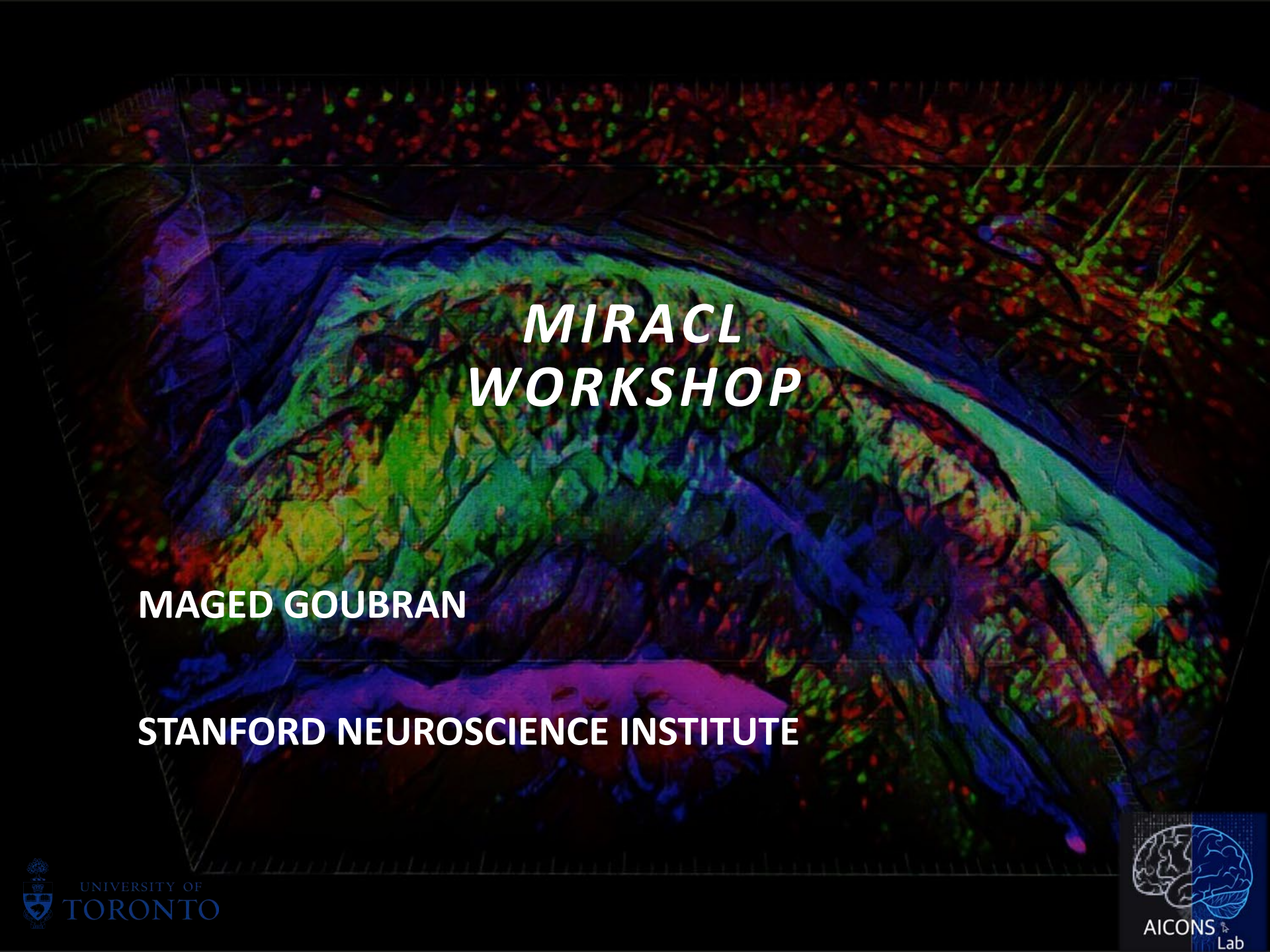


MIRACL WORKSHOP

MAGED GOUBRAN

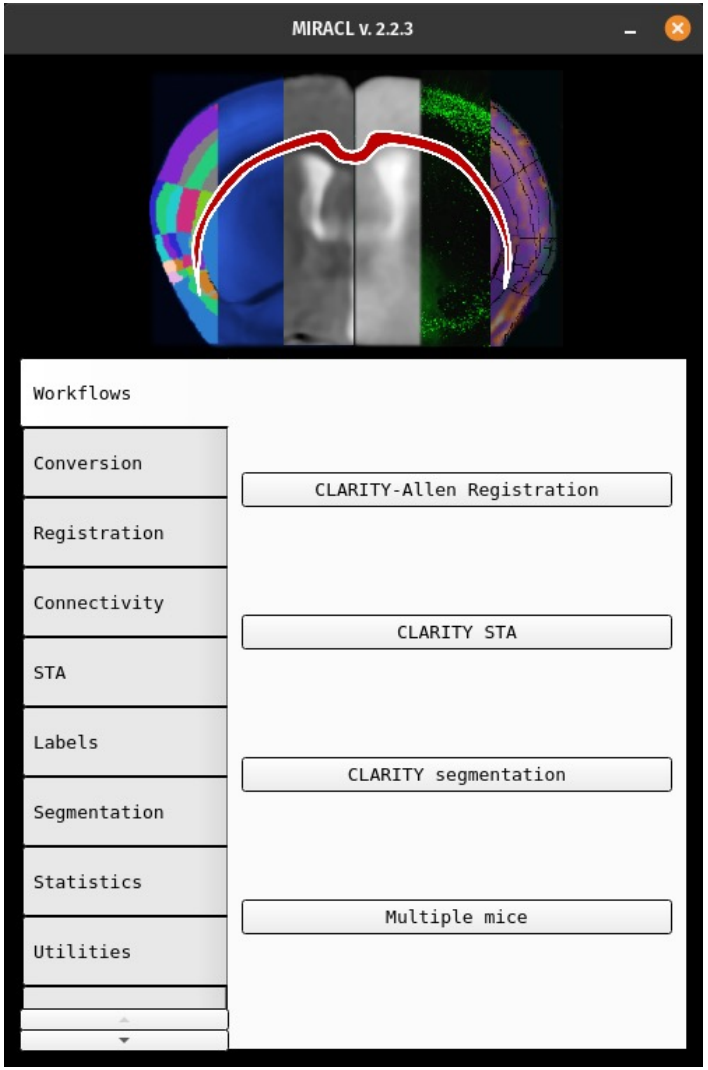
STANFORD NEUROSCIENCE INSTITUTE

UNIVERSITY OF TORONTO

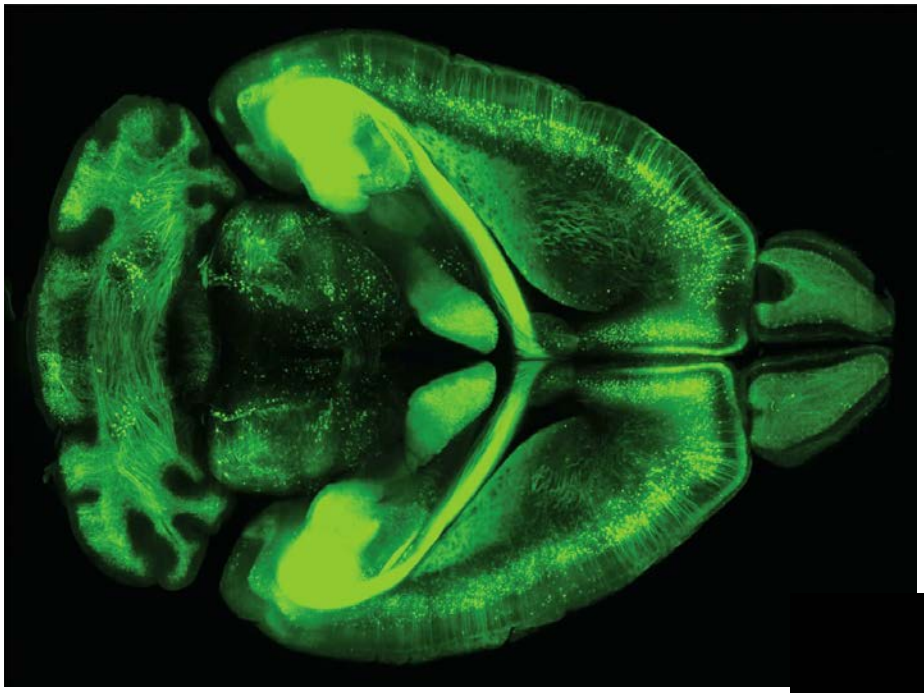
AICONS Lab

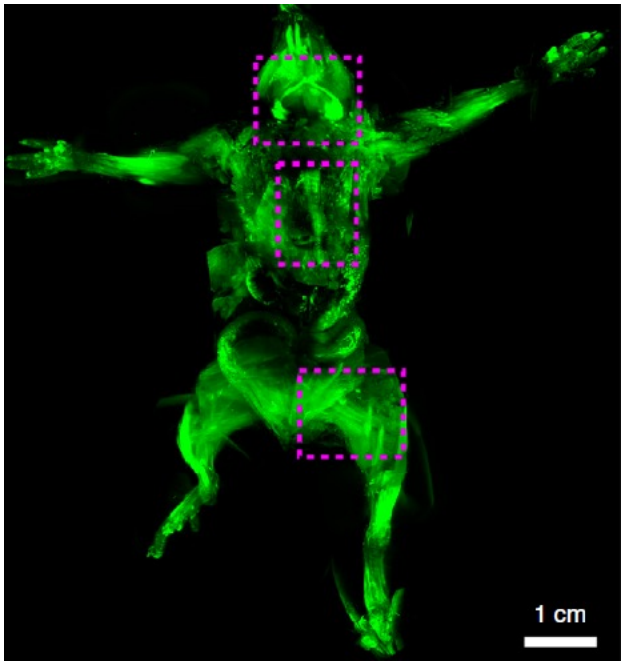
WORKSHOP OVERVIEW

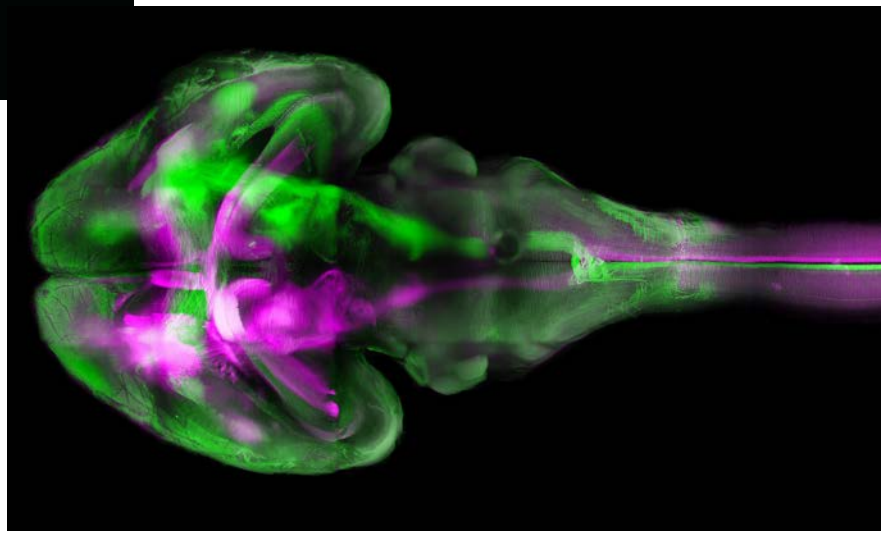
- Background & motivation behind MIRACL
- Overview of different functionality
- Example work using MIRACL
