基因產物在細胞中之定位技術
Distributions of Cellular Proteins

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前言

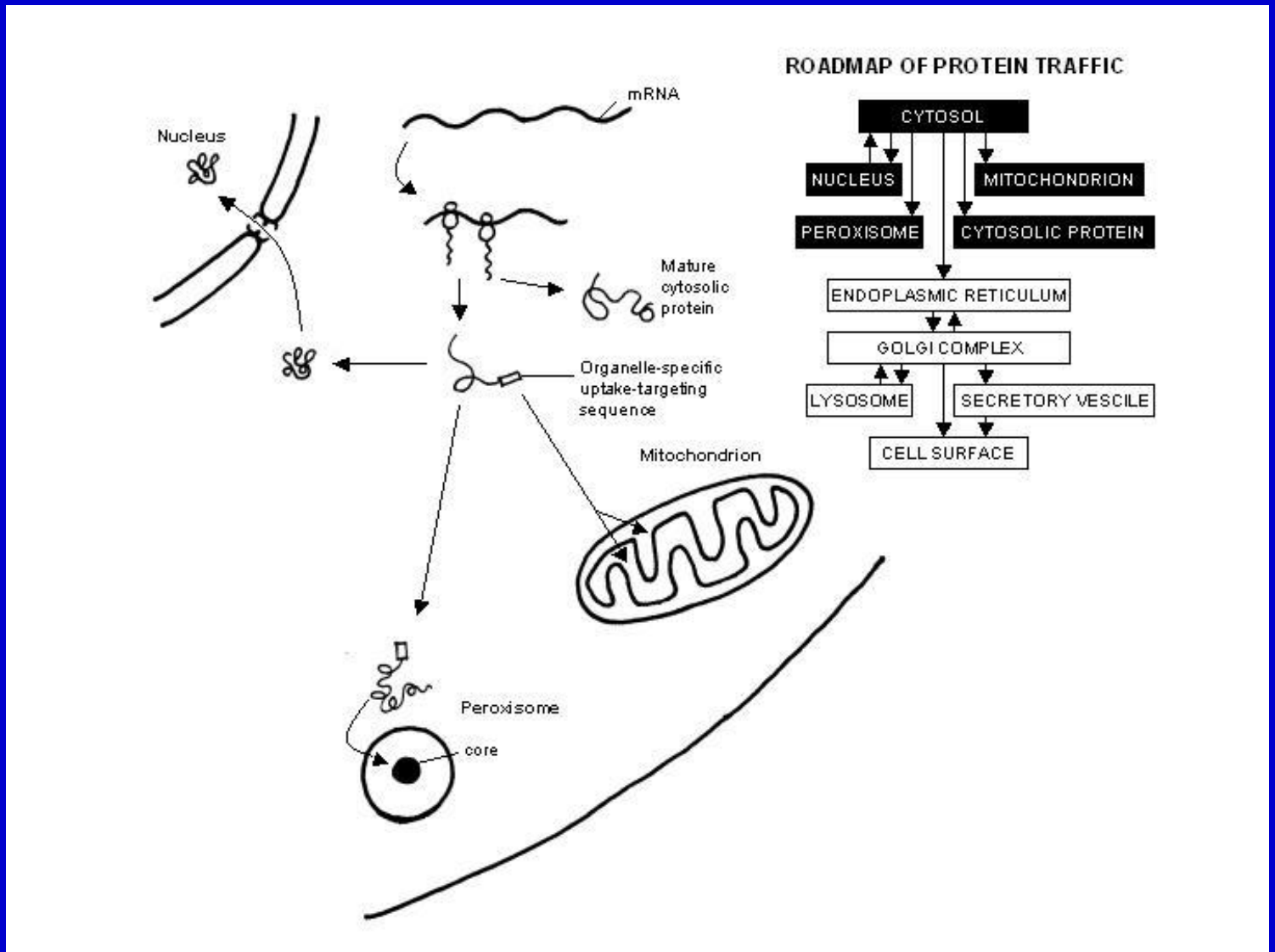
- 由於後基因體時代的來臨，新發現且具有功能的基因產物蛋白質在細胞中的分佈，即成為近日研究的重要課題。

- 特殊蛋白質從細胞、組織、乃至於生命個體中的生理功能，常可由其在細胞的生成與分佈而推斷得知。

- 細胞胞器類別，分別介紹蛋白質，在各胞器分佈的可能機制。並介紹一些目前研究蛋白質的常用細胞生物學方法，以期運用在基因體學研究，奠定並釐清功能性蛋白質的學理基礎。

細胞核 (Nucleus) (Protein sorting, movie)

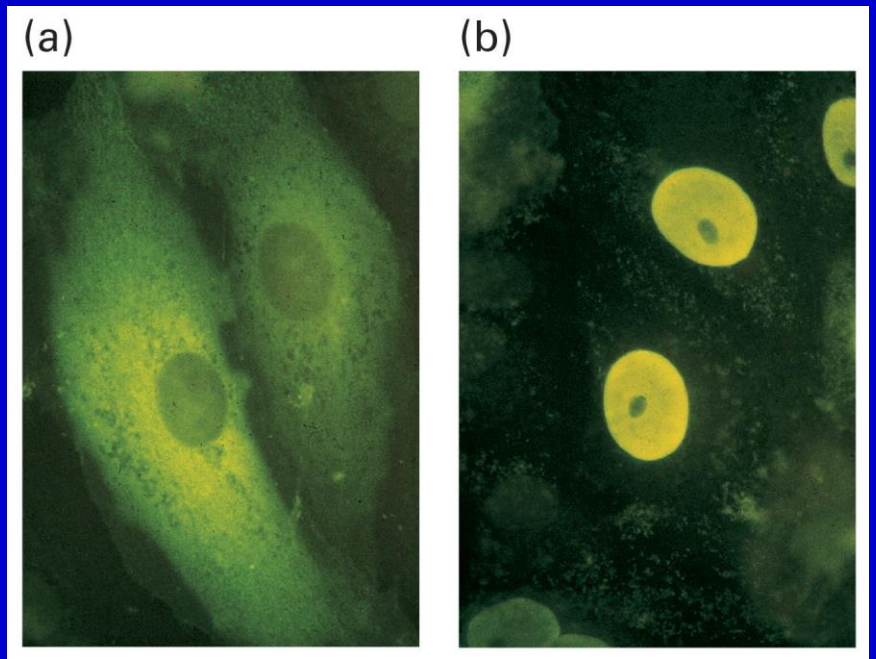
・大多數的核內功能性蛋白質由細胞質中的核醣體合成再運送至細胞核中，如調控細胞基因表現的轉錄因子(transcriptional factors)。

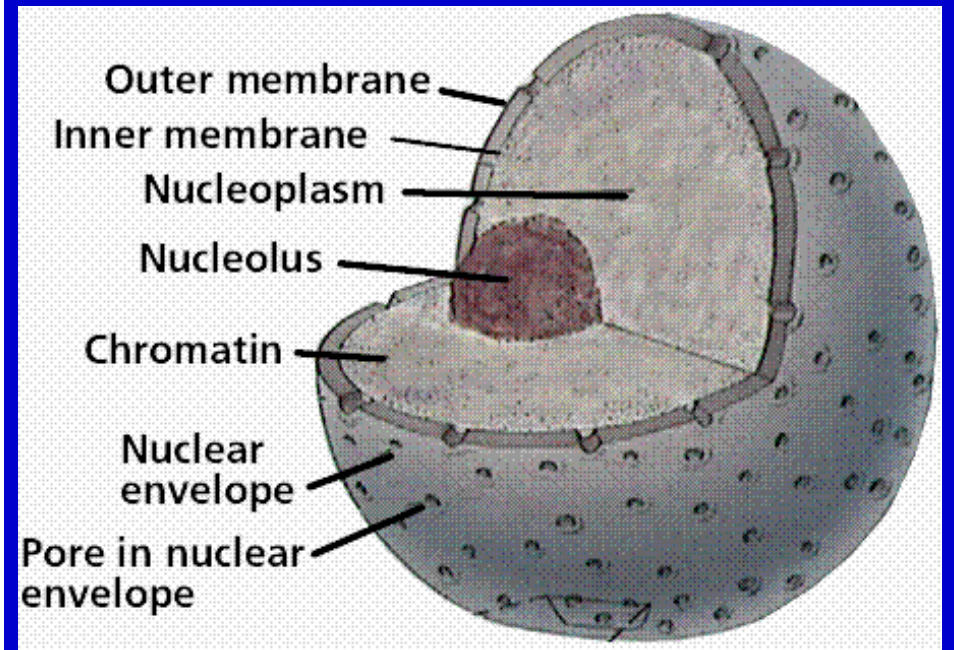


細胞核 (Nucleus)

• 目前已知許多核內功能性蛋白質在其氨基酸序列上均具有一段特殊的訊息序列如 –Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-，稱為核轉置訊息(Nuclear Location Signal, NLS)。如果在新發現的蛋白質中，藉由序列比對找到此NLS訊息序列，即可推斷此蛋白質合成後可能會被送入細胞核中。

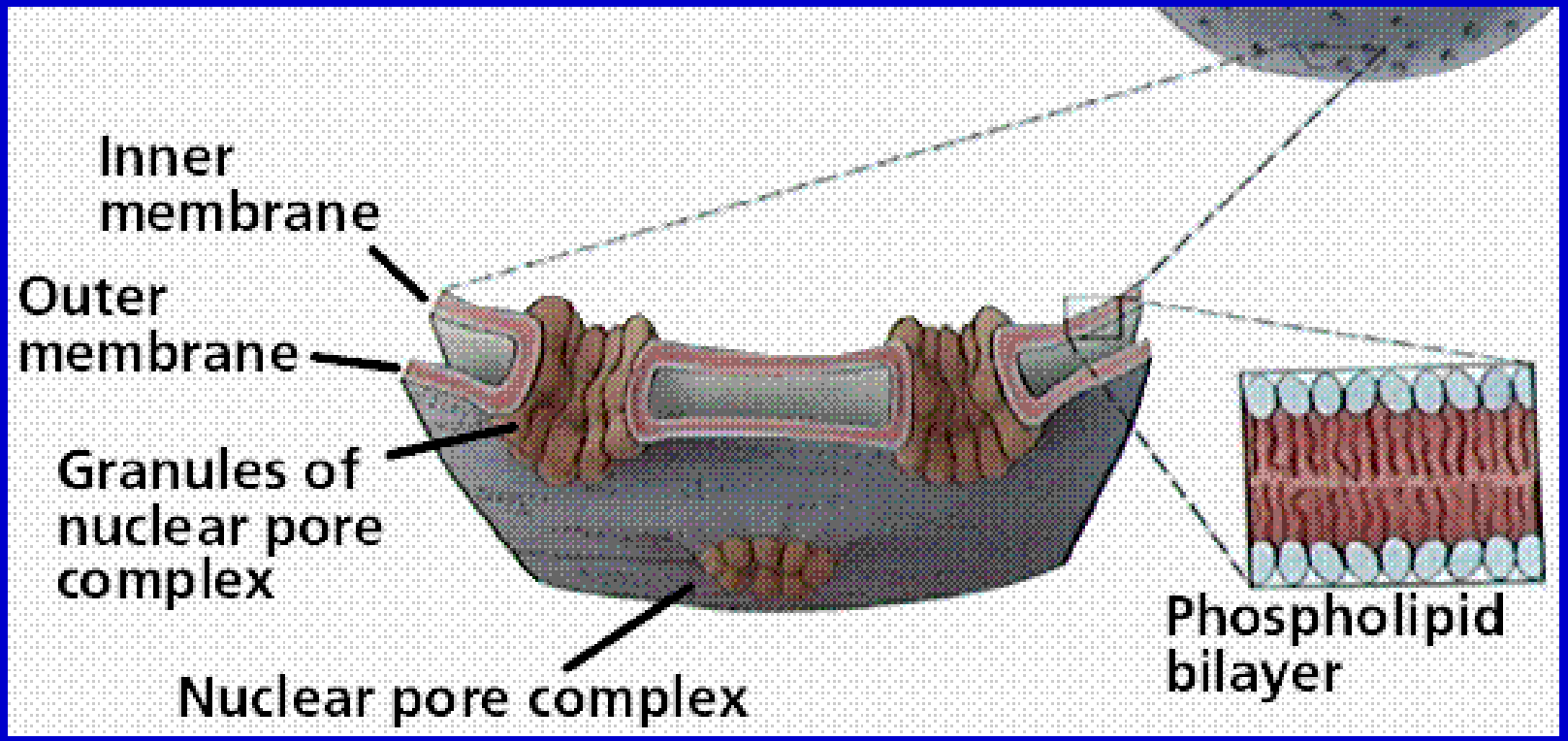
• 此分佈機制只是針對較大分子的蛋白質，這些蛋白質須要靠核輸入接受器 (nuclear import receptors) 來辨識NLS訊息序列，進而與特殊運送蛋白(cargo proteins)結合，藉由主動運輸(active transport)將其穿過核孔(nuclear pores)而送入細胞核中。





細胞核 (Nucleus)

• 對於一些較小分子的蛋白質，則不須要具備此NLS訊息序列與特殊運送蛋白，可藉由擴散或是其他運送機制穿過核孔而送入細胞核中。基本上，蛋白分子大於60,000 daltons即不太可能藉由擴散自行穿過核孔而進入細胞核中；對於較小分子的蛋白質的擴散，如5,000 daltons以下的蛋白質，核孔是完全通透的。



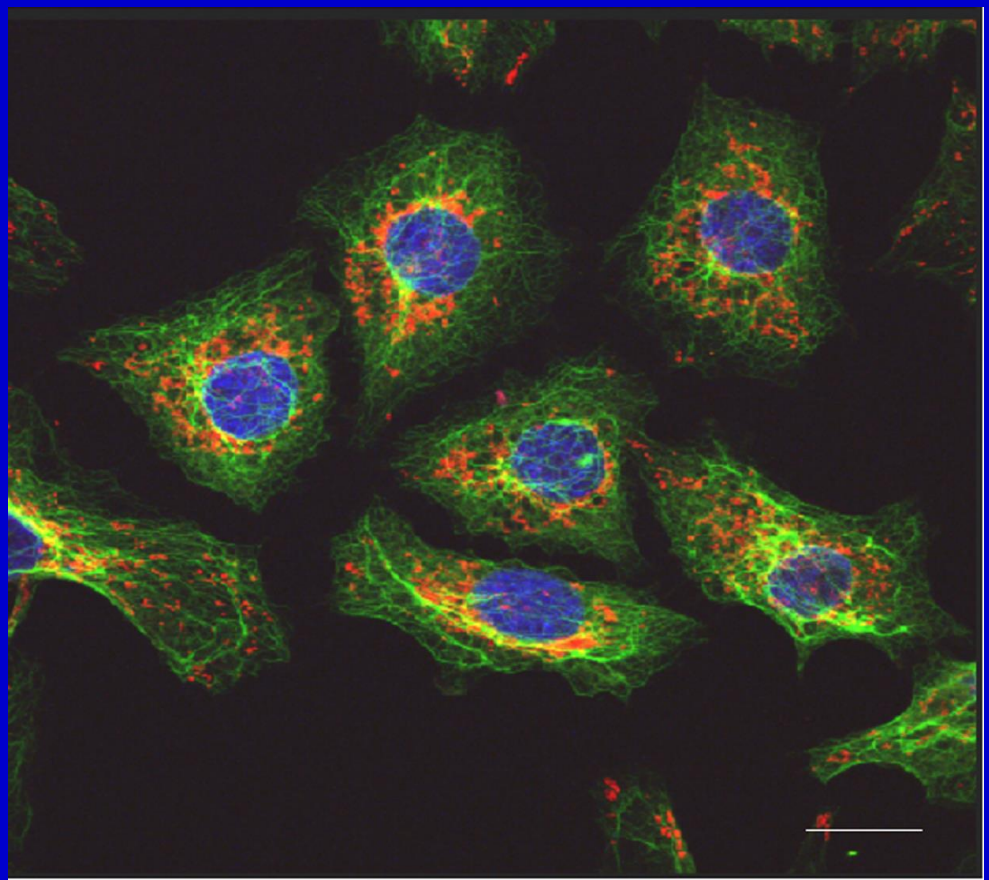
細胞核 (Nucleus)定位

1. 為瞭解新發現的蛋白質是否真能送入細胞核中，可藉由細胞轉殖實驗(cell transfections)或免疫細胞化學(immunocytochemistry)染色 (如有很好的專一性抗體)。

2. 再與DNA特殊染色如DAPI (激發光譜約為359 nm；與DNA接合後釋放光譜約為461 nm)或是Hoechst33342 (激發光譜約為343 nm；與DNA接合後釋放光譜約為483 nm)等染色來做對比，即可將標示蛋白質是否在細胞核中。

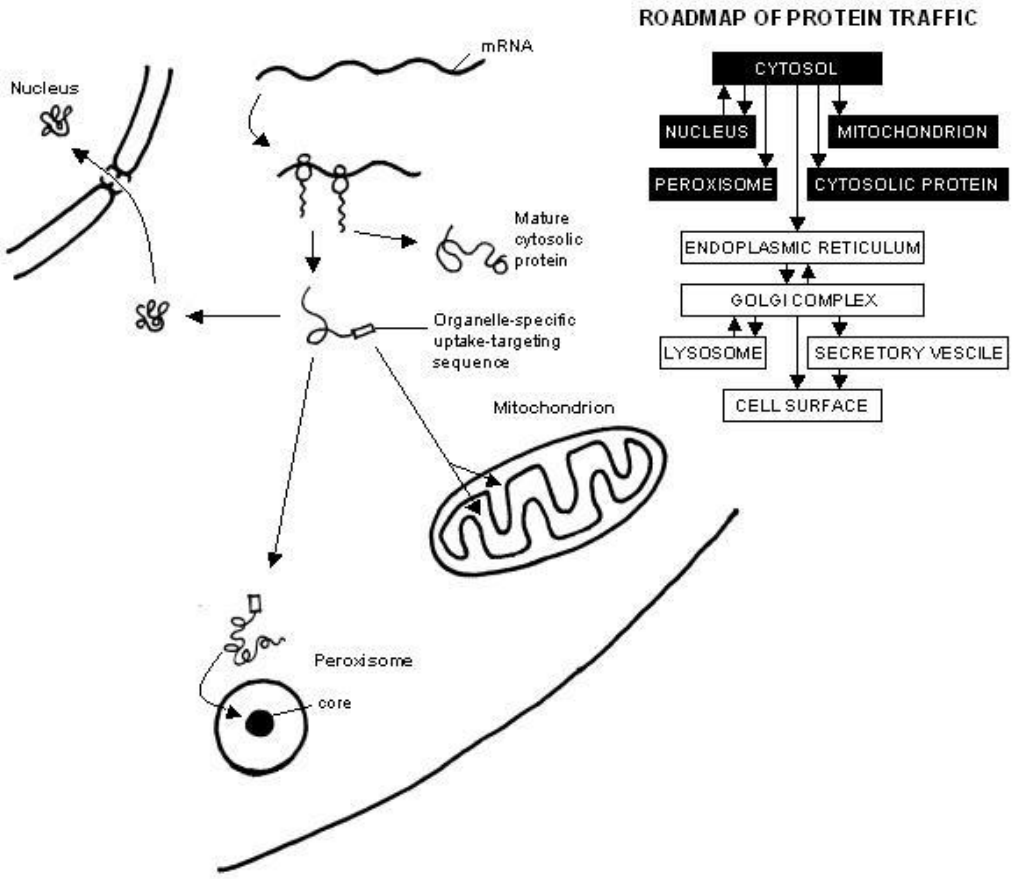
膀胱癌T24細胞螢光染色

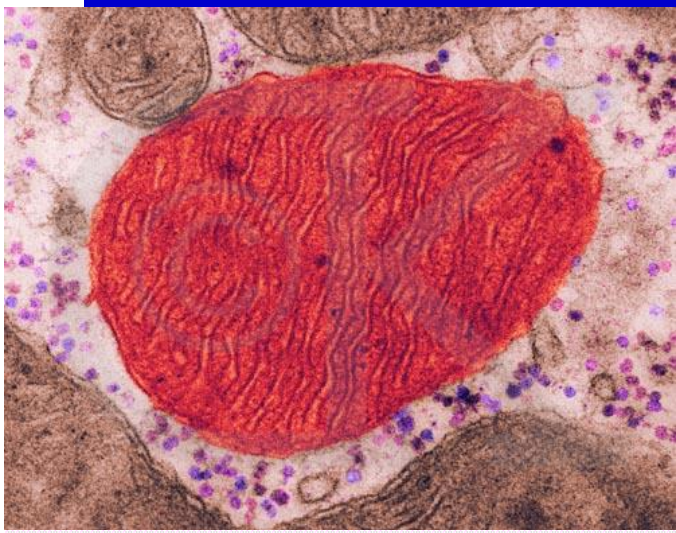
Hoechst 33342標示細胞核呈藍色，在細胞質中MitoTracker標示粒線體呈紅色， Keratin 18中間絲蛋白則呈綠色絲狀分佈。Scale bar = 20μm 。

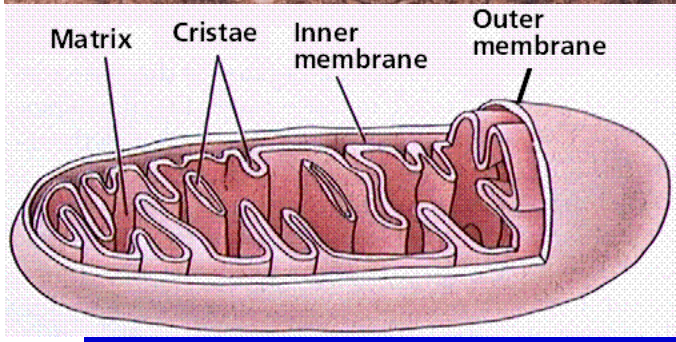


粒線體 (Mitochondria)

粒線體的蛋白質合成除了少部份由粒線體自身基因體(mitochondrial genome)轉錄轉譯而成外，大部份的蛋白質仍由細胞核的功能基因表現訊息RNA (mRNA)，藉由細胞質中的核醣體合成蛋白後再運送至粒線體內膜或基質(matrix)中。

