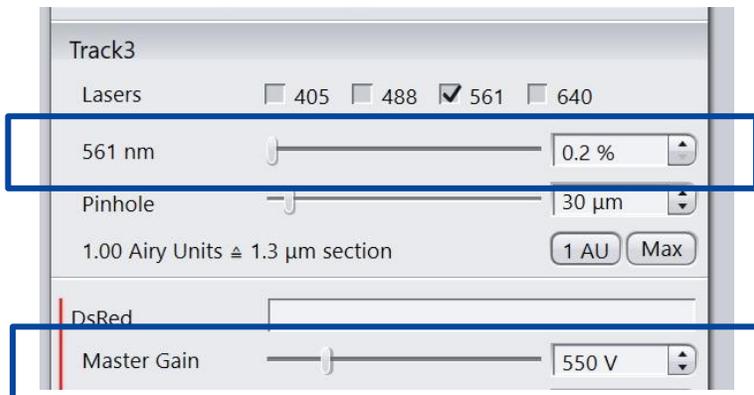
	Name		Offset (μm)
	Ch1-T1		0
✓	<u>FITC-T1</u>	Ref.	0
	T-PMT-T1		0
✓	DAPI-T2		-5.89
	Ch2-T2		-5.89
	T-PMT-T2		-5.89

Set as Reference Channel

雙擊填入焦距誤差值

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



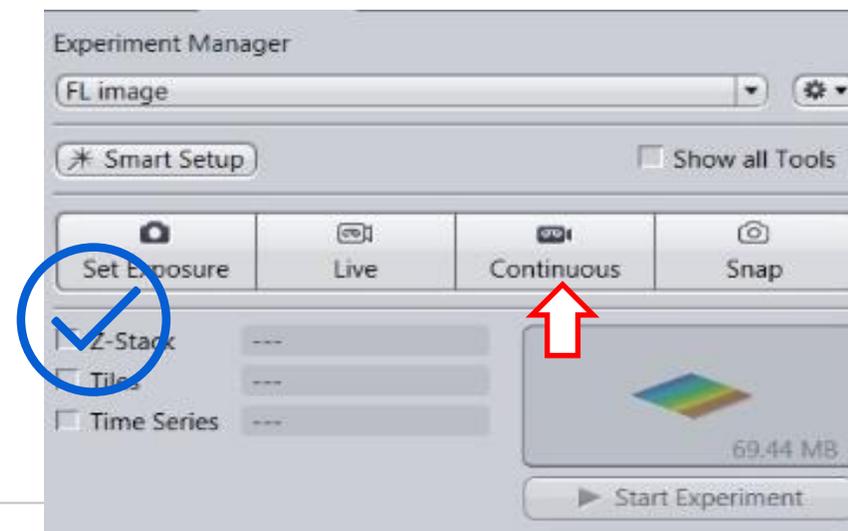
2 選擇一個Track



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

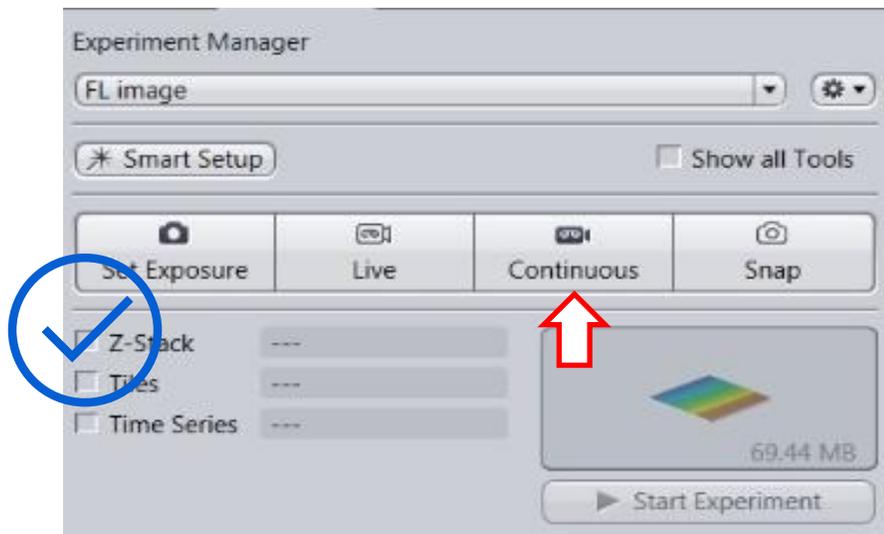


4 Continuous with higher frame rate (ex: 512² @ speed 7)

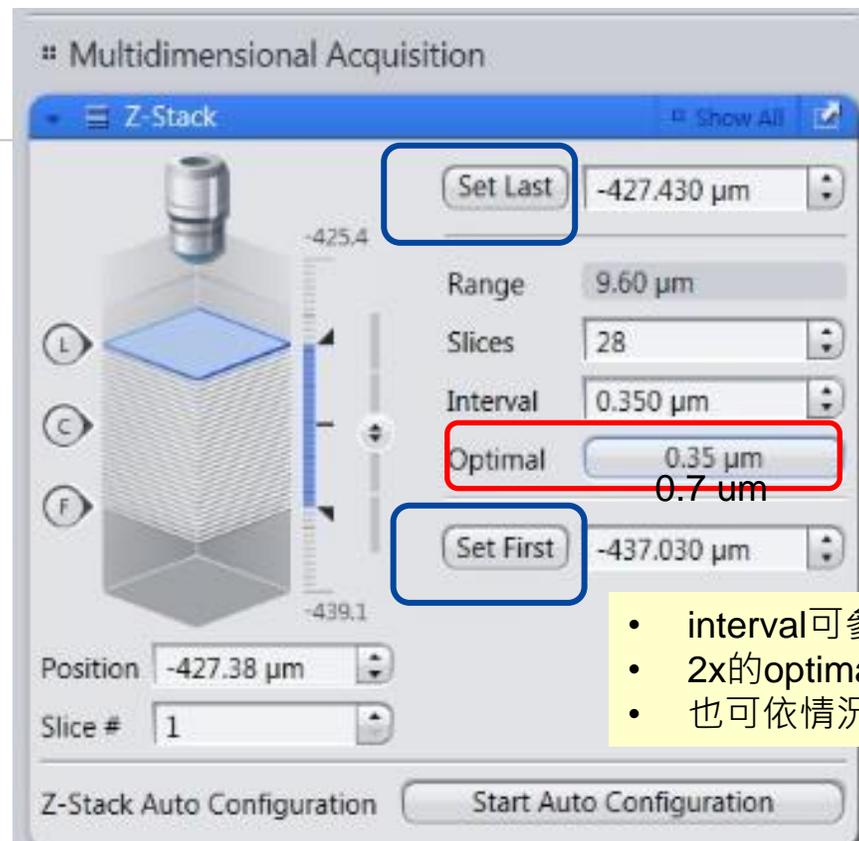


Multichannel Image Acquisition 7 3D Image - Z Stack Acquisition

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



- interval可參考optimal
- 2x的optimal $0.35 * 2 = 0.7$ 也很好
- 自行決定間隔



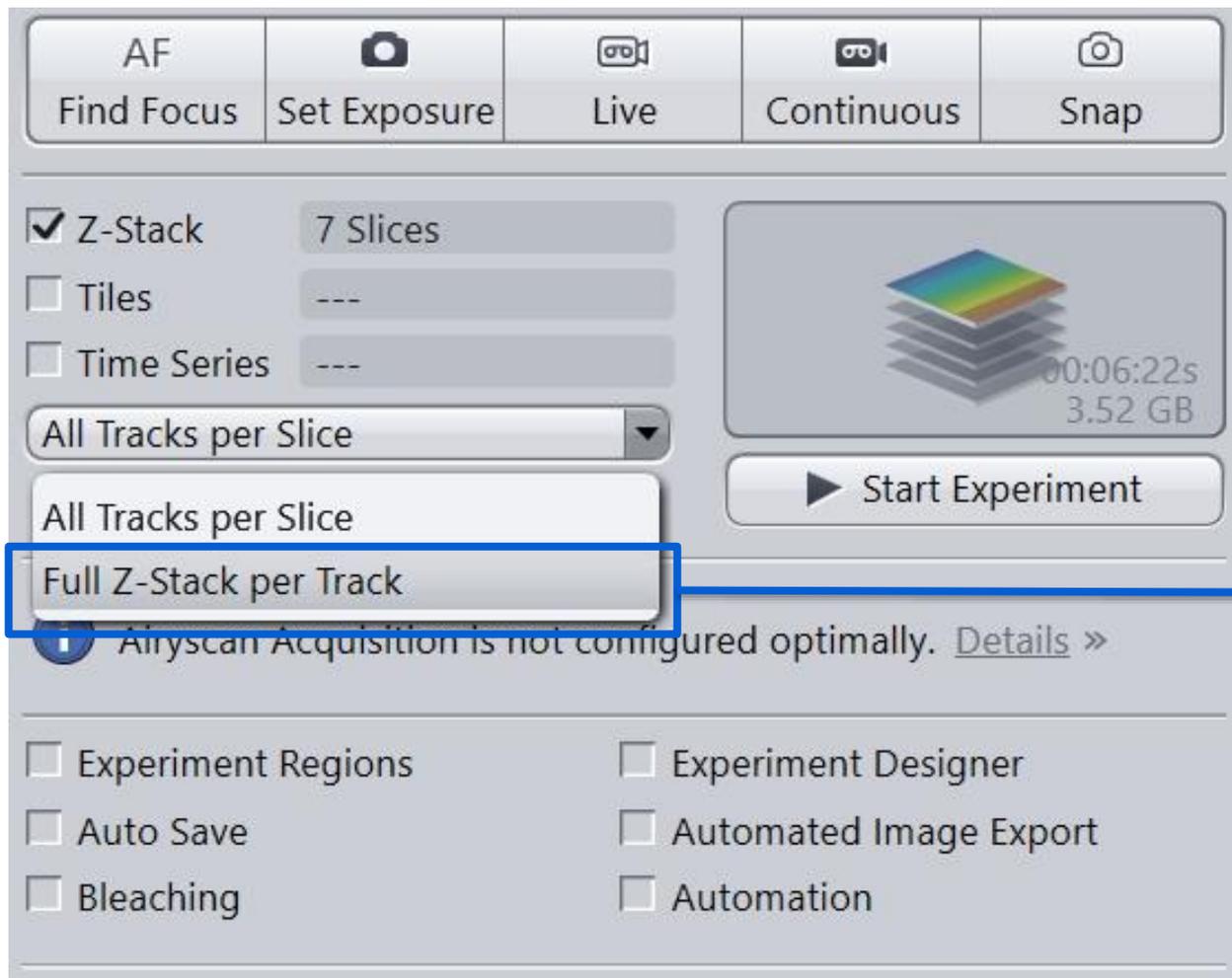
- interval可參考optimal
- 2x的optimal $0.35 * 2 = 0.7$ 也很好
- 也可依情況自行決定間隔

設訂Z stack的上下界限

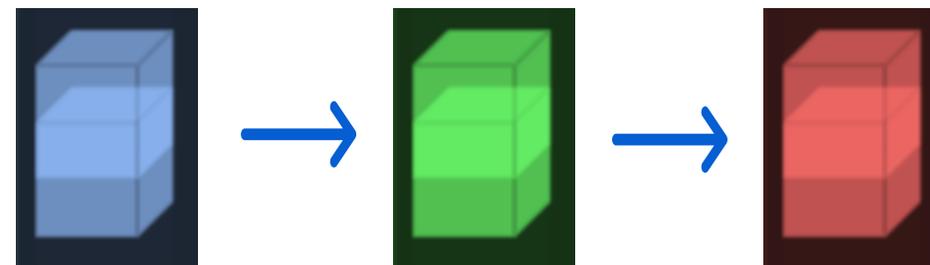
- 選擇一個channel > Continuous
- 搭配Z stack視窗 > Z-stack
- 找到樣品焦距起點 > Set First
- 找到樣品焦距終點 > Set Last

Multichannel Image Acquisition 8

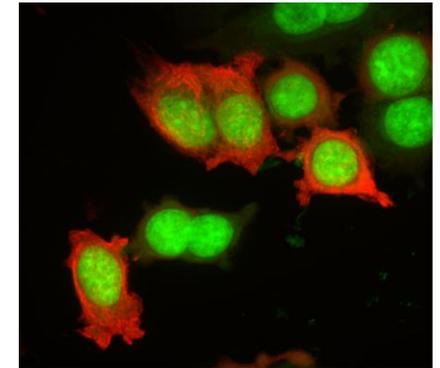
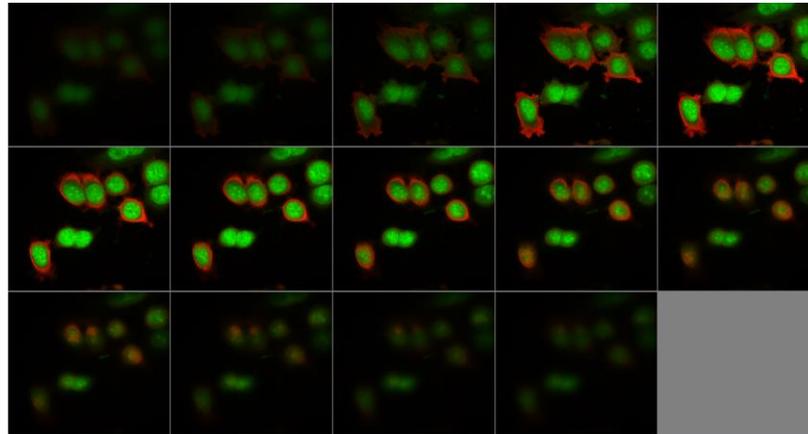
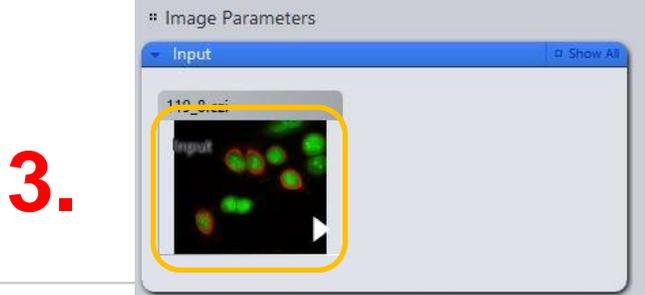
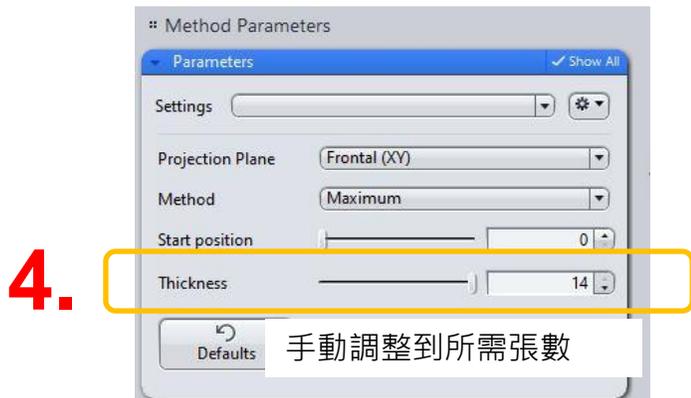
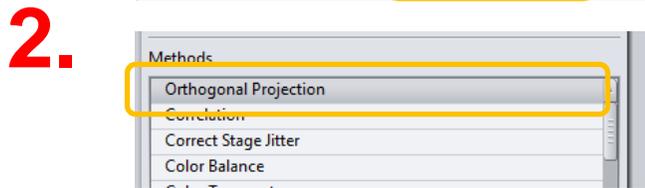
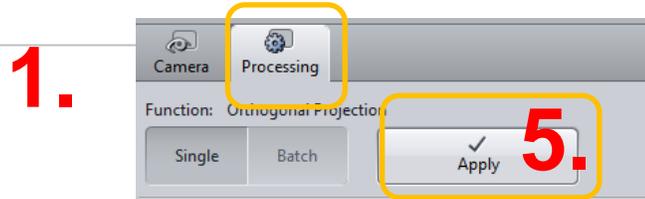
3D Image - Z Stack Acquisition



- 每次一個Channel的Z Stack 掃完才換下一個Channel 的Z Stack
- 可再搭配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



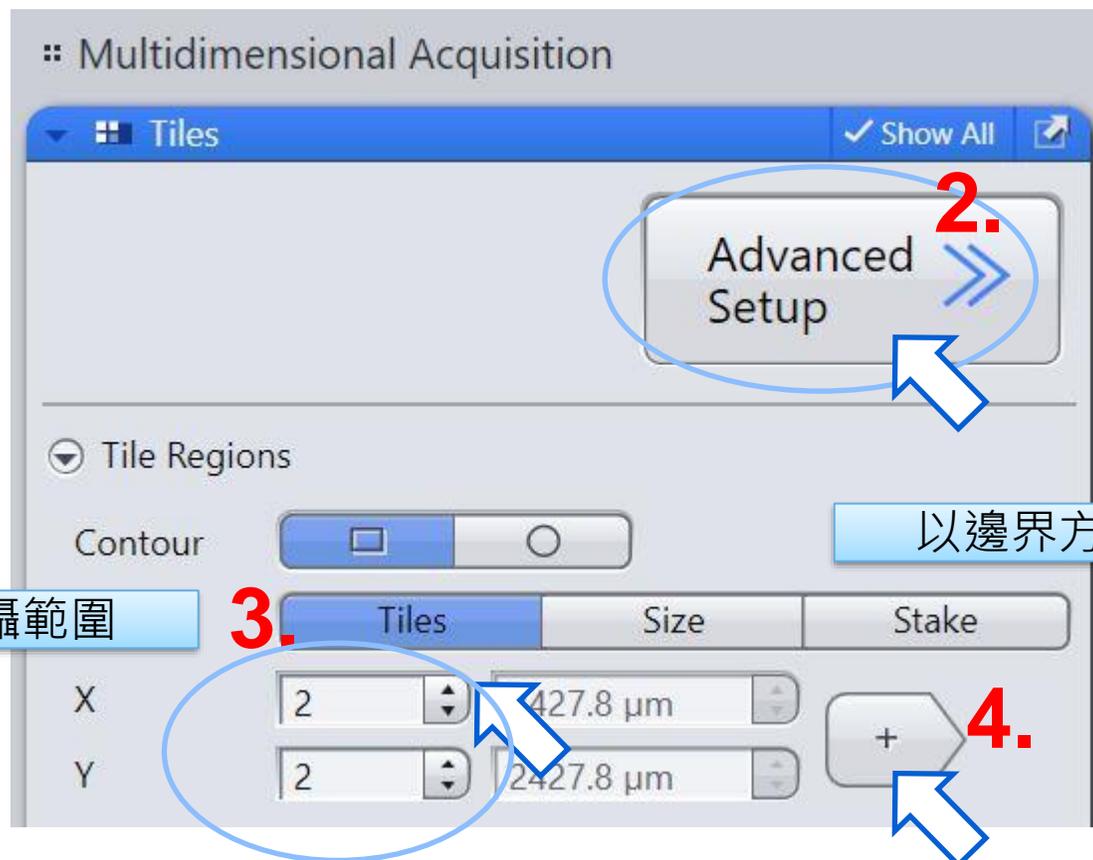
Tile Scan Imaging Setup

以目前視野為中心點做拼圖



1. 完成設定，正式開始掃圖

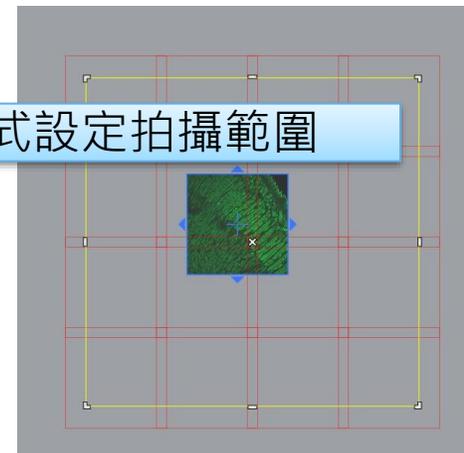
5.



