

ZEISS Lightsheet Z.1

大视野多角度光片显微成像技术



Carl Zeiss Microscope
Application Specialist
Dr. Lu Xi

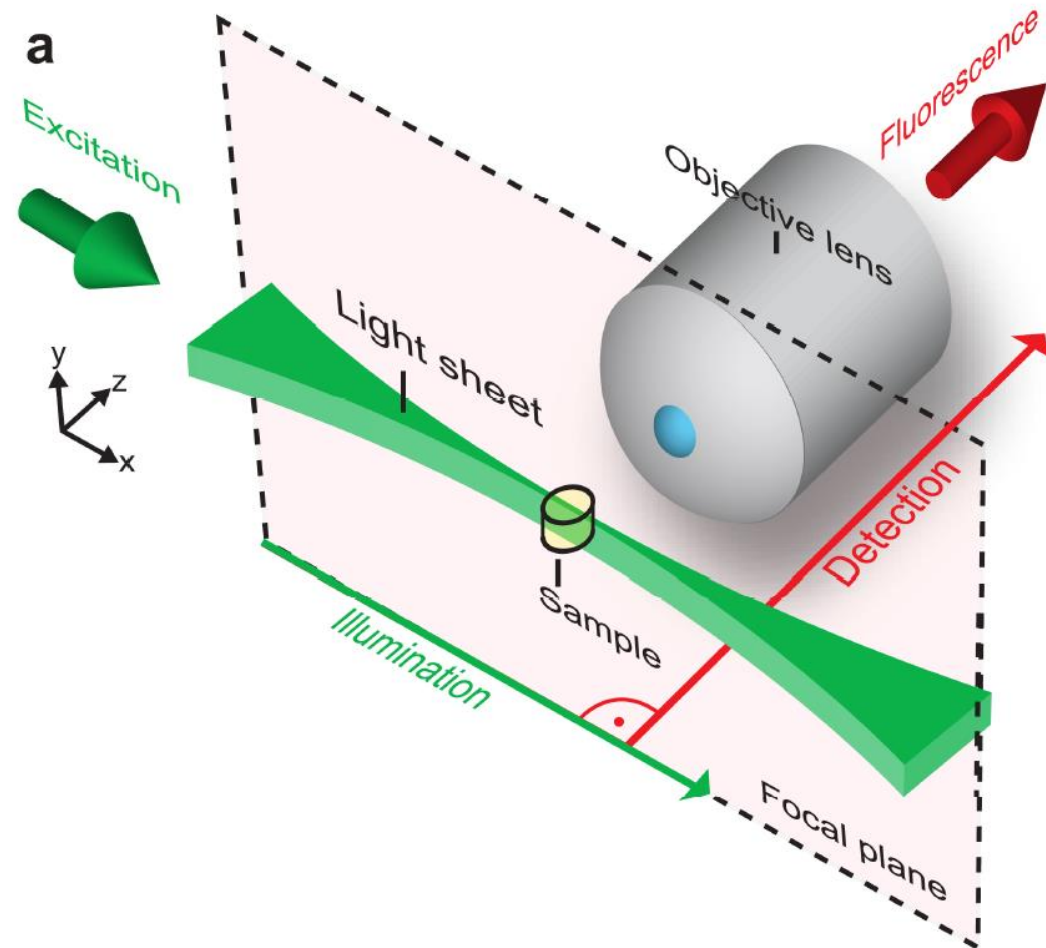


Lightsheet Z.1

Zeiss Lightsheet Z.1 Microscope

激光片层扫描显微镜



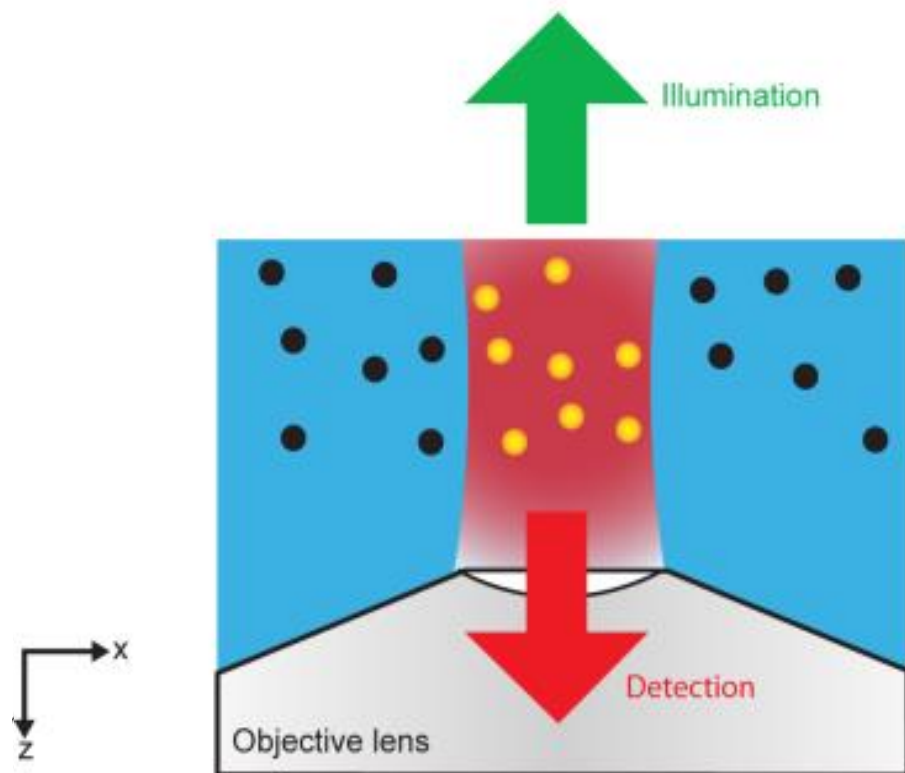


Jörg Ritter, Dissertation, Universität Bonn (2011)

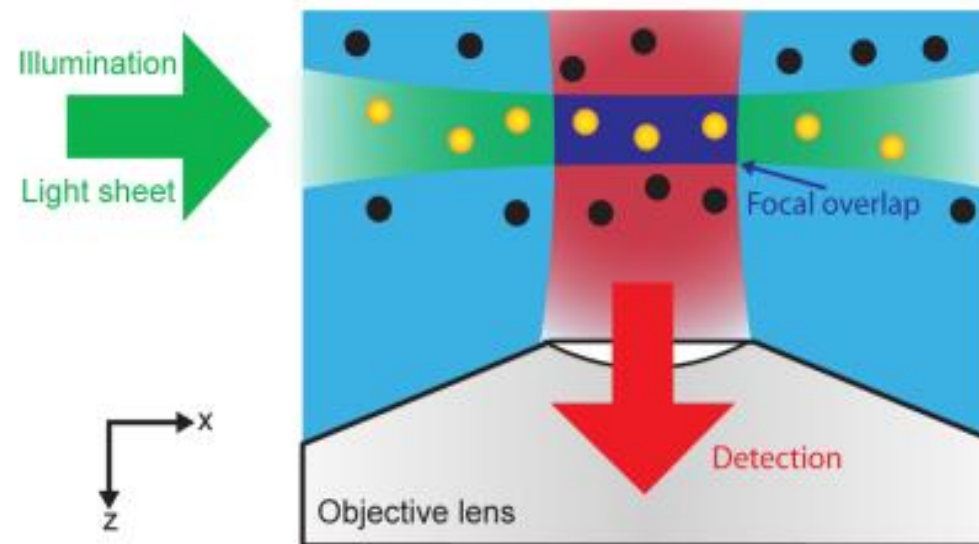
普通显微镜同向照明 vs Lightsheet垂直照明



普通显微镜

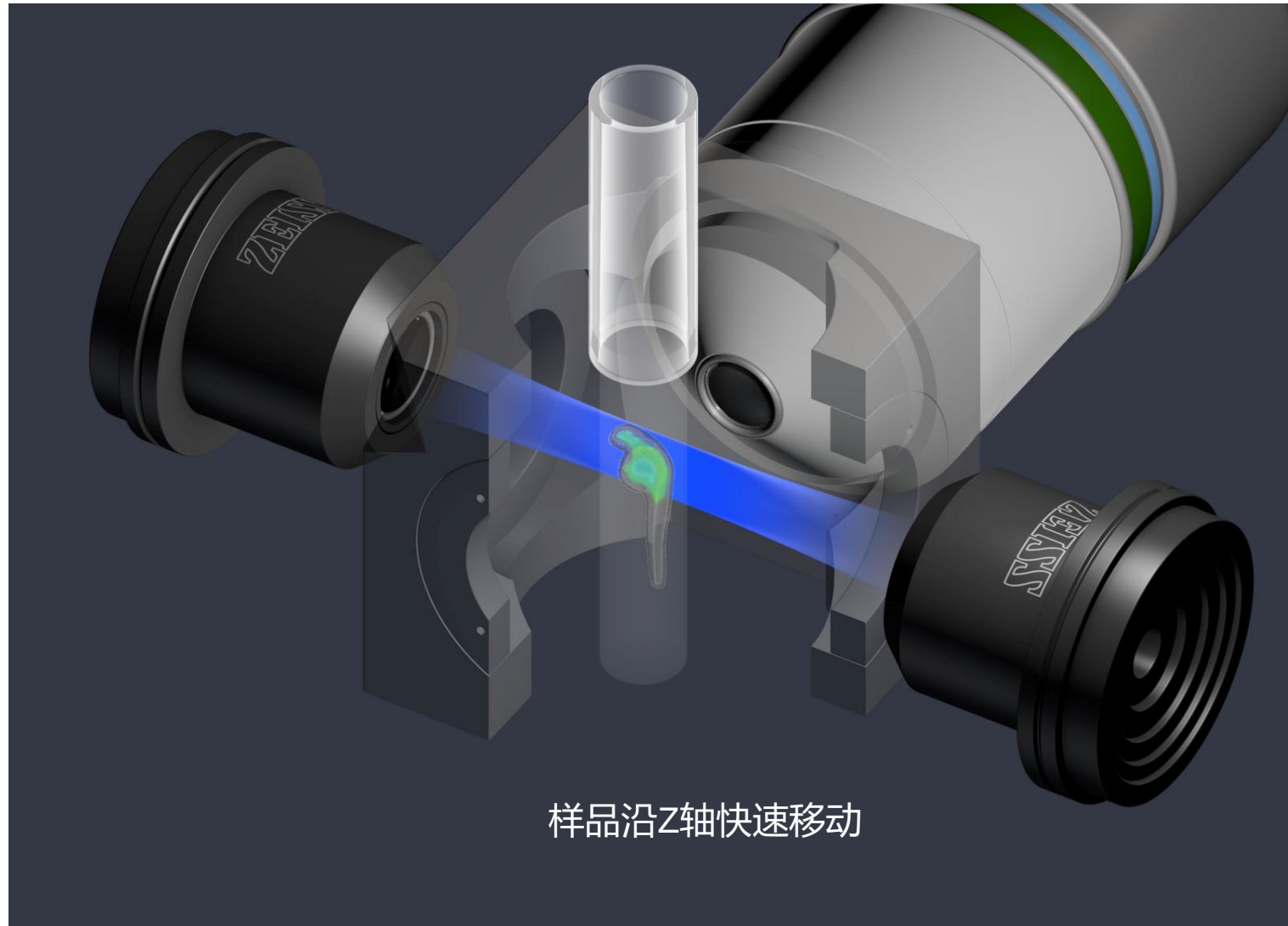


Lightsheet



Lightsheet激发光为光片，自带光切技能，不激发非焦平面的荧光

快速的Z轴移动实现高速3D成像

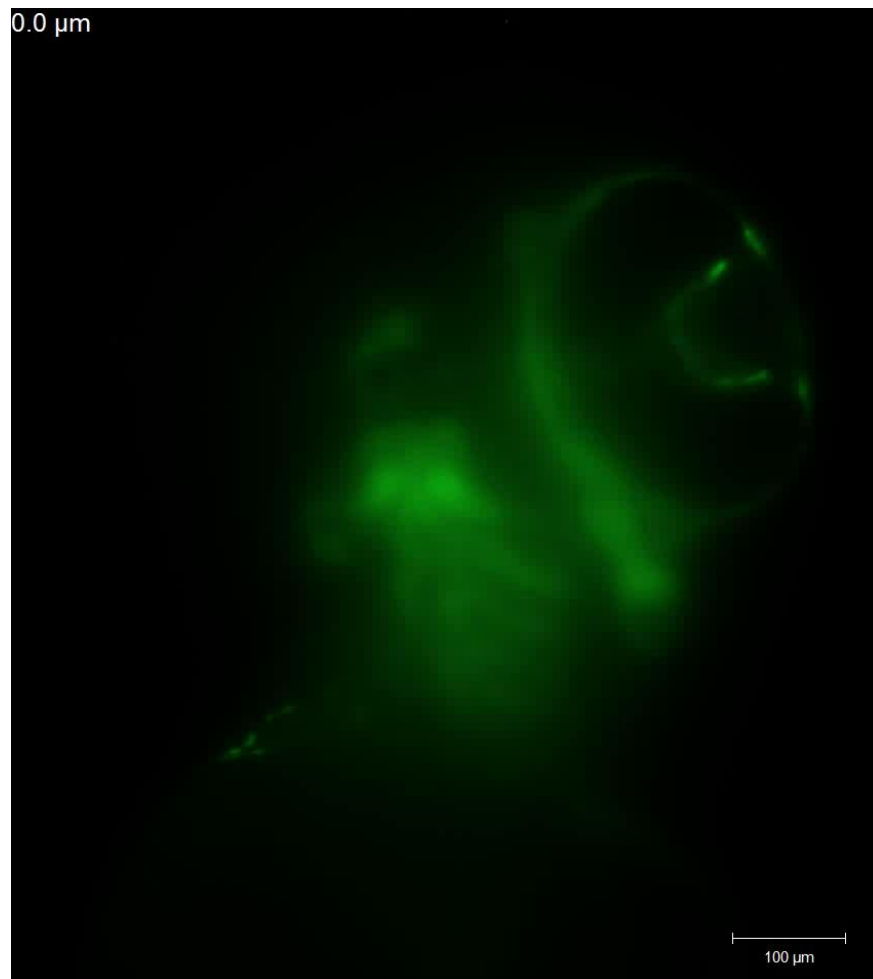


样品沿Z轴快速移动

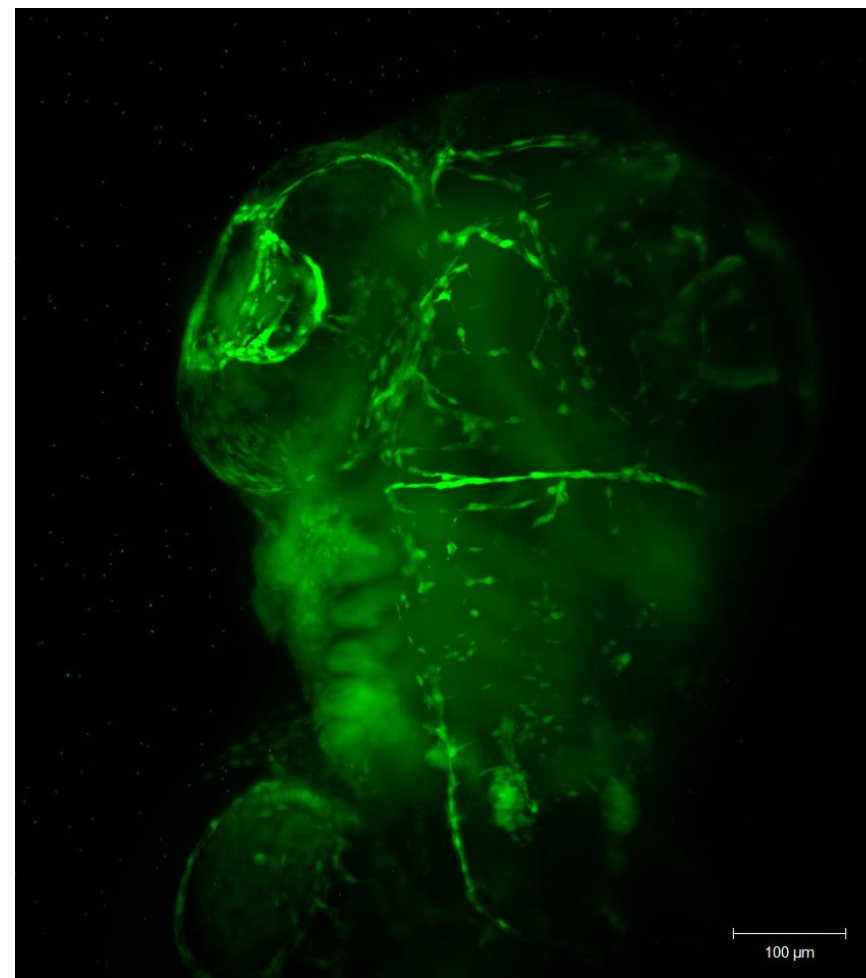
Lightsheet Z.1快速的Z轴运动实现高速3D成像



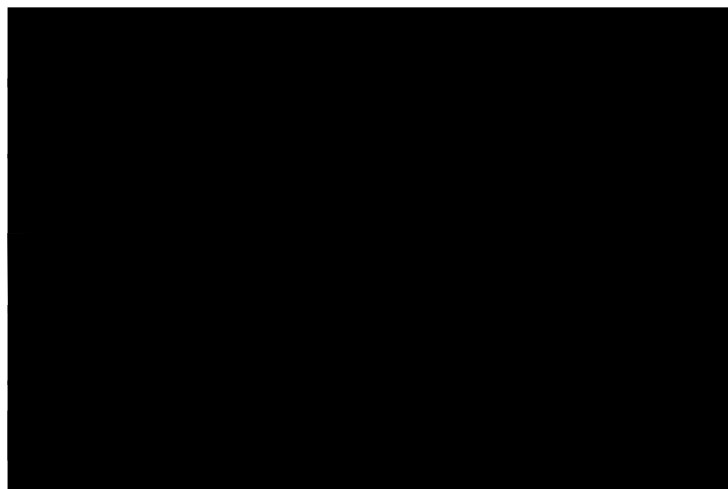
Z轴连续拍摄 (30 fps)



