Cytation 5
自動化影像系統暨多功能光學檢測儀
教育訓練

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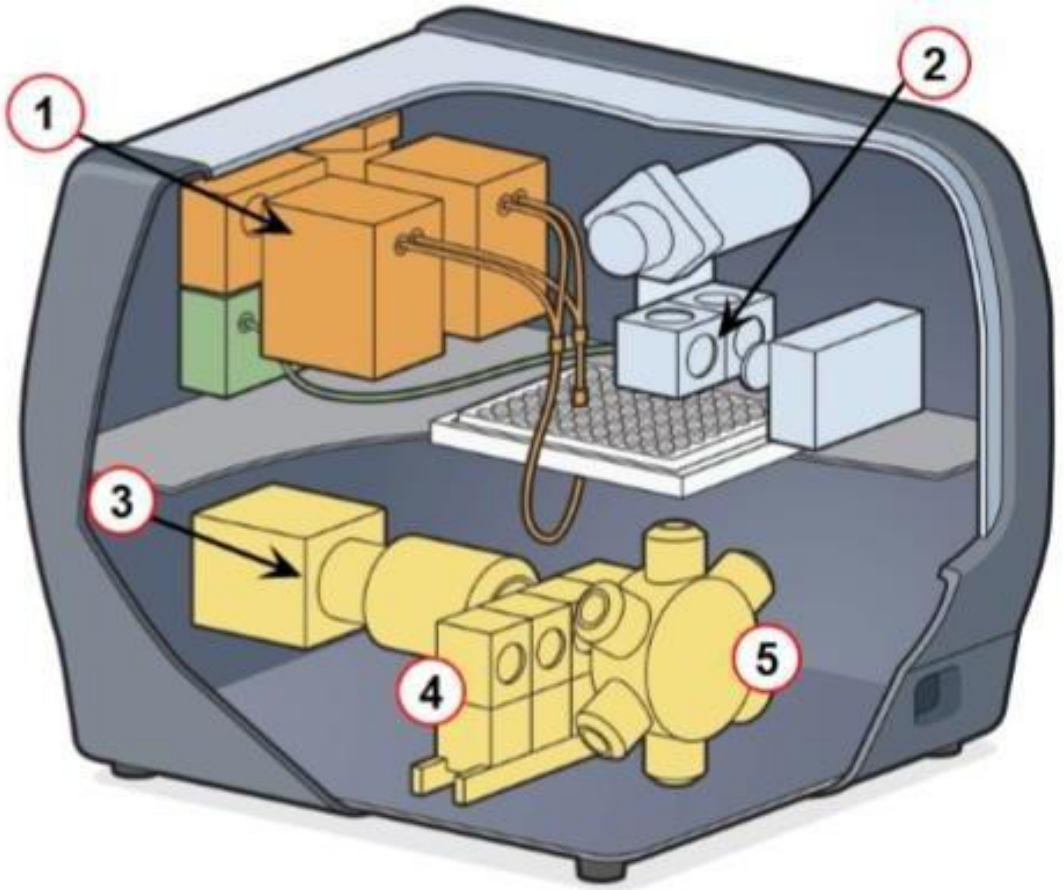
level
Discovering new boundaries

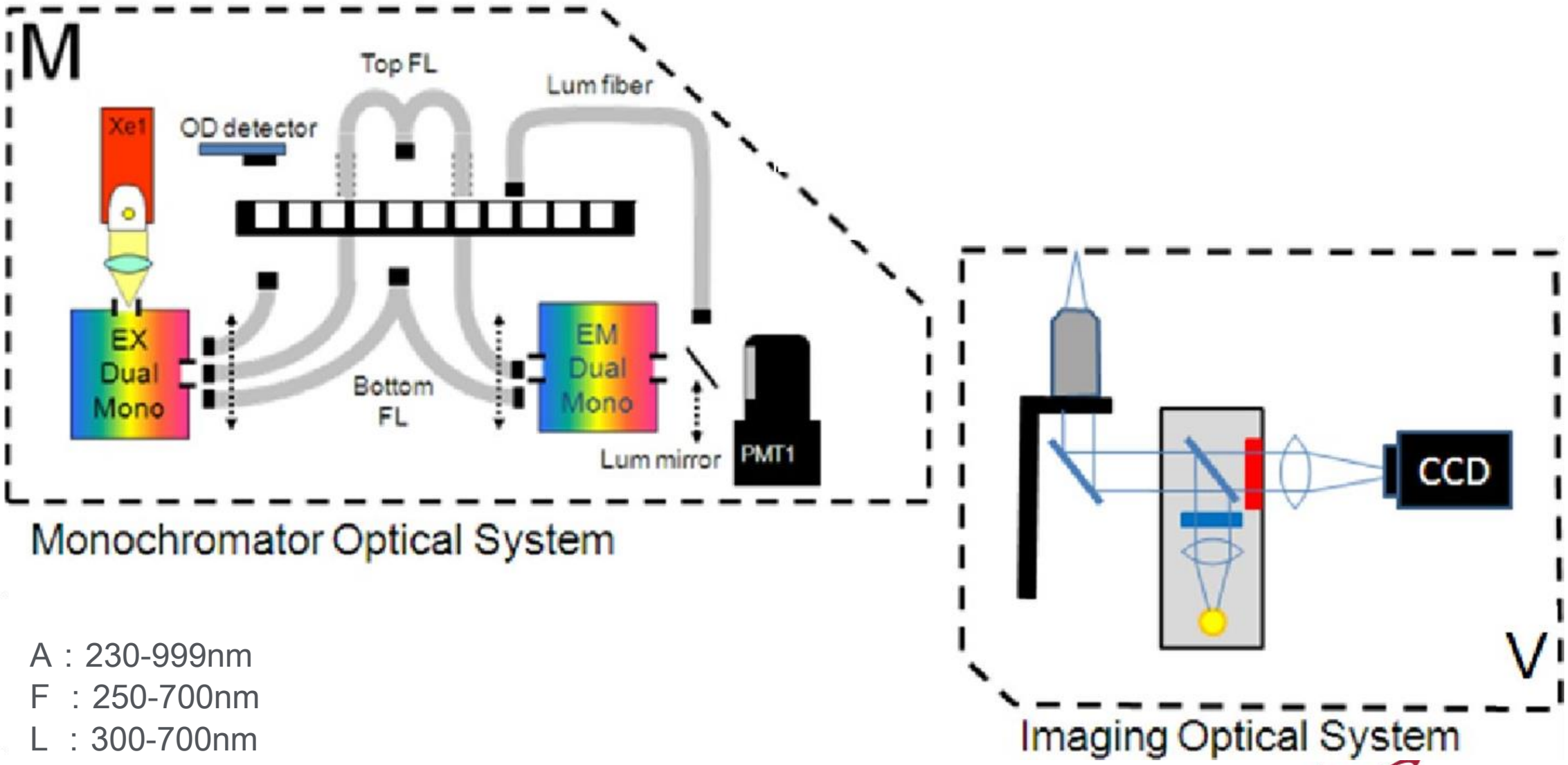
進 階 生 物 科 技
Level Biotechnology Inc.

Configuration: CYT5MPV

1. 全波長 Reader: 吸收光 螢光 冷光
2. 相位差、明視野、彩色明視野
3. 相機
4. 影像LED和濾鏡組
5. 物鏡旋轉台



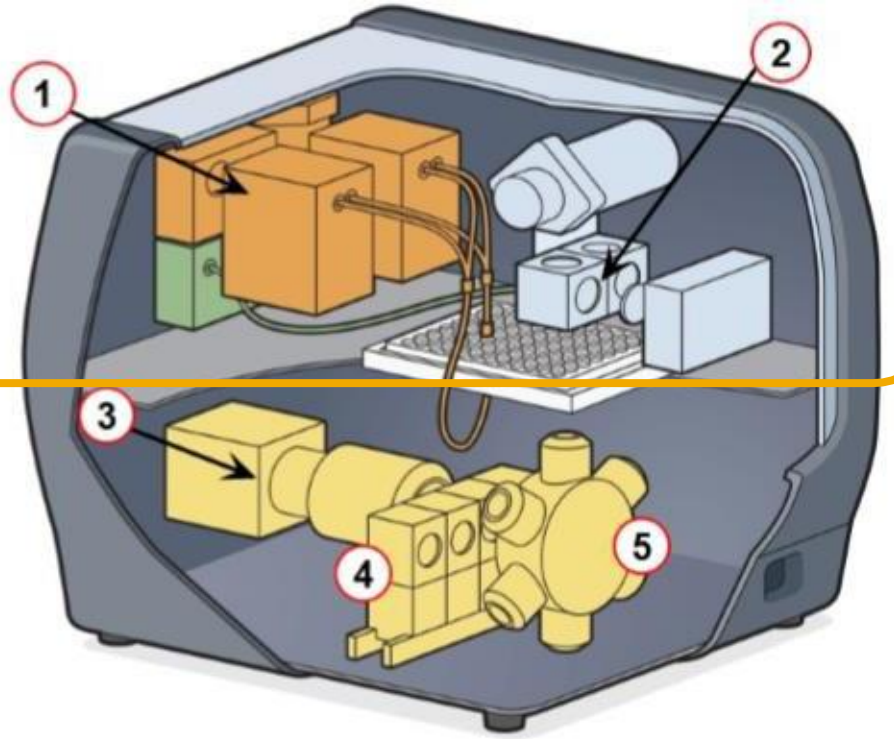
光學路徑



A：230-999nm
F ：250-700nm
L ：300-700nm

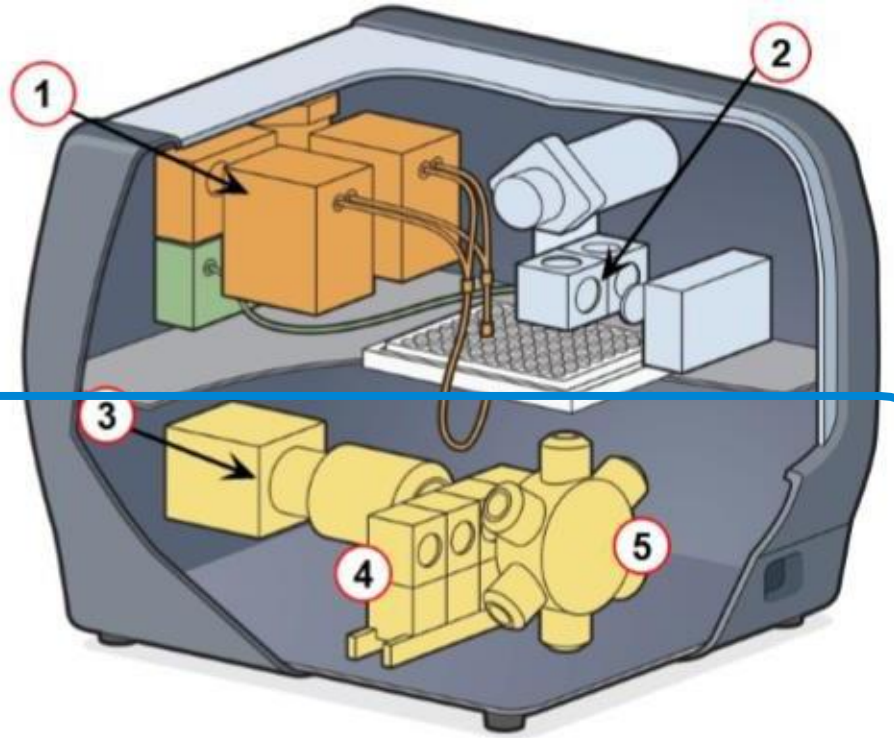
Reader

Microplate reader

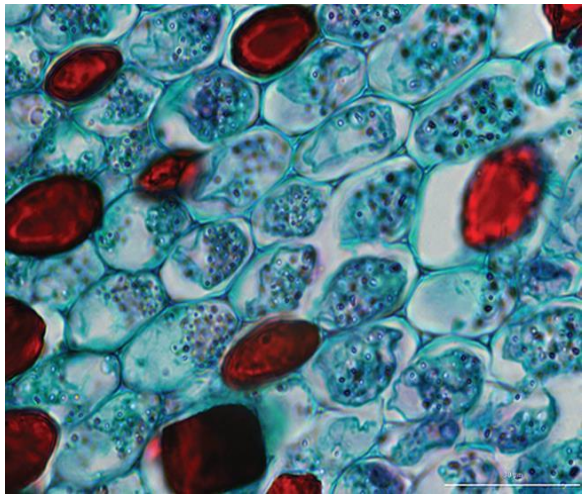


Imaging System

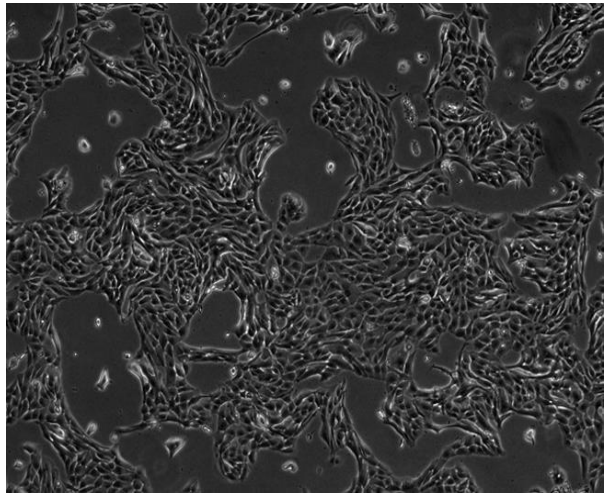
Imaging System



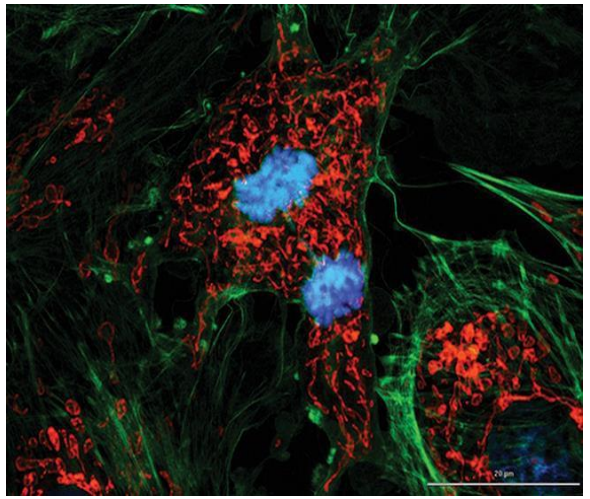
拍攝模式 Capture Modes



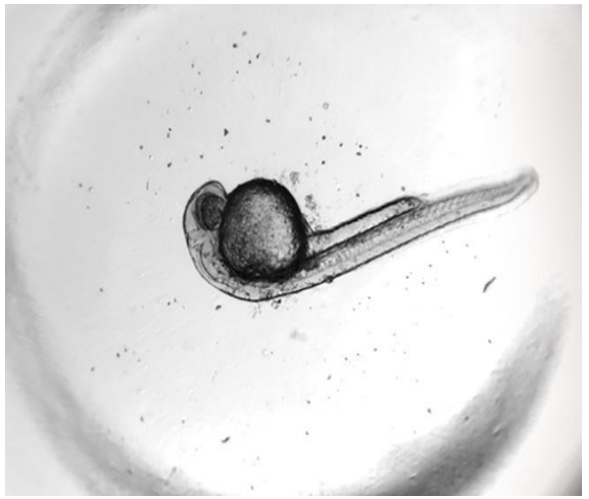
彩色明視野



相位差

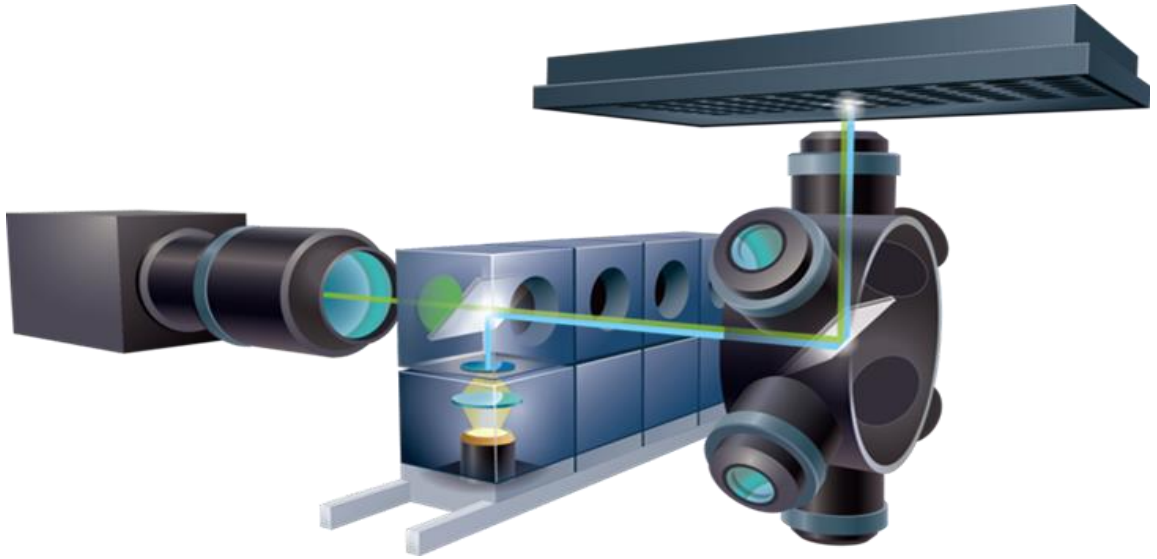


螢光



明視野

倒立螢光顯微鏡

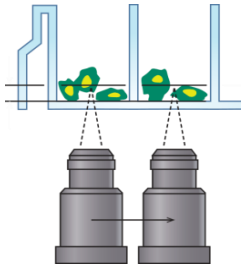


- 自動化物鏡載台
- 物鏡: 4X、10X、20X、40X 空氣鏡
- 影像自動對焦、雷射自動對焦
- 螢光濾鏡組：DAPI、 GFP、CY5、Texas Red、PE
- Gen5™ image prime Software

物鏡旋轉台

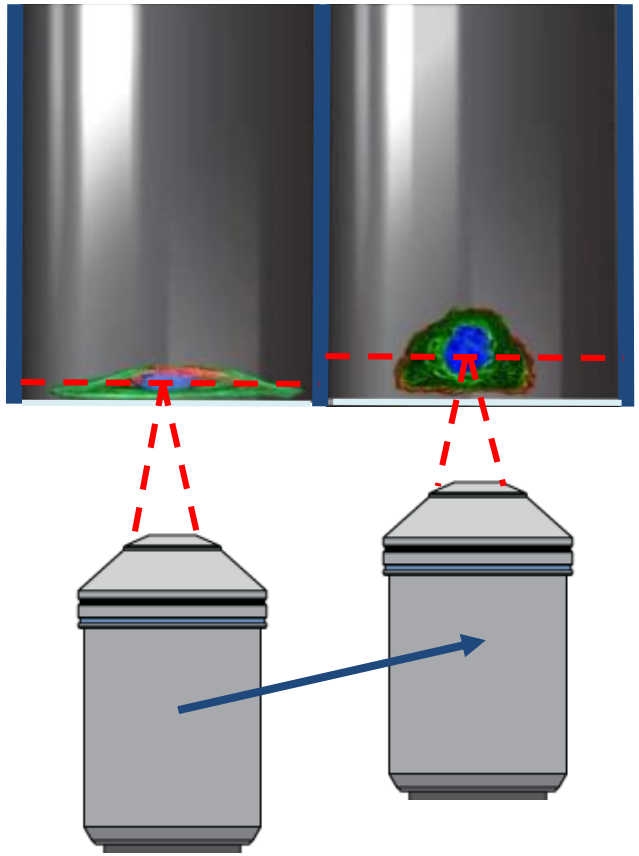
高倍物鏡
配有校正環

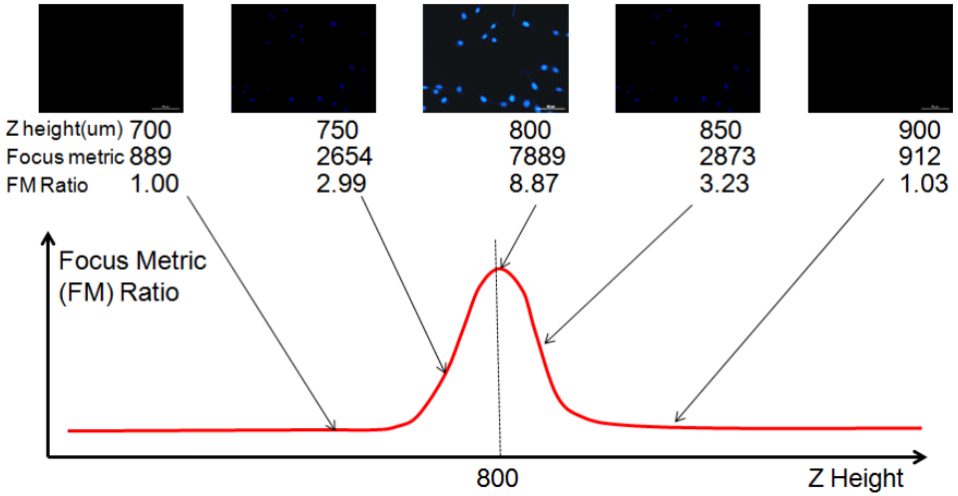
LED/Filter 套組



雷射自動對焦 (專利)

影像自動對焦
Imaging-based Autofocus (Default)





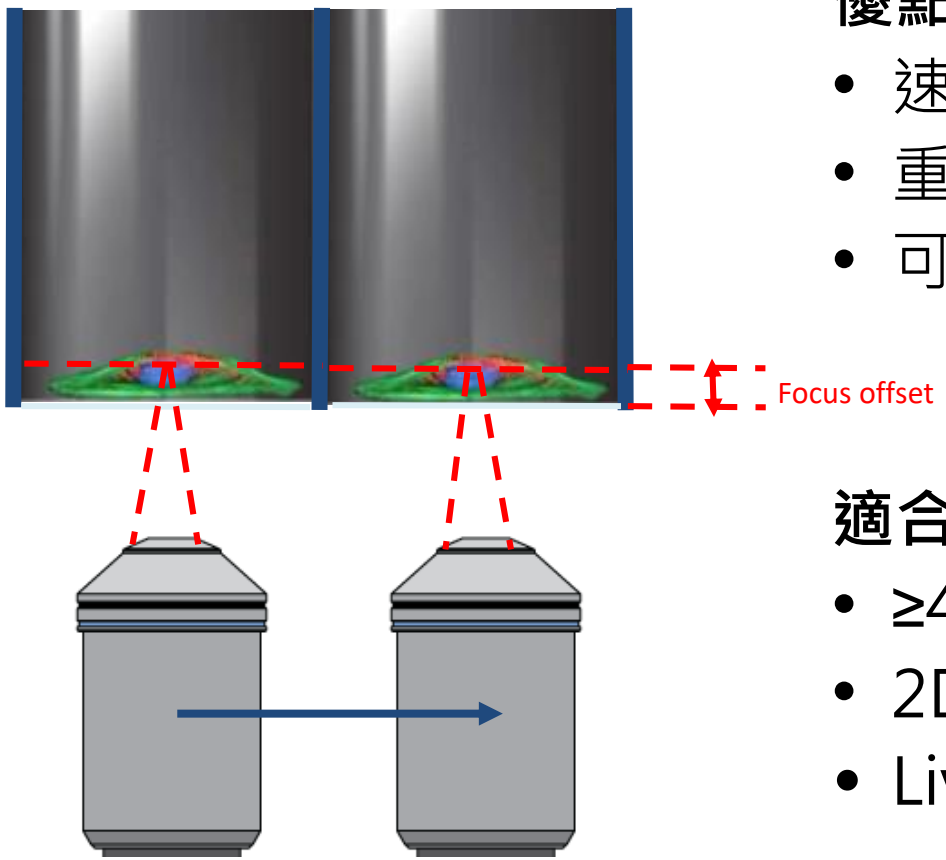
優點

- 優化生物樣本影像的彈性度較高
- 較佳的空間分辨力

適合

- 3D cell culture
- Thick biology
- Kinetic studies of 3D biology
- Moving biology
- Fixed samples

雷射自動對焦 Laser Autofocus



記憶盤底的折射率

優點

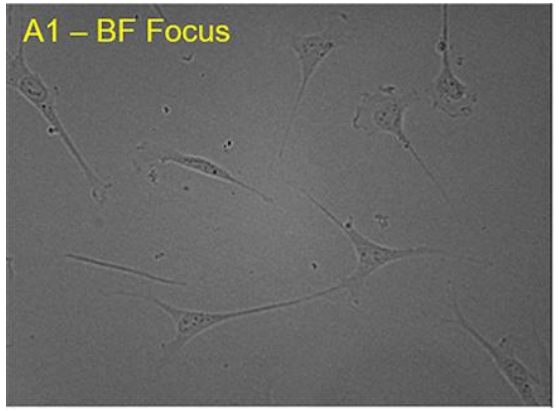
- 速度快 (比影像自動對焦快約50%)
- 重複性與再現性佳
- 可避免光漂白和光毒性

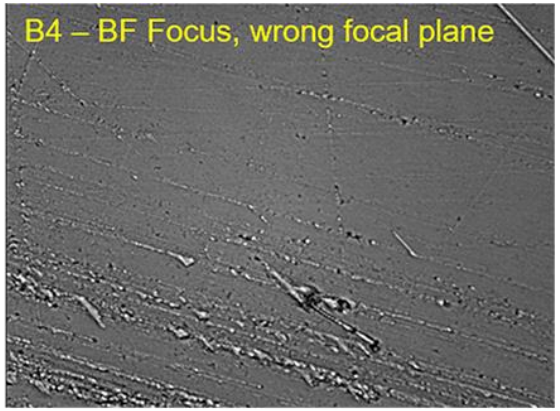
適合

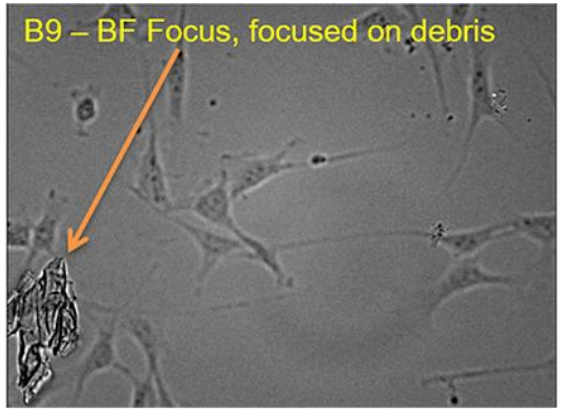
- ≥4x imaging
- 2D cell culture
- Live cell biology
- High throughput screening
- Kinetic studies
- Red emitting dyes

