Leica
MICROSYSTEMS

Vision, Measurement and Analysis

MetaMorph®

Live Cell Imaging

Major Instruments Co., Ltd.

MetaMorph® MetaFluor® MetaVue™

Leica MICROSYSTEMS

- Outline -

Hardware –

Inverted Microscope : LeicaDMI4000

Incubation Chamber

Acquisition Device : Roper HQ2

Software –

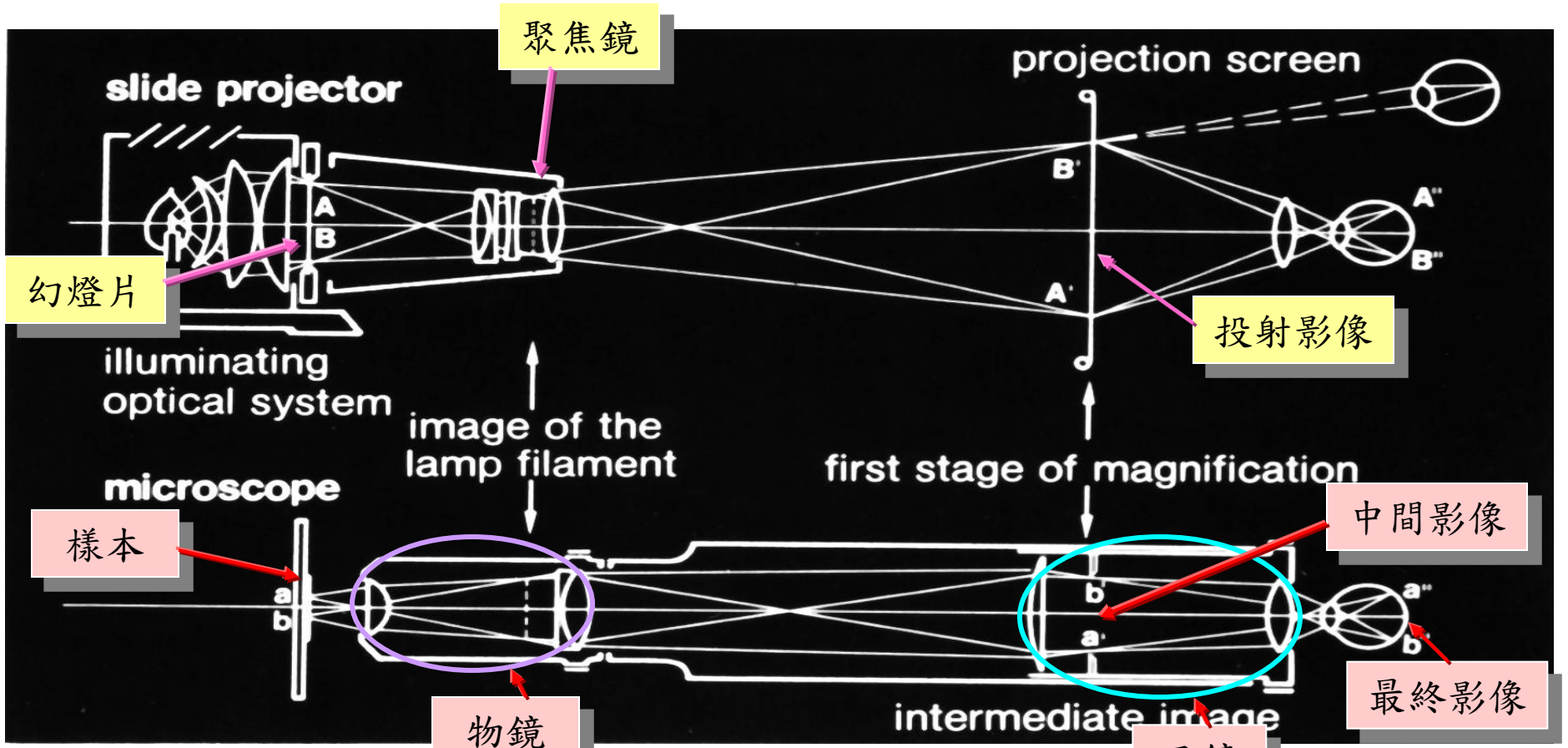
MetaMorph

Light Microscopy

- **Image formation**
- **Optics**
  - **Aberration**
  - **Objectives**
- **Bright Field, Phase Contrast**
- **Fluorescence Microscopy**

❖顯微鏡的成像

- 二次放大(*物鏡, 目鏡*)與中間影像(*Intermediate Image*)



❖影像的形成

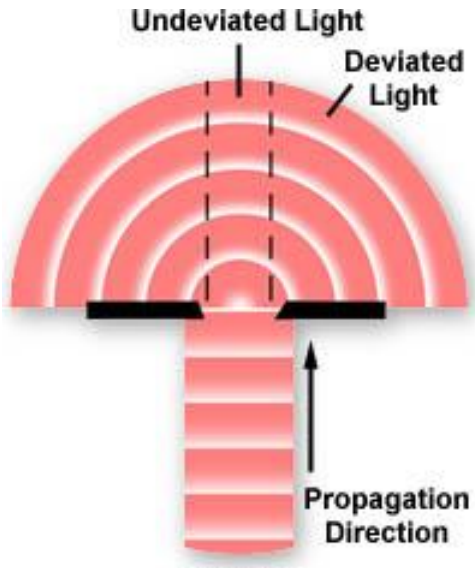
- 光線在透過物體時， 因物體的阻擋而產生二種情況：

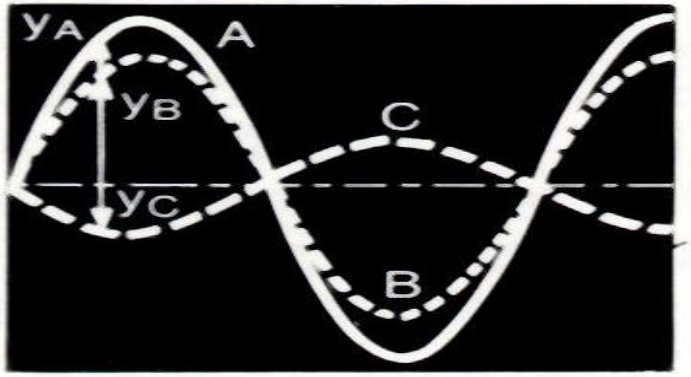
  Undeviated light (Direct light) – 即為背景光亮。

  Deviated light (Diffracted light) – 因通過樣品而產生。 } ***1/2λ or 180 degree out of phase***

- 二種光線產生破壞性的干擾 (destructive interference)而觀察到物體的影像。

  ⇒ ***Image = Direct light + Diffracted light***





YA = direct light

YC = diffracted light

YB = Image

YB = YA + YC

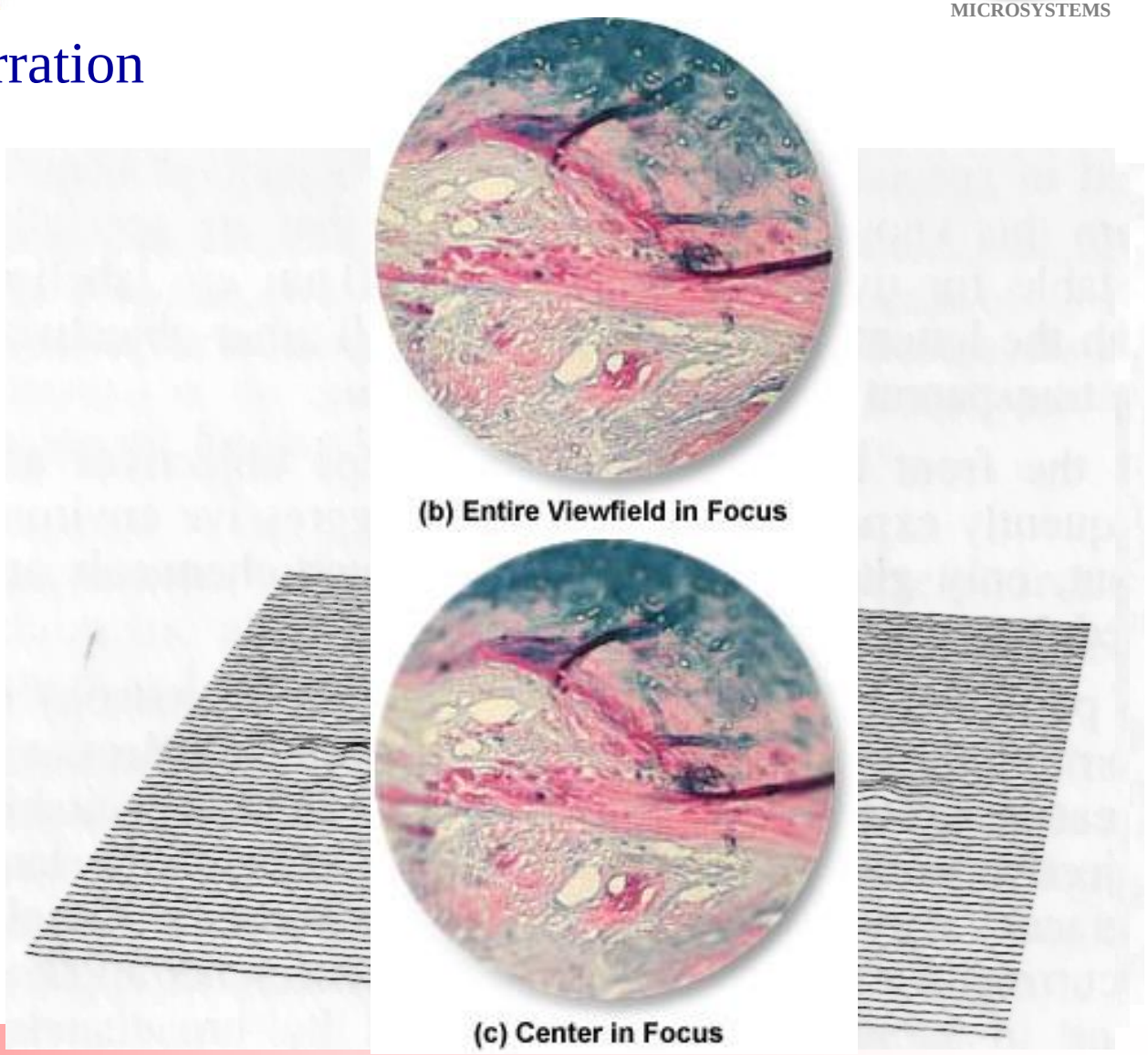
❖ Monochromatic aberration

Spherical aberration

Coma

Astigmatism

Image curvature



❖ *Chromatic aberration*

Image lenses are never free of aberrations, but most of these can be eliminated by a combination of lenses.

Axial chromatic aberration – colored fog

Lateral chromatic aberration – color frignes

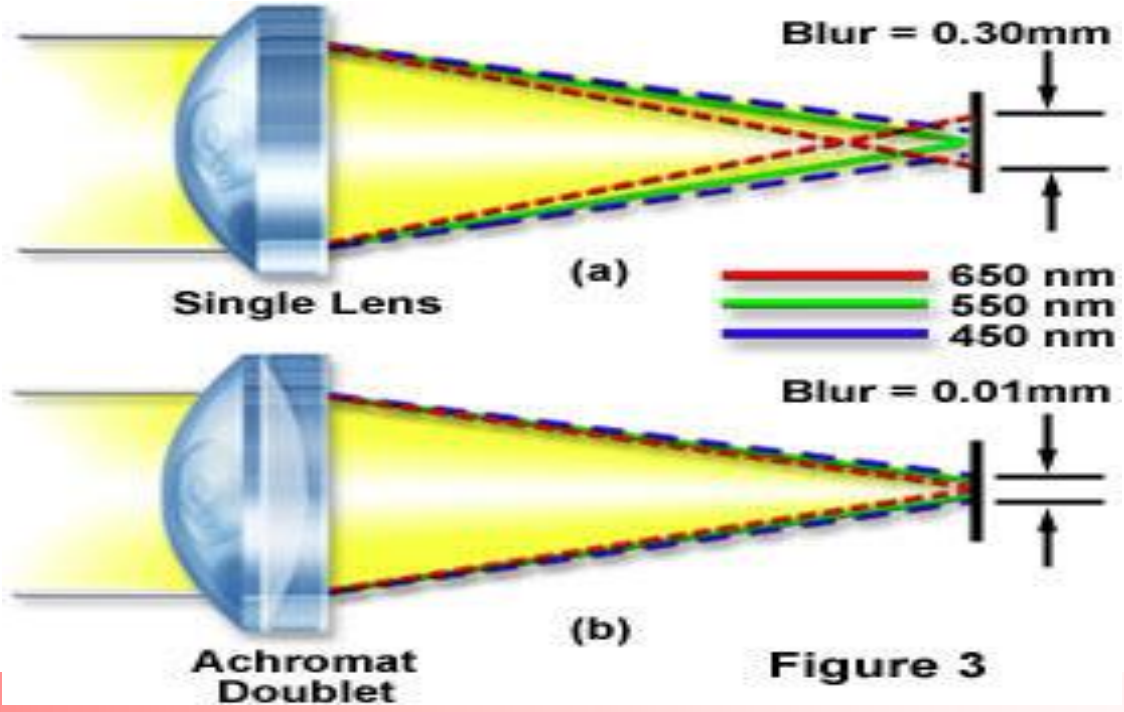


Figure 3

Objectives

- C PLAN: Achromatic objectives with a field performance up to 20 mm.
  Magnification: from 4x to 100x oil

- N PLAN: Planachromatic objectives with a field performance up to 22 mm.
  Magnification: from 2.5x to 100x oil

- PL FLUOTAR: Semi-apochromats with a field performance of at least 25 mm.
  Magnification: from 1.6x to 100x oil.

- PL APO: Plan apochromats with a field performance of up to 28 mm,.
  Magnification: from 10x to 100x oil, 150x and 250x are for semiconductor.

- Special Designed Objectives – HCX PL APO CS:
  Some HCX PL APO objectives are re-calibrated for confocal system.

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❖ How to "read" your objectives?

- ∞/0.17/D
  - ∞: Infinite tube length objectives.
  - 0.17: 0.17mm蓋玻片 (0: Coverglass thickness 0; -:With or without coverglass; 0-2:for 0-2mm coverglass.)
  - A,B1,B2,C,D: DIC 稜鏡種類

- HC PL FLUOTAR

- 100x/1.40 OIL PH3
  - 100x: 物鏡倍率.
  - 1.40: 物鏡 NA 值
  - OIL：油鏡
  - PH3 ：Phase Contrast
  - (CORR: Continuous setting to coverglass thickness)

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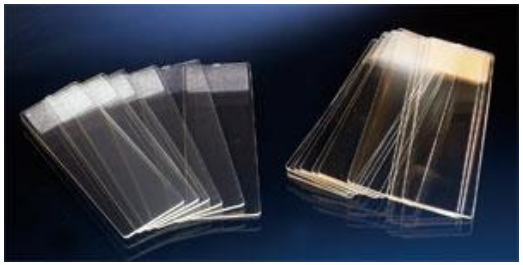
正立顯微鏡

倒立顯微鏡



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Two kind of beam path

- Transmission Light
  明視野　Bright Field
  相位差　Phase Contrast

- Incident Light
  螢光　Fluorescent

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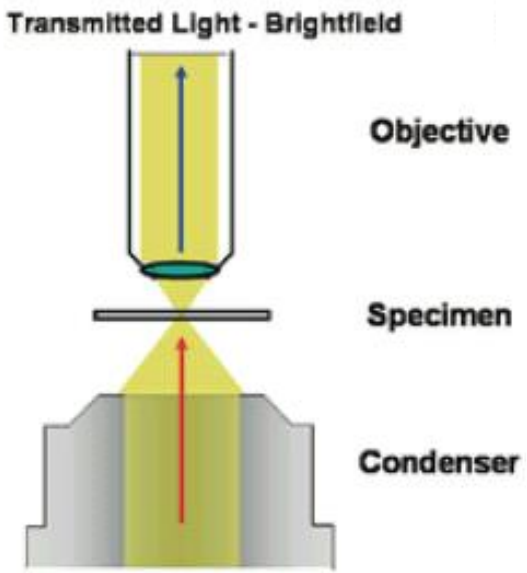
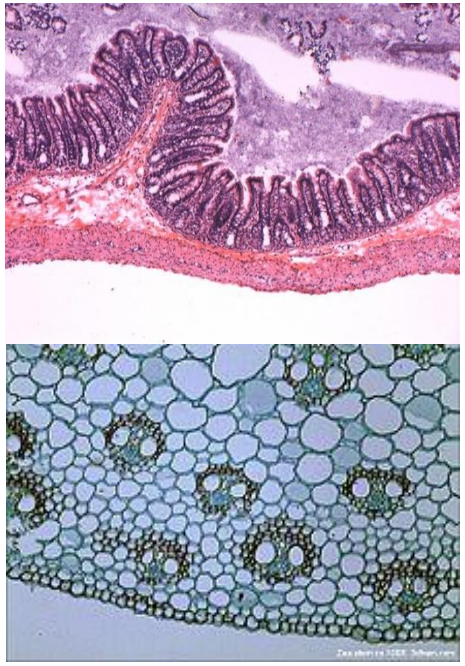
- Bright-Field Microscopy -

- Bright Field – 適用於已染色、強對比的樣本。





為什麼要有Phase Contrast?

❖ *觀察的限制*

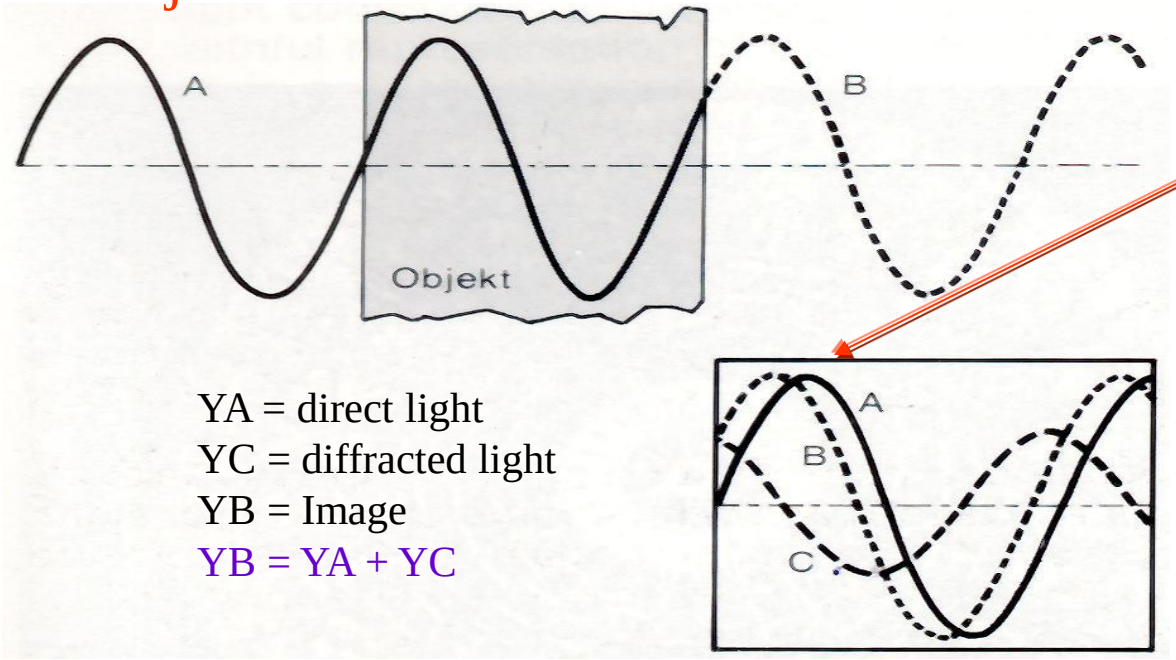
- 受觀測物體可區分為：**Amplitude objects** – 已染色或吸光率高的高對比樣品。
  Phase objects – 未染色之活細胞。
- 人的眼睛只能觀察到：1. 顏色的變化 – 波長wavelength.
  2. 光強度的改變 – 振幅wave amplitude.
- 未染色之活細胞通常不能吸收光線，他們只是改變入射光線約1/4λ的位相差，而這樣變化人眼是幾乎無法察覺的.....

⇒我們只能觀察Amplitude objects 而無法看到Phase objects。

❖ *解決的方法*

- 將原本只有phase變化的物體，藉由顯微技術的設計，將其轉換為人眼可觀察的amplitude變化，此即為***Phase Contrast Microscopy.***
- 1930年代，Fritz Zernike成功的將phase objects的影像以amplitude方式展示出來而可被人眼容易的觀察，奠定了Phase contrast microscopy的基礎，Zernike也因此於1953年獲得諾貝爾物理獎。

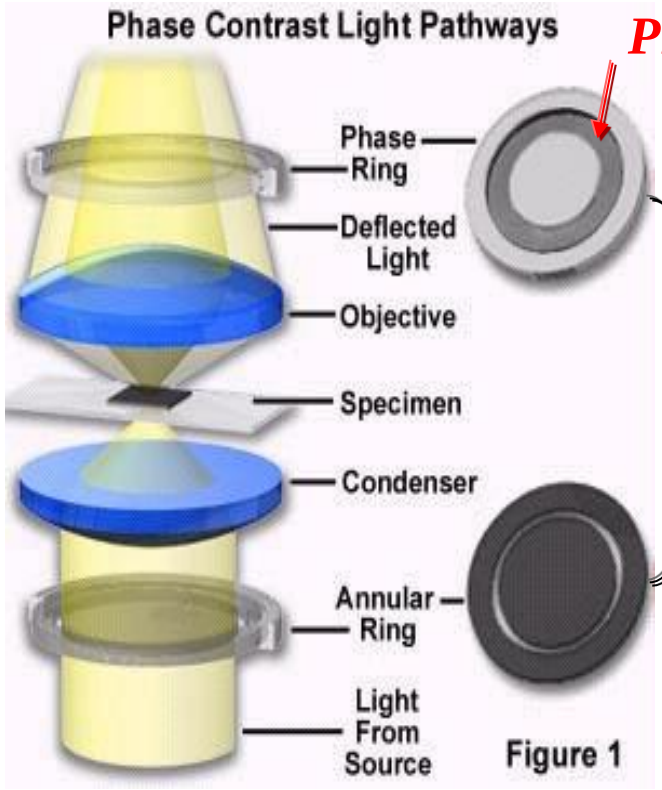
Phase object



Speed up direct light A
1/4λ to make image visible

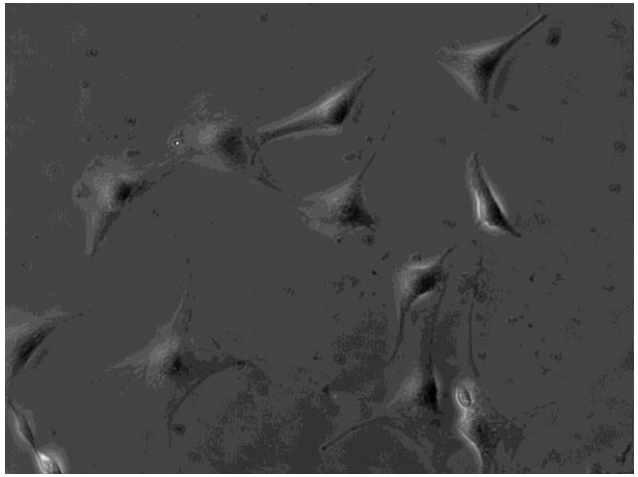
YA = direct light
YC = diffracted light
YB = Image
YB = YA + YC

- 依據Abbe的成像理論，物體影像的產生是由direct light (0th order)與diffracted light干擾加成而產生。
- 因此，direct light與diffracted light呈現180度（1/2λ）的位相差時，物體影像對比將最好。
- 在Phase的樣本中，藉由加快或減慢direct light 1/4λ，使phase的樣本轉換為amplitude的形式而可被觀察。
- 加快1/4λ ⇒ Positive or dark phase contrast。 減慢1/4λ ⇒ negative or bright phase contrast。