Z- Stack 最大强度投影

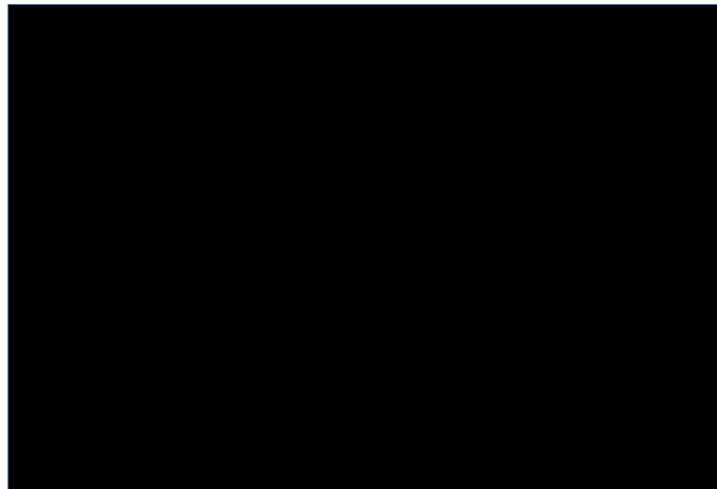


点扫描 (共聚焦, 双光子)



PMT采集图像, 逐点扫描

Lightsheet



sCMOS采集图像, 整张拍摄

扫描速度:

66fps @640x640

57fps @960x960

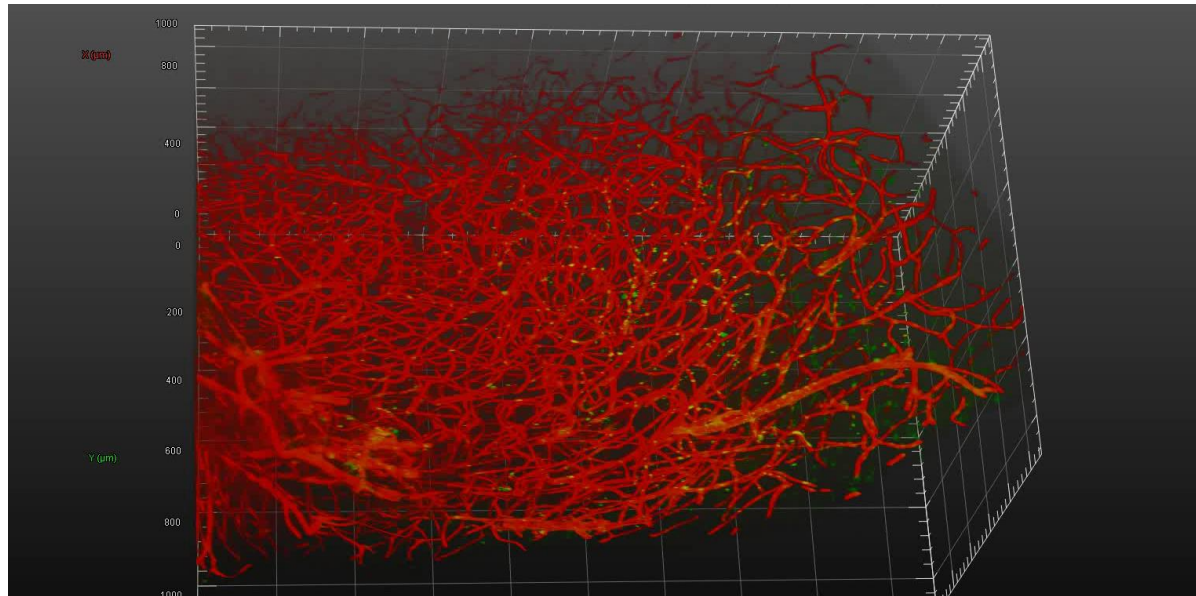
30fps @1000x1000

20fps @1920x1920

大体积活体或固定样品的3D成像

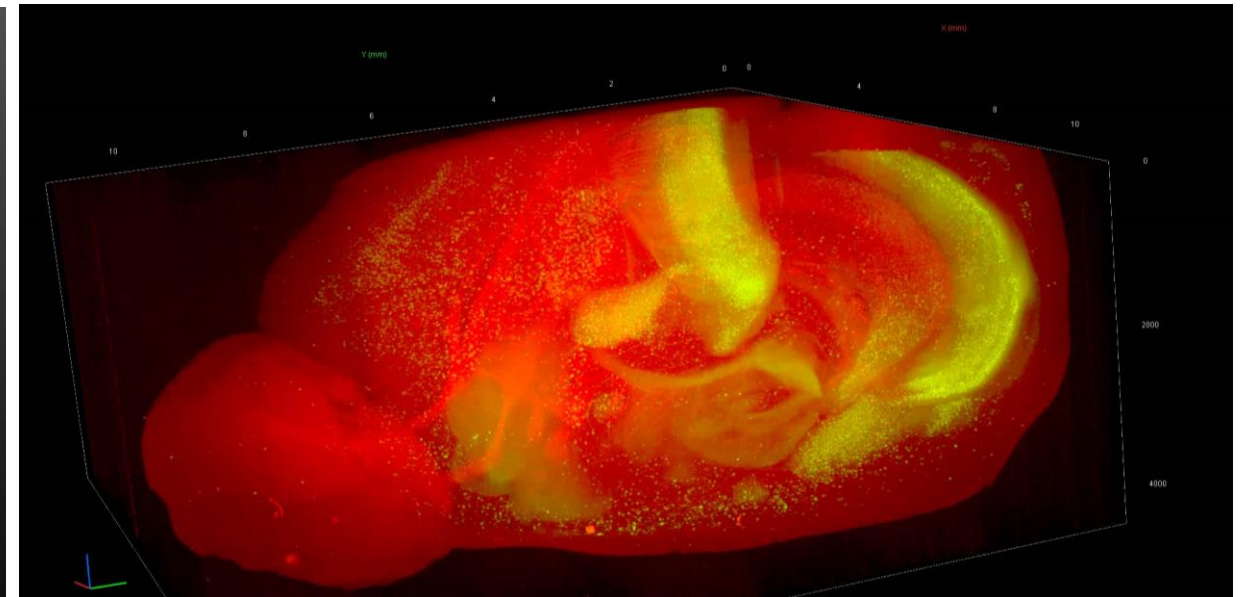


小鼠脑血管 (CLARITY)



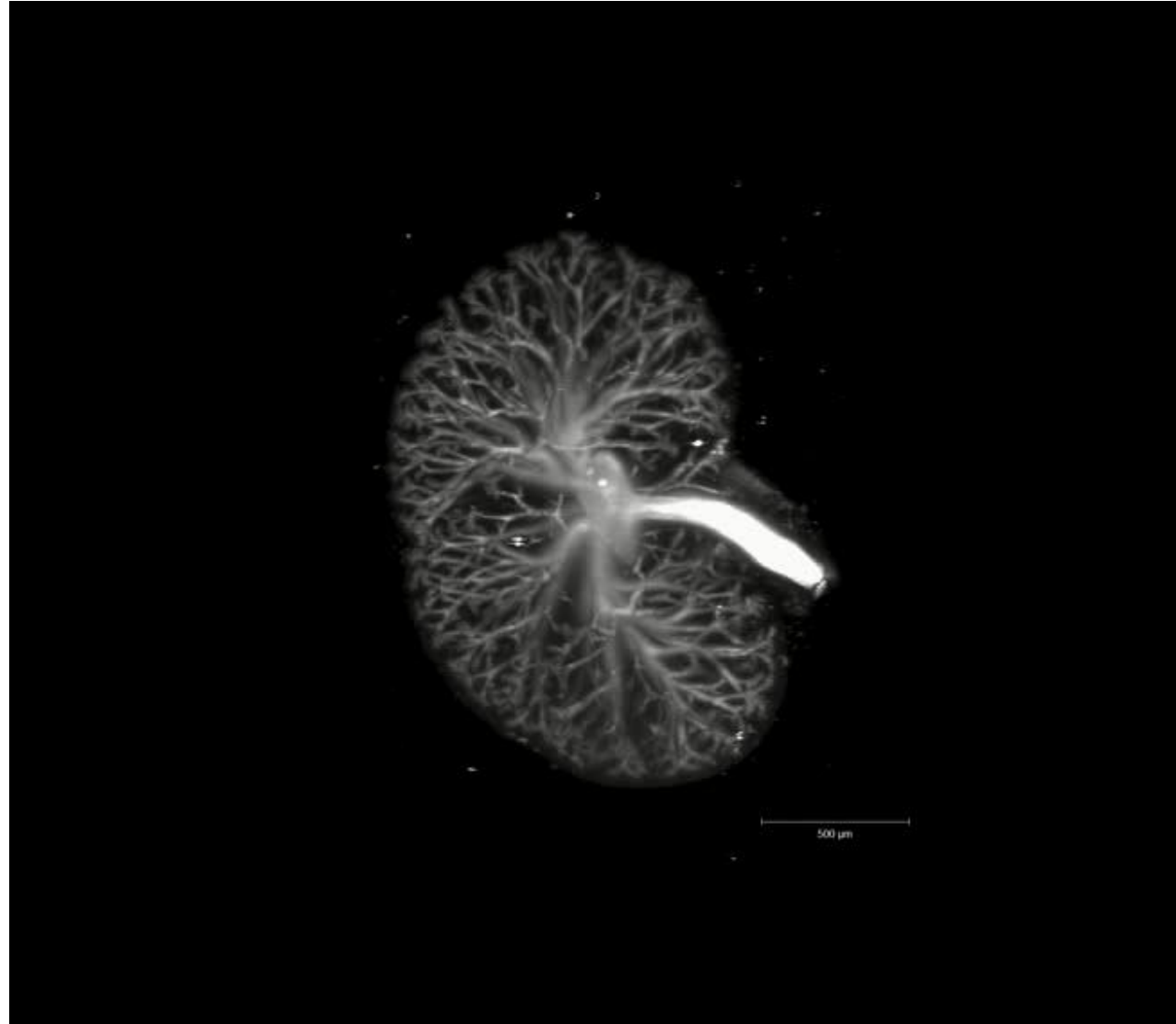
Jean-Marie Vanderwinden, Université Libre de Bruxelles, ULB Neuroscience Institute - Neurophysiology Lab

小鼠大脑神经元 (LUMOS)



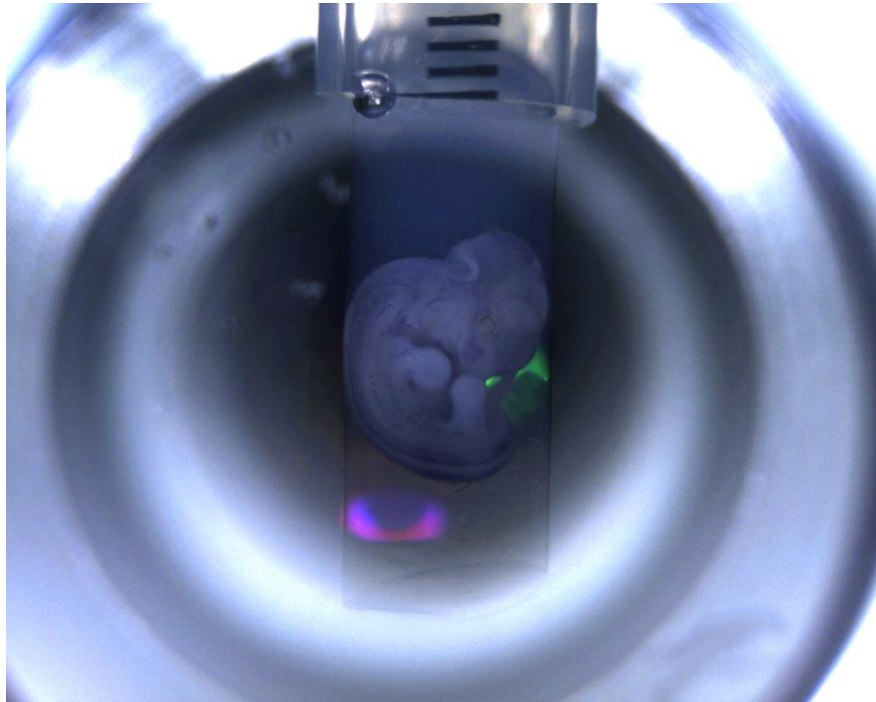
Data and Clearing Method by O.Efimova National Research Center "Kurchatov Institute", Moscow, Russia