- ACE: New AI model & sub-regional analyses
- MRI analyses & diffusion comparison
- Future work
- Google collab notebooks
  - Registration
  - ACE
  - Regional & sub-regional stats
  - Tract tracing

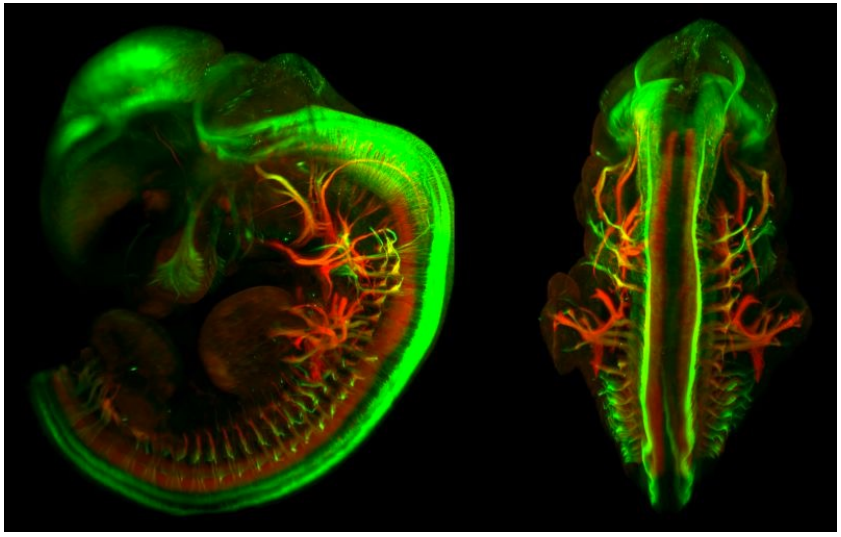


TISSUE CLEARING







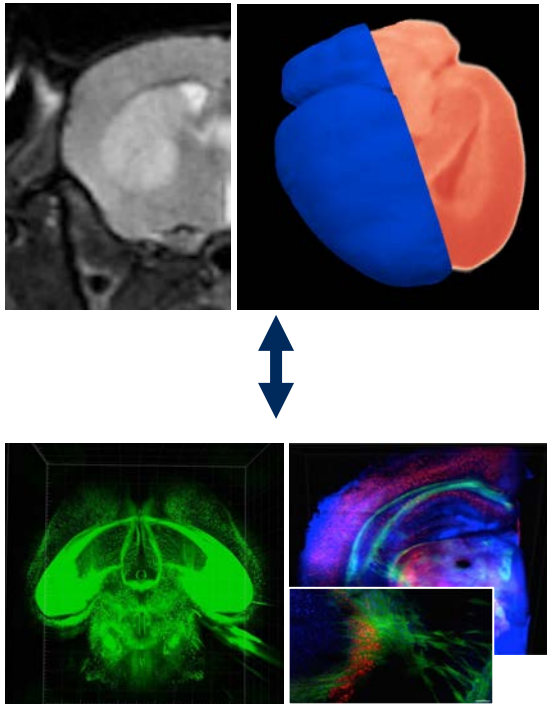


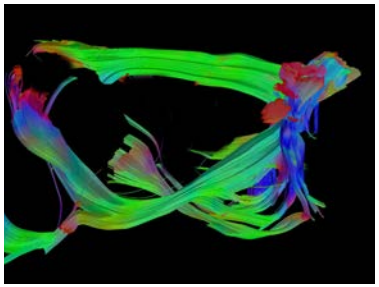
Park et al. *Nature Biotechnology 2019*
Pan et al. *Nature Methods 2016*
Chung et al. *Nature 2015*