影像自動對焦 vs 雷射自動對焦

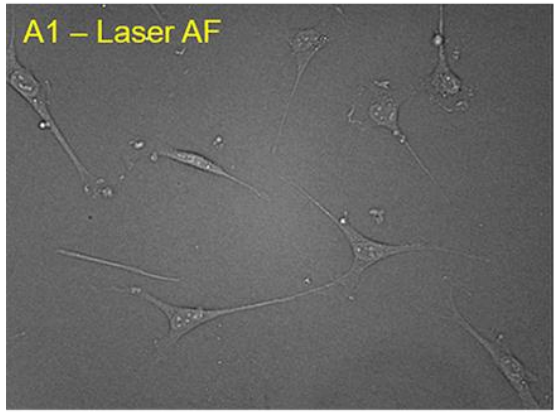
影像自動對焦

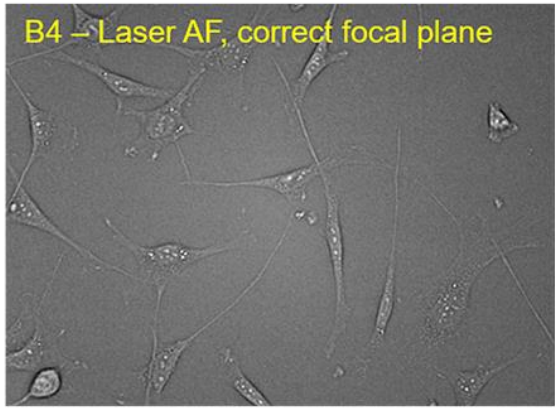


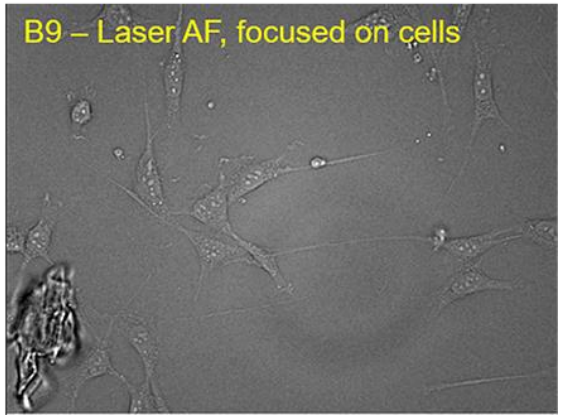




雷射自動對焦



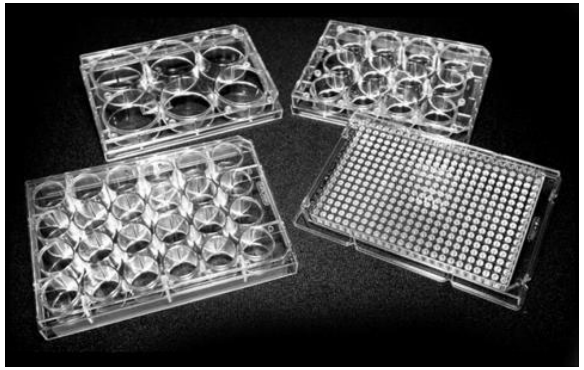




Imaging System Filter Set

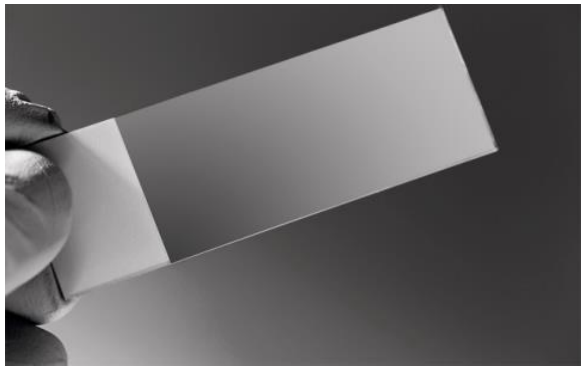
Fluorophore	Excitation	Emission	BioTek Filter set	
Hoechst 33342	352	461	DAPI Filter/LED Set	★
DAPI	359	461	DAPI Filter/LED Set	
Fluorescein, FITC, GFP	490	513	GFP Filter/LED Set	★
Alexa Fluor 488	493	517	GFP Filter/LED Set	
YFP	514	527	YFP Filter/LED Set	
Cy3	550	565	RFP Filter/LED Set	
TRITC (Rhodamine), RFP	550	570	RFP Filter/LED Set	
Alexa Fluor 594	588	613	Texas Red Filter/LED Set	
Texas Red	596	623	Texas Red Filter/LED Set	
Cy5	652	667	Cy5 Filter/LED Set	
Alexa Fluor 647	650	668	Cy5 Filter/LED Set	★
Cy 7	743	767	Cy7 Filter/LED Set	

Labware

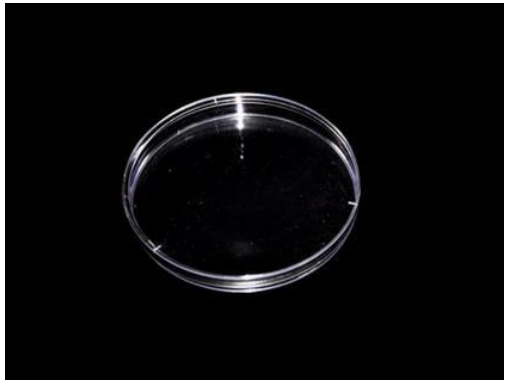


6- to 1536-well microplates

T25 flasks



Slides



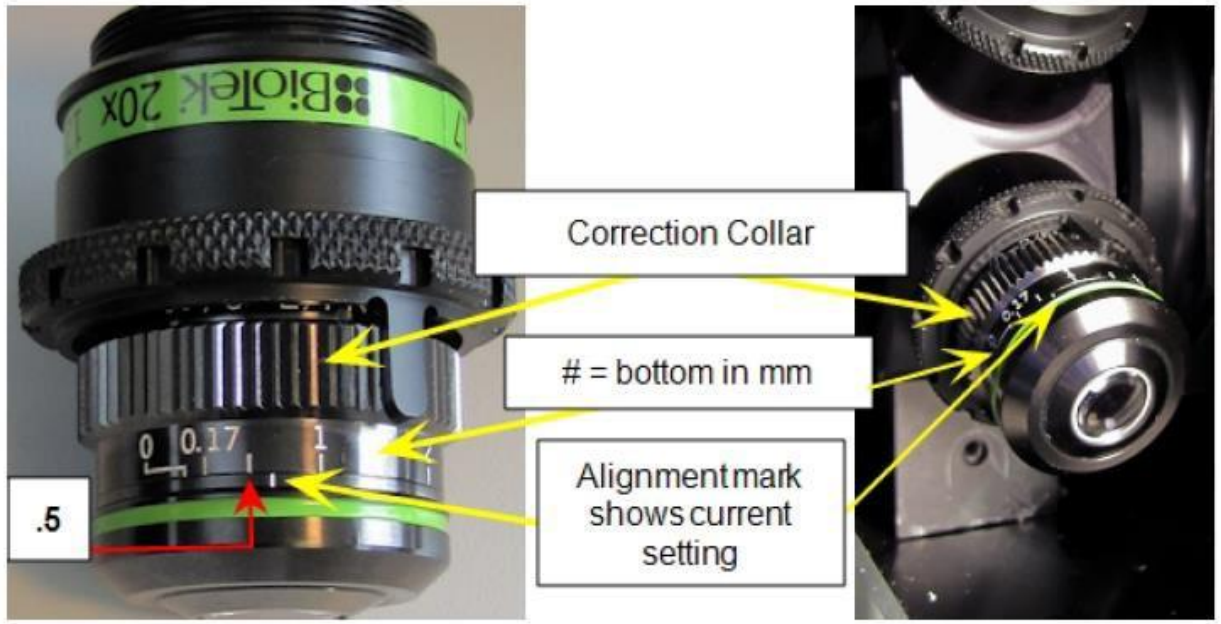
Cell Culture dishes
(10cm)

Take3 Micro-Volume plates

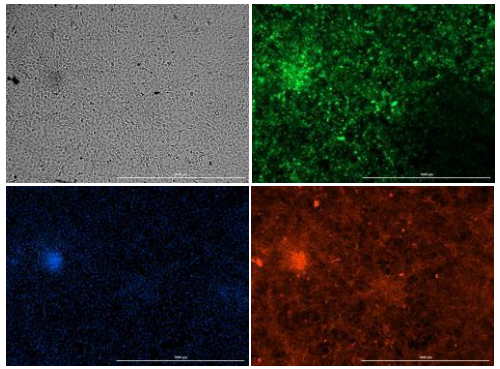
物鏡- Correction collar

20X, 40X 物鏡，需依據plate thickness 調整。

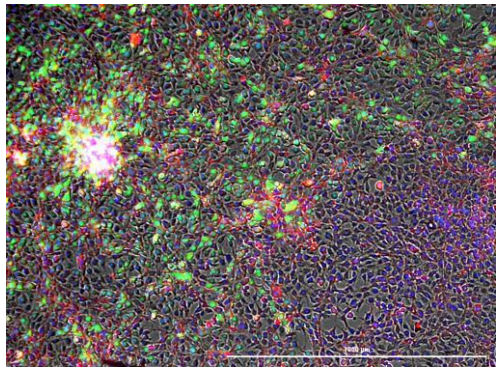
Plate Type	Typical Bottom Thickness
Glass-bottom microplate	0.17 mm
Cover slip	0.17 mm
Plastic microplate (e.g. 96-, 384-well)	0.5 mm
Low-density microplate (e.g. 6-, 12-well)	up to 2.0 mm



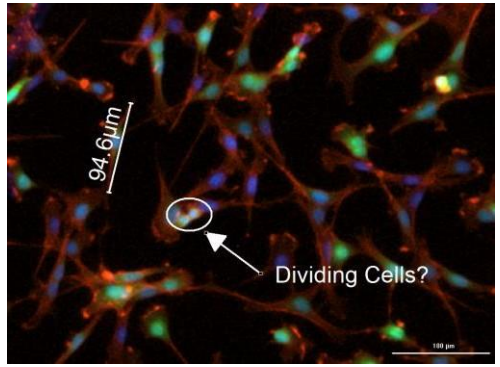
Imaging Workflow



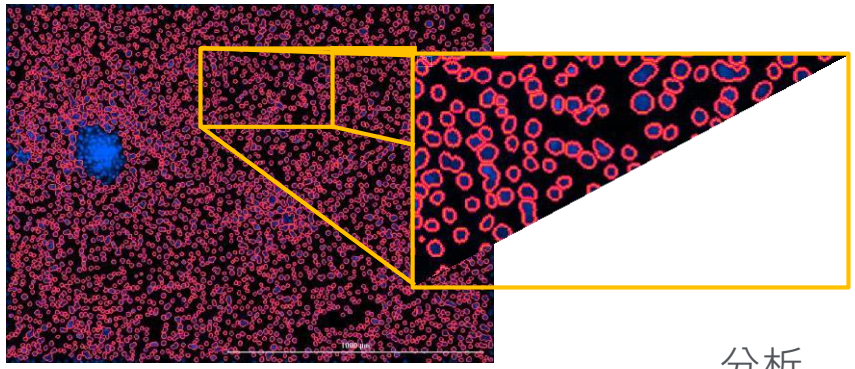
4-Channel Capture



影像處理
Process

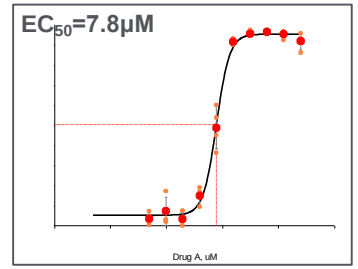


編輯與註解
Edit & Annotate

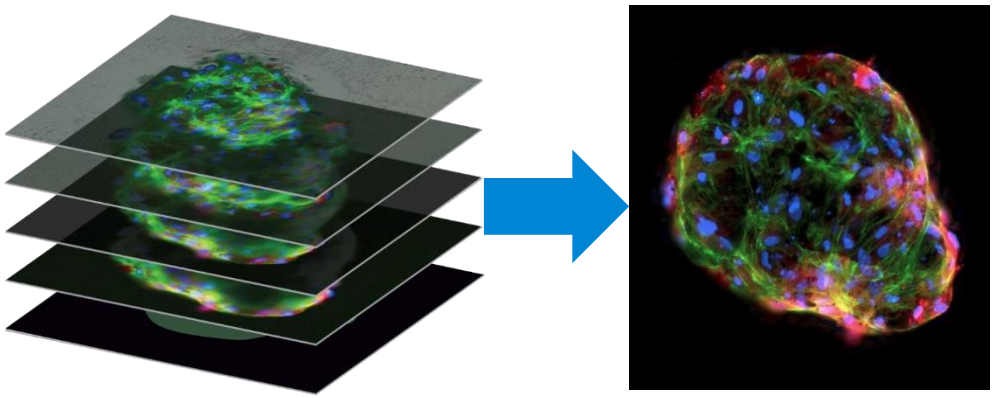


分析　　發表
Analyze & Publish

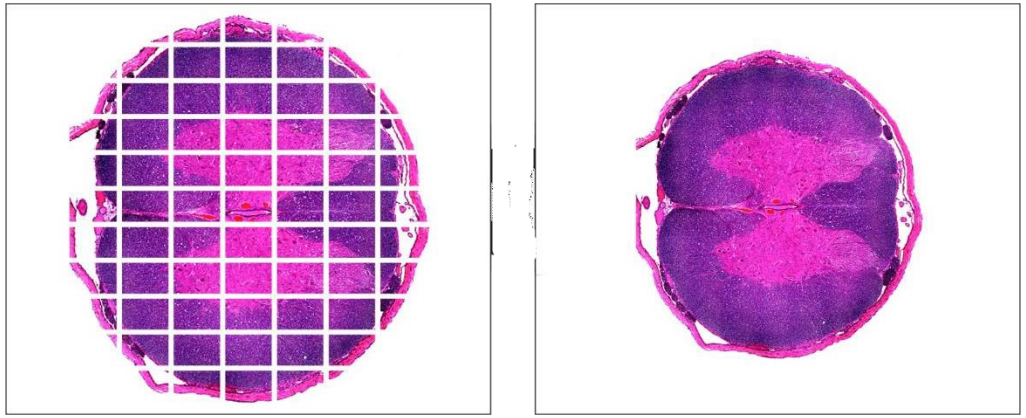
Cell Count	3408
Object Area	269
Object Circularity	0.661



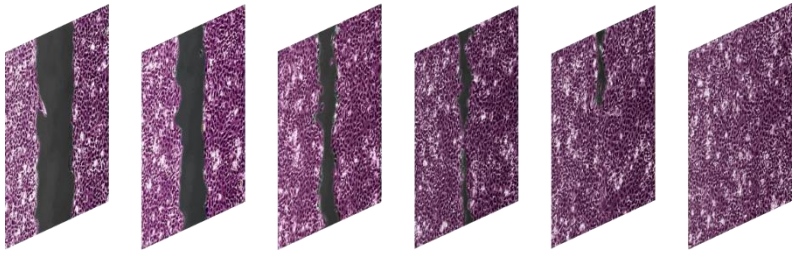
影像拍攝方式



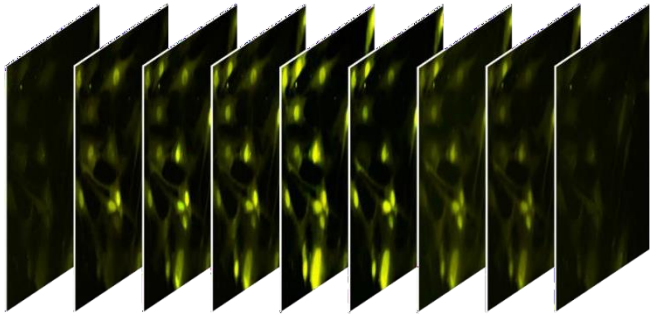
Z軸疊圖與投影
Z-stack



大圖拼接與縫圖
montage

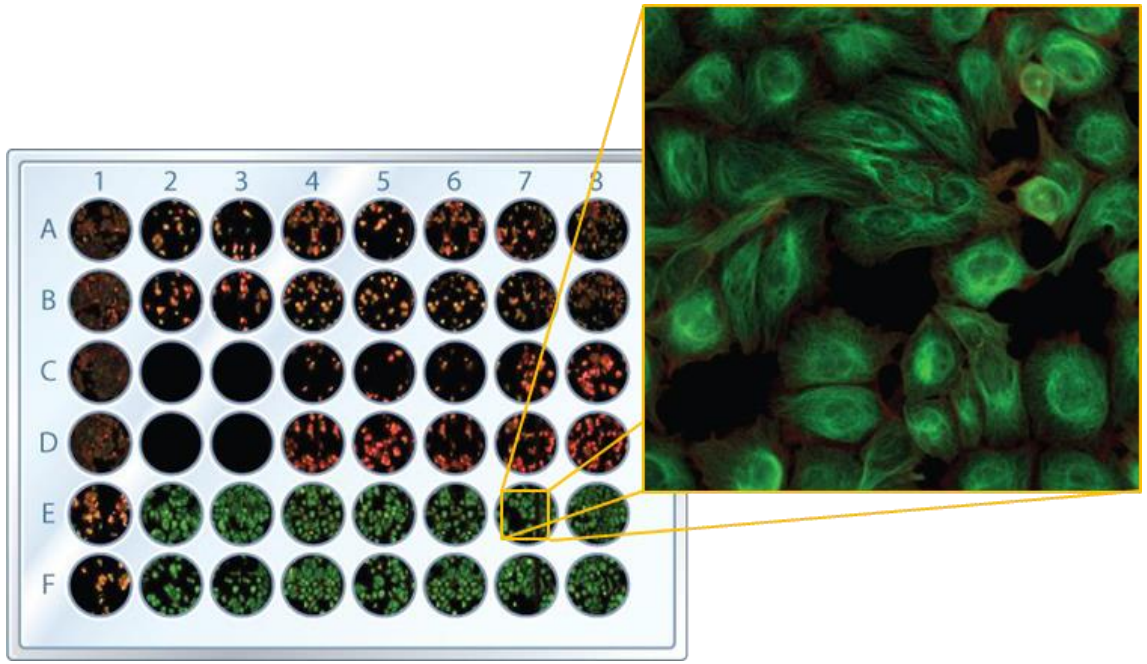


長時間拍攝
應用： 傷口癒合、細胞增生

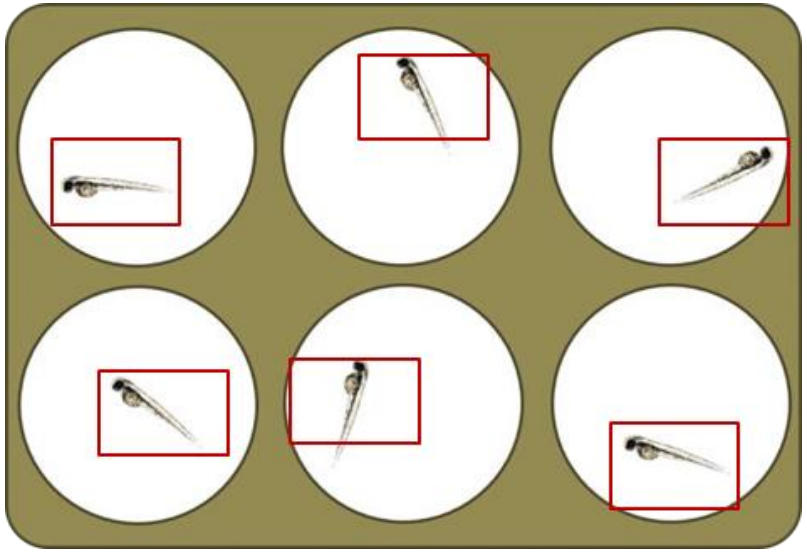


高速動力學拍攝
應用： 鈣流和其他動力學實驗

影像拍攝方式

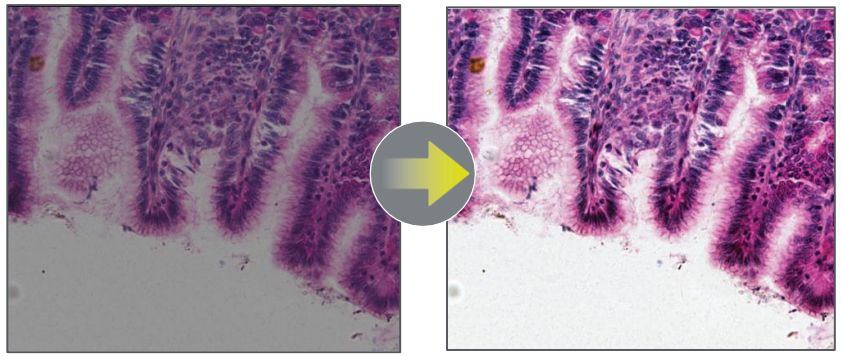


批次拍攝 Batch Mode

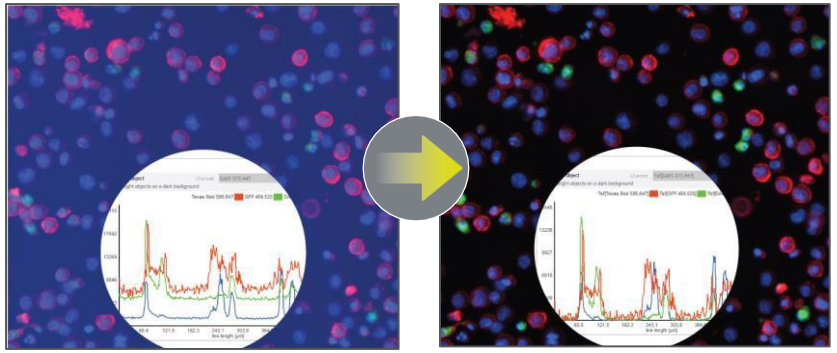


定位拍攝 Beacon Mode

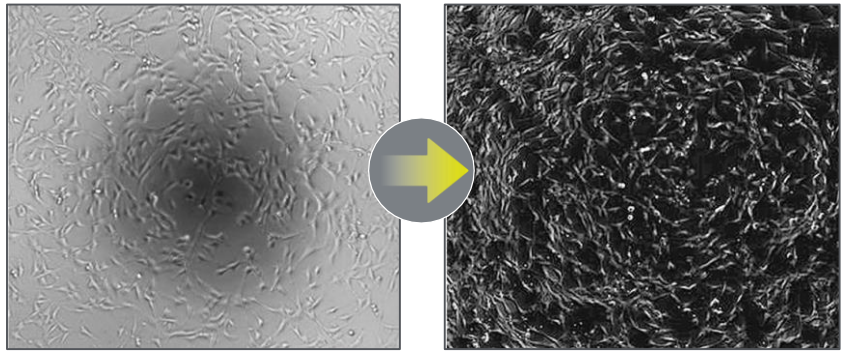
Image Processing影像處理



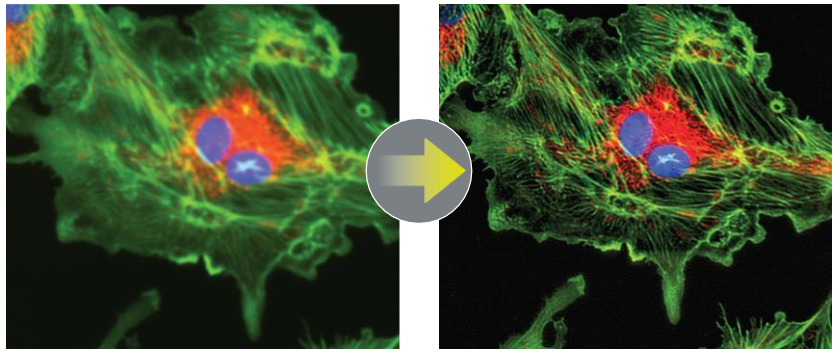
明亮度與對比
Brightness and contrast



去背景值
Background flattening



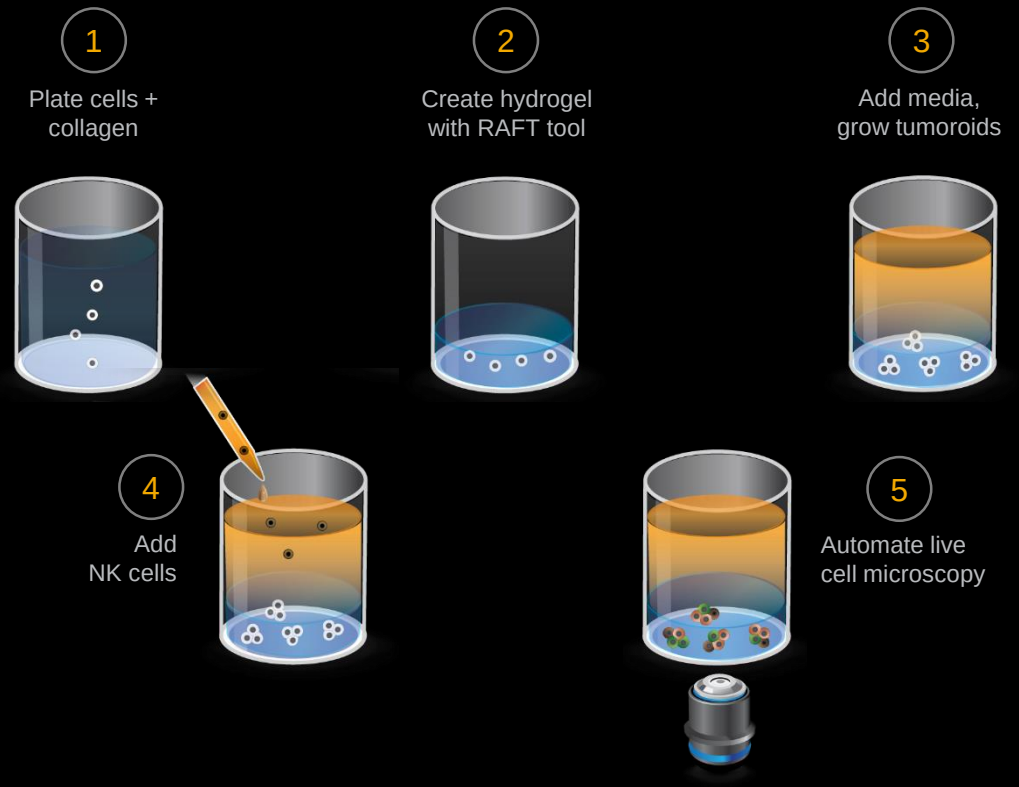
數位相位差
Digital phase contrast



圖像銳化
Deconvolution

Applications

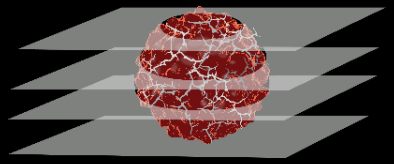
3D Kinetic NK Cell Cytotoxicity Assay



- Using Lonza's RAFT system, cancer cells are suspended in the hydrogel and propagate to form 3D tumoroids.
- Natural killer (NK) cells are then introduced and apoptotic and necrotic induction within cancer cells measured over 120 hours.

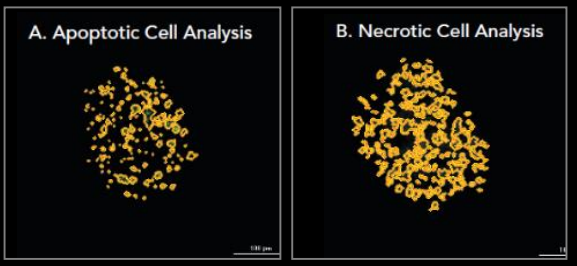
Augmented microscopy

Capture
Three-color, z-stacked images of tumoroids are captured in each well over 120 hours.

