

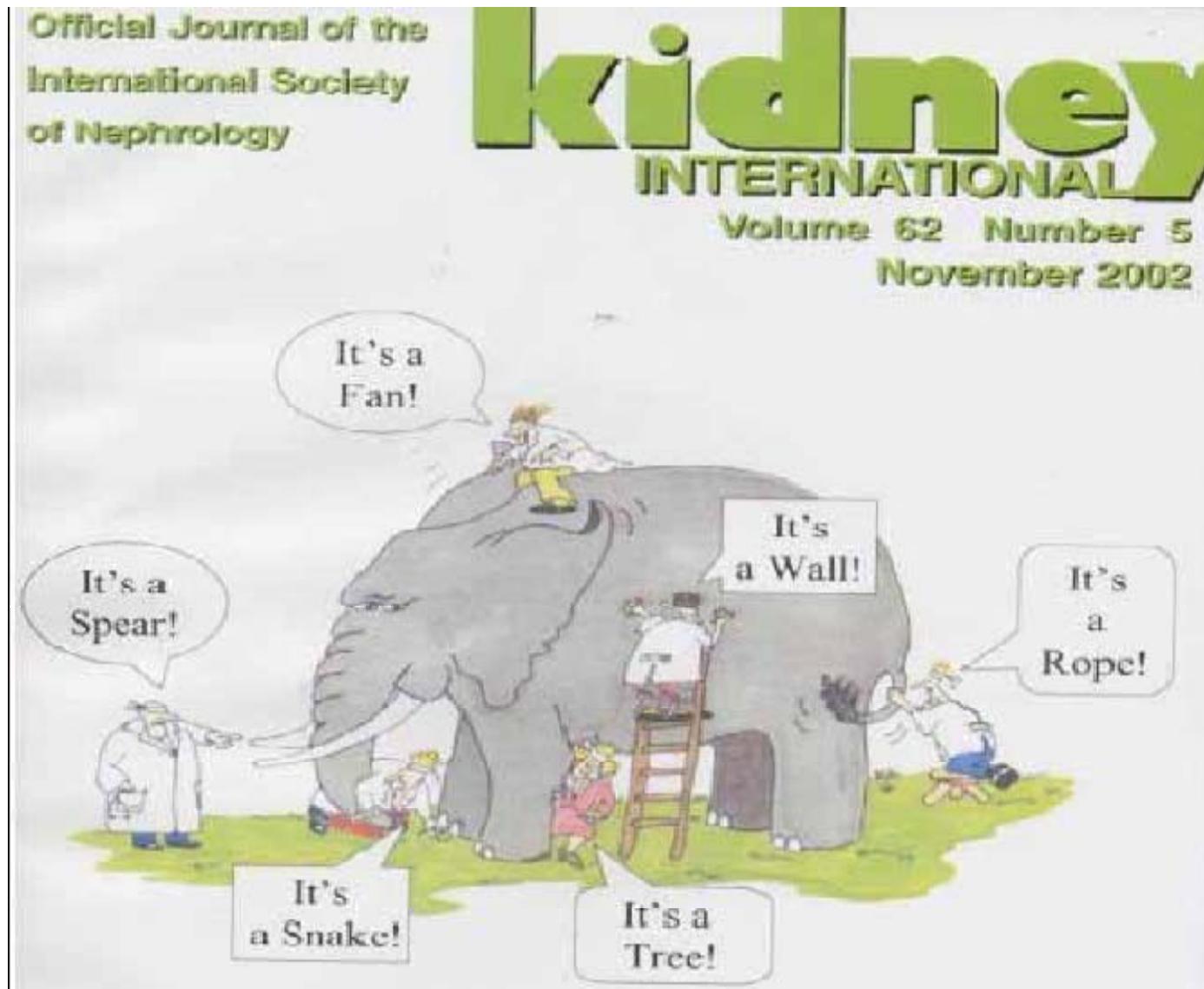
Neuronal Cellular Morphology

神经细胞形态学

Yan Zhang

张研

Reductionism & Hypothesis 还原论与假设



Basic morphology of the neuron

神经元的基本形态

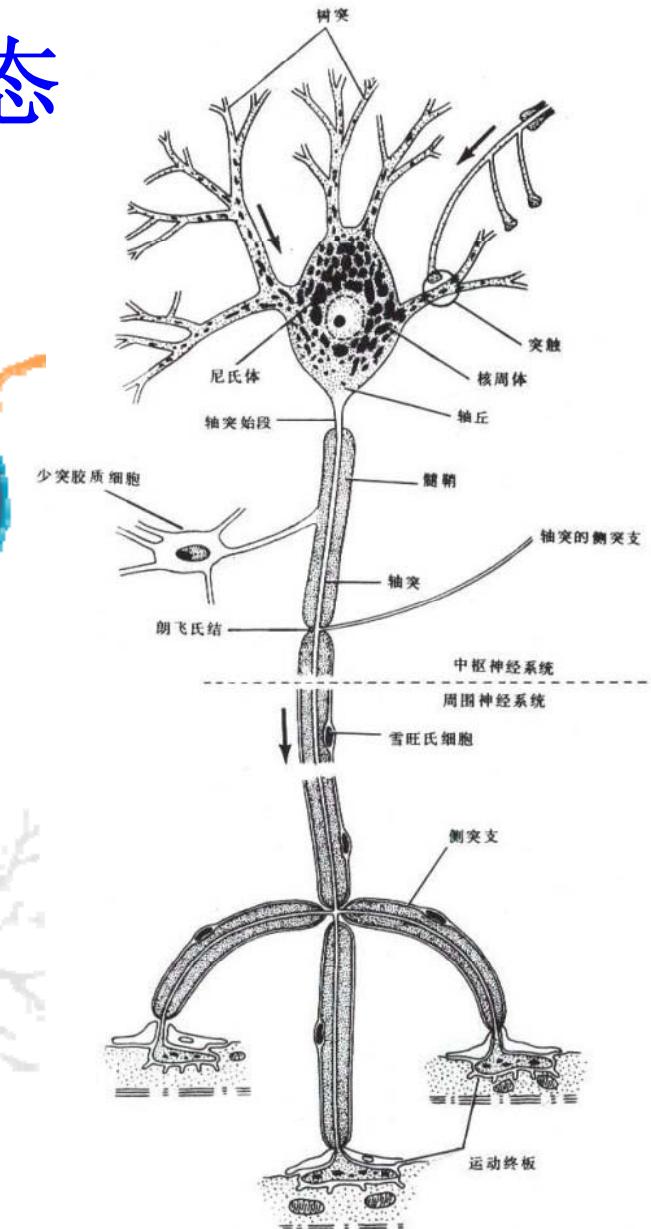
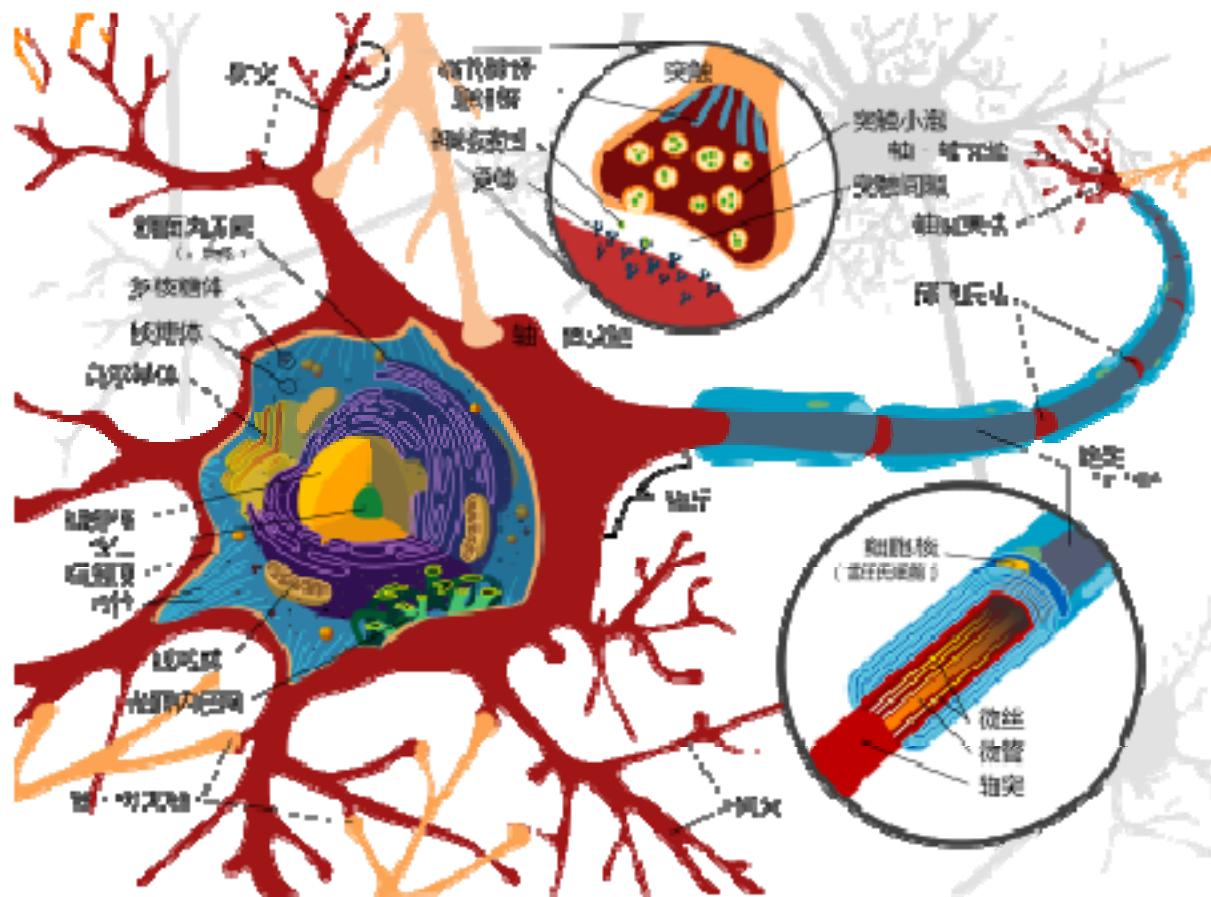
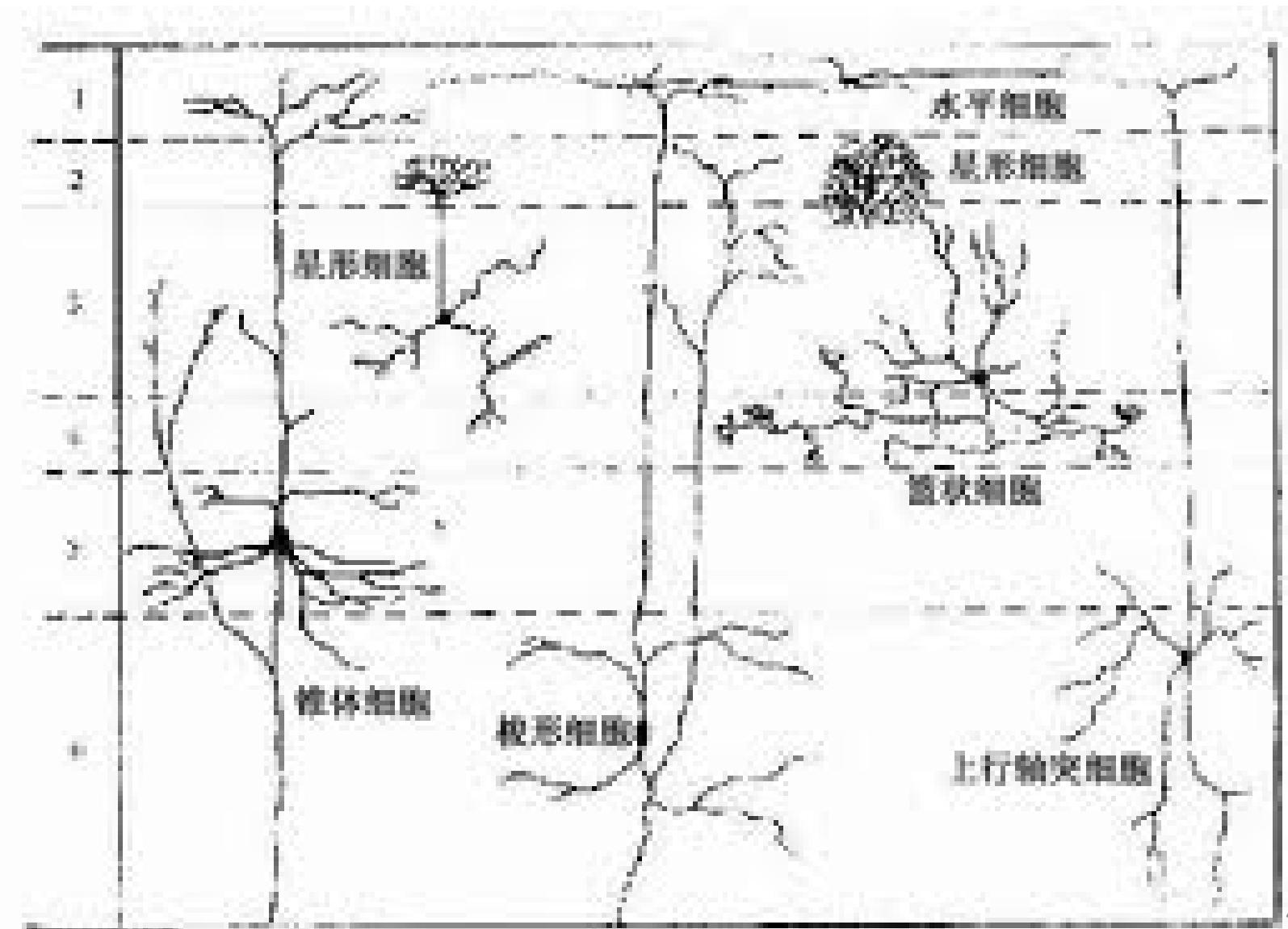
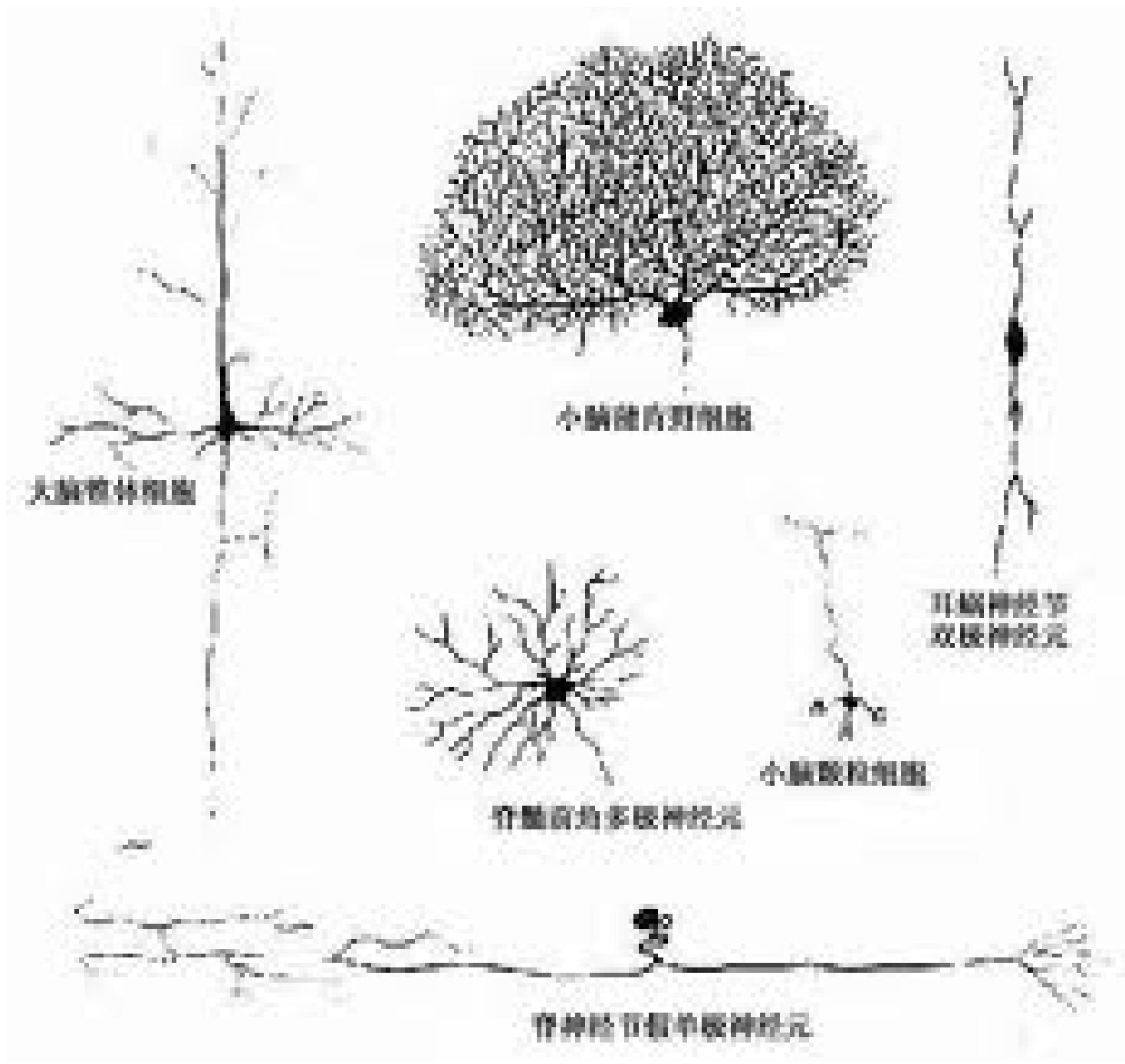


图 3 ~ 85 运动神经元示意图

Diversity of neuronal morphology

神经元形态的多样性





The principle of optical microscoped

光学显微镜原理

显微镜之所以能将被检物体进行放大，是通过透镜来实现的。单透镜成像具有像差，严重影响成像质量。因此显微镜的主要光学部件都由透镜组合而成。从透镜的性能可知，只有凸透镜才能起放大作用，而凹透镜不行。

Optical microscopes utilize a system of lenses to make enlarged images of samples. Aberrations in single-lens imaging systems have a negative effect on imaging quality. Thus, the main optical components are comprised of a set of lenses. The convex lens is able to enlarge things while the concave lens is not due to the property of the lens.

1673年荷兰人列文虎克
(Antoni van Leeuwenhoek) 用自己制造的显微镜观察到了被他称为“小动物”的微生物世界。列文虎克原本只是个荷兰德尔夫市政府的看门人。他利用看门之余，磨制了许多镜片。他发现了杆菌、球菌和原生动物，因为这个伟大的发现，他当上了英国皇家学会的会员。



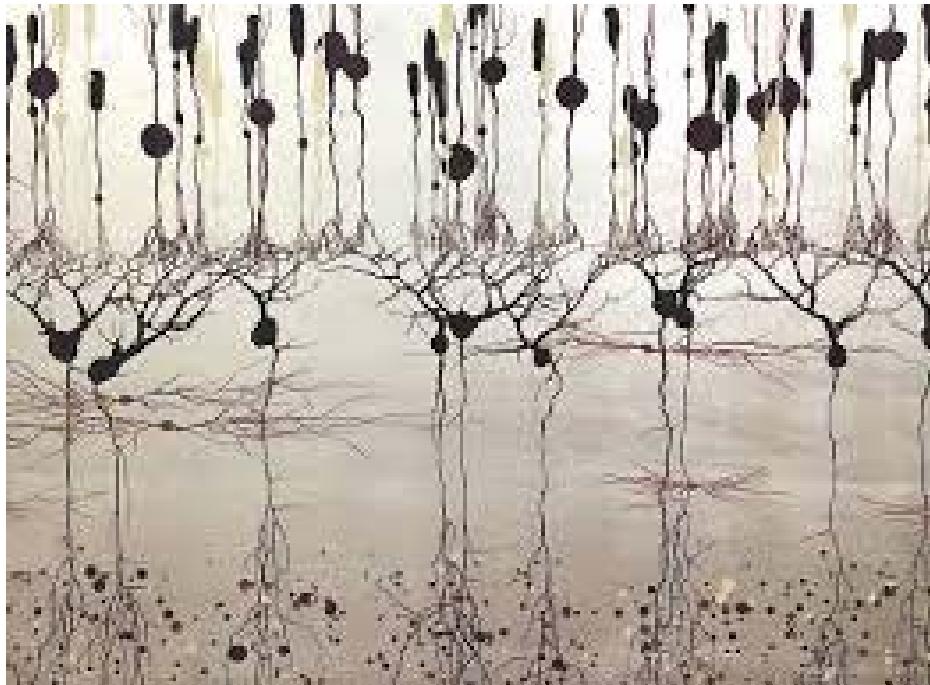
In 1673, Antoni van Leeuwenhoek in Holland became the first to observe and describe microorganisms using his handcrafted microscopes, which he originally referred to as animalcules. He used to be a door keeper in Delft government. In his free time, he made plenty of lens and discovered bacillus, coccus and protists. He became a member of the Royal Society due to these discoveries.

Golgi staining 高尔基染色

Camillo Golgi (1844-1926) , 意大利医生、神经学家、组织学家。帕维亚大学教授。1906年获得诺贝尔生物学奖。

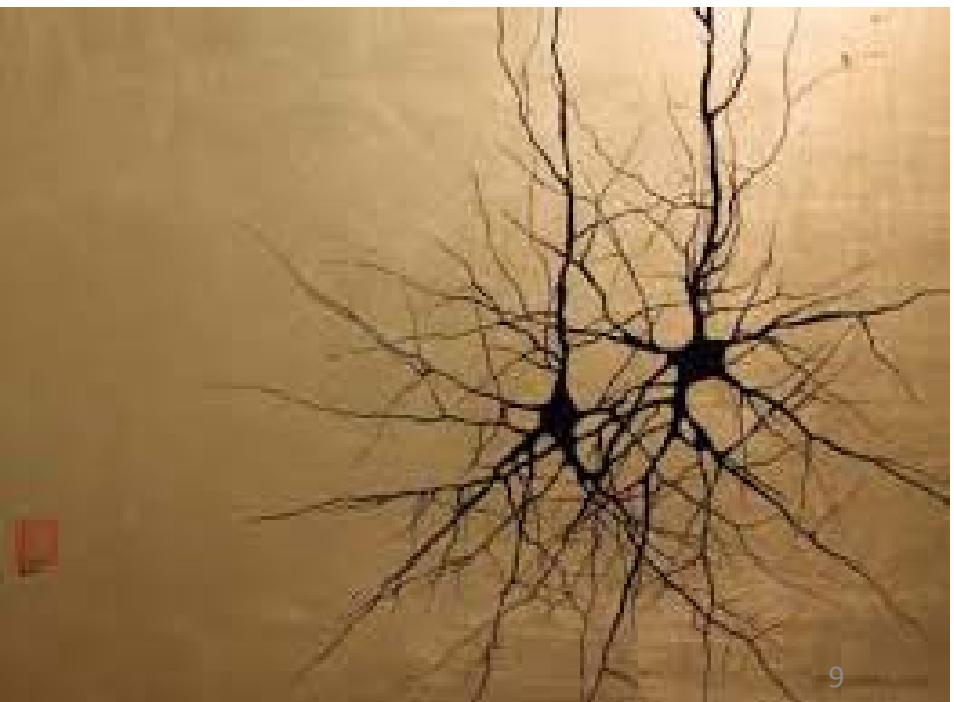
Camillo Golgi(1844 – 1926)
Italian doctor, neuroscientist and
histologist, professor in the University
of Pavia, received the Nobel Prize in
Physiology or Medicine in 1906.





1898年观察到神经细胞质中的硝酸银染色区域，后称“高尔基体”或“高尔基器”。

In 1898, Golgi observed regions stained with silver nitrate in the neuronal cytoplasm. Golgi identified the intracellular reticular apparatus bearing his name, the Golgi body or Golgi apparatus.



1873 年， Golgi 在《 *Gazzetta Medica Italiana* 》杂志上发表了一篇题为“脑灰质结构（ On the structure of the brain grey matter ）”的短文，文中介绍了他经过长时间系列的尝试性研究，终于找到了一种用金属浸染（ metallic impregnations ）的方法能清楚地观察到神经组织的成分，这就是“黑色反应”（ Black reaction ）的发现。



In 1873, Golgi published an article "On the structure of the brain grey matter" on the journal *Gazzetta Medica Italiana*. This paper introduced his long tentative research to an approach in use of metallic impregnations which made it possible to clearly observe neural tissue components (black reaction).

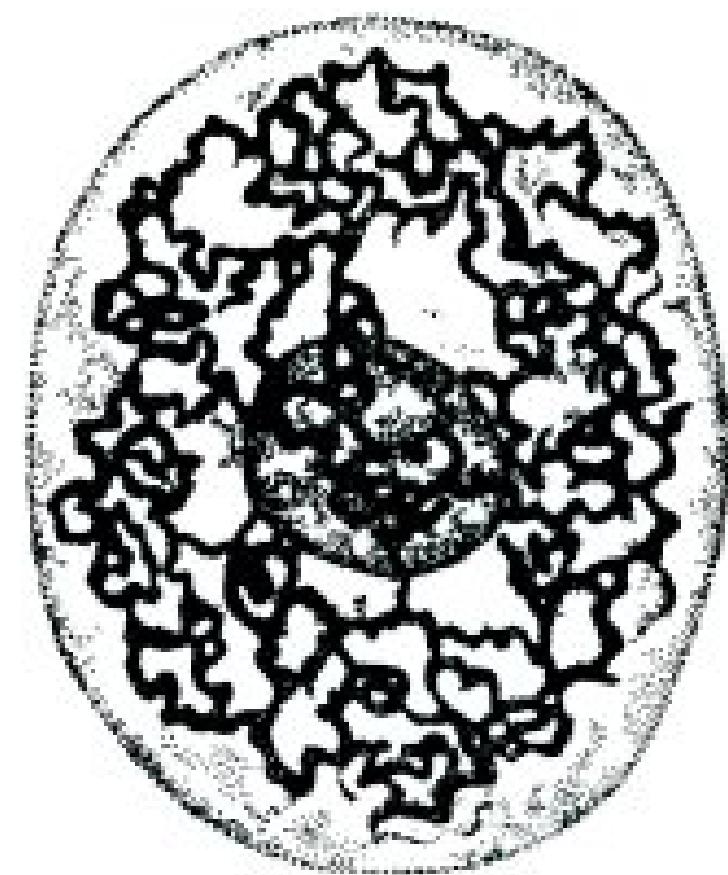
这种染色法是将神经组织在重铬酸钾溶液中被固定，并以硝酸银沉着于神经组织而使之显色。这一染色法至今仍被广泛应用，并被称为 Golgi 染色法（Golgi staining）或 Golgi 浸染法（Golgi impregnation）。这一方法能使少量神经细胞的胞体及其全部突起随机地（其原因至今仍不清楚）被浸染而清晰地显示出来。这是人类历史上第一次在显微镜下观察到了神经细胞和神经胶质细胞。



The staining method is to make nervous tissues fixed in potassium dichromate solution, silver nitrate subsided in the nervous tissues and finally colored, which is still widely used and is called Golgi staining or Golgi impregnation. This method makes the cell body and neurites of a few nerve cells randomly stained (the reason is still not clear) and clearly visualized. This is the first time in human history to observe nerve cells and glial cells under a microscope.

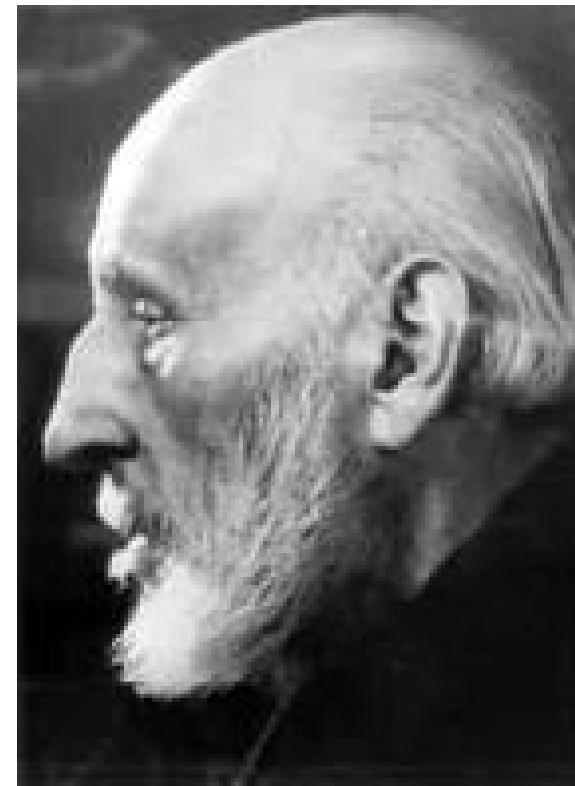
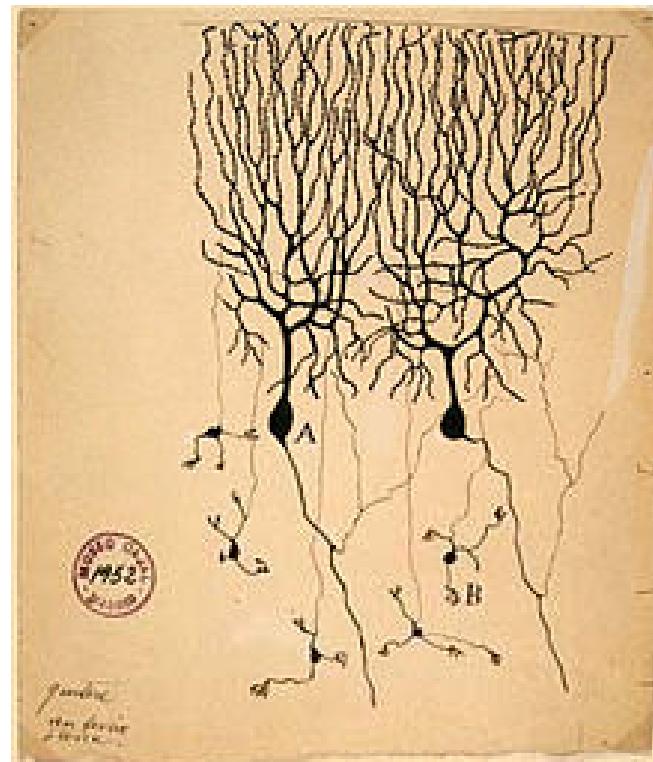
1897年，高尔基就是利用他所发明的染色法，在猫头鹰和猫的小脑神经细胞（浦肯野细胞）中发现了一种巨大的黑色网状结构，并将其命名为内网器，1898年人们便将其更名为高尔基体。20世纪初期，一开始大家都认为这所谓的黑色结构只不过是由于固定和染色而产生的一种认为假象。直到50年代，电镜的出现使直接观察细胞超微结构成为可能，A.J.Dalton和M.D.Felix等人等工作才最终证实了高尔基体的存在。

In 1897, Golgi found a huge black mesh structure in owl and cat cerebellum neuronal cells (Purkinje cells) by this staining method and named it the internal reticular apparatus. In 1898 it was renamed as the Golgi apparatus. In early 20th century, it was widely accepted at first that the black structure was just artificial due to fixation and staining. Until the 1950 s, the invention of electron microscope made it possible to observe the cell ultrastructures directly, and A.J.D Alton and M.D.Felix finally confirmed the existence of the Golgi apparatus.



