



Nikon C2⁺ Confocal System

教育訓練

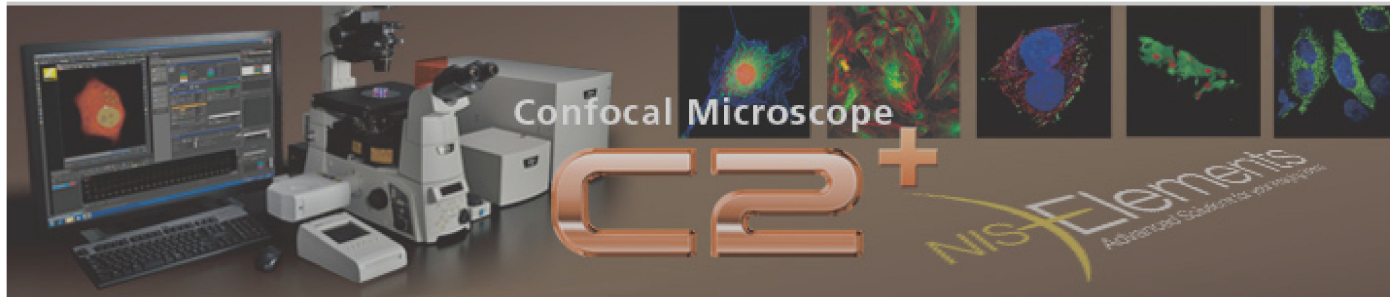


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SCIENTIFIC INSTRUMENTS
科學儀器部門

謝欣倫
2014.12.22

Basic Concepts of Confocal Microscopy



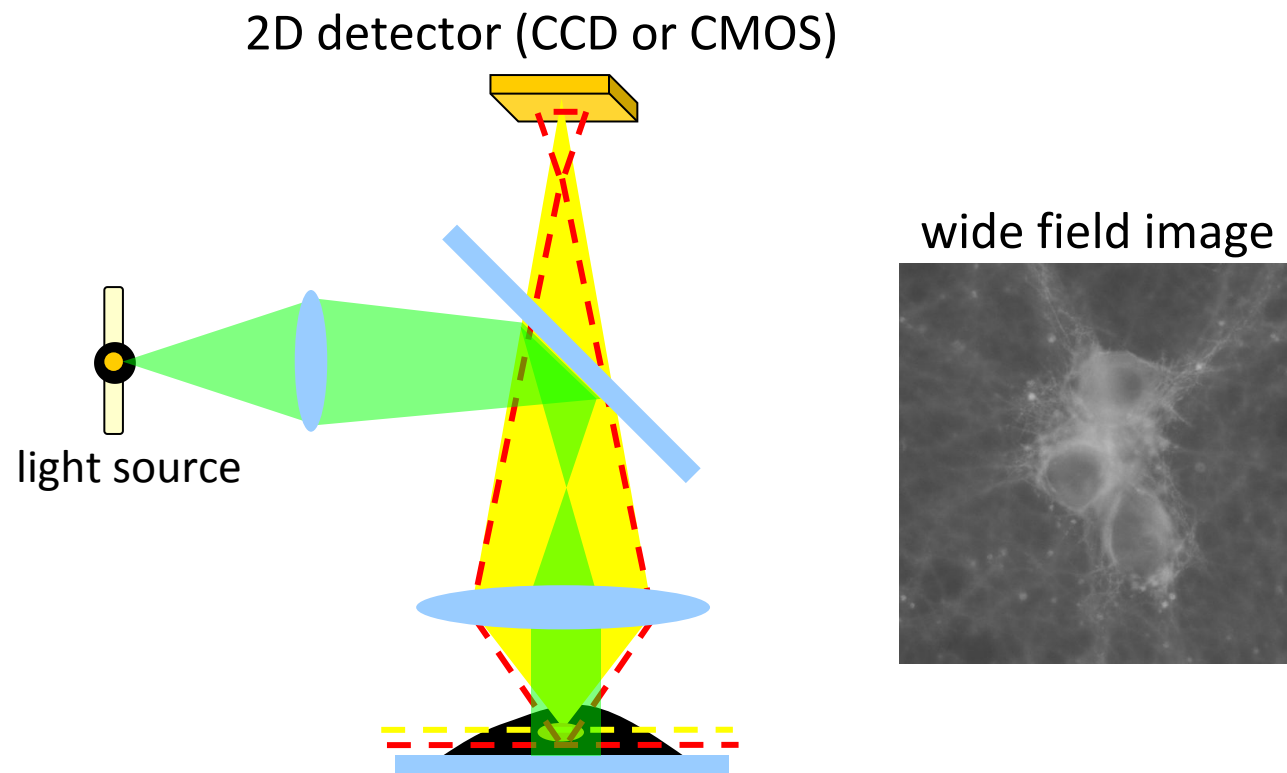


Advantages of Confocal Microscopy

	Fluorescence Microscopy	Confocal Microscopy
Image quality	Low SNR	High SNR
Magnification	Changed by optics	Variable by scan zoom
Brightness	Change exposure time	Variable by PMT gain
Multi-color	Slow multiple	Fast Multiple



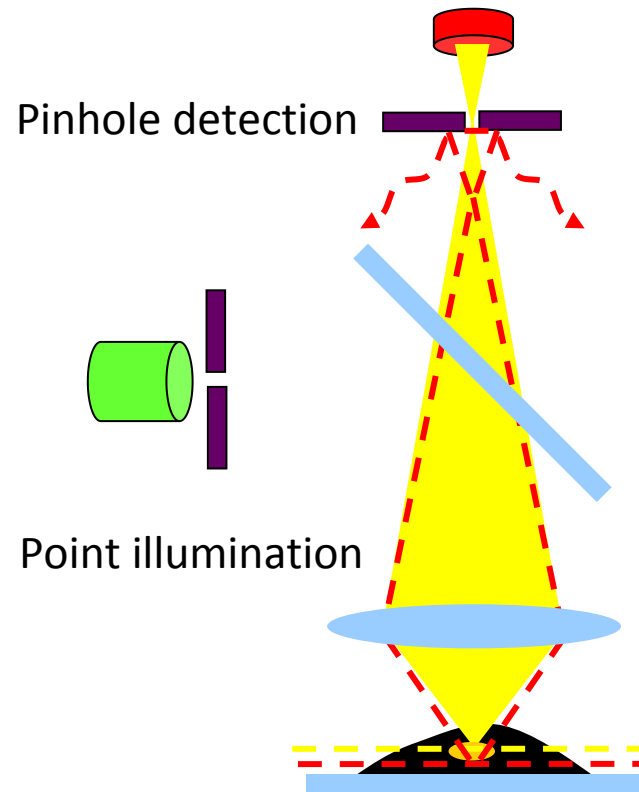
Widefield Fluorescence Microscopy





Confocal Microscope

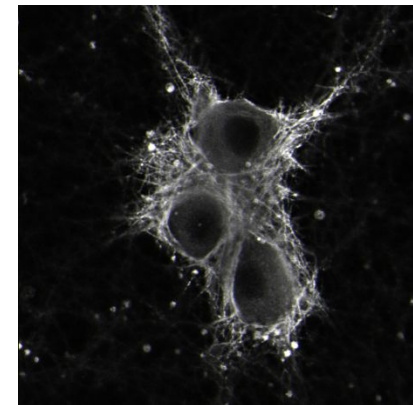
Confocal Microscopy relies on the presence of apertures, called **pinholes** in the light path that restrict emission light from non-focal planes