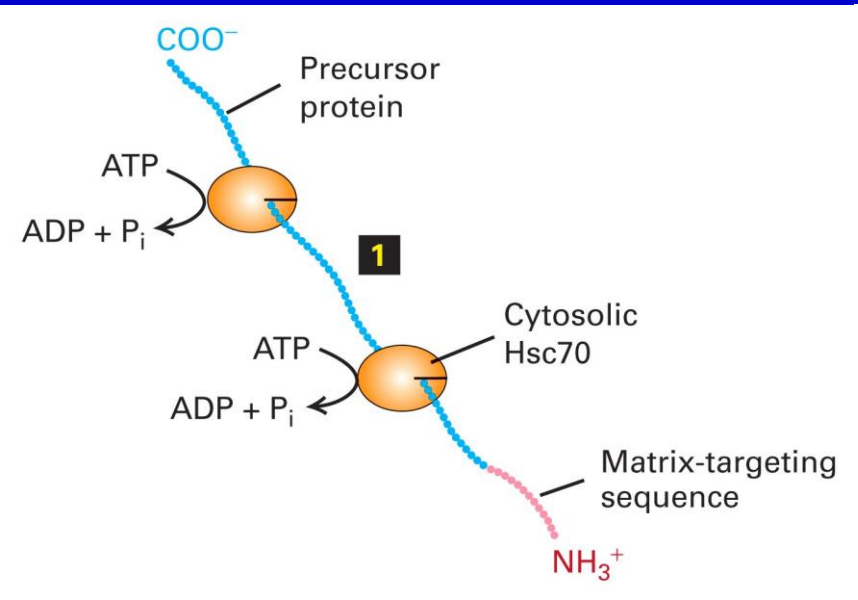


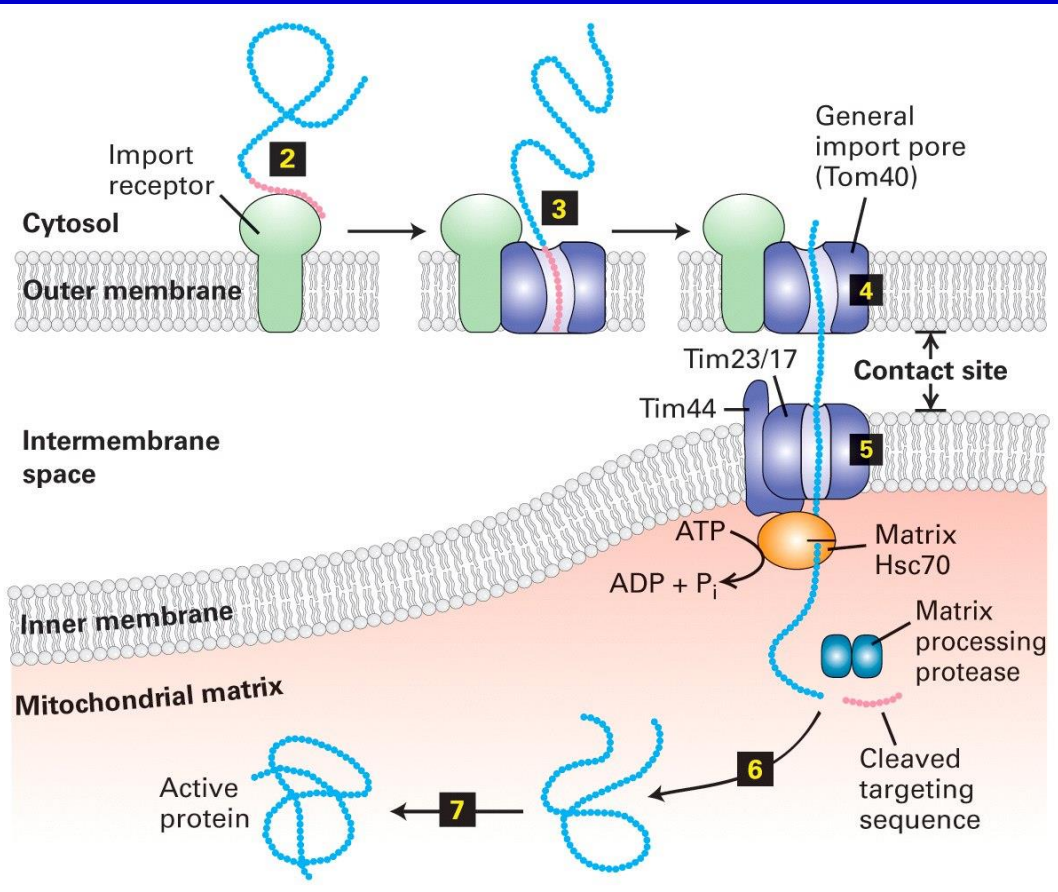




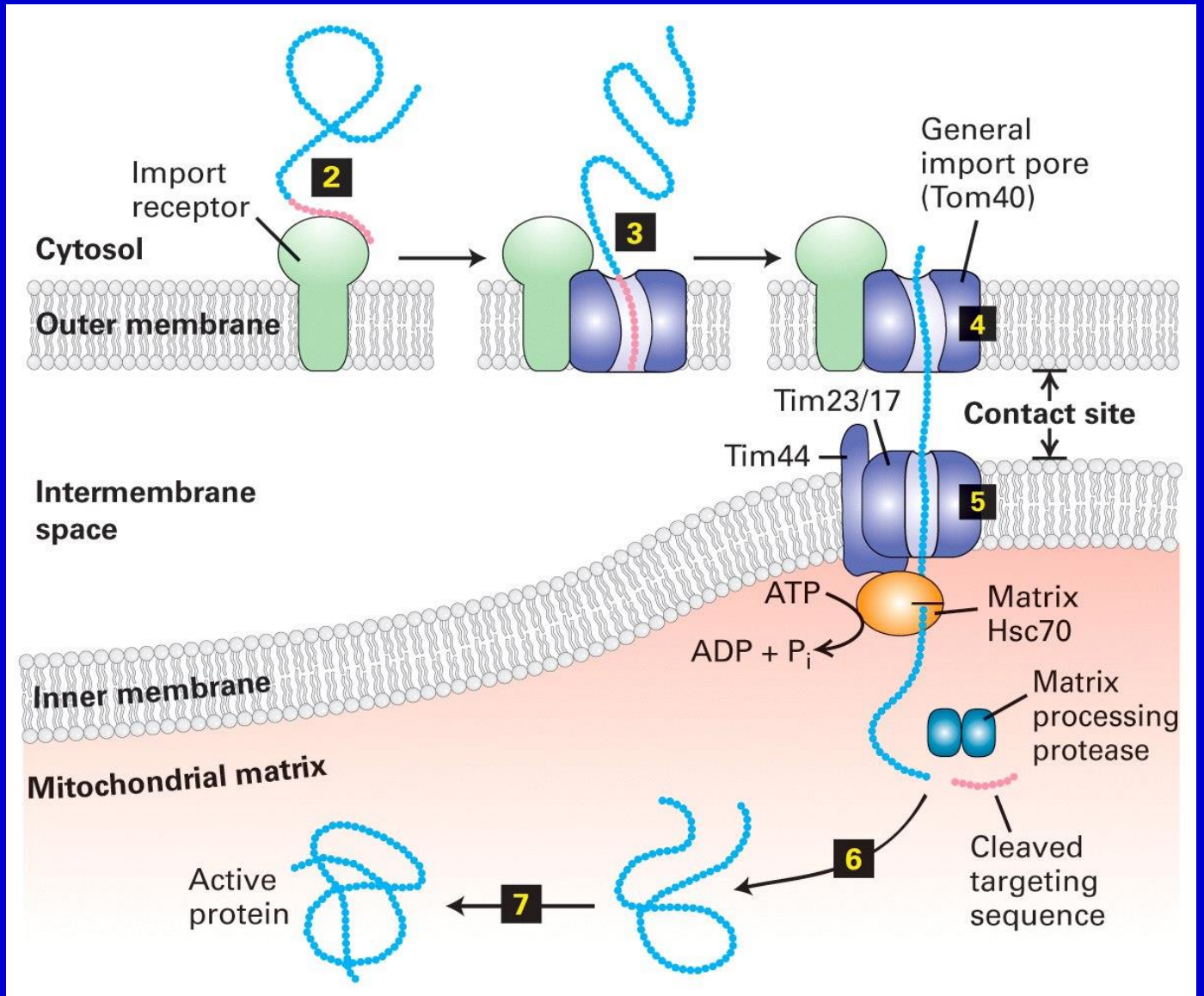
粒線體 (Mitochondria)

1. 如同核蛋白，粒線體的蛋白質亦具有特殊的訊息序列(signal sequence)供粒線體外膜上的特殊接受器辨識，這訊息序列多在蛋白的氨基端(N-terminus)： $+H_3N$ –Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-，而這序列常構成α-helix螺旋結構供粒線體外膜上的接受器辨識，進而藉由位於粒線體內膜上的三種不同的蛋白轉置者(protein translocators)運送至粒線體基質或嵌合在內膜中。





2. 粒線體的蛋白質運送的過程非常複雜，基本上須需要ATP能量，與特殊的熱誘蛋白70 (Heat shock protein, hsp 70)參與。而訊息序列在蛋白質被送入粒線體後，立即被特殊的訊息酶(Signal peptidase)切離分解。



粒線體 (Mitochondria)定位

1. 利用免疫細胞化學染色標示細胞內粒線體分佈位置時，常用粒線體特有蛋白的抗體，如細胞色素C氧化酶 (cytochrome c oxidase)等參與粒線體能量代謝的蛋白的抗體。

2. 然而亦可利用一些對於粒線體呼吸膜電位變化非常敏感的螢光化學物，如 rhodamine 123 (激發光譜約為504 nm；釋放光譜約為534 nm)來標示活細胞中的粒線體分佈位置。

3. 也有一些經過改良的螢光染劑如Mito Tracker dyes (Molecular Probes)等，可染完活細胞後再將細胞固定(fixed)，仍然可以觀察到染上的粒線體螢光。當然這些螢光化學物的濃度不能太高(小於1 mM)否則將照造成粒線體的傷害。

粒線體 (Mitochondria)定位

- Mito Tracker dyes (Molecular Probes)等，可染完活細胞後再將細胞固定(fixed)，仍然可以觀察到染上的粒線體螢光。

膀胱癌T24細胞螢光染色

Hoechst 33342標示細胞核呈藍色，在細胞質中MitoTracker標示粒線體呈紅色， Keratin 18中間絲蛋白則呈綠色絲狀分佈。
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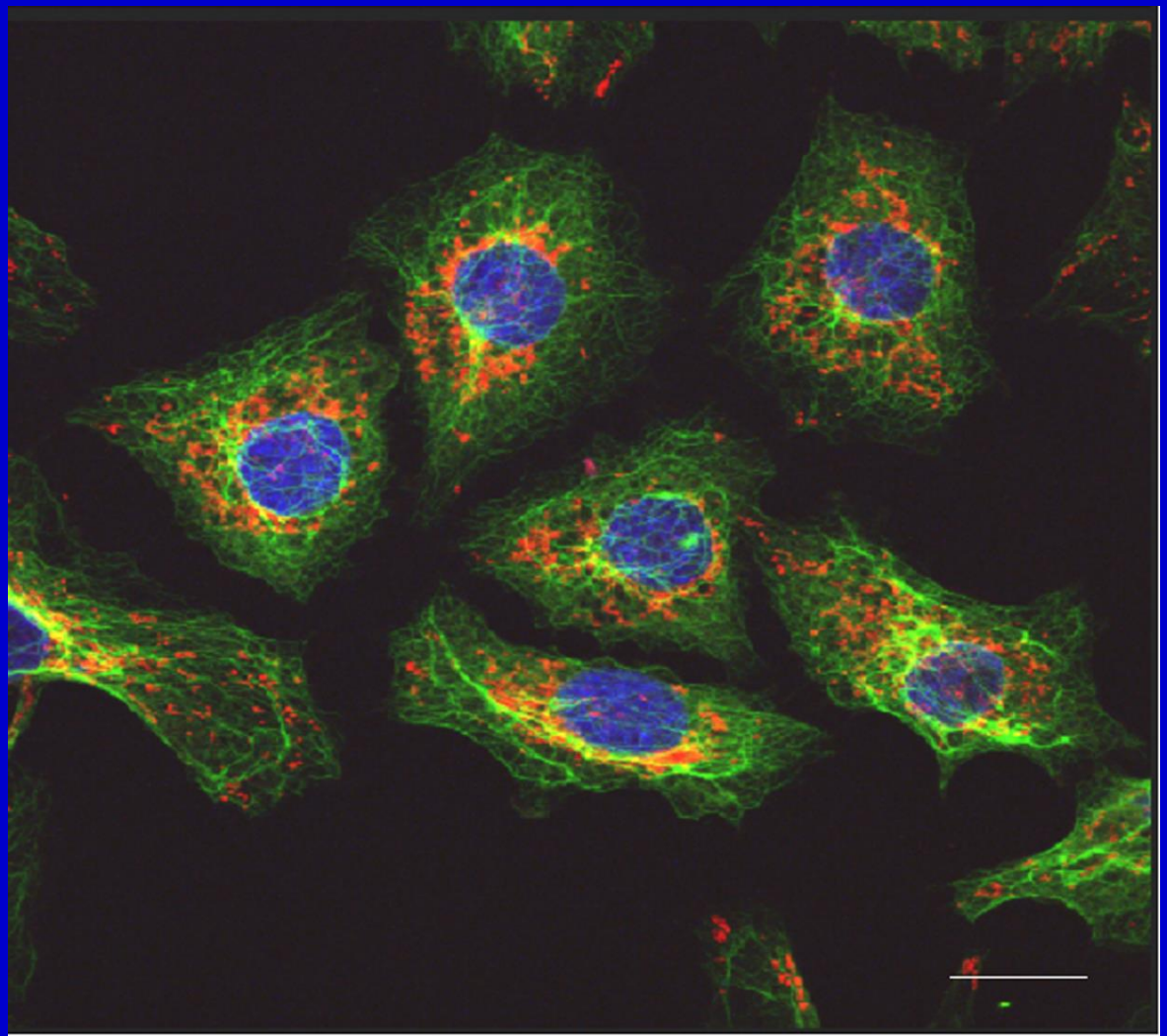
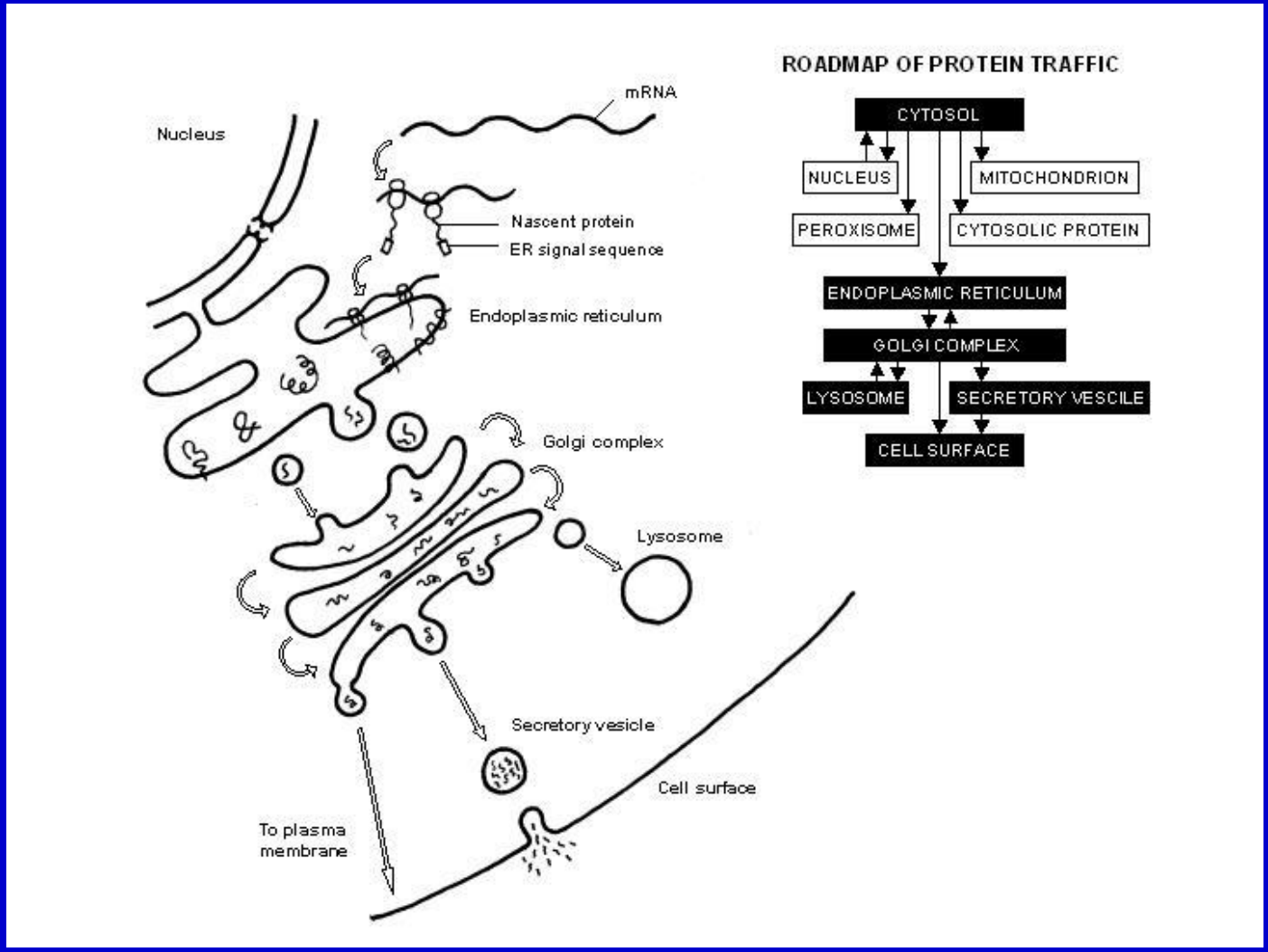


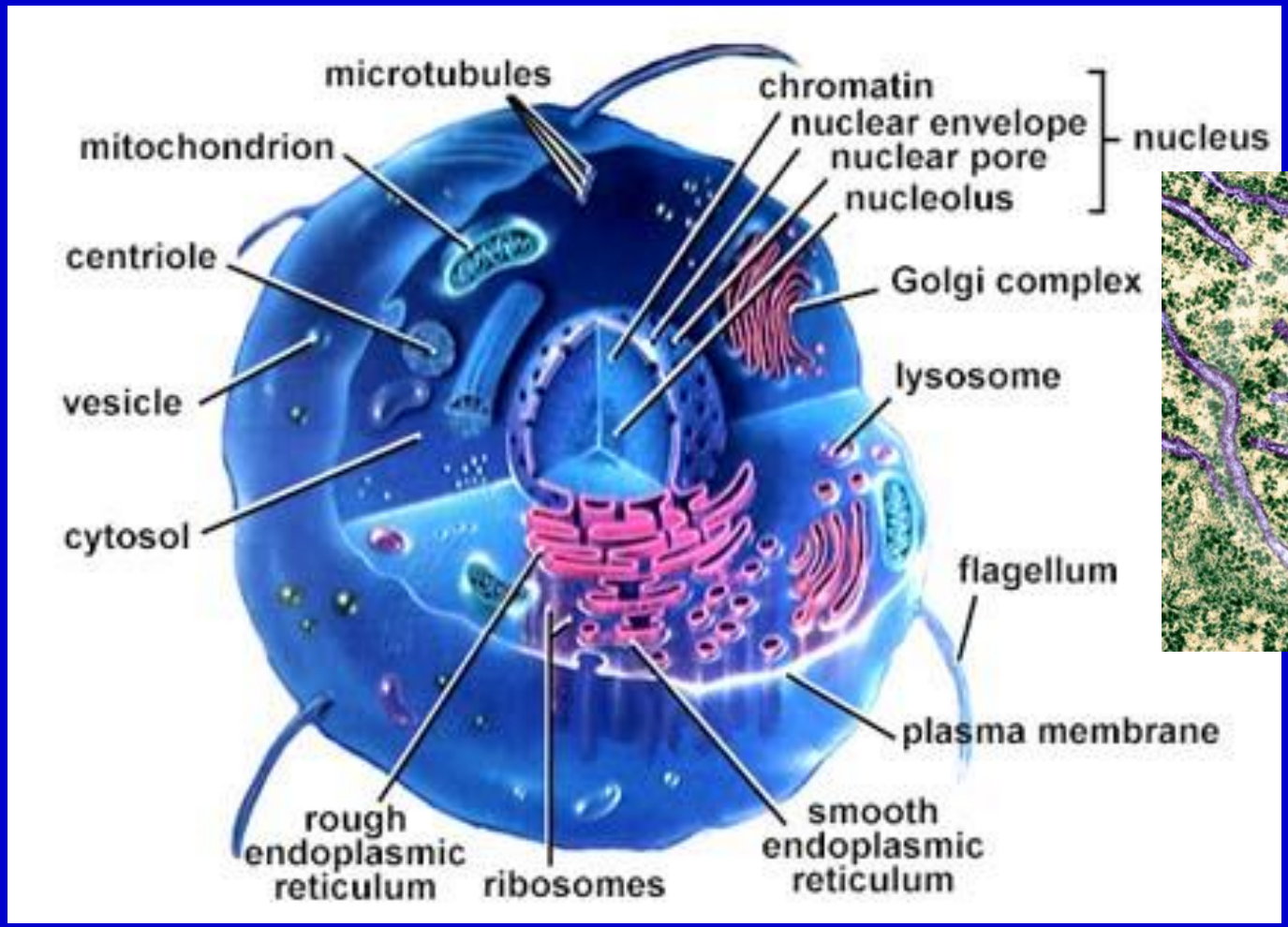
顆粒性內質網 (rER)與高爾基氏體 (Golgi apparatus)

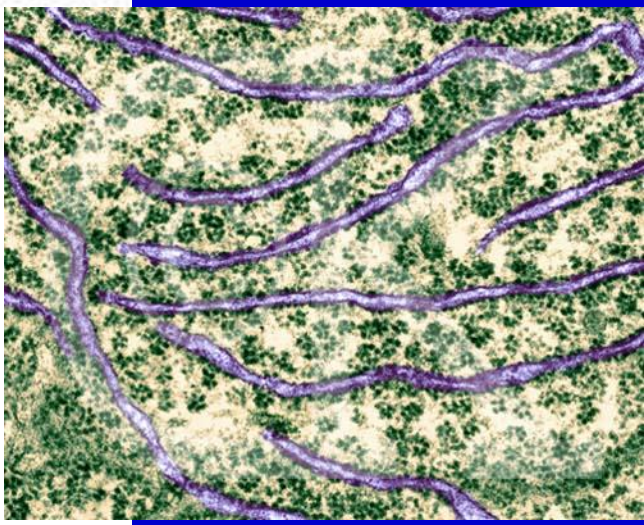


內質網內合成蛋白的運送途徑。蛋白質在內質網合成及在高爾基氏體修飾後，可分別送至溶酶體 (Lysosome)、分泌小泡 (Secretory vesicles)及細胞膜(Plasma membrane) 。Movie

顆粒性內質網 (rER)

細胞中許多與細胞膜相關的蛋白或是分泌至細胞外的部分蛋白，其合成與轉譯後修飾(Post-translational modifications)主要均在顆粒性內質網 (rough endoplasmic reticulum, rER)與高爾基氏體 (Golgi apparatus) 。

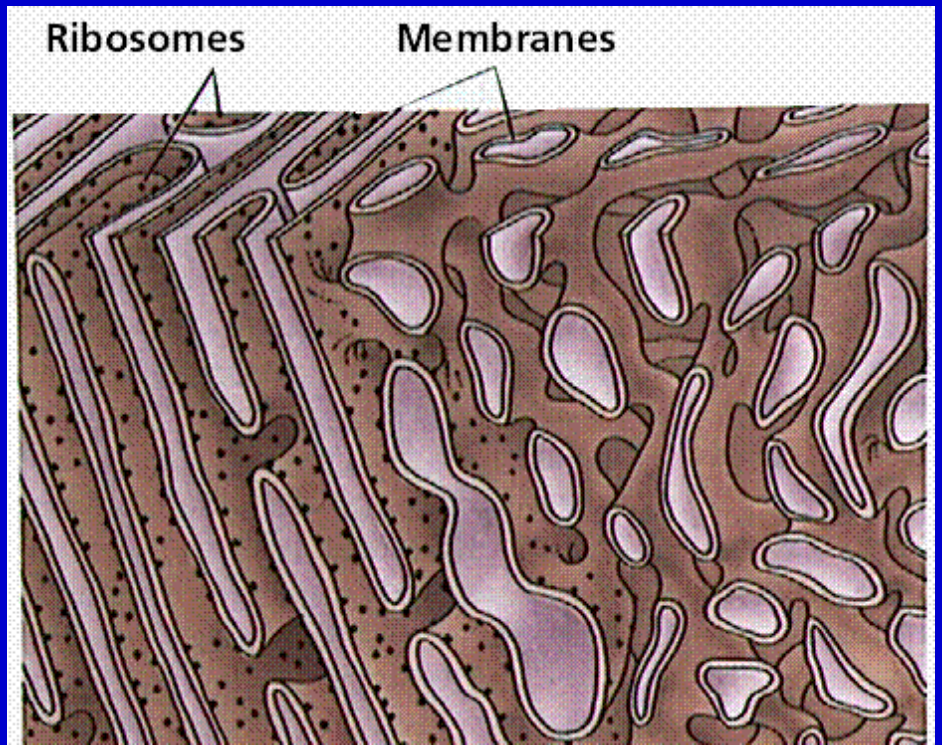


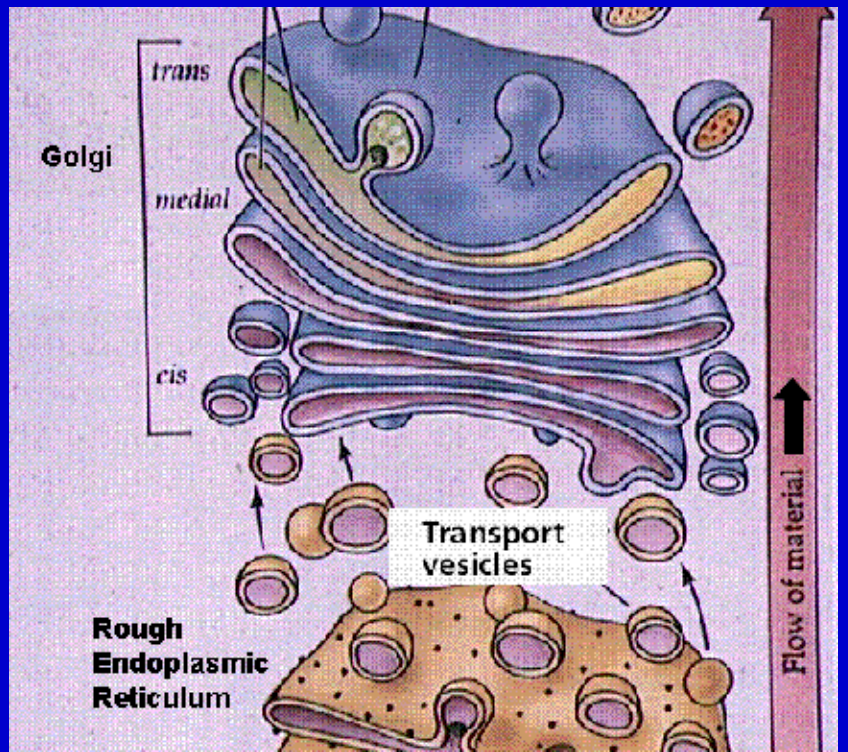


顆粒性內質網 (rER)

而顆粒性內質網與高爾基氏體內的蛋白質常常有動態的變化，因此欲定位新發現的蛋白質是否只限於顆粒性內質網或高爾基氏體內，就比較不容易界定。

然而在蛋白質的氨基端 (N-terminus)起始訊息序列若具備：
+H_3N-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln- 內質網訊息序列，蛋白質即可被運送至內質網腔(ER lumen)或嵌合在內質網膜上。大底上蛋白質在內質網經一些修飾，如醣化作用(glycosylation) 後，再送往高爾基氏體。





