

活體螢光蛋白在生物醫學之應用

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Fluorescence probes in the living cells

Fluorescent proteins (GFP, YFP, CFP, and DesRed) are ideal for monitoring gene expression and protein localization *in vivo*, *in situ*, and in real time.

Fluorescent proteins do not require additional proteins, substrates, or cofactors for detection.

NEW PRODUCTS

Living Colors® Red Fluorescent Protein

The only red fluorescent protein for expression studies

- Exclusively available from CLONTECH
- Ideal for *in vivo*, multiple color labeling
- Virtually eliminates background fluorescence
- Highly specific antibody available



CLONTECH introduces **Living Colors® Red Fluorescent Protein**—the only commercially available red fluorescent protein (RFP) for expression studies. This unique protein (DsRed) was isolated from the IndoPacific sea

Figure 1. Bright fluorescence of DsRed and GFP.

Living Colors® Red Fluorescent Protein...continued

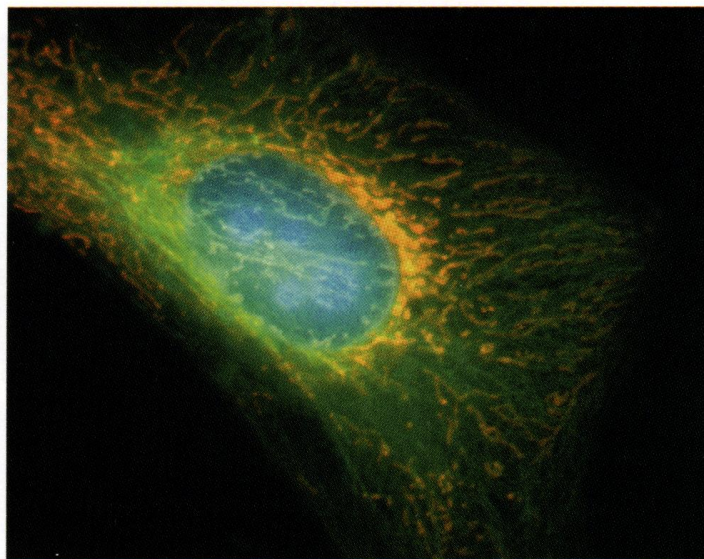


Figure 2. Triple labeling with DsRed1, ECFP, and EYFP. HeLa cells were transiently transfected with pECFP-Nuc (#6904-1), pEYFP-Tub (#6118-1), and pDsRed1-Mito (n/a), which label the nucleus, tubulin, and mitochondria, respectively. The cells were incubated at 37°C for 48 hr, fixed in 3.7% formaldehyde in PBS, and observed by fluorescence microscopy using a Zeiss Axioskop. The images were taken with Omega filter sets XF35 (propidium iodide) for DsRed1-Mito, XF104 for EYFP-Tub, and XF114 for ECFP-Nuc, a cooled CCD camera (MicroMax Interline Transfer Camera, Roper Scientific), and MetaMorph Software (Universal Imaging Corp.). Individual images were overlaid and pseudocolored. The overlap of EYFP and DsRed gives a bright yellow color.

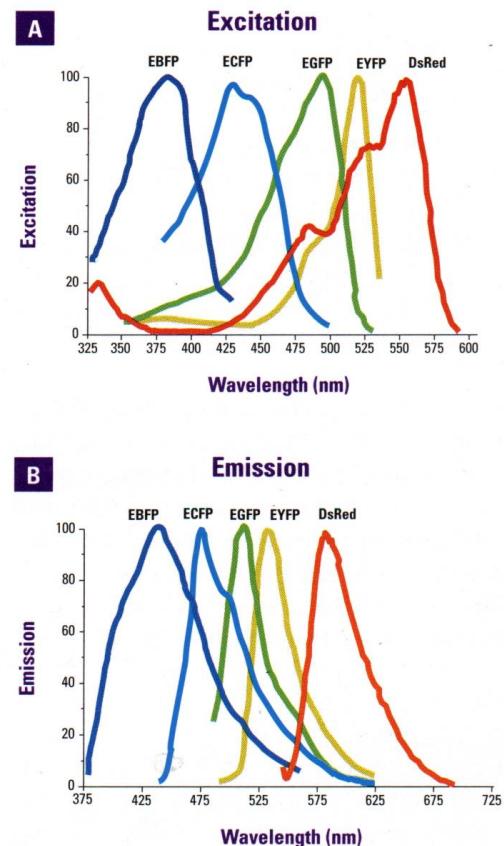


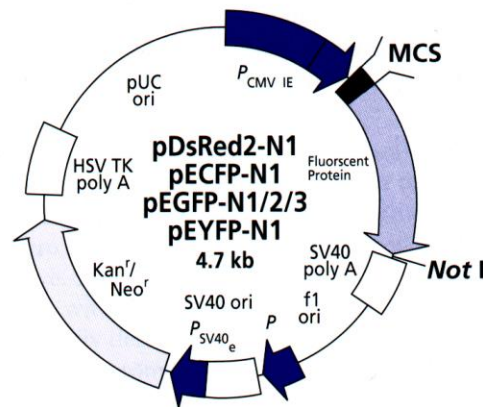
Figure 3. Excitation and emission spectra for all Living Colors® fluorescent proteins.

Some commercial available expression vectors for fluorescent proteins: (from BD Biosciences Clontech)

*N-terminal Protein Fusion expression vectors: pDsRed2-N1, pECFP-N1, pEGFP-N1, N2, N3, and pEYFP-N1

N-Terminal Enhanced Fluorescent Protein Vectors

Journal
Citation



N1 MCS

G CTA GCG CTA CCG GAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG CAG TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GTC GCC ACC ATG GTG
 Nhe I Eco47 III Bgl II Xho I Sac I Hind III EcoR I Pst I* Sal I Kpn I Apa I Xma I BamH I Age I**
 Acc I Asp718 I Sac II Bsp120 I Sma I

N2 MCS

STOP
 GC TAG CGC TAC CGG ACT CAG ATC TCG AGC TCA AGC TTC GAA TTC TGC AGT CGA CGG TAC CGC GGG CCC GGG ATC CAC CGG CCG GTC GCC ACC ATG GTG
 Nhe I Eco47 III Bgl II Xho I Sac I Hind III EcoR I Pst I Sal I Kpn I Apa I BamH I Eag I†
 Acc I Asp718 I Sac II Bsp120 I Xma I Sma I

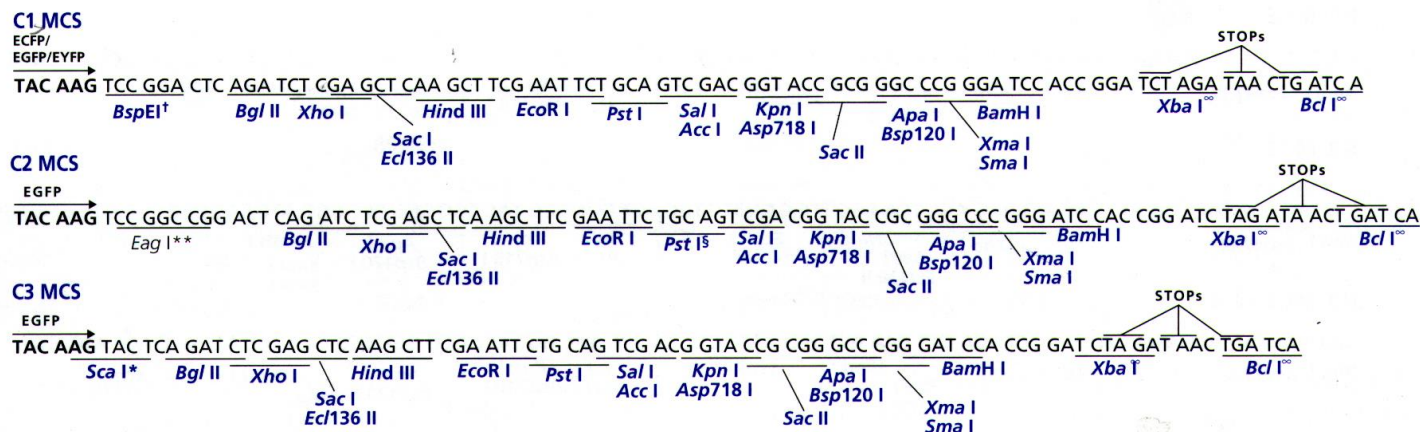
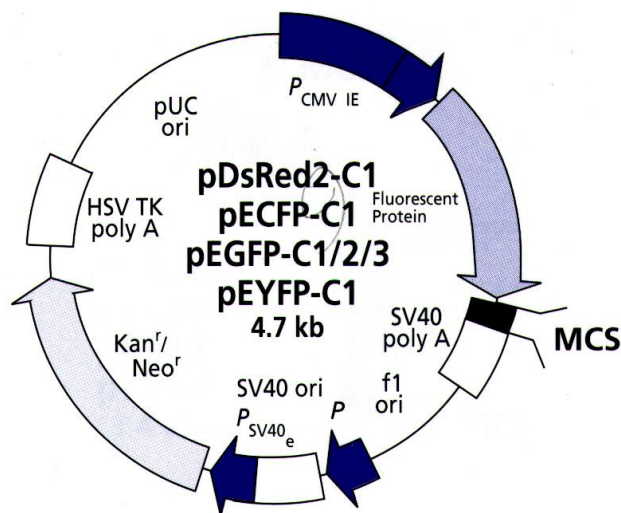
N3 MCS

GCT AGC GCT ACC GGA CTC AGA TCT CGA GCT CAA GCT TCG AAT TCT GCA GTC GAC GGT ACC GCG GGC CCG GGA TCC ATC GCC ACC ATG GTG
 Nhe I Eco47 III Bgl II Xho I Sac I Hind III EcoR I Pst I Sal I Kpn I Apa I BamH I Xcm I‡
 Acc I Asp718 I Sac II Bsp120 I Xma I Sma I

C-terminal Protein Fusion expression vectors: pDsRed2-C1, pECFP-C1, pEGFP-C1, C2, C3, and pEYFP-C1



C-Terminal Fluorescent Protein Vectors



Living Colors™ Subcellular Localization Vectors

- Localize fluorescence to specific organelles or structures in living cells
- Visualize biological processes as they occur
- Dual- or triple-label cells with different fluorescent proteins

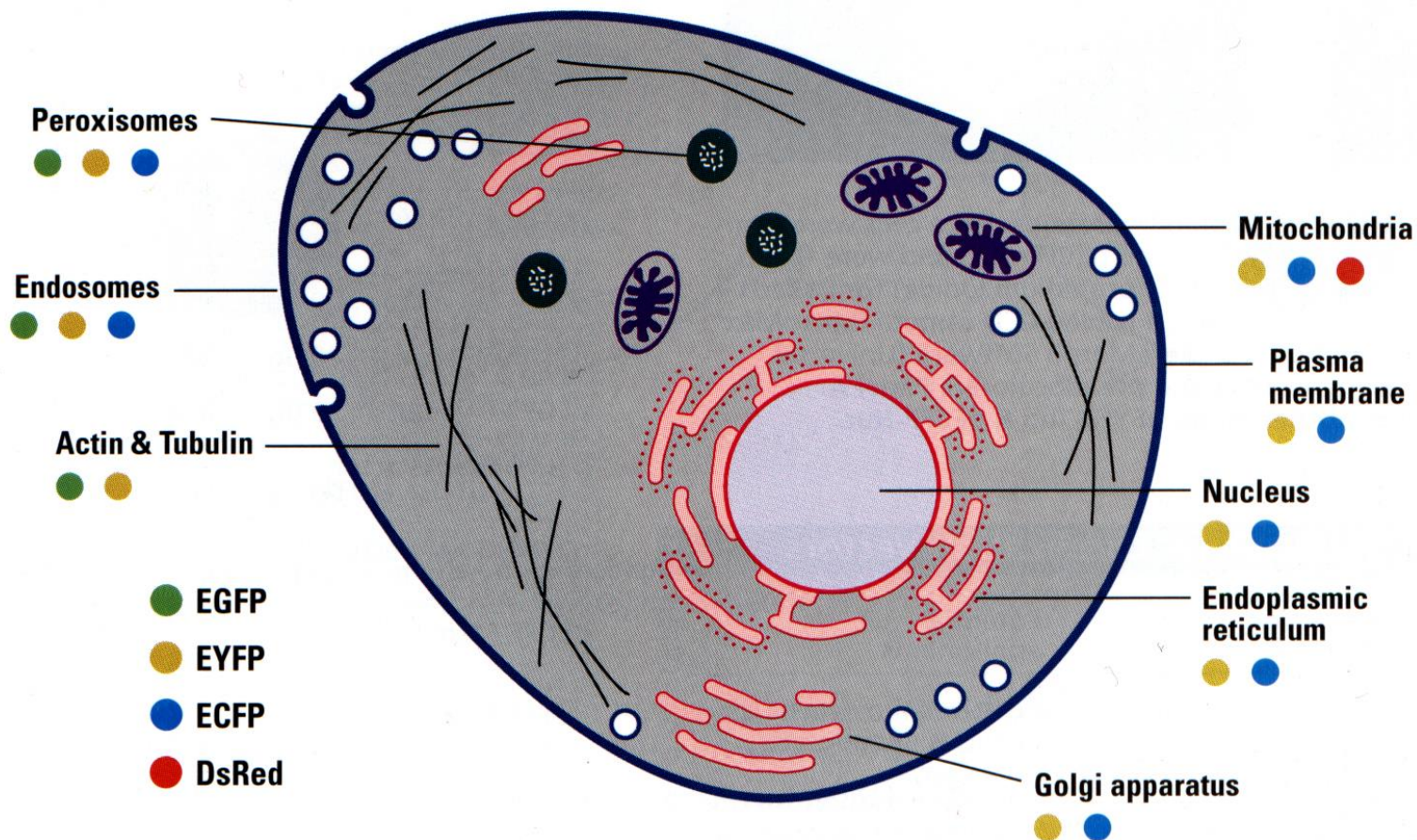


Figure 1. Organelles targeted by Living Colors™ Subcellular Localization Vectors.