Optical section

Eliminate out-of-focus light

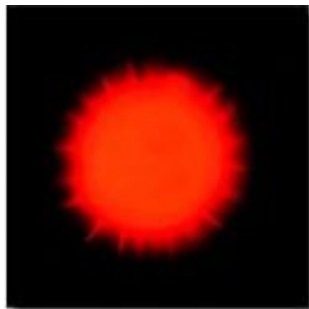
Confocal image



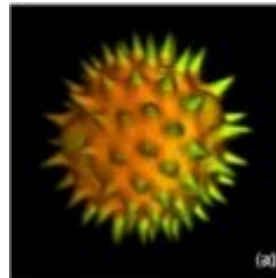
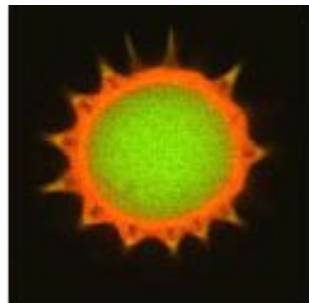


3D Reconstruction of Confocal Microscopy

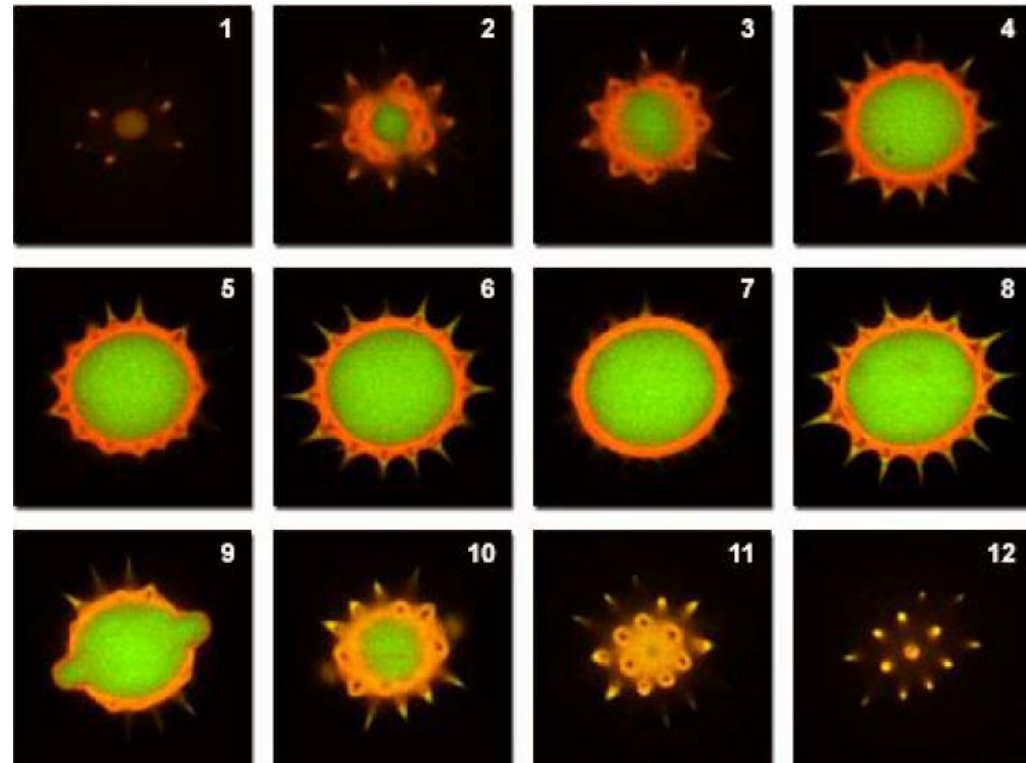
Widefield Fluorescence
Microscopy



Confocal
Microscopy



3D Reconstruction
with z-stack





Advantages of Confocal Microscopy

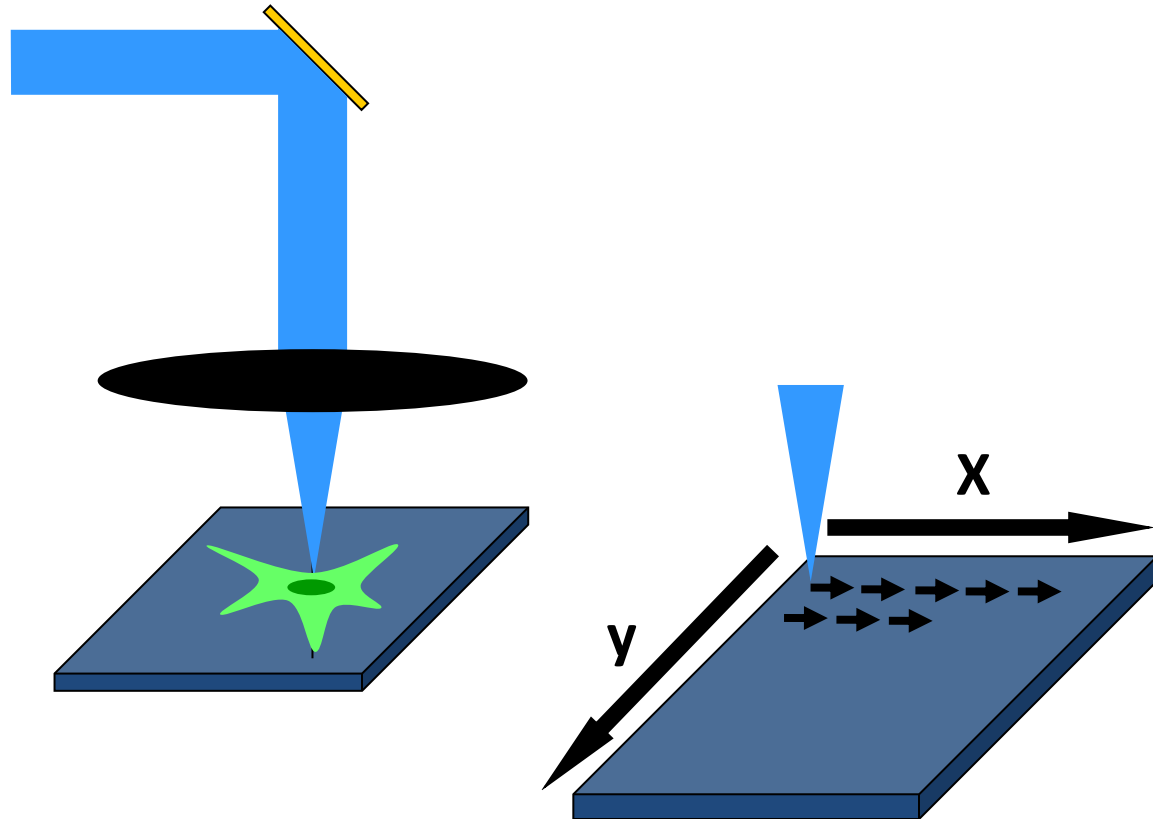
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Extremely High quality image

