Nephron number and new imaging techniques for histology specimens

Norbert Gretz

Medical research center

Klinikum Mannheim

University Heidelberg

MRI experience

Combining new tools to assess renal function and morphology: a holistic approach to study the effects of aging and a congenital nephron deficit.

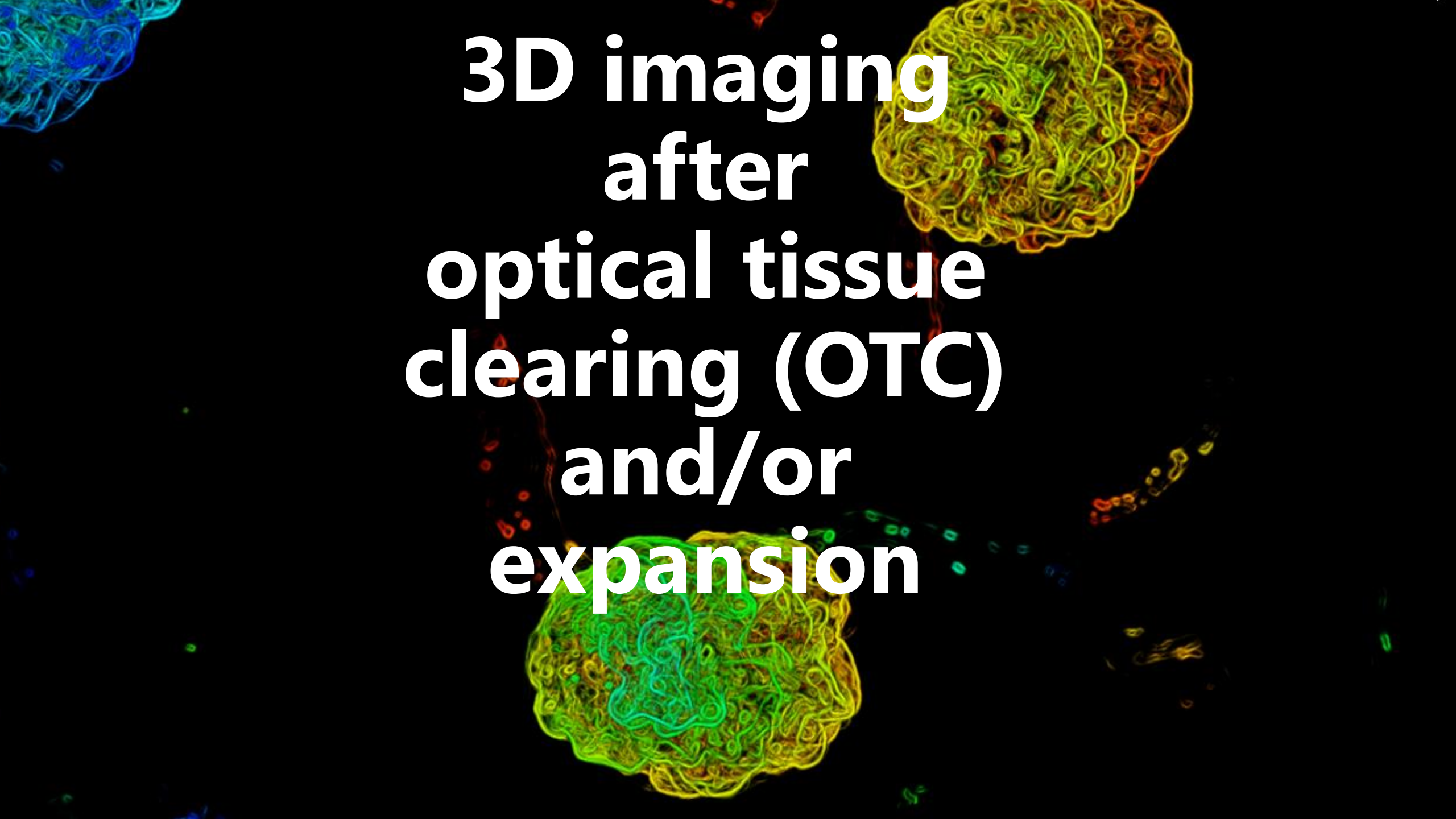
Geraci S, Chacon-Caldera J, Cullen-McEwen L, Schad LR, Sticht C, Puelles VG, Bertram JF, Gretz N.

Am J Physiol Renal Physiol. 2017 Sep 1;313(3):F576-F584.

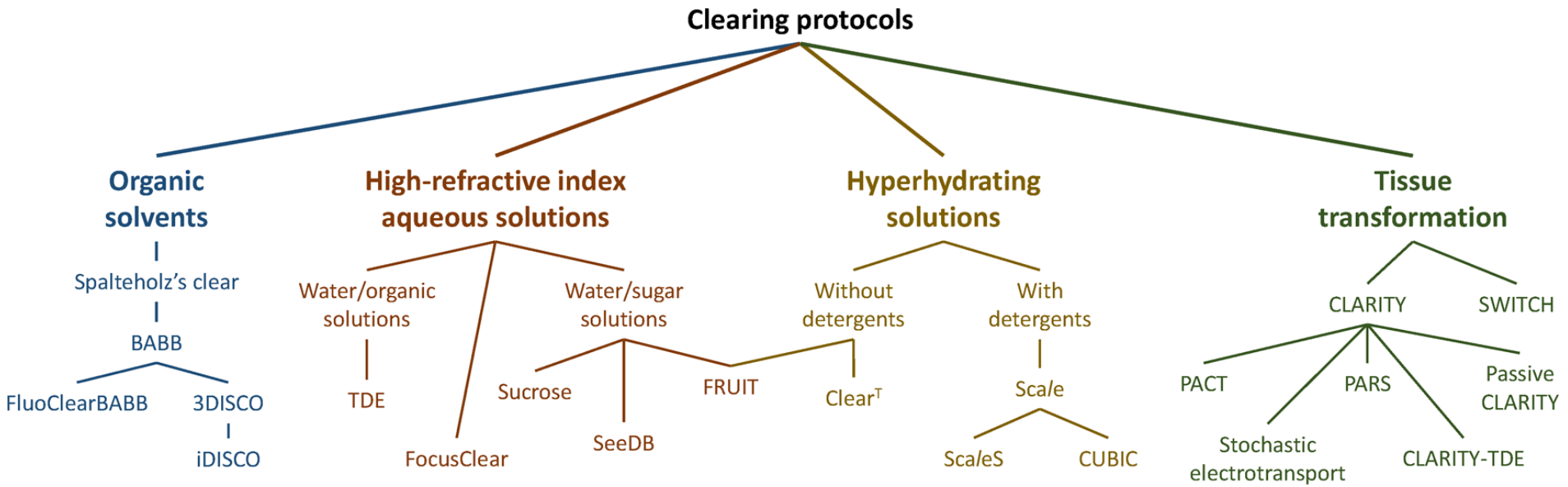
Quantification of glomerular number and size distribution in normal rat kidneys using magnetic resonance imaging.

Heilmann M, Neudecker S, Wolf I, Gubhaju L, Sticht C, Schock-Kusch D, Kriz W, Bertram JF, Schad LR, Gretz N.

Nephrol Dial Transplant. 2012 Jan;27(1):100-7.


3D imaging
after
optical tissue
clearing (OTC)
and/or
expansion

Optical tissue clearing methods



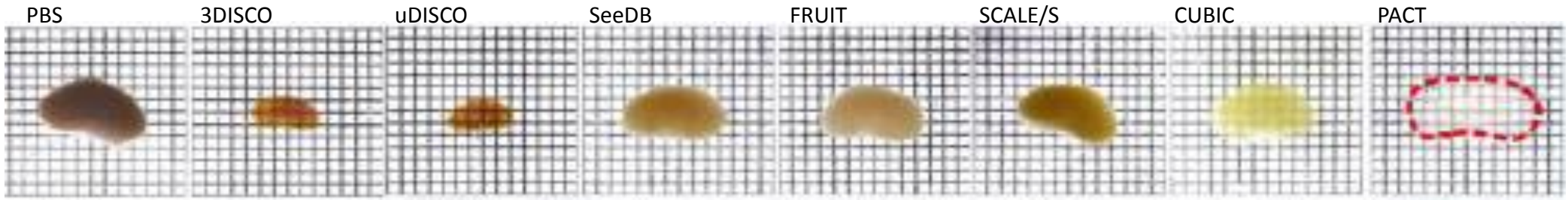
Silvestri et al. J. Biomed. Opt. (2016). doi:10.1117/1.JBO.21.8.081205

Principal: removal of lipids + refractive index adjustment

(how fast light propagates through material)

OTC methods

Strategy	Methods	Time to clear	Morphological alterations	Immunstaining demonstrated
Solvent based	• BABB • 3DISCO • iDISCO	• Days • Hours-days • Hours-days	• Shrinkage • Shrinkage • Shrinkage	• Yes • Limited • Yes
Acqueus solvent	• CLEAR T • SeeDB • TDE	• Hours-days • Days • Days-weeks	• No • No • No	• Yes • No • Yes
Hyperhydration	• Scale/A • Scale/S • CUBIC	• Weeks • Days • Days	• Expansion • No • Expansion	• Yes • Yes • Yes
Gel embedding	• CLARITY • PACT • PARS	• Days • Days-weeks • Days	• Slight expansion • Slight expansion • No	• Yes • Yes • Yes



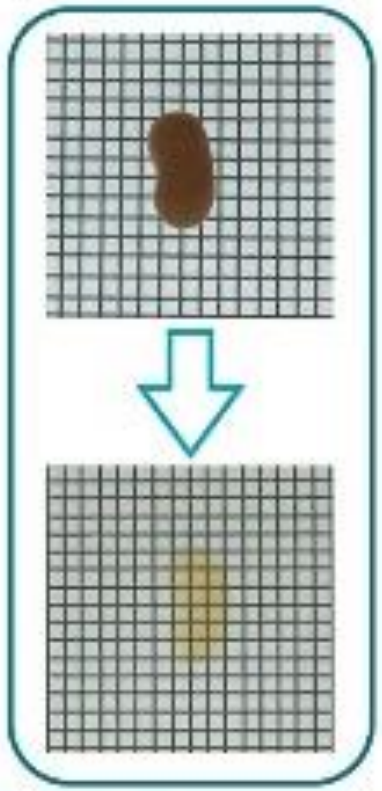
Adapated from: Xu et al., J Biophotonics (2019)