2. 以邊界方式設定拍攝範圍

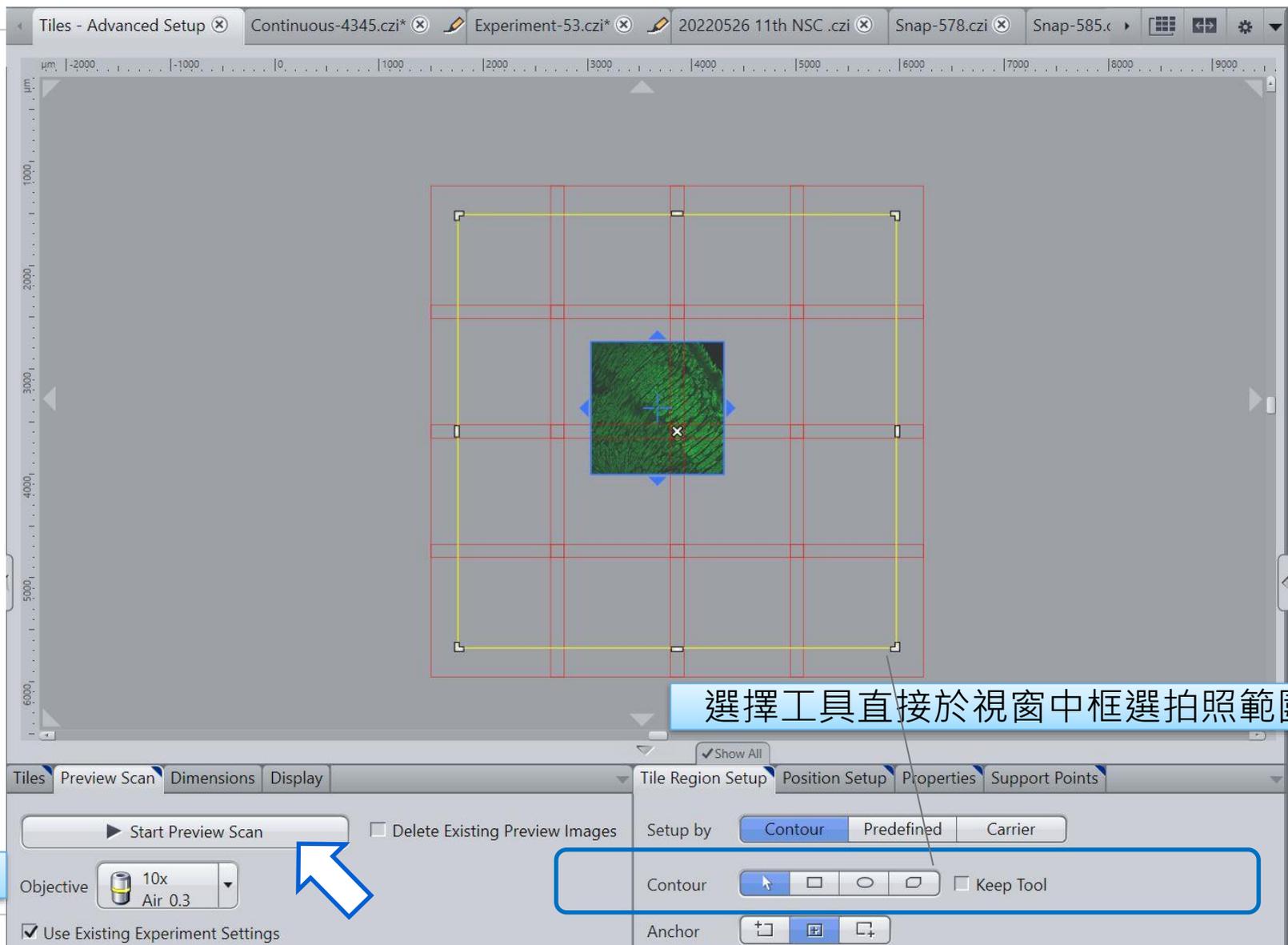
3. 中心點模式設定拍攝範圍

4. 按下之後右方視窗會出現大視野拼圖，如下頁所示

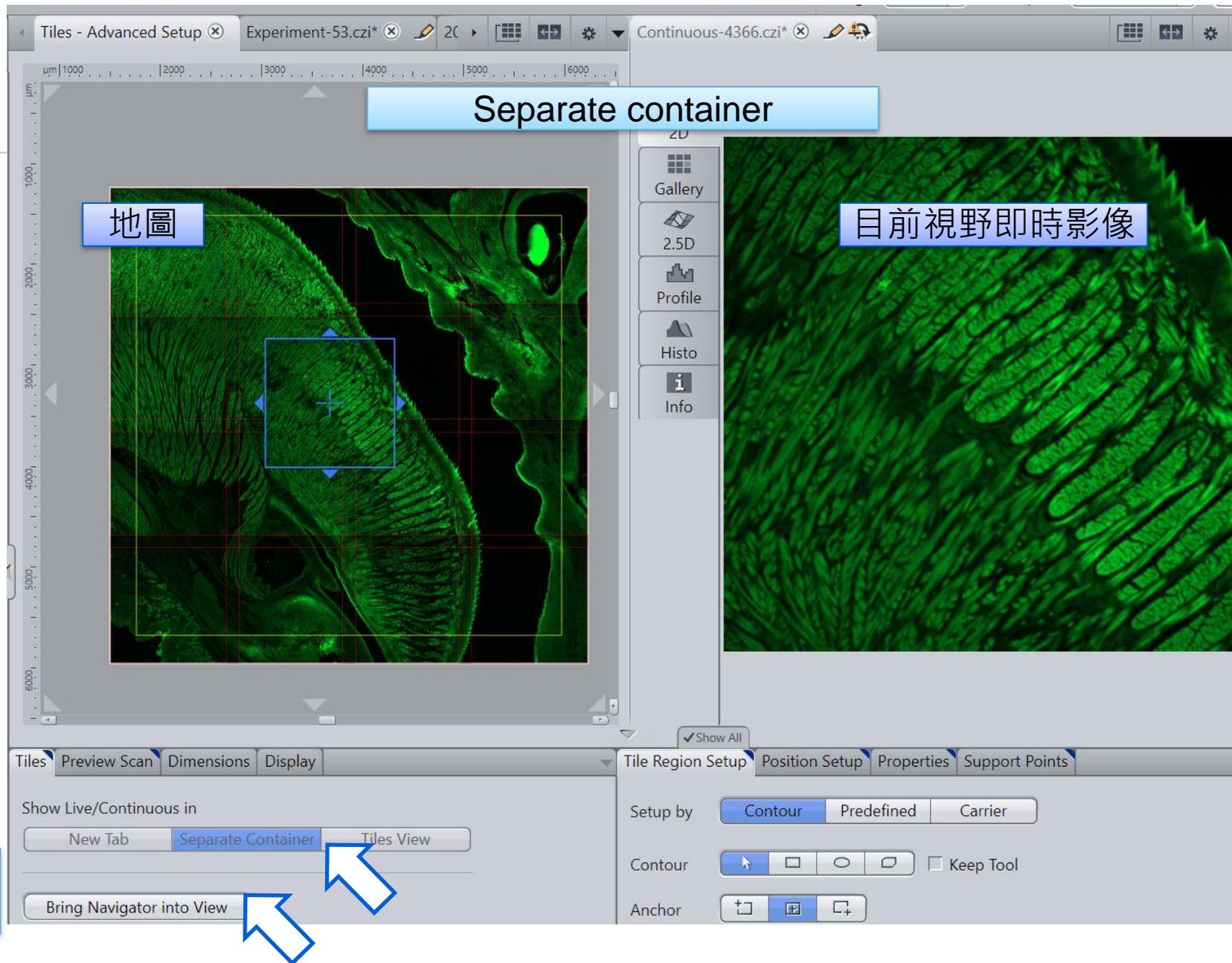


Tile Scan Imaging Setup

拼圖範圍設定 補充1

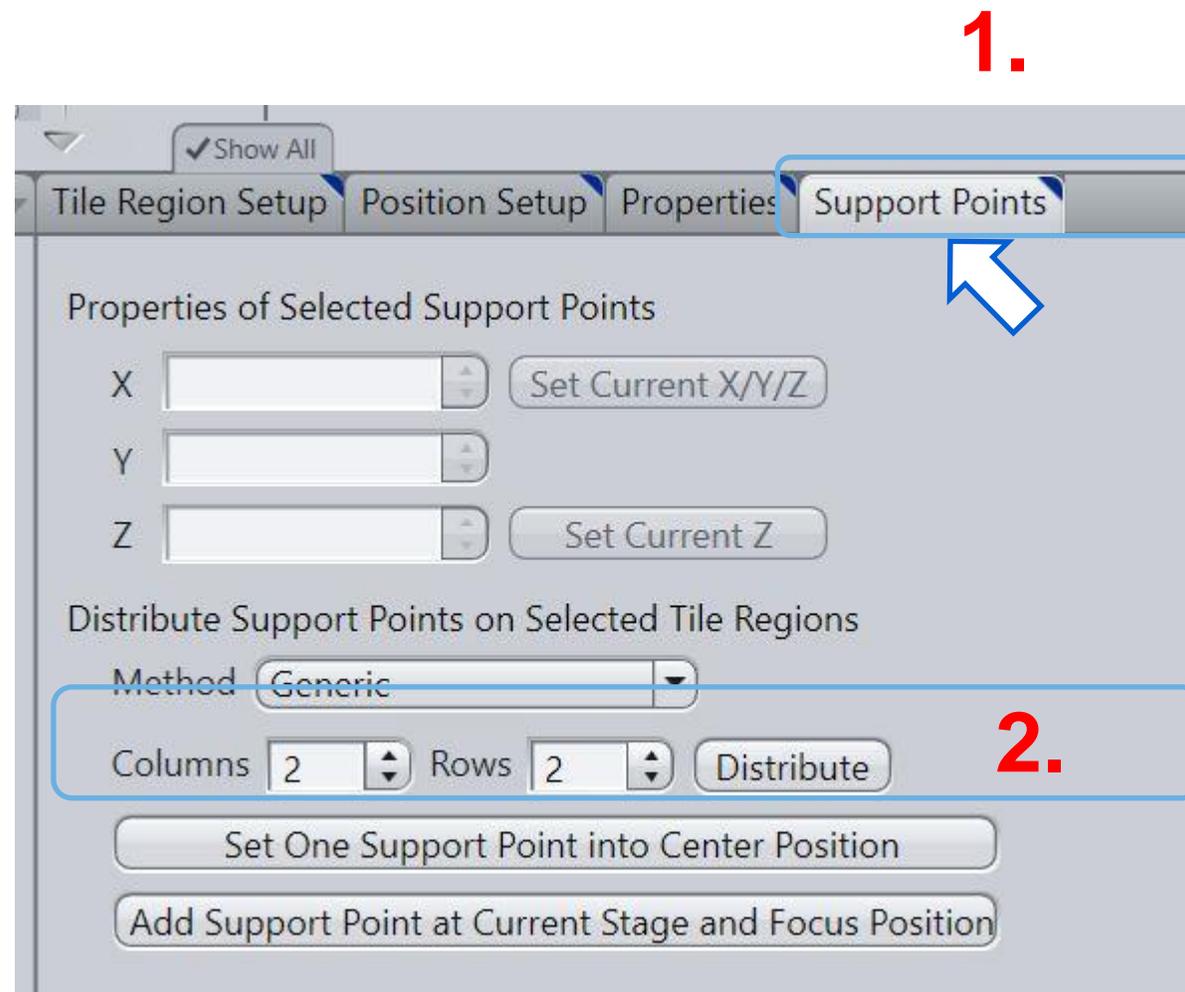
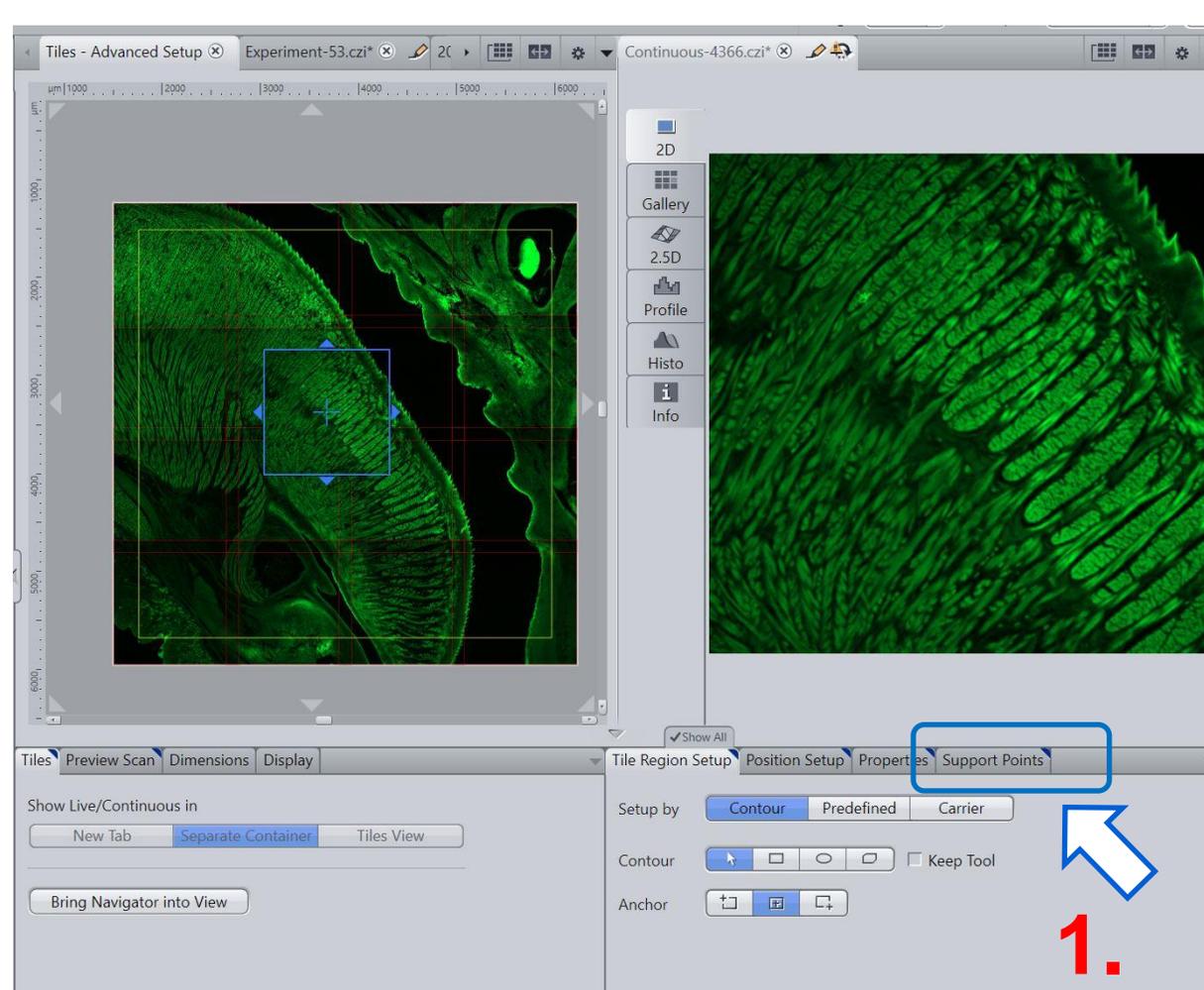


Tile Scan Imaging Setup 拼圖視窗設定 補充2



Bring Navigator into View
將視目前視野置中於視窗

Tile Scan Imaging Setup 拼圖設定對焦輔助點 補充3



Tile Scan Imaging Setup

拼圖設定對焦輔助點 補充4

黃圈為support points

1.

2.

3.

4點或9點對焦法

Support Points of Selected Tile Region: TR1			
X (μm)	Y (μm)	Z (μm)	
4354.2	-5981.7	-128.5	
3592.3	-5514.7	-113.8	
3630.1	-6469.9	-123.9	

Interpolation Degree	
1 - Tilted Plane (at least 4 support points)	
0 - Horizontal Plane from Mean Z (at least 1 support point)	
1 - Tilted Plane (at least 4 support points)	
2 - Parabolic Saddle Surface (at least 9 support points)	
3 - Parabolic Saddle Surface (at least 15 support points)	
4 - Parabolic Saddle Surface (at least 22 support points)	
5 - Parabolic Saddle Surface (at least 35 support points)	
6 - Parabolic Saddle Surface (at least 51 support points)	

Tile Scan Imaging Setup

拼圖設定對焦輔助點 補充5



為每個Support point手動調整焦距

Verify Tile Regions/Positions

	Name	Z (μm)	Tile Region	Array
✓	SP	612.9	TR1	
✓	SP	612.4	TR1	
✓	SP	613.8	TR1	
✓	SP	604.9	TR1	
✓	SP	600.6	TR1	
✓	SP	602.8	TR1	
✓	SP	589.5	TR1	
	SP	606.7	TR1	
	SP	606.7	TR1	

Autofocus (AF) Select Verification Helper Method

Move to Current Point Current Stage X/Y \neq Current Point
 Include Z when Moving to Points

Set Z & Move to Next Current Z 589.5 μm

Run AF and Set Z Use AF to Verify the Remaining

Not all points have been verified.