Image acquired **ONLY** from focusing plane ---> High S/N Ratio Image

Point illumination
w/ laser beam



x-y scanning generated
2D image

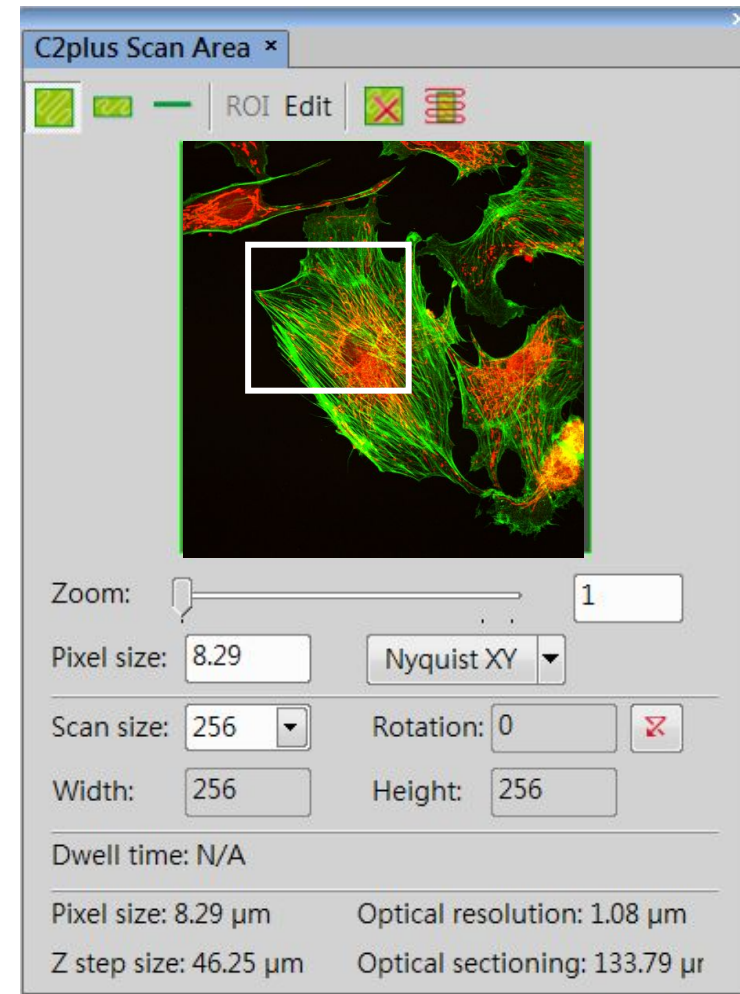
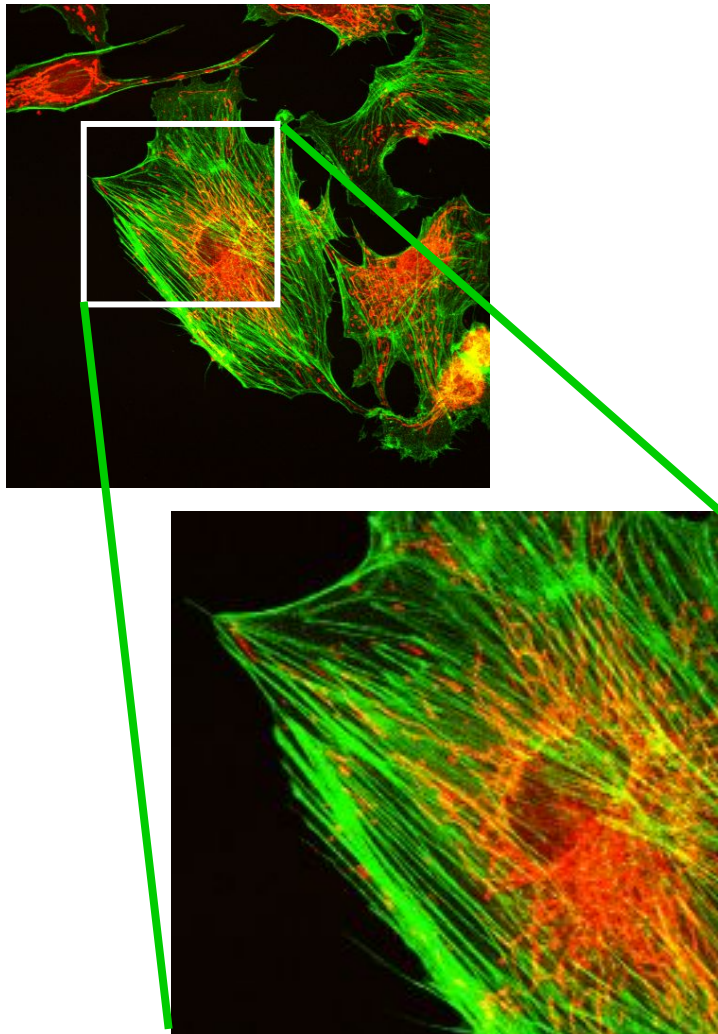


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Zooming just by changing scanning area





Advantages of Confocal Microscopy

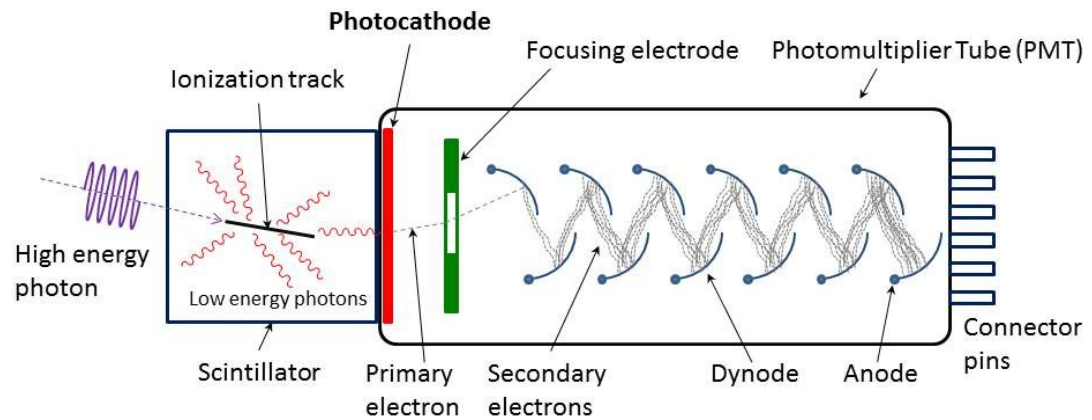
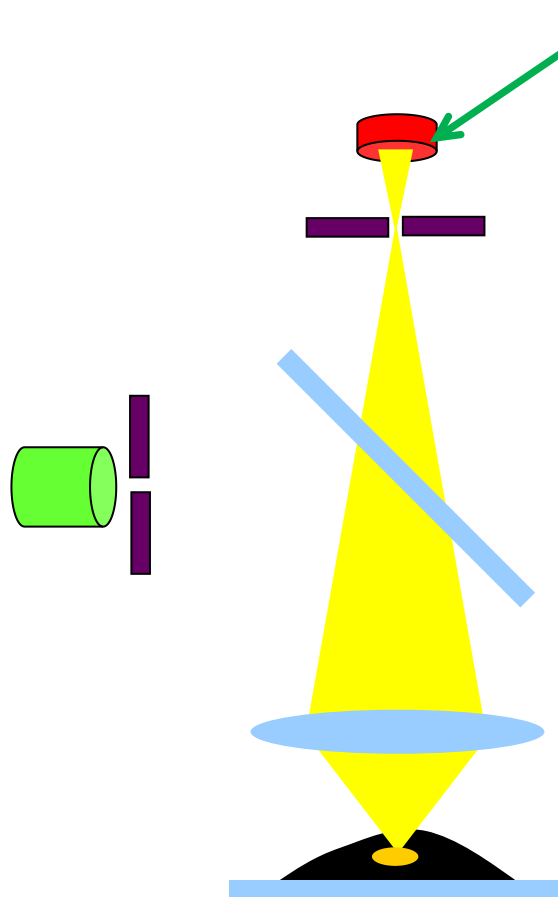
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Variable of PMT gain

Photo Multiplier Tube:

Signal amplification can be changed by changing High Voltage of the PMT



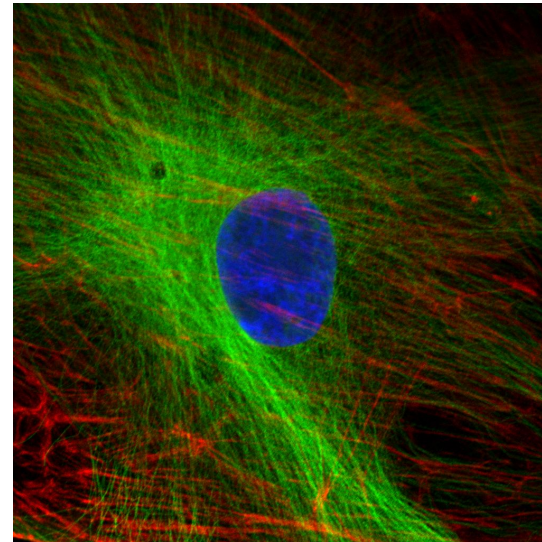
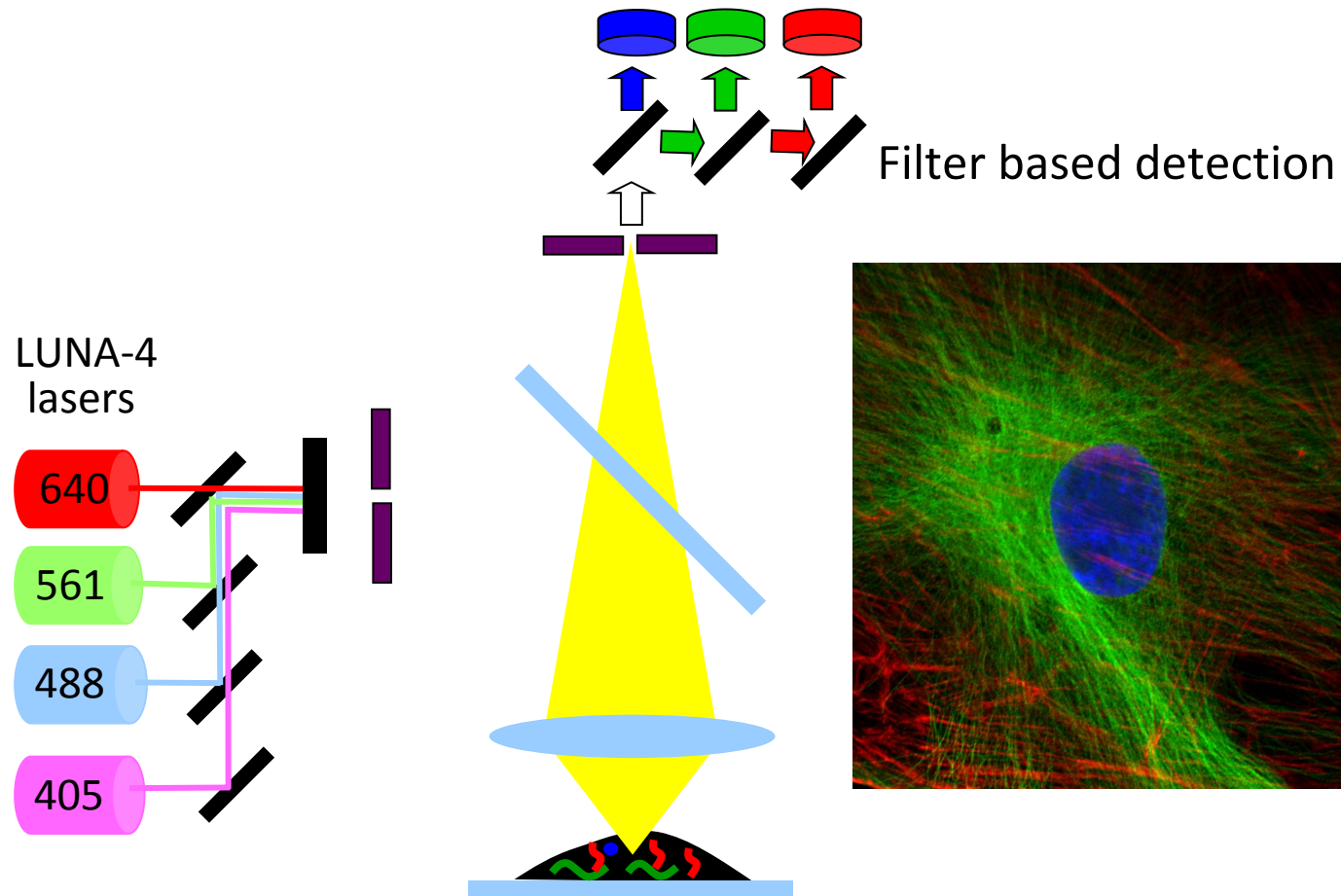


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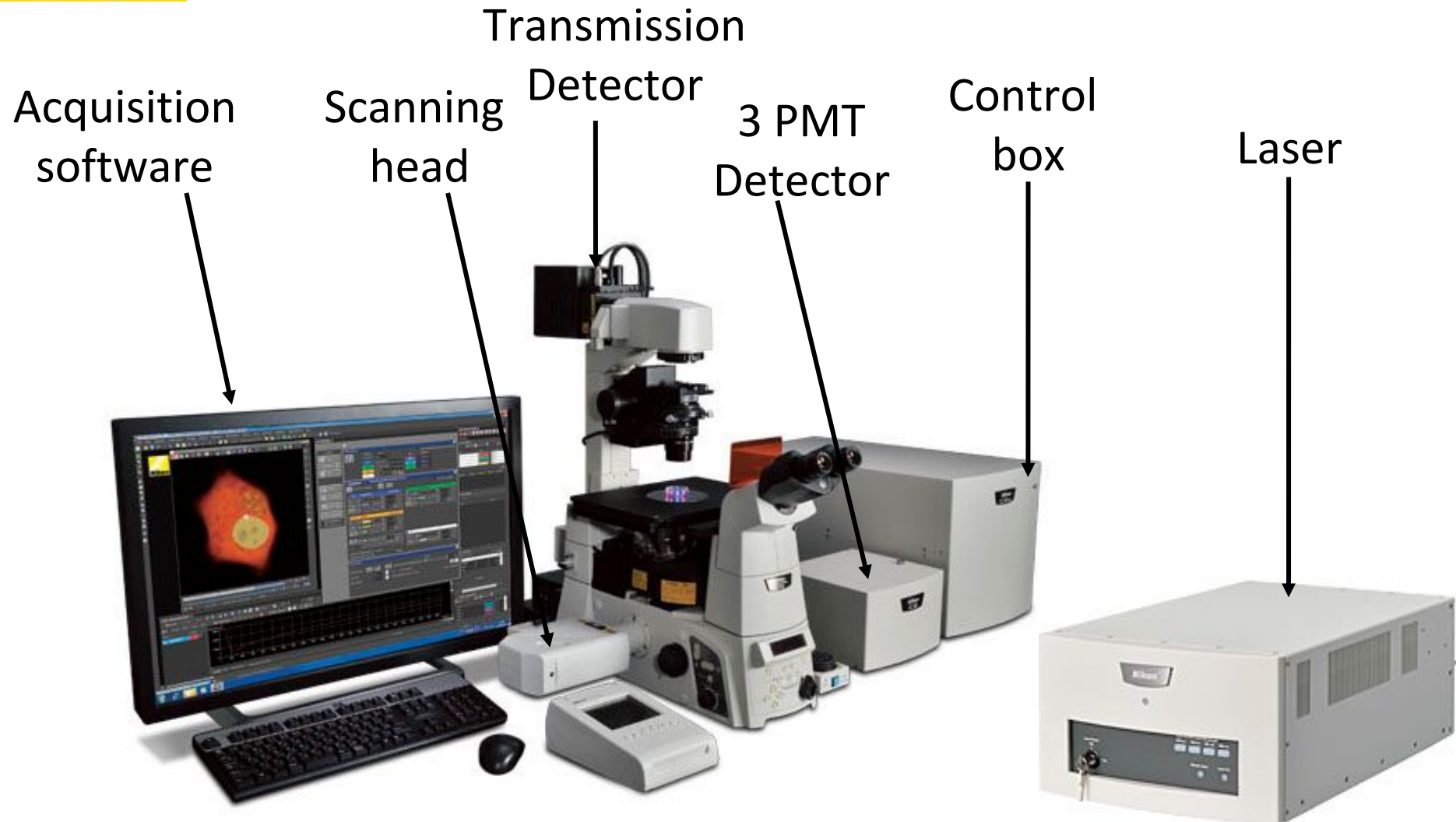


