

# Cytation 5

## 自動化影像系統暨多功能光學檢測儀

### 教育訓練



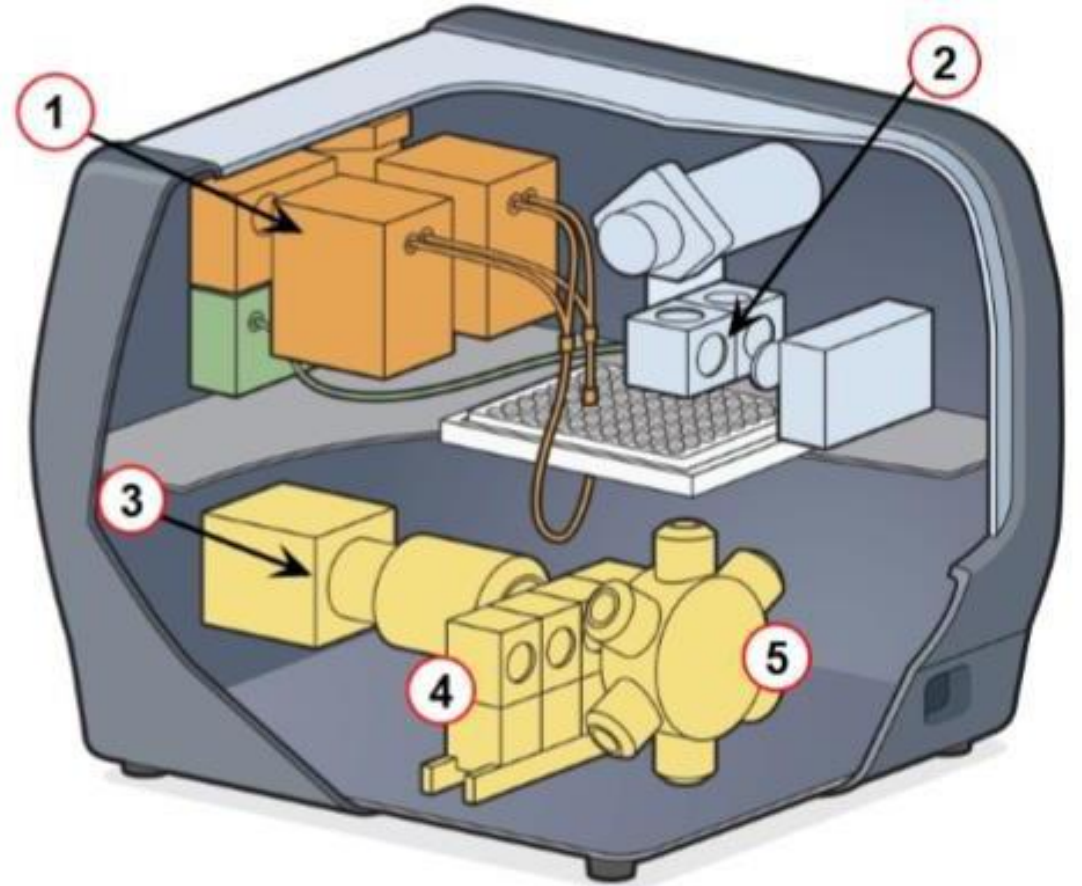
莊惠文 Helen Chuang  
Product Specialist  
[helenchuang@mail.level.com.tw](mailto:helenchuang@mail.level.com.tw)



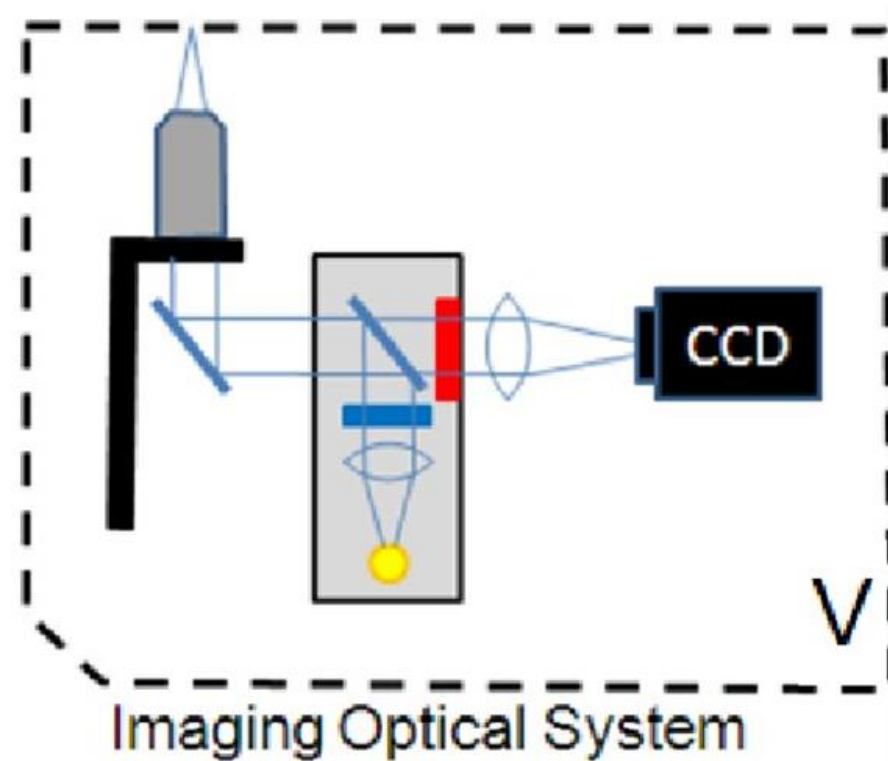
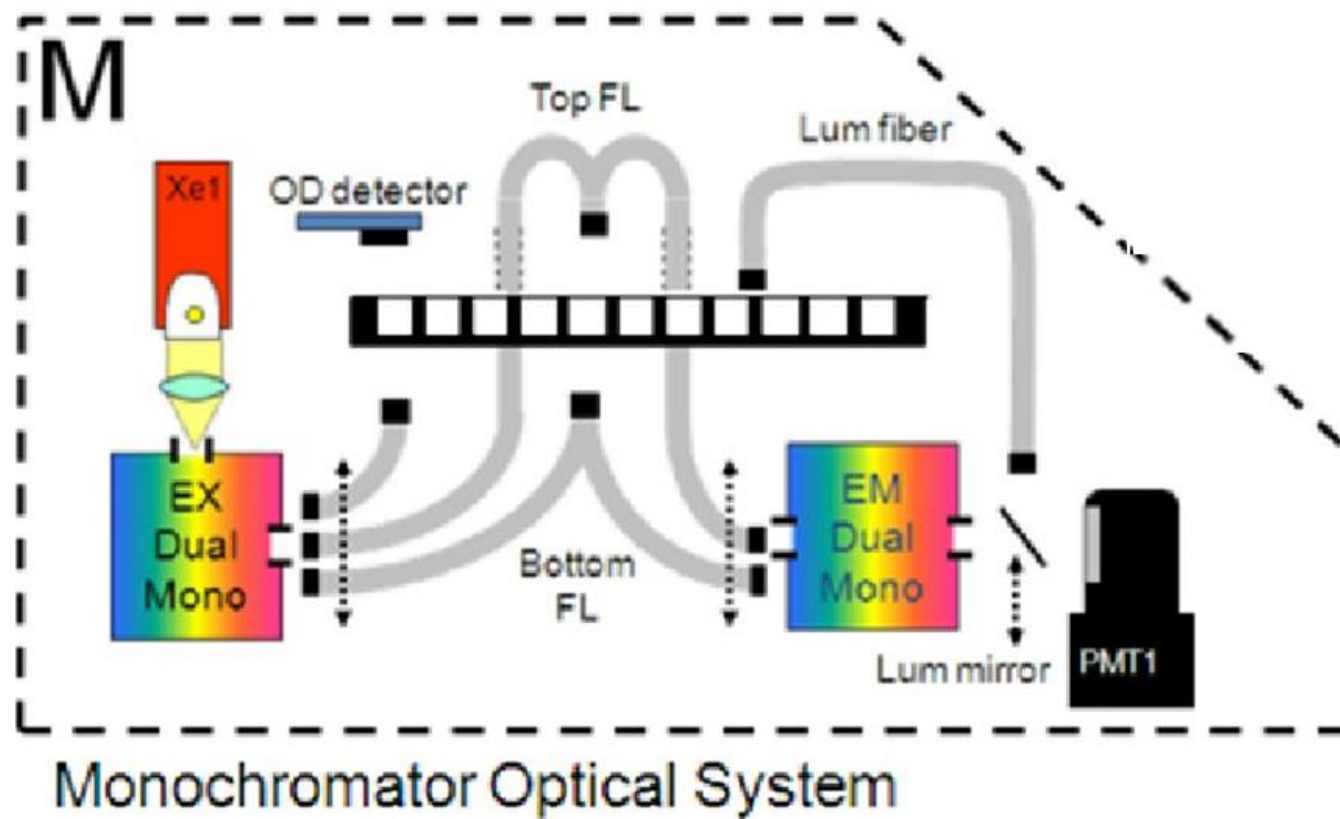
進階生物科技  
Level Biotechnology Inc.

# 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

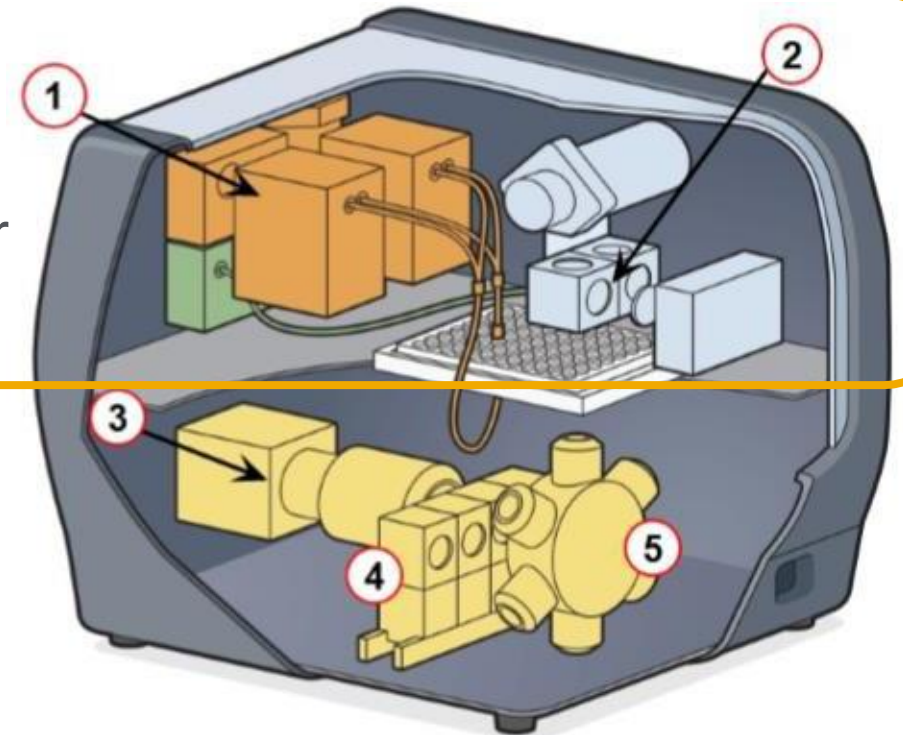
F : 250-700nm

L : 300-700nm

# Reader



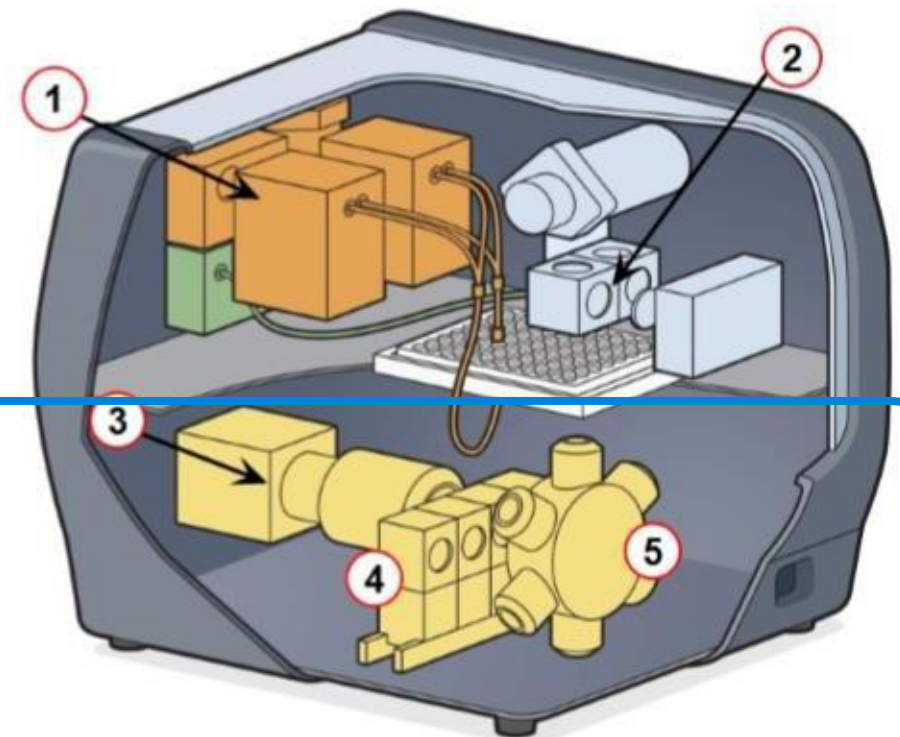
Microplate reader



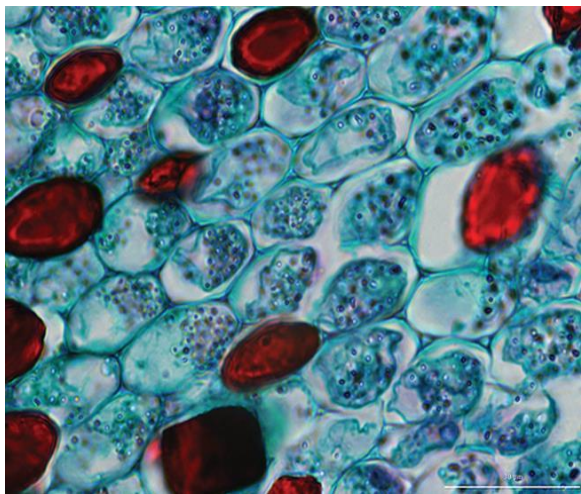
# Imaging System



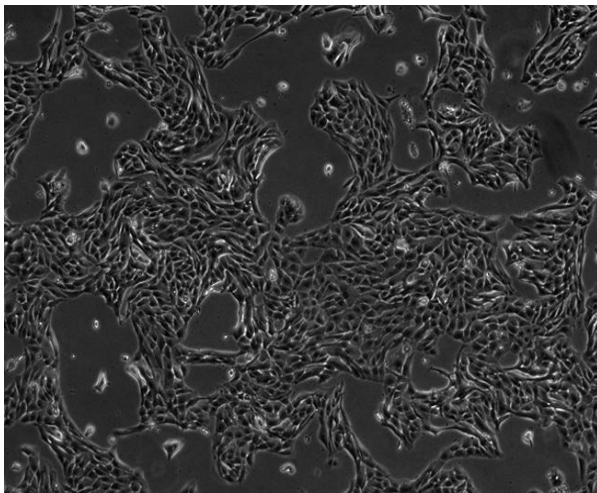
Imaging System



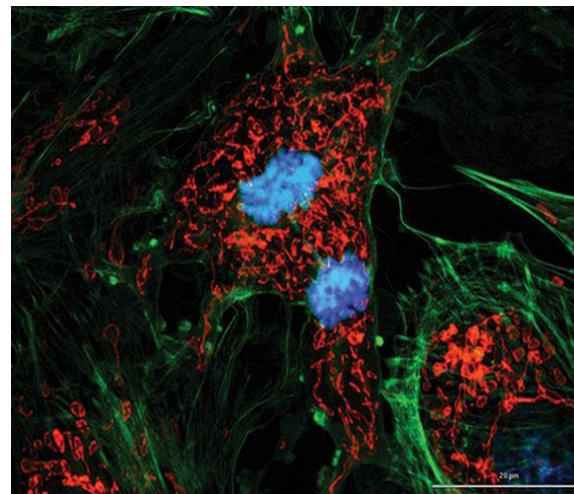
# 拍攝模式 Capture Modes



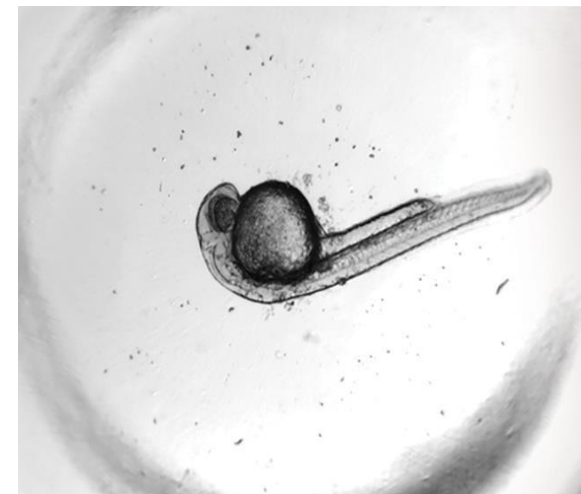
彩色明視野



相位差

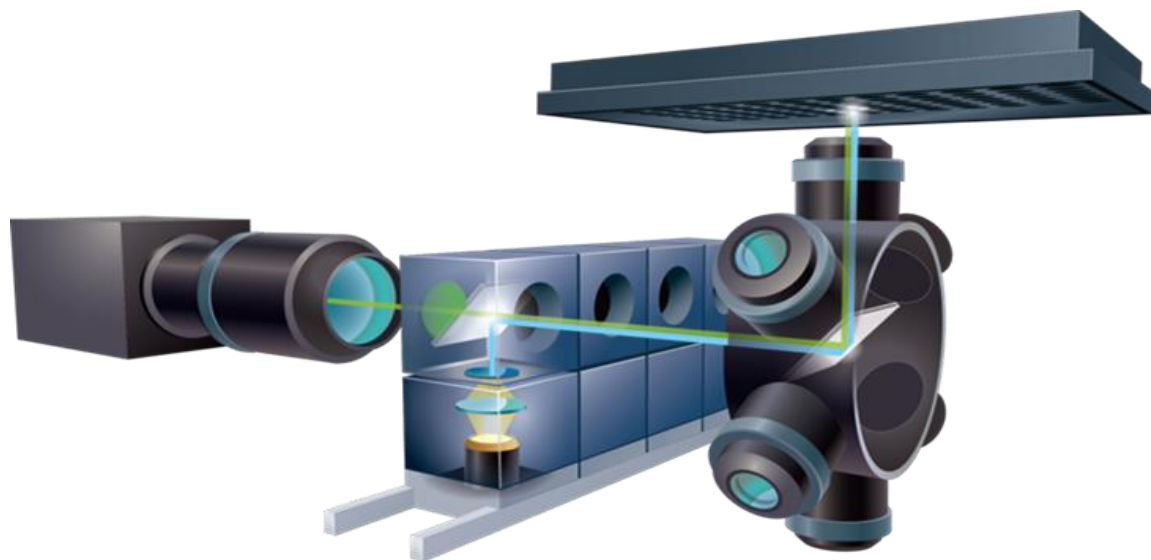


螢光



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



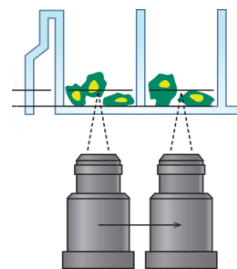
物鏡旋轉台



高倍物鏡  
配有校正環



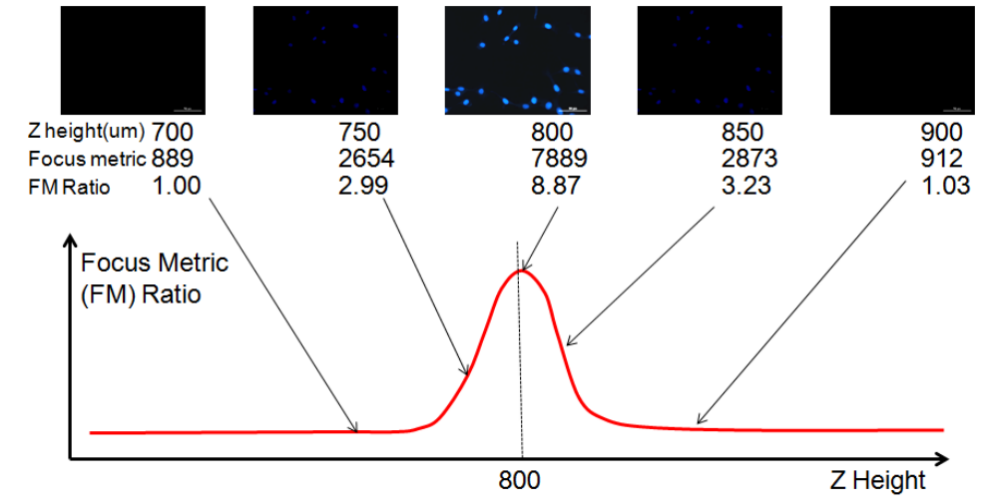
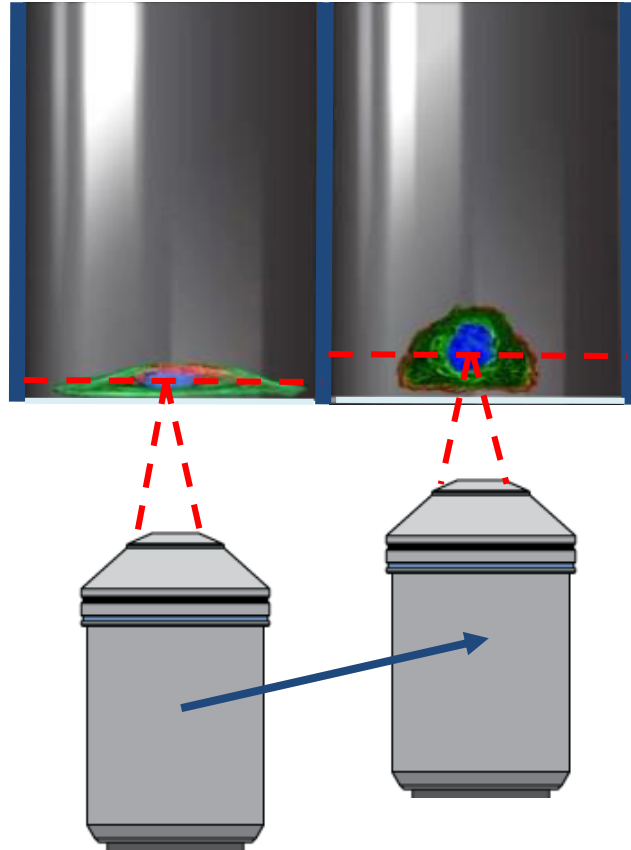
LED/Filter 套組