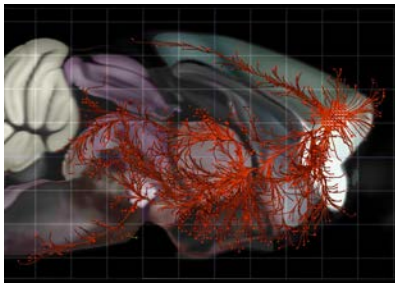
Sunnybrook HEALTH SCIENCES CENTRE

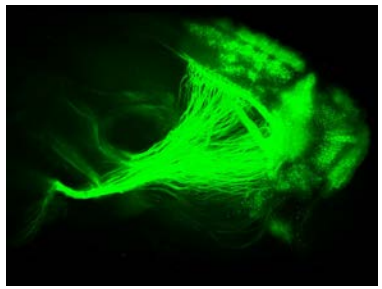
MOTIVATION / CHALLENGES

- Accurate mapping between mesoscopic & microscopic domains
- Accurate cellular & fiber quantification
- Automated network & tract-level connectivity analysis
- Automated analysis of cellular alterations across the whole-brain





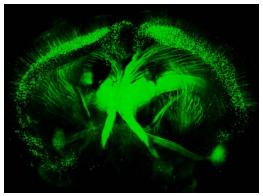




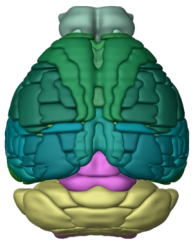
PIPELINE OVERVIEW

with Jennifer McNab, Karl Deisseroth & Michael Zeineh

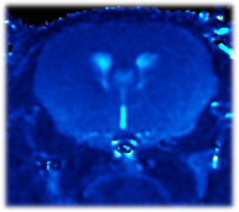
Multi-modal **I**mage **R**egistration **A**nd **C**onnectivity ana**L**ysis



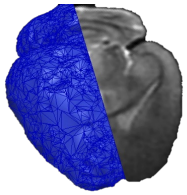
CLARITY (3D histology)

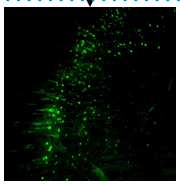


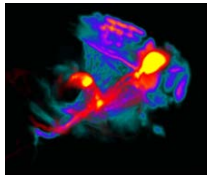
Allen Atlas (map)



