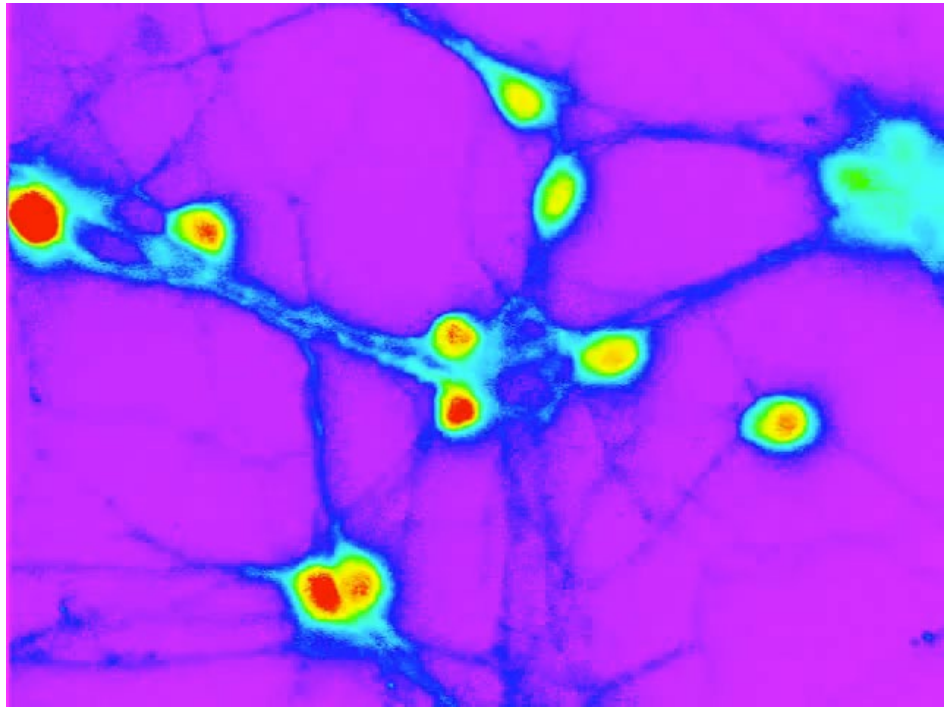
元利儀器
YUAN LI INSTRUMENT CO., LTD.

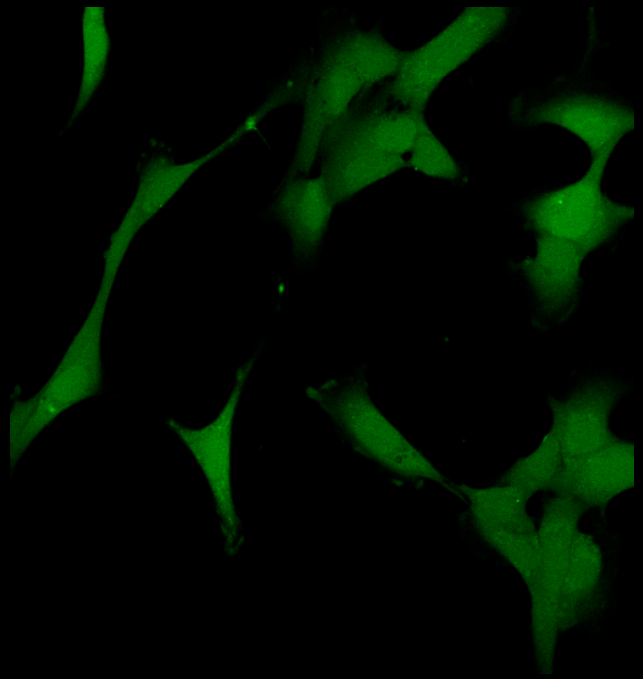
OLYMPUS Cell^R

細胞內離子偵測系統

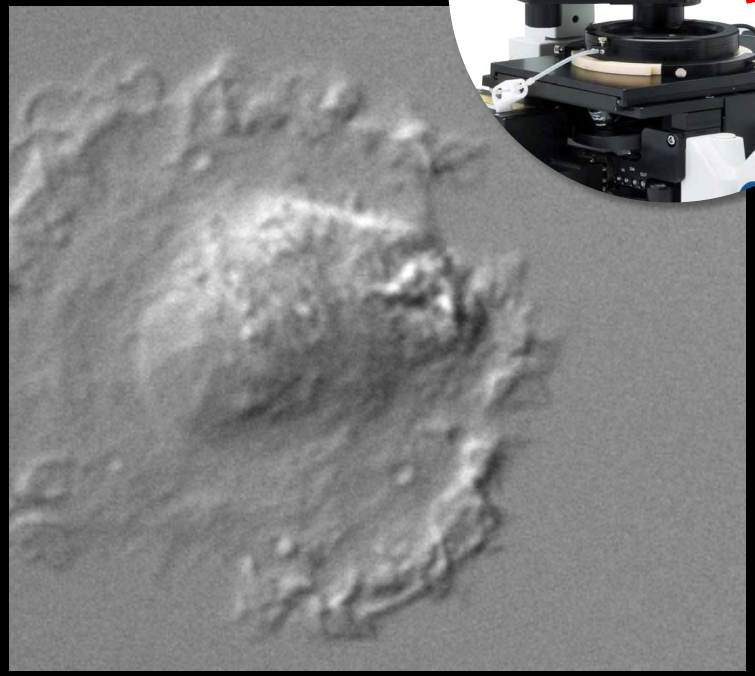


Speaker: 洪鳳嬬 Jill Hung

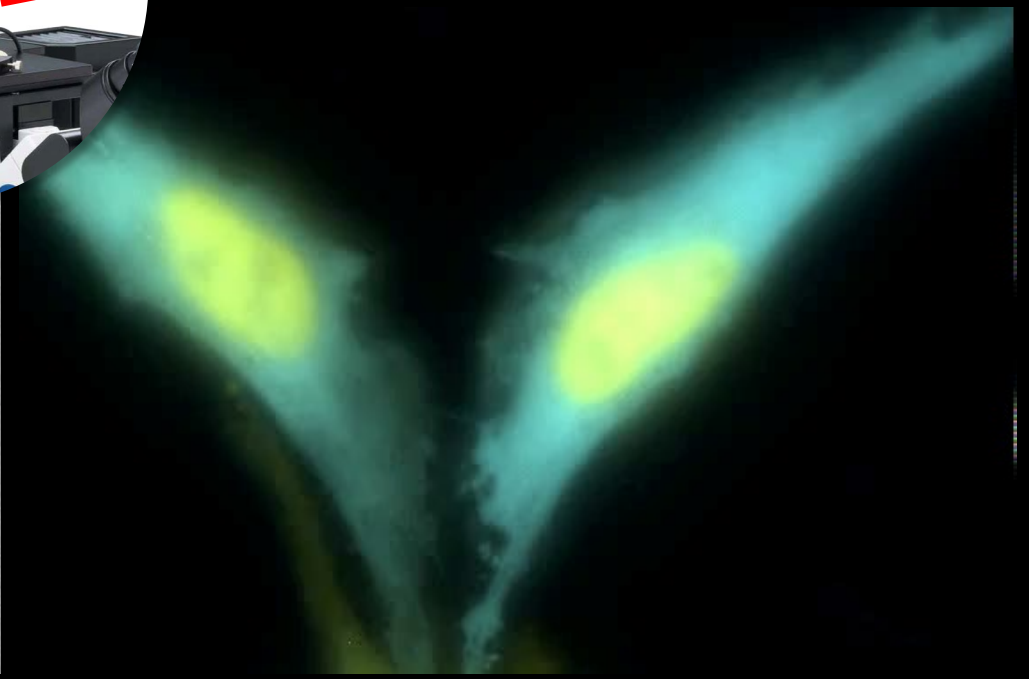
Cell^R Xcellence System



觀測細胞螢光影像



備有小型細胞培養箱，可
供長時間細胞影像之記錄



可偵測即時之細胞影像變化

大綱

元利儀器
YUAN LI INSTRUMENT CO., LTD.

硬體架構

軟體特色

系統應用

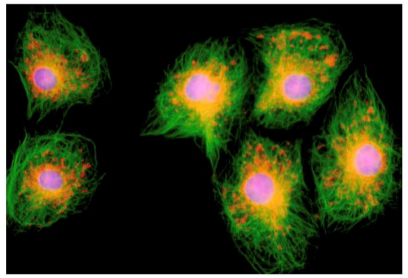
樣本製備

影像分析

樣本製備-染色方法分享

元利儀器
YUAN LI INSTRUMENT CO., LTD.

Olympus Protocols
for
Fluorescence Staining
of Cells



OLYMPUS

nature protocols
REVIEW ARTICLE
https://doi.org/10.1038/s41596-020-0313-9
Check for updates

Tutorial: guidance for quantitative confocal microscopy

James Jonkman[1✉], Claire M. Brown[2], Graham D. Wright[3], Kurt I. Anderson[4] and Alison J. North[5]

When used appropriately, a confocal fluorescence microscope is an excellent tool for making quantitative measurements in cells and tissues. The confocal microscope's ability to block out-of-focus light and thereby perform optical sectioning through a specimen allows the researcher to quantify fluorescence with very high spatial precision. However, generating meaningful data using confocal microscopy requires careful planning and a thorough understanding of the technique. In this tutorial, the researcher is guided through all aspects of acquiring quantitative confocal microscopy images, including optimizing sample preparation for fixed and live cells, choosing the most suitable microscope for a given application and configuring the microscope parameters. Suggestions are offered for planning unbiased and rigorous confocal microscope experiments. Common pitfalls such as photobleaching and cross-talk are addressed, as well as several troubling instrumentation problems that may prevent the acquisition of quantitative data. Finally, guidelines for analyzing and presenting confocal images in a way that maintains the quantitative nature of the data are presented, and statistical analysis is discussed. A visual summary of this tutorial is available as a poster (https://doi.org/10.1038/s41596-020-0307-7).

Confocal microscopes offer a modest advantage over regular 'widefield' (epifluorescence) microscopes in resolution, but their main advantage is the ability to generate high-contrast images through optical sectioning. In a widefield microscope, the acquired images are a superposition of sharp features from the focal plane and blurry features from outside of the focus. A confocal microscope blocks the latter, resulting in a sharp image from the focal plane alone (Fig. 1a,b). With a high-resolution objective lens, a confocal microscope can generate optical sections thinner than 1 µm without having to physically slice the sample. It can therefore be used to quantify the intensities and investigate the spatial arrangement of fluorescent molecules with high precision, which is useful for assigning the localization of molecules to specific cellular compartments or assessing the colocalization of different molecules. A single confocal image (or 'slice') may be sufficient for quantification if it is representative of the entire thickness of the sample, but one can also take a series of confocal images while changing the focus to produce a 3D dataset (or 'z-stack'), enabling the reconstruction and quantification of the entire sample volume (Fig. 1c,d).

The essential component common to all confocal microscopes is one or more strategically placed pinhole apertures. Figure 2a shows a schematic of the main components and the lightpath in the classic confocal laser-scanning microscope (CLSM). A laser beam is focused into a specimen, where it excites fluorescent molecules throughout the entire cone of illumination. The light emitted by these excited fluorescent molecules (i.e., fluorescence) is collected by the objective lens and focused by a second lens through a carefully aligned pinhole. The pinhole ensures that only fluorescence that originates at the focal point is captured by the detector; fluorescence emission from above or below the focal plane is blocked. The name 'confocal' derives from the position of the pinhole(s) in the microscope's lightpath, in a CONjugate FOCAL plane with the sample. As the CLSM collects fluorescence from only one focal point at a time, scanning mirrors are used to sweep the laser beam across the specimen, generating an image pixel by pixel. Since you typically must dwell for ~1 µs on each pixel to collect enough fluorescence, it takes ~1 s to generate a modest 1,024 × 1,024 pixel (1 megapixel) image. To capture fast dynamics in live specimens, small regions of interest can be scanned, or a different confocal geometry might be needed. One such alternative is a spinning-disk confocal microscope (SD), which illuminates the sample using an array of pinholes arranged in a special pattern on a disk, creating hundreds of focused beams (Fig. 2b). The fluorescence is then collected back through the pinholes (creating the optical section) and detected using a digital camera—effectively parallelizing multiple confocal lightpaths. The disk spins to rapidly sweep the pattern of

[1]Advanced Optical Microscopy Facility (AOMF), University Health Network, Toronto, Ontario, Canada. [2]Advanced BioImaging Facility (ABIF), McGill University, Montreal, Quebec, Canada. [3]A*STAR Microscopy Platform (AMP), Skin Research Institute of Singapore, A*STAR, Singapore, Singapore. [4]Crick Advanced Light Microscopy Facility (CALM), The Francis Crick Institute, London, UK. [5]Bio-Imaging Resource Center, The Rockefeller University, New York, NY, USA. ✉e-mail: james@aomf.ca

樣本製備-容器與物鏡校正環

元利儀器
YUAN LI INSTRUMENT CO., LTD.

