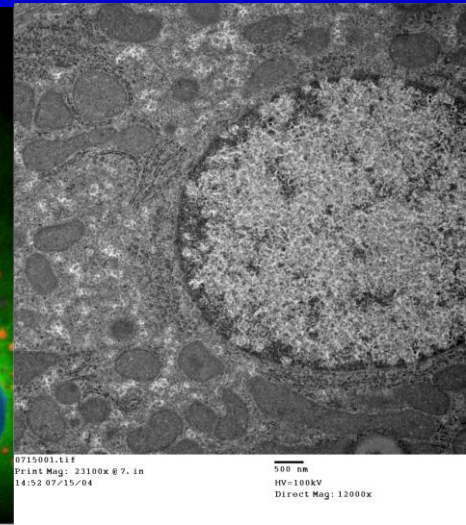
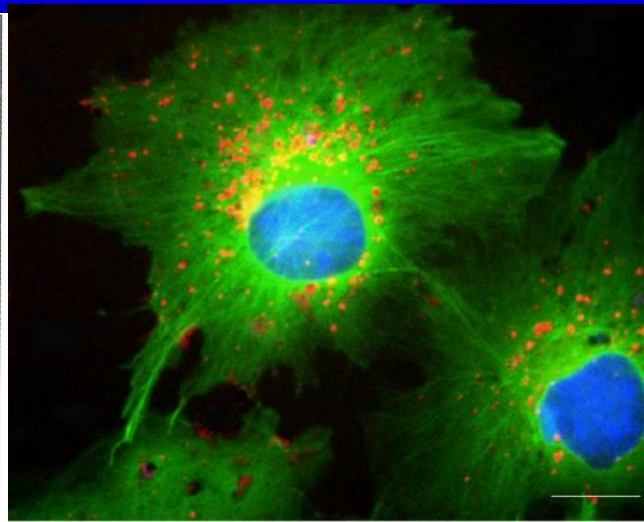
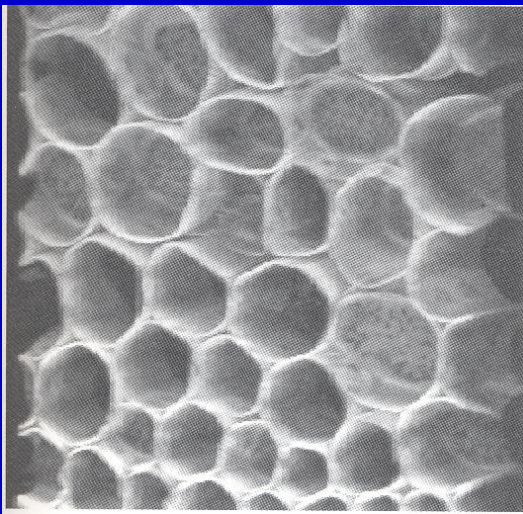


# 共軛焦顯微鏡與電子顯微鏡 在醫學影像之運用

Chung-Liang Chien 錢宗良

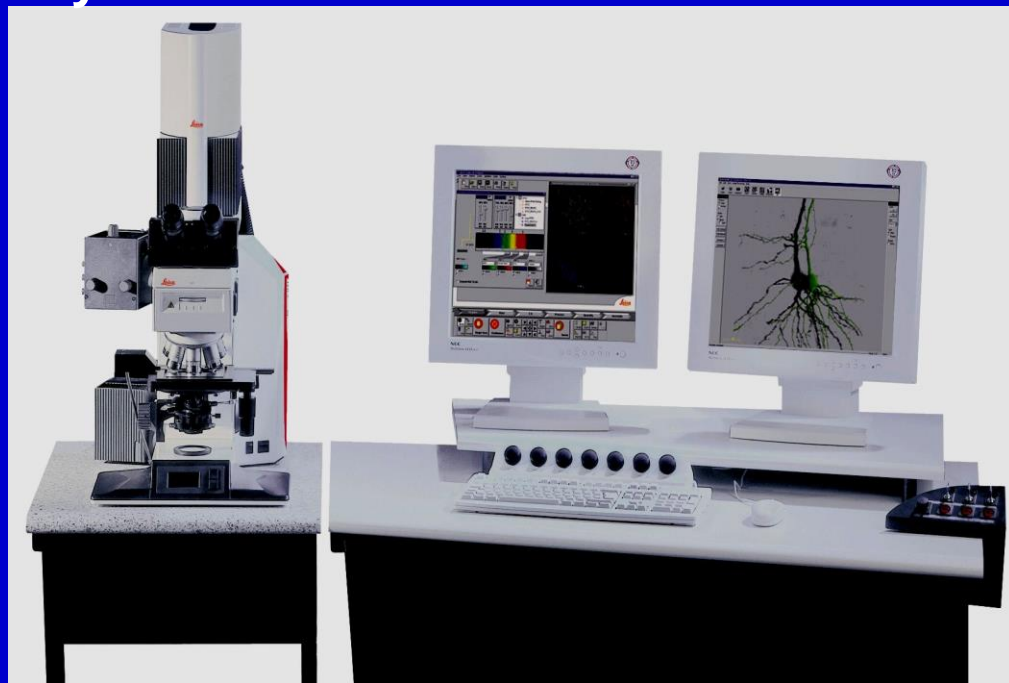
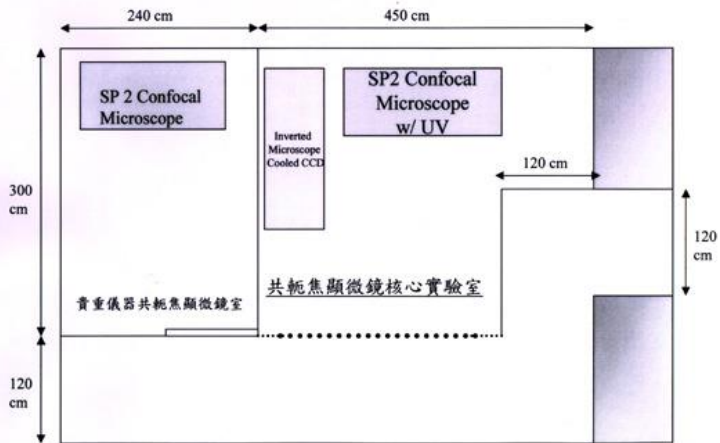
Department of Anatomy and Cell Biology  
College of Medicine, National Taiwan University



# 雷射掃描光譜共軛焦顯微鏡核心實驗室

Leica TCS SP2 Spectral Confocal System

基礎醫學大樓14樓1410室



實驗室配備

DM RE HC upright Microscope  
Multiple Laser Merge System

1. UV Laser 351 nm/ 364 nm

2. Ar Laser 458 nm / 488 nm

3. He-Ne Laser 543 nm

4. He-Ne Laser 633 nm

# 服務項目

## 1. 組織或細胞染色技術

- Multiple fluorescence staining
- Immunohistochemistry & Immunocytochemistry
- Cell nucleus staining
- Organelle staining

