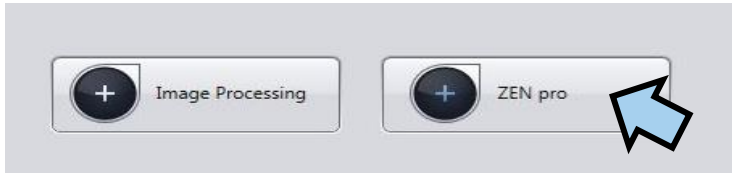
Carl Zeiss Axio Observer / ZEN blue

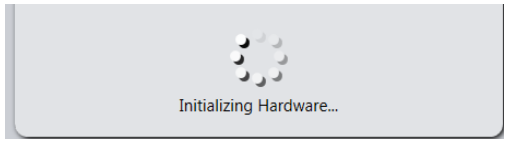
Quick Guide

啟動軟體 ZEN

1 進入ZEN BLUE

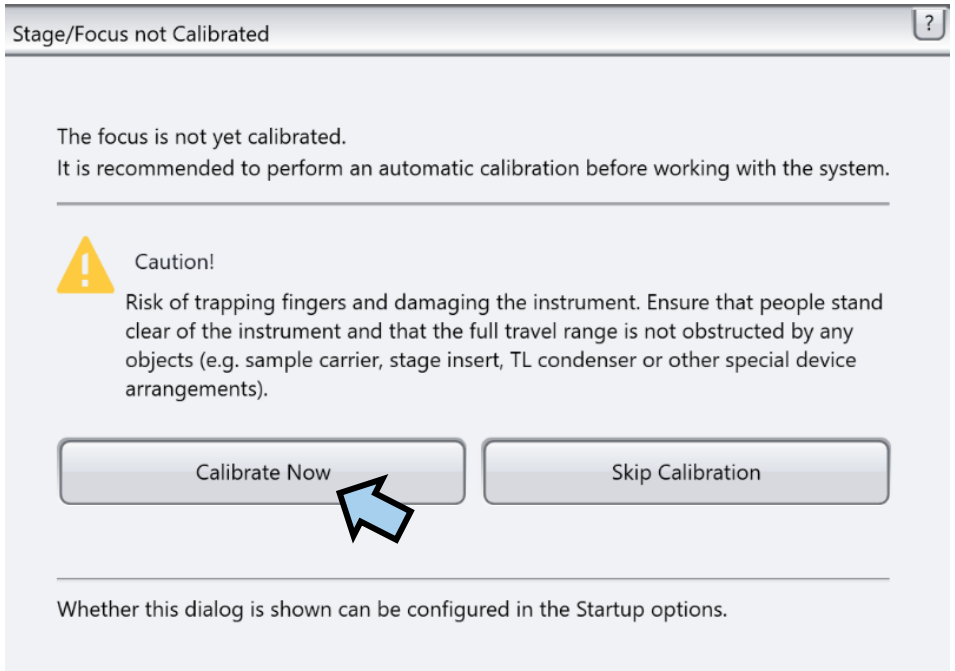
2 ZEN pro
連接硬體 啟動拍照功能





等待約一分鐘

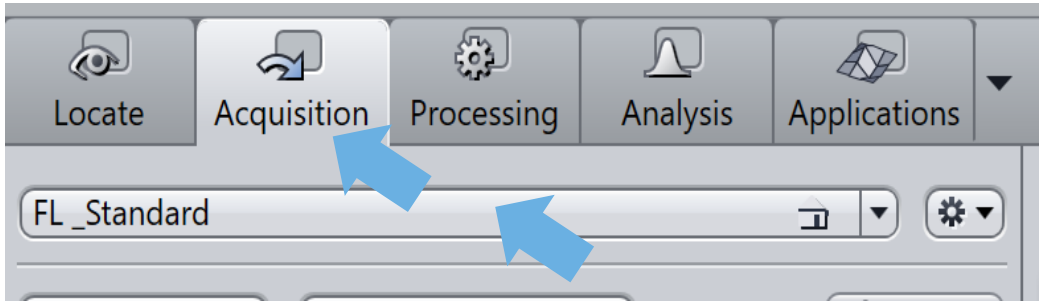
3 [可略過] 需先清空載物台，先不放樣品



拍攝螢光 1.
套用拍攝參數experiment manager

套用拍攝參數

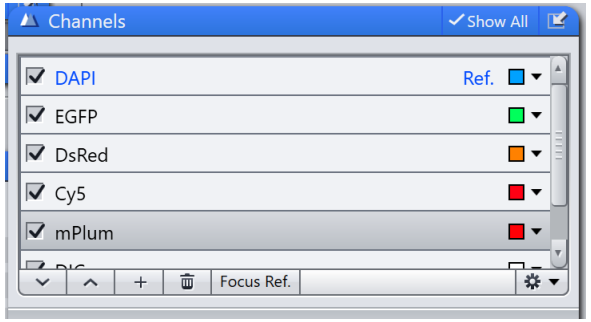
拍攝螢光請選擇FL image (Axiocam 712 mono)



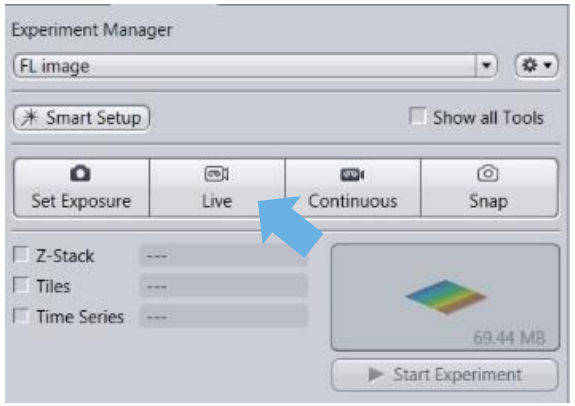
拍攝螢光 2.

1

選擇需要的channel

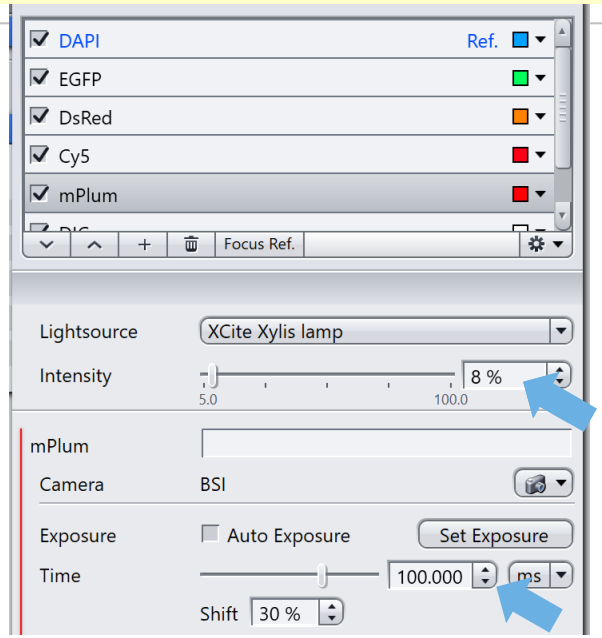


2



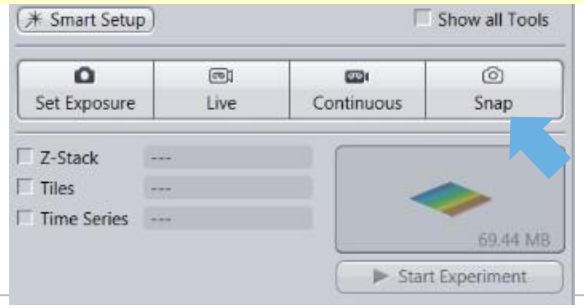
3

調整所有channel的light source intensity
與曝光時間



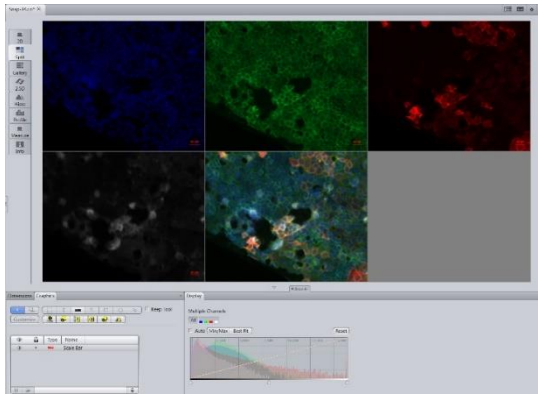
4

按下snap
系統會自動拍下所有已打勾的channels



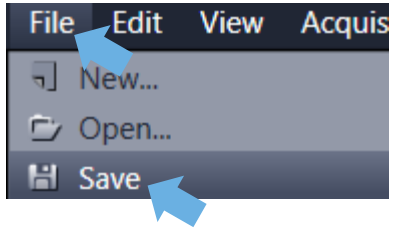
5

得到完整影像



6

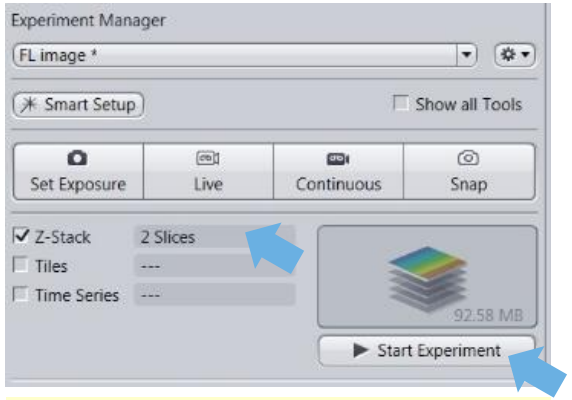
存檔



詳見下頁

拍攝multichannel+ Z stack

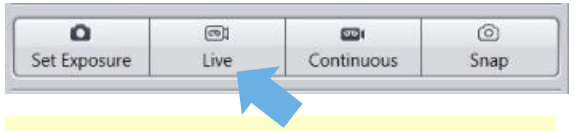
1



4

勾選z stack選項

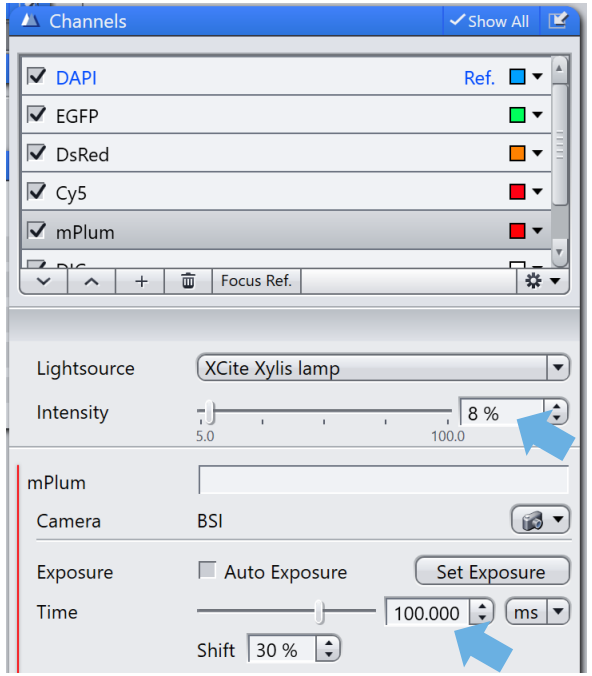
2



Live
調整所有channel的lightsource intensity 與曝光時間

3

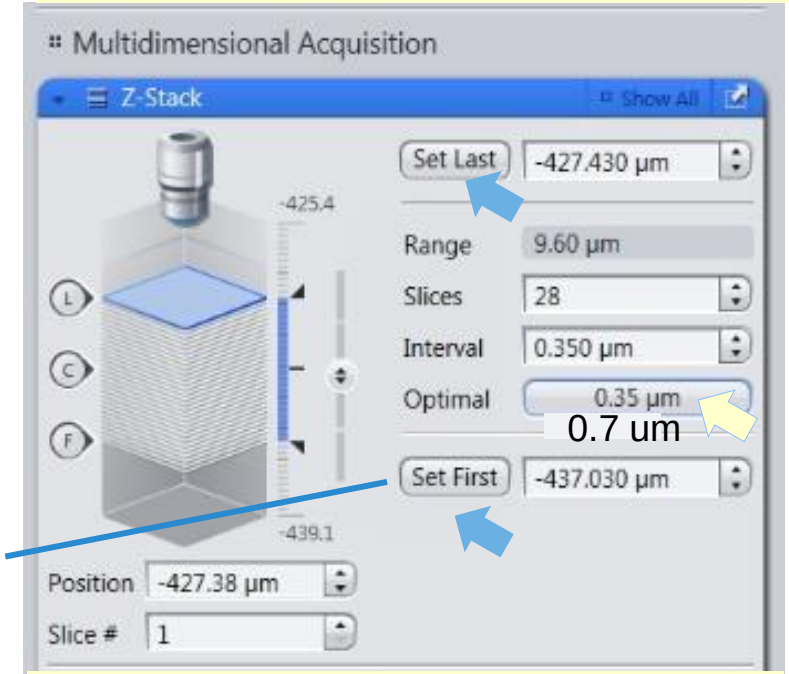
Live
調整所有channel的light source intensity 與曝光時間



4

Live→開始設訂Z stack的上下界限

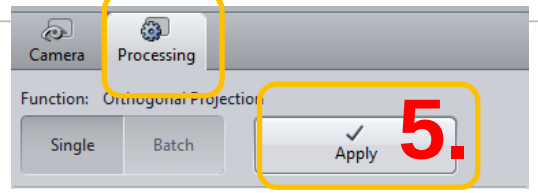
- 選擇一個channel>live
- 搭配Z軸調整視窗 > Z-stack
- 找到樣品焦距起點 >Set First
- 找到樣品焦距終點> Set Last



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

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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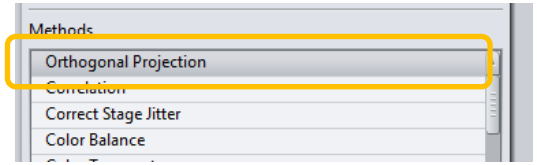
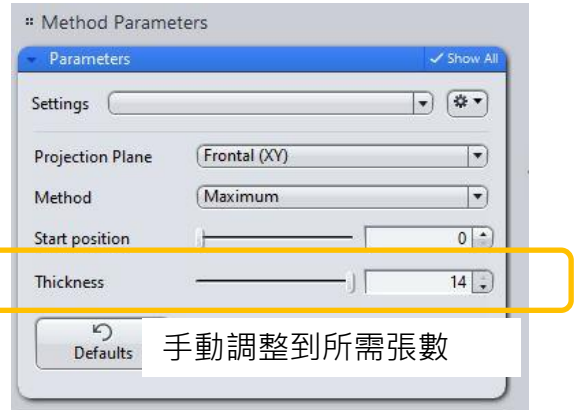
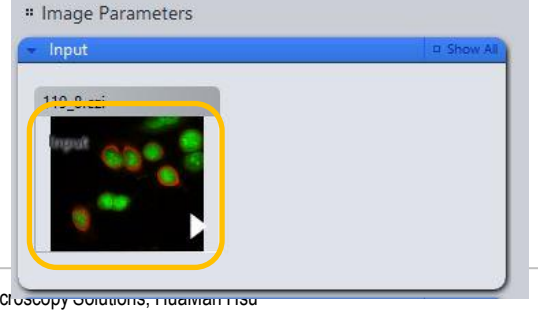


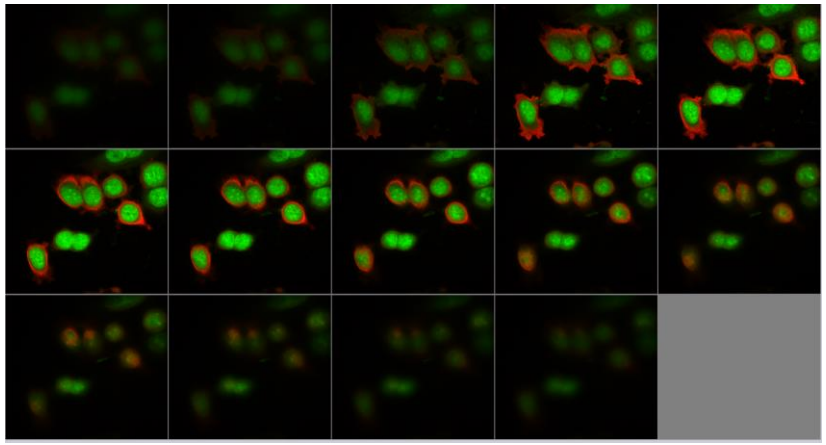
4.

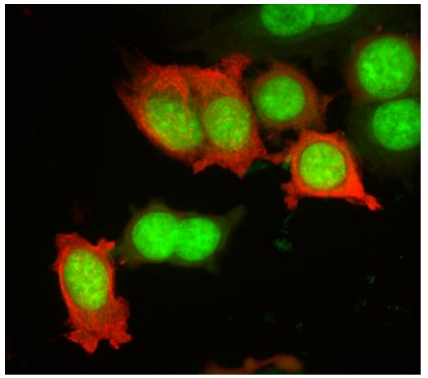


手動調整到所需張數

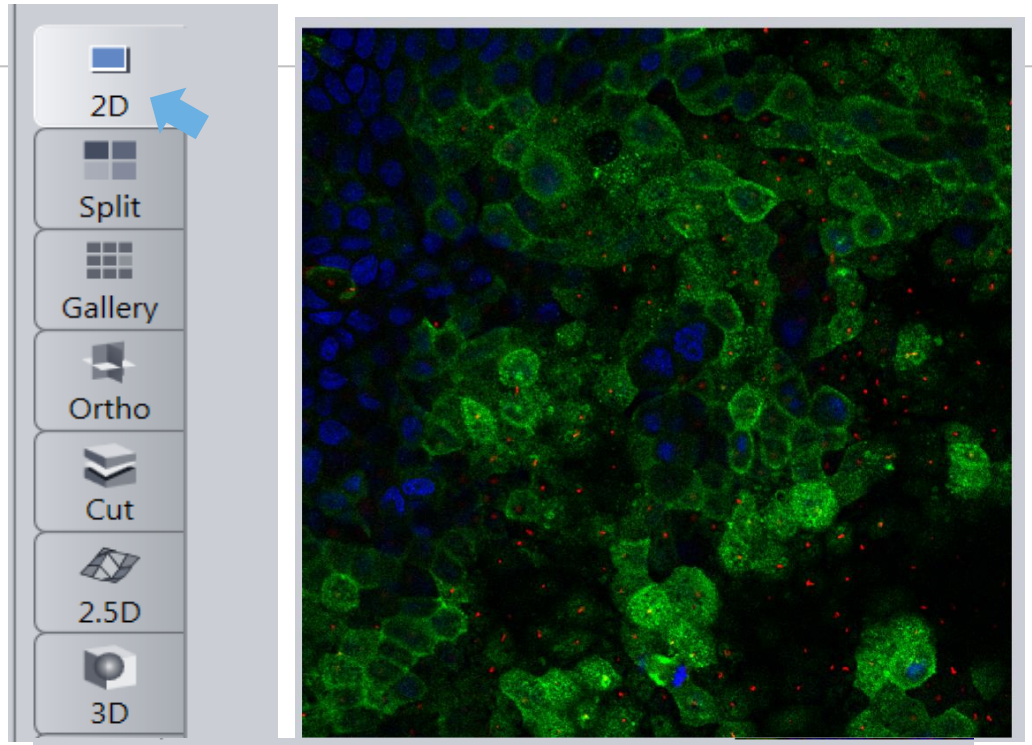
3.

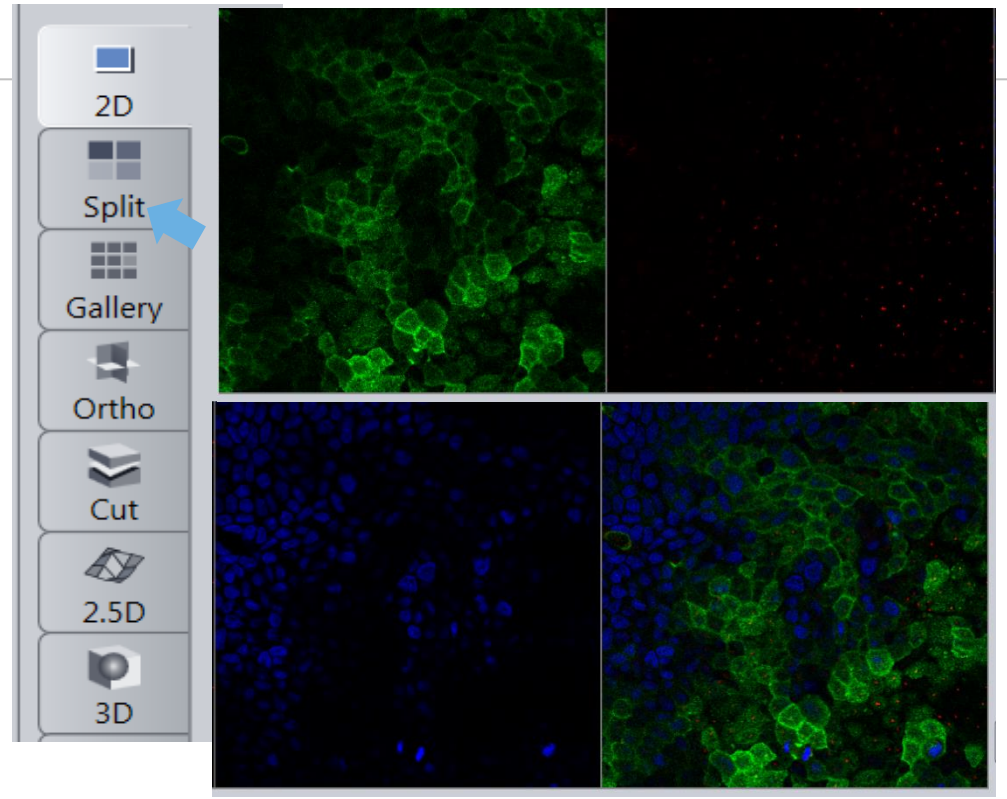


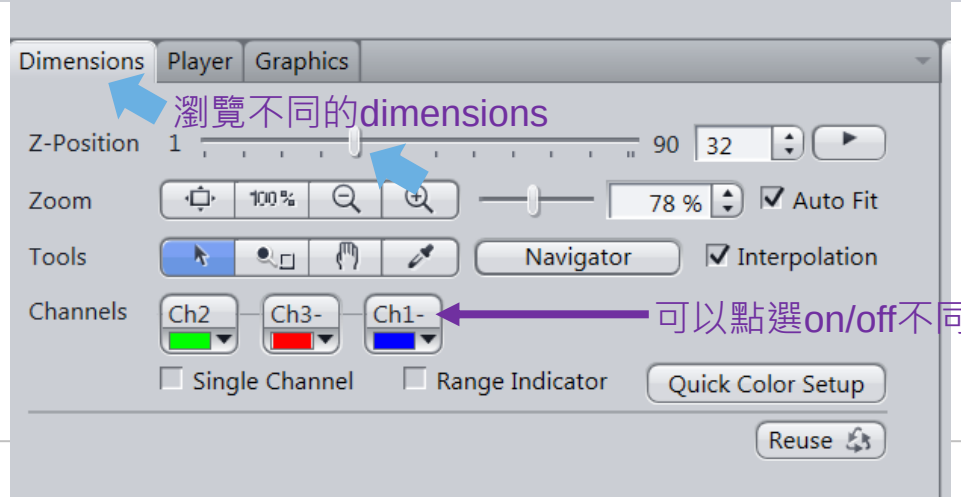


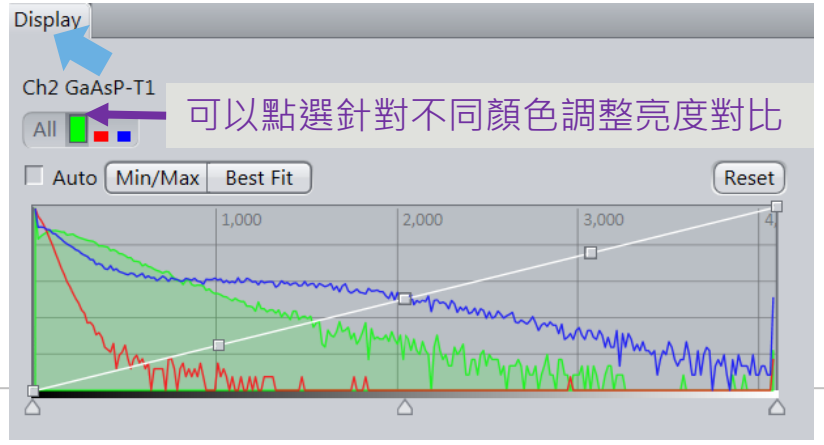


影像視窗2D/Split & Dimensions/Display



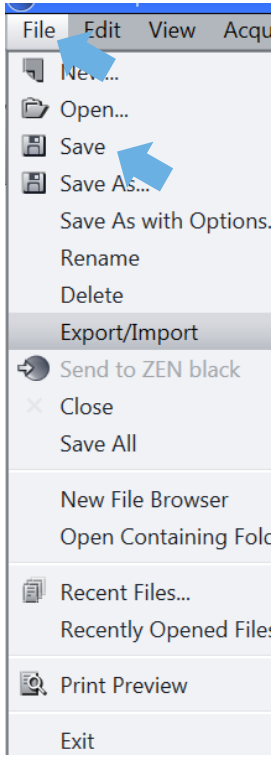






存檔

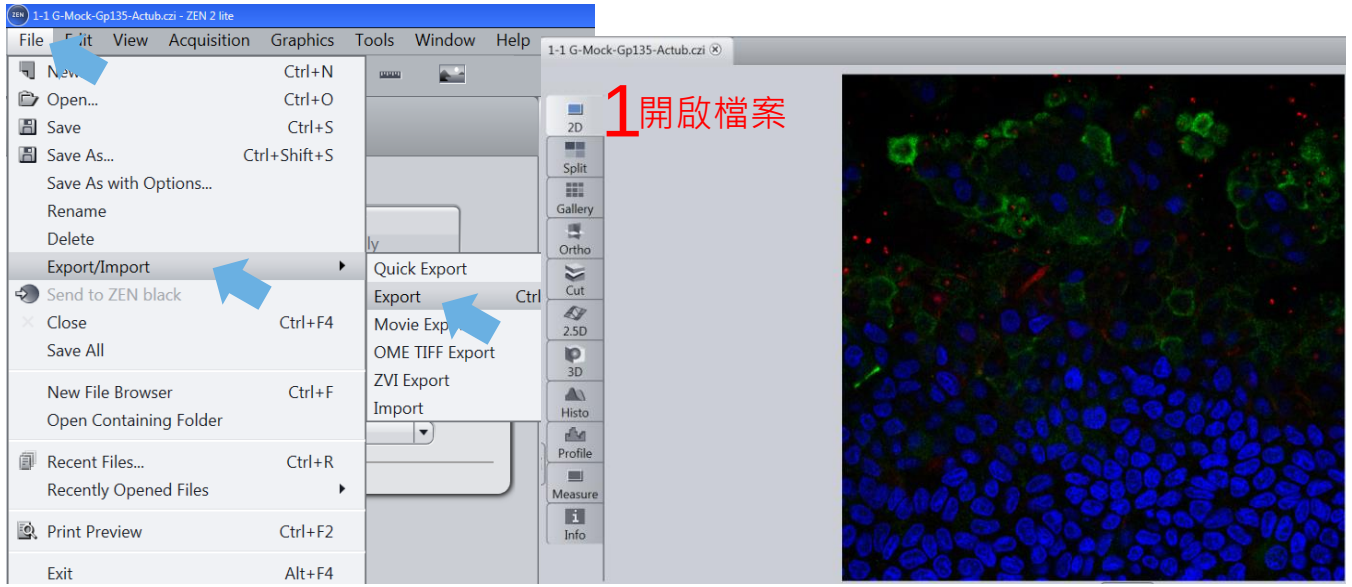
5



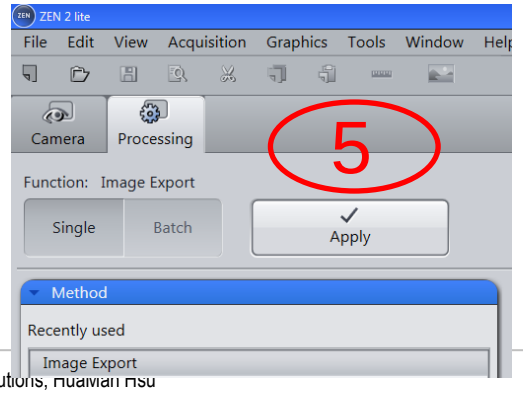
- 請選擇*.czi格式存檔，此格式為原始檔
- *.czi 日後可reuse
- 請勿直接存成tif/jpg檔，以免日後無法定量及添加尺規
- 需要純圖檔請進行圖檔輸出export或大量批次轉檔batch export，詳見下頁

