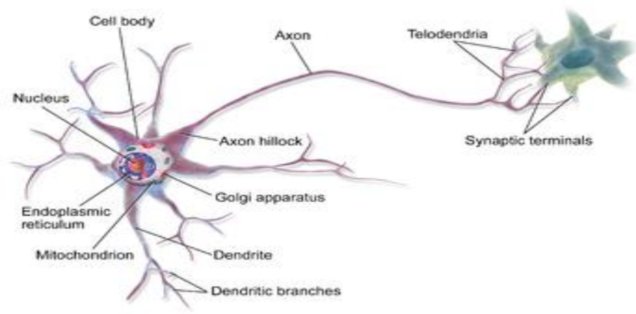
Mouse Brain Connectivity Atlas

ALLEN **Mouse Brain Connectivity Atlas**



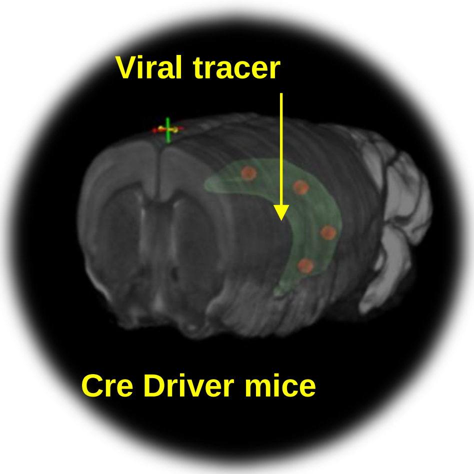
Stereotaxic Injections
(300-500 brain regions, >100 cell-type specific Cre mice)

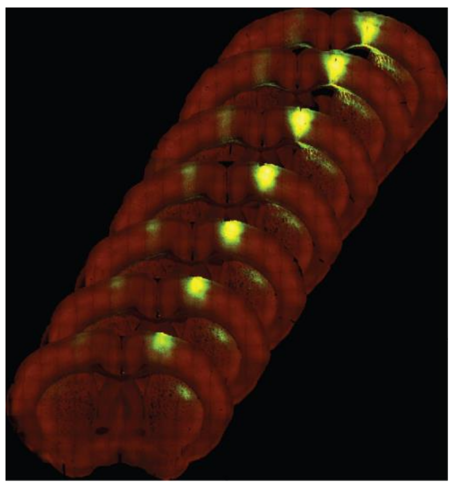
→

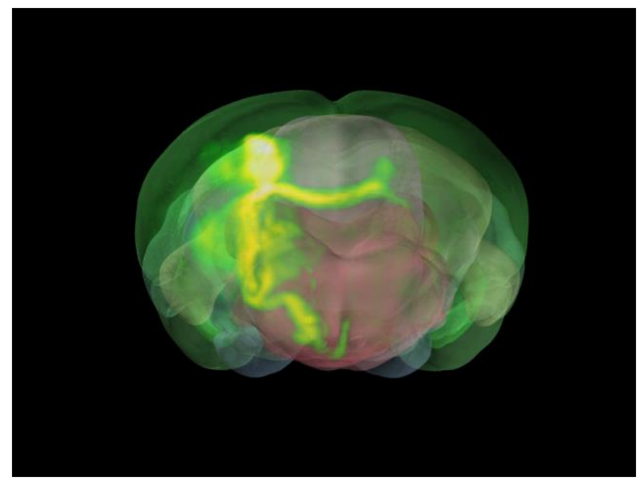
High-throughput Fluorescence Imaging

→

3D Connectivity Map & Model







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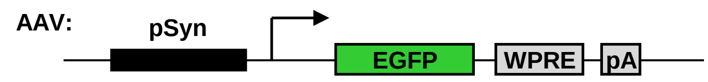
Components of the Connectivity Atlas

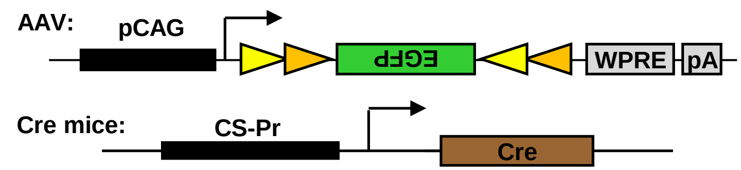
Phase I: Regular rAAV as tract tracer (non-Cre-dependent)

- Mapping all axonal projections from injection sites (300-500 sites covering the entire brain)
- Comparison with conventional tracer BDA



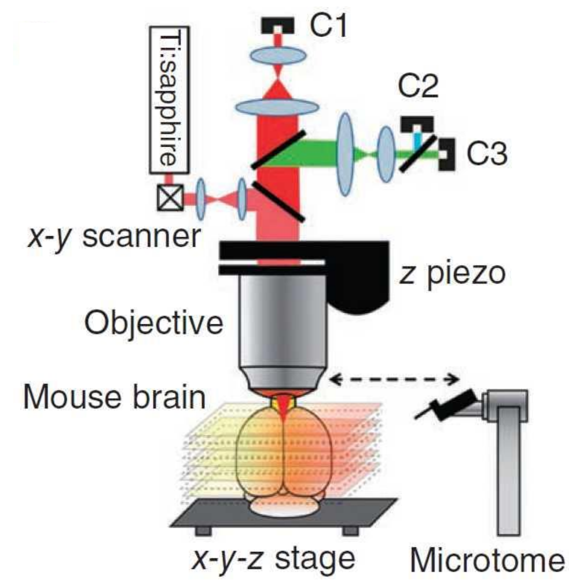
Phase II: Cre lines + Cre-dependent rAAV

- Cell-type-specific mapping of projections from injection sites
- Use >100 Cre lines



Imaging: Serial Two-Photon (STP) Tomography

- Automated two-photon serial imaging coupled with vibratome sectioning
- Whole-brain fluorescent scanning at sub-µm resolution of coronal cross-sections
- 100 µm overall sampling density between sections
- Maximized signal-detection sensitivity
- Throughput: imaging 1 brain per microscope per day



Ragan, … Osten, 2012

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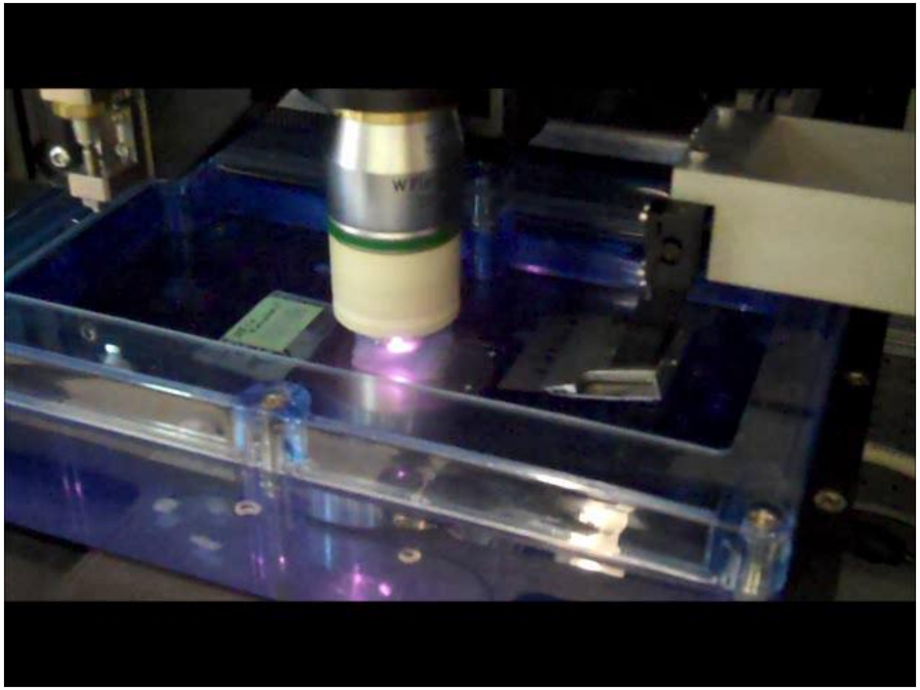
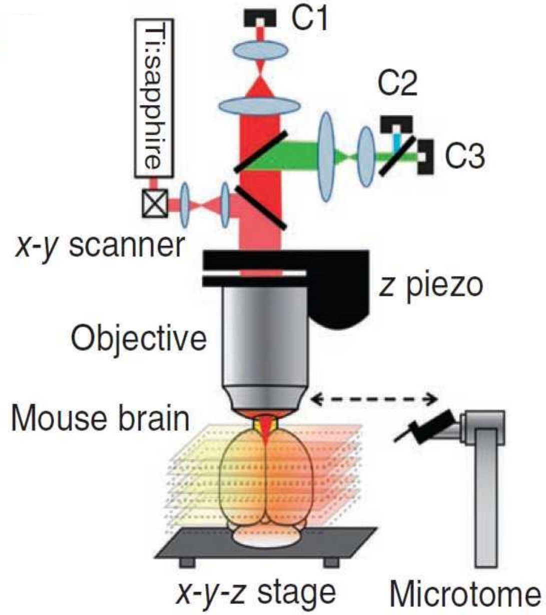
Allen Mouse Brain Connectivity Atlas



TissueCyte imaging system

Serial Two-Photon (STP) Tomography



Ragan, … Osten, 2012

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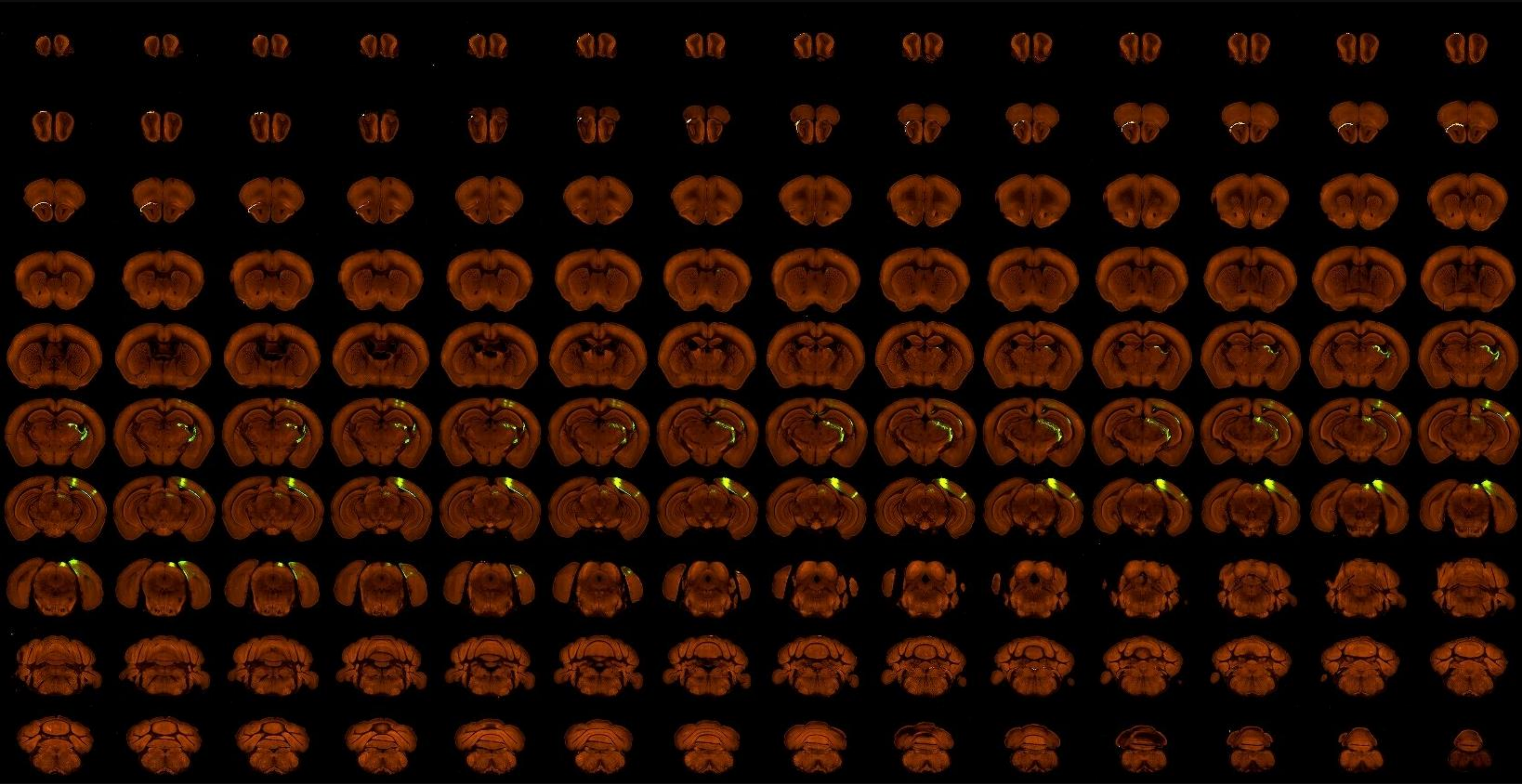
A Single Connectivity Experiment



hSyn-EGFP-WPRE injection VISp, 21 day survival.
140 serial 100 μm vibratome sections, imaged with 2P at 20X, one optical section (z) per slice.
TissueCyte1000, TissueVision (Ragan et al., 2012, Nature Methods)

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Data included in the Connectivity Atlas

Experimental Image Series (140 coronal images)