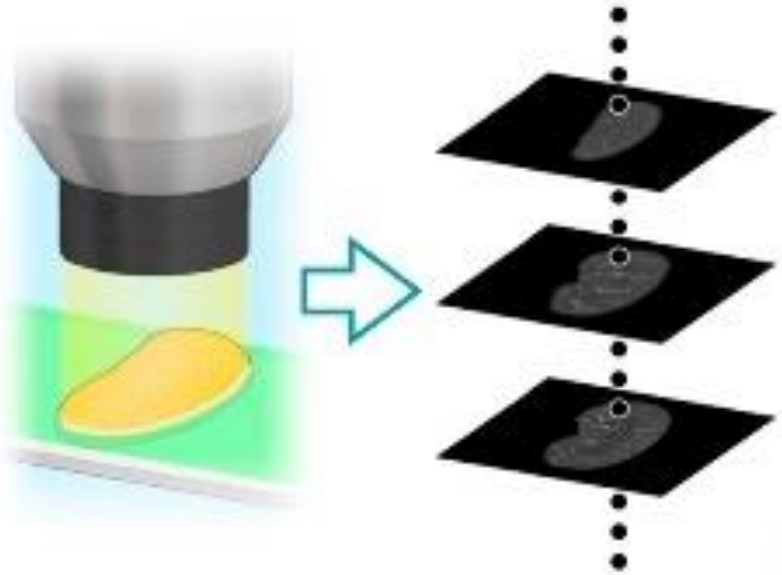
Comprehensive three-dimensional analysis (CUBIC-kidney) reveals abnormal renal sympathetic nerves after ischemia-reperfusion injury

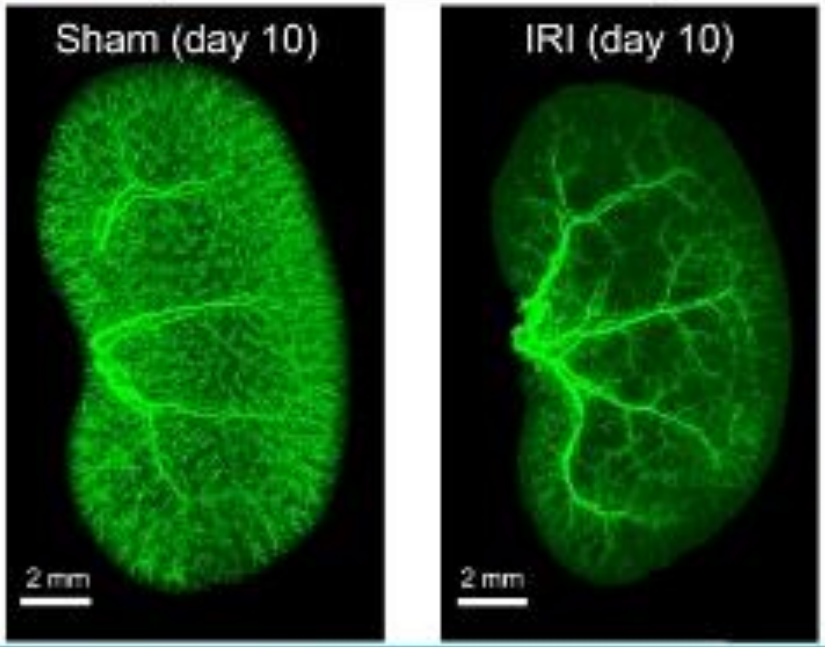
Tissue clearing (CUBIC)

Image acquisition (LSFM)

Sympathetic nerves (3D reconstruction)







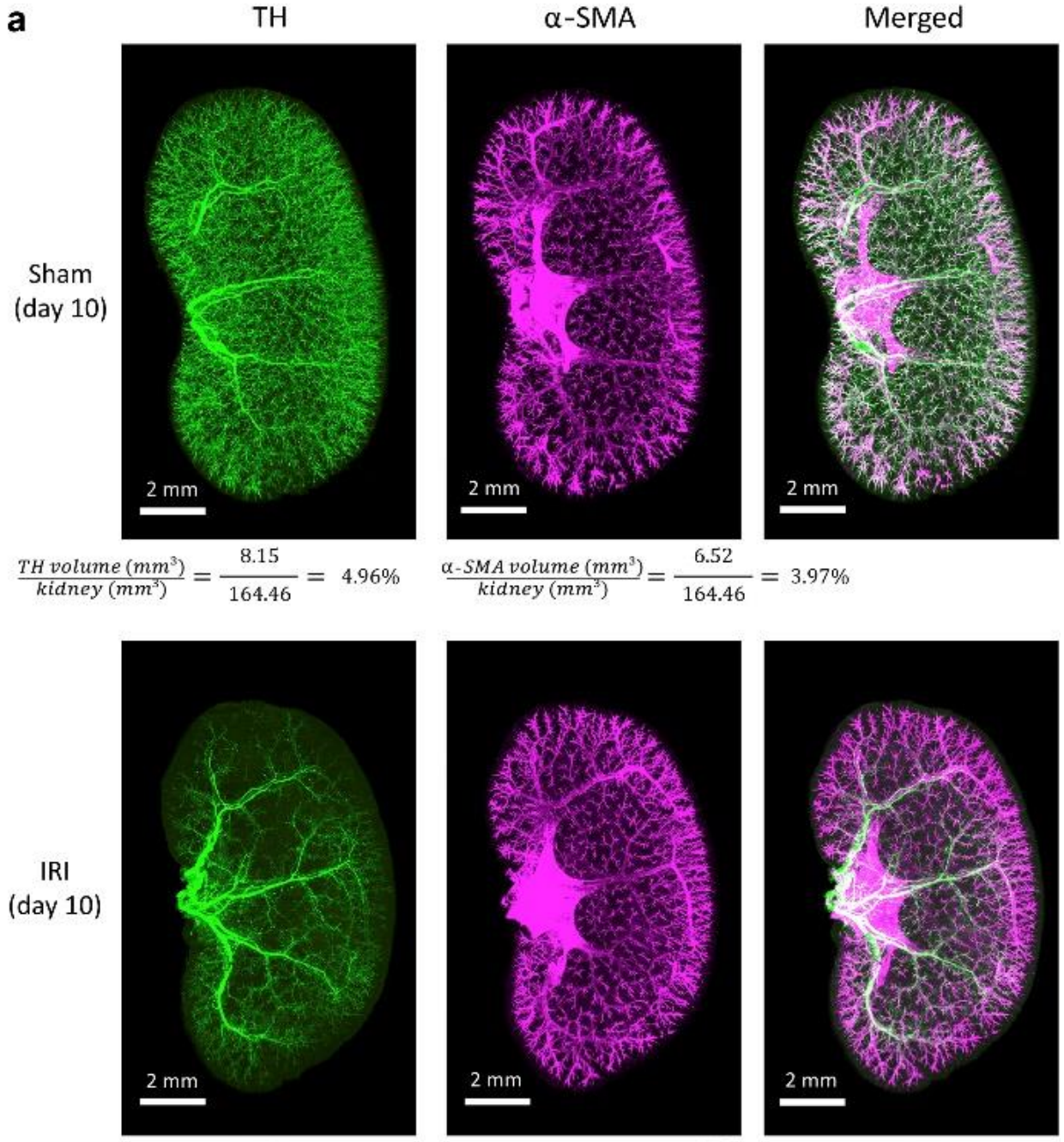
CONCLUSION:

CUBIC-kidney clearly visualized the 3D structure of renal sympathetic nerves and showed sympathetic denervation after IRI.

ISN kidney INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL SOCIETY OF NEPHROLOGY

Hasegawa et al., 2019

a



$$\frac{TH\ volume\ (mm^3)}{kidney\ (mm^3)} = \frac{8.15}{164.46} = 4.96\%$$

$$\frac{\alpha\text{-}SMA\ volume\ (mm^3)}{kidney\ (mm^3)} = \frac{6.52}{164.46} = 3.97\%$$

$$\frac{TH\ volume\ (mm^3)}{kidney\ (mm^3)} = \frac{2.75}{131.63} = 2.09\%$$

$$\frac{\alpha\text{-}SMA\ volume\ (mm^3)}{kidney\ (mm^3)} = \frac{6.48}{131.63} = 4.92\%$$

Recent publications from our group

A Novel Optical Tissue Clearing Protocol for Mouse Skeletal Muscle to Visualize Endplates in Their Tissue Context.

Williams MPI, Rigon M, Straka T, Hörner SJ, Thiel M, Gretz N, Hafner M, Reischl M, Rudolf R.

Front Cell Neurosci. 2019;13:49

- MYOCLEAR

A cationic near infrared fluorescent agent and ethyl-cinnamate tissue clearing protocol for vascular staining and imaging.

Huang J, Brenna C, Khan AUM, Daniele C, Rudolf R, Heuveline V, Gretz N.

