双光子显微镜在小动物活体成像中的应用及进展
--天坛云课堂

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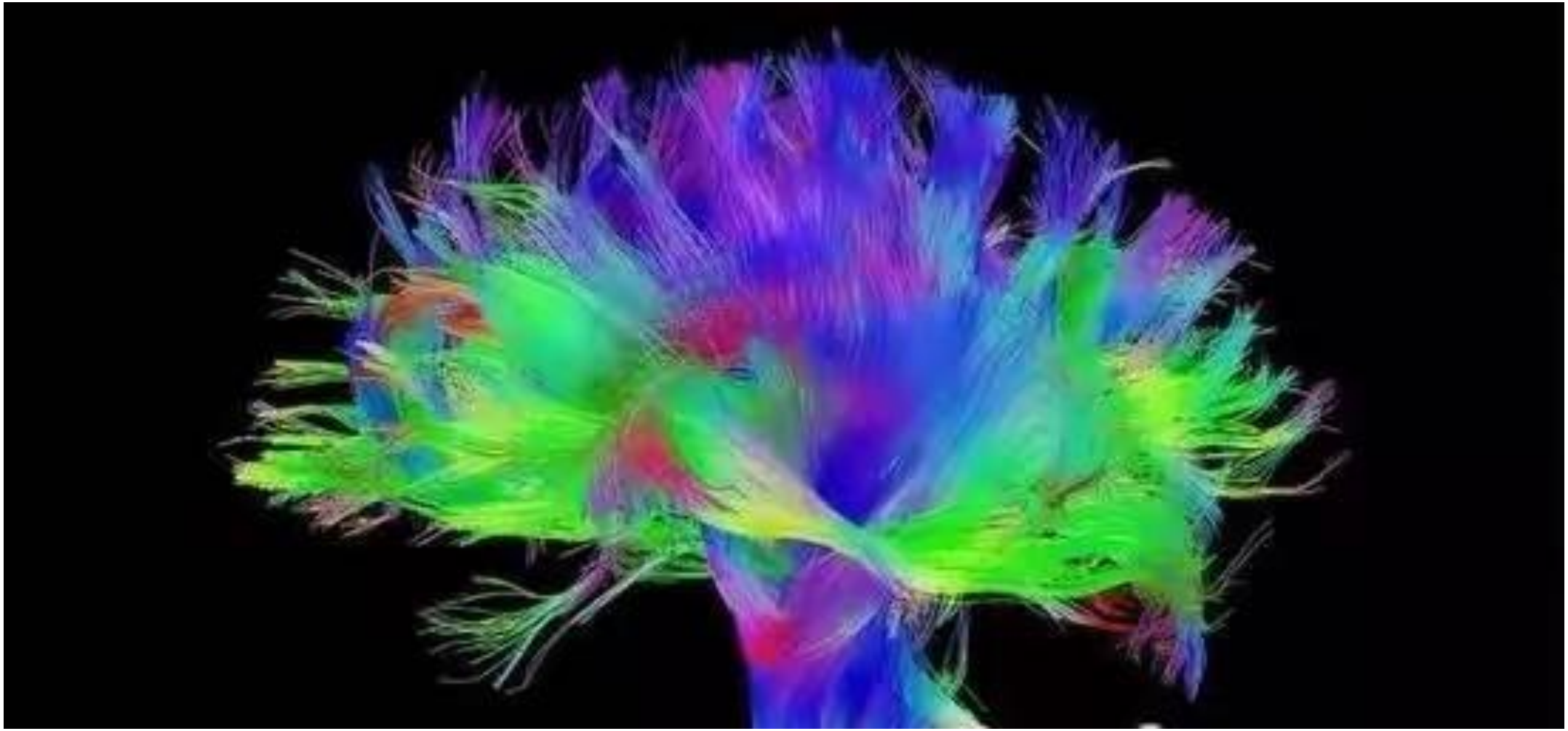
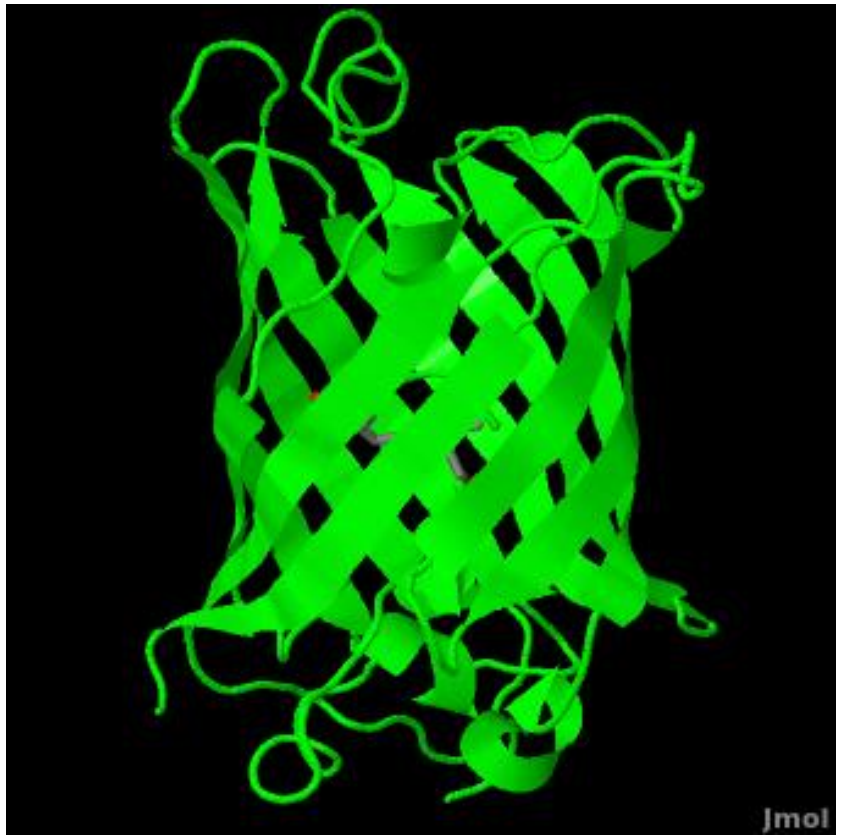
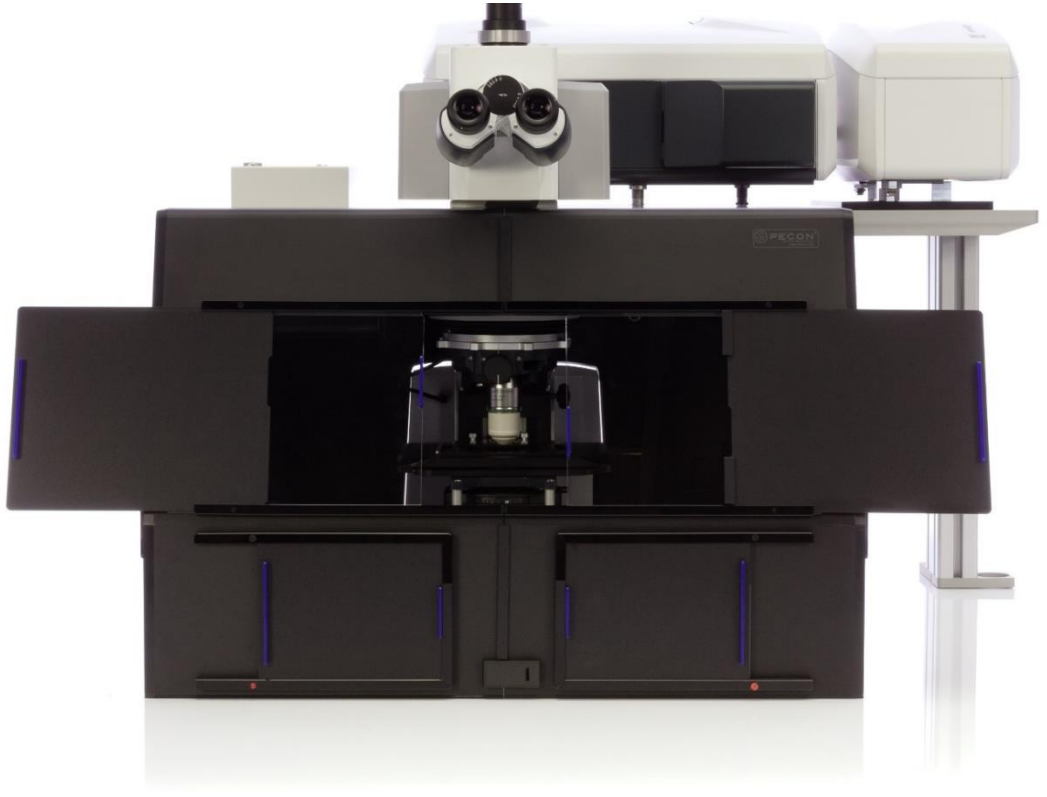
See what you were missing …