Ramn y Cajal (1852~1934) 西班牙神经组织学家，神经解剖学家。1903年他改进了神经组织学的染色方法(主要是创建了还原硝酸银染色法)，并用以研究胚胎和幼小动物脑和脊髓神经细胞的微细结构和细胞间的连拉关系，同时阐明了视网膜的微细结构。

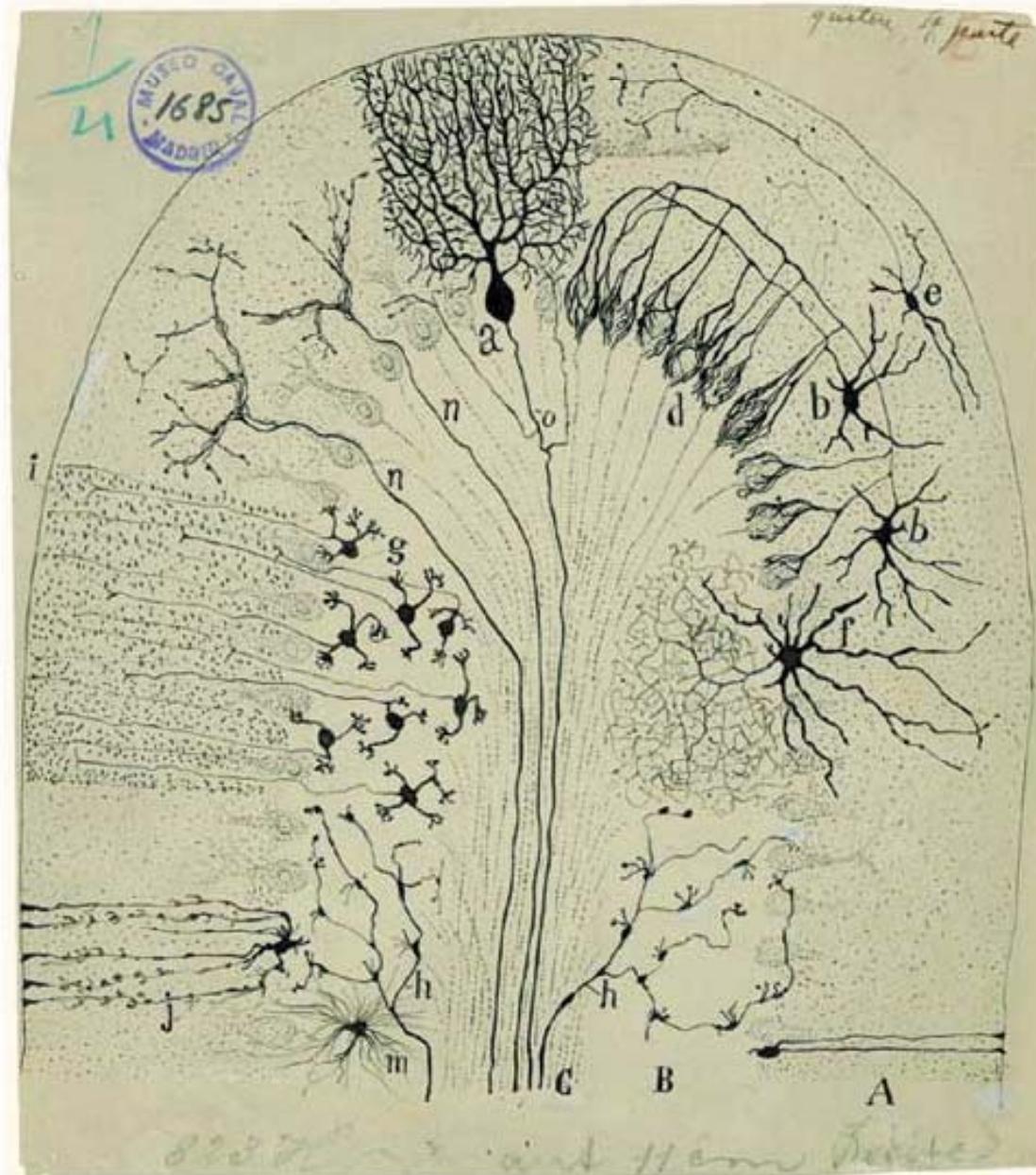
Ramn y Cajal (1852 ~ 1934) Spanish neural histologist and neuroanatomist. In 1903, he improved neural histological staining method (mainly by invention of reduced silver nitrate staining method) to study fine structures of nerve cells in the brain and spinal cord and neuronal connections of embryo and young animals and illustrated the fine structure of the retina.



拉蒙·伊·卡哈尔, S.

19世纪80年代他对神经细胞是神经系统基本结构单位提出许多有力证据，认为每个神经细胞都是独立的，它的轴突末端以不同的形态与其他神经细胞接触。他阐明了神经细胞间的真正关系，对神经元学说的确立有重要贡献。1906年他与意大利生物学家C.高尔基同获诺贝尔生理学或医学奖。

In the 1880s Cajal put forward many strong evidences of the hypothesis that nerve cells are basic structure units of the nervous system and every nerve cell is independent and its axon terminal interacts with other nerve cells in different forms. He clarified the relationship between nerve cells and made great contributions to the establishment of the neuron doctrine. In 1906 he won the Nobel Prize in physiology or medicine sharing with the Italian biologist C.Golgi.

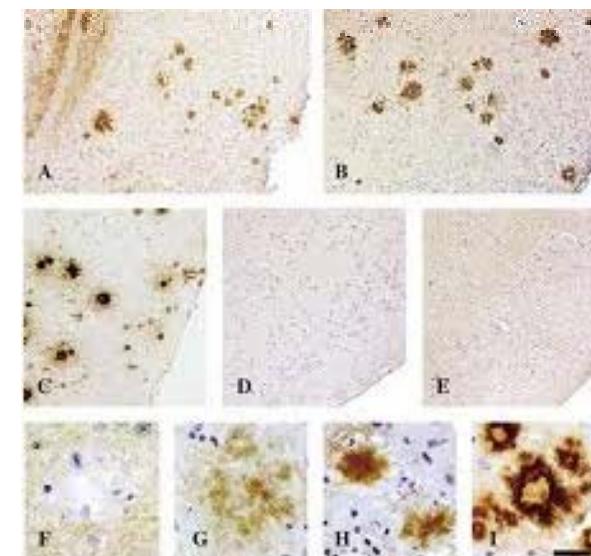
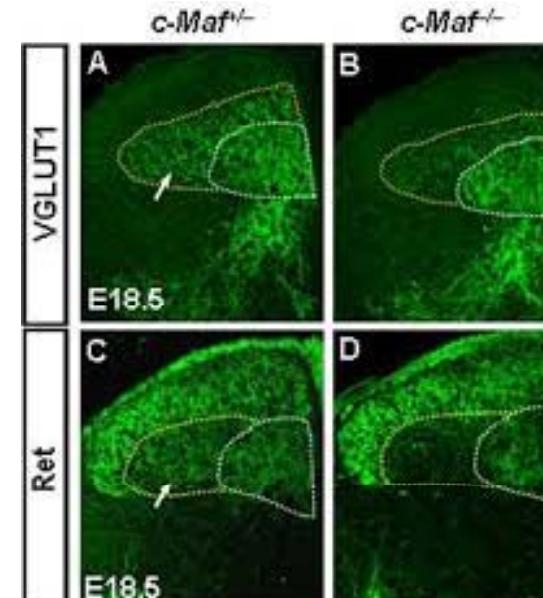
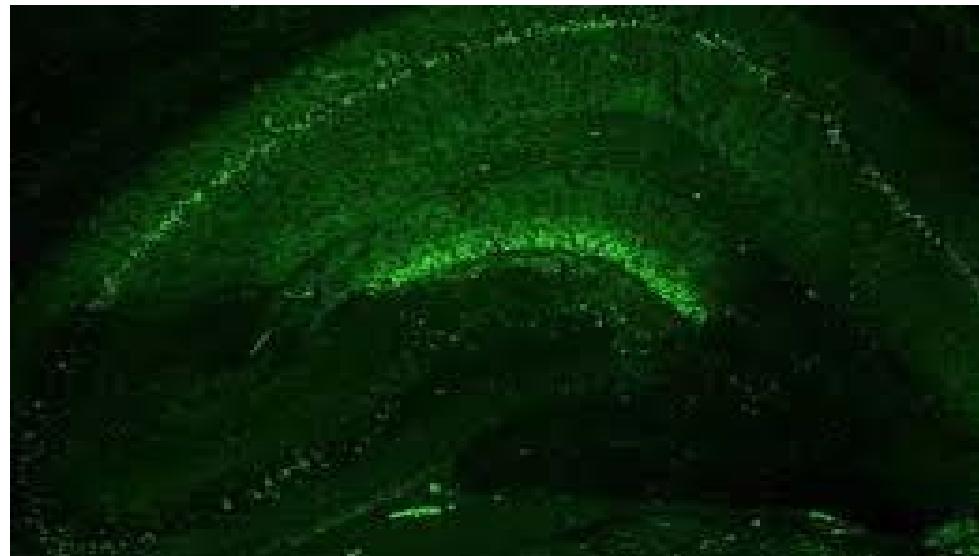


"Neuron doctrine" stands as the foundation of modern neuroscience. In the debate of the neural network theories (neuron theory, reticular theory) Ramón y Cajal was a fierce defender of the neuron theory. He discovered the axonal growth cone and dendrite spines.

“神经元学说”奠定了现代神经科学的基础，在与“神经网络学说”之争中，Ramón y Cajal是“神经元学说”的不屈捍卫者，他发现了轴突生长锥和树突棘。

Labeling methods in tissues

组织中标记方法



The story of fluorescent protein

荧光蛋白的故事

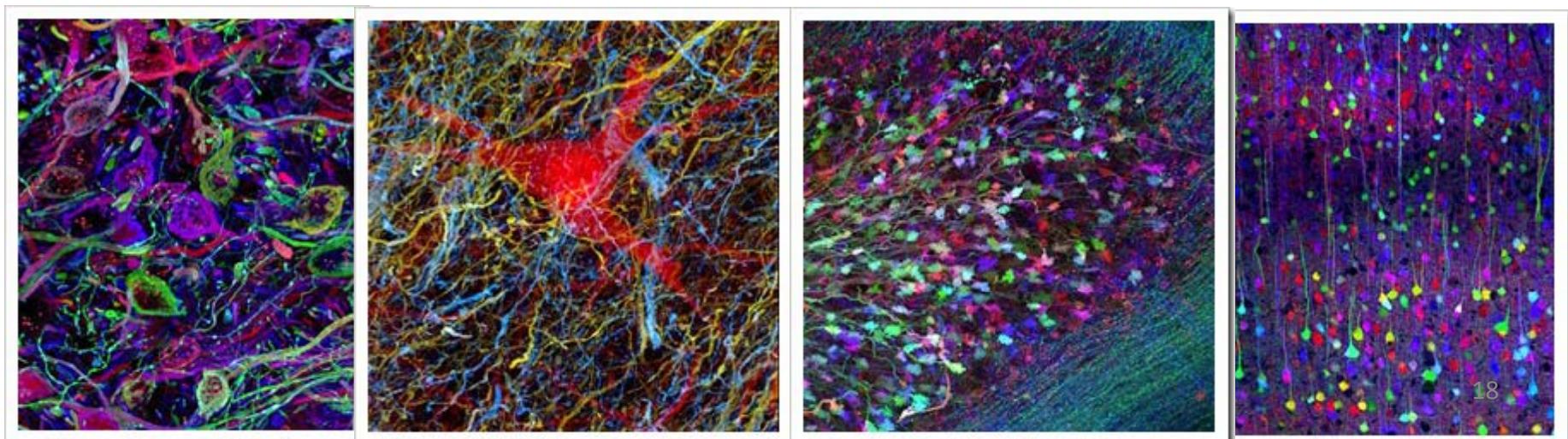
首先发现绿色荧光蛋白的是生于1928年的下村修。下村修出生于日本京都府，1960年获得名古屋大学理学博士学位，曾先后在美国普林斯顿大学、波士顿大学和伍兹霍尔海洋生物实验所工作。他1962年从一种水母中发现了荧光蛋白，被誉为生物发光研究第一人。他1962年从生活在美国西海岸近海的一种水母身上分离出了绿色荧光蛋白。



Green fluorescent protein (GFP) was first discovered in 1928 by Osamu Shimomura. He was born in Kyoto and got his Ph.D. in Nagoya University and later worked in Princeton University, Boston University and Woods Hole Oceanographic Institution. He was the first to discover bioluminescence due to his discovery and isolation of the green fluorescent protein in jellyfish living in west coast of the US.

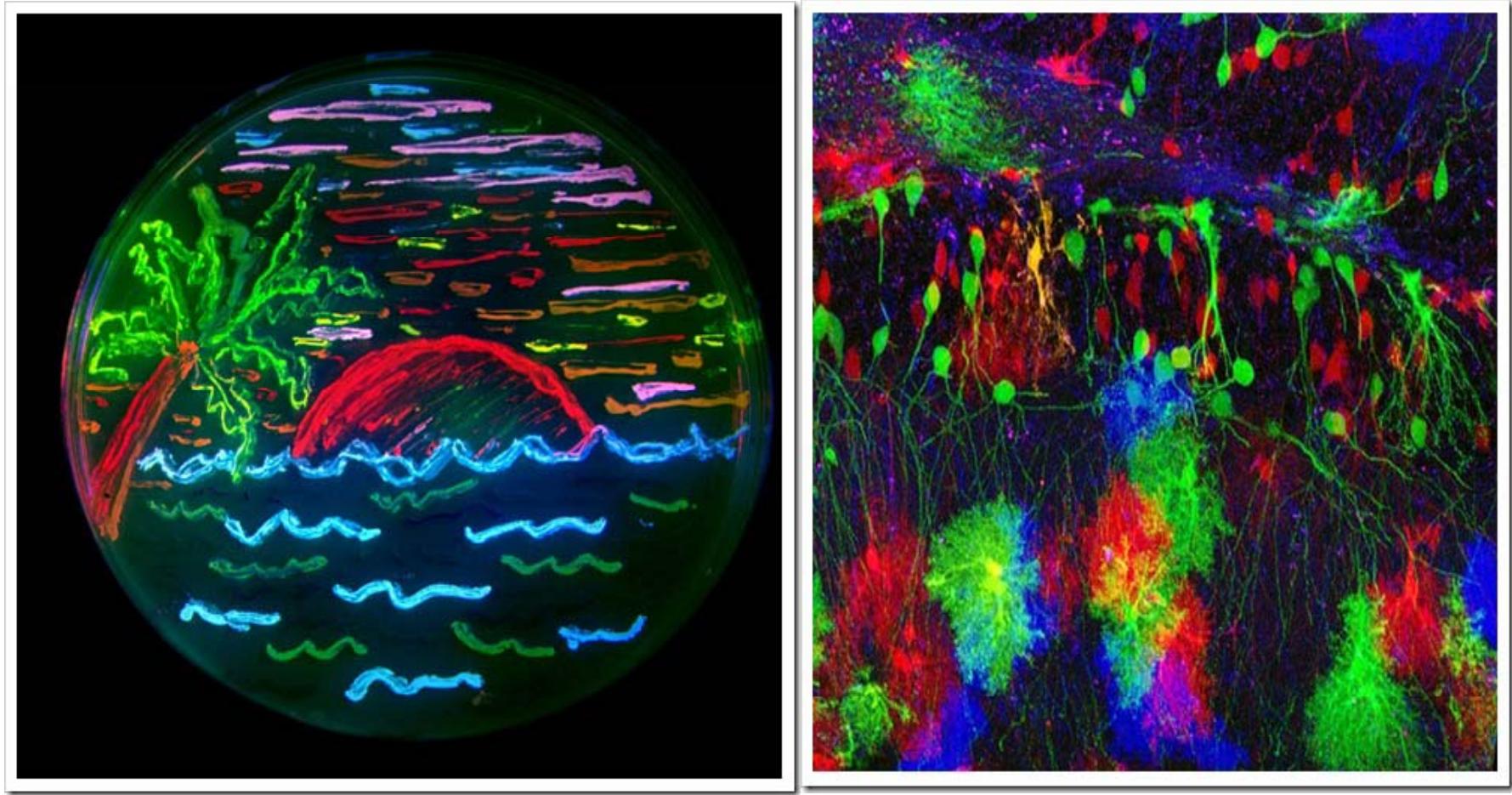
钱永健走出的可说是绿色荧光蛋白发展历程的“最后一步”，他在下村修与沙尔菲研究的基础上进一步搞清楚了绿色荧光蛋白特性。他改造绿色荧光蛋白，通过改变其氨基酸排序，造出能吸收、发出不同颜色光的荧光蛋白，其中包括蓝色、青色和黄色，并让它们发光更久、更强烈。世界上目前使用的荧光蛋白大多是钱永健实验室改造后的变种。

Roger Tsien took the last step of GFP development. Based on Shimomura and Chalfie's researches, he took further steps to clarify the characteristics of GFP. By changing its amino acid sequence, he produced fluorescent proteins with different absorbing and emitting colors including blue, cyan and yellow and with longer and stronger fluorescence. Currently most of the fluorescent proteins used worldwide are variant proteins of Tsien's lab.

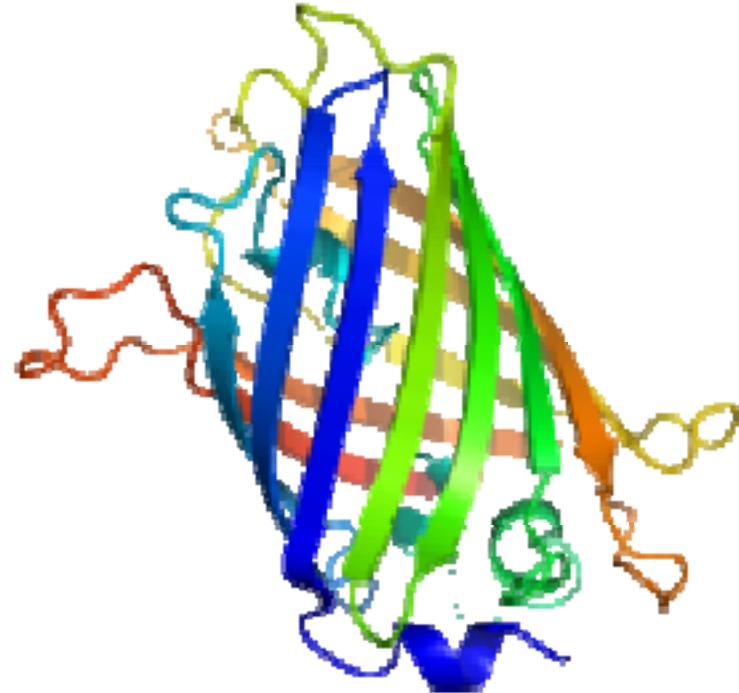


钱永健1952年生于纽约，现为美国加州大学圣迭戈分校生物化学及化学系教授、美国国家科学院院士、国家医学院院士，2004年沃尔夫奖医学奖得主。主要贡献是利用水母发出绿光的化学物来追查实验室内进行的生物反应，他被认为是这方面的先驱。

Roger Tsien was born in New York in 1952 and is now a professor of biochemistry and chemistry in the university of California, San Diego and a member of the National Academy of Sciences and the Institute of Medicine and won Wolf prize in Medicine in 2004. His major contribution is to trace biological reactions by use of glowing green chemicals found in jellyfish and he was regarded as the pioneer of this field.



photoconvertible fluorescent protein 显色可变的荧光蛋白
Split-GFP 分裂绿色荧光蛋白



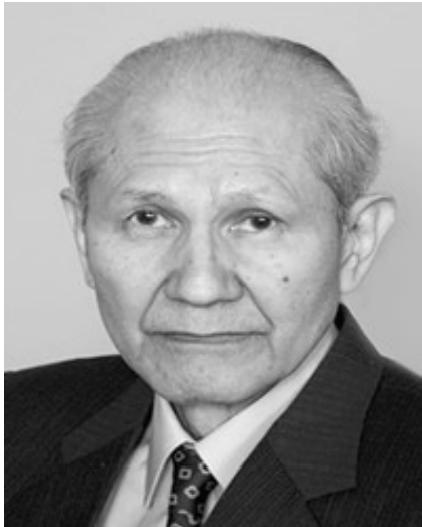
2008年10月8日，日本科学家下村脩(Osamu Shimomura)、美国科学家马丁·查尔菲(Martin Charfie)和钱永健(Roger Tsien)因为发现和改造绿色荧光蛋白而获得了当年的诺贝尔化学奖。

On October 8th, Japanese marine biologist Osamu Shimomura, American scientist Martin Charfie and Roger Y. Tsien shared the 2008 Nobel Prize in Chemistry for the discovery and development of the green fluorescent protein (GFP).



GFP-based sculpture *Steel Jellyfish* (2006). The image shows the stainless steel sculpture on display at Friday Harbor Laboratories on San Juan Island (Wash., USA), the place of GFP's discovery.

以GFP结构为基础的雕塑——钢化水母这个不锈钢雕塑陈列于美国华盛顿区圣胡安岛的星期五港实验室，GFP发现的地方。



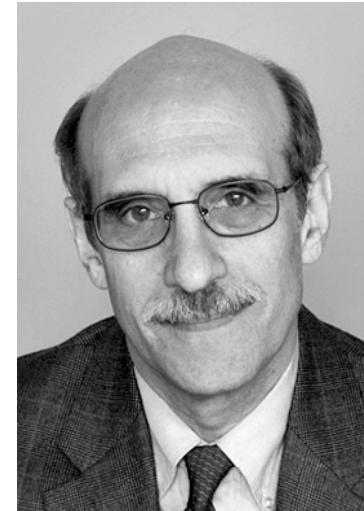
Osamu Shimomura **Douglas C. Prasher**

下村修
从luciferin到
aequorin，发现荧
光蛋白是钙离子敏
感的。

From luciferin to
aequorin, he found
that fluorescent
proteins are
sensitive to
calcium ions.



找到并克隆了编码
aequorin的基因。最先意
识到可以通过基因工程的
方法将这种荧光蛋白连接
到细胞内的各种分子上
*Found and cloned the
gene encoding aequorin.
First to realize that
genetic engineering can
be adopted to link this
fluorescent protein with all
sorts of molecules.*



Martin Charfie

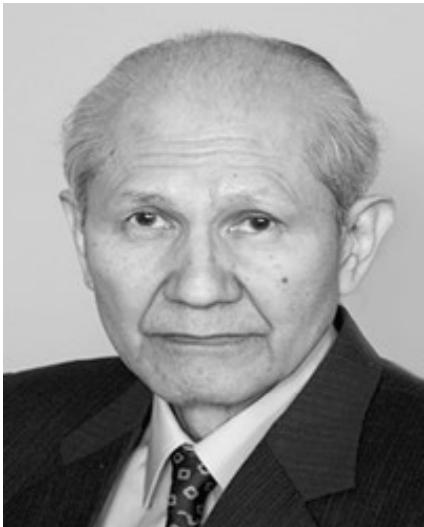
GFP最终发出荧
光是否需要经过
水母体内特殊物
质的加工?
*Does the GFP
fluoresce
eventually
require special
materials
processed in the
jellyfish?*



Roger Tsien

运用巧夺天工
的设计开始了
对GFP的改造
*Used
wonderful
design to
transform GFP*

Some Nobel Prizes are late....有些诺奖是迟来的

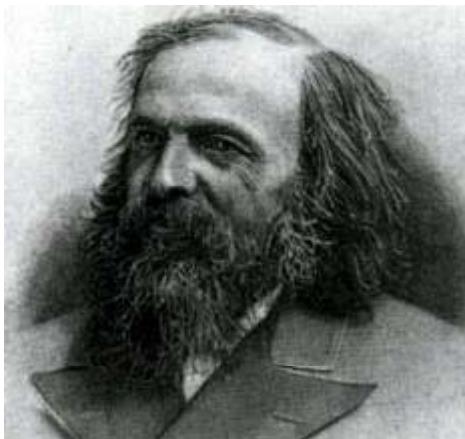


Osamu Shimomura



Barbara McClintock

Some Nobel Prizes are wrong.... Some Nobel Prizes are unfortunate....
有些诺奖是错误的
有些诺奖是遗憾的



Motier Lev



Douglas C. Prasher



Rosalind Franklin

Organic fluorescent probes 有机荧光探针

最传统的荧光探针有：荧光素、若丹明、吖啶橙、碘化丙啶（PI）、4',6-二脒基-2-苯基吲哚（DAPI）和Hoechst染料。这些染料大多由紫外光或者蓝光激发。

异硫氰酸荧光素（Fluorescein isothiocyanate, FITC）是一种基于氧杂蒽的荧光染料。由于其高信噪比，它在宽场和共聚焦荧光显微镜中都广泛应用。但是，荧光素的激发强度对于pH的变化是非常敏感的，并且其激发光谱比较宽，会与其他荧光探针的激发光谱重叠。这就使得双重或者三重染料标记显得比较困难。

The most traditional fluorescent probes are: fluorescein, Rhodamine, acridine orange, propidium iodide (PI) ,4',6-2 amidino-2-phenyl indole (DAPI) and Hoechst. Most of these dyes are stimulated by UV or blue light.

Fluorescein isothiocyanate(FITC) is a kind of fluorescent dye based on xanthene. Due to its high signal-to-noise ratio, it is widely used in the wide field and confocal fluorescence microscope. However, fluorescein intensity of excitation is very sensitive to variations of pH, and its wide excitation spectrum overlaps with other fluorescent probes, which makes double or triple dye staining harder.

罗丹明是另外一种小分子有机探针，随着吸收和发射的微小改变也有许多变种。与荧光素相比，若丹明衍生物对于外部环境的依赖更小，因此也更适于多重标记实验。

吖啶橙是一种能插入DNA碱基对，或者与RNA和单链DNA结合的染料。它能在细胞膜上自由扩散，在溶酶体中积累。

碘化丙啶也能与DNA结合，而且还与双链RNA有亲和力，通常用于双重或三重标记试验中对细胞核的染色。**DAPI**和**Hoechst**染料能与DNA双螺旋特异性的结合，同样也是一种非常常用的多重标记实验中的细胞核染色剂。

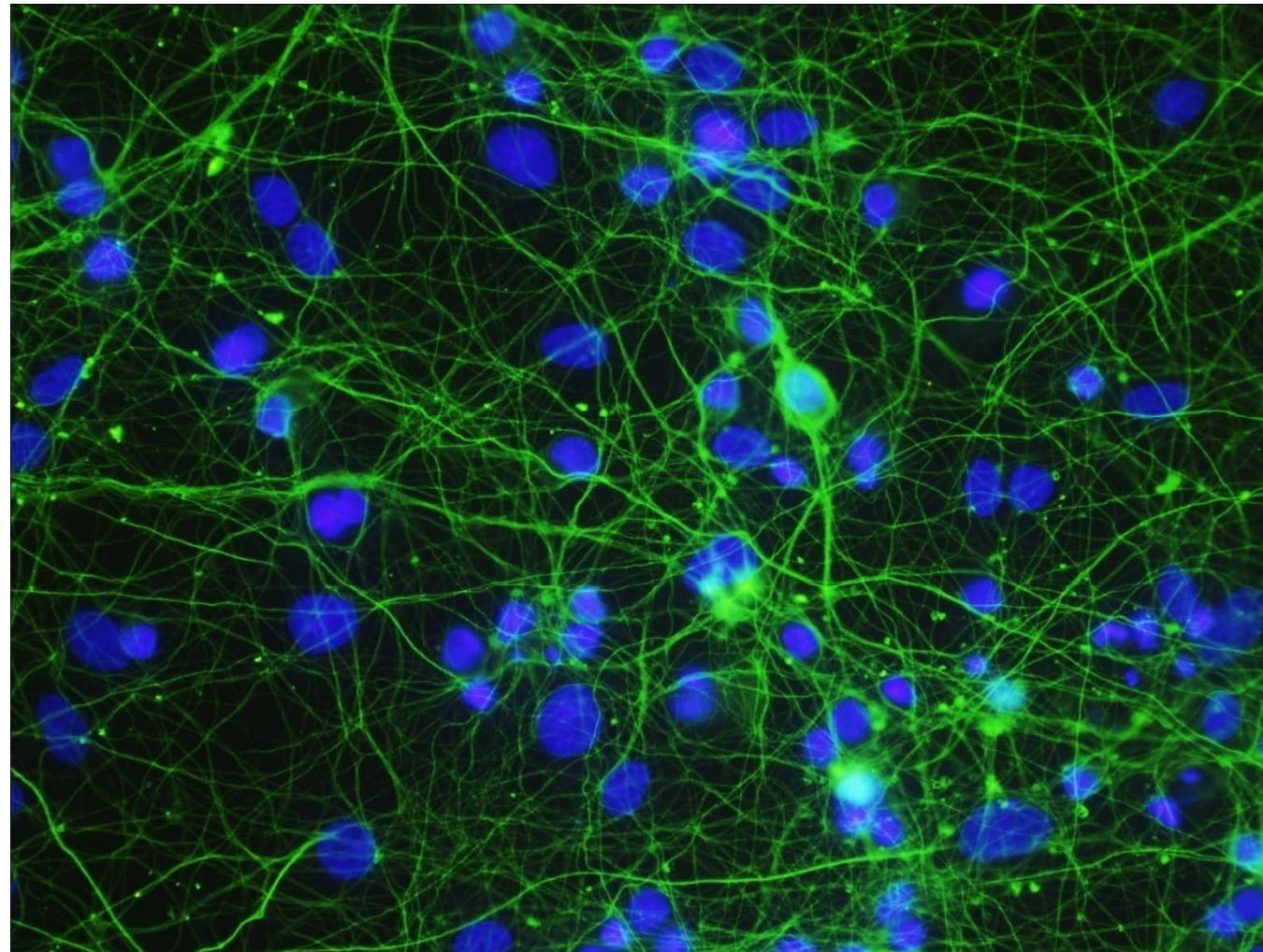
Rhodamine is another small-molecule organic probe with many variations of tiny alterations of absorption and emission. Compared with fluorescein, rhodamine derivatives hardly depend on external environments and is more suitable for multiple staining.

Acridine orange is able to insert in DNA base pairs and to bind RNA or single-stranded DNA. It freely diffuses through the cellular membrane and accumulates in the lysosome.

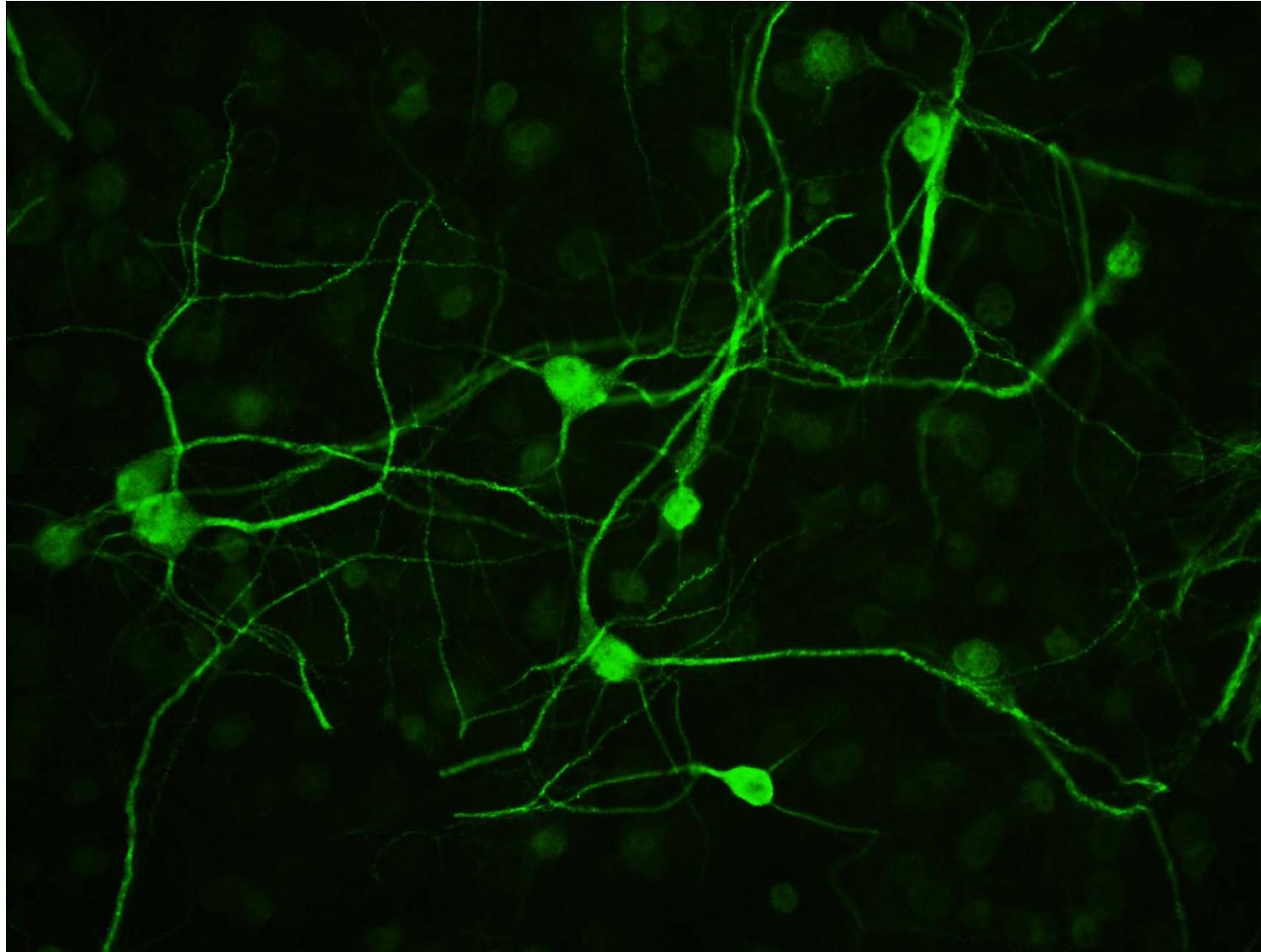
Propidium iodide is also able to bind DNA and double-stranded RNA and is widely used in double or triple staining to display the nucleus. **DAPI** and **Hoechst** dyes bind DNA double helixes specifically and is commonly used in multiple staining to show the nucleus.

