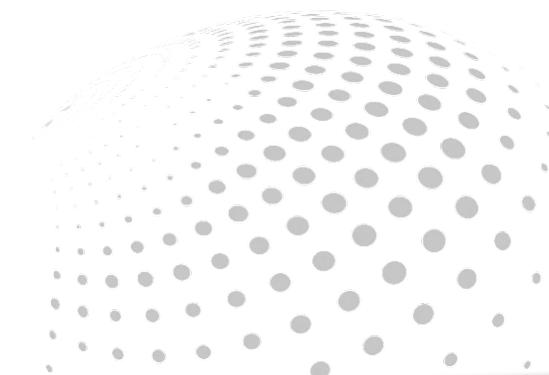


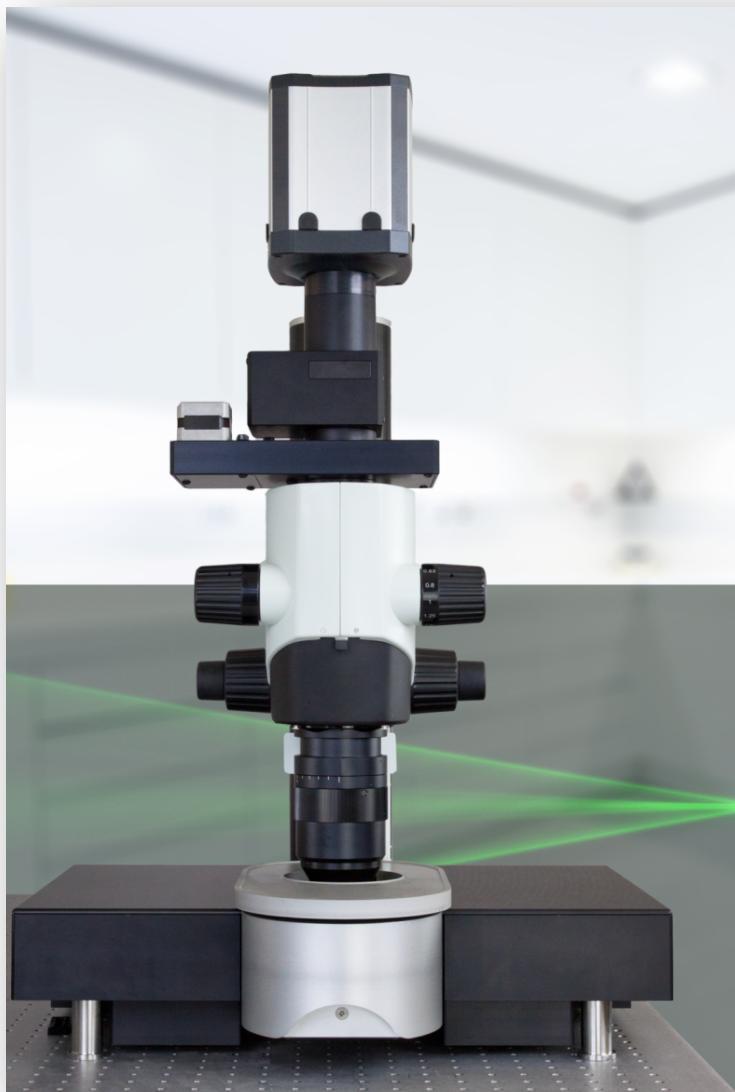
# Light sheet imaging and clearing

March 2017, Frankfurt a. M.

*Uwe Schroer*



# LaVision BioTec GmbH - History



**2014** UltraMicroscope, 2<sup>nd</sup> generation.

**2012** ZIM award for the UltraMicroscope.

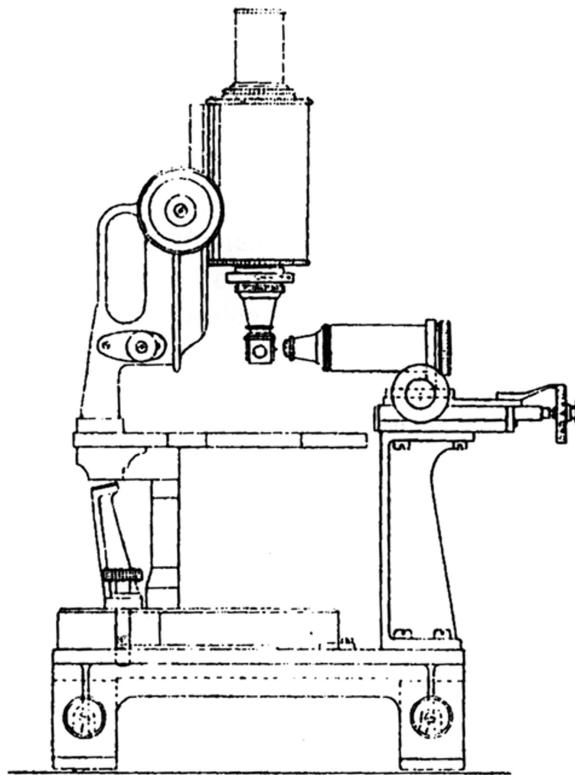
**2009** First commercial Lightsheet microscope.

**2005** First commercial OPO microscope for deep imaging.

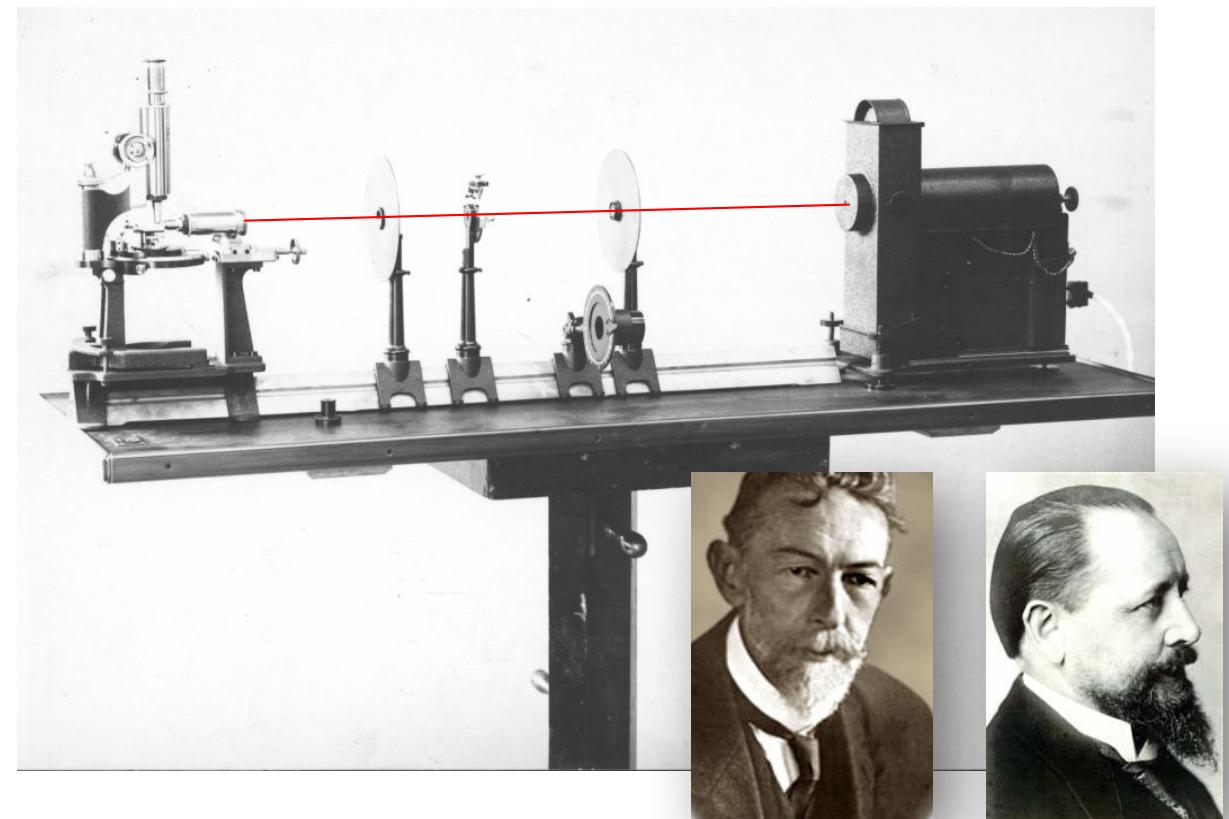
**2001** First multiphoton microscope with 64-beam technique.

**2000** Company foundation.

## 1903 - The first light sheet microscope



Peter A. Santi J  
*Histochem Cytochem* 2011;59:129-138



This is Figure 3 from Siedentopf and Zsigmondy's (1903) article and shows part of their light sheet microscope with an upright microscope containing a specimen holder that appears to be mounted to its objective lens and orthogonal illumination at 90° from what appears to be an illuminating objective.

# An epitome of the light sheet microscope history

## 1903

First light sheet by Siedentopf and Zsigmondy: The ultramicroscope described here was used for the visualization of particles below the diffraction limit in a solution by means of a non-coherent light sheet.

## 1964 -1993

The concept of orthogonal, planar illumination was used mainly in the field of flow cytometry.

## 1993

First application of light sheet illumination to a biological specimen by Voie et al. He made use of the light sheet technology known as orthogonal-plane fluorescence optical sectioning (OPFOS) for the characterization of cochlea.

## 2004

In 2004, the OPFOS concept was again reported in an article by Huisken. In this, researchers used a light sheet microscope for the visualization of live samples.

## 2007

This time the use of light sheet technology was combined in a work by Dodt with sample-clearing, as described by Spalteholz in 1914.

## 2009

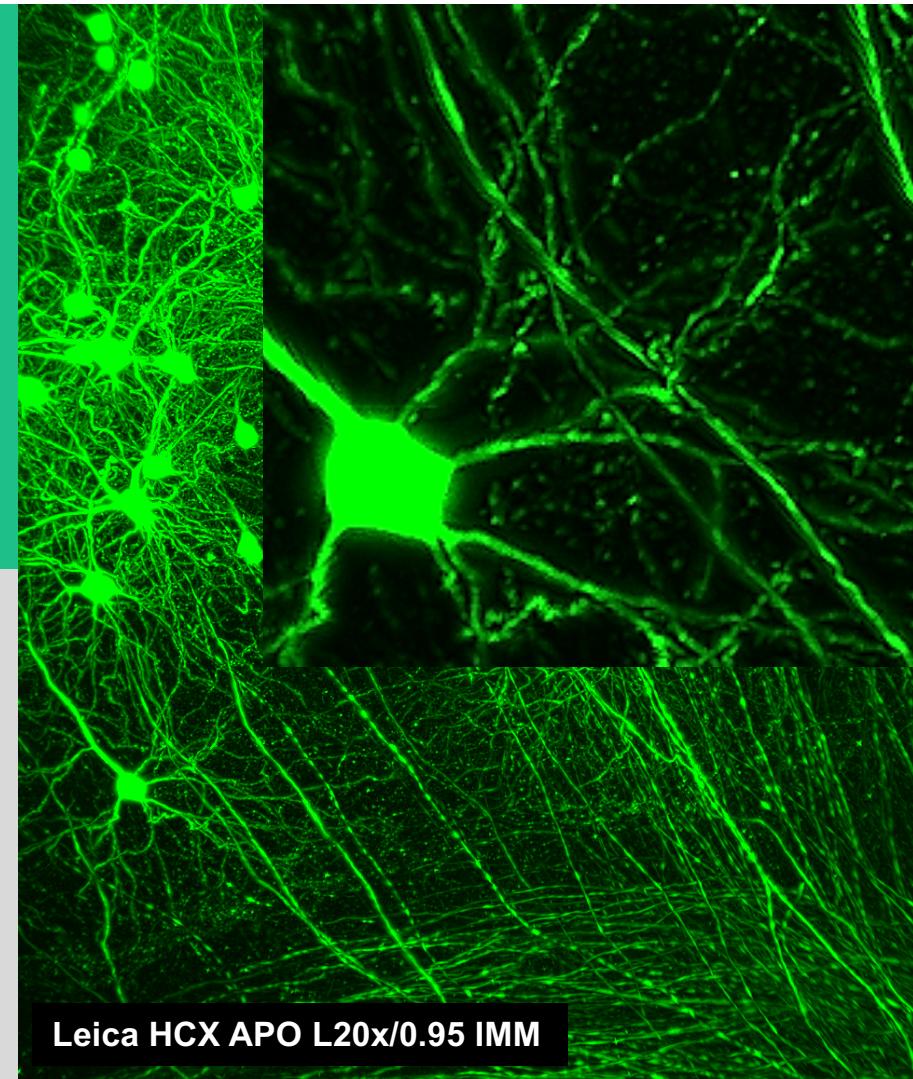
The UltraMicroscope - The first commercial light sheet fluorescence microscope

## 2016 - The new UltraMicroscope



# UltraMicroscope II – Setup infinity corrected objective lens

Setup for infinity corrected third party objective lenses or for LVBT 4x multi immersion objective lens



## UltraMicroscope II - Accessories



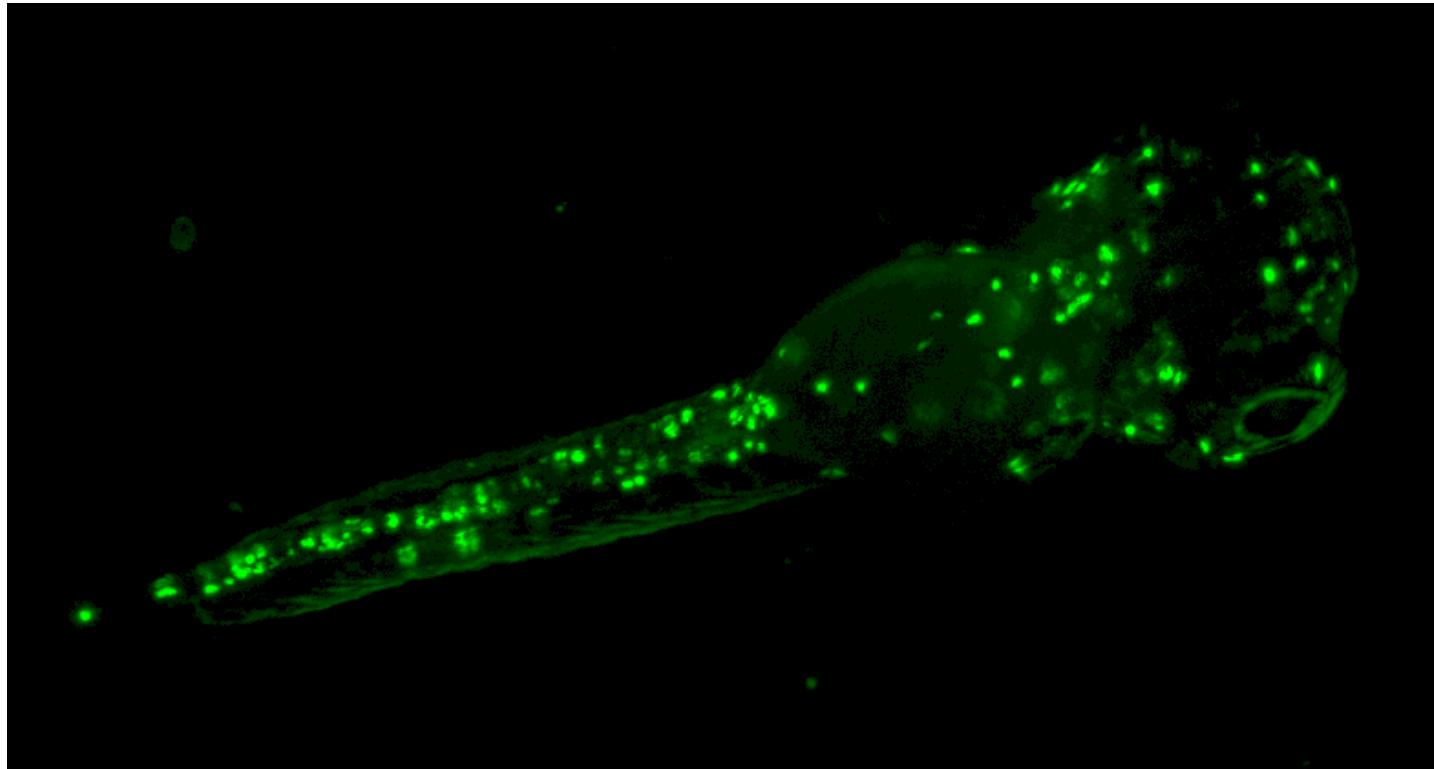
### Dipping caps, 2<sup>nd</sup> generation