Close

Tile Scan Setup

ZEN 3.3補充設定 (多位置焦距support points)



The screenshot displays the ZEISS ZEN 3.3 software interface with several key components highlighted and numbered:

- 1:** The 'Tiles' checkbox in the 'Smart Setup' panel is checked.
- 2:** The 'Show Advanced Tiles Viewer' button is highlighted.
- 3:** The 'Add Tile Region' button and the 'Tiles' configuration area (Size X 3, Y 3) are highlighted.
- 4:** The 'Preview' window showing a grid of 9 tiles is highlighted.
- 5:** The 'Add Tile Region' button in the top toolbar is highlighted.
- 6:** The 'TR1' tile region entry in the 'Tile Regions' table is highlighted.
- 7:** The 'Start Experiment' button is highlighted.
- 8:** The 'Sample Carrier' section is highlighted.
- 8.1:** The 'Add Multiple Support Points' section, including the 'Method' dropdown and 'Columns'/'Rows' settings, is highlighted.
- 8.2:** The 'Verify Support Points' section, including the table of support points and the 'Verify' button, is highlighted.

The 'Support Points of Selected Tile Region: TR2' table is as follows:

	X (μm)	Y (μm)	Z (μm)
○	-1741.9	-15729.6	1025.5
○	1743.7	-15729.6	1025.5
○	-1741.9	-13169.8	1025.5
○	1743.7	-13169.8	1025.5

Additional interface elements include the 'Imaging Setup' panel with 'Bright' and 'DIC' channels, and the 'Dimensions' panel with a 'Center to Stage Position' button highlighted in yellow.

視窗中心回正顯示

Tile Scan Setup

ZEN 3.3補充設定 (多位置焦距support points)



Focus Surface and Support Points

Selected Tile Regions: TR1

Add Single Support Point

Current Position Center of Tile Region

Add Multiple Support Points

Method: Generic **1. 依需求選擇** **3.**

Columns: 2 Rows: 2 Distribute Distributes s

Local (per Tile Region)

Support Points of Selected Tile Region: TR1

	X (μm)	Y (μm)	Z (μm)	
⊕	-4300.3 μm	-11492.6 μm	-779.9 μm	
⊕	-3385.4 μm	-11492.6 μm	-779.9 μm	
⊕	-4300.3 μm	-10743.9 μm	-779.9 μm	4
⊕	-3385.4 μm	-10743.9 μm	-779.9 μm	

+ 4 Points ⚙

2.

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

拼圖結果融合處理

Processing → Stitching / Fuse

1.

2.

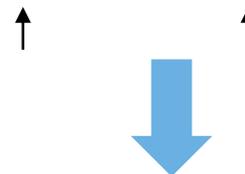
3.

4.

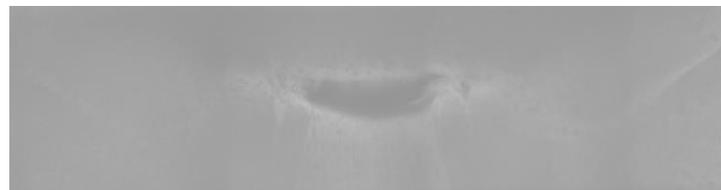
5.

建議選擇Basic即可

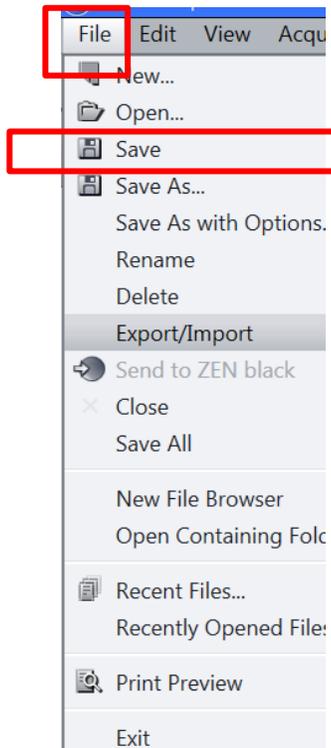
Before



After



5



存檔

檔案名稱(N): shNME3-R2-d-3

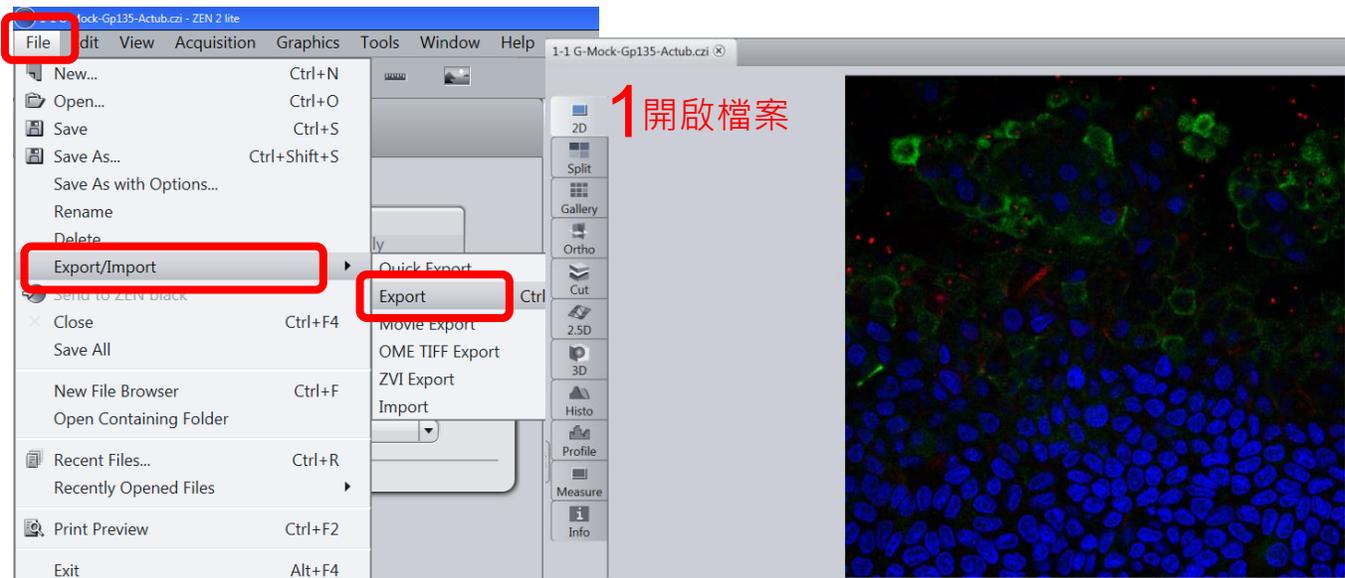
存檔類型(T): Carl Zeiss Image (*.czi)

- 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)

4

2



Method Parameters

Parameters: Show All

Settings: STD

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

Show all處點擊 打勾可顯示更多選項

建議下拉選擇STD
自動套用以下參數

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
產生個別channel影像

產生所有xyz影像

產生個別xyz影像 · 例如不要merge
穿透光影像請由此設定

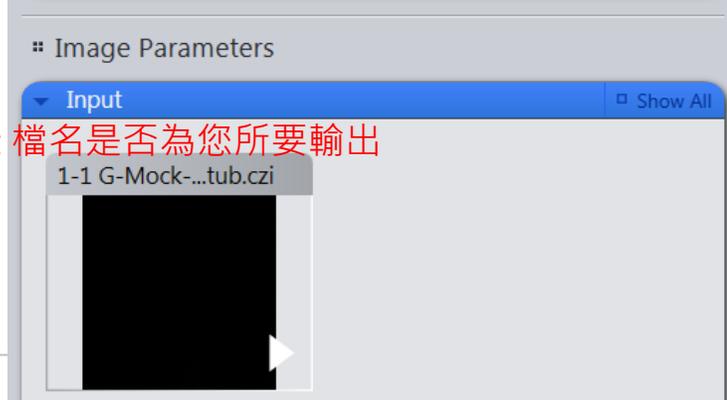
請選擇自己的資料夾位置

產生資料夾

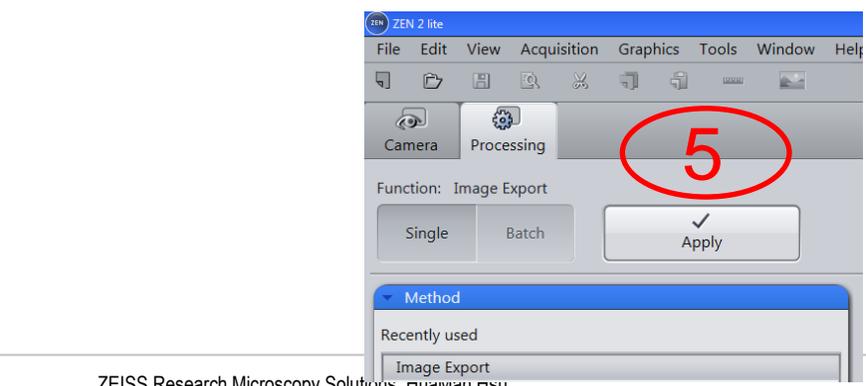
其餘設定請參考左圖
Prefix為預設檔名

3

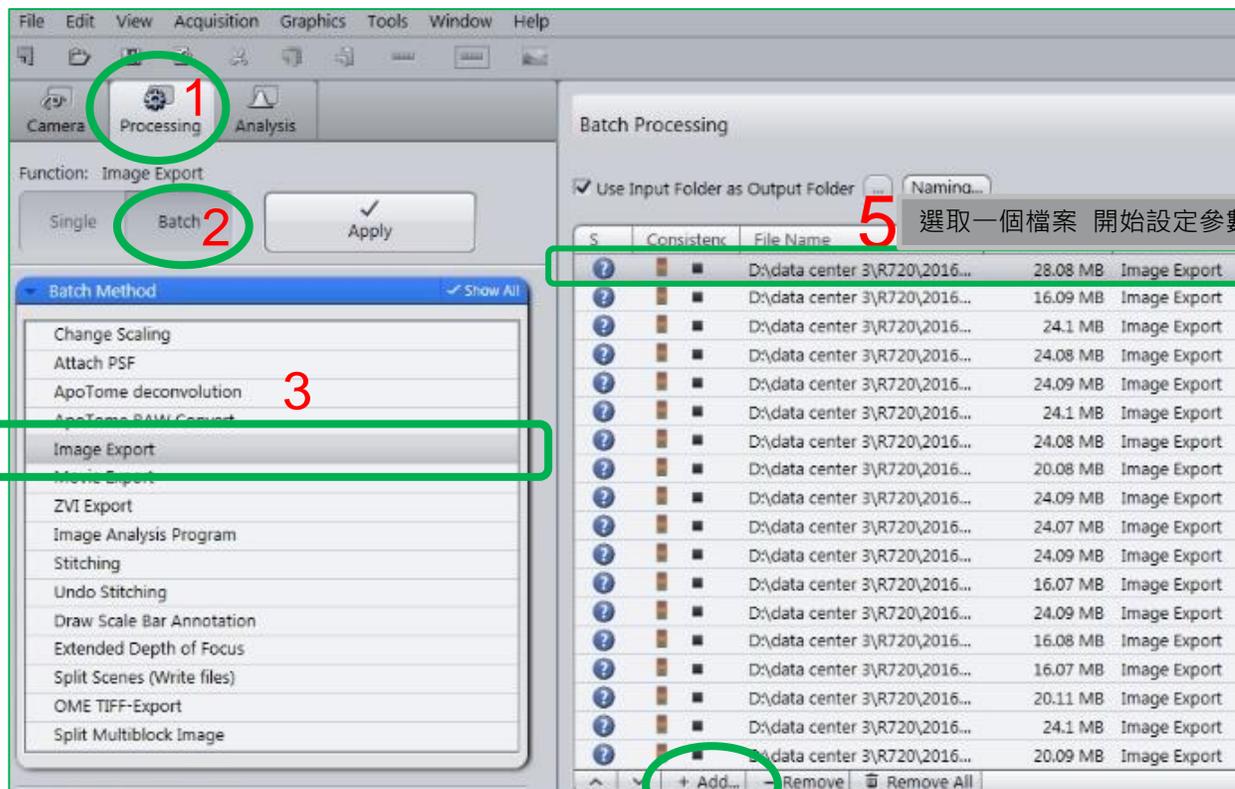
確認input檔名是否為您所輸出的檔案



2

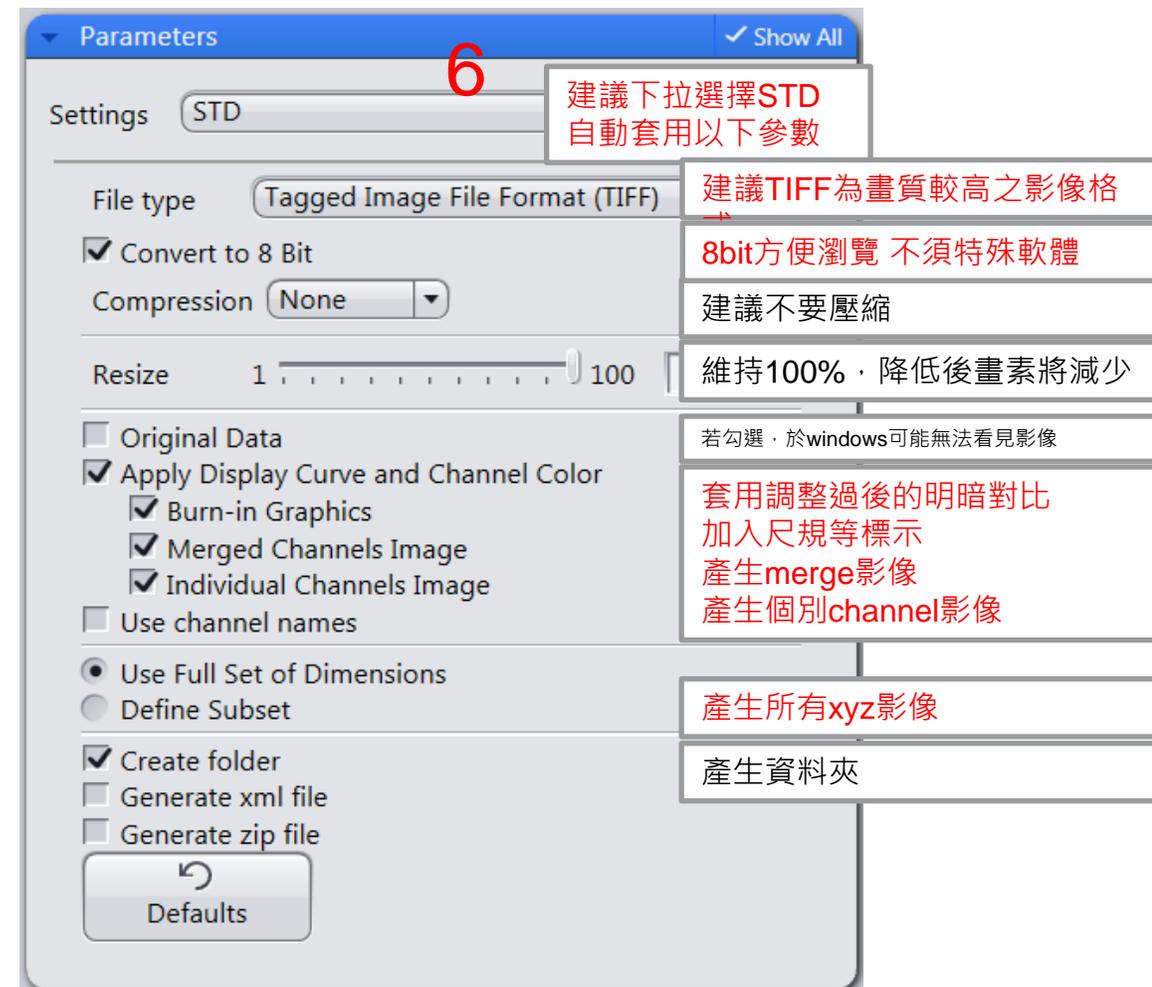


大量批次轉檔batch export 1



5 選取一個檔案 開始設定參數

4 加入你所想要轉檔的項目



6 建議下拉選擇STD 自動套用以下參數

建議TIFF為畫質較高之影像格式

8bit方便瀏覽 不須特殊軟體

建議不要壓縮

維持100% · 降低後畫素將減少

若勾選 · 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 產生個別channel影像

產生所有xyz影像

產生資料夾

大量批次轉檔batch export 2



Batch Processing 輸出至原檔案同一資料夾

Function: Image Export

Single Batch

Apply 12

Batch Method

- Change Scaling
- Attach PSF
- ApoTome RAW Convert
- Image Export
- Movie Export
- ZVI Export
- Draw Scale Bar Annotation
- Split Scenes (Write files)
- OME TIFF-Export

Batch Processing

Use Input Folder as Output Folder ... Naming... 11

Copy Parameters 8 Paste Parameters 10 Check All Run Selected

S	Consistenc	File Name	Size	Method	Output Name	Output Storage Path
?		E:\DEMO and analyze imag...	468.22 MB	Image Export		E:\DEMO and analyze image\20160202 siGal8-Gp135
?		E:\DEMO and analyze imag...	600.25 MB	Image Export		E:\DEMO and analyze image\20160202 siGal8-Gp135
?		E:\DEMO and analyze imag...	744.28 MB	Image Export		E:\DEMO and analyze image\20160202 siGal8-Gp135
?		E:\DEMO and analyze imag...	942.34 MB	Image Export		E:\DEMO and analyze image\20160202 siGal8-Gp135
?		E:\DEMO and analyze imag...	348.2 MB	Image Export		E:\DEMO and analyze image\20160202 siGal8-Gp135
?		E:\DEMO and analyze imag...	312.17 MB	Image Export		E:\DEMO and analyze image\20160202 siGal8-Gp135
?		E:\DEMO and analyze imag...	342.17 MB	Image Export		E:\DEMO and analyze image\20160202 siGal8-Gp135
?		E:\DEMO and analyze imag...	540.24 MB	Image Export		E:\DEMO and analyze image\20160202 siGal8-Gp135
?		E:\DEMO and analyze imag...	612.26 MB	Image Export		E:\DEMO and analyze image\20160202 siGal8-Gp135

7

9 按住SHIFT鍵選取剩下的檔案

Load List... Save List...

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

Import stage marks



1.

Tiles 3 Positions
 Time Series 10 Cycles
 All Tile Regions per Time Point

5.

6.

7.

Single Positions	Position Arrays
<input checked="" type="checkbox"/>	<input type="checkbox"/>
Name	X (μm)
Y (μm)	Z (μm)
Cate	
There are no single positions defined. Use the F10 key or the Advanced Tile viewer to define new positions.	