## 2. 共軛焦顯微鏡基本操作指導

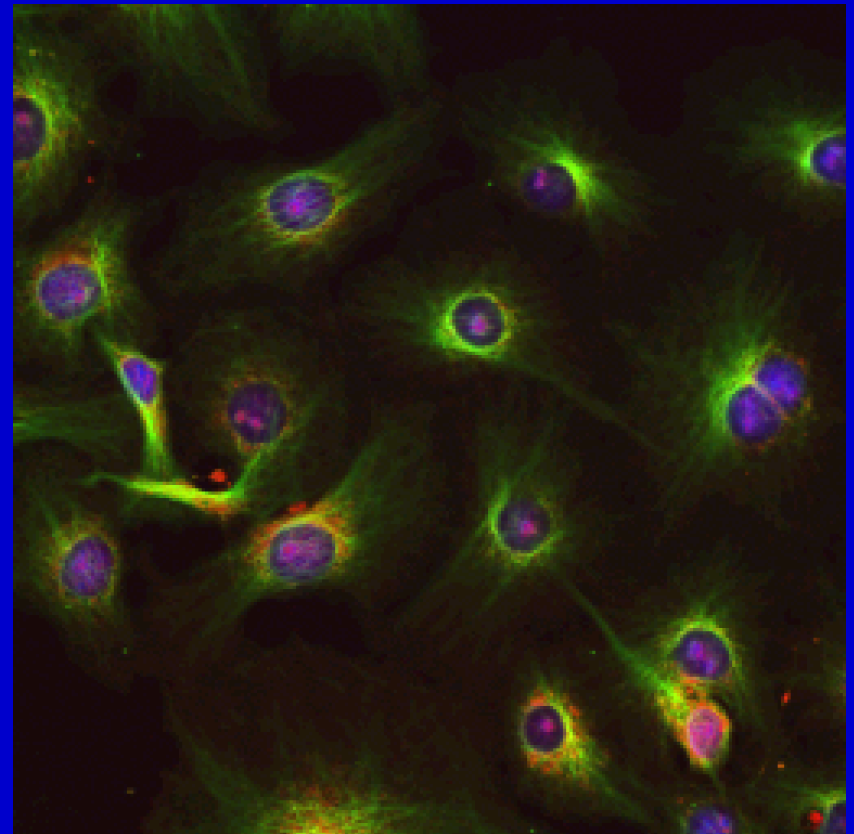
- Basic microscopy
- Image acquisition
- Z sectioning for 3 D

技術支援：錢宗良 老師

(02) 23123456 分機：8193

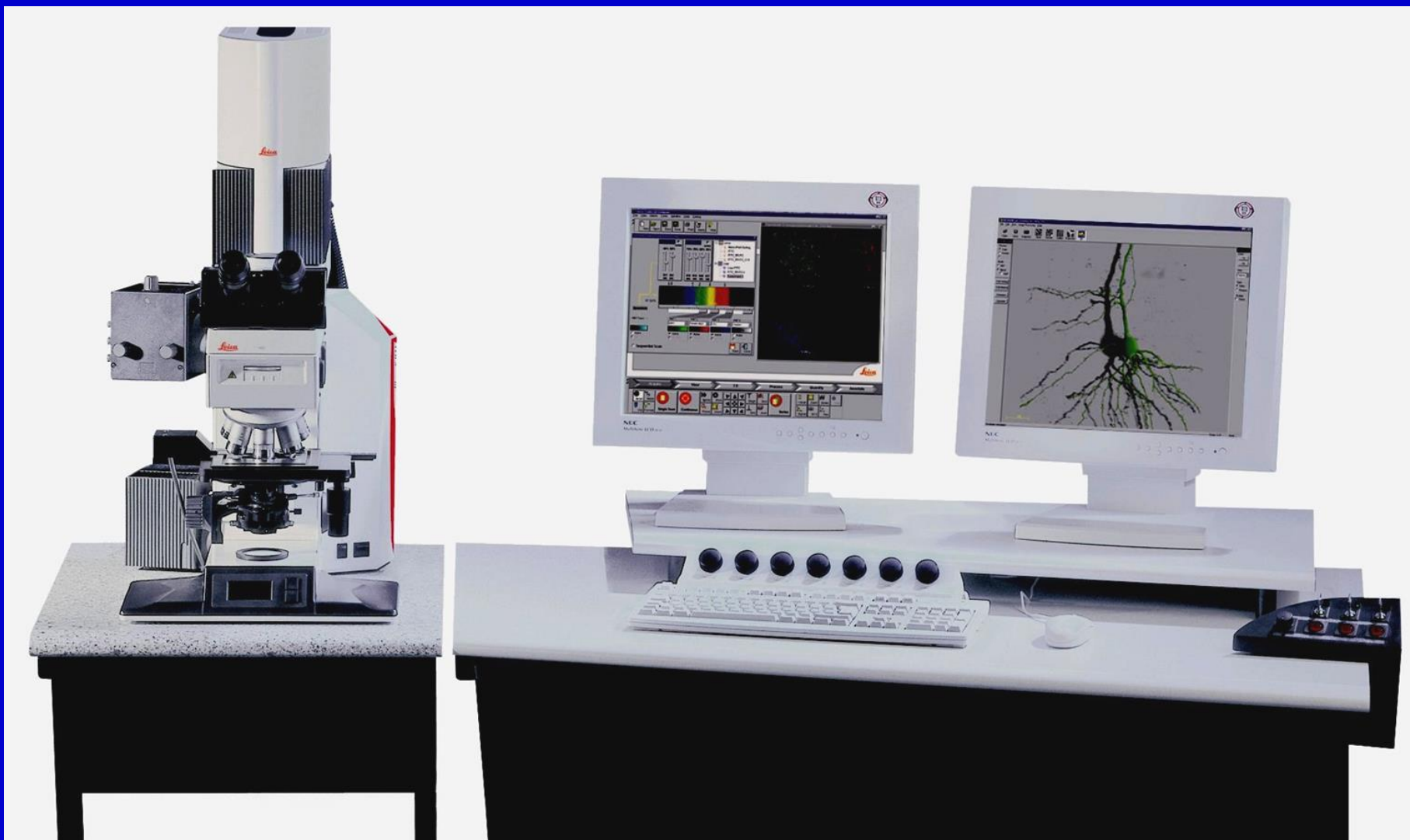
E-mail address: [clc@ha.mc.ntu.edu.tw](mailto:clc@ha.mc.ntu.edu.tw)