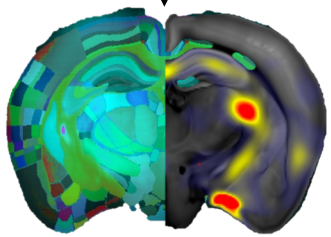
MRI





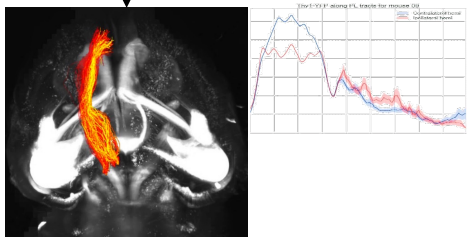


Advanced image analysis



Localized / regional analyses

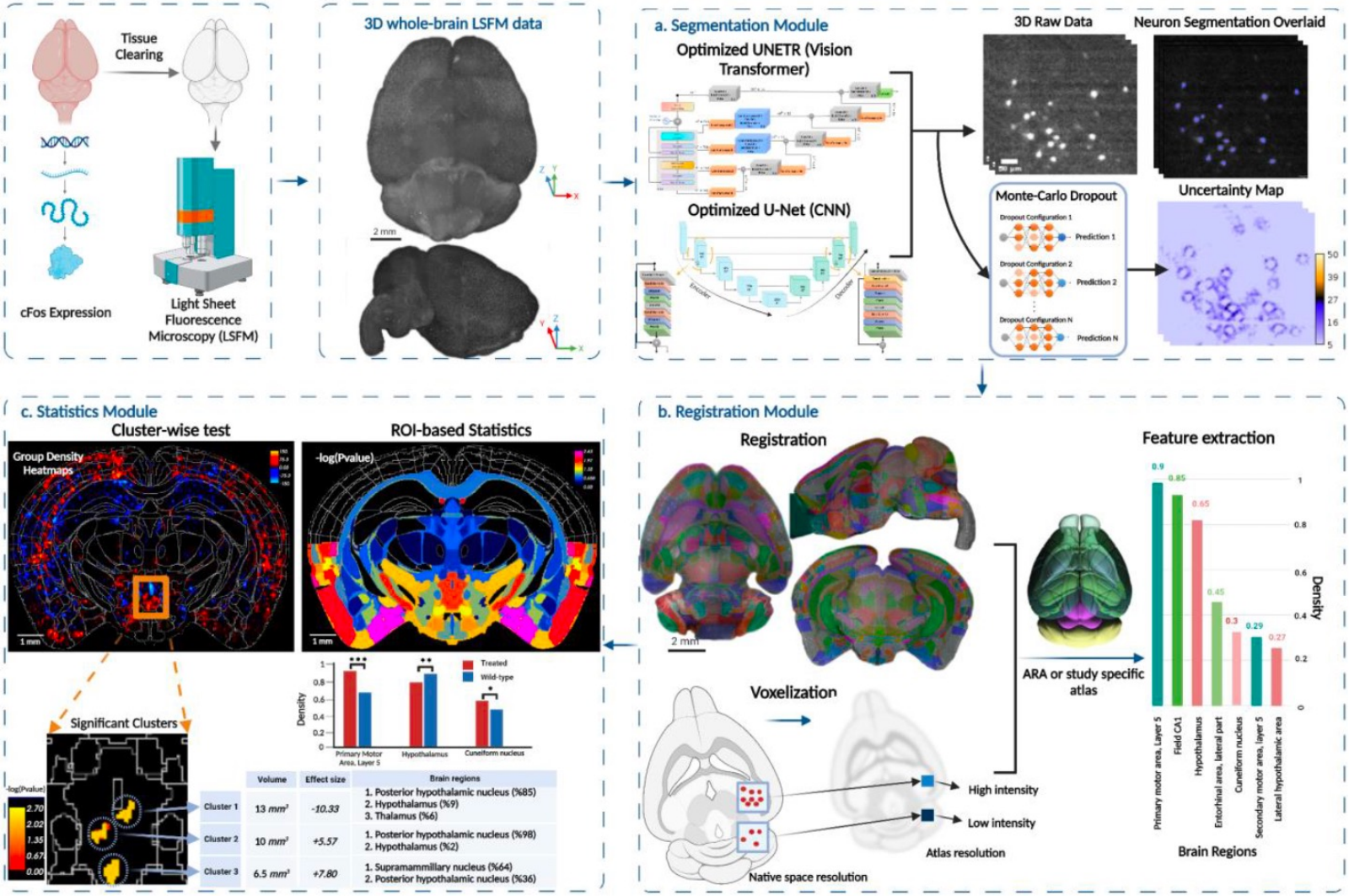
https://miracl.readthedocs.io



Connectivity analyses

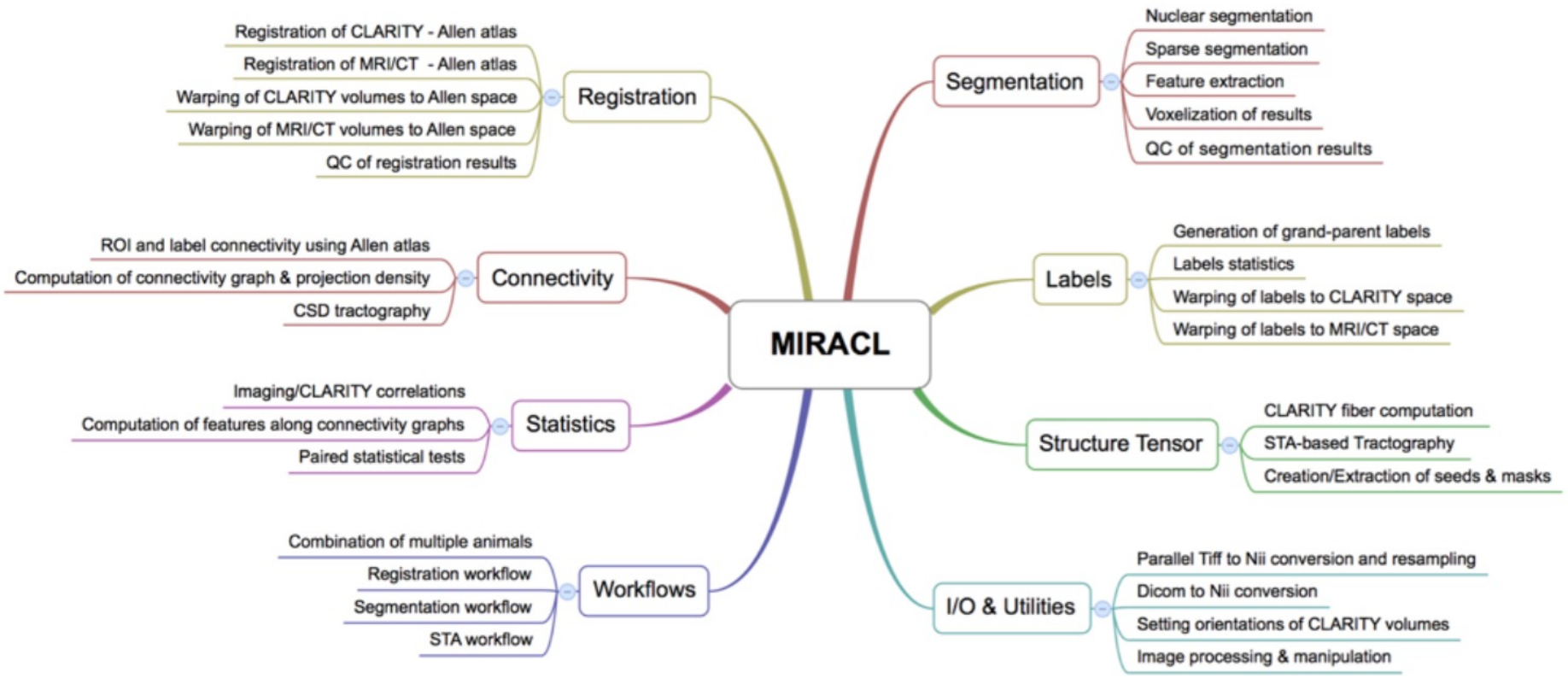
Goubran et al. *Nature communications*. 2020

ACE: AI-BASED CARTOGRAPHY OF (NEURONAL) ENSEMBLES



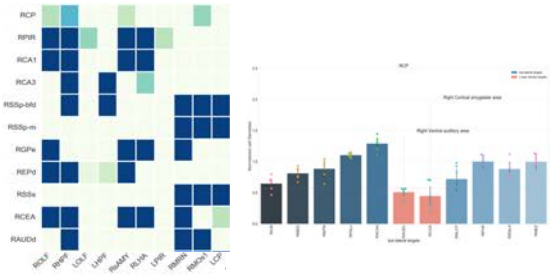
Attarpour… Goubran. *Nature Methods.* Under review

