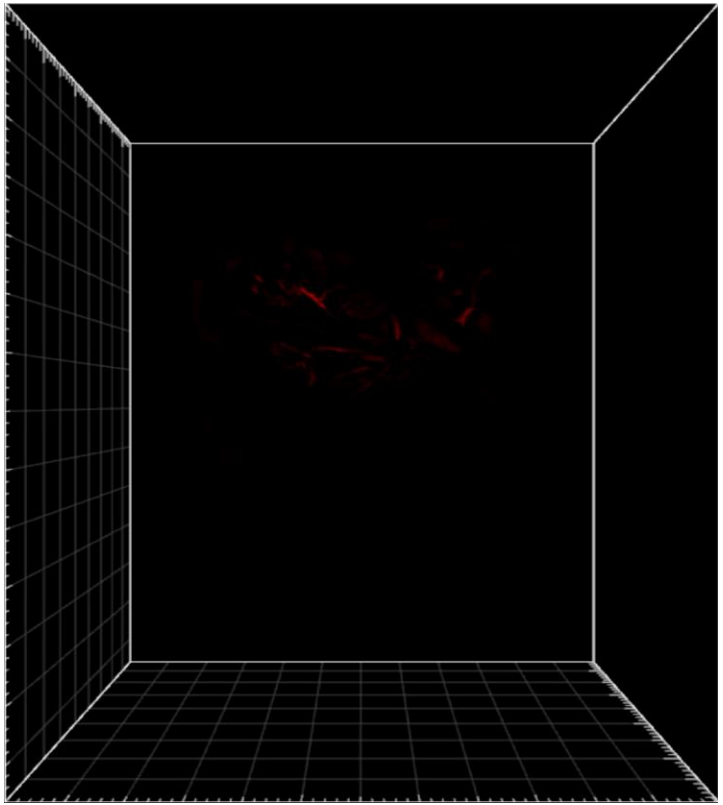
Enhancing your research with new imaging technologies

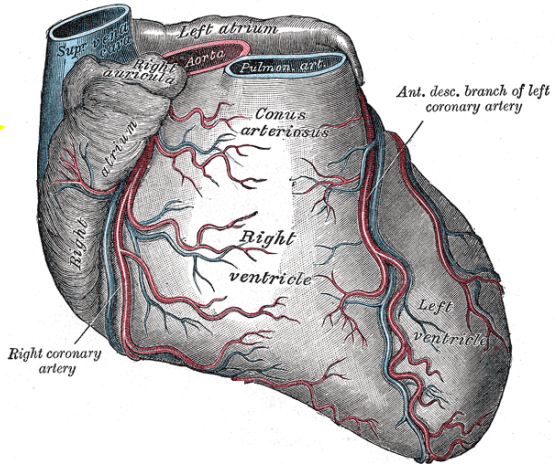


Coronary arteries
Adult mouse heart
Rendered in Imaris

Brian Raftrey
Lindsey Hamilton

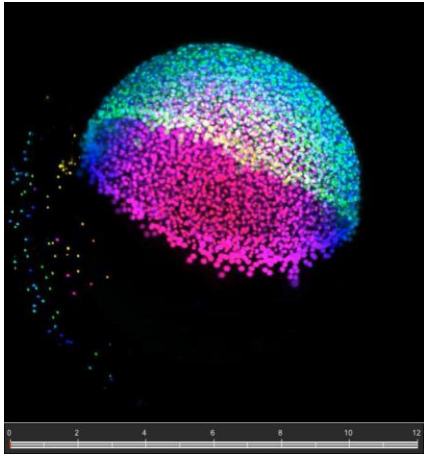
Tissue sectioning:
a powerful technique for studying tissue biology
that can miss details of three dimensional structures

?



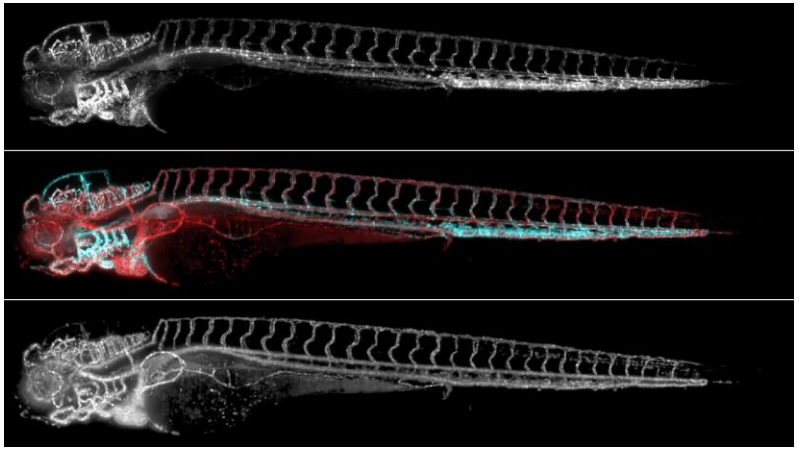
Amazing biological insights have been discovered from organisms amenable to extensive whole organ imaging

Zebrafish gastrulation



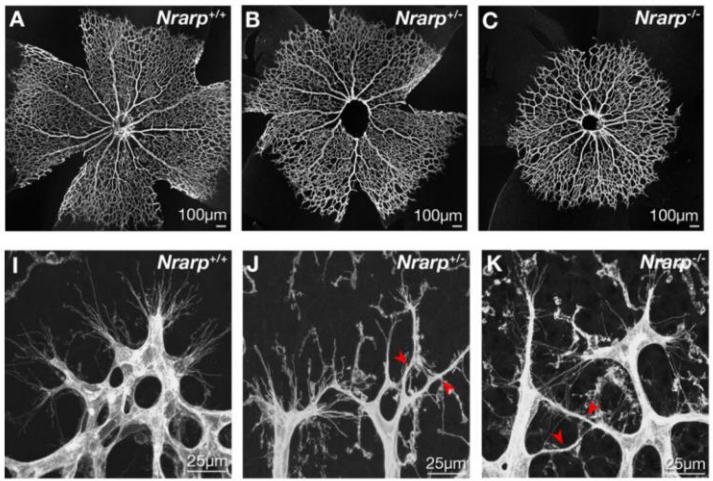
Gopi Shah, https://vimeo.com/131417079

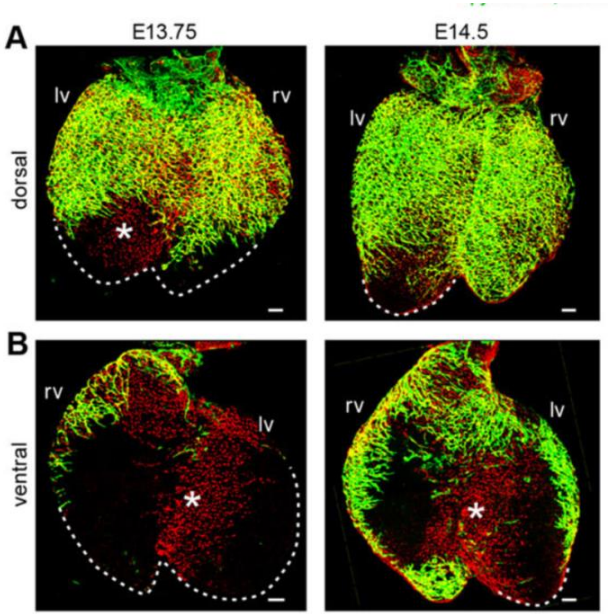
Zebrafish cardiovascular system



Huisken laboratory, https://vimeo.com/131417081

These techniques were brought to developmental biology in other organisms where tissues were relatively small



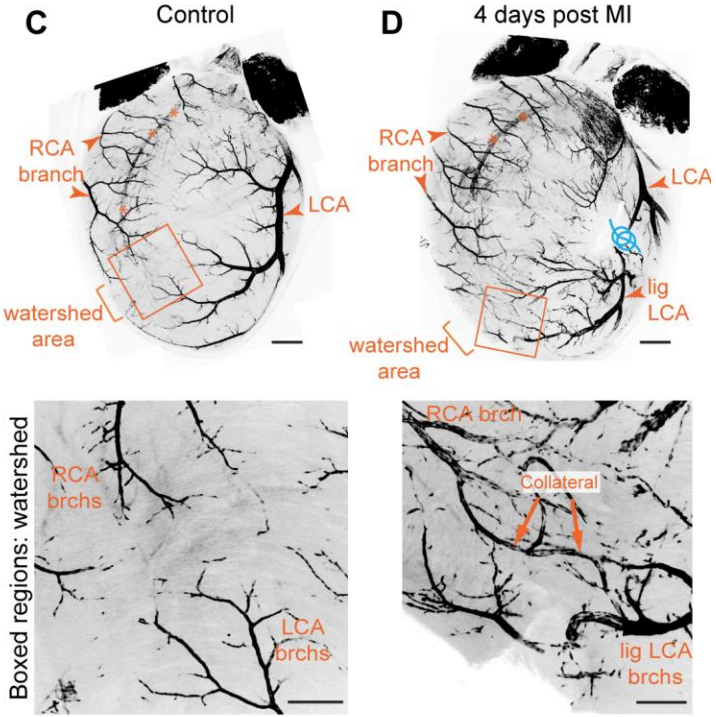


Phng et al., 2009, *Dev Cell*; Chen, Sharma et al., 2014, *Develop*

What happens when you want to expand your 3D investigations into more complex or larger tissues?

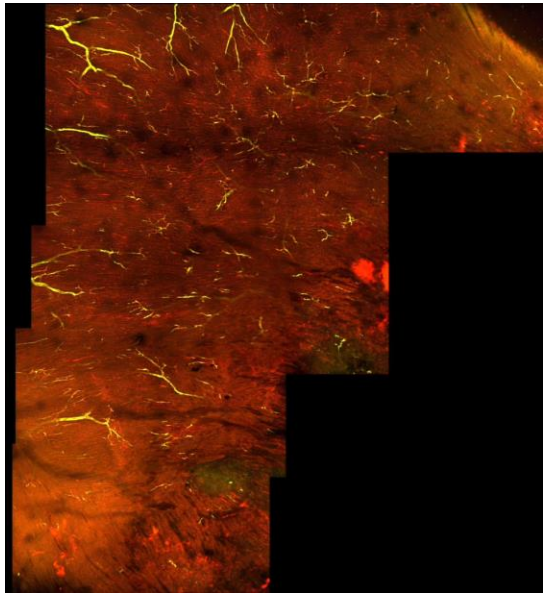
What happens when you want to expand your 3D investigations into more complex or larger tissues?

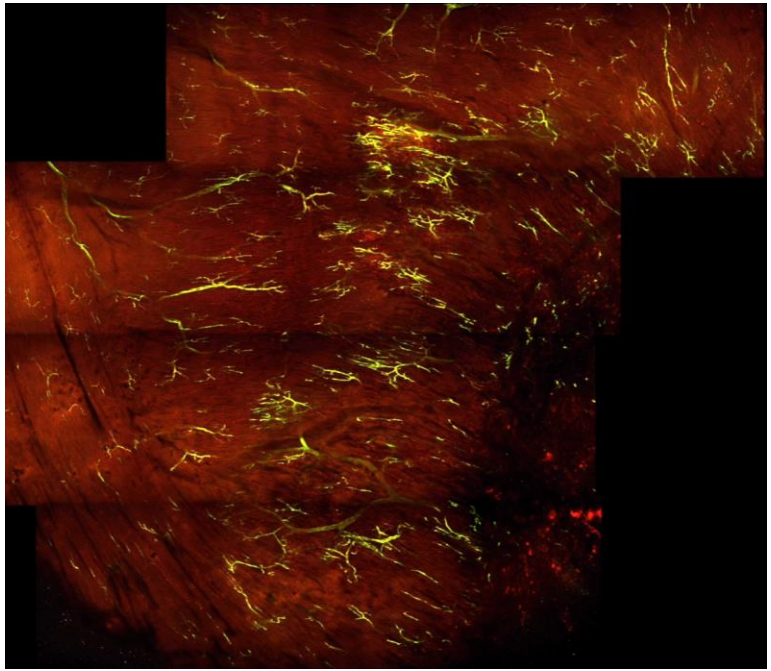
Use smaller versions such as the neonatal organs



What happens when you want to expand your 3D investigations into more complex or larger tissues?

For adults, cut out tissue chunks and
get crazy with the confocal and stitching . . . . . time intensive!





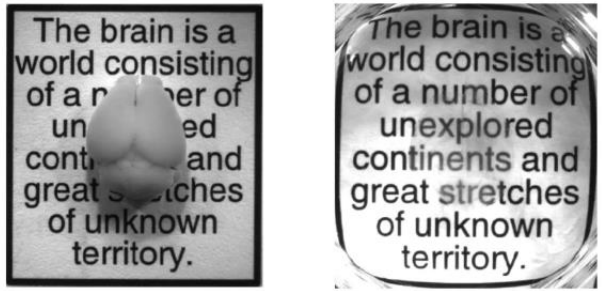
Attempting to visualize large organs by chemically clearing tissues has been around, but recently refined for the brain

The New York Times

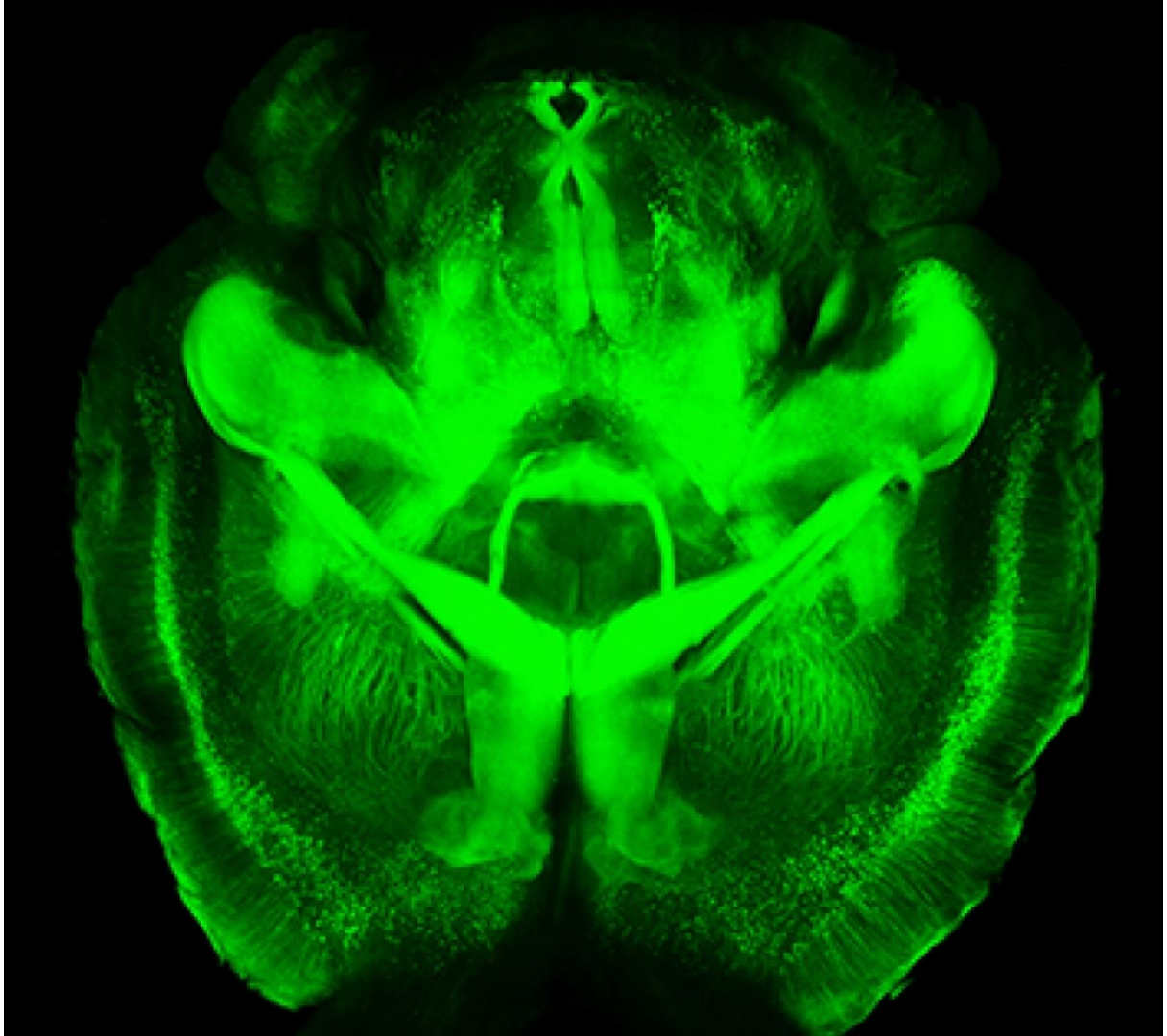
Brains as Clear as Jell-O for Scientists to Explore

By James Gorman

April 10, 2013

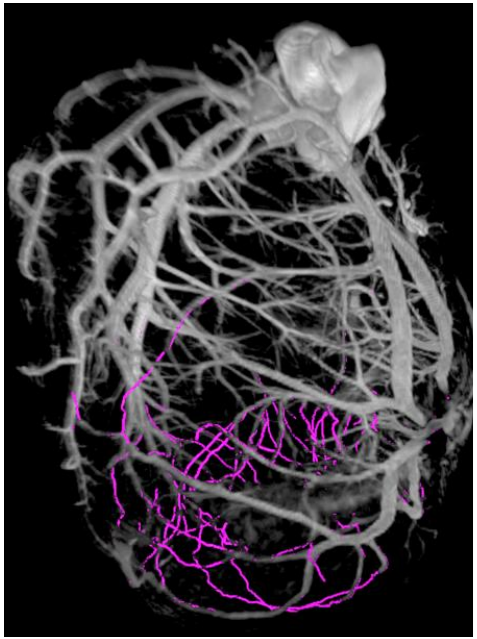


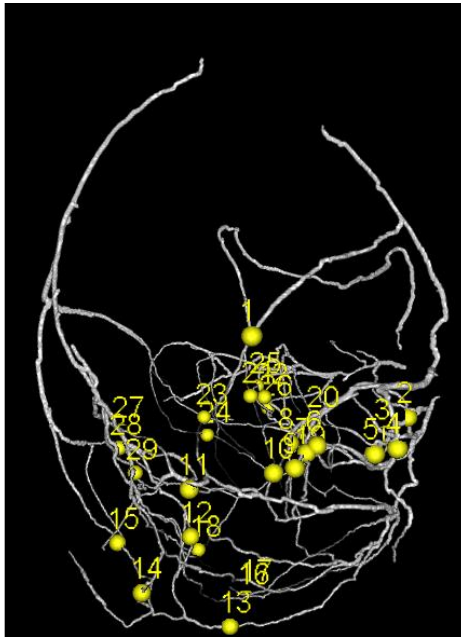
Two views of the same intact adult mouse brain, before, at left, and after a new technique developed by researchers at Stanford University was applied to it to make its tissue transparent. Deisseroth Lab



Adoption of brain clearing and imaging methods for other organs solves experimental roadblocks

Quickly identify and quantify collateral arteries

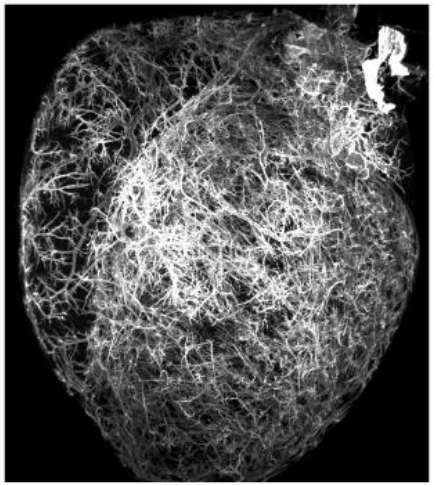


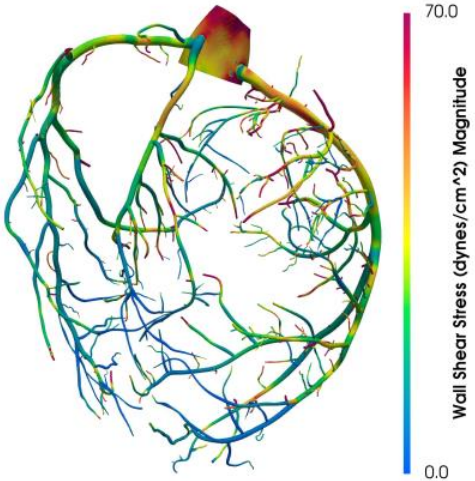


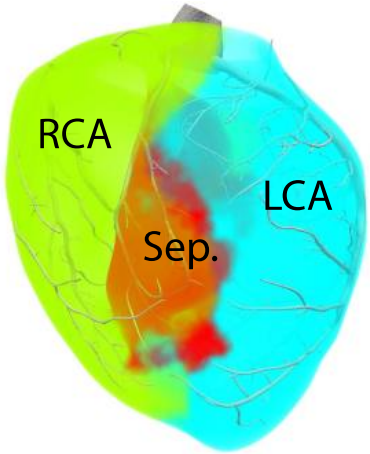
Pam Rios

Adoption of brain clearing and imaging methods for other organs solves experimental roadblocks

Intact vasculature for computational flow modeling



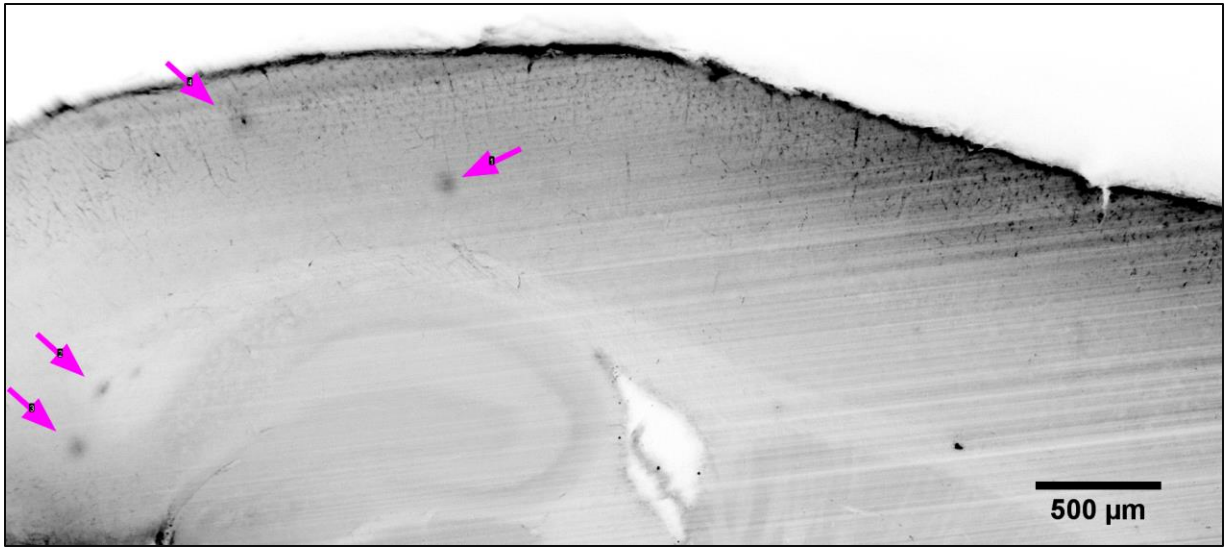




Suhaas Anbazhakan

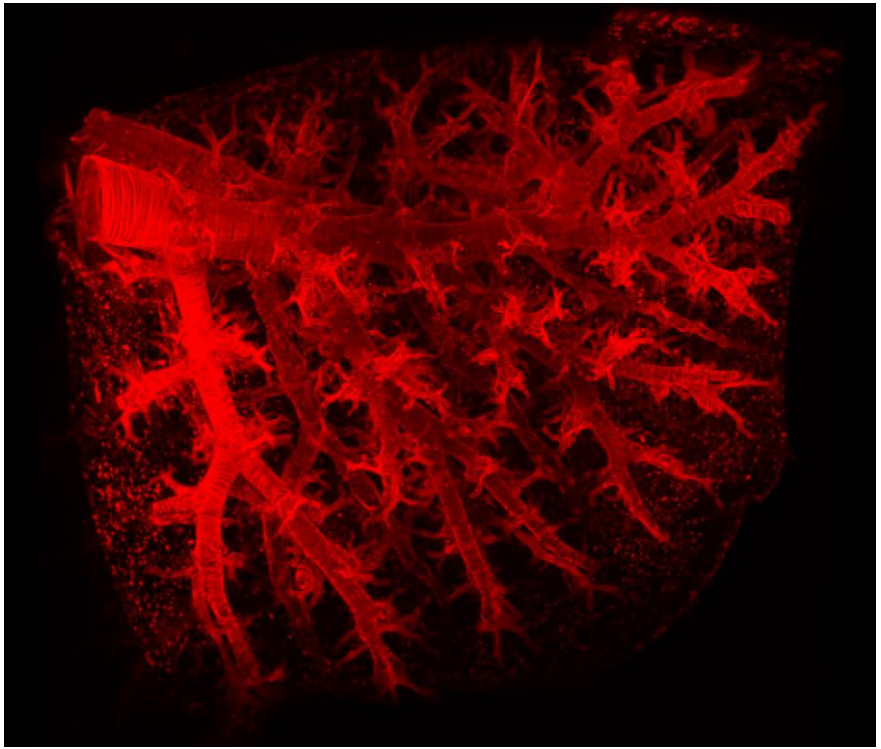
Adoption of brain clearing and imaging methods for other organs solves experimental roadblocks