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

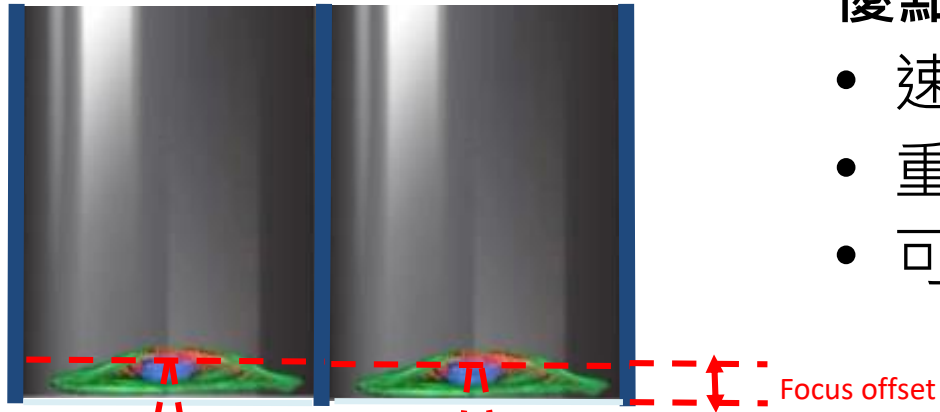
- 3D cell culture
- Thick biology
- Kinetic studies of 3D biology
- Moving biology
- Fixed samples

# 雷射自動對焦 Laser Autofocus



## 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

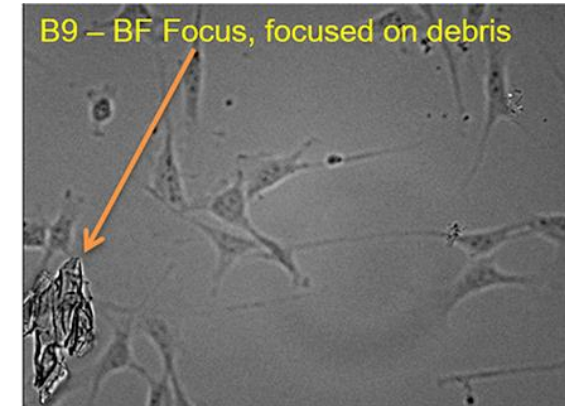
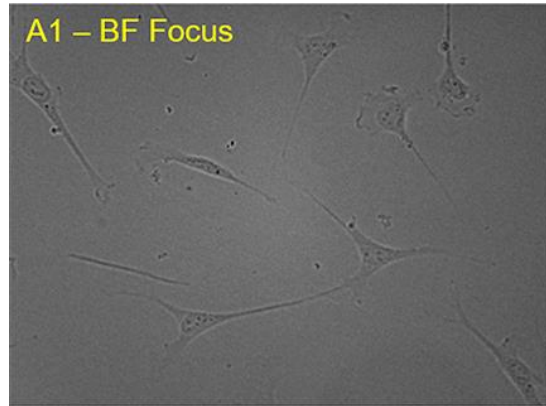
- $\geq 4\times$  imaging
- 2D cell culture
- Live cell biology
- High throughput screening
- Kinetic studies
- Red emitting dyes



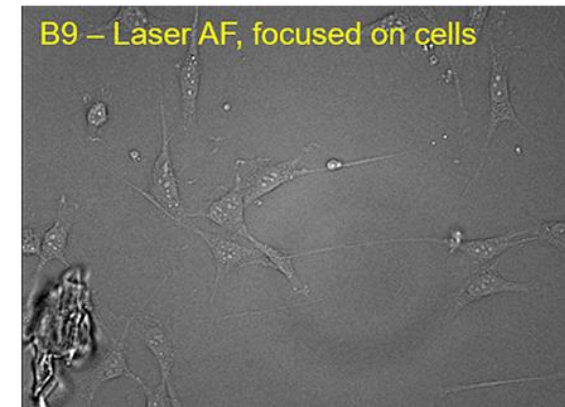
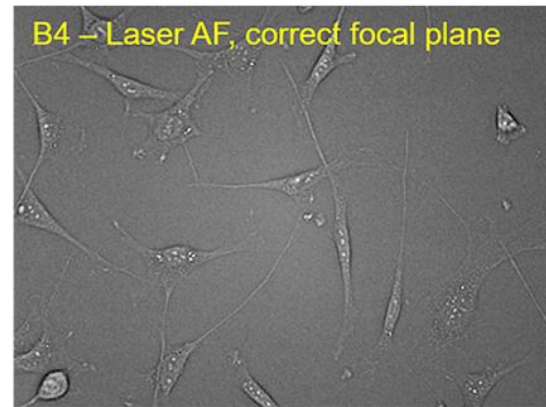
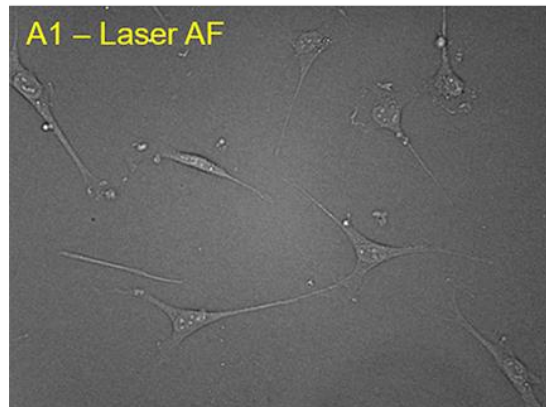
記憶盤底的折射率

# 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

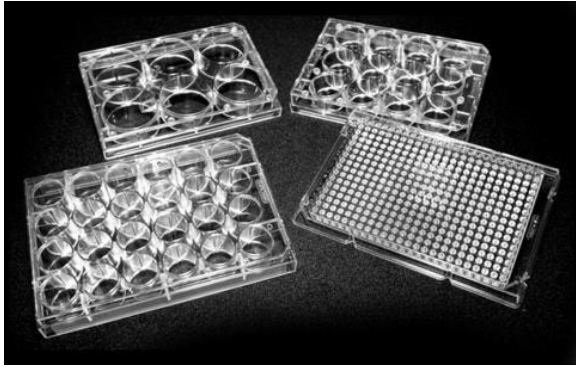


# Imaging System Filter Set

| 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),<br>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       |



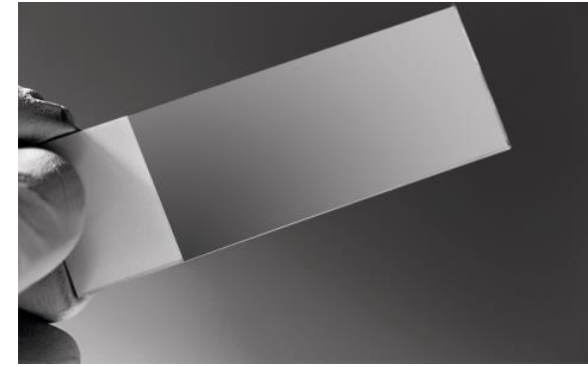
# Labware



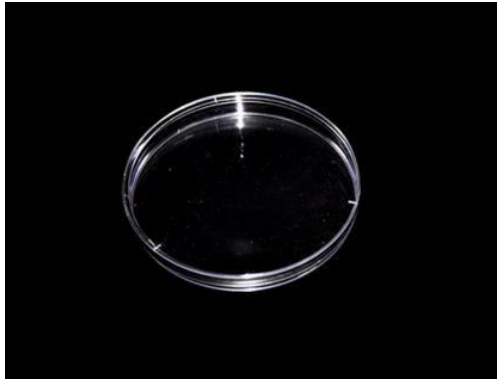
6- to 1536-well microplates



T25 flasks



Slides



Cell Culture dishes  
(10cm)

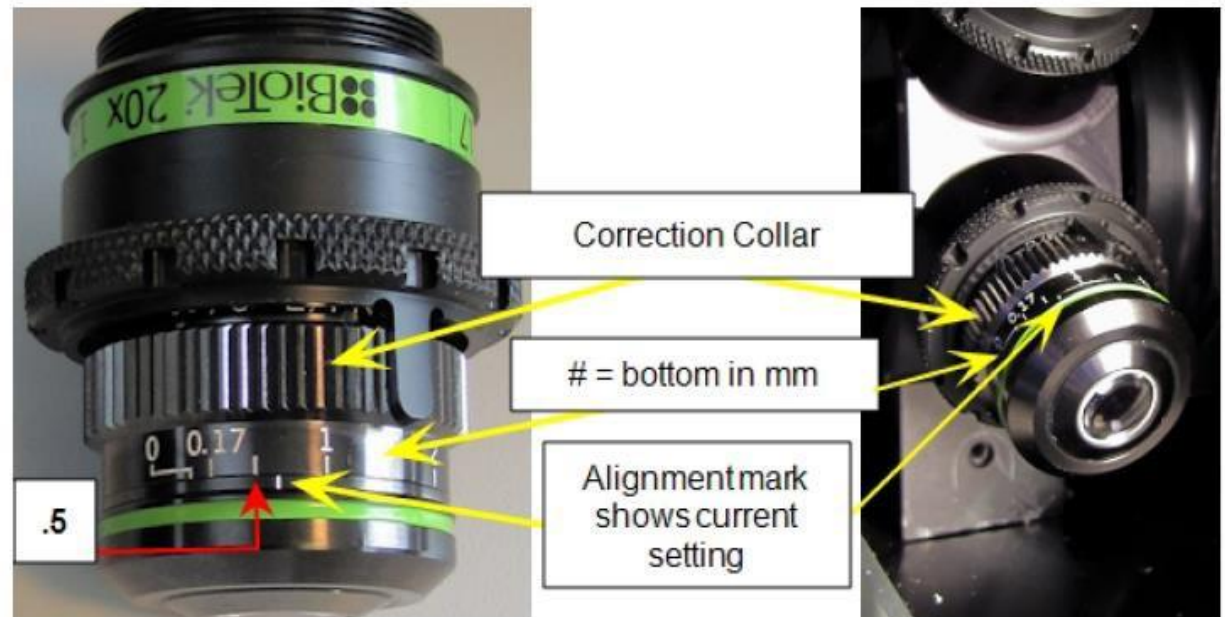


Take3 Micro-Volume plates

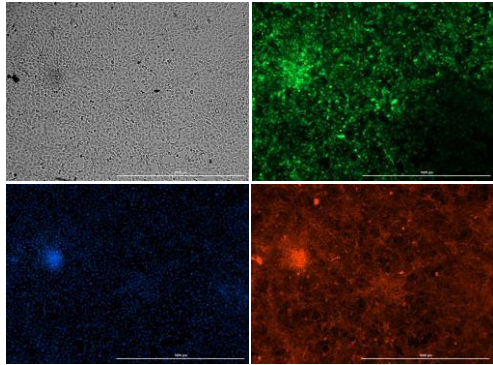
# 物鏡- Correction collar

20X, 40X 物鏡，需依據plate thickness  
調整。

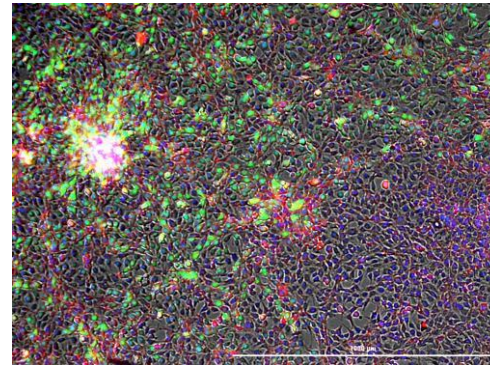
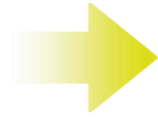
| Plate Type                                | Typical Bottom Thickness |
|-------------------------------------------|--------------------------|
| Glass-bottom microplate                   | 0.17 mm                  |
| Cover slip                                | 0.17 mm                  |
| Plastic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



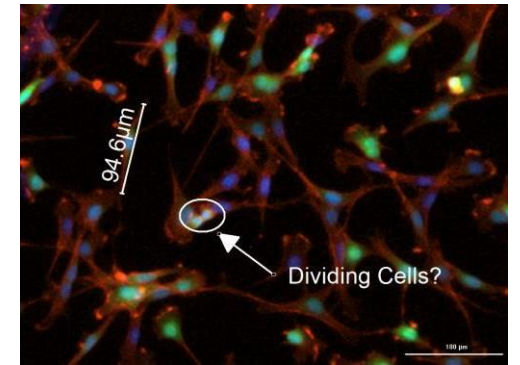
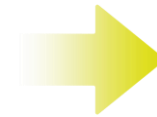
# Imaging Workflow



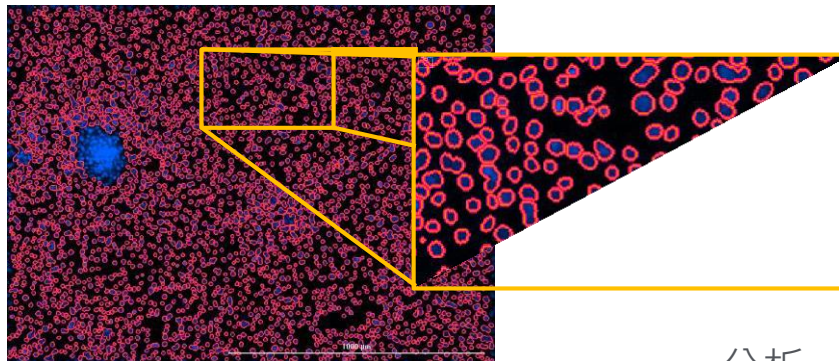
4-Channel Capture



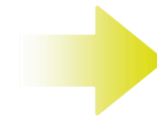
影像處理  
Process



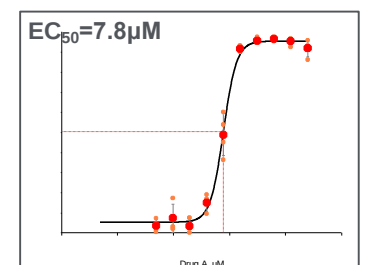
編輯與註解  
Edit & Annotate



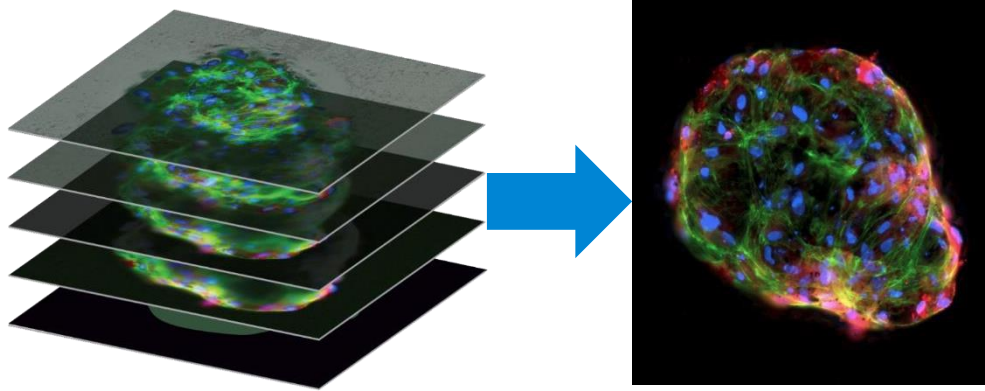
分析 發表  
Analyze & Publish



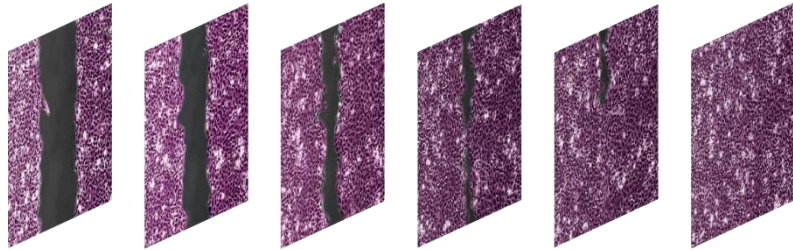
|                    |       |
|--------------------|-------|
| Cell Count         | 3408  |
| Object Area        | 269   |
| Object Circularity | 0.661 |