Sci Rep. 2019;9:521

(a) Retrograde perfusion

Saline/Heparin	6 min
A cationic dye solution	10 min
Saline/Heparin	1 min
4% PFA/PBS	6 min
Postfix in 4% PFA/PBS	30 min
Total	1 hour

Jun-Air compressor

A perfused kidney

(b) Tissue clearing

Dehydration in 50% EtOH	30 min
Dehydration in 80% EtOH	30 min
Dehydration in 100% EtOH	30 min
Dehydration in 100% EtOH	30 min
ECi clearing	120 min
Total	4 hours

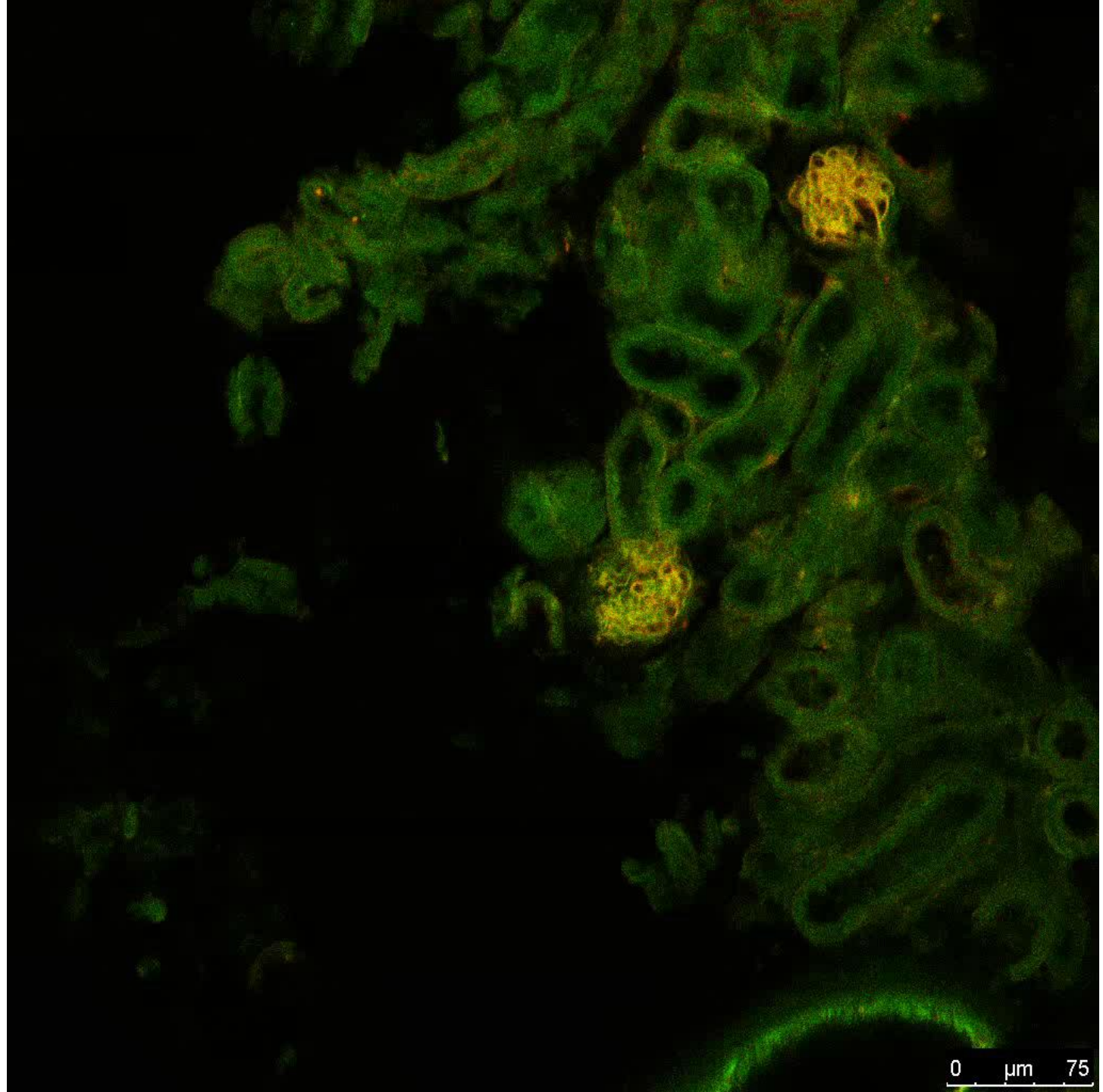
Automatic tissue processor

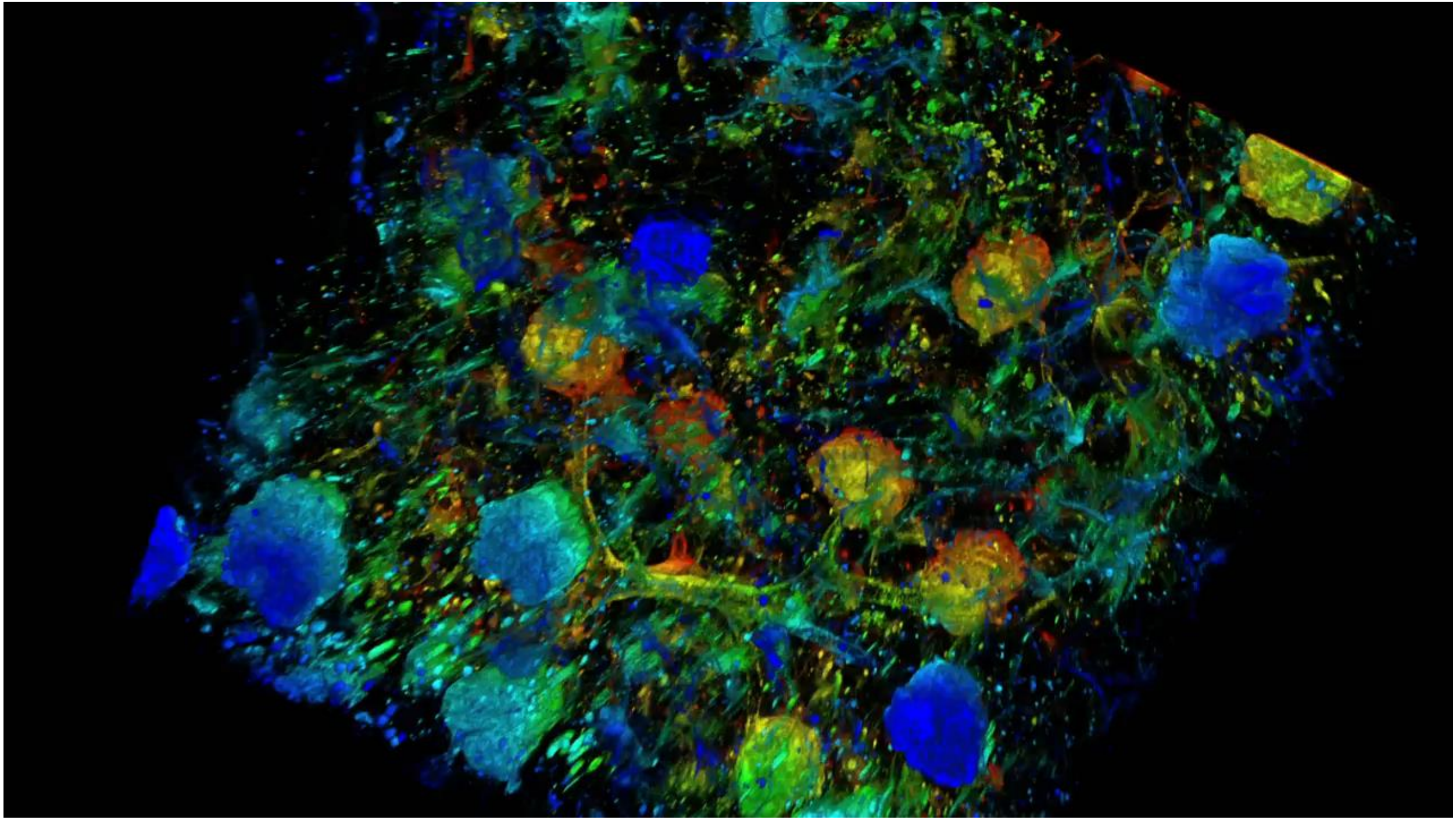
A cleared kidney

(c) Imaging of cleared tissues

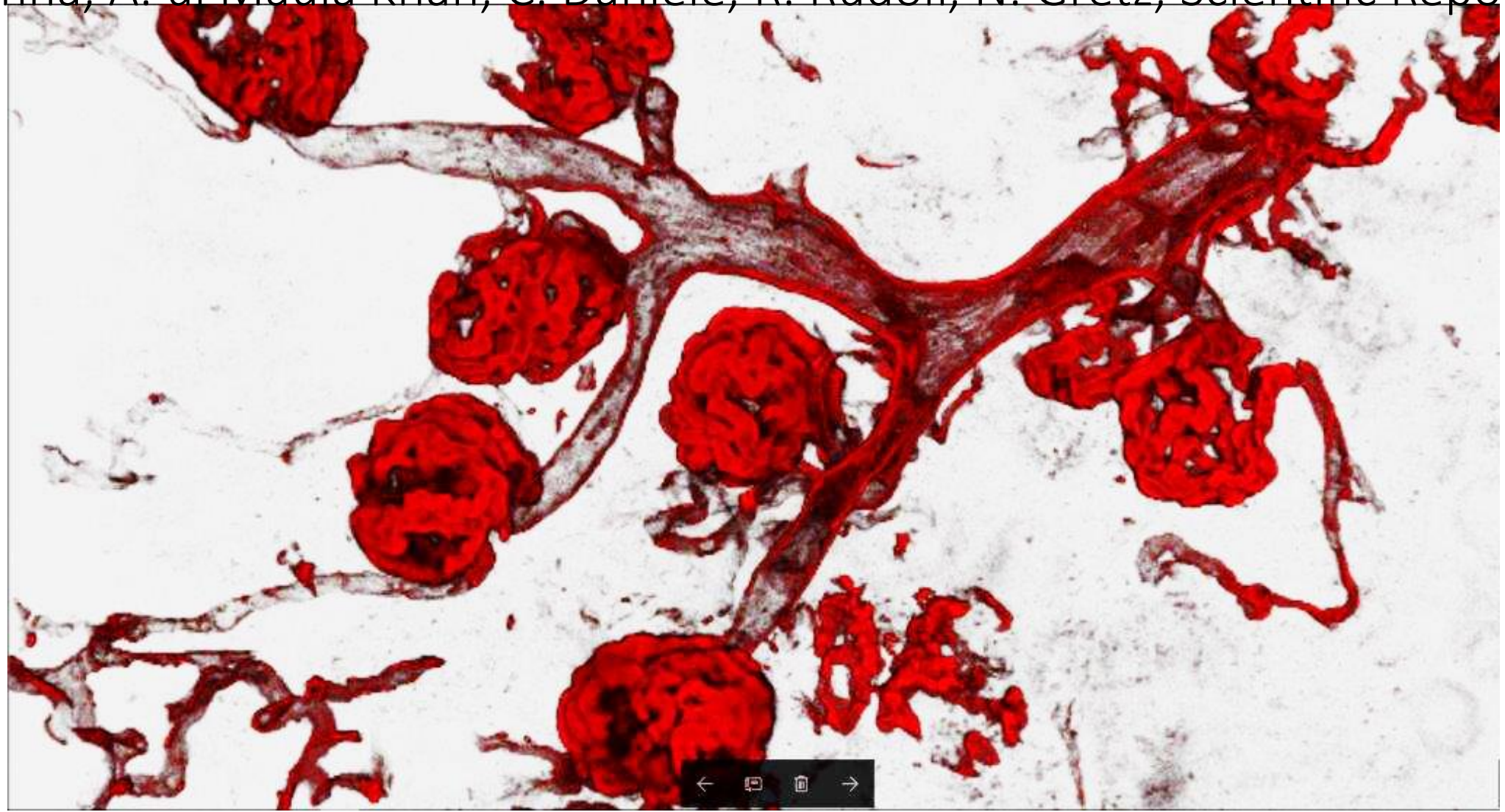
Confocal or light-sheet microscopy

