



Carl Zeiss Axio Observer / ZEN blue

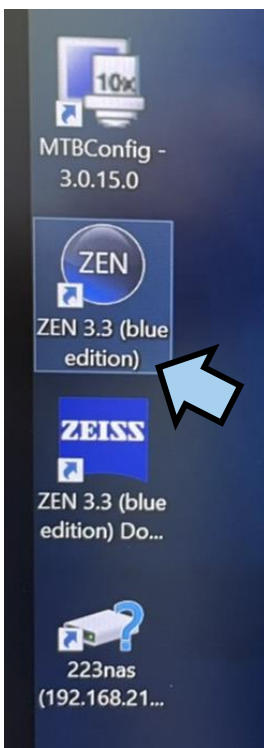
Quick Guide



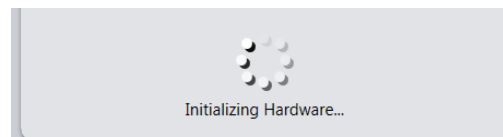
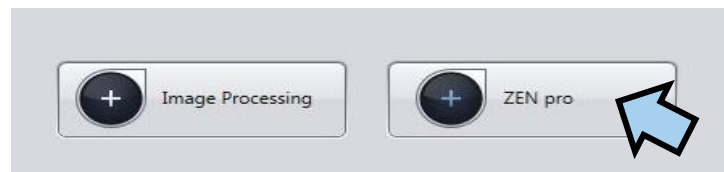
啟動軟體 ZEN



1 進入ZEN BLUE

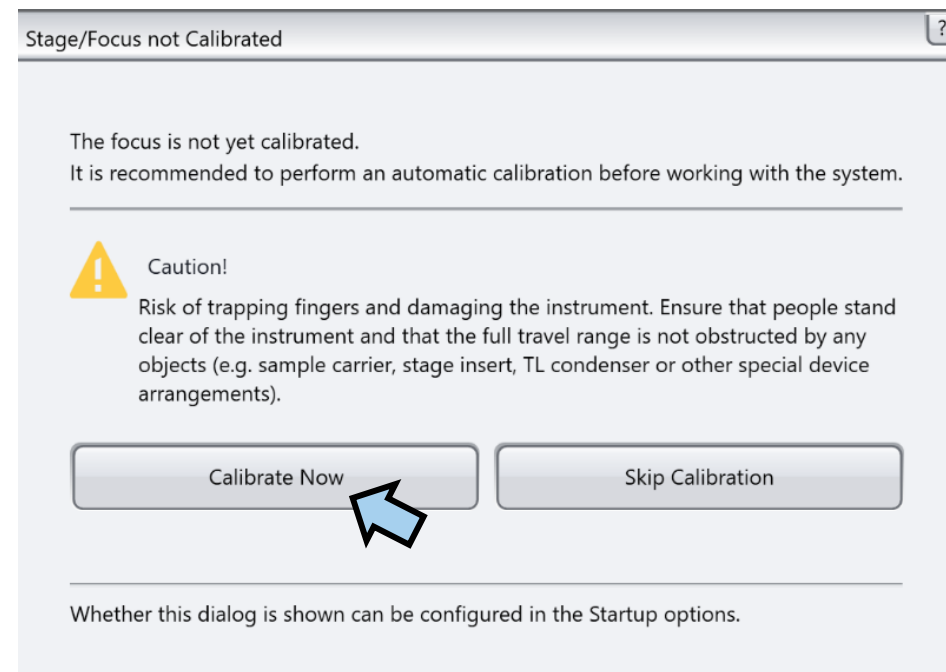


2 ZEN pro 連接硬體 啟動拍照功能



等待約一分鐘

3 [可略過] 需先清空載物台，先不放樣品

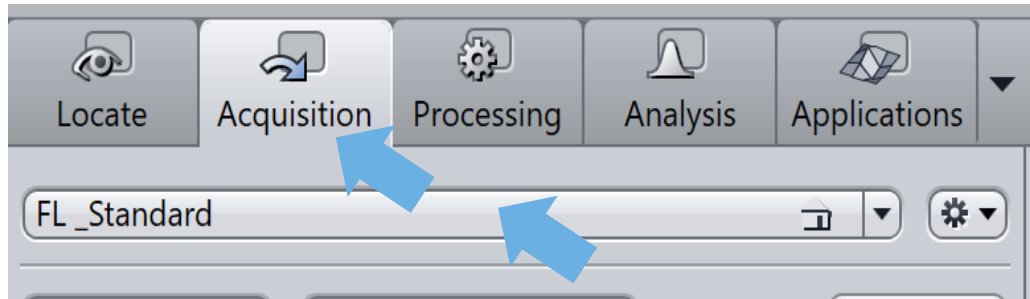


拍攝螢光 1. 套用拍攝參數experiment manager



套用拍攝參數

拍攝螢光請選擇FL image (Axiocam 712 mono)

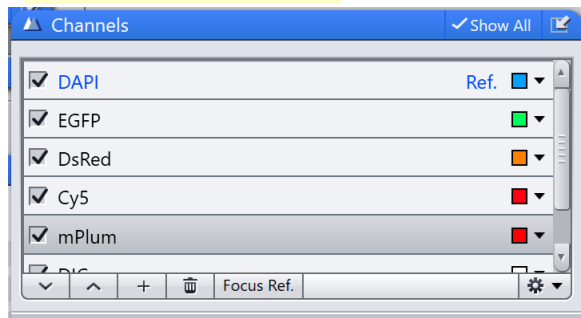


拍攝螢光 2.

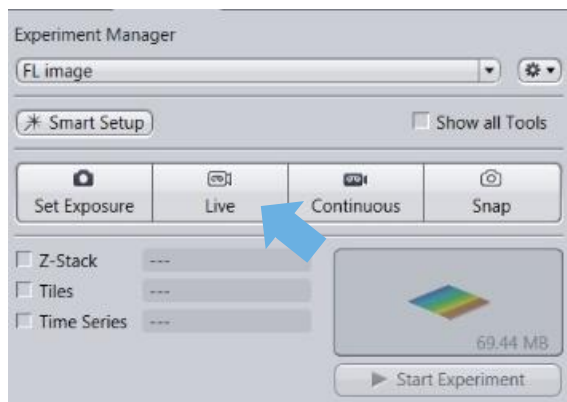


1

選擇需要的channel

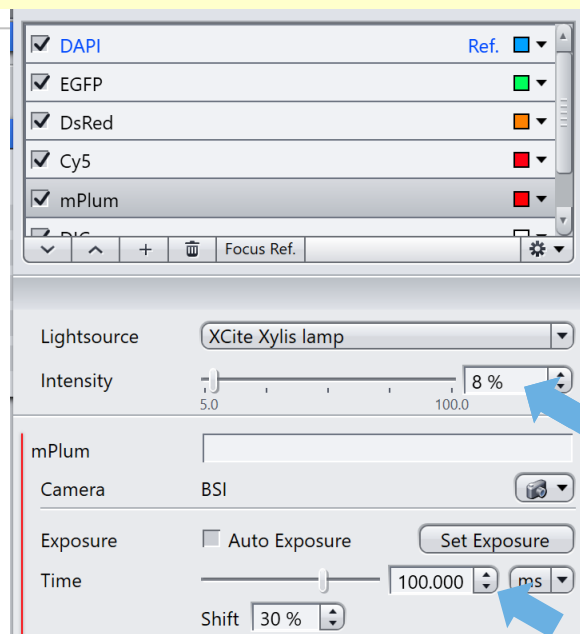


2



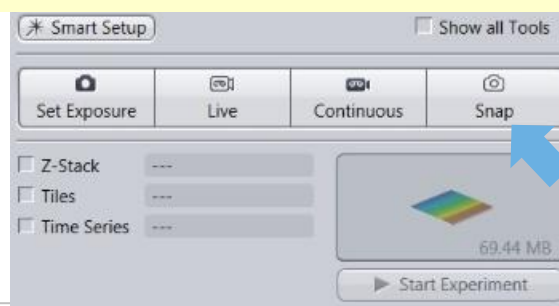
3

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



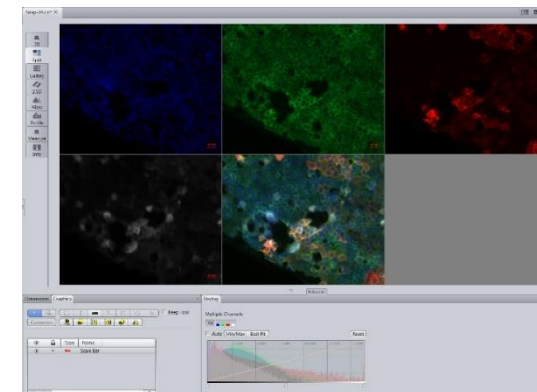
4

按下snap
系統會自動拍下所有已打勾的channels



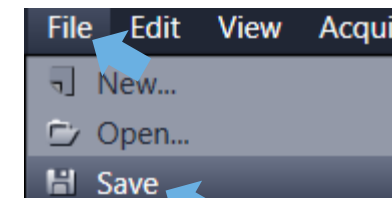
5

得到完整影像



6

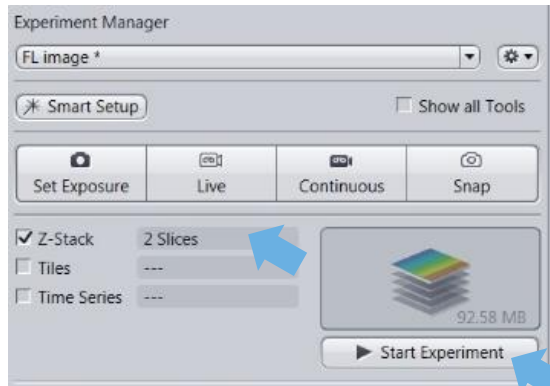
存檔



詳見下頁

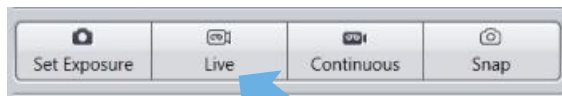
拍攝multichannel+ Z stack

1



勾選z stack選項

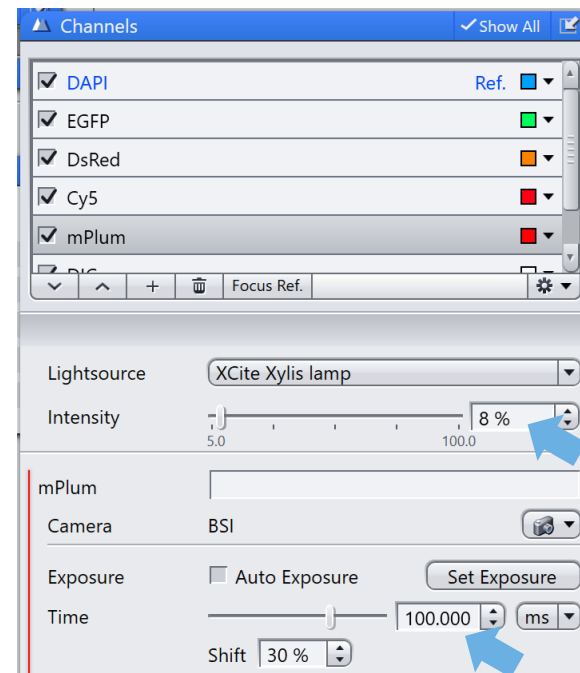
2



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

3

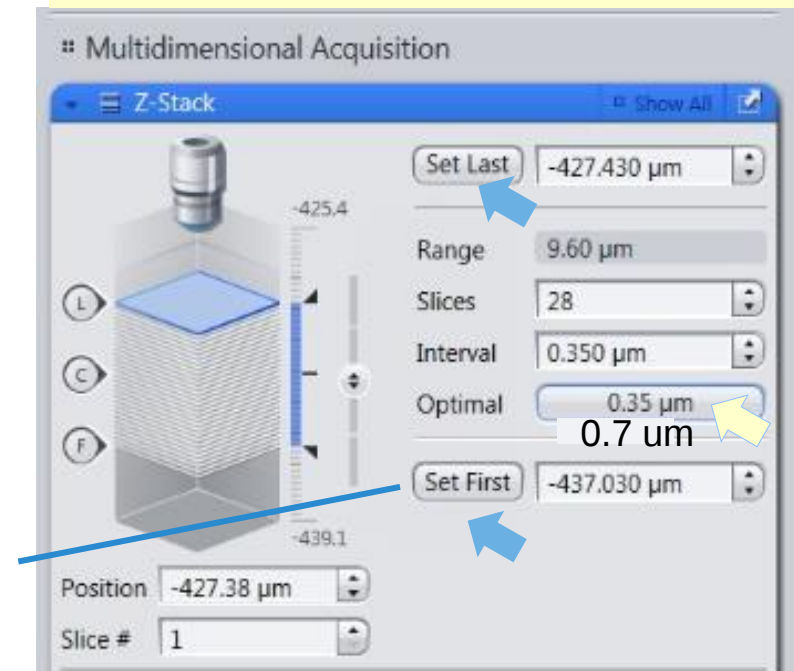
Live
調整所有channel的light source
intensity 與曝光時間



4

Live→開始設訂Z stack的上下界限

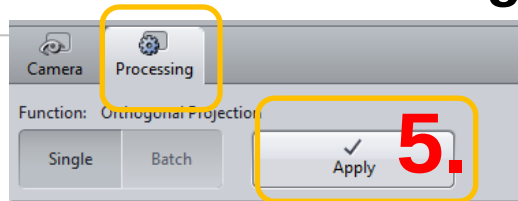
- 選擇一個channel>live
- 搭配Z軸調整視窗 > Z-stack
- 找到樣品焦距起點 > Set First
- 找到樣品焦距終點> Set Last



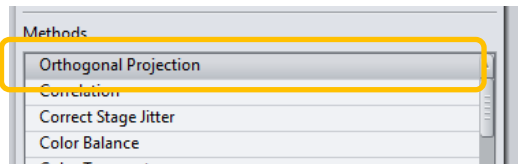
- interval可參考optimal
- 2x的optimal $0.35 \times 2 = 0.7$ 也很好
- 自行決定間隔

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

1.



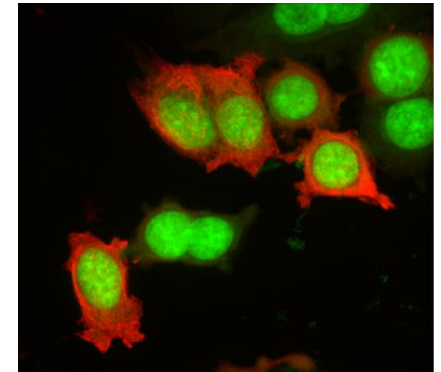
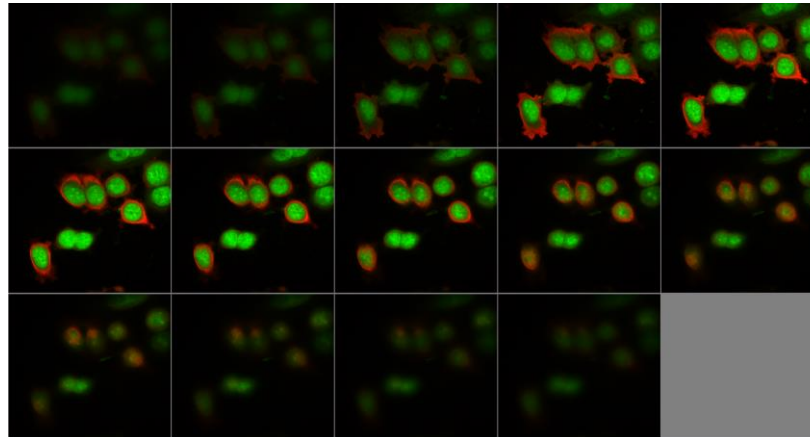
2.



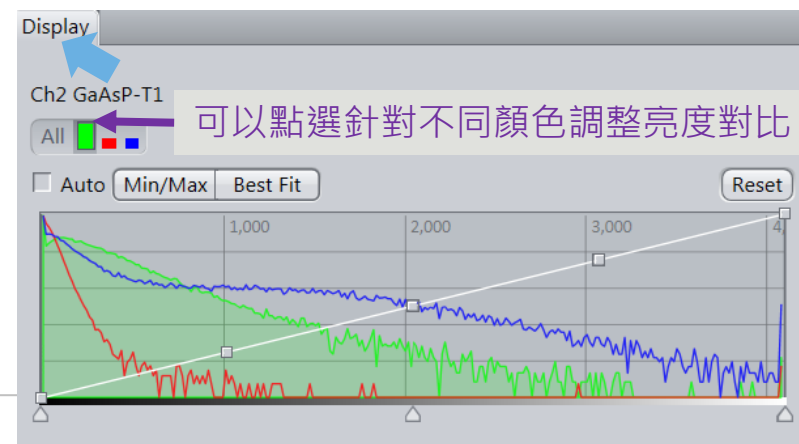
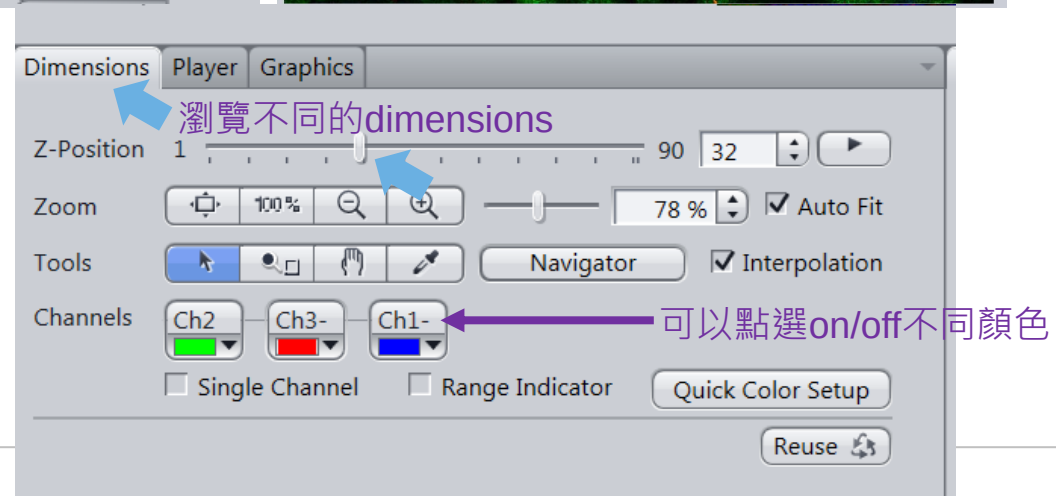
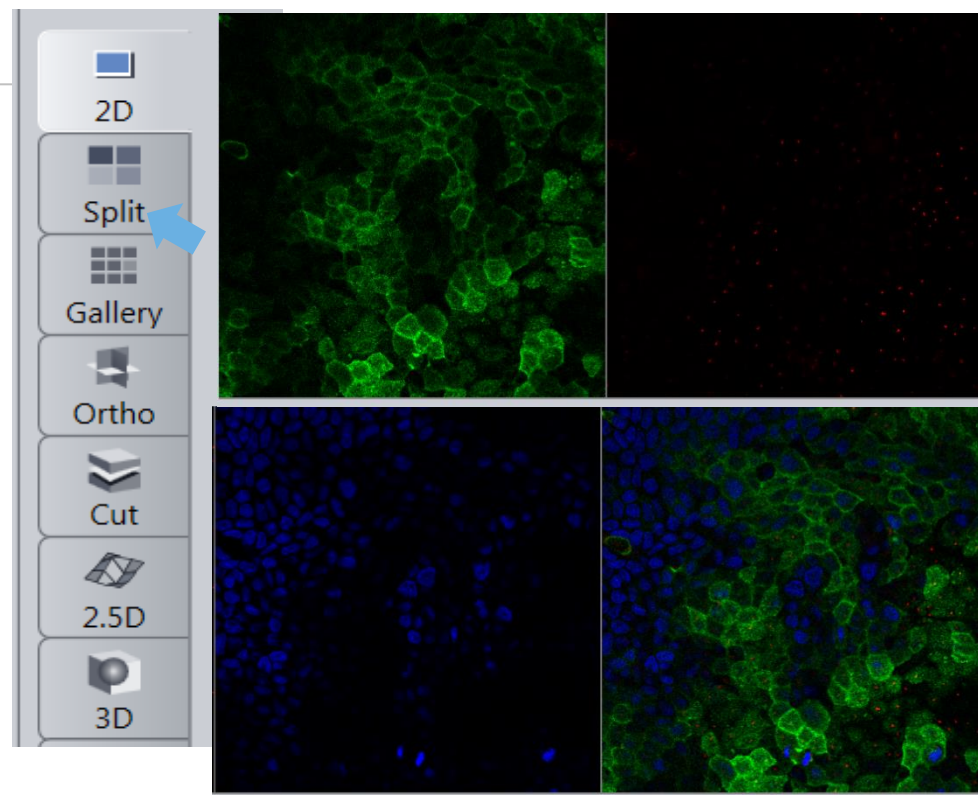
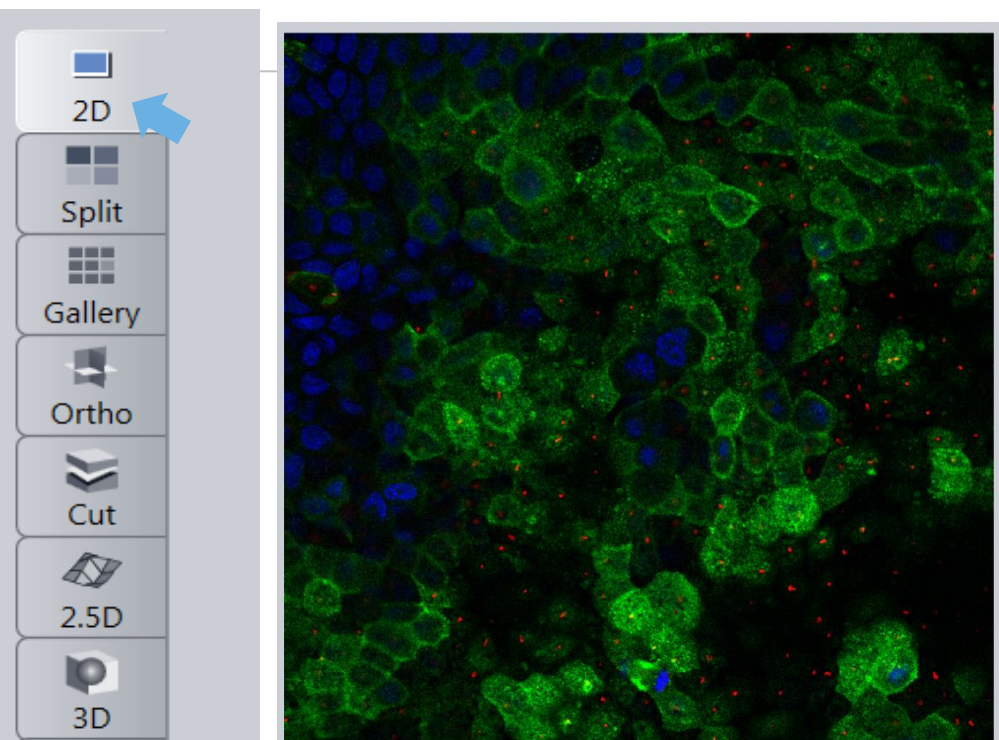
4.



