深耕計畫 - 高雄醫學大學培育特色研發人才系列活動

【特色研發技術培訓班】
細胞分子影像分析研發技術培訓班

課程代碼：FY

課程日期：2018/7/23-27

開課單位：高雄醫學大學研究資源整合發展中心

高雄醫學大學-培育特色研發人才-特色研發技術培訓班

【細胞分子影像分析人才培訓營】

◆ 課程目標

培育具備建構細胞分子影像與分析之生醫人才，以提高其就業及工作競爭力。

◆ 課程規劃

藉由細胞蛋白質分子的螢光偵測實驗，學習(1)基本細胞生物學觀念，(2)免疫螢光染色原理，(3)共軛焦顯微鏡原理與操作，(4)3D 影像分析，以達到培育生醫研究人才之目的。

◆ 支援設備/軟體

1. 研資中心高階雷射共軛焦顯微鏡(Olympus FV 1000 及 Zeiss LSM 700)

   共軛焦顯微鏡能提升傳統螢光顯微影像之品質，其可提供螢光標示之生物影像處理。其有一針孔狀遮蔽罩(Pinhole)，阻擋非焦面的螢光雜光進入感測器中，解決傳統螢光顯微鏡在非焦面雜光干擾下的模糊影像，產生螢光清晰的光學切片效果，提升螢光顯微影像品質。可應用在螢光標示之生物及非生物材料光切片影像處理、不同時間螢光變化影像分析、生物活體觀察、雙光束光調控刺激觀察、螢光定量分析。

2. Imaris 立體空間影像分析軟體

   此軟體可直接對 30 多種不同的影像格式檔案進行分析，並重建三維影像，可進一步分析其空間與時間變化，包含：細胞運動軌跡、分子於細胞內分佈與位移、病毒運作紀錄、神經結構與生長...等。學員藉由此培訓，將有益於增強研究結果的深度與廣度，同時可提升其對於資訊與智慧應用軟體之能力。

◆ 課程內容

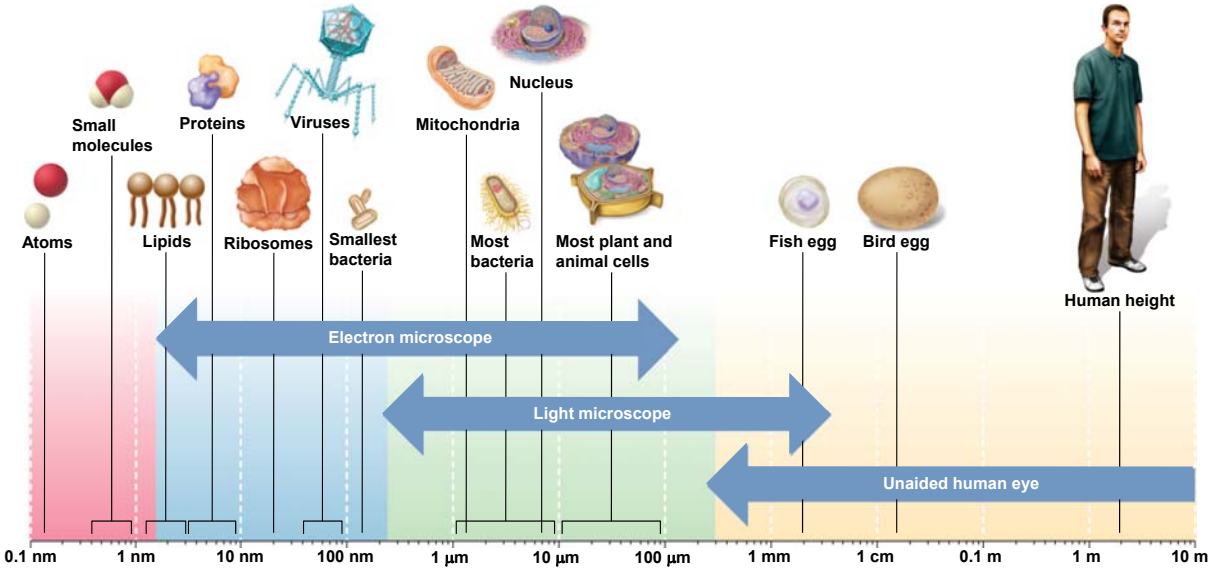
日期	上課時間	內容	講師	備註
7/23 Mon (FY1)	10:00-12:00	細胞分子結構簡介	鄭智美 副教授 高醫 生物系	集中上課 (IR202)
	2:00-4:00	螢光顯微鏡技術在細胞生物學的應用	蔡克勵 副教授 高醫 生理學科	集中上課 (IR202)
7/24 Tue (FY2)	10:00-12:00	螢光免疫染色的原理及應用	孫昭玲 副教授 高醫 醫研所	集中上課 (IR202)
	2:00-4:00	光學顯微鏡的介紹及操作	張永福 副教授 高醫 生物系	集中上課後實機操作光學顯微鏡 (IR202、IR8F 樓生物醫學影像核心實驗室)
7/25 Wed (FY3A-3D)	10:00-12:00	螢光共軛焦顯微鏡的介紹	1. 蔡博全 先生 (元利儀器股份有限公司) 2. 蔡克勵 副教授 3. 蘇湘涵 助教	集中上課後實機操作光學顯微鏡 (IR202、IR8F 樓生物醫學影像核心實驗室)
	1:00-1:30	紙筆測驗	朱家瑩 技佐 研資中心	(IR202)
	1:30-3:00 (第一梯次) 3:00-4:30 (第二梯次) 4:30-6:00 (第三梯次)	實驗操作: 1.細胞螢光免疫染色 2.螢光共軛焦顯微鏡操作	1. 孫昭玲 副教授 2. 蘇湘涵 助教	實驗操作依報名人數預計分為三梯次進行 (IR8F 樓生物醫學影像核心實驗室)
7/26 Thu (FY4A-4F)	8:30-10:00 (第一梯次) 10:00:11:30 (第二梯次) 11:30-1:00 (第三梯次)	1.細胞螢光免疫染色 2.螢光共軛焦顯微鏡操作	1. 鄭智美 副教授 2. 蘇湘涵 助教	實驗操作依報名人數預計分為三梯次進行 (IR8F 樓生物醫學影像核心實驗室)
	1:30-3:00 (第一梯次) 3:00-4:30	1.細胞螢光免疫染色 2.螢光共軛焦顯微	1. 鄭智美 副教授 2. 蘇湘涵 助教	實驗操作依報名人數預計分為三梯次進行

	(第二梯次) 4:30-6:00 (第三梯次)	鏡操作		(IR8F 樓生物醫學影像核心實驗室)
7/27 Fri (FY5A-5B)	10:00-12:00	Imaris 3D 影像分析軟體介紹及應用	1. 林政賢先生 (美嘉儀器股份有限公司) 2. 鄭智美 副教授	集中上課 (IR202)
	1:30-4:30	軟體操作: Imaris 3D 影像分析軟體操作與完成實驗數據分析	1. 林政賢先生 2. 孫昭玲 副教授	IR8F 樓生物醫學影像核心實驗室
	4:30-5:30	成果報告及討論	孫昭玲 副教授	

細胞分子結構

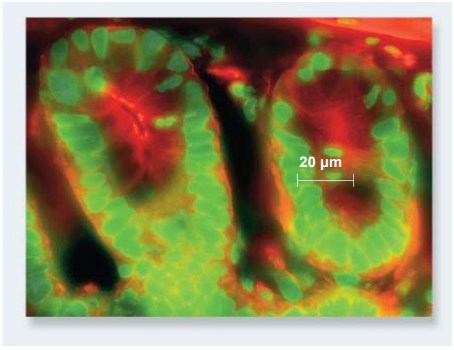
Molecular Structure of Cells

1. **All living organisms are composed of one or more cells**
2. **Cells are the smallest units of life**
3. **New cells come only from pre-existing cells by cell division**

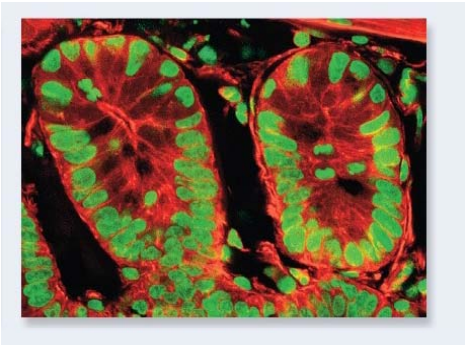


Fluorescent Microscope

Standard (wide-field) fluorescence microscopy.



Confocal fluorescence microscopy.



Cell Structure

- **Prokaryotes**
  - Simple cell structure
  - No nucleus
- **Eukaryotes**
  - More complex cells
  - DNA enclosed within membrane-bound nucleus
  - Internal membranes form organelles

Prokaryotic cells

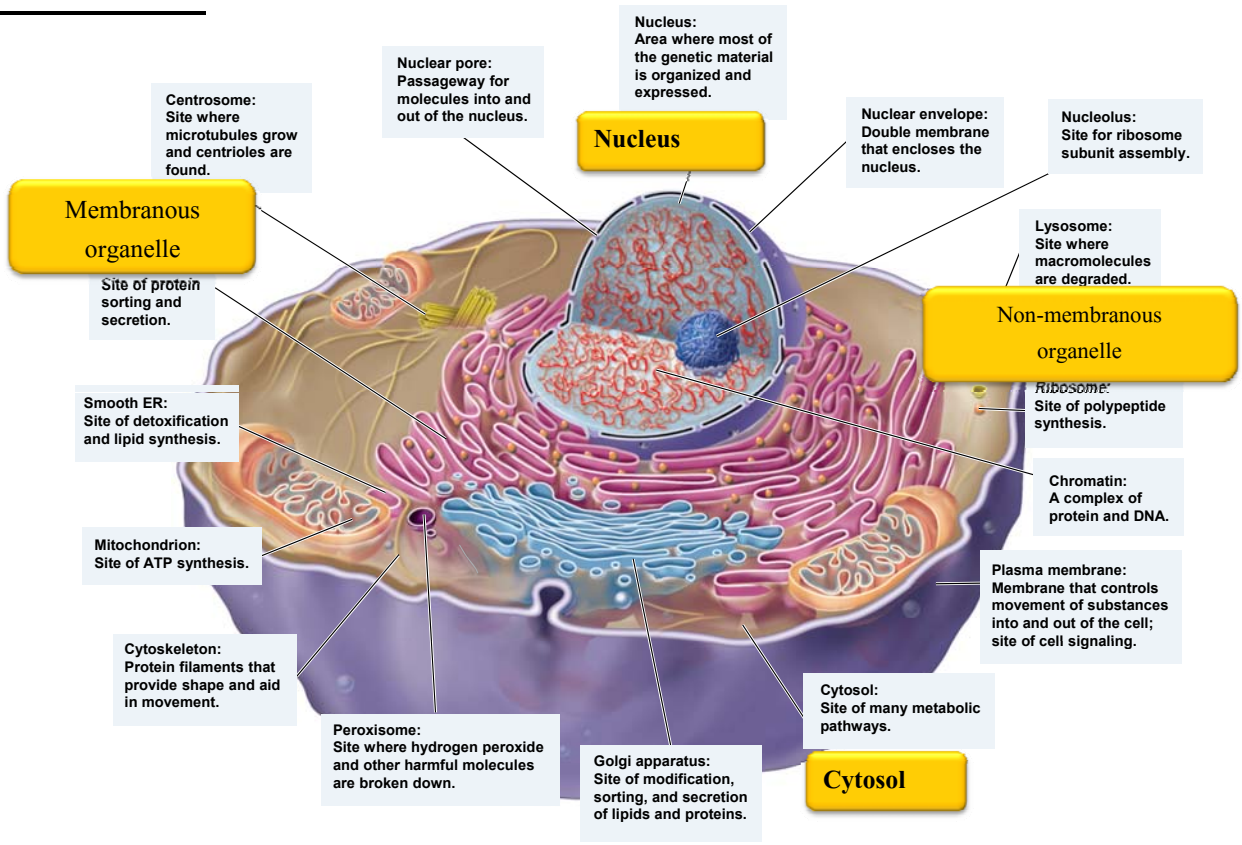
Two categories of prokaryotes:

- **Bacteria**
  - Small cells, 1 μm – 10 μm in diameter
  - Very **abundant** in environment and our bodies
  - Vast majority are not harmful to humans
  - Some species cause disease
- **Archaea**
  - Also small cells, 1 μm – 10 μm in diameter
  - Less common
  - Often found in **extreme environments**

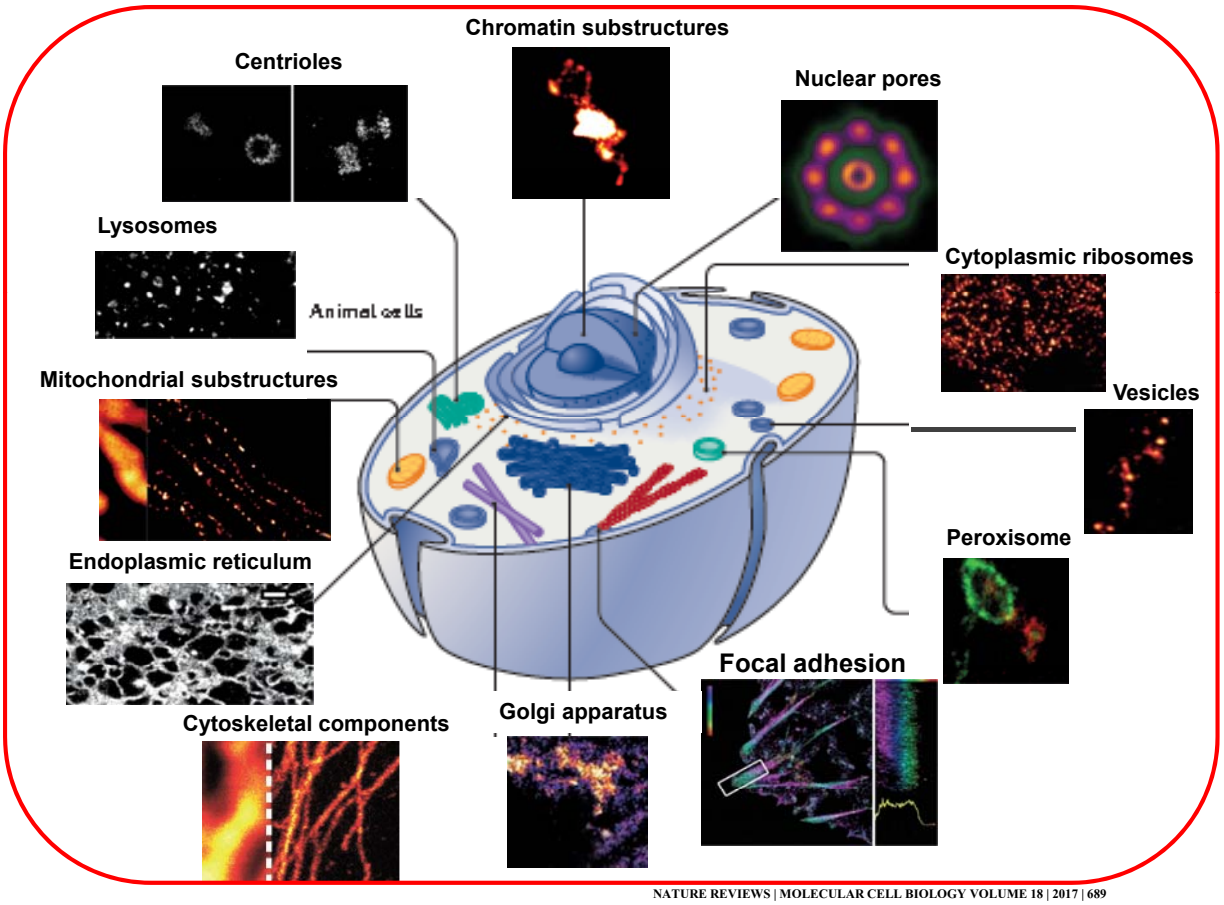
Eukaryotic cells

- DNA is housed inside membrane-bound **nucleus**
- **Compartmentalized functions**
- **Organelles**
  - Membrane-bound compartments
  - Each has a unique structure and function
- **Variety**
  - Shape, size, and organization of cells vary considerably
  - Differences between **species**
  - Differences between **specialized cell types**

Animal cell



Dissecting animal cells with fluorescence nanoscopy



NATURE REVIEWS | MOLECULAR CELL BIOLOGY VOLUME 18 | 2017 | 689

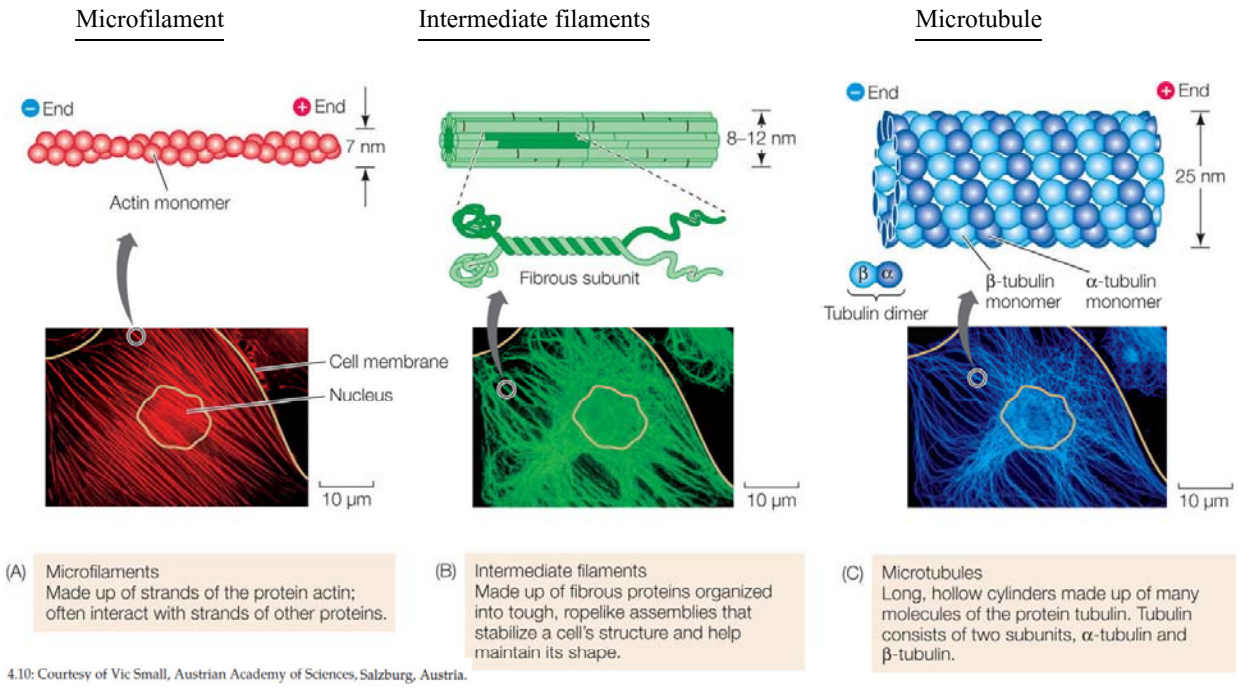
The Cytosol

- Region of a eukaryotic cell that is outside the cell organelles but **inside the plasma membrane**
- Cytoplasm includes **everything** inside the plasma membrane
  - **Cytosol**
  - **Endomembrane system**
  - **Semiautonomous organelles**

Cytoskeleton: the Cell skeleton

Network of **three types of protein filaments**

- **Microtubules**
  - Long, hollow cylindrical structures
  - Dynamic instability
- **Intermediate filaments**
  - Intermediate in size
  - Form twisted, ropelike structure
- **Actin filaments**
  - Also known as microfilaments
  - Long, thin fibers



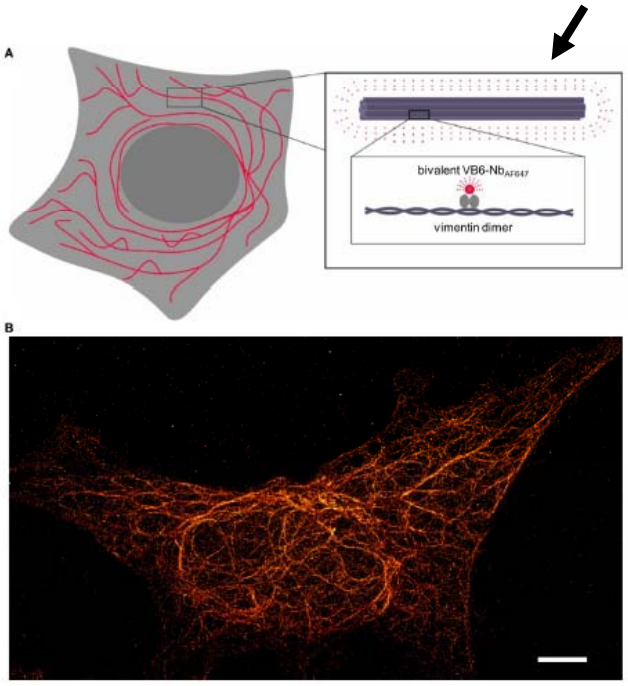
4.10: Courtesy of Vic Small, Austrian Academy of Sciences, Salzburg, Austria.

http://www.macmillanhighered.com/BrainHoney/Resource/6716/digital_first_content/trunk/test/hillis2e/hillis2e_ch04_5.html

Vimentin network in HeLa cell

(Intermediate filament)

bivalent VB6 (bivVB6)-Nb-labeled vimentin network



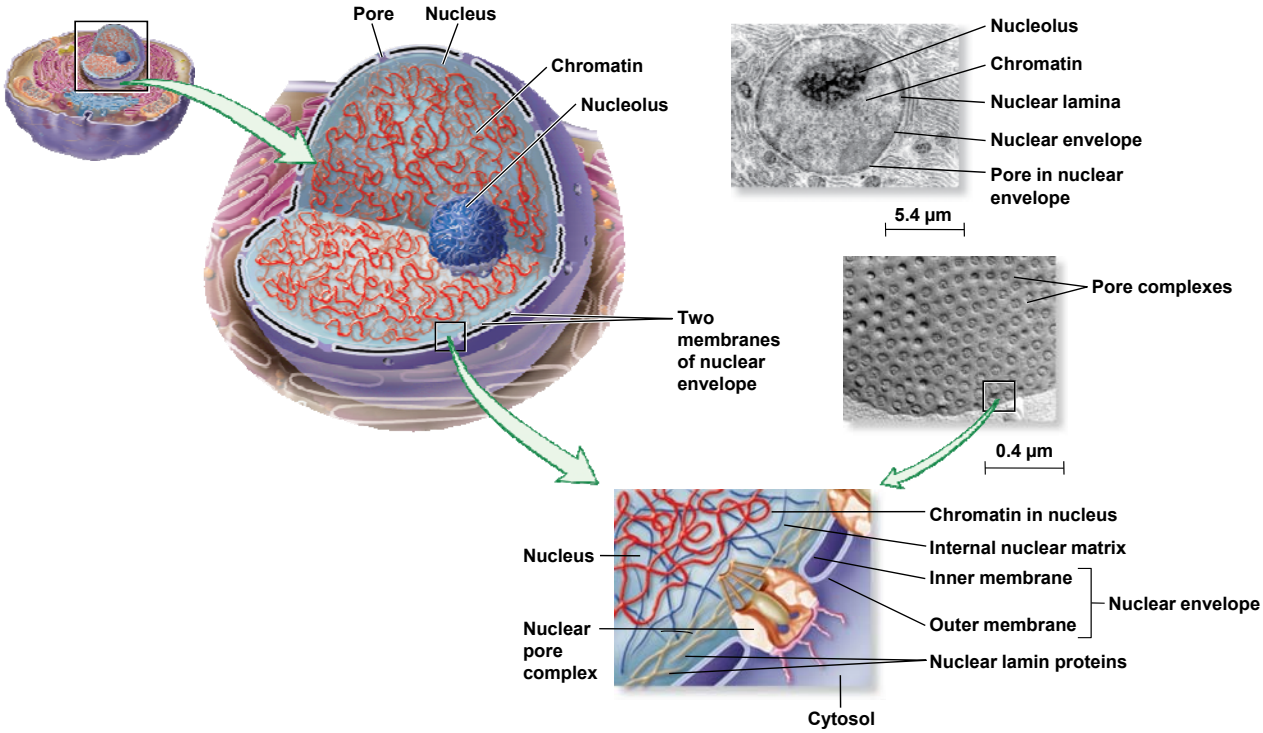
1 August 2017 | Volume 8 | Article 1030 doi: 10.3389/fimmu.2017.01030

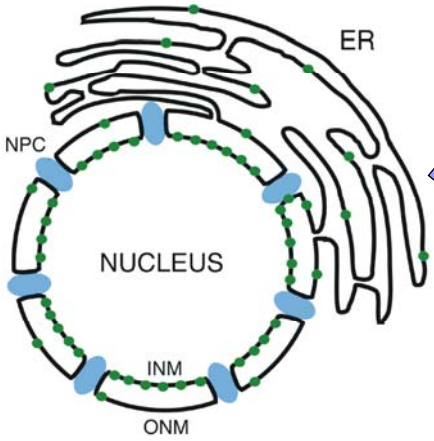
The Nucleus and Endomembrane System

- **Network of membranes** enclosing the nucleus, endoplasmic reticulum, Golgi apparatus, lysosomes, and vacuoles
- Also **includes plasma membrane**
- May be **directly connected** to each other or pass materials via **vesicles**

Nuclear envelope

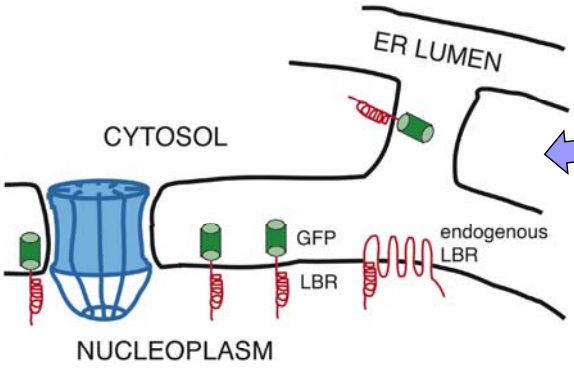
- Double-membrane structure enclosing nucleus
- Outer membrane of the nuclear envelope is continuous with the ER membrane
- Nuclear pores provide passageways
- Materials within the nucleus are *not* part of the endomembrane system





*NE and ER membrane continuities

* Outer nuclear membrane: shares its proteins and functional properties with the ER, whose lumen is continuous with the perinuclear space



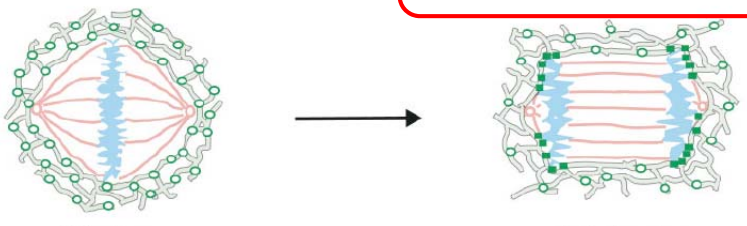
* **The inner nuclear membrane has unique characteristics. It contains a distinct set of membrane proteins, i.e. Lamin B receptor(LBR)**

The Journal of Cell Biology, Volume 138, Number 6, September 22, 1997 1193–1206

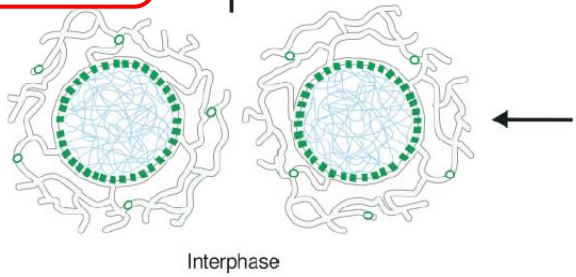
Model of nuclear envelope reassembly.

LBR diffuses freely within an interconnected ER network that surrounds the spindle apparatus

Immobilization of LBR receptor at contact sites between ER and chromatin.



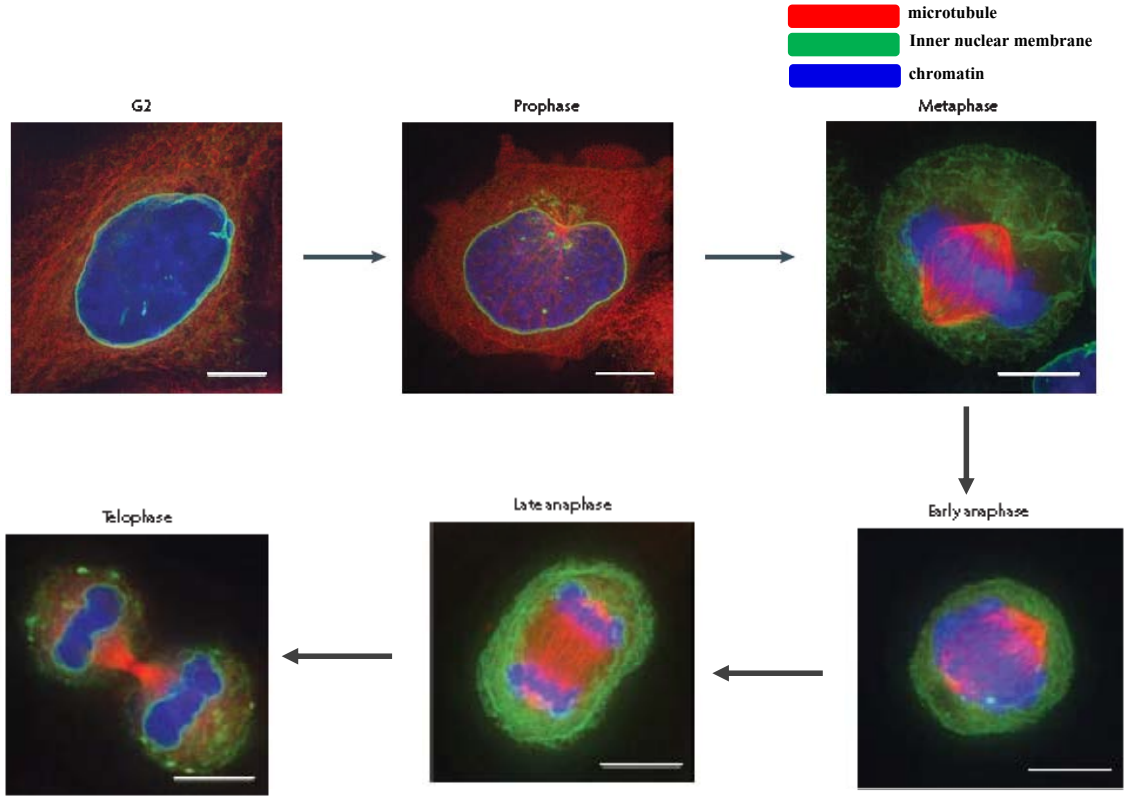
Equilibration of the mobilized LBR molecules within the ER/NE system



more binding sites become exposed as the spindle retracts, trapping more LBR molecules and forcing ER membranes to wrap around the chromatin

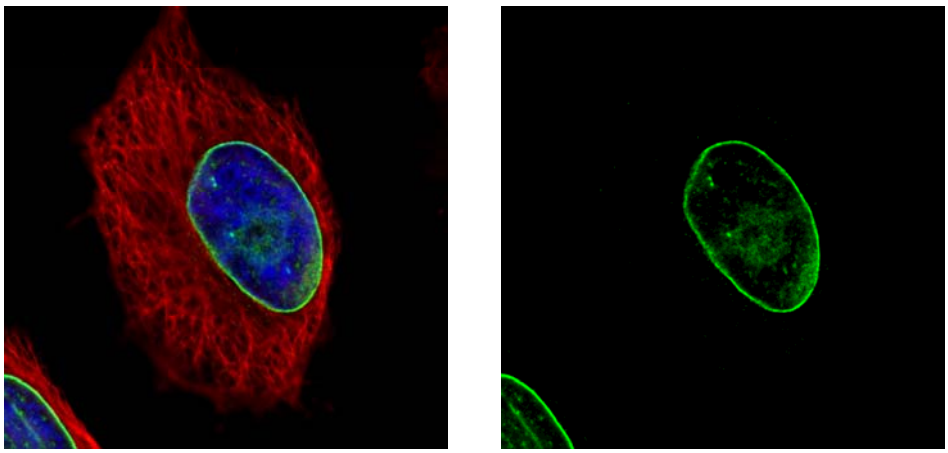
Diffusion of LBR from ER to inner nuclear membrane and immobilize by binding to chromatin

Nuclear envelope reassembly after mitosis



NATuRE REVIEWS | Molecular cell Biology VOluME 10 | MARCH 2009 | 181

Nuclear Envelope (Lamin A + C)

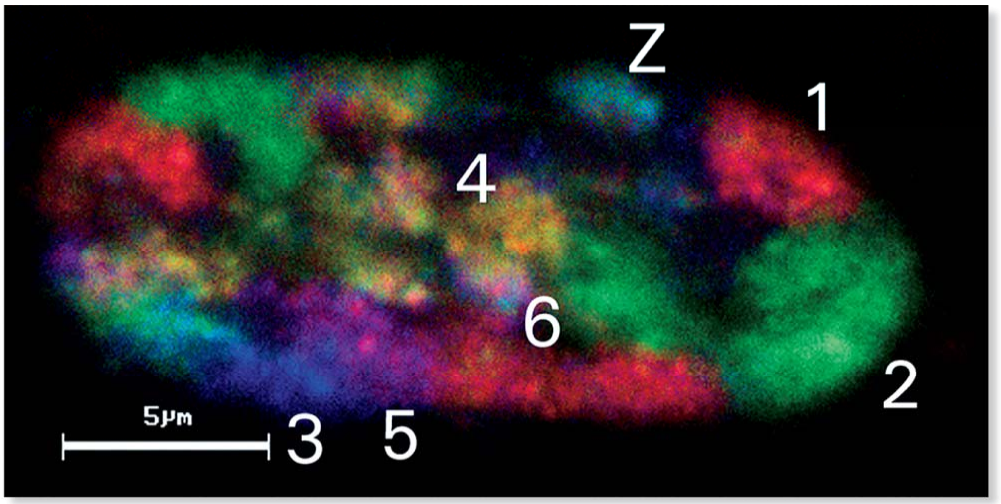


Nuclear lamins A/C control chromatin organization, gene transcription, DNA replication, DNA damage responses, cell cycle progression, cell differentiation, and cell polarization during migration

Nucleus. 2014;5(5):396-401

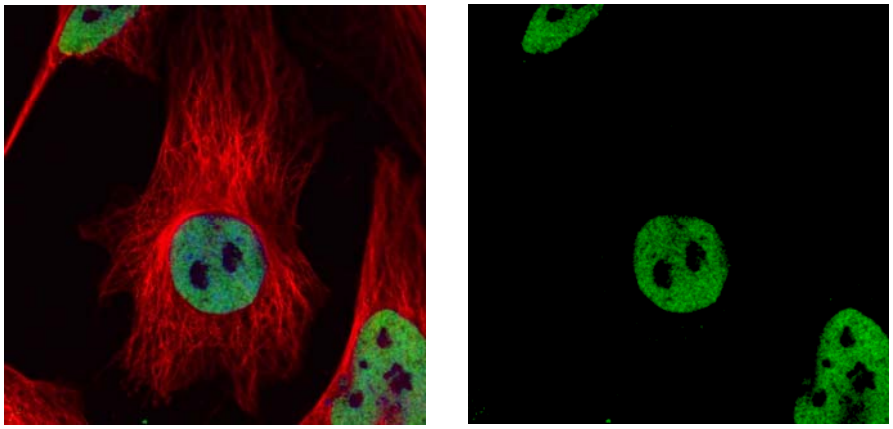
Nucleus

- **Chromosomes**
  - **Composed of DNA and proteins = chromatin**
- **Nuclear matrix**
  - **Filamentous network**
  - **Organizes chromosomes**
- **Ribosome assembly occurs in the nucleolus**



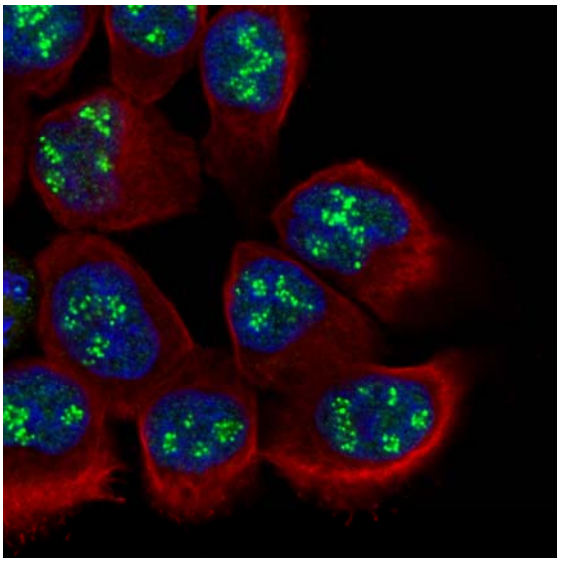
Courtesy of Felix A. Habermann

Nucleus - HDAC2 (histone deacetylase 2)



* Responsible for the deacetylation of lysine residues of core histones (H2A, H2B, H3 and H4)
* Forms transcriptional repressor complexes by associating with YY1, a mammalian zinc-finger transcription factor.
* Plays an important role in transcriptional regulation, cell cycle progression and developmental events

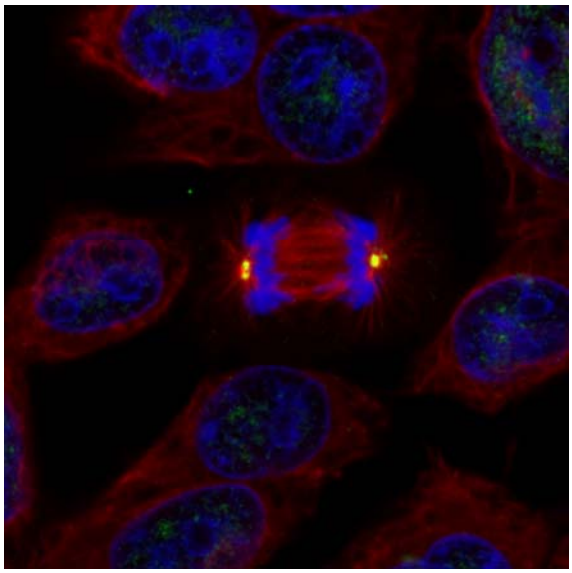
Nucleolus - PAF49



* A subunit of RNA polymerase I
* Tyr phosphorylation of CAST/hPAF49 is important for signaling during T-cell activation

Panov, K. I., Panova, T. B., Gadal, O., Nishiyama, K., Saito, T., Russell, J., & Zomerdijk, J. C. B. M. (2006). RNA Polymerase I-Specific Subunit CAST/hPAF49 Has a Role in the Activation of Transcription by Upstream Binding Factor. Molecular and Cellular Biology, 26(14), 5436–5448. http://doi.org/10.1128/MCB.00230-06

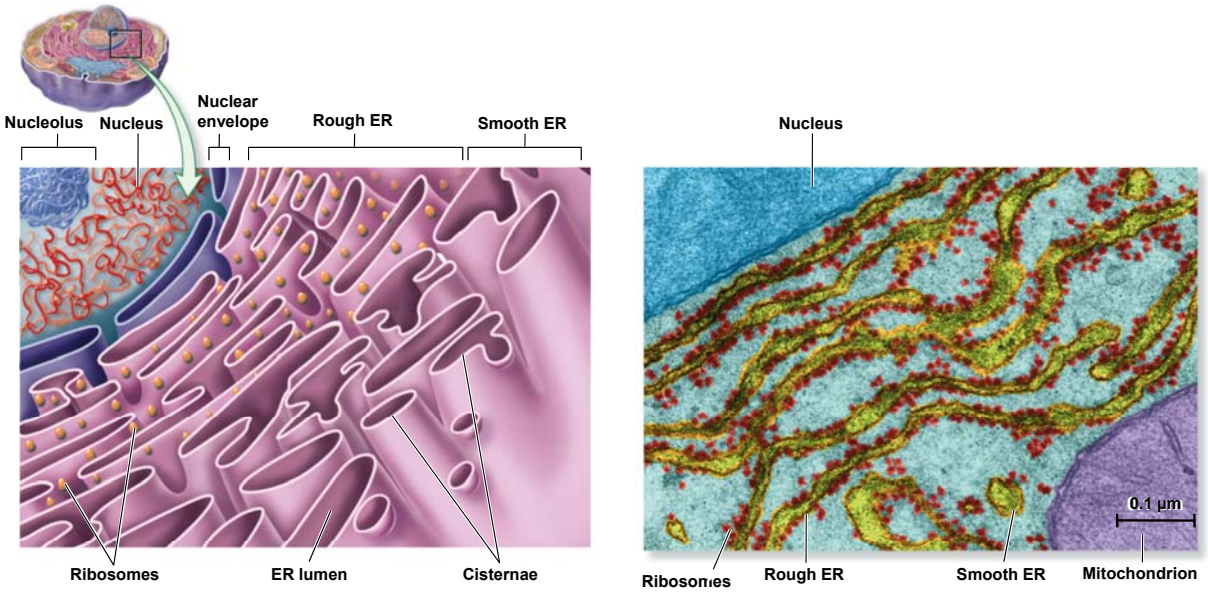
Centromere –gamma Tubulin



spindle microtubules are all made from the same α/β-tubulin heterodimers

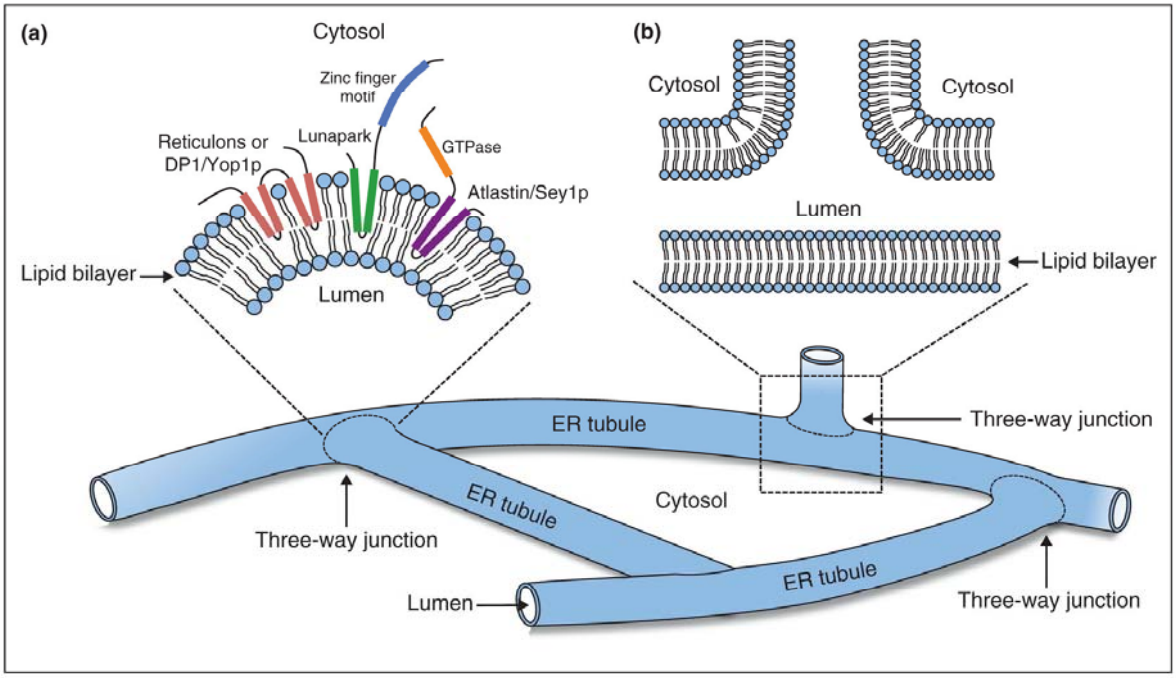
Endoplasmic reticulum

- **Network of membranes** that form flattened, fluid-filled tubules or cisternae
- ER membrane encloses a single compartment called the **ER lumen**
- **Rough endoplasmic reticulum** (rough ER)
  - Studded with ribosomes
  - Involved in protein synthesis and sorting
- **Smooth endoplasmic reticulum** (smooth ER)
  - Lacks ribosomes
  - Detoxification, carbohydrate metabolism, calcium balance, synthesis, and modification of lipids



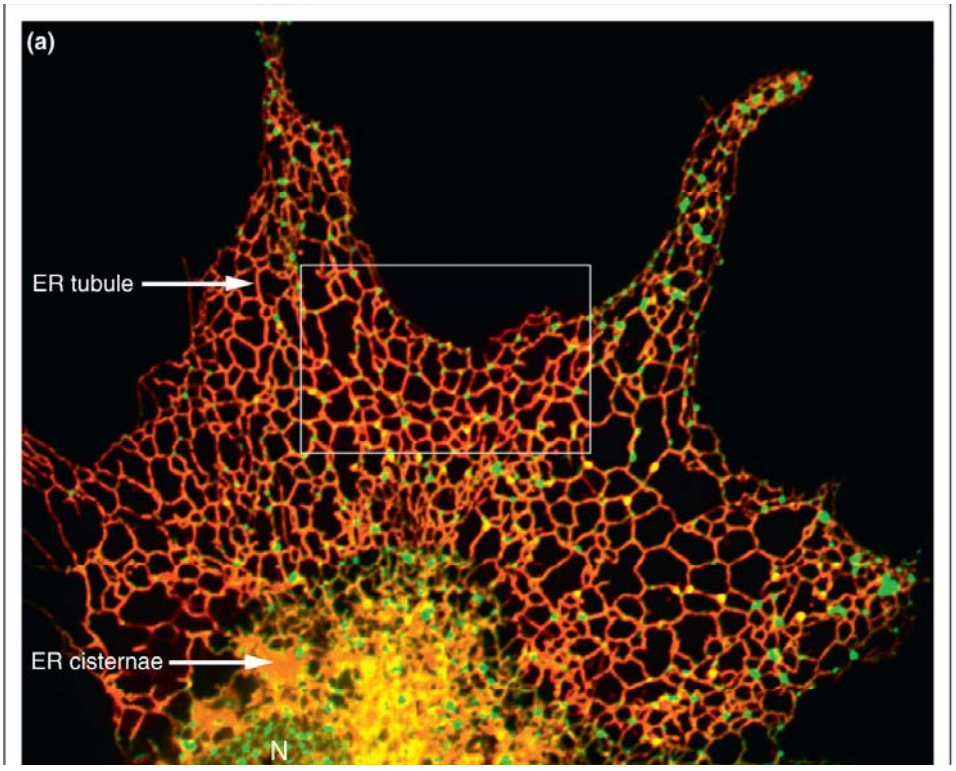
27

The ER shaping proteins at three-way junctions



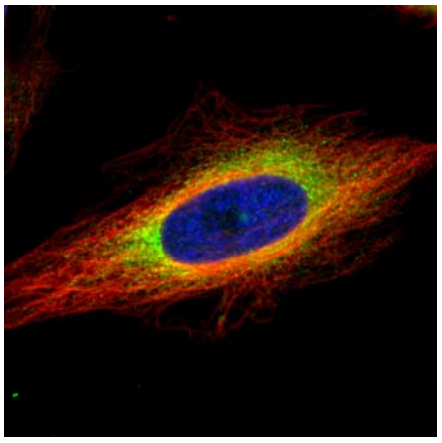
Curr Opin Cell Biol. 2013 August ; 25(4): 428–433. doi:10.1016/j.ceb.2013.02.006.

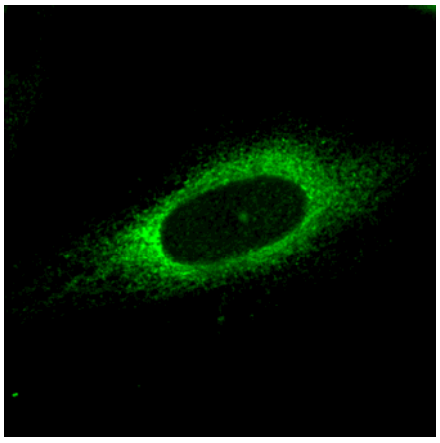
28



Curr Opin Cell Biol. 2013 August ; 25(4): 428–433. doi:10.1016/j.ceb.2013.02.006.