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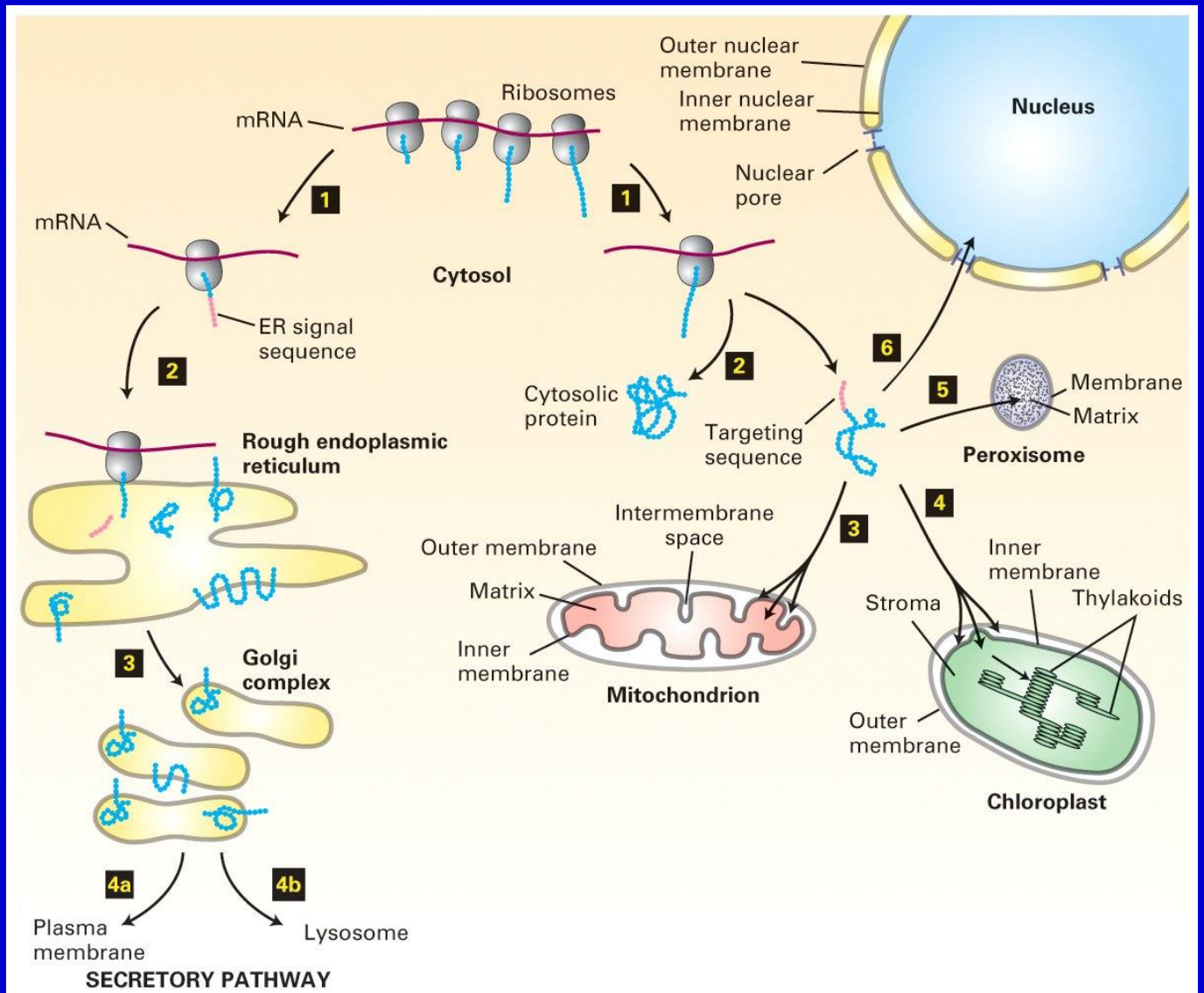
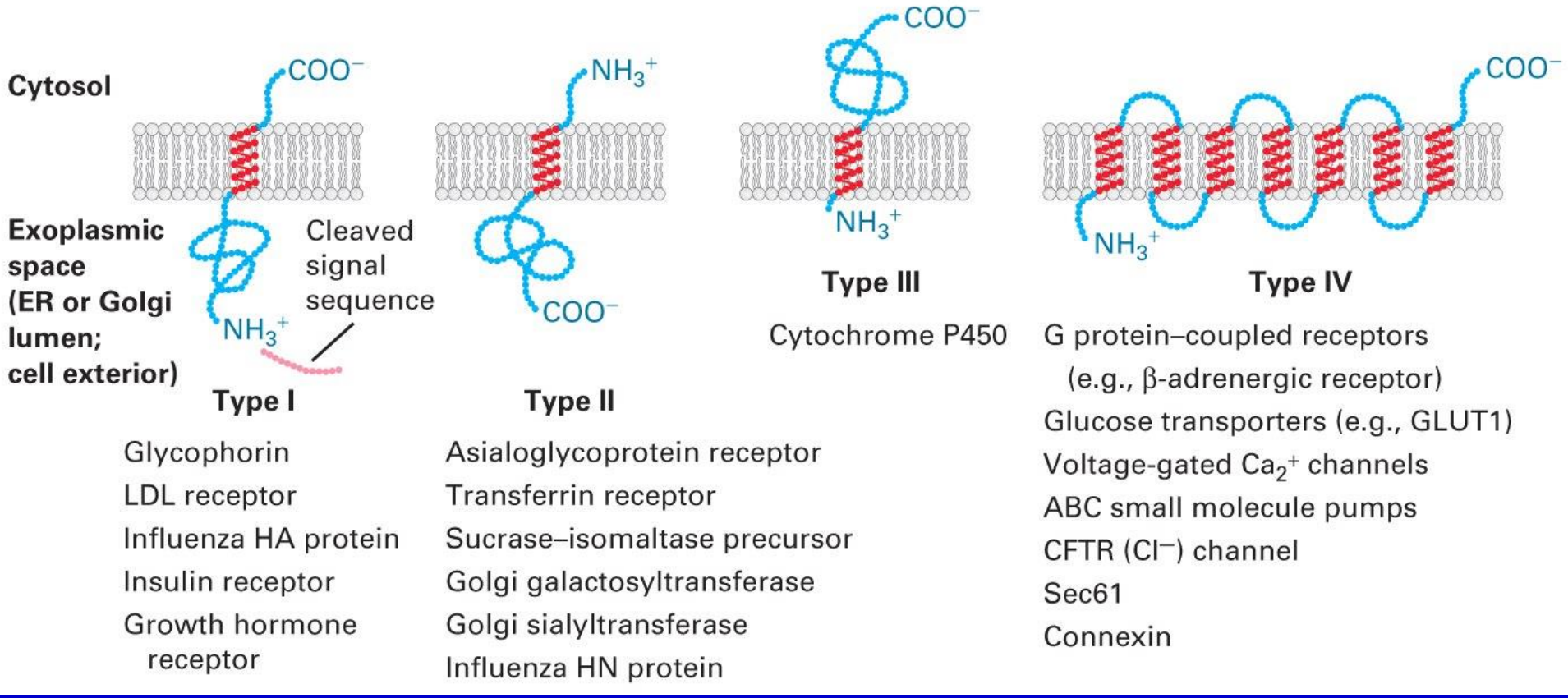
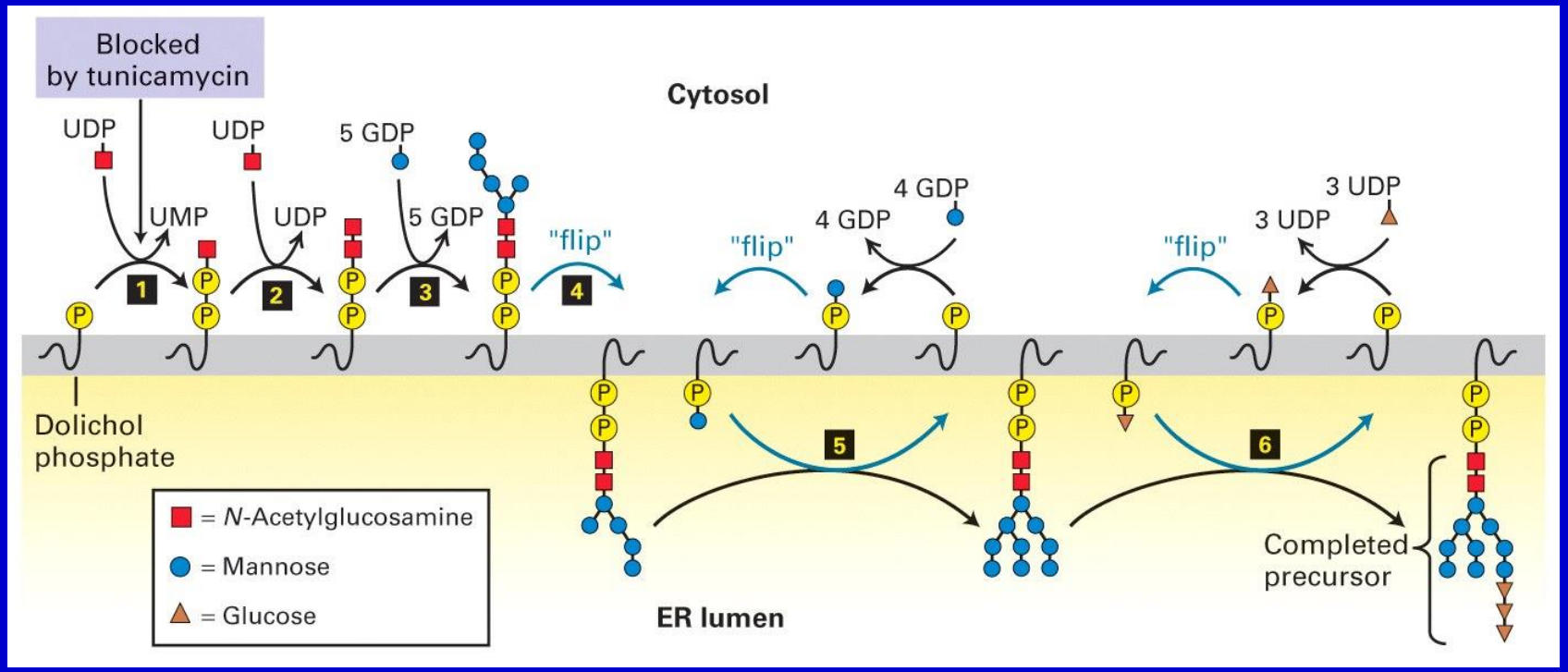
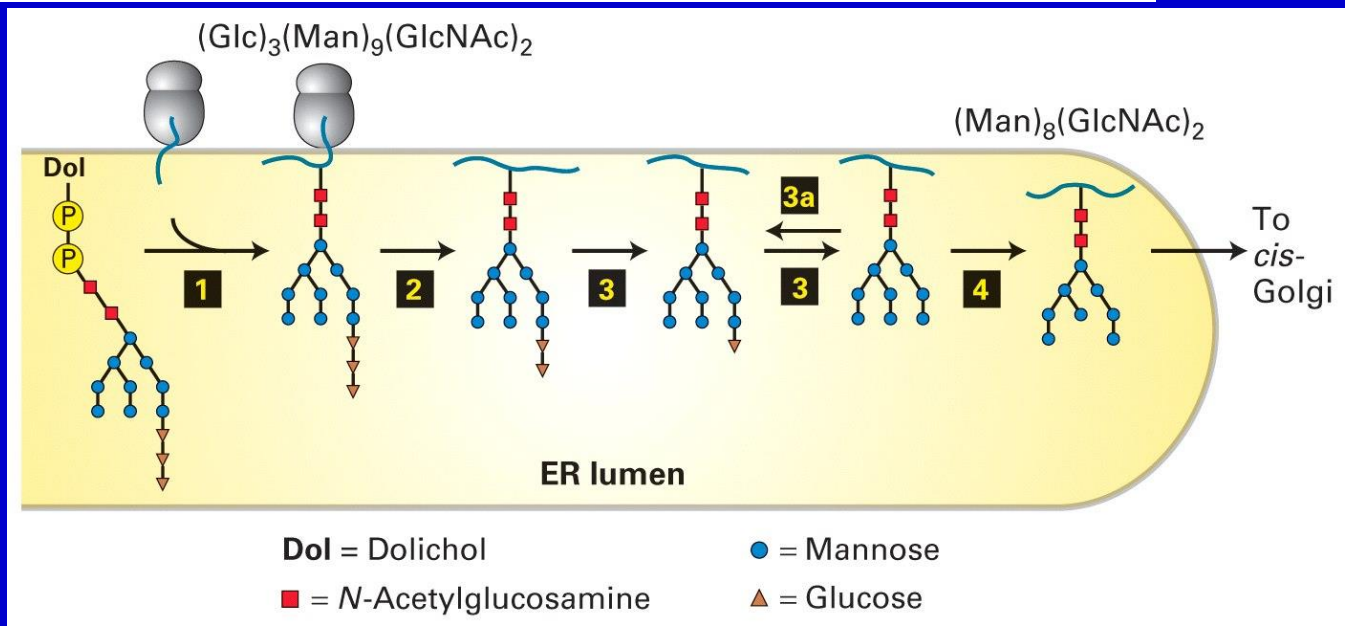


Major topological classes of integral membrane proteins



蛋白質在內質網經一些修飾，如glycosylation 後，再送往高爾基氏體





顆粒性內質網 (rER)定位

• 嘗試利用免疫細胞化學染色來標示出在細胞內顆粒性內質網新合成的蛋白質，有許多技術上的考量，例如抗體的辨識區段是否有經修飾？

• 假若專一抗體的辨識區段是屬未經修飾的氨基酸序列，則可能無法在細胞中正確標示經過轉譯後修飾的最終蛋白產物。抗原標的(epitope)是在內質網腔或在內質網膜上？

• 而假若抗原標的(epitope)的蛋白質若是屬於在內質網腔內的分泌性蛋白，則可能轉置到高爾基氏體並形成分泌小泡(secretory vesicles) 後，再與細胞膜融合而排放至細胞外。

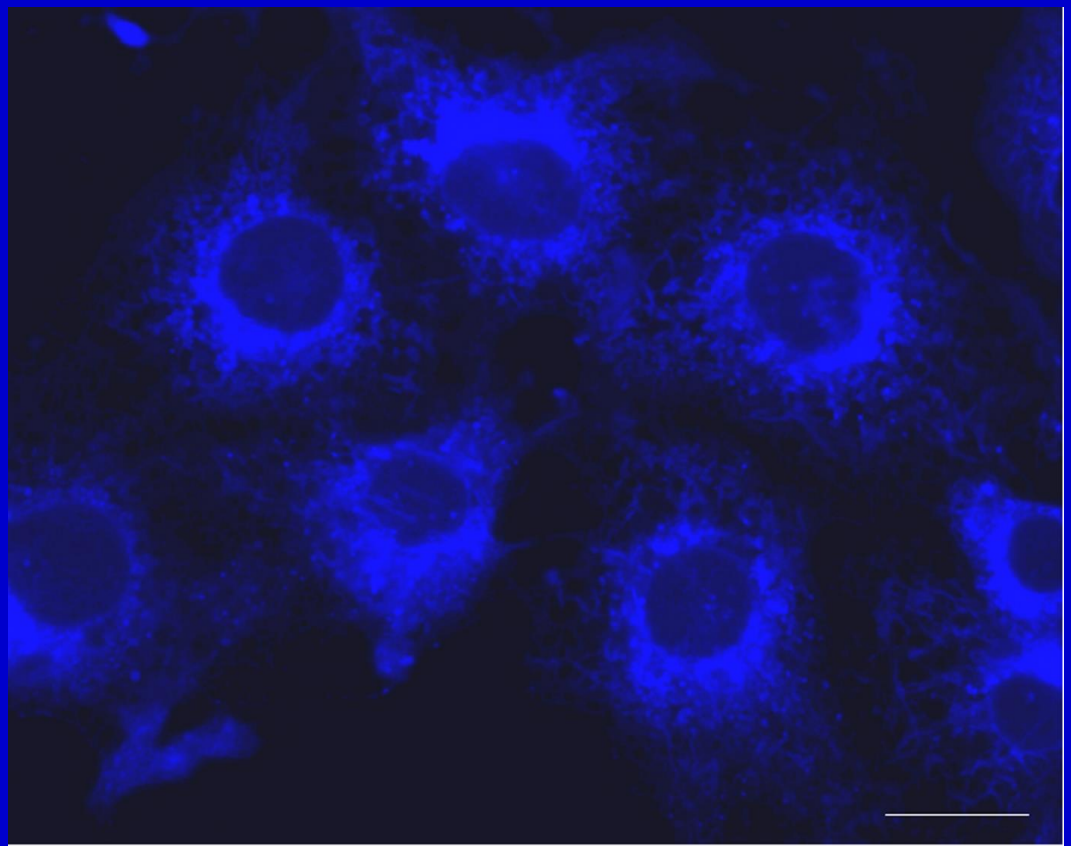
• 相對的抗原標的(epitope)的蛋白質若嵌合在內質網膜上，則可能在與其他膜性胞器 (membranous organelles) 融合後仍共同存在於各種胞器膜的結構中。綜合釐清相關技術上的問題再來做專一染色定位就較為可靠。

顆粒性內質網 (rER)

當然如同粒線體一般，有一些具有螢光的物質可用來標定活細胞中的內質網，例如DiO-C_6(3) (m.w. 573)螢光物質 (激發光譜約為484 nm；釋放光譜約為501 nm)[5]。然而DiO-C_6(3)亦會標示粒線體，導致其專一性不足。近來廠商研發出一種 ER Tracker Blue-White DPX染劑 (Molecular Probes)，其激發光譜約為374 nm；釋放光譜可在430 nm到640nm之間，較DiO-C_6(3)更具ER專一性，可供標示活細胞中內質網的分佈。

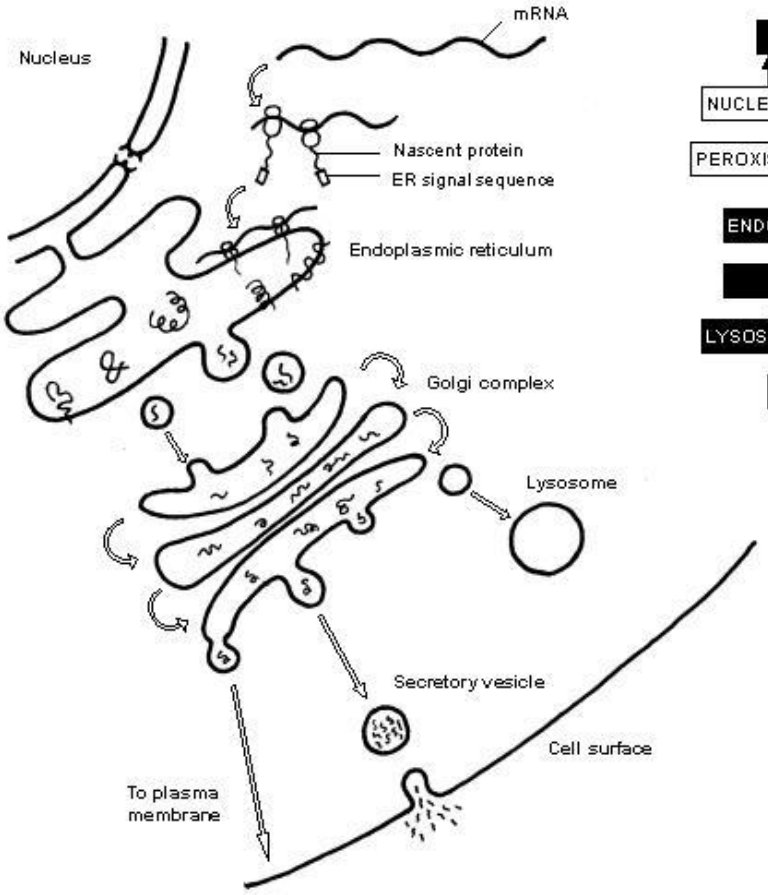
COS7活細胞內的質網染色

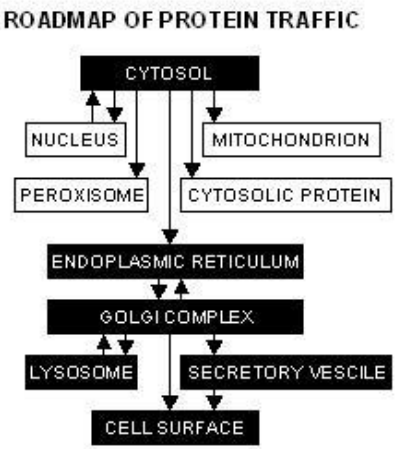
ER Tracker Blue-White DPX
標示散佈在細胞質中的內質網。
Scale bar=20μm

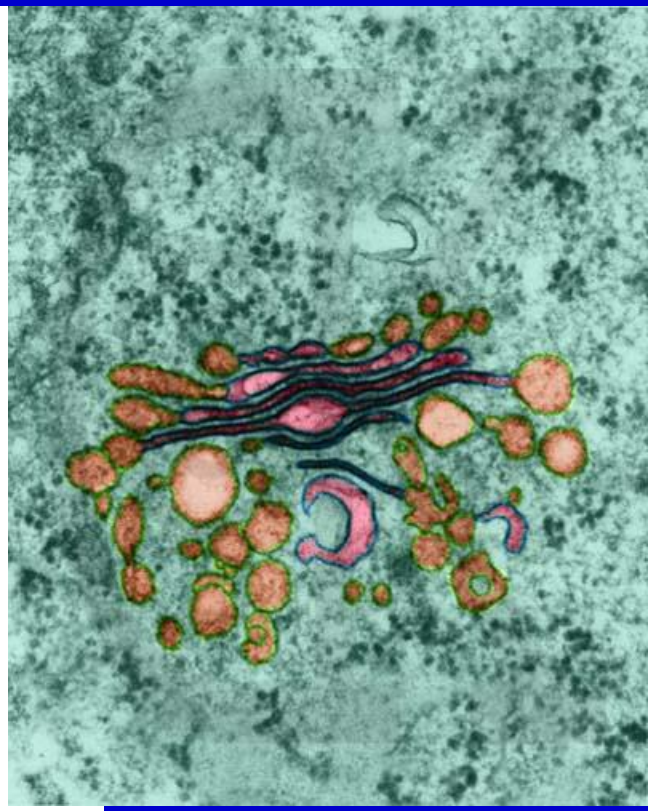


高爾基氏體 (Golgi apparatus)

高爾基氏體形態較為特殊，一般而言是由數層盤狀膜囊構成，較靠近細胞核。蛋白質在高爾基氏體的修飾作用相當複雜，依其可能的轉置胞器的不同，就有不同功能性的分類修飾。大底上高爾基氏體轉置出去可到達溶酶體 (lysosome)、分泌小泡 (secretory vesicles)及細胞膜 (plasma membrane)。

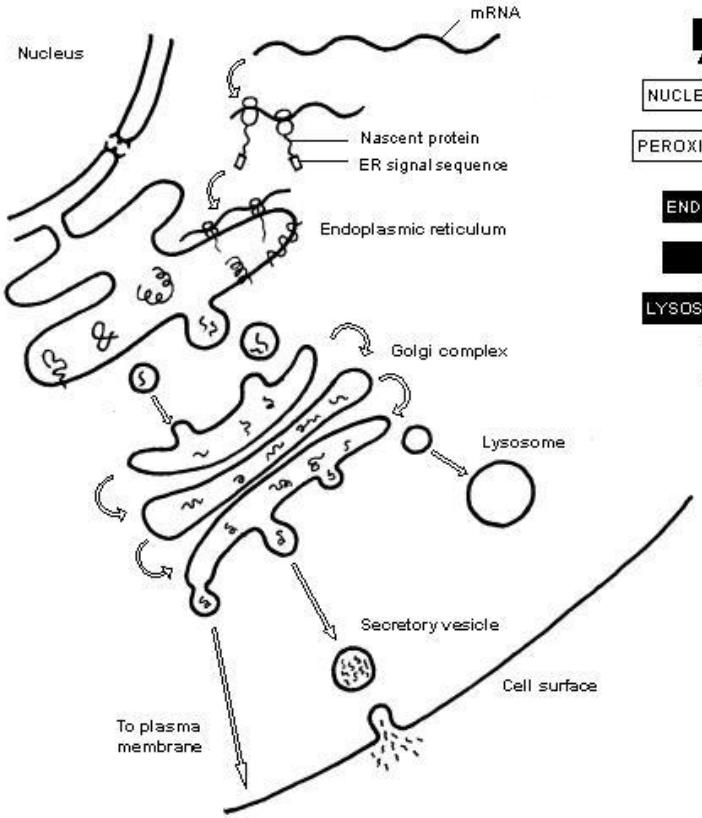


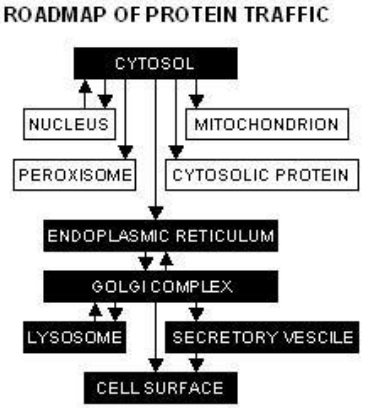


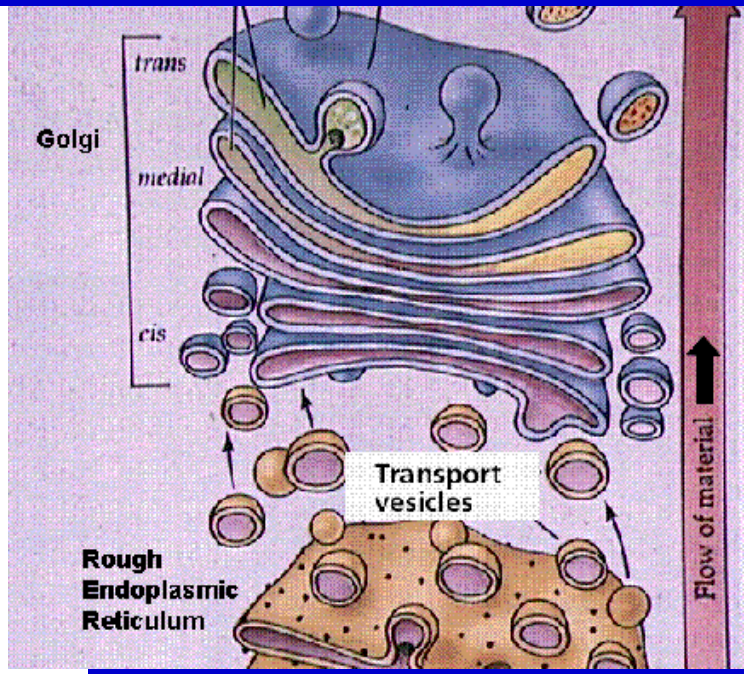


高爾基氏體 (Golgi apparatus)