圖檔輸出export (單一檔案)

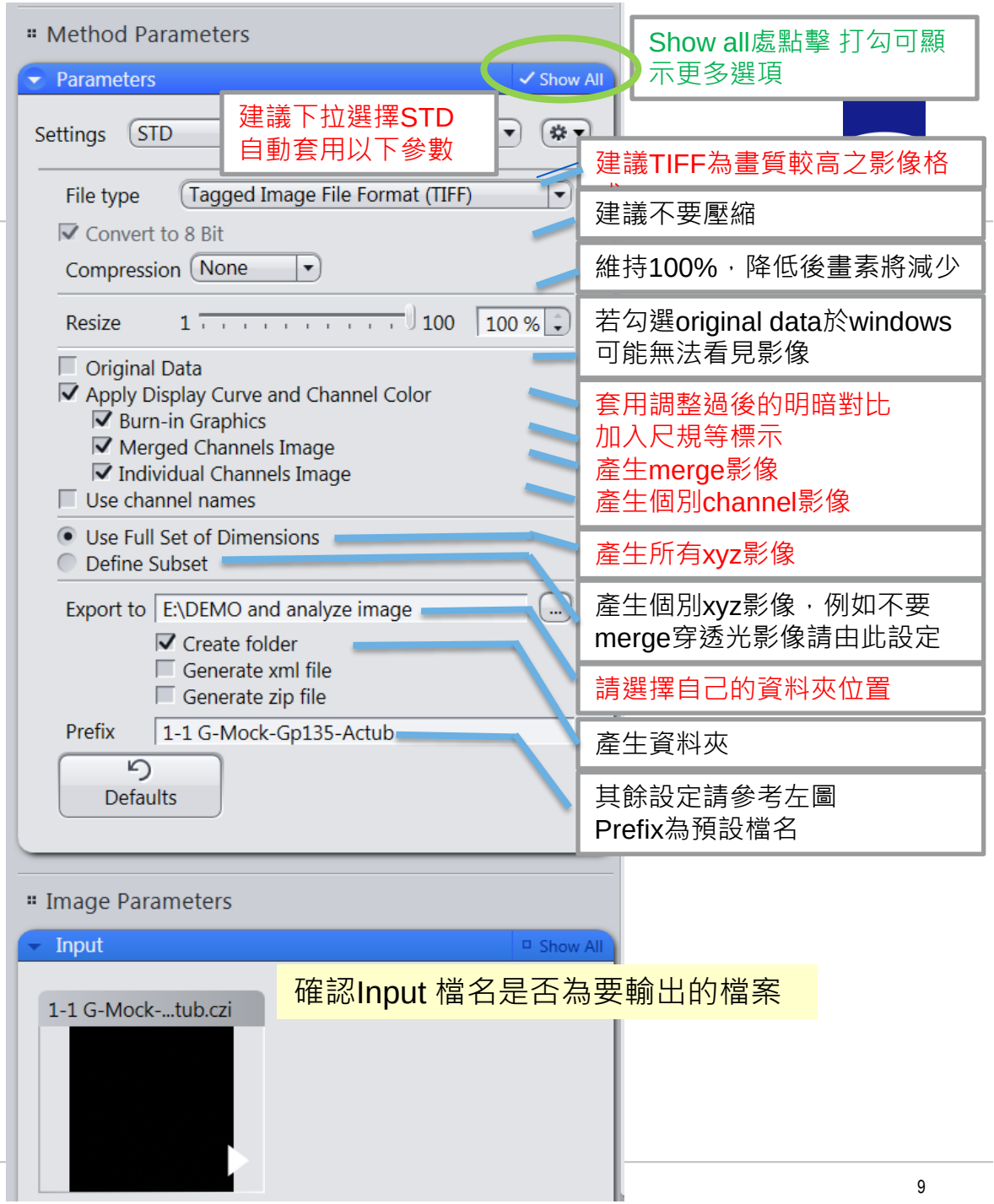
2



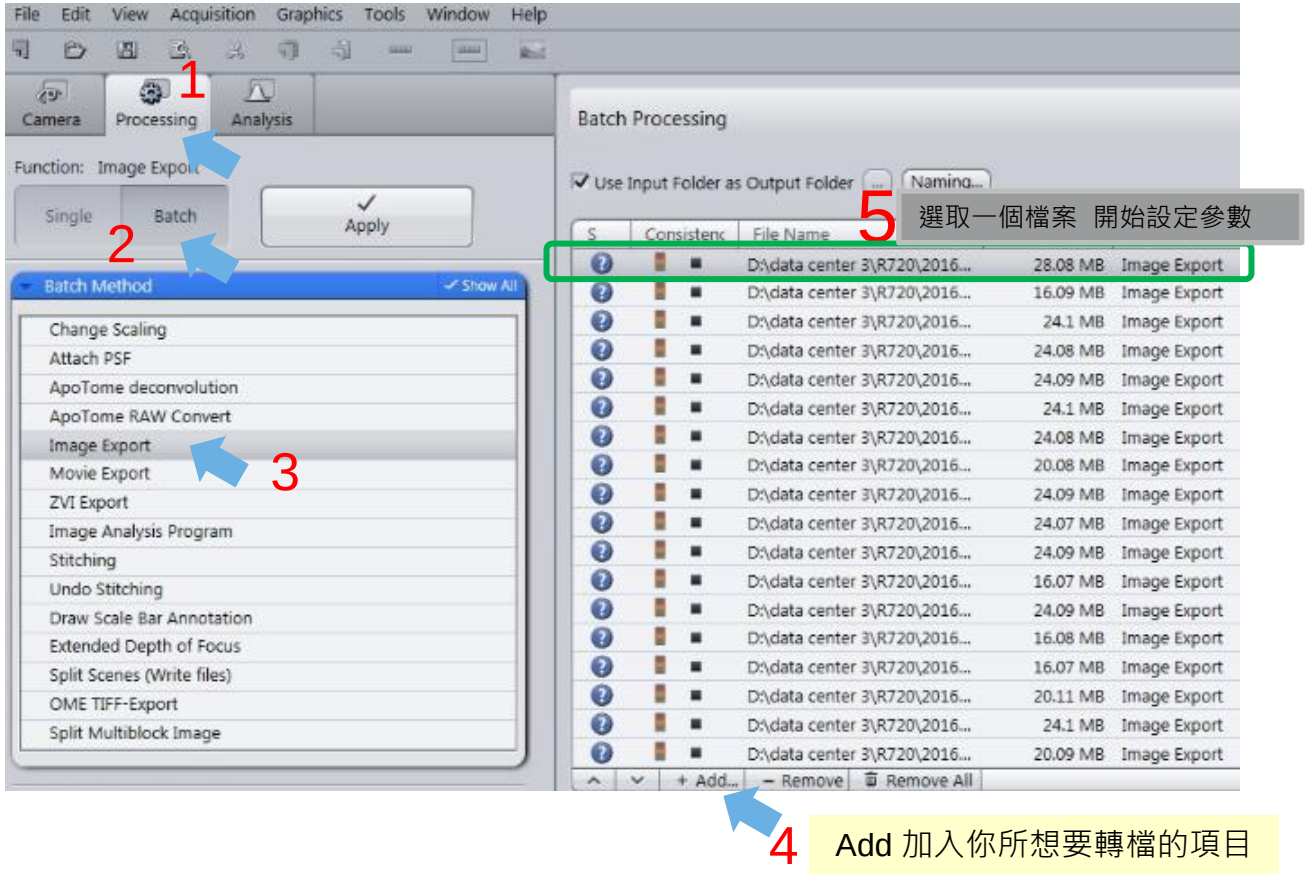
3

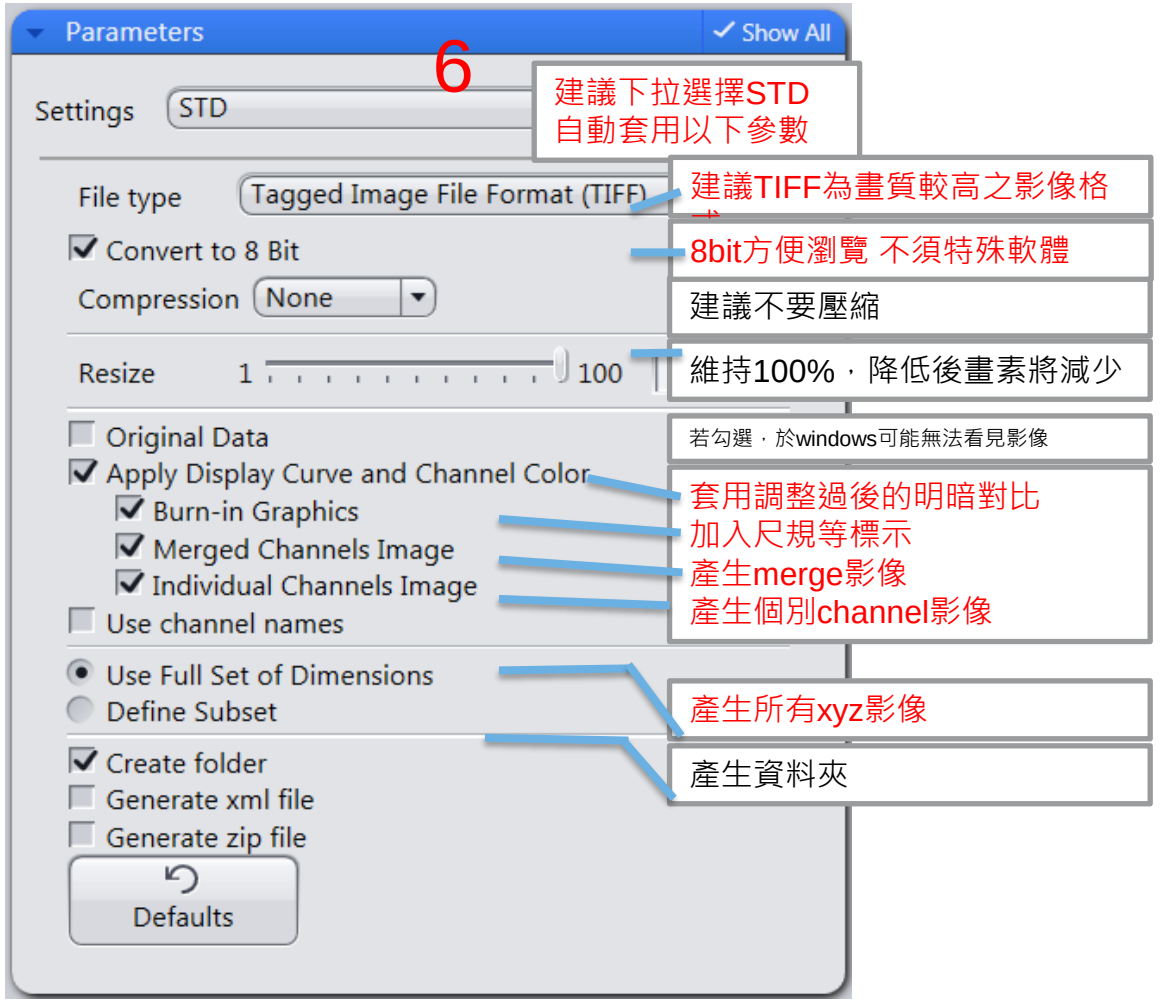


4

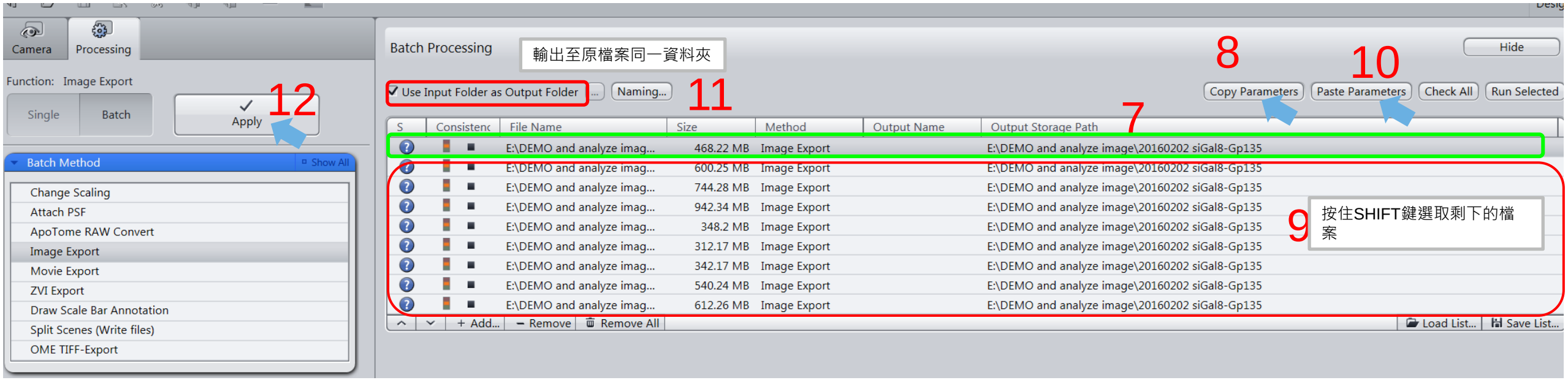


大量批次轉檔Batch export 1



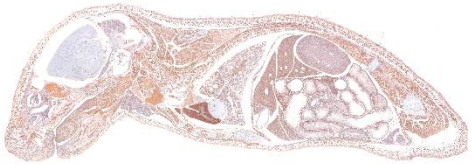


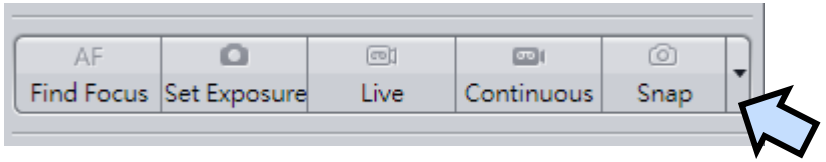
大量批次轉檔batch export 2



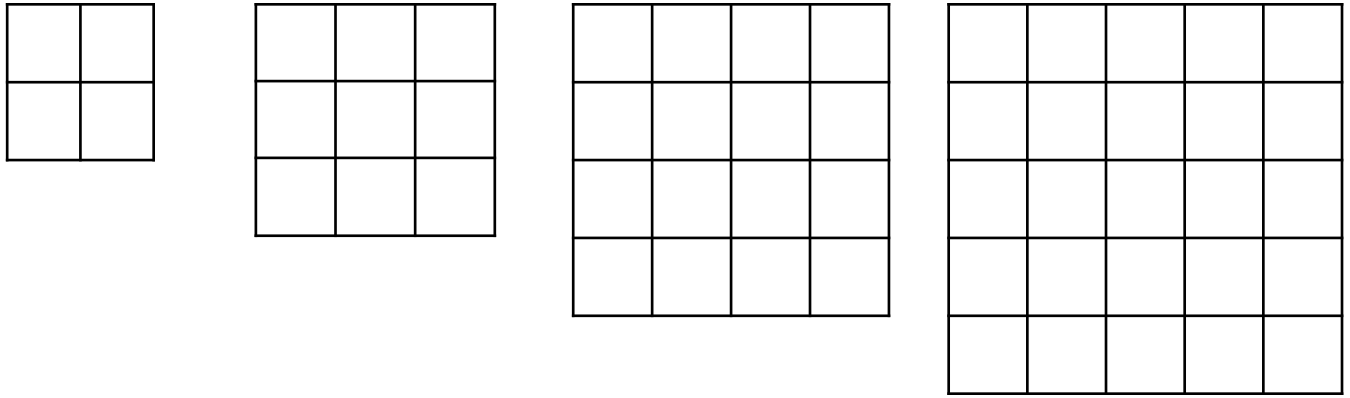
7~10 將設訂好的參數貼至其餘檔案當中，若沒有做paste parameter的動作，可能會失敗喔!!

拍攝拼圖
簡易拼圖 快捷鍵

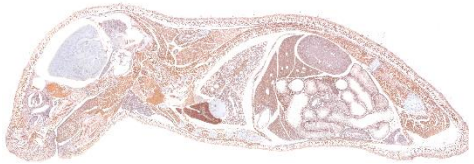


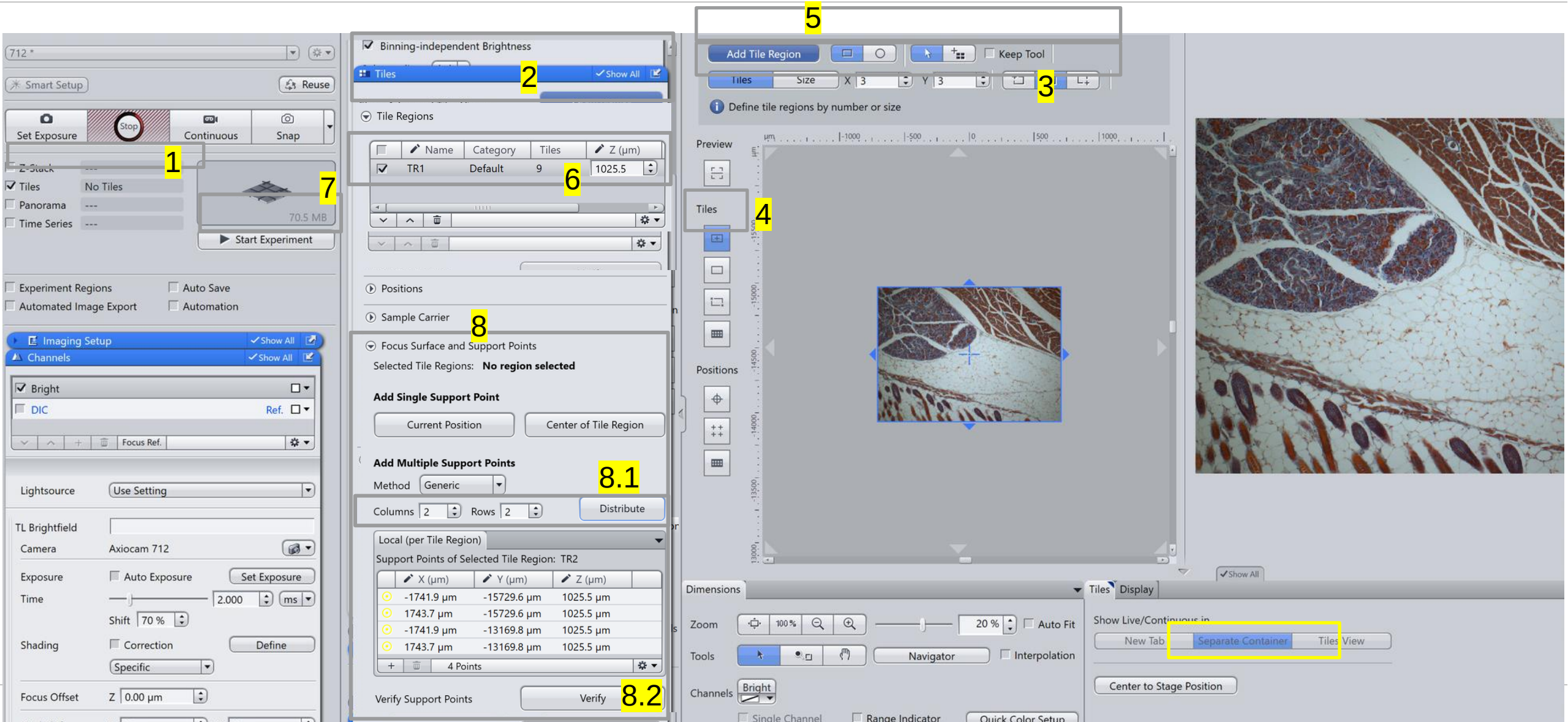


2x2
3x3
4x4
5x5

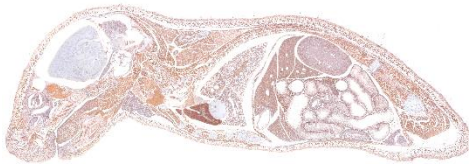


Tile Scan Setup





TILE Image stitching / fuse 拼圖結果融合處理



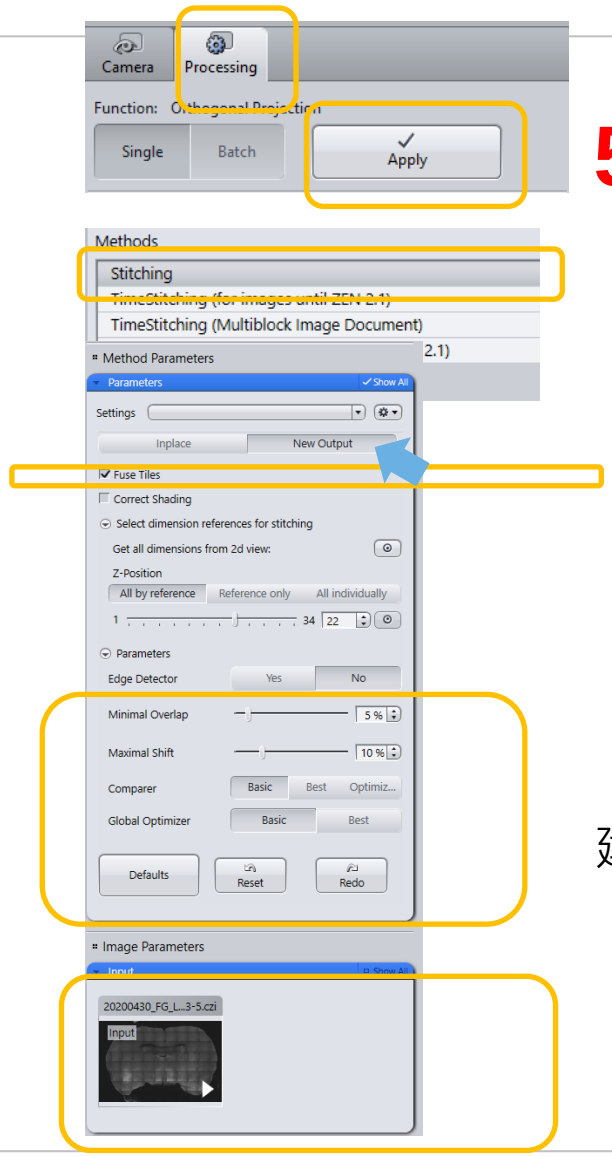
1.

2.

4.

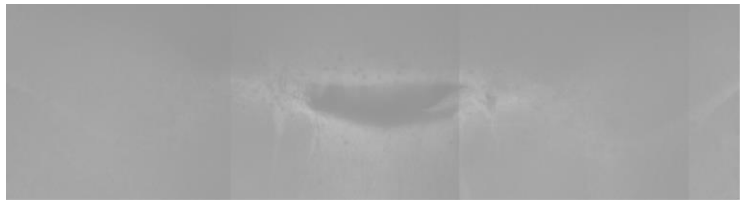
3.

5.

