I big data nella microscopia ad alta risoluzione per la mappatura del cervello

Giacomo Mazzamuto

Databeers Tuscany
4 ottobre 2023 – Firenze

Microscopy Imaging: acquisition and processing (old days)

Sample

Image acquisition

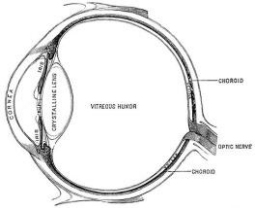
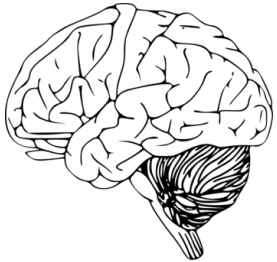


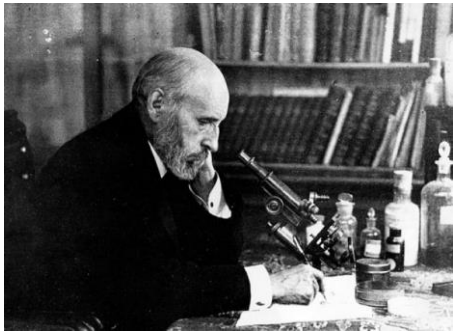
Image processing



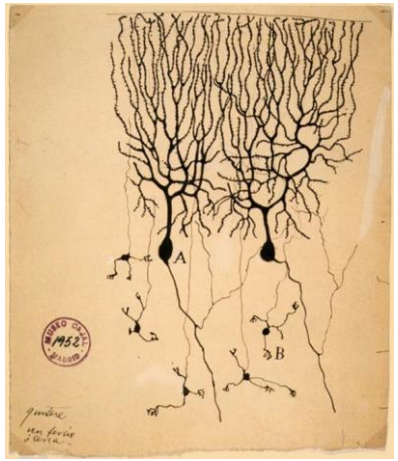
Visualization and storage

Camillo Golgi

1906



Santiago Ramón y Cajal



Microscopy Imaging: acquisition and processing (today)

Sample

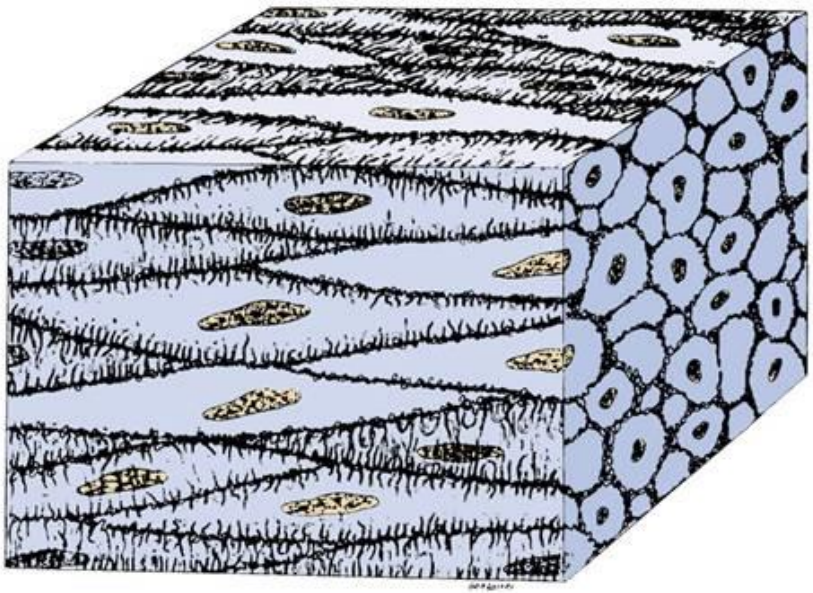
Image acquisition

Storage

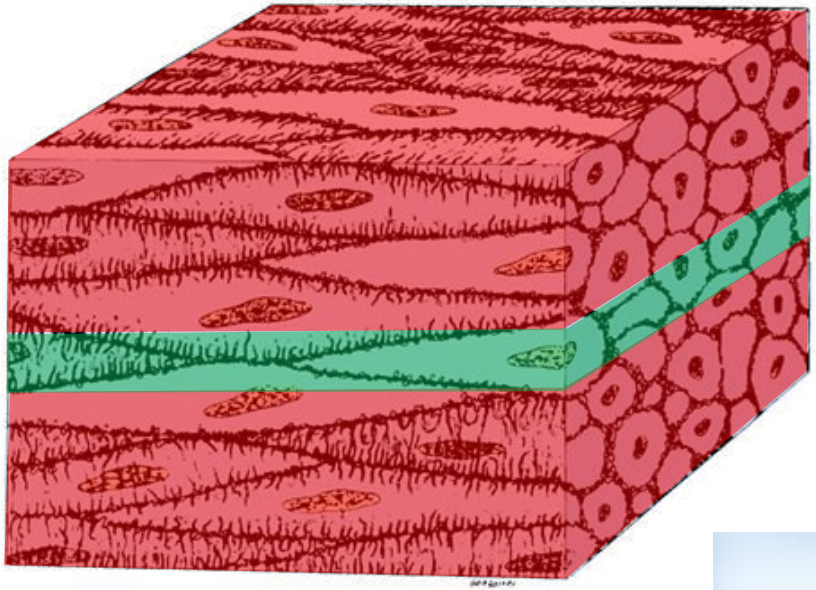
Visualization

Image processing

Biological samples are inherently 3D…



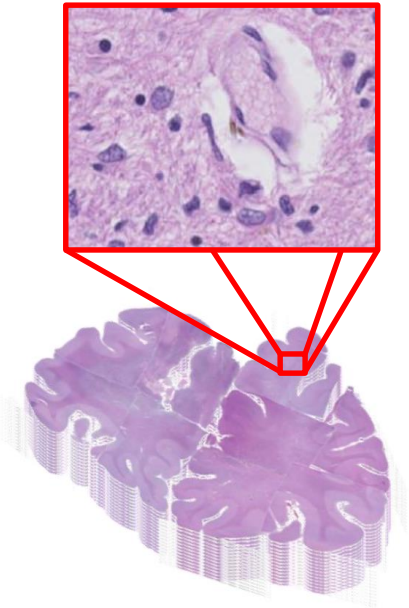
Physical sectioning

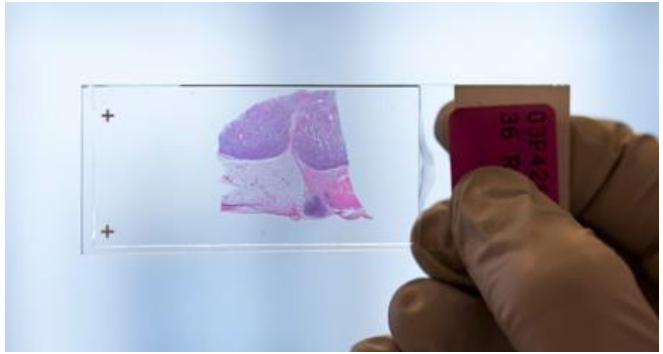


Out of focus

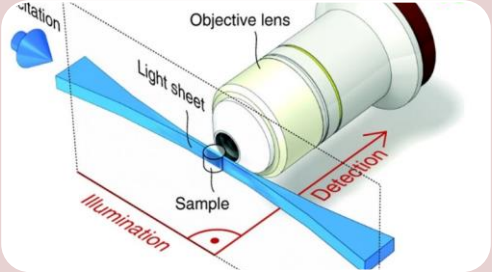
In focus

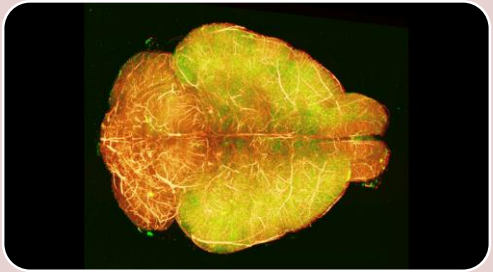
Out of focus





The three pillars of selective plane illumination microscopy





Tissue preparation:

optical clearing

Instruments:

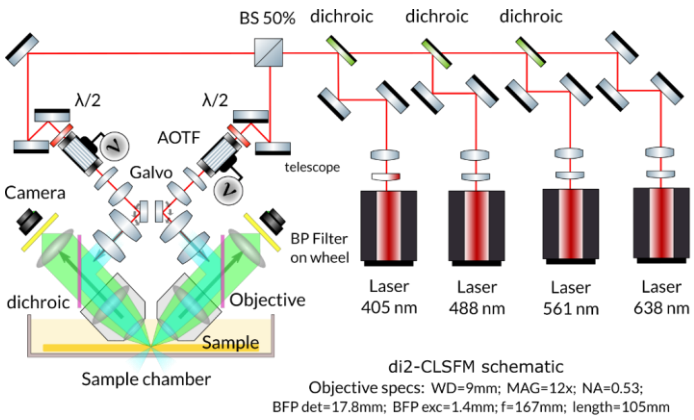
Light-Sheet Microscopy

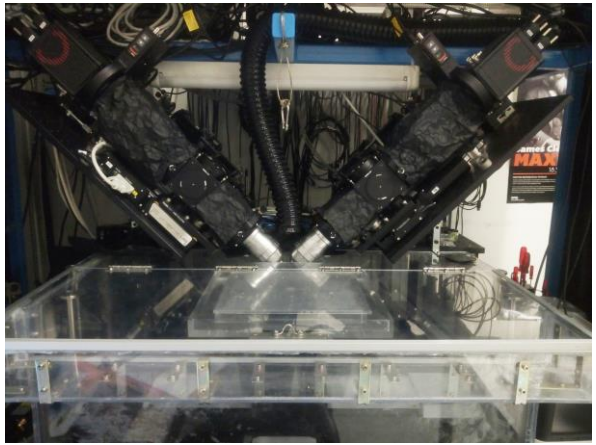
Image processing

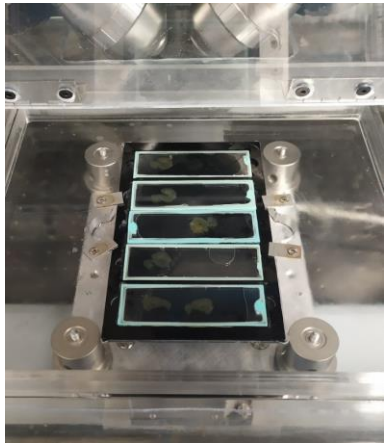
&

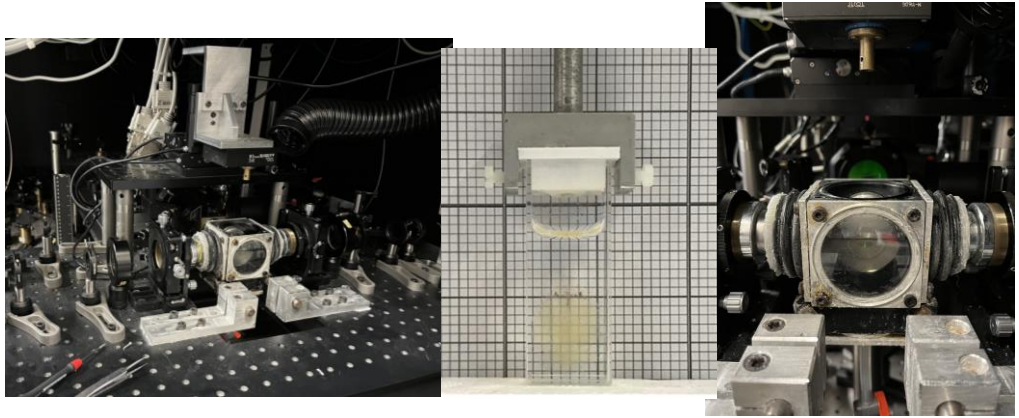
Data analysis

Dual view inverted Light Sheet Fluorescence Microscope







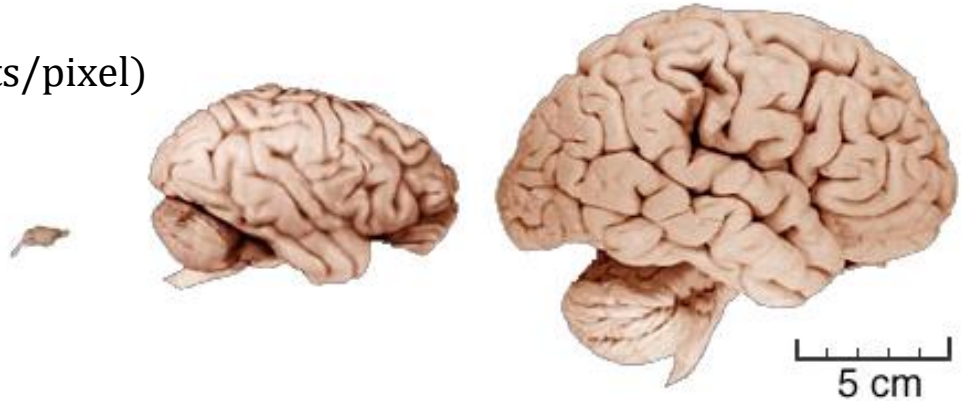


- dual-view inverted SPIM apparatus
  - Isotropic 1 um resolution
  - Up to 4 channels
  - Designed to image 1 mm thick sample
  - High data throughput (up to 1 GB/s, one channel)
  - Volumetric rate: 0.1 mm^3/s.