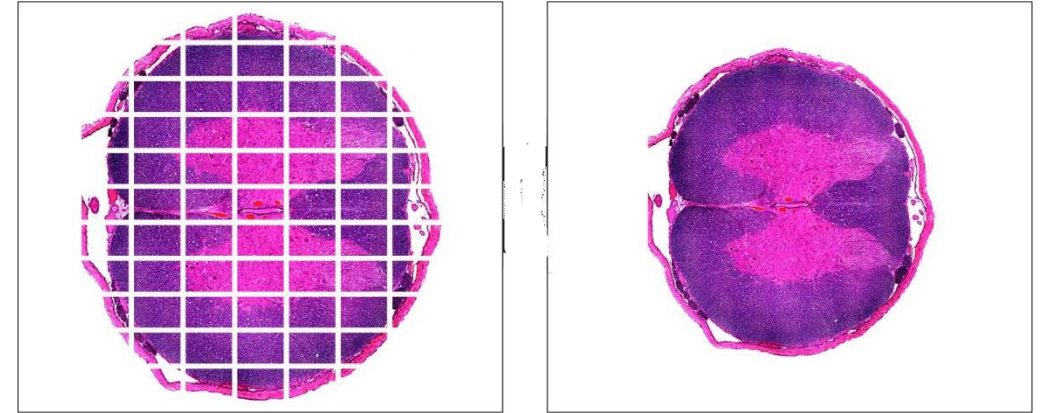
# 影像拍攝方式



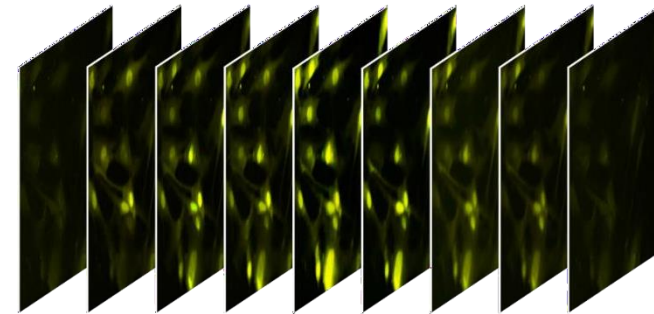
Z軸疊圖與投影  
Z-stack



長時間拍攝  
應用：傷口癒合、細胞增生

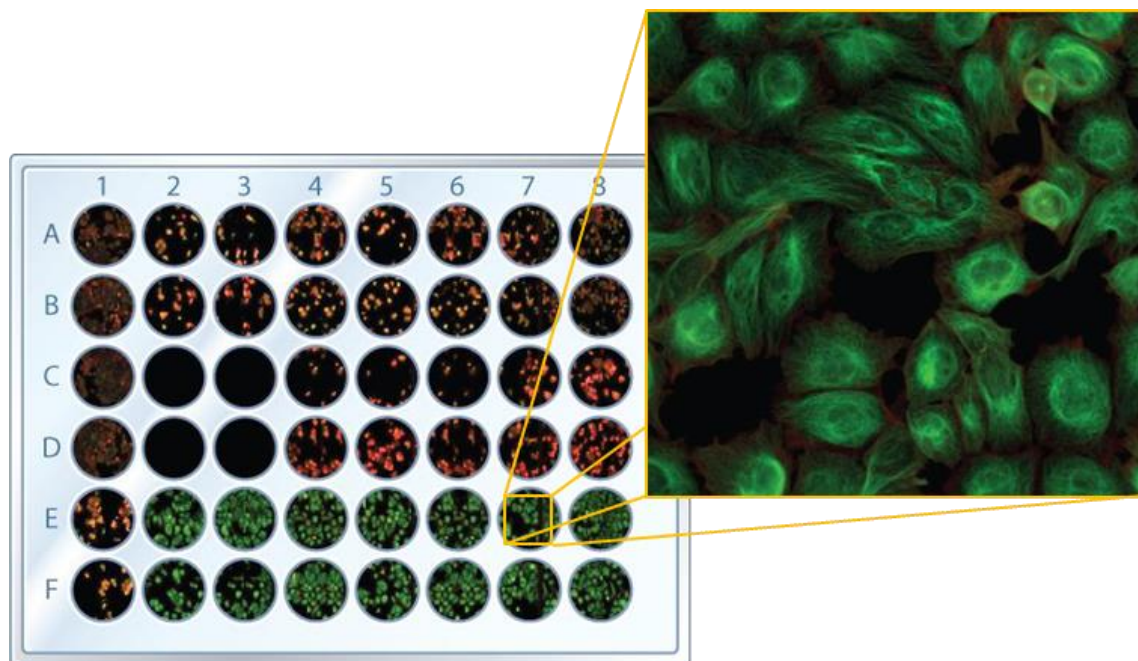


大圖拼接與縫圖  
montage

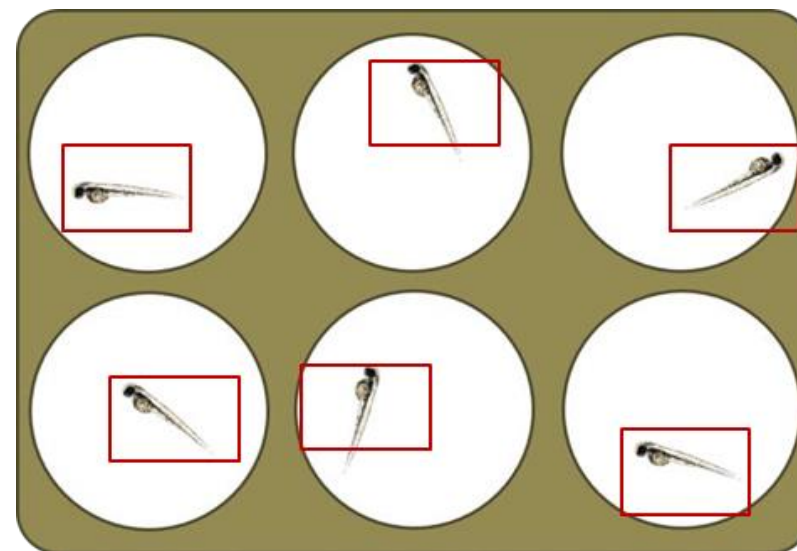


高速動力學拍攝  
應用：鈣流和其他動力學實驗

# 影像拍攝方式

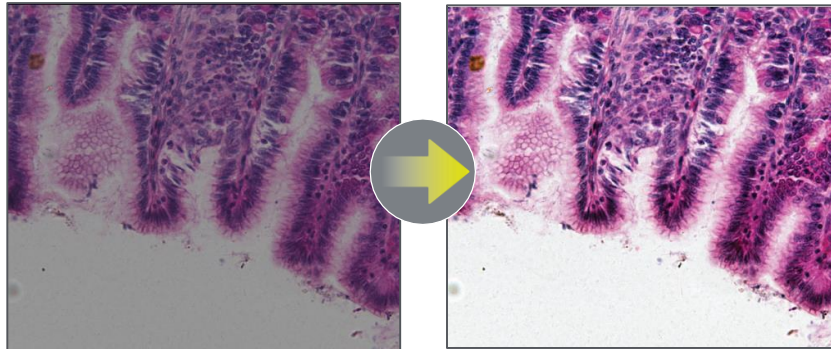


批次拍攝 Batch Mode

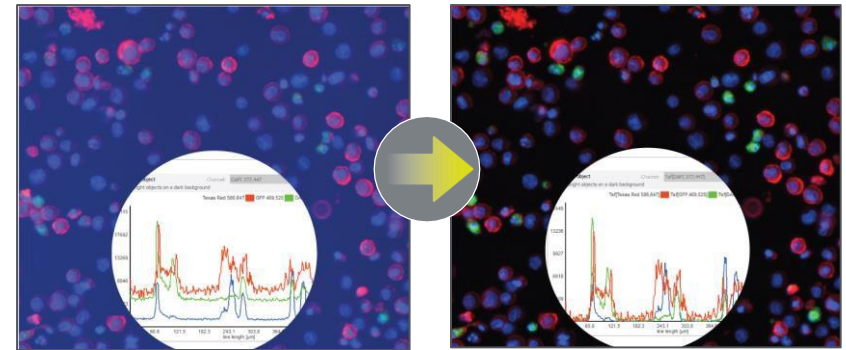


定位拍攝 Beacon Mode

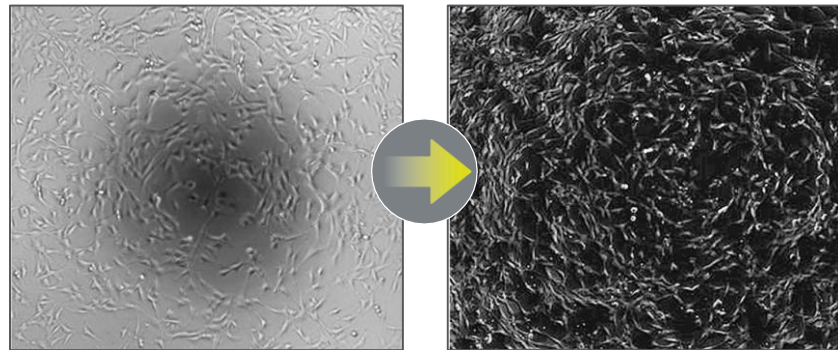
# Image Processing影像處理



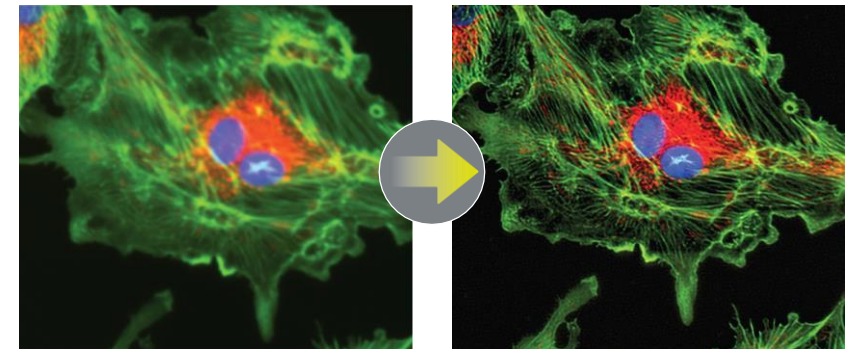
明亮度與對比  
Brightness and contrast



去背景值  
Background flattening



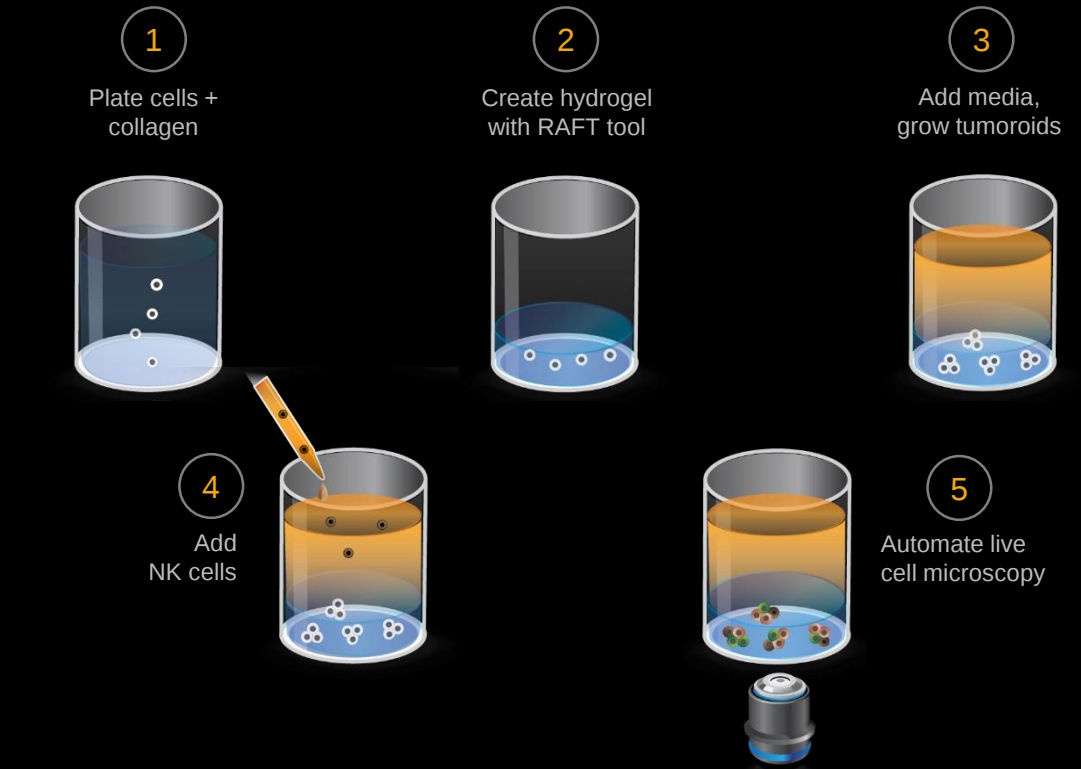
數位相位差  
Digital phase contrast



圖像銳化  
Deconvolution

# Applications

# 3D Kinetic NK Cell Cytotoxicity Assay



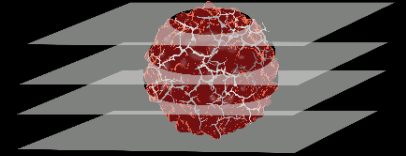
- Using Lonza's RAFT system, cancer cells are suspended in the hydrogel and propagate to form 3D tumoroids.

- 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.



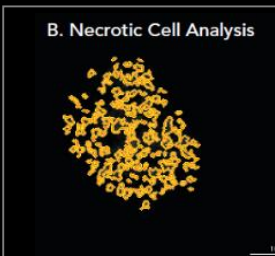
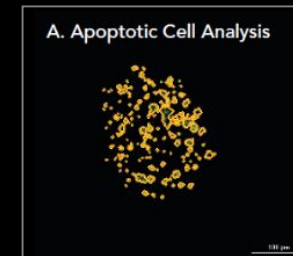
### Process

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



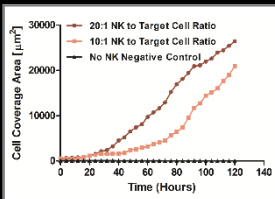
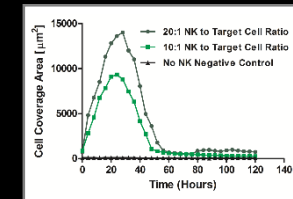
### Analyze

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

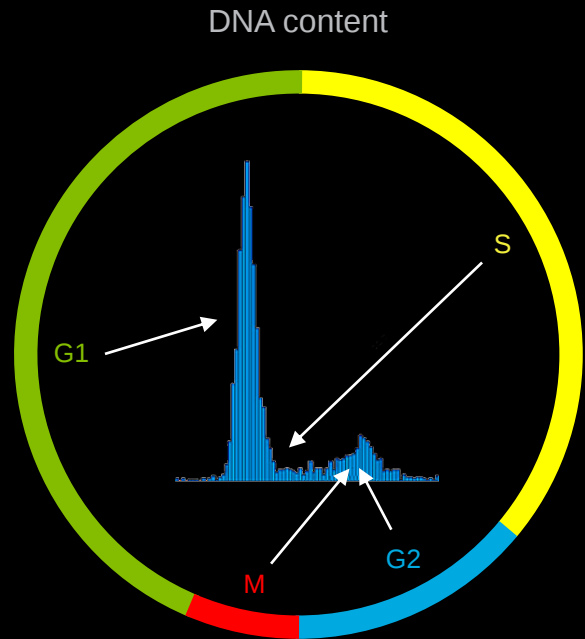


### Publish

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



# Cell Cycle Analysis Using a Nuclear Stain

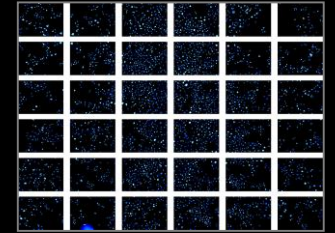


- Cell cycle progression is a tightly regulated process that involves the duplication of nuclear DNA content prior to cell division.
- A nuclear stain such as DAPI can be used to quantify this process since fluorescence intensity doubles as cells progress from G1 to G2 phase.

Augmented microscopy

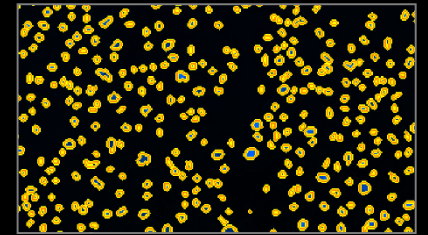
## Capture

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



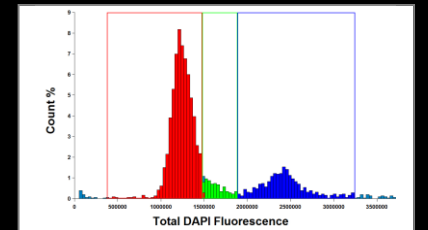
## Process

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



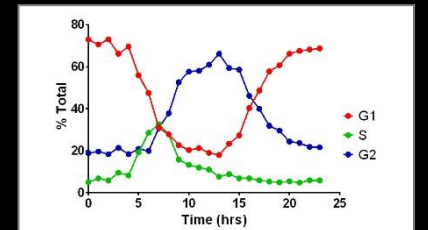
## Analyze

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

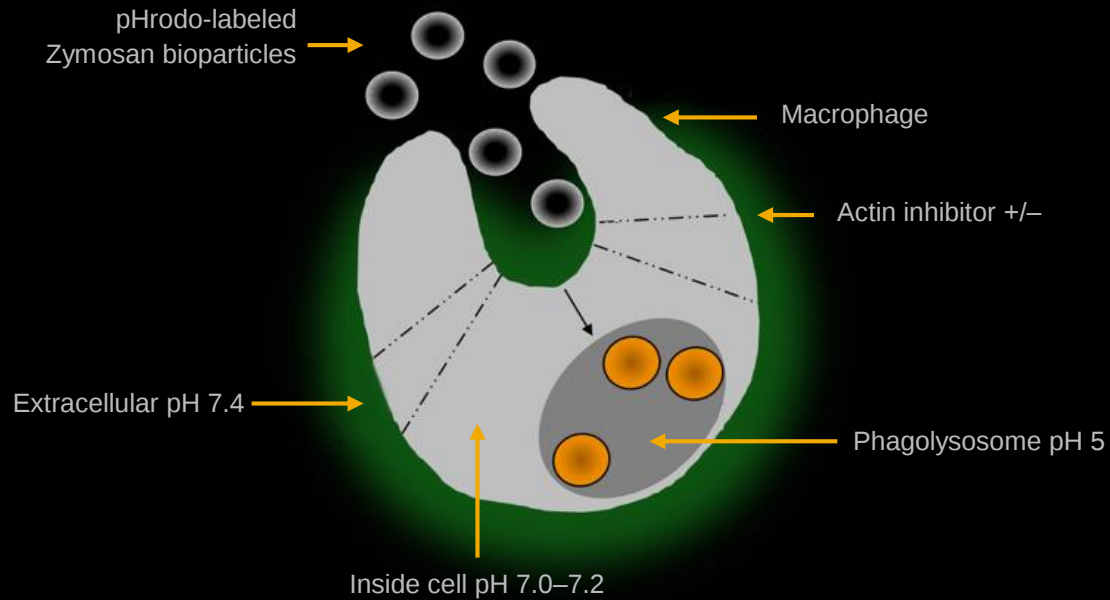


## Publish

Cell cycle progression of synchronized PC-3 cells.



# Phagocytosis Assay

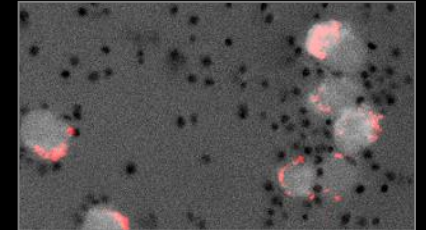


- Macrophages are specialized cells that consume and digest foreign matter through phagocytosis.
- pH-sensitive bioparticles are a useful tool to study phagocytosis as particles fluoresce in response to acidic environment of phagolysosomes.
- Cellular actin enables unique physical changes necessary for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



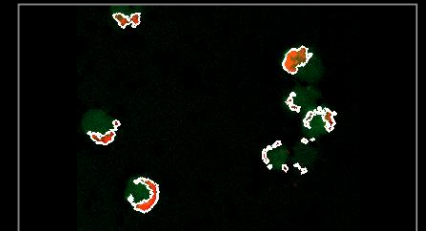
### Process

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



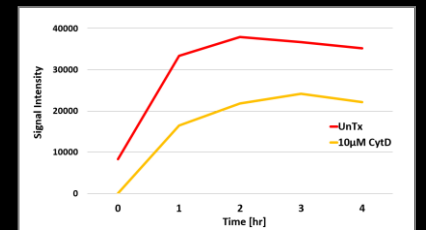
### Analyze

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).

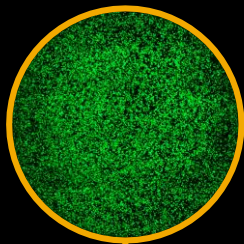


# Plaque Assay

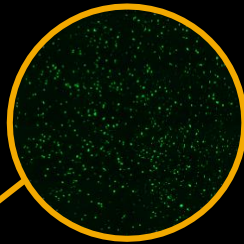
A1: Negative control



A2: Positive control



A3: Sample 1

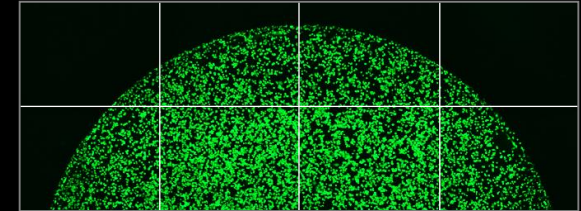


- A cell monolayer is treated with a dilution of a GFP-expressing virus.
- The cells are then coated with agar, which limits the virus spread to only neighboring cells.
- After incubation, GFP+ circles of infected cells are counted for virus titration.

## Augmented microscopy

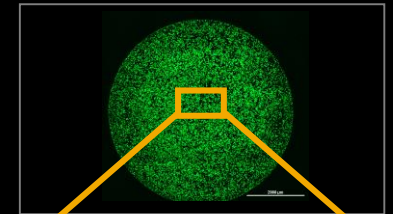
### Capture

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



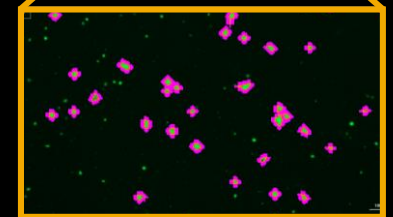
### Process

The resulting 20 images are stitched together by the software.



### Analyze

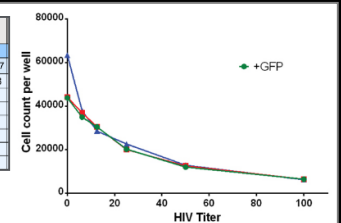
The stitched image is analyzed; plaque objects are identified using intensity and size thresholds.



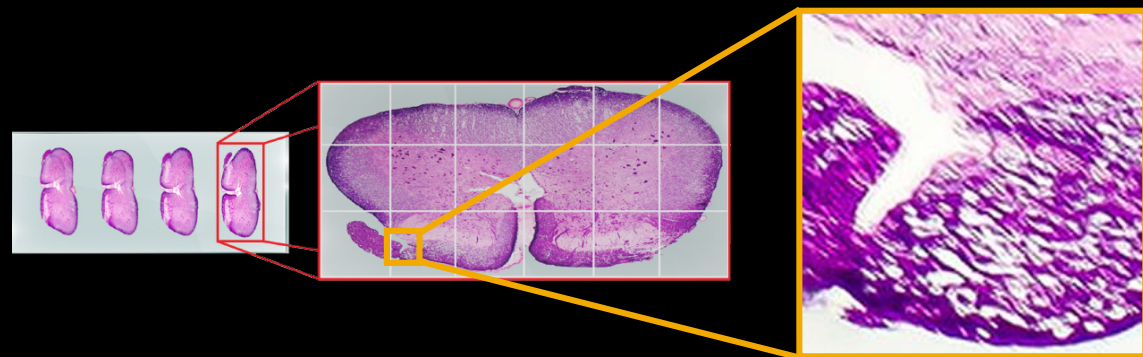
### Publish

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 | 1888 | 1038 | 1117 |  |  |  |  |
| B | 3   | 13317 | 7710 | 624  | 483  | 464  | 517  | 448  | 348  |  |  |  |  |
| C | 12  | 13208 | 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.



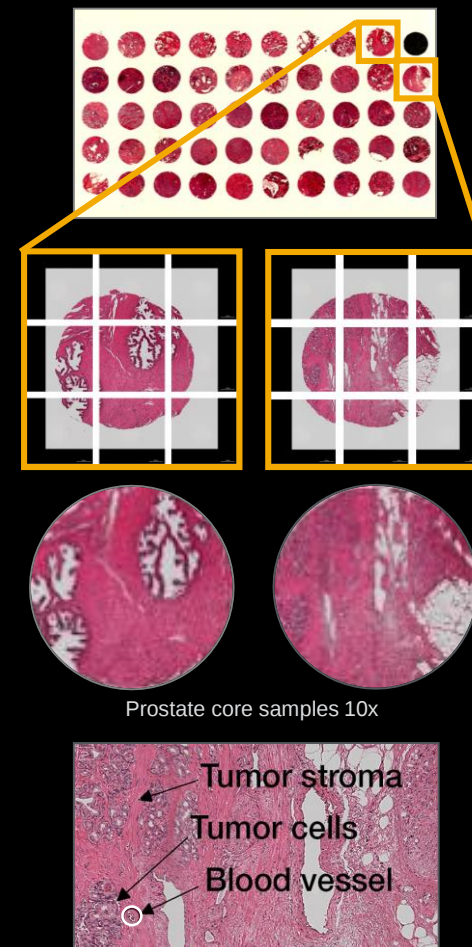
### Analyze

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



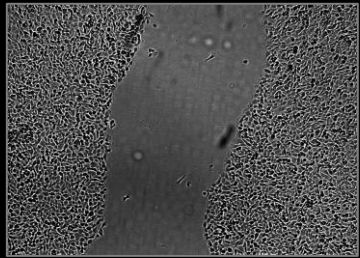
### Publish

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

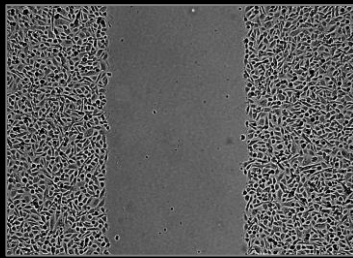


# Automated Wound Creation for Scratch Wound Healing Assays

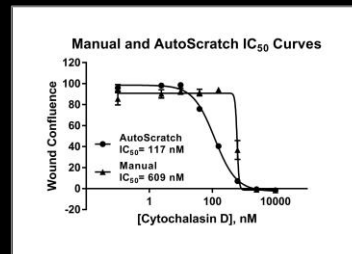
## Automated wound healing assay procedure



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.