Web address: <http://info.ntu.edu.tw/biotech/main.htm>

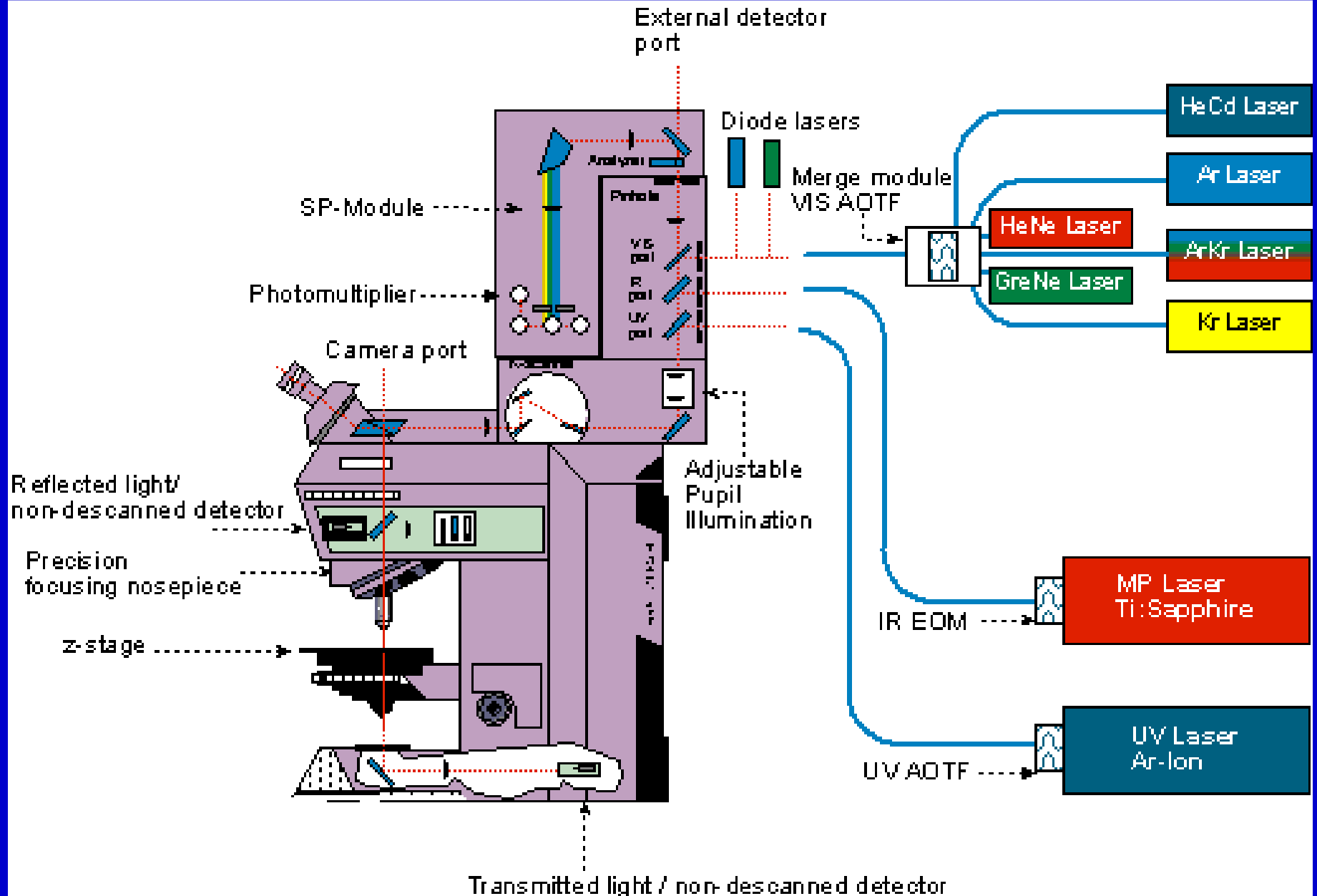


**Leica TCS SP2**

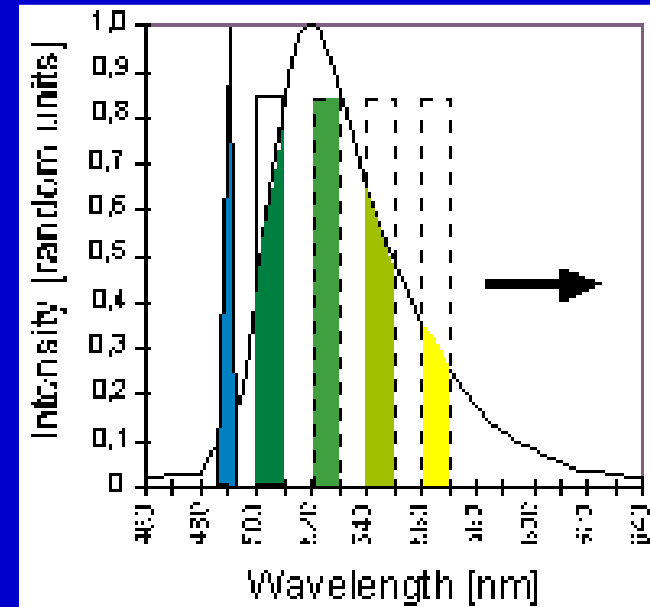
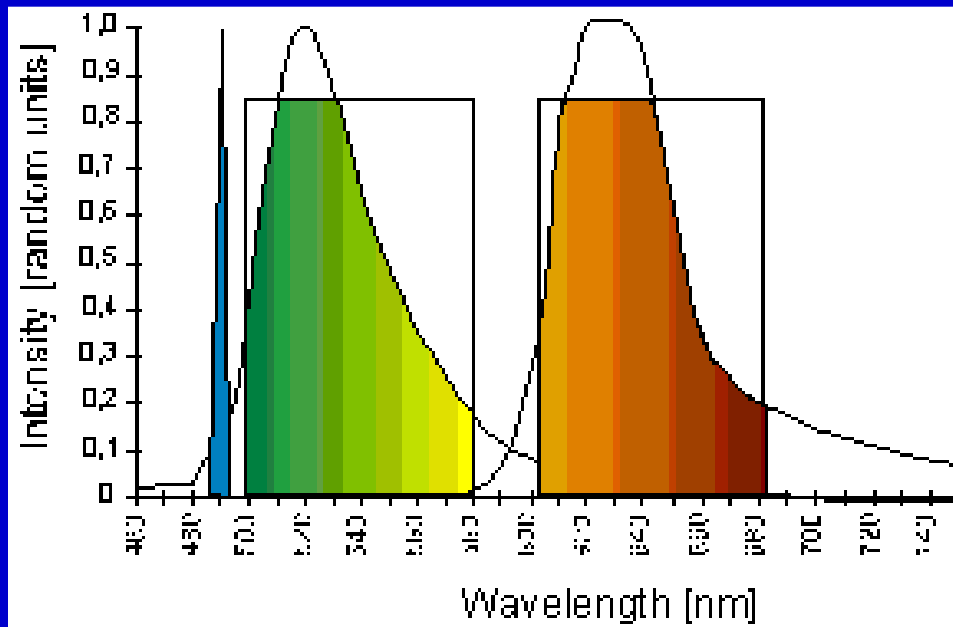
**Confocal Spectral Microscope ( UV-VIS )**