Can quickly scan through large tissues for rare events



Merlini et al. Neuron. 2019

Adoption of brain clearing and imaging methods for other organs solves experimental roadblocks



Smooth muscle actin
Adult mouse lung

Ke Yuan
De Jesus Perez Lab

Outline of workshop (slides will be linked on our website)

1. Overview of clearing methods ***Andrew Chang***
   Ghosting tissues, not people

2. Successful use of iDISCO clearing ***Pam Rios***
   Trick or Treat

3. Microscopy for large cleared tissues ***Suhaas Anbazhakan***
   Fright Sheet microscopy

4. Image processing ***Suhaas Anbazhakan***
   Terror bytes of data!!

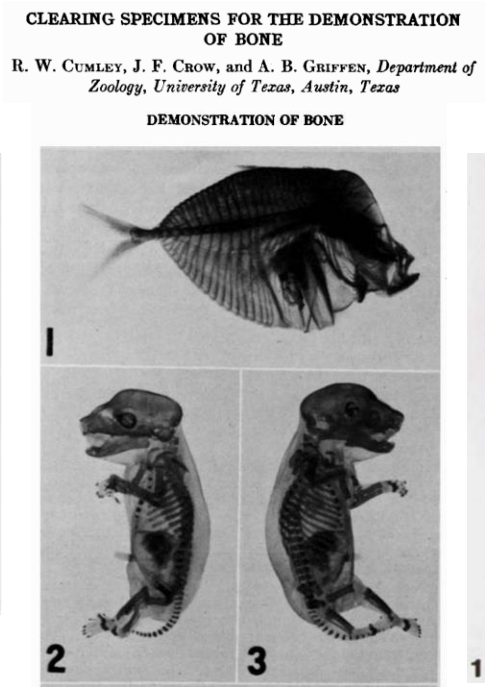
5. Miltenyi presentation of LaVision Light Sheet Microscope ***David Castaneda***

6. Breakout DEMONstrations:
   1. LaVision Ultramicroscope II
   2. Imaris processing software
   3. Computational Fluid Modeling- SimVascular
   4. Q&A for sample preparation and image analysis

Overview of clearing methods (Andrew)

Tissue clearing to visualize bone structure

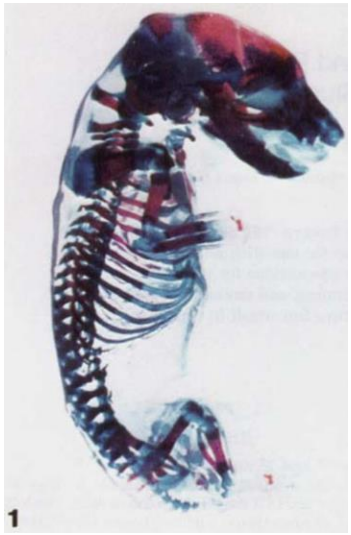
Spalteholz, 1914


CLEARING SPECIMENS FOR THE DEMONSTRATION OF BONE

R. W. Cumley, J. F. Crow, and A. B. Griffen, *Department of Zoology, University of Texas, Austin, Texas*

DEMONSTRATION OF BONE

Cumley, Crow, Griffen, 1939



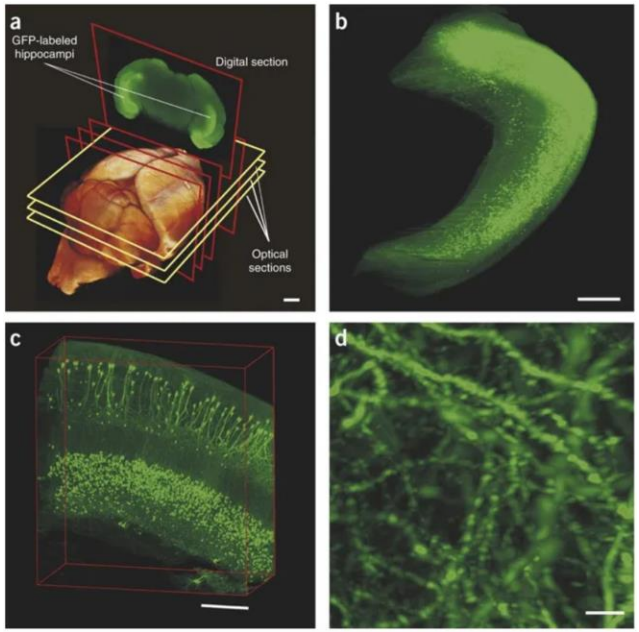
McLeod, 1980

Fernandez et al., 2011

- Fixation in 95% ethanol, treated with 1% KOH
- Stained with Alizarin Red and Alcian Blue
- Cleared in glycerin (weeks)

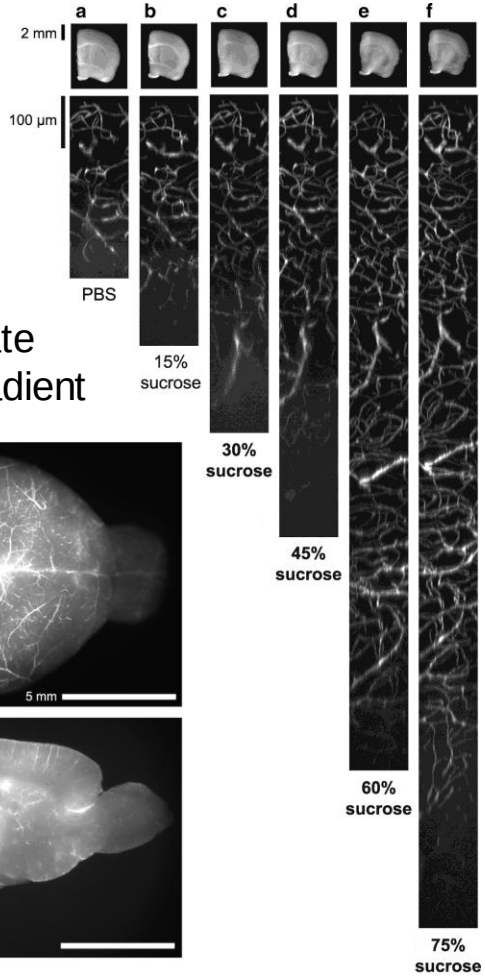
Revisiting clearing methods

Renewed interest in volume imaging with the incorporation of fluorescent labeling

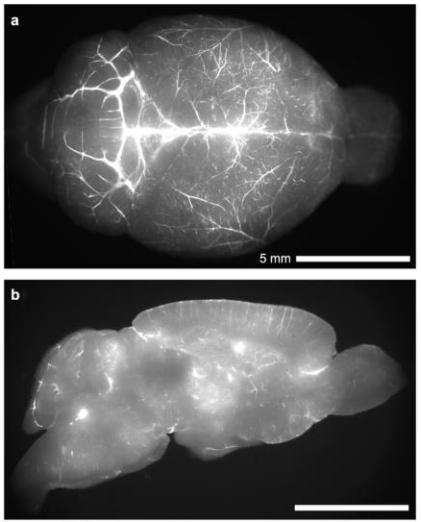


Dodt et al, 2007

- Endogenous GFP
- Dehydrated with ethanol
- Cleared in Benzylalcohol-Benzylbenylzoate (1:2 BABB)



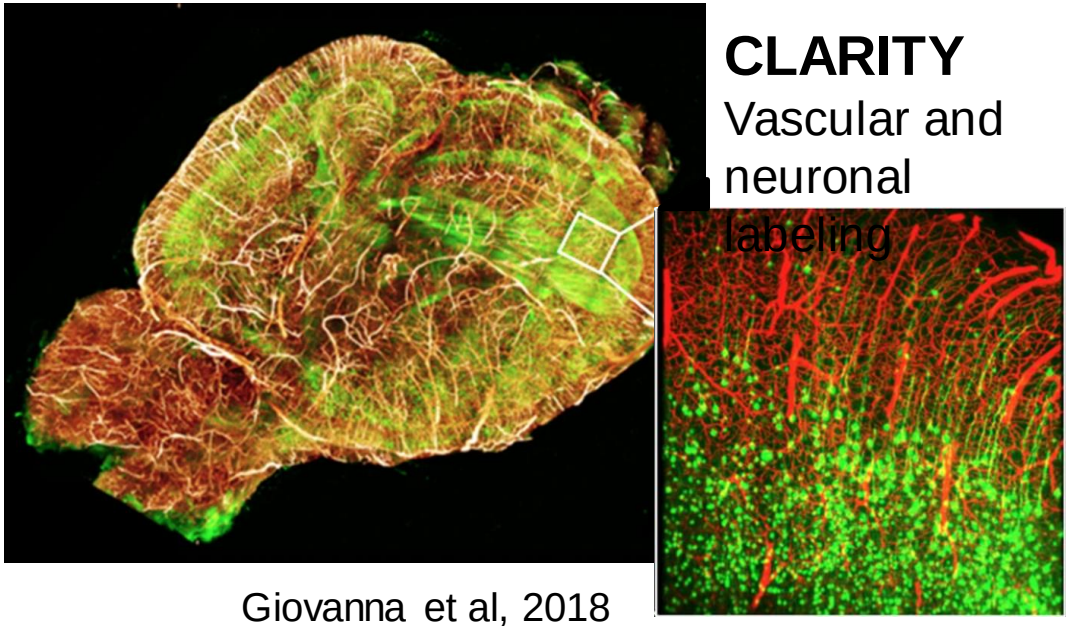
Vascular labeling by fluorescent gel perfusate
Cleared in sucrose gradient



Tsai et al, 2009

As of 2019…

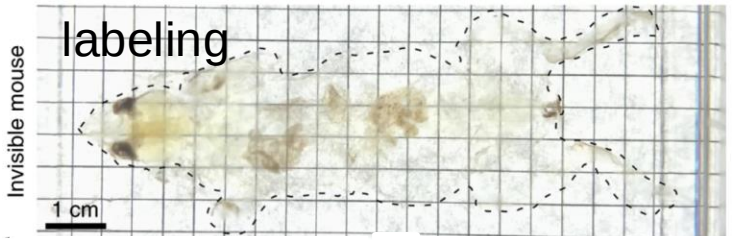
Improvements in clearing techniques and computational power pushed recent advances

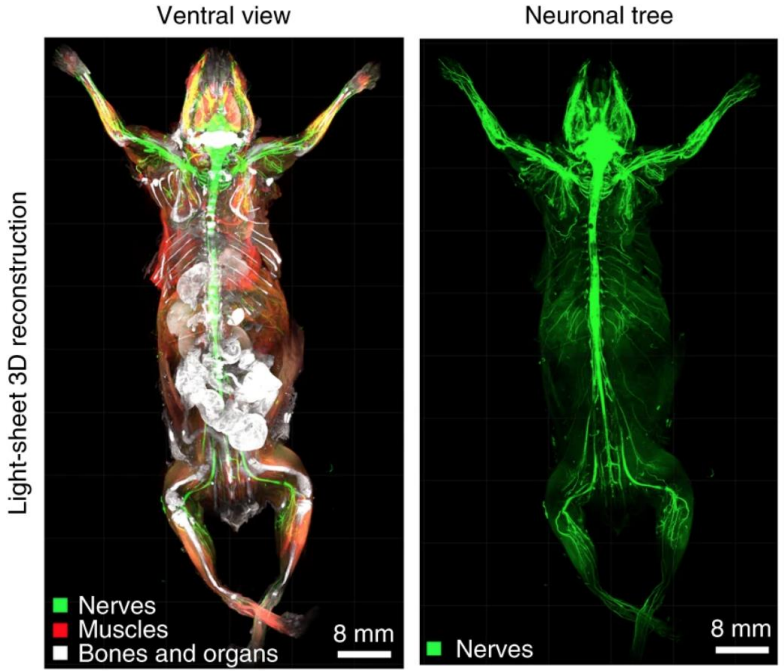


Giovanna et al, 2018

CLARITY
Vascular and neuronal labeling

vDISCO – Whole animal labeling





Cai et al, 2019

But which protocol to pick?

TECHNICAL REPORTS

nature neuroscience

SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction

Meng-Tsen Ke[1,2], Satoshi Fujimoto[1] & Takeshi Imai[1–3]

SCIENCE ADVANCES | RESEARCH ARTICLE

RESEARCH METHODS

FDISCO: Advanced solvent-based clearing method for imaging whole organs

Yisong Qi[1,2]*, Tingting Yu[1,2]*, Jianyi Xu[1,2], Peng Wan[1,2], Yilin Ma[1,2], Jingtan Zhu[1,2], Yusha Li[1,2], Hui Gong[1,2], Qingming Luo[1,2], Dan Zhu[1,2†]

Cell

Shrinkage-mediated imaging of entire organs and organisms using uDISCO

Chenchen Pan[1,2,6], Ruiyao Cai[1,2,6], Francesca Paola Quacquarelli[1,6], Alireza Ghasemigharagoz[1], Athanasios Lourbopoulos[1], Paweł Matryba[1,5], Nikolaus Plesnila[1–3], Martin Dichgans[1–4], Farida Hellal[1,3] & Ali Ertürk[1–3]

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

Nicolas Renier,[1,3] Zhuhao Wu,[1,3] David J. Simon,[1] Jing Yang,[1] Pablo Ariel,[2] and Marc Tessier-Lavigi

[1]Laboratory of Brain Development and Repair
[2]Bio-Imaging Resource Center
The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
[3]Co-first author
*Correspondence: marctl@rockefeller.edu
http://dx.doi.org/10.1016/j.cell.2014.10.010

SCIENTIFIC REPORTS

OPEN

Large-scale tissue clearing (PACT): Technical evaluation and new perspectives in immunofluorescence, histology, and ultrastructure

Received: 02 June 2016
Accepted: 12 September 2016
Published: 29 September 2016

SCIENTIFIC REPORTS

OPEN

ACT-PRESTO: Rapid and consistent tissue clearing and labeling method for 3-dimensional (3D) imaging

Received: 08 September 2015
Accepted: 23 November 2015
Published: 11 January 2016

Eunsoo Lee[1], Jungyoon Choi[1], Youhwa Jo[1], Joo Yeon Kim[1], Yu Jin Jang[1], Hye Myeong Lee[2], So Yeun Kim[3], Ho-Jae Lee[4], Keunchang Cho[4], Neoncheol Jung[4], Eun Mi Hur[5,6], Sung Jin Jeong[2], Cheil Moon[3], Youngshik Choe[2], Im Joo Rhyu[1], Hyun Kim[1] & Woong Sun[1]

PROTOCOL

Advanced CLARITY for rapid and high-resolution imaging of intact tissues

Raju Tomer[1–3], Li Ye[1–3], Brian Hsueh[1,3] & Karl Deisseroth[1–4]

TECHNICAL REPORTS

nature neuroscience

ScaleS: an optical clearing palette for biological imaging

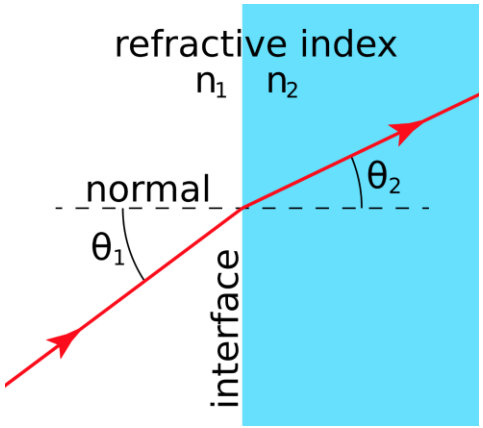
Hiroshi Hama[1], Hiroyuki Hioki[2], Kana Namiki[1], Tetsushi Hoshida[3], Hiroshi Kurokawa[1], Fumiyoshi Ishidate[1], Takeshi Kaneko[2], Takumi Akagi[4], Takashi Saito[5], Takaomi Saido[5] & Atsushi Miyawaki[1,3]

Why is tissue clearing needed?

- Refractive index: Descriptor of how much light bends while passing through a medium

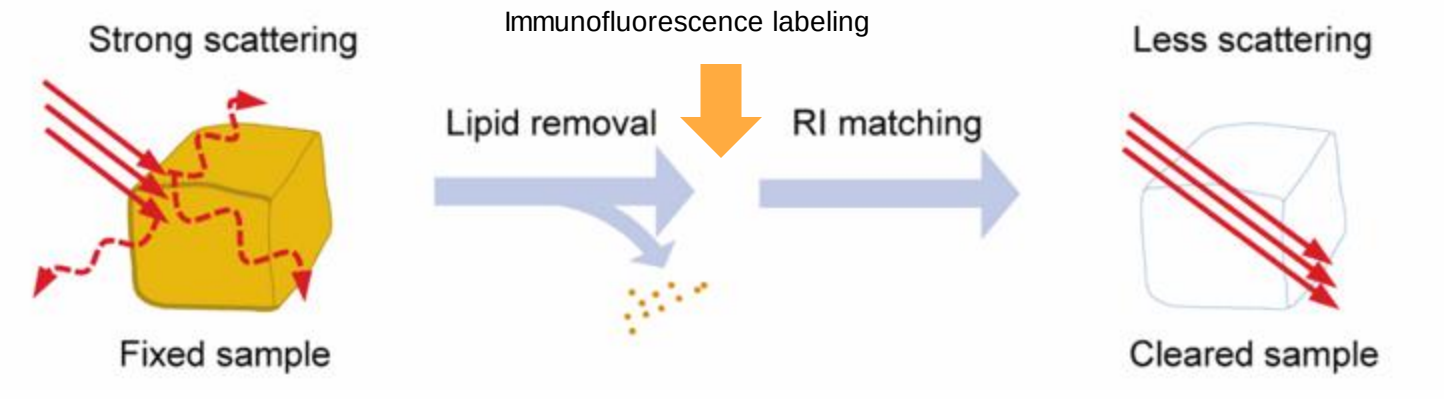
$$n = \frac{c}{v}$$

index of refraction; c: velocity of light in vacuum; v: velocity of light in the medium



Medium	Refractive Index
Air	1.0003
Water	1.33
Glycerin	1.47
Organic tissue	~1.4-1.5
Diamond	2.42

Why is tissue clearing needed?

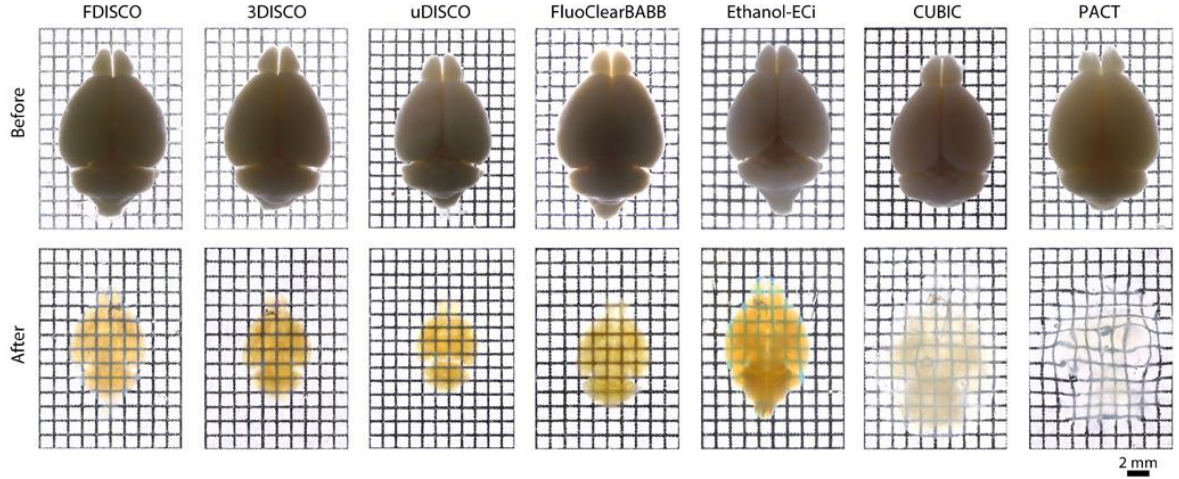


- Lipids have a high RI (~1.44) and form granular structures, leading to strong light scattering
- Immersion of specimen in medium with the same RI as protein (~1.43)
    - Note: Dehydrated proteins RI is ~1.5

Seo et al., 2016

Considerations for clearing method

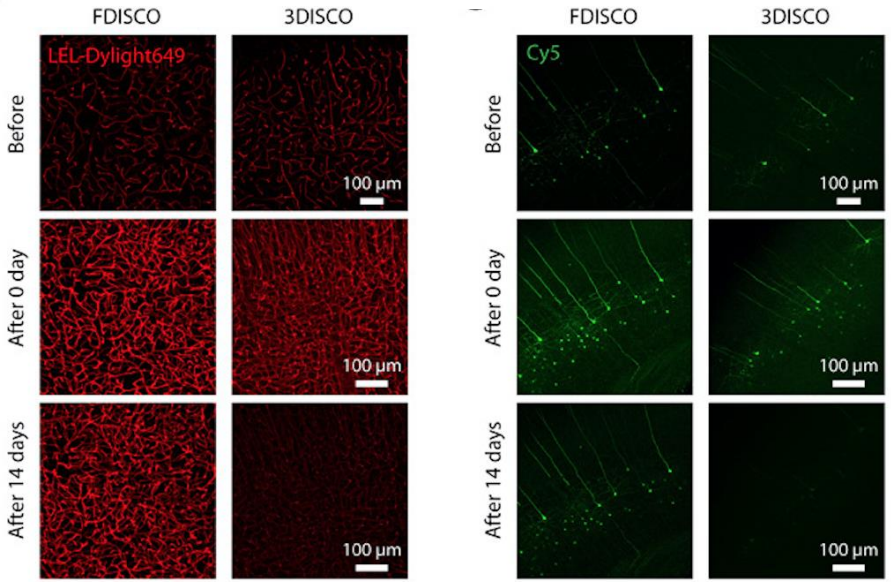
- Clearing capacity
- Changes to tissue size (expansion/shrinkage)
- Compatibility with fluorescence proteins and immunolabeling
- Ease of tissue and chemical handling
- Time of clearing process