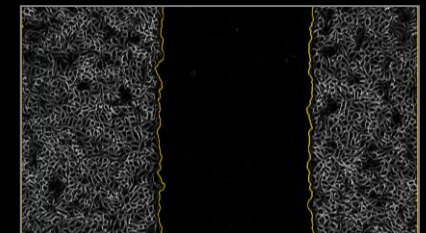
### Wound

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



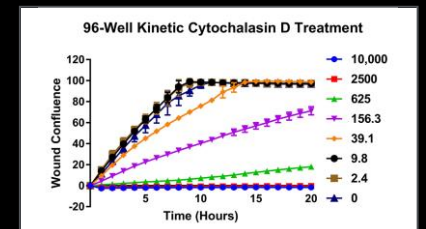
### Image and Analysis

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

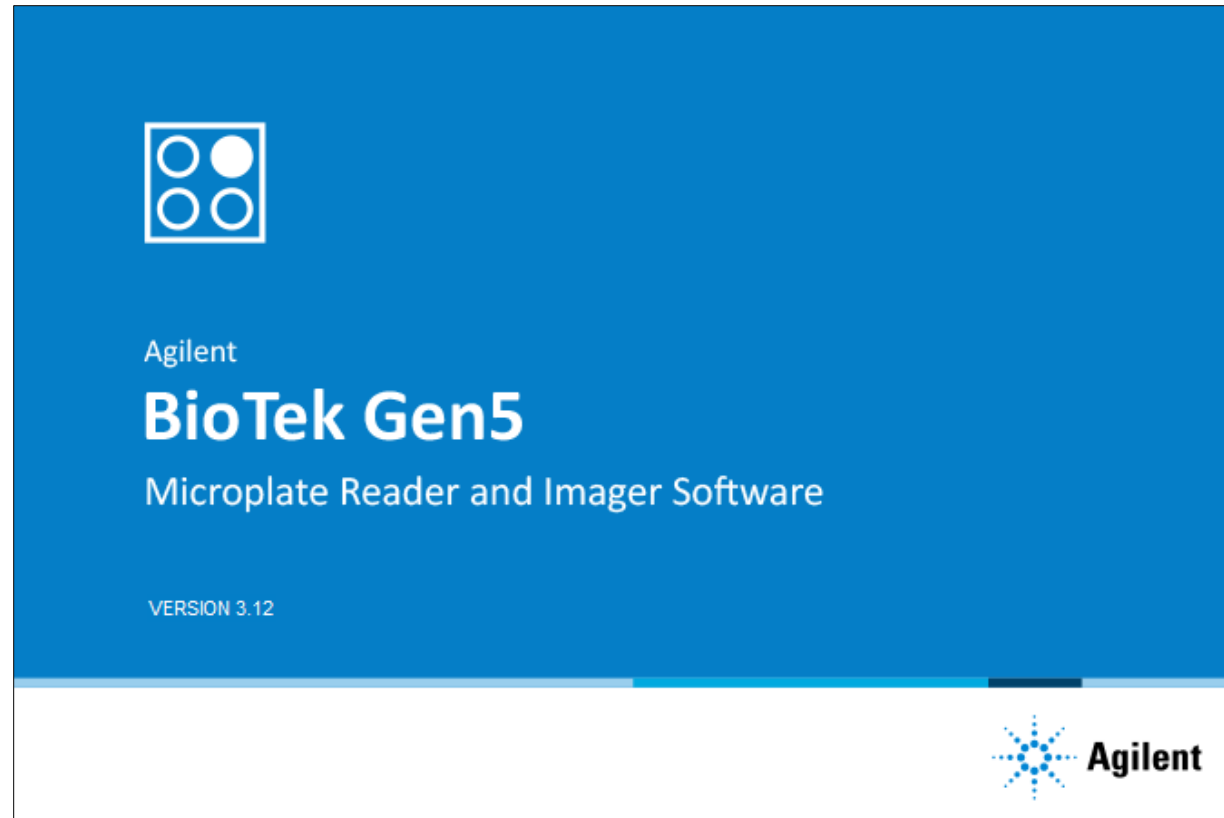


### Publish

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



# Gen5 image prime 教育訓練

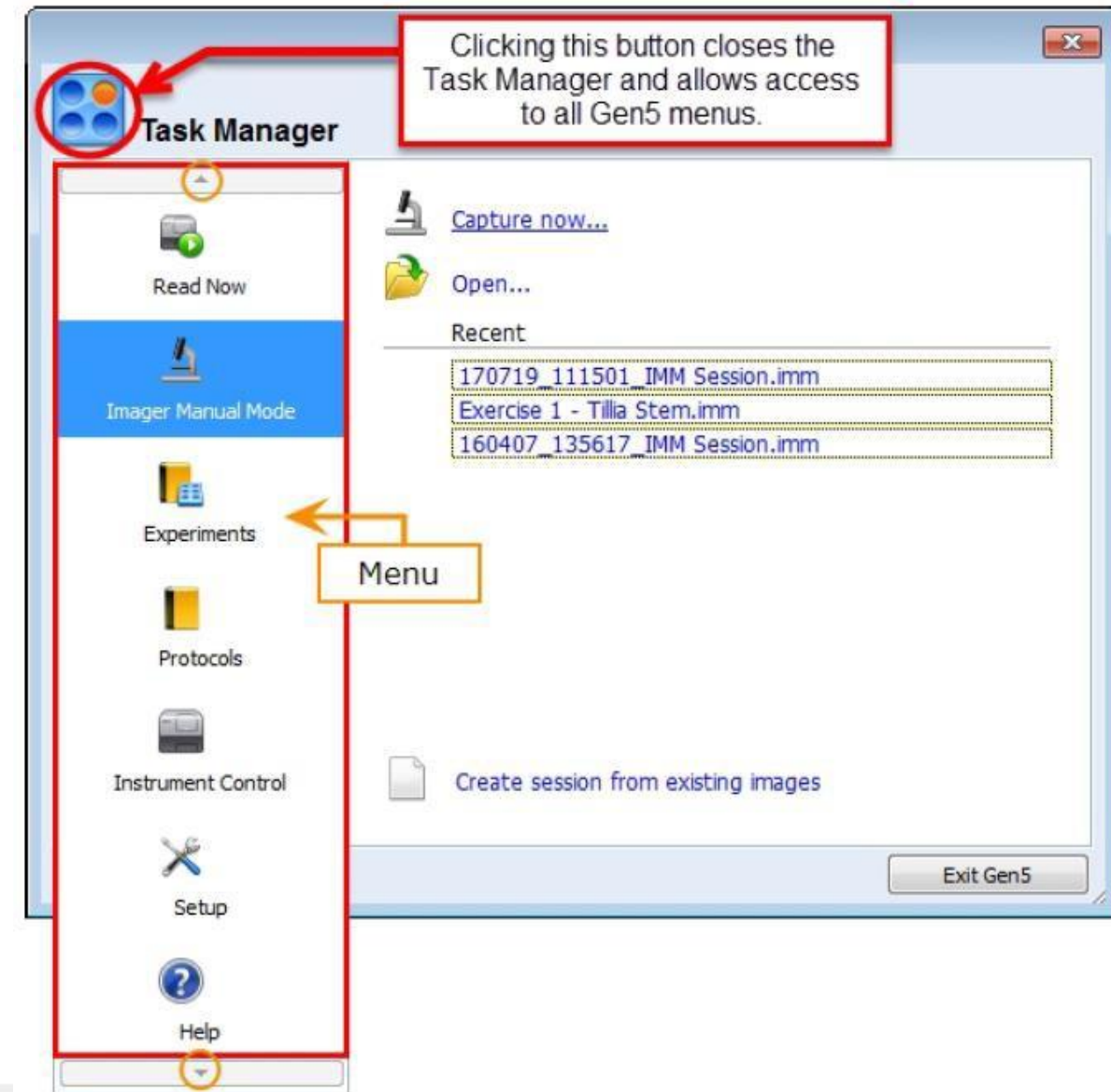


# Before You Begin

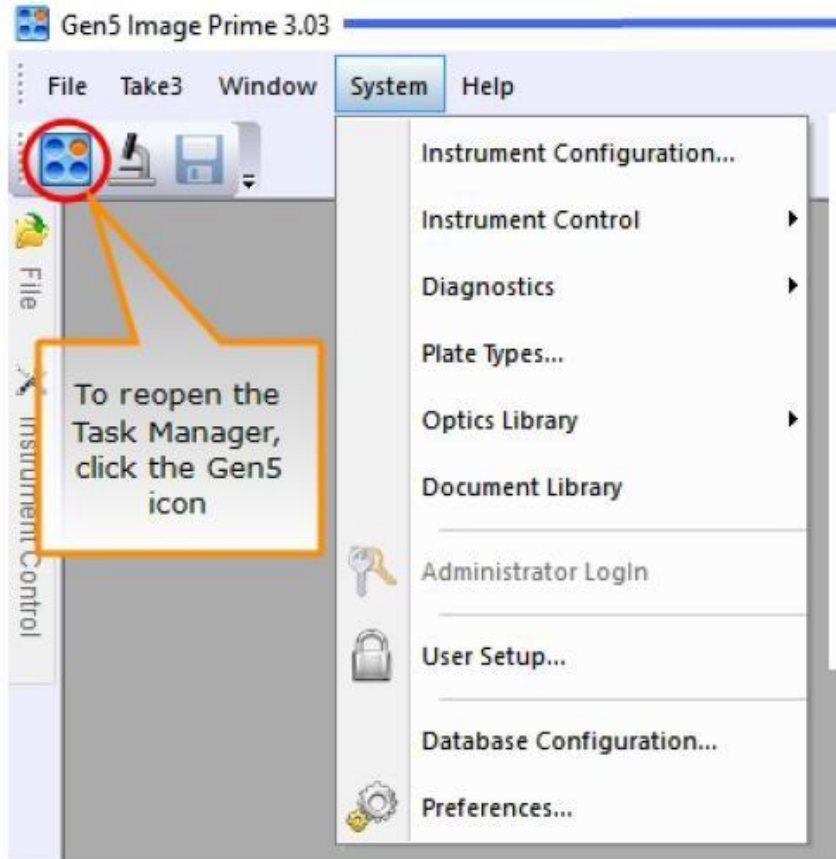
- 了解Gen5 Task Manager
- Manual Mode VS Experiment Mode
- 檔案儲存位置
- 盤型定義
- 高倍物鏡使用

# 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                                                                          |
|----------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| <b>Image Capture</b> | 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                 |
| <b>Analysis</b>      | 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**

**Image Save Options**

Gen5 Image Library

Pathname:  Browse...

Experiment Folder

Name:

Example:

Plate Folder

Name:

Example:

Image File Naming Convention

Name:

Example:

Image files are stored in the Gen5 Image Library in a user-specified location in the Windows File System

# 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

**Select System > Plate Types**

**Plate Types**

Plate Types:

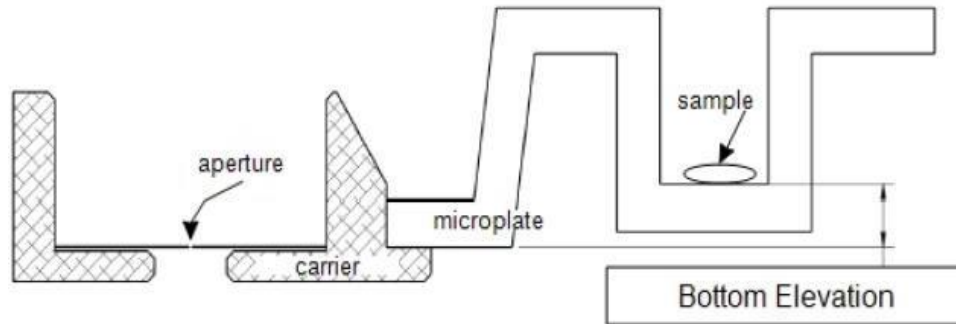
| Favorite                            | Labware | Name                          |
|-------------------------------------|---------|-------------------------------|
| <input type="checkbox"/>            |         | Default; 6 WELL PLATE         |
| <input type="checkbox"/>            |         | Default; 12 WELL PLATE        |
| <input type="checkbox"/>            |         | Default; 24 WELL PLATE        |
| <input type="checkbox"/>            |         | Default; 48 WELL PLATE        |
| <input type="checkbox"/>            |         | Default; 60 WELL PLATE TERA   |
| <input type="checkbox"/>            |         | Default; 72 WELL PLATE TERA   |
| <input checked="" type="checkbox"/> |         | Default; 96 WELL PLATE        |
| <input type="checkbox"/>            |         | Default; 96 WELL PLATE HELMA  |
| <input type="checkbox"/>            |         | Default; 96 WELL PLATE METRIC |
| <input type="checkbox"/>            |         | Default; 96 WELL PLATE TERA   |
| <input type="checkbox"/>            |         | Default; BIOTEK BIOCELL PLATE |
| <input type="checkbox"/>            |         | Default; 384 WELL PLATE       |
| <input type="checkbox"/>            |         | Default; 1536 WELL PLATE      |
| <input type="checkbox"/>            |         | 1222520                       |
| <input type="checkbox"/>            |         | 1222520_AF                    |
| <input type="checkbox"/>            |         | 384 Standard                  |
| <input type="checkbox"/>            |         | 384 Well Plate SBS dimensions |

**Copy a plate type to use as a template**

**View/Modify a plate type to change its settings**

Buttons: Add Plate ..., Add Labware..., Copy..., View/Modify..., View/Compare..., Delete, Export..., Import...

# Bottom elevation: 自動對焦起始點



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

Plate Description

Name: Greiner 96 Black Flat Bottom Fluorac Catalogue #: 655073-655079

Manufacturer: Greiner

Number of Rows: 8 Number of Columns: 12

Plate Width: 85480  $\mu\text{m}$  Plate Length: 127760  $\mu\text{m}$

Plate Height: 14400  $\mu\text{m}$  Stacked Height:  $\mu\text{m}$

Plate Lid adds: 3500  $\mu\text{m}$  ☐ Include Lid Parameters

Wells

Top Left Y: 11290  $\mu\text{m}$  Top Left X: 14380  $\mu\text{m}$

Bottom Right Y: 74290  $\mu\text{m}$  Bottom Right X: 113380  $\mu\text{m}$

Well Diameter: 6960  $\mu\text{m}$

☐ Slide Holder

Side View

Plate Description - Imaging Parameters

Bottom Elevation (1): 3500  $\mu\text{m}$  (Distance from top of carrier to bottom elevation)

☒ Define Accessible Bottom Focus Region

X/Y Positions (3)

Generate default values from existing well definitions

Top Left Y: 1500  $\mu\text{m}$  Top Left X: 1500  $\mu\text{m}$

Bottom Right Y: 83980  $\mu\text{m}$  Bottom Right X: 126260  $\mu\text{m}$

Bottom thickness (2): 100  $\mu\text{m}$  (Distance from bottom elevation to lowest position in bottom region)

Max distance below carrier: 0  $\mu\text{m}$  (Max distance below the top of the carrier outside the bottom region)

Update Display

OK Cancel Help

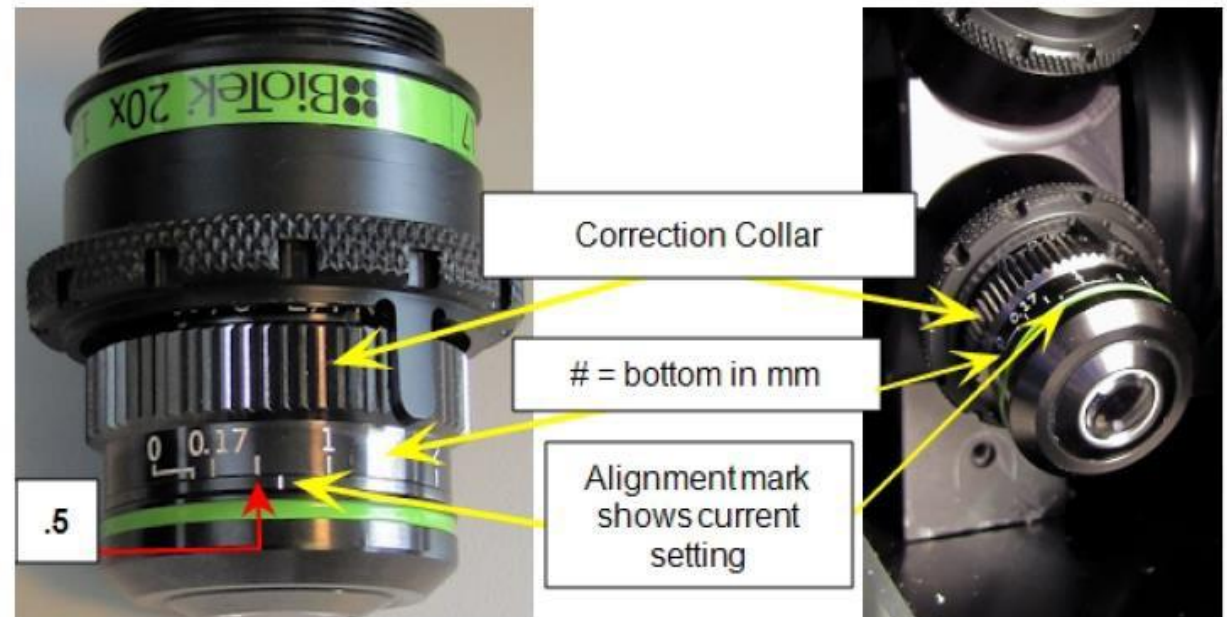
Click here first

then, change these parameters

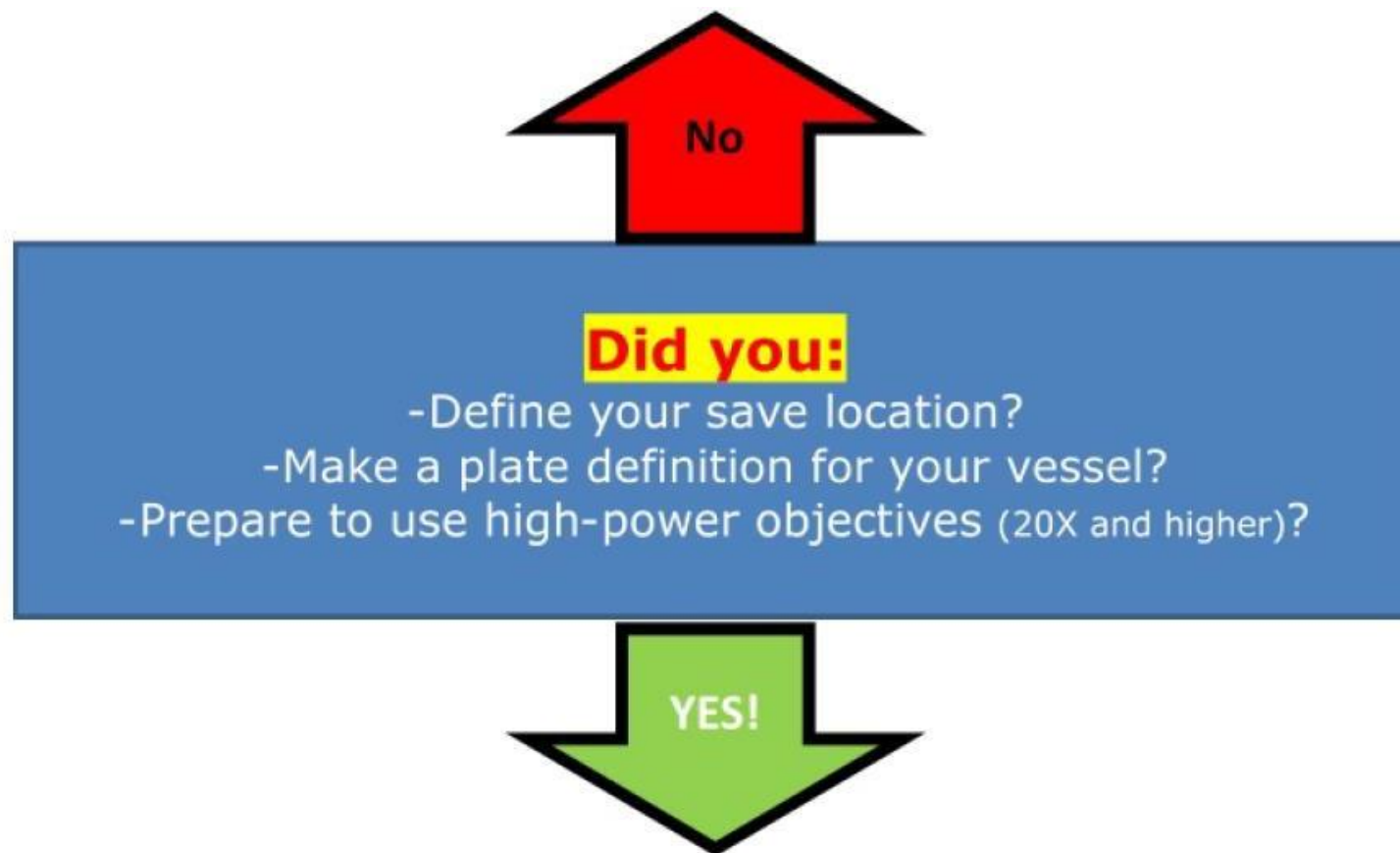
# 物鏡- Correction collar

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

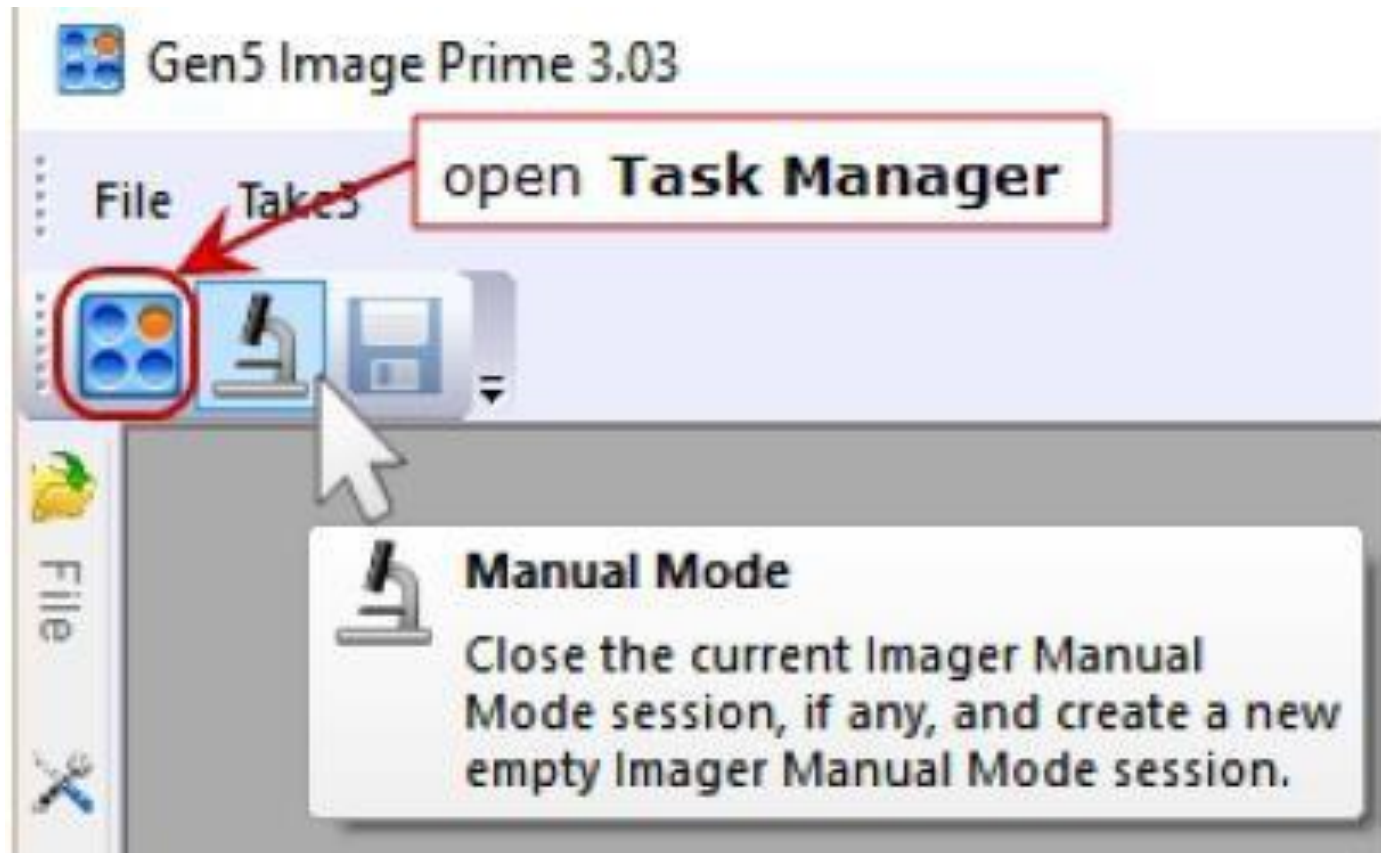
條件確認/少量樣本/單次拍攝/一般顯微鏡操作



# 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”

From the: **Task Manager**

1 Read Now

Imager Manual Mode

2 Capture now...

Open...