Proteins specifically expressed in neurons

神经元中特异表达的蛋白



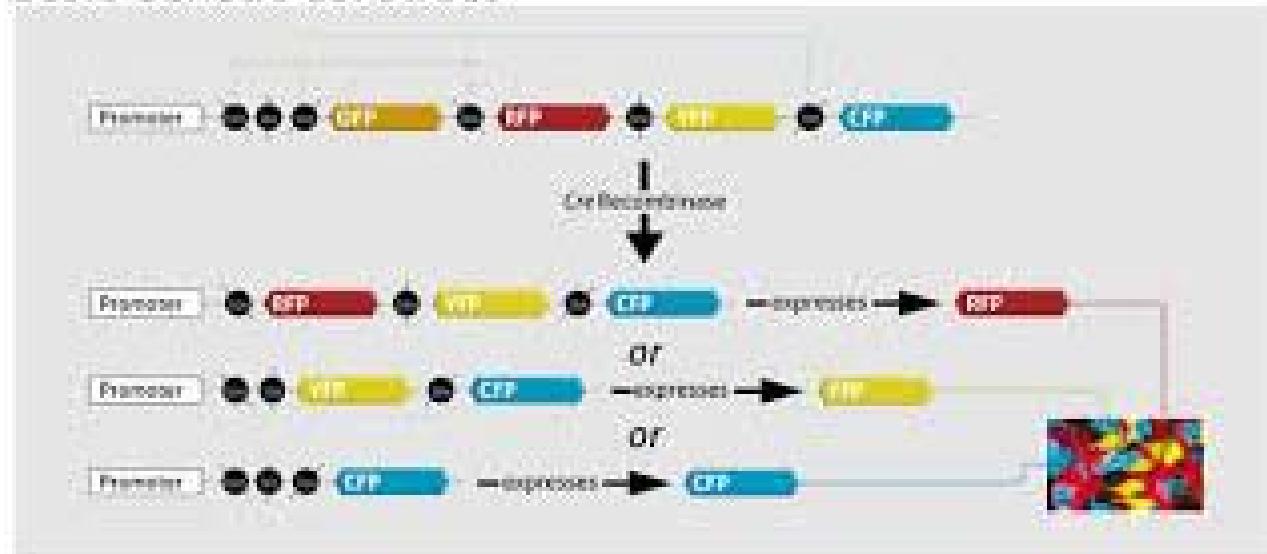
MAP2: dendrites and soma
MAP2: 树突和胞体



tau: axons
Tau: 轴突

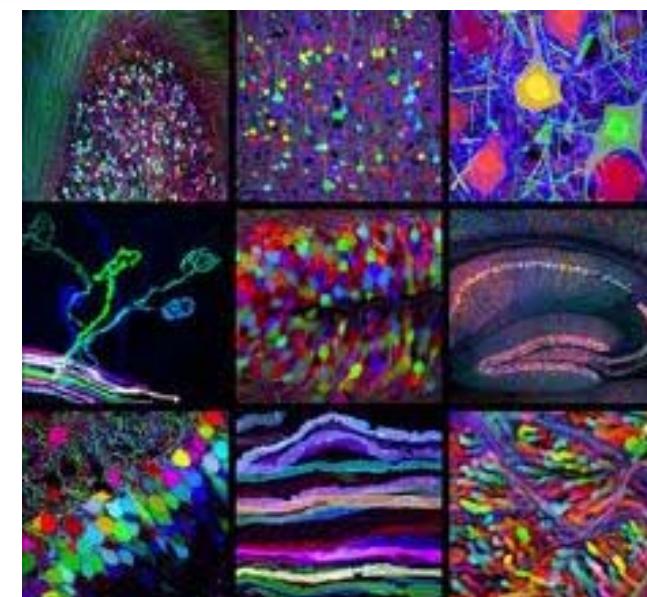
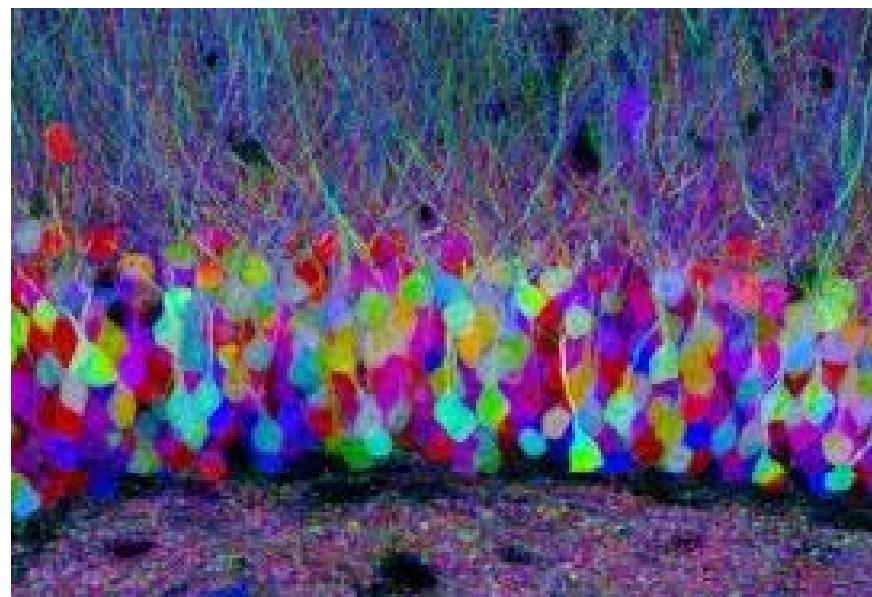
Brainbow 脑虹

Basic Genetic Construct



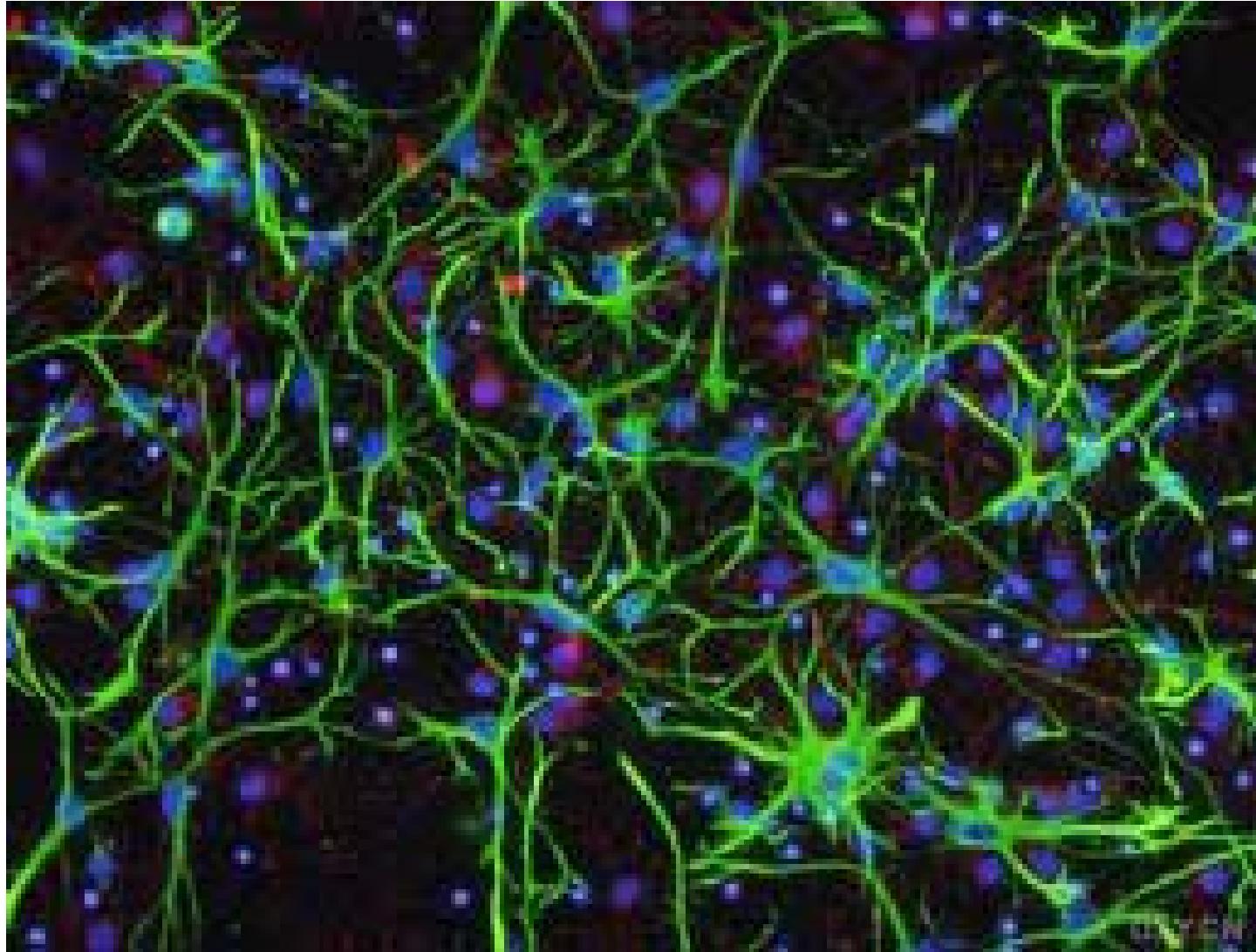
Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system

Jean Livet, Tammy A. Weissman,
Hyuno Kang, Ryan W. Draft, Ju
Lu, Robyn A. Bennis, Joshua R.
Sanes
& Jeff W. Lichtman



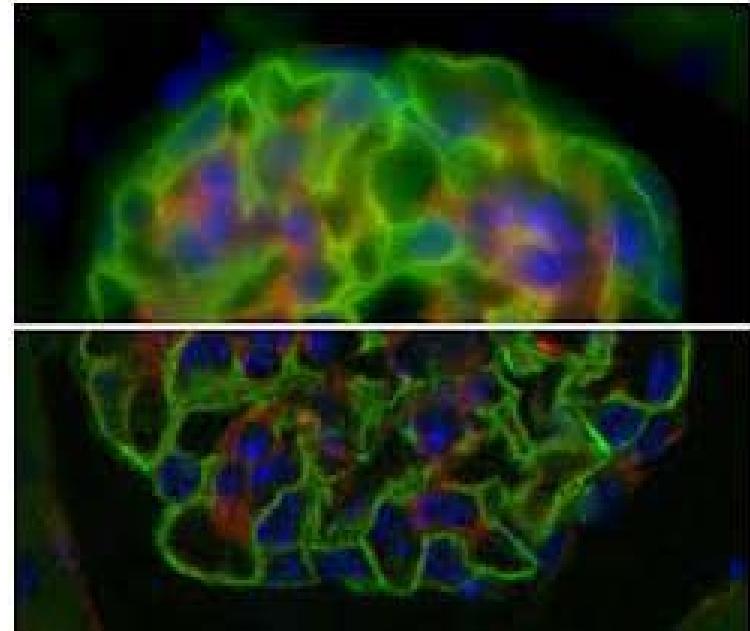
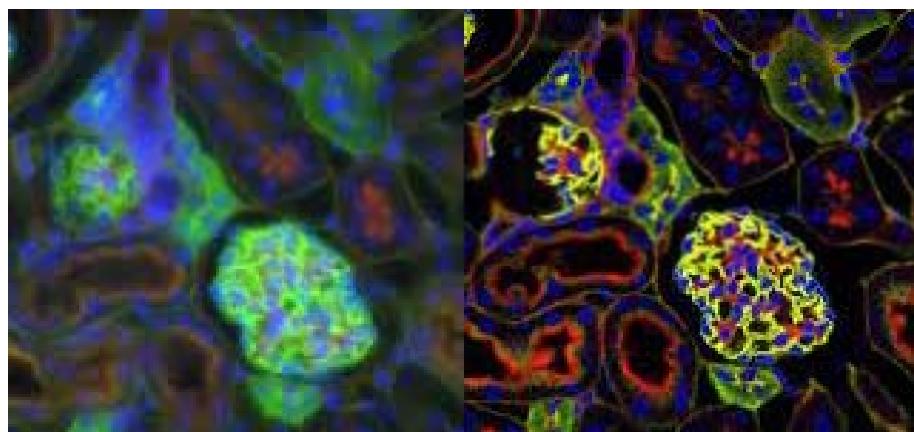
Neurons in isolated culture

离体培养神经元

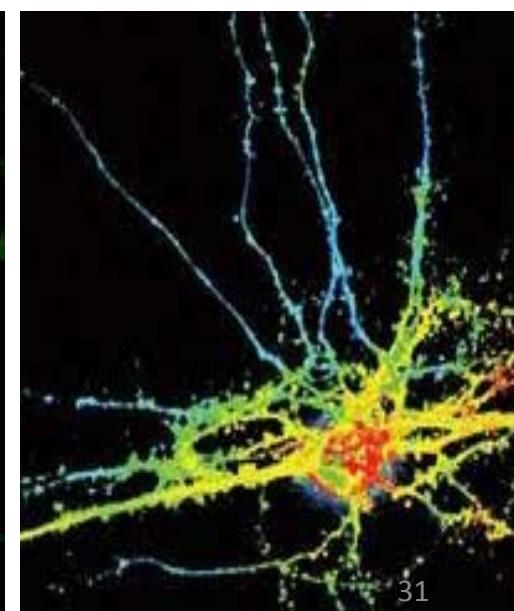
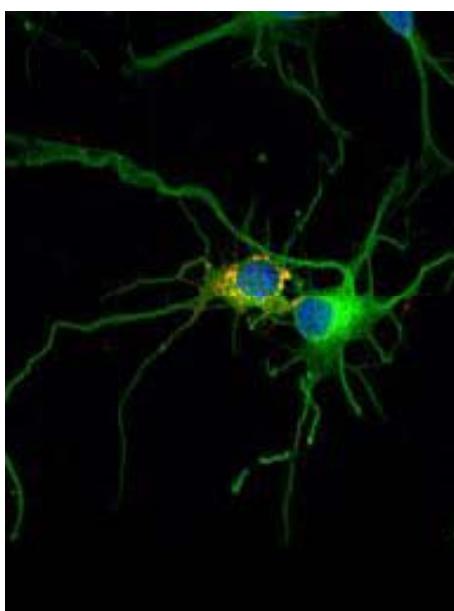
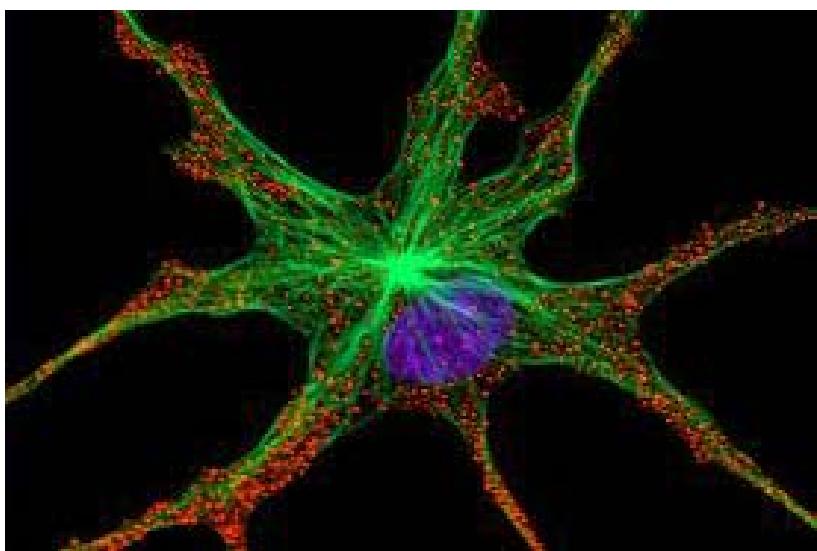
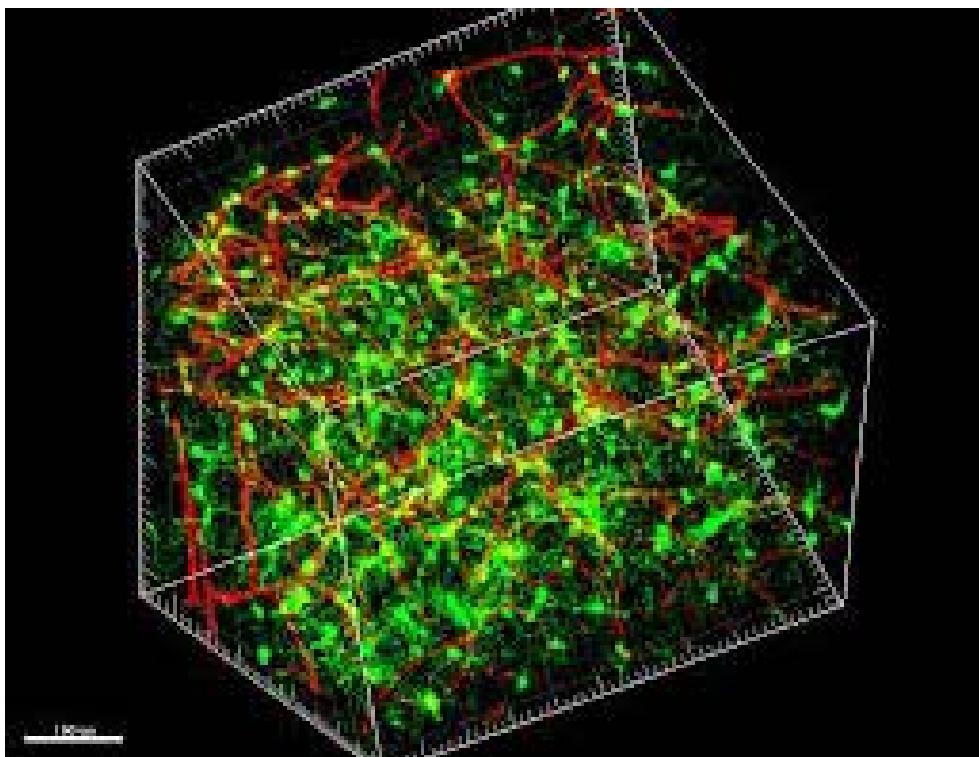
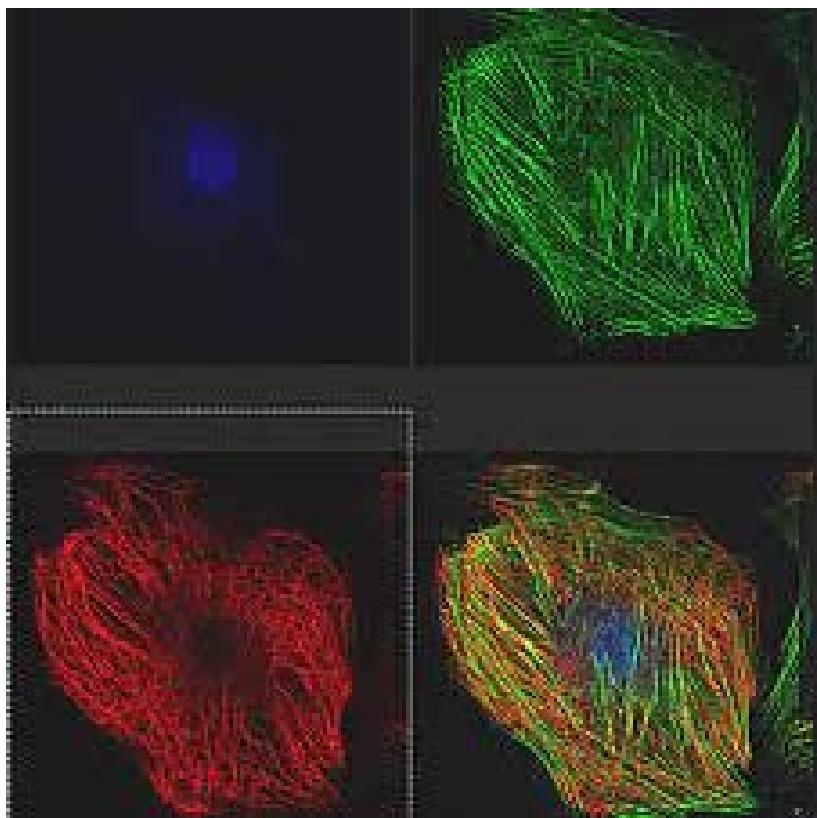


Confocal microscopy 共聚焦显微成像

Confocal microscopy is an optical imaging technique used to increase optical resolution and contrast by using point illumination and a spatial pinhole to eliminate out-of-focus light in specimens that are thicker than the focal plane. It enables the reconstruction of three-dimensional structures from the obtained images. However, as much of the light from sample fluorescence is blocked at the pinhole, this increased resolution is at the cost of decreased signal intensity – so long exposures are often required.



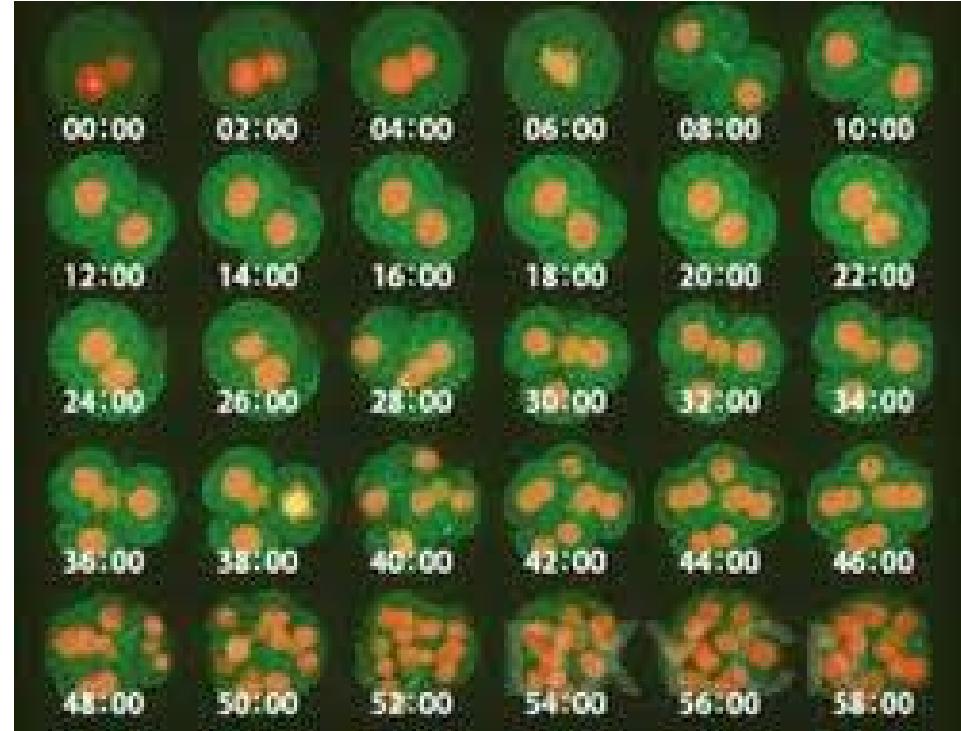
激光扫描共聚焦显微镜利用光学成像技术显微拍照提高分辨率和对比度，通过点照明和空间孔来消除比样本厚的非焦面光束，可实行对拍得照片进行三维重建。由于样本荧光大部分会被针孔阻拦，提高分辨率以降低信号强度为代价，故长时间曝光就成为了必需。



Live cell imaging 活细胞成像

维持细胞健康

在活细胞成像实验中的首要大事是在细胞内维持一个正常的代谢状态。在没有培养基或者适宜温度的条件下，细胞只能维持几分钟的正常活动。对于短期成像来说，维持细胞的健康就显得不那么重要了，但是对于长达几个小时或者几天的成像来说，就急需要去注意那些能引起代谢功能改变的因素。细胞外环境的变量包括:pH值、湿度、氧气、气压、温度和渗透压。



To maintain cell health:

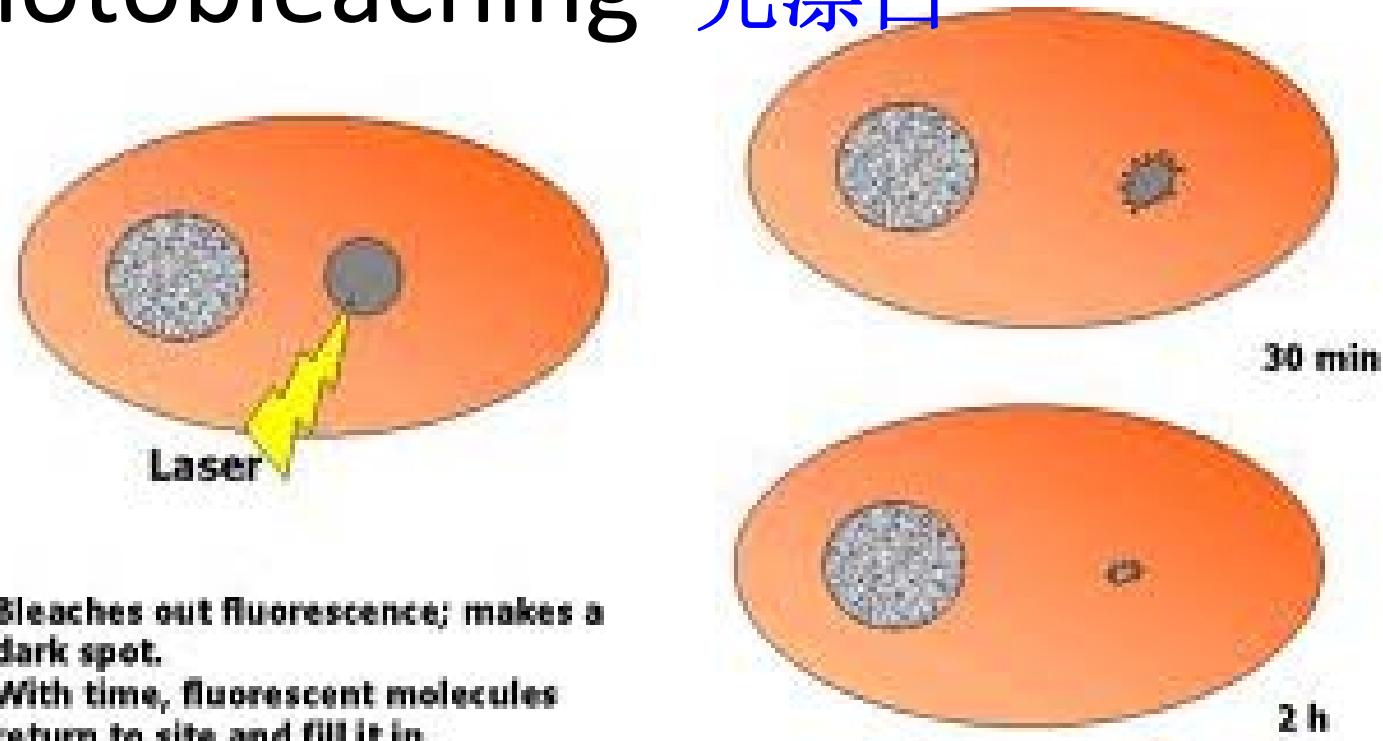
The most vital issue in live cell imaging is to maintain a normal metabolism state. In the absence of culture medium or the optimum temperature conditions, cells can only maintain normal activities for a few minutes. For short-term imaging, maintaining cell health is less important, while for imaging for a few hours or days, it's necessary to pay attention to factors that may cause metabolic function changes. Extracellular environment variables include: pH, humidity, oxygen, air pressure , temperature and osmotic pressure.

Live cell imaging devices 活细胞成像装置

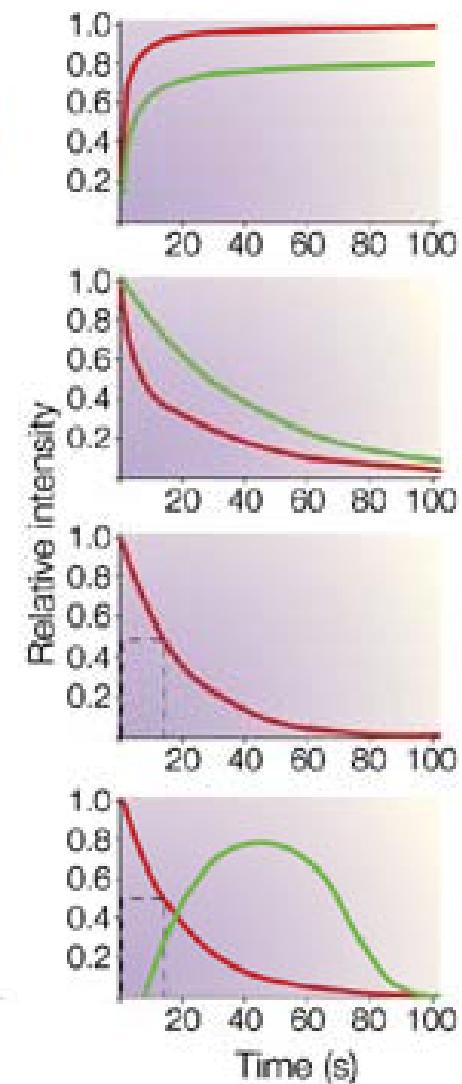
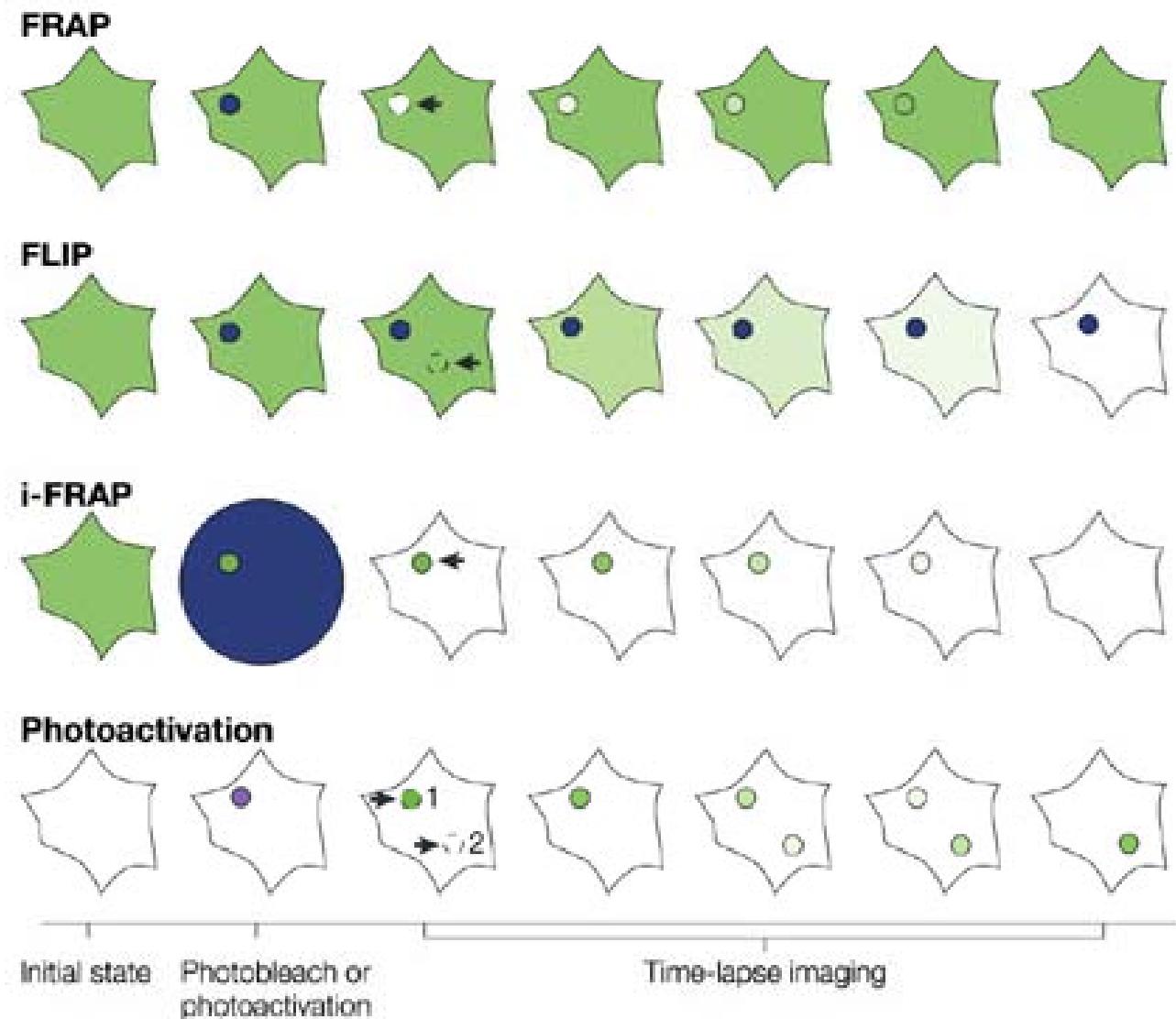


Photobleaching 光漂白

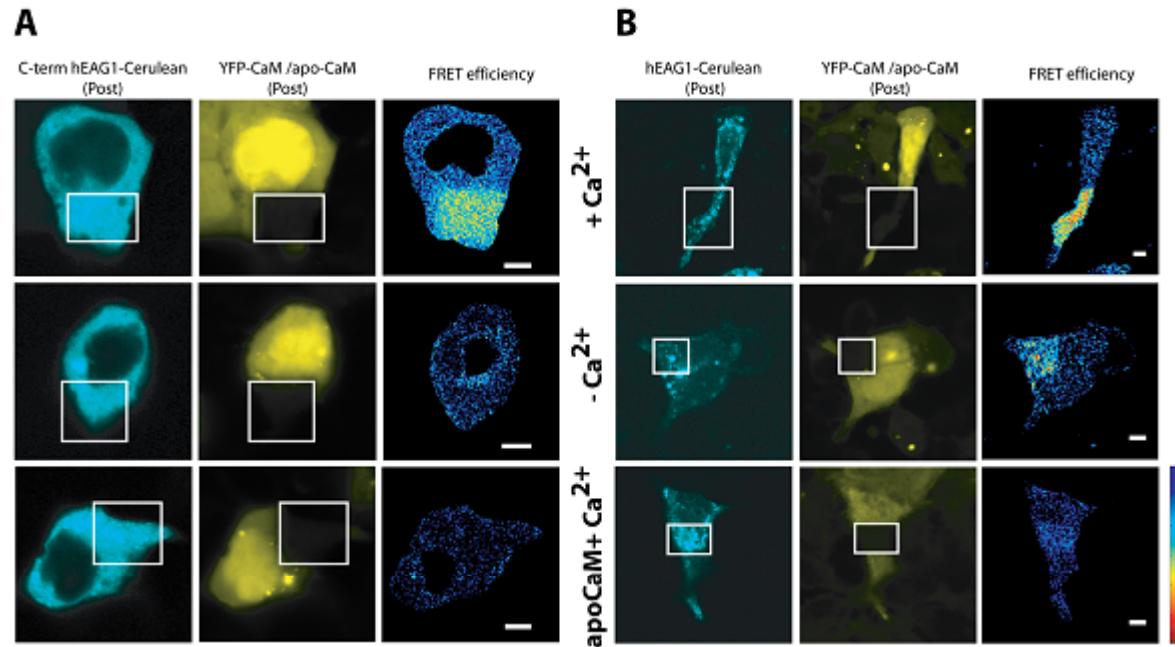
指在光的照射下荧光物质所激发出的荧光强度随着时间推移逐步减弱乃至消失的现象。荧光成像的质量很大程度上依赖于荧光信号强度，提高激发光强度固然可以提高信号强度，但激发光的强度不是可以无限提高的，当激发光的强度超过一定限度时，光吸收就趋于饱和，并不可逆地破坏激发态分子，这就是光漂白现象。



The fluorescence intensity gradually fades and even disappears with time under the irradiation of light. The quality of fluorescence imaging heavily relies on the fluorescence signal intensity. Signal strength can be improved by increase of excitation light intensity, but the intensity can not be increased unlimitedly. Light absorption will tend to be saturated and excited molecules will be damaged irreversibly when exciting light intensity reaches a limit and this is called photobleaching.