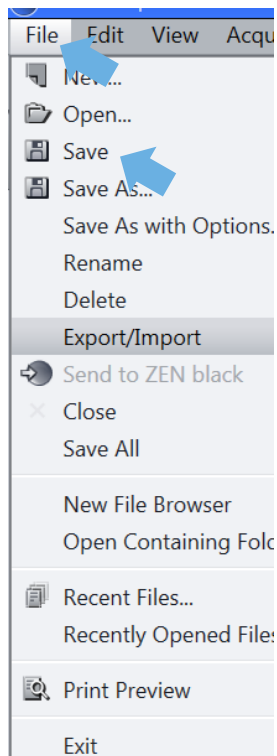
3.



影像視窗2D/Split & Dimensions/Display

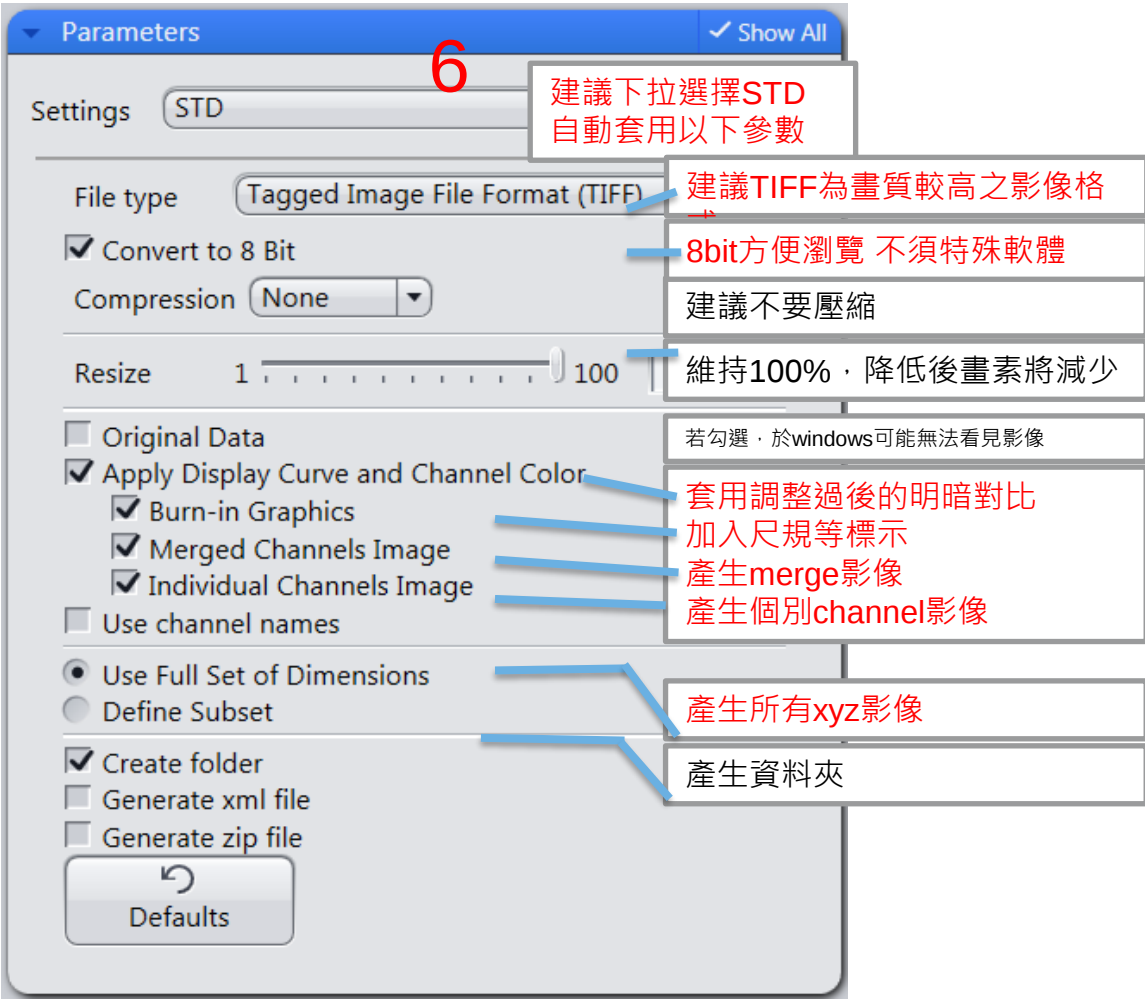
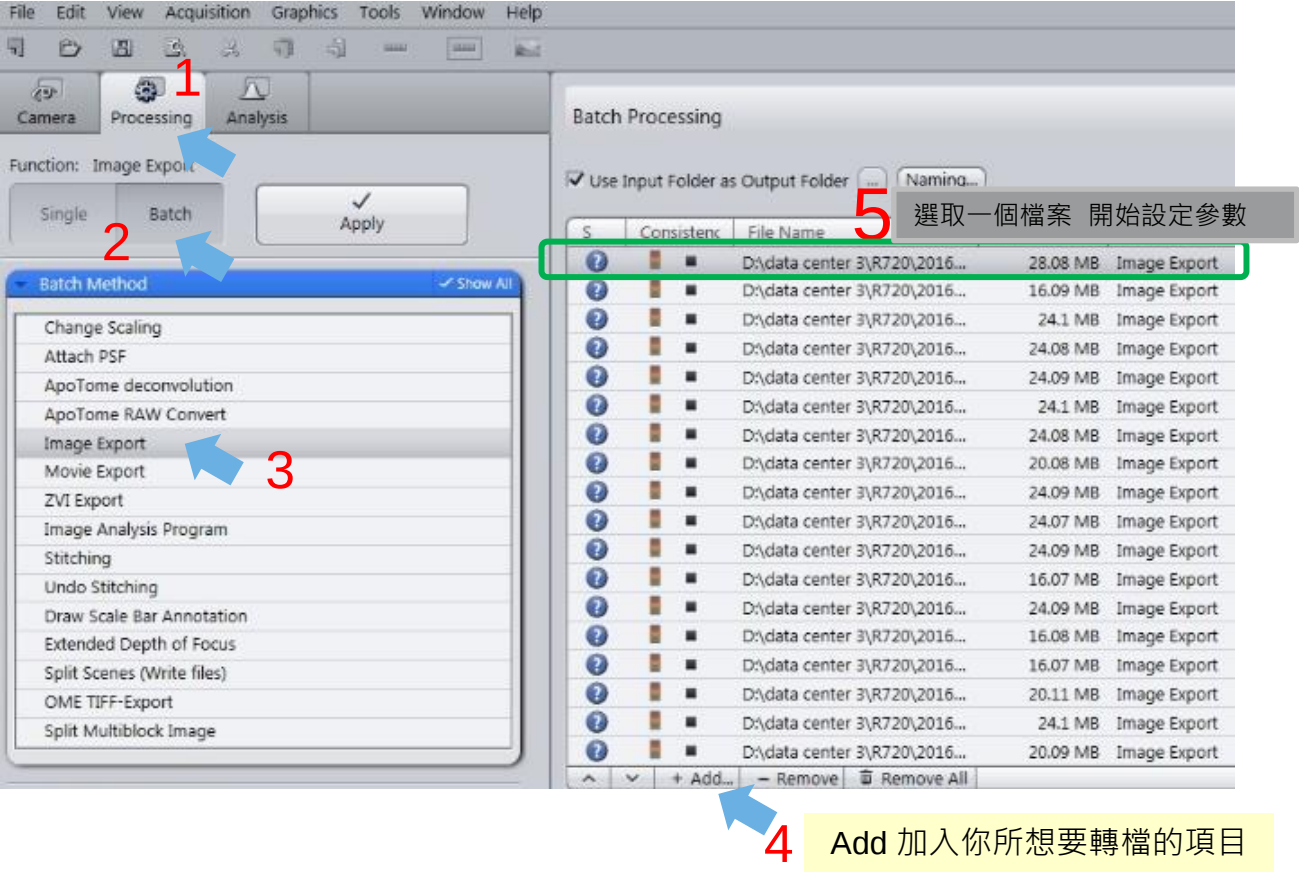


5



- 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

大量批次轉檔Batch export 1



大量批次轉檔batch export 2



Camera

Processing

Function: Image Export

Single

Batch

✓

Apply

Batch Method

Show All

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

輸出至原檔案同一資料夾

✓

Use Input Folder as Output Folder

...

Naming...

7

8

10

Copy Parameters

Paste Parameters

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

+

Add...

-

Remove

Remove All

Load List...

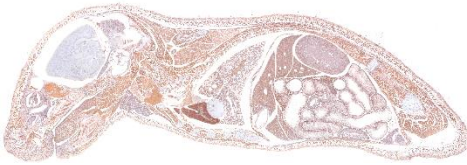
Save List...

9

按住SHIFT鍵選取剩下的檔案

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



AF

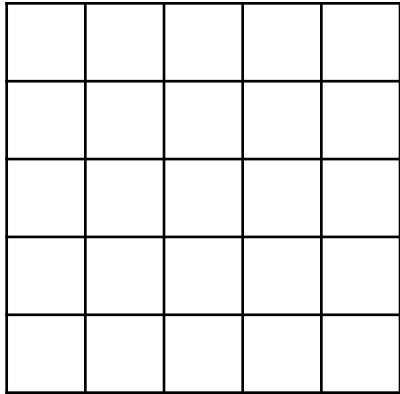
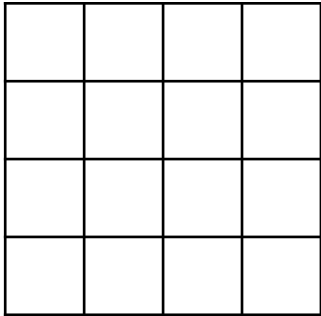
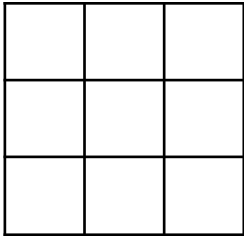
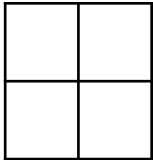
Find FocusSet ExposureLiveContinuousSnap

2x2

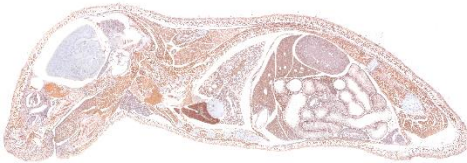
3x3

4x4

5x5



Tile Scan Setup



712 *

Smart Setup Reuse

Set Exposure **Stop** Continuous Snap

1

70.5 MB

Start Experiment

Experiment Regions Auto Save Automated Image Export Automation

Imaging Setup Channels

Bright DIC Ref.

Lightsource Use Setting

TL Brightfield

Camera Axiocam 712

Exposure Time 2.000 ms

Shading Correction Define

Focus Offset Z 0.00 µm

2

3

4

5

6

8

8.1

8.2

Tile Regions

Name	Category	Tiles	Z (µm)
TR1	Default	9	1025.5

Positions

Sample Carrier

Focus Surface and Support Points

Selected Tile Regions: No region selected

Add Single Support Point

Current Position Center of Tile Region

Add Multiple Support Points

Method Generic

Columns 2 Rows 2 Distribute

Local (per Tile Region)

Support Points of Selected Tile Region: TR2

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

Verify Support Points Verify

Preview

Define tile regions by number or size

Dimensions

Zoom 100% 20% Auto Fit

Tools Navigator Interpolation

Channels Bright

Single Channel Range Indicator Quick Color Setup

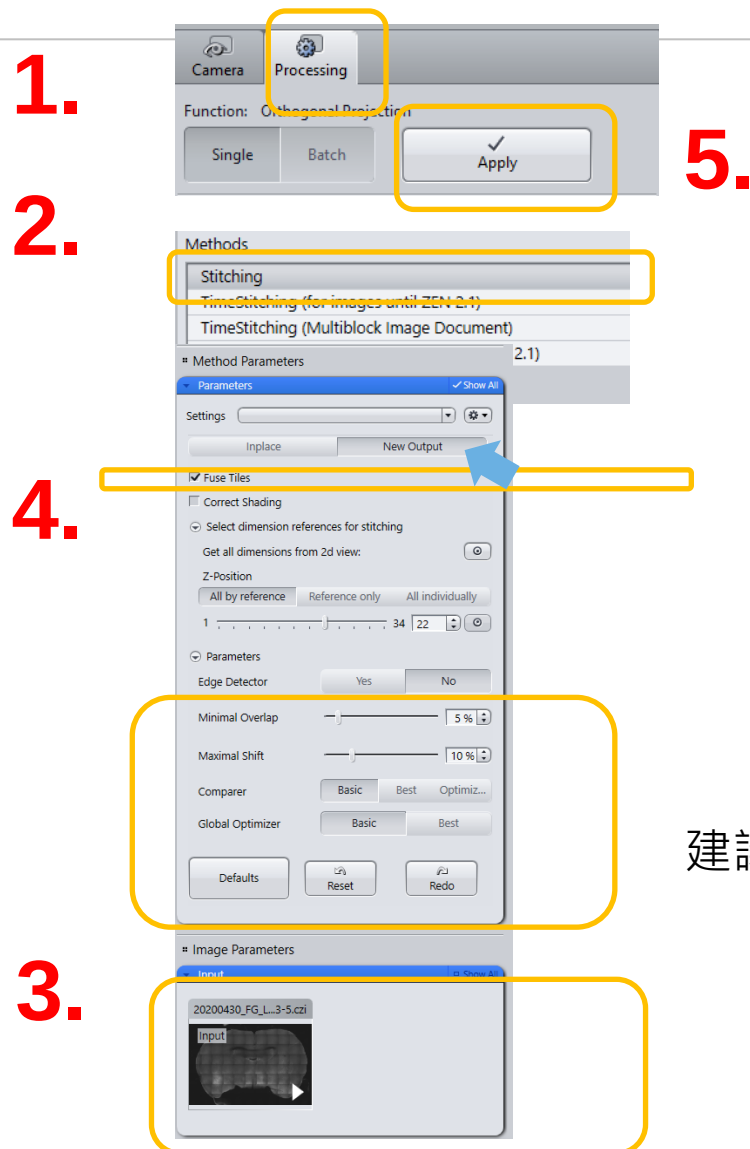
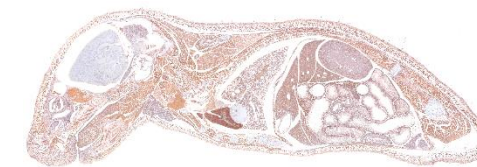
Display

Show Live/Continuous in

New Tab Separate Container Tiles View

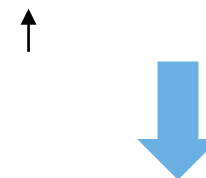
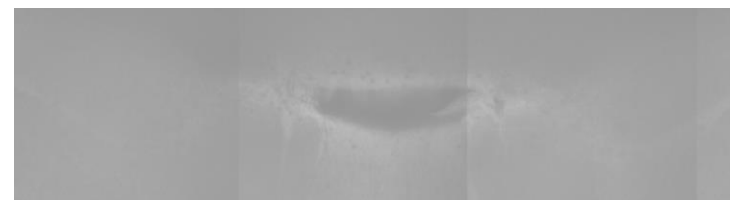
Center to Stage Position

TILE Image stitching / fuse 拼圖結果融合處理



建議選擇Basic即可

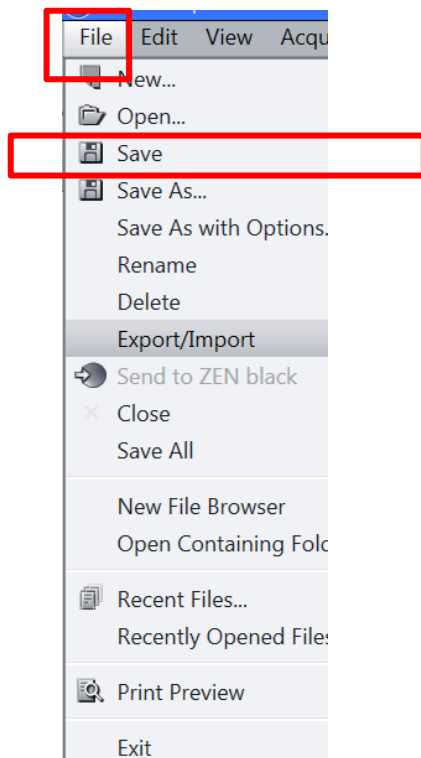
Before



After

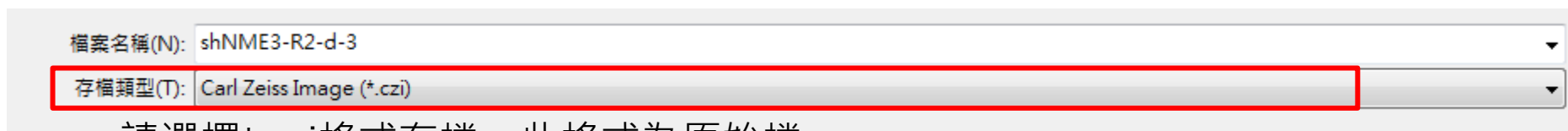


5



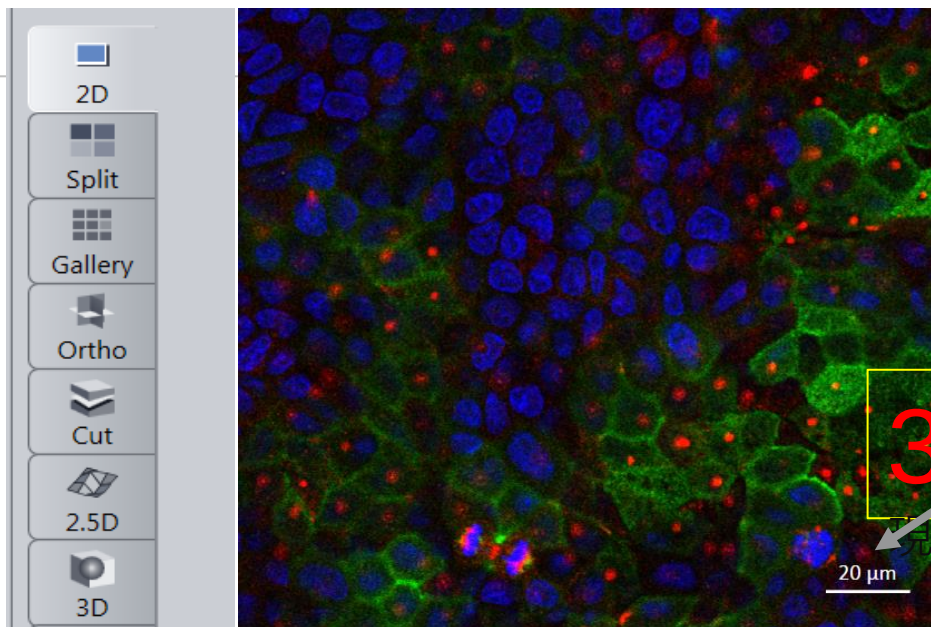
存檔位置請選擇D槽 data pool，請勿直接設定存於外面的data center中。

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!