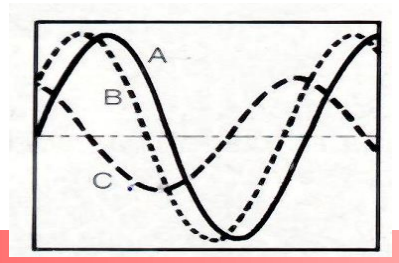
Phase shifter

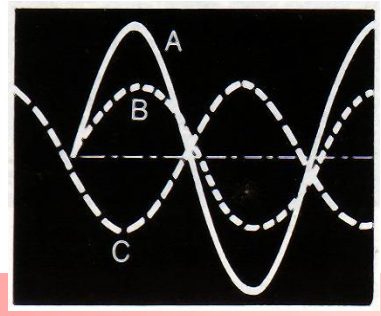
Conjugate



❖ *Phase Contrast Microscopy 應用上的限制*

- 解析度限制。.
- Phase影像周圍的光暈現象。
- 不適合用於過厚的樣品觀察。
- 使用 時應加入綠光濾片 。





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什麼是螢光？

- Incident Light

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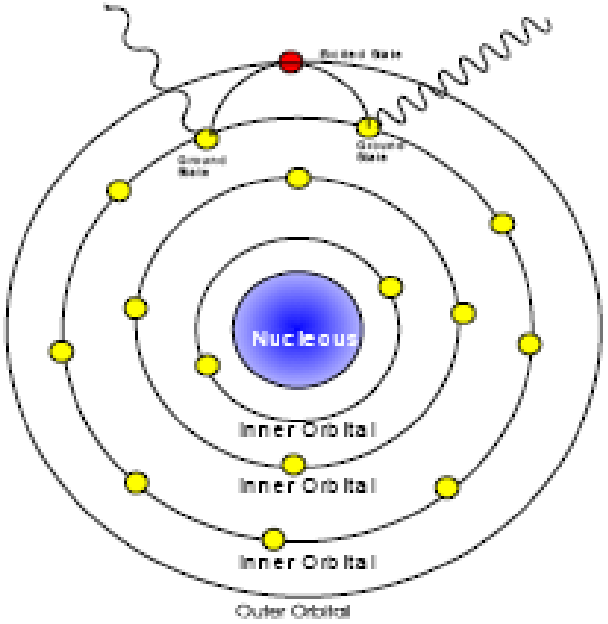
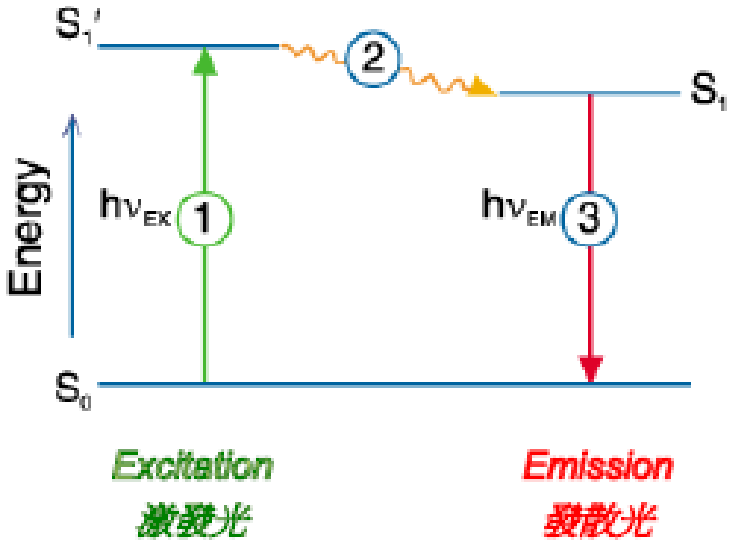
Fluorescence





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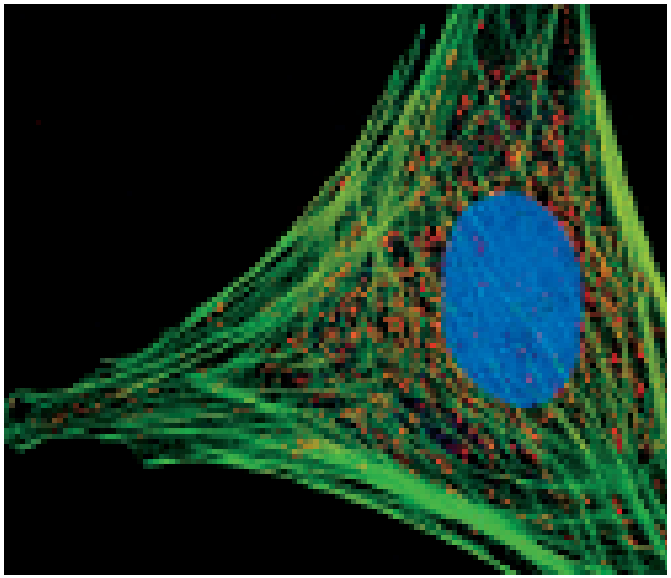
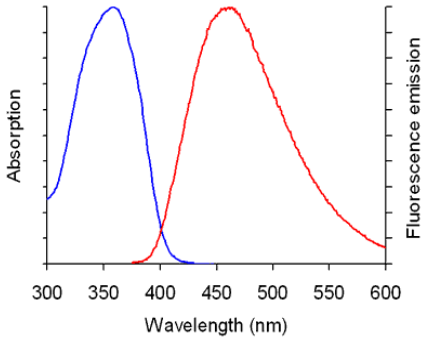
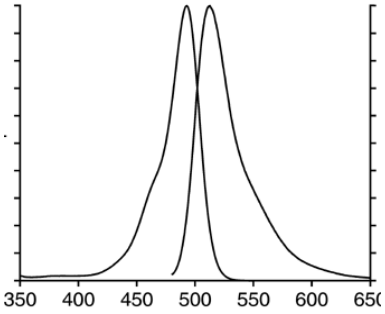
- Fluorescence 螢光



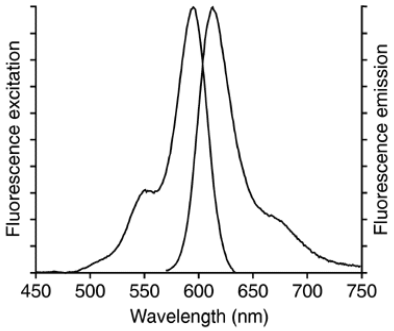
DAPI



Fluorescein



Texas Red



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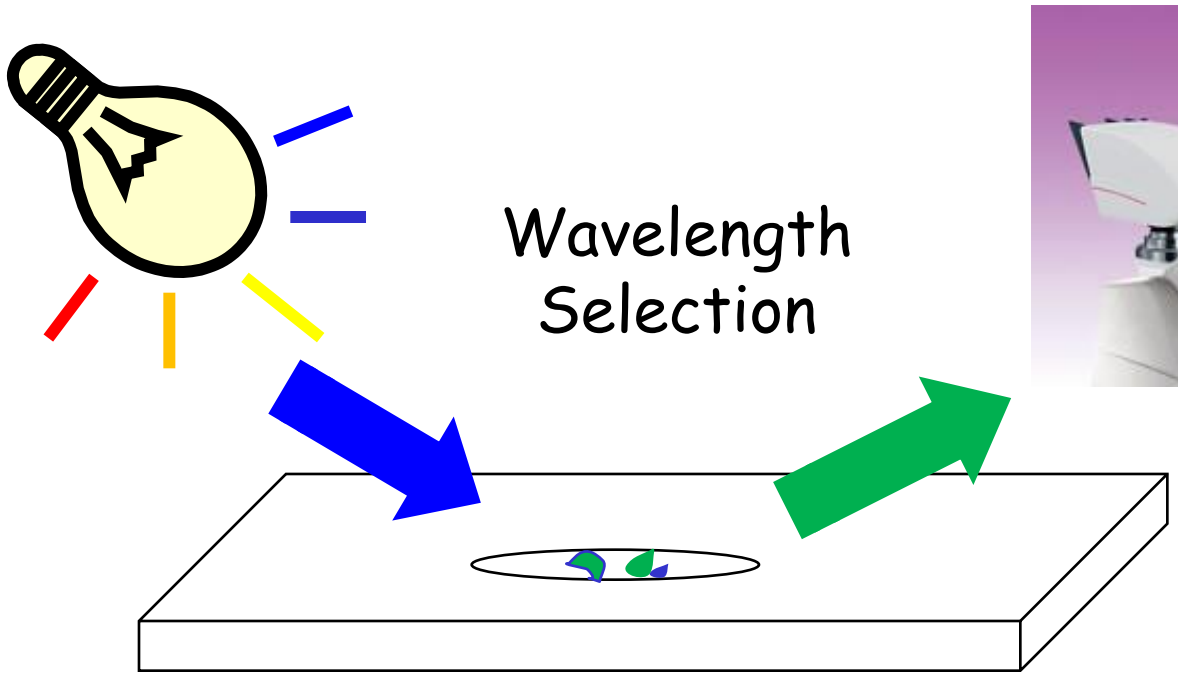
- Fluorescence 螢光

Illumination System
(Excitation)

Detection System
(Emission)



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- Conventional Fluorescence Microscope

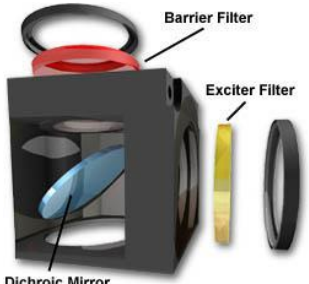
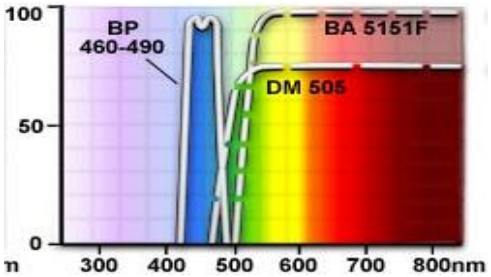
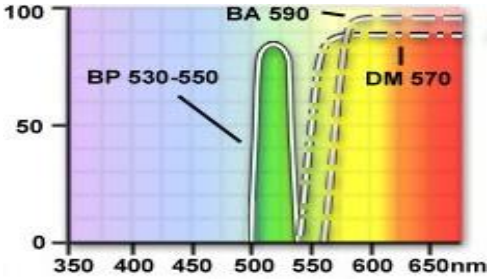
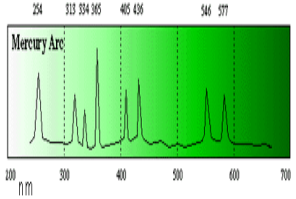


Figure 4

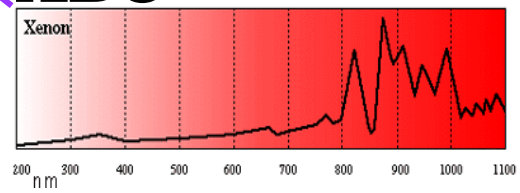




HBO



XBO



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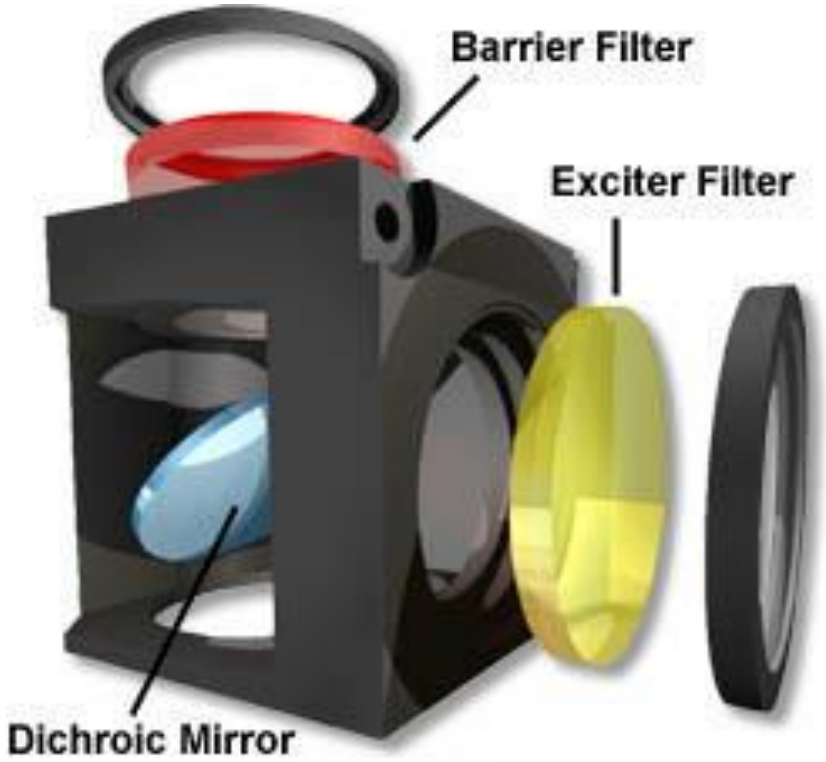
- Conventional Fluorescence Microscope

Wavelength Selection -- Fliter



Short Pass

Long Pass

Band Pass

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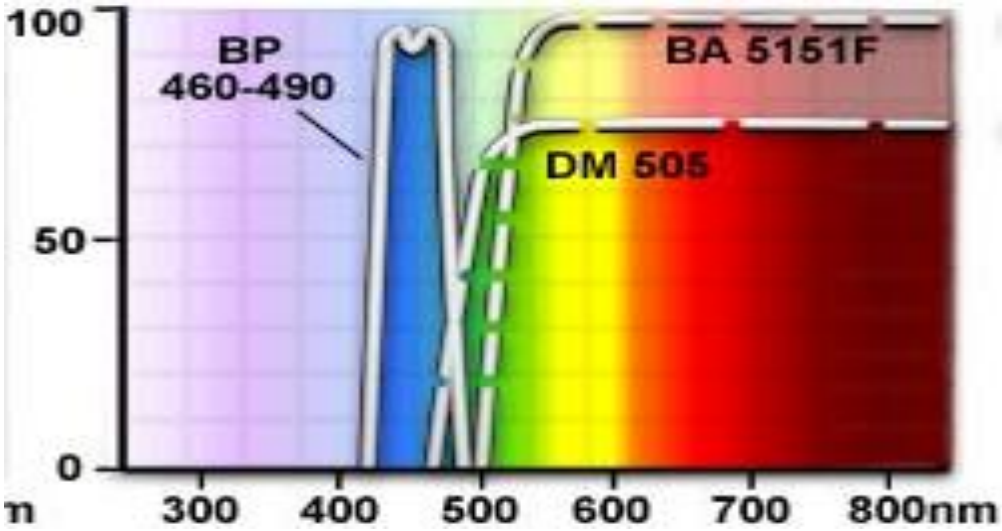
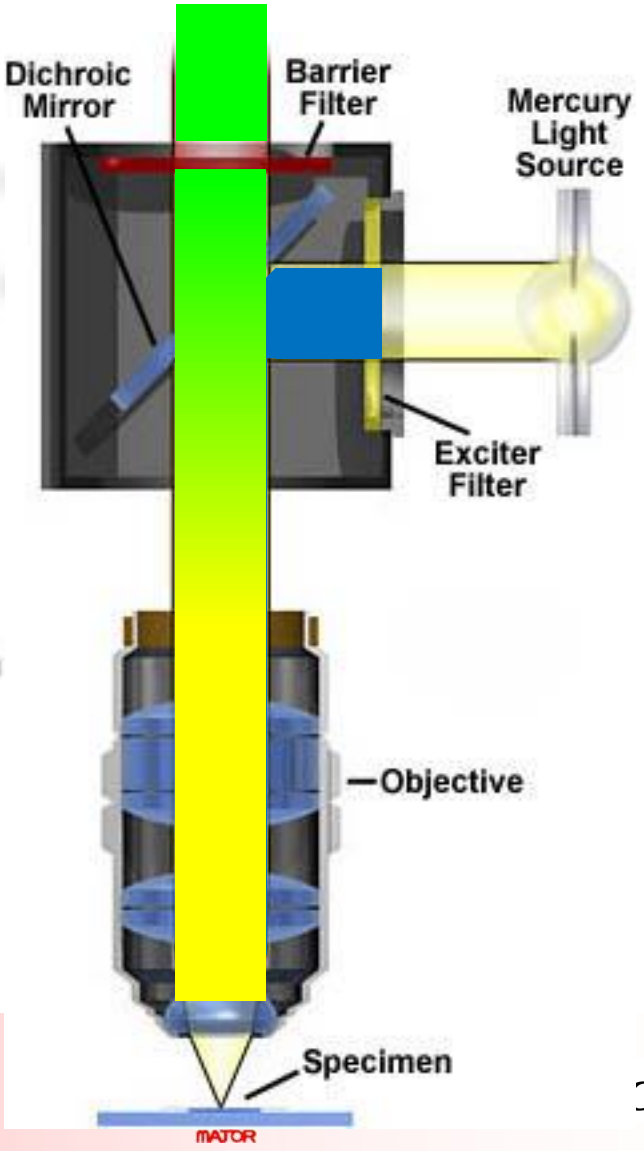
- Conventional Fluorescence Microscope





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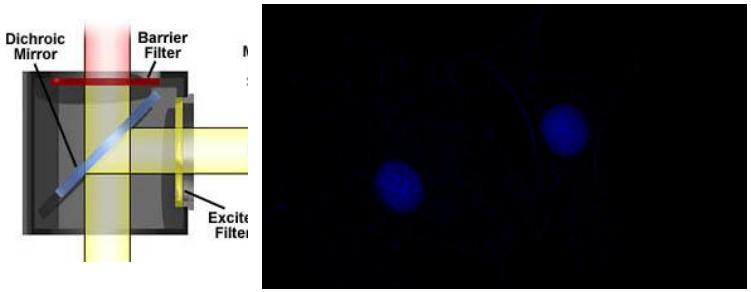

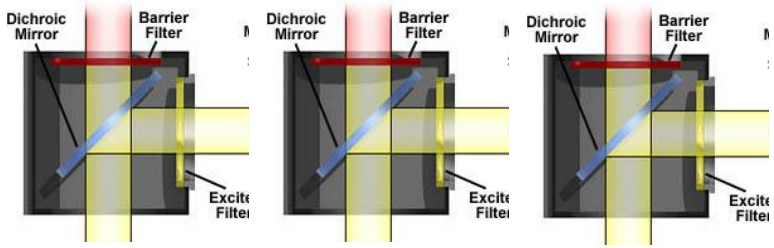

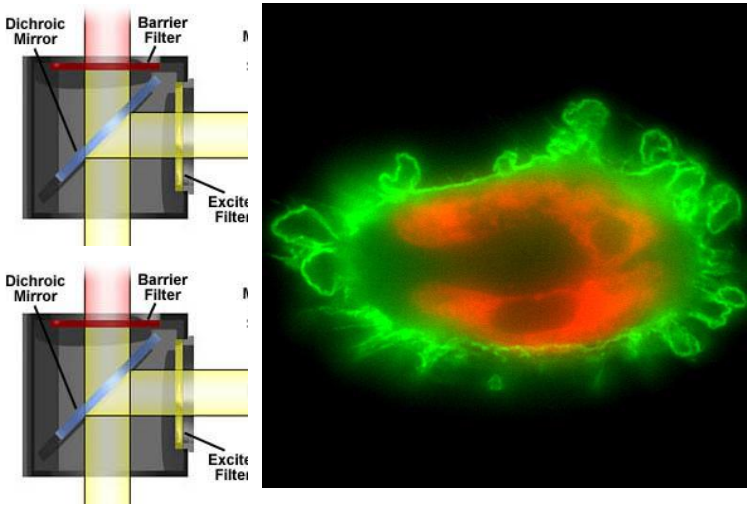

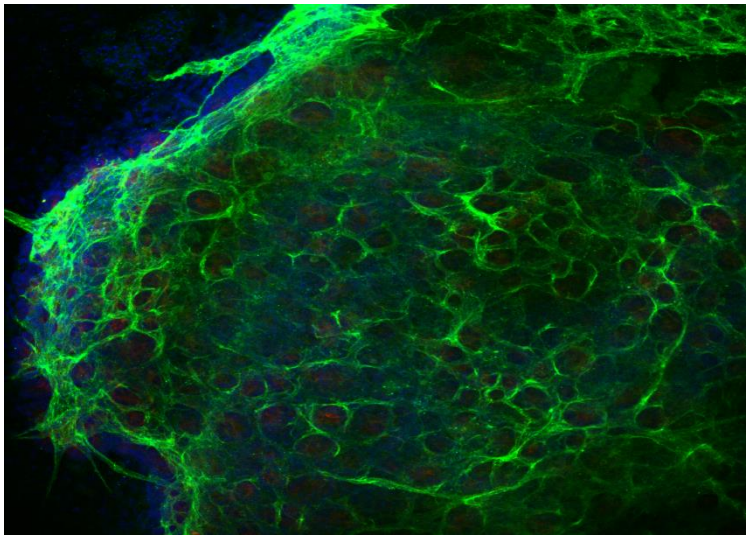

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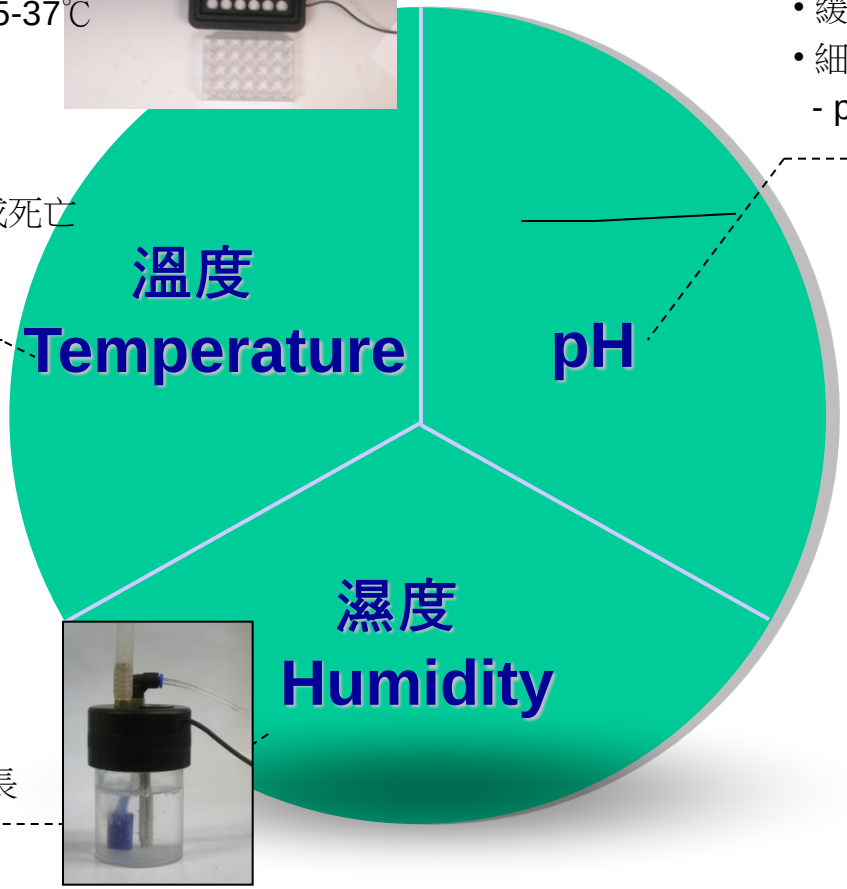
Incubation

溫度需維持在35-37℃.

- 大部分哺乳動物細胞需生長於35-37℃
- 溫度控制不良：
  - 代謝失調
  - 溫度過低可能造成生長遲緩
  - 溫度過高會造成 heat-shock 或死亡
    38.5-39.5℃數小時即會死亡



需維持5%CO_2

- 緩衝系統 CO_2/HCO_3^-
- 細胞培養適宜環境 pH 7.4.
  - pH 低於6.8常會抑制生長

需保持適當濕度

- 培養液蒸發
  - 細胞受損或死亡
  - 培養液濃度改變 影響細胞生長

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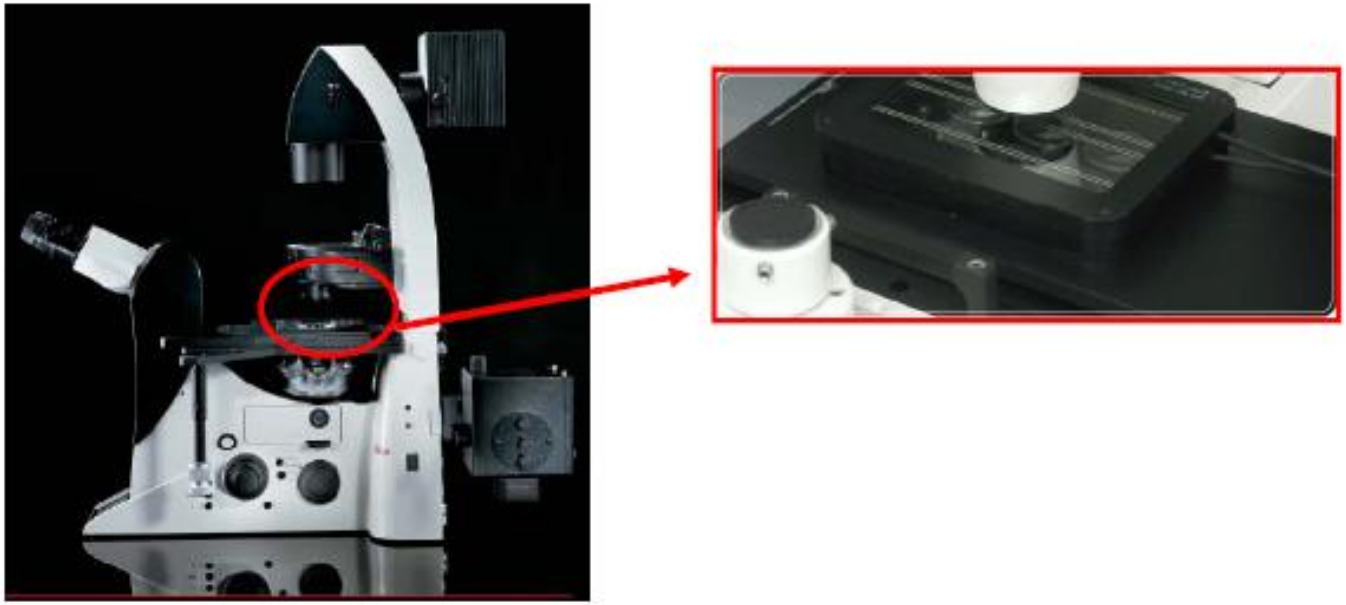
顯微鏡上細胞影像觀察



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+

Leica

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=

Reach High !