Endoplasmic Reticulum - GRP94



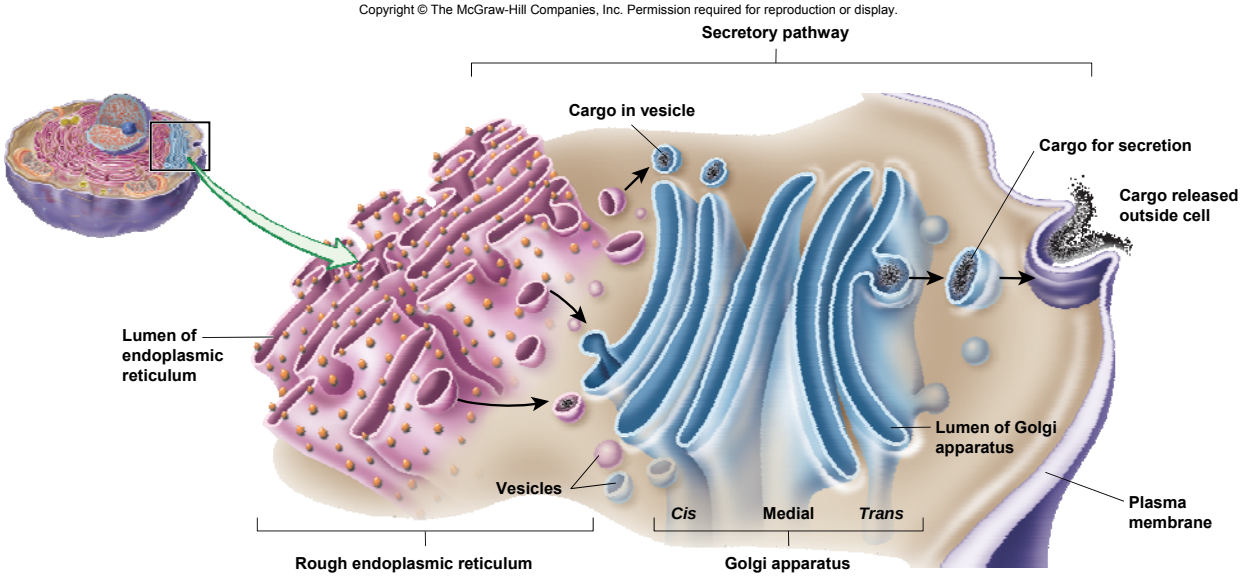


* **Heat Shock Protein (HSP) 90 family – GRP94**
* **Induction upon glucose deprivation**
* **The only HSP90-like protein that resides in the ER.**
* **Up-regulation is often used as a hallmark of responses to ER stress**

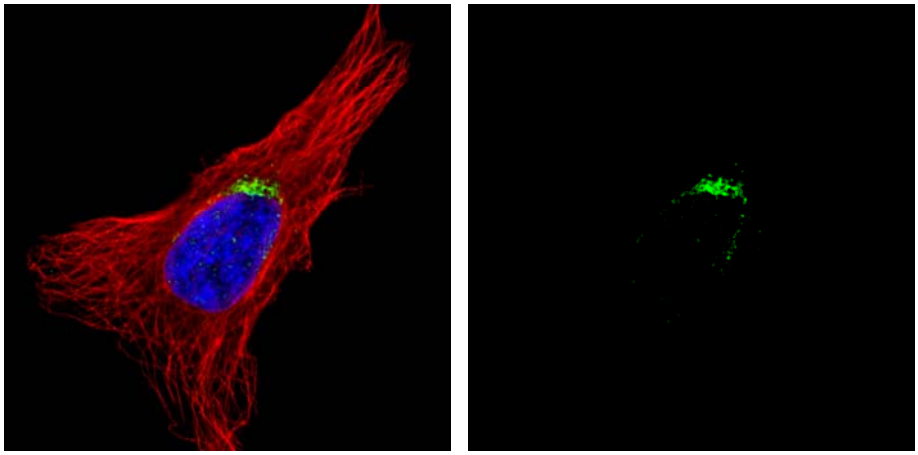
Eletto, D., Dersh, D., & Argon, Y. (2010). GRP94 in ER Quality Control and Stress Responses. Seminars in Cell & Developmental Biology, 21(5), 479–485. http://doi.org/10.1016/j.semcdb.2010.03.004

Golgi apparatus

- Also called the Golgi body, Golgi complex, or simply Golgi
- **Stack** of flattened, membrane-bounded compartments
- **Vesicles** transport materials between stacks
- Three overlapping functions
  - **Secretion, processing,** and **protein sorting**



Golgi Apparatus - GOLGA5

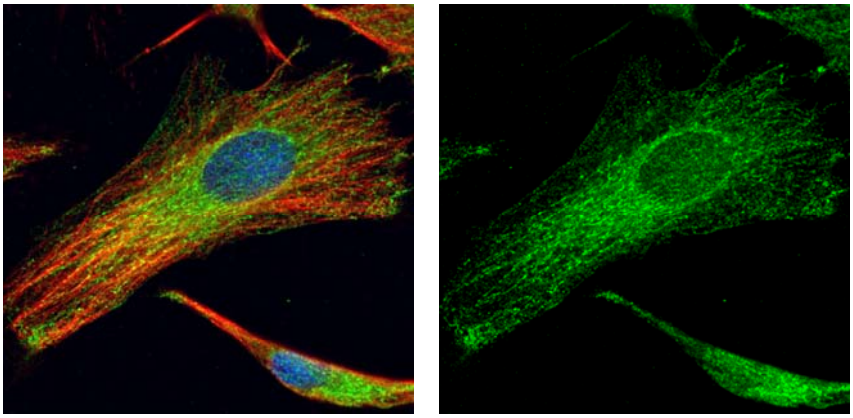


* **A coiled-coil membrane protein**
* **Play a role in vesicle tethering and docking**
* **Translocations involving this gene and the ret proto-oncogene have been found in tumor tissues**

Lysosomes

- Contain **acid hydrolases** that perform hydrolysis
- Many **different types** of acid hydrolases to break down proteins, carbohydrates, nucleic acids, and lipids
- **Autophagy**
  - Recycling of worn-out organelles through endocytosis

Lysosome - LAMP2



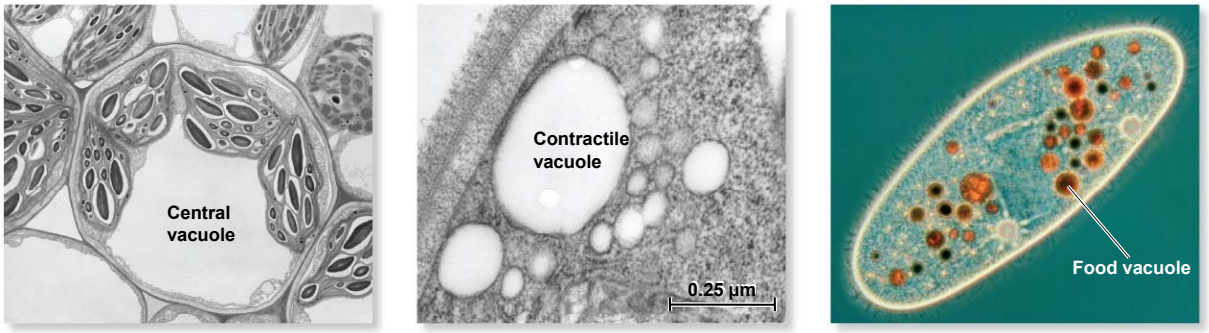
* Major protein components of the lysosomal membrane.
* LAMP-2 is required for the maturation of autophagosomes by fusion with lysosomes
* Danon disease(cardiomyopathy disease) are thought to be mediated by loss of the LAMP-2B isoform

Acta Neuropathol (2015) 129:391–398

35

Vacuoles

- Functions are extremely **varied,** and they differ among cell types and environmental conditions
- **Central vacuoles** in plants for storage and support
- **Contractile vacuoles** in protists for expelling excess water
- **Phagocytic vacuoles** in protists and white blood cells for degradation

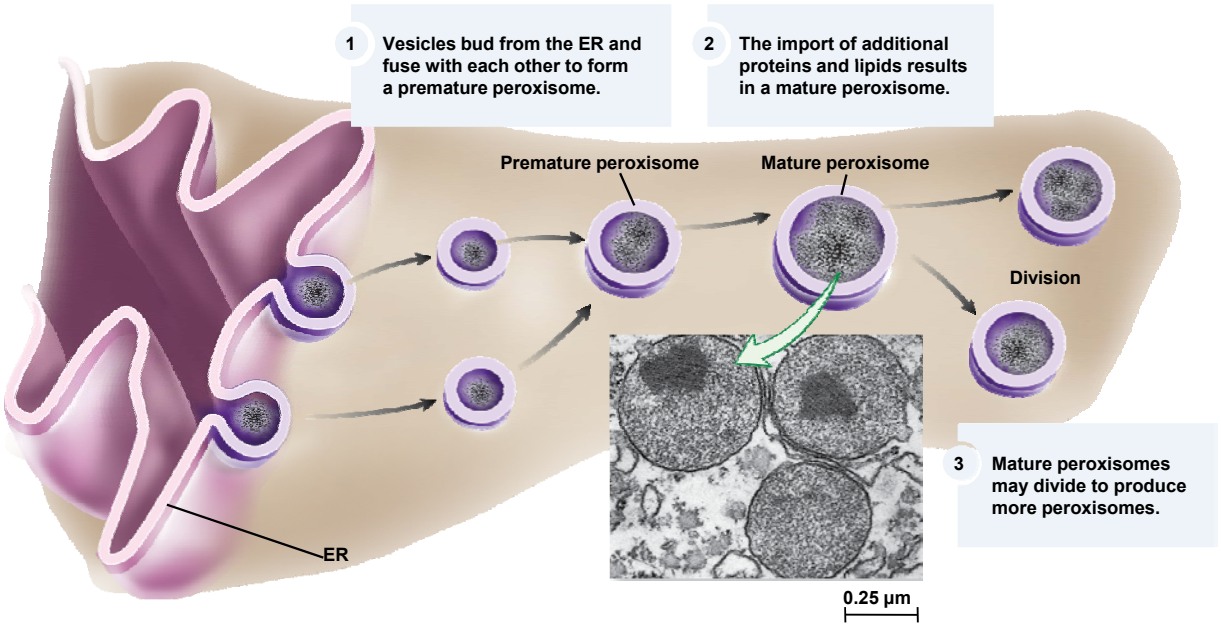


(a) Central vacuole in a plant cell (b) Contractile vacuoles in an algal cell (c) Food vacuoles in a paramecium

a: © E.H. Newcomb & S.E. Frederick/Biological Photo Service; b: Courtesy Dr. Peter Luykx, Biology, University of Miami;
c: © Dr. David Patterson/Photo Researchers, Inc.

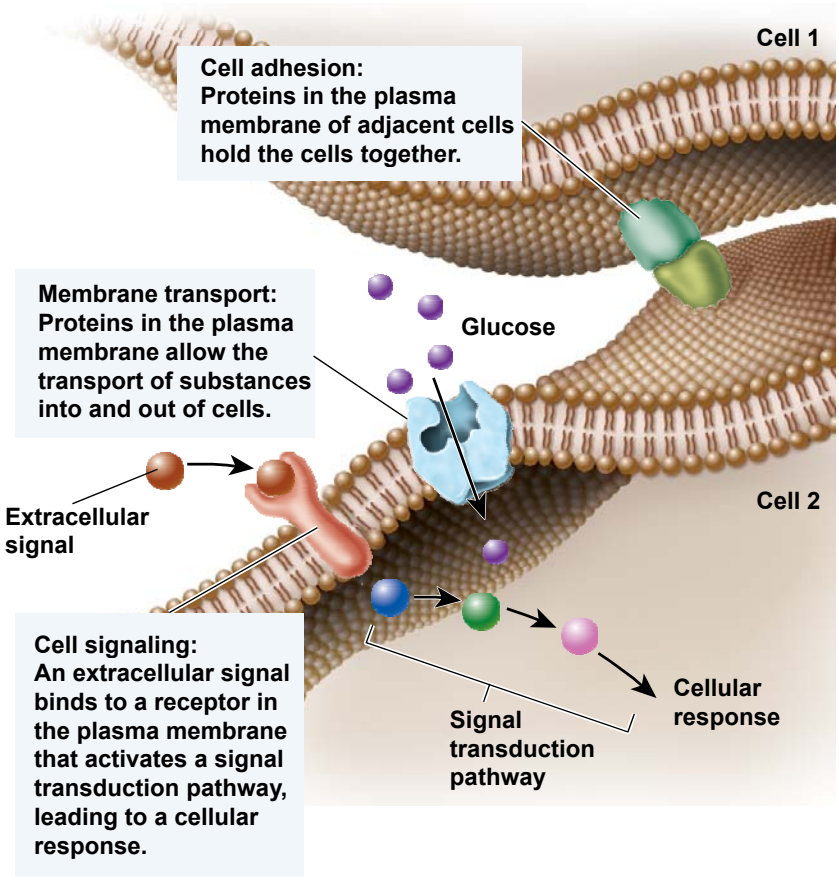
Peroxisomes

- **Catalyze** certain reactions that break down molecules by removing hydrogen or adding oxygen
- **Hydrogen peroxide** (H_2O_2) is a byproduct
- **Catalase** breaks down dangerous H_2O_2 into water and oxygen



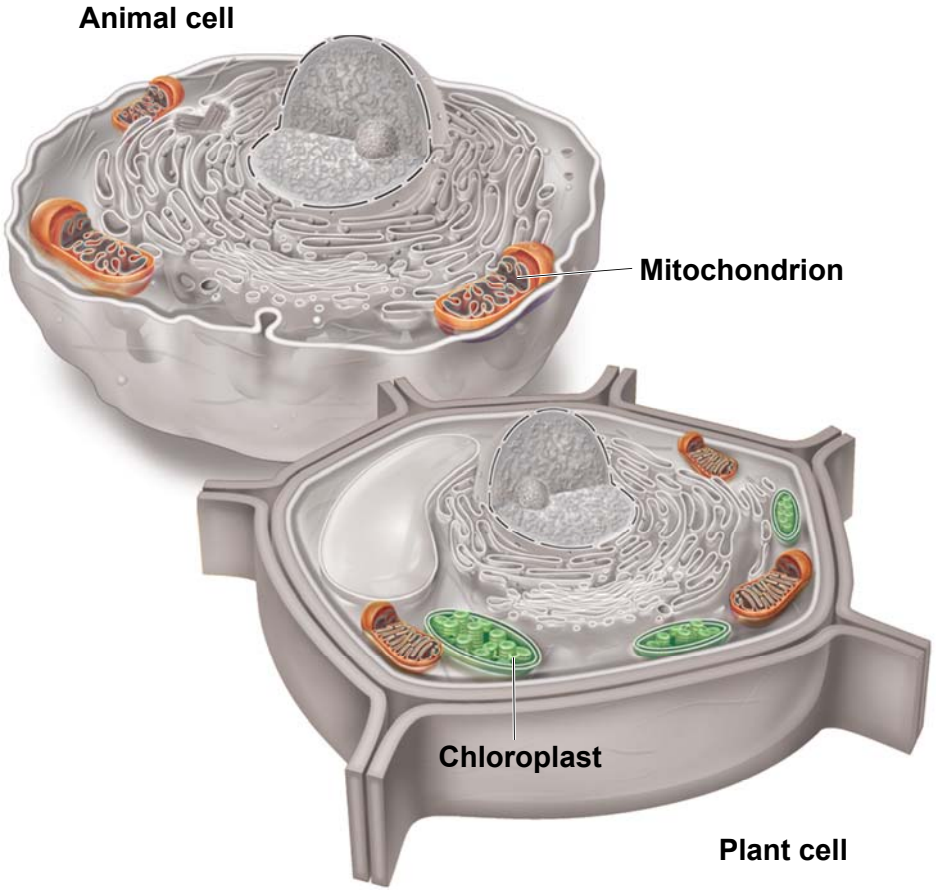
Plasma membrane

- Boundary between the cell and the extracellular environment
- Functions
  - **Membrane transport** in and out of cell, with selective permeability
  - **Cell signaling** using receptors
  - **Cell adhesion**



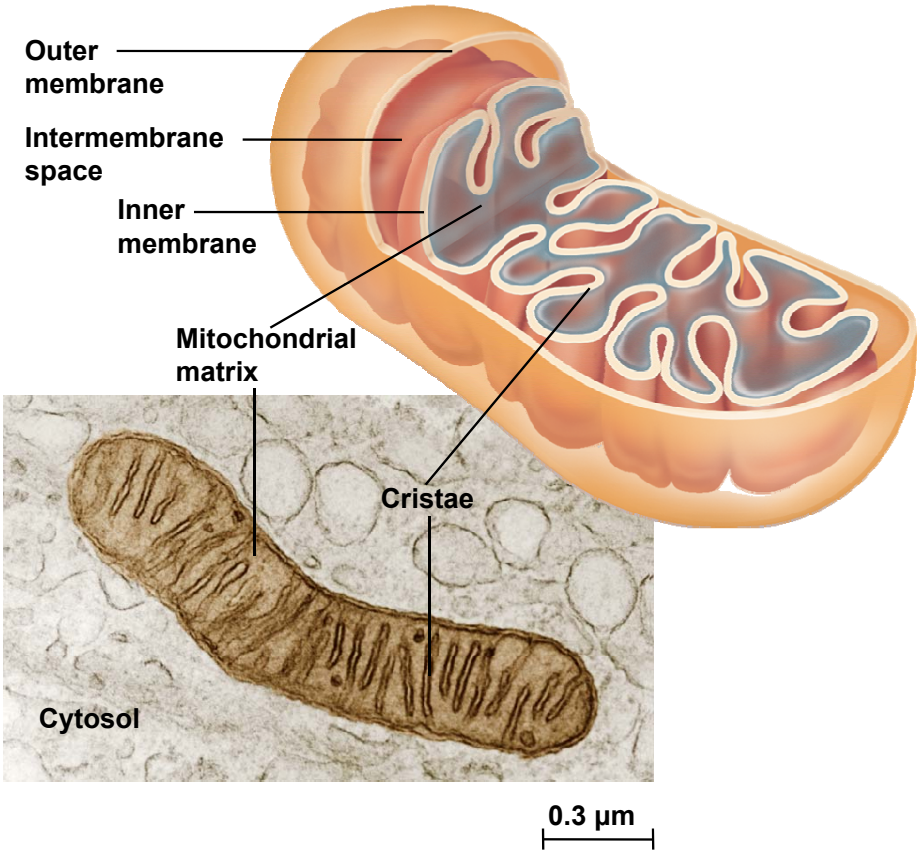
Semiautonomous Organelles

- **Mitochondria** and **chloroplasts**
- Grow and divide to **reproduce** themselves
- They are not completely autonomous because they depend on the cell for synthesis of **internal components**

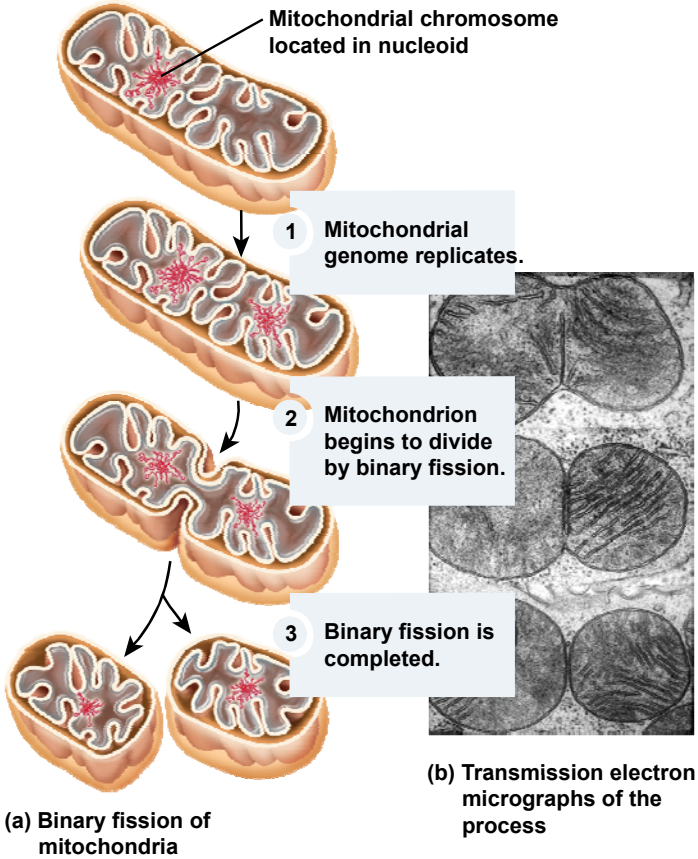


Mitochondria

- Primary role is to **make ATP**
- Outer and inner membrane
  - Intermembrane space and mitochondrial matrix
- Also involved in the synthesis, modification, and breakdown of several types of cellular molecules
- Contain their own DNA, divide by binary **fission**



© Dr. Donald Fawcett/Visuals Unlimited

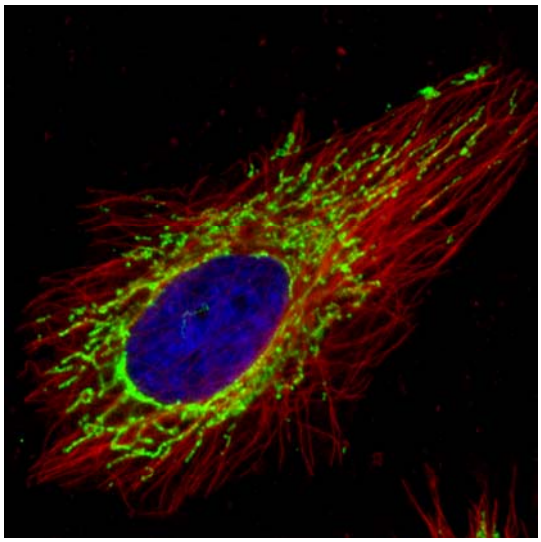


(a) Binary fission of mitochondria

(b) Transmission electron micrographs of the process

© T. Kanaseki and Dr. Donald Fawcett/Visuals Unlimited

Mitochondria - COX4 (cytochrome c oxidase subunit IV)



Cytochrome C oxidase assembly and stabilization is dependent on the presence of Cox4 and on Zn(II) coordination in Cox4

The Journal of Biological Chemistry 282, 8926-8934

Fluorescence Microscopy Applications in Cell Biology

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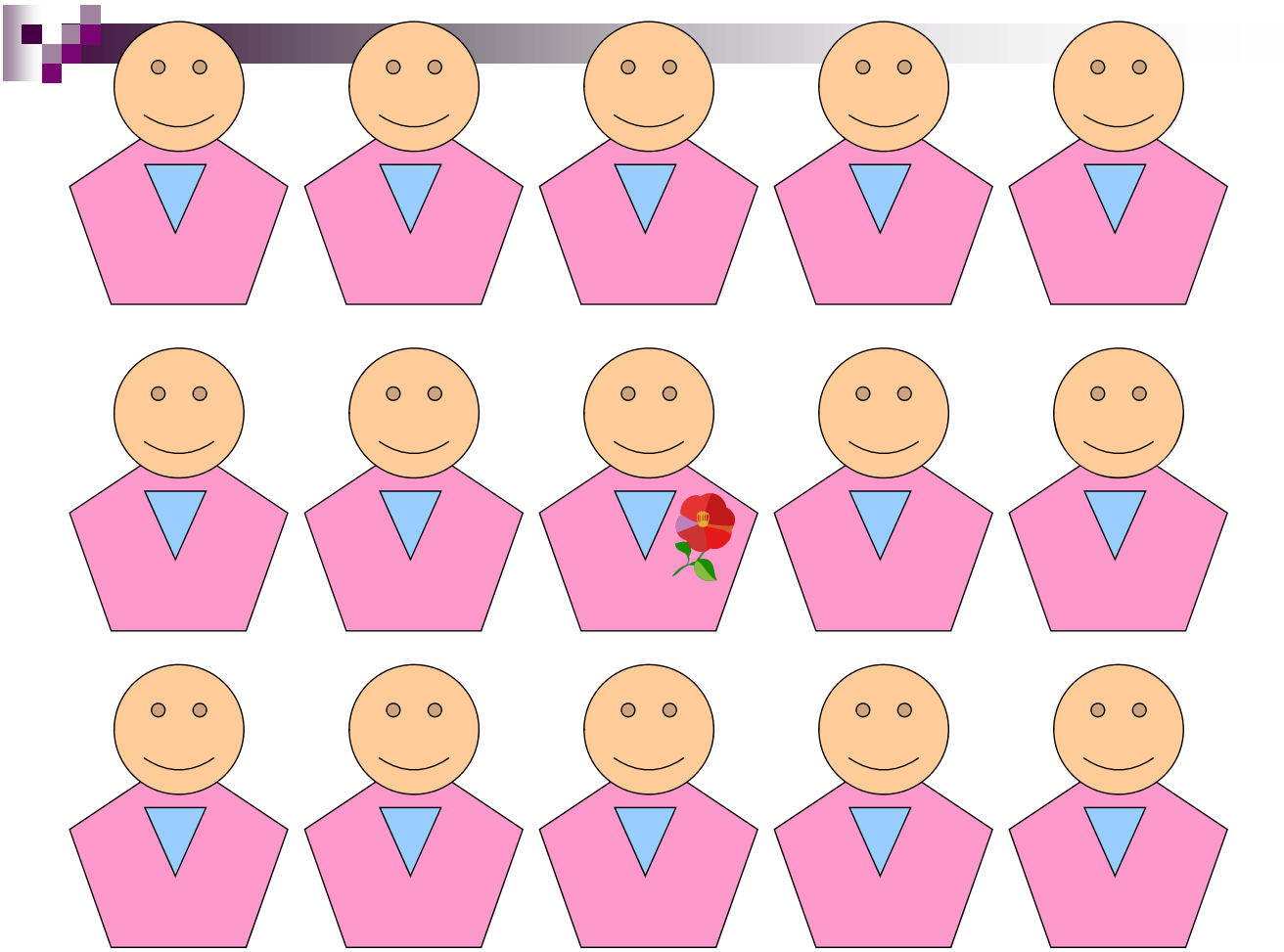
<kelitsai@cc.kmu.edu.tw> 校內分機 2244

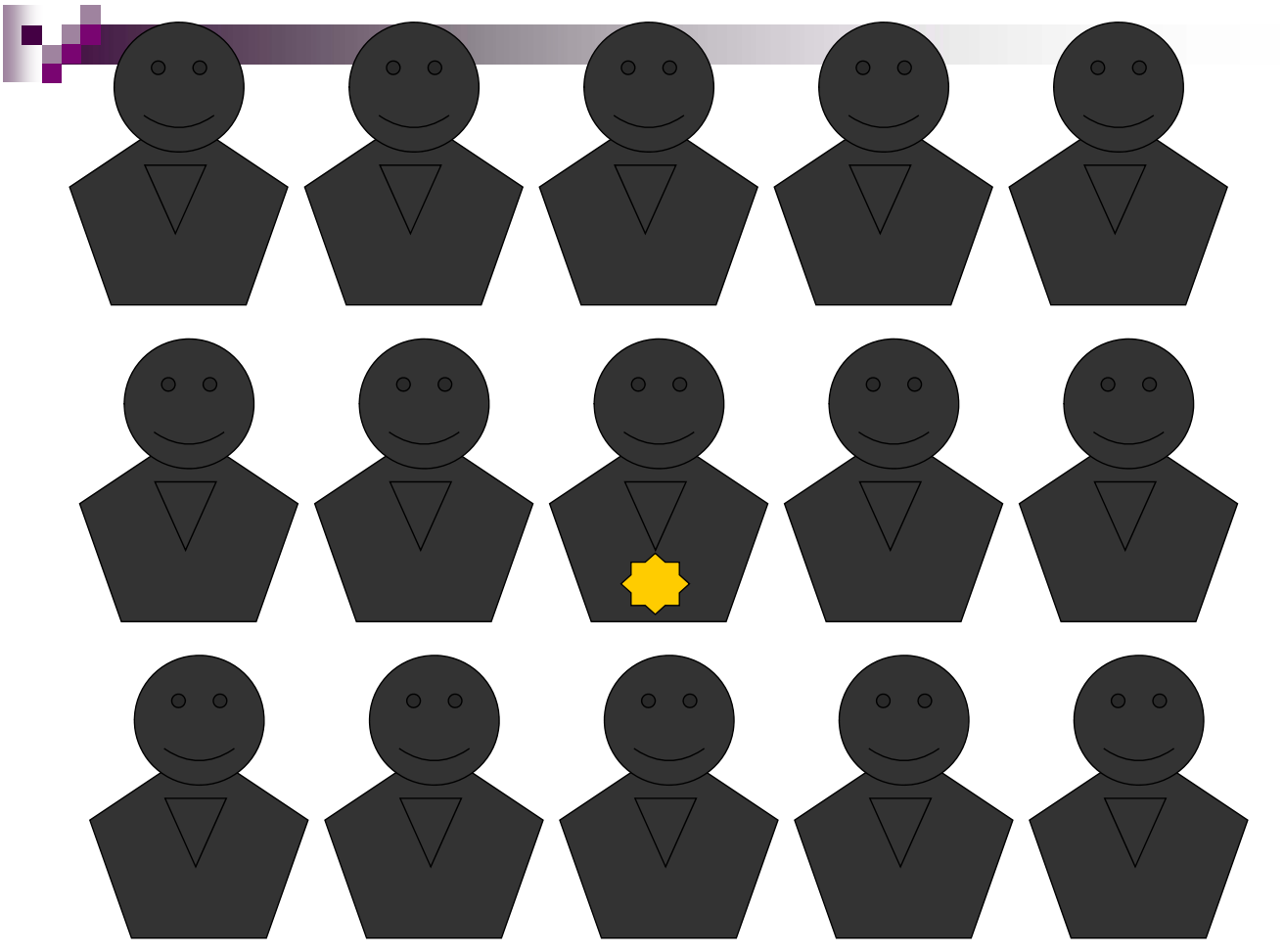
Principle of Fluorescence

Biological Imaging of the Living Cell

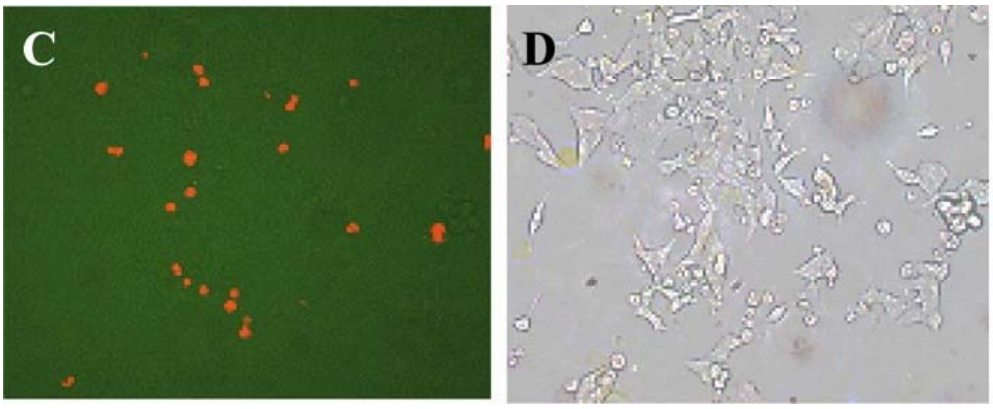
- An *in vivo* approach is required to study transport or signalling of biomolecules in living cells.
- So far, microscopy combined with fluorescence technique remains a major tool in cellular biological studies.

Science **300** (5616) 4 April, 2003





How to find the guy (or biomolecule) of your interest?

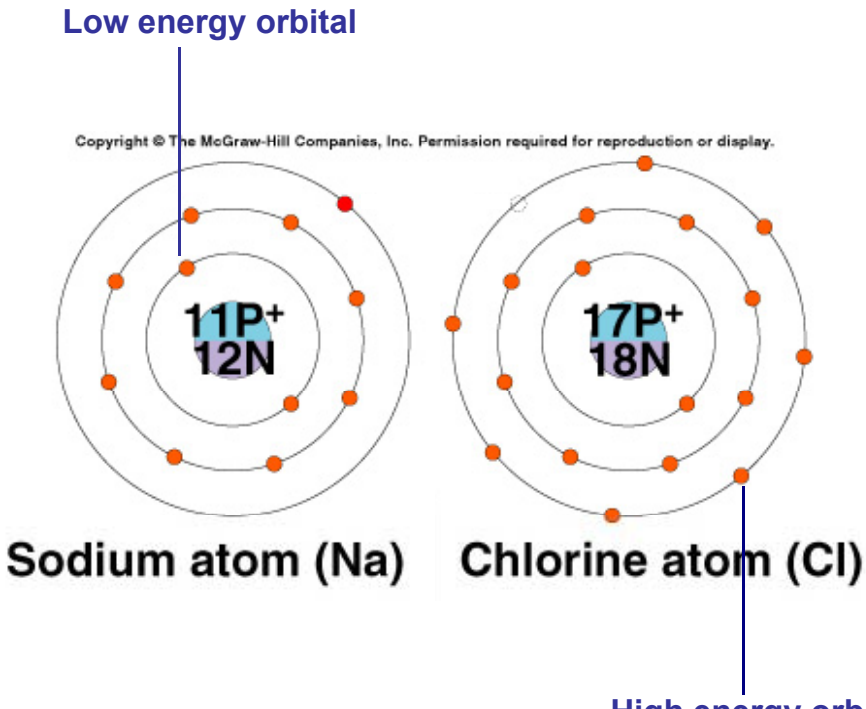


Fluorescence helps you to find the guy (or biomolecule)!

Cao et al. (2005). *Am. J. Physiol.* **289**: E543–E550

Physical Basis of Fluorescence

Electron Orbitals of Atom



Fox (2004)

Electron Transition between Energy States

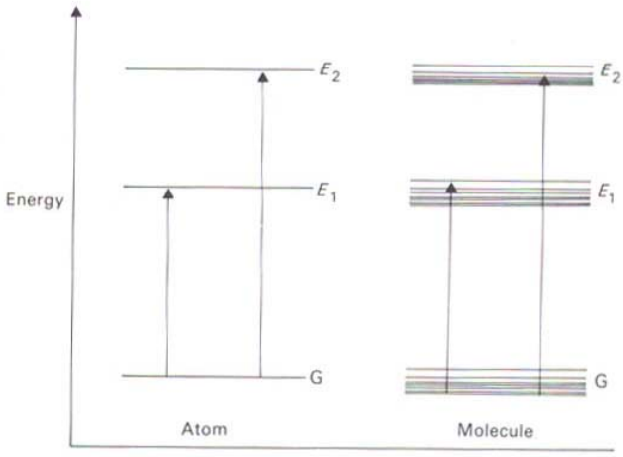


Fig. 9.25. Comparison of electronic energy states in atoms and molecules. G is the ground state and E_1 and E_2 are two possible excited states. In molecules the total number of energy states is increased because of molecular rotations and vibrations.

Excited state 2

Excited state 1

Ground state

Single atom **Molecule**

Duncan (1990)

Excitation and Relaxation of Electron Energy

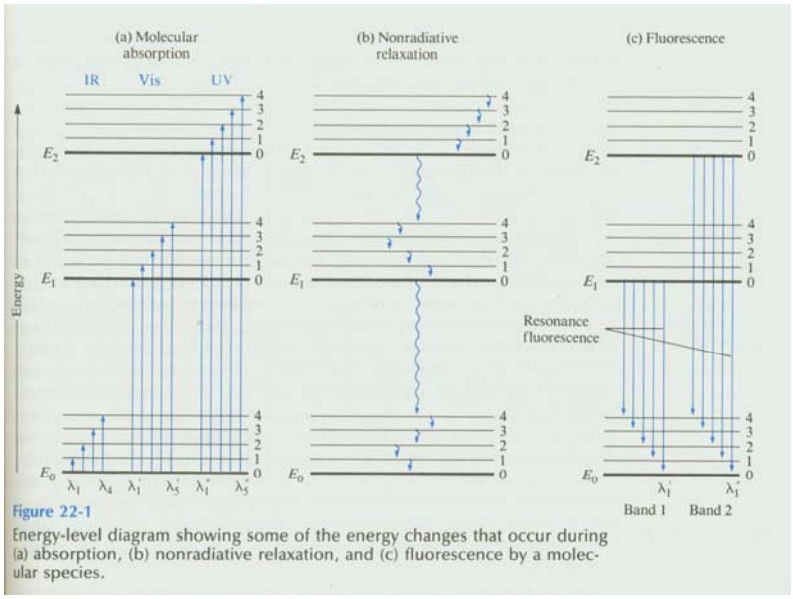


Figure 22-1
Energy-level diagram showing some of the energy changes that occur during (a) absorption, (b) nonradiative relaxation, and (c) fluorescence by a molecular species.

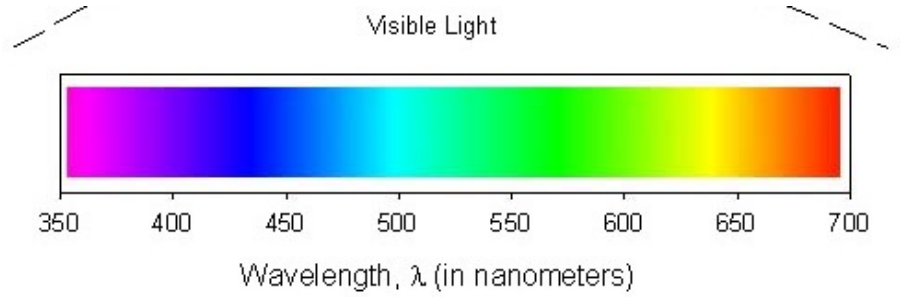
Fluorescence is the result of releasing excess energy of electrons

Skoog (1992)

Light as a Form of Energy

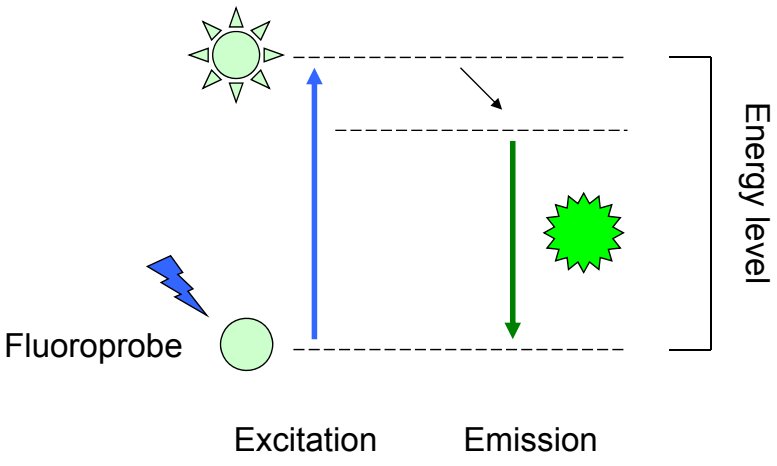
$$E = h\upsilon = \frac{h \cdot c}{\lambda}$$

E: energy of light
h: Planck's constant
c: light speed
u: frequency
λ: wave length



Shorter wavelength means higher energy

Fluorescence Mechanism



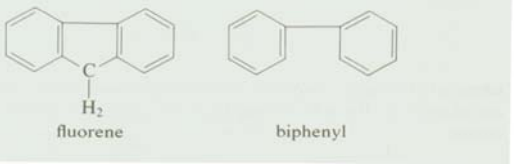
Jablonski diagram for fluorescence mechanism

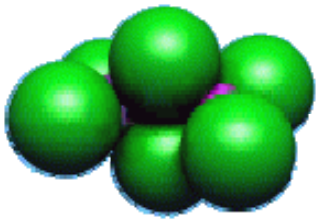
Fluorochrome

- **Substance that can be excited to emit fluorescence is a fluorescent dye, or fluorochrome**

- **Most biological fluorochrome have rigid ring structure, therefore limiting non-radiative energy transfer (e.g. vibration)**

The Effect of Structural Rigidity
It is found experimentally that fluorescence is particularly favored in rigid molecules. For example, under similar conditions of measurement, the quantum efficiency of fluorene is nearly 1.0, whereas that of biphenyl is about 0.2:





Skoog (1992)

Efficiency of Fluorescence Emission

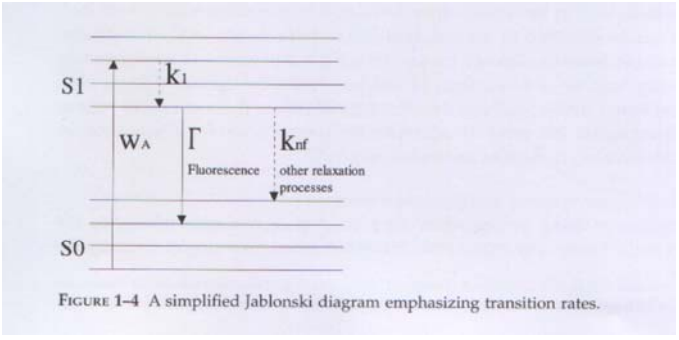


FIGURE 1–4 A simplified Jablonski diagram emphasizing transition rates.

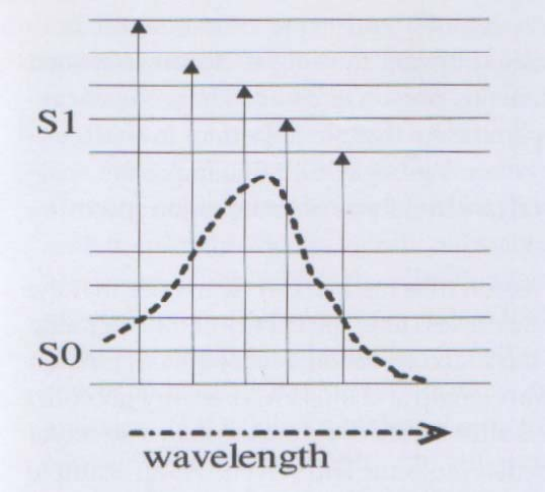
$$Q\,(\text{quantum yield}) = \frac{\Gamma}{\Gamma + k_{nf}}$$

Higher quantum yield means brighter fluorescence

Periasamy (2001)

Absorption of Light Energy and Electron Transition

Absorption spectrum



Light energy absorbed is used in the promotion of electron energy state

Periasamy (2001)

Excitation and Emission Spectra

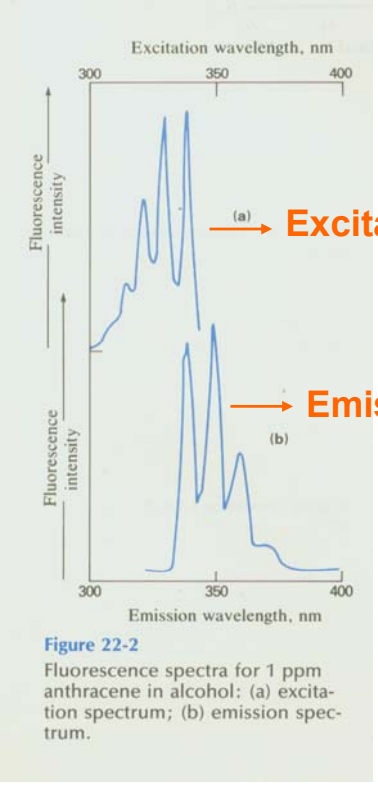
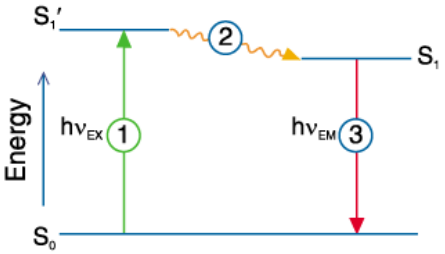


Figure 22-2
Fluorescence spectra for 1 ppm anthracene in alcohol: (a) excitation spectrum; (b) emission spectrum.



$$E = h\upsilon = \frac{h \cdot c}{\lambda}$$

υ: frequency
λ: wave length

Excitation wavelength is always shorter than emission wavelength

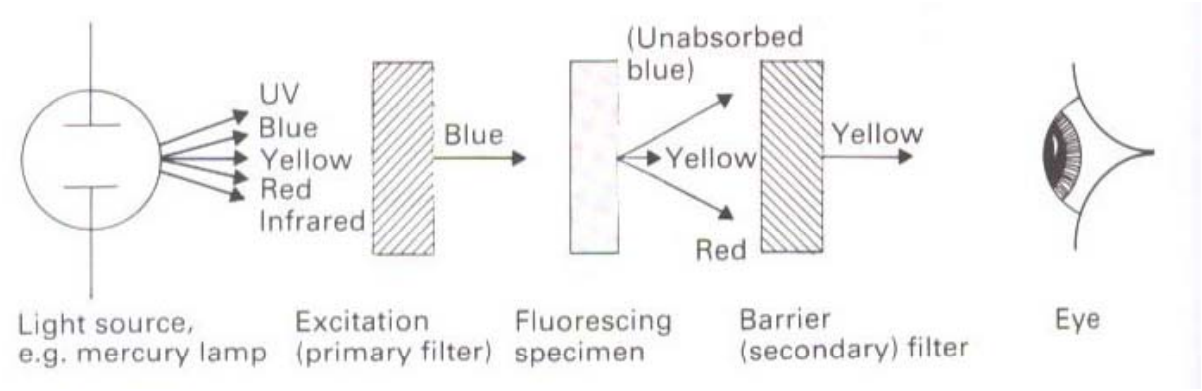
Skoog (1992)

Introduction to Fluorescence and Cellular Imaging Methods

Fluorescence and Cellular Imaging Methods

- Epi-fluorescence Microscopy and Microflurospectrometry
- Cellular Imaging
- Confocal Laser Scanning Microscopy

Basic Components in a Fluorescence Instrument



1. **Light source**
2. **Excitation filter**
3. **Fluorescing specimen (fluorochrome)**
4. **Emission filter**

Duncan (1990)

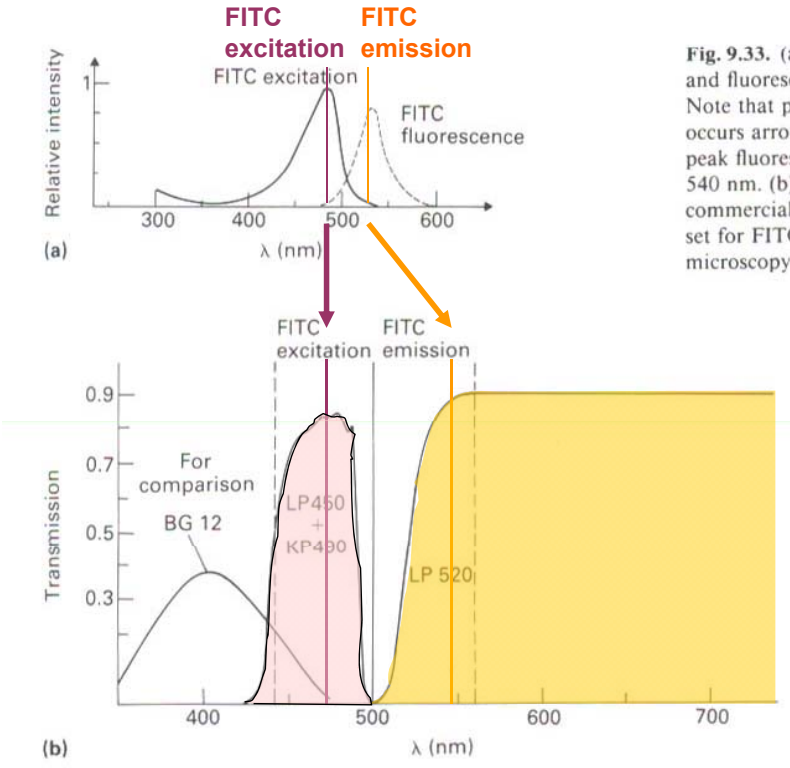


Fig. 9.33. (a) FITC excitation and fluorescence characteristics. Note that peak excitation occurs arround 480 nm while peak fluorescence occurs at 540 nm. (b) Typical commercially available filter set for FITC fluorescence microscopy (Zeiss).

Filter types
1. **Band-pass**
2. **Long-pass**
3. **Short-pass**

Excitation filter (Band -pass)

Emission filter (Long- pass)

Duncan (1990)

Dichromatic Mirror

Long-pass Dichromatic Mirror (Beam Splitter)

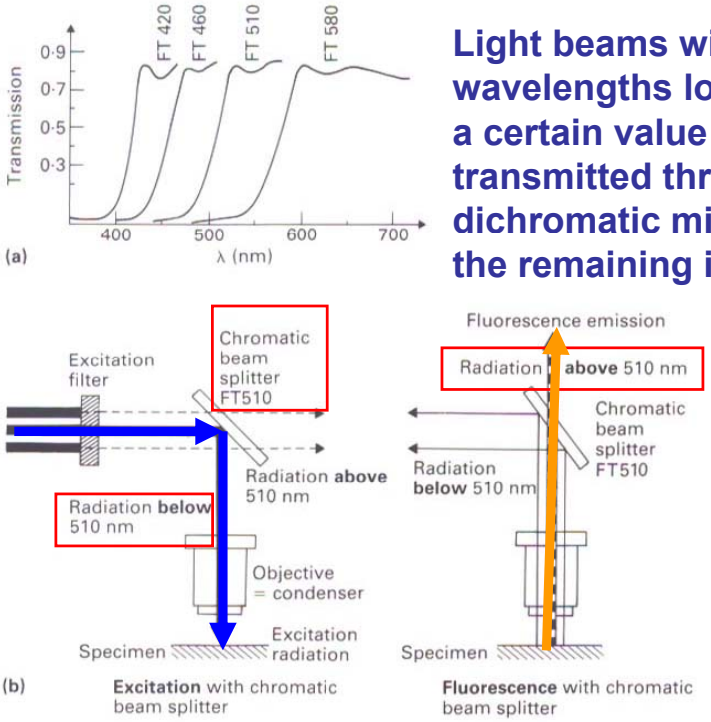
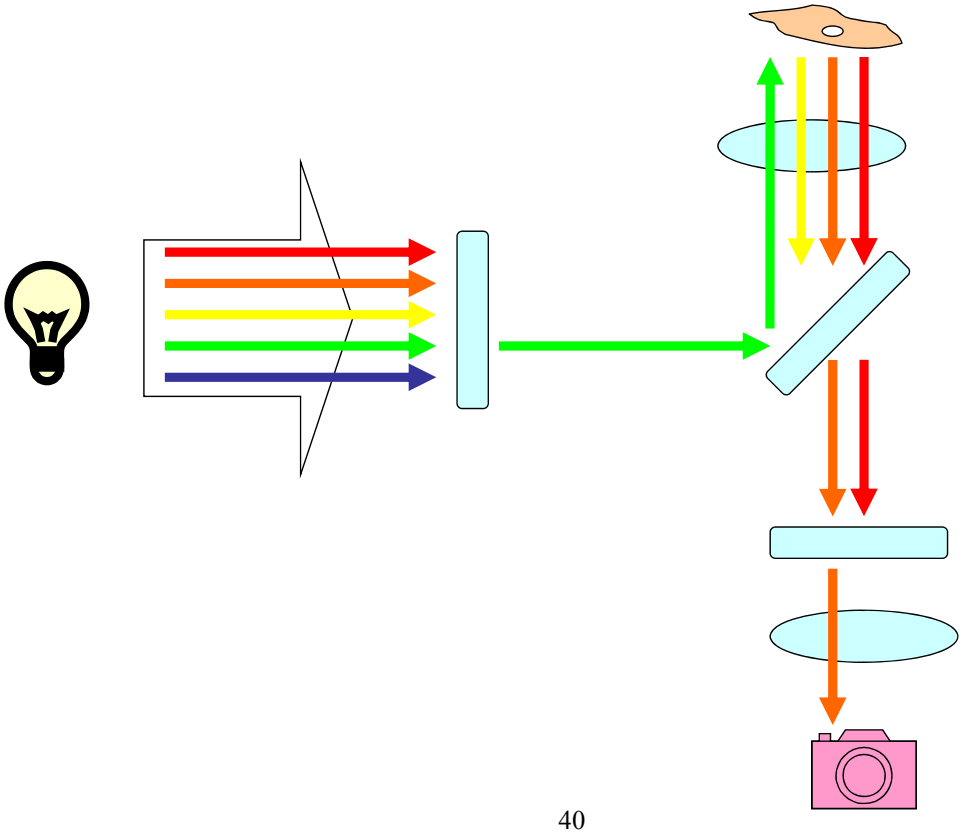


Fig. 9.34. (a) Examples of transmission curves of commercially available chromatic beam splitters. The *reflection* curves for each are the mirror-inverted images of the above. (b) Epifluorescence system for FITC-labelled specimens (after Holz, 1977).

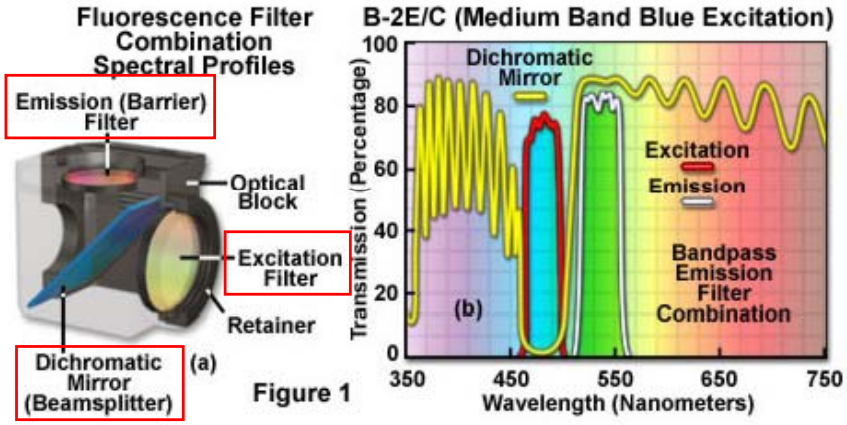
Light beams with wavelengths longer than a certain value is transmitted through the dichromatic mirror, while the remaining is reflected

Duncan (1990)

Epi-fluorescence Microscope Set-up



Fluorescence Filter Combination Cube



Fluorescence filter combination cube consists of:

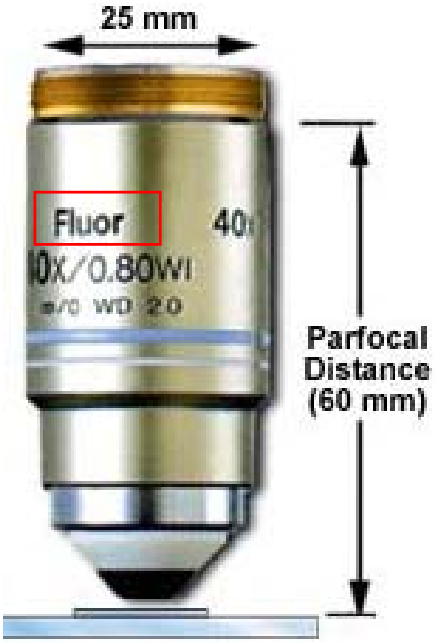
1. **Excitation filter**
2. **Dichromatic mirror (beam splitter)**
3. **Emission filter**

Nikon Microscope

Fluorescence Microscope

高雄醫學大學・醫研部精密儀器室

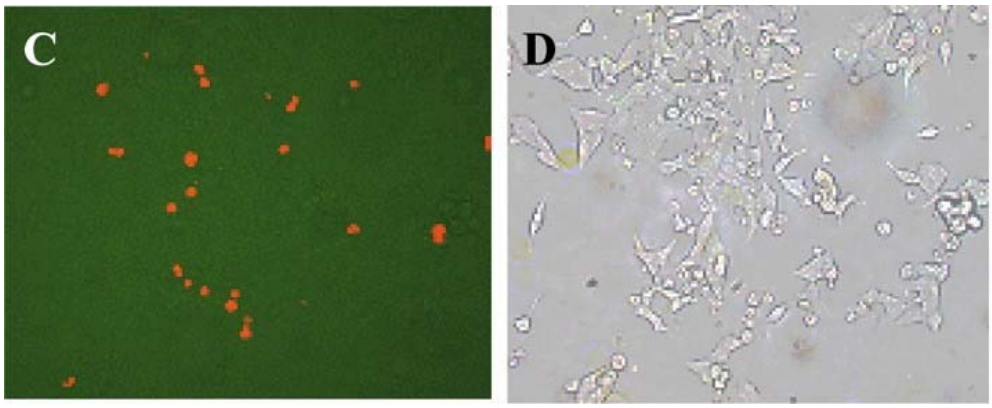
Objective Lens for Fluorescence Microscopy



Fluorescence objective lens permits ultra-violet light to pass

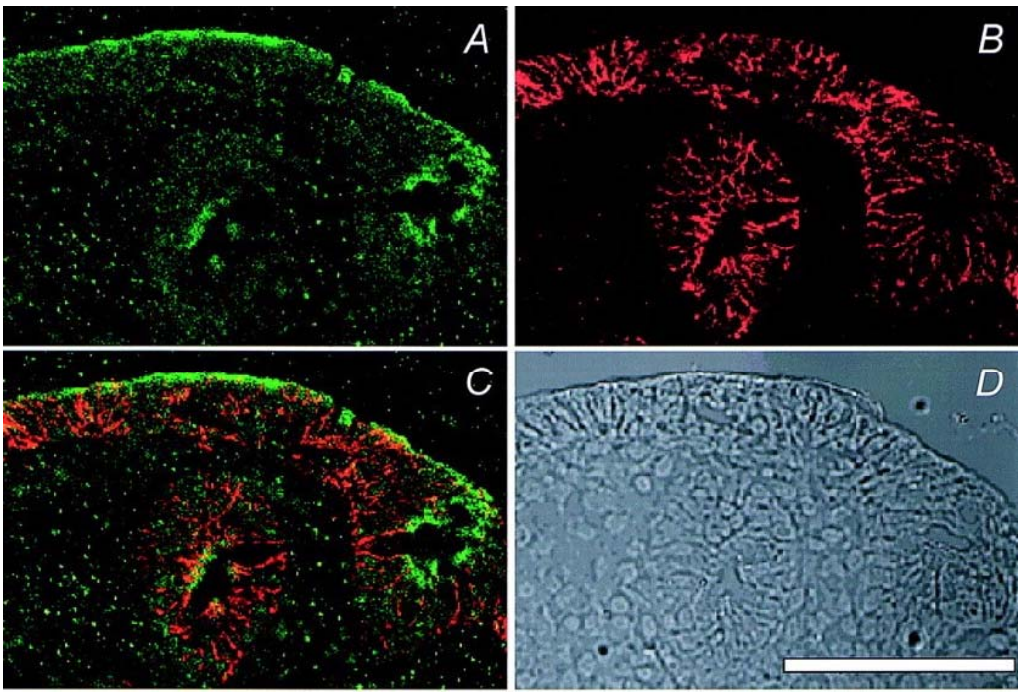
Molecular Expressions

Fluorescence **Bright-field**



Cao et al. (2005). *Am. J. Physiol.* **289**: E543–E550

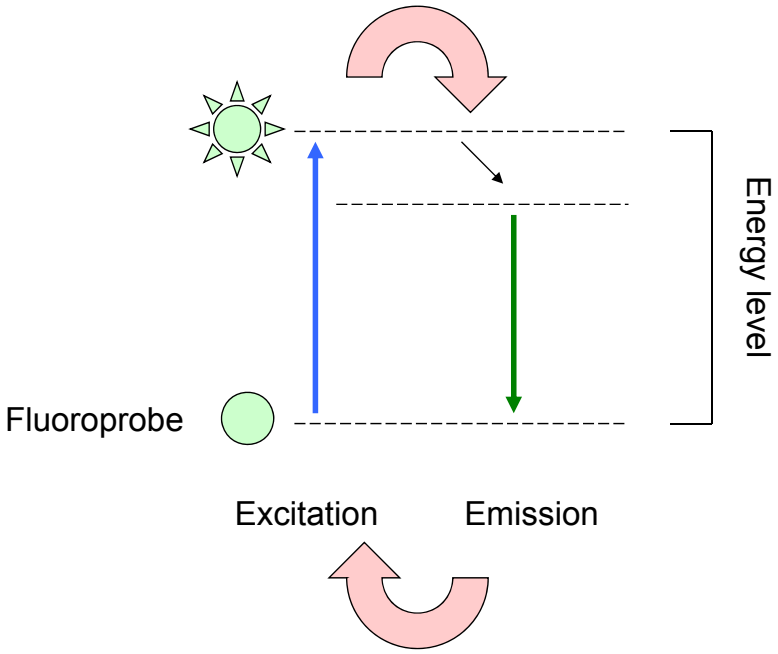
X molecule (Green Fluorescence) Y molecule (Red Fluorescence)



Merge Image Bright-field

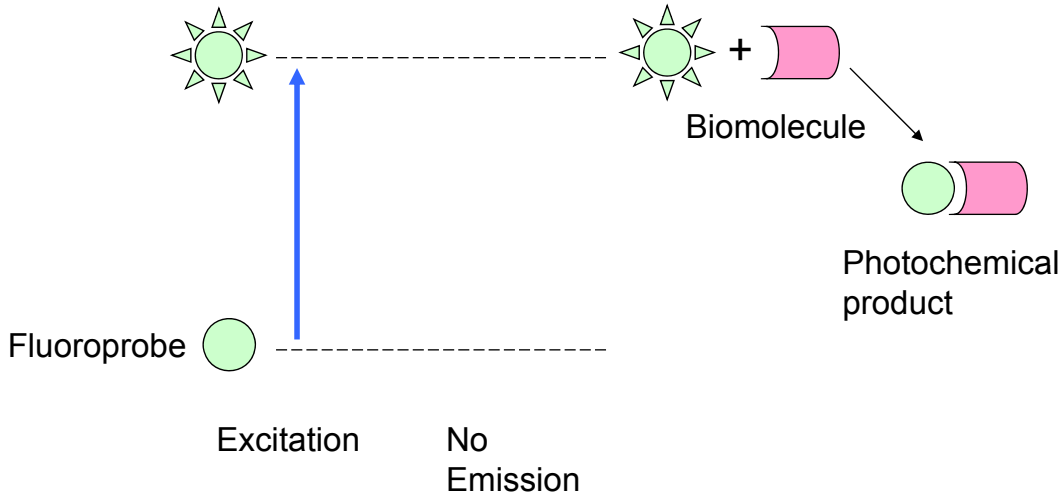
Gallardo et al. (2001). *Am. J. Physiol.* **281**: G856-G861

Ideal Fluorescence Performance



Photobleaching

Excited fluoroprobe reacts with biomolecules in the specimen, causing a decay of fluorescence.



