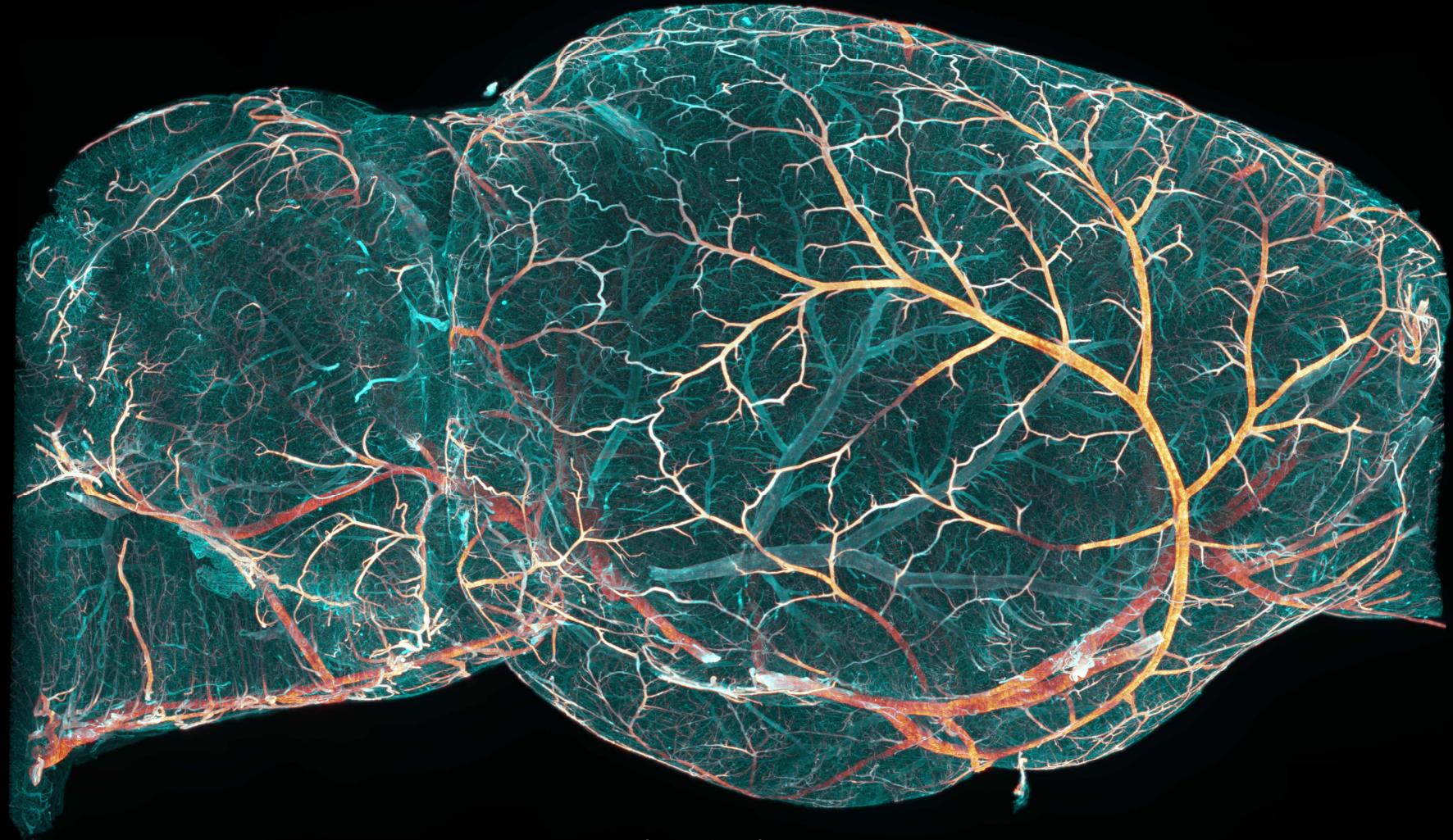


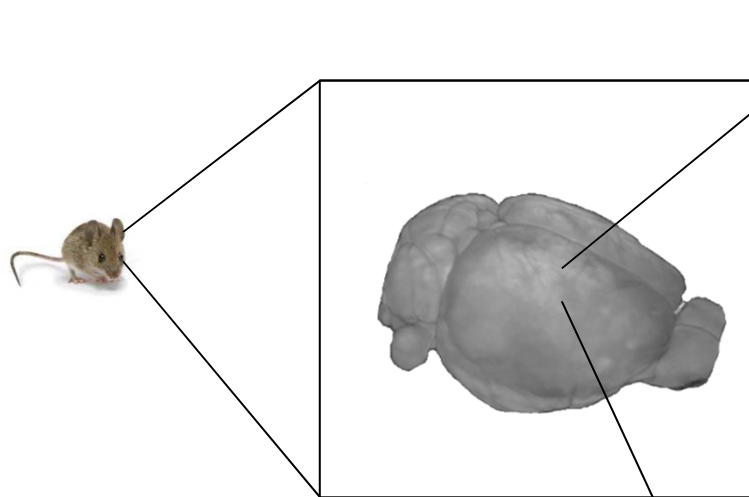
# Mapping brain activity and structure: iDISCO+ and ClearMap



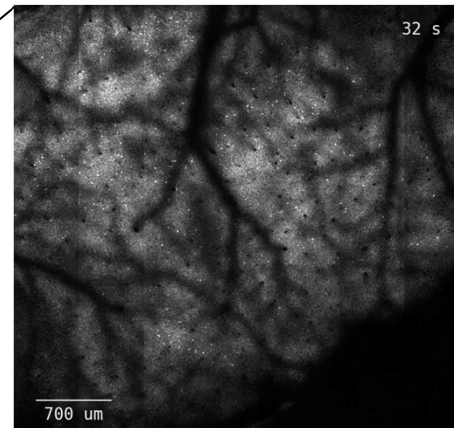
Christoph Kirst

University of California, San Francisco and Lawrence Berkeley National Laboratory

# Complex system brain

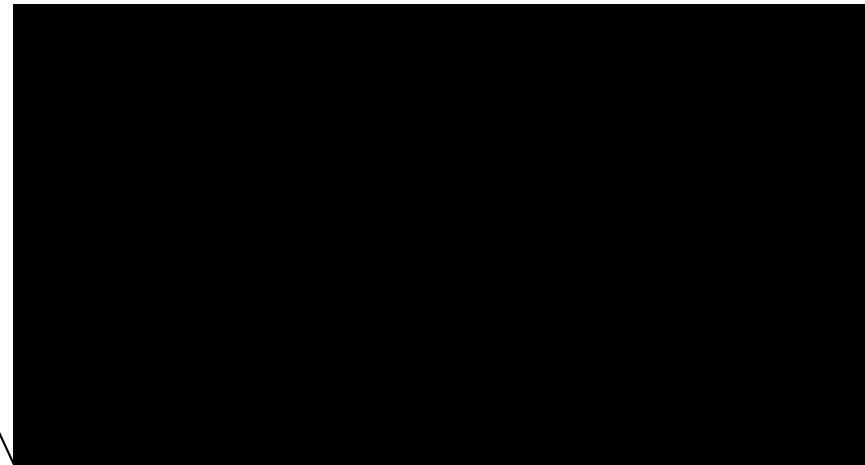


Brain activity



[Sofroniew, ..., Svoboda, 2016]

Brain structure



[Renier\*, Adams\*, Kirst\*, Wu\*, et al. 2016]

How do brain networks compute, enable cognition and generate intelligent behavior ?

How is brain activity and structure altered in diseases, by genetics, or via drugs ?

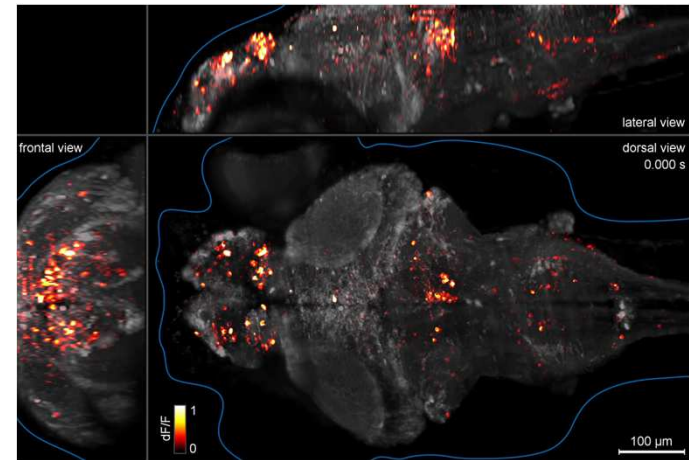
# Neuroscience and large data

+ microscopy and method development

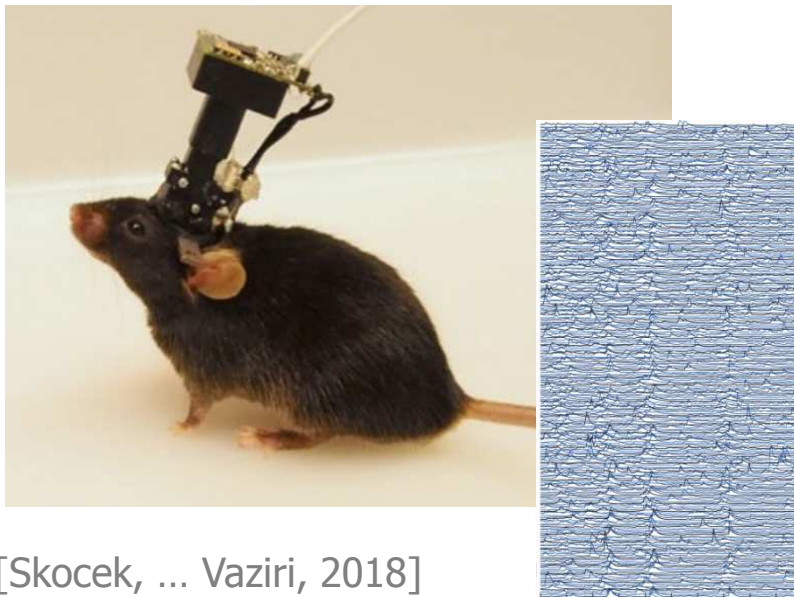
+ Morse law for computational power

= boost in scale and data size

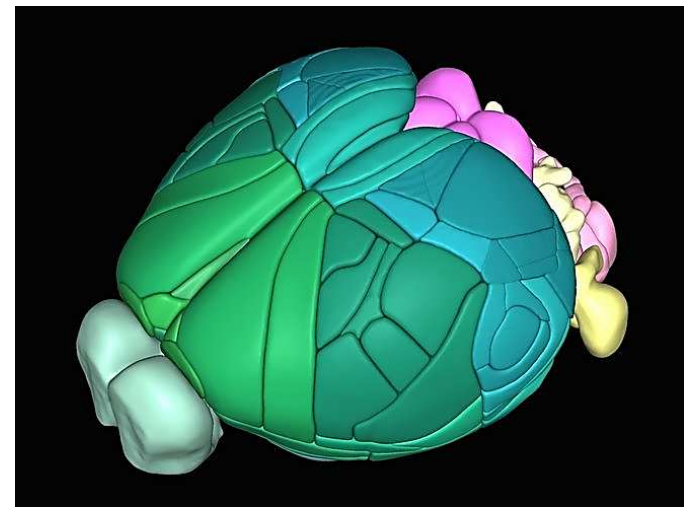
→ understand circuit function  
on the brain-wide level



