

Gene Manipulation for Bio-systems

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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

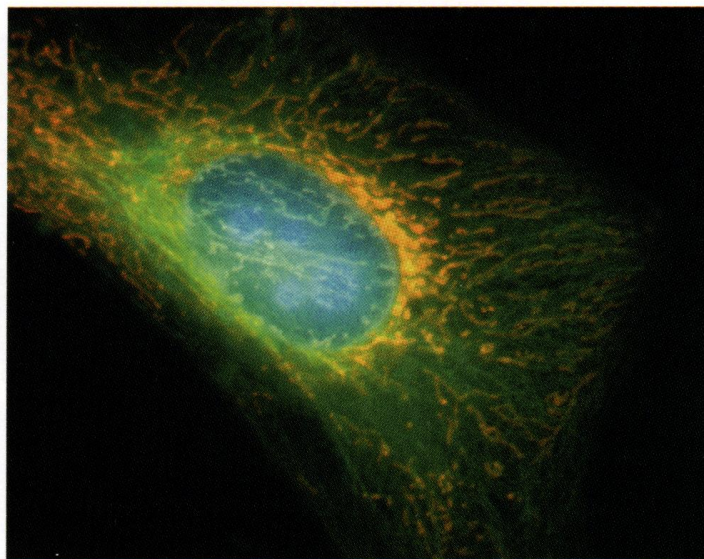


Figure 2. Triple labeling with DsRed1, ECFP, and EYFP. HeLa cells were transiently transfected with pECFP-Nuc (#6904-1), pEYFP-Tub (#6118-1), and pDsRed1-Mito (n/a), which label the nucleus, tubulin, and mitochondria, respectively. The cells were incubated at 37°C for 48 hr, fixed in 3.7% formaldehyde 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.

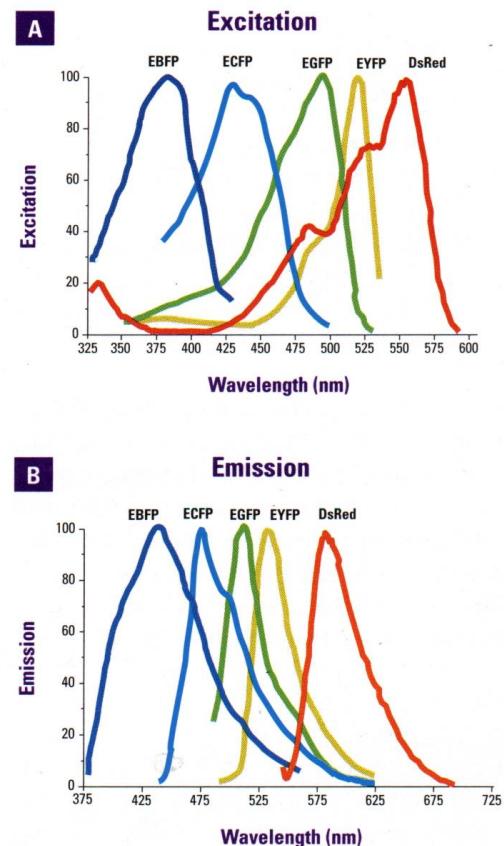


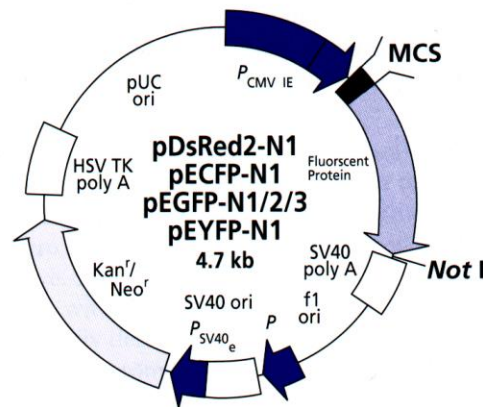
Figure 3. Excitation and emission spectra for all Living Colors® fluorescent proteins.

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

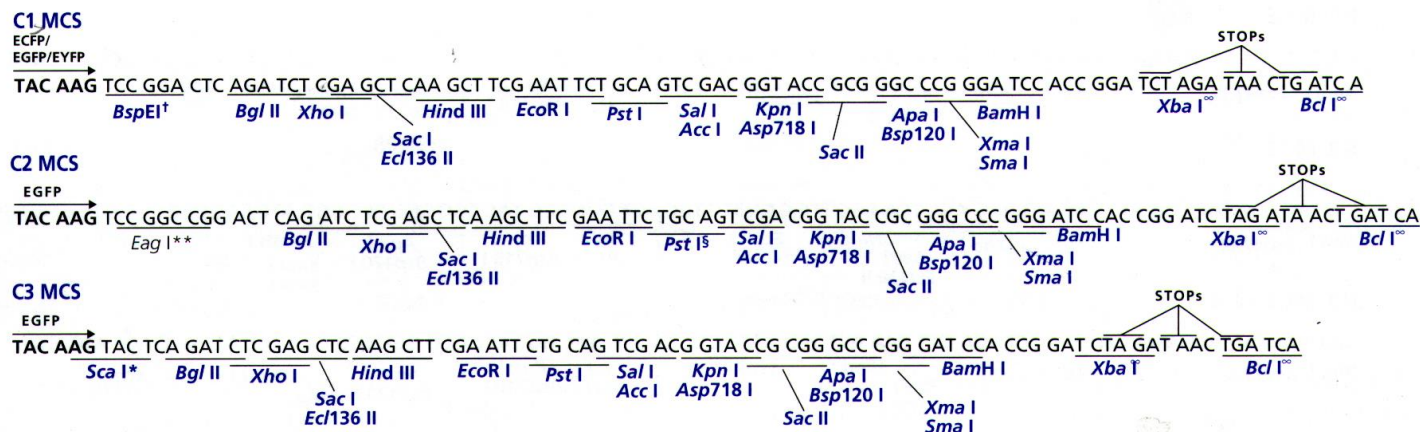
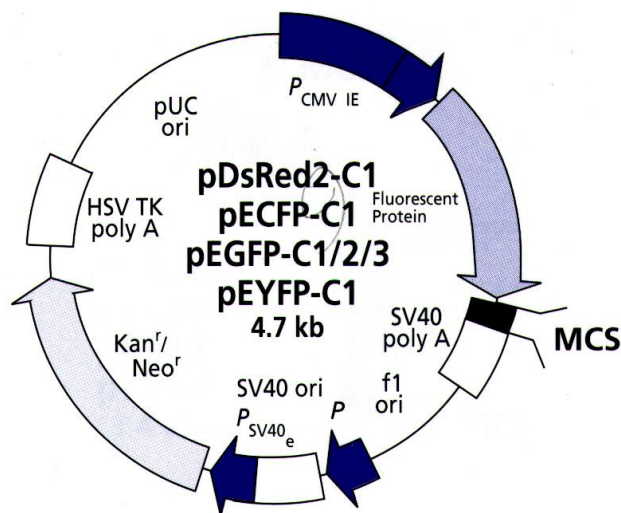
N3 MCS

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

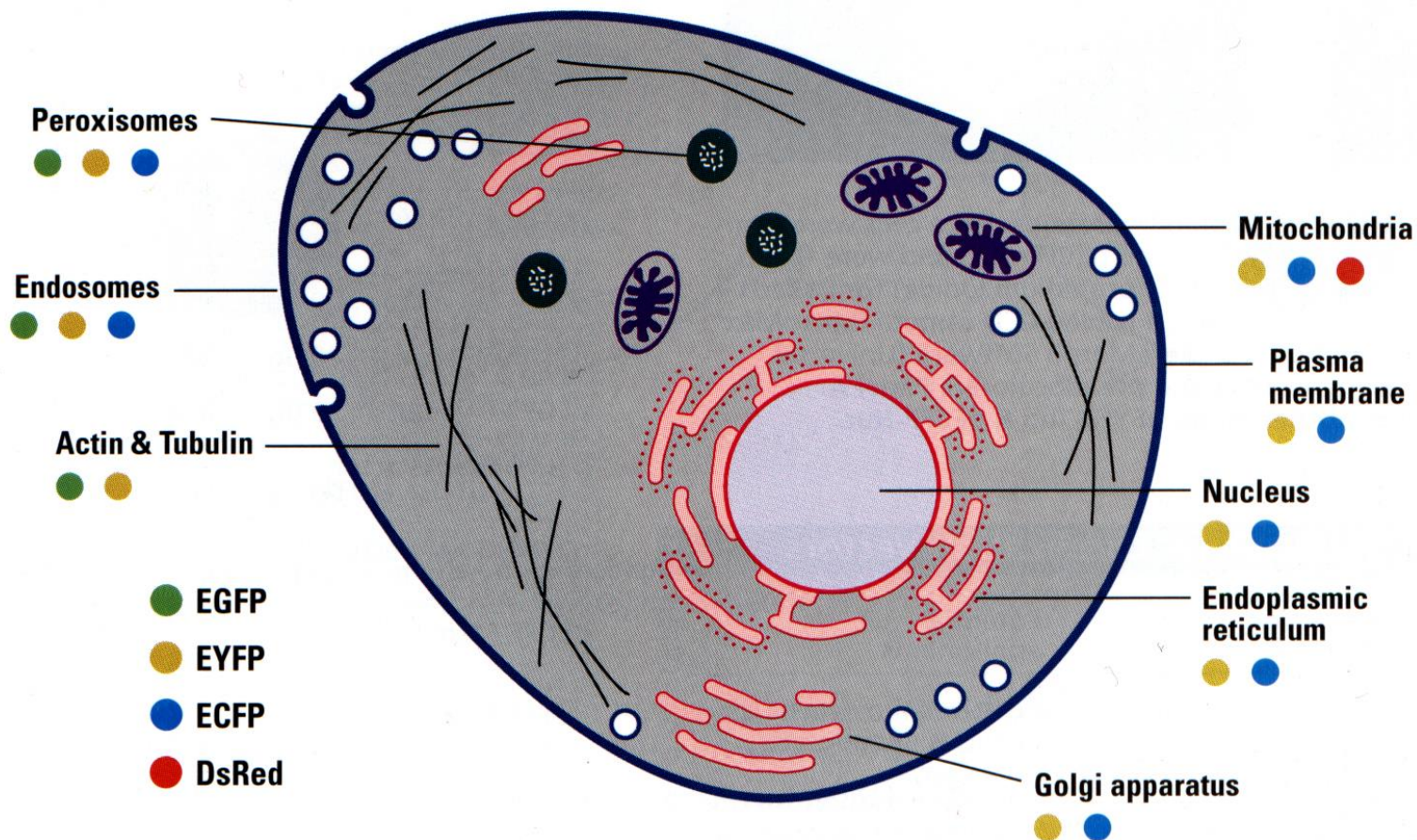


Figure 1. Organelles targeted by Living Colors™ Subcellular Localization Vectors.

Living Colors™ Subcellular Localization Vectors continued

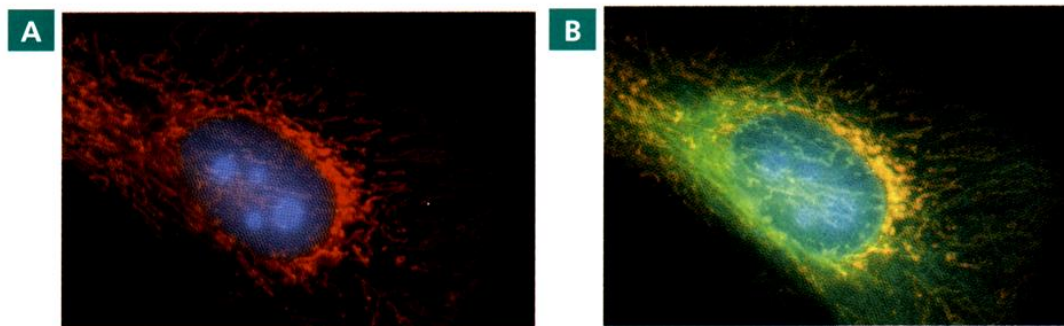
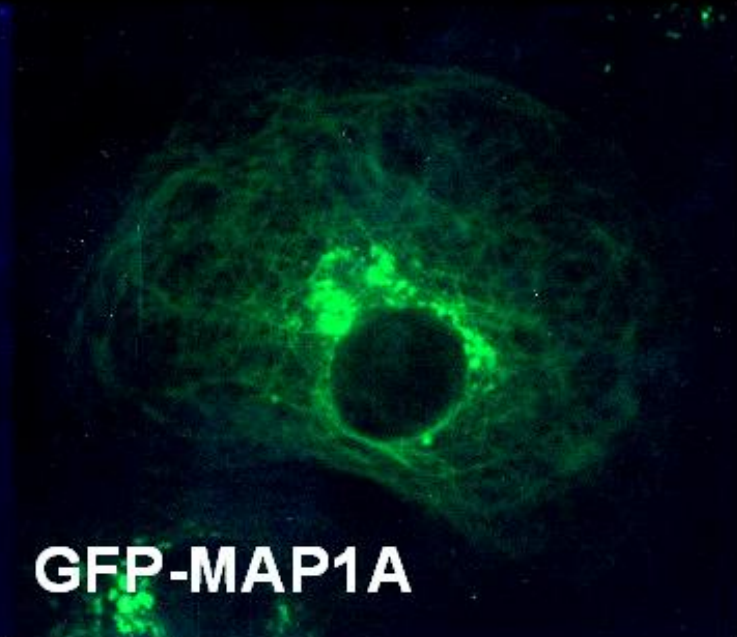
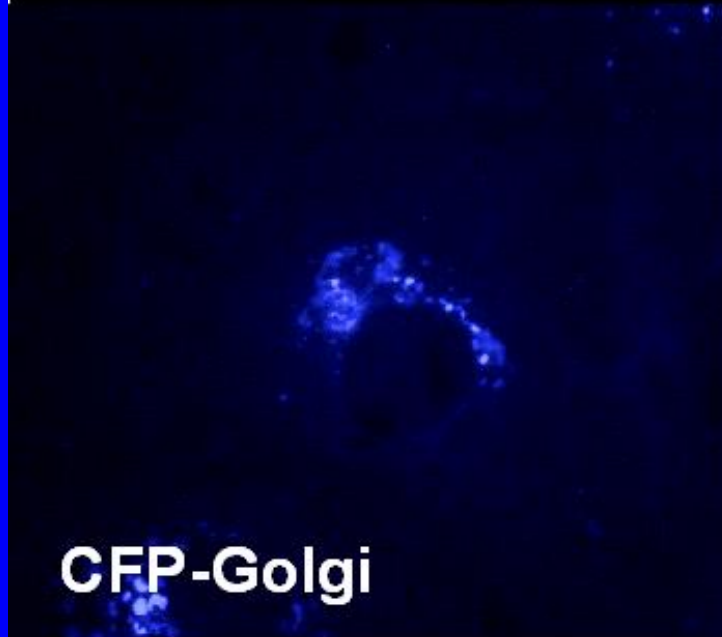
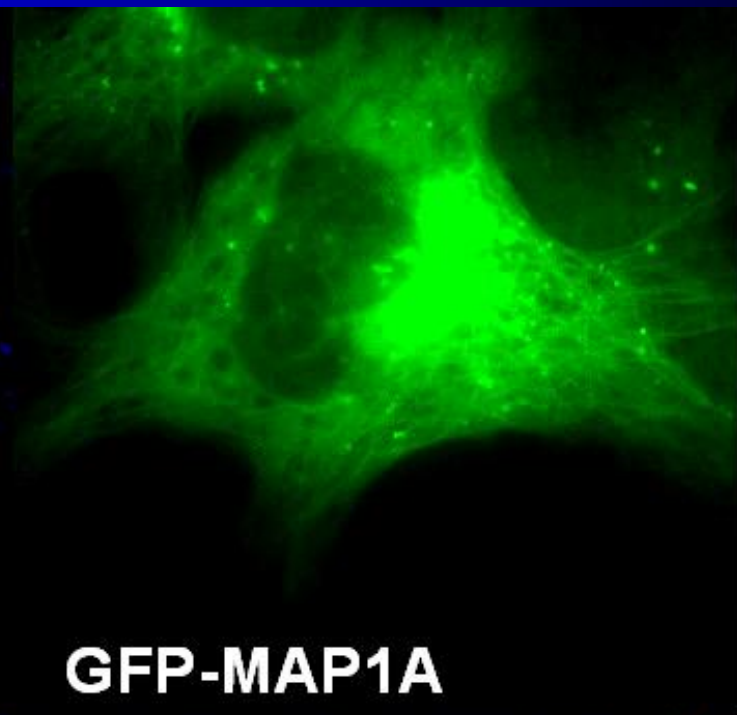
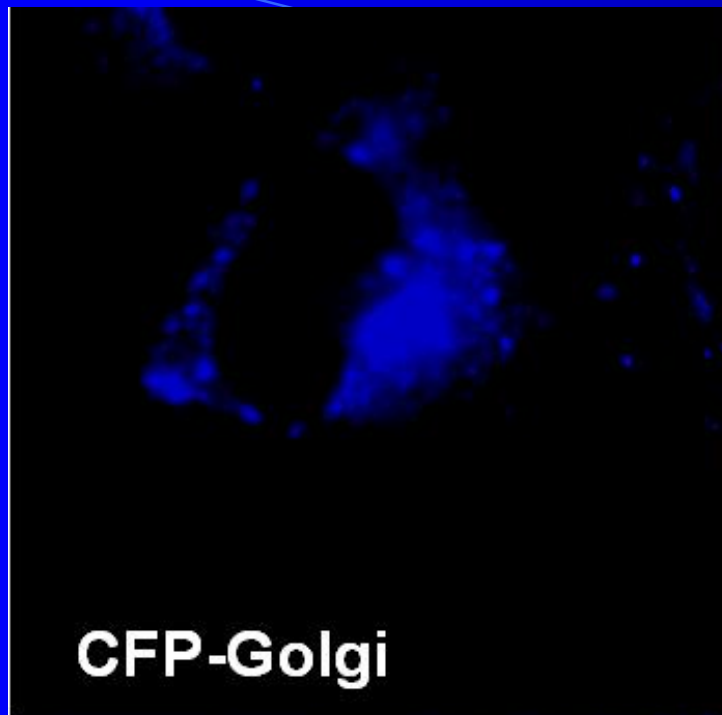


Figure 2. Dual and triple labeling with Living Colors™ proteins. HeLa cells were transiently transfected with pDsRed1-Mito, pEYFP-Tub, and pECFP-Nuc, and were fixed. The images were taken using Omega filter sets XF35 (propidium iodide) for DsRed1-Mito, XF104 for EYFP-Tub, and XF114 for ECFP-Nuc; a cooled 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.

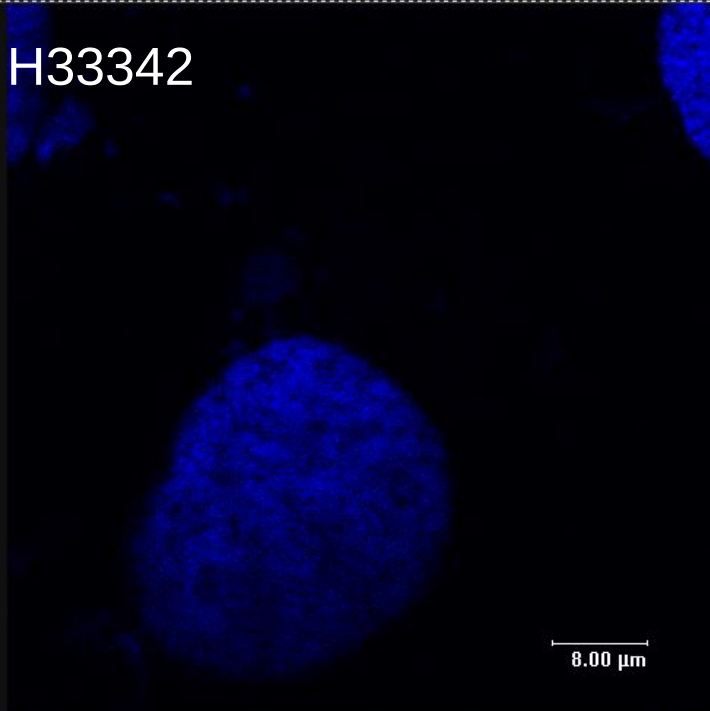
Table I: Living Colors™ Subcellular Localization Vectors

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

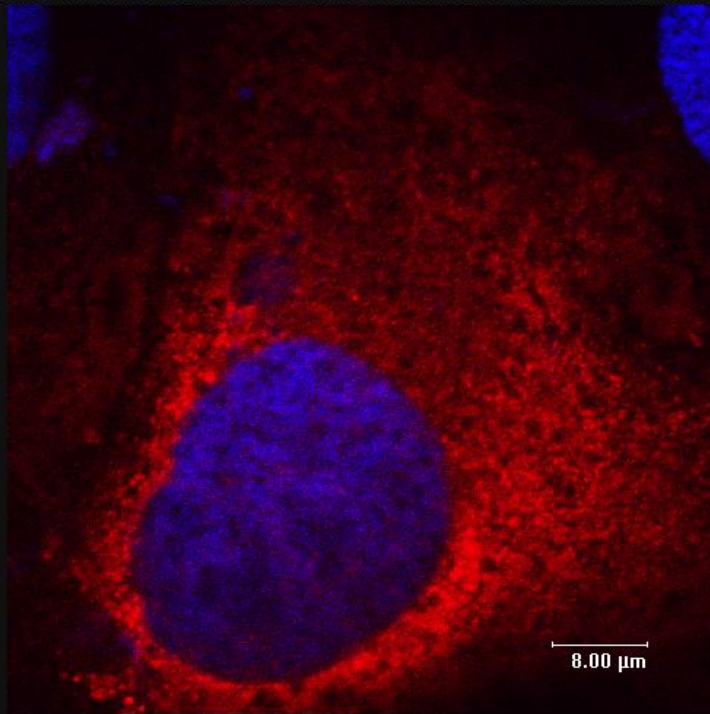
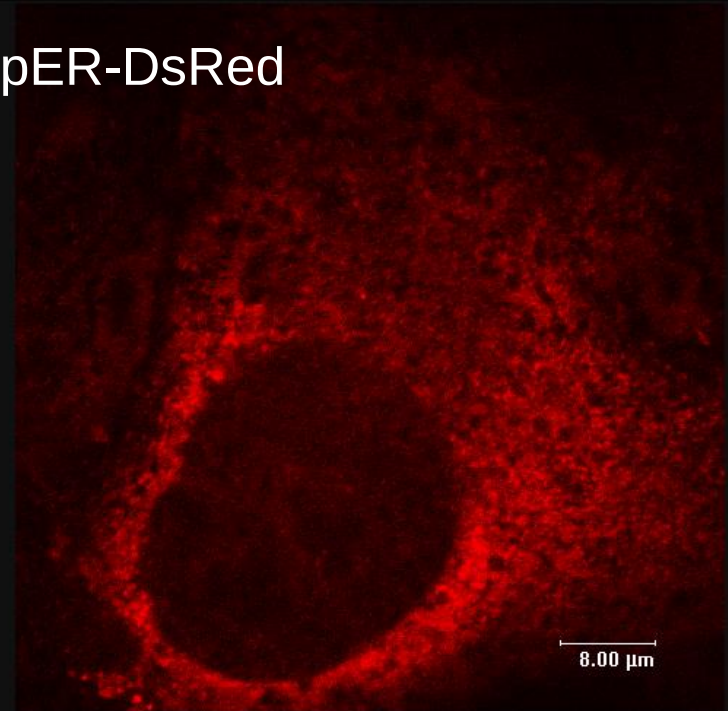
Product	Size	Cat. #
pEGFP-Actin Vector	20 µg	6116-1
pEYFP-Actin Vector	20 µg	6902-1
pECFP-Endo Vector	20 µg	6934-1
pEGFP-Endo Vector	20 µg	6935-1
pEYFP-Endo Vector	20 µg	6936-1
pECFP-ER Vector	20 µg	6907-1
pEYFP-ER Vector	20 µg	6906-1
pEGFP-F Vector	20 µg	6074-1
pECFP-Golgi Vector	20 µg	6908-1
pEYFP-Golgi 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