Verify Tile Regions	Verify
<input checked="" type="checkbox"/>	<input type="checkbox"/>
Name	X (μm)
Y (μm)	Z (μm)
M1	10.0
M2	50.0
M3	120.0

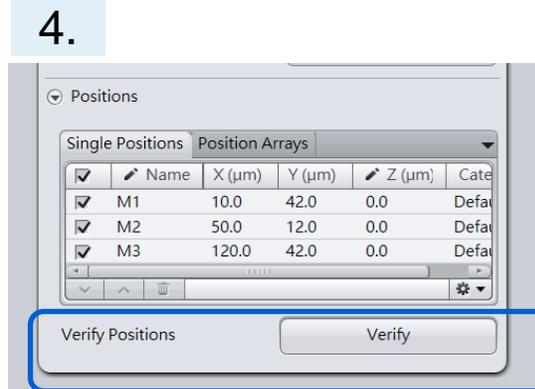
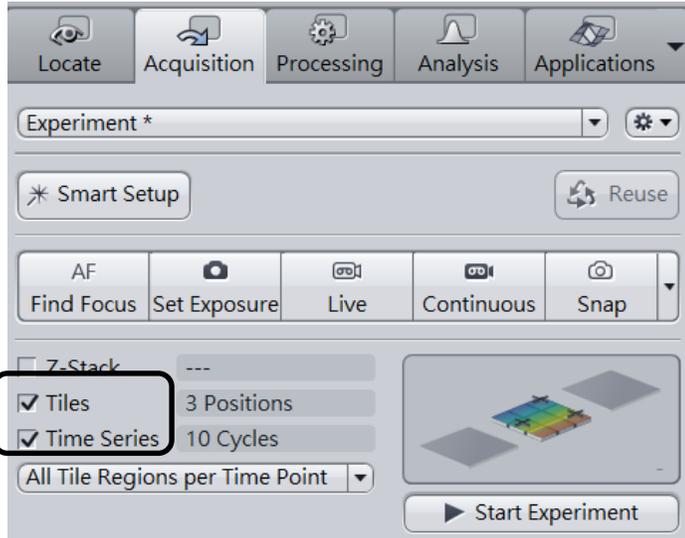
2. Stage → Marks

3.

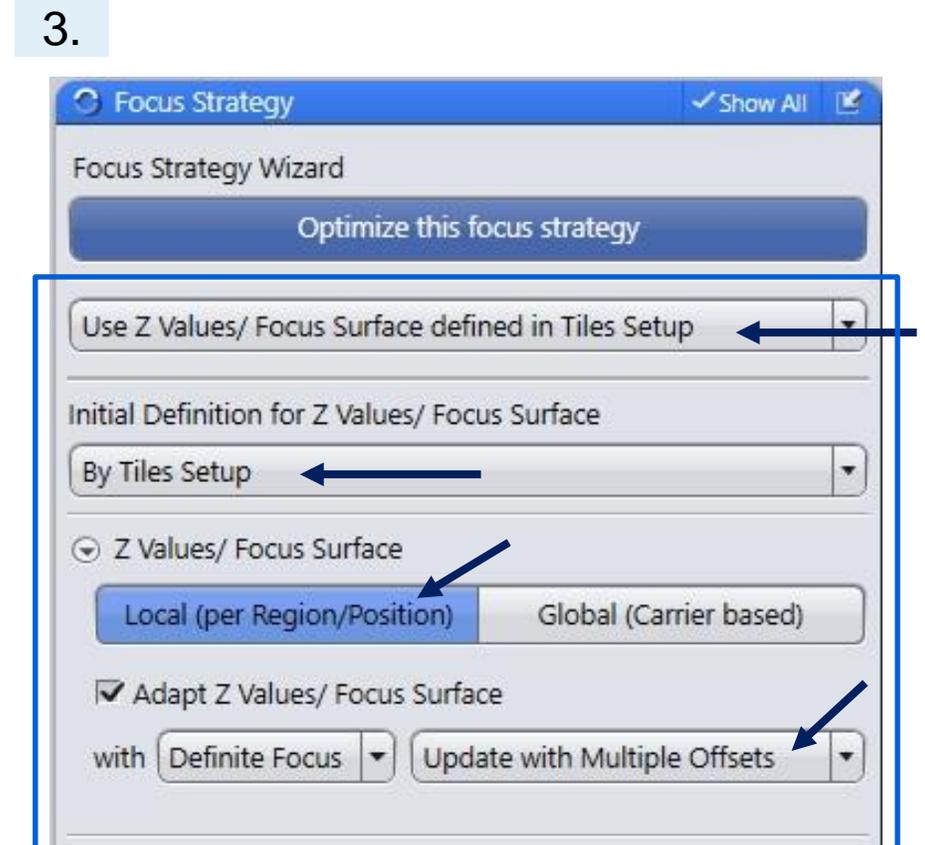
4. At the positions you would like to proceed imaging, click "+".

#	Name	X (μm)	Y (μm)
1		10.0	42.0
2		50.0	12.0
3		120.0	42.0

Multi-position Time Lapse Imaging 2. Definite Focus Focus Strategy 長時間自動追焦

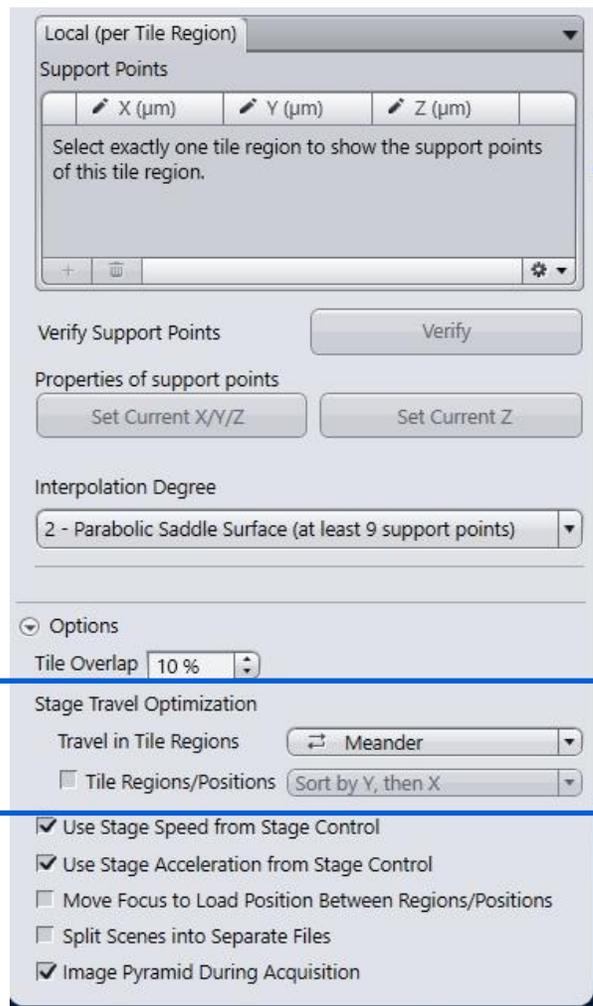


5. • 所有位置焦距設定完畢
• 按下Start Experiment後軟體會自動設Multiple Offsets



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

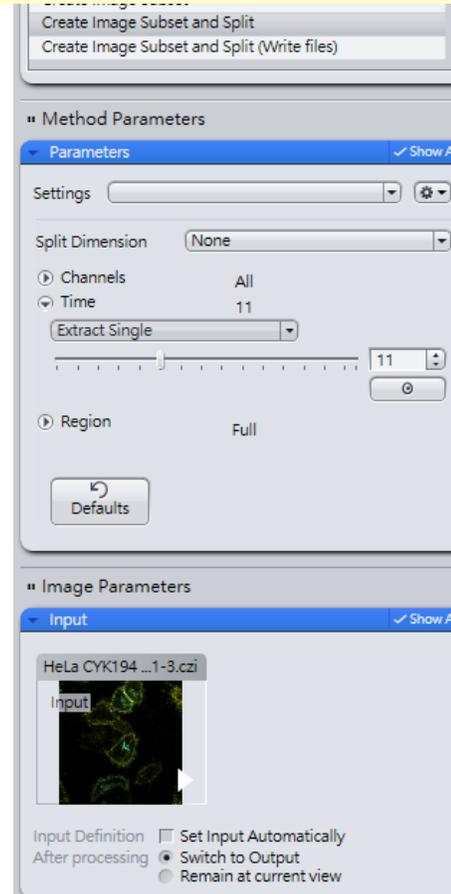


Processing → Split Scenes/ Create Subsets

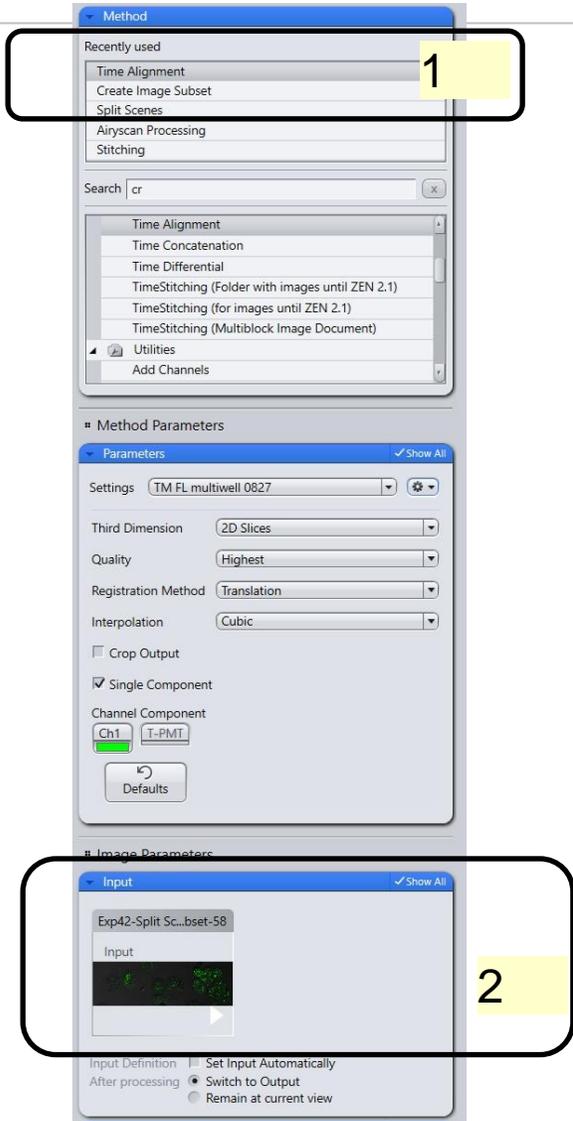


每只需要某個位置的結果獨立出來,可一個個分別存檔

只需要某個位置或時間的結果獨立出來



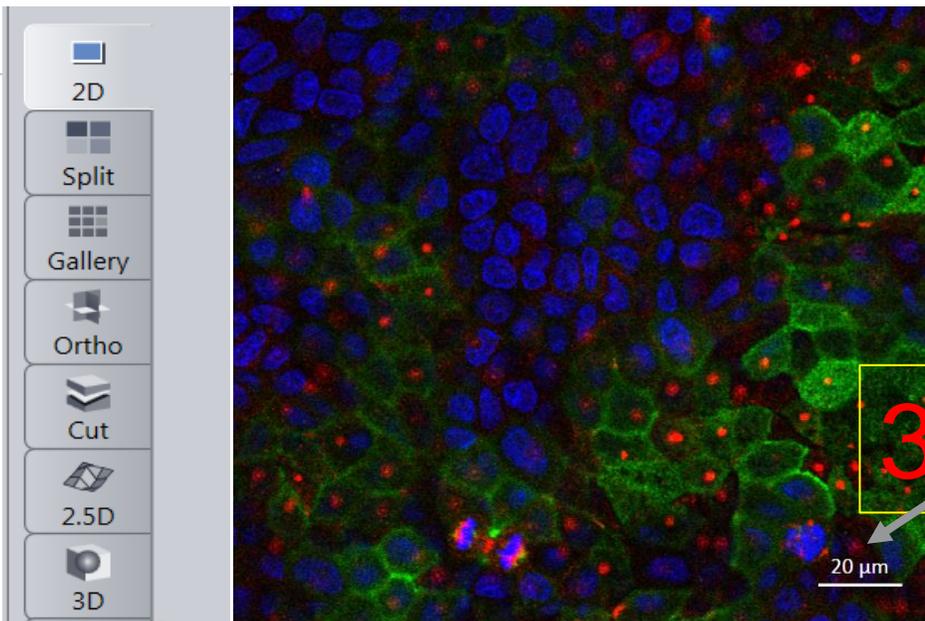
多位置拍攝長時間晃動問題 Processing → Time lapse Alignment



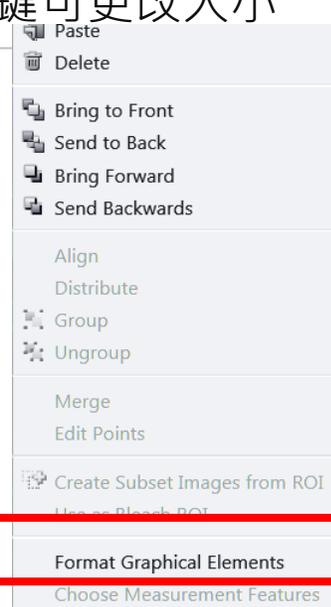
關於連續撥放影片晃動

- 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

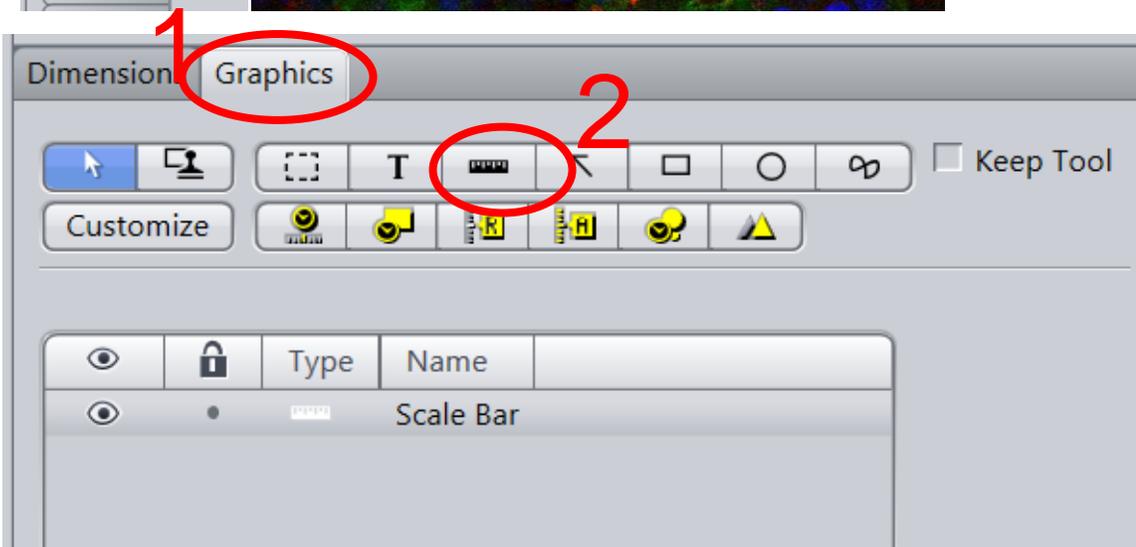
Add Scale Bar加入尺規



4 於scale bar按右鍵可更改大小
顏色粗細等格式

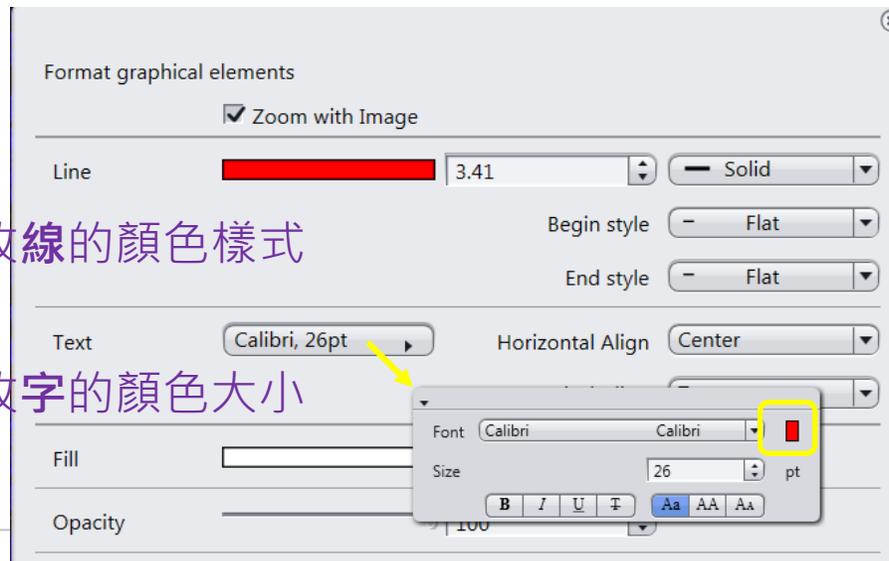


3 scale bar會自動出現

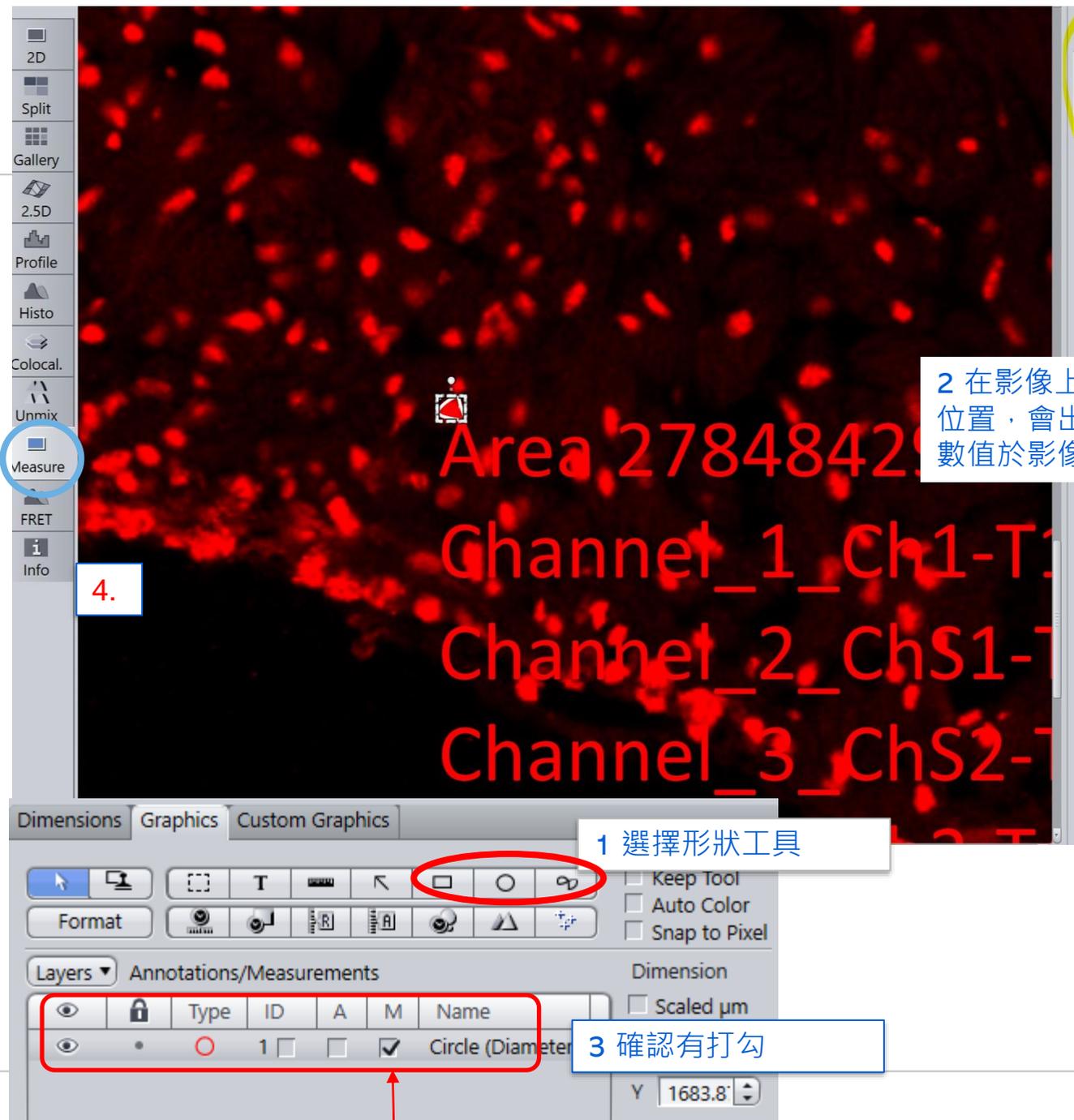


更改線的颜色樣式

更改字的颜色大小



量測area & intensity



2 在影像上框選有興趣的位置，會出現面積及亮度數值於影像上

4.