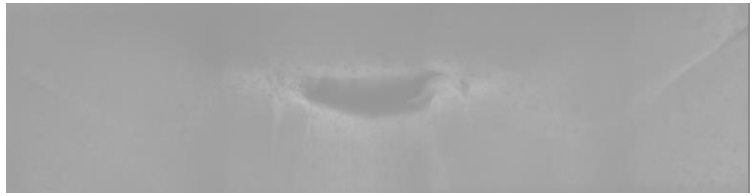


建議選擇Basic即可

Before

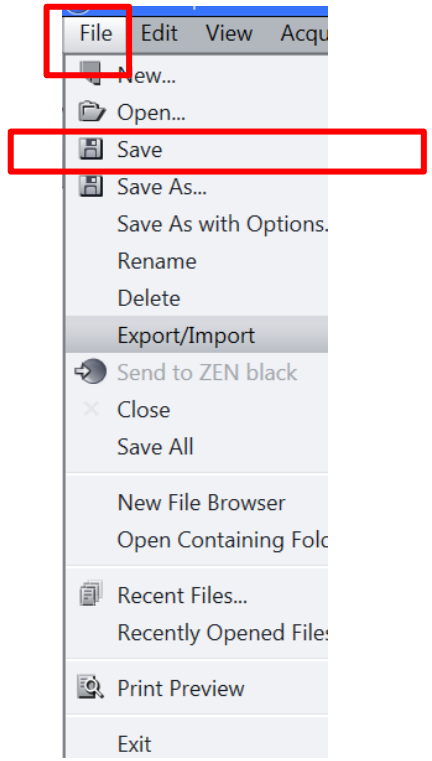


After



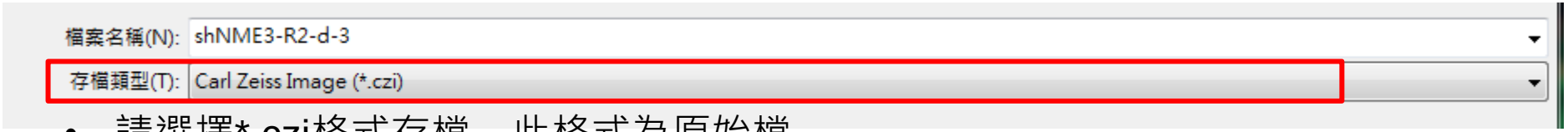
存檔

5



存檔位置請選擇D槽 data pool，請勿直接設定存於外面的data center中。

由於藉由網路傳輸，存檔速度會變得非常緩慢或者您的檔案可能會有損壞的風險。已發生過多起不幸~~切記!



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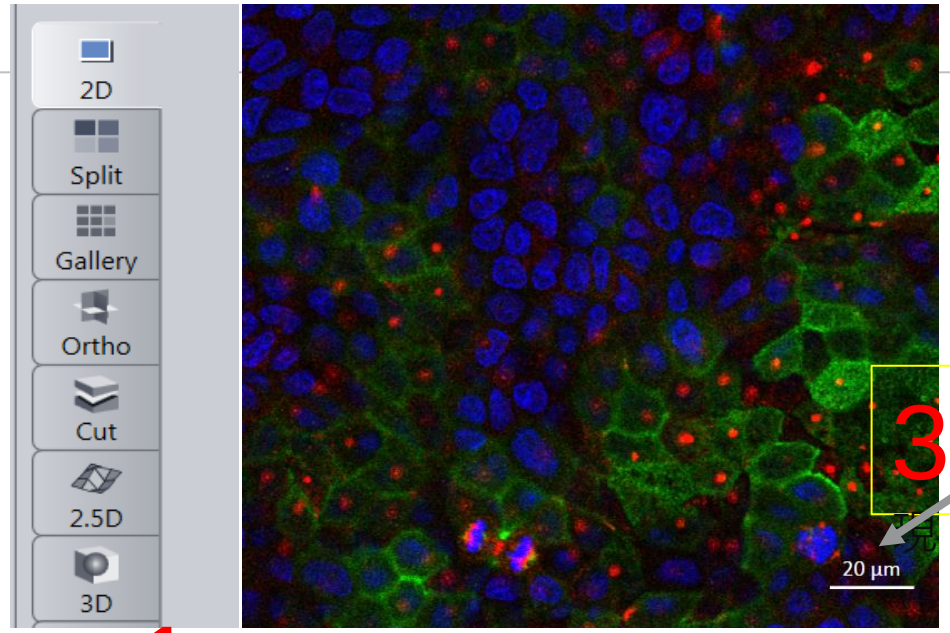
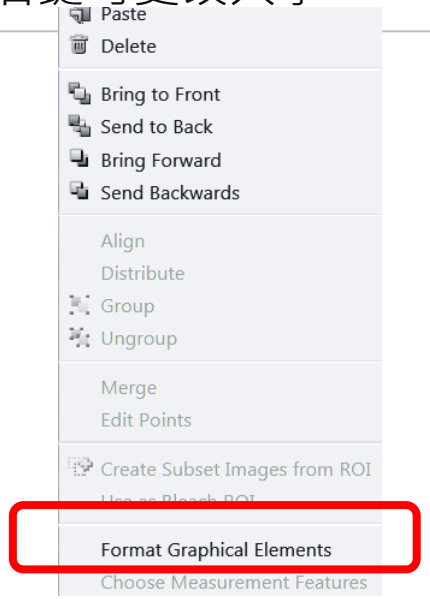
- 請勿直接存成tif/jpg檔，以免日後無法定量及添加尺規
- 需要純圖檔請進行圖檔輸出export或大量批次轉檔batch export，詳見下頁

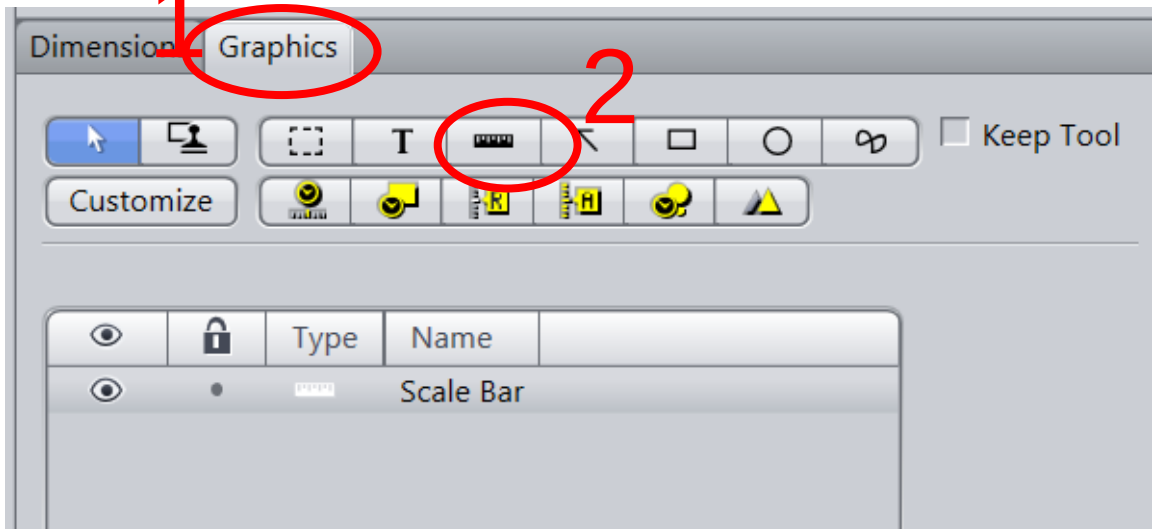
加入尺規



3 scale bar會自動出

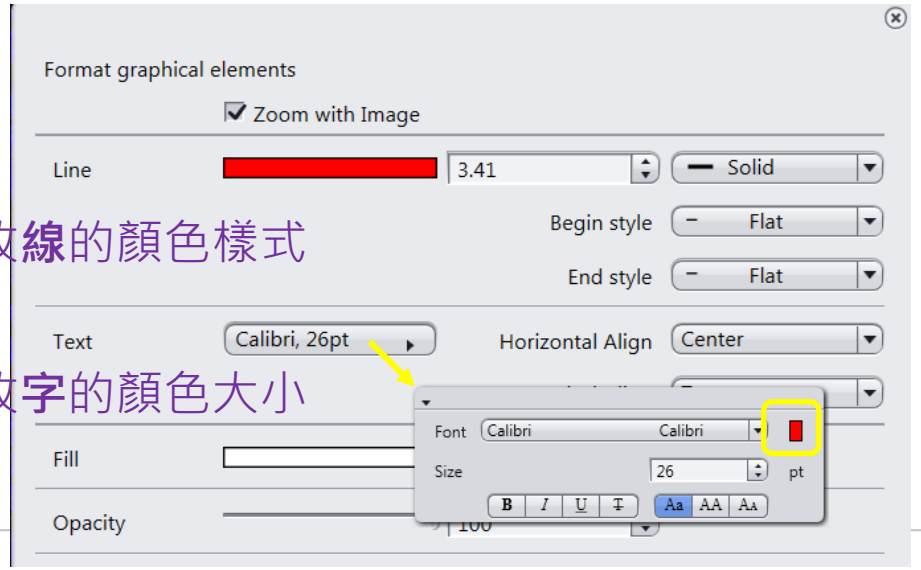
4 於scale bar按右鍵可更改大小顏色粗細等格式



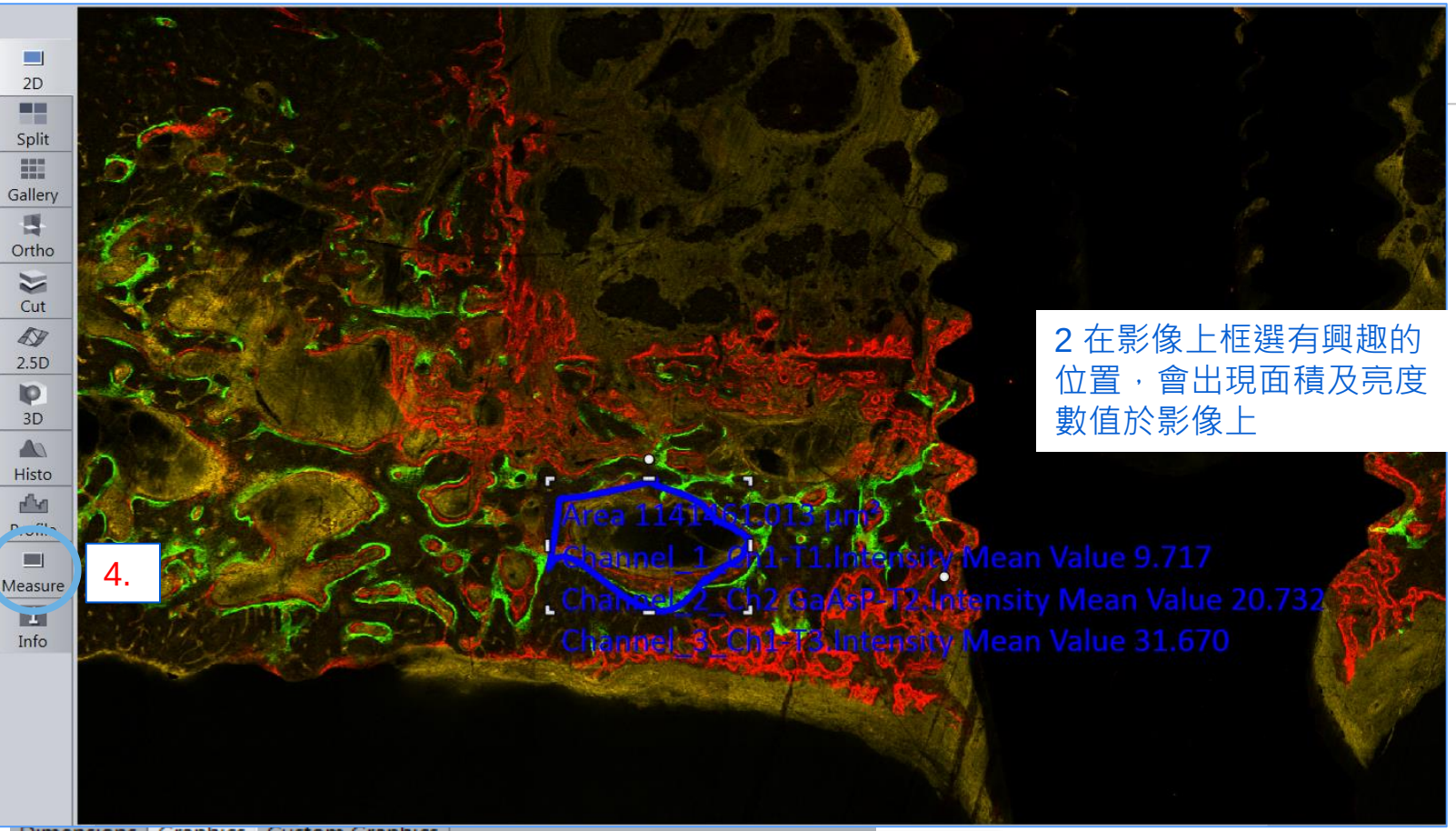


更改線的顏色樣式

更改字的顏色大小



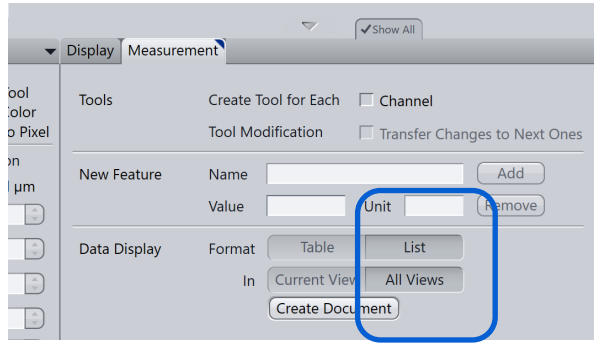
量測intensity

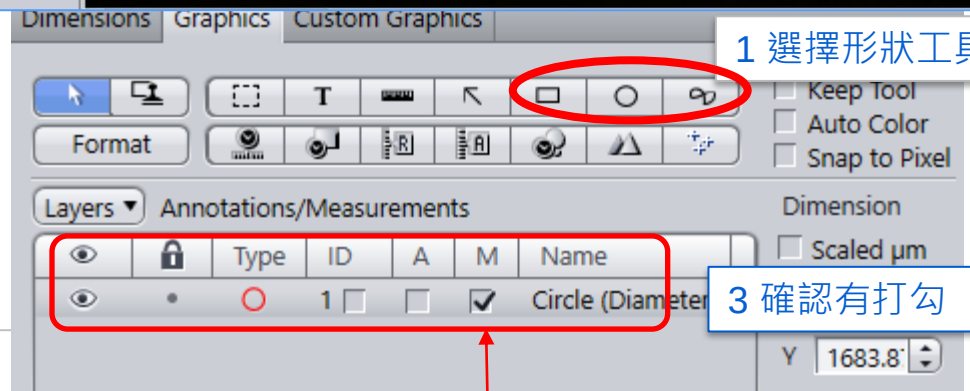


2 在影像上框選有興趣的位置，會出現面積及亮度數值於影像上

4.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	

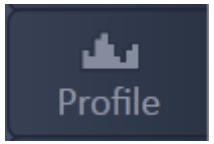




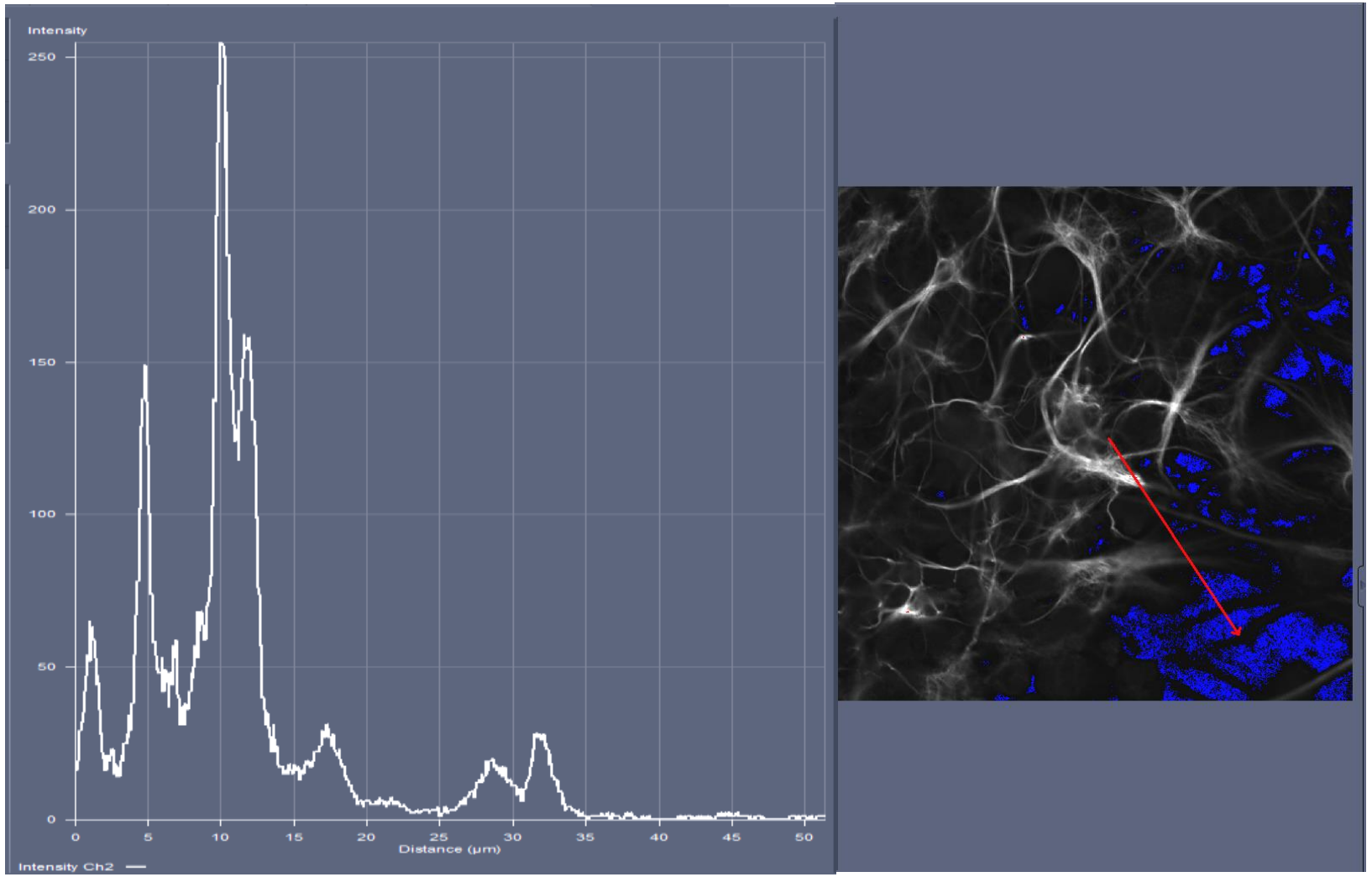
1 選擇形狀工具

3 確認有打勾

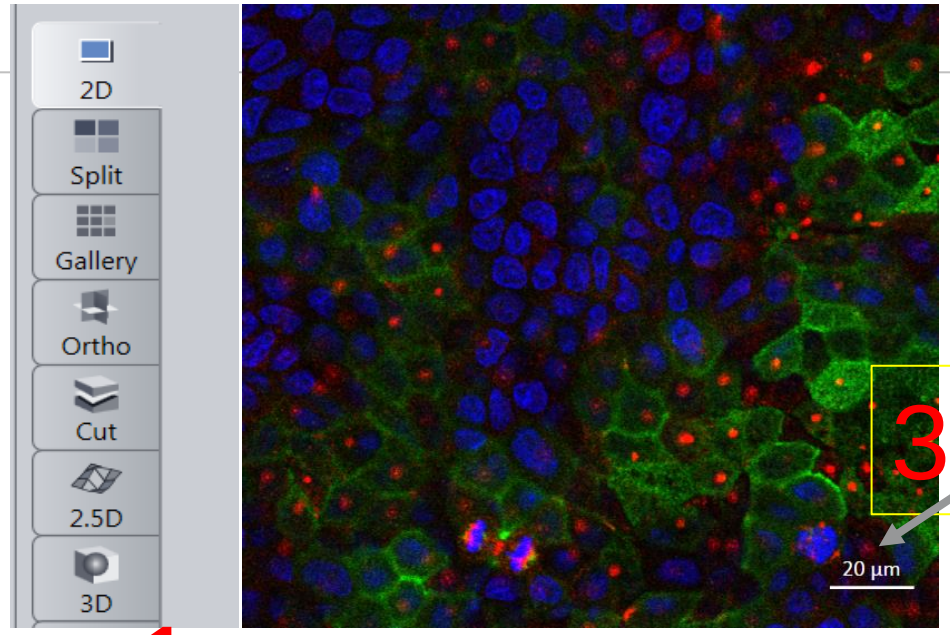
Profile
Intensity distribution along the line