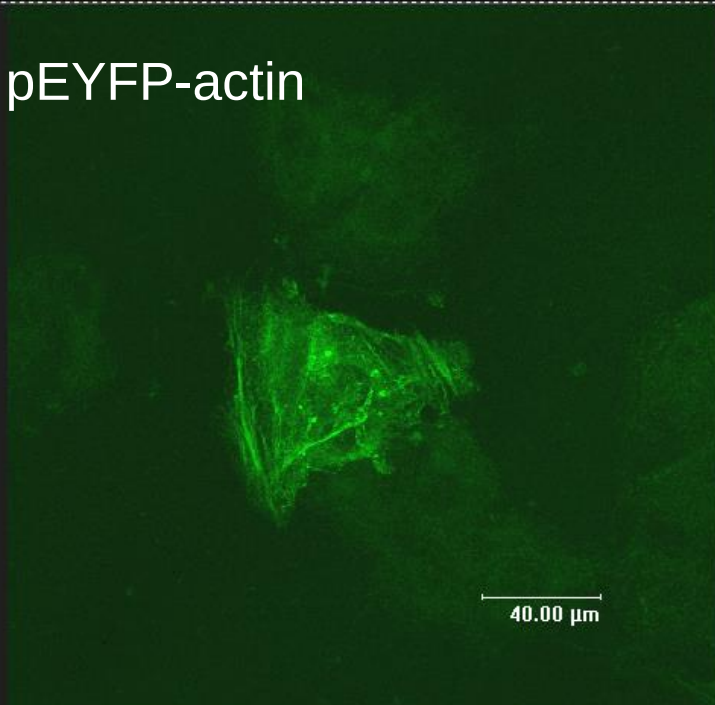
H333342



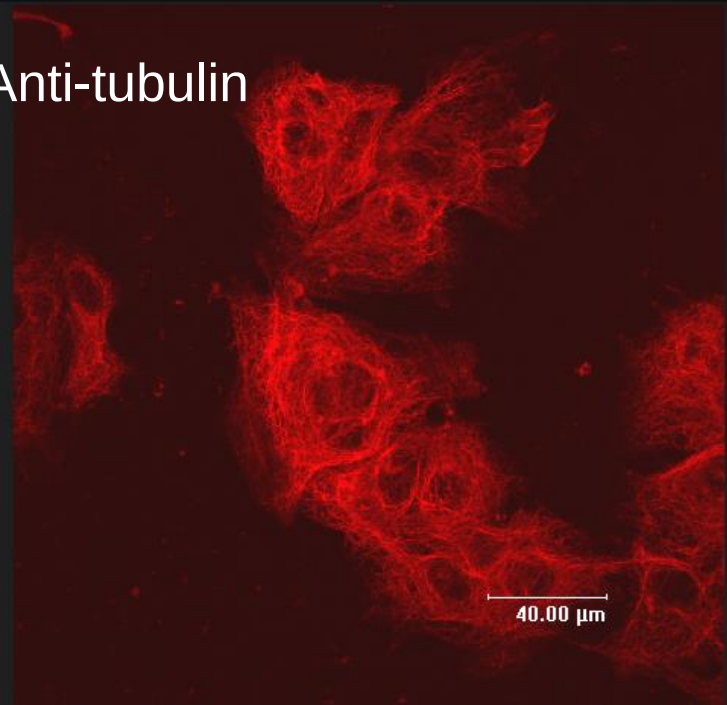
pER-DsRed



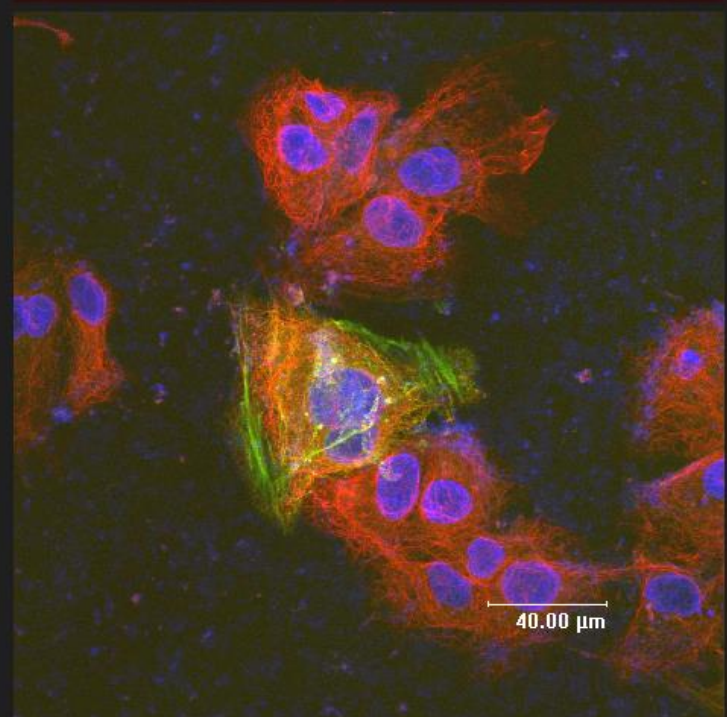
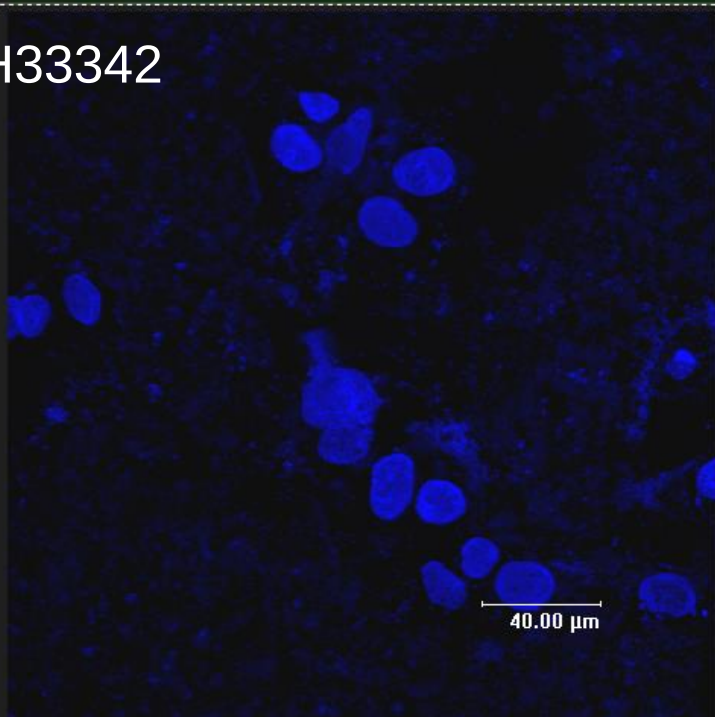
pEYFP-actin



Anti-tubulin



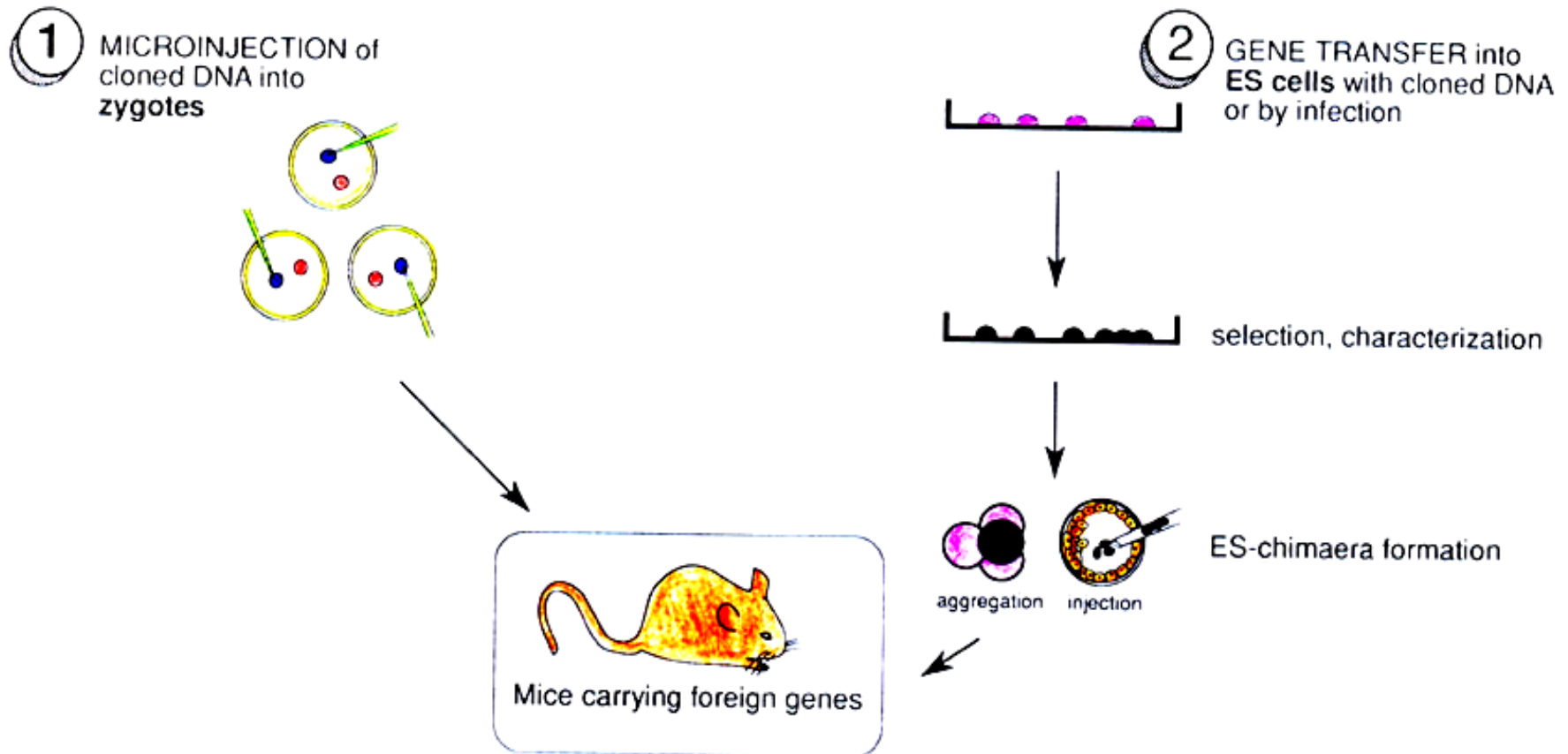
H33342



Fluorescent Organisms:

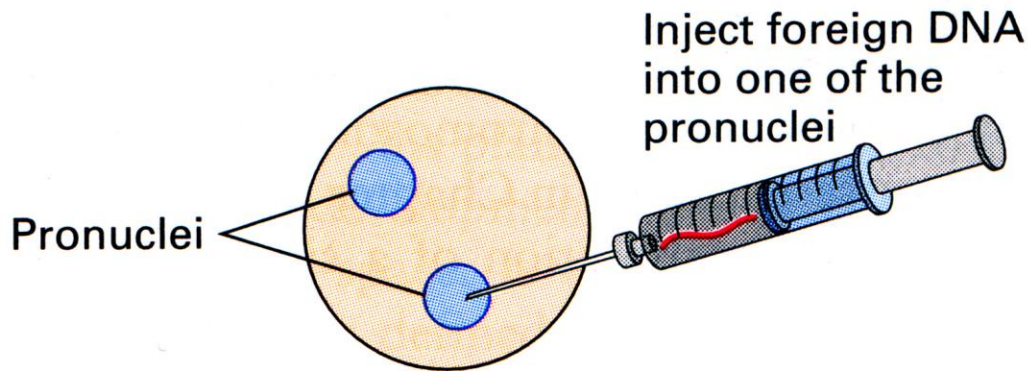
Transgenic GFP mouse

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



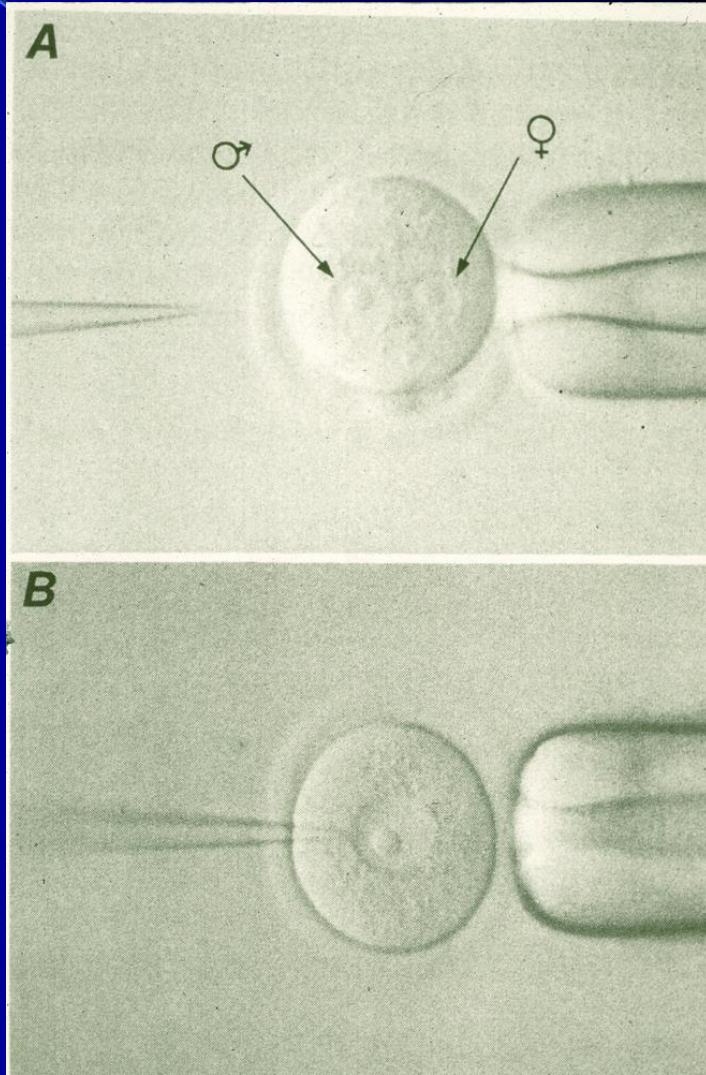
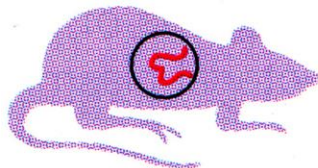
傳統基因轉殖：

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



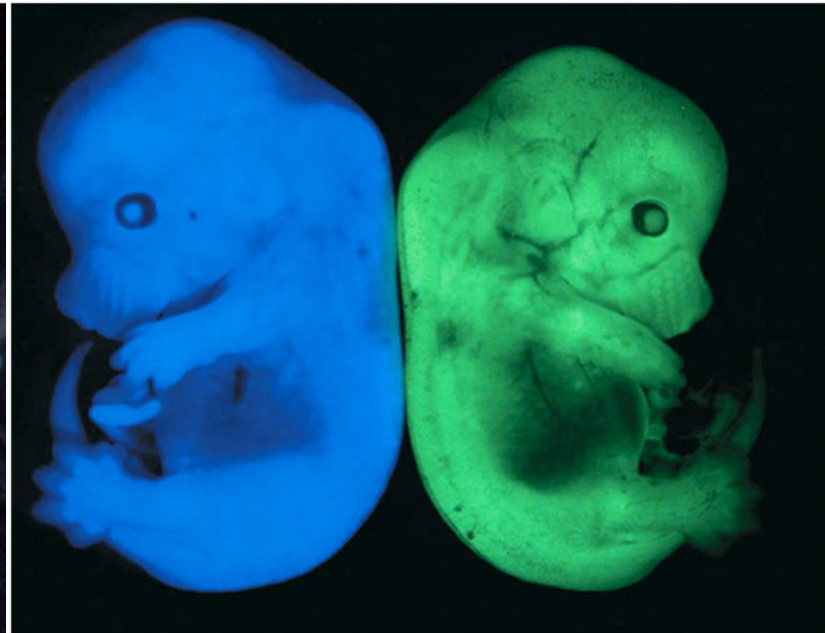
Transgenic GFP mouse

(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亮的飼養觀賞魚，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！

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

山常識

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。

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

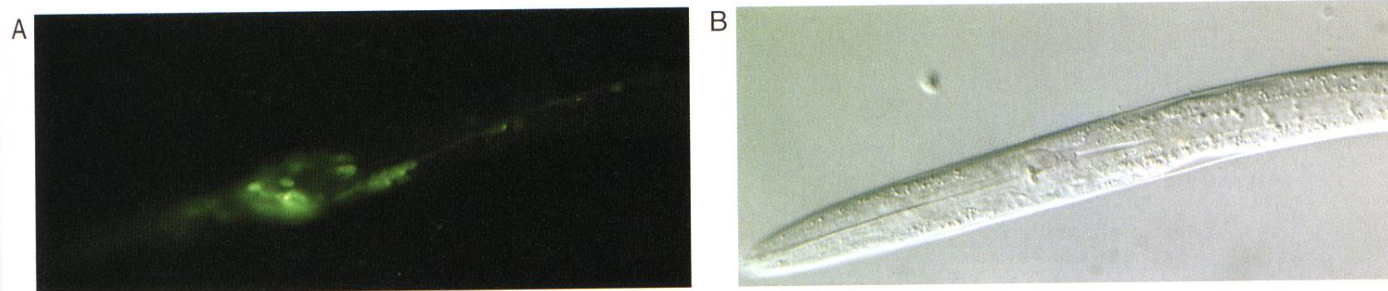
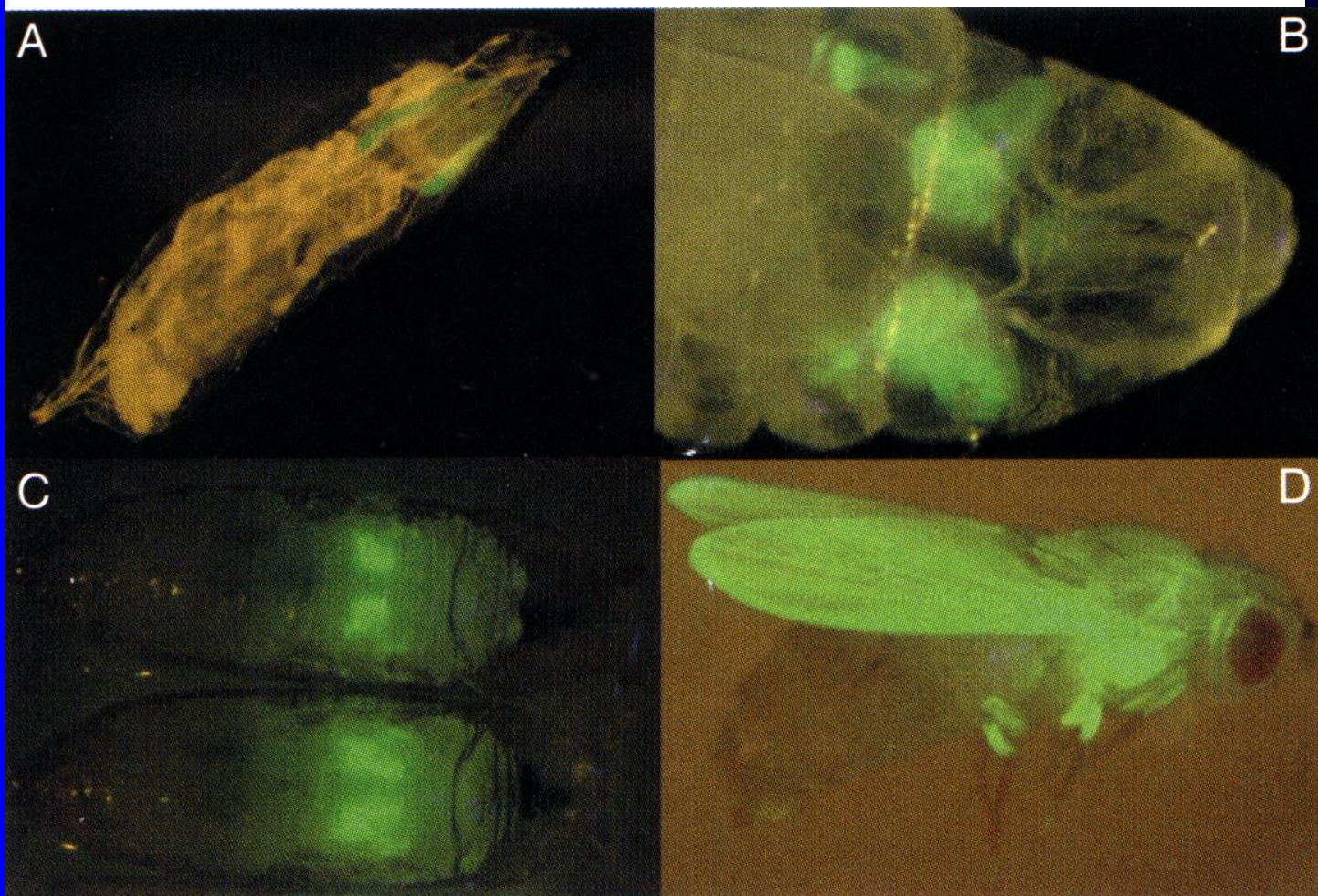
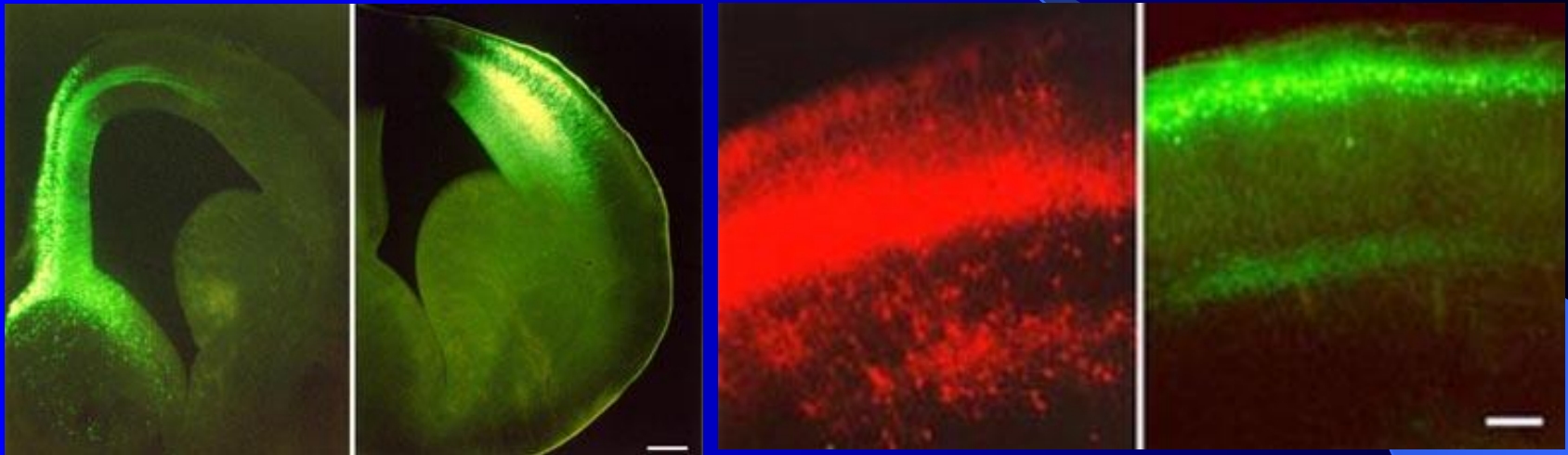