1 選擇形狀工具

3 確認有打勾

4.圖表位於右方視窗

	Name	Feature	Value	Unit
	A	B	C	D
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	

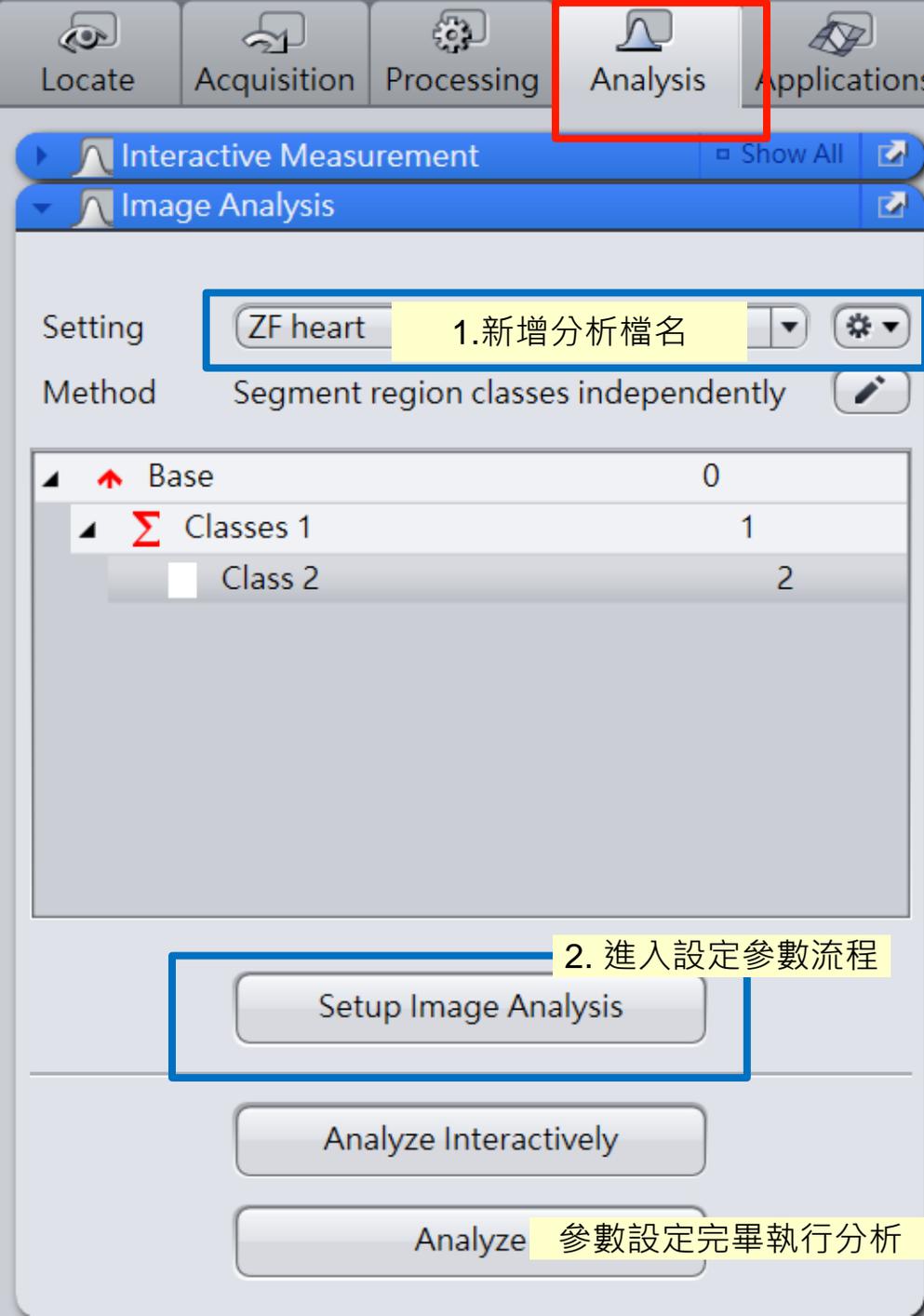
Display Measurement Show All

Tools Create Tool for Each Channel
 Tool Modification Transfer Changes to Next Ones

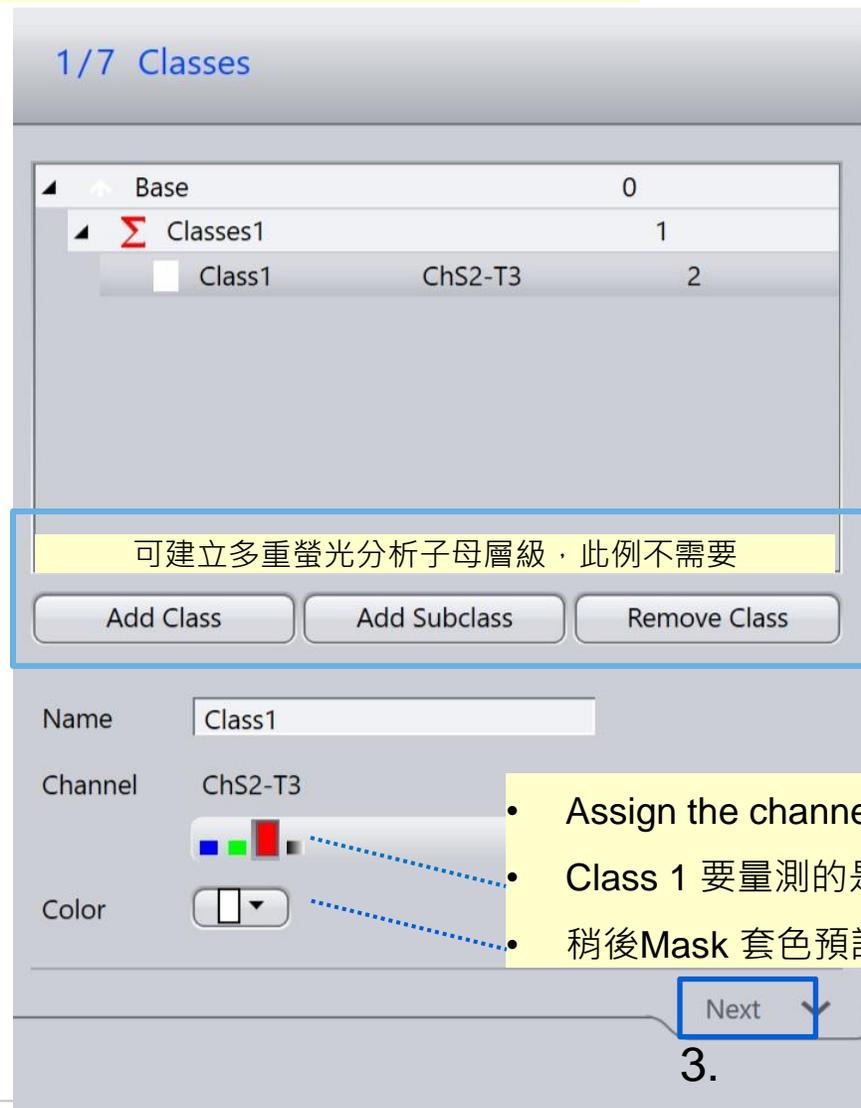
New Feature Name Add
 Value Unit Remove

Data Display Format
 In

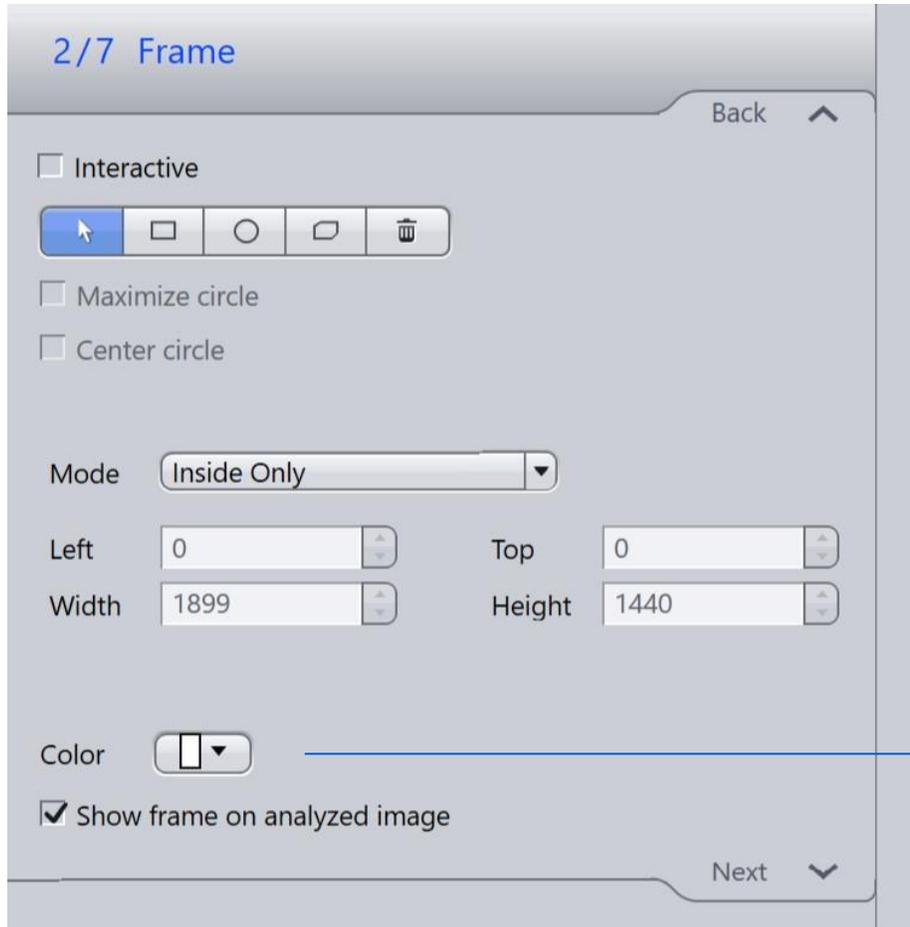
自動量測 Image Analysis Module 1.



以量測前頁影像內的紅色細胞核數目為例



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.

Execute Interactive

Base	0
Classes 1	1
Class 2 ChS2-T3	2

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

Segment by global

Smoothing: Median

Size: 3

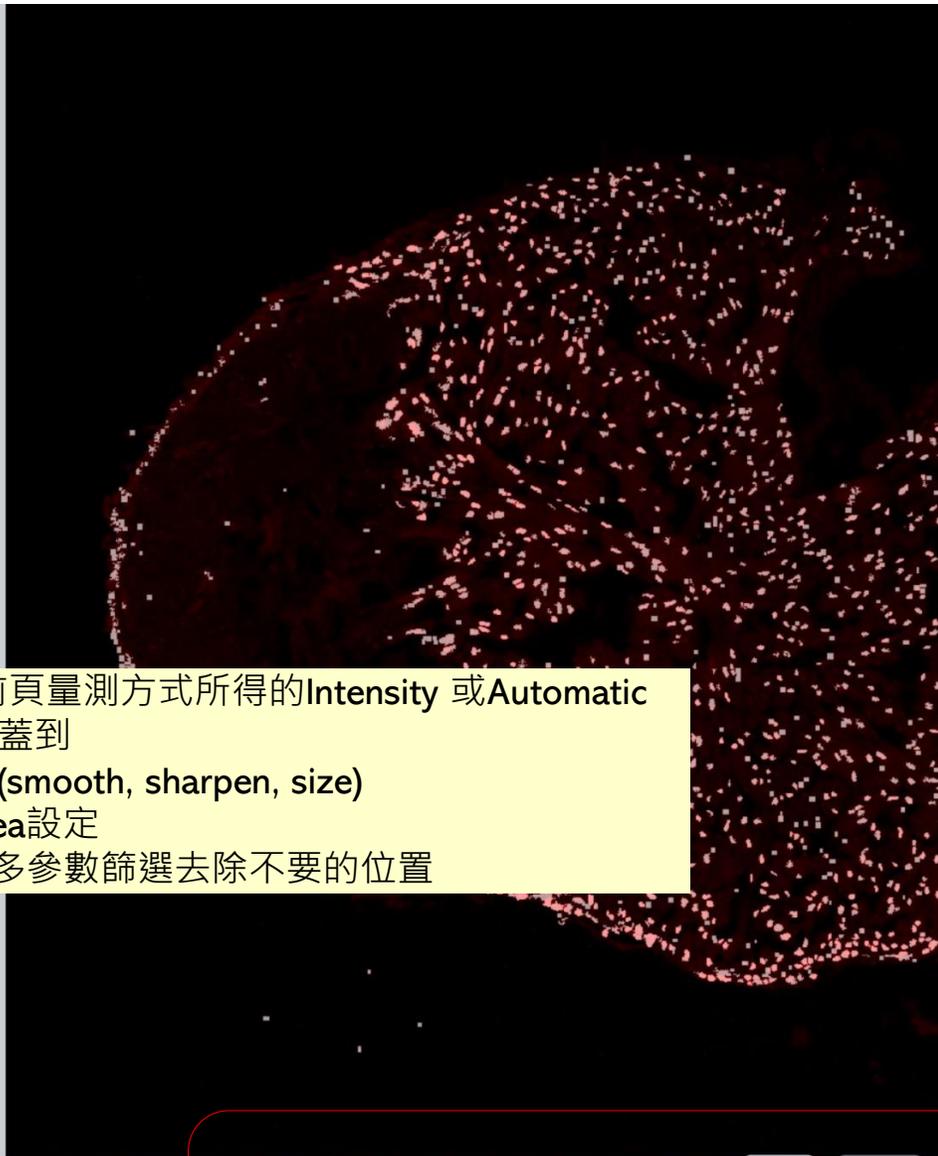
Sharpen: Unsharp Masking

Strength: 1.4

Threshold

Histogram

- 設定Threshold，數值可參考前頁量測方式所得的Intensity 或Automatic
- 務必使需要的位置都被mask覆蓋到
- 依mask結果來回調整所有參數(smooth, sharpen, size)
- 有些小區塊未能滿意可以在Area設定
- 後續步驟進行region filter有更多參數篩選去除不要的位置



Open

None

Open

Close

Dilate

Erode

區域分割 融合 演算

區域切割 搭配count

Method: Otsu Threshold (Light Regions)

Minimum Area: 11

Min. Hole Area: 1

Fill Holes: On

Binary: None

Separate: Watersheds

Count: 20

Suppress Invalid: Off

Next

Zoom: 76%

Tools

Channels: Ch1-T1, ChS1-T2, ChS2-T3, Ch2-T4

Single Channel Range Indicator

Analysis

Show Objects Fill Opacity

Show All Classes

Base	0
Classes 1	1
Class 1 ChS2-T3	2

Mask顯示輔助

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

Name	Minimum	Maximum	
Area	<input checked="" type="checkbox"/> 100000.0	<input type="checkbox"/> 95000000	And
Fibrelength	<input checked="" type="checkbox"/> 1.000	<input type="checkbox"/> 0.000	And
Intensity Me	<input checked="" type="checkbox"/> 3.000	<input type="checkbox"/> 255.000	And
Perimeter	<input type="checkbox"/> 3000.000	<input type="checkbox"/> 1000.000	