# Leica TCS SP2/MP2: System Optics Overview



# TCS SP/SP2: Prism Spectrophotometer Benefits

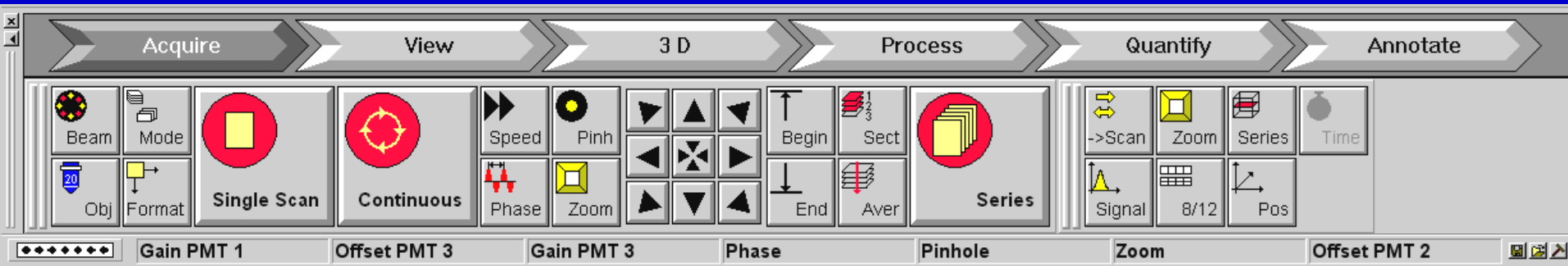


- Maximize efficiency
- Maximize flexibility
- Minimize crosstalk

- Analyze the spectrum

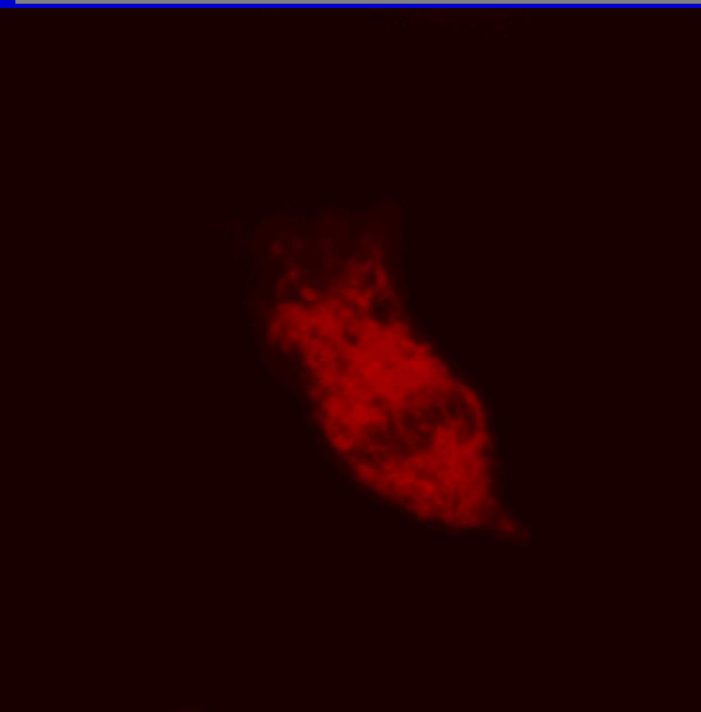


# Mode : Scanning and Image Capture



Mode	Functions
xyz	An image stack is recorded from xy-sections in z-direction. ( 3D )
xzy	An image stack is recorded from xz-sections in y-direction.
xt	A line is recorded several successive times.
xyt	An xy-section is recorded several successive times.
xzt	An xz-section is recorded several successive times.
xyzt	An image stack is recorded from xy-sections in z-direction several successive times.
xyl	An xy-section is recorded at different wavelengths. ( wavelength )
xzl	An xz-section is recorded at different wavelengths.

# 3D (xyz) series Continuous scanning



Series Scan Overview

Mode: xyz

Y 512  
X 512  
Z 166.68  $\mu\text{m}$

Pos: -7.14  $\mu\text{m}$   
Begin: 39.87  $\mu\text{m}$   
End: -50.25  $\mu\text{m}$   
Total: 90.12  $\mu\text{m}$

Begin  
 End

Close

1  
2  
3  
4  
6  
8  
10  
14  
16  
18  
20  
25  
30  
40  
50  
Others...  
Close

1  
2  
3  
Sect

Number of optic sections

Acquire View 3 D Process Quantify Annotate

Beam Mode Single Scan Continuous Speed Pinh Begin Sect Series  
Obj Format Phase Zoom End Aver

Gain PMT 1 Offset PMT 3 Gain PMT 3 Phase Pinhole Zoom Offset PMT 2



## Leica DM IRE2 microscope

*enclosed within a computerized CO<sub>2</sub>-incubator for indispensable thermal and mechanical stability*



**CO<sub>2</sub> controller**

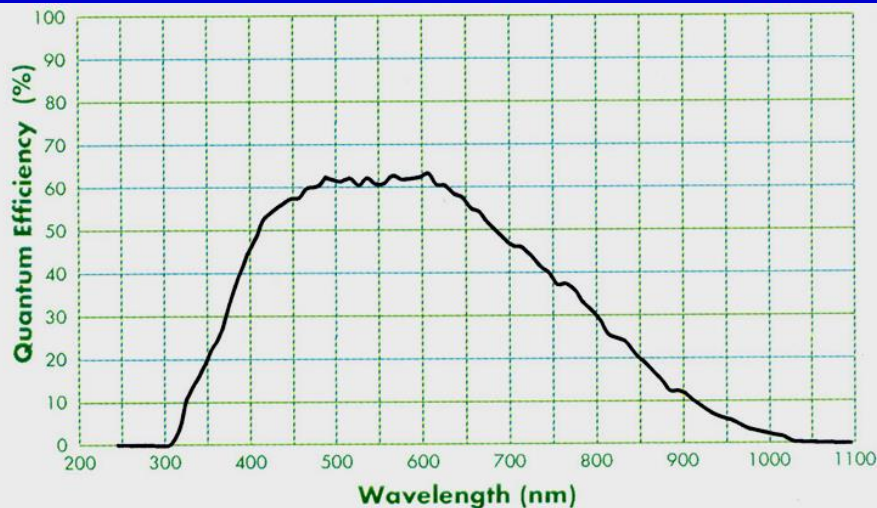


**Microincubation  
Imaging-Chamber**

# Roper Scientific Cooled CCD Camera Cool SNAP-HQ

## High sensitivity, High Resolution, High Speed

- Interline Progressive Scan 1392 x 1040 pixels
- Pixel size: 6.45 x 6.45  $\mu\text{m}$
- Low read-out noise: 6 e-/sec at 10 MHz, 8 e-/sec at 20 MHz
- Electronic shuttering, “*full speed overlapped*” read-out
- programmable read-out capabilities (*subregion, binning*)
- - 30 °C Cooling – reduce noise