Difficulties in Microspectrofluorimetry

Photobleaching

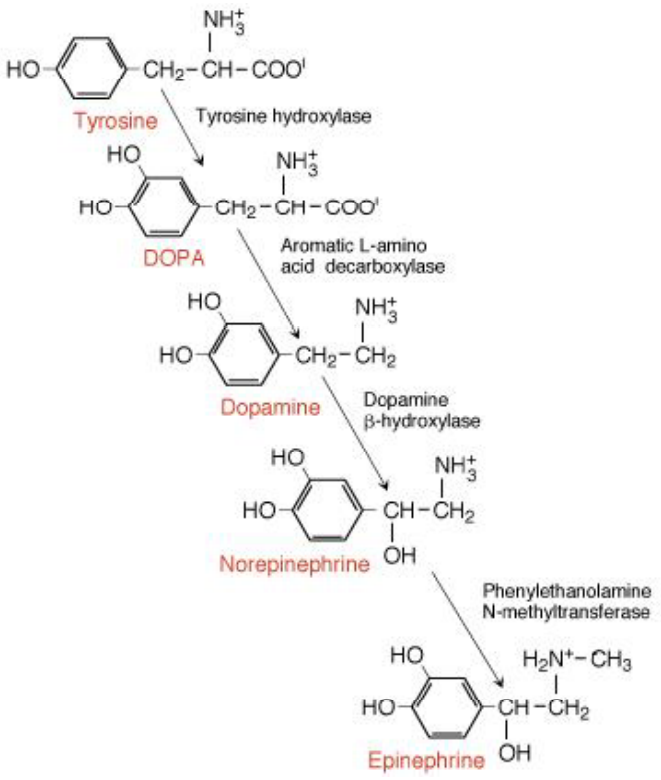
- Shorten exposure to excitation light
- Reduce excitation light intensity
- Lower working temperature

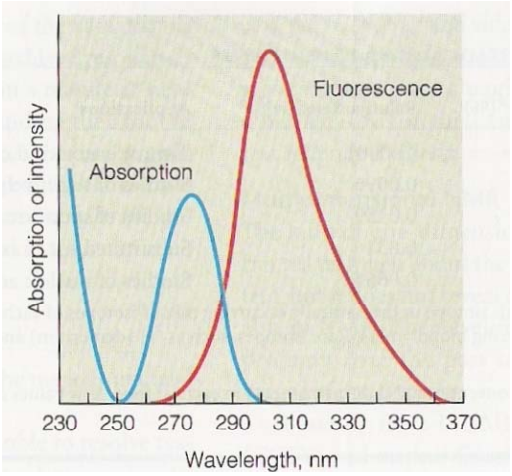
Difficulties in Microspectrofluorimetry

Weak fluorescence

- Increase excitation light (Cons: photobleaching)
- Increase amplification of PMT (Cons: noise is simultaneously amplified)
- Choose fluoroprobe with high quantum yield

Autofluorescence of Catecholamines



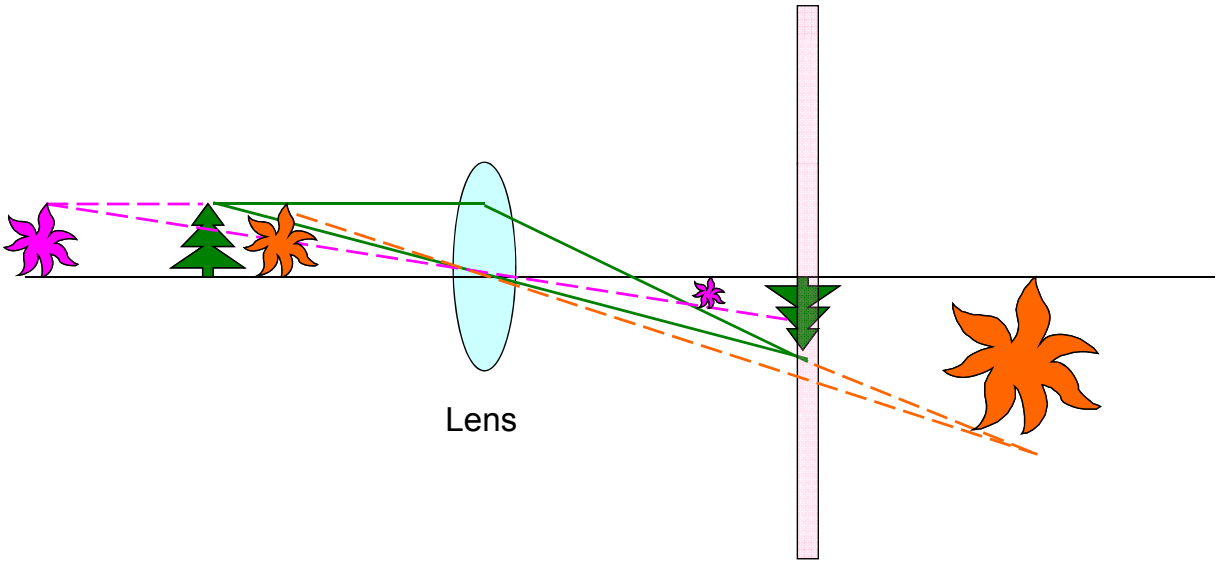


Tyrosine spectra

Confocal Laser Scanning Microscope

Dimensional illusion

Out-of-focus Image



Optical Sectioning of Thick Specimen

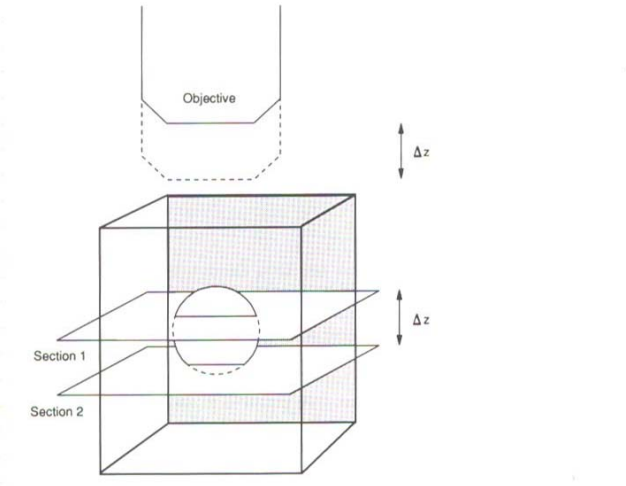


FIGURE 5.1: *Optical sectioning. Two optical sections are formed by focusing up through the specimen by a distance Δz.*

3-D microscopy enhances optical sectioning for observation.

Rawlins (1992)

Confocal Laser Scanning Microscope

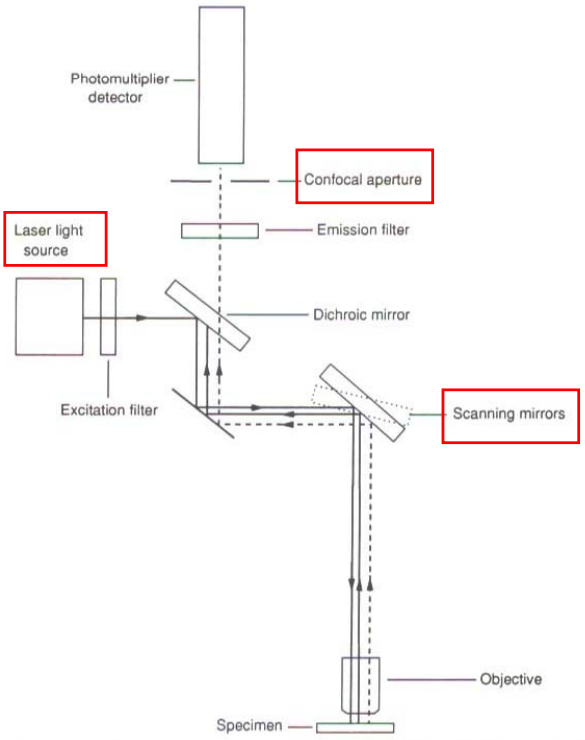
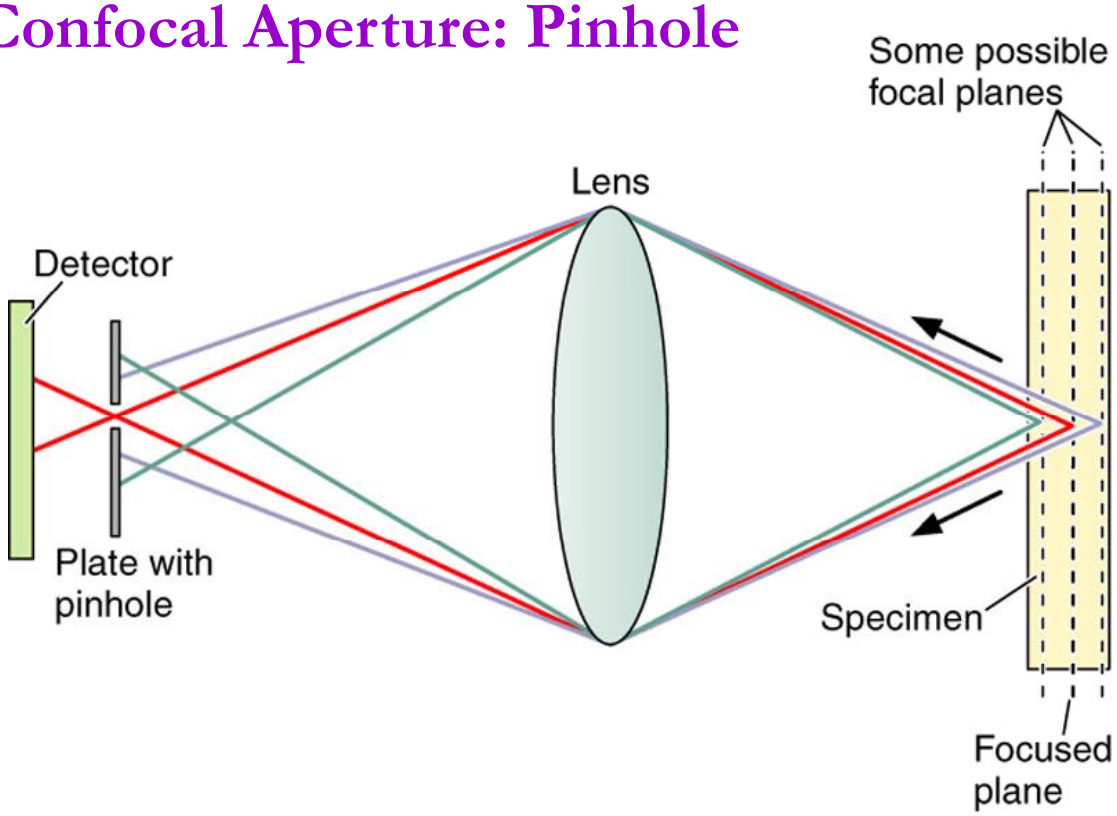


FIGURE 5.3: *The components of a confocal laser scanning microscope (CLSM).* *Rawlins (1992)*

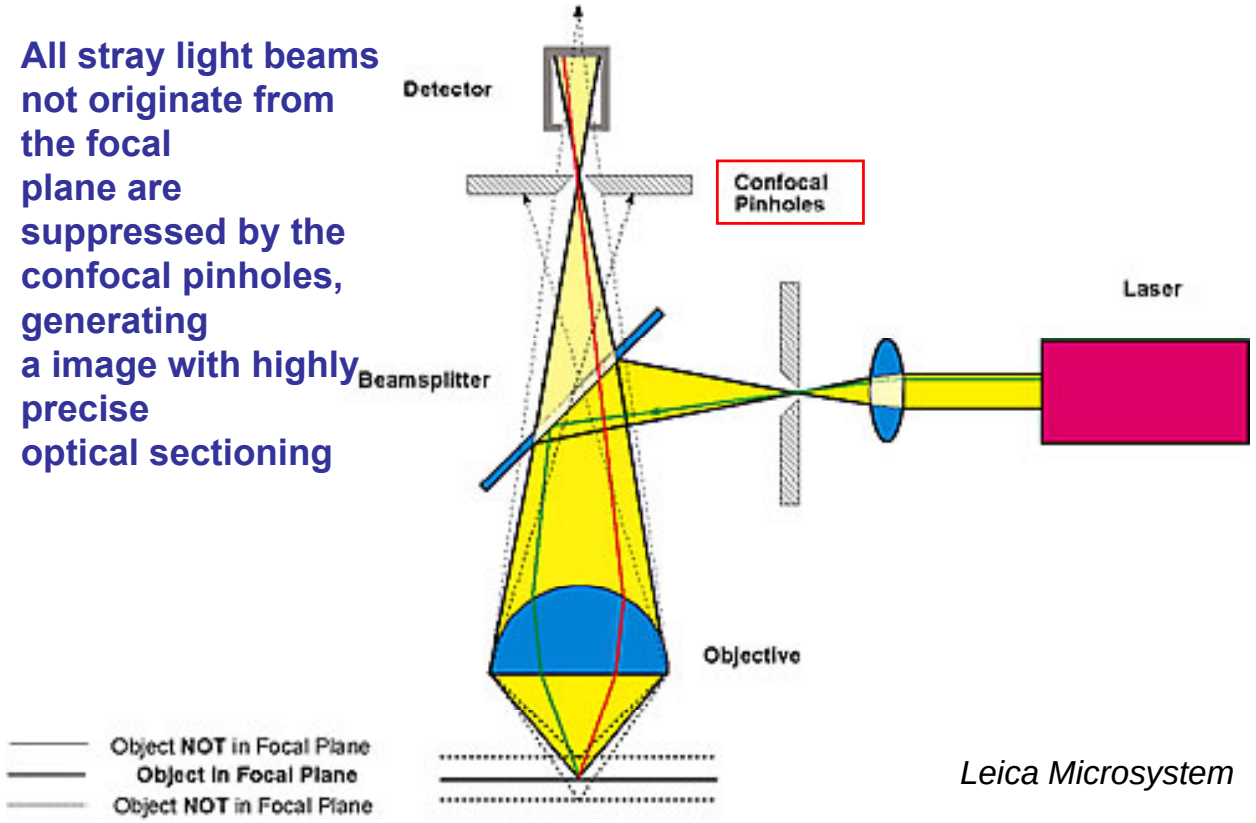
Confocal Aperture: Pinhole



Confocal microscope has high precision in focusing, so it is better in optical sectioning than traditional light microscope.

Confocal: Conjugate Focus

All stray light beams not originate from the focal plane are suppressed by the confocal pinholes, generating a image with highly precise optical sectioning



Leica Microsystem

Laser Light Source

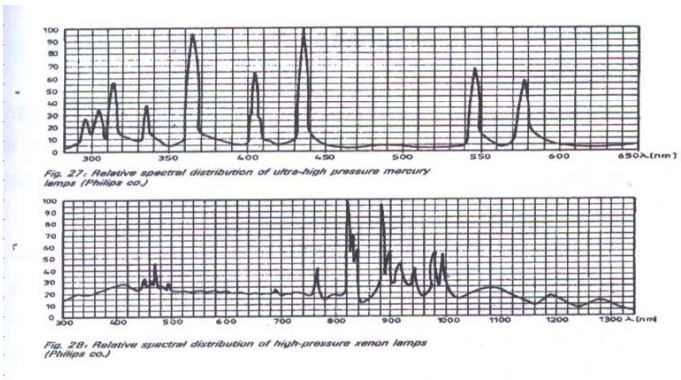
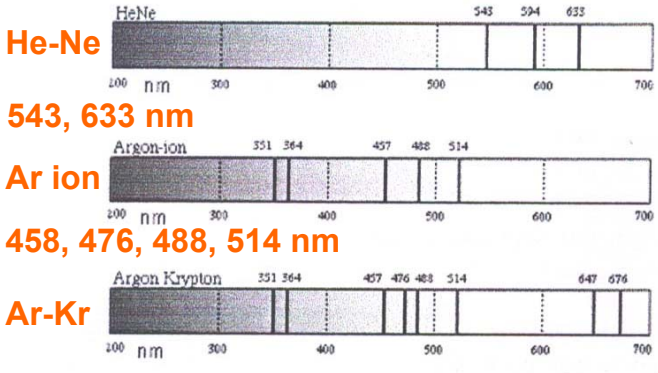


Fig. 27: Relative spectral distribution of ultra-high pressure mercury lamps (Philips co.)

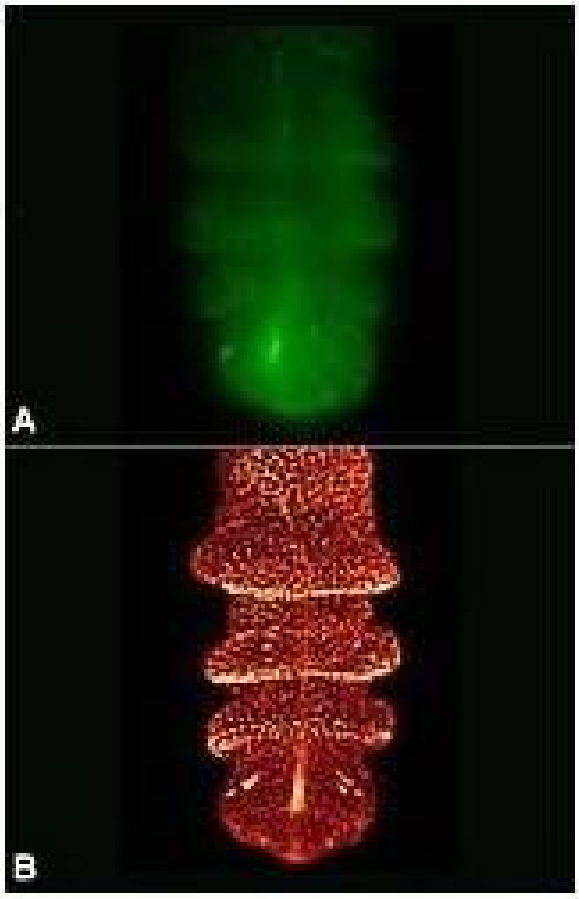
Fig. 28: Relative spectral distribution of high-pressure xenon lamps (Philips co.)

Xenon lamp used in traditional epi-fluorescence microscope is a multi-wavelength light source



Laser light source has discrete wavelengths, it can be used as "single wavelength" light

Leica Microsystem

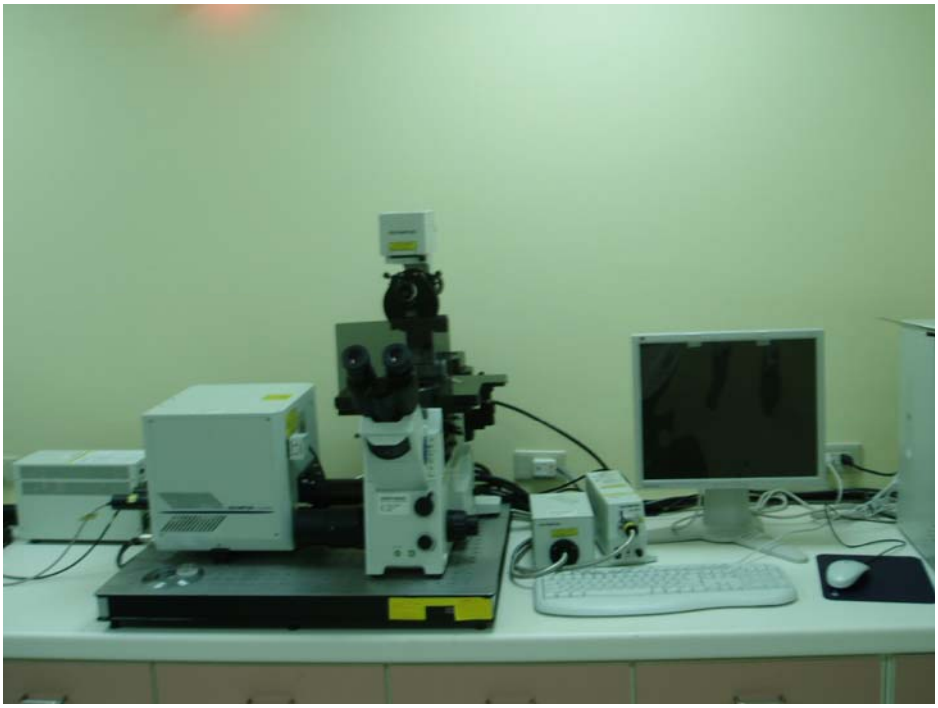


Traditional fluorescence microscope image

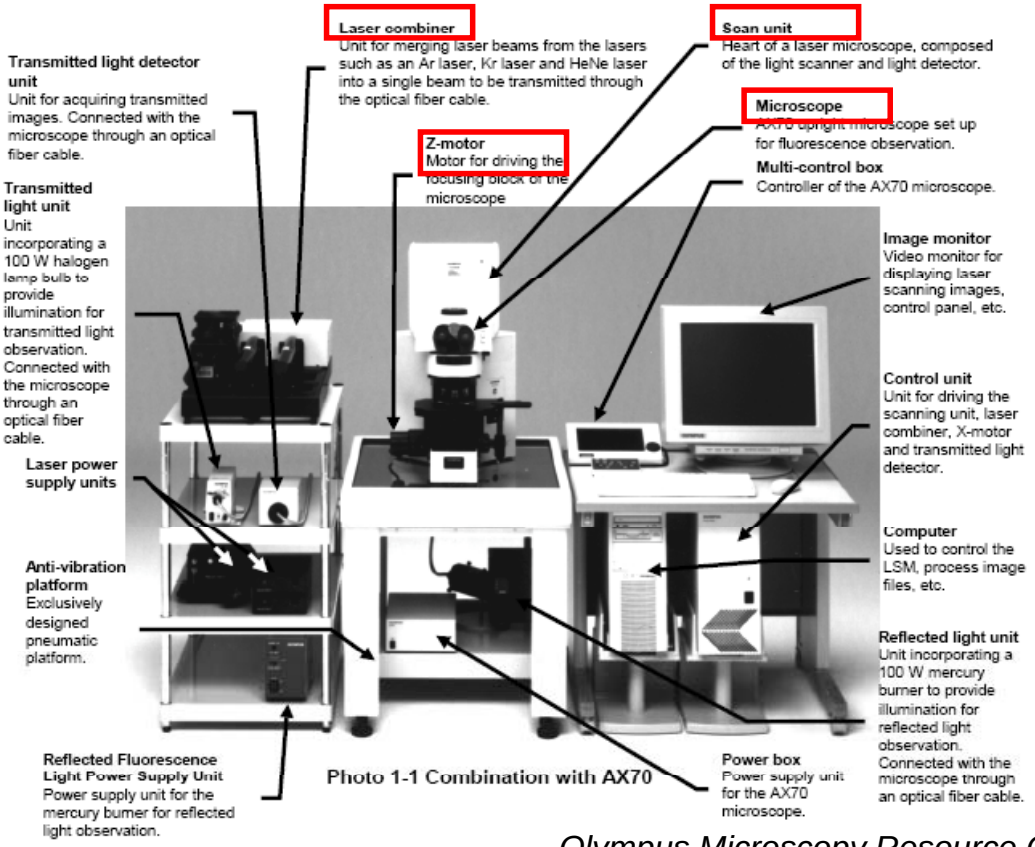
Confocal microscope image

Leica Microsystem

Confocal Laser Scanning Microscope



高雄醫學大學・醫研部精密儀器室



Olympus Microscopy Resource Center

Scanning Apparatus

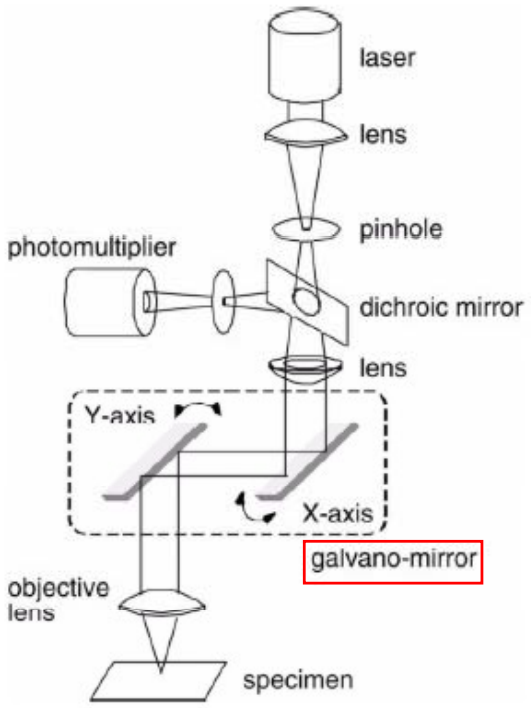


Fig. 1. Conventional confocal laser scanning microscope. Laser beam that passes though a pinhole is focused on the specimen as a light point. This point is scanned over X- and Y-axis by a mechanical way, i.e. action of mirrors (galvano-mirror method). Emitted fluorescence is detected by a photomultiplier and reconstructed to a 2-D image by a computer. The demerit of this method is the slowness of scanning.

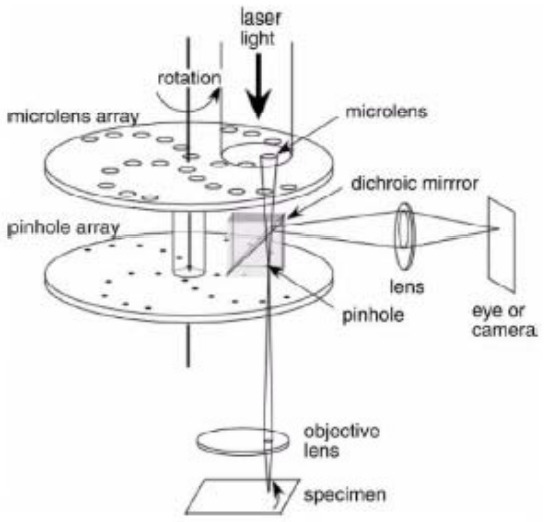


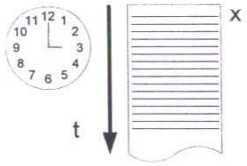
Fig. 2. Nipkow disk confocal laser scanning microscope with microlens. The Nipkow disk method utilizes a spinning disk with multiple pinholes. The problem of irradiation efficiency is markedly improved by introduction of another disk with microlenses (Yokogawa patents). This method has enabled scanning at as fast as 1000 frames/sec. Since the light axis never moves during scanning, fluorescent signals produce a real image, which can be directly viewed by eye or captured by camera.

Rawlins (1992)

Common Scanning Mode Used in Laser Scanning Confocal Microscopy

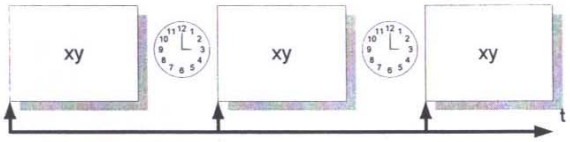
- Line-Mode „xt“

  Line scan



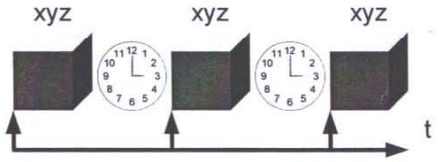
- Frame-Mode „xyt“

  Frame scan



- Stack-Mode „xyzt“

  3-D scan

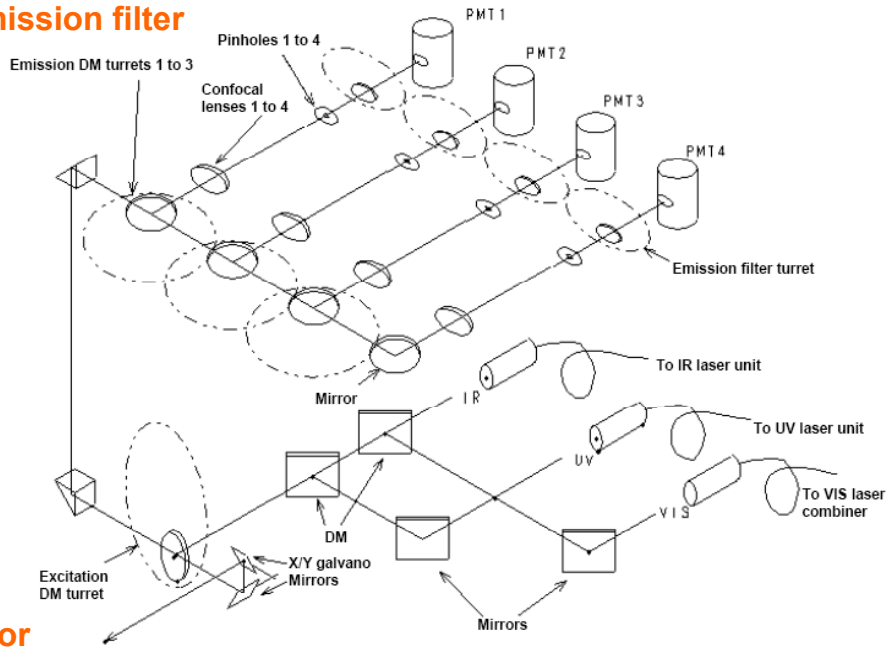


Leica Microsystem

Light Path in Laser Scanning Confocal Microscopy

Photomultiplier tube

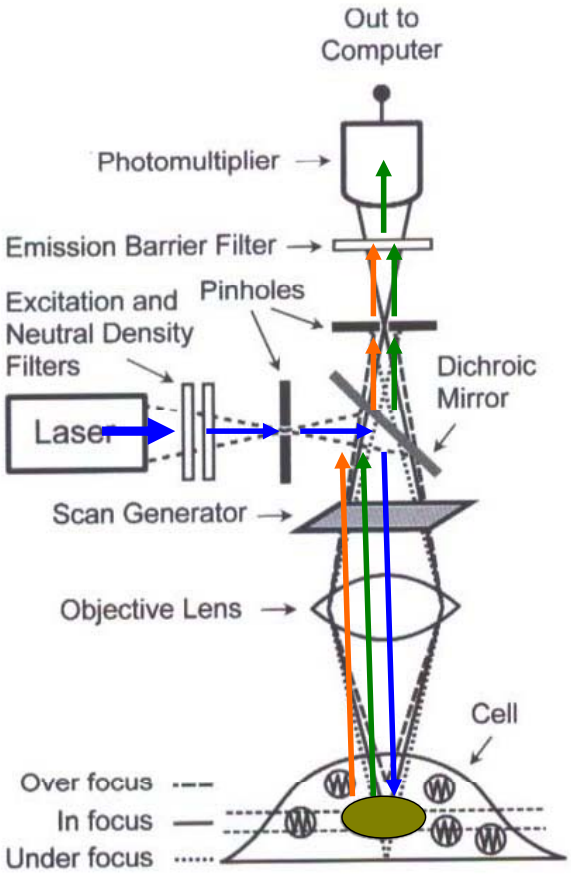
Emission filter



Dichroic mirror

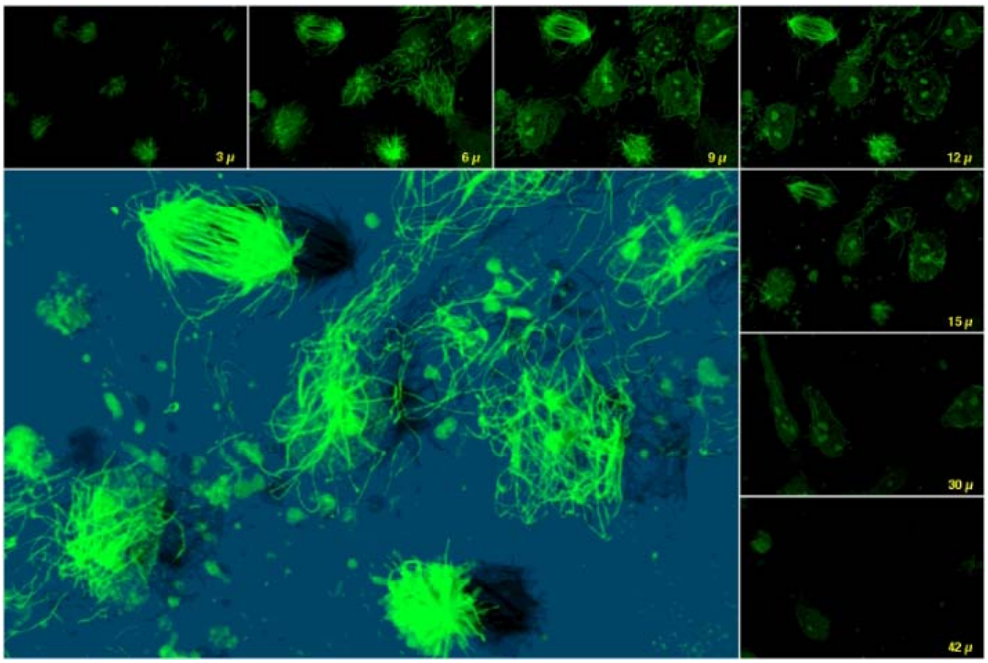
Scanner (Galvano-mirror)

Olympus Microscopy Resource Center



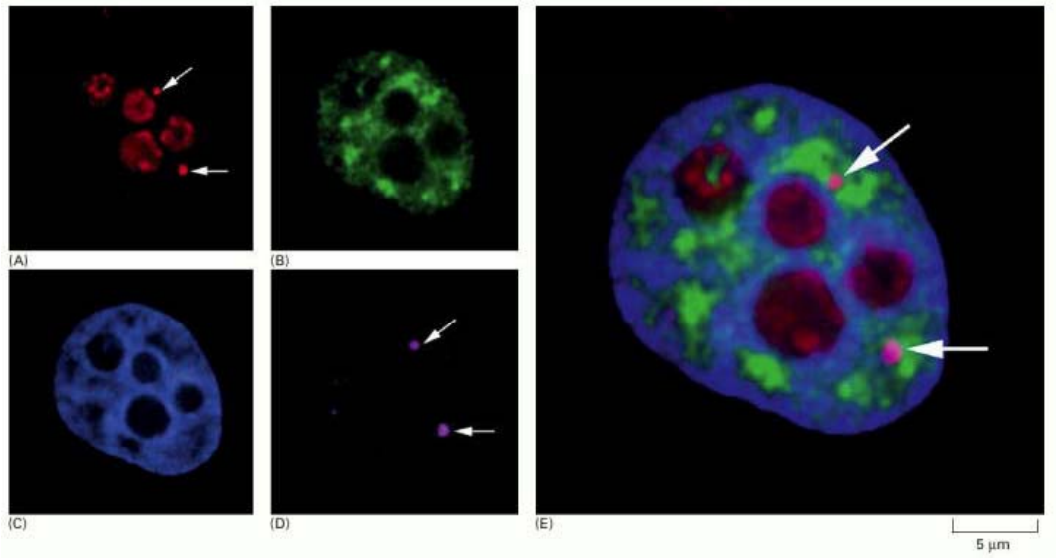
Periasamy (2001)

XYZ Scanning

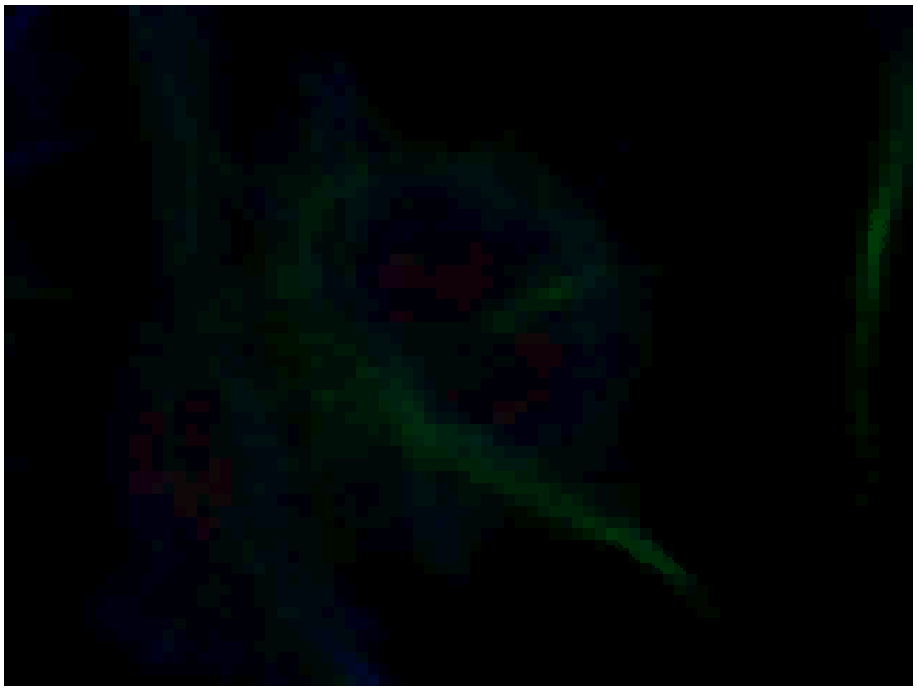


M.E. Müller Institute for Microscopy

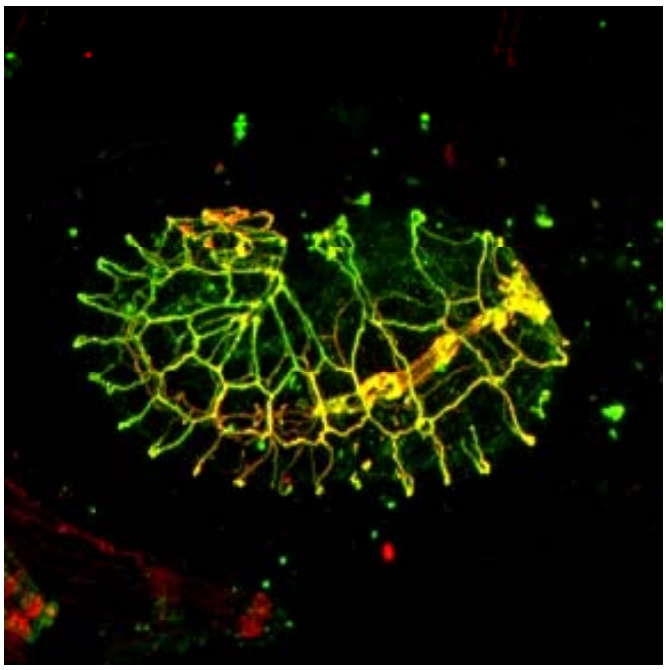
Determination of Subcellular Localisation of Biomolecules



Alberts et al. (2002)



XYZT Scanning of Cell



Combination and rotation of 3-D images

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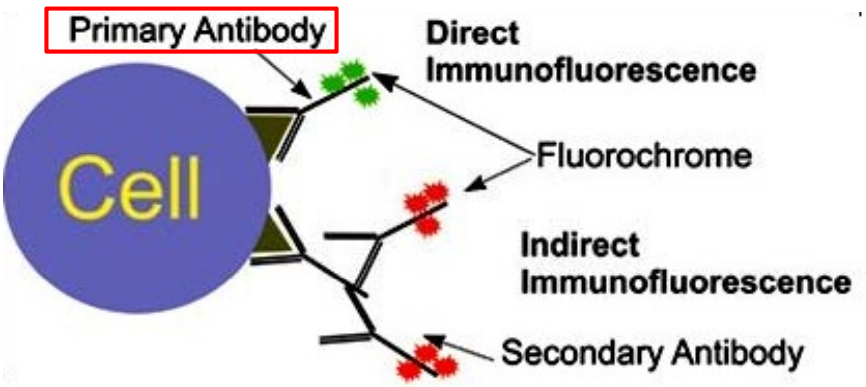
Source of Images

- Carl Zeiss Micro Imaging (http://www.zeiss.com/micro)
- Junqueira & Carneiro, *Basic Histology*, 10th edn, McGraw Hill, 2003
- Leica Microsystem (http://www.leica-microsystems.com)
- Molecular Expressions (http://micro.magnet.fsu.edu/index.html)
- PEMED Home (http://www.pemed.com)
- Nikon Microscope (http://www.microscopyu.com)
- Olympus Microscopy Resource Center (http://www.olympusmicro.com)
- M.E. Müller Institute for Microscopy (http://www.mih.unibas.ch)

免疫螢光染色
原理及應用

孫昭玲 副教授
研資中心主任
醫研所

ImmunoFluorescence
免疫螢光染色法

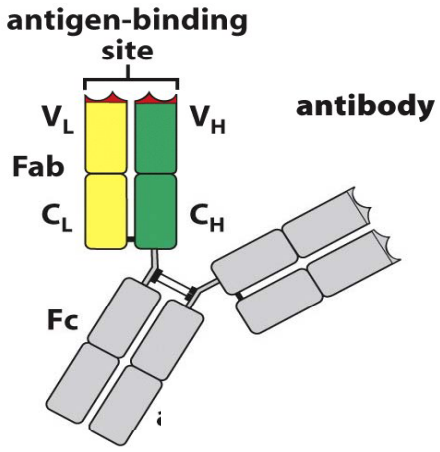


Oral Surgery, Oral Medicine, Oral Radiology, 2014, Vol. 2, No. 1

Immuno-Fluorescence (IF)

- Principle of Immuno-fluorescence
- Antibody
  - Monoclonal vs. Polycolonal antibody
  - Host/isotype
  - Non-specific binding
  - Cross-reactivity
- Fluorochrome
- IF protocol
- IF pictures

Antibody (Ab) structure 抗體



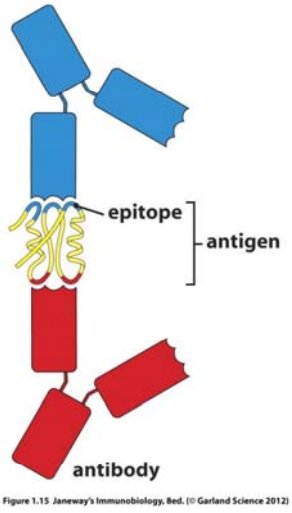


Figure 1.15 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Computer simulation of Ag-Ab interaction

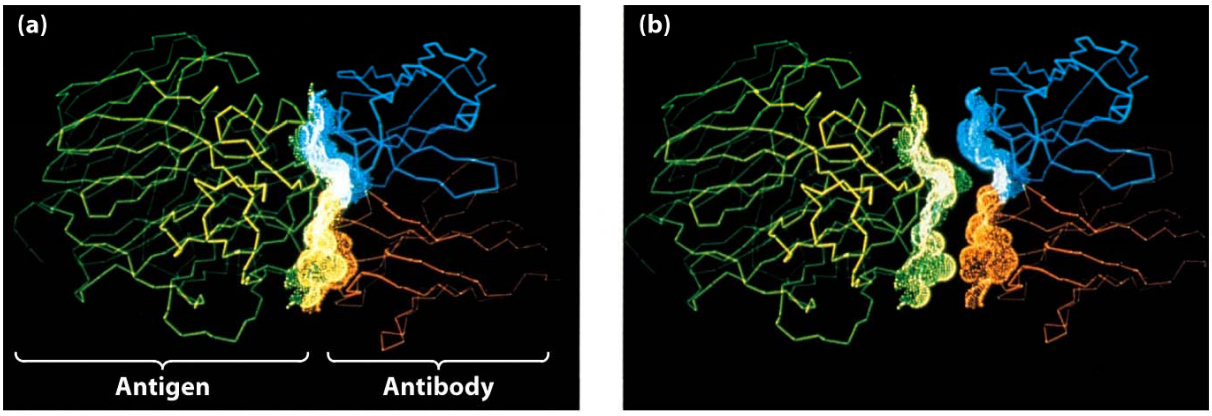


Figure 3-26
Kuby Immunology, Seventh Edition
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Ab preparation

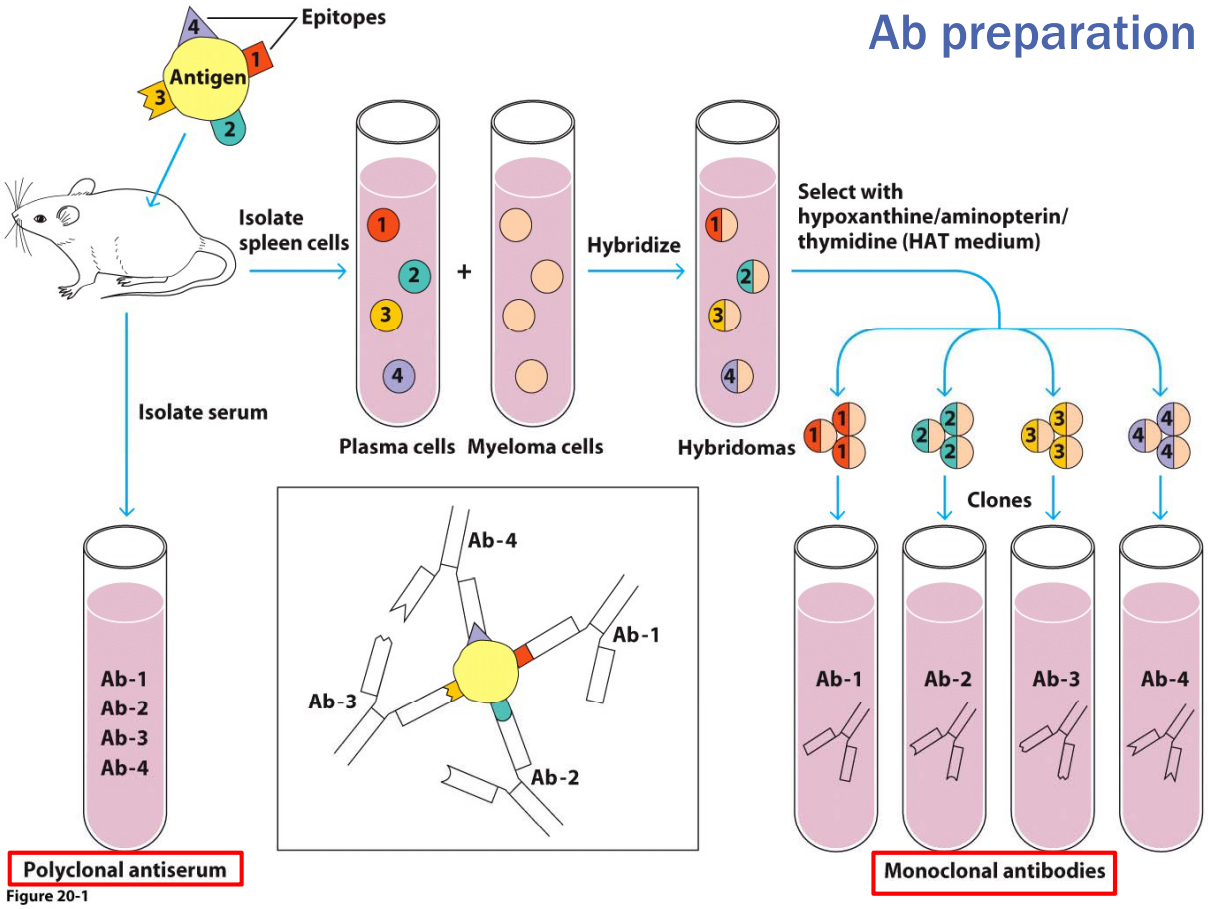

Figure 20-1
Kuby Immunology, Seventh Edition
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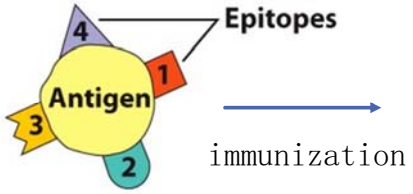
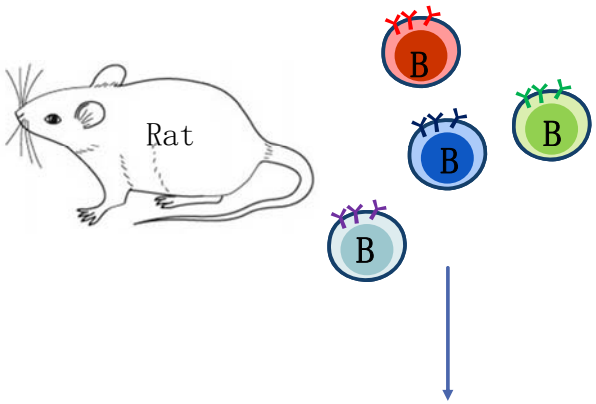
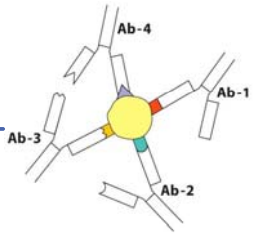
Polyclonal Ab

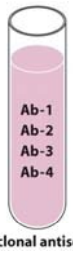


immunization



Mouse AhR (mAhR)