Mouse Kidney 小鼠肾脏

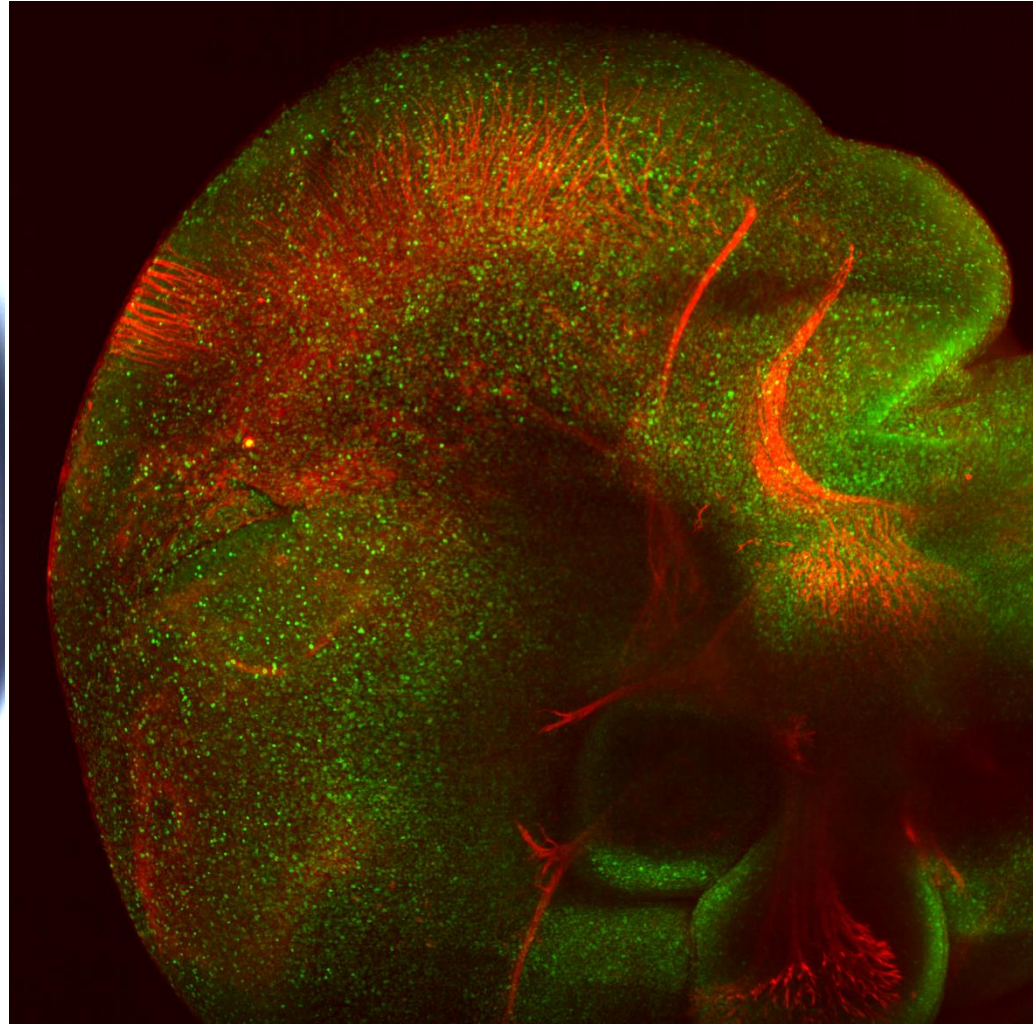


Sample and Data by Renata-Prunskate

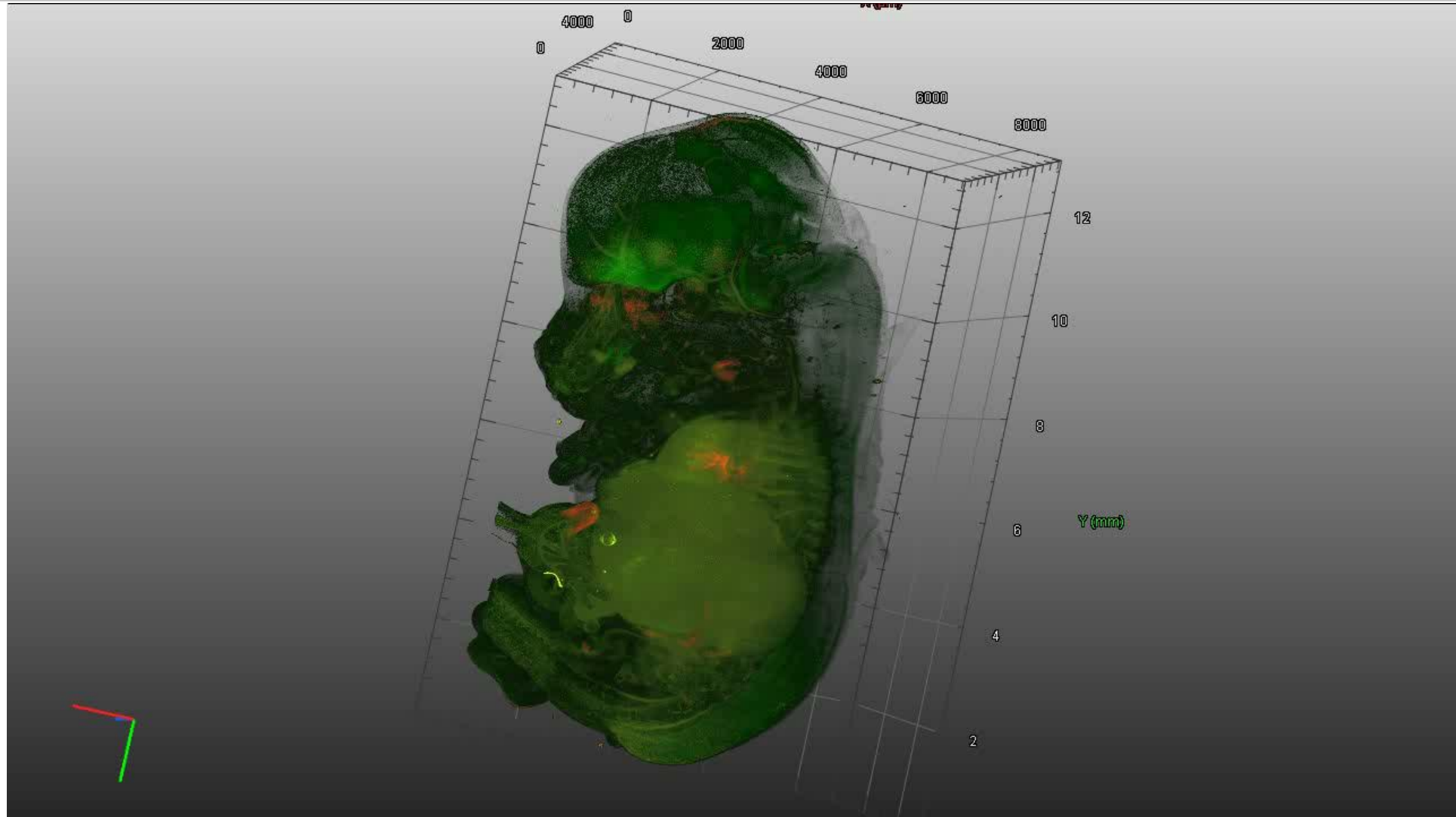
小鼠胚胎



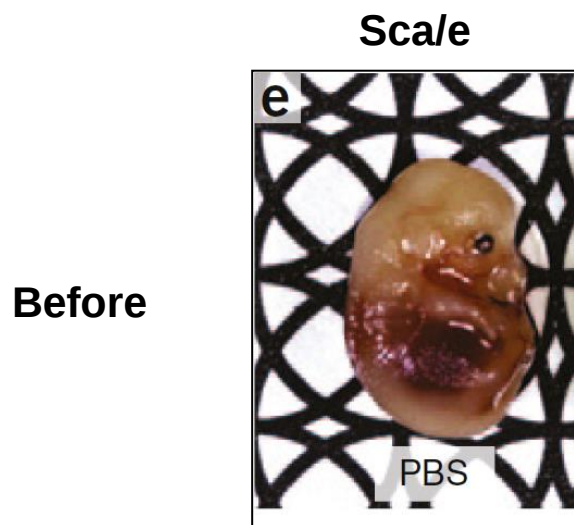
Data by Zeiss, germany



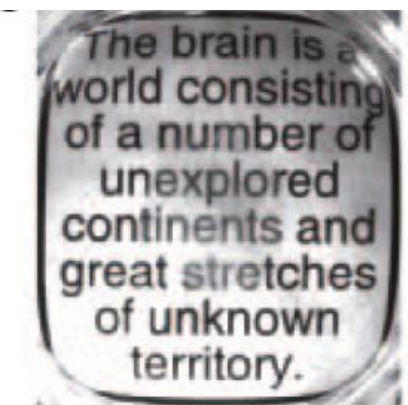
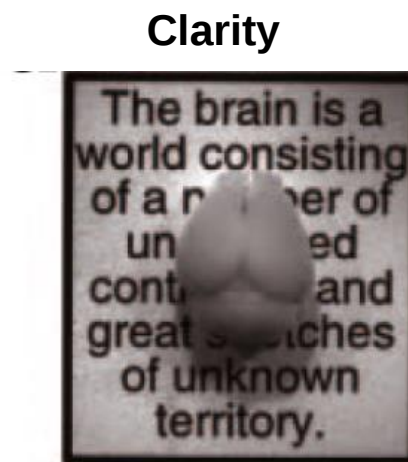
小鼠胚胎



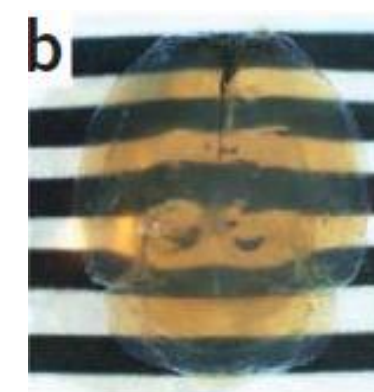
透明化处理：减少样品光散射，实现深层成像



Hama et al, Nat Neuroscience 2011



Chung et al , Nature 2013



Ertürk et al, Nature Methods, 2012

透明化技术：适用于不同的组织器官



Lee et al. *BMC Developmental Biology* 2014 14:781
DOI 10.1186/s12861-014-0048-3



METHODOLOGY ARTICLE

Open Access

Improved application of the electrophoretic tissue clearing technology, CLARITY, to intact solid organs including brain, pancreas, liver, kidney, lung, and intestine

Hyunsu Lee¹, Jae-Hyung Park², Incheol Seo³, Sun-Hyun Park^{2,5*} and Shin Kim^{4*}

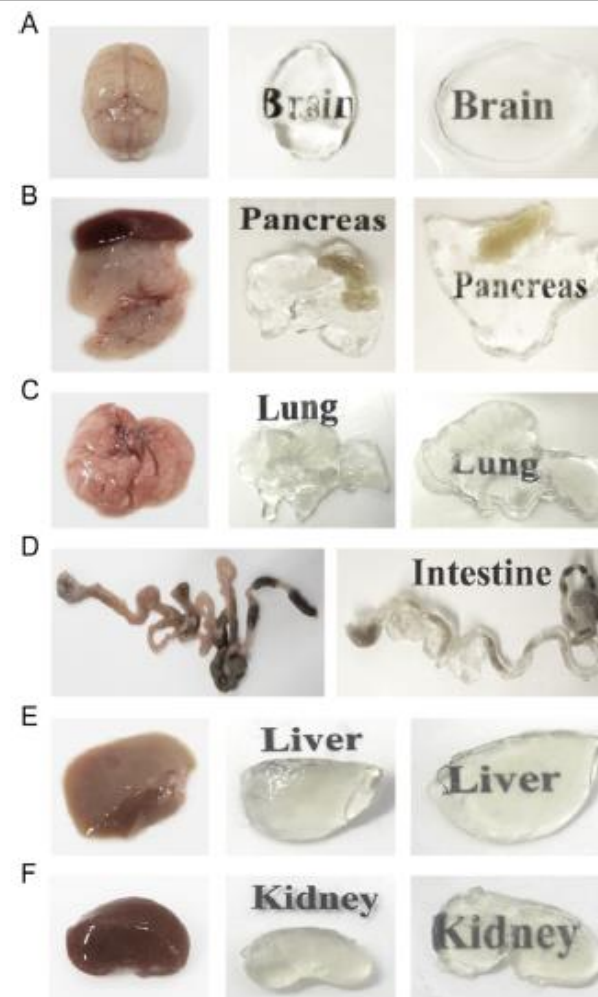
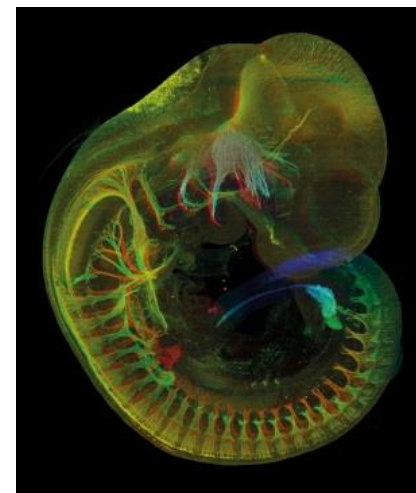
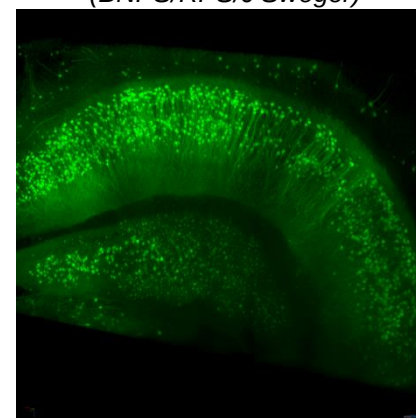


Figure 1 CLARITY of intact adult mouse tissues. In adult mouse tissues (12 weeks old), imaging was performed before and/or after CLARITY. (A) Brain. (B) Pancreas. (C) Lung. (D) Intestine. (E) Liver. (F) Kidney.

- **神经科学:** 果蝇、小鼠、大鼠、猴以及人类的脑组织成像
- **发育生物学:** 斑马鱼、爪蟾以及小鼠胚胎组织或器官的成像
- **药理学及医疗领域:** 组织检查、组织、器官以及3D培养细胞(> 100 μm)的成像



(BNPS/RPS/J Swoger)



O.Efimova National Research Center
"Kurchatov Institute", Moscow, Russia

基于有机溶剂的透明化方法

基于有机溶剂的透明化方法

BABB

PegaSOS

3Disco

vDisco

iDisco

FDisco

uDisco

BABB

α -羟基甲苯 (BA) & 安息香酸苯甲酯 (BB)

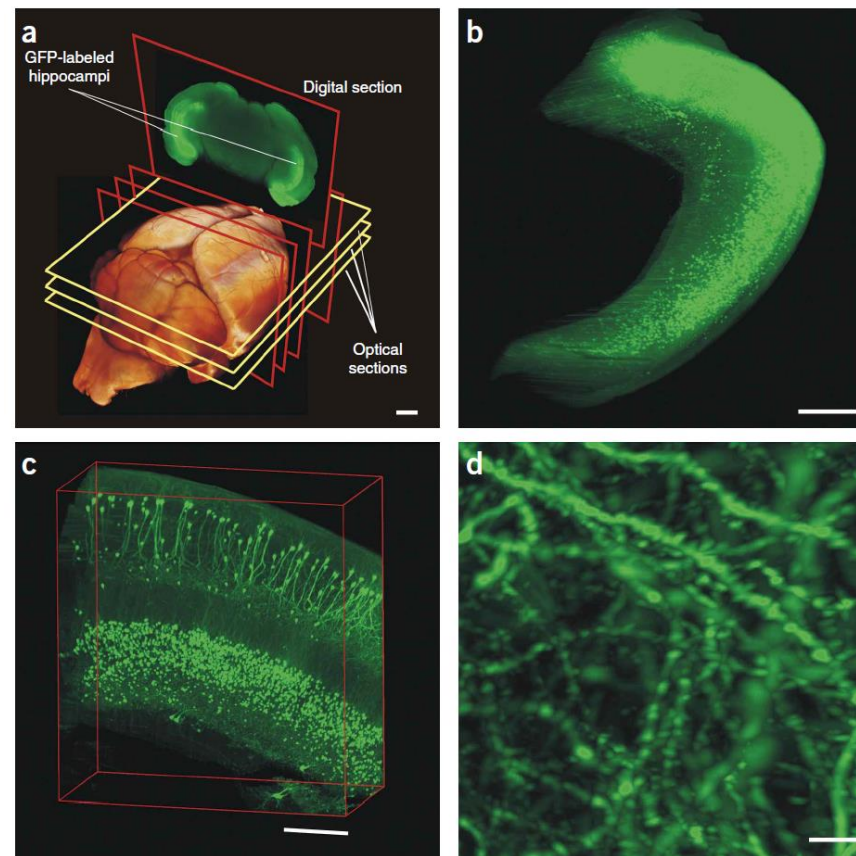


➤ 最早的透明化方法之一

- 1914年 Werner Spalteholz 用 BB 进行透明
- 2007年 Hans – Ulrich Dodt 用 BABB 对鼠脑、鼠胚胎、果蝇等进行透明化

➤ 步骤:

- 梯度甲醇脱水
 - BABB中孵育至组织透明
- ✓ 适用样品广泛
 - ✓ 透明化非常快速
 - ✓ 便宜
- ❑ 透明剂在很短时间内淬灭荧光蛋白
 - ❑ 很难透明有髓鞘的组织
 - ❑ **有毒试剂并且易挥发**
 - ❑ 使组织发生收缩和畸变



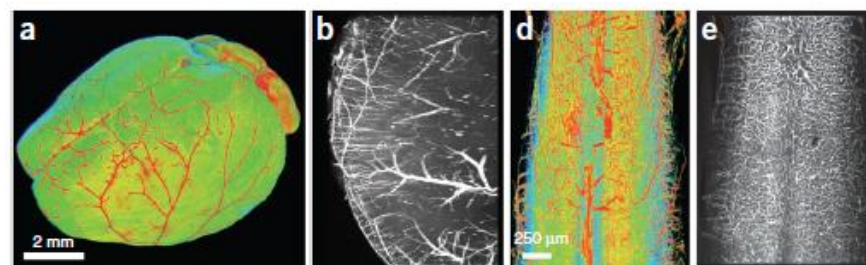
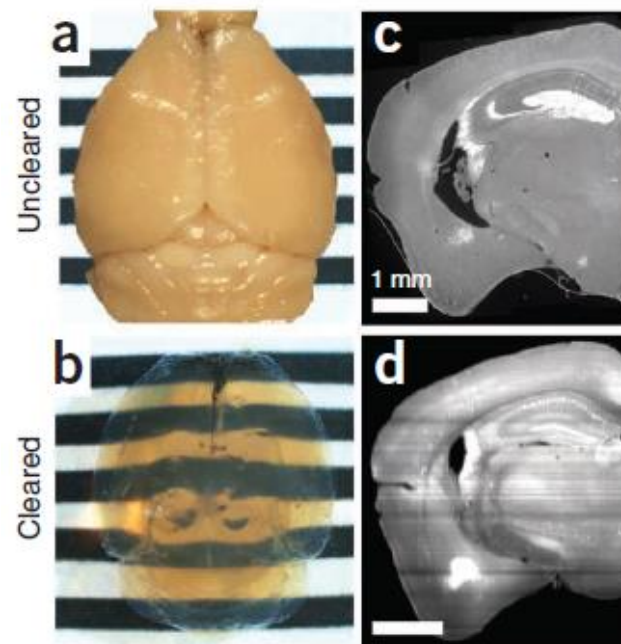
Hans – Ulrich Dodt, et, al. *Nature Method.* 2007