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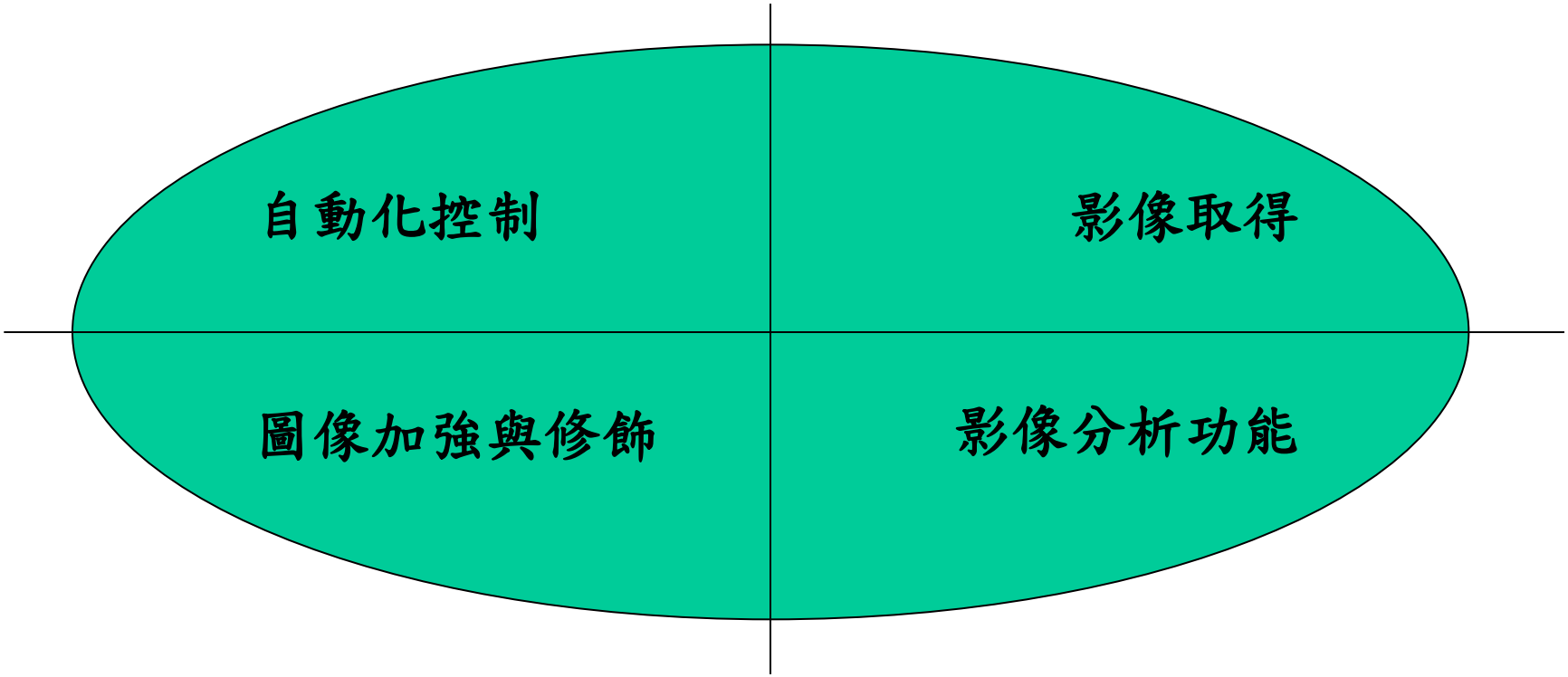
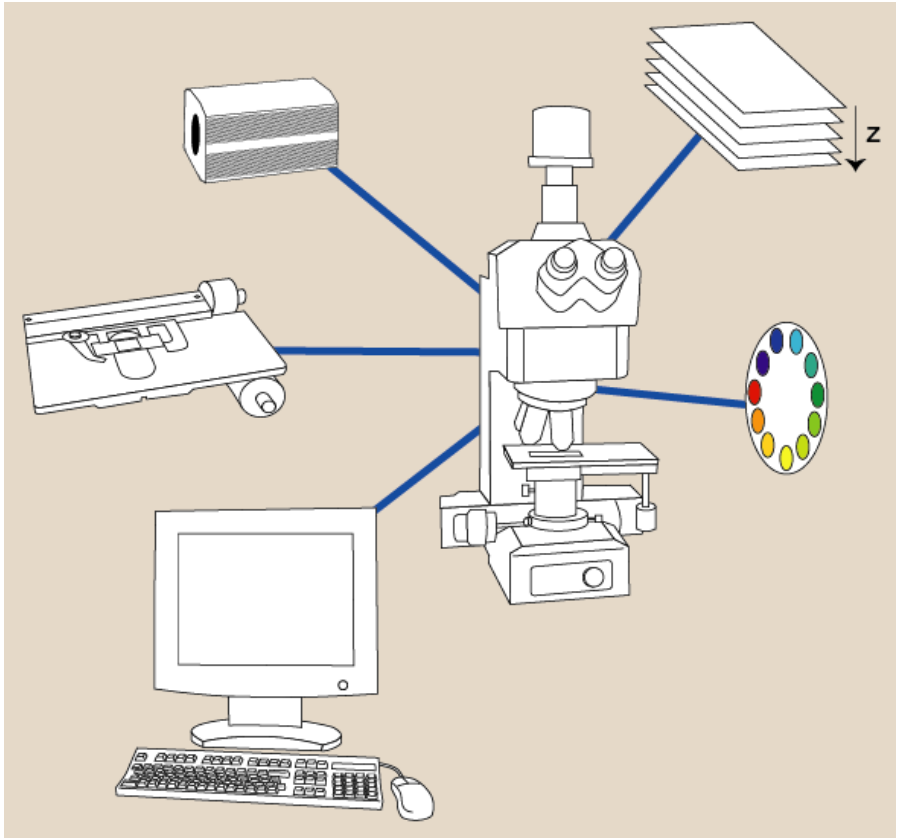
MetaMorph® imaging system



自動化控制

- 顯微鏡系統
- 影像CCD
- 電動XYZ三軸控制
- 光源選擇
- 共阨焦模組
- TTL訊號



自動化控制

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PHOTOMETRICS a division of Roper Scientific, Inc.

SUTTER INSTRUMENT

ANDOR TECHNOLOGY

Ludl Electronic Products Ltd.

ZEISS

Q IMAGING

OLYMPUS

YOKOGAWA

DIAGNOSTIC instruments, inc.

Nikon

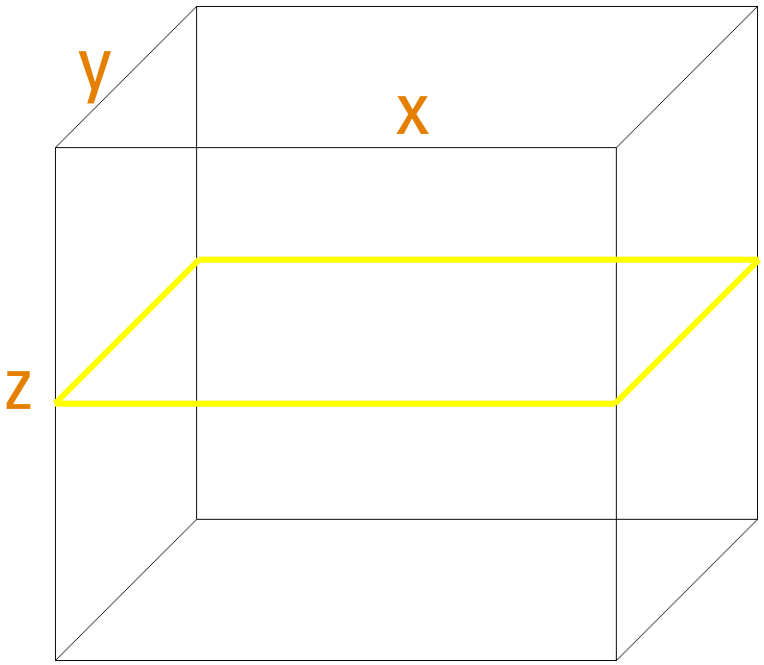
HAMAMATSU

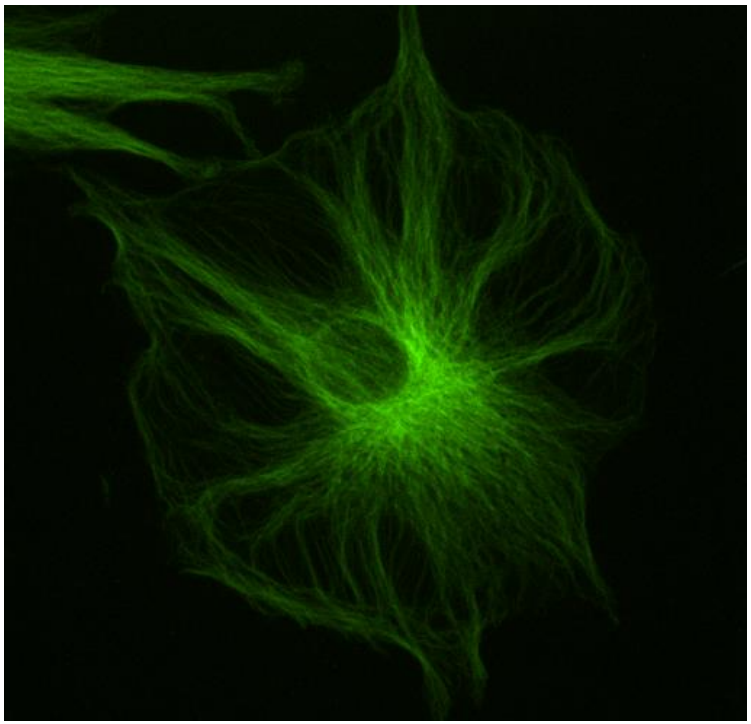
….and etc.

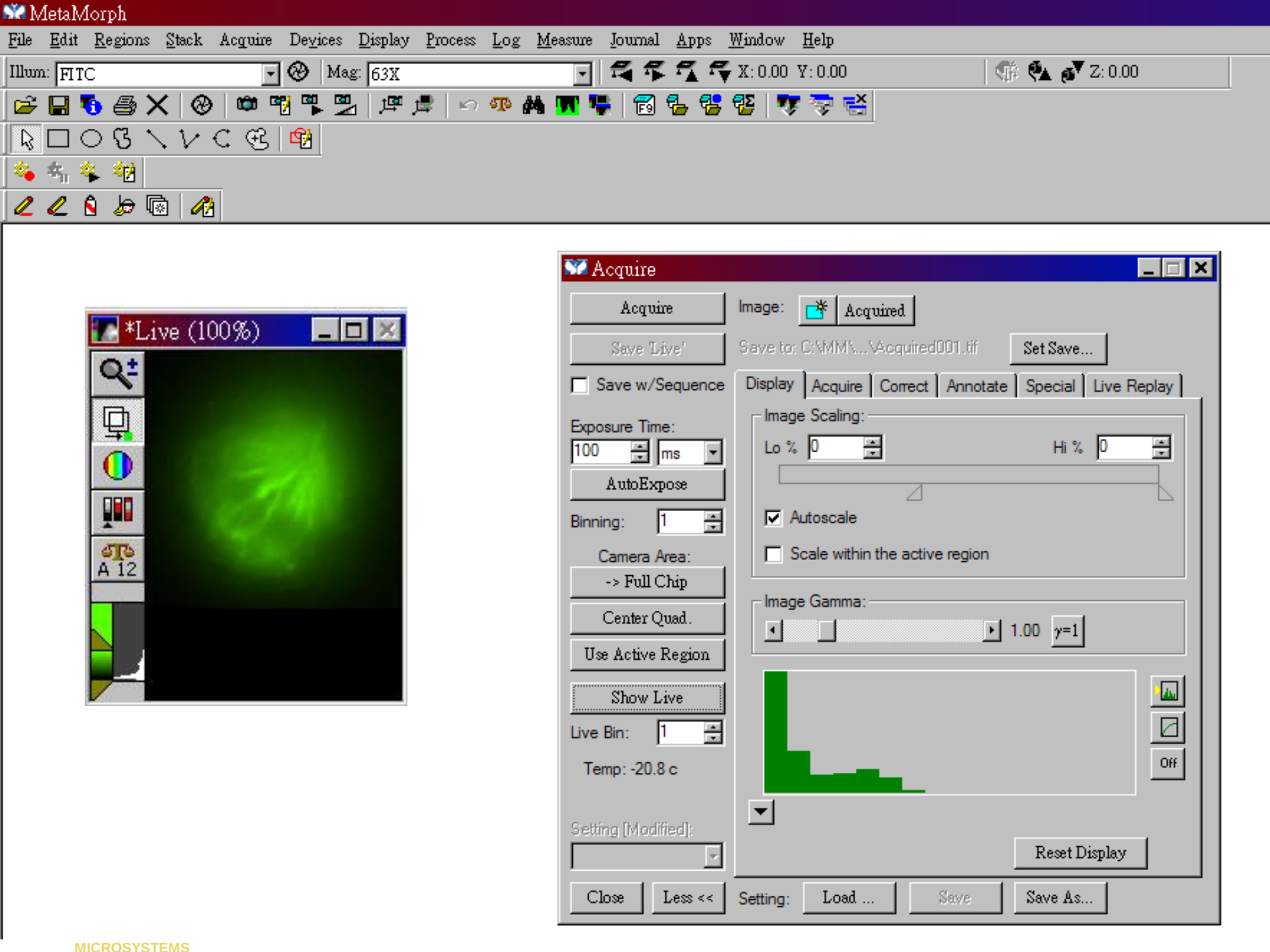
影像取得

- 單一圖層擷取(XY)
- Z軸序列擷取(XYZ)
- 時間序列擷取(XYT)
- 多波長擷取(XYλ)
- 位置移動擷取(XYP)
- 多維合併序列擷取

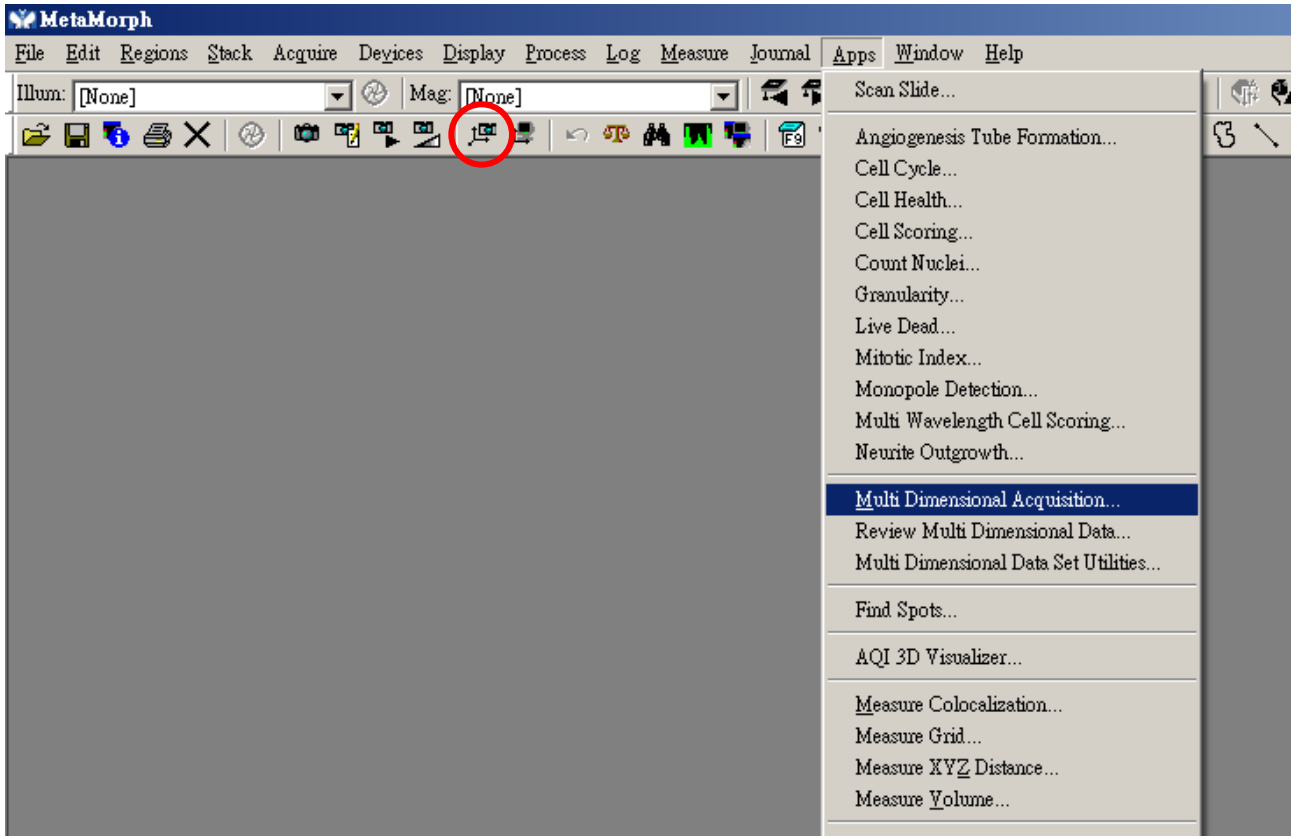
一般平面



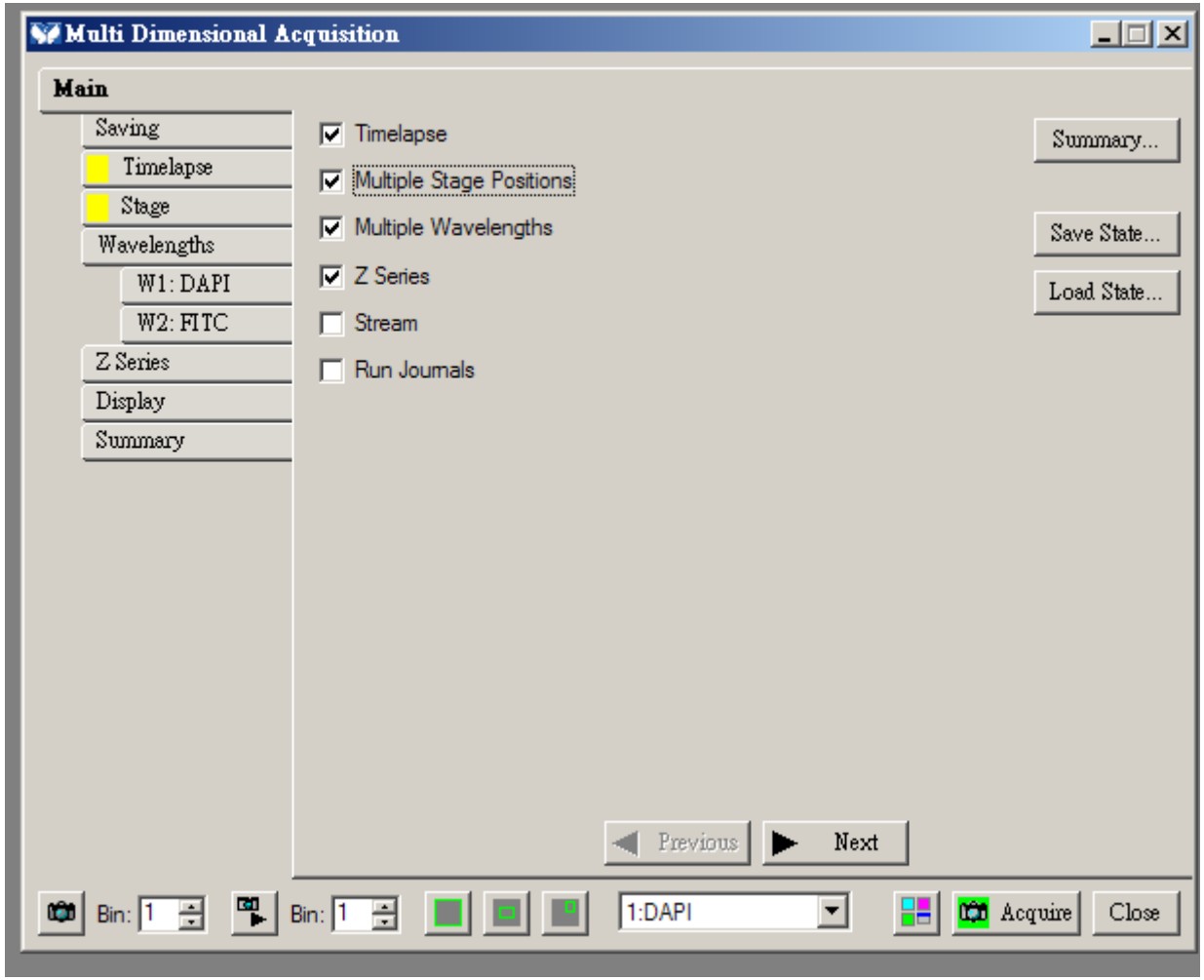



MetaMorph
File Edit Regions Stack Acquire Devices Display Process Log Measure Journal Apps Window Help
Illum: FITC
Mag: 63X
X: 0.00 Y: 0.00
Z: 0.00
*Live (100%)
A 12
Acquire
Acquire
Image:
Acquired
Save 'Live'
Save to: C:\MM\...\Acquired001.tif
Set Save...
Save w/Sequence
Display
Acquire
Correct
Annotate
Special
Live Replay
Exposure Time:
100
ms
AutoExpose
Binning:
1
Camera Area:
-> Full Chip
Center Quad.
Use Active Region
Show Live
Live Bin:
1
Temp: -20.8 c
Setting [Modified]:
Image Scaling:
Lo %
0
Hi %
0
Autoscale
Scale within the active region
Image Gamma:
1.00
γ=1
Off
Reset Display
Close
Less <<
Setting:
Load ...
Save
Save As...
MICROSYSTEMS