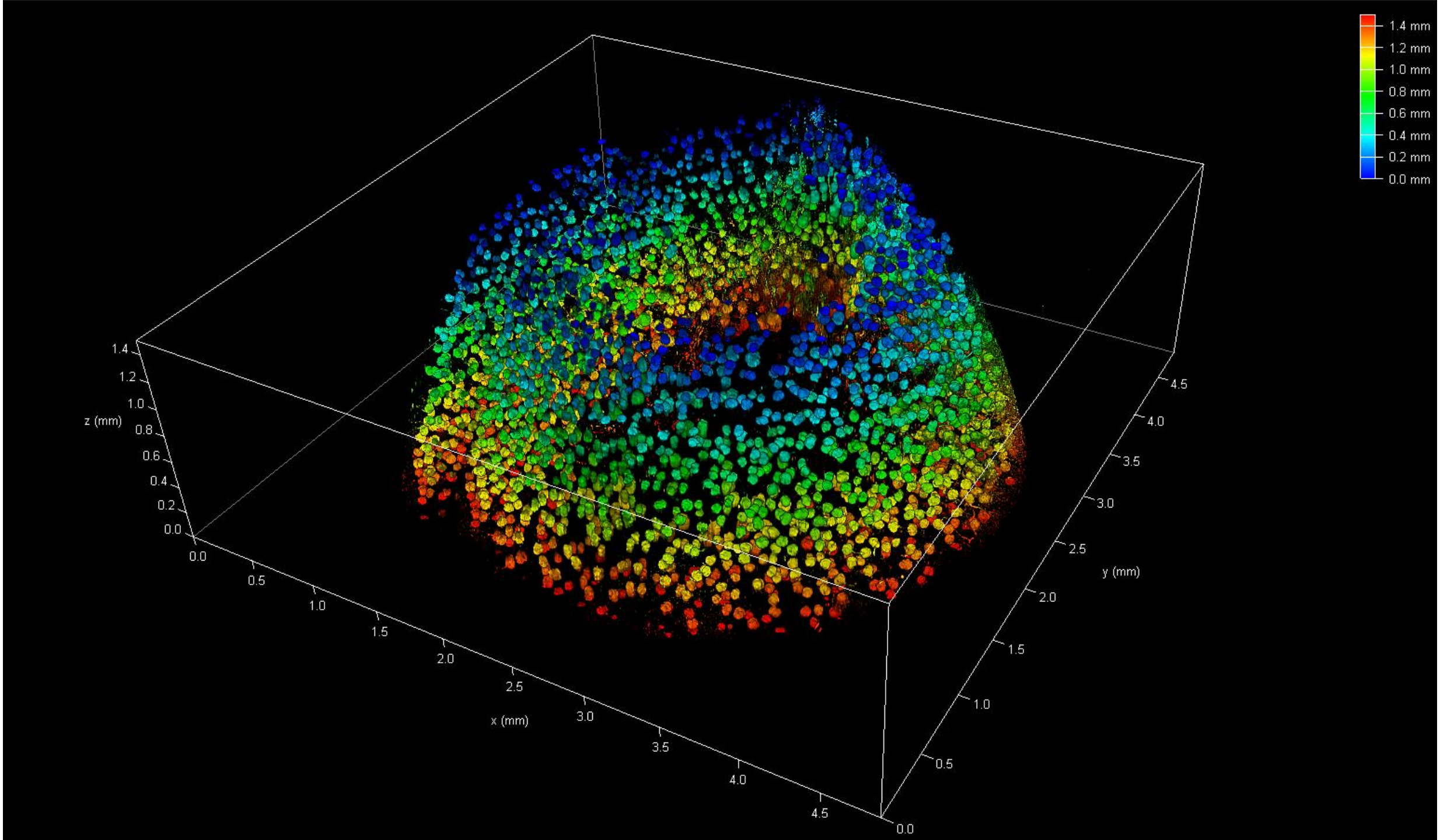
Confocal microscopy


0 µm 75



A cationic near infrared fluorescent agent and ethyl-cinnamate tissue clearing protocol for whole body vascular imaging. J. Huang, C. Brenna, A. ul Maula Khan, C. Daniele, R. Rudolf, N. Gretz, Scientific Reports2019

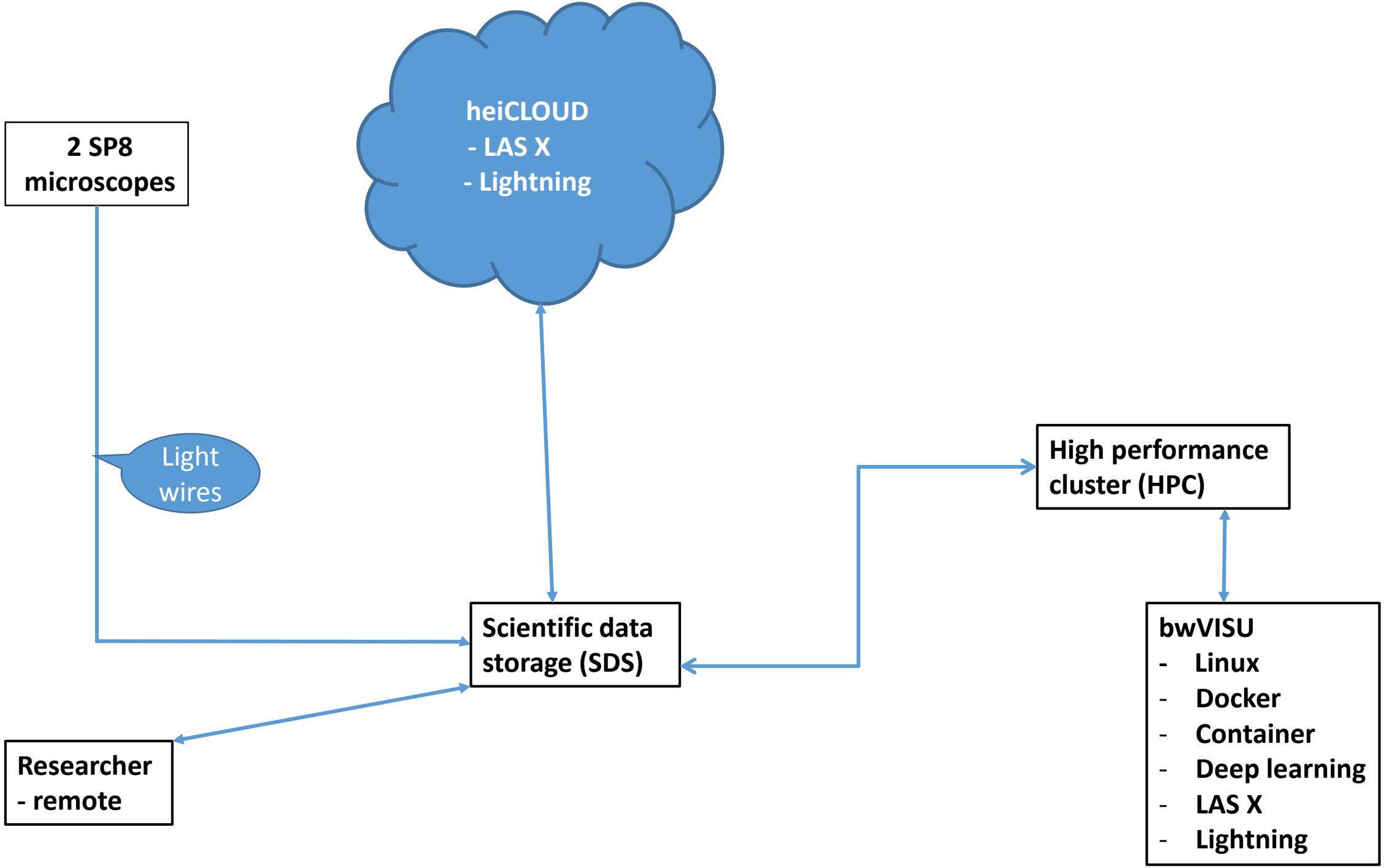



1.4 mm
1.2 mm
1.0 mm
0.8 mm
0.6 mm
0.4 mm
0.2 mm
0.0 mm
z (mm)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
x (mm)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
y (mm)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5

scanning: confocal, light sheet (2x SP8)