神秘的大脑



帅酷的双光子
LSM880 Airyscan NLO

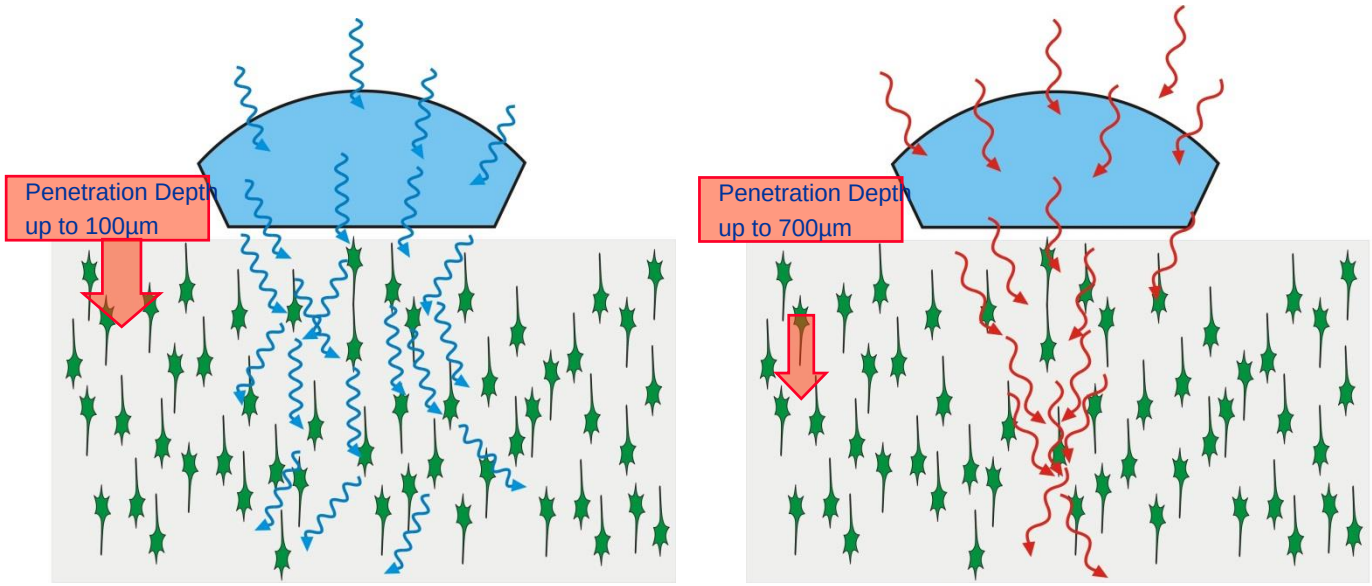




Agenda

1. 为什么需要双光子
2. 双光子优势
3. 双光子在小动物活体成像中的应用

为什么需要双光子



Scattering of short wavelength photons in brain tissue. From a certain depth on, focusing of light will not be efficient any more.

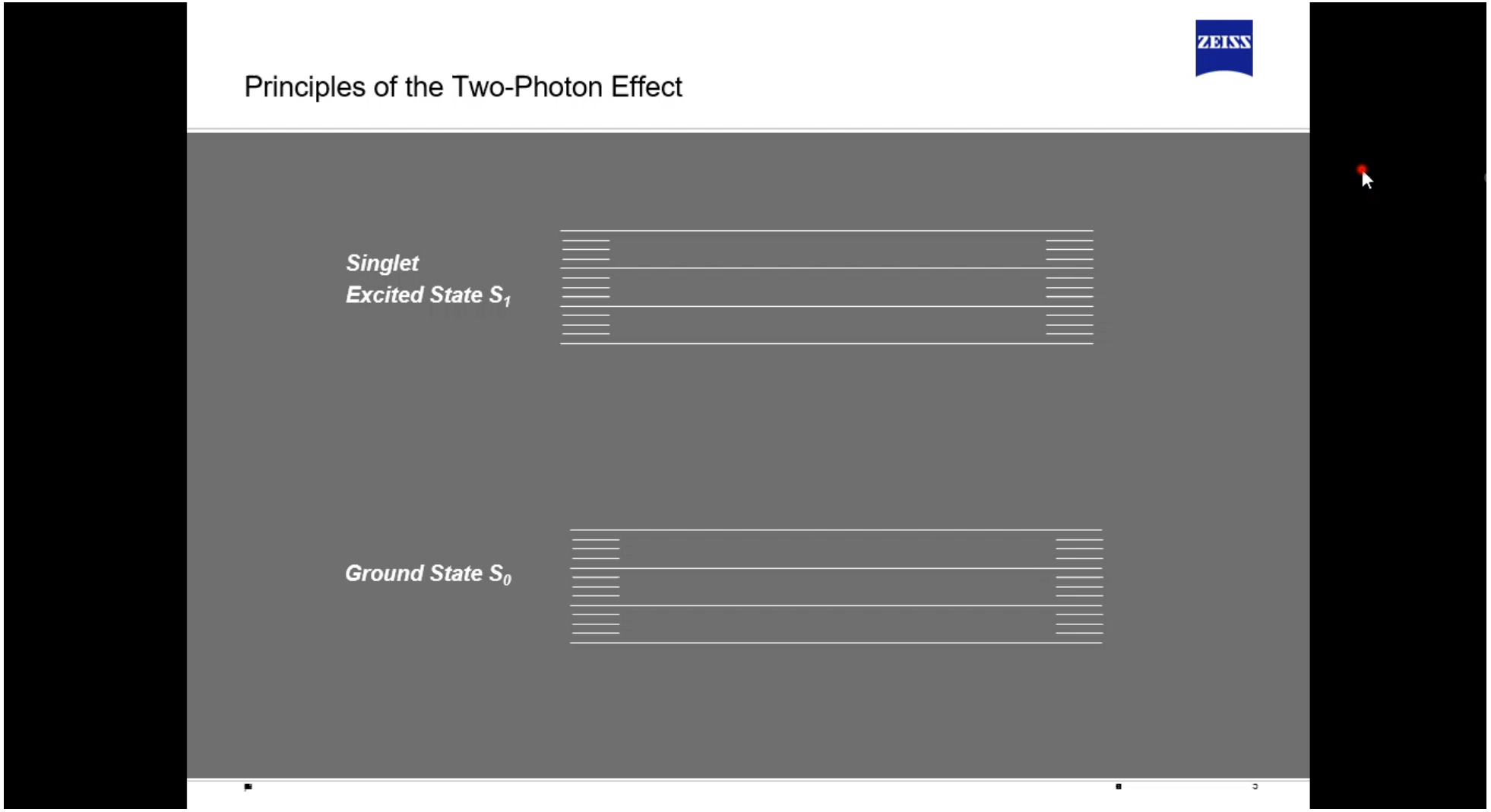
Infrared Photons are less scattered if compared to blue ones. An efficient focus can be created in deep tissue layers.

→ Human and animal tissue exhibit an optical window for infrared light.

→ This window is used to create a multi-photon excitation focus up to 700 µm deep in neuronal tissue.

长波长光穿透能力更强

为什么需要双光子


Principles of the Two-Photon Effect
Singlet
Excited State S_1
Ground State S_0

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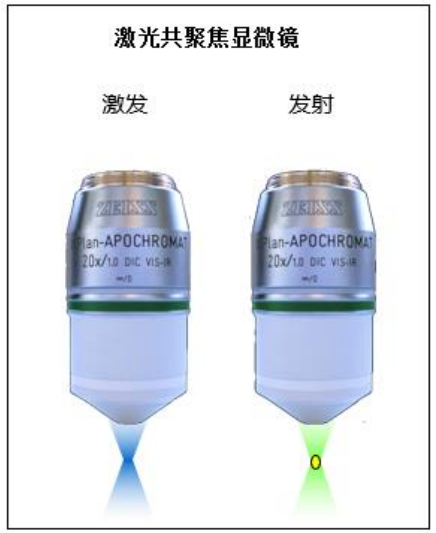
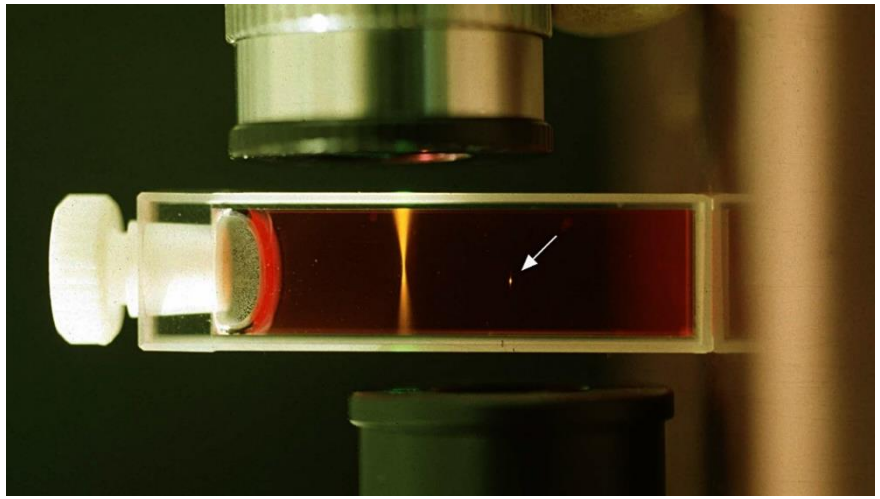
双光子优势