Gives measurements
of grey values along a line

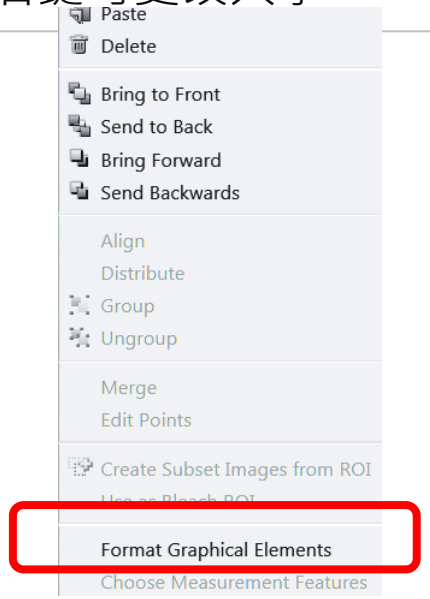


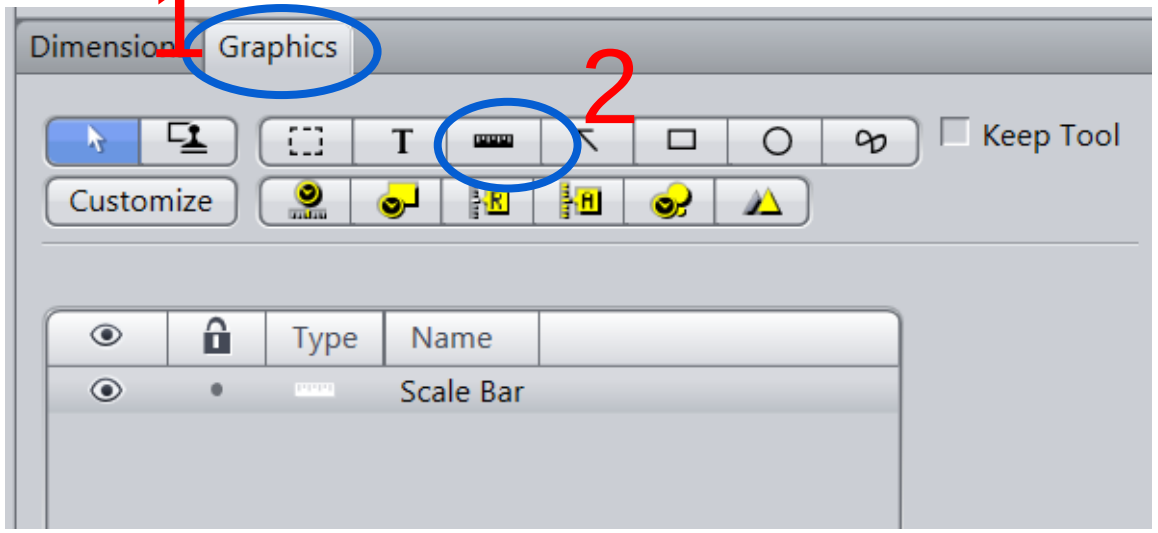
Add Scale Bar加入尺規



3 scale bar會自動出現

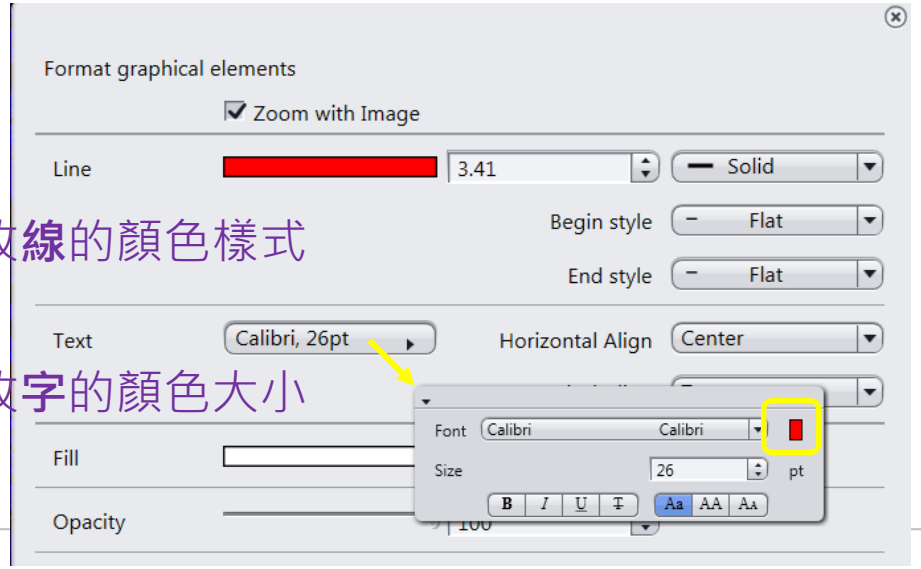
4 於scale bar按右鍵可更改大小顏色粗細等格式



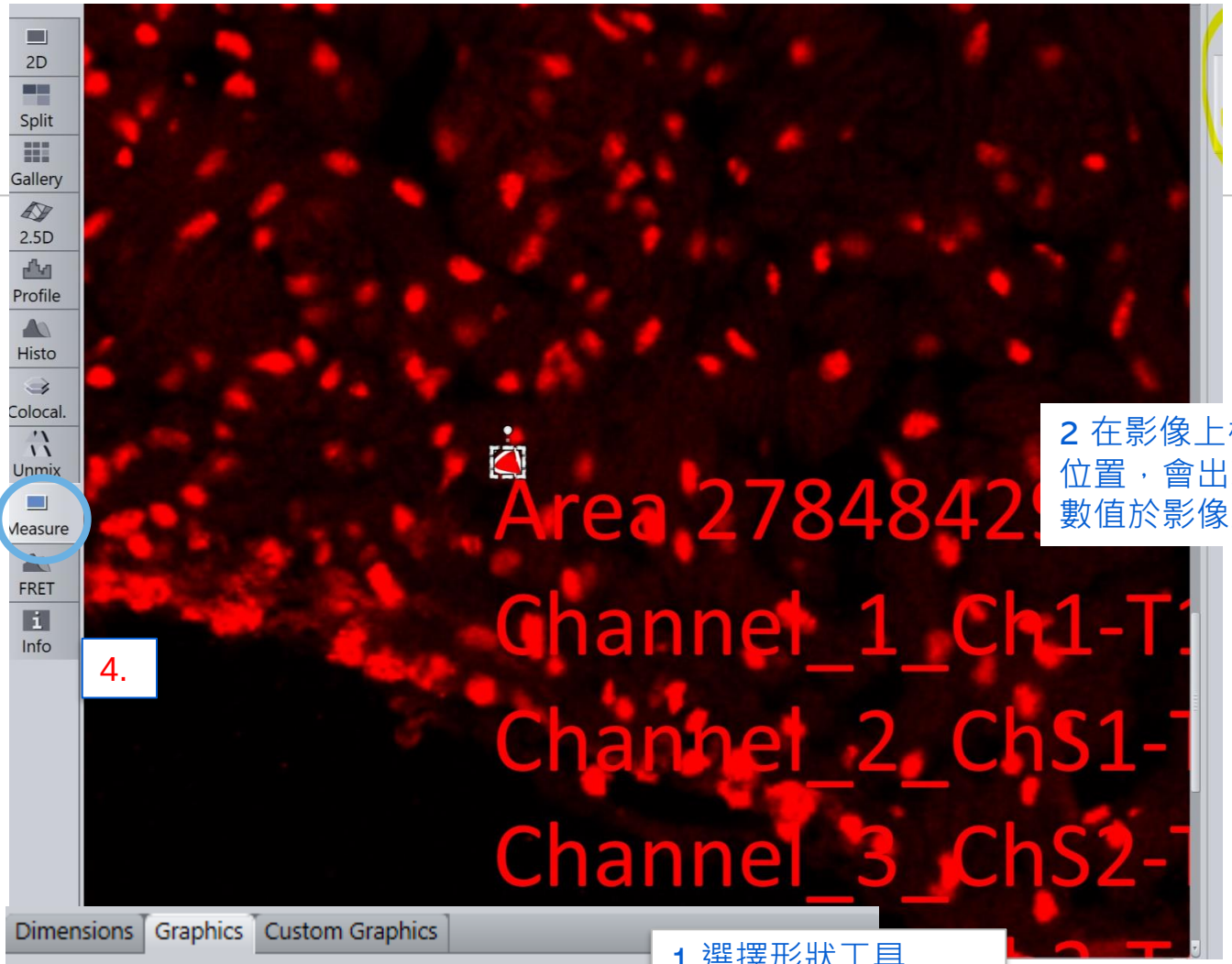


更改線的顏色樣式

更改字的顏色大小



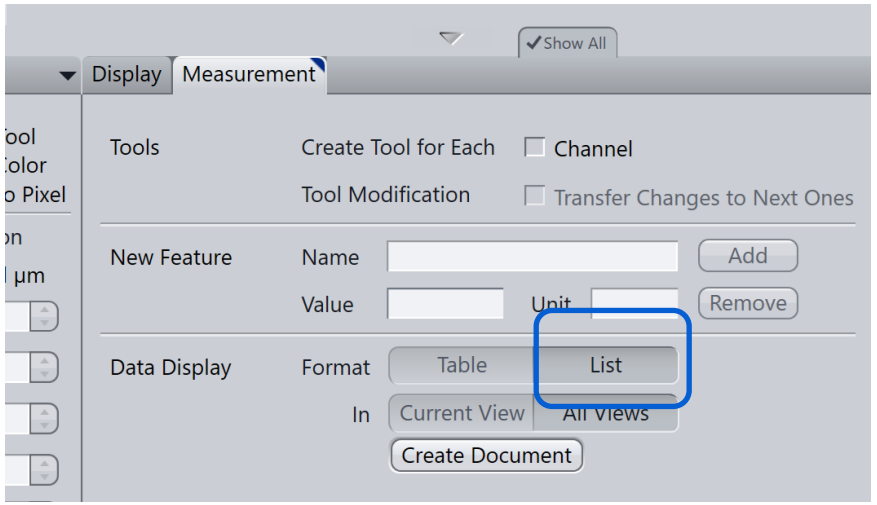
量測area & intensity

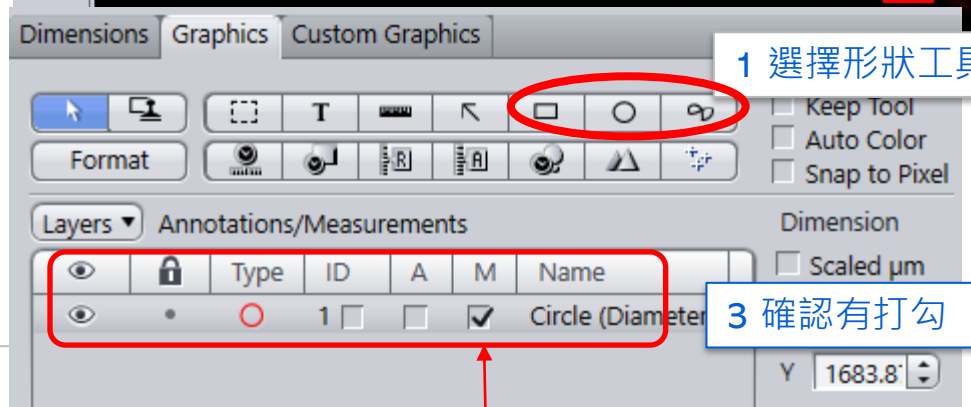


2 在影像上框選有興趣的位置，會出現面積及亮度數值於影像上

4.圖表位於右方視窗

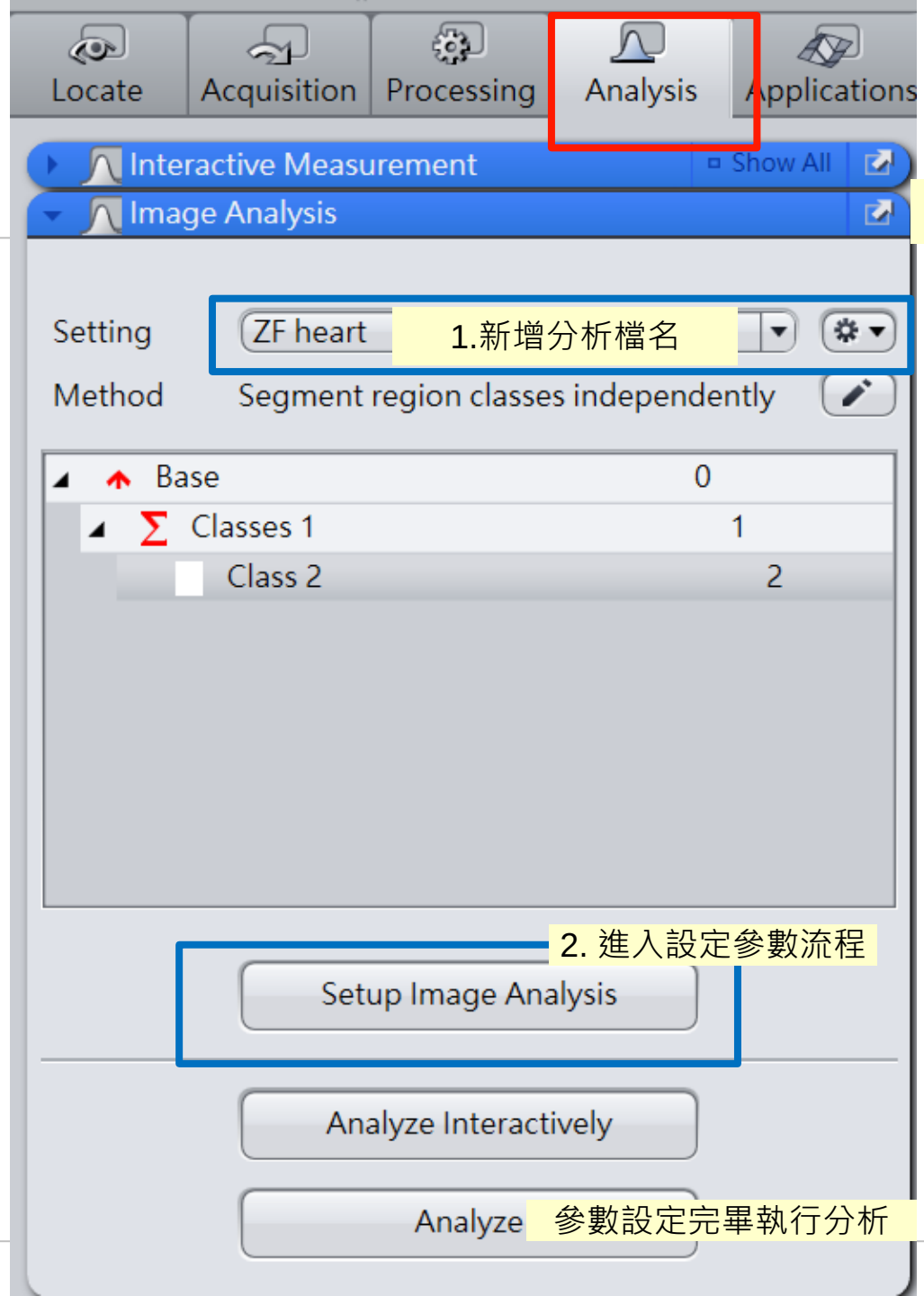
	Name	Feature	Value	Unit
	A	B	C	D
1	Circle (Diameter)	Area	1,146,259.648	µm²
2	Circle (Diameter)	Channel_3_mPlum.Intensity Mean Value	8,855.551	
3	Circle (Diameter)	Channel_2_EGFP.Intensity Mean Value	5,622.191	
4	Circle (Diameter)	Channel_1_DAPI.Intensity Mean Value	3,224.520	
5	Circle (Diameter)	Diameter	1,208.082	µm
6	Circle (Diameter)	Channel_4_Cy5.Intensity Mean Value	808.316	

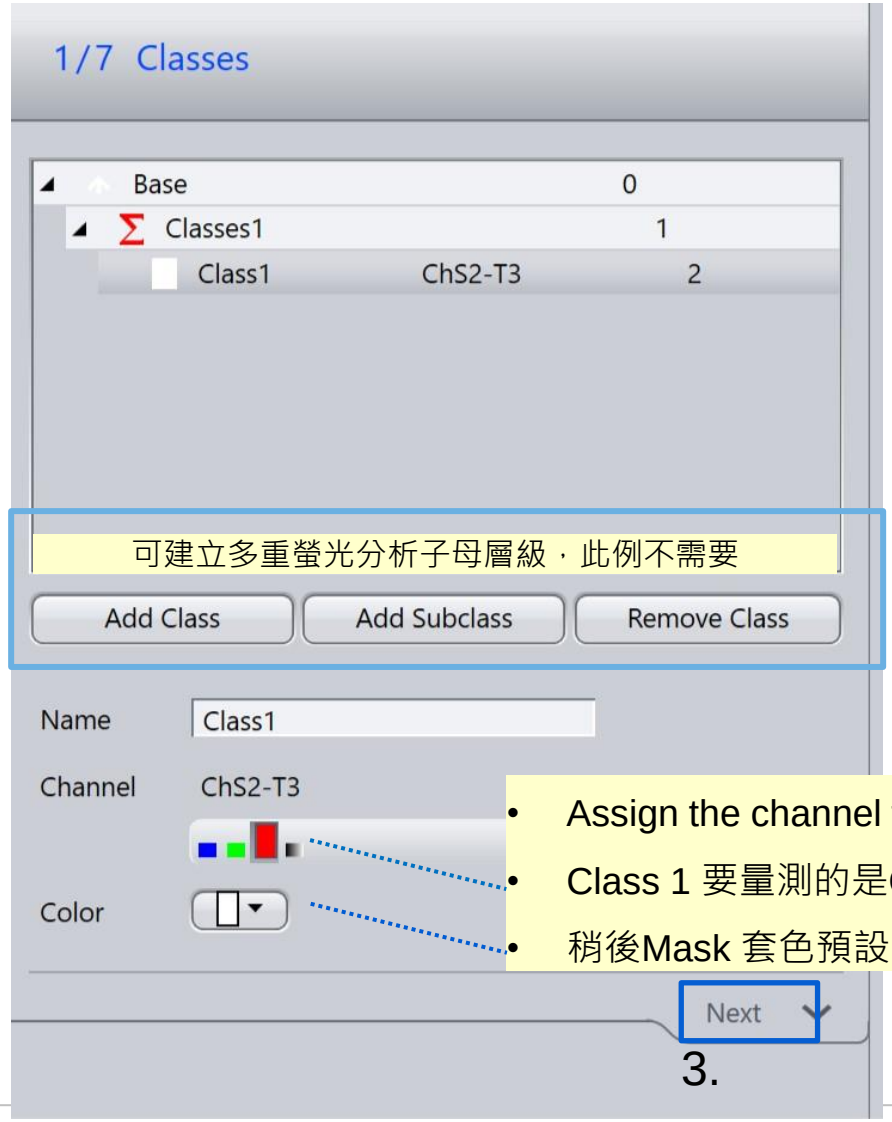




自動量測 Image Analysis Module 1.

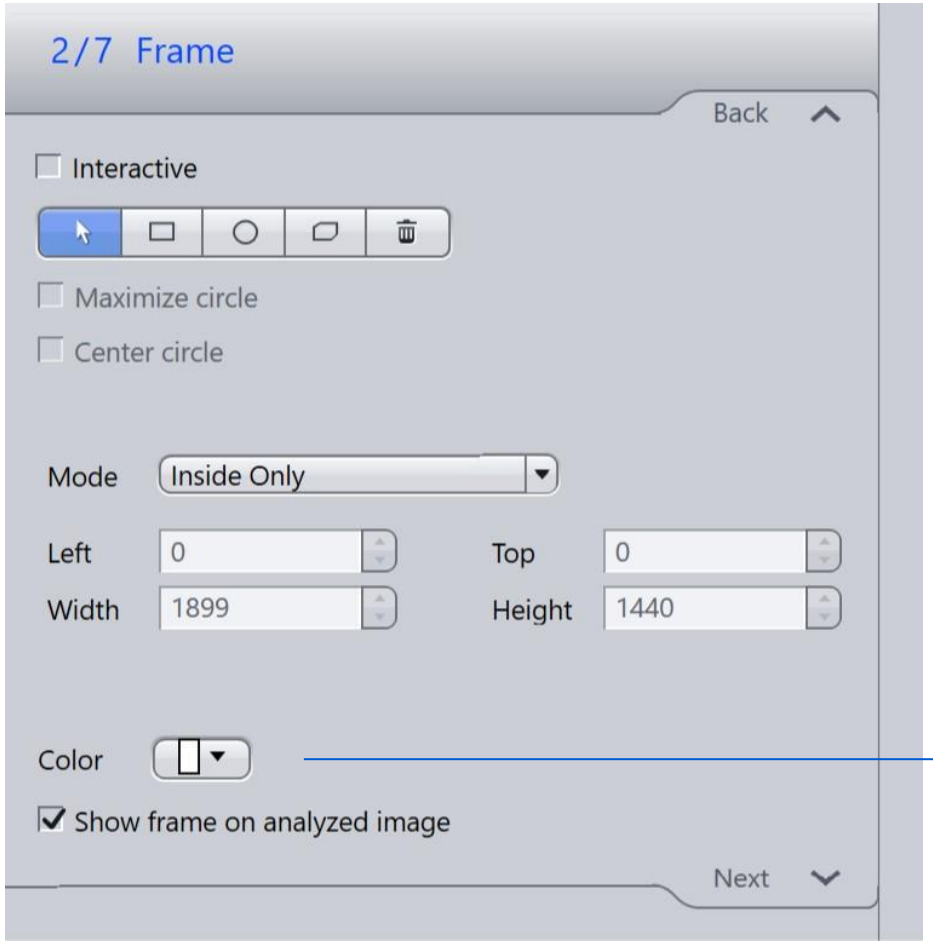
以量測前頁影像內的紅色細胞核數目為例





- Assign the channel to the Class
- Class 1 要量測的是Chs2-T3
- 稍後Mask 套色預設白色

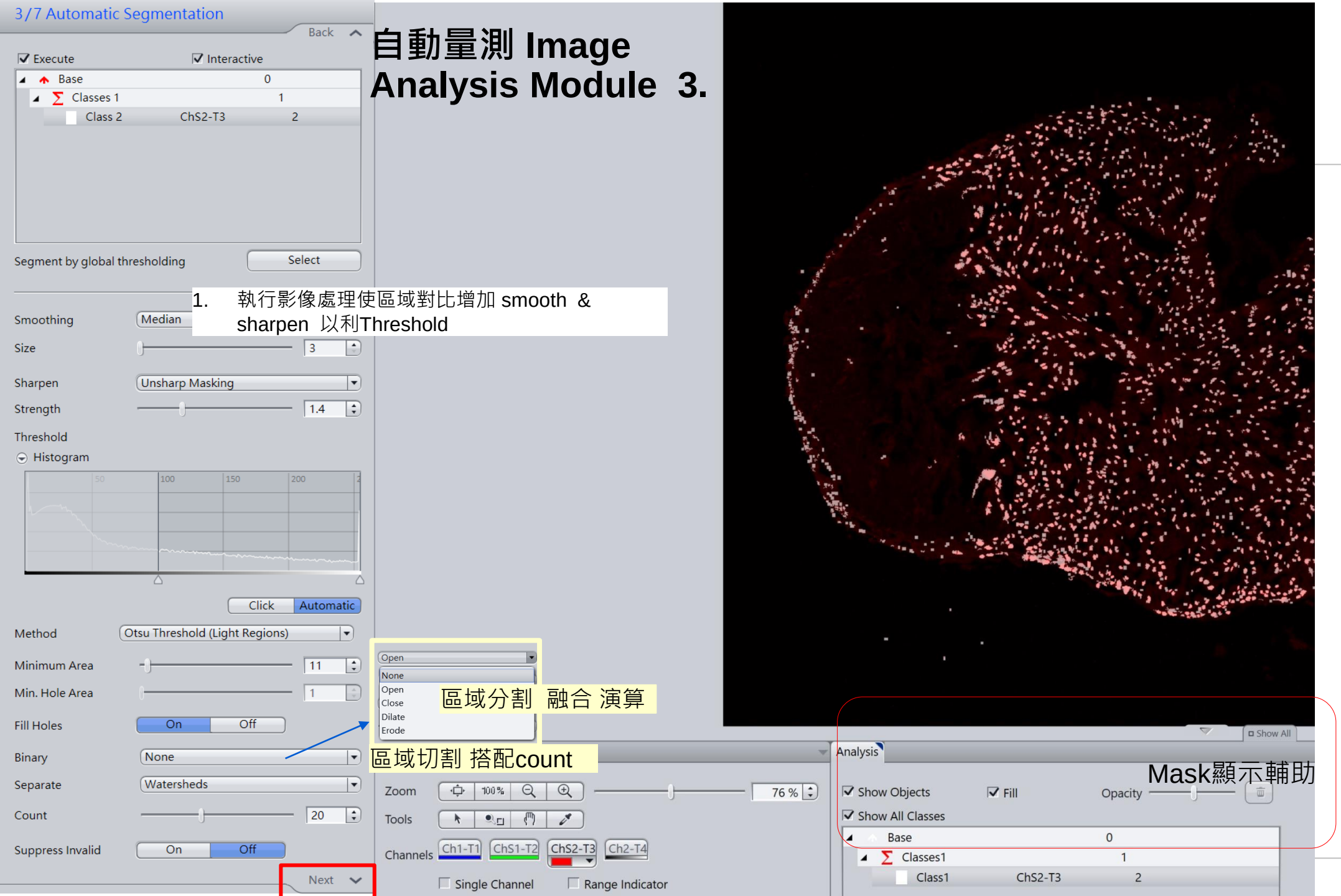
自動量測 Image Analysis Module 2.
圈選分析範圍，可略過



- 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

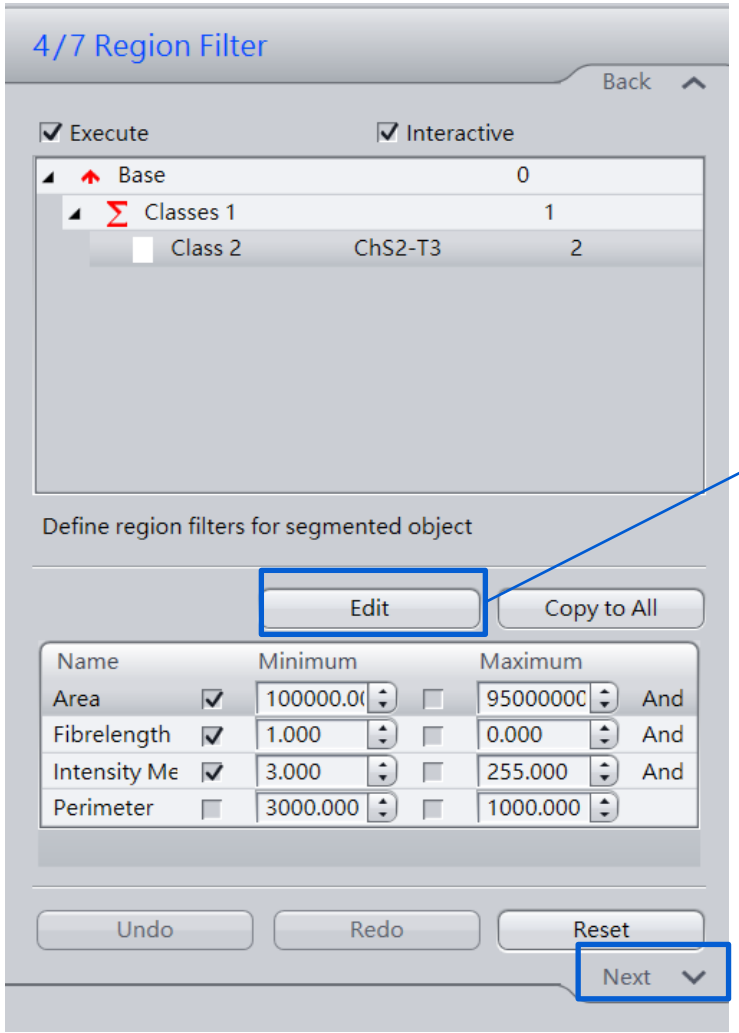
- 量測區域外框顏色

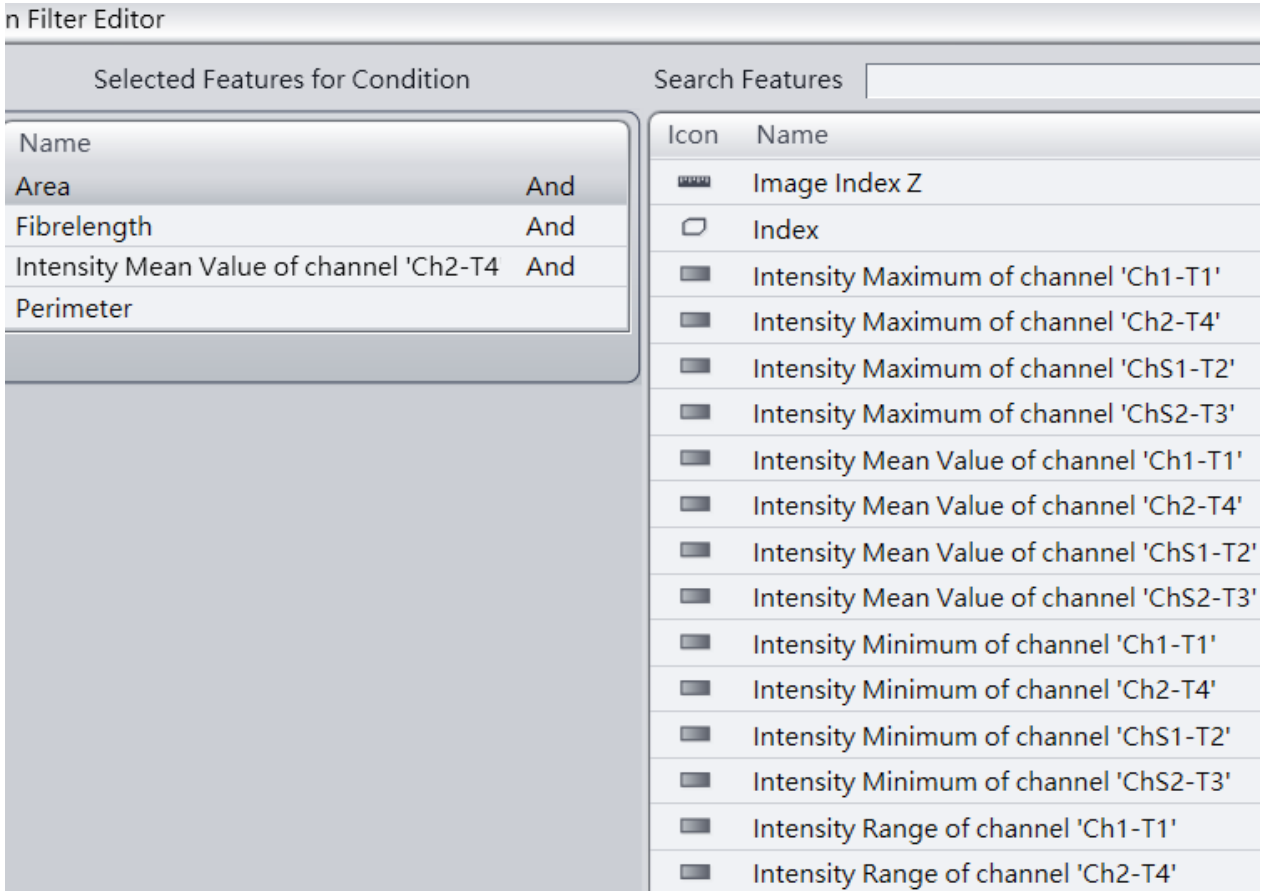
自動量測 Image Analysis Module 3.