高爾基氏體在細胞內的結構是與內質網相連接的，與內質網相連最近的部份稱為順氏高爾基氏網 (*cis* Golgi network)，相對的較為遠端的結構則稱為反式高爾基氏網 (*trans* Golgi network)。從內質網往反式高爾基氏網方向，膜內腔的pH梯度越來越小，內含蛋白質的周遭酸性程度因而增加。

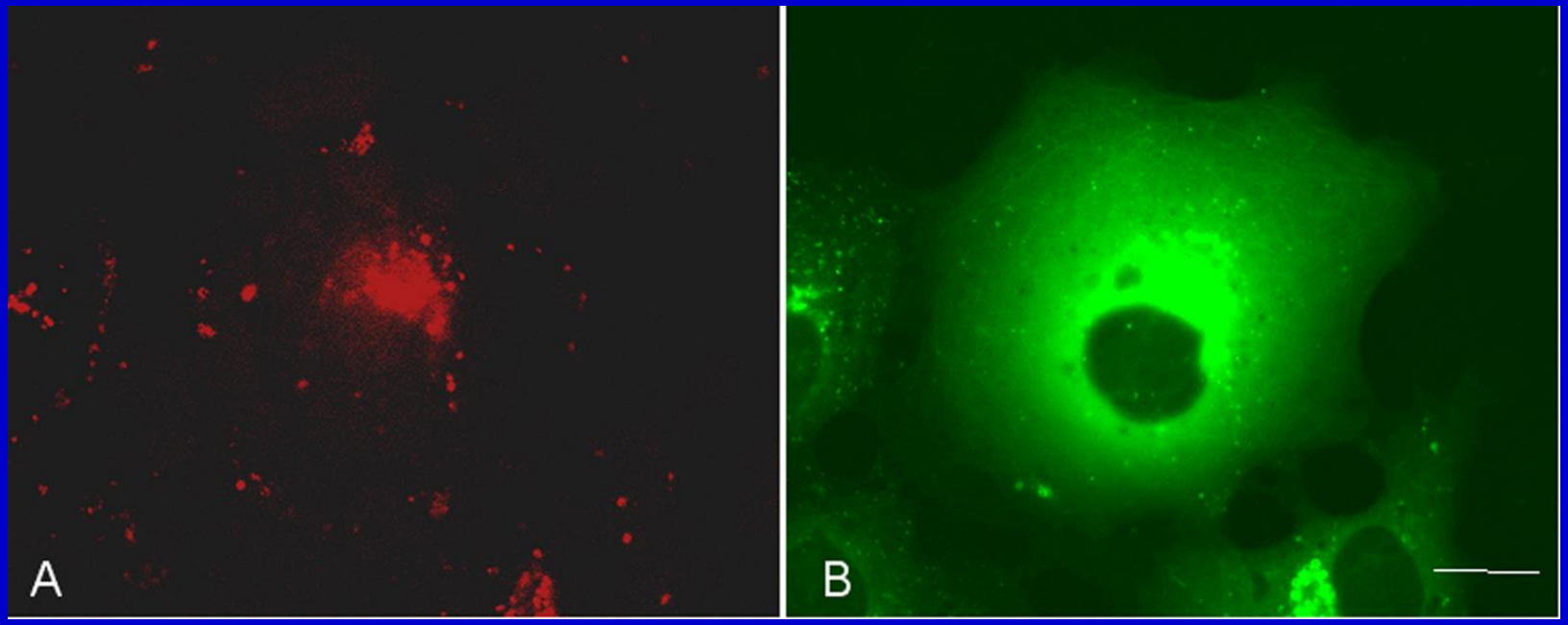






高爾基氏體 (Golgi apparatus)定位

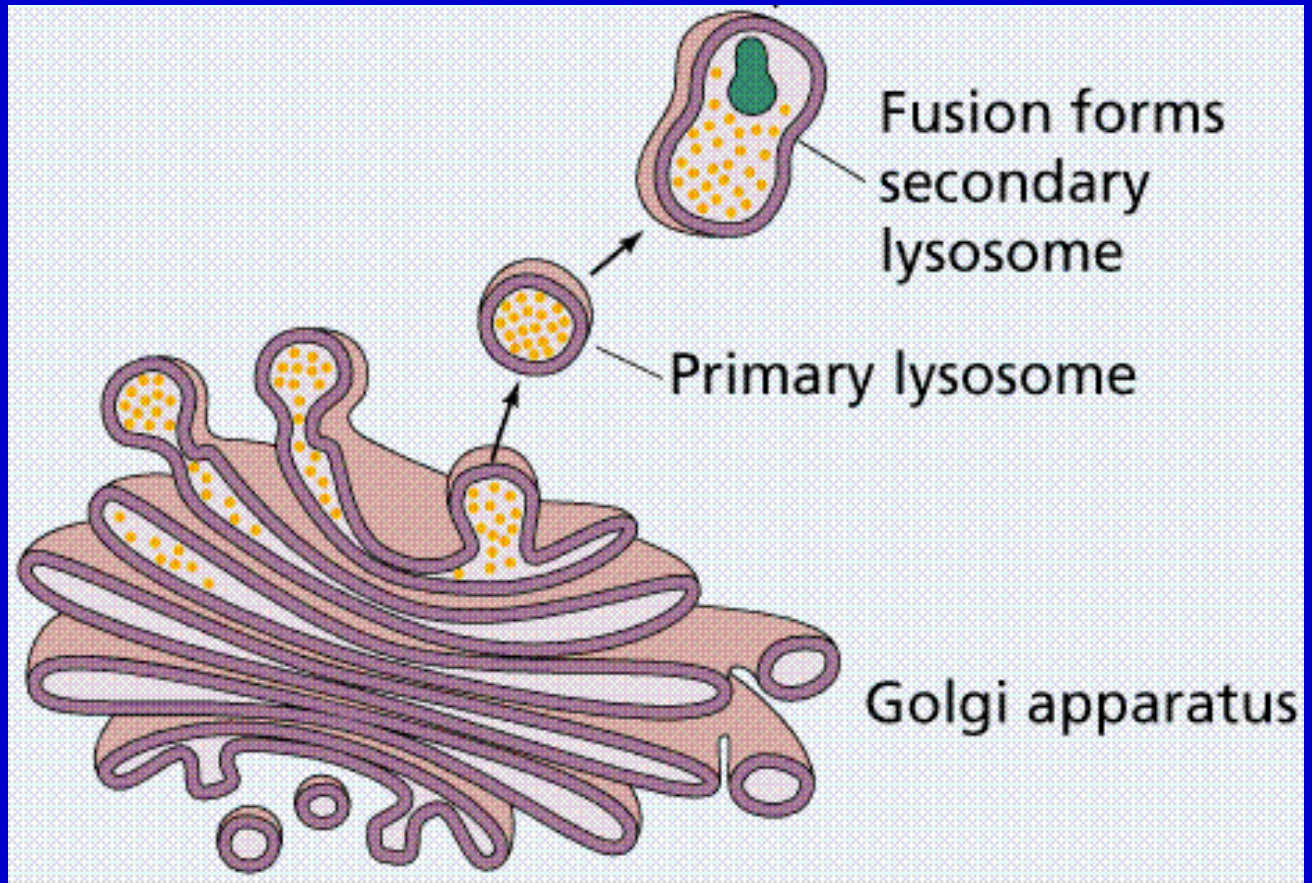
1. 利用組織化學染色(Histochemical stains)即可區分出不同的高爾基氏體區間，例如酸性磷酸酶(acid phosphatase)會在反式高爾基氏網 (*trans* Golgi network)，只要加入酸性磷酸酶的呈色受質即可染色定位。當然，在溶酶體中亦有酸性磷酸酶的存在。
2. 欲在細胞中單純的定位完整的高爾基氏體，則可利用高爾基氏體修飾蛋白質時的加成作用，即加入含醯基鞘氨醇(Ceramide) 受質的螢光化學物，如 BODIPY-FL-ceramide (M.W. 576)(Molecular Probes) 來標示活細胞的高爾基氏體。BODIPY-FL-ceramide，其激發光譜約為464 nm；釋放光譜約為532 nm。



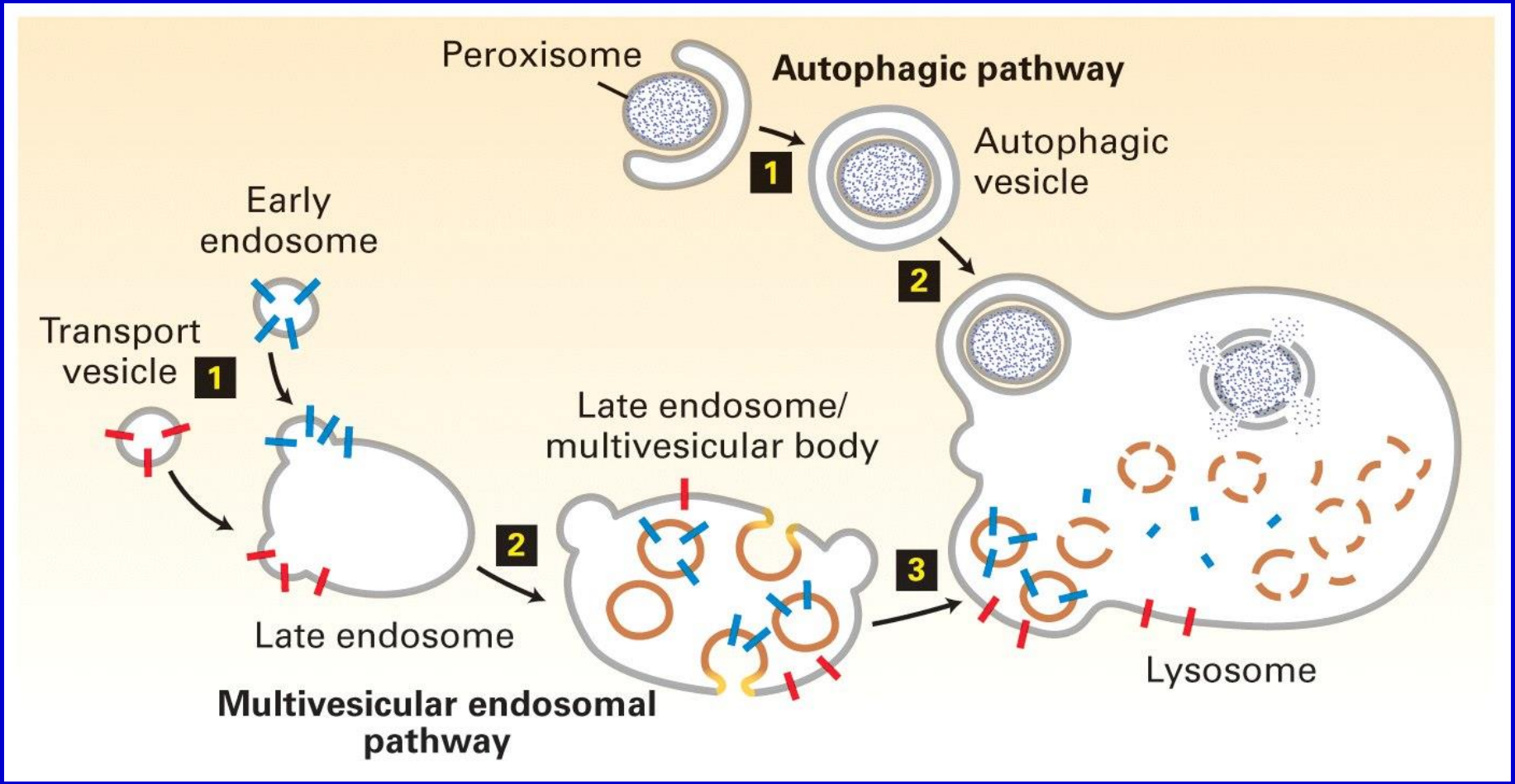
表現綠色螢光蛋白(GFP)的COS7活細胞的高爾基氏體染色。 BODIPY-FL-ceramide標示分佈在核周圍的高爾基氏體(A)，GFP自體螢光散佈在細胞質(B)。Scale bar = 20 μm

溶酶體 (Lysosome)

細胞內的溶酶體大小與形狀會因細胞生理情況不同而異。由於確知在溶酶體中含有數十種酸性水解酶 (acid hydrolases)。因此可利用溶酶體內在酸性環境的特性，來標示細胞內溶酶體，如化學螢光染劑Neutral red及acridine orange等。以Neutral red而言，染活細胞的酸性胞器包括溶酶體的結果，很容易觀察，其激發光譜約為541 nm；釋放光譜約為640 nm。



細胞內的溶酶體大小與形狀會因細胞生理情況不同而異。在溶酶體中含有數十種酸性水解酶 (acid hydrolases)。

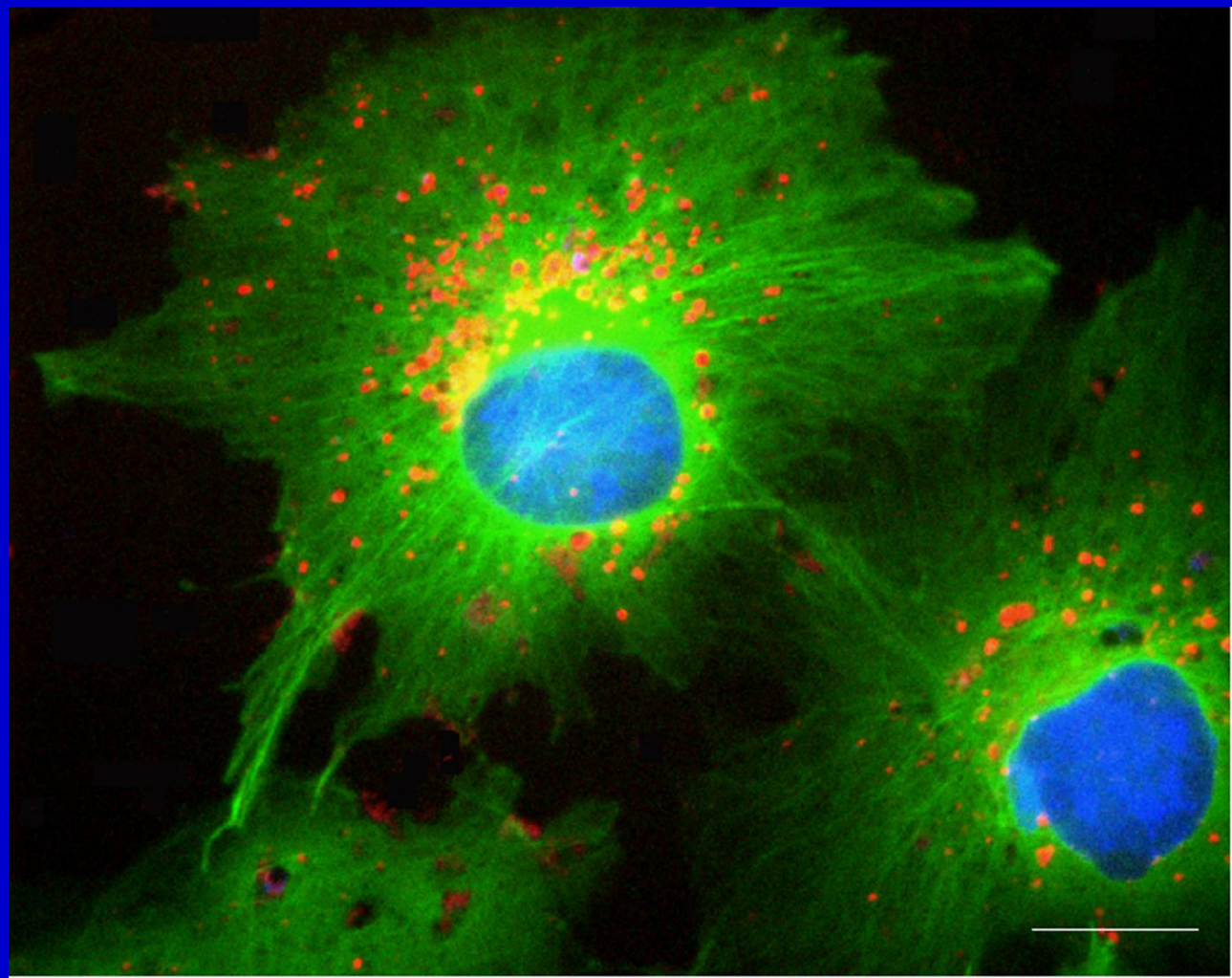


溶酶體 (Lysosome)

Neutral red及acridine orange兩種化學螢光染劑均不具備溶酶體專一性，因此廠商利用另一種化學物N-(3-[2,4-dinitrophenyl0amino]propyl)-N-(3-aminopropyl) methylamine (DAMP)，將之修飾裝接不同螢光光譜染劑而設計出LysoTracker及LysoSensor probes (Molecular Probes)，可更精確的標示不同酸性的溶酶體。

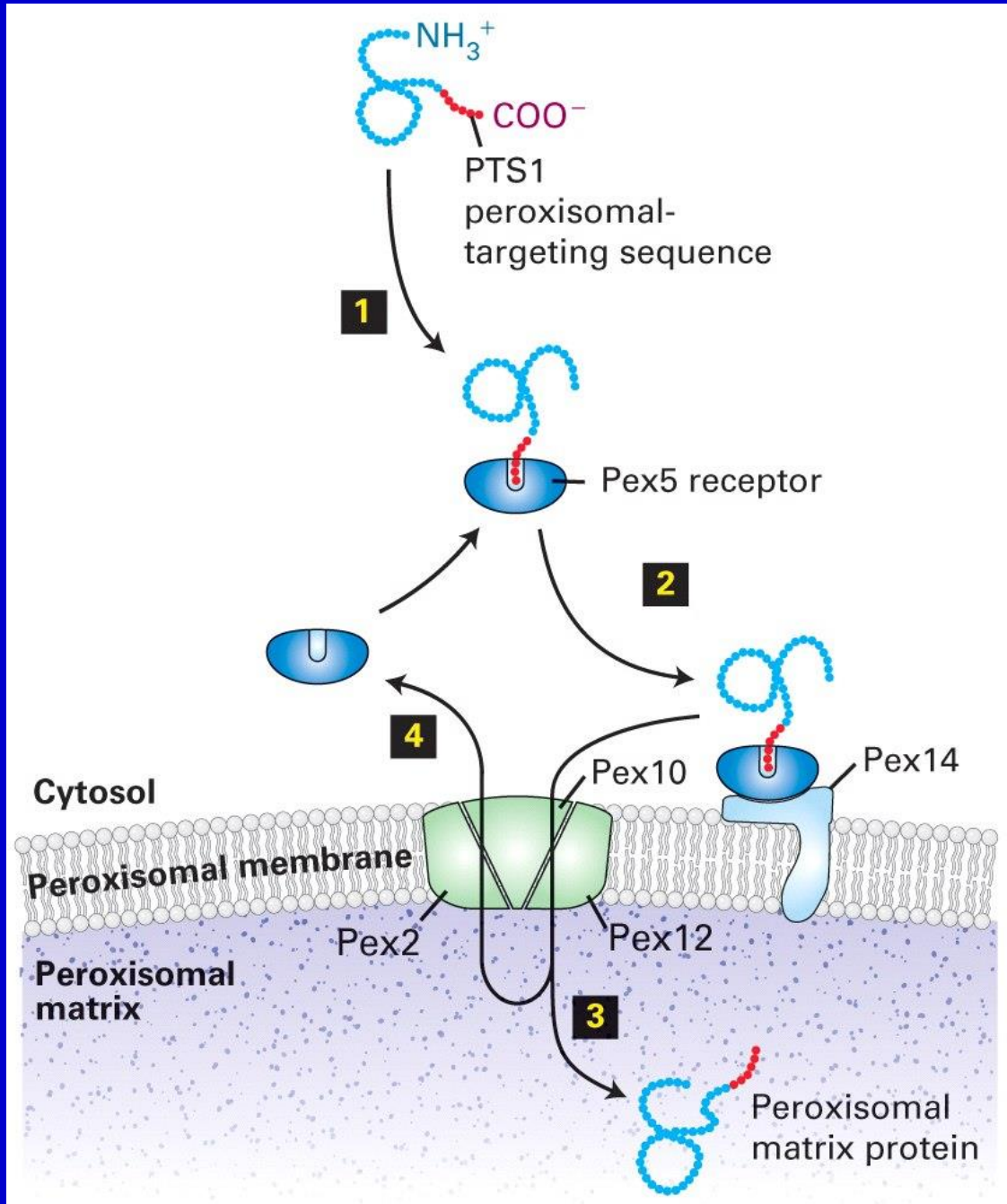
表現YFP-EB1的COS7活細胞 。

以Hoechst 33342標示細胞核呈藍色。在細胞質LysoTracker標示溶酶體呈紅色，而 YFP-EB1 蛋白則與微小管接合呈現綠色絲狀分佈。Scale bar = 20 μm



過氧化酶體 (Peroxisome)

1. 在蛋白質的碳酸基端 (C-terminus) 若具備訊息序列：-Ser-Lys-Leu-COO$^-$ 即有可能被運送至過氧化酶體。



過氧化酶體 (Peroxisome)

1. 在蛋白質的碳酸基端 (C-terminus) 若具備訊息序列：-Ser-Lys-Leu-COO^- 即有可能被運送至過氧化酶體。

2. 已知許多氧化酶(oxidative enzymes)如catalase及 urate oxidase均大量聚集在過氧化酶體中。經由過氧化酶體內作用，過氧化氫(hydrogen peroxide)的釋放，進而分解或氧化細胞質內的有害物質如醛類(aldehyde)及酚類(phenol)。

3. 目前過氧化酶體的功能仍有許多未知，然而藉由已知的過氧化酶體酵素蛋白的抗體，仍可以免疫細胞化學染色的方法，定位出過氧化酶體在細胞內的分佈。

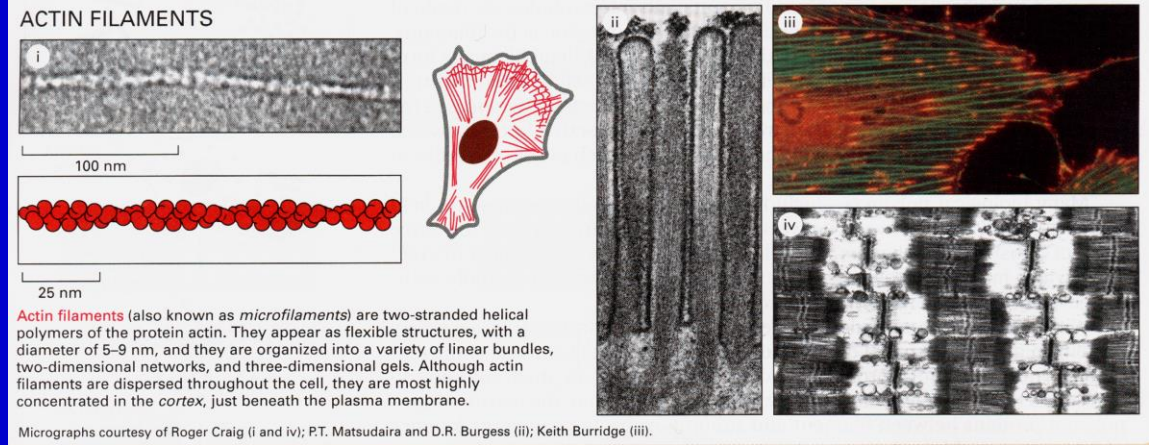
細胞骨架蛋白 (Cytoskeletons)

大多數構成細胞骨架的蛋白質及相關的蛋白質是由細胞質中的多核醣體 (polyribosome) 合成後，藉由特殊的運送蛋白轉置至細胞各部位。細胞骨架蛋白基本上均需聚合成多分子結構才能構成所謂的“細胞骨架”。根據聚合而成的絲狀粗細大略可分為三類：

1. 微小絲 (Microfilaments)

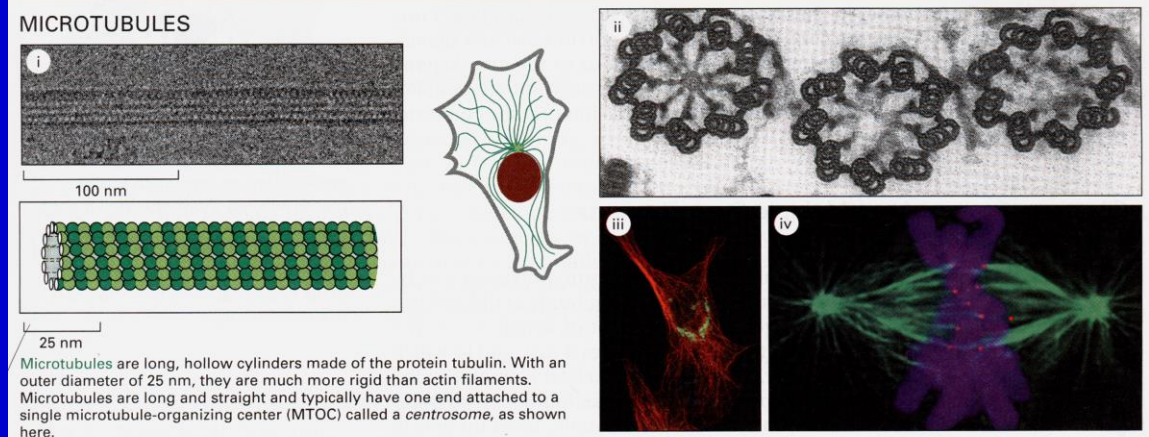
2. 中間絲蛋白 (Intermediate filaments)

3. 微小管(Microtubule)



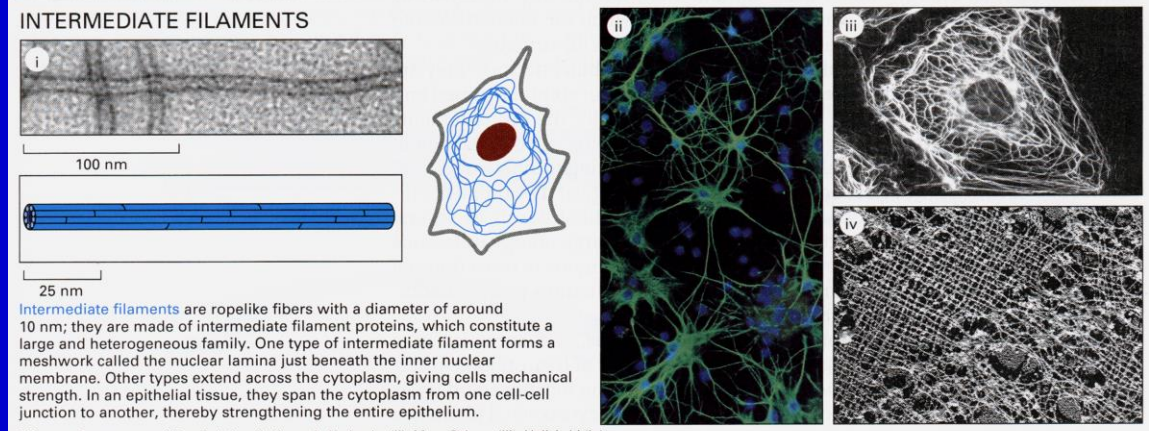
Actin filaments (also known as *microfilaments*) are two-stranded helical polymers of the protein actin. They appear as flexible structures, with a diameter of 5–9 nm, and they are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane.