蓋玻片 NO.1.5H

MARIENFELD

High Precision Cover Glass,Sc

- 硼矽酸玻璃材質
- 適用於體外診斷
- 與蔡司和SCHOTT技術合作
  特別適用於高倍率解析顯微鏡
- 厚度:0.170mm±0.005mm
- 光學特性:ne值1.524~1.52
  阿貝係數(Abbe)Ve=55
- 建議使用於下列光學值目鏡
  浸水式目鏡:NA≧1.0;甘油式
  油式目鏡:NA≧1.3
- 最小訂購量1cs

貨號	描述
AP-0107032	18x18mm,200e
AP-0107052	22x22mm,200e
AP-0107222	24x50mm,100e
AP-0107242	24x60mm,100e

螢光樣本上機 建議

正確使用物鏡校正環

樣本製備-容器與物鏡校正環

元利儀器 YUAN LI INSTRUMENT CO., LTD.

蓋玻片 NO.1.5H 方形 高精度

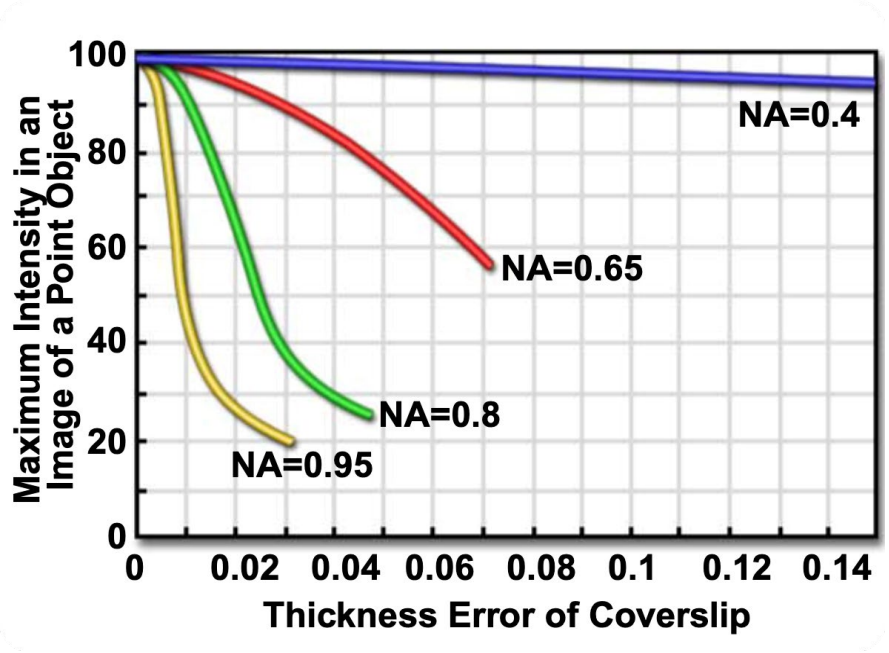
MARIENFELD

High Precision Cover Glass,Square,NO.1.5H

- 硼矽酸玻璃材質
- 適用於體外診斷
- 與蔡司和SCHOTT技術合作，特別適用於高倍率解析顯微鏡
- 厚度:0.170mm±0.005mm
- 光學特性:ne值1.524~1.527(於波長546.07nm);阿貝係數(Abbe)Ve=55
- 建議使用於下列光學值目鏡:乾式目鏡:NA≧0.7,浸水式目鏡:NA≧1.0;甘油式目鏡:NA≧1.2,礦物油式目鏡:NA≧1.3
- 最小訂購量1cs

貨號	描述	bx/cs
AP-0107032	18x18mm,200ea/bx	10
AP-0107052	22x22mm,200ea/bx	10
AP-0107222	24x50mm,100ea/bx	10
AP-0107242	24x60mm,100ea/bx	10

螢光樣本上機 建議使用1.5H蓋玻片



蓋玻片厚度對於螢光亮度的影響

正確使用物鏡校正環

大綱

元利儀器
YUAN LI INSTRUMENT CO., LTD.

硬體架構

軟體特色

系統應用

樣本製備

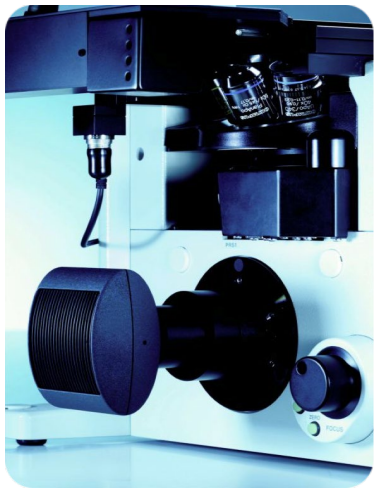
影像分析

硬體架構

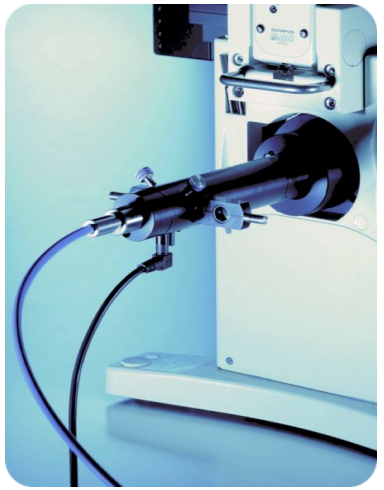
元利儀器
YUAN LI INSTRUMENT CO., LTD.

倒立式顯微鏡與軟體

照明系統



黑白CCD



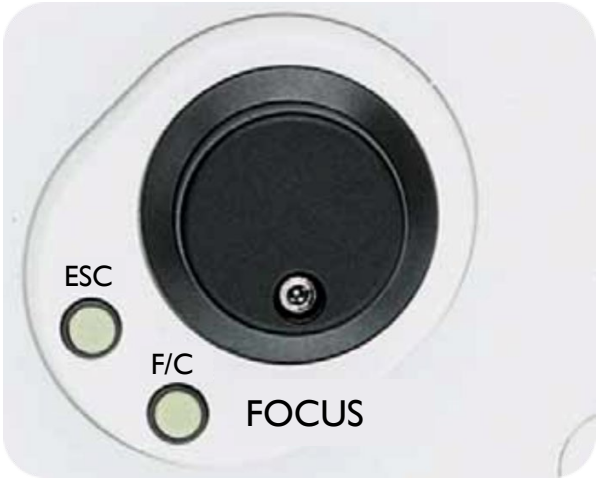
螢光投射管

Linux控制器

硬體架構-電動顯微鏡IX81

元利儀器
YUAN LI INSTRUMENT CO., LTD.

- 觀察法：螢光, DIC(微分干涉差), 明視野
- 螢光濾片組: DAPI, GFP, RFP, Fura2
- DIC: 10X, 20X, 40X, 60X, 100X



ESC 快速換片

F/C 粗細切換

倍率切換

硬體架構-高解析物鏡組

元利儀器
YUAN LI INSTRUMENT CO., LTD.

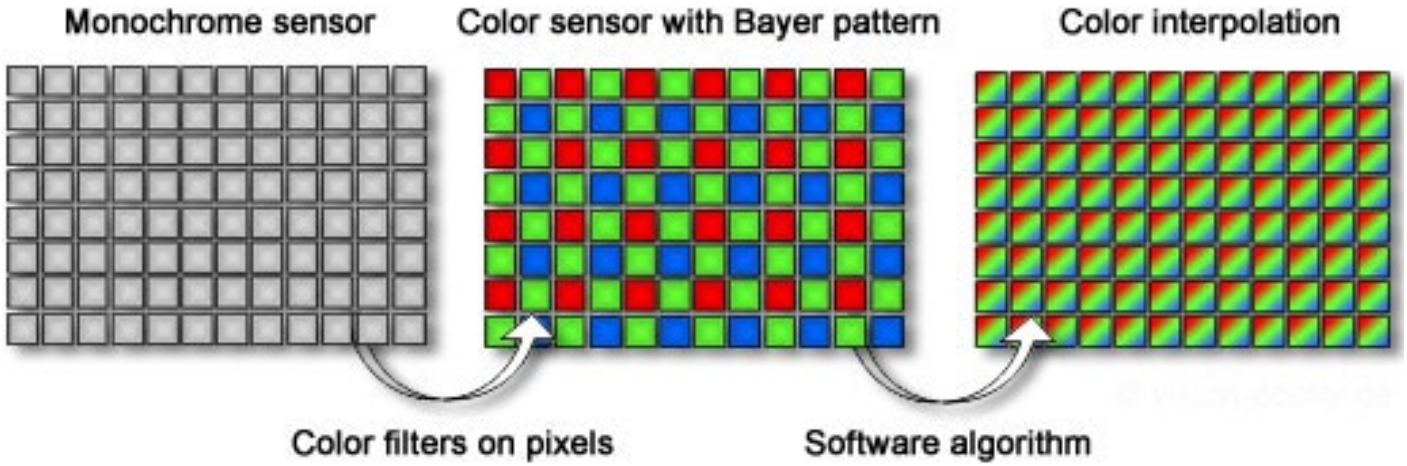
	倍率	應用	備註
空氣鏡	10X 20X(APO)	廣視野或較大組織	勿沾到**水或油**
油鏡	40X(FL)	廣視野高解析	請用**玻璃容器**
油鏡	60X 100X(APO)	單一細胞高解析影像	請用**玻璃容器**

硬體架構-黑白CCD

元利儀器
YUAN LI INSTRUMENT CO., LTD.

高靈敏照相機

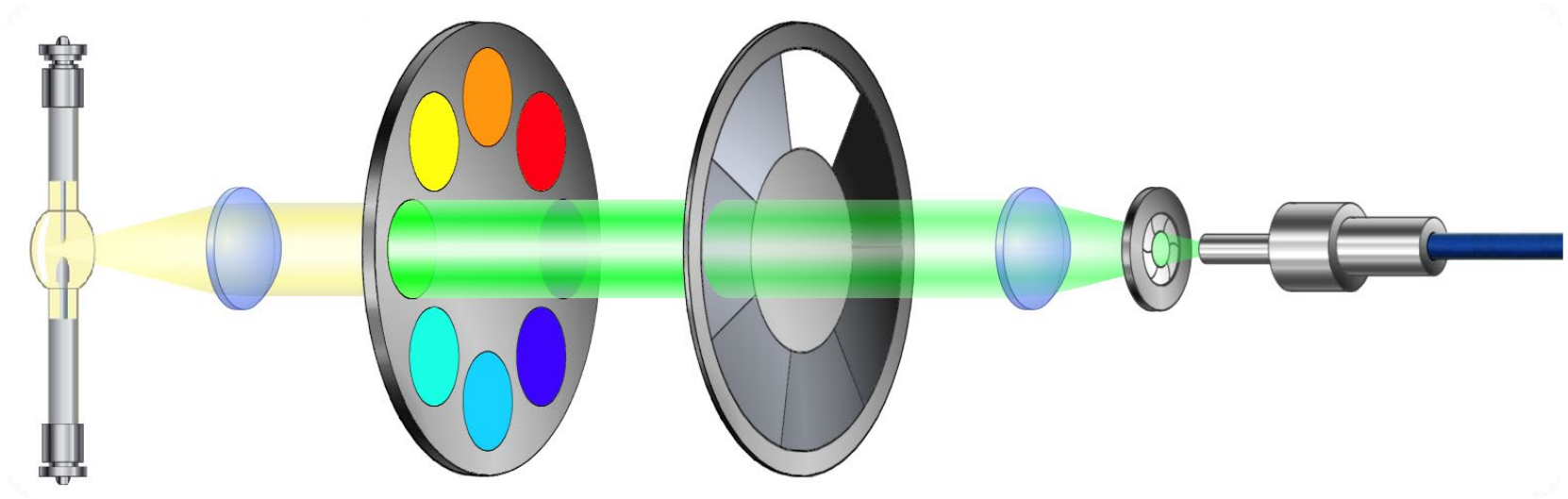
- 解析度: 1,376 x 1,032 pixels
- Peltier-cooling CCD chip
- 收光範圍: 450-700nm



硬體架構-螢光照明系統MT20

元利儀器
YUAN LI INSTRUMENT CO., LTD.