FRET (Forster resonance energy transfer) 荧光共振能量转移



荧光共振能量转移 (Forster resonance energy transfer, FRET) 显微成像是一种同时使用两种荧光探针的成像技术。

Forster resonance energy transfer (FRET) microscopy is an imaging technique using two kinds of fluorescent probes at the same time.

其中一个荧光探针的发射谱与另一个荧光探针的激发谱重叠。前者被称为**FRET**供体，后者被称为受体。

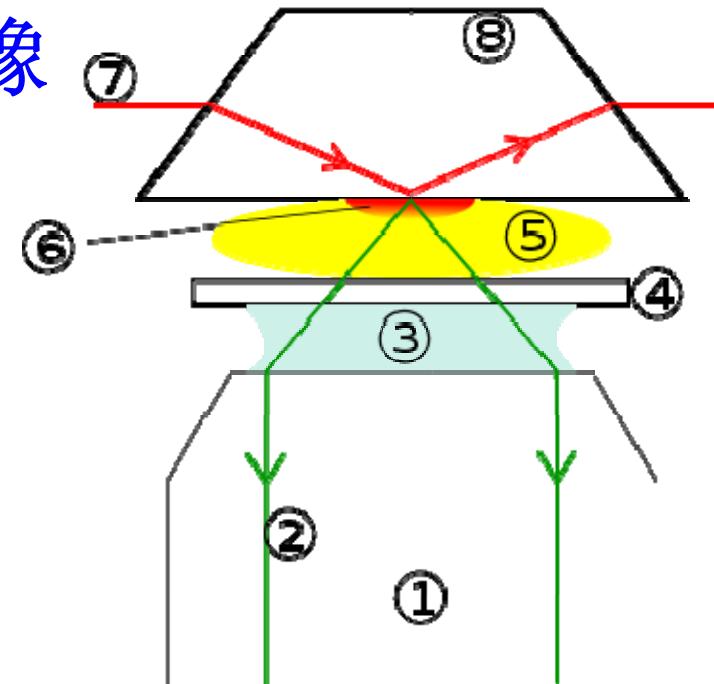
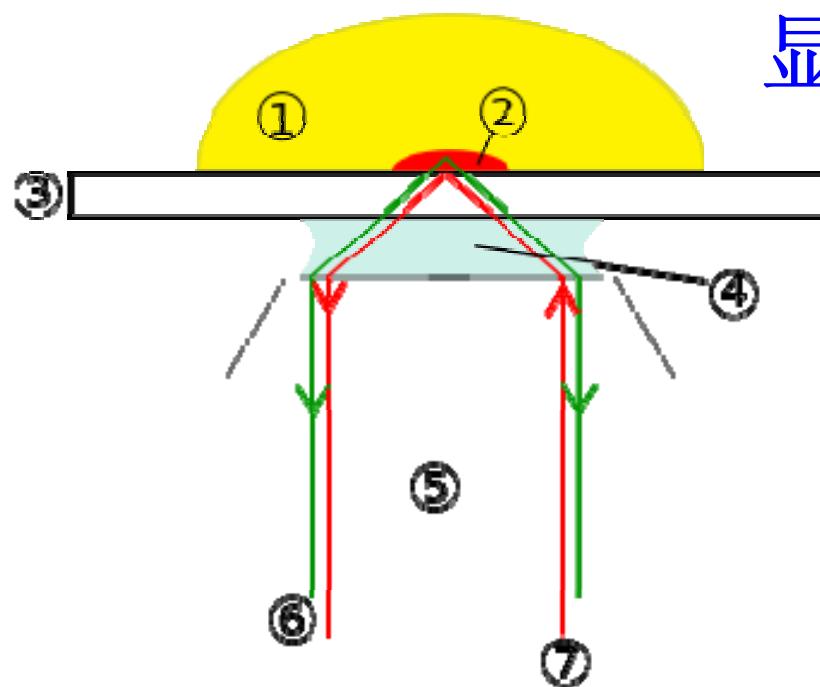
当供体和受体分子之间的距离小于10nm的时候，对于供体探针的激发会引起能量转移导致受体的发射。在**FRET**成像中**ECFP**和**EYFP**通常被作为一个荧光探针对来使用。**FRET**现象可以用于目的分子构象改变的研究：将受体分子和供体分子设计到一个能和目的分子发生相互作用的荧光蛋白内。当目标分子被激活并且其构象产生改变时，受体和供体探针就能够靠的足够近以产生**FRET**信号。

The emission spectrum of one fluorescent probe overlaps with excitation spectrum of another fluorescent probe. The former is called FRET donor and the latter is called receptor.

When the distance between the donor and receptor molecule is less than 10 nm, the donor probe excitation leads to energy transfer causing the emission of the receptor. In FRET imaging, **ECFP** and **EYFP** are often used as a fluorescent probe pair. FRET may be used to detect molecular conformational changes: the receptor and the donor are designed to insert into a fluorescent protein molecule able to interact with the target molecule. When the target molecule is activated and its conformation is altered, the receptor and donor probes are close enough to generate FRET signals.

Microimaging

显微成像



A **total internal reflection fluorescence microscope (TIRFM)** is a type of microscope with which a thin region of a specimen, usually less than 200 nm can be observed. Thus the TIRFM enables a selective visualization of surface regions such as the basal plasma membrane (which are about 7.5 nm thick) of cells as shown in the figure above.

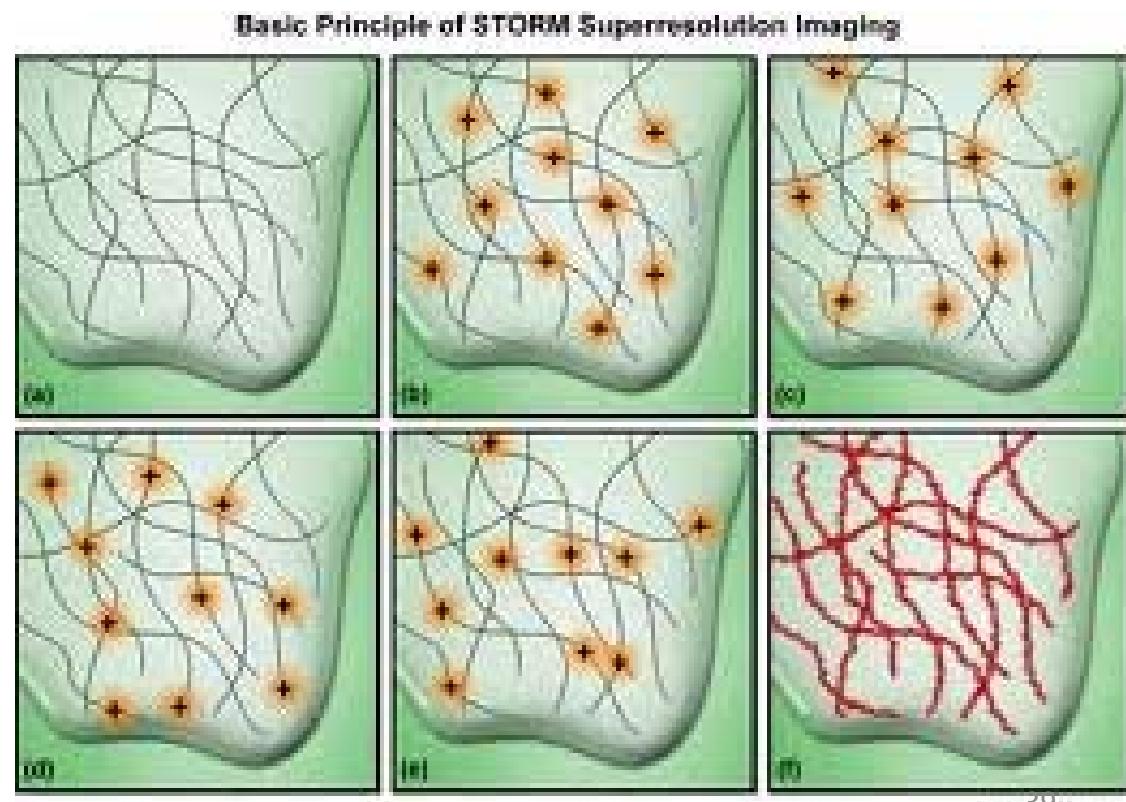
全内反射荧光显微镜是一种可以观察样本薄区域（一般少于200 nM）的显微镜。因此，它可以选择性观察区域表面，比如上图显示的细胞质膜的基底部（大约7.5nm厚）。

Micrography 显微成像

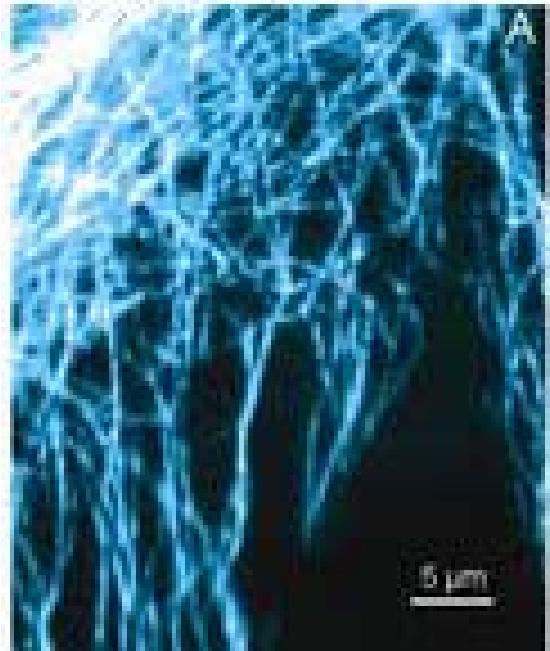
Traditional optical microscopy is limited in the wavelength of light and is unable to distinguish objects under 200 nm. Although the resolution of the electron microscope reaches nanoscale, samples are still limited due to easy current damage. Although molecular biologists may use fluorescent tags to label target proteins, the proteins are still crowded and hard to distinguish under the microscope. Super-resolution imaging: PALM, STORM, SIM

传统光学显微镜受限于光的波长，对于200nm以下的物体无法分辨。虽然电子显微镜可以达到纳米级的分辨率，但电流容易造成样品破坏，因此能观测的样本也相当有限。分子生物学家虽然可以把若干目标蛋白贴上荧光标记，但这些蛋白还是经常挤在一块，在显微镜下难以分辨开来。

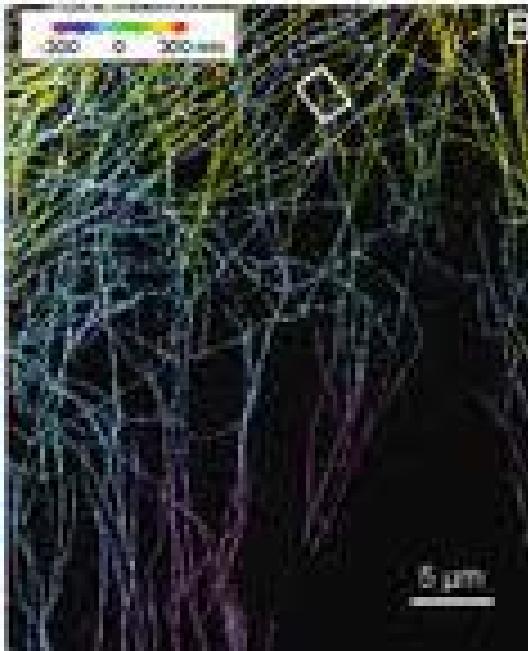
超高分辨成像：PALM, STORM, SIM



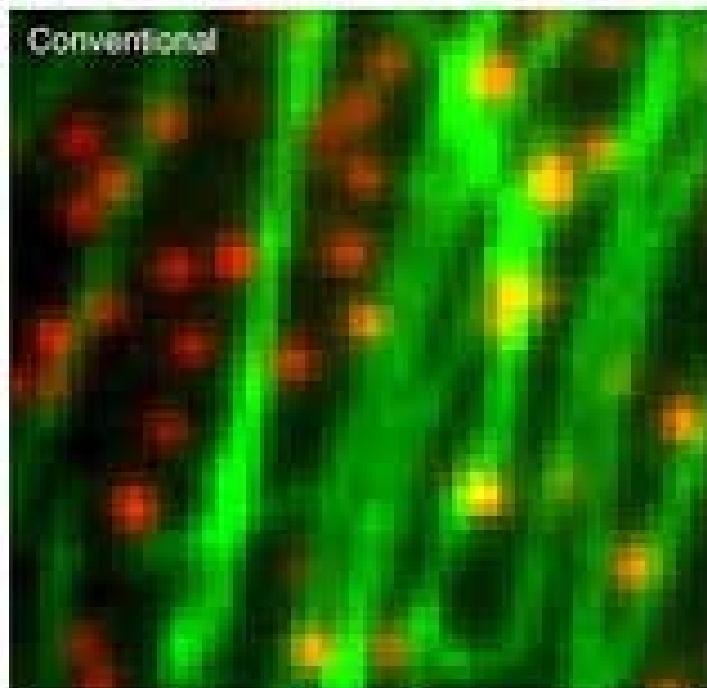
Conventional



STORM



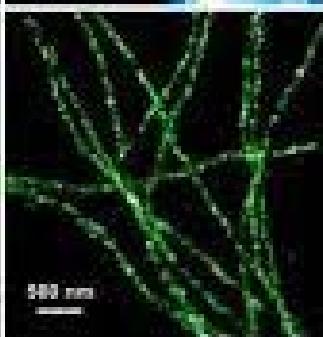
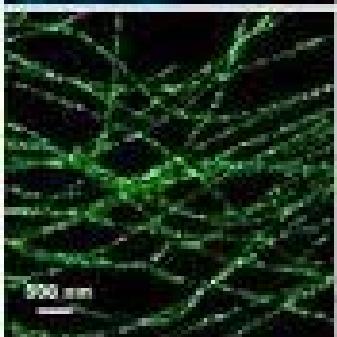
Conventional



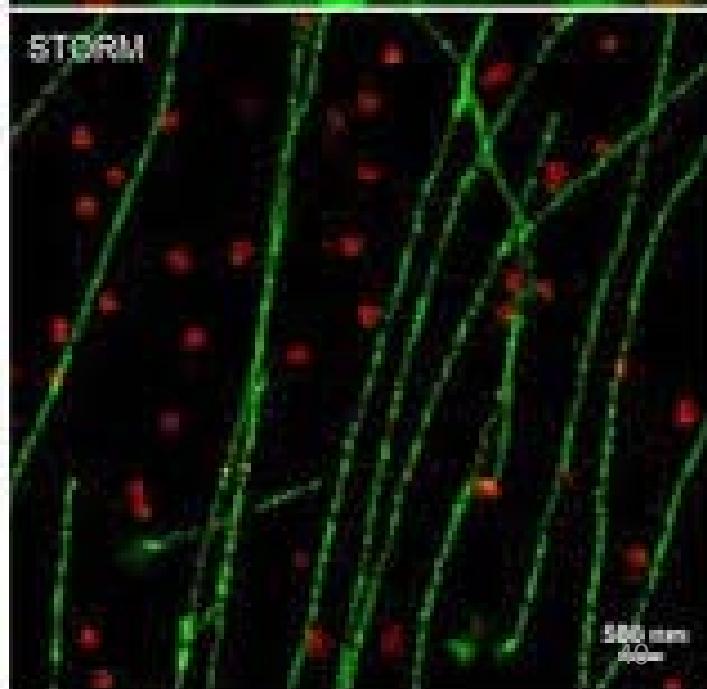
Conventional



STORM

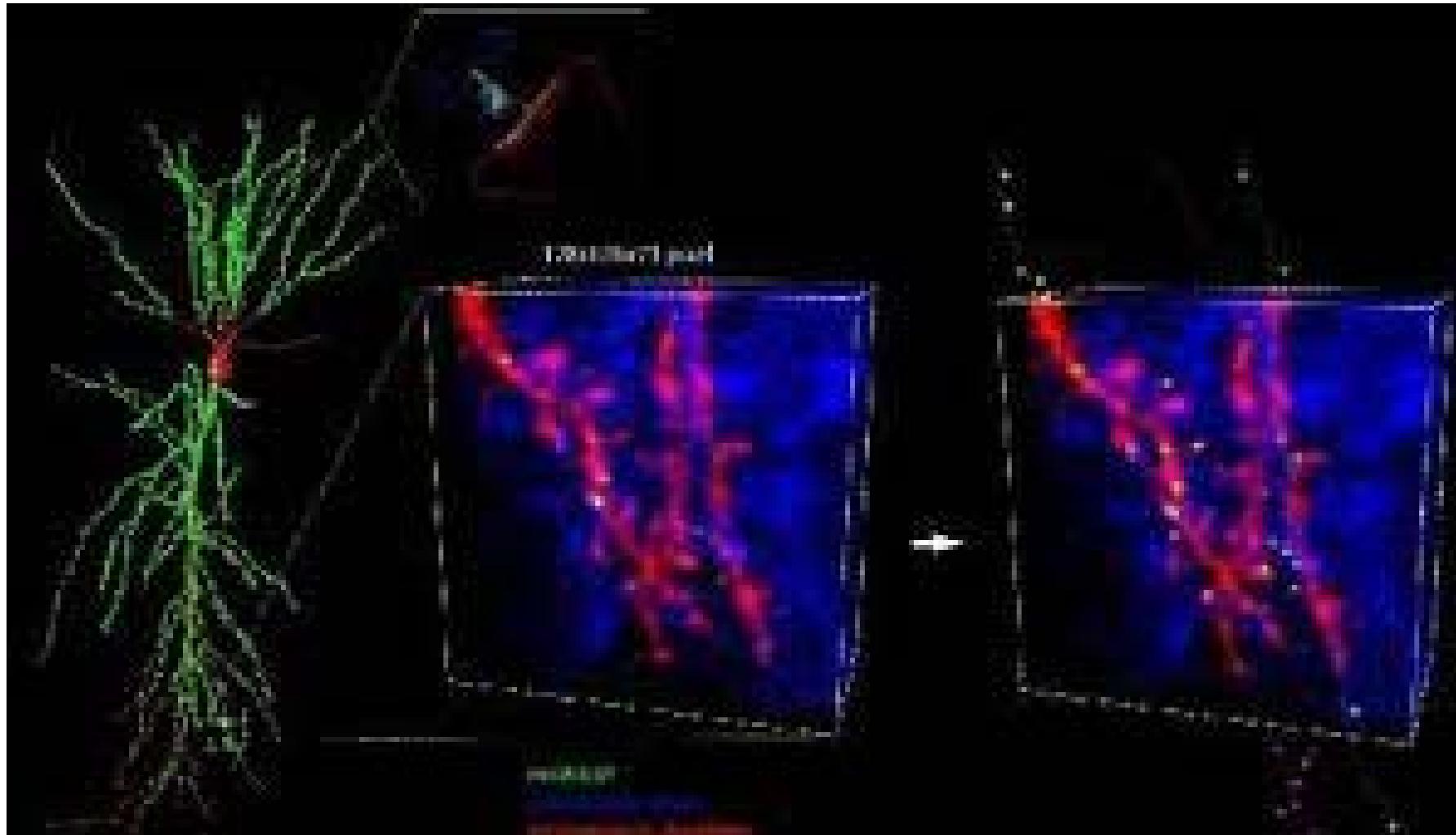


STORM



Two-photon imaging

双光子成像



Two-photon imaging 双光子成像

优势:

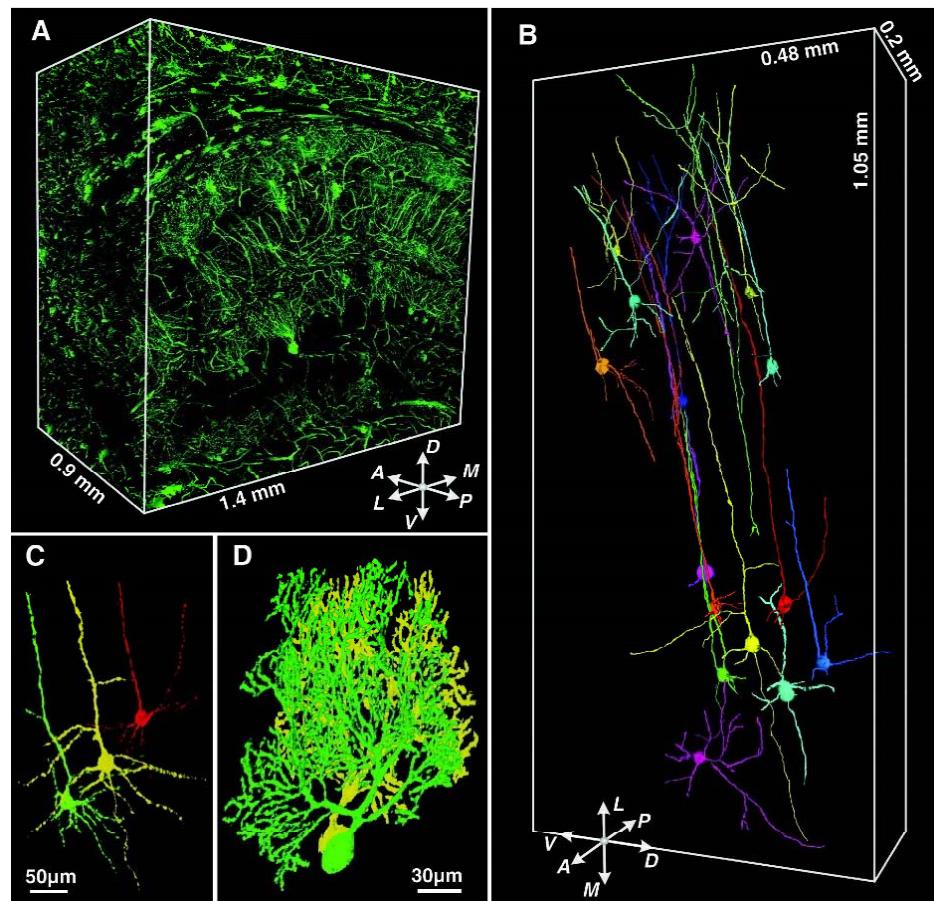
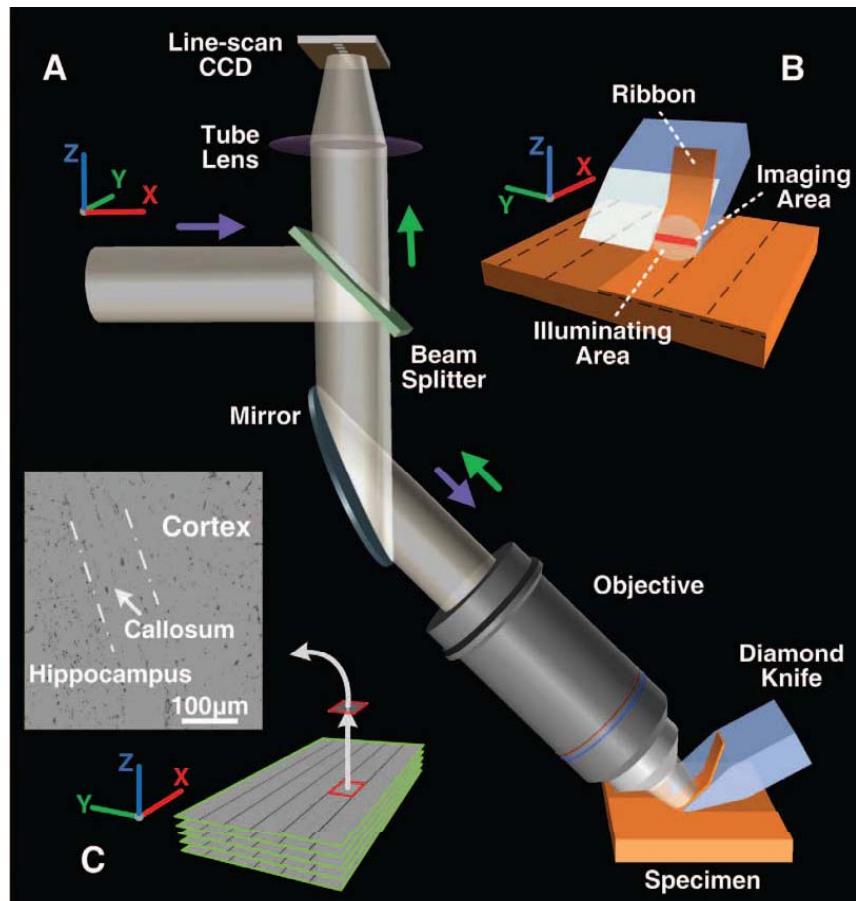
- 1) 长波长的光比短波长的光受散射影响较小容易穿透标本;
- 2) 焦平面外的荧光分子不被激发使较多的激发光可以到达焦平面, 使激发光可以穿透更深的标本;
- 3) 长波长的近红外光比短波长的光对细胞毒性小;
- 4) 使用双光子显微镜观察标本的时候, 只有在焦平面上才有光漂白和光毒性。所以, 双光子显微镜比单光子显微镜更适合用来观察厚标本、更适合用来观察活细胞、或用来进行定点光漂白实验。

Advantages:

- 1) Longer wavelengths are less affected by scattering than short wavelengths and easily pass through the specimen;
- 2) Fluorescent molecules outside the focal plane are not excited and more exciting light reaches the focal plane and penetrates deeper specimens;
- 3) Near-infrared wavelength light is less toxic to cells than the short wavelength light;
- 4) When using two-photon microscopy to observe specimens, photobleaching and light toxicity only happen on the focal plane. Thus, the two-photon microscope is more suitable for observation of thick specimens, living cells and fixed-point photobleaching compared with single photon microscope.

MOST显微光学切片断层成像系

显微光学切片断层成像系统（Micro-optical Sectioning Tomography, MOST）
和荧光显微光学切片断层成像系统（fluorescence Micro-optical Sectioning
Tomography, fMOST）



Scale 透明体



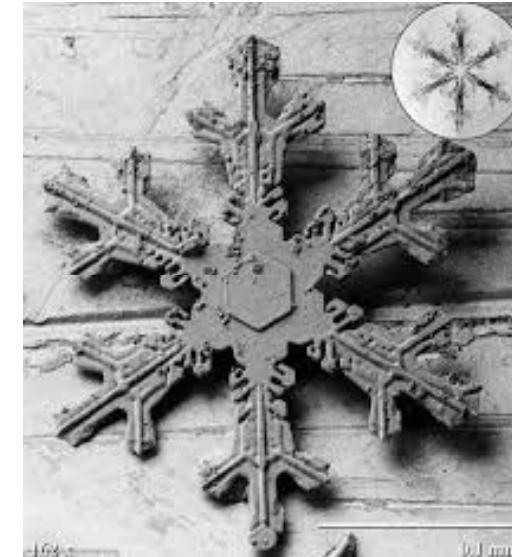
Scale试剂是日本理化学研究所开发出一种新的透明试剂，可以让活体组织变透明，实验用的老鼠胚胎，在泡过这种试剂后，变成透明果冻状，这项研究发布在了2011年8月30日的《自然—神经科学》（Nature Neuroscience）杂志上。

Scale reagent is a new transparent reagent developed by RIKEN, which makes a living tissue transparent. Murine embryos immersed in the reagent become transparent and jelly. This study was published in Nature Neuroscience in August 30th, 2011.