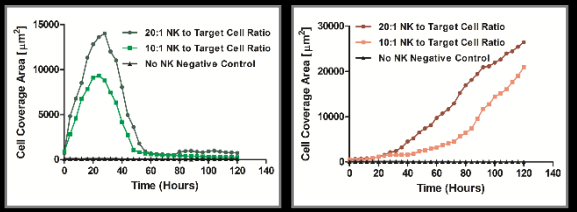


Process
Each set of z-stacked images is z-projected at each time point for analysis of apoptosis (green fluorescence) or necrosis (red fluorescence).

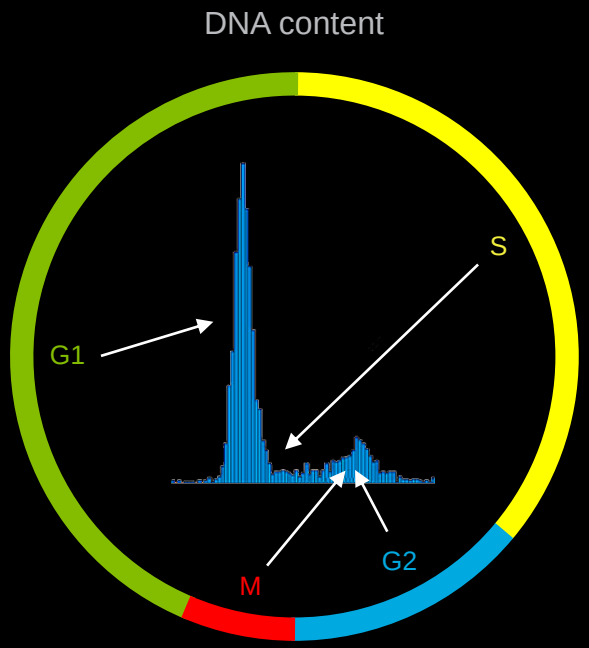
Analyze
Image analysis quantifies apoptosis (green fluorescence) and necrosis (red fluorescence).



Publish
Apoptotic and necrotic induction are plotted over time for each condition.



Cell Cycle Analysis Using a Nuclear Stain

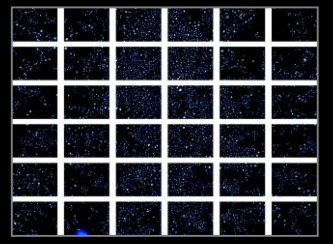


- Cell cycle progression is a tightly regulated process that involves the duplication of nuclear DNA content prior to cell division.
- A nuclear stain such as DAPI can be used to quantify this process since fluorescence intensity doubles as cells progress from G1 to G2 phase.

Augmented microscopy

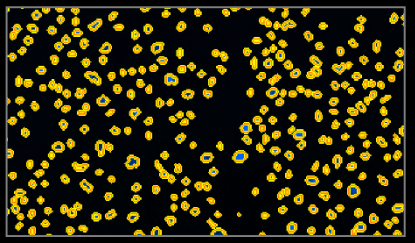
Capture

DAPI montage (6 × 6) image using 10x objective (one tile expanded).



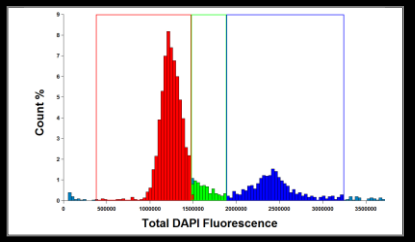
Process

Stitched and background subtracted, montage image with cell nuclei identified (zoom shown, about 3,000 cells per well counted on final montage).



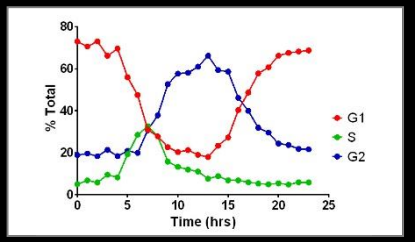
Analyze

Determination of G1, S, and G2 subpopulations using histogram analysis of object total DAPI fluorescence.

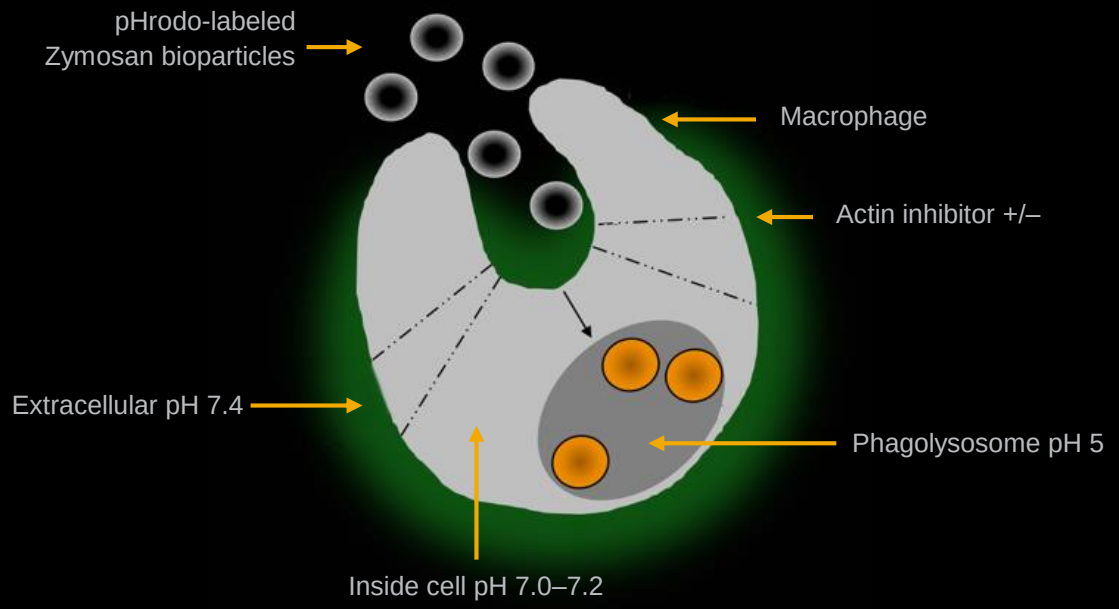


Publish

Cell cycle progression of synchronized PC-3 cells.



Phagocytosis Assay



- Macrophages are specialized cells that consume and digest foreign matter through phagocytosis.
- pH-sensitive bioparticles are a useful tool to study phagocytosis as particles fluoresce in response to acidic environment of phagolysosomes.
- Cellular actin enables unique physical changes necessary for phagocytosis.
- This assay analyzed effects of actin disruption on bioparticle phagocytosis.

Augmented microscopy

Capture

A two-channel image shows black extracellular bioparticles in contrast to the red fluorescence of phagocytized bioparticles (RFP).

Process

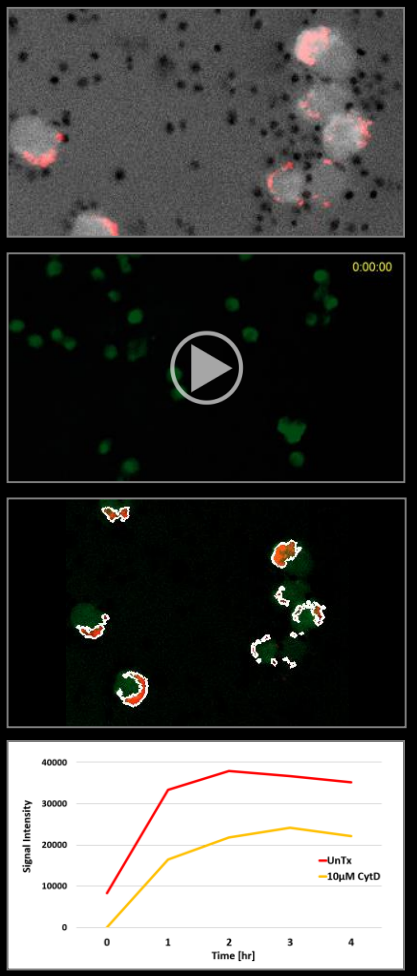
This movie of kinetic movie shows an increase in bioparticle phagocytosis over time (orange).

Analyze

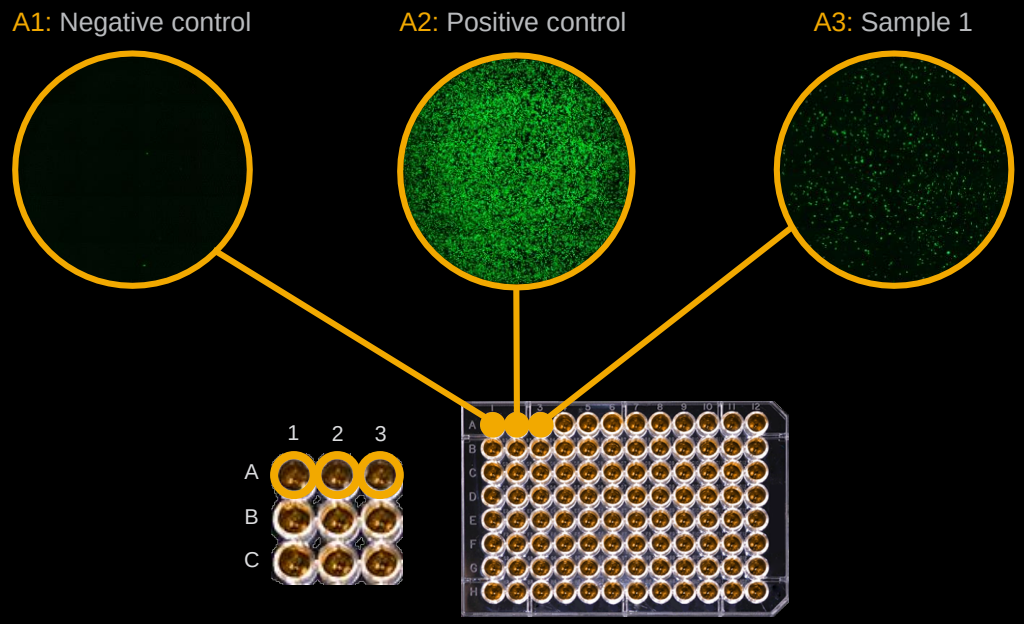
A primary mask on bioparticle phagocytosis is applied to all kinetic images.

Publish

Compared to untreated macrophages (red) actin disruption causes decreased bioparticle phagocytosis (yellow).



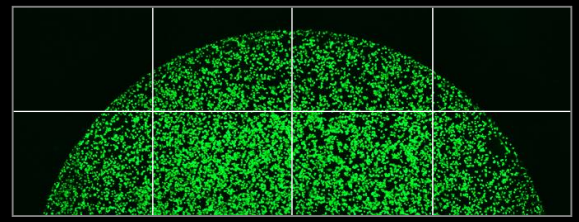
Plaque Assay



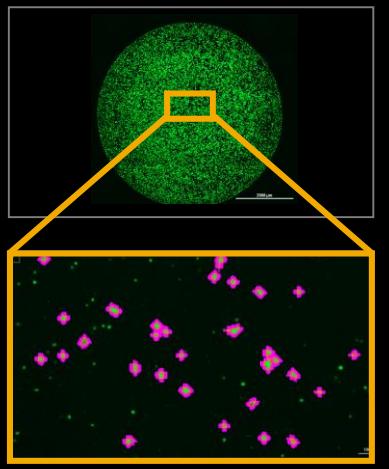
- A cell monolayer is treated with a dilution of a GFP-expressing virus.
- The cells are then coated with agar, which limits the virus spread to only neighboring cells.
- After incubation, GFP+ circles of infected cells are counted for virus titration.

Augmented microscopy

Capture
Each well is automatically imaged at 4x as a 5 x 4 montage.



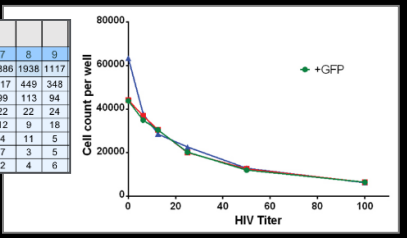
Process
The resulting 20 images are stitched together by the software.



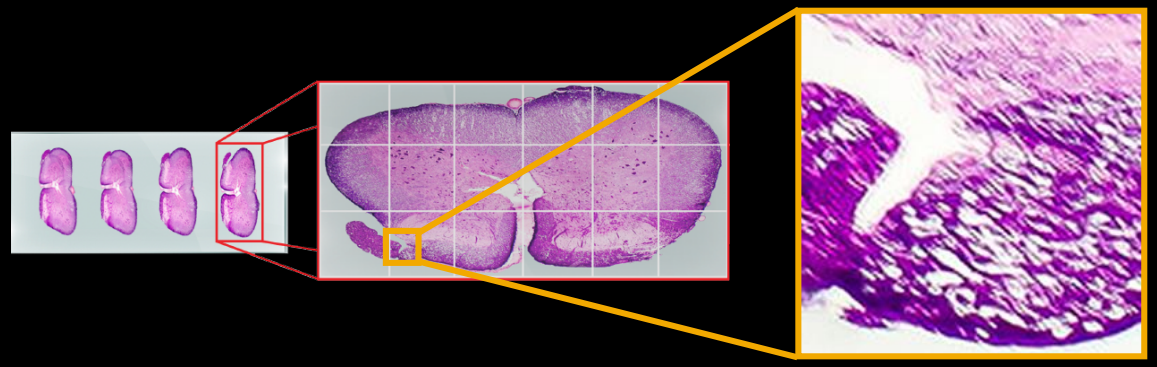
Analyze
The stitched image is analyzed; plaque objects are identified using intensity and size thresholds.

Publish
Objective, quantitative plaque count data can be published.

	NEG	POS							
	1	2	3	4	5	6	7	8	9
A	6	12083	2752	2562	2081	1721	1886	1938	1117
B	3	13317	710	624	483	464	517	449	348
C	12	13205	139	147	102	108	99	113	94
D	5	4663	31	43	29	26	22	22	24
E	2	1049	6	15	16	10	12	9	18
F	17	284	8	3	6	3	4	11	5
G	7	69	8	12	5	5	7	3	5
H	4	15	7	2	11	8	2	4	6



Whole-Slide Imaging: Regions of Interest



- Whole-slide imaging is a means to transform physical specimens on a microscopy slide into a digital medium for analysis, sharing, and archiving.
- Augmented microscopy is the method used to locate regions of interest (ROIs) followed by automated image acquisition and subsequent image analysis.

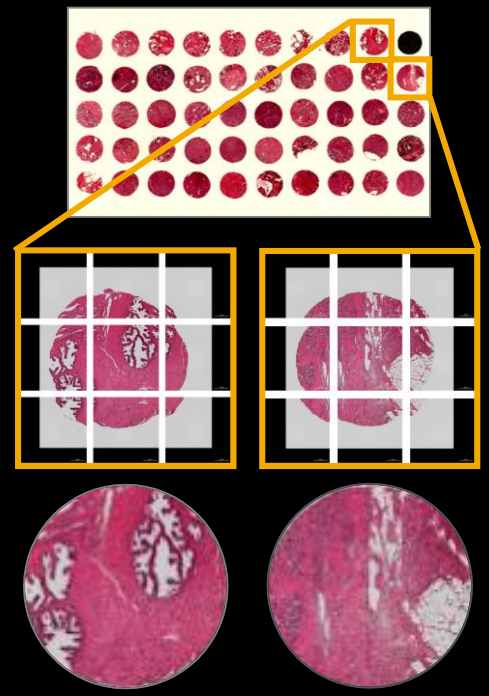
Augmented microscopy

Capture 1
Whole-slide imaging of prostate core samples at low resolution using upright microscope. ROIs selected.

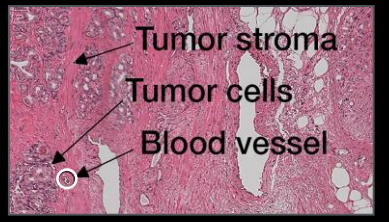
Capture 2
A montage of each ROI was imaged at higher magnification (10x) using the inverted microscope.

Analyze
Each ROI montage was stitched resulting in a high-resolution image for downstream analysis.

Publish
The digitized, high-resolution images can be analyzed, annotated, and easily shared or archived for future use.

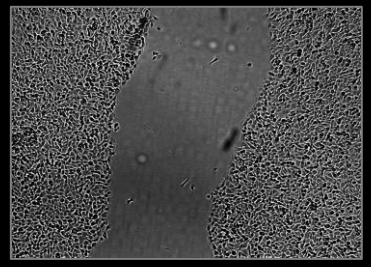


Prostate core samples 10x

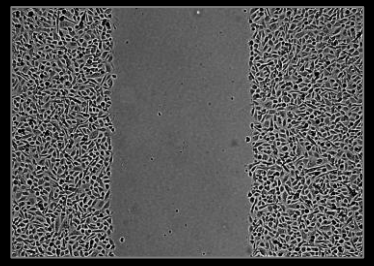


Automated Wound Creation for Scratch Wound Healing Assays

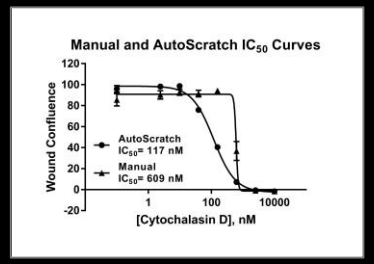
Automated wound healing assay procedure



Wound created manually using P200 pipette tip



Wound created using the Agilent BioTek AutoScratch wound making tool



Comparison of HT-1080 wound healing inhibitor curves from manually and AutoScratch generated wounds

- Automated wound creation decreases variability within and between wounds, improving repeatability of generated cell migration data.
- Combined with kinetic image capture and analysis, a robust method to generate high-quality scratch wound healing results is created.

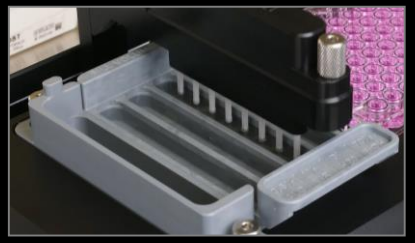
AutoScratch wound making tool

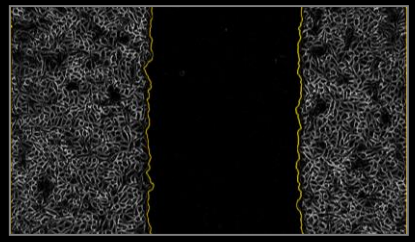
Clean
Walk-away procedure cleans and sterilizes pins before and after wound creation.

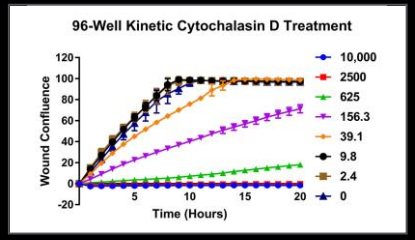
Wound
Repeatable wounds created automatically in 24- or 96-well plates.

Image and Analysis
Kinetic images captured, processed, and analyzed to determine wound width, % wound confluence and max wound healing rate.

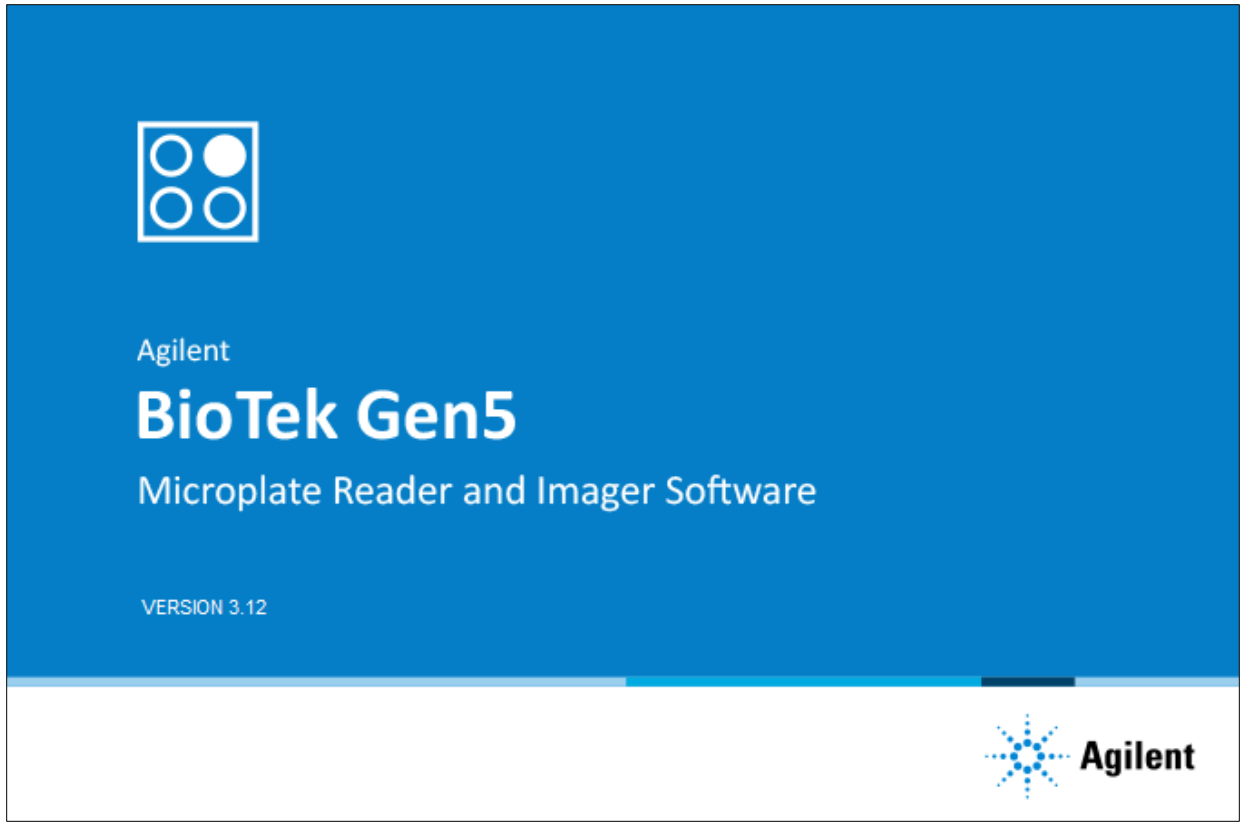
Publish
Kinetic wound healing curves allow accurate decisions to be made regarding cell models and test molecules.







Gen5 image prime 教育訓練

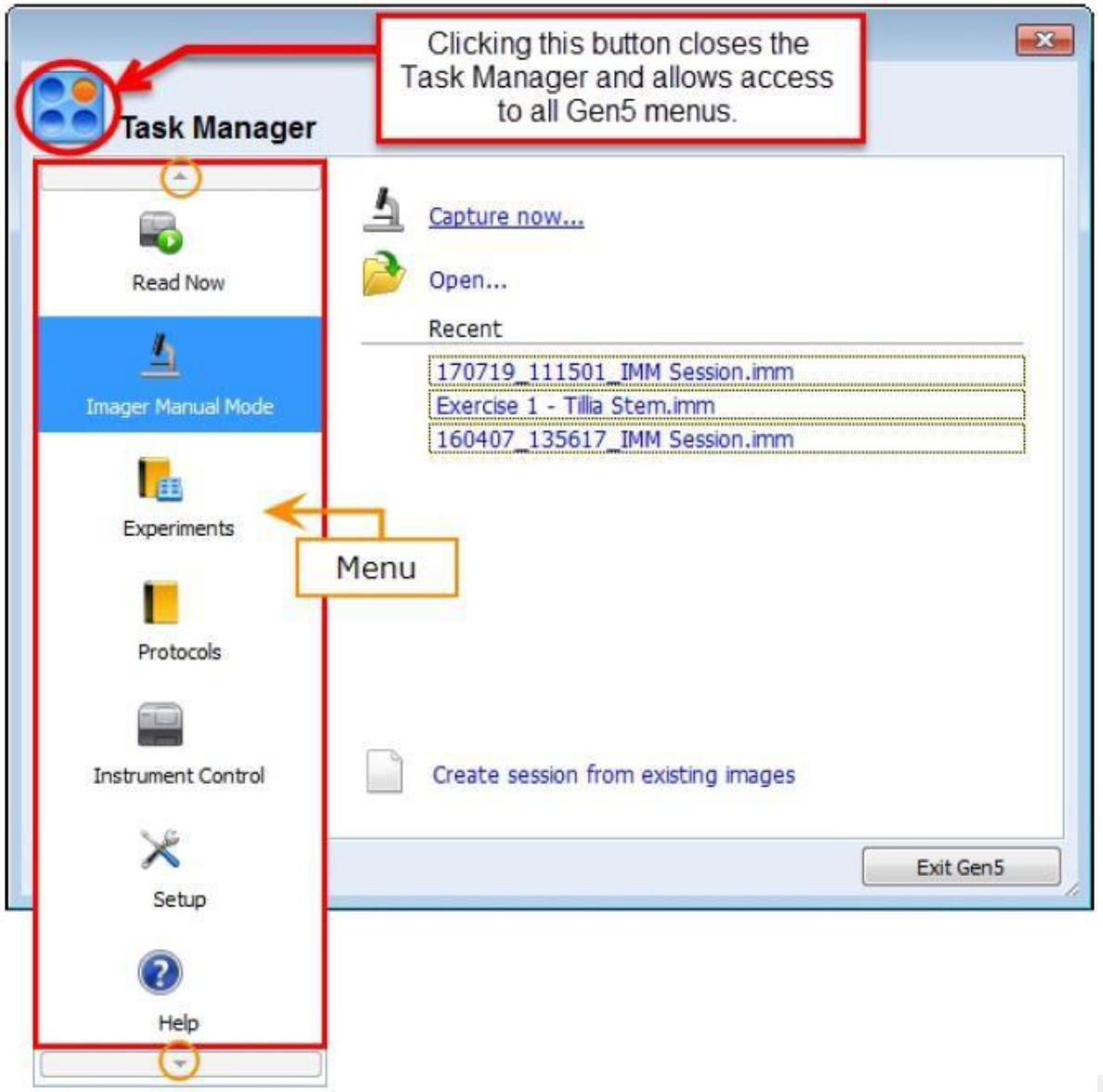


Before You Begin

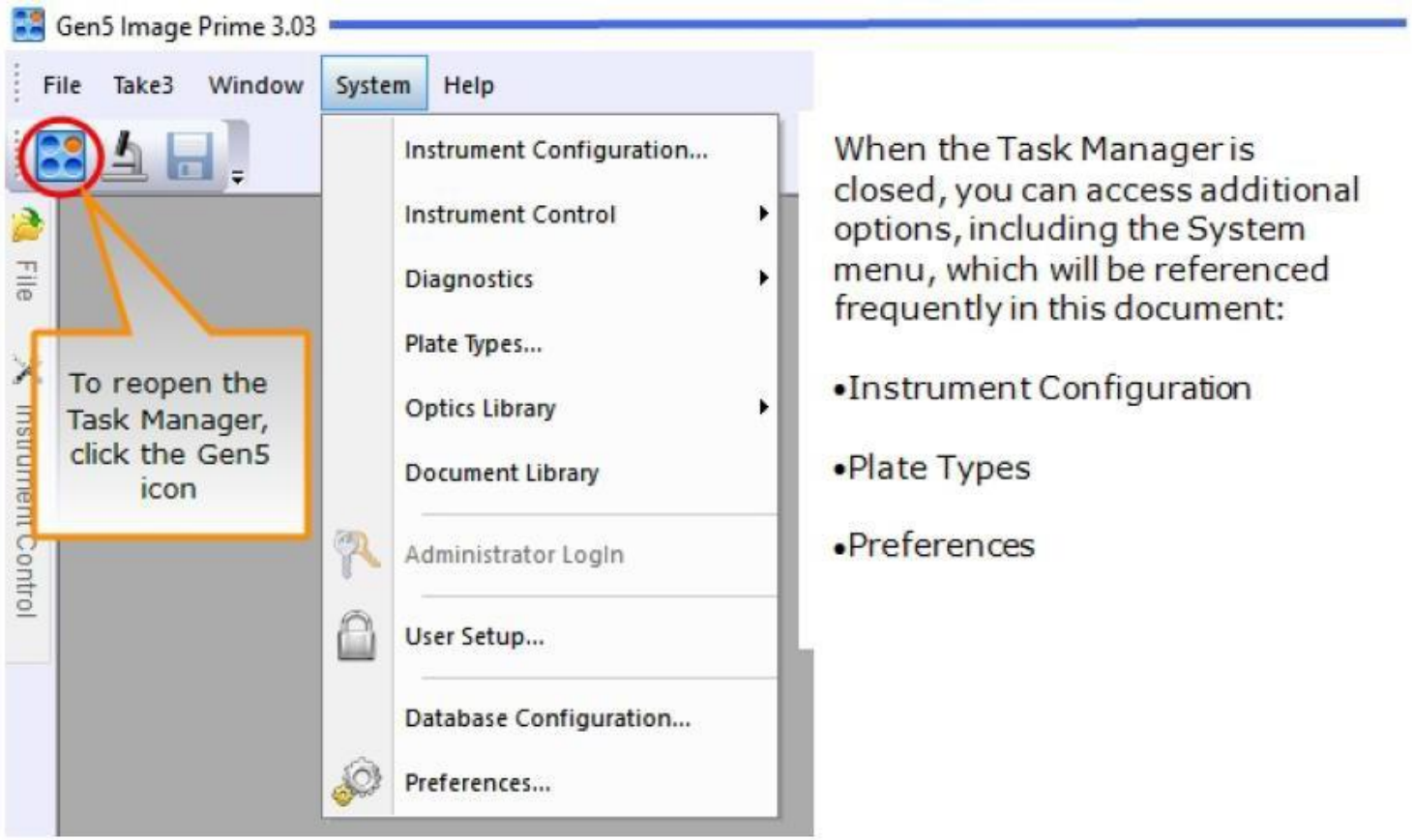
- 了解Gen5 Task Manager
- Manual Mode VS Experiment Mode
- 檔案儲存位置
- 盤型定義
- 高倍物鏡使用