▾

n Filter Editor

Selected Features for Condition

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

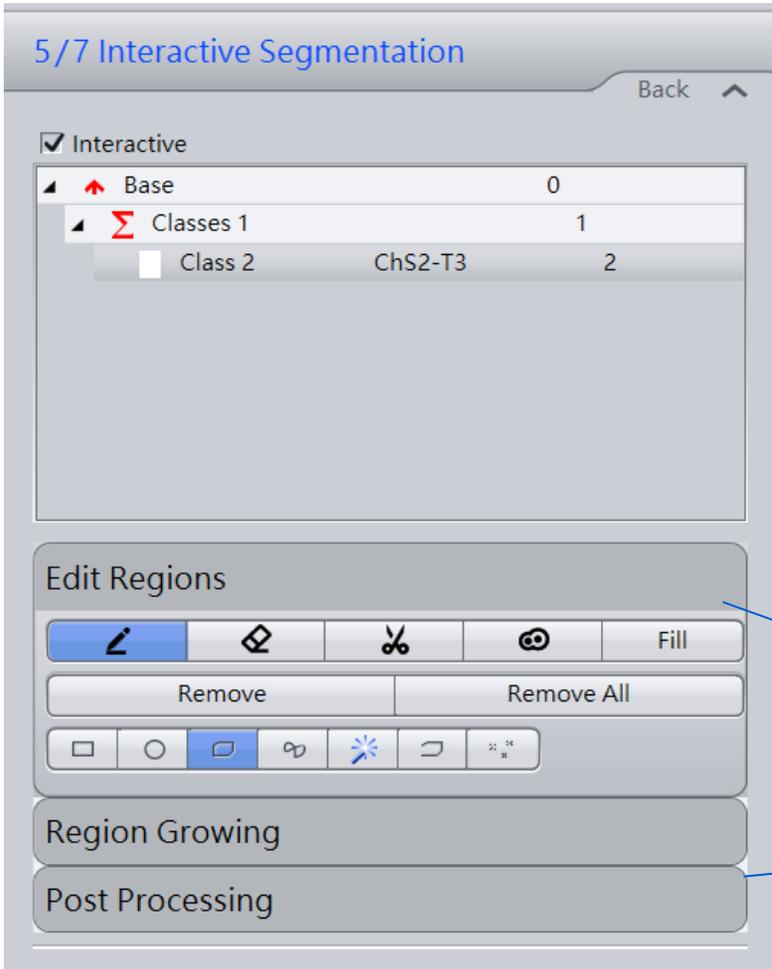
Search Features

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

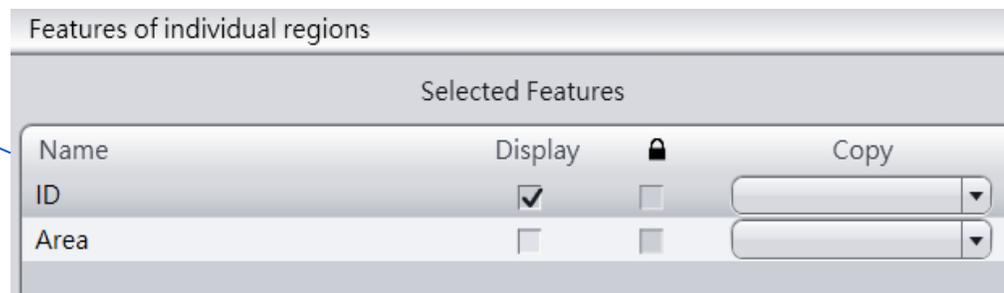
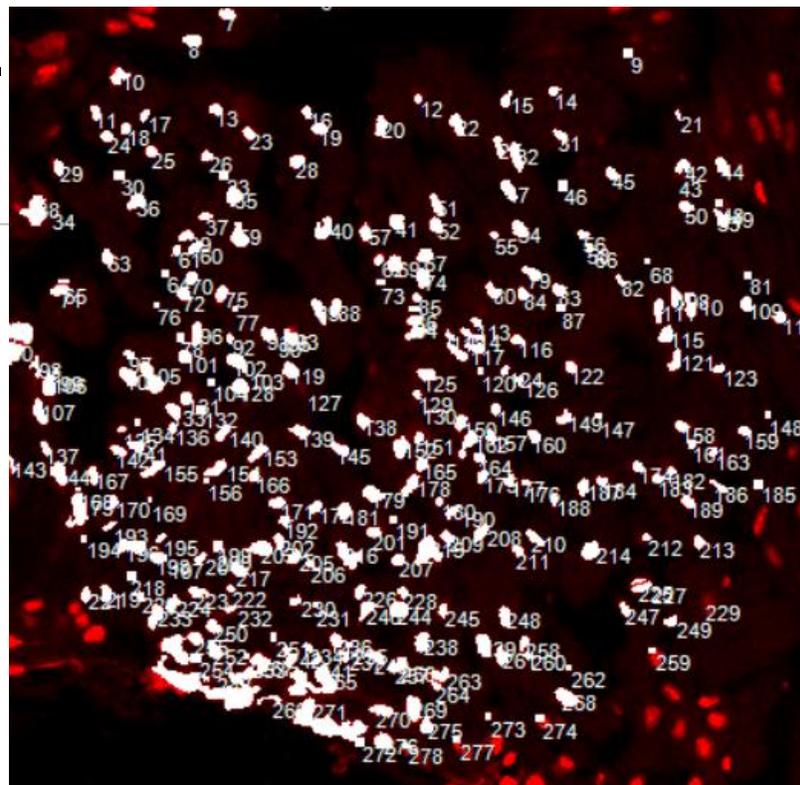
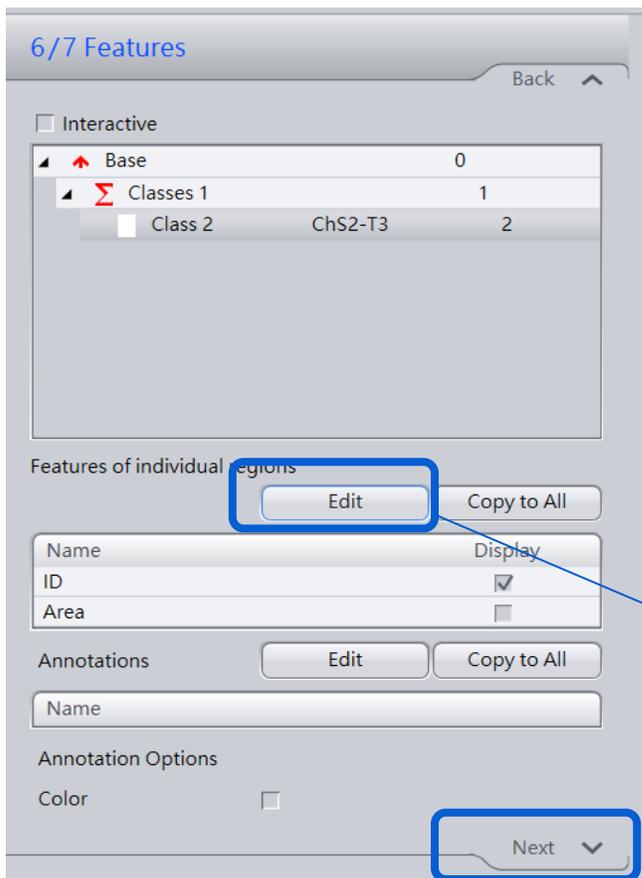
批次處理需求請略過此步



- 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



7/7 Results Preview

Base 0

Classes 1

Class 2 ChS2-T3 2

Highlight Box

Color []

Line Width 1

Enable chart

Chart Type [] []

X-Axis ID

Y-Axis Area

Please note that the data list is only a preview and results may differ from the final analysis.

< Back Next > Finish Cancel

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

結果預覽

此時尚無法輸出結果

按下，儲存以上各步驟分析設定

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



1. Select the **Batch** button in the Function: Image Analysis Program section.

2. Select the **Image Analysis Program** method in the Batch Method list.

3. Select the desired file location in the Batch Processing window.

4. Select the analysis method in the Method Parameters dialog.

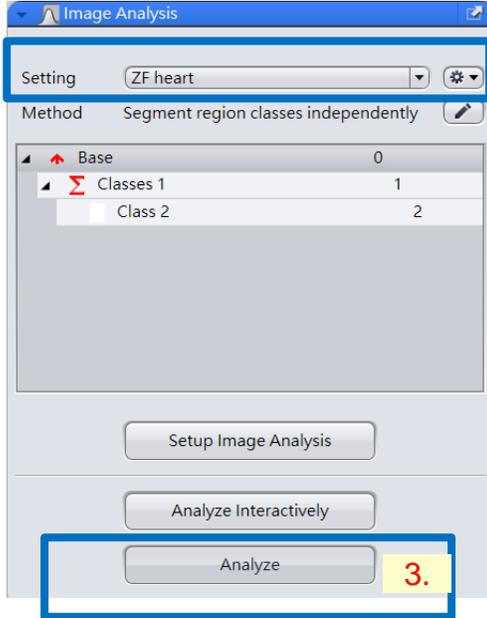
5. Click the **Apply** button to confirm the settings.

S	Consisten	File Name	Size	Method	Output Name	Output Storage Path
?		E:\00 Temporary image analy...	41.87 MB	Image Analysis Program		E:\00 Temporary image analysis\IBS ZF heart
?		E:\00 Temporary image analy...	41.96 MB	Image Analysis Program		E:\00 Temporary image analysis\IBS ZF heart
?		E:\00 Temporary image analy...	42.08 MB	Image Analysis Program		E:\00 Temporary image analysis\IBS ZF heart
?		E:\00 Temporary image analy...	41.86 MB	Image Analysis Program		E:\00 Temporary image analysis\IBS ZF heart

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

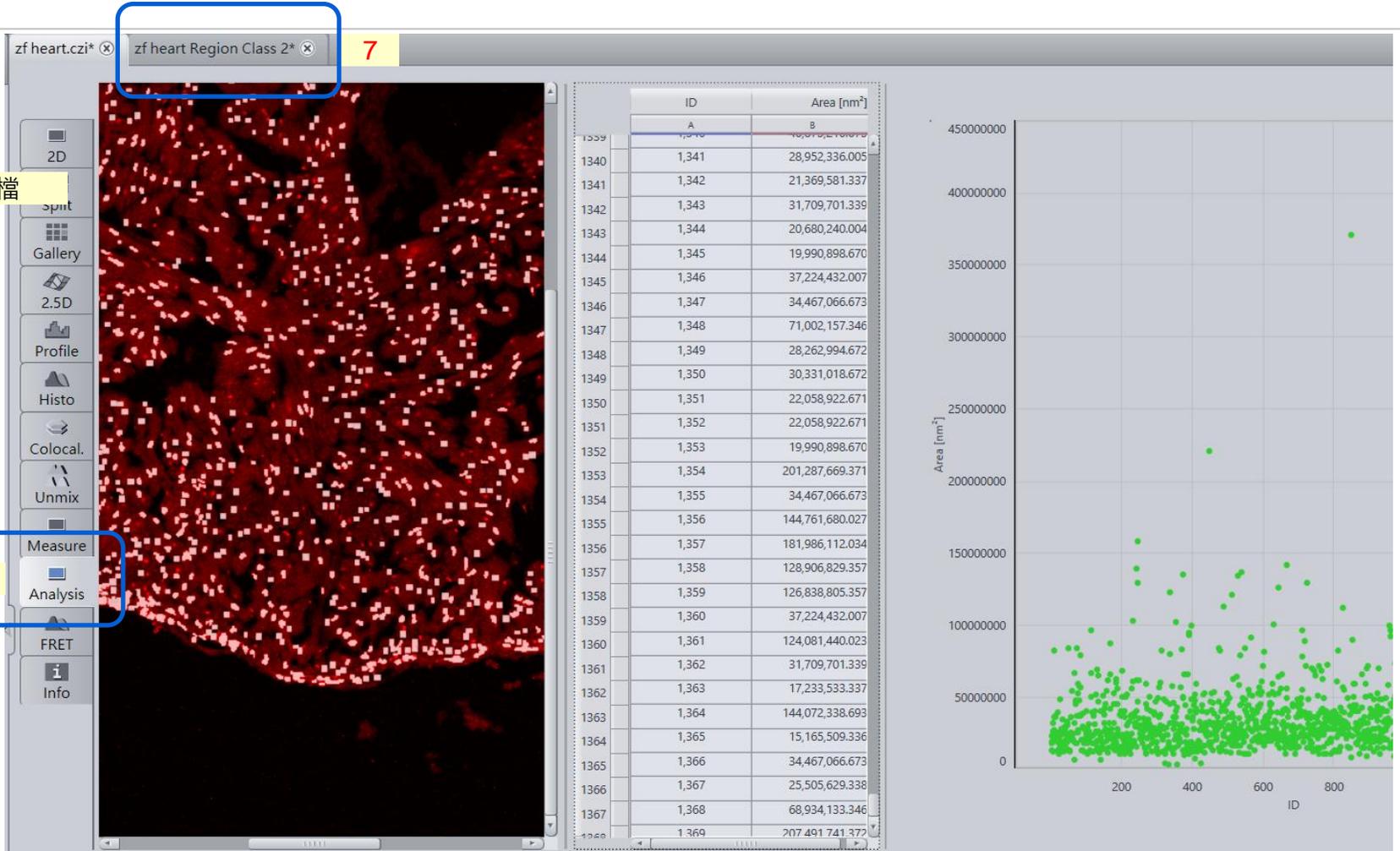


1 打開原圖 或其他同系列要分析的圖



2. 選擇設定檔

4



7

輸出圖表

輸出量測結果表格
會自動產生新檔案於視窗中

6

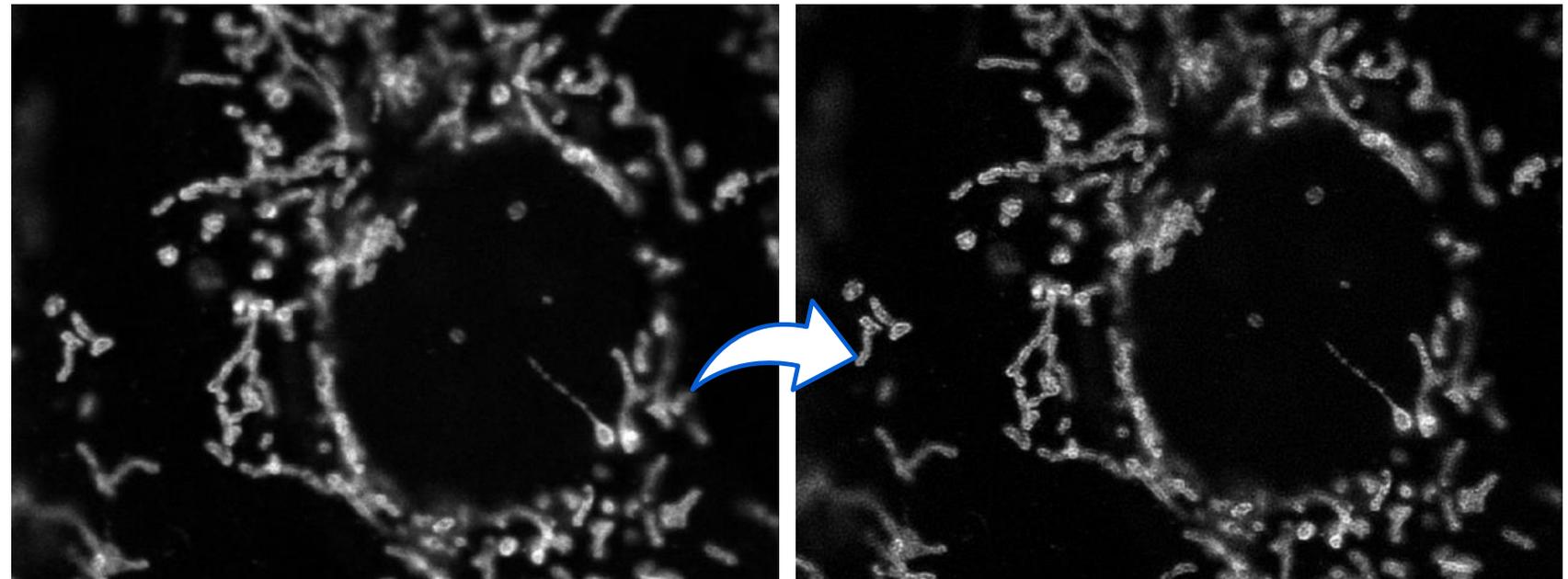
ZEN 3.3 Image Processing

Image Clearing Option 1: 2D DCV (defaults)

The screenshot shows the ZEN 3.3 software interface. The 'Deconvolution' menu is expanded, with 'Deconvolution (defaults)' highlighted and marked with a yellow '1'. Below the menu, the 'Method Parameters' section shows three options: 'Simple, very fast (Nearest Neighbor)', 'Better, fast (Regularized Inverse Filter)', and 'Good, medium speed (Fast Iterative)', with the latter marked with a yellow '3'. The 'Image Parameters' section shows the 'Input' field with a preview of a green image, marked with a yellow '2'.