Electron Microscope

电子显微镜成像

电子显微镜（electron microscope，简称：电镜）是利用电子与物质作用所产生之讯号来监定微区域晶体结构，微细组织，化学成份，化学键结和电子分布情况的电子光学装置。常用的有透射电子显微镜和扫描电子显微镜。与光学显微镜相比电子显微镜用电子束代替了可见光，用电磁透镜代替了光学透镜并使用荧光屏将肉眼不可见电子束成像。



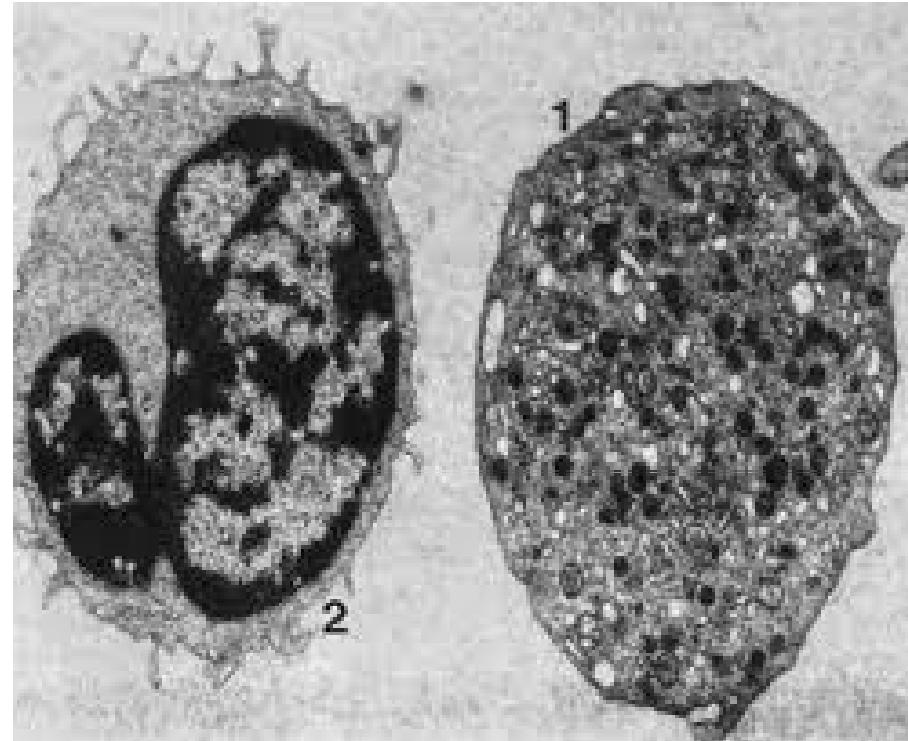
The electron microscope is an electrooptic device to identify the crystal structure of micro-regions, fine structures, chemical components, chemical bonds and electron distributions through the interaction between electrons and sample materials, including [transmission electron microscope](#) and [scanning electron microscope](#). Compared with optical microscopes, the electron microscope replaces visible light with beams of accelerated electrons and replaces optical lenses with electromagnetic lenses to make invisible electrons visible on a fluorescent screen.



透射电子显微镜

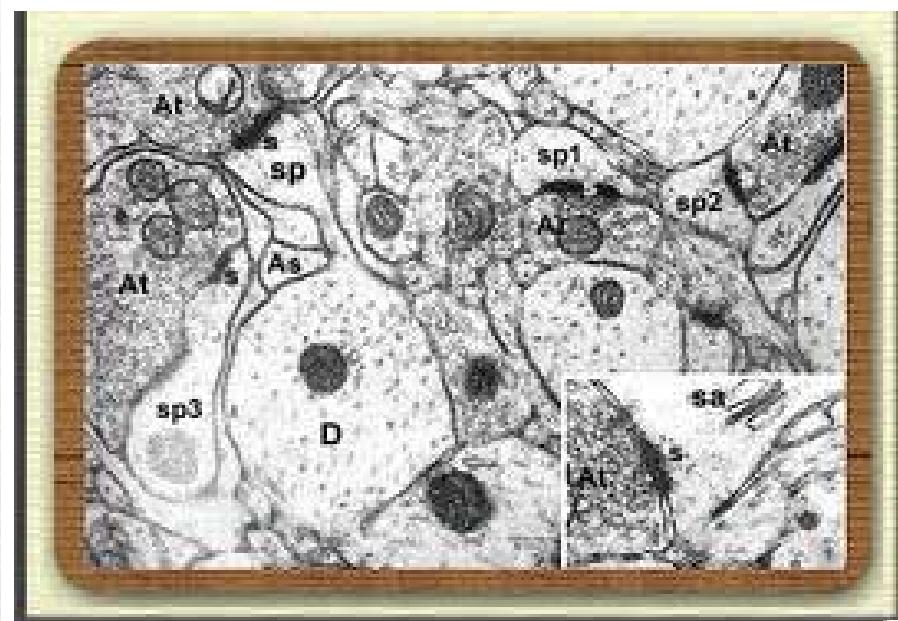
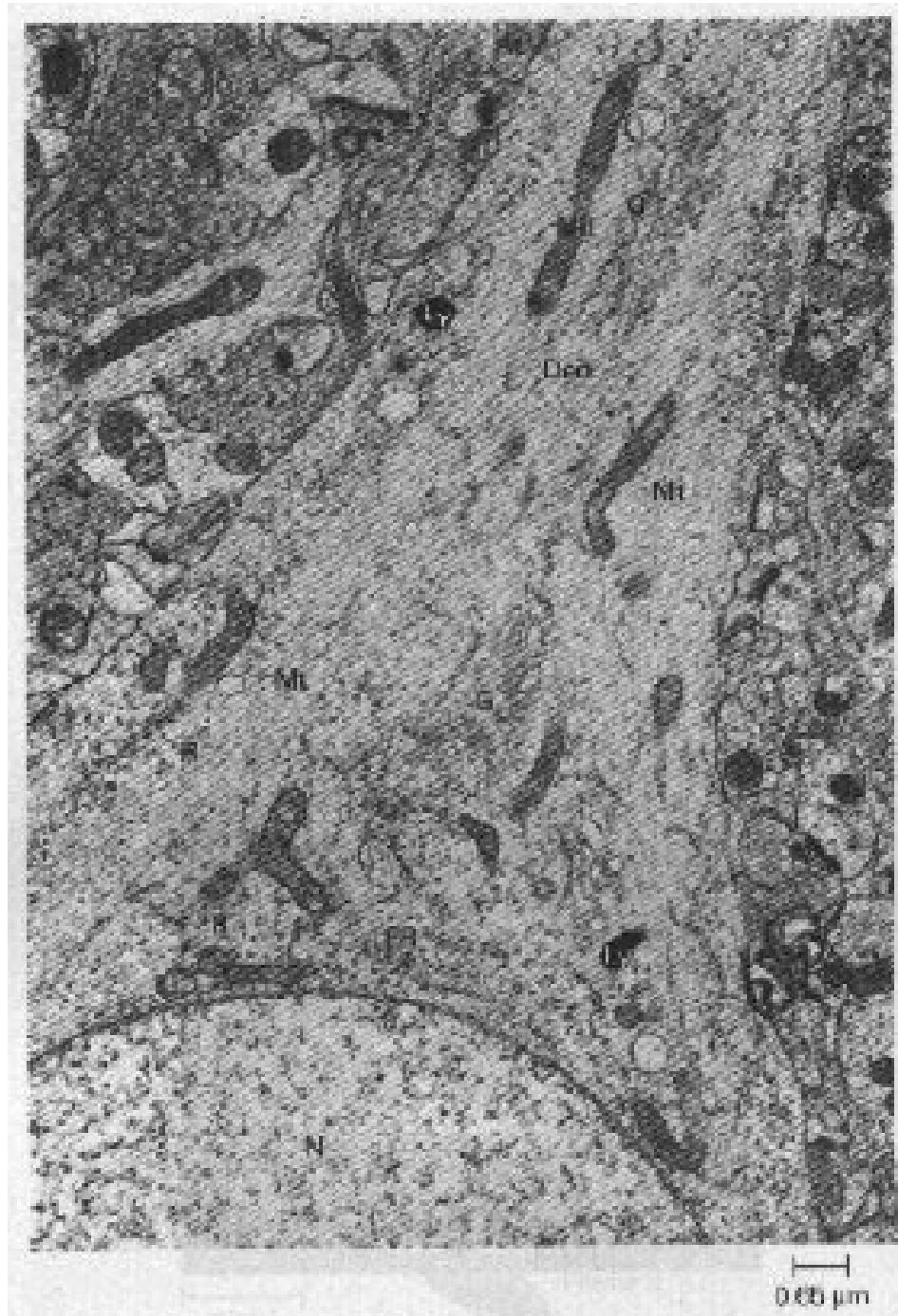
因电子束穿透样品后，再用电子透镜成像放大而得名。它的光路与光学显微镜相仿，可以直接获得一个样本的投影。

在这种电子显微镜中，图像细节的对比度是由样品的原子对电子束的散射形成的。由于电子需要穿过样本，因此样本必须非常薄。样本的厚度可以从数纳米到数微米不等。样品较薄或密度较低的部分，电子束散射较少，这样就有较多的电子通过物镜光栏，参与成像，在图像中显得较亮。反之，样品中较厚或较密的部分，在图像中则显得较暗。



TEM (Transmission electron microscopy)

It's named TEM because electron beams first transmit through the sample and then electron lenses obtain the magnified images. Its light path is similar to that of a optical microscope and a sample projection can be obtained directly. In this electron microscope, the image contrast is produced by scattering of the electron beams due to atom blocks of the sample. Samples must be thin enough for electrons to pass through, possibly ranging from nanometers to micrometers. In thinner parts, the scattering is less severe and more electrons pass through, so these parts looks brighter and vice versa.



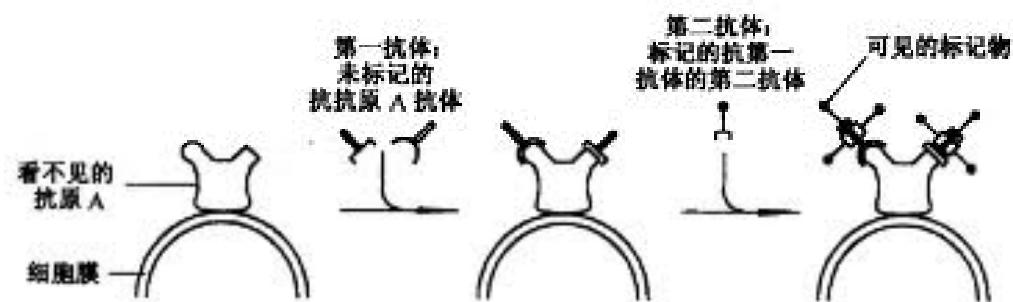
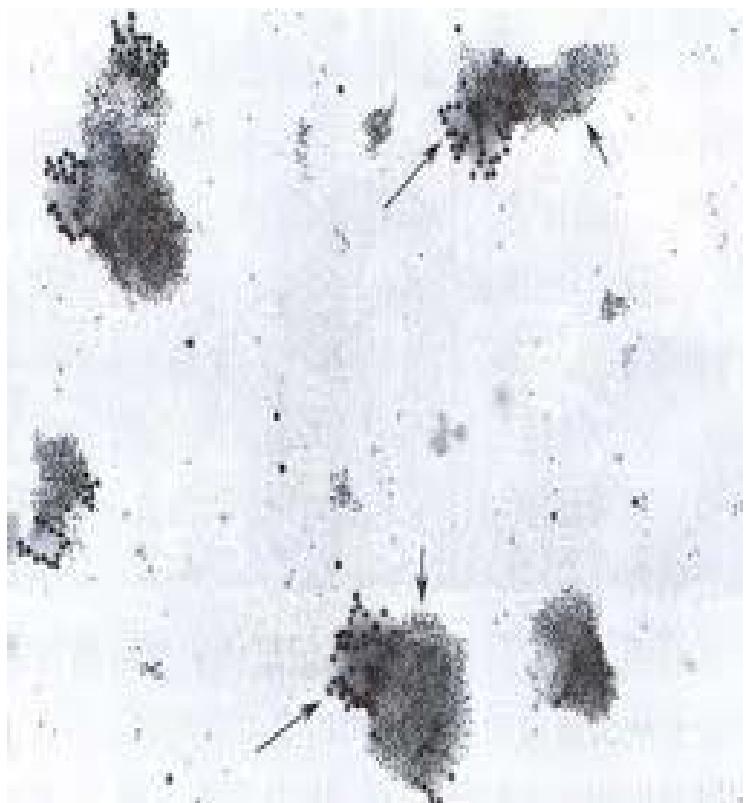
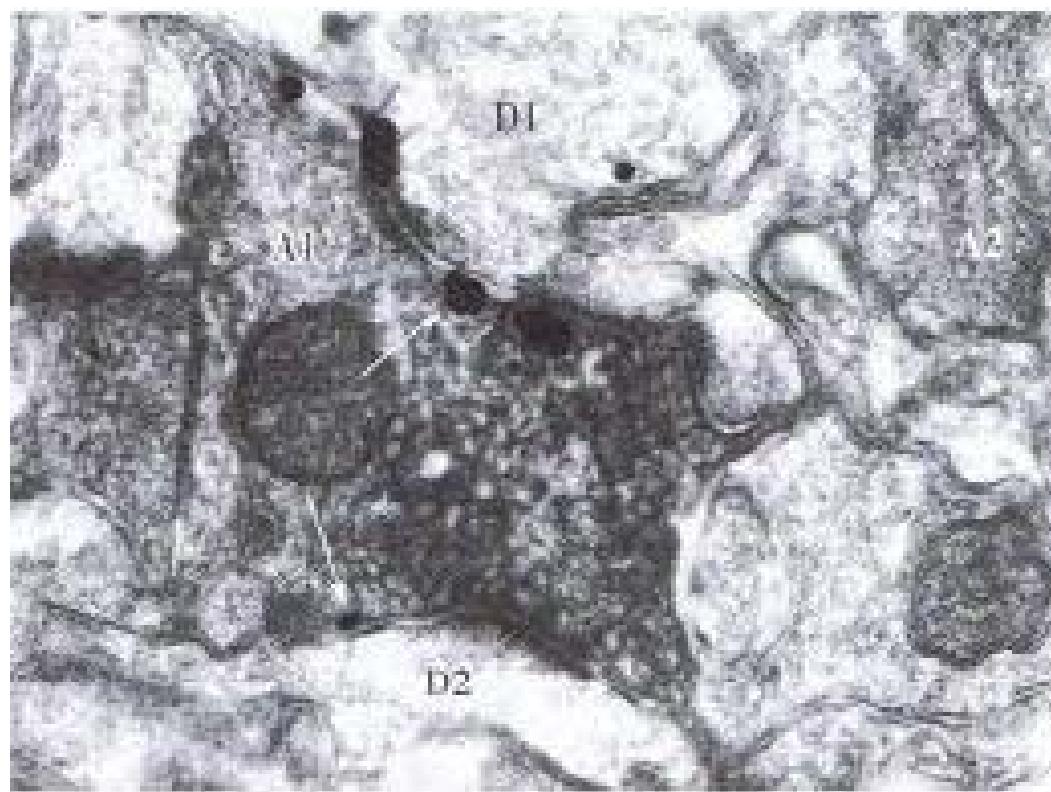
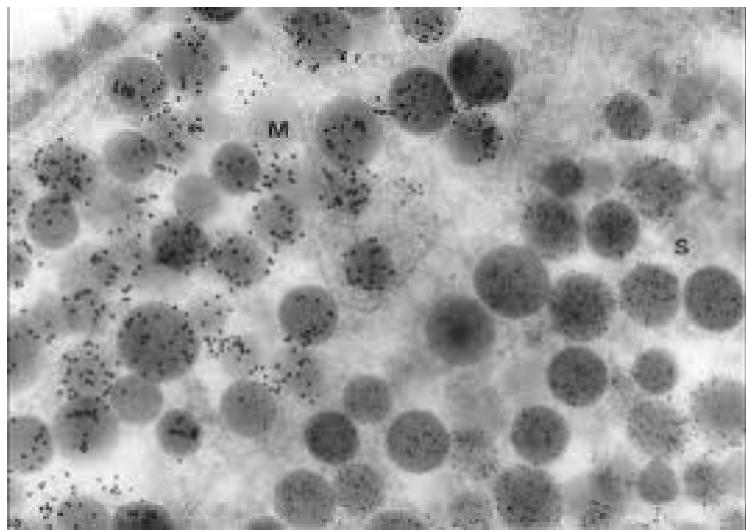
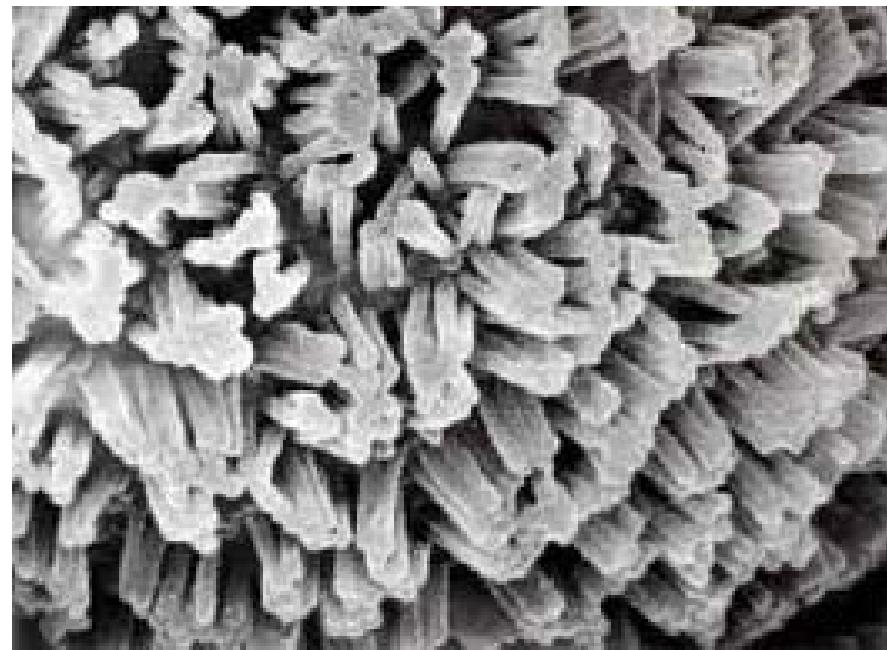
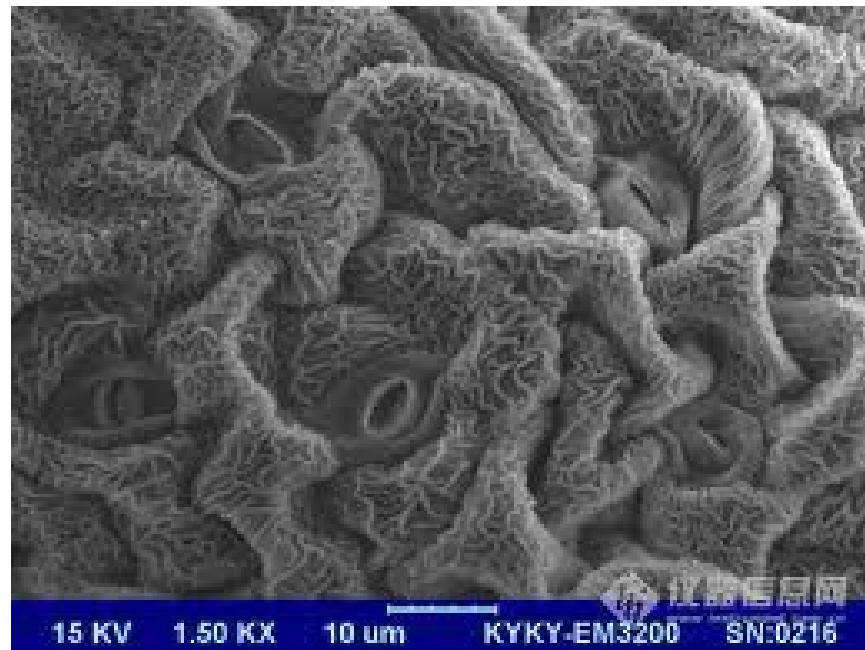


图 2-4 间接法检测抗原示意图

扫描电子显微镜扫描电子显微镜的电子束不穿过样品，仅以电子束尽量聚焦在样本的一小块地方，然后一行一行地扫描样本。入射的电子导致样本表面被激发出次级电子。图像为立体形象，反映了标本的表面结构。

SEM (Scanning electron microscope)

Electron beams don't pass through the sample but only focus on a small piece and then scan the sample line by line. Incident electrons lead to the excitation of secondary electrons. Images of the sample surface are stereoscopic, reflecting the surface structure.



缺点

1. 在电子显微镜中样本必须在真空中观察，因此无法观察活样本。随着技术的进步，环境扫描电镜将逐渐实现直接对活样本的观察。
2. 在处理样本时可能会产生样本本来没有的结构，这加剧了此后分析图像的难度；
3. 由于电子散射能力极强，容易发生二次衍射等；
4. 由于为三维物体的二维平面投影像，有时像不唯一；

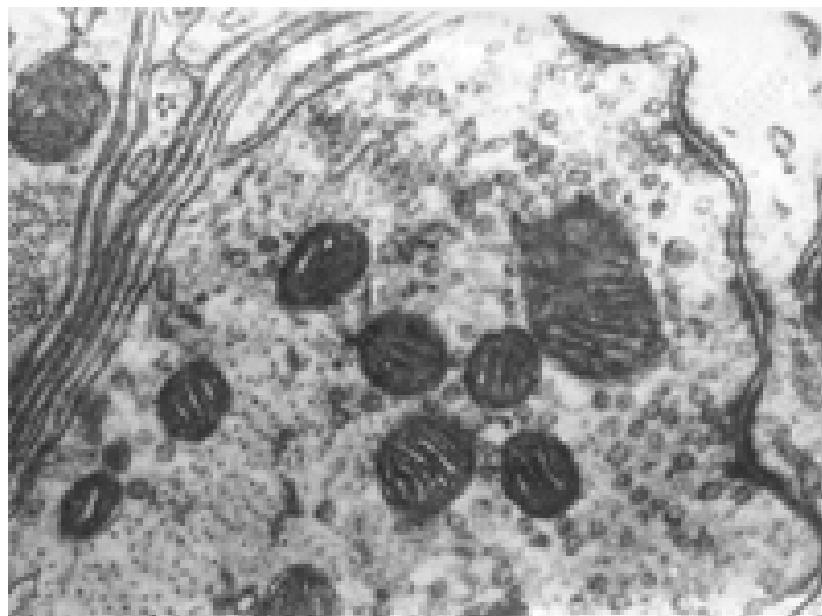
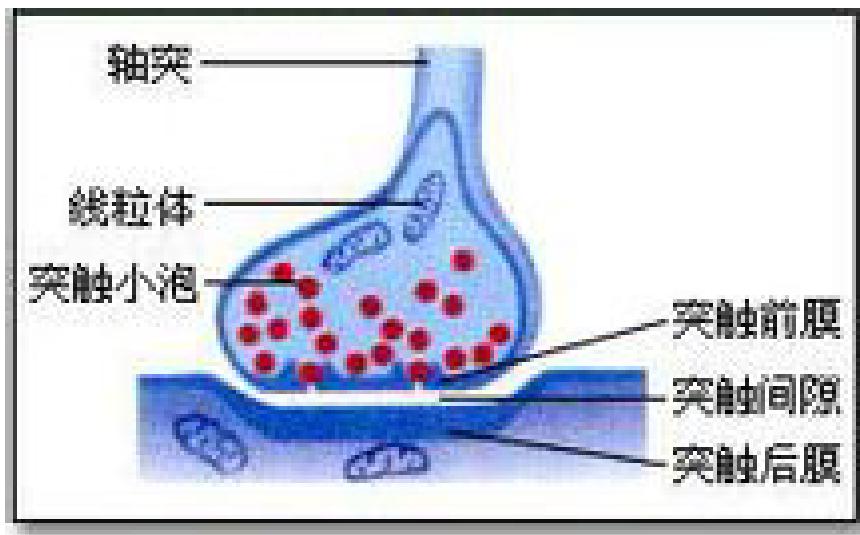
Disadvantages:

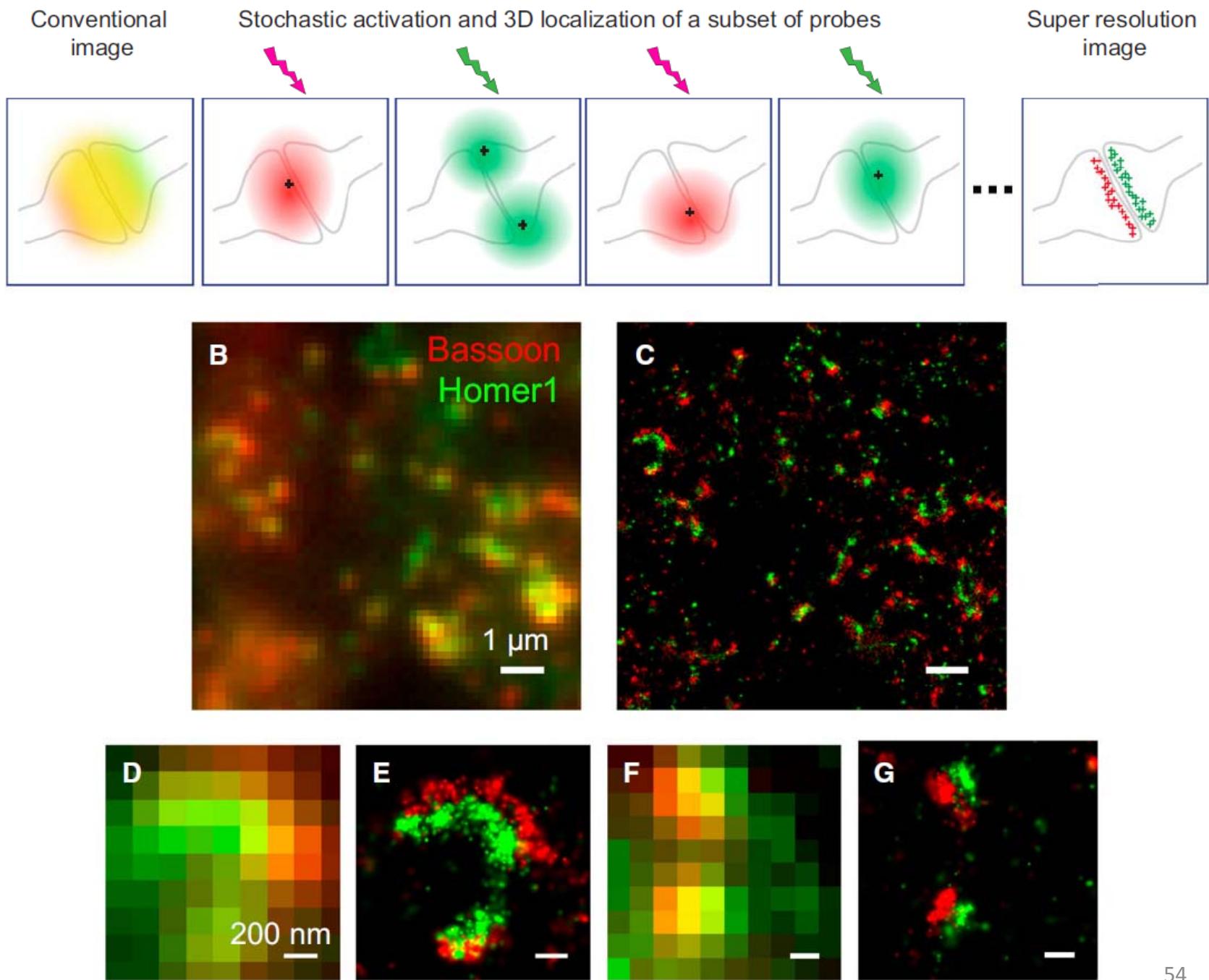
1. Samples must be observed in vacuum and thus live samples are not suitable. As technology advances, environmental scanning electron microscopy may gradually achieve to direct observations of live samples.
2. Inexistent structures may be generated when dealing with samples, enhancing difficulty of the analysis of images.
3. Secondary diffractions may occur because electrons are easy to scatter.
4. 2-D planar projections of a 3-D object are sometimes not exclusive.

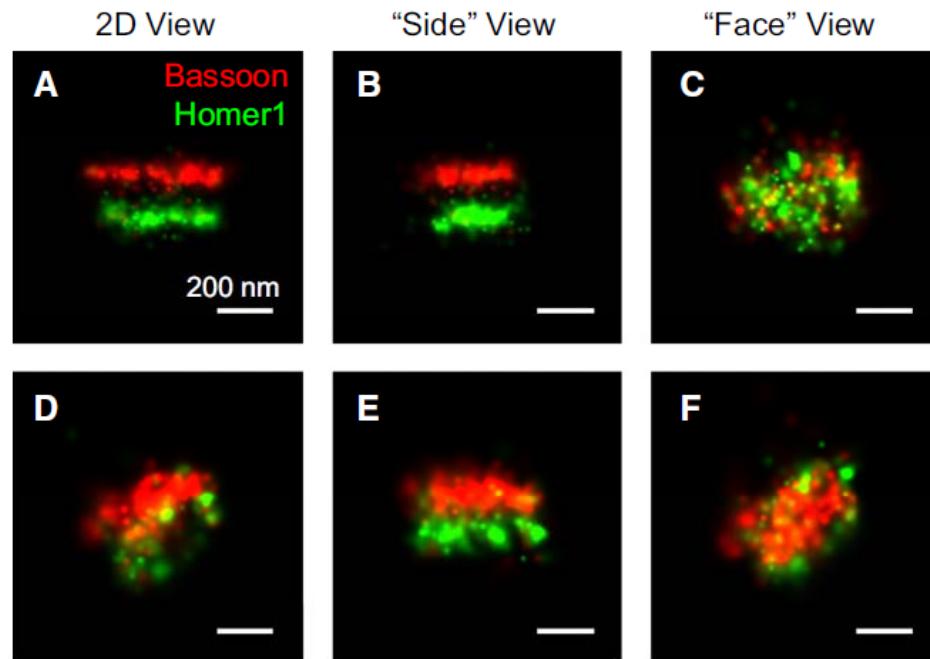
- 5.由于透射电子显微镜只能观察非常薄的样本，而有可能物质表面的结构与物质内部的结构不同；
- 6.超薄样品（100纳米以下），制样过程复杂、困难，制样有损伤；
- 7.电子束可能通过碰撞和加热破坏样本；
- 8.此外电子显微镜购买和维护的价格都比较高。

- 5.TEM is only suitable for thin samples and the surface structure may be different from the internal structure.
- 6.Ultra-thin sample (under 100 nm) preparation is complexed and complicated and samples may be damaged;
- 7.Electron beams may damage samples by collision and heating.
- 8.The cost to purchase and maintain electron microscopes is high.