図1 線虫頭部におけるUNC-18/EGFP融合タンパク質の蛍光／微分干渉観察像
A：蛍光観察像，B：Aと同一視野の微分干渉観察像。



In vivo electroporation

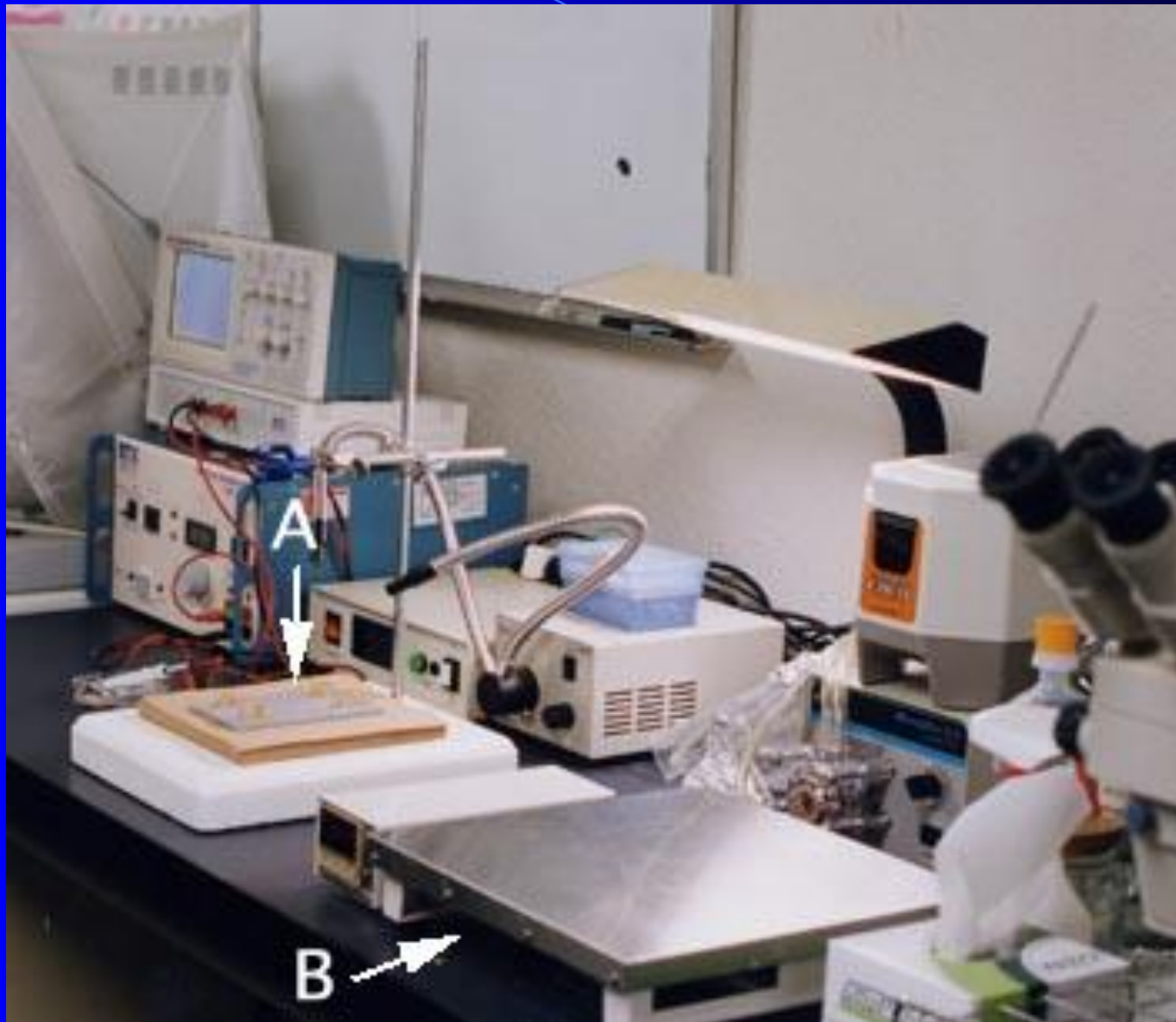
Effective gene transfer into the embryonic mouse brain using *in vivo* electroporation (Developmental Biology 240:230-246, 2001)



http://www.frontier.kyoto-u.ac.jp/rc01/in_vivo_electroporation.html

- Transfection Efficiency
- Apparatus
- Procedures

In vivo electroporation •Apparatus



In vivo electroporation

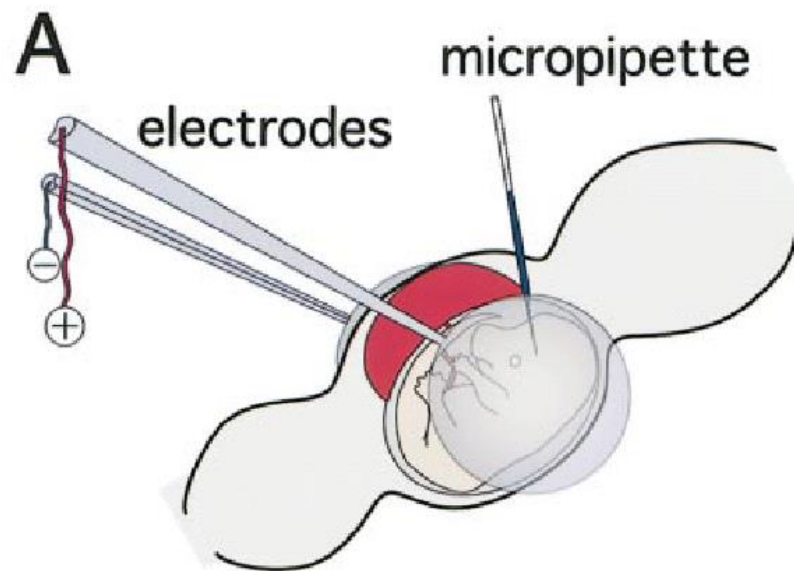
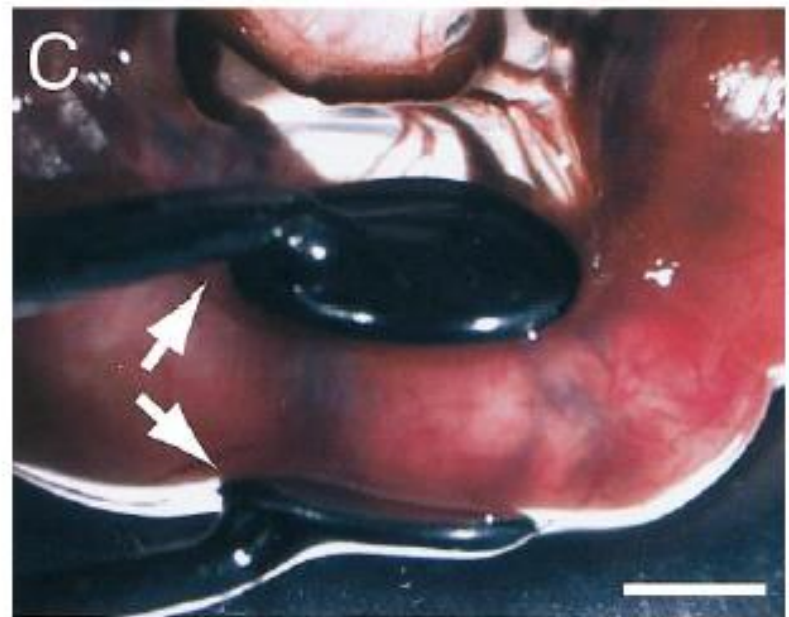


TABLE 1

Effect of Voltage and Pulse Numbers on Survival and EYFP-Positive Rate

Voltage (V)	No. of pulses	Embryo survival (%)	EYFP ⁺ embryos (%)	No. of operated embryos (litters)
0	0	93.3	0	15 (1)
20	5	93.3 ± 0.0	32.2 ± 3.6	30 (2)
30	5	92.8 ± 1.1	53.9 ± 0.6	28 (2)
40	5	90.7 ± 4.7	68.5 ± 3.5	42 (3)
	8	87.5 ± 0.0	71.5 ± 7.2	32 (2)
50	3	76.1 ± 5.8	58.0 ± 4.0	43 (3)
	5	70.6 ± 3.6	84.3 ± 7.9	44 (3)
	8	61.5 ± 7.7	74.6 ± 3.2	26 (2)
60	5	63.9 ± 19.5	71.9 ± 1.7	37 (3)
70	3	58.8 ± 12.6	60.0 ± 10.0	27 (2)
	5	41.7 ± 8.4	73.4 ± 6.7	27 (2)



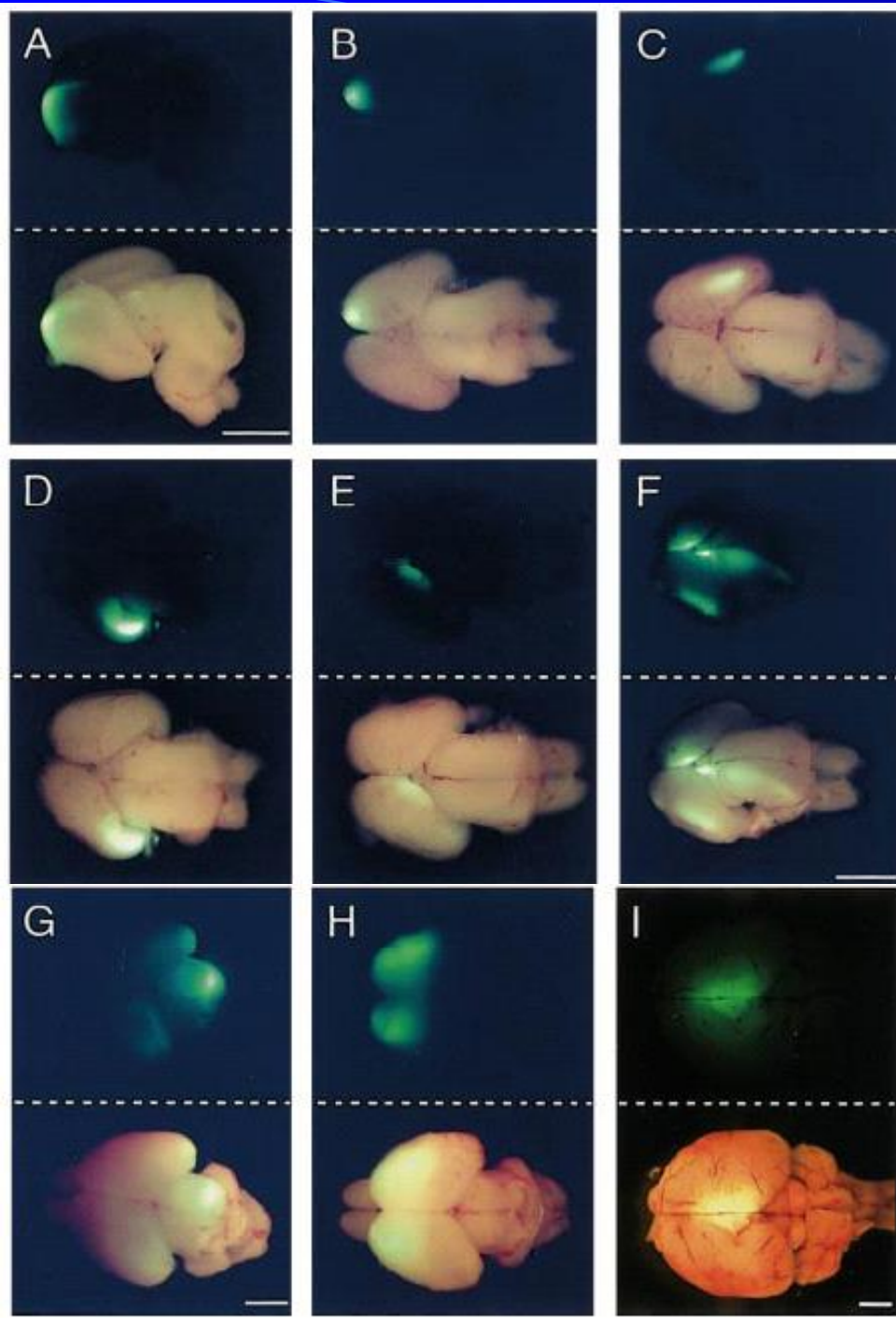


FIG. 3. *EYFP* expression in restricted regions of the brain.

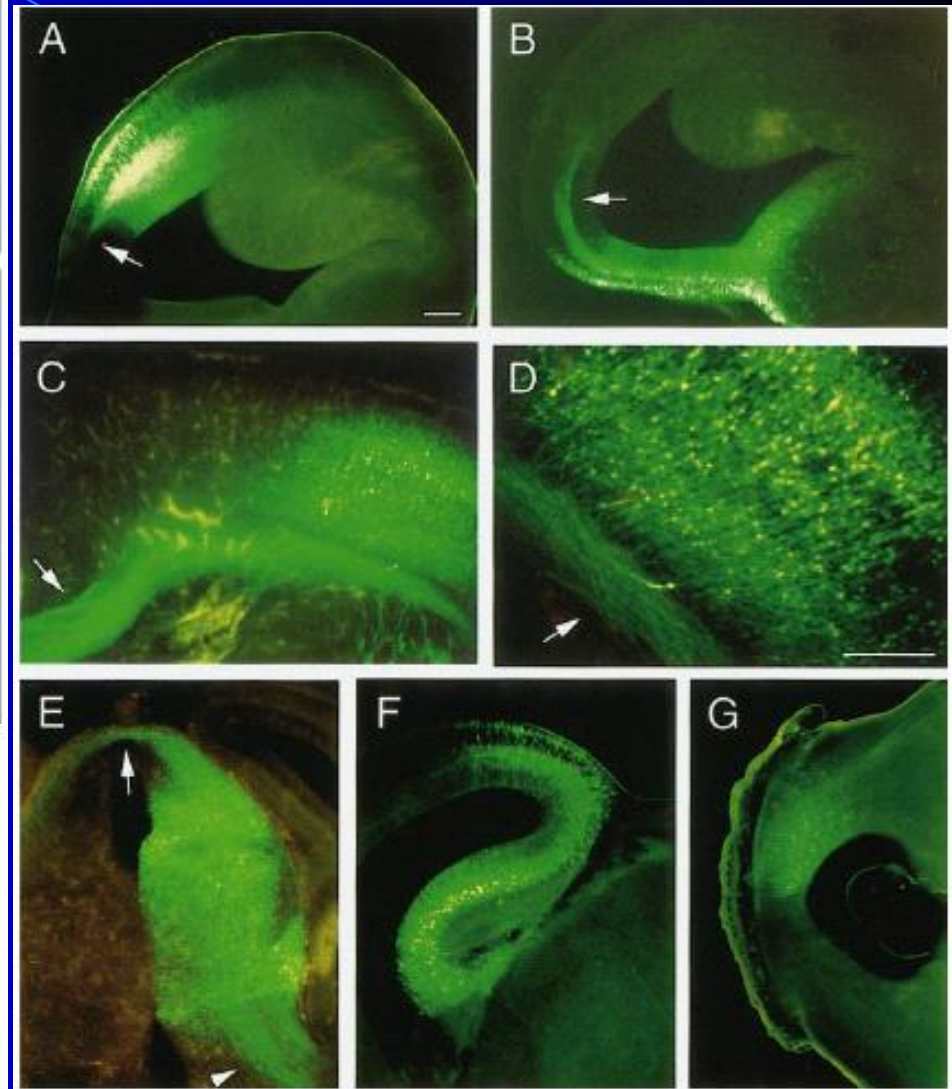


FIG. 4. *EYFP*-expressing cells in electroporated brains.

活體動物體內螢光觀察

Cell~vizio

> Molecular imaging on living animals is now possible **at the cellular level**

Mauna Kea Technologies, leader in the field of ultra-high resolution molecular imaging systems, has developed a revolutionary product - Cell~vizio™ - based on its 'Fibered Confocal Fluorescence Microscopy' (FCFM) proprietary technology. Cell~vizio is designed to visualize cellular structures in living animals and quantify molecular events. This unique imaging system is the result of technological breakthroughs in different fields leading to the **3 main elements of Cell~vizio**:

Laser Scanning Unit (LSU)

1

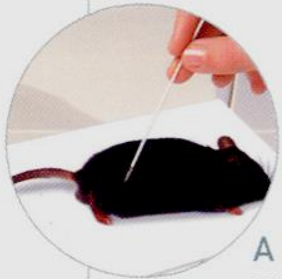
Range of ProFlex fibered imaging microprobes

2

ImageCell software

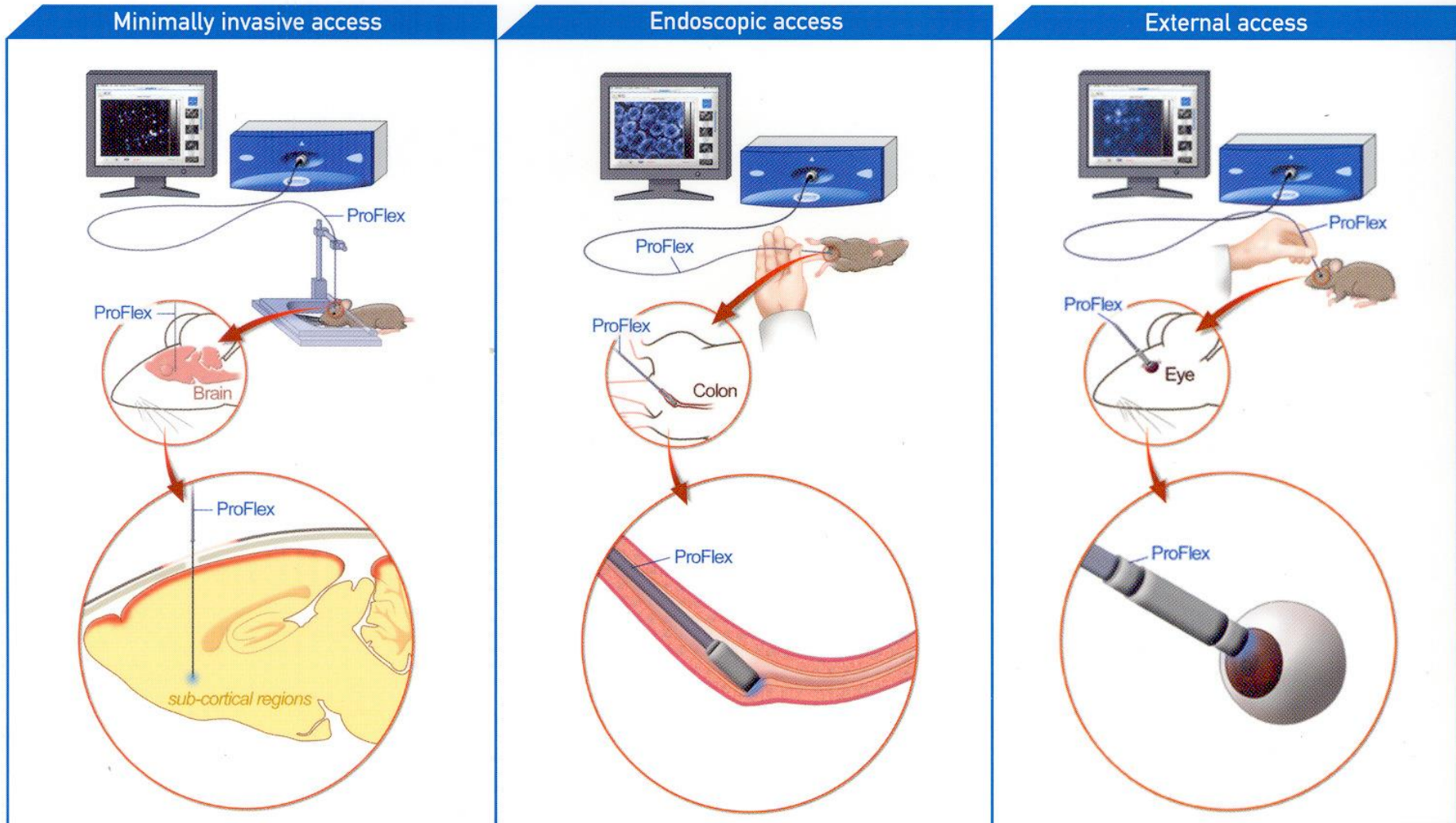
3

A simple and direct contact with the tip of a ProFlex provides **real time images at the cellular level**.