Big data challenges in whole-brain imaging

		Mouse	Monkey (Rhesus)	Human
Approx volume		1 cm^3	100 cm^3	1500 cm^3
Raw data size	1 channel, 10 µm res.	2 GB	200 GB	3 TB
	3 channels, 10 µm res.	6 GB	600 GB	9 TB
	1 channel, 1 µm res.	2 TB	200 TB	3000 TB
	3 channels, 1 µm res.	6 TB	600 TB	9000 TB

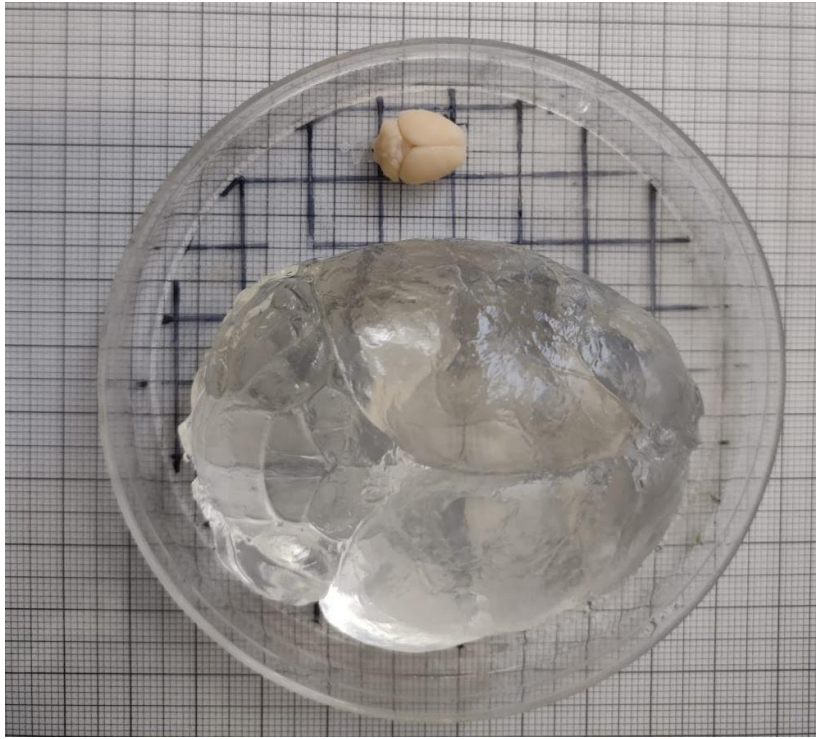
$$\frac{1\ \mathrm{cm}^3}{(10\ \mu\mathrm{m})^3} = 10^9\ \mathrm{voxels} = 2\mathrm{GB}\ \ (@\ 16\mathrm{bits/pixel})$$

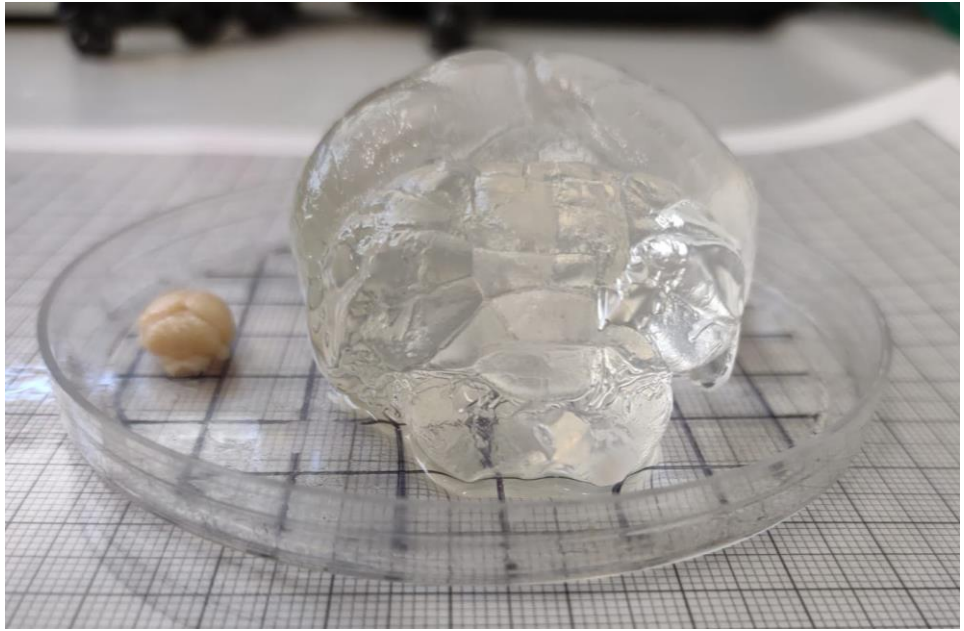


mouse monkey human

Expansion Microscopy

- Pioneered by Fei Chen, Paul W. Tillberg and Edward S. Boyden
  Science, 30 Jan 2015 Vol 347, Issue 6221 pp. 543-548