Living Colors™ Subcellular Localization Vectors continued

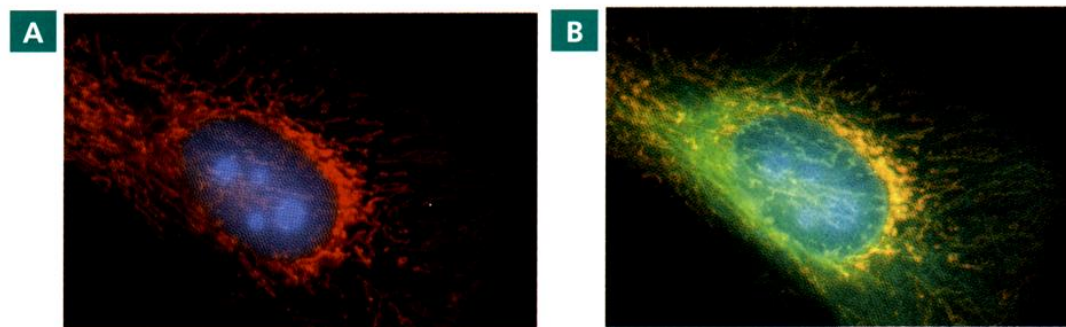
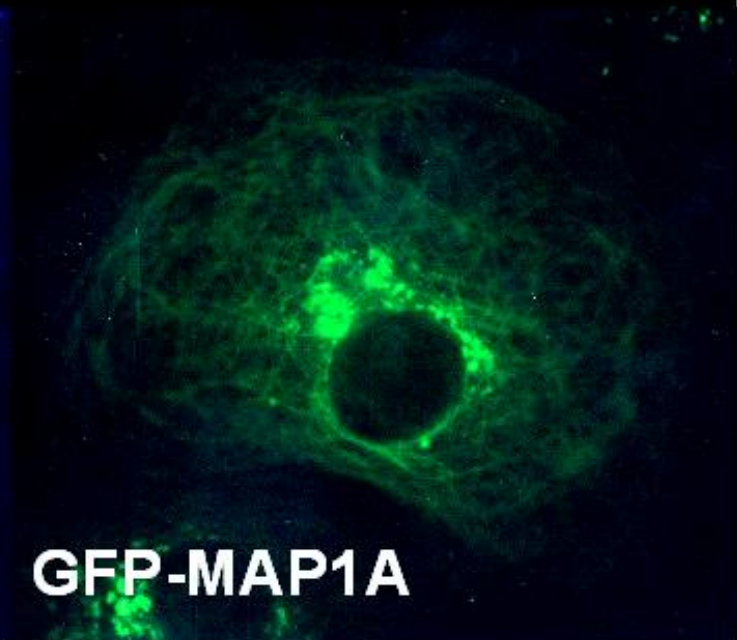
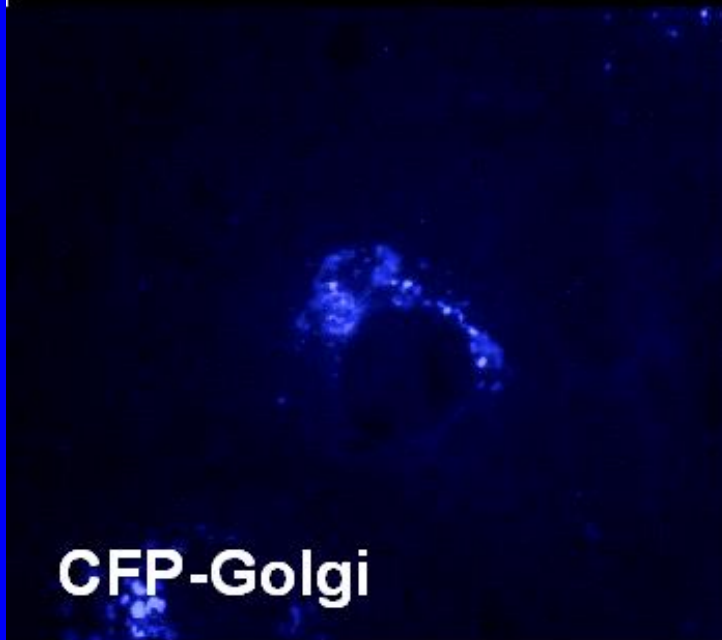
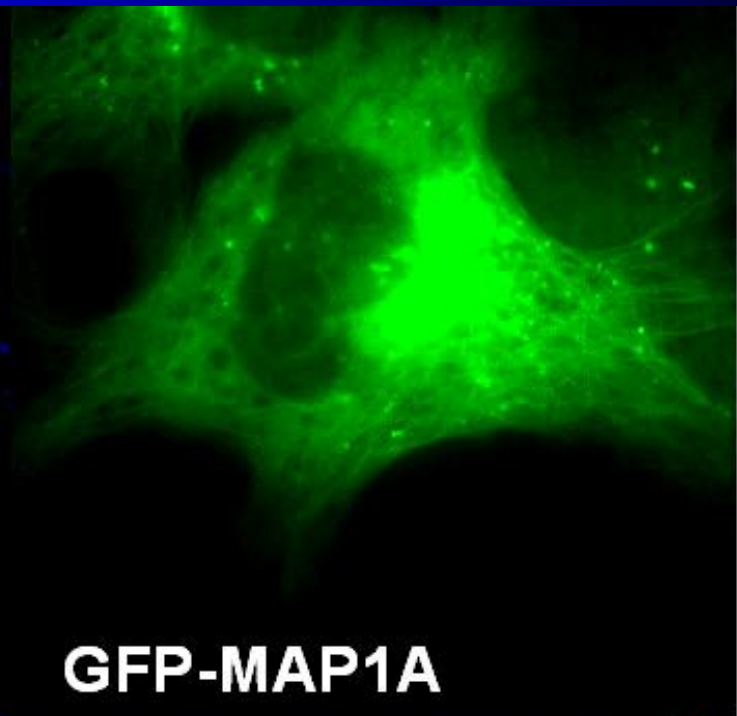
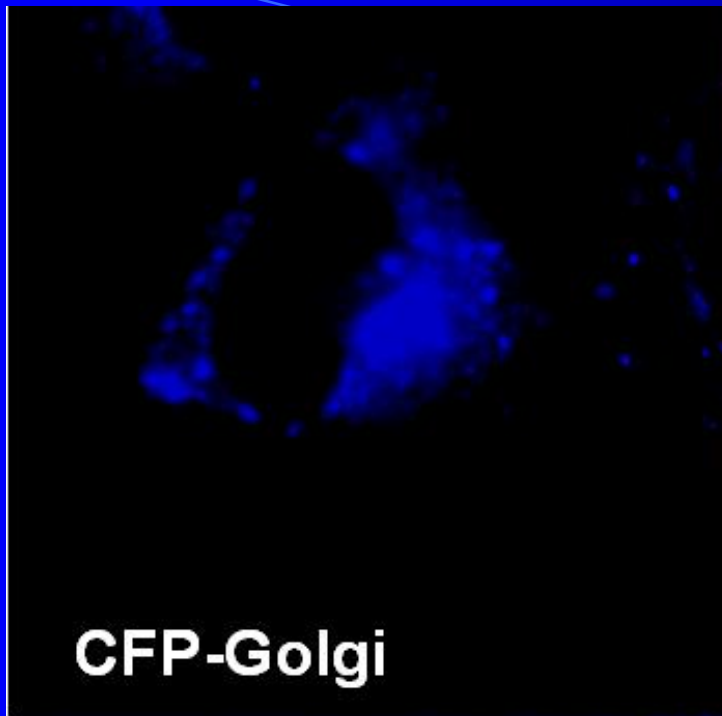


Figure 2. Dual and triple labeling with Living Colors™ proteins. HeLa cells were transiently transfected with pDsRed1-Mito, pEYFP-Tub, and pECFP-Nuc, and were fixed. The images were taken using Omega filter sets XF35 (propidium iodide) for DsRed1-Mito, XF104 for EYFP-Tub, and XF114 for ECFP-Nuc; a cooled CCD camera (MicroMax Interline Transfer Camera, Roper Scientific); and MetaMorph Software (Universal Imaging Corp.). Individual images were overlaid and pseudocolored. **Panel A.** pDsRed1-Mito & pECFP-Nuc. **Panel B.** pDsRed1-Mito, pEYFP-Tub & pECFP-Nuc.

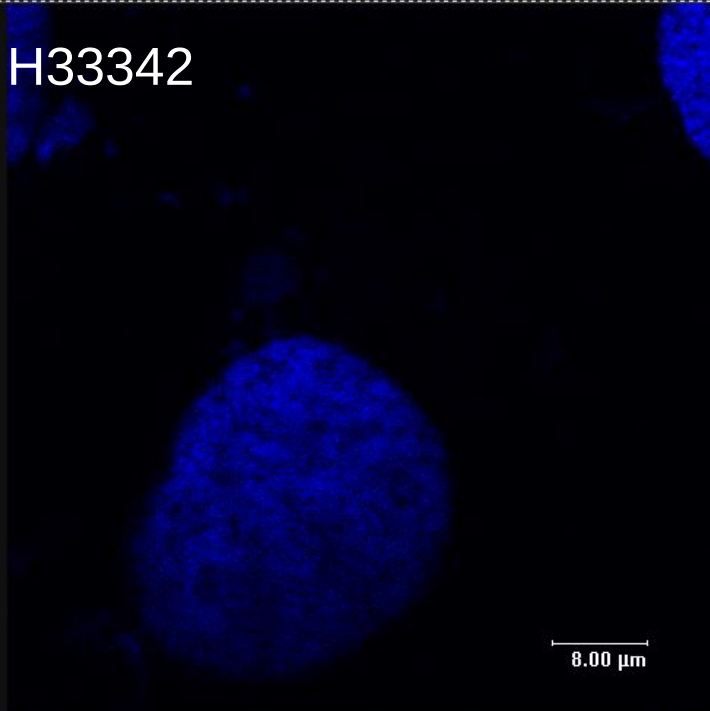
Table I: Living Colors™ Subcellular Localization Vectors

Targeted subcellular structure	Color variants available	Localization tag or gene	Potential applications
Endosomes	Green, cyan, yellow	RhoB	• Observe movement of vesicles of endocytic pathway • Monitor endocytosis of labeled receptors or ligands
Mitochondria	Cyan, yellow	Targeting sequence from subunit VIII of cytochrome c oxidase	• Study normal & disease state • Track mitochondrial dynamics
Nucleus	Cyan, yellow	SV40 T-antigen NLS*; 3 tandem repeats	• Study nuclear import • Track cell lineage • Monitor cell growth & division