[Vladimirov, ..., Ahrens, 2014]



[Skocek, ... Vaziri, 2018]



[Allen Brain Atlas]

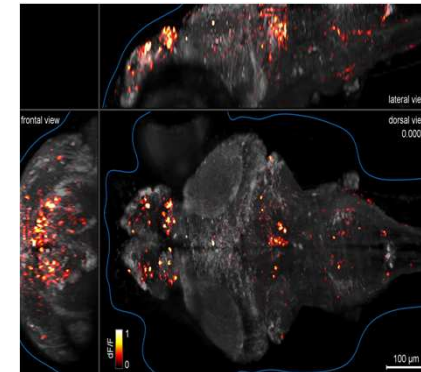
# Brain wide activity mapping

- EEG, MEG, fMRI
  - + fast, non-invasive
  - low spatial resolution

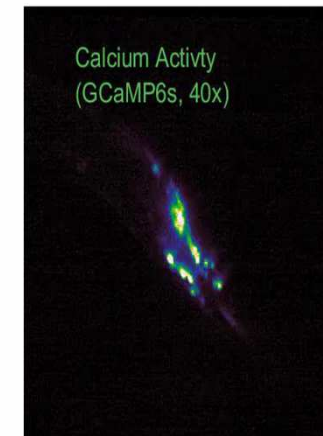
- multi-channel silicon probes
  - + fast
  - limited number of channels

- opto-genetics
  - + larger populations & dynamics
  - challenging for deeper structures / limited field of view

⇒ not achieved in mammalian brain



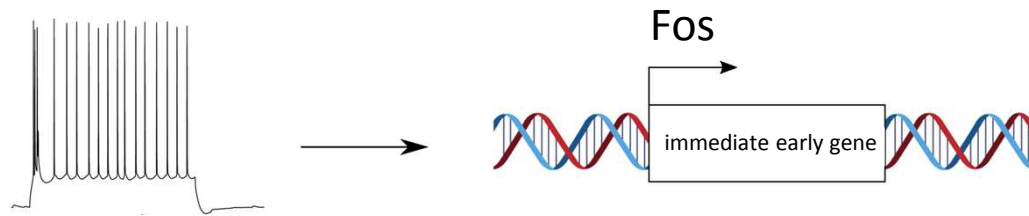
[Vladimirov, ..., Ahrens, Nat Meth 2014]



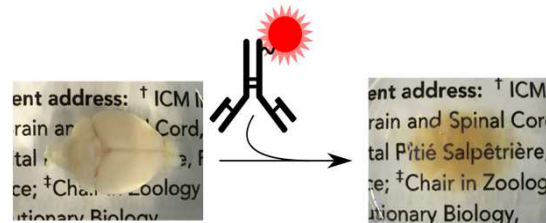
[Nguyen, ..., Leifer, PNAS 2014]

# Mapping whole brain activity at cellular resolution

- ▶ neuronal activity drives Fos expression



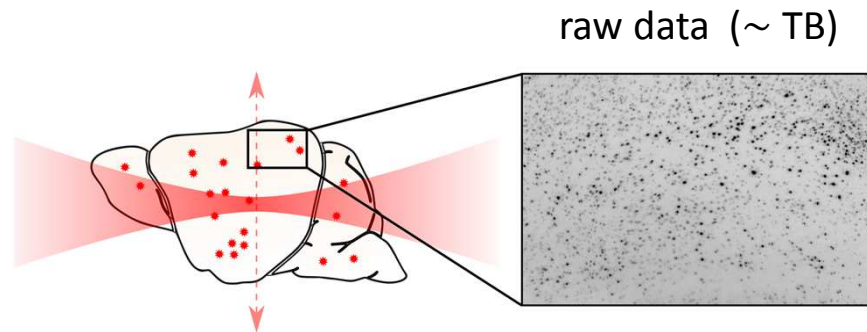
- ▶ vasculature clearing



iDISCO+



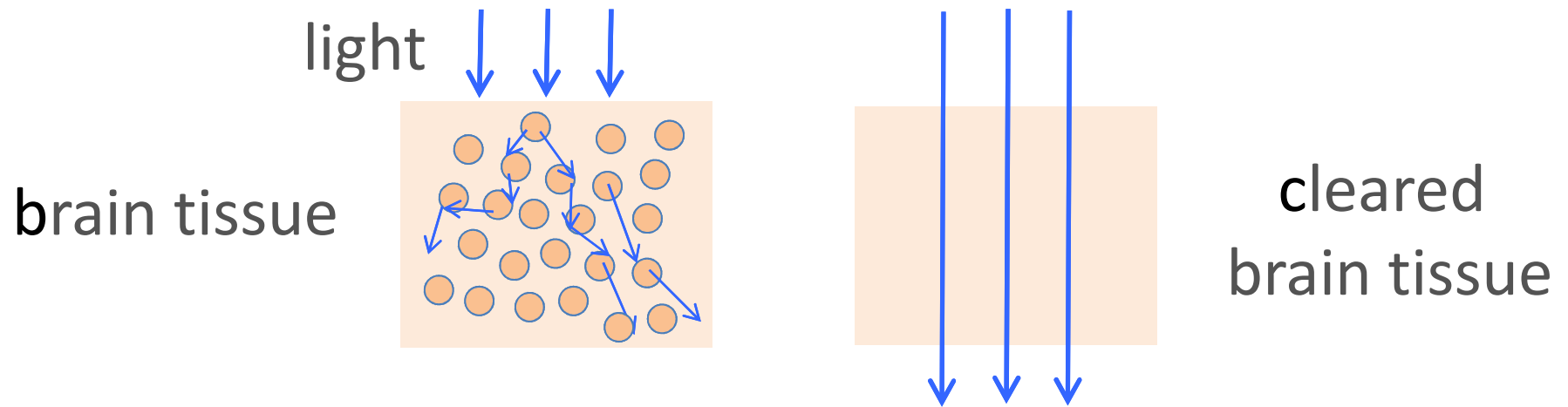
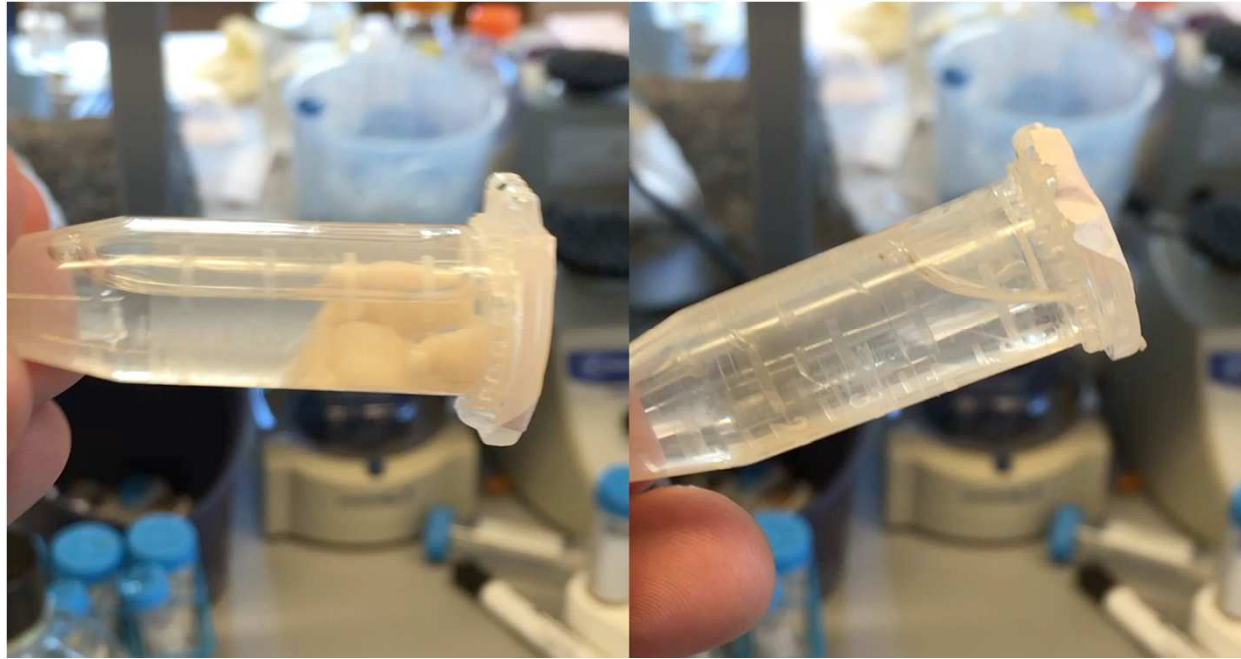
- ▶ light sheet imaging and data analysis