Synapse 突触

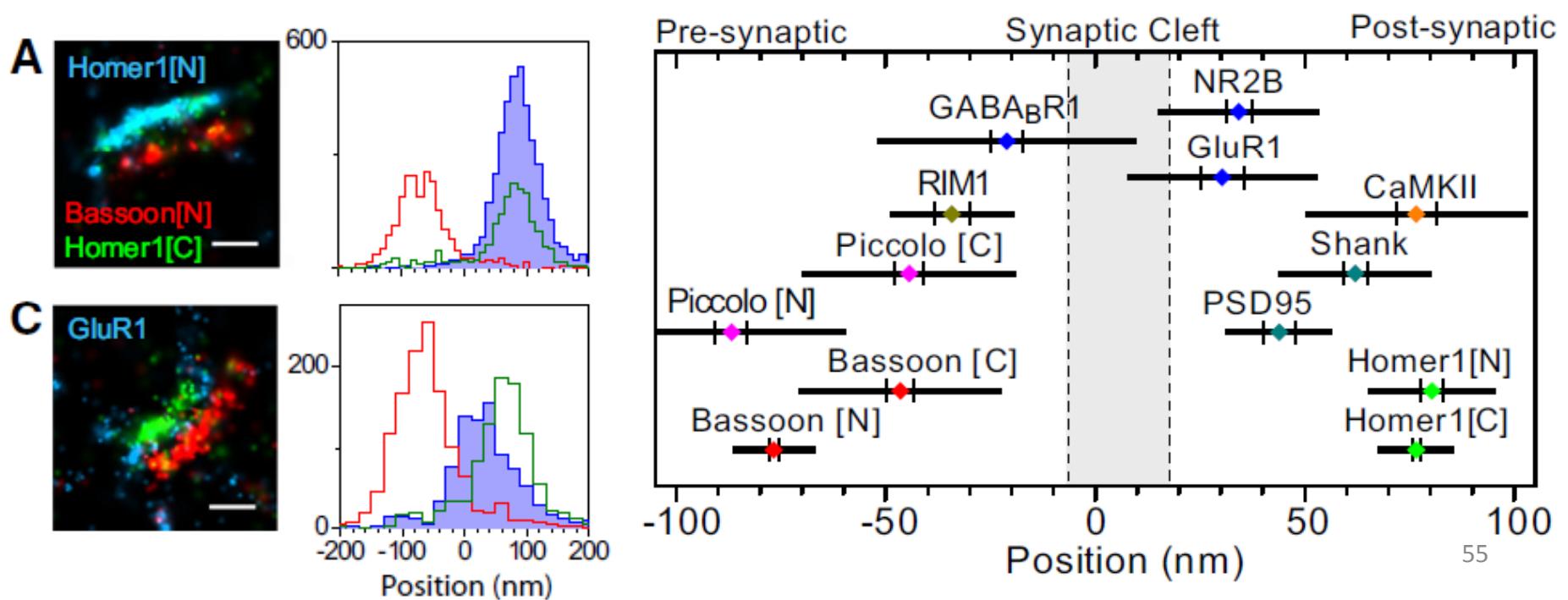






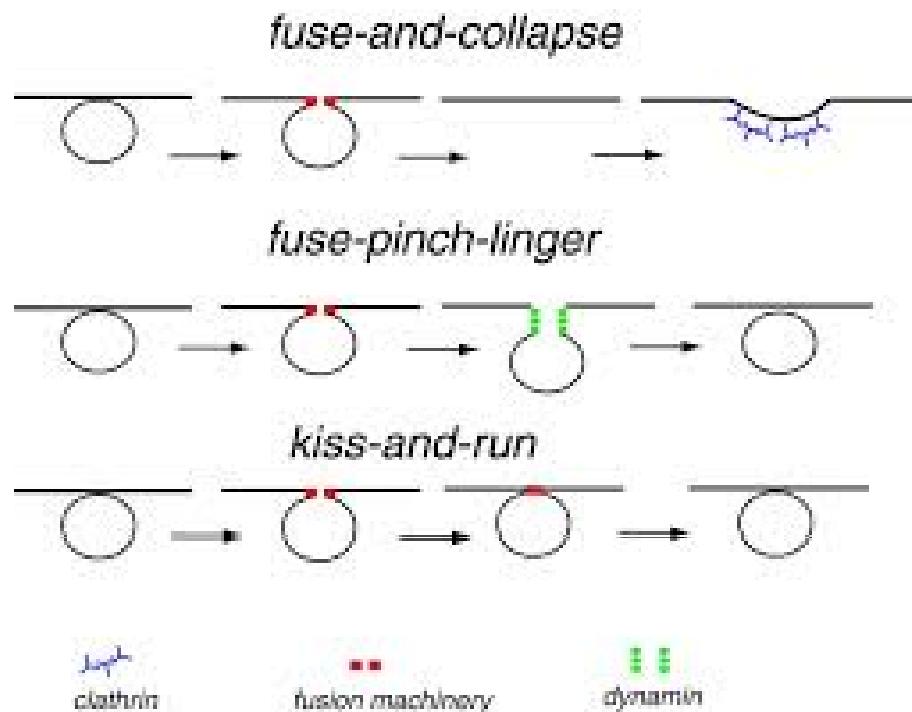
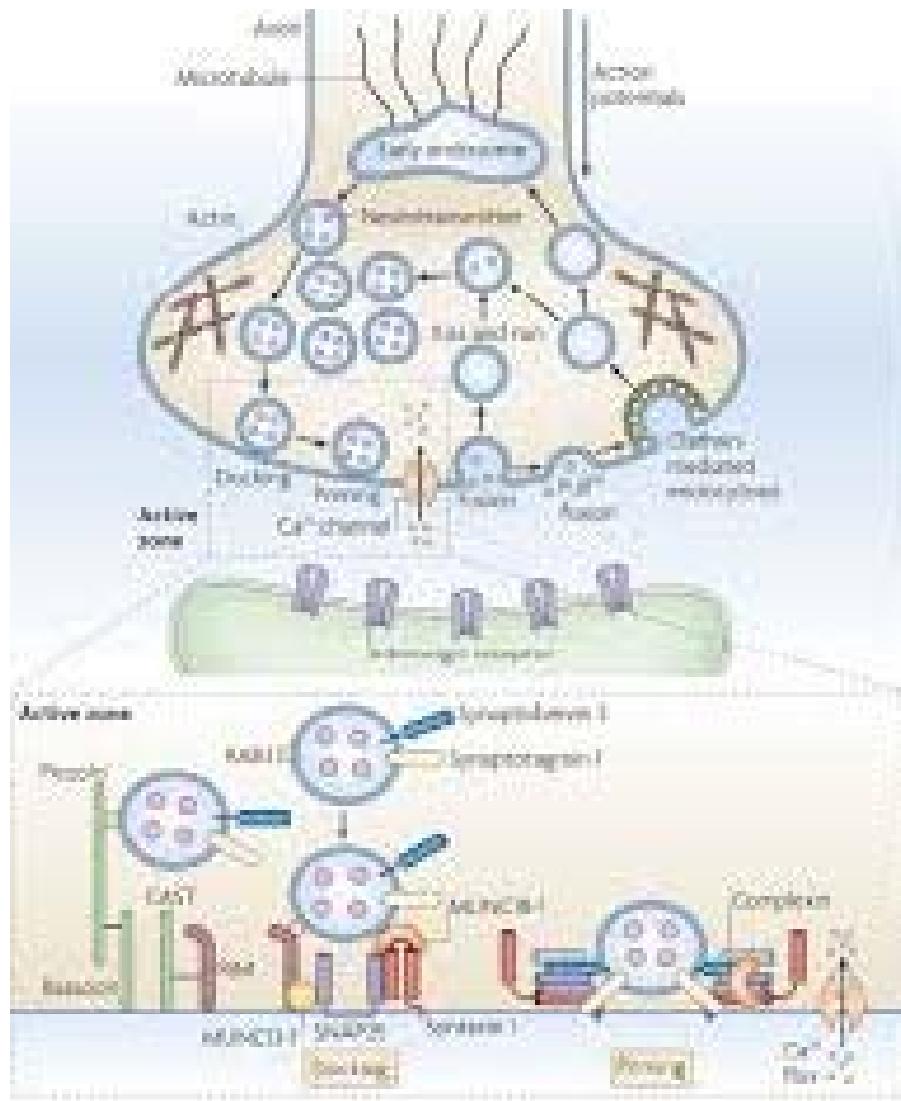
Superresolution Imaging of Chemical Synapses in the Brain

Adish Dani, Bo Huang, Joseph
Bergan, Catherine Dulac,* and
Xiaowei Zhuang



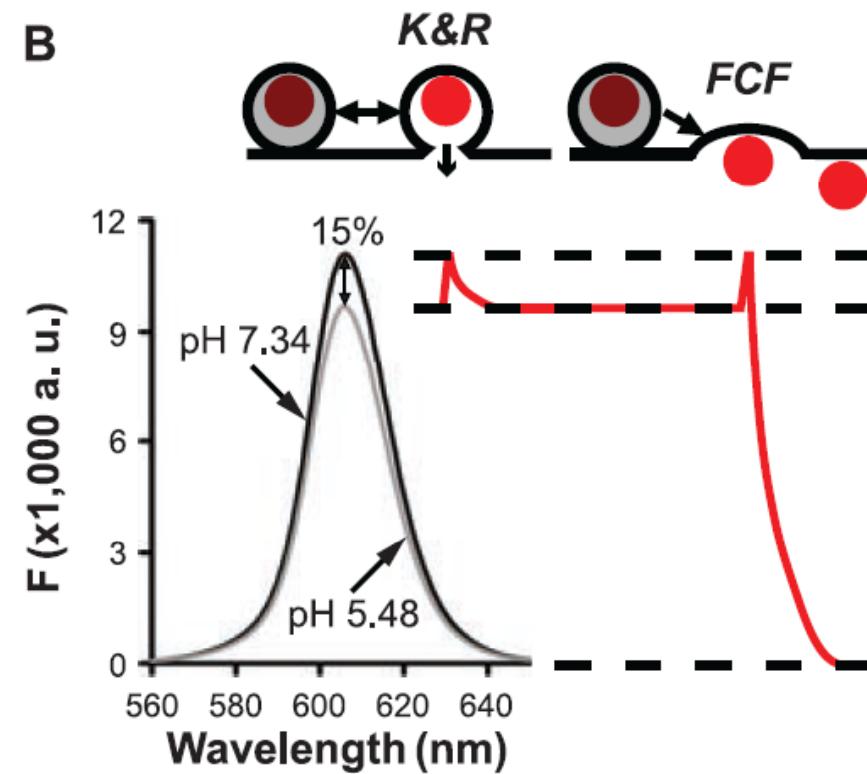
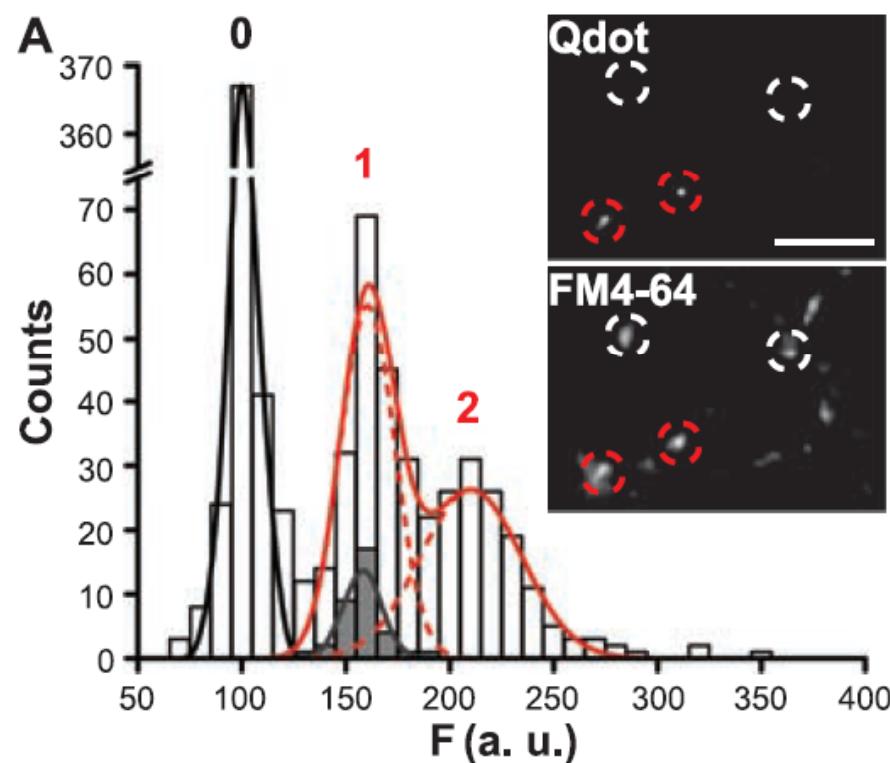
Synaptic vesicles dynamics

突触小泡运动动力学

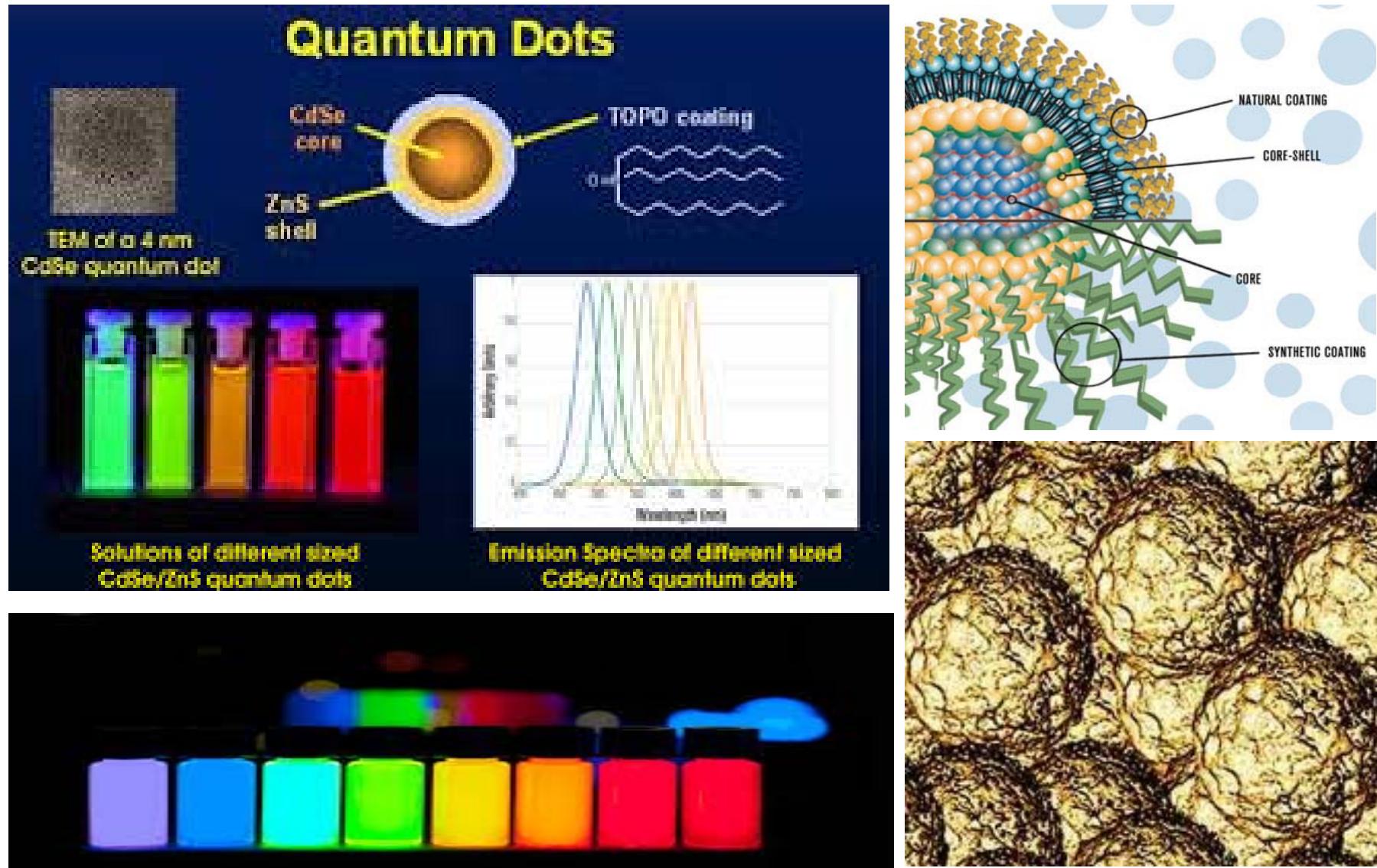


The Dynamic Control of Kiss-And-Run and Vesicular Reuse Probed with Single Nanoparticles

Qi Zhang, Yulong Li, Richard W. Tsien



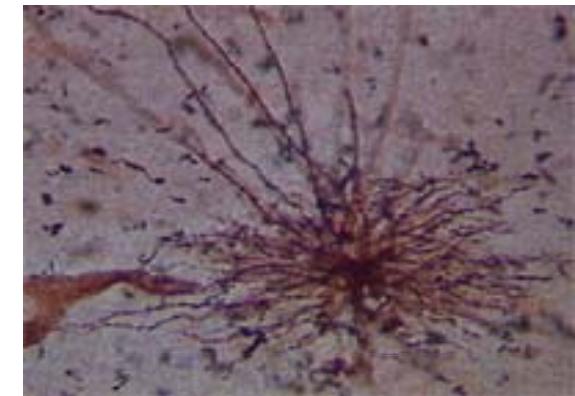
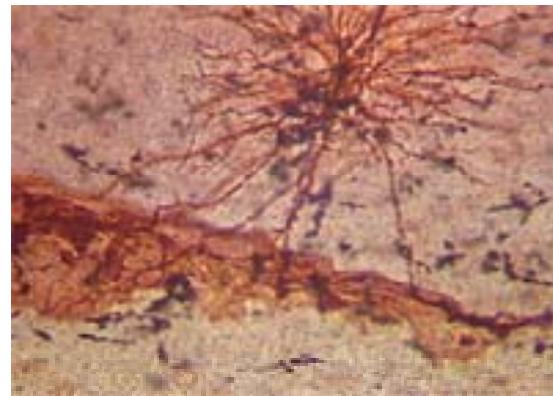
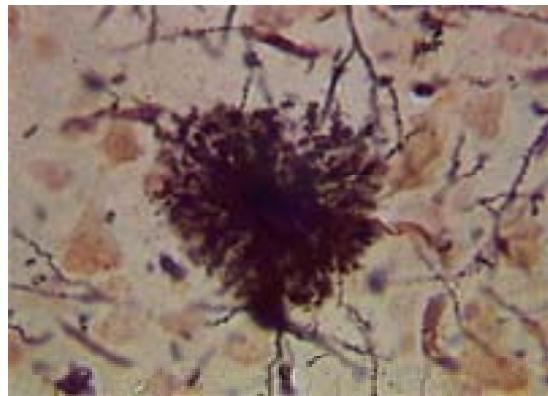
Quantum dots 量子点



Glial cells 胶质细胞

也称神经胶质，是广泛分布于神经系统内的，除了神经元以外的所有细胞。具有支持、滋养神经元的作用，也有吸收和调节某些活性物质的功能。胶质细胞虽有突起，但不具轴突，也不产生动作电位。神经胶质细胞有分裂的能力，还能够吞噬因损伤而解体破碎的神经元，并能修补填充、形成瘢痕。大脑和小脑发育中细胞构筑的形成都有赖胶质细胞作前导，提供原初的框架结构。神经轴突再生过程必须有胶质细胞的导引才能成功。

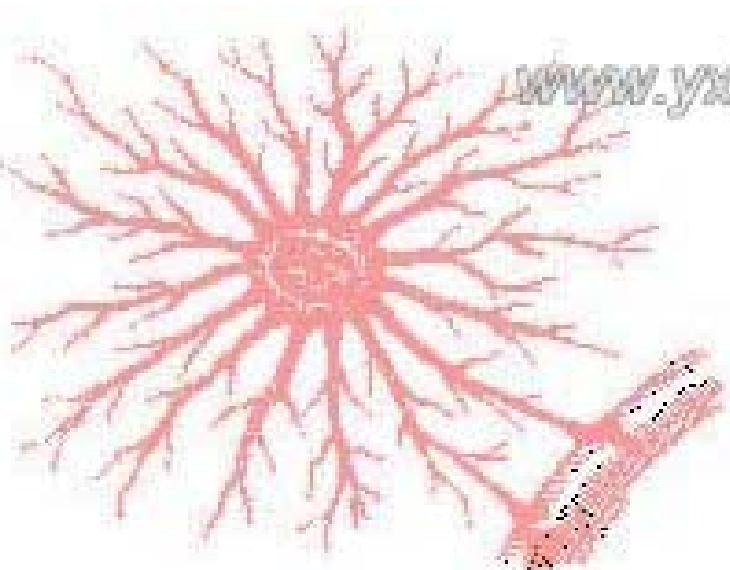
Glial cells are also called neuroglia and are cells widely distributed in the nervous system except neurons. Function to support and nourish neurons and absorb and regulate certain substances. Glial cells have neurites but not axons and they don't generate action potentials. Glial cells are able to divide and swallow the collapses of damaged and broken neurons and are able to repair and form scars. In the development of the brain and cerebellum, cellular constructions rely on the guidance of glial cells to provide primary frame structures. Neuronal axon regeneration process requires guidance of glial cells.



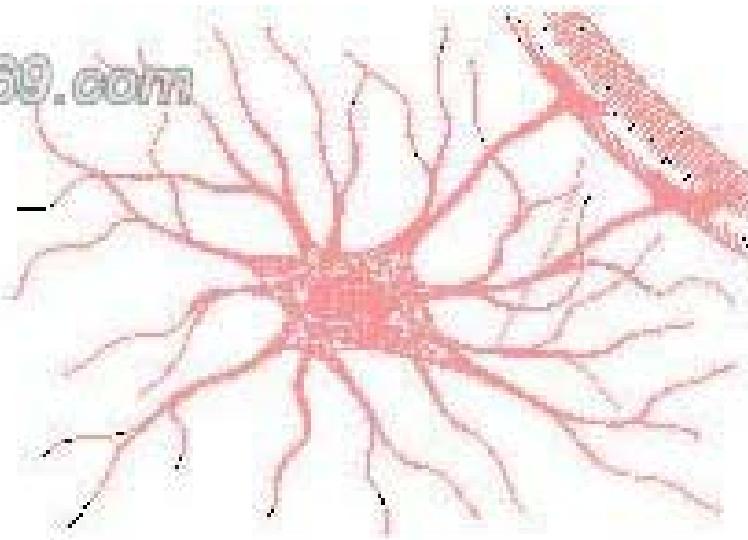
中枢 CNS: 星形胶质细胞 (astrocyte) (纤维性星形胶质细胞 fibrous astrocyte、原浆性星形胶质细胞 protoplasmic astrocyte) 、少突胶质细胞 (oligodendrocyte) 、小胶质细胞 (microglia) 、室管膜细胞 (ependymal cell) 四种。

外周 PNS: 神经膜细胞 (Schwann cell, 施万细胞) 、卫星细胞 (satellite cell) 。

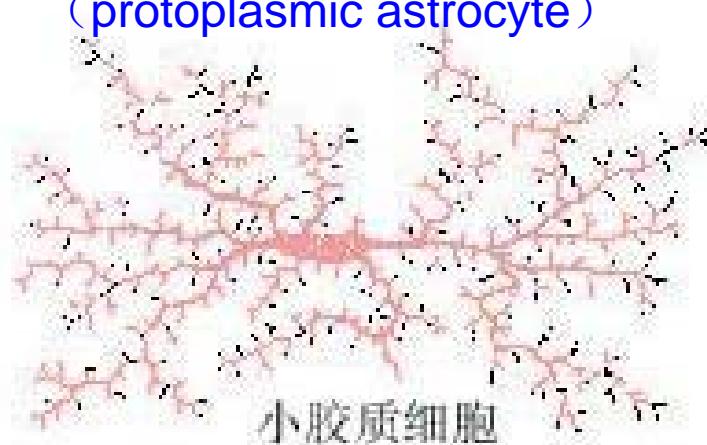
Glial cells 胶质细胞



原浆性星形胶质细胞
(protoplasmic astrocyte)



纤维性星形胶质细胞
(fibrous astrocyte)



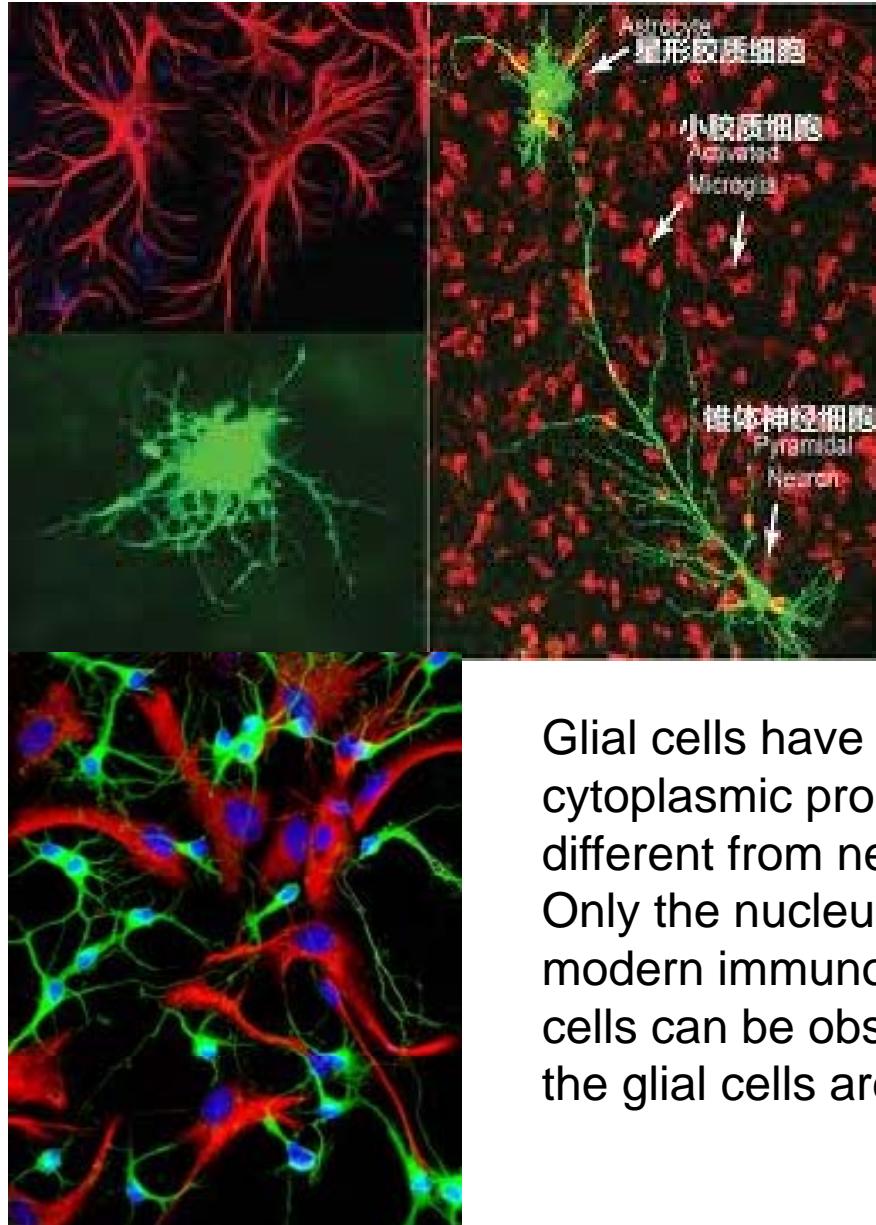
小胶质细胞
(Microglia)



少突胶质细胞
(oligodendrocyte)

The morphological characteristics of glial cells

胶质细胞的形态特点

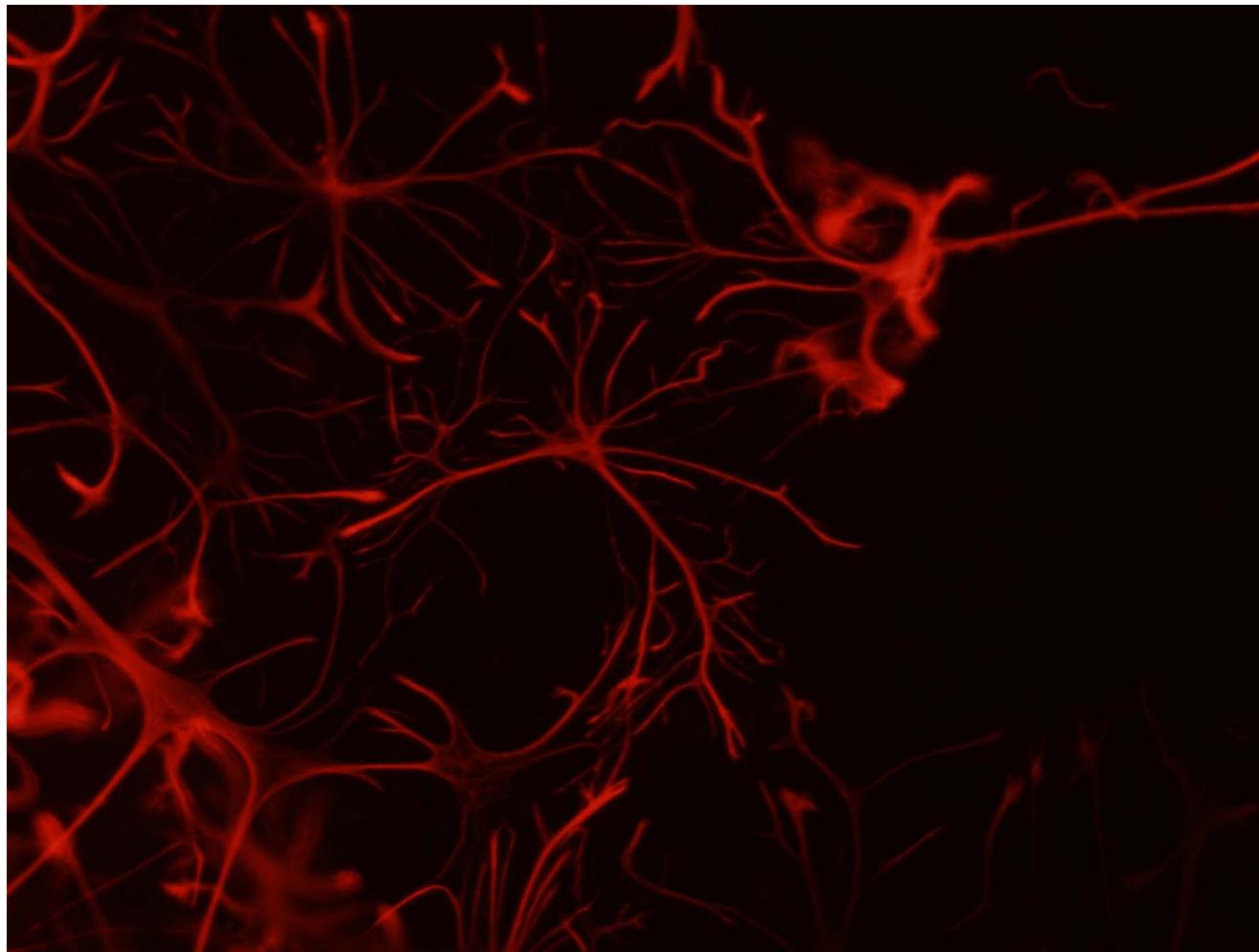


胶质细胞与神经元一样也具有细胞突起，但其胞质突起不分树突和轴突。它与神经元不同，可终身具有分裂增殖的能力。常规染色标本上只能看到细胞核，用现代免疫细胞化学方法可在光镜下观察胶质细胞的整体形态，电镜下可发现在胶质细胞之间存在着低电阻通路的缝隙连接（gap junction）。

Glial cells have neurites similar to that of neurons, but their cytoplasmic processes are not dendrites and axons. They are different from neurons due to their lifelong proliferating ability. Only the nucleus is visible in conventional staining, while by modern immunocytochemistry the whole morphology of glial cells can be observed. Low-resistance gap junctions between the glial cells are found by EM observation.