多維影像拍攝 Multi Dimentional Acquisition



Multi Dimensional Acquisition



MetaMorph

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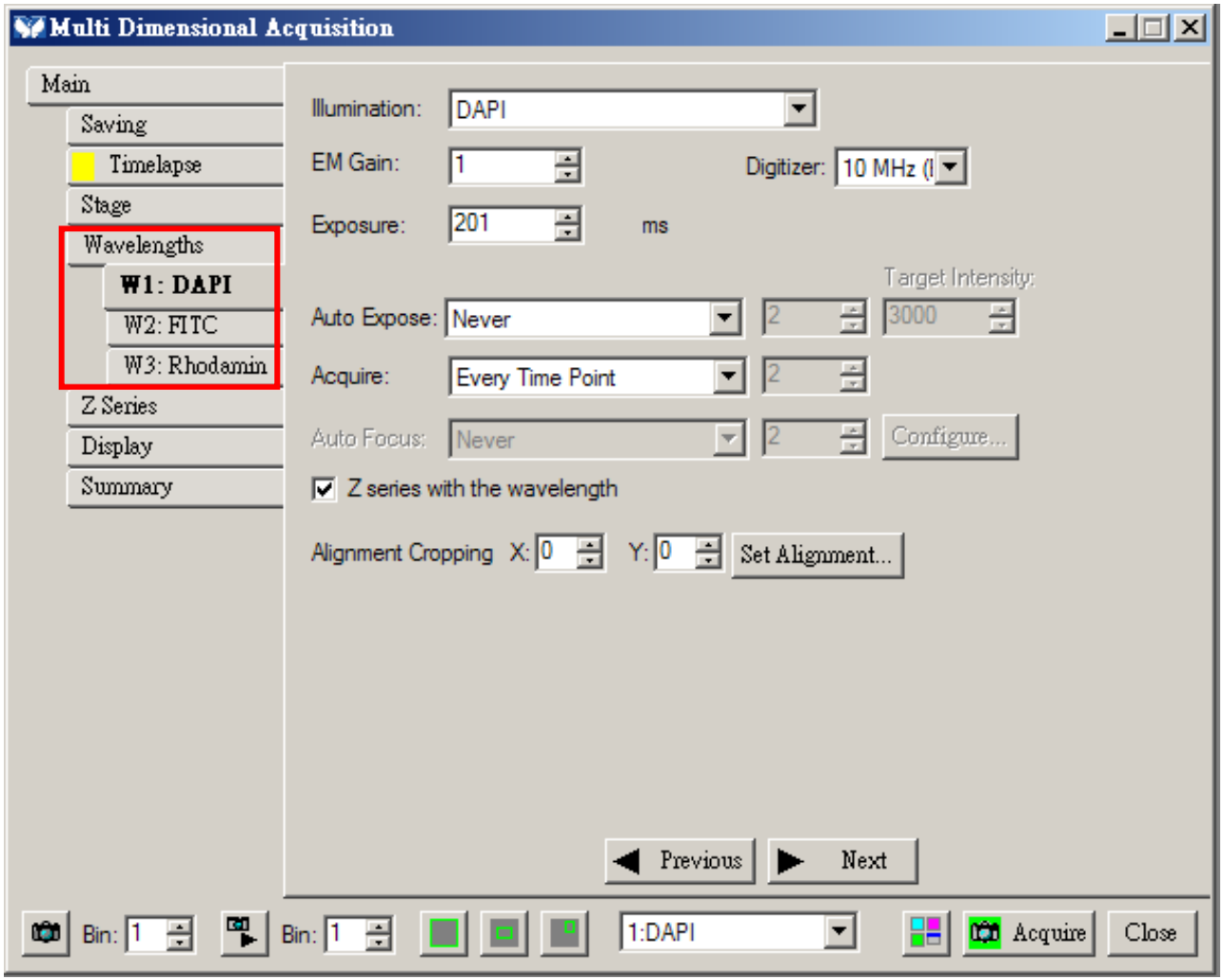
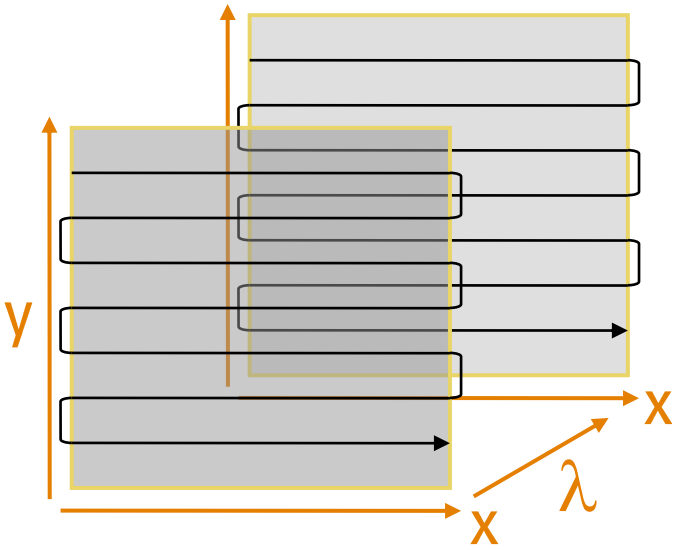
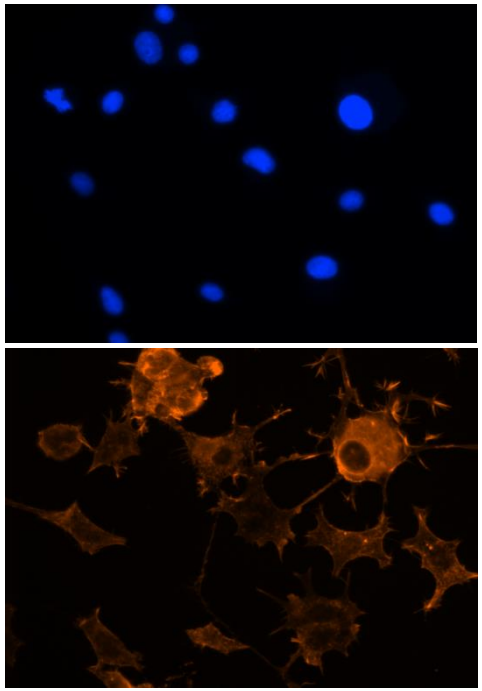
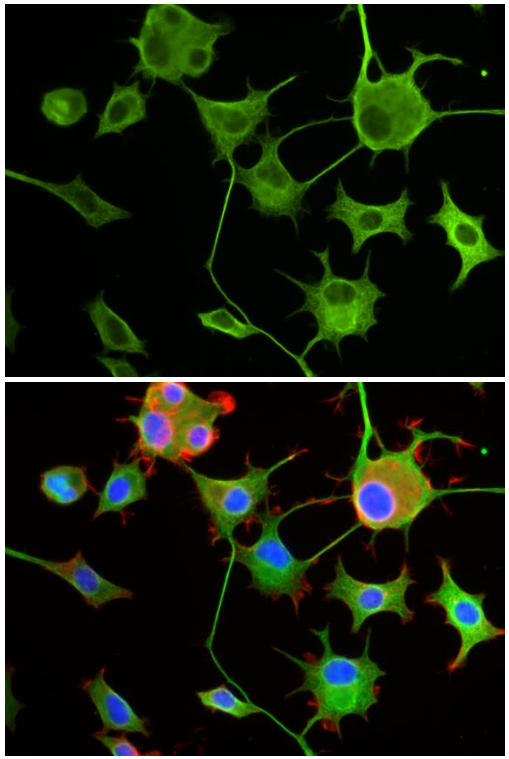
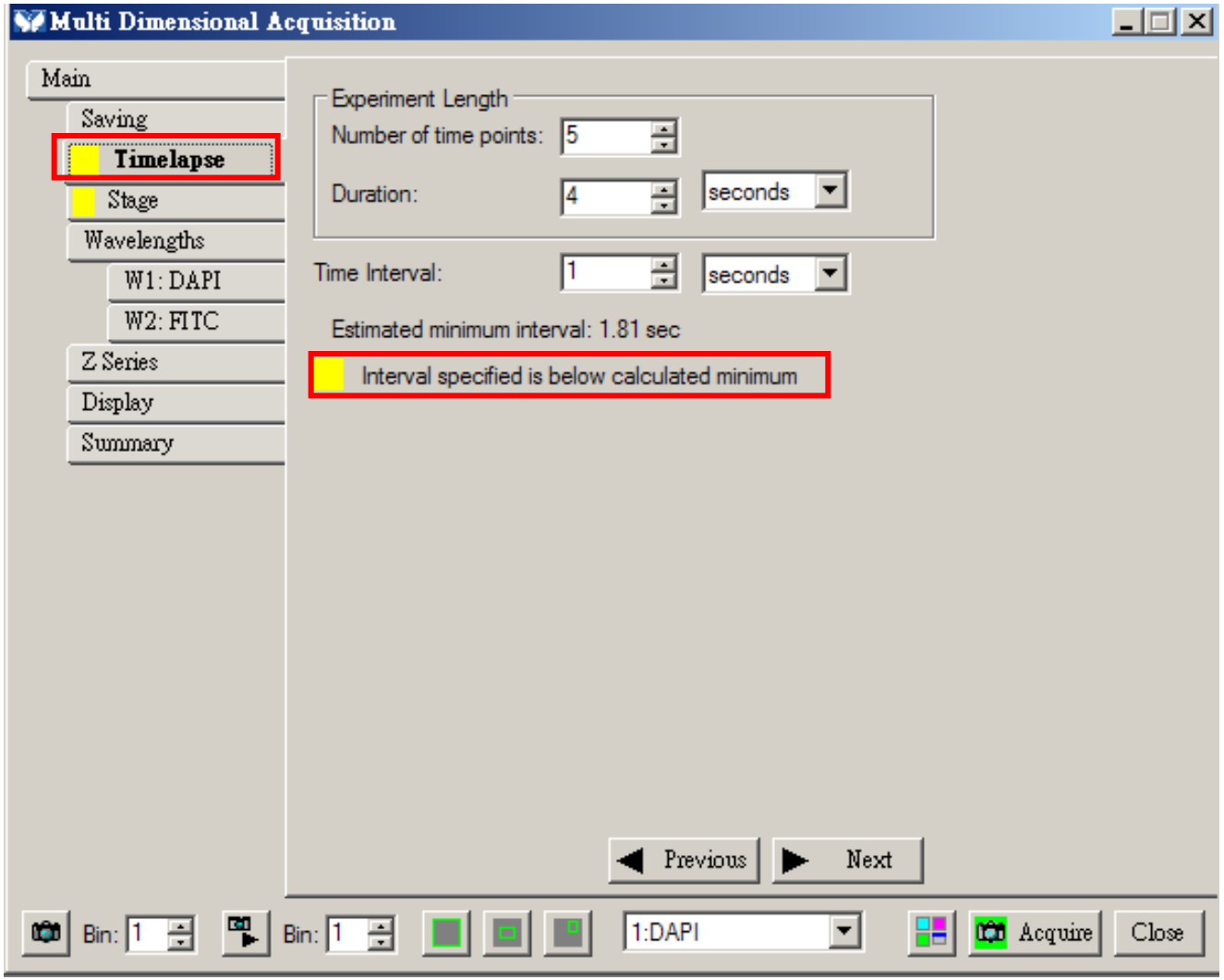
多維影像攝影---多重螢光


Multi Dimensional Acquisition
Main
Saving
Timelapse
Stage
Wavelengths
W1: DAPI
W2: FITC
W3: Rhodamin
Z Series
Display
Summary
Illumination: DAPI
EM Gain: 1
Digitizer: 10 MHz (l
Exposure: 201 ms
Target Intensity:
Auto Expose: Never 2 3000
Acquire: Every Time Point 2
Auto Focus: Never 2 Configure...
Z series with the wavelength
Alignment Cropping X: 0 Y: 0 Set Alignment...
Previous
Next
Bin: 1
Bin: 1
1:DAPI
Acquire
Close


y
x
x
λ







MetaMorph

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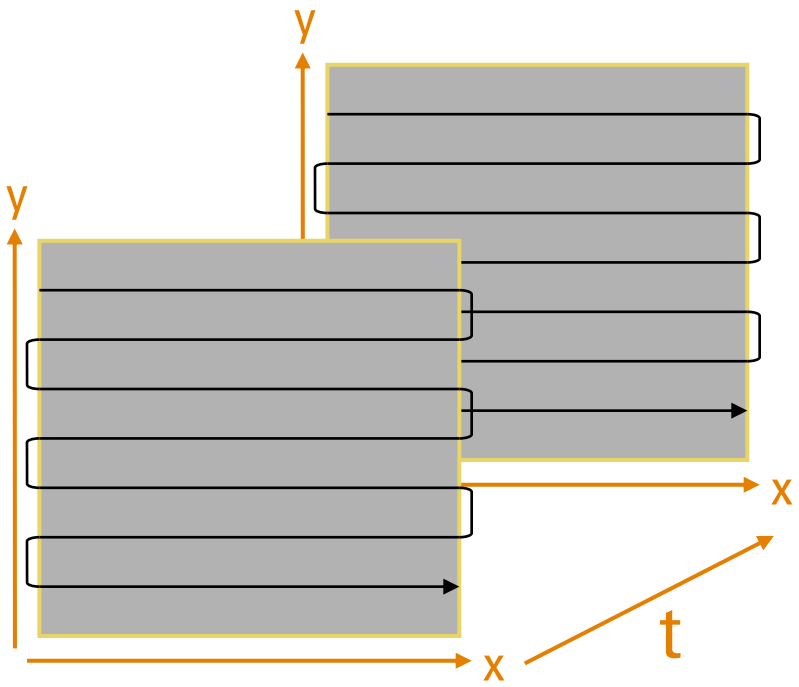
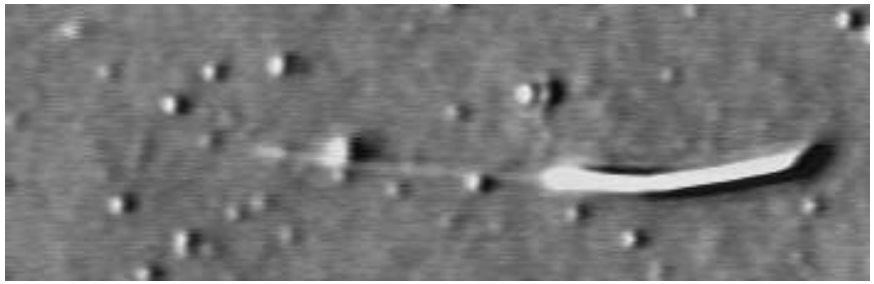
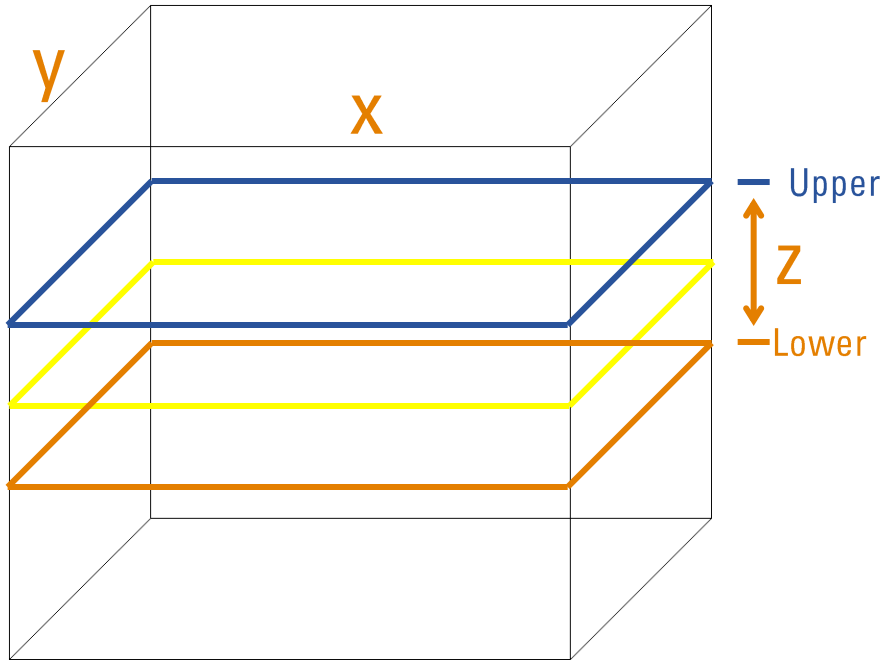
多維影像攝影---時間序列