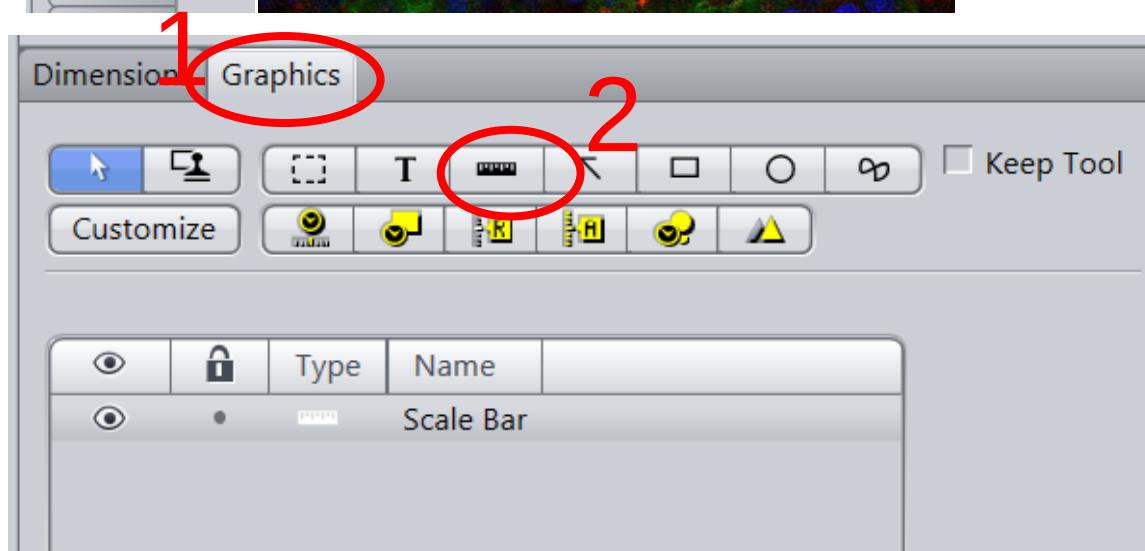
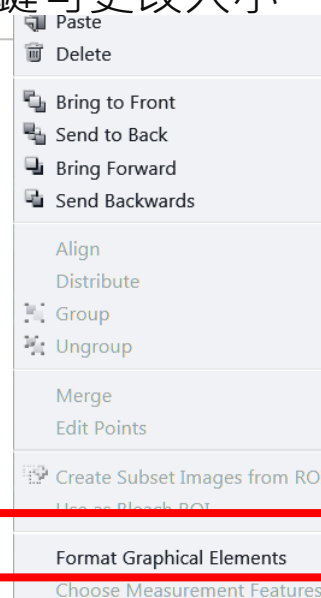
- 請選擇*.czi格式存檔，此格式為原始檔
- 請勿直接存成tif/jpg檔，以免日後無法定量及添加尺規
- 需要純圖檔請進行圖檔輸出export或大量批次轉檔batch export，詳見下頁

加入尺規



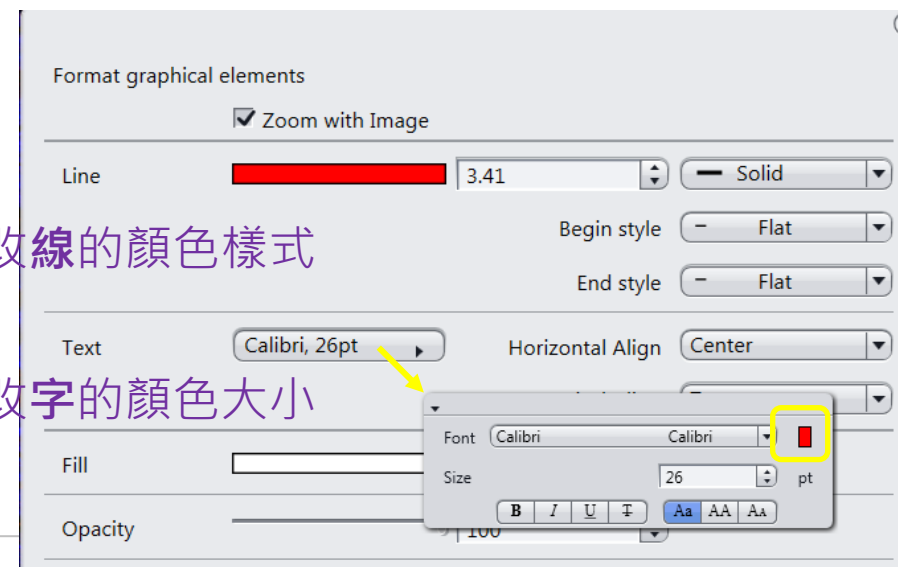
3 scale bar會自動出

4 於scale bar按右鍵可更改大小
顏色粗細等格式

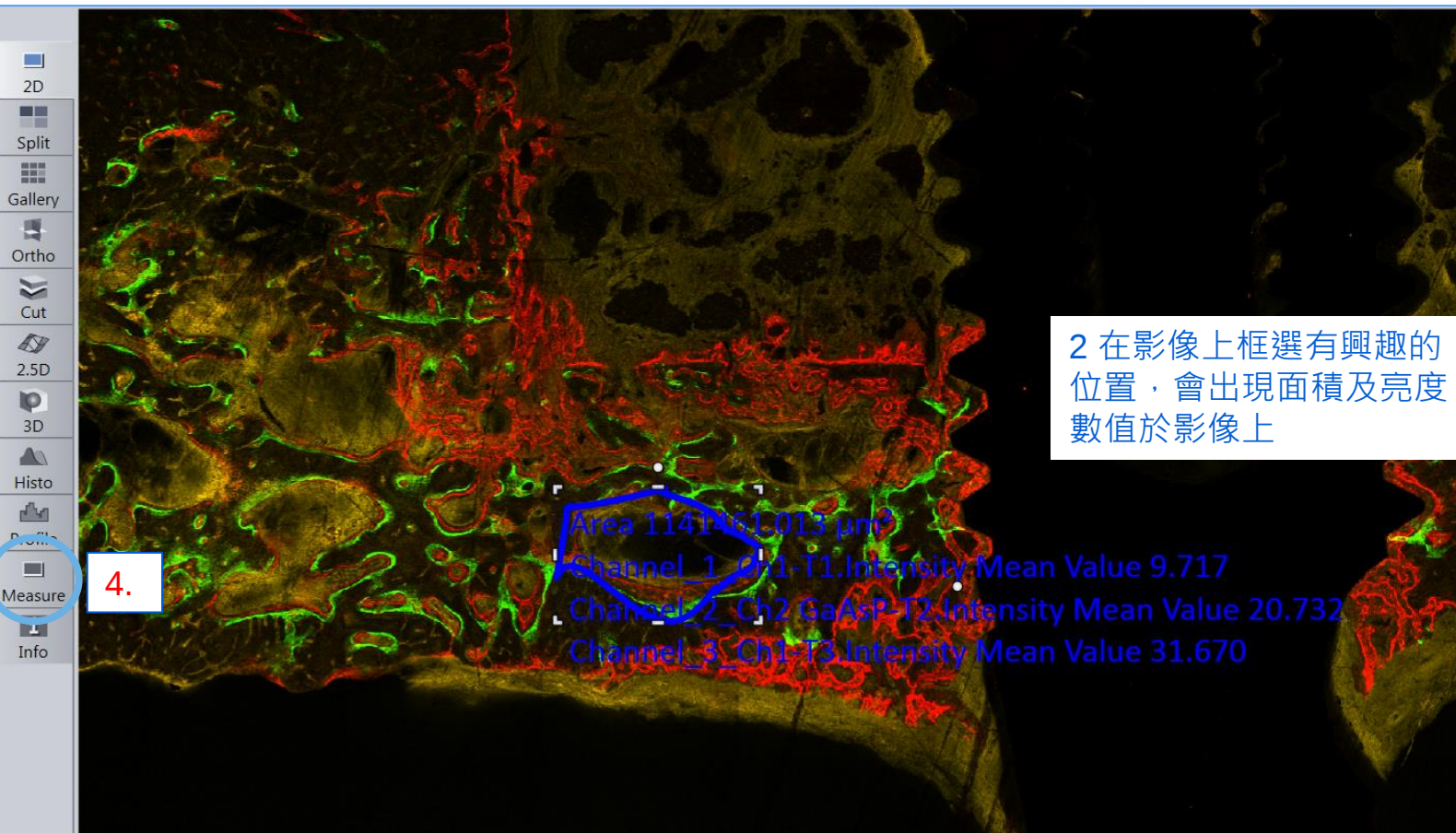


更改線的颜色樣式

更改字的颜色大小



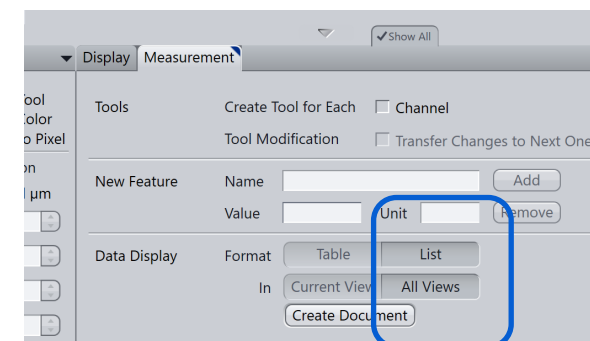
量測intensity



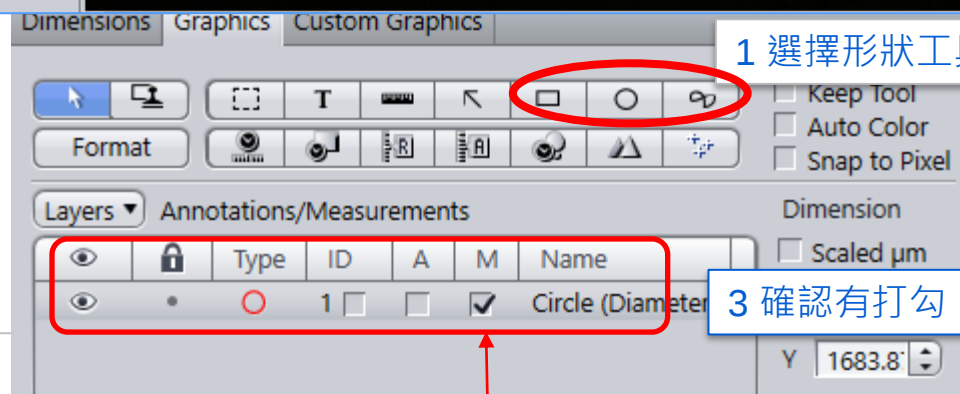
2 在影像上框選有興趣的位置，會出現面積及亮度數值於影像上

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	μm^2
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	



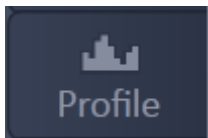
1 選擇形狀工具



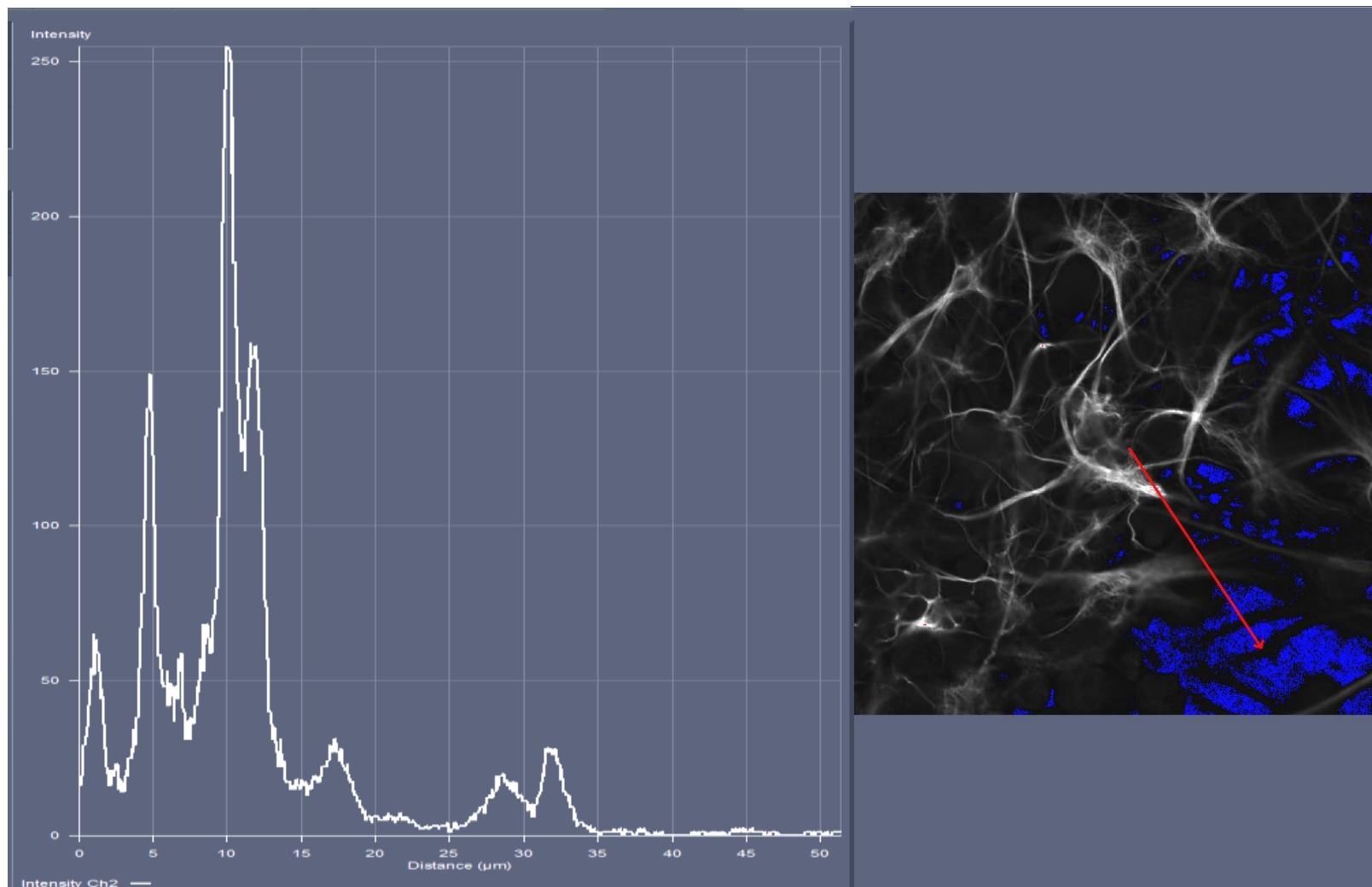
3 確認有打勾

Profile

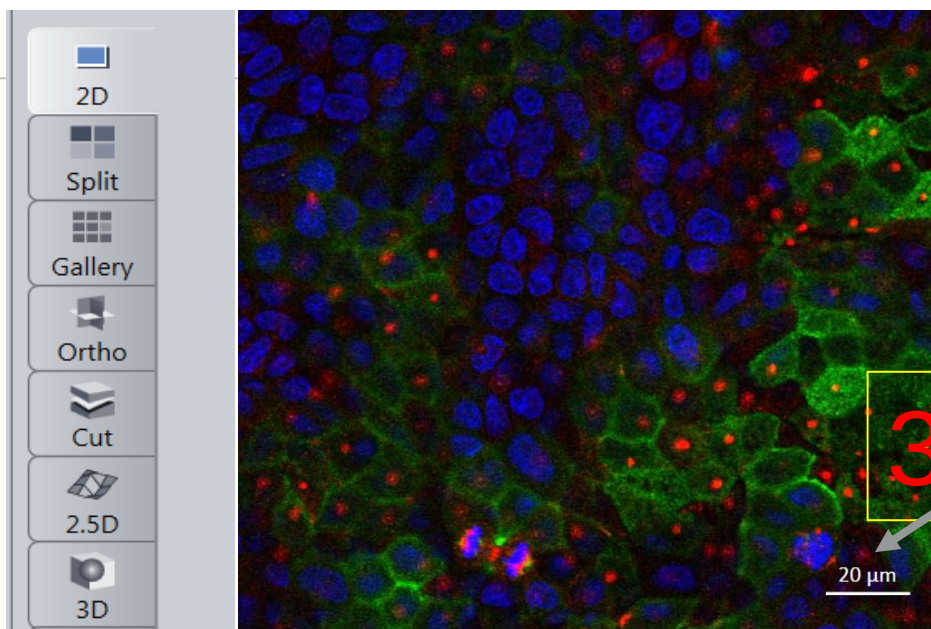
Intensity distribution along the line



Gives measurements
of grey values along a line

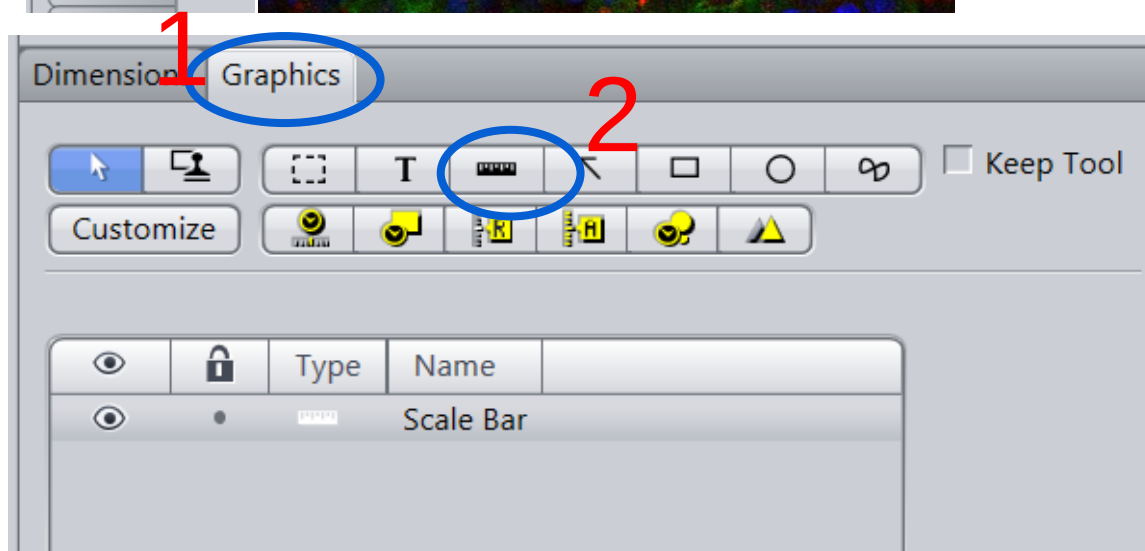
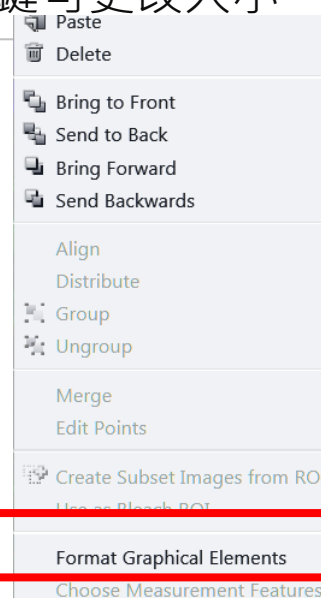