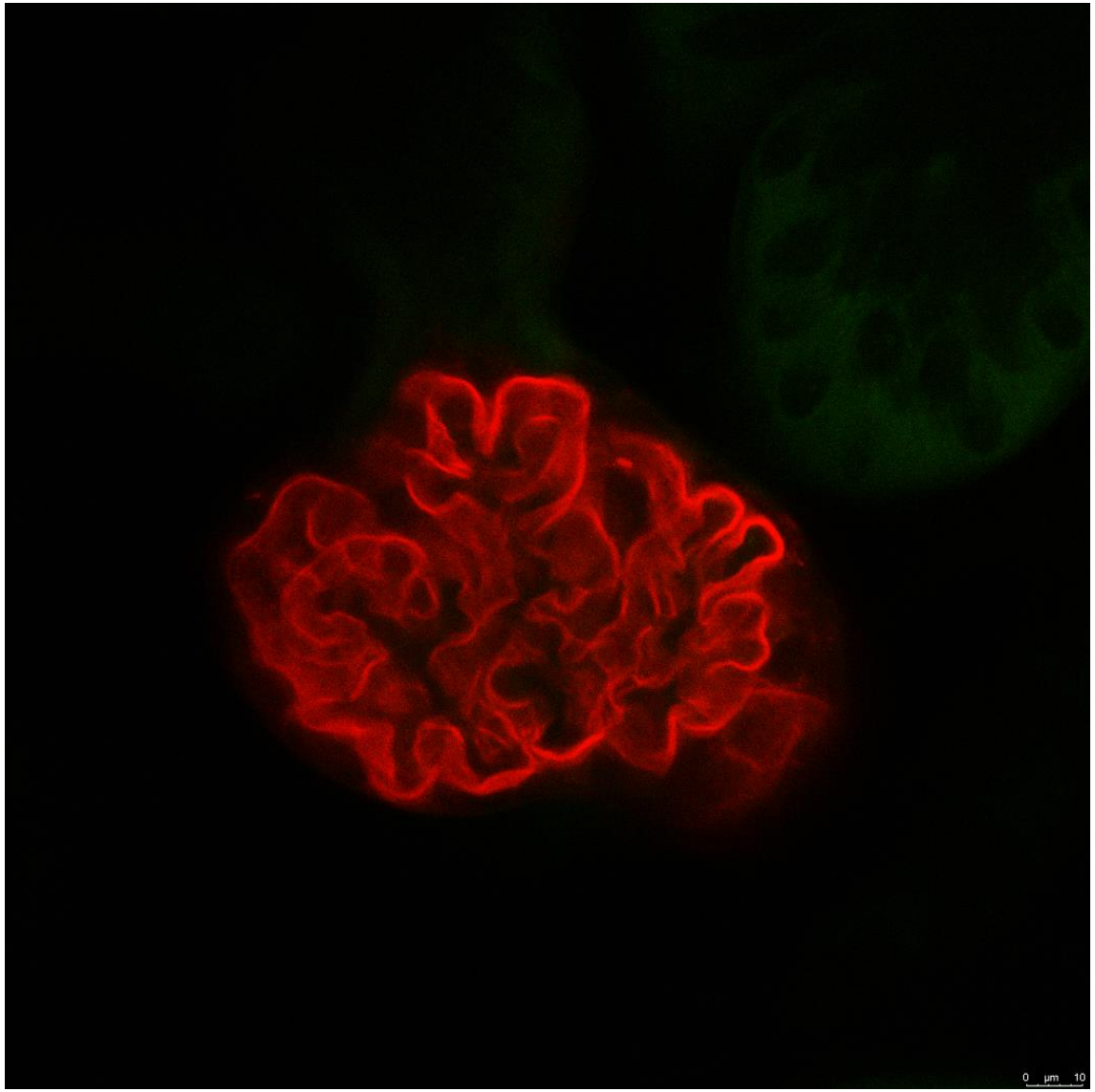
- Microscopy (1 mouse kidney)
  - confocal special objectives (60 h)
  - light sheet (23 min)

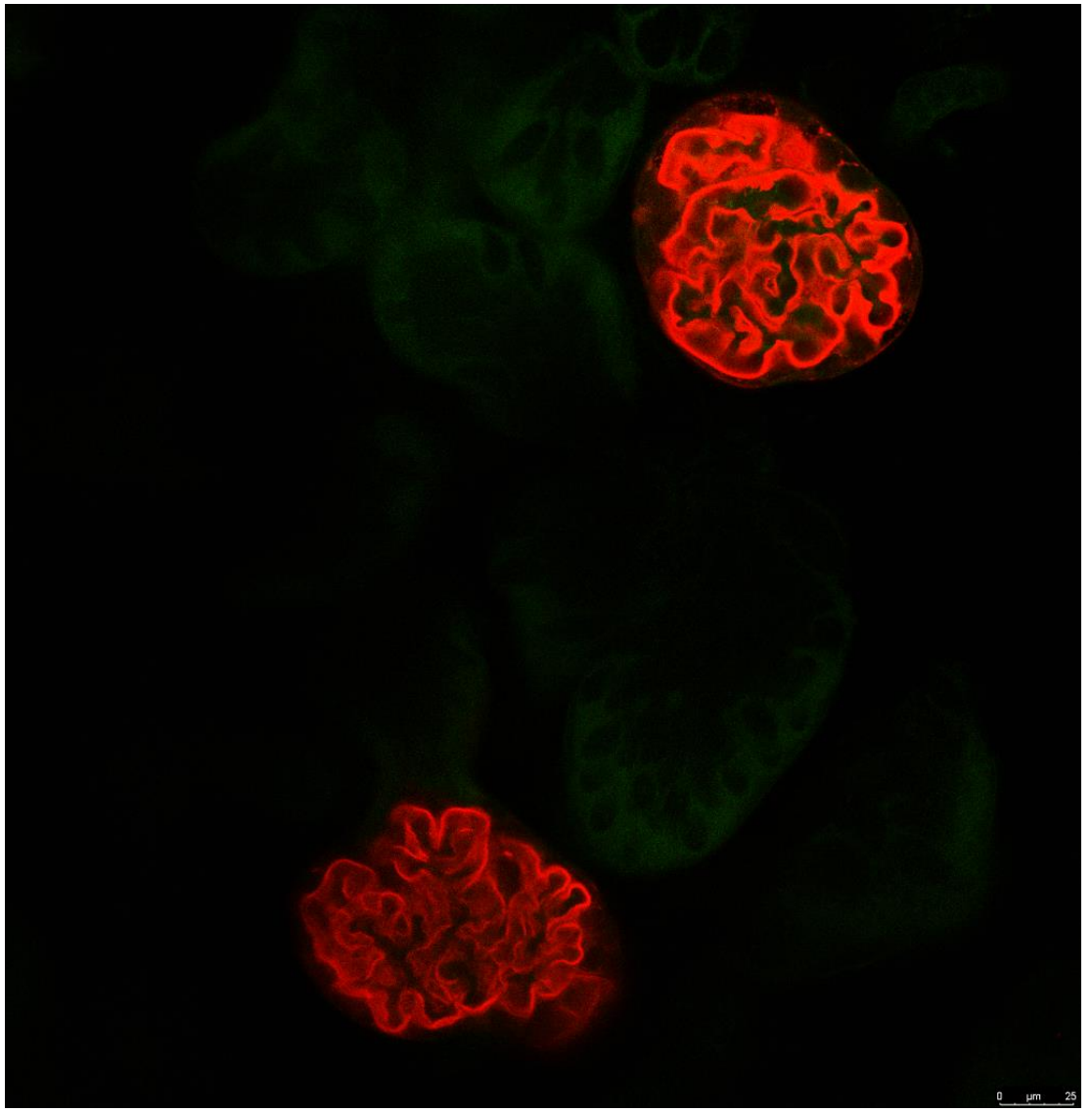
- Data volume
  - (voxel size) (1 mouse kidney)
  - 200 **nm** 83 **TB**
  - 1 **µm** 756 **GB**
  - 10 **µm** 770 **MB**


heiCLOUD
- LAS X
- Lightning
2 SP8 microscopes
Light wires
Scientific data storage (SDS)
High performance cluster (HPC)
bwVISU
- Linux
- Docker
- Container
- Deep learning
- LAS X
- Lightning
Researcher
- remote

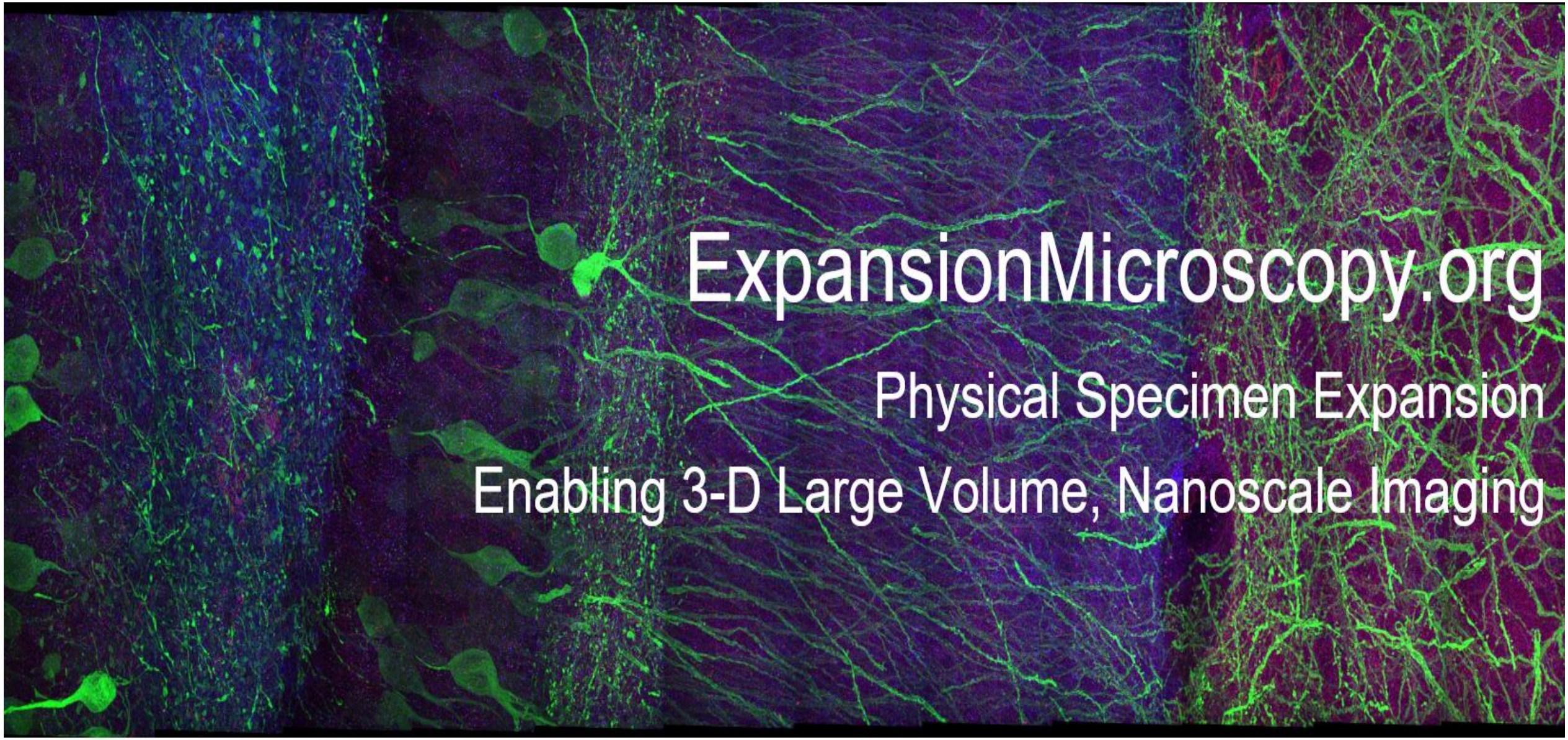
3D ANTIBODY STAINING AFTER ECi TISSUE CLEARING