Multi-staining images you can get

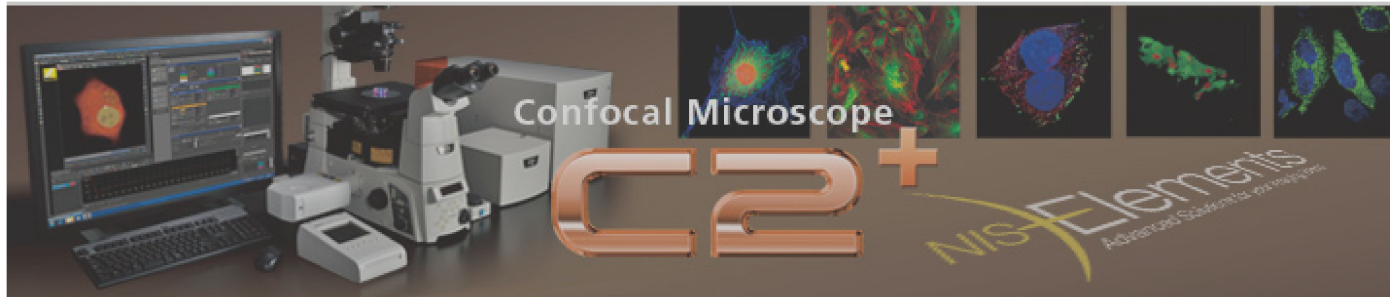




C2+ product concept



The Concepts of C2+ Confocal System





C2+ product concept

	規格	備註
DETECTOR	3 PMT Epi 1 PMT TD	可同時接收三組螢光影像及 一組可見光DIC影像
LASER	405 /488 /561/640	固態雷射光源，隨開即用，穩定
RESOLUTION	2048*2048	依目的調整影像解析
ZOOM	1x - 1,000x	軟體設有最佳建議Zoom數值
SPEED	8 fps (512*512) 100 fps (512*32)	標準掃描模式 快速掃描模式
AUTO GAIN	Auto Gain	軟體自動搜尋最佳拍攝條件
AUTO INTENSITY Z	Z intensity control	可隨樣品深度調整雷射能量強度
PINHOLE	六段式調整	圓形設計可擷取最多焦距面訊號
SOFTWARE FUNCTION	1. 5D影像擷取(X,Y,Z,T, Multi-channel) 2. 3D立體影像重組, 多重模式顯示 3. 螢光共位分析(Co-localization)	



Light Source - LUN-4 Laser Module

- LD 405nm, 15mW
- LD 488nm, 15mW
- DPSS 561nm, 15mW
- LD 640nm, 15mW

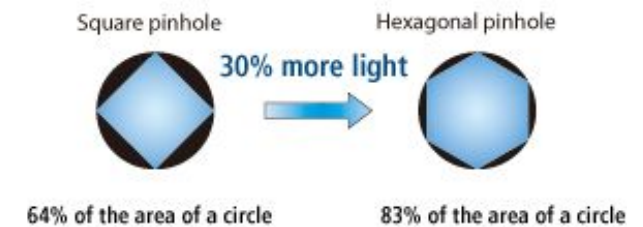
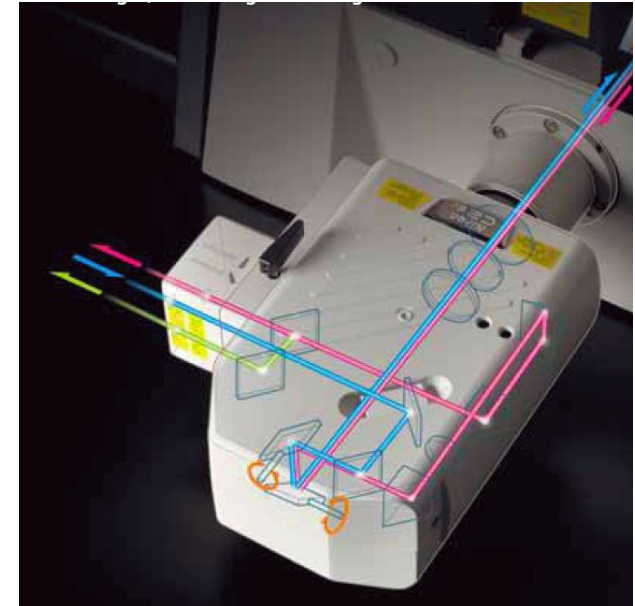


- 優點：
 - 固態雷射光源，隨開即用，穩定
 - 具備AOTF，可由軟體調控雷射波長及輸出能量，並作高速開關，降低樣品褪色的機會
 - 免校正！降低維護成本以及系統停擺的損失



C2+ Scanner

- 2048 x 2048 max.
- 8 fps@512x512
- 100 fps@512 x 32
- 圓形Pinhole設計，使收光效率最大化





Detector - PMT

- 3 PMT for Fluorescence by standard
- 1 PMT for Transmission light
- 可同時接收三組螢光訊號與一組穿透光訊號
- (DAPI, FITC, TRITC, Transmitted light)
- 利用多次掃描的方式可以收到4色螢光