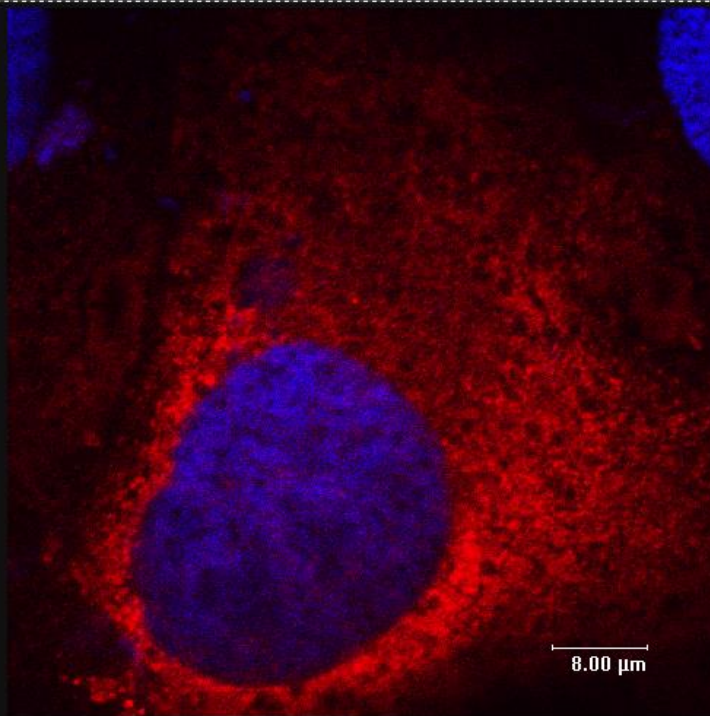
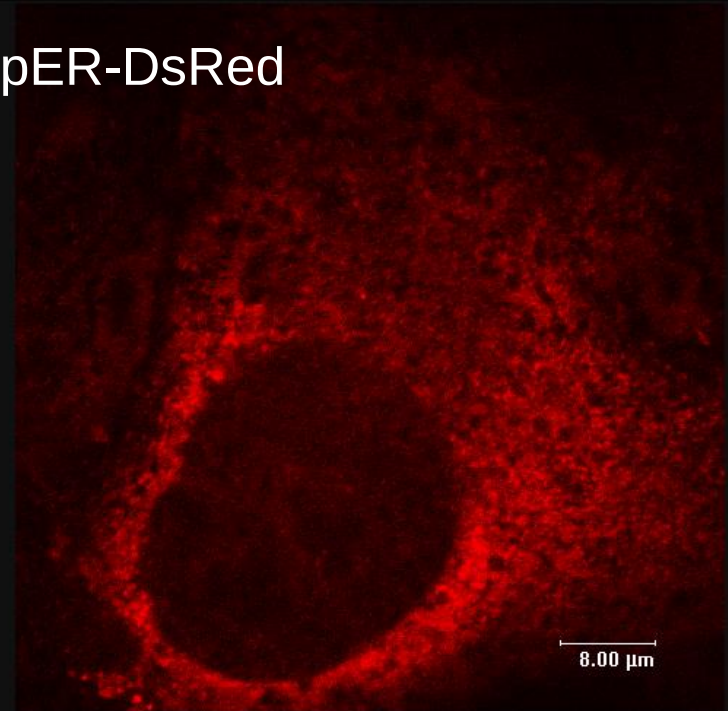
Product	Size	Cat. #
pEGFP-Actin Vector	20 µg	6116-1
pEYFP-Actin Vector	20 µg	6902-1
pECFP-Endo Vector	20 µg	6934-1
pEGFP-Endo Vector	20 µg	6935-1
pEYFP-Endo Vector	20 µg	6936-1
pECFP-ER Vector	20 µg	6907-1
pEYFP-ER Vector	20 µg	6906-1
pEGFP-F Vector	20 µg	6074-1
pECFP-Golgi Vector	20 µg	6908-1
pEYFP-Golgi Vector	20 µg	6909-1
pECFP-Mem Vector	20 µg	6918-1
pEYFP-Mem Vector	20 µg	6917-1
pECFP-Mito Vector	20 µg	6903-1
pEYFP-Mito Vector	20 µg	6115-1
pECFP-Nuc Vector	20 µg	6904-1
pEYFP-Nuc Vector	20 µg	6905-1
pEGFP-Peroxi Vector	20 µg	6932-1
pECFP-Peroxi Vector	20 µg	6931-1



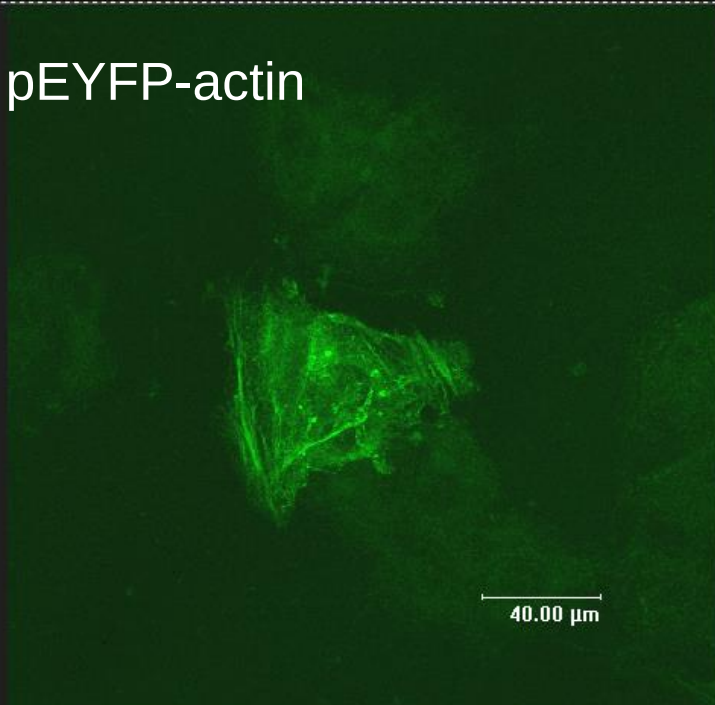
H333342



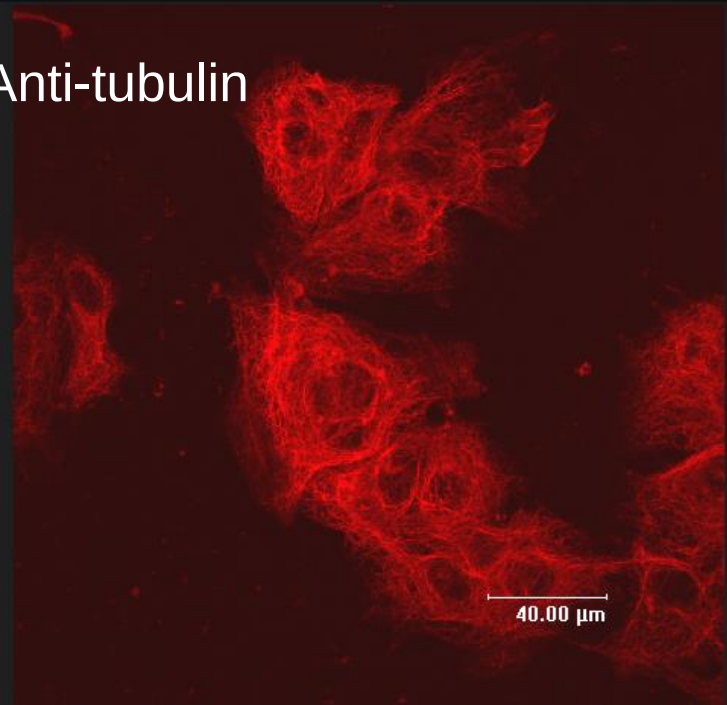
pER-DsRed



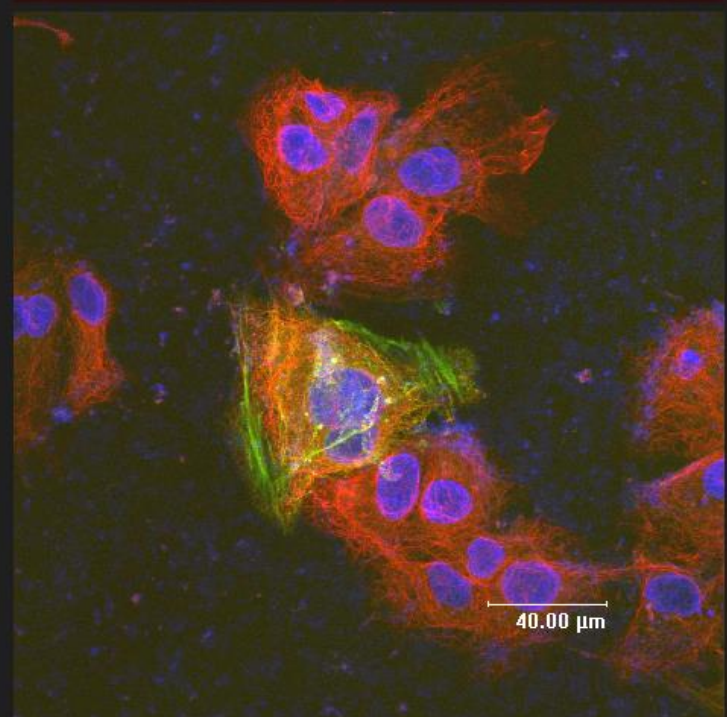
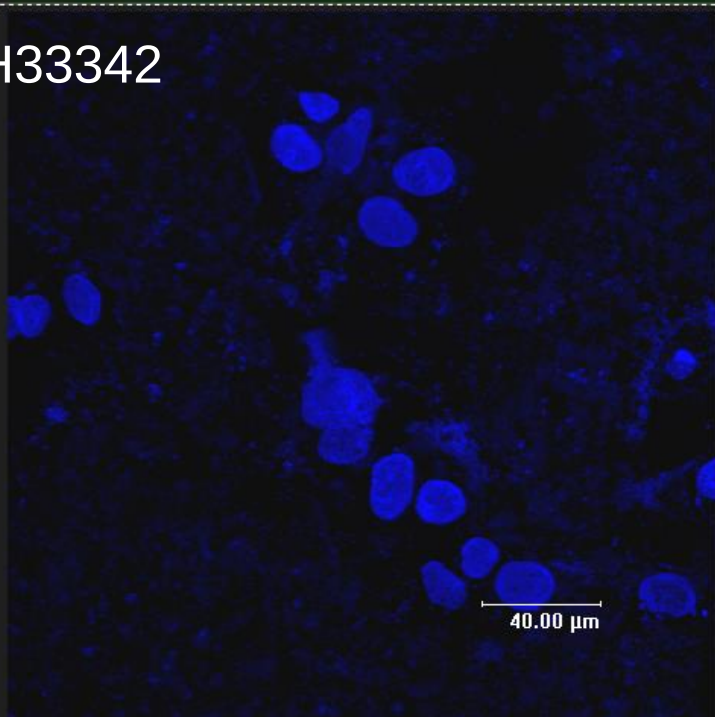
pEYFP-actin