Add Scale Bar加入尺規



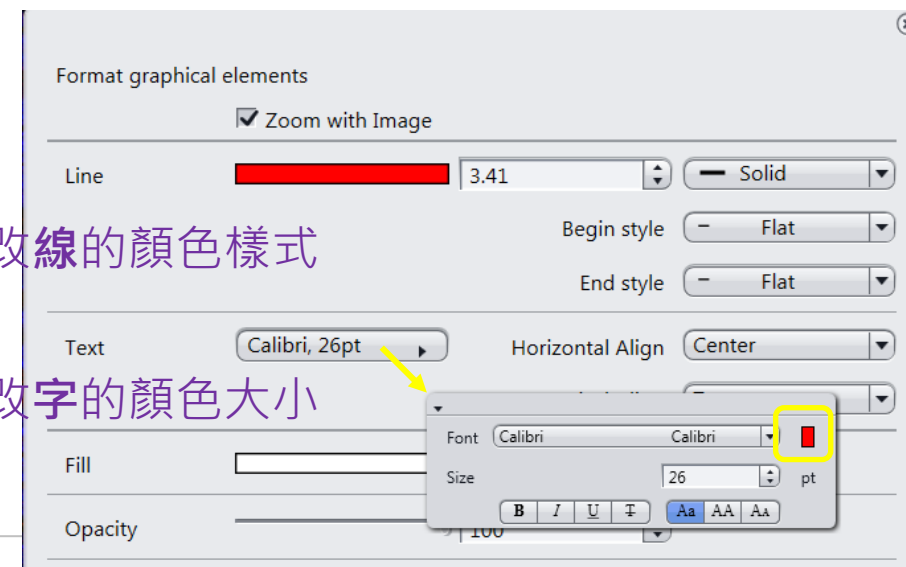
3 scale bar會自動出現

4 於scale bar按右鍵可更改大小
顏色粗細等格式

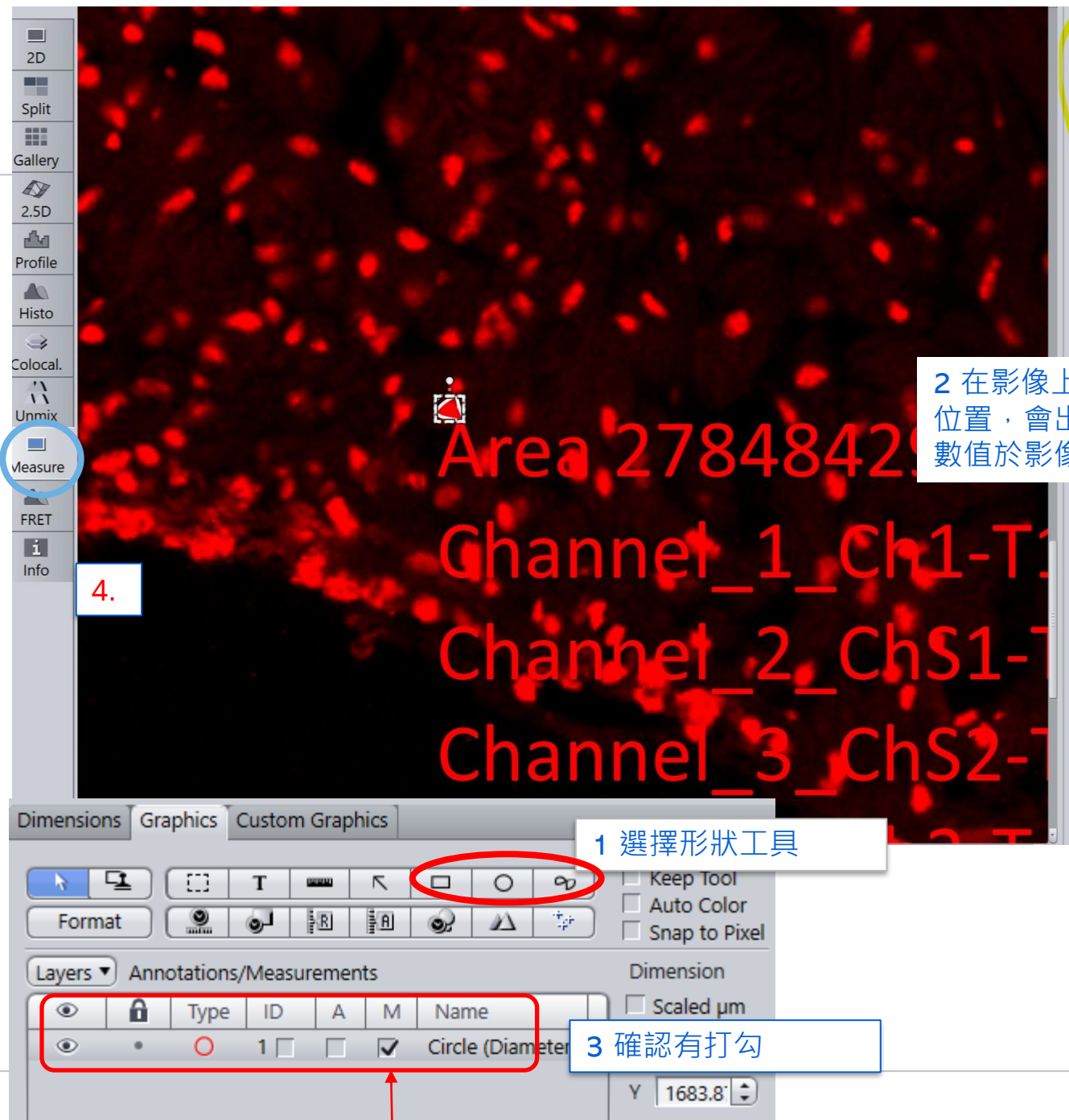


更改線的颜色樣式

更改字的颜色大小



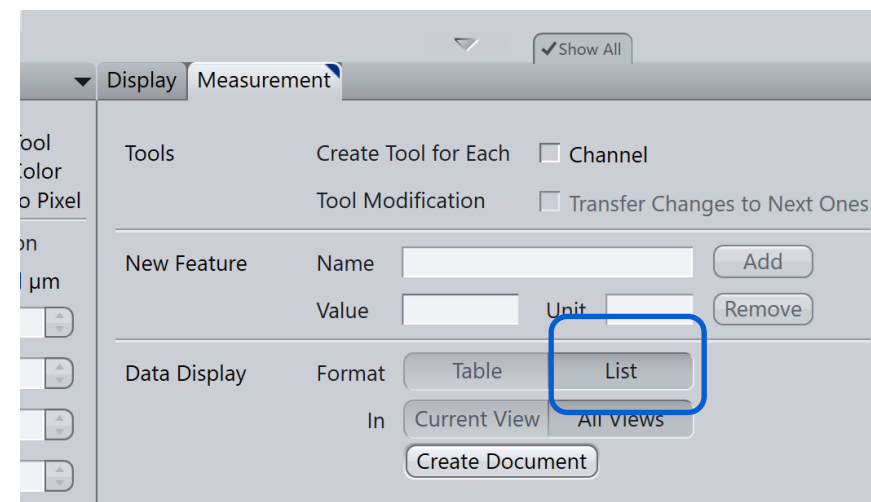
量測area & intensity

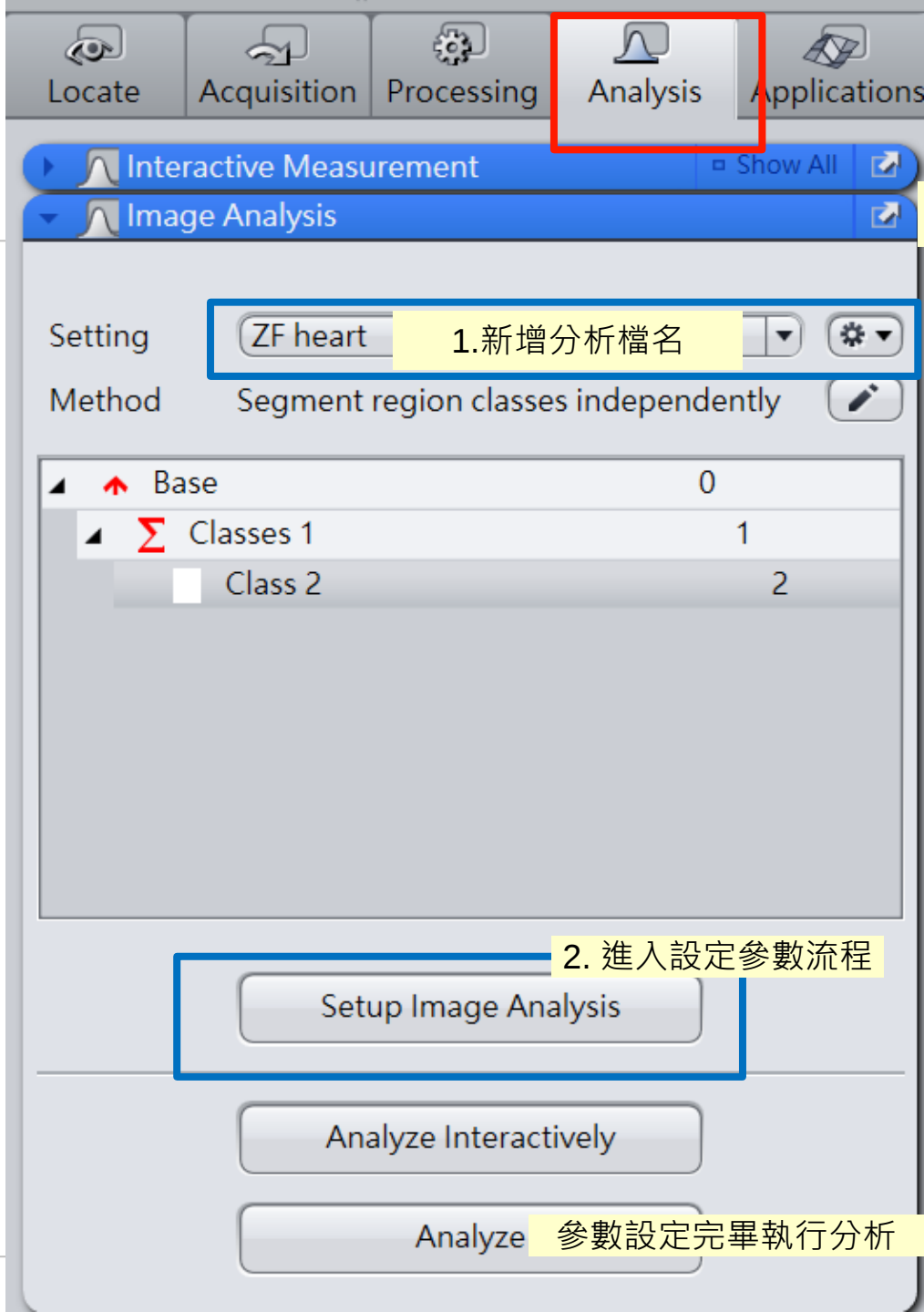


2 在影像上框選有興趣的位置，會出現面積及亮度數值於影像上

4.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	

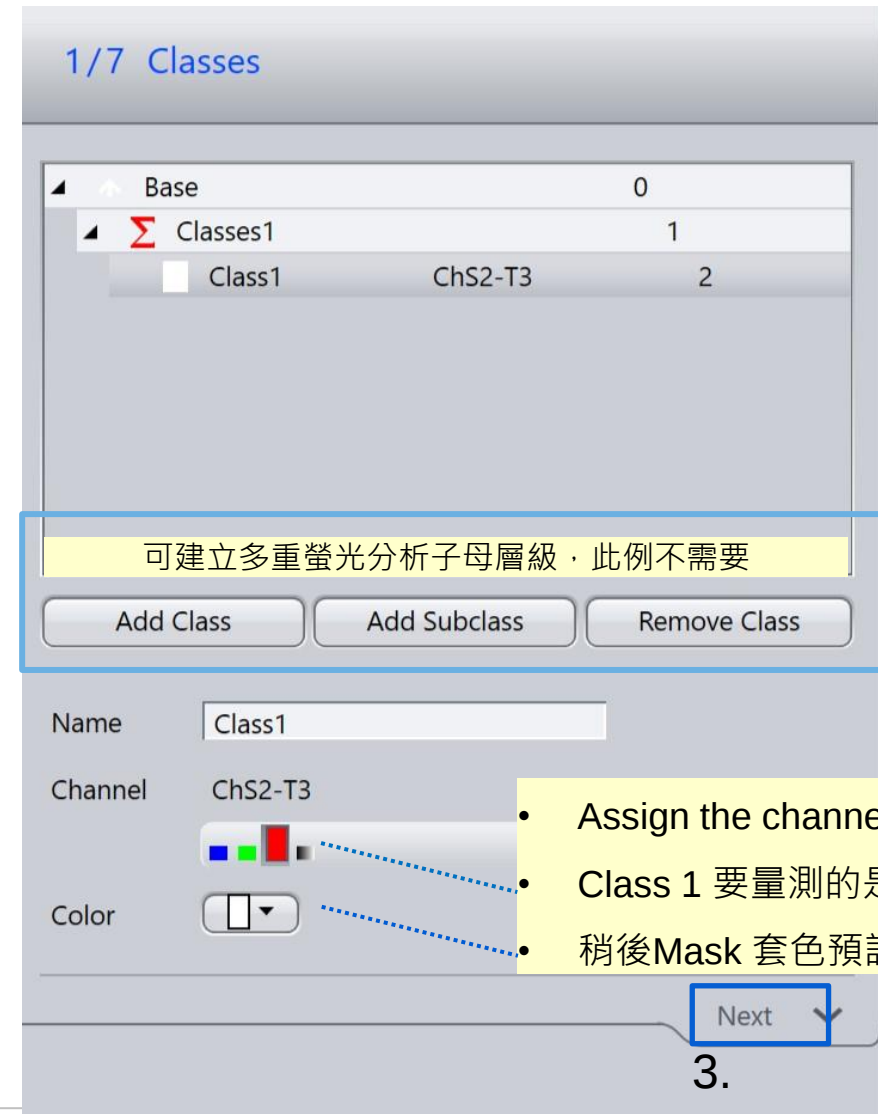




自動量測 Image Analysis Module 1.



以量測前頁影像內的紅色細胞核數目為例



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



2/7 Frame

Back ^

☐ Interactive

☐ Maximize circle

☐ Center circle

Mode Inside Only

Left 0 Top 0

Width 1899 Height 1440

Color

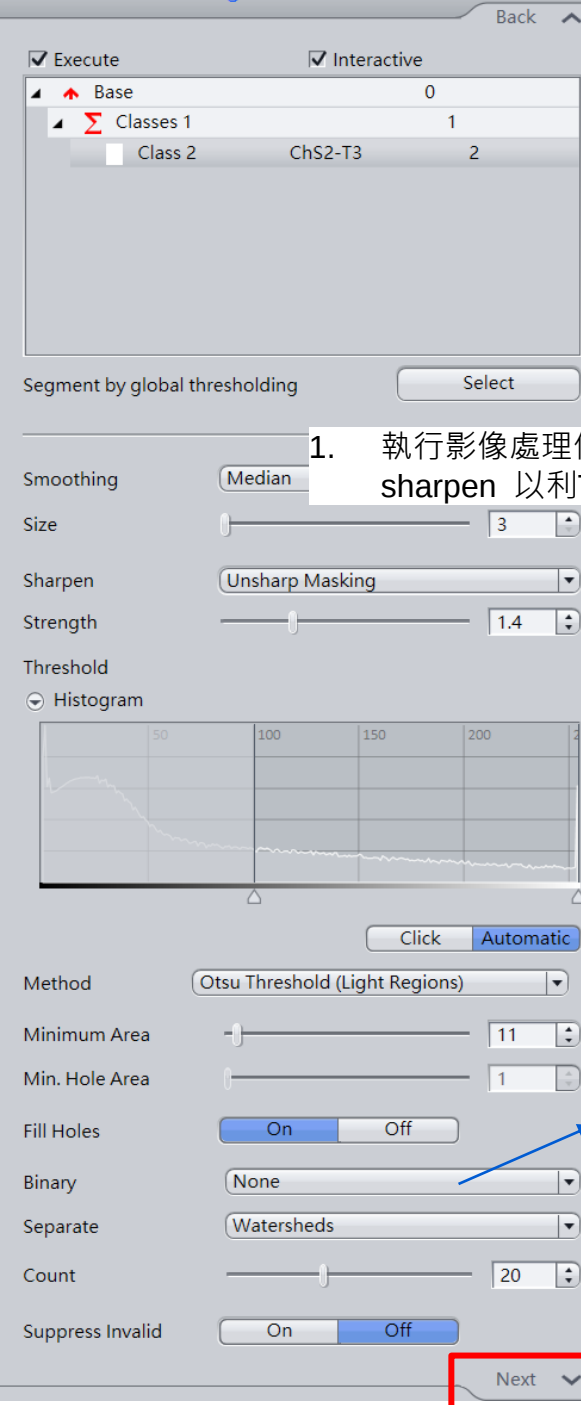
☒ Show frame on analyzed image

Next v

- 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

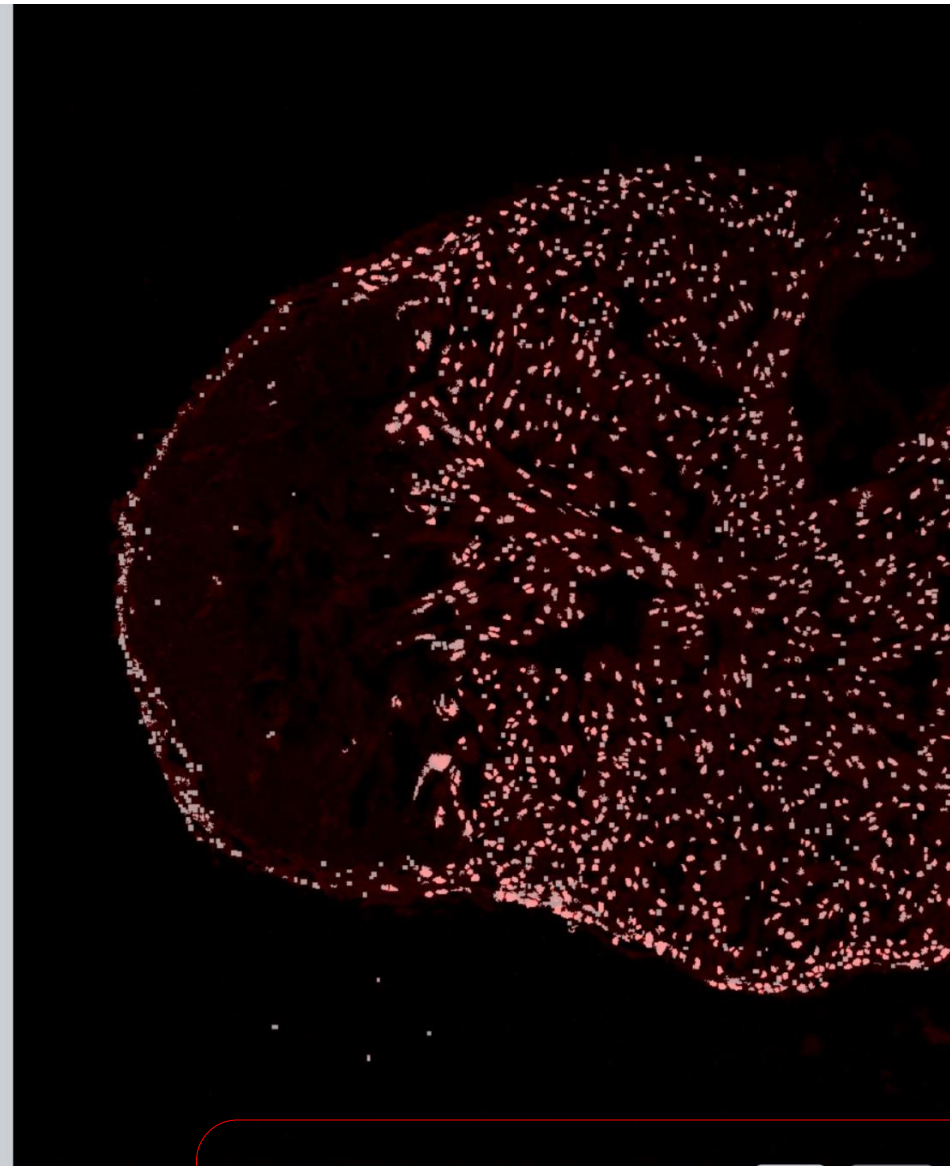
自動量測 Image Analysis Module 3.



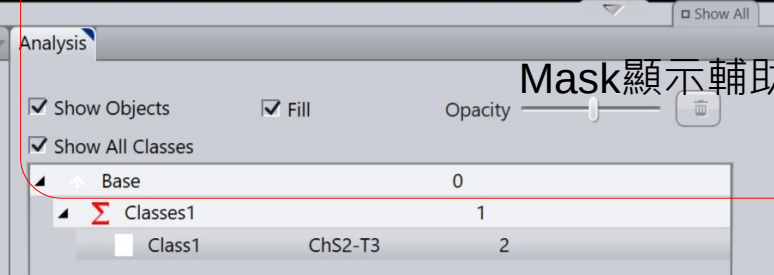
1. 執行影像處理使區域對比增加 smooth & sharpen 以利 Threshold

區域分割 融合 演算

區域切割 搭配 count

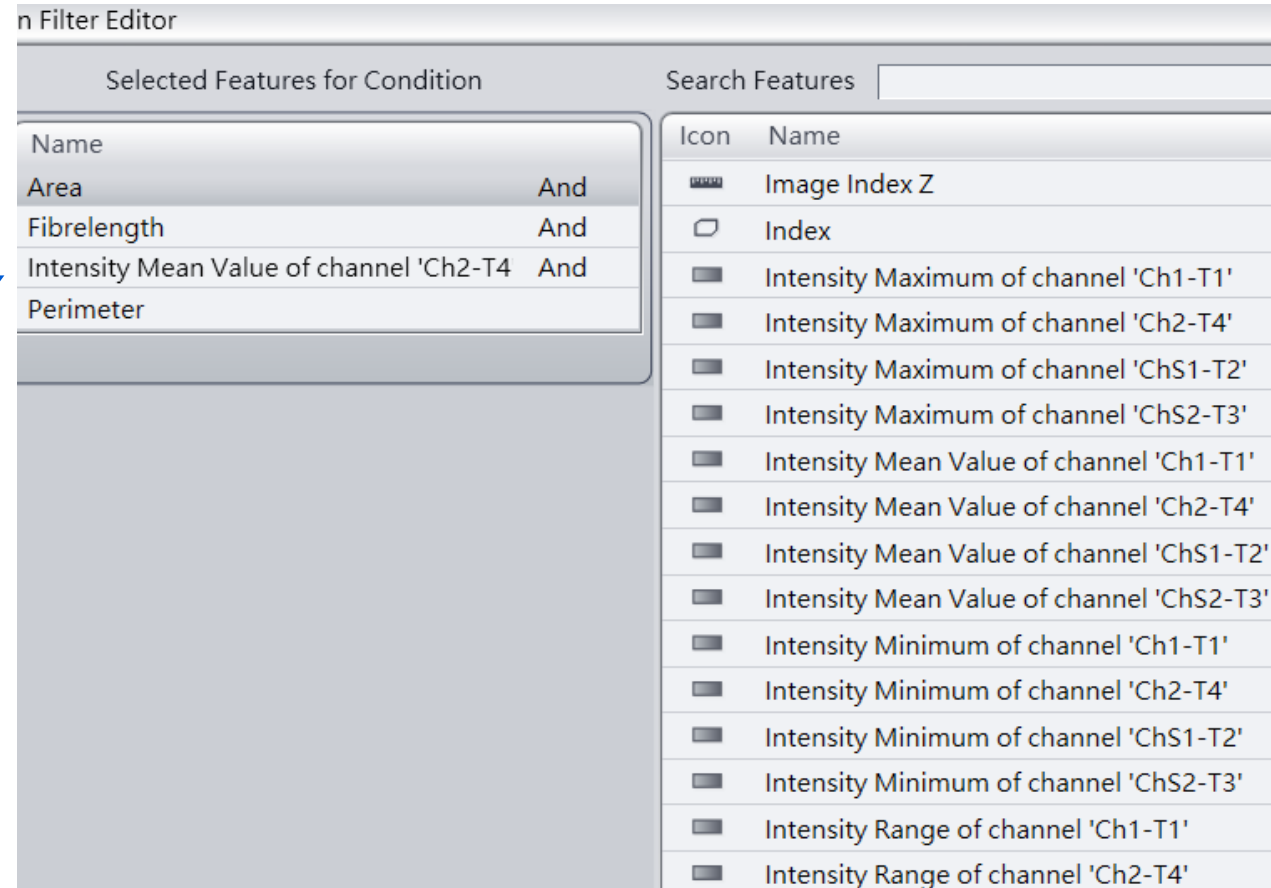
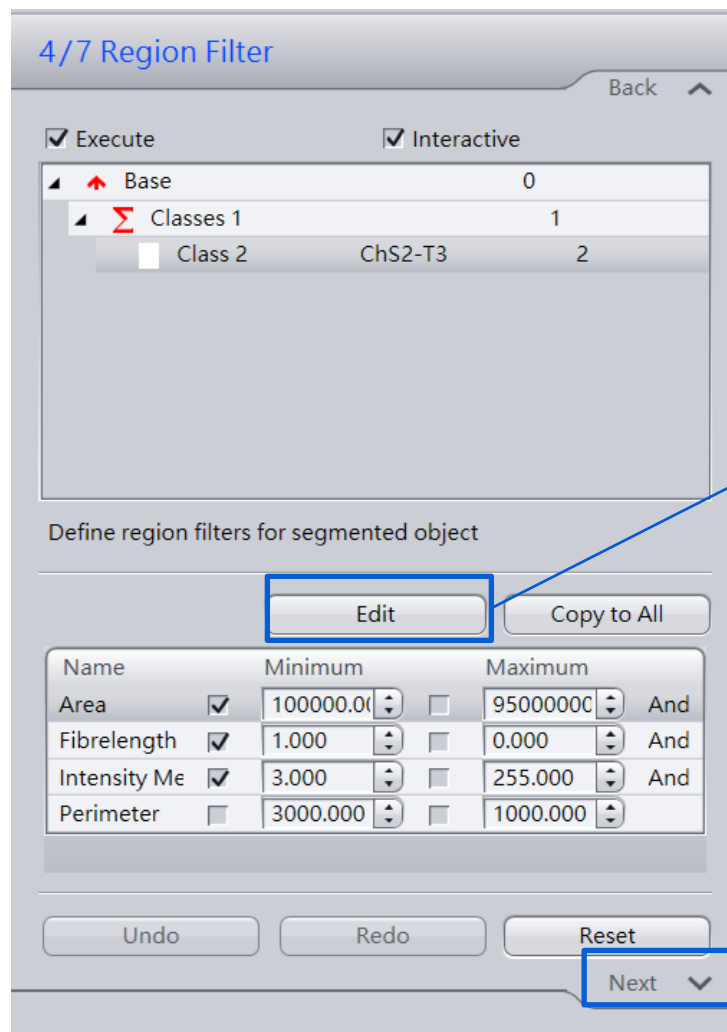


Mask顯示輔助



自動量測 Image Analysis Module 4.