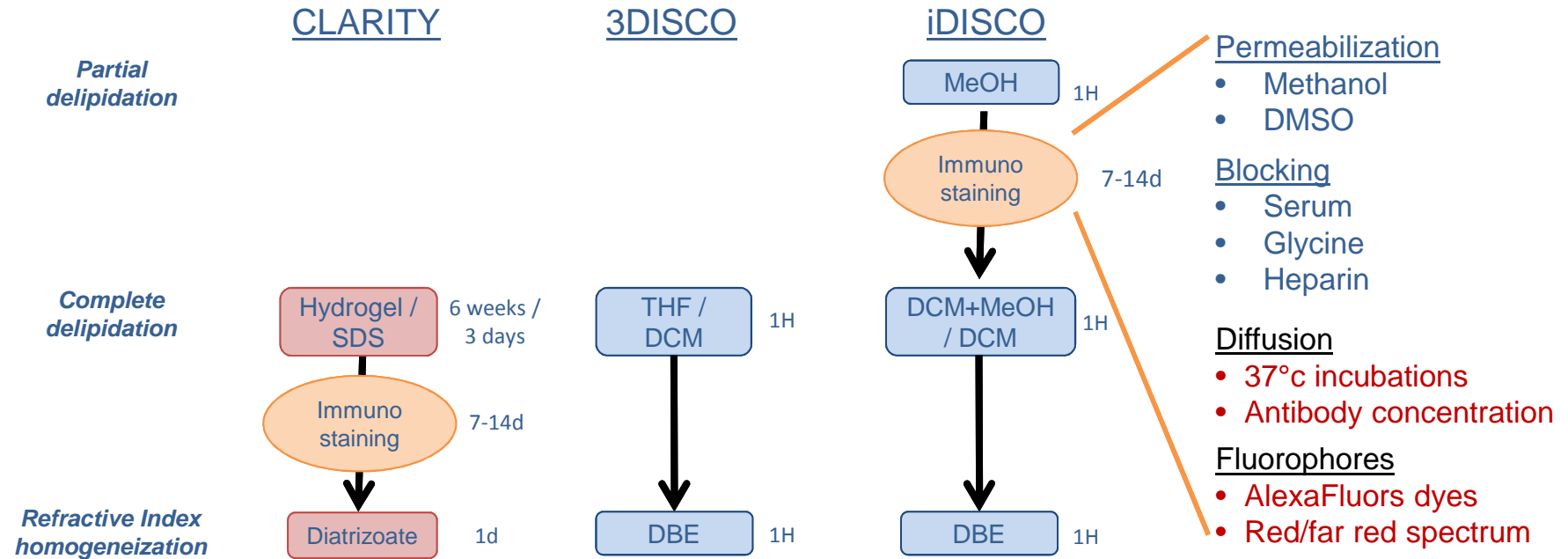
ClearMap



# iDISCO+ tissue clearing



# Tissue clearing: iDISCO

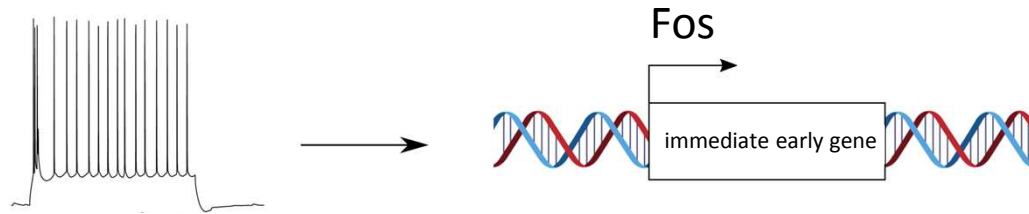


Enhanced whole-mount labeling protocol + 3DISCO clearing =  
**iDISCO: immunolabeling-enabled 3 dimensional clearing of solvent cleared organs**

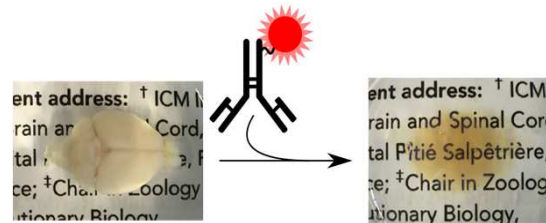


# Mapping whole brain activity at cellular resolution

- ▶ neuronal activity drives Fos expression



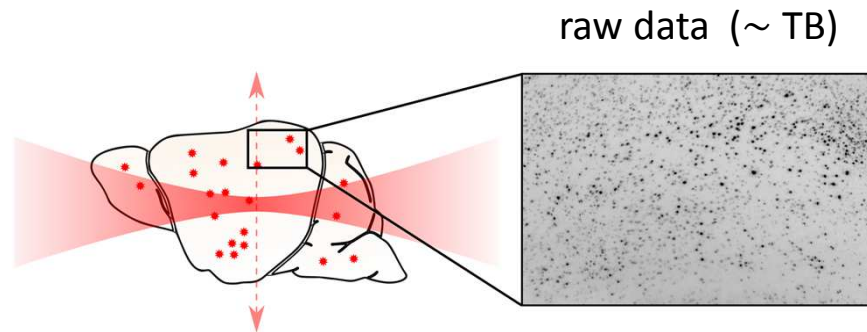
- ▶ vasculature clearing



iDISCO+

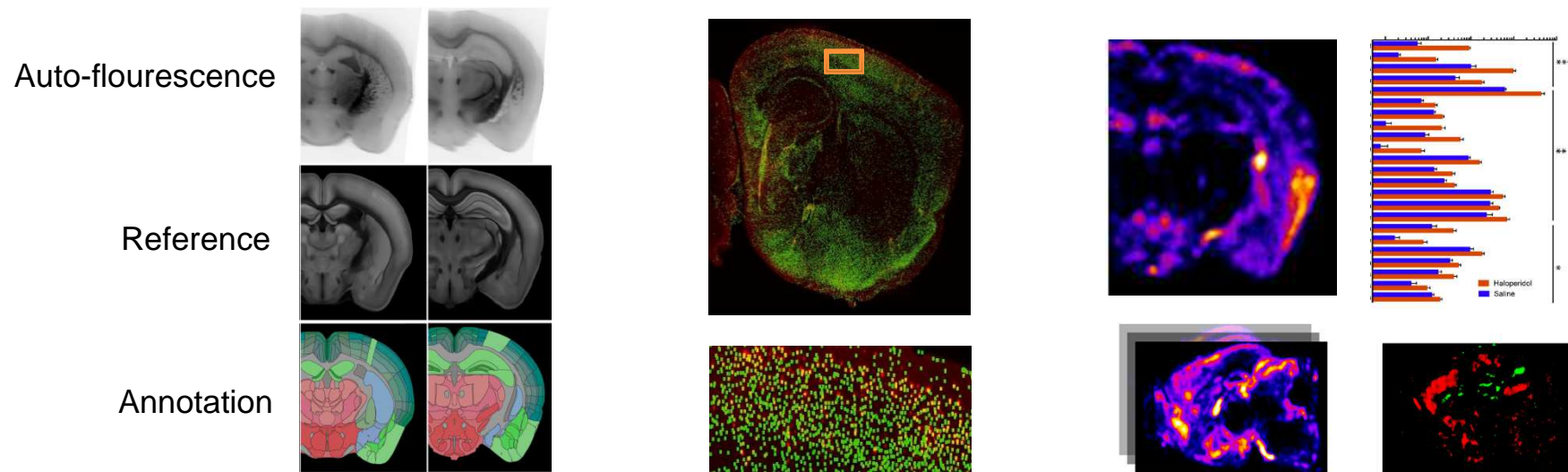
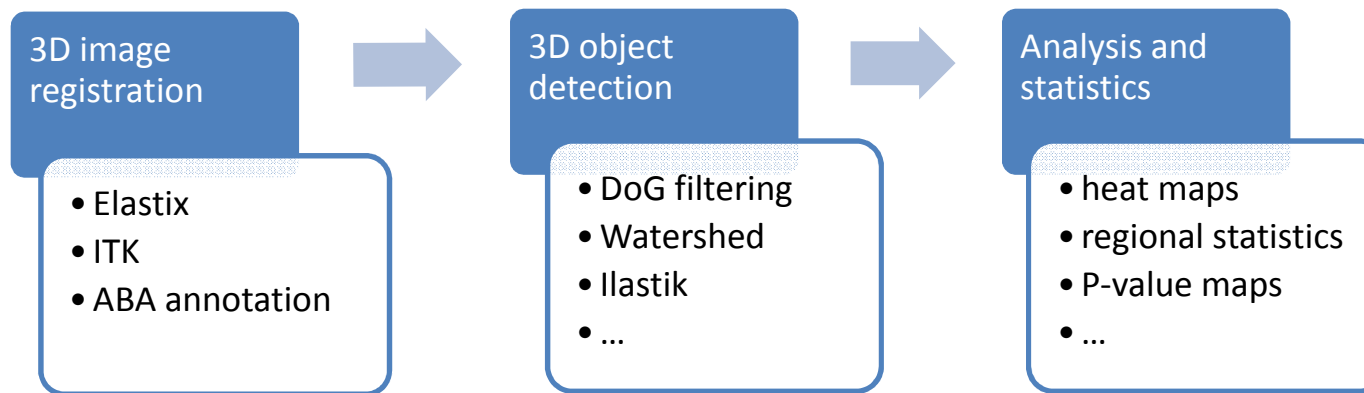