高度整合
快速照明系統



燈泡

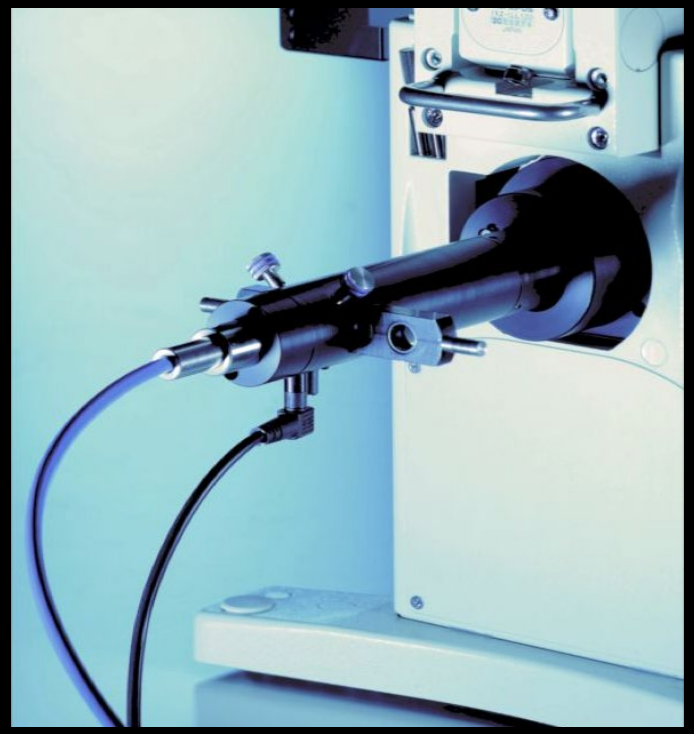
激發光
濾片轉盤

減光片
轉盤

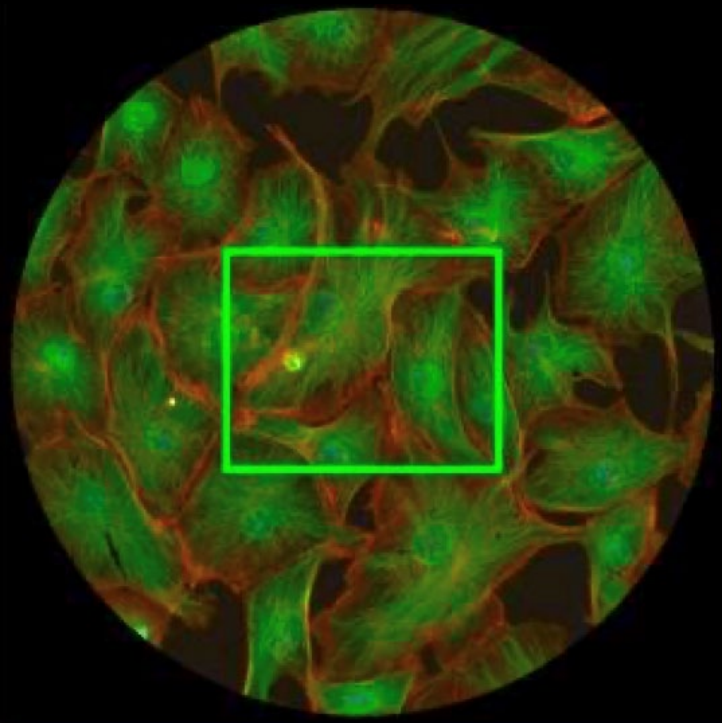
高速
Shutter

光纖

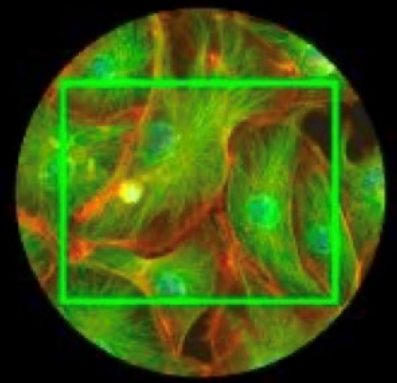
硬體架構-螢光投射管



螢光投射管



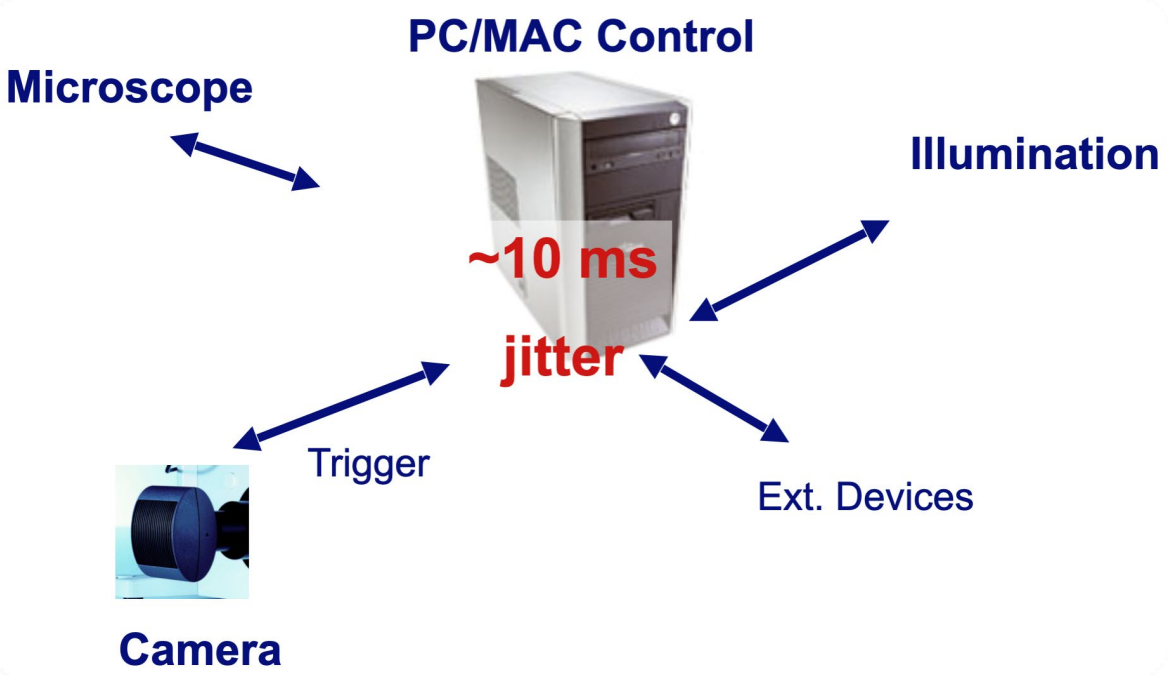
傳統投射管形成
多餘激發範圍

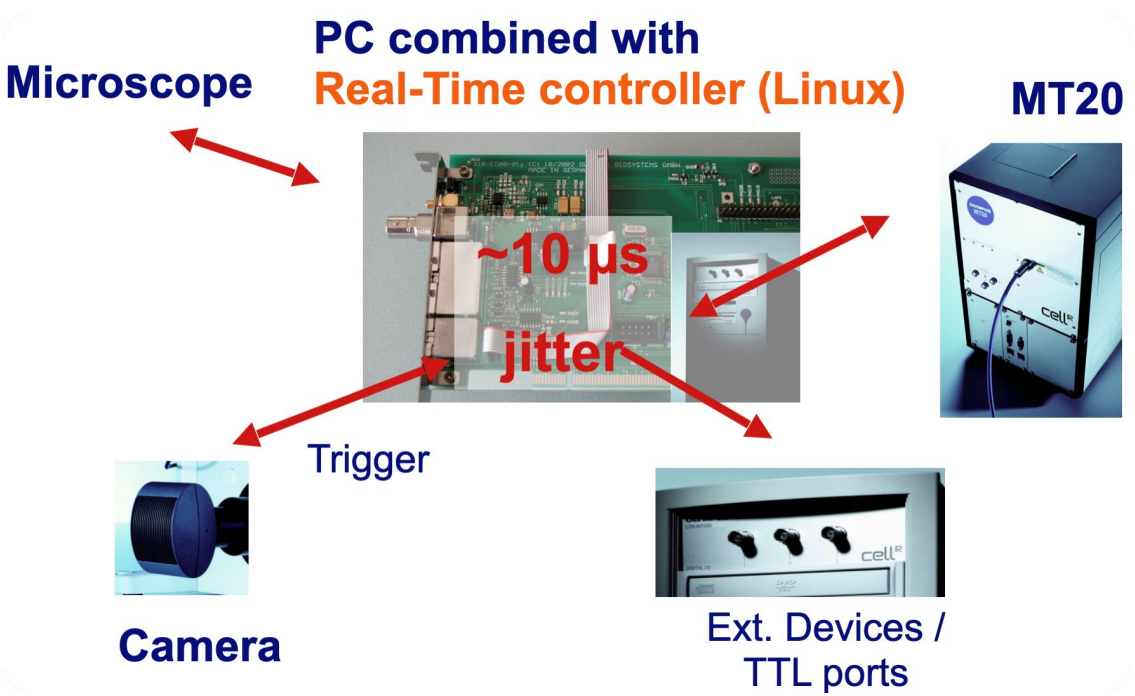


cell^R依照相設備
取最小激發範圍

硬體架構-Real-Time controller

元利儀器 YUAN LI INSTRUMENT CO., LTD.





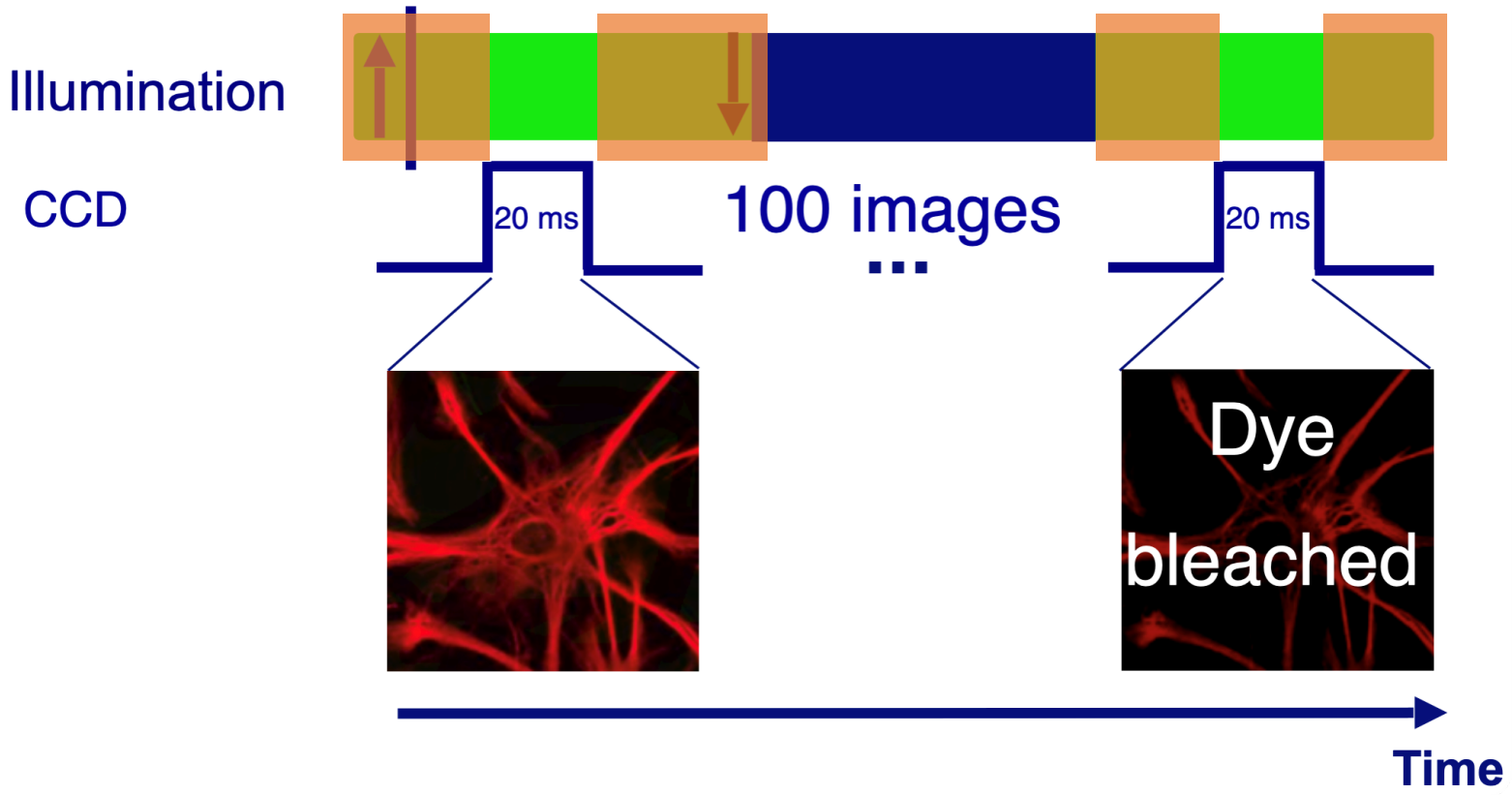
一般電腦架構採用序列式處理
造成額外等待時間(jitter time)

Linux架構採用平行式處理
可同步控制所有硬體

硬體架構-Real-Time controller

元利儀器
YUAN LI INSTRUMENT CO., LTD.