Task Manager 功能

- Read now: 立即讀取實驗
- Image mode: 影像手動模式
- Experiment:自動化拍攝使用
- Protocol: 建立實驗範本使用
- Instrument: 儀器控制區



Task Manager



When the Task Manager is closed, you can access additional options, including the System menu, which will be referenced frequently in this document:

- Instrument Configuration
- Plate Types
- Preferences

Instrument Configuration: 儀器連線時使用
Plate type: 定義盤型
Preference: 定義圖片儲存位置 (勿動)

Image Mode VS Experiment

	Manual Mode	Comments	Experiment Mode	Comments
Image Capture	Single Image Capture	Low volume samples; Some tissue section and chamber well slide work; Oil objectives	Multi-well, multi-image capture; Full automation of image acquisition	Screening, high density plates, high throughput image acquisition
Analysis	Cell counts and other cellular analysis metrics; Subpopulations from each image	No ratios, custom formulas or transformation steps	Cell counts and other cellular analysis metrics; Subpopulations from each image/well/ full plate/ full vessel	Ratios, custom formulas and transformation steps applied to the full plate/vessel

Image Mode 手動模式

- 顯微鏡概念
- 少量樣本
- 確認樣本曝光與對焦位置

Experiment Mode 自動模式

- 自動拍攝(曝光/對焦)
- 大量樣本
- 長時間拍攝
- 大批次圖片/影片輸出/數據分析

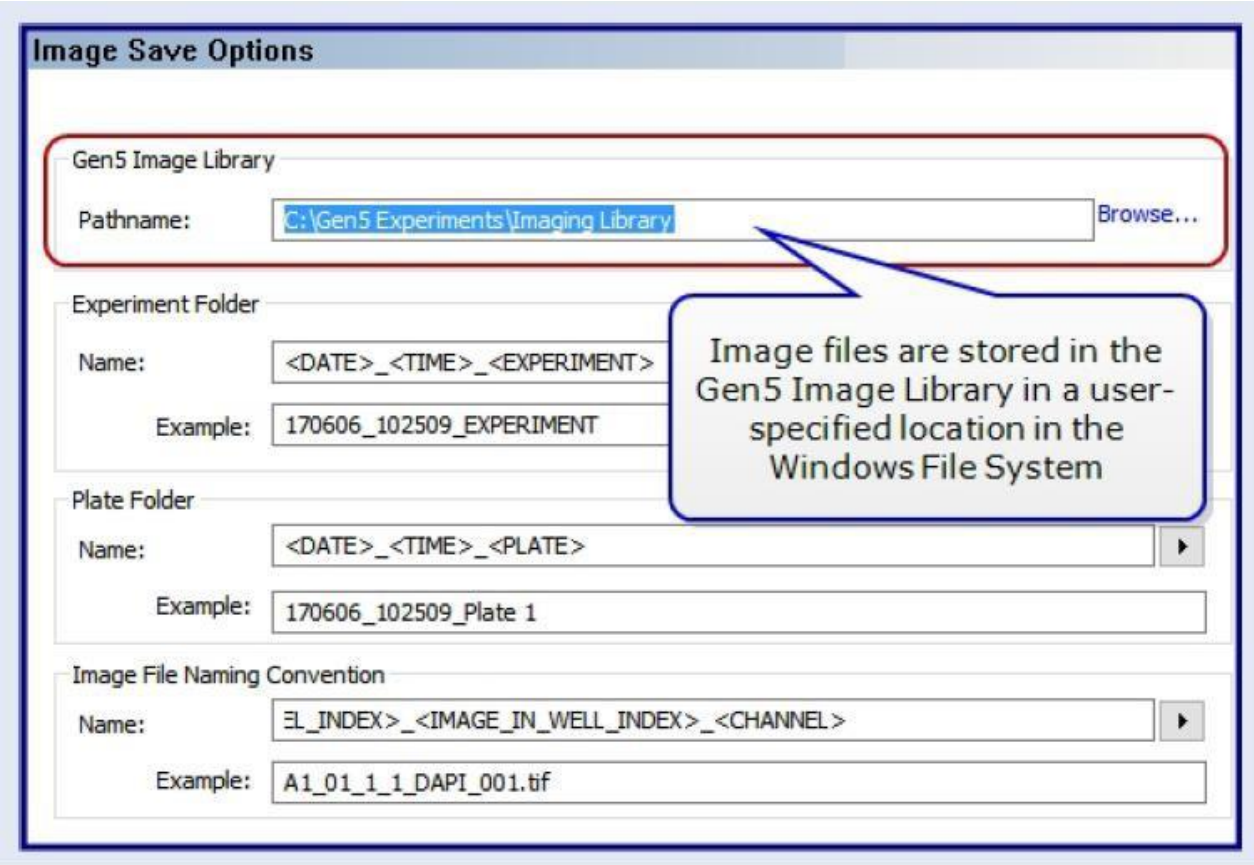
檔案儲存位置

Gen5 file 儲存位置

- Gen5 Experiment (.xpt) 或是Protocol檔案 (.prt)
- Default儲存位置是在. C>使用者>公用>公用文件>Experiment or Protocol

圖片檔案儲存位置

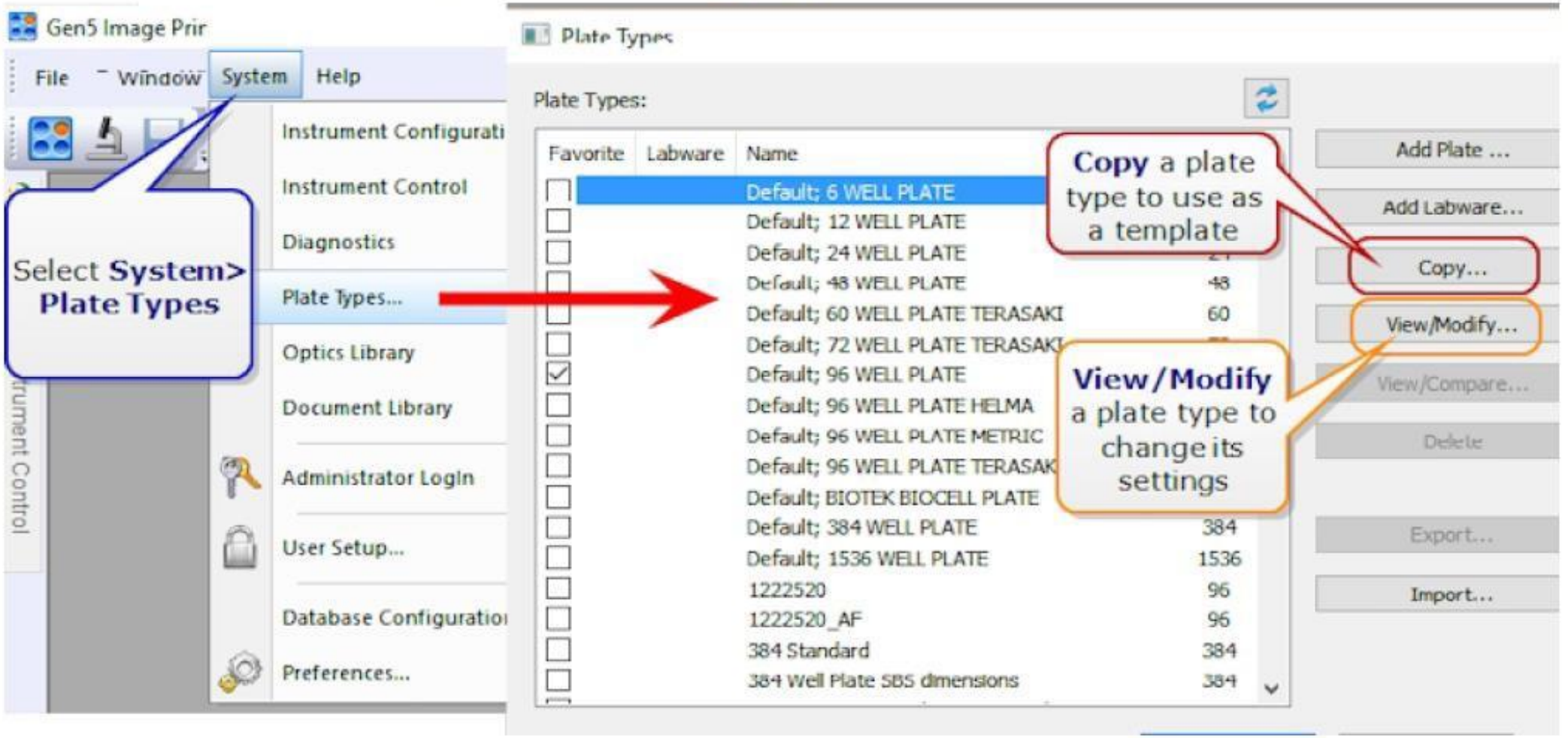
- 硬碟 (可指定)



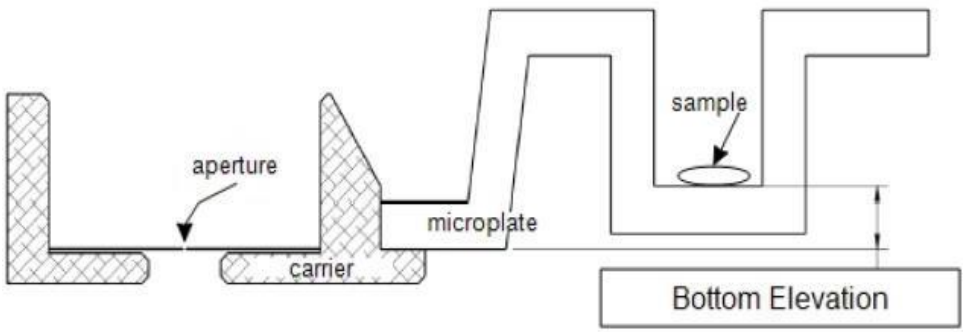
- **Gen5 Experiment 和圖片資料夾互相搭配使用**
- **若需要將檔案存到另一電腦使用，則此兩個檔案 都必須一同移過去**

Plate Type 盤型定義

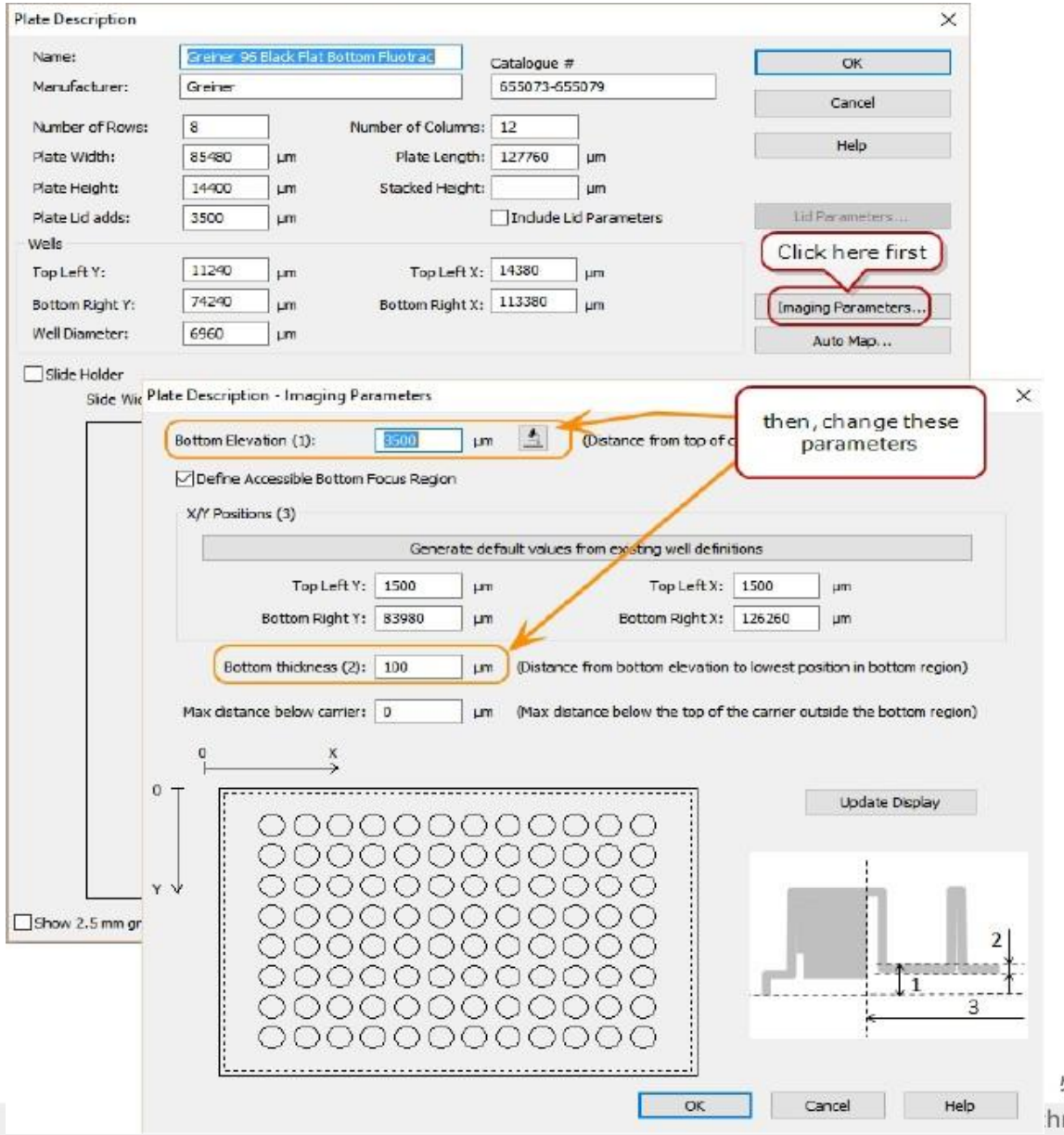
自動化影像拍攝(Experiment)時使用
需告訴儀器對焦位置起始點



Bottom elevation: 自動對焦起始點



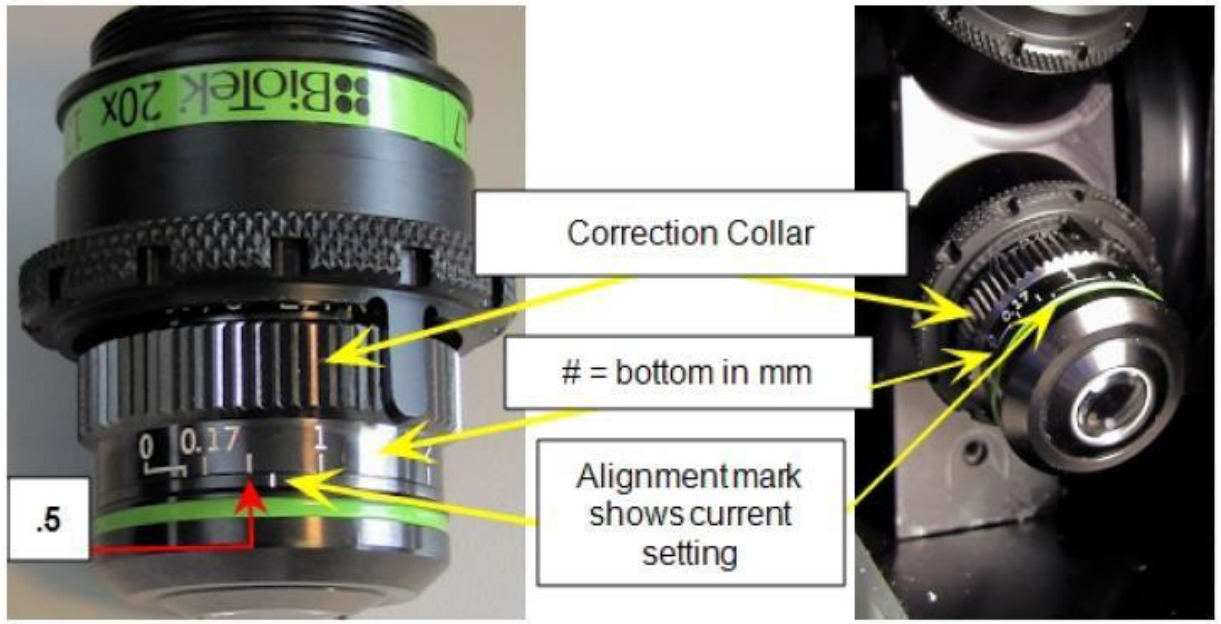
- Bottom elevation: 找到對焦的位置
- Bottom thickness: 盤子規格



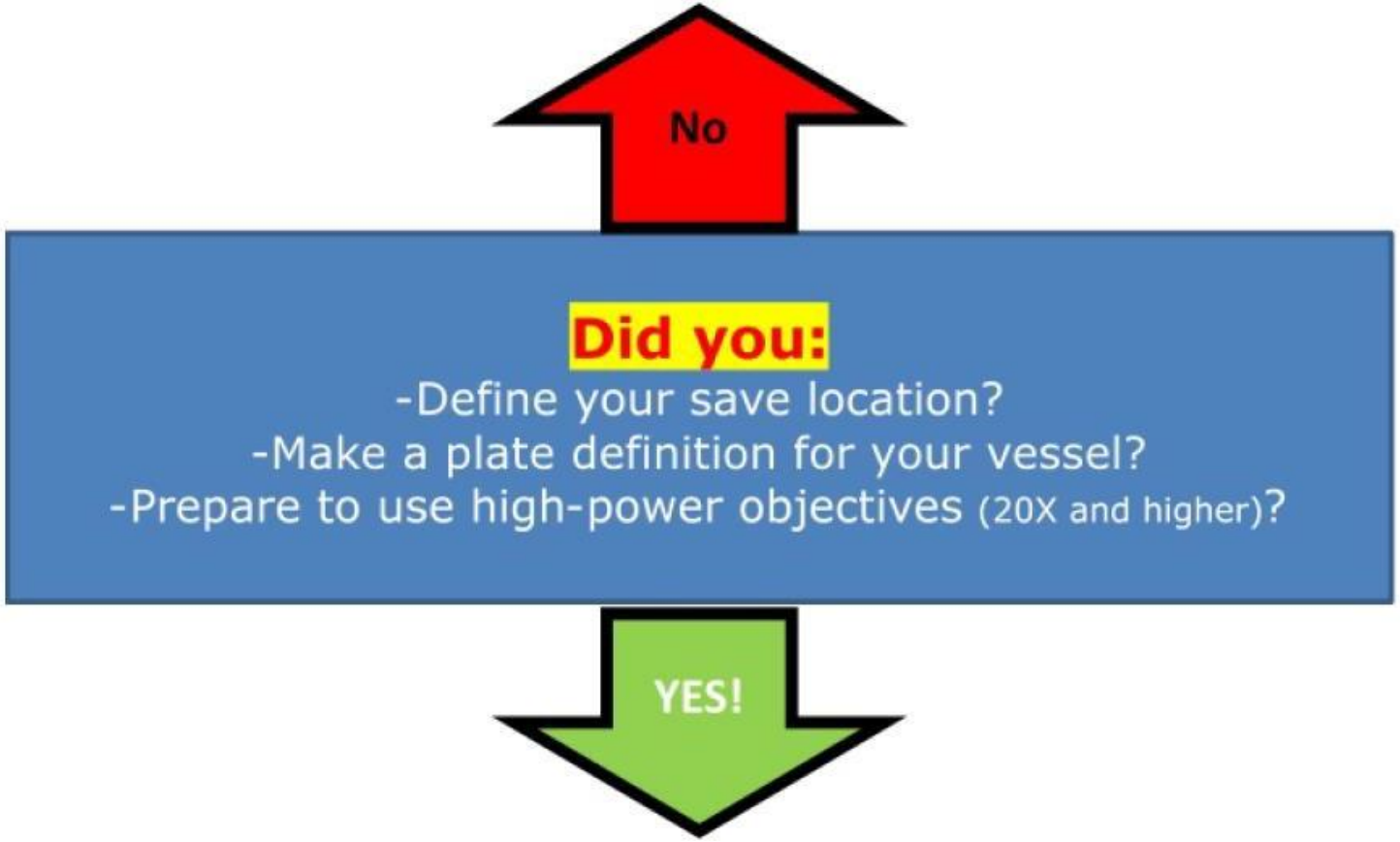
物鏡- Correction collar

20X, 40X 物鏡，需依據plate thickness 調整。

Plate Type	Typical Bottom Thickness
Glass-bottom microplate	0.17 mm
Cover slip	0.17 mm
Plastic microplate (e.g. 96-, 384-well)	0.5 mm
Low-density microplate (e.g. 6-, 12-well)	up to 2.0 mm



以上資訊是否都確認完整?

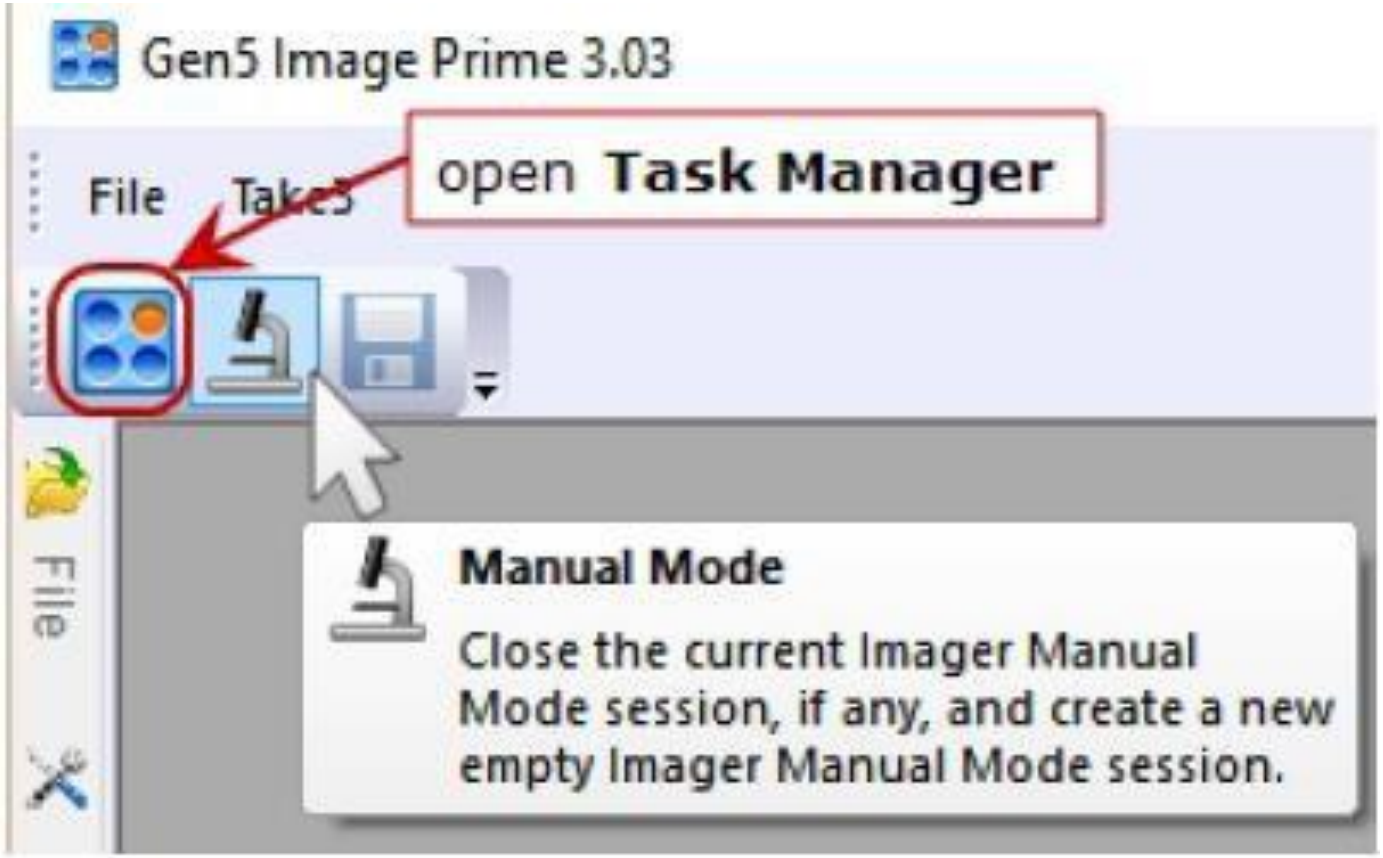


Manual mode

條件確認/少量樣本/單次拍攝/一般顯微鏡操作

從哪進入manual mode?