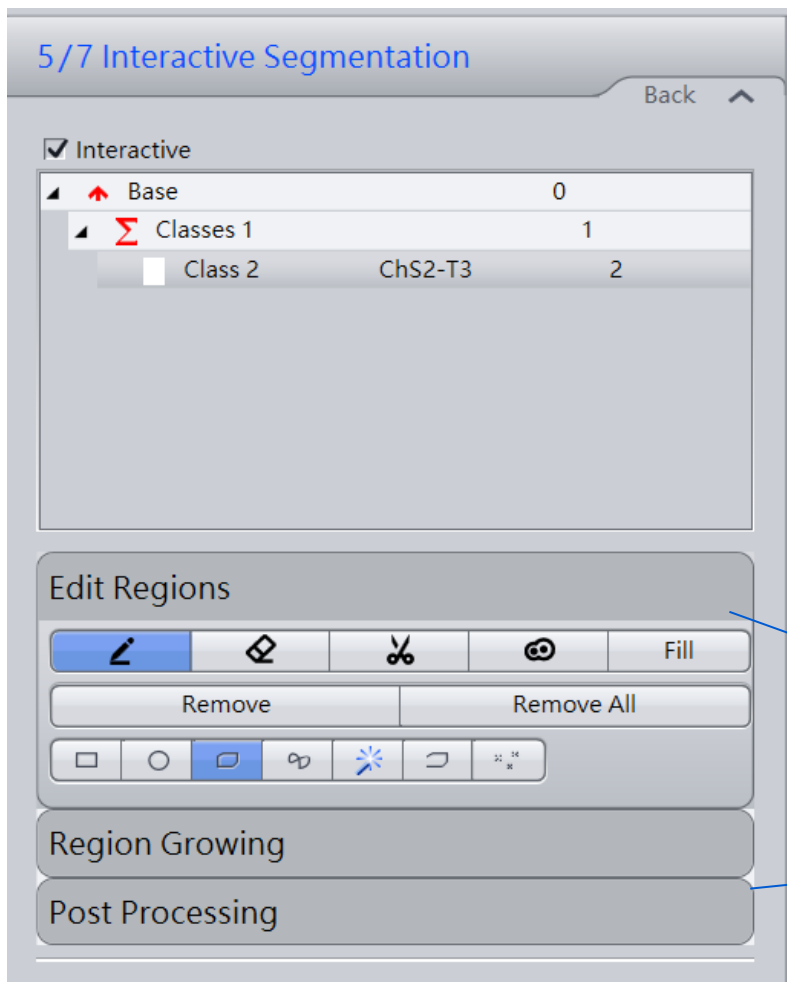
若仍有不需計算的位置，此步驟可進一步篩出需要的位置，依需求加入各式range filter



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

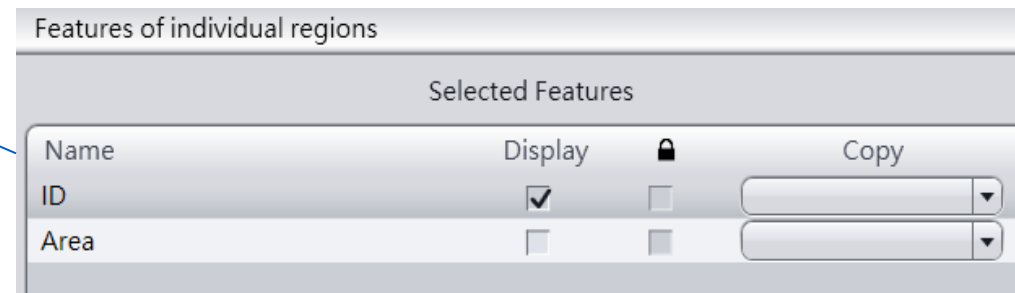
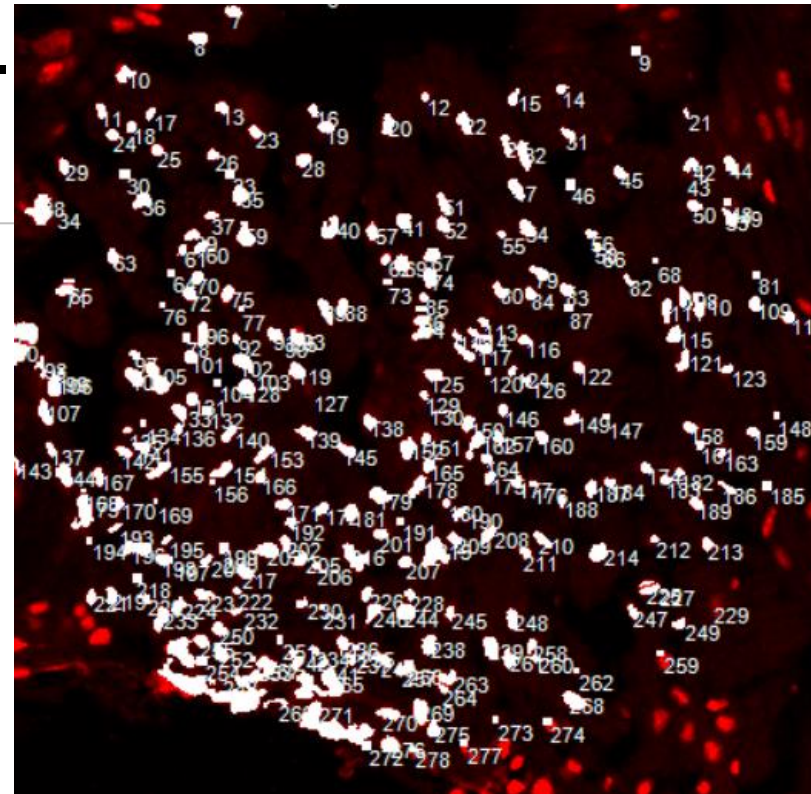
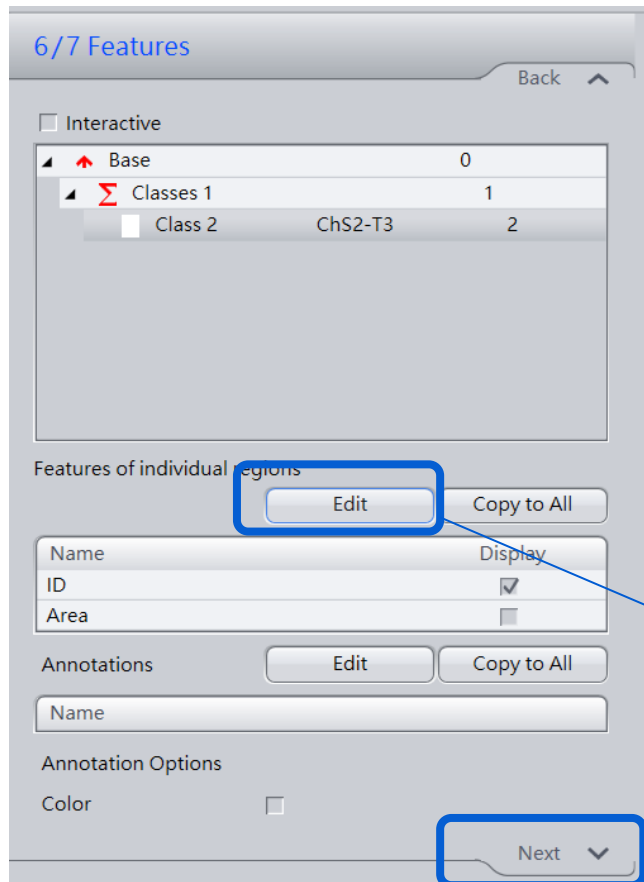
批次處理需求請略過此步



- 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

Back

Base0

Classes 11

Class 2ChS2-T32

Highlight Box

Color

Line Width1

Enable chart

Chart Type

X-AxisID

Y-AxisArea

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

< Back

Next >

Finish

Cancel

Navigator

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

按下，儲存以上各步驟分析設定

- 結果預覽
- 此時尚無法輸出結果

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



Function: Image Analysis Program

Single Batch Apply 5

1

Batch Method Show All

- Change Scaling
- Attach PSF
- ApoTome deconvolution
- ApoTome RAW Convert
- Image Export
- Movie Export
- ZVI Export
- Image Analysis Program 2

Settings

Setting Apply

Base User Documents

- dapi area 2
- dapi area
- Setting
- ZF heart all channel
- ZF heart

4. 選擇分析法

Batch Processing Hide

Use Input Folder as Output Folder Naming...

Copy Parameters Paste Parameters Check All Run Selected

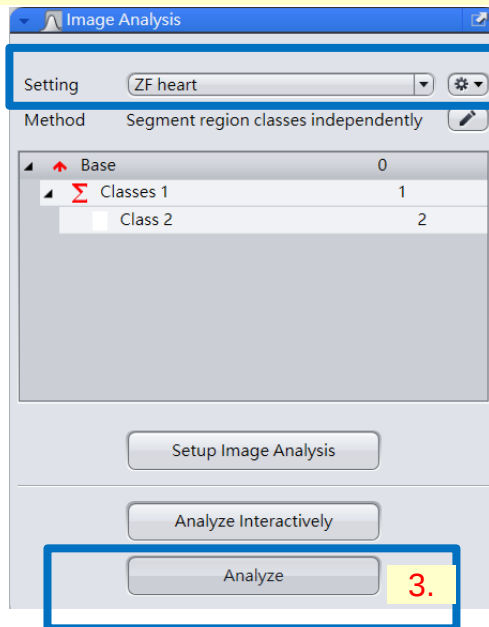
S	Consisten	File Name	Size	Method	Output Name	Output Storage Path
?		E:\00 Temporary image analy...	41.87 MB	Image Analysis Program		E:\00 Temporary image analysis\IBS ZF heart
?		E:\00 Temporary image analy...	41.96 MB	Image Analysis Program		E:\00 Temporary image analysis\IBS ZF heart
?		E:\00 Temporary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

+ Add... Remove Remove All Load List... Save List...

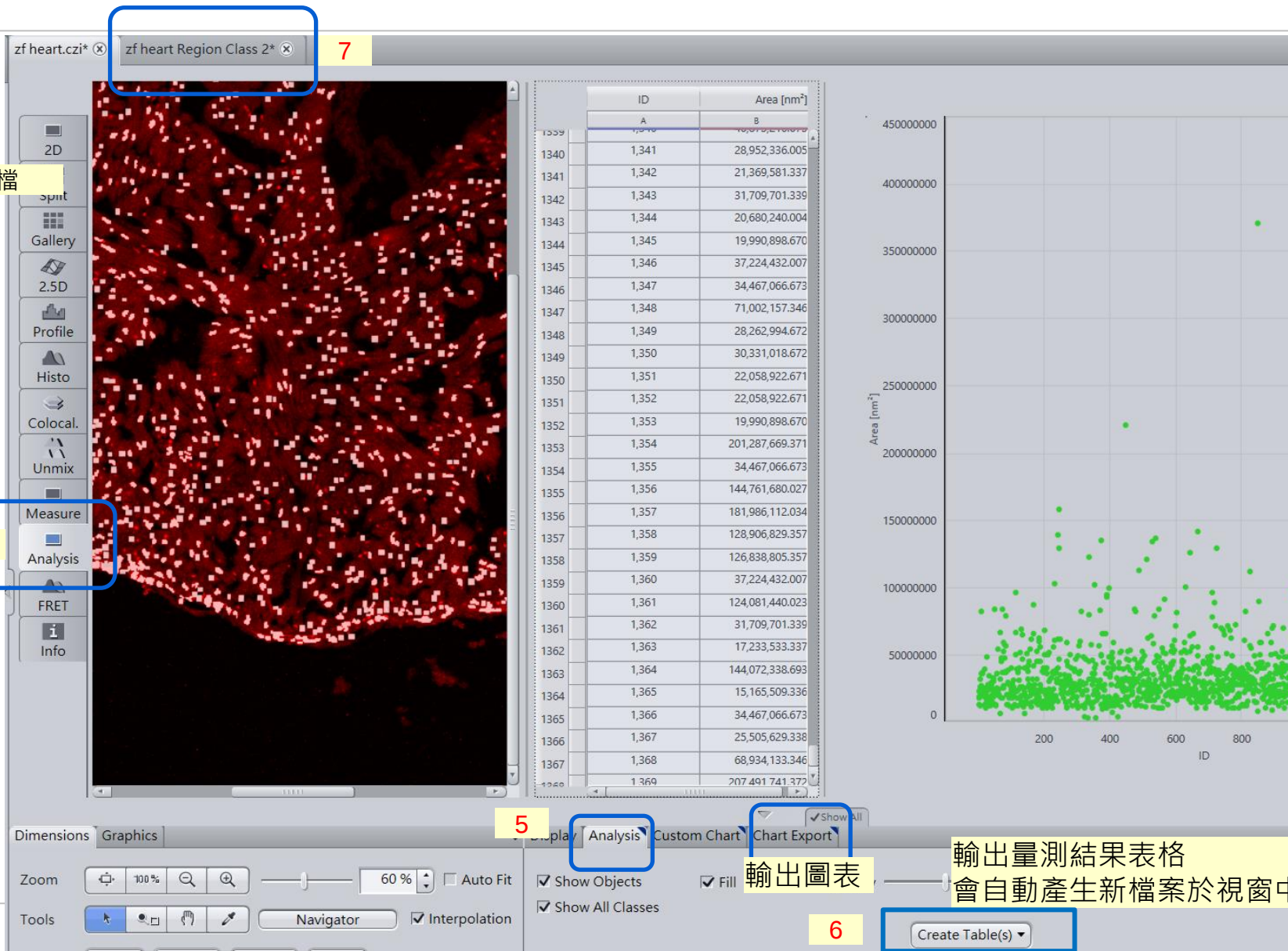
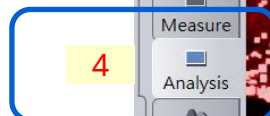
3. 選擇欲分析之圖檔位置

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

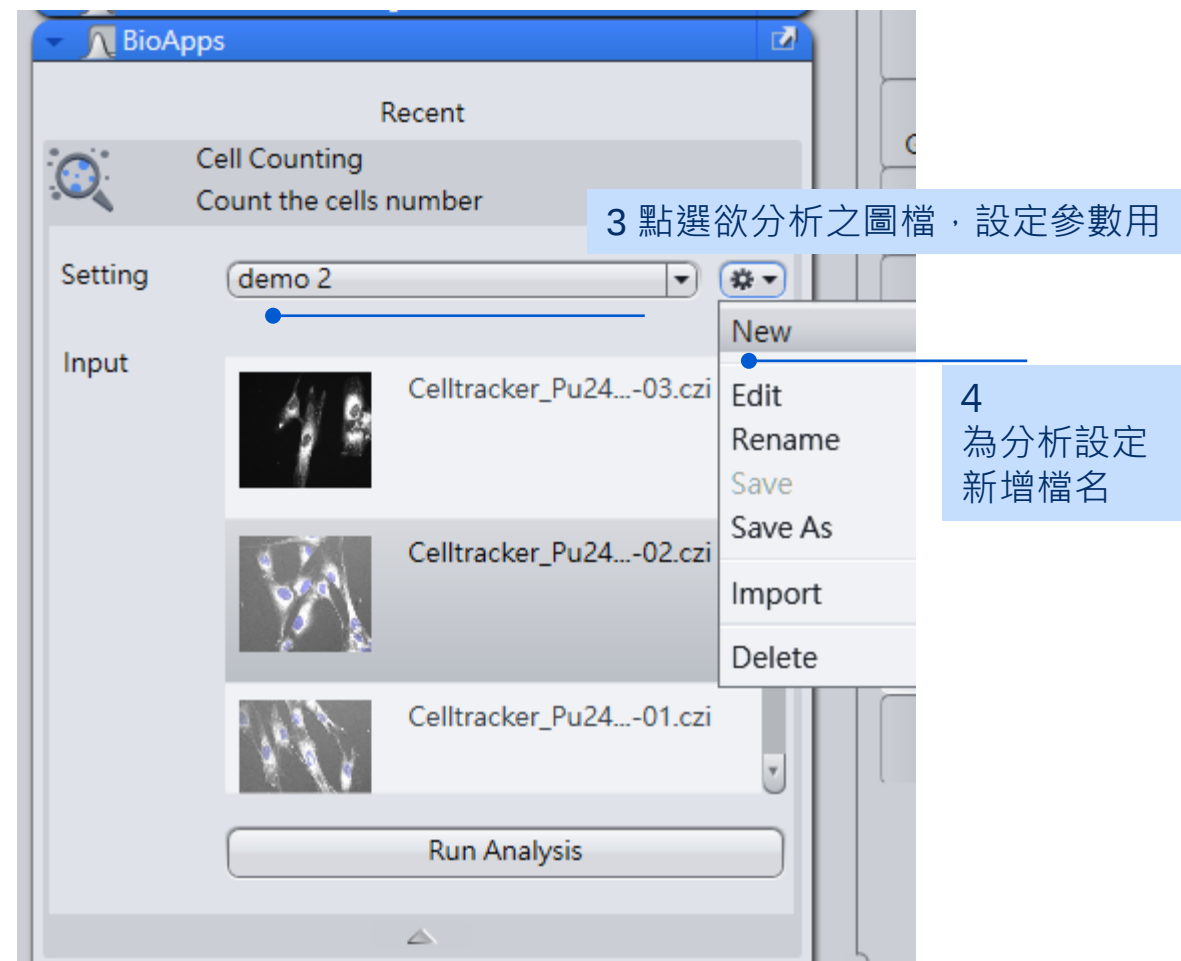
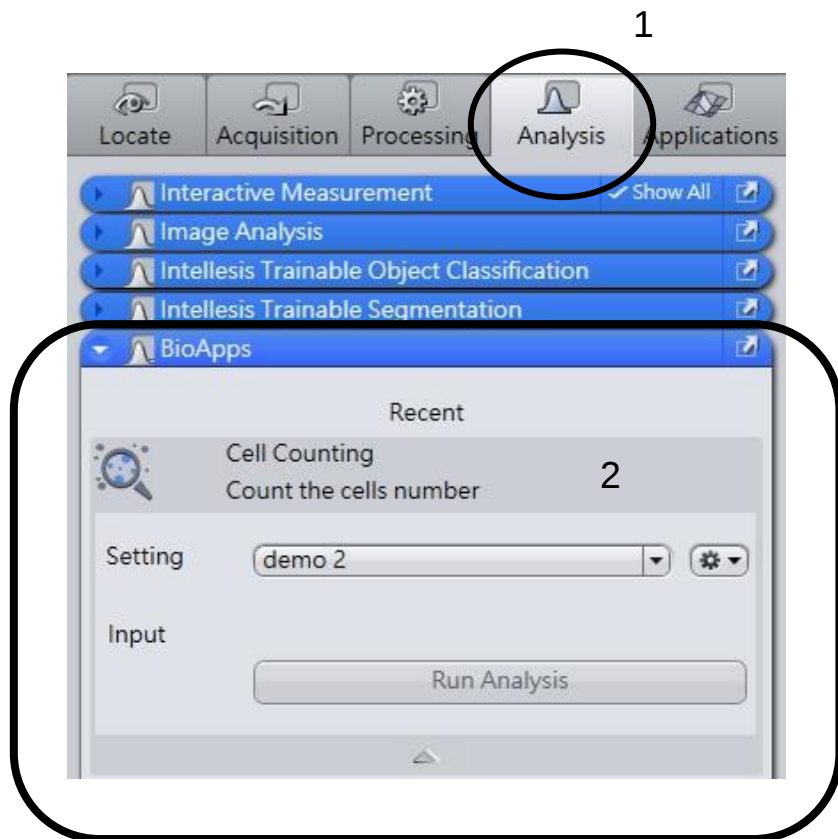


輸出量測結果表格
會自動產生新檔案於視窗中

BioApps- Cell Counting



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



BioApps- Cell Counting 參數設定



Define the cells you would like to count.

Name: Cell

Channel: DAPI

Masking Color: [Red]

Please use a different color than the channel color to make the masked cells visible.