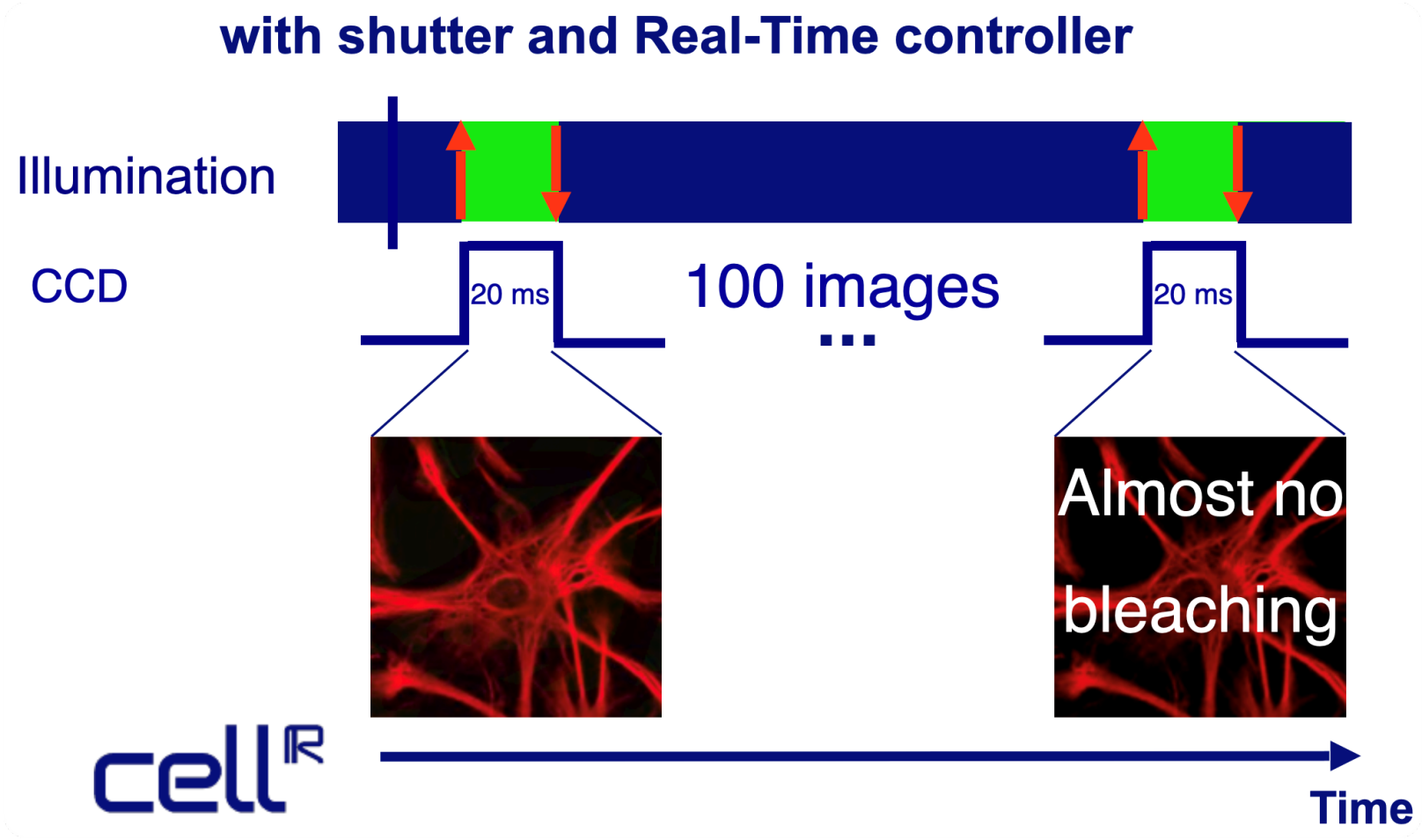
Typical Imaging System plus shutter:



多餘激發時間造成樣本褪色,活細胞死亡

硬體架構-Real-Time controller

元利儀器
YUAN LI INSTRUMENT CO., LTD.



cell^R系統精準控制激發時間,降低褪色與光毒性效應

大綱

元利儀器
YUAN LI INSTRUMENT CO., LTD.

硬體架構

軟體特色

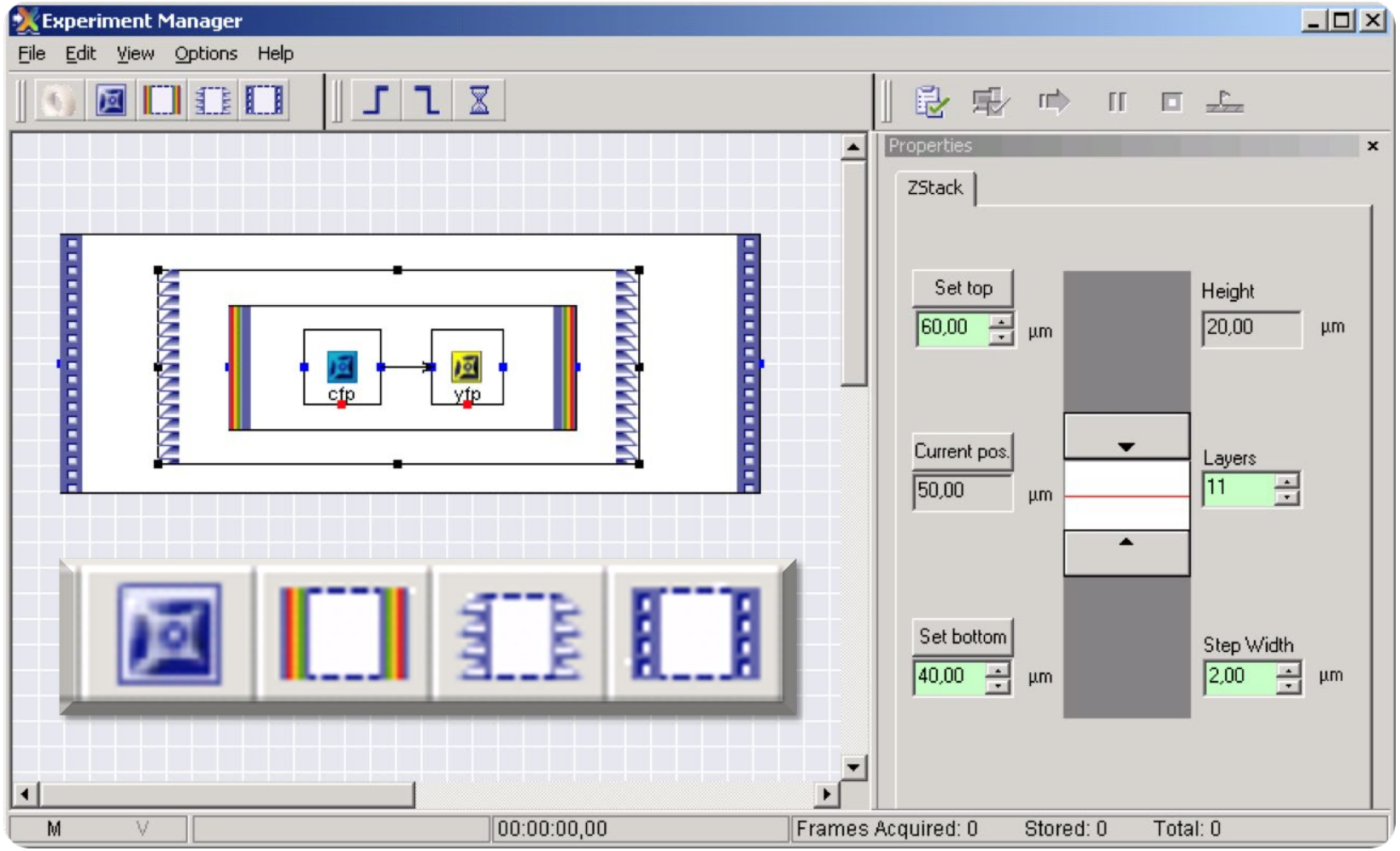
系統應用

樣本製備

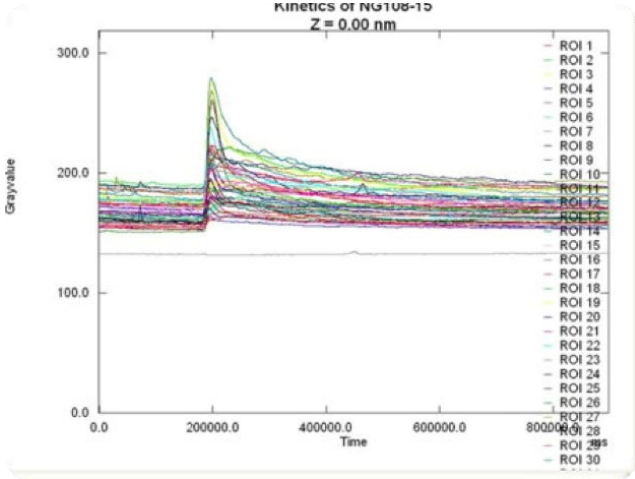
影像分析

軟體特色-Experiment manager

元利儀器 YUAN LI INSTRUMENT CO., LTD.

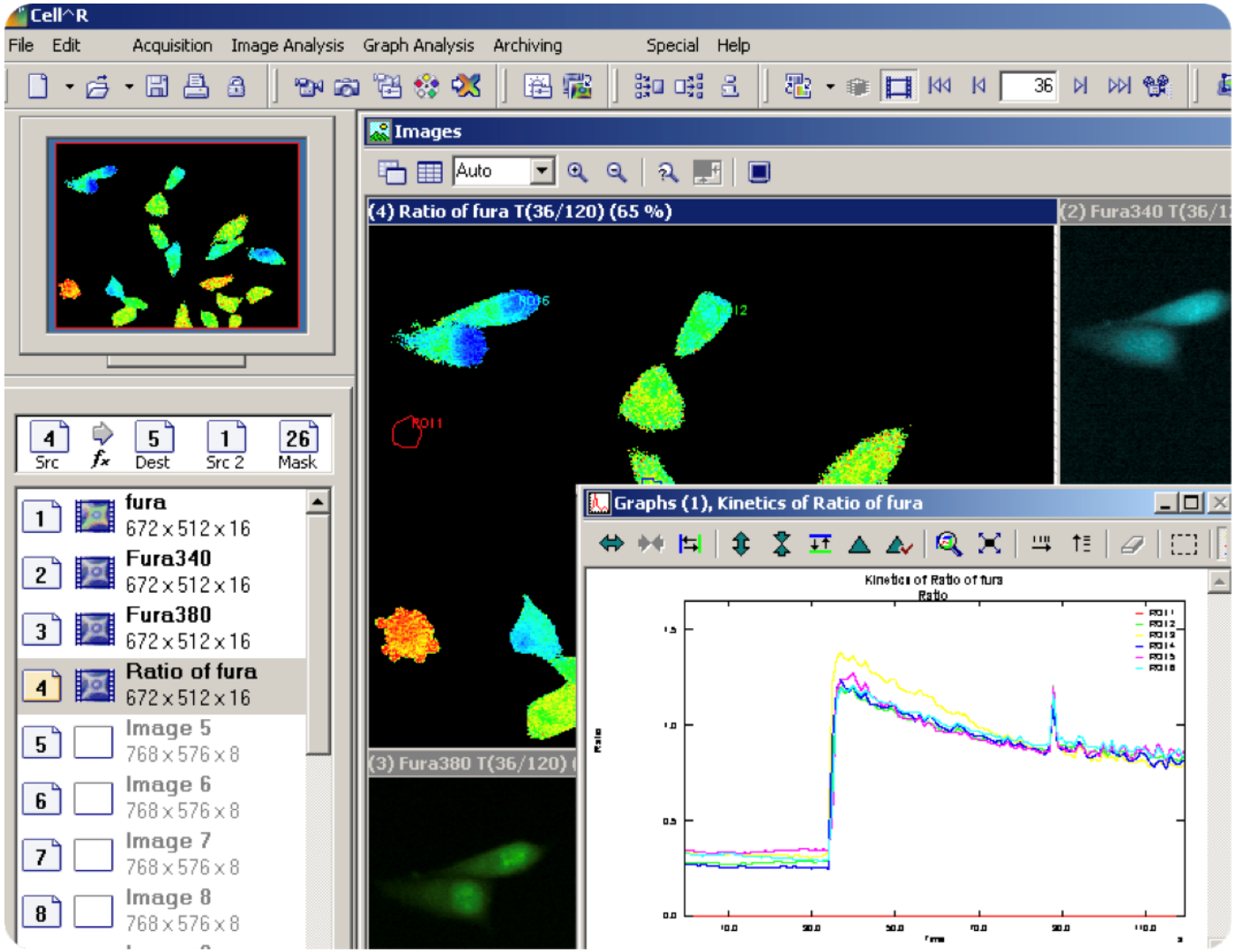


- 圖像式序列介面
- 輕鬆完成多維影像
- 支援複雜條件實驗
- 即時繪製螢光圖表



軟體特色-Database

元利儀器
YUAN LI INSTRUMENT CO., LTD.