- 利用长波长激发样品，穿透能力强；
- 只有焦平面的荧光分子被激发，不需要共聚焦的针孔去掉非焦平面的信息；





- 光损伤小；
- 高分辨率、高信噪比

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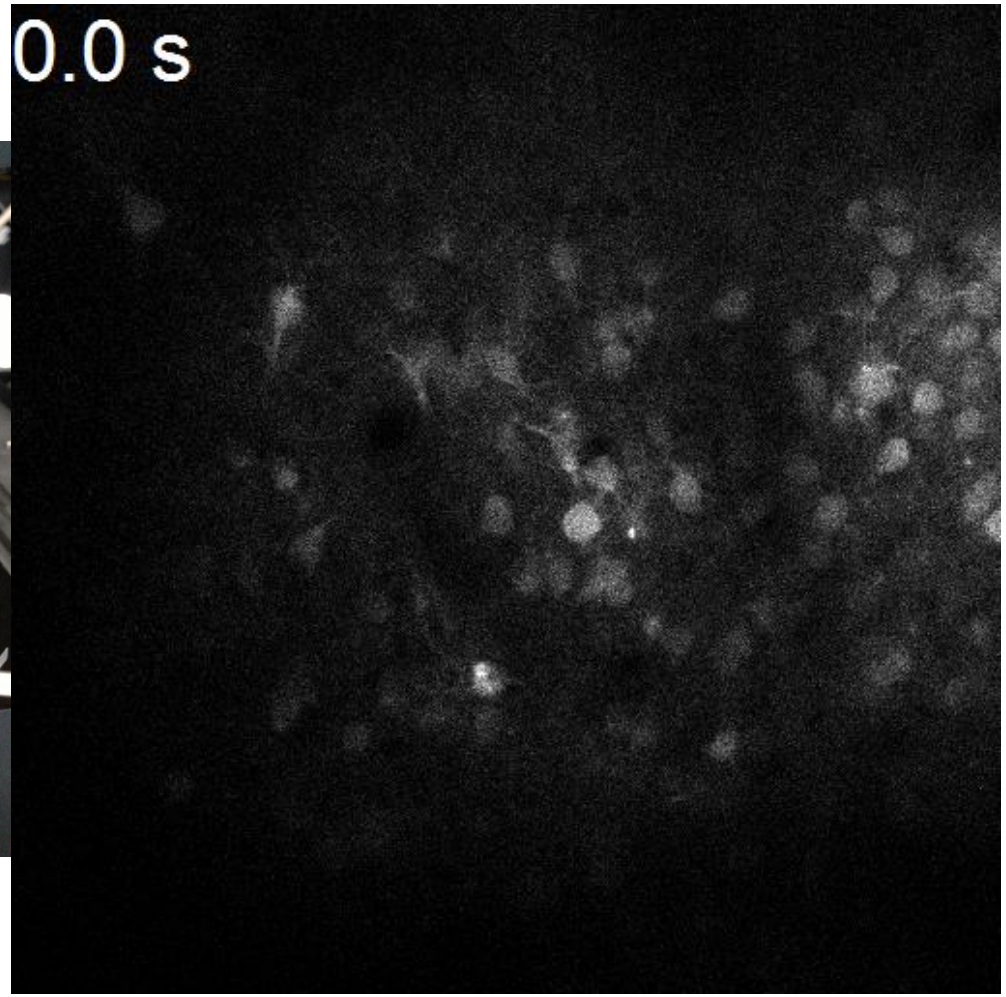
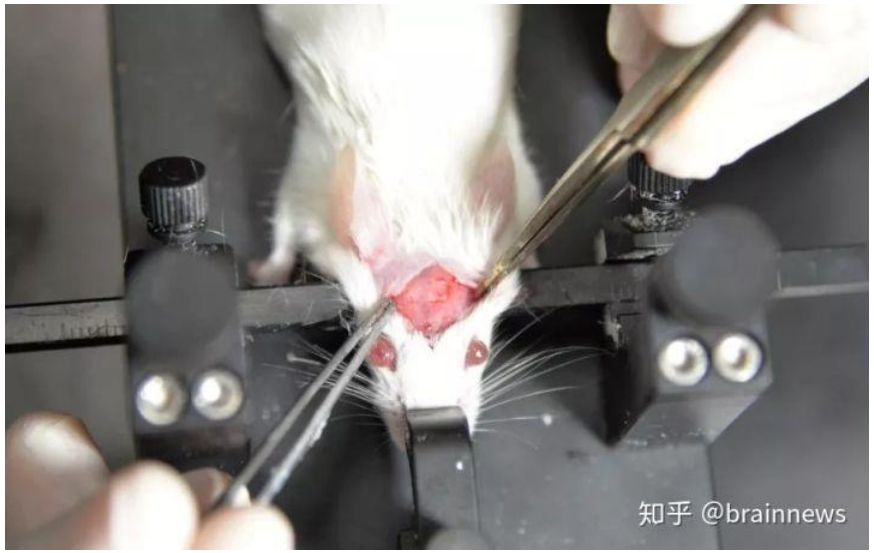
双光子在活体成像中的应用