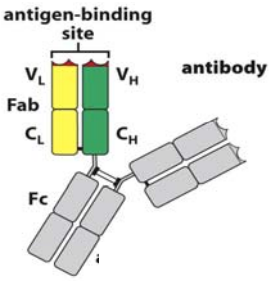
Polyclonal antiserum

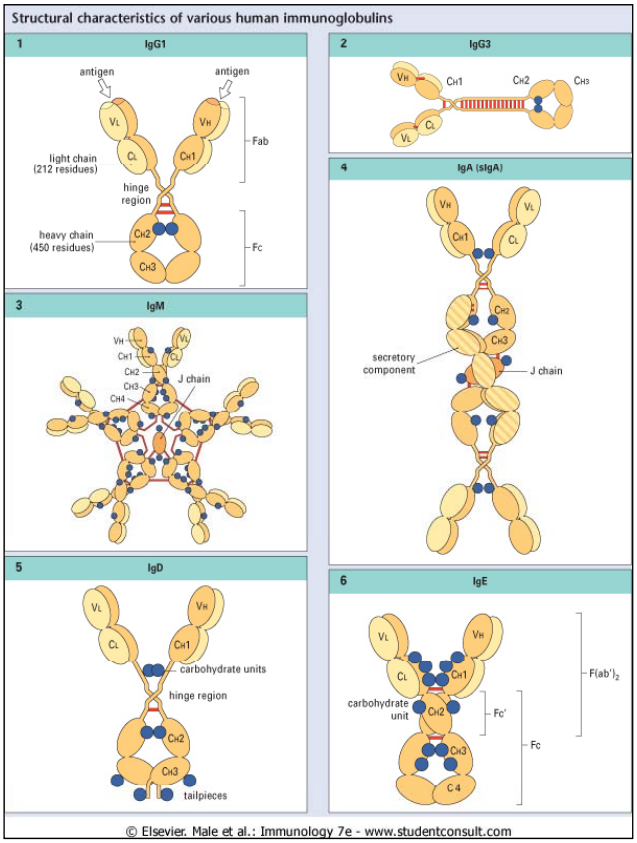
Rat anti - mouse AhR polyclonal IgG

Host Ag isotype

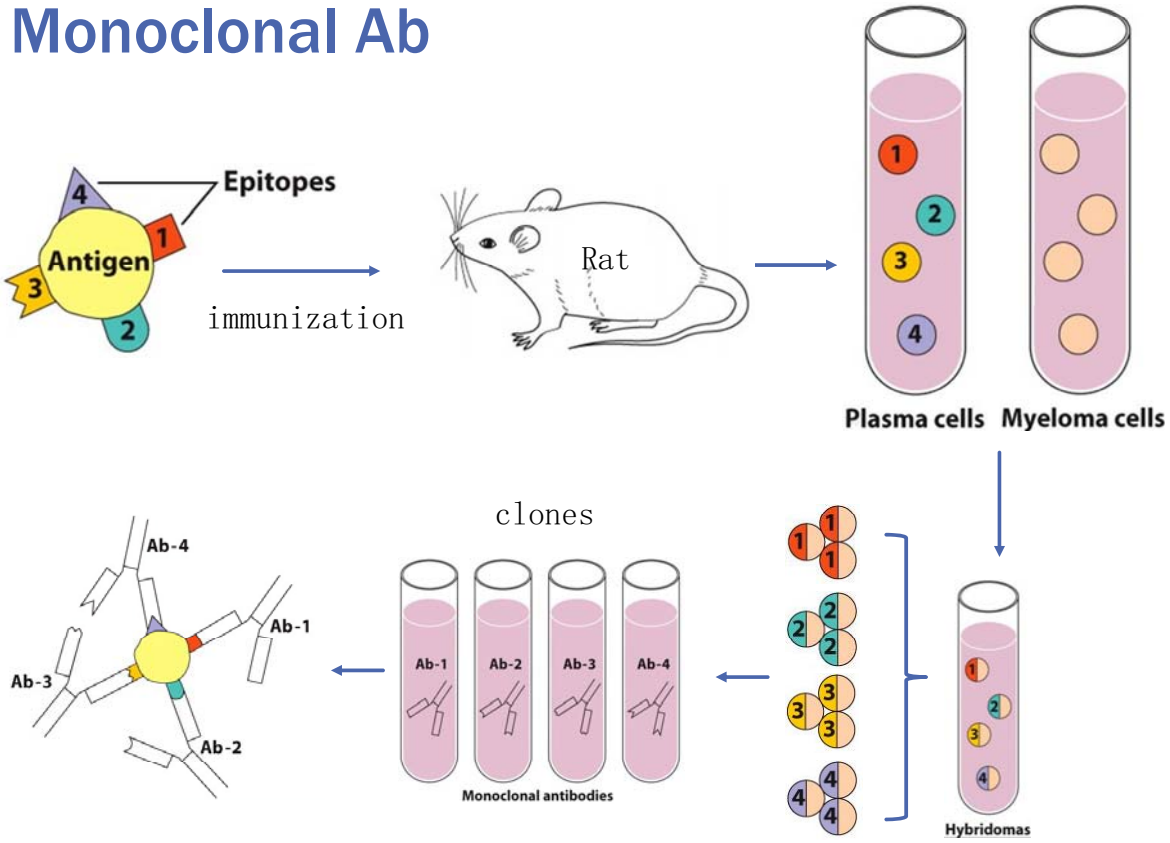
Primary Ab

- Isotype
  - IgG1, IgG2a (最常見)
  - IgM
  - IgA (少見)
  - IgE (少見)





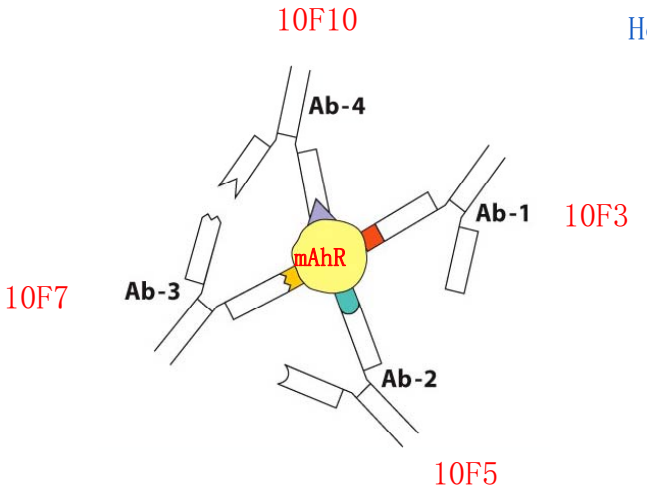
Monoclonal Ab



Monoclonal Ab

Rat anti - mouse AhR monoclonal IgG

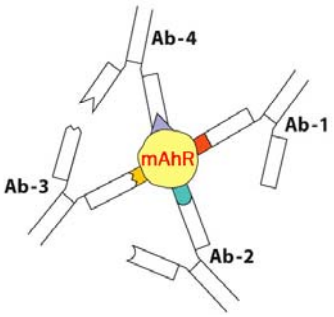
Host Ag isotype



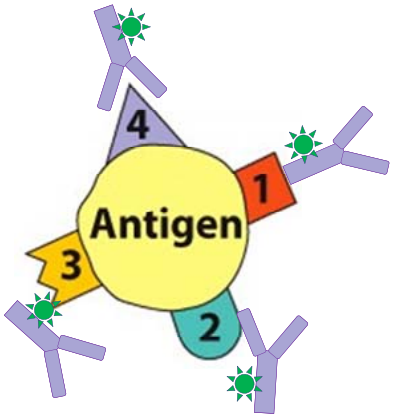
Clone name

Primary Ab

- Monoclonal Ab
  - Ab-1 or Ab-2 or Ab-3 or Ab-4
- Polyclonal Ab
  - Ab-1+Ab-2+Ab-3+Ab-4
- Host and isotype
  - Ab from Rat (以slide 6為例)
  - IgG, IgM, IgA, IgE, IgD
- Clone names
  - Different clone name, against different epitope



Non-specific binding

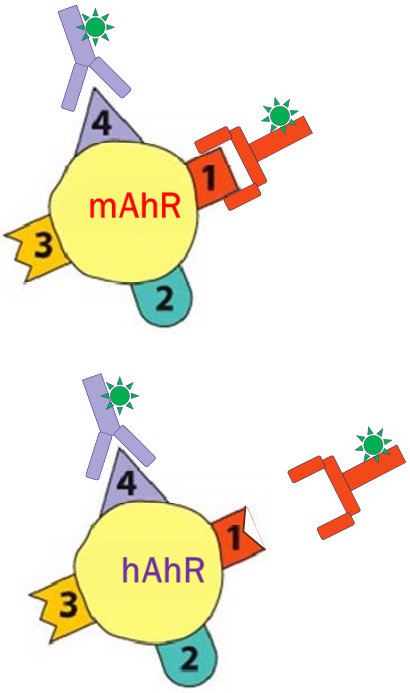


抗原1, 2, 3皆被抗體 non-specific binding

Experimental controls

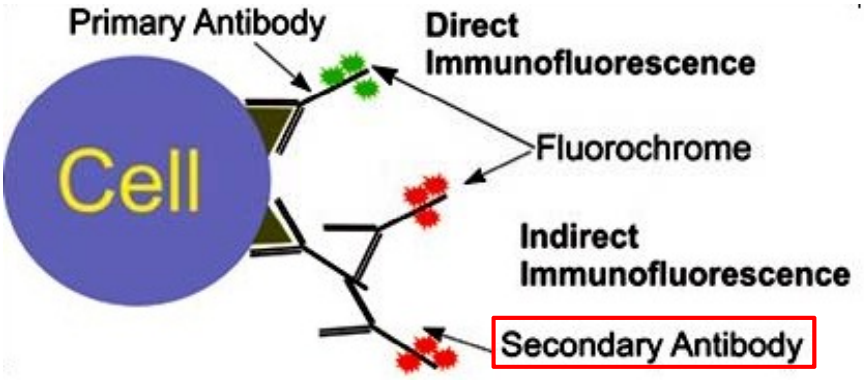
- Isotype control
  - The same isotype
  - The same dye
  - The same company
- Blocking control
  - Purified Ag + 1st Ab ➔ Ag-Ab complex ➔ staining
- Fc blocking
  - Anti-CD16/CD32 mAb pretreatment

Cross reactivity

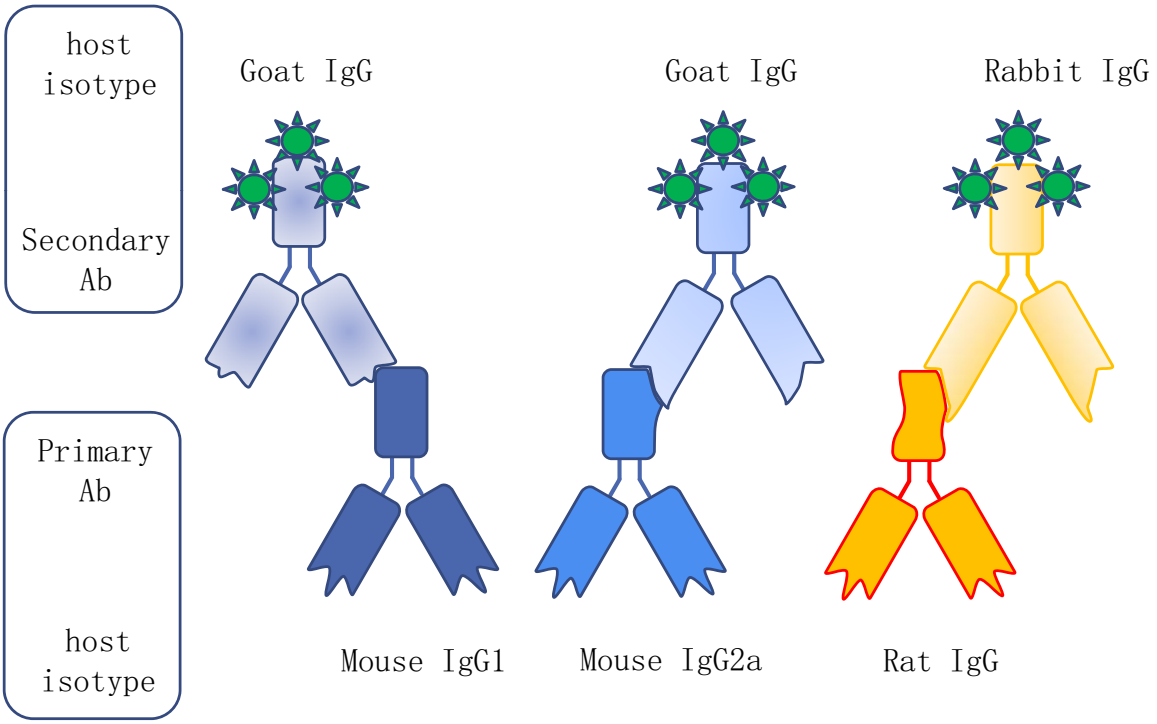


- Ab-4 can bind mouse AhR, also cross-react with human AhR.
- Ab-1 specifically recognizes mouse AhR, but not human origin.

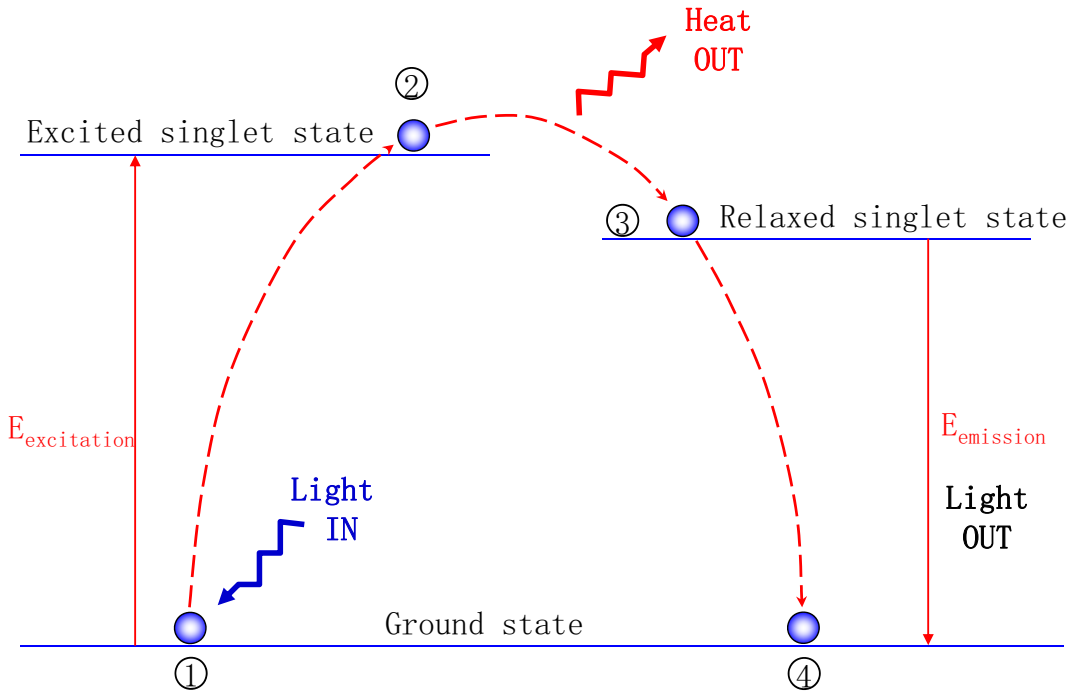
ImmunoFluorescence



Secondary Ab (二抗)

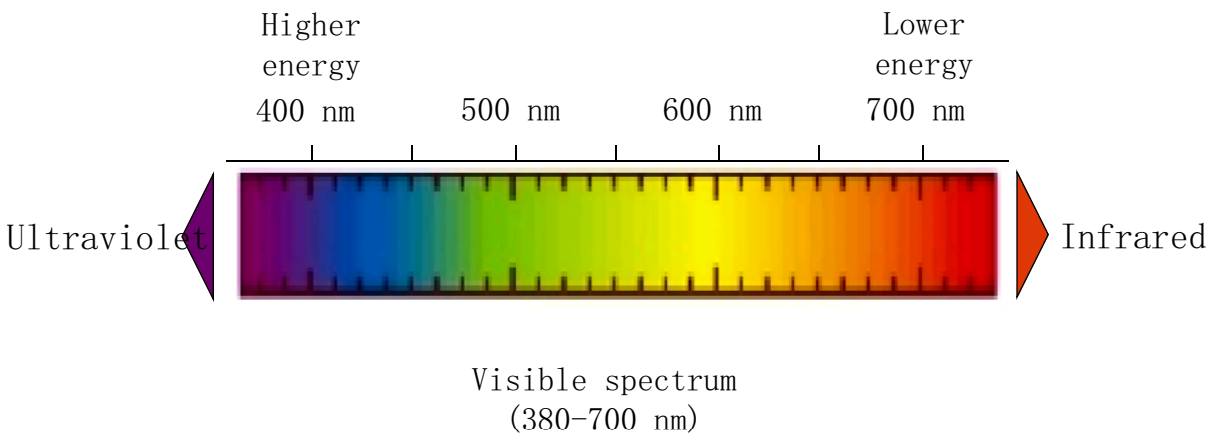


Principle of fluorescence



16

The electromagnetic spectrum



17

Excitation and emission wavelengths of some commonly used fluorochromes		
Probe	**Excitation (nm)**	**Emission (nm)**
R-phycoerythrin (PE)	480; 565	578
Fluorescein	495	519
PerCP	490	675
Texas Red	589	615
Rhodamine	550	573

Figure A.17 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

研資中心共軛焦顯微鏡之螢光染劑選擇

Laser type	Dye	Emission
405nm	DAPI, Alexa405, BFP	藍
440 nm	CFP	藍
488 nm	Alexa488, FITC , Fluo-4, GFP	綠
515 nm	YFP	黃
559 nm	Alexa 546, Alexa 555, Alexa 568, Cy3, TRITC, Texas Red, Rhodamine, DsRed	紅
635 nm	Cy5, Cy7, Alexa633	遠紅

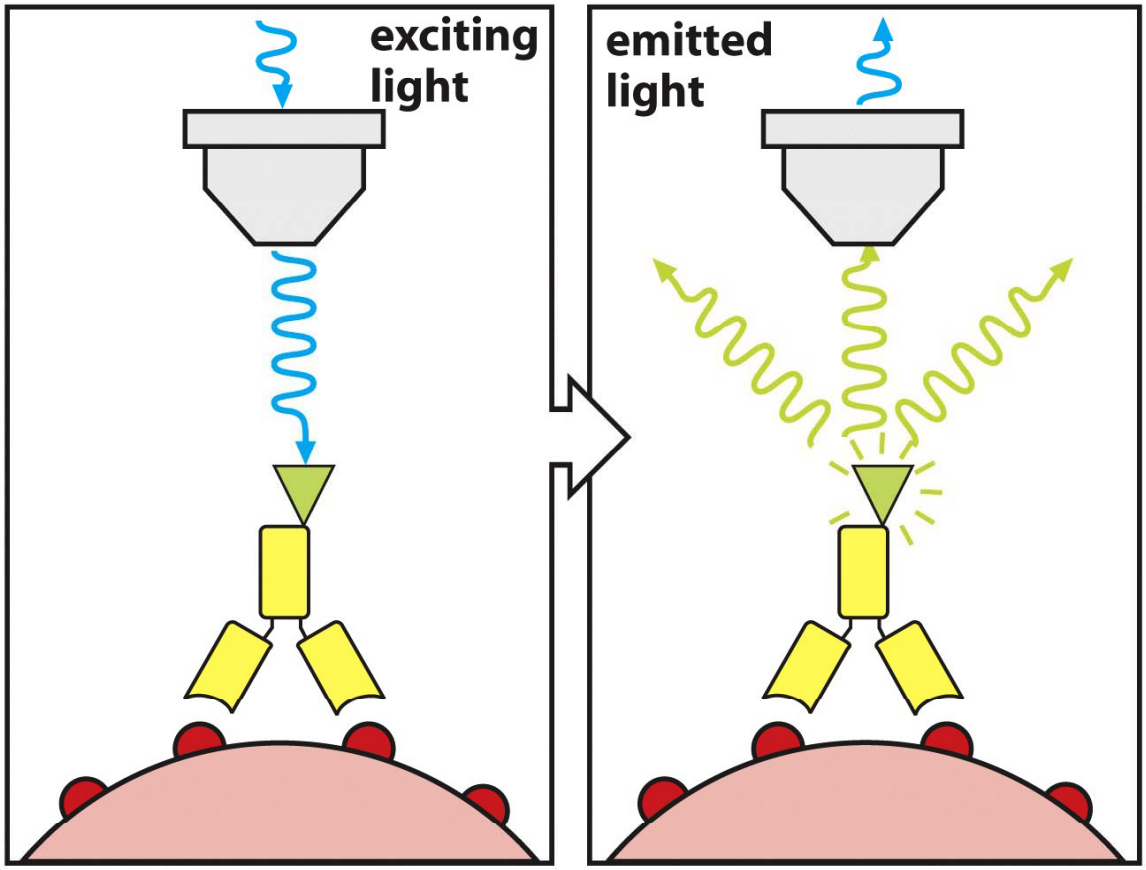


Figure A.18 part 1 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Immunofluorescence methods

Method	Direct	Indirect	Combined
Step 1 (Primary Ab)	✓ FITC-mouse anti-hA ✓ Cy3-mouse anti-hB	✓ mouse anti-hA ✓ rabbit anti-hB	✓ mouse anti-hA ✓ rabbit anti-hB
Step 2 (Secondary Ab)	-	✓ FITC-goat anti-mouse IgG ✓ Cy3-goat anti-rabbit IgG	✓ FITC-goat anti-mouse IgG ✓ Cy3-goat anti-rabbit IgG
Step 3			Cy5-mouse anti-hC

Modified from https://biotium.com/support/tech-tips/tech-tip-combined-direct-and-indirect-immunofluorescence-using-primary-antibodies-from-the-

Immunofluorescence methods

Method	Direct	Indirect	Combined
Advantages	✓ Rapid staining ✓ Multiple 1st Ab from same host	Strong signal	Strong signal
Disadvantages	✓ Weak signal	2nd Ab from different hosts	Take long time for staining

Modified from https://biotium.com/support/tech-tips/tech-tip-combined-direct-and-indirect-immunofluorescence-using-primary-antibodies-from-the-

For example

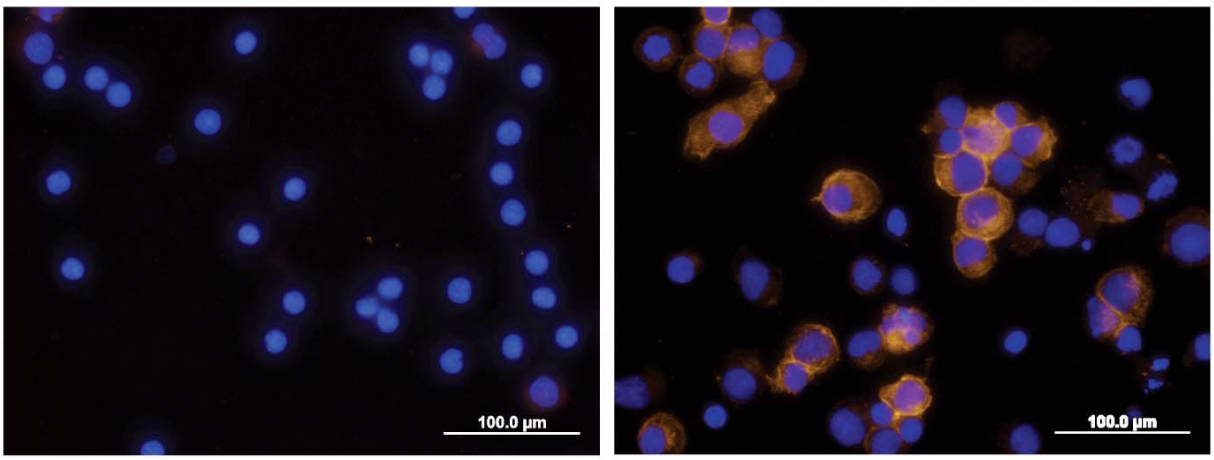


Figure 3-3
Kuby Immunology, Seventh Edition

For example

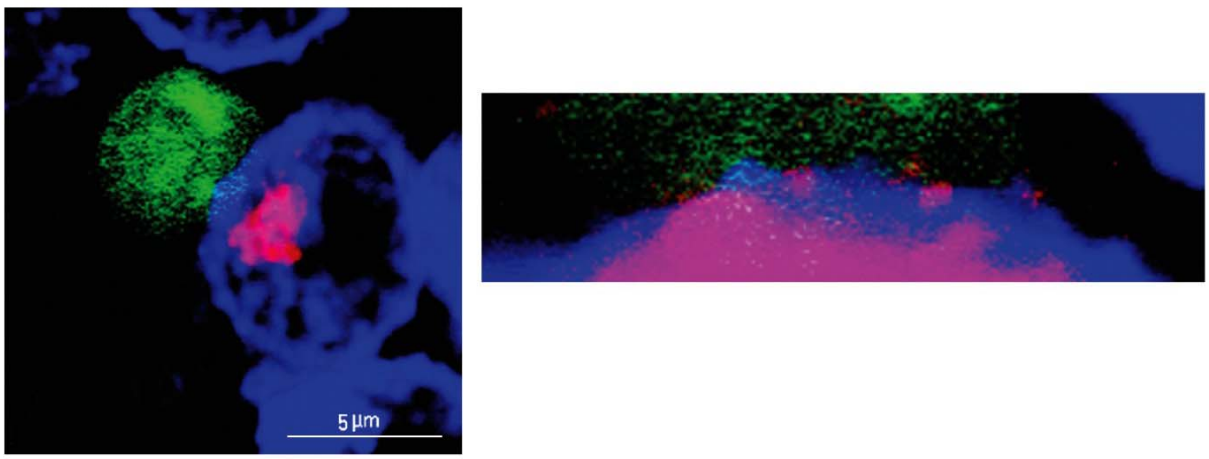


Figure 3-4
Kuby Immunology, Seventh Edition

TABLE 20-1 Sensitivity of various immunoassays

Assay	Sensitivity* (μg antibody/ml)
Precipitation reaction in fluids	20–200
Precipitation reaction in gels	
Ouchterlony double immunodiffusion	20–200
Agglutination reactions	
Direct	0.3
Agglutination inhibition	0.006–0.06
Radioimmunoassay (RIA)	0.0006–0.006
Enzyme-linked immunosorbent assay (ELISA)	~0.0001–0.01
ELISA using chemiluminescence	~0.00001–0.01†
Immunofluorescence	1.0
Flow cytometry	0.006–0.06

*The sensitivity depends on the affinity of the antibody used for the assay as well as the epitope density and distribution on the antigen.

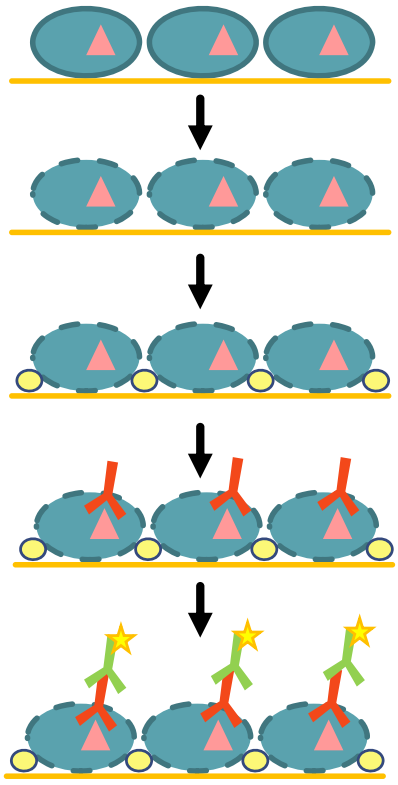
†Note that the sensitivity of chemiluminescence-based ELISA assays can be made to match that of RIA.

Source: Updated and adapted from N. R. Rose et al., eds., 1997, *Manual of Clinical Laboratory Immunology,* 5th ed., Washington, DC: American Society for Microbiology.

Table 20-1
Kuby Immunology, Seventh Edition

本次實驗過程

Mouse epithelial cell line



Fix cells in 4 % paraformaldehyde

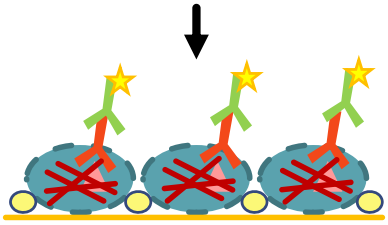
0.1 %Triton-X-100 Permeabilization

1 % BSA Blocking

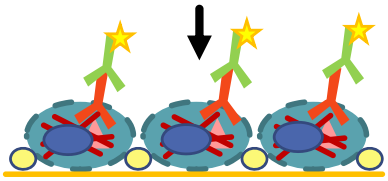
AhR 轉錄因子

Primary antibody mAb mouse anti-AhR IgG

Secondary antibody Alexa Fluor 488-polyclonal Goat anti-mouse IgG



Alexa Fluor 568-Phalloidin
for F-actin staining



DAPI for nuclear DNA staining

Seeding cell (NL-20) on cover slip, overnight

↓

Treatment

↓

1X PBS Wash, 5 mins

↓

4 % paraformaldehyde fix cell, 30 mins

↓

1X PBS Wash, 5 mins X3

↓

0.1 % Triton-X-100 for permeabilization, 10 mins

↓

1X PBS Wash, 5 mins

↓

Blocking in 1 % BSA, 1 hour

↓

Primary antibody (anti-AhR, 1:200) in 1 % BSA, 4℃ overnight

1X PBS Wash, 5 mins

↓

0.5 % Tween 20 Wash, 10 mins

↓

1X PBS Wash, 5 mins X3

↓

Blocking in 1 % BSA, 30 mins

↓

Secondary antibody (anti-mouse IgG Alexa Fluor 488, 1:1000) in 1 % BSA, 45mins

↓

1X PBS Wash, 5 mins X3

↓

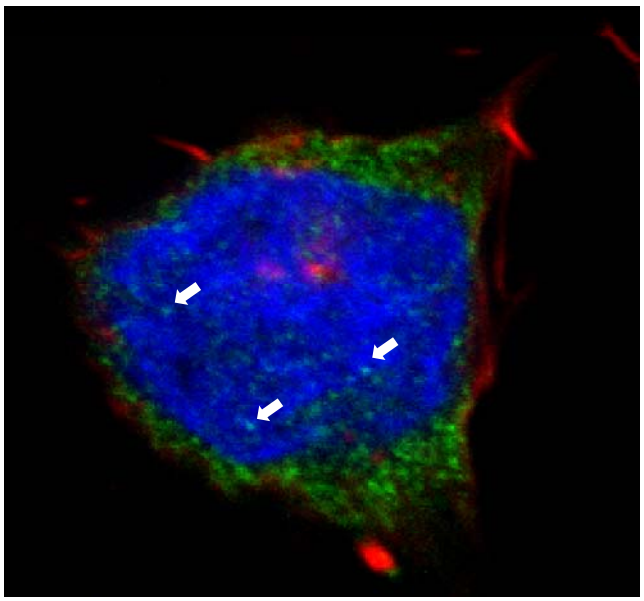
Alexa Fluor 568 phalloidin (1:100) in 1 % BSA, 20 mins

↓

1X PBS Wash, 5 mins X3

↓

Fluoromount-G with DAPI to cover slip



光學顯微鏡的介紹及操作　　高醫 生物系　張永福 20180621

實驗目的

顯微鏡是生物學發展上很基本又重要的儀器。學會熟練的操作光學顯微鏡是細胞生物學研究的基本要求，能嫻熟的操作光學顯微鏡，日後才能快速駕馭更複雜的其他顯微鏡，如螢光顯微鏡技與共軛焦顯微鏡。本實驗目的是瞭解顯微鏡使用的正確方法並經由觀察各種細胞而熟練地操作光學顯微鏡。

實驗原理

A.光學顯微鏡結構

雙目鏡複式光學顯微鏡（Binocular compound microscope）(圖 1)

1.接目鏡：放大倍率為 10×。可調整目鏡間的距離以符合各人的雙眼寬度。接目鏡上有焦距調節輪，可因應各人焦距來調整最佳呈像。

2.鏡筒：介於接目鏡與接物鏡之間。

3.旋轉盤：可以旋轉藉以更換不同倍數的接物鏡。

4.接物鏡：一般由低倍到高倍 4×、10×、40×、油鏡 100× 四種倍率。

5.載物台：可放置標本，上面有玻片夾固定玻片。

6.集光器 (Condenser)：由許多透鏡組合而成，用以集合光線。

7.光圈(虹膜) (Iris diaphragm)：可用以調整光圈孔徑大小，增加標本的明暗對比。

8.照明器：提供觀察所需之光源。

9.鏡臂：供握取顯微鏡。

10.調節輪：可調整載物台的升降，以供對焦。

11.鏡座：有光源調整鈕，可調整照明器內光源亮度；後方接有電源線，有的廠牌尚有光源開關。

B.顯微鏡的取用

1. 提取顯微鏡時，一手緊握鏡臂，另一手托住鏡座。
2. 將顯微鏡置於距桌緣約一個姆指距離。調整坐椅高度以輕鬆使用顯微鏡。
3. 將載物台降至最低點，轉動旋轉盤使低倍鏡位於鏡筒正下方。打開光源。
4. 將所要觀察的玻片放在載物台上，以玻片夾夾好並調整玻片位置。轉動粗調節輪。調整載物台製最高即無法再上升為止。

5. 由接目鏡觀察，轉動粗調節輪使載物台下降至看得到觀察物，再以細調節輪微調，直到影像清晰為止，並將目標部位調整至視野中央。若視野太亮或太暗，可調整光源調整鈕及光圈，使視野中之亮度適當。
6. 轉換高倍鏡觀察時，將高倍鏡移至標本上方，旋轉細調節輪至取得標的物清晰影像。

C.注意事項

1.嚴忌單手提取顯微鏡。
2.若須在桌上移動顯微鏡，將顯微鏡提起再放至適當位置，嚴忌推動顯微鏡。
3. 坐椅調至適當的高度應，觀察時兩眼同時觀察，否則長時間觀察易感覺疲勞。
4. 接目鏡與鏡筒的承接鈕切勿任意旋開，以免目鏡掉落。
5. 切勿在載物台染色或其他任何操作，以免染劑或其他液體傷及顯微鏡內部。
6. 更換玻片時，務必將載物台下降至最低點。
7. 欲觀察的玻片上，不應殘留任何水滴或藥劑，以免污染鏡頭。
8. 擦拭顯微鏡鏡頭時只能用拭鏡紙（Lens paper），切勿用其他紙張或手指接觸鏡頭。擦拭時應沿單一直線方向輕拭，不可旋轉磨擦。
9. 用畢顯微鏡應將載物台下降至最低點，並將低倍鏡對準載物台中央圓孔處。

實驗步驟

1. 英文字母“e”方位變化的觀察：將字母“e”放在載玻片上，以低倍觀察之。比較玻片及顯微鏡中前後及左右方位的平面呈相。
2. 觀察三條不同顏色的線相交於一點的情形:在載玻片上放置三種不同色的線，相交於一點，於低倍鏡下轉動調節輪觀察。比較玻片及顯微鏡中三條線的上中下的立體呈相。
3. 色素細胞(Chromatophore)觀察: 取魚鱗片置載玻片上，加一滴水，覆上蓋玻片，置於低倍鏡下，觀察細胞內色素顏色。
4. 澱粉粒(Starch grain)觀察:以刀片刮取少許馬鈴薯截面，塗抹於載玻片上，加水覆上蓋玻片，置顯微鏡下觀察。可見許多圓形或卵圓形澱粉粒後，改用高倍鏡觀察，可發現澱粉粒在較小的一端有臍(Hilum)；以臍為中心，其周圍有輪紋。
5. 蛙的精子細胞(The sperm of frog)觀察:自燒杯內吸取一小滴蛙睪丸碎片的液體，覆上蓋玻片，然後置顯微鏡下觀察細胞的形態及是否能夠運動。
6. 肝細胞(Liver cell)觀察:以刀片刮取少許豬肝切面，塗抹後，加一滴甲基藍液，覆以蓋玻片，觀察其細胞核的數目。
7. 脂肪細胞(Fat cell)觀察:刮取肥肉少許在載玻片上塗成薄層，加蘇旦三號染色，覆上蓋玻片，細胞內染成紅色部分者即為油滴。
8. 蛙的血球(Blood cell)觀察:將自然乾後蛙的血液，加上瑞特氏染料，染 1-2 分鐘後，用水輕輕洗去染料，然後將載玻片置濾紙內以吸去水滴，待乾後置顯微鏡下觀察蛙的紅血球與白血球，並注意細胞核的形狀。

9. 雜色體(Chromoplast)觀察:切取紅辣椒表皮薄片，置顯微鏡下觀察。細胞中有黃紅色小顆粒，即為雜色體。
10. 針狀結晶(Raphide)觀察:將鴨跖草莖縱切一薄片，置顯微鏡下觀察。可看到許多針狀結晶位於細胞中或散置細胞外。此亦為草酸鈣結晶。
11. 鐘乳體(Cystolith)觀察: 切取印度橡膠樹葉薄片，置顯微鏡下觀察。在上表皮的一些細胞中可看到葡萄穗狀的碳酸鈣結晶，即為鐘乳體。

問題

1. e 顯示顯微鏡中前後及左右方位的平面呈相是否改變?
2. 三條線顯示顯微鏡中上中下的立體呈相是否改變?
3. 魚鱗色素細胞有幾種顏色?
4. 欲清楚觀察澱粉粒的臍及蛙的精子，應調整顯微鏡何結構?
5. 肝細胞細胞核的顏色及數目為何?
6. 脂肪細胞看得到細胞核嗎?
7. 青蛙血球的種類及數目?
8. 雜色體的數目?
9. 針狀結晶及鐘乳體的顏色?

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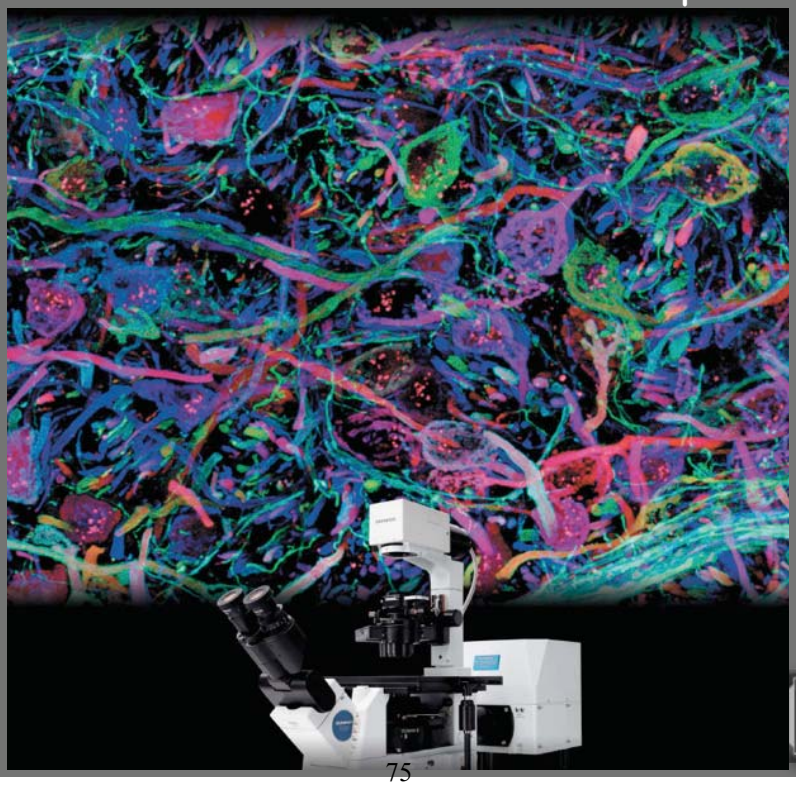
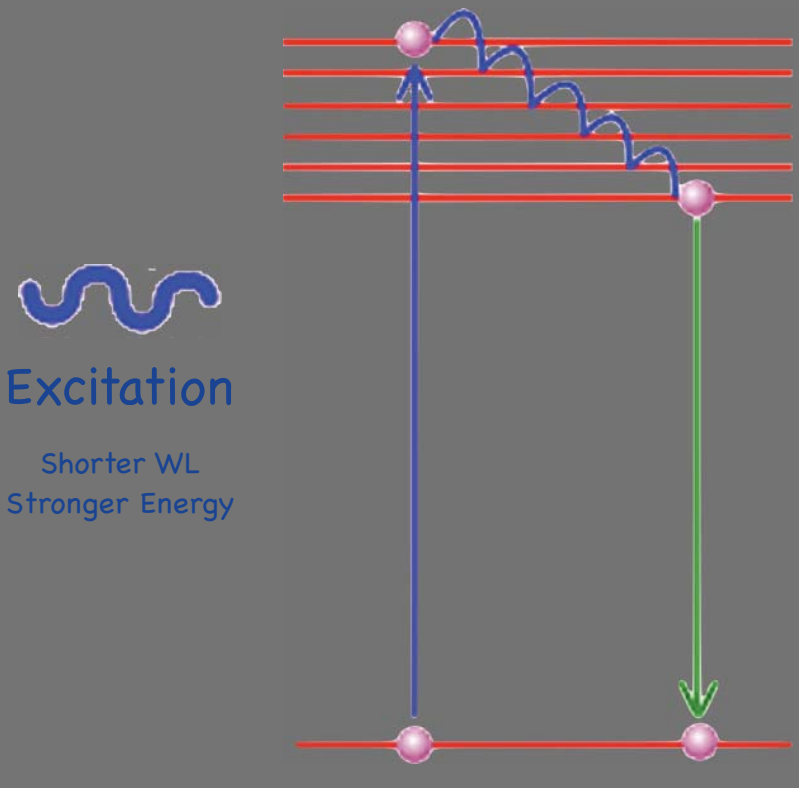
FV1000 cLSM

-concept



What is Fluorescence (Epi)



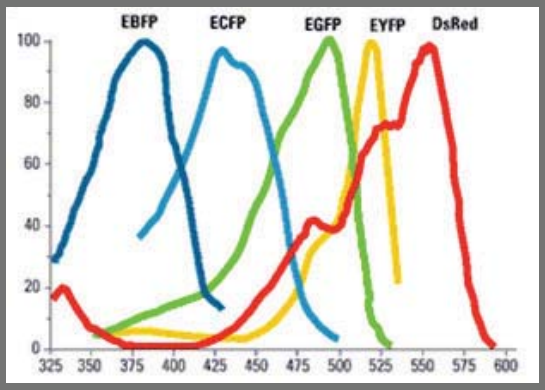
Excitation

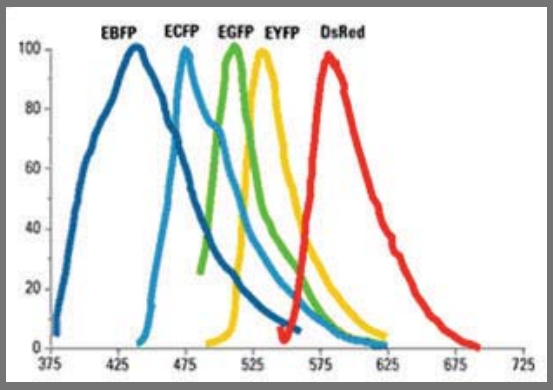
Shorter WL
Stronger Energy

Emission

Longer WL
Lower Energy

What Should We Notice!



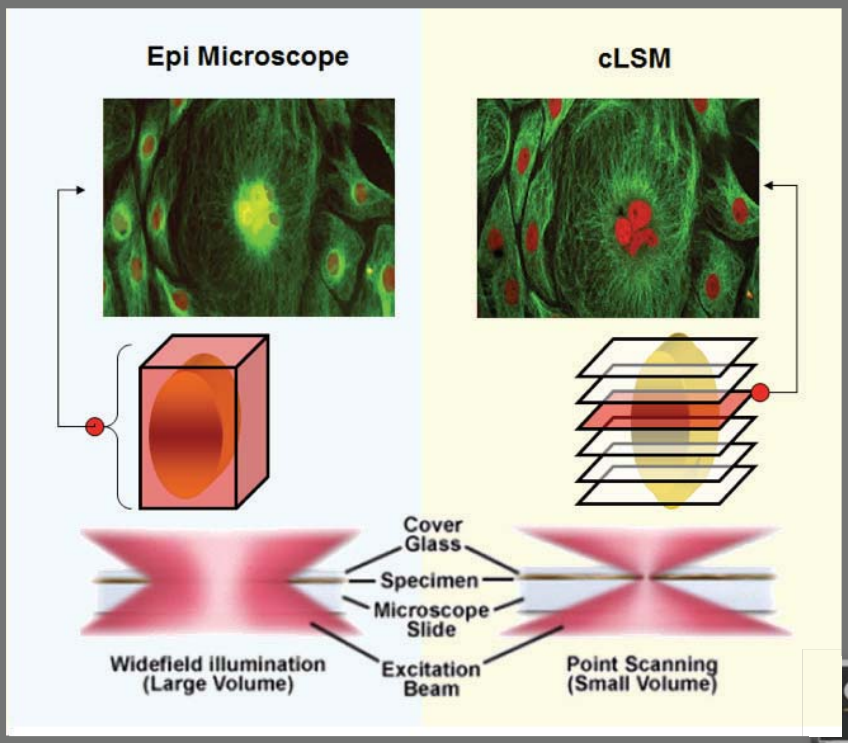


Excitation WL

Emission WL

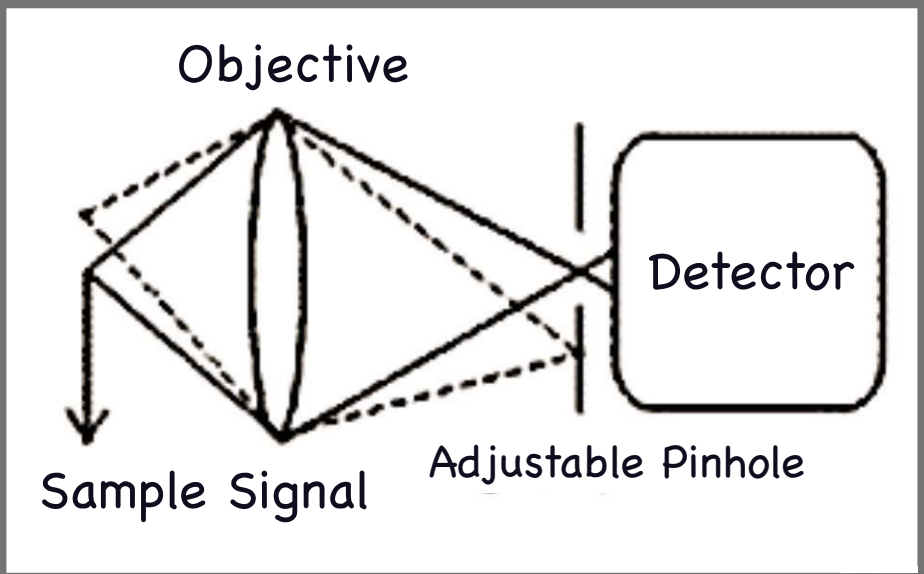
How Confocal System Reduce Blur Signal

-Laser (CW or Pulse)



How Confocal System De-blur

-Pinhole



Optical Resolution Under cLSM System

-XY, Z

$$FWHM_r = \frac{0.36\bar{\lambda}}{NA}\sqrt{\frac{\lambda_{ex}^2+\lambda_{em}^2}{\lambda_{em}^2+\frac{\lambda_{ex}^2}{1+\left(\frac{1.552\,NA\cdot PH_D}{\lambda_{em}Mm}\right)^2}}}$$

XY Direction

Z Direction

$$FWHM_z = \sqrt{\left(\frac{0.67\bar{\lambda}}{n-\sqrt{n^2-NA^2}}\right)^2\left(\frac{\lambda_{ex}^2+\lambda_{em}^2}{\lambda_{em}^2+\frac{\lambda_{ex}^2}{1+\left(\frac{1.55NA\cdot PH_D}{\lambda_{em}Mm}\right)^2}}\right)+\left(\frac{0.90n}{MmNA}PH_D\right)^2}$$

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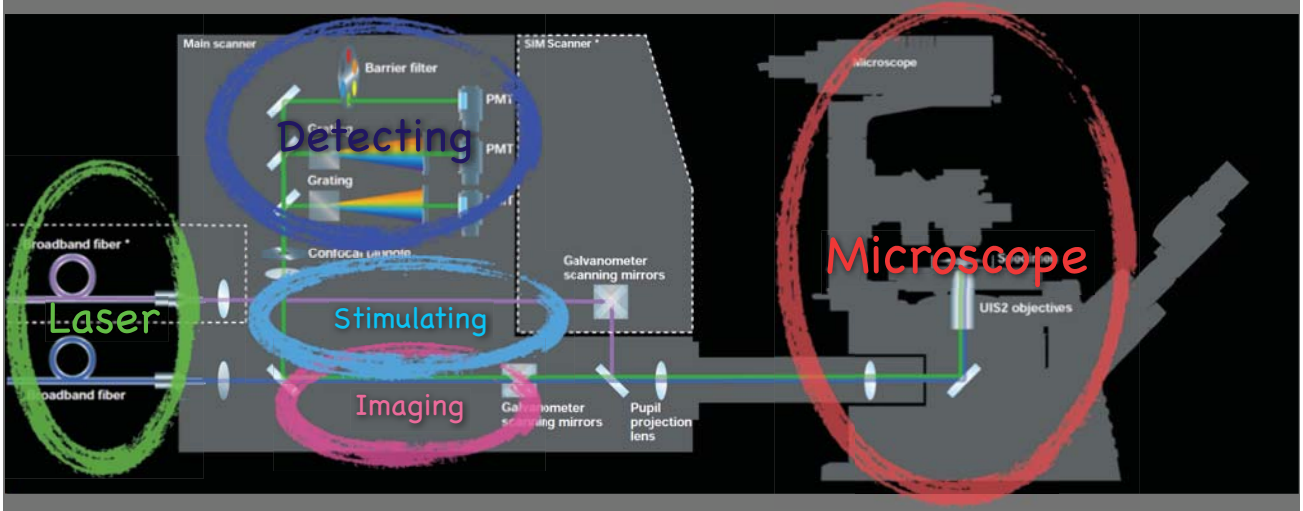

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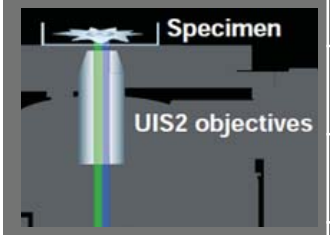
Objective



Objective	Model	Immersion Medium	N.A.	W.D.	Correction Ring
10X	UPLSAPO	Air	0.4	3.1	X
20X	UPLSAPO	Air	0.75	0.6	X
40X	UPLFLN	Oil	1.3	0.2	V
60X	UPLSAPO	Oil	1.35	0.15	V
100X	UPLSAPO	Oil	1.4	0.13	V

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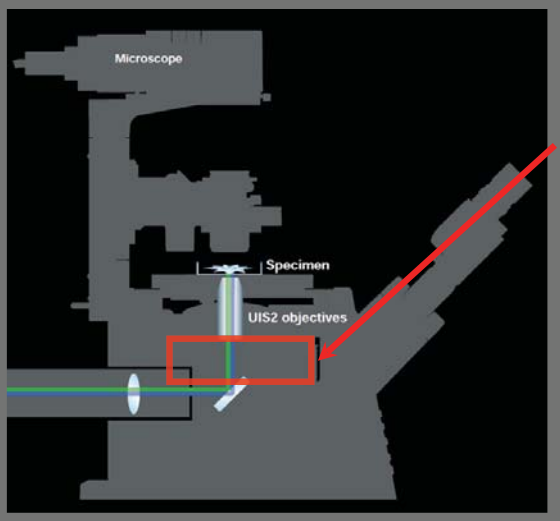
-How to OBSERVE Our Sample

Epi filter set



	Ex.	DM	Em.
Blue	330-385	400	420
Green	470-495	505	510-550
Red	530-550	570	575
NIR	595-645	655	660-745