- Corrects chromatic aberrations
- Stainless steel

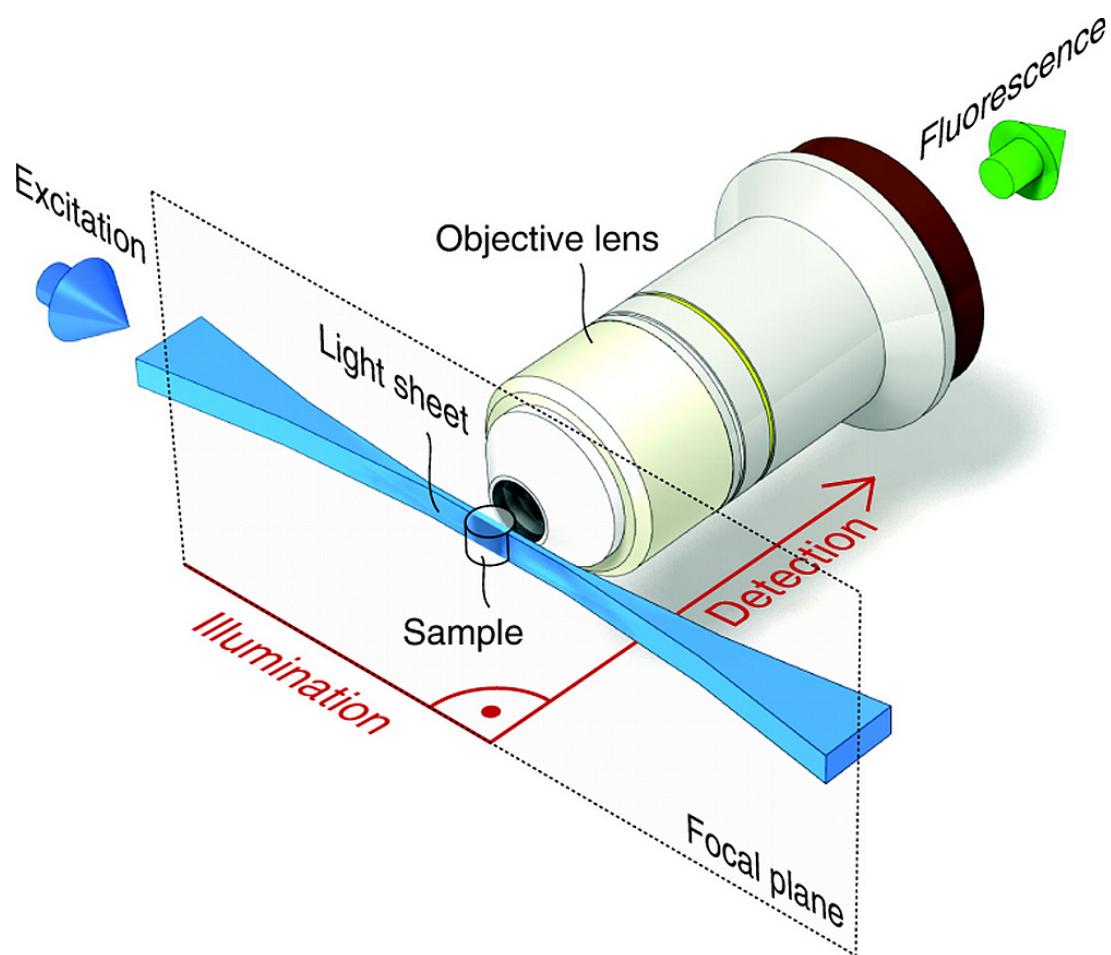
### LVBT Multi-Immersion lens

- RI-correction from 1.33 to 1.56
- for water and organic solvents

## *in vivo* imaging



# 3D Microscopy Concepts

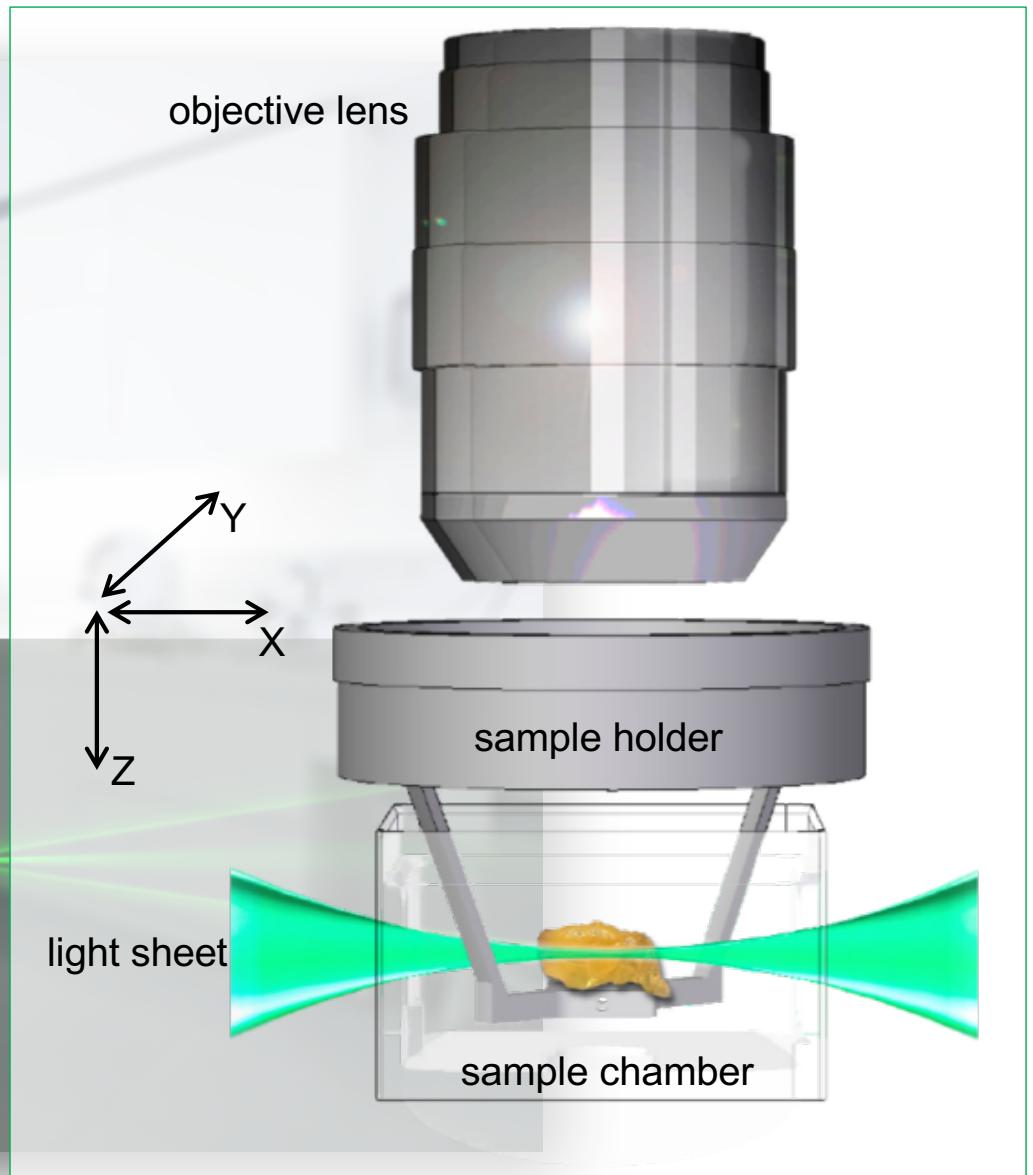
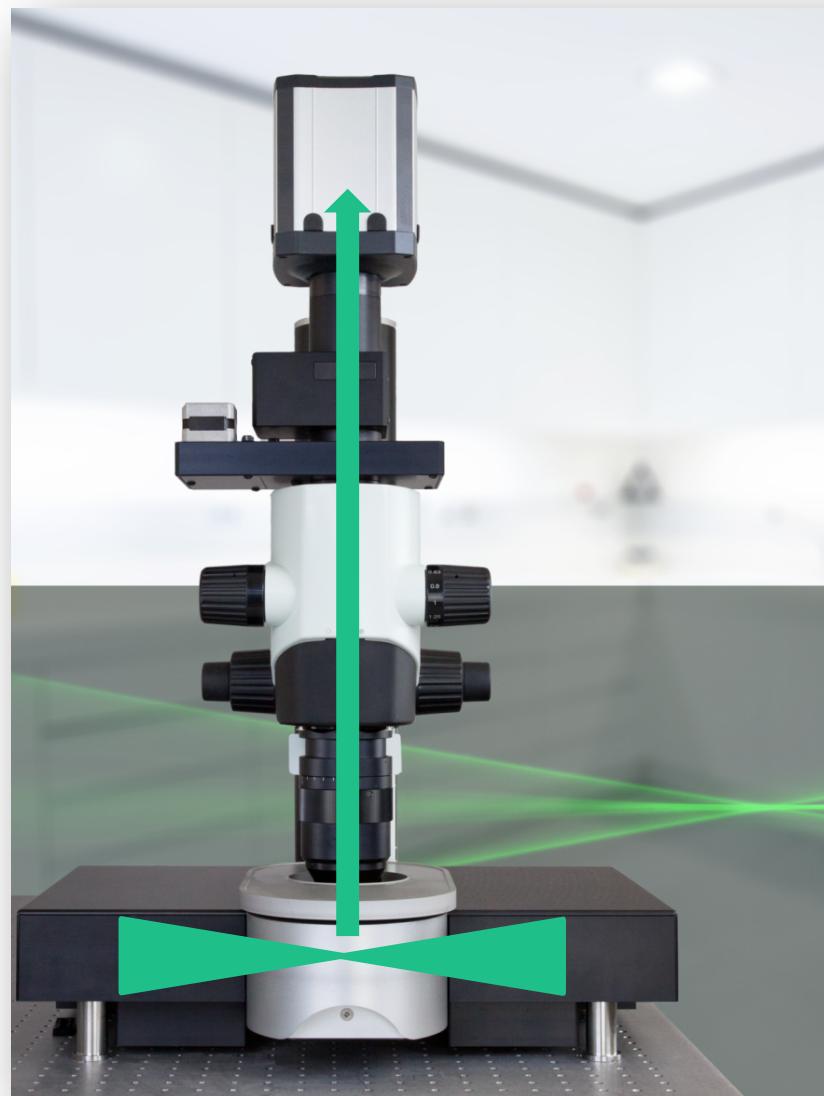


Selective plane illumination microscopy techniques in developmental biology

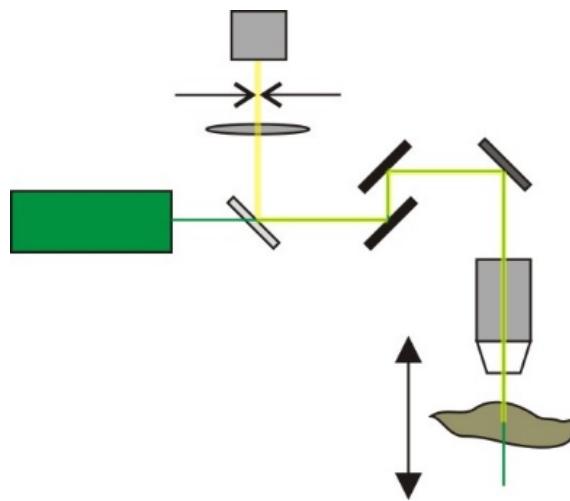
Jan Huisken, Didier Y. R. Stainier

Development 2009 136: 1963-1975; doi: 10.1242/dev.022426

# UltraMicroscope II - Setup

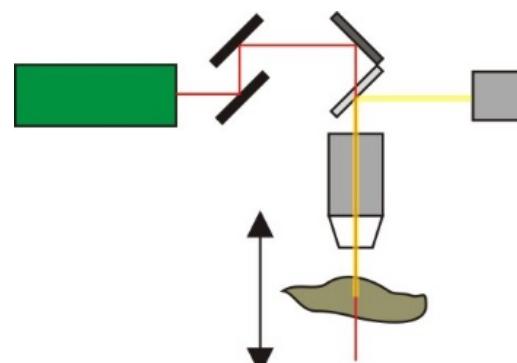


# 3D imaging concepts



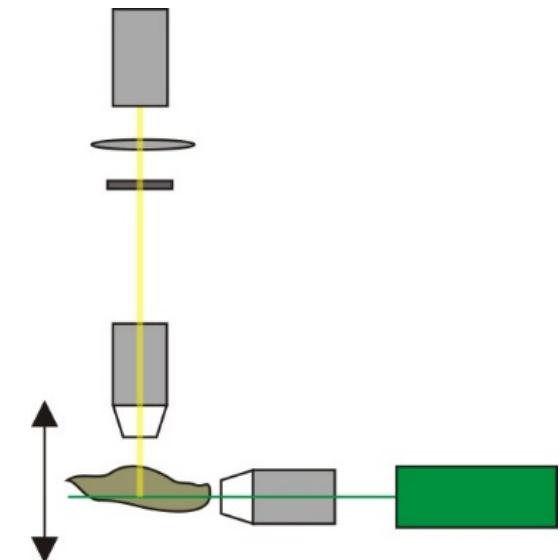
**Confocal Microscope**

- + 3D resolution
- High bleaching rate
- Penetration depth
- Slow



**2-Photon Microscope**

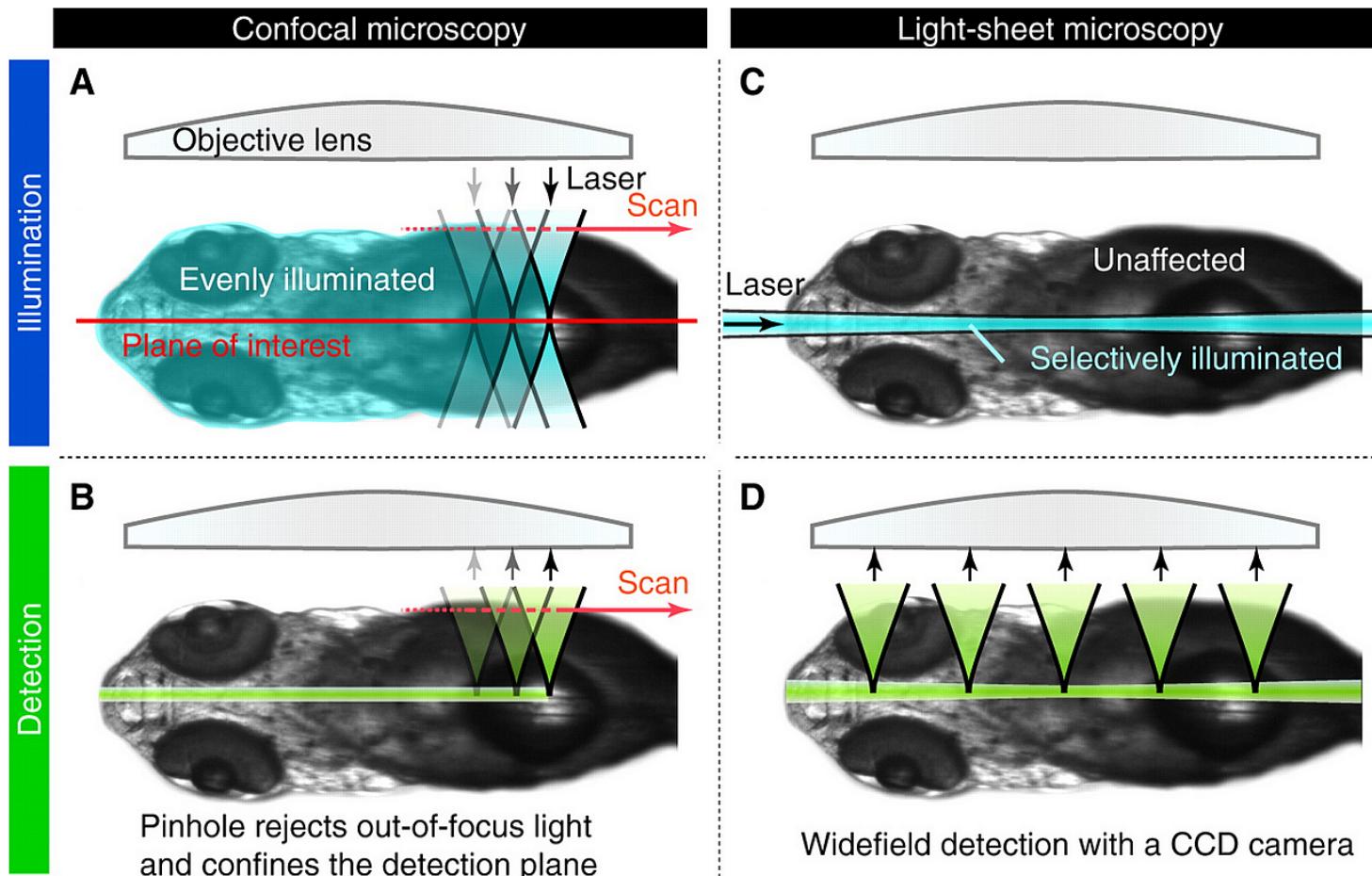
- + 3D resolution
- + Low bleaching rate
- + Penetration depth
- Slow



**Light Sheet Microscope**

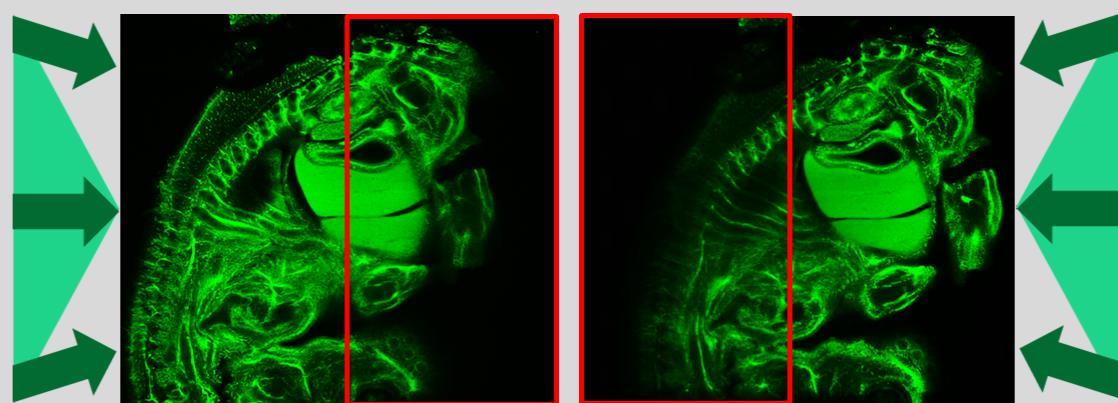
- + 3D resolution
- + Low bleaching rate
- + Penetration depth
- + Fast

# 3D Microscopy Concepts



Selective plane illumination microscopy techniques in developmental biology  
 Jan Huisken, Didier Y. R. Stainier  
 Development 2009 136: 1963-1975; doi: 10.1242/dev.022426

# UltraMicroscope – Setup excitation – Bidirectional illumination



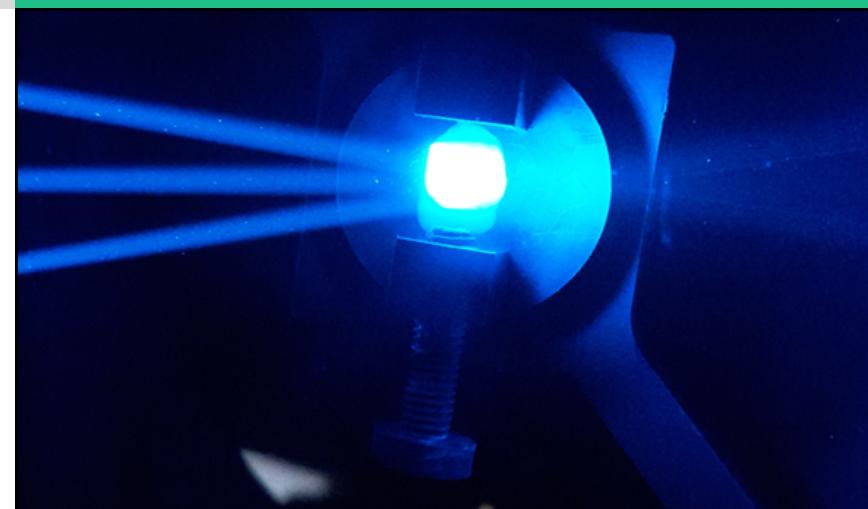
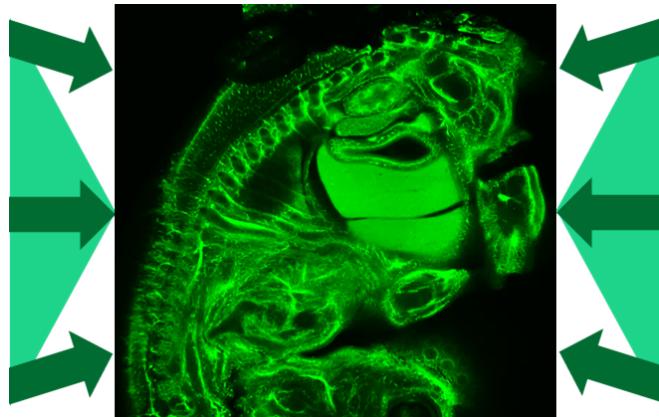
## Excitation

Set of 3 light sheets (uni- or bidirectional)

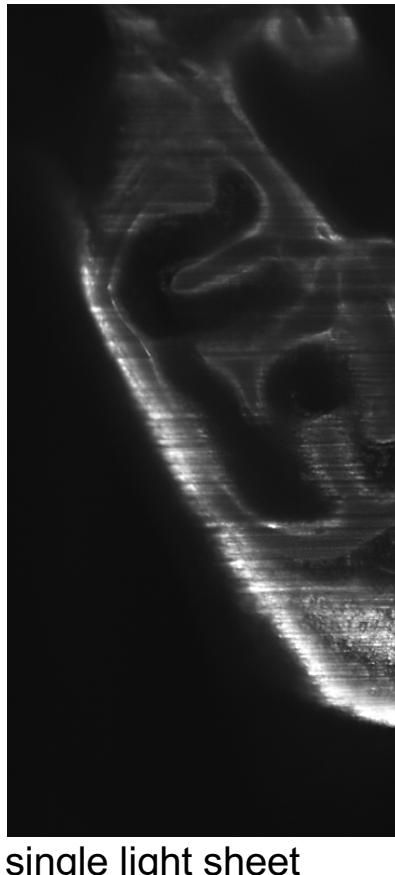
- Thickness 4 µm
- Width 1-20 mm

## Light sources

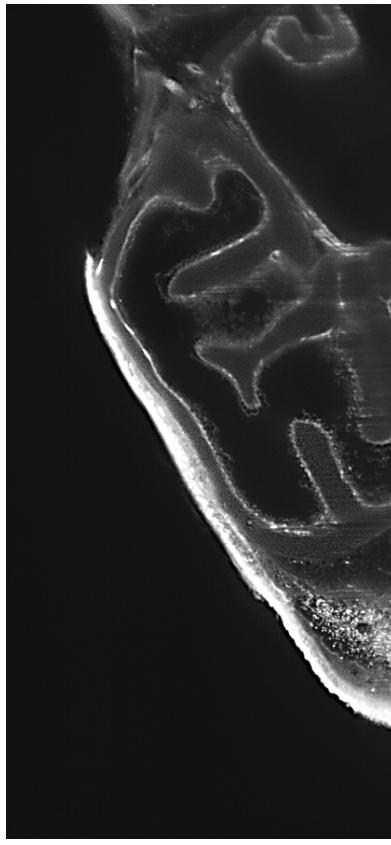
- 1 to 5 solid state lasers or laser diodes
- White light laser combined with 8 position filterwheel



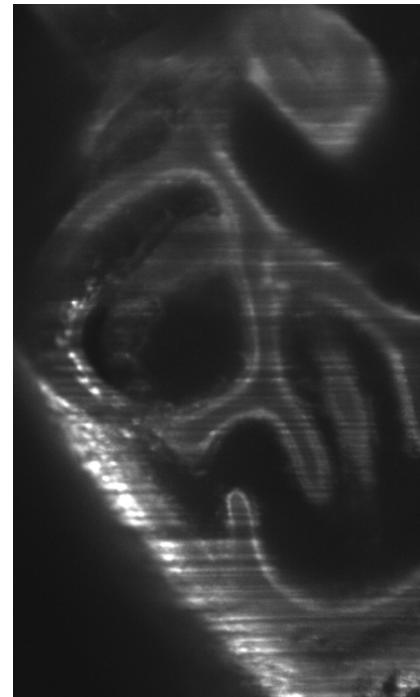
## UltraMicroscope – Multiple light sheet illumination



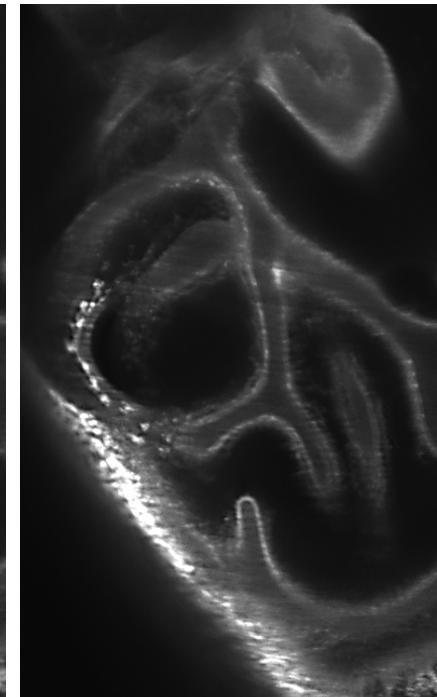
single light sheet



triple light sheet



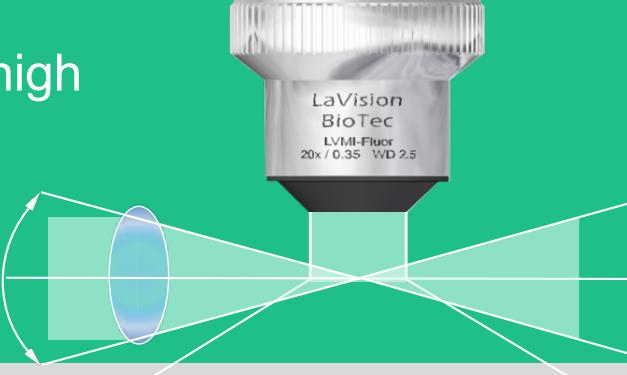
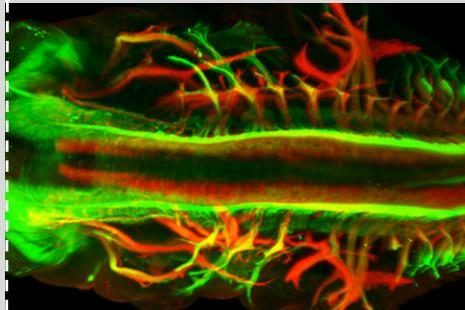
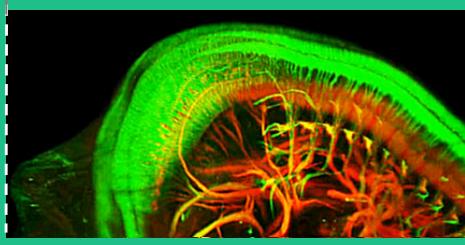
single light sheet