- Mouse Kidney - 1mm slice;
- Removal of ECi by ethanol and rehydration after ECi clearing;
- Antibody staining (1st: Antibody against Nephrin; 2nd: Alexa Fluo 647) ;
- Imaging by Confocal Microscope with 20x and 88% glycerol as mounting solution



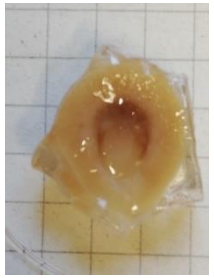


C.Brenna – Images acquired by Confocal Microscope

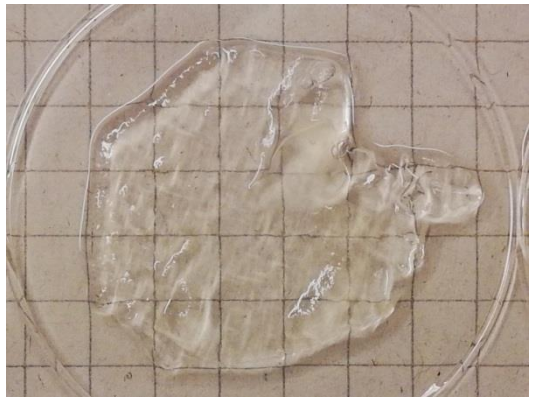

ExpansionMicroscopy.org
Physical Specimen Expansion
Enabling 3-D Large Volume, Nanoscale Imaging