Anti-tubulin

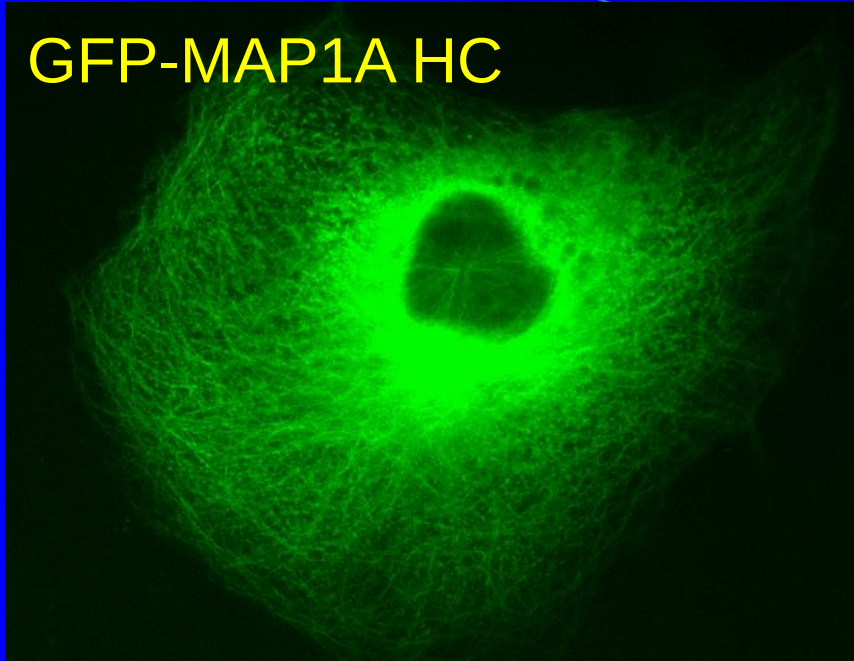


H33342

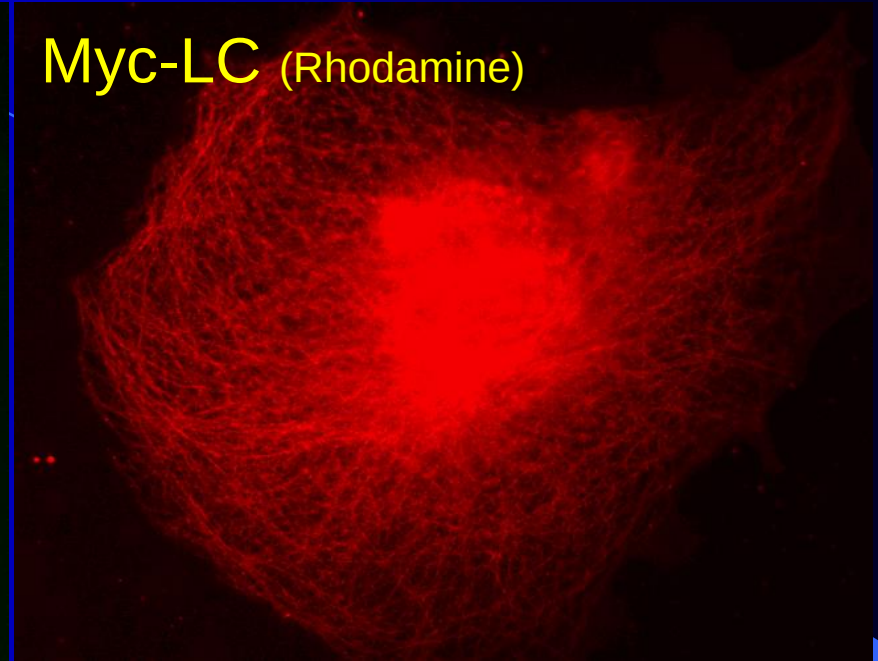


Immunocytochemical staining of transfected COS7 cells

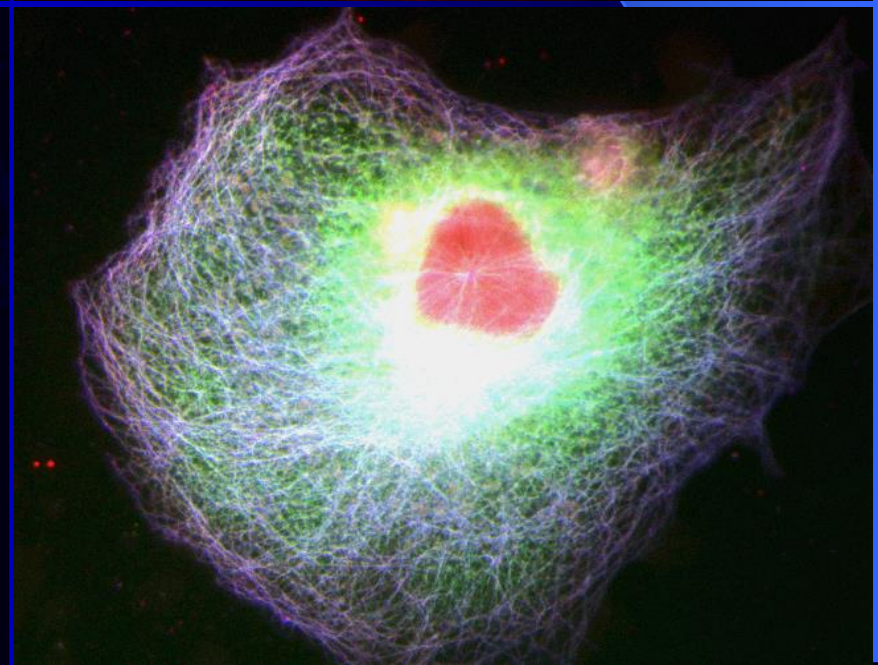
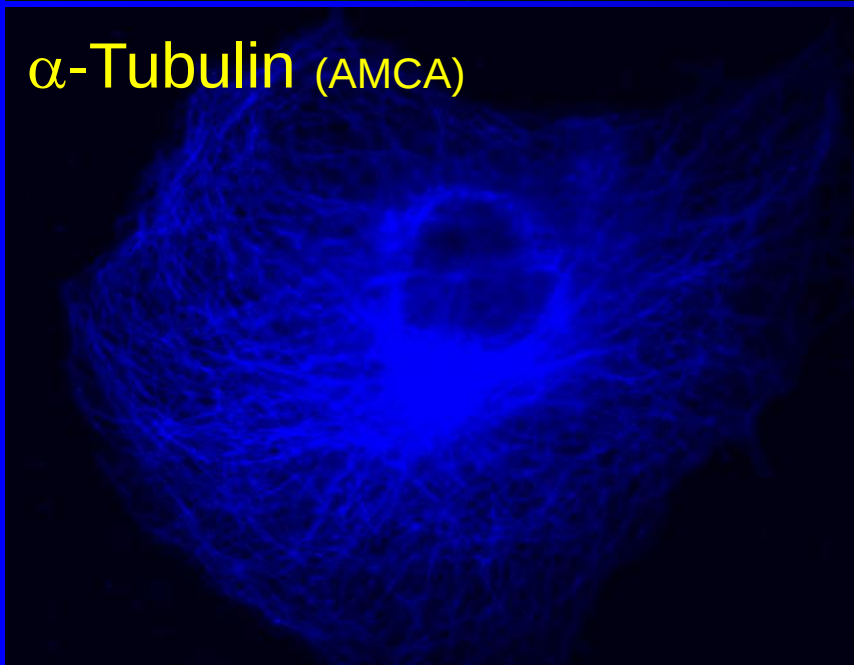
GFP-MAP1A HC



Myc-LC (Rhodamine)

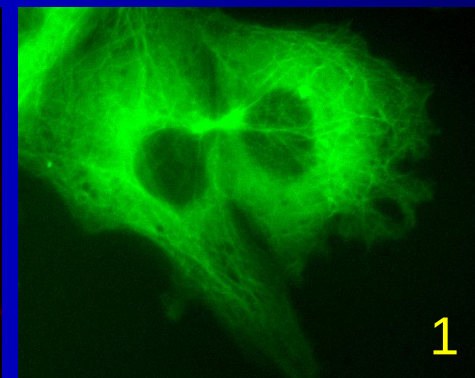
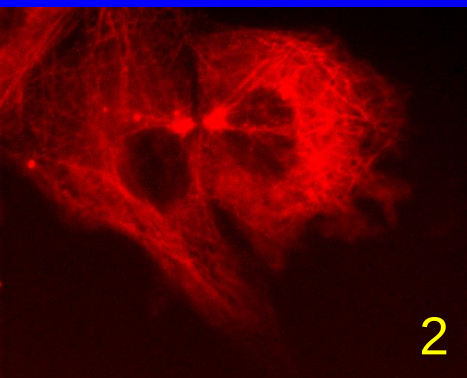
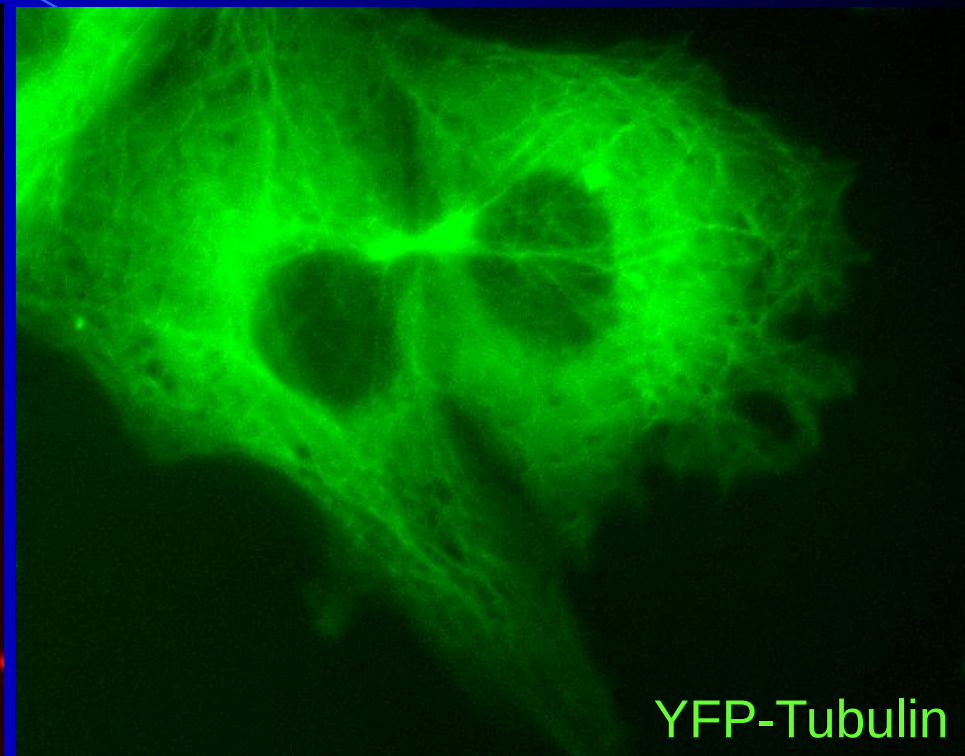
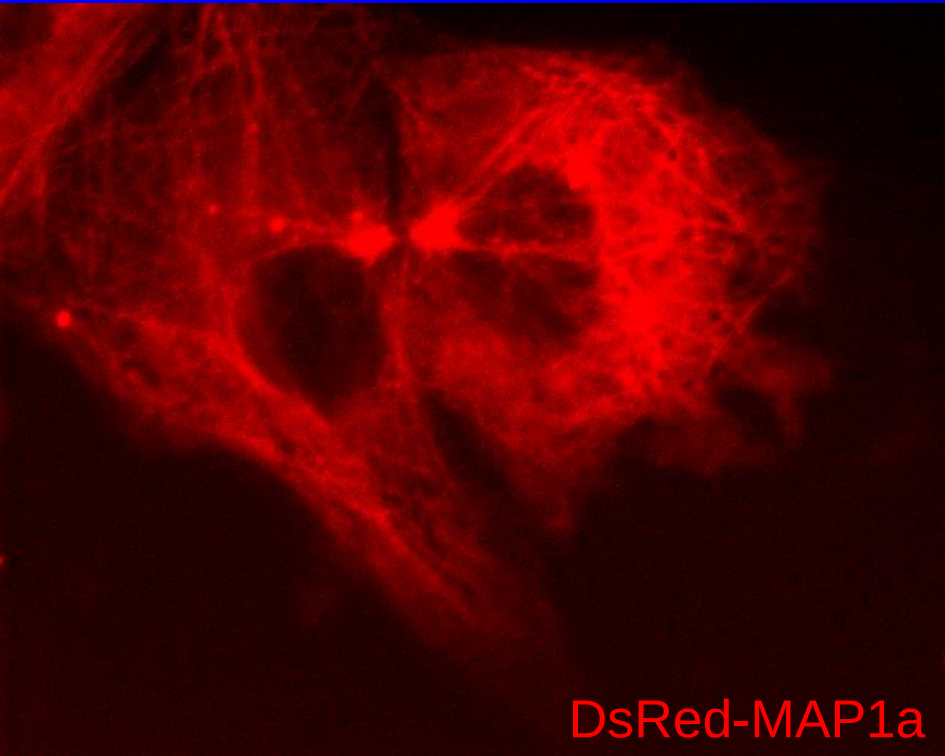


α -Tubulin (AMCA)



Nocodazol treated COS 7 cells co-transfected **DsRed-MAP1a** +
YFP-Tubulin

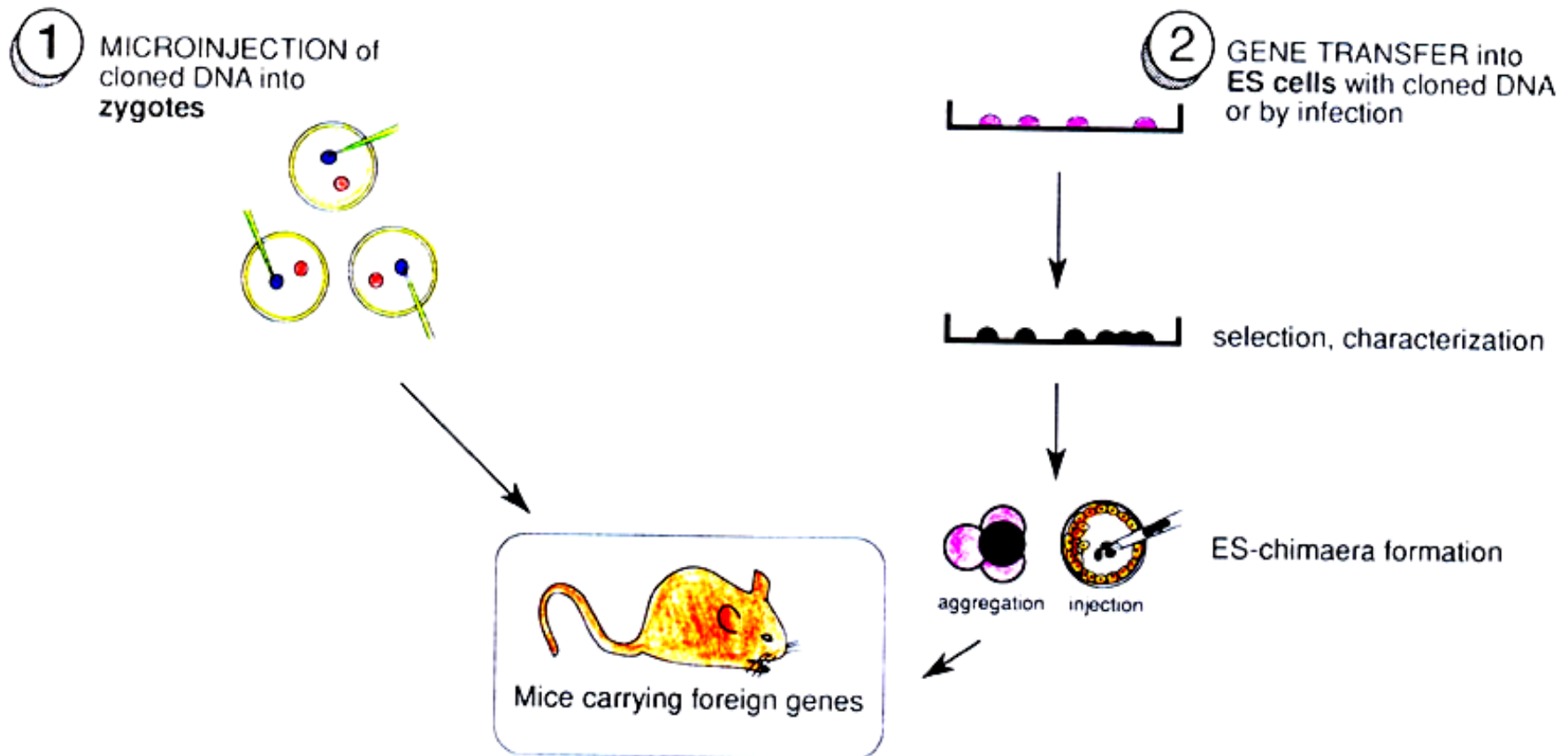
10 second / frame, total 216 frames, 36 min