Qi et al, 2019

Considerations for clearing method

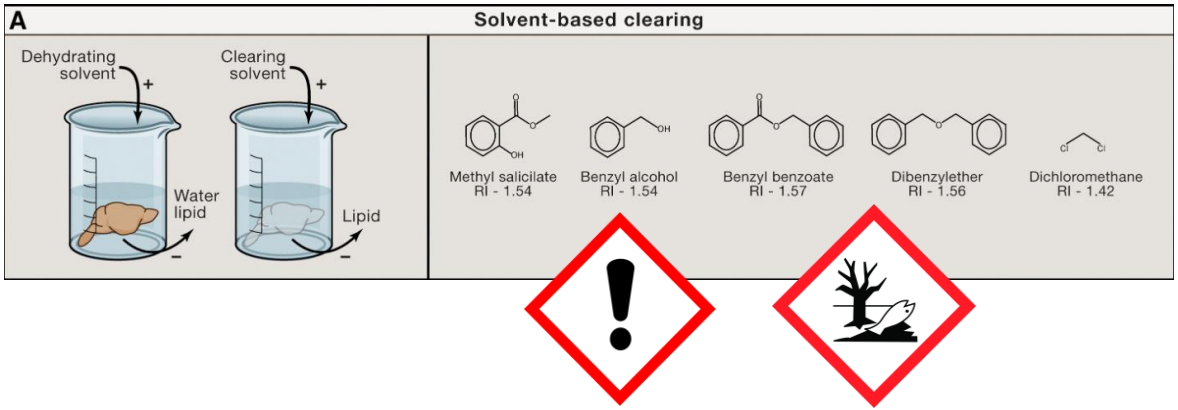
- Clearing capacity
- Changes to tissue size (expansion/shrinkage)
- Compatibility with fluorescence proteins and immunolabeling
- Ease of tissue and chemical handling
- Time of clearing process



Qi et al, 2019

Considerations for clearing method

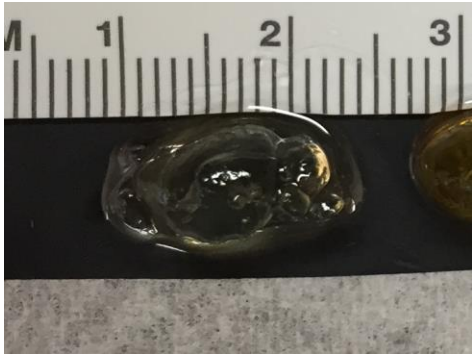
- Clearing capacity
- Changes to tissue size (expansion/shrinkage)
- Compatibility with fluorescence proteins and immunolabeling
- Ease of tissue and chemical handling
- Time of clearing process



Richardson & Litchman, 2015

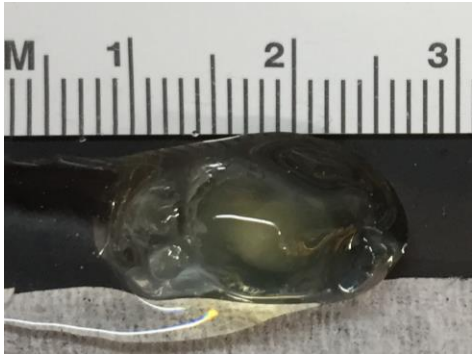
Tissue hardens

iDISCO



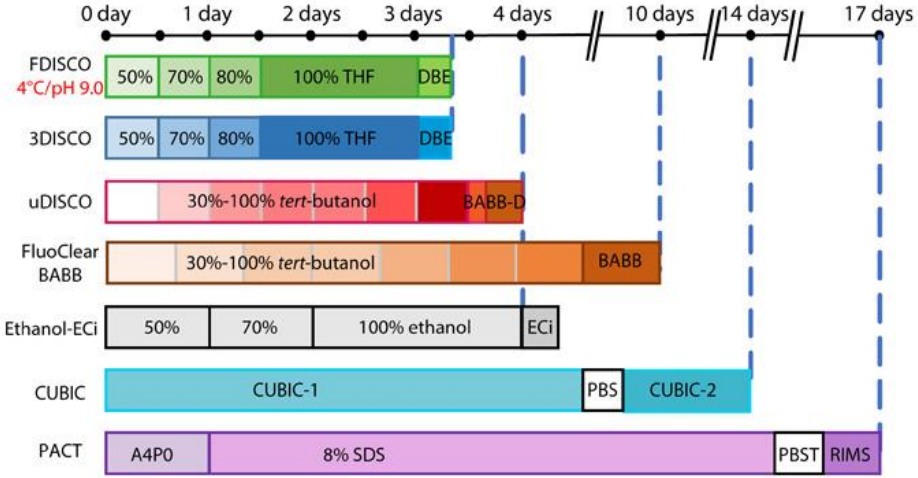
Tissue softens

CUBIC



Considerations for clearing method

- Clearing capacity
- Changes to tissue size (expansion/shrinkage)
- Compatibility with fluorescence proteins and immunolabeling
- Ease of tissue and chemical handling
- Time of clearing process



Qi et al, 2019

Considerations for clearing method

- Clearing capacity
- Changes to tissue size (expansion/shrinkage)
- Compatibility with fluorescence proteins and immunolabeling
- Ease of tissue and chemical handling
- Time of clearing process

Mol. Cells 2016; 39(6): 439-446
http://dx.doi.org/10.14348/molcells.2016.0088

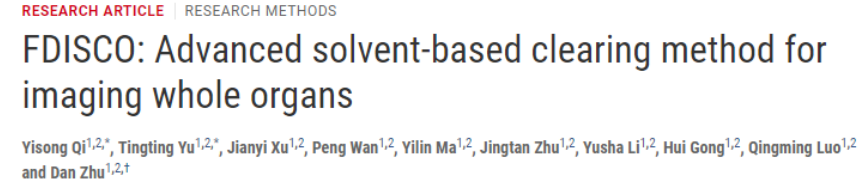
Molecules and Cells
http://molcells.org
Established in 1990

Minireview

Clearing and Labeling Techniques for Large-Scale Biological Tissues

Jinyoung Seo[1,5], Minjin Choe[2,5], and Sung-Yon Kim[1,2,3,4,*]

Seo et al, 2016

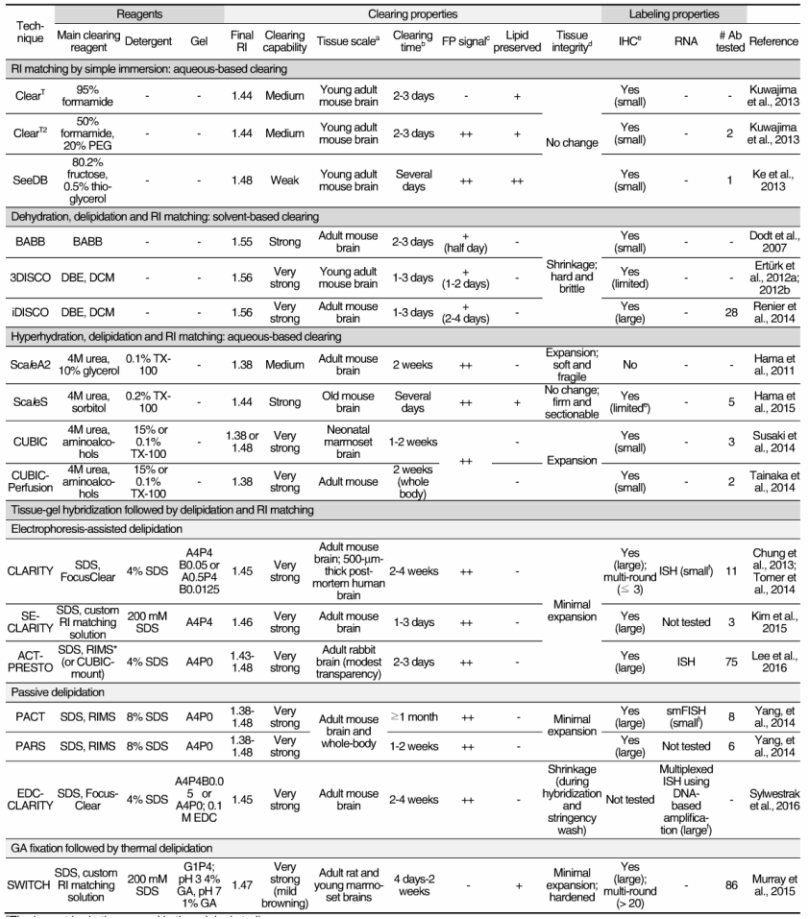


RESEARCH ARTICLE | RESEARCH METHODS

FDISCO: Advanced solvent-based clearing method for imaging whole organs

Yisong Qi[1,2,*], Tingting Yu[1,2,*], Jianyi Xu[1,2], Peng Wan[1,2], Yilin Ma[1,2], Jingtan Zhu[1,2], Yusha Li[1,2], Hui Gong[1,2], Qingming Luo[1,2] and Dan Zhu[1,2,†]

Qi et al, 2019



Tech-nique	Reagents: Main clearing reagent	Reagents: Detergent	Reagents: Gel	Clearing properties: Final RI	Clearing properties: Clearing capability	Clearing properties: Tissue scale[a]	Clearing properties: Clearing time[b]	Clearing properties: FP signal[c]	Clearing properties: Lipid preserved	Clearing properties: Tissue integrity[d]	Labeling properties: IHC[e]	Labeling properties: RNA	Labeling properties: # Ab tested	Reference
RI matching by simple immersion: aqueous-based clearing														
Clear[T]	95% formamide	-	-	1.44	Medium	Young adult mouse brain	2-3 days	-	+	No change	Yes (small)	-	-	Kuwajima et al., 2013
Clear[T2]	50% formamide, 20% PEG	-	-	1.44	Medium	Young adult mouse brain	2-3 days	++	+		Yes (small)	-	2	Kuwajima et al., 2013
SeeDB	80.2% fructose, 0.5% thio-glycerol	-	-	1.48	Weak	Young adult mouse brain	Several days	++	++		Yes (small)	-	1	Ke et al., 2013
Dehydration, delipidation and RI matching: solvent-based clearing														
BABB	BABB	-	-	1.55	Strong	Adult mouse brain	2-3 days	+ (half day)	-	Shrinkage; hard and brittle	Yes (small)	-	-	Dodt et al., 2007
3DISCO	DBE, DCM	-	-	1.56	Very strong	Young adult mouse brain	1-3 days	+ (1-2 days)	-		Yes (limited)	-	-	Ertürk et al., 2012a; 2012b
iDISCO	DBE, DCM	-	-	1.56	Very strong	Adult mouse brain	1-3 days	+ (2-4 days)	-		Yes (large)	-	28	Renier et al., 2014
Hyperhydration, delipidation and RI matching: aqueous-based clearing														
ScaleA2	4M urea, 10% glycerol	0.1% TX-100	-	1.38	Medium	Adult mouse brain	2 weeks	++	-	Expansion; soft and fragile	No	-	-	Hama et al., 2011
ScaleS	4M urea, sorbitol	0.2% TX-100	-	1.44	Strong	Old mouse brain	Several days	++	+	No change; firm and sectionable	Yes (limited[f])	-	5	Hama et al., 2015
CUBIC	4M urea, aminoalco-hols	15% or 0.1% TX-100	-	1.38 or 1.48	Very strong	Neonatal marmoset brain	1-2 weeks	++	-	Expansion	Yes (small)	-	3	Susaki et al., 2014
CUBIC-Perfusion	4M urea, aminoalco-hols	15% or 0.1% TX-100	-	1.38	Very strong	Adult mouse	2 weeks (whole body)		-		Yes (small)	-	2	Tainaka et al., 2014
Tissue-gel hybridization followed by delipidation and RI matching														
Electrophoresis-assisted delipidation														
CLARITY	SDS, FocusClear	4% SDS	A4P4 B0.05 or A0.5P4 B0.0125	1.45	Very strong	Adult mouse brain; 500-μm-thick post-mortem human brain	2-4 weeks	++	-	Minimal expansion	Yes (large); multi-round (≤ 3)	ISH (small[f])	11	Chung et al., 2013; Tomer et al., 2014
SE-CLARITY	SDS, custom RI matching solution	200 mM SDS	A4P4	1.46	Very strong	Adult mouse brain	1-3 days	++	-		Yes (large)	Not tested	3	Kim et al., 2015
ACT-PRESTO	SDS, RIMS* (or CUBIC-mount)	4% SDS	A4P0	1.43-1.48	Very strong	Adult rabbit brain (modest transparency)	2-3 days	++	-		Yes (large)	ISH	75	Lee et al., 2016
Passive delipidation														
PACT	SDS, RIMS	8% SDS	A4P0	1.38-1.48	Very strong	Adult mouse brain and whole-body	≥1 month	++	-	Minimal expansion	Yes (large)	smFISH (small[f])	8	Yang, et al., 2014
PARS	SDS, RIMS	8% SDS	A4P0	1.38-1.48	Very strong		1-2 weeks	++	-		Yes (large)	Not tested	6	Yang, et al., 2014
EDC-CLARITY	SDS, Focus-Clear	4% SDS	A4P4B0.05 or A4P0; 0.1 M EDC	1.45	Very strong	Adult mouse brain	2-4 weeks	++	-	Shrinkage (during hybridization and stringency wash)	Not tested	Multiplexed ISH using DNA-based amplifica-tion (large)	-	Sylwestrak et al., 2016
GA fixation followed by thermal delipidation														
SWITCH	SDS, custom RI matching solution	200 mM SDS	G1P4; pH 3 4% GA, pH 7 1% GA	1.47	Very strong (mild browning)	Adult rat and young marmo-set brains	4 days-2 weeks	-	+	Minimal expansion; hardened	Yes (large); multi-round (> 20)	-	86	Murray et al., 2015

But which protocol to pick?

TECHNICAL REPORTS

nature neuroscience

SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction

Meng-Tsen Ke[1,2], Satoshi Fujimoto[1] & Takeshi Imai[1–3]

SCIENCE ADVANCES | RESEARCH ARTICLE

RESEARCH METHODS

FDISCO: Advanced solvent-based clearing method for imaging whole organs

Yisong Qi[1,2]*, Tingting Yu[1,2]*, Jianyi Xu[1,2], Peng Wan[1,2], Yilin Ma[1,2], Jingtan Zhu[1,2], Yusha Li[1,2], Hui Gong[1,2], Qingming Luo[1,2], Dan Zhu[1,2†]

Cell

Shrinkage-mediated imaging of entire organs and organisms using uDISCO

Chenchen Pan[1,2,6], Ruiyao Cai[1,2,6], Francesca Paola Quacquarelli[1,6], Alireza Ghasemigharagoz[1], Athanasios Lourbopoulos[1], Paweł Matryba[1,5], Nikolaus Plesnila[1–3], Martin Dichgans[1–4], Farida Hellal[1,3] & Ali Ertürk[1–3]

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

Nicolas Renier,[1,3] Zhuhao Wu,[1,3] David J. Simon,[1] Jing Yang,[1] Pablo Ariel,[2] and Marc Tessier-Lavigi

[1]Laboratory of Brain Development and Repair
[2]Bio-Imaging Resource Center
The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
[3]Co-first author
*Correspondence: marctl@rockefeller.edu
http://dx.doi.org/10.1016/j.cell.2014.10.010

SCIENTIFIC REPORTS

OPEN

Large-scale tissue clearing (PACT): Technical evaluation and new perspectives in immunofluorescence, histology, and ultrastructure

Received: 02 June 2016
Accepted: 12 September 2016
Published: 29 September 2016

SCIENTIFIC REPORTS

OPEN

ACT-PRESTO: Rapid and consistent tissue clearing and labeling method for 3-dimensional (3D) imaging

Received: 08 September 2015
Accepted: 23 November 2015
Published: 11 January 2016

Eunsoo Lee[1], Jungyoon Choi[1], Youhwa Jo[1], Joo Yeon Kim[1], Yu Jin Jang[1], Hye Myeong Lee[2], So Yeun Kim[3], Ho-Jae Lee[4], Keunchang Cho[4], Neoncheol Jung[4], Eun Mi Hur[5,6], Sung Jin Jeong[2], Cheil Moon[3], Youngshik Choe[2], Im Joo Rhyu[1], Hyun Kim[1] & Woong Sun[1]

PROTOCOL

Advanced CLARITY for rapid and high-resolution imaging of intact tissues

Raju Tomer[1–3], Li Ye[1–3], Brian Hsueh[1,3] & Karl Deisseroth[1–4]

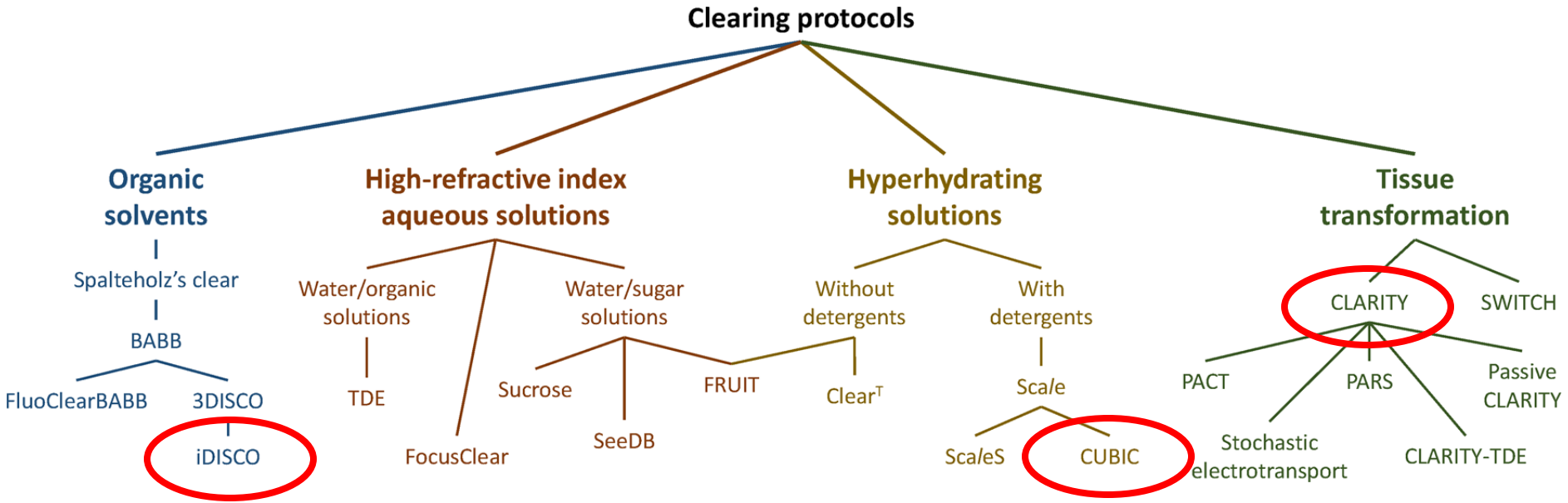
TECHNICAL REPORTS

nature neuroscience

ScaleS: an optical clearing palette for biological imaging

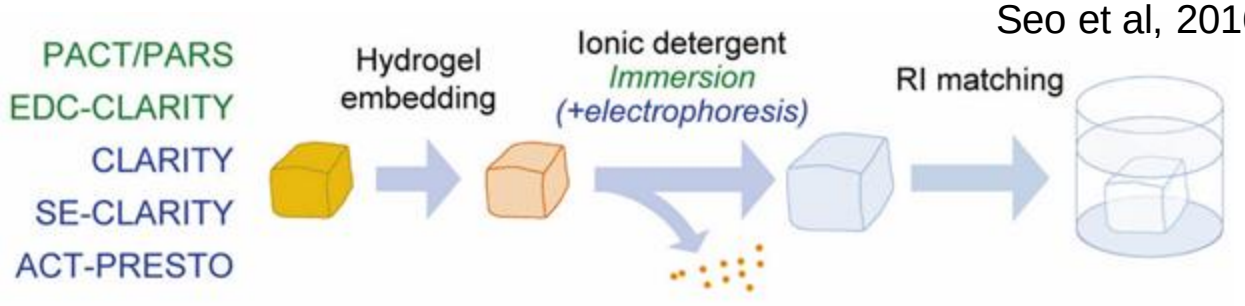
Hiroshi Hama[1], Hiroyuki Hioki[2], Kana Namiki[1], Tetsushi Hoshida[3], Hiroshi Kurokawa[1], Fumiyoshi Ishidate[1], Takeshi Kaneko[2], Takumi Akagi[4], Takashi Saito[5], Takaomi Saido[5] & Atsushi Miyawaki[1,3]

Different methods of clearing

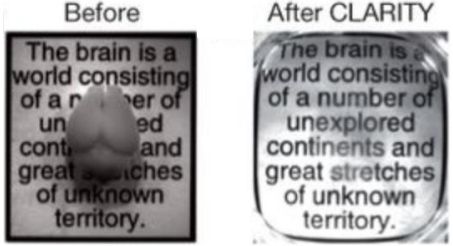


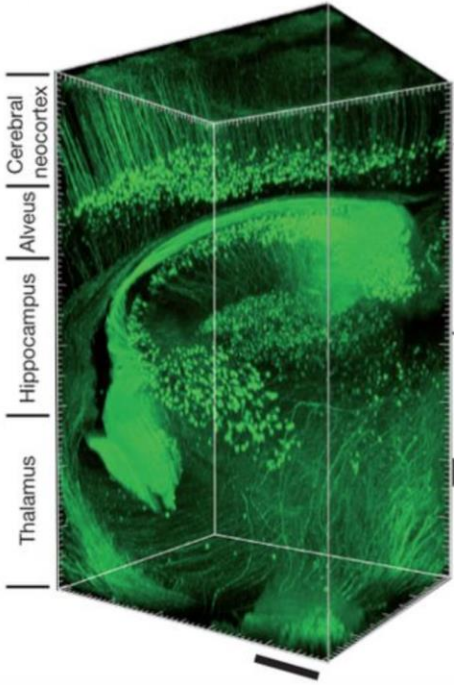
Silvestri et al, 2016

Tissue-gel hybrid formation followed by delipidation and RI matching



Seo et al, 2016





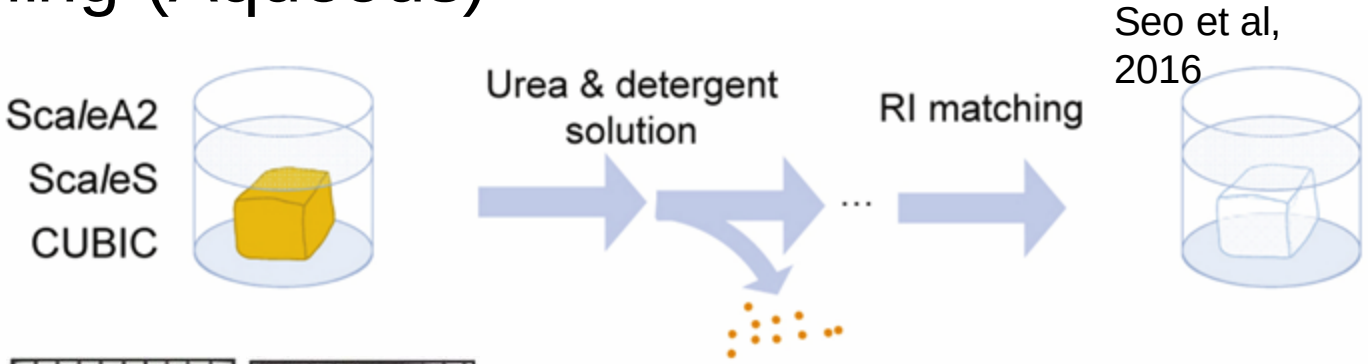
Chung et al, 2013

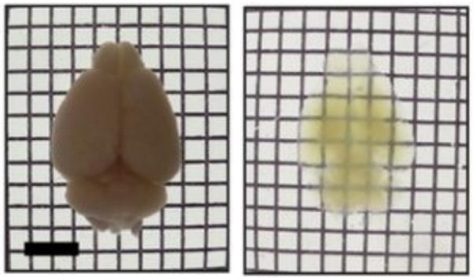
Time: Active: ~2 days-4 weeks; Passive: > month