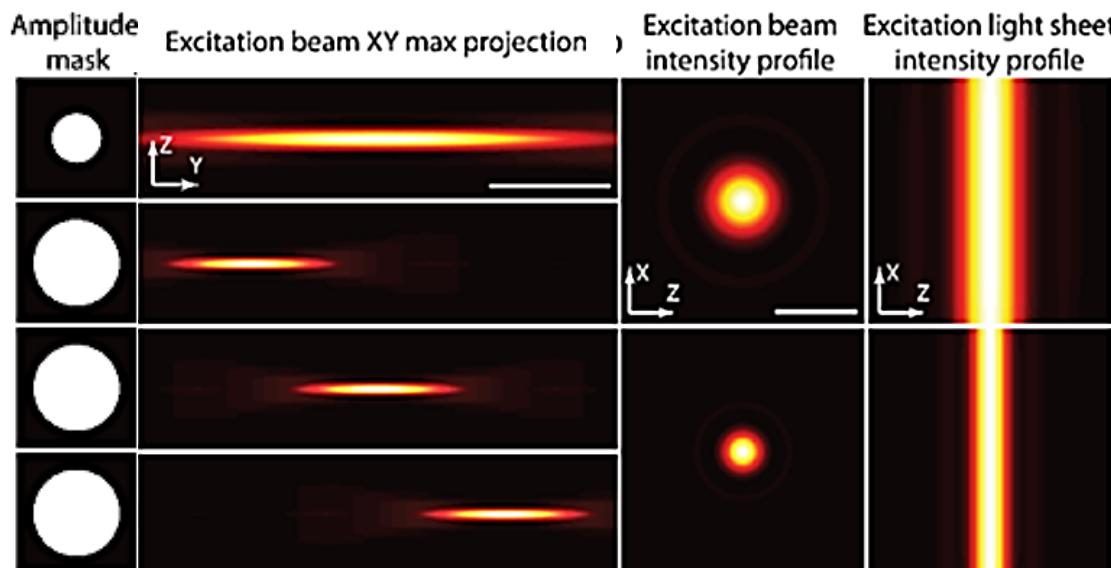
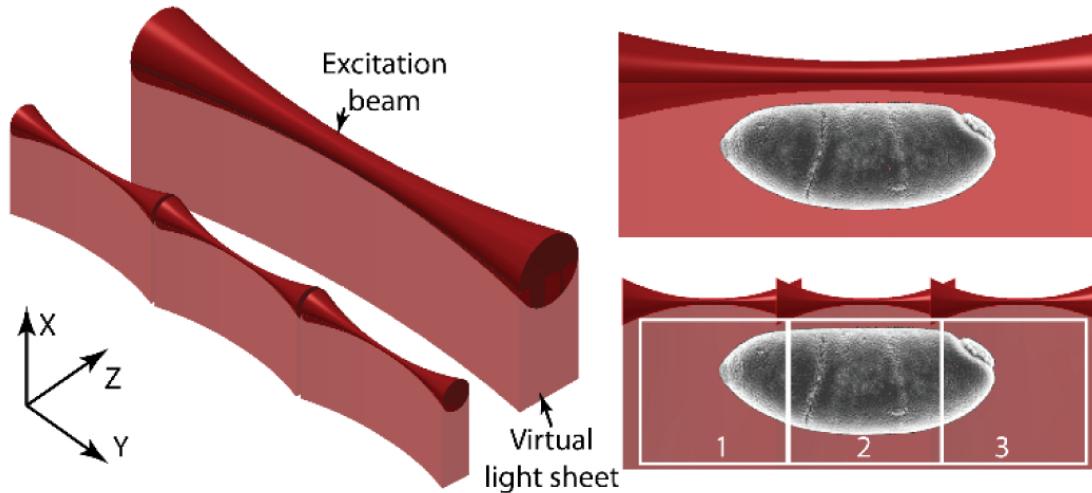
triple light sheet

Multiple light sheet illumination for de-striping and removing shadows  
(embryonal zebra finch head, autofluorescence)

# Light sheet NA & Z resolution

Light Sheet NA	high	
X,Y high contrast area (Rayleigh length)	entire FOV	
Z Resolution	high	
Light Sheet	thin	

## Dynamic horizontal focus



### Citation

Liang Gao, "Extend the field of view of selective plan illumination microscopy by tiling the excitation light sheet," Opt. Express **23**, 6102-6111 (2015);

<https://www.osapublishing.org/oe/abstract.cfm?uri=oe-23-5-6102>

Image © 2015 Optical Society of America and may be used for noncommercial purposes only. Report a [copyright concern regarding this image](#).

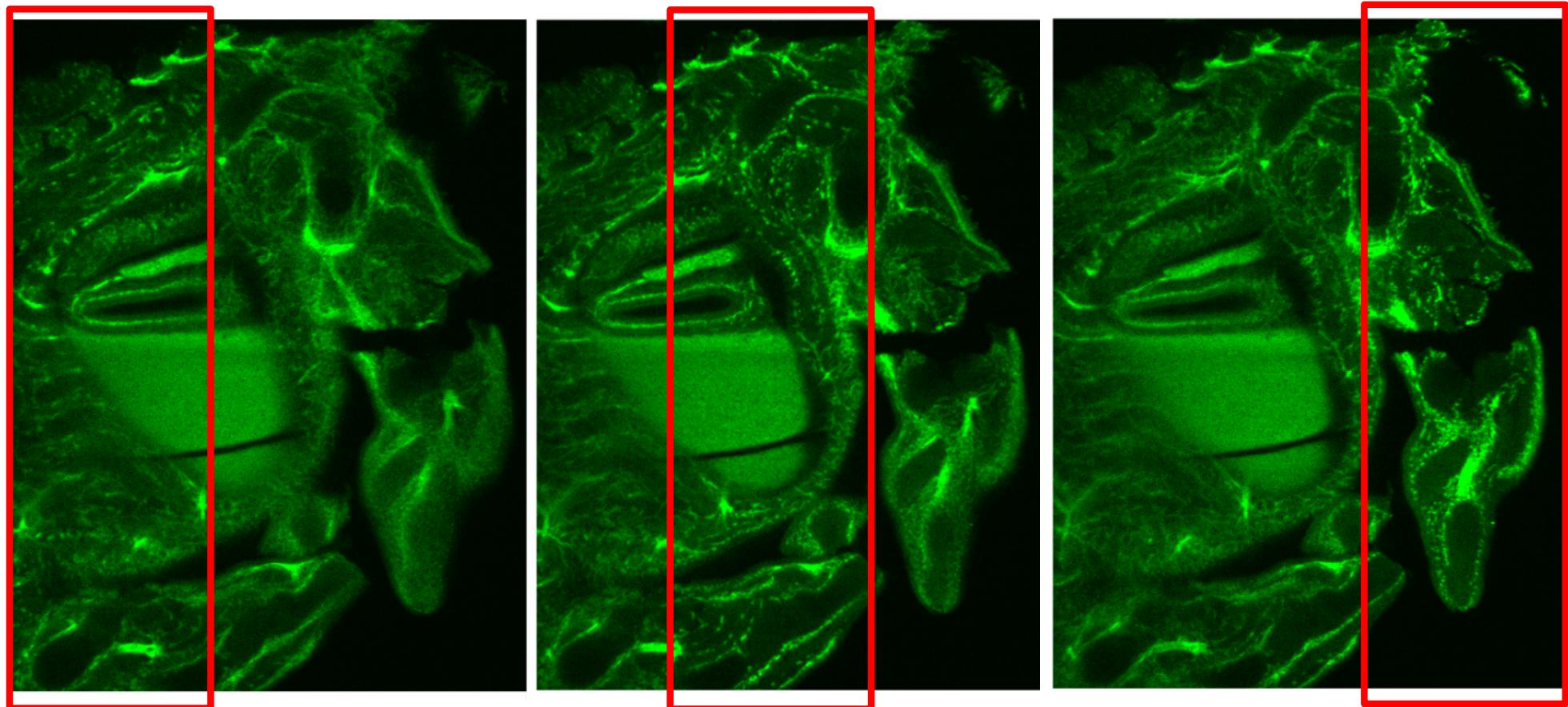
### Citation

Liang Gao, "Extend the field of view of selective plan illumination microscopy by tiling the excitation light sheet," Opt. Express **23**, 6102-6111 (2015);

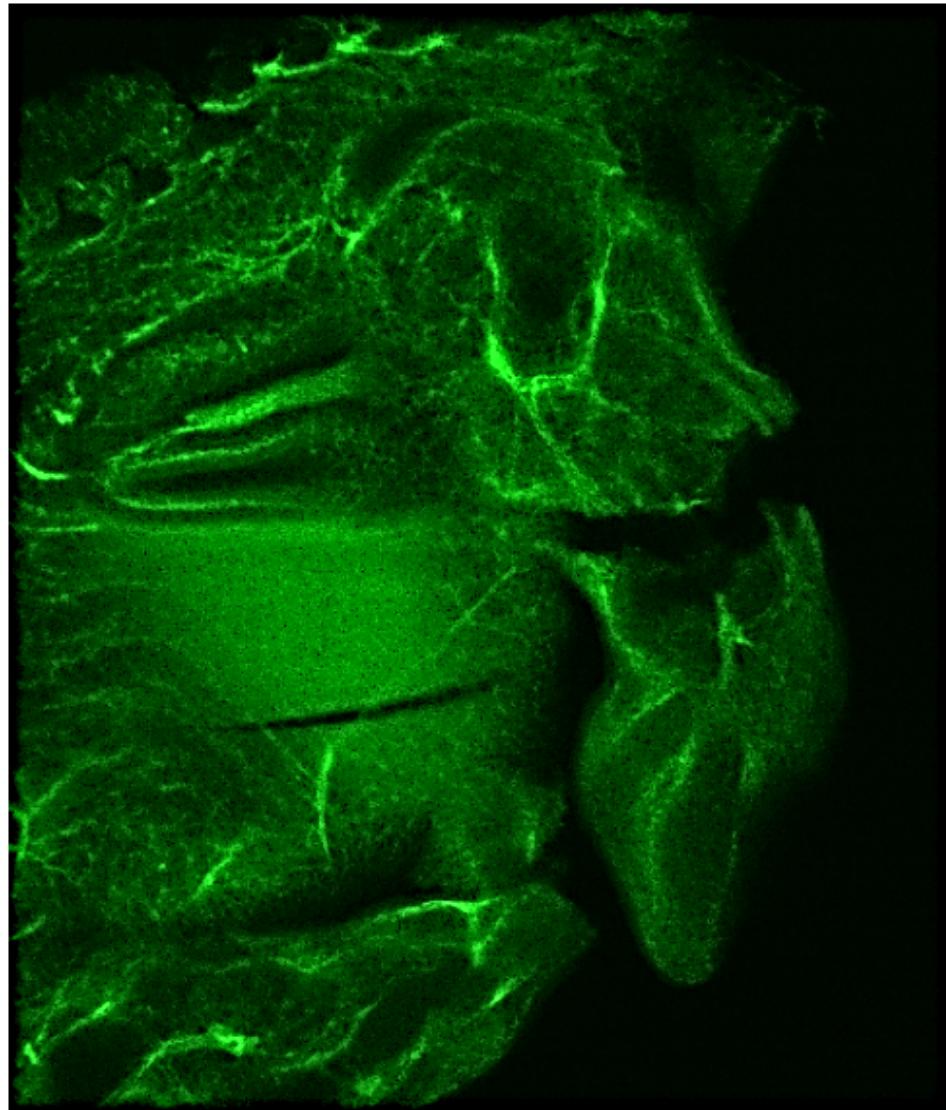
<https://www.osapublishing.org/oe/abstract.cfm?uri=oe-23-5-6102>

Image © 2015 Optical Society of America and may be used for noncommercial purposes only. Report a [copyright concern regarding this image](#).

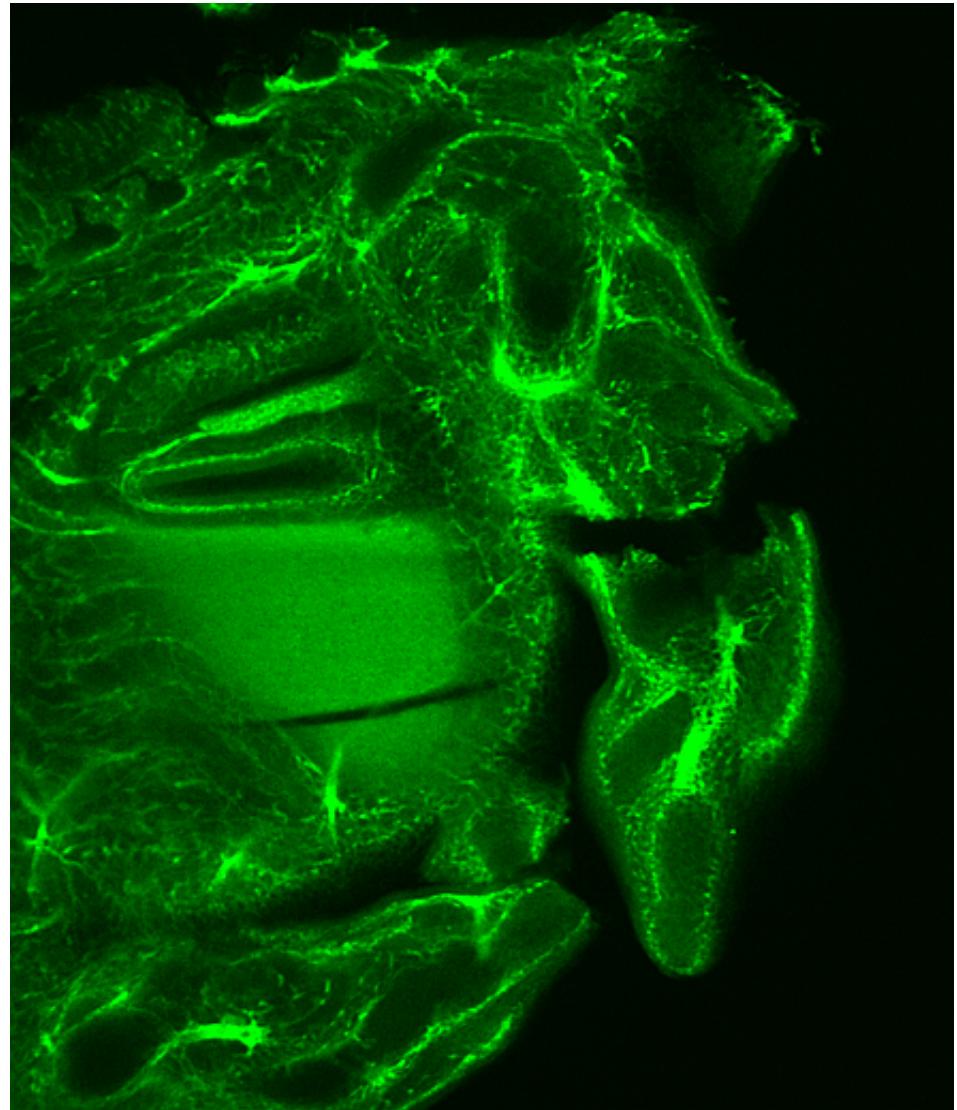
## Dynamic horizontal focus



## Dynamic horizontal focus

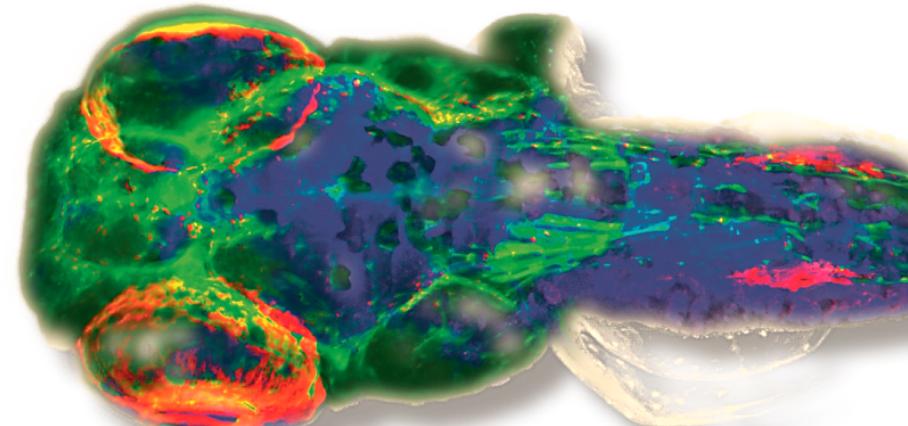
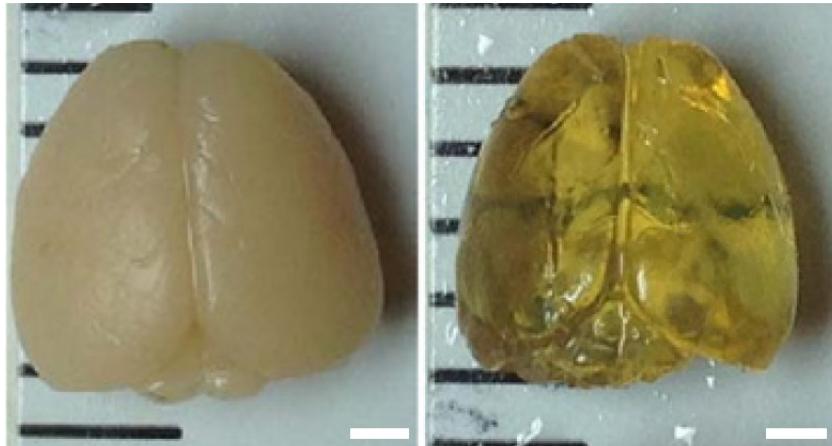


low NA, no dynamic horizontal focus

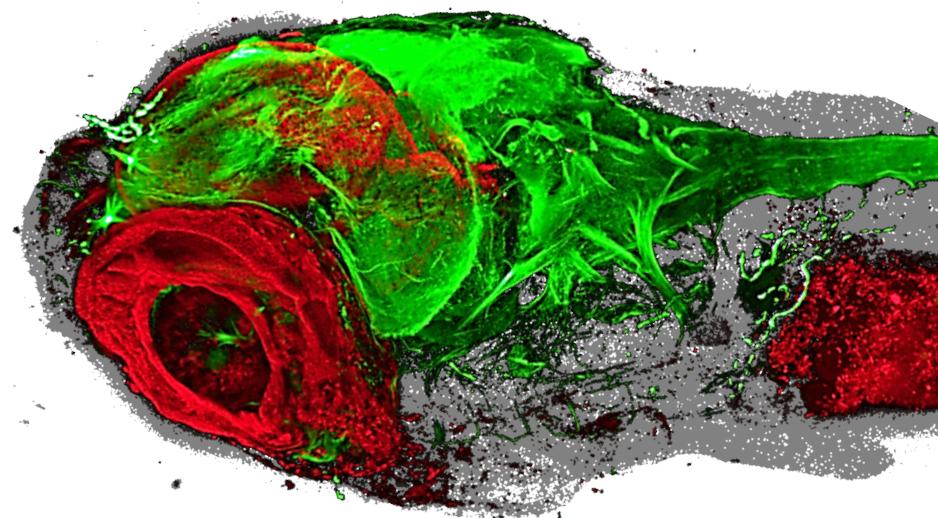


high NA, with dynamic horizontal focus

# CLEARING



Autofluorescence & GFP



Autofluorescence & Alexa 488

- iDISCO <http://idisco.info/>
- CLARITY <http://clarityresourcecenter.org/>
- CUBIC <http://cubic.riken.jp/>
- SWITCH <http://www.chunglabresources.com/sw1>
- SeeDB2 <https://sites.google.com/site/seedbresources/>

# Clearing

Protocol	Author	Principle
Methylsalicylat	Spalteholz, 1911	Solvent
BABB	Dodt et al., 2007	Solvent
THF/DBE	Dodt et al., 2012	Solvent
3DISCO	Ertürk et al., 2012	Solvent
iDISCO	Renier et al., 2014	Solvent
iDISCO+	Renier et al., 2016	Solvent
uDISCO	Pan, Ertürk et al, 2016	Solvent
FluoClearBABB	Schwarz et al., 2015	Solvent
Eci	Klingberg et al., 2016	Solvent
CLARITY	Chung & Deisseroth, 2013	Hydrogel
PACT	Yang et al., 2014	Hydrogel
PARS	Yang et al., 2014	Hydrogel
SE-CLARITY	Kim et al., 2015	Hydrogel
SWITCH	Murray et al., 2015	Hydrogel
EDC-CLARITY	Sylwestrak et al., 2016	Hydrogel
ACT-PRESTO	Lee et al., 2016	Hydrogel
Chloralhydrat	Amann, 1899	Immersion
FocusClear	Chiang et al., 2002	Immersion
TDE	Staudt et al., 2007	Immersion
Sucrose	Tsai et al., 2009	Immersion
ClearT	Kuwajima et al., 2013	Immersion
ClearT2	Kuwajima et al., 2013	Immersion
SeeDB	Ke et al., 2013	Immersion
FRUIT	Hou et al., 2015	Immersion
SeeDB2	Ke et al., 2016	Immersion
Scale	Hama et al., 2011	Hyperhydration
ScaleA2	Hama et al., 2011	Hyperhydration
CUBIC	Susaki et al., 2014	Hyperhydration
ScaleS	Hama et al., 2015	Hyperhydration