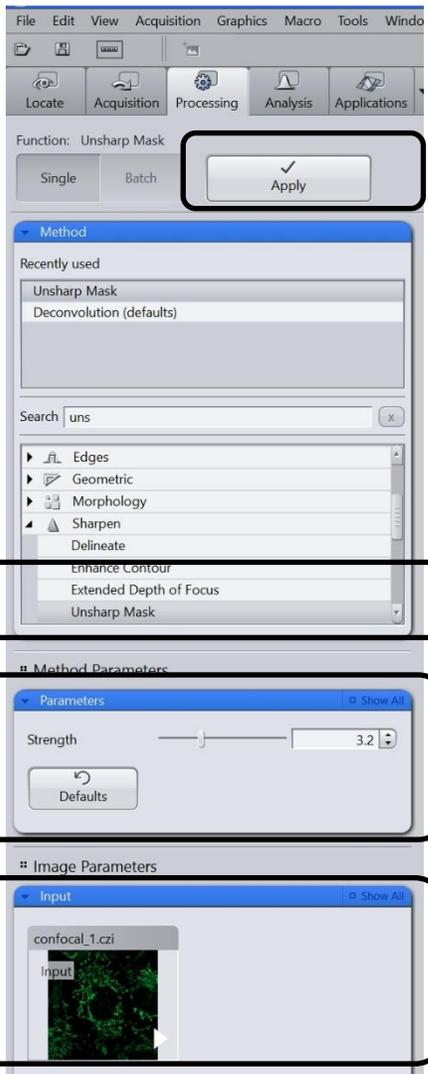
Before

After



ZEN Image Processing

Image Clearing Option 2: Unsharp masking

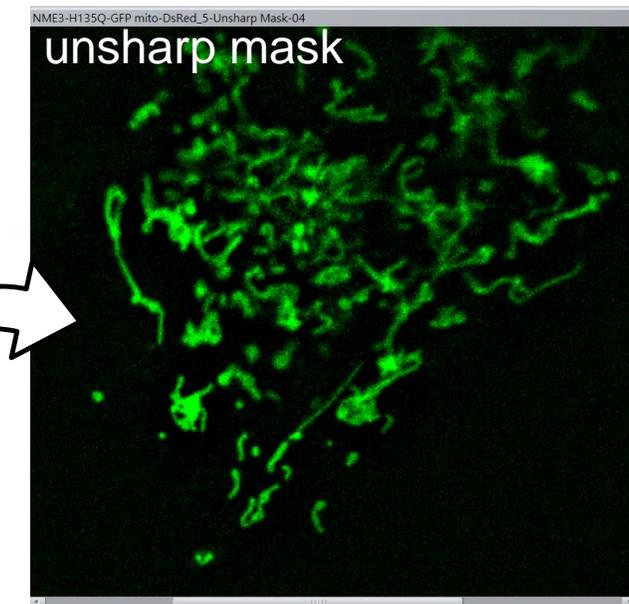
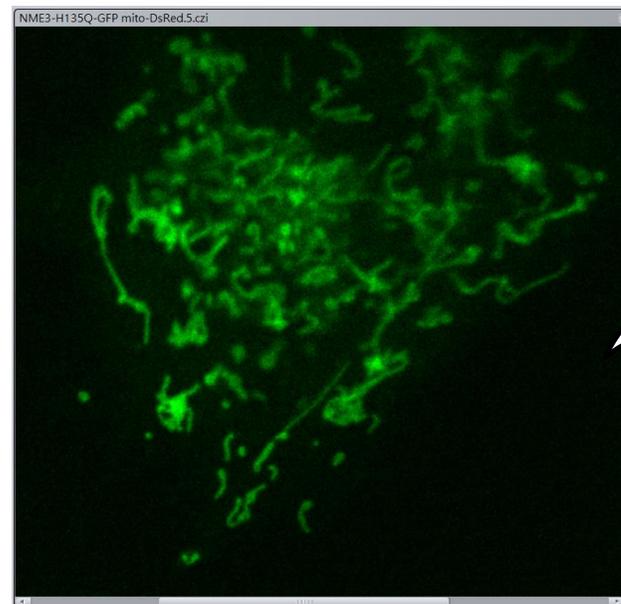
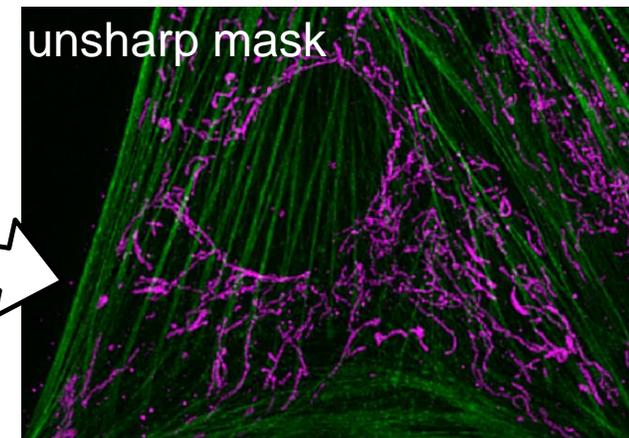
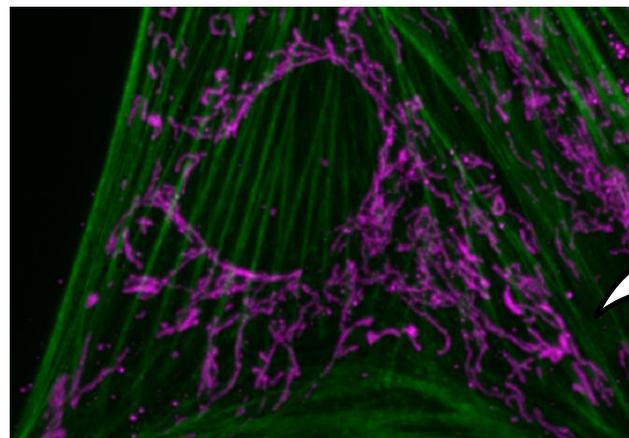


4

1

3

2



ZEN 3.3 Image Processing

Image Clearing Option 3: Background subtraction

Methods

Background subtraction **1**

Method Parameters

Parameters **3**

Radius: 1

Create background only

Do smoothing beforehand

Light background

Defaults

Image Parameters

Input **2**

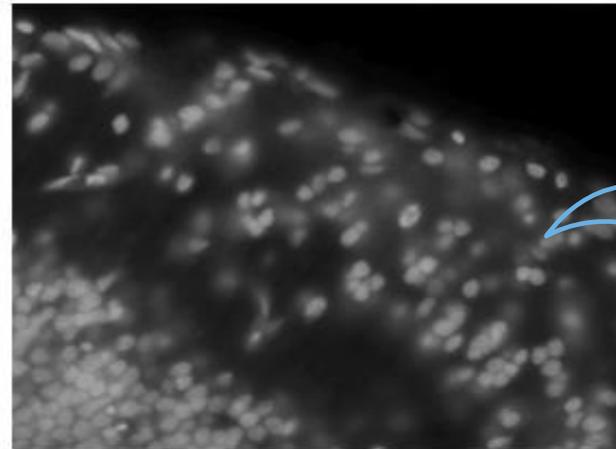
Cell leading edg...63E.tif

Input

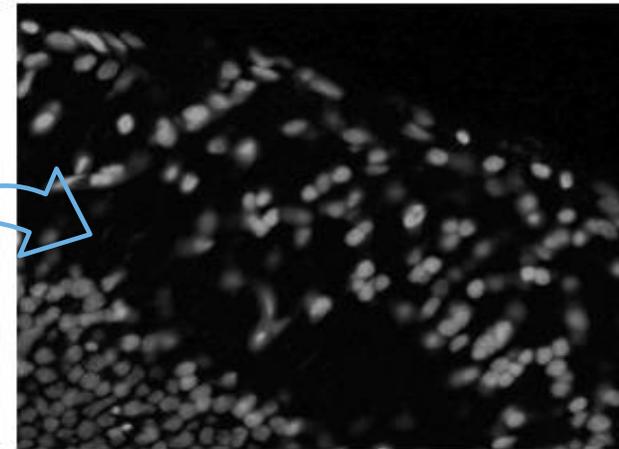
Output

Single
Plane

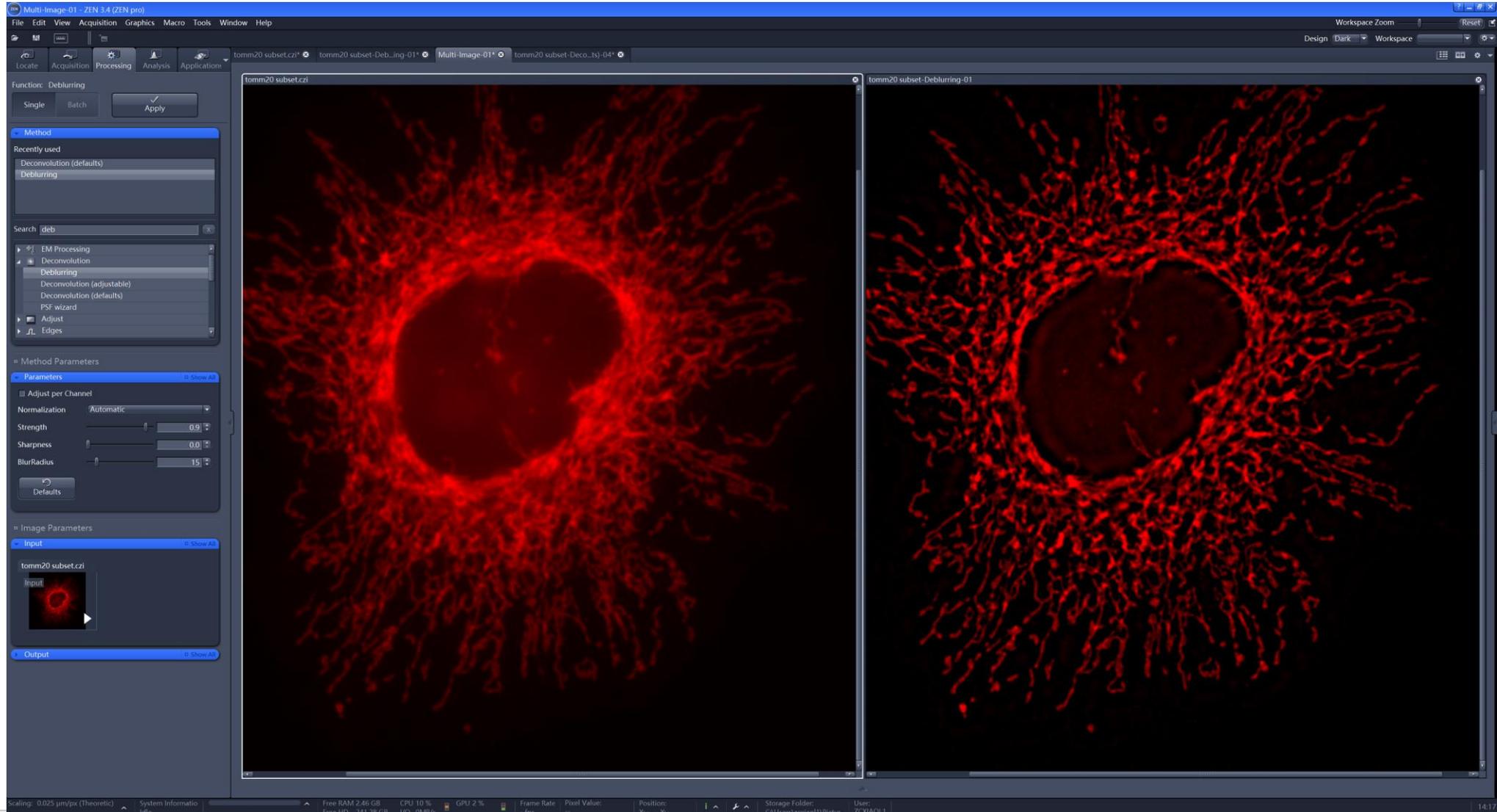
Widefield (Original)



Background Subtraction

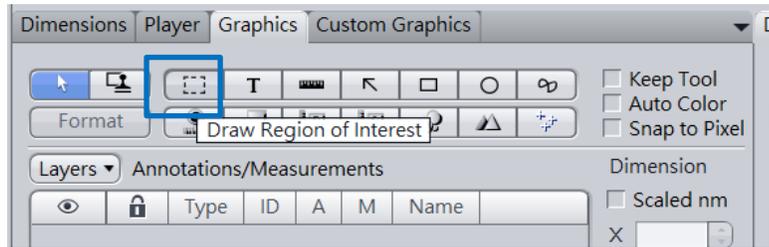


Option 4: ZEN 3.4 WF vs Deblurring/2D DCV

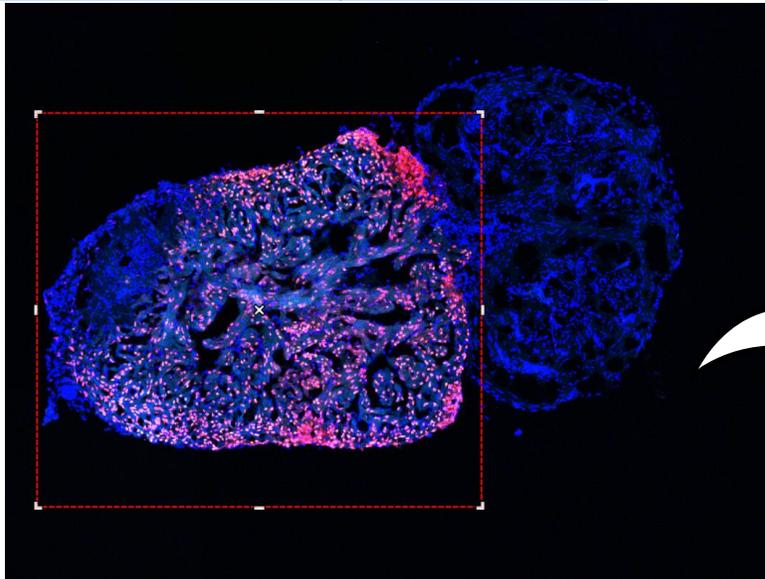


裁圖，複製選取範圍影像

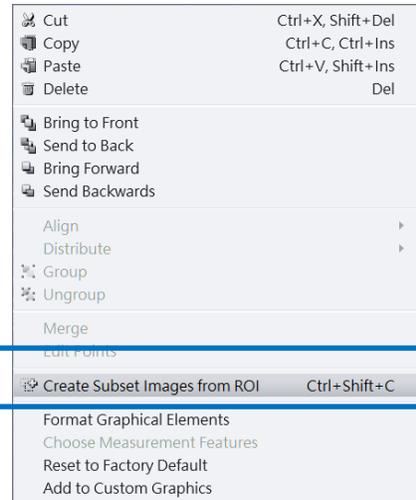
1.



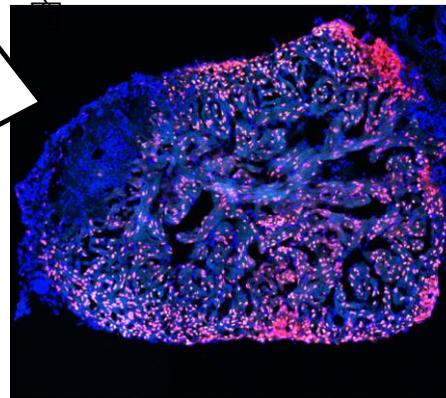
2. Draw an area and mouse right click



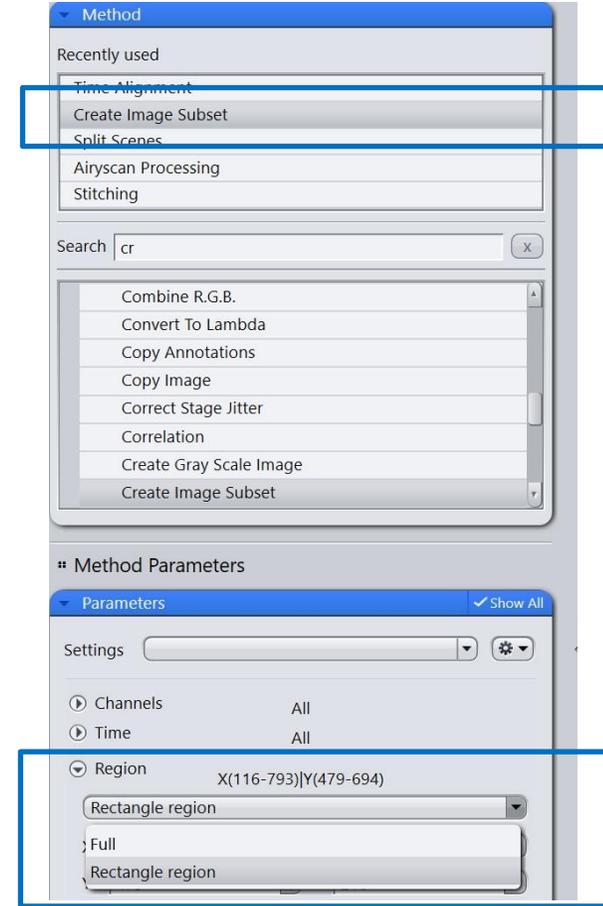
3. Create Subset Images from ROI



4. 獲得裁切範圍於新視



3. 或者
Processing → Create Image Subset

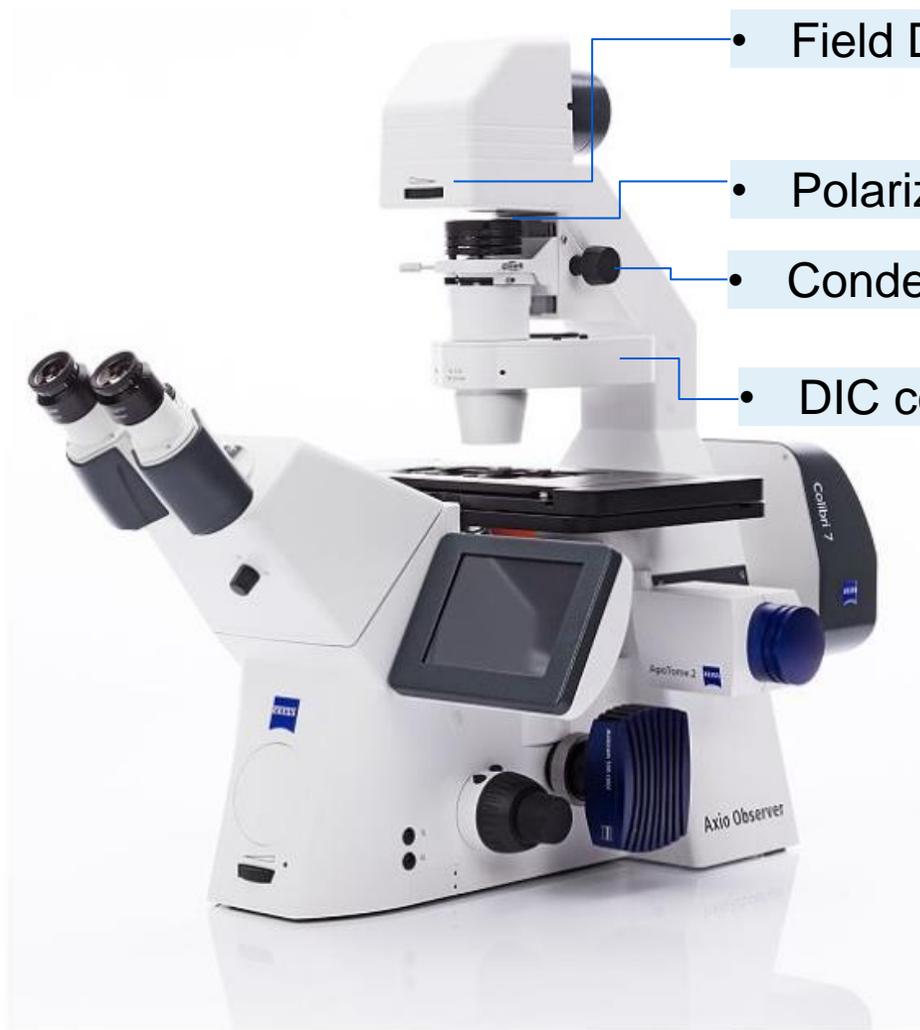


補充: 穿透光 Bright Field / DIC Observation



先於Ocular(目鏡)下調整最佳DIC 對比

Bright Field / DIC Observation Microscope setting for DIC



• Field Diaphragm

• Polarizer

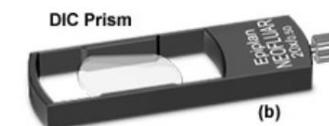
• Condenser focusing

• 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
5. Swing the polarizer holder in
6. Choose condenser turret position for DIC
7. Insert the objective DIC prism and adjust the knob