Fluorescent Organisms:

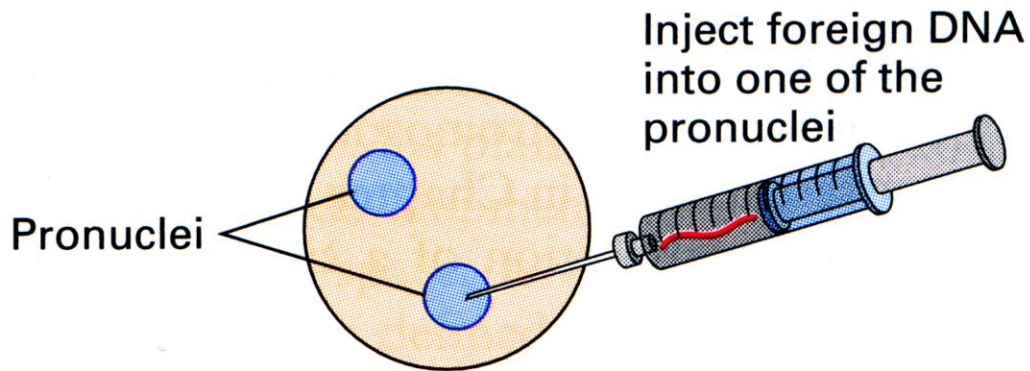
Transgenic GFP mouse

Method for introducing genes into mouse embryos:
DNA injection into fertilized eggs
(over-expression, multiple copies of GFP transgene)



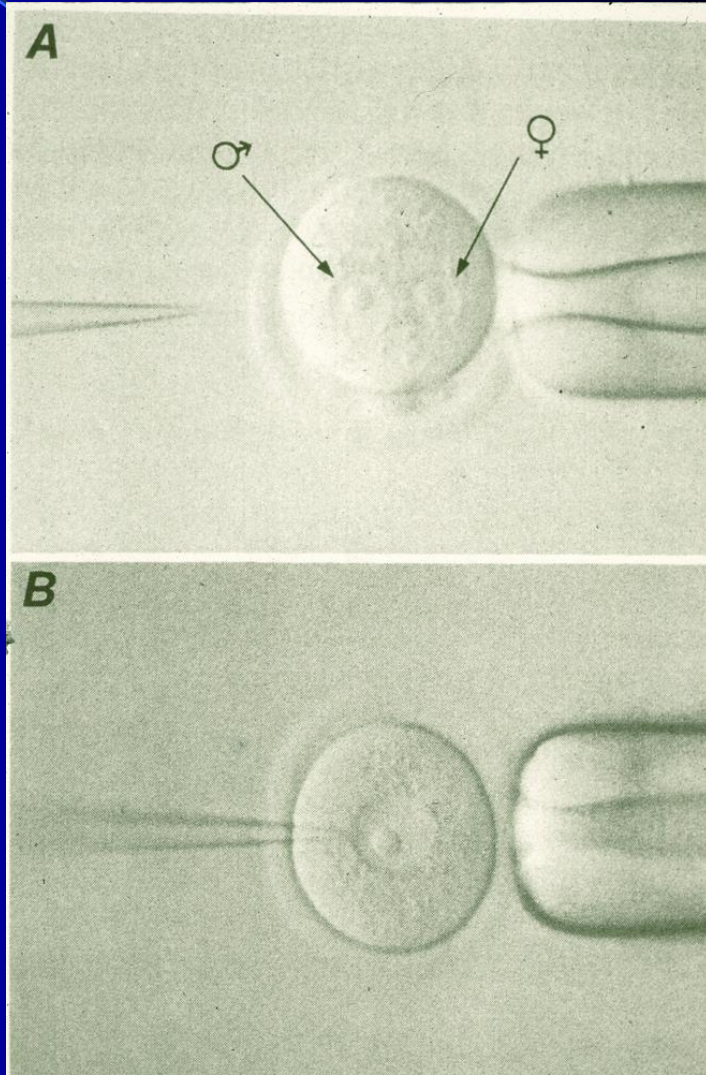
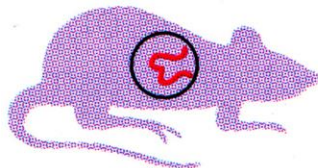
傳統基因轉殖：

將欲探討的基因直接打入動物的受精卵。



Fertilized mouse egg prior to fusion of male and female pronuclei

Transfer injected eggs into foster mother



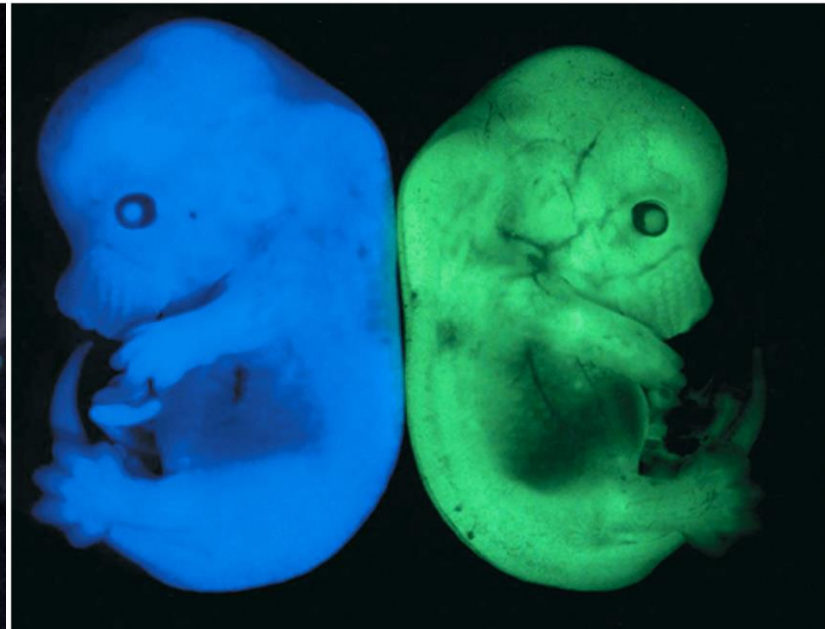
Transgenic GFP mouse

(cited from Dr. Nagy's Homepage: <http://www.mshri.on.ca/nagy/visual.html>)
Fluorescence Stereomicroscopy (Leica MZ FLIII)



GFP-5

Transgenics



進階生物科技的螢光鼠

<http://www.level.com.tw>



水中夜明珠 螢光魚

邨港生技的螢光魚 基因轉殖全身發光

市面上有許多看似會發光的飼養觀賞魚，像日魚、紅蓮燈魚等，因色素細胞非螢光的關係，燈一暗就不會產生任何顏色，而螢光魚絕非利用化學螢光劑注射，而是利用轉殖技術，使魚生下來就有螢光色彩，不僅飼養簡單，即不燈照，也能釋放的光芒。

報導／劉詩韻 攝影／吳曉

看我的螢光魚閃閃發亮，像許多小天使一樣！
(設計對白)

螢光魚小檔案

習性：性情溫和，以浮游生物為食，喜歡棲息在平緩溪流中，水域表層到底層均分布。

繁殖：卵生，一年多產，屬体外受精型。

食性：雜食性，紅蟲、絲蚯蚓、動物性浮游生物、人工飼料。

壽命：約2-3年。

變身前青鱗魚

變身後的特色



★利用顯微注射方式，把外來水母的綠螢光基因及紅珊瑚螢光基因，植入野生種的青鱗魚之胚胎內，經長時間培養、篩選所得的基因轉殖魚。

★不止在外表會發綠或紅色螢光，甚至於產下的卵、胚



胎、稚魚及仔魚全身也都有螢光色出現。

★在一般的日光燈照射下，螢光基因魚也會顯出螢光色；特別在短波長燈光下，則更加顯出其特殊的綠系或紅系螢光體色。

目前利用基因轉殖研發成功的有，螢光綠及螢光紅兩種顏色，未來還會陸續有紅、綠、紫、黃…等色，讓這些魚在長黑燈及藍燈的照射下相互輝映，就能真正展現出五彩繽紛的螢光世界！



山常繼

什麼是基因轉殖

螢光基因魚是運用基因工程及基因轉殖兩項生物科技所完成的新品種，基因轉殖簡單的說，就是把一段外來的基因片斷，經顯微注射的方式迫使它進入胚胎的細胞核中或細胞質內，好讓外來基因片斷能在於胚胎中繼續複製，使宿主體內表現出該基因所帶來的遺傳特性。

超級好養



環境

★有稻田魚之稱的青鱗魚，適應力很強，不管是淡水缸還是海水缸，都可以飼養。

★水溫則是26-28℃最適合。



餵食

可以飼紅蟲、絲蚯蚓、綠藻水、孵化的豐年蝦及小型淡水魚吃食的人飼料外，也可飼專用飼料「魚漢堡」，呈浮水性顆粒狀，兼顧成長、增肥、瘦等功能，可永遠綻放耀眼光彩。

小叮嚀

2呎以上的大缸約1個月換1次水，而在要換水之前，可以用清缸棉將水族箱壁的表面附著物刮除後，再進行換水；換水時也不用將魚撈離水族箱，只需要以虹吸管把

水吸取三分之一，再將要添加的自來水，先行滴入適量的水質安定劑及硝化菌後再倒入缸中即可，或是可選擇免換水的活菌系列及過濾系統，就可以不用換水。

看缸

市面上有為螢光魚量身打造的家，從過濾器到造景，全部都有，但最重要的是

燈光的選擇，為了突顯螢光魚美麗動身影，以及散發出來的獨特光，就必須選擇黑燈管或藍燈管來照射。



■一般白光燈管，白天效果，螢光魚身上螢光體色依舊不變。

■還可切換一黑一白燈管套缸，可看螢光魚白天與夜晚風采。

炫麗效果，更襯托螢光魚的風采。

掛式過濾器，過濾棉可隨時更換新即可。

風水建議

綠色螢光魚不僅可招來財運，還可以防小人，因為他就好比古代的夜明珠，在黑夜中發光，紅色螢光魚可使人緣變好，如果能再請風水師至家中勘查，在一定的時間，放在對的位置，其效果更佳。



風水專家 陳正倫

以大運來看，魚缸建議擺放位置為東方、東南及西南方較適合，且魚缸兩旁最好都有牆壁，可有收住錢財的功效，而且要注意不要讓傢俱擋住魚缸即可。



專家說

林經理說此次有別於前年誕生的綠螢光魚，今年所研發創造的也是全球第一條全身型的紅螢光基因魚，其全身包括眼睛、內臟，均散發出桃紅色螢光魅力，不僅可產生新的能量，創造絕佳磁場，對喜愛紅色代表喜氣的國人來說，真可謂是最佳的風水招財魚。



邨港科技研發經理 林學廉

蔡教授說，在家中，若飼養一缸精心布置的螢光魚，是讓小孩子認識基因工程的最好教材，藉由螢光魚讓他們對生物科技產生興趣，達到教育的功能，而利用基因轉殖技術不僅可保護海洋生態，更將生技發展的技术實際運用在休閒生活之上。