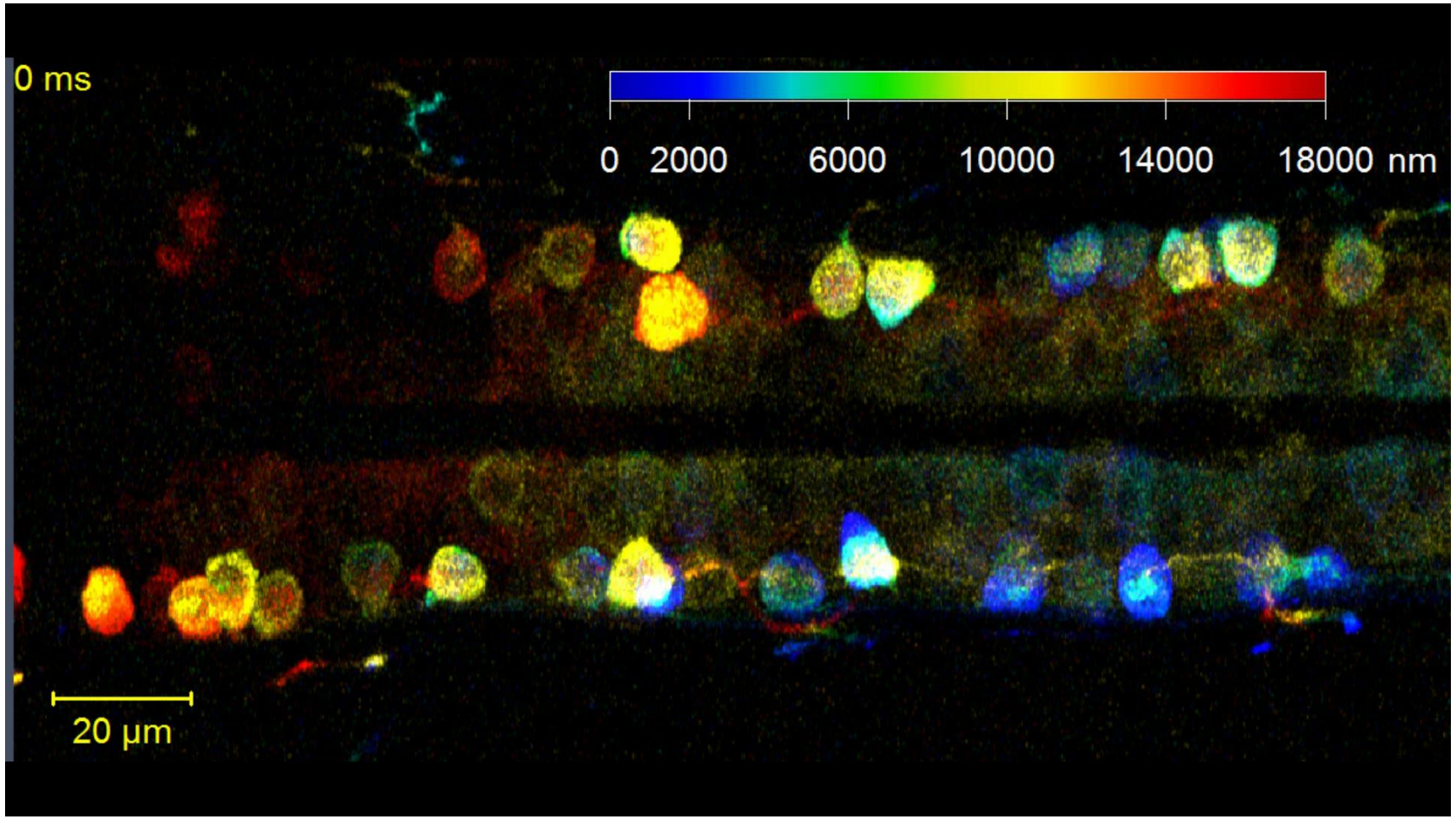
小鼠运动区神经元，GCaMP3 标记

20x 1.0 W Plan-Apo

Dr. Wake , NIPS, Matsuzaki Lab

微信链接-活体小鼠成像之背部篇
https://mp.weixin.qq.com/s/wf1vgwf5DMNvfkXirwZ1BA

双光子在活体成像中的应用



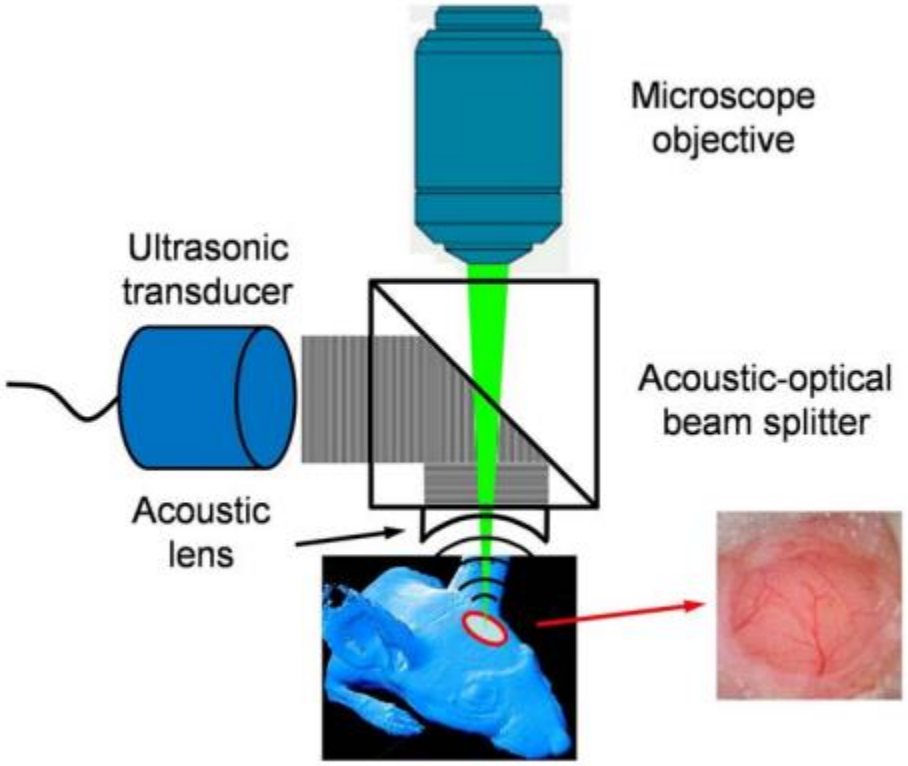
如图，GCaMP5标记斑马鱼，可以看到随着时间钙信号的快速变化。

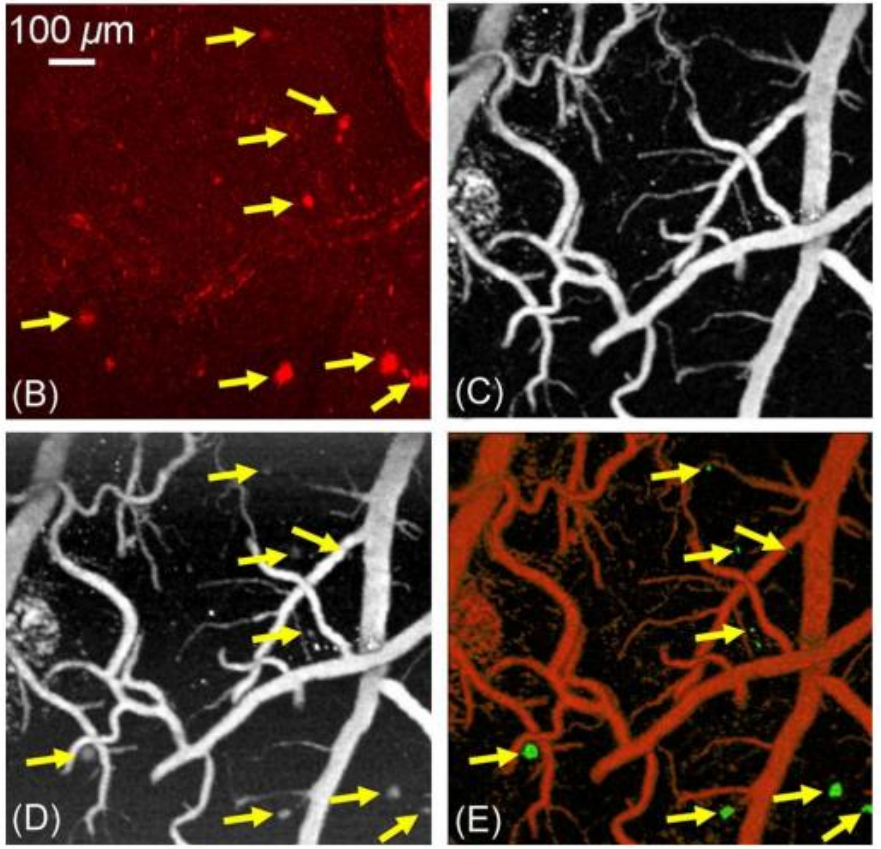
斑马鱼 GCaMP5标记 @ 920 nm Excitation
40X 1.0 W Plan Apochromat
Sample Courtesy of Drew Friedmann, University of California Berkeley

双光子显微镜对转基因小鼠模型中的淀粉样斑块进行活体成像

Resource

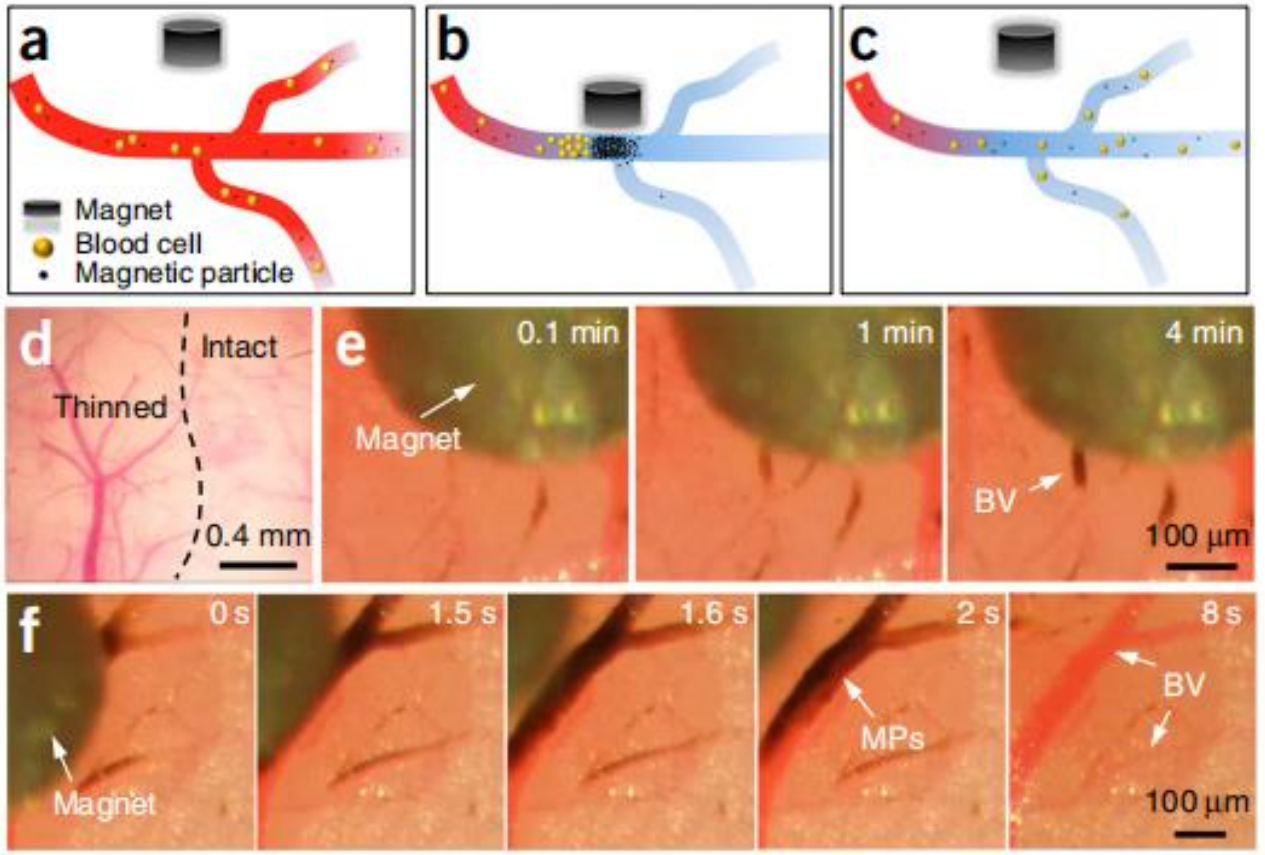
Intravital imaging of amyloid plaques in a transgenic mouse model using optical-resolution photoacoustic microscopy，OPTICS LETTERS, *2009*