Micrographs courtesy of Roger Craig (i and iv); P.T. Matsudaira and D.R. Burgess (ii); Keith Burridge (iii).



Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and typically have one end attached to a single microtubule-organizing center (MTOC) called a *centrosome*, as shown here.

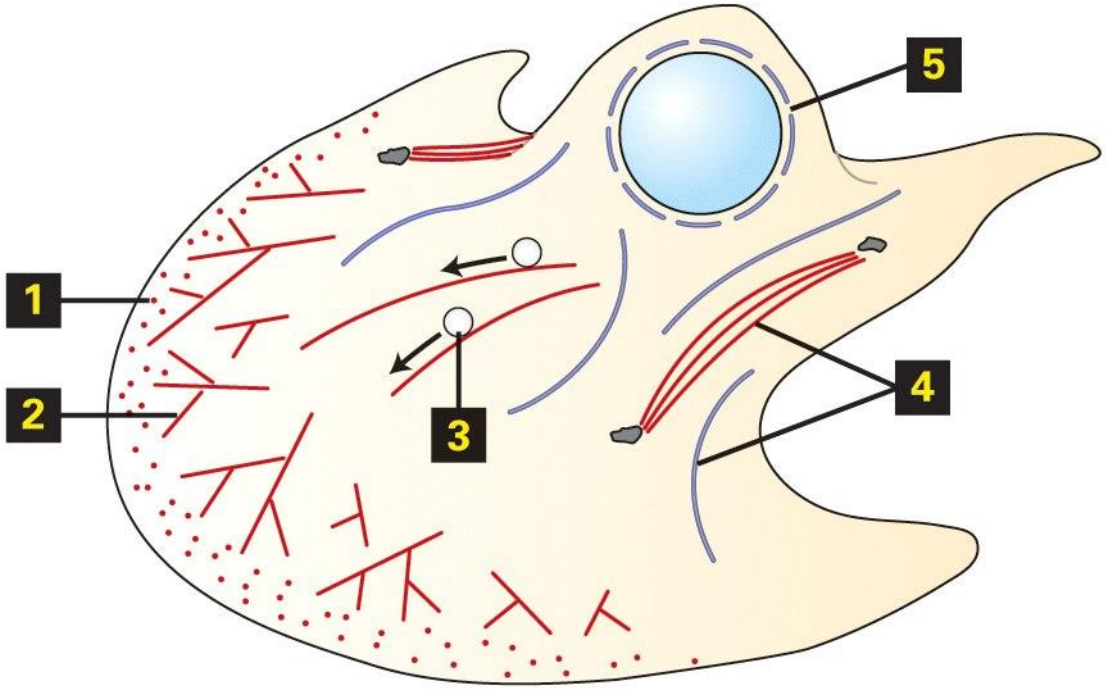
Micrographs courtesy of Richard Wade (i); D.T. Woodrow and R.W. Linck (ii); David Shima (iii); A. Desai (iv).



Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell-cell junction to another, thereby strengthening the entire epithelium.

Micrographs courtesy of Roy Quinlan (i); Nancy L. Kedersha (ii); Mary Osborn (iii); Ueli Aebi (iv).

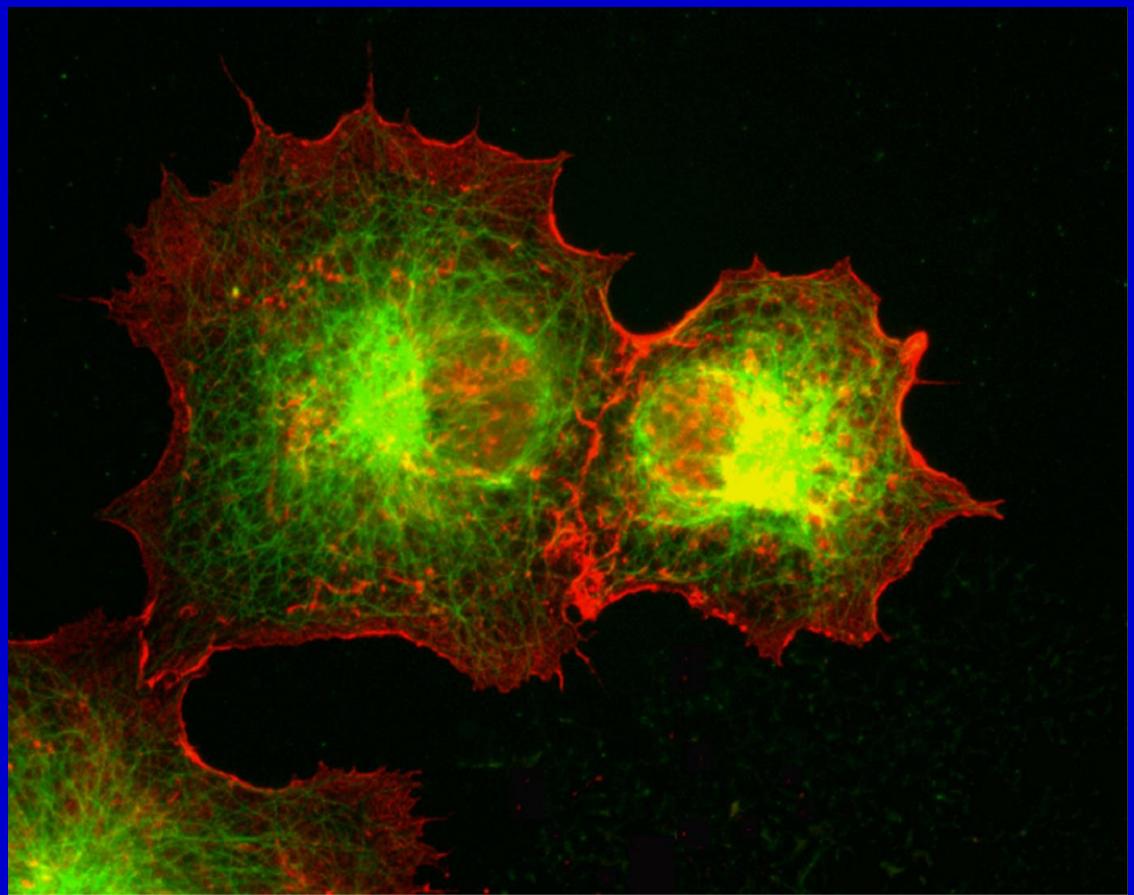
微小絲 (Microfilaments) and 中間絲蛋白 (Intermediate filaments)



	CYTOSKELETAL COMPONENT	CELL FUNCTION
1	Actin dynamics	Membrane extension
2	Filament networks: bundles	Cell structure
3	Myosin motors	Contractility and vesicle transport
4	Actin bundles and intermediate filaments	Cell adhesion
5	Lamin network	Nuclear structure

微小絲 (Microfilaments)：

細胞微小絲的主要成份為肌動蛋白(actin)，而會與肌動蛋白直接或間接接合的蛋白不下數十種。為探討新發現的蛋白質是否會與肌動蛋白作用或接合，首先就必須將成為絲狀的肌動蛋白(F-actin)標示出來。最常用來標示絲狀肌動蛋白的化學物就是phallotoxins中的phalloidin，因為phallotoxins對於絲狀肌動蛋白具有非常高的親合性。因此，只要將具有螢光的染劑 (如Rhodamine；激發光譜約為495 nm；釋放光譜約為520 nm)先接合在phalloidin上，然後再來染細胞，細胞內絲狀的肌動蛋白均可被染出。

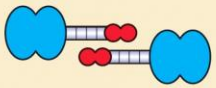
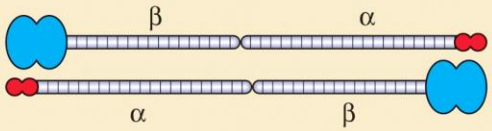
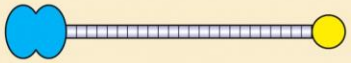
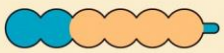


COS7細胞骨架的螢光染色

綠色免疫螢光染色標示微小管，與紅色Rhodamine Phalloidin標示肌動蛋白。Scale bar = 20μm

微小絲 (Microfilaments)：細胞微小絲的主要成份為肌動蛋白(actin)，而會與肌動蛋白直接或間接接合的蛋白不下數十種。

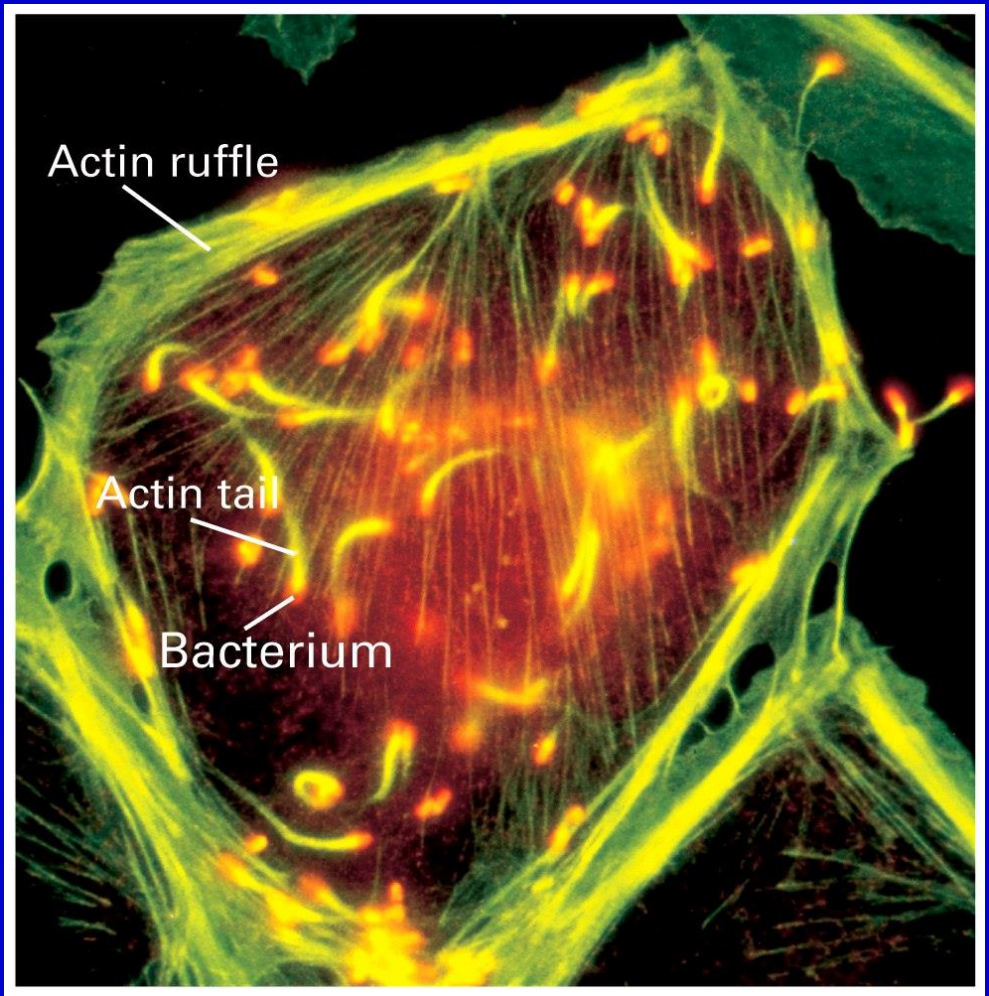
TABLE 19-1 Selected Actin-Binding Proteins

Protein	MW	Domain Organization*	Location
CH-DOMAIN SUPERFAMILY			
Fimbrin	68,000		Microvilli, stereocilia, adhesion plaques, yeast actin cables
α-Actinin	102,000		Filopodia, lamellipodia, stress fibers, adhesion plaques
Spectrin	α: 280,000 β: 246,000–275,000		Cortical networks
Dystrophin	427,000		Muscle cortical networks
Filamin	280,000		Filopodia, pseudopodia, stress fibers
OTHERS			
Fascin	55,000		Filopodia, lamellipodia, stress fibers, microvilli, acrosomal process
Villin	92,000		Microvilli in intestinal and kidney brush border

*Blue = actin-binding domains; red = calmodulin-like Ca^{2+}-binding domains; purple = α-helical repeats; green = β-sheet repeats; orange = other domains.

微小絲 (Microfilaments)：

最常用來標示絲狀肌動蛋白的化學物就是phallotoxins中的phalloidin，因為phallotoxins對於絲狀肌動蛋白具有非常高的親合性。因此，只要將具有螢光的染劑(如Rhodamine；激發光譜約為495 nm；釋放光譜約為520 nm)先接合在phalloidin上，然後再來染細胞，細胞內絲狀的肌動蛋白均可被染出。



中間絲蛋白(Intermediate filaments)：細胞中間絲蛋白聚合成多分子結構後，其絲狀結構直徑約10 nm。組成中間絲蛋白的蛋白質大抵會依不同組織的分佈而有所區別[14]，例如在上皮組織細胞即以角質蛋白(keratins)為主的中間絲蛋白；中胚層衍生的結締組織則以vimentin中間絲蛋白為主，肌肉組織含desmin中間絲蛋白，而神經組織亦含有其特殊的神經元中間絲蛋白。

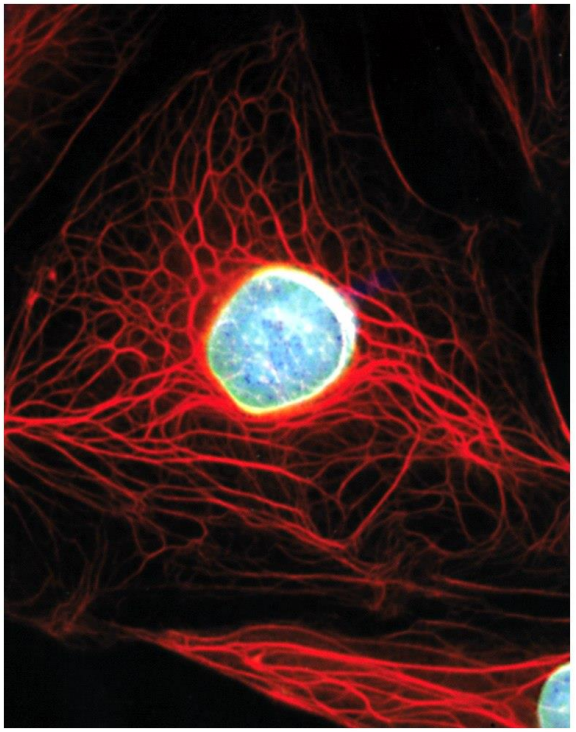
TABLE 19-4 Primary Intermediate Filaments in Mammals

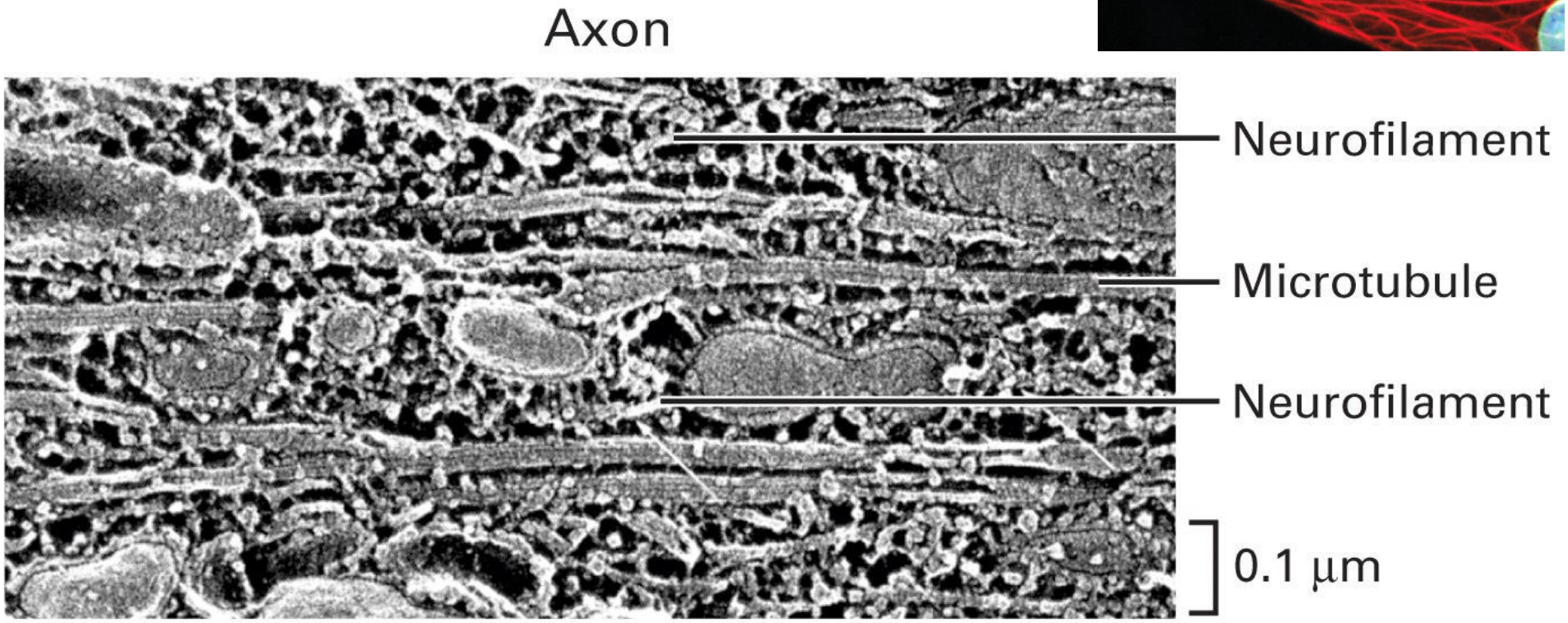
IF Protein	MW (10^{-3})*	Filament Form	Tissue Distribution
NUCLEAR LAMINS			
Lamin A	70	Homopolymer	Nucleus
Lamin B	67	Homopolymer	Nucleus
Lamin C	67	Homopolymer	Nucleus
KERATINS†			
Acidic keratins	40–57	Heteropolymers	Epithelia
Basic keratins	53–67	Heteropolymers	Epithelia
TYPE III INTERMEDIATE FILAMENTS			
Vimentin	57	Homo- and heteropolymers	Mesenchyme (fibroblasts)
Desmin	53	Homo- and heteropolymers	Muscle
Glial fibrillary acidic protein	50	Homo- and heteropolymers	Glial cells, astrocytes
Peripherin	57	Homo- and heteropolymers	Peripheral and central neurons
NEUROFILAMENTS			
NF-L	62	Homopolymers	Mature neurons
NF-M	102	Heteropolymers	Mature neurons
NF-H	110	Heteropolymers	Mature neurons
Internexin	66	—	Developing CNS

*Intermediate filaments show species-dependent variations in molecular weight (MW).
†More than 15 isoforms of both acidic and basic keratins are known.

中間絲蛋白 (Intermediate filaments)：

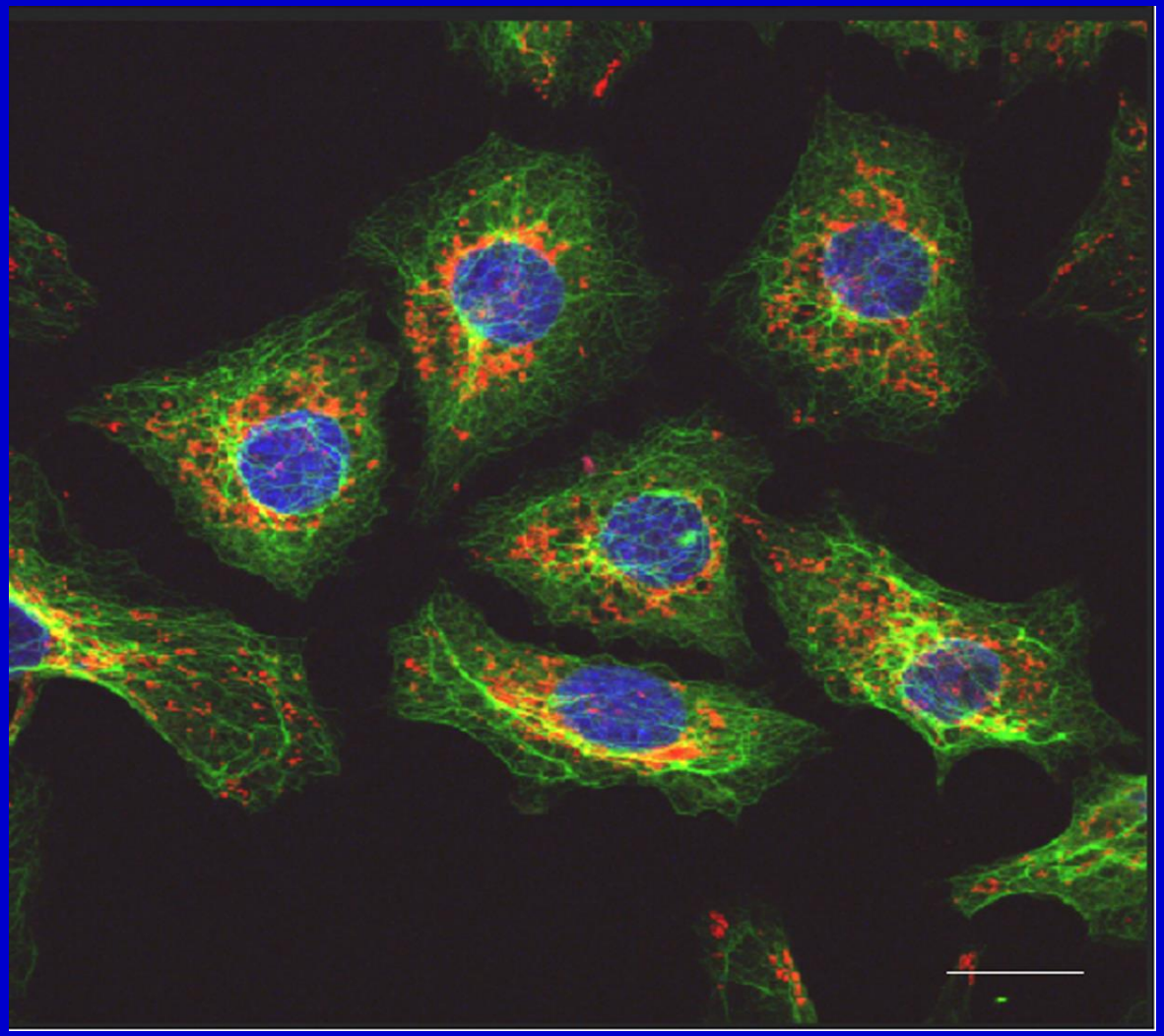
要探討新發現的蛋白質是否會與中間絲蛋白作用或接合，先初步瞭解此蛋白質在何種組織或細胞表現，再選用具組織特性的中間絲蛋白的抗體，即可探討新蛋白質的定性與定位。





中間絲蛋白(Intermediate filaments)：

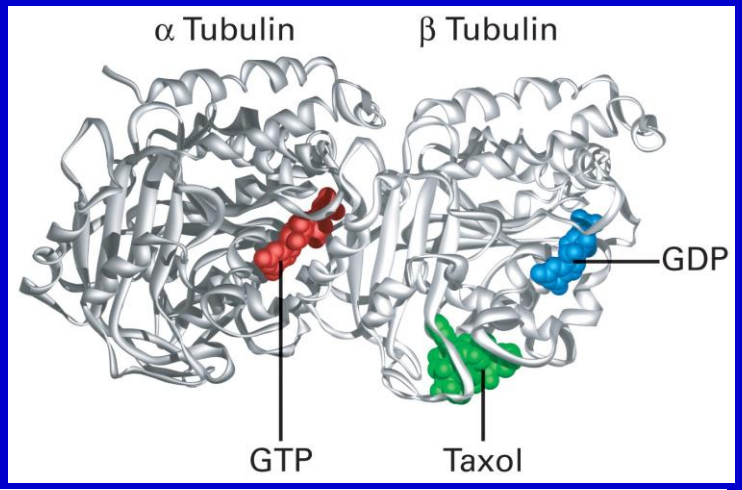
要探討新發現的蛋白質是否會與中間絲蛋白作用或接合，先初步瞭解此蛋白質在何種組織或細胞表現，再選用具組織特性的中間絲蛋白的抗體，即可探討新蛋白質的定性與定位。