台大生科院分子細胞生物研究所所長 蔡懷植

哪裡買

螢光魚套缸約1990-8888元，除配備外還加送3-10隻螢光魚。購買10隻以上螢光魚，1隻99元。

如果是新的魚缸，建議購買套缸會比較划算。

請上網或電話洽詢全省螢光魚直營門市各據點：0800-833-311或2763-2680，www.szoo.com.tw

綠色螢光豬：將綠色螢光基因轉殖於早期豬胚原核中，
產製出全身均能表現綠色螢光之基因轉殖豬
By 台灣大學動物科技學系助理教授吳信志博士



Multi-dimensional Live-cell Imaging System

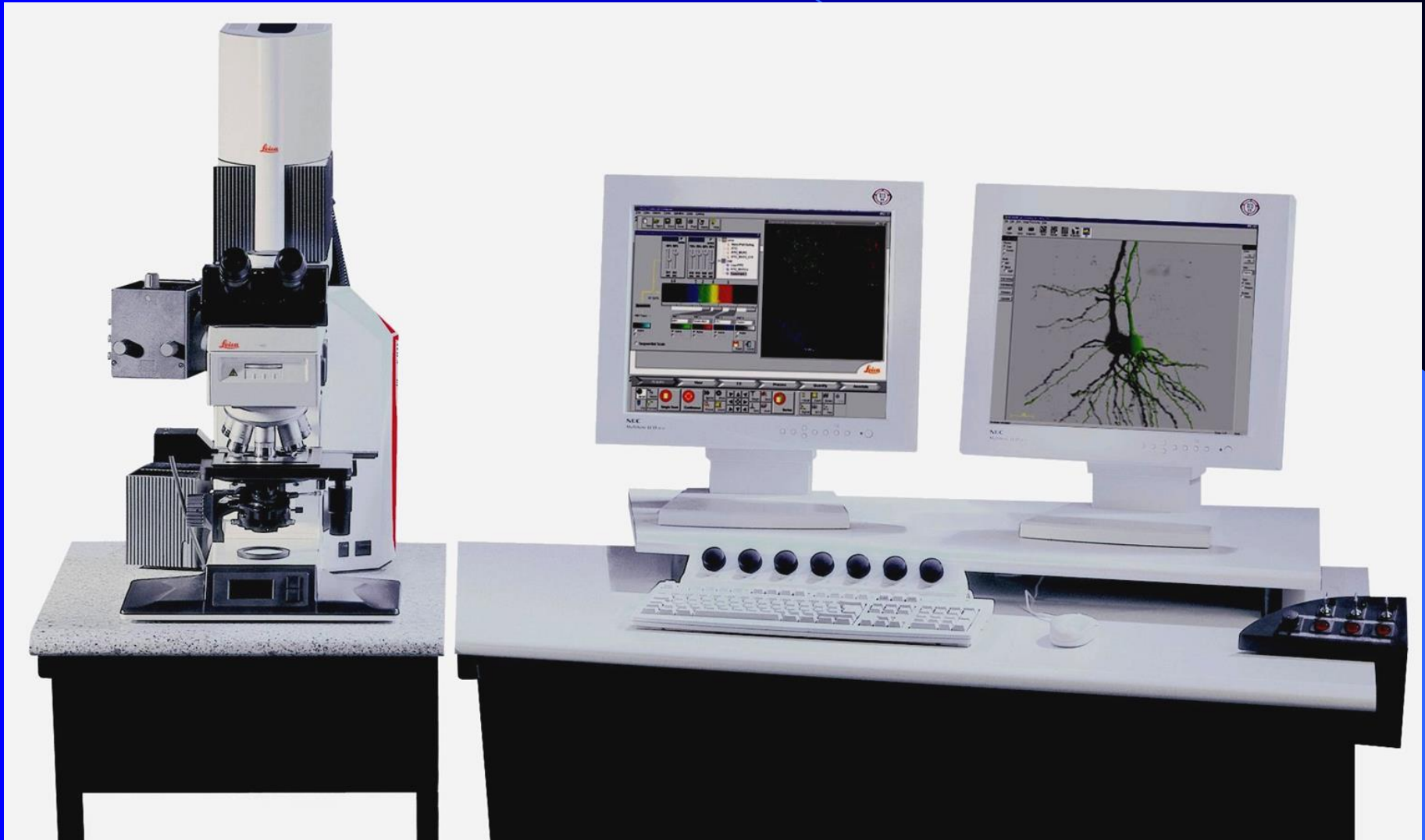
Functions:

1. Provide non-invasive ways to observe and measure the *in situ* behavior of gene products.
2. Analysis of the dynamics of proteins association/dissociations at cellular structures.

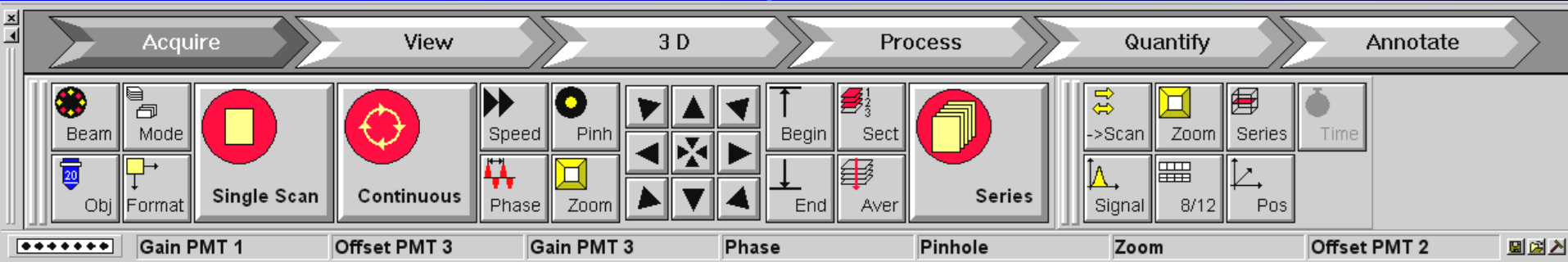


Leica TCS SP2

Confocal Spectral Microscope (UV-VIS)



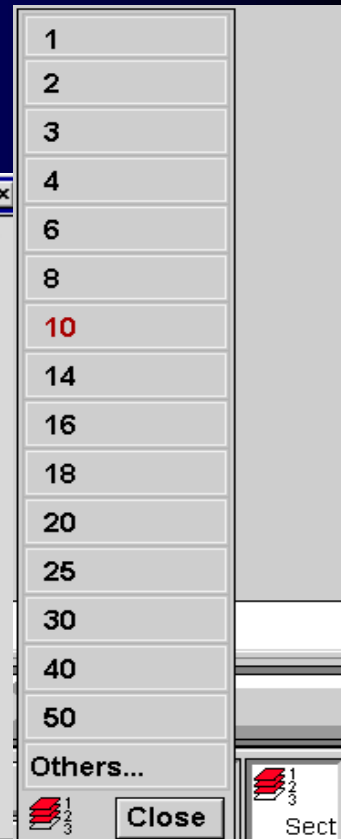
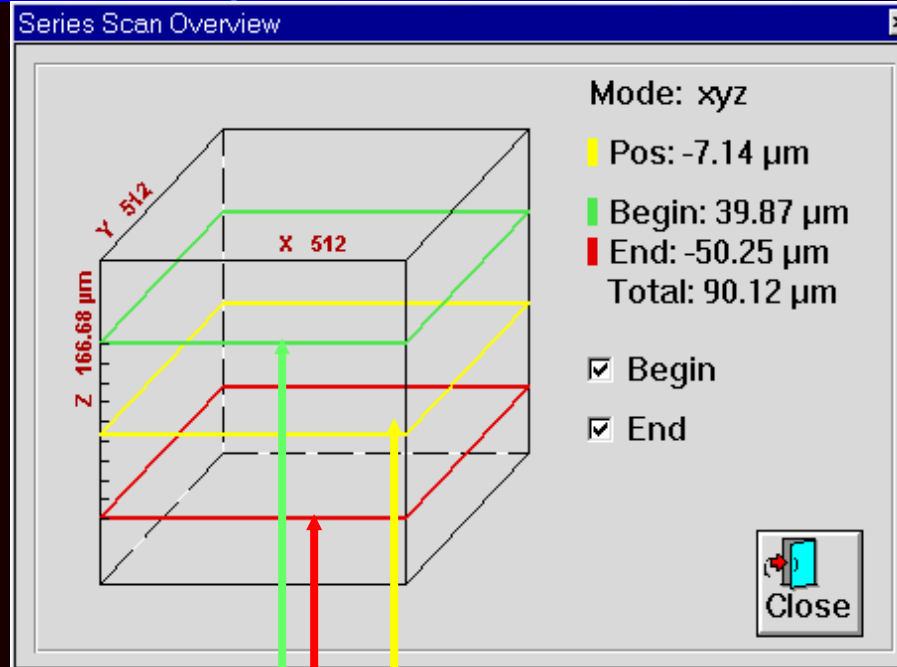
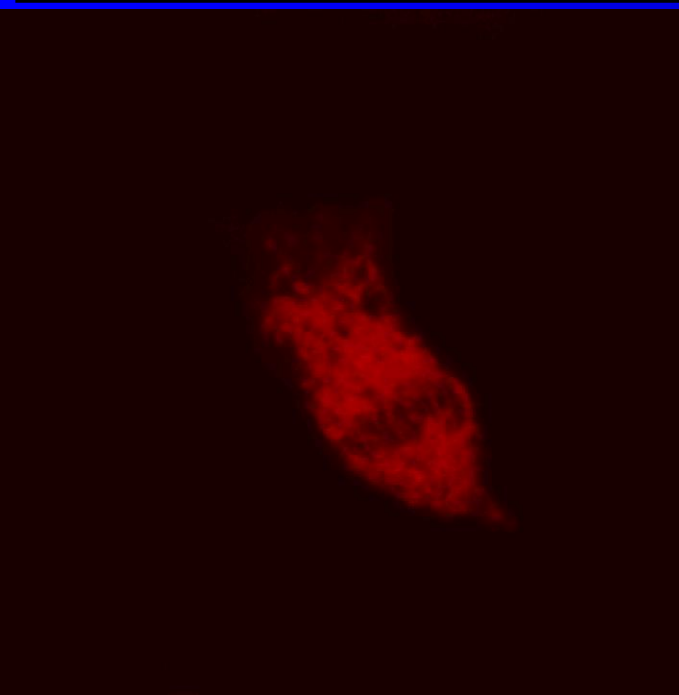
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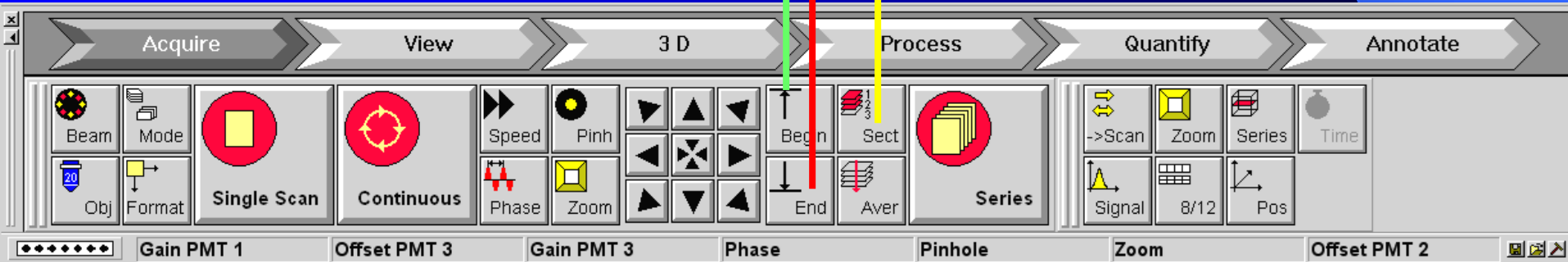
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xzy	An image stack is recorded from xz-sections in y-direction.
xt	A line is recorded several successive times.
xyt	An xy-section is recorded several successive times.
xzt	An xz-section is recorded several successive times.
xyzt	An image stack is recorded 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

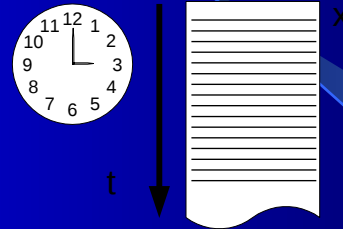


Number of optic sections

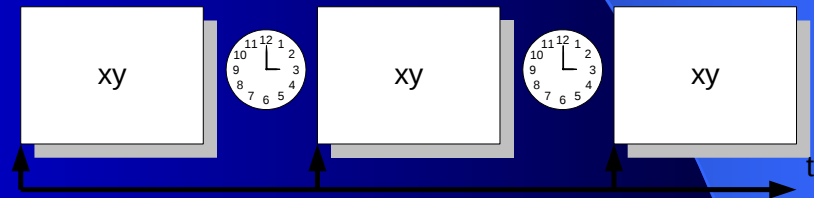


Time-Series

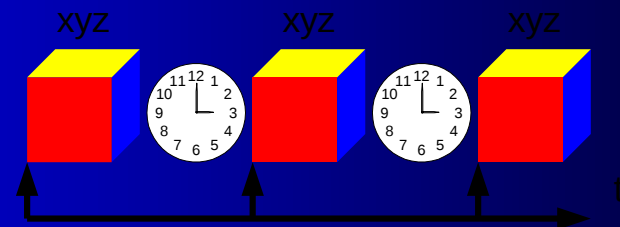
- Line-Mode „xt“



- Frame-Mode „xyt“



- Stack-Mode „xyzt“



Frame-Mode xyt Configuration

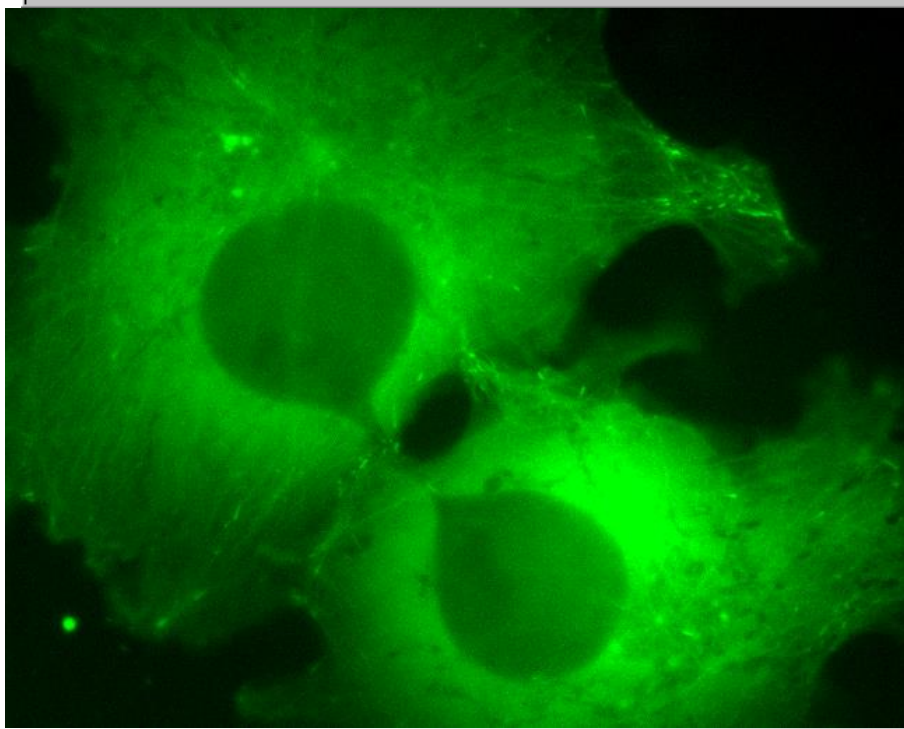
Time-lapse vs. Real Time (movie)