- Task Manager
- 顯微鏡符號
- .imm file

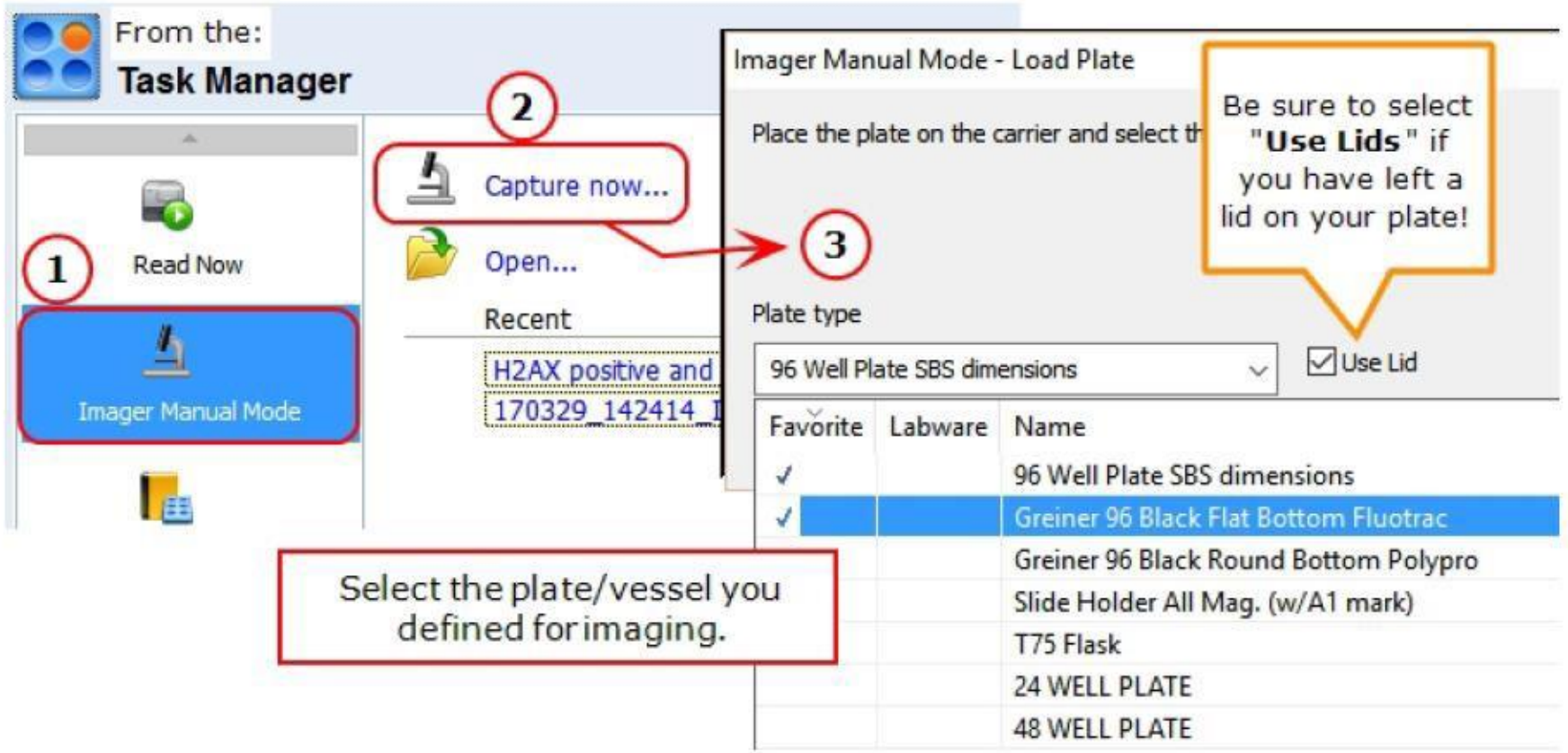


Manual Mode

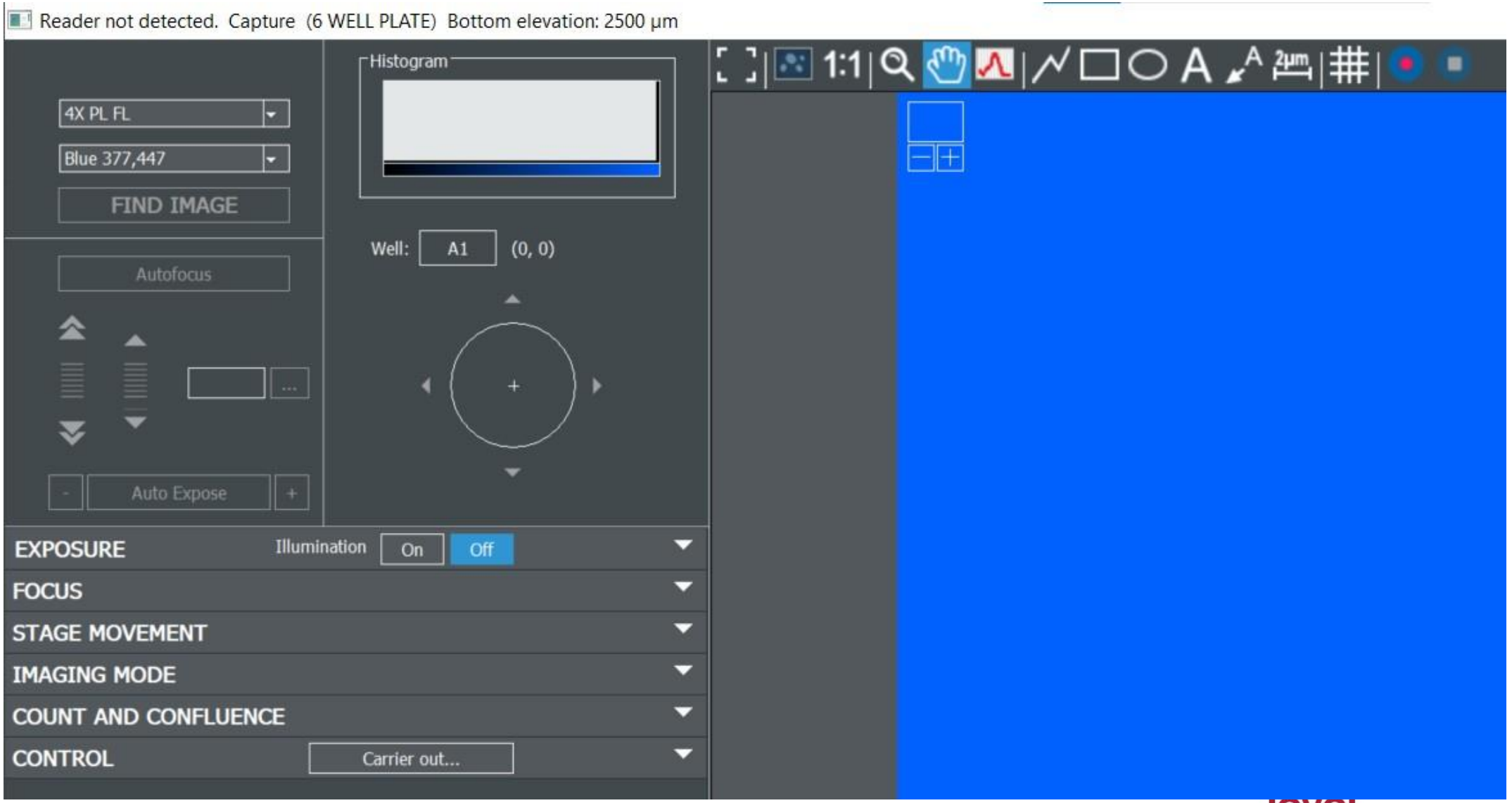
Capture now

選擇適當的盤型

若有含上蓋拍攝，請勾選“ Use Lid”



Manual Mode

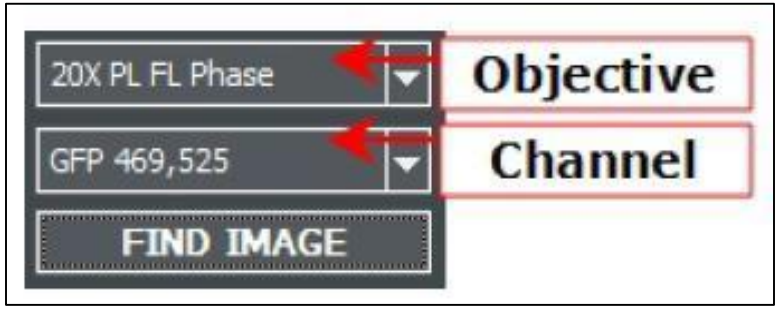


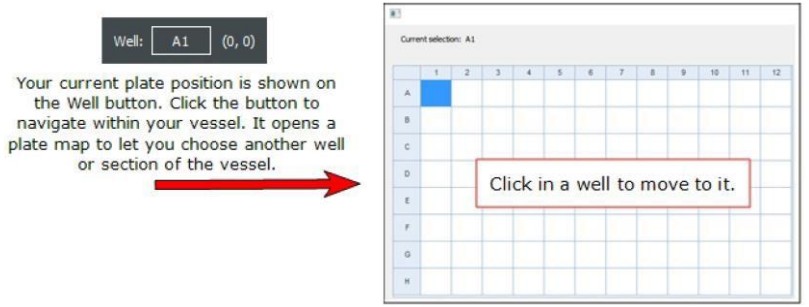
1.變換物鏡/拍攝模式與拍攝位置

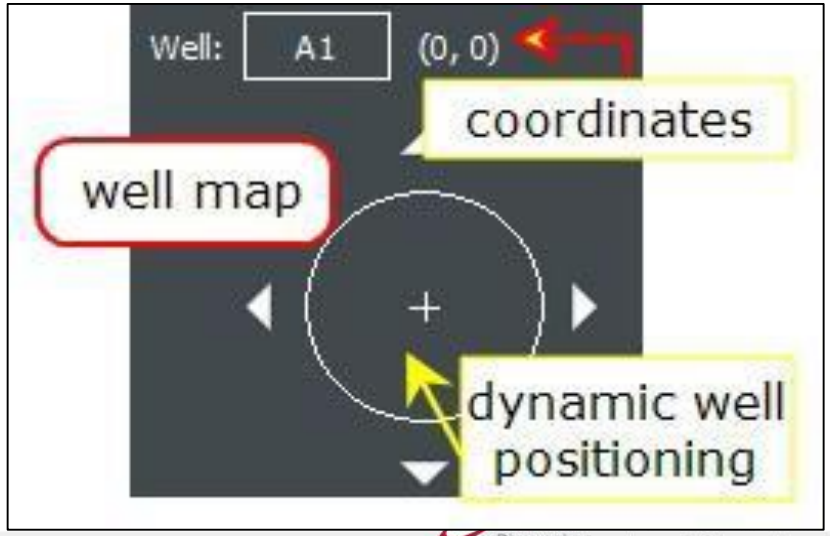
2.調整曝光(自動或手動調整)

3.調整對焦(自動或手動調整)

4.擷取影像

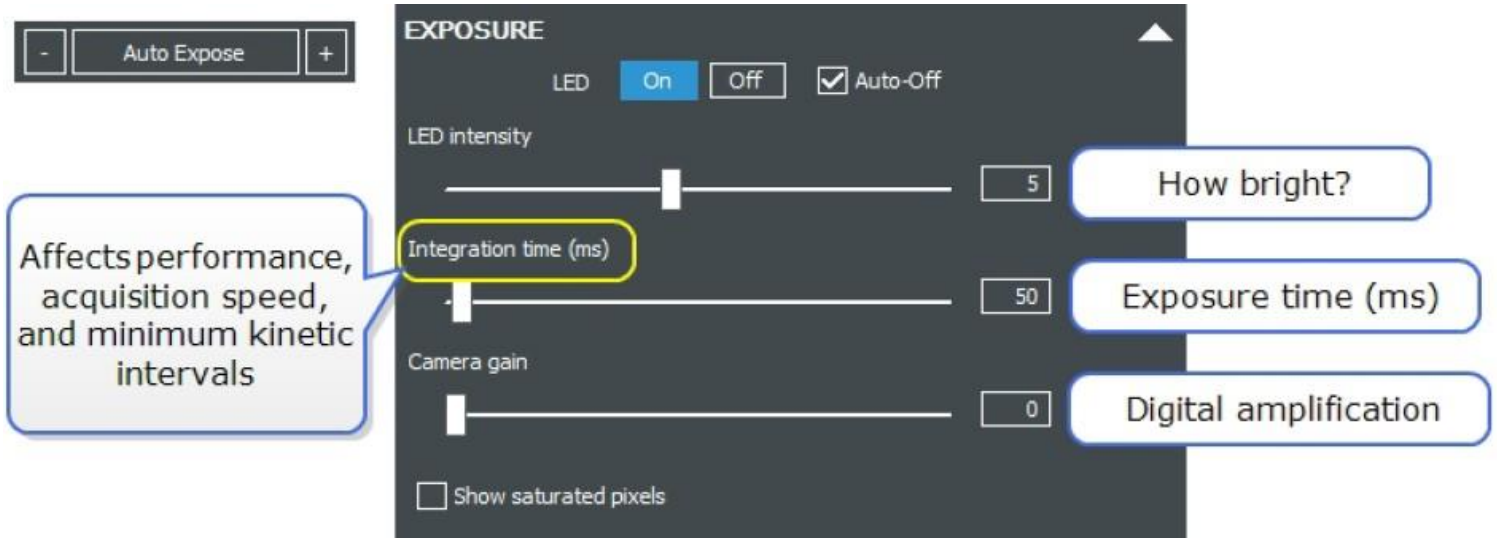


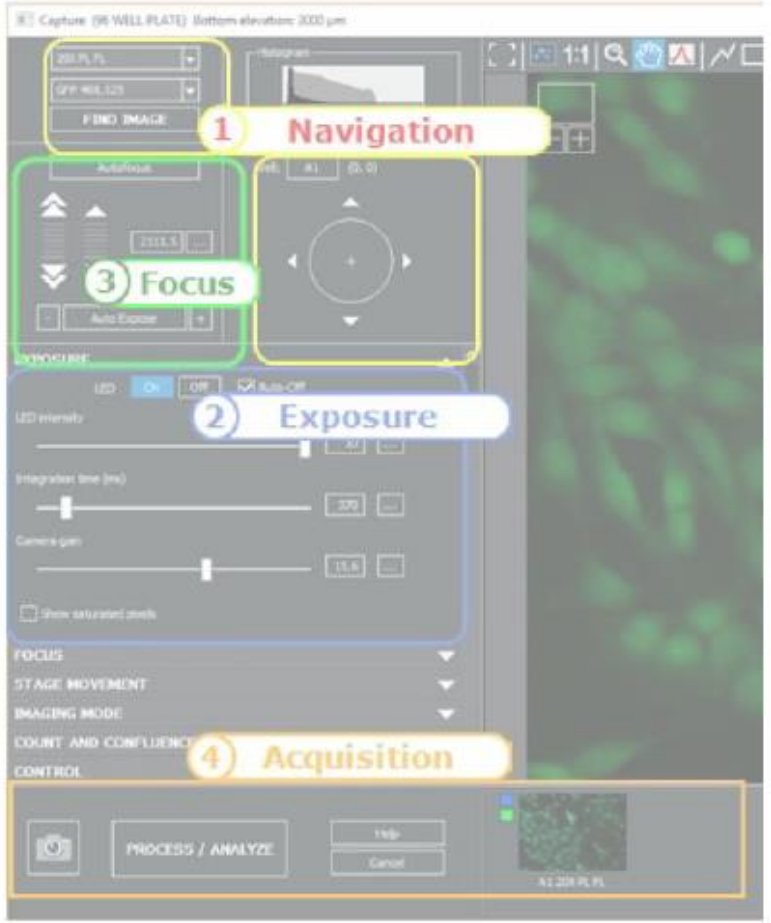




Exposure 曝光

自動或是手動調整



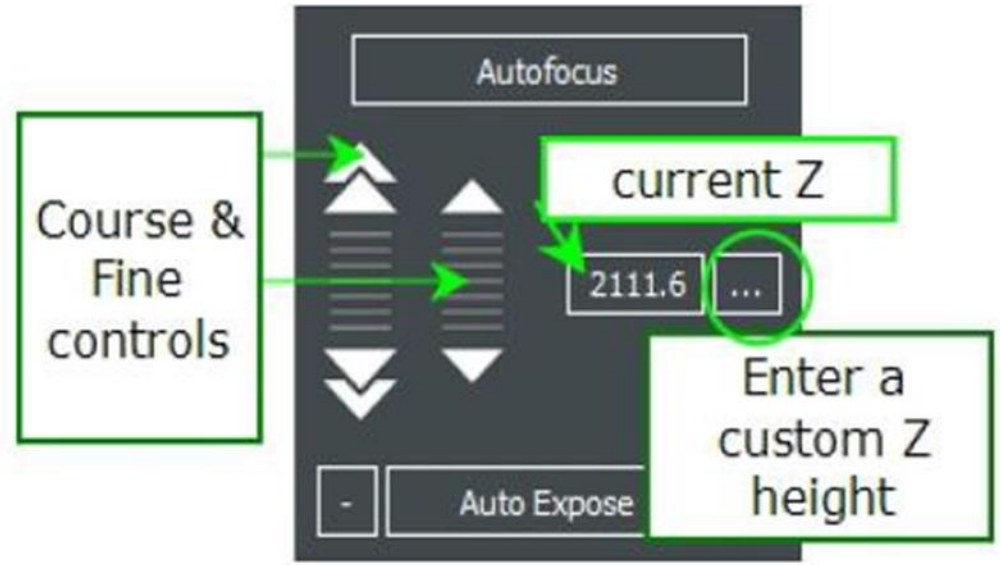


Exposure Parameter	Benefit of high setting	Drawback of high setting
1. LED Intensity	Sensitivity	Photo-bleaching
2. Integration time	Higher image quality	Significantly slower imaging speed
3. Camera gain	Faster read speed	Lower image quality

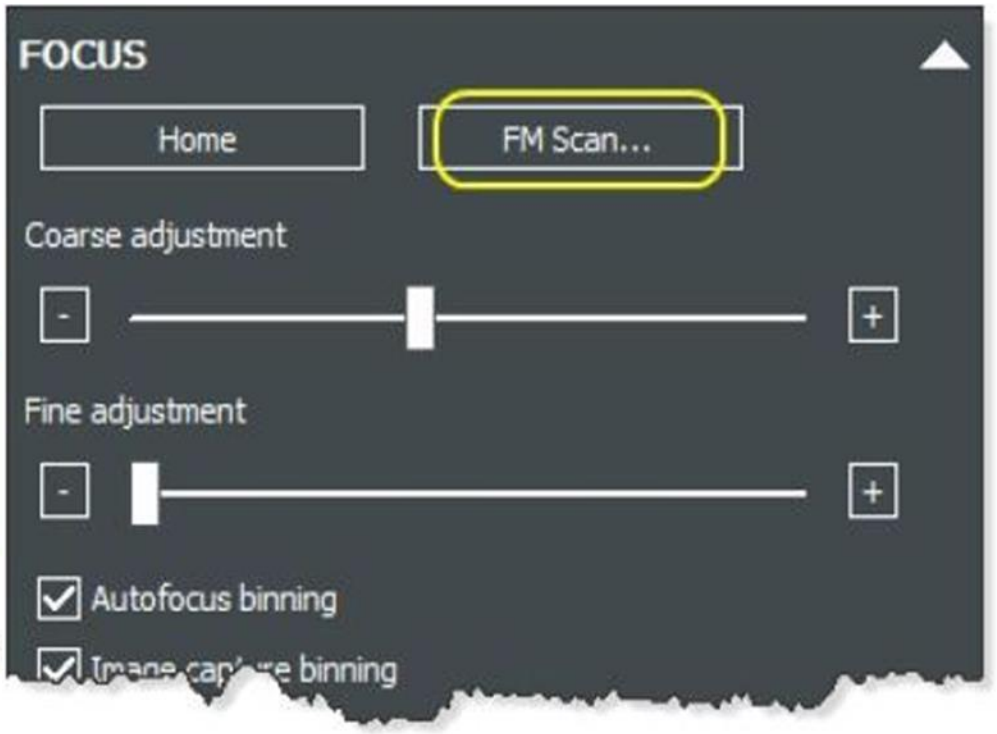
Auto focus 對焦

可點選Autofocus 利用箭頭上下微調

Current Z position 等於 Plate Bottom elevation之數據



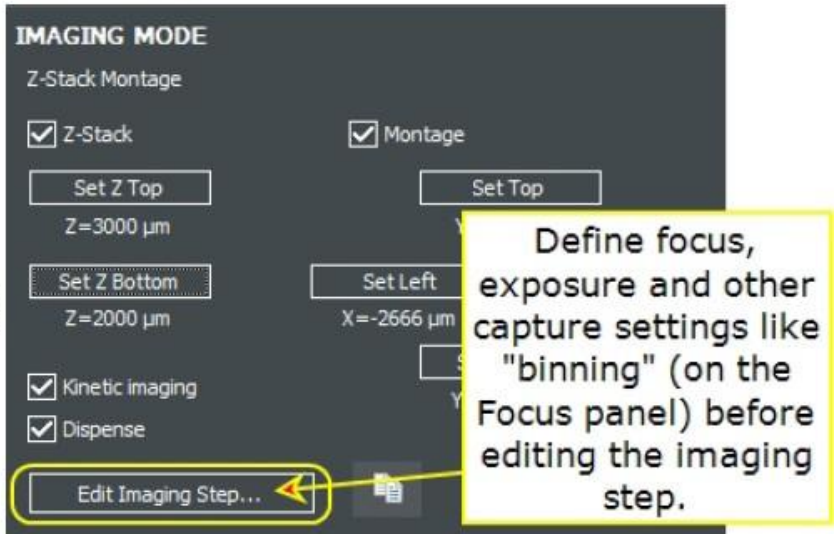
The focus controls help you position the objective for the best image acquisition.

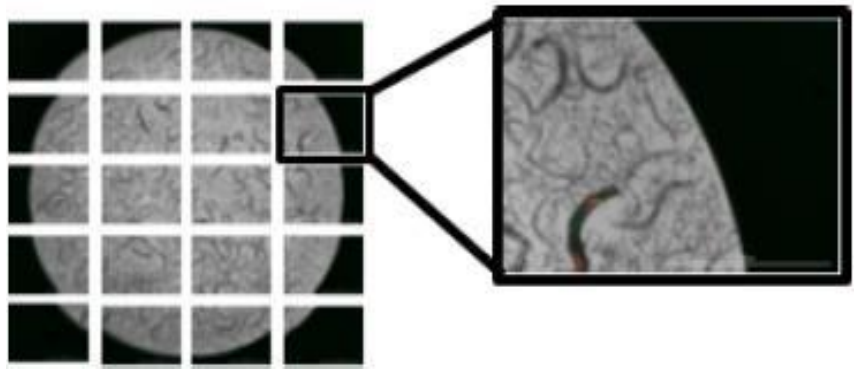


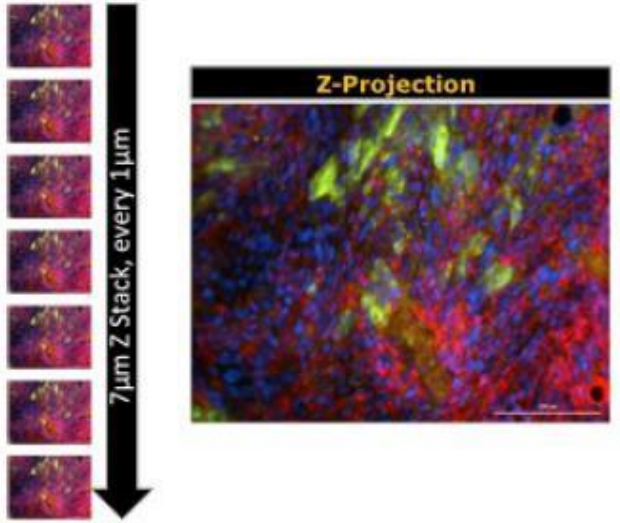
進階設定: Image Mode

IMAGING MODE The **Imaging Mode** panel in Manual Mode lets you define additional options for image acquisition, including:

- **Kinetic Imaging**: to capture live biology in real time or set up time-lapse imaging.
- **Dispensing**: if the optional Reagent Injector accessory is set up, dispense steps can be built into your assay.
- **Advance Imaging**: to acquire single vs multiple images in the X/Y (Montage) or Z (Z-Stack) plane.