3DISCO

有机溶剂的三维成像



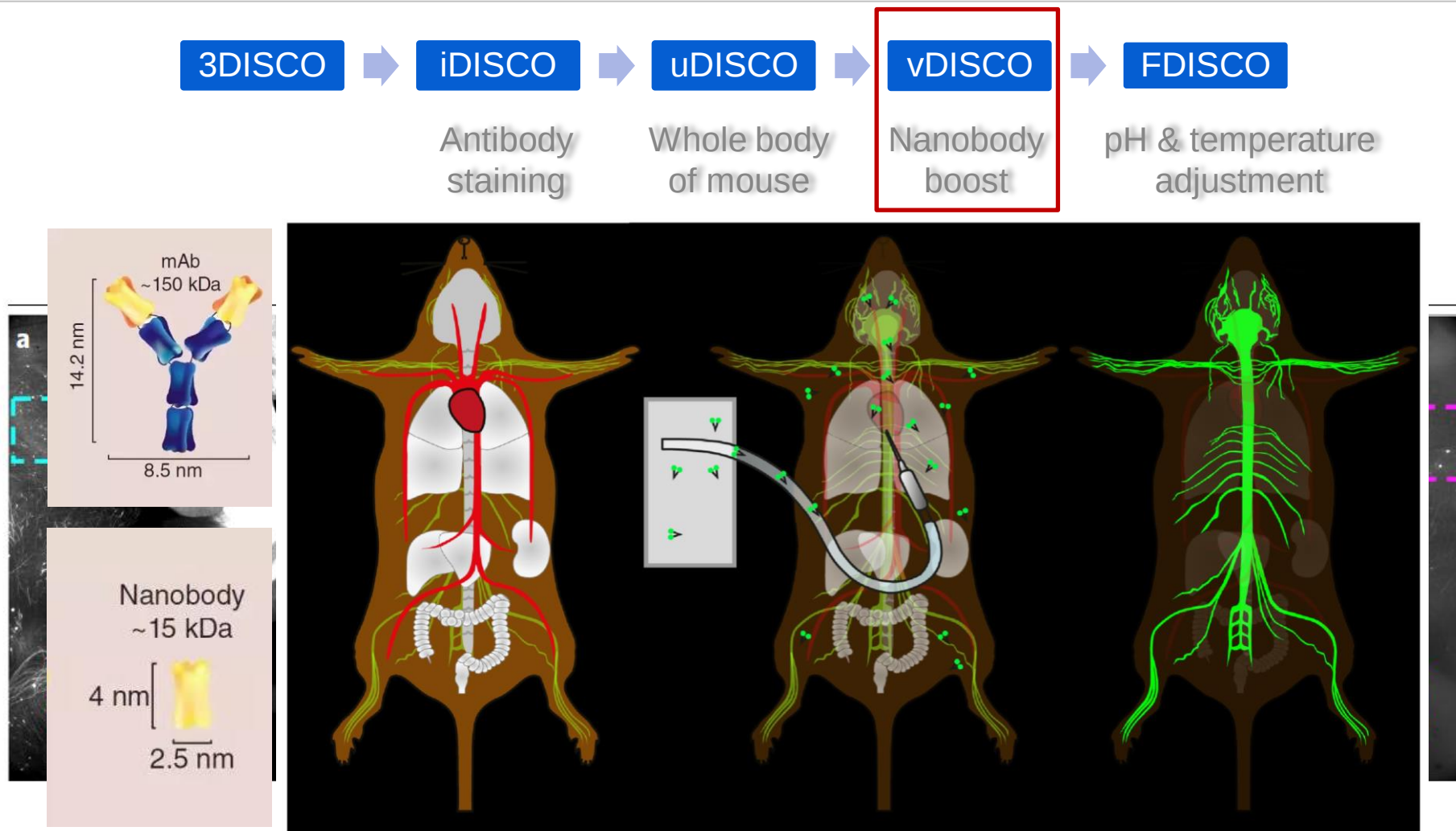
- 四氢呋喃(THF) 和 联苳基醚(DBE)
- 步骤:
 - PFA固定
 - THF脱水, 用DBE透明
 - 在DBE溶液中成像
- ✓ 适用样品广泛- 未切片的大脑, 脊髓和肺淋巴结等
- ✓ 透明化非常快速
- ✓ 便宜
- ✓ 荧光信号保存比BABB好
- ❑ 透明剂在很短时间内淬灭荧光蛋白
- ❑ 透明化组织无法保存 – 必须立即成像
- ❑ 有限制的抗体穿透深度 – 长时间孵育
- ❑ **有毒试剂**



Erturk, A, et al, Nature Methods, 7:(11) 2012

iDISCO – uDISCO – vDISCO - FDISCO

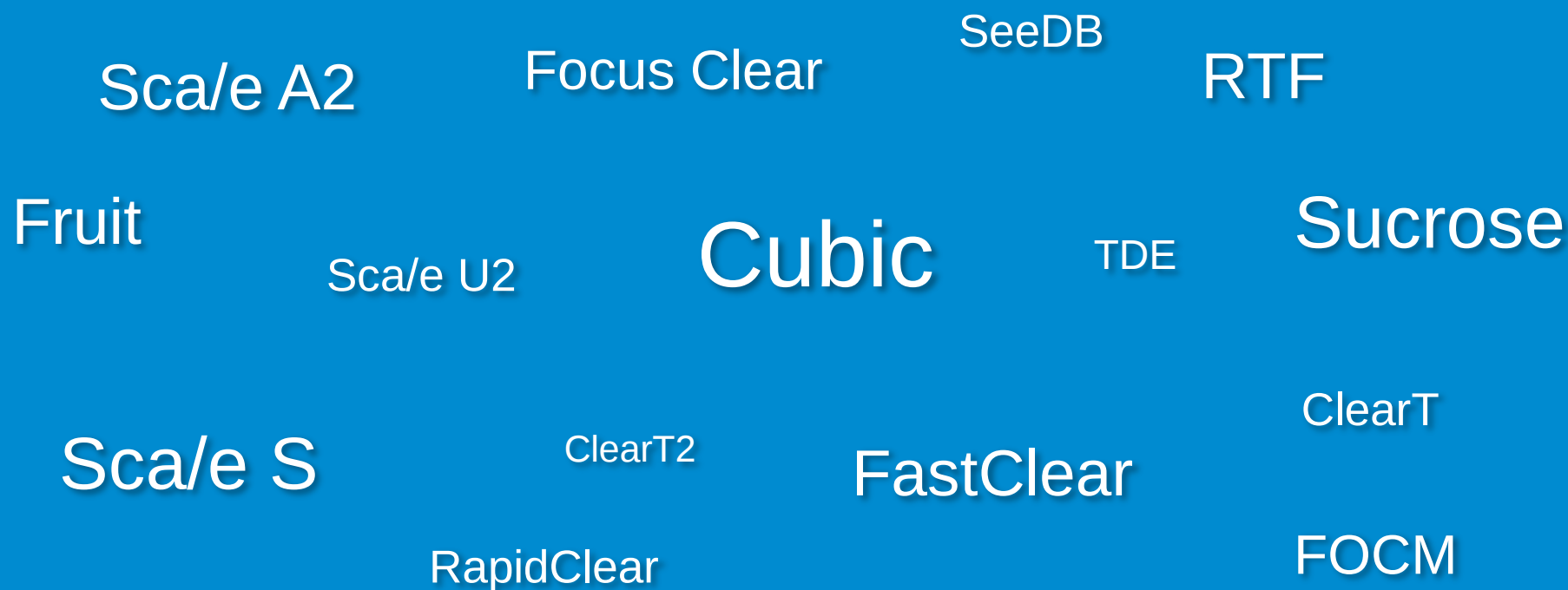
进阶的3DISCO



Ruiyao Cai, et, al. *Nature Neuroscience* 2018

基于水溶液的透明化方法

基于水溶液的透明化方法



SeeDB

看见更深的大脑



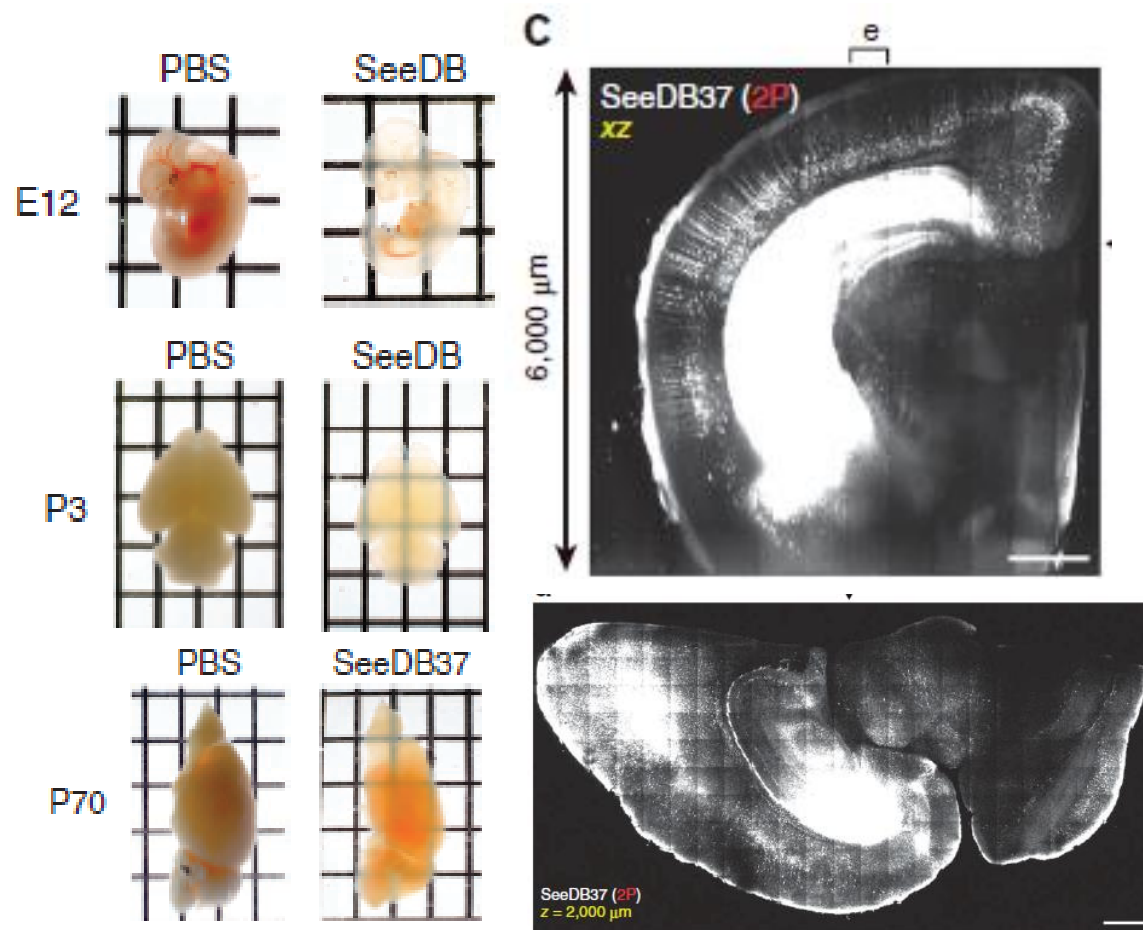
➤ 基于高浓度糖溶液的透明化方法

➤ 步骤:

- PFA固定
- 80.2% 果糖和 α -硫代甘油

- ✓ 水溶液
- ✓ 保护荧光蛋白信号
- ✓ 便宜的
- ✓ 胚胎和脑组织
- ✓ 少于1周透明时间
- ✓ 兼容其它液体染料

- 组织棕色化, 带有自发荧光
- 37°C 孵育防止沉淀
- 厚样品解决方案
- 大块样品很难做到均匀透明



Ke, M. et al. H, *Nature Neuroscience*, 16, (8) 1154-1161, 2013

Scale

水溶剂透明化



➤ 基于尿素的透明化方法

➤ 步骤:

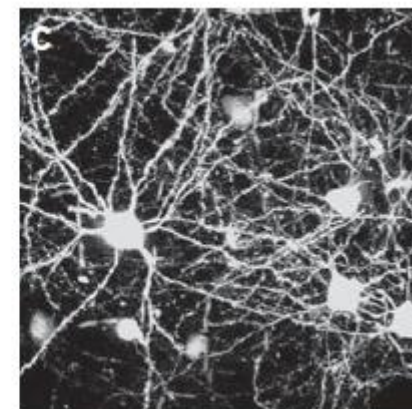
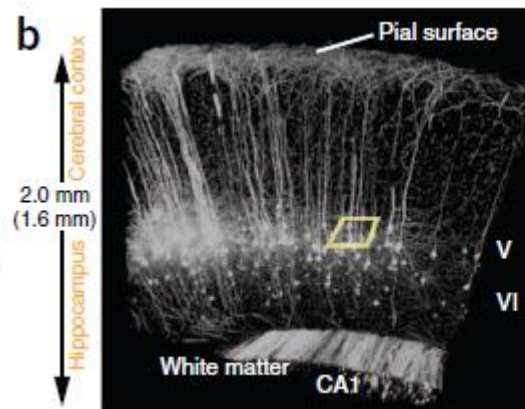
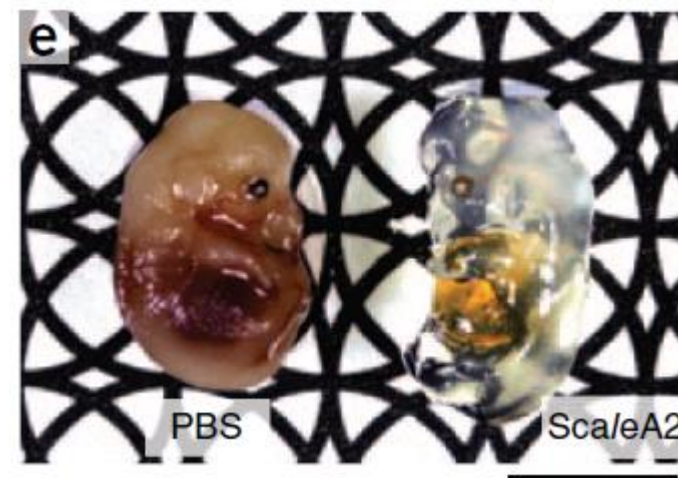
- 使用8M的尿素混合PFA固定
- 4M尿素处理
- 10%甘油和Triton X-100处理

- ✓ 水溶液
- ✓ 保护荧光蛋白信号
- ✓ 便宜的
- ✓ 胚胎和脑组织

- ❑ 组织肿胀和易碎性
- ❑ 尿素使某些蛋白质变性
- ❑ 需要很长时间来透明 >2周



聚偏二氟乙烯膜 (PVDF)



Hama, H, *Nature Neuroscience*, 14, 1481-1488, 2011

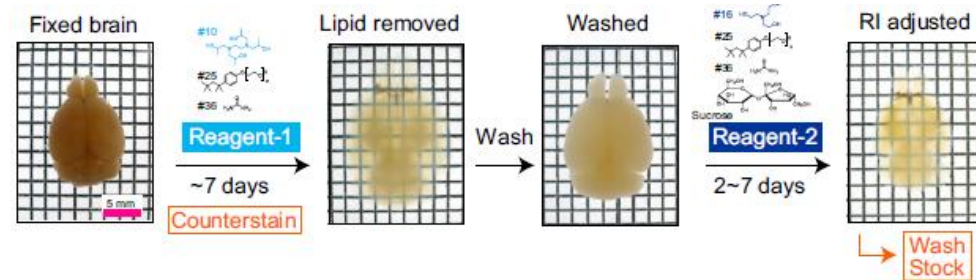
CUBIC

像调鸡尾酒一样透明组织

➤ 基于Sca/e的透明化方法

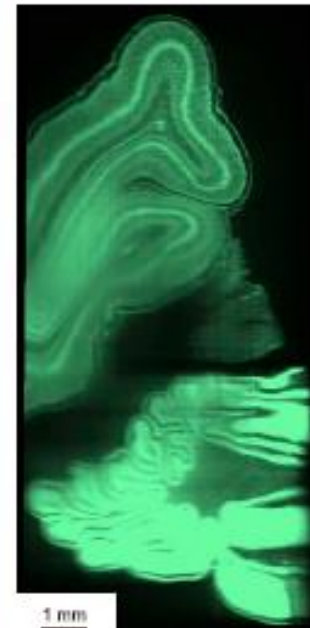
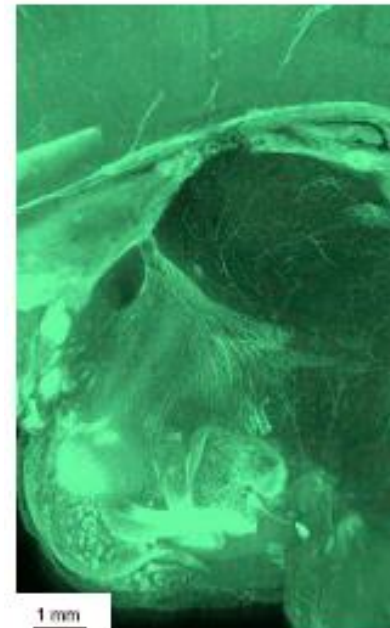
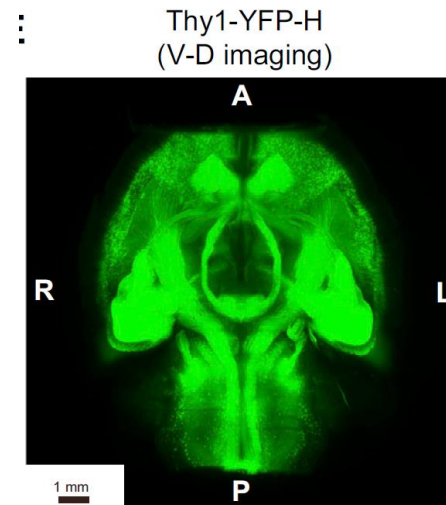
➤ 步骤:

- PFA固定
- Reagent 1溶液处理（尿素，乙二胺，Triton X-100）
- PBS清洗组织
- Reagent 2 溶液处理（尿素，蔗糖，三乙醇胺）



- ✓ 保护荧光蛋白信号
- ✓ 适用多种组织
- ✓ 组织肿胀但逐步恢复
- ✓ 可用于抗体染色
- ✓ 可用于灵长类动物透明化
- ✓ 价格便宜

- ❑ 透明化时间2周
- ❑ 去垢剂可能丢失信号
- ❑ 溶液粘度大



Susaki, E., A., *Cell*, 157, 726-739, 2014

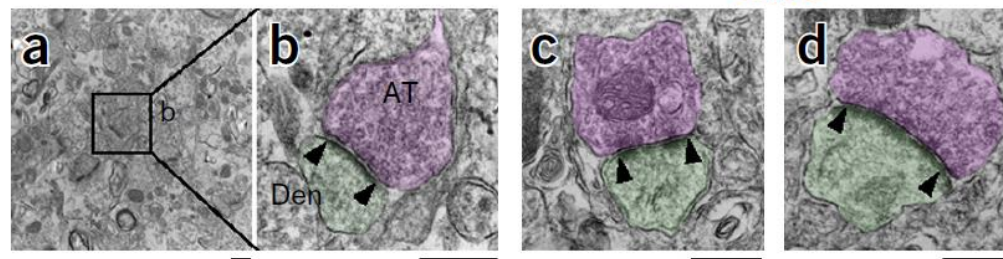
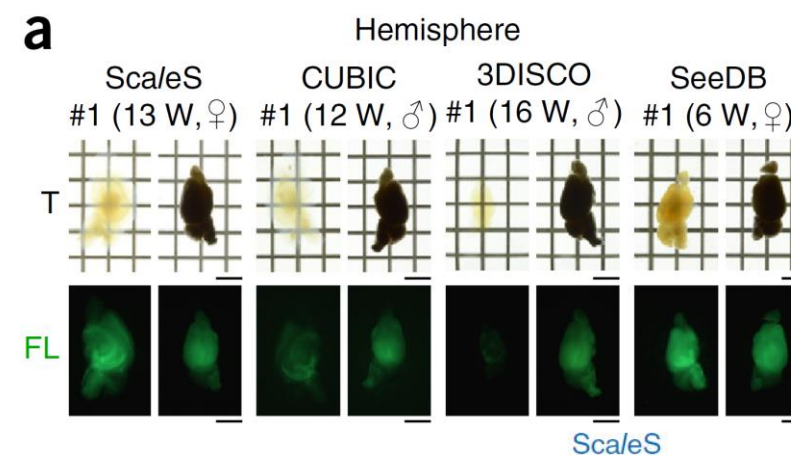
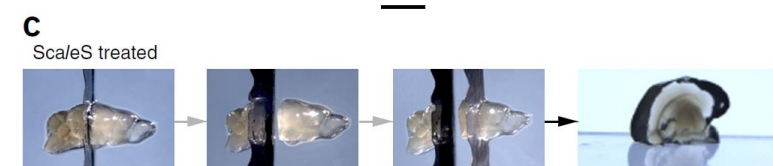
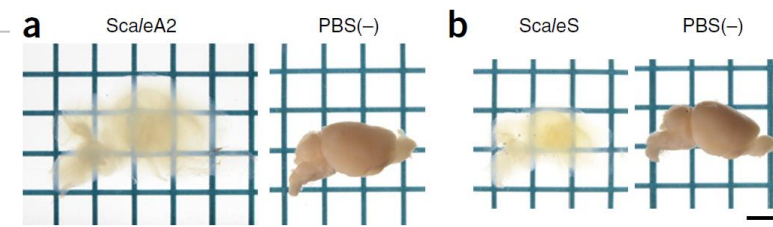
Scale S

同时保留组织结构和荧光信号



- 利用山梨糖醇进行透明化
- 步骤
 - 梯度山梨糖醇处理组织
- ✓ 水溶液，粘度小，易操作
- ✓ 荧光蛋白信号好，且可长久保存（1年）
- ✓ 组织膨胀小
- ✓ 组织硬度高
- ✓ 透明化后的组织还可进行电镜成像
- ✓ 兼容抗体染色
- ✓ 减少去垢剂的使用，减少信号丢失
- ✓ 透明化时间短

Action	Reagent
Fixation	4% PFA/PBS(-) (4°C)
Adaptation	ScaleS0 (~12 hrs, 37°C)
Permeabilization	ScaleS1 (~12 hrs, 37°C) ScaleS2 (~12 hrs, 37°C) ScaleS3 (~12 hrs, 37°C)
DeScaling	PBS(-) (~12 hrs, 4°C)
Clearing	ScaleS4 (~12 hrs, 37°C)
Mounting	ScaleS4 (RT)

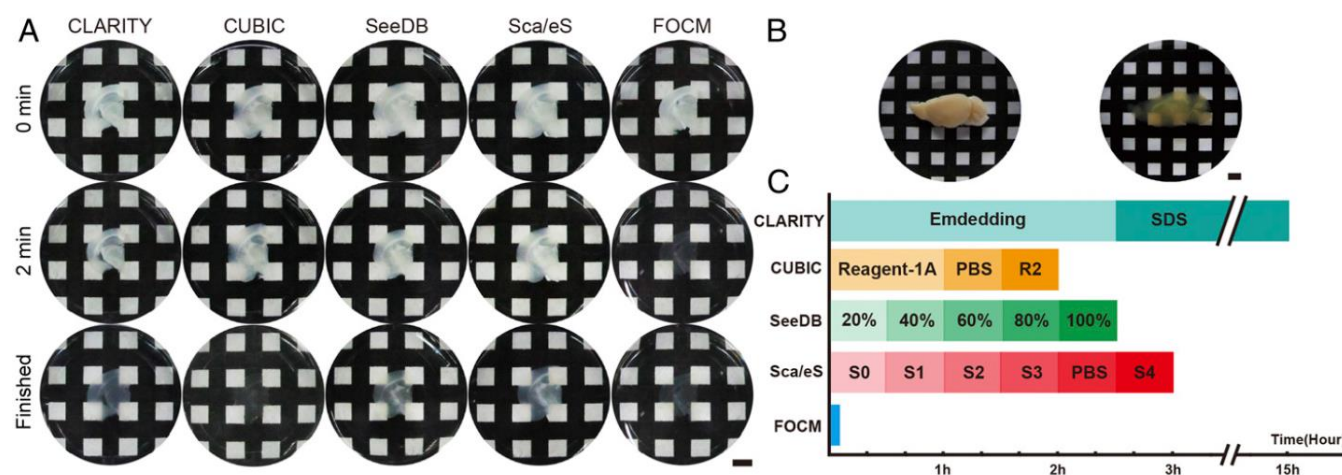
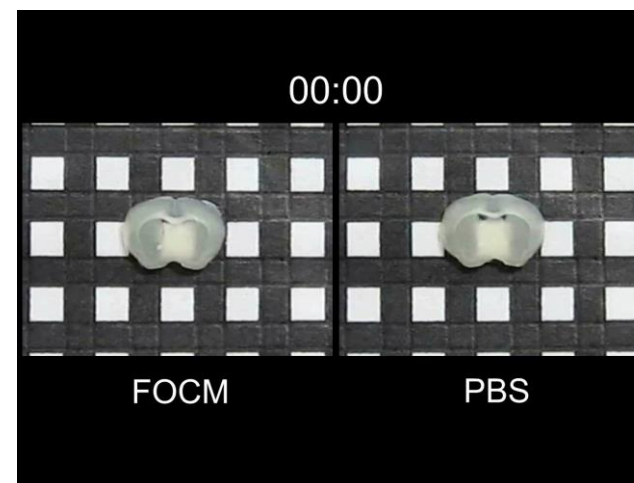


FOCM

超快速透明化方法



- 立等可见的透明化方法
- 步骤
 - 尿素、山梨糖醇、甘油、DMSO处理组织
- ✓ 水溶液
- ✓ 透明化速度非常快
- ❑ 适用于脑片等薄样品透明化
- ❑ 厚样品透明化效果未知



Xinpei Zhu, et, al. *PNAS*, 2019

基于水凝胶的透明化方法

基于水凝胶的透明化方法

PARS

CLARITY

PACT

RIMS

CLARITY

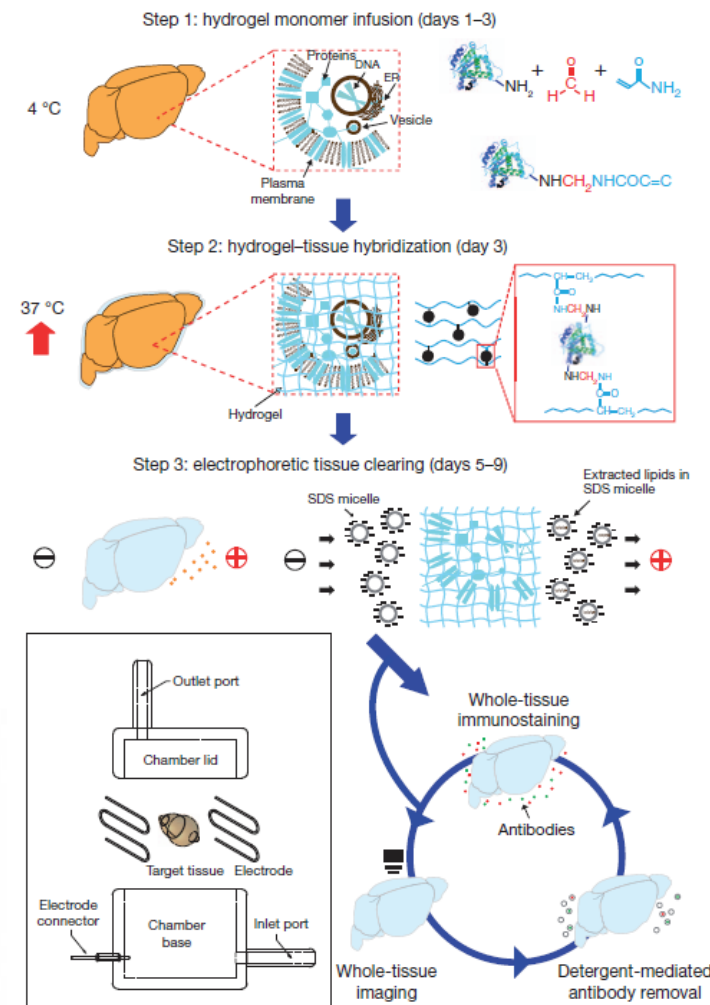
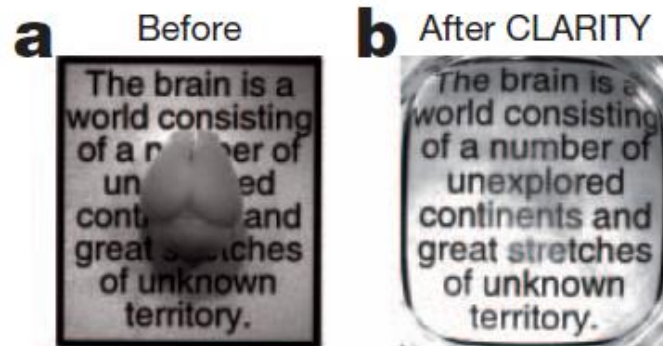
基于电泳的透明化方法



- 水凝胶 + 电泳的透明化方法
- 步骤:
 - PFA固定, 灌流小的带有共价交联剂和热触发多聚体的水凝胶单体到完好的大脑(1-3天) °C
 - 水凝胶杂交(3天) 37°C
 - 电泳组织透明(ETC) 在离子去垢剂下全脑电泳去除脂类 (5-8天)
 - 在相应介质的聚焦溶液中成像

- ✓ 水溶液
- ✓ 保护荧光蛋白信号
- ✓ 适用于骨组织透明化
- ✓ 组织坚固耐用
- ✓ 有商品化设备和试剂
- ✓ 兼容免疫荧光标记

- ❑ 部分试剂昂贵
- ❑ 效率上有些易变
- ❑ ETC较复杂



Chung, K., C., et al, *Nature*, 16;497(7449):332-7, 2013

PACT and PARS

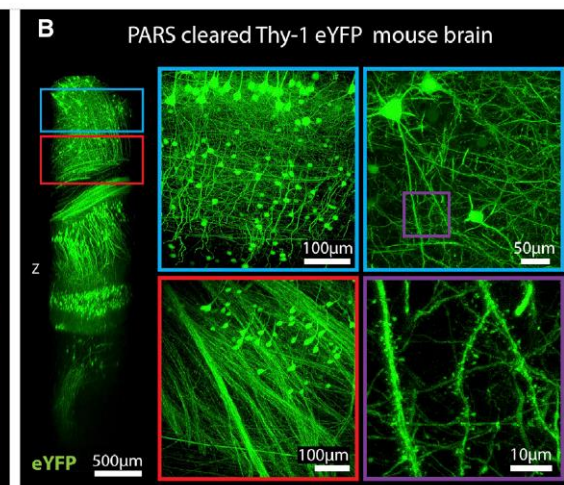
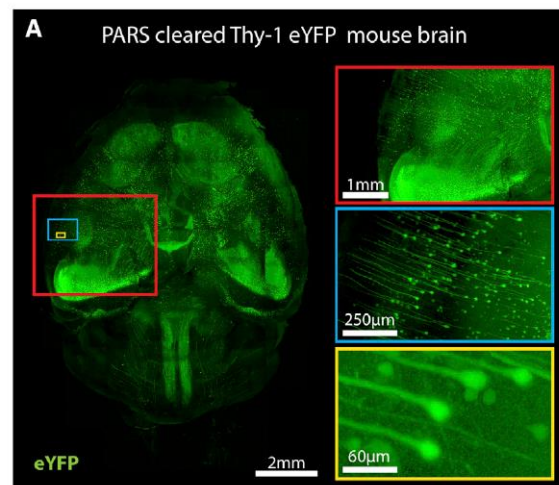
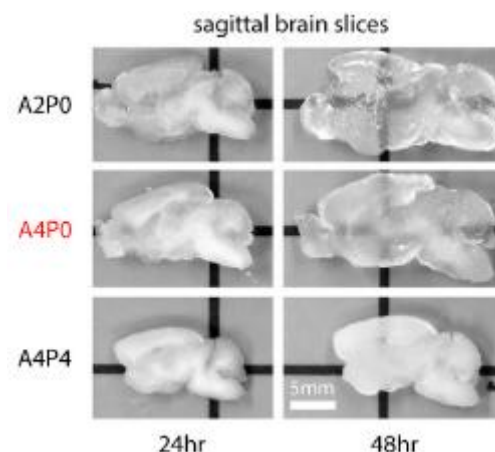
被动透明化方法



- 无需电泳，被动的透明方法
- 步骤:
 - 组织交联(丙烯酰胺) 和杂交
 - 水凝胶单体处理
 - 用离子去垢剂提取液体
 - 嵌入RIMS中成像及保存

- ✓ RIMS (Refractive Index Matching Solution)
折射率匹配溶液
- ✓ 全身透明化及免疫荧光标记
- ✓ 保护荧光蛋白信号
- ✓ 相对CLARITY价格便宜