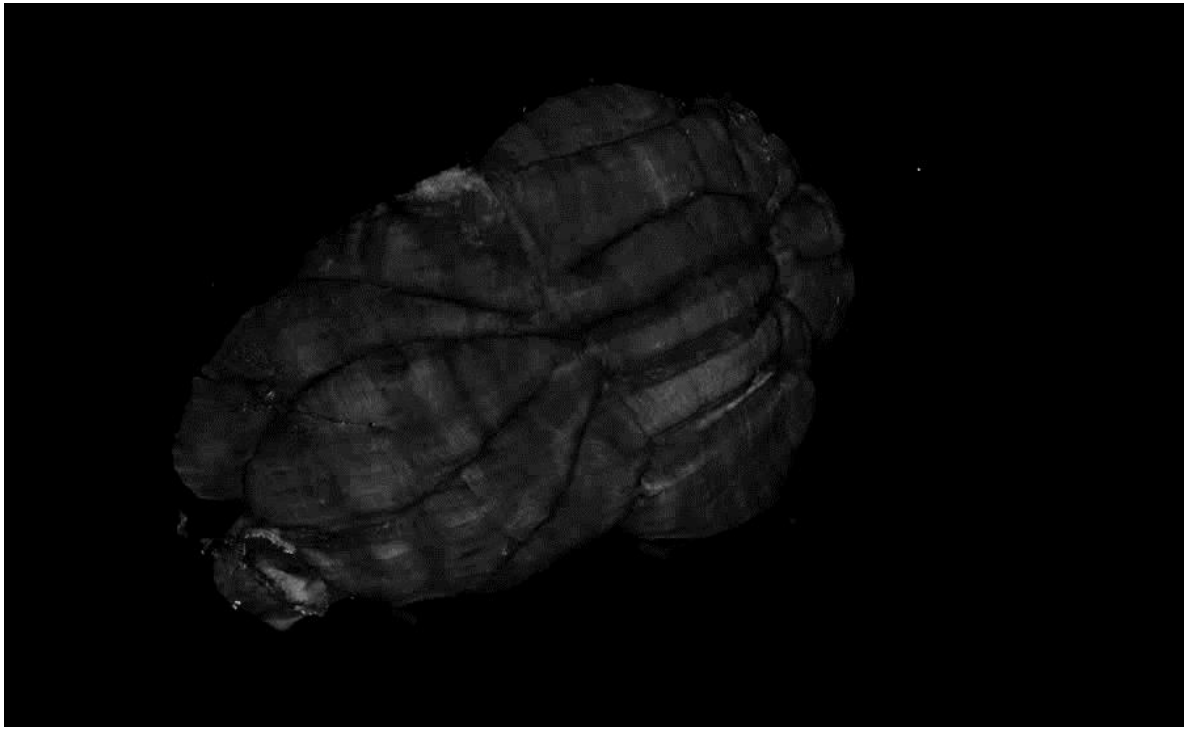


Niamh Brady, Marta Rojas

Light-sheet microscopy

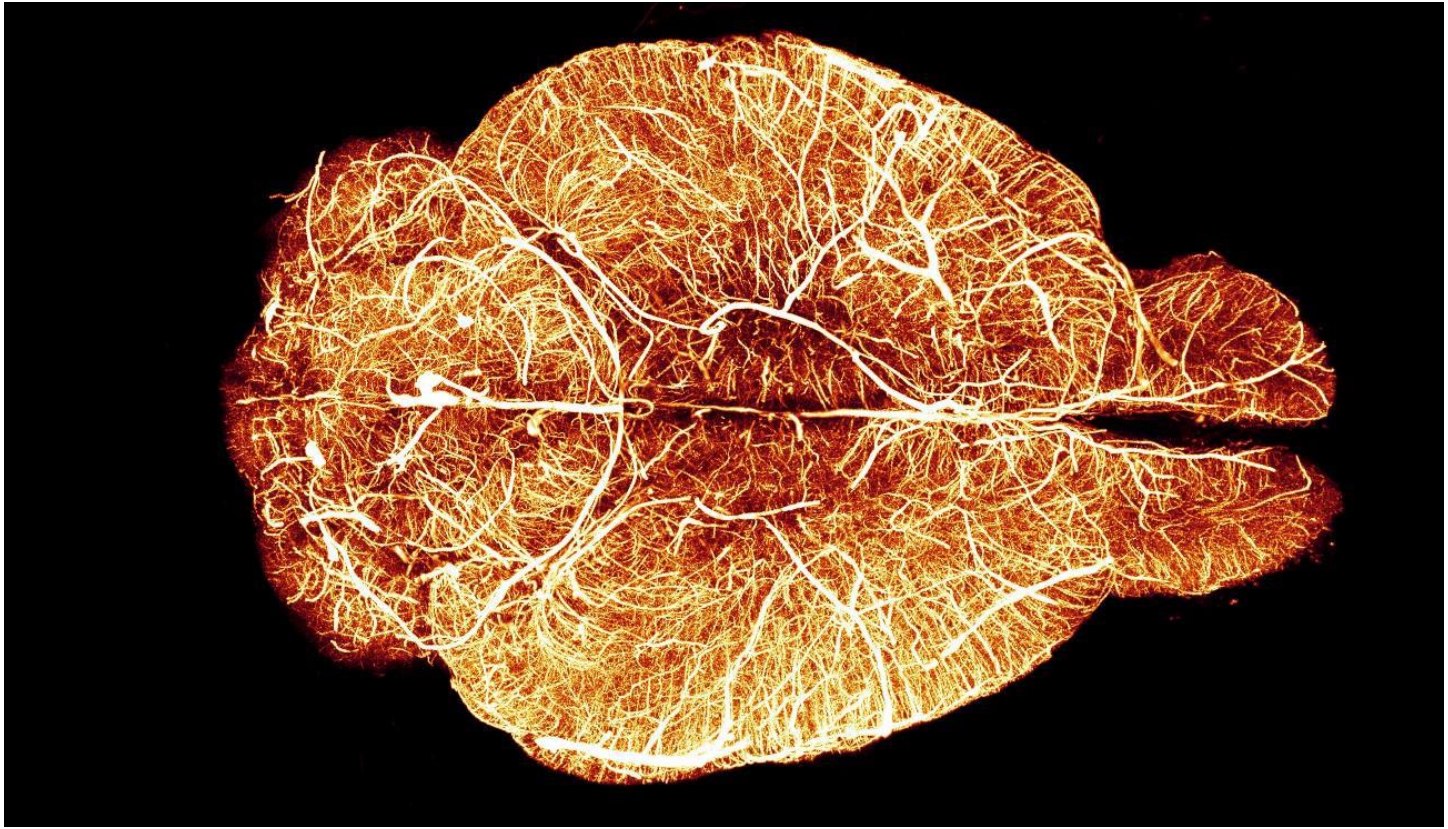
Very high throughput (camera-limited)
Very low photobleaching

Suitable for imaging of large volumes, and for long-term volumetric imaging



Whole cerebellum of a mouse brain

Whole mouse brain tomography with LSFM: vasculature

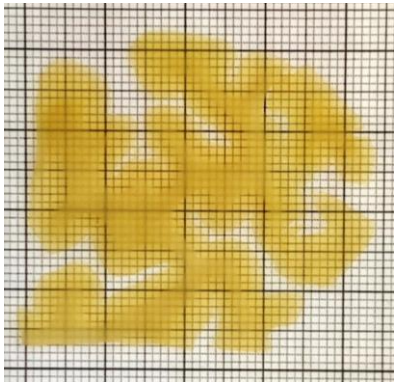


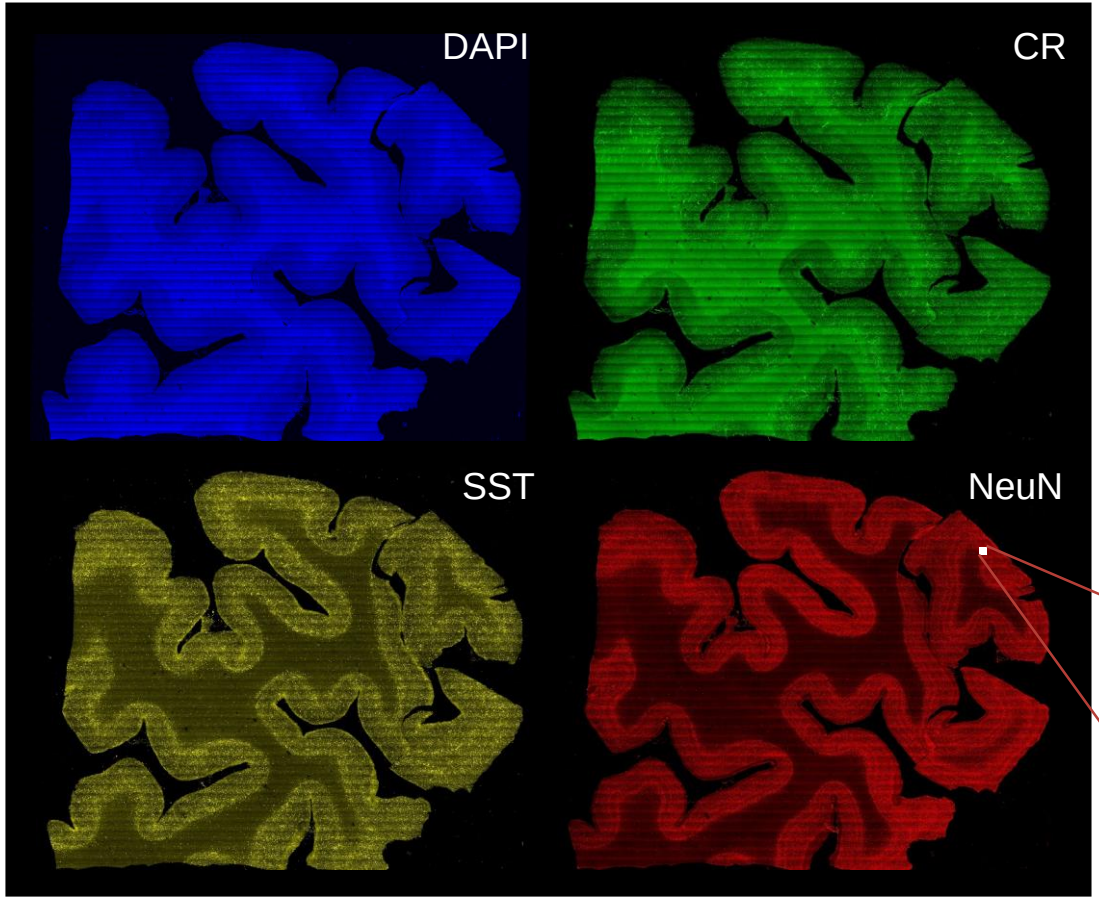
A.P. Di Giovanna et al, Scientific Reports volume 8, Article number: 12573 (2018)