	Region		
	1392 x 1040	512 x 512	256 x 256
1 x 1	10	19	30
2 x 2	18	30	44
3 x 3	24	38	51
4 x 4	29	43	56

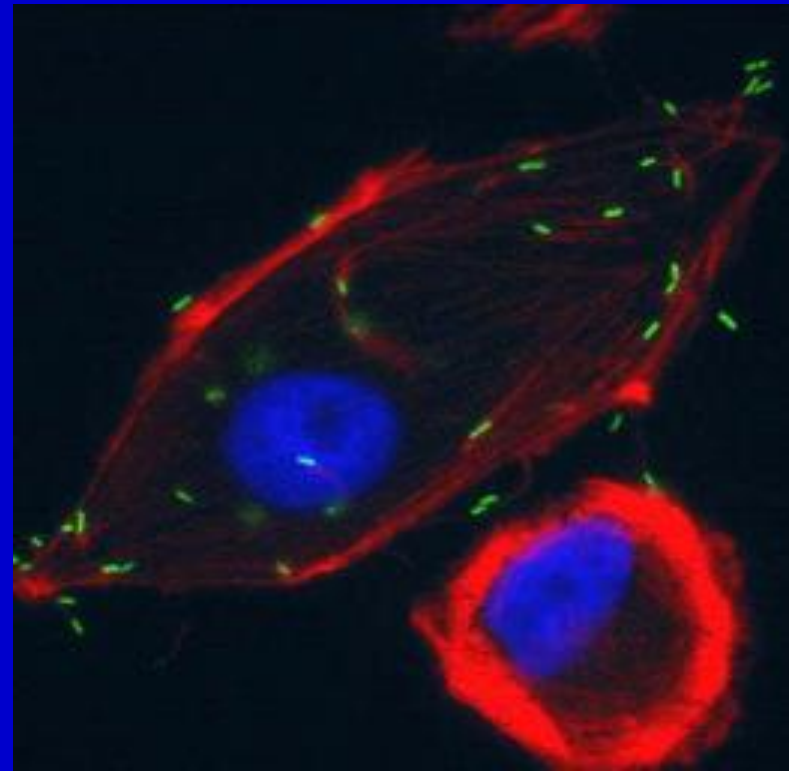
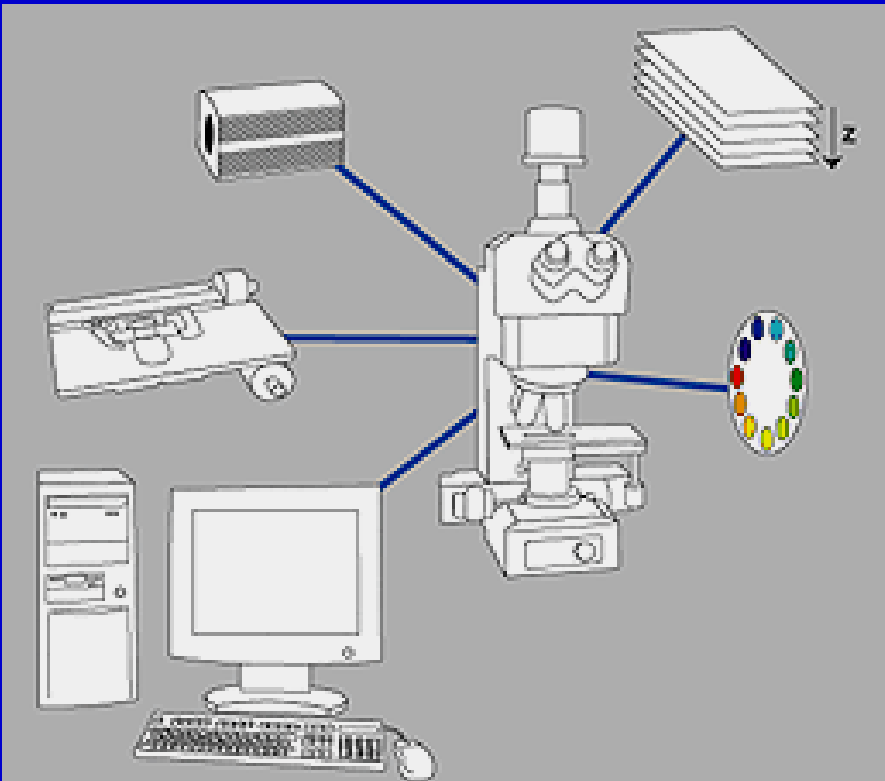
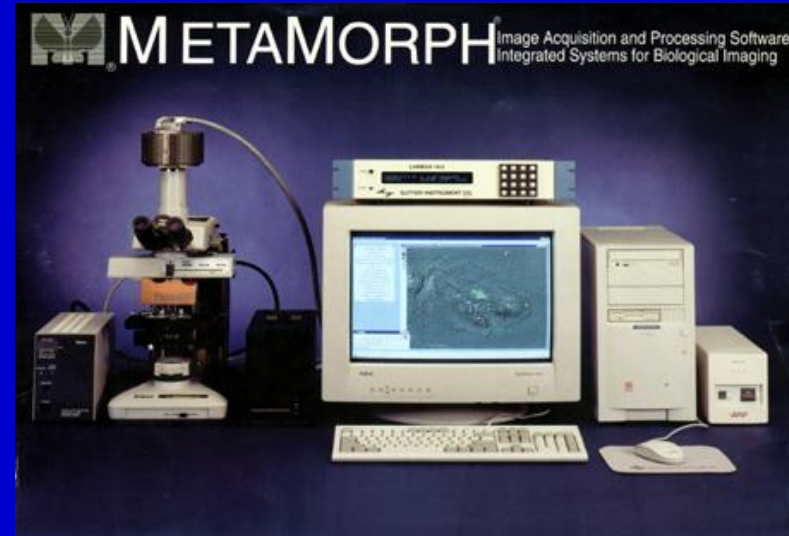
(Frames per second)

Note: Frame rates are measured at 20 MHz with 0-second exposure times.