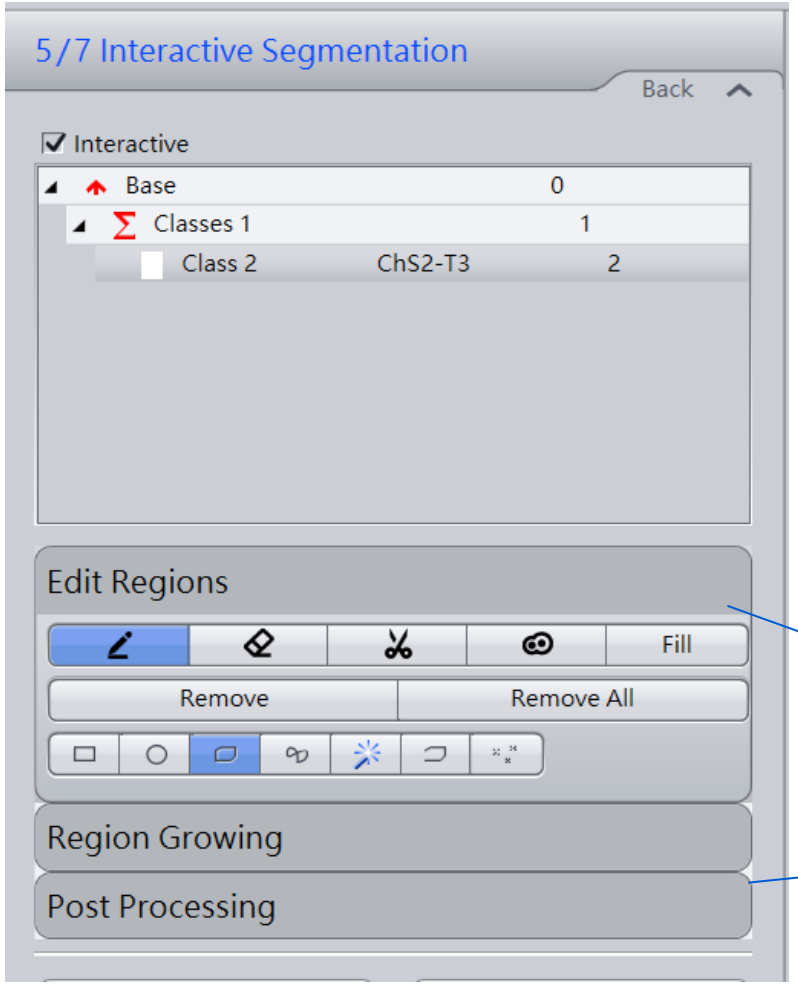
1. 執行影像處理使區域對比增加 smooth & sharpen 以利Threshold

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





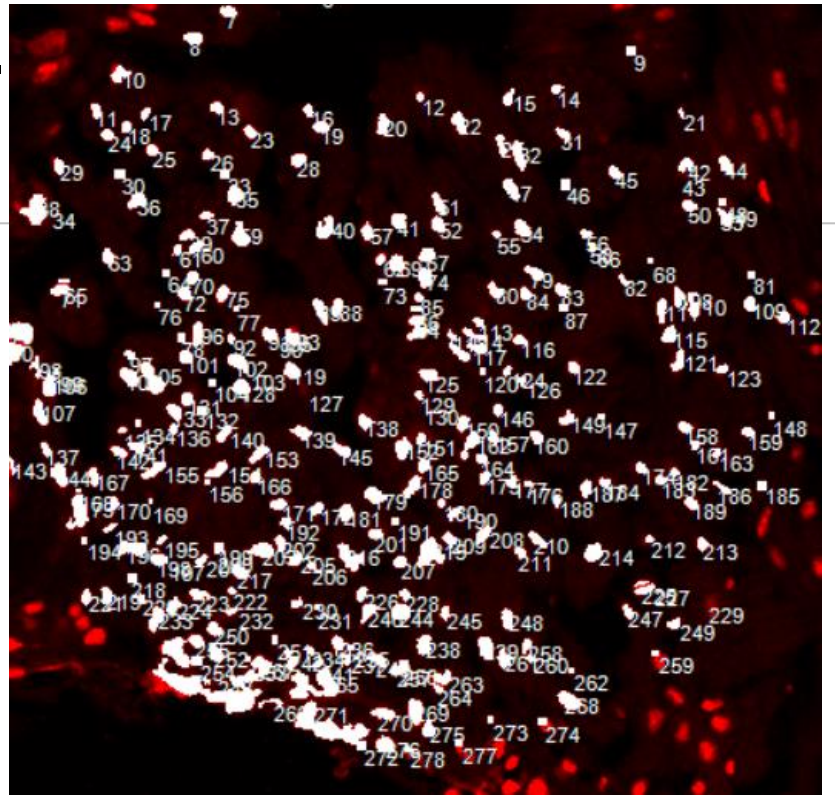
自動量測 Image Analysis Module 5.
互動式調整量測區域 增加 減少 或 切割 縫合
批次處理需求請略過此步

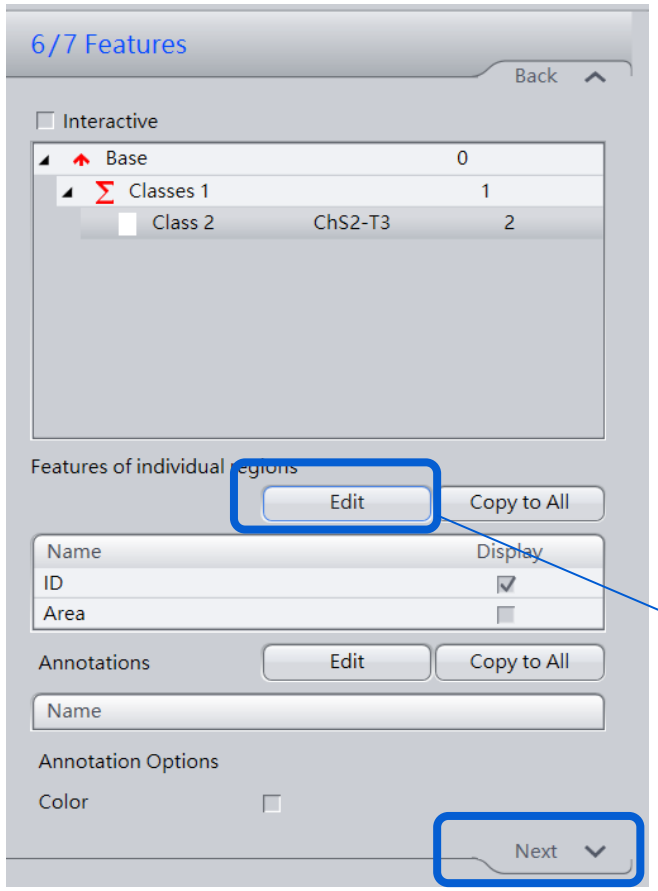


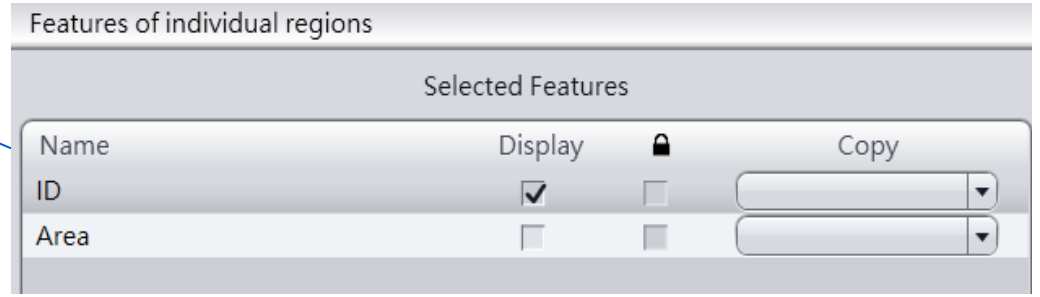
- 手動增加/刪除區域
- 手動切割/接合區域
- 若要進行自動大量圖檔批次分析，請避免使用此步驟

- 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

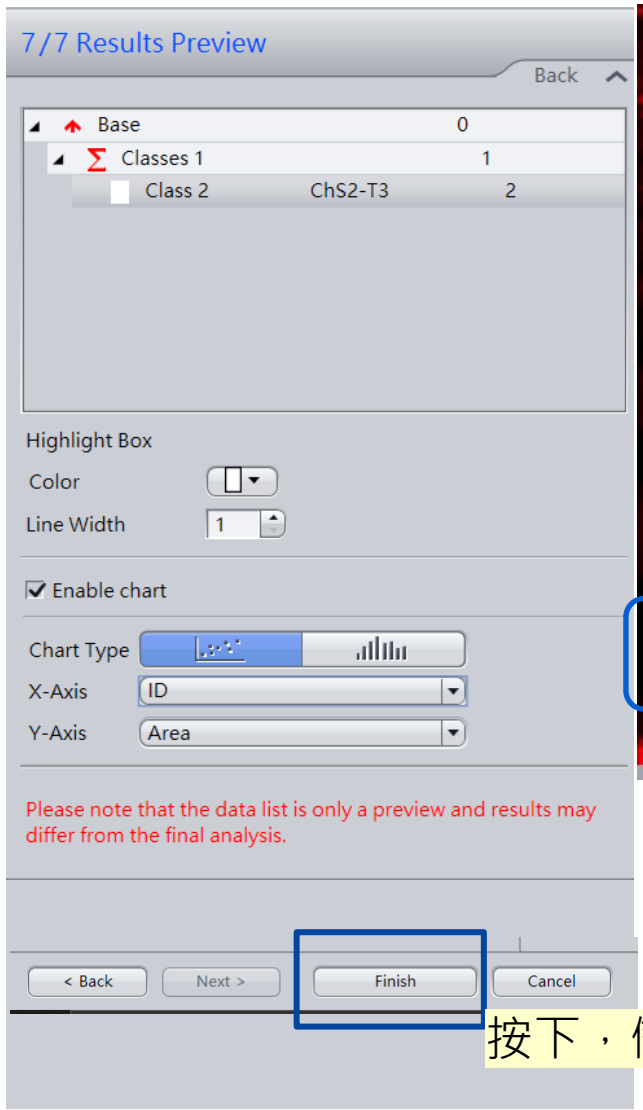


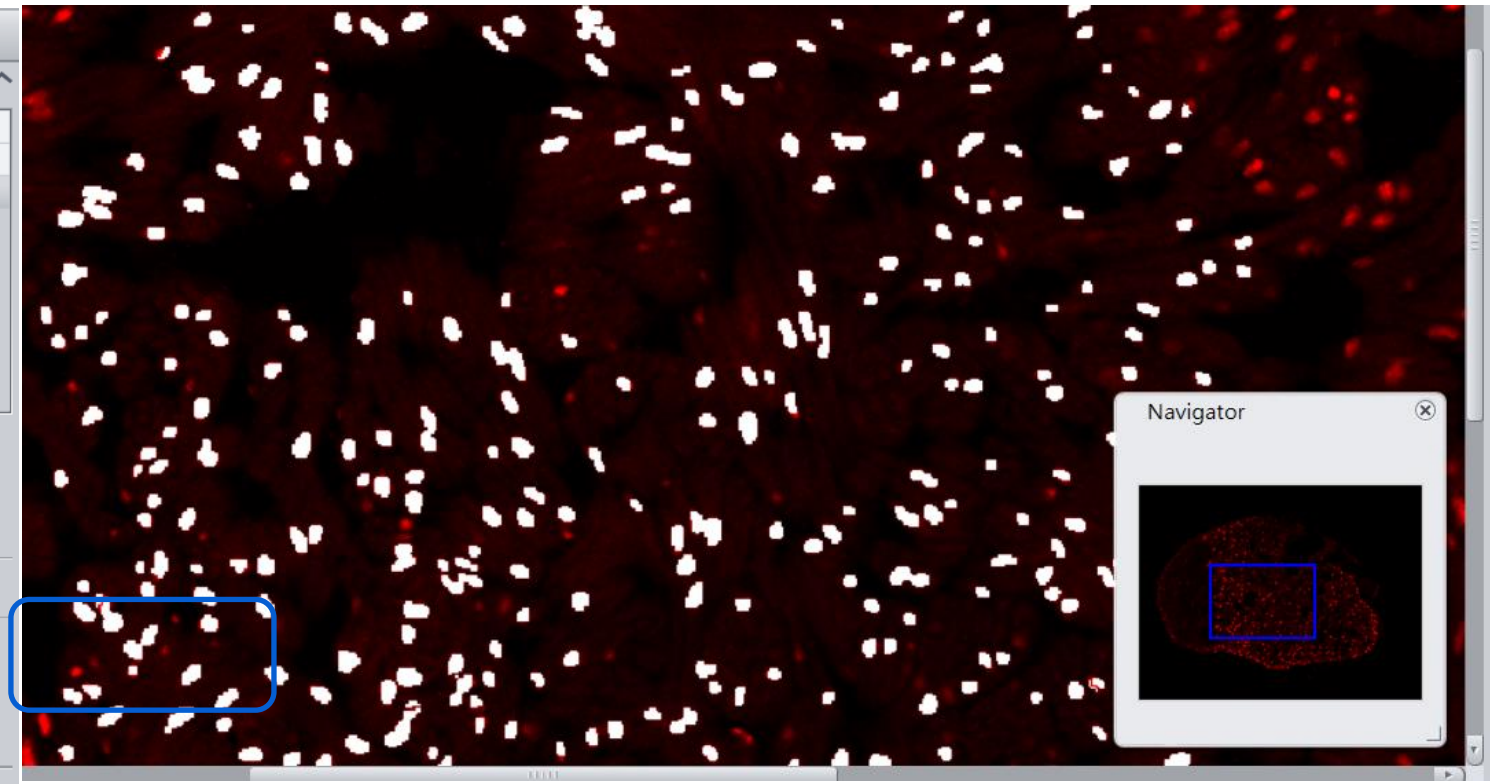




- 圖上若不需要顯示量測結果不要勾Display
- 量測結果顯示設定 ， 如果密密麻麻的mask 請避免使用，以免影響觀察量測區域

自動量測 Image Analysis Module 7.
參數設定完畢，結果預覽



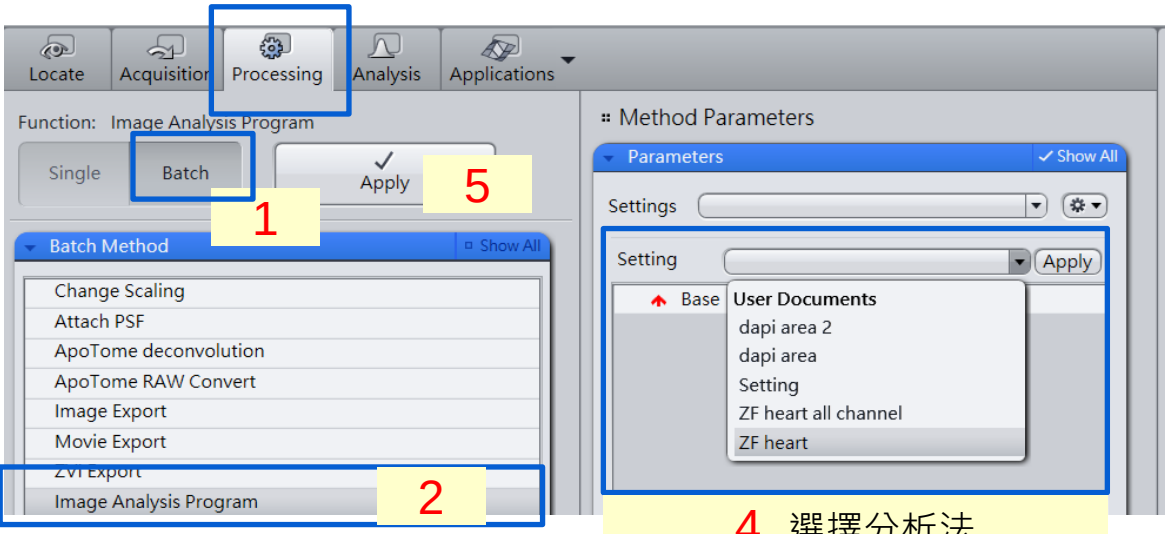


10	11	12,408,144.002
11	12	16,544,192.003
12	13	19,990,898.670
13	14	15,854,850.670
14	15	18,612,216.003
15	16	15,854,850.670
16	17	39,981,797.341
17	18	51,700,600.010
18	19	23,437,605.338
19	20	31,709,701.339
20	21	15,854,850.670
21	22	26,194,970.672
22	23	22,748,264.004
23	24	37,913,773.340
24	25	17,922,874.670
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

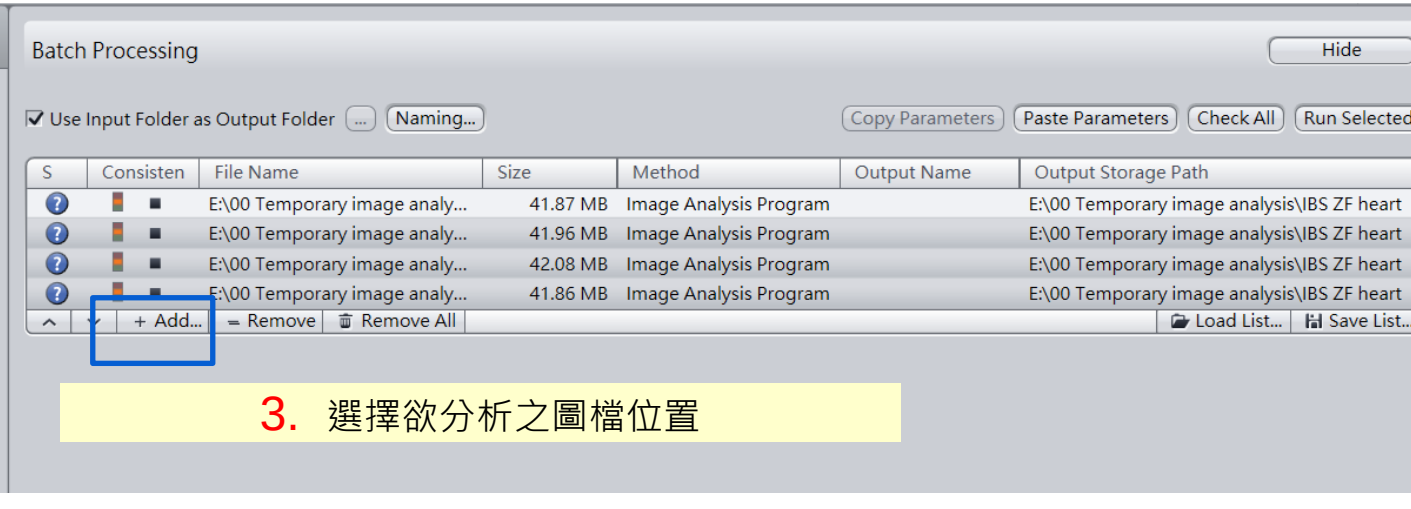
- 結果**預覽**
- 此時尚無法輸出結果

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



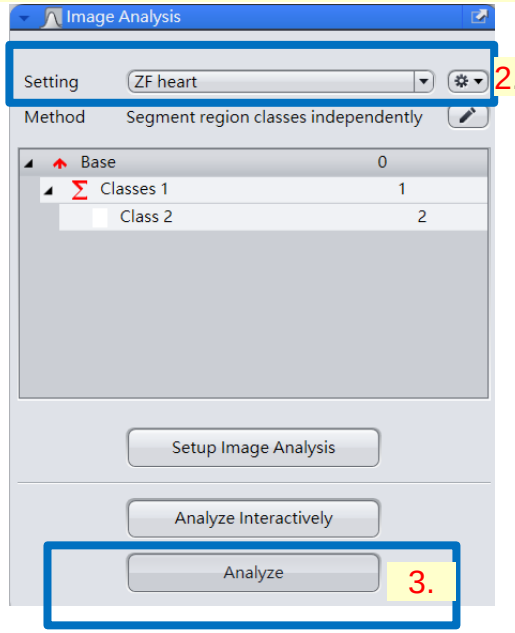
4. 選擇分析法



3. 選擇欲分析之圖檔位置

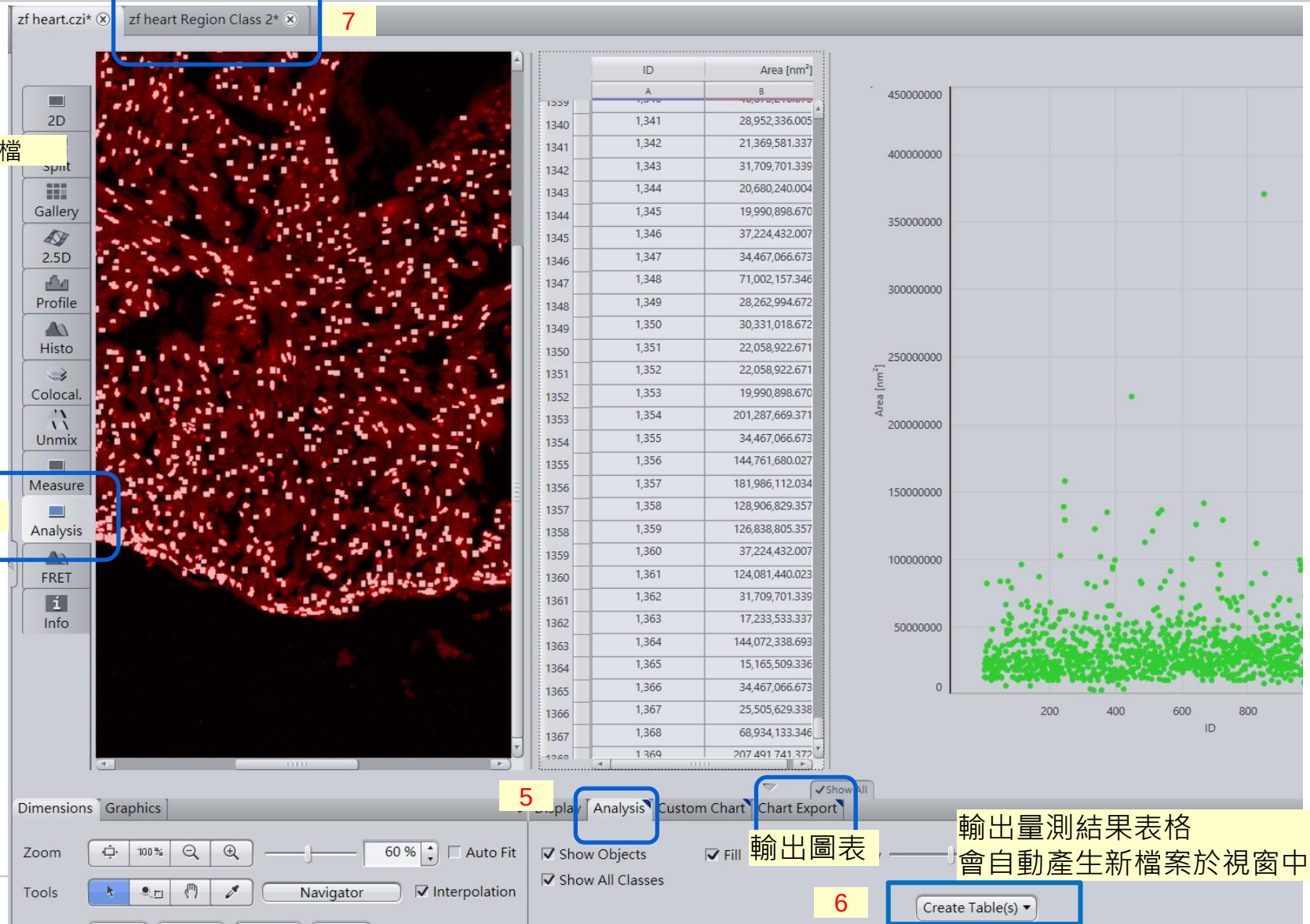
自動量測 Image Analysis Module 9.
Analyze the Image and Export the Data