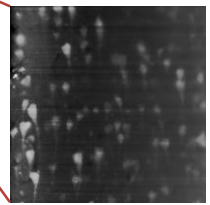
A bigger challenge: human Broca's area (4x4x2cm^3, 48 slices)

Fixed tissue

Cleared tissue



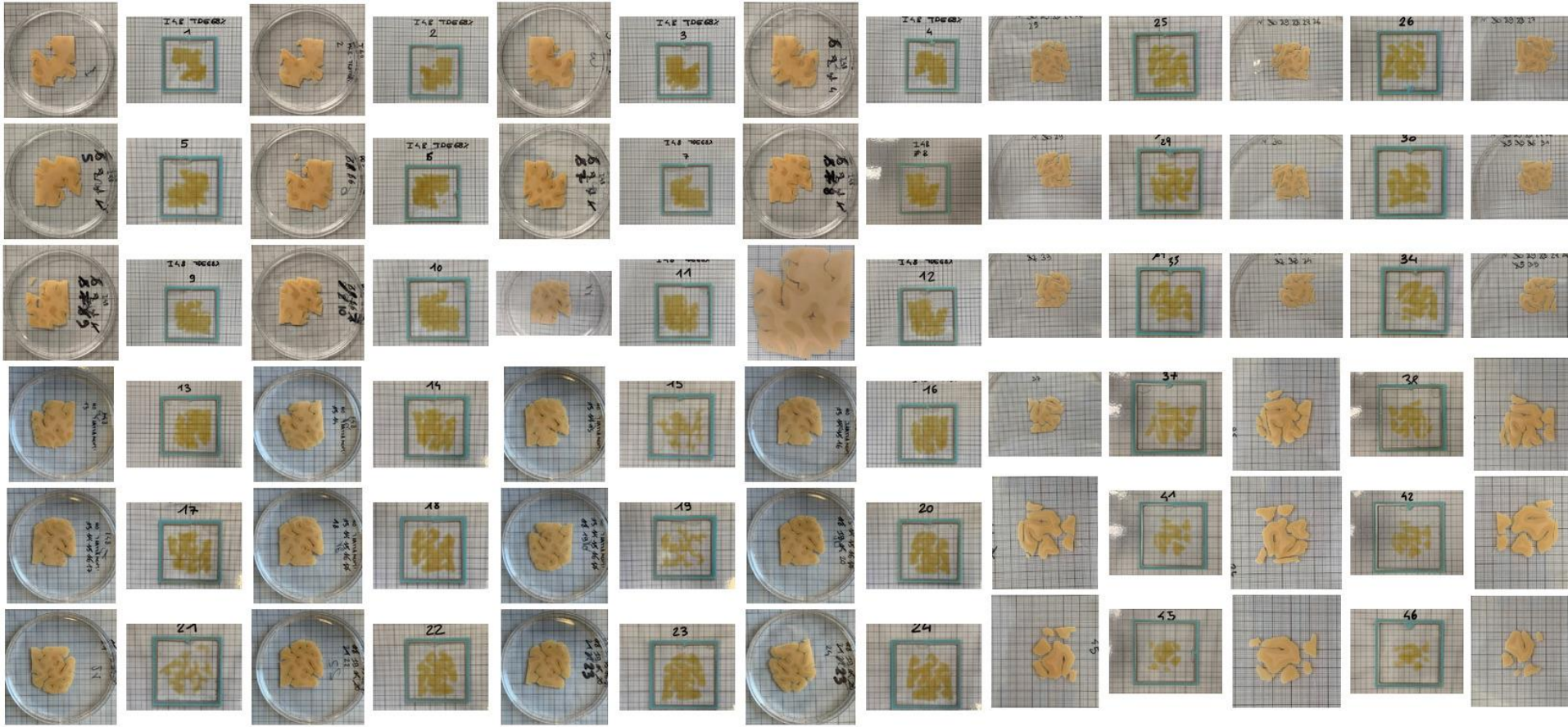




Brain Initiative Cell Census Network (BICCN)

Supported by The General Hospital Corporation Center of the National Institutes of Health, award number 1U01MH117023-01

A bigger challenge: human Broca's area (4x4x2cm^3, 48 slices)



Some numbers

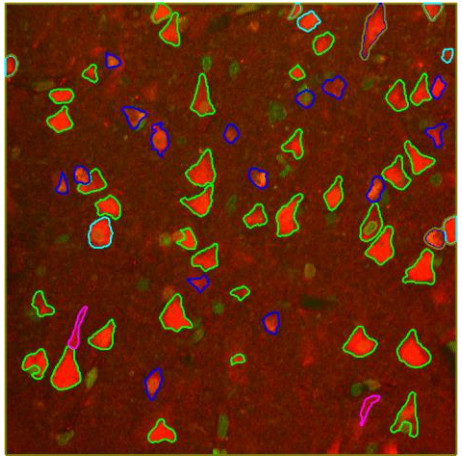
August 2021

4 channels, 48 slices

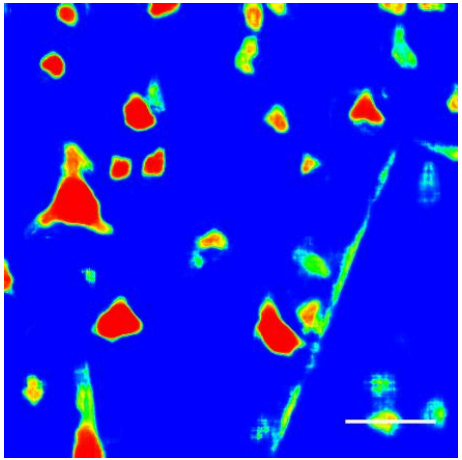
Number of files	8602
Raw data	507 TiB
Lossy Compression, JPEG2000, original resolution (0.5x0.5x3.5µm)	25 TiB
Resliced (lower res, 3.5µm isotropic)	10.4 TiB
Resliced (lossless compression, 3.5µm, delivered)	4.2 TiB