# Software: MetaMorph System

integrated imaging system for maximized control

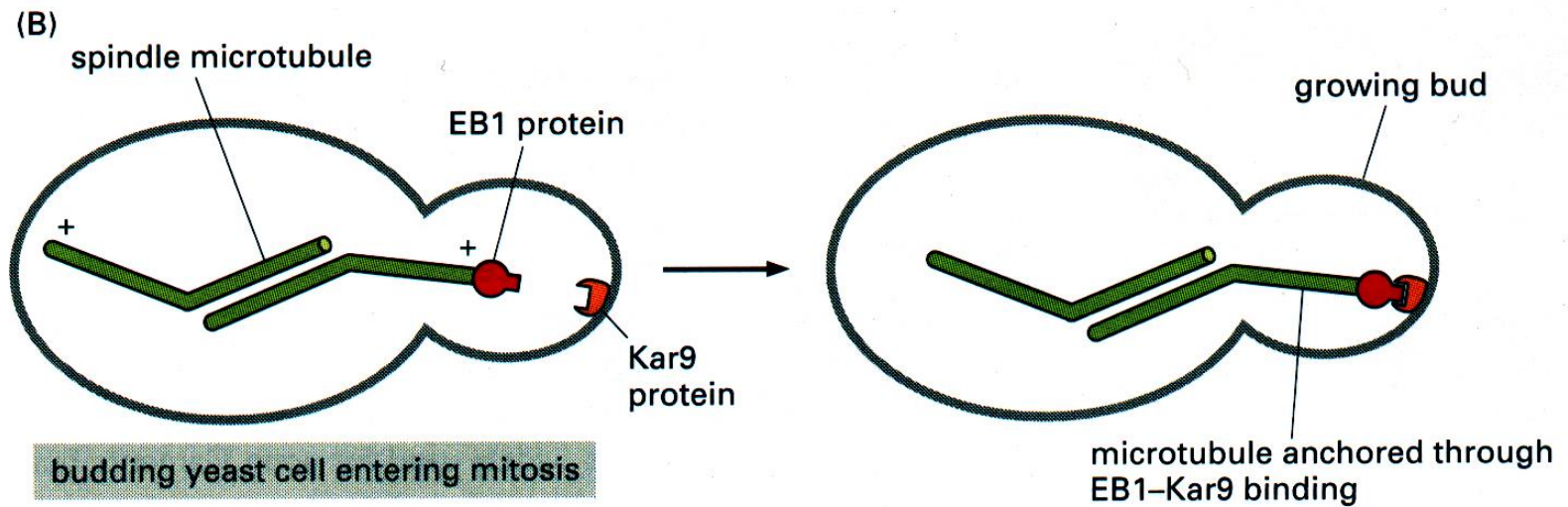
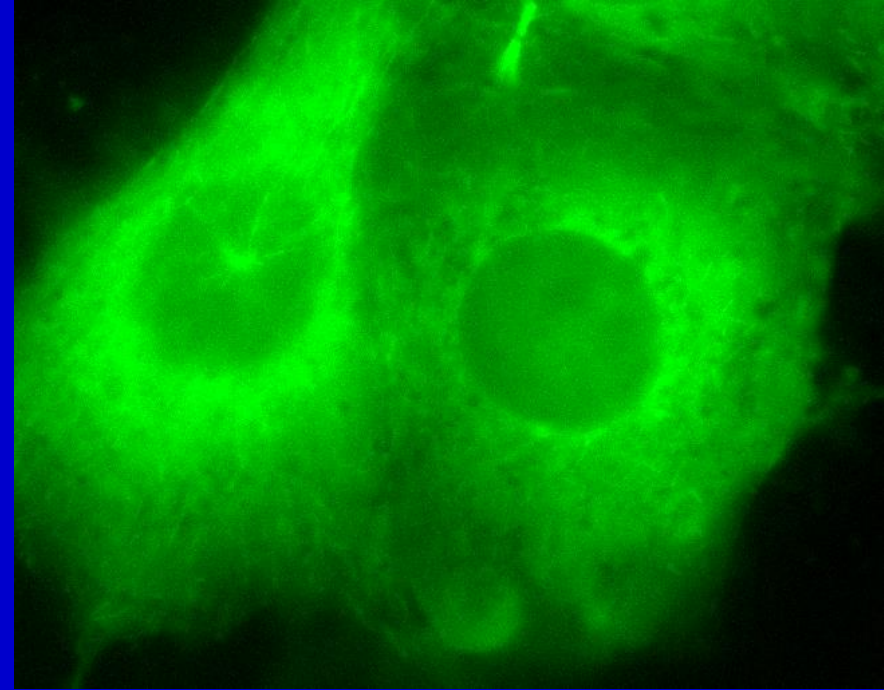
1. Multi-dimensional imaging
2. 3D reconstruction/ Deconvolution
3. Time lapse recording
4. Z-series acquisition
5. Morphometry: Cell counting