1 打開原圖 或其他同系列要分析的圖



2. 選擇設定檔

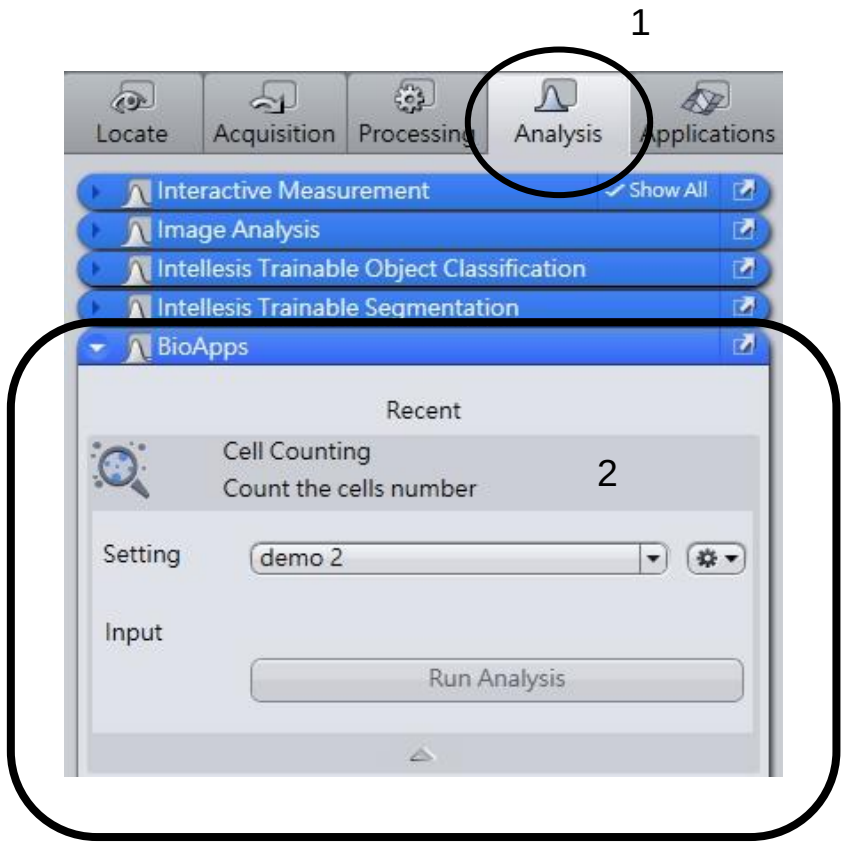
4

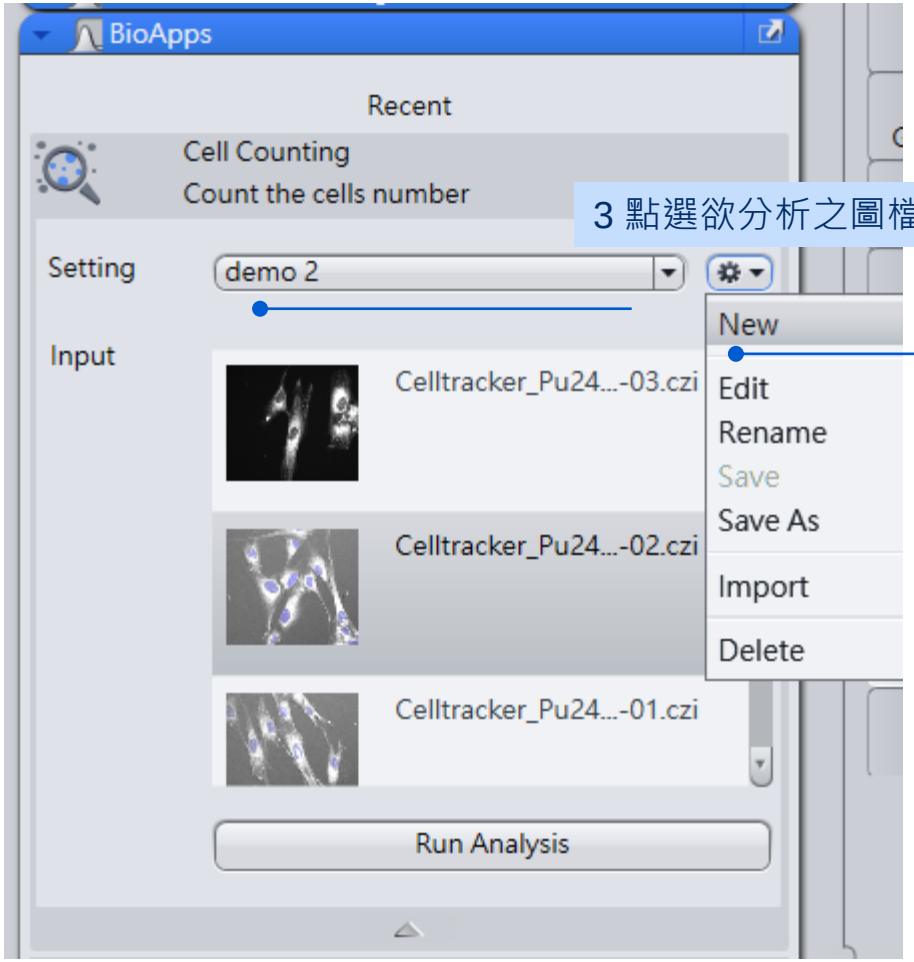


輸出量測結果表格
會自動產生新檔案於視窗中

BioApps- Cell Counting

只能分析平面圖檔，z stack請先做Extended Focus或Orthogonal Projection

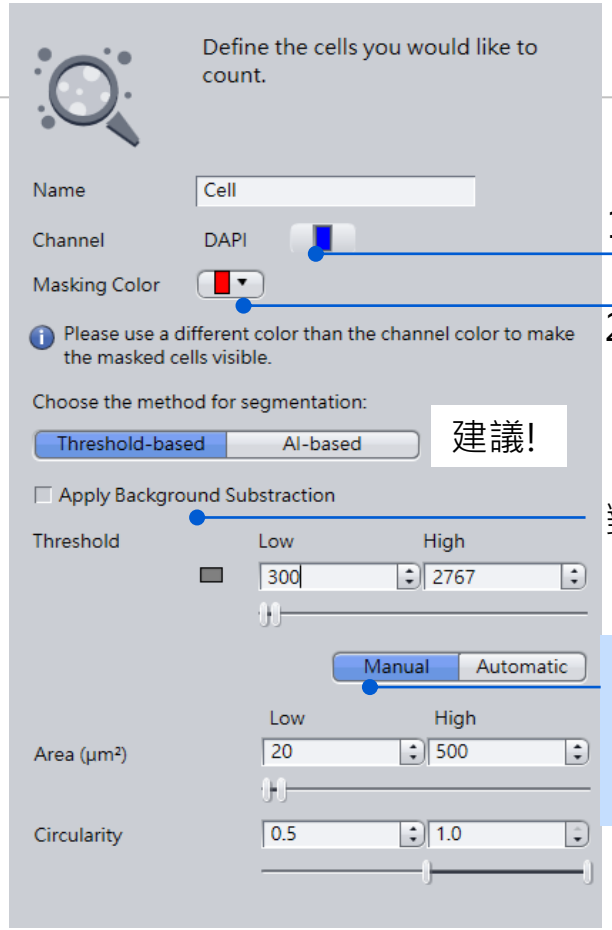




3 點選欲分析之圖檔，設定參數用

4
為分析設定
新增檔名

BioApps- Cell Counting 參數設定

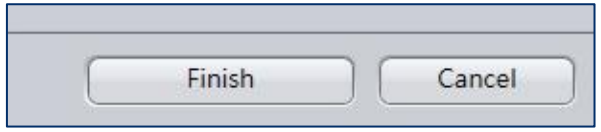


1 選擇欲分析的channel

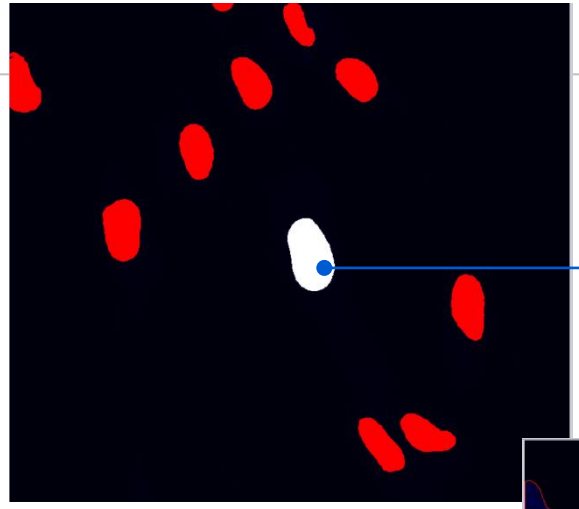
2 選擇mask顏色

對比好的圖不需勾選

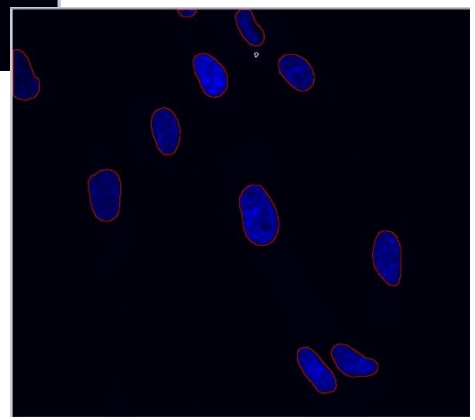
3 於圖中以滑鼠直接點選圖中目標區域，自動調整參數，接著依mask結果手動調整數值

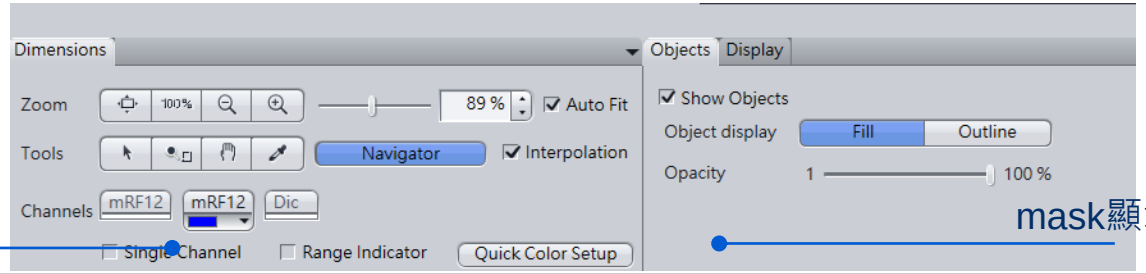


5 結束，上面參數會自動存檔



4 尚未進入圈選範圍，需再調整數值，例如面積域值擴大

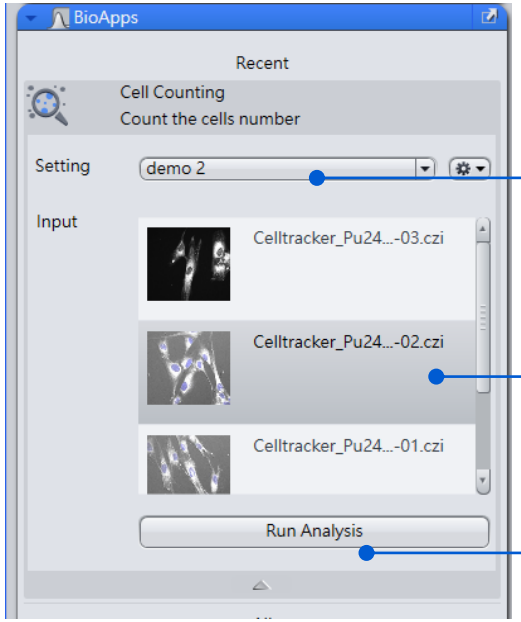




關閉沒有要量測的channel 顯示

mask顯示設定

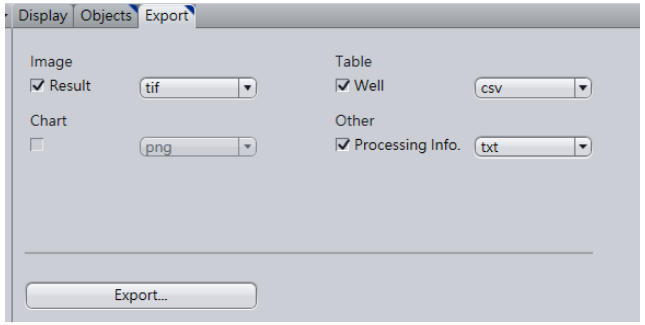
BioApps- Cell Counting 結果呈現與輸出



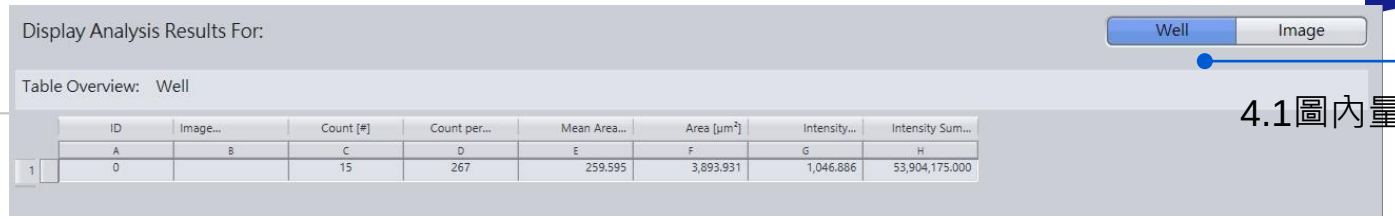
1.選擇參數

2.點選圖檔

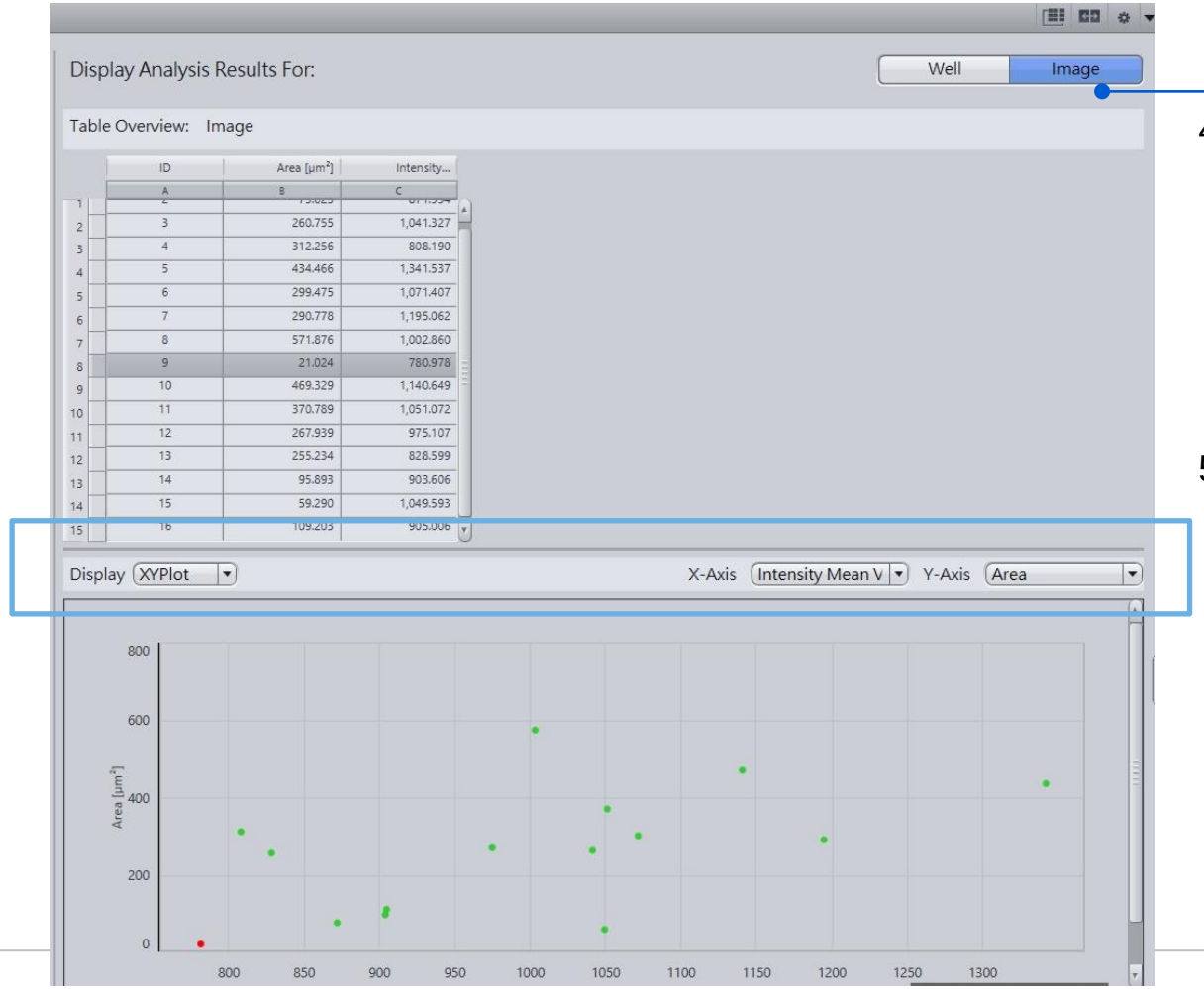
3.執行分析



6.圖/表/參數輸出



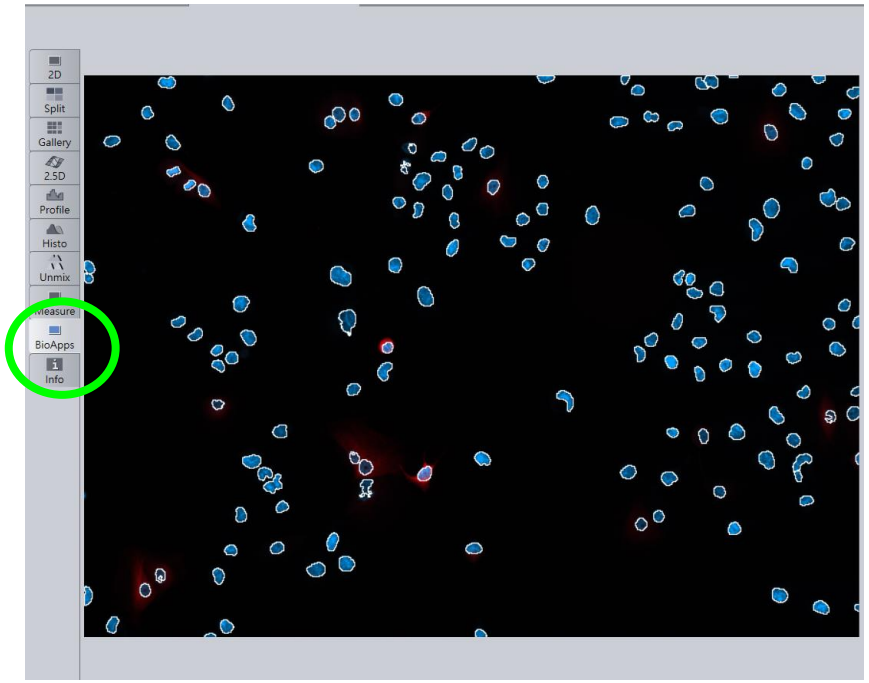
4.1圖內量測**總和**



4.2**個別**物件

5.依需求選擇

BioApps- ZEN 3.4 Cell Counting 支援多孔盤量測結果呈現與輸出

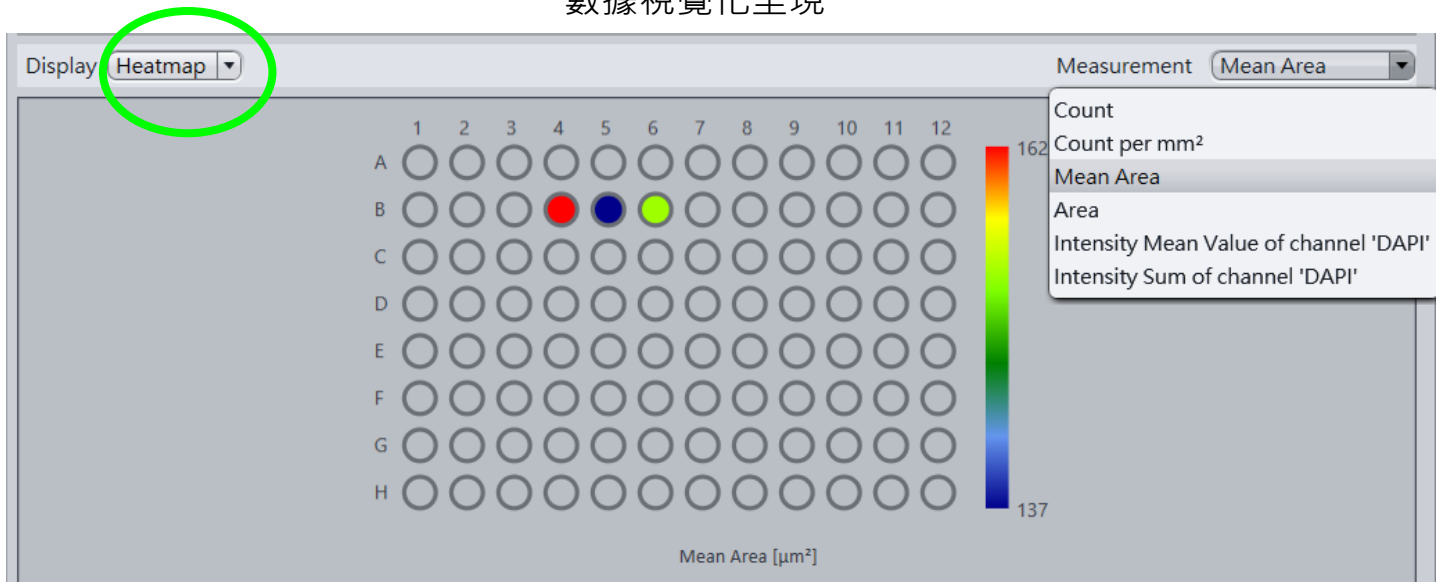


Display Analysis Results For: Well Image

Table Overview: Well

	Name	Image...	Count [#]	Count per...	Mean Area...	Area [µm²]	Intensity...	Intensity Sum...
	B	C	D	E	F	G	H	I
1	B4		146	255	161.97	23,647.89	7,320.70	849,786,882.00
2	B5		57	99	137.15	7,817.77	6,069.10	240,861,288.00
3	B6		209	366	153.24	32,026.51	6,645.92	1,075,314,176.00

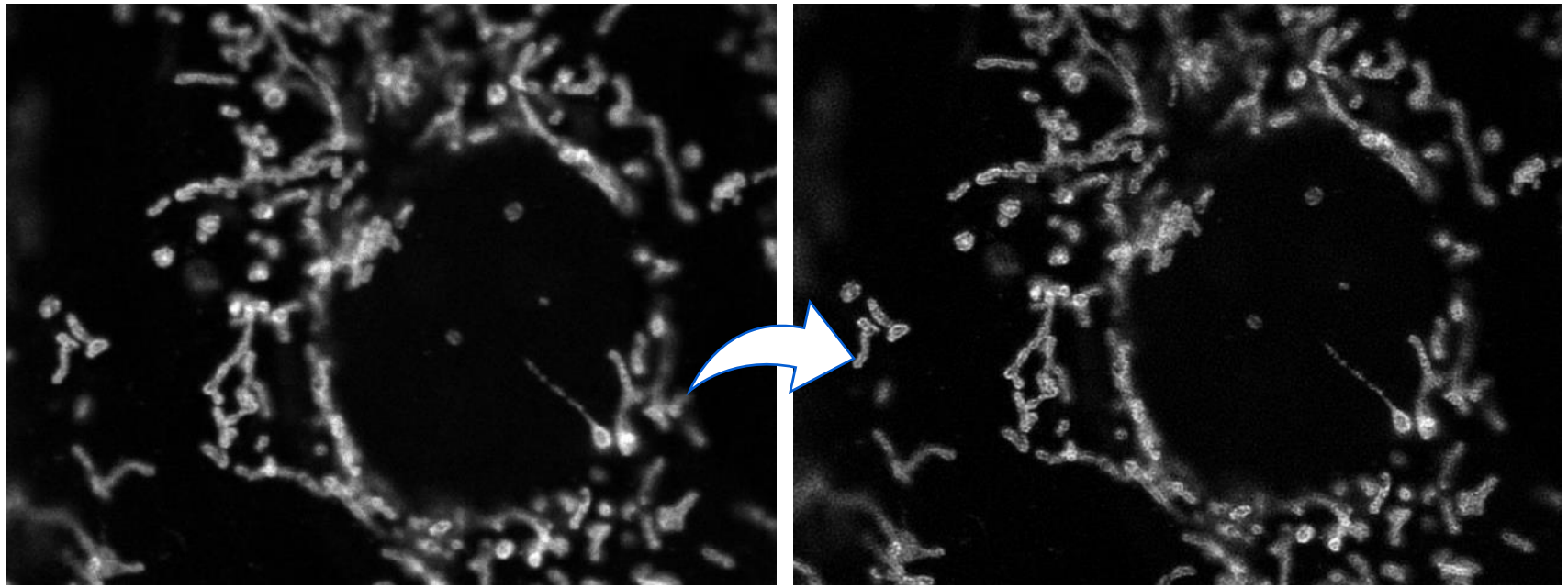
數據視覺化呈現



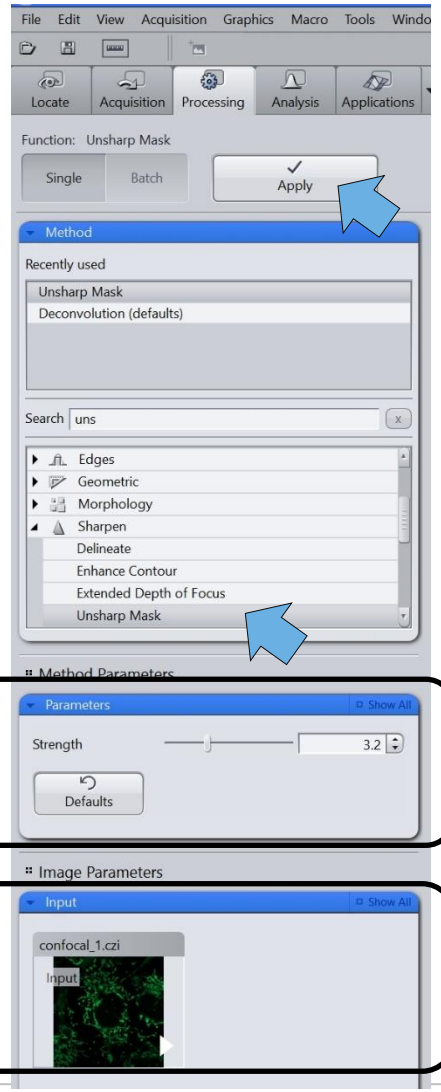
ZEN 3.3 Image Processing
Image Clearing Option 1:
2D DCV (defaults)