- 使用RIMS或PBS时有组织肿胀



Yang, B., et al, *Cell* 158, 945-958, 2014

Zeiss的透明化解决方案



Technology	Method	Final R.I.	Main Agents	Time to Clear	Index of Refraction	Hydrogel Embedding	Clearing Solution	Perfused	Toxic	Reference	Objective to be used	Light-sheet	Single- and Multi-Photon
Simple Immersion													
	Sucrose	1.44	sucrose	1 day	+	-	-	-	-	Tsai et al., 2009b	Clr Plan-NEOFLUAR 20x/1.0 Corr nd=1.45 or Lightsheet Z.1 Detection Optic 5x/0.16 nd=1.45	+	+
	Focus Clear	1.43 – 1.47	diatrizoic acid (hypaque)	several days	+	-	-	-	-	Chiang et al., 2002	Clr Plan-NEOFLUAR 20x/1.0 Corr nd=1.45 or Lightsheet Z.1 Detection Optic 5x/0.16 nd=1.45	+++	++
	ClearT	1.44	formamide	2 – 3 days	+	-	-	-	+	Kuwajima et al., 2013	"Lightsheet Z.1 Detection Optic 5x/0.16 nd=1.45 Confocal: LCI Multi-Immersion objectives should be working with coverslips"	+	+
	ClearT2	1.44	formamide/PEG	2 – 3 days	+	-	-	-	+	Kuwajima et al., 2013	"Lightsheet Z.1 Detection Optic 5x/0.16 nd=1.45 Confocal: LCI Multi-Immersion objectives should be working with coverslips"	+	+
	SeeDB	1.48 – 1.50	fructose/thioglycerol	several days	-	-	-	-	-	Ke et al., 2013	"Lightsheet Z.1 Detection Optic 5x/0.16 nd=1.45 Confocal: LCI Multi-Immersion objectives should be working with coverslips"	+	++
	FRUIT	1.48	fructose/thioglycerol/urea	days	-	-	+	-	-	Hou et al., 2015	Clr Plan-NEOFLUAR 20x/1.0 Corr nd=1.45 or Lightsheet Z.1 Detection Optic 5x/0.16 nd=1.45	++	++
	TDE	1.42	2,2'-thiodiethanol	days – weeks	+	optional	-	-	-	Costantini et al., 2015; Aoyagi et al., 2015; Staudt et al., 2007	Clr Plan-NEOFLUAR 20x/1.0 Corr nd=1.45 or Lightsheet Z.1 Detection Optic 5x/0.16 nd=1.45	++	++
Hyperhydration													
	ScaleS	1.44	urea/sorbitol	days	+	-	-	-	-	Hama et al., 2015	Clr Plan-NEOFLUAR 20x/1.0 Corr nd=1.45 or Lightsheet Z.1 Detection Optic 5x/0.16 nd=1.45	+++	+++
	Scale A2	1.38	4M urea, 10% glycerol	2 weeks	-	-	-	-	-	Hama et al., 2011	Clr Plan-APOCHROMAT 20x/1.0 Corr nd=1.38	+++	+++
	Scale U2	1.38	4M urea, 30% glycerol	months	-	-	-	-	-	Hama et al., 2012	Clr Plan-APOCHROMAT 20x/1.0 Corr nd=1.38	+++	+++
	CUBIC	CUBIC1, 1.38; CUBIC2, 1.48	4M urea, 50% sucrose	1 – 2 weeks	+	-	-	-	-	Susald et al., 2014	Clr Plan-APOCHROMAT 20x/1.0 Corr nd=1.38 or Clr Plan-NEOFLUAR 20x/1.0 Corr nd=1.45 or Lightsheet Z.1 Detection Optic 5x/0.16 nd=1.45	+++	+++
	Whole-Body-CUBIC (perfusion)	1.45	4M urea	2 weeks	+	-	+	-	-	Tainaka et al., 2014	Clr Plan-APOCHROMAT 20x/1.0 Corr nd=1.38	+++	+++
Hydrogel Embedding													
	CLARITY	1.45	SDS, boric acid, FocusClear / 80 % glycerol	2 – 4 weeks	+	+	-	-	-	Chung et al., 2013	Clr Plan-NEOFLUAR 20x/1.0 Corr nd=1.45 or Lightsheet Z.1 Detection Optic 5x/0.16 nd=1.45	+++	+++
	PACT	1.38 – 1.48	Histodenz, SDS	2 – 4 weeks	+	+	-	-	-	Yang et al., 2014	Clr Plan-APOCHROMAT 20x/1.0 Corr nd=1.38 or Clr Plan-NEOFLUAR 20x/1.0 Corr nd=1.45 or Lightsheet Z.1 Detection Optic 5x/0.16 nd=1.45	+++	++
	PARS	1.38 – 1.48	Histodenz, SDS	1 – 2 weeks	+	+	+	-	-	Yang et al., 2014	Clr Plan-APOCHROMAT 20x/1.0 Corr nd=1.38 or Clr Plan-NEOFLUAR 20x/1.0 Corr nd=1.45 or Lightsheet Z.1 Detection Optic 5x/0.16 nd=1.45	+++	++
Solvent Based													
	Spalteholz	1.55	Benzyl benzoat/ Methyl salicylate	months	-	-	-	-	+	Spalteholz 1914	LS: Simax glass enclosed samples only (see Whitepaper); MP: Tests for dipping objective resistance towards chemical compounds have to be done	(+)	(+)
	BABB	1.55	Benzyl alcohol/ Benzyl benzoat	3 days	+	-	-	-	+	Dotd et al., 2007	LS: Simax glass enclosed samples only (see Whitepaper); MP: Tests for dipping objective resistance towards chemical compounds have to be done	(+)	(++)
	3DISCO	1.56	Dichloromethane/ Dibenzylether	3 days	-	-	-	-	+	(DBE) Ertürk et al., 2012a, 2012b	LS: Simax glass enclosed samples only (see Whitepaper); MP: Tests for dipping objective resistance towards chemical compounds have to be done	(+)	(++)
	IDISCO	1.56	Dichloromethane/ Dibenzylether	3 days	+	-	-	-	+	(DBE) Renier et al., 2014	LS: Simax glass enclosed samples only (see Whitepaper); MP: Tests for dipping objective resistance towards chemical compounds have to be done	(+)	(++)

(+)=glass enclosed

透明化处理的样品 (LUMOS)



LUMOS透明化处理的小鼠全脑

- Thy1-EGFP M-line mouse whole brain, optically cleared in LUMOS clearing agent, co-stained with Propidium Iodide
- Imaging Parameters: *ZEISS 5x/0.16 lens in RI=1.45 (WD= 5.6 mm),*
- Voxel Size: (x:y:z) approx. $1.3\ \mu\text{m} \times 1.3\ \mu\text{m} \times 1.75\ \mu\text{m}$
- Volume Size: 11.1 x 11 x 4.5mm (8600 x 8500 pixel and 2588 z-sections)

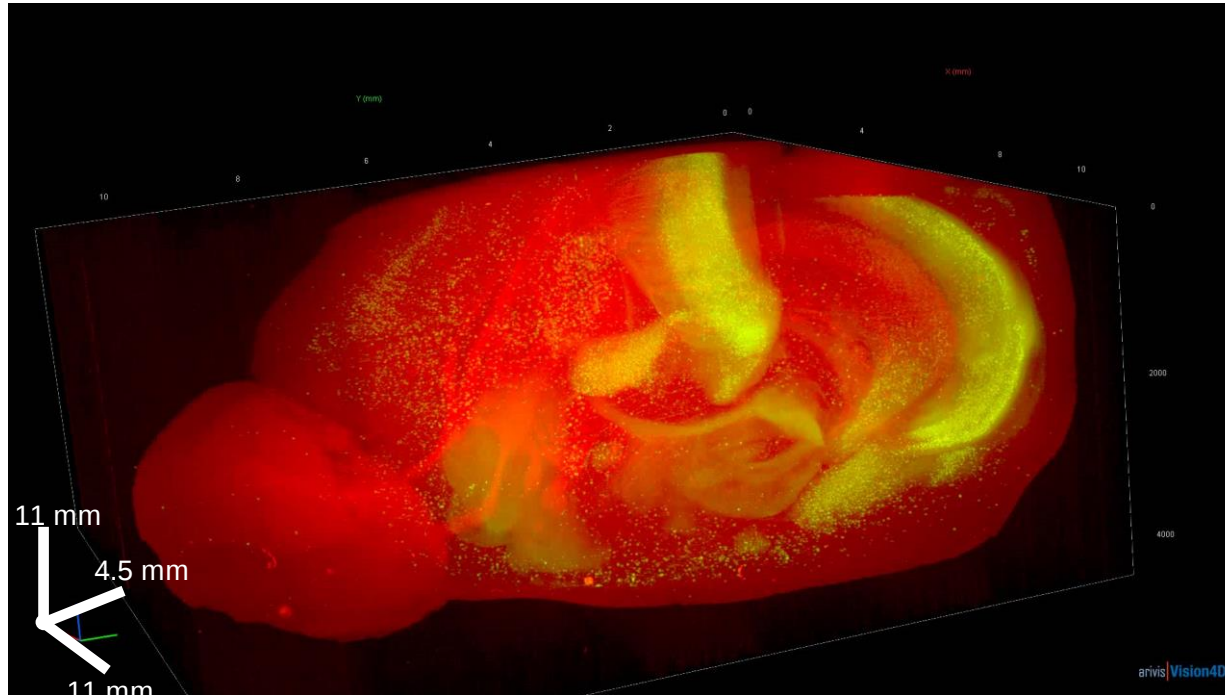
Data by O.Efimova National Research Center
"Kurchatov Institute", Moscow, Russia



透明化处理的样品 (LUMOS)



- Thy1-EGFP M-line mouse whole mount, optically cleared in LUMOS clearing agent, co-stained with Propidium Iodide
- Volume Size: 11.1 x 11 x 4.5 mm (8600 x 8500 pixel and 2588 z-sections)



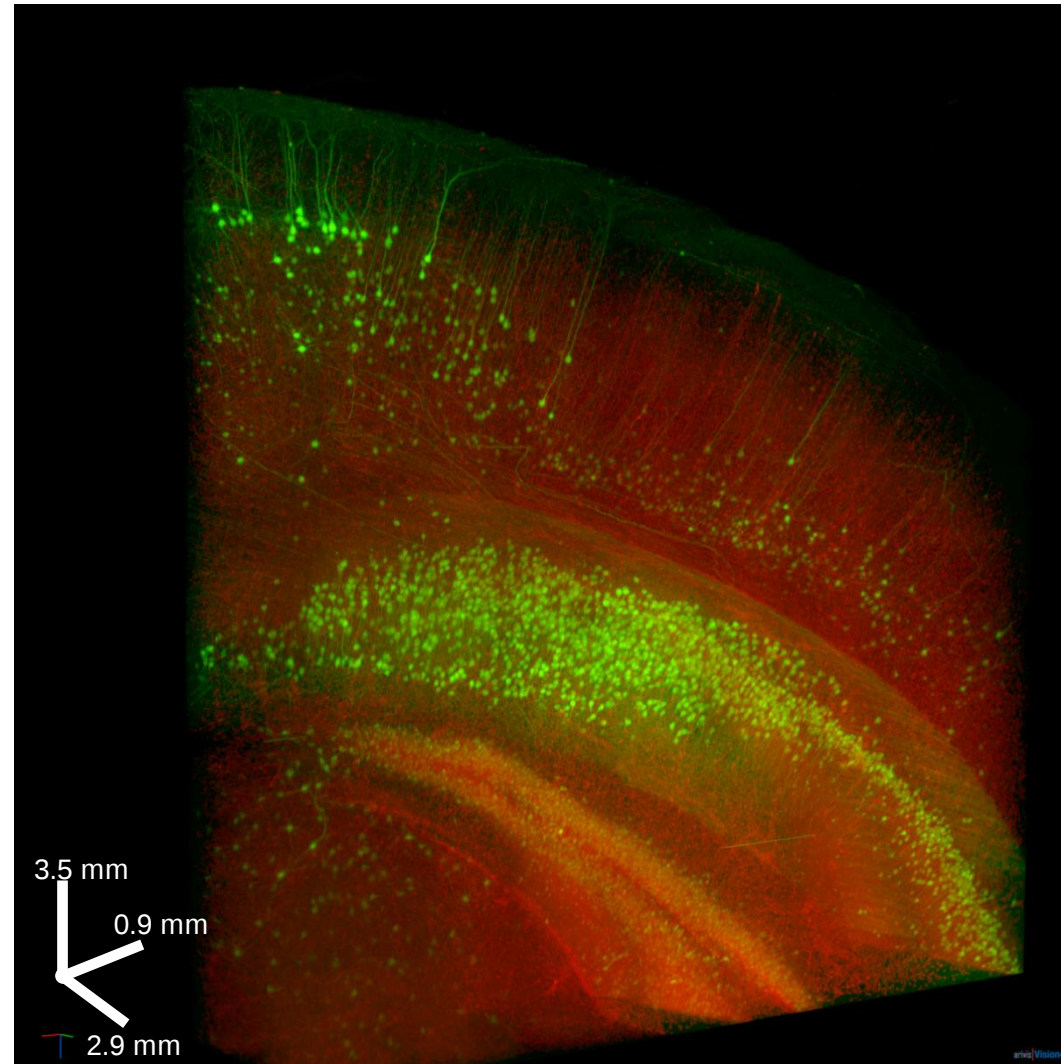
Data by O.Efimova National Research Center "Kurchatov Institute", Moscow, Russia

透明化处理的样品 (LUMOS)



- Thy1-EGFP M-line mouse whole mount, optically cleared in LUMOS clearing agent, co-stained with Propidium Iodide
- Imaging Parameters: ZEISS 20x/1.0 immersion lens for RI=1.45 (WD= 5.6 mm),
- Voxel Size: (x:y:z) 330 x 330 x 500 nm
- Volume Size: 2.9 x 0.9 x 3.25 mm (8950 x 2755 pixel and 6500 z-sections)

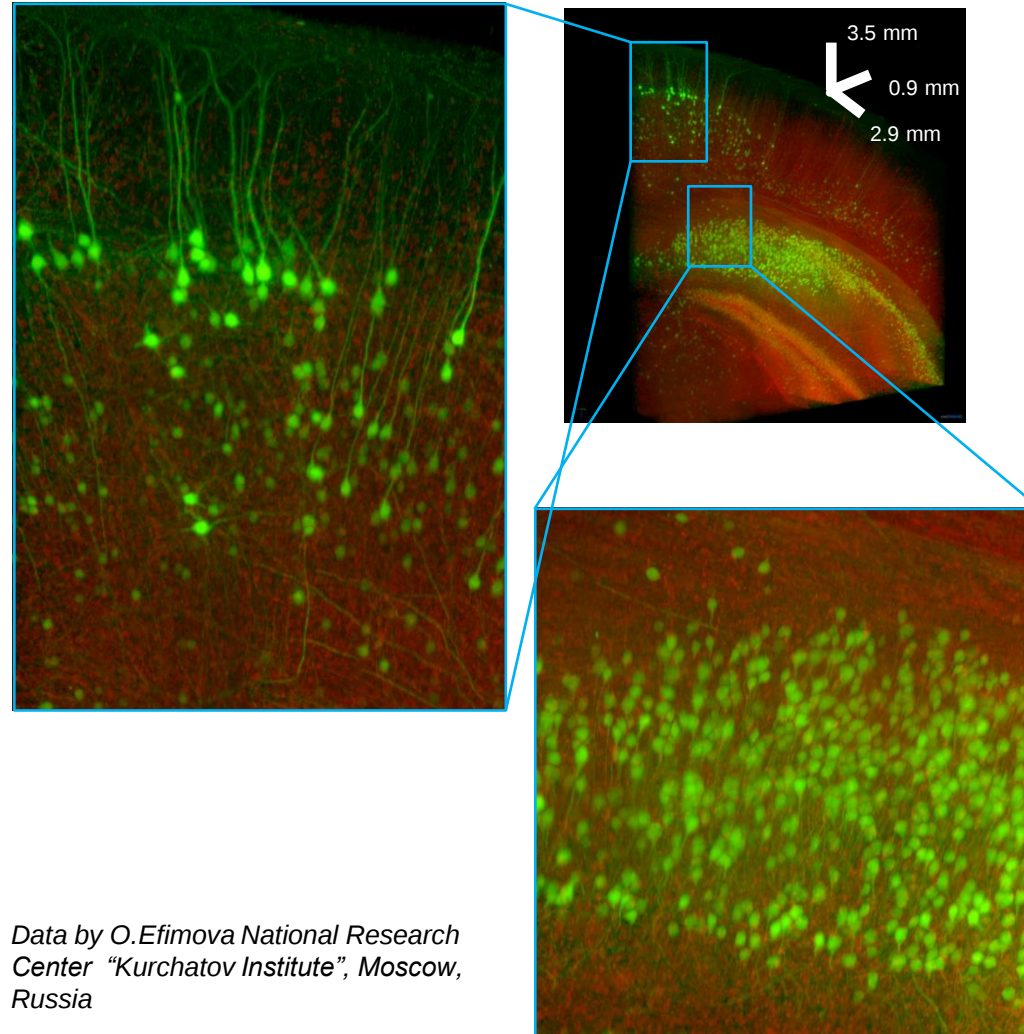
Data by O.Efimova National Research Center
"Kurchatov Institute", Moscow, Russia