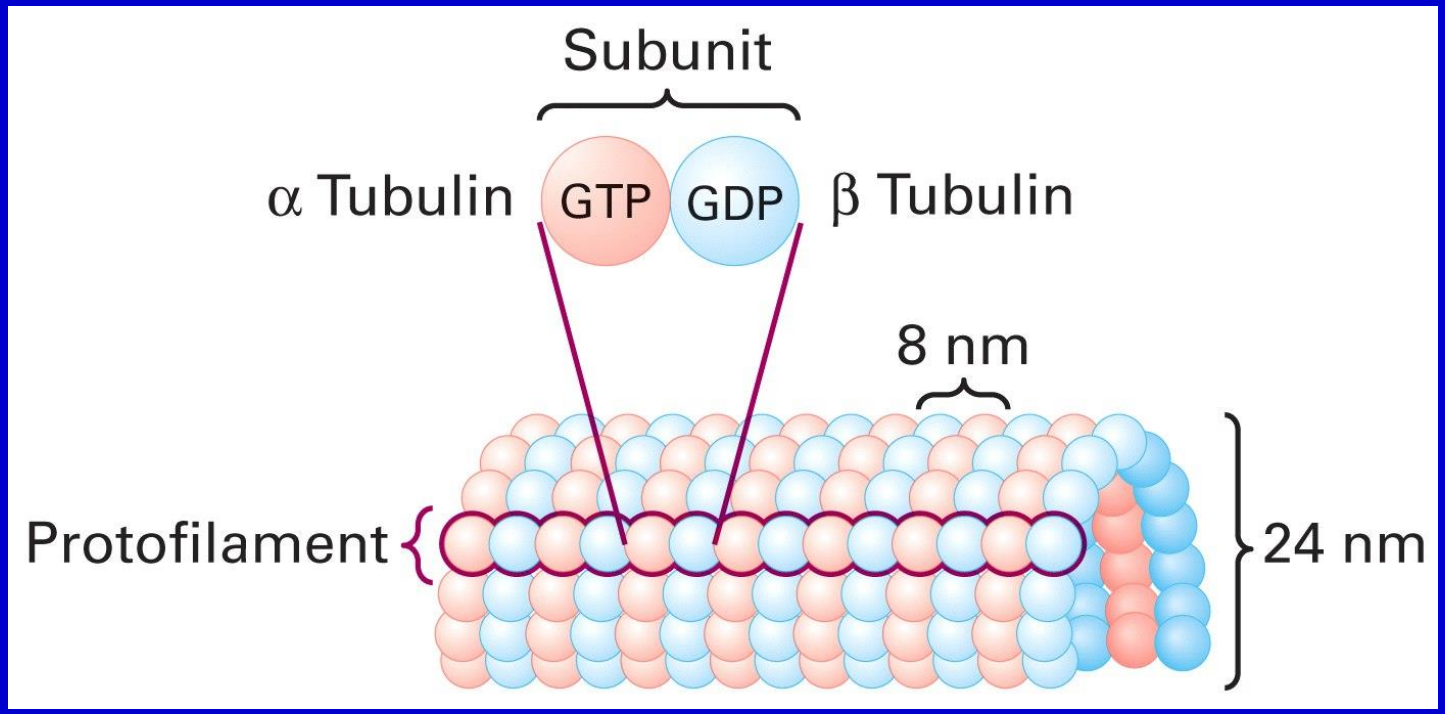


膀胱癌T24細胞螢光染色: Hoechst 33342標示細胞核呈藍色，在細胞質中MitoTracker標示粒線體呈紅色， Keratin 18中間絲蛋白則呈綠色絲狀分佈。Scale bar = 20μm

微小管(Microtubule)：

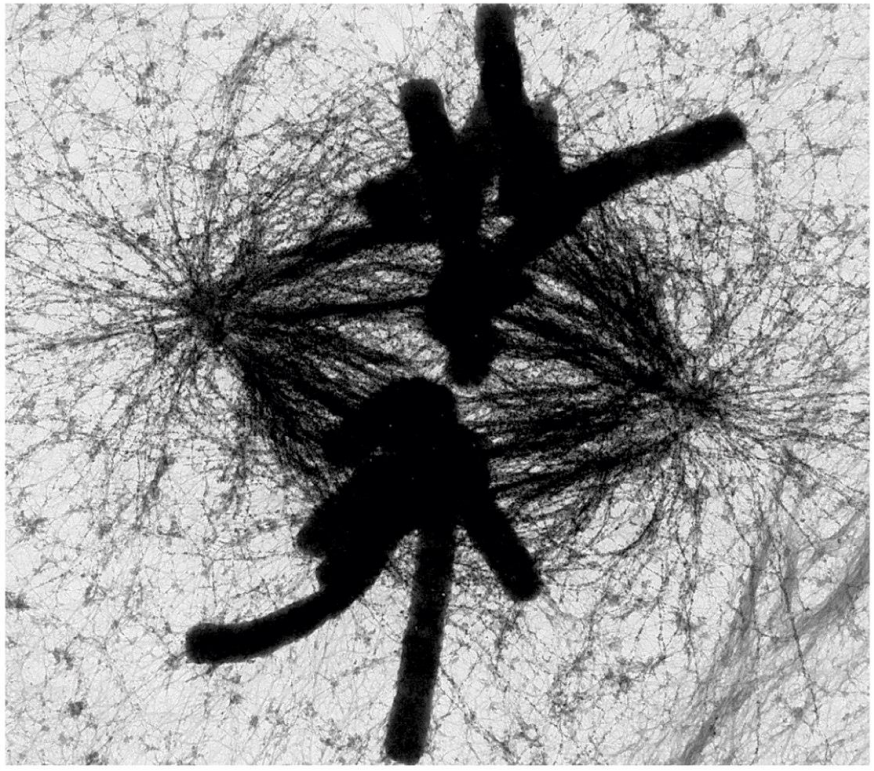
細胞中有許多由微管素 (tubulin) 聚合成多分子結構的微小管結構，其絲狀結構直徑約23 -24 nm。

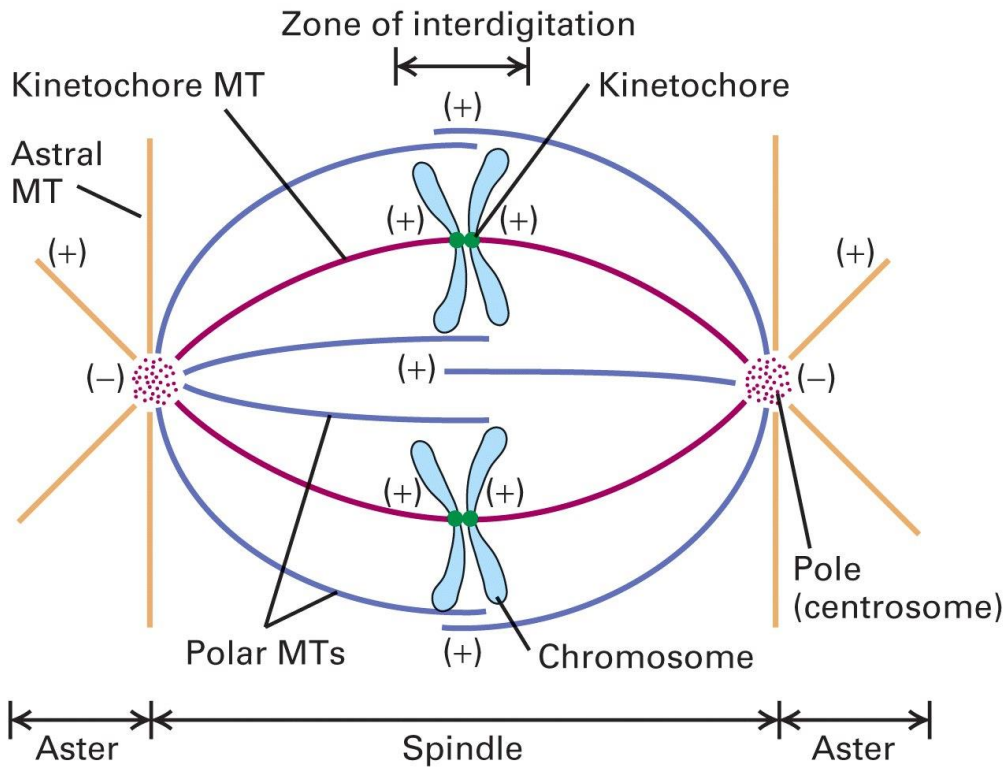




微小管(Microtubule)：

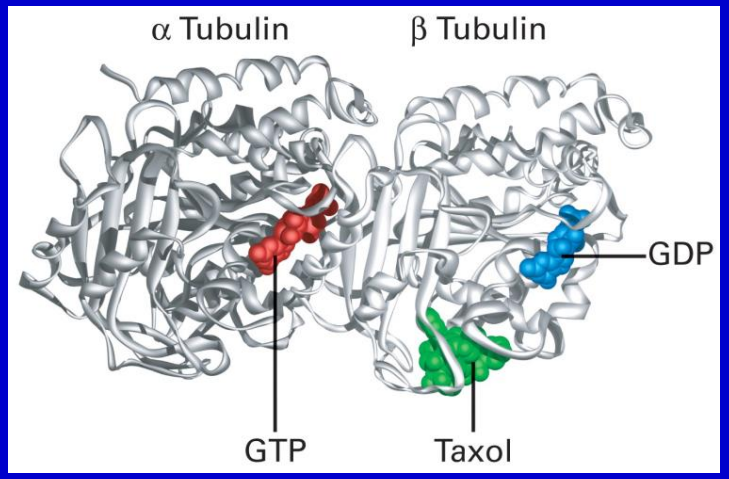
細胞有絲分裂的紡錘絲(Mitotic spindle) 亦是由微小管所構成。就因為微小管參與許多細胞生理的動態變化，因此標示微管素的化學物質可能直接影響細胞的正常生理功能。目前常用來標示微小管的紫杉醇 Paclitaxel (Taxol)，即有穩定微小管的功能，導致細胞無法形成正常的有絲分裂紡錘絲，也因此紫杉醇可用來治療部分癌症，抑制癌細胞的增殖[16]。利用紫杉醇與微管素的高親合性，將具有螢光的染劑(如FITC)先接合在Paclitaxel上，再來染細胞，就可染出細胞內絲狀的微小管結構。一般培養的細胞經由紫杉醇處理，其微小管會聚集成絲束狀結構，藉由微管素抗體標示可明顯觀察到細胞微小管的變化 。

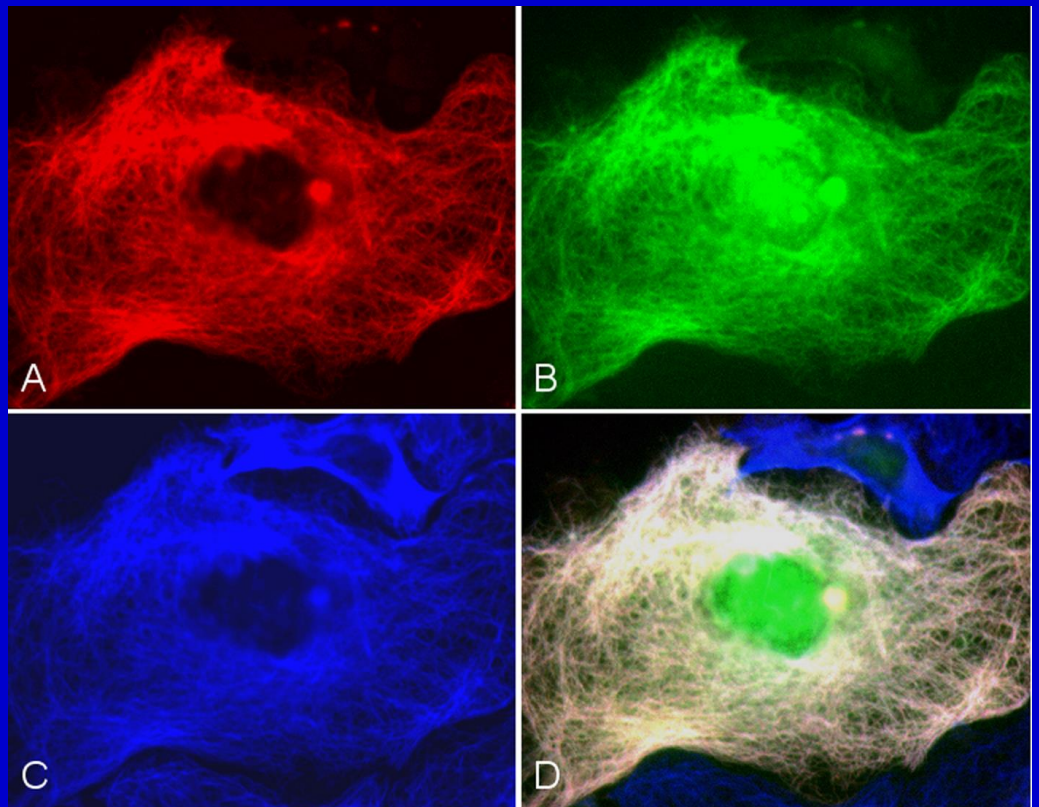




微小管(Microtubule)：

目前常用來標示微小管的紫杉醇 Paclitaxel (Taxol)，即有穩定微小管的功能，導致細胞無法形成正常的有絲分裂紡錘絲，也因此紫杉醇可用來治療部分癌症，抑制癌細胞的增殖[16]。利用紫杉醇與微管素的高親合性，將具有螢光的染劑(如FITC)先接合在Paclitaxel上，再來染細胞，就可染出細胞內絲狀的微小管結構。一般培養的細胞經由紫杉醇處理，其微小管會聚集成絲束狀結構，藉由微管素抗體標示可明顯觀察到細胞微小管的變化 。





經紫杉醇處理後的COS7螢光形態

細胞經固定後，紅色微小管相關蛋白MAP1A重鏈(DsRed-HC, A)與綠色MAP1A輕鏈(GFP-LC, B)仍然存在。再以藍色AMCA免疫螢光標示微小管 (C)。細胞內的微小管和微小管相關蛋白MAP1A均受紫杉醇的作用，而產生束狀的聚集(D)。Scale bar = 20μm

Summary

1.細胞核: DNA特殊染色如DAPI (激發光譜約為359 nm；與DNA接合後釋放光譜約為461 nm)或是Hoechst33342 (激發光譜約為343 nm；與DNA接合後釋放光譜約為483 nm)等染色

2.粒線體: 螢光染劑如Mito Tracker dyes (Molecular Probes)等，可染完活細胞後再將細胞固定(fixed)，仍然可以觀察到染上的粒線體螢光。

3.內質網: ER Tracker Blue-White DPX染劑 (Molecular Probes)，其激發光譜約為374 nm；釋放光譜可在430 nm到640nm之間，較DiO-C_6(3)更具ER專一性，可供標示活細胞中內質網的分佈。

4.高爾基氏體: BODIPY-FL-ceramide (M.W. 576)(Molecular Probes) 來標示活細胞的高爾基氏體。BODIPY-FL-ceramide，其激發光譜約為464 nm；釋放光譜約為532 nm。

5. 溶酶體: N-(3-[2,4-dinitrophenyl0amino]propyl)-N-(3-aminopropyl) methylamine (DAMP)，將之修飾裝接不同螢光光譜染劑而設計出LysoTracker及LysoSensor probes (Molecular Probes)，可更精確的標示不同酸性的溶酶體。

6. 微小絲: 具有螢光的染劑 (如Rhodamine；激發光譜約為495 nm；釋放光譜約為520 nm)先接合在phalloidin上，然後再來染細胞，細胞內絲狀的肌動蛋白均可被染出。

7. 微小管: 利用紫杉醇與微管素的高親合性，將具有螢光的染劑(如FITC)先接合在Paclitaxel上，再來染細胞，就可染出細胞內絲狀的微小管結構。

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*

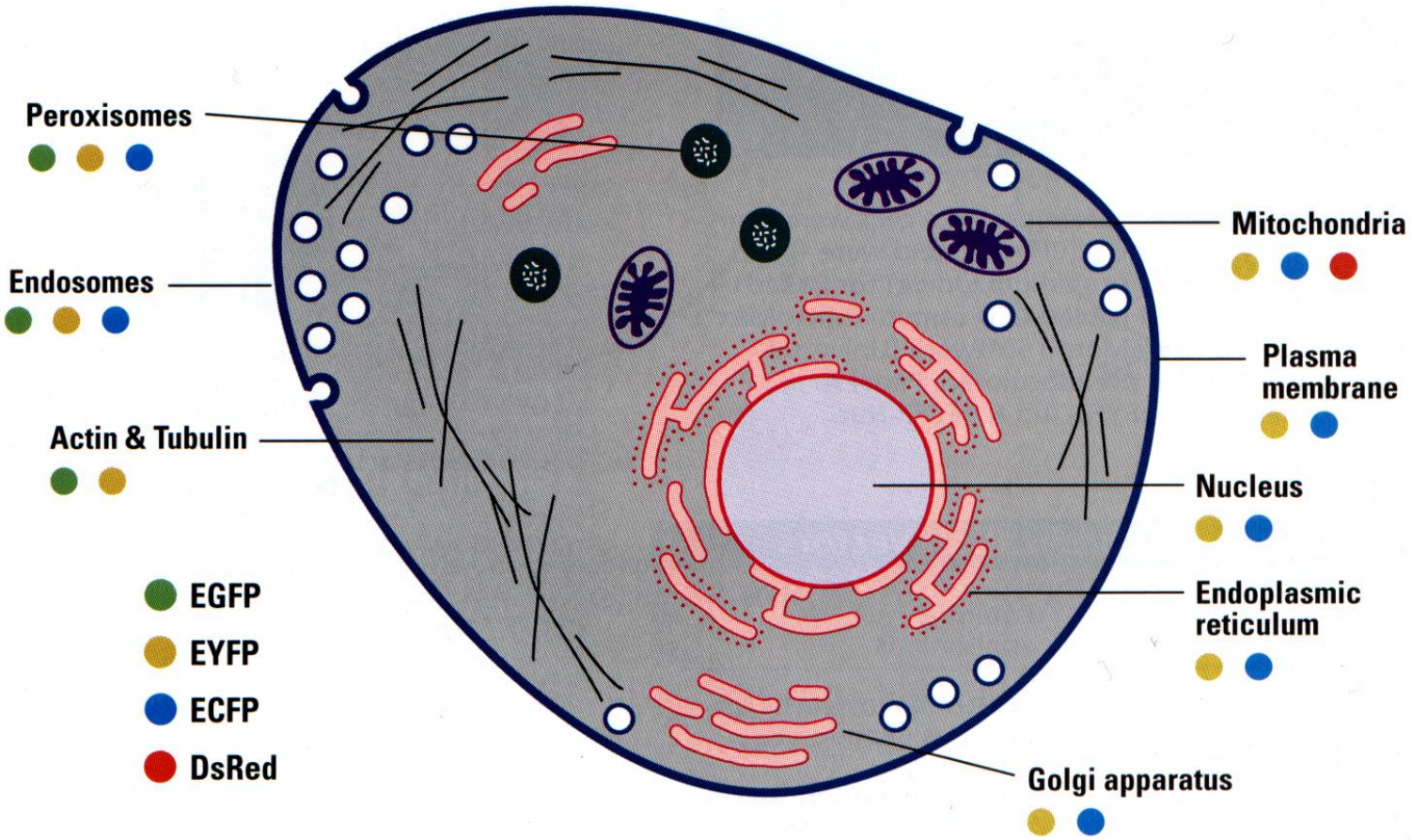


Figure 1. Organelles targeted by Living Colors™ Subcellular Localization Vectors.

Living Colors™ Subcellular Localization Vectors continued

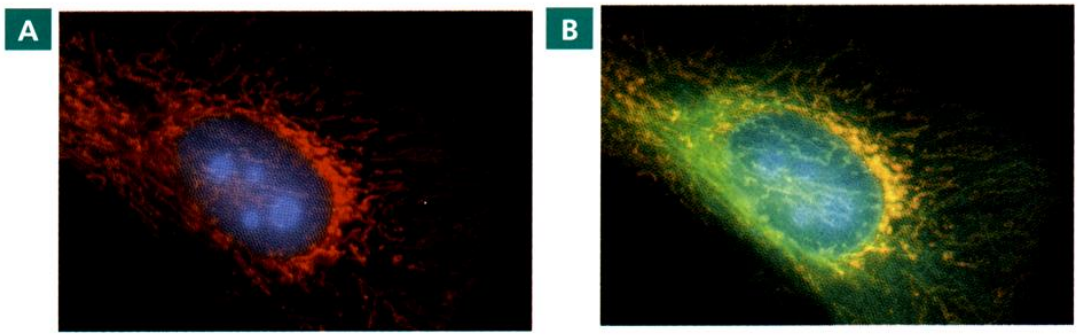
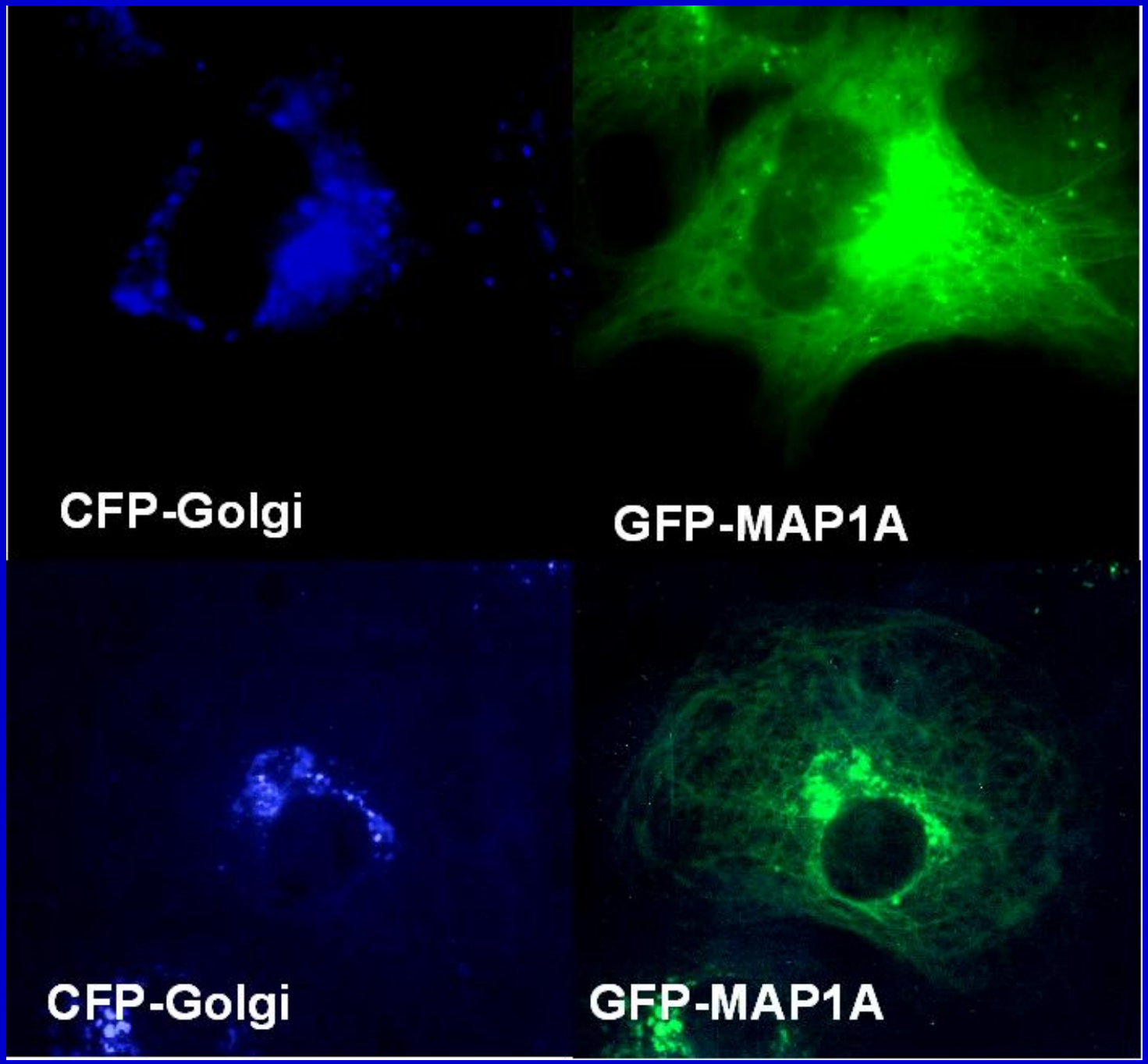