Tips for good confocal images



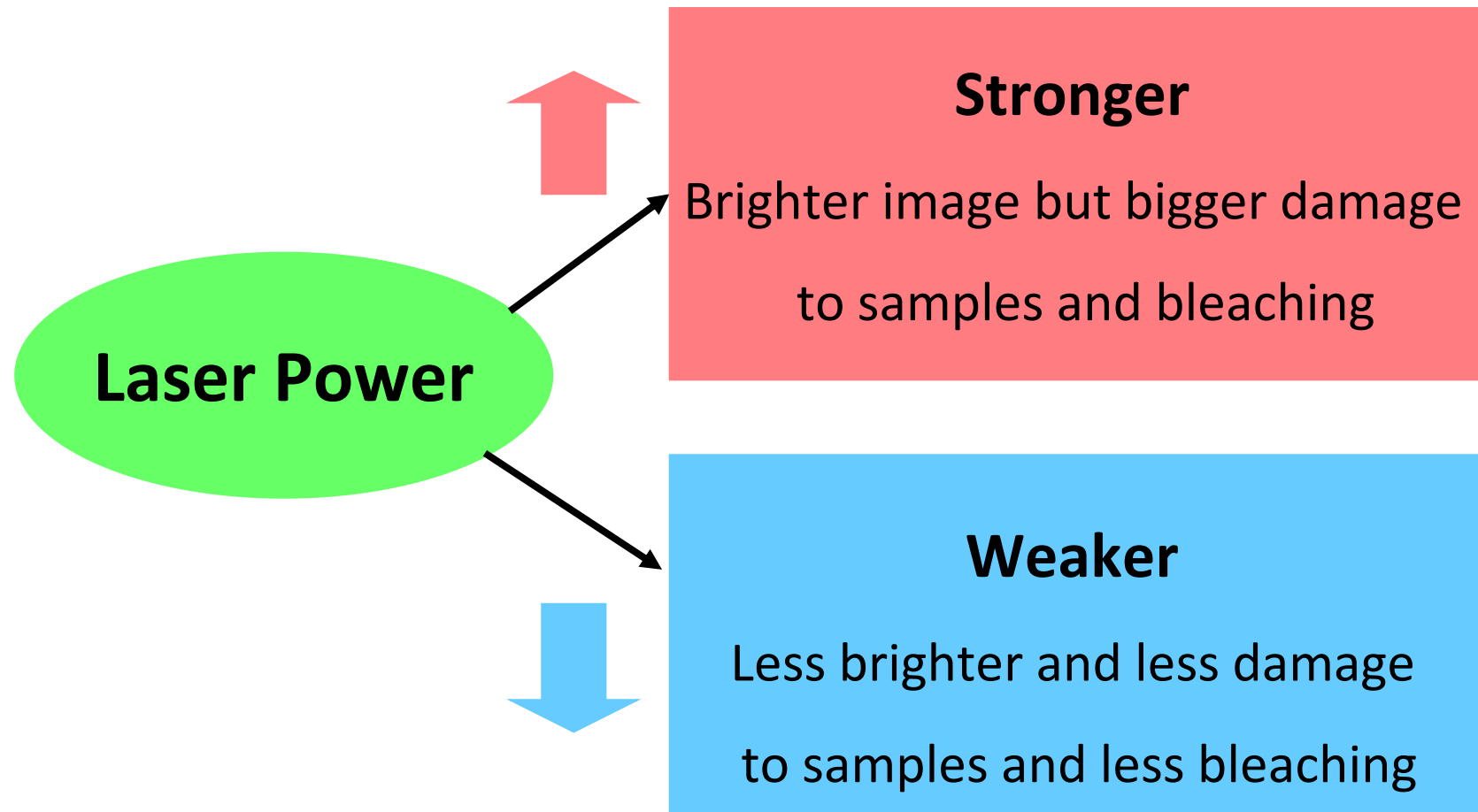


Tips for good confocal images

- Laser Power
- Scan Speed
- Pinhole Size
- Detector (PMT) gain

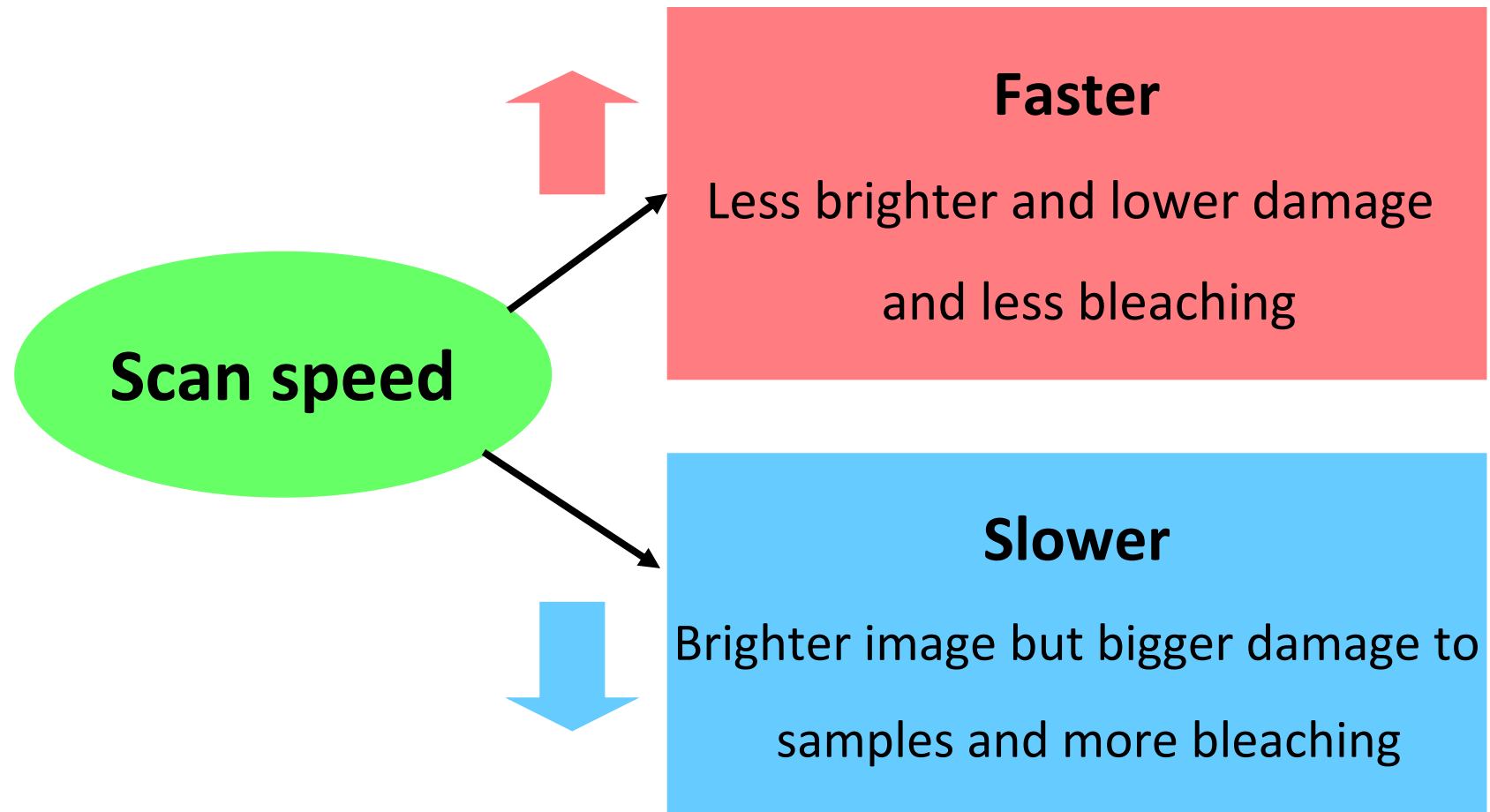


Tips #1. Laser Power



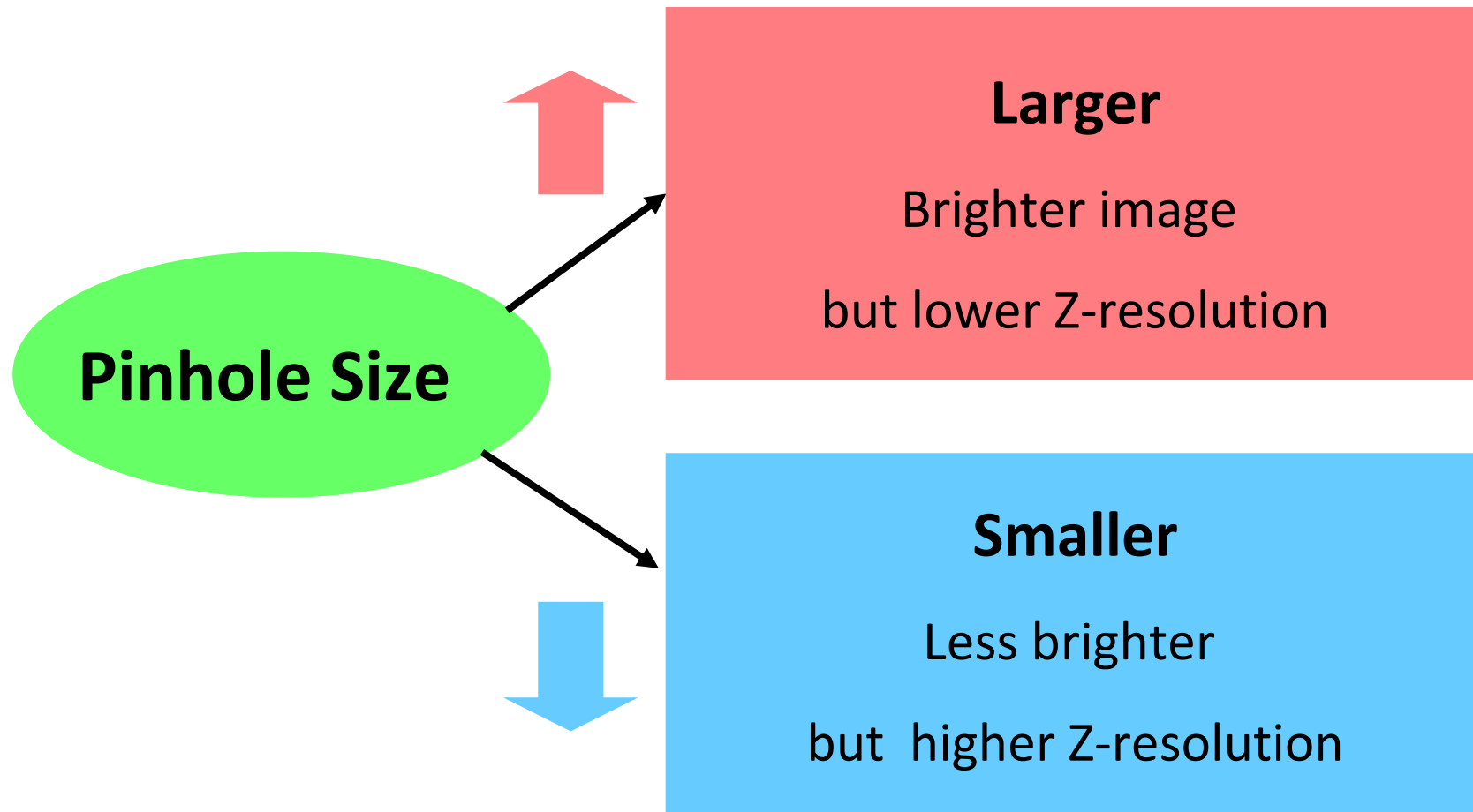


Tips #2. Scan Speed



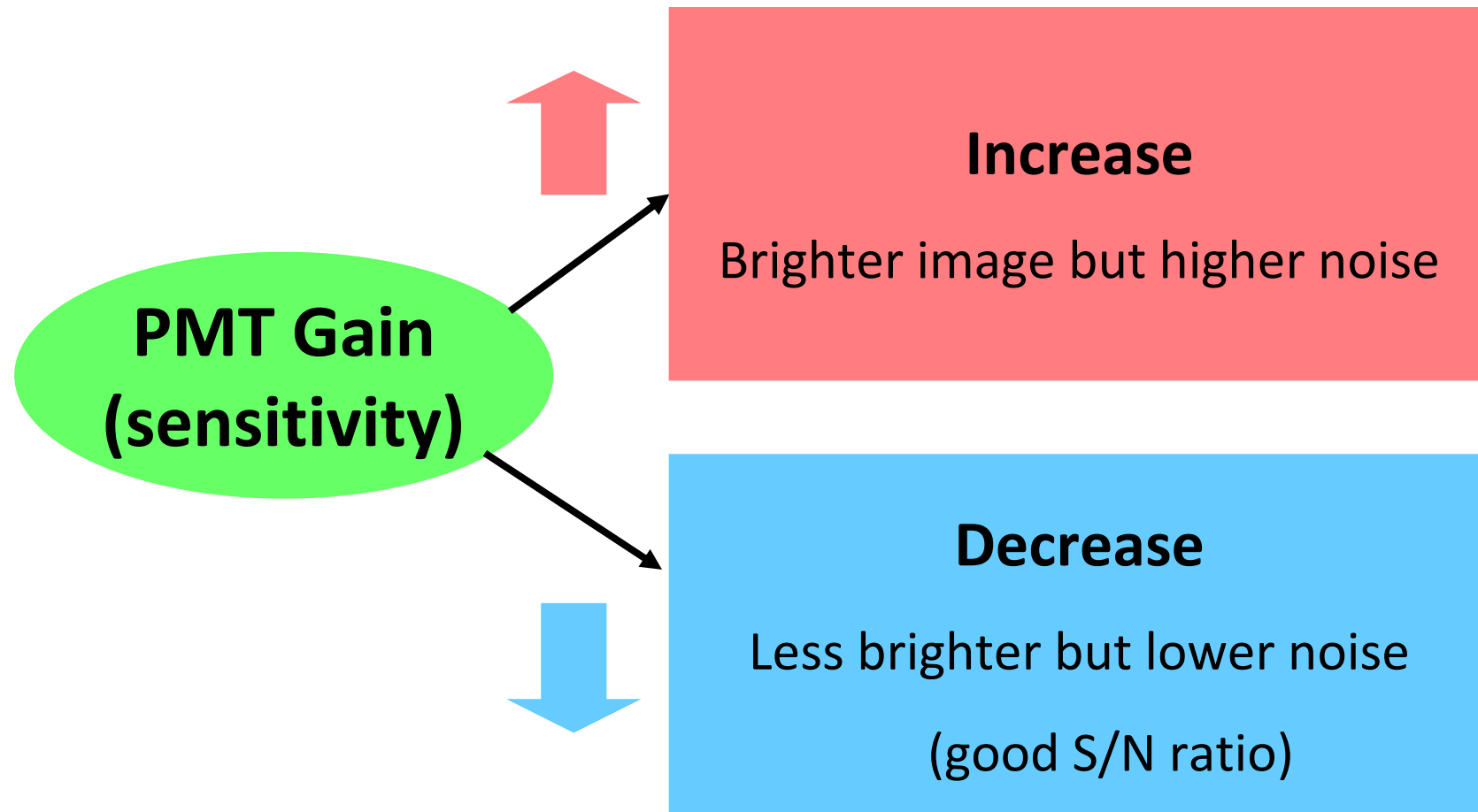


Tips #3. Pinhole Size





Tips #4. Detector (PMT) gain



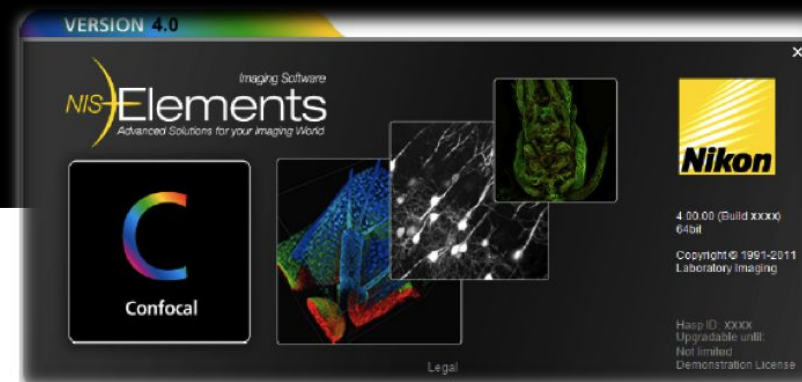
Interface of Confocal Software