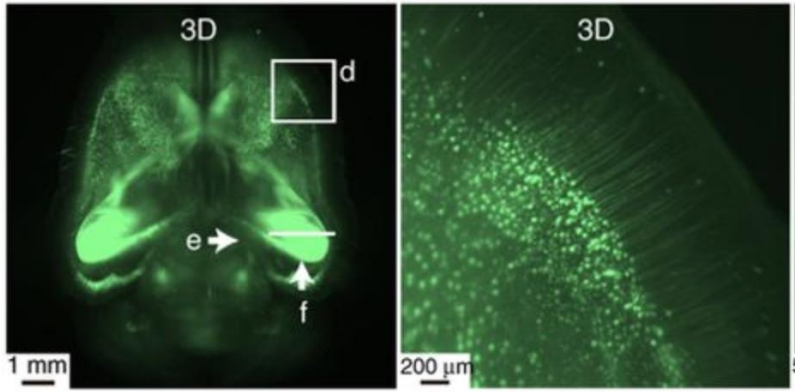
Advantages: Min size change, preserves endo FP, min protein loss, allows for IHC

Disadvantages: Requires special equipment or very long immersion time

Delipidation and hyperhydration followed by RI matching (Aqueous)





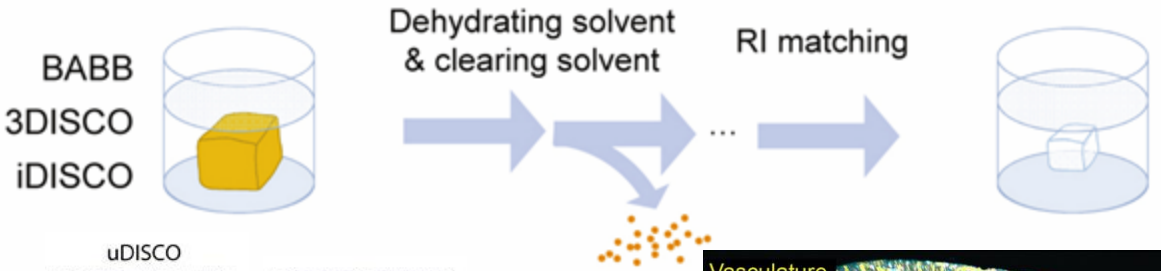


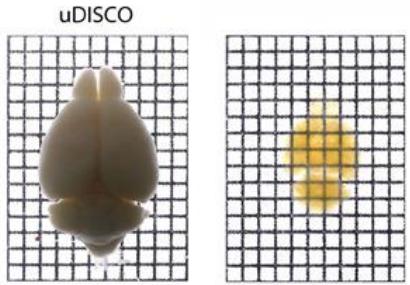
Susaki et al, 2015

Time: 2 weeks
Advantages: Preserves endo FP, safe, cheap
Disadvantages: Tissue expansion, IHC only in small samples, protein denaturation

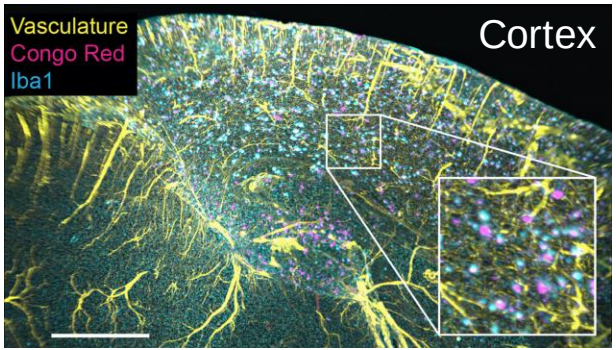
Delipidation and dehydration followed by RI matching (organic solvent)

Seo et al, 2016





Qi et al, 2019



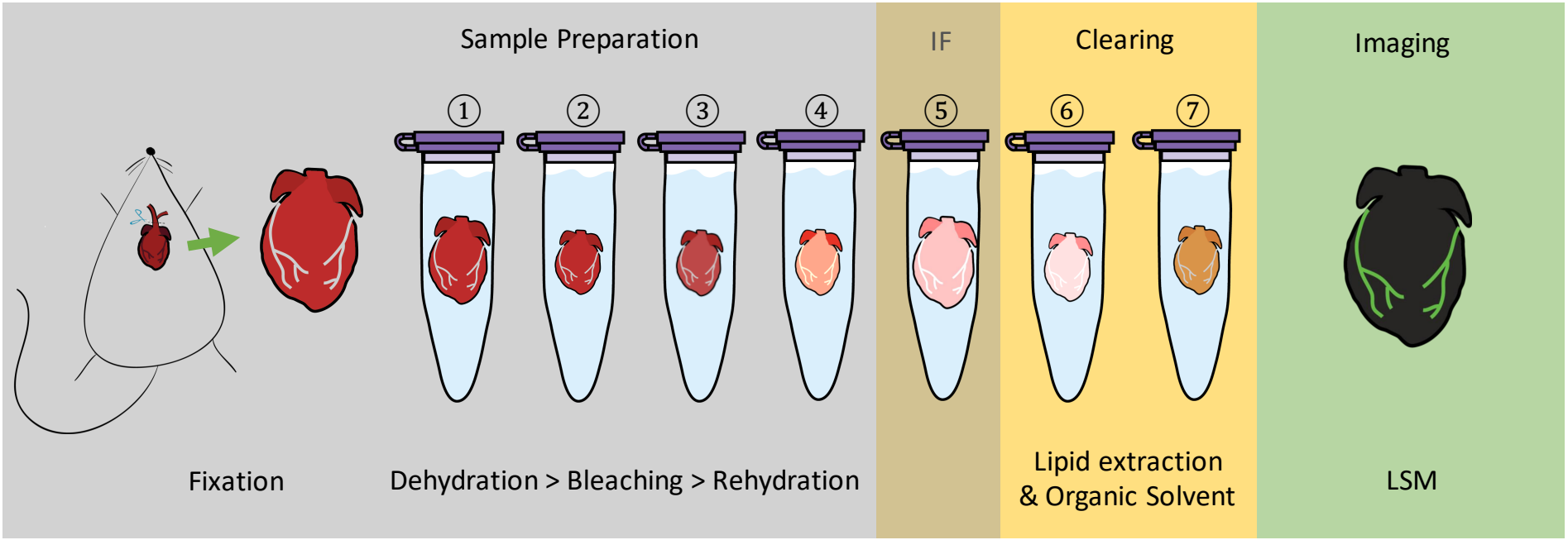
Leibmann et al, 2016

Renier et al, 2016

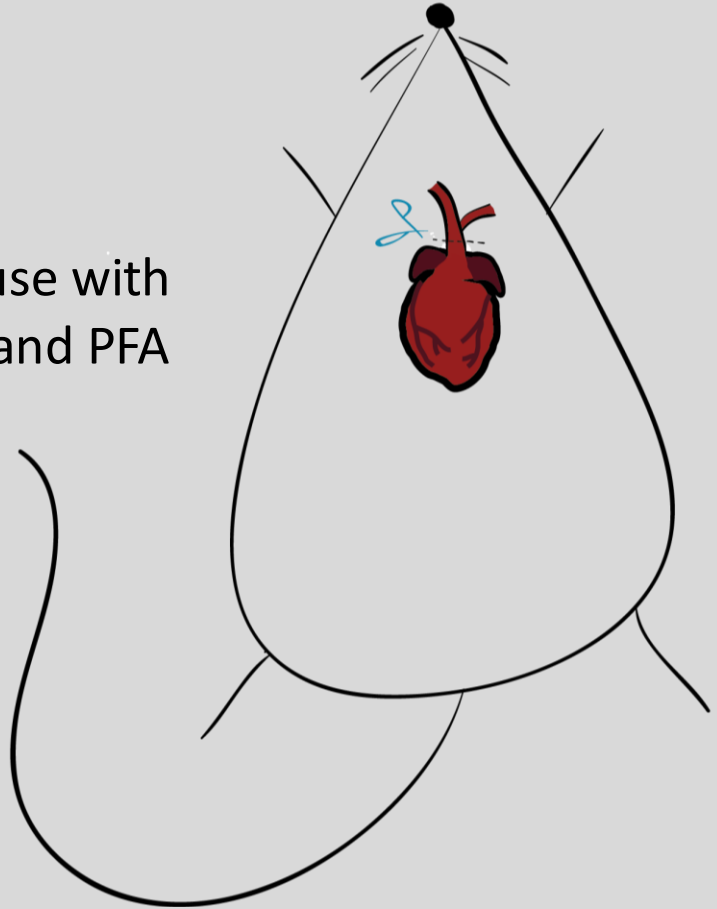
Time: 4 days, up to 3 weeks with IHC
Advantages: Easy, cheap, allows for robust IHC
Disadvantages: Tissue shrinkage, endo FP quenching, toxic solvent

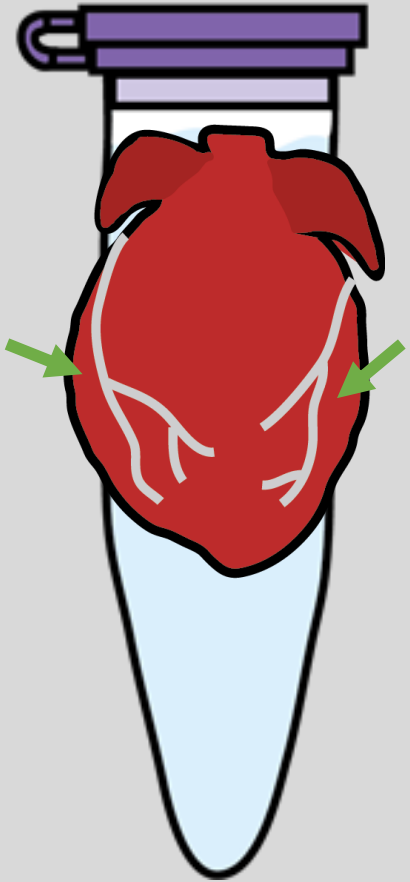
Successful use of iDISCO clearing (Pam)

Clearing the heart with iDISCO

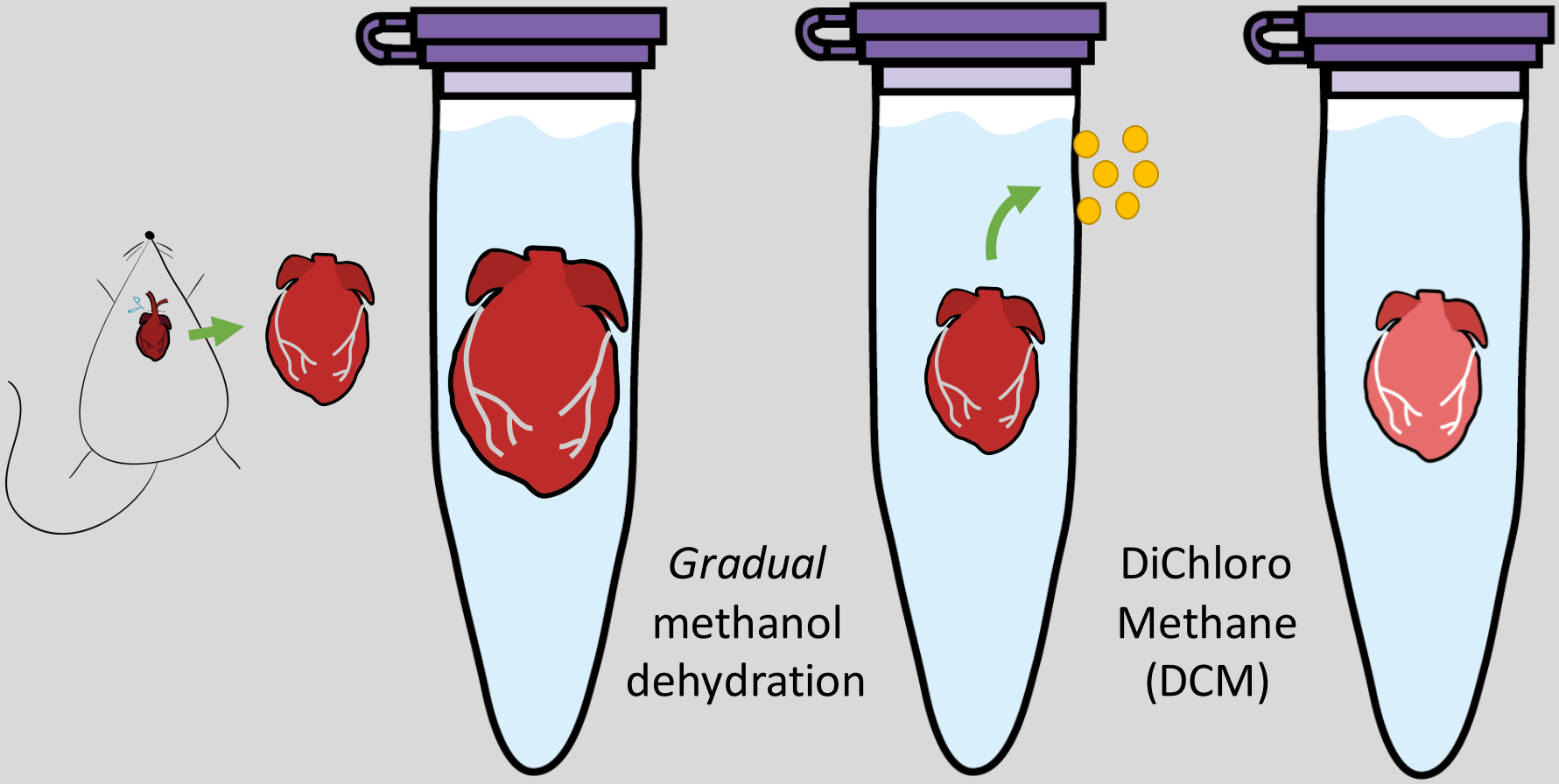


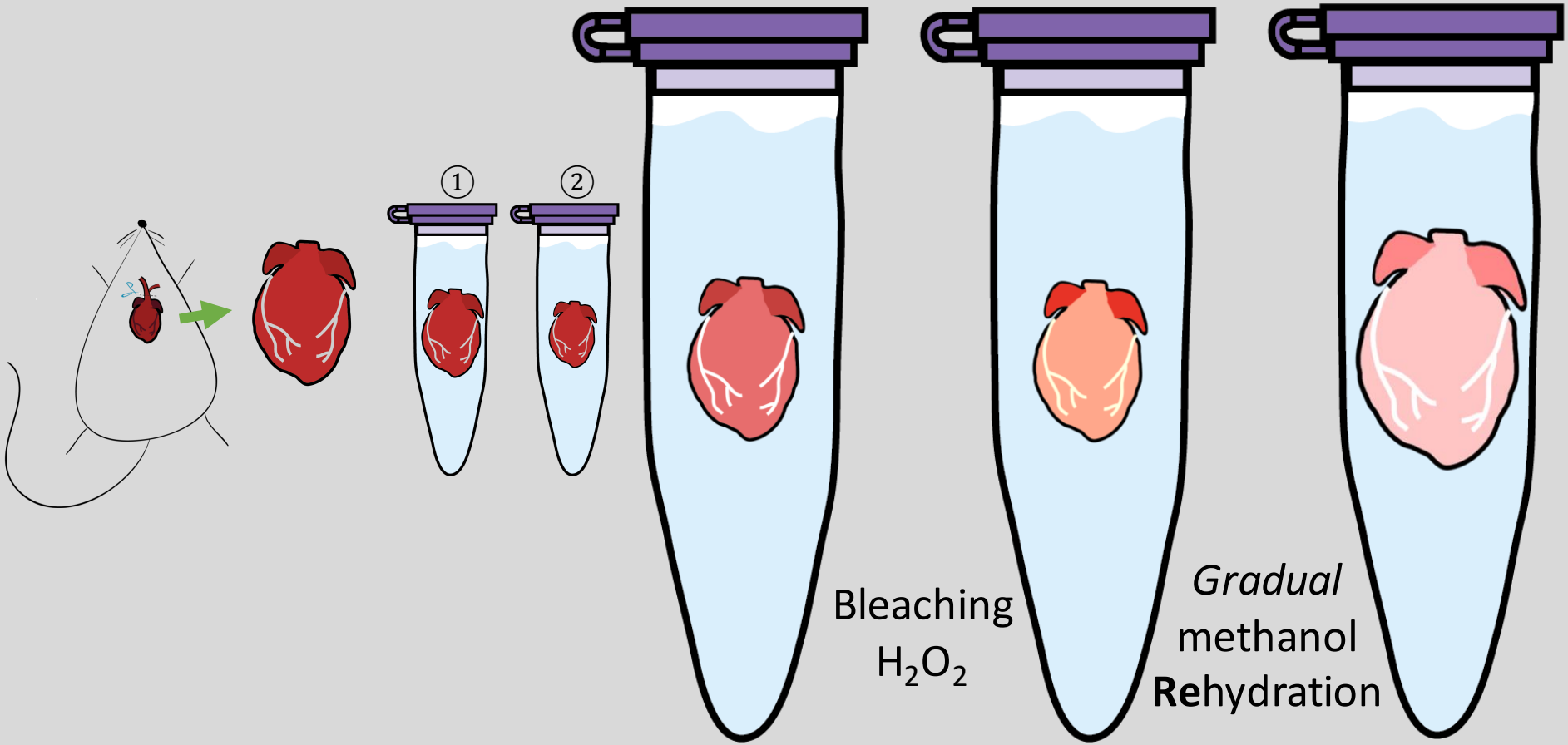
Perfuse with
PBS and PFA

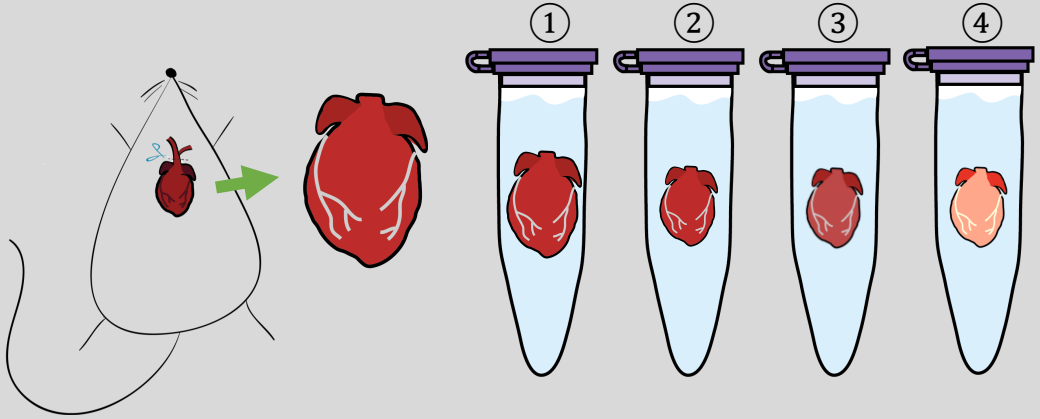


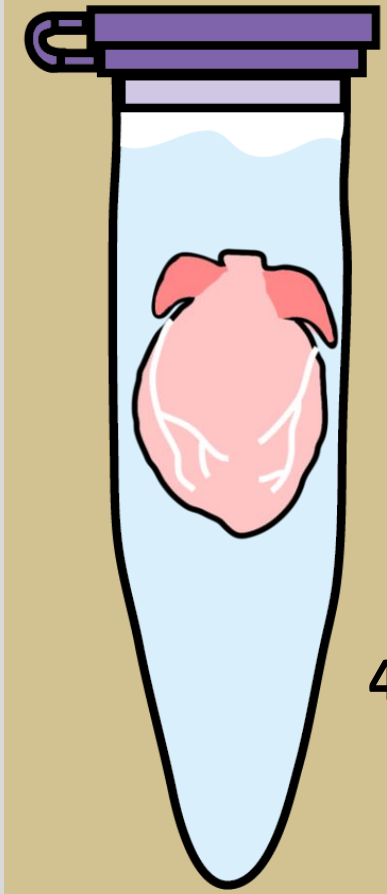


Incubate in
PFA for 1 to 48
hours
Rinse tissue
with PBS


Gradual
methanol
dehydration
DiChloro
Methane
(DCM)


①
②
Bleaching
H_2O_2
Gradual
methanol
Rehydration





Immunolabeling:

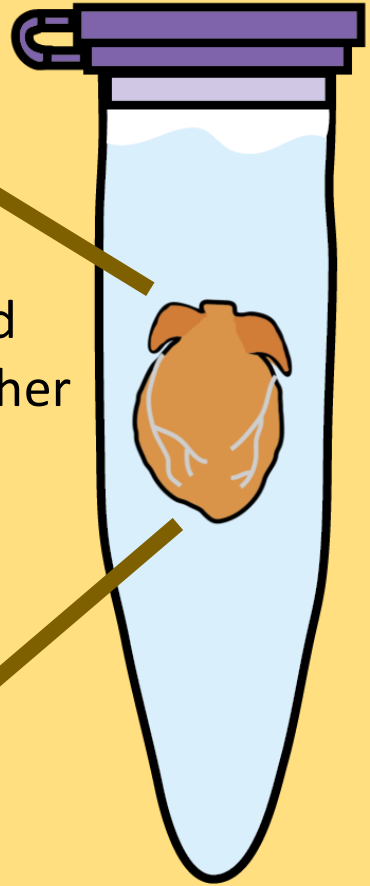
- Permeabilization
- Blocking
- Primary antibody
- Secondary antibody

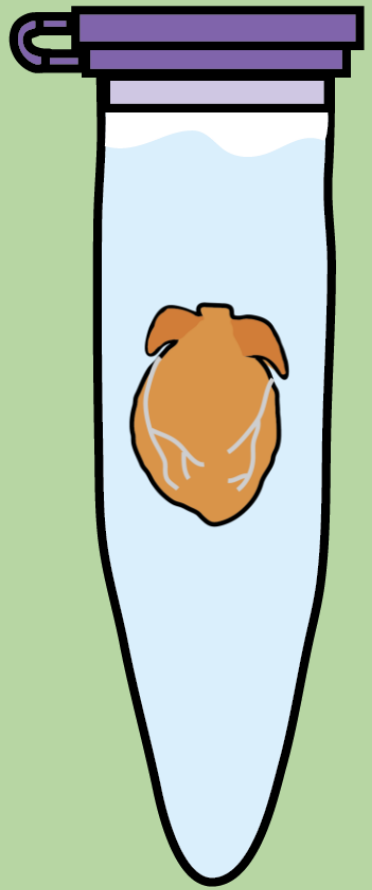
4 day to 3 weeks

Jorryt Tichelaar (Nael Nadif Kasri Lab)

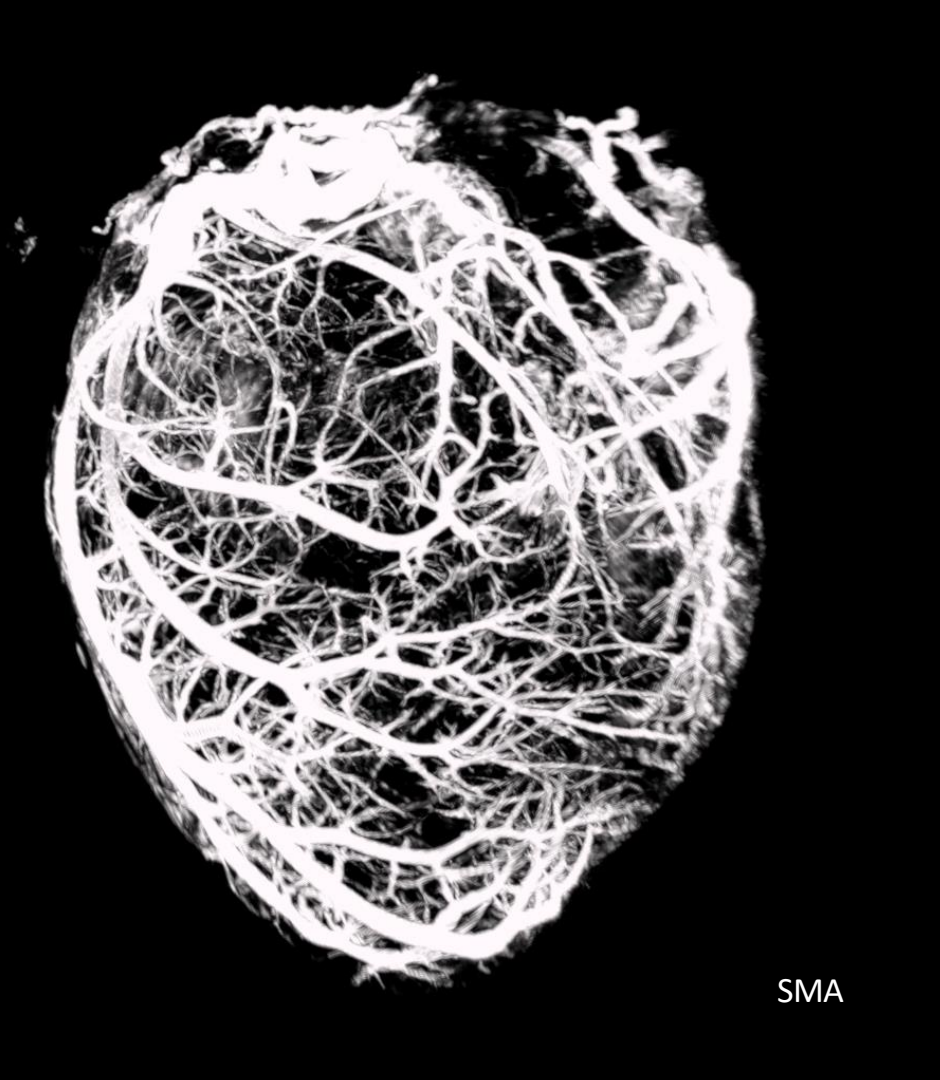
IDisco clearing of half a brain hemisphere.
Speed 256x.
Added liquid is DiBenzylEther.