ExM – Kidney - Expansion Factor

Gelated kidney

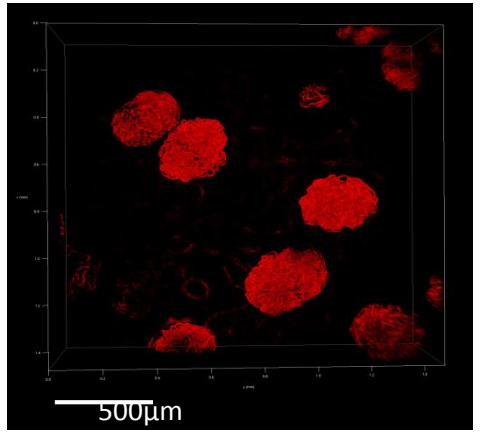


denaturated

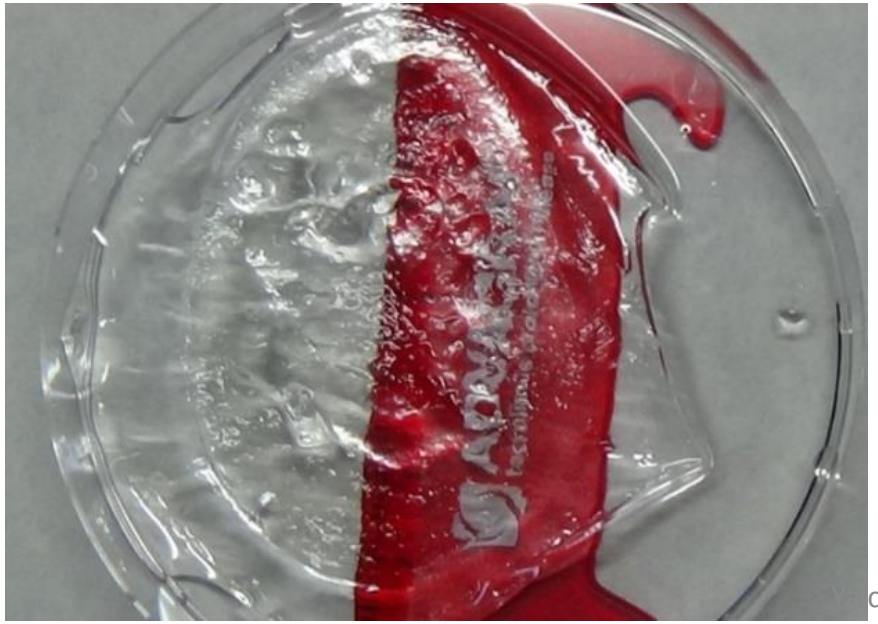


3 cm

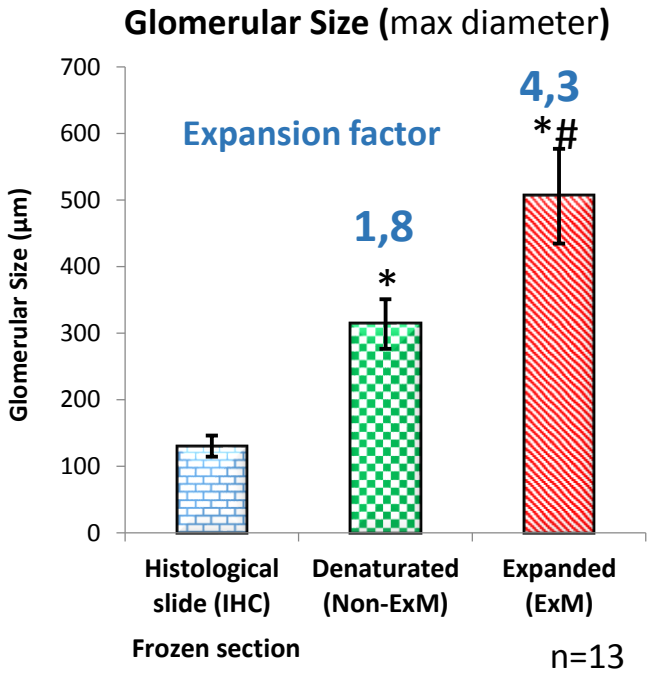
Podocin - IHC

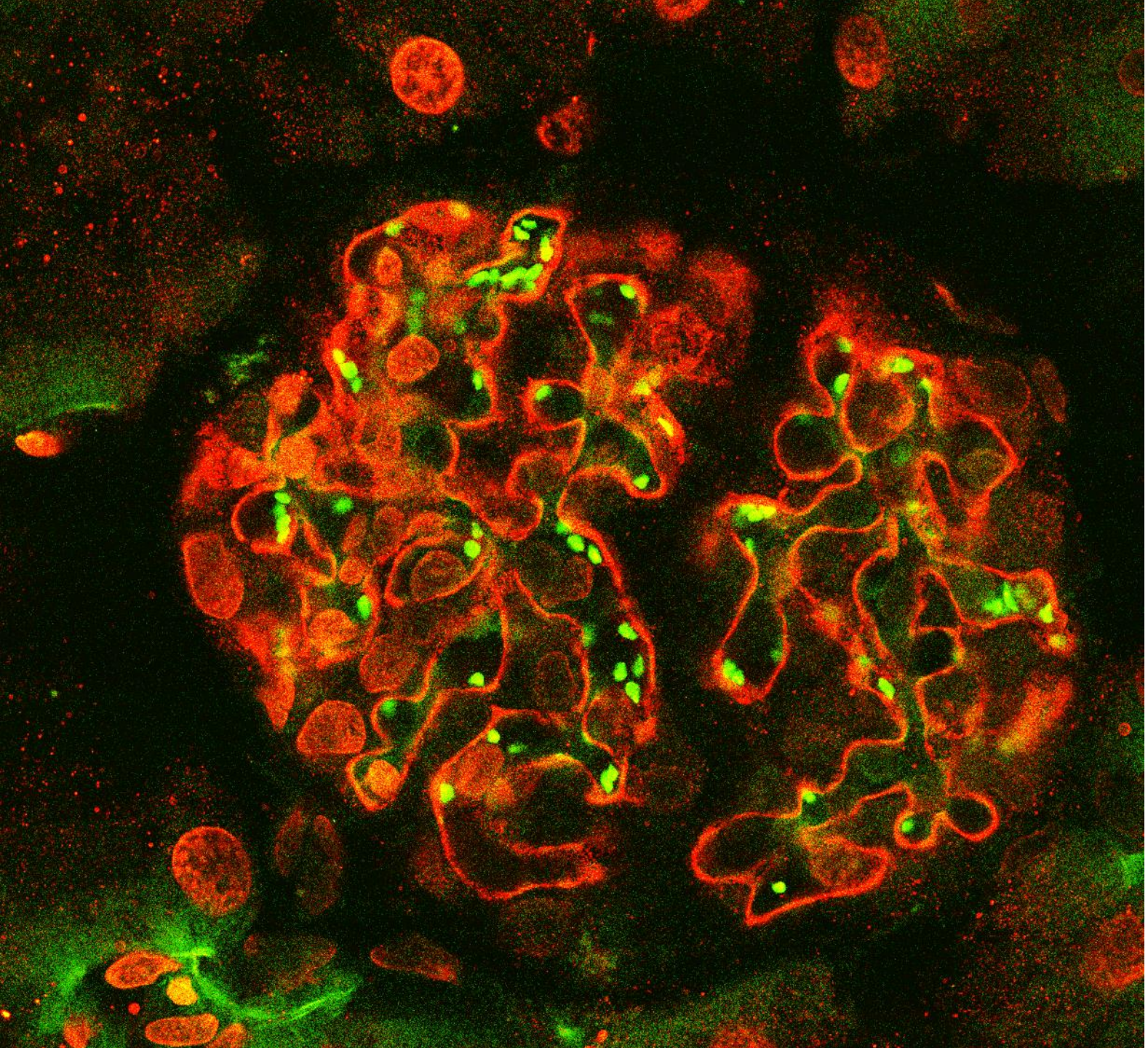


expanded



10 cm



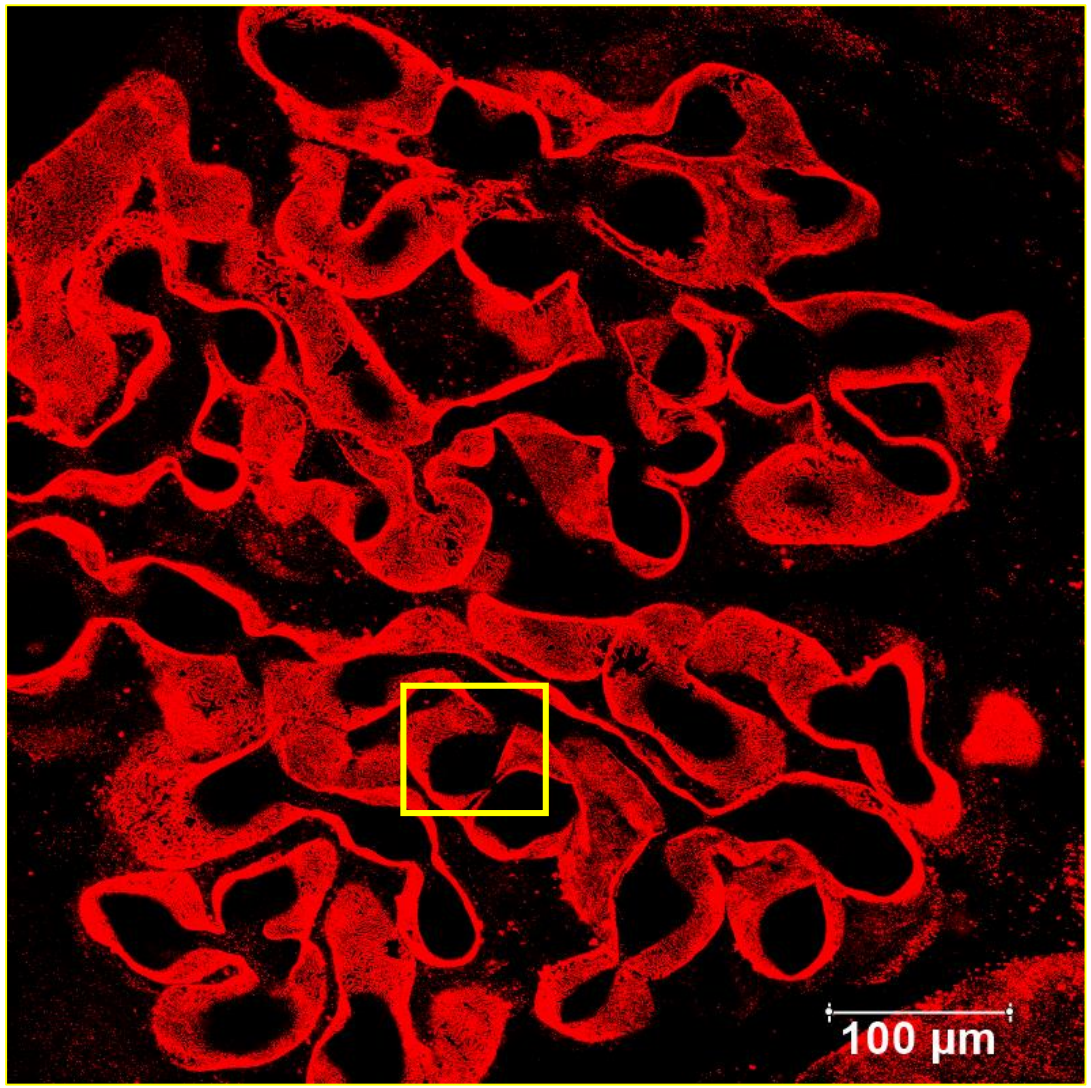


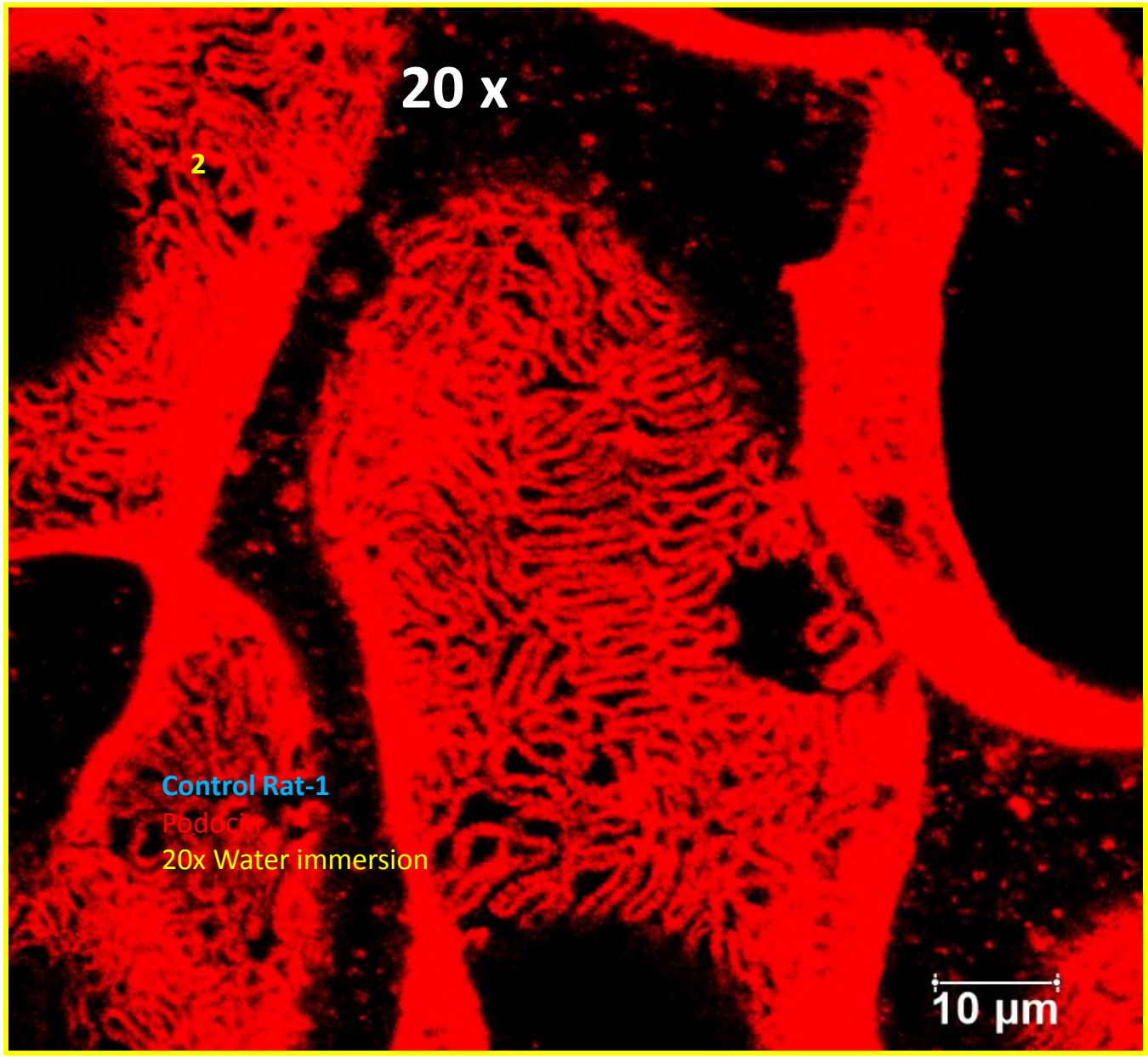
Nephrin Staining

There are also 3D images

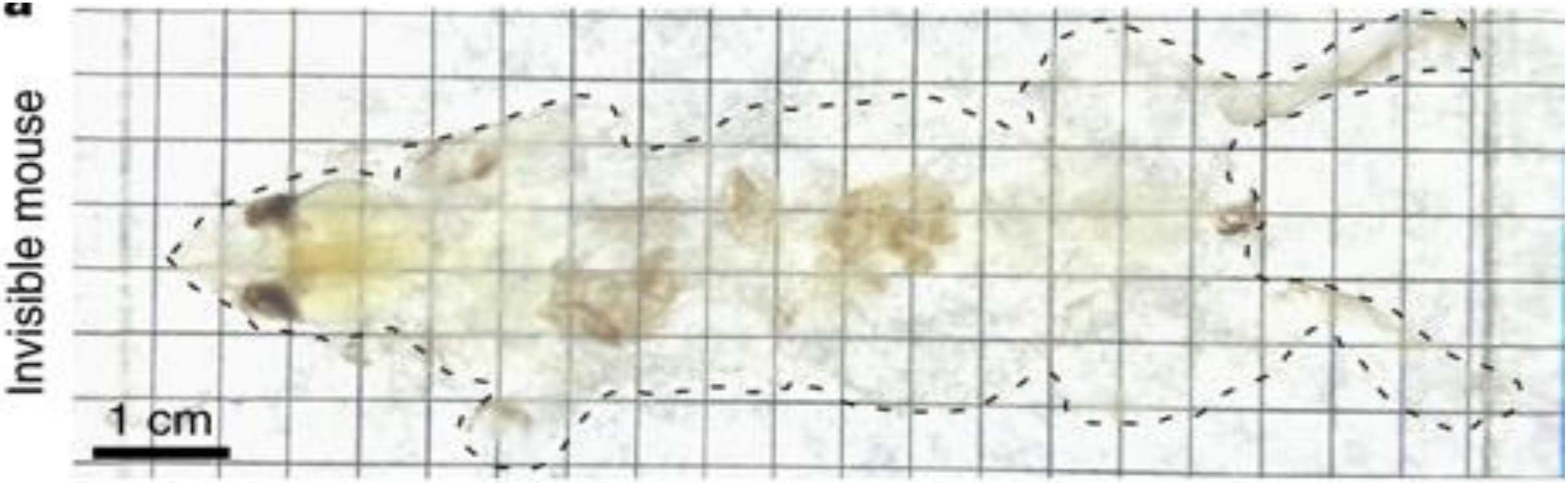
See Original file: **Z:\Yalcin Kuzay\Expansion Microscopy\Eosin-Hematoxylin-Methyl Green- Perfused Rat\228-Hematoxylin-PFA**