T Configuration

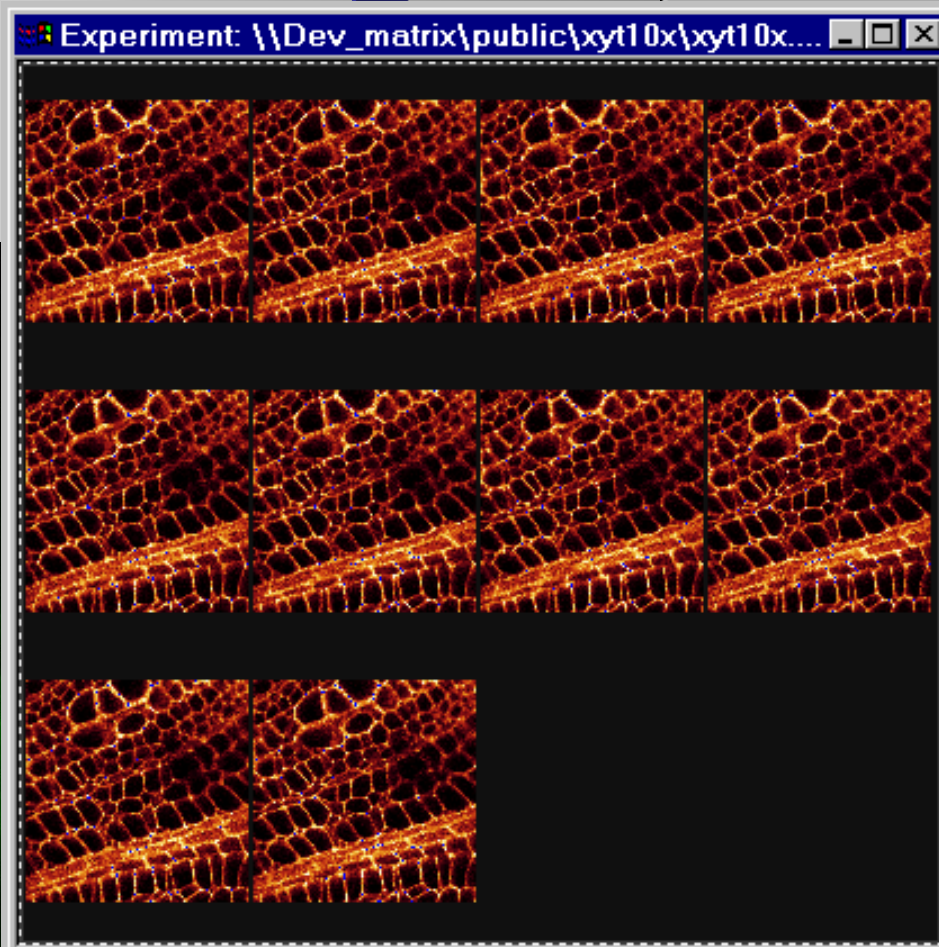
☐ ΔT : h min s ms

☐ Frames:

☒ Complete Time: h min s ms



64 * 64
100 * 100
200 * 200
512 * 32
512 * 512
640 * 512
1024 * 1024

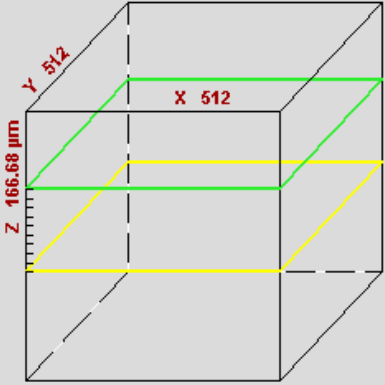


Stack-Mode xyzt Configuration

Series Scan Overview

64 * 64
100 * 100
200 * 200
512 * 32
512 * 512
640 * 512
1024 * 1024
2048 * 2048
4096 * 4096

Close



Mode: xyzt

Pos: -16.34 μm

Begin: 34.78 μm

End: -16.51 μm

Total: 51.29 μm

☒ Begin

☒ End

Close

Others...

Close

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

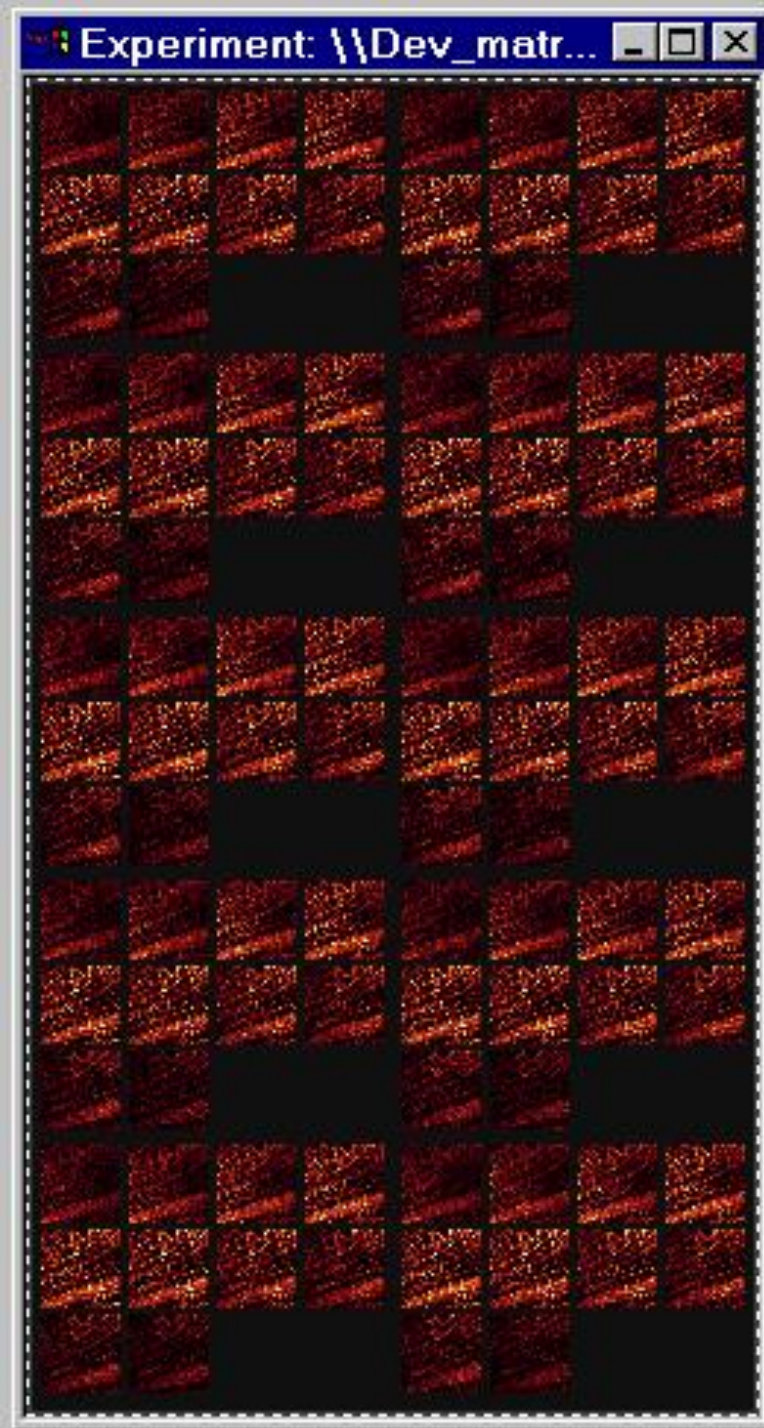
T Configuration

☐ ΔT: 0 h 1 min 0 s 0 ms

☐ Stacks: 10

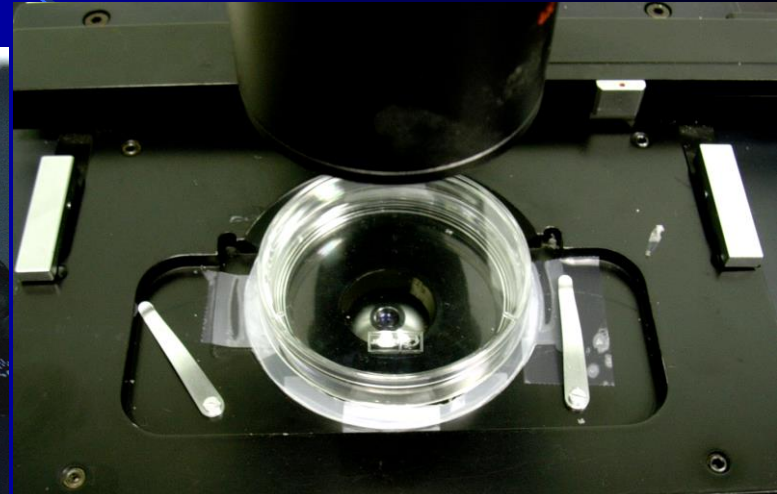
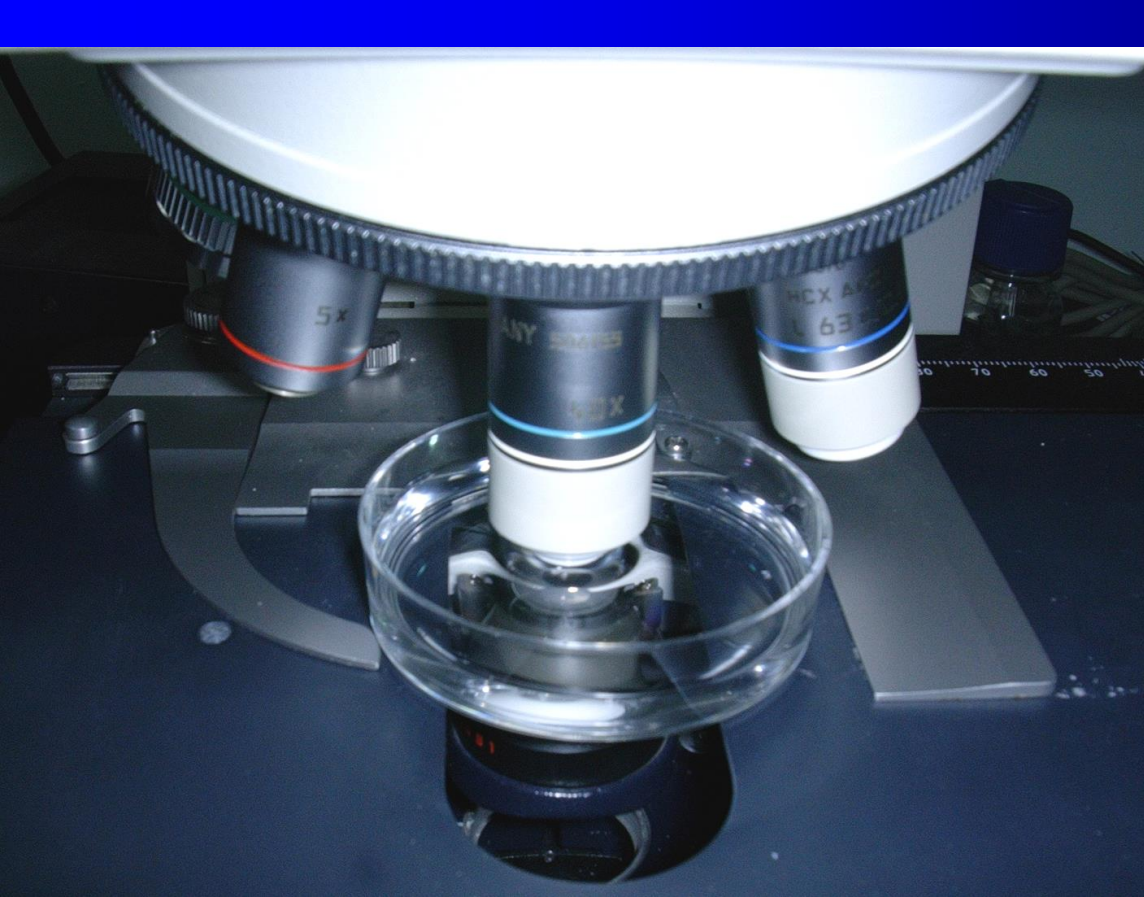
☒ Complete Time: 0 h 9 min 0 s 0 ms