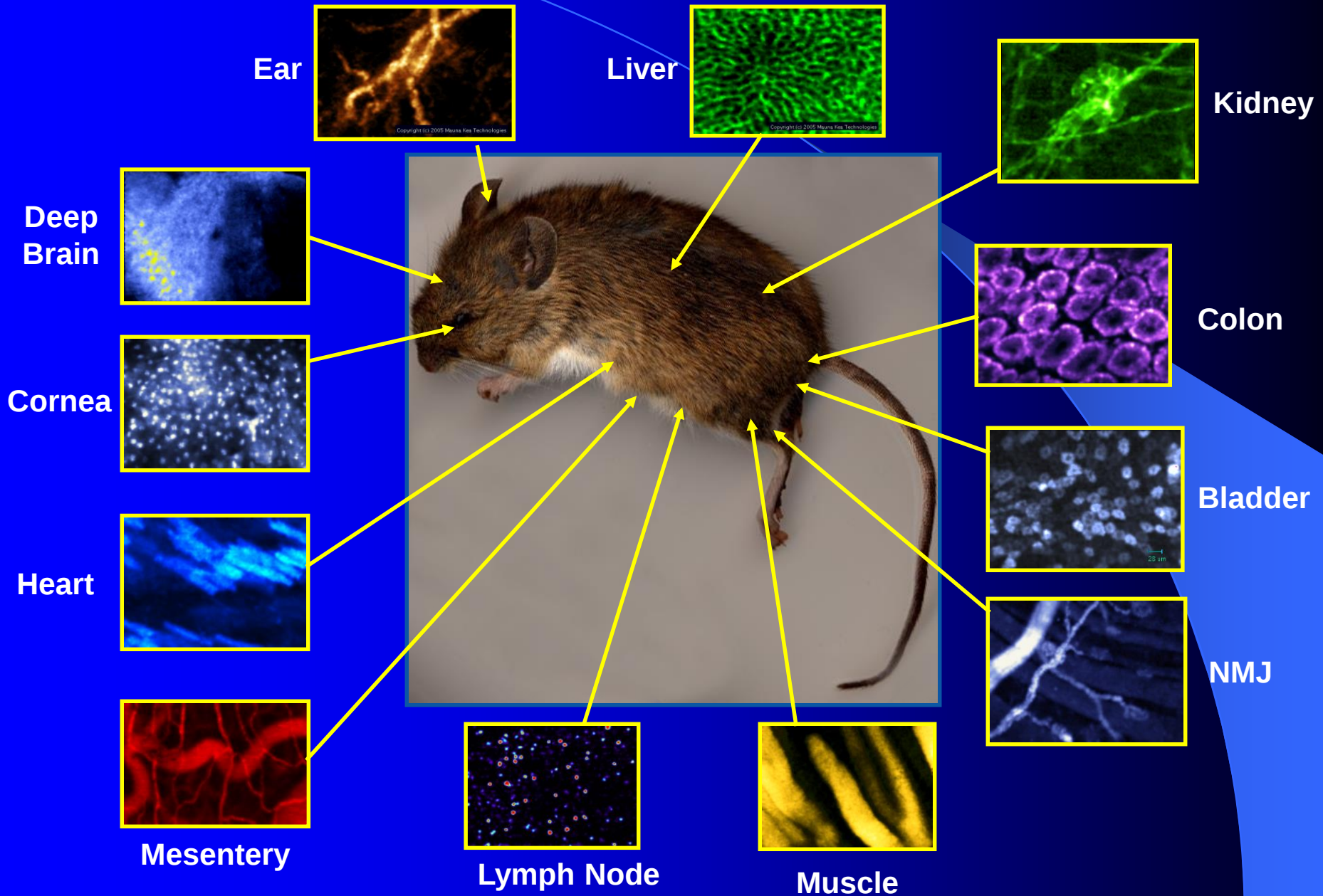


活體動物體內螢光觀察

> The fiber-based technology allows **unique *in situ* access** resulting in a whole new range of possibilities

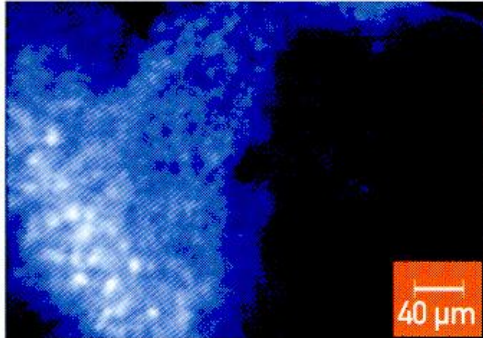


A World of Possibilities

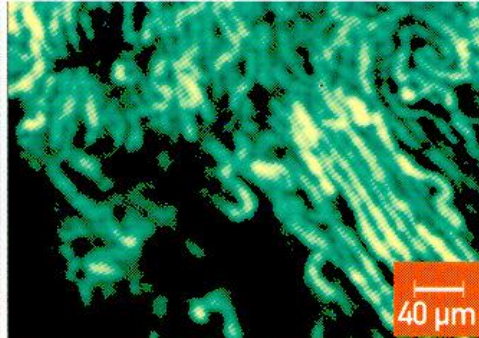


活體動物體內螢光觀察

Neuroscience Research



GFP neurons in the striatum

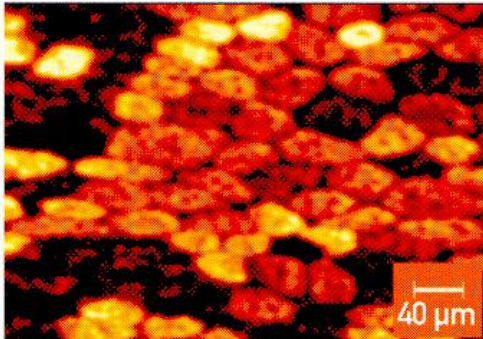


Proximal end of cut sciatic nerve in YFP transgenic mouse

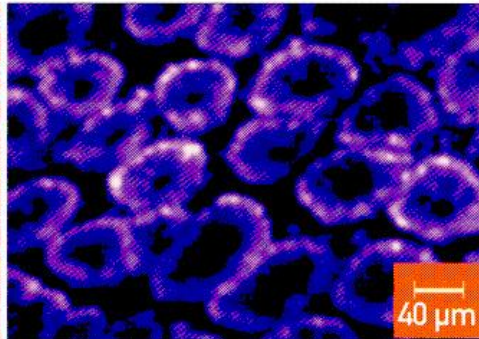
- Deep brain imaging
- Postnatal neurogenesis
- Neurodegenerative diseases
- Stem cells & regenerative therapies
- Peripheral neuropathies & neural repair
- Neuromuscular diseases

Applications

Cancer Research



Prismatic superficial cells of the rat bladder



Glandular organization of the mouse colon

- *In vivo* histological observations
- Tumor angiogenesis
- Immune response & immunotherapies
- Orthotopic tumors observation
- Aberrant crypt foci characterization
- Mouse model of colorectal cancer

Applications

Cell Vizio by Mauna Kea Technologies

三典科技代理

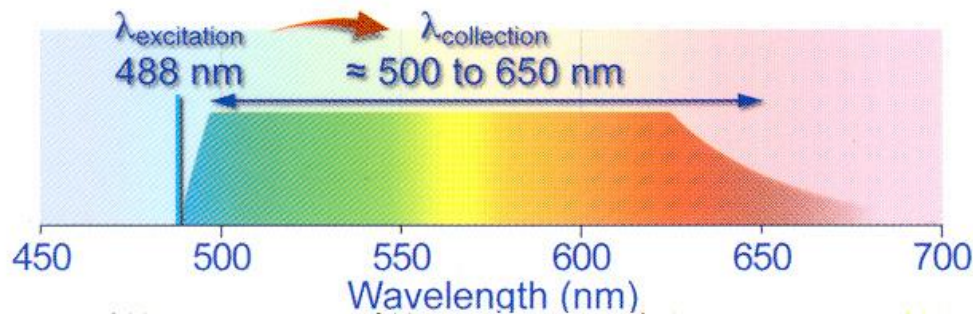
ProFlex Specifications

	Part Number	ProFlex diameter (mm)	Working distance (μm)	Axial resolution (μm)	Lateral resolution (μm)	Max field of view (μm)	Sensitivity
S series	S-0300-5.0	0.3	0	15	5	300 x 300	++
	S-0350-5.0	0.35	0	15	5	330 x 330	++
	S-0650-5.0	0.65	0	15	5	600 x 500	++
	S-1500-5.0	1.5	0	15	5	600 x 500	++
HD series	HD-1800-2.5 / 30	1.8	30	20	2.5	240 x 200	+
	HD-1800-2.5 / 50	1.8	50	20	2.5	240 x 200	+
	HD-1800-2.5 / 80	1.8	80	20	2.5	240 x 200	+

Laser Scanning Unit (LSU) Specifications

Excitation wavelength	488 nm
Collection bandwidth	500 - 650 nm
Frame rate	12 frames/sec.
Signal encoding	13 bits
Image export format	.png, .bmp, .jpeg, .pbm, .pgm, .ppm, .xbm
Movie export format	.mpeg, .mhd (raw format)
Power requirements	150 W (110 - 240 V)
Dimensions	480 x 180 x 500 mm
Weight	20 kg

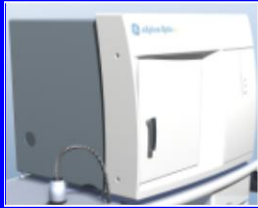
The Laser Scanning Unit™, LSU-488, is specifically developed to meet the needs of *in vivo* and real-time confocal imaging through fiber bundles. The excitation wavelength at 488 nm makes it compatible with numerous fluorophores (Syto13, Yoyo1, FITC, CellTracker Green, Calcein, Rhodamine 123 or 6G, Cy3,...) and GFP or YFP transgenic animals.



The ImageCell™ software controls the image acquisition and real-time display, at 12 frames per second, and performs on-the-fly image processing. The standard version offers analytical or quantification tools and allows standard export formats. It operates with Mac OS X.

eXplore Optix Imager from GE

Optix



Optical

Vista



PET

Locus SP



uCT Specimen

Locus



uCT in vivo

Locus Ultra

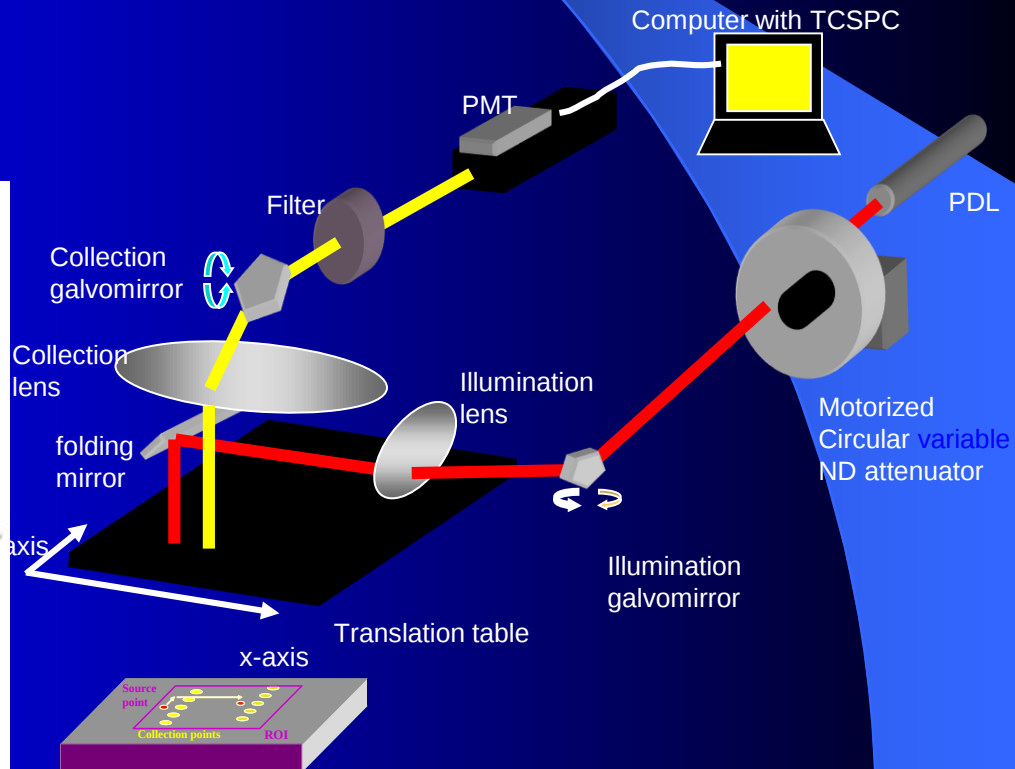


Volume CT

Advantage Workstation



Viewing workstation



Fluorescence Optical Applications

Area

Activity

Requirements

Oncology

- Tumor detection
- Tumor size
- Tumor localization
- Angiogenesis

- ✓ Sensitivity
- ✓ Quantitation
- ✓ Tomography

Disease biology

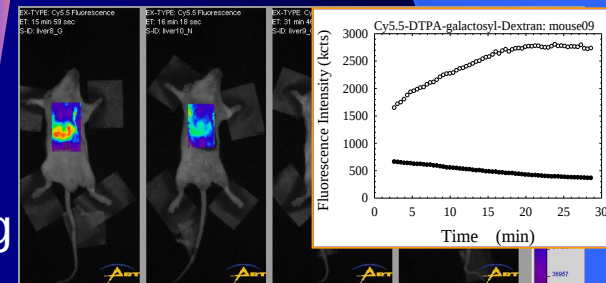
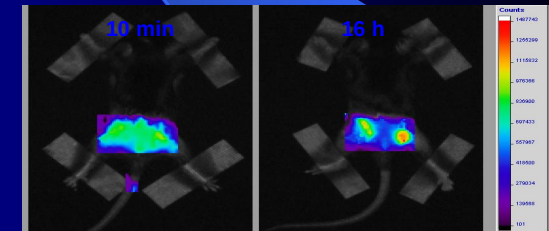
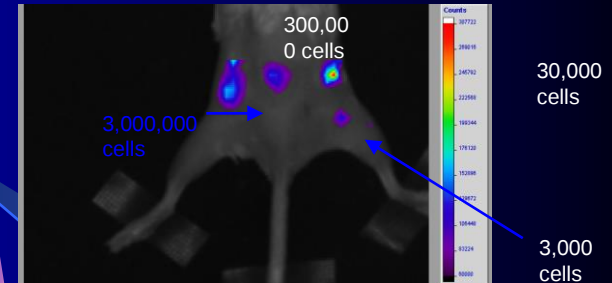
- Disease progression
- Gene expression
- Protein-protein interaction
- Receptor studies
- Antibody labeling

- ✓ Deep tissue
- ✓ Sensitivity
- ✓ Quantitation
- ✓ Multiplexing

ADME/Tox

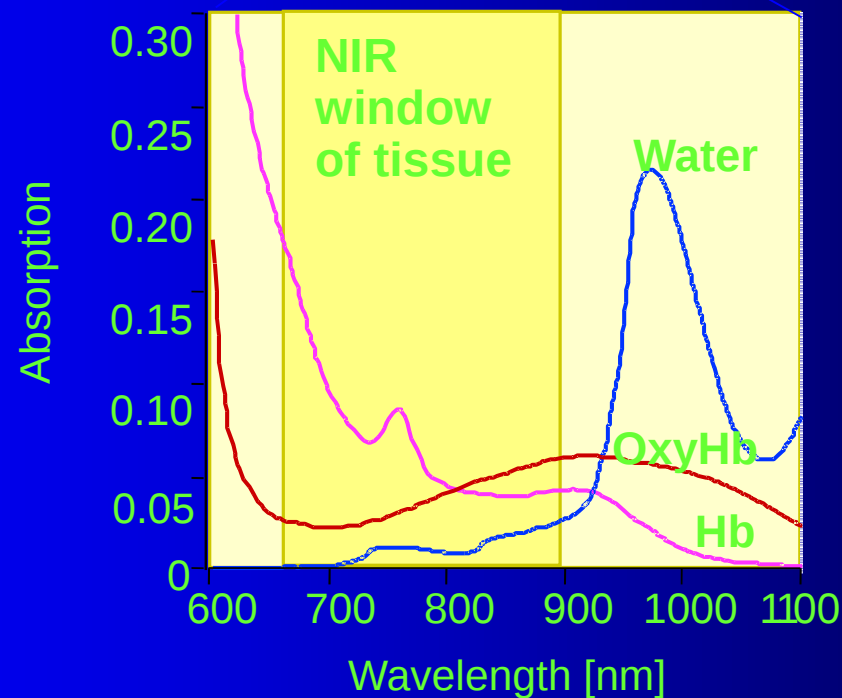
- Kinetics
- Gastric Emptying
- Organ screening

- ✓ Sensitivity
- ✓ Quantitation
- ✓ Dynamic imaging



NIR Tissue Optical Window

Low absorption in the near-IR spectrum (650-950nm)

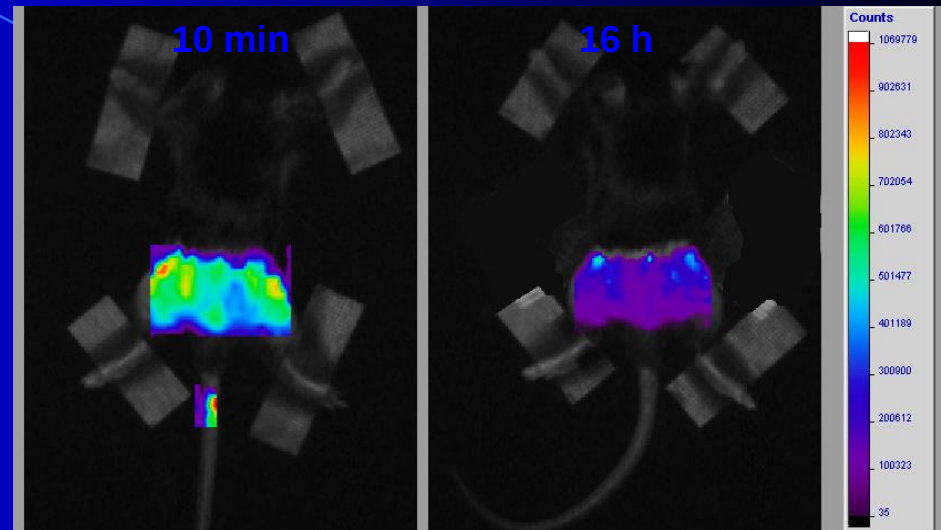


Tumor Biology

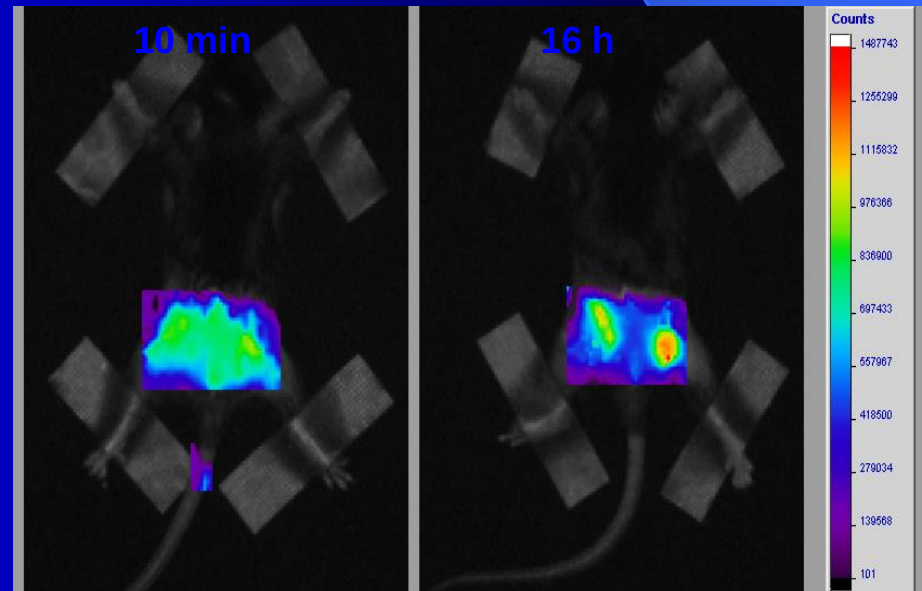
Imaging cell surface antigens

- Mice with Lewis Lung Carcinoma subcutaneous growing tumors
- Injected with Cy5 labeled Control (RDG) and Targeted (RGD) Agents (100 nm/kg)
- Scanned 10 min and 16 h post injection
- Images acquired using the eXplore Optix system

CONTROL AGENT

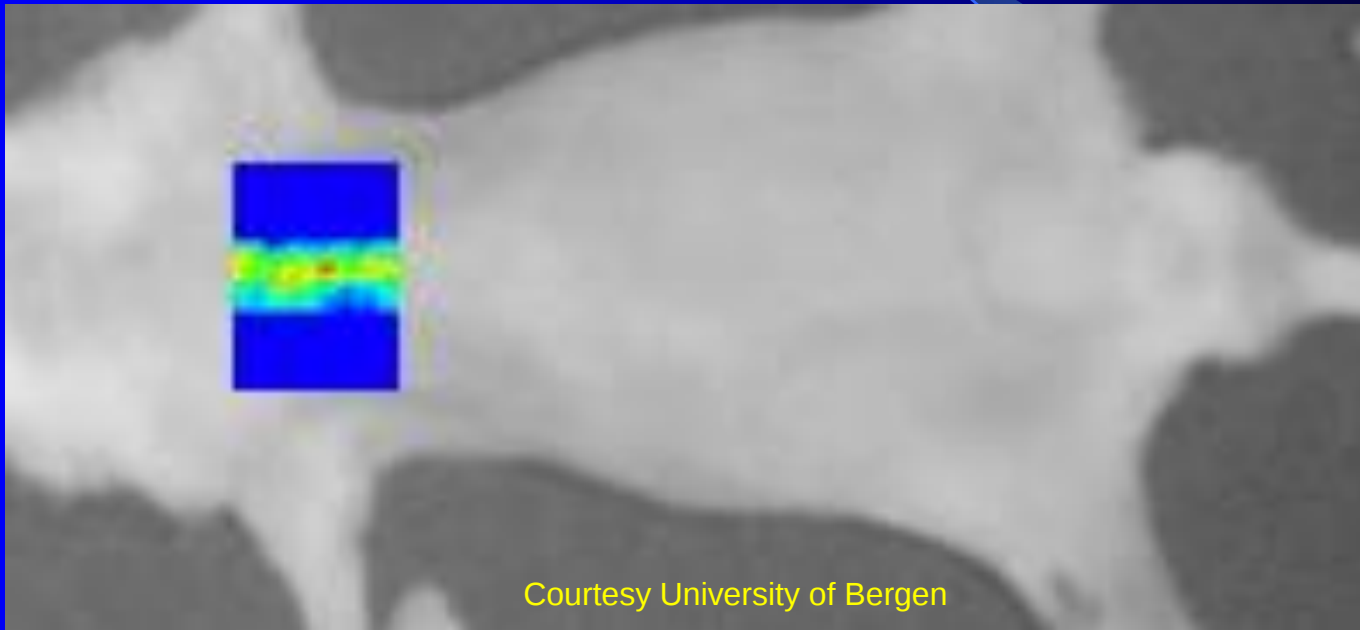


TARGETED AGENT



Sternum Leukocytes of Tumor Model w/Cy5.5

Colonies of leukocytes confirmed ex-vivo

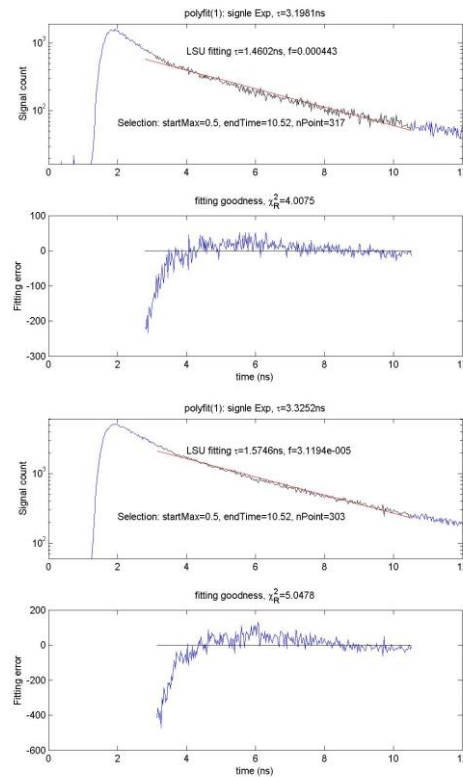
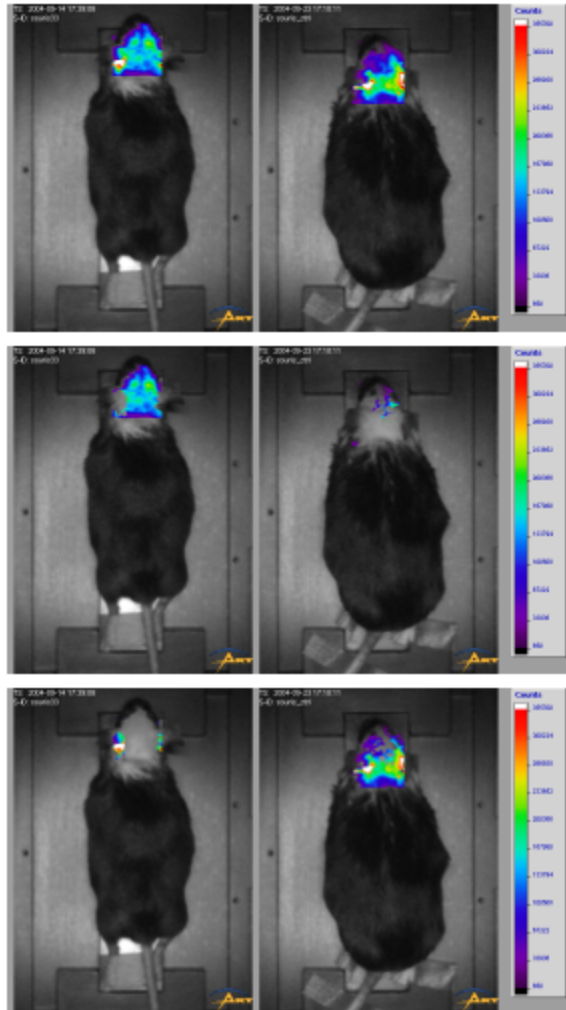