大圖拍攝: Montage

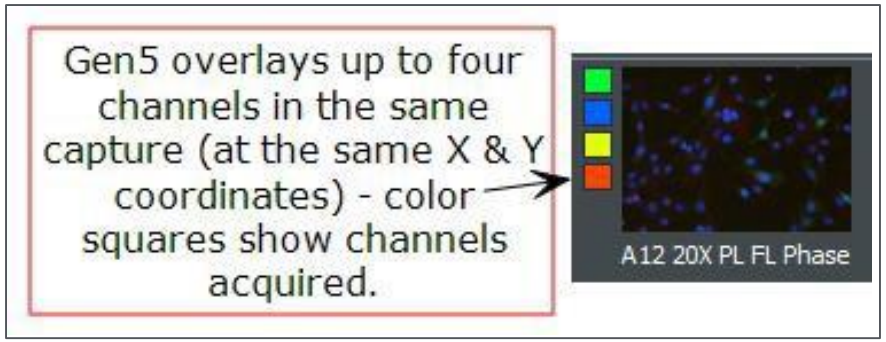
立體樣本: Z stacking

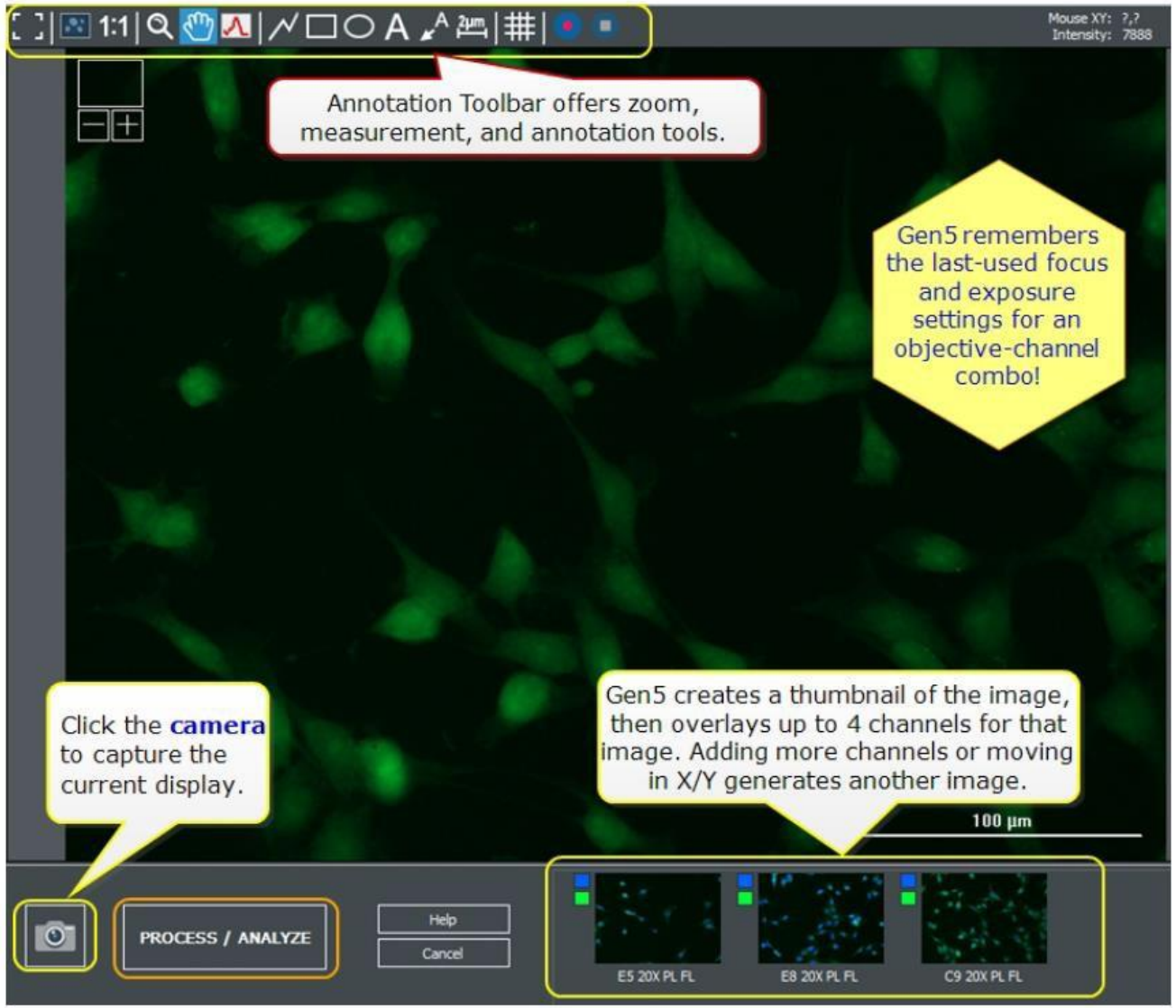
Kinetic imaging and dispensing: Fast kinetic assay

Edit imaging step

圖片拍攝

Process/Analyze 之後可編輯圖片





Manual Mode基本操作

1.選擇Objective與螢光

2.找到想拍攝的位置

3.先調整”曝光“，再調整”對焦”

4.確認無誤之後，即可拍攝

5.若需要繼續拍照，可持續移動位置、Objective與Channel拍攝。

6.若需要圖片後製與分析，點選” Process/Analyze”

Experiment
長時間拍攝/樣本量多/大圖拍攝/multi-channel拍攝

Experiment 設定

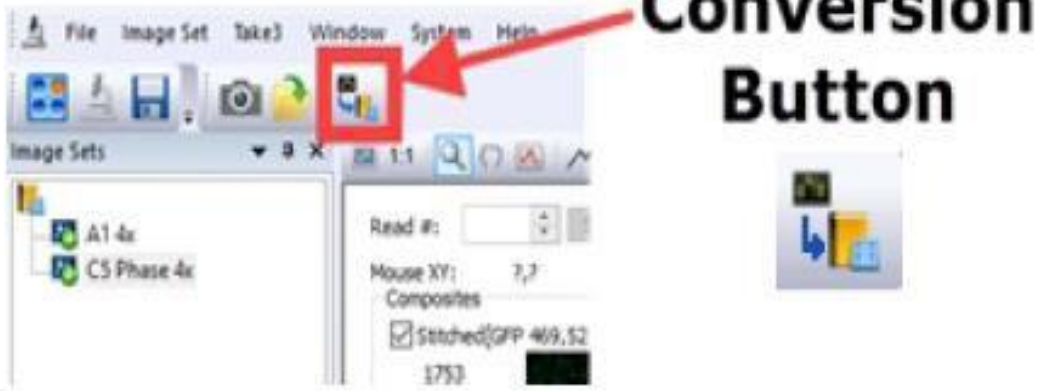
若你已經使用Manual mode 確認了

1.曝光/對焦條件

2.Bottom elevation 位置

3.並有大量樣品拍攝需求

可直接將Manual mode 的所有設定條件，轉到experiment做更進一步的設定



Conversion Button

1. Select the image of interest.
2. Click "**Create experiment from an image set**" button.

You can simply click the "Read New" button to begin acquisition.
To edit the protocol, see below.

Experiment: Procedure 設定

設定讀取條件

螢光/明視野/相位差/彩色明視野

曝光條件

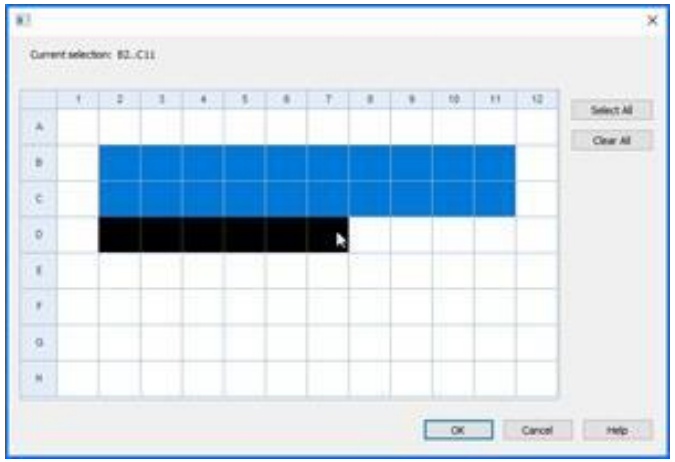
對焦方法

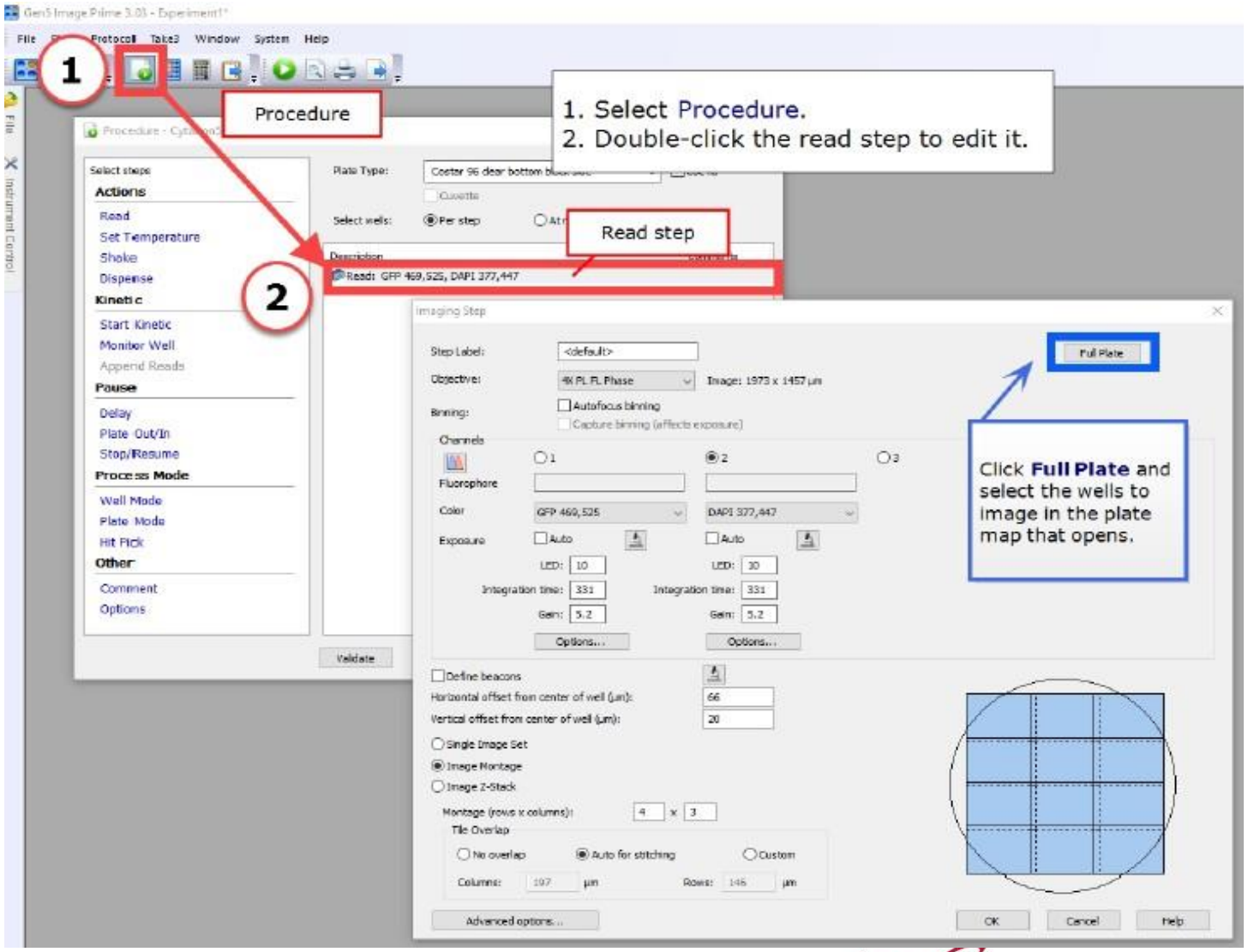
讀取位置

大圖拍攝

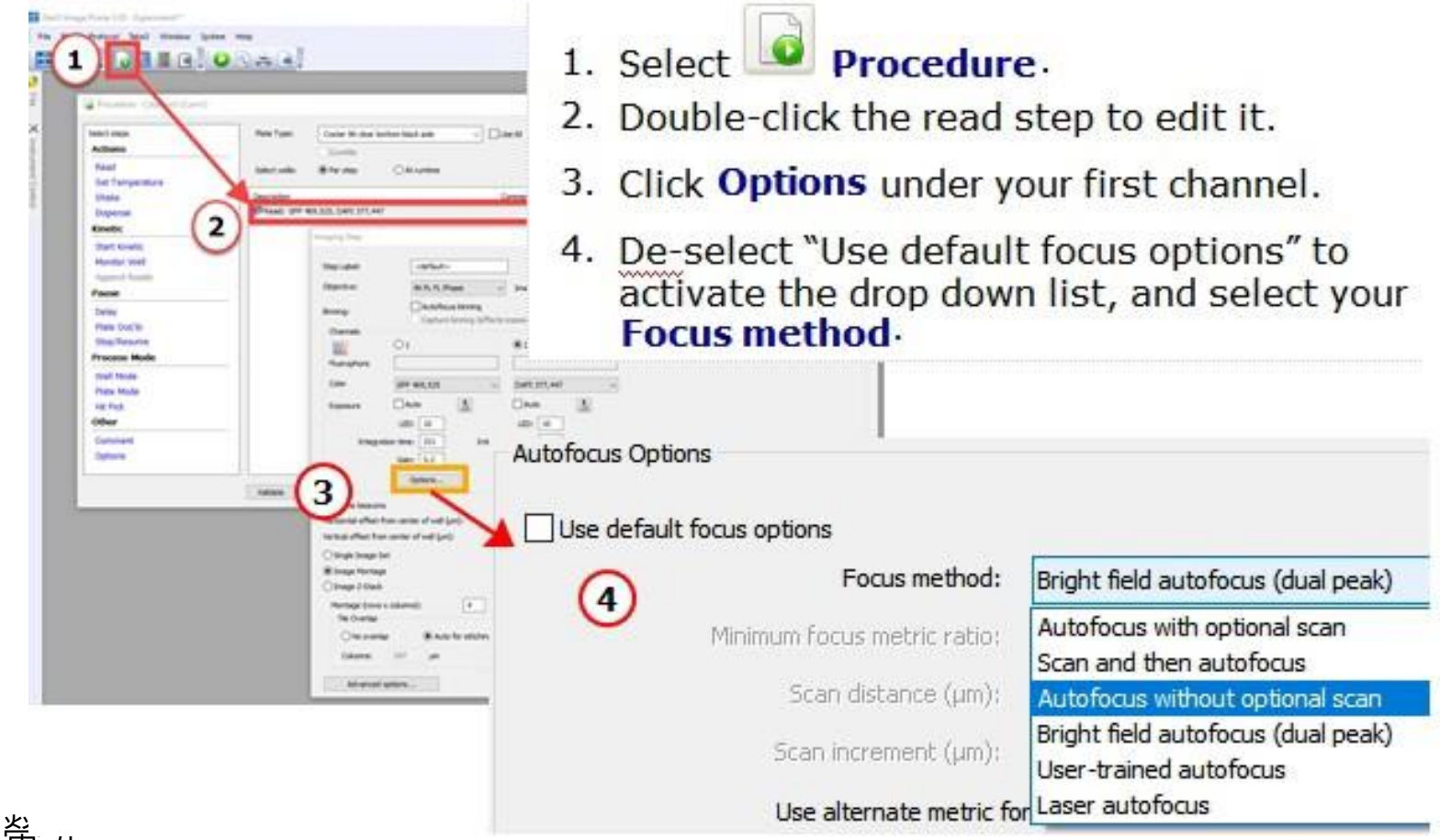
立體細胞拍攝

活細胞長時間影像拍攝





Autofocus 條件選擇



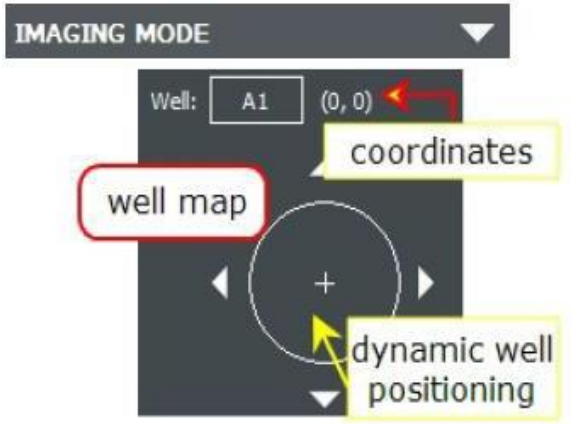
若同時拍攝三色螢光

Autofocus method 會follow第一個channel的對焦位置。

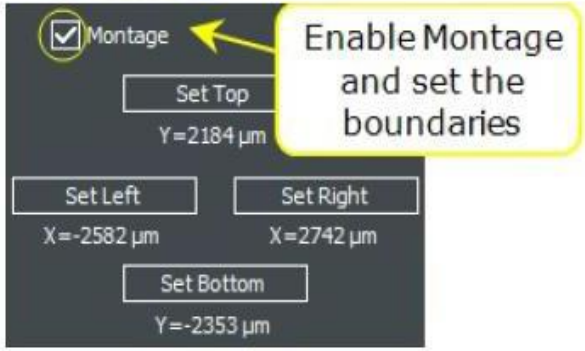
若三色螢光對焦位置皆有差異，則每個channel可自行定義Autofocus method

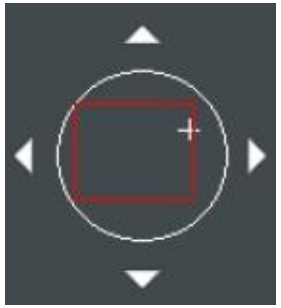
Montage: Manual VS Experiment

Manual

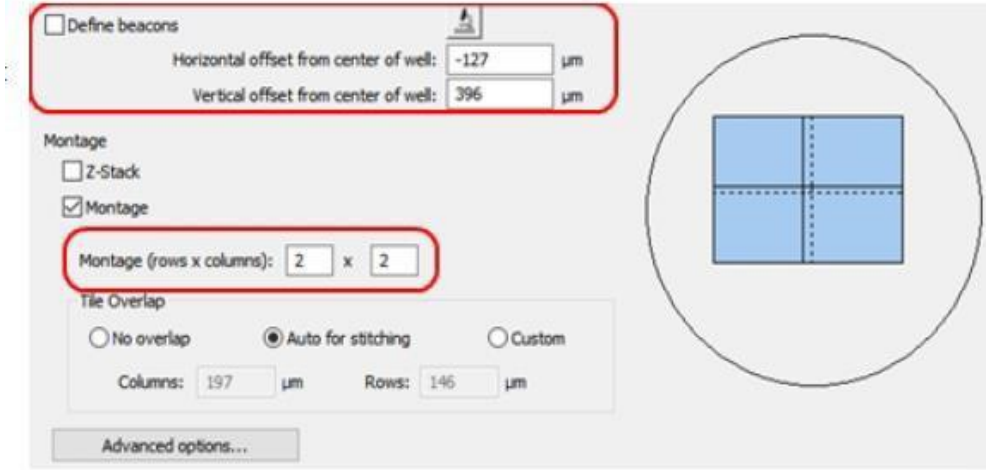


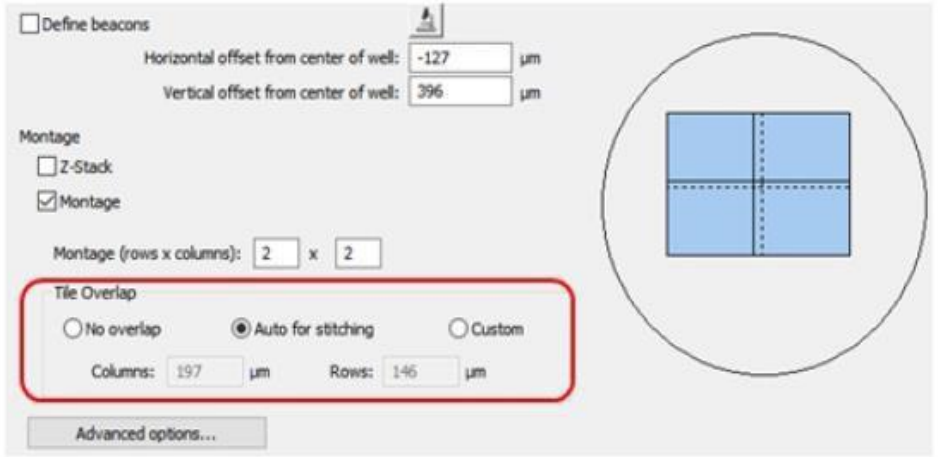
Expand the **Imaging Mode** panel.