Recent

H2AX positive and  
170329 142414 I

Imager Manual Mode - Load Plate

Place the plate on the carrier and select the

3

Plate type

96 Well Plate SBS dimensions

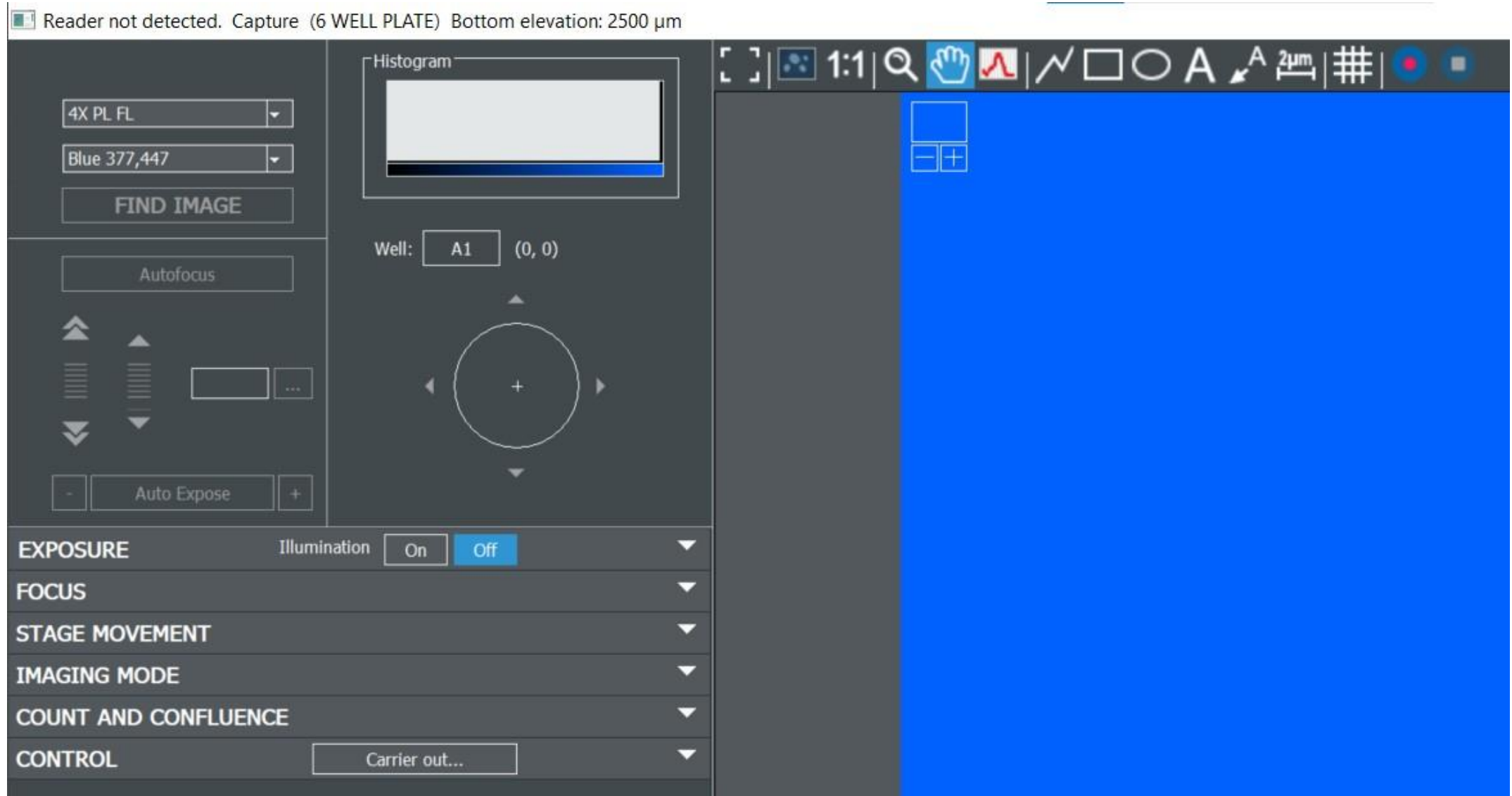
☒ Use Lid

| Favorite | Labware | Name                                  |
|----------|---------|---------------------------------------|
| ✓        |         | 96 Well Plate SBS dimensions          |
| ✓        |         | Greiner 96 Black Flat Bottom Fluotrac |
|          |         | Greiner 96 Black Round Bottom Polypro |
|          |         | Slide Holder All Mag. (w/A1 mark)     |
|          |         | T75 Flask                             |
|          |         | 24 WELL PLATE                         |
|          |         | 48 WELL PLATE                         |

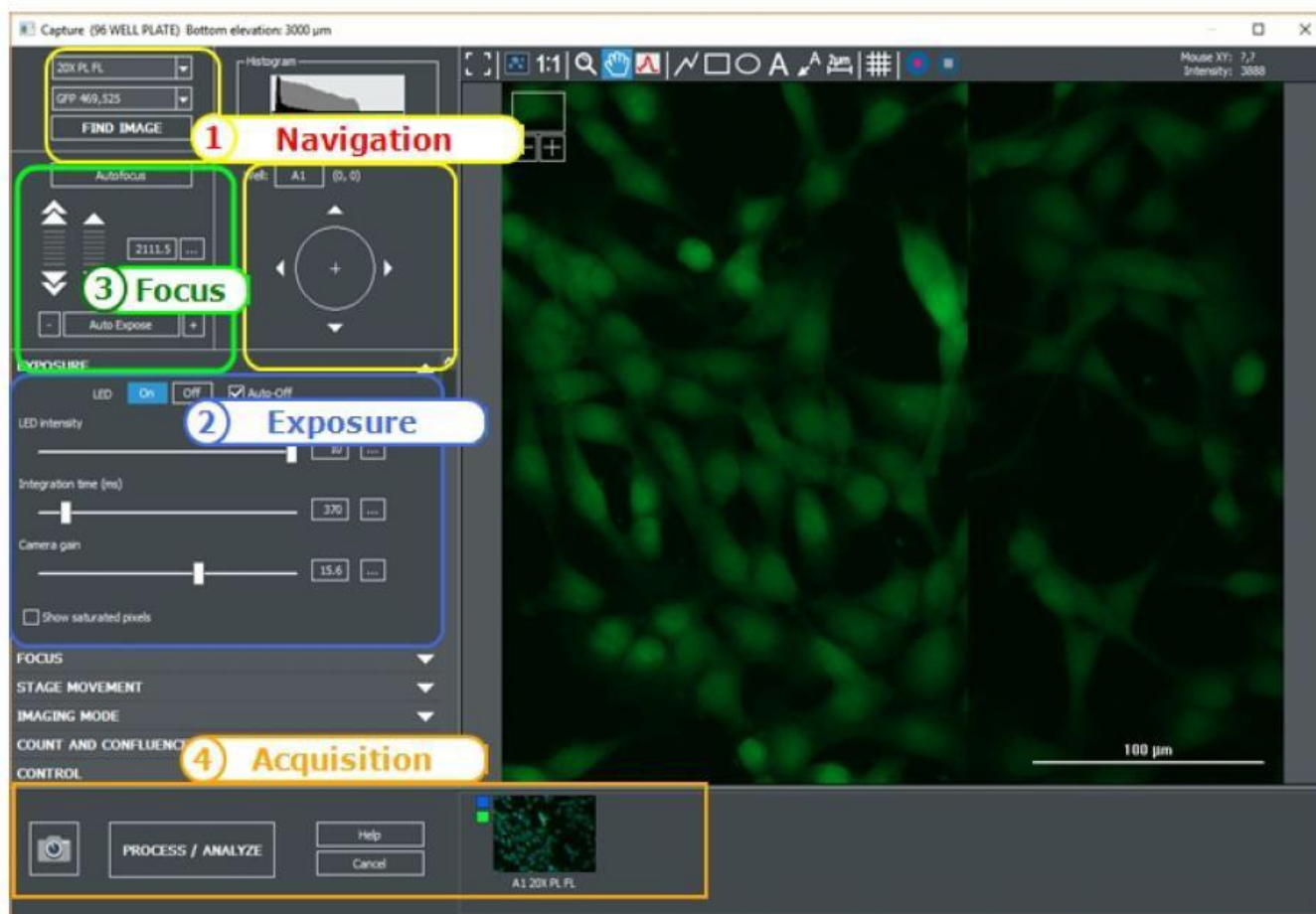
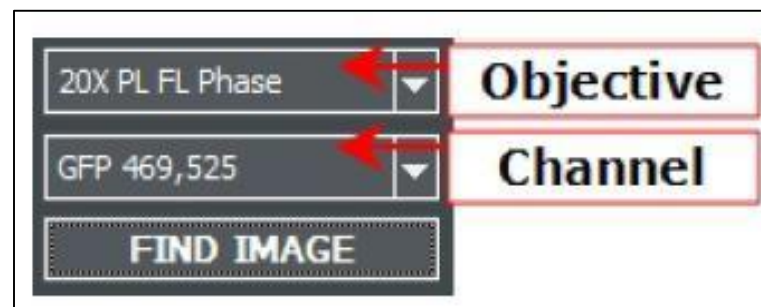
Select the plate/vessel you defined for imaging.

Be sure to select "Use Lids" if you have left a lid on your plate!

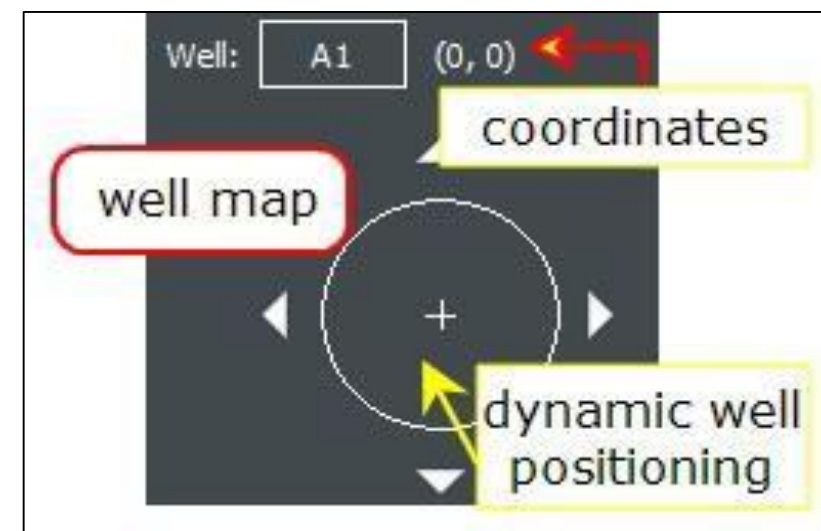
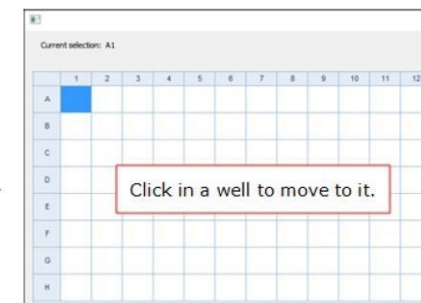
# Manual Mode



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



Your current plate position is shown on the Well button. Click the button to navigate within your vessel. It opens a plate map to let you choose another well or section of the vessel.



# Exposure 曝光

自動或是手動調整

- Auto Expose +

EXPOSURE

LED On Off ☒ Auto-Off

LED intensity 5

Integration time (ms) 50

Camera gain 0

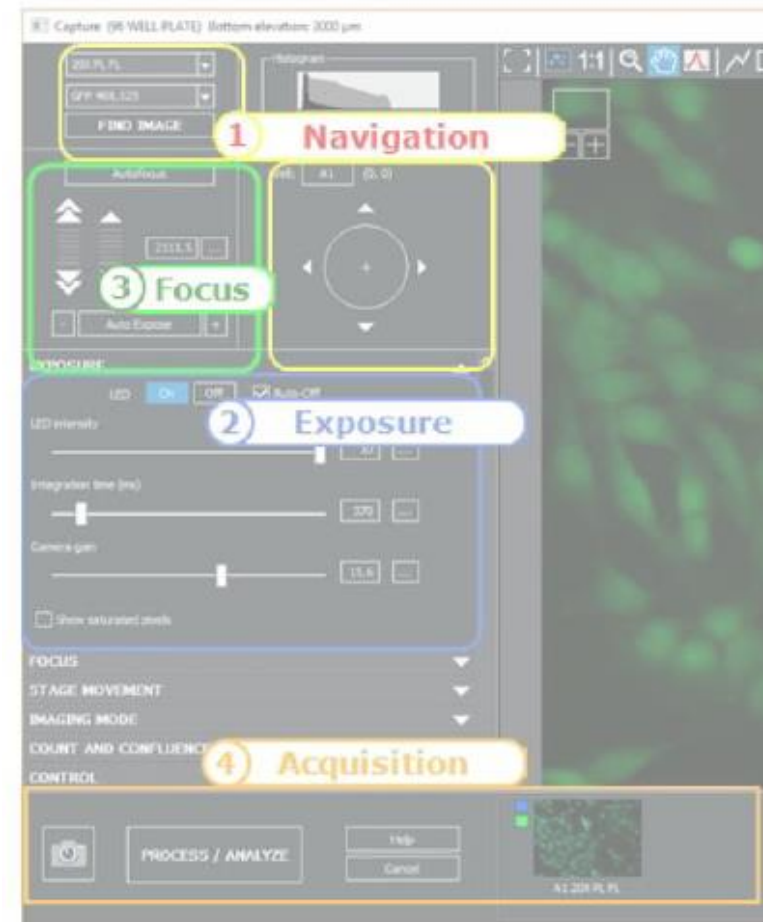
☐ Show saturated pixels

How bright?

Exposure time (ms)

Digital amplification

Affects performance, acquisition speed, and minimum kinetic intervals

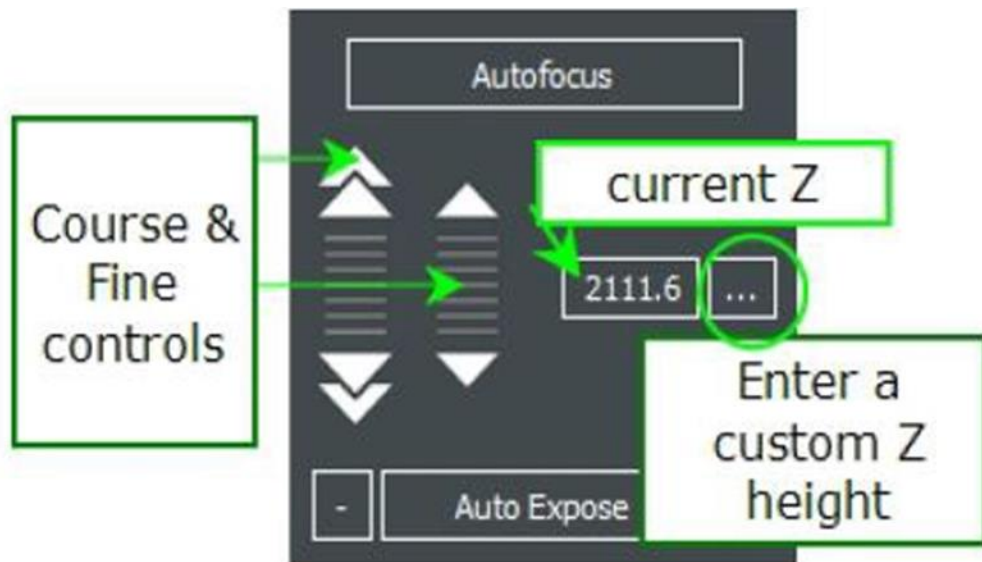


| Exposure Parameter  | Benefit of high setting | Drawback of high setting           |
|---------------------|-------------------------|------------------------------------|
| 1. LED Intensity    | Sensitivity             | Photo-bleaching                    |
| 2. Integration time | Higher image quality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

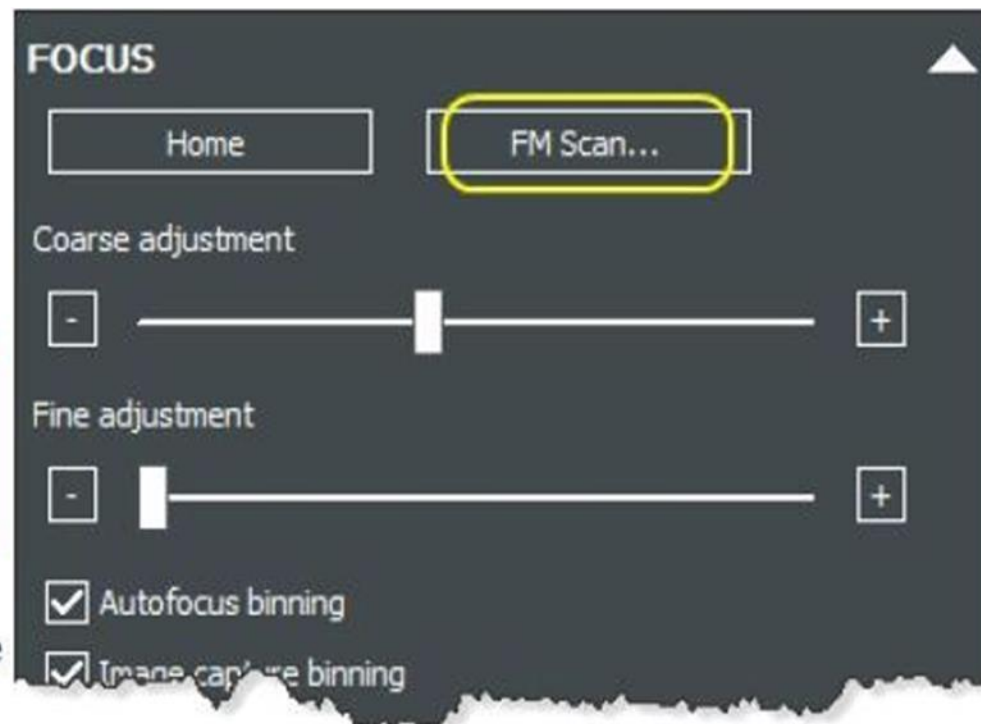
# Auto focus 對焦

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.