双光子活体人脑脑片成像

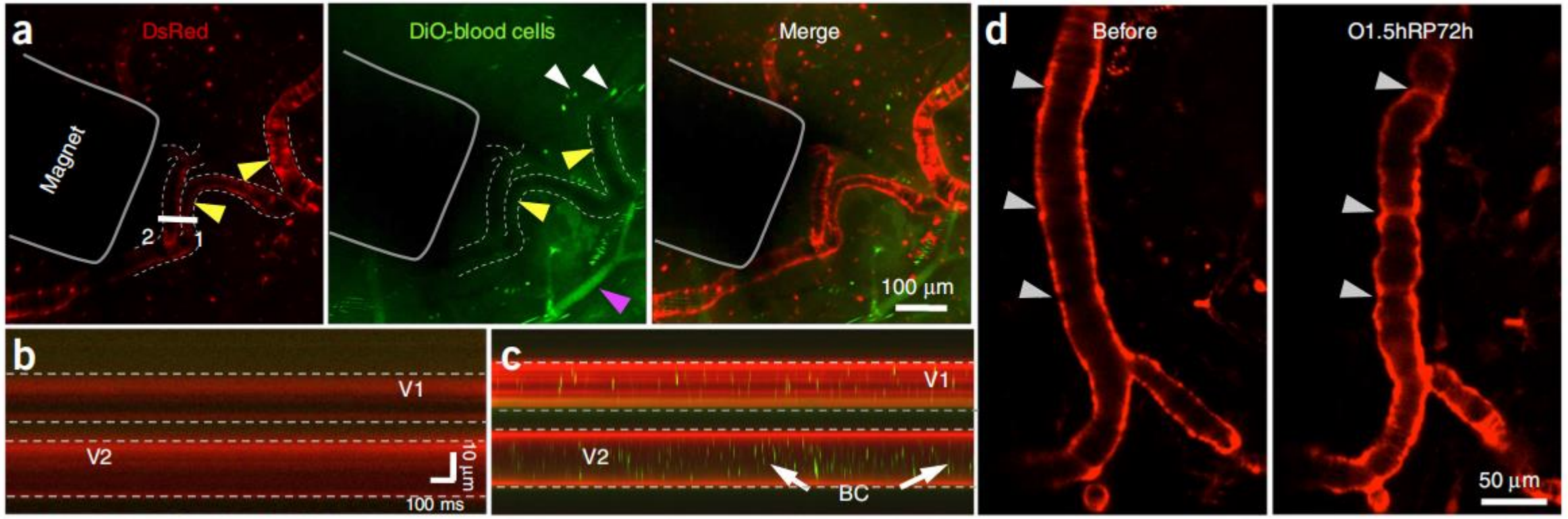
Resource	Control of cerebral ischemia with magnetic nanoparticles，nature methods, *2016*



双光子观察磁性纳米粒子控制脑缺血

Resource

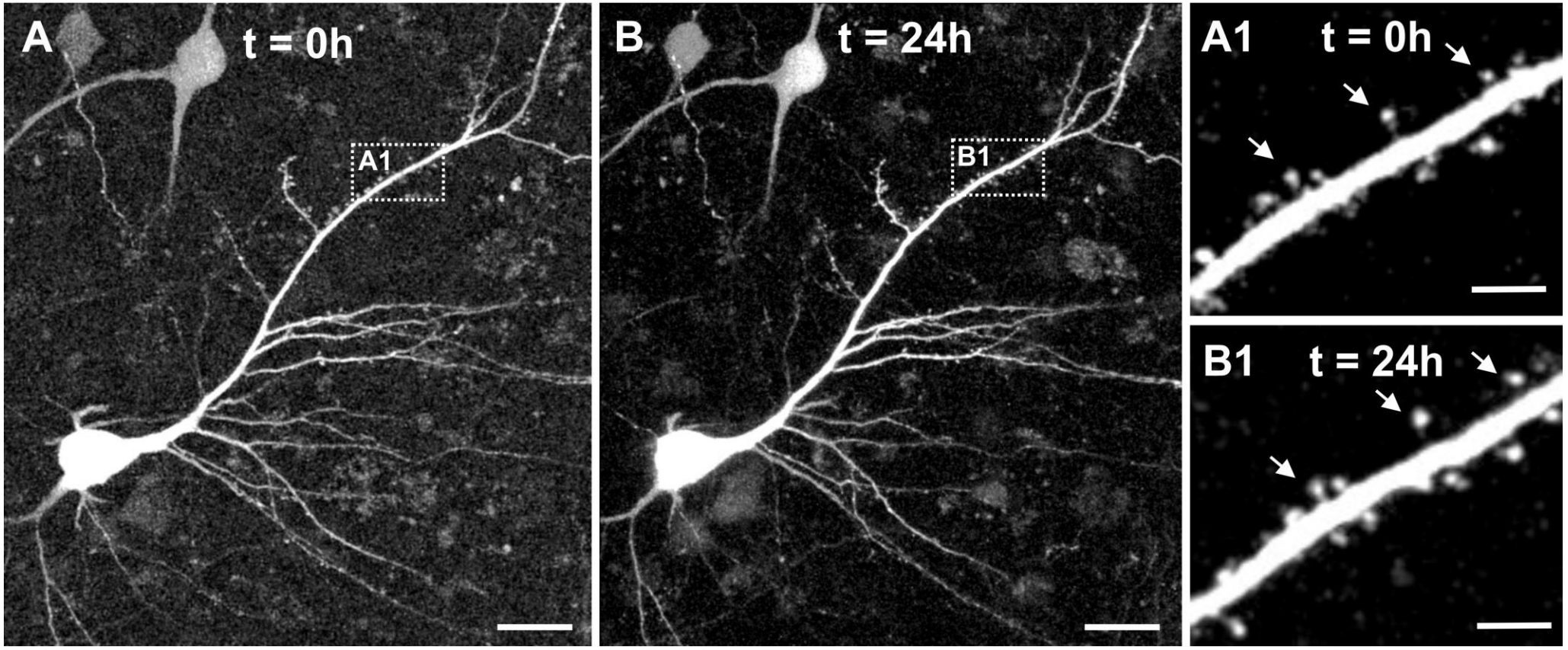
Control of cerebral ischemia with magnetic nanoparticles，nature methods, *2016*



双光子活体人脑脑片成像

Resource

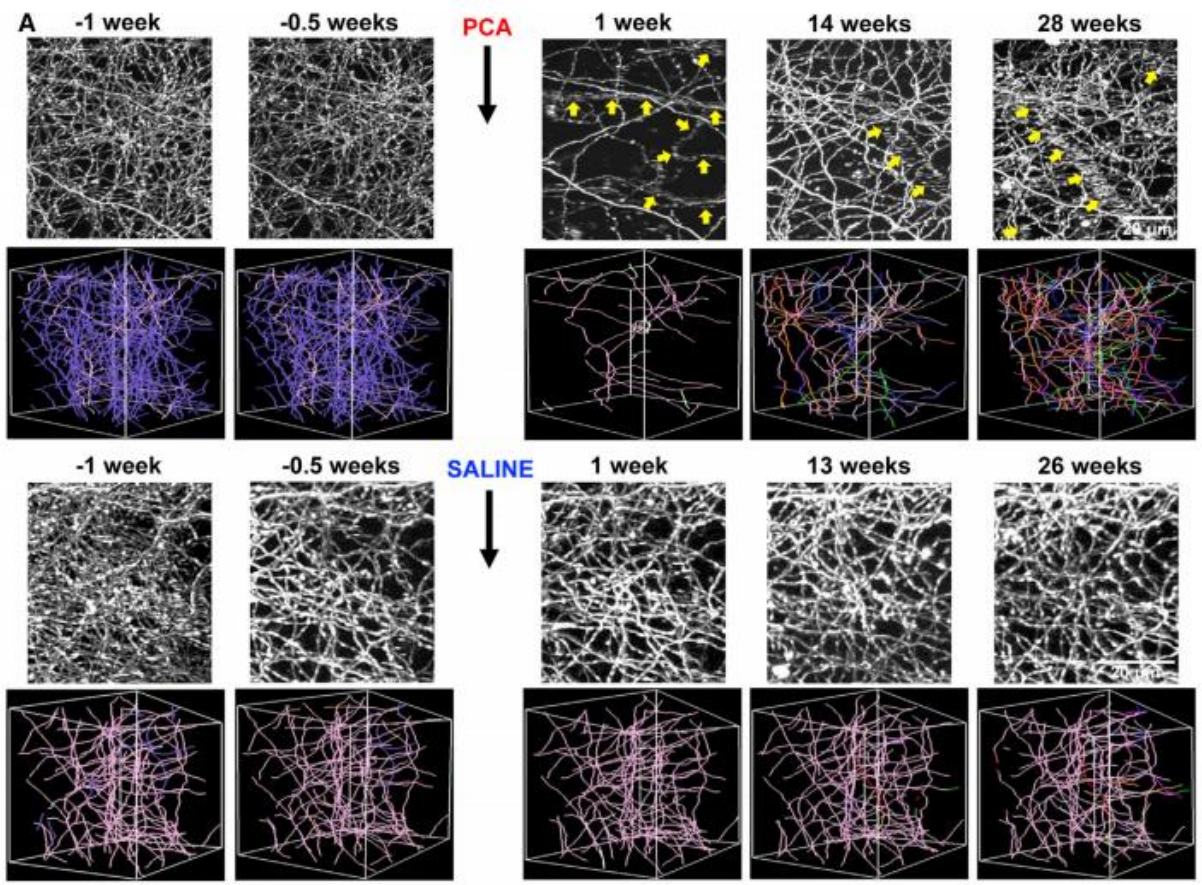
Long-term adult human brain slice cultures as a model system to study human CNS circuitry and disease，eLIFE, 2019