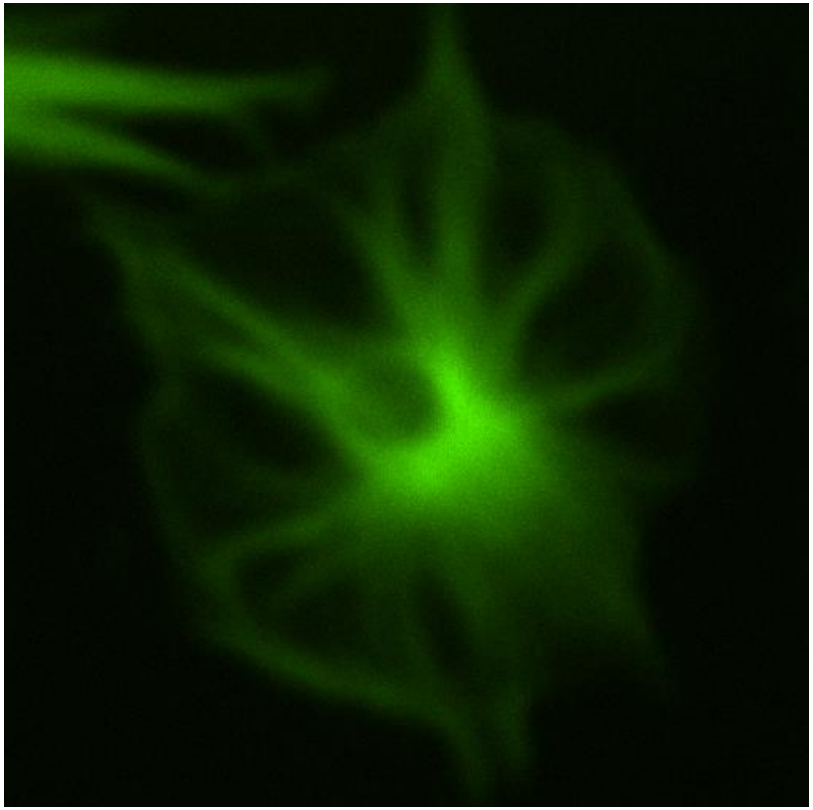




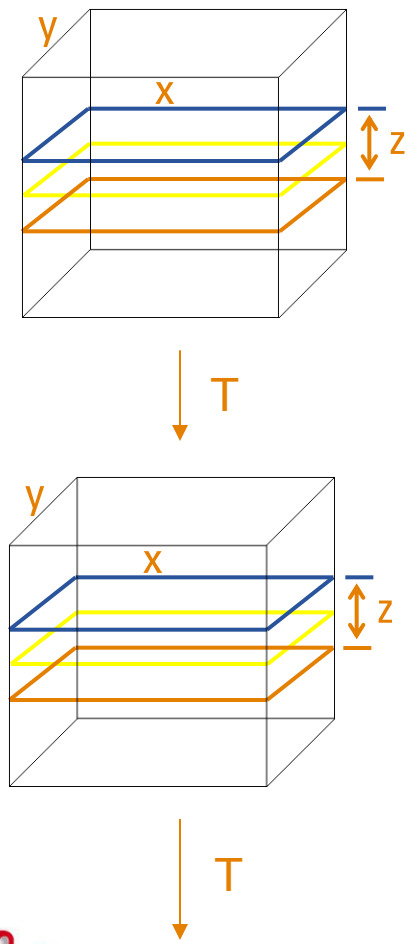
Microtubule based motility

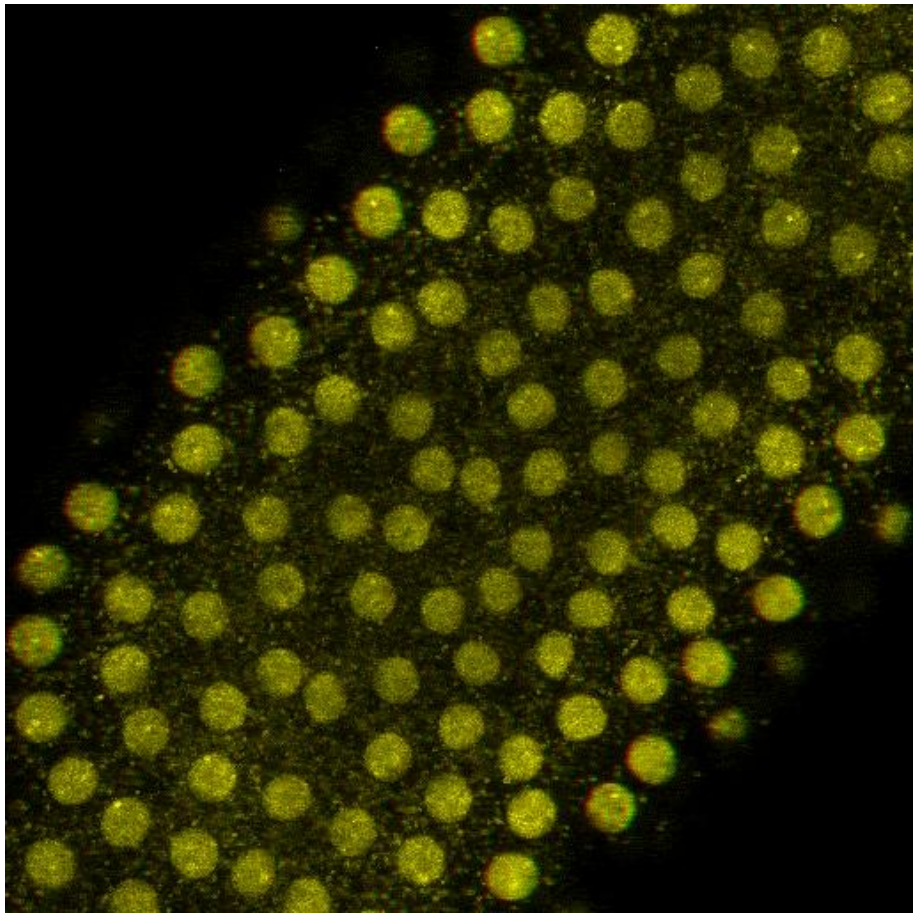
多維影像攝影--- Z軸拍攝



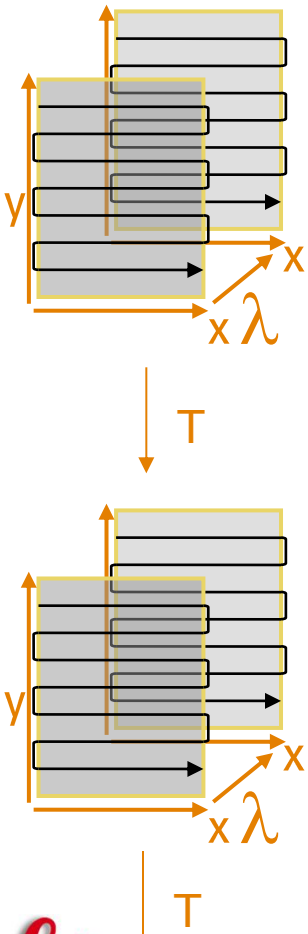


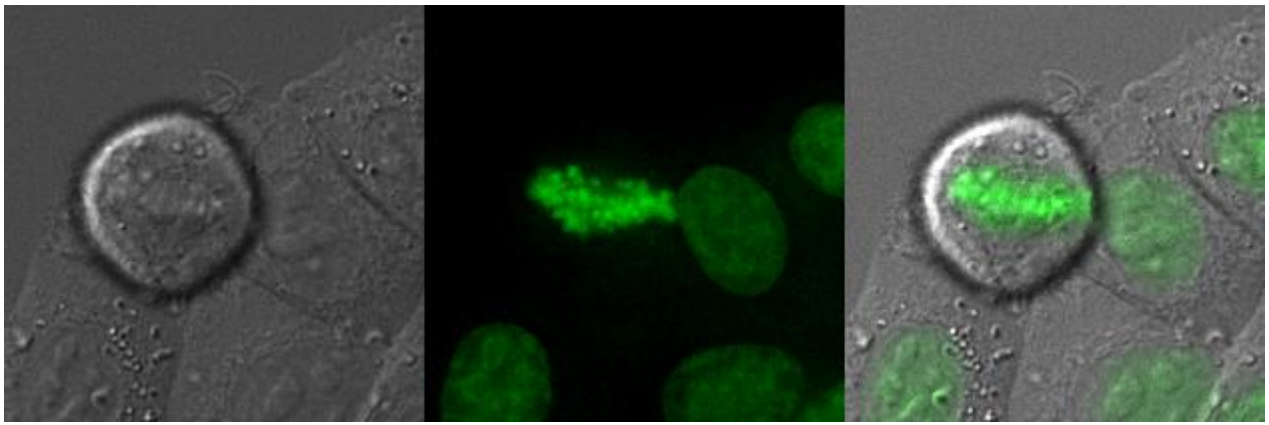
多維影像攝影---Z軸時間序列



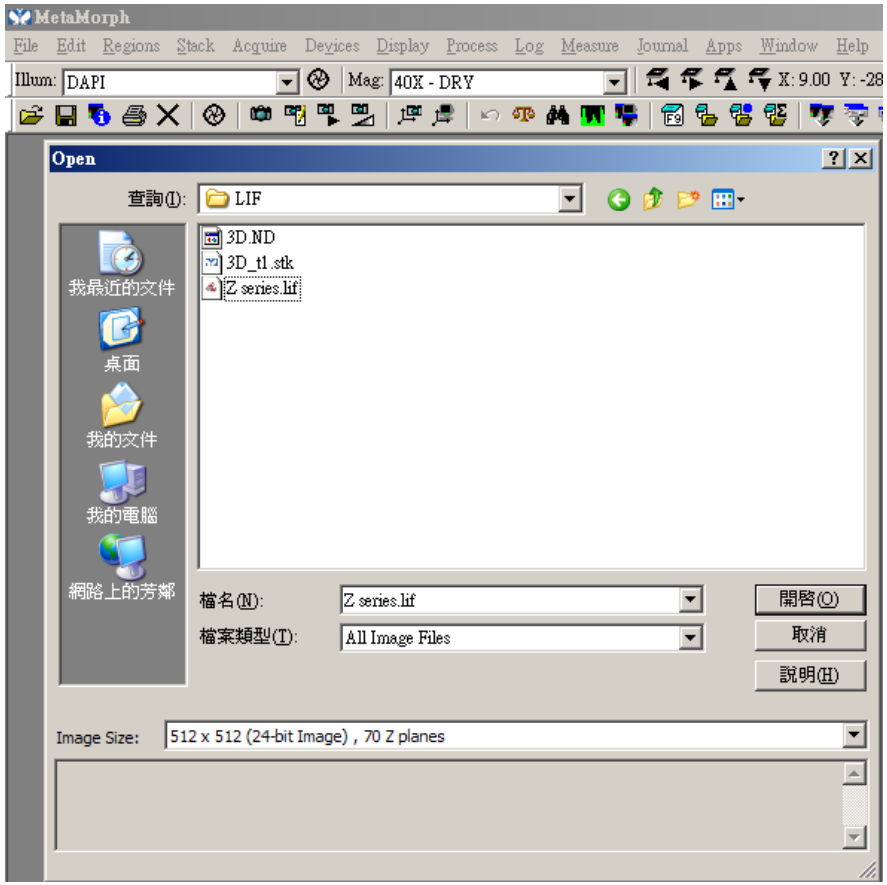


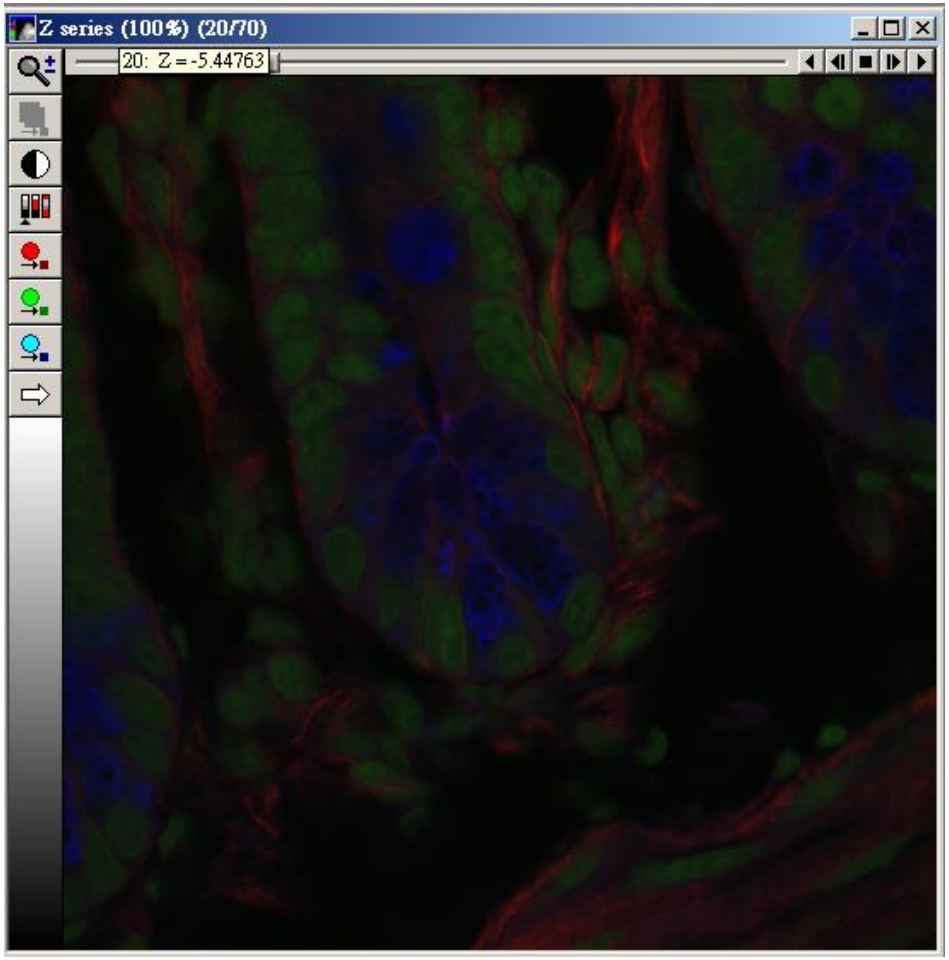
多維影像攝影---多重螢光時間序列





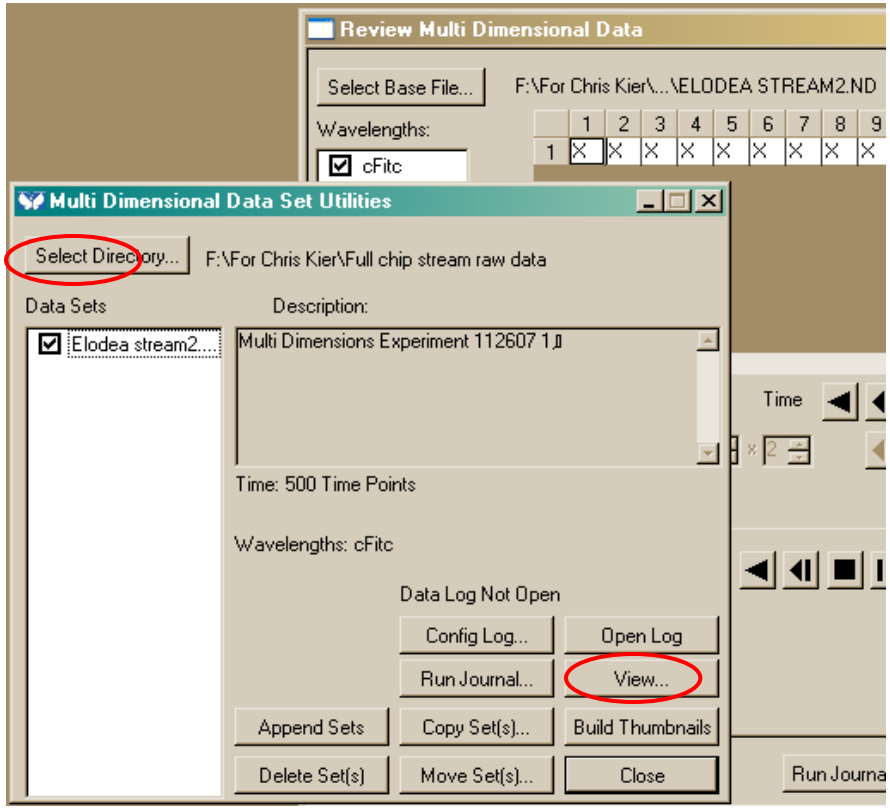
File → Open (.tif, jpg, bmp,)



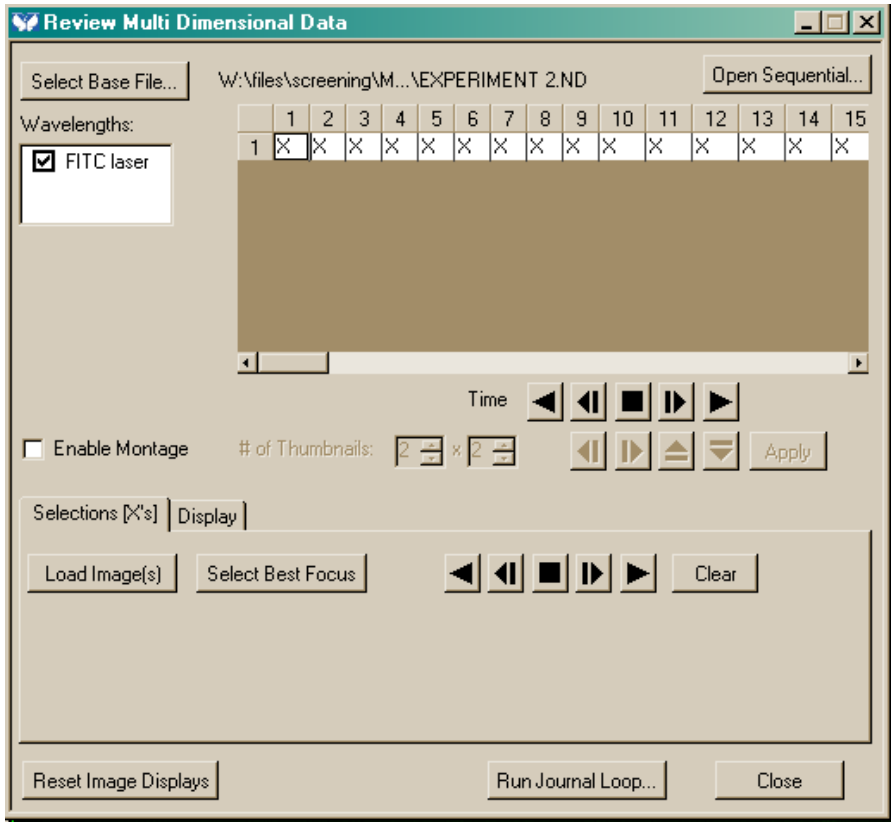


Apps → Review Multidimensional Data

使用於觀看,輸出,分析,在MetaMorph Multi Dimensional Data 具有(*.nd)的影像序列檔案



Review Multidimensional Data



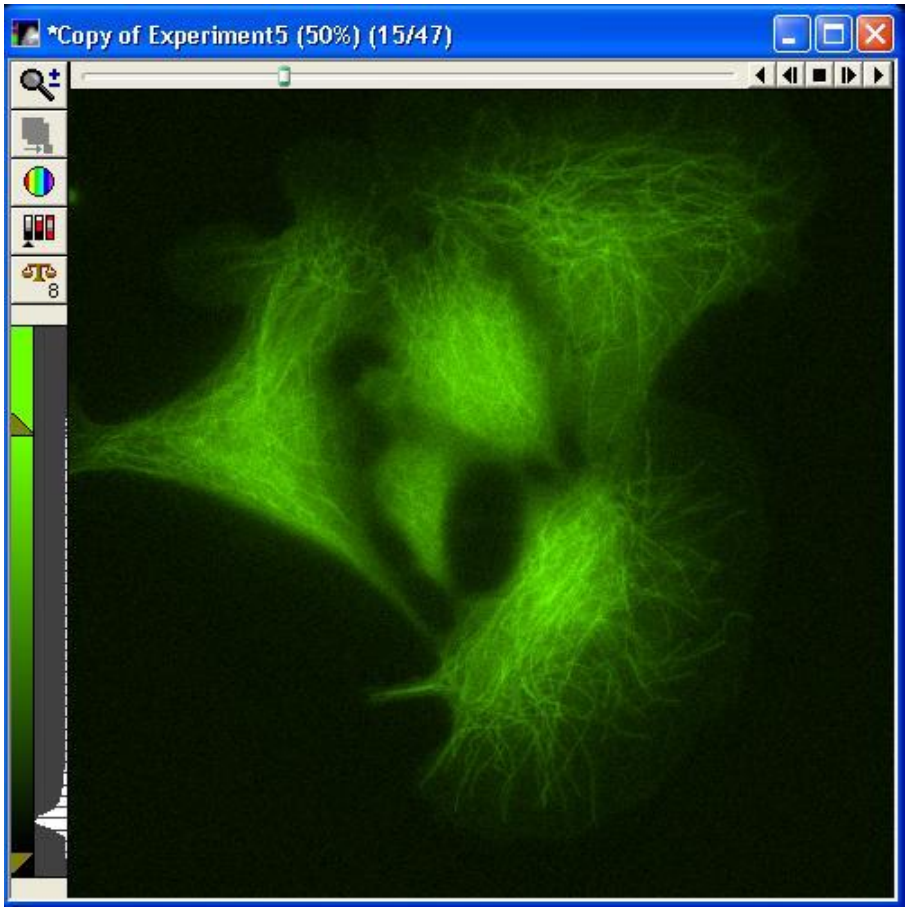
- Load Image

  勾選要觀察的波長與時間,Z軸的影像序列,按下Load可整個輸出

- Select Best Focus

  在拍攝多時間與Z軸影像的檔案中,可用此選項幫助選出最佳聚焦平面.

Stacks



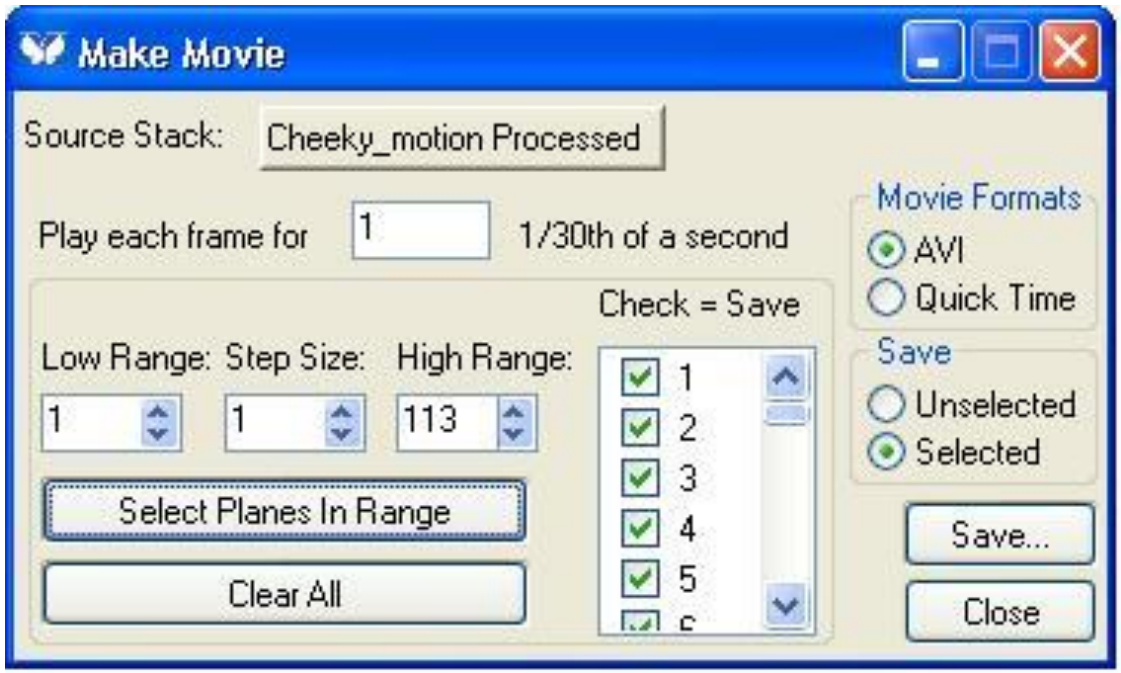
- What are Stacks?

  單一檔案包含多張影像

- Time Series
  - Z Series
  - Wavelength Series

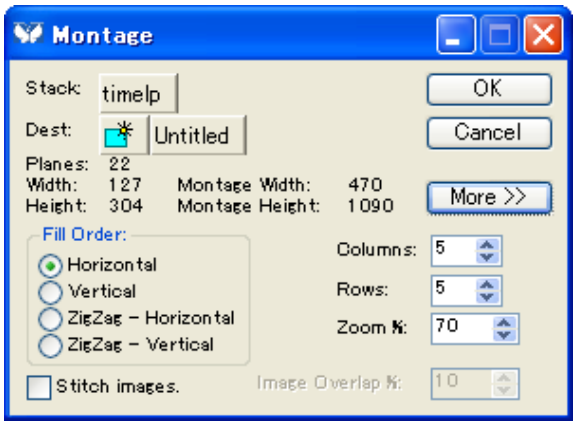
Make Movie

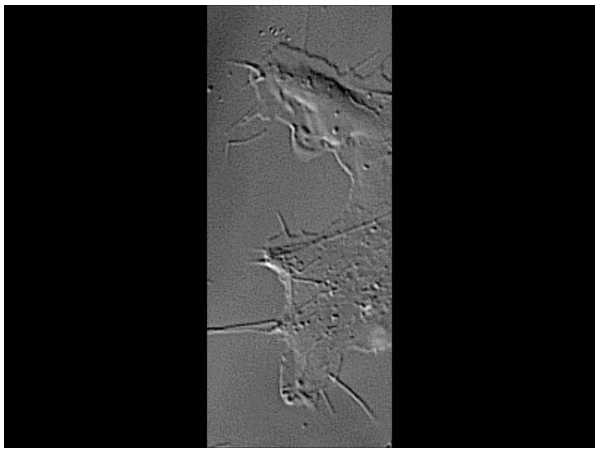
- Stack → Make Movie
- AVI or Quick Time movie
- 可自訂影片播放時間



Working with Stacks

- Stack → Montage





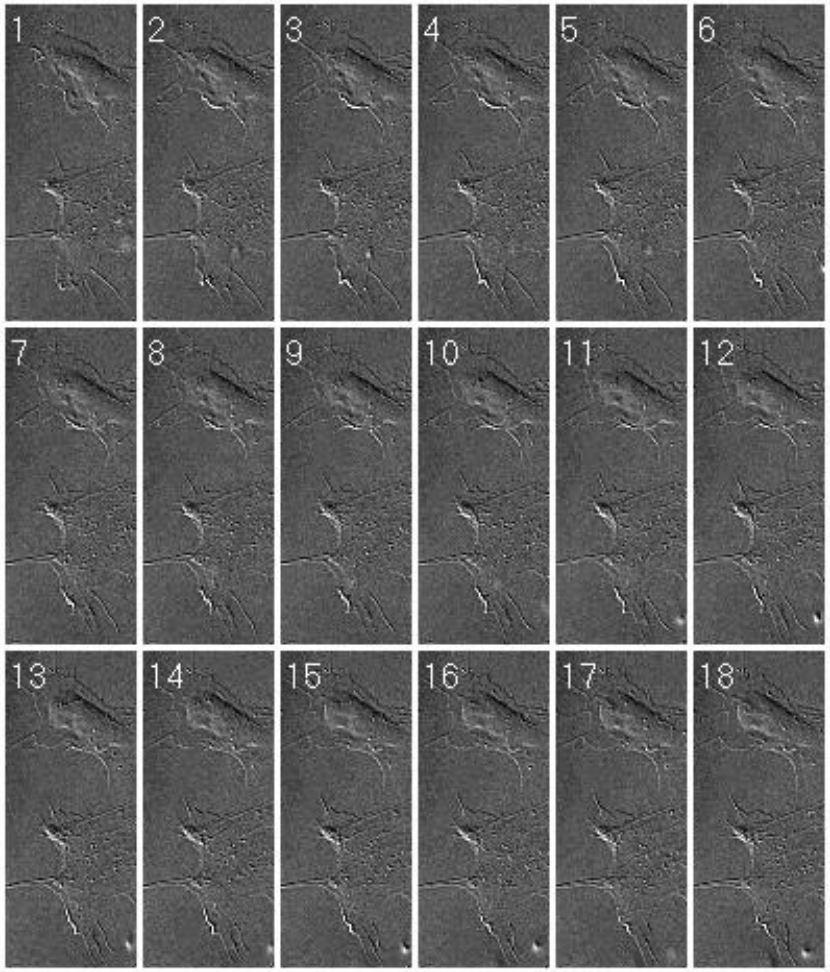


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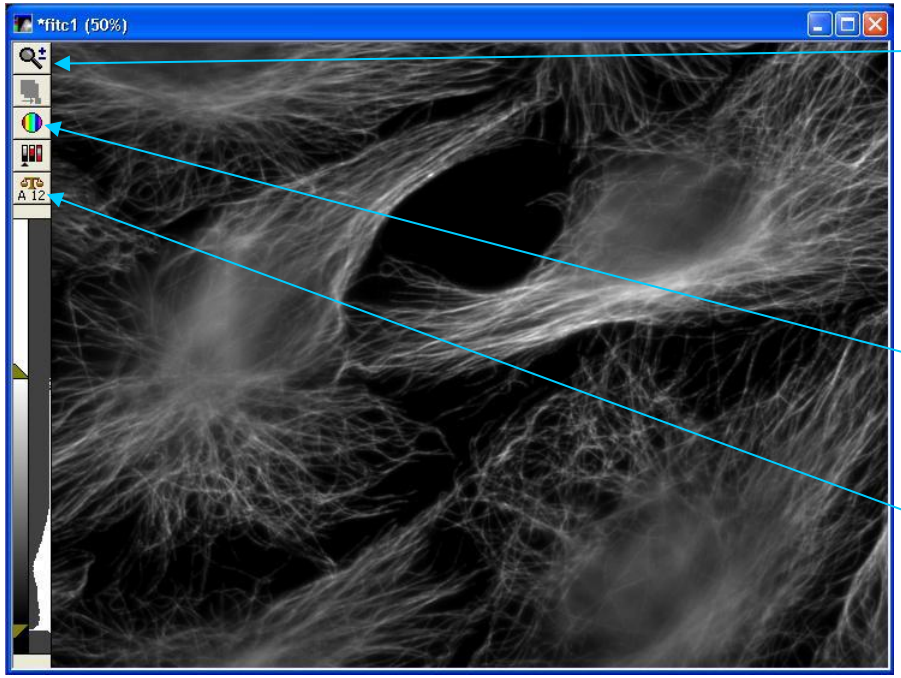
Image Window Tools



- Zoom ±

  調整影像視窗放大或縮小,
  也可利用滑鼠的滾輪調整

- Look Up Table(LUT)擬色

  可將單色的影像做不同顏色套色

- Scale Image

Image Information

- Edit → Image Info or Alt-I or 

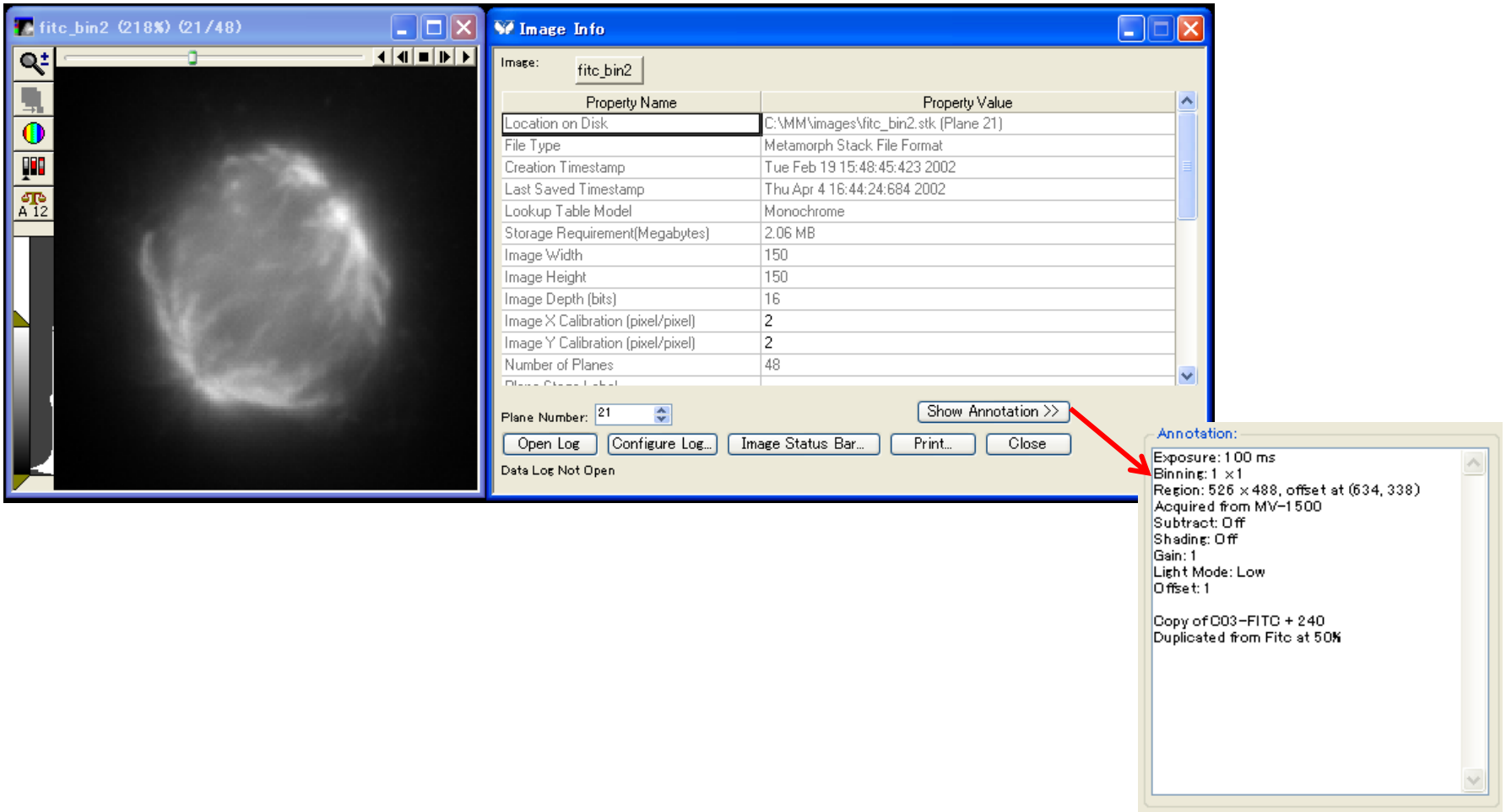


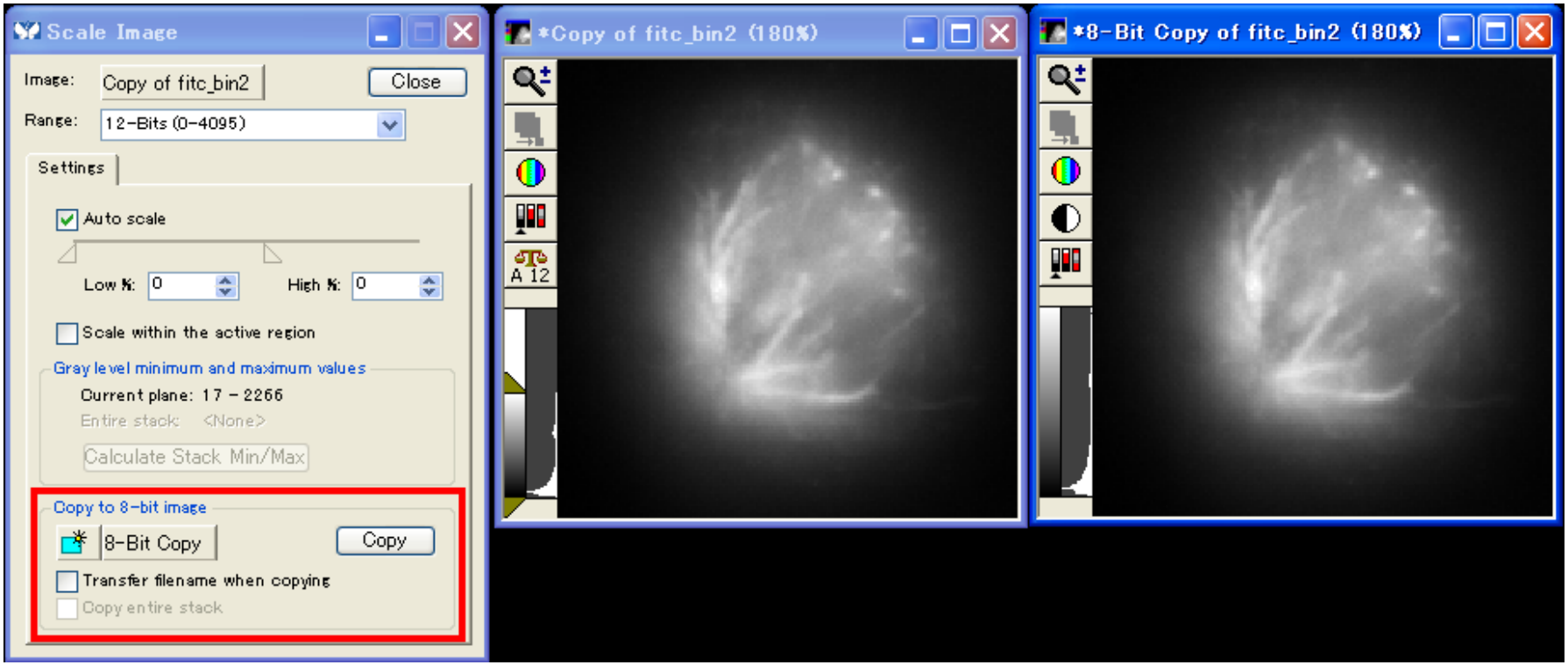
Image Bit Depths

- Bit depth defines the number of values available to each **pixel** within an image
- Function of the camera electronics and software