- 1772 Projection experiments
- 70 BDA/rAAV double injection experiments

Manual Annotation

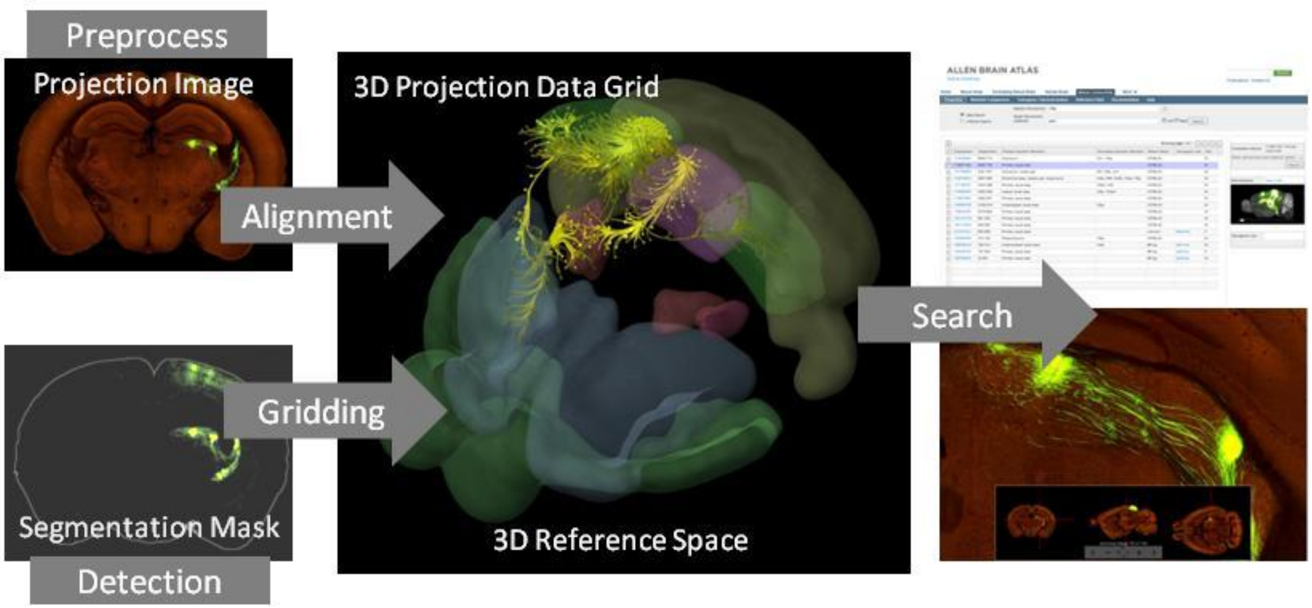
- Injection sites

Reference Datasets

	Staining Type	Stain	Target	Staining Pattern
	Histological staining	Nissl	Nissl substances	Pan-cellular, cell bodies and dendrites
	Histological staining	AchE	Synaptic protein Acetylcholinesterase	Axons and cell bodies of cholinergic neurons
	Immunohistochemistry	Pvalb	Ca2+ binding protein Parvalbumin	Cell-type marker: a subset of GABAergic interneurons (fast-spiking)
		SMI-32	Neurofilament-H (non-phosphorylated)	Cell bodies, dendrites and thick axons
	Immunohistochemistry	Calb1	Ca2+ binding protein Calbindin, 28 kDa	Cell-type marker: a subset of GABAergic Interneurons
		SMI-99	Myelin basic protein	Myelinated axons, oligodendrocytes
	Immunohistochemistry	NeuN	Nuclear protein Fox3	Cell-type marker: Pan-neuronal
		NF-160	Neurofilament-M	Cell bodies, dendrites and axons

Allen Mouse Brain Connectivity Atlas

Registration of Fluorescent Data to a Reference Atlas



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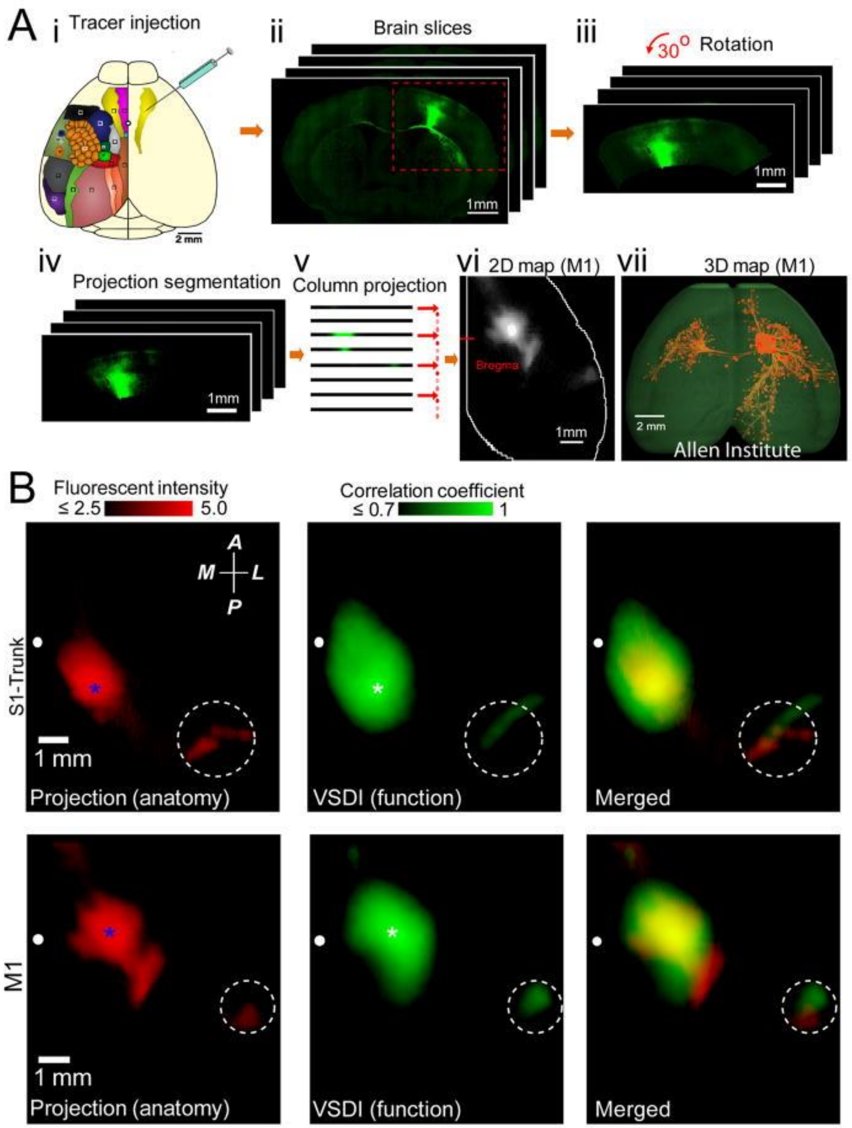
Using the Connectivity Atlas

Inspecting specific experiments (biologists)

- Source Search – Injections by structure
- Target Search – Injections by structure AND high signal density in a target structure
- Spatial Search – Injections/Signal by voxel
- Brain Explorer
- Quantified Projection Signal Summary
- High Resolution Image Viewer
- Composite Projection Viewer

[Allen Mouse Brain Connectivity Atlas]

From our users…

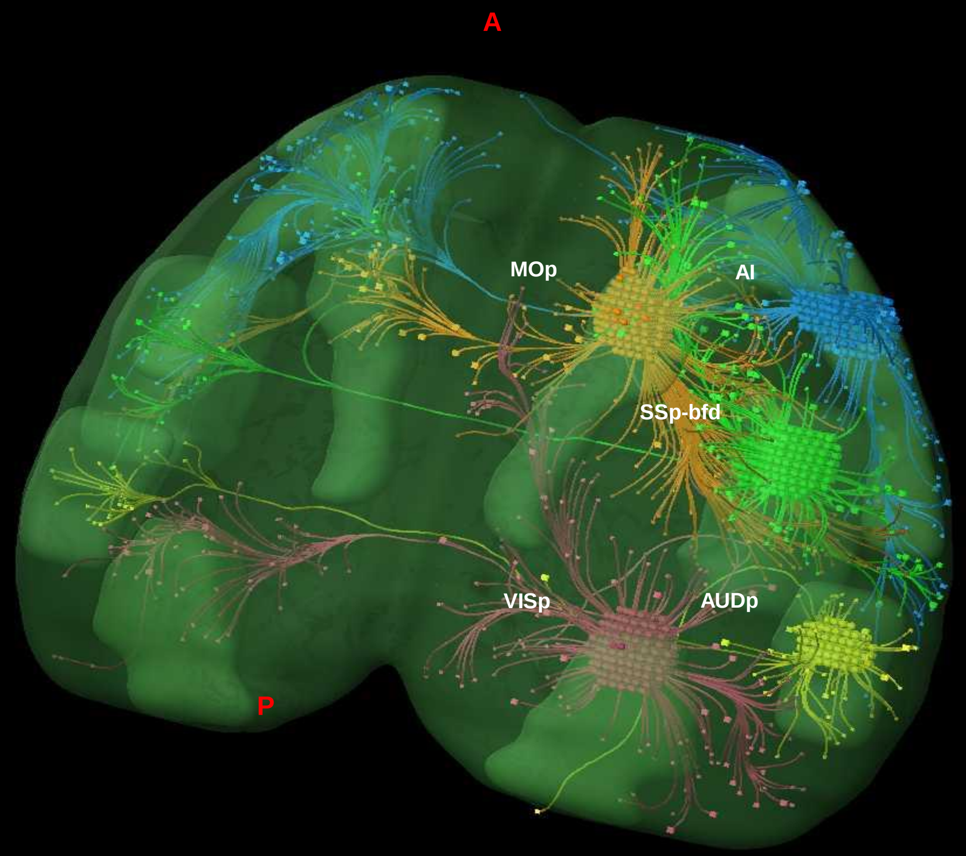


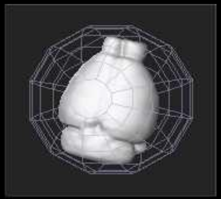
Spontaneous cortical activity alternates between motifs defined by regional axonal projections

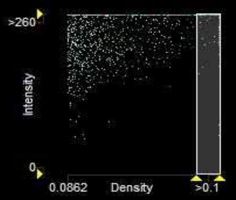
Majid H. Mohajerani, et. al.

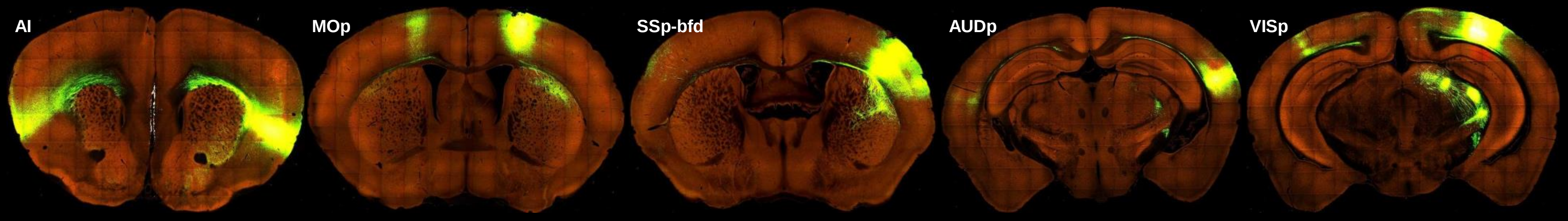
Nat Neurosci. 2013 October ; 16(10): 1426–1435.

Cortical Projection Map

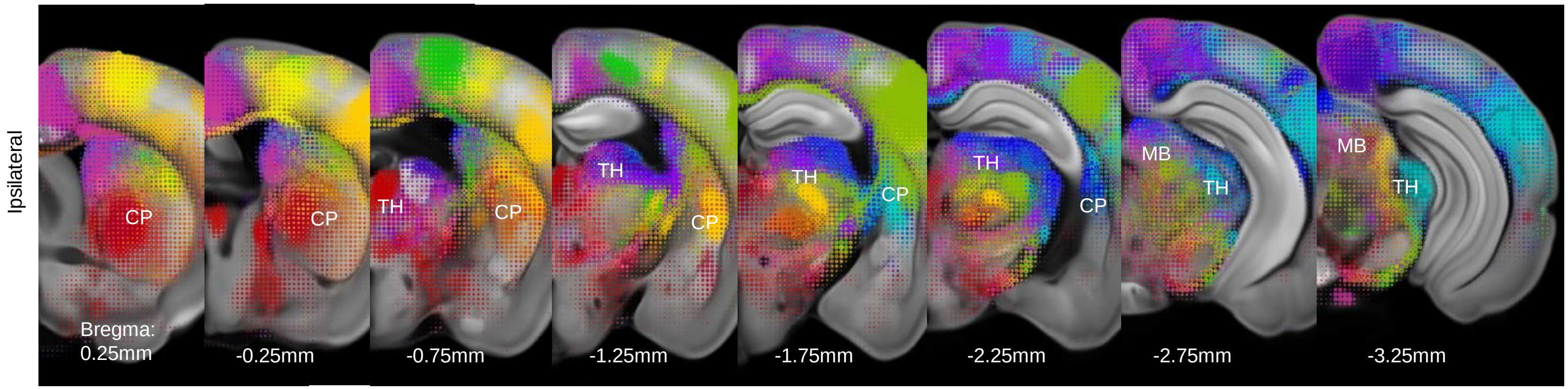




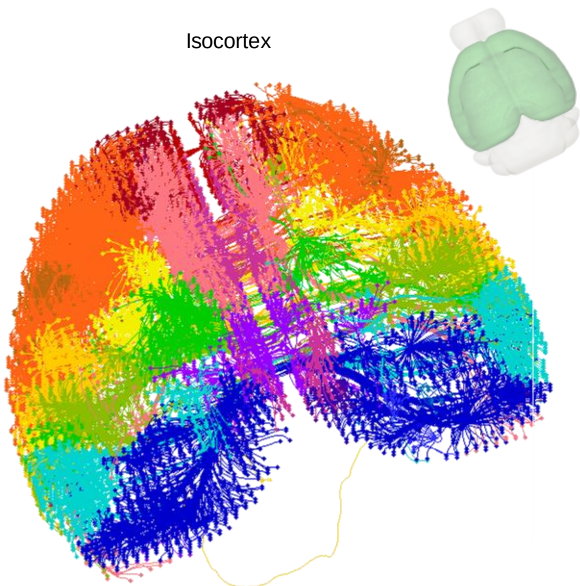




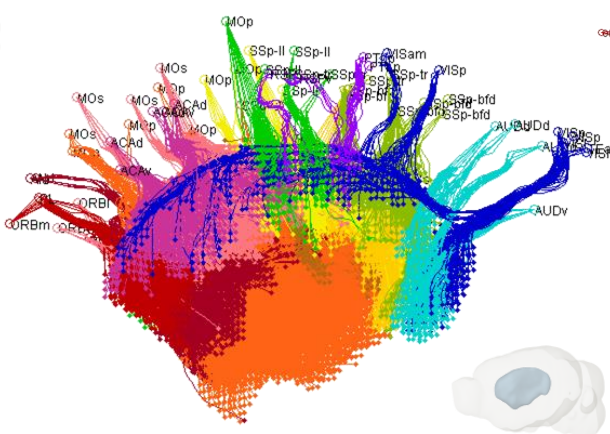
Topography of Cortico-subcortical Projections