- ▶ light sheet imaging and data analysis





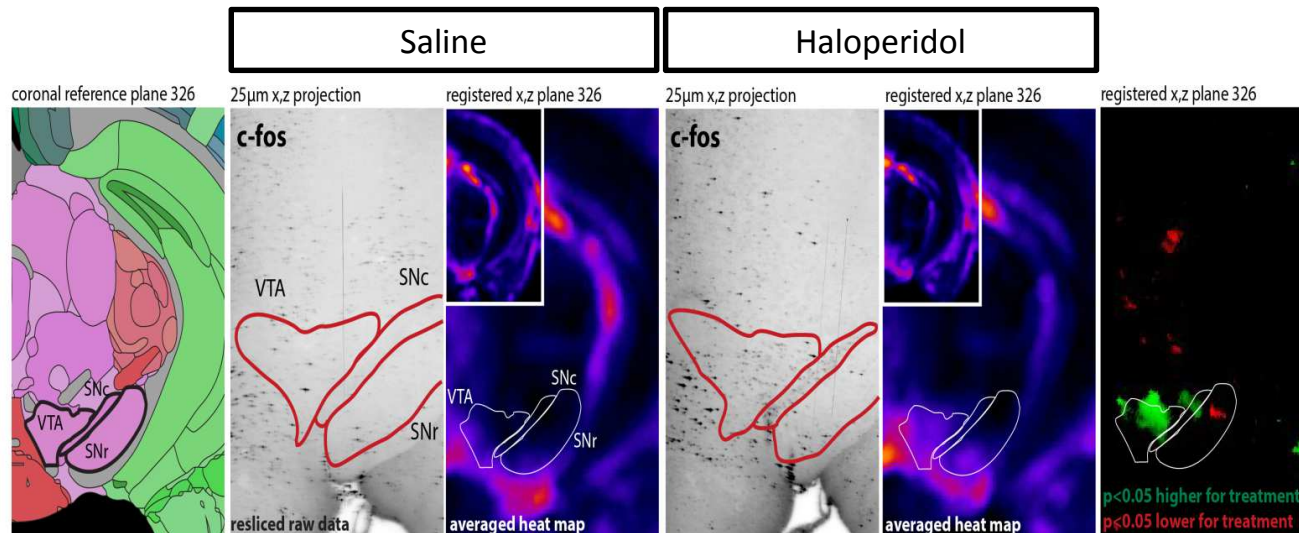
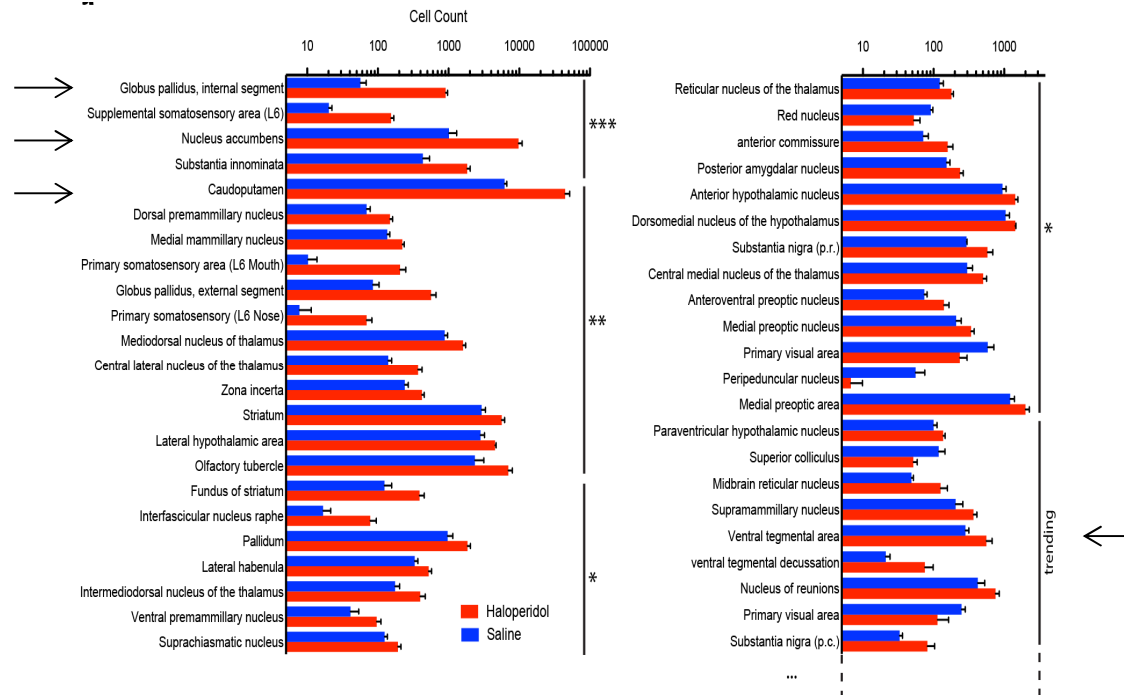
# ClearMap: A pipeline to detect and register cells in 3D



- 20k+ lines python & c++
- open source

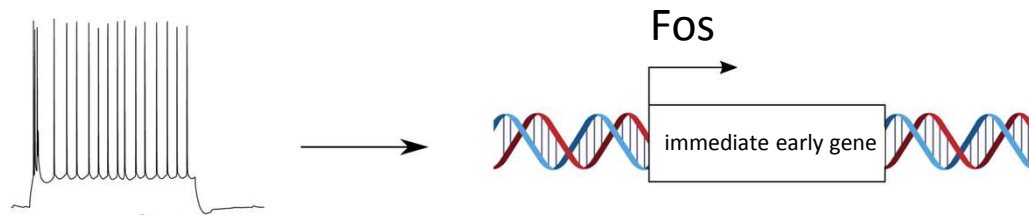
- widely used (Harvard, MIT, Princeton, Stanford, Yale, Columbia, CalTech, IMP Vienna, Rockefeller, CNRS, ...)

# Mapping the effects of psychoactive drugs

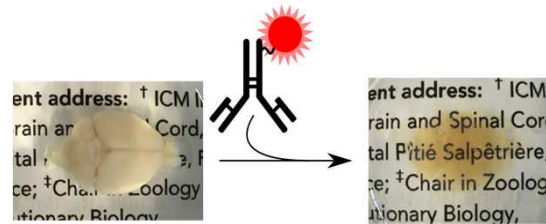


# Mapping whole brain activity at cellular resolution

- ▶ neuronal activity drives Fos expression



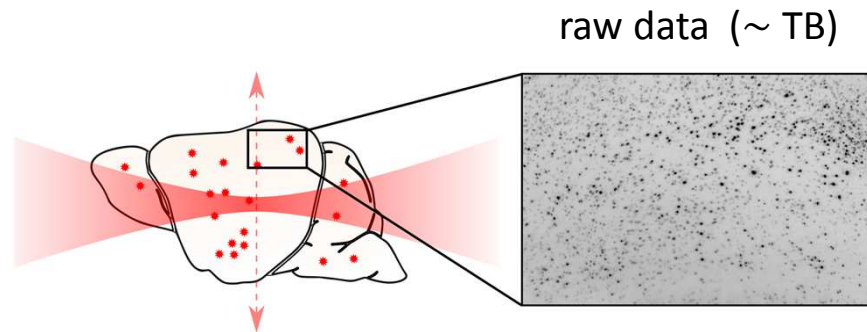
- ▶ vasculature clearing



iDISCO+



- ▶ light sheet imaging and data analysis

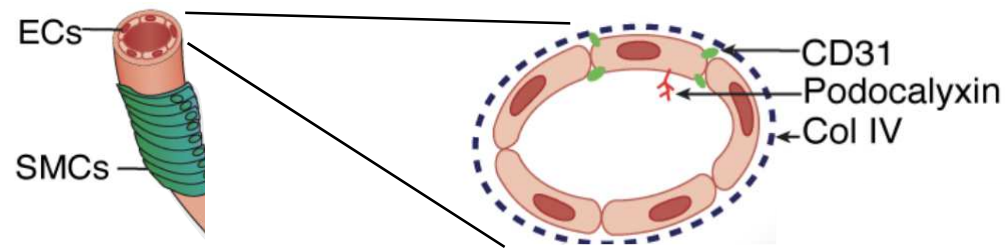


ClearMap

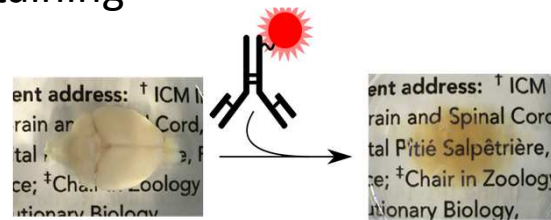


# Mapping whole brain structure at cellular resolution

- ▶ vasculature network via tissue markers



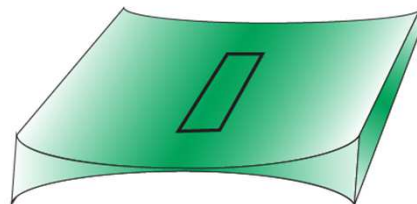
- ▶ tissue clearing & immunostaining



iDISCO+



- ▶ light sheet imaging and data analysis



raw data (~ TB)



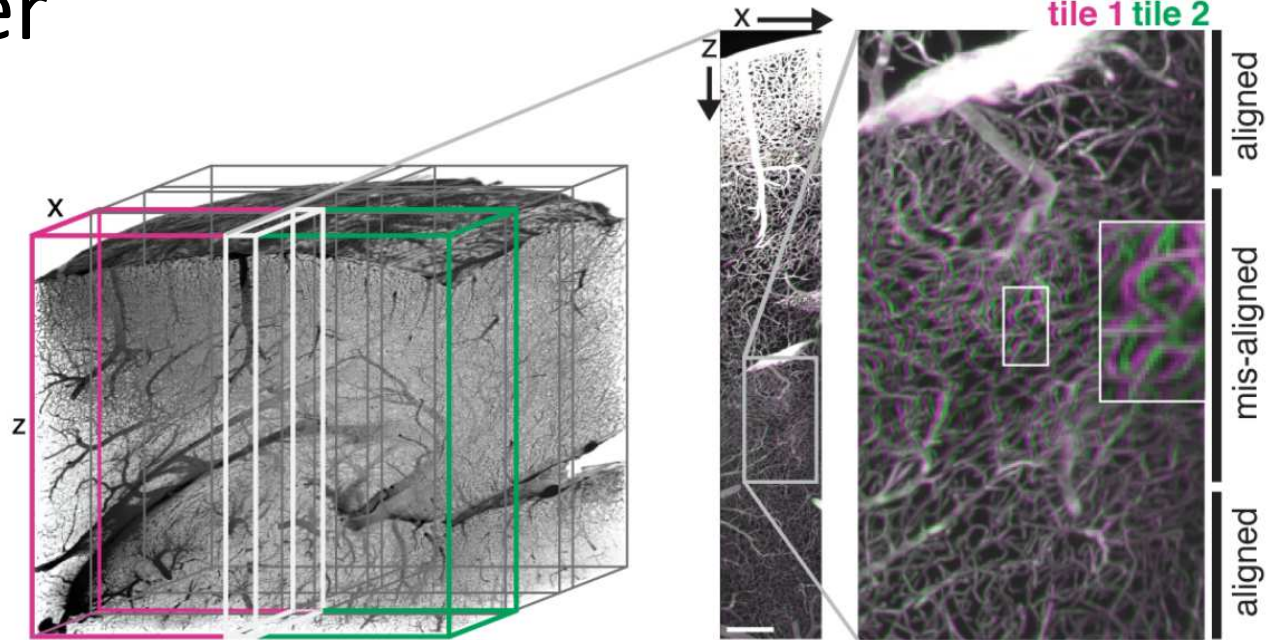
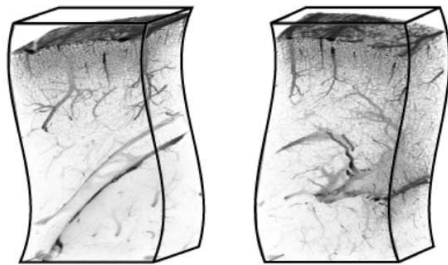
ClearMap