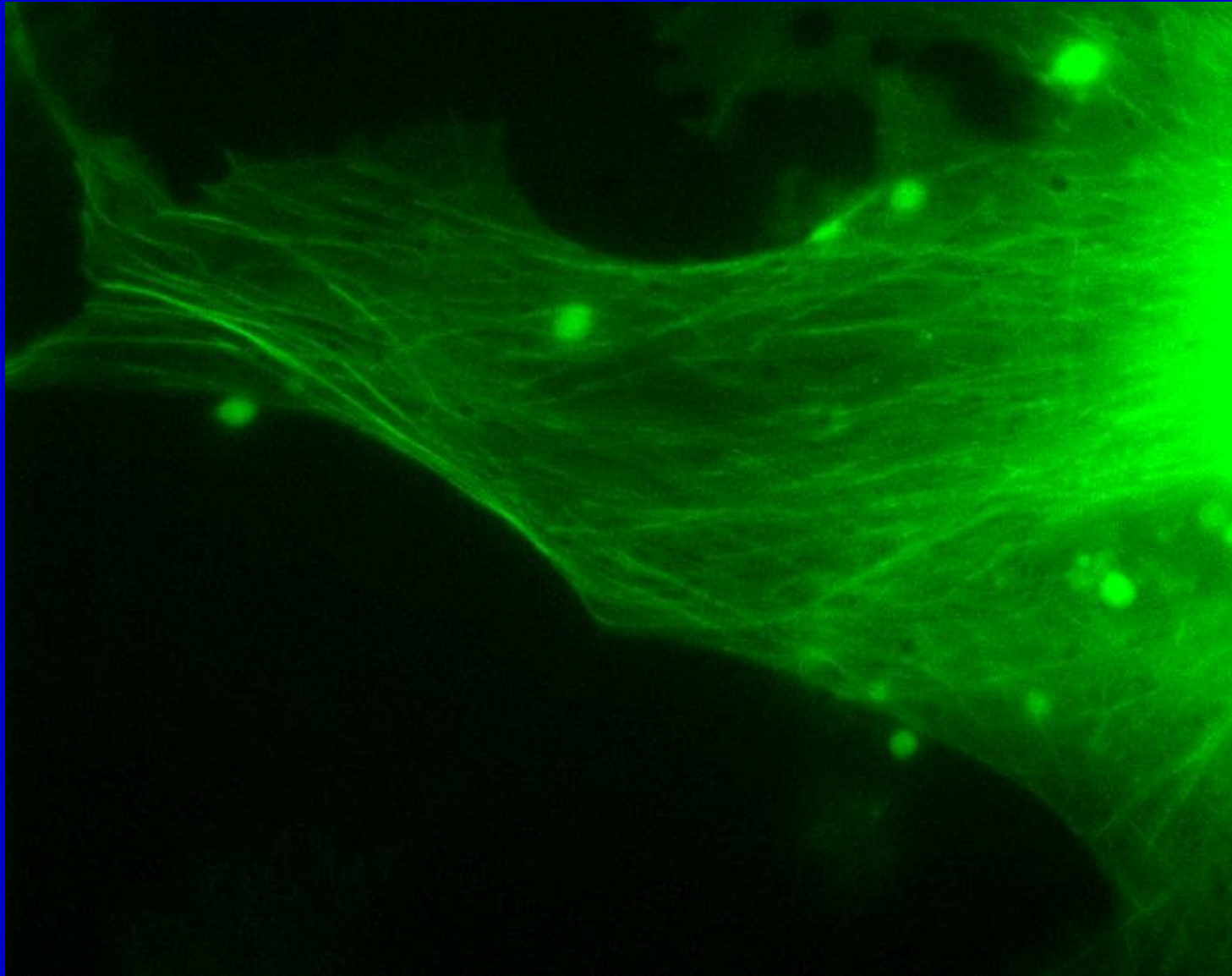
# EB-1: microtubule capping protein

Microtubule dynamics:  
plus end capped with GFP-EB-1

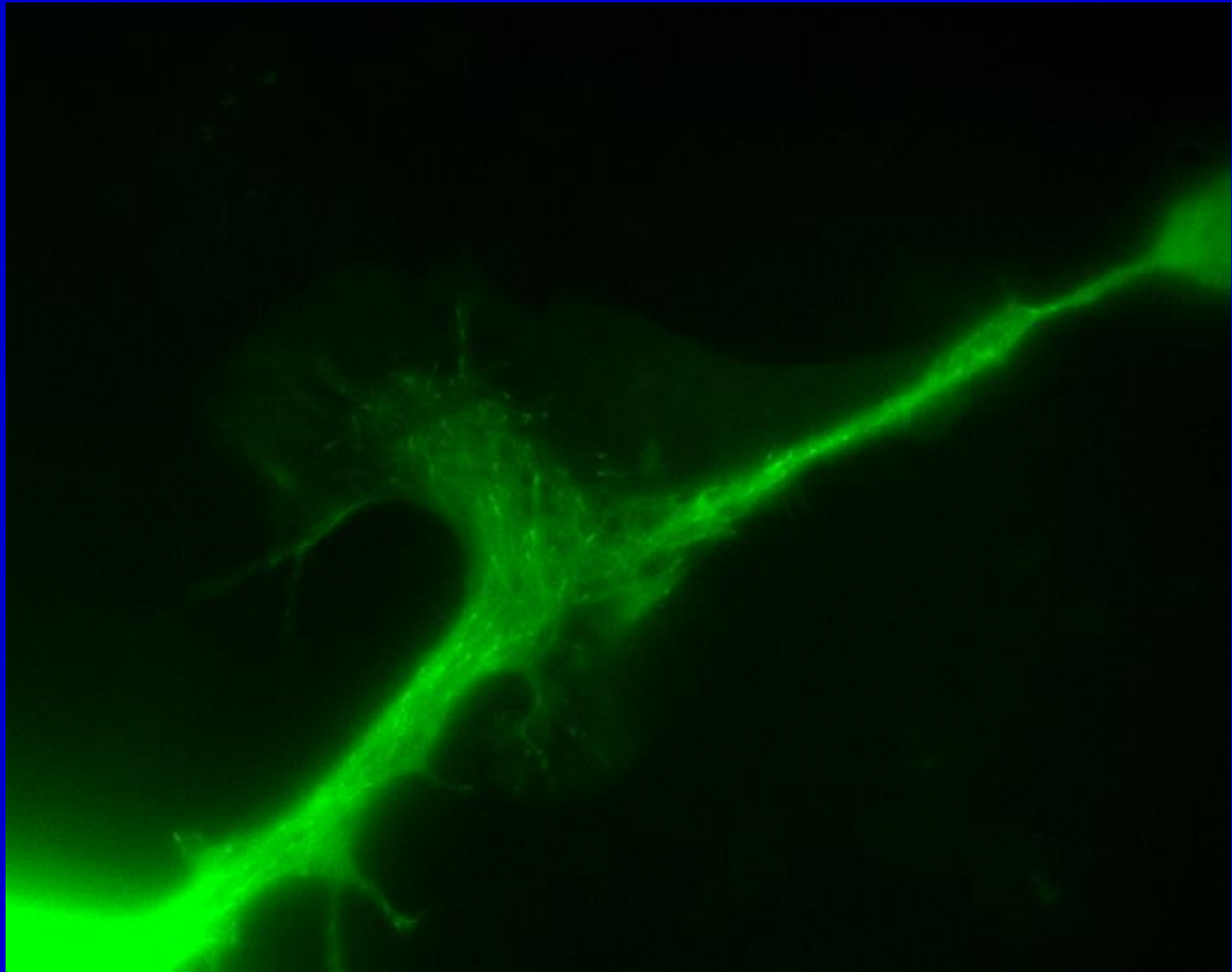


## EB-1-YFP in process of COS 7 cell

EB-1-YFP is a gift from Professor Hirokawa (University of Tokyo)