Imaging Reporter Genes

Autofluorescence Differentiation

GFP

Control



GFP

$\tau = 2.7 \text{ ns}$

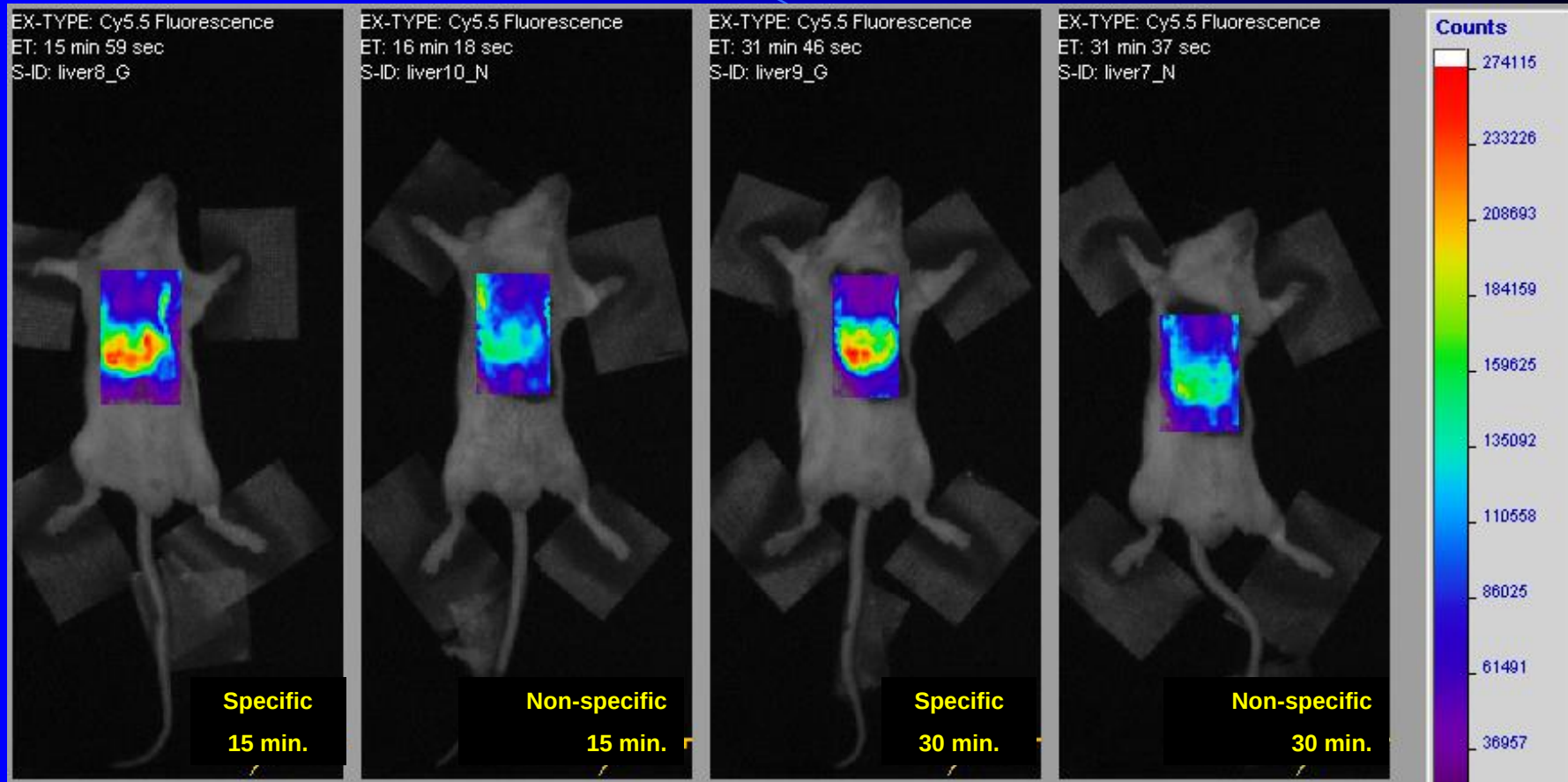
Autofluorescence

$\tau > 3.0 \text{ ns}$

Kinetic Study

Liver targeted agent

Courtesy Mattrey and Vera, UCSD

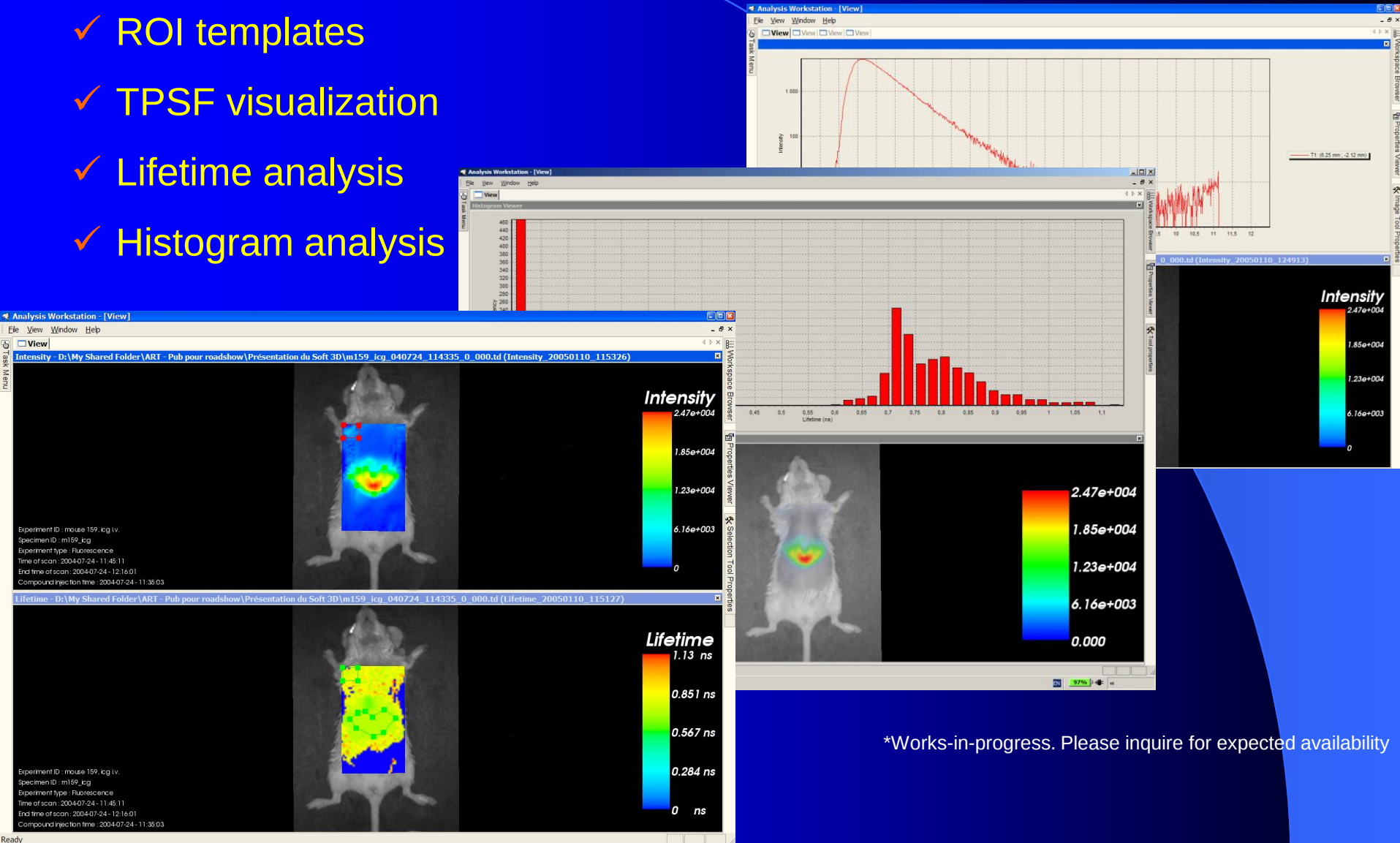


Fluorescently labeled ligands, specific and non-specific for a receptor type on hepatocytes, were injected & imaged. ROIs around heart the liver used to generate organ-specific fluorescence lifetime and intensity.

Data Analysis Capability*

Extract advanced information from 2D and 3D datasets

- ✓ ROI templates
- ✓ TPSF visualization
- ✓ Lifetime analysis
- ✓ Histogram analysis



*Works-in-progress. Please inquire for expected availability

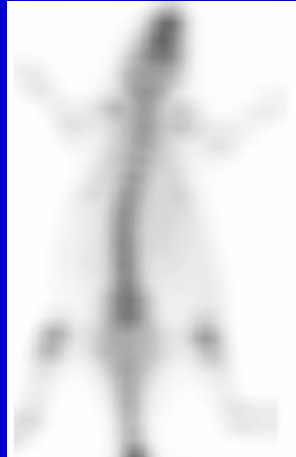
What's next? Anatomy with Function...

CT



+

PET

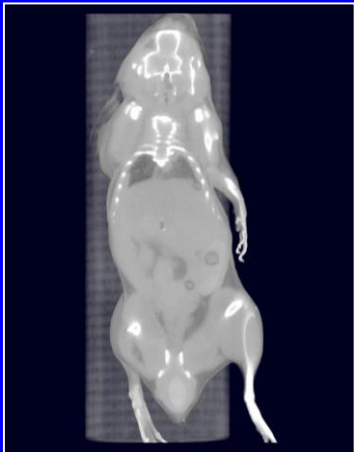


=

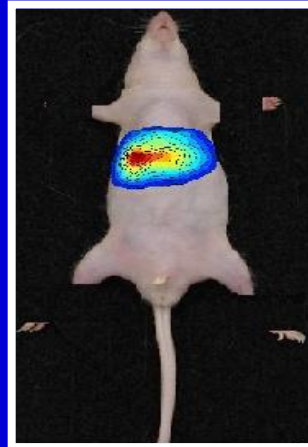
Specificity



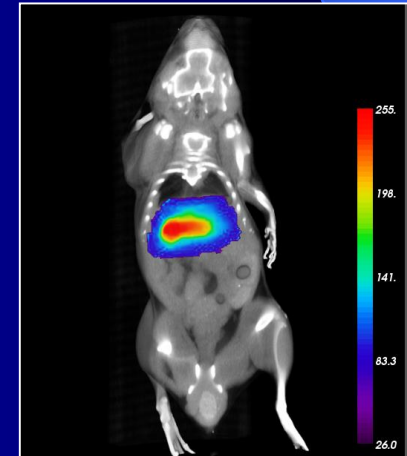
Optical



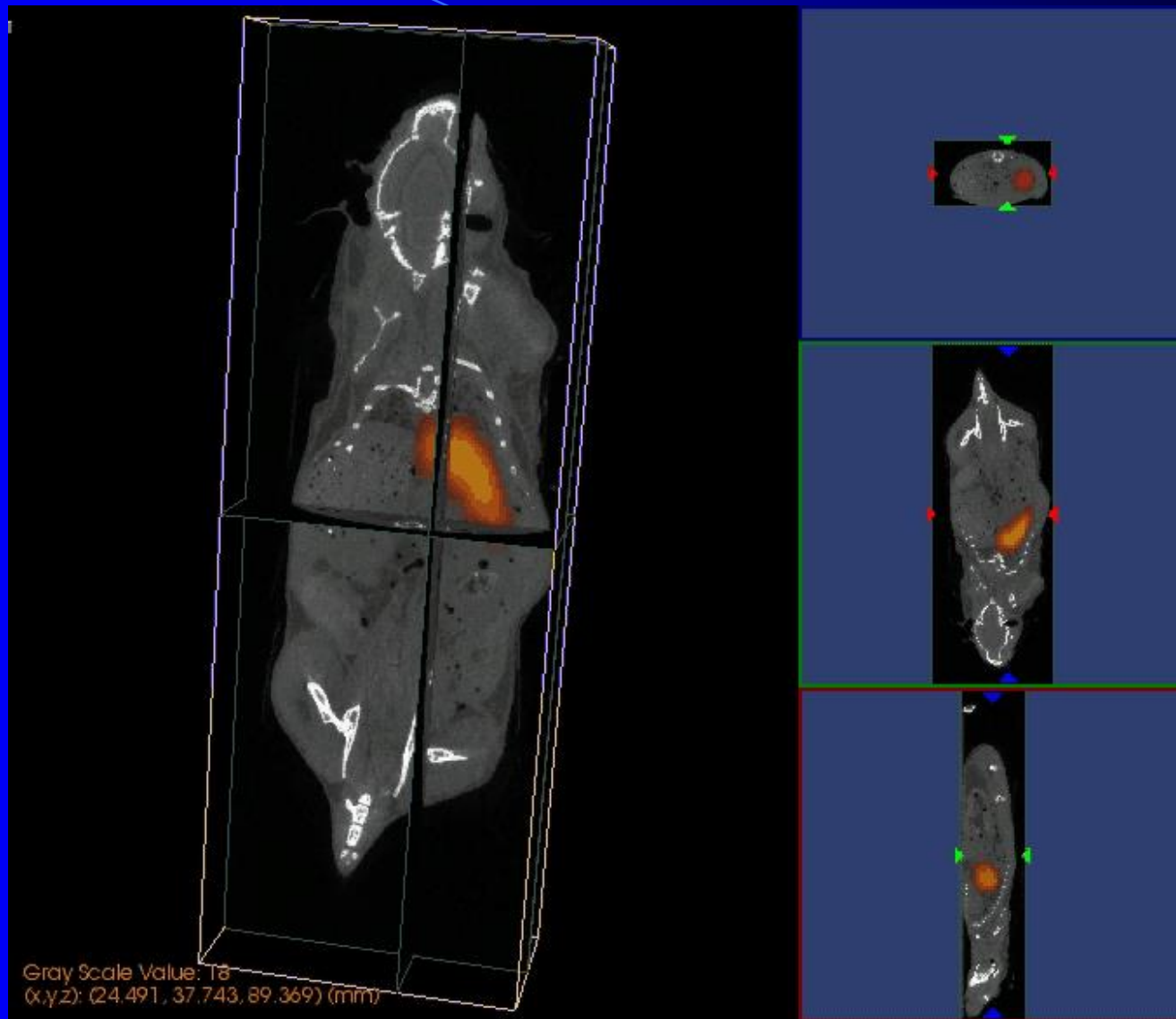
+



=



Co-register w/ CT for Precise Anatomical Localization*



*Works-in-progress. Please inquire for expected availability

eXplore Optix Summary

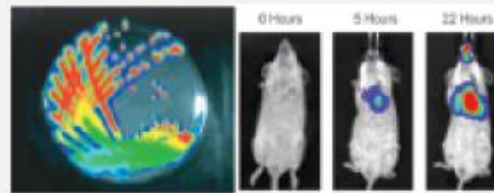
3D localization & Quantitation Accuracy

<u>Benefit</u>	<u>Feature</u>	<u>Why it Matters</u>
Sensitivity & Tissue Penetration	▪ NIR Optimization ▪ Time Correlated Single Photon Counting(TCSPC) approach	✓ Less auto-fluorescence... lower background... greater SNR ✓ Greater tissue penetration...enables internal organ measurements ✓ Earlier disease/response detection
Quantitation	▪ TSCPC ▪ Depth analysis tool	✓ Depth localization ✓ Flourophore concentration estimation accuracy ✓ Platform for tomography.. Absolute concentration quantitation
Fluorescence Lifetime	▪ TCSPC	✓ Endogenous fluorecence differentiation. ✓ Enables multi tracer & interaction applications ✓ Enables micro-environment interrogation

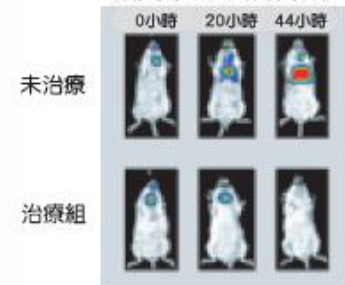


XENOVEN IVIS system

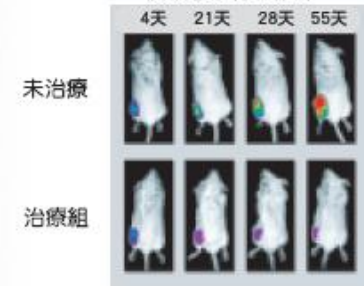
非侵入式活體分子影像系統



肺炎葡萄球菌動物感染模式
(葡萄球菌具冷光表現)



前列腺癌小鼠動物模式
(癌細胞表現冷光)



IVIS (In vivo Imaging System)是針對實驗動物所開發之活體影像系統。利用冷光酵素(luciferase)產生生物冷光的原理，將冷光酵素標定於細胞或特定基因，藉以觀察與分析同一隻動物於特定時間點的反應。建立新的實驗方法流程及搭配先進的軟硬體設計，讓動物實驗獲得更多且高品質的資訊。

IVIS (In vivo Imaging System)是針對實驗動物所開發之活體影像系統。利用冷光酵素(luciferase)產生生物冷光的原理，將冷光酵素標定於細胞或特定基因，藉以觀察與分析同一隻動物於特定時間點的反應。建立新的實驗方法流程及搭配先進的軟硬體設計，讓動物實驗獲得更多且高品質的資訊。

IVIS® 的 4大優點

- ◆ 高通量動物檢測，降低實驗動物花費，加速實驗速度
- ◆ 可收集及分析大量偵測資料
- ◆ 藉由觀察同一隻動物在不同偵測時間的訊號以降低個體差異所造成的誤差
- ◆ 改善動物試驗方法加速新藥開發效率

IVIS® 的 5大應用

- ◆ 新藥開發
- ◆ 藥理/毒理測試
- ◆ 腫瘤醫學研究
- ◆ 感染疾病研究
- ◆ 功能性基因體學研究



進階生物科技股份有限公司
Level Biotechnology Inc.

電話: 02-26959935 免付費專線: 0800-251302
www.level.com.tw

Microtubule Associated Protein (MAP) probes

Cell mitosis

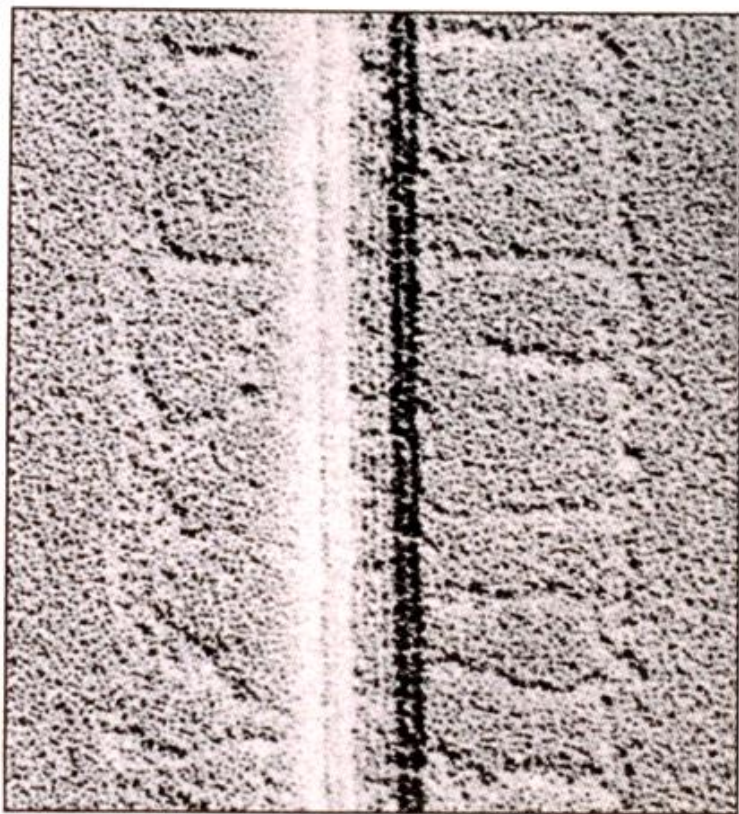
Axon outgrowth

TABLE 19-1 Major Microtubule-Associated Proteins

Protein	MW	Domain Organization*	Location
TYPE I MAP1A	300,000 heavy chain		Dendrites and axons
MAP1B	255,000		Dendrites and axons
TYPE II MAP2a	280,000		Dendrites
MAP2b	200,000		Dendrites
MAP2c	42,000		Embryonic dendrites
MAP4	210,000		Non-neuronal cells
Tau	55,000–62,000		Dendrites and axons

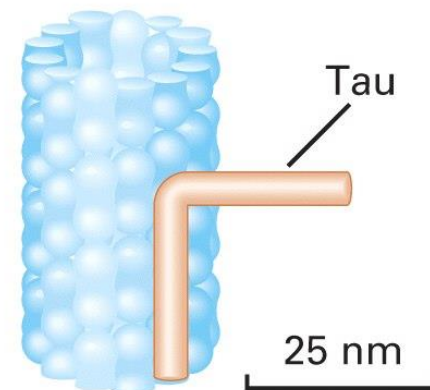
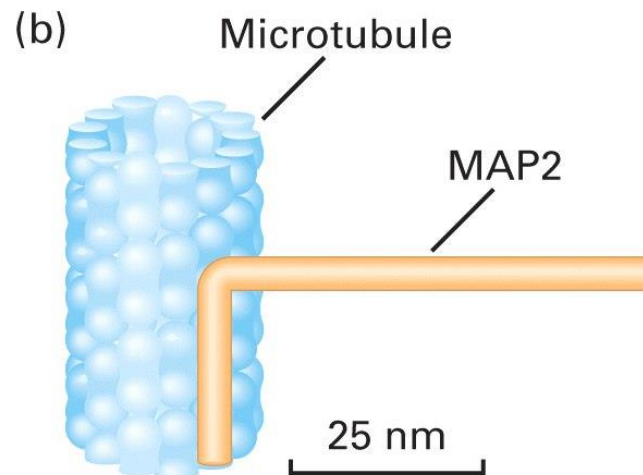
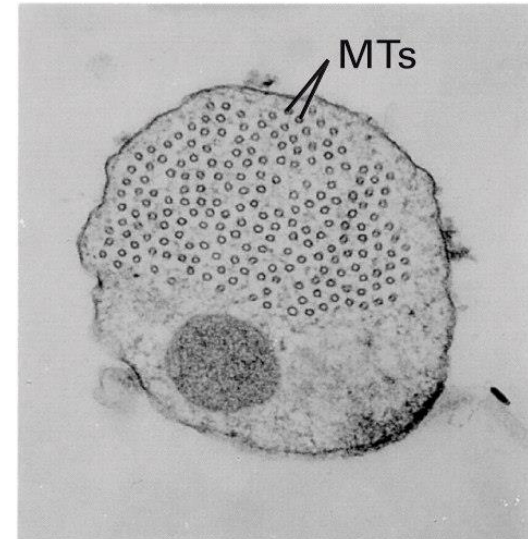
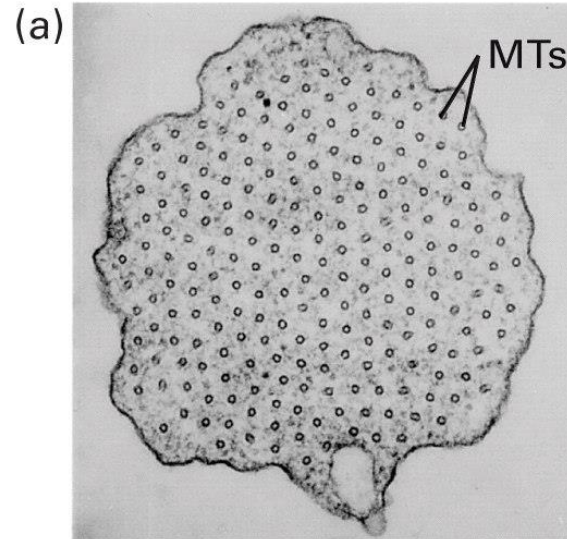
*Yellow, microtubule-binding domain; pink, projection domain; green, 18 amino acid repeats.