- 創建自己的Database
- 圖像自動存檔,方便管理
- 輕鬆再現拍照條件

大綱

元利儀器
YUAN LI INSTRUMENT CO., LTD.

硬體架構

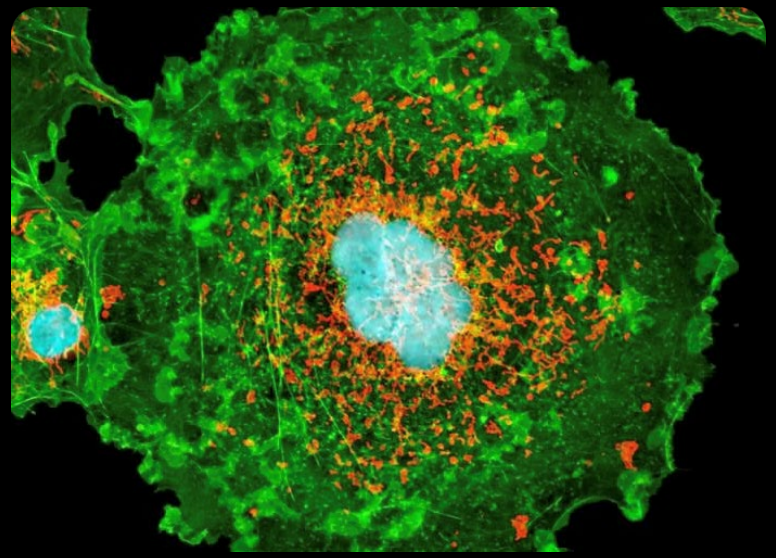
軟體特色

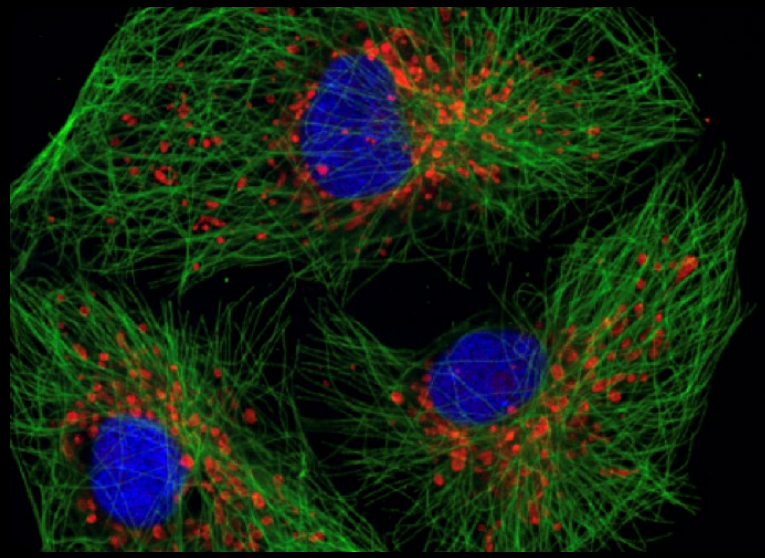
系統應用

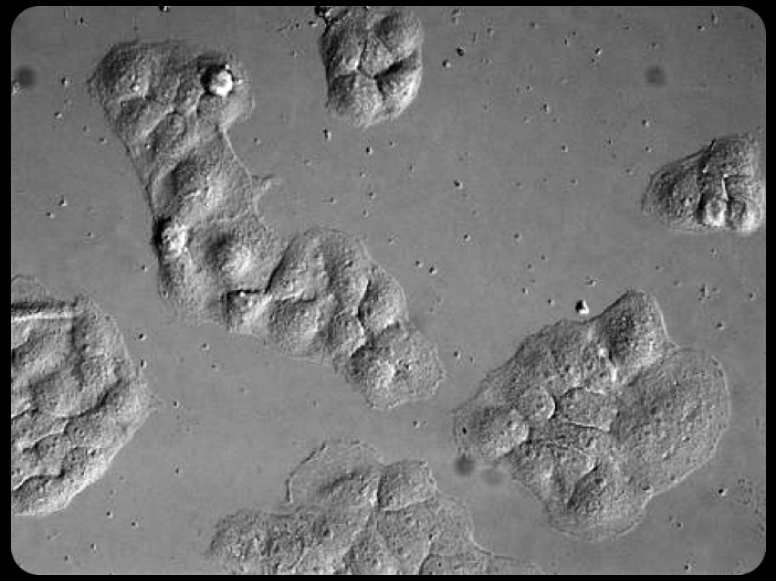
樣本製備

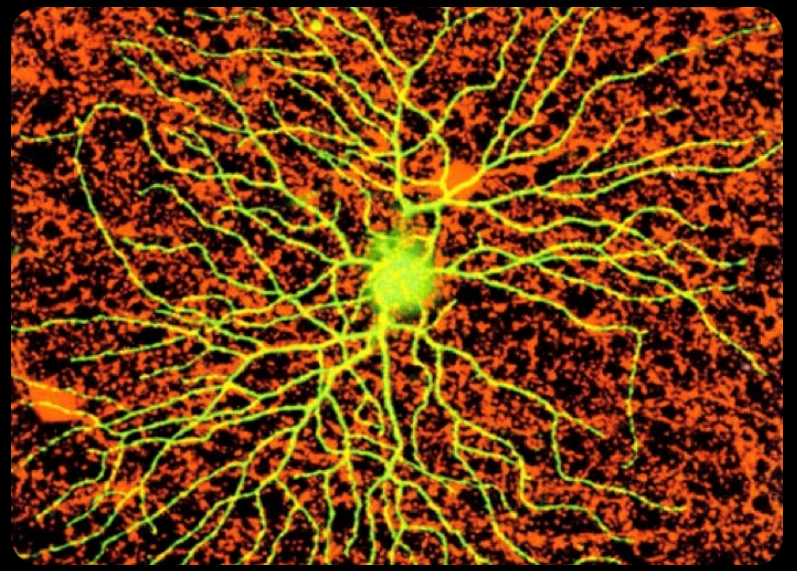
影像分析

系統應用-XY

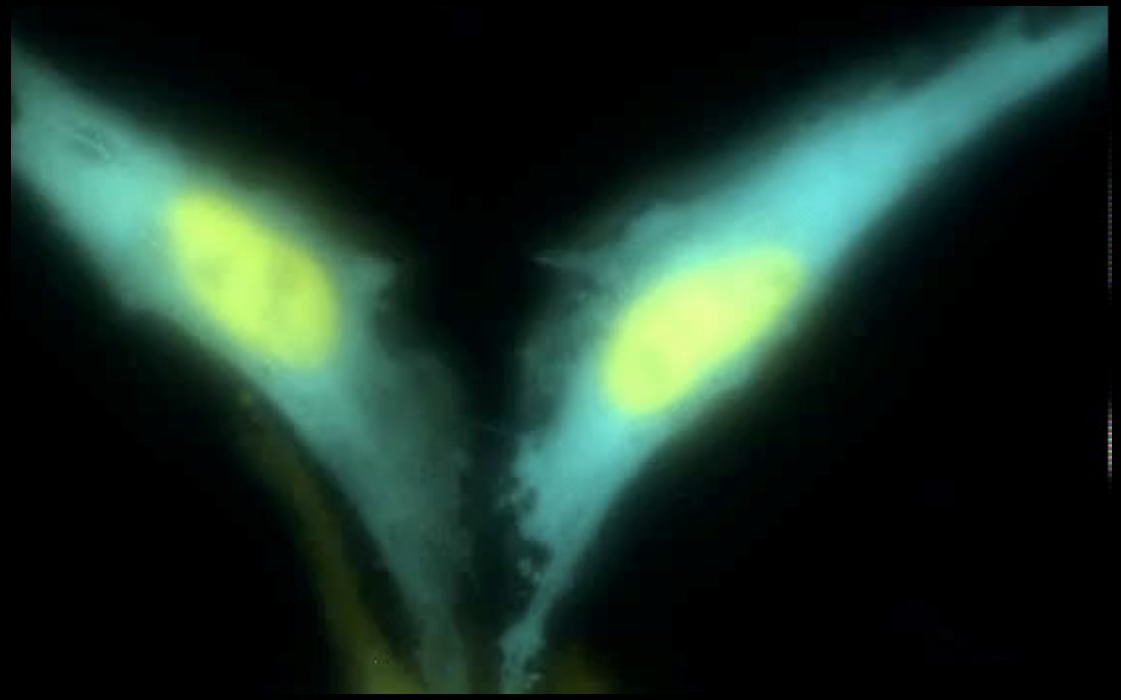




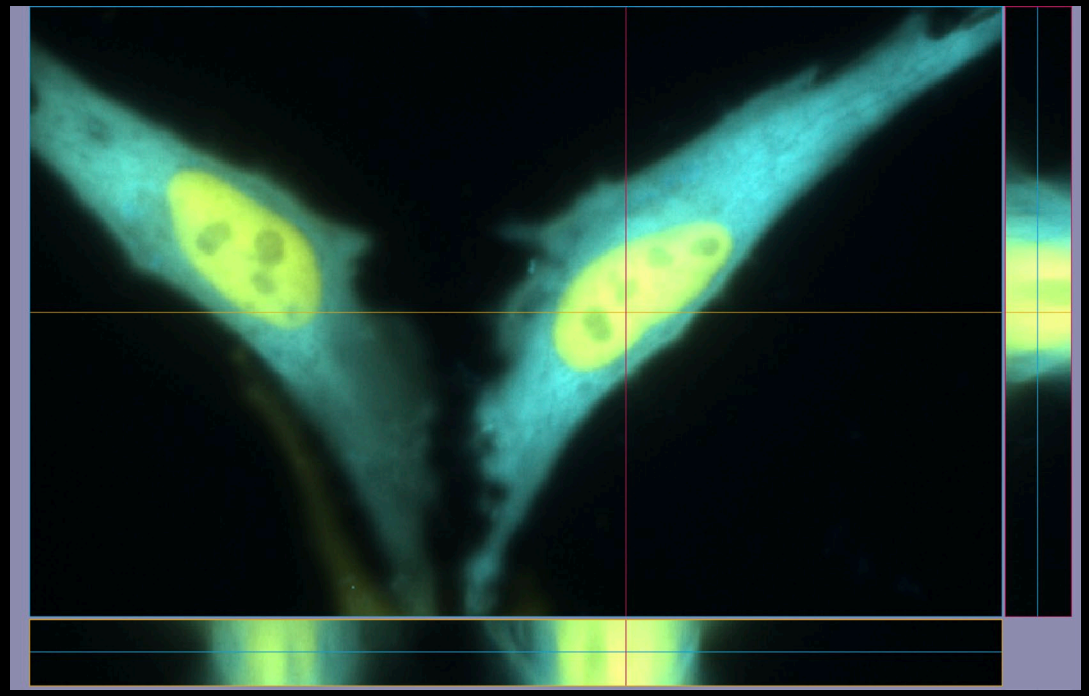




系統應用-XYZ

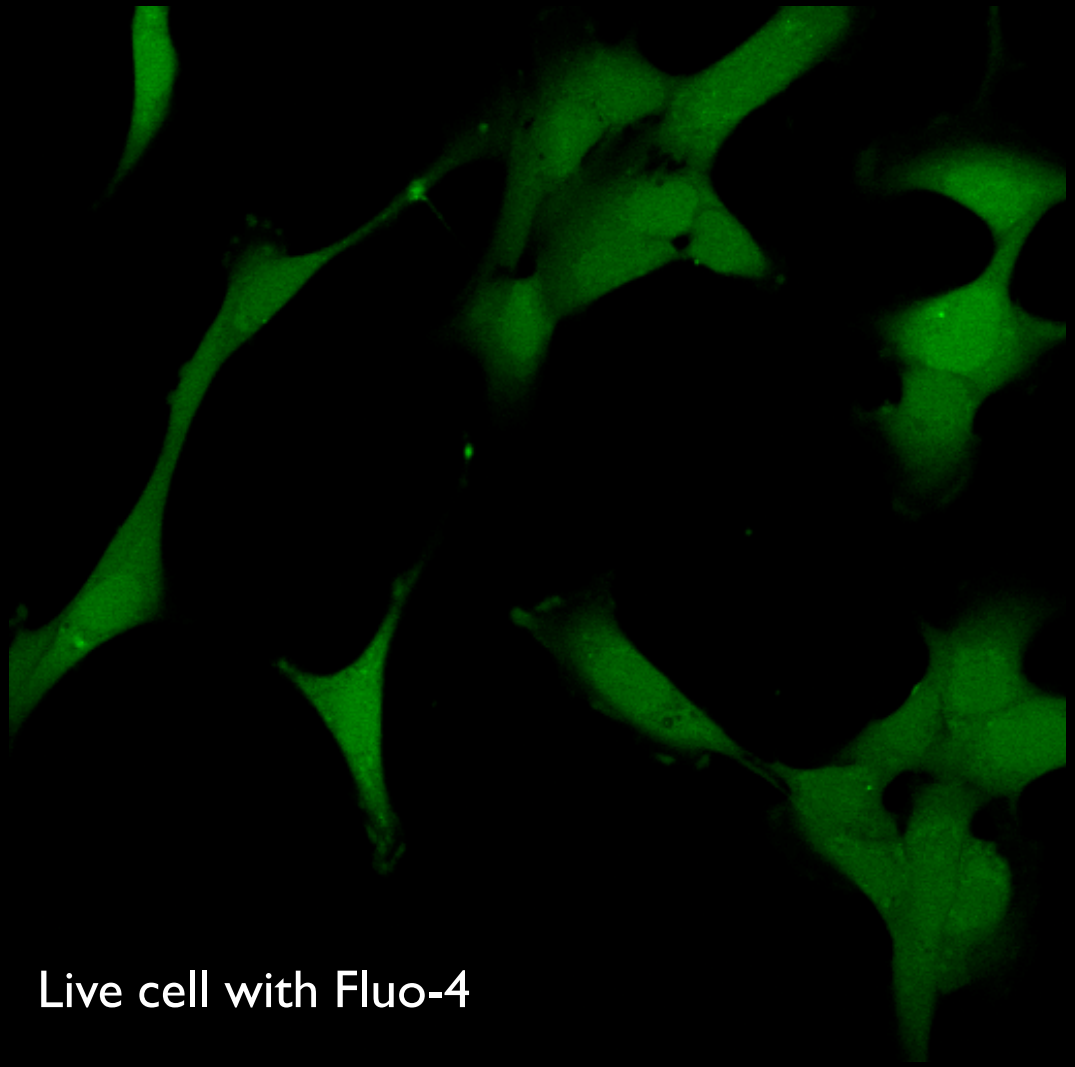


60X油鏡. 0.2um. 21 slices



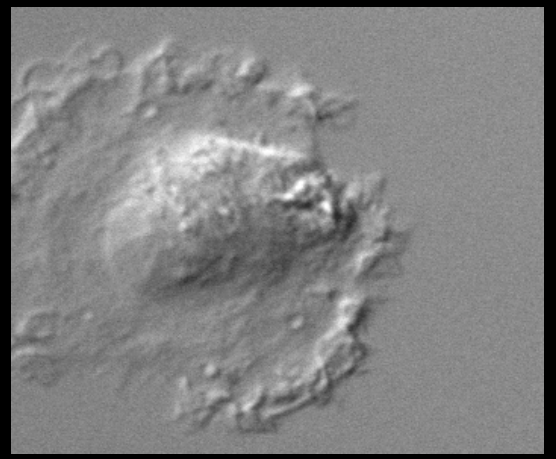
XY. XZ. YZ. 切面