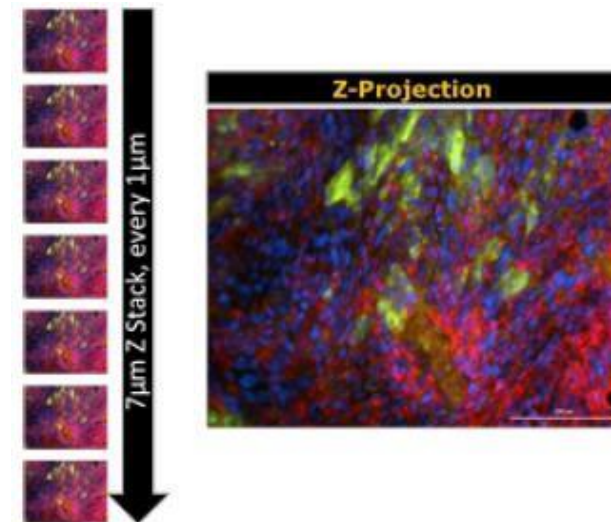
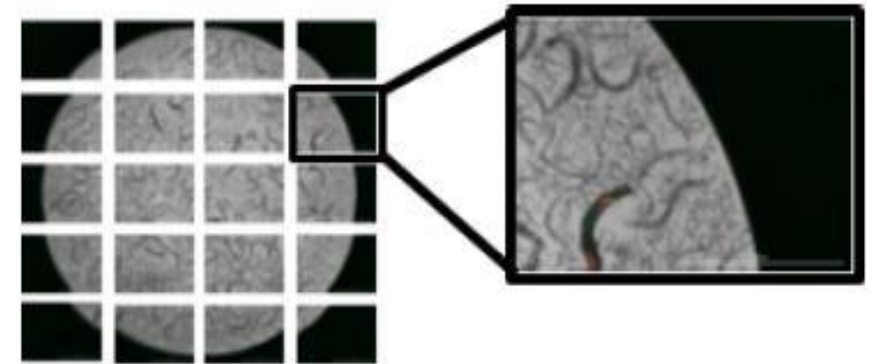
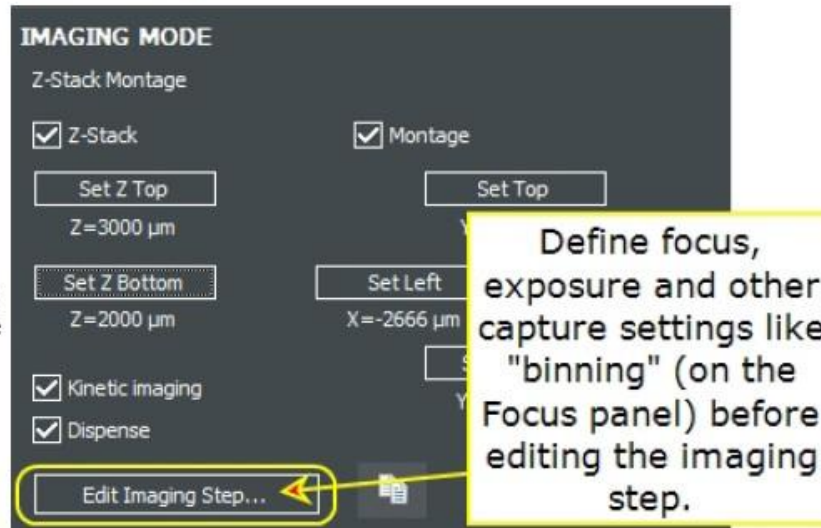
# 進階設定: Image Mode



## 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

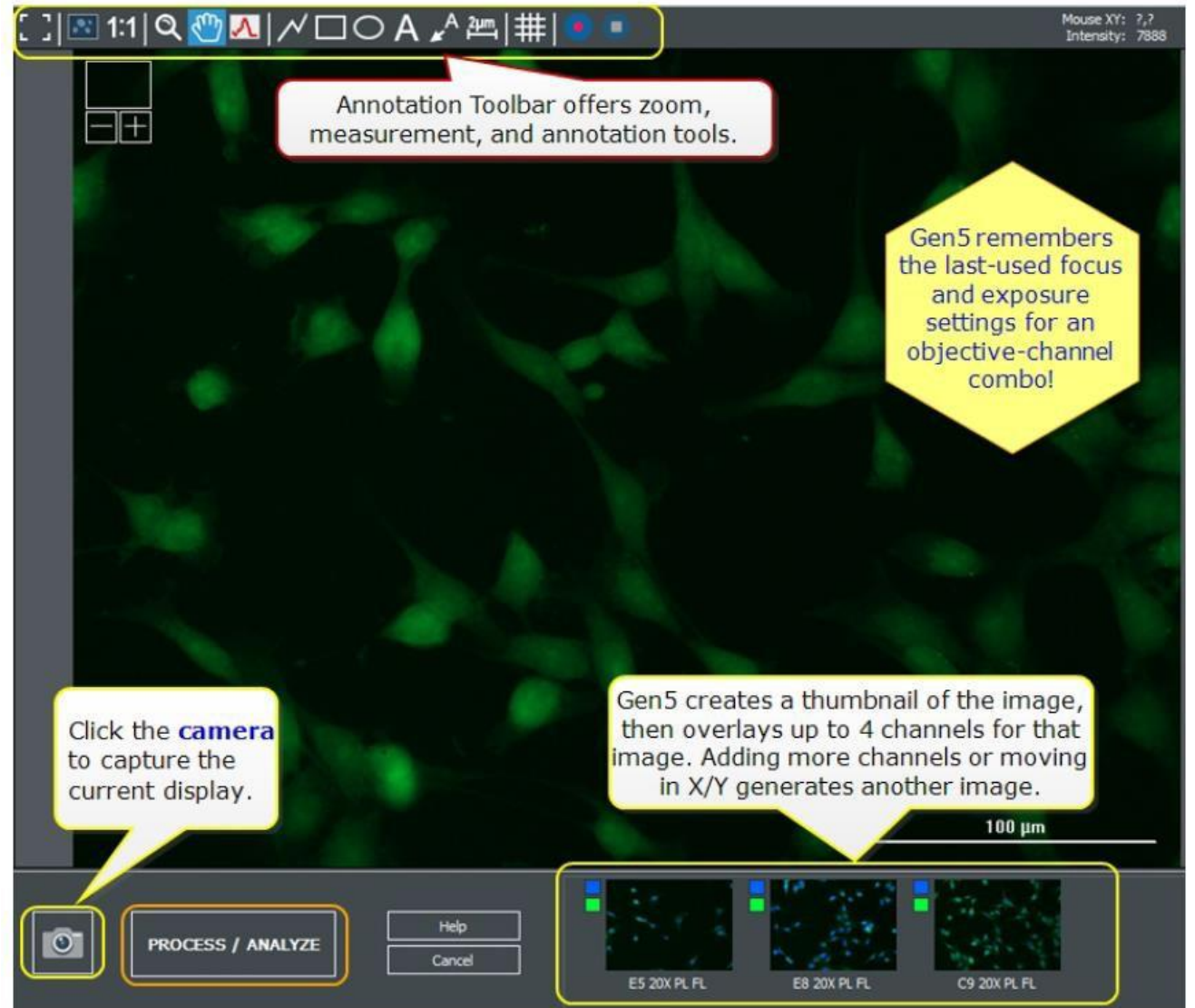
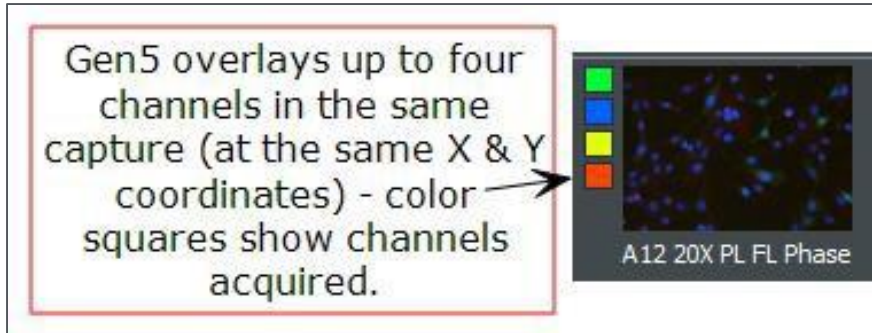
立體樣本: Z stacking

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

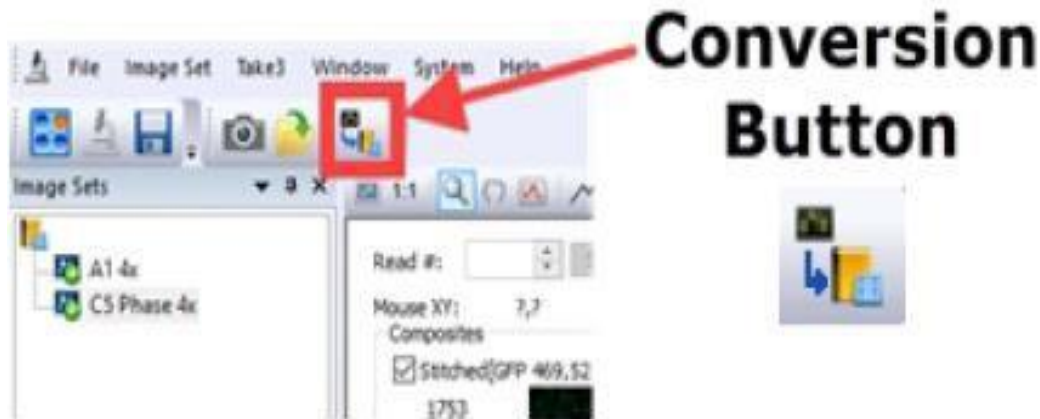
長時間拍攝/樣本量多/大圖拍攝/multi-channel拍攝

# 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



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

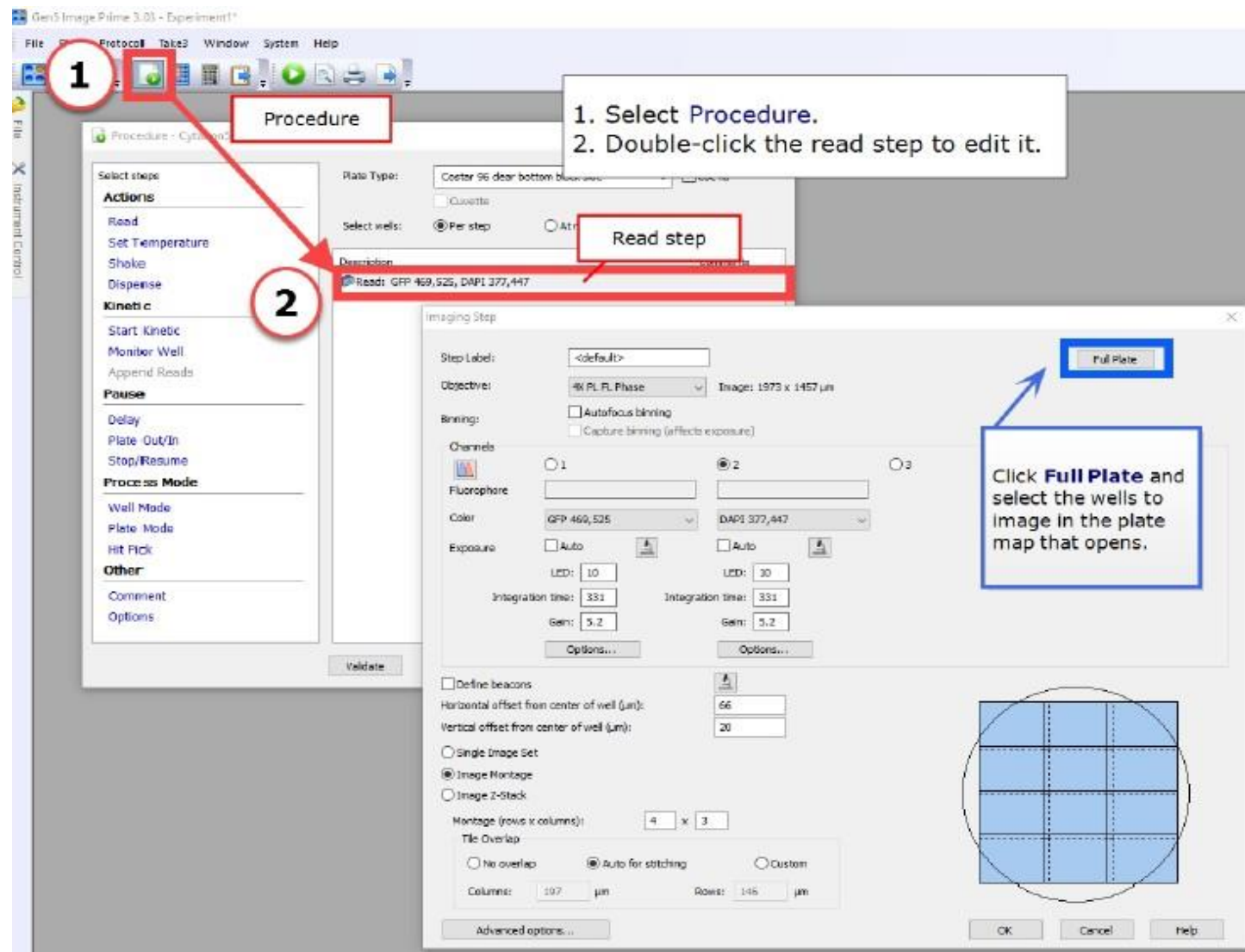
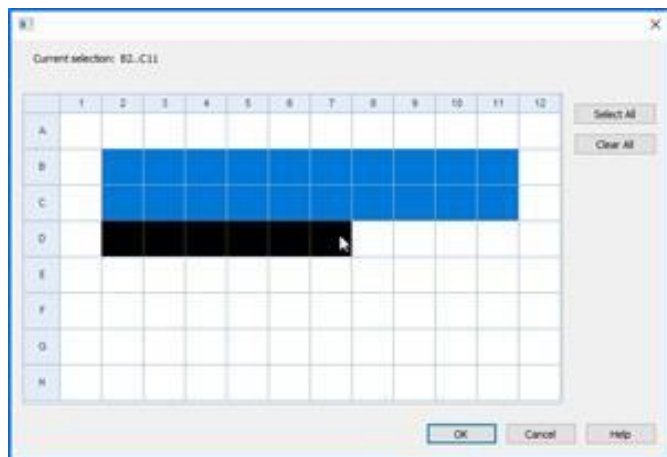
對焦方法

讀取位置

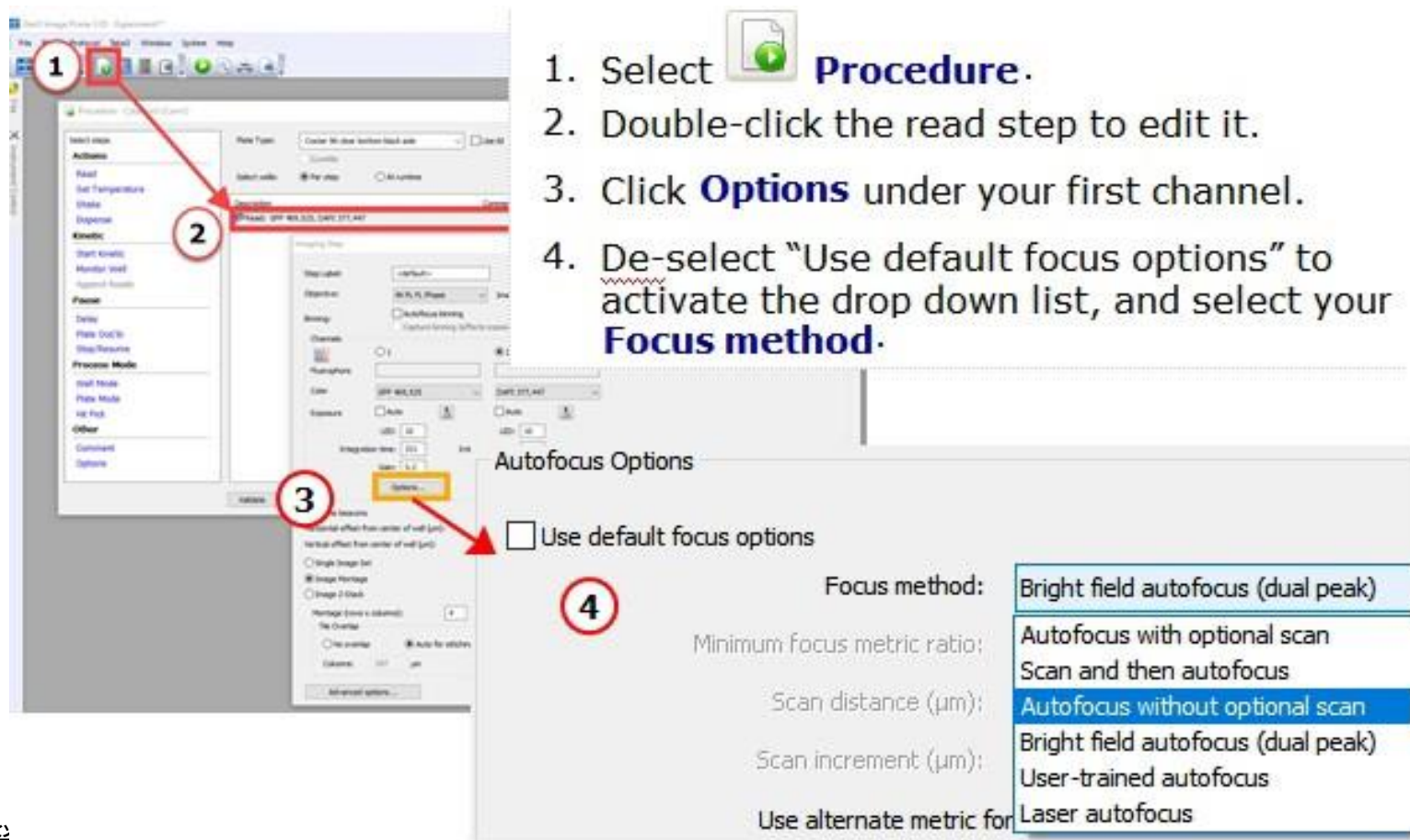
大圖拍攝

立體細胞拍攝

活細胞長時間影像拍攝



# Autofocus 條件選擇



1. Select  **Procedure**.
2. Double-click the read step to edit it.
3. Click **Options** under your first channel.
4. De-select "Use default focus options" to activate the drop down list, and select your **Focus method**.

Autofocus Options

☐ Use default focus options

Focus method:

- Bright field autofocus (dual peak)
- Autofocus with optional scan
- Scan and then autofocus
- Autofocus without optional scan
- Bright field autofocus (dual peak)
- User-trained autofocus
- Laser autofocus

Minimum focus metric ratio:

Scan distance (μm):

Scan increment (μm):

Use alternate metric for

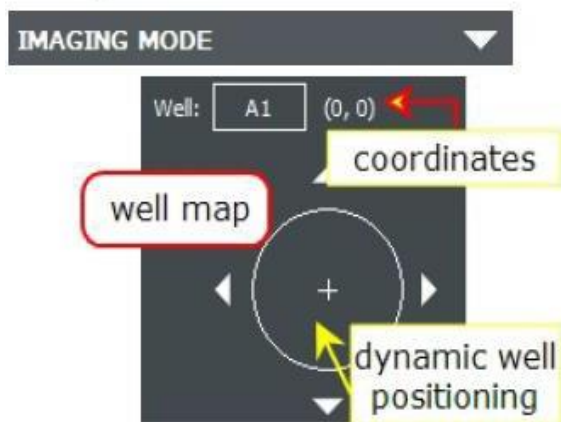
若同時拍攝三色螢光

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

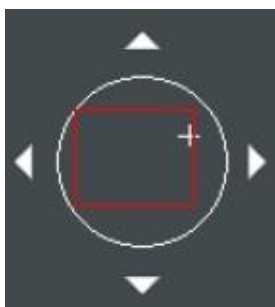
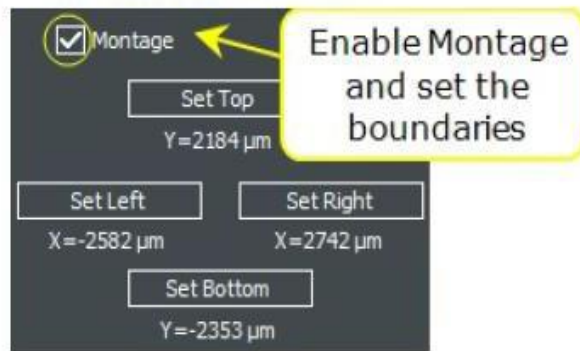
若三色螢光對焦位置皆有差異，則每個channel可自行定義Autofocus method

# Montage: Manual VS Experiment

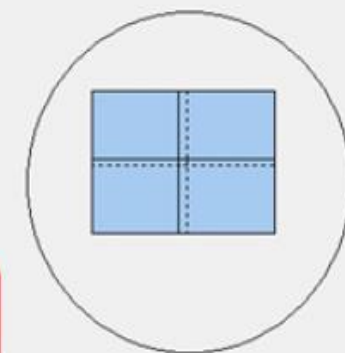
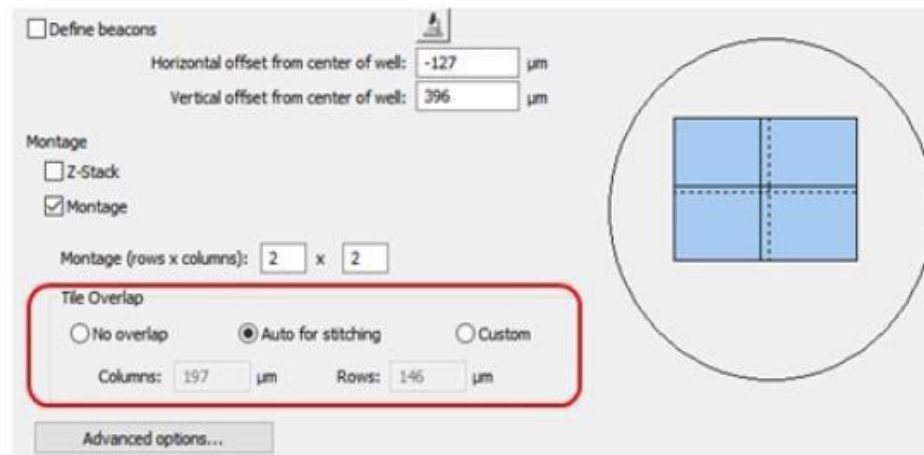
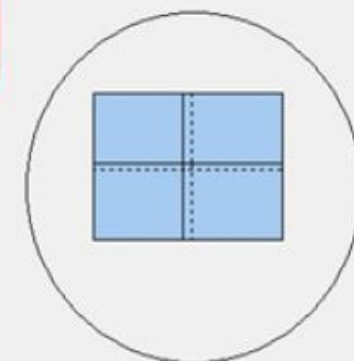
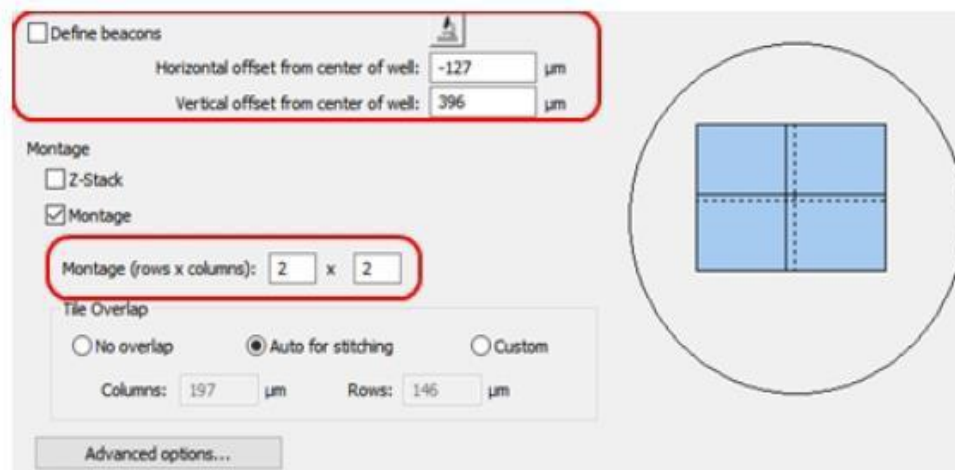
## Manual



Expand the **Imaging Mode** panel.