Microtubule Associated Protein (MAP)



(A)

100 nm
100 nm



Taxol-
treatment

GFP-MAP1A

A

Tubulin

B

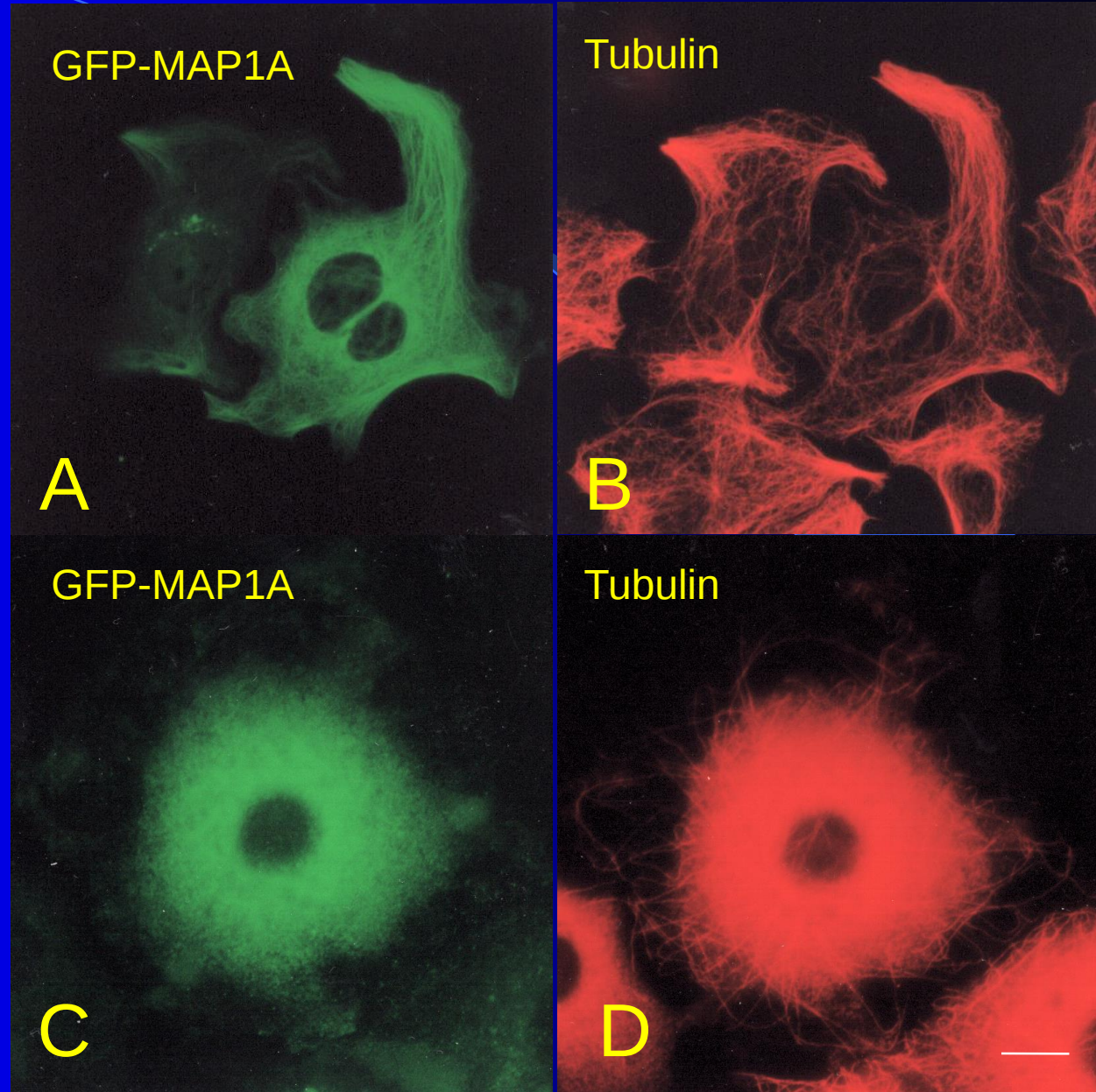
Nocodazol-
treatment

GFP-MAP1A

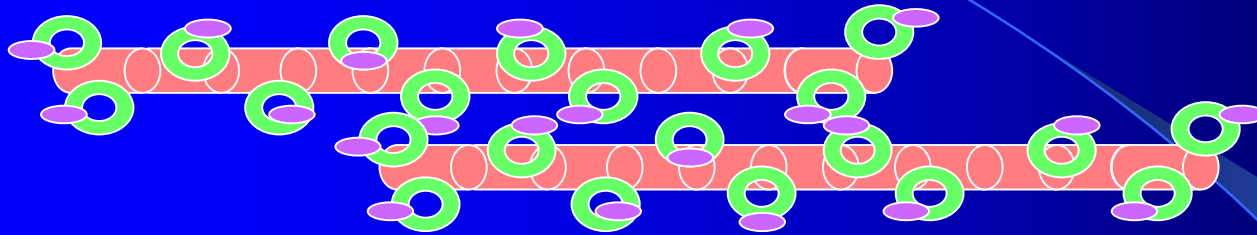
C

Tubulin

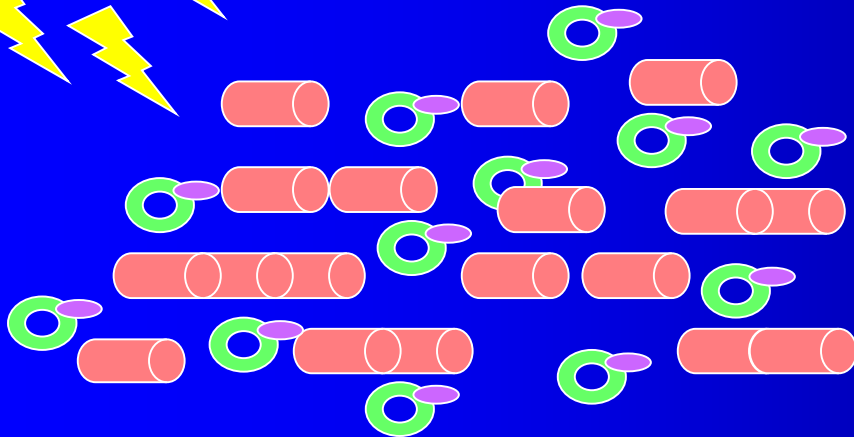
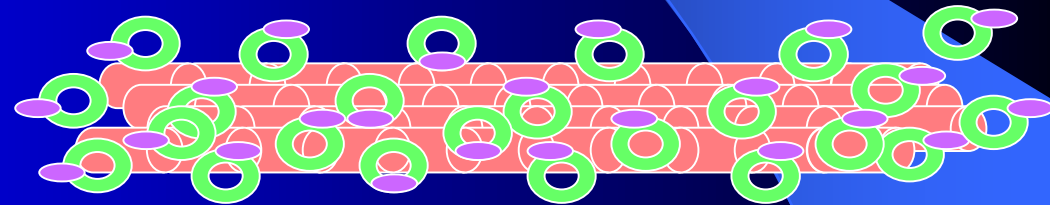
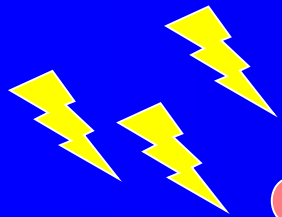
D



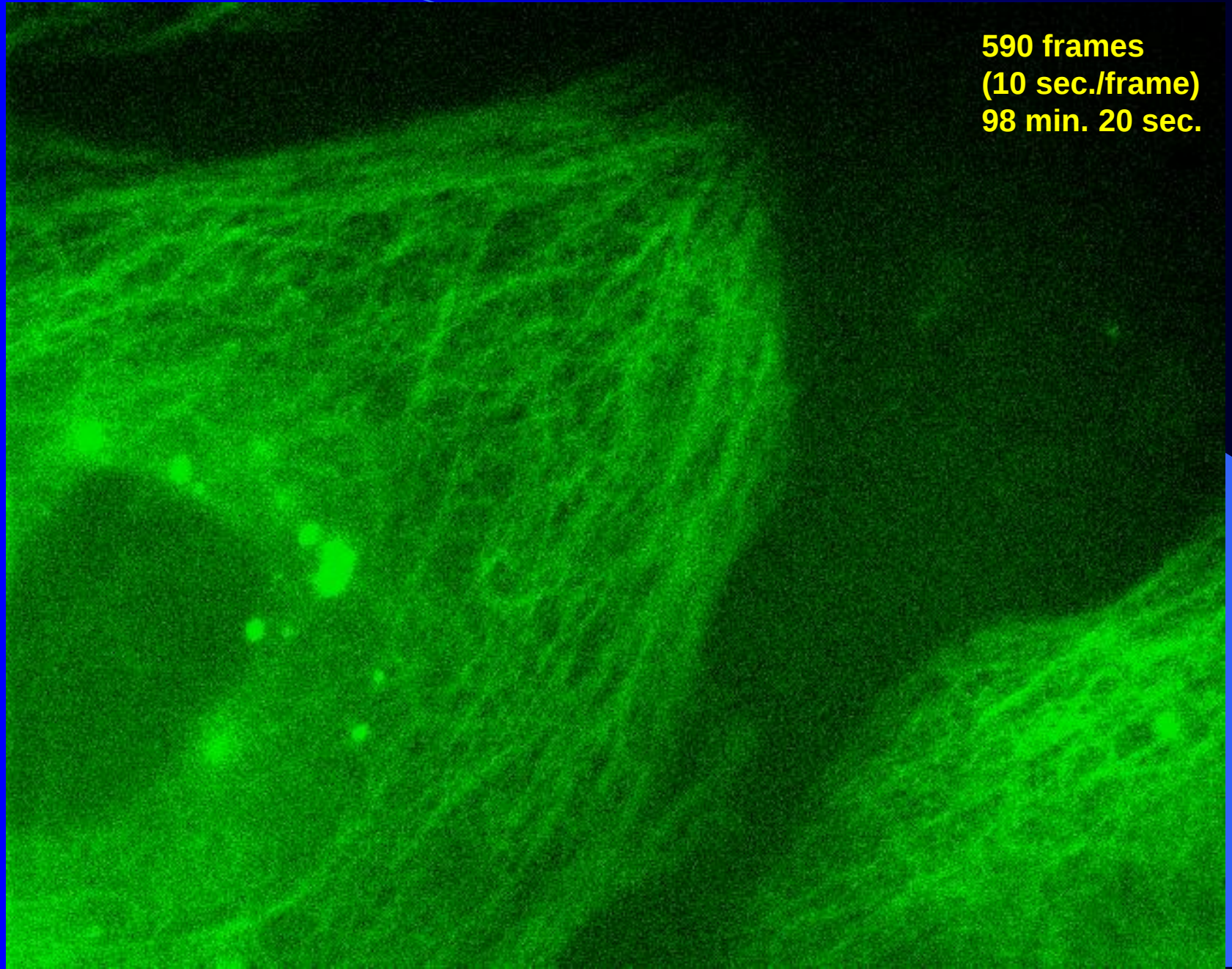
○ MAP1A-HC ● MAP1A-LC 〰 Tubulin ☀ Taxol ⚡ Nocodazol



Nocodazol



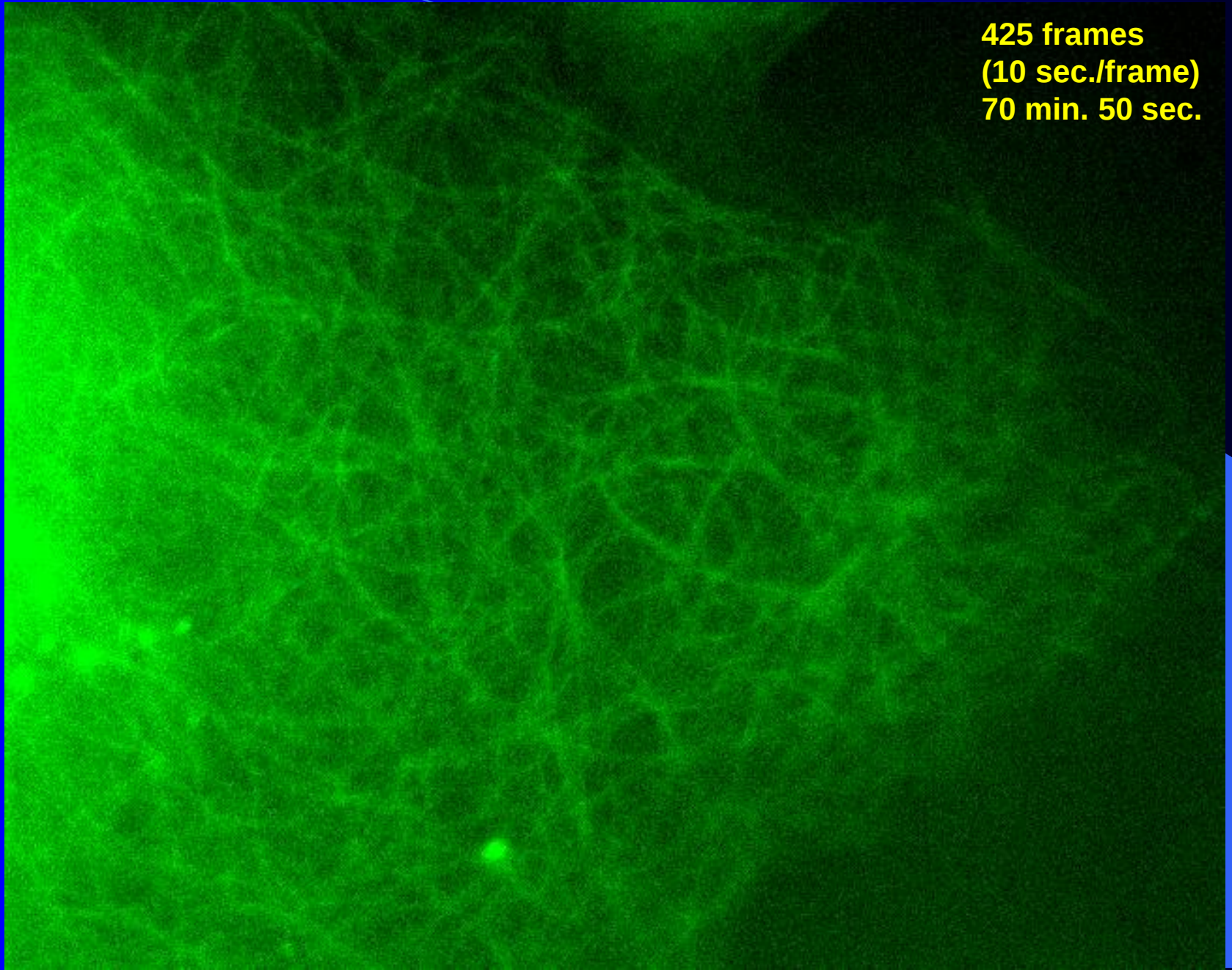
GFP-MAP1A in COS 7 (Taxol 10 μ M)

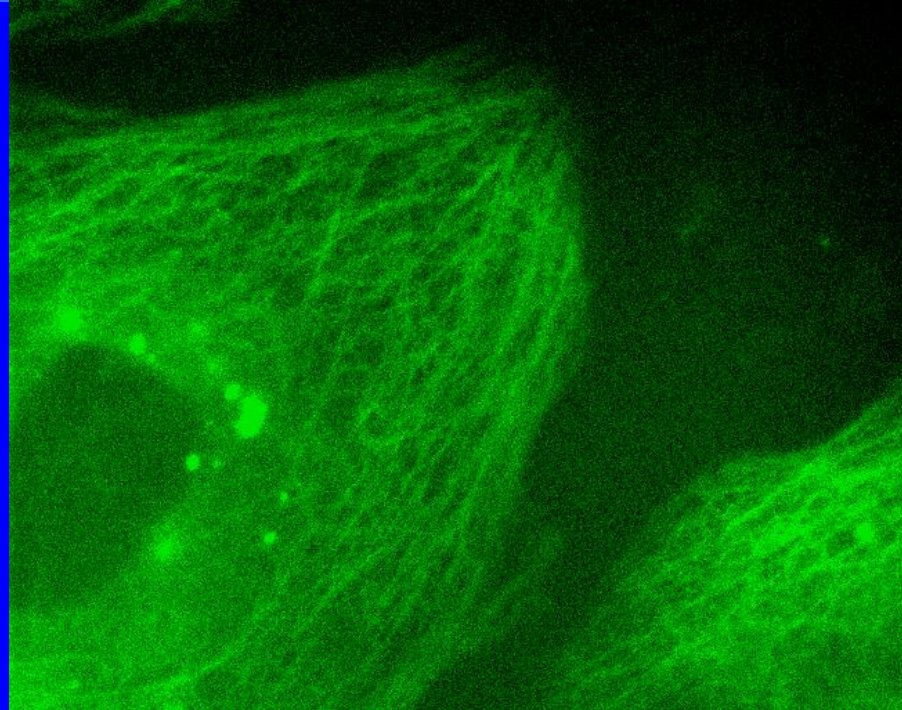


590 frames
(10 sec./frame)
98 min. 20 sec.

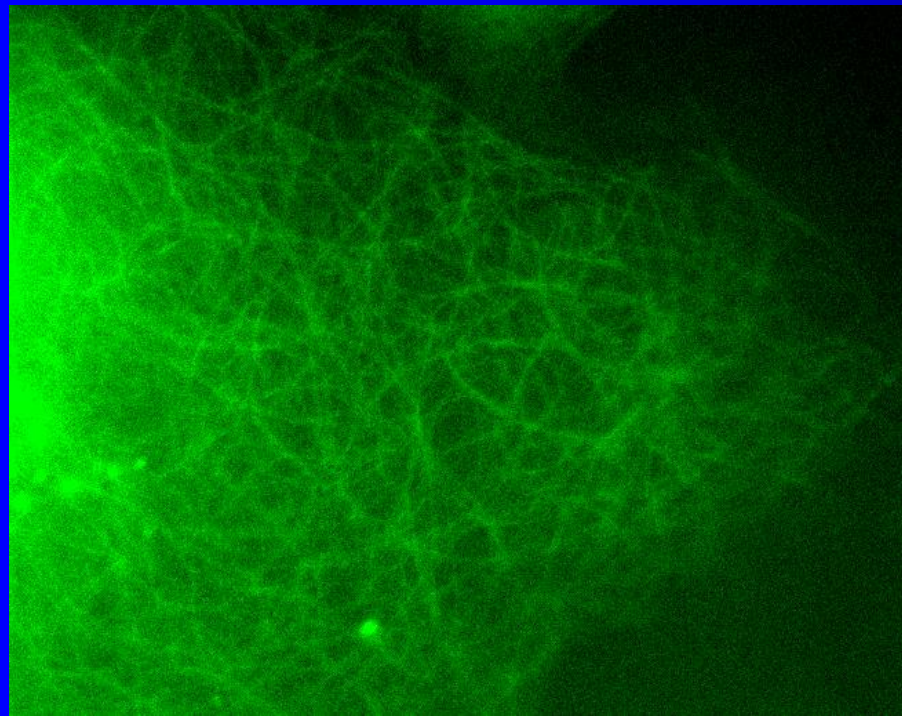
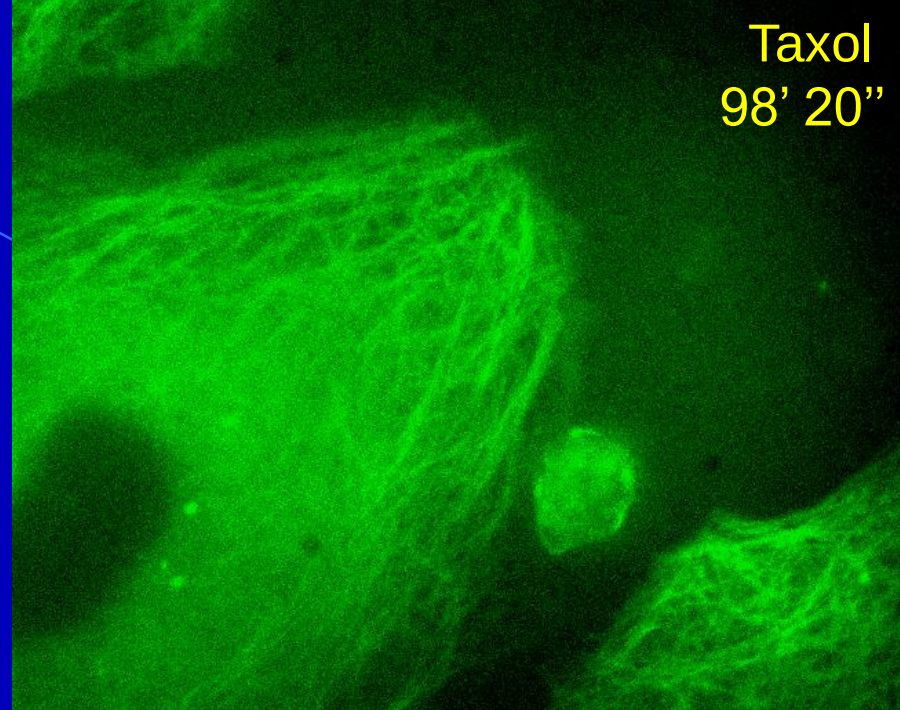
GFP-MAP1A in COS 7 (Nacodazol 10 $\mu\text{g/ml}$)

425 frames
(10 sec./frame)
70 min. 50 sec.