Polarizer



DIC Prism

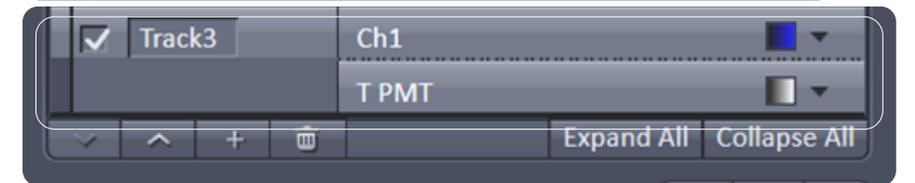
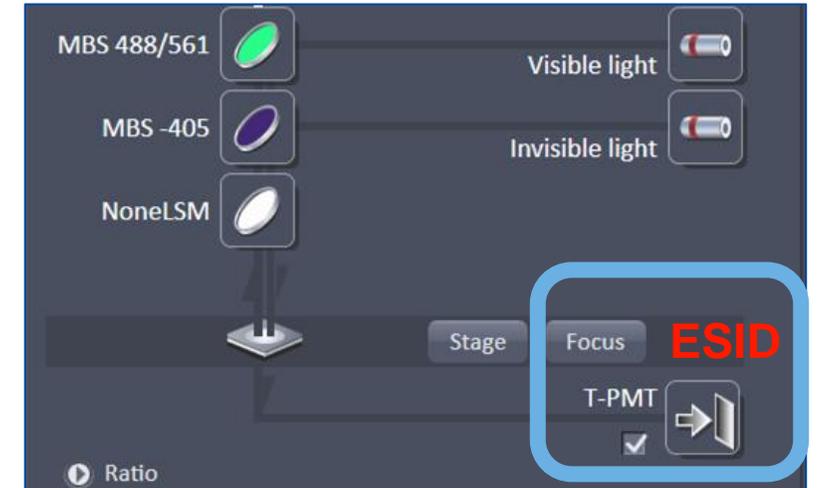
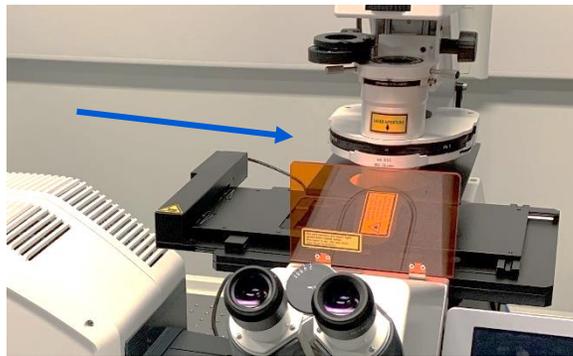
(b)

拍圖補充: DIC 影像

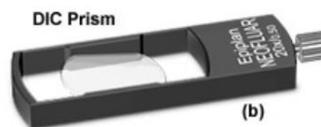


拍攝DIC影像

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. 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) 。



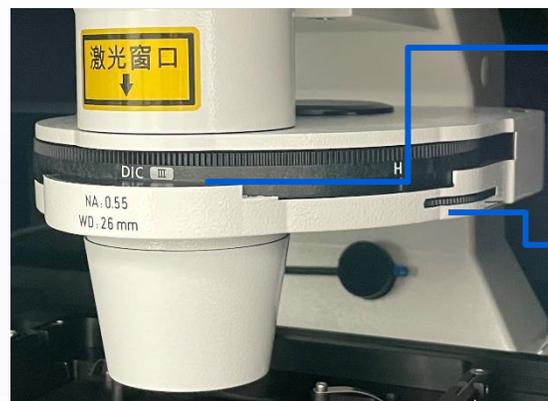
Bright Field / DIC Observation Microscope setting for DIC



DIC prism可能會造成訊號誤差，AiryScan時建議拔出



1. Focus the sample with objective
2. Adjust the condenser height by condenser focus knob (標記線對齊).
3. Check the condenser center position by closing the field diaphragm and reopen it after focusing the condenser.
4. Choose the DIC filter position (螢光濾片轉盤)
5. Swing the polarizer holder in
6. Choose condenser turret position for DIC(油鏡:轉到 DICIII)
7. Adjust aperture diaphragm (油鏡:全開)
8. Insert the objective DIC prism and adjust the knob

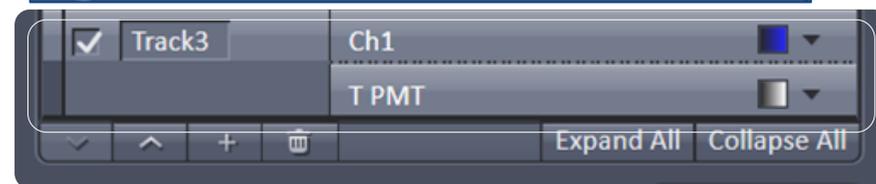
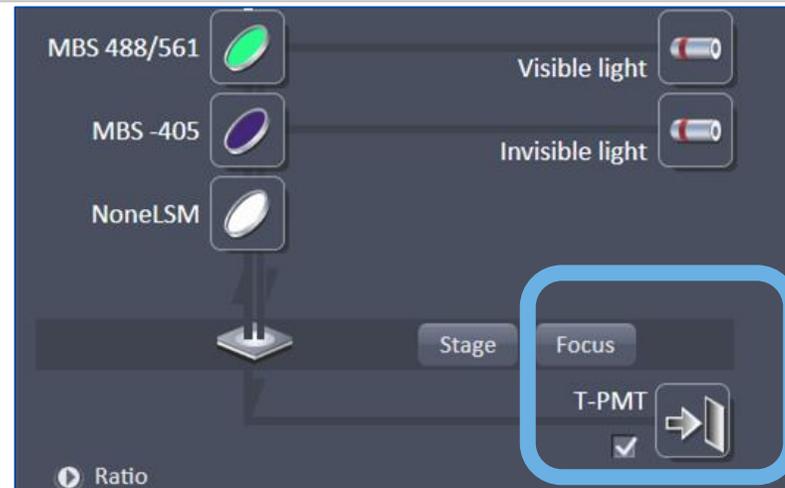
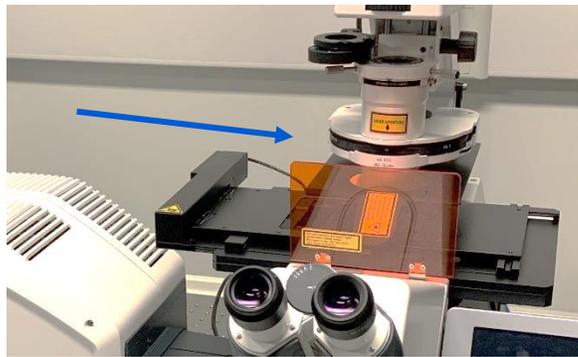


拍圖補充: DIC 影像



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. 將螢光設定好最後再開啟T-PMT(或ESID)
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) 。



40x/ 1.1水鏡 請用專用鏡油

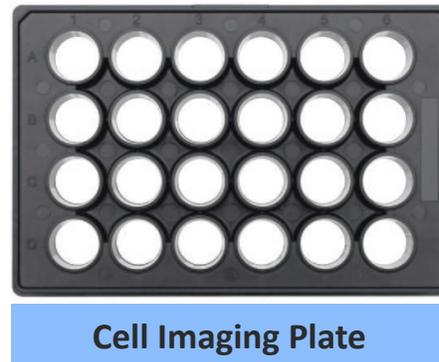
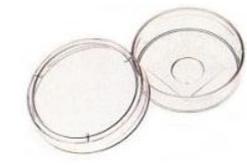


認明瓶蓋與瓶身標示

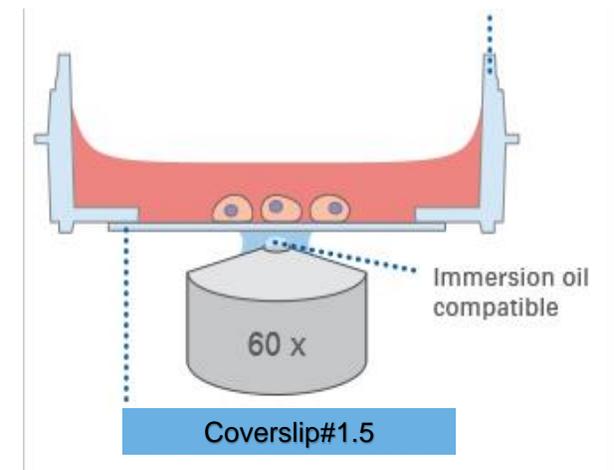
勿與其他鏡油混合使用或取代
(可以dd H2O 取代，需注意蒸發問題)

- 室溫請參考黑線位置
- 37度加熱時參考紅線

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**

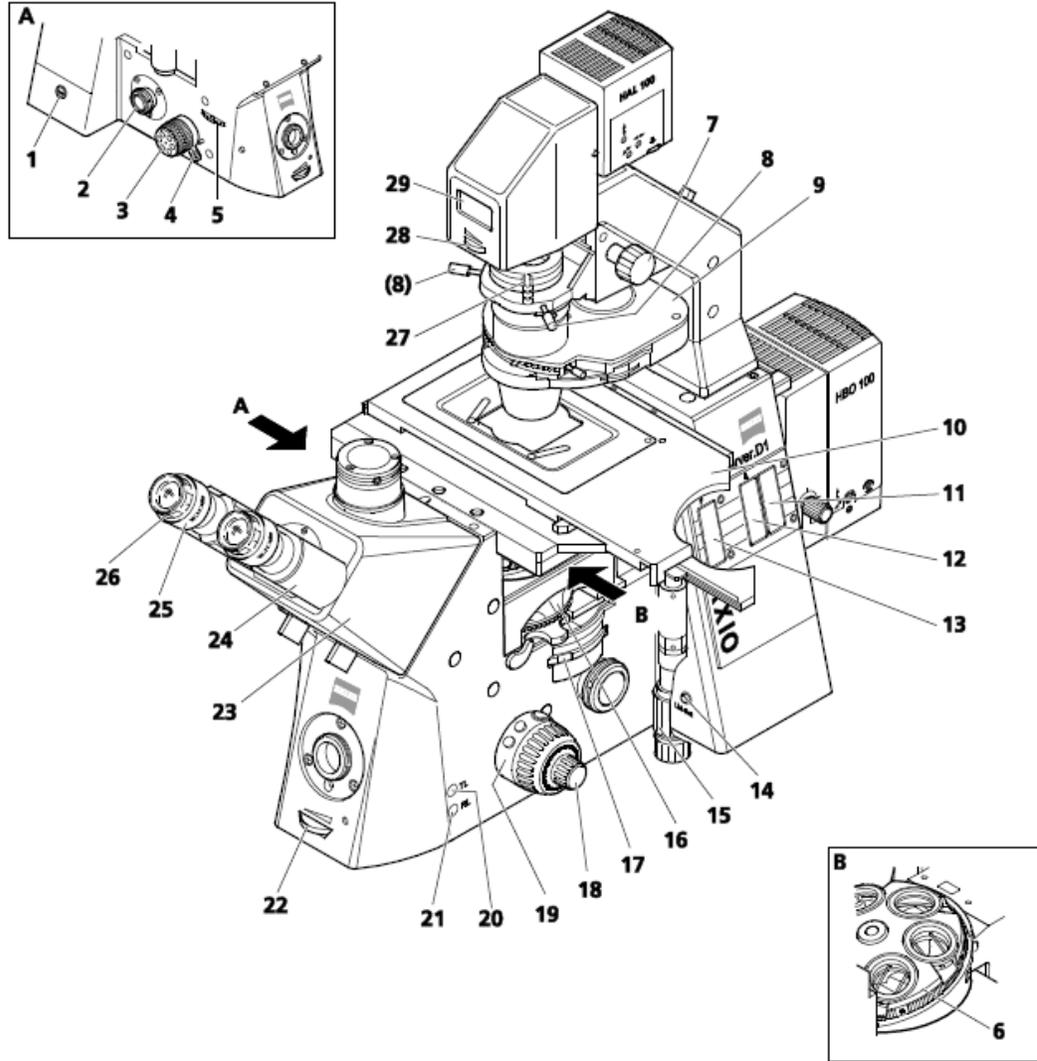


Turn off the system



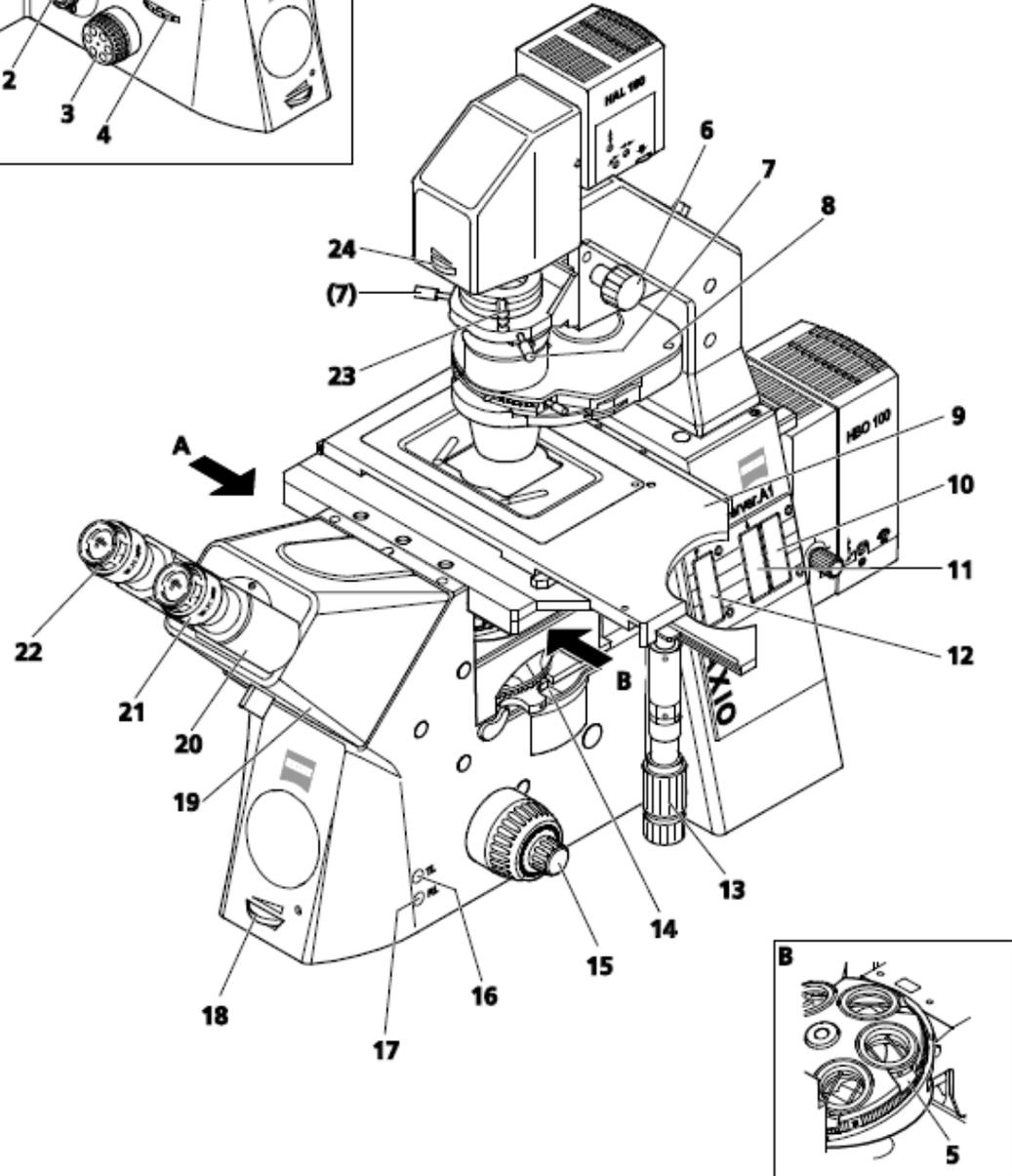
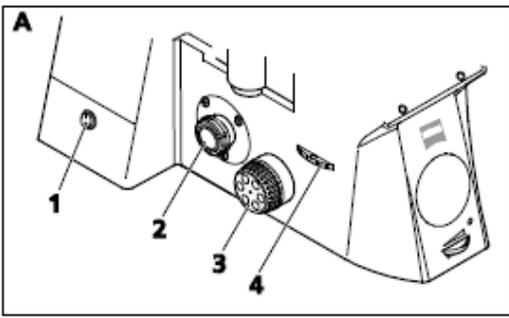
- ➔ • Exit ZEN
- ➔ • 如果有使用活細胞培養裝置，TFT面板內Incubation 頁面OFF 所有 (Incubator 及Heating insert)溫控
 - 關閉C02，鋼瓶鎖緊
 - 加濕水瓶倒除
- ➔ 4
- ➔ 3
- ➔ 2
- ➔ 1





- 1 On / off button
- 2 Left Sideport
- 3 Coarse / fine focus drive with fine drive, flat
- 4 Vertical stop for focus drive
- 5 Light path switching control
- 6 Objective nosepiece
- 7 Vertical adjustment knob for condenser
- 8 Condenser centering screw
- 9 Condenser
- 10 Microscope stage
- 11 3-position filter slider slot
- 12 Slot for FL attenuator
- 13 Slot for iris stop slider as reflected light luminous-field stop
- 14 LM set button
- 15 Drive knobs for controlling XY positioning of the mechanical stage
- 16 Reflector turret
- 17 Optovar turret control wheel
- 18 Control ring, left
- 19 Control ring, right
- 20 TL button for transmitted light shutter
- 21 RL button for reflected light shutter on and off
- 22 Halogen illumination intensity control
- 23 Binocular tube
- 24 Binocular section of the binocular tube
- 25 Eyepiece
- 26 Eyepiece adjustment ring
- 27 Polarizer D with 2-position filter changer or 3-position filter changer
- 28 Luminous-field stop control
- 29 LCD display

Fig. 4-2 Axio Observer.D1 components and controls (coded, semi-motorized)



- 1 On / Off switch
- 2 Left Sideport
- 3 Coarse / fine focus drive (left side) with fine drive, flat
- 4 Light path switching control (leftSideport / vis)
- 5 Objective nosepiece
- 6 Vertical adjustment knob for condenser
- 7 Condenser centering screw
- 8 Condenser
- 9 Microscope stage
- 10 3-position filter slider slot
- 11 Slot for iris stop slider as reflected light aperture stop or FL attenuator
- 12 Slot for iris stop slider as reflected light luminous-field stop
- 13 Drive knobs for controlling XY positioning of the mechanical stage
- 14 Reflector turret
- 15 Focus drive coarse / fine
- 16 TL button for switching the transmitted light halogen illuminator on and off or for opening and closing the transmitted light shutter
- 17 RL button for switching fluorescence shutter on and off
- 18 Halogen illumination intensity control
- 19 Binocular tube
- 20 Binocular section of the binocular tube
- 21 Eyepiece
- 22 Eyepiece adjustment ring
- 23 Polarizer D with filter changer
- 24 Luminous-field stop control

Fig. 4-1 Axio Observer.A1 components and controls (manual)