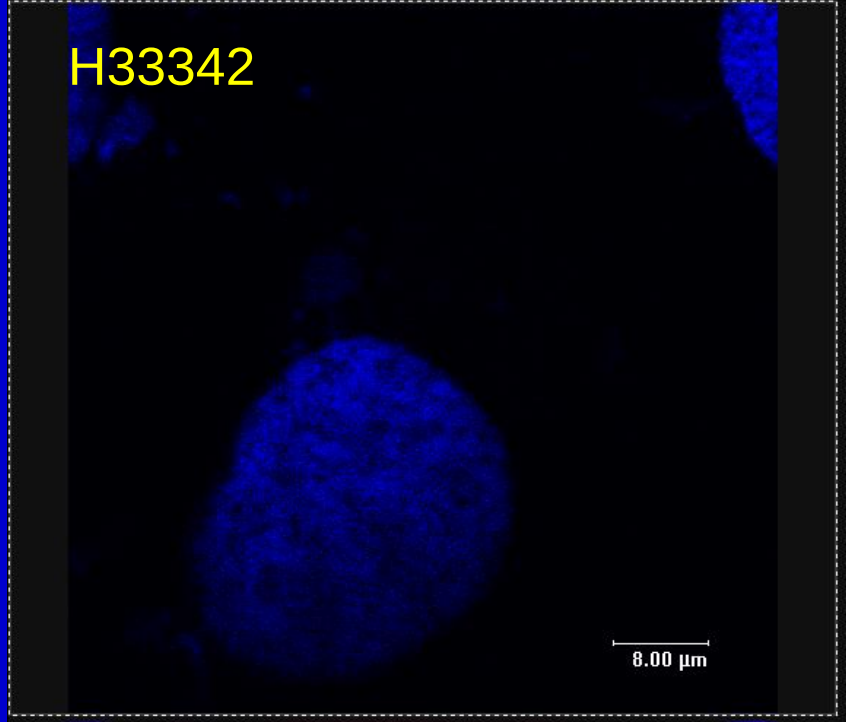
Figure 2. Dual and triple labeling with Living Colors™ proteins. HeLa cells were transiently transfected with pDsRed1-Mito, pEYFP-Tub, and pECFP-Nuc, and were fixed. The images were taken using Omega filter sets XF35 (propidium iodide) for DsRed1-Mito, XF104 for EYFP-Tub, and XF114 for ECFP-Nuc; a cooled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

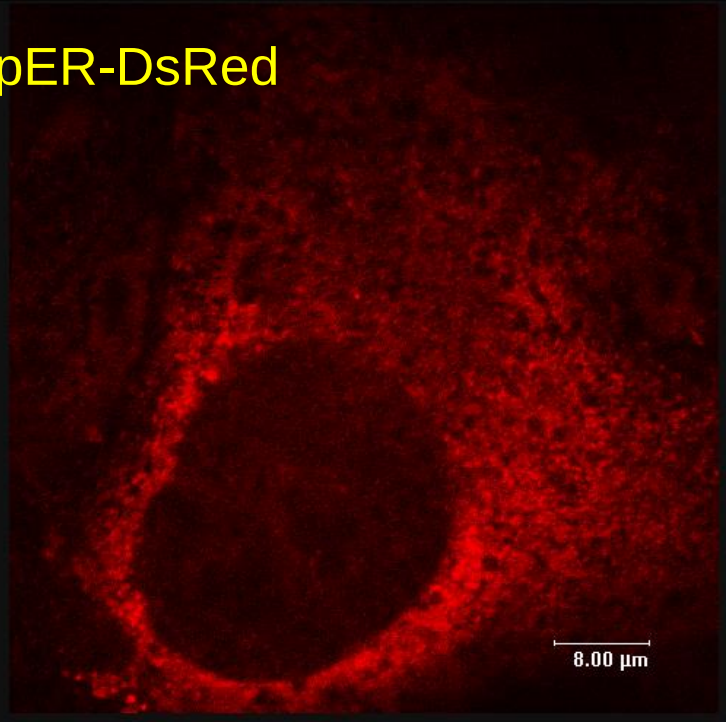
Table I: Living Colors™ Subcellular Localization Vectors

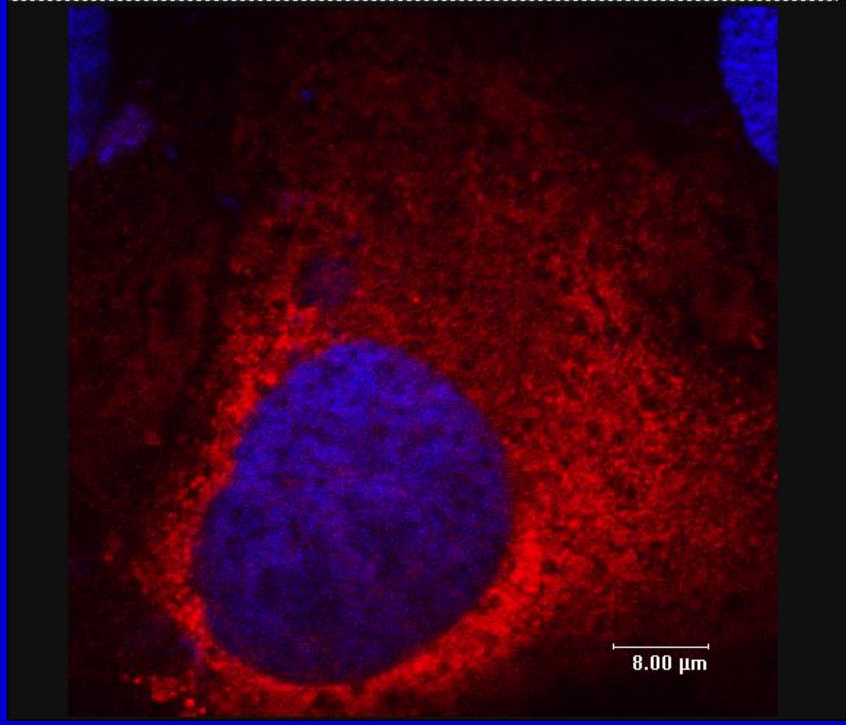
Targeted subcellular structure	Color variants available	Localization tag or gene	Potential applications
Endosomes	Green, cyan, yellow	RhoB	• Observe movement of vesicles of endocytic pathway • Monitor endocytosis of labeled receptors or ligands
Mitochondria	Cyan, yellow	Targeting sequence from subunit 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

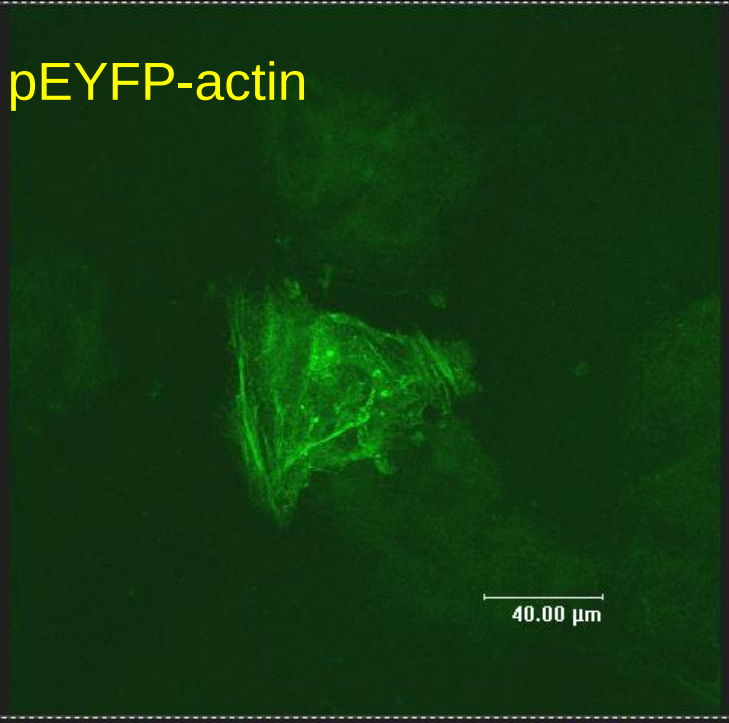

CFP-Golgi
GFP-MAP1A
CFP-Golgi
GFP-MAP1A


H33342
8.00 µm

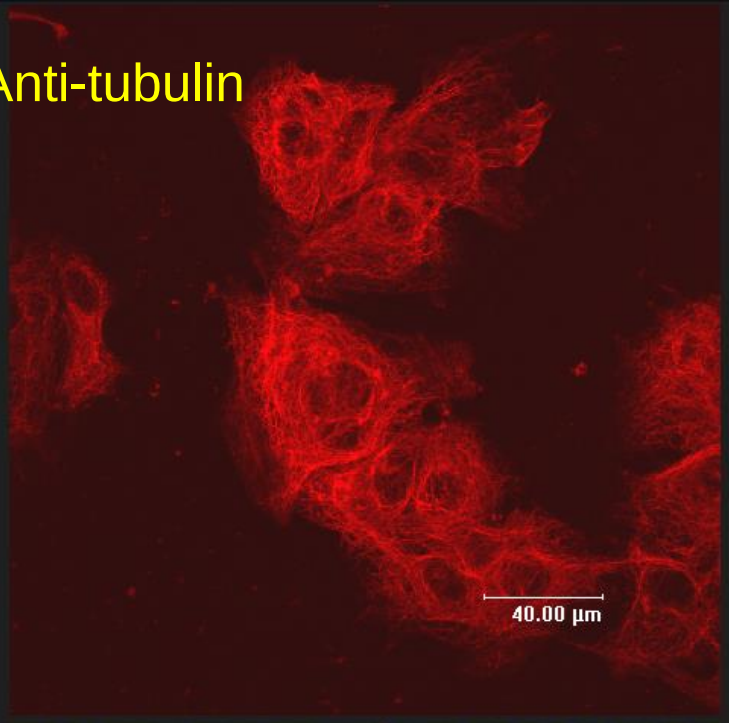

pER-DsRed
8.00 µm


8.00 µm

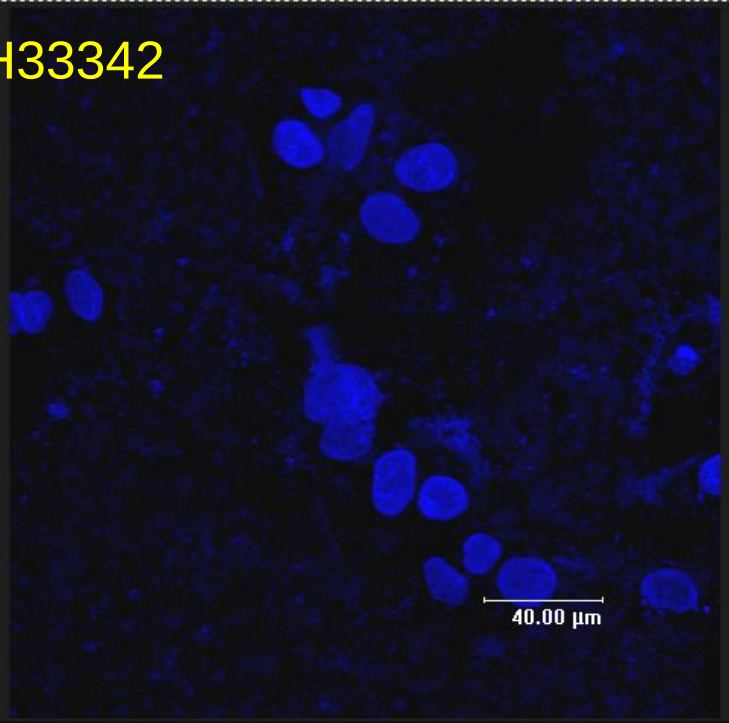
pEYFP-actin

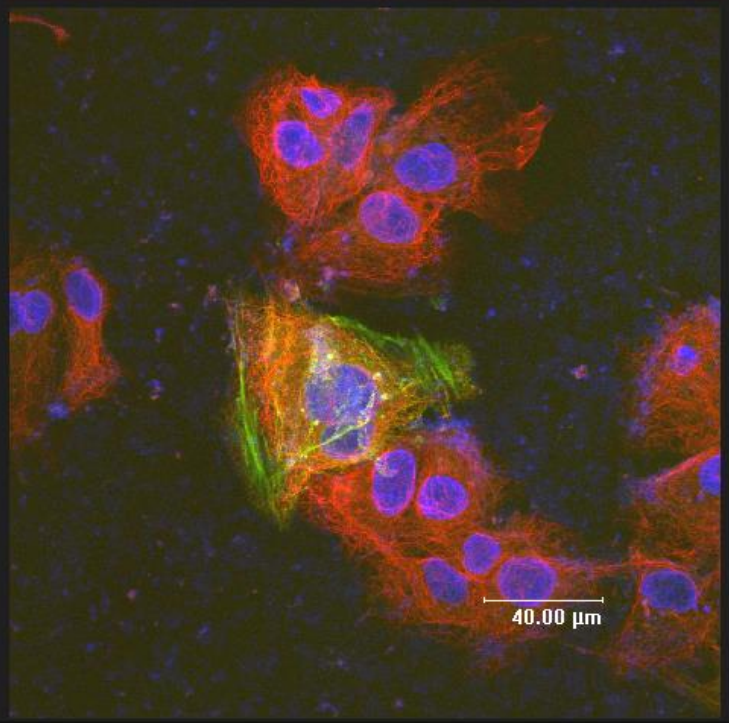


Anti-tubulin



H33342



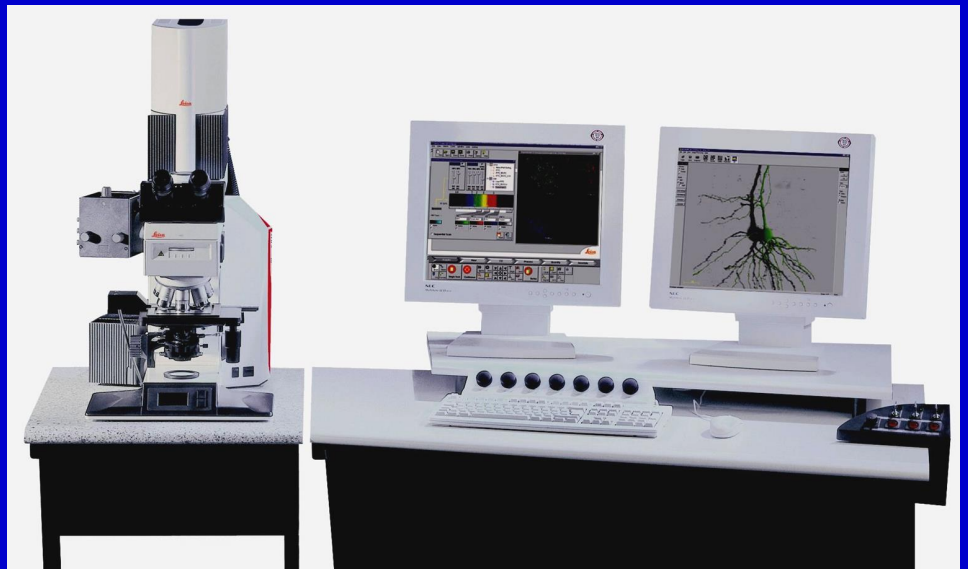


Multi-dimensional Live-cell Imaging System

Functions:

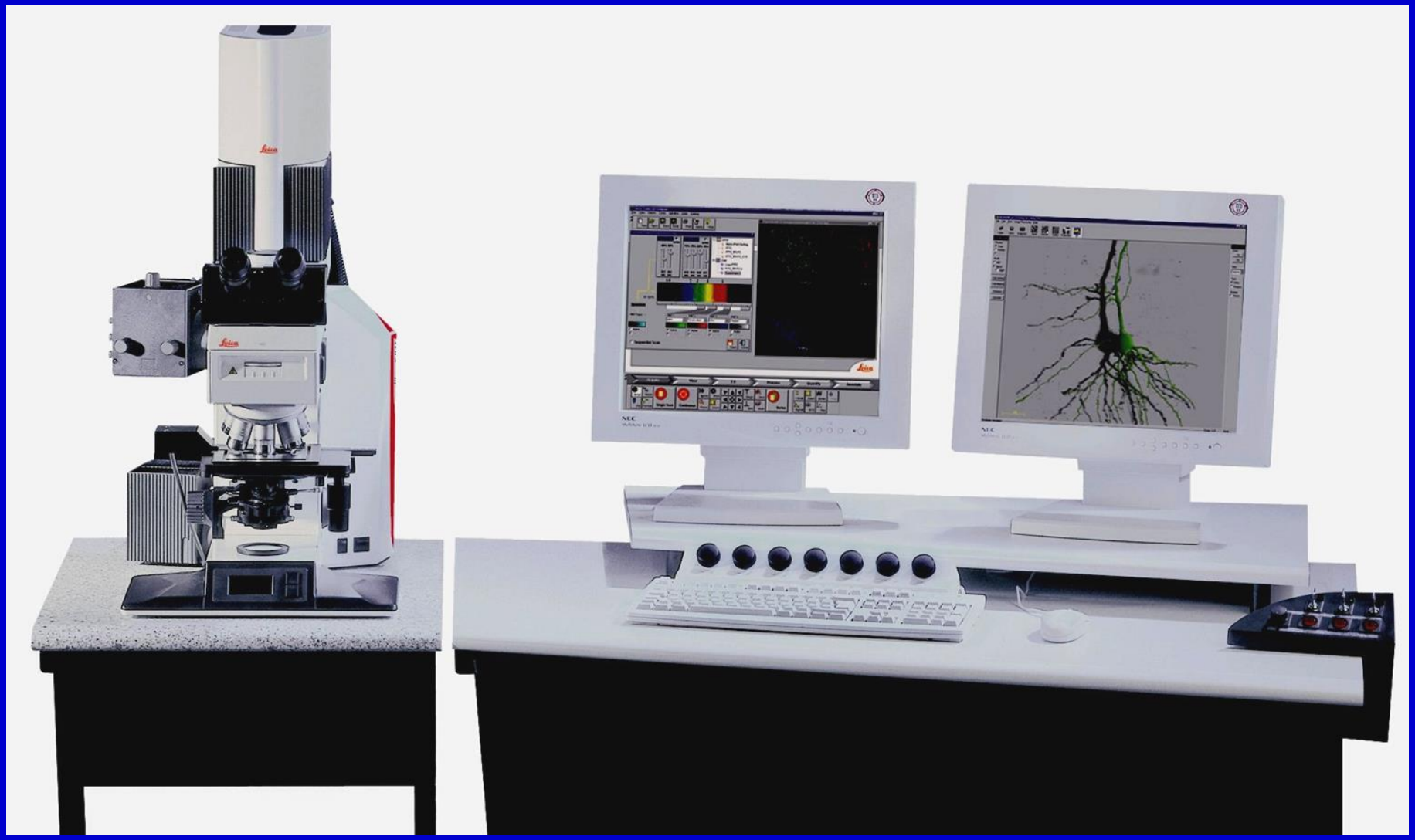
1. Provide non-invasive ways to observe and measure the *in situ* behavior of gene products.

2. Analysis of the dynamics of proteins association/dissociations at cellular structures.

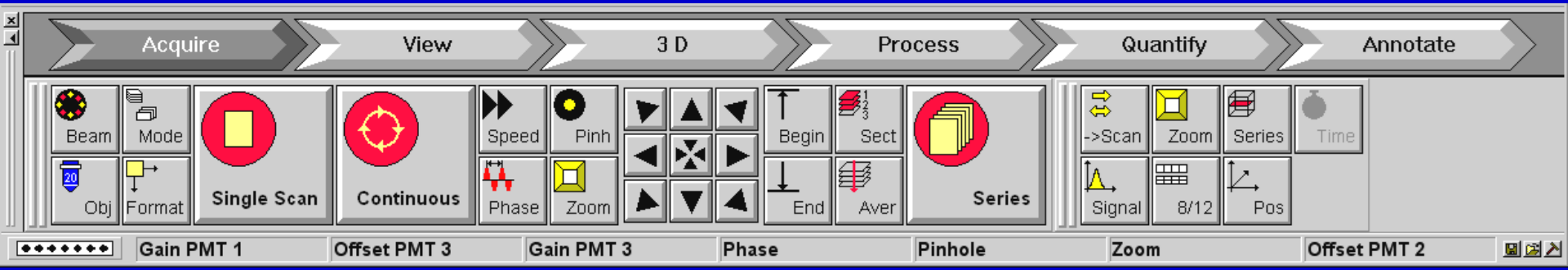


Leica TCS SP2

Confocal Spectral Microscope (UV-VIS)

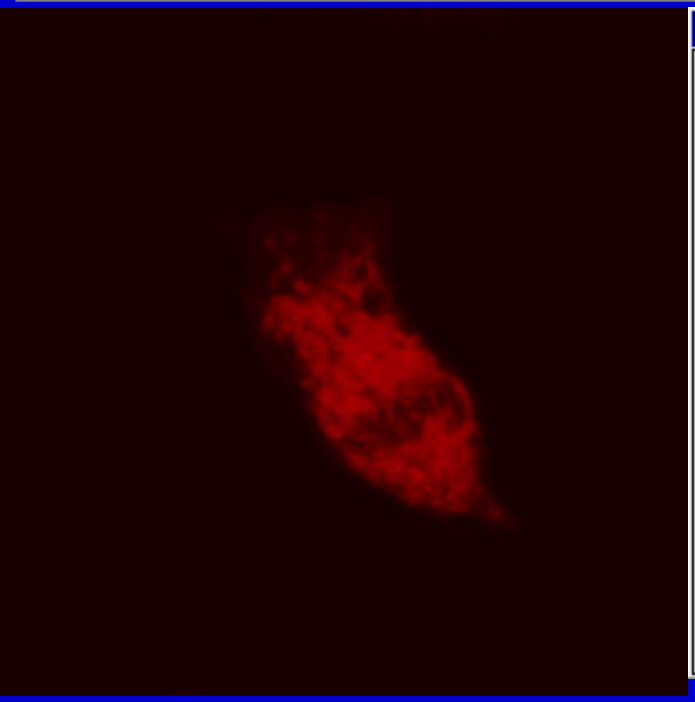


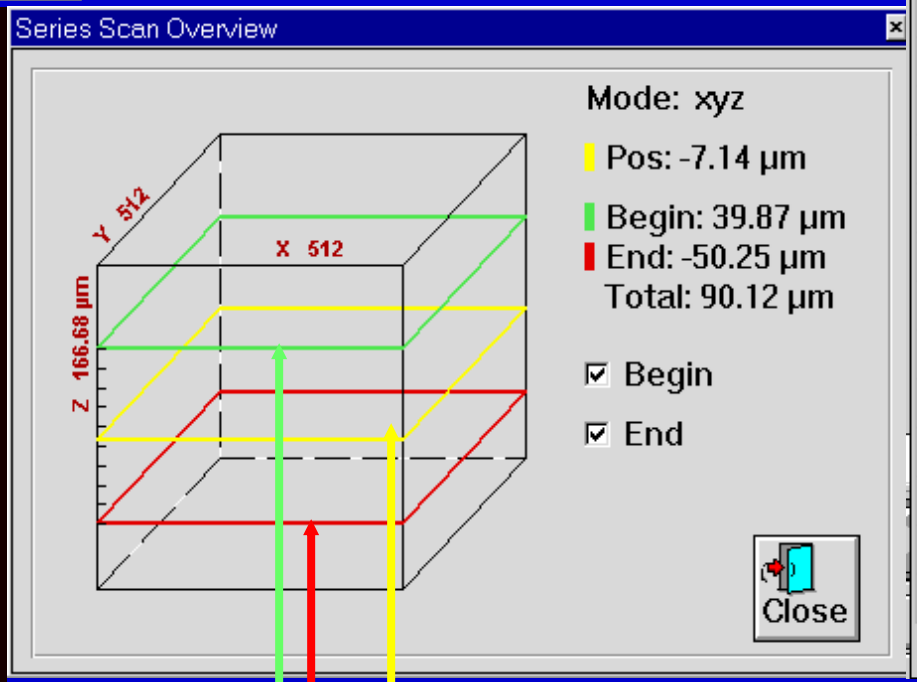
Mode : Scanning and Image Capture

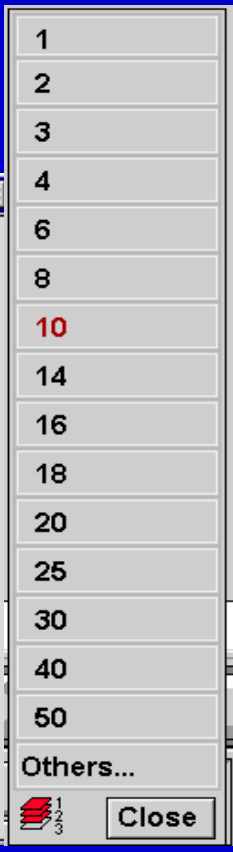


Mode	Functions
xyz	An image stack is recorded from xy-sections in z-direction. (3D)
xzy	An image stack is recorded from xz-sections in y-direction.
xt	A line is recorded several successive times.
xyt	An xy-section is recorded several successive times.
xzt	An xz-section is recorded several successive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

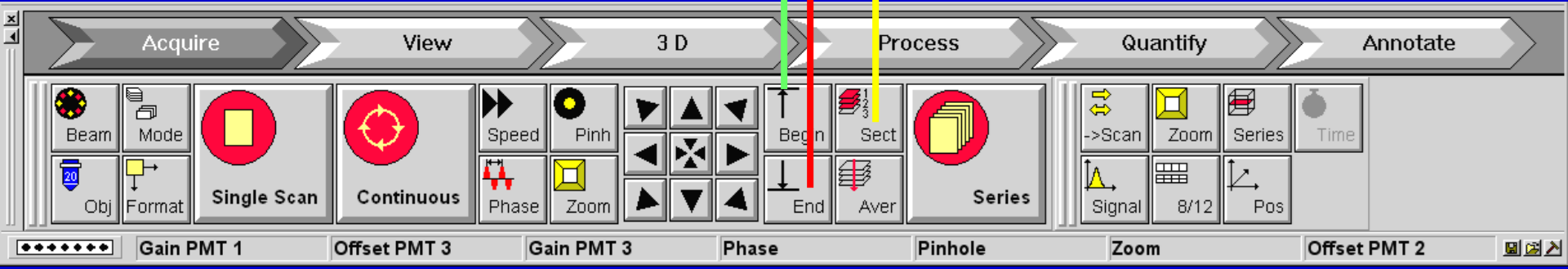
3D (xyz) series
Continuous scanning







Number of optic sections



Frame-Mode xyt Configuration

Time-lapse vs. Real Time (movie)