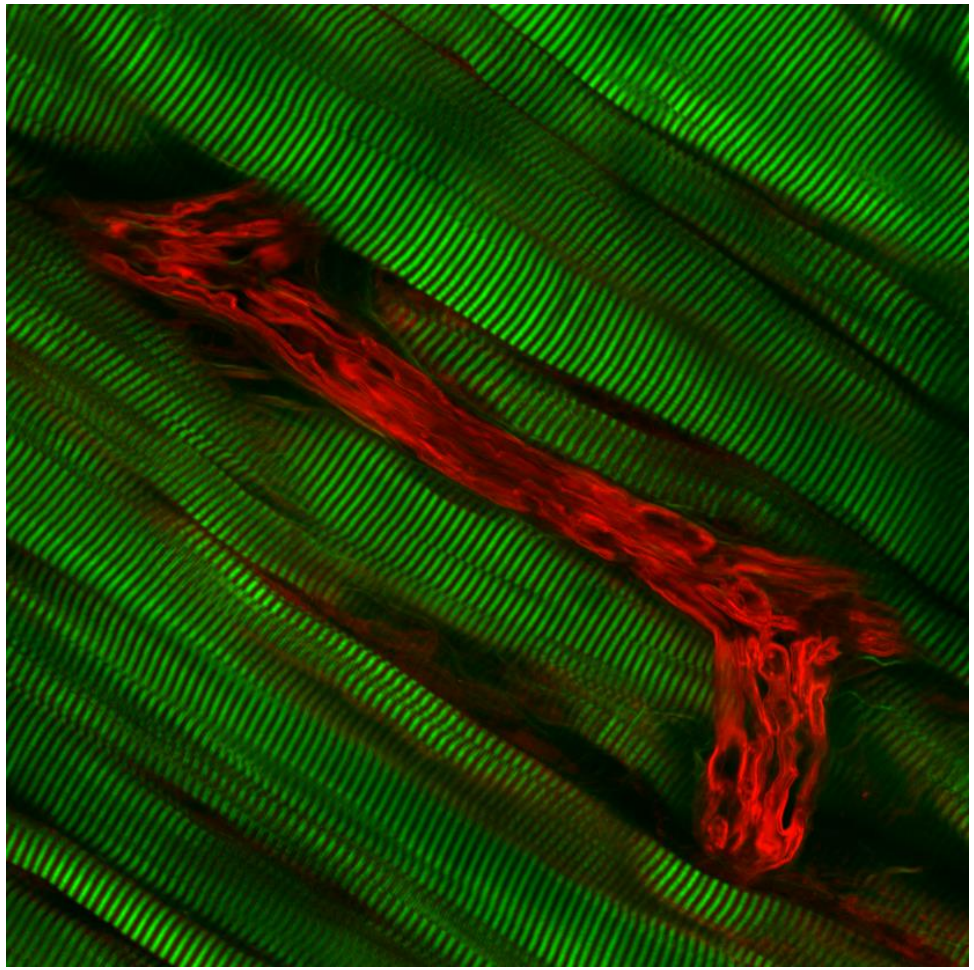
双光子观察损伤后成年小鼠大脑中5-羟色胺轴突的再生

Resource

Regrowth of Serotonin Axons in the Adult Mouse Brain Following Injury ，Neuron*, 2016*



双光子的应用



二次/三次谐波成像

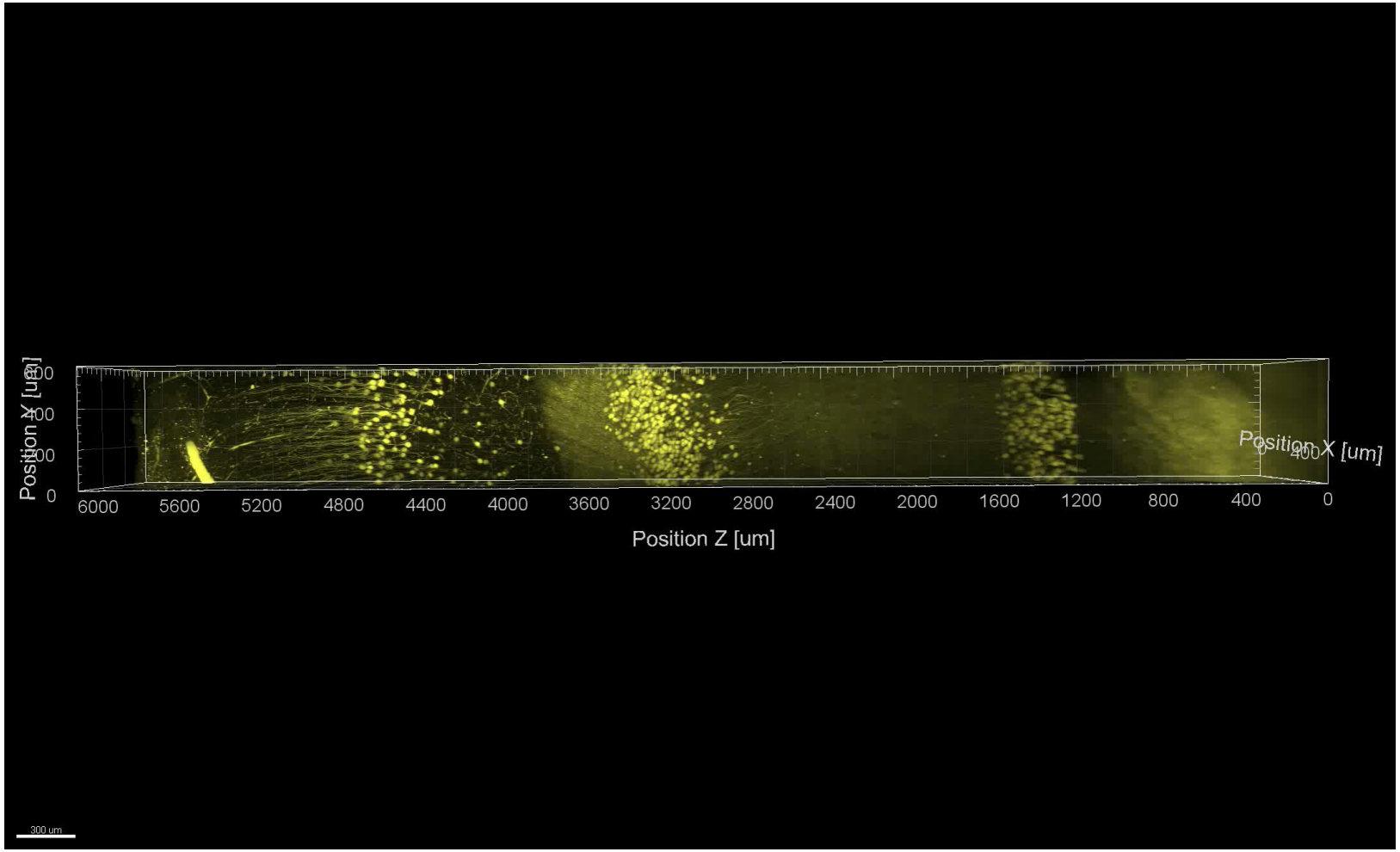
二次/三次谐波即 利用特定波长激发无染色的特殊样品，获得激发光1/2或1/3波长的发射光。

小鼠提睾肌

绿色:横纹肌二次谐波信号；红色: 神经髓鞘三次谐波信号

S. Dietzel, LMU München, Germany

双光子的应用



透明化样品

如图，透明化小鼠脑成像，成像深度达到5.6mm。

YFP标记透明化小鼠脑 @ 920 nm Excitation
20x Scale lens
Sample and data set: Hiroshi Hama, Fumiyoshi Ishidate, Atsushi Miyawaki, RIKEN BSI, Wako, Japan

Suitable dyes for Multi-Photon

Conventional dyes have a broad excitation spectrum when excited with pulsed NIR lasers. The excitation spectra of different dyes may largely overlap.

This table provides an overview of the excitation spectra obtained with different dyes. The data are based on empirical measurements; they may slightly vary with specimen treatment. (See also: Xu, et al. (1996), PNAS, 93, 10763)

Fluorochrome	Absorption	Emission
Alexa Fluor 350	720-800	440
Alexa Fluor 488	720-800	515
Alexa Fluor 546	720-840	569
Alexa Fluor 568	720-840	596
Alexa Fluor 594	720-850	610
Alexa Fluor 633	720-900	647
AMCA	780-800	444
bis-MSB	680-750	420
Bodipy	900-950	512
Calcium Crimson	900	615
Calcium green	780-850	531
Cascade Blue	750-800	420
Coumarin 307	780-800	530
CY2	780-800	506
CY3	780	565, 615
CY5	780-820	670
Dansyl Hydrazine	700-750	440
DAPI, Hoechst	700-820	455, 478
DiA	800-860	580
DID	780-820	670
DiO	780-830	510
eCFP	800-900	476
eGFP	820-950	509
eYFP	860-950	532
Fluorescein	780 - 820	519
Indo-1 free	690-720	490
Indo-1 Ca2+	690-720	400
Lucifer Yellow	860-890	533
Mito Tracker red	750-840	600
Nile Red	810	640
Oregon Green	780-860	526
Propidium Iodide (PI)	820-850	617
Rhodamin B	800-840	600
Rhodamine 123	780-860	550
Sytox Green	740-760 or 880-940	524
TRITC	800-840	572

We make it visible.