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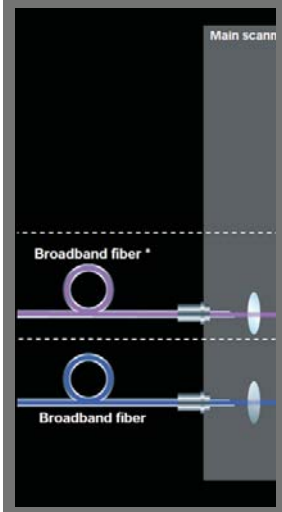
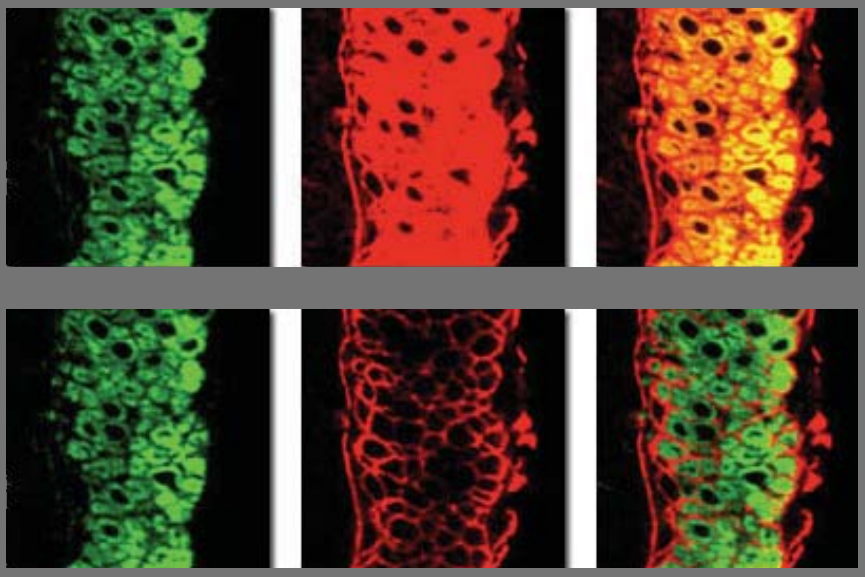
-Laser Scanning



Laser Type	Suitable Dye
LD Laser 635nm	Cy7, TOTO3
LD Laser 559nm	MitoTracker, DsRed
Multi Ar Laser 488 515nm	Alexa488, GFP
LD Laser 440nm	CFP
LD Laser 405nm	DAPI, Photobleach

WHAT! Cross Talk

-Epi Emission Bleed-Through



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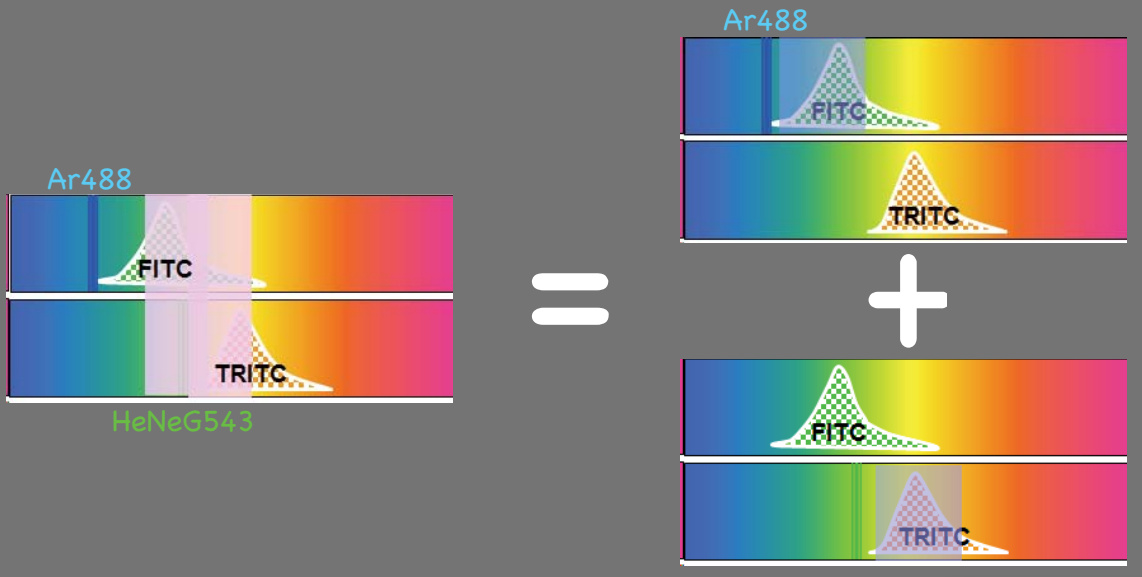
How to Solve Cross Talk

-Excitation Sequential Scanning (AOTF)



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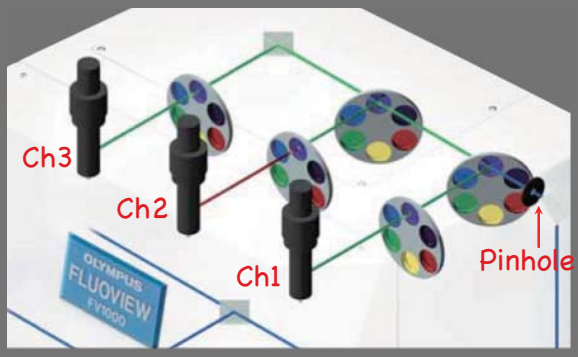
PMT Filter set

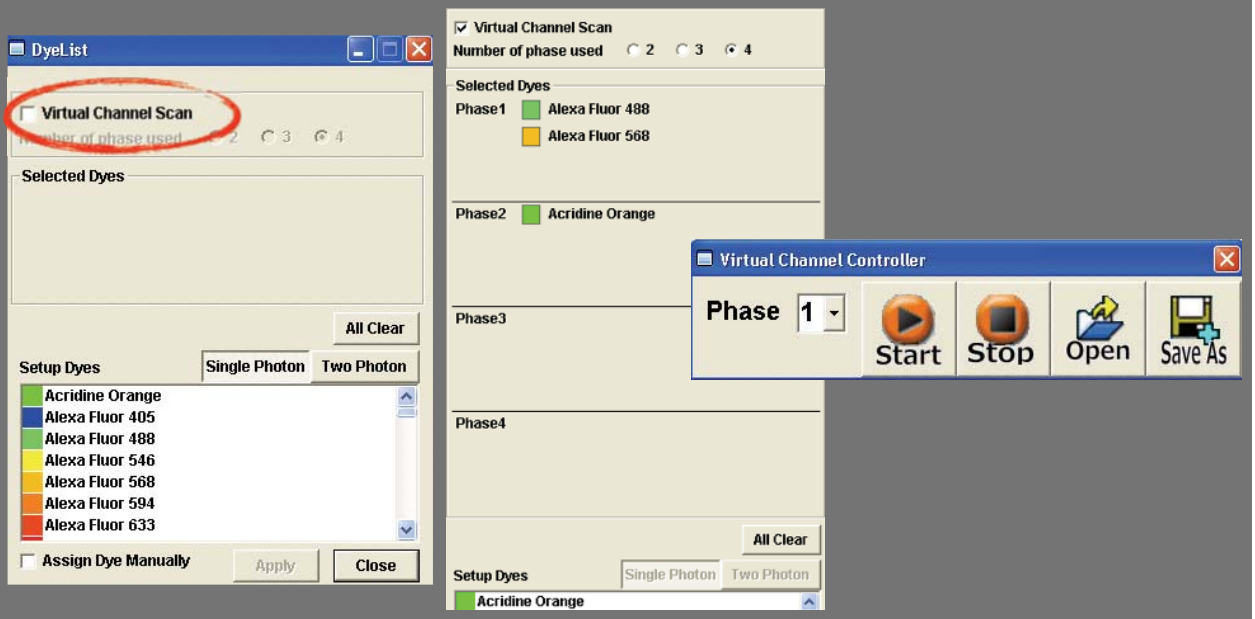


DM for PMT	1st PMT	2nd PMT	3rd PMT
Pos. 1	Mirror	Mirror	Mirror
Pos. 2	Glass	Glass	Glass
Pos. 3	SDM560	SDM640	
Pos. 4	SDM510	SDM560	
Pos. 5	SDM490		
Pos. 6			

Em for PMT	1st PMT	2nd PMT	3rd PMT
Pos. 1	465-495	505-605	655-755
Pos. 2	505-540	575-620	575-675
Pos. 3	480-495	535-565	
Pos. 4	430-470	505-540	
Pos. 5			
Pos. 6			

Virtual Channel

-more than 3 dye



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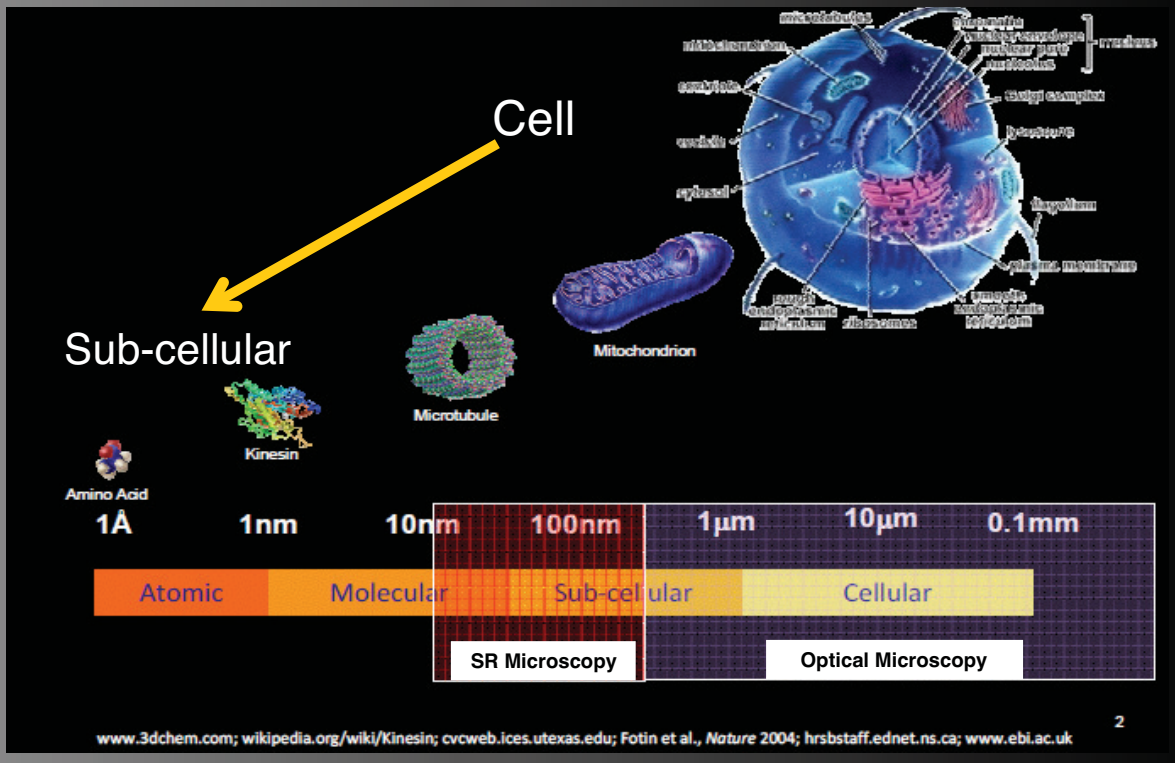
Confocal Based Super-resolution

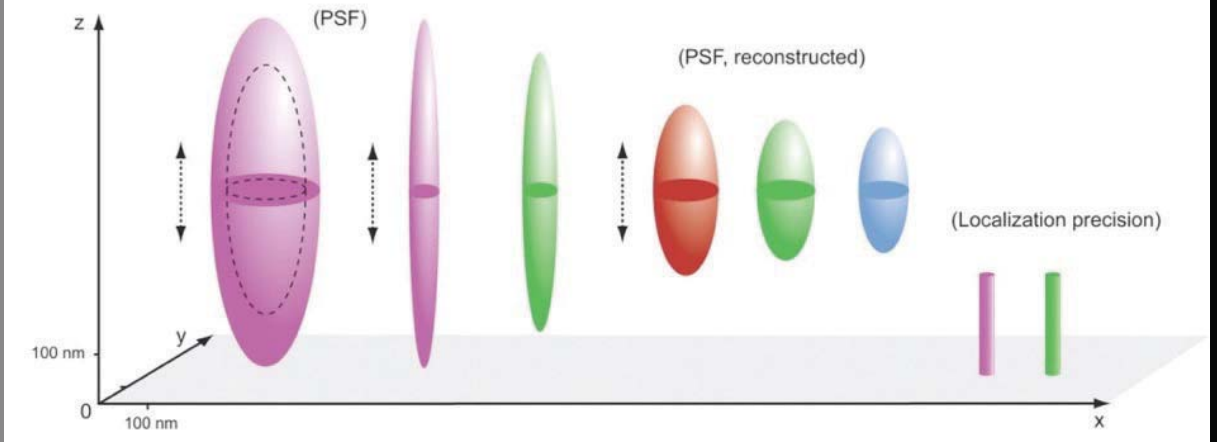
About Super-Resolution

-2014 Nobel Prize in Chemistry



About Super-Resolution

	CLSM	STED	CW-STED	3D-SIM			PALM/STORM	
~λ_{em} [nm]	460-670	670	520	620	520	460	670	520



~$D_{x,y}$ [nm]	180-250	60	70	130	110	100	30
~D_z [nm]	500-700	700	560	340	280	250	140 (TIRF-range)
~$V_{x,y,z}$ [*10^{-3} μm^3]	10-23	1.3	1.5	3.0	1.8	1.3	0.1

J Cell Biol. 2010, 190(2):165-75.

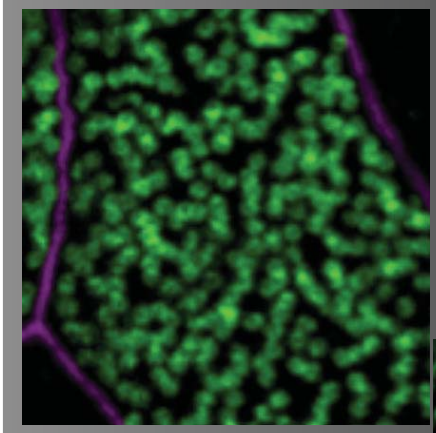
About Super-Resolution

Techniques	Strong points	Weak points
SIM	Enable to use most of dyes. Fast imaging (several fps)	No optical sectioning Not able to apply to deep plane
STED	Resolution (up to 50nm)	Not able to apply to deep plane Some restrictions in dyes Multi color imaging
PALM/STORM	Resolutions (up to 10nm)	Sampling time (>10min) Need special dyes Only for sample surface

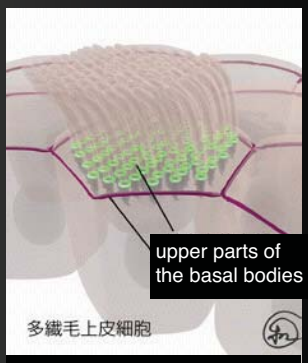
There are no perfect technique for super resolution

Confocal Based Super-Resolution

-OLYMPUS Super-Resolution (OSR)

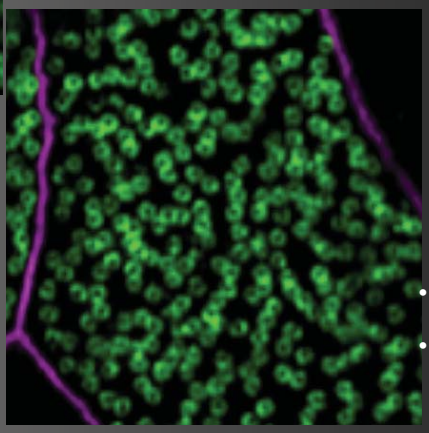


confocal



Trachea epithelial cells

Approx. 250~300nm diameter



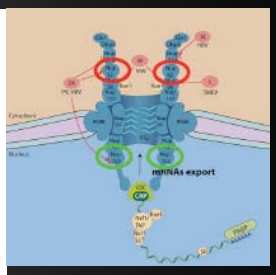
FV-OSR

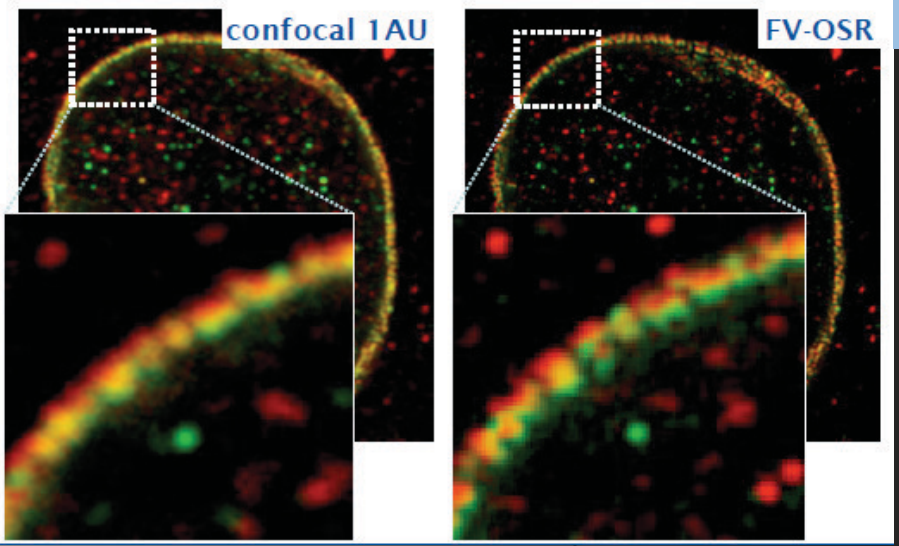
- **FV1200/UPLSAPO60XS**
- **ex 473/559 nm**

Confocal Based Super-Resolution

-OLYMPUS Super-Resolution (OSR)

Nuclear pore of HeLa cell
Green : Nup153(Alexa488)
Red: Nup62(Alexa555)
Image data courtesy of:
Prof. Kosako, H (TOKUSHIMA Univ.)

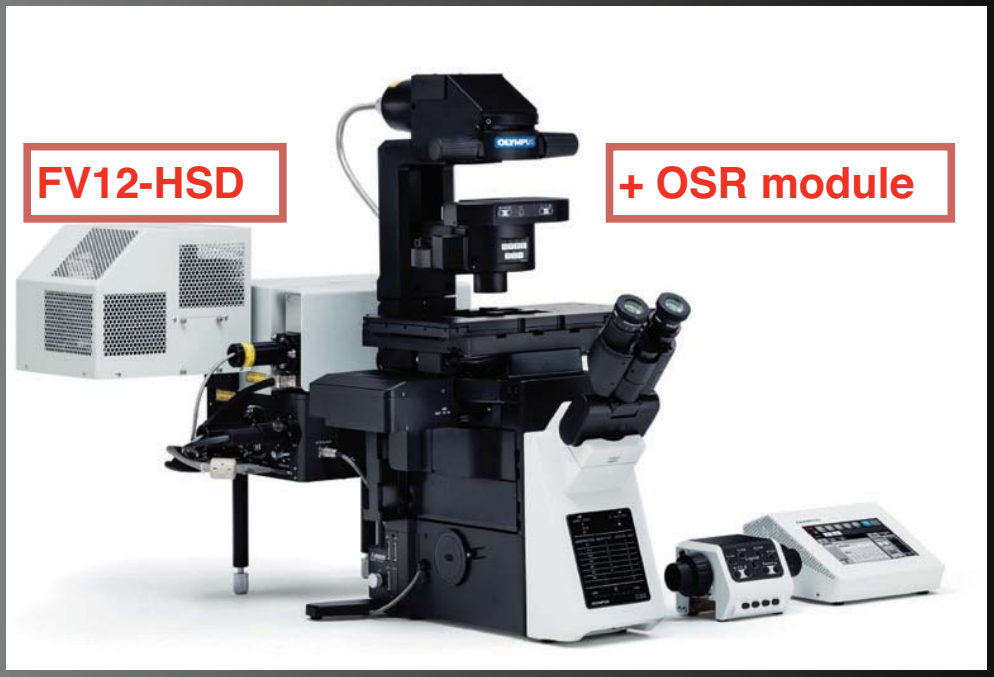




Confocal Based Super-Resolution
-FV-OSR

Techniques	Strong points	Weak points
FV-OSR	**Enable to use most of dyes.** **Enable to get the image in deep plane.** **Optical sectioning for thick samples**	**Sampling time (compare to SIM)** **Resolutions (compare to STED, PALM/STORM)**
SIM	Enable to use most of dyes. Fast imaging (several fps)	No optical sectioning Not able to apply to deep plane
STED	Resolution (up to 50nm)	Not able to apply to deep plane Some restrictions in dyes Multi color imaging
PALM/STORM	Resolutions (up to 10nm) Z resolutions	Sampling time (around 10min) Need special dyes Only able to observe surface of sample.

Confocal Based Super-Resolution
-FV-OSR



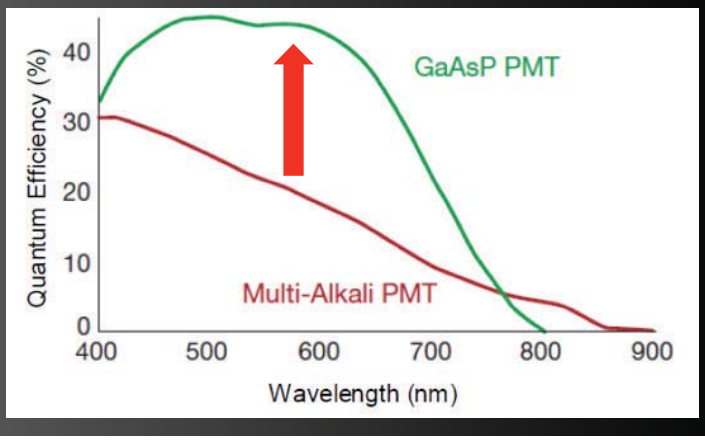
Confocal Based Super-Resolution: FV-OSR –Why Cooling GaAsP Detector?

FV12-HSD

Higher sensitivity →

Better detail structure and less laser excitation →

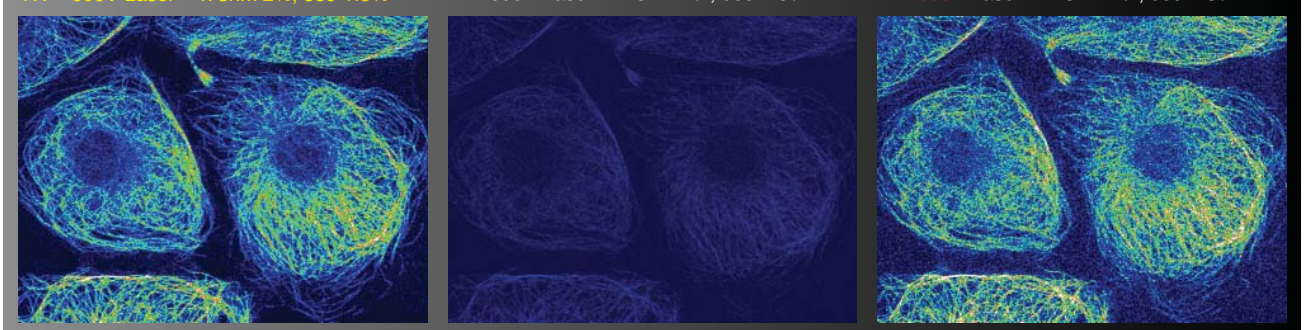
Less signal bleach and photo-toxicity



Confocal Based Super-Resolution: FV-OSR –Why Cooling GaAsP Detector?

GaAsP-PMT | Multi Alkali-PMT | Multi Alkali-PMT

HV：600V Laser：473nm 2%, 559 1.3% | HV：600V Laser：473nm 2%, 559 1.3% | HV：900V Laser：473nm 2%, 559 1.3%



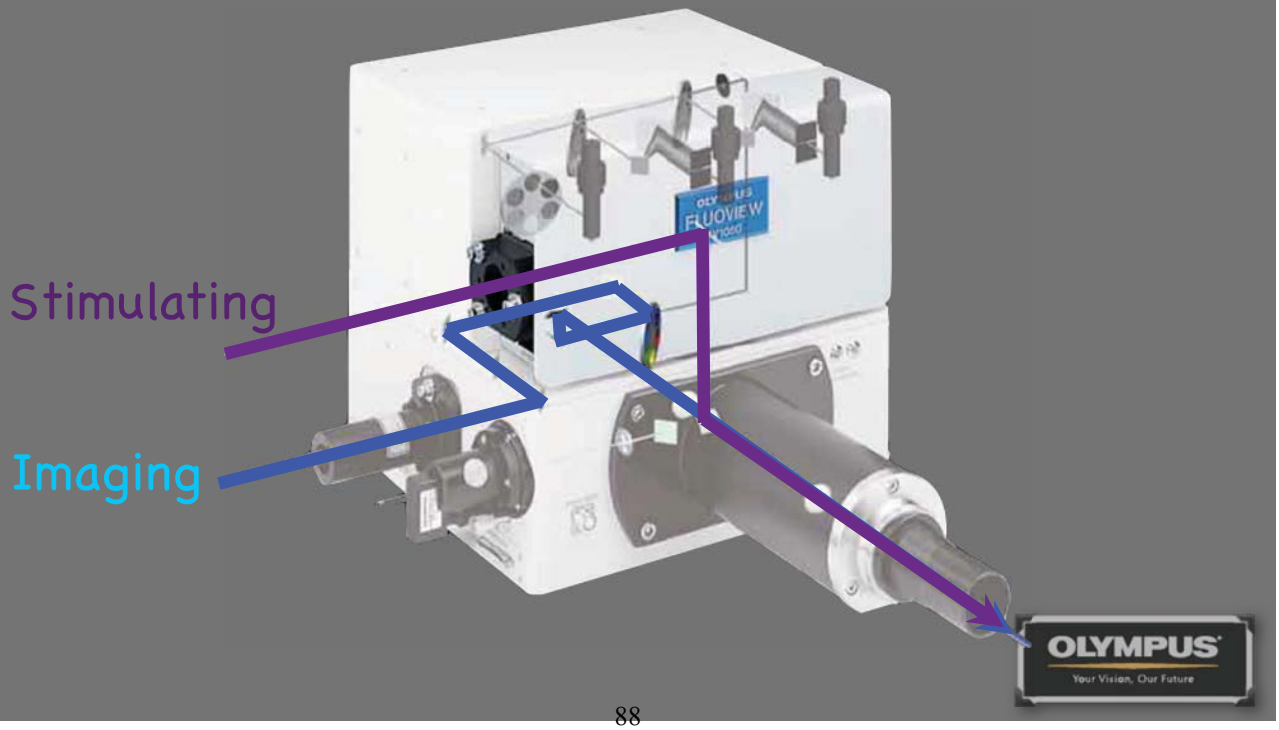
Confocal Based Super-Resolution: FV-OSR –Resolution relay on objective NA

Table : Objective lens and resolution

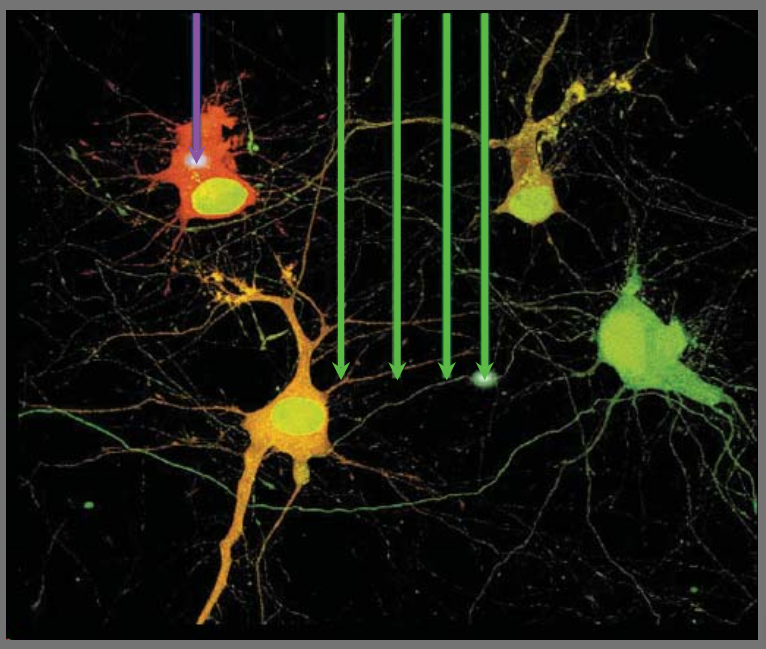
Objective lens	NA	Resolution(with high contrast mode) *2
UPLSAPO60XW	1.2	144nm
UPLSAPO60XO	1.35	128nm
UPLSAPO60XS	1.3	134nm
UPLSAPO60XS2	1.3	134nm
UPLSAPO100XO	1.4	121nm
UPLSAPO100XS	1.35	129nm
PLAPON60XO	1.42	120nm
PLAPON60XOSC	1.4	124nm
PLAPON60XOSC2	1.4	124nm
APON60XOTIRF *1	1.49	119nm
APON100XHOTIRF *1	1.7	111nm
UAPON100XOTIRF *1	1.49	117nm

Except Imaging??

-SIMultaneous Photobleaching



What's SIM

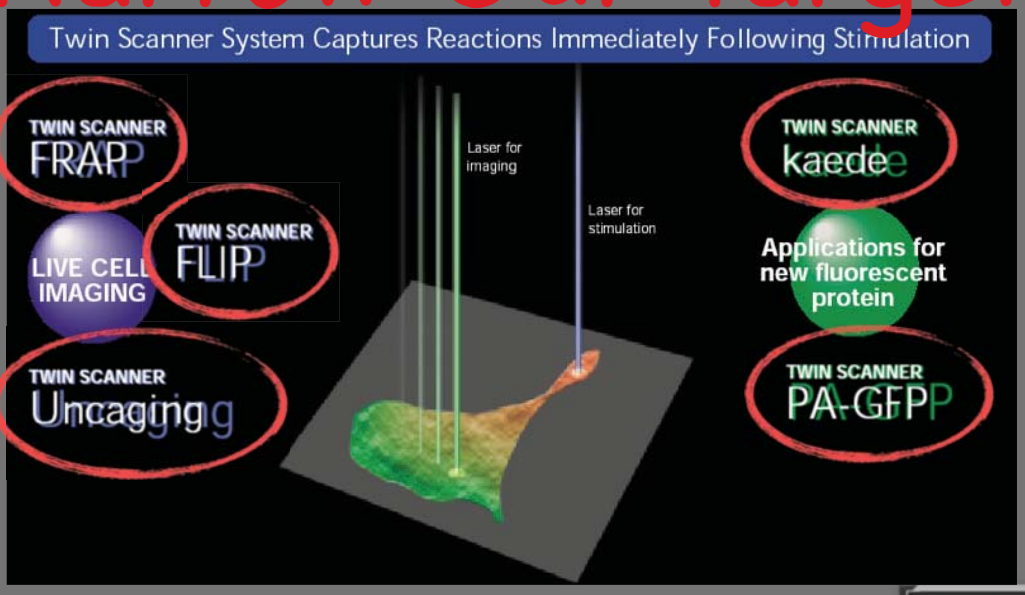


OLYMPUS
Your Vision, Our Future

Why SIM

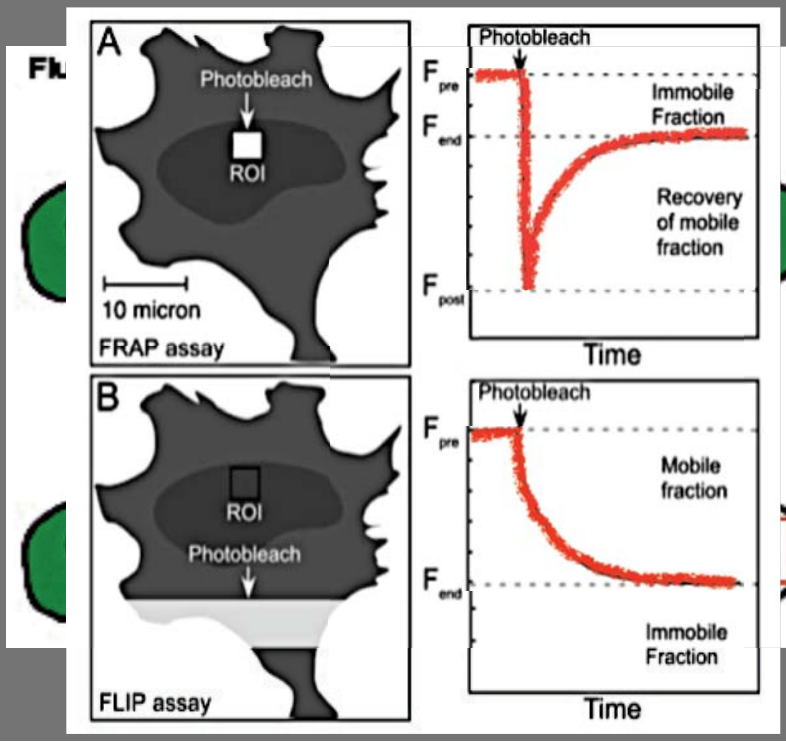
-Application of SIM

Narrow Our Target



OLYMPUS
Your Vision, Our Future

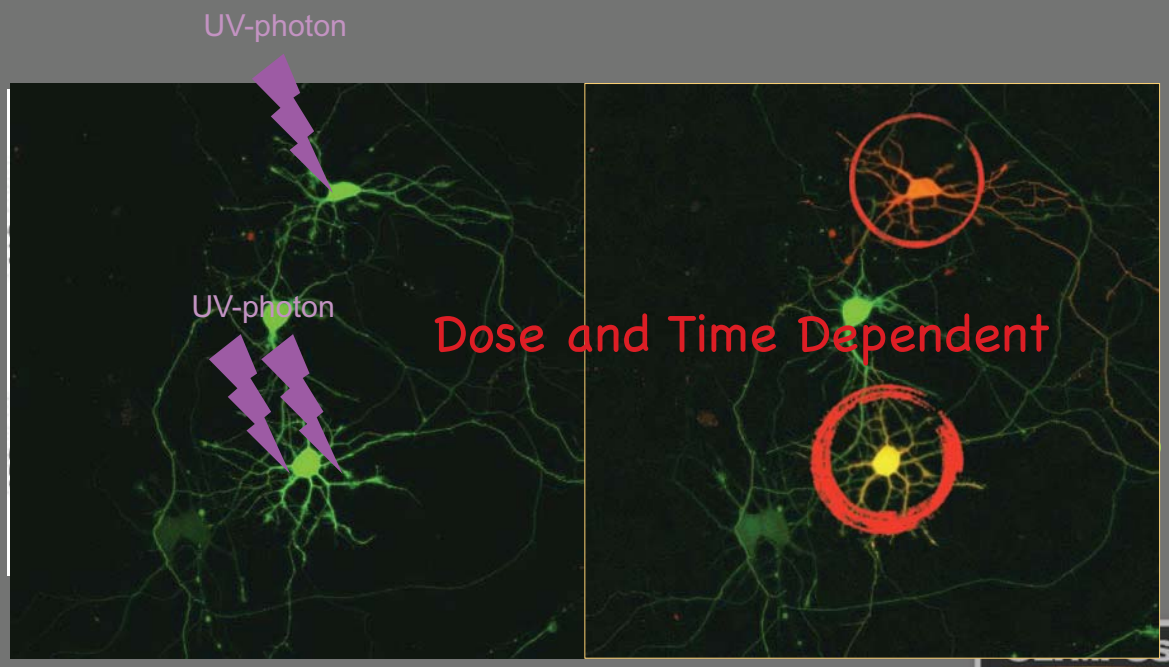
Fluorescence Recovery After Photobleach
Fluorescence Loss In Photobleach



Uncaging
-Bullseye

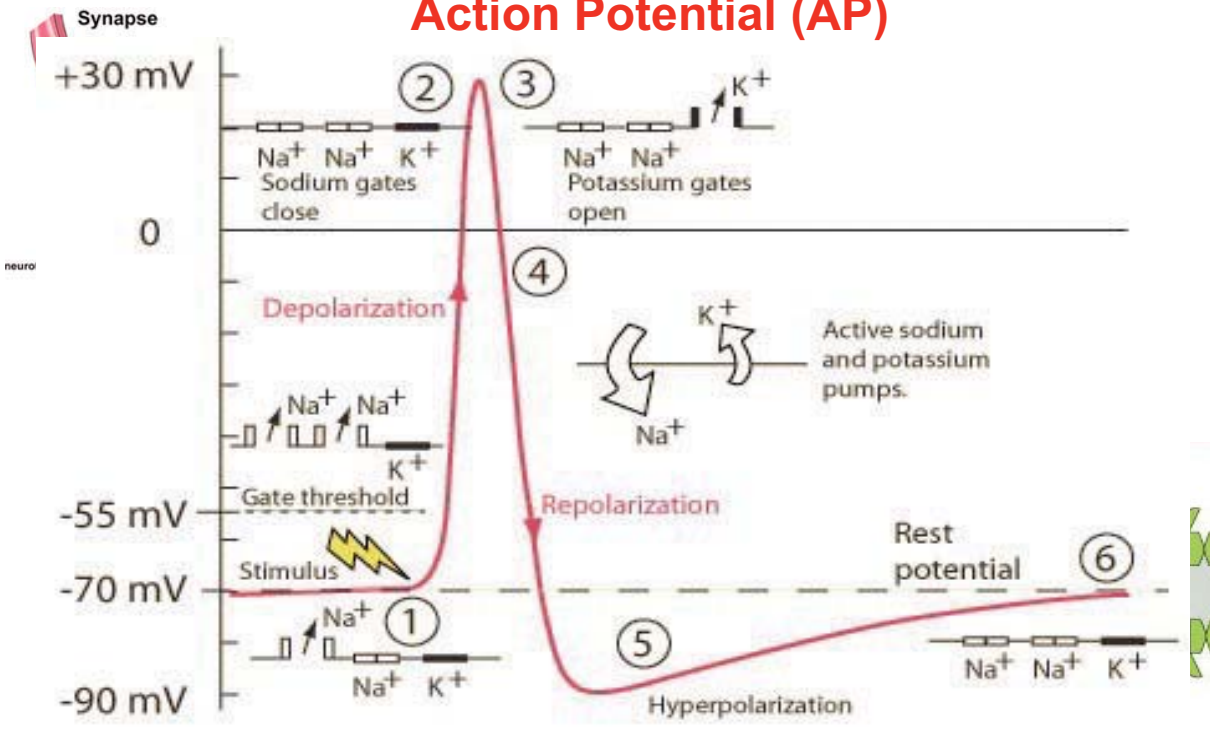
Photo-Conversion

-PA-GFP and Kaede

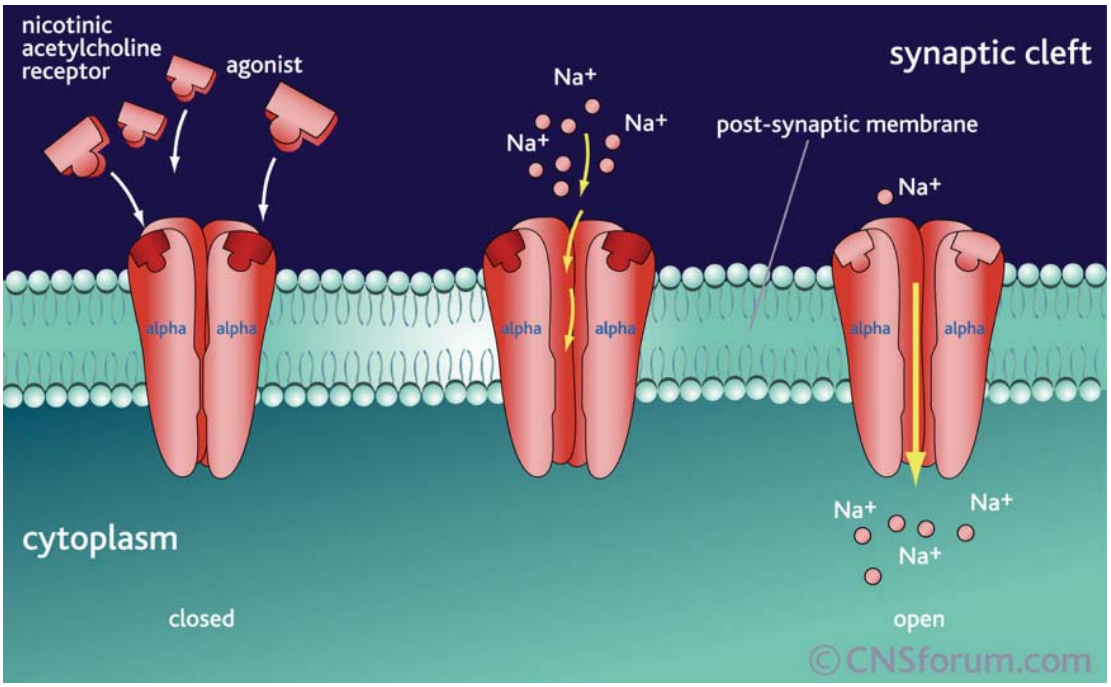


Fire or Not Fire

Action Potential (AP)



Channel Mediated Ion Concentration



Optogenetics (Optical and Genetics)-

photo-sensitive channel

- The Method of 2010- Nature Method
- Optogenetics: Breakthroughs of the Decade- Science

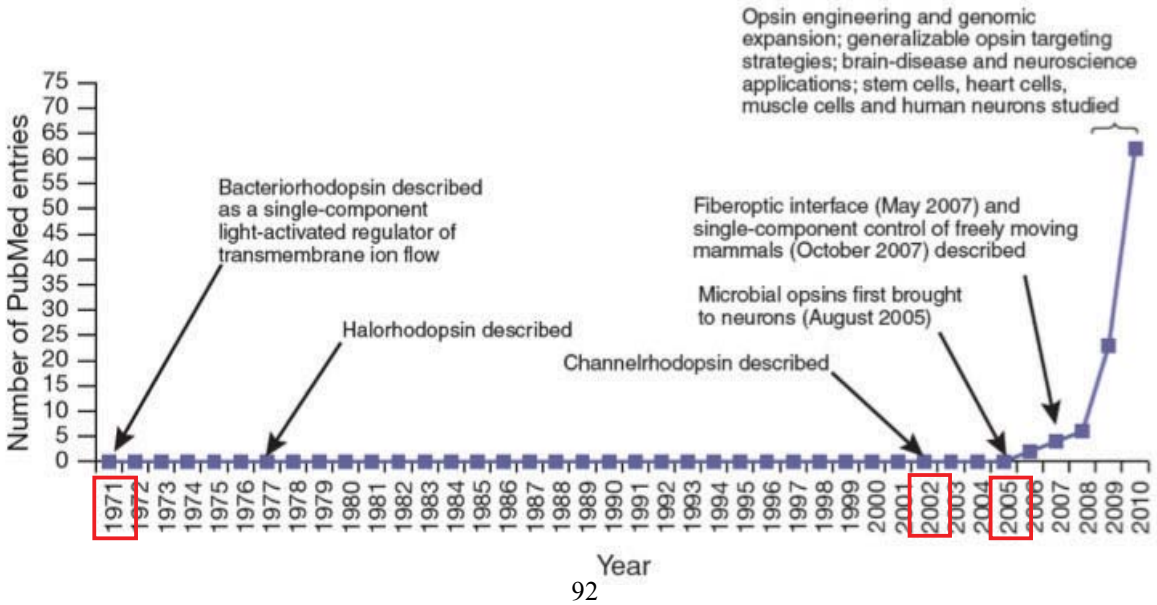
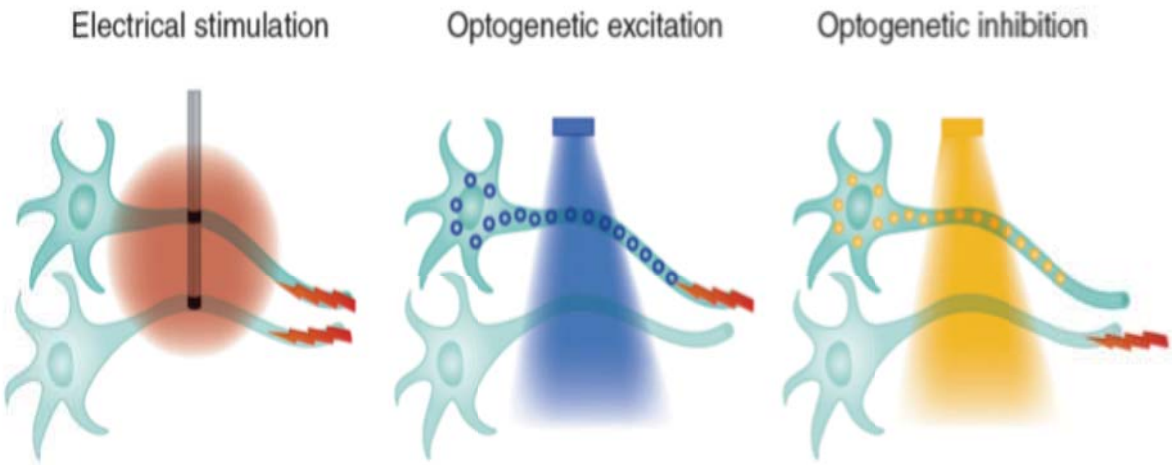
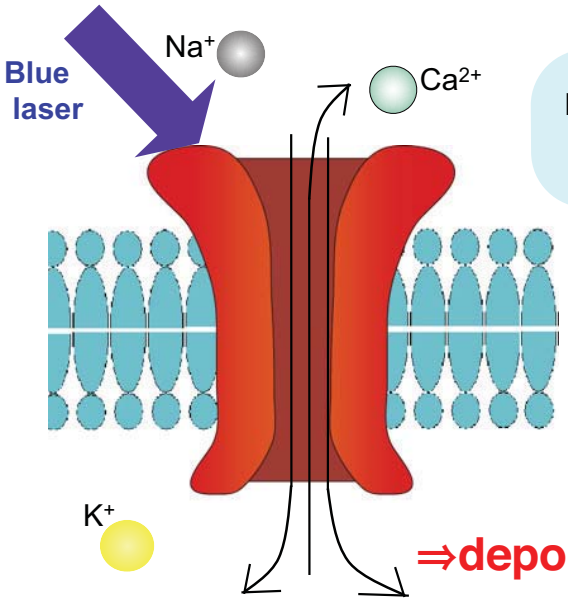


Photo- Excitatory or Inhibitory



Control cell activation using LIGHT

ChR2 — Channelrhodopsin-2 —



light-sensitive protein **Rhodopsin**

Origin of Chlamydomonas reinhardtii

+

Cation channel

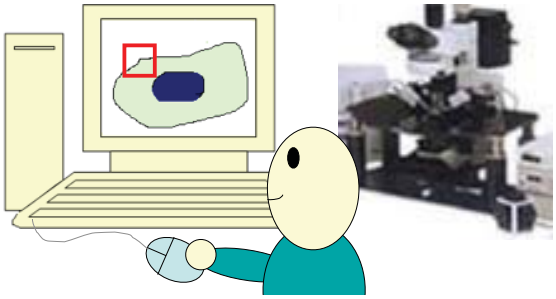
Blue laser irradiated to make Channels open, allow cation (Positive Ion) into the cell.