Organic solvent clearing

Aqueous buffer clearing

# Aqueous buffer clearing – CLARITY – Hydrogel group

## CLARITY, (PACT-PARS)

Nature 2013 June

### CLARITY for mapping the nervous system

Kwanghun Chung & Karl Deisseroth

#### Pro:

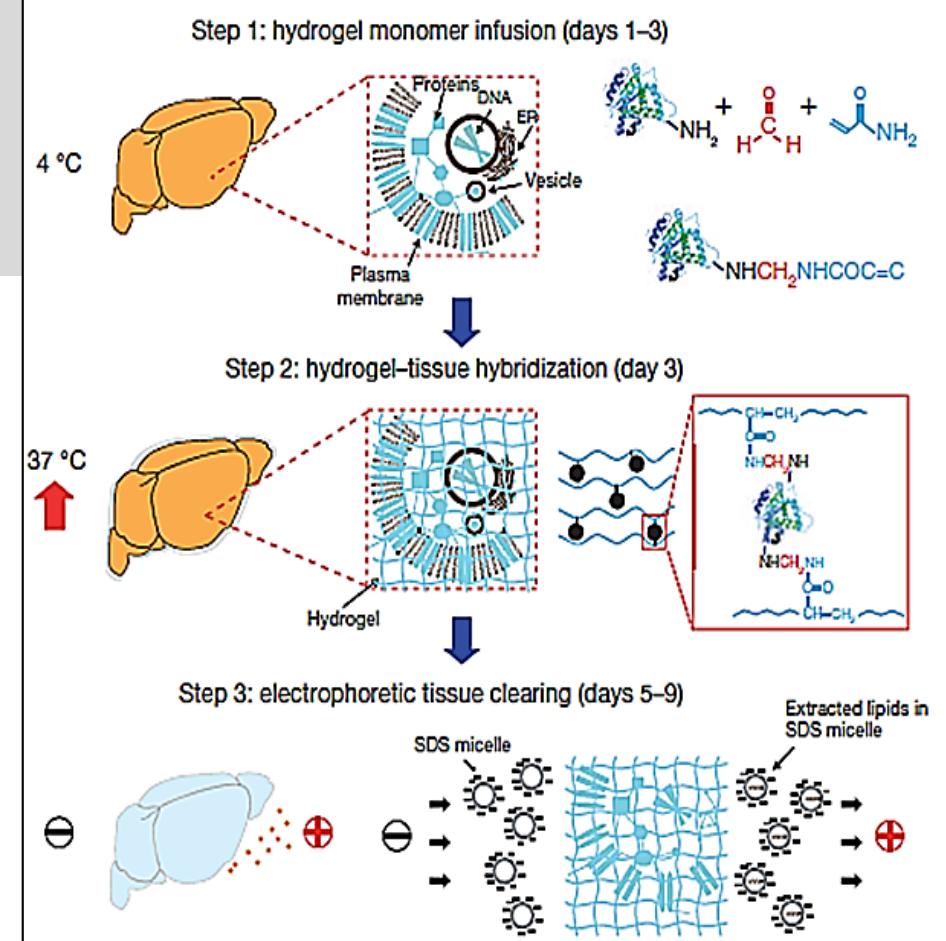
- XFP preserved

#### Cons:

- Complex protocol
- Samples can be easily burned by the electrophoresis
- Passive protocol takes a long time to get samples cleared
- Jelly like structure of the sample
- Difficult to fix the sample for imaging
- Sample may swell

#### Imaging buffers:

- RIMS
- sRIMS
- SDS CLARITY buffer
- Glycerol + DABCO
- PBS
- 64% - 73% TDE

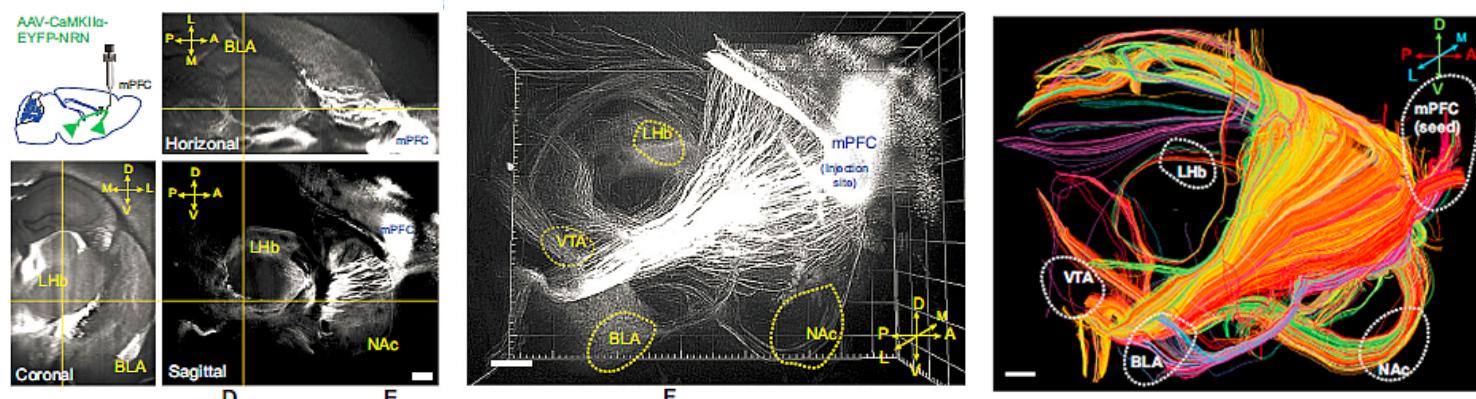


# Aqueous buffer clearing – CLARITY – Hydrogel group

Cell. 2016 June 16; Volume 165, Issue 7, p1776–1788.

## Wiring and Molecular Features of Prefrontal Ensembles Representing Distinct Experiences

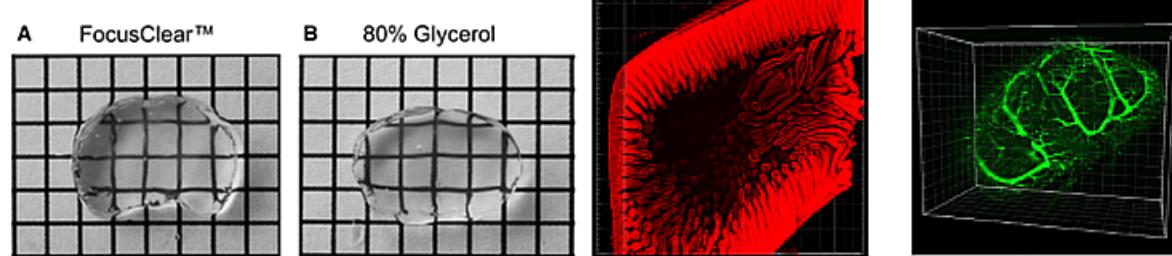
Li Ye, William E. Allen, Kimberly R. Thompson, Qiyuan Tian, Brian Hsueh, Charu Ramakrishnan, Ai-Chi Wang, Joshua H. Jennings, Avishek Adhikari, Casey H. Halpern, Ilana B. Witten, Alison L. Barth, Liqun Luo, Jennifer A. McNab, Karl Deisseroth



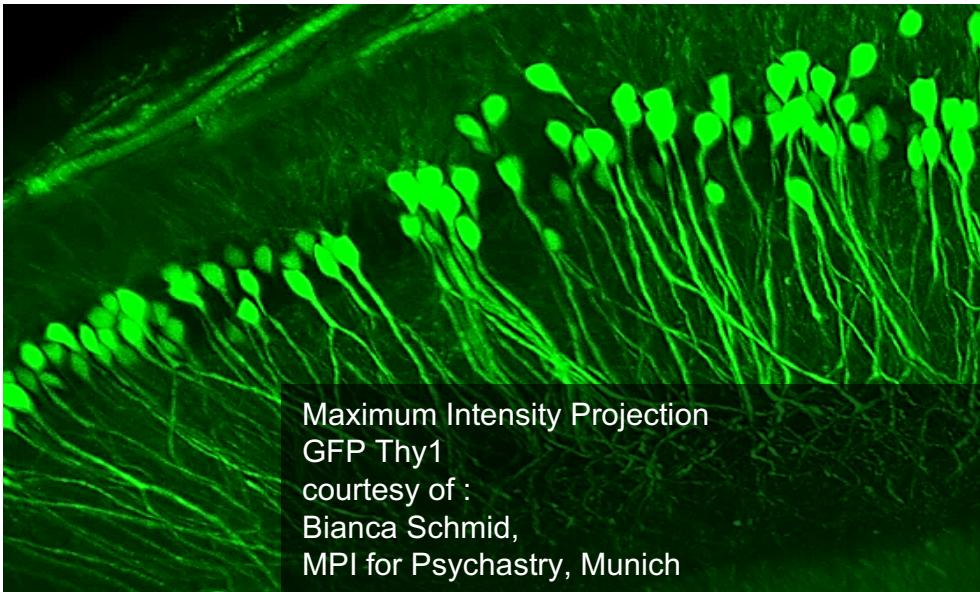
DOI: 10.1523/ENEURO.0022-15.2015

## Optimization of CLARITY for Clearing Whole-Brain and Other Intact Organs

Jonathan R. Epp, Yosuke Niibori, Hwa-Lin (Liz) Hsiang, Valentina Mercaldo, Karl Deisseroth, Sheena A. Josselyn, Paul W. Frankland



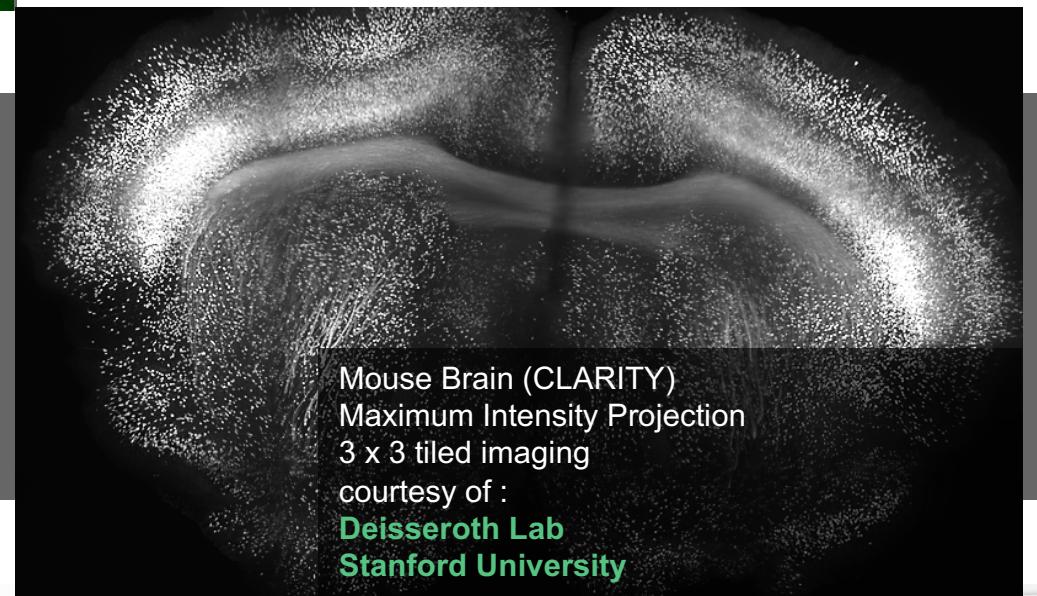
# Aqueous buffer clearing – CLARITY – Hydrogel group



CLARITY  
PACT  
PARS  
SE-CLARITY  
SWITCH  
EDC-CLARITY  
ACT-PRESTO

sRIMS  
63% TDE  
mineral/silicone oil

Glycerol  
RIMS  
Focus Clear



# Aqueous buffer clearing – CUBIC – Hyperhydration group

Nat Protoc. 2015 Nov;10(11):1709-27. doi: 10.1038/nprot.2015.085. Epub 2015 Oct 8.

## Advanced CUBIC protocols for whole-brain and whole-body clearing and imaging

Susaki EA, Tainaka K, Perrin D, Yukinaga H, Kuno A, Ueda HR.

Cell 2014 Nov

## Whole-Body Imaging with Single-Cell Resolution by Tissue Decolorization

Kazuki Tainaka, Shimpei I. Kubota, Takeru Q. Suyama, Etsuo A. Susaki, Dimitri Perrin, Maki Ukai-Tadenuma, Hideki Ukai, and Hiroki R. Ueda

RI= 1.48 – 1.49

- XFP preserved
- No complex protocol
- Less swelling of samples
- De-colorization

CUBIC – web page  
<http://cubic.riken.jp/>

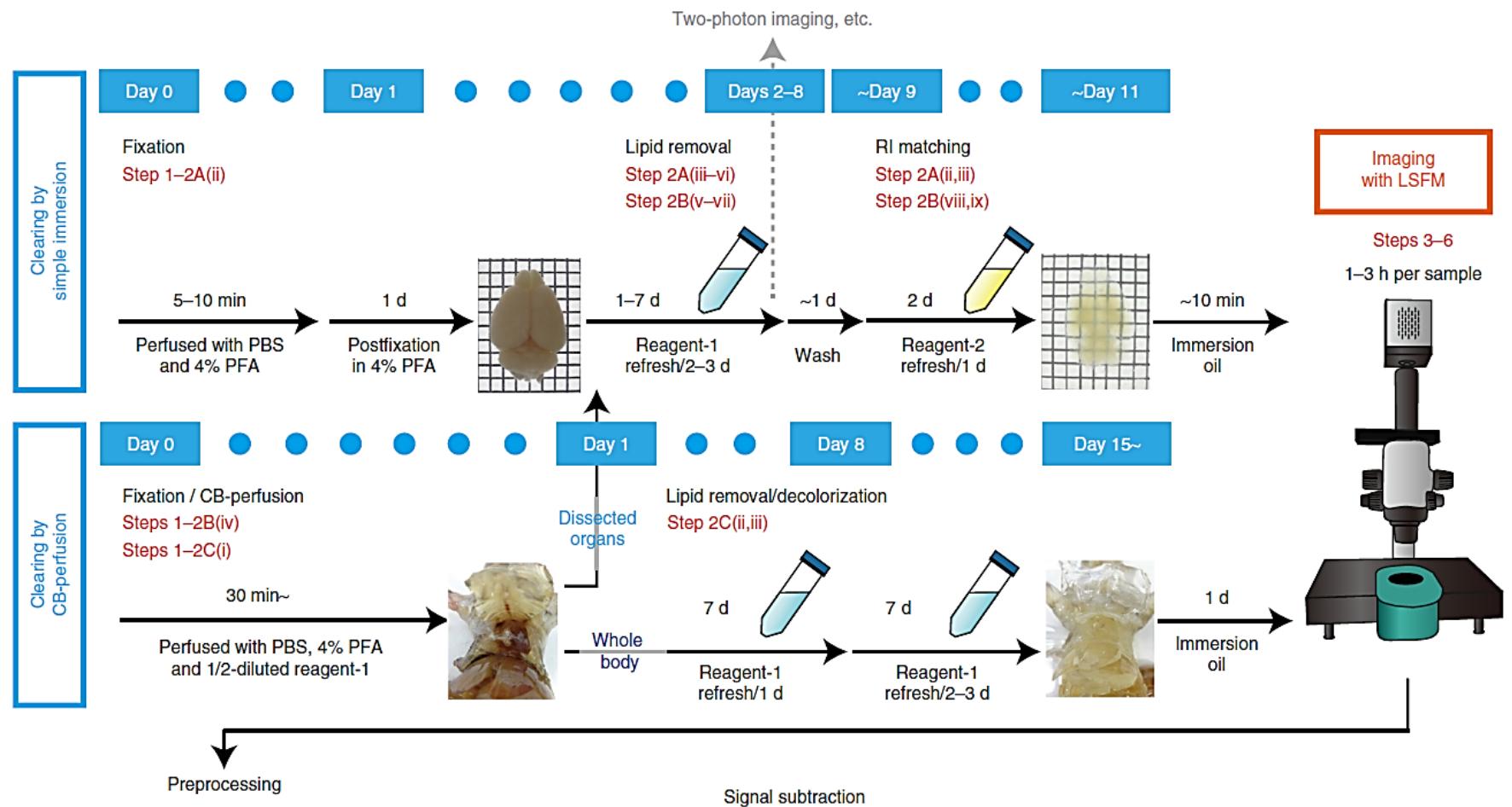


# Aqueous buffer clearing – CUBIC – Hyperhydration group

## Advanced CUBIC protocols for whole-brain and whole-body clearing and imaging

Nat Protoc. 2015 Nov;10(11):1709-27. doi: 10.1038/nprot.2015.085. Epub 2015 Oct 8.

Susaki EA, Tainaka K, Perrin D, Yukinaga H, Kuno A, Ueda HR.



# Aqueous buffer clearing – Scale – Hyperhydration group

## Scale

**Scale: a chemical approach for fluorescence imaging and reconstruction of transparent mouse brain**

*Hiroshi Hama, Hiroshi Kurokawa, Hiroyuki Kawano, Ryoko Ando, Tomomi Shimogori,*

*Hisayori Noda, Kiyoko Fukami, Asako Sakaue-Sawano & Atsushi Miyawaki*

Nature Neuroscience August 2011

Clearing based on highly concentrated Urea buffer. RI= 1.38

Scale composed of 4 M urea, 30% glycerol  
and 0.1% Triton X-100.

### Pro:

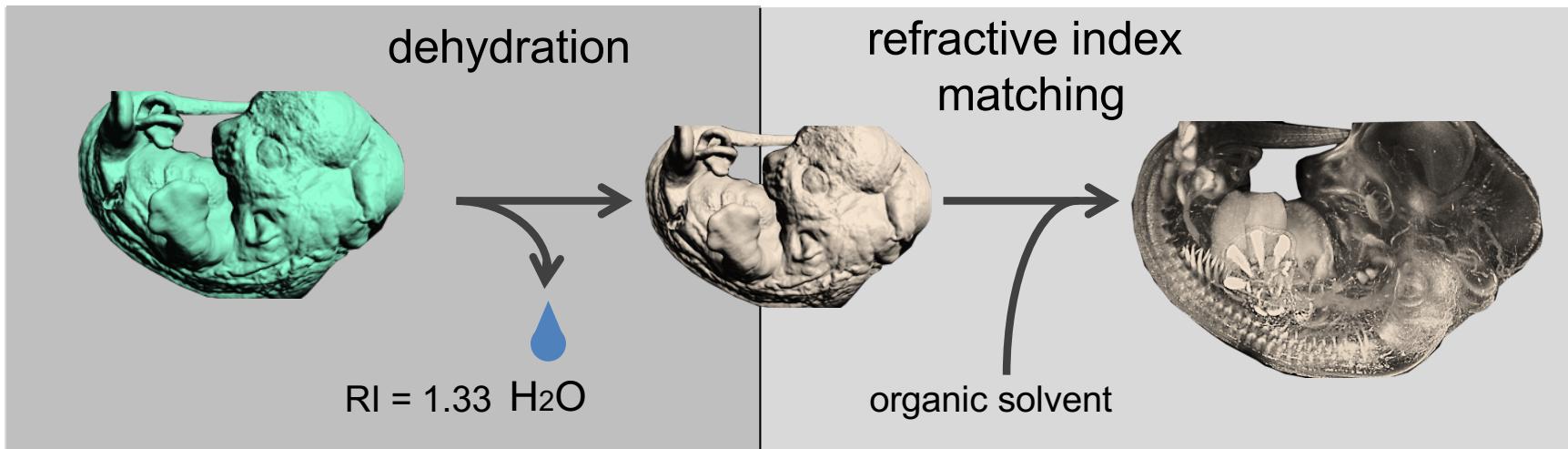
- XFP preserved

### Cons:

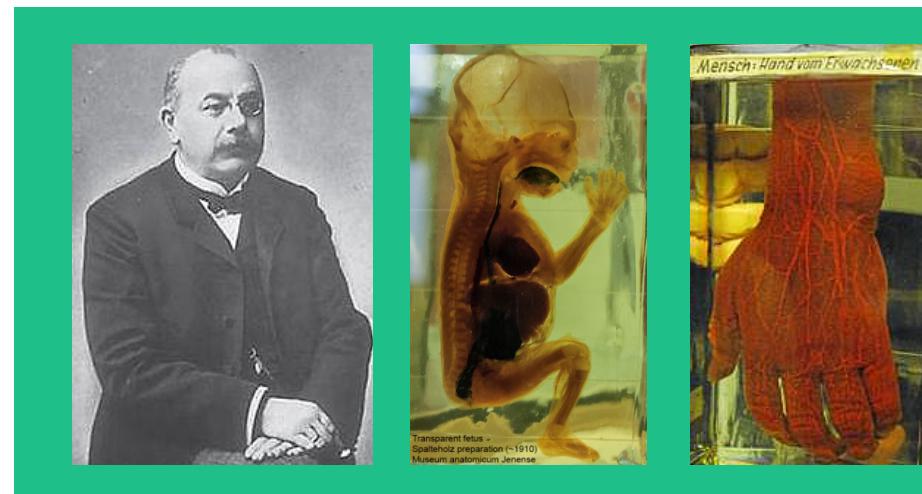
- Extended incubation times (several weeks depending on sample size)
- Samples swell to double of the original size.
- Jelly like structure of the sample (stranded-whale-syndrome), sample collapse due to its own weight
- Difficult to fix the sample for imaging
- Sample not absolutely transparent