NIS-Element C





NIS-Element C

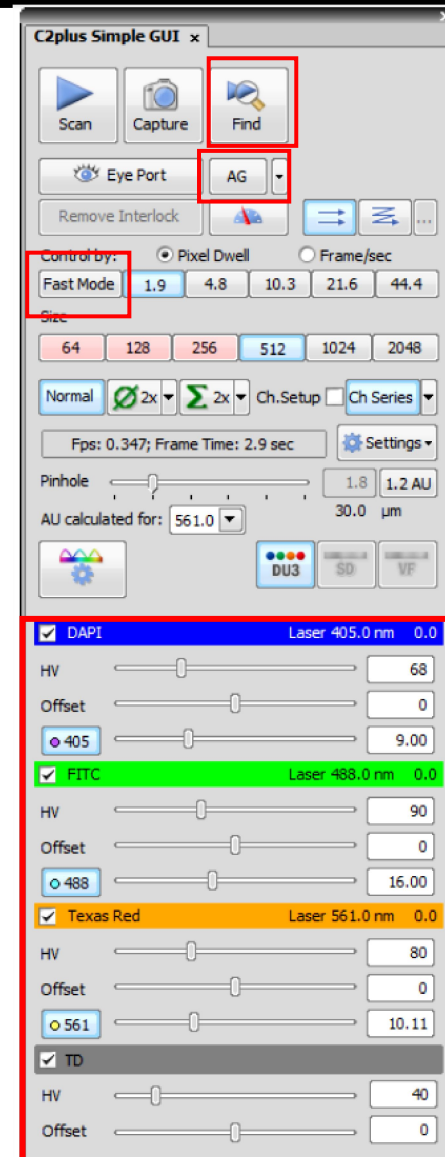


功能	應用
Auto Gain	依據樣品訊號強弱，軟體自動判斷最佳的Gain值
Z intensity control	可隨樣品深度調整雷射能量及Gain值，確保影像品質
6D experiment design	可支援進行X,Y,Z,Time, Multi-color, Multi-point實驗
3D reconstruction	可進行3D影像重組，以不同顯示方式呈現並製作影片輸出
Co-localization	螢光共位分析，可分析兩種螢光訊號的交互重疊範圍
Time Measurement	可隨時間即時觀測螢光蛋白強度變化
Automatic Optimization	軟體自動提供最佳Resolution, Pinhole, Zooming, Z step size數值
Re-Use function	圖檔可將拍照條件記下，下次只需要將圖檔再次叫出即可套用相同拍照條件