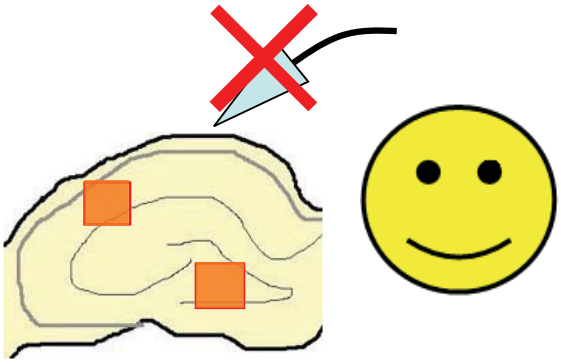
⇒depolarization ⇒Action Potential

Control cell activation

activated by LIGHT

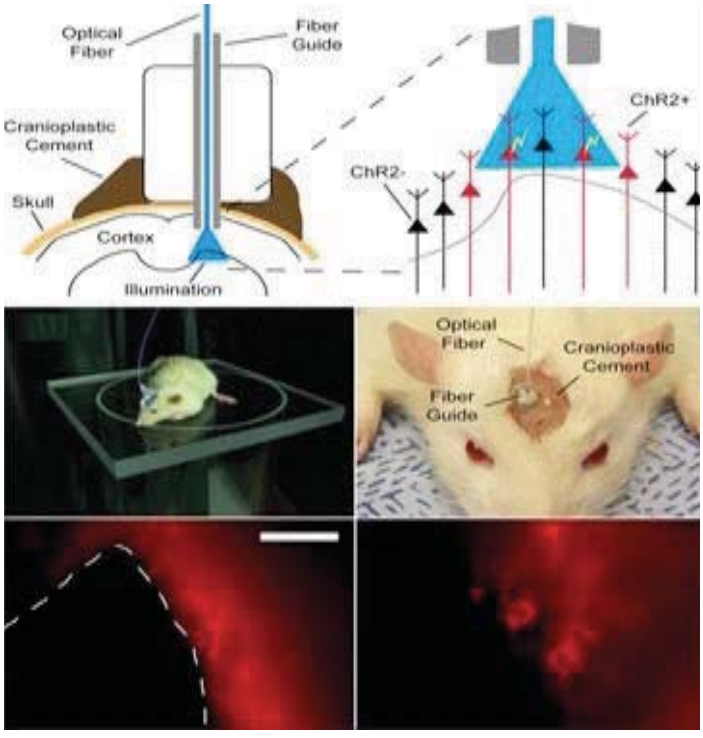
Activation in ChR2/NpHR using Blue/Yellow laser…

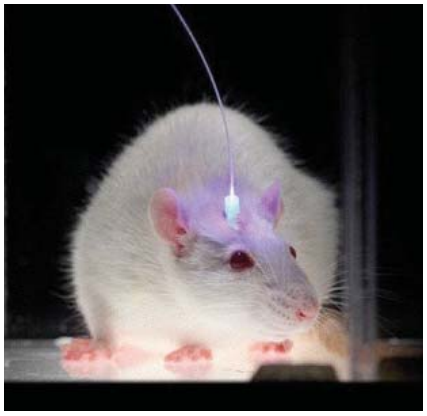




- **No need to use electrode!** Don't take time to set.
- **It is possible to select stimulus area freely.**

in vivo Model





Behavior recording

How to Operate

- Turn on system
- Light-path adjust
- Observation
- Image Scan
- Z-section
- Time-lapse
- Dual Beam operation

Turn on system

填寫登記簿

開啟AR雷射

開啟559雷射

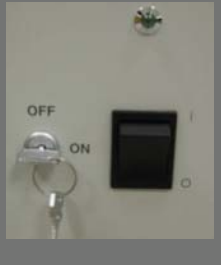
開啟雷射總控制模組

開啟電動載物臺控制器

開啟螢光燈

開啟電動顯微鏡控制器

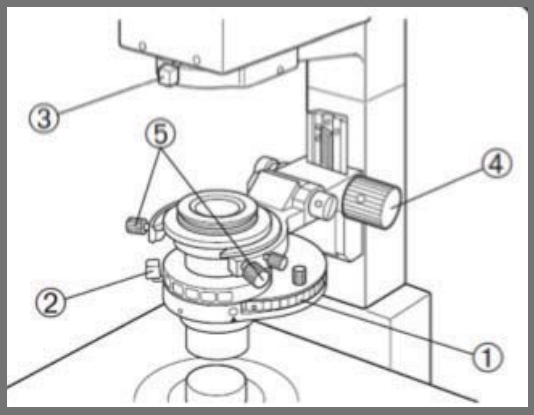
開啟掃描控制器



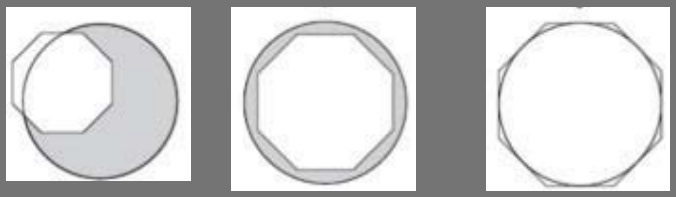
開啟電腦及軟體
(Cleaning……)

Turn on system

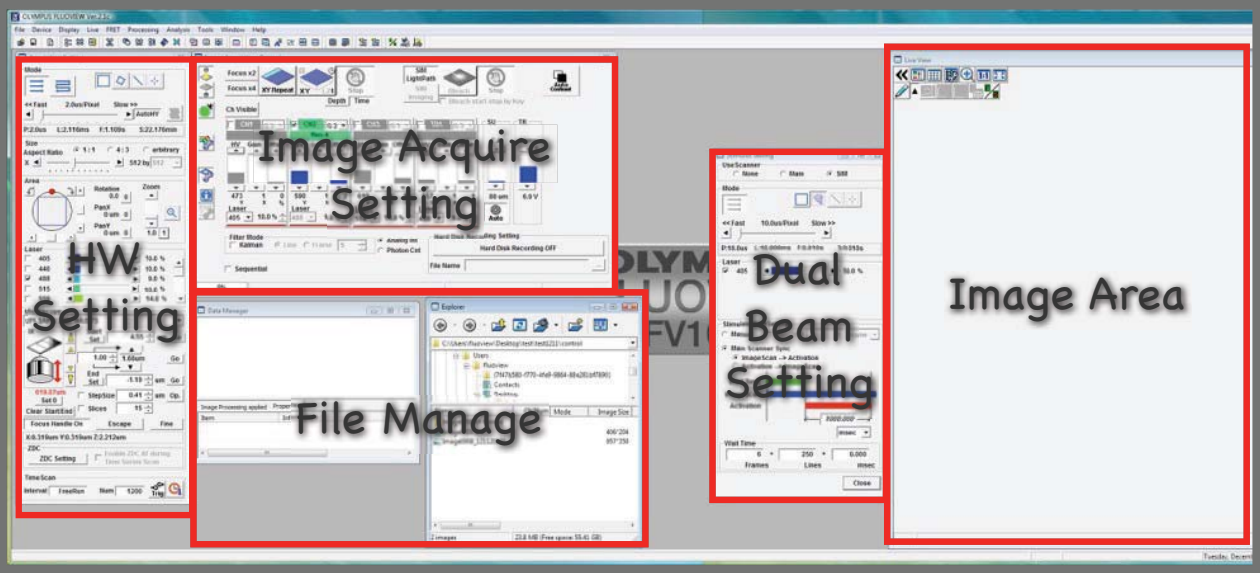
Light-path adjust



- 以10倍鏡頭找到焦距
- 將3關到最小
- 調整4到八角型最清晰
- 調整5光圈中心
- 將3開回最大原位



SW Interface



Observation

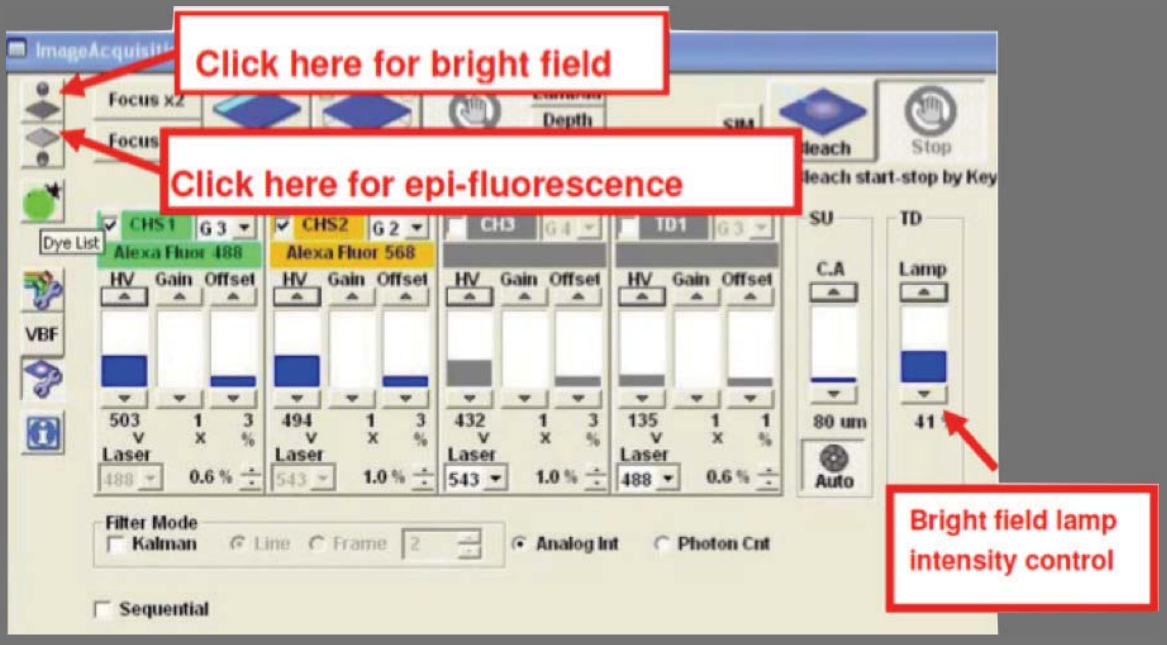


Image Scan

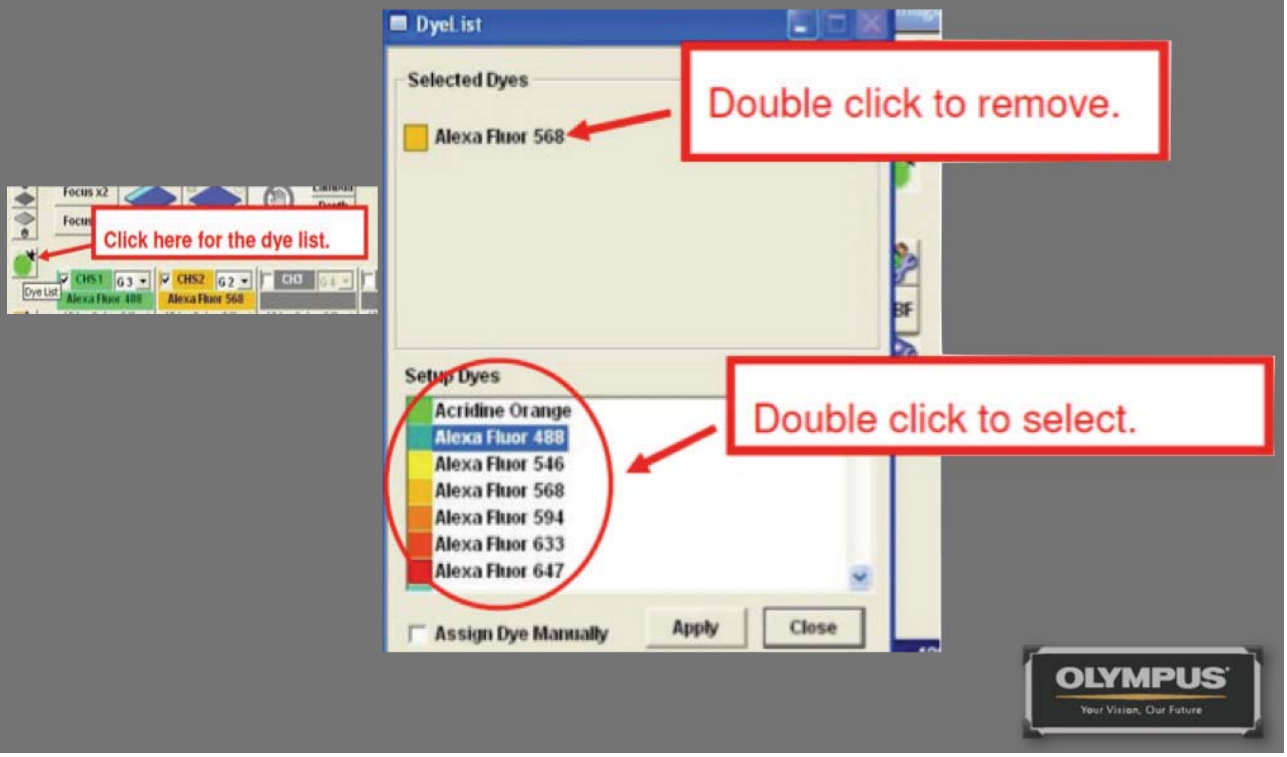


Image Scan

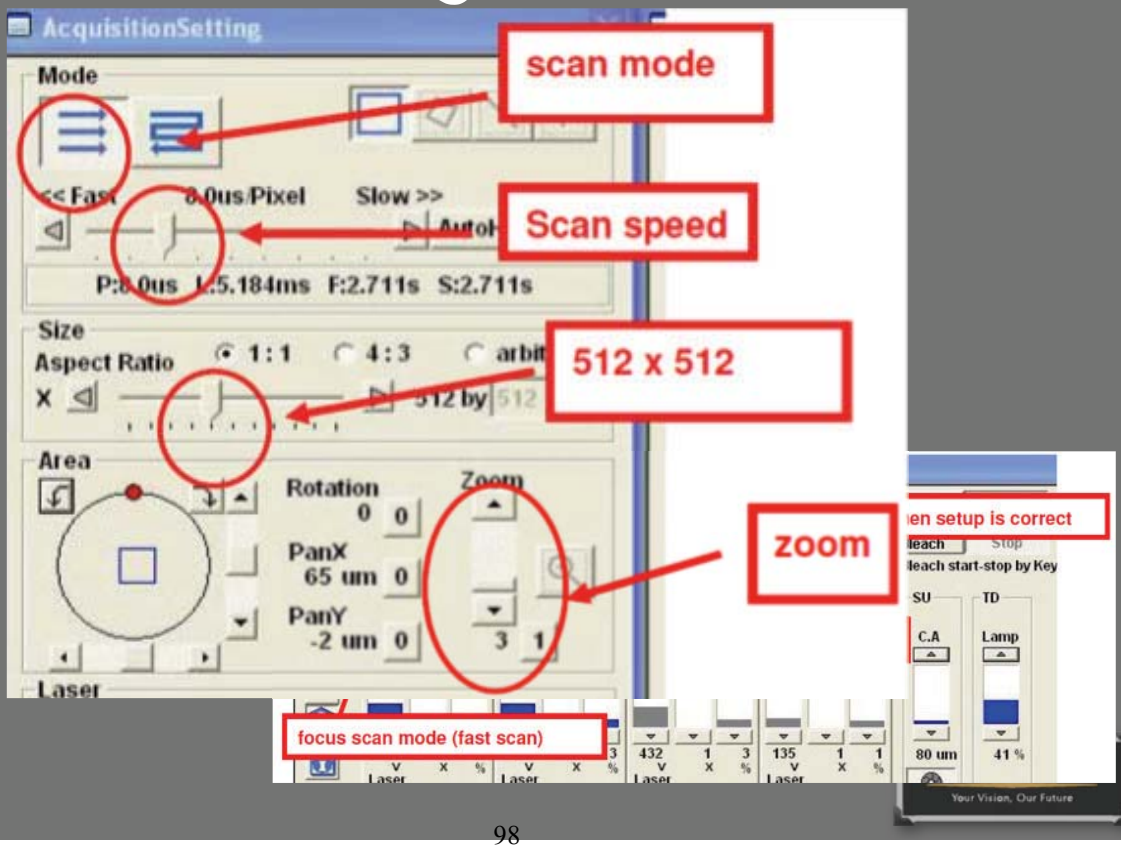


Image Scan

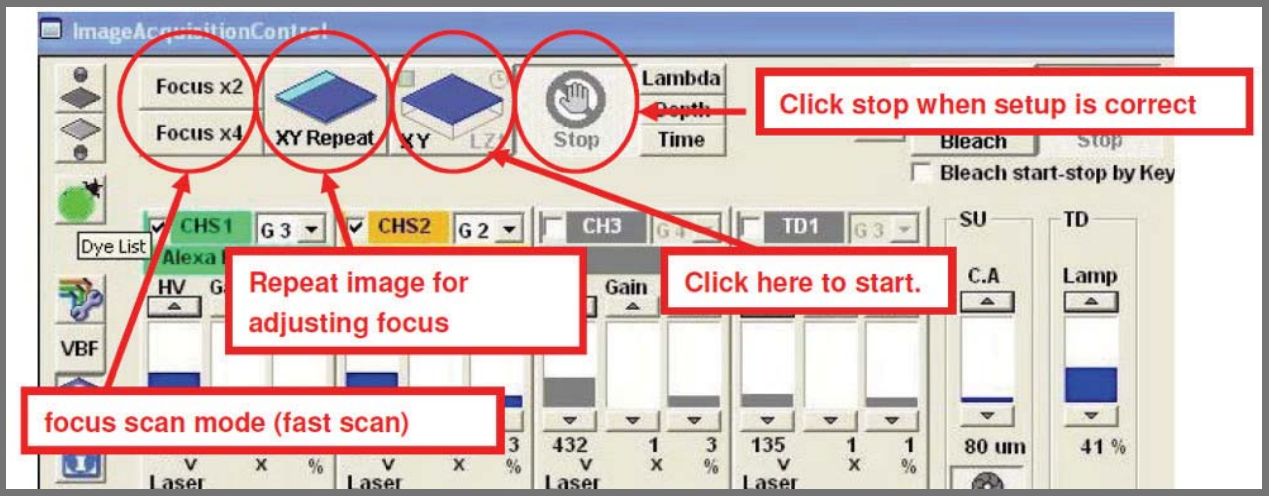
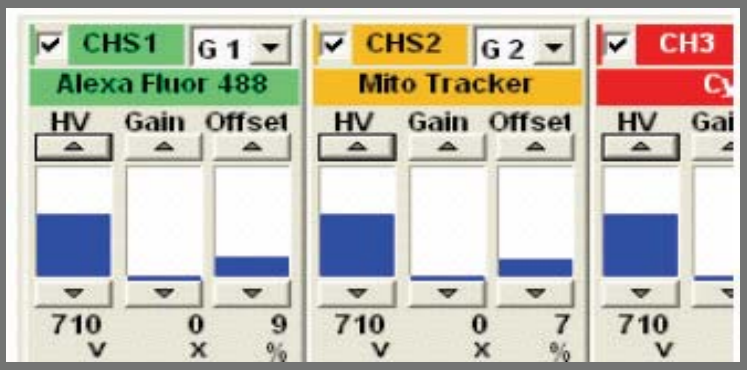


Image Scan

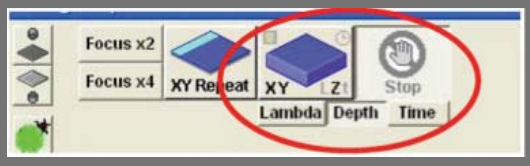


HV = PMT 電壓，增加 HV 會提高檢測敏感度，但圖像噪音也會隨之增加

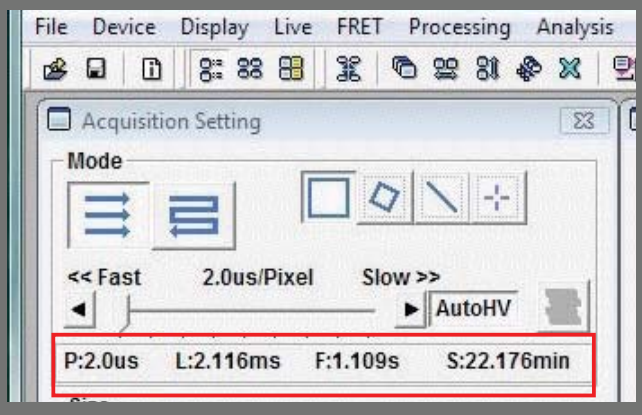
Gain = 後期信號放大，在圖像信號極低的情況下可以適當調節

Offset = 影像位準調整，Offset 越高，圖像背景變暗，但一些位準以下的螢光信號也可能被同時扣除

Z-Section

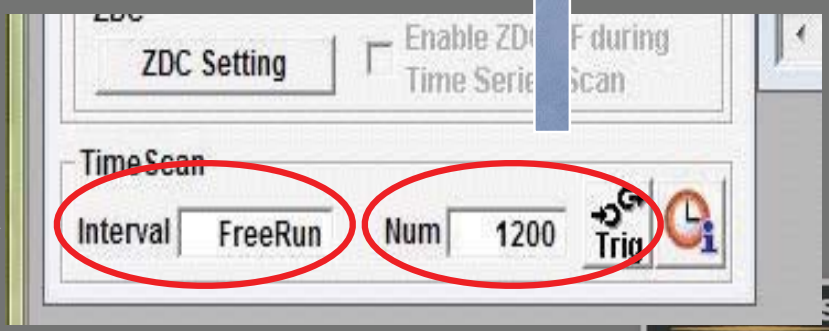


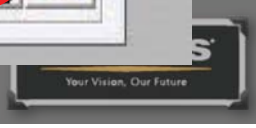
Time Lapse



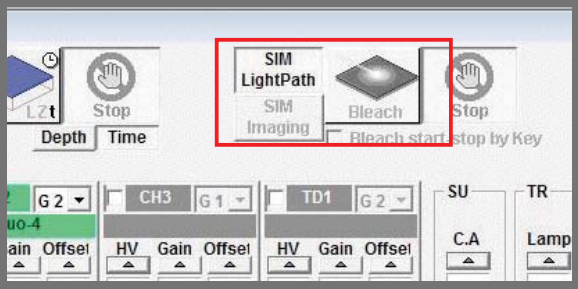
重複次數

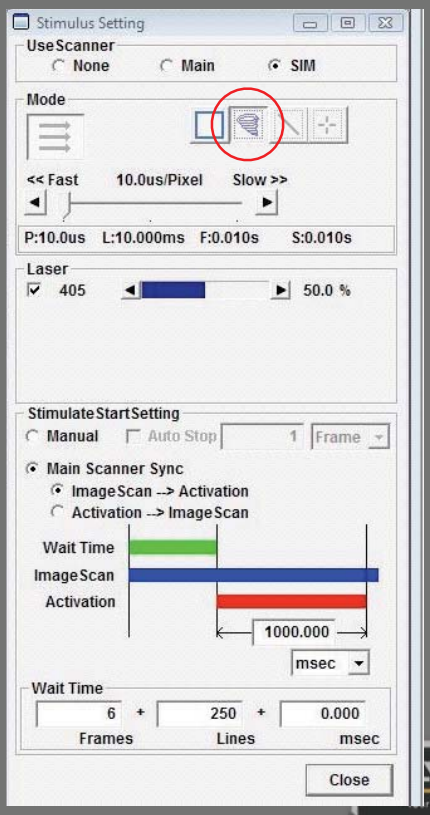
時間間隔



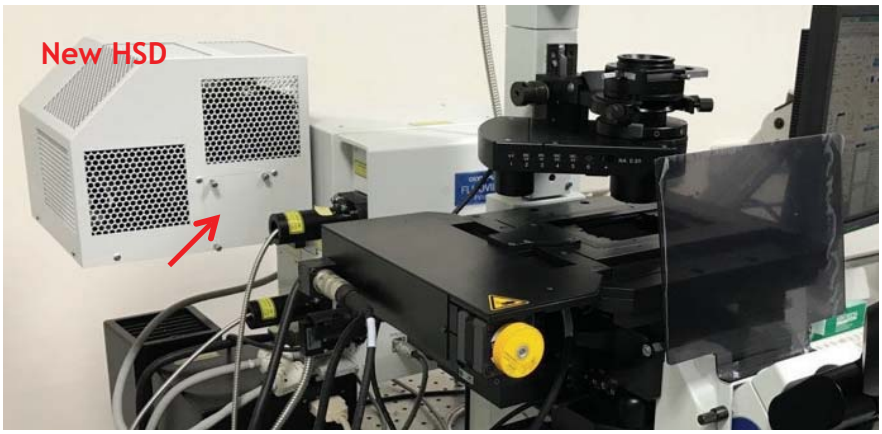


Dual Beam Operation





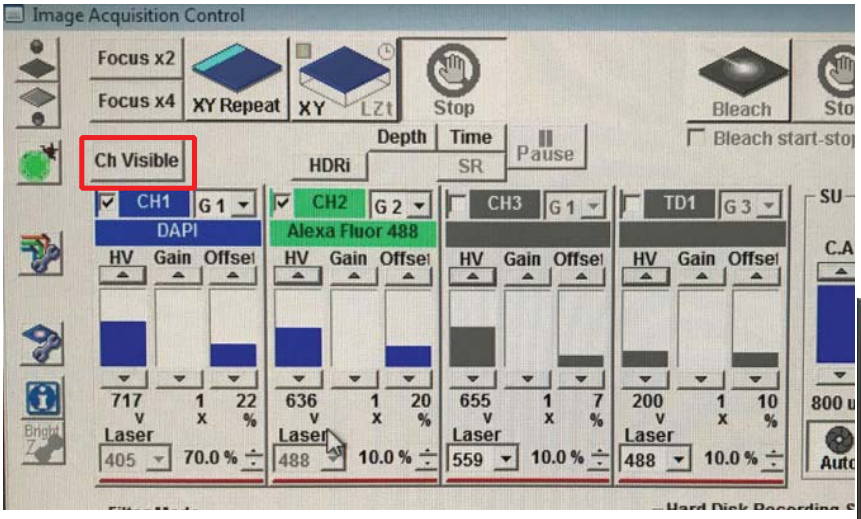
HSD 的使用

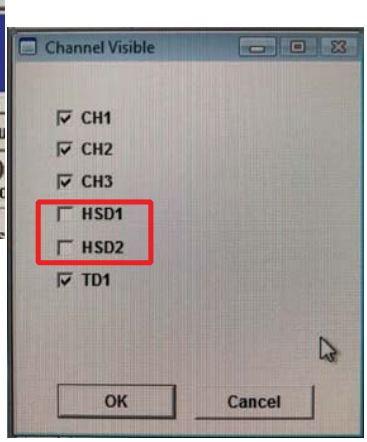


1. 開關機請依照原本的方式即可。

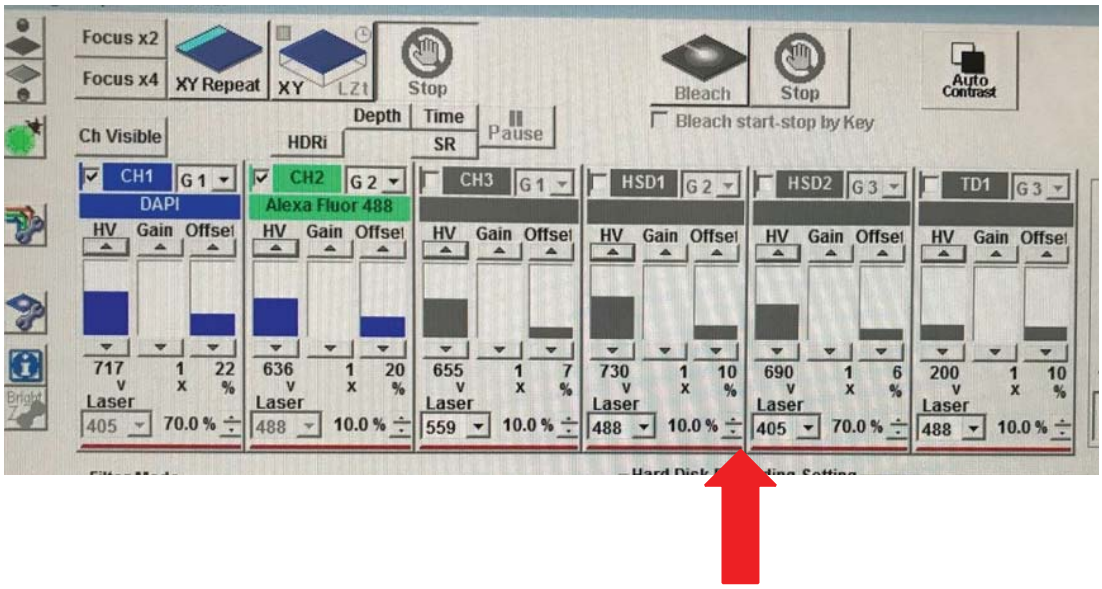
2. 更換濾片請將紅色箭頭所指處的外殼卸下 (四顆螺絲，手轉即可)→放鬆濾片匣固定螺絲後將濾片匣取出→更換濾片匣→鎖緊濾片匣固定螺絲後蓋上外殼，完成更換。

選取HSD 偵測器

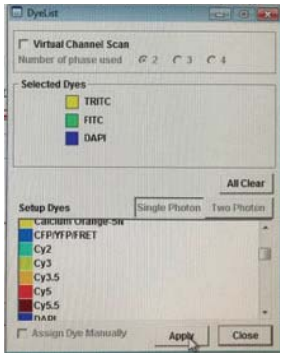




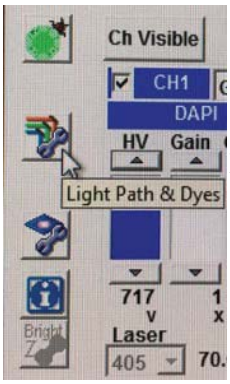
若是開啟軟體之後看不到 HSD 的選項，請選擇 Ch Visible，選取 HSD1 及 HSD2 即可看到 HSD1 及 HSD2。



HSD 光路設定

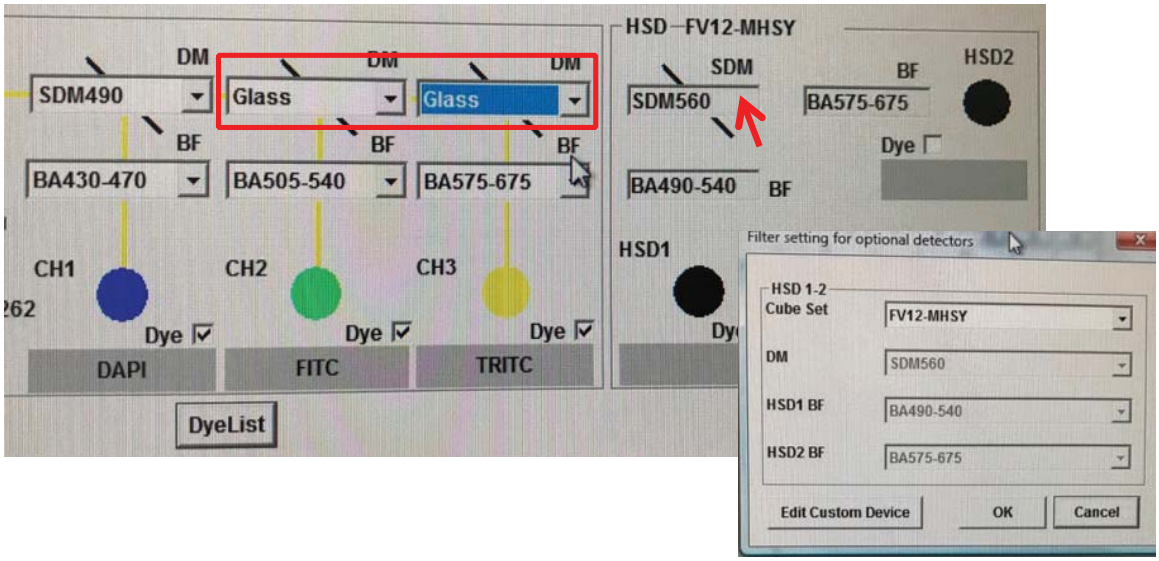


1. 一開始請照舊，選擇 Dye List，並選擇樣本的螢光種類。在此以 DAPI，FITC & TRITC 為例。



2. 選擇 Light Path & Dyes，進行光路設定

HSD 光路設定



3. 在此我們將 DPAI 維持原本的 PMT，將 FITC & TRITC 設定以 HSD1 及 HSD2 擷取影像。因此將紅框處更改為 Glass ，讓訊號可以通過進入 HSD1 & HSD2。

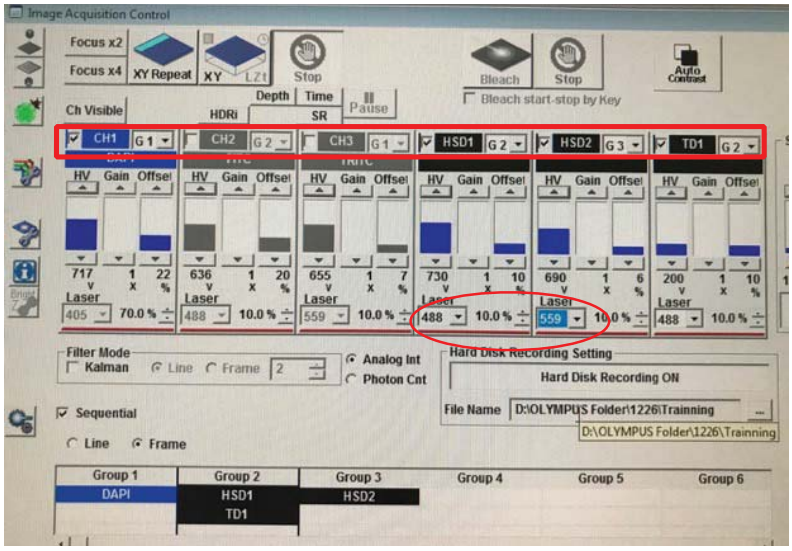
4. 於紅色箭頭處點一下滑鼠左鍵，選取我們所安裝的濾片匣。

HSD 濾片匣選項

目前共有 4 組 HSD 的濾片匣，收光波長範圍請參閱下表

濾片名稱	HSD1 範圍	SDM	HSD2 範圍
FV12-MHBVE	*480-495*	*510*	*535-565*
FV12-MHBY	*505-540*	*559*	*575-675*
FV12-MHSY	*490-540*	*559*	*575-675*
FV12-MHYR	*575-620*	*635*	*655-755*

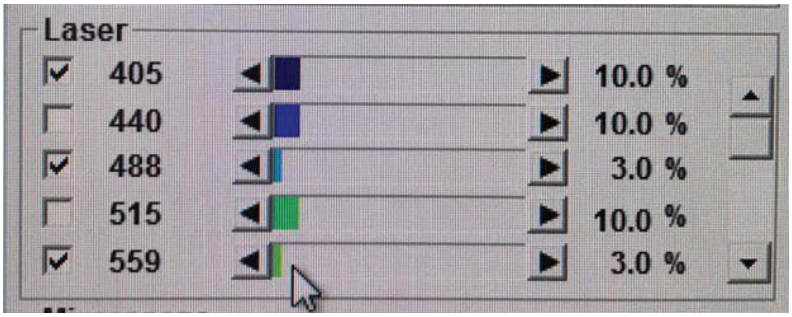
HSD 光路設定



5. 回到原視窗，確認要使用的偵測器是否被勾選，以及 HSD1 & HSD2 的雷射設定是否正確。

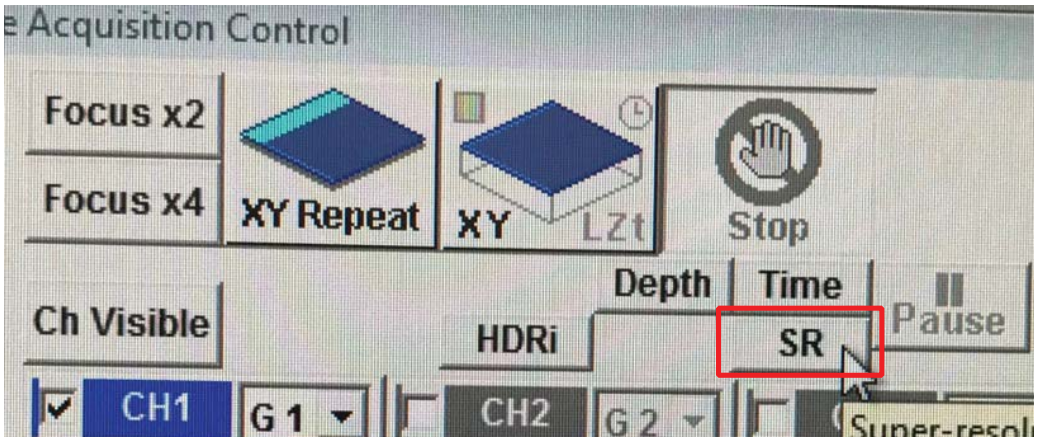
6. 其餘的設定以及影像調整均與之前操作方式相同。可開始進行掃描。

HSD 使用注意事項

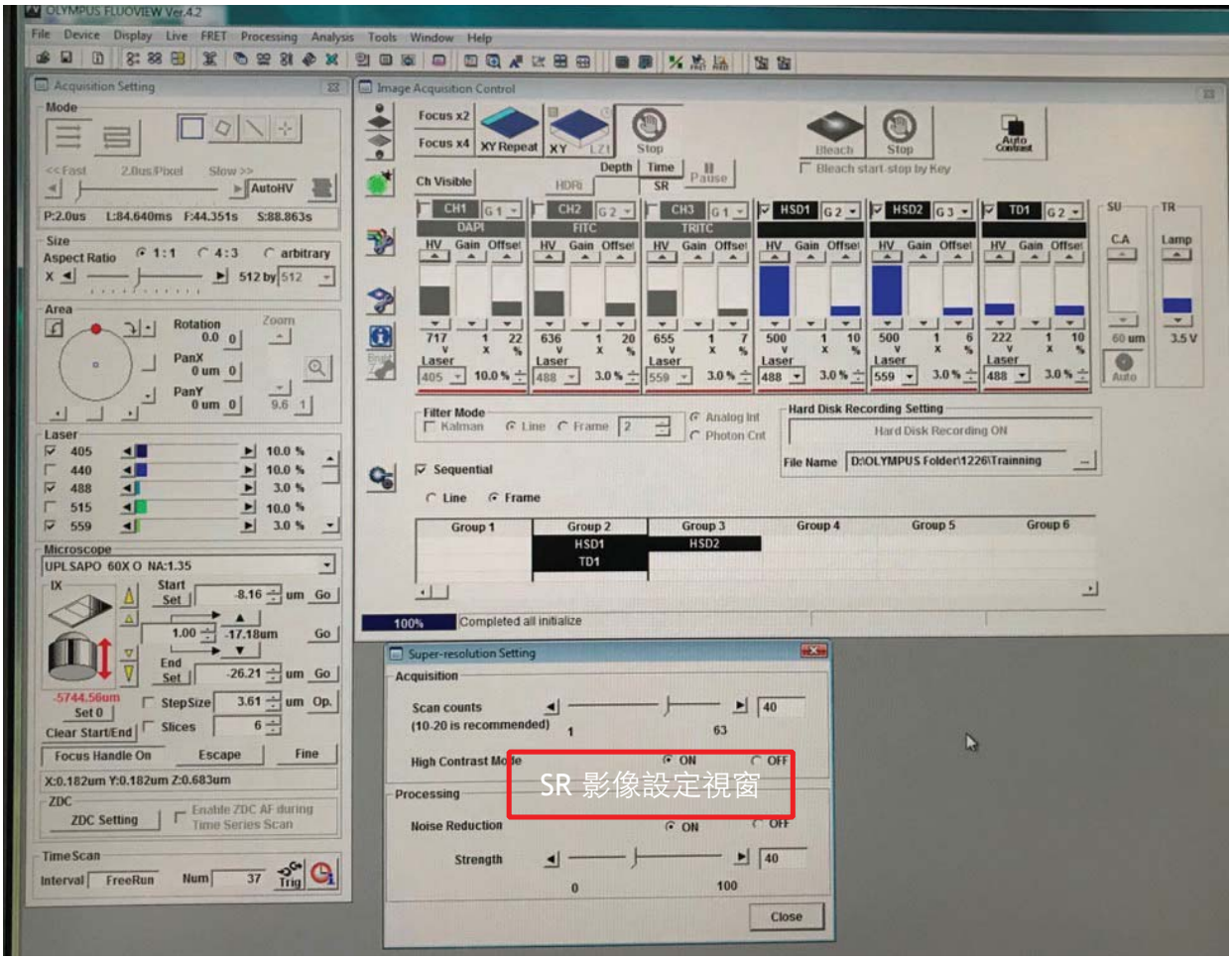


由於 HSD 的敏感度相當高，所以雷射盡量由低輸出開始掃圖，
再慢慢地提高雷射輸出，避免影響 HSD 的使用壽命。

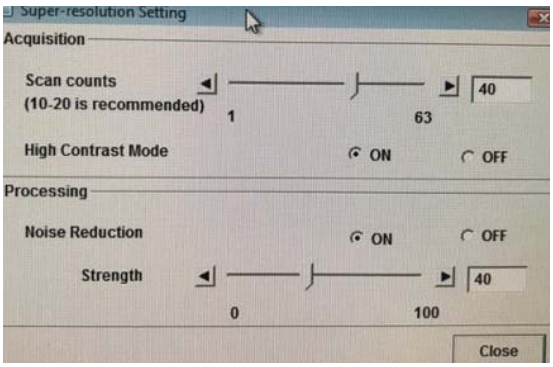
OLYMPUS　Super Resolution (OSR) 使用方式



若要使用 OSR 功能，請先選取 SR 選項，就會跳出 SR 影像的控制視窗，如下頁



SR 影像注意事項

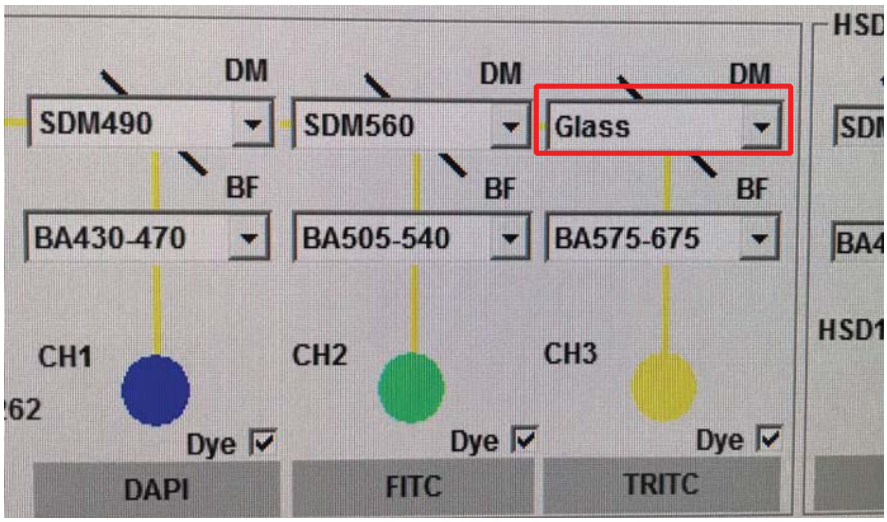


在此可以設定 SR 影像的擷取張數及影像處理參數，建議先嘗試預設參數， Scan counts: 20, Strength: 40。

1. 在 SR 影像模式時，只能用 HSD 擷取影像。
2. 在 SR 影像模式時，掃描速度，pinhole，HSD 的 HV 值等參數會固定無法調整。
3. 在 SR 影像模式時，掃描範圍 (Zoom in) 會隨這著影像大小設定而變動。

Image Size	128	256	320	512	640	800	1024	1600	2048	4096
Zoom factor	***38.5***	***19.3***	***15.4***	***9.6***	***7.7***	***6.2***	***4.8***	***3.1***	***2.4***	***1.2***
Speed per Frame	*7.507*	*17.173*	*22.985*	*44.351*	*61.767*	*87.221*	*129.870*	*276.319*	*427.162*	*1524.147*

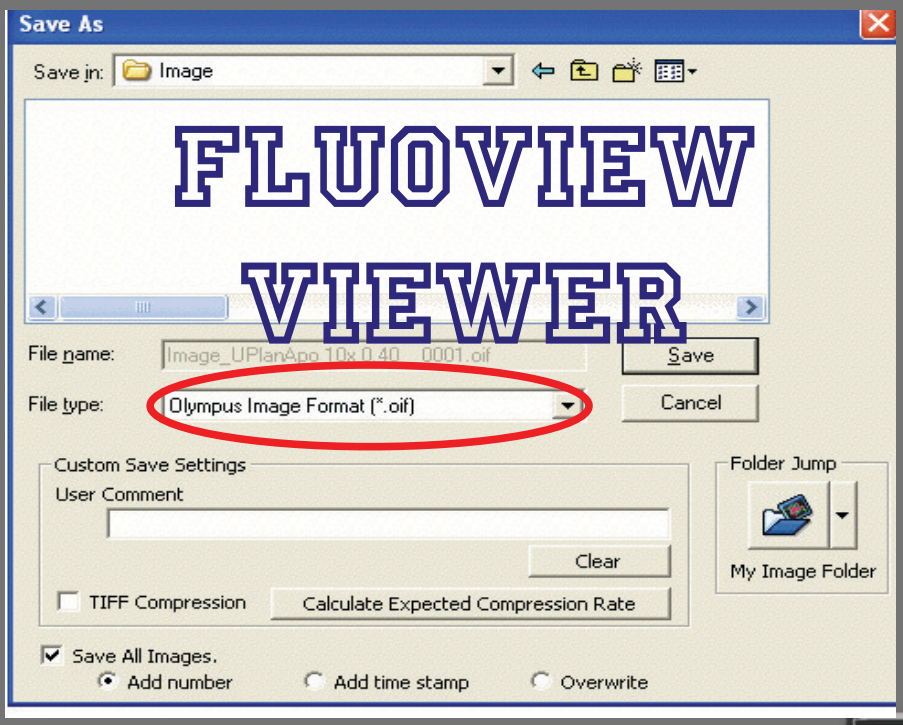
使用完畢後.....



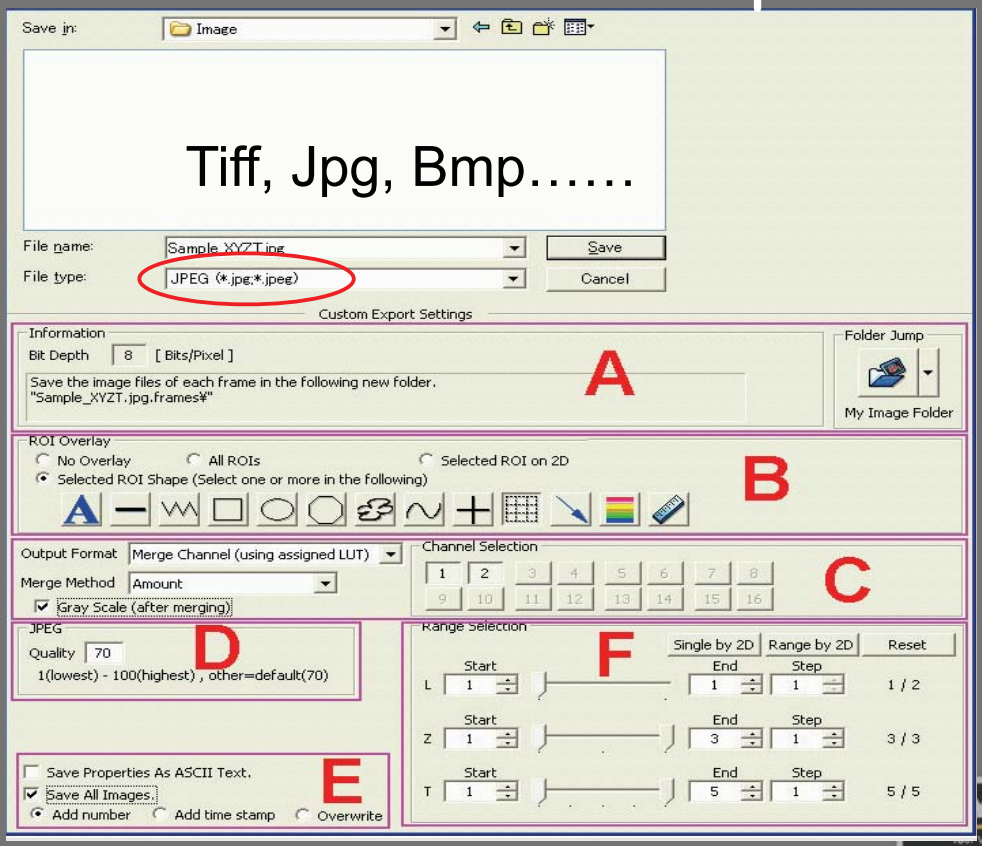
使用 HSD 或 SR 之後，請回歸預設參數，請選擇 Dye List ，在按一次 Apply，讓光路設定回歸預設參數即可。

不過，紅色框位置，請各位手動變更為 Mirror，謝謝大家。

File Save and Export



File Save and Export



OLYMPUS®

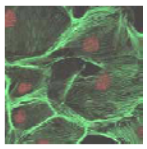
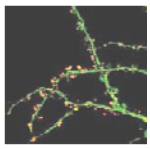
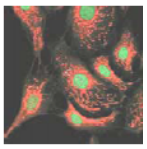
Your Vision, Our Future

Olympus FV 1000 雙光束雷射掃描共軛焦顯微鏡

儀器使用規則及認證辦法說明

研究資源整合中心

107 年 7 月 25 日

管理者：蔡克勵
技術員：朱家瑩

Olympus FluoView 1000
雙光束雷射掃描共軛焦顯微鏡

儀器特色

- 特色
  - 雙光束雷射光源
  - 可進行最多四色掃描
  - 波長－ 405 nm, 440nm, 488 nm, 515 nm, 559 nm, 635 nm
  - 倒立式顯微鏡，便於活細胞觀察
  - 物鏡放大倍率－ 5x, 10x, 20x（以上空氣鏡）；40x, 60x, 100x（以上油鏡）
  - 可進行活細胞即時紀錄 (real-time recording)

儀器沿革

- 沿革
  - 裝機－
  - 測試－ 99 年 12 月 1 日
  - 第一階段種籽教師訓練－ 99 年 12 月 15 日
  - 驗收－ 99 年 12 月 23 日
  - 制定使用規則－100 年 1 月
  - 開放全校使用－100 年 2 月 1 日
  - 開始收費－100 年 4 月
  - 加裝升級套件－107 年 4 月

使用者

- 欲使用本儀器者，必須完成以下四項程序，方能成為合格使用者：
  - 參加訓練課程
  - 通過筆試
  - 上機認證
  - 種籽教師陪同第一次使用儀器

- 法規依據
  - 高雄醫學大學雙光束雷射掃描共軛焦顯微鏡使用規則（100 年 7 月 15 日本校共軛焦顯微鏡管理委員會修訂通過）
  - 研究資源整合中心相關規範

訓練課程

- 全校性共軛焦顯微鏡教育訓練課程
  - 本校現有兩台共軛焦顯微鏡： Zeiss LSM 700 及 Olympus FV1000
  - 本中心原則上每三個月舉辦一次共軛焦顯微鏡教育訓練，輪流訓練兩台共軛焦顯微鏡之使用者

訓練課程時程表

時間＼日期	第一天	第二天	第三天
8:30-9:30	1. 儀器使用規則介紹 2. 儀器原理及操作介紹 3. 筆試	一對二教學 一對二認證	一對二教學 一對二認證
9:30-10:00			
10:00-11:00		一對二教學 一對二認證	一對二教學 一對二認證
11:00-11:30			
11:30-12:30		一對二教學 一對二認證	一對二教學 一對二認證
12:30-13:00			
13:00-14:00	分組上機示範操作	一對二教學 一對二認證	一對二教學 一對二認證
14:00-14:30			
14:30-15:30		一對二教學 一對二認證	一對二教學 一對二認證
15:30-16:00			
16:00-17:00		一對二教學 一對二認證	補考
17:00-17:30			

訓練課程：報名

- 本儀器教育訓練課程
  - 107 年 7 月 25-27 日
- 報名
  - 正取 20 名，候補 3 名。
  - 每個實驗室（以實驗室主持人為單位）最多錄取 2 名。

訓練課程：筆試

- 筆試
  - 訓練課程授課完畢後，隨堂舉行筆試測驗。
  - 筆試成績達 70 分為通過，通過名單稍後公布於本中心網頁。
  - 候補者 3 名可參與上午之課程及筆試，若有正取者筆試未通過，候補者可以依序遞補。
  - 若有候補者筆試成績通過，卻未能遞補上，則可保留筆試成績到下一次訓練時間，擁有優先報名權，以一次為限（亦即下次若無參加上機訓練，則本次筆試成績作廢）。

訓練課程：上機認證

- 上機認證
  - 正取及遞補之候補者通過筆試後，下午分組觀摩上機示範操作。
  - 第二天起，由種籽教師以一對二方式進行實機教學及認證。
  - 若有認證不通過者，集中於最後一天下午補考，以一次為限。

第一次使用儀器

- 種籽教師陪同使用儀器
  - 訓練課程結束後，使用者第一次使用儀器時，須由種籽教師陪同。若確認使用無誤，種籽教師簽名核可之後，日後即可獨立上機。
  - 通過上機認證之使用者，請於半年內實際上機使用儀器，否則將喪失使用者資格，須重新報名訓練課程。

預約使用儀器

- 預約申請方式：撥電話（分機 2371#27）至本中心向朱家瑩技術員預約使用時間，並至本中心網站下載申請表，填妥後於上機前交給技術員，完成預約申請。

- 合格使用者必須完成前述程序，方能自行操作儀器。未通過前述程序者，可以與技術員約定時間，提供樣本委任技術員代為上機，費用另計。

預約使用儀器

- 使用者可於實驗 2 個月前至一週前預約（即可預約當月下週起至下個月）。以實驗室負責人為單位，每個實驗室每月最多預約三次。
- 每次使用時段為 3 小時，若之後無其他使用者預約，可彈性延長半小時。若超過 1 小時以上，則視作另開時段收費。
- 萬一因為突發狀況必須取消實驗，須於一天前通知本中心取消預約，否則仍照扣儀器使用費。
- 若有急需使用者，可先以 E-mail 向技術員申請後補使用，並用電話告知。一旦有空餘時段，本中心即可通知其來使用。此種後補使用不受每月最多三次之限制。

開放時段

- 一般開放時段為週一至週五 8:00~11:00、11:00~14:00、14:00~17:00，每天三個時段，每 3 小時為一時段。限合格使用者才可自行操作儀器。
- 開放預約實驗安排：週一及週二為雙光束 (two beam) 實驗時間，週二原則上以雙光束實驗預約優先，若無人預約則亦可開放單光束 (one beam) 實驗。週三至週五為單光束實驗時間。
- 平日晚間及假日只限種籽教師本人使用。

收費標準

- 對校內合格使用者，每三小時為一時段，每一時段收費 1200 元。

- 連續使用兩個時段以上，費用以八折計算。若連續使用超過三個時段，當日（含夜間）最高以三個時段計費，隔日第一時段開始，則繼續累進計費。

- 為鼓勵新使用者儘速實際使用儀器，並提升儀器使用率，凡是完成訓練課程之新使用者，於上機認證後得享有兩次免費使用之優惠，三個月內須使用完畢。

收費標準

- 校外研究合作者視同校內人員收費，但預約申請時須註明校內合作人員，並簽署「高雄醫學大學跨校合作共軛焦顯微鏡使用同意書」。共同發表研究成果時，亦須明列校內合作人員。

- 委任技術員上機服務，每三小時為一時段，每一時段收費 2300 元。

- 其它無研究合作關係之校外人士，每三小時為一時段，每一時段使用收費 6000 元。若委任技術員上機服務，每一時段使用收費 7000 元。

影像存取

- 禁止使用隨身碟或外接硬碟存取。
- 由於電腦硬碟空間有限，為了維持儀器之正常運作，請自備光碟片燒錄儲存個人實驗資料。每月 1 日定期清空電腦硬碟所有資料夾中所有使用者檔案。若因此遺失實驗資料，本中心無法負責。
- 電腦僅供測量及分析數據使用，請勿在此電腦進行無關實驗之操作。

數據分析

- 為了避免影響其他使用者拍攝顯微影像，使用者在擷取影像後若欲利用軟體進一步做數據分析，請用離線工作專用電腦進行。
- 使用者若使用共軛焦顯微鏡一個時段（三小時），則可在離線電腦上使用同樣時間。欲使用離線工作專用電腦分析數據者，請事先預約。

使用者須知

- 請使用者愛護儀器及相關設備，並保持周邊環境整潔。
- 使用前務必先申請預約，使用完畢後，必須依照使用情況，確實填寫使用登記簿。
- 使用儀器時請注意自身安全，並正確使用儀器。若因不當使用而造成損壞，將取消儀器使用權利。
- 使用者上機時，須負維護儀器之責任。若有任何使用上的問題，請向技術員或管理者反應以尋求協助。切勿自行嘗試進行光路校正及拆卸鏡頭、顯微鏡，或更改基本軟體設定。

使用者須知

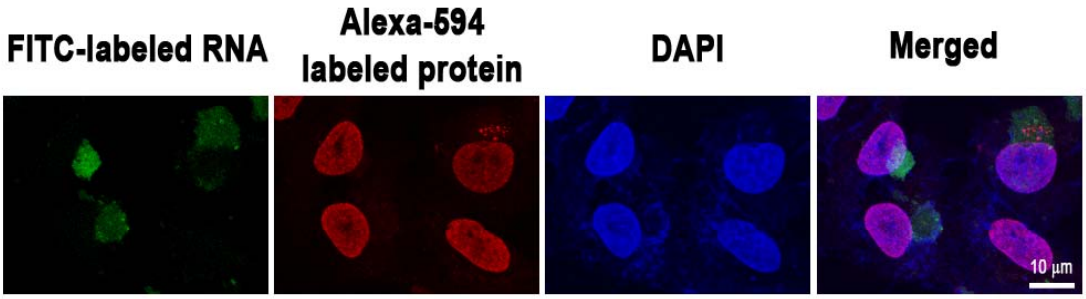
- 樣本的準備須符合本儀器的使用要件，方可上機進行觀察。本儀器禁用放射性物質 (radioactive isotope)。
- 使用油鏡時，應注意鏡油勿沾污其他鏡頭。油鏡使用後，應以拭鏡紙將油液擦拭乾淨。限用本中心提供之鏡油與拭鏡紙。
- 若使用者有①違反使用規則②未確實登記儀器使用情形③未清洗乾淨器具④未保持週邊環境清潔等行為：
  - 第一次發生，本中心提醒並記錄
  - 第二次發生，本中心暫停其使用儀器一個月，並通知所屬實驗室負責人
  - 第三次發生，本中心取消其使用儀器權利，並通知所屬實驗室負責人

成果發表

- 使用本儀器獲得之實驗結果，請在發表文獻之致謝詞 (ackowldegement) 中註明「感謝高雄醫學大學研究資源整合發展中心提供儀器協助」或 “We thank the Center for Research Resources and Development at Kaohsiung Medical University for the instrumental support of confocal microscope”。

- 校外人員若使用本儀器與本校研究人員合作研究，亦請比照辦理。

使用實例

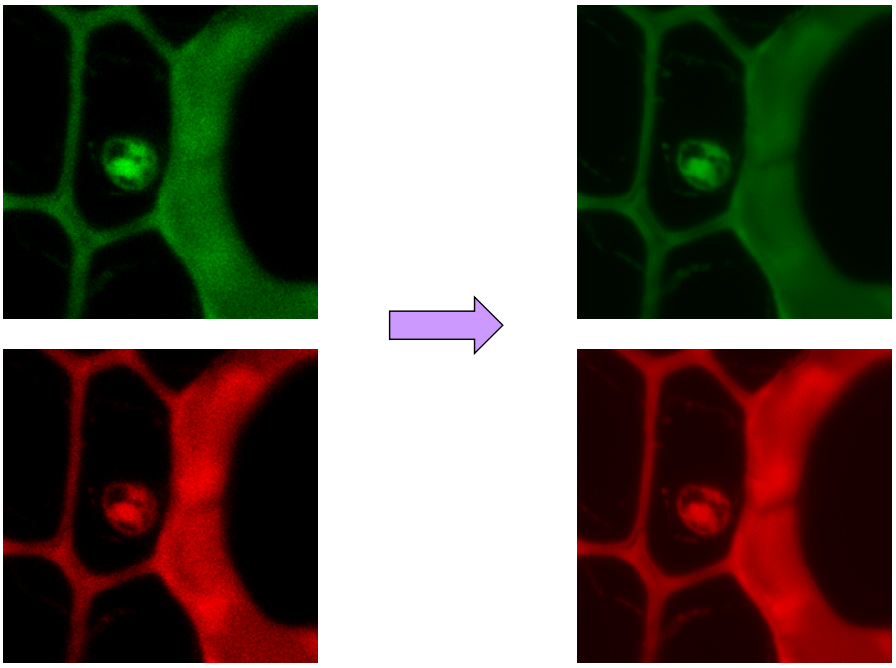


Laser:
405 nm for DAPI (nucleus)
488 nm for FITC (RNA)
635 nm for Alexa-594 (protein)

Scan speed: 400 Hz
Scan resolution: 1024 x 1024
Sample: Huh7 cells
Method: Immunofluorescent combined-FISH (fluorescent in situ hybridization)

感謝臨醫所洪詩雅小姐提供測試結果

升級套件



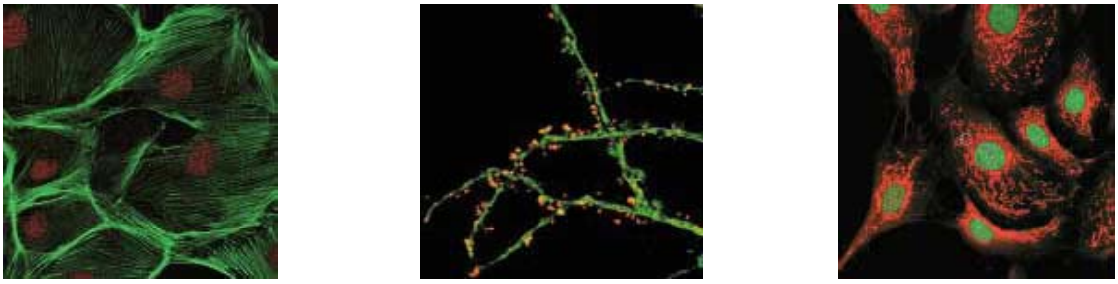
升級前影像

High Sensitivity-Spectral Detector (HSD) ＋
Olympus Super Resolution (OSR)

感謝元利儀器蔡博全先生提供測試結果

感謝聆聽！

祝實驗成功！



Olympus 提供

Protocol for immunofluorescence

Materials

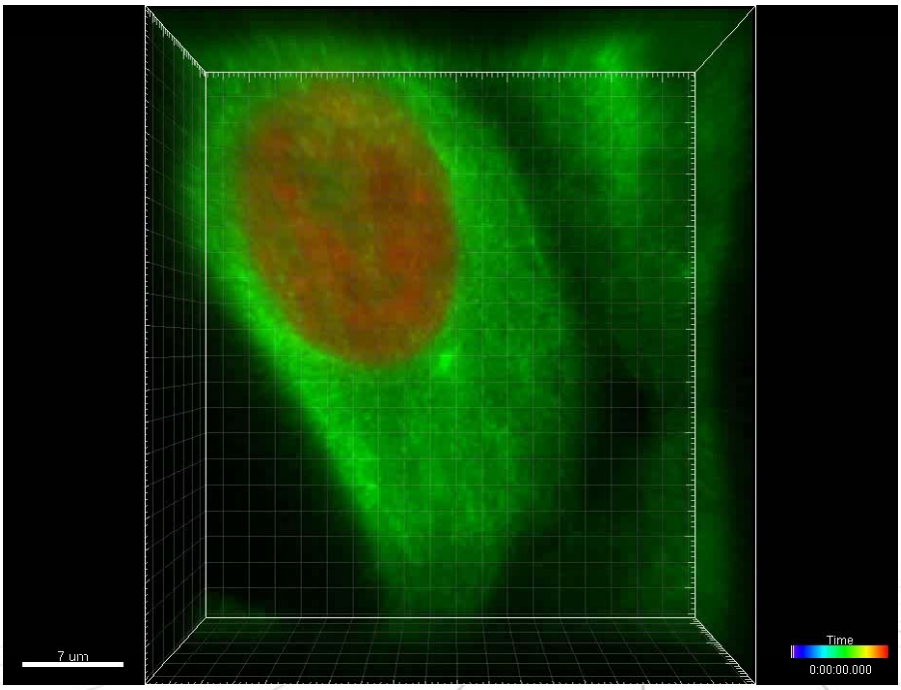
1. Human lung epithelial cell line (NL-20)
2. 1X PBS
3. 4 % Paraformaldehyde in PBS
4. 0.1 % Triton-X-100 in PBS
5. 1 % BSA in PBS
6. Monoclonal mouse anti-mouse AhR antibody (clone: RPT1)
7. Polyclonal Goat anti-mouse IgG Alexa Fluor 488 antibody
8. Alexa Fluor 568 Phalloidin
9. Fluoromount-G with DAPI

Methods

1. Seeding cells on cover slip (attached cell) /overnight
2. Treatment
3. Wash slip in PBS for 5 minutes.
4. 4 % Paraformaldehyde (in PBS) fix for 30 minutes.
5. Wash slip 3 times in PBS for 5 minutes each.
6. 0.1 % Triton-X-100 (in PBS) permeabilize for 10 minutes.
7. Wash slip in PBS for 5 minutes.
8. Blocking slip in 1 % BSA (in PBS) for 1 hour.
9. Apply diluted primary antibody (anti-mouse AhR, 1:200) in blocking buffer, 4℃, overnight.
10. Wash slip in PBS for 5 minutes.
11. Wash slip in 0.5 % Tween 20 (in PBS) for 10 minutes.
12. Wash slip 3 times in PBS for 5 minutes each.
13. Blocking slip in 1 % BSA (in PBS) for 30 minutes.
14. Incubate in conjugated secondary antibody (Goat anti-mouse IgG Alexa Fluor 488, 1:1000) diluted in blocking Buffer for 45 minutes at room temperature in dark.
15. Wash slip 3 times in PBS for 5 minutes each.
16. Incubate slip in Alexa Fluor 568 phalloidin (1:100) diluted in blocking Buffer for 20 minutes at room temperature in dark.
17. Wash slip 3 times in PBS for 5 minutes each.
18. Cover slip in Fluoromount-G with DAPI.
19. For long term storage, store slides flat at 4°C protected from light.