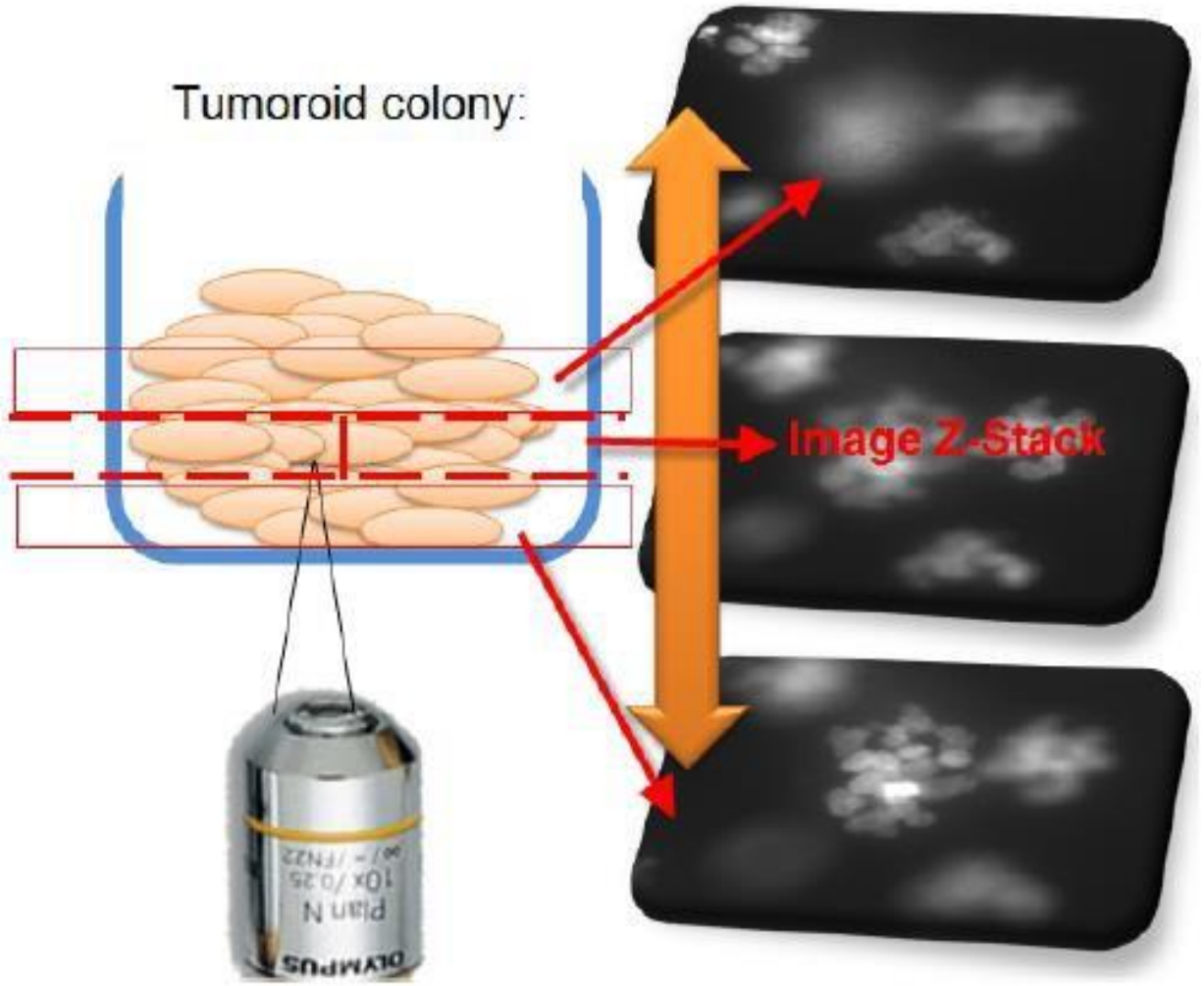
Experiment





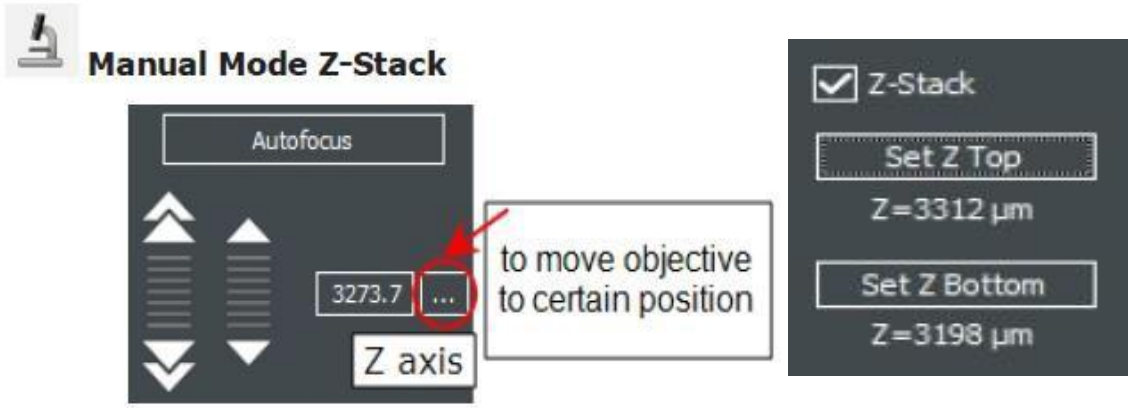
Next step: Stitching

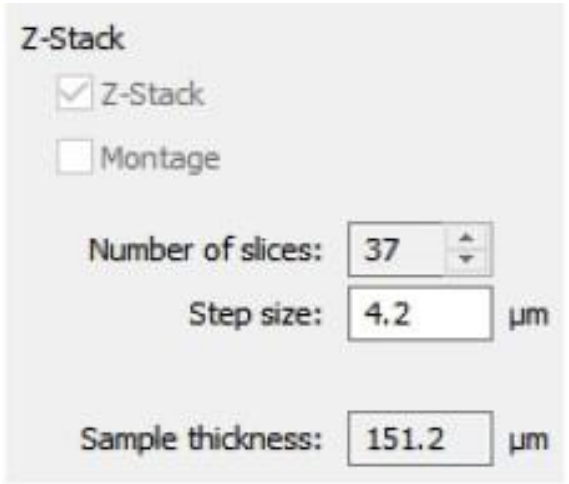
Z Stacking: Manual VS Experiment



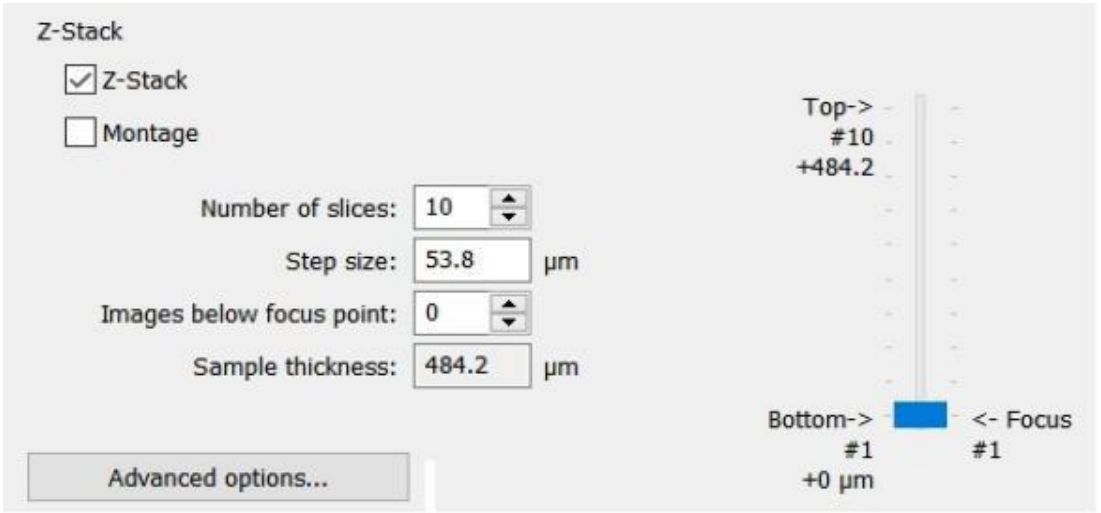
Z Stacking: Manual VS Experiment

Manual

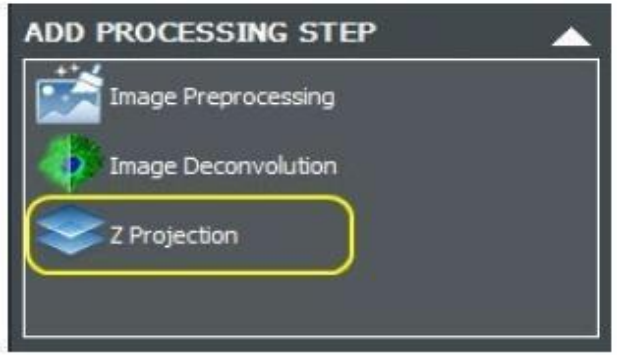




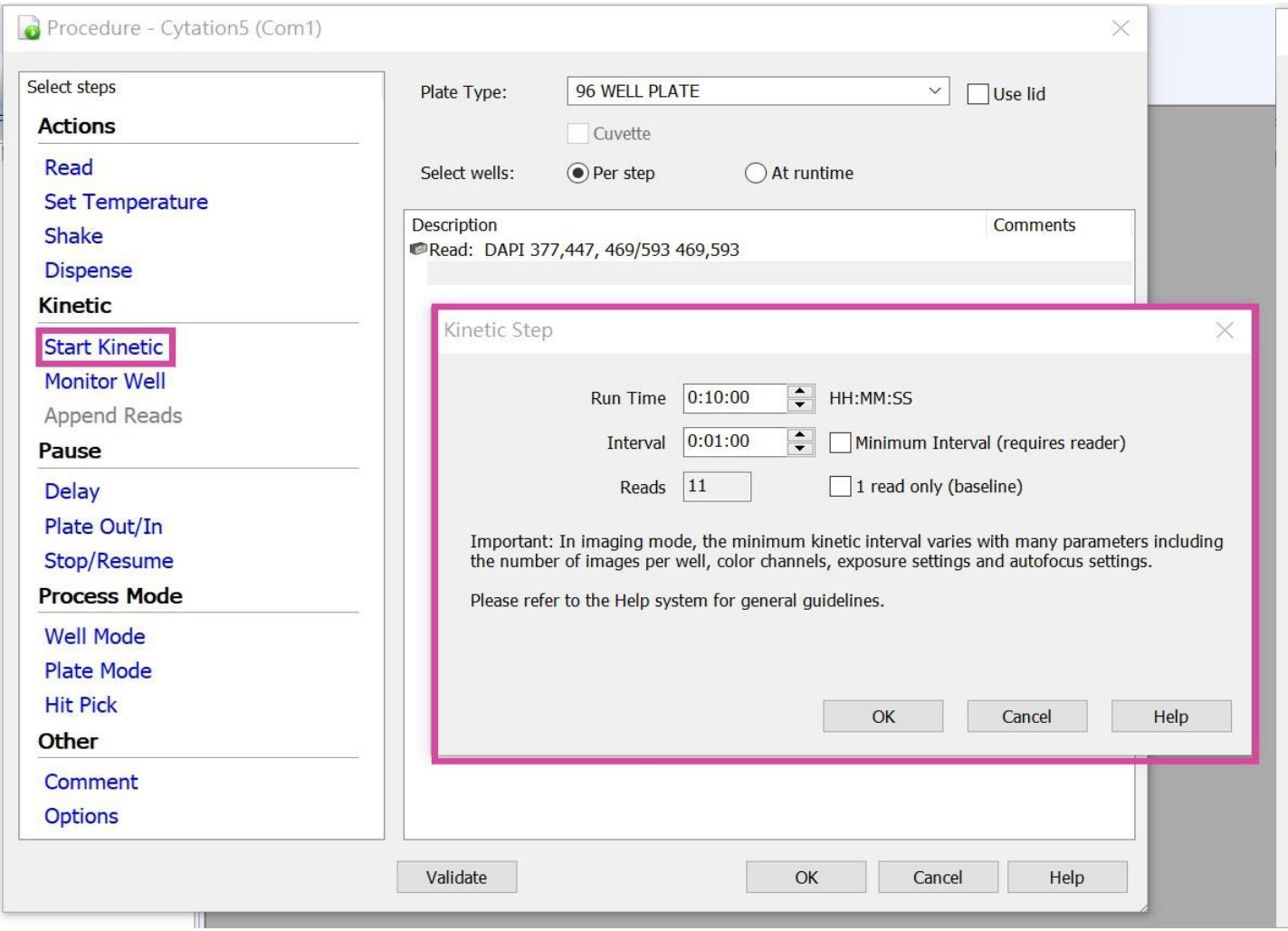
Experiment

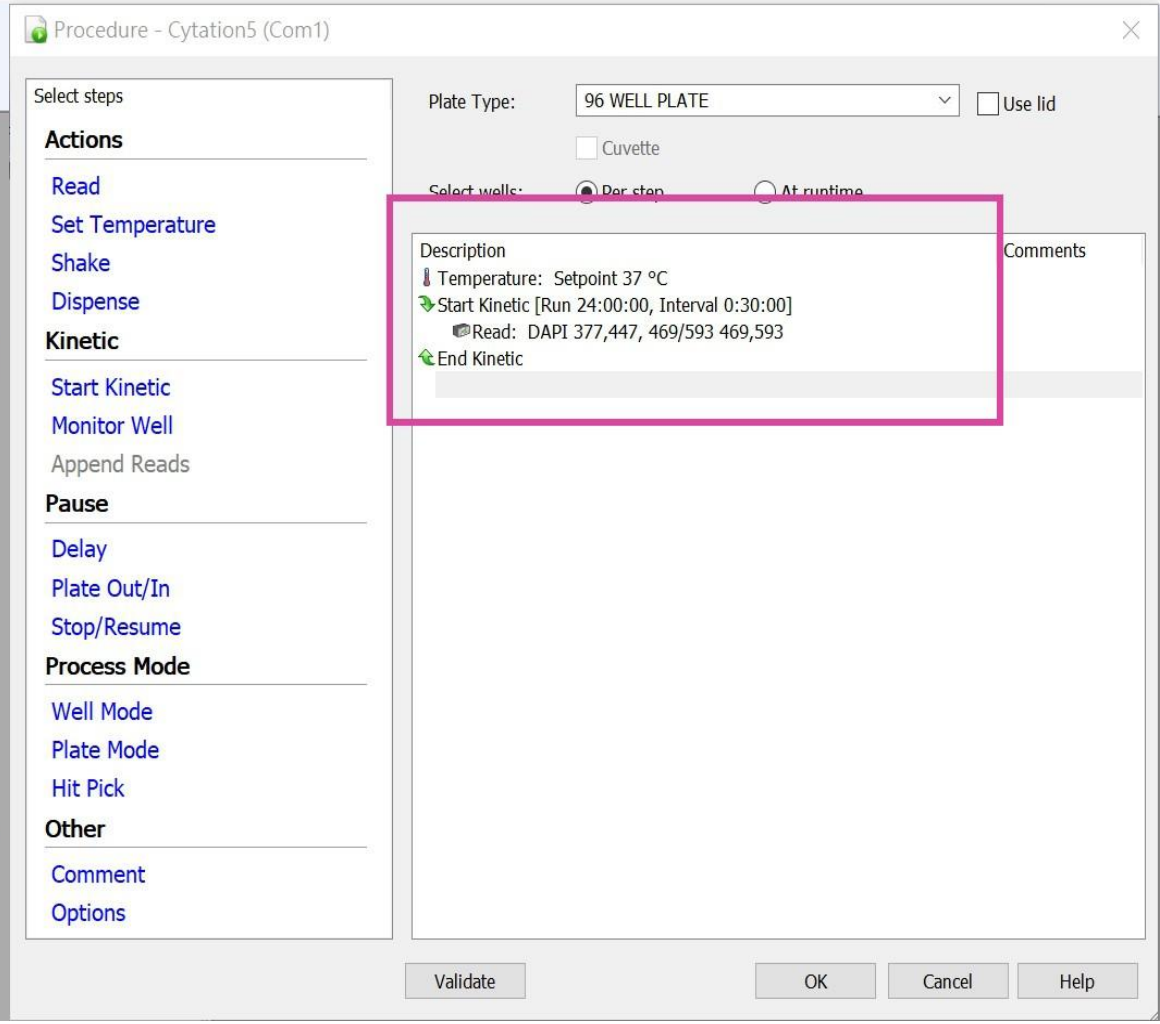


Next step: Projection

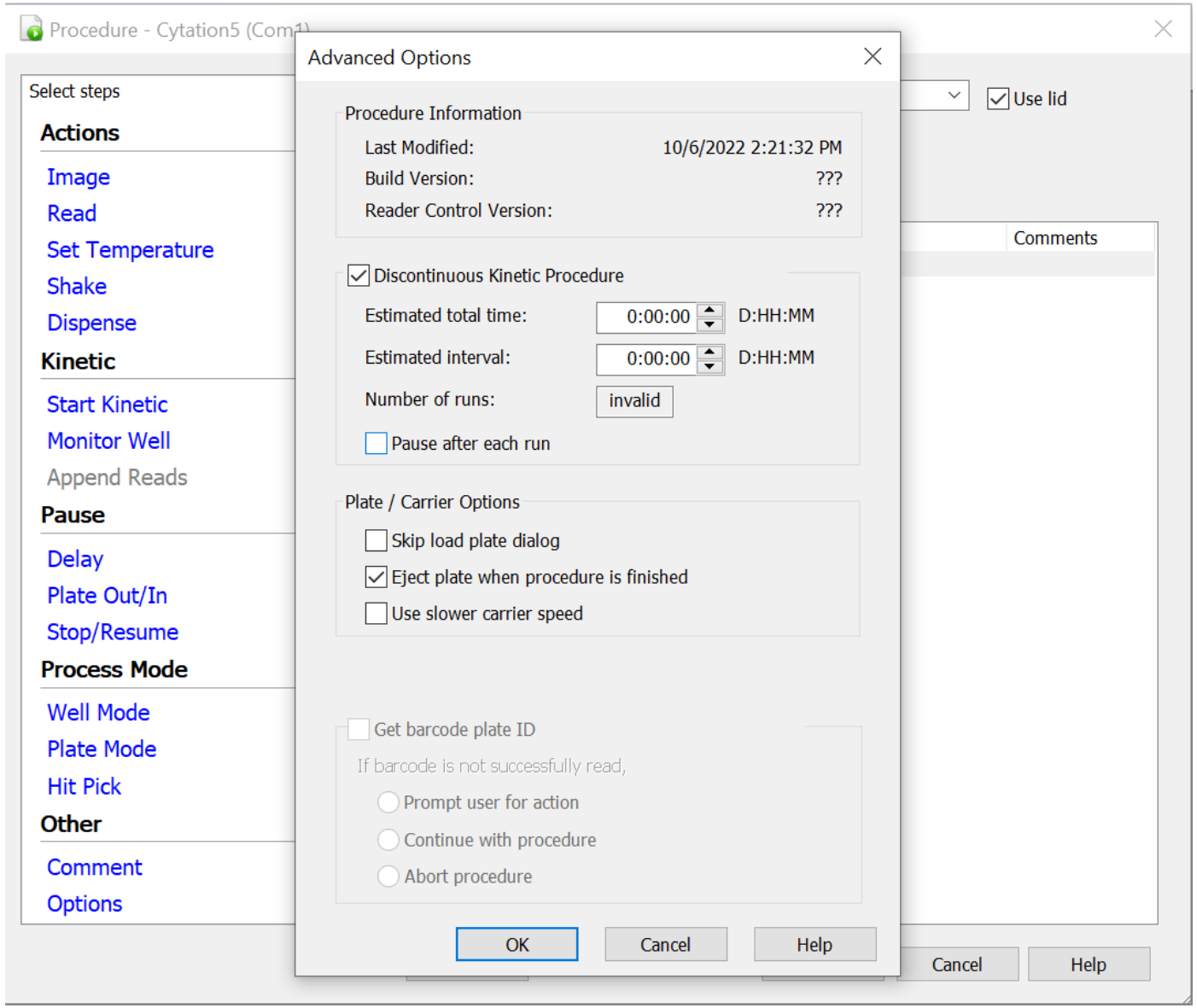


活細胞影像觀察: Kinetic Image Capture (無法暫停)





活細胞影像觀察: Discontinuous Kinetic (可暫停)



推薦的盤子品牌與型號

Recommended microplates:

- Greiner® µClear TC-treated 655090 96-well & 781091 384-well black polystyrene
- Greiner µClear cell-repellent 655976 96-well & 781976 384-well black
- Greiner 655892 96-well & 781892 384-well glass bottom
- Corning® 4850 96-well half-area and 4851 384-well glass bottom

Get best image quality when using high-power objectives using glass-bottom plates. Oil and low WD objectives require glass vessels.

20x, 40x倍物鏡→使用玻璃底

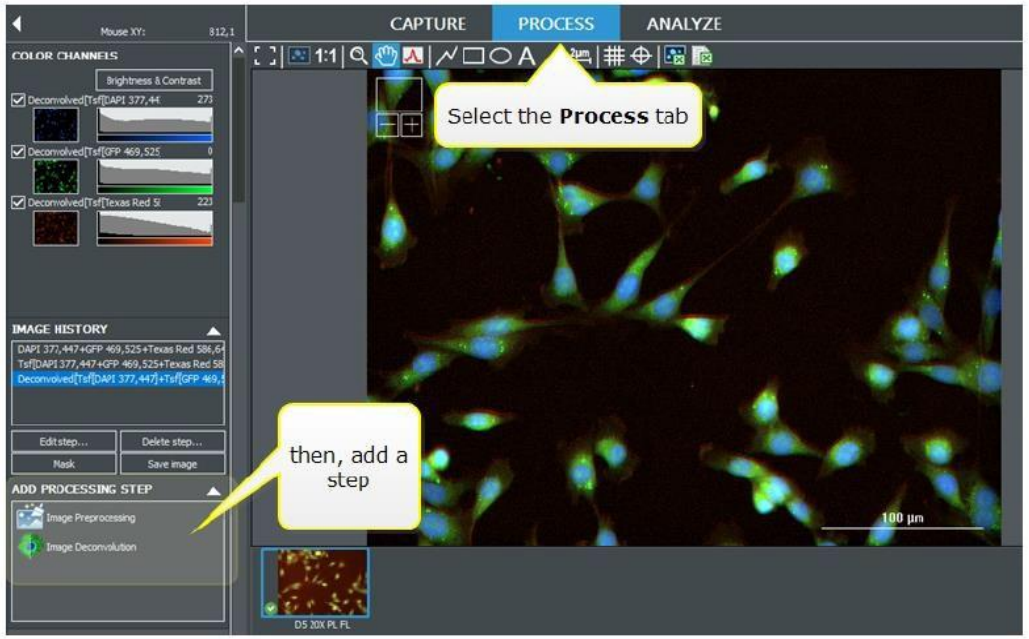
無法拍到對焦照片?

- Plate Type是否選擇正確?
- Bottom Elevation 是否設定正確?
- 高倍物鏡之Correction collar是否正確?
- 不同的Autofocus method
- 盤底清潔
- 適當的細胞數量 (40-70% confluency)
- 曝光條件調整