Zoom-in from glomeruli to podocyte foot processes

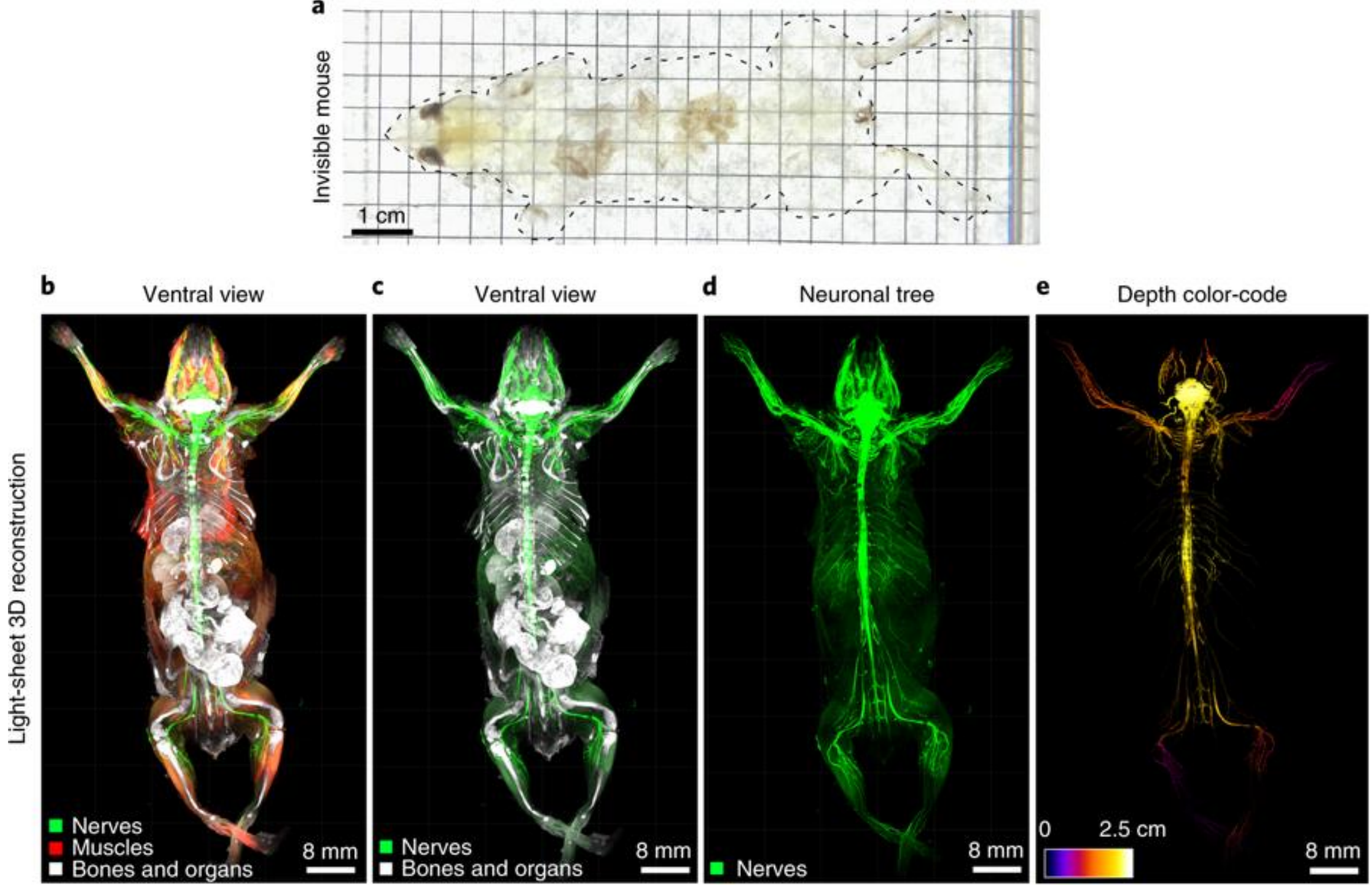


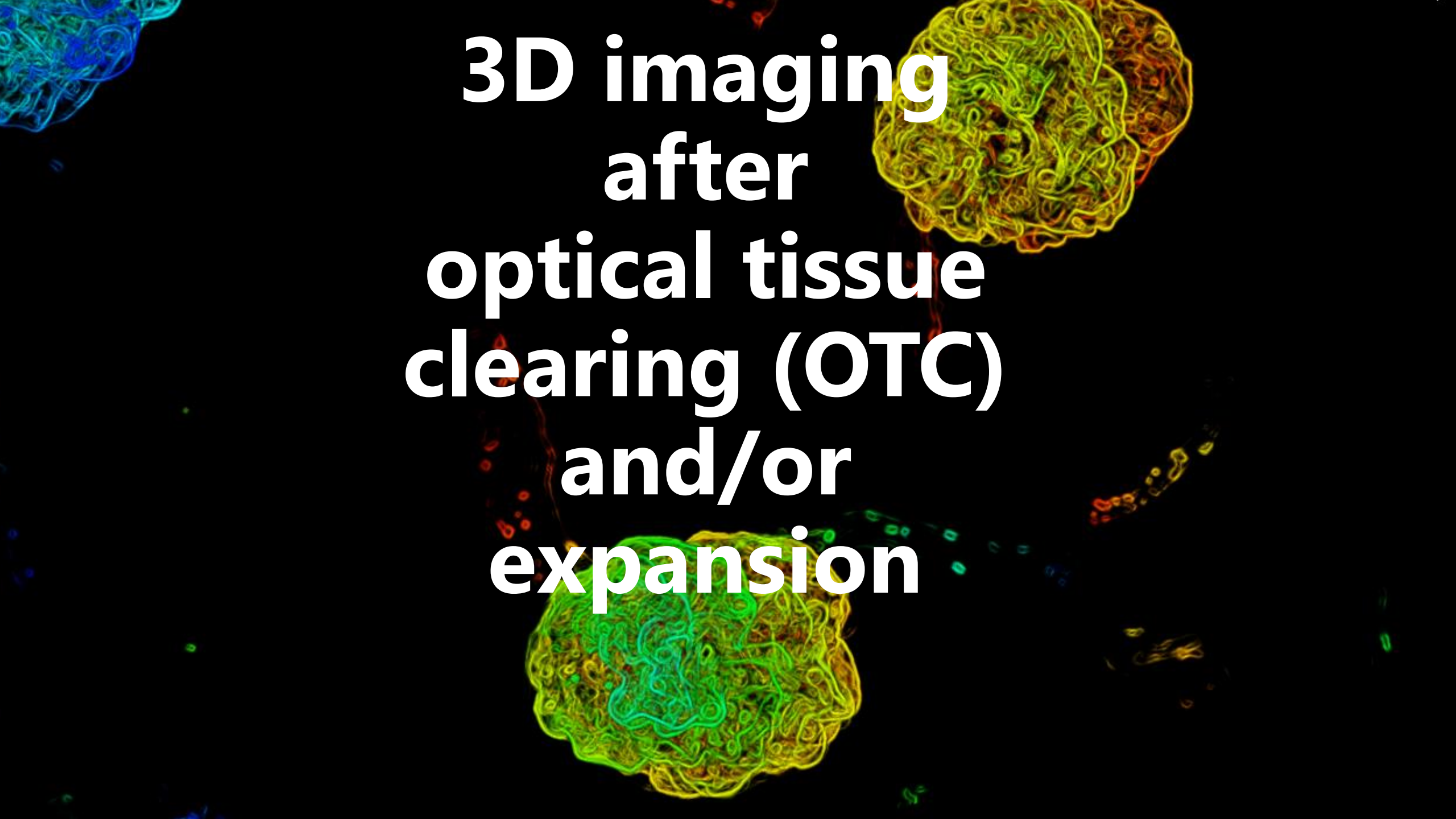


3D imaging of a mouse as a whole



Cai, R. et al. (Ertürk group)
Panoptic imaging of transparent mice reveals whole-body neuronal projections and skull–meninges connections.
Nat. Neurosci. 22, 317–327 (2019).


a
Invisible mouse
1 cm
b
Ventral view
c
Ventral view
d
Neuronal tree
e
Depth color-code
Light-sheet 3D reconstruction
Nerves
Muscles
Bones and organs
8 mm
Nerves
Bones and organs
8 mm
Nerves
8 mm
0
2.5 cm
8 mm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