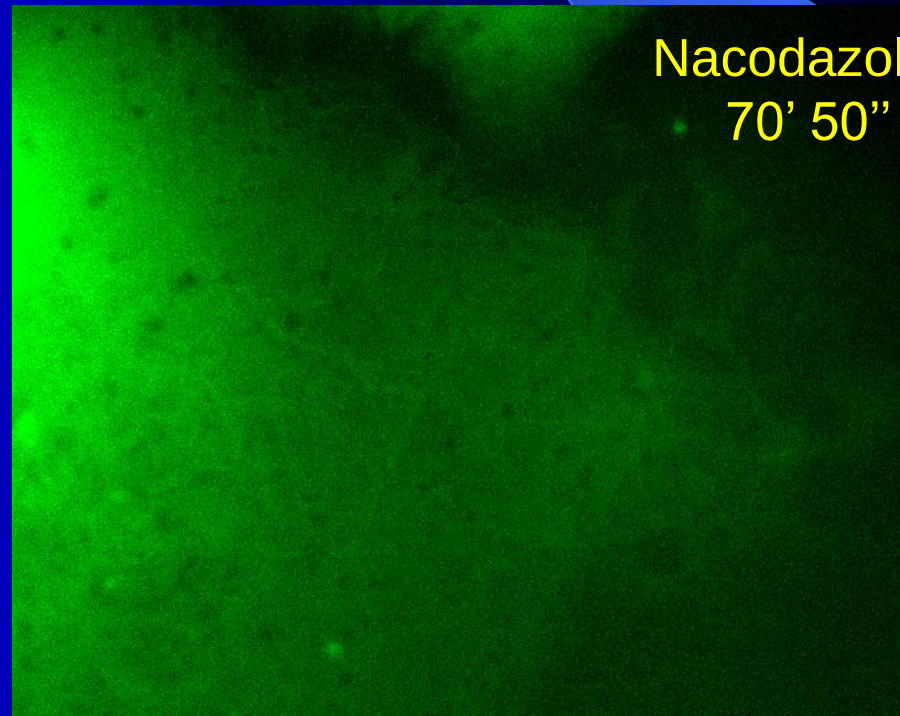




Taxol
98' 20"

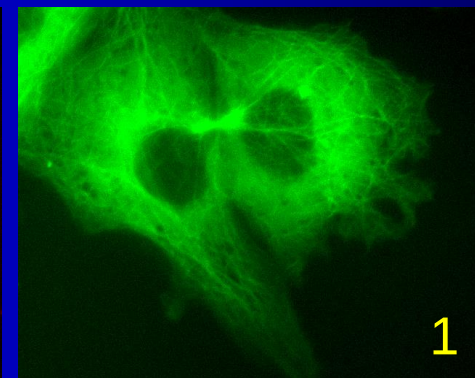
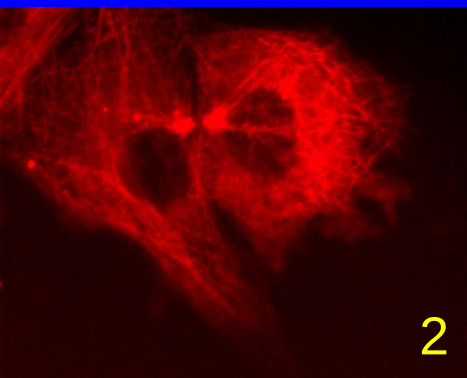
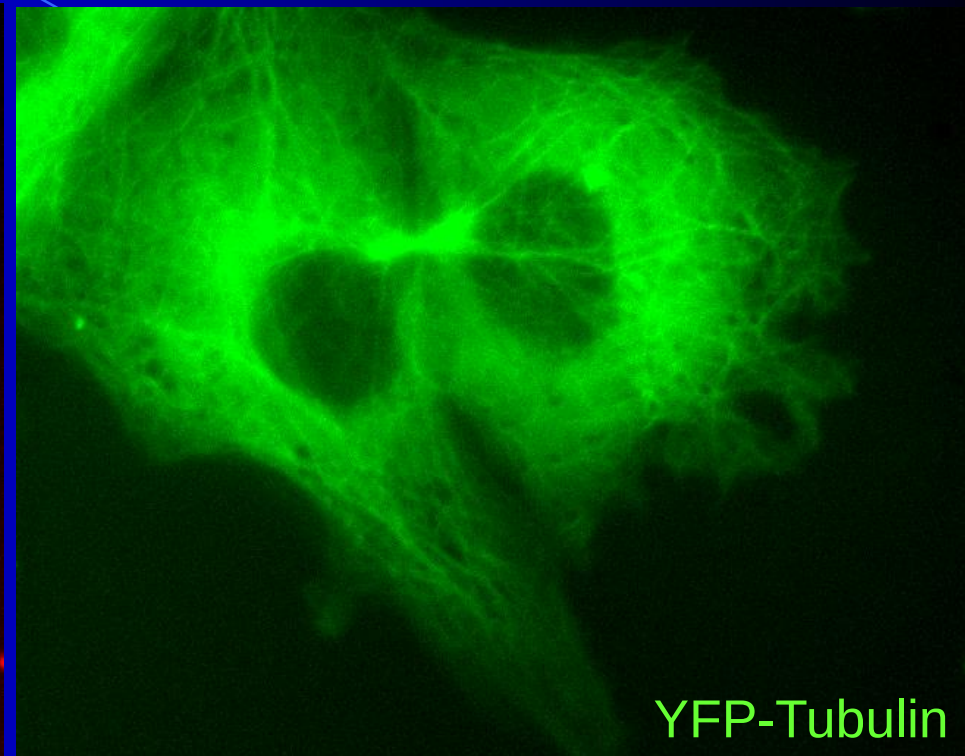
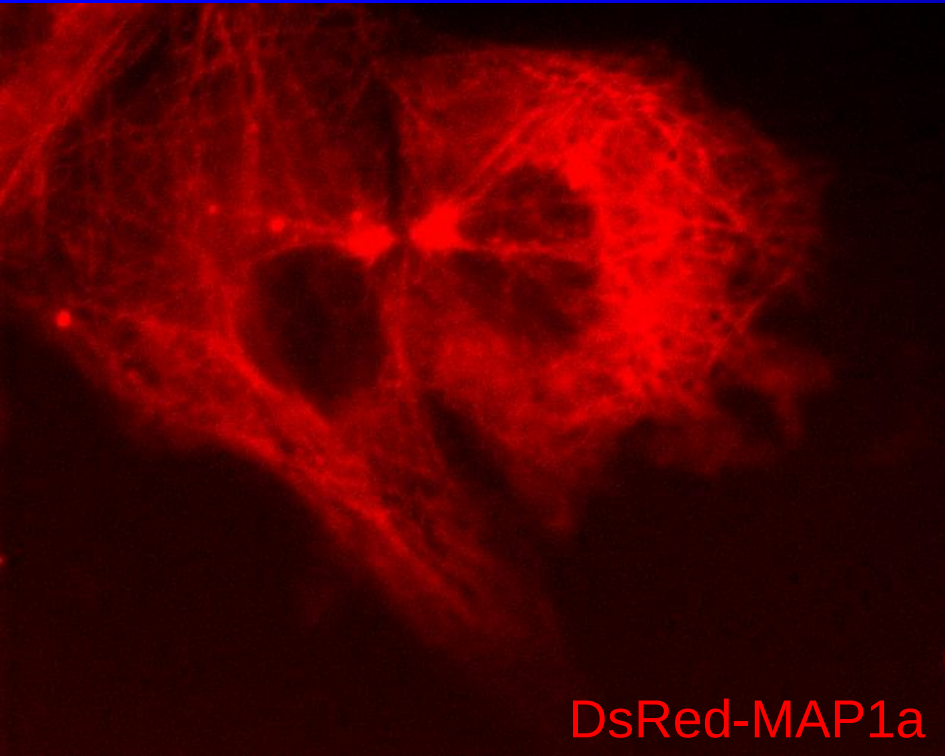


Nacodazol
70' 50"



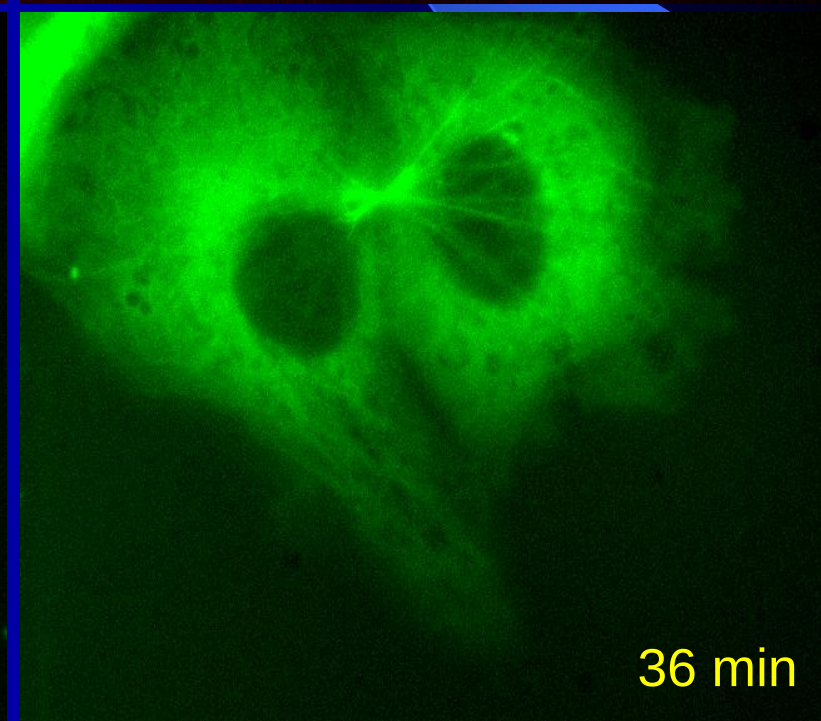
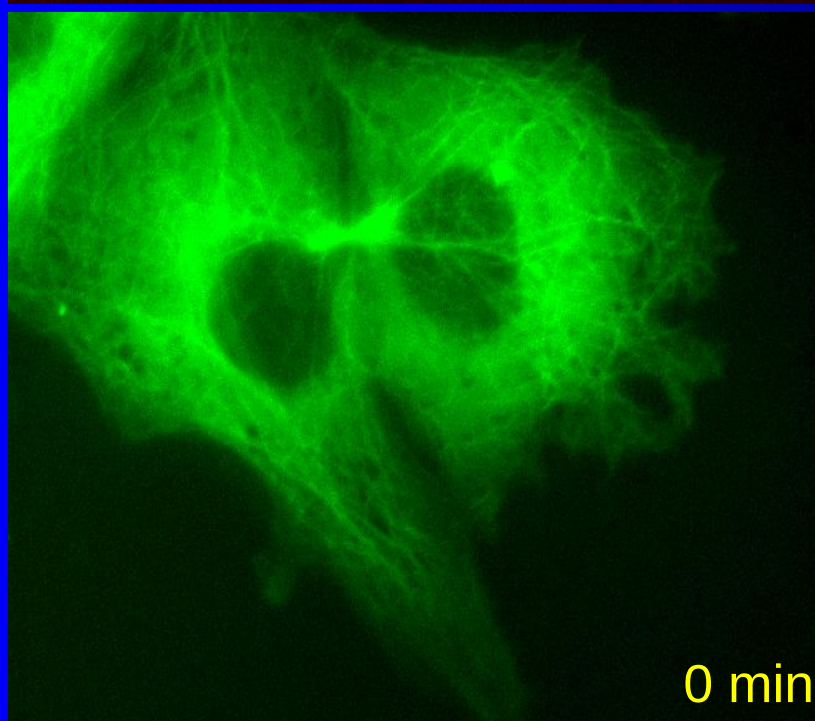
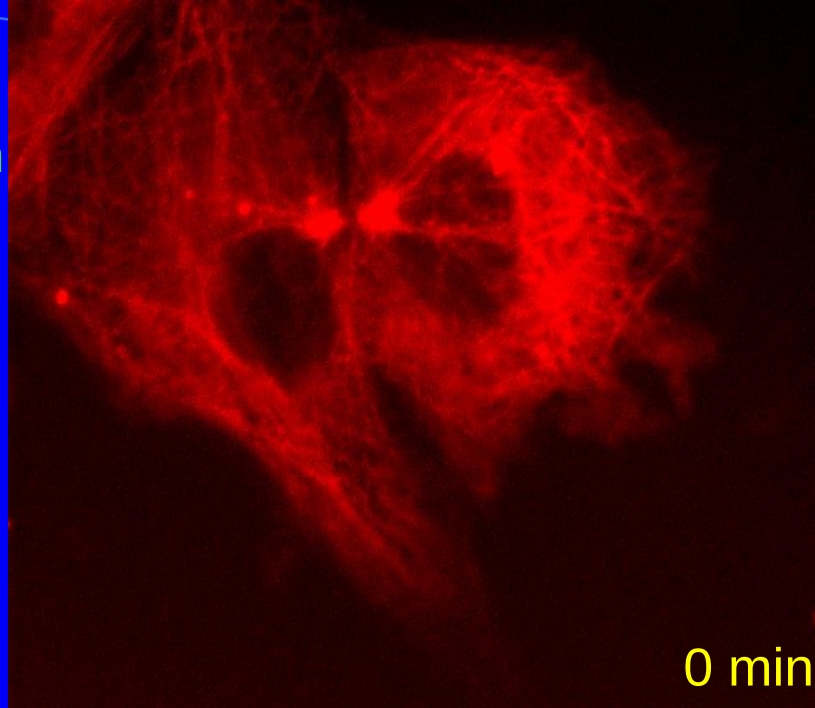
Nocodazol treated Cotransfected DsRed-MAP1a + YFP-Tubulin

10 second / frame, total 216 frames, 36 min

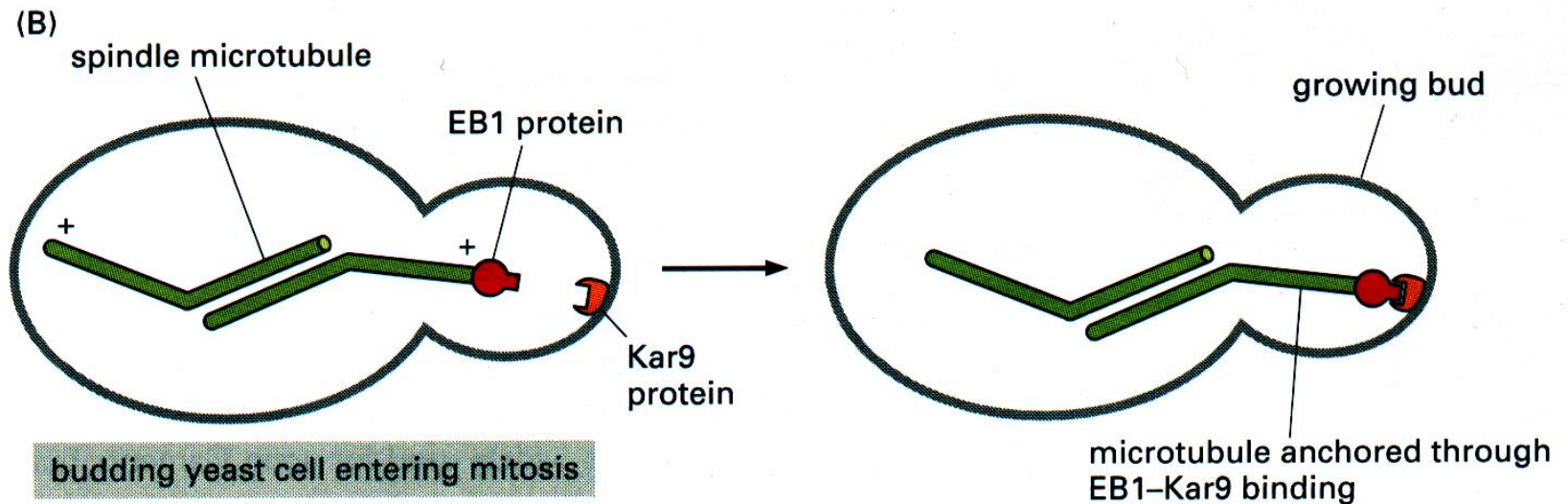


DsRed-
MAP1a
YFP-Tubulin
in
Nacodazol-
treated
COS7 cell

10 s / frame,
total 216
frames, 36 min



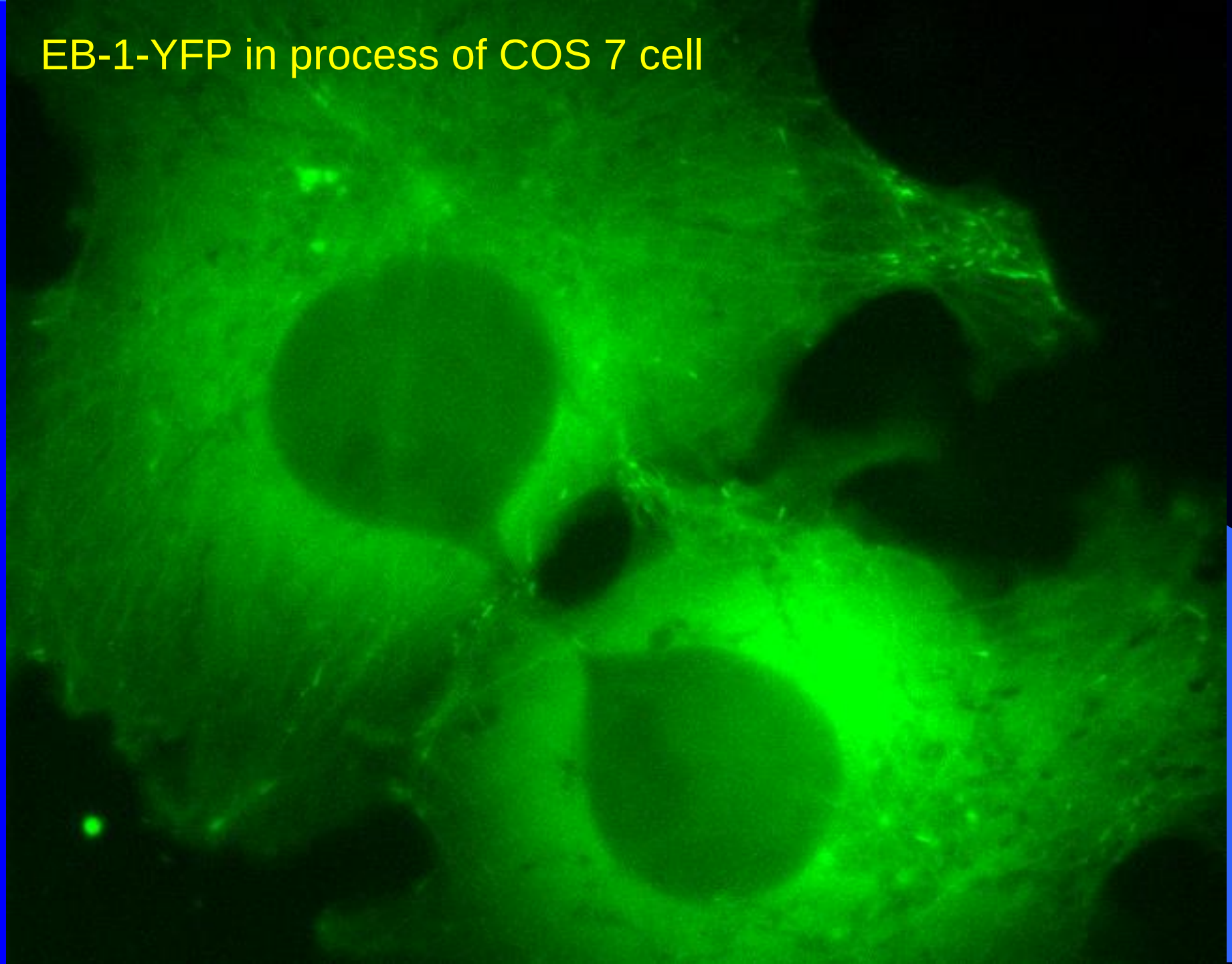
EB-1 microtubule capping protein



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

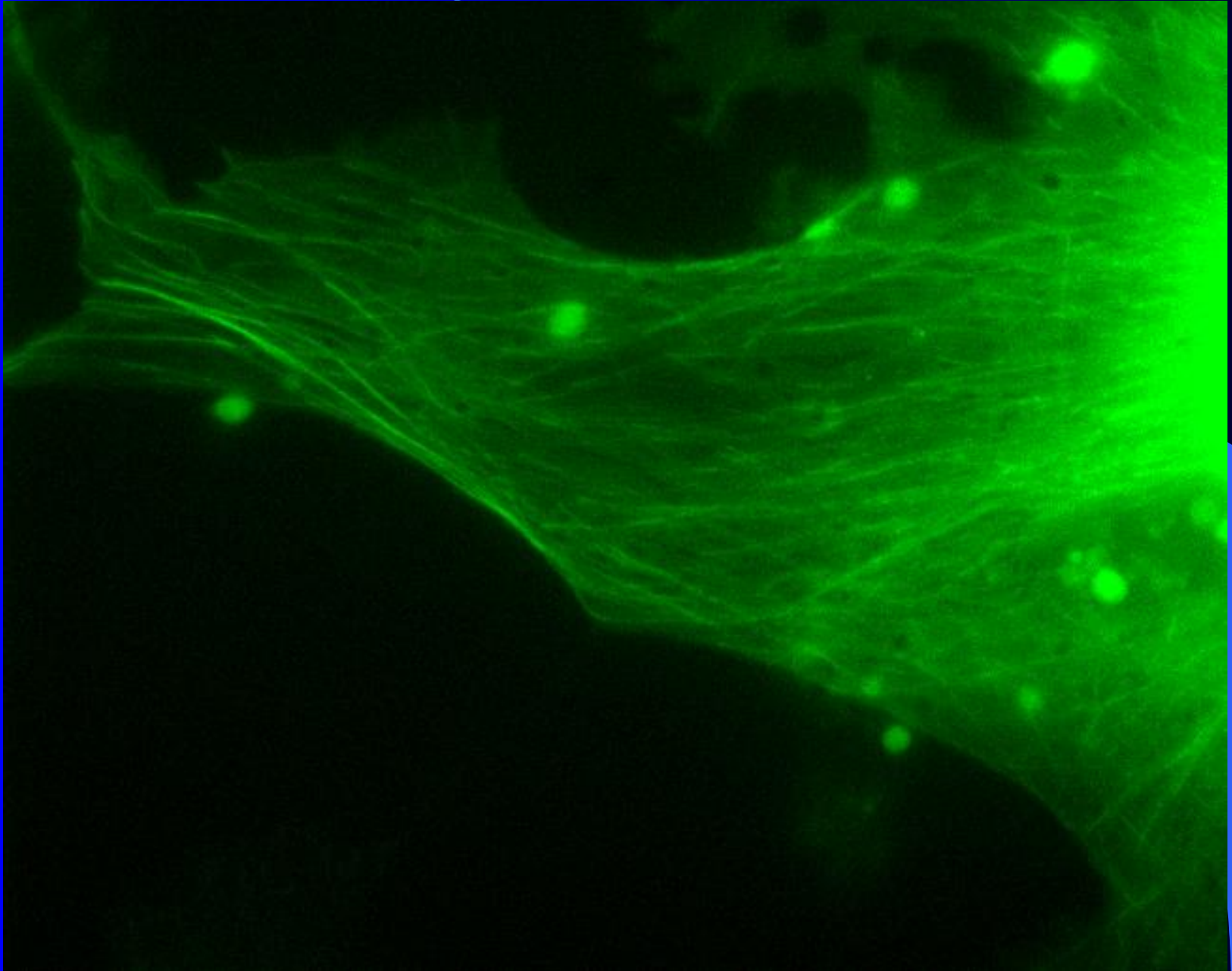
(movie from Molecular Biology of The Cell, 4th Ed. 2002)