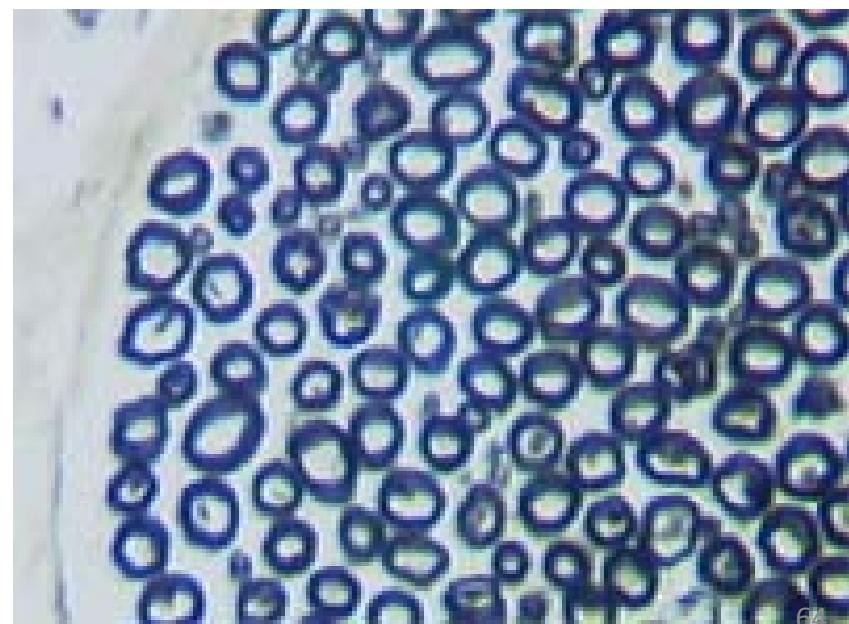
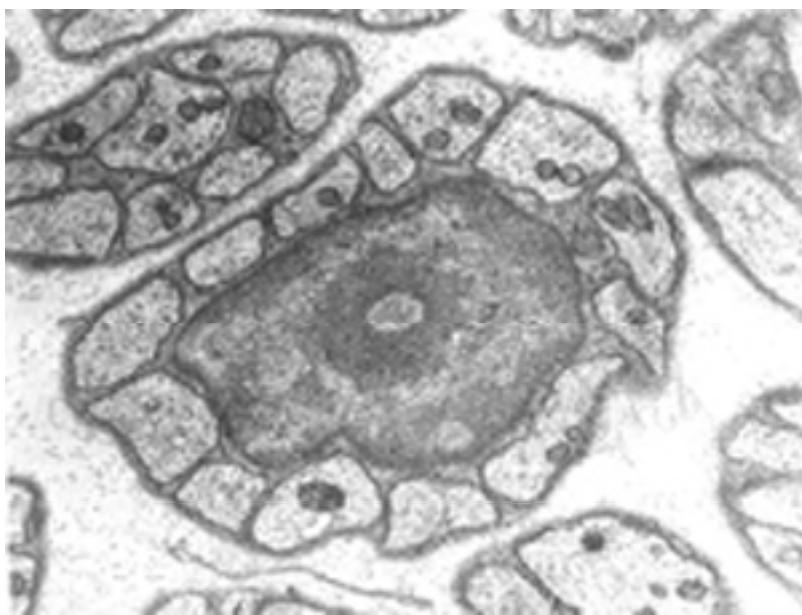
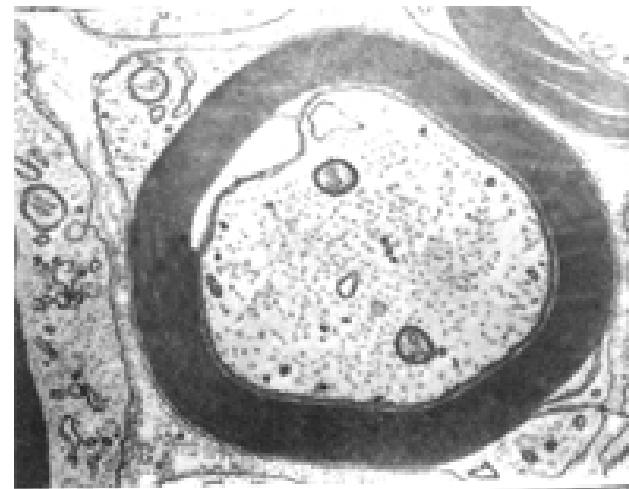
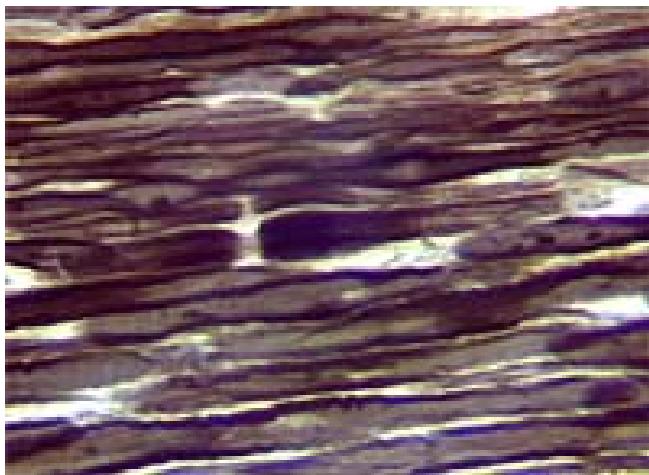
Proteins specifically expressed in glial cells

胶质细胞中特异表达的蛋白



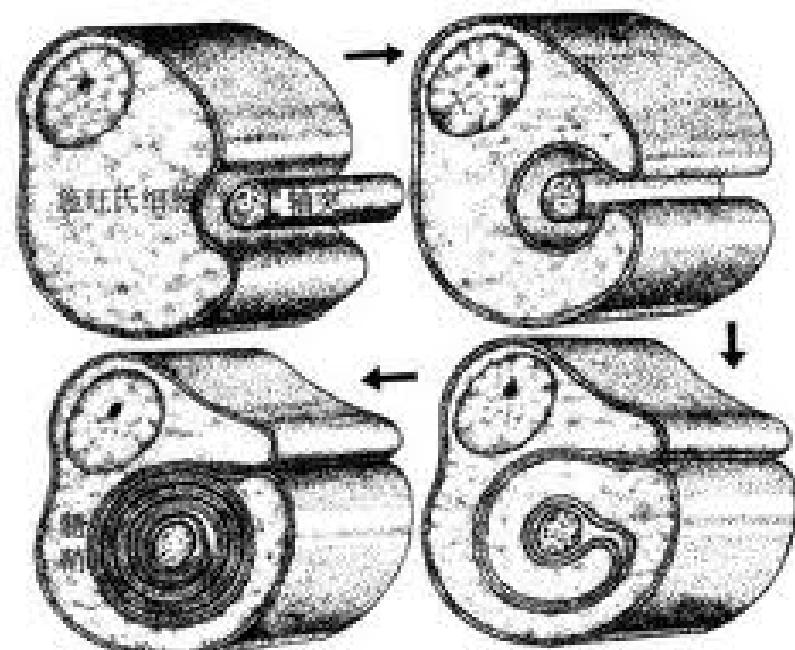
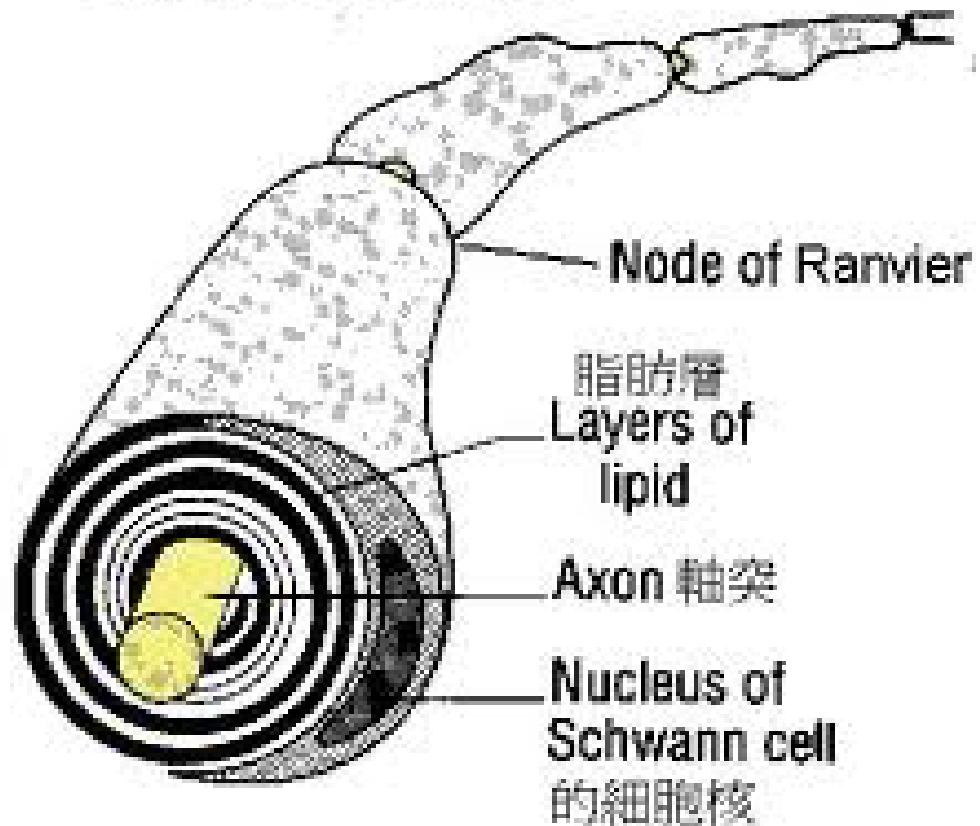
GFAP

Nerve fibers 神经纤维



Myelin sheath 體鞘

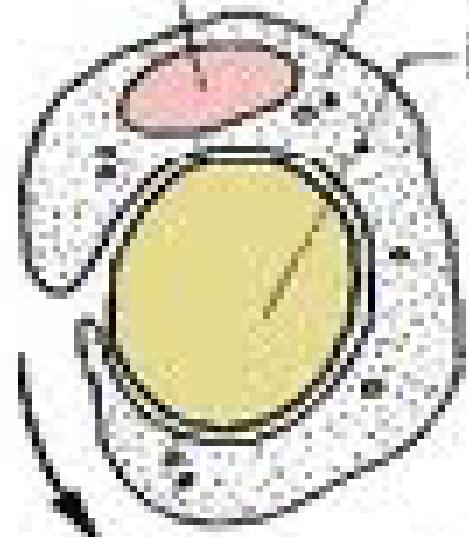
有髓鞘神经纤维外觀



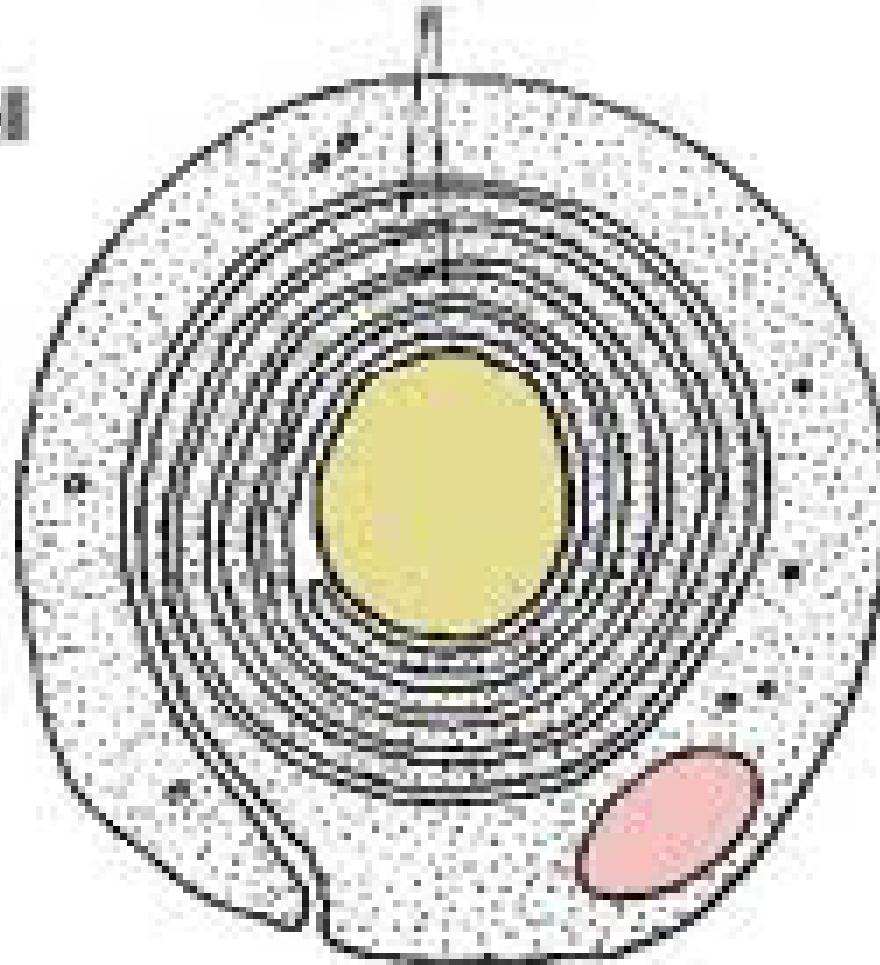
髓鞘的層次

細胞核
Schwann cell

軸突

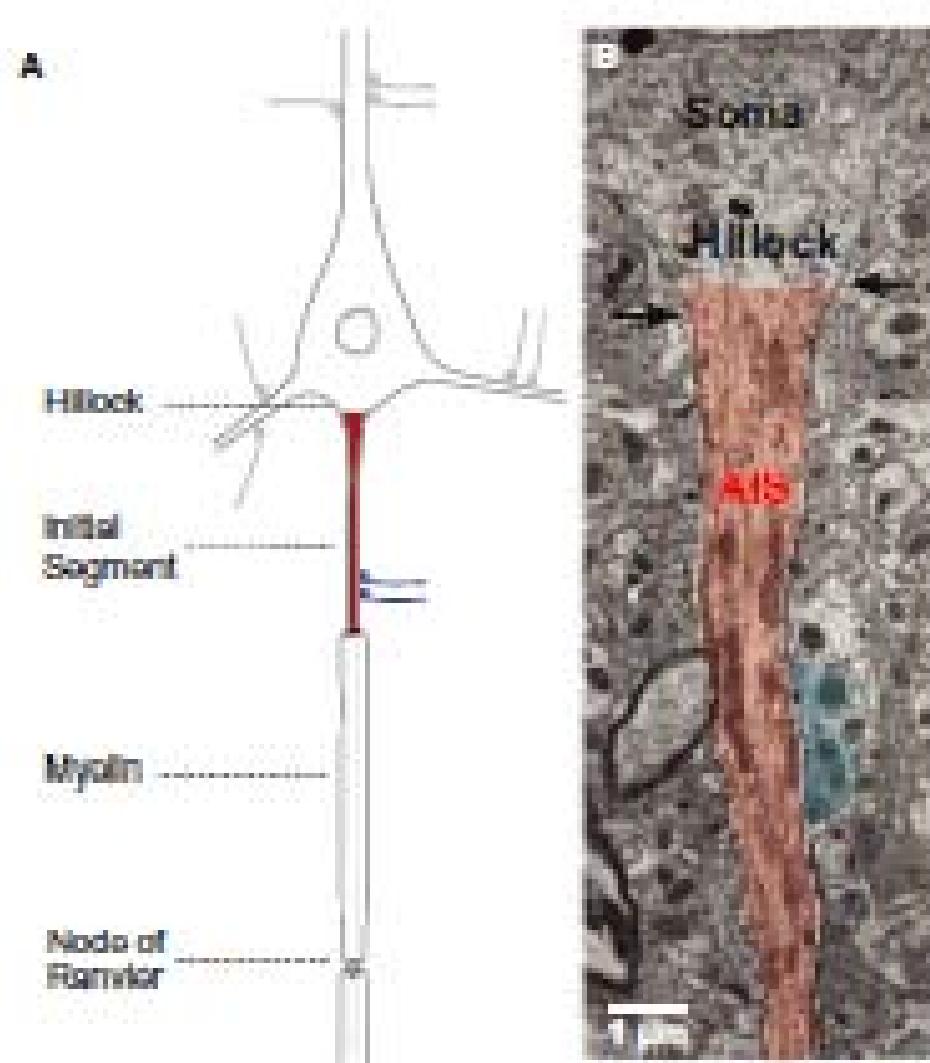


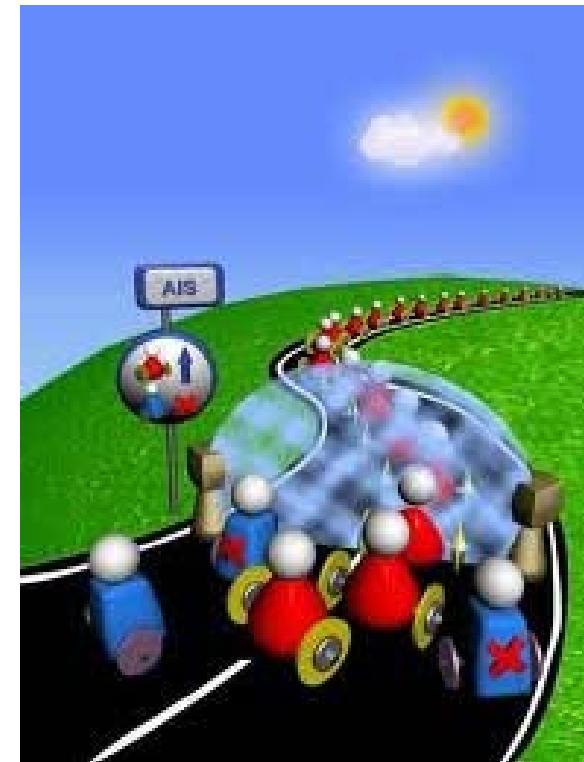
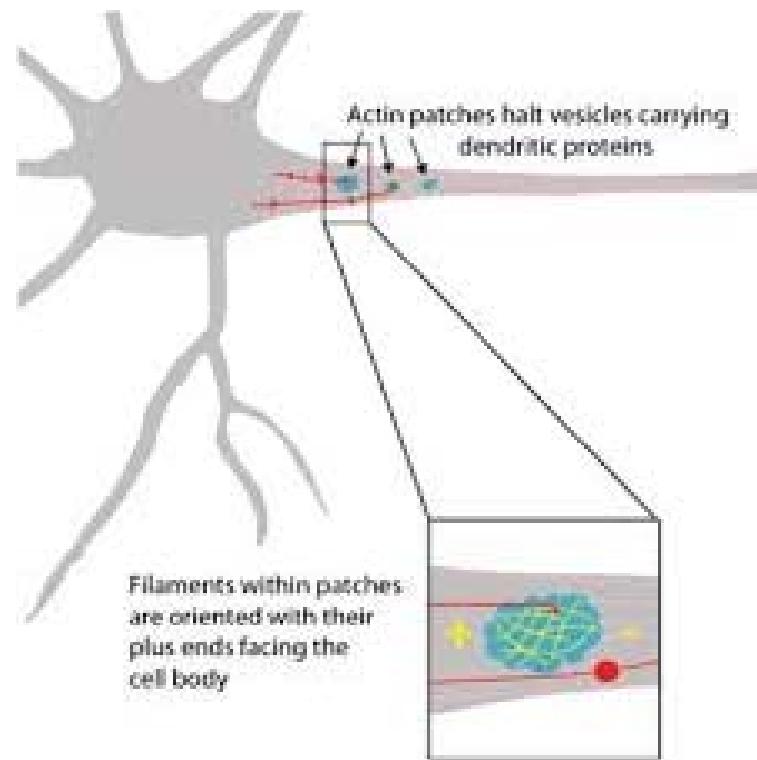
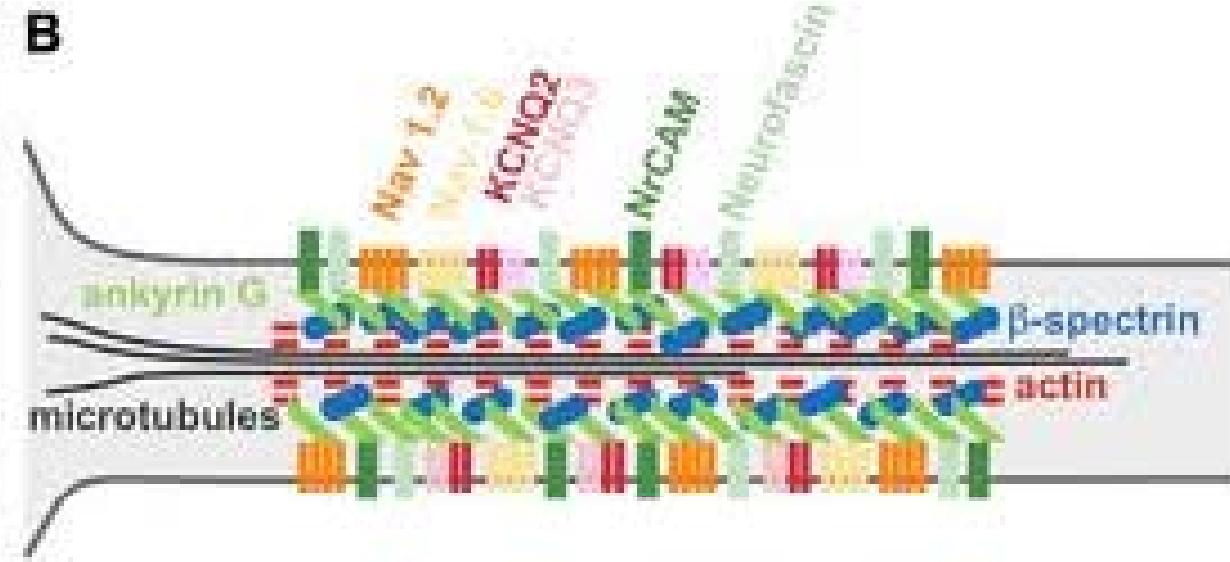
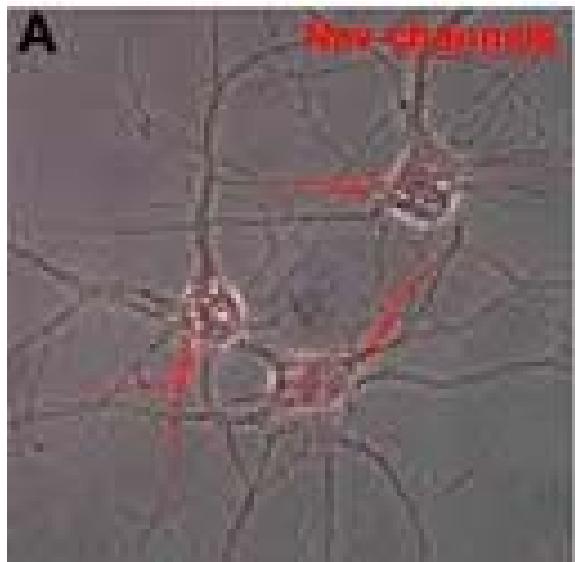
Schwann cell
繞著軸突旋轉



髓鞘的產生

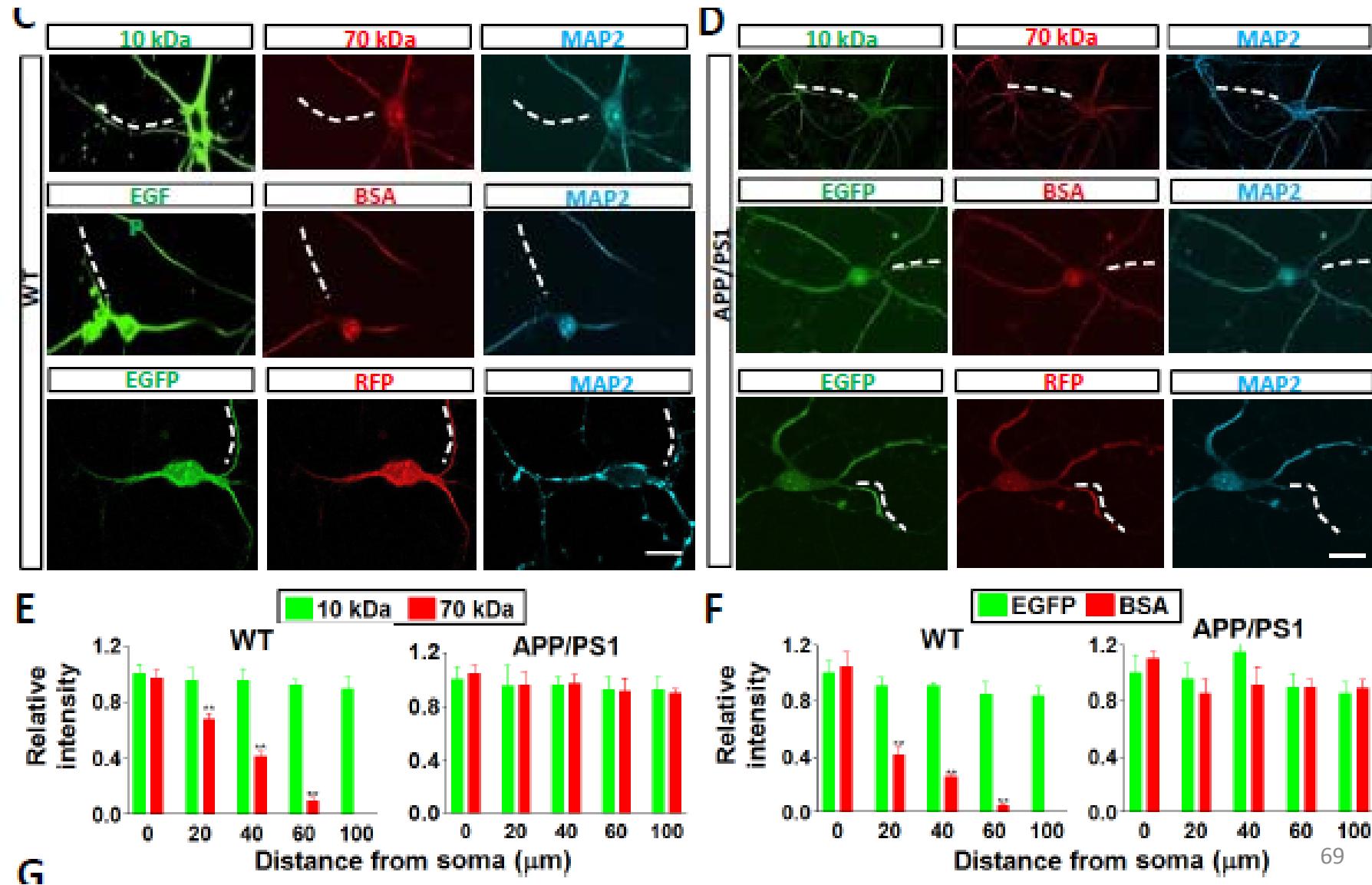
Axon initial segment 神经元轴突起始节





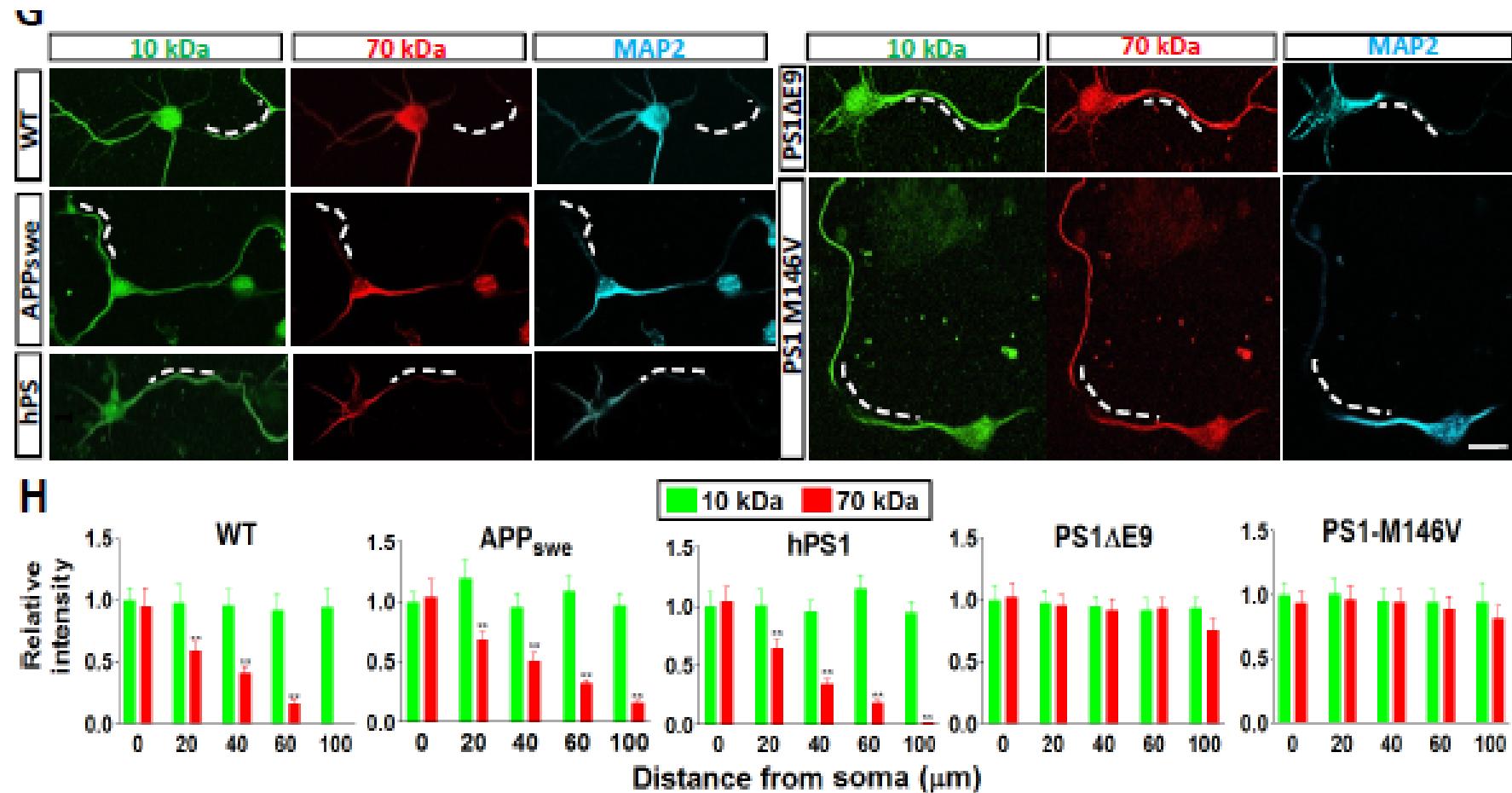
A selective filter located at the AIS

轴突起始节的选择性滤网



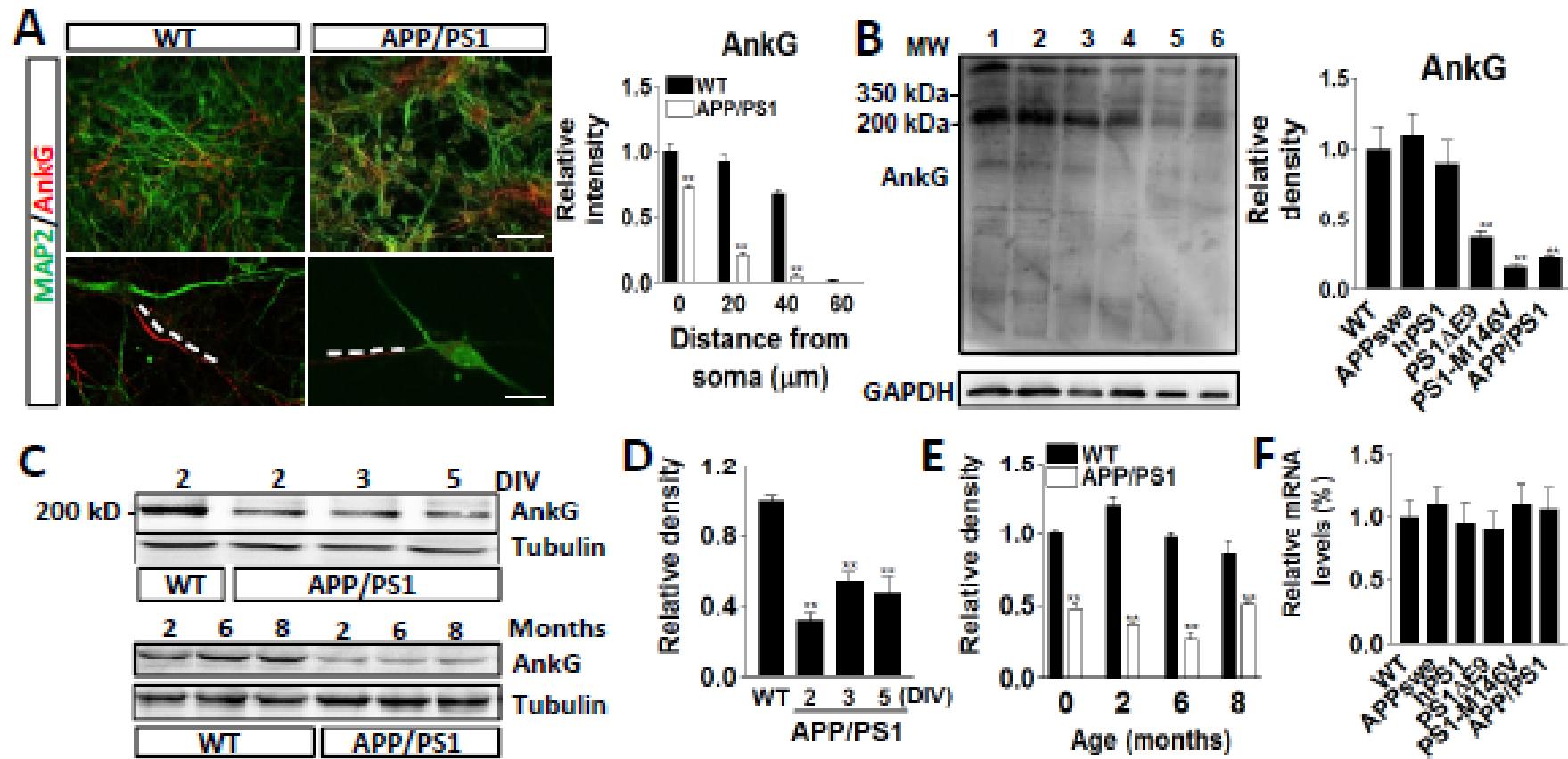
The selective filter is impaired in PS1 mutant lines