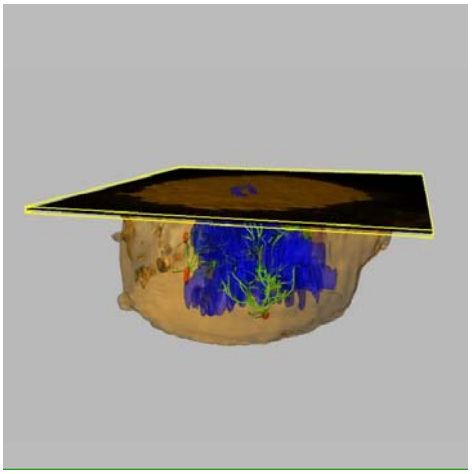
BITPLANE
Scientific Software

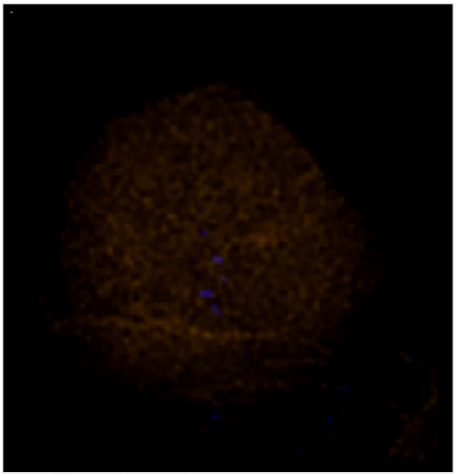
Or Even 4D…



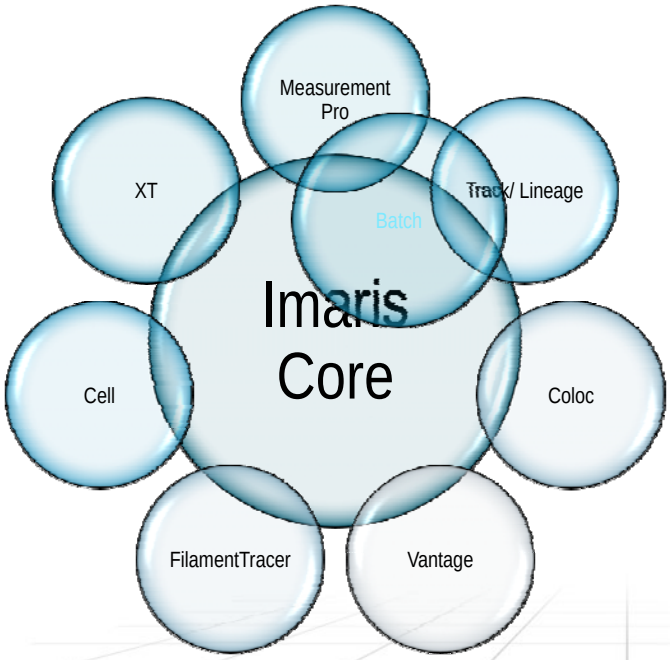
How do you reconstruct a z-stack?

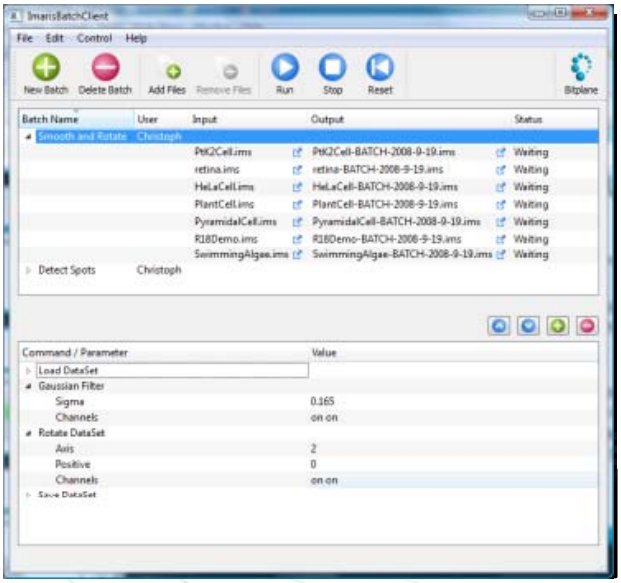
Optical sections are acquired at different focal planes and assembled into a volume of data. Each pixel from the 2D sections becomes a 3D voxel.



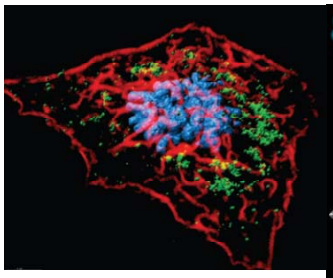


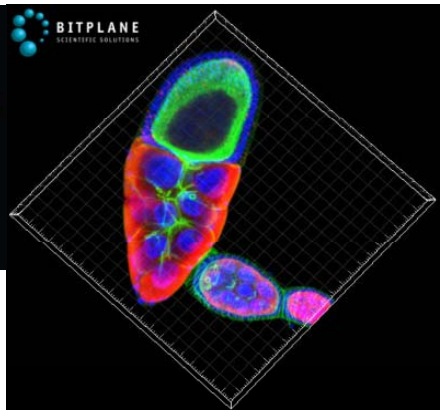
Imaris: a modular software





Core





Imaris Core

生命科學領域裡最領先的圖像分析軟體

作為Imaris軟體家族的核心模組，

Imaris是顯微鏡圖像快速交互展示的基本工具。

- 可直接讀取30多種格式的檔，幾乎涵蓋了所有顯微鏡廠家的圖像格式。
- 輕鬆處理50 GB 甚至更大的3D/4D文件
- 提供Slice, Section, Gallery 等觀察模式，並提供MIP 投影和混合投影模式。
- 在Supass 觀察方式下，各種交互工具結合展示（Volume rendering, iso-surface rendering, clipping planes, spots,slices）
- 採取易於操作的關鍵幀（key-frame）工具創建內容豐富的電影。
- 通過多種分離工具，自動識別並分離數以千計的物體。
- 通過多個CPU，GPU，及多解析度渲染模式提高Imaris 的分析能力，提高渲染速度。
- Bitplane 的專利觀察模式 InMotion 使3D 空間中物體的選擇及操作變得非常容易。

美嘉儀器股份有限公司

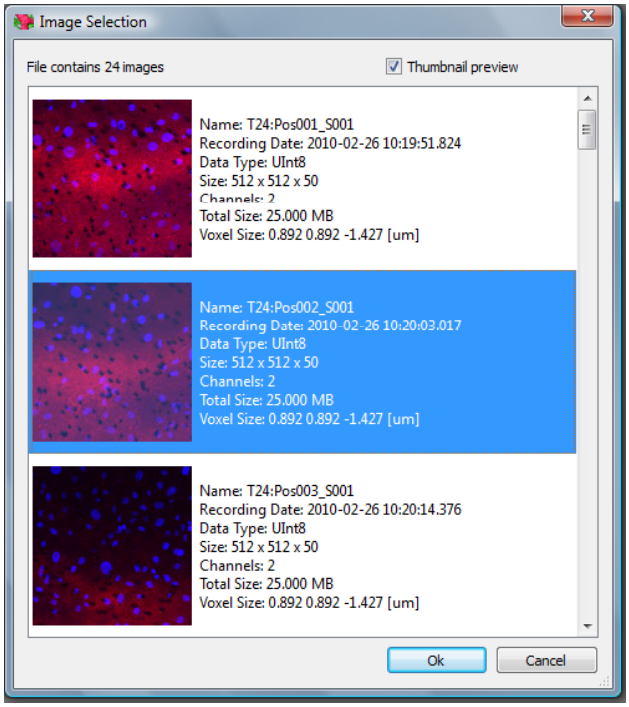
Supported files

- Andor: Multi-Tiff (Series) (*.tiff, *.tif)
- Andor: iQ ImageDisk (*.kinetic)
- Applied Precision DeltaVision (*.r3d, *.d3d, *.dv)
- Biorad MRC 1024, 600 Series (*.pic)
- Biovision: Ivision (.ipm)
- Bitplane Scene File (*.imx)
- Hamamatsu/Compix SimplePCI (*.cxd)
- BMP (adjustable file series) (*.bmp)
- Huygens and Huygens Compatible Nikon ICS File (*.ics, *.ids)
- Gata Digitial Micrograph (*.dm3)
- Imaris 2.7, Imaris 3, and Imaris 5.5 (*.ims)
- IMOD binary file (*.imod, *.mod), object scene file
- Leica Image Format LIF (*.lif)
- Leica LCS (*.tif, *.tiff, *.lei, *.raw)
- Leica Series (*.tif, *.tiff, *.inf, *info)
- Leica TCS-NT (*.tif, *.tiff)
- Molecular Devices (Formerly Universal Imaging now MDS Analytical Technologies) Metamorph Stack (*.stk)
- Micro Manager (*.tif *.tiff *.txt)
- MRC (*.mrc, *.st, *.rec)
- Nikon ND2 (*.ND2)
-Perkin Elmer: Ultraview (*.tim, *.zpo)
-Prairie Technologies (*.xml *.tif)
- Olympus Cell^R 1.1 (*.tif, *.tiff)
- Olympus FluoView (*.tif, *.tiff)TIFF
- Olympus OIB (*.oib)
- Olympus OIF (*.oif)
- Olympus VSI (*.vsi)
- Open Microscopy Environment Tiff (*.tiff, *.tif)
- Open Microscopy Environment XML (*.ome)
- OpenLab LIFF (*.liff)
- OpenLab Raw (*.raw)
- Quick Palm (.quickpalm, .tif)
- Scanalytics: IPLAB (*.ipl)
- SlideBook Slide (.sld)
- TIFF (adjustable file series) (*.tiff)
- TILLvisION (*.rbinf)
- Ziess Zen (*.czi)
- Zeiss Axiovision (*.zvi)
- Zeiss LSM410, LSM310 (*.tif, *.tiff)
- Zeiss LSM510, LSM 710 (*.lsm)

File Opening Special Cases: Leica *.lif

- The *.lif file can contain any number of 2D images, 3D images, 4D images, projections, or movies.
- Check Thumbnail Preview to see a projection of the data
- Click on each “file name” in the image list and the properties area below will tell you details about the particular image so you can choose the right one.

Imaris will only read the first file, if the user does not choose another one from the list.



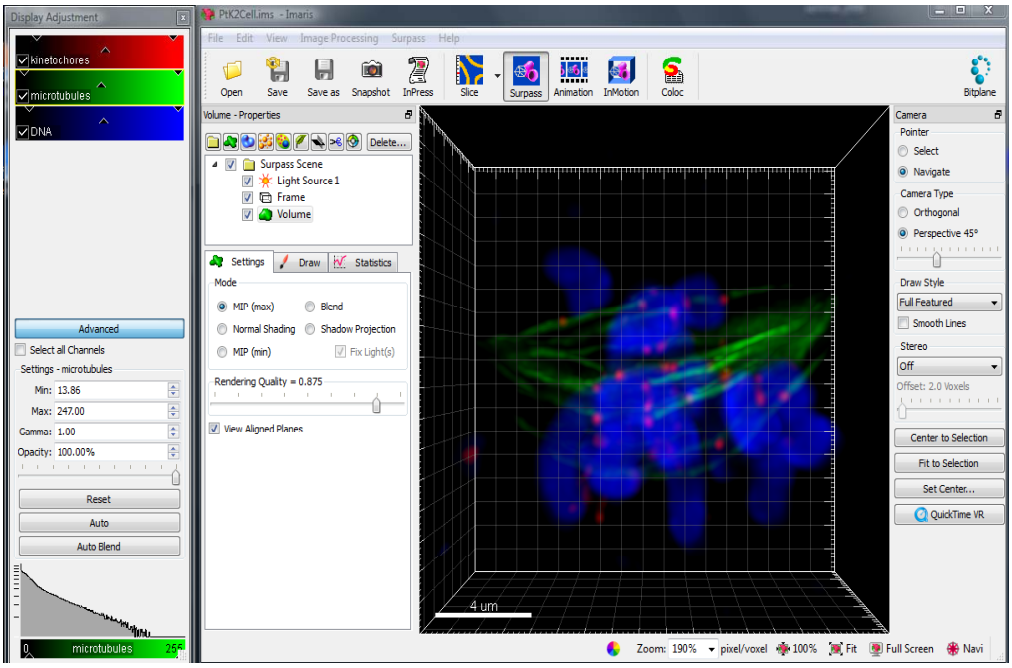
Imaris Interface

Tree Structure
All components in the scene are represented here in a tree hierarchy

Properties
Analysis, Wizards, statistics....

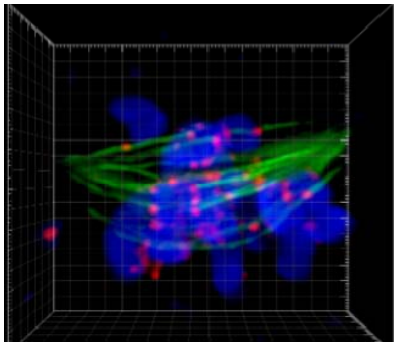
Imaris Scene
Workspace area for navigation, selection and interaction

Display Adjustment
Max/Min intensity, Opacity, Gamma

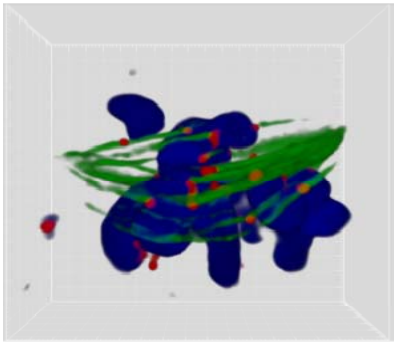


Imaris Volume Rendering

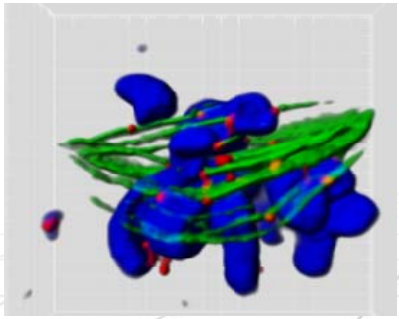
MIP



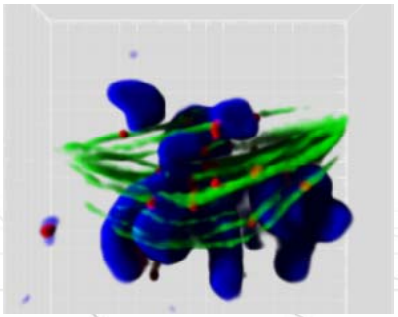
Blend



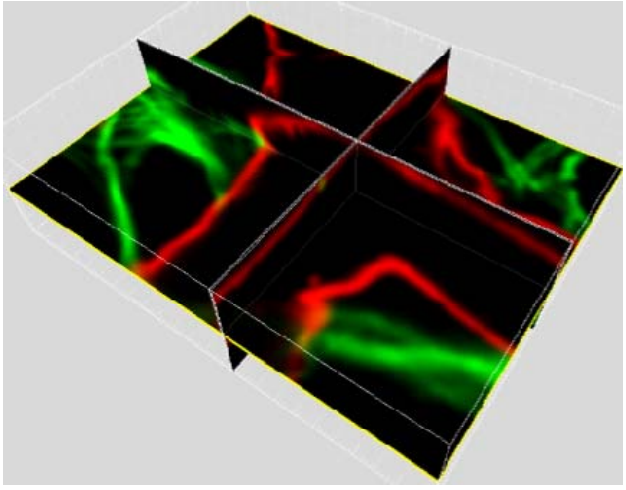
Normal Shading

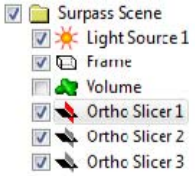


Shadow Projection

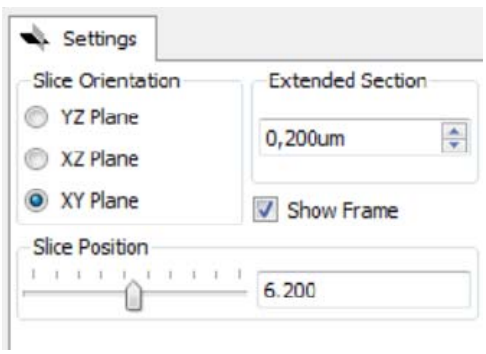


OrthoSlicer

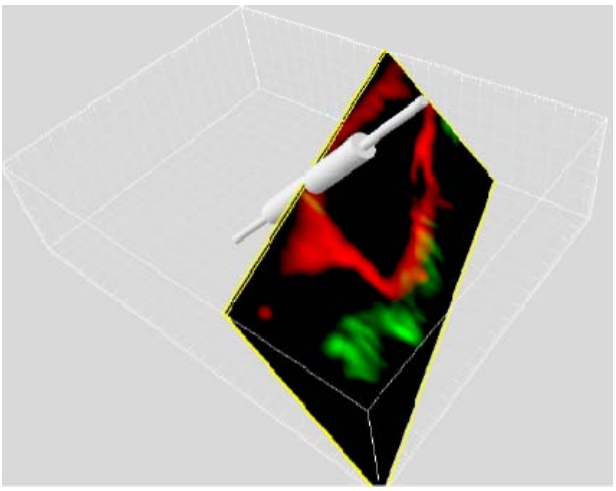


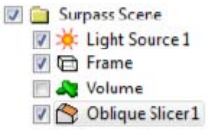


- Orthogonal plane on which original data are projected
- Can be moved within the dataset
- “Extended section” adjusts slices thickness

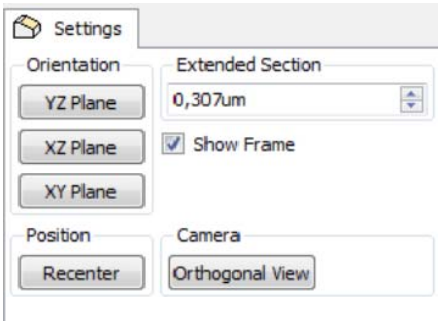


Oblique Slicer



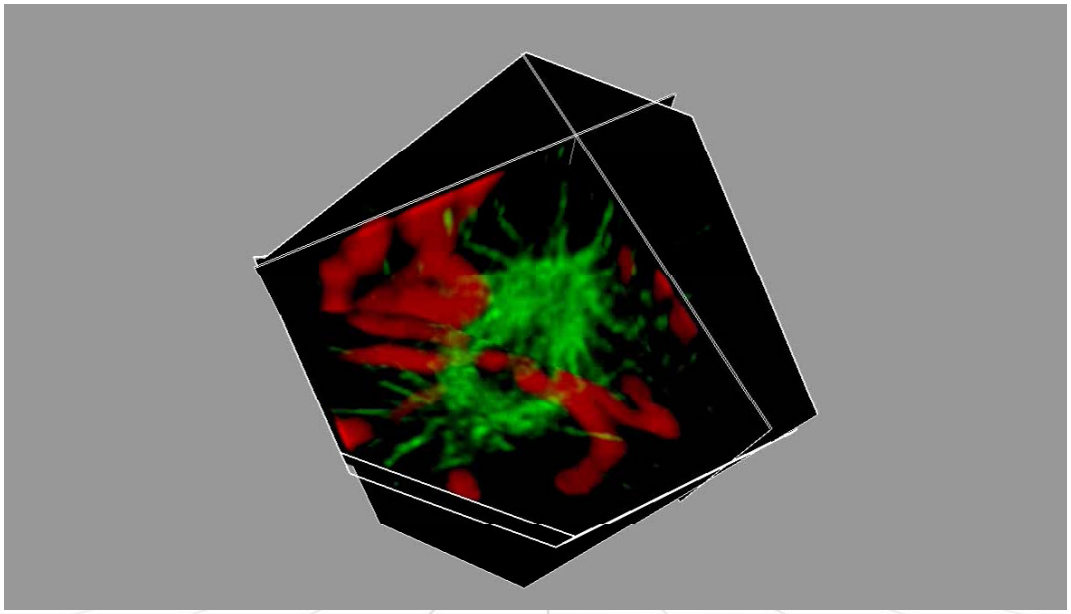


- Plane on which original data are projected
- Can be freely moved and rotated within the dataset (similar to Clipping Plane)
- “Extended section” adjusts slices thickness

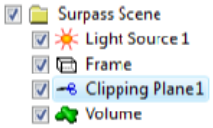


Explore the Next Dimension

ortho slicer and oblique slicer view

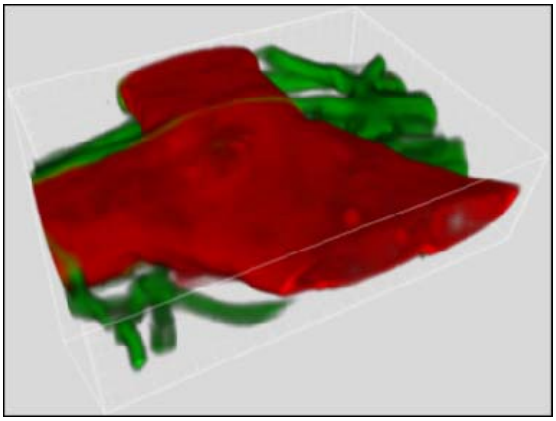


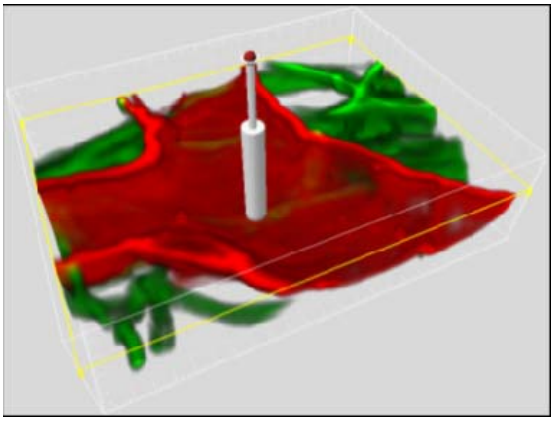
Explore the Next Dimension

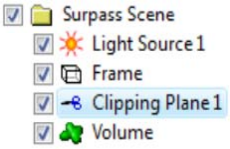


Clipping Plane

- Cuts away objects on one side of the plane
- Allows you to look inside any object
- Can be freely rotated

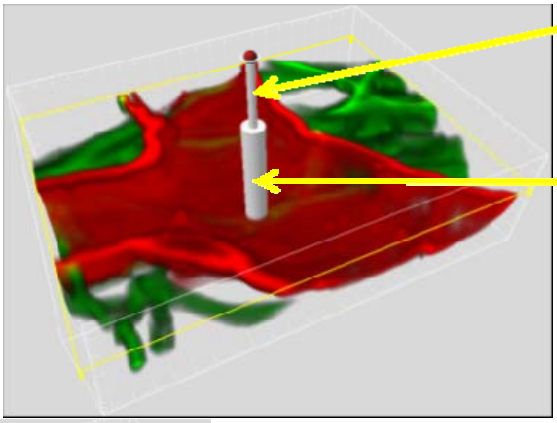






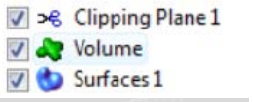
Clipping Plane

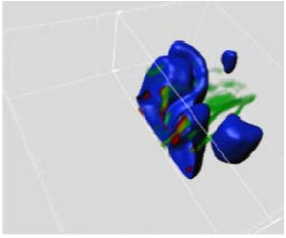
Only cuts objects lower in Imaris tree

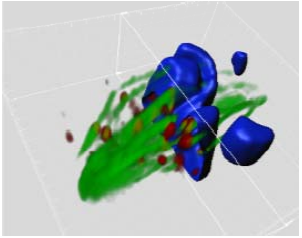


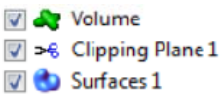
Use this rod to freely rotate the plane

Use this rod to change the position of the plane

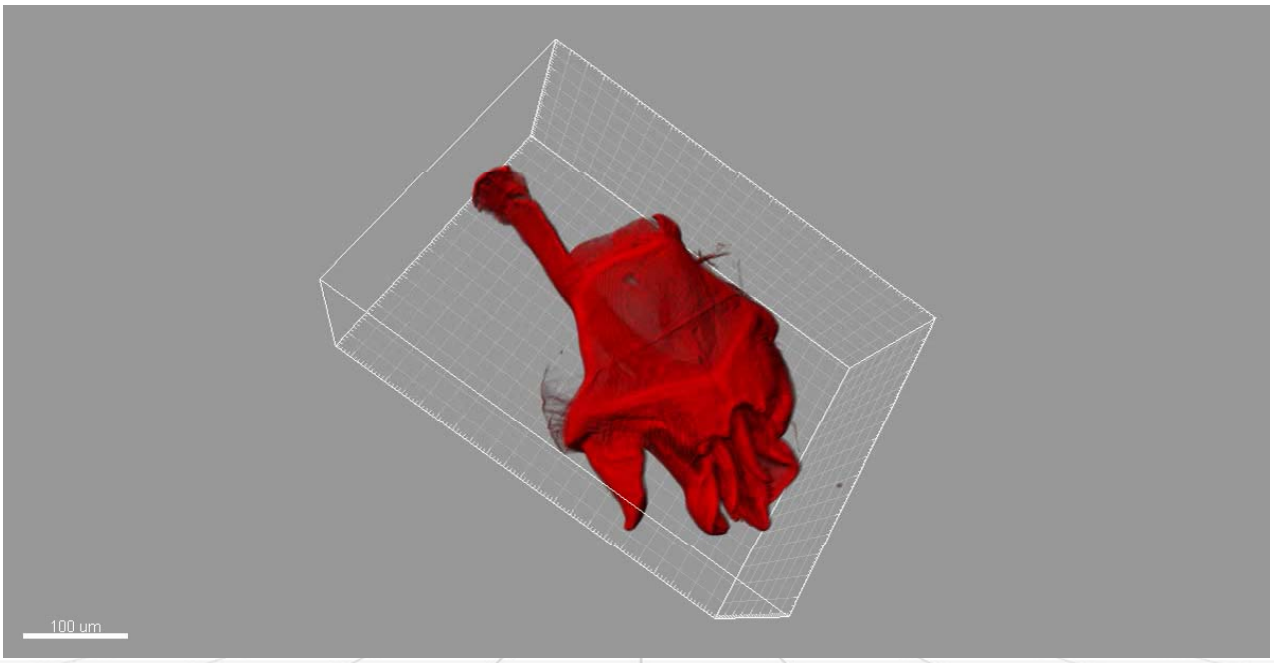


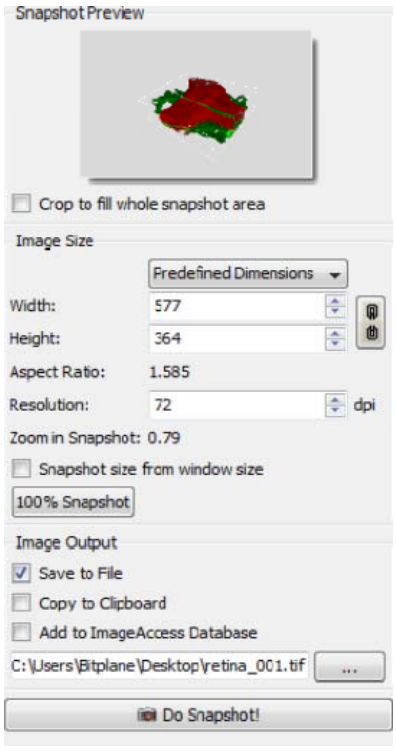






Clipping Plane



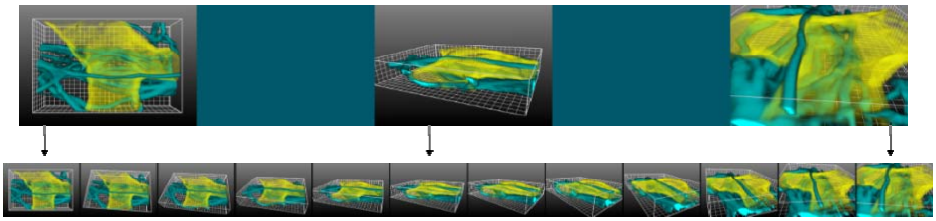


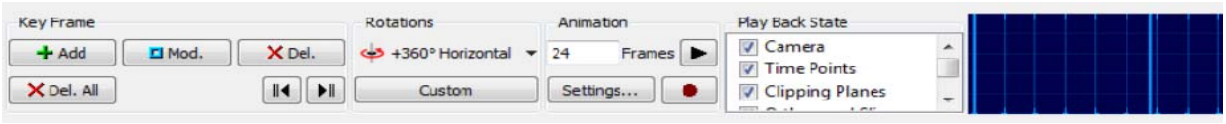
Snapshot

- Automatic naming: takes the data set name and adds a running number for the snapshot name
- Must choose where you want the Image Output to go or nothing will happen
  - Save to File
  - Copy to Clipboard
  - Add to ImageAccess Database
  - Combination
- After determining save location, remember to click Do Snapshot!

Movie Creation: Keyframe Animation

- Define special Keyframes with defined settings (zoom, rotation,...)
- Imaris calculates the frames between the defined positions





Setup the Animation

1. Turn the image view to the starting position.
   - Klick “Add” to add a keyframe
   - Change the image view
   - Klick “Add”...
   - Choose the total number of frames of the movie
   - In the settings window you can choose the frame rate of the movie
   - Press the Play button to play the movie
   - Press the Record button to export the movie

Core

BITPLANE Scientific Software

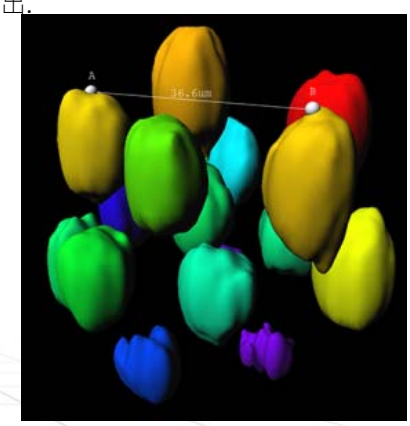
MP

Imaris MeasurementPro

圖像定量分析的重要工具

通過Imaris MeasurementPro模組進行幾何和強度測量，從而得到定量結果!

- 得到點和表面的體積,表面積,橢圓性參數,球化率等結果,並即時展示和輸出.
- 精確測量每個Channel多個物體的強度值
- 提取或刪除感興趣物體以及相關的定量參數
- 進行基於統計結果的排序和分類
- 在感興趣物體內任意位置添加多個點,可以得到他們之間的距離
- 基於2D的感興趣區域可以生成3D視圖,並同時得到3D測量結果.



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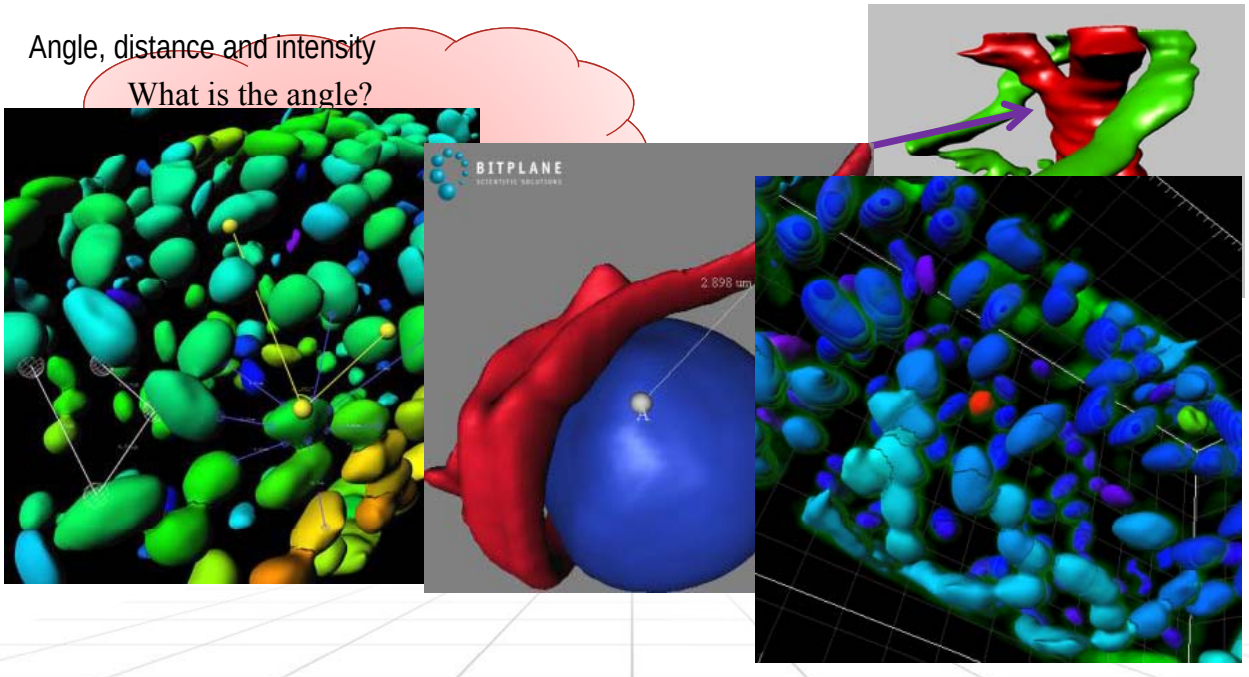
Explore the Next Dimension

BITPLANE Scientific Software

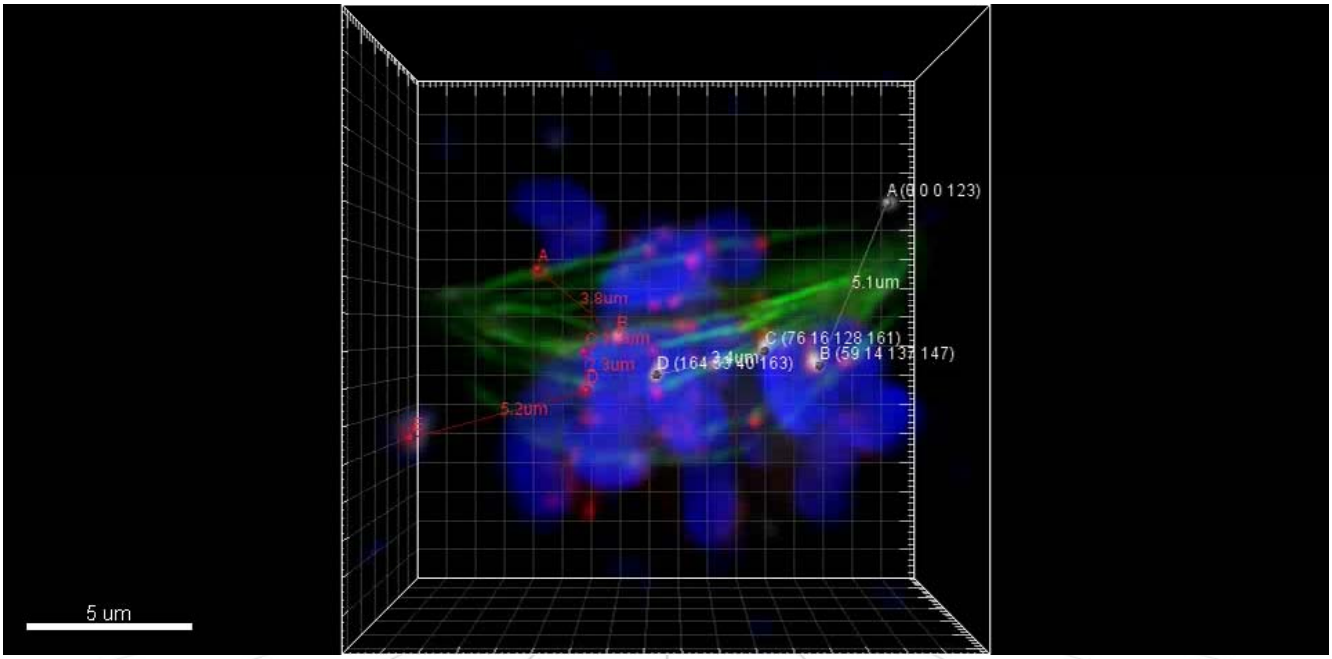
3D Measurement



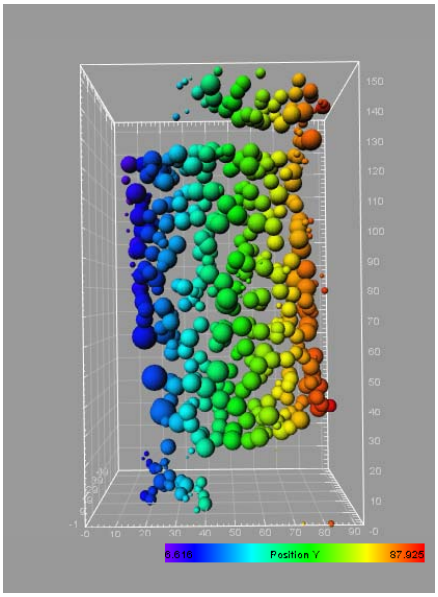
Explore the Next Dimension

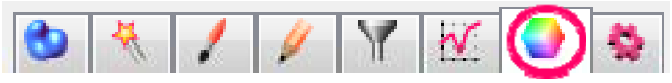
MP

Imaris MeasurementPro -- 立體空間上距離、角度、強度量測

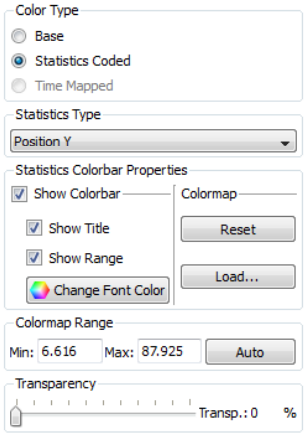


Color Code Objects by Statistics





Surfaces/Spots/Tracks

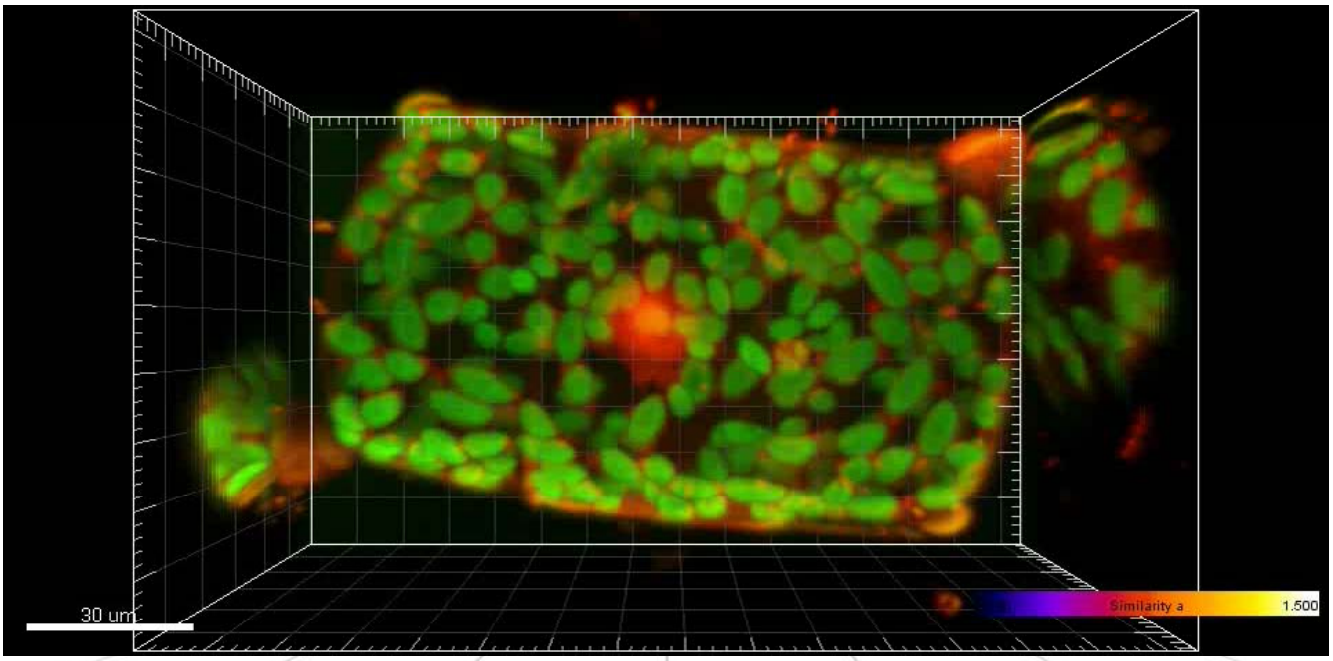


BITPLANE Scientific Software

MP

Imaris MeasurementPro

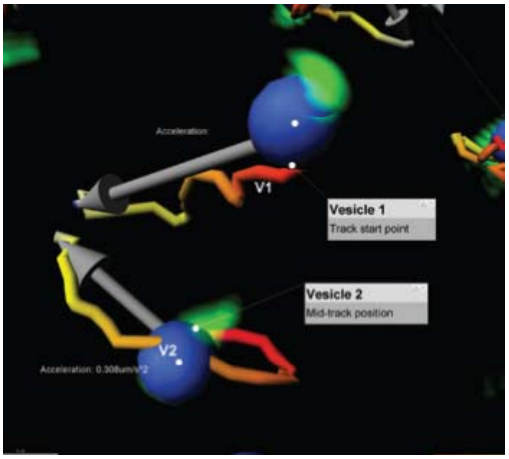
-- Statistics Coded 直接將結果數值轉換成顏色套用於分析影像上



BITPLANE Scientific Software

Track

ImarisTrack/ Lineage



探索運動的意義

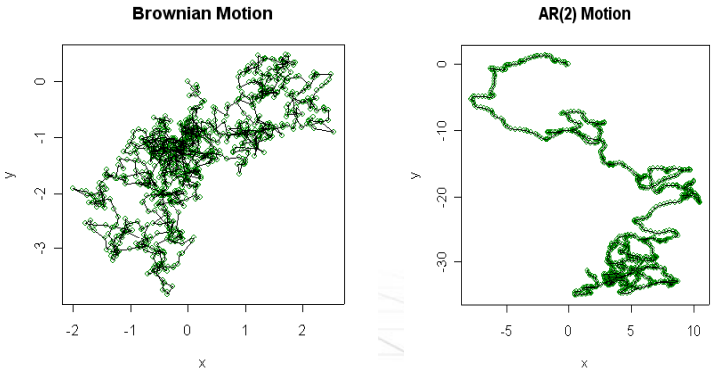
Imaris Track是用於3D/4D圖像追蹤的前沿科學分析方法

- . 提供多種追蹤演算法優化分析結果
- 實現數以千計的大規模即時追蹤
- 方便的手動修改創建軌跡
- 基於物體的大小,強度,形狀,速度,運動方向,運動曲度等多種參數實現自動追蹤
- 運動軌跡以路徑,運動方向,"Dragon Tails"等多種方式展示,排序及分類
- 自動跟蹤3D+時間序列的運動物體
- 與ImarisXT模組結合實現更強大的特異性追蹤

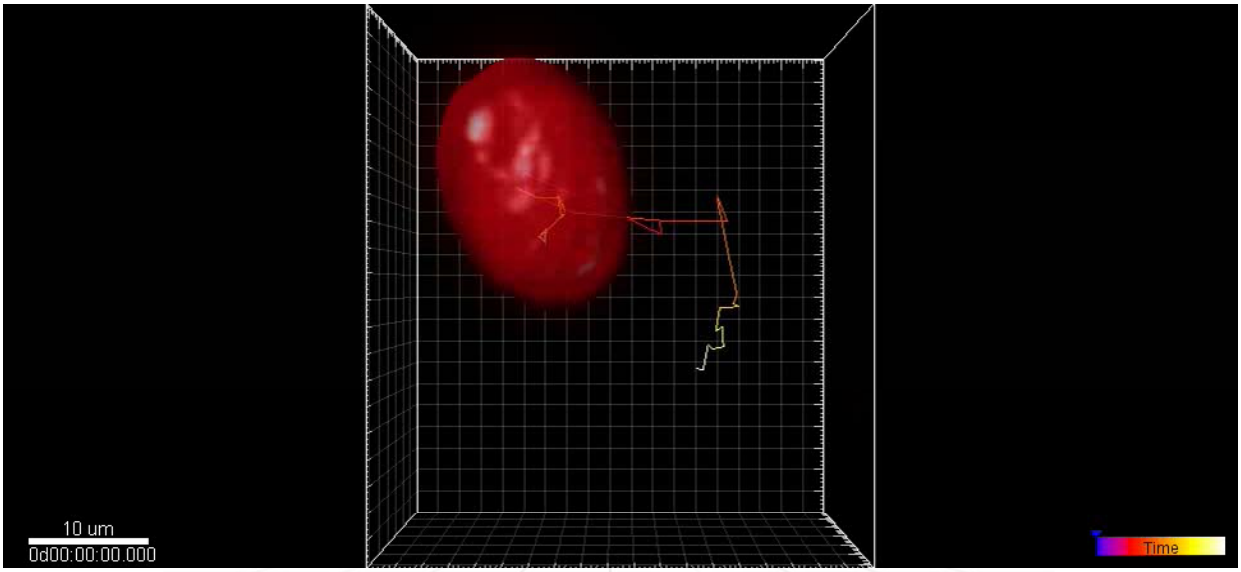
Track

Multiple tracking algorithms

- Brownian Motion
- Autoregressive Motion
- Autoregressive Motion Expert
- Connected Components
- Lineage