# Organic solvent clearing



Methylsalicylate	(Spalteholz, 1911)
BABB	(Dodd et al., 2007)
THF/DBE	(Dodd et al., 2012)
3DISCO	(Ertürk et al., 2012)
iDISCO	(Renier et al., 2014)
FluoClearBABB	(Giese et al., 2015)
sDISCO	(Dodd, SfN 2015)
iDISCO+	(Renier et al., 2016)
uDISCO	(Ertürk et al., 2016)
ECi	(Klingberg et al. 2016)



# Organic solvent clearing – THF/DBE

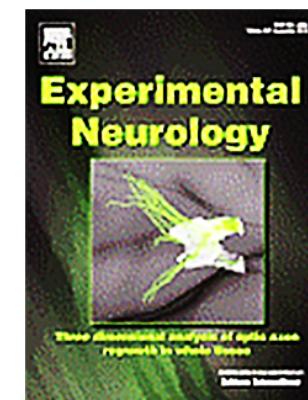
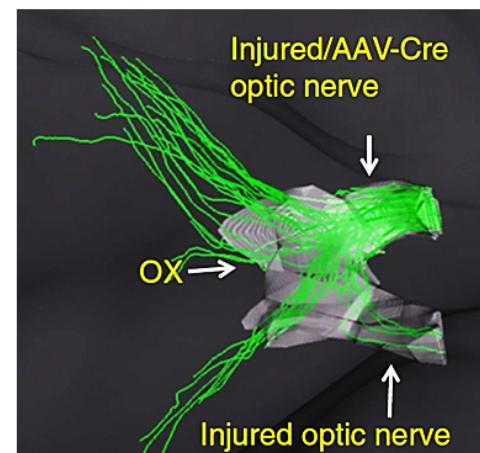
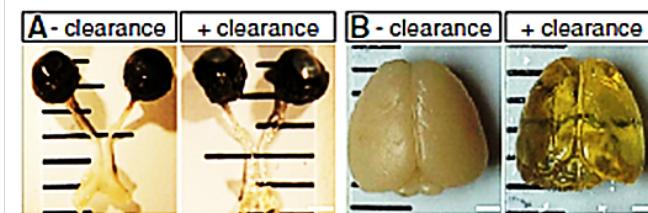
## Tetrahydrofuran (THF) & Benzylether (DBE)

Three-dimensional evaluation of retinal ganglion cell axon regeneration and pathfinding in whole mouse tissue after injury

Xuetong Luo, Yadira Salgueiro, Samuel R. Beckerman, Vance P. Lemmon, Pantelis Tsoufas, Kevin K. Park \*

Miami Project to Cure Paralysis, University of Miami, Miller School of Medicine, Miami, FL, 33136, USA

Department of Neurosurgery, University of Miami, Miller School of Medicine, Miami, FL, 33136, USA



# Organic solvent clearing – 3DISCO

## Tetrahydrofuran (THF) & Benzylether (DBE), 3DISCO

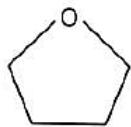
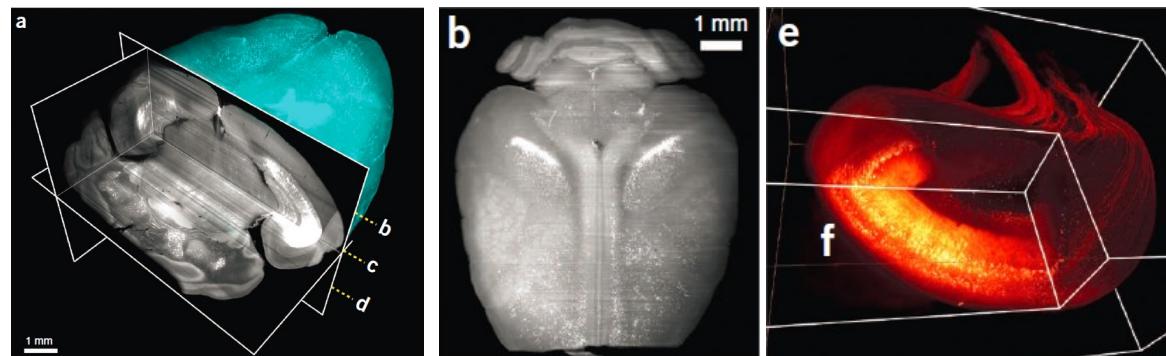
Chemical Clearing and Dehydration of GFP Expressing Mouse Brains, Dodt et al., 2011  
 Three-dimensional imaging of solvent-cleared organs using 3DISCO, Erturk et al., 2012

### Pro:

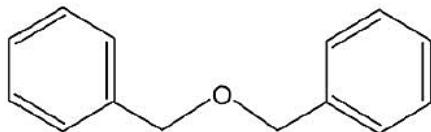
- Very clear samples
- Useful for dense samples
- Simple protocol
- Low costs
- Fast

### Cons:

- GFP not preserved



Tetrahydrofuran (THF)



Dibenzyl ether (DBE)

Reagents	Mammary gland, lymph node	Spinal cord, lung, spleen	Brain stem	Brain
50% (vol/vol) THF	20 min	30 min	1 h	1 h
70% (vol/vol) THF	20 min <sup>a</sup>	30 min <sup>a</sup>	1 h <sup>a</sup>	1 h
80% (vol/vol) THF	20 min	30 min	1 h	1 h
100% (vol/vol) THF	3 × 20 min	3 × 30 min	2 × 1 h	1 h, overnight, 1h
DCM	15 min	20 min	45 min	—
DBE	≥15 min	≥15 min	≥30 min	≥3 h

# Organic solvent clearing – iDISCO

## iDISCO

Cell. 2014 Nov 6;159(4):896-910

**iDISCO: A Simple, Rapid Method to Immunolabel Large Tissue Samples for Volume Imaging.**

Renier N, Wu Z, Simon DJ, Yang J, Ariel P, Tessier-Lavigne M.

Cell Rep. 2014 Nov 20;9(4):1191-201.

**A Simple Method for 3D Analysis of Immunolabeled Axonal Tracts in a Transparent Nervous System.**

Belle M, Godefroy D, Dominici C, Heitz-Marchaland C, Zelina P, Hellal F, Bradke F, Chédotal A

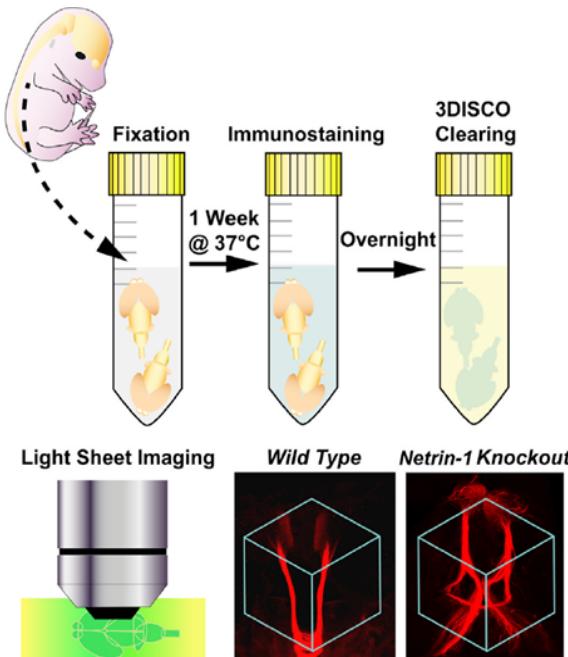
### Pro:

- Very clear samples
- Useful for dense samples
- Immunolabeling

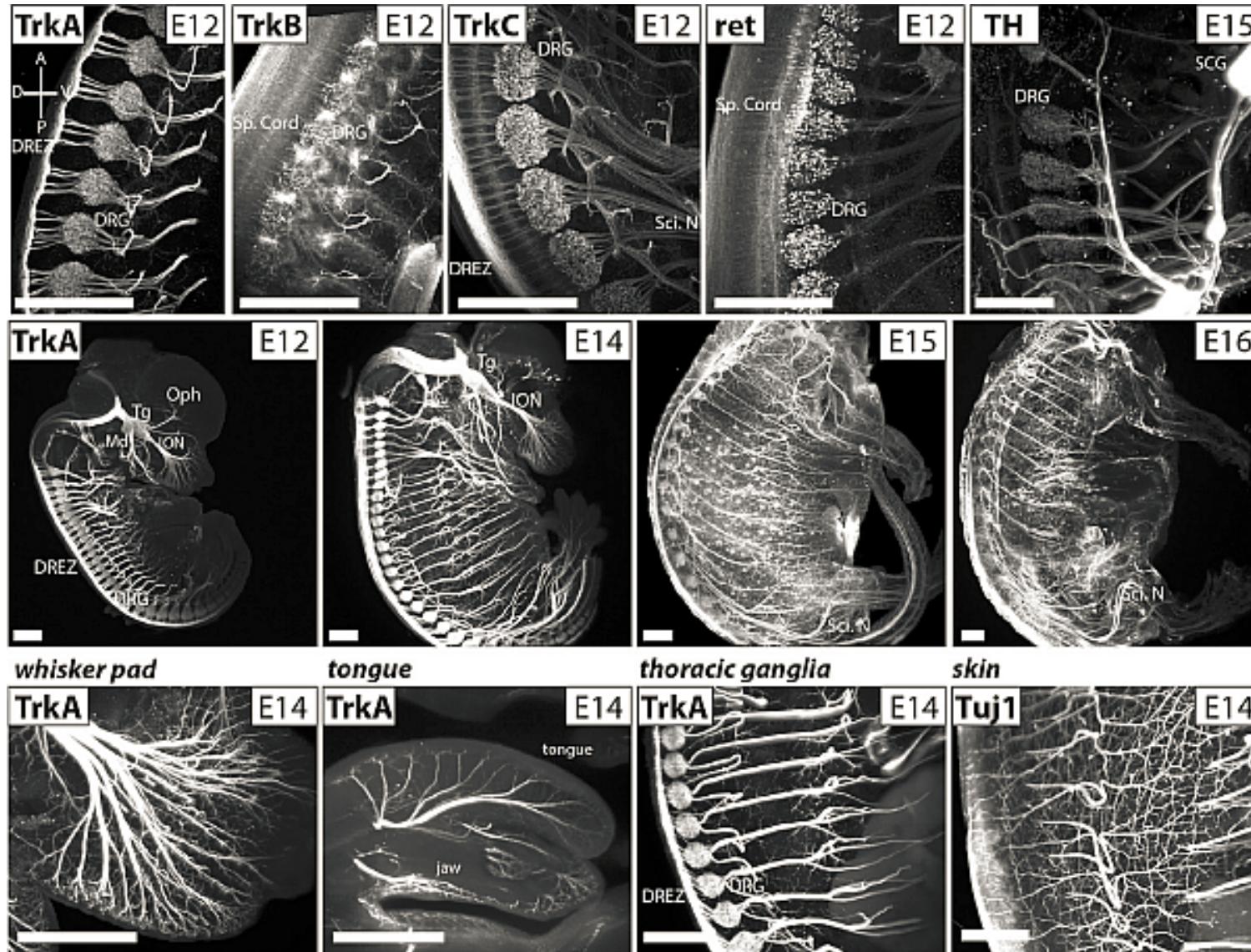
### Cons:

- GFP not preserved
- Extended protocol

<http://idisco.info/>



# Organic solvent clearing – iDISCO

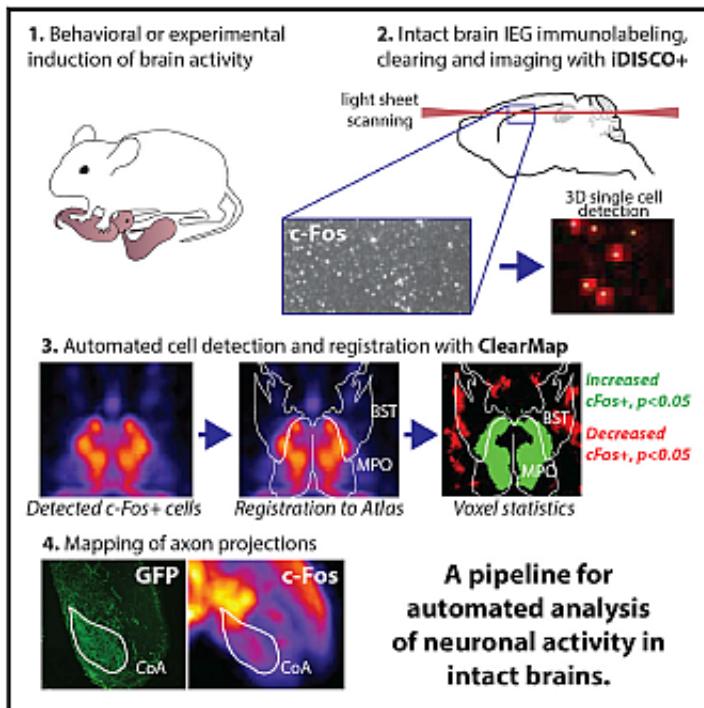


# Organic solvent clearing – iDISCO+

## Cell

### Mapping of Brain Activity by Automated Volume Analysis of Immediate Early Genes

#### Graphical Abstract



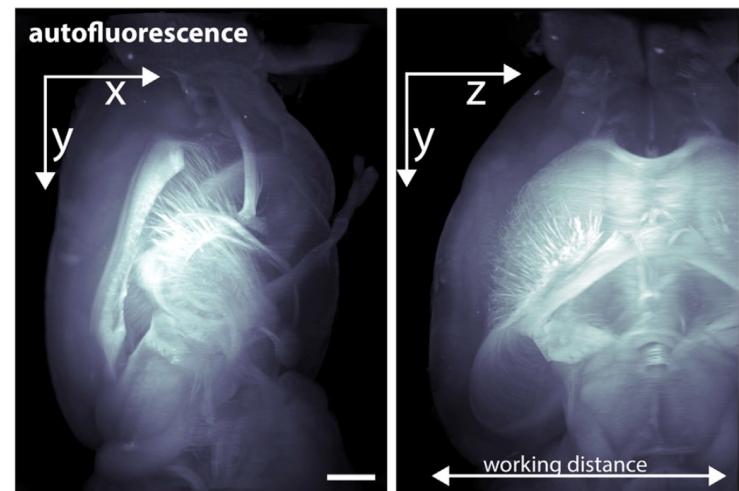
#### Authors

Nicolas Renier, Eliza L. Adams,  
Christoph Kirst, ..., Catherine Dulac,  
Pavel Osten, Marc Tessier-Lavigne

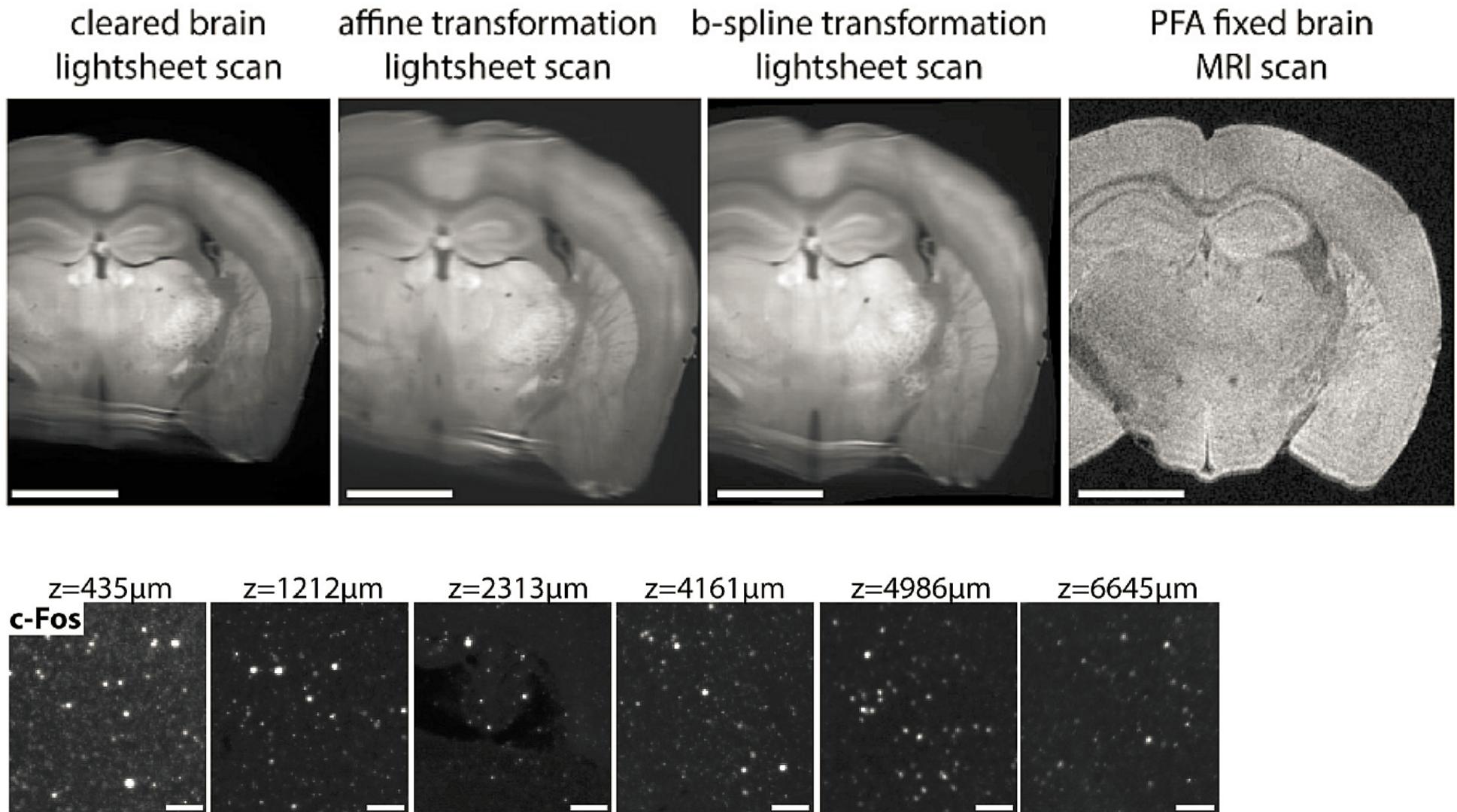
**Correspondence**  
marctl@rockefeller.edu