PS1突变品系选择性滤网受损



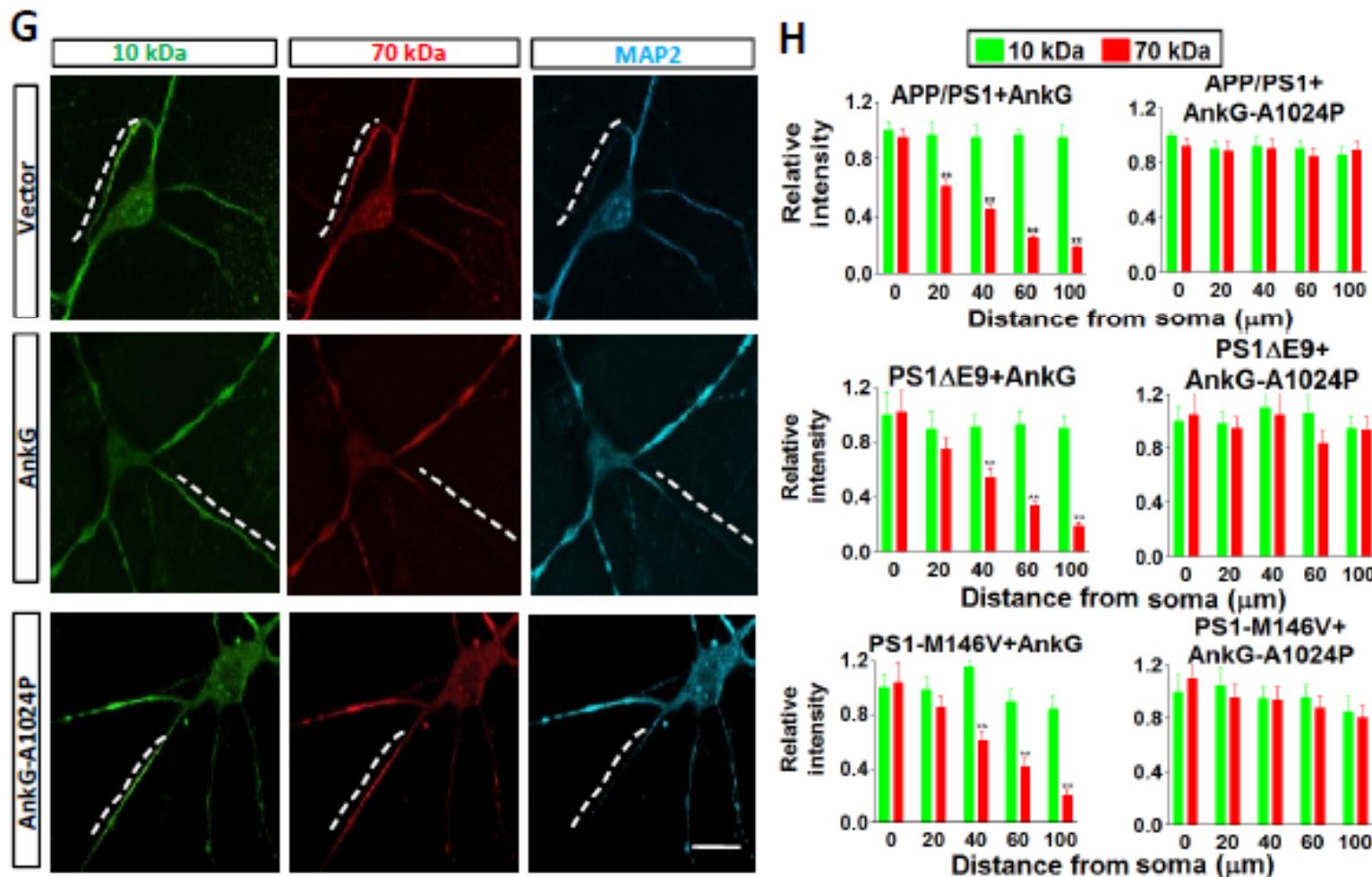
AnkG is decreased in PS1 mutant lines

PS1突变品系中AnkG表达量降低



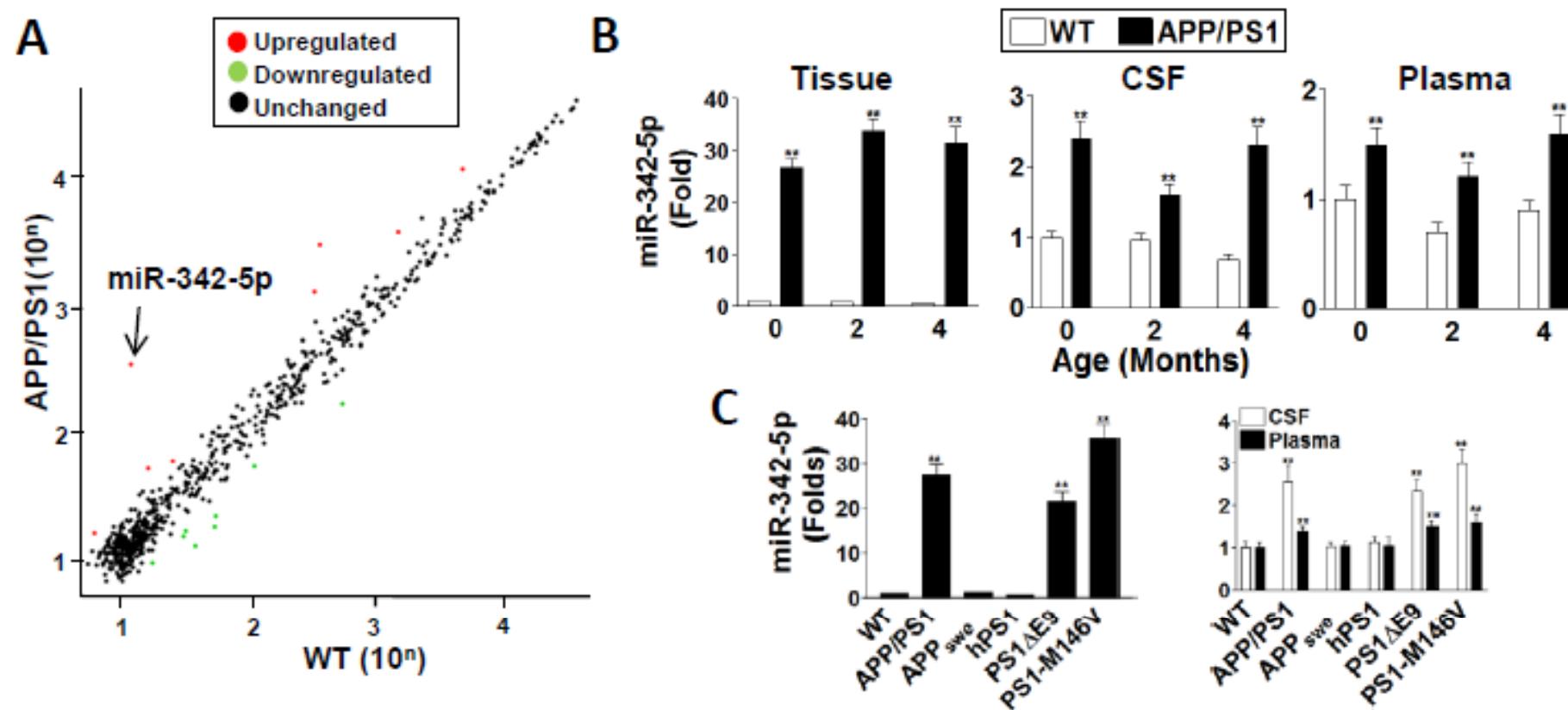
AnkG rescues the impaired filtering at the AIS in AD models

AD模型鼠中AnkG可以恢复AIS处受损滤网



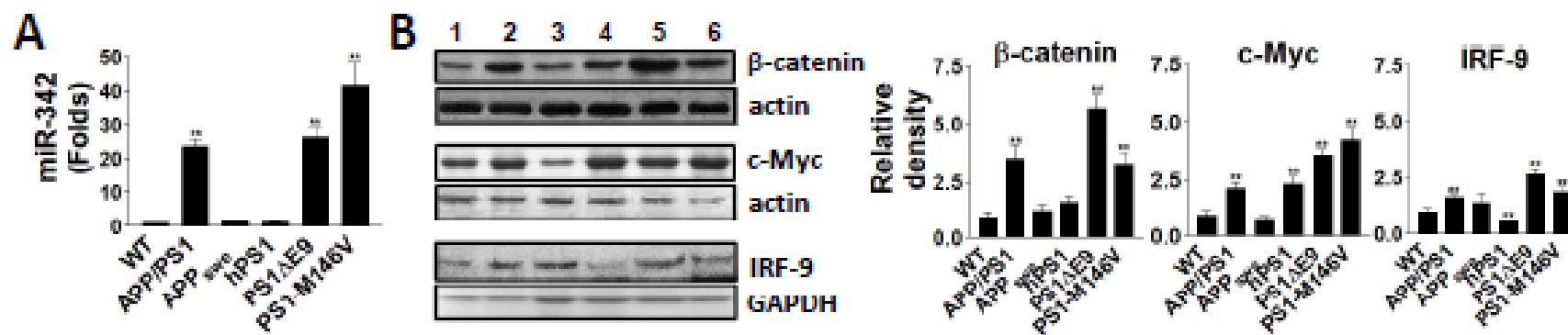
miR-342-5p is increased in PS1 mutant lines

PS1突变品系中miR-342-5p含量升高



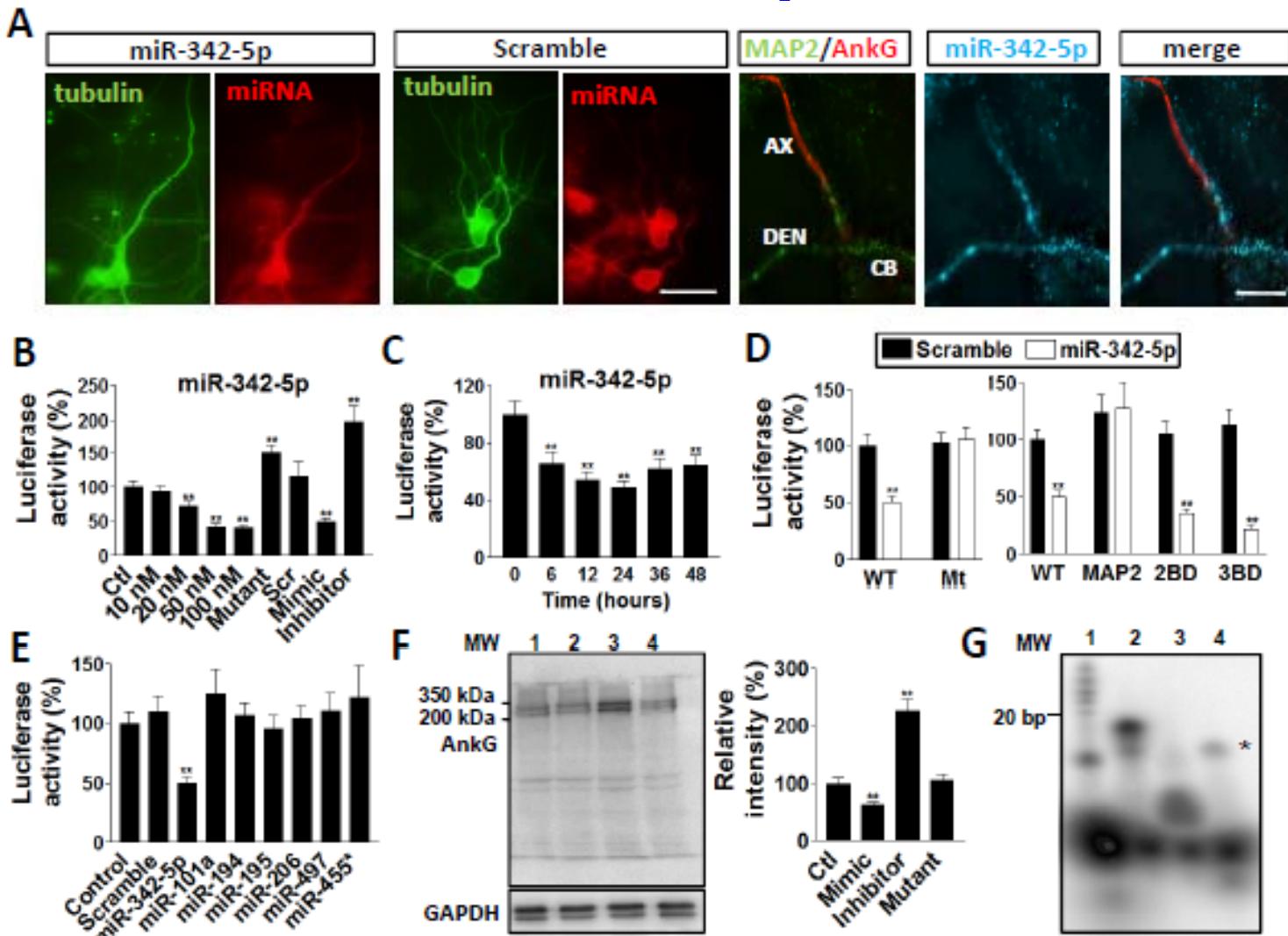
PS1 mutants induce upregulation of miR-342-5p

PS1突变引起 miR-342-5p表达上调



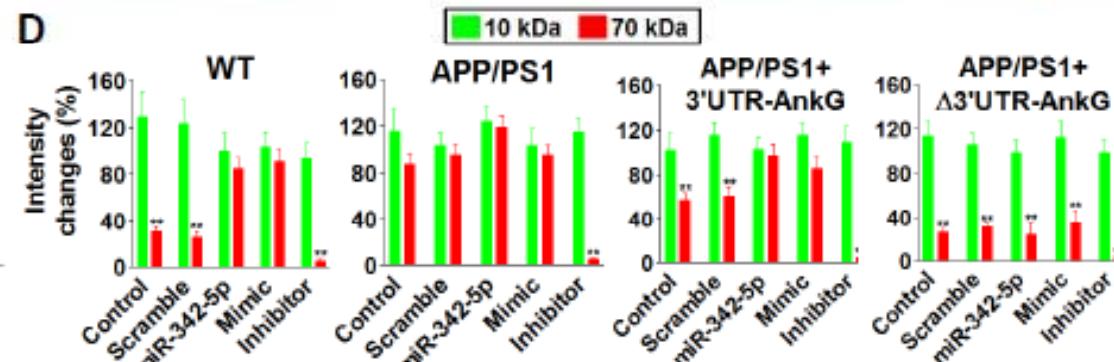
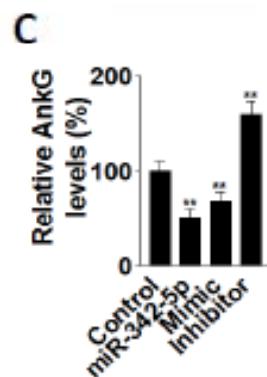
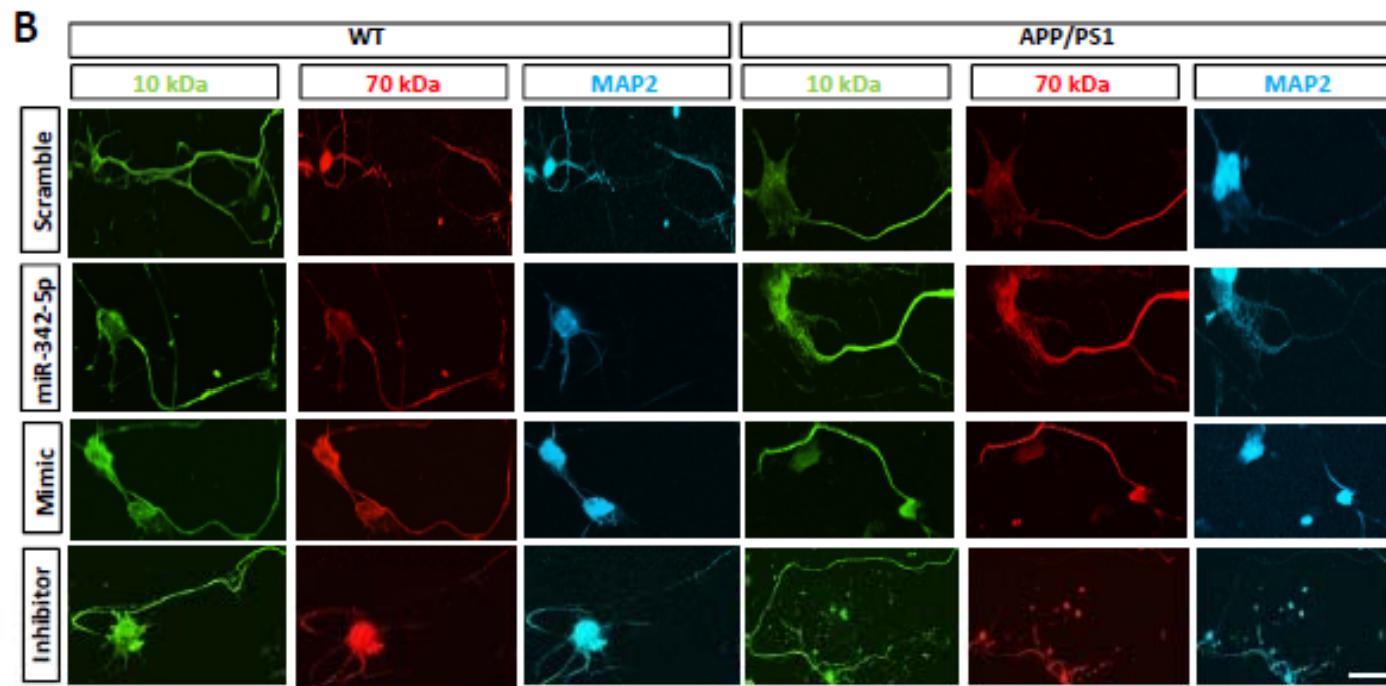
AnkG mRNA is one of the targets for miR-342-5p

AnkG mRNA是miR-342-5p的作用靶点之一



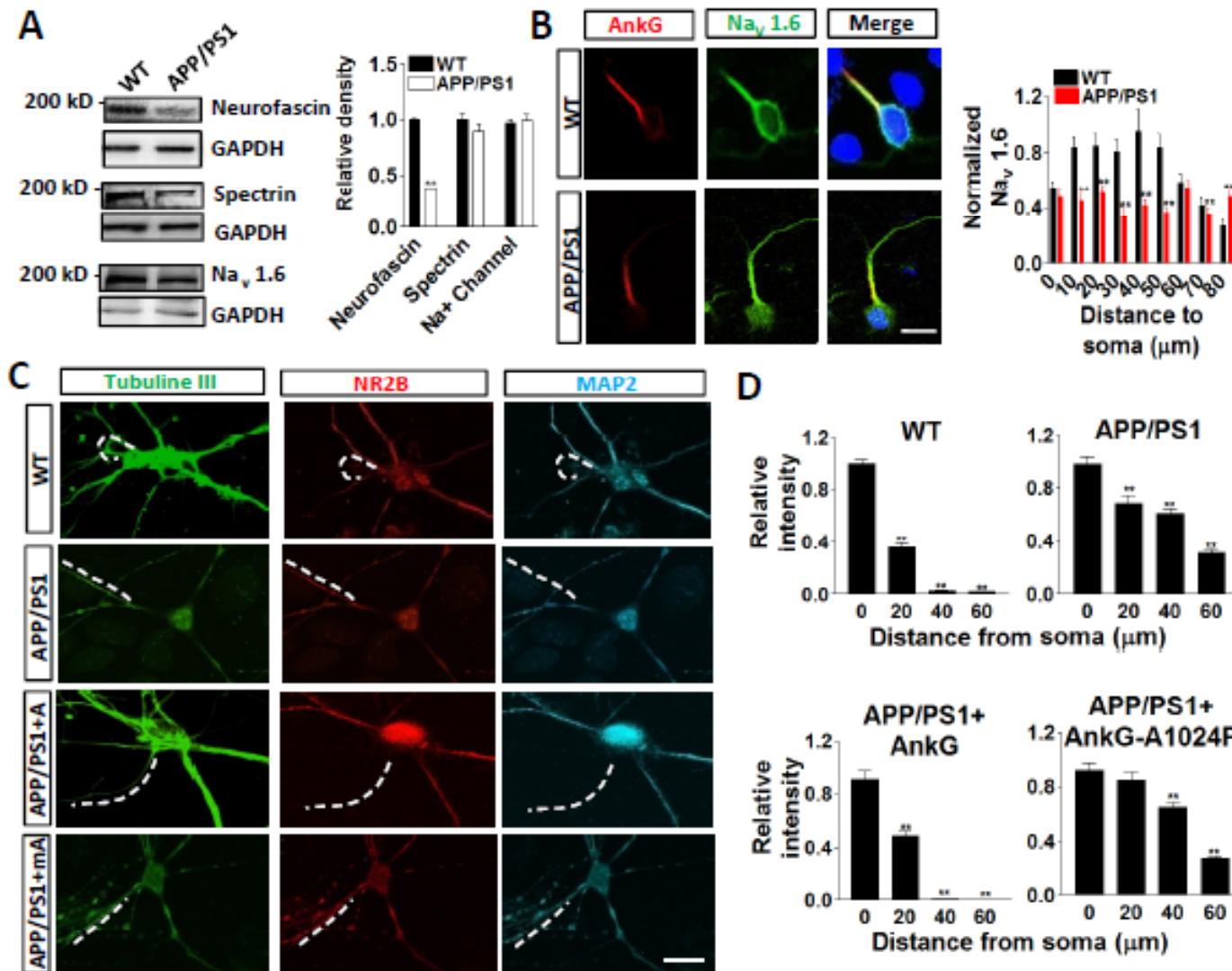
miR-342-5p rescues the impaired filtering at the AIS

miR-342-5p可以恢复AIS处受损滤网



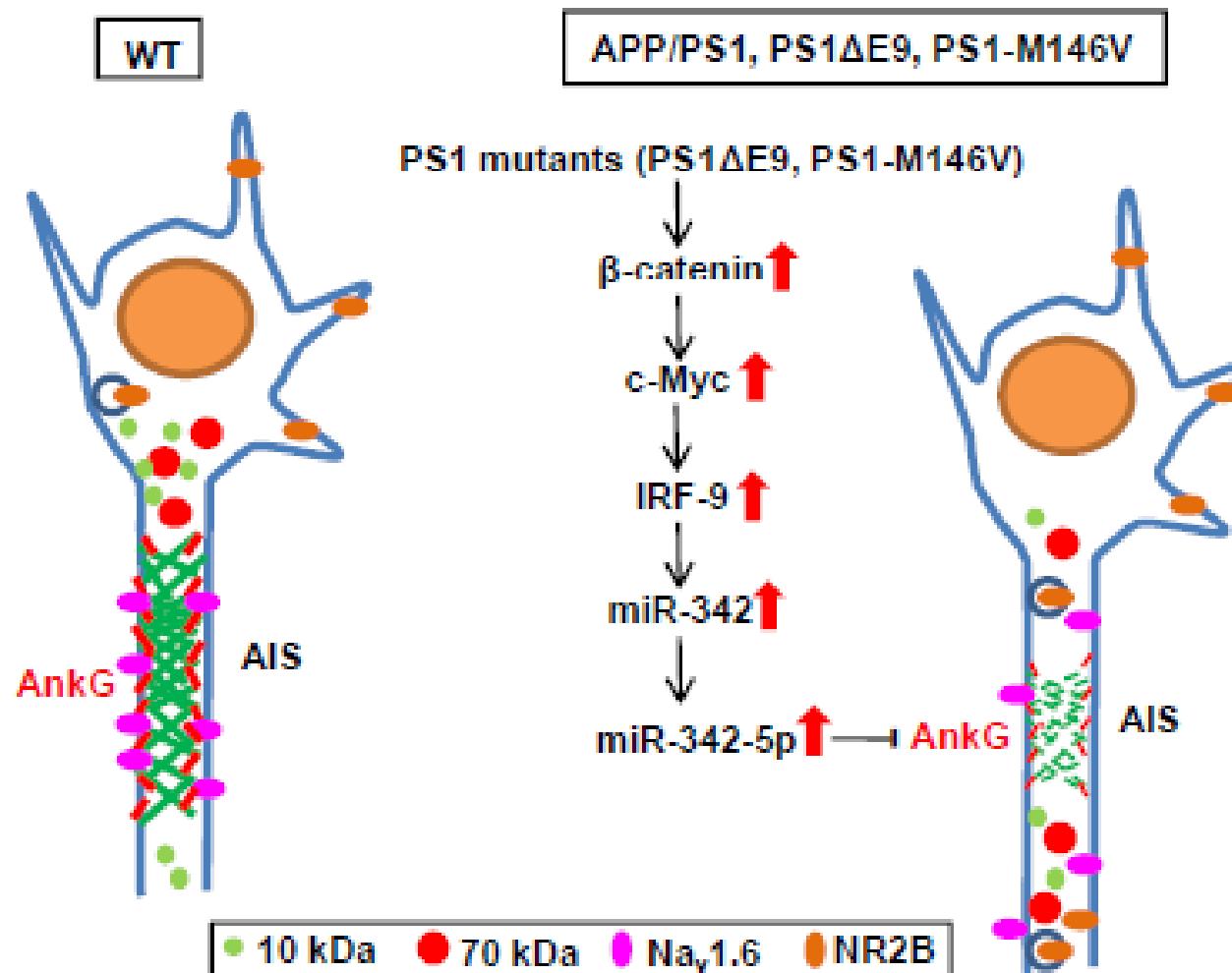
Functional implications of impaired filtering at the AIS

AIS滤网受损指征功能

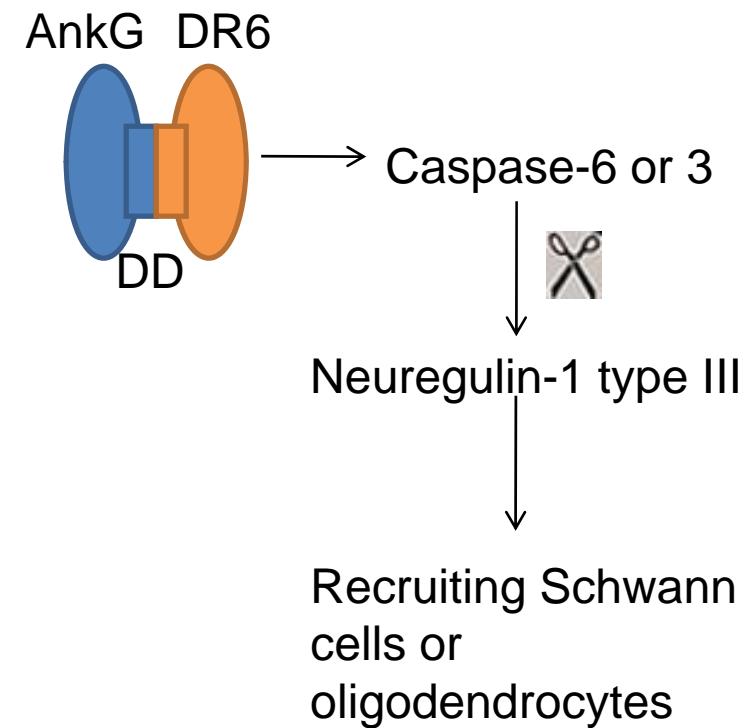
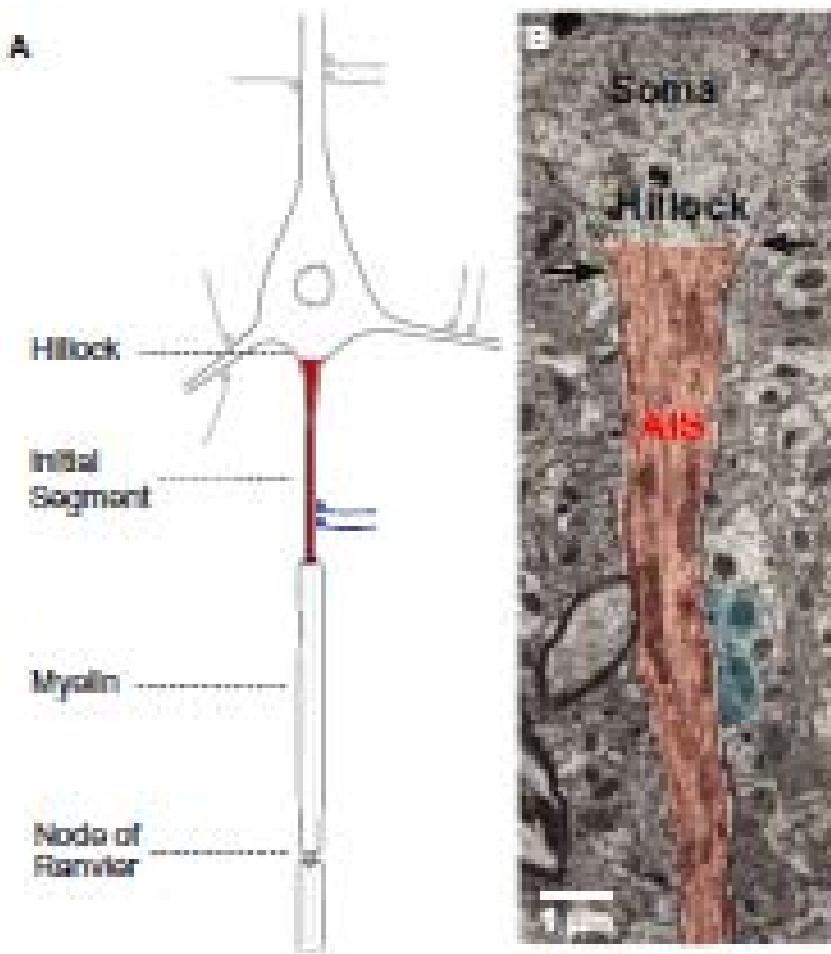


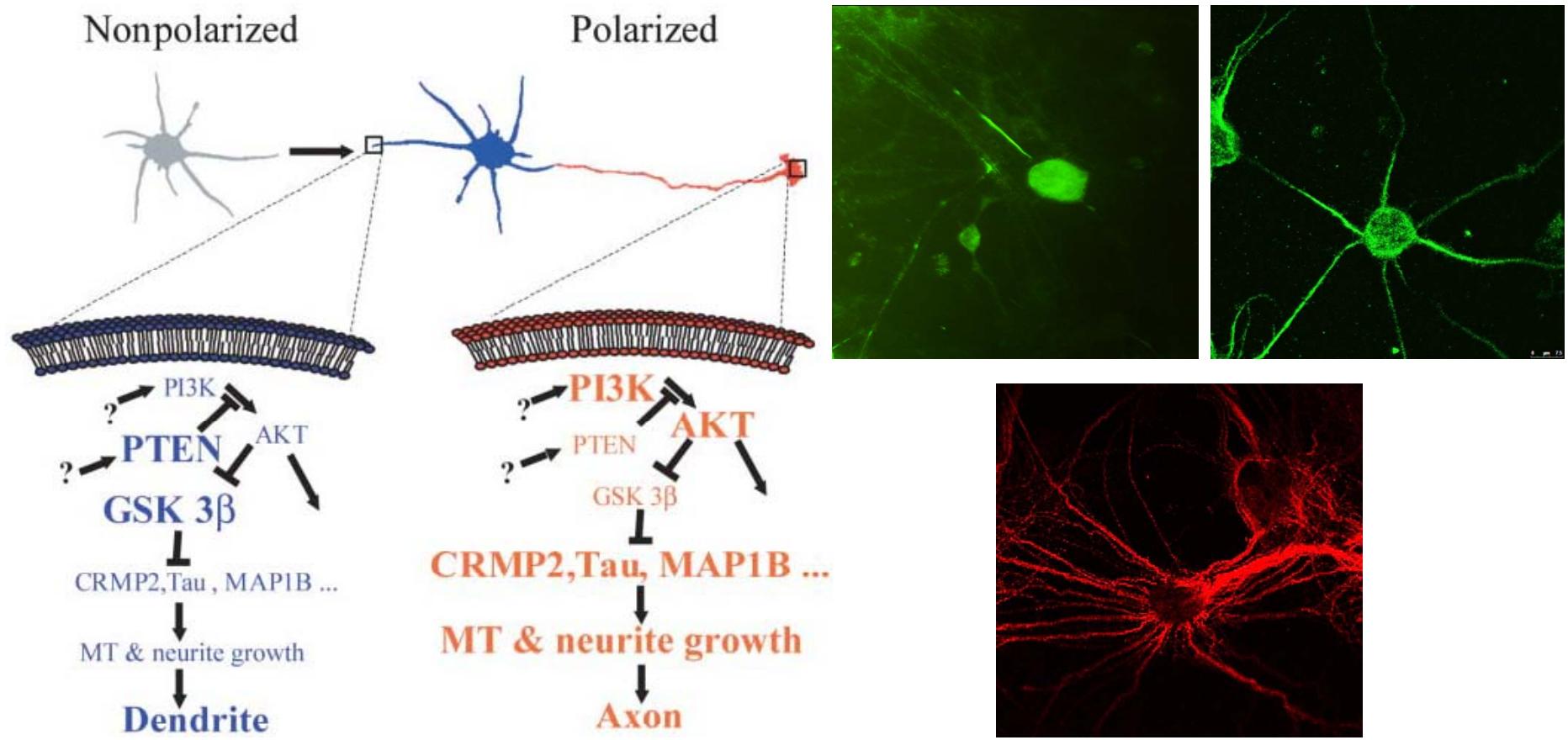
miR-342-5p regulates AIS function through regulation of AnkG

miR-342-5p通过调节AnkG来调节AIS的功能



Why AIS is not myelinated? AIS为何不被髓鞘化？





Electrophysiology of neurons with multiple axons or no axons
多轴突及无轴突神经元的电生理现象

Neural circuitry of neurons with multiple axons or no axons
多轴突及无轴突神经元的神经环路