Neuron segmentation in the human cortex

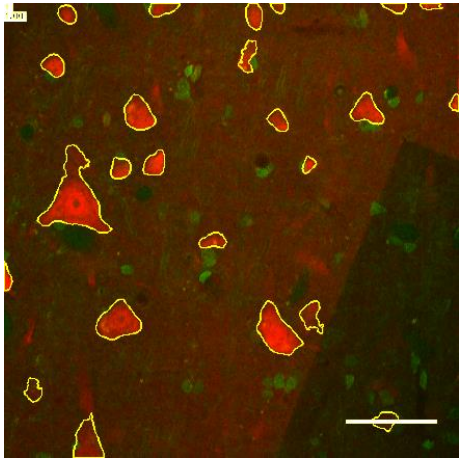
Manual annotation (ground truth)



Probability heatmap



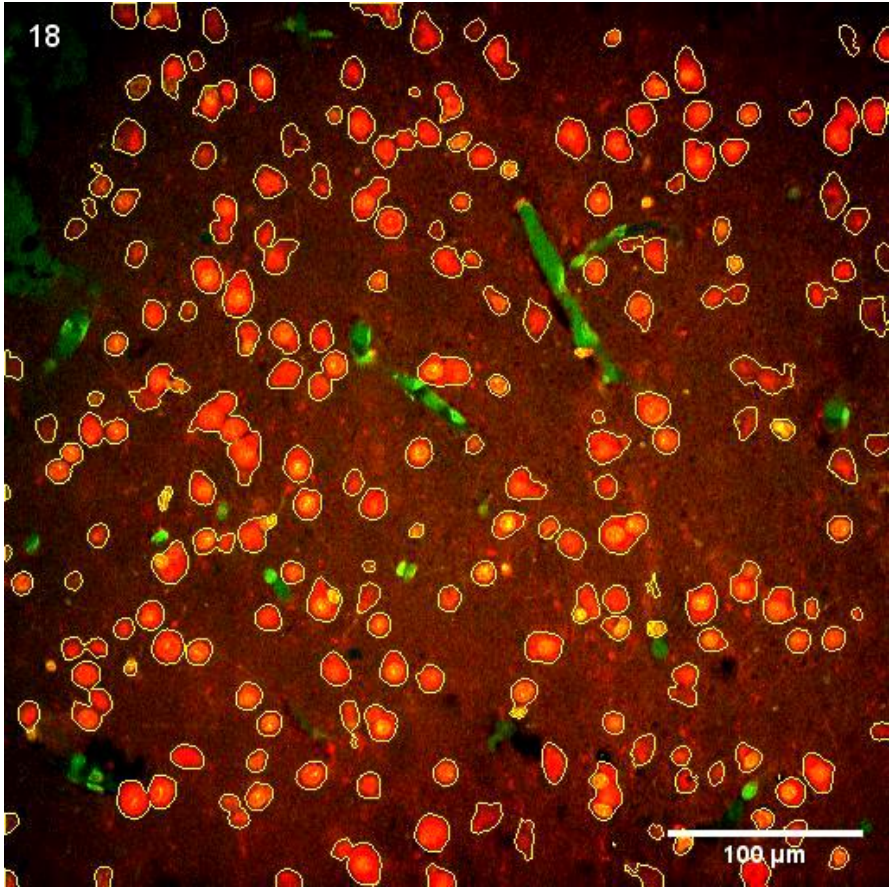
Automatic segmentation



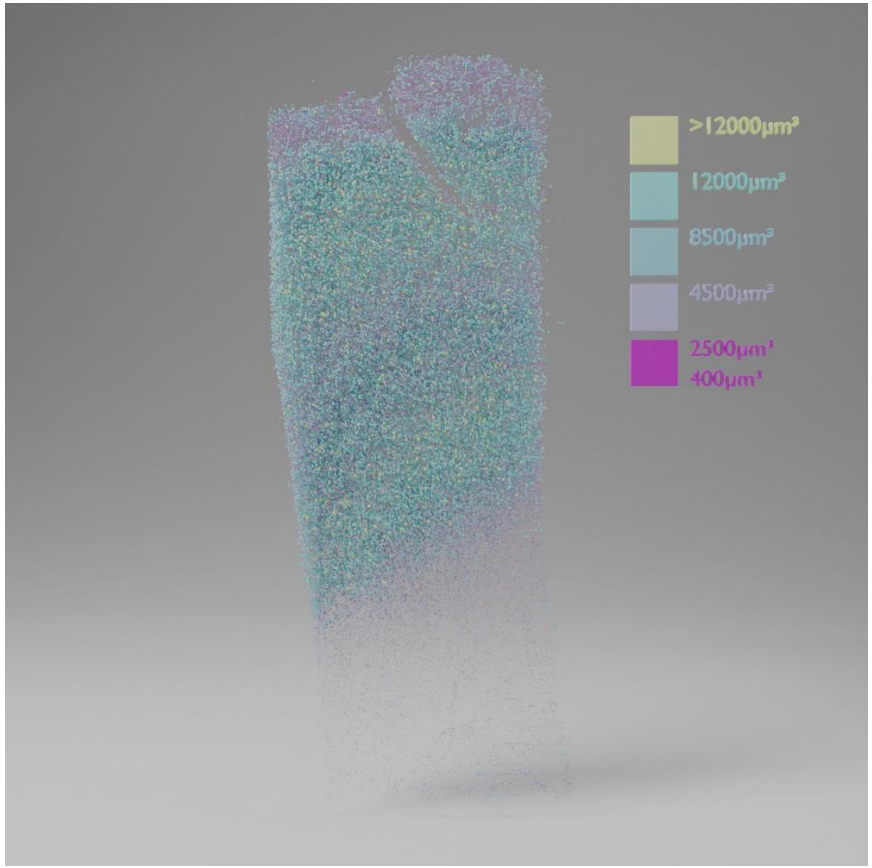
Supervised convolutional network on 2D images