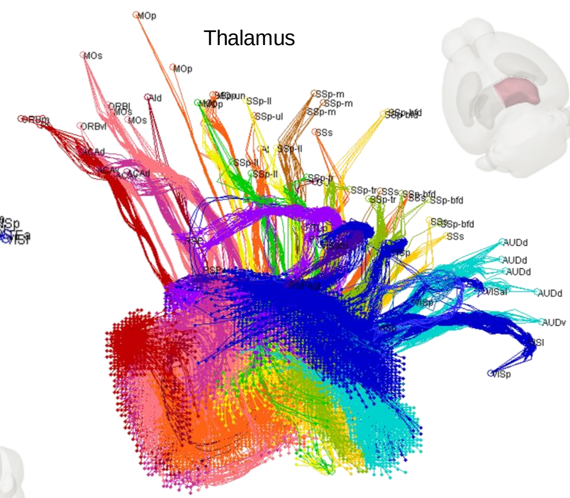
Isocortex



Caudoputamen



Thalamus



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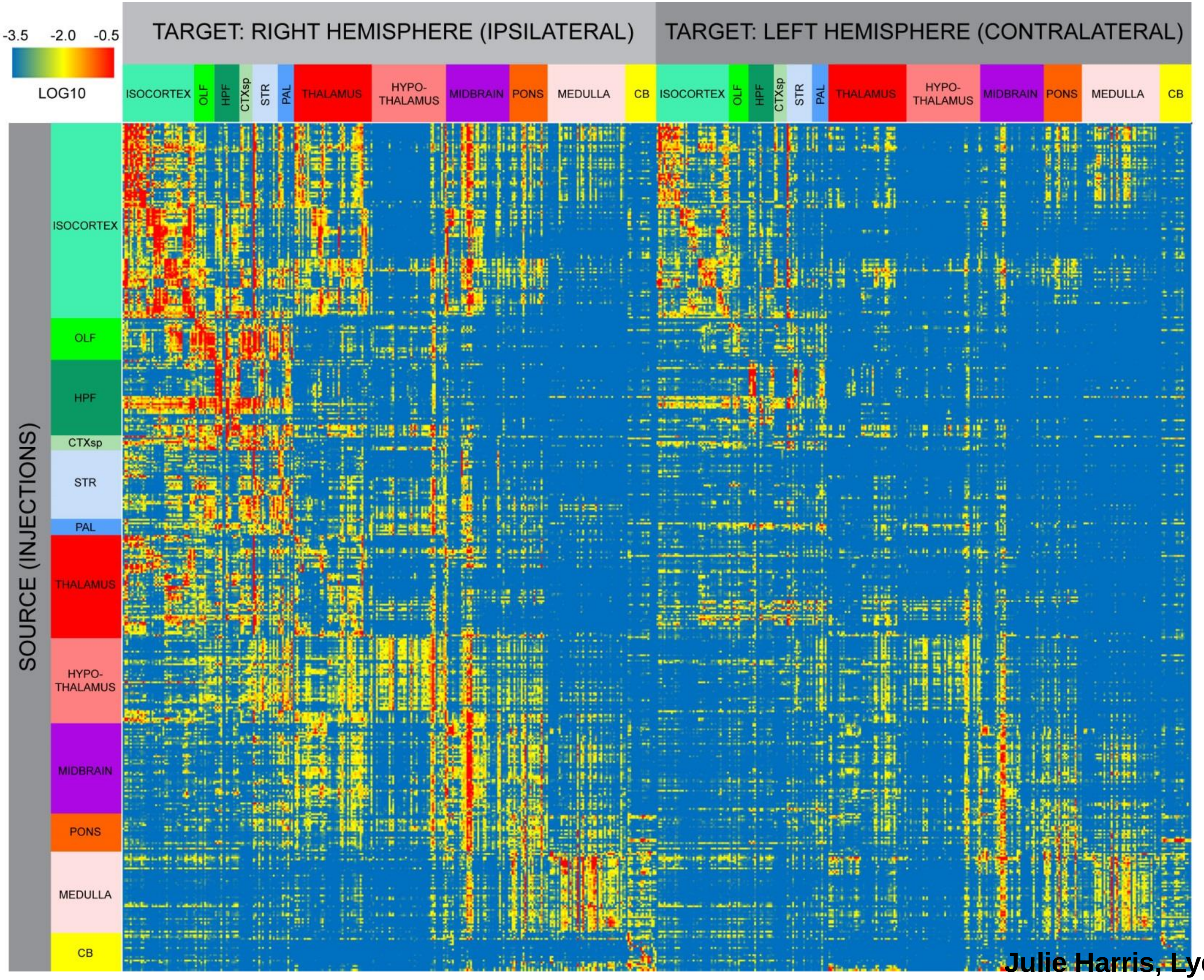
Lydia Ng

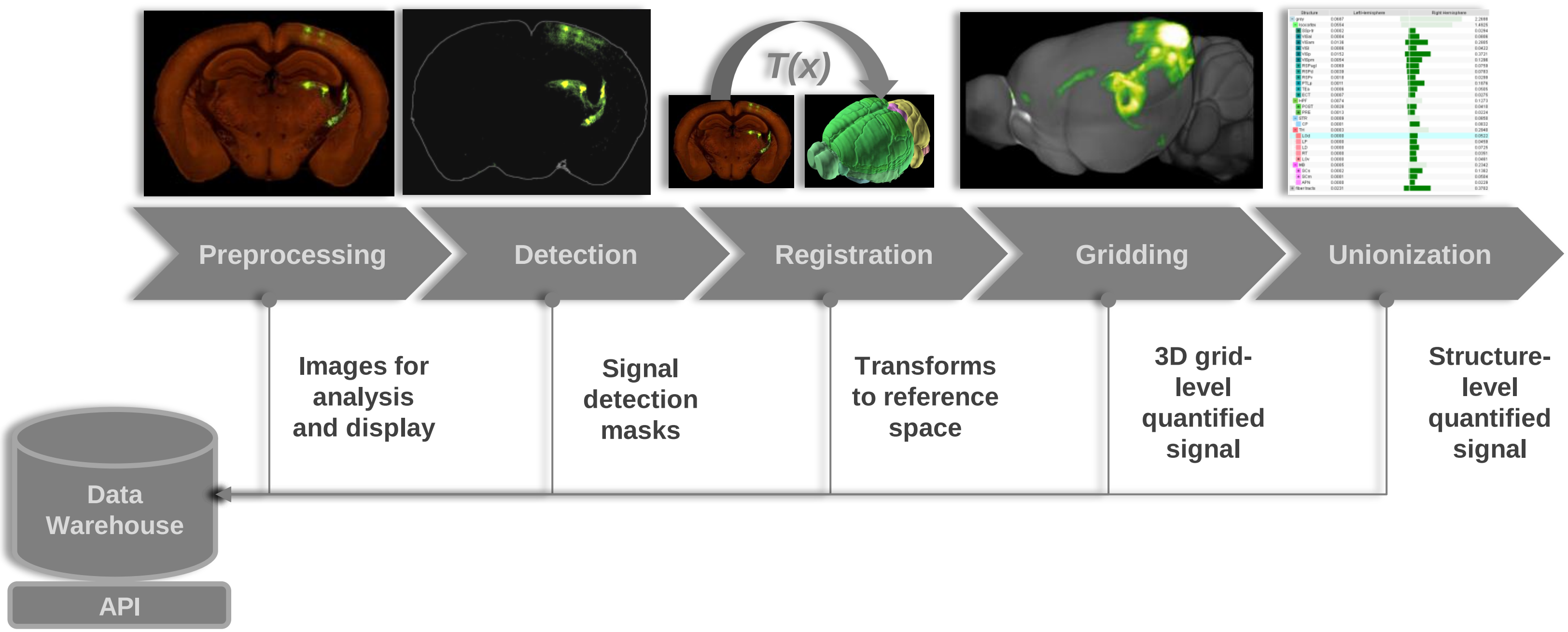
Connectivity Matrix for the Entire Mouse Brain



Julie Harris, Lydia Ng

Connectivity Informatics

Image data is processed through an automated informatics processing pipeline



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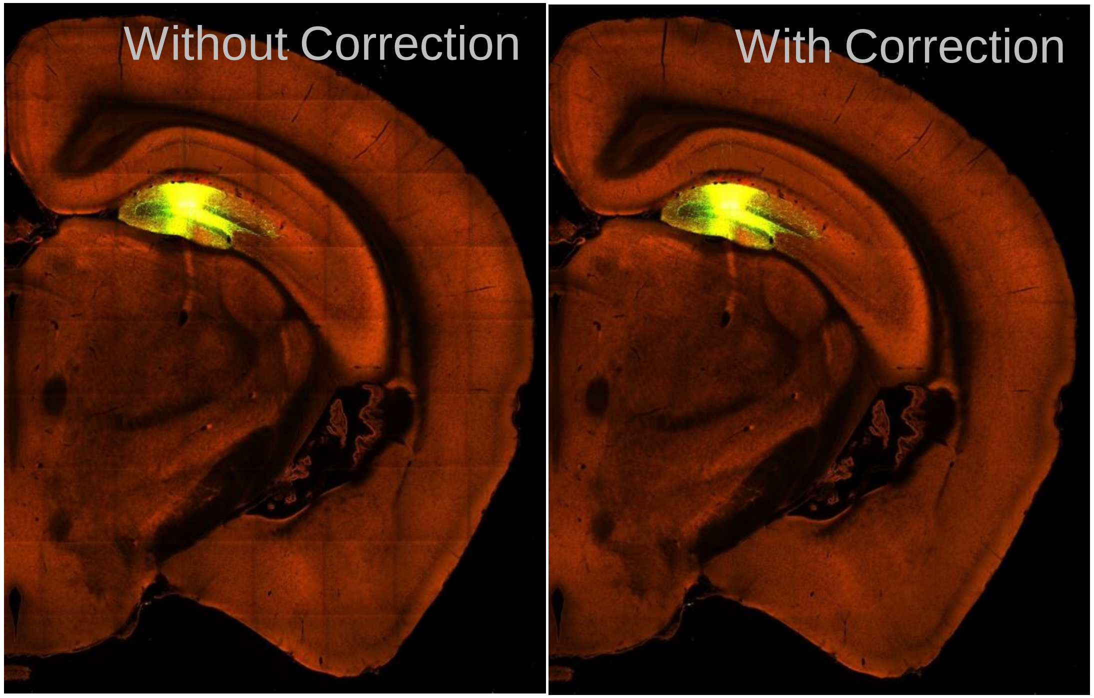
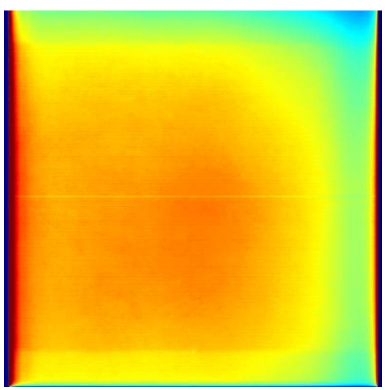
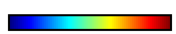
Tile inhomogenity correction