EB-1-YFP in process of COS 7 cell

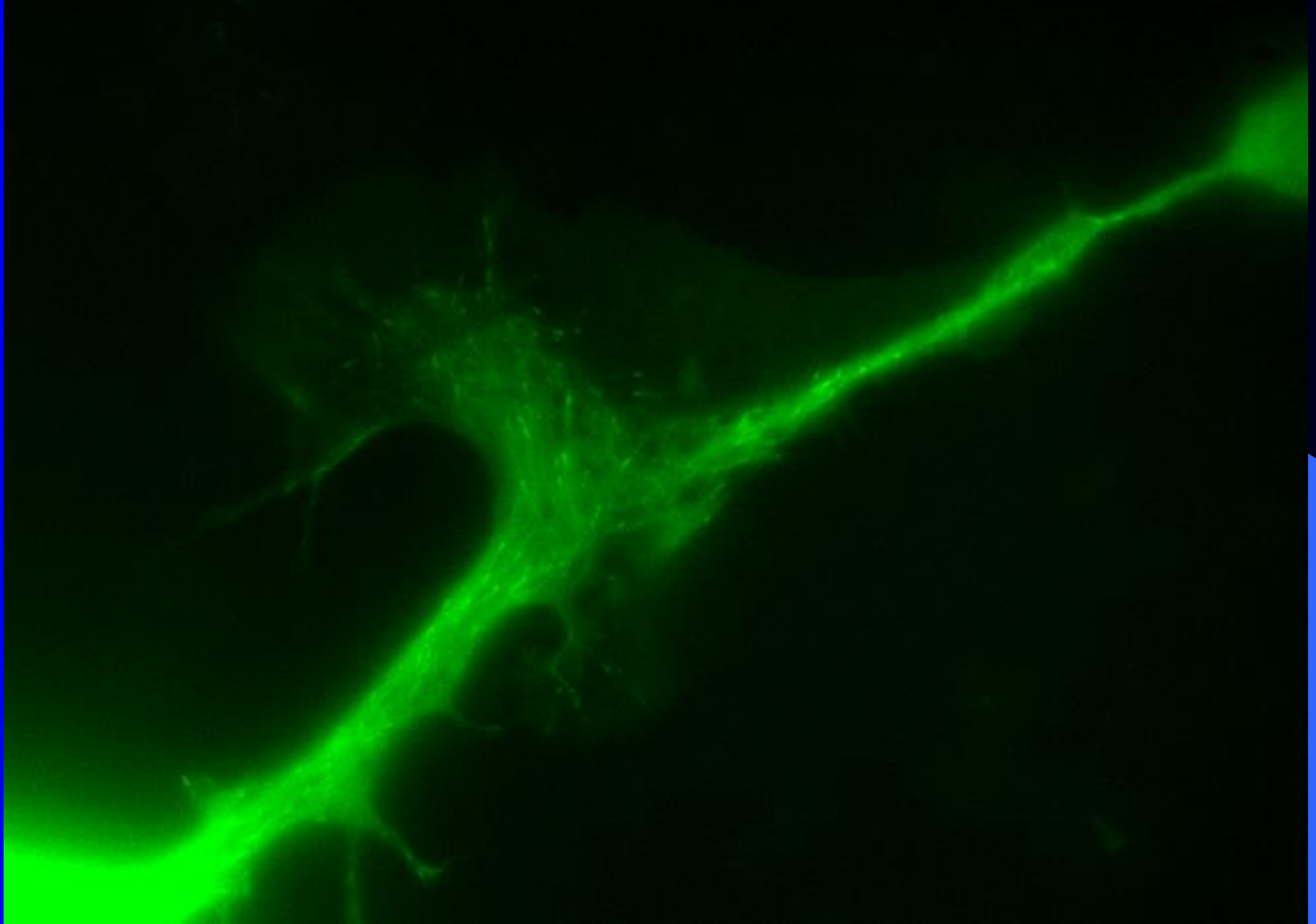


EB-1-YFP in COS7 cell

2 second / frame, total 151 frames, 5 min



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



Frame-Mode xyt Configuration

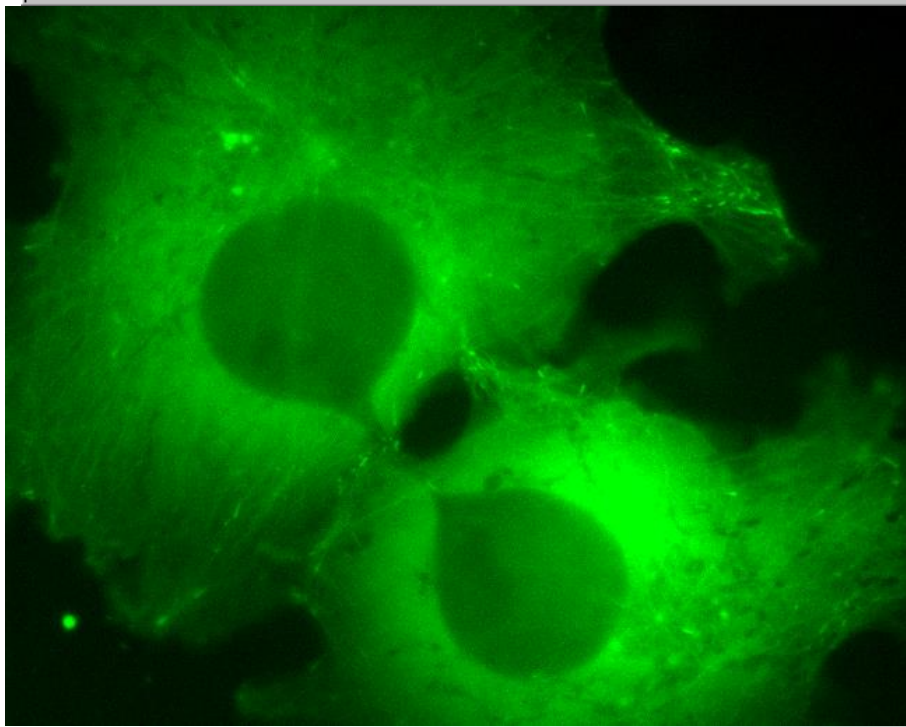
Time-lapse vs. Real Time (movie)

T Configuration

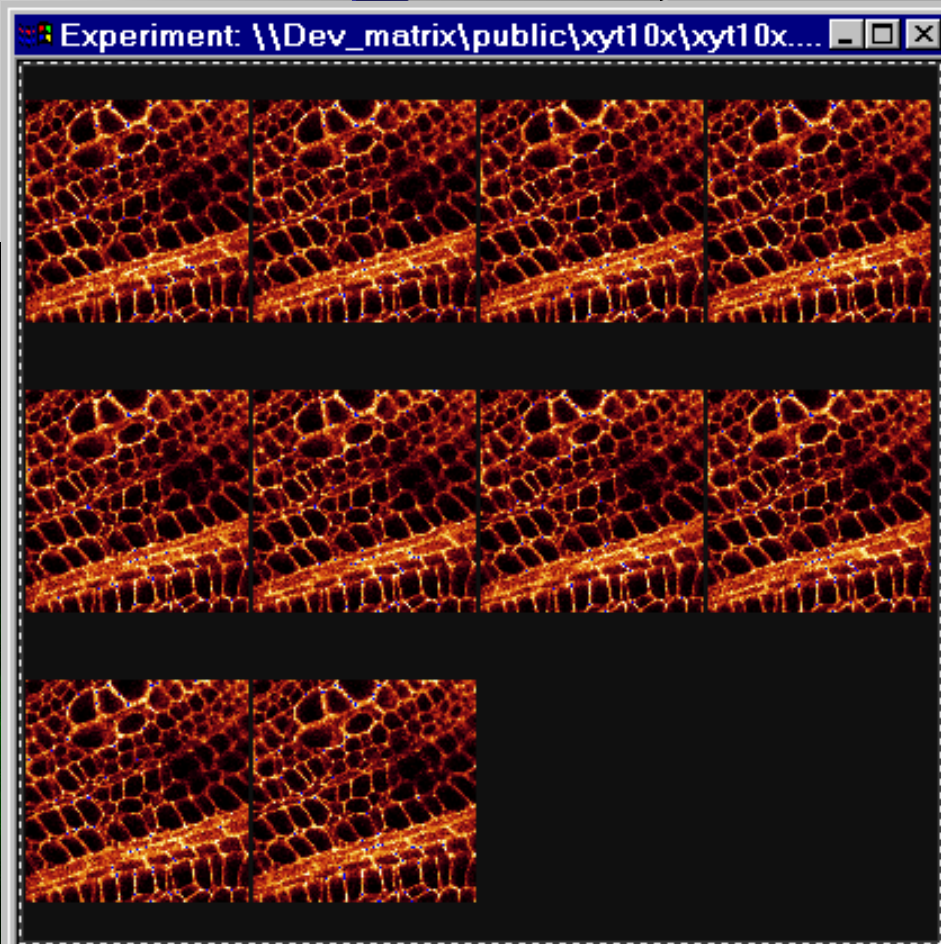
☐ ΔT : h min s ms

☐ Frames:

☒ Complete Time: h min s ms



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



Leica DM IRE2 microscope

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



CO₂ controller

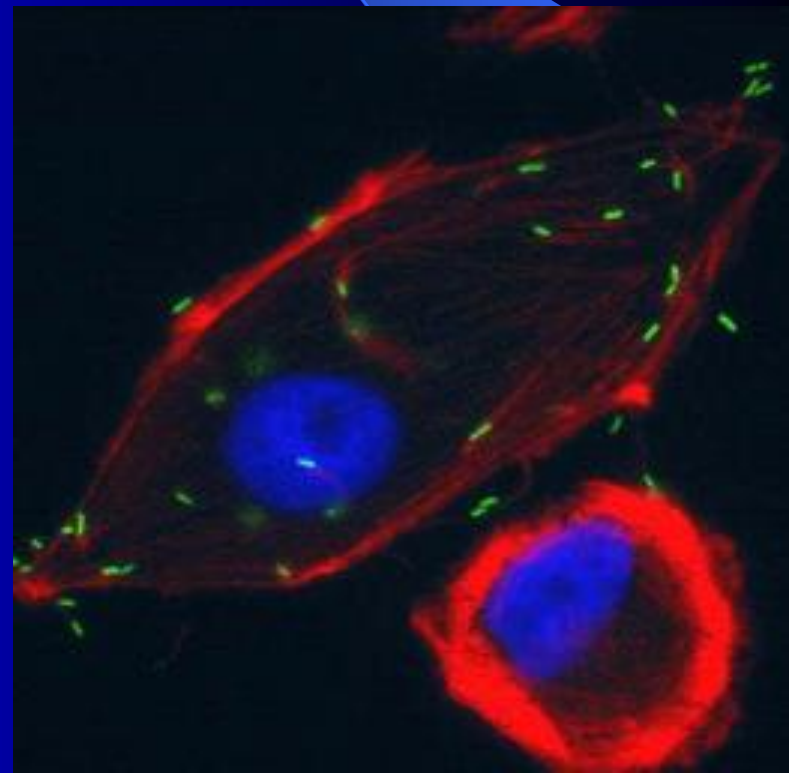
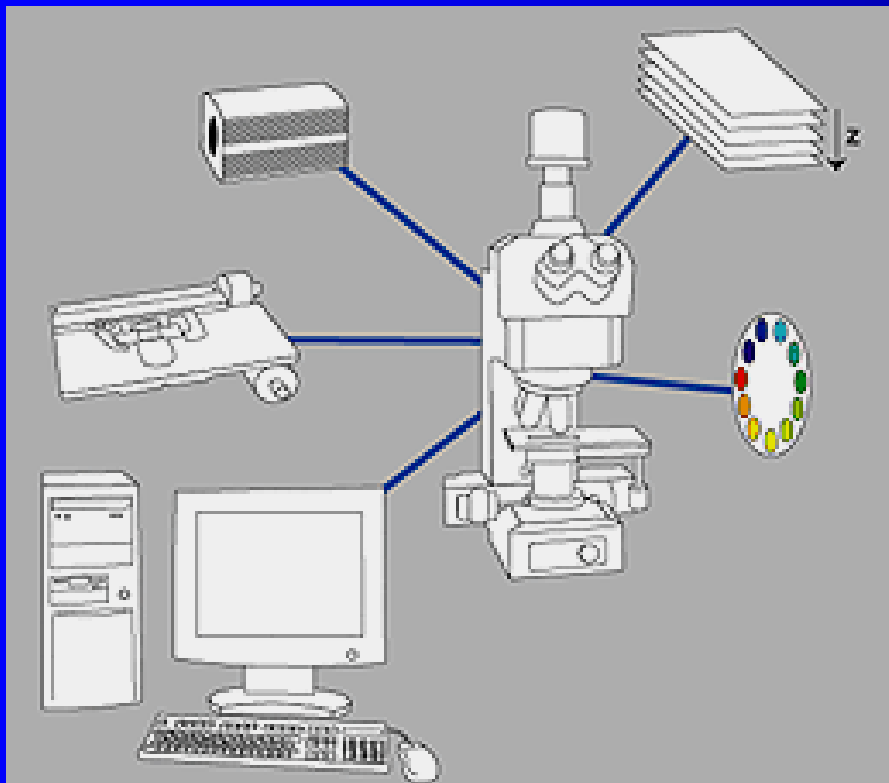
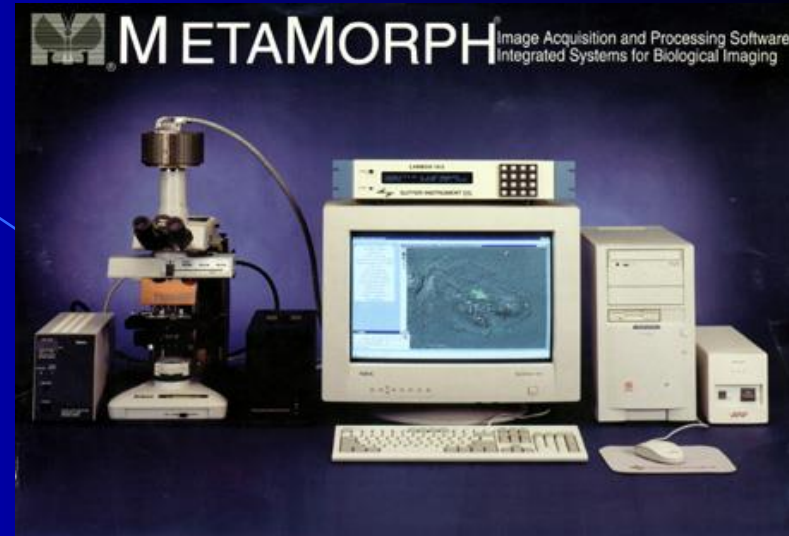


**Microincubation
Imaging-Chamber**

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





Real-Time Cultured Cell Monitoring System

— Supporting the Challenge of Discovery —



Designed to Fit...

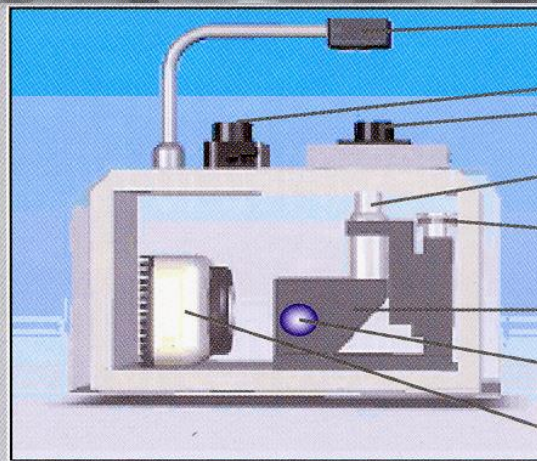
Designed to Resist...

Designed to Discover...



Microscope Now Rests in Incubator!!

Stem cell proliferation
Long-term recording
(movie)



White LED

Sample Stage Dial

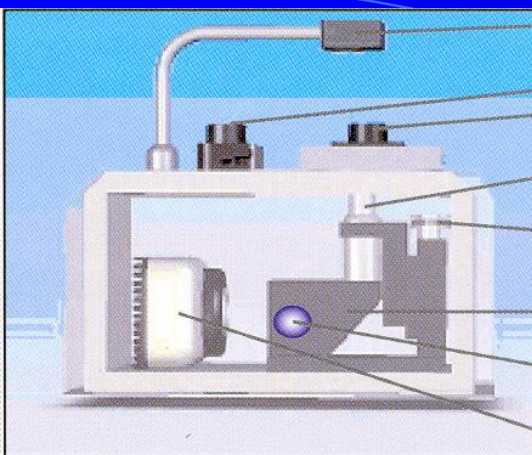
Objective Lens

Motorized Focus

Fluorescence Filter Unit

Blue LED

CCD Camera



ASTEC **Real-Time** **Cultured Cell** **Monitoring** **System**

弘優科技代理

	CCM-330F	CCM-500F
Resolutions	3.3 Mega Pixels (2048x1536)	5.0 Mega Pixels (2560x1920)
Camera / Chip Size	Cooled CCD / 1/2 Inch	Cooled CCD / 2/3 Inch
Cooling	Peltier Divice RT-10℃	Peltier Divice RT-10℃
Pixel Size	3.45μm x 3.45μm	3.4μm x 3.4μm
Field of View (Objective X10)	707 x 530 μm	870 x 650 μm
Exposure Time	1.6μs x 17.9min	1.6μs x 17.9min
Capturing Interval	1min - 24h	1min - 24h
Image Format	TIFF / BMP	TIFF / BMP
Objective Lens (Standard)	X 10 / NA0.22	X 10 / NA0.22
Integrated magnification (17" LCD monitor)	X 440	X 360
Light Source (VIS)	White LED	White LED
Light Source (FL)	Blue LED	Blue LED
Excitation Filter	472.5nm Half band width 30nm	472.5nm Half band width 30nm
Fluorescence Filter	520nm Half band width 35nm	520nm Half band width 35nm
Dichroic Mirror	503nm - 730nm	503nm - 730nm
Focus Adjustment	Remote Control from the Controller	Remote Control from the Controller
PC	WindowsXP Professional SP2	WindowsXP Professional SP2
CPU	Intel Pentium4, 3.0GHz 512MB and up	Intel Pentium4, 3.0GHz 512MB and up
Standard Display	SXGA 17" LCD display	SXGA 17" LCD display
Camera Unit Dimensions	W165 x D275 x H165 (8.0kg)	W165 x D275 x H165 (8.0kg)
Controller Dimensions	W220 x D260 x H120 (6.0kg)	W220 x D260 x H120 (6.0kg)

Real-Time Cultured Cell Monitoring System

ASTEC

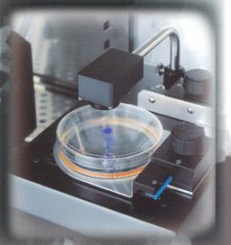
— Supporting the Challenge of Discovery —



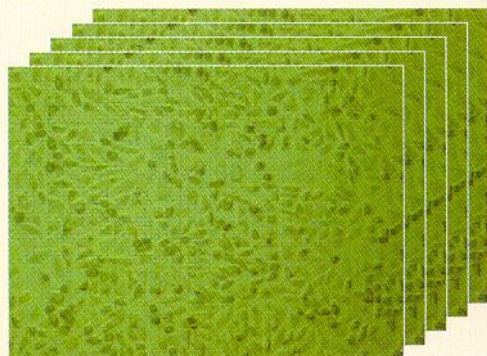
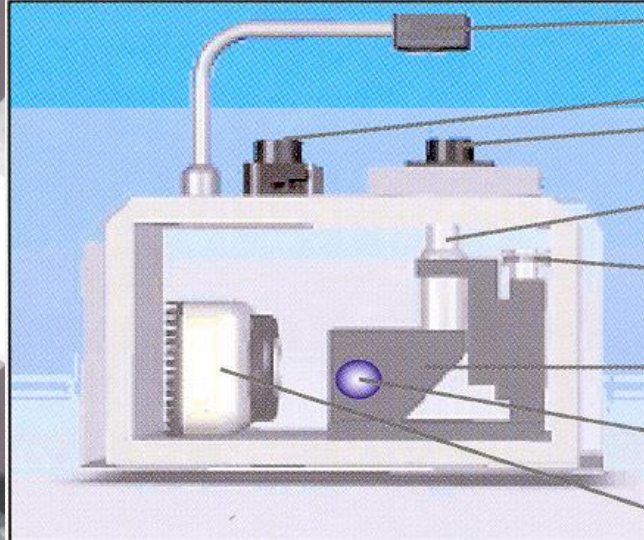
Designed to Fit...

Designed to Resist...

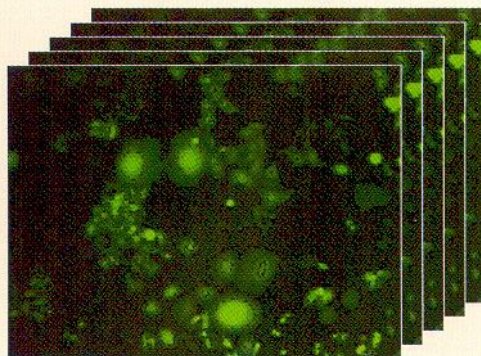
Designed to Discover...



Microscope Now Rests in Incubator!!



形態観察画像



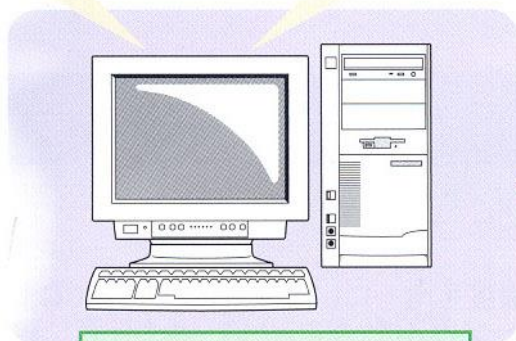
蛍光観察画像

CO₂インキュベーター
(37°C 95%RH 以上)



カメラユニット

・撮影



・画像取込 ・編集



コントロールユニット

・ライトコントロール
・エアーポンプ
・フォーカス