Choose the method for segmentation:

Threshold-based AI-based

Apply Background Subtraction

Threshold: Low High

Area (μm^2): Low High

Circularity: 0.5 1.0

Finish Cancel

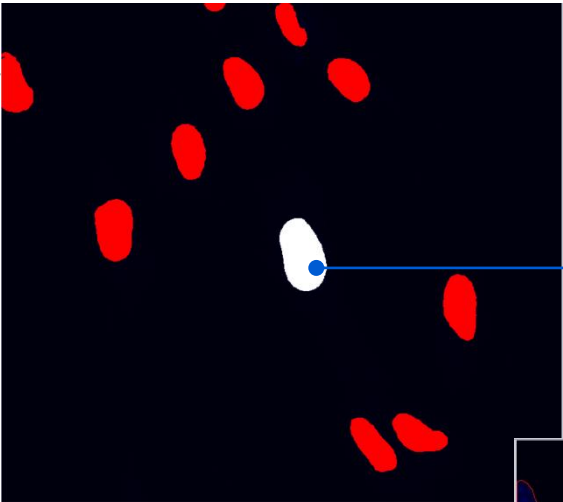
1 選擇欲分析的channel

2 選擇mask顏色

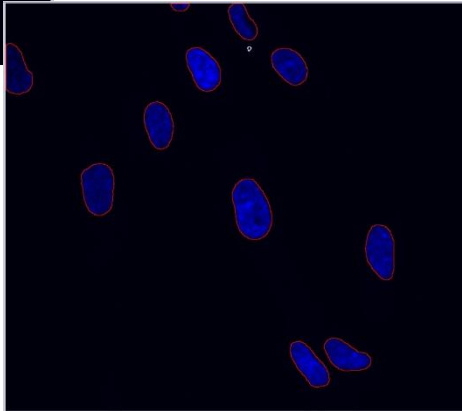
對比好的圖不需勾選

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

Dimensions

Zoom: 100% 89%

Tools: Navigator

Channels: mRF12 mRF12 Dic

Objects Display

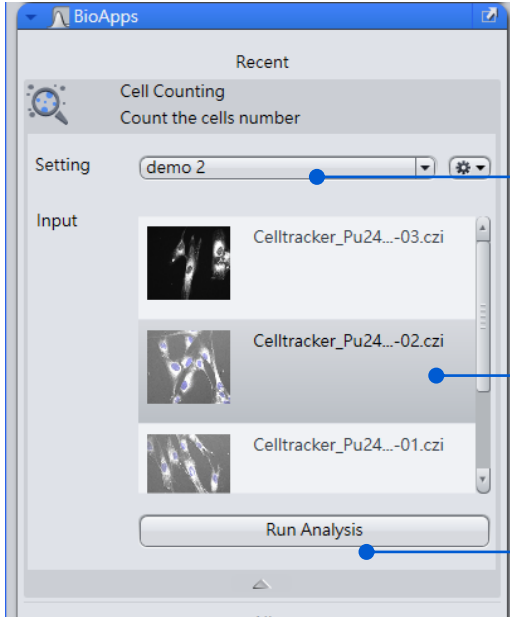
Show Objects

Object display: Fill Outline

Opacity: 1 100%

mask顯示設定

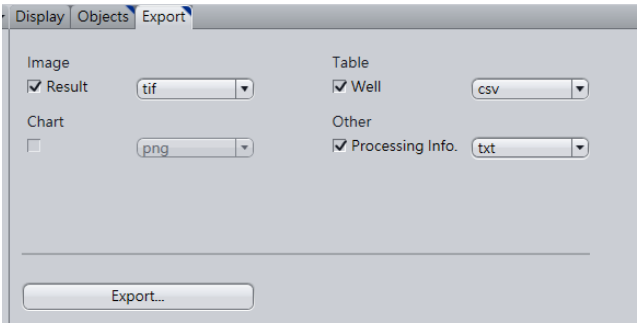
BioApps- Cell Counting 結果呈現與輸出



1. 選擇參數

2. 點選圖檔

3. 執行分析



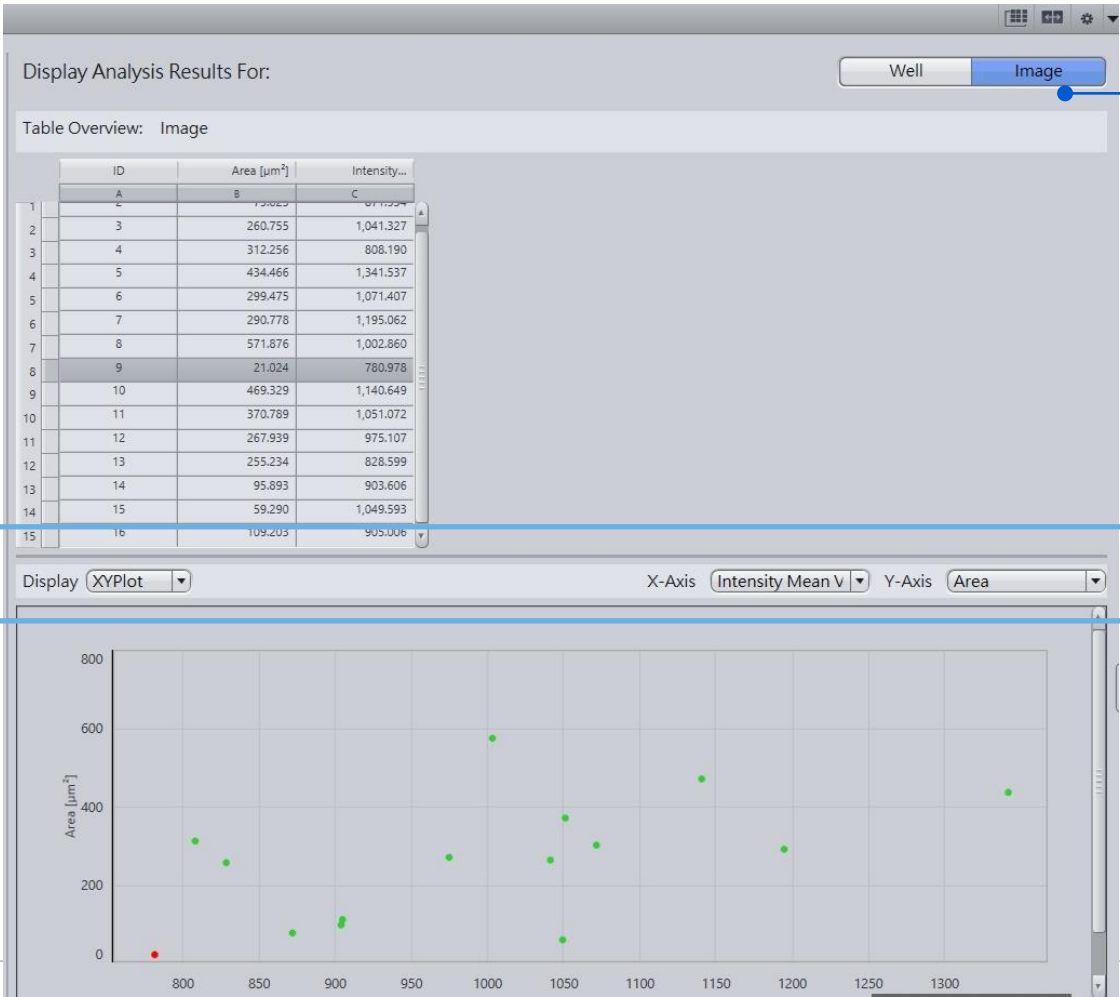
6. 圖/表/參數輸出

Display Analysis Results For: Well

Table Overview: Well

ID	Image...	Count [#]	Count per...	Mean Area...	Area [μm^2]	Intensity...	Intensity Sum...
A	B	C	D	E	F	G	H
1	0	15	267	259.595	3,893.931	1,046.886	53,904,175.000

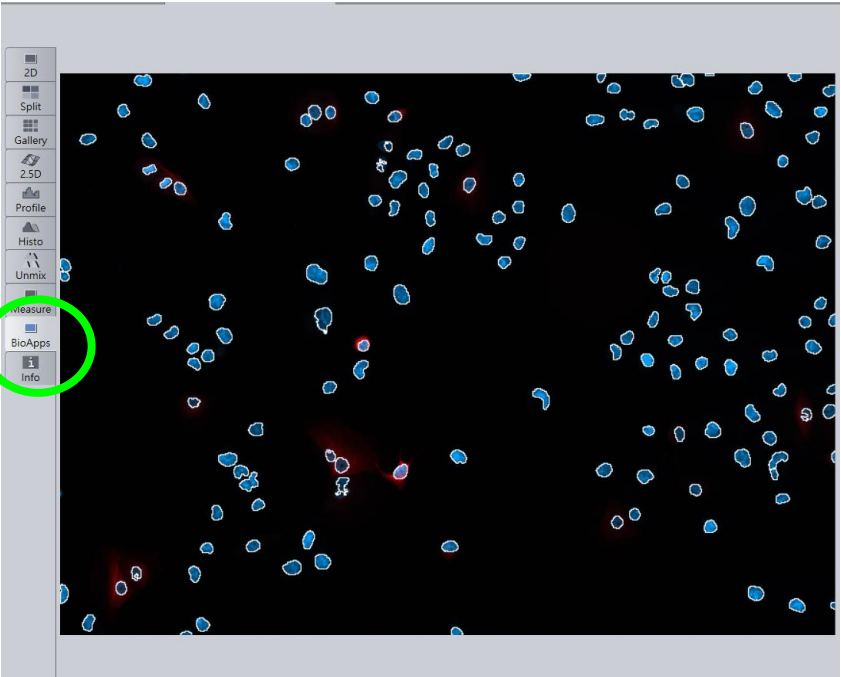
4.1 圖內量測總和



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

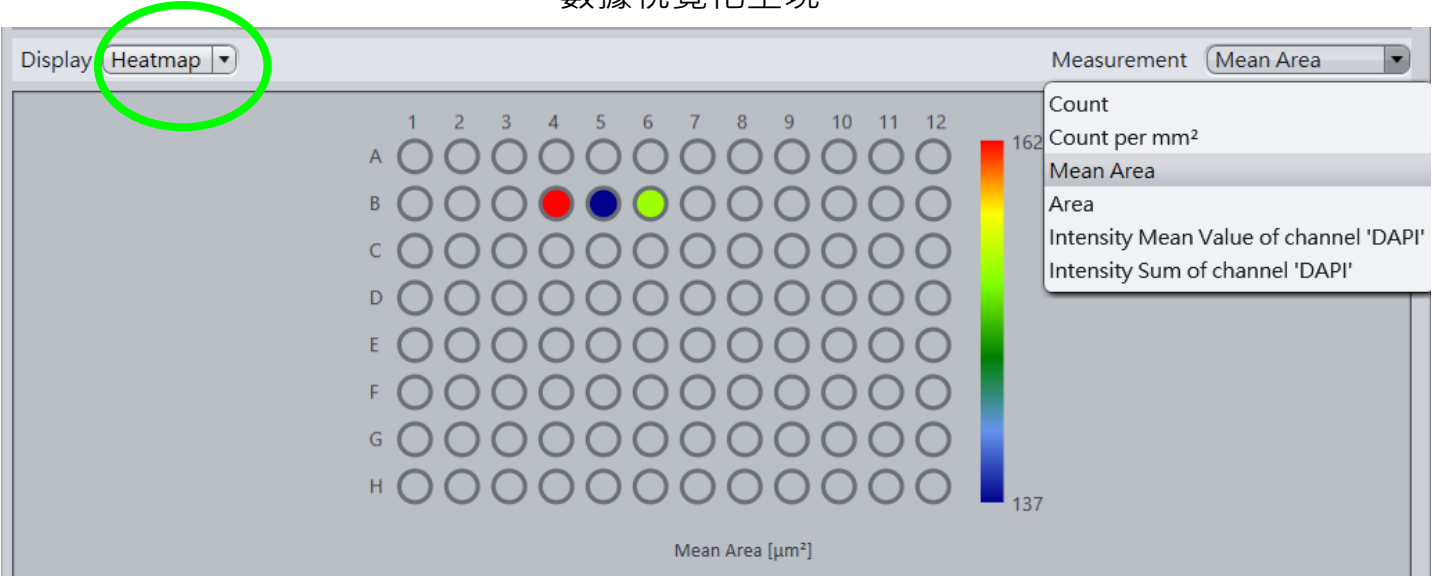


Display Analysis Results For: Well Image

Table Overview: Well

	Name	Image...	Count [#]	Count per...	Mean Area...	Area [μm^2]	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



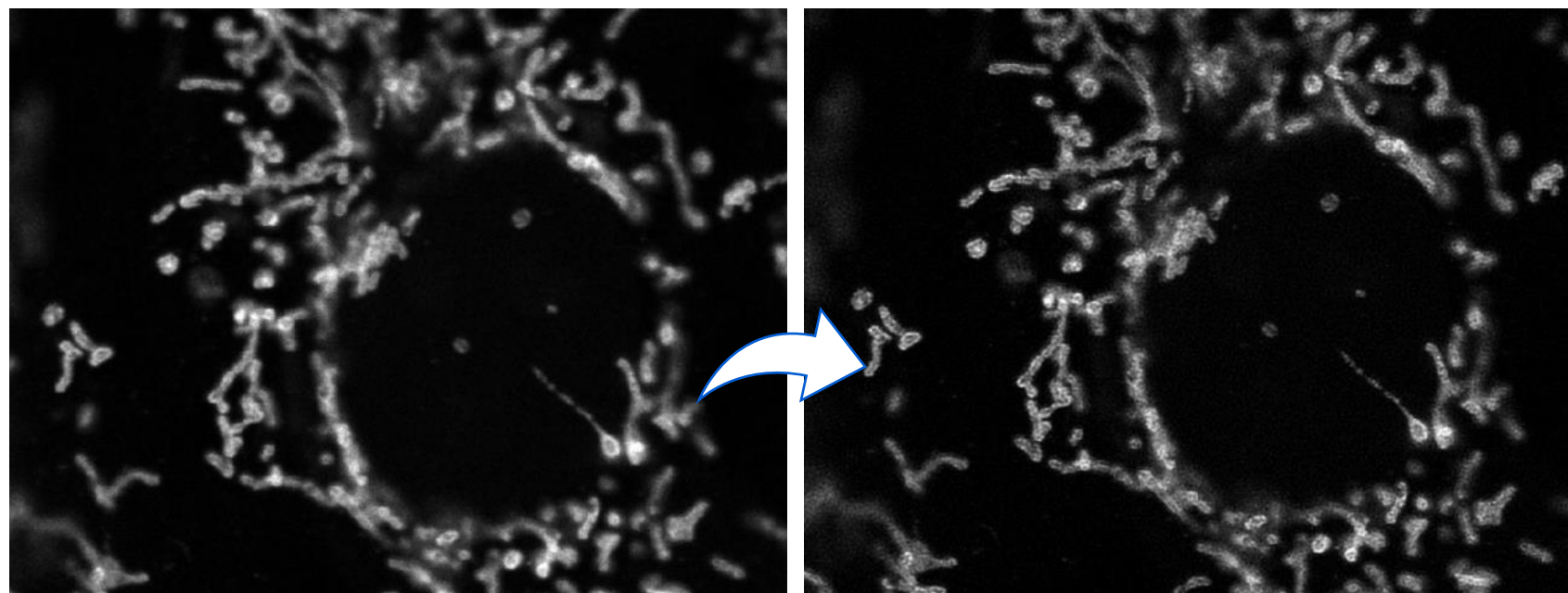
ZEN 3.3 Image Processing

Image Clearing Option 1: 2D DCV (defaults)



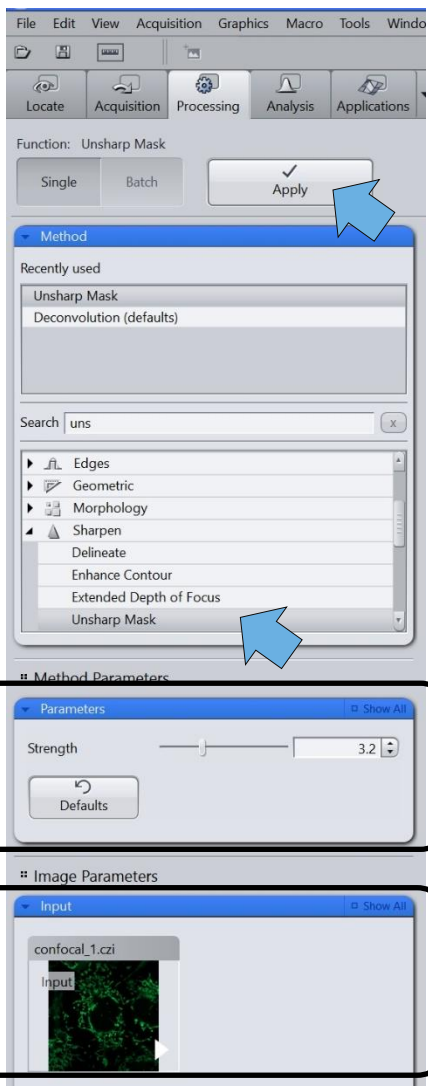
Before

After



ZEN Image Processing

Image Clearing Option 2: Unsharp masking

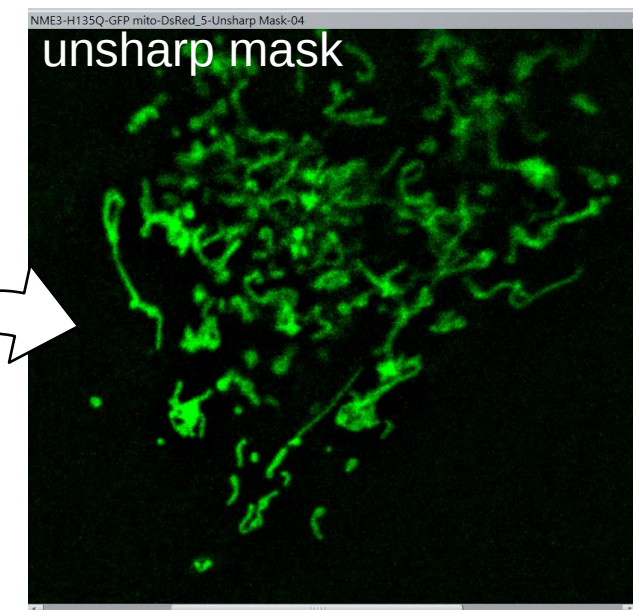
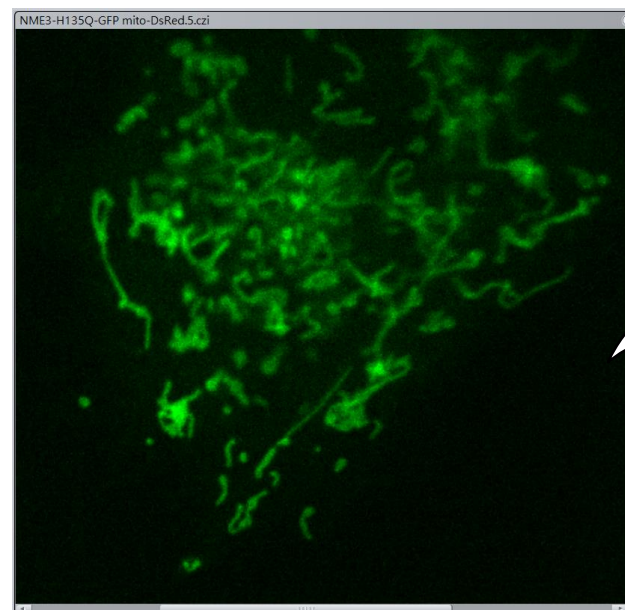
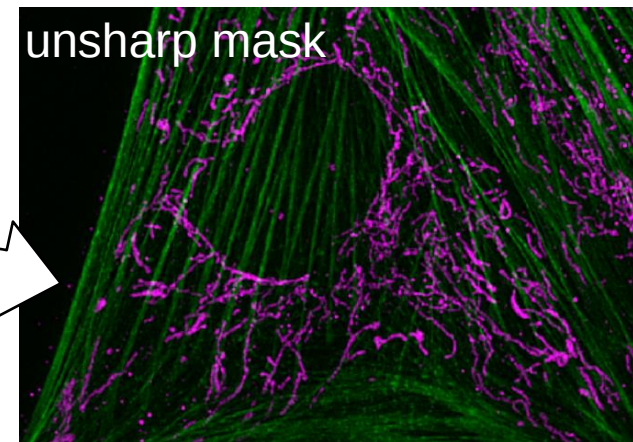
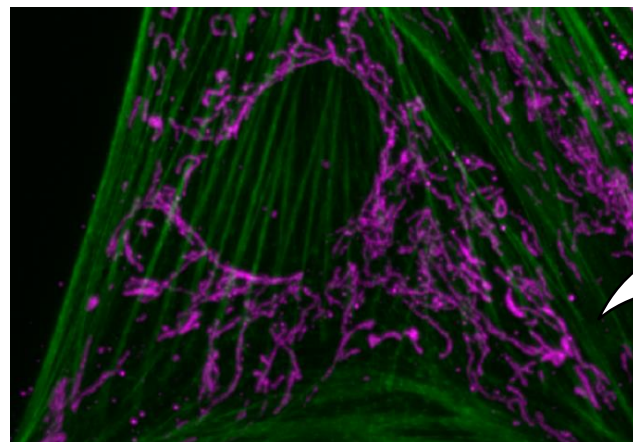