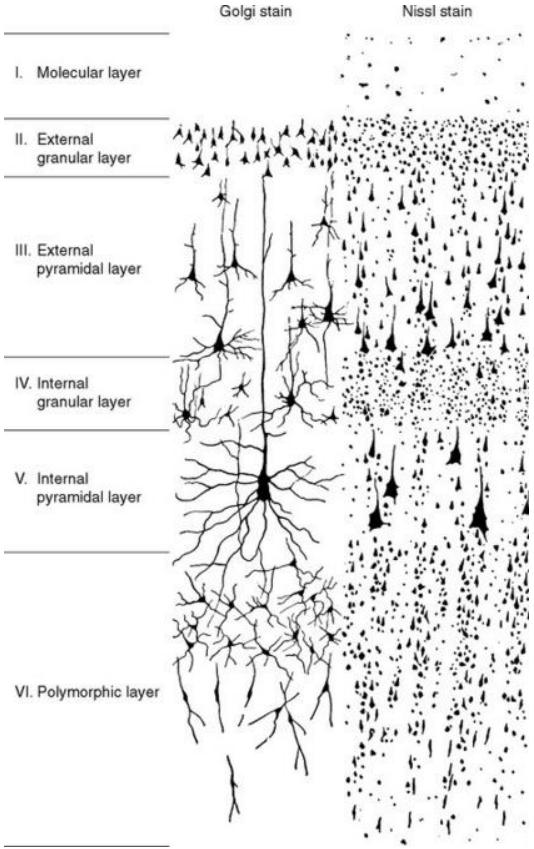
Contour finding

Contours



3D digital histology

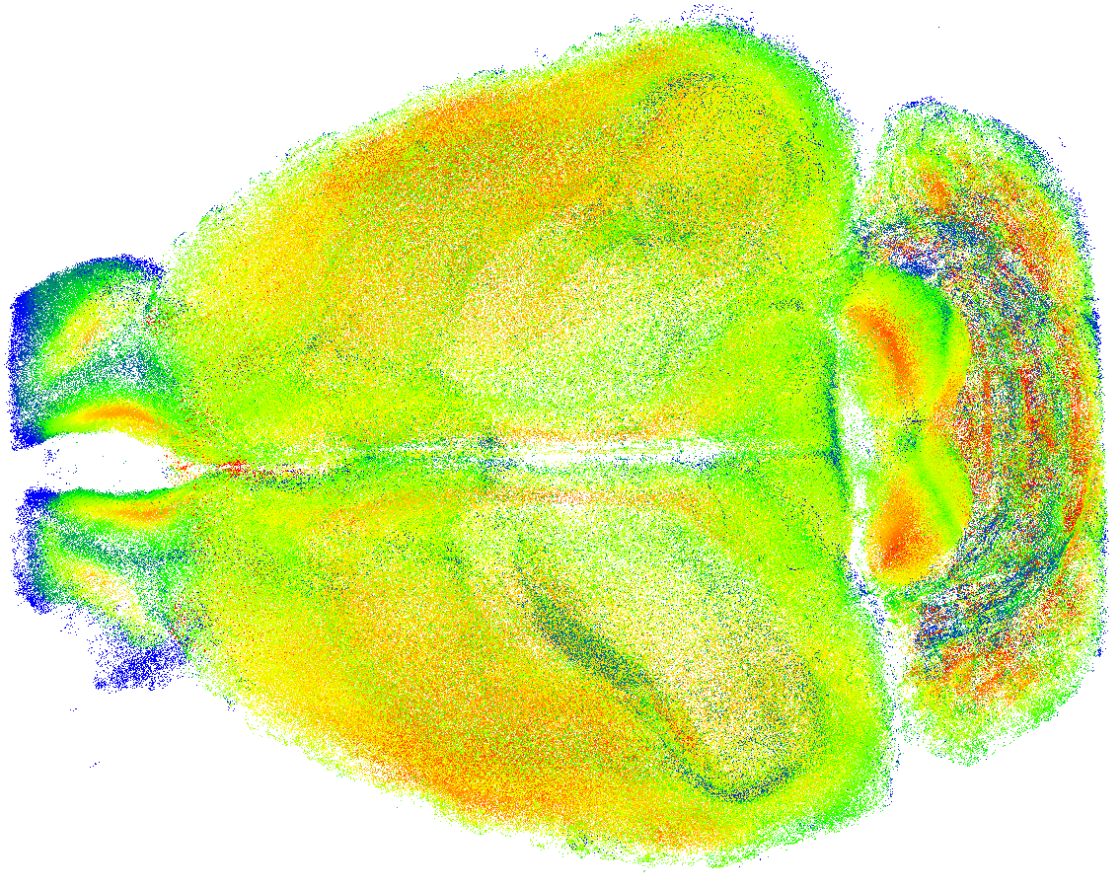




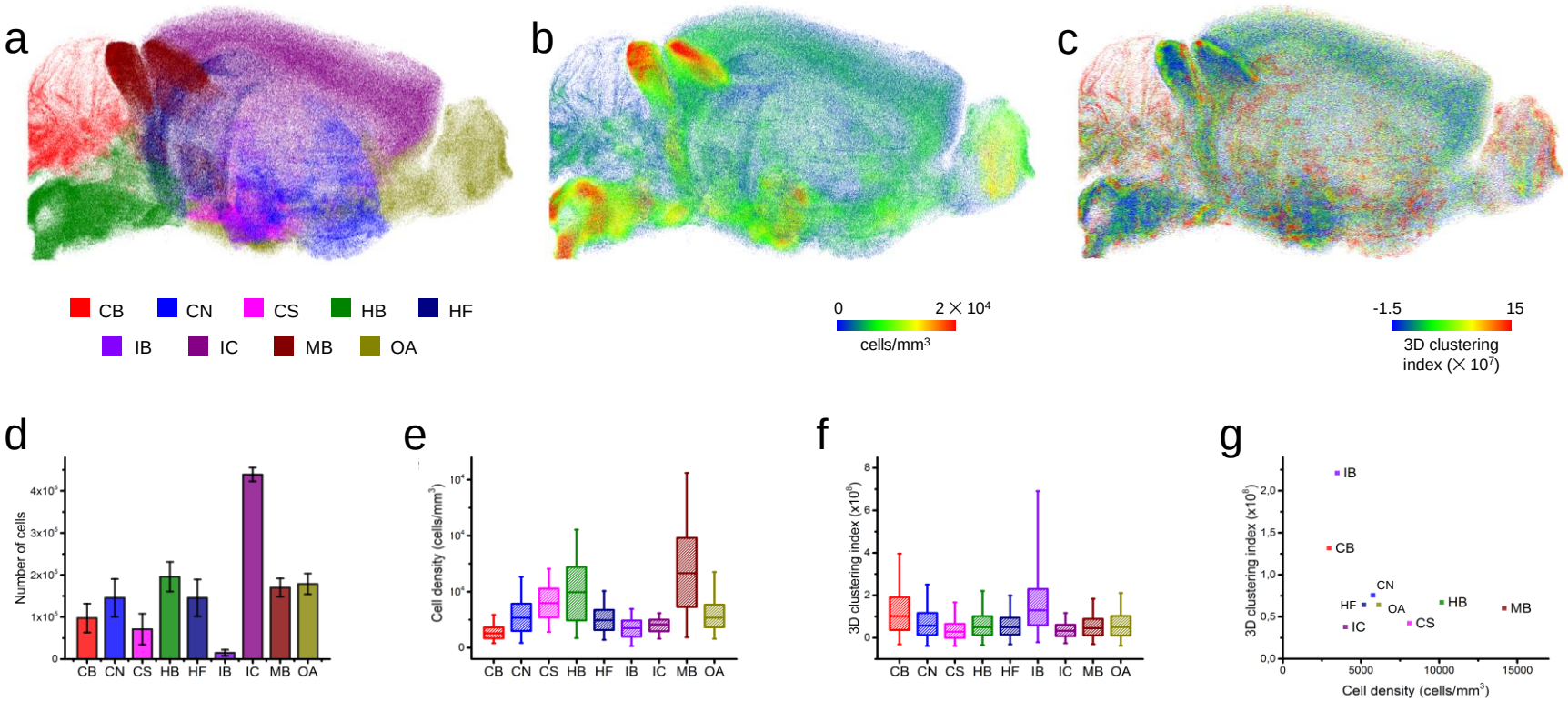
Whole-brain neuron localization

SST-positive cells

About 2.3×10^6 neurons
Precision 80%
Recall 95%



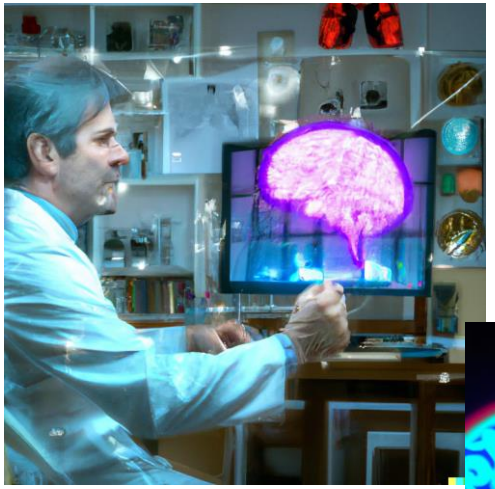
Quantitative data analysis

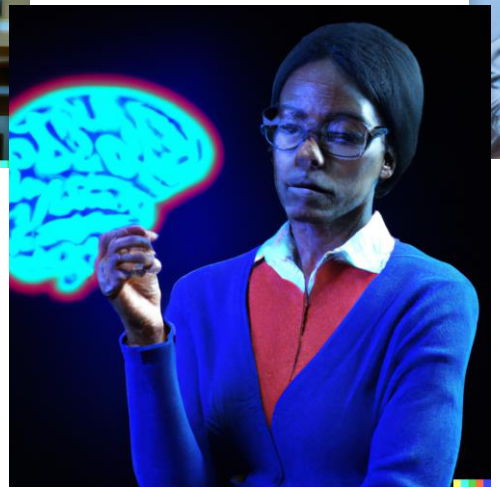


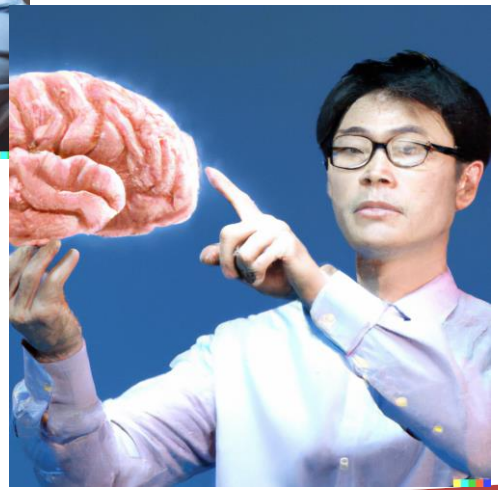
Silvestri, L. *et al. Nat Methods* **18,** 953–958 (2021).