Bit depth	Number of gray levels
1 – bit	Black and white (on or off) “Binary”
8 – bits	256 gray scale
12 – bits	4,096 gray scale
16 – bits	65,536 gray scale
24 – bits	Color image 8bits Red, 8 bits Green, 8 bits Blue

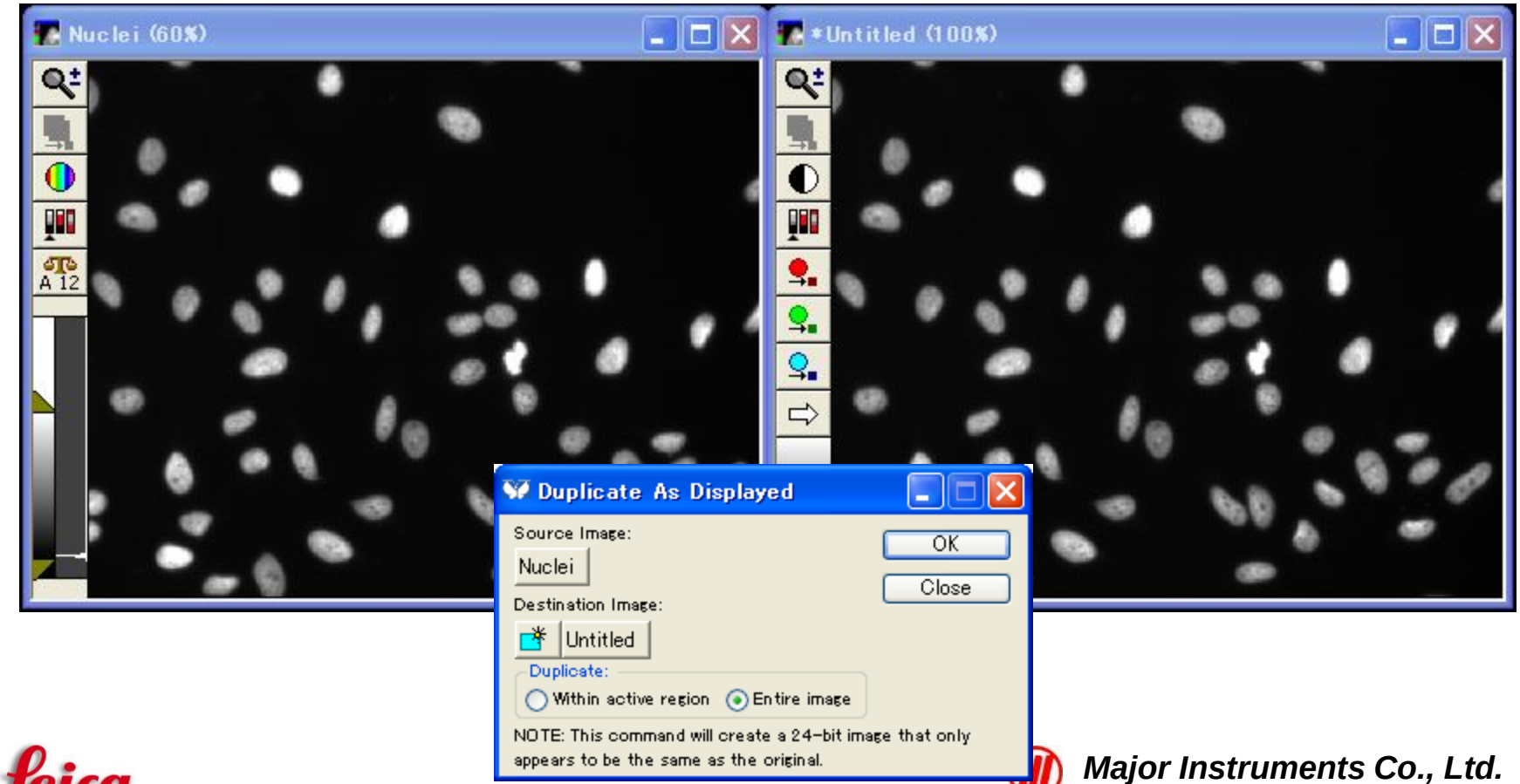
Presentation Image

- 8 Bit Copy: Create an 8 bit copy
- 影像的原始資訊會被改變！



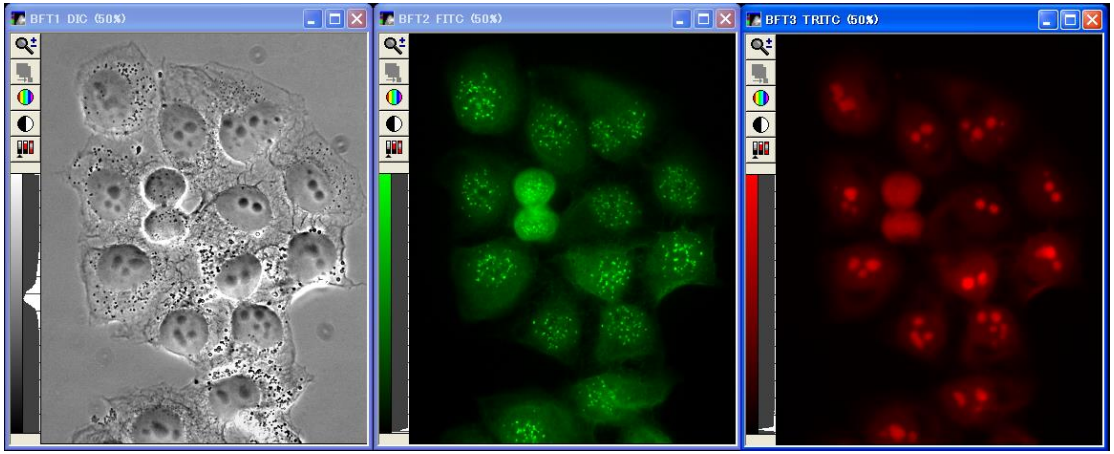
Presentation Image

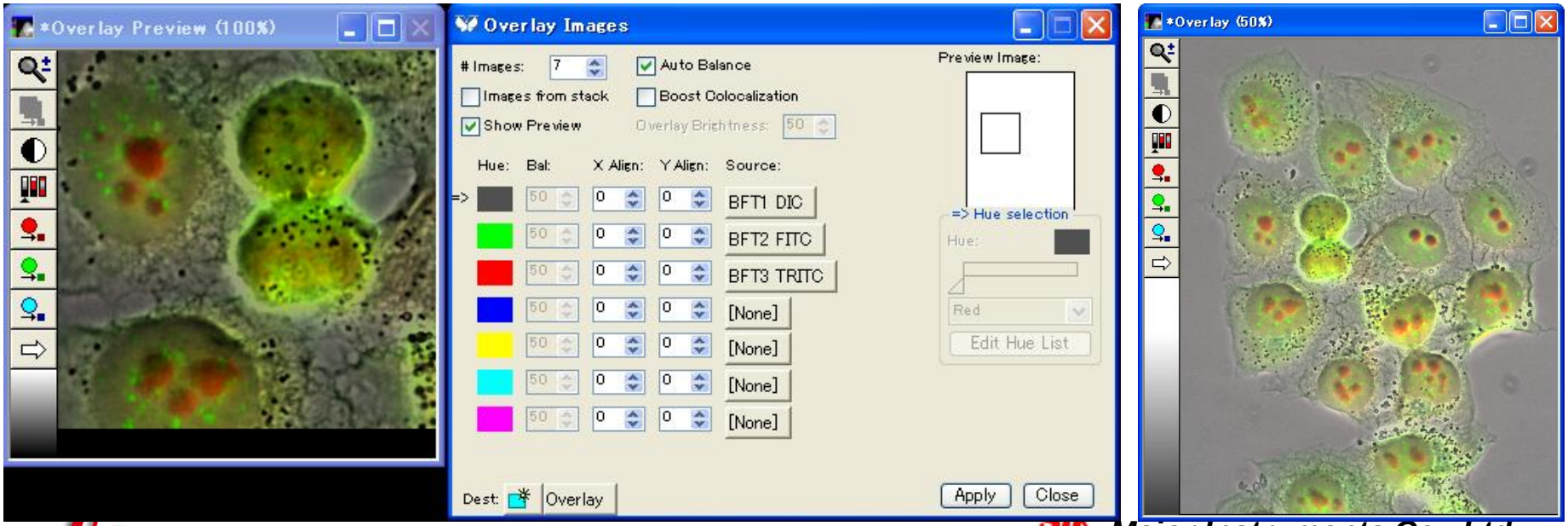
- **24 Bit Copy: Edit menu →Duplicate As Displayed**
  LUT, measurement overlays, scaling…
- 影像原始資訊會被改變！



Color Overlays (Overlay Image)

- Display → Overlay Images
- 最多可重疊7 個不同顏色
- 可預覽
- 可作XY align





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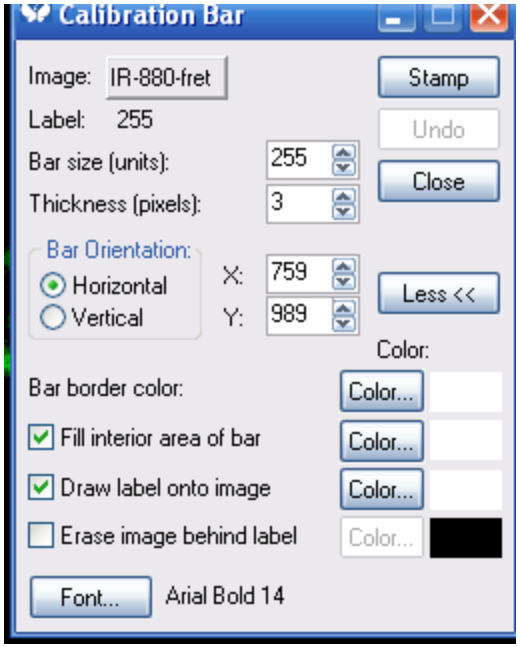
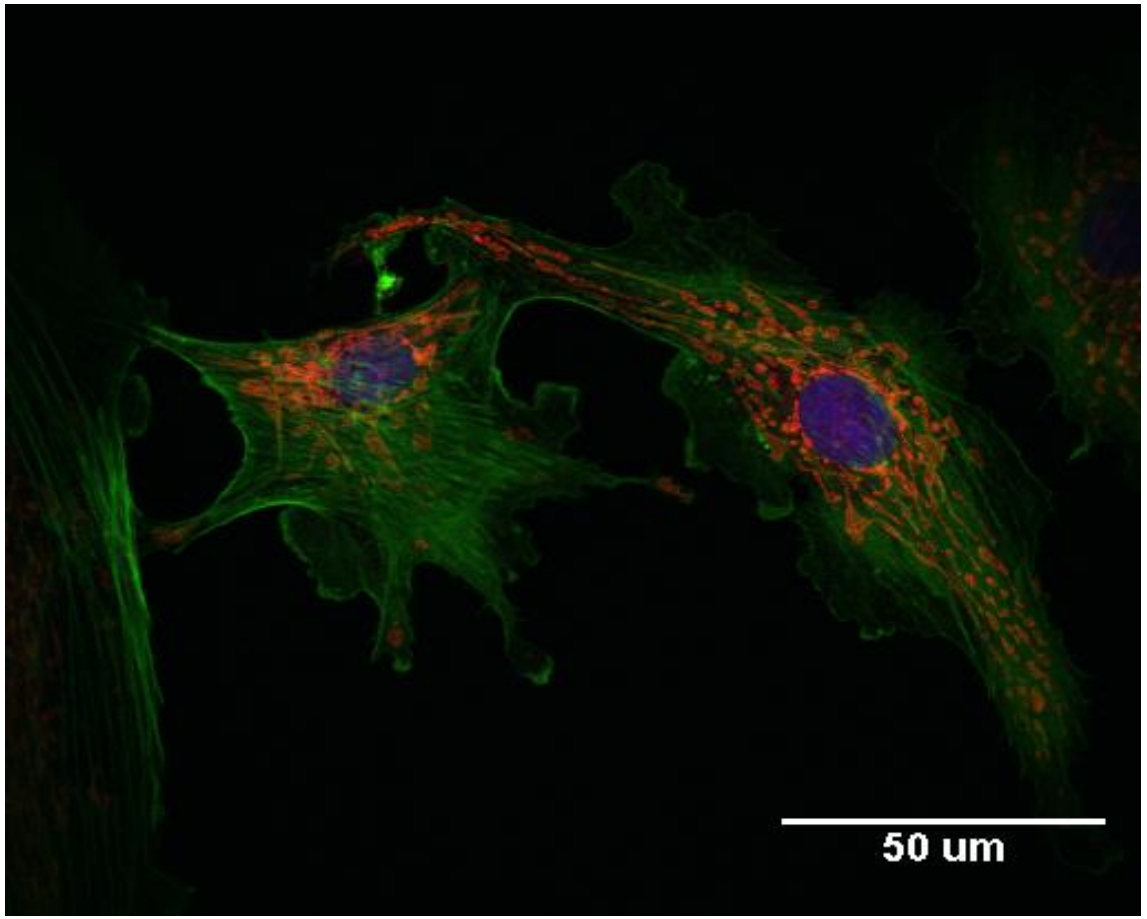
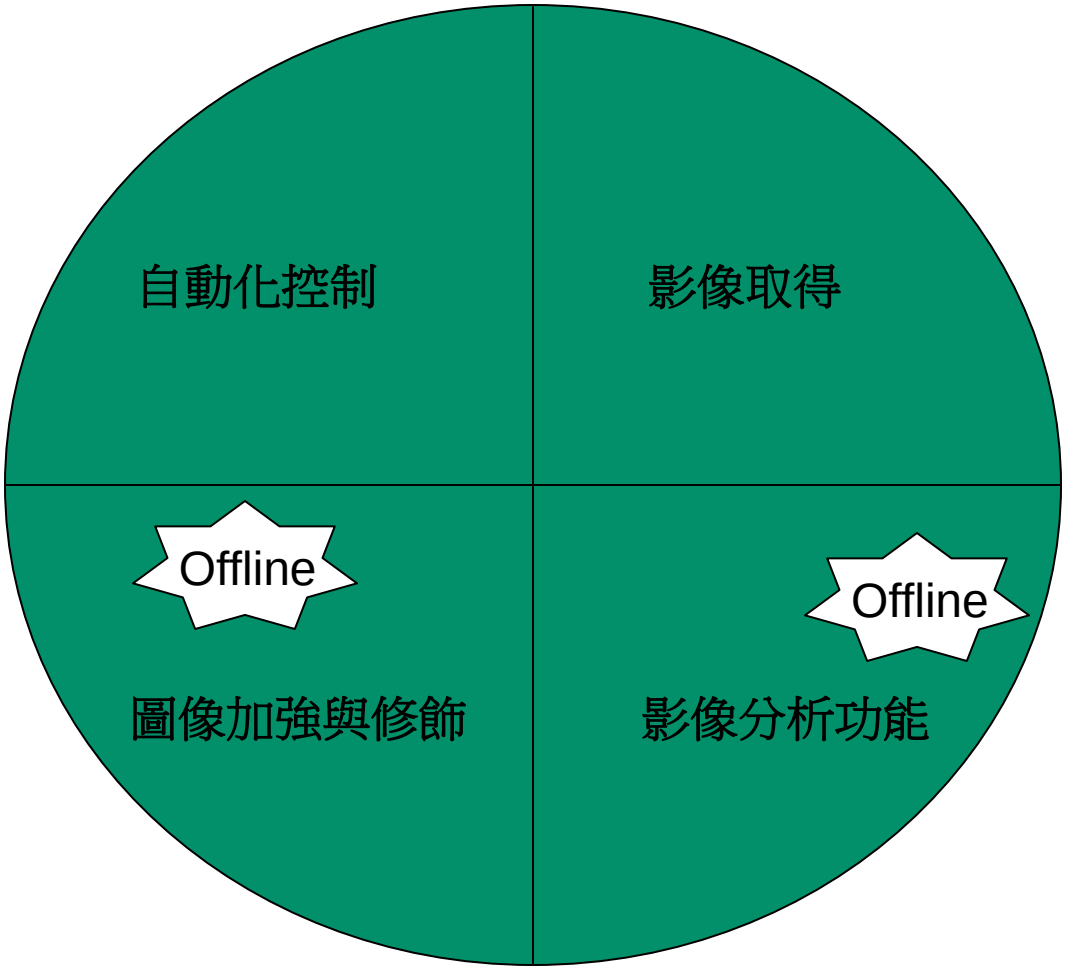
Graphics

- Display→Graphics → Calibration Bar





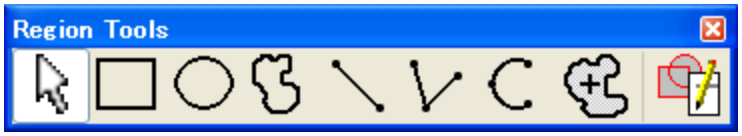
MetaMorph® imaging system



Basic Measurements

- Calibrate Distances
- Region Measurements
- Linescan
- Integrated Morphometry Analysis

Measure region -- Region of Interest (ROI)



Locator

Rectangular

Ellipse

Trace

Single Line

Multi-Line

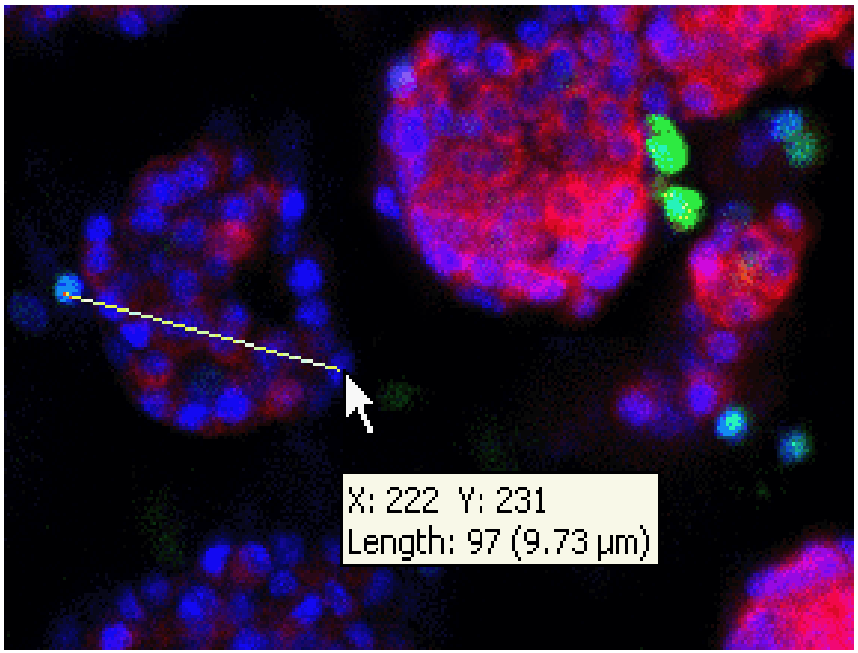
Traced Line

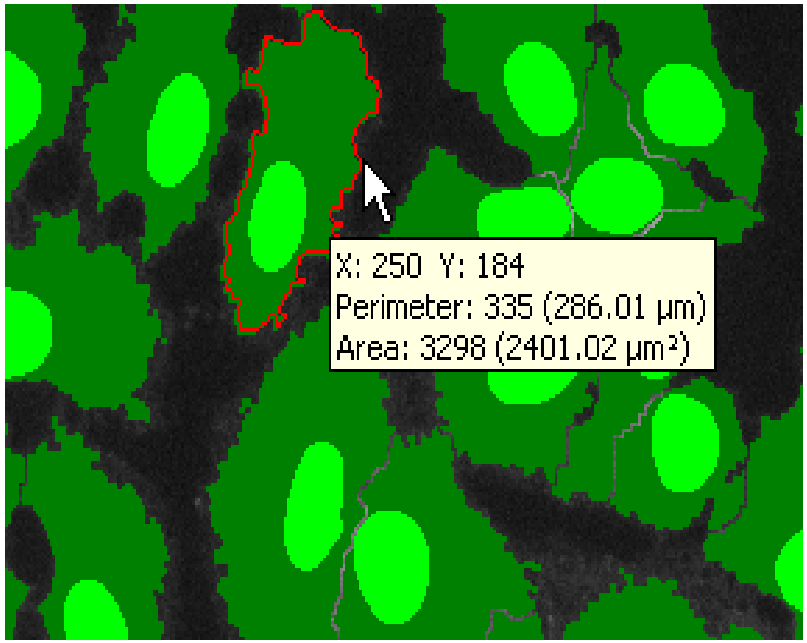
Auto-Trace

Region Tool Properties

MetaMorph®

Region Tooltips Info

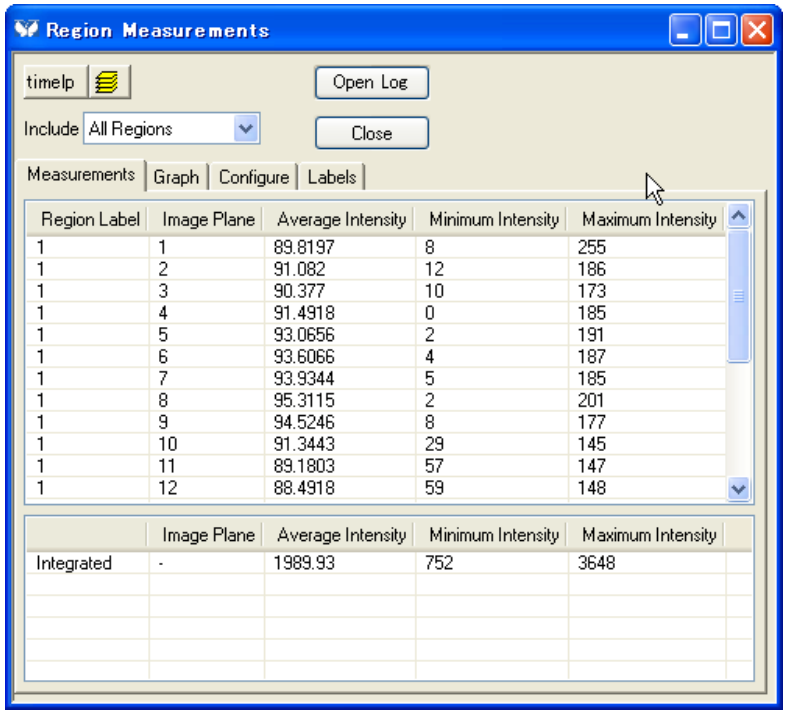


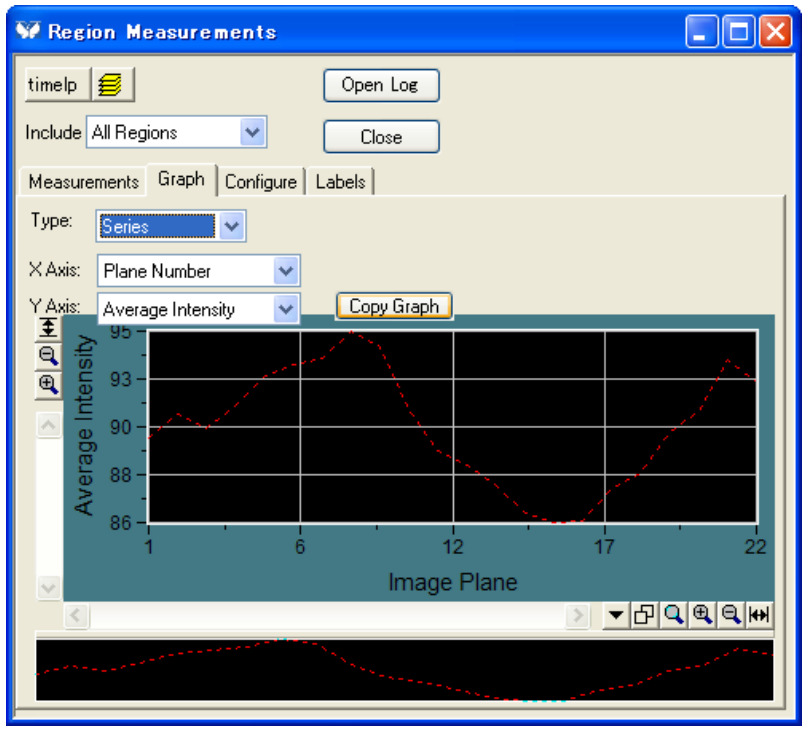


長度和面積以校正後之單位顯示

Region Measurements

- Measure → Region Measurements
- 可同時量測多個圈選區域
- 對於單張影像或是多個影像堆疊檔都可進行量測
- 量測數據顯示在介面上或是可用圖表顯示