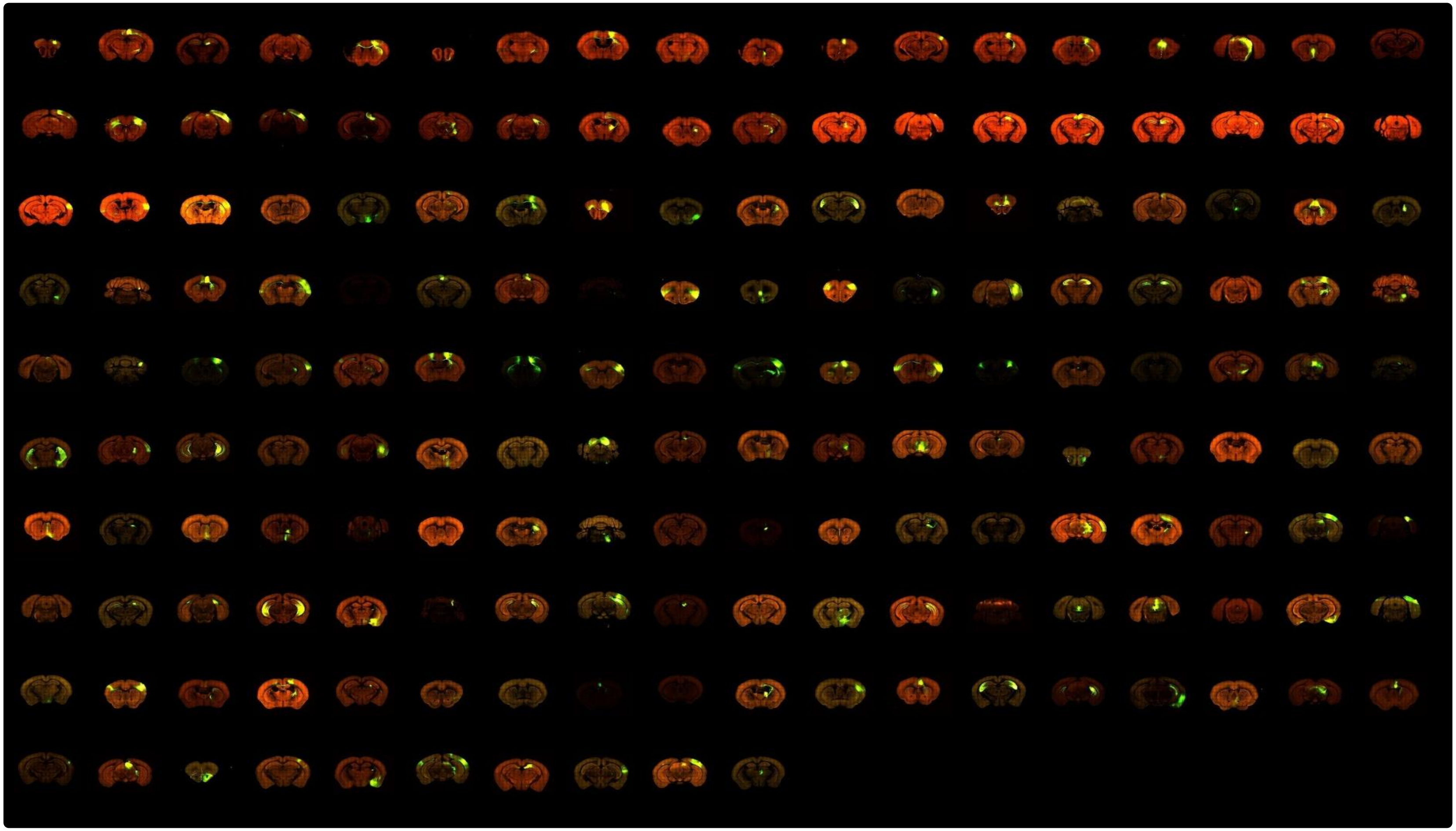


Averaged green channel tile
in “rainbow” color-scale



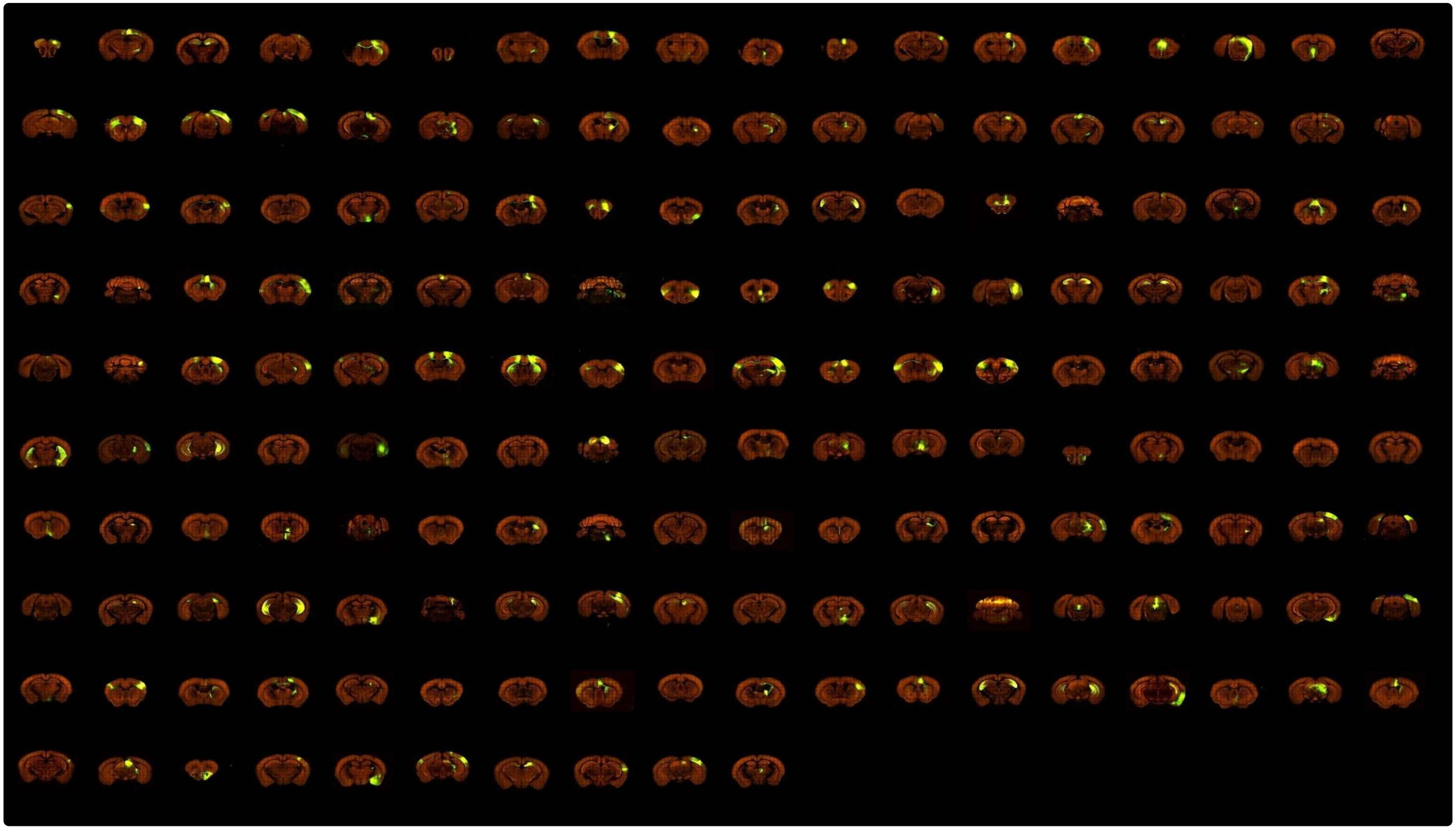
- Goal: to reduce per-tile intensity inhomogenity
- For each brain, generate the average tile for each channel using every tile from every section
- During image stitching, divide each tile by the average tile to remove systematic intensity inhomogenity
- Ragan et al. *Nature Methods* 2012

Handling intensity variation



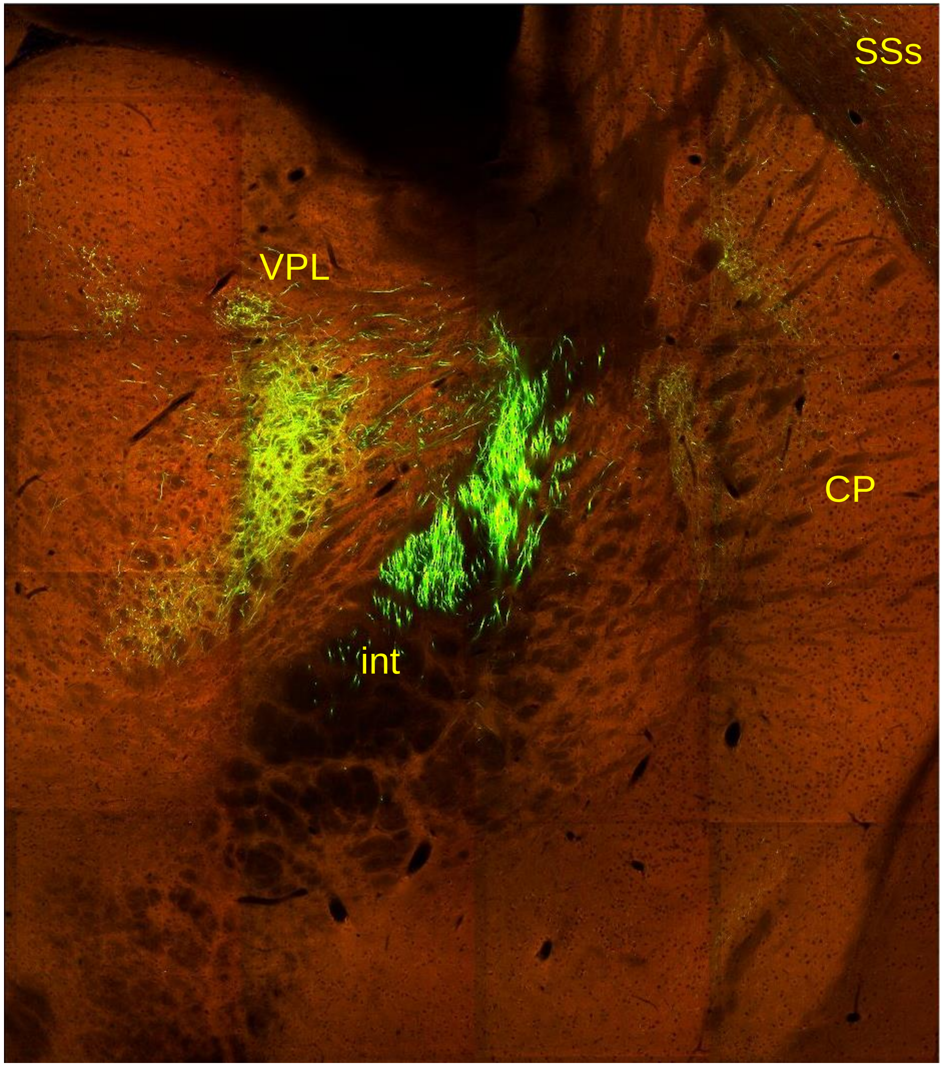
162 data sets displayed with same thresholds: (821,1280,4095)

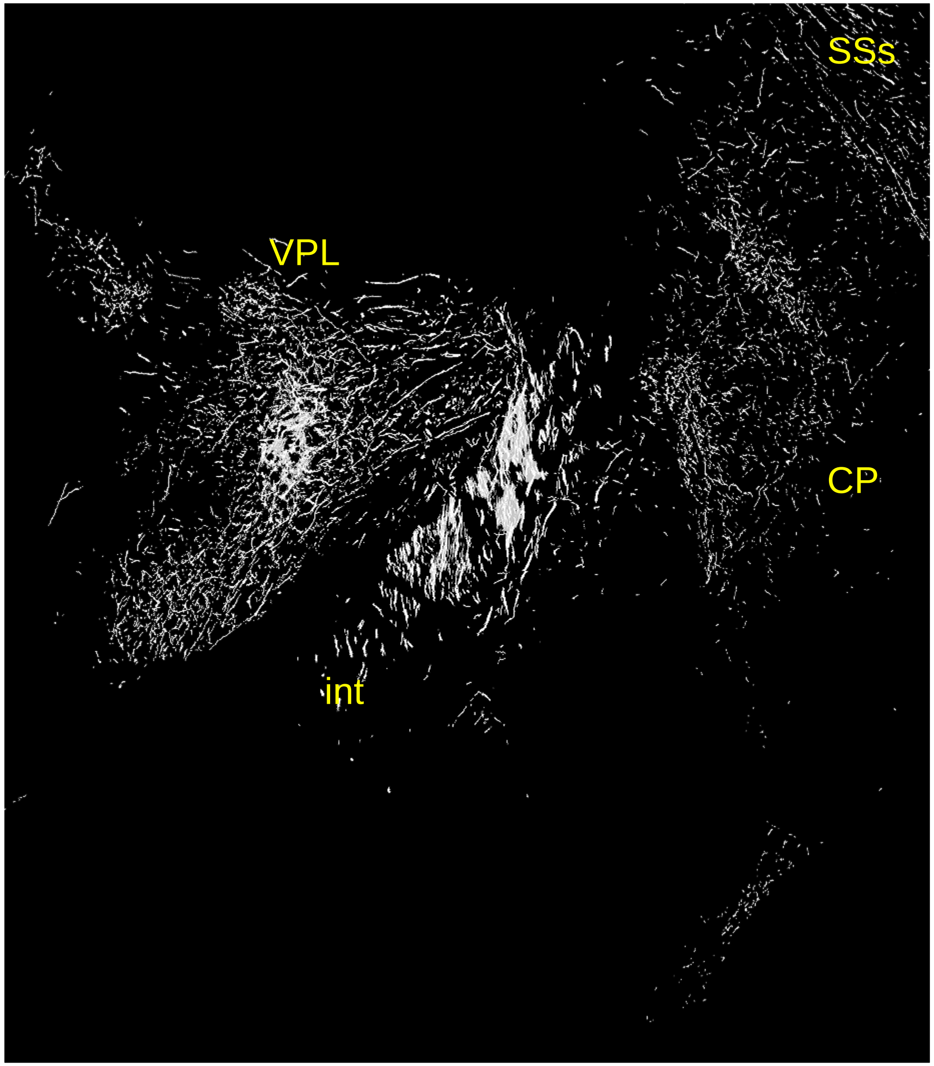
Threshold (window/level) computation



162 data sets displayed with computed thresholds
Red threshold = 2.33 * (95th percentile red value)
Green threshold = 6.33 * (95th percentile green value)

Signal detection





Intensity scaling, noise reduction, edge and dense cloud signal detection

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Annotated 3D reference space

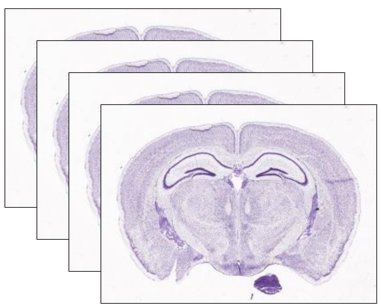
3D Reference Space

Symmetric, Annotated

Registration to a canonical space enables comparison of information within and across datasets