常见双光子染料的激发及发射波长。

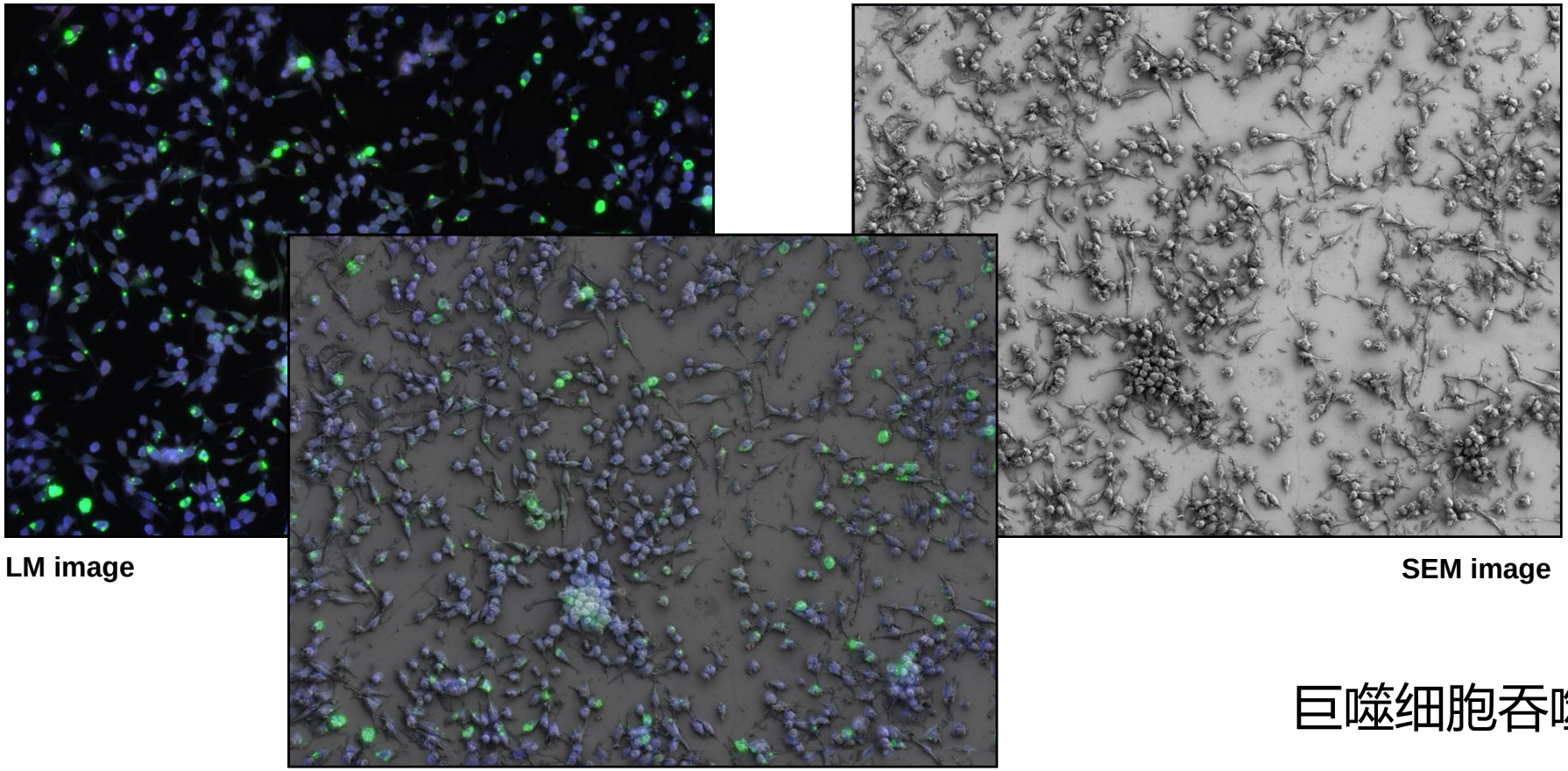
Future

最新成像技术
光电联用

Stereo LM | Sub-micron XRM | Widefield LM | ConfocalLM | Super resolution LM | Nanoscale XRM | C-SEM | FE-SEM | FIB-SEM | Helium Ion Microscope

1 µm | 700 nm | 250 nm | 200 nm | 20 nm | < 50 nm | < 2 nm | < 1 nm | < 1 nm | < 0.5 nm