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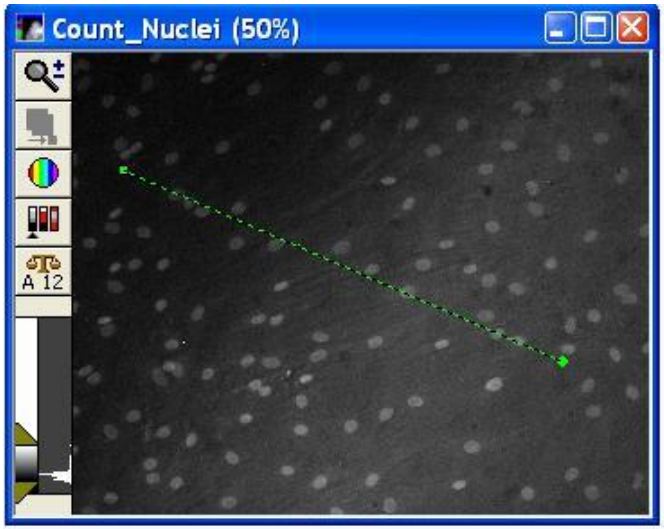
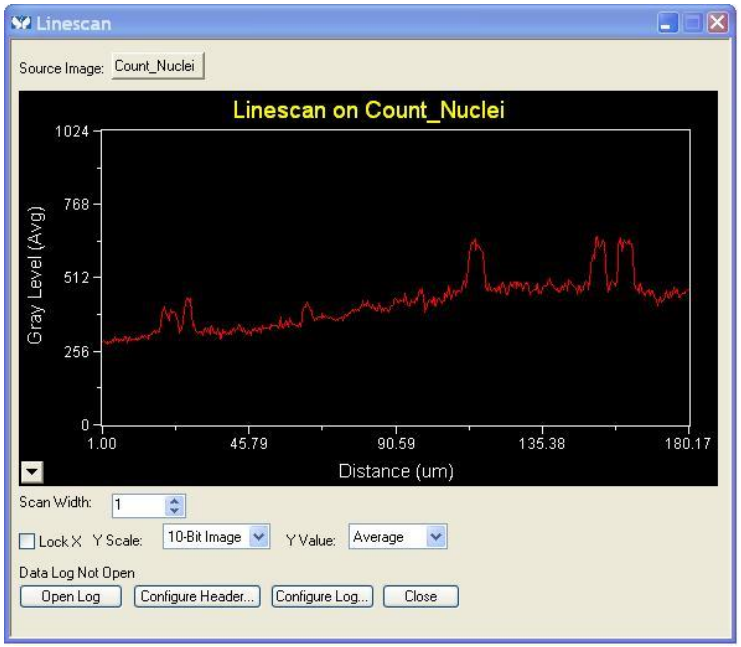
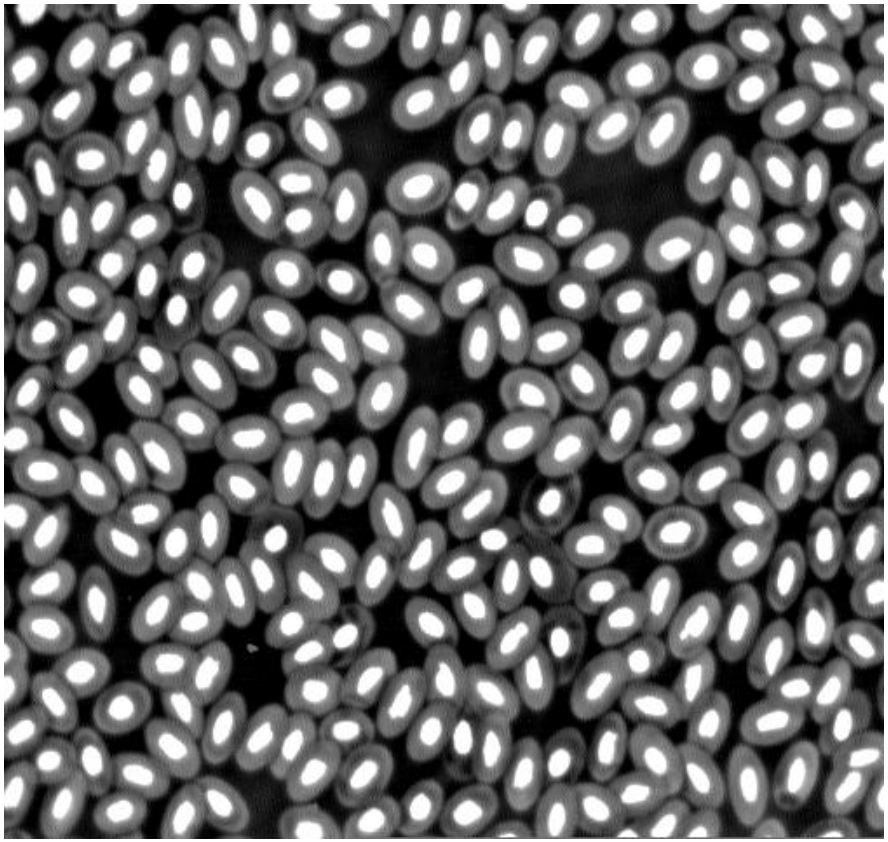
Line Scan

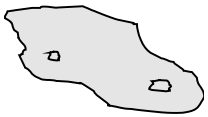
- Measure → Line Scan
- 拖曳的線條可顯示經過的強度分布
- 可方便使用於區分背景與物體的強度差異
- 一次只能畫一條線





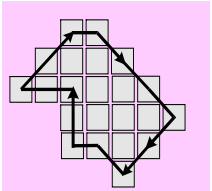
Integrated Morphology Analysis





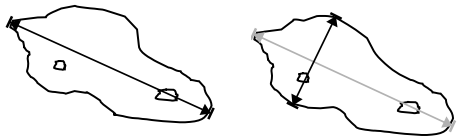
Area

Hole Area

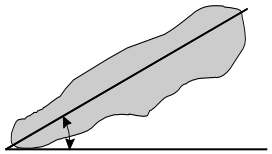


Perimeter

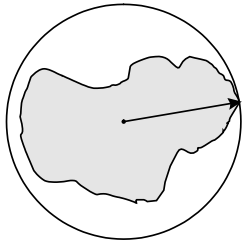
Width and Height



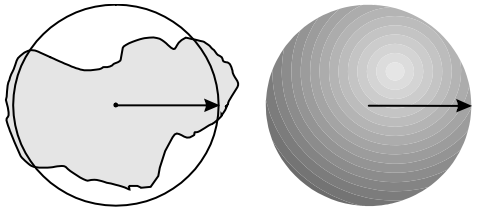
Length and Breadth



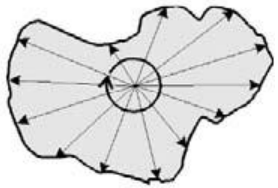
Orientation



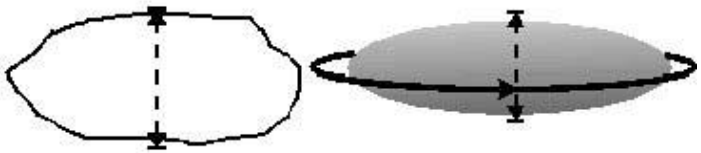
Outer radius



Equivalent sphere volume



Mean Radius



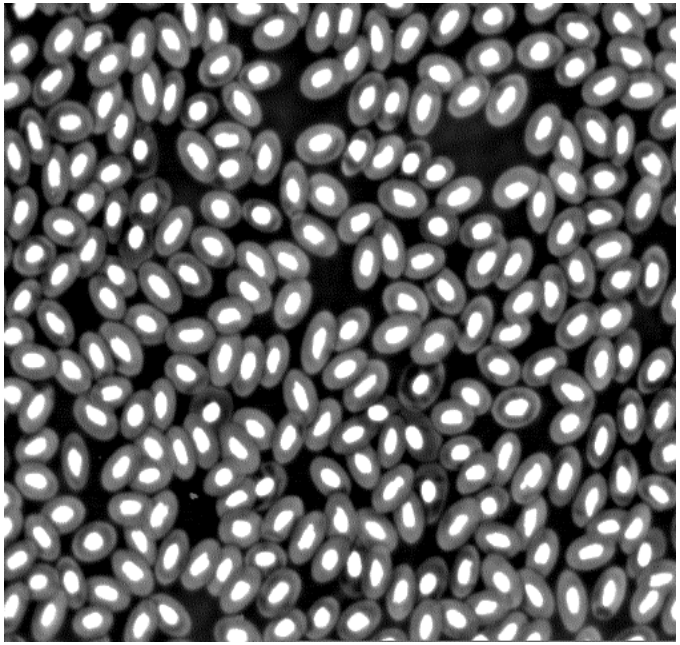
Equivalent Oblate Volume

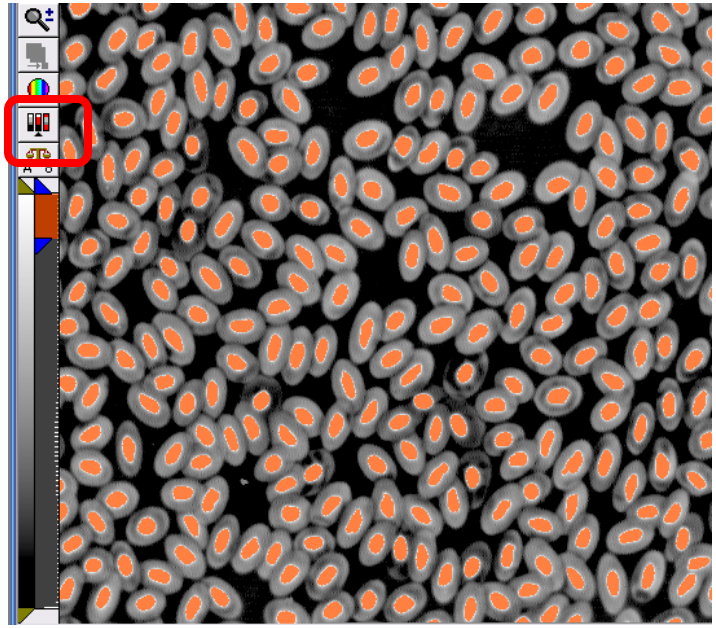
$$OD = \log_{10}\left(1 / \text{transmittance}\right)$$

$$\text{Where transmittance} = \frac{\text{transmitted light}}{\text{incident light}}$$

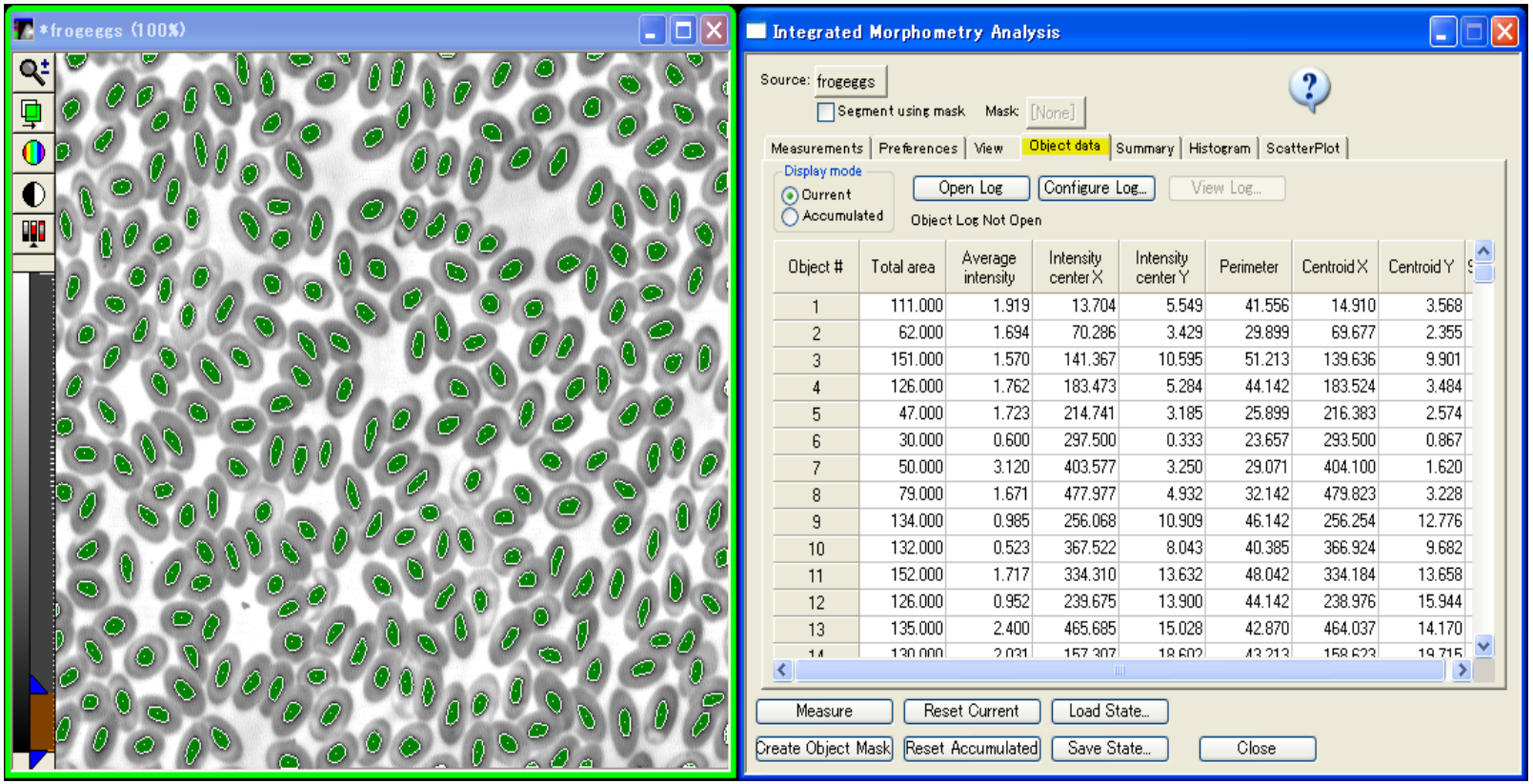
Thresholding

- Measure → Threshold Image
- 以Intensity 篩選欲觀測的區域

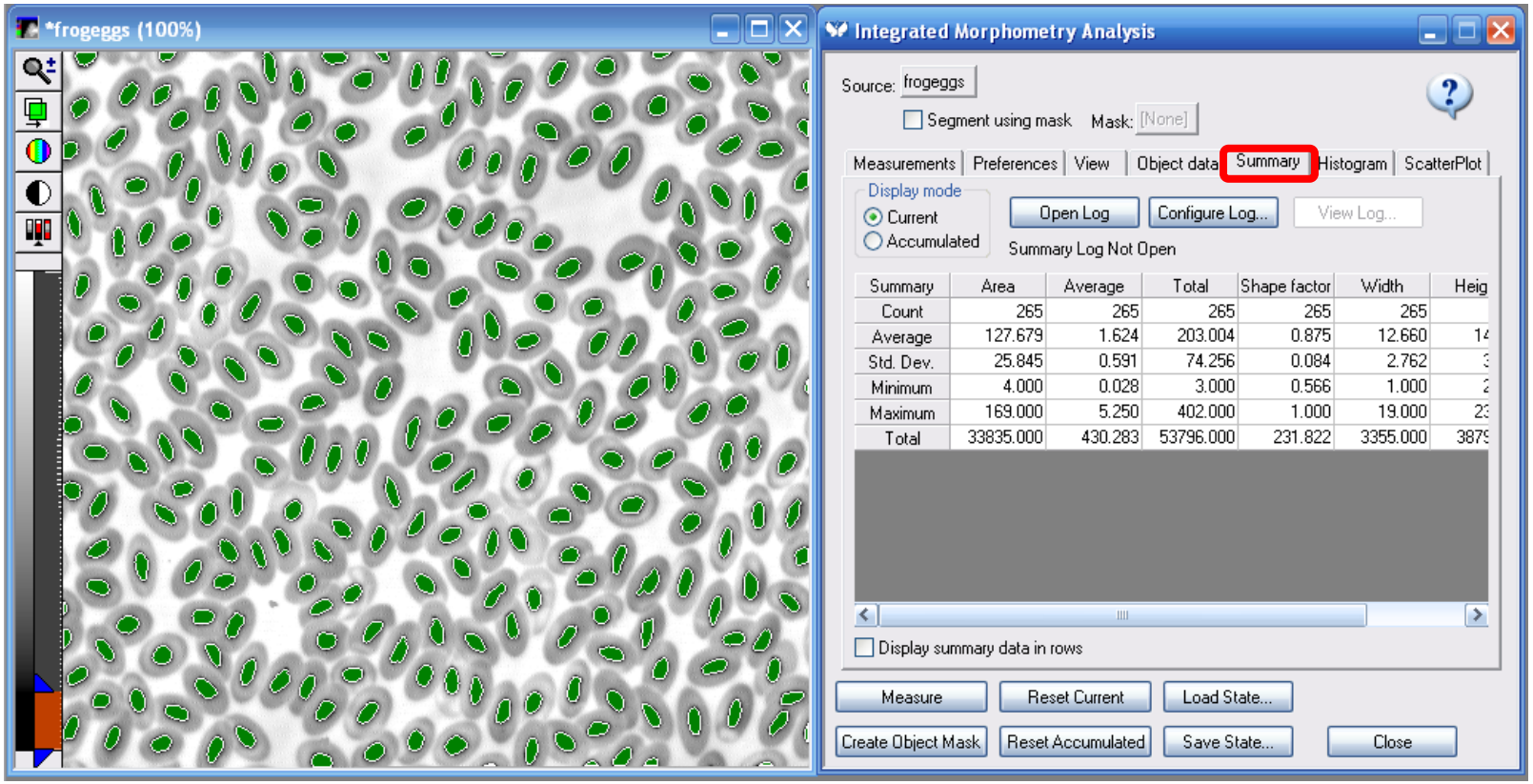




Integrated Morphometry Analysis



Integrated Morphometry Analysis



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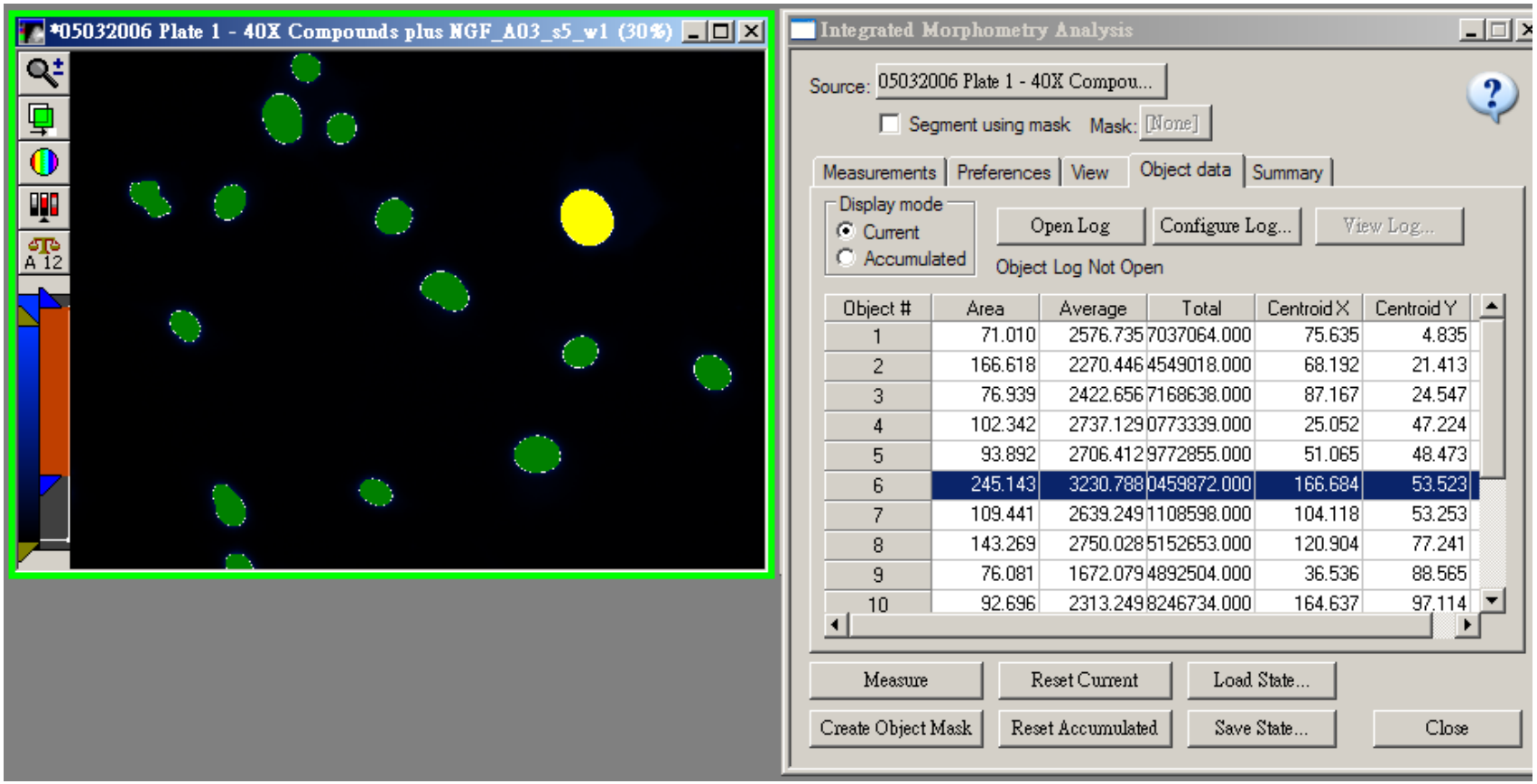
Integrated Morphometry Analysis



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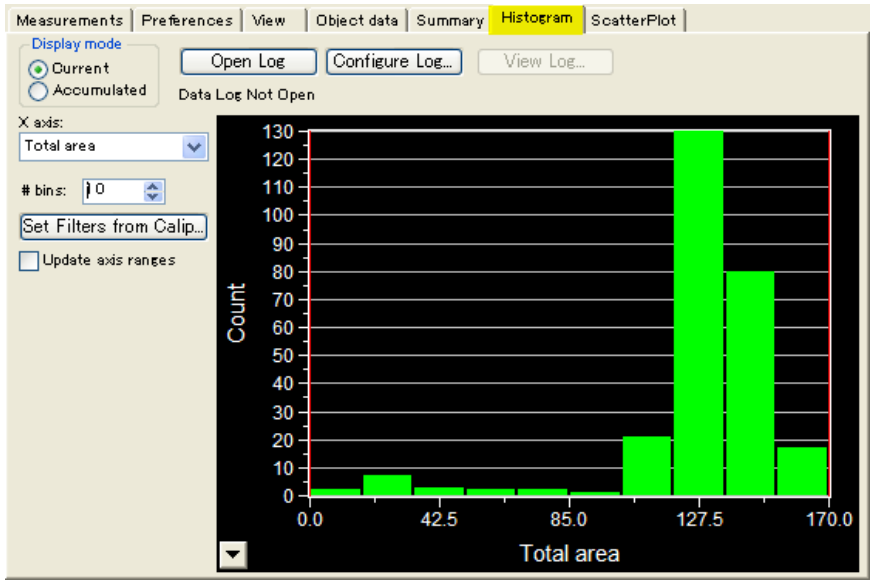
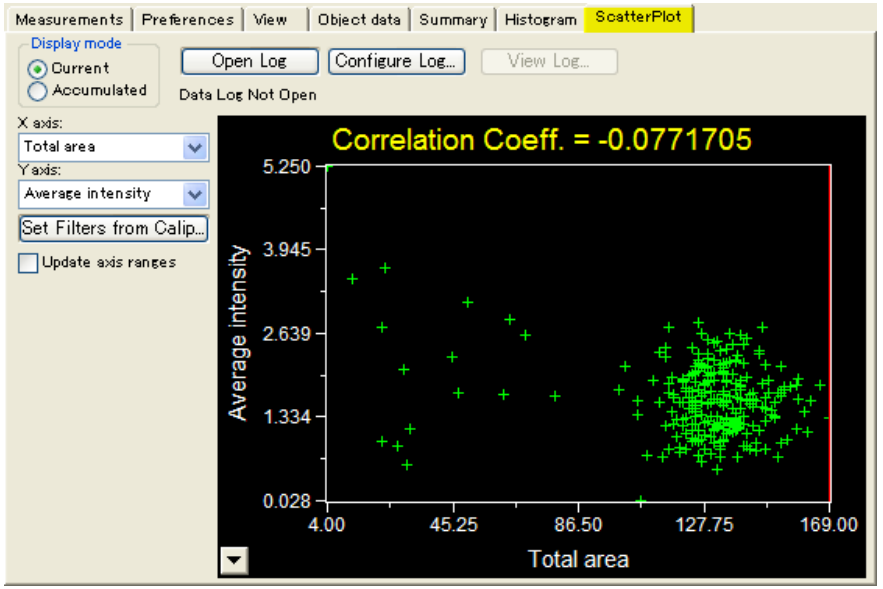
Histogram