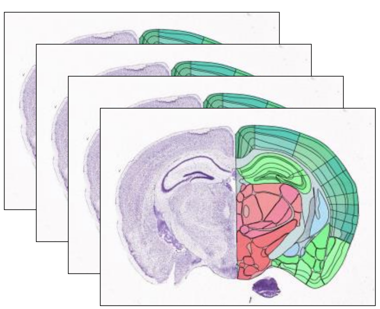
Allen Reference Atlas

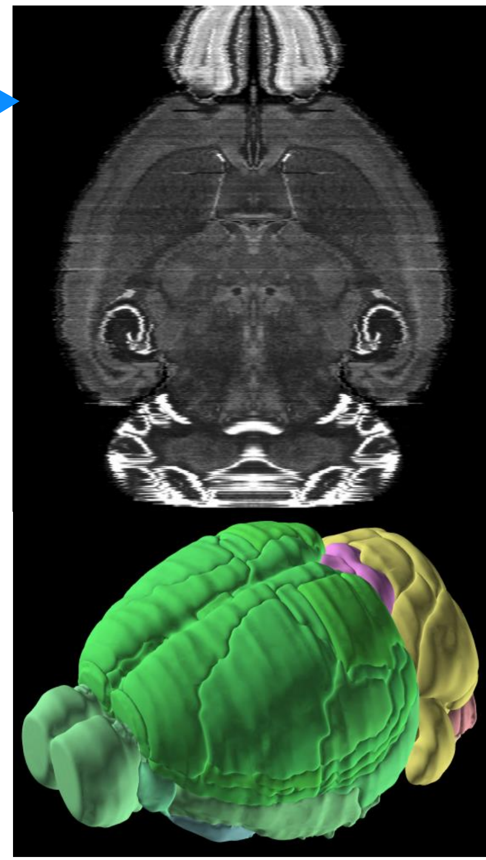
~500 Nissl sections



132 annotated sections

~800 structures





Gene Expression Data

Projection Data

API

External Spaces

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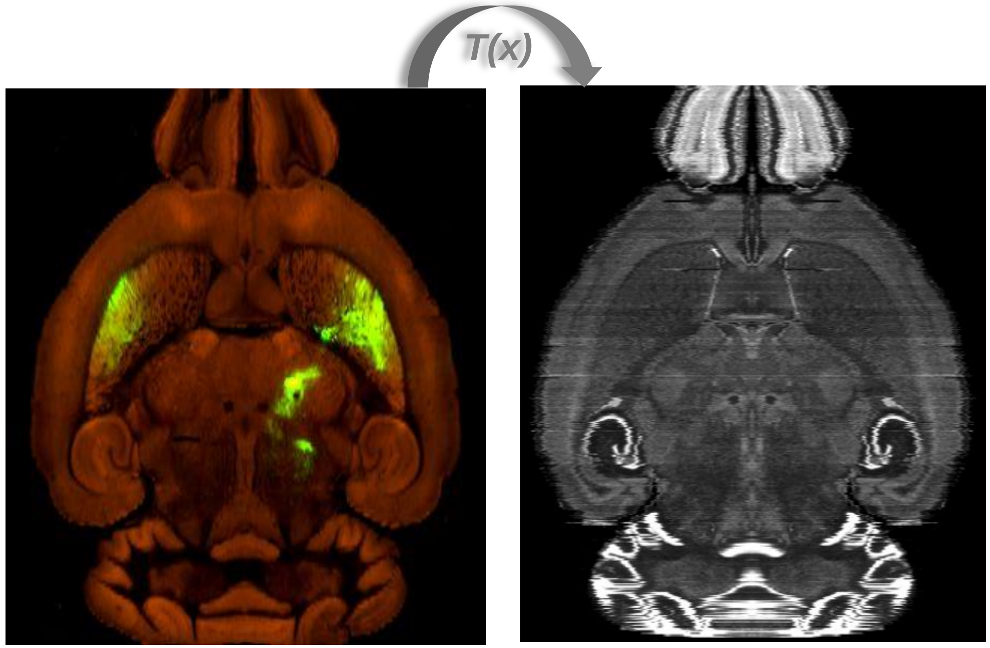
Image Registration

Perfused brain

2P Tomography Imaging

Background autofluorescne

Sections 100µm apart



Fresh frozen brain

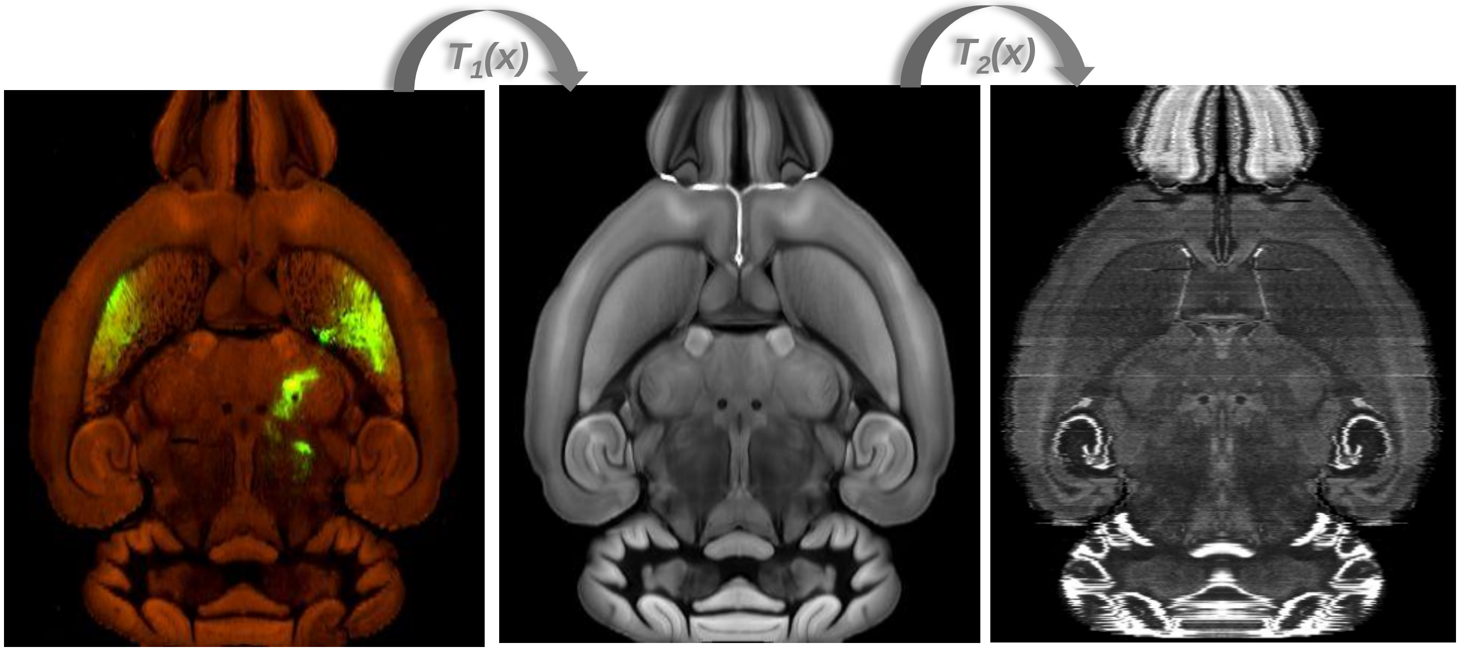
Reconstructed from Nissl sections

25µm thick sections

Elements of a registration algorithm:

Goodness of fit metric	Type of transformation	Optimization method	Multiscale Strategy

Averaged brain as registration template

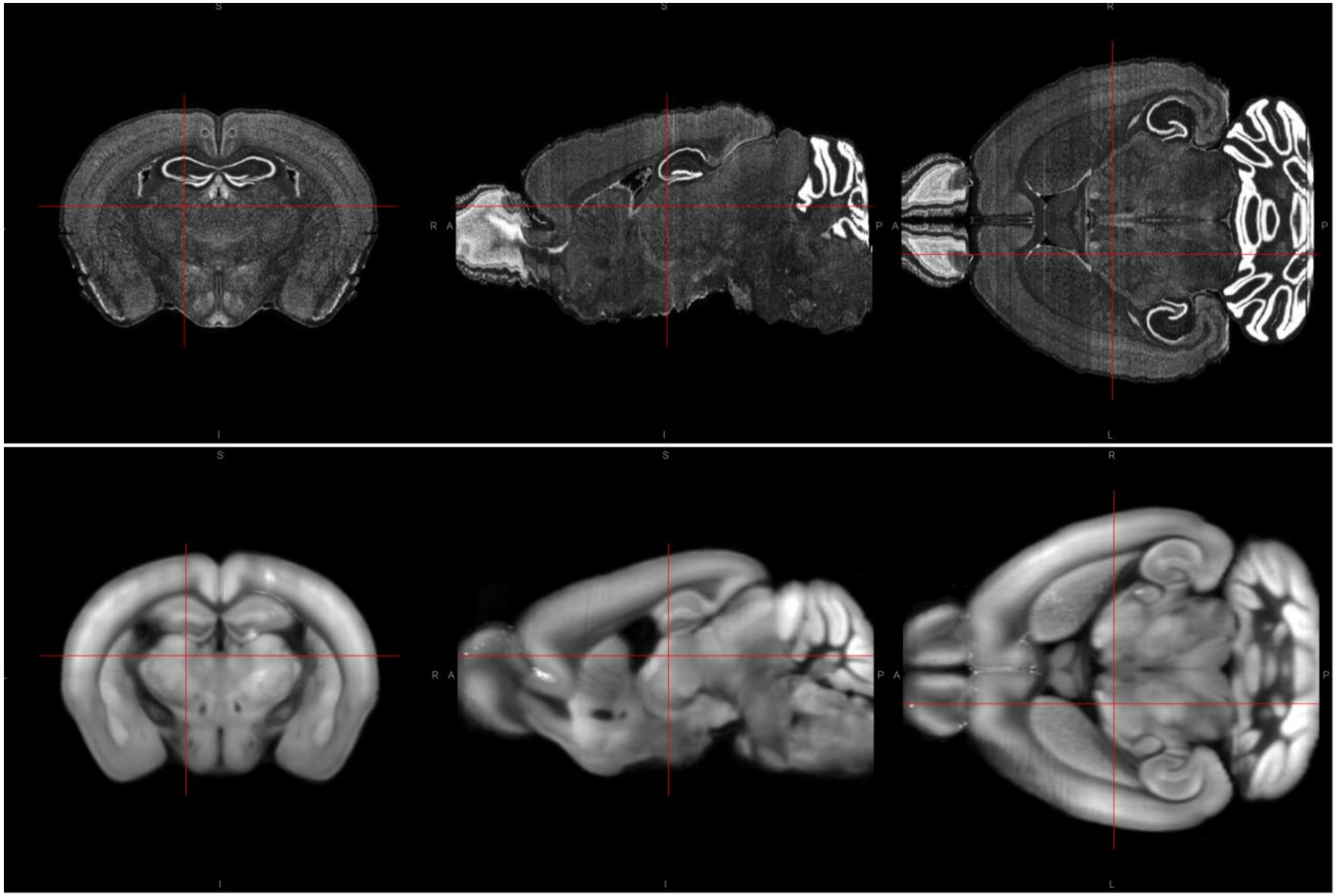


Chicken or the egg?

You need a good registration to get a good averaged brain
You need a good averaged brain to get good registration

Start with a rough template and iteratively improve both

Evolution of the average template



41 brains were globally (affine) mapped to the 3D Nissl and averaged to form the first registration template

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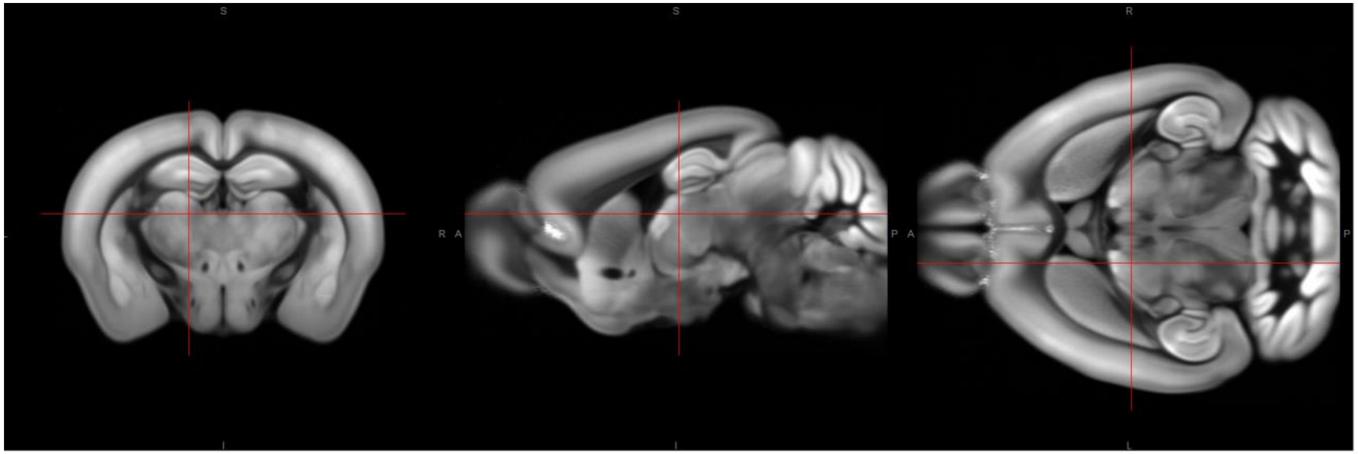
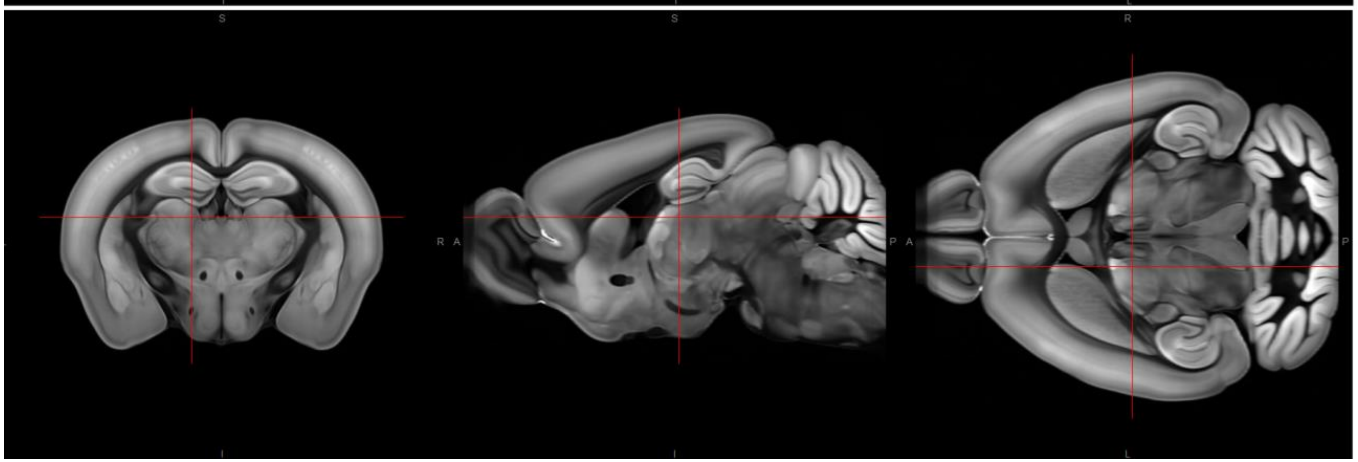
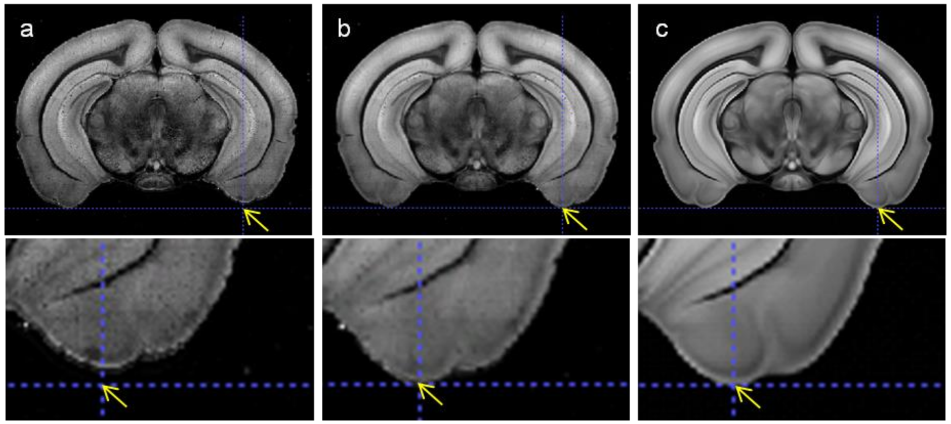