# EB-1-YFP in process of Neuro 2A cell



# 電子顯微鏡實驗室：(負責人:盧國賢教授)

穿透式電子顯微鏡、掃描式電子顯微鏡及超薄切片機、冷凍超薄切片機與暗房設備



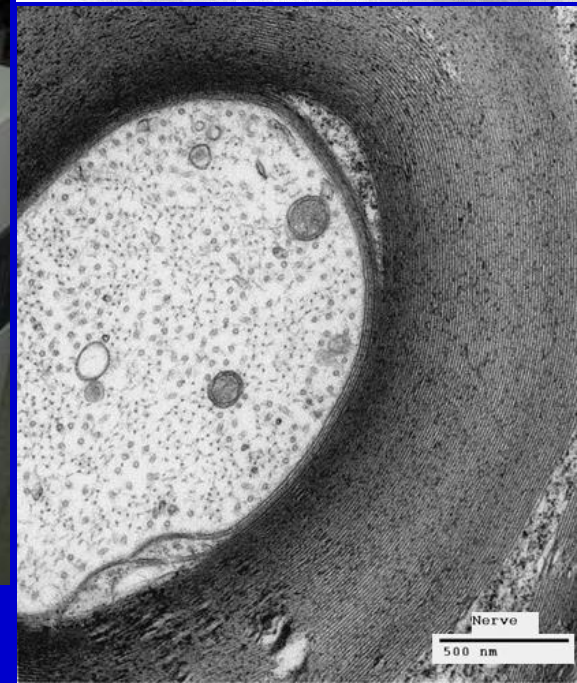
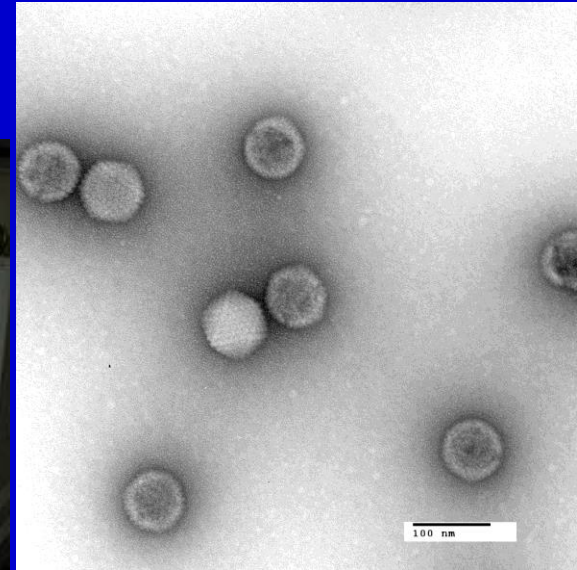
掃描式電子顯微鏡 (JEOL 330A)



穿透式電子顯微鏡 (Hitachi 7100)

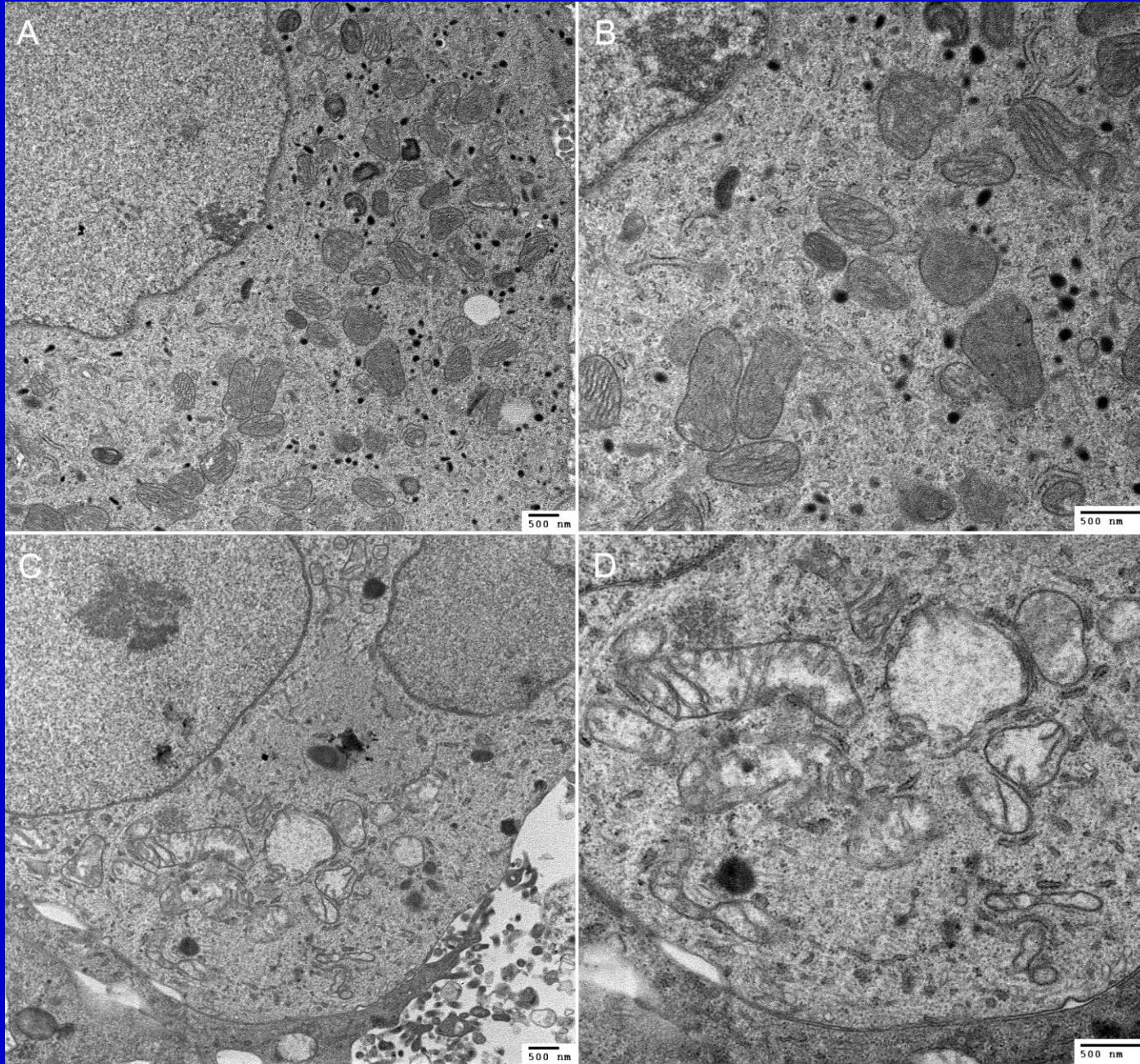


# Digital CCD System for TEM





# Early Apoptosis: Mitochondrial Swelling





# 【Impact & Contribution】

共軛焦光譜顯微鏡核心實驗室自90年4月24日起啟用服務之至今，共舉辦七次應用講習與操作實習會。七次應用講習會共計已有471名研究人員參與學習操作。93年10月21日舉辦的第八次應用講習會與操作實習，仍吸引超過60人參與，每位參與操作實習人員均有機會上機操作，並取得日後使用預約登記權。



# What we could do!?

1. 為配合奈米科技與其他材料科學在生物醫學的運用，藉由研究計畫之分工合作，提供細胞階層的醫學影像技術支援。
2. 定期舉辦技術研習會，為本校區研究同仁培訓研究人員，利用共軛焦顯微鏡與電子顯微鏡技術，順利執行相關研究計畫。
3. 利用網路之便捷性，提供共軛焦顯微鏡與電子顯微鏡相關技術之最新研究資訊，實驗設計及操作程序，藉以推廣提升相關研究。