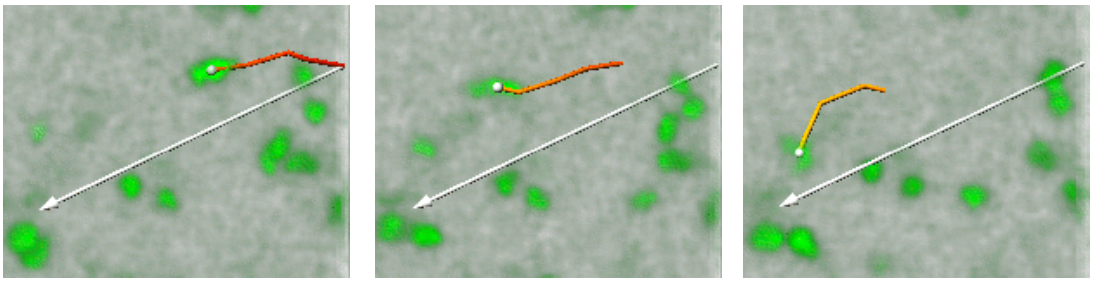


Track

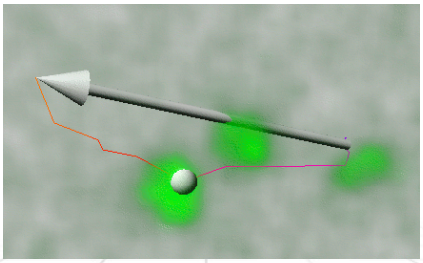


Track

Results Visualization



• Dragon Tail



•Displacement

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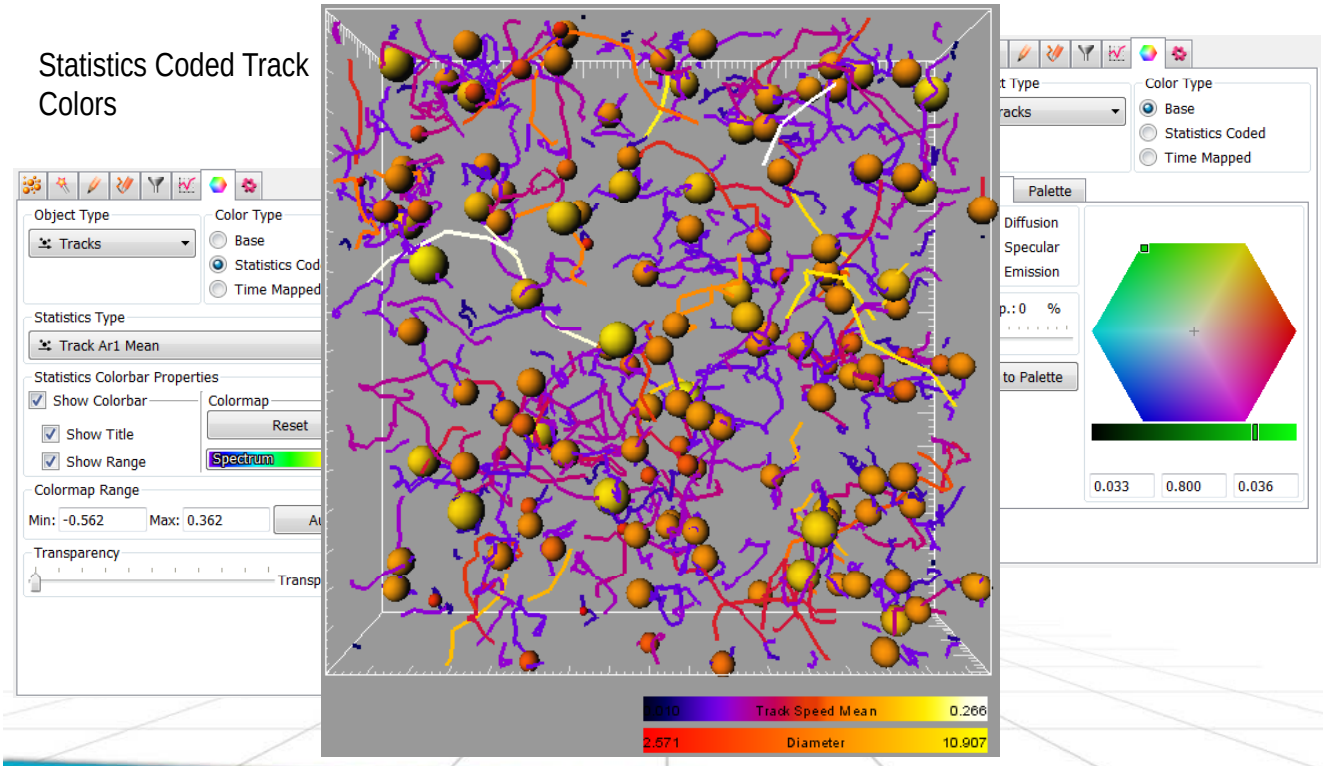
Track

Results Visualization

Statistics Coded Track Colors

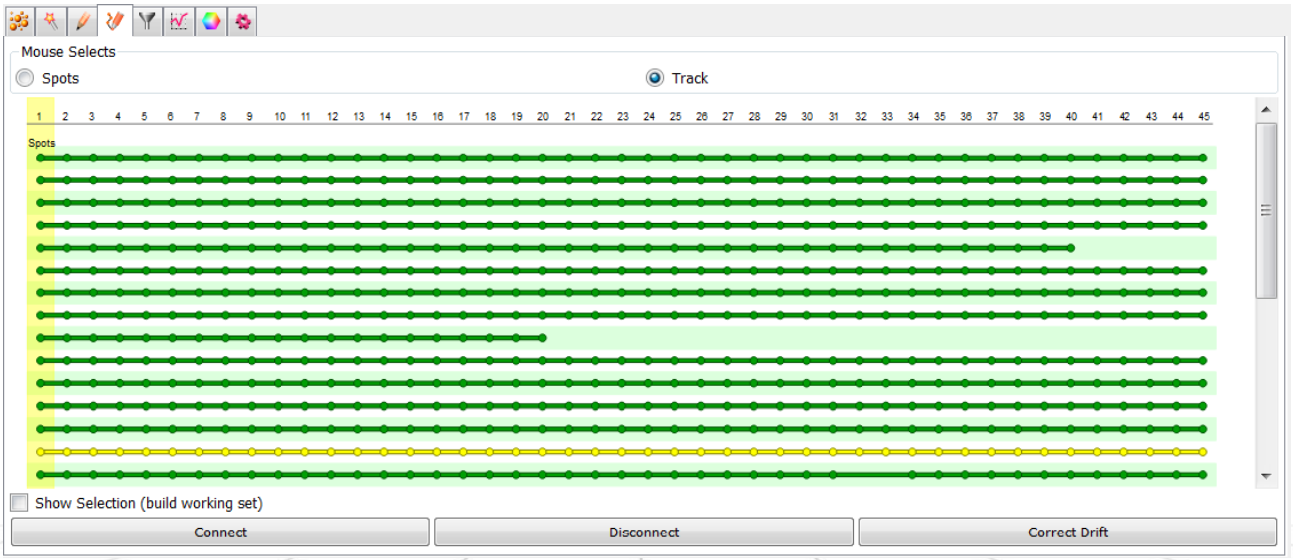


Explore the Next Dimension

Track

Edit

- Drift Correction
- Track Editing
- Single Spot Object Tracking



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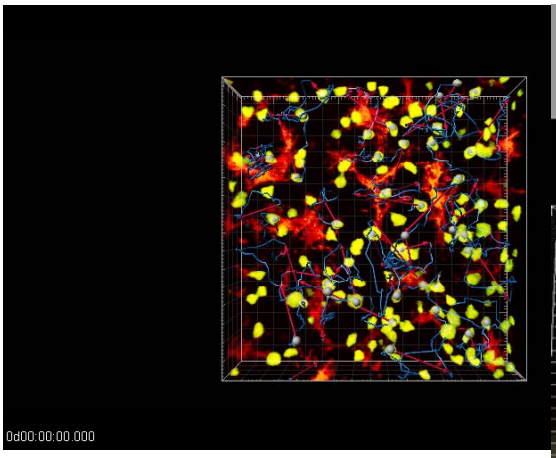
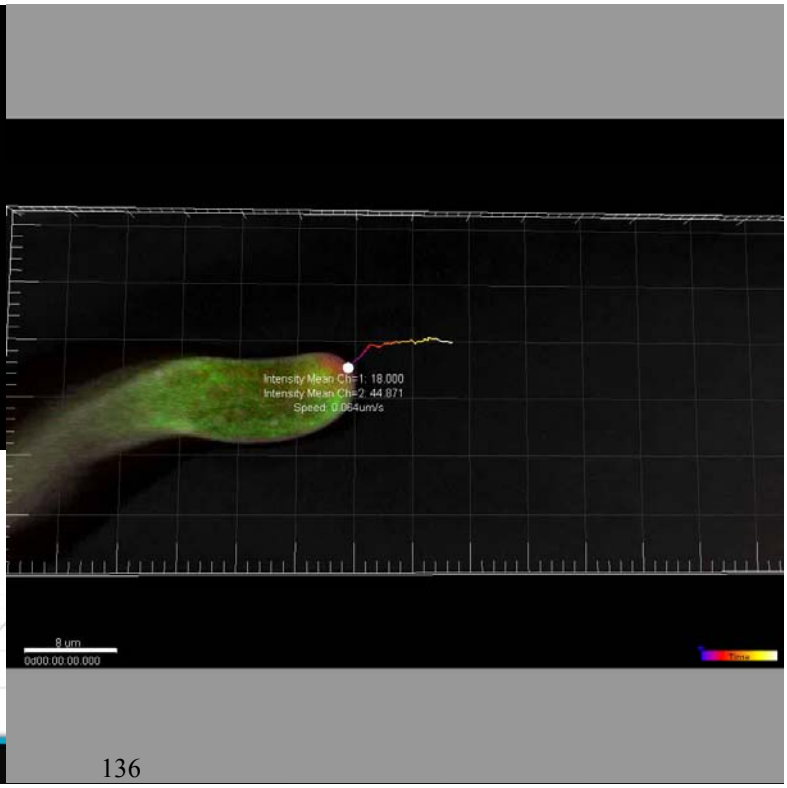
Track





Pollen tube

Explore the Next Dimension

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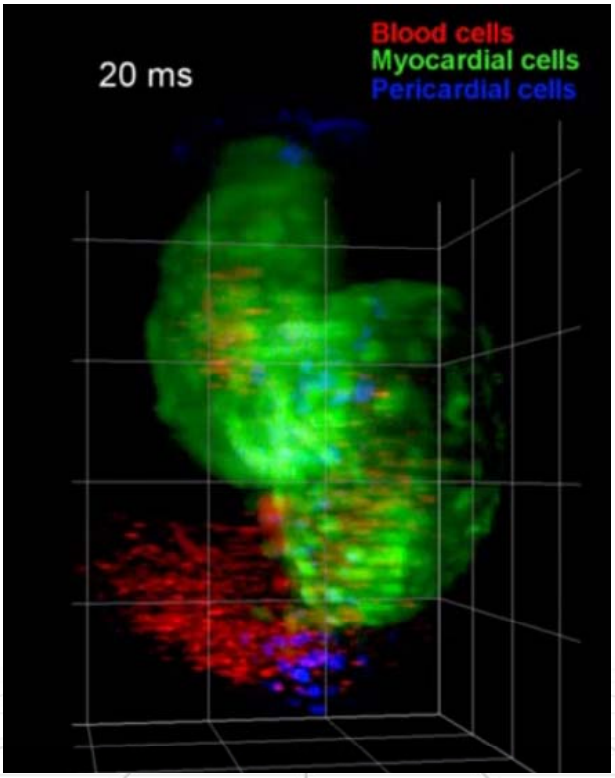
Scientific Software

Track

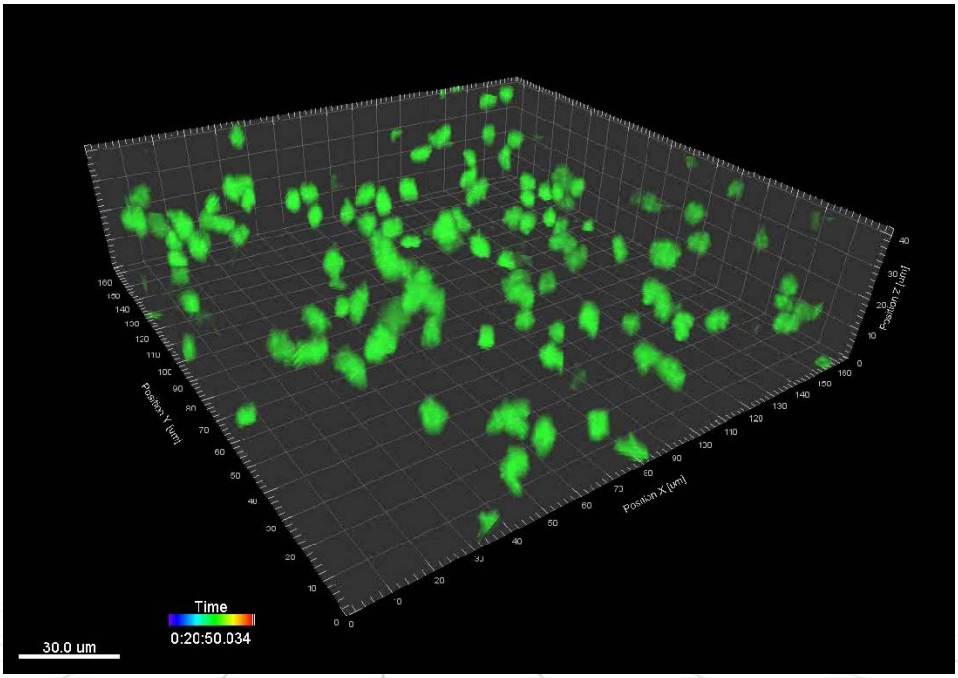
ImarisTrack



BITPLANE
Scientific Software

Track

ImarisTrack



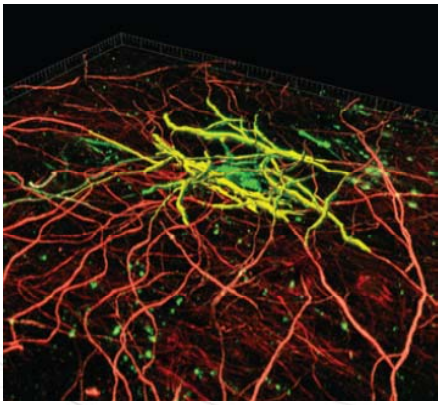
Coloc

ImarisColoc

揭秘3D/4D空間位置關係

ImarisColoc模組說明使用者獲得3D/4D空間共定位定量資訊，並進行空間視圖展示

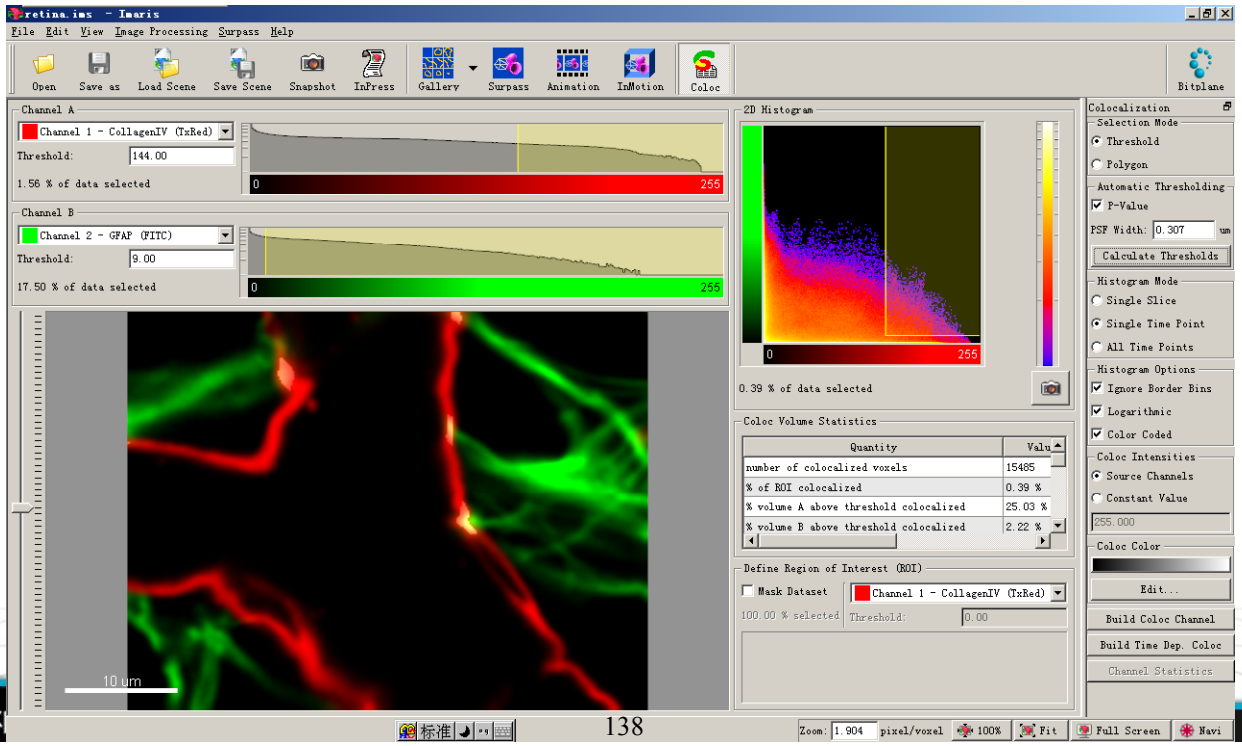
- 提供多種空間共定位區域的選定方法
- 根據國際標準自動計算共定位閾值
- 即時獲得共定位資料,得到的結果可進行進一步分析
- 空間共定位結果可以展示為新的Channel,並進行進一步的渲染分析



Coloc

Selection of co-localized voxels

- Co-localization in 2-D, 3-D and 4-D images
- Possible of >2 channels
- Histogram
- Intensity scaling



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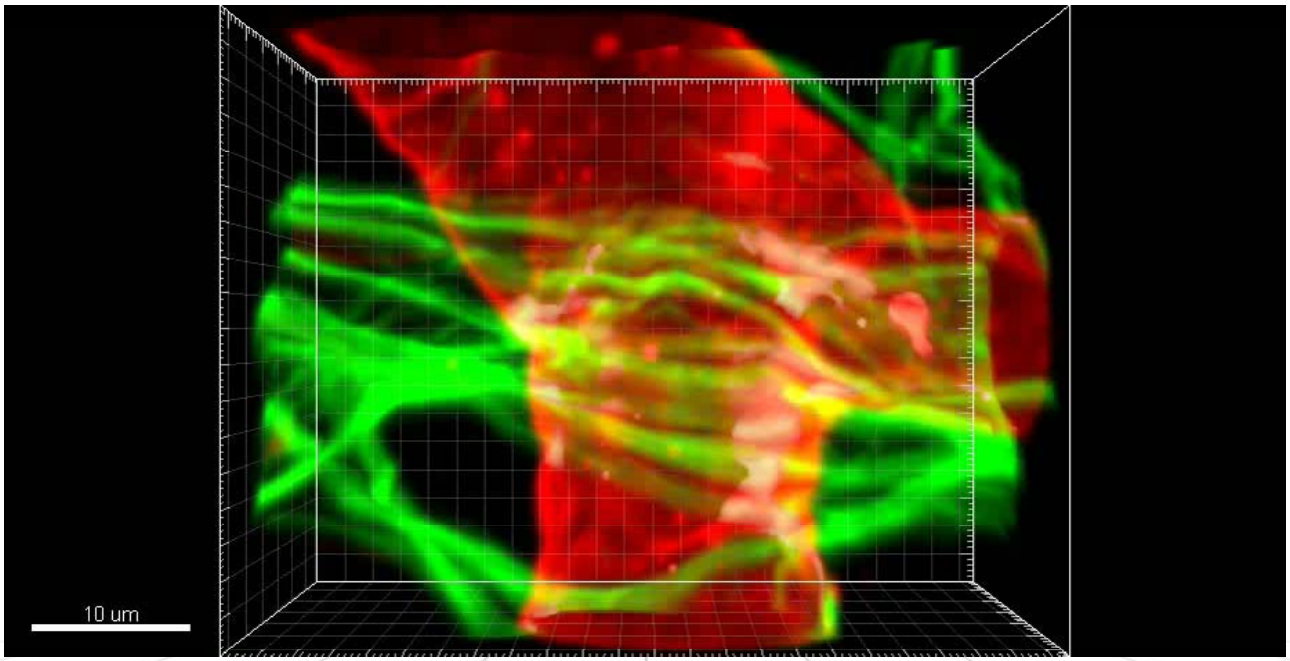
Coloc

ImarisColoc – 可額外產生 Colocolization channel



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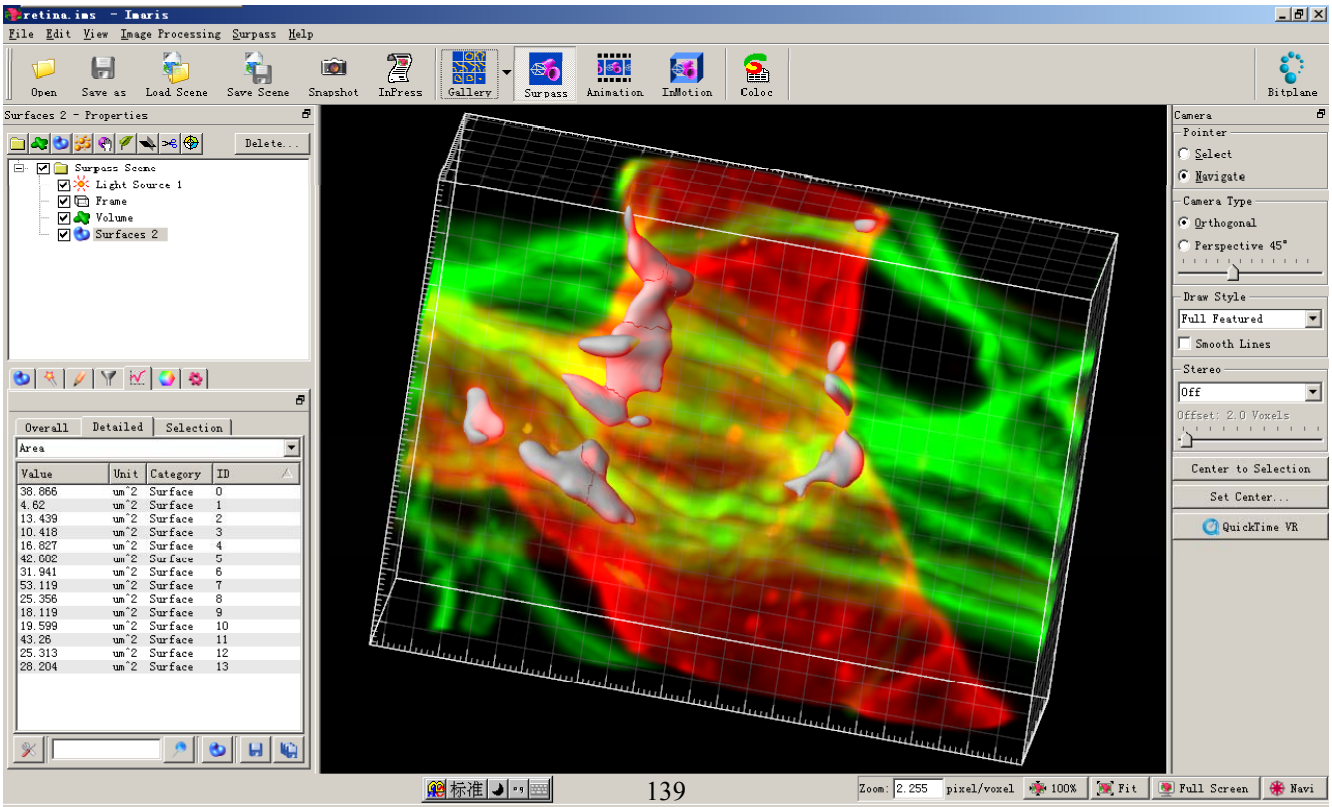
Coloc

Create co-localized channel



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Coloc

Numerical Data Output

- Pearson's Correlation Coefficient
- Manders' Correlation Coefficients
- Colocalization percentage

Explore the Next Dimension

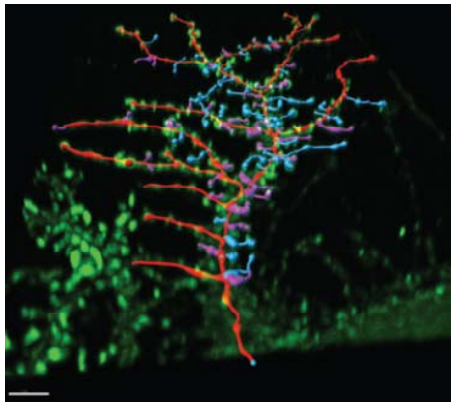
BITPLANE Scientific Software

FT

Filament Tracer

對神經以及絲狀結構進行智慧分析和視覺化

Filament Tracer模組對絲狀結構自動檢測，示蹤和結構分析



- 使用全自動,手動,AutoPath以及AutoDepth等方法實用有效的神經示蹤.
- 獲得分支長度,直徑,體積及神經拓撲學等眾多神經專業分析參數
- 對手動畫出的分支進行自動中心定位,並與相鄰的區域進行自動連接
- 實用的多種分支選擇方法
- 使用先進的專業展示功能能夠實現絲狀體和非絲狀結構的同時展示
- FilamentTracer的資料結果可用於進一步分析
- 結合更多模組,實現複雜的絲狀體資料處理

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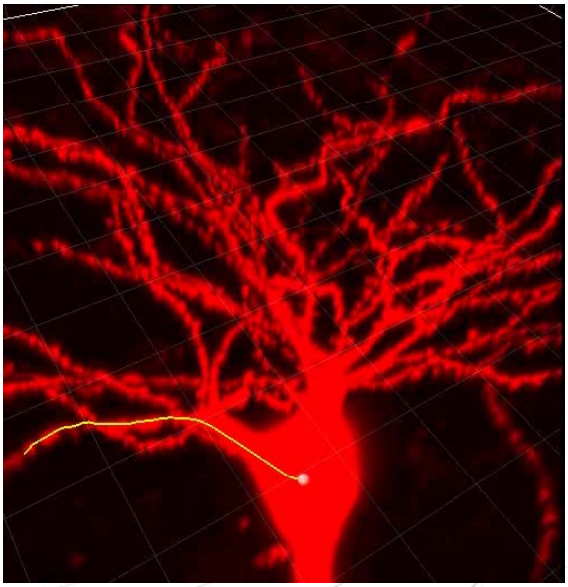
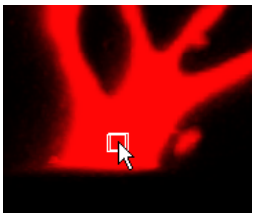
Explore the Next Dimension

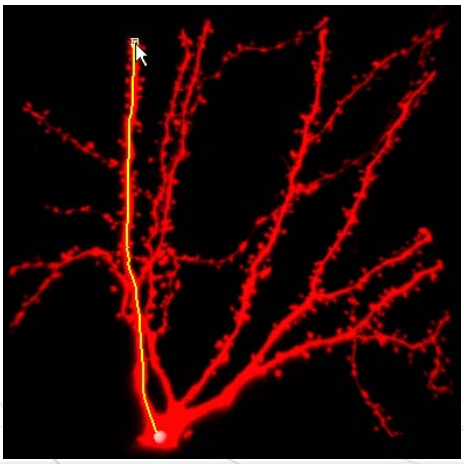
FT

Semi-automatic filamentous structure detection



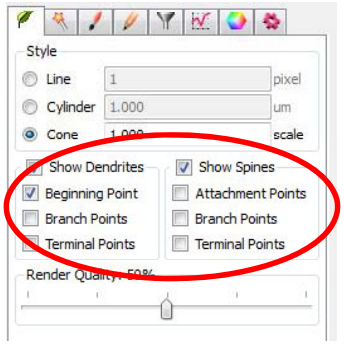


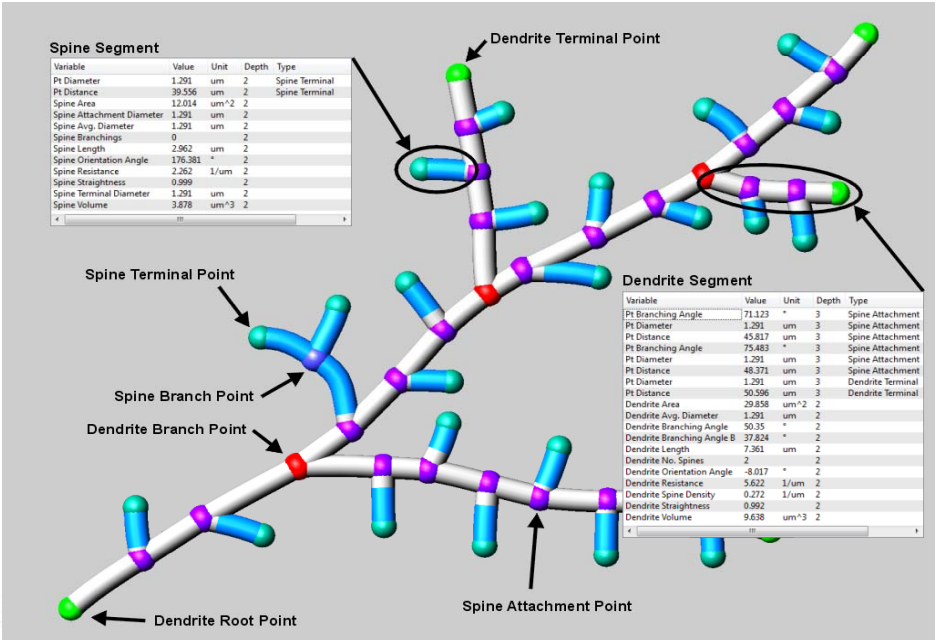
Starting point



FT

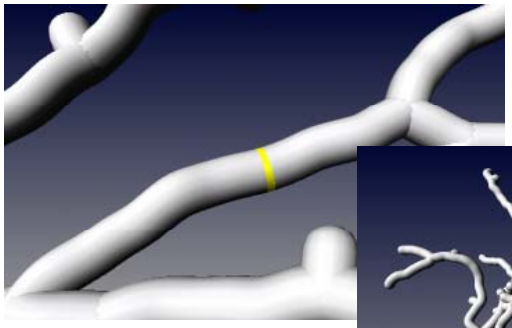
Filament Element Visualization



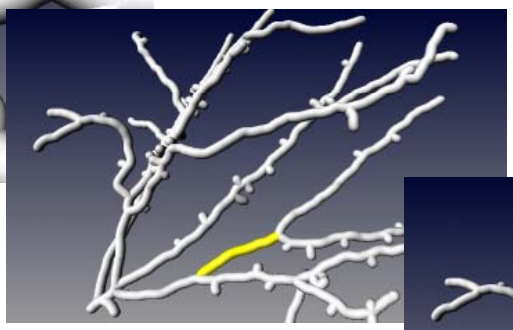


FT

Filament Editing Selection

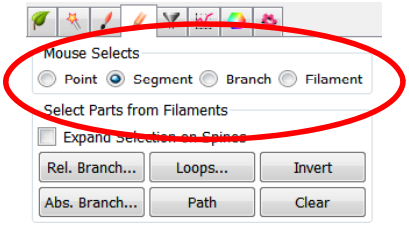


Point



Segment

Branch

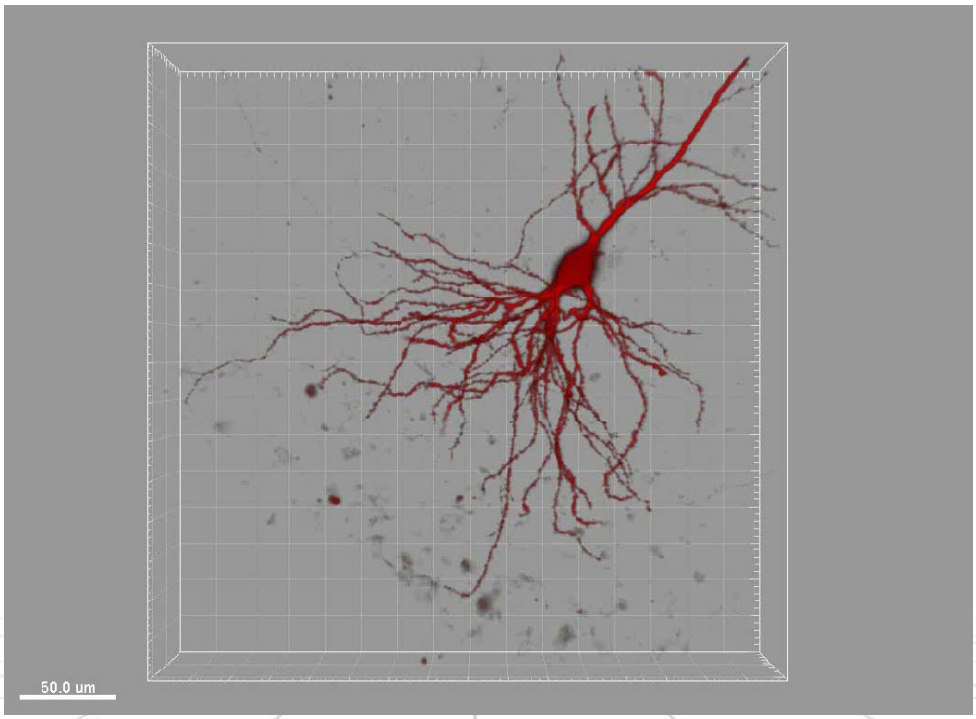


FT

Filament Tracer

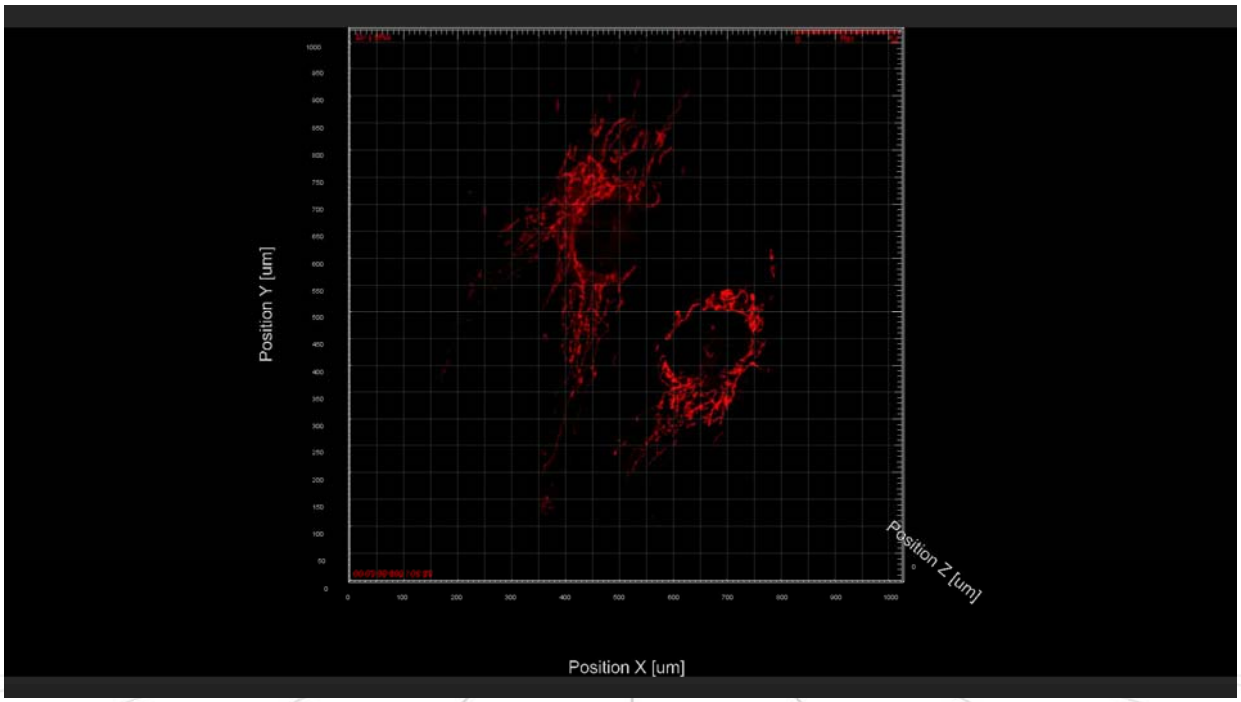
FT

Filament Tracer



FT

Filament Tracer



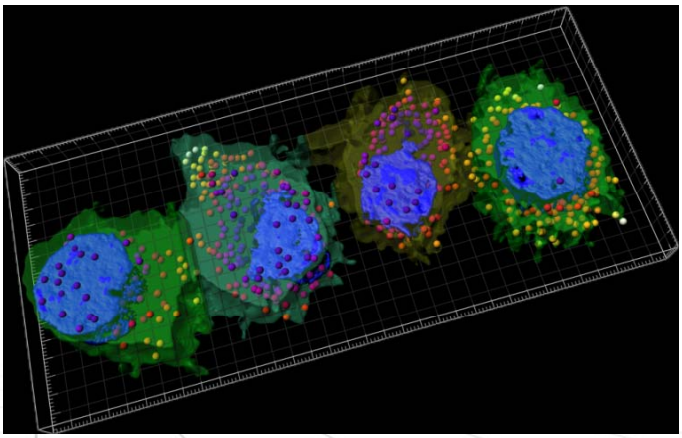
Cell

Imaris Cell

揭秘細胞間和細胞內的交流

Imaris Cell模組對每個單獨的細胞進行個體研究，從而分析多個細胞間或者細胞內的交流狀態。

- 揭秘細胞間和細胞內細胞器的關係!
- 揭秘細胞內細胞器等級的關係!
- 對有獨立生物學意義的細胞或者器官進行分析
- 自動展示和分割細胞膜
- 對大量的囊泡狀物體進行自動檢測和分類
- 對3D/4D圖像進行細胞等級的研究
- 對細胞功能,如胞間通訊,進行機制和結構研究
- 智慧導航讓您輕而易舉的操作.



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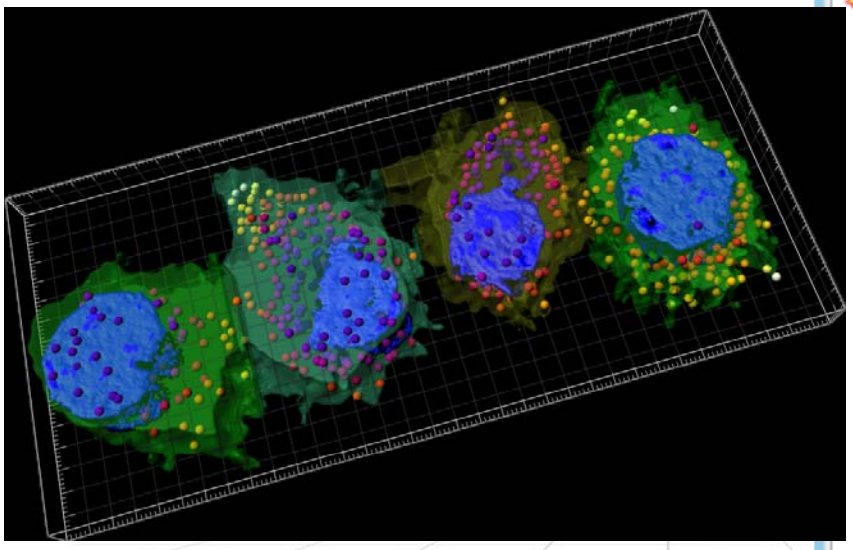
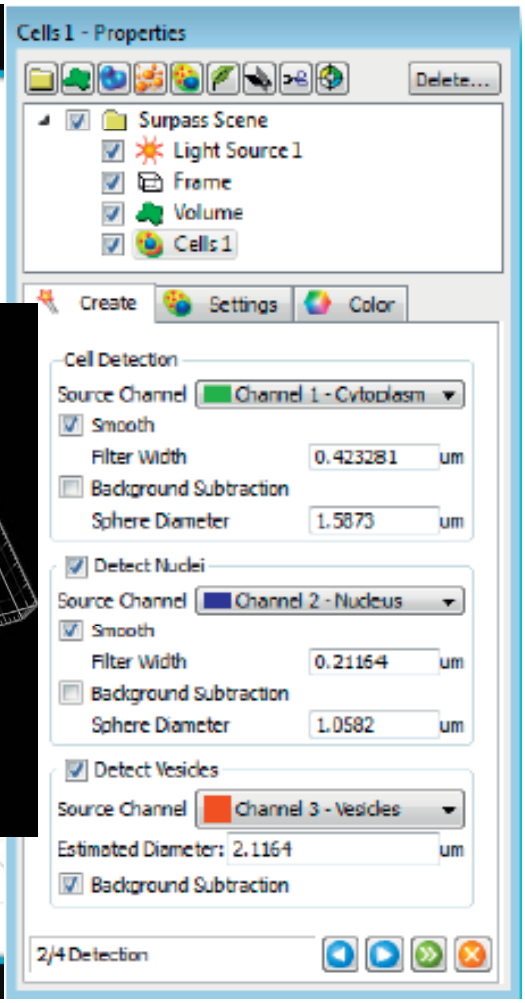
Cell

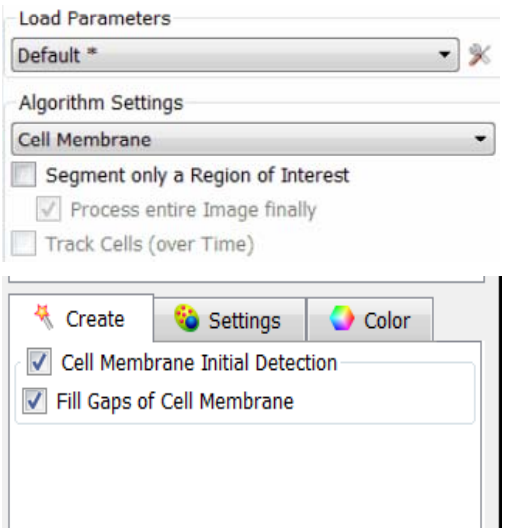


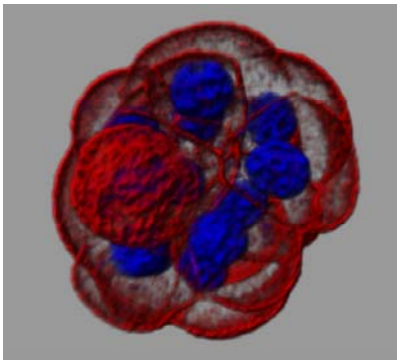


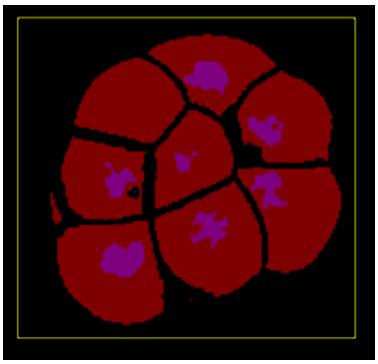
Explore the Next Dimension

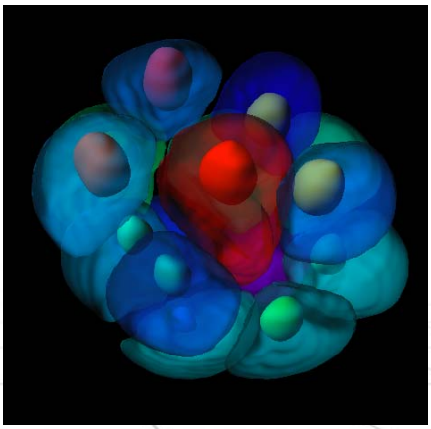
Cell

Cell Membrane detection









Cell

Imaris Cell

Cell

Imaris Cell

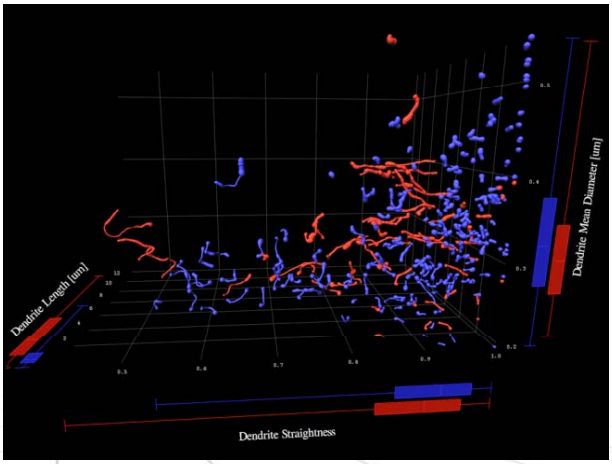
Vantage

ImarisVantage

超越視覺展示圖像效果

Imaris Vantage採用資料與立體視圖結合展示的模式，清晰的展現隱藏在多維圖像資料中複雜的相互關係

- 實現您預想的多維資料展示模式
- 對2D-5D資料進行立體視圖展示
- 最大限度的視圖展示統計結果之間的關聯
- 對軌跡按照一定屬性進行分析和排列
- 在任意視圖中自由添加注釋
- 在發表文章和展示時方便的添加分析處理結果



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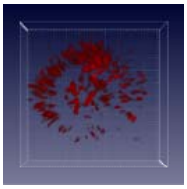
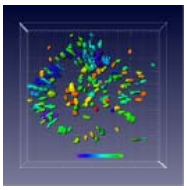
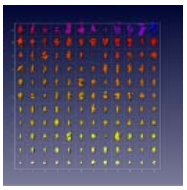
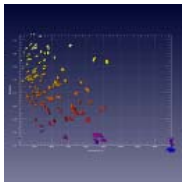
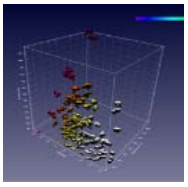
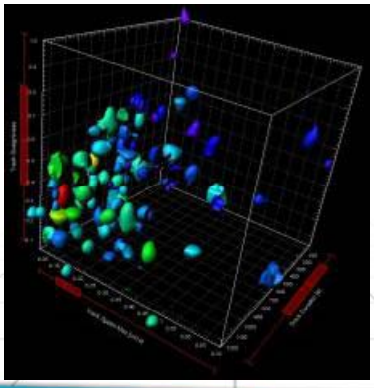
Vantage

ImarisVantage

Unique visualization of statistics and object data plotted in many different ways;

- Color–coded volume vs. Position
- Object gallery sorted by size
- 2D object scatter plot
- 3D object scatter plot
- Scatter plots with box plots

Vantage

ImarisVantage

BITPLANE Scientific Software

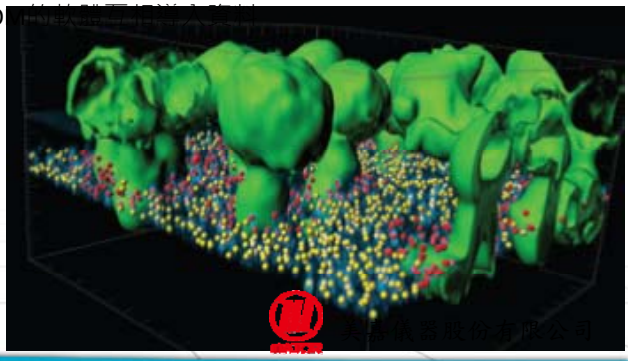
XT

ImarisXT

顯微圖像計算方法的革新

ImarisXT通過與其它經典軟體的交互調用，實現了多功能拓展, 為您的實驗提供更多的計算方法!

- 探測前沿科研未知參數的工具，回答您最想要知道的問題
- 在影像處理,分離,分類以及報告等方面增加更多的計算方法.
- 新功能與常用介面無縫整合,方便用戶調用
- 流暢的與Matlab,Java,C++,C#,Visual Basic以及其他經由CO
- 模組內置了其他人員開發的常用計算方法
- 實現電腦技術與生物研究的完美整合



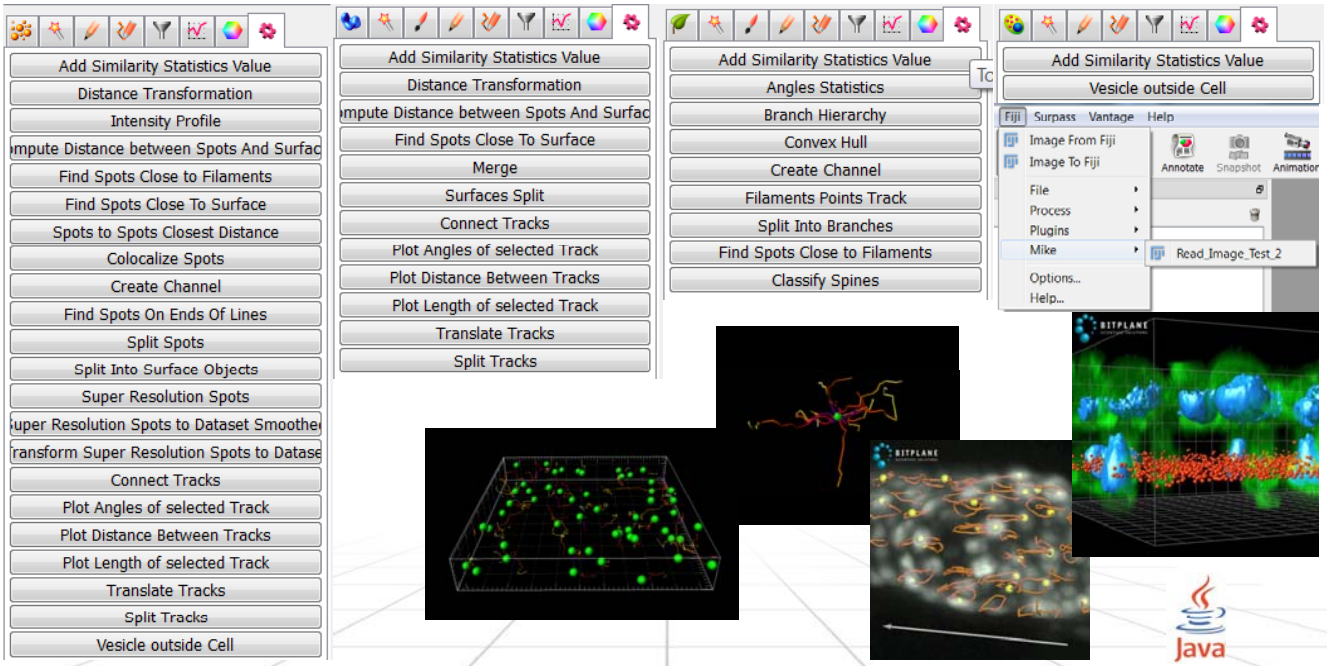
Explore the Next Dimension

53

BITPLANE Scientific Software

XT ImarisXT

C# C++



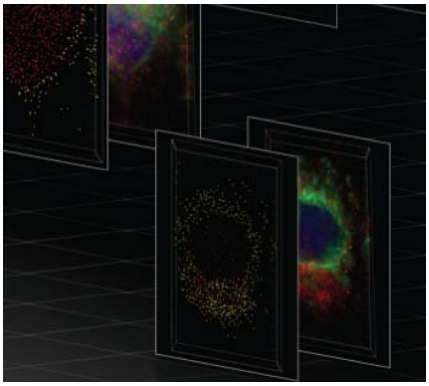
Explore the Next Dimension

54

Batch

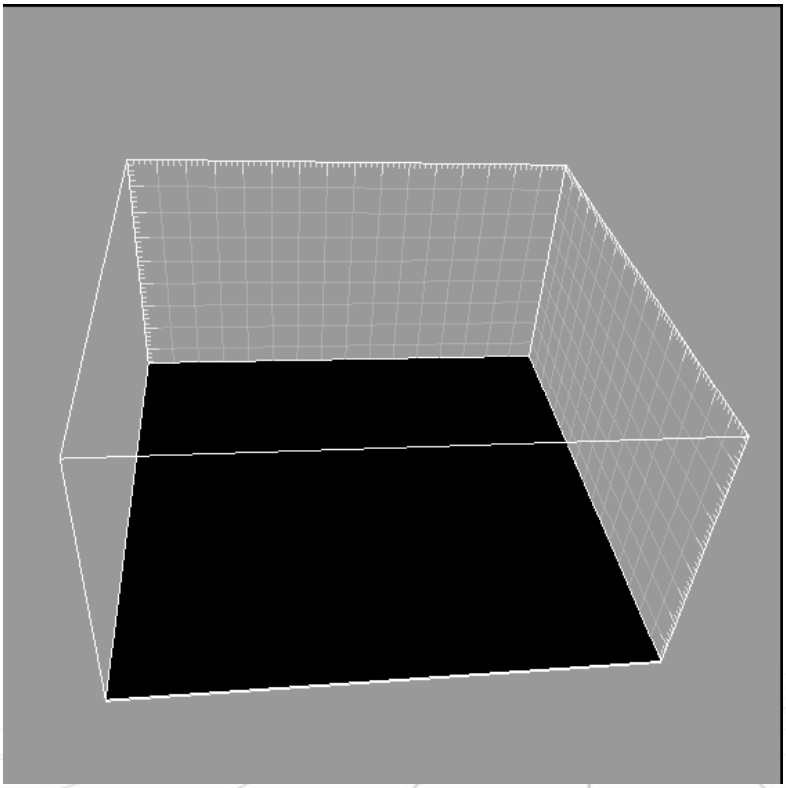
ImarisBatch

批量自動執行圖像的分析處理



Imaris Batch可以自動對2D/3D+時間序列圖像進行批量處理。

- 自動重複多種分析,批量得到圖像結果,為您節省後期分析圖像的時間
- 無需不同模組間的功能交交交互操作,批次處理可以快速得到最終結果
- 在批次處理過程中,根據電腦的使用狀況,智慧進行最有效率的分配
- 在批次處理過程中,平行任務自動分配給不同的處理器以提高效率.
- 可以更改參數達到更好的處理效果



Thank you!