Average of 700+ globally (affine) mapped brains

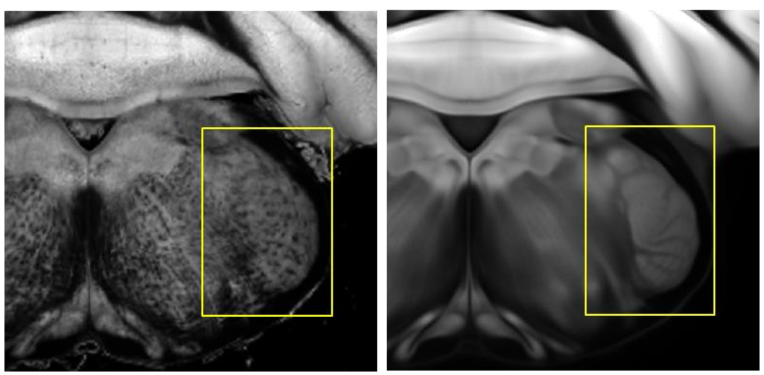


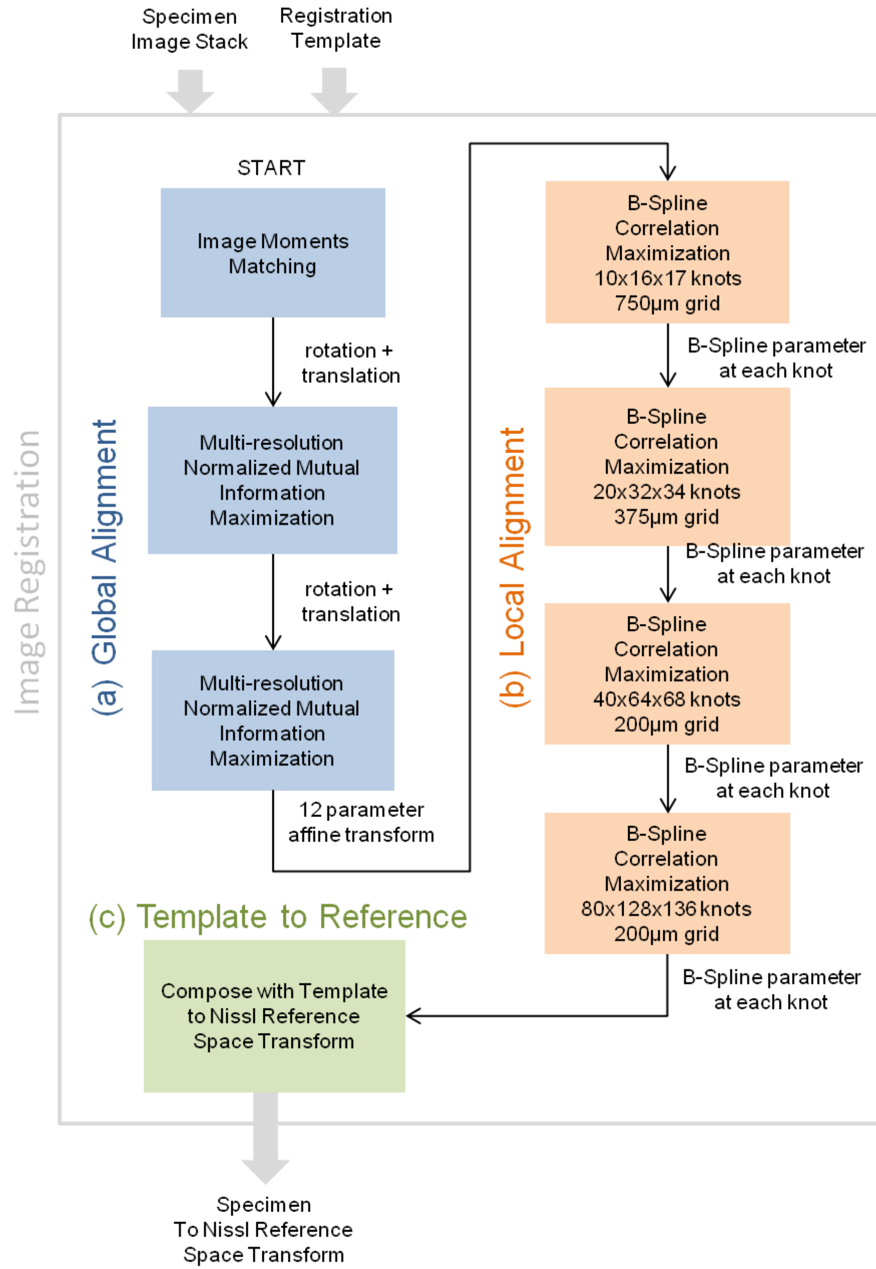
Average of 1200+ brains locally (deformable) mapped after 4 generations

Registration algorithm



The whole is greater than the sum of its parts





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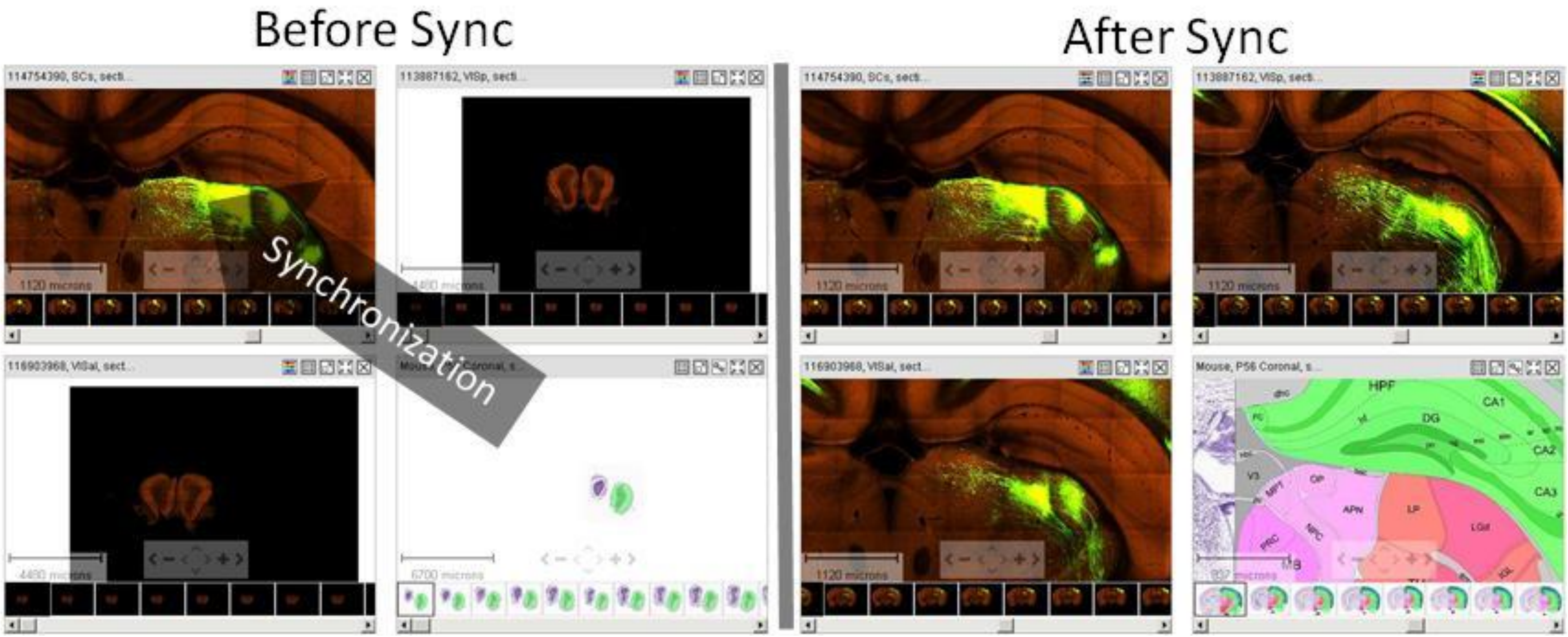
Synchronicity!



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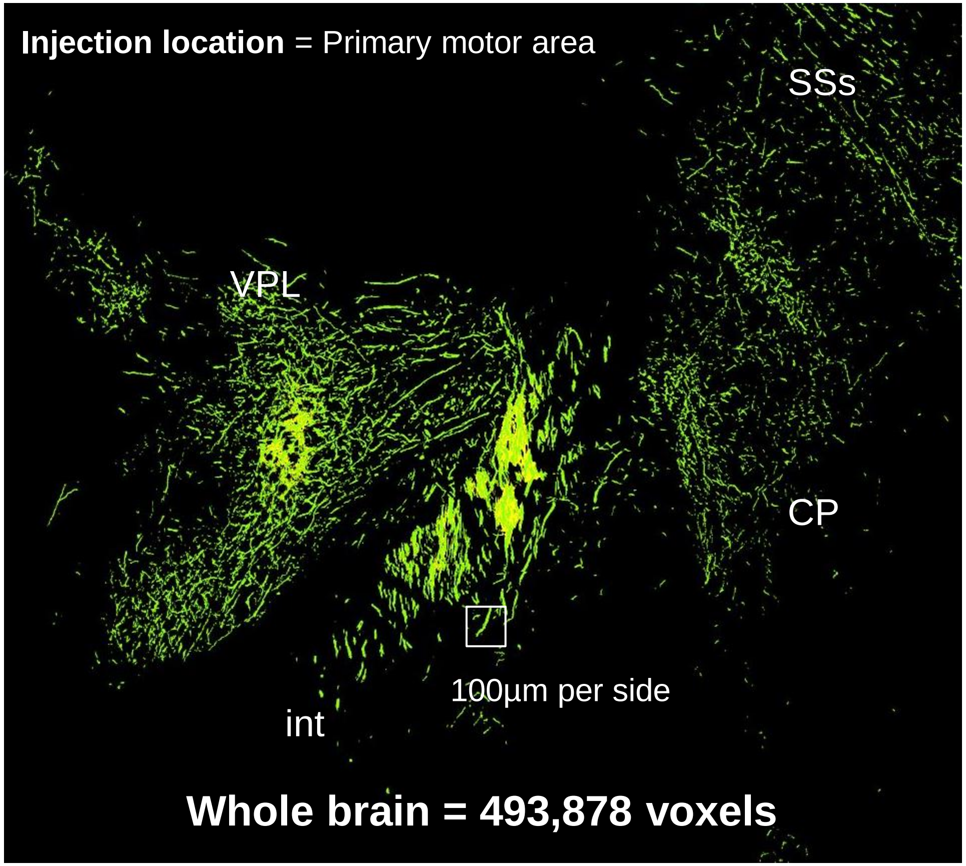
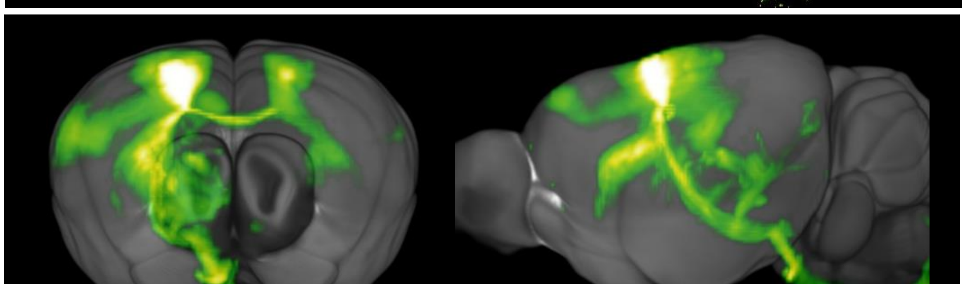
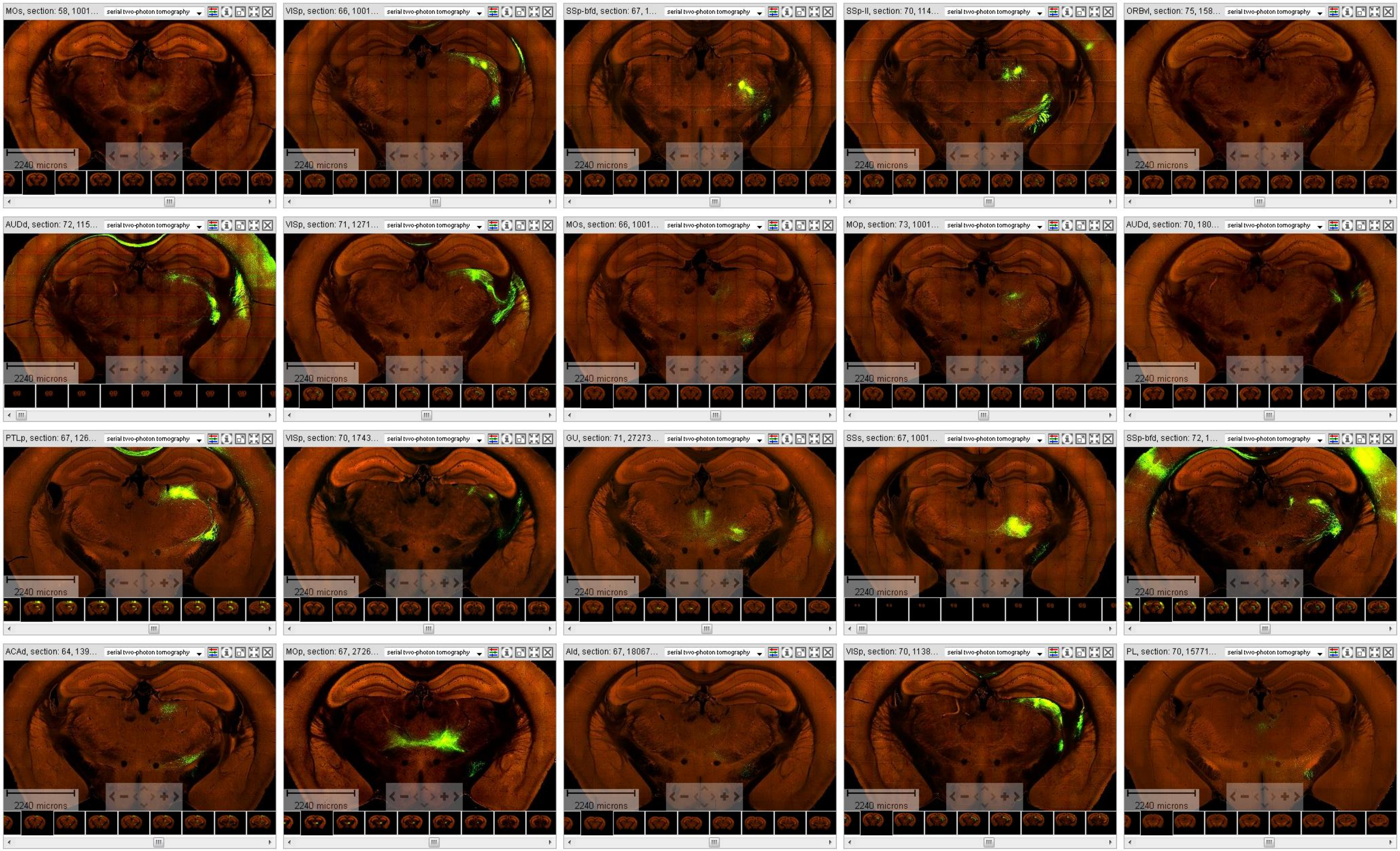
Voxelizing the data