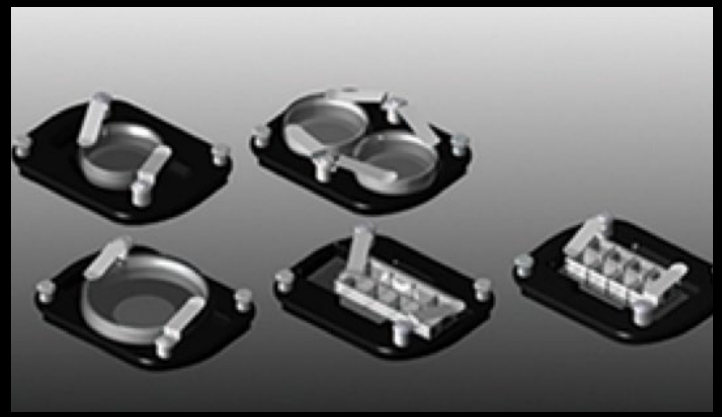
系統應用-XYT



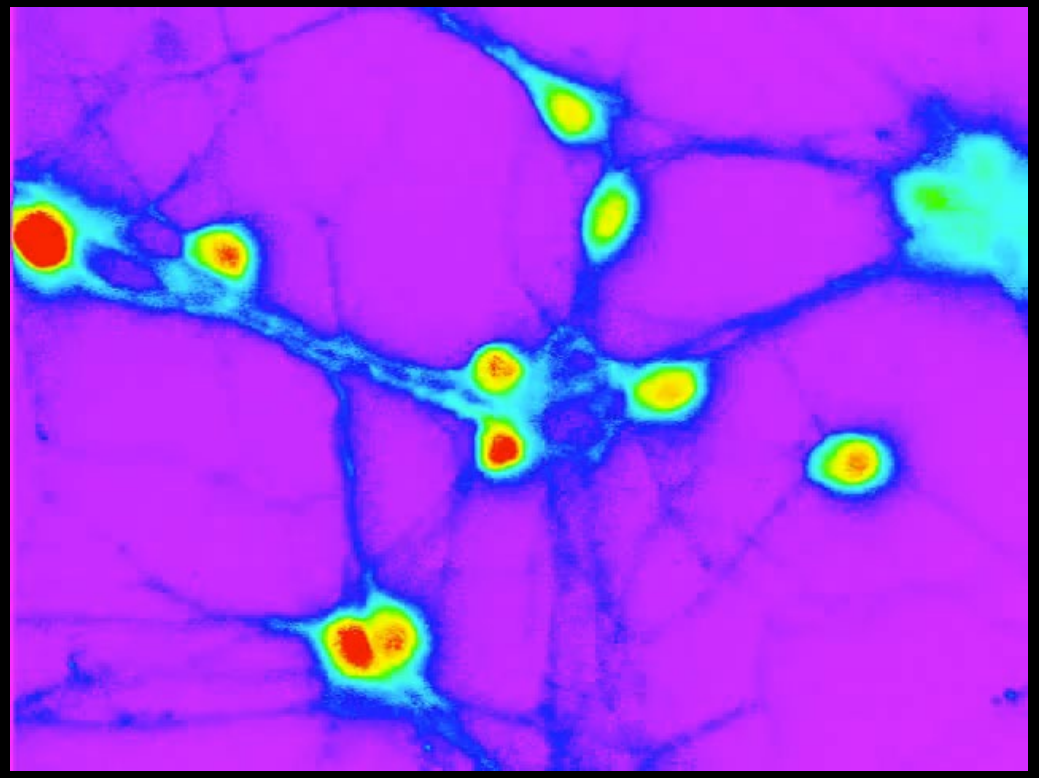
Live cell with Fluo-4

Live cell DIC video

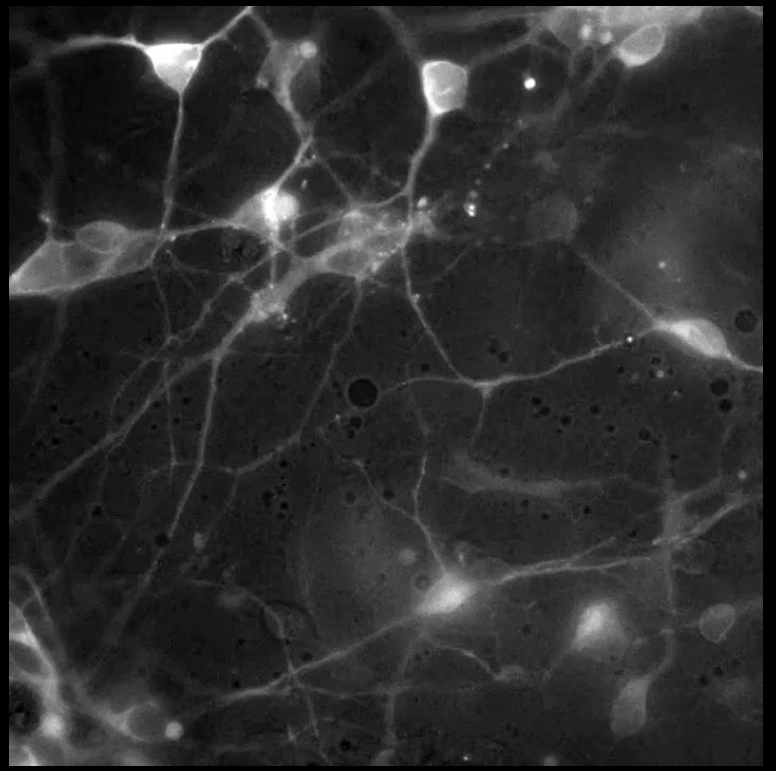




系統應用-高速鈣離子影像



Fura2 ratio video



GCaMP high speed video

大綱

元利儀器
YUAN LI INSTRUMENT CO., LTD.

硬體架構

軟體特色

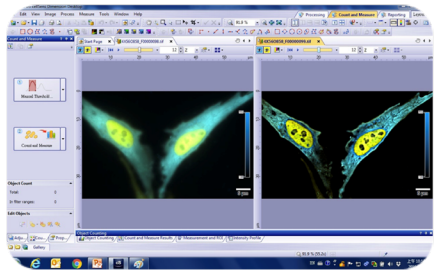
系統應用

樣本製備

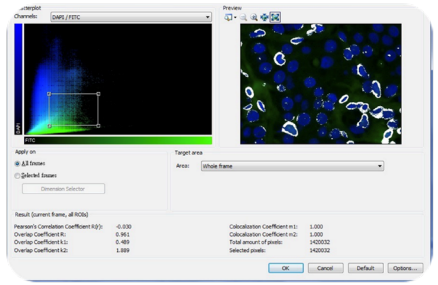
影像分析

影像分析-cellSens Dimension

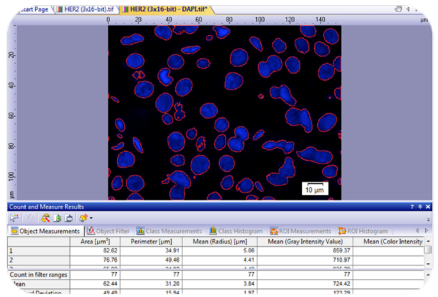
元利儀器
YUAN LI INSTRUMENT CO., LTD.