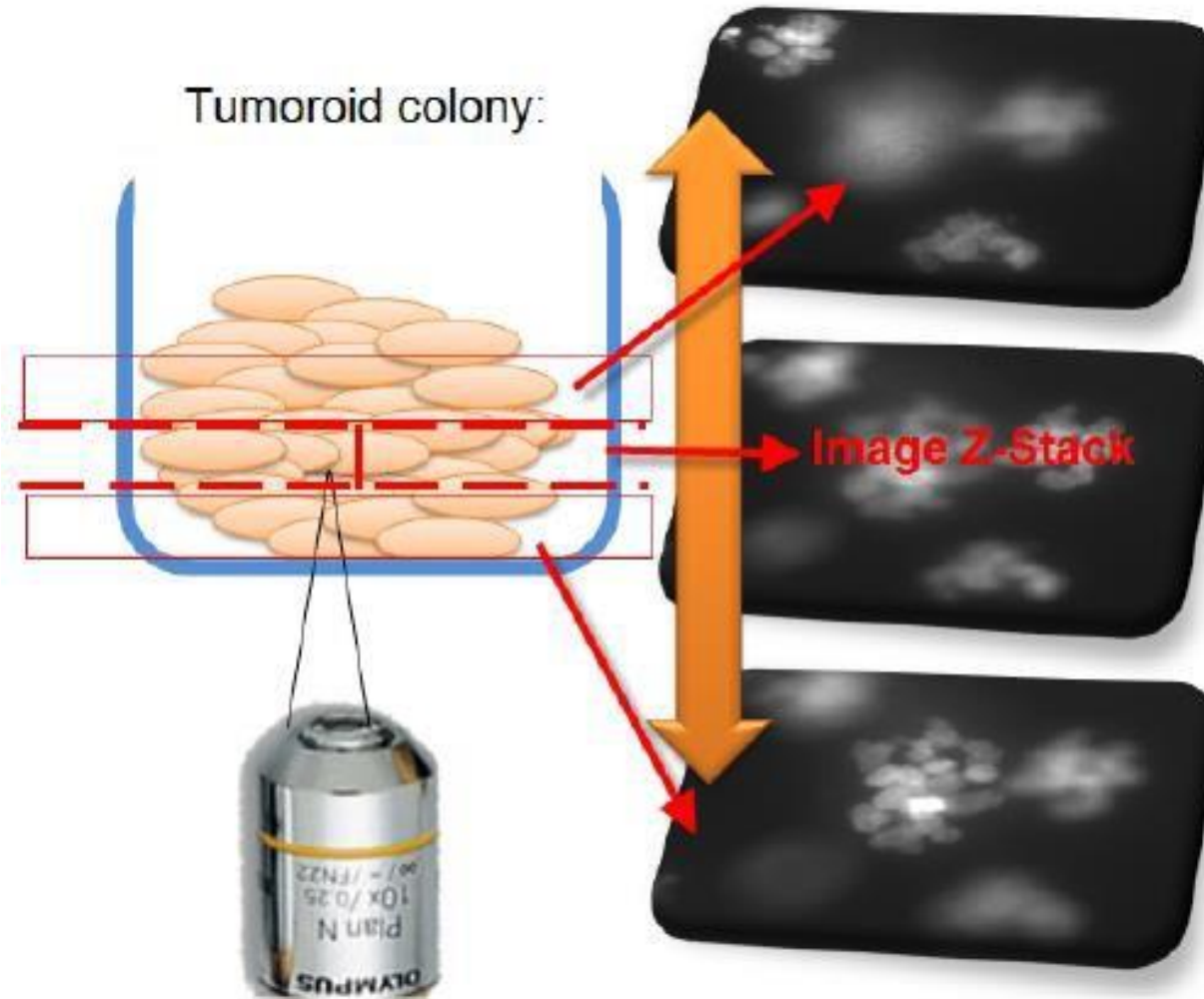


## Experiment



Next step: Stitching

# Z Stacking: Manual VS Experiment



# Z Stacking: Manual VS Experiment

## Manual

 **Manual Mode Z-Stack**

Autofocus

3273.7 ... to move objective to certain position

Z axis

☒ Z-Stack

Set Z Top

Z=3312  $\mu\text{m}$

Set Z Bottom

Z=3198  $\mu\text{m}$

**Z-Stack**

☒ Z-Stack

☐ Montage

Number of slices: 37

Step size: 4.2  $\mu\text{m}$

Sample thickness: 151.2  $\mu\text{m}$

## Experiment

**Z-Stack**

☒ Z-Stack

☐ Montage

Number of slices: 10

Step size: 53.8  $\mu\text{m}$

Images below focus point: 0

Sample thickness: 484.2  $\mu\text{m}$

Advanced options...

Top-> #10 +484.2

Bottom-> #1 +0  $\mu\text{m}$  <- Focus #1

**ADD PROCESSING STEP**

Image Preprocessing

Image Deconvolution

**Z Projection**

Next step: Projection

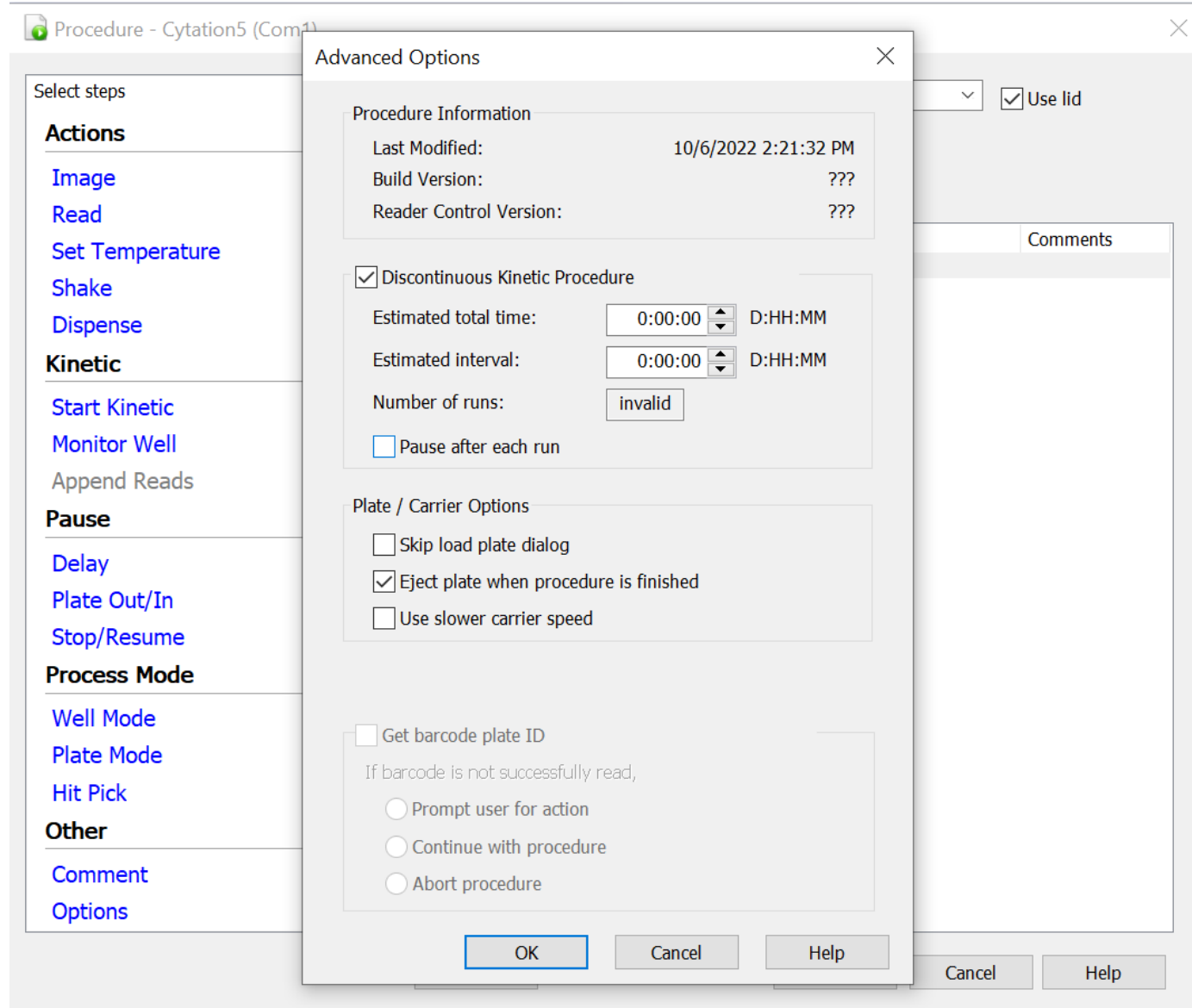
# 活細胞影像觀察: Kinetic Image Capture (無法暫停)

The image displays two screenshots of the Cytation5 software interface, illustrating the setup for Kinetic Image Capture.

**Left Screenshot:** The 'Procedure - Cytation5 (Com1)' window shows the 'Kinetic Step' dialog box. The 'Run Time' is set to 0:10:00, the 'Interval' is 0:01:00, and the 'Reads' are set to 11. The 'Description' field contains 'Read: DAPI 377,447, 469/593 469,593'. The 'Important' note states: 'Important: In imaging mode, the minimum kinetic interval varies with many parameters including the number of images per well, color channels, exposure settings and autofocus settings. Please refer to the Help system for general guidelines.'

**Right Screenshot:** The 'Procedure - Cytation5 (Com1)' window shows the 'Start Kinetic' step in the procedure list. The 'Description' field contains 'Temperature: Setpoint 37 °C', 'Start Kinetic [Run 24:00:00, Interval 0:30:00]', and 'Read: DAPI 377,447, 469/593 469,593'. The 'End Kinetic' step is also visible.

# 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

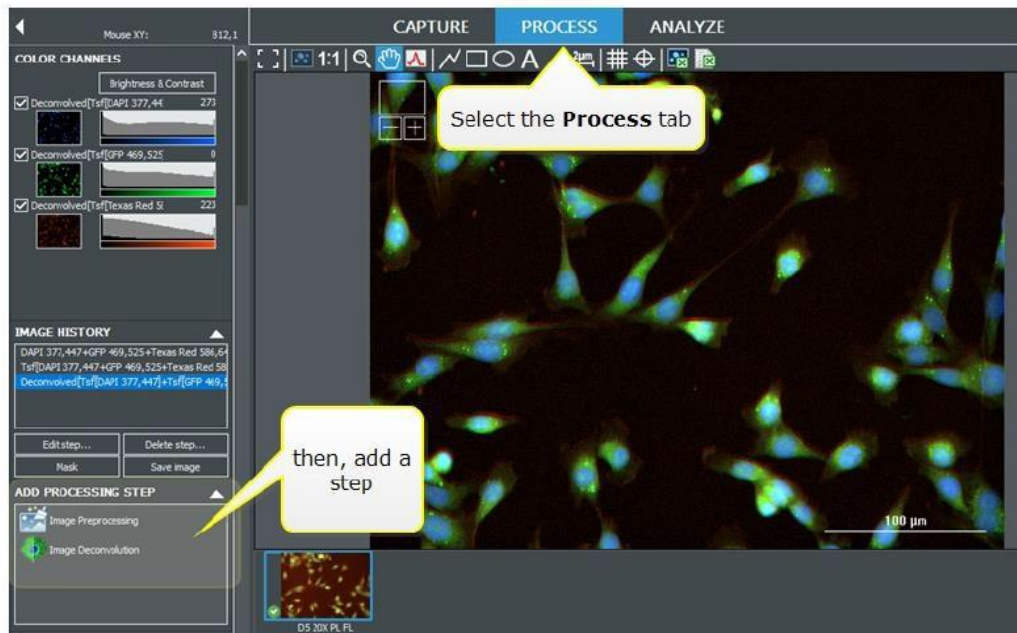
# 無法拍到對焦照片？

- 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

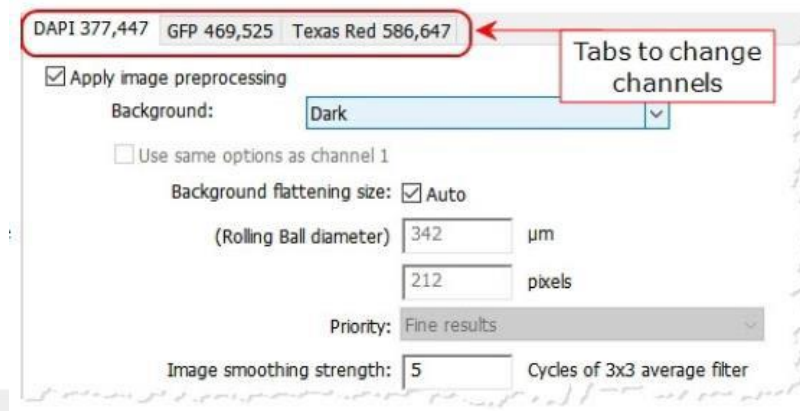
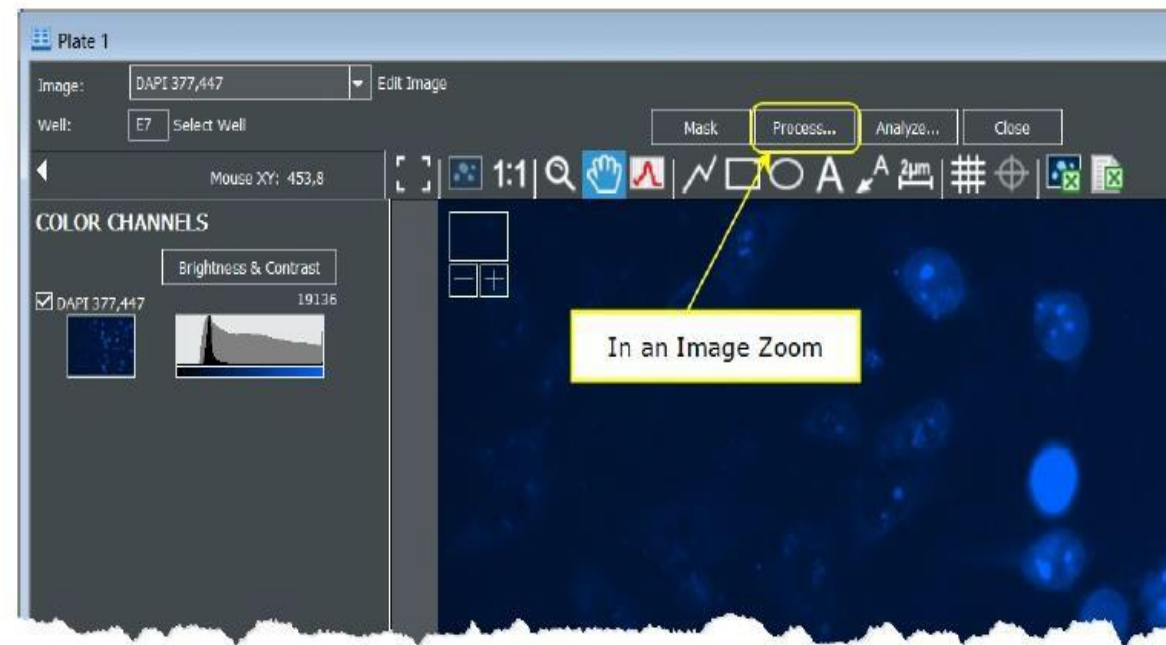
# Optimize your data: Process