Before

After



ZEN Image Processing
Image Clearing Option 2:
Unsharp masking

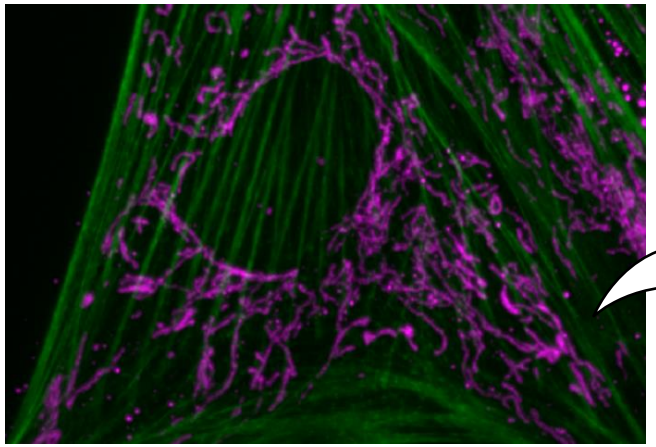


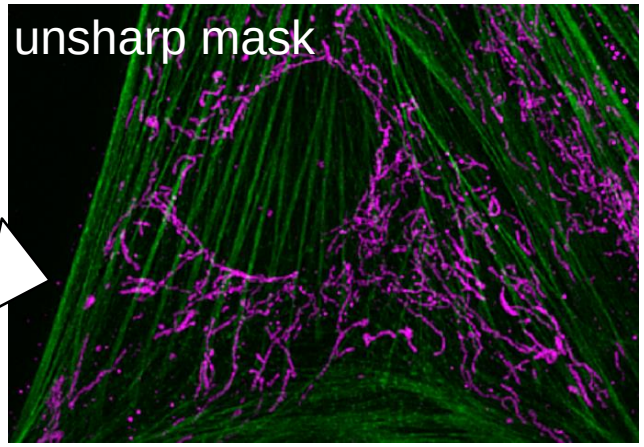
4

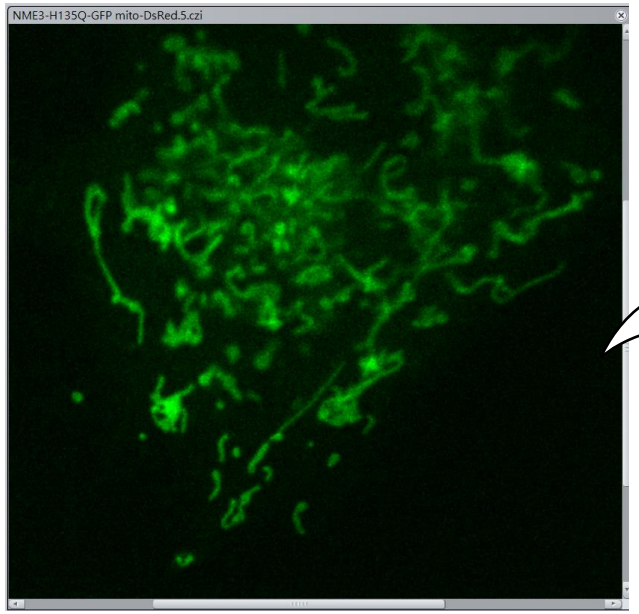
1

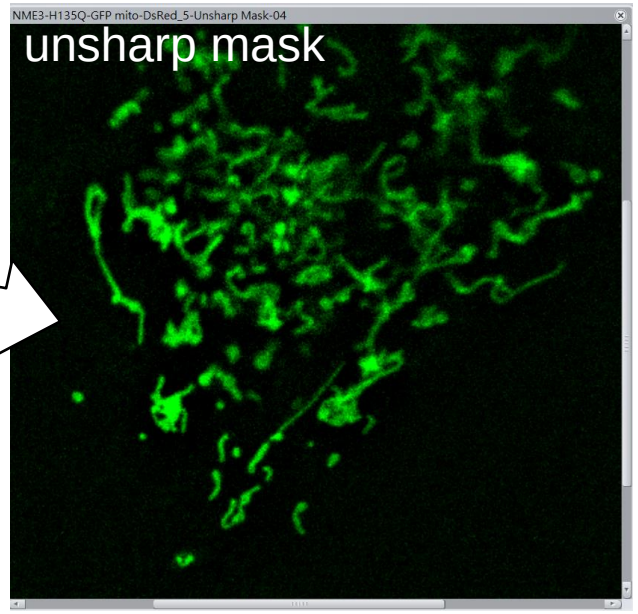
3

2

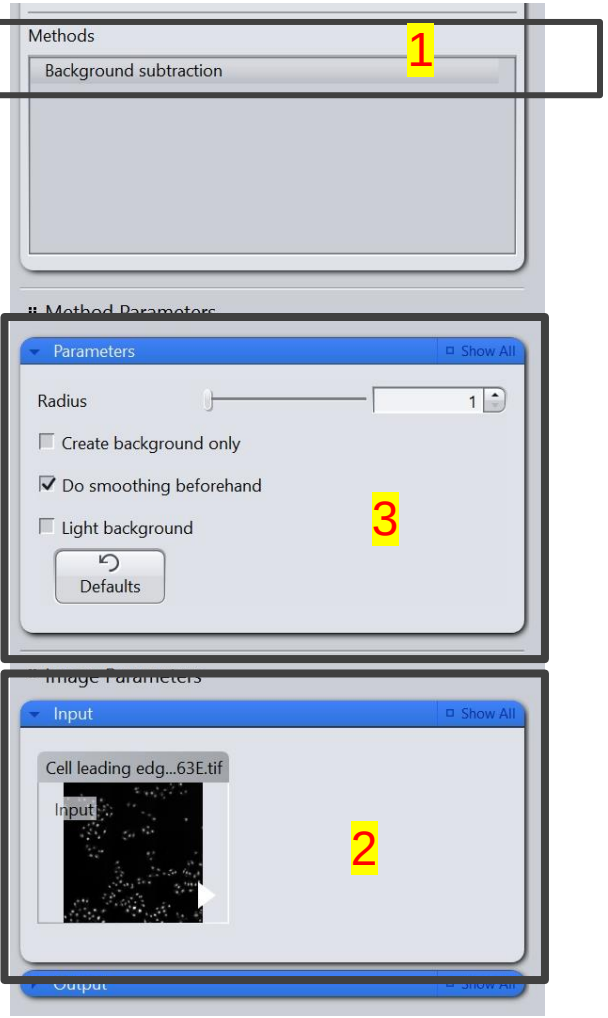


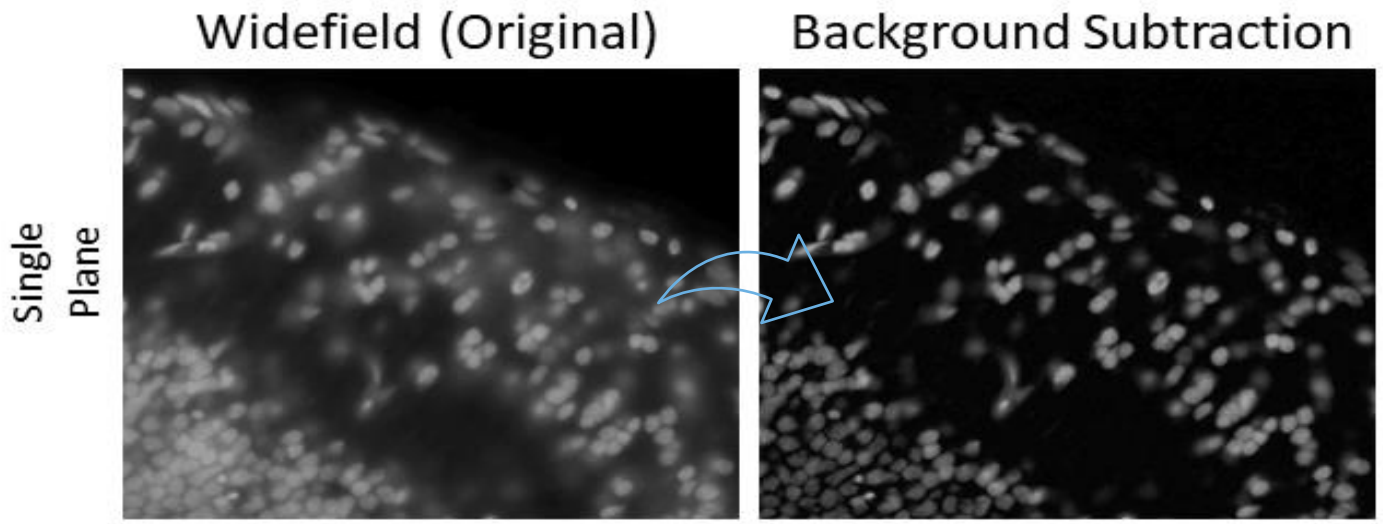




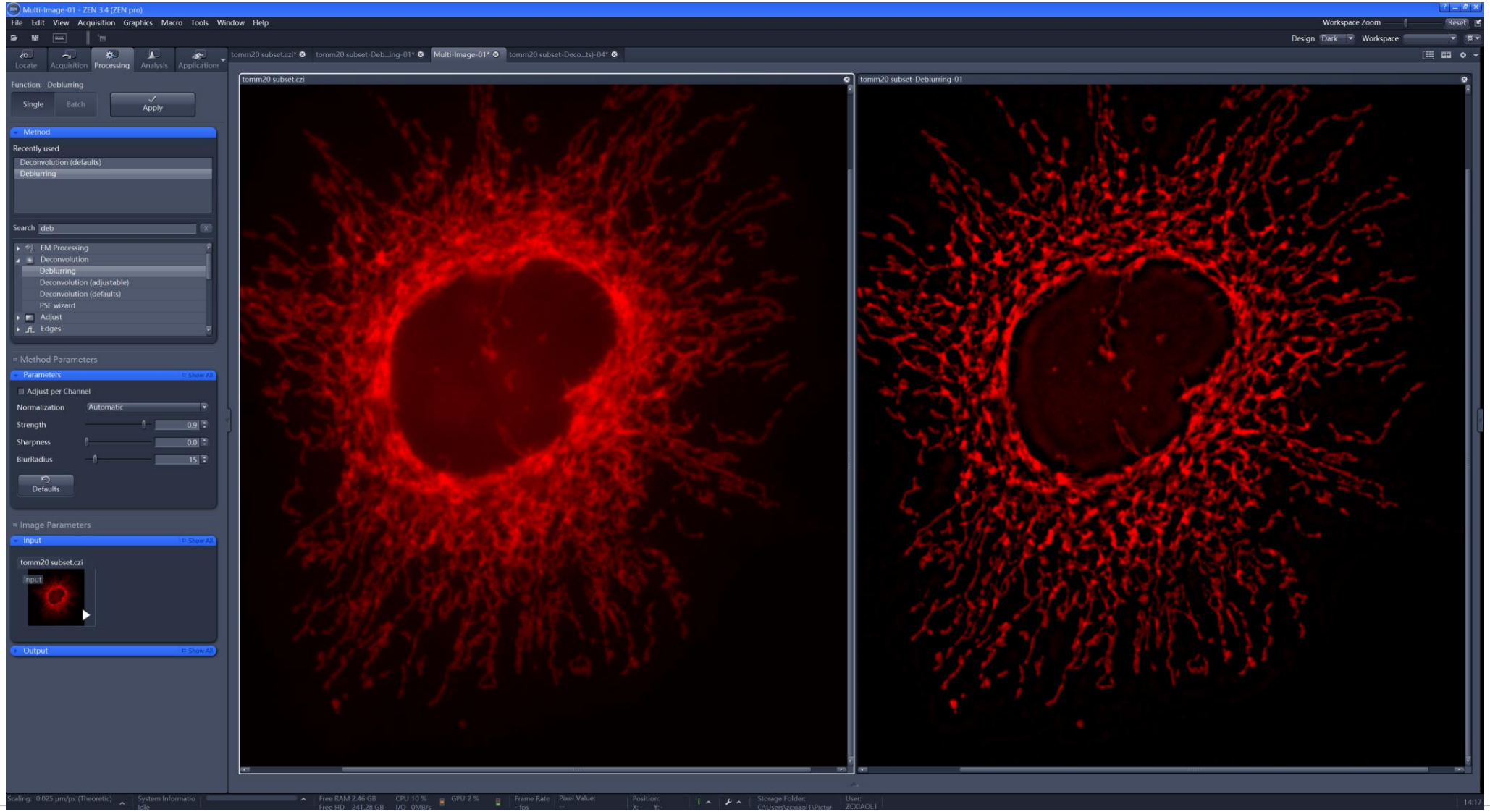


ZEN 3.3 Image Processing
Image Clearing Option 3:
Background subtraction



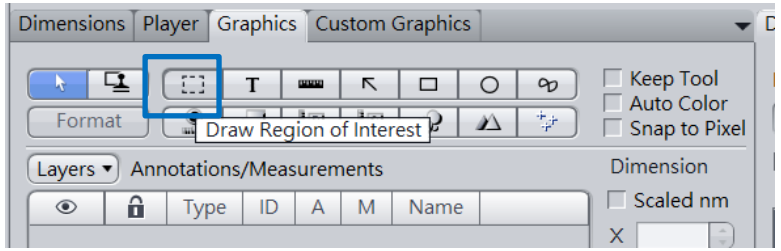


Option 4:
ZEN 3.4
WF vs Deblurring/2D DCV

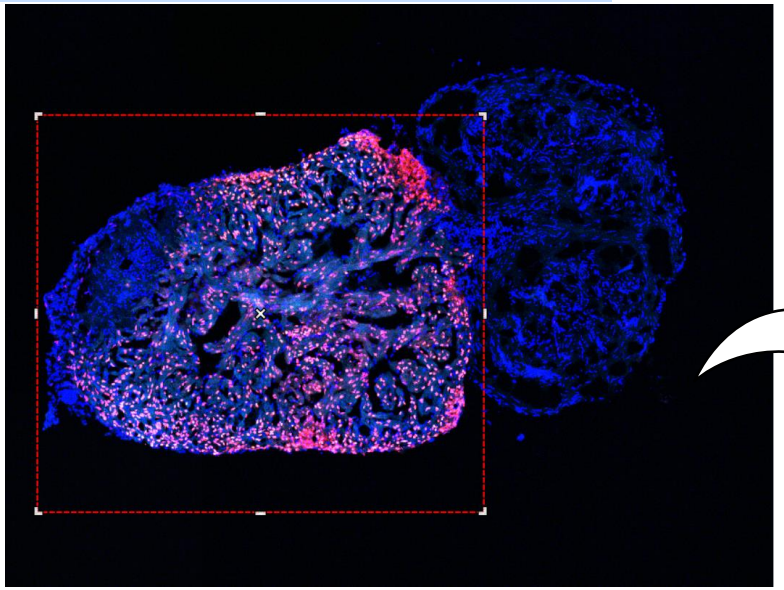


裁圖，複製選取範圍影像

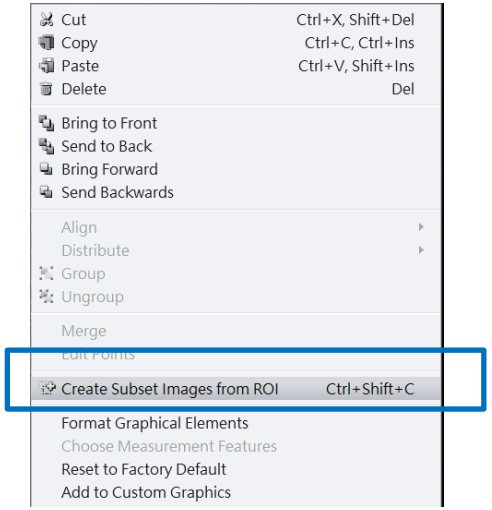
1.



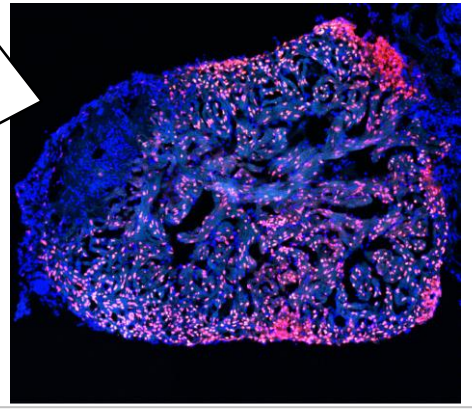
2. Draw an area and mouse right click



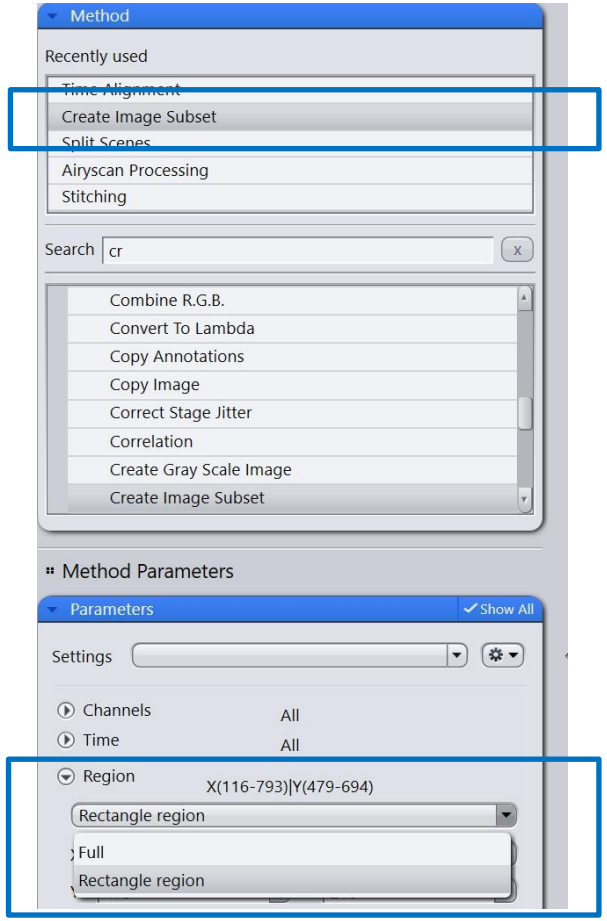
3. Create Subset Images from ROI



4. 獲得裁切範圍於新視窗



3. 或者
Processing → Create Image Subset



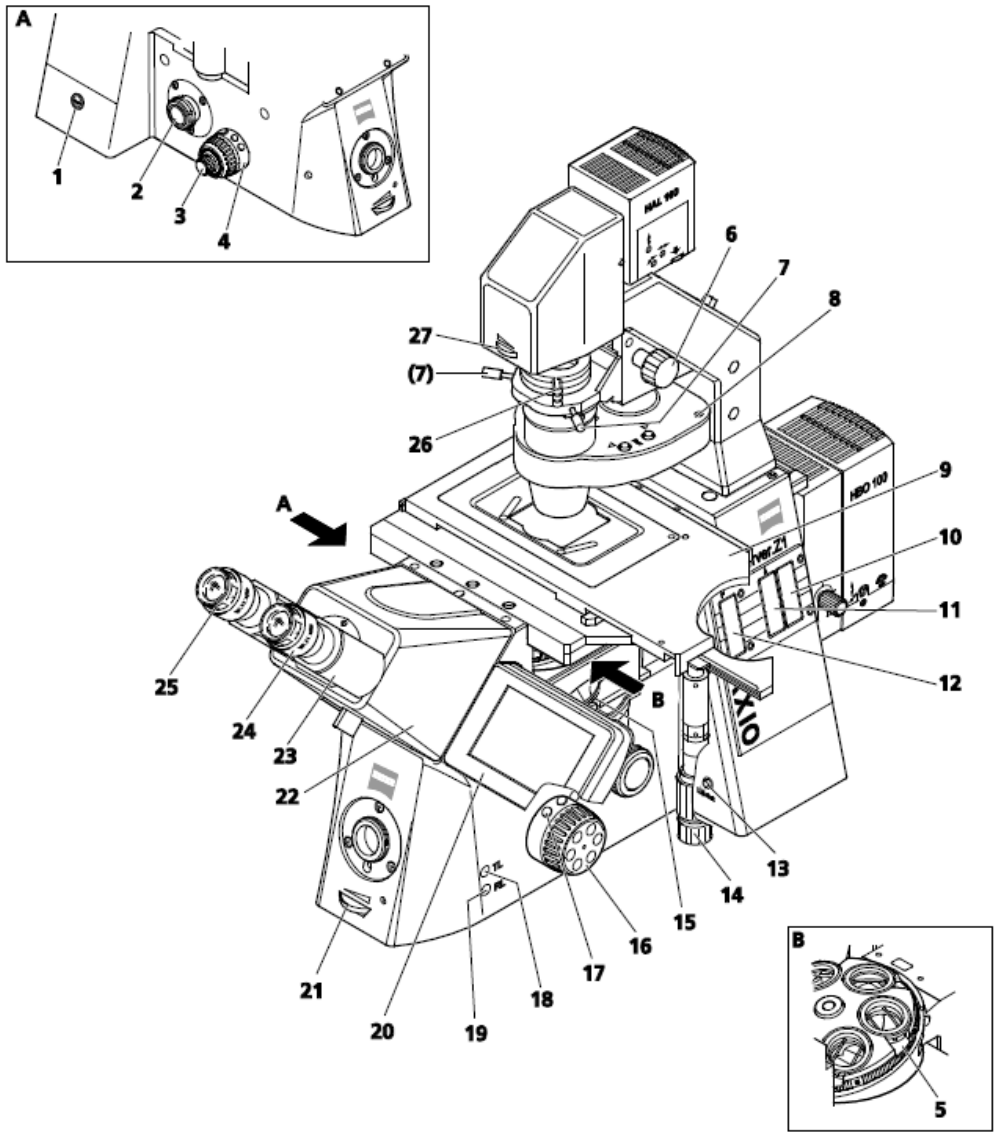


Fig. 4-3 Axio Observer.Z1 components and controls (motorized)

1 Standby button
2 Left Sideport
3 Focus drive coarse / fine (left side)
4 Control ring, left
5 Objective nosepiece
6 Vertical adjustment knob for condenser
7 Condenser centering screw
8 Condenser (manual or motorized)
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 LM set button
14 Drive knobs for controlling XY positioning of the mechanical stage
15 Reflector turret (coded or motorized)
16 Coarse / fine focus drive with fine drive, flat (right side)
17 Control ring, right
18 TL button for switching the transmitted light halogen illuminator on and off or for opening and closing the transmitted light shutter
19 RL button for switching the reflected light shutter on and off
20 TFT display
21 Halogen illumination intensity control
22 Binocular tube
23 Binocular section of the binocular tube
24 Eyepiece
25 Eyepiece adjustment ring
26 Polarizer D with 2-position filter changer or 3-position filter changer
27 Luminous-field stop control

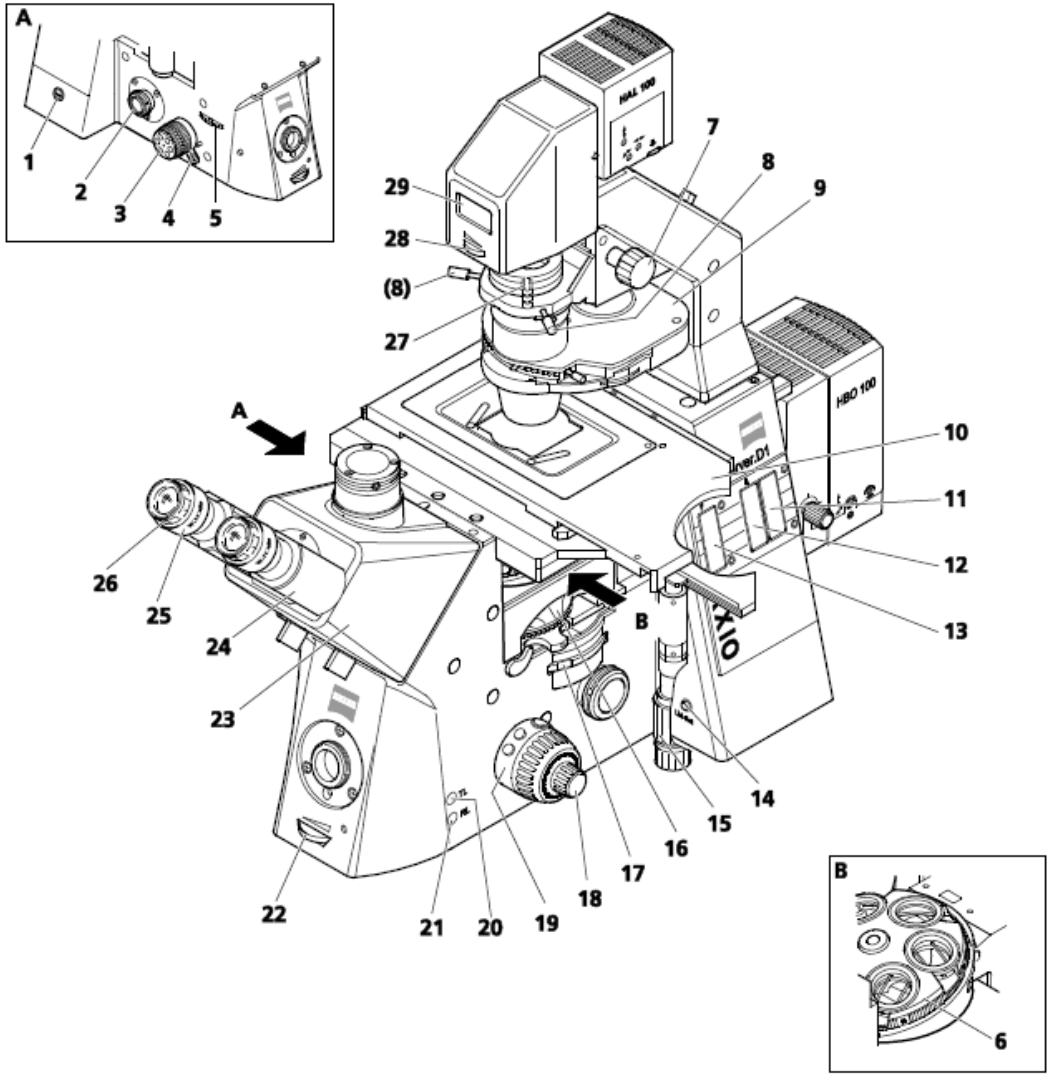


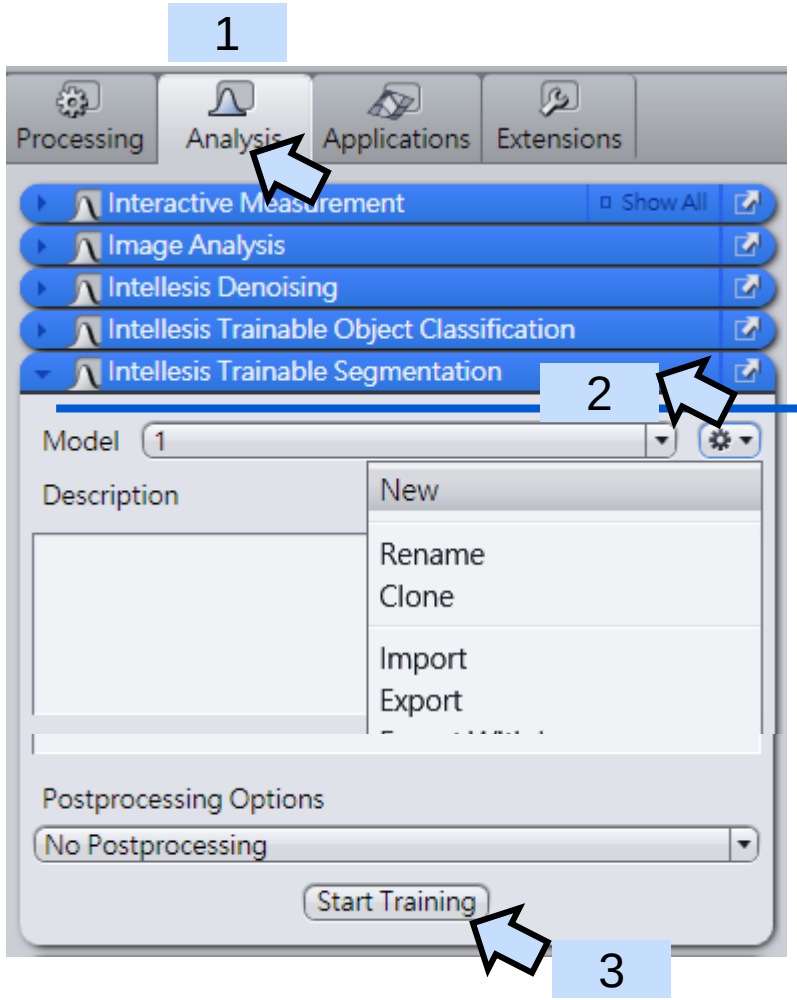
Fig. 4-2 Axio Observer.D1 components and controls (coded, semi-motorized)

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
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

Intellesis Trainable Segmentation

- 目的在產生正確遮罩
- 使用於難以用threshold做mask 的影像
- pixel classifier
- 需要手工筆刷塗抹，訓練
- 日後訓練好的model可以再套用於其他類似圖

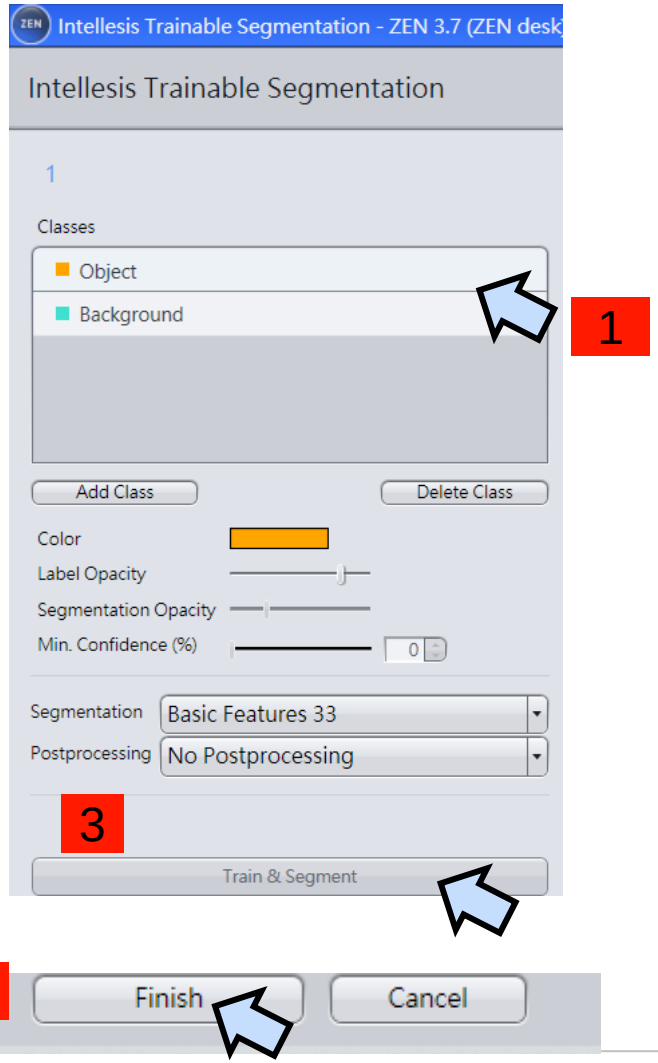
Intellesis Trainable Segmentation Setups 1.