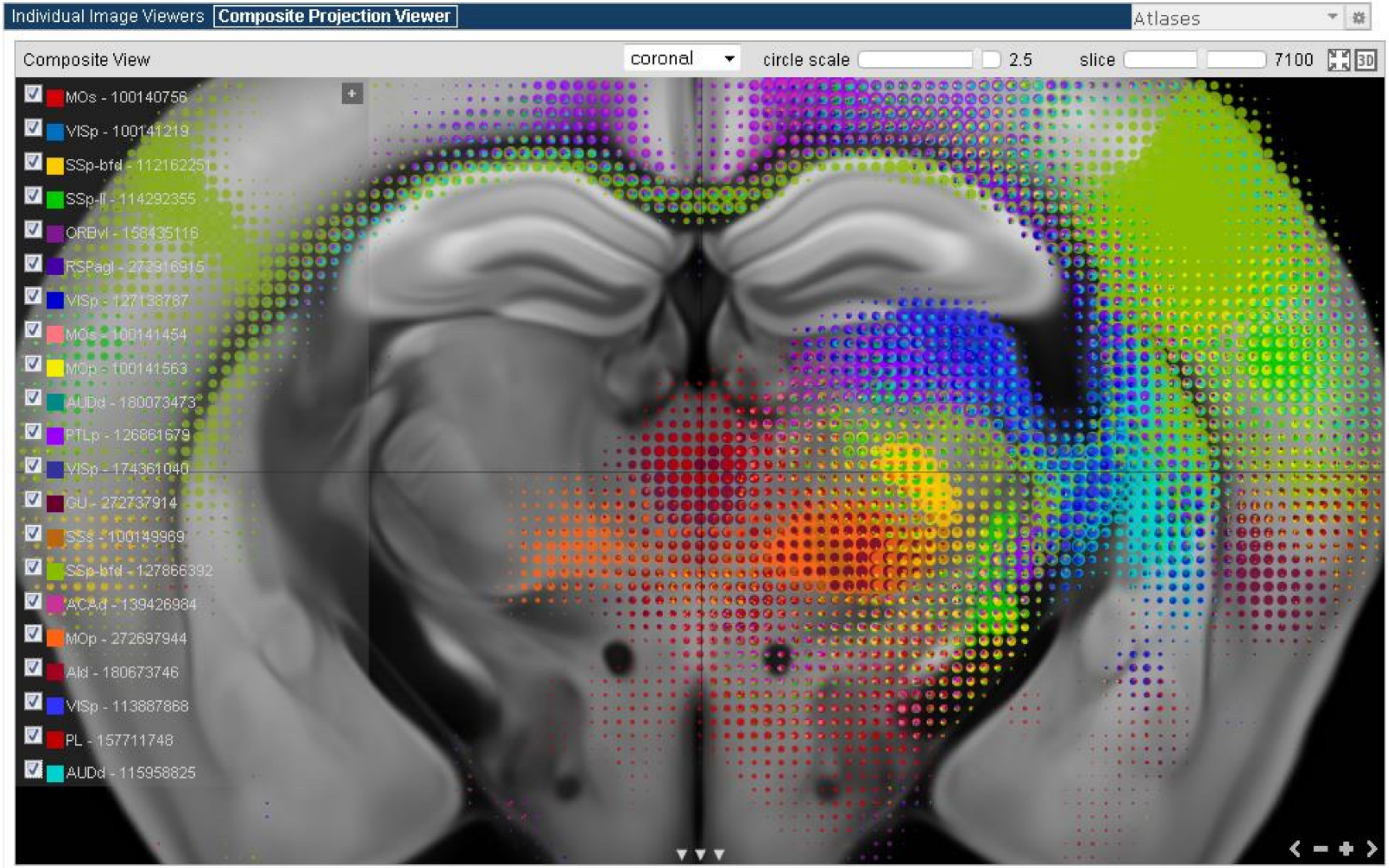
Maximum density projection of grid data

- Compute signal statistics in a 100µm per side grid voxels
- Projection density = sum of signal positive pixels / sum of pixels
- Injection site size quantification
- Resample into reference space
- Search services:
  - Correlation Search
  - Target Search
  - Spatial Search
  - Injection Search
- Analysis
  - Cortical projection topography
  - Linear model / network analysis
  - *Add yours here!*

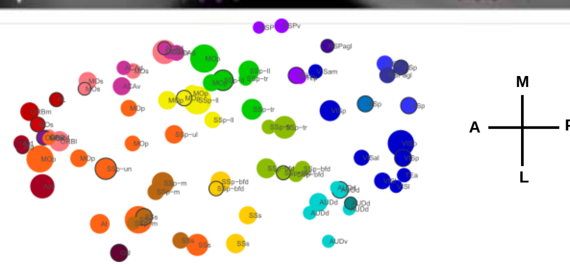


20 wild-type injections spanning the isocortex

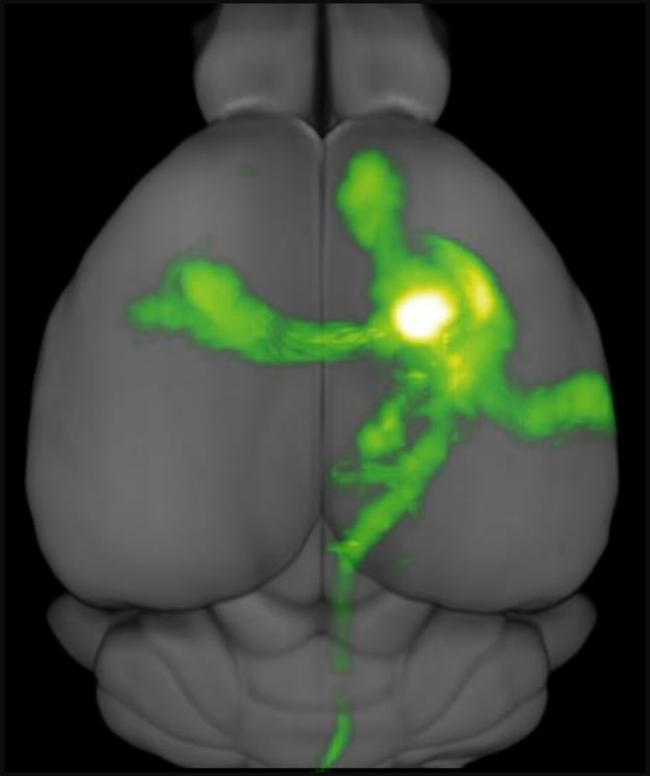
| preprocess | detection | alignment | **gridding** | unionization |

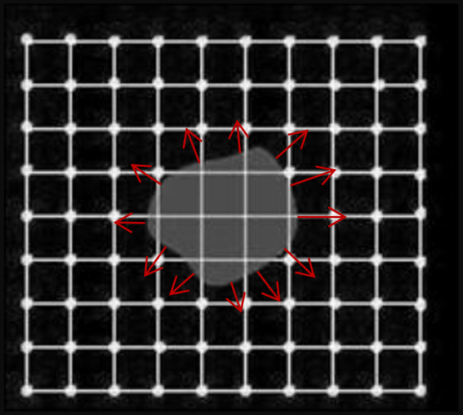


21 wild-type injections spanning the cortex



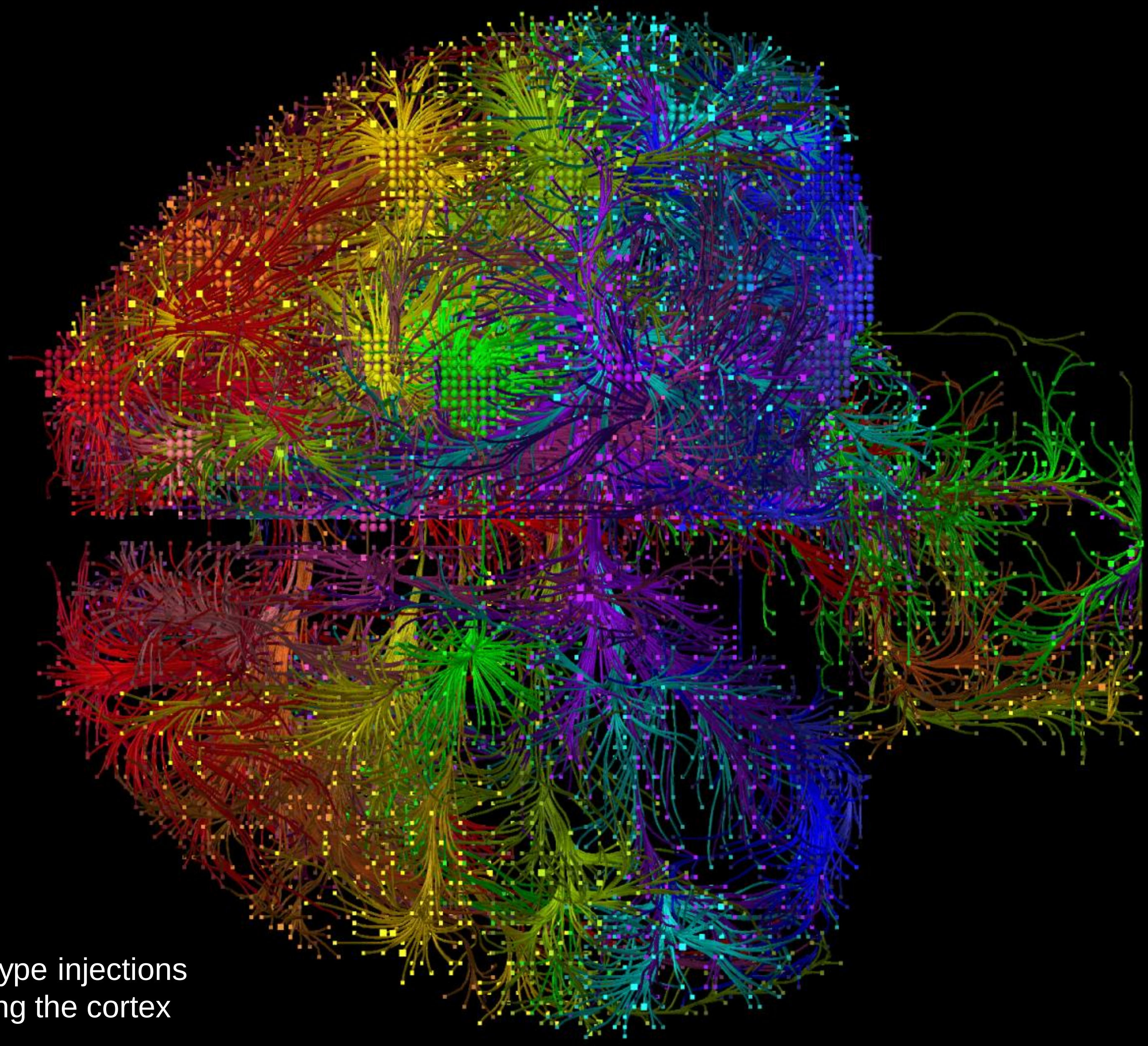
Virtual tractography





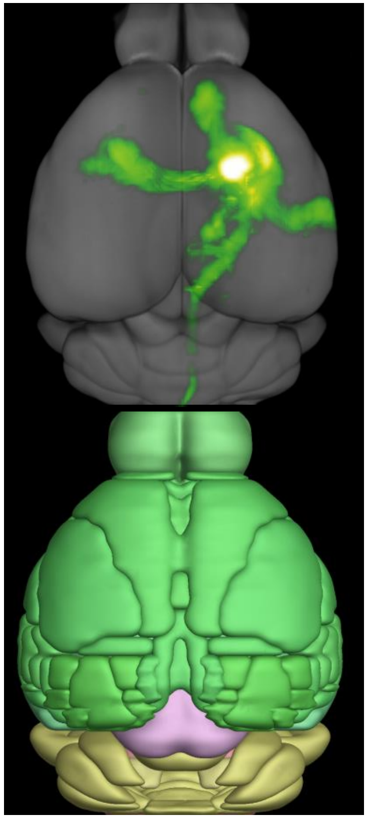
Fast marching algorithm

- Starting from injection site, evolve 3D surface conditioned by signal density
- Record the time it takes for the surface to cross each point on the image grid
- Upwind vectors of the arrival time form paths back to the injection site
- Brain Explorer display a sub-sample of paths for visualization
- All paths available through API