OVERVIEW OF FUNCTIONALITY

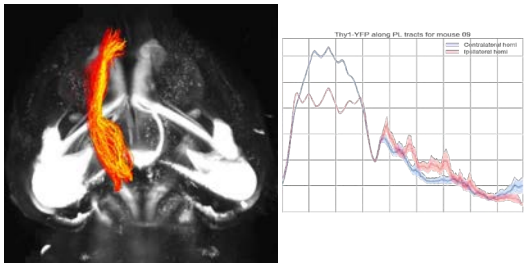


EXAMPLES OF FUNCTIONALITY / OUTPUTS

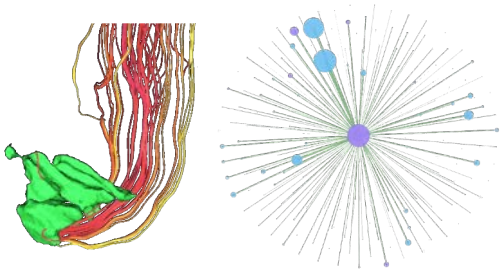
Tract & network-based analyses



Features across network

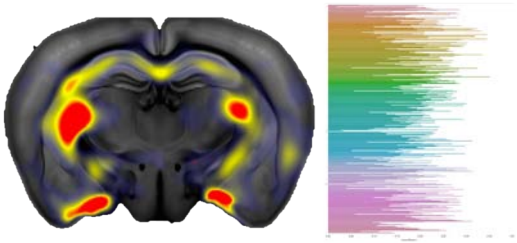


Tract-level histological analyses

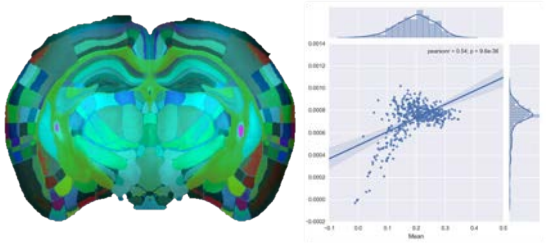


Terminals-based connectivity analyses

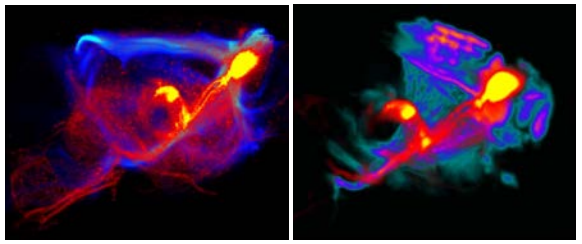
Label & voxel-based analyses



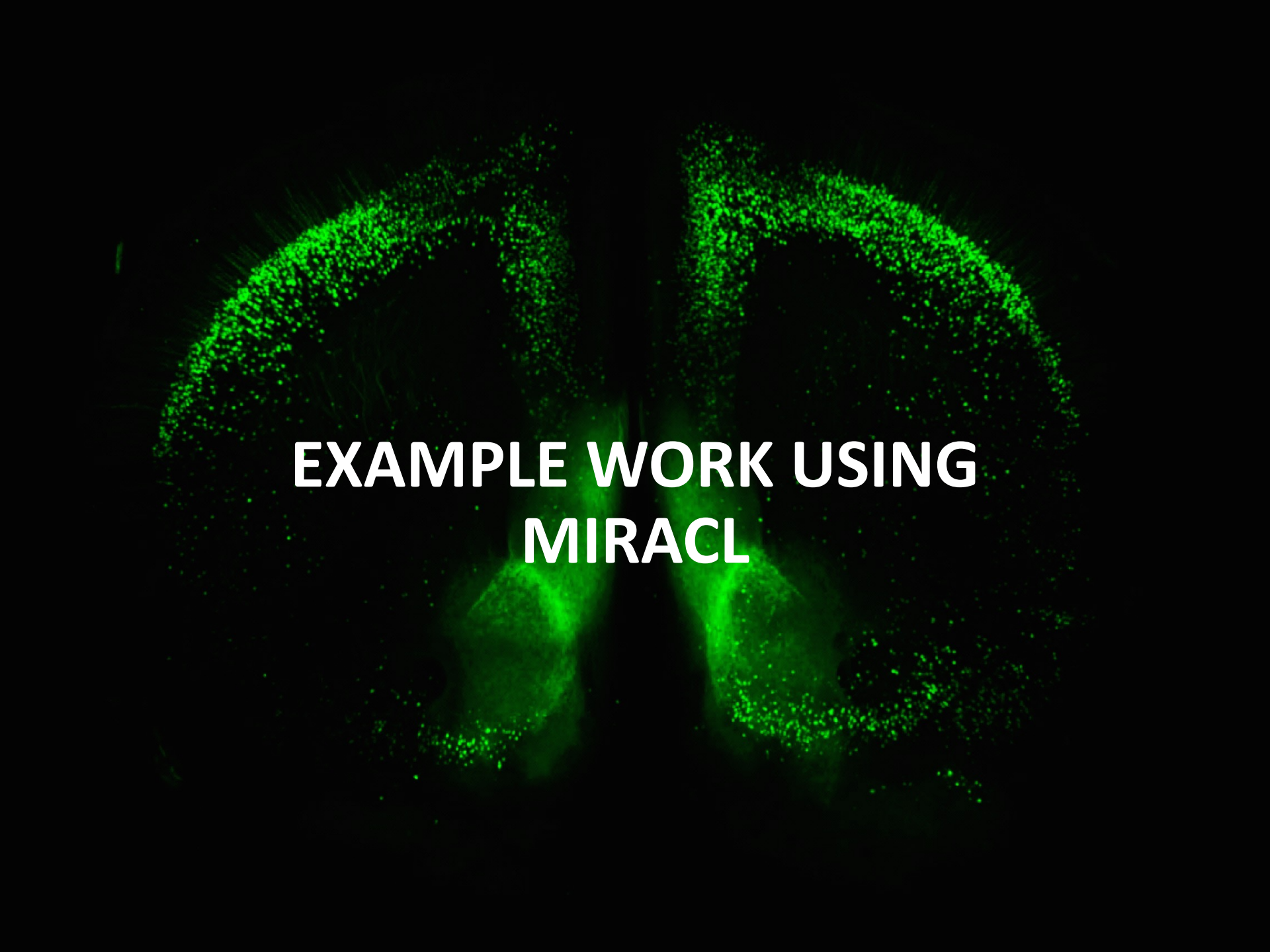
Multimodal group-level statistics



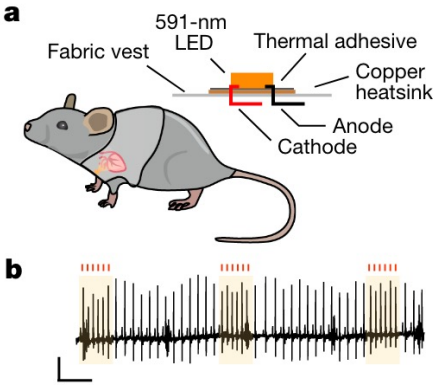
Correlations between modalities

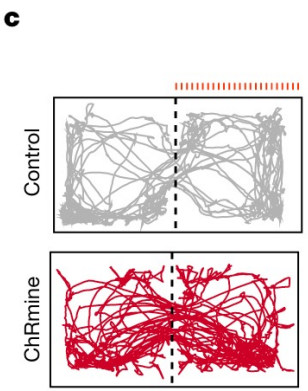


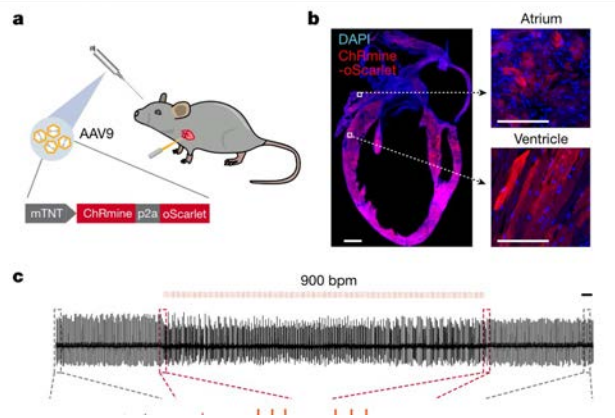
Connectivity maps comparison across scales