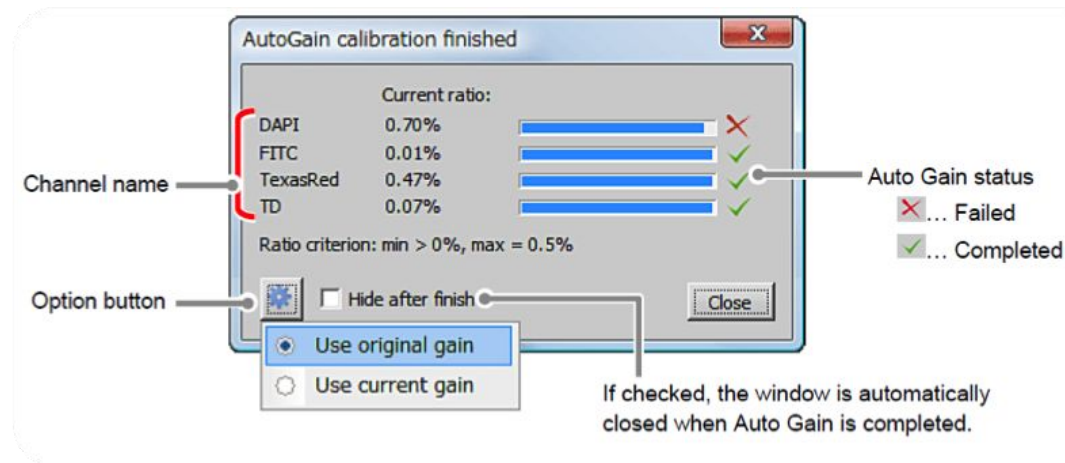
Simple software interface

- Find mode
- Auto Gain
- Fast mode
- Average
- Laser Intensity
- PMT Gain

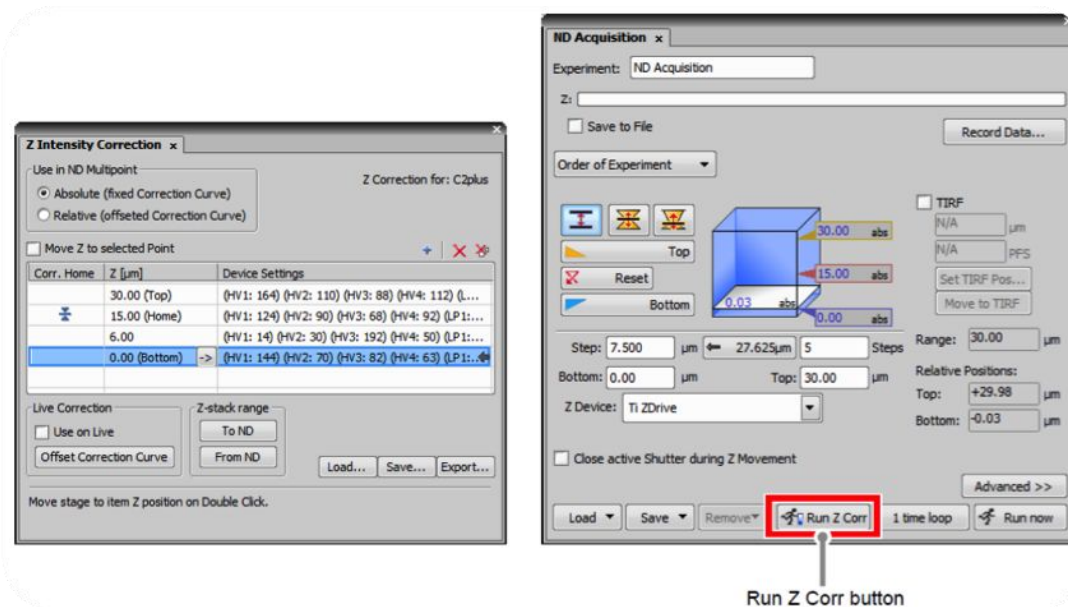




■ Auto Gain



■ Control of Laser Power to be matched with Z-axis intensity

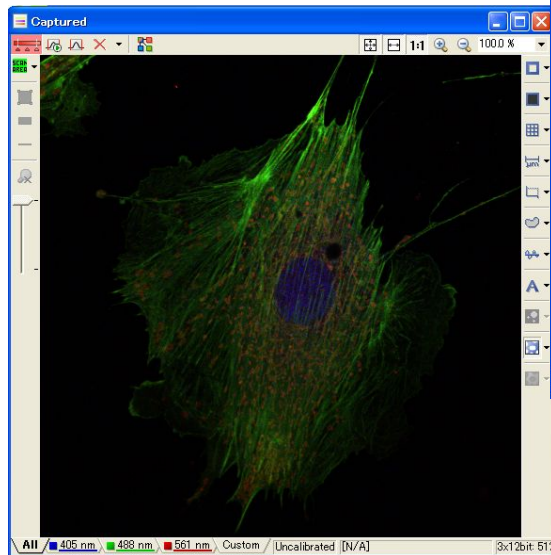




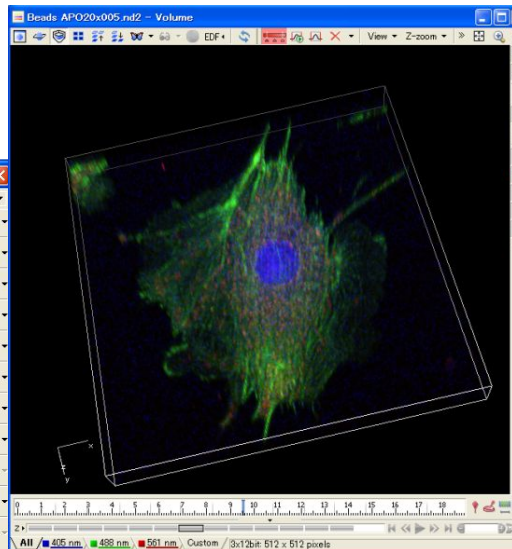
NIS-Element C software

Acquisition & Analysis

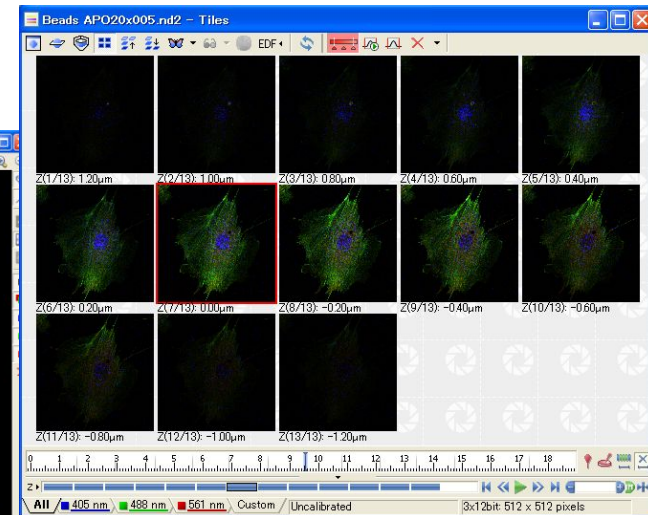
2D



3D volume



Tiling





Thanks for your attention !!!