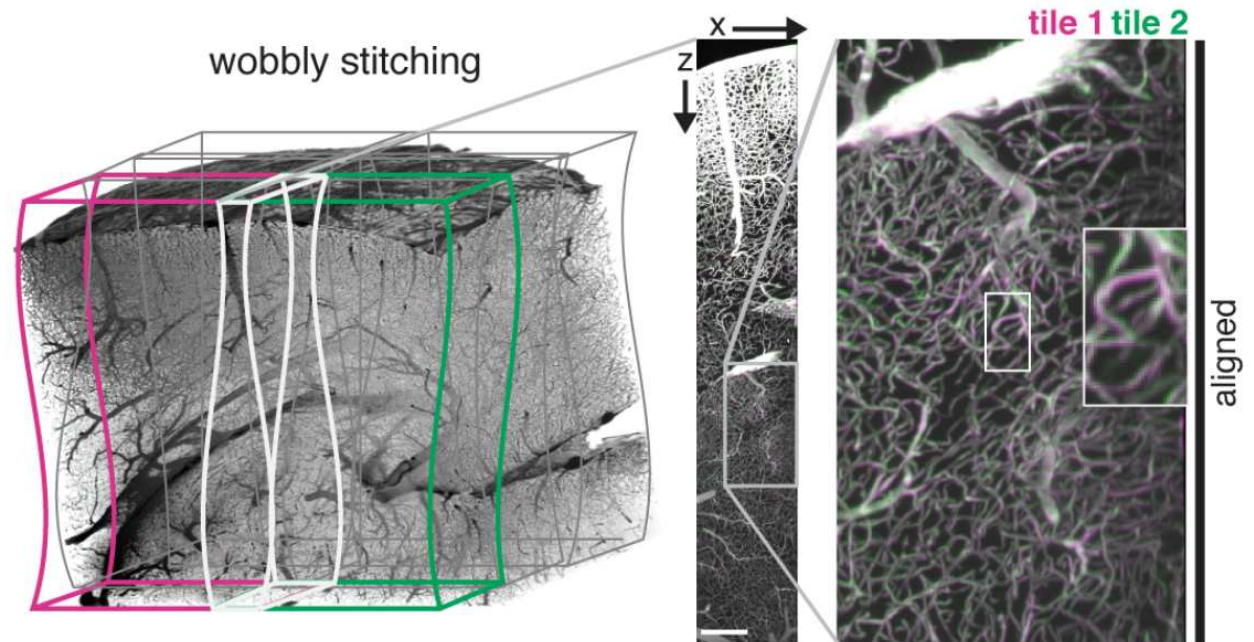
# Wobbly Stitcher

- rigid stitching fails

'wobbly' image stacks  
(oscillations along stack axis)

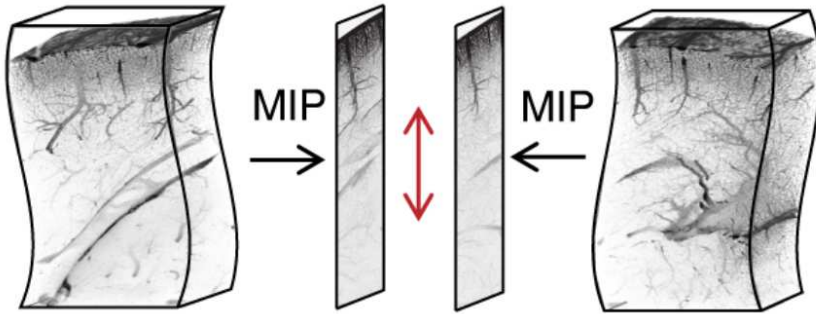


- wobbly stitching

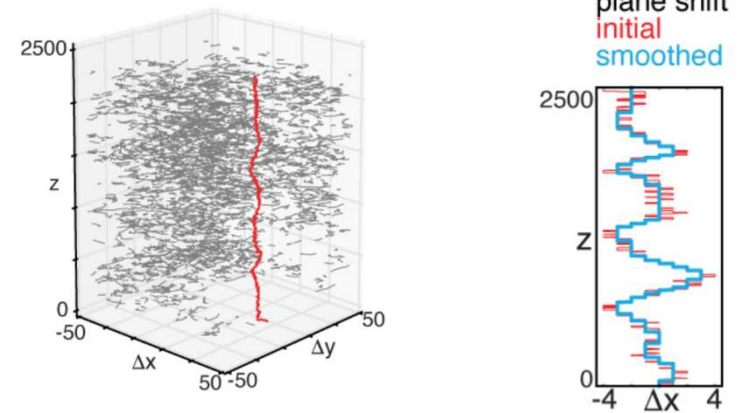


# Wobbly Stitcher

1. z-alignment via MIP projections



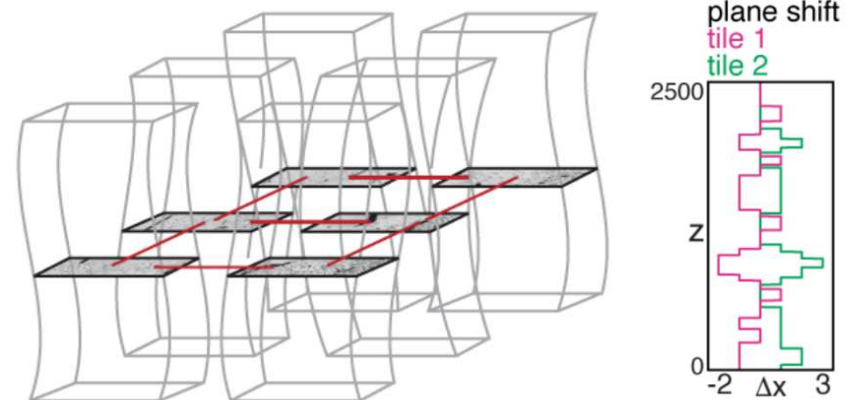
3. alignment tracking



2. plane-wise x-y alignment



4. global optimal placement



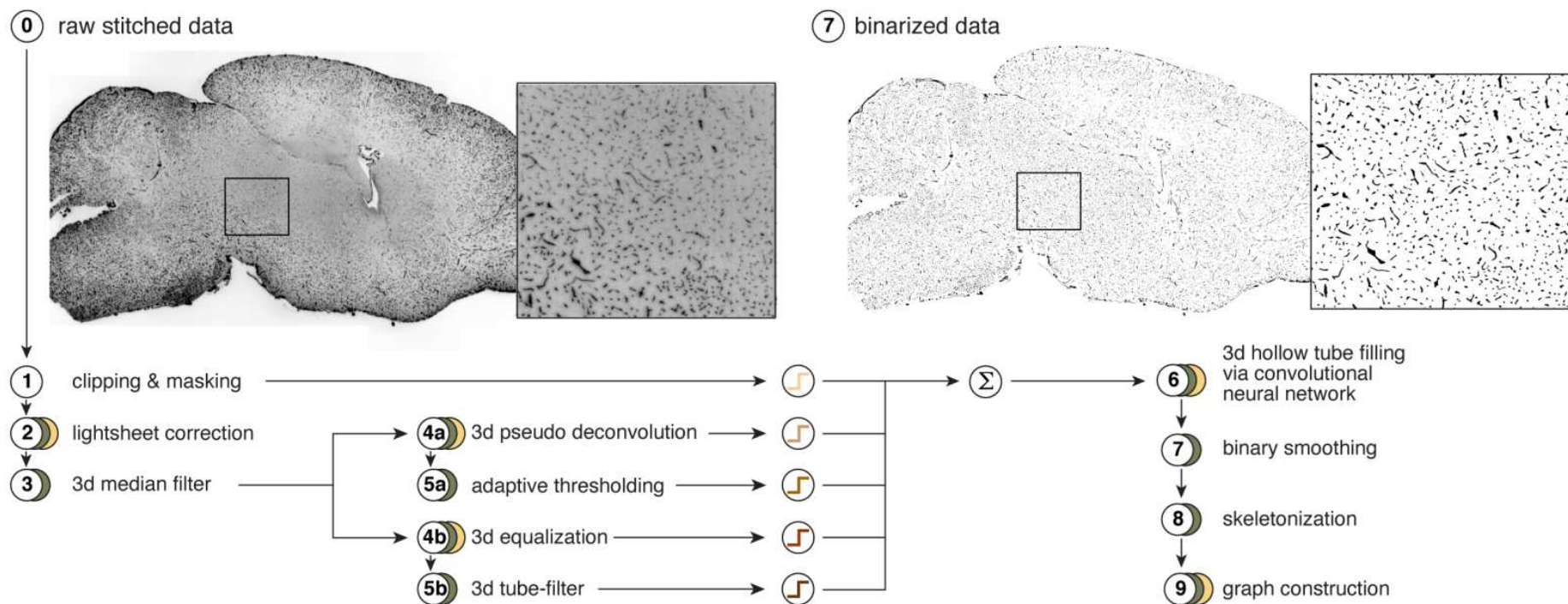
⇒ ~15min for 100 giga pixel volume (~ 3000 planes, ~100 individual stacks)

A 3D reconstruction of a mouse brain's vasculature, showing a dense network of blood vessels in yellow and orange against a blue background. The vessels are highly branched and complex, covering the entire brain structure. The text is overlaid in white on a semi-transparent dark blue band across the center of the image.