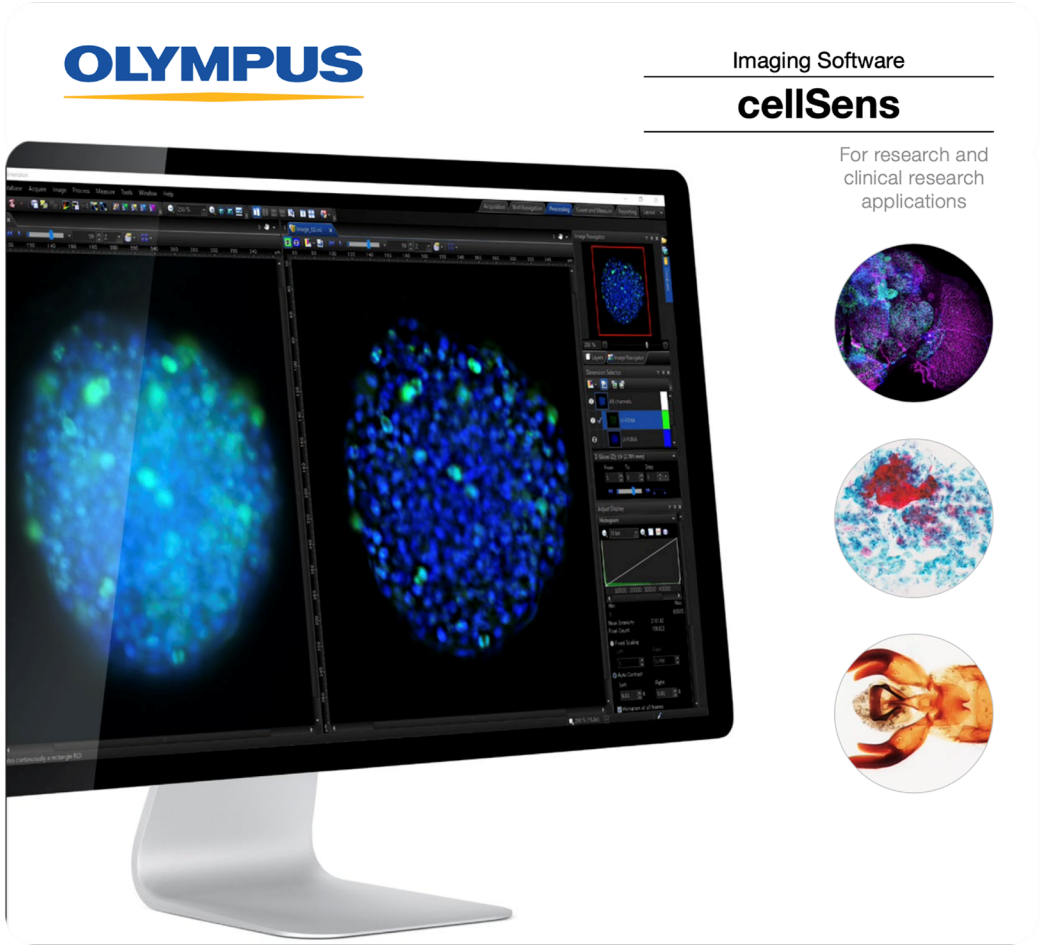
去離焦影像

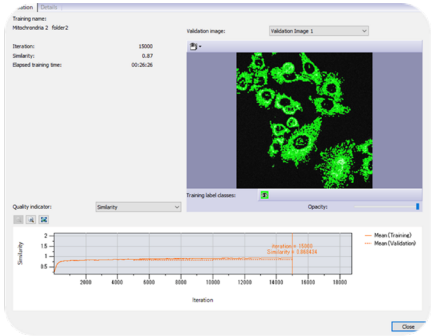


螢光共位分析

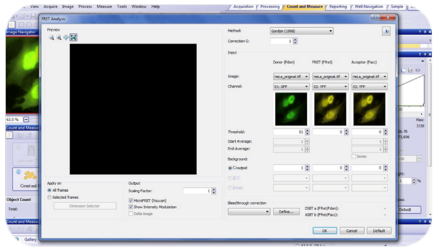


細胞自動計數

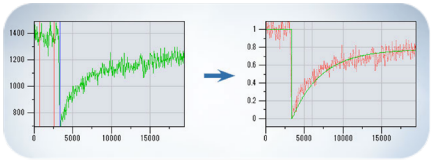




深度學習模組



FRET分析模組



FRAP分析模組

影像分析-自動計數

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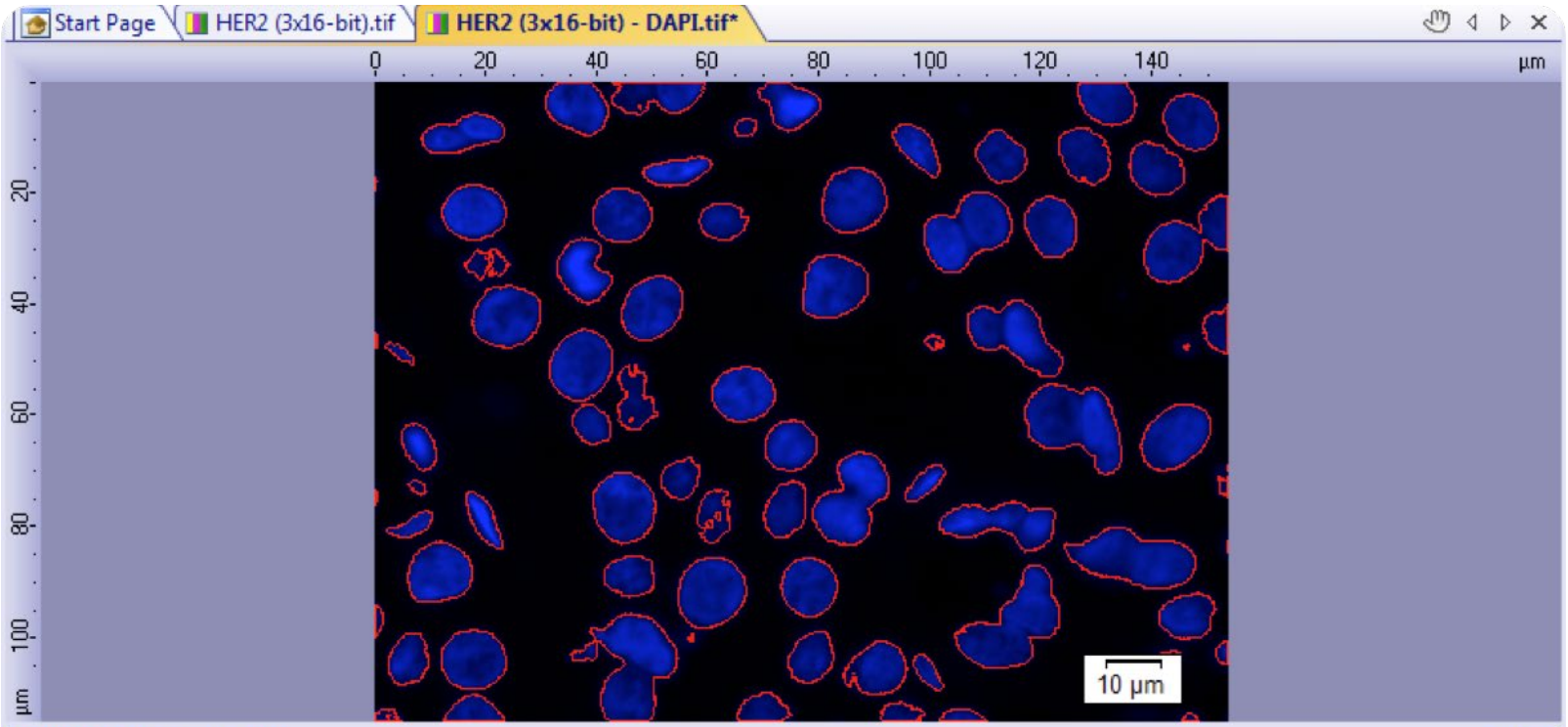


Count and Measure Results

Object Measurements | Object Filter | Class Measurements | Class Histogram | ROI Measurements | ROI Histogram

	Area [µm²]	Perimeter [µm]	Mean (Radius) [µm]	Mean (Gray Intensity Value)	Mean (Color Intensity
1	82.62	34.91	5.06	859.37	
2	76.76	49.46	4.41	710.97	
Count in filter ranges	77	77	77	77	
Mean	62.44	31.20	3.84	724.42	
Standard Deviation	49.49	15.94	1.97	123.29	

- 自動計數模組

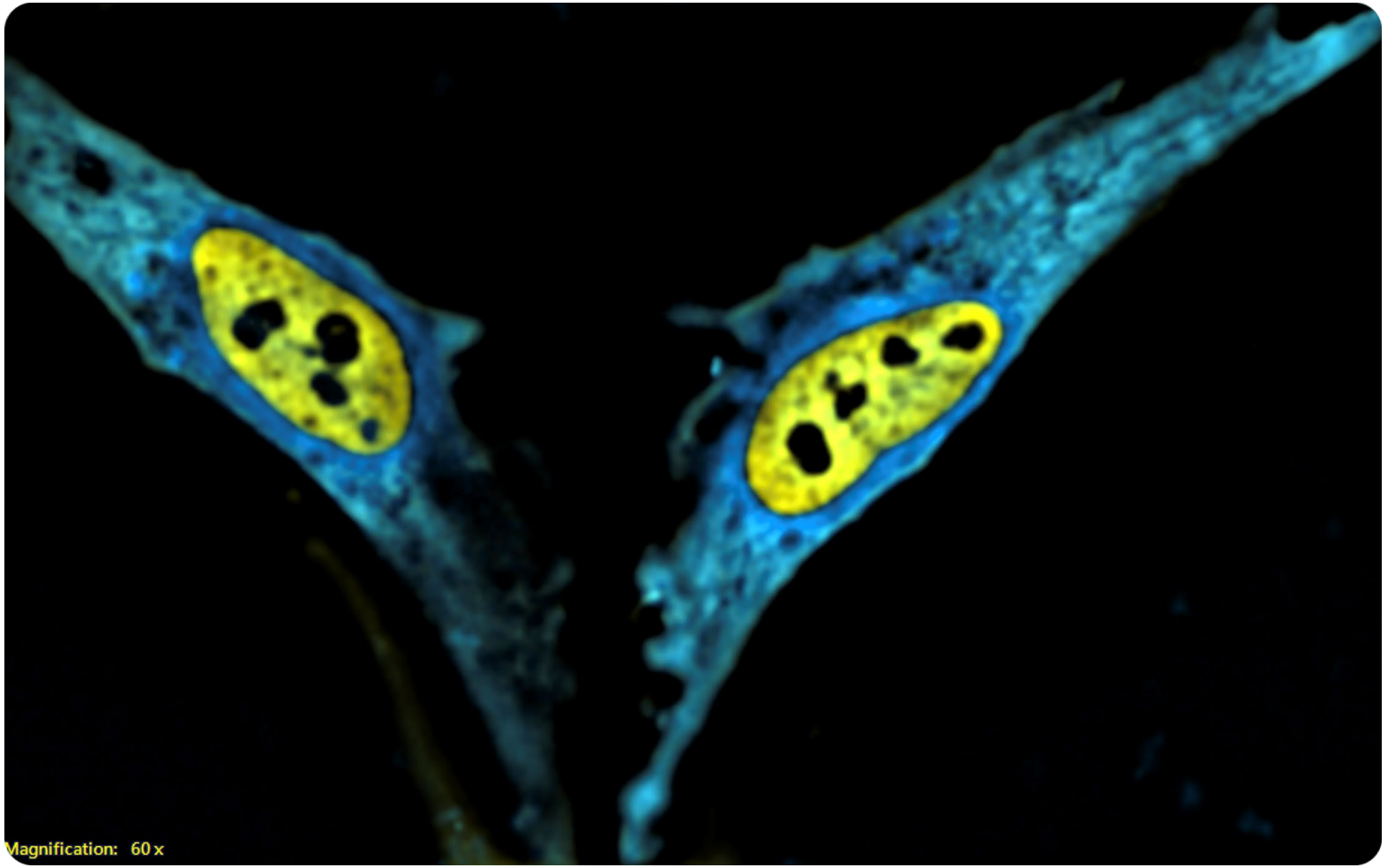
-細胞/核/胞器計數

-面積/周長/螢光強度

-圓度/群組分類...etc

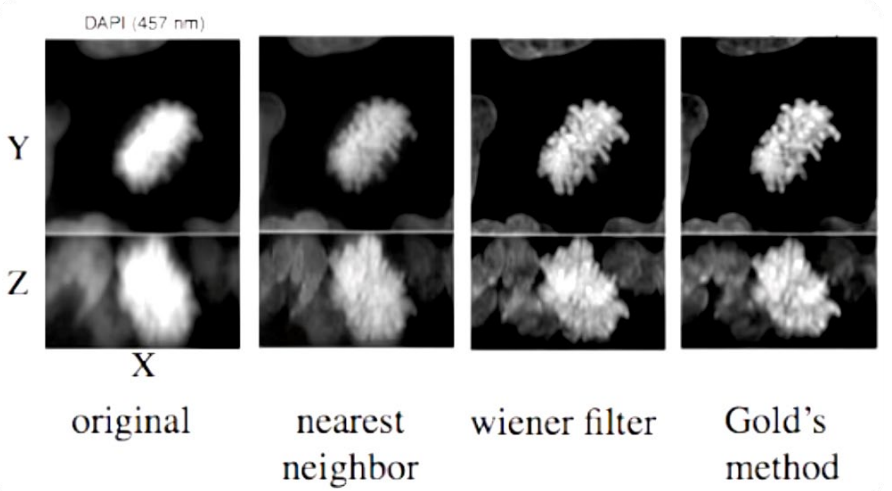
影像分析-去離焦模組

元利儀器
YUAN LI INSTRUMENT CO., LTD.



- Deconvolution

提供多種演算法

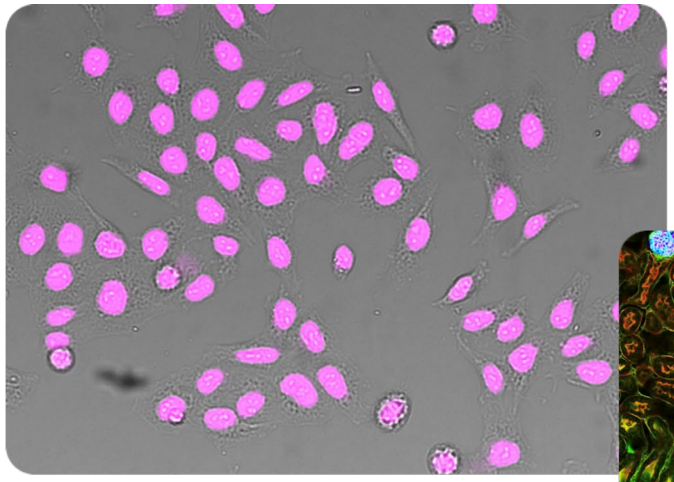


影像分析-AI深度學習

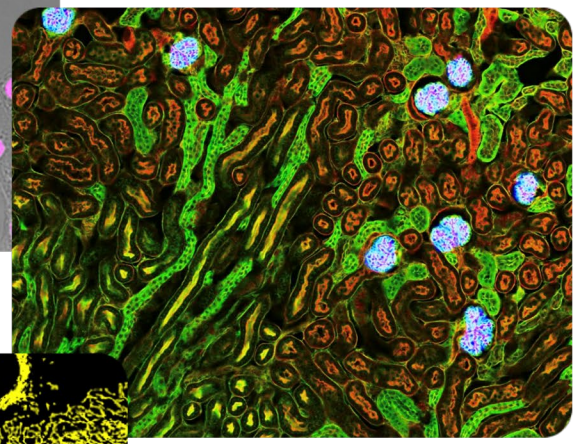
元利儀器
YUAN LI INSTRUMENT CO., LTD.