#### In Brief

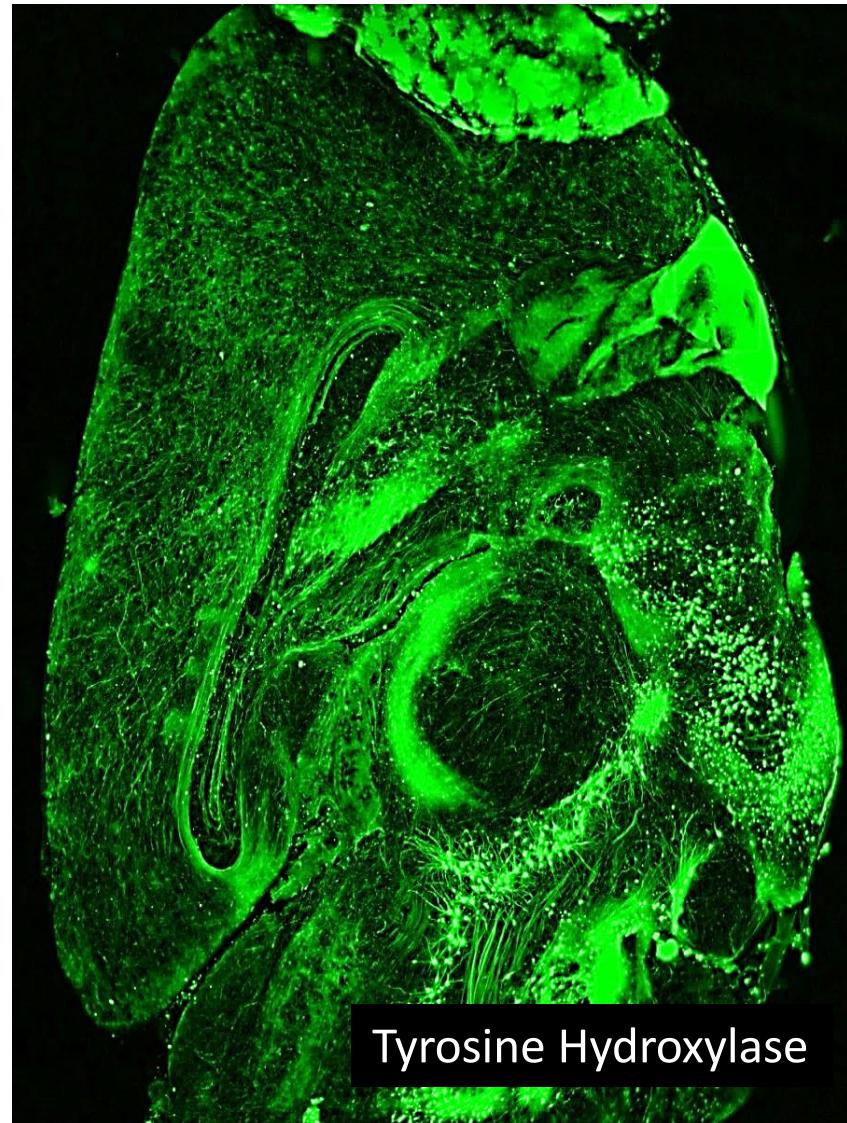
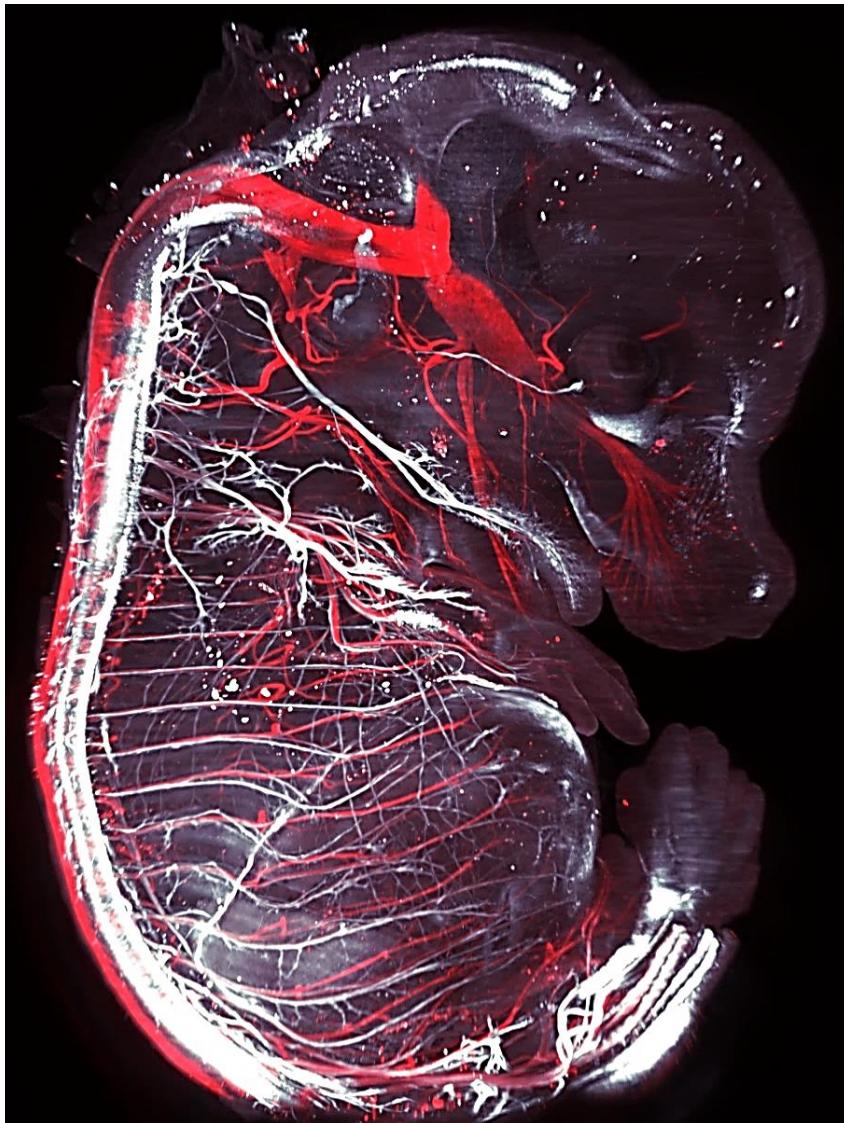
The neural circuits in mice involved in complex behaviors, such as parenting, can be visualized by combining tissue clearing with activity mapping in an automated platform called ClearMap.



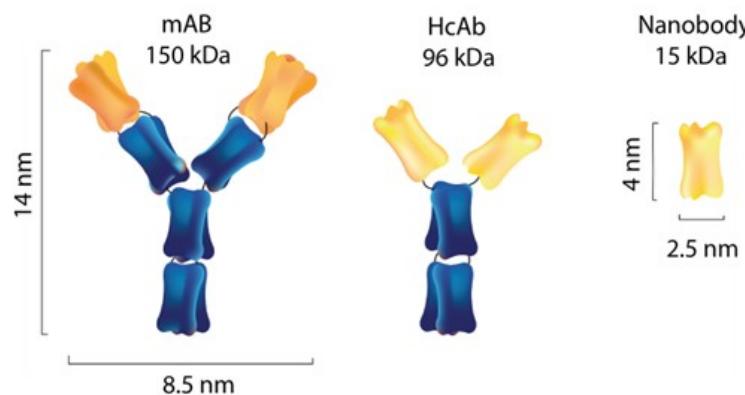
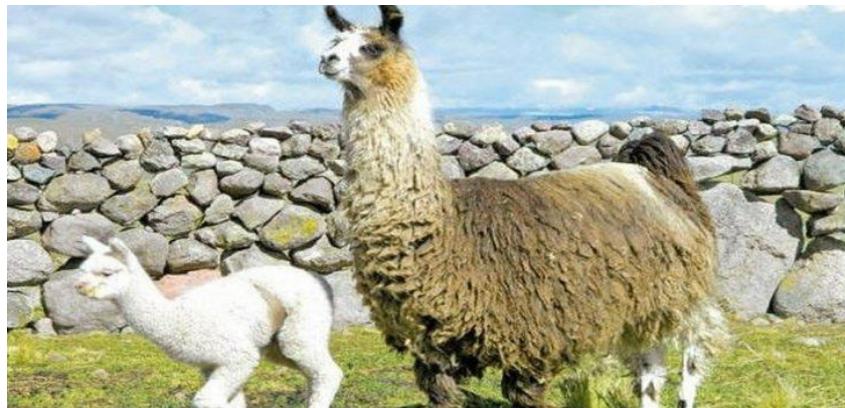
## Organic solvent clearing – iDISCO+



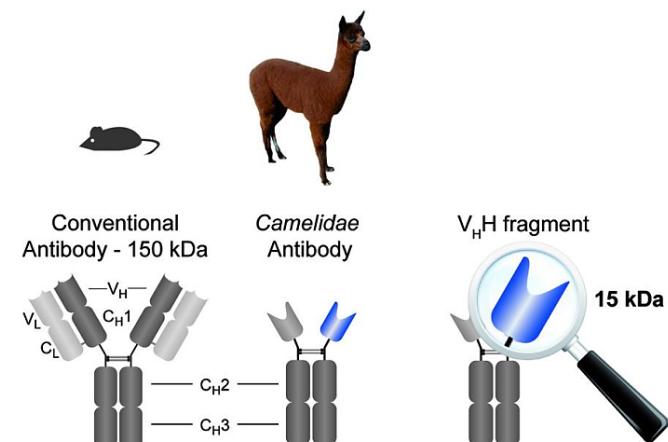
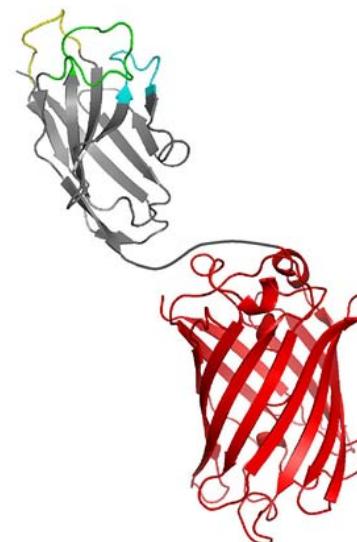
## Organic solvent clearing – iDISCO+



# Organic solvent clearing – iDISCO+



[http://cellbiology.science.uu.nl/wp-content/uploads/2014/12/unf\\_nanobody.jpg](http://cellbiology.science.uu.nl/wp-content/uploads/2014/12/unf_nanobody.jpg)  
Utrecht Nanobody Facility (UNF)



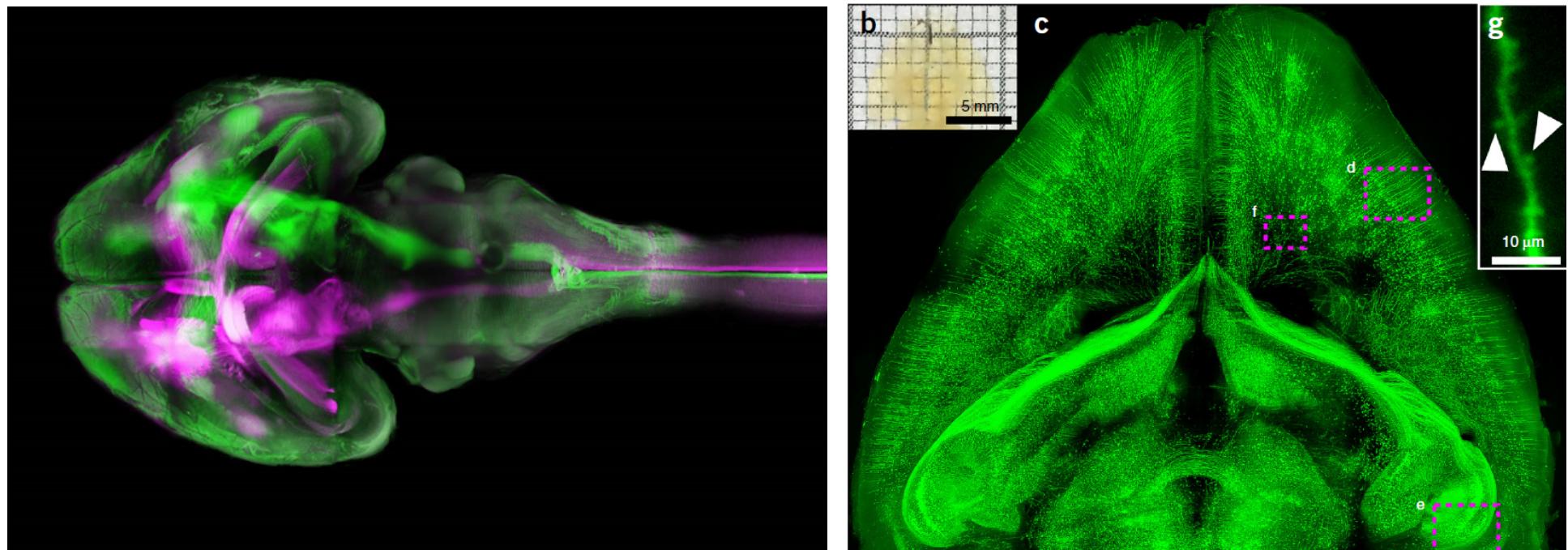
<http://www.chromotek.com/about-us/the-alpaca-antibody-advantage/>

# Organic solvent clearing – uDISCO

Nat Methods. 2016 Aug 22. doi: 10.1038/nmeth.3964. PubMed PMID: 27548807

**Shrinkage-mediated imaging of entire organs and organisms using uDISCO.**

Pan C, Cai R, Quacquarelli FP, Ghasemigharagoz A, Lourbopoulos A, Matryba P, Plesnila N, Dichgans M, Hellal F, Ertürk A.



Including protocol:

Butanol for dehydration

Mixture of BABB and DPE for clearing

# Organic solvent clearing – uDISCO



# The New York Times

Friday, August 26, 2016 | [Today's Paper](#) | [Video](#) | 77°F | DAX -0.19% ↓

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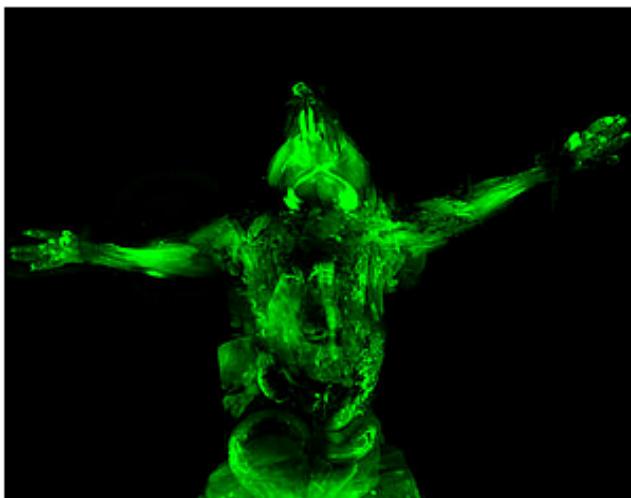
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## SCIENCE

### *Seeing Through to a Mouse's Nervous System*

#### Trilobites

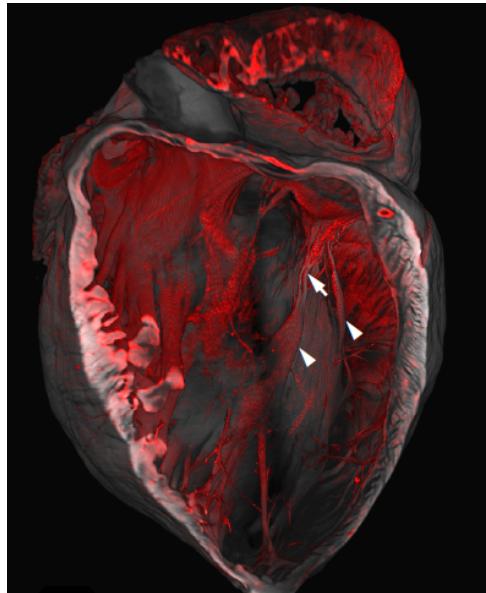
By NICHOLAS ST. FLEUR AUG. 22, 2016



Neuroscientists have developed a way to turn an entire mouse, including its muscles and internal organs, transparent while illuminating the nerve paths that run throughout its body.

The process, called uDisco, provides an alternate way for researchers to study an organism's nervous system without

# Organic solvent clearing – ECI



## Fully Automated Evaluation of Total Glomerular Number and Capillary Tuft Size in Nephritic Kidneys Using Lightsheet Microscopy

Anika Klingberg,\* Anja Hasenberg,\* Isis Ludwig-Portugall,† Anna Medyukhina,‡ Linda Männ,\* Alexandra Brenzel,\* Daniel R. Engel,\* Marc Thilo Figge,‡§ Christian Kurts,† and Matthias Gunzer\*

\*Institute for Experimental Immunology and Imaging, University Hospital, University Duisburg-Essen, Essen, Germany;

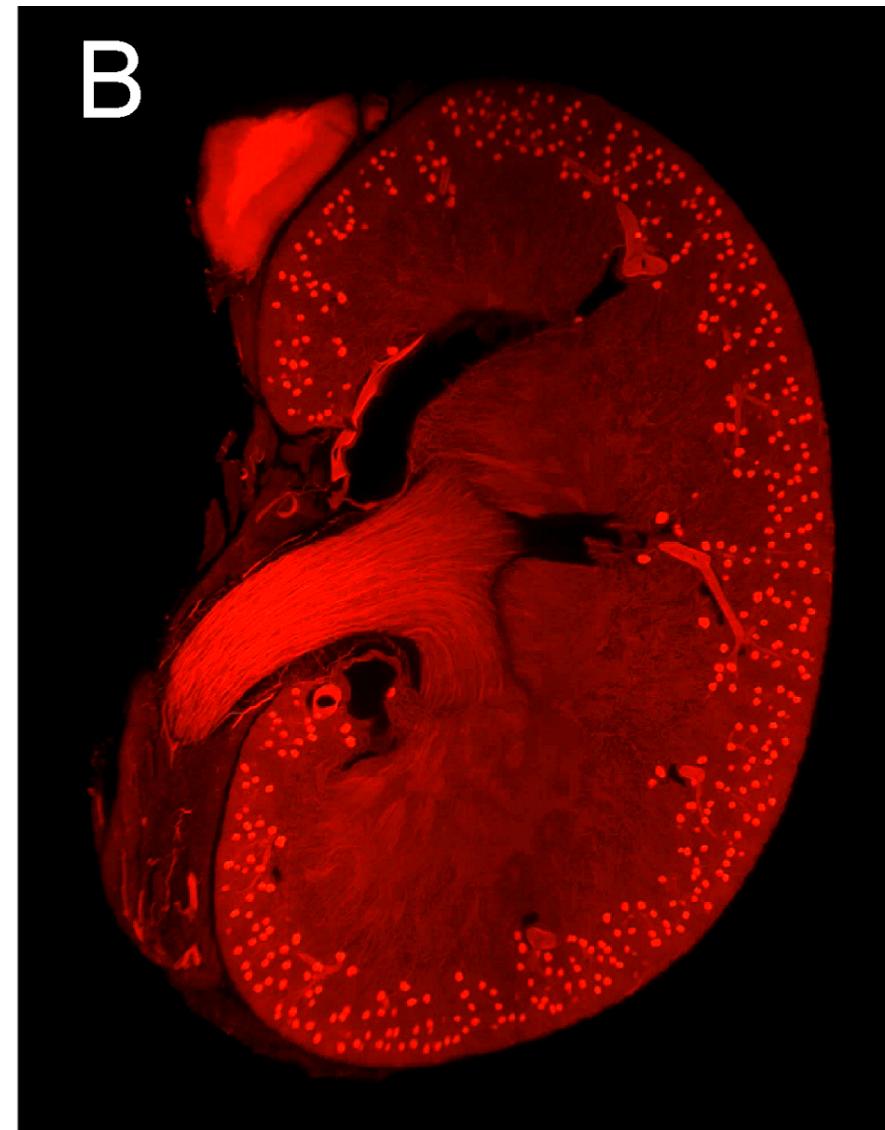
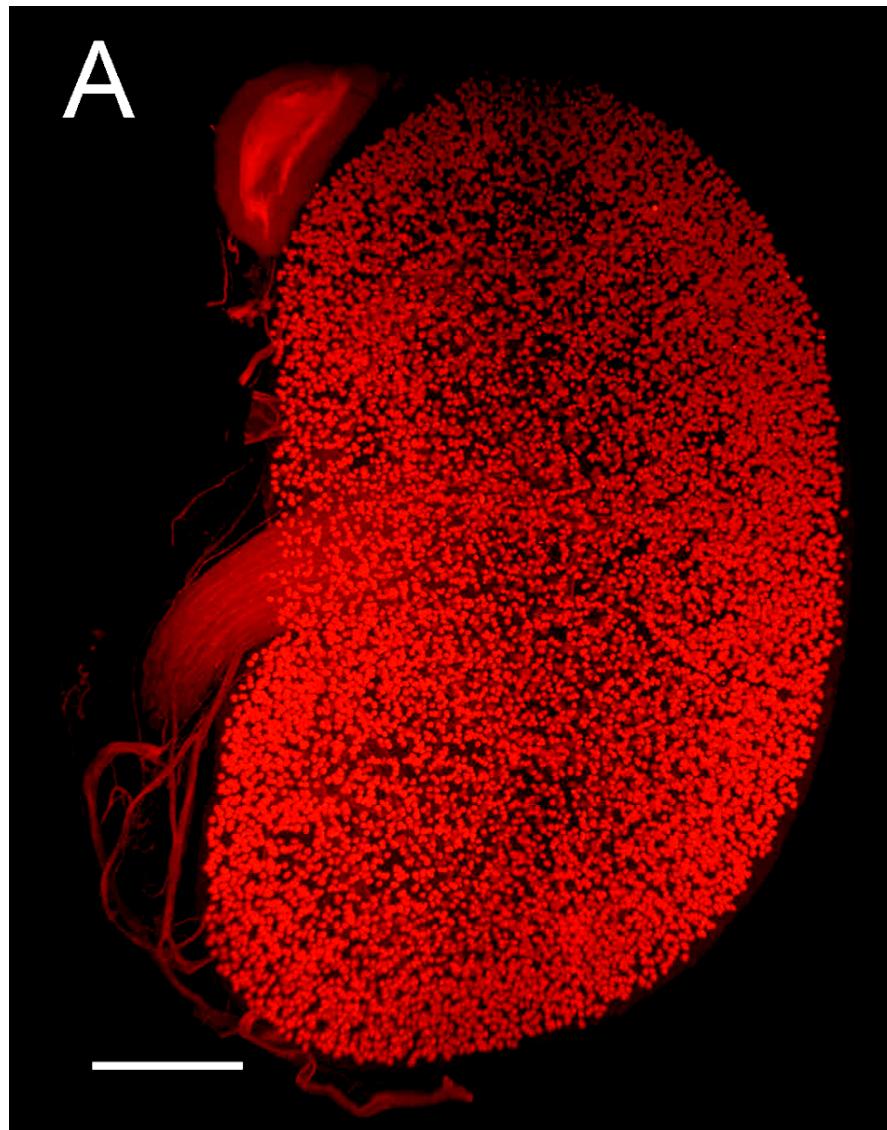
†Institute for Experimental Immunology, Rheinische-Friedrichs-Wilhelms University of Bonn, Bonn, Germany;

‡Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Leibniz-Association, Jena, Germany; and §Friedrich Schiller University Jena, Jena, Germany