透明化处理的样品 (LUMOS)

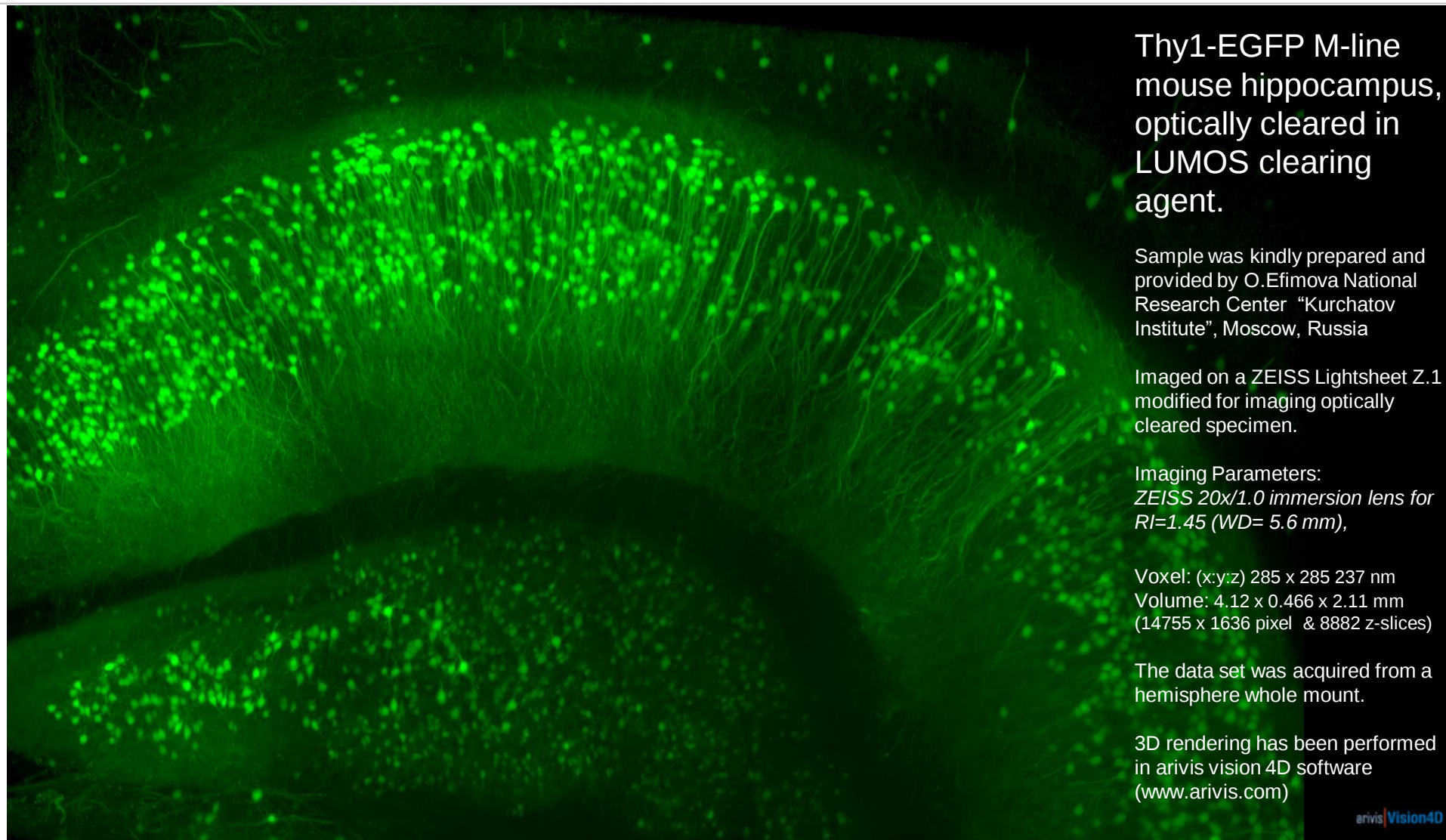


- Thy1-EGFP M-line mouse whole mount, optically cleared in LUMOS clearing agent, co-stained with Propidium Iodide
- Imaging Parameters: ZEISS 20x/1.0 immersion lens for RI=1.45 (WD= 5.6 mm),
- Voxel Size: (x:y:z) 330 x 330 x 500 nm
- Volume Size: 2.9 x 0.9 x 3.25 mm(8950 x 2755 pixel and 6500 z-sections)



Data by O.Efimova National Research Center "Kurchatov Institute", Moscow, Russia

透明化处理的样品 (LUMOS)



Thy1-EGFP M-line
mouse hippocampus,
optically cleared in
LUMOS clearing
agent.

Sample was kindly prepared and
provided by O.Efimova National
Research Center "Kurchatov
Institute", Moscow, Russia

Imaged on a ZEISS Lightsheet Z.1
modified for imaging optically
cleared specimen.

Imaging Parameters:
*ZEISS 20x/1.0 immersion lens for
RI=1.45 (WD= 5.6 mm),*

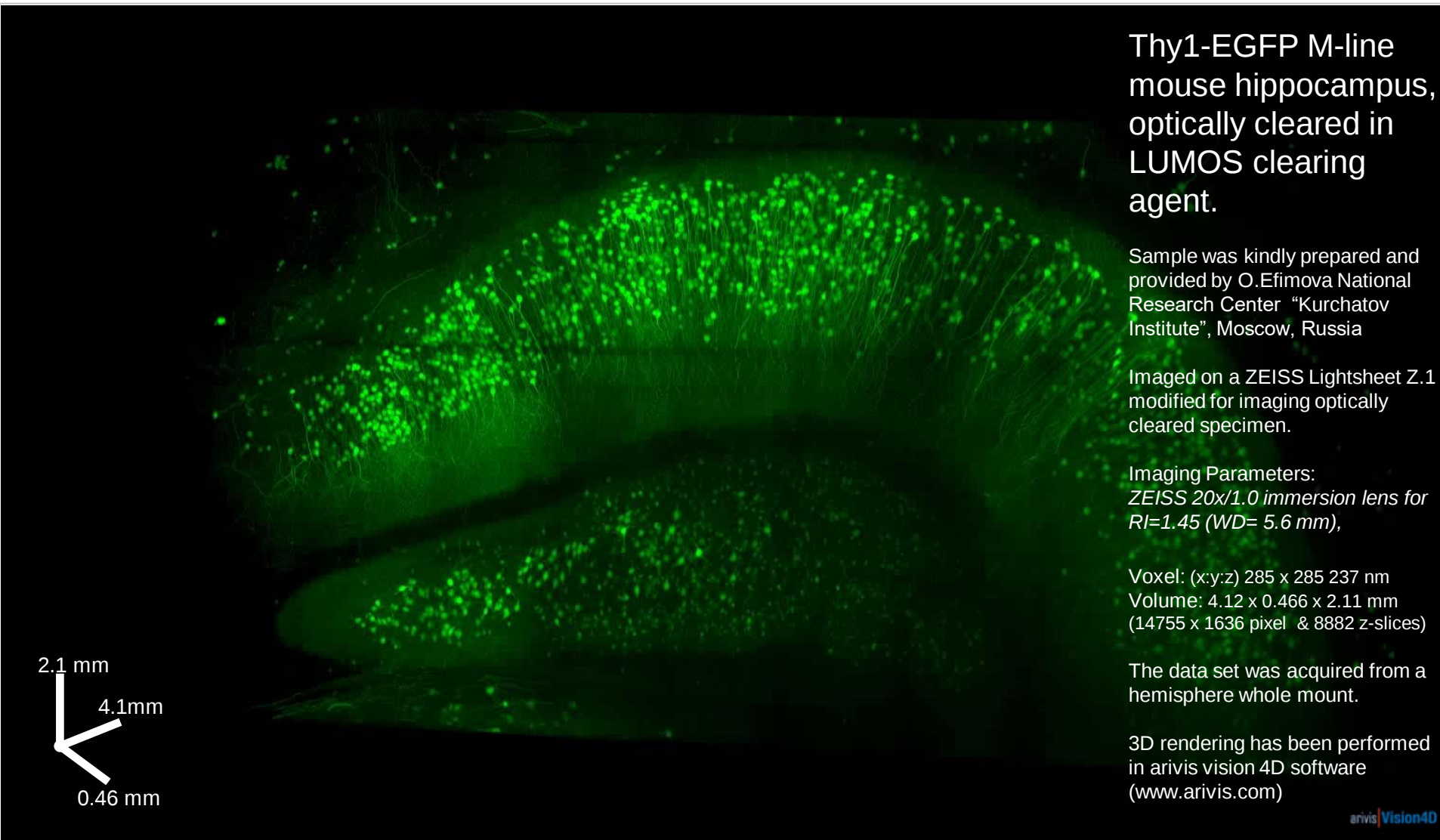
Voxel: (x:y:z) 285 x 285 237 nm
Volume: 4.12 x 0.466 x 2.11 mm
(14755 x 1636 pixel & 8882 z-slices)

The data set was acquired from a
hemisphere whole mount.

3D rendering has been performed
in arivis vision 4D software
(www.arivis.com)

arivis Vision4D

透明化处理的样品 (LUMOS)



Thy1-EGFP M-line
mouse hippocampus,
optically cleared in
LUMOS clearing
agent.

Sample was kindly prepared and
provided by O.Efimova National
Research Center "Kurchatov
Institute", Moscow, Russia

Imaged on a ZEISS Lightsheet Z.1
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Imaging Parameters:
*ZEISS 20x/1.0 immersion lens for
RI=1.45 (WD= 5.6 mm),*

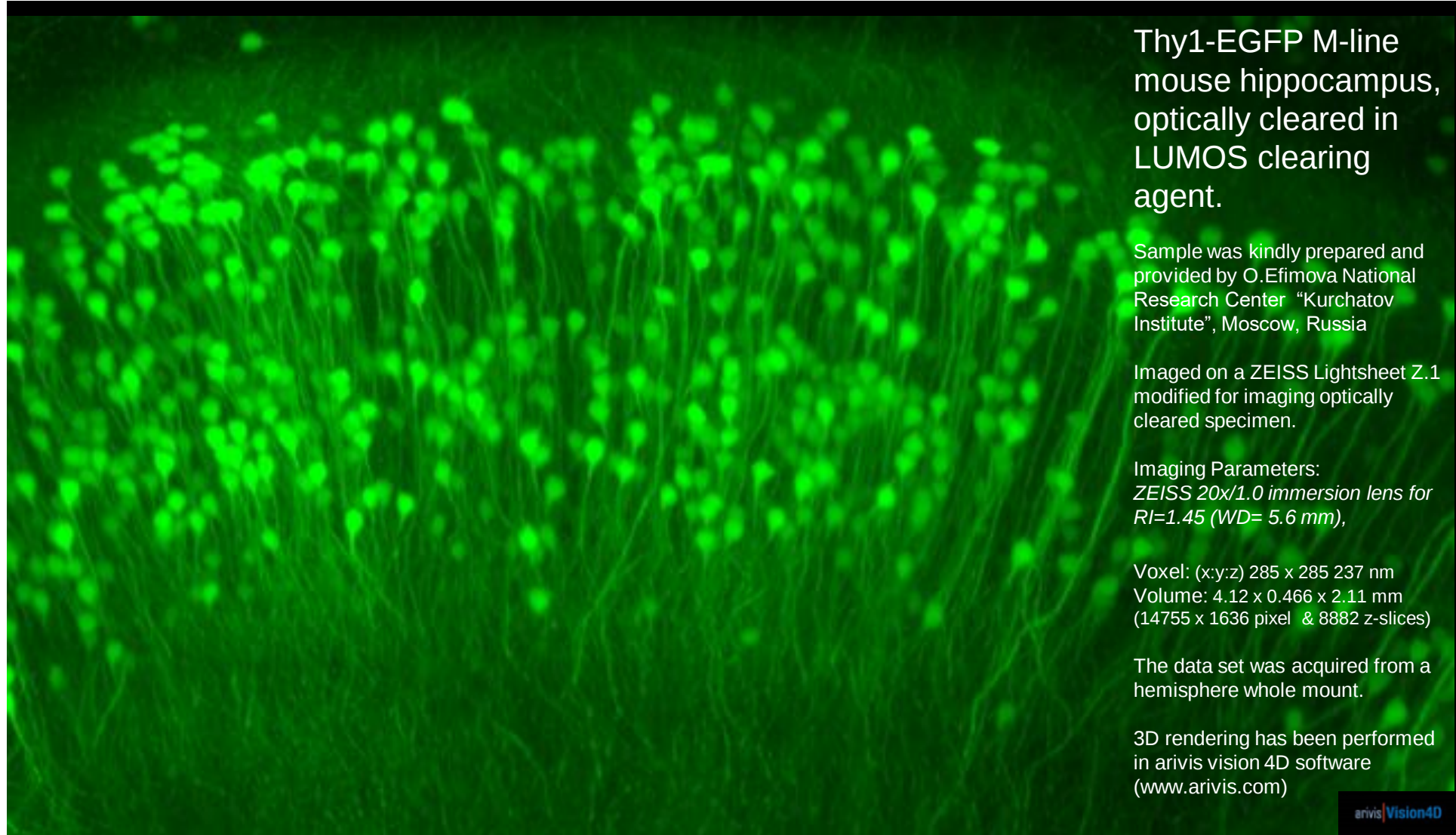
Voxel: (x:y:z) 285 x 285 237 nm
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arivis Vision4D

透明化处理的样品 (LUMOS)



Thy1-EGFP M-line
mouse hippocampus,
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*ZEISS 20x/1.0 immersion lens for
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(www.arivis.com)

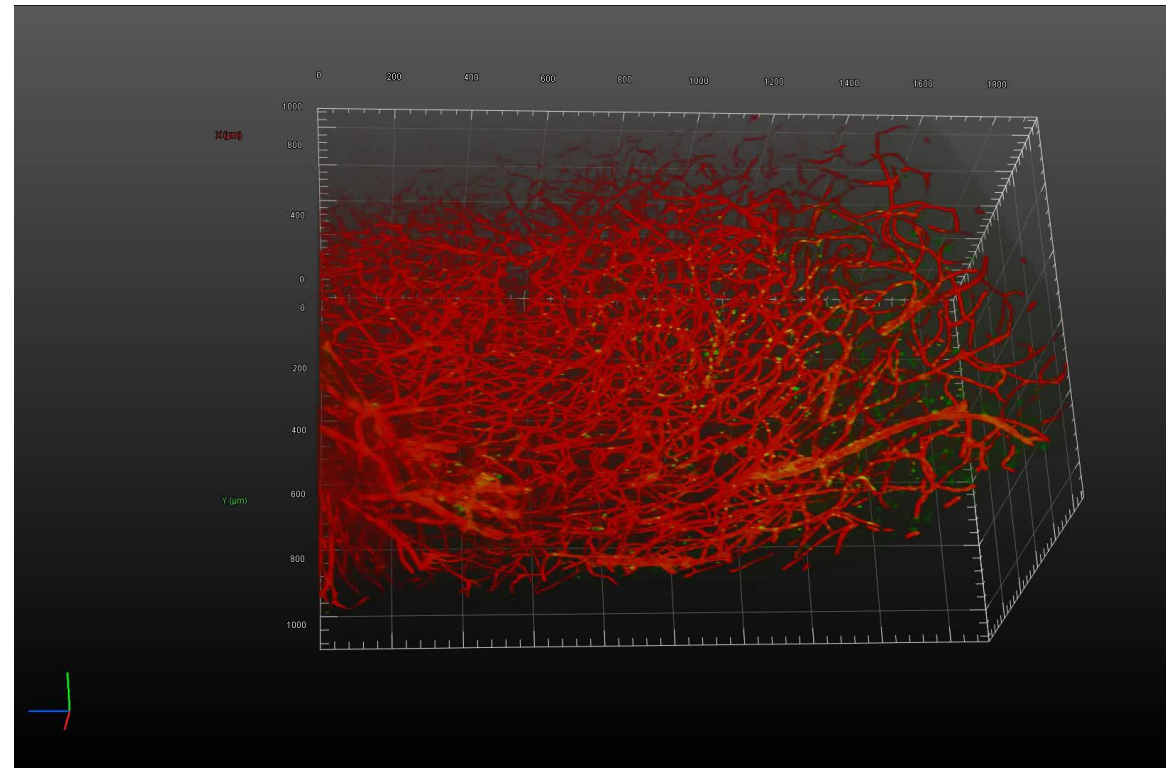


透明化处理的样品 (CLARITY)