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

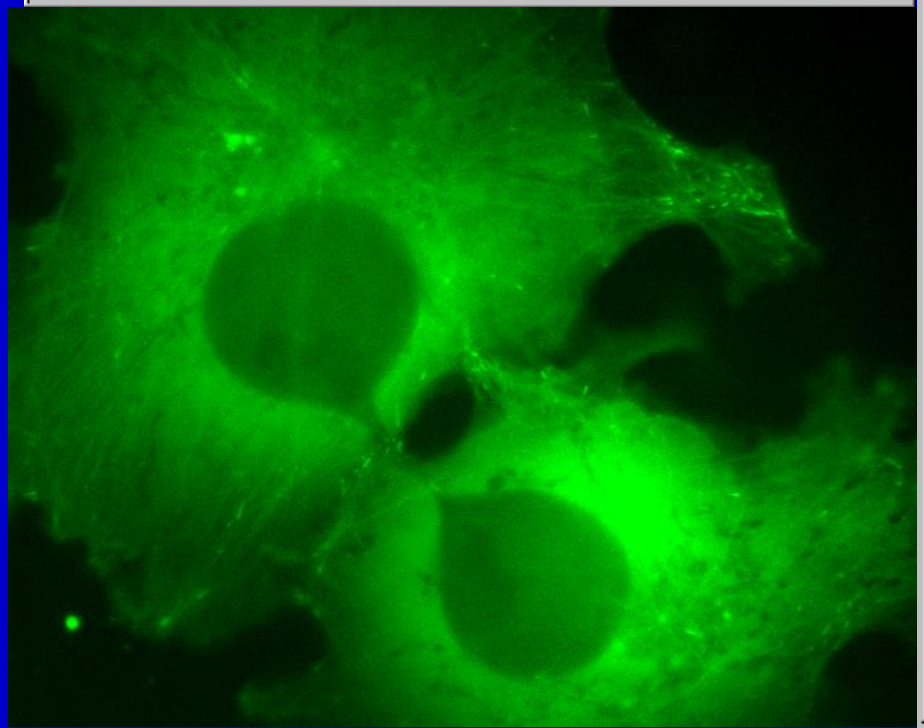
T Configuration

ΔT: 0 h 0 min 3 s 0 ms

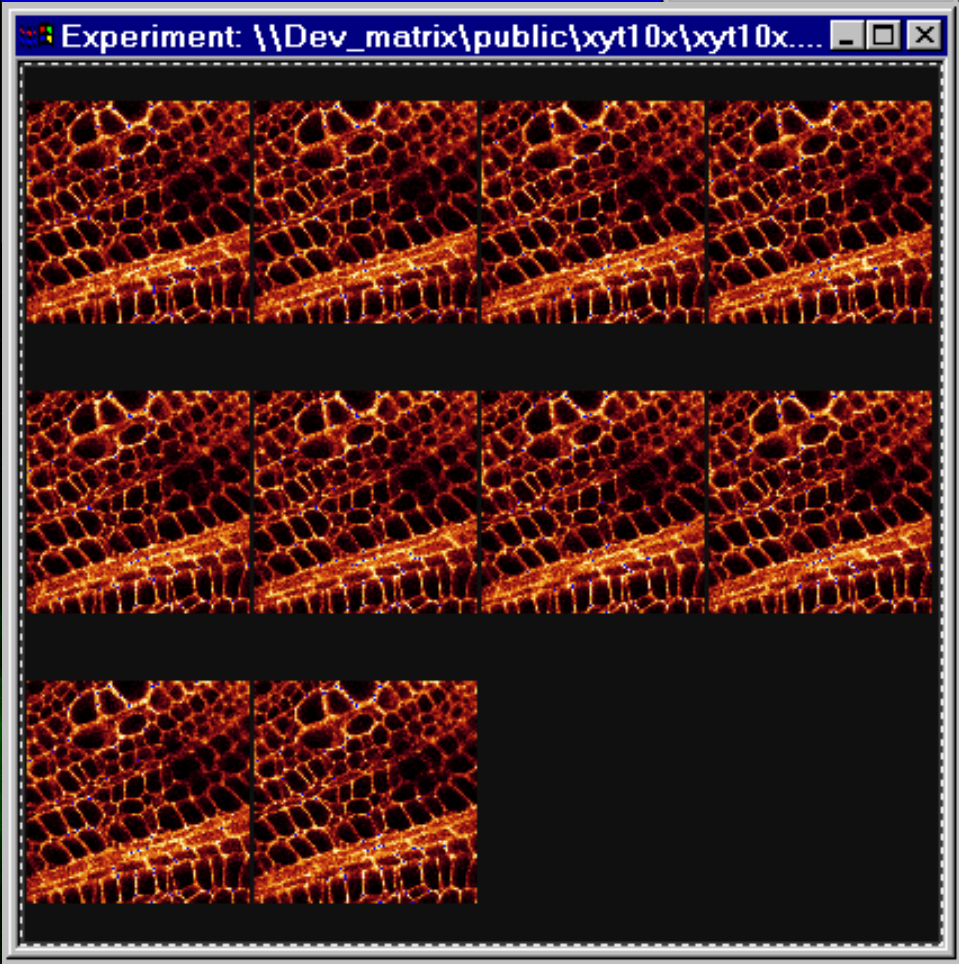
Frames: 10

Complete Time: 0 h 0 min 27 s 0 ms

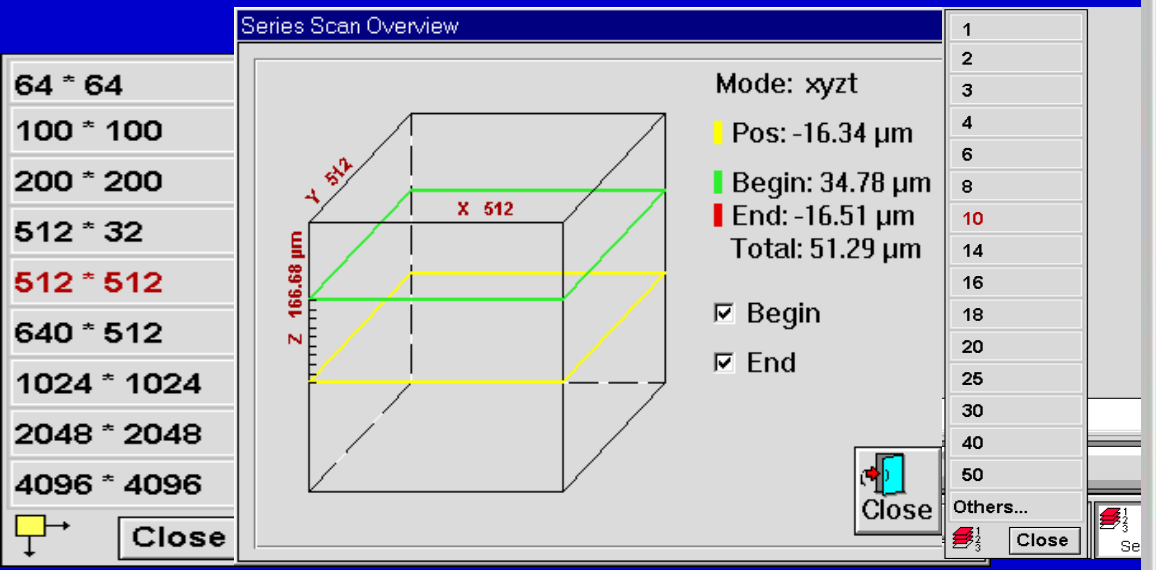
Reset Apply Close

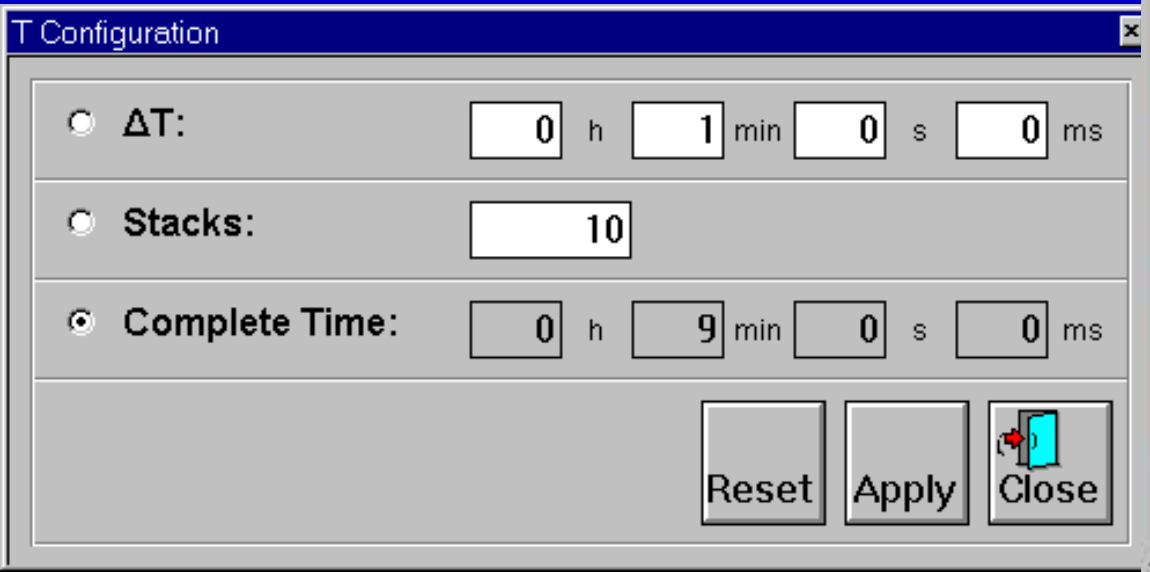


Experiment: \\Dev_matrix\public\xyt10x\xyt10x....



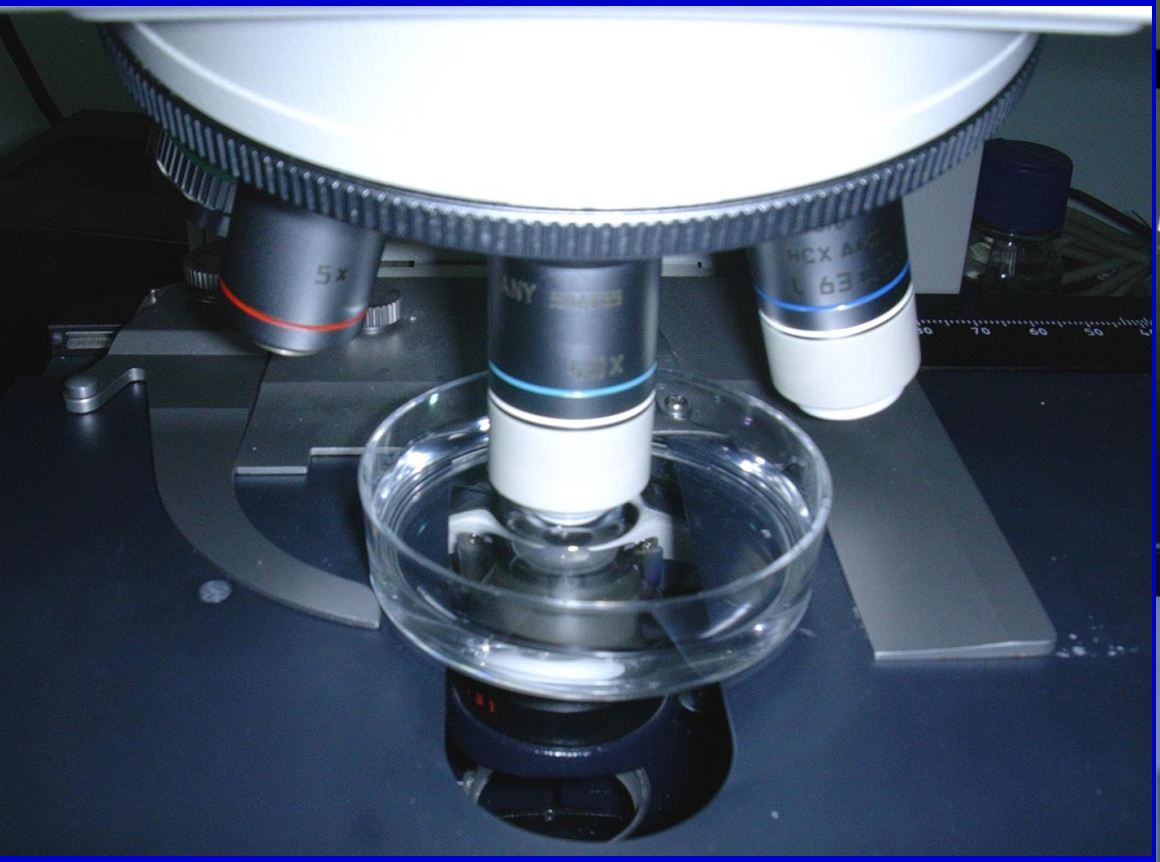
Stack-Mode xyzt Configuration

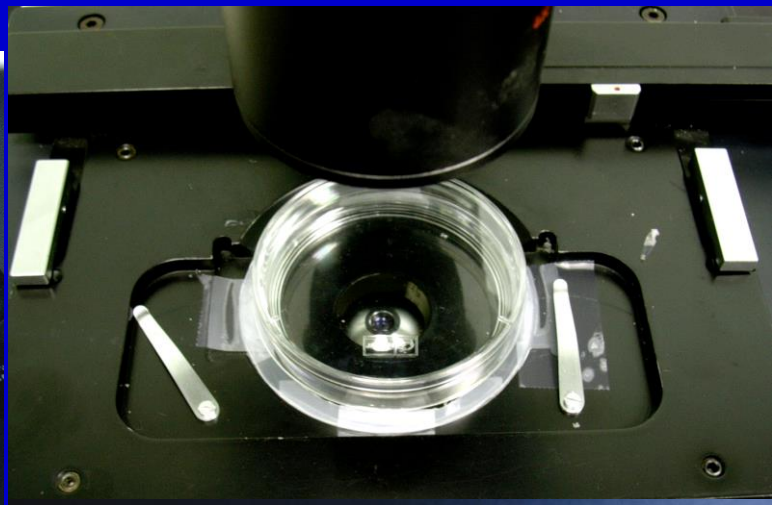




Traditional Live Cell Observation

Up-right microscope with Water Lens or Inverted microscope





Leica DM IRE2 microscope

enclosed within a computerized CO_2-incubator for indispensable thermal and mechanical stability

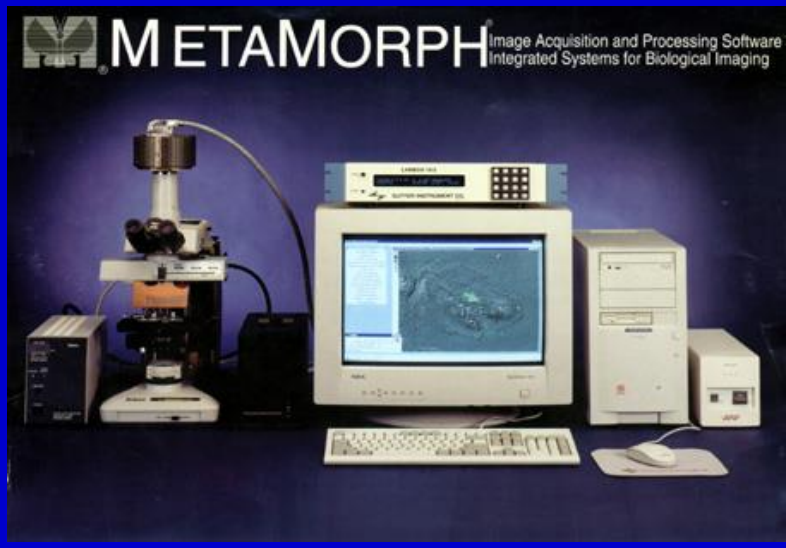
CO_2 controller

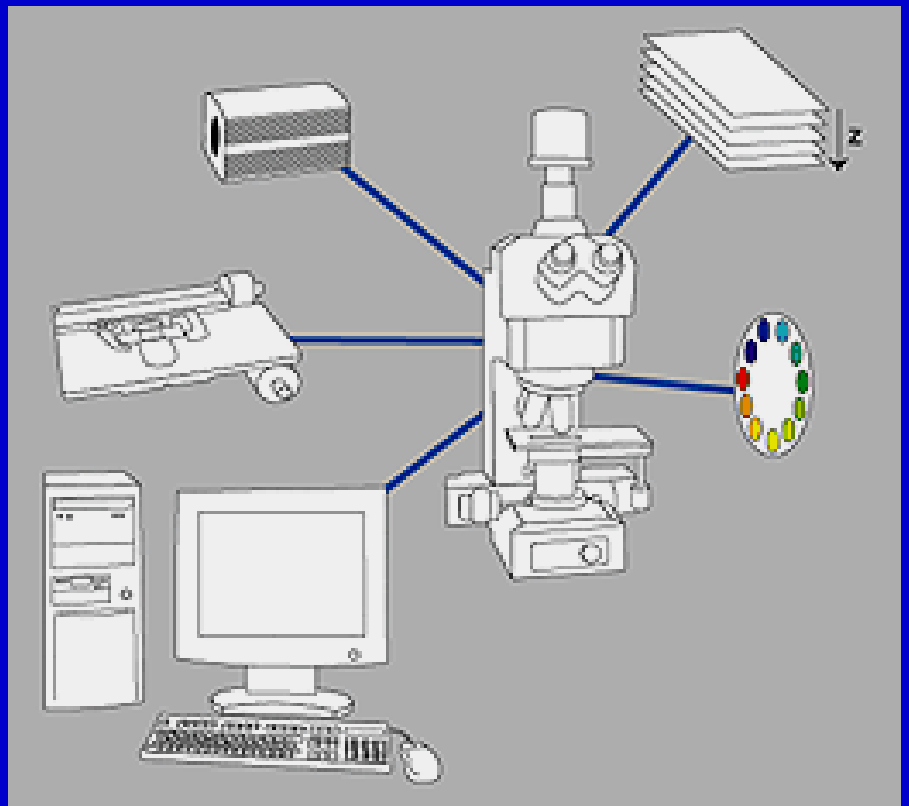
Microincubation Imaging-Chamber

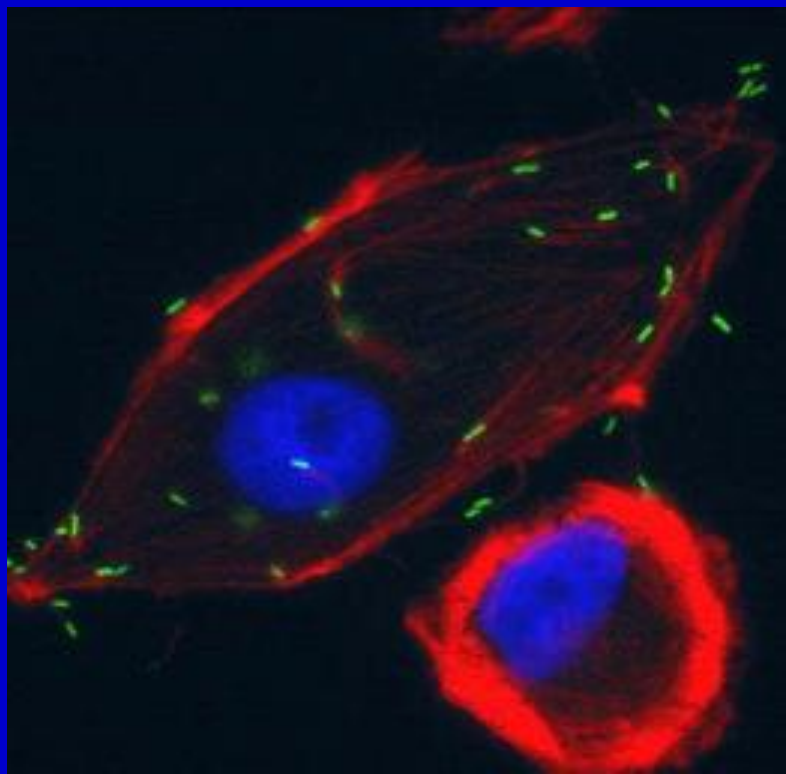
Software: MetaMorph System

integrated imagining system for maximized control

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







ASTEC

Real-Time Cultured Cell Monitoring System

—Supporting the Challenge of Discovery—

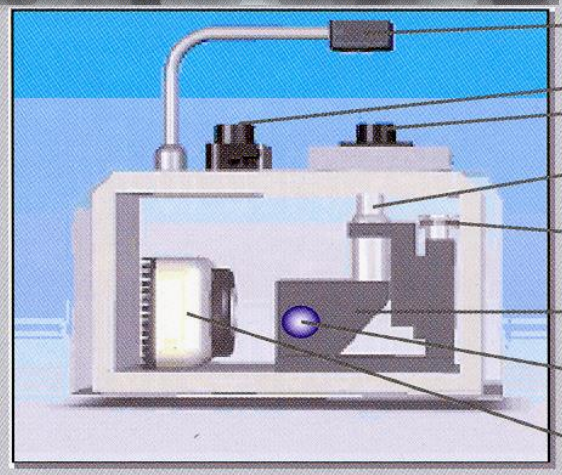
Designed to Fit...

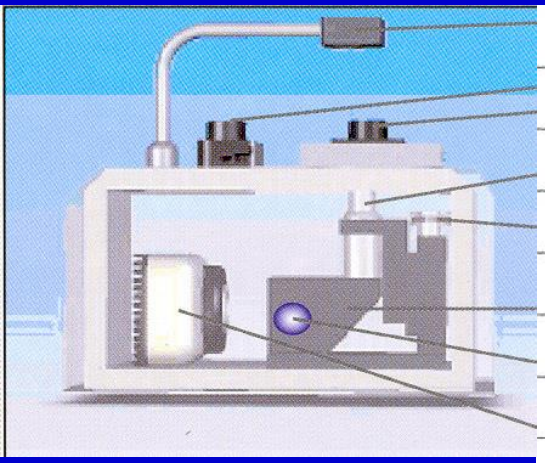
Designed to Resist...

Designed to Discover...

Microscope Now Rests in Incubator!!

Stem cell proliferation
Long-term recording
(movie)





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)

ASTEC

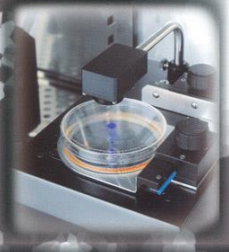
Real-Time Cultured Cell Monitoring System

—Supporting the Challenge of Discovery—

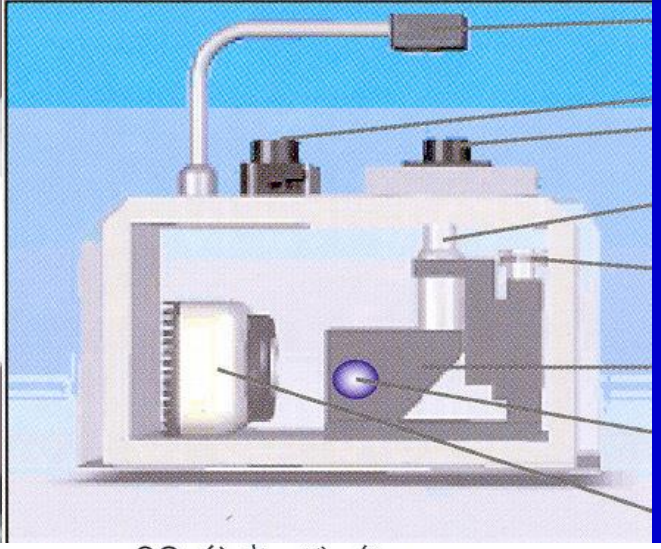
Designed to Fit...

Designed to Resist...

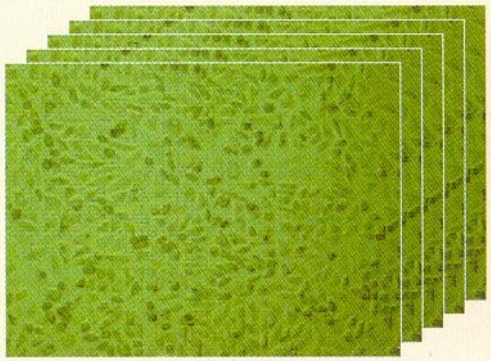
Designed to Discover...



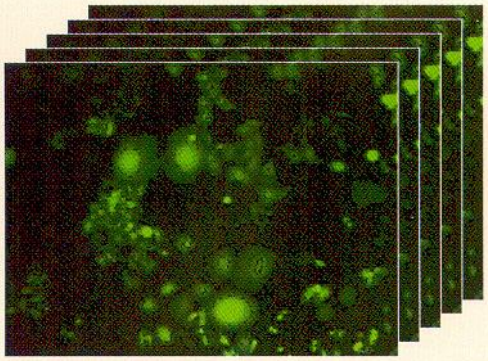
Microscope Now Rests in Incubator!!



使用設置例



形態観察画像

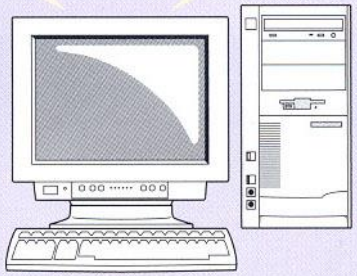


蛍光観察画像

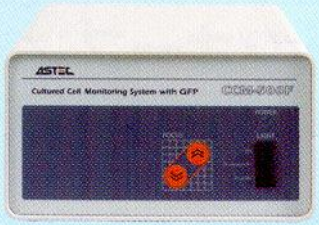
CO_2インキュベーター
(37℃ 95%RH 以上)

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