# 去背景值: Image Preprocessing

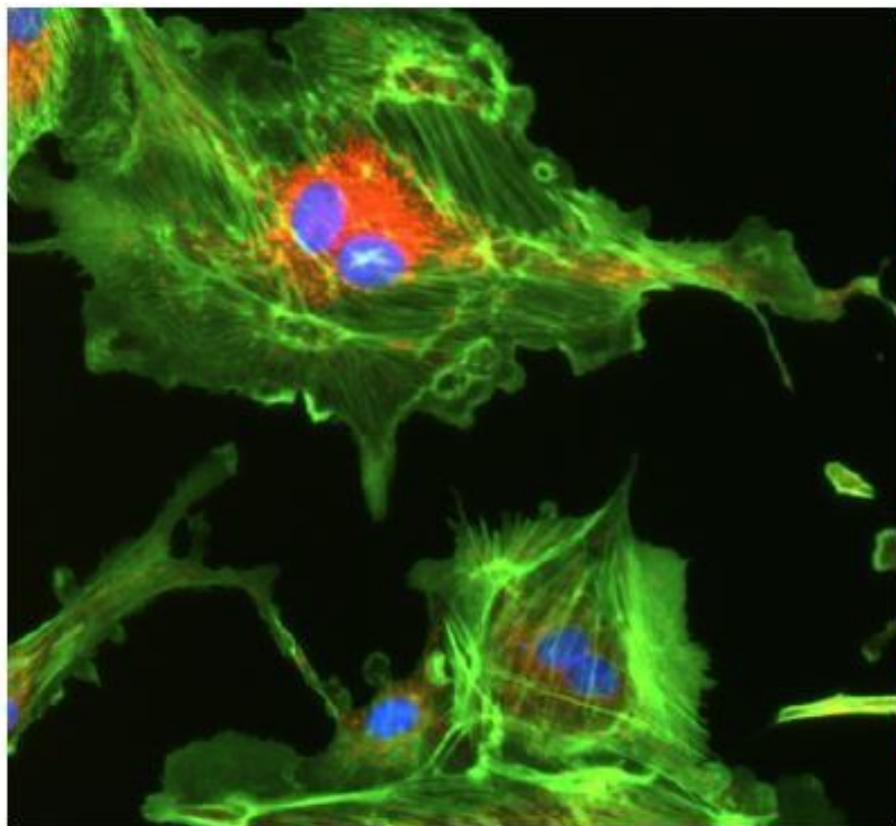
Manual



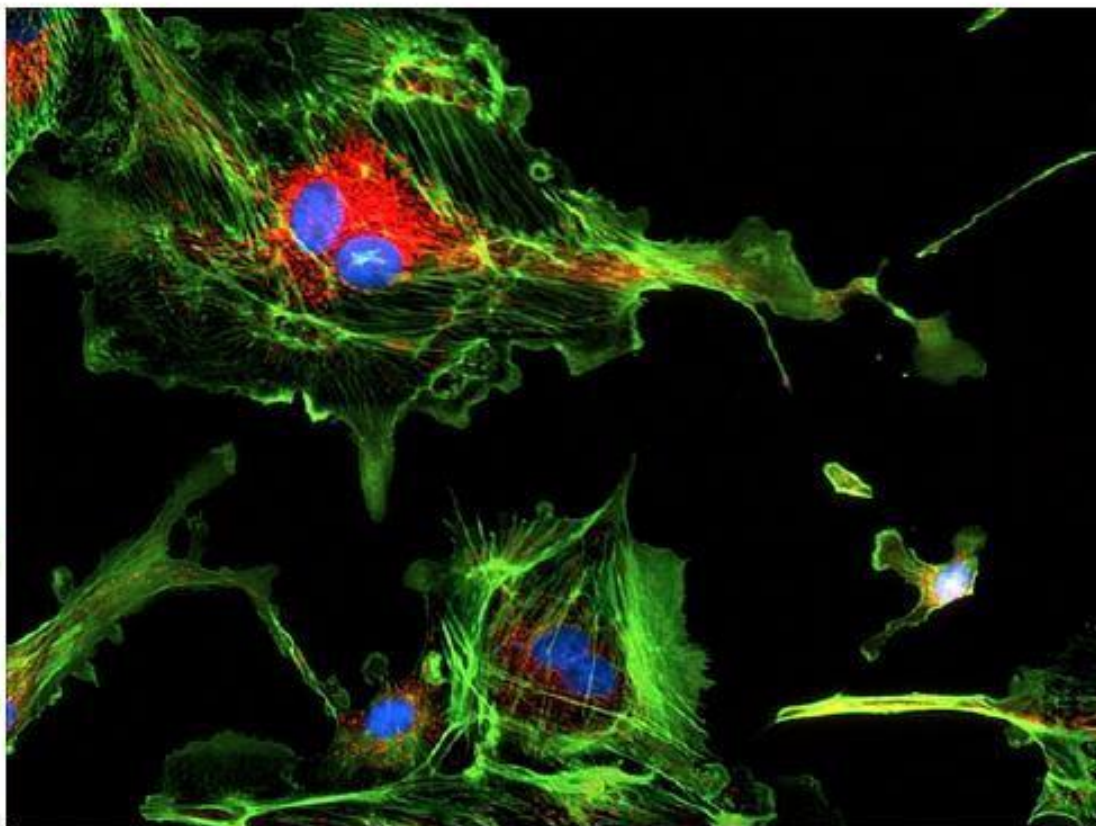
Experiment



# 線條銳利化: Deconvolution



Before deconvolution



After deconvolution

# Basic Image Analysis

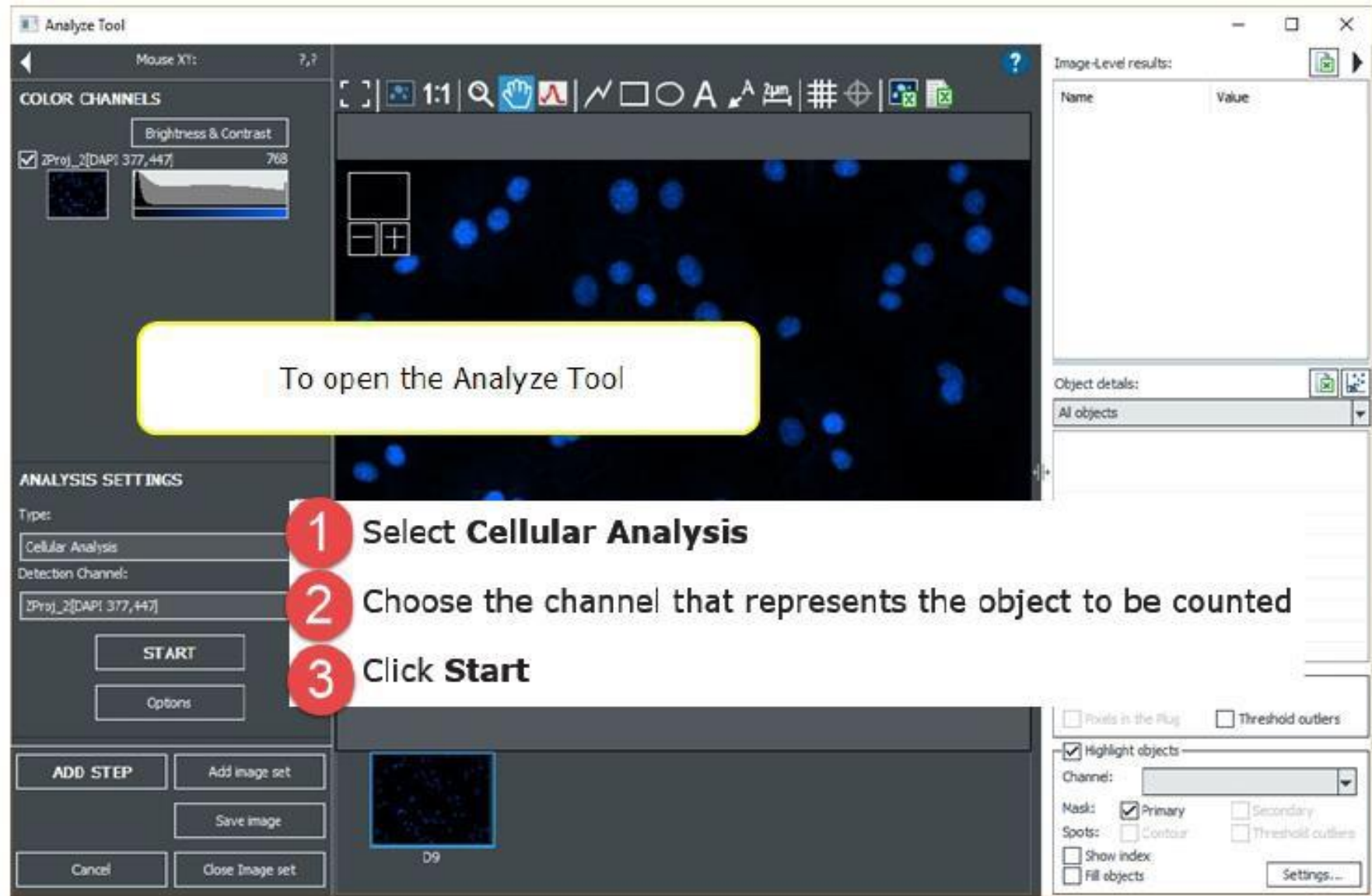
# Basic image analysis

選擇image statistics 或cellular analysis

The screenshot displays the software interface with the **ANALYZE** tab selected. The interface includes several panels and a main image area.

- COLOR CHANNELS:** A panel on the left showing three channels: ☒ Deconvolved[Tsf[DAPI 377,447], ☒ Deconvolved[Tsf[GFP 469,525], and ☒ Deconvolved[Tsf[Texas Red 568]. A callout box points to the checkboxes, stating: "View one channel at a time using the checkbox to show or hide the channel in the current image."
- IMAGE HISTORY:** A panel below the color channels showing a list of data sets. A callout box points to the list, stating: "Highlight the data set to use for the analysis."
- ADD ANALYSIS STEP:** A panel at the bottom left with two options: ☒ Image Statistics and ☐ Cellular Analysis. A callout box points to the **Image Statistics** option, stating: "Click an option and then click **START**".
- NAVIGATION:** A panel at the bottom left with a **Navigation** button.
- Main Image:** A large central area showing a fluorescence image of cells. A callout box points to the **ANALYZE** tab, stating: "After selecting the **ANALYZE** tab".
- Scale Bar:** A scale bar in the bottom right corner of the main image, labeled "100  $\mu$ m".
- Image Selection:** Two small image thumbnails at the bottom center, labeled "EB 20X PL FL\*" and "C7 20X PL FL\*". A callout box points to the "C7 20X PL FL\*" thumbnail, stating: "Select the captured image to analyze."

# 細胞計數: Cellular Analysis



# 細胞計數: Cellular Analysis

Mouse XY: 1,7

COLOR CHANNELS

Brightness & Contrast

Ts[DAPI 377,447]

28

Ts[GFP 469,525]

63

IMAGE HISTORY

DAPI 377,447+GFP 469,525

Ts[DAPI 377,447+GFP 469,525]

Mask Save Image

ADD ANALYSIS STEP

Image Statistics

Cellular Analysis

NAVIGATION

CAPTURE

PROCESS

ANALYZE

1:1

Q

Hand

Line

Rectangle

Circle

Area

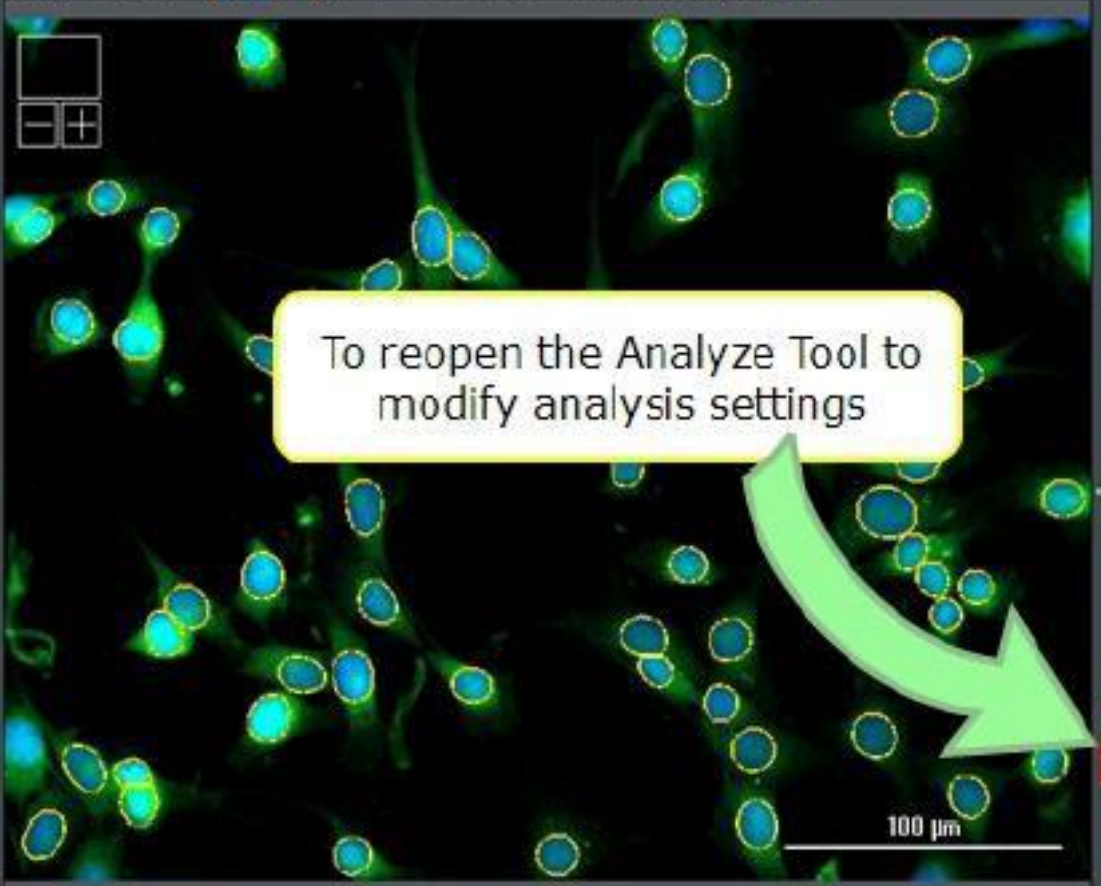
2µm

Grid

Zoom

Reset

Close



To reopen the Analyze Tool to modify analysis settings

100 µm

C9 20X P, FL \*

Image Review

Kinetic Analysis

Image-Level results:

Cellular Analysis[Ts[DAPI 377,447]]

|                               | Value    |
|-------------------------------|----------|
| Cell Count                    | 59       |
| Object Size                   | 14.3     |
| Object Area                   | 161      |
| Object Sum Area               | 9.40E+03 |
| Object Mean[Ts[DAPI 377,447]] | 17499    |

Object details:

All objects

| #  | Size | Area | Mean(Ts[DAPI 377,447]) | Area(Ts[DAPI 377,447]) | Mean(Ts[GFP 469,525]) |
|----|------|------|------------------------|------------------------|-----------------------|
| 1  | 12.9 | 130  | 18724                  | 130                    | 15329                 |
| 2  | 12.7 | 127  | 22031                  | 127                    | 22429                 |
| 3  | 15.6 | 190  | 18602                  | 190                    | 12093                 |
| 4  | 17.0 | 226  | 15358                  | 226                    | 9994                  |
| 5  | 15.8 | 194  | 12987                  | 194                    | 1460                  |
| 6  | 14.4 | 162  | 23538                  | 162                    | 17648                 |
| 7  | 12.3 | 118  | 19168                  | 118                    | 14392                 |
| 8  | 14.2 | 157  | 18666                  | 157                    | 14833                 |
| 9  | 11.6 | 103  | 24614                  | 103                    | 23105                 |
| 10 | 17.2 | 224  | 20885                  | 224                    | 13554                 |
| 11 | 12.2 | 117  | 19297                  | 117                    | 16127                 |

Edit step... Delete step...

Highlight points

☐ Saturated pixels

☐ Pixels in the Rug

☐ Threshold outliers

☒ Highlight objects

Channel: Ts[DAPI 377,447]

Mask: ☒ Primary ☐ Secondary

Spots: ☐ Contour ☐ Threshold outliers

☐ Show index

☐ Fill objects

Settings...

# Cellular Analysis

Cellular Analysis

Primary Mask and Count Secondary Mask Calculated Metrics Subpopulation Analysis

Channel: Tsf[DAPI 377,447]

Threshold 關值設定

☒ Auto

Include less   Include more

Background: Dark

☒ Split touching objects

☒ Fill holes in masks

[Advanced detection options...](#)

Object selection

Min. object size: 5  $\mu\text{m}$

Max. object size: 100  $\mu\text{m}$

☐ Include primary edge objects

☒ Analyze entire image

欲分析之細胞大小設定

欲分析之範圍設定，如同image stastics 分析

# Secondary Mask

Cellular Analysis

Primary Mask and Count Secondary Mask Calculated Metrics Subpopulation Analysis

Tsf[GFP 469,525]

☒ Measure within a Primary mask

Use Primary mask

☒ Measure within a Secondary mask

Type: ☒  

Distance from Primary mask:   $\mu\text{m}$  Ring width:   $\mu\text{m}$

☒ Threshold

☒ Auto Include less   Include more  Neutral

Background:  Dark ☐ Fill holes in the mask

Method:  Propagate mask

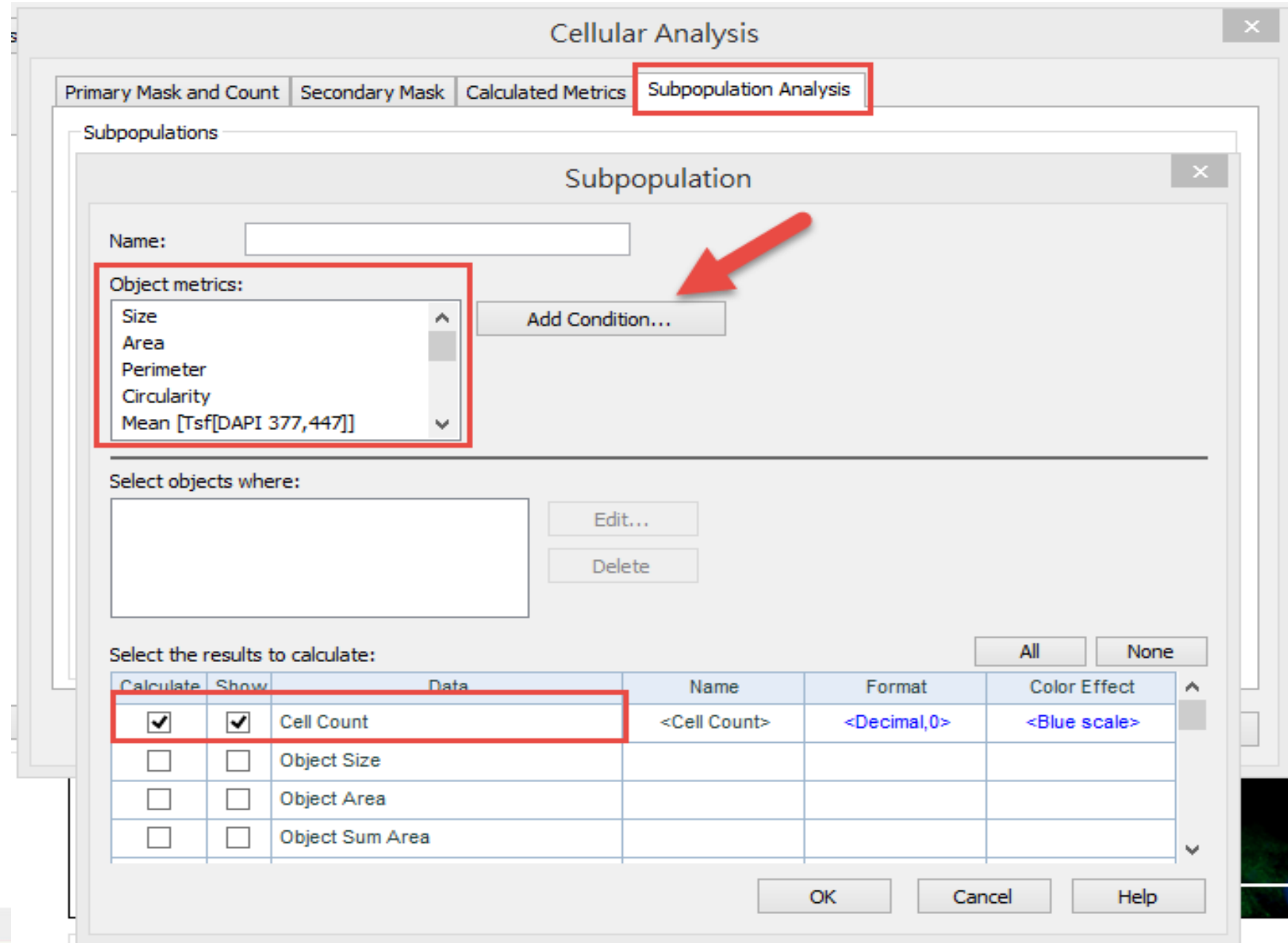
Apply OK Cancel Help

分析核以外區域

分析整顆細胞

設定範圍大小

# 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**

**Image Save Options**

Gen5 Image Library

Pathname:  Browse...

Experiment Folder

Name:

Example:

Plate Folder

Name:

Example:

Image File Naming Convention

Name:

Example:

Image files are stored in the Gen5 Image Library in a user-specified location in the Windows File System

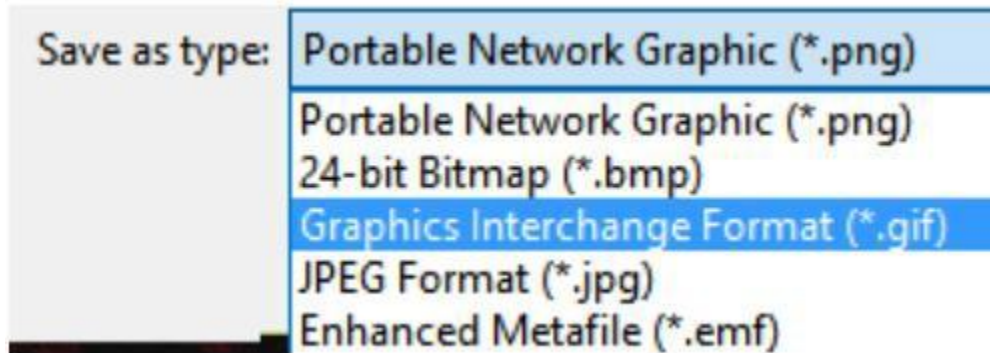
# Save Image

手動模式：

滑鼠右鍵> Save> Save Picture for presentation

實驗模式：

Plate > Batch Image Save



## Image Save Options

### ☒ Save image raw data

Save raw, uncompressed gray-scale 16-bit image in TIFF format. This option preserves the entire image information (including metadata) and the saved file can be used for further image processing and analysis.

#### Multi-channel image

- ☒ Save channels as separate files
- ☐ Stack all images in a single TIFF file

### ☐ Save picture for presentation

Save for use as a picture file in Office-type documents. This option can preserve color, scale bar and zoom level, but some image information is lost and the file should not be used for image processing and analysis.

#### Dimension

- ☐ Save entire image (1 camera pixel resolution)
- ☒ Save current display (WYSIWYG)

#### Include

- ☒ Scale bar
- ☐ Zoom position in image

# Thank you for listening



進 階 生 物 科 技  
Level Biotechnology Inc.

# Gen5 Image Prime 影像分析

細胞計數

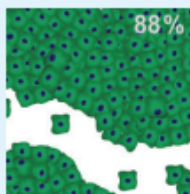
Cell Count



|                       |                                                                 |
|-----------------------|-----------------------------------------------------------------|
| Cell count            | Spot count (liposomes, mitochondria, endosomes, autophagosomes) |
| Cell viability        | Toxicology (hepatotoxicity, genotoxicity, necrosis)             |
| Micronuclei Formation | Toxicity in whole organisms                                     |
| Proliferation         |                                                                 |

覆蓋率

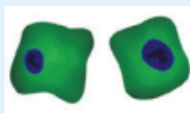
Confluence



|                     |                       |                              |
|---------------------|-----------------------|------------------------------|
| 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       |

螢光強度

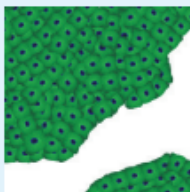
Total Intensity



|                     |                       |                                 |
|---------------------|-----------------------|---------------------------------|
| Cell viability      | Syncytia formation    | Proliferation assays            |
| Colony formation    | Microtubule formation | Signal appearance/disappearance |
| Cytotoxicity assays | Neurotoxicity         |                                 |

總面積

Total Area



|                     |                       |                      |
|---------------------|-----------------------|----------------------|
| Cell viability      | Syncytia formation    | Neurite outgrowth    |
| Colony formation    | Microtubule formation | Neurotoxicity        |
| Cytotoxicity assays | Hypertrophy           | Proliferation assays |

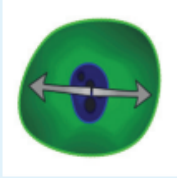
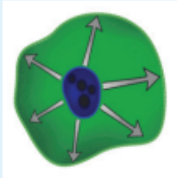
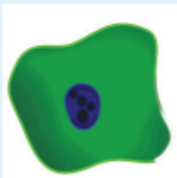
# Gen5 Image Prime 影像分析

細胞大小

細胞面積

細胞周長

細胞圓度

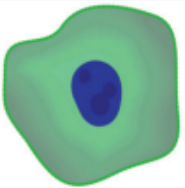
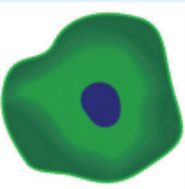
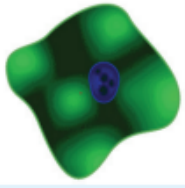
| Gen5 Object-Level Parameters and Applications                                                                    |  |                               |                               |                                         |
|------------------------------------------------------------------------------------------------------------------|--|-------------------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

# Gen5 Image Prime 影像分析

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

| Gen5 Object-Level Parameters and Applications                                                                                     |  |                          |                                         |
|-----------------------------------------------------------------------------------------------------------------------------------|--|--------------------------|-----------------------------------------|
| Object-Level Parameter                                                                                                            |  | Possible Targets         |                                         |
| <b>Object Mean Intensity</b><br>                 |  | Biomarker classification | Gene expression                         |
|                                                                                                                                   |  | Cell cycle               | Genotoxicity                            |
|                                                                                                                                   |  | Cell signaling           | Glutathione depletion                   |
|                                                                                                                                   |  | Cell survival            | Ion channel studies (Ca <sup>2+</sup> ) |
|                                                                                                                                   |  | Cell viability           | Organelle health                        |
|                                                                                                                                   |  | Target colocalization    | Oxidative stress                        |
|                                                                                                                                   |  | ER stress                | Protein expression                      |
| <b>Object Integral Intensity</b><br>            |  | Hypertrophy              | Morphological changes                   |
|                                                                                                                                   |  |                          | COMET assay                             |
| <b>Object Intensity Standard Deviation</b><br> |  | Cell cycle               | Phenotypic analysis                     |
|                                                                                                                                   |  | Translocation            | Stem cell differentiation               |