鼠脑中的血管 (CLARITY透明化)

- Volume size: 1,1 x 1,1 x 1,8 mm



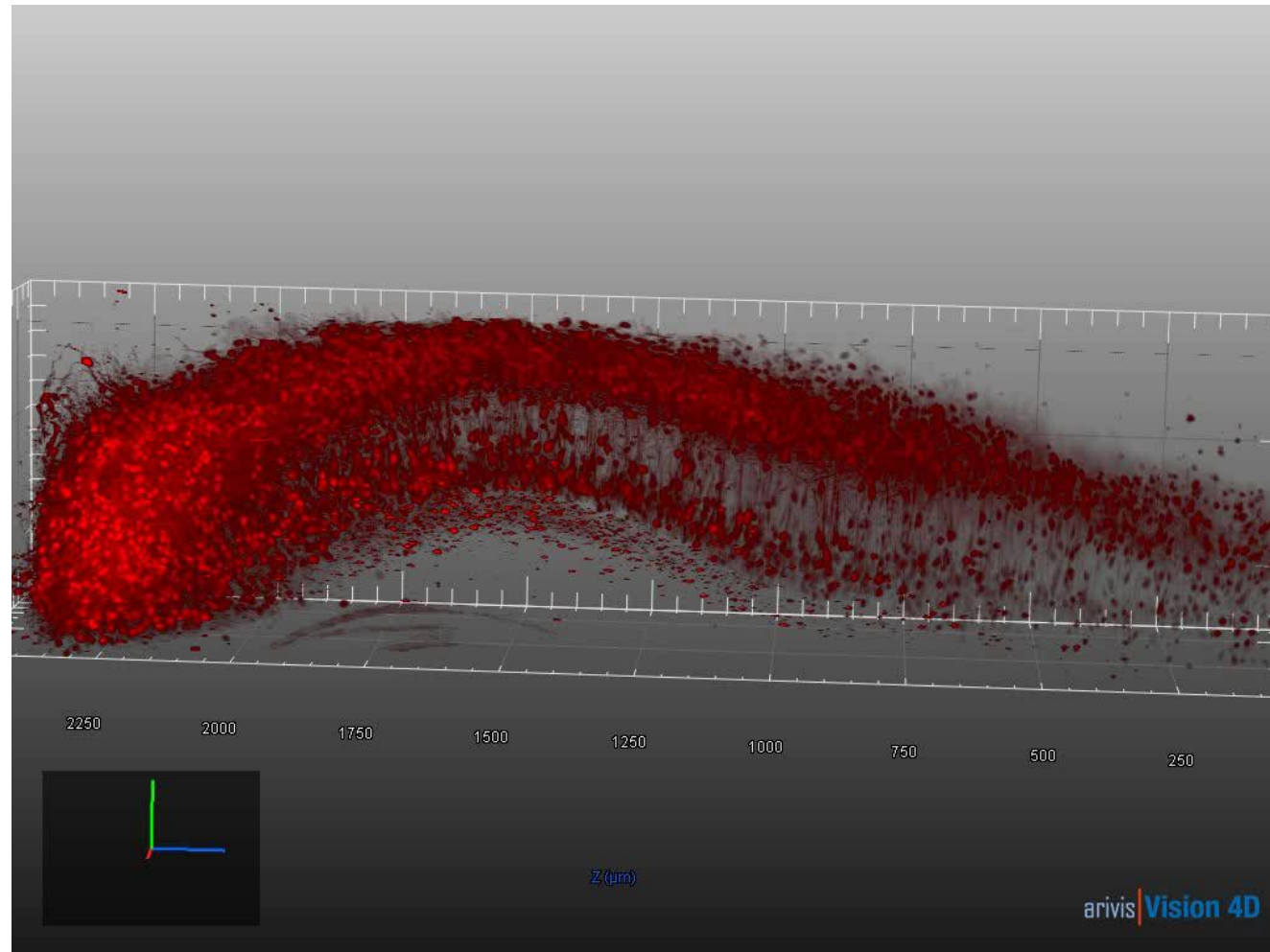
*Data by: Jean-Marie Vanderwinden, Université Libre de Bruxelles,
ULB Neuroscience Institute - Neurophysiology lab*

透明化处理的样品 (CLARITY)



小鼠大脑 (CLARITY)

- protocol based on CLARITY (Chung et al. 2013)

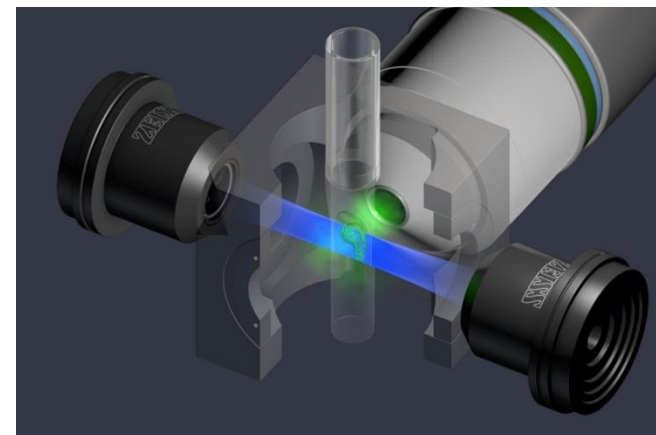


Data by Joe Bergan, Catherine Dulac Lab, Harvard University, USA

Zeiss Lightsheet Z.1 Microscope



- ✓ 几乎没有光漂白
- ✓ 采集速度快 (时间序列 & Z轴扫描)
- ✓ 多视角图像采集, 样品固定方式灵活
- ✓ XYZ 多维度高分辨率高对比度 (NA=1)
- ✓ 大容积样品槽



适合**大体积固定样品**成像

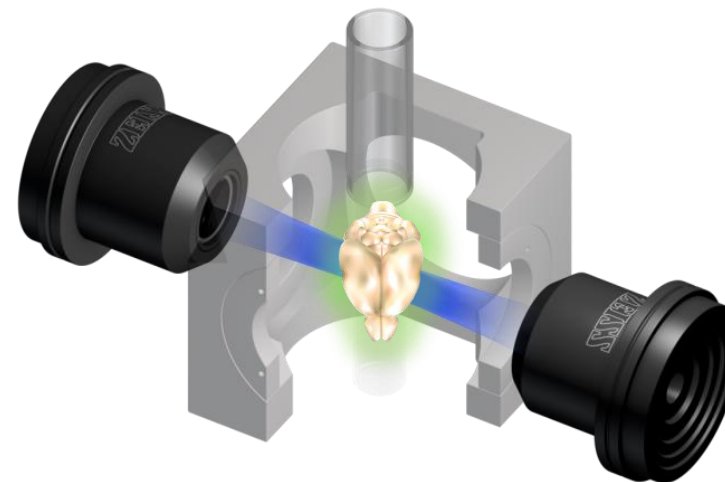




Lightsheet Z.1 适合透明化样品成像



- 大体积样品高速图像采集
- 几乎没有光漂白
- 高分辨率图像
- 样品角度自由旋转
- 样品尺寸: 可达1 x 1 x 2 cm
- 成像深度: 可达5.6 mm



专门配备适合透明化的样品槽和物镜

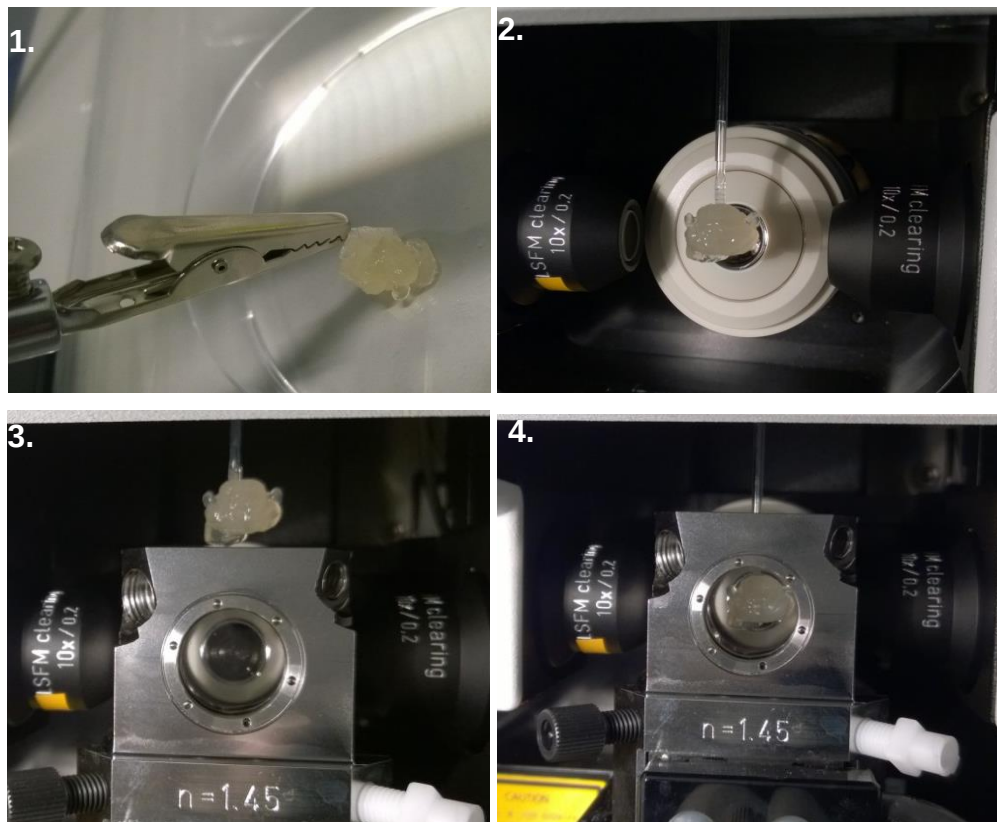
5.6 mm 自由工作距离:

- 20x N.A. 1.0 for $n = 1.45 (\pm 0.03)$
- 5x N.A. 0.16 for $n = 1.45$

透明化样品固定方法



- 样品体积: 1 x 1 x 2 cm
- 灵活多样的样品夹



样品可以360°任意旋转



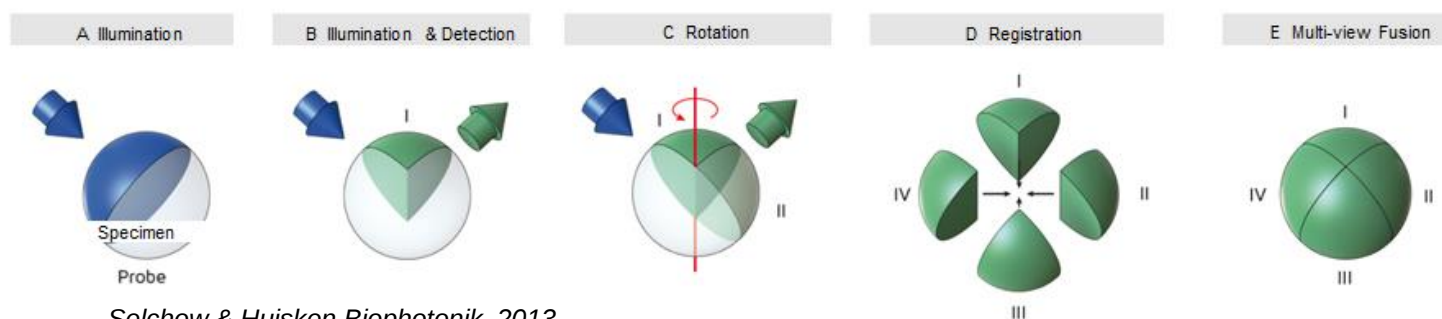
可旋转样品夹



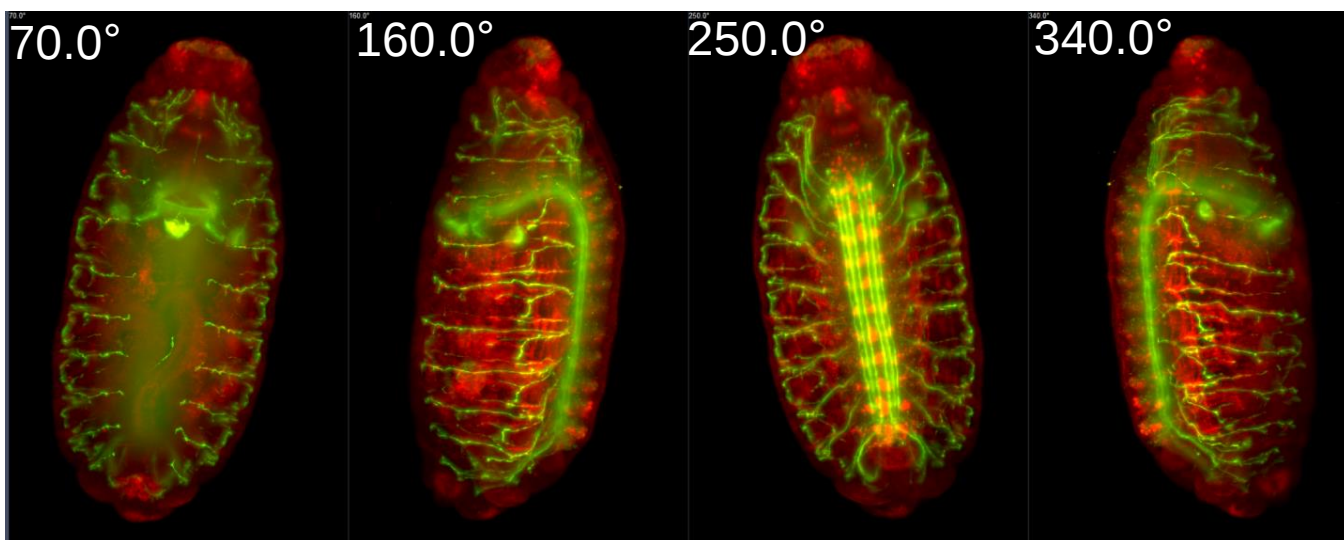
多角度图像合成



通过样品旋转，在不同角度拍摄Z轴图像，并进行图像合成。
提升3D图像的分辨率

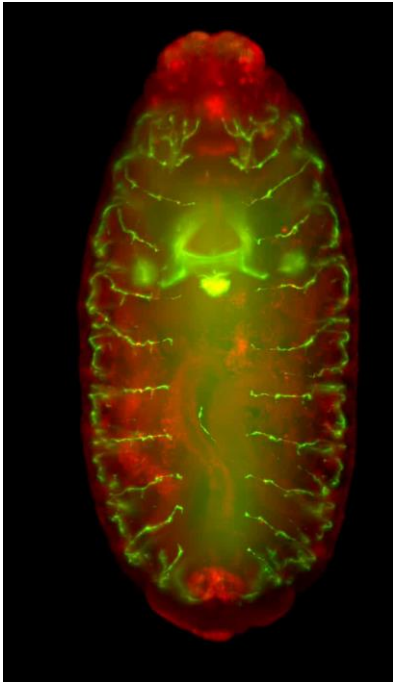


Selchow & Huisken *Biophotonik*, 2013

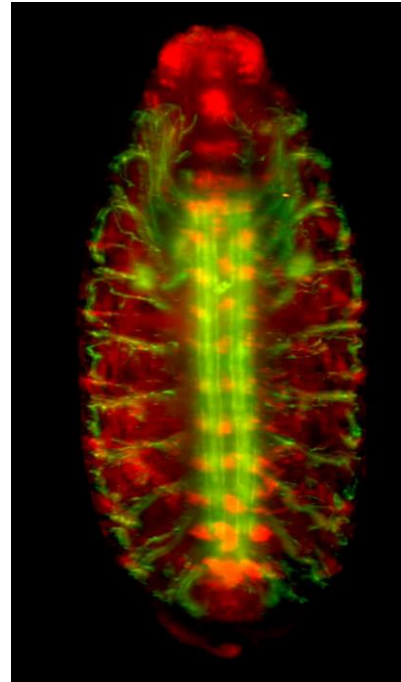


Sample and Data by Dr. Cecilia Lu, OIST, Okinawa, Japan

View 1



对齐和融合



View 3



Sample and Data by Dr. Cecilia Lu, OIST, Okinawa, Japan