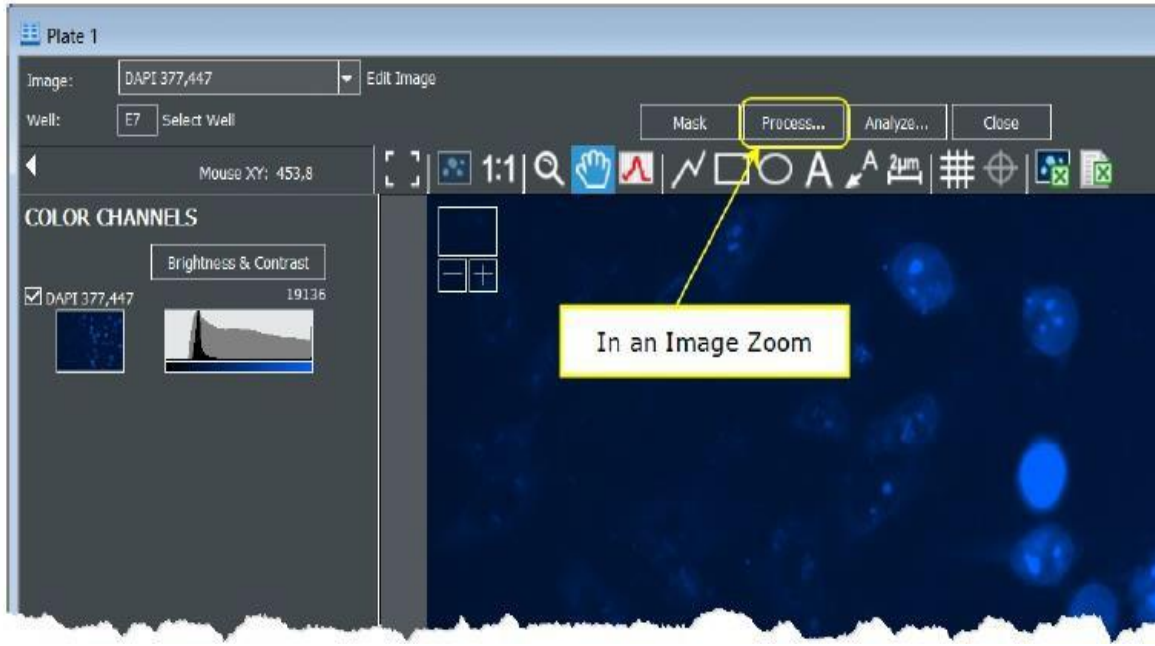
Optimize your data: Process

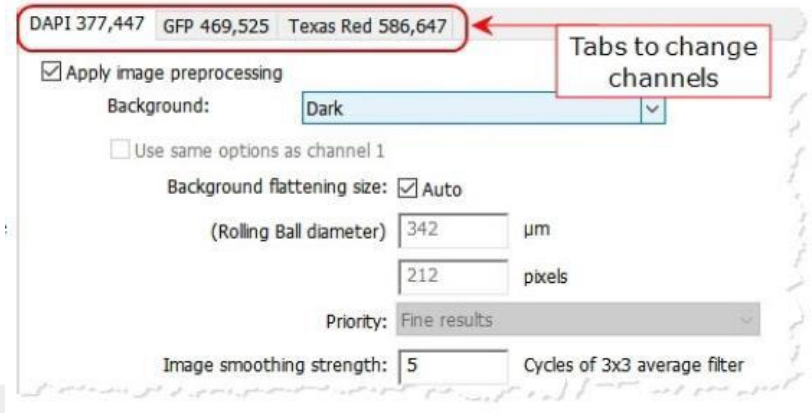
去背景值: Image Preprocessing

Manual

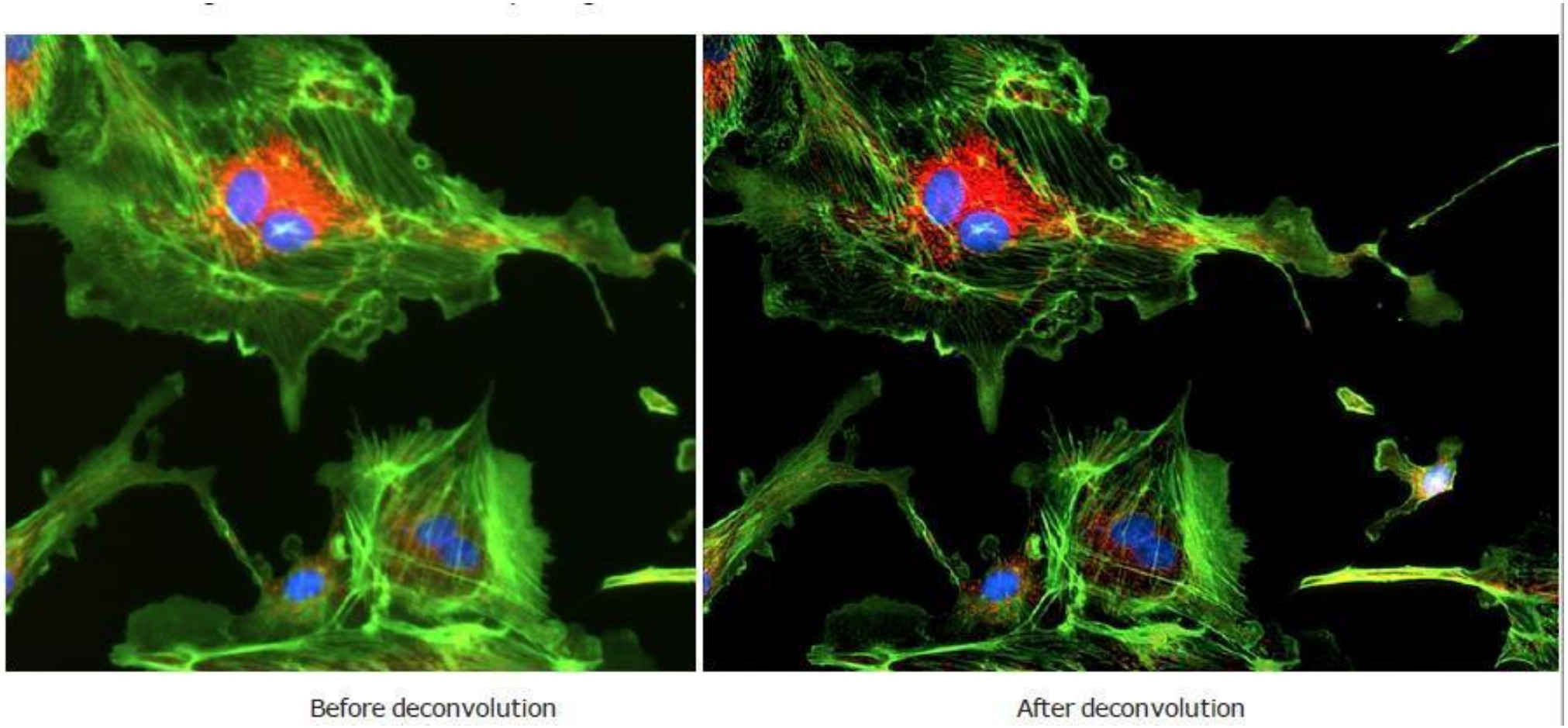


Experiment





線條銳利化: Deconvolution



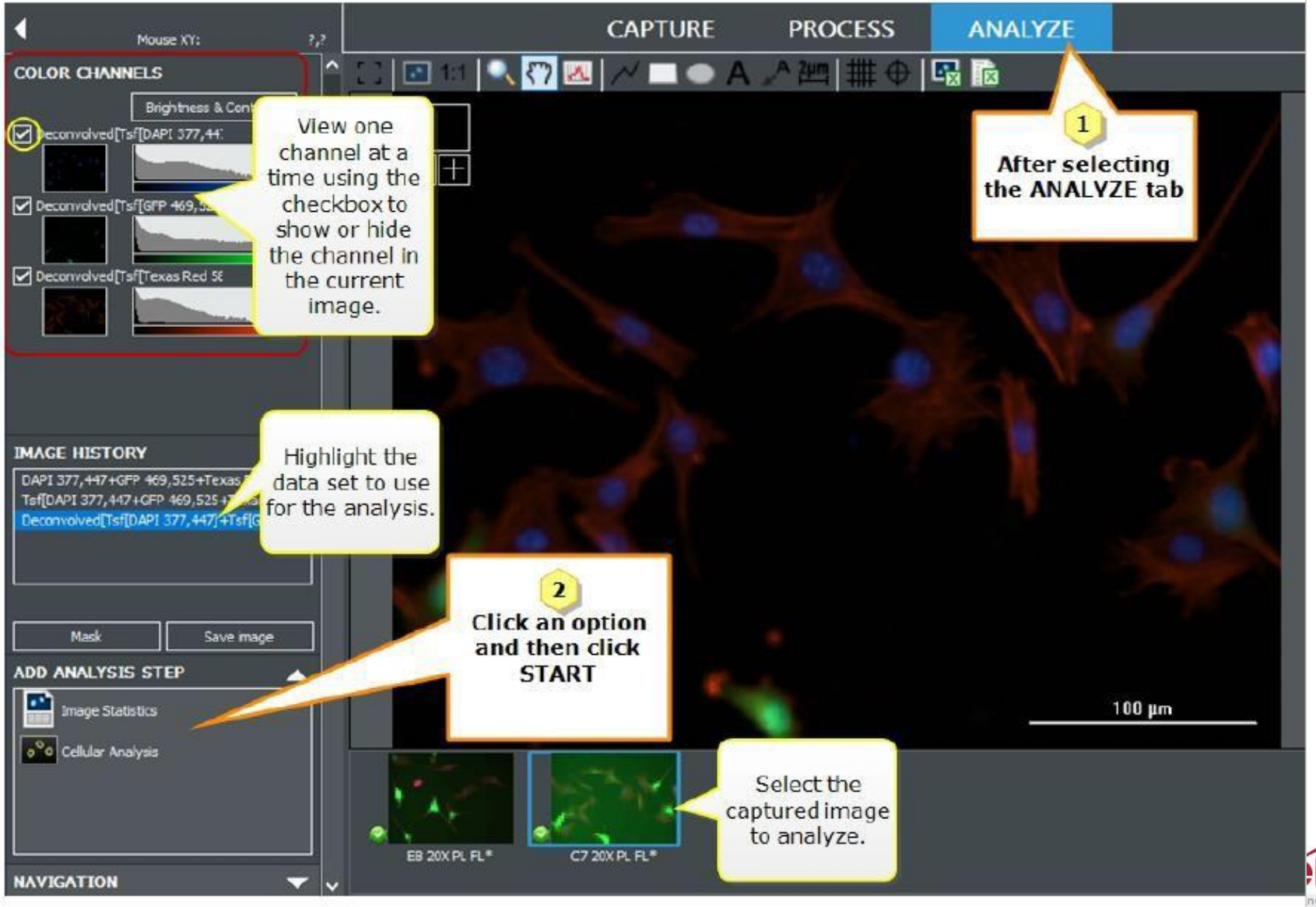
Before deconvolution

After deconvolution

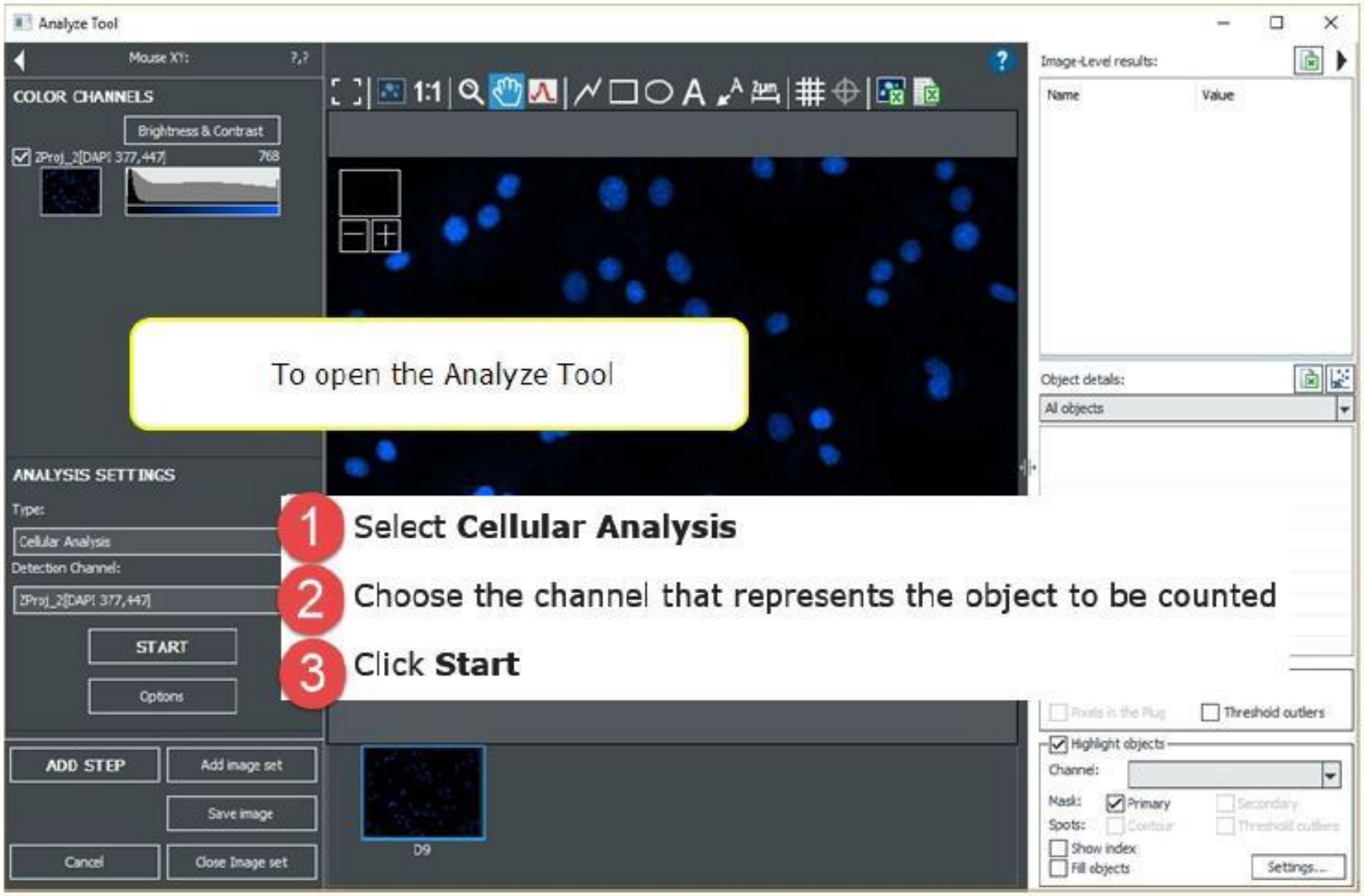
Basic Image Analysis

Basic image analysis

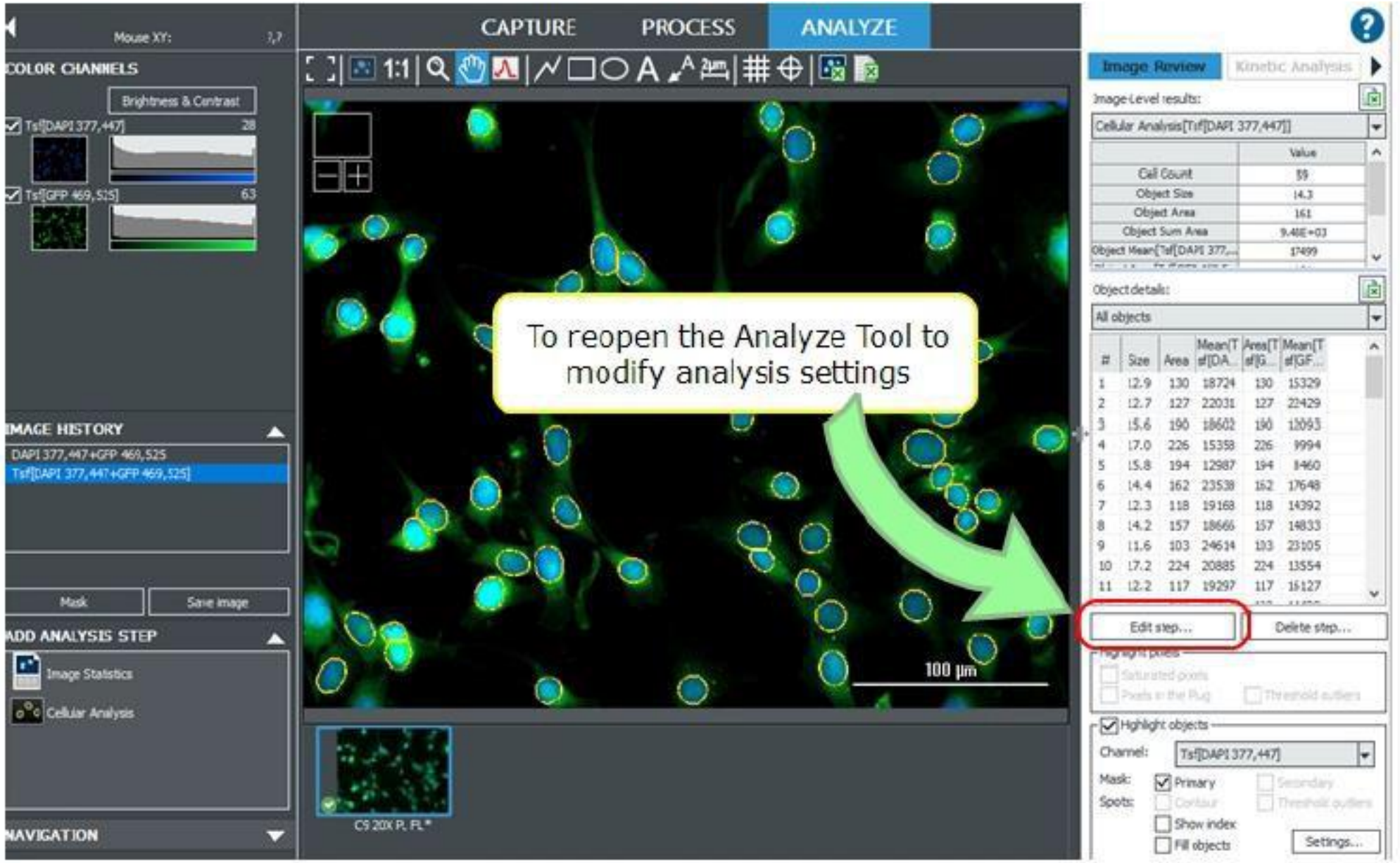
選擇image statistics 或cellular analysis



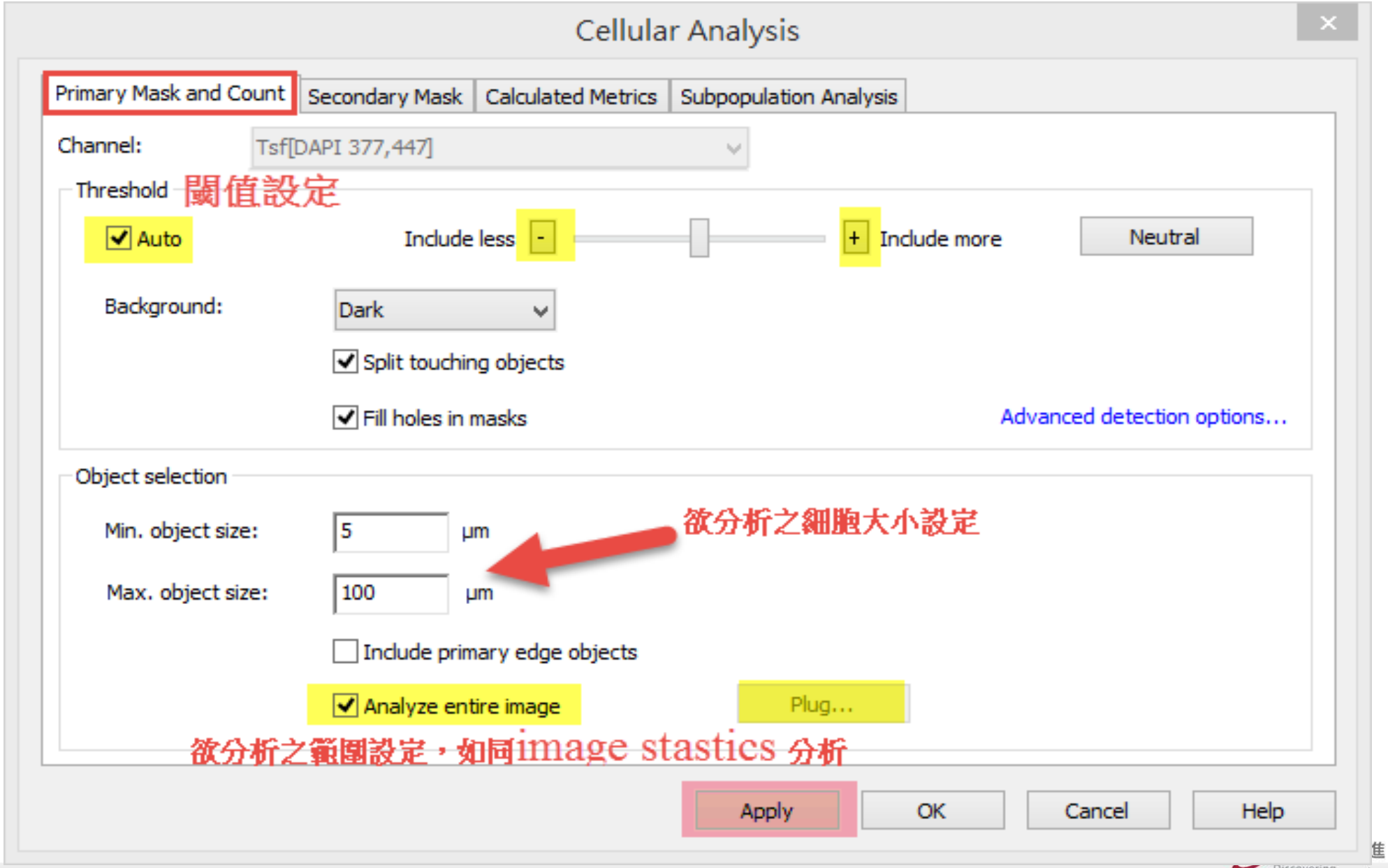
細胞計數: Cellular Analysis



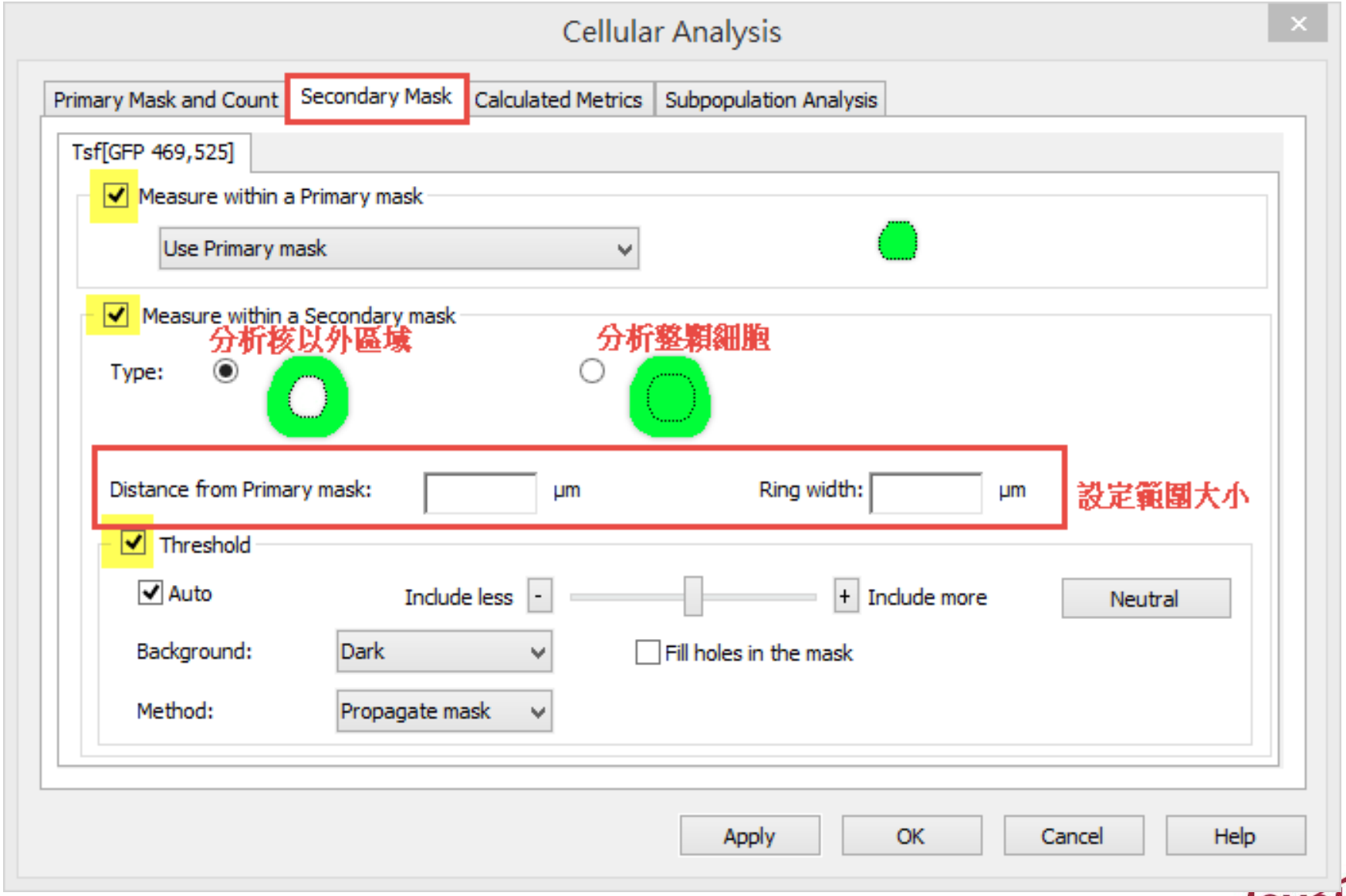
細胞計數: Cellular Analysis



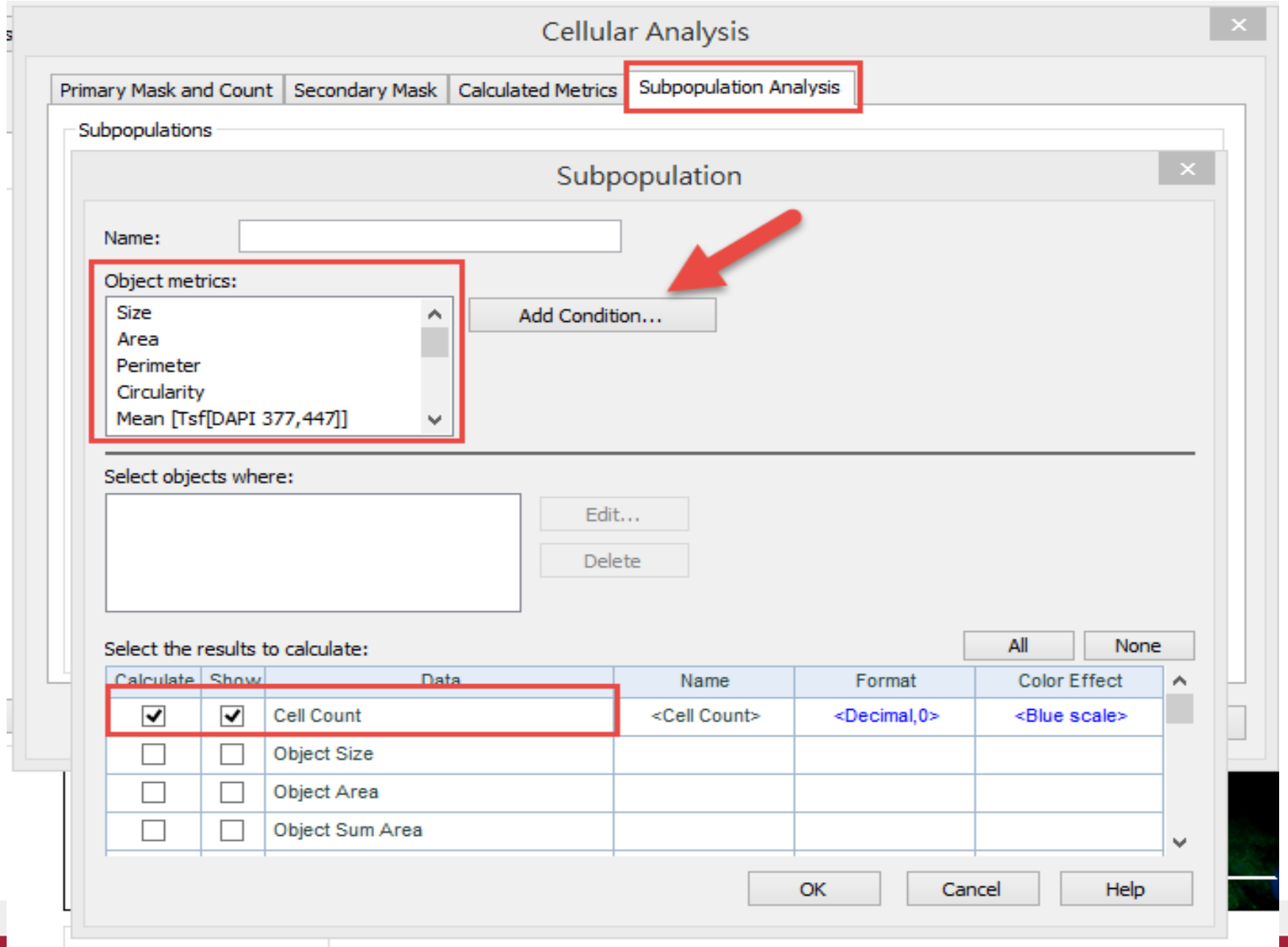
Cellular Analysis



Secondary Mask



Subpopulation 分析：細胞分群



Save Images

檔案儲存位置

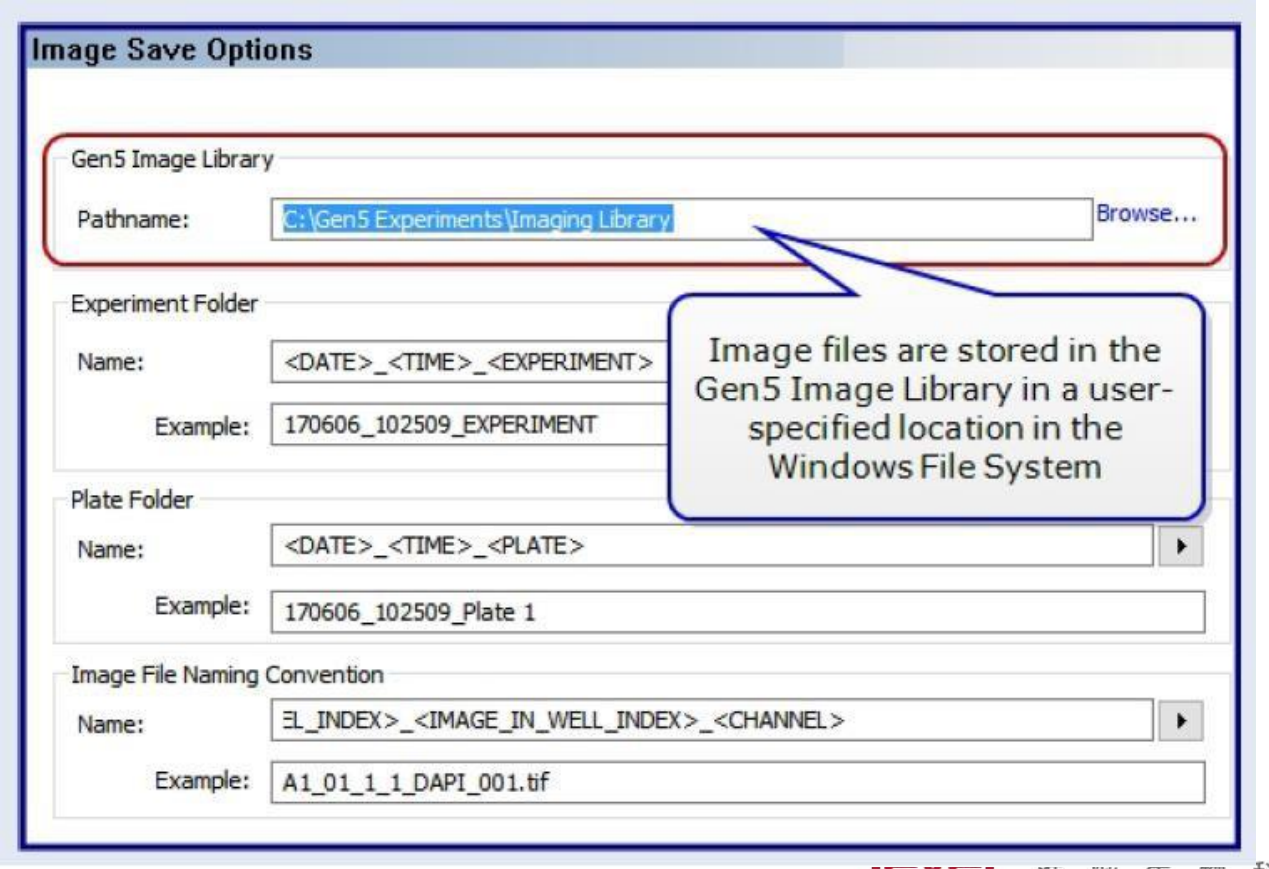
Gen5 file 儲存位置

- Gen5 Experiment (.xpt) 或是Protocol檔案 (.prt)
- Default儲存位置是在. C>使用者>公用>公用文件>Experiment or Protocol

圖片檔案儲存位置

- 硬碟 (可指定)

- **Gen5 Experiment 和圖片資料夾互相搭配使用**
- **若需要將檔案存到另一電腦使用，則此兩個檔案都必須一同移過去**



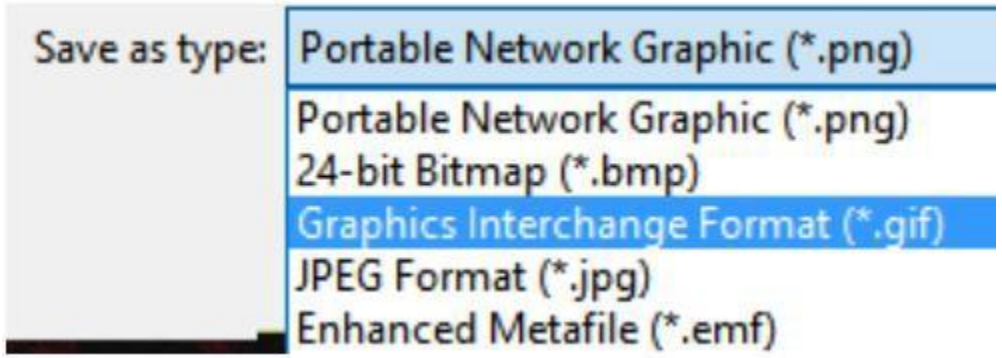
Save Image

手動模式：

滑鼠右鍵> Save> Save Picture for presentation

實驗模式：

Plate > Batch Image Save



Thank you for listening

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Discovering
new boundaries

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Level Biotechnology Inc.

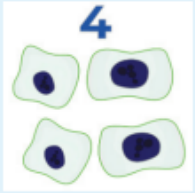
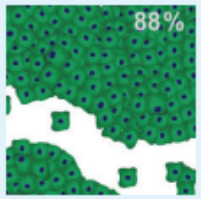
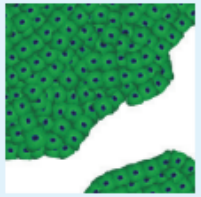
Gen5 Image Prime 影像分析

細胞計數

覆蓋率

螢光強度

總面積

Gen5 Image-Level Parameters and Applications			
Image-Level Parameter	**Possible Targets**		
Cell Count 	Cell count	Spot count (liposomes, mitochondria, endosomes, autophagosomes)	
	Cell viability	Toxicology (hepatotoxicity, genotoxicity, necrosis)	
	Micronuclei Formation	Toxicity in whole organisms	
	Proliferation		
Confluence 	Cell confluence	Syncytia formation	Neurotoxicity
	Cell viability	Hypertrophy	Proliferation assays
	Colony formation	Microtubule formation	Scratch/wound healing assays
	Cytotoxicity assays	Neurite outgrowth	Immune cell activation
Total Intensity 	Cell viability	Syncytia formation	Proliferation assays
	Colony formation	Microtubule formation	Signal appearance/disappearance
	Cytotoxicity assays	Neurotoxicity	
Total Area 	Cell viability	Syncytia formation	Neurite outgrowth
	Colony formation	Microtubule formation	Neurotoxicity
	Cytotoxicity assays	Hypertrophy	Proliferation assays

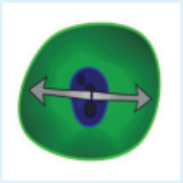
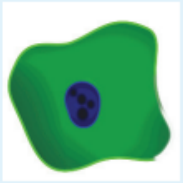
Gen5 Image Prime 影像分析

細胞大小

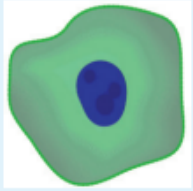
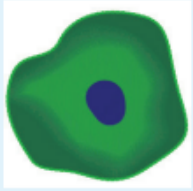
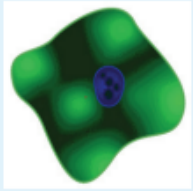
細胞面積

細胞周長

細胞圓度

Gen5 Object-Level Parameters and Applications			
Object-Level Parameter	**Possible Targets**		
Object Size 	Apoptosis/necrosis	Cytoskeletal changes	Organelle health
	Cell cycle	Colonies	Toxicity
	Cell morphology	Hypertrophy	Toxicity in whole organisms
	Clusters	Lipid formation	Microtubule formation
Object Area 	Apoptosis/necrosis	Syncytia formation	Neurotoxicity
	Cell cycle	Hypertrophy	Nuclear health
	Cell viability	Microtubule formation	Scratch/wound healing assays
	Colony formation	Neurite outgrowth	
Object Perimeter 	Cell cycle	Hypertrophy	Nuclear health
	Cell death type	Microtubule Fformation	Phenotypic change due to drug treatment
	Colony formation	Neurite outgrowth	Syncytia formation
Object Circularity 	Cell health	Cytoplasmic phenotypic change	Nuclear health
	COMET assay	Mitosis	Nucleus/cell identification
	Apoptotic cell identification		

Gen5 Image Prime 影像分析

Gen5 Object-Level Parameters and Applications			
Object-Level Parameter	**Possible Targets**		
Object Mean Intensity 	Biomarker classification	Gene expression	Receptor activation
	Cell cycle	Genotoxicity	Oxidative stress
	Cell signaling	Glutathione depletion	siRNA studies
	Cell survival	Ion channel studies (Ca^{2+})	Stem cell differentiation
	Cell viability	Organelle health	Stem cell pluripotency
	Target colocalization	Oxidative stress	Transfection efficiency
	ER stress	Protein expression	Translocation
Object Integral Intensity 	Hypertrophy	Morphological changes	COMET assay
Object Intensity Standard Deviation 	Cell cycle	Phenotypic analysis	Organelle health
	Translocation	Stem cell differentiation	

細胞平均螢光亮度

細胞螢光亮度總和

細胞螢光亮度標準差