M and
zylEther
)BE)

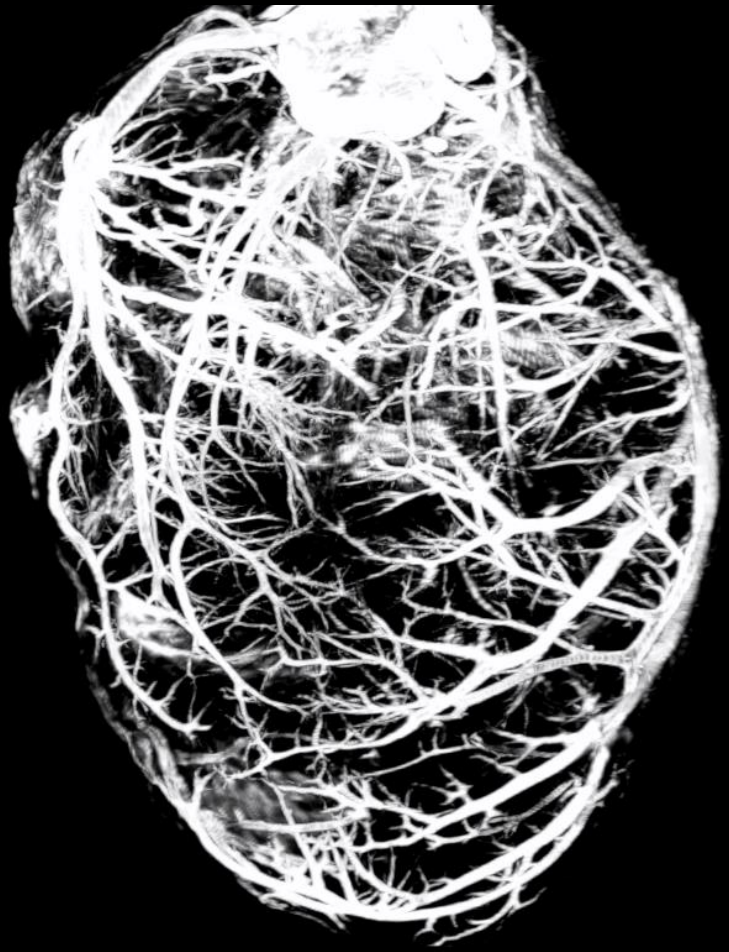




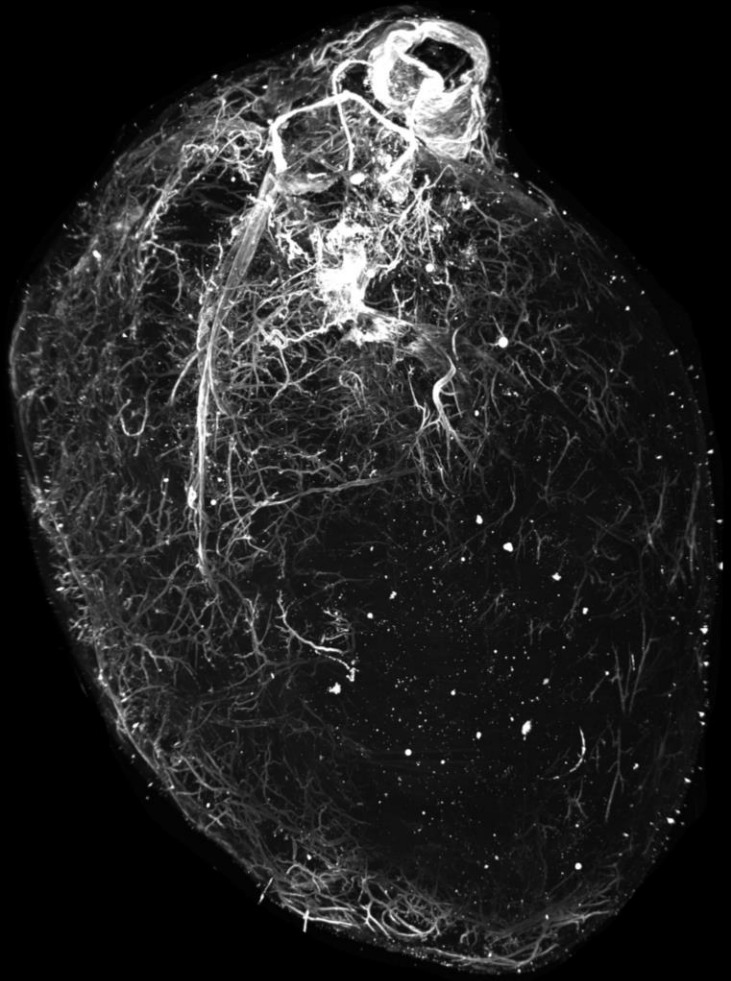
Ready to image!!



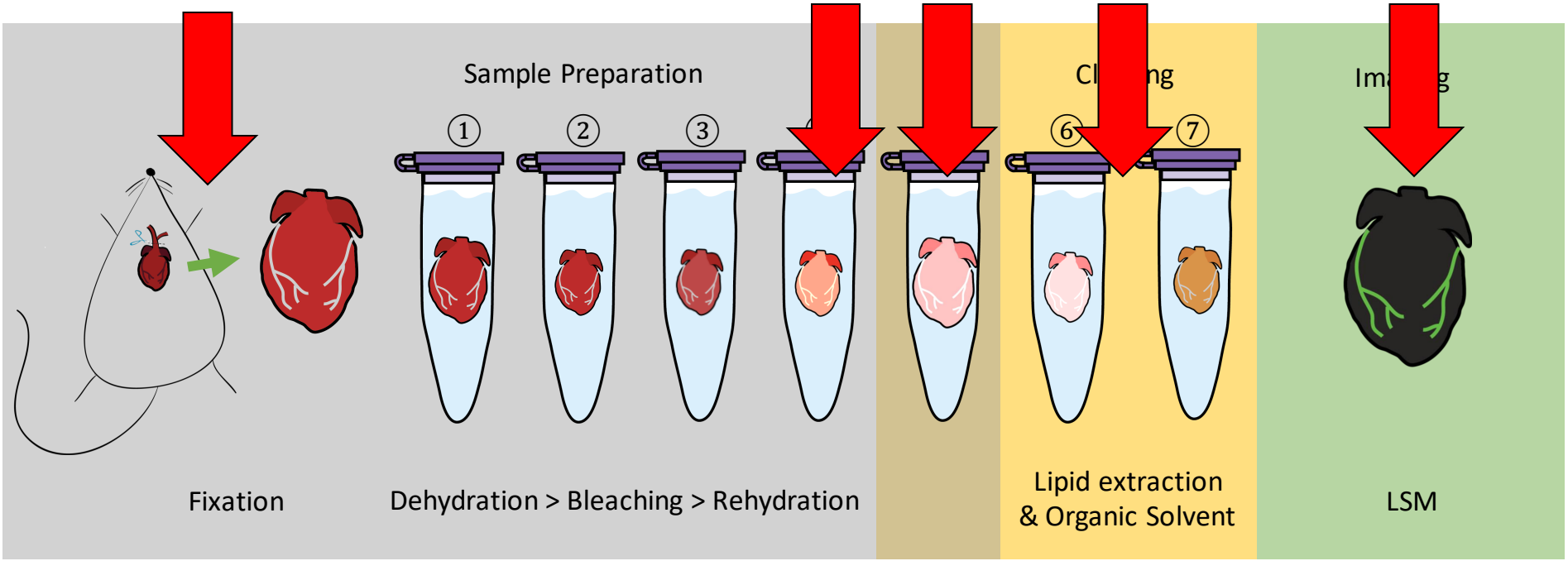
SMA



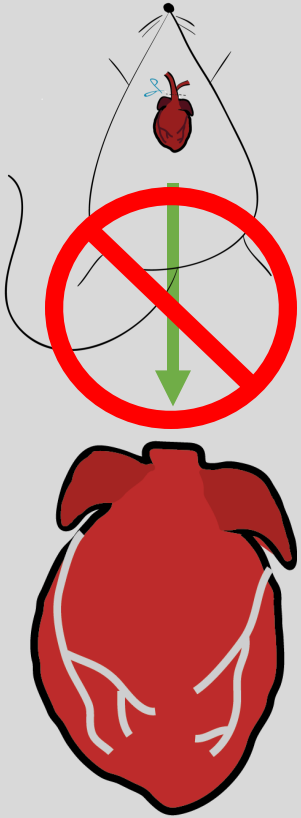
Expectation
vs
Reality



What went wrong?