4

1

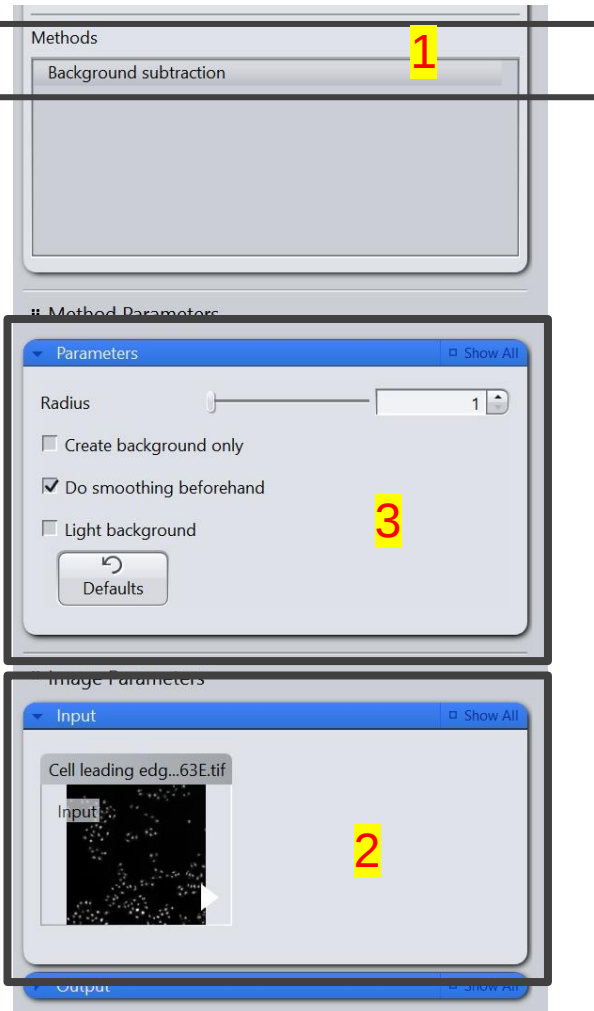
3

2



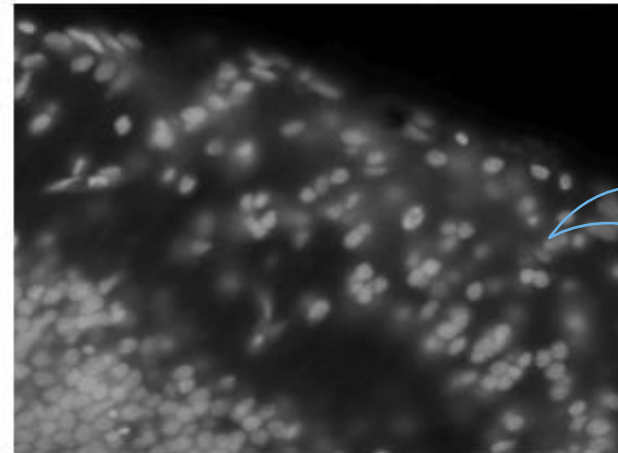
ZEN 3.3 Image Processing

Image Clearing Option 3: Background subtraction

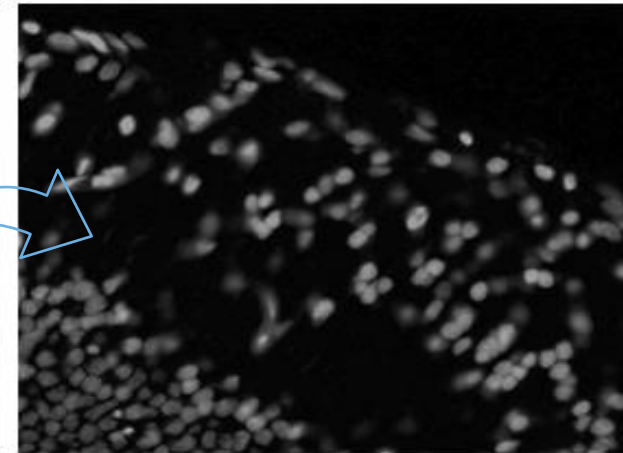


Single
Plane

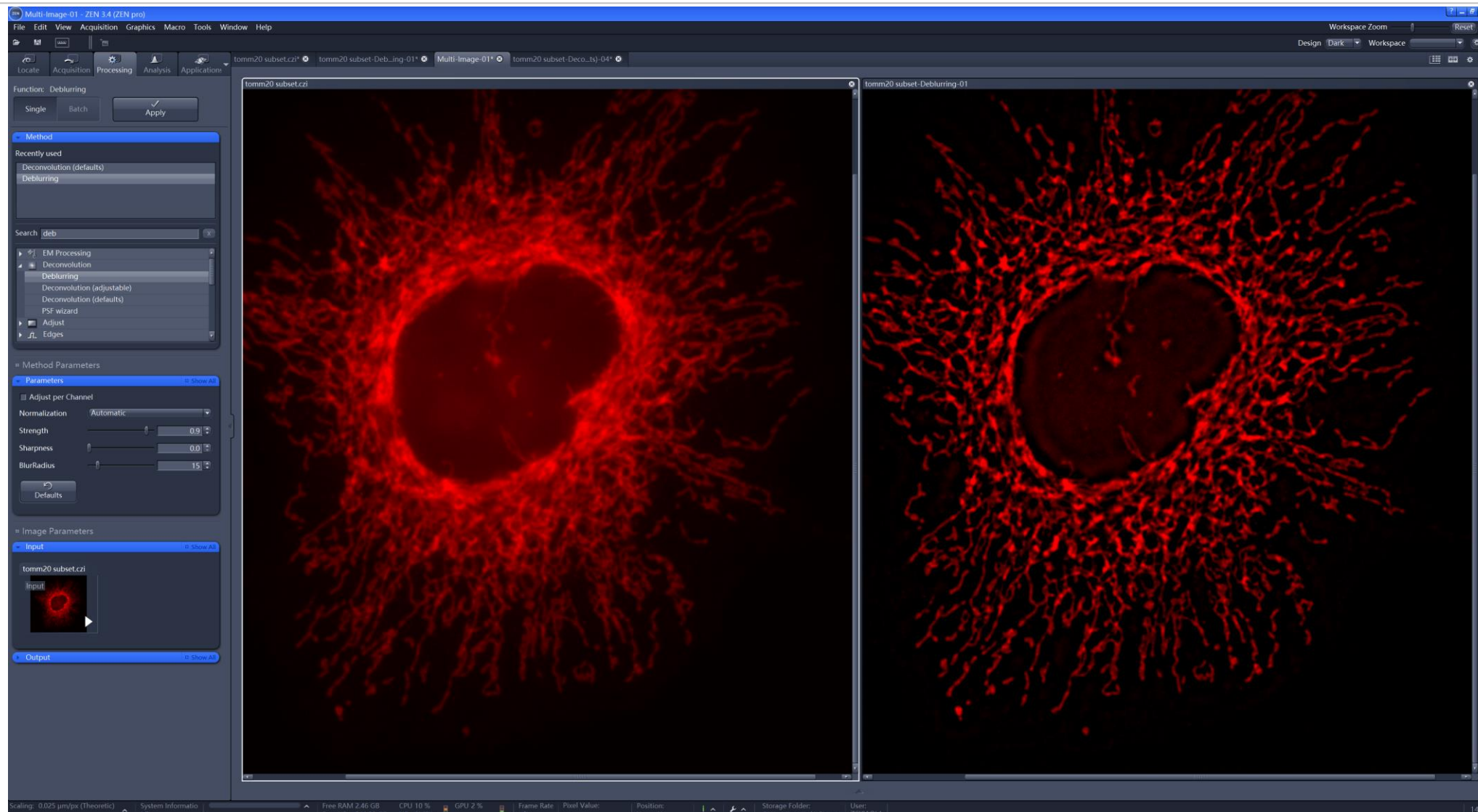
Widefield (Original)



Background Subtraction

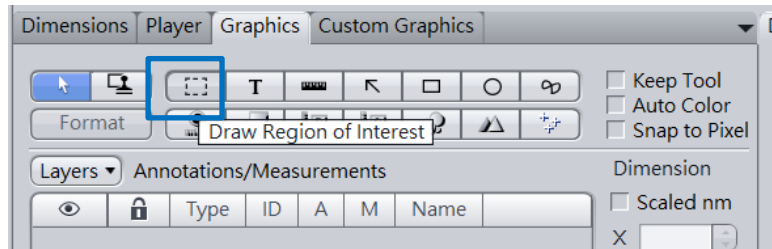


Option 4: ZEN 3.4 WF vs Deblurring/2D DCV

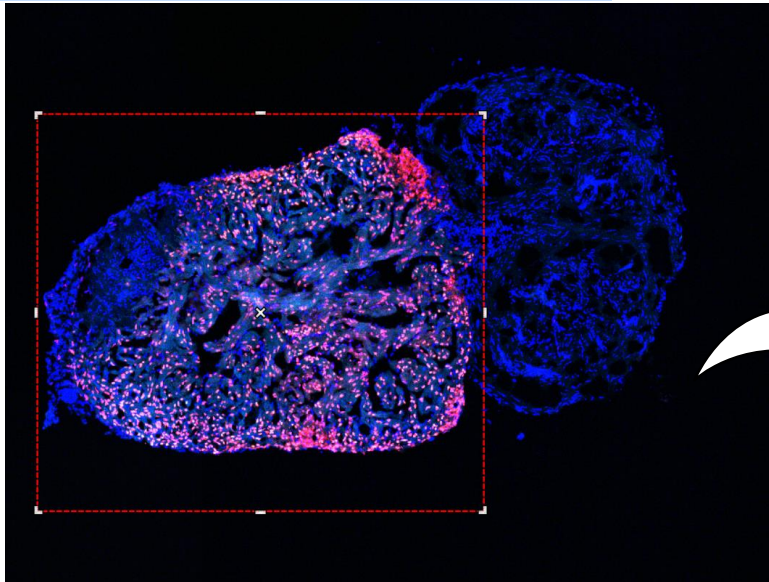


裁圖，複製選取範圍影像

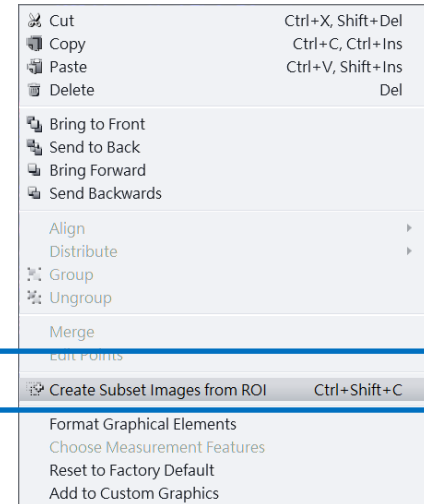
1.



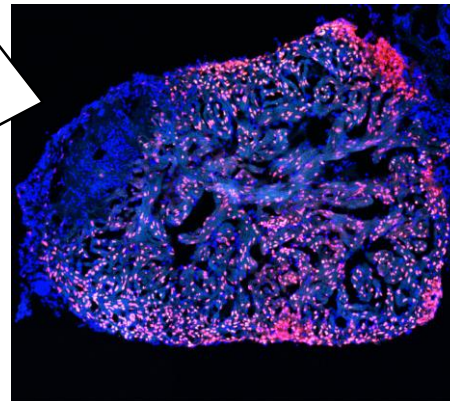
2. Draw an area and mouse right click



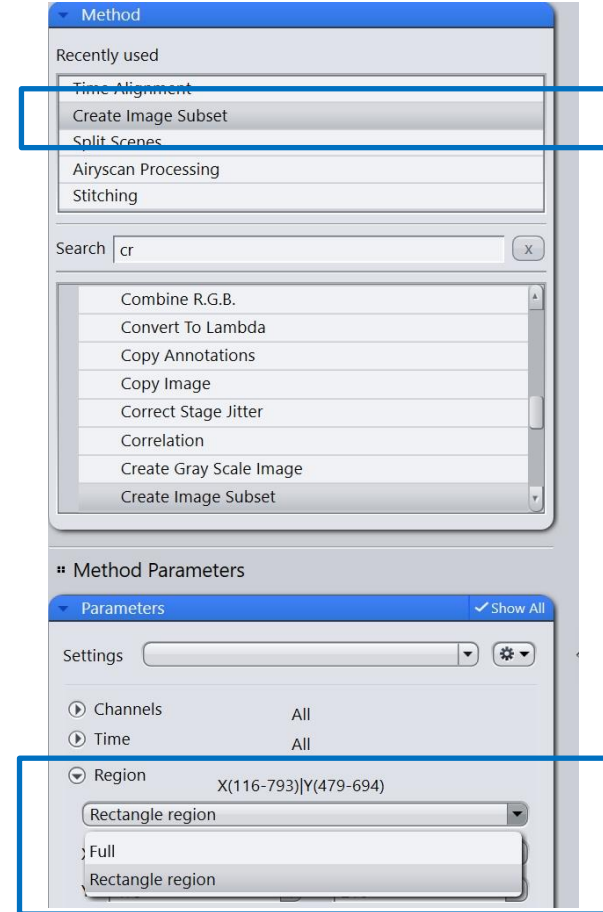
3. Create Subset Images from ROI



4. 獲得裁切範圍於新視窗



3. 或者
Processing → Create Image Subset



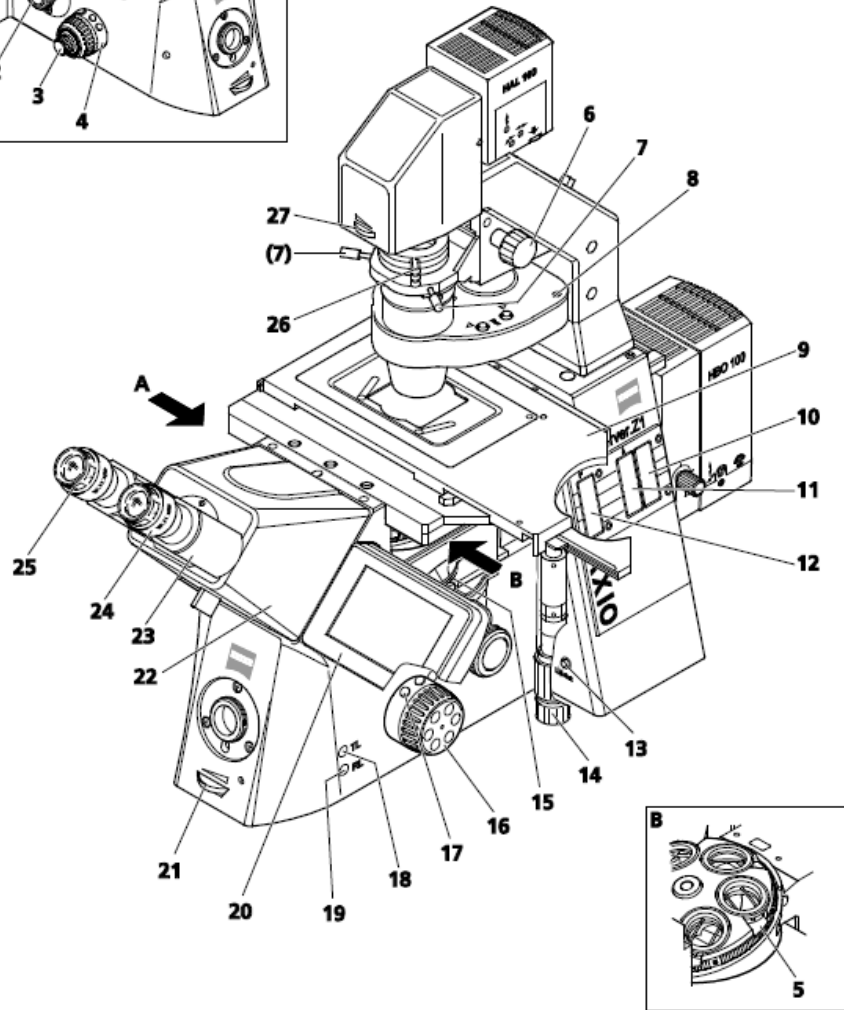
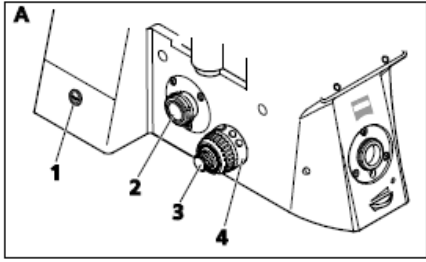


Fig. 4-3 Axio Observer.Z1 components and controls (motorized)

- 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

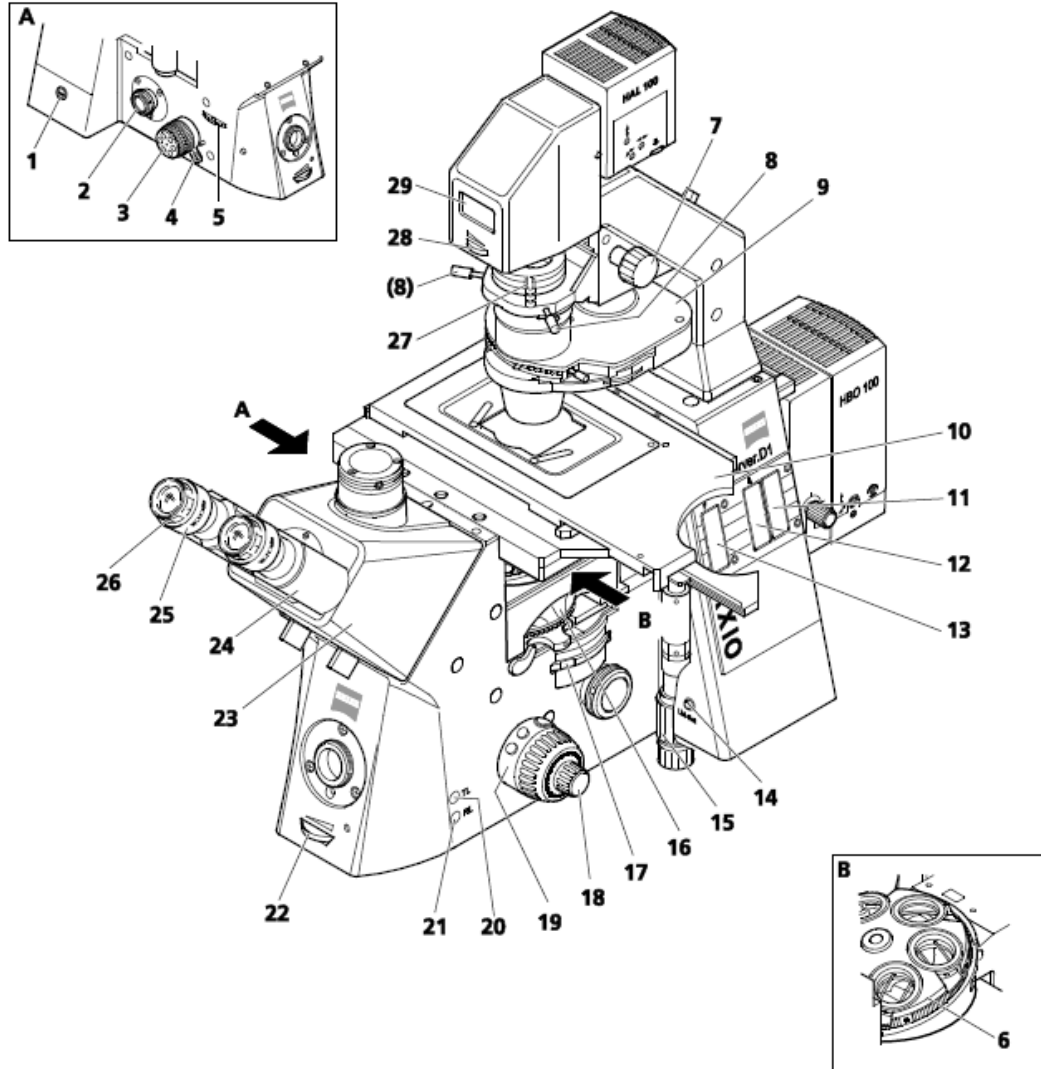


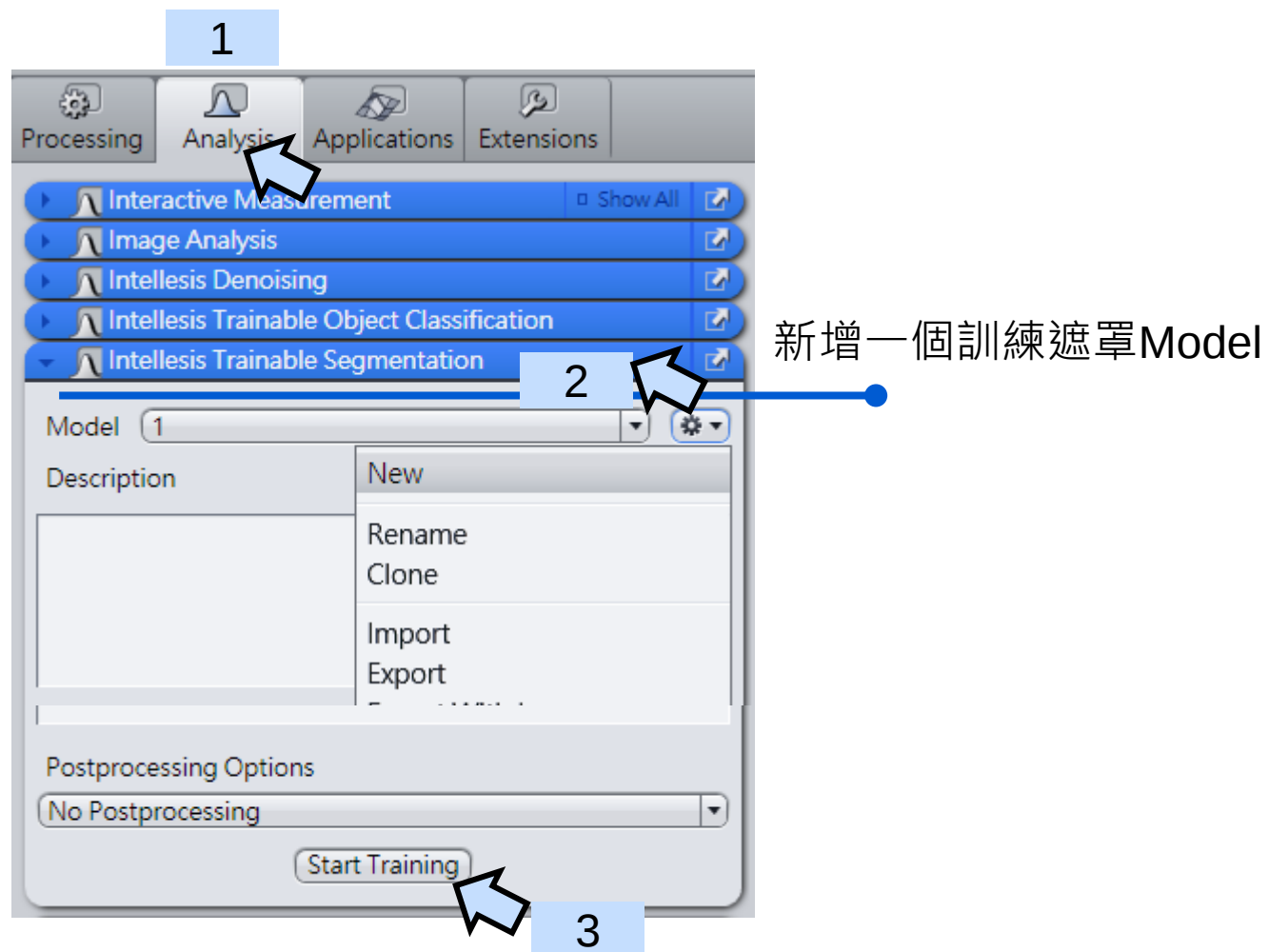
Fig. 4-2 Axio Observer.D1 components and controls (coded, semi-motorized)

- 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

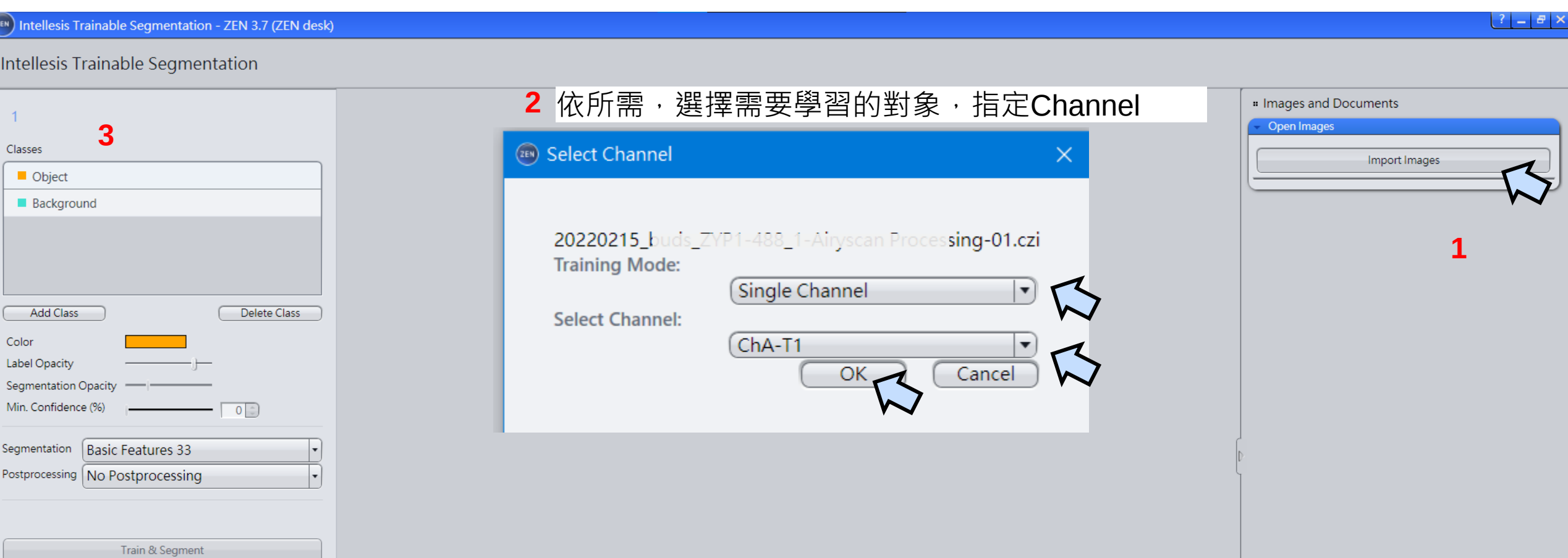
Intellesis Trainable Segmentation

- 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.