21 wild-type injections
spanning the cortex

Structural Unionization



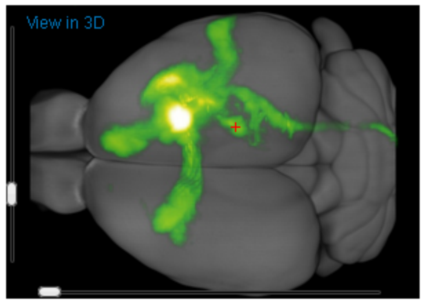
Grid data summed up to structures using 3D reference atlas

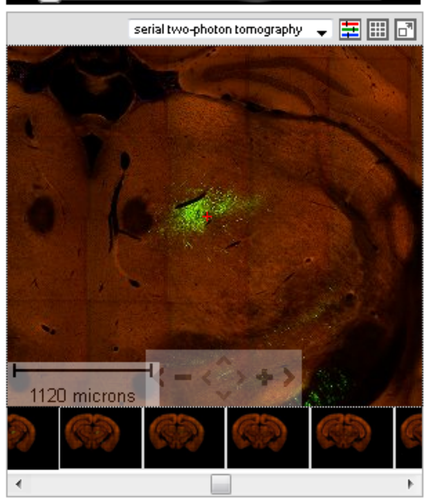
Manual Injection Summary

Primary Structure	**Primary motor area**
Secondary Structures	**SSp-ll**
Coords. (AP,DV,ML,∠)	**Bregma(0.14, 0.45, 1.75, 0)**
Mouse Strain	**C57BL/6J**

Quantified Injection Summary

MOp	SSp-ll





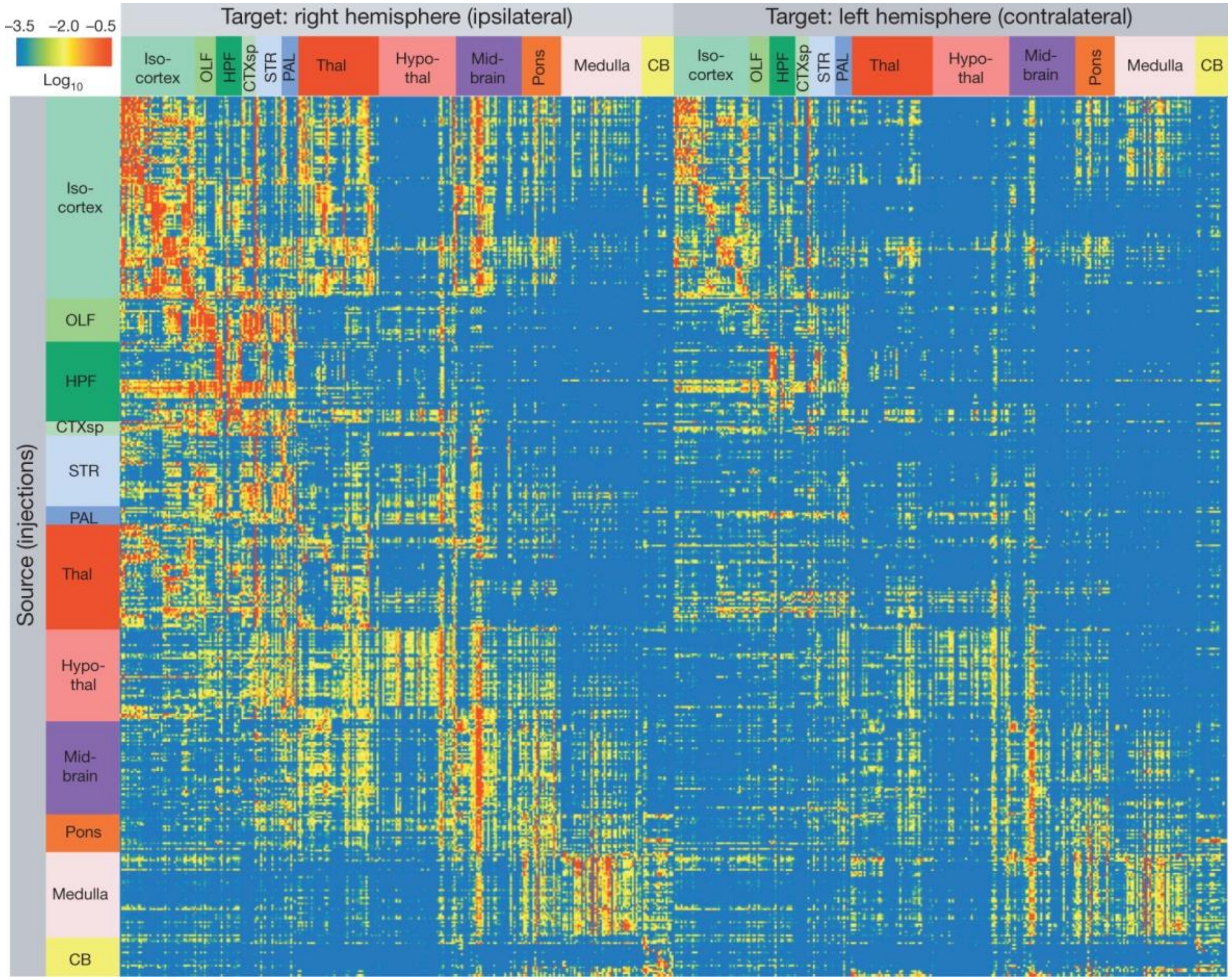
Projection Volume ▼ 0.0050 / 2.7946 mm^3

PF - Parafascicular nucleus

Structure	Left Hemisphere	Right Hemisphere
grey	0.4034	2.5260
Isocortex	0.2869	1.6767
MOp	0.1152	0.4384
MOp5	0.0315	0.1641
MOs	0.0082	0.2441
MOs2/3	0.0031	0.0879
SSp-bfd	0.0058	0.0523
SSp-ll	0.1027	0.3419
SSp-ul	0.0267	0.2329
SSp-ul5	0.0033	0.0576
SSp-tr	0.0039	0.1573
SSs	0.0055	0.0507
AUDd	0.0017	0.0415
AUDp	0.0025	0.0312
AUDv	0.0040	0.0449
ACAd	0.0004	0.0089
PTLp	0.0002	0.0067
TEa	0.0021	0.0065
STR	0.1069	0.5414
CP	0.1056	0.5343
PAL	0.0002	0.0156
GPe	0.0001	0.0128
TH	0.0001	0.1086
VAL	0.0000	0.0135
VPL	0.0000	0.0165
VPM	0.0000	0.0129
PO	0.0000	0.0196
PF	0.0000	0.0116
RT	0.0000	0.0137
HY	0.0001	0.0348
ZI	0.0000	0.0297
MB	0.0007	0.1137
SNr	0.0000	0.0144
MRN	0.0002	0.0501
SCm	0.0000	0.0156
PAG	0.0003	0.0072
APN	0.0000	0.0091
P	0.0010	0.0200

This data is also available as XML.

Adult mouse brain connectivity matrix



Each row shows the quantitative projection signals from one of the 469 injected brains to each of the 295 non-overlapping target regions (in columns) in the right (ipsilateral) and left (contralateral) hemispheres.

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The ALLEN **Institute API**

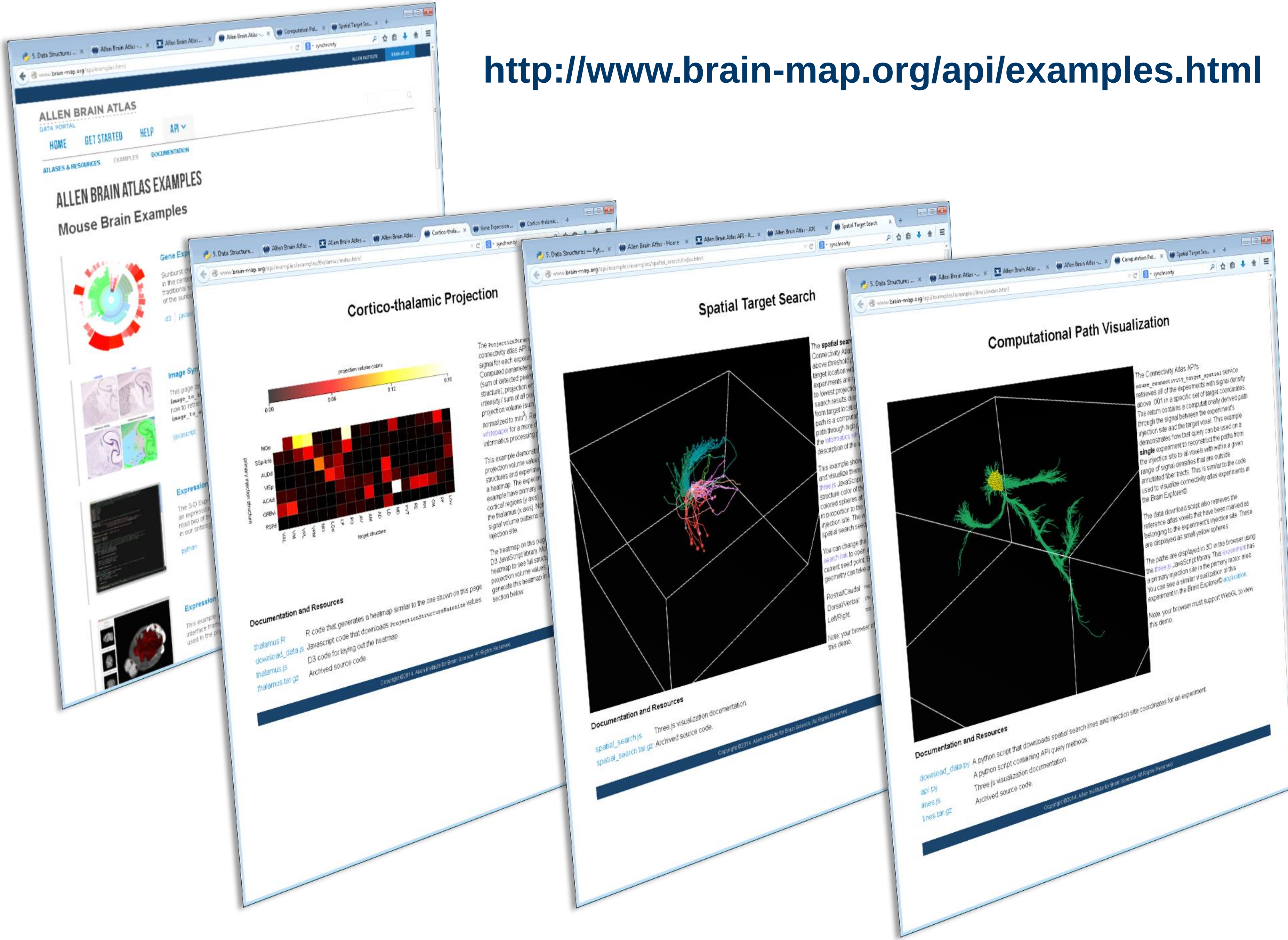
http://www.brain-map.org/api/index.html

http://help.brain-map.org/display/mouseconnectivity/API

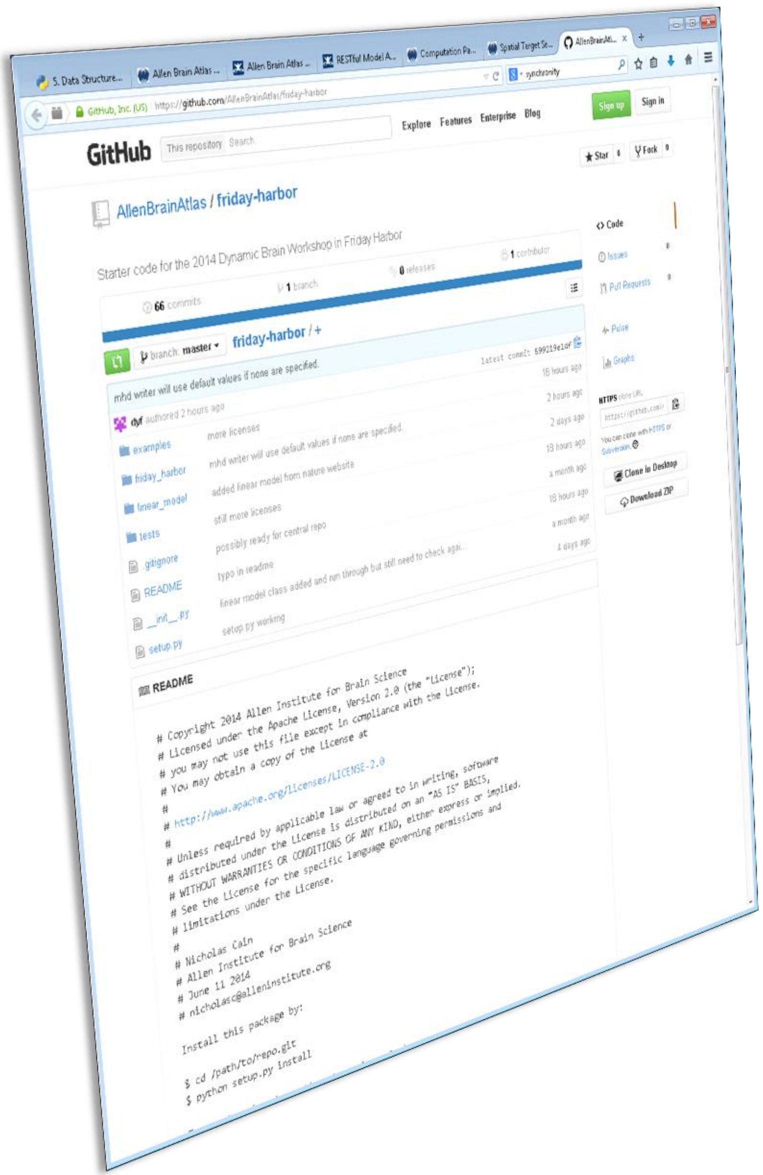
Tutorial on how to use the API to get all things related to the Connectivity Atlas

- Download images at different resolution and for specific ROI
- Download voxelized data and 3D template and atlas
- Download quantified projection value for experiment and structure
- Download virtual tractography data
- Query the source, target, spatial and correlative search services
- Query the image synchronization service

http://www.brain-map.org/api/examples.html



https://github.com/AllenBrainAtlas/friday-harbor



Why are we having a python bootcamp?

Nick Cain and David Feng have written a python library wrapper to the API to let you quickly get started with the data

Easy access to voxel and injection information

Voxelized and tractography data have also been pre-downloaded onto a portable drive

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