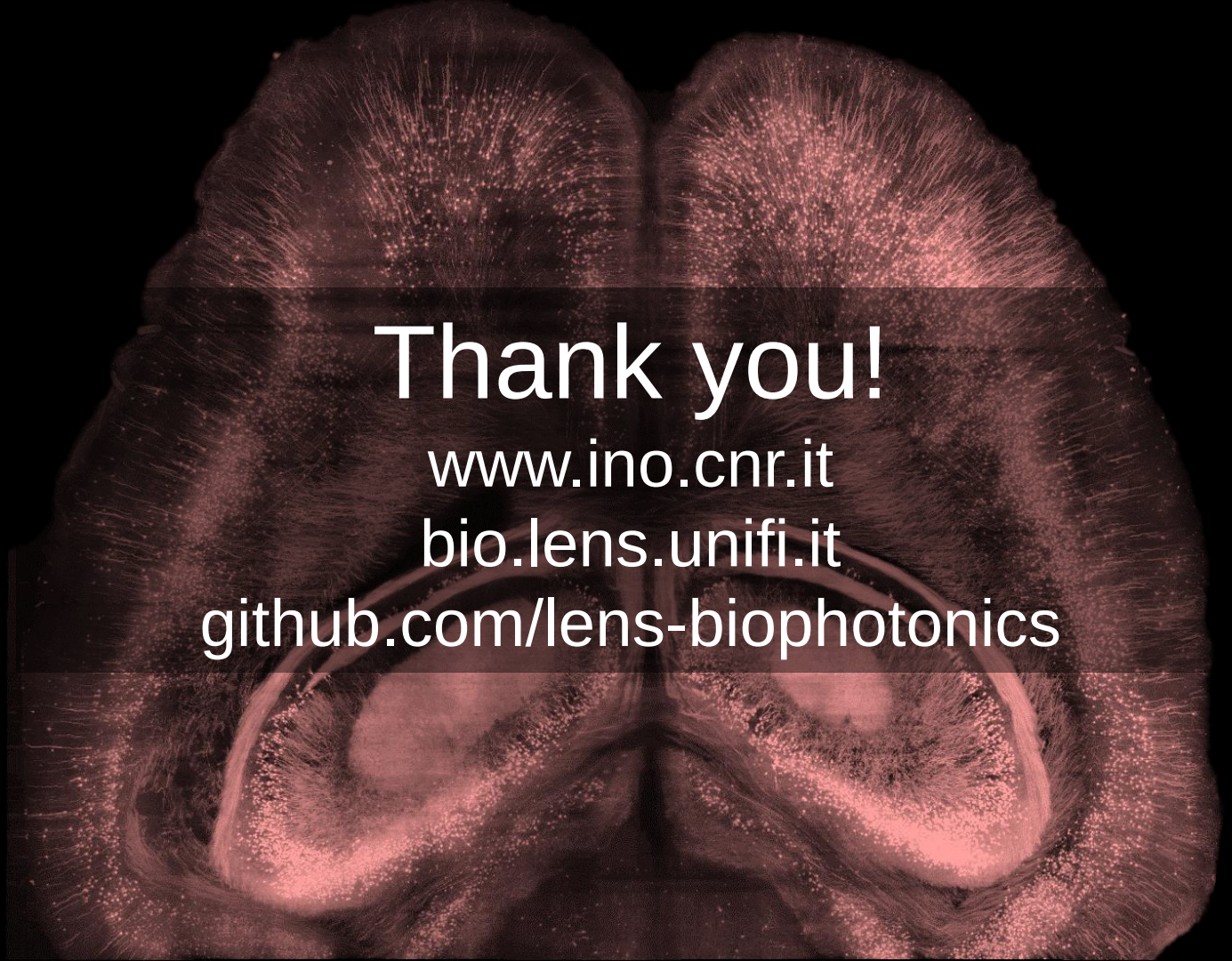
OpenAI DALL·E2

“Neuroscientists finally discover how the human brain works”









Thank you!

www.ino.cnr.it

bio.lens.unifi.it

github.com/lens-biophotonics

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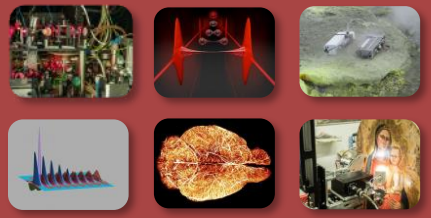
ISTITUTO NAZIONALE DI OTTICA

CONSIGLIO NAZIONALE DELLE RICERCHE



ALLIES of LIGHT

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