Perfusion is key

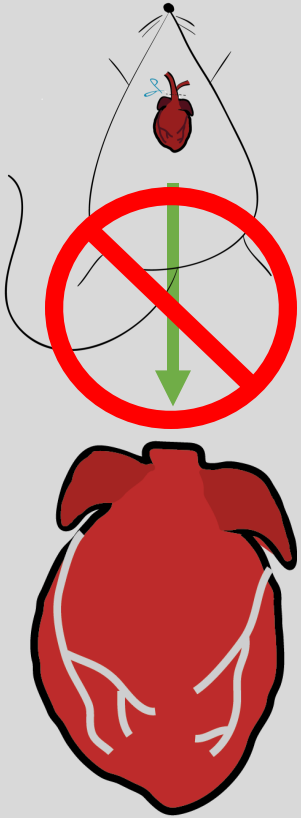


Max Projection

Optical slide

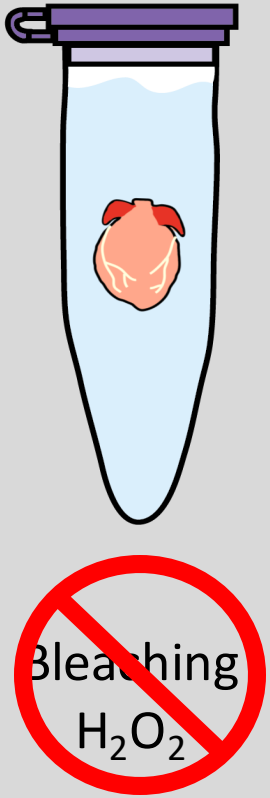
SM-MHC

Perfusion is key



Smooth muscle heavy chain

Smooth muscle Actin

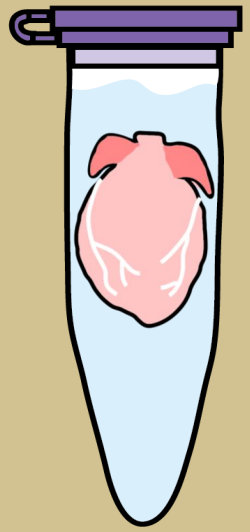


Max Projection

Optical slide

SM-MHC

Know your antibodies: Fixation times

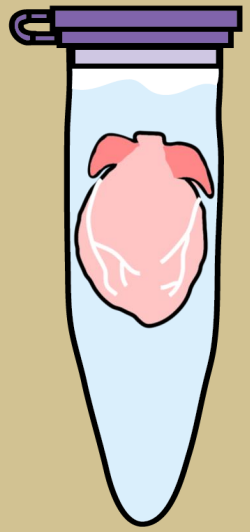


Short (1hr) Fixation

O/N Fixation

CX40

Know your antibodies: Fixation times

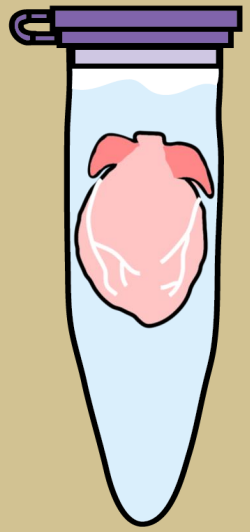


Short (1hr) Fixation

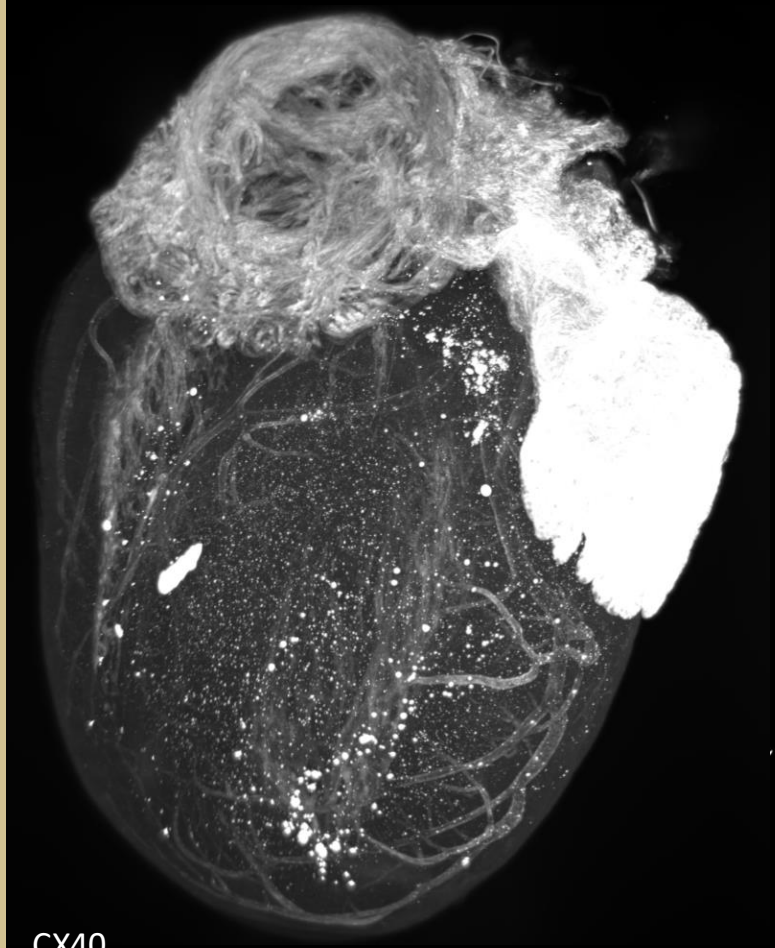
O/N Fixation

SM-MHC

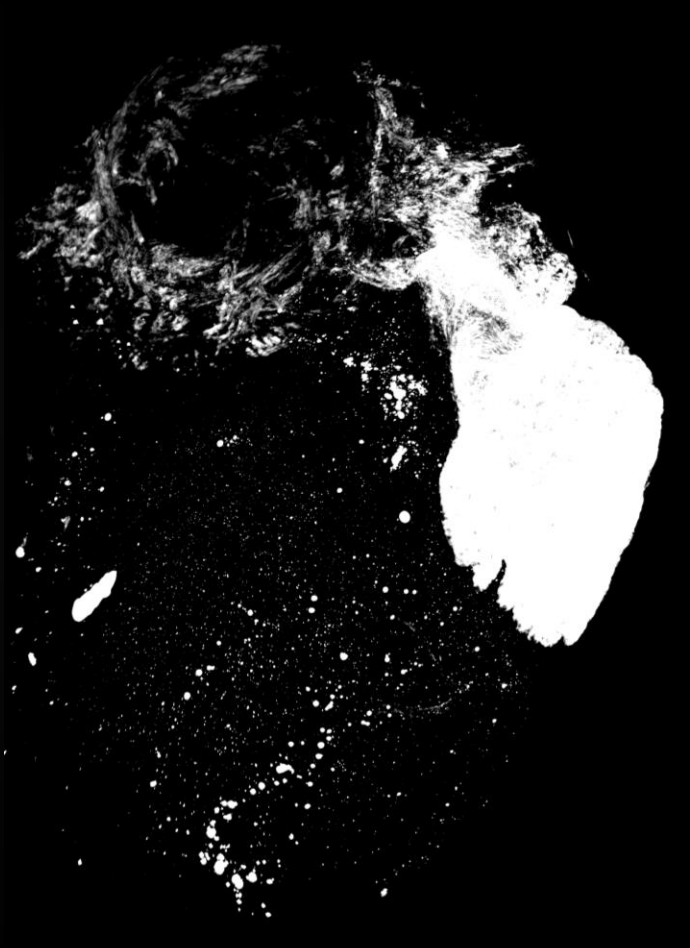
Know your antibodies: Concentrations



Max Projection

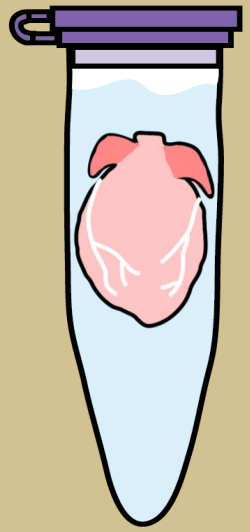


Precipitate Max Projection



CX40

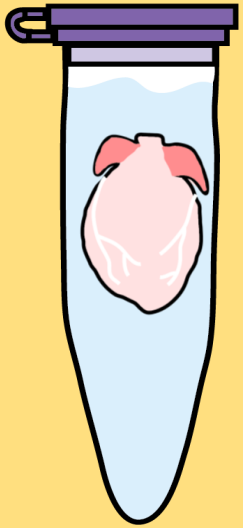
Know your antibodies: Secondaries



CX40 + Secondary A

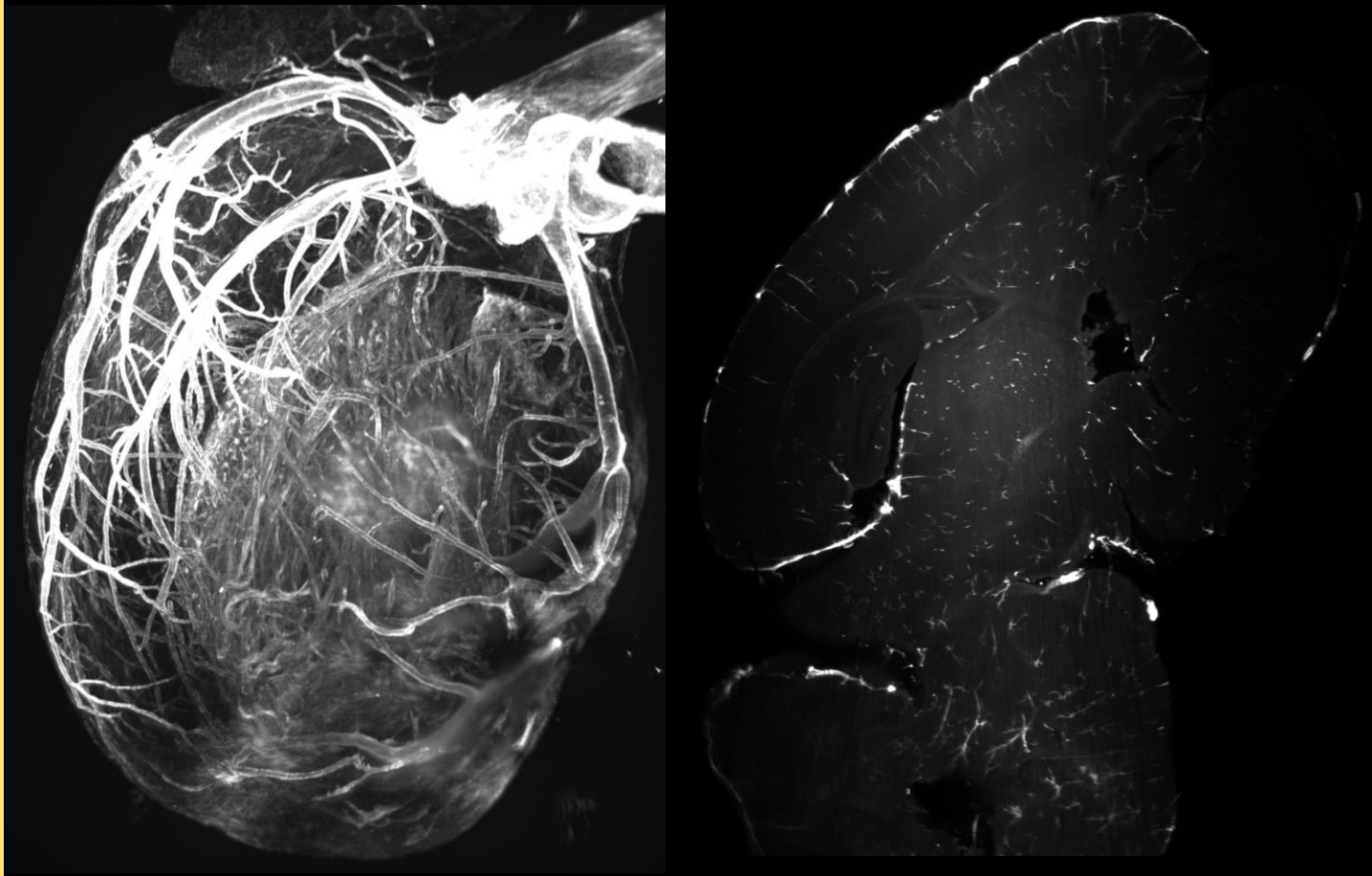
CX40 + Secondary B

DCM Steps



Heart

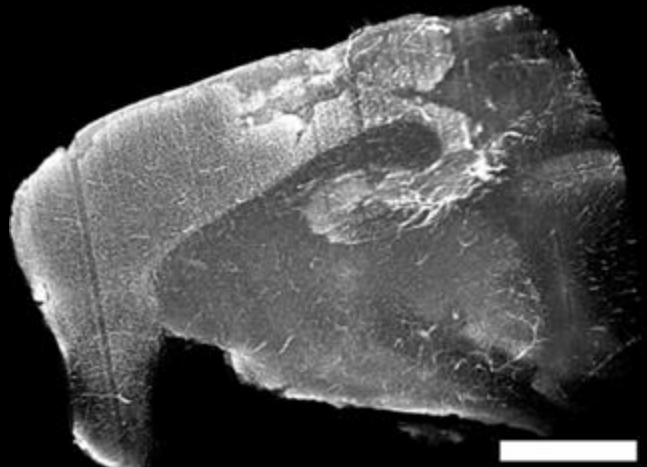
Brain



SMA

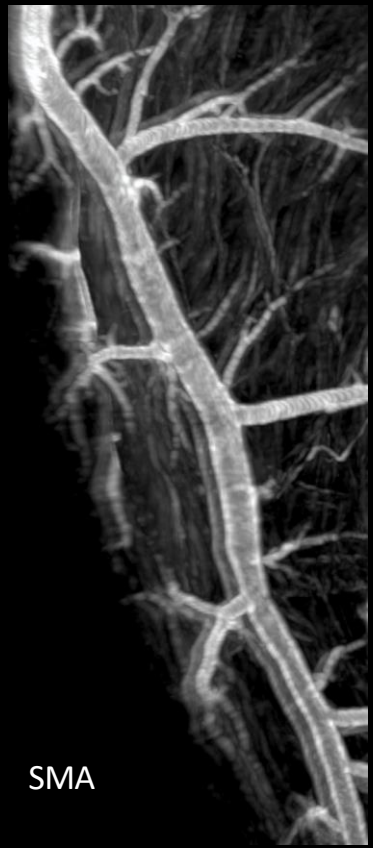
Equipment matters

Single laser LSM



CD31

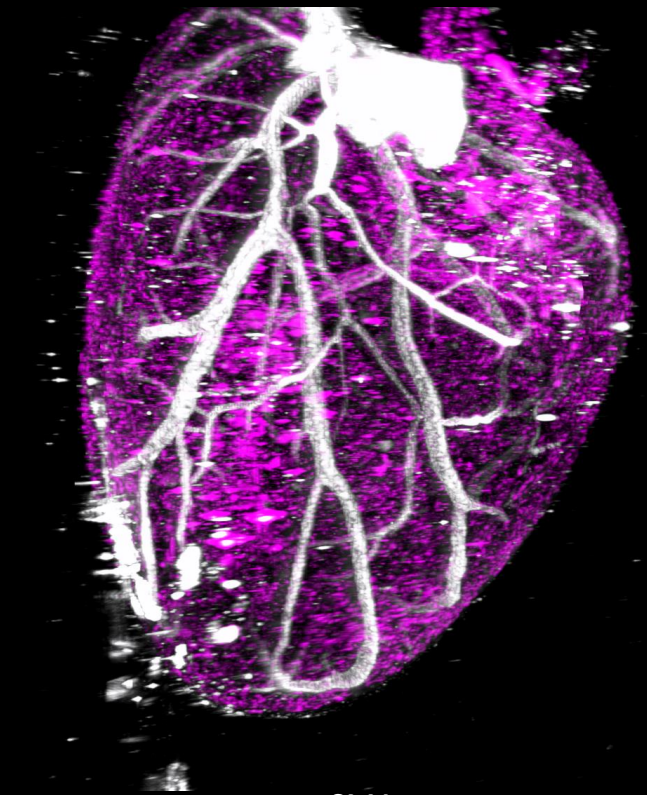
Miss-aligned dual laser LSM



SMA

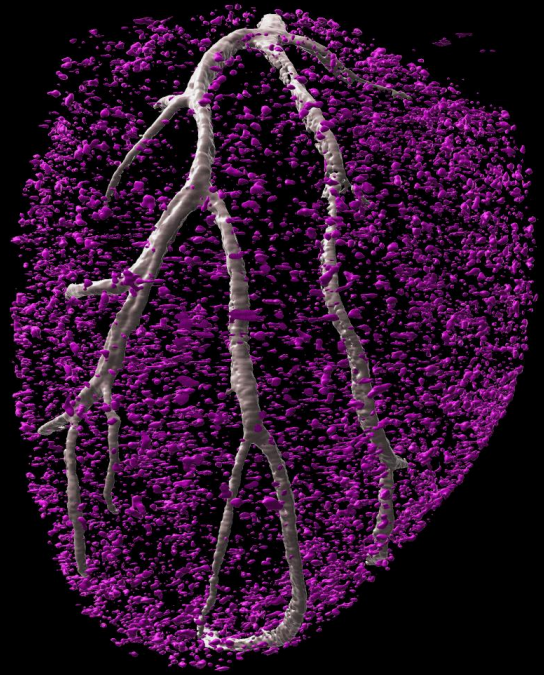
Merlini et al. Neuron. 2019

Post-processing can mend a broken heart



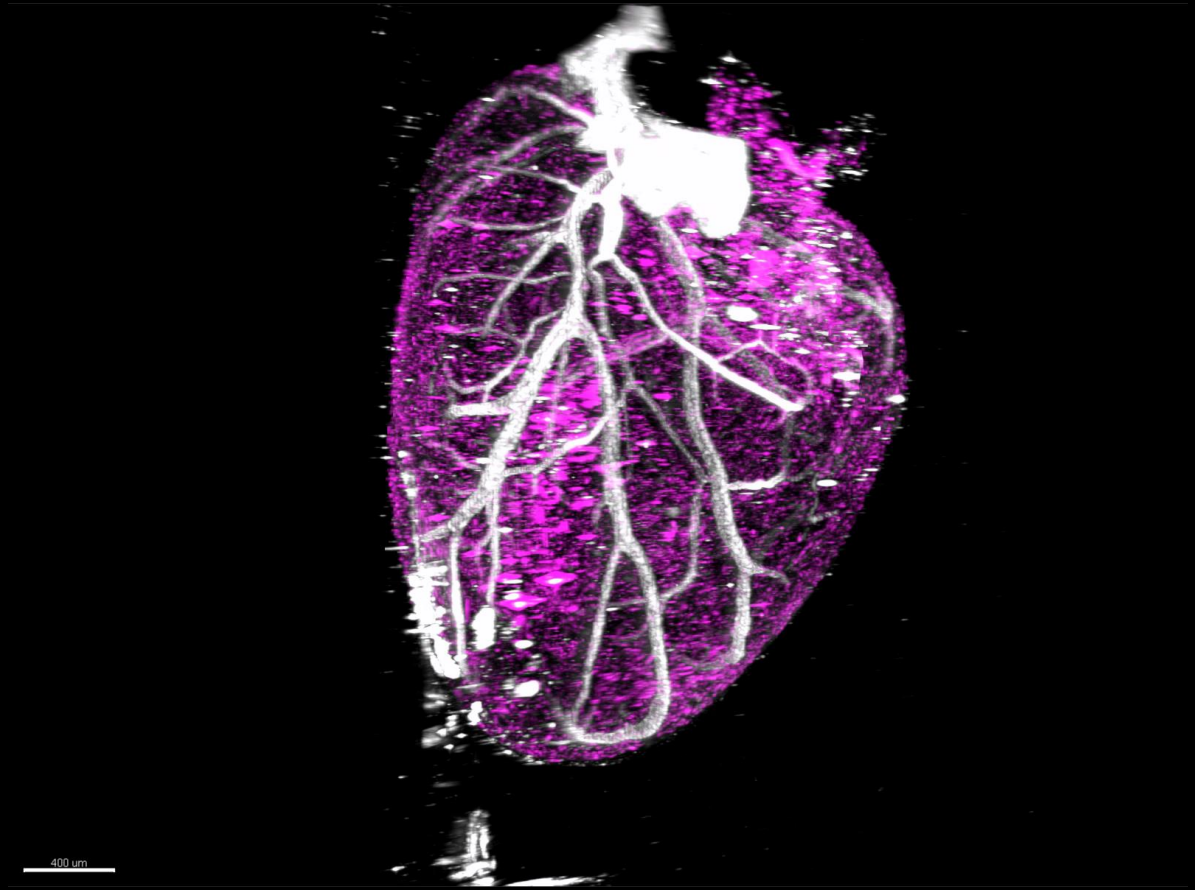
SMA
PH3

Masked channels



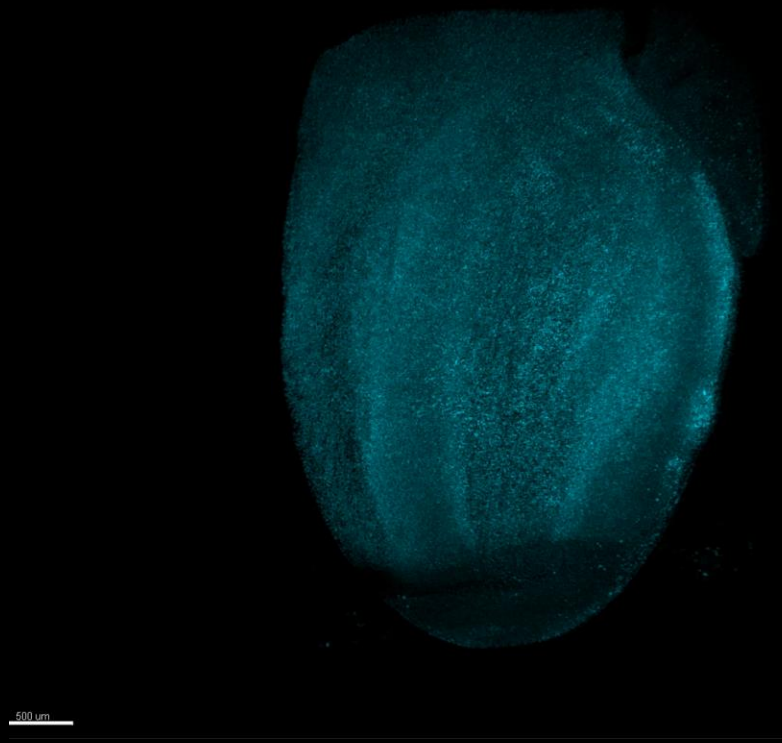
Surface rendered

Post-processing can mend a broken heart



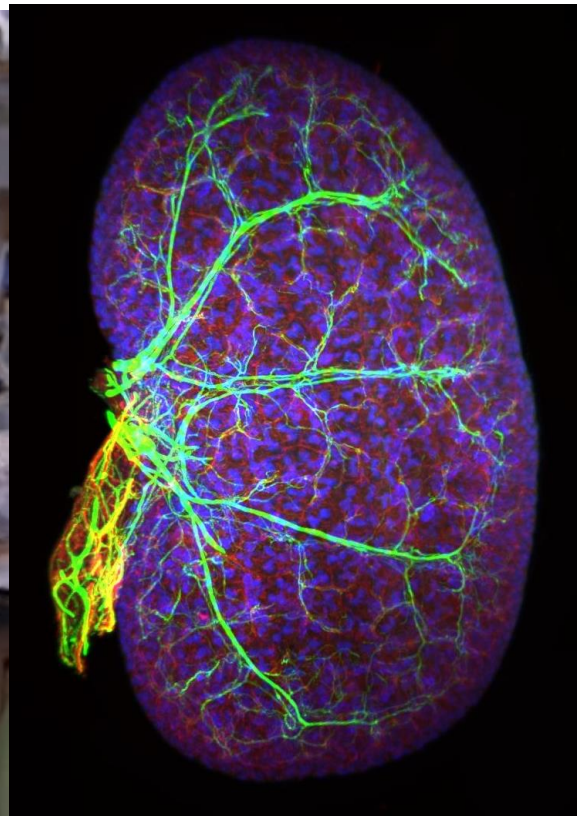
Post-processing can mend a broken heart

PH3

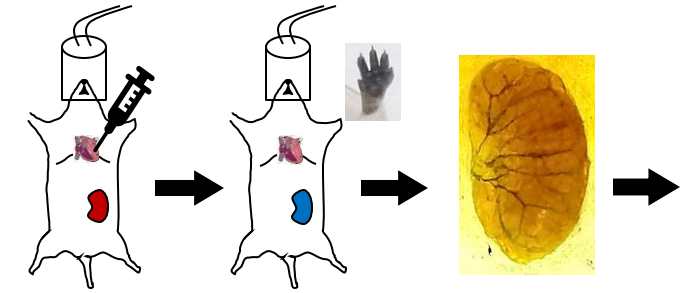


PH3 (masked channel)

Lori O'Brien Lab and Evans blue-vasculature labeling



Evans blue for labeling vasculature

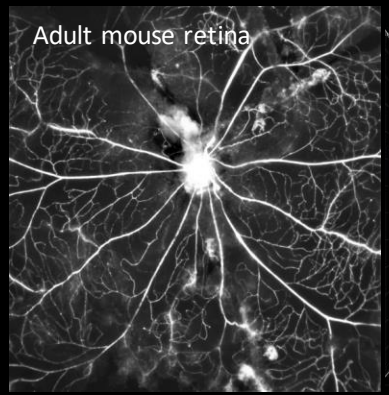


1mg Labeled tomato lectin: $145 (10 injections)

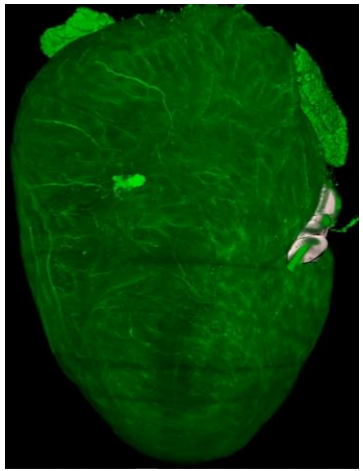
50g Evans blue=$202.00 (13,888 injections)

Kidney vasculatule

P6 mouse lung

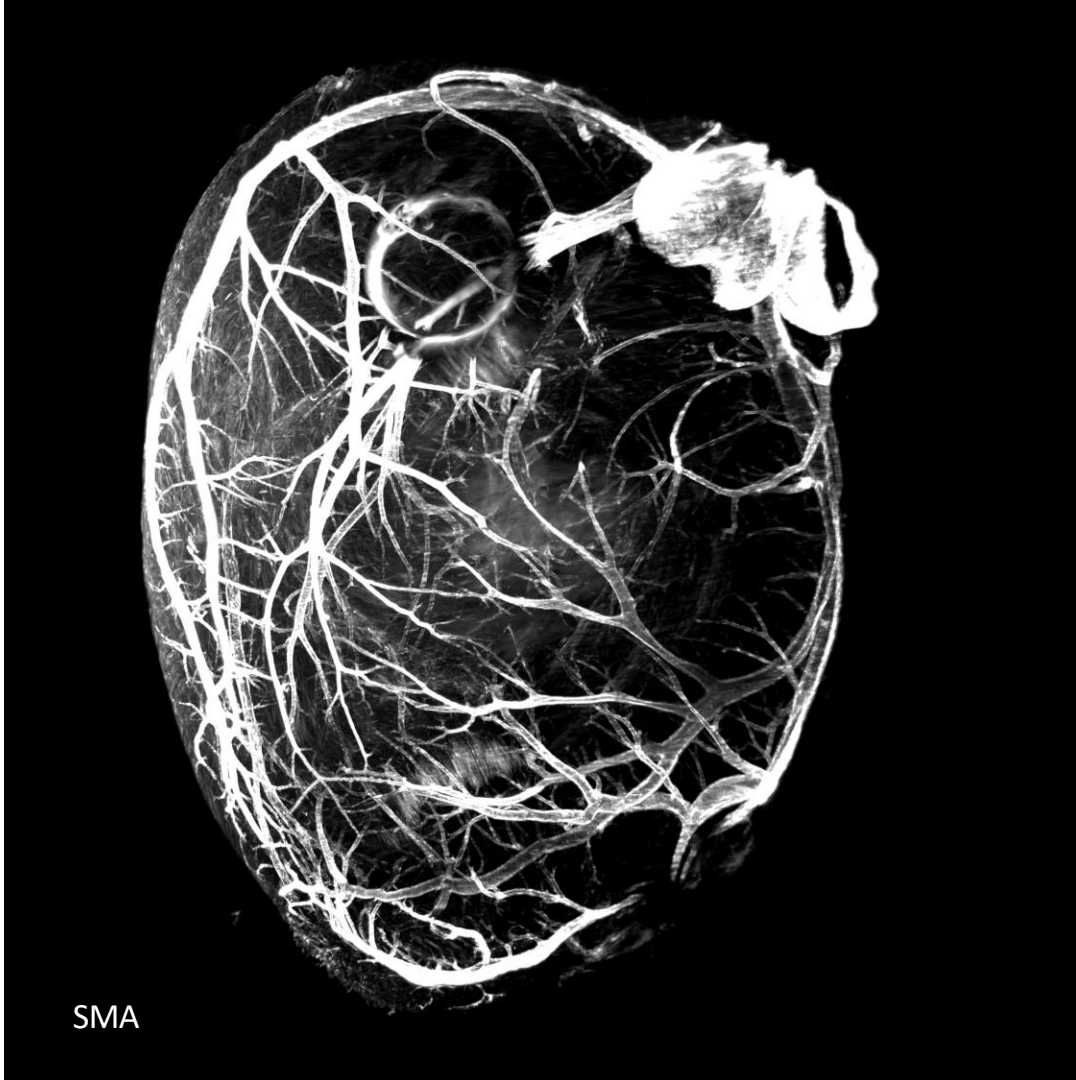


Non-toxic alternative to organic solvents: Ethiethyl cinnamate (ECi)



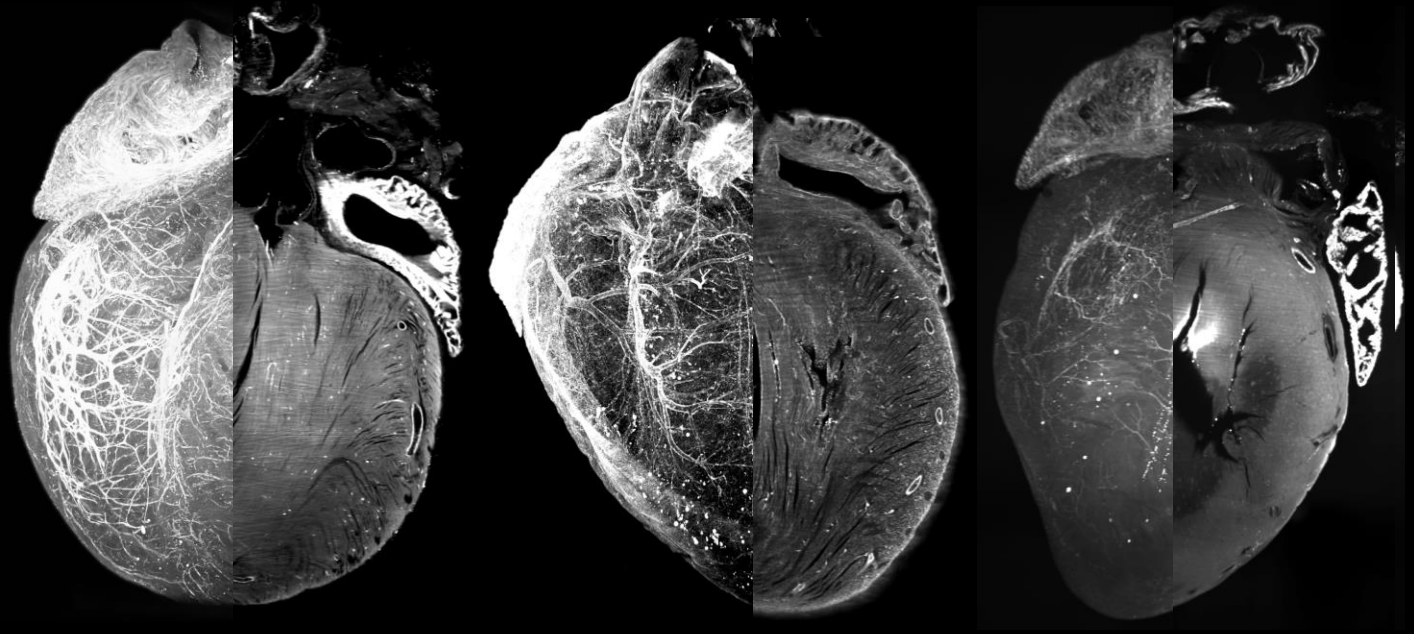
in vivo CD31 immunolabeling

ECi: Merz et al. **2019**. Nat Comm.