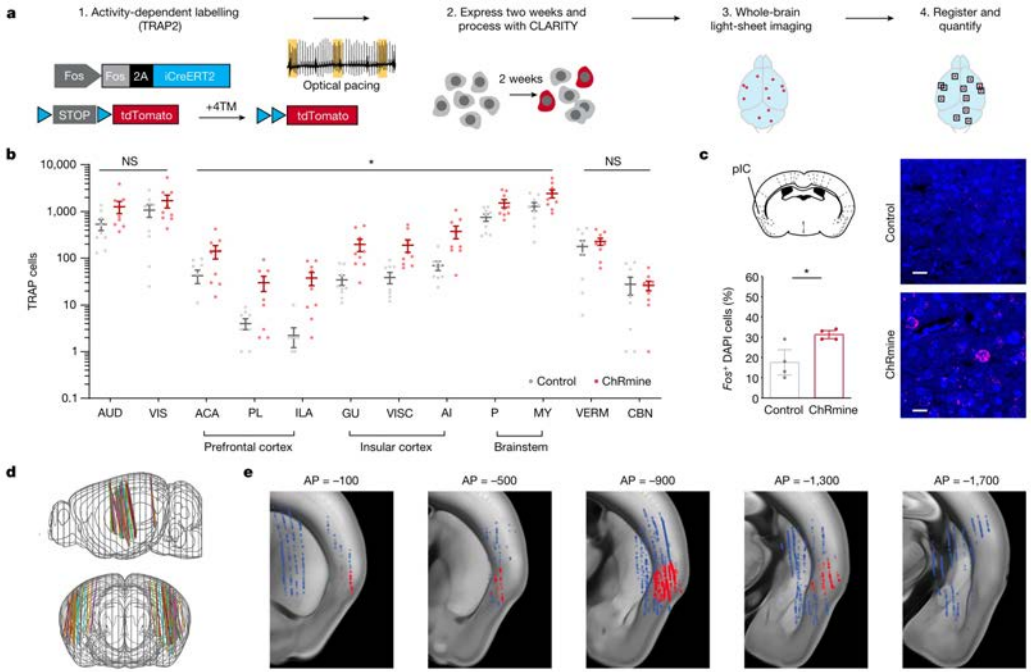

EXAMPLE WORK USING
MIRACL

BRAIN REGIONS ORCHESTRATING ANXIETY





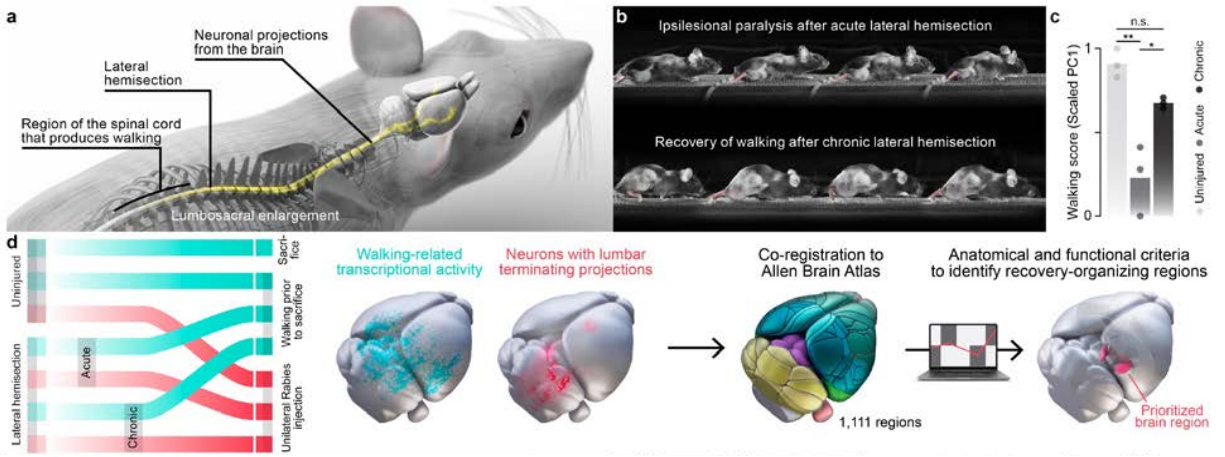


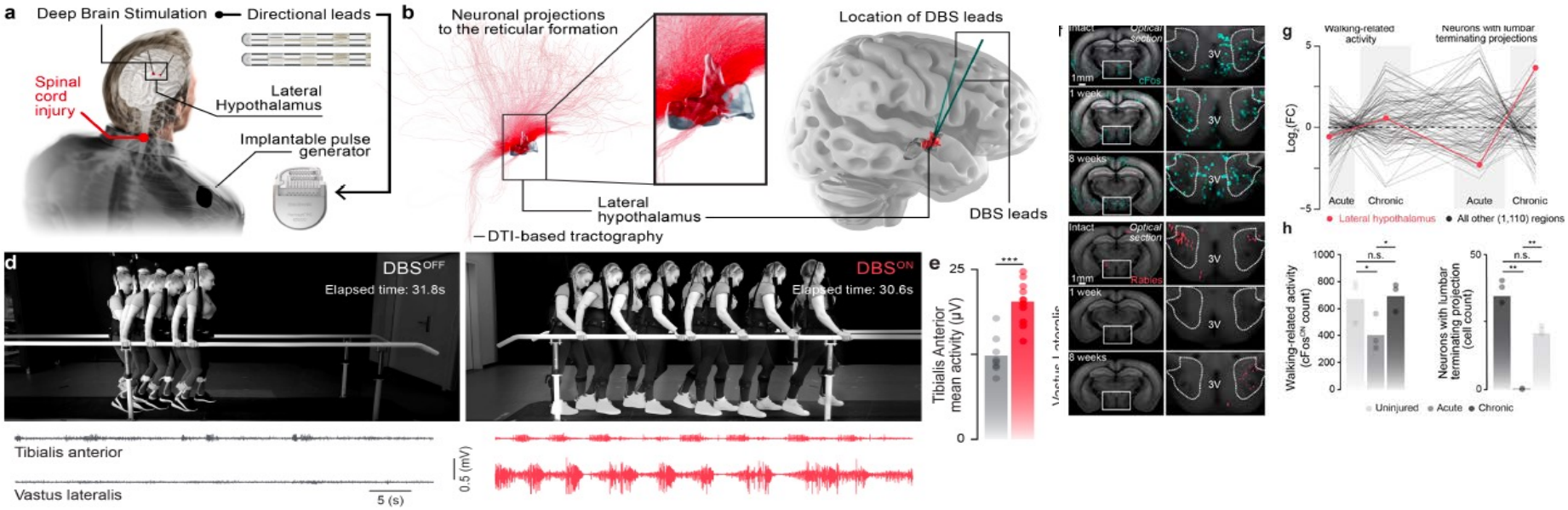


Hsueh et al. *Nature* 2023

RECOVERING WALKING AFTER SPINAL CORD INJURY