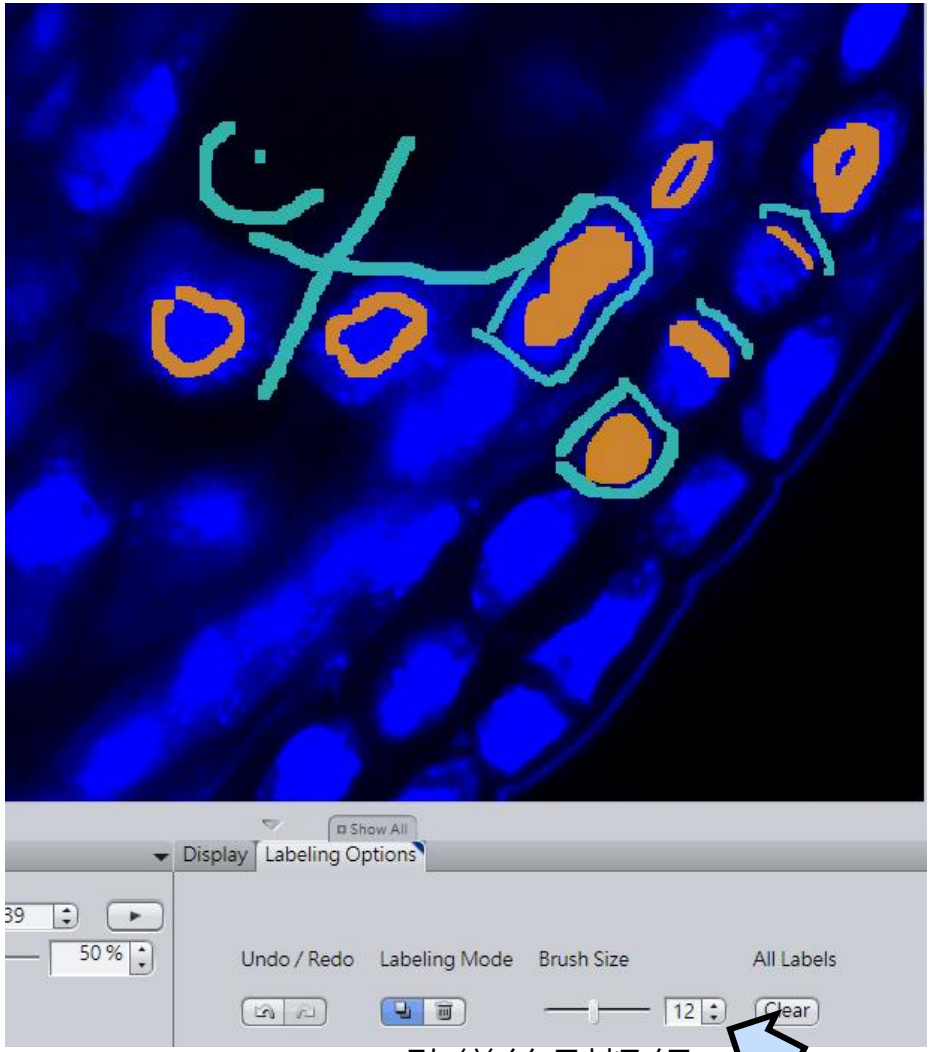
新增一個訓練遮罩Model

Intellesis Trainable Segmentation Setups 2.

Intellesis Trainable Segmentation setups 3.
Use brush tool , Assign some pixel example as Object and Background

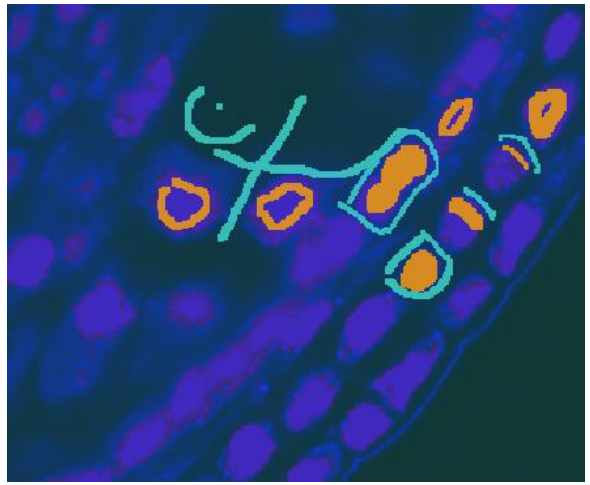


2 使用工具筆刷塗抹於物件與背景

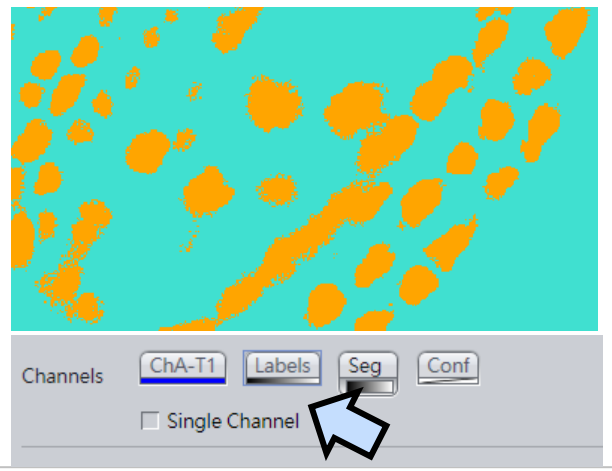


改變筆刷粗細

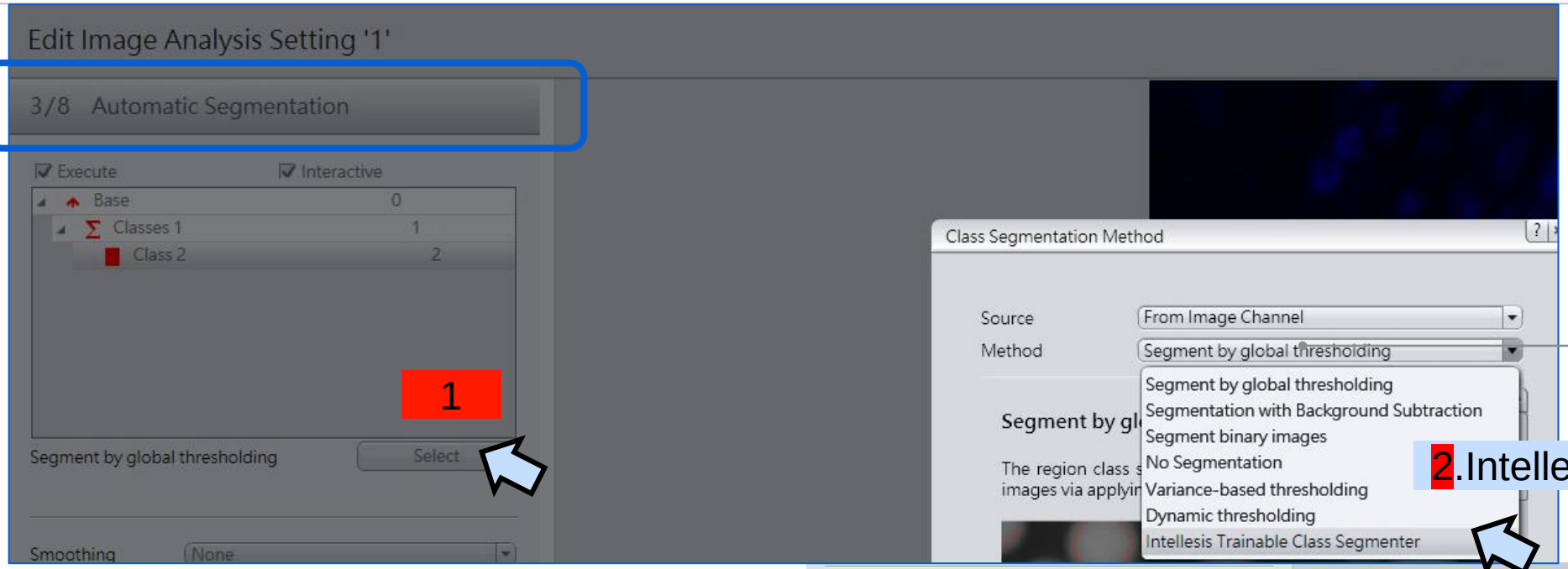
4 預覽結果



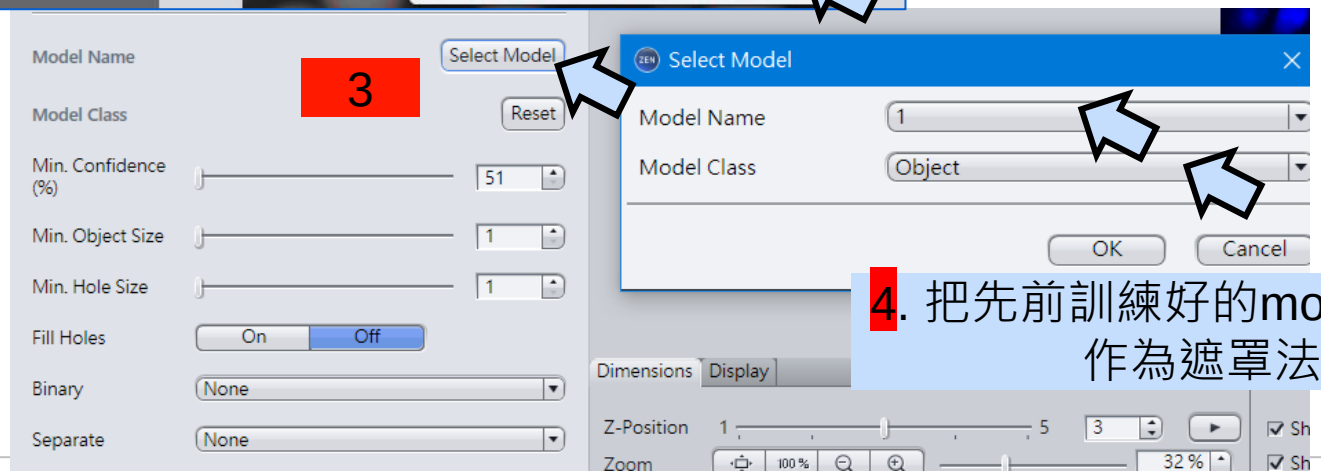
善用channel on /off預覽結果



訓練好的model使用時機 1
Image Analysis

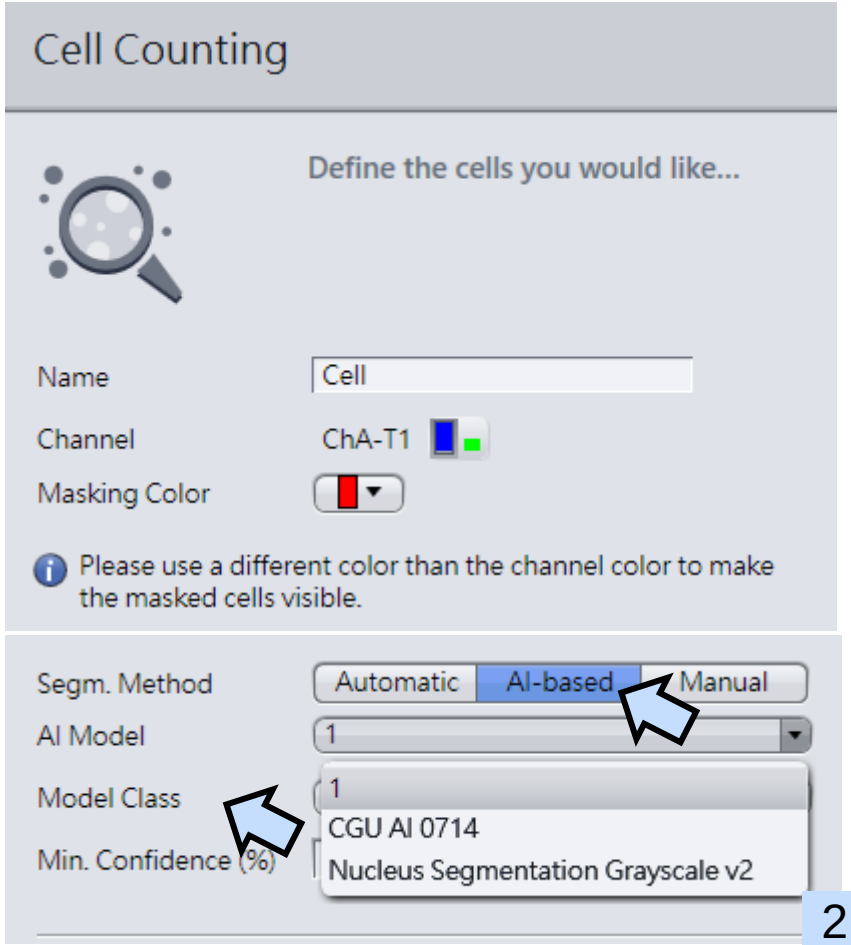


2.Intellesis Trainable Class Segmenter



4. 把先前訓練好的model套用，作為遮罩法

訓練好的model使用時機 2
BipApps，以Cell counting 為例



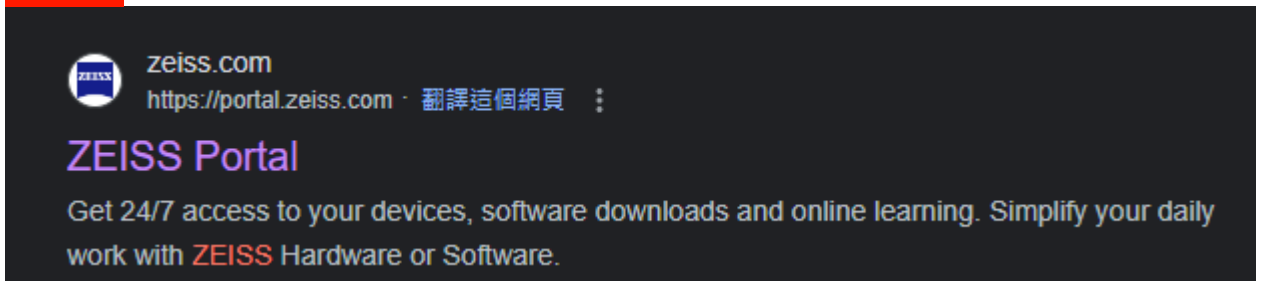
1

2. 把先前訓練好的model套用，作為遮罩法

下載離線軟體 ZEN lite 1

1

2

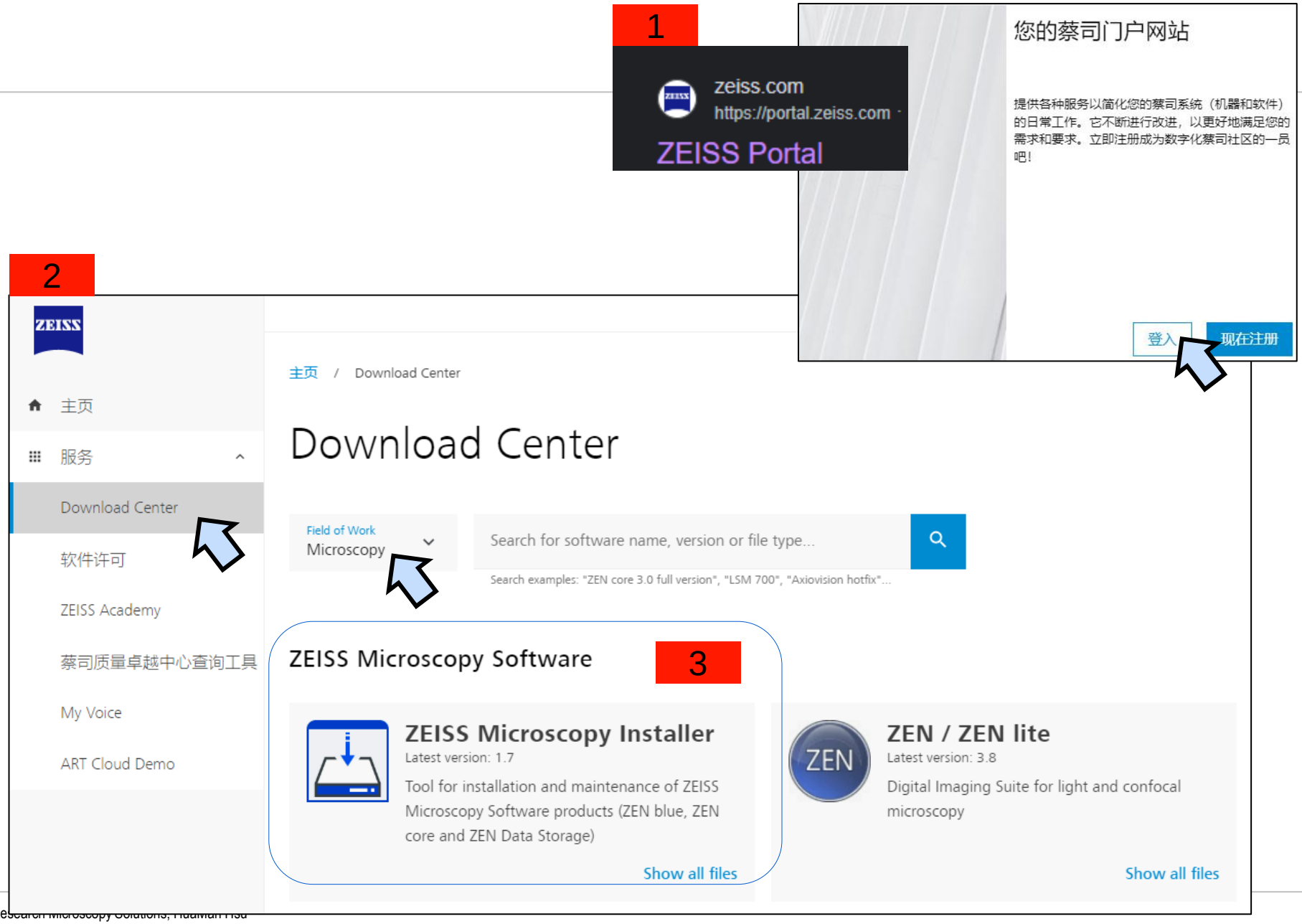


3

4

信箱收註冊確認信，沒有收到請製垃圾桶中找尋

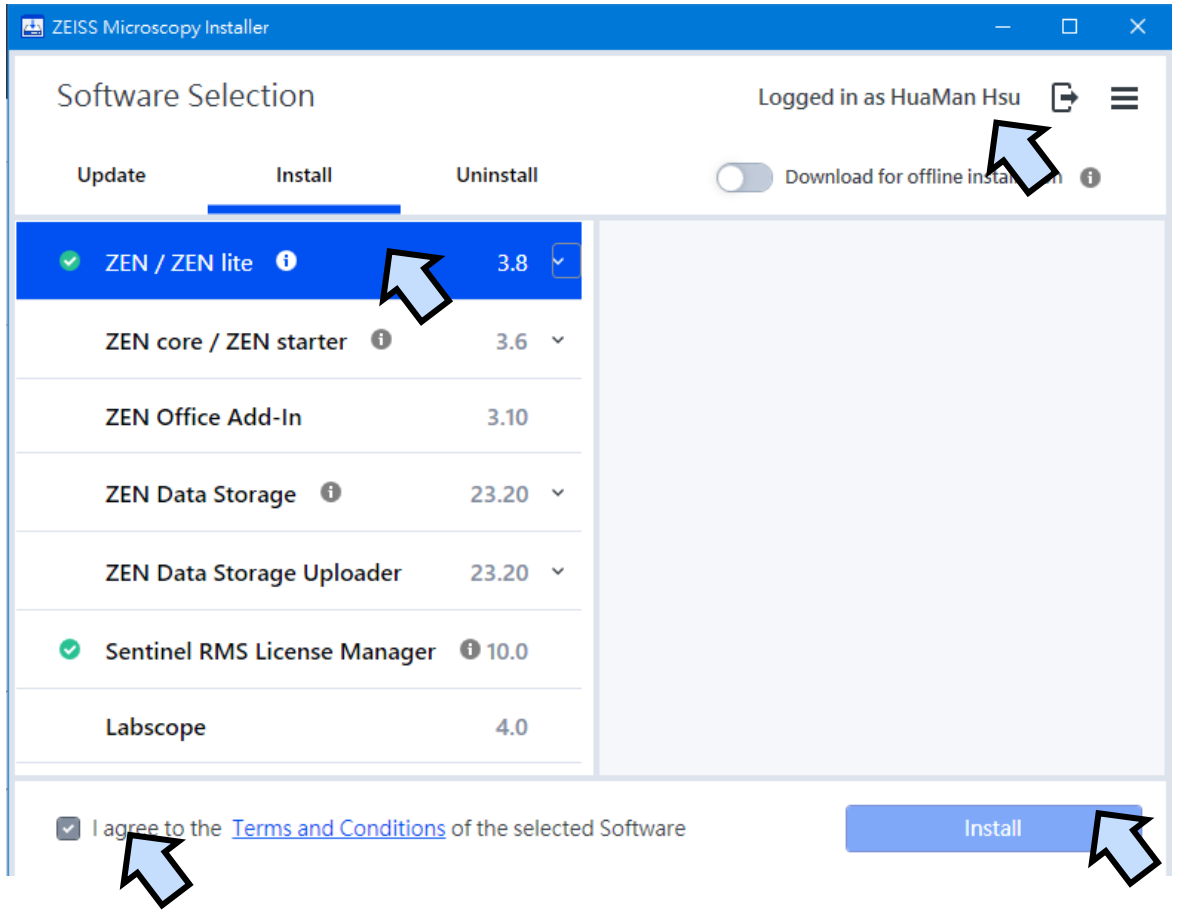
下載離線軟體 ZEN lite 2



下載離線軟體 ZEN lite 3

1

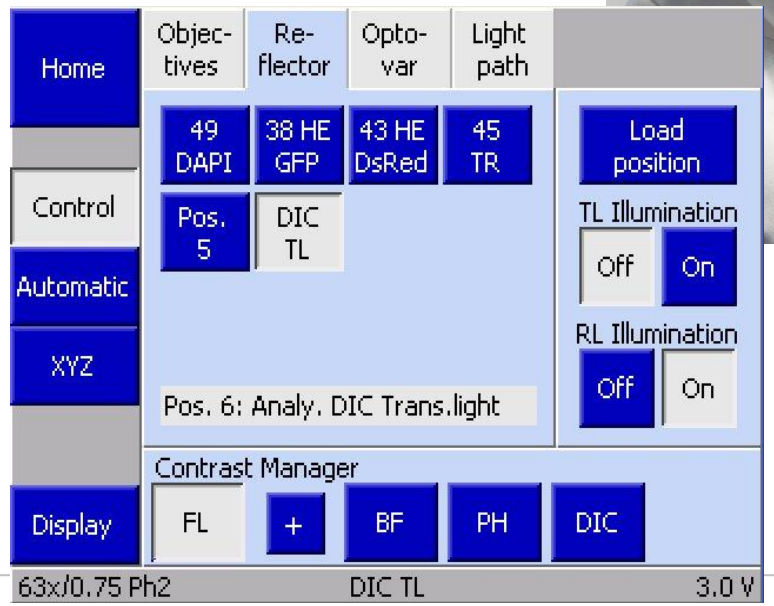
2




ZEISS

Axio Observer Z1
Improved Workflow – The TFT Touchscreen

- Input and Monitoring Station at the same time
- All functions merged radically
- Includes control and monitoring of incubation



The Cell Observer – New Incubation Generation

- **Incubation perfectly integrated**

- **Control and Monitoring via TFT or Axiovision SW**