ScatterPlot



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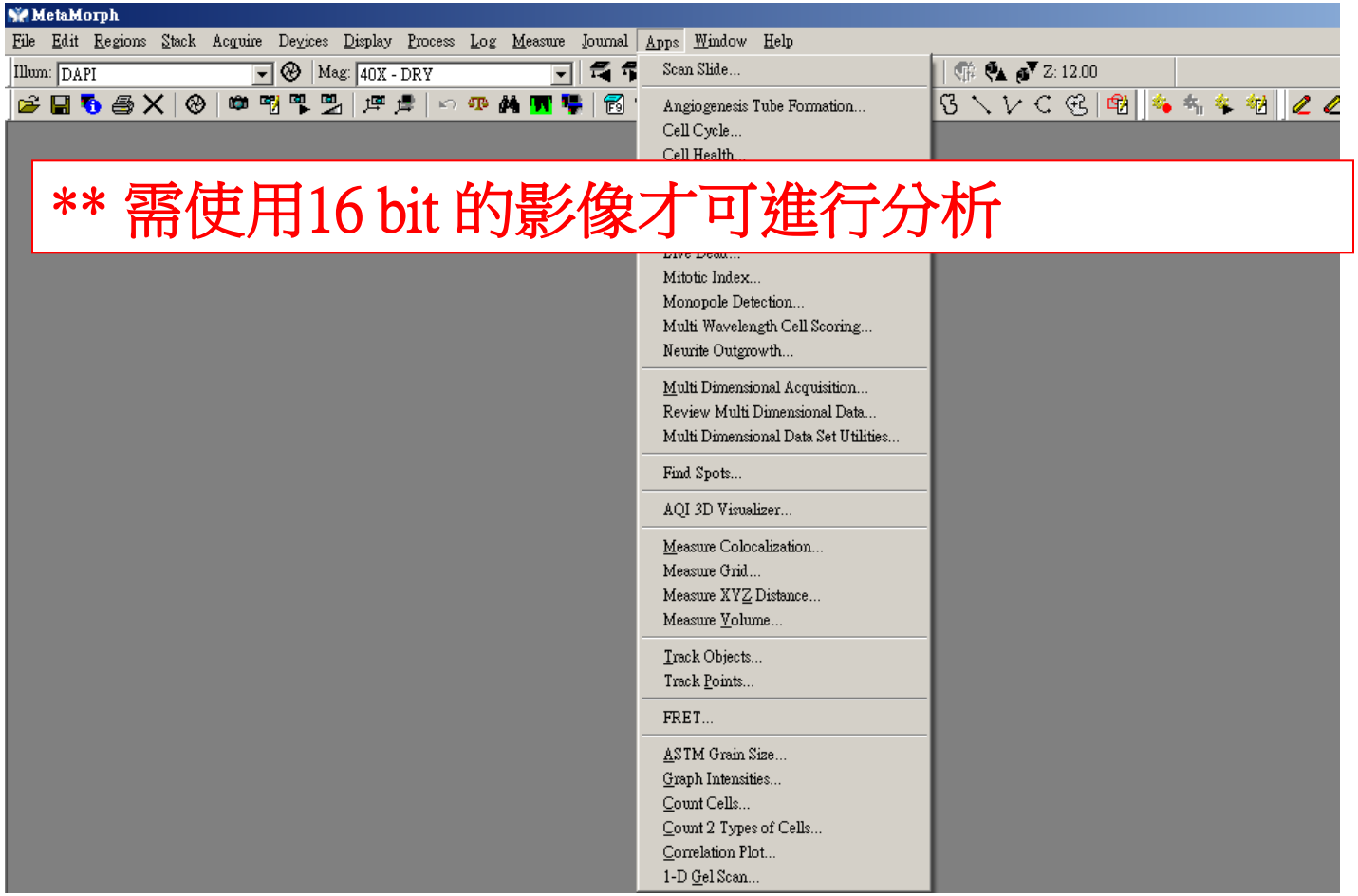
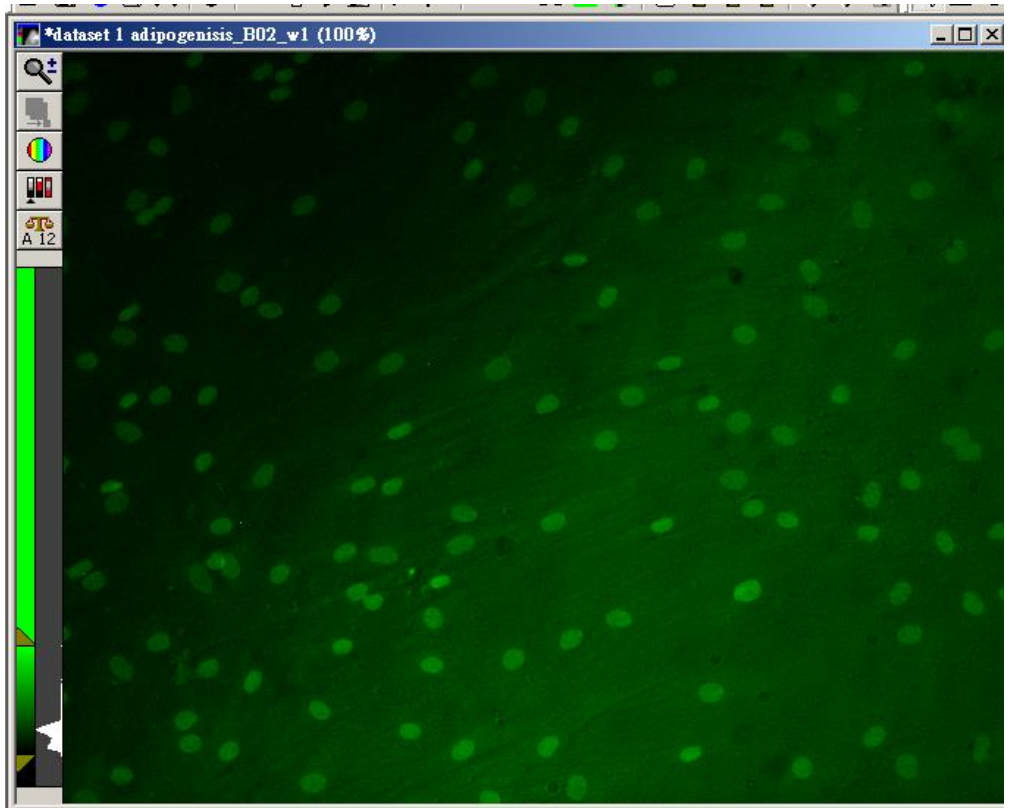
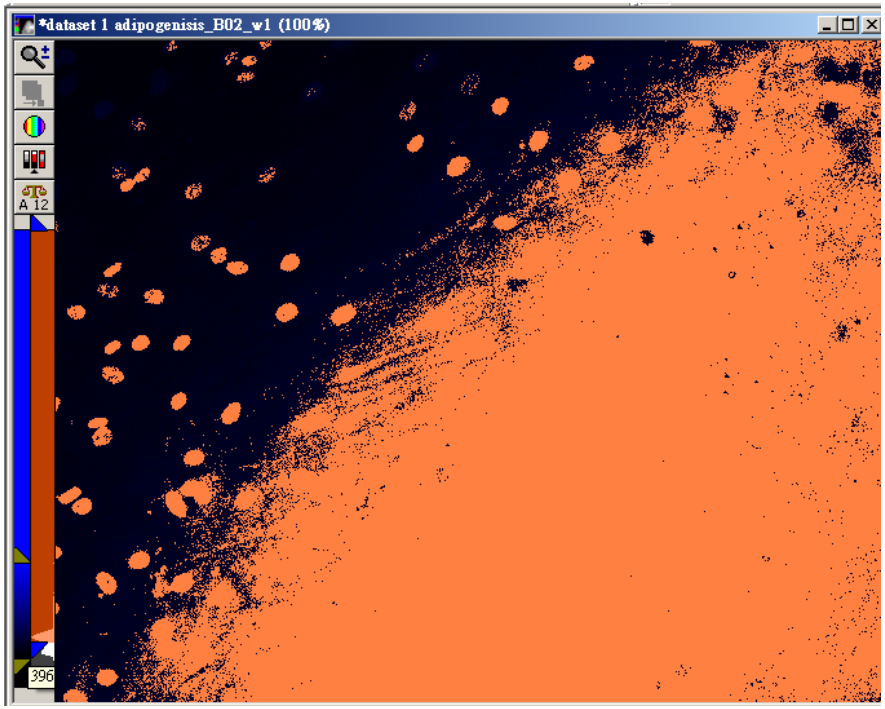
Application modules

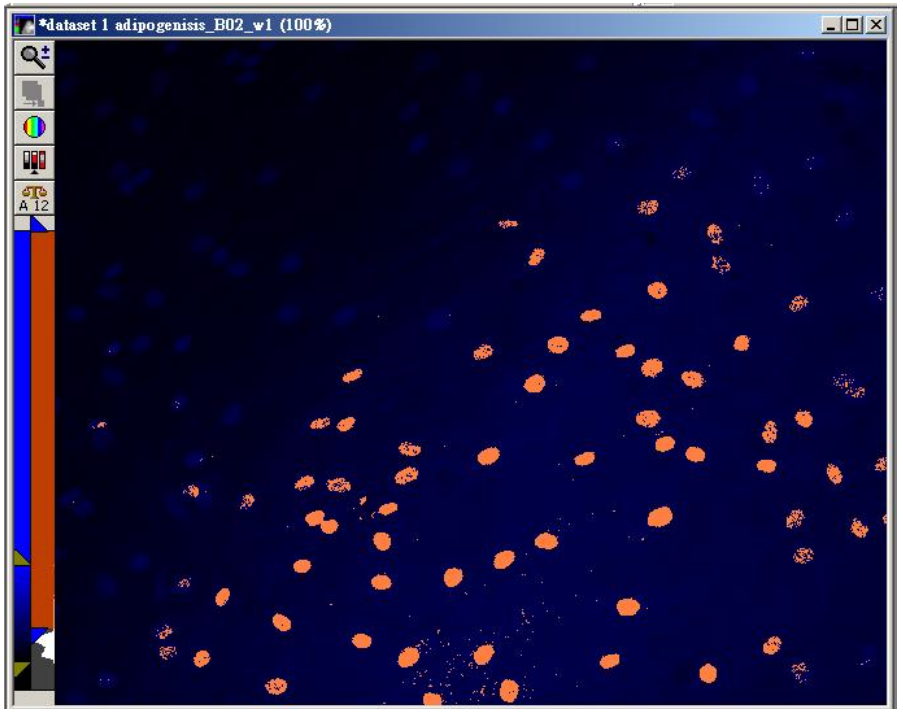


** 需使用16 bit 的影像才可進行分析

Count Nuclei







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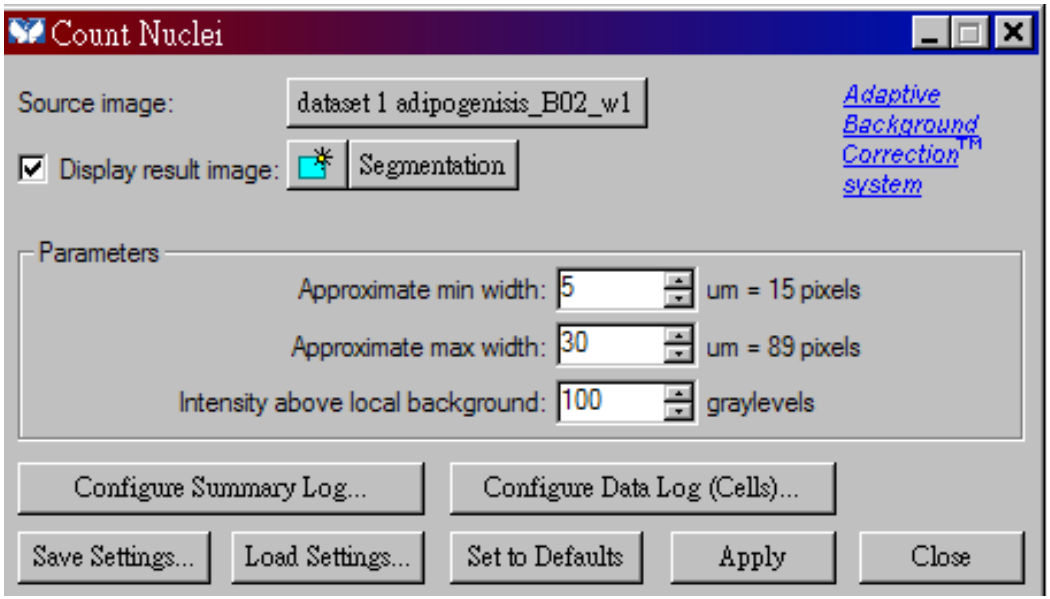
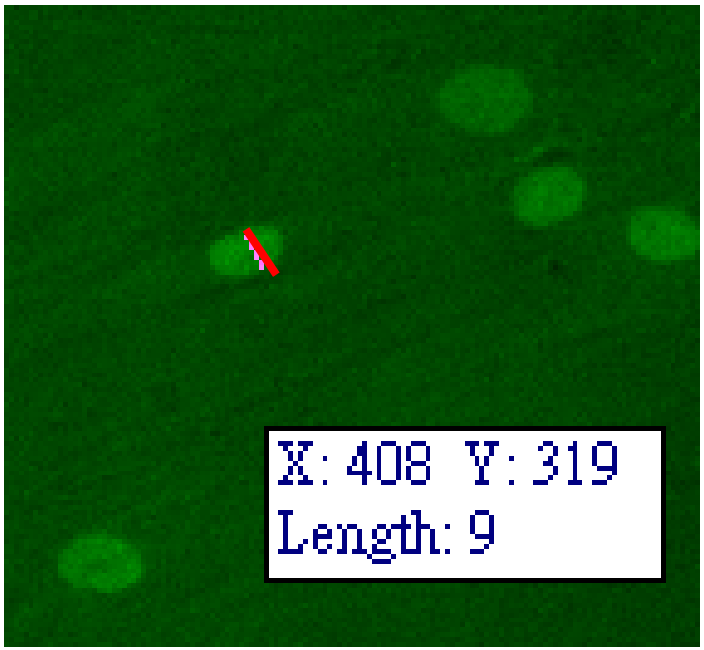
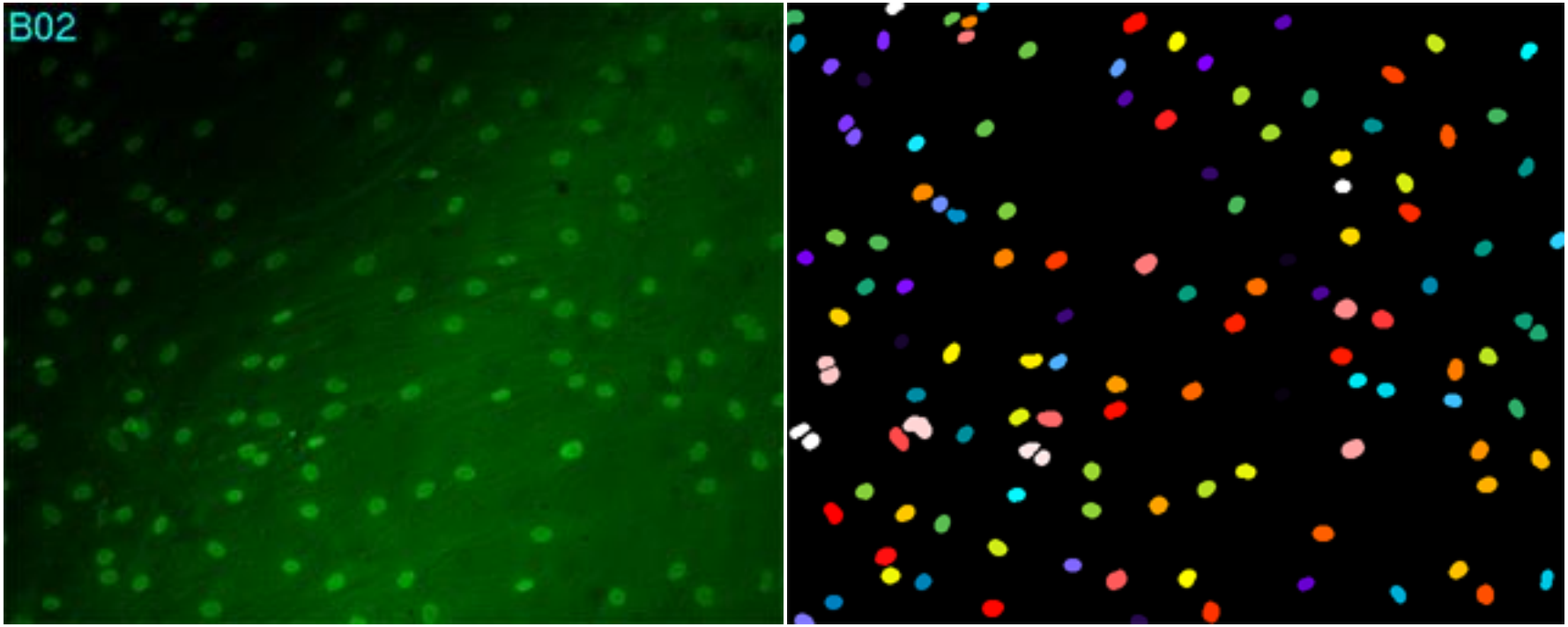

Count Nuclei
Source image:
dataset 1 adipogenisis_B02_w1
Display result image:
Segmentation
Adaptive Background Correction™ system
Parameters
Approximate min width: 5 um = 15 pixels
Approximate max width: 30 um = 89 pixels
Intensity above local background: 100 graylevels
Configure Summary Log...
Configure Data Log (Cells)...
Save Settings...
Load Settings...
Set to Defaults
Apply
Close


X: 408 Y: 319
Length: 9


B02

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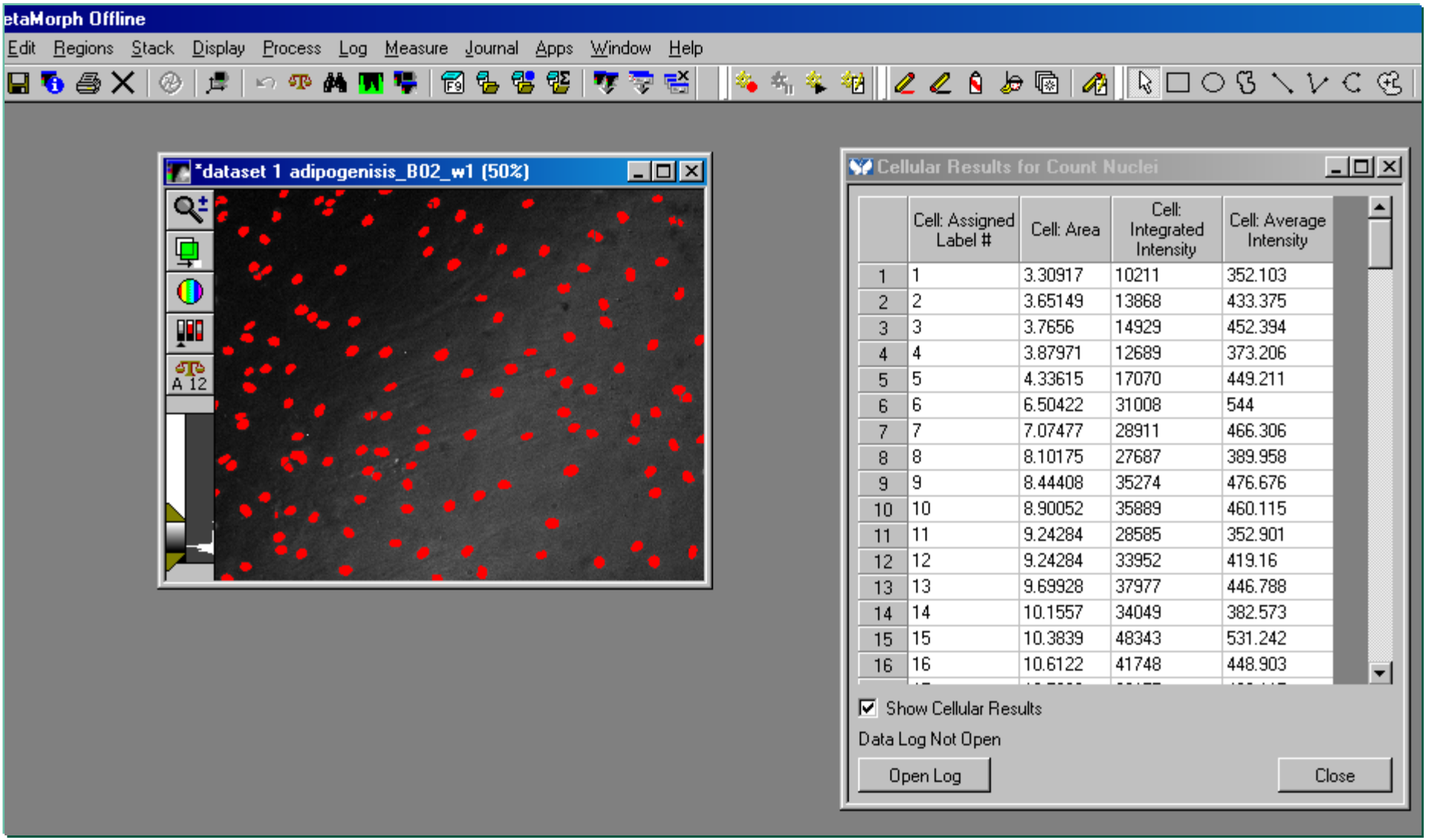

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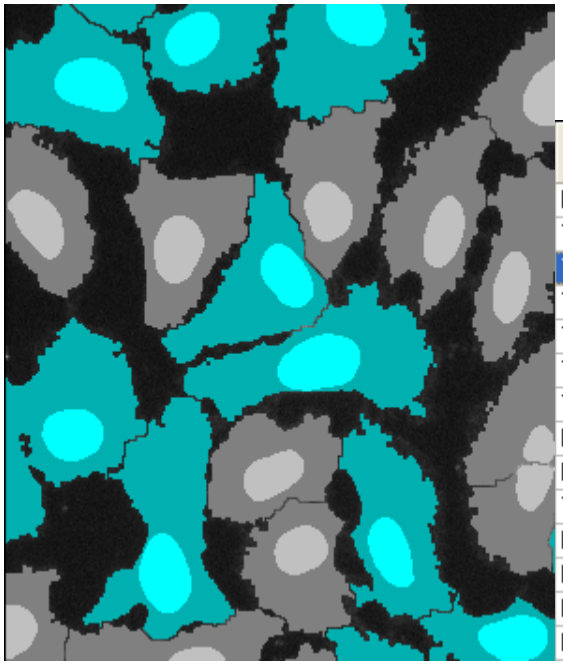
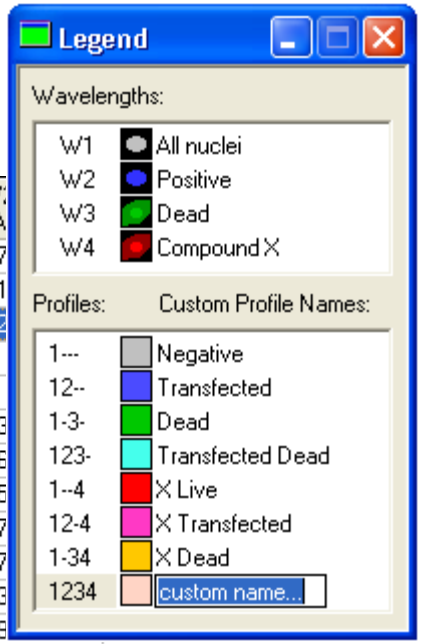
Multi Wavelength Cell Scoring Application Module

- 用途
  - *Your* research— 不需要寫程式或其他巨集就可設計多樣化的模組
  - 可使用1到7種不同的波長
  - 分析結果可針對每一個細胞及每一個波長
- 特性
  - 每一波長都可獨立進行預視
  - 針對不同研究設定波長及名稱
  - 用互動式的圖形來連結每一個獨立波長及所有波長之間的關係



Cell: Custom Profile Name	Cell: Total Area	Cell: W1 Stained Area	Cell: W2 A	
Negative	1685.37	180.549	1685.37	
Transfected	3627.01	426.621	3627.01	
Transfected	2920.82	433.173	2920.82	
Transfected	4011.4	769.519	4011.4	
Transfected	2992.9	208.942	2992.9	
Transfected	3627.73	535.096	3627.73	
Transfected	3418.06	540.92	3418.06	
Negative	2323.85	546.016	2323.85	
Negative	1708.67	475.398	1708.67	
Transfected	3033.67	584.602	3033.67	
Negative	1887.03	394.588	1887.03	
Negative	1815.69	422.253	1815.69	
Negative	2476.73	421.525	2476.73	377.003
Negative	2363.89	428.077	2363.89	389.5



MetaMorph®

MetaFluor®

MetaVue™

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Thank You