Brain regions that augment walking in spinal cord injury

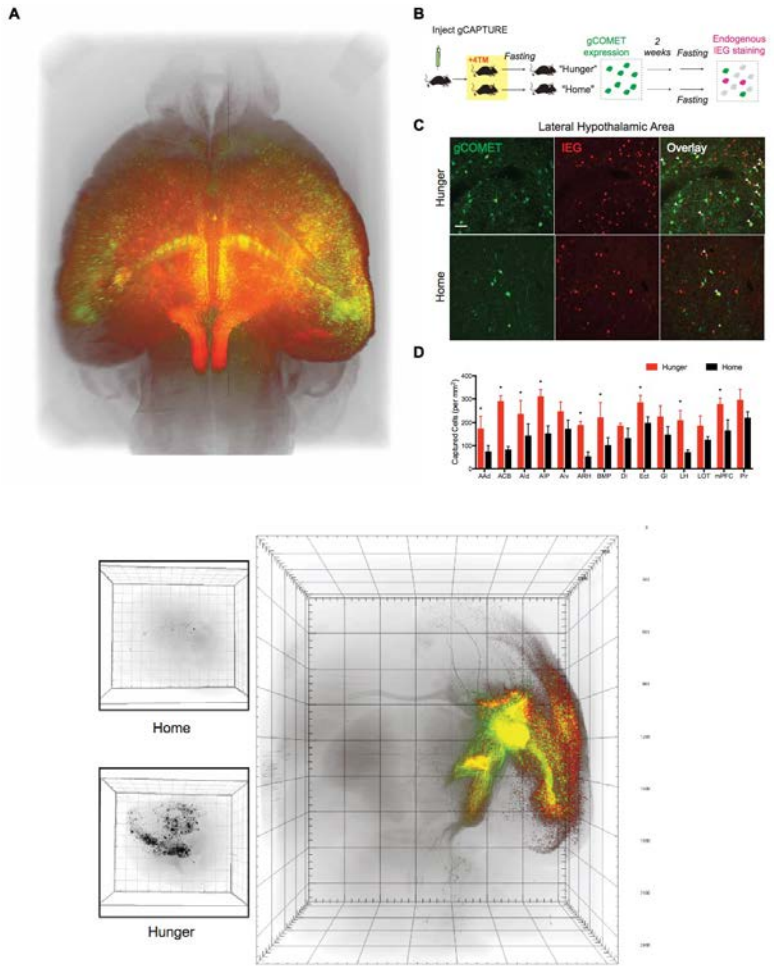




Cho et al. *Nature.* Under review

OTHER EXAMPLE STUDIES

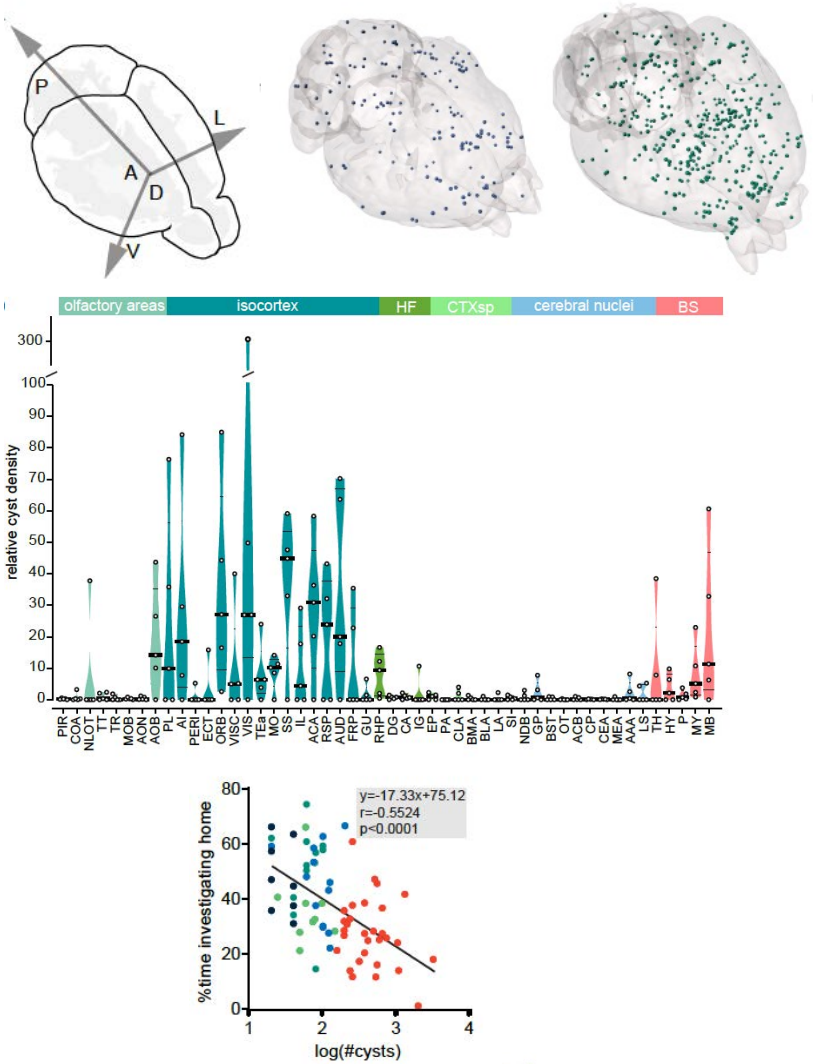
Projection mapping / Tractography



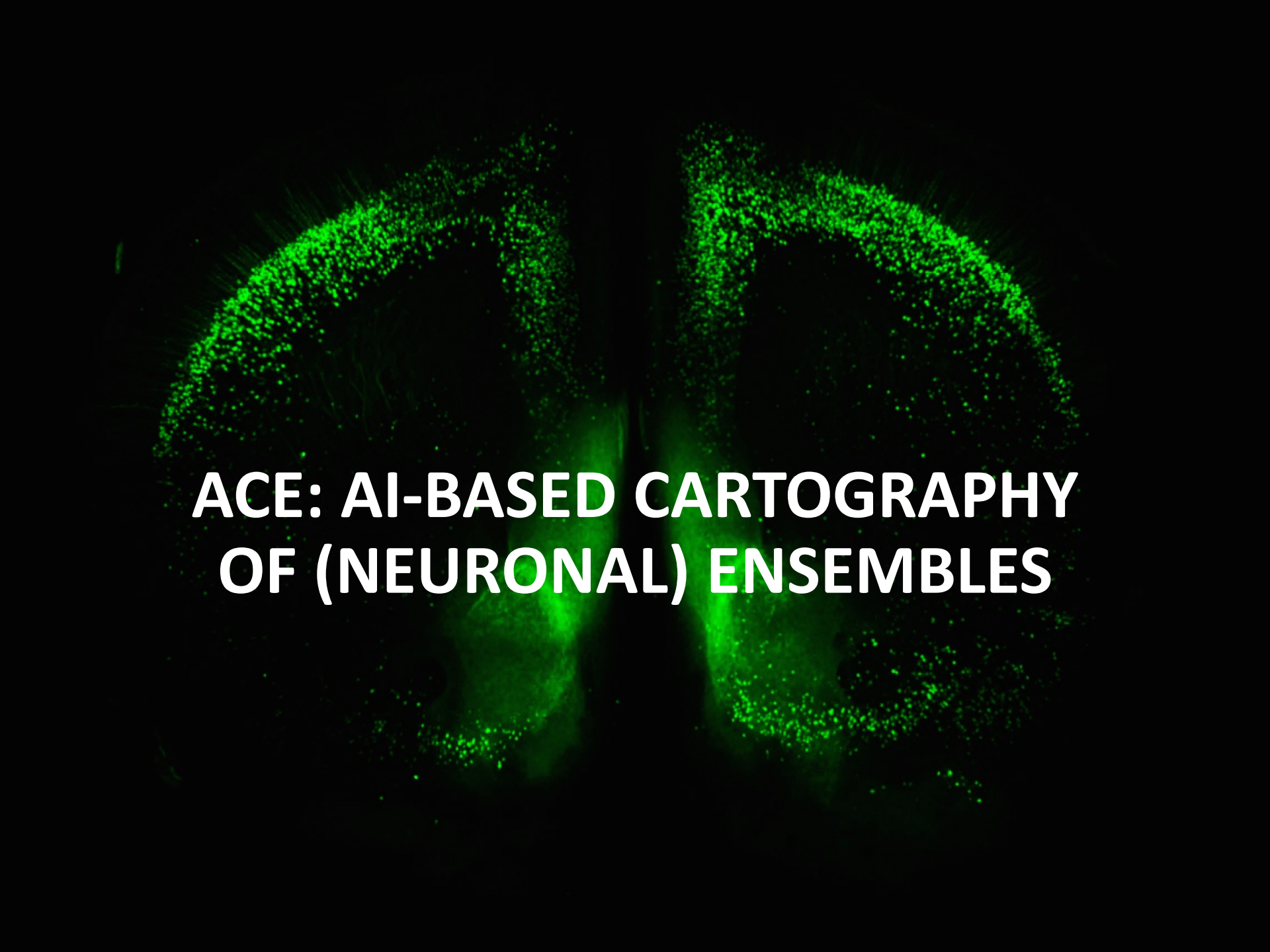
Leuze*, Goubran* et al. *Neuroimage*. 2021