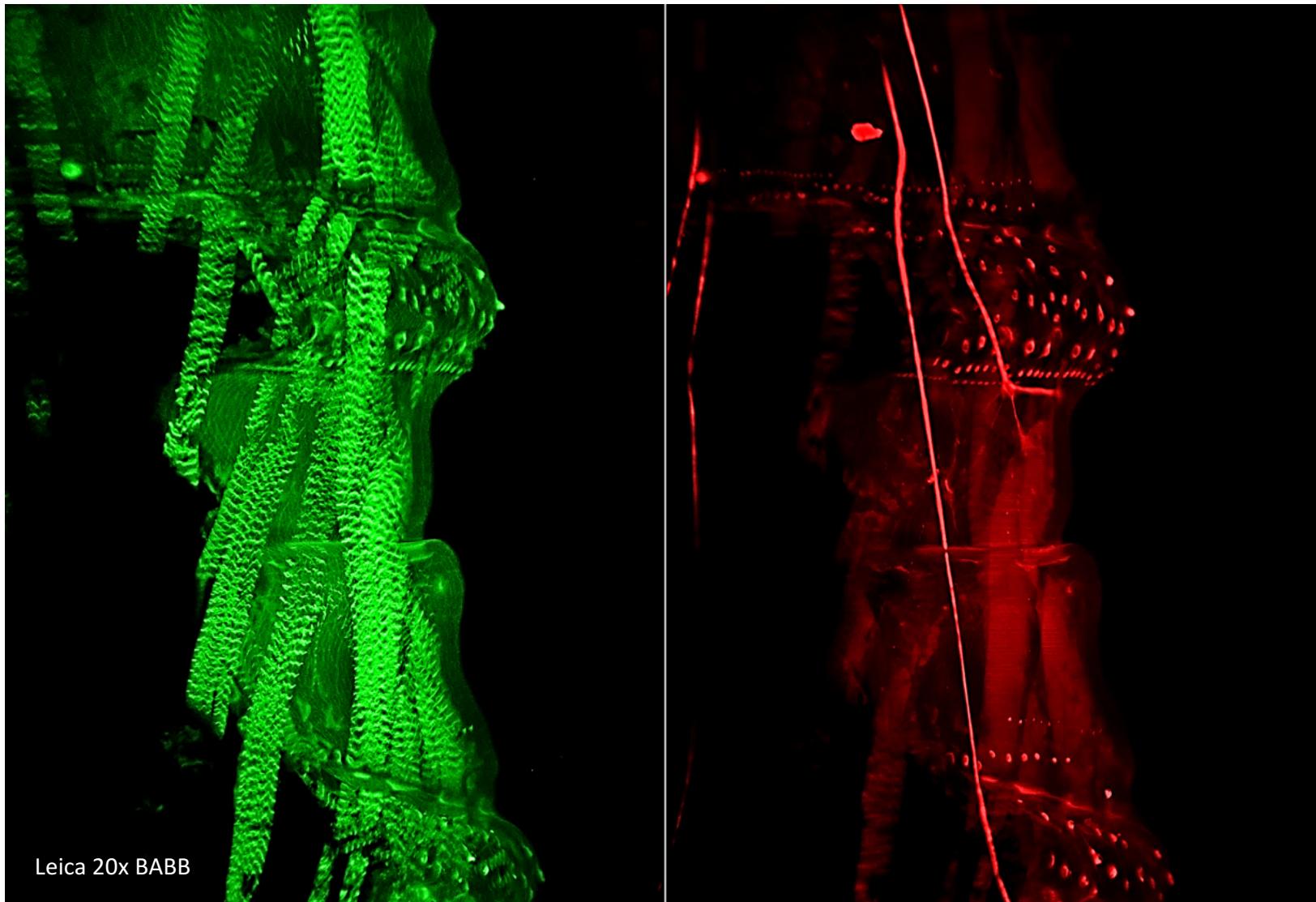
$$RI = 1.558$$



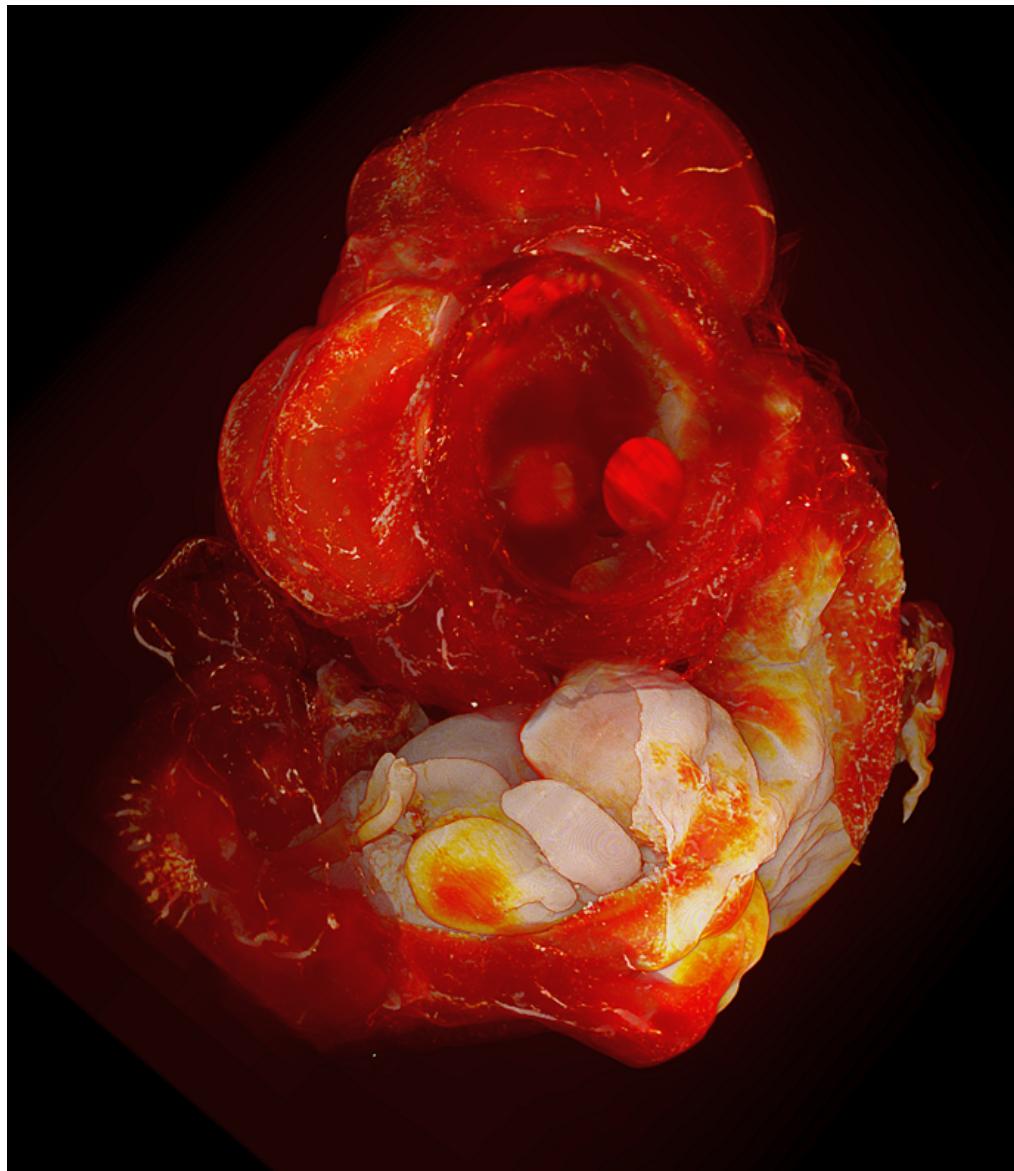
## Organic solvent clearing – ECI



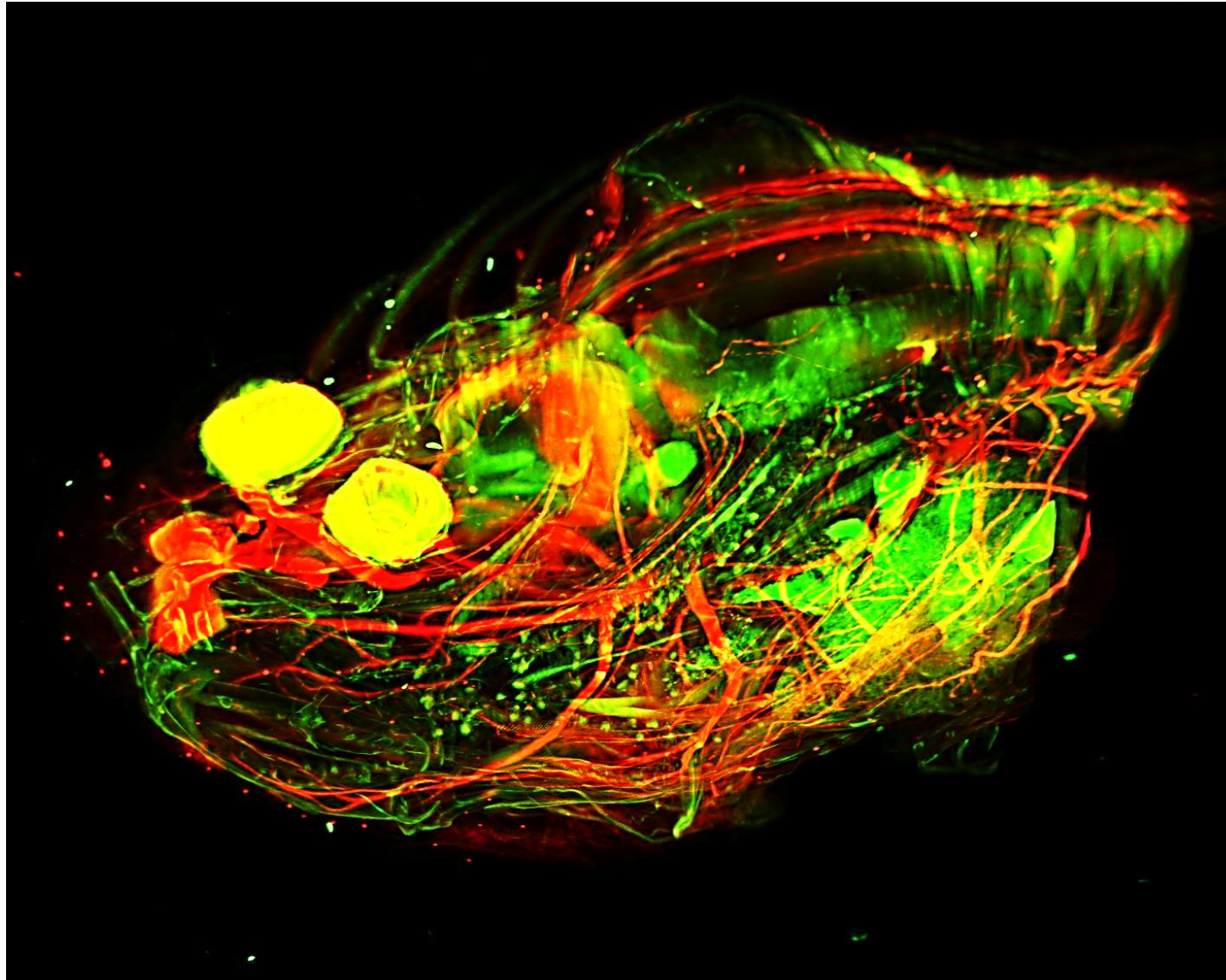
## Drosophila melanogaster larva



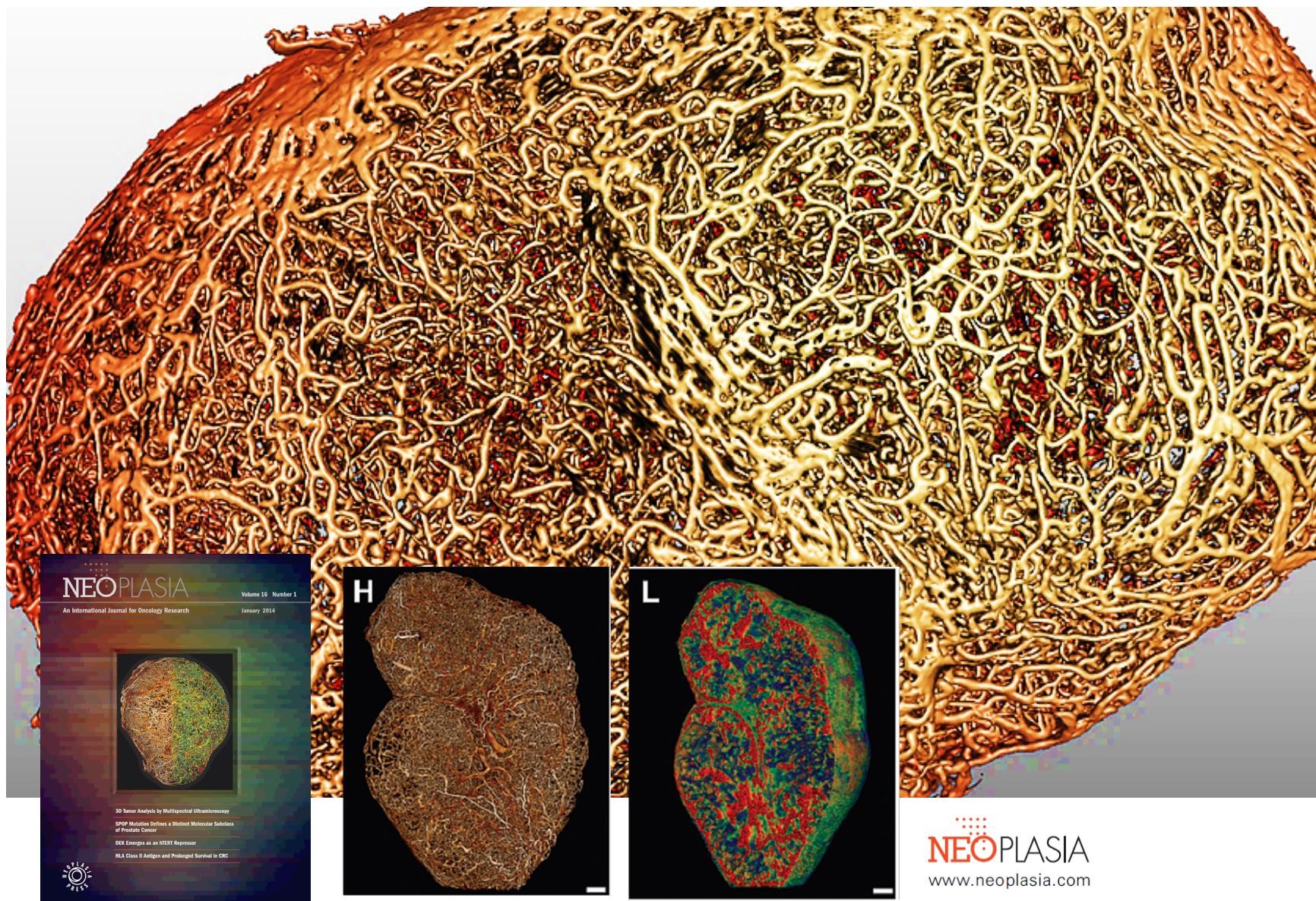
# 3D developmental imaging course 2016



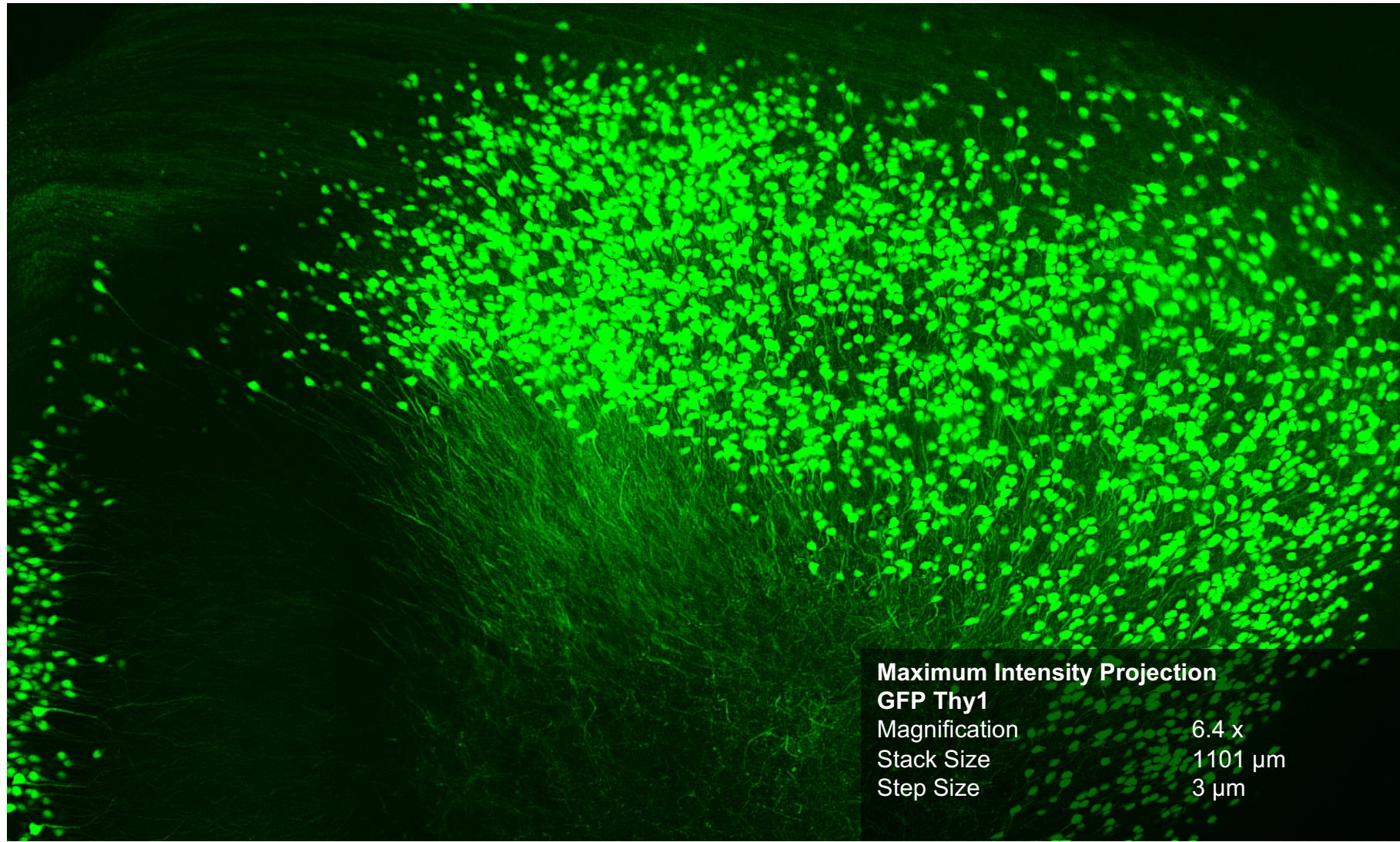
# 3D developmental imaging course 2016



# Tumor vascularization



## Mouse spinal cord / hippocampus GFP



Maximum Intensity Projection

GFP Thy1

Magnification

6.4 x

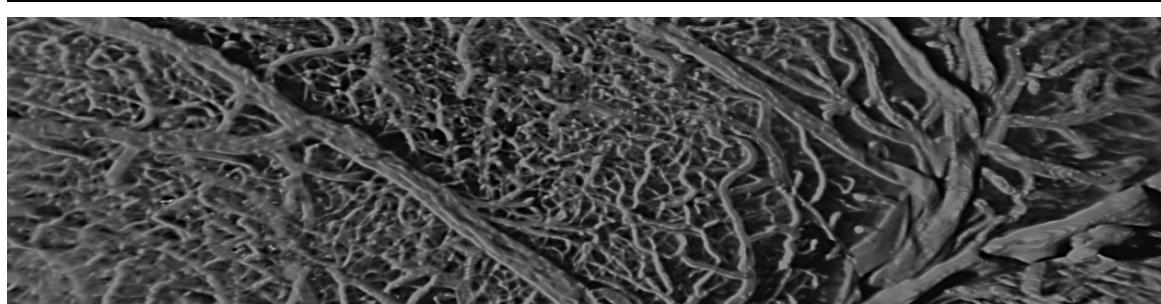
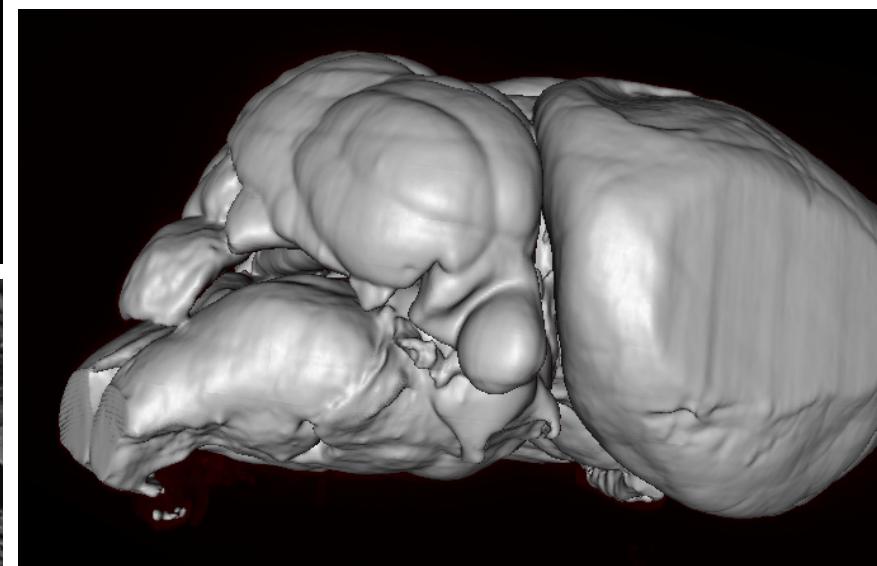
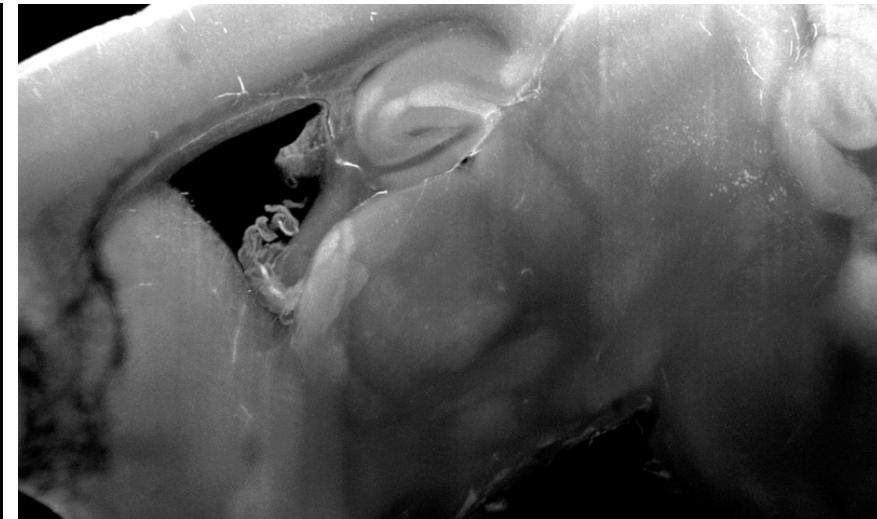
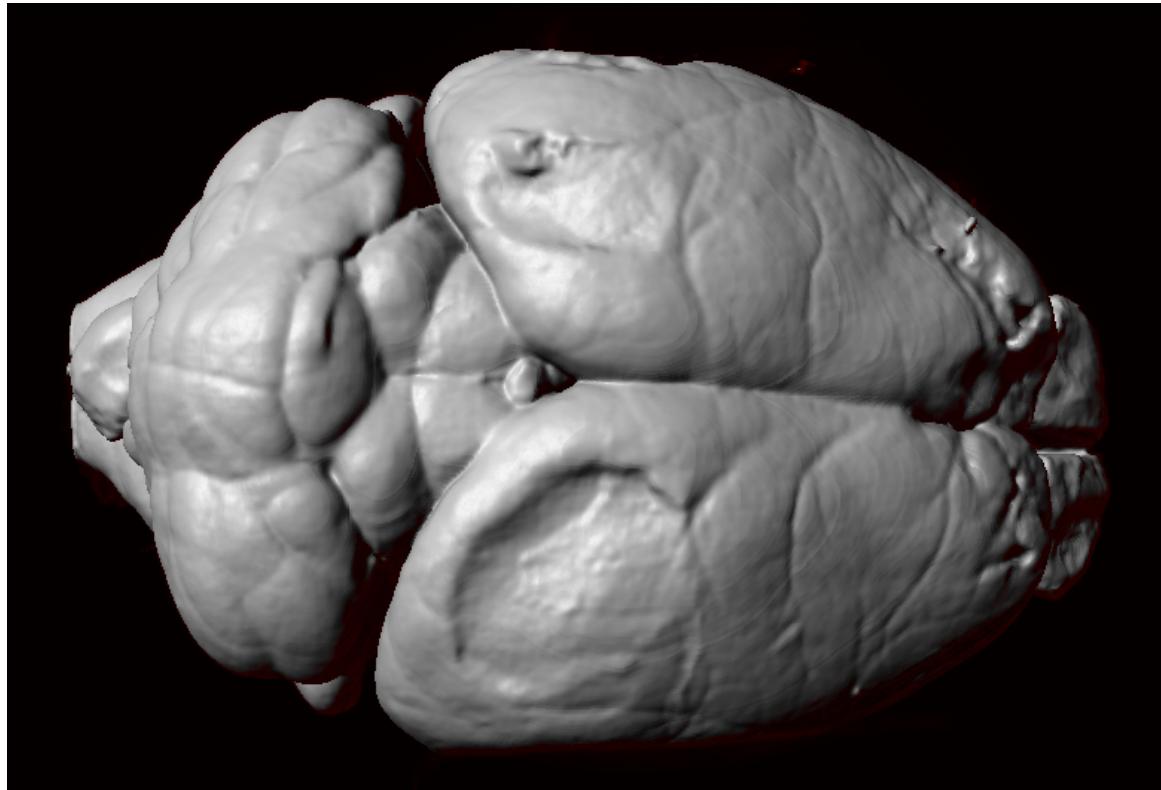
Stack Size

1101 µm

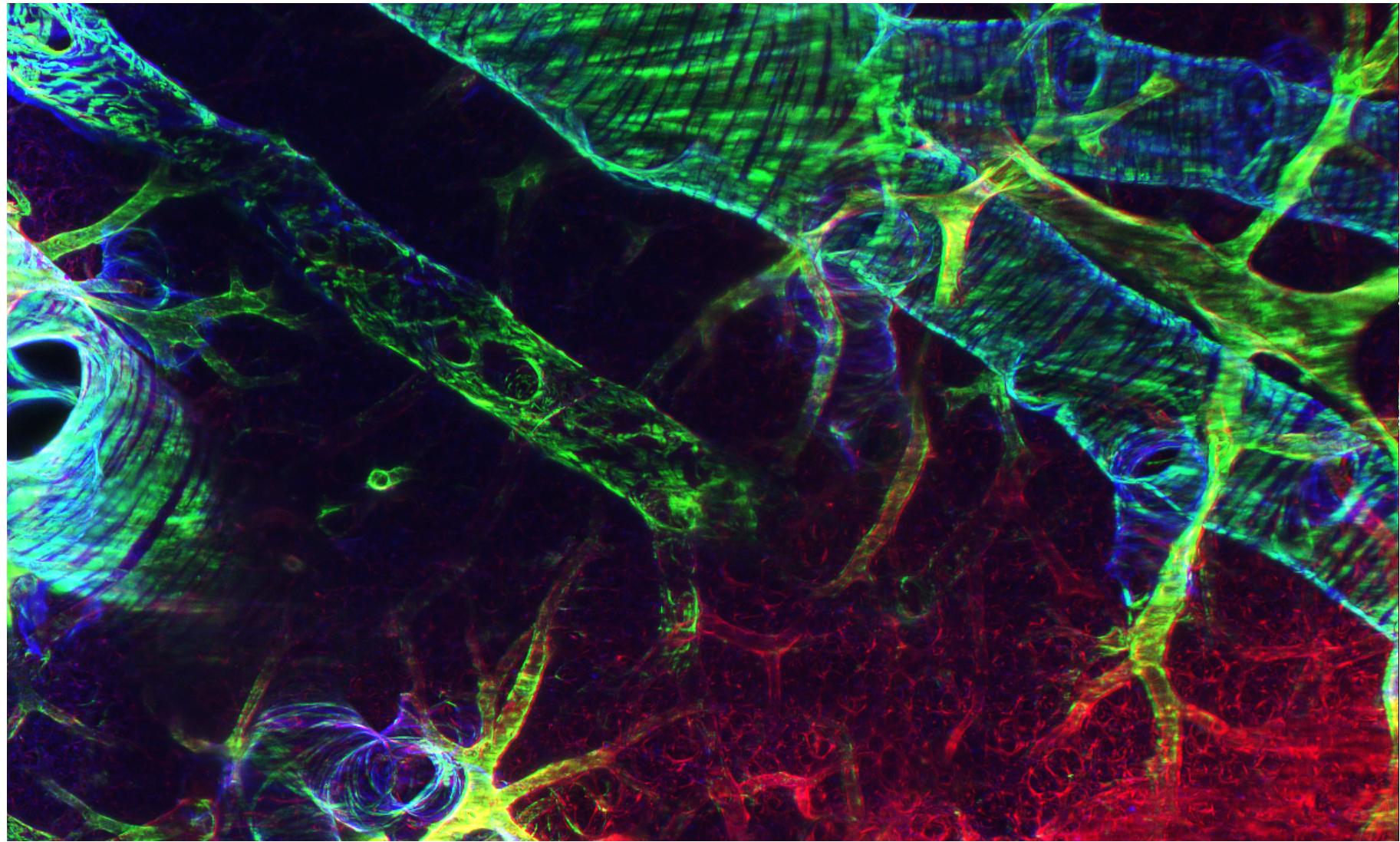
Step Size

3 µm

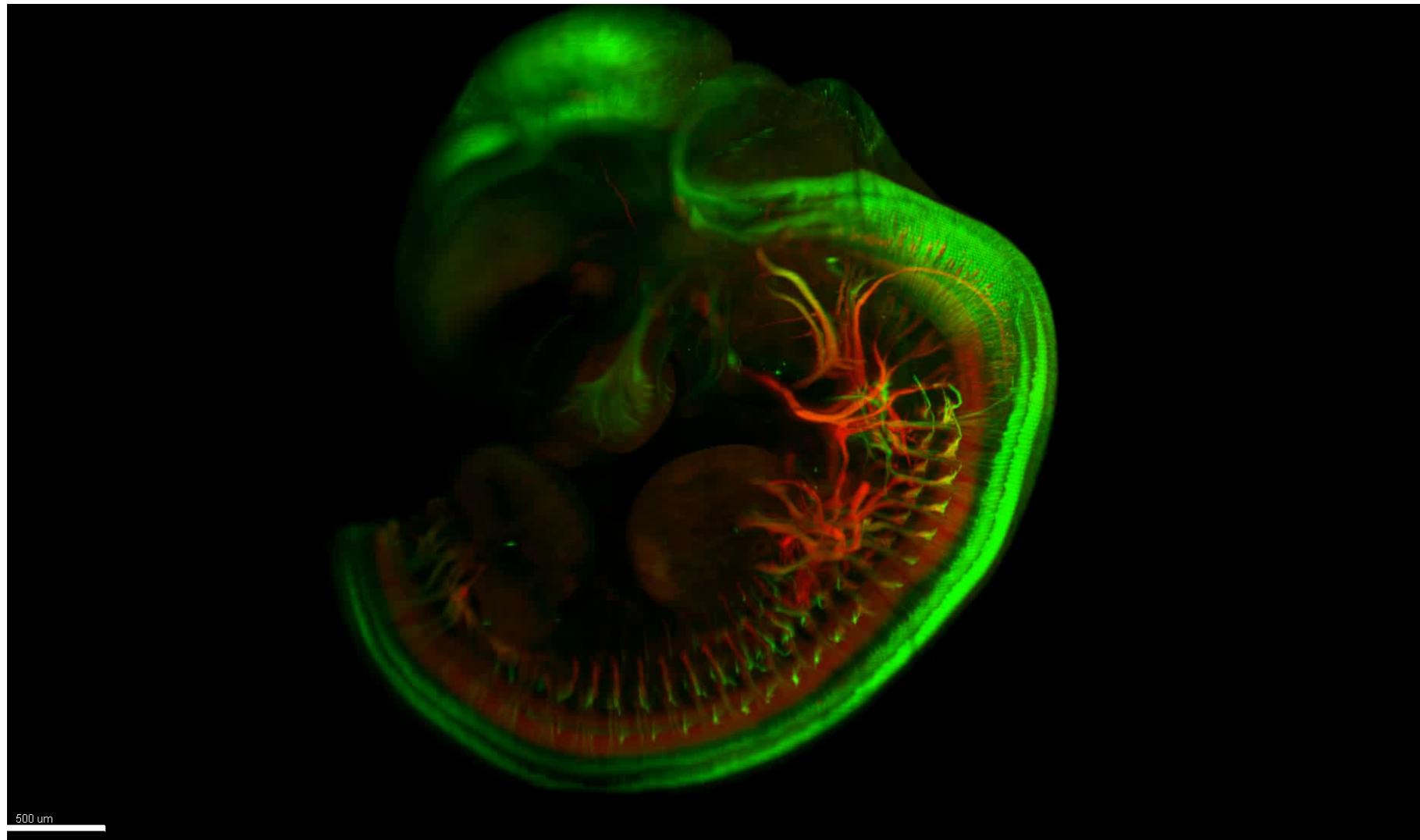
## Mouse brain



## Mouse Lung

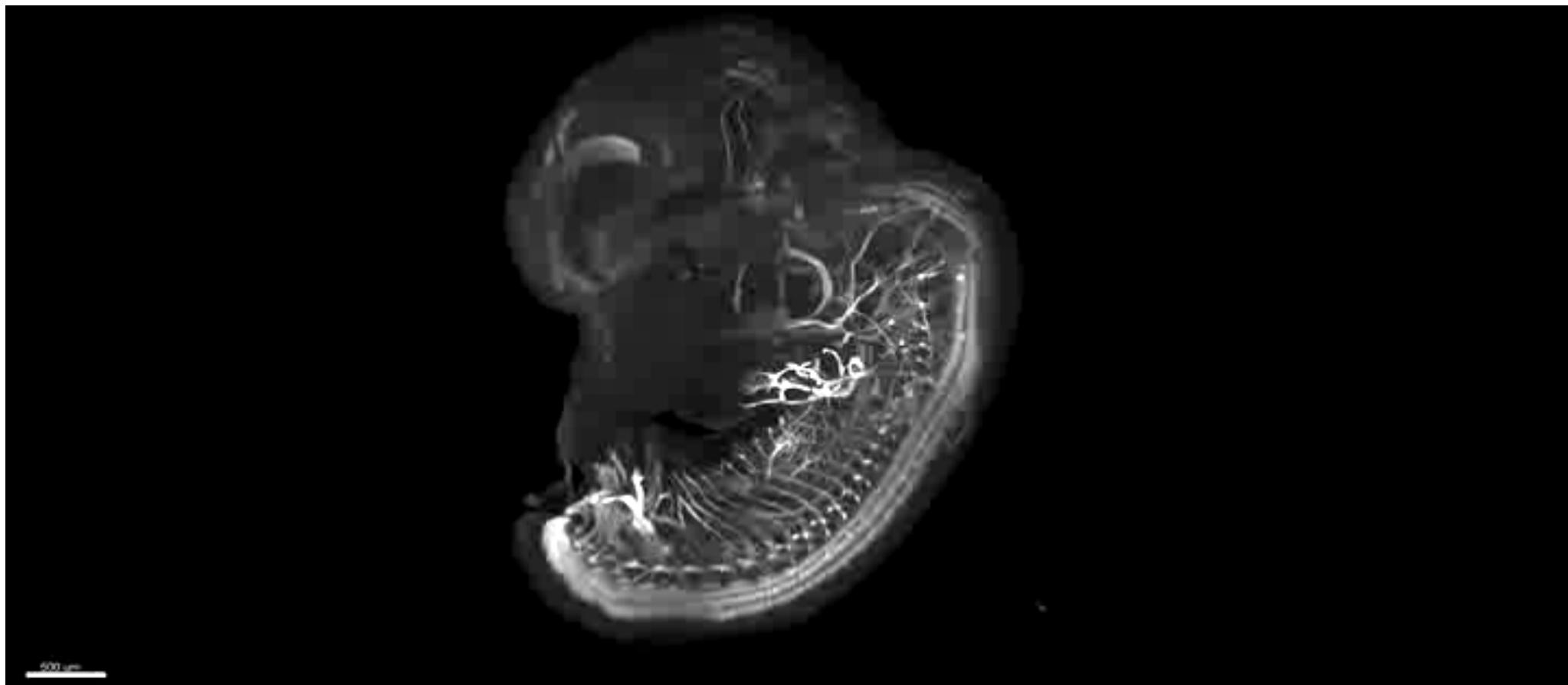


# Mouse Embryo - Institute de la Vision, Paris



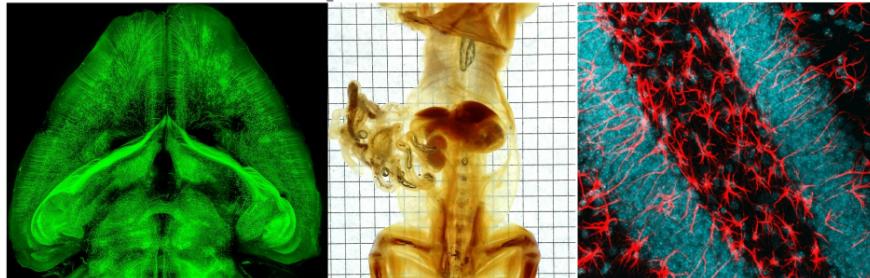
[chloe.dominici@inserm.fr](mailto:chloe.dominici@inserm.fr); [alain.chedotal@inserm.fr](mailto:alain.chedotal@inserm.fr)

## Mouse Embryo - Institute de la Vision, Paris



# Tissue Clearing and 3D imaging

## Workshop, summer 2017



The workshop demonstrates:

- 3DISCO and uDISCO clearing of organs, rodents and large human tissues
- Imaging of entire organs and rodents with light-sheet microscope
- Advantages of DISCO clearing in your research