Reset Apply Close



Traditional Live Cell Observation

Up-right microscope with Water Lens or Inverted microscope



Computerized Fluorescence Inverted Microscope

Leica DM IRE 2 HC



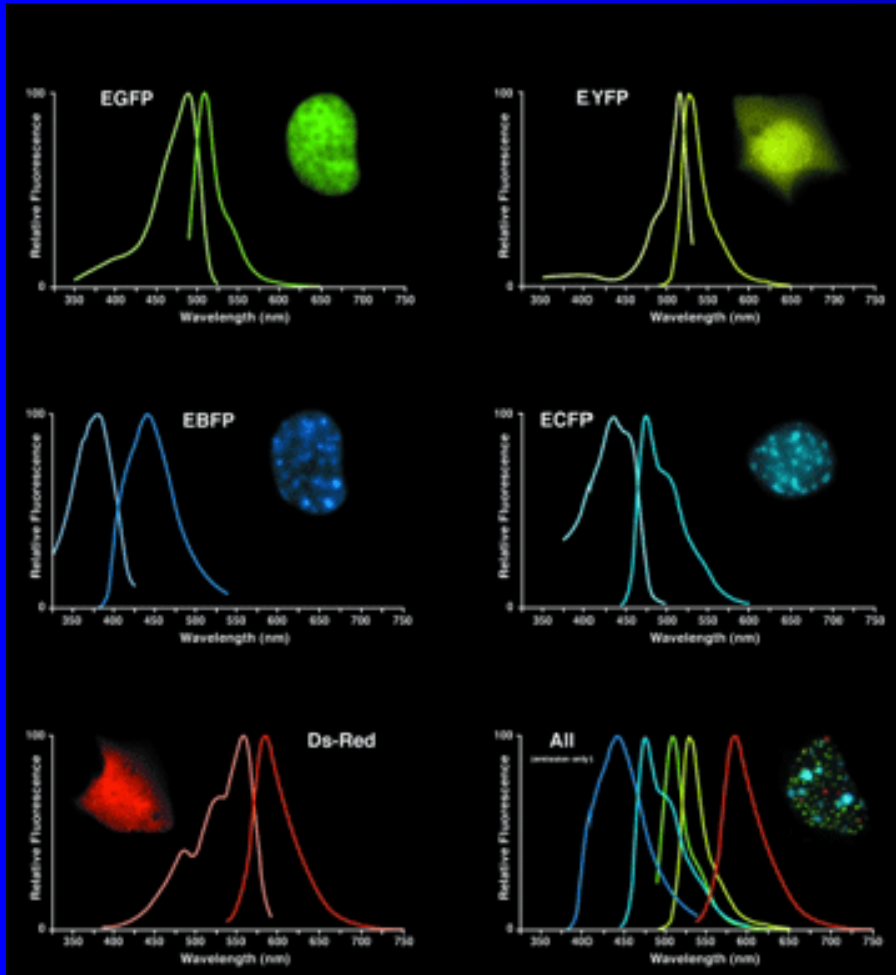
**Universal Microscope Controller
with
Remote Control Knob**

Objectives :
The best axial and lateral resolution
Optimized correction for cell-imaging

HCX PL-APO 10x/0.40 Ph 1
HCX N Plan L20x/0.40 Ph 1, 0-2 mm corr
HCX PL-Fluotar L40x/0.60 Ph 2, 0-2 mm corr
HCX PL-APO 100x/1.35 OIL Ph 3

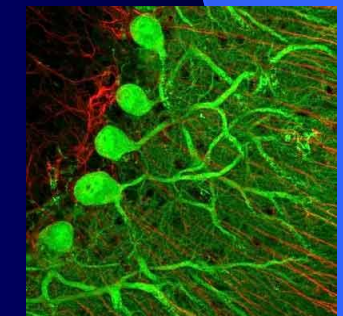
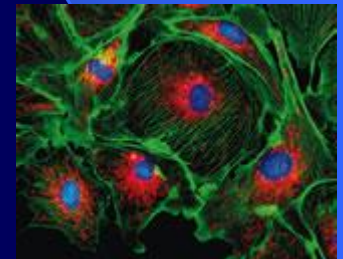
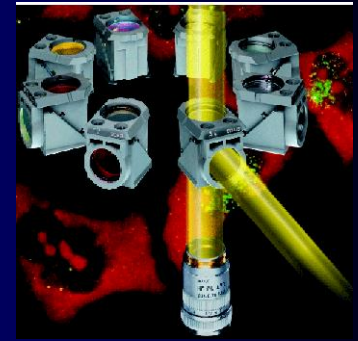
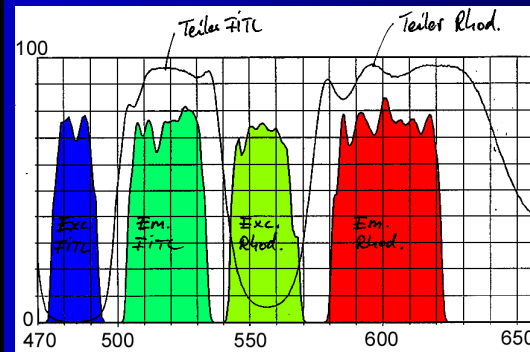
Computerized Fluorescence Inverted Microscope

Fluorescence Filters



Built-in Four Microscope Filters

- **GFP**
- **CFP**
- **YFP**
- **DsRed**



Leica DM IRE2 microscope

enclosed within a computerized CO₂-incubator for indispensable thermal and mechanical stability



CO₂ controller

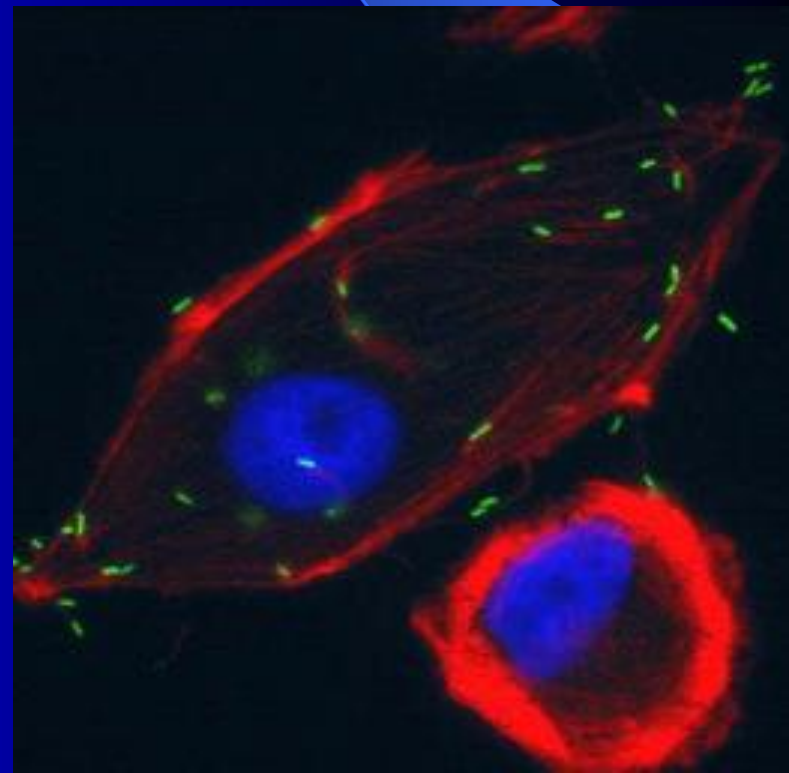
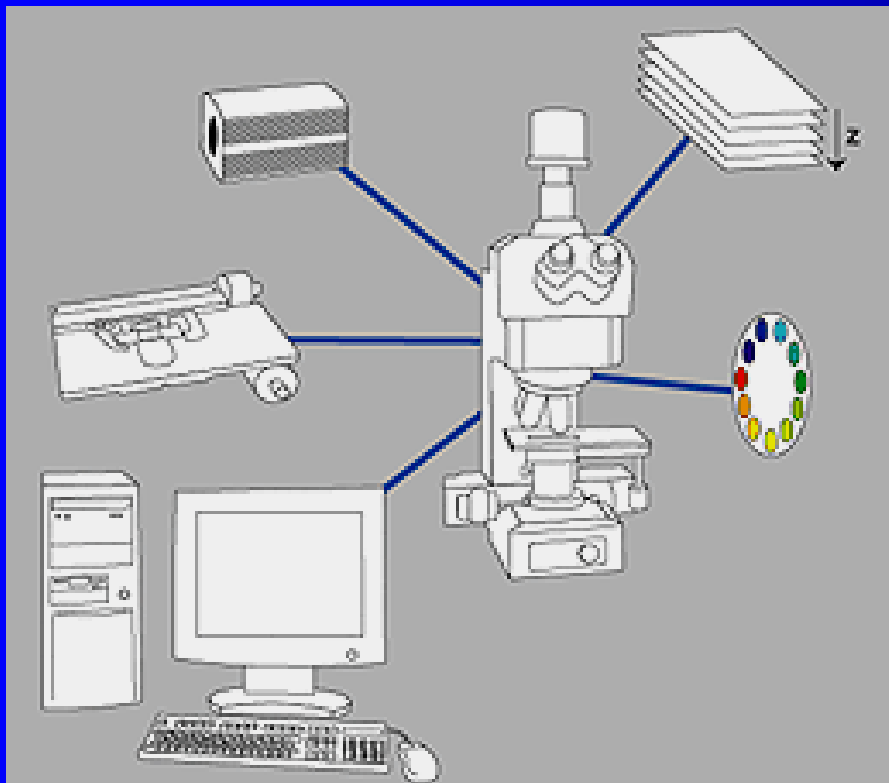
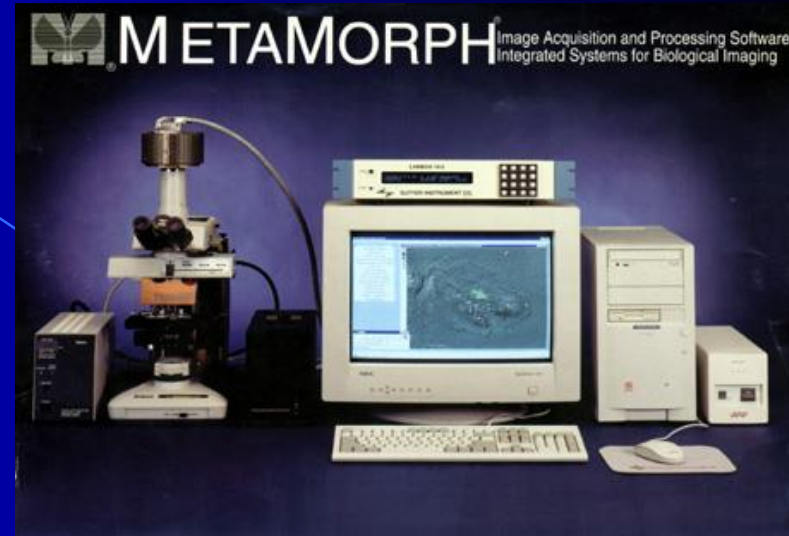


**Microincubation
Imaging-Chamber**

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



雷射掃描光譜共軛焦顯微鏡核心實驗室

Core Laboratory for Confocal Scanning Microscope

<http://host.cc.ntu.edu.tw/biotech/core3-1.htm>

1. the manual for confocal microscopes
2. protocols for immunocytochemistry and cell staining
3. Service regulations

臺灣大學基因體醫學研究中心：教學資源中心

<http://www.cgm.ntu.edu.tw/chinese/9/index.html>

1. Training Workshops
2. Vectors
3. Virtual Cell Bank

本中心支援雷射掃描光譜共軛焦顯微鏡核心實驗室

共軛焦顯微鏡核心實驗室自90年啟用服務之至今，共舉辦十一次應用講習與操作實習會。共計已有798名研究人員參與學習操作。

應用講習與操作實習會	參與人數
第一次講習會（90年4月）	66
第二次講習會（90年10月）	63
第三次講習會（91年4月）	63
第四次講習會（91年10月）	64
第五次講習會（92年4月）	60
第六次講習會（92年10月）	69
第七次講習會（93年4月）	85
第八次講習會（93年10月）	79
第九次講習會（94年4月）	84
第十次講習會（94年10月）	115
第十一次講習會（94年4月）	50



教學資源中心服務記錄表(至95年9月總計 149人次)：

系所單位	主持人	提供物品明細
生命科學院分細所	吳益群	pGolgi-CFP vector
生命科學院分細所	周子賓	pDsRed monomer N-1
工學院醫工所	楊台鴻	H33342 dye, pEYFP-tubulin
工學院醫工所	林峰輝	FITC/TRITC 2nd Antibodies
皮膚科	廖怡華	Neuro2A cell line
醫技所	林淑華	pPNT, pEYFP, pECFP vectors
病理科	黃佩欣	pEYFP, pECFP vectors
眼科	王一中	Golgi-CFP vector
口生所	張百恩	pTimer vector
生化所	張富雄	pDesRed2 vector
病理科	許輝吉	pEYFP-tubulin vector
分醫所	呂勝春	pEYFP-tubulin vector
生理學科	湯志永	pGolgi-CFP vector
生化所	張智芬	pEYFP-tubulin vector
藥學系	楊雅雯	pEYFP, pECFP vectors
生化所	林榮耀	pEYFP-tubulin vector
內科	楊泮池	pEYFP-actin vector