**Reconstruction of the mouse brain vasculature  
with iDISCO<sup>+</sup> and TubeMap**

# ClearMap 2.0: A toolbox for 3d image processing

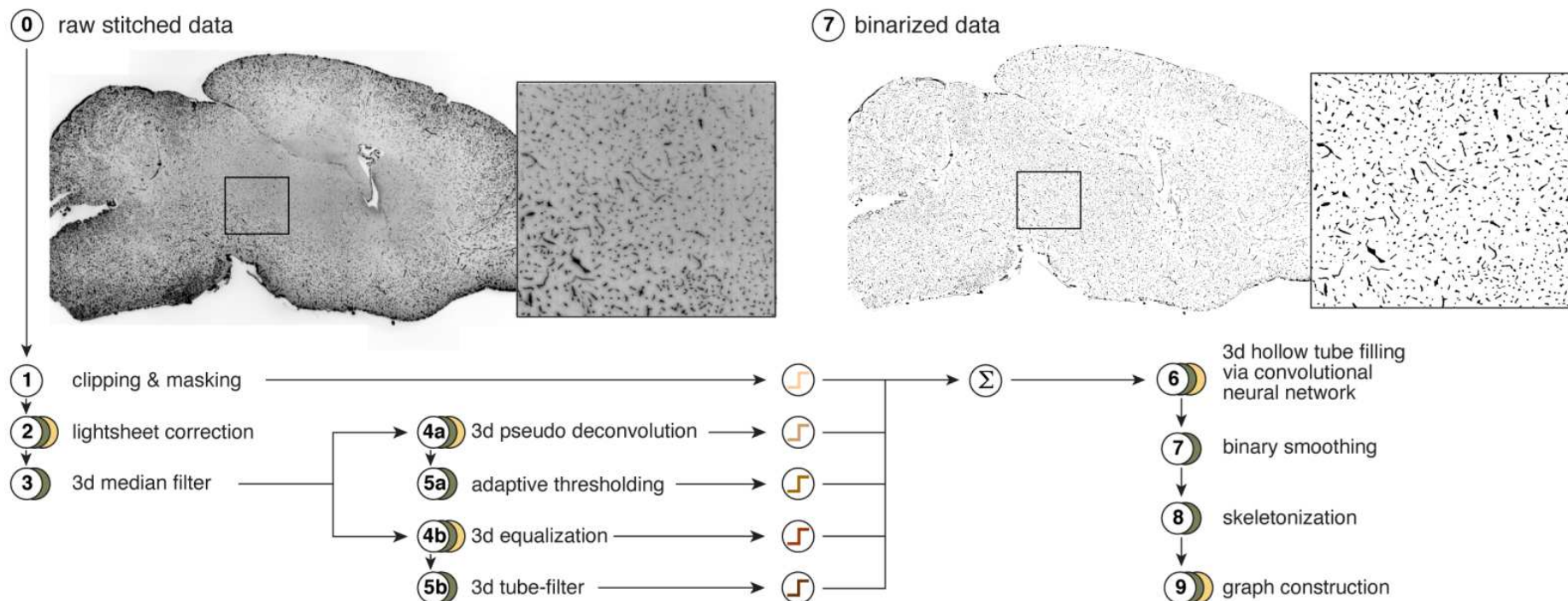
- Toolbox for fast 3d image processing methods of TB sized light sheet microscopy data.
- TubeMap: full vasculature network reconstruction





# ClearMap 2.0: a toolbox for 3d image processing

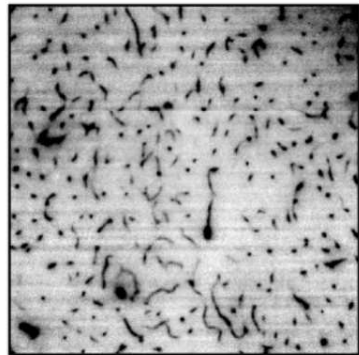
- Toolbox for fast 3d image processing methods of TB sized light sheet microscopy data.
- TubeMap: full vasculature network reconstruction



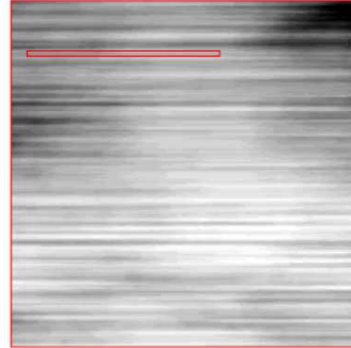
# ClearMap 2.0: a toolbox for 3d image processing

## 2) lightsheet correction

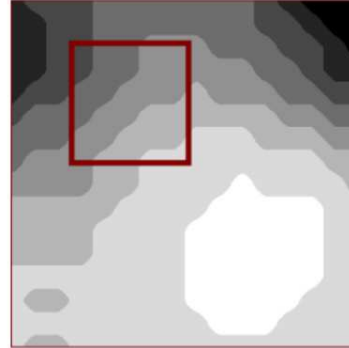
clipped data



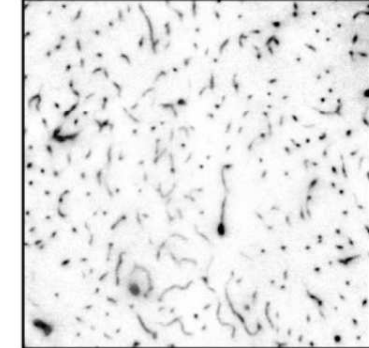
elongated percentile



square percentile

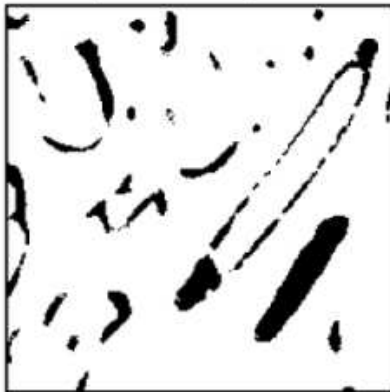


light sheet corrected



## 6) 3d hollow tube filling convolutional neural network

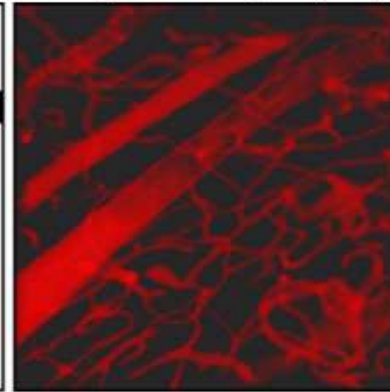
binary mask input



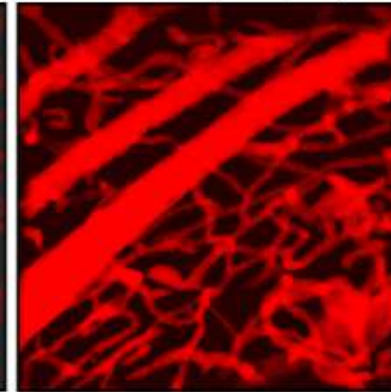
network output



binary mask input (3D)

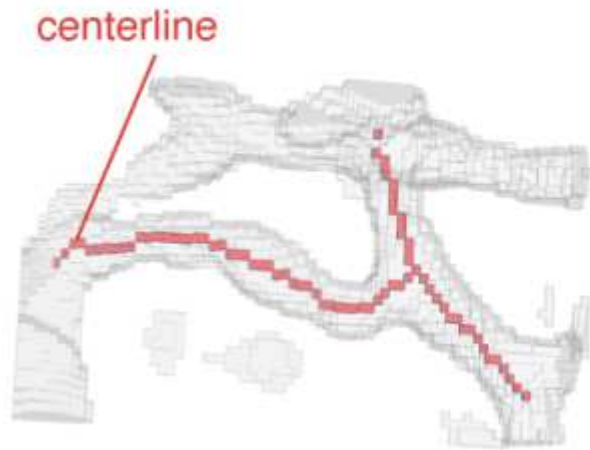


network output (3D)

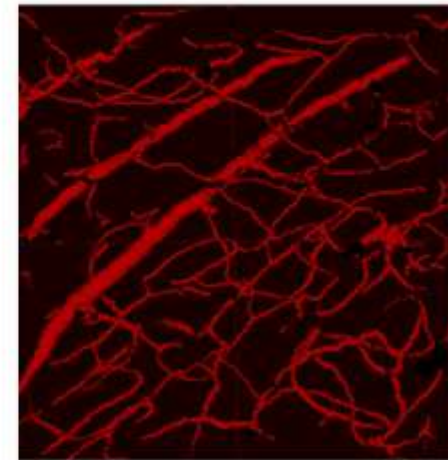
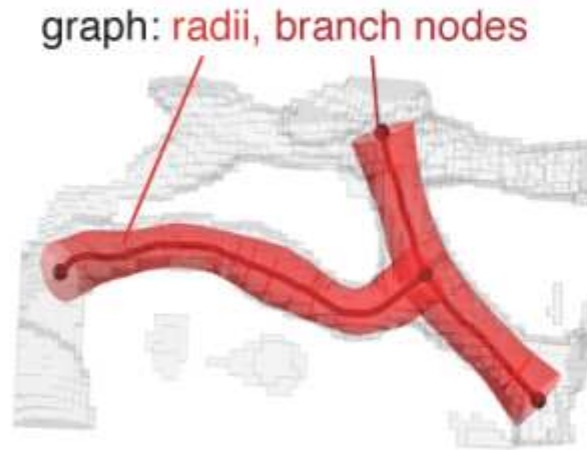


# ClearMap 2.0: a toolbox for 3d image processing

⑧ skeletonization

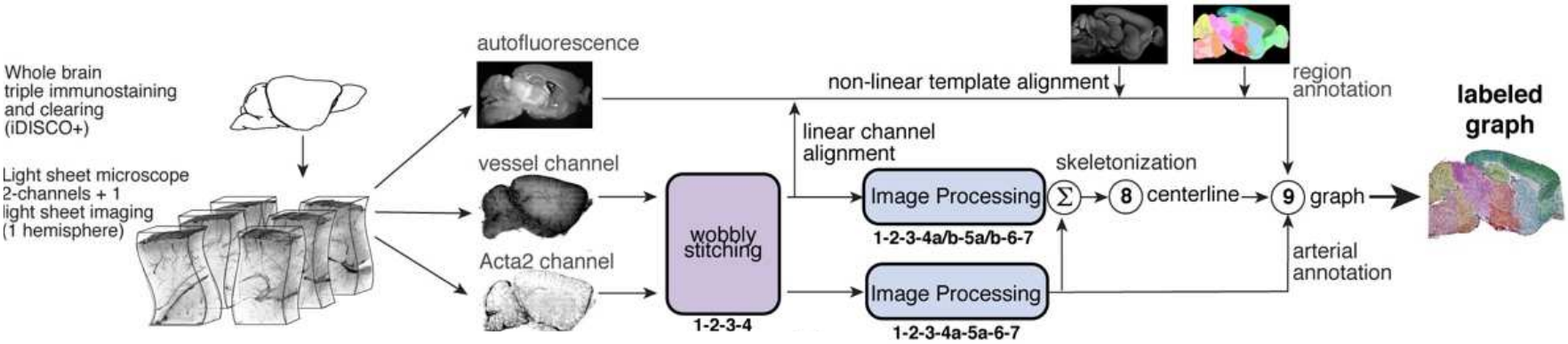


⑨ graph construction



# ClearMap 2.0: a toolbox for 3d image processing

TubeMap pipeline:



# ClearMap 2.0: a toolbox for 3d image processing

## Alignment methods:

- 3d resampling
- 3d alignment to reference atlases
- wobbly stitching (see above)
- Allen Brain Atlas modules

## Image processing methods:

- clipping and normalization
- binary filling
- discrete topology based binary smoothing
- 3d local gradients and Hessian matrices
- 3d tube filter and tubeness measures
- 3d rank filter library (>30 filters)
- skeletonization via parallel thinning
- 3d tracing
- fast calculation of 3d local image statistics
- equalization methods
- hysteresis and seeded thresholding
- 3d adaptive and local image statistics based thresholding
- light-sheet artifact removal
- fast pseudo deconvolution
- deep convolutional neuronal network based image processing
- expert processing pipelines for specific applications

## Graph analysis:

- graph preprocessing and cleanup
- graph branch reduction
- graph annotation
- graphs embedded 3d space
- 3d graphs with 3d edge geometry
- graph processing
  - morphological operations on edges and vertices
  - sub-graph extraction and spatial slicing
  - network analysis

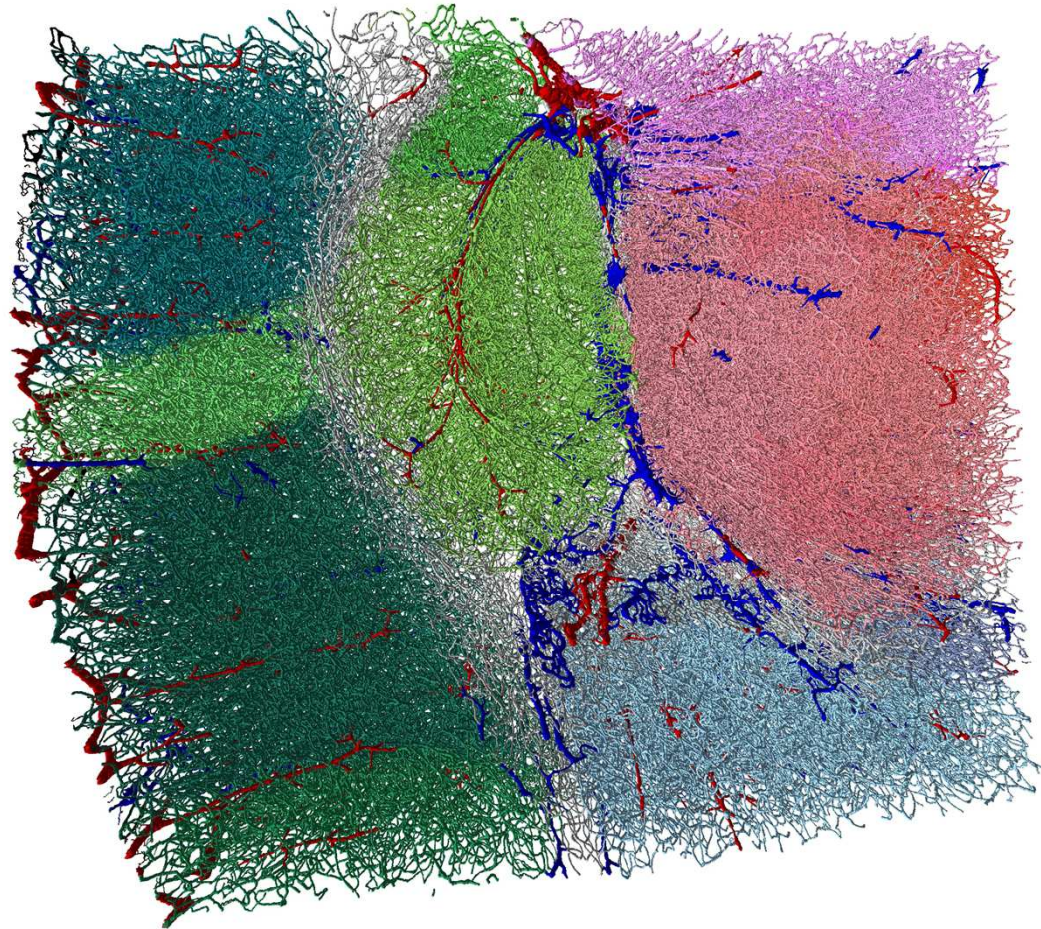
## Visualization tools for 3d images:

- fast interactive 2d slice plotting of 3d TB data sets
- overlays and/or synchronized window display
- interactive image processing pipeline
- 3d volume rendering
- 3d list and line plots

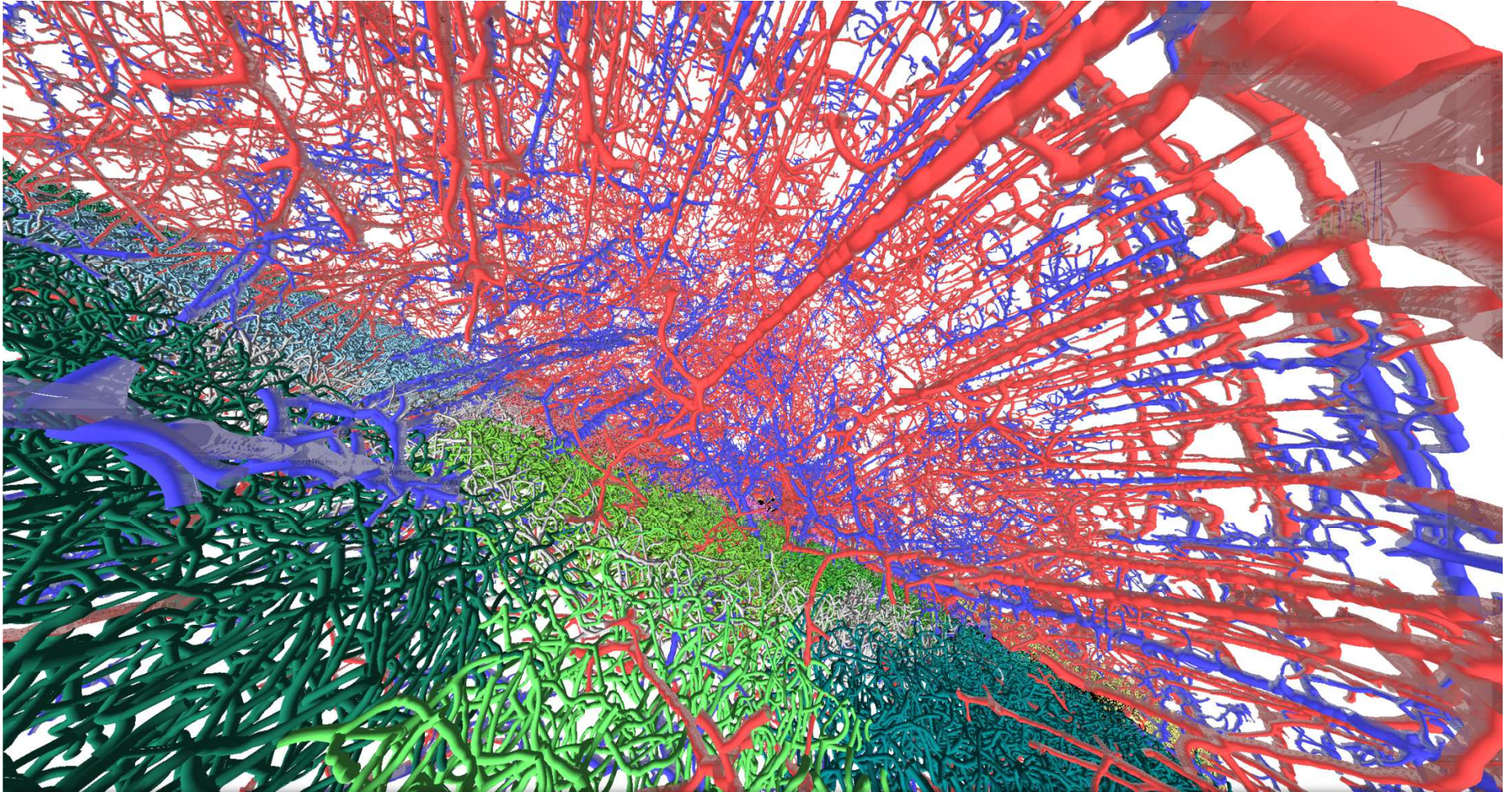
## Visualization tools for 3d graphs:

- 3d line plots of 3d graphs
- 3d mesh plots of 3d vasculature graphs

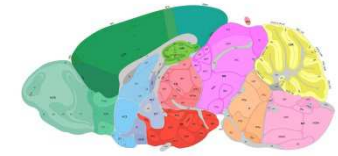
# Vasculature graph reconstruction



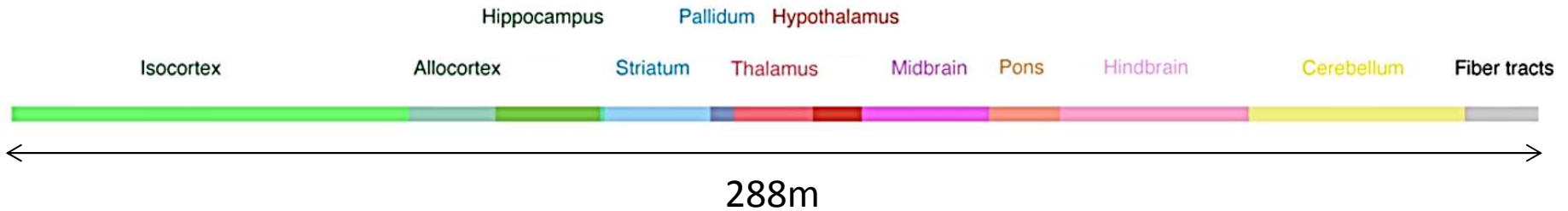
# Vasculature graph reconstruction



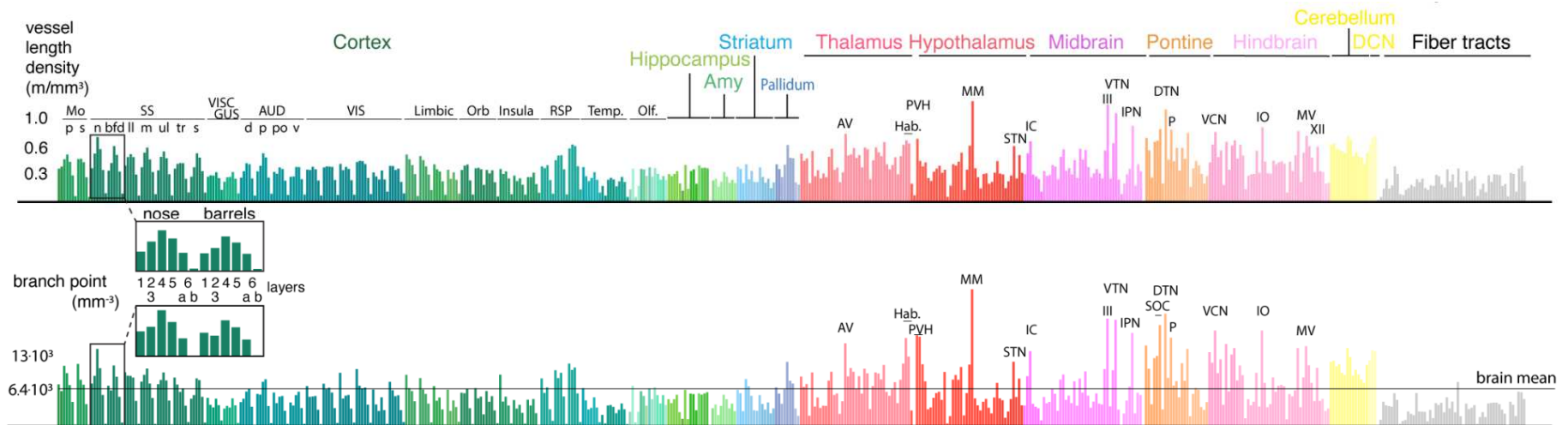
# Vasculature Features



total vasculature vessel length

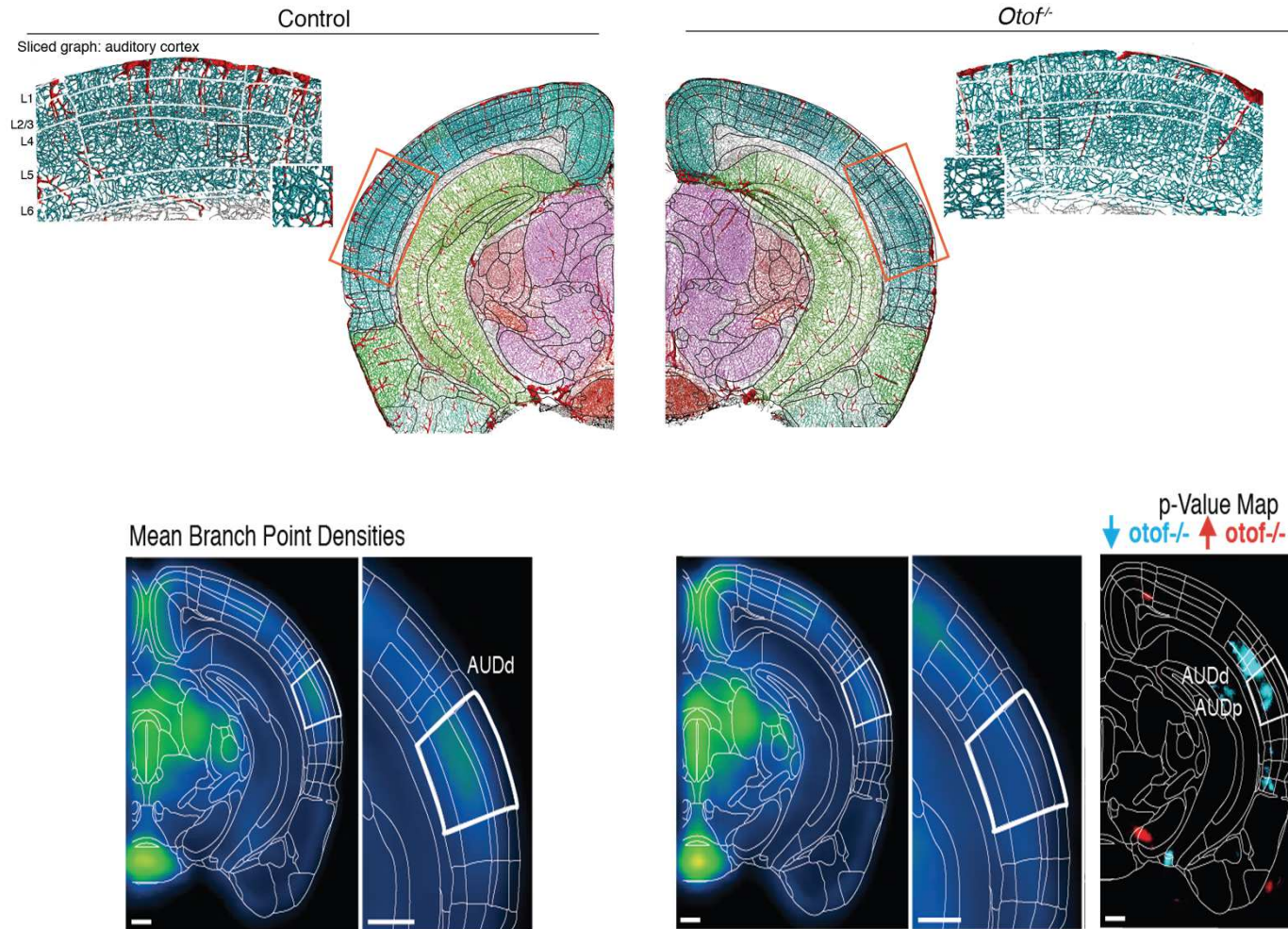


Vessel length density

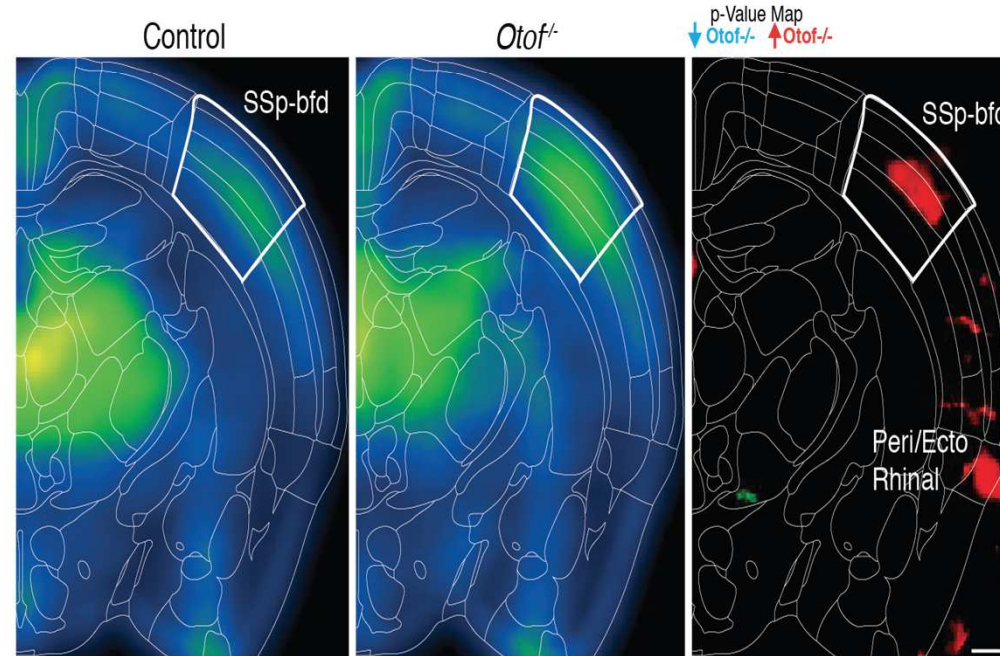




# Brain wide alterations to the vasculature in congenitally deaf mice



# Brain wide alterations to the vasculature in congenitally deaf mice



# iDISCO+ and ClearMap 1.0 Resources

- iDISCO+  
[www.idisco.info](http://www.idisco.info)
- Brain activity mapping:  
[www.github.com/ChristophKirst/ClearMap](https://www.github.com/ChristophKirst/ClearMap)
- Structure mapping:  
[www.github.com/ChristophKirst/ClearMap2](https://www.github.com/ChristophKirst/ClearMap2)

The image shows two overlapping web pages. The background page is the iDISCO+ website, titled "iDISCO method" with the subtitle "Resources for whole-mount immunostaining and volume imaging". It features a navigation menu with "HOME", "IDISCO PROTOCOL", "VALIDATED ANTIBODIES", "CLEARMAP", and "FAQ AND TROUBLESHOOTING". The main content area includes a "Home" section with a welcome message and a "July 2016 update" section listing "Training datasets for ClearMap" with a link to the "project page". A large 3D visualization of a brain with neural structures is visible on the left side of the page.

The foreground page is the "ClearMap iDISCO+ Toolbox Documentation" page. It has a navigation menu with "home", "search", and "documentation". The main content area is titled "ClearMap" and describes it as a toolbox for the analysis and registration of volumetric data from cleared tissues. It mentions that ClearMap is designed to analyze large 3D image stacks obtained with Light Sheet Microscopy of iDISCO+ cleared mouse brains. The page also includes a "Table Of Contents" with links to "Overview of ClearMap", "Installation", "Tutorial", "ClearMap Image Analysis Tools", "Roadmap", and "Issues". A "Quick search" box is located at the bottom right of the page.

[Renier\*, . Adams\*, Kirst\*, Wu\*, et al., **Cell** 2016]

[Kirst et al. **Cell** 2020]

# Acknowledgments

- Laboratory of Structural Plasticity, ICM Paris



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Thomas Topilko



Alba Vieites Prado

pipeline development:



Sophie Skriabine



Paul Bertin

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