SMA

Bonus: Antibodies that work with iDISCO



GFP

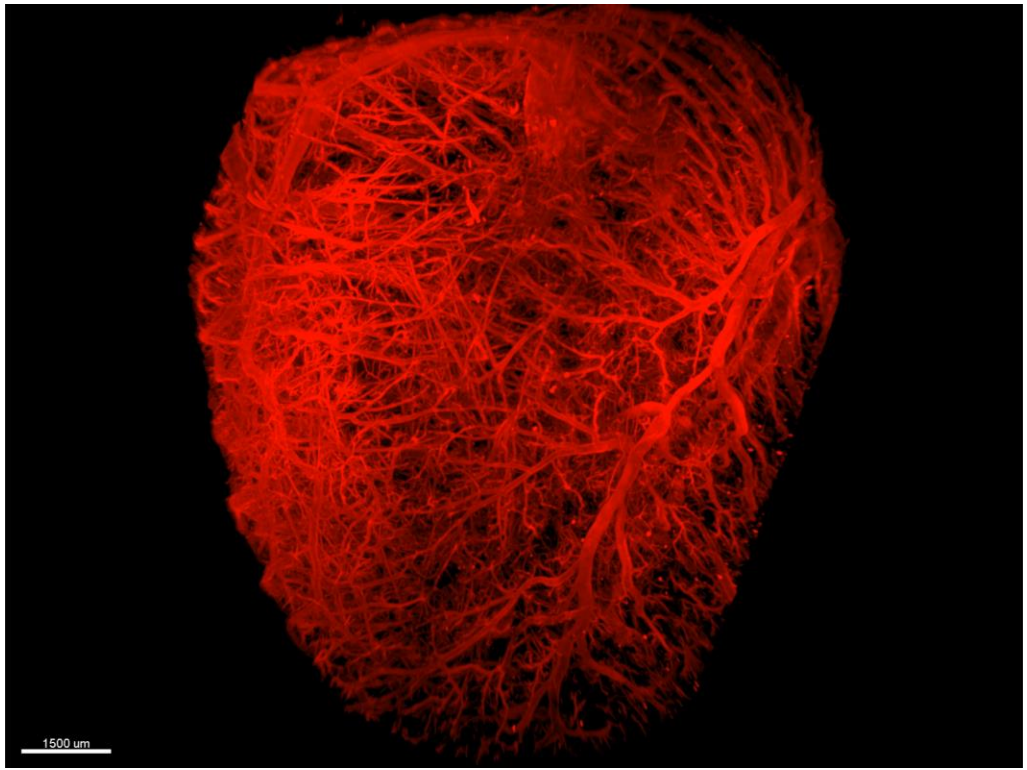
PECAM

RFP

ERG
VEGFR2
JAGGED-1
PROXY1
PDGFR

Tips “Treats” and Tricks

- Fresh Solutions
- Troubleshoot antibodies
  - ALTERNATIVE Method
- ECi non-toxic
- Plan ahead
- Be adventureous

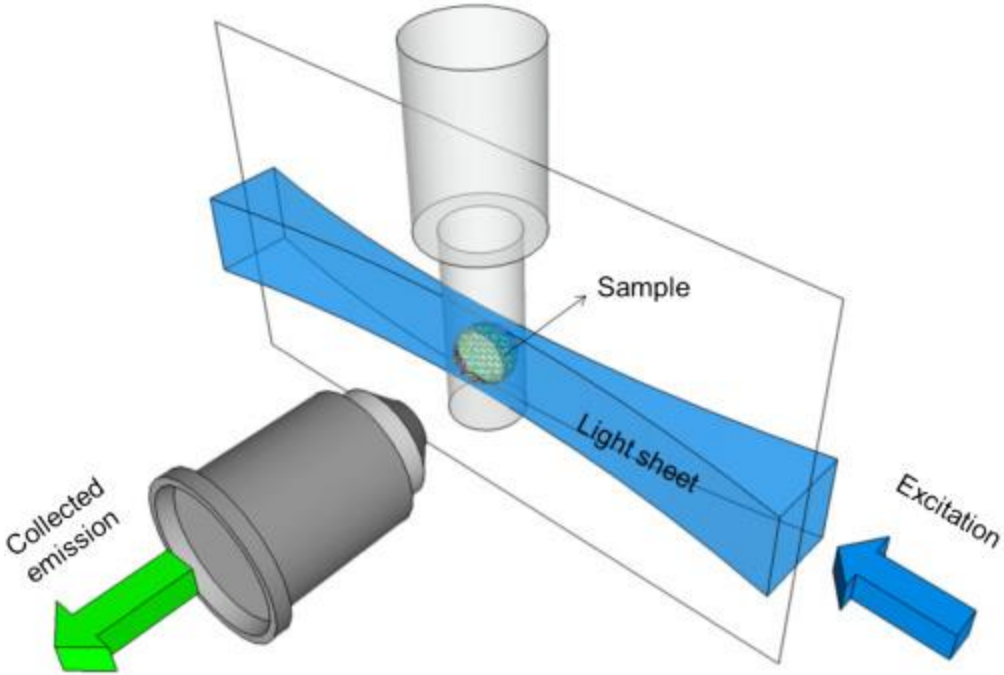


Brian Raftrey

Microscopy for large cleared tissues (Suhaas)

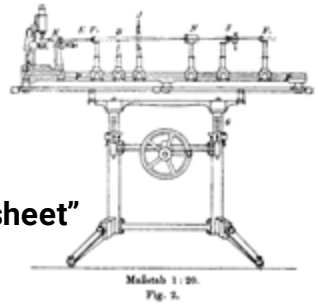
Many names, same concept

LSM, light-sheet microscopy
LSFM, light-sheet fluorescence microscopy
OPFOS, orthogonal-plane fluorescence optical sectioning
TLSM, thin-light sheet microscopy
SPIM, selective or single-plane illumination microscopy
mSPIM, multidirectional selective plane illumination microscopy
HROPFOS, high-resolution orthogonal-plane fluorescence optical sectioning
OPM, oblique plane microscopy
OCPI, objective-coupled planar illumination
DSLIM, digital scanned laser light sheet microscope
TSLIM, thin-sheet laser illuminating microscopy



Olarte O et al. Adv. Opt. Photon. 10, 111-179 (2018)

Light-sheet microscopy then and now



First "Light-sheet" microscope

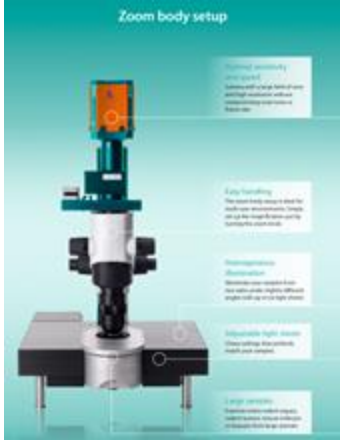
Siedentopf H. and Zsigmondy R.

First to use for zebrafish embryos

SPIM/mSPIM - Huisken et al. 2004, Huisken and Stainier 2007

First Commercial Light-sheets

LaVision, then Zeiss



1902

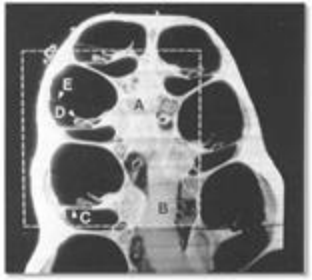
1993

2004

2007

2009, 2012

2014



Guinea-pig cochlea, Voie *et al* (1993)

Modern application

Voie et al. 1993; Voie and Spelman 1995; Voie 2002

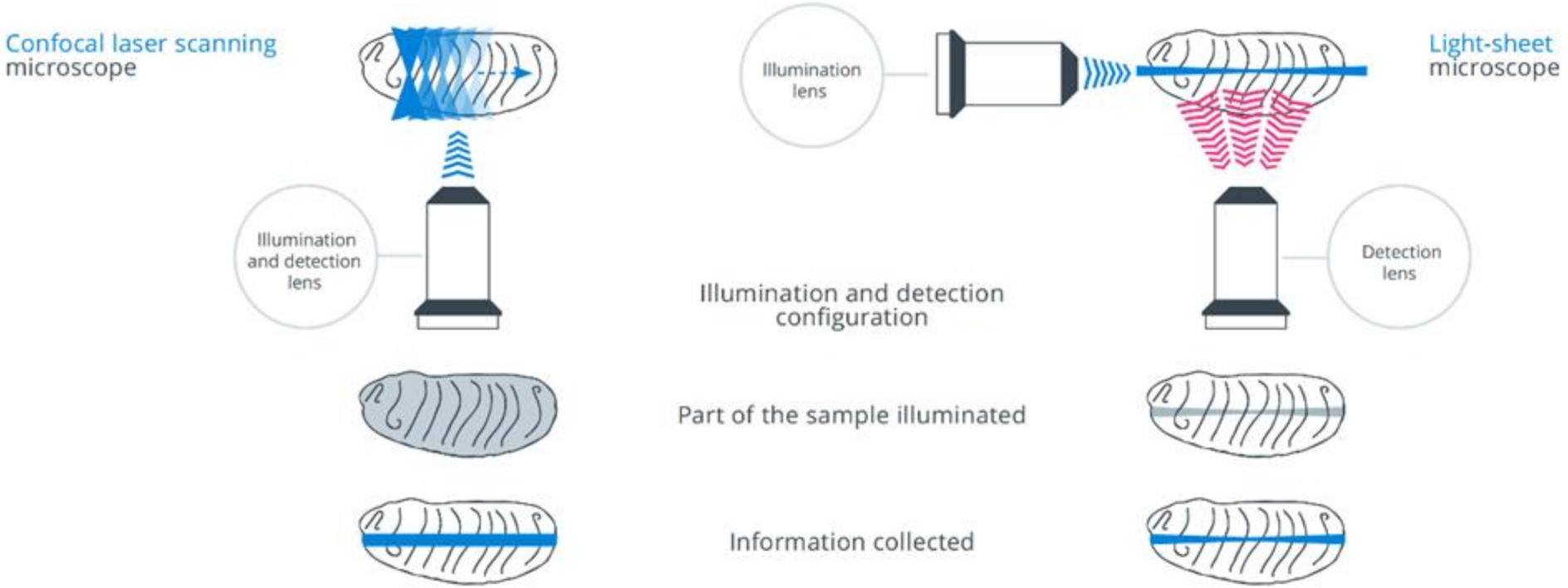
Imaging large samples (Brains)

Dual-sided illumination
Dodt et al. 2007

Lattice Light-sheet

Chen, B; Legant, W; Wang, K. Science 2014

Santi, P. J Histochem Cytochem. 2011



https://luxendo.eu/technology-details/

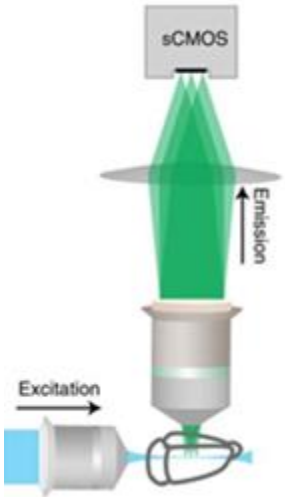
Pros and Cons of Light-sheet imaging

Pros:

- Volumetric imaging (up to 1-2cm thick)
- Fast acquisition
- Low phototoxicity/photobleaching

Cons:

- Requires sample-specific mounting techniques
- Relies on good clearing for larger samples
- Huge file sizes and data analysis



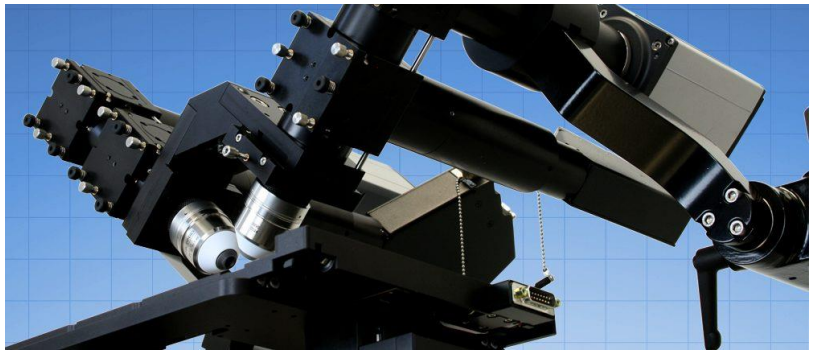
Olarte, O. et al. Adv. in Opt. and Phot. (2018)

Current options for Lightsheet

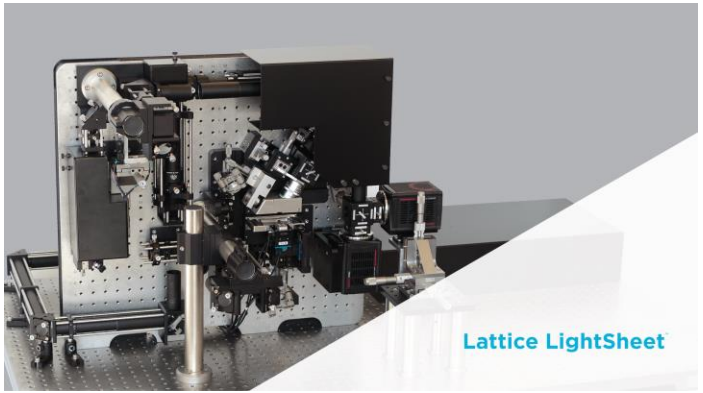
Luxendo MuVi SPIM

…and many more DIY
and custom options!

Leica TCS Digital Lightsheet



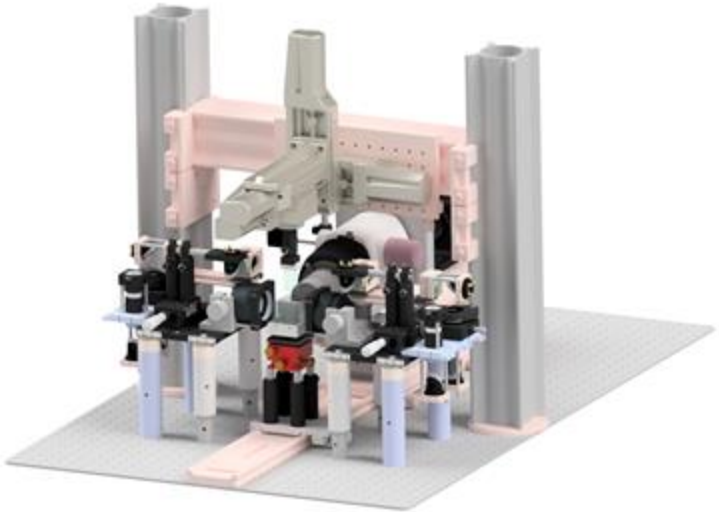
Applied Scientific Instrumentation CT-DSPIM



3i Lattice Lightsheet

Do-It-Yourself Light-sheet microscopes

MesoSPIM: open-source



Custom built

K. McDole, L. et al. Dev. Cell (2018)

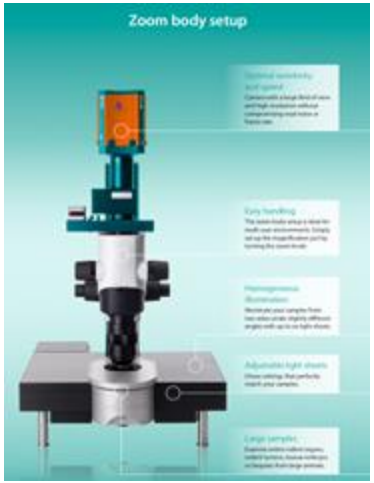
Most Popular Commercial Options

Zeiss Z.1

- Great for live imaging of small samples
- Multiview reconstruction
- More complex mounting methods

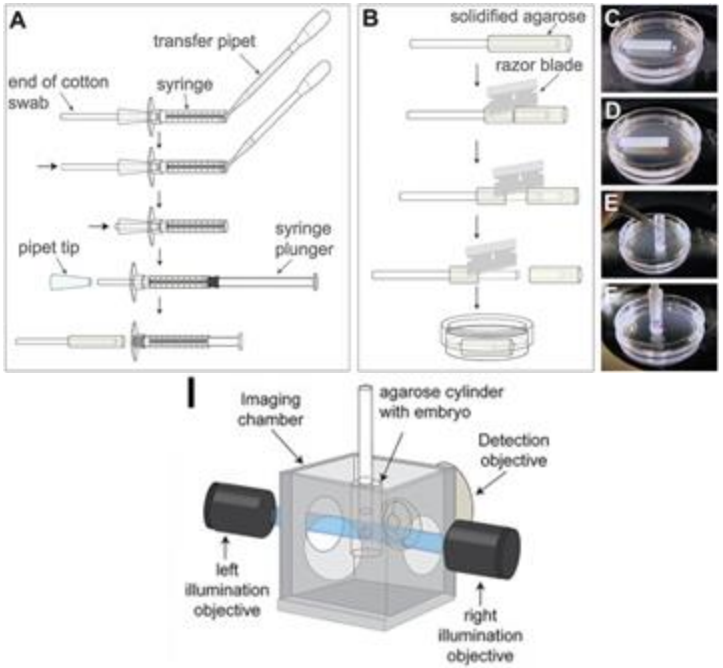
LaVision Ultramicroscope II

- Large FOV great for whole organs
- Organic solvent dipping objective available
- No motorized rotation of sample



Mounting examples

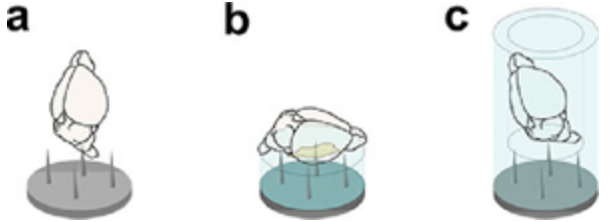
Zeiss Mounting



Udan R et al. Development (2014)

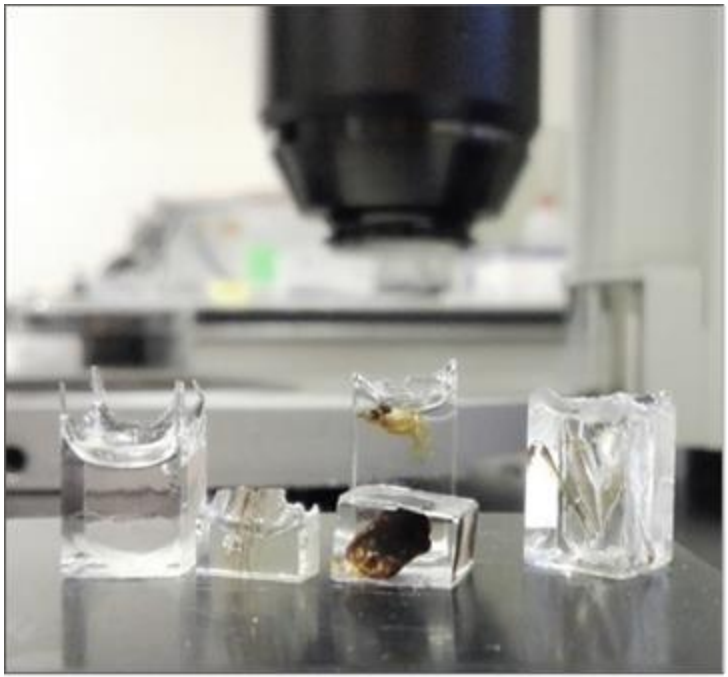
LaVision Mounting

Provided by Miltenyi



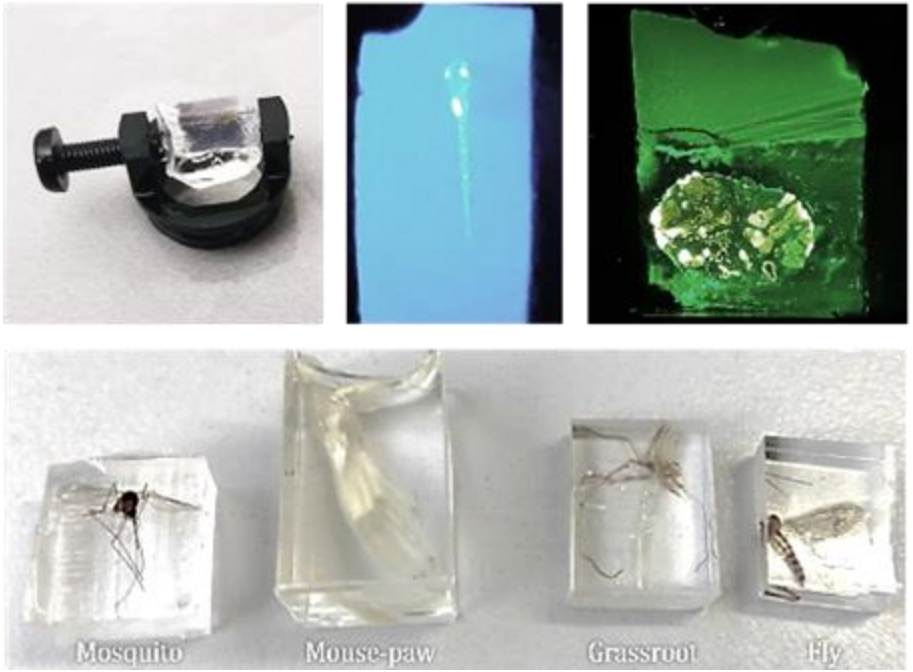
Silvestri L et al. J. Vis. Exp. (2013)

Mounting with Gels



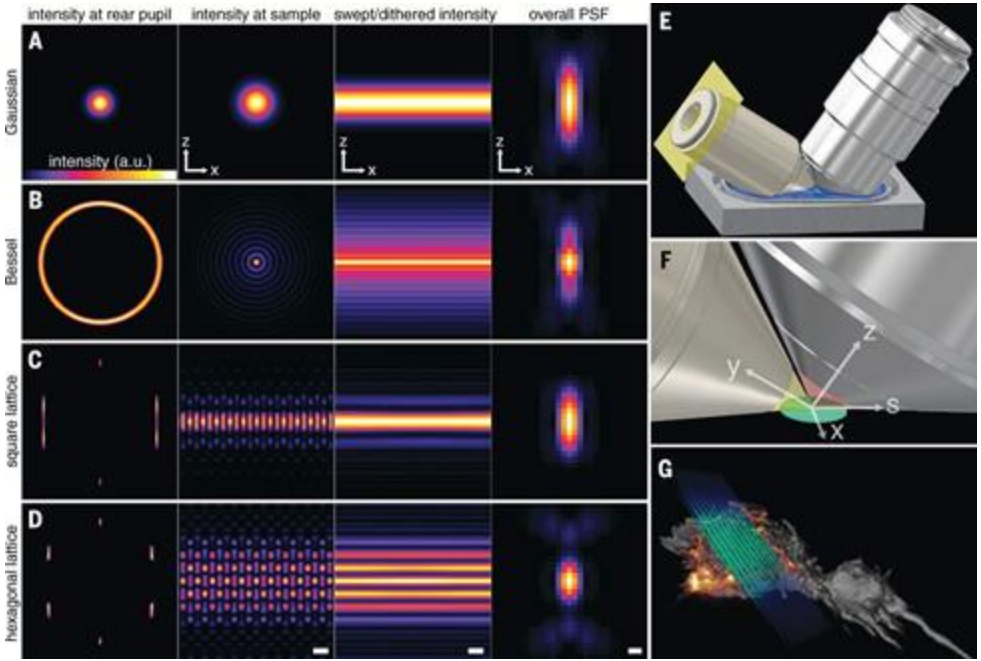
Agarose, PhytaGel, BioDur

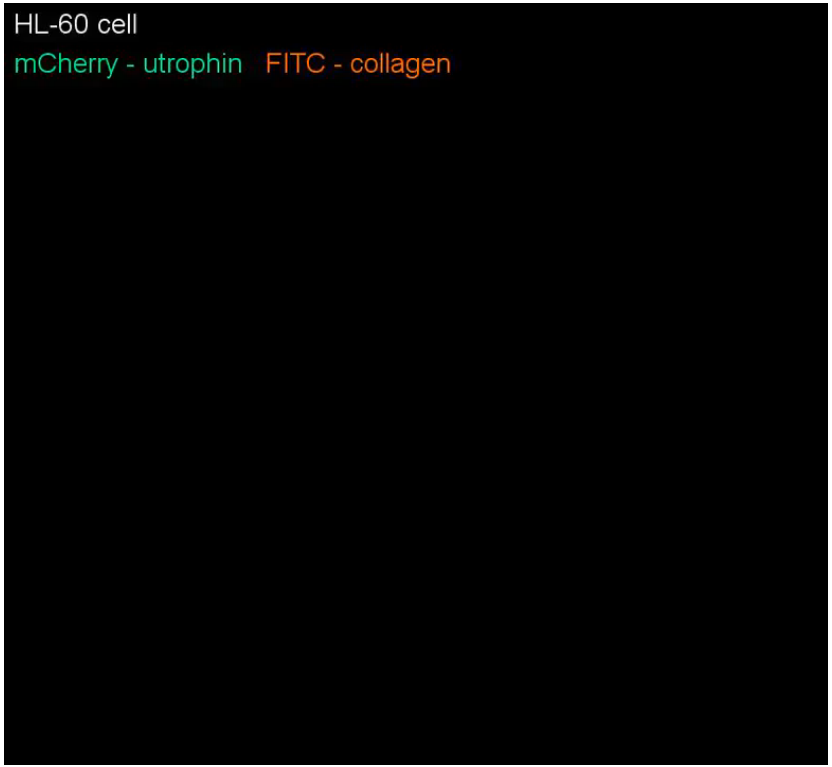
For small samples and preservation:



Provided by Miltenyi

Lattice Lightsheet Imaging





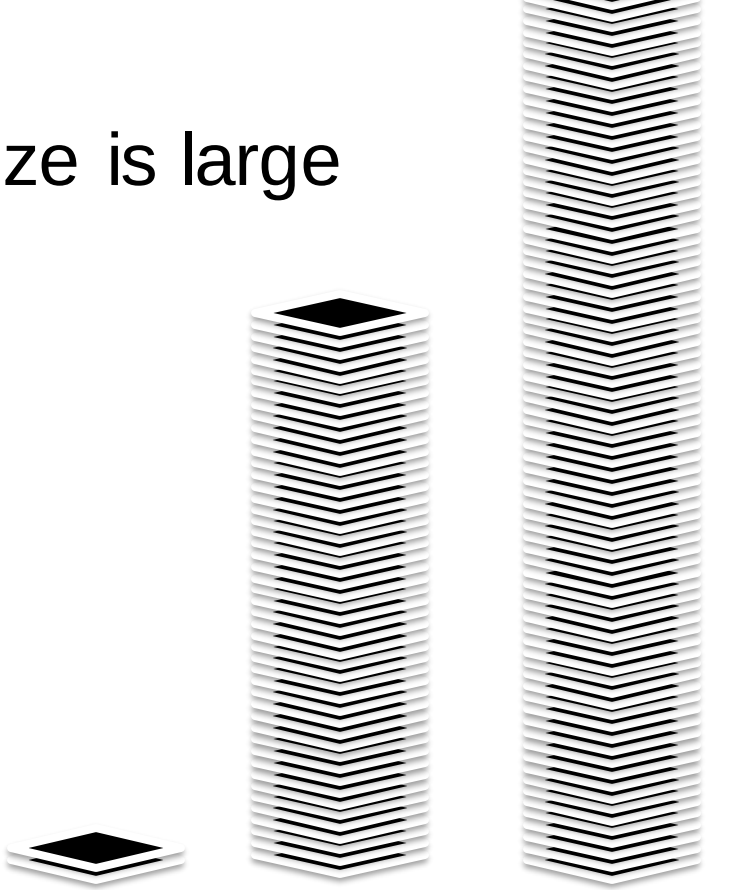
Chen B, Legant W, Wang K et al. Science (2014)