- iDISCO antibody labeling of organs for 3D imaging
- How to optimize your particular tissue of interest and application
- 3D analysis of the data
- Demo: light-sheet microscopy by Lavision BioTec and Zeiss
- Demo: 3D image analysis software by FEI (Amira), Bitplane (Imaris) and MBF bioscience (Neurolucida), Arivis (immersive view with 3D glasses)

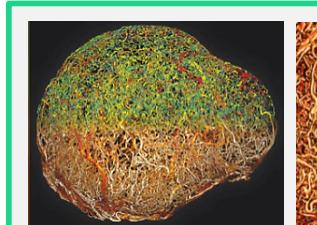
1 session for 4 days (July 24th-27th), maximum 20 participants

Stockholm workshop  
5<sup>th</sup> – 7<sup>th</sup> September 2017

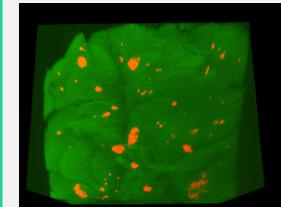
uDISCO, ECi, light sheet imaging,  
data processing (Imaris)



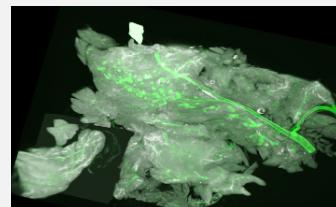
Organized by Ertürk lab in Munich, resources available at <http://www.erturk-lab.com>



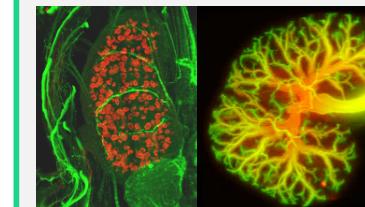
Oncology: tumour vascularization & drug delivery



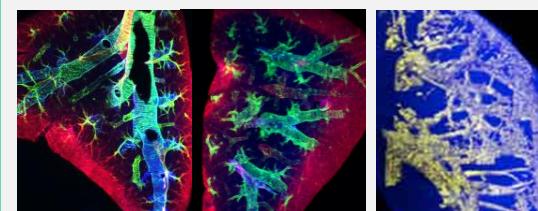
Diabetes: insulin production



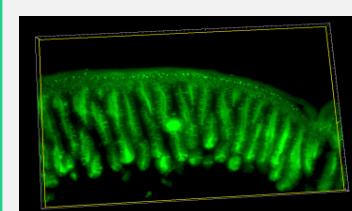
Diabetes: pancreatic pathologies



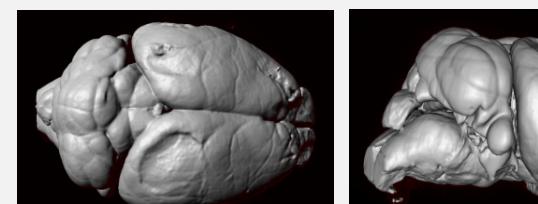
Renal disease & hypertension



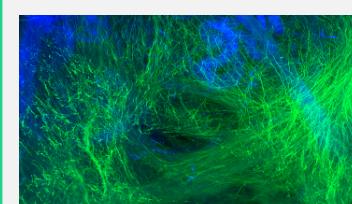
Oncology: metastatic spread



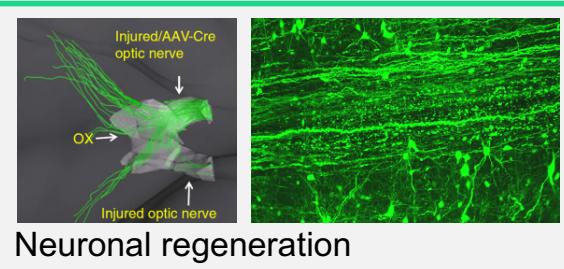
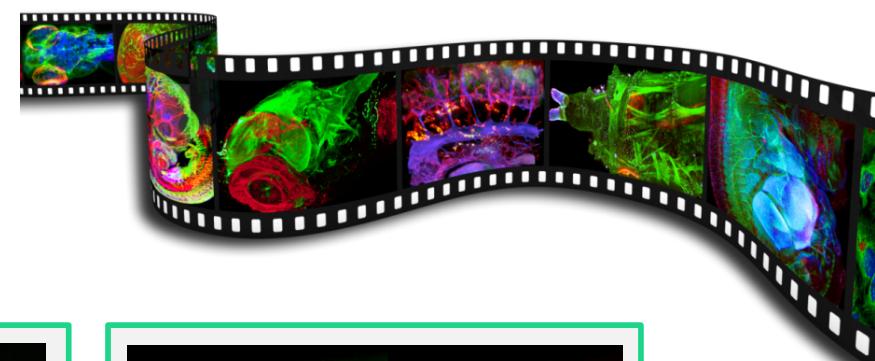
Metabolic diseases



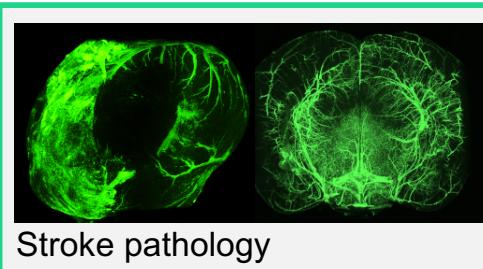
Traumatic brain injury (tbi)



Adiposity



Neuronal regeneration



Stroke pathology



Tissue repair & dermatology

*Thank You!*