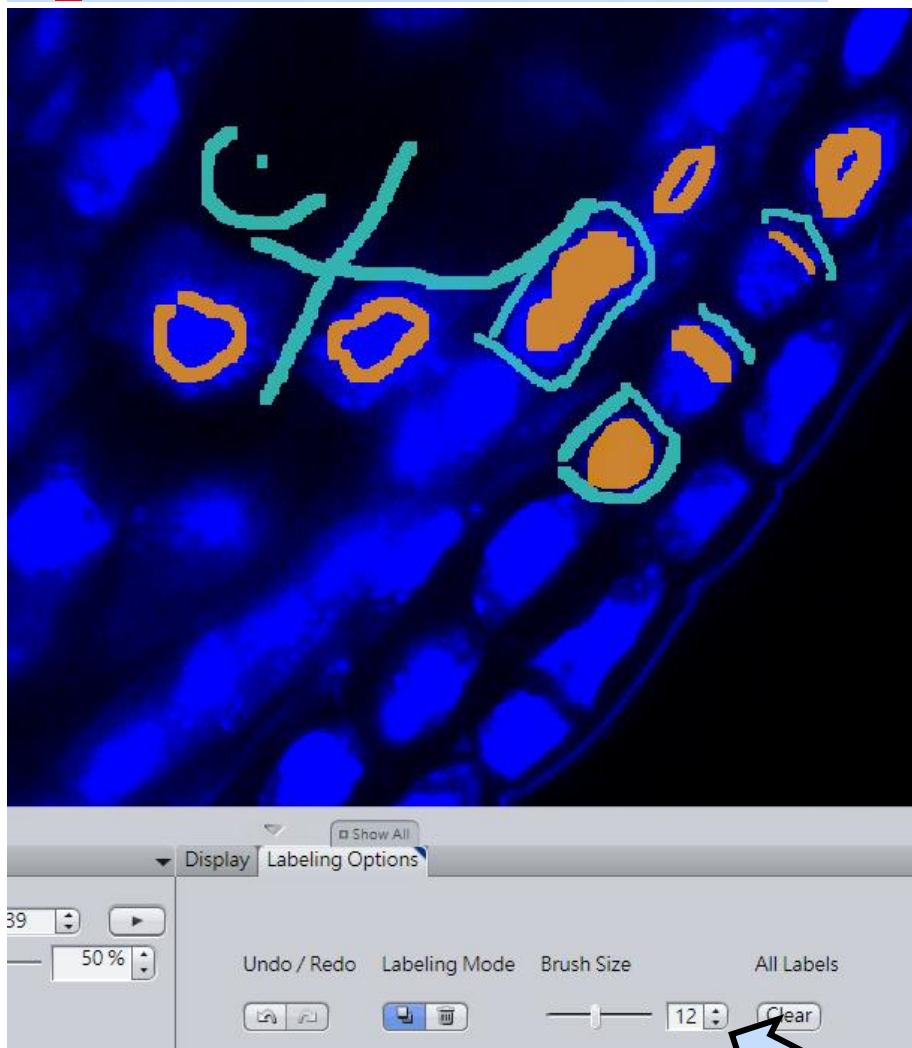
Intellesis Trainable Segmentation Setups 2.



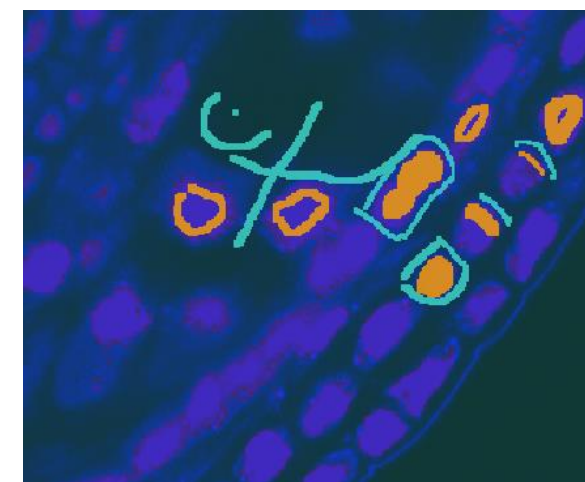
Intellesis Trainable Segmentation setups 3.

Use brush tool , Assign some pixel example as Object and Background

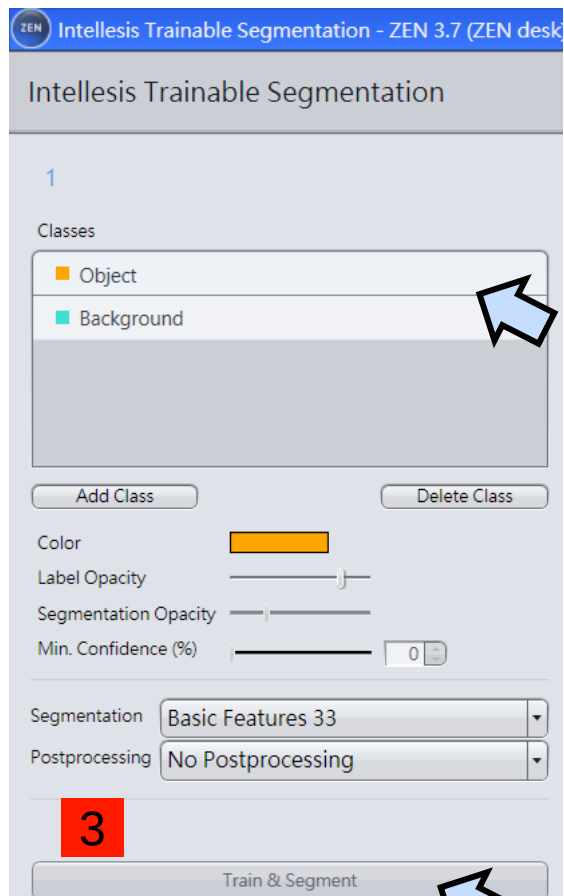
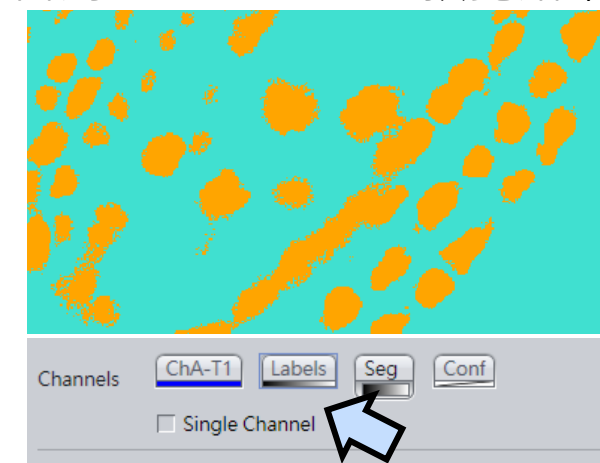
2 使用工具筆刷塗抹於物件與背景



4 預覽結果



善用channel on /off預覽結果



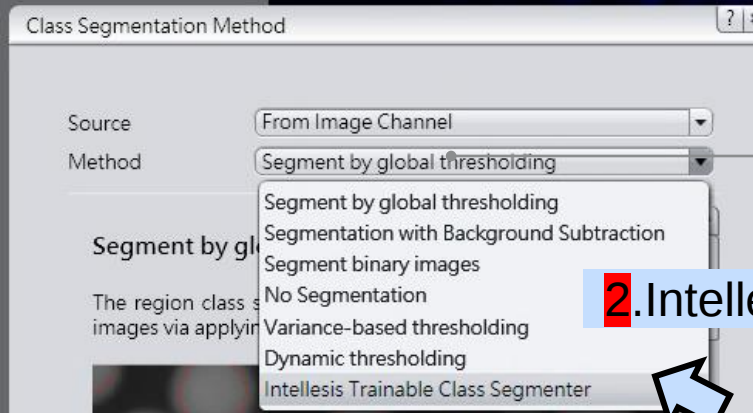
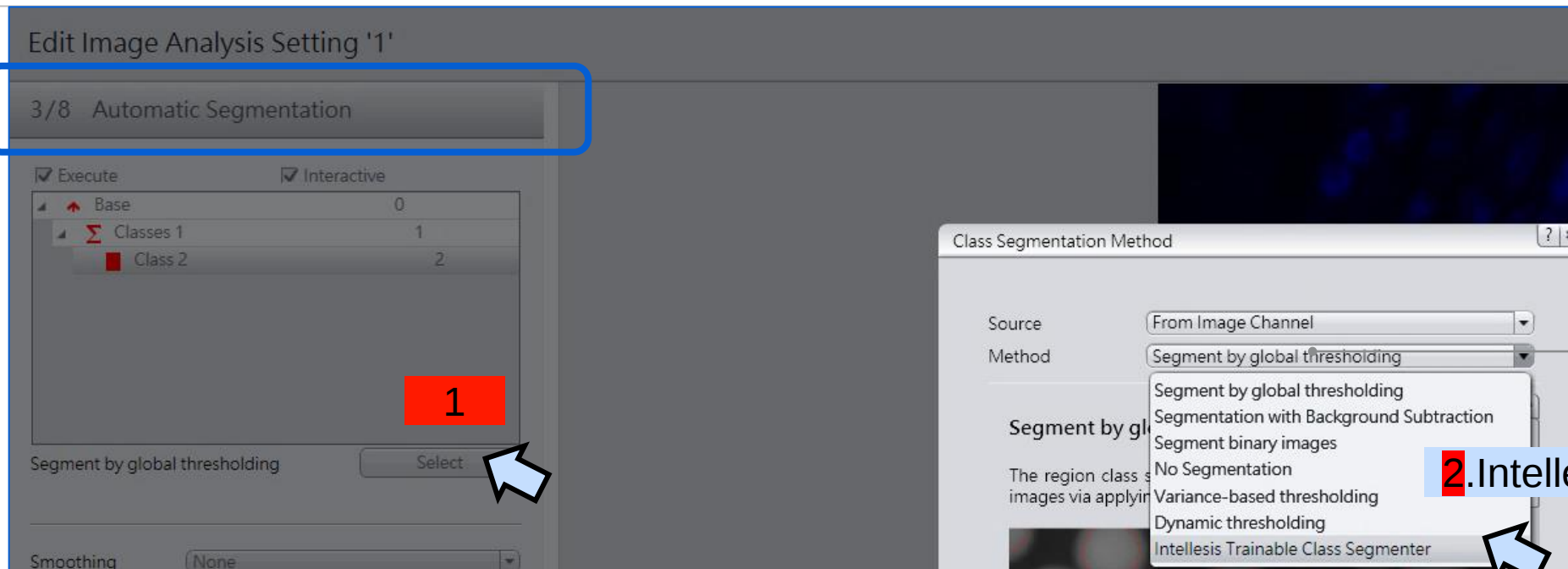
5



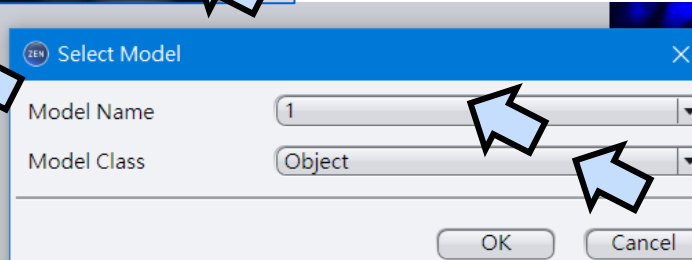
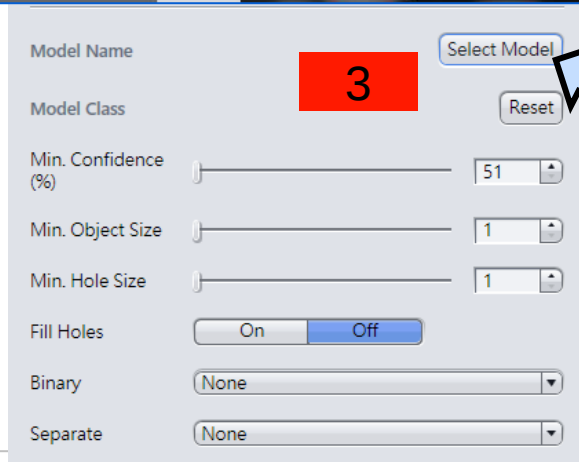
改變筆刷粗細

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



Cell Counting

Define the cells you would like...



Name

Channel ChA-T1 

Masking Color 

 Please use a different color than the channel color to make the masked cells visible.

Segm. Method ☐ Automatic ☒ AI-based ☐ Manual

AI Model

Model Class  1

Min. Confidence (%)

1

2. 把先前訓練好的model套用，
作為遮罩法

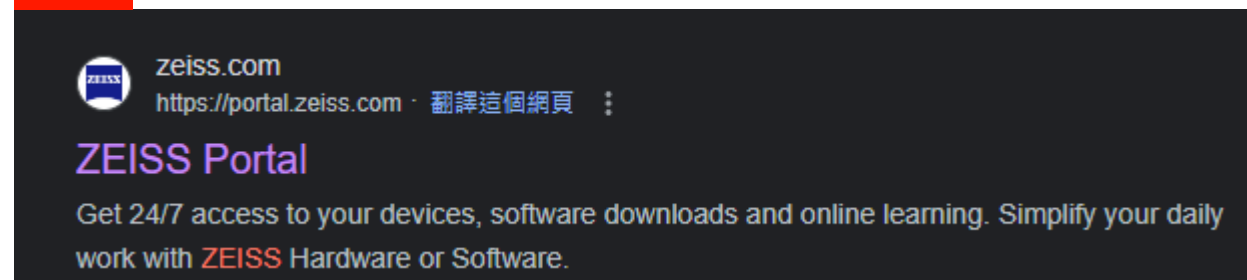
下載離線軟體 ZEN lite 1



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



1

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https://portal.zeiss.com

ZEISS Portal

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提供各种服务以简化您的蔡司系统（机器和软件）的日常工作。它不断进行改进，以更好地满足您的需求和要求。立即注册成为数字化蔡司社区的一员吧！

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蔡司质量卓越中心查询工具

My Voice

ART Cloud Demo

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Field of Work
Microscopy

Search for software name, version or file type...

Search examples: "ZEN core 3.0 full version", "LSM 700", "Axiovision hotfix"...

3

ZEISS Microscopy Software

Latest version: 1.7

Tool for installation and maintenance of ZEISS Microscopy Software products (ZEN blue, ZEN core and ZEN Data Storage)

Show all files

ZEN / ZEN lite

Latest version: 3.8

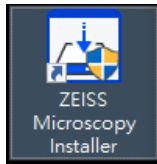
Digital Imaging Suite for light and confocal microscopy

Show all files

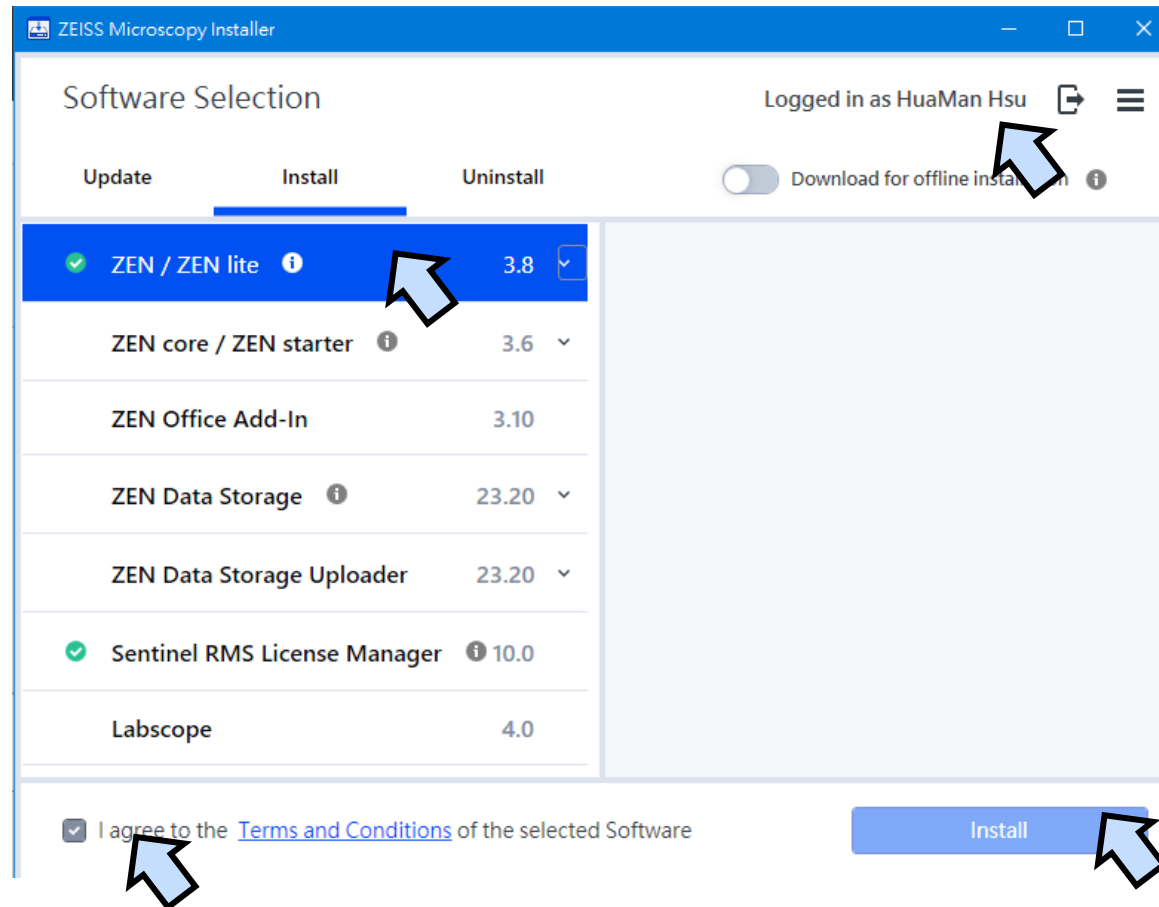
下載離線軟體 ZEN lite 3



1



2

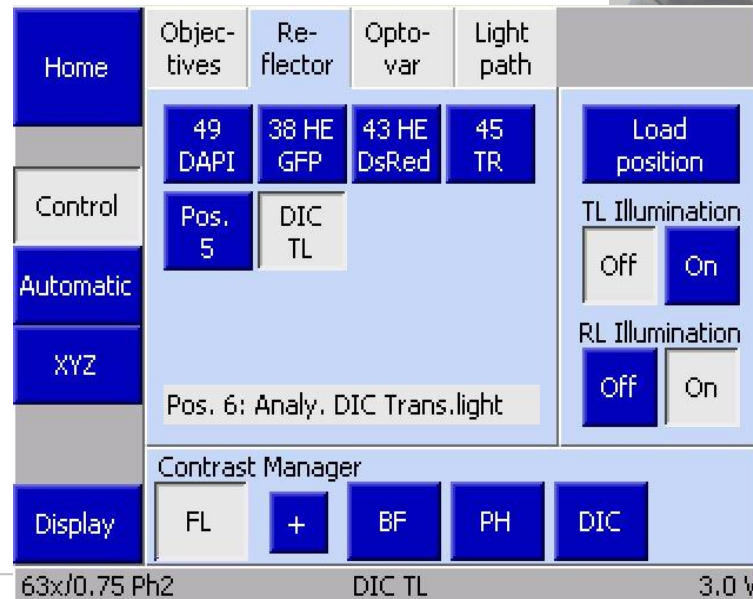




Axio Observer Z1

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