Image Analysis (Suhaas)

Why is it an issue? - Raw file size is large

- The whole folder size can range from 10's of Gb to 1-10's Tb

First challenge is to open these images



Confocal
~50 z-slices
~100MB

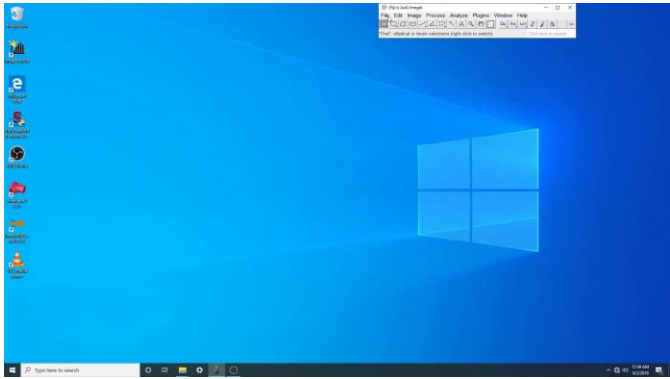
Lightsheet
~1500 z-slices
~10GB

Time-lapse Lightsheet
>10000 z-slices
>500GB

Handling the large image data sets

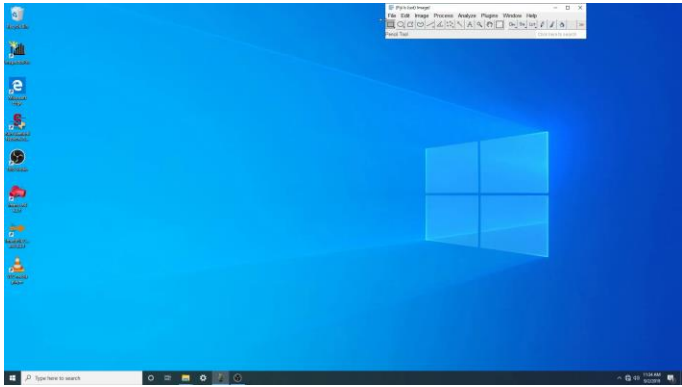
- Saving in big data formats dramatically improves loading time
- Does not decrease the file size/resolution
- Most computers may still not have the capacity to load in the entire dataset

Image stack



>4 minutes

Hdf5 file

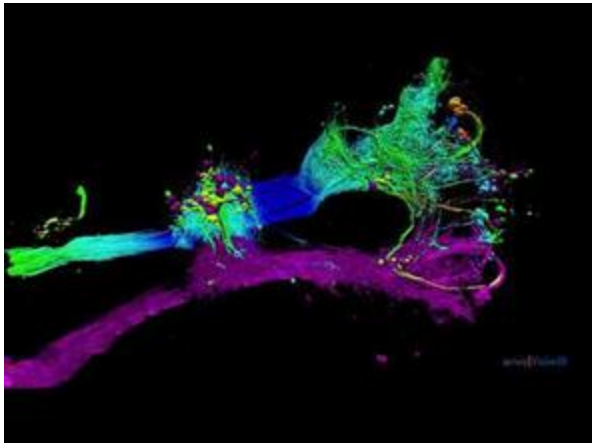


<30 seconds

Want something more powerful

Imaris

Arivis



Huygens Software Suite

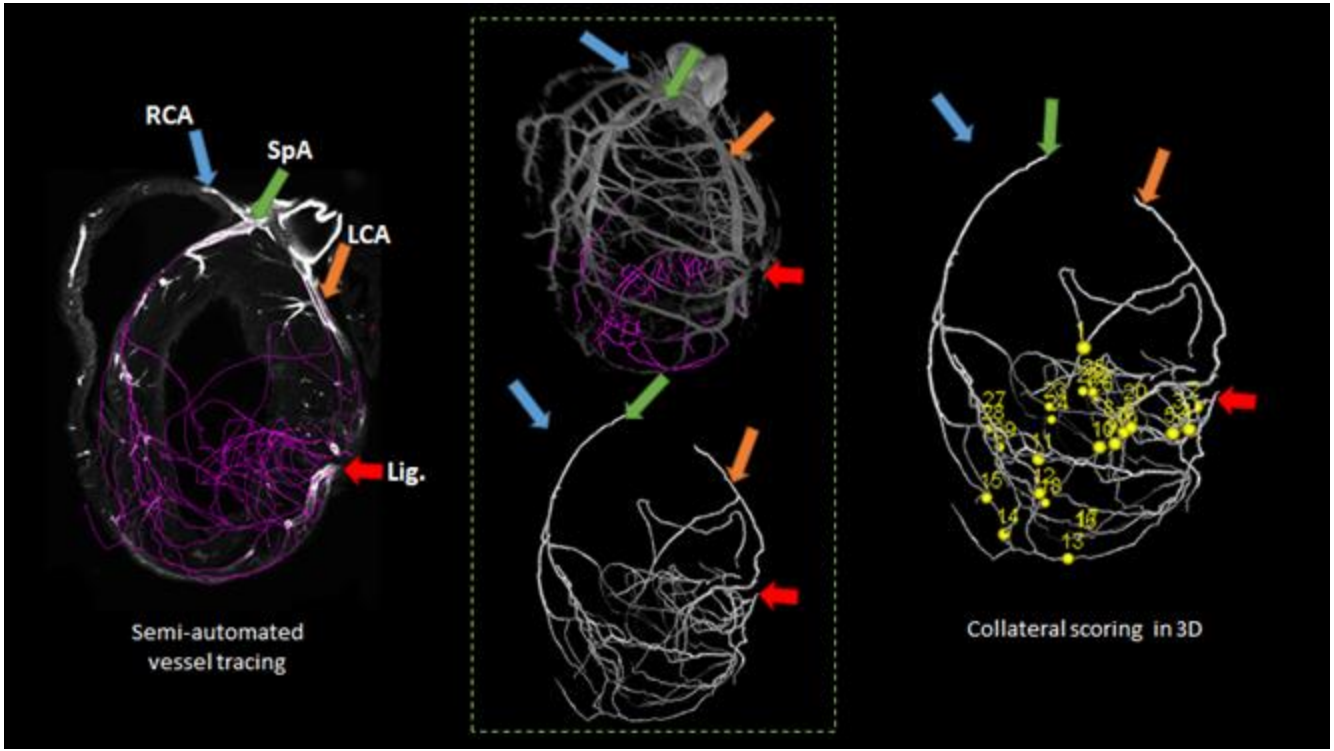
Volocity

Tips to reduce data size

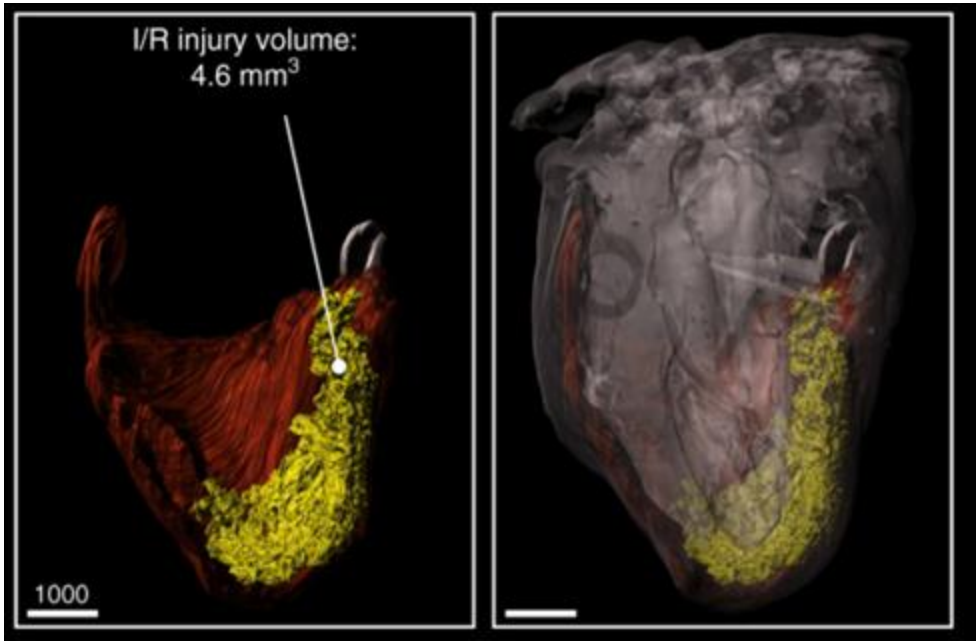
- After imaging:
  - Lower the resolution of the dataset
  - Convert to 8-bit (usually 16-bit is the default)
  - Only save a subset of the stack
- Archiving/compressing:
  - Can compress the image in ImageJ

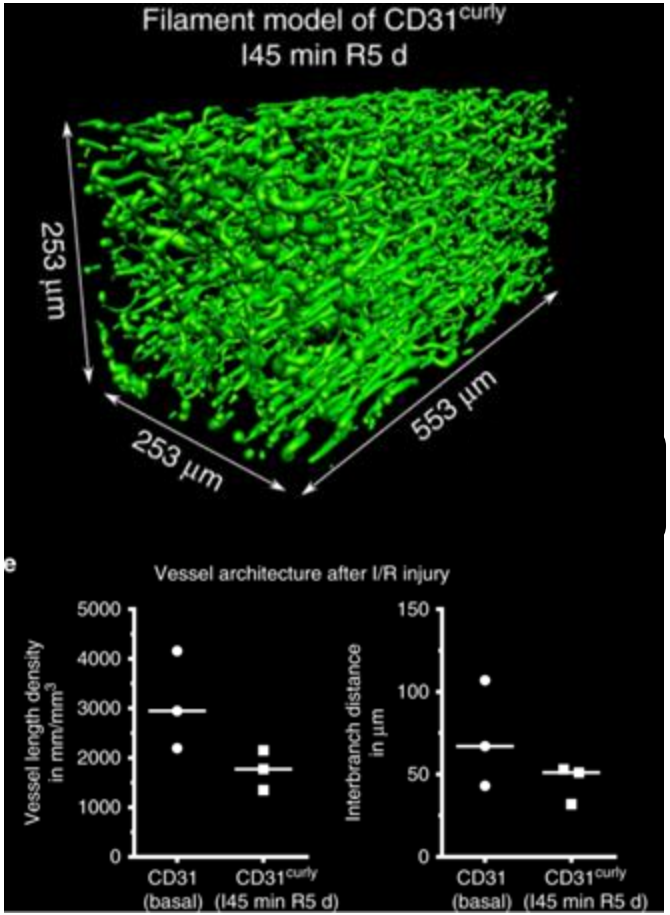
How can we get quantitative data?

Simple Neurite (Vessel) Tracer in ImageJ



Imaris features

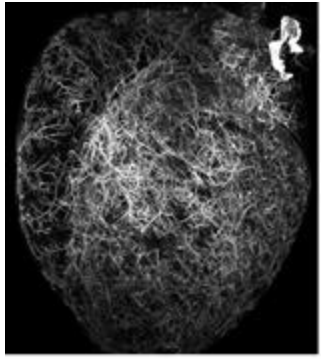




Merz S. et al. Nature Comm. (2019)

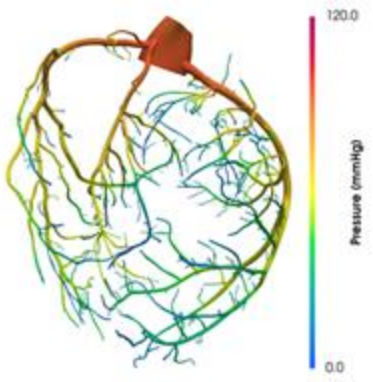
Adult mouse modeling

Light-sheet image

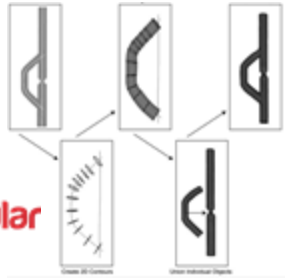


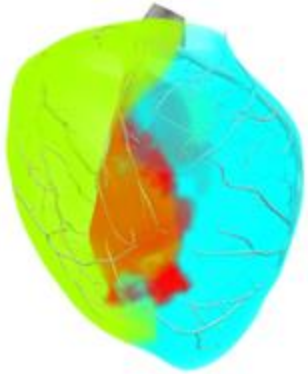
3D Segmentation

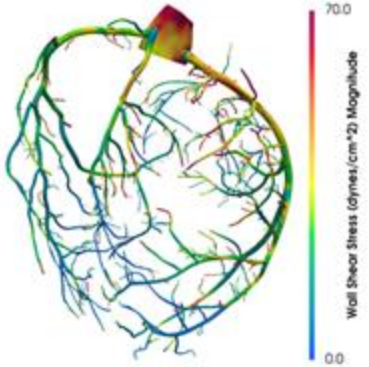
CFD Results



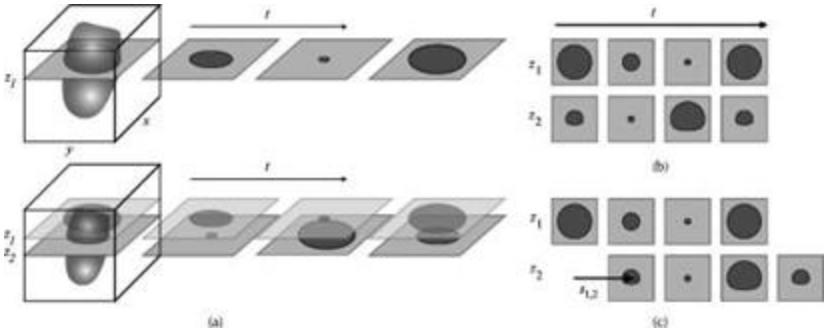
Simvascular



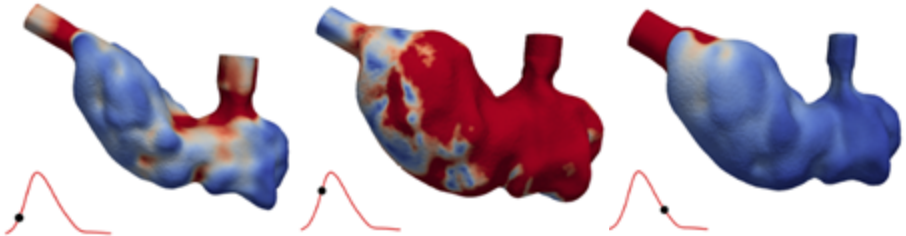


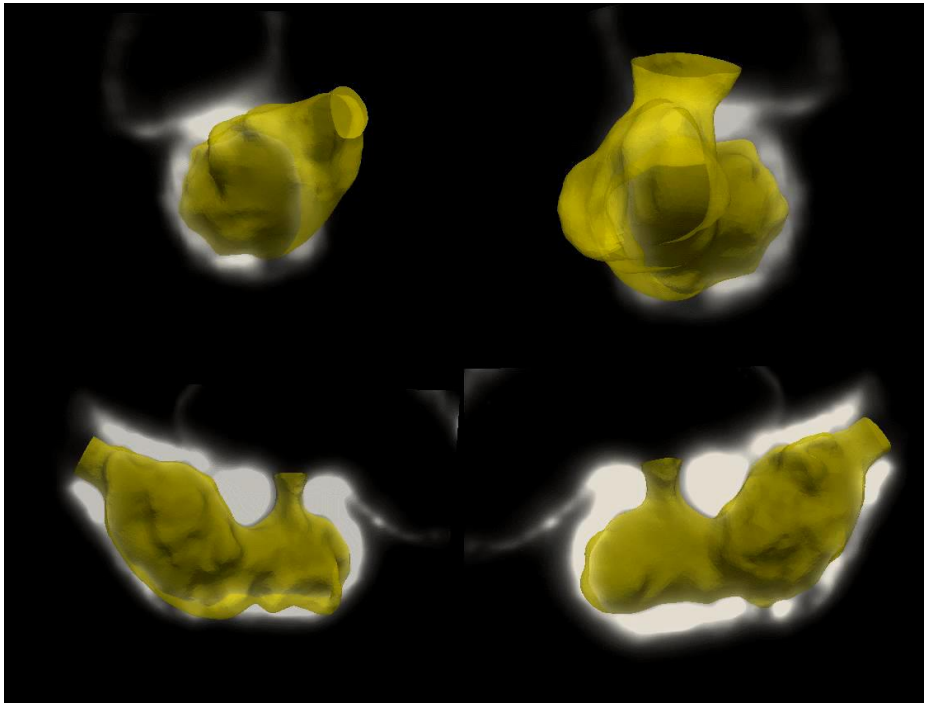


Using 4D lightsheet images for cardiac modeling



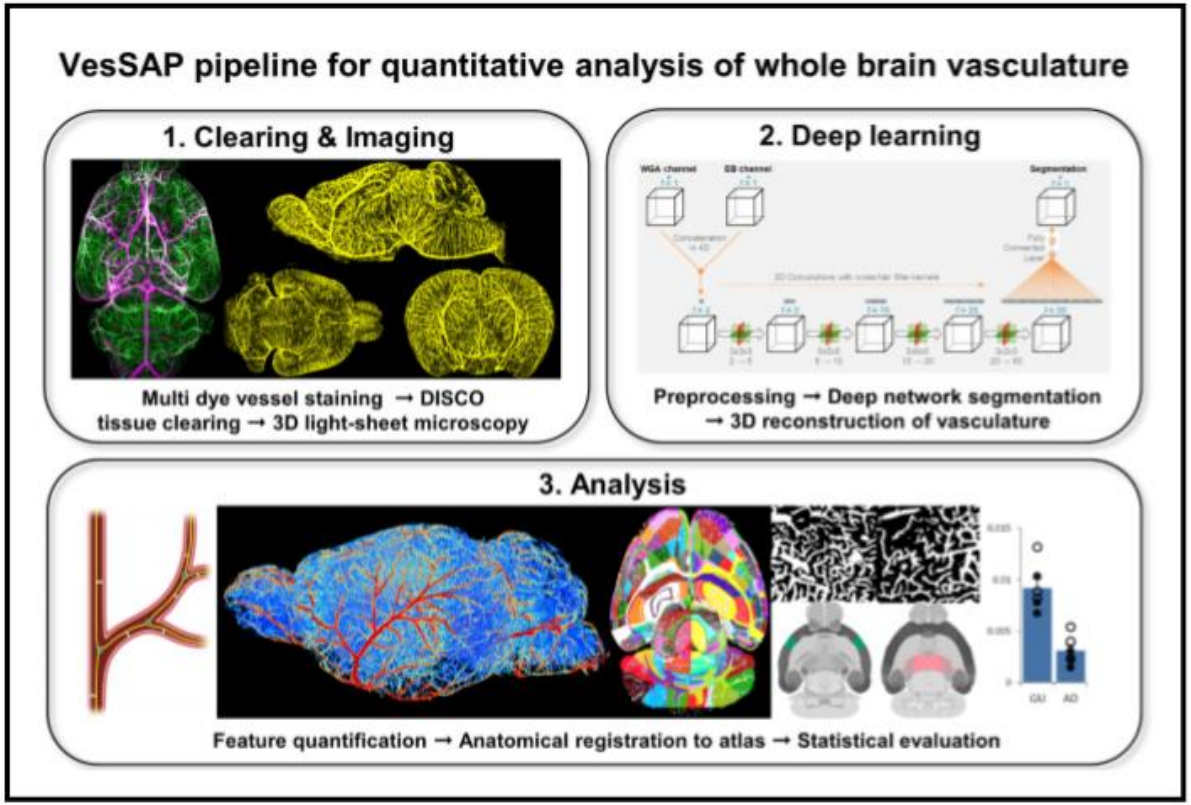
Liebling M, et al. J. of Biomedical Optics (2005)





Vedula V, et al. PLOS Com. Bio. (2017)

Deep Learning to Segment Brain Vasculature



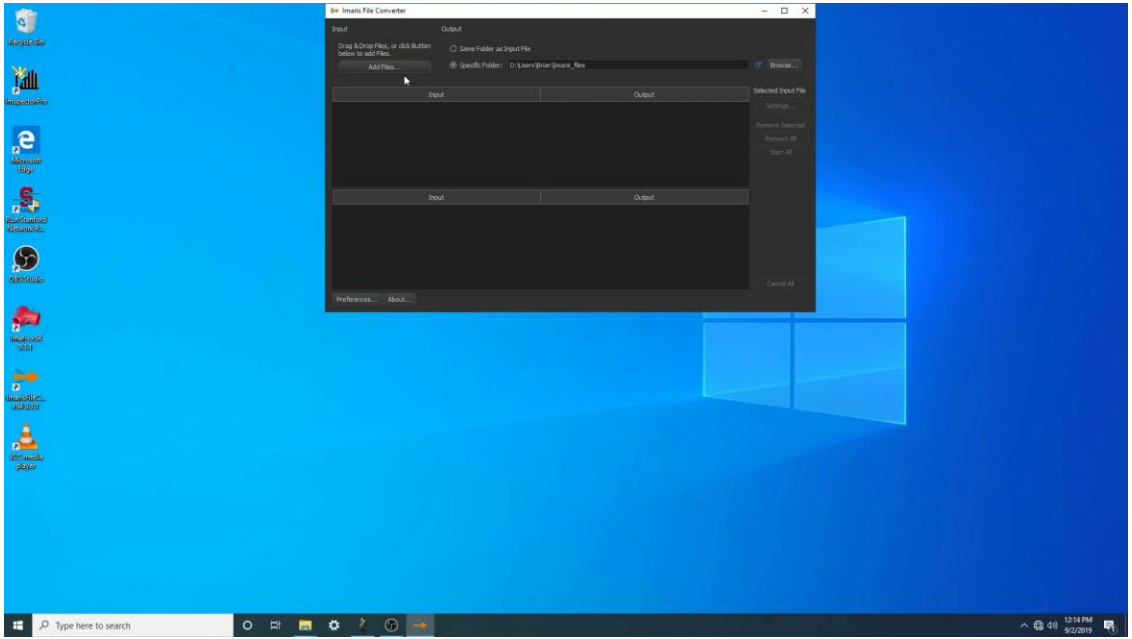
Todorov, M and Paetzold, J et al. bioRxiv 2019

Miltenyi presentation of LaVision Light Sheet Microscope (David Castaneda)

Appendix

Using Imaris converter

- Easily convert many image stacks into .ims files for imaris to read
- Once converted .ims files are much faster to load into Imaris
- Free tool - good for pre-converting data before using Imaris at a core facility



Best Practices

- Secure your sample to prevent micro-vibrations that will distort your image
- Keep the microscope glove free to prevent organic solvents getting on the scope
- Make sure sample will fit within the working distance of the microscope

Saving data as Hdf5 file