最新成像技术
光电联用



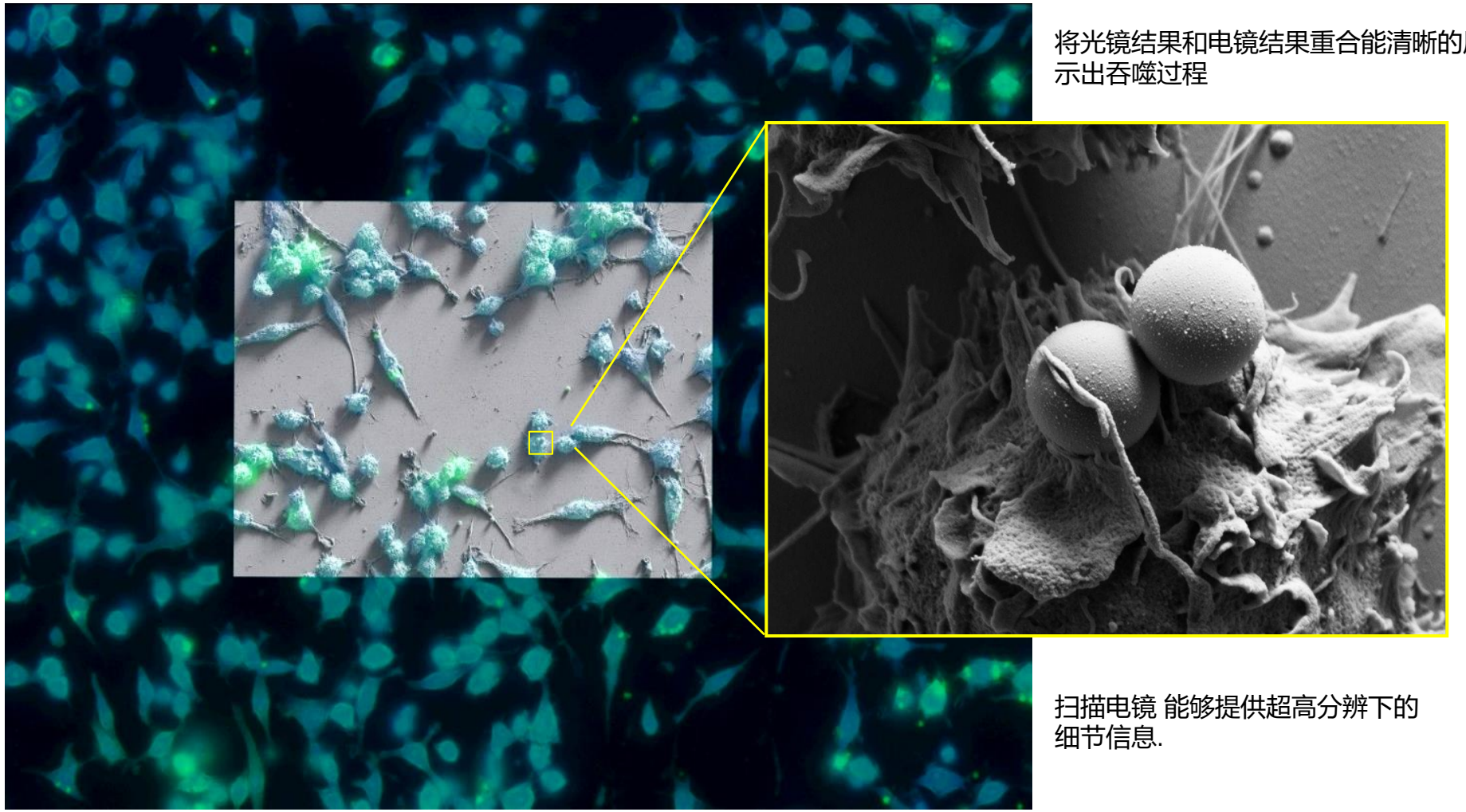
LM image

SEM image

Correlated LM + SEM image

巨噬细胞吞噬荧光小球

最新成像技术
光电联用

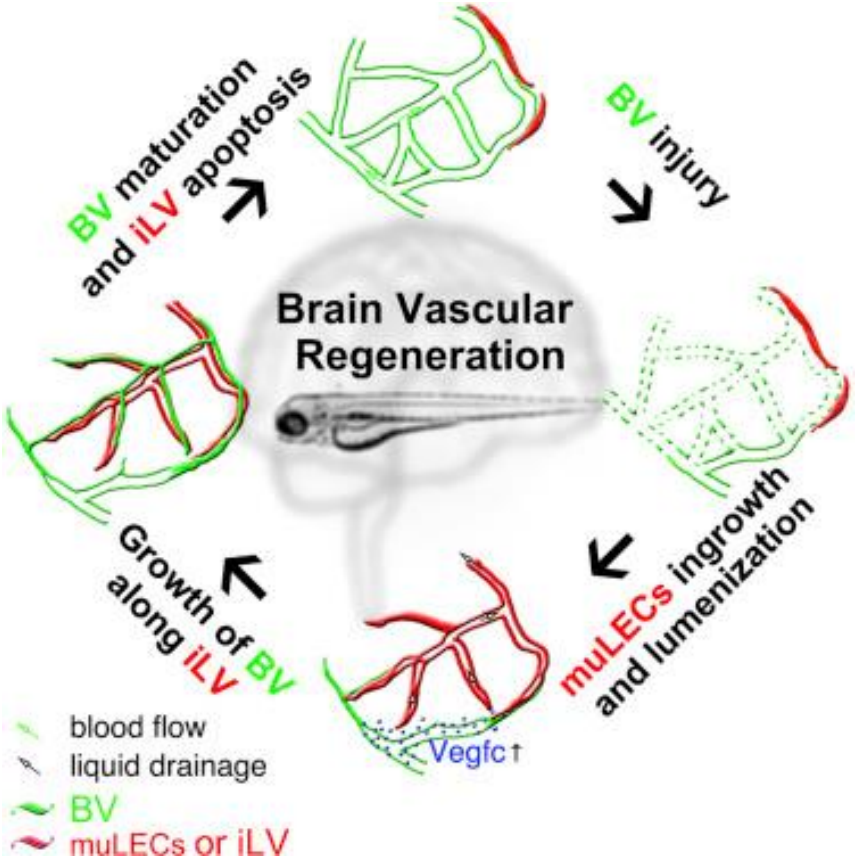


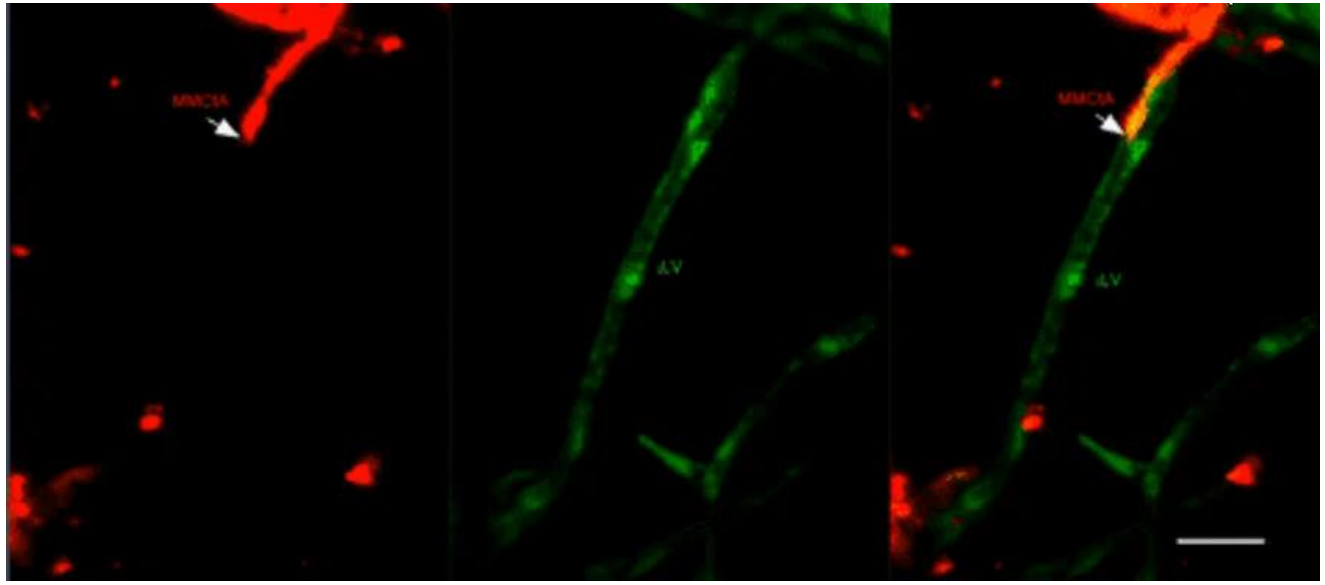
将光镜结果和电镜结果重合能清晰的展示出吞噬过程

扫描电镜 能够提供超高分辨下的细节信息.

最新成像技术
光电联用

Resource	Cerebrovascular Injuries Induce Lymphatic Invasion into Brain Parenchyma to Guide Vascular Regeneration in Zebrafish，Developmental Cell, *2019*





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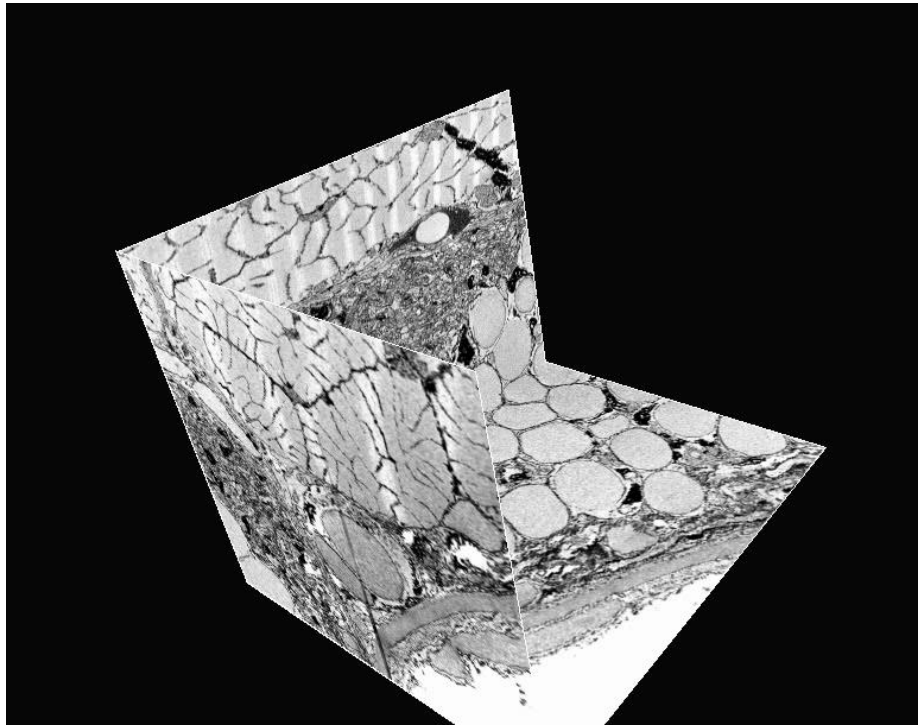
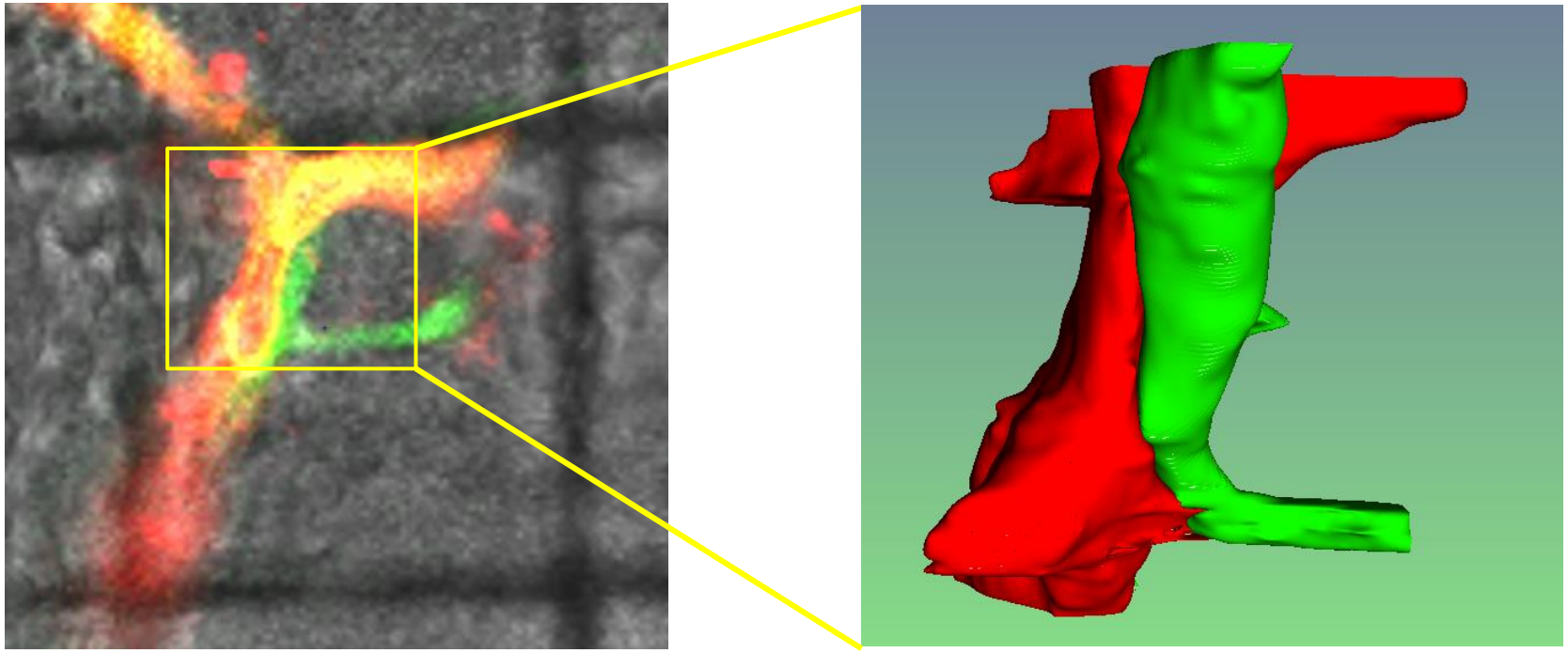


最新成像技术
光电联用



www.biomart.cn/infosupply/15185822.htm

Seeing beyond