TruAI

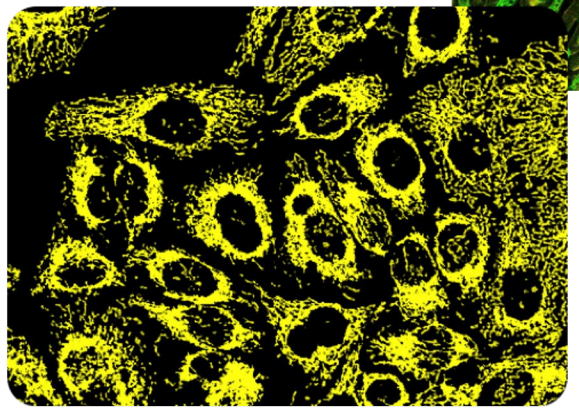
深度學習技術
實現更為快速精確的分析



免標定核辨識

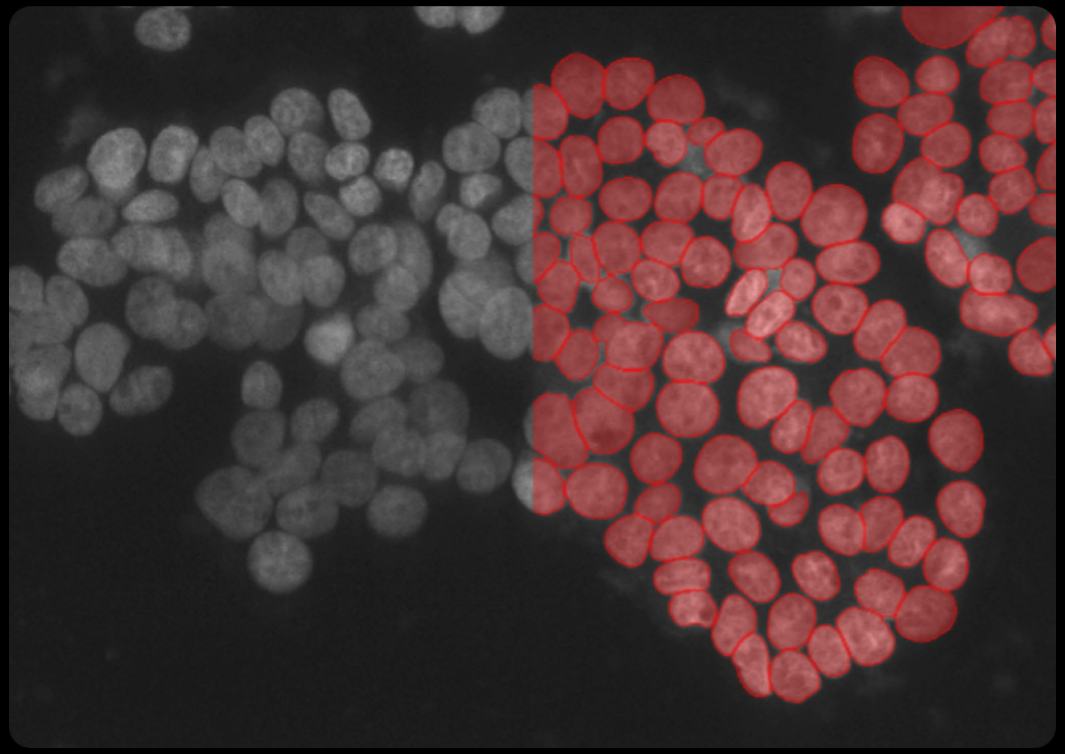


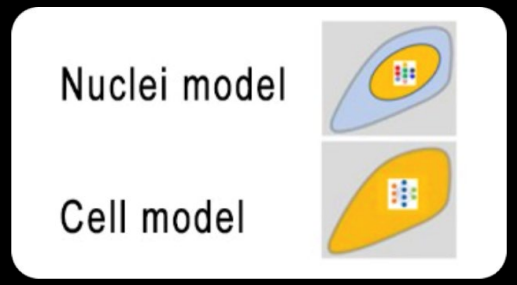
腎絲球判定

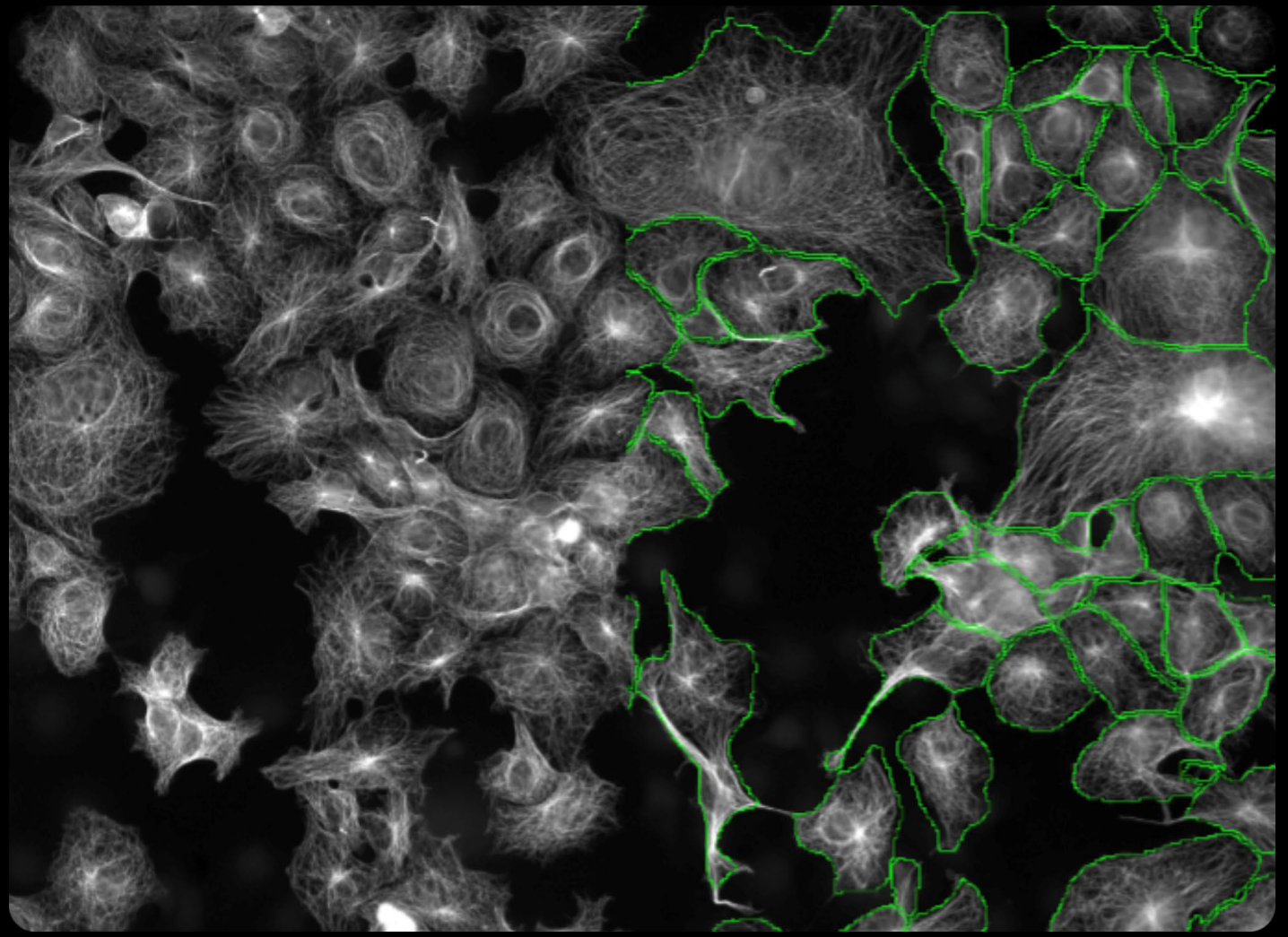


粒線體分析

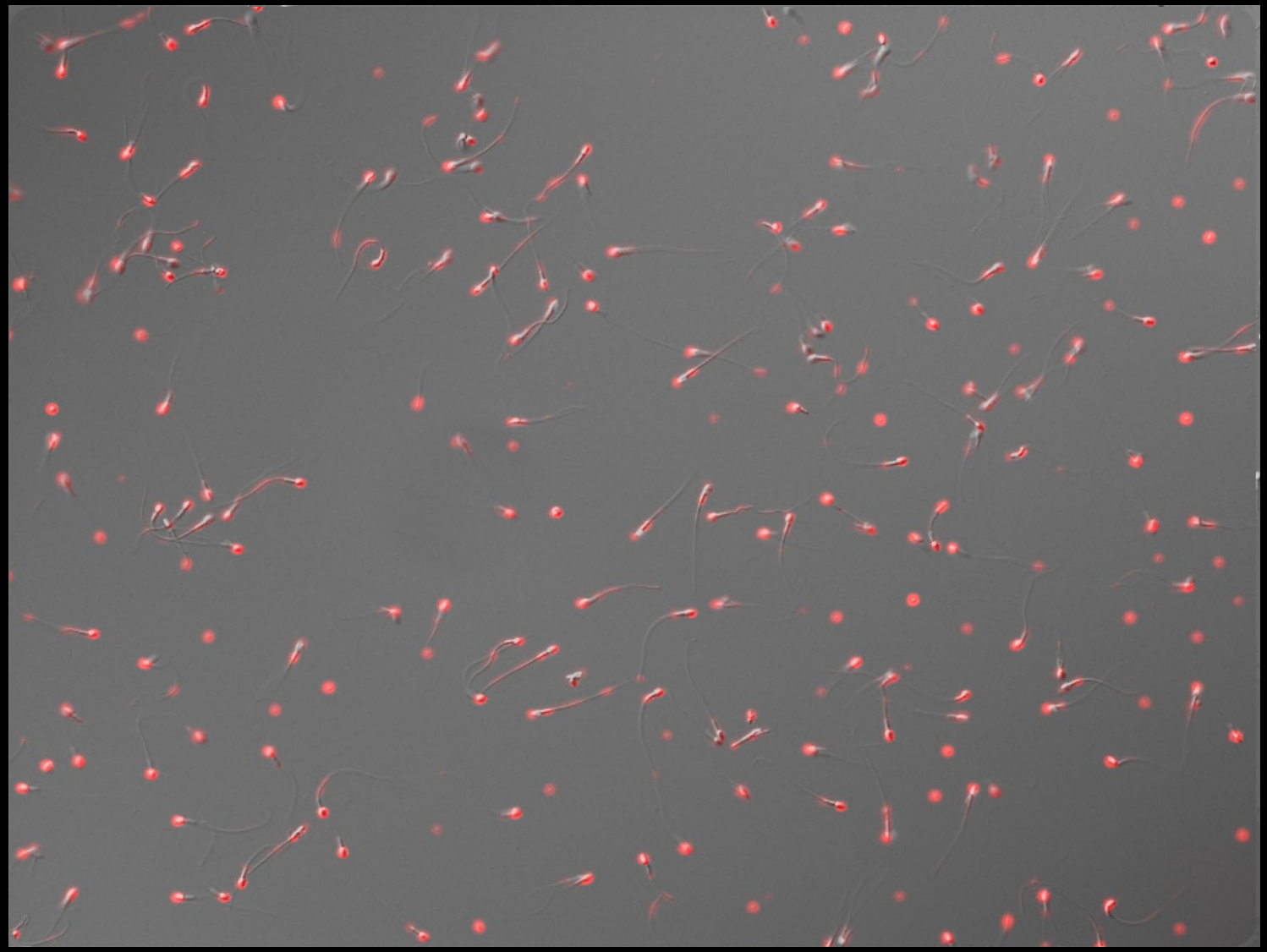
影像分析-AI深度學習







影像分析-AI深度學習實例



總結

元利儀器
YUAN LI INSTRUMENT CO., LTD.

- 活細胞螢光, 易褪色樣本, 長時間觀察最適用系統
- 完整倍率, 10X~100X
- DAPI, GFP, RFP, Fura2 Experiment manager, database
- 可利用最新的**cellSens軟體**,加速分析效率

Thank You

元利儀器
YUAN LI INSTRUMENT CO., LTD.

50 YEARS SINCE 1970

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