Ye, Goubran et a . under review

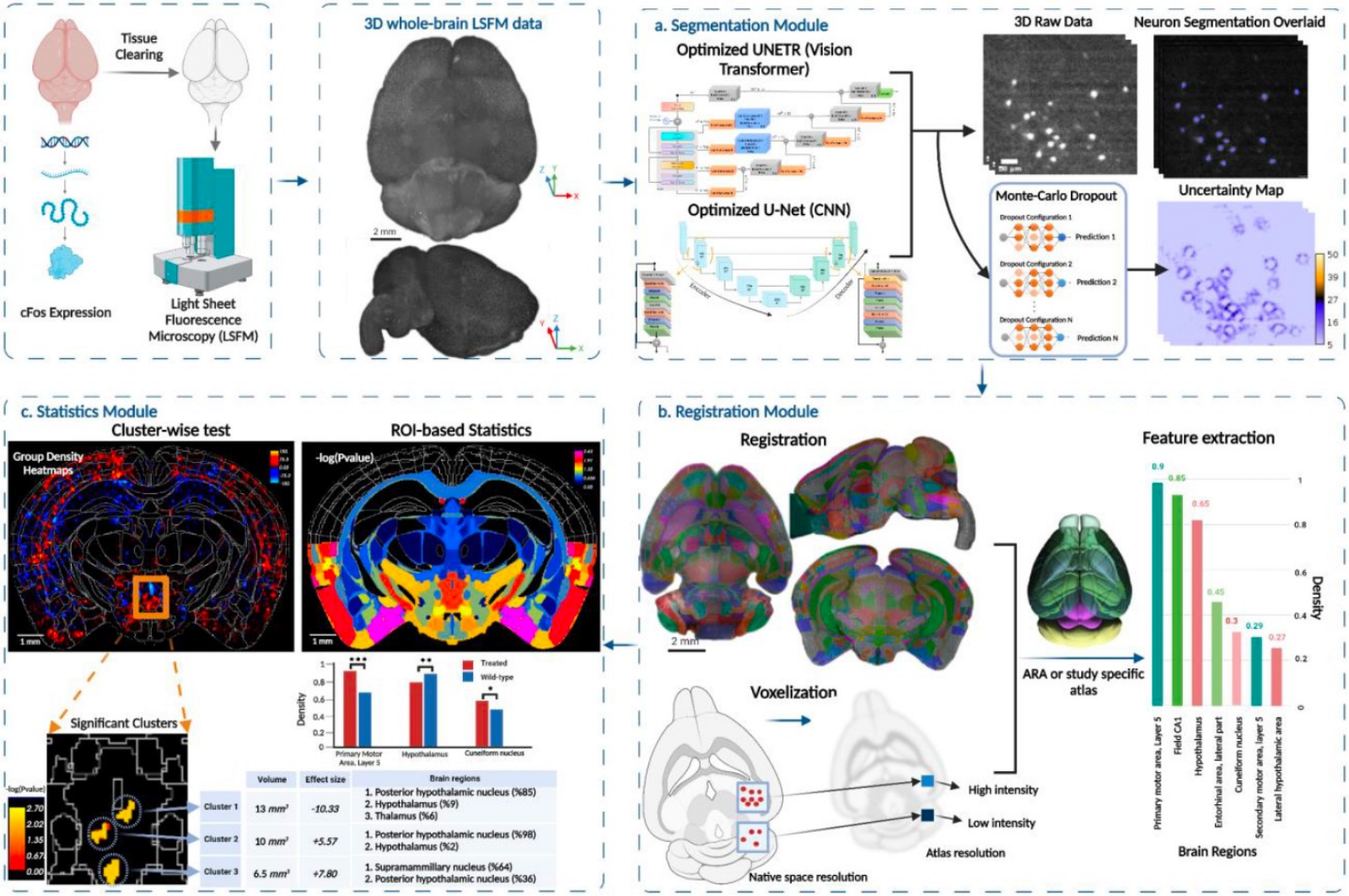
Regional mapping / Cartography



Boillat et al. *Cell reports*. 2020


ACE: AI-BASED CARTOGRAPHY
OF (NEURONAL) ENSEMBLES

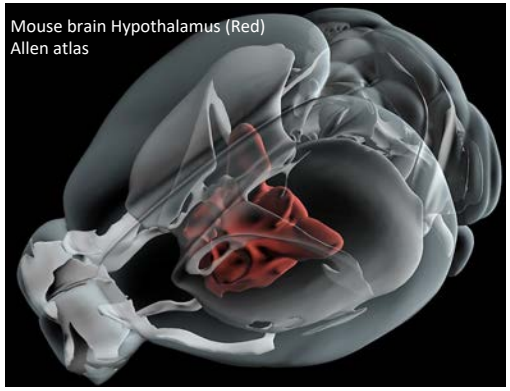
ACE: AI-BASED CARTOGRAPHY OF (NEURONAL) ENSEMBLES

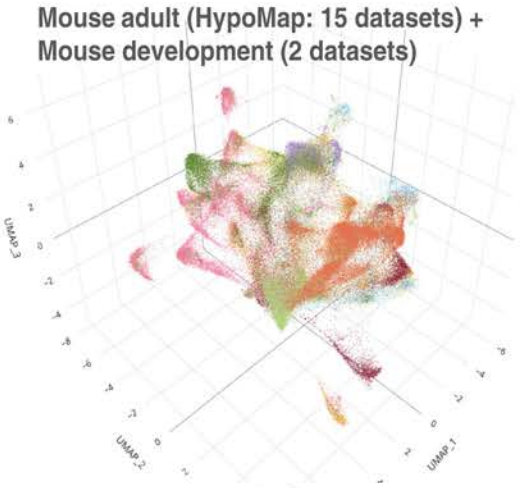


Attarpour… Goubran. *Nature Methods.* Under review

MOTIVATION BEHIND ACE

- Methodological gaps:
  - Current computational pipelines are focused on *regional* analysis, yet
  - Within-region activity is quite heterogeneous (*cf.* results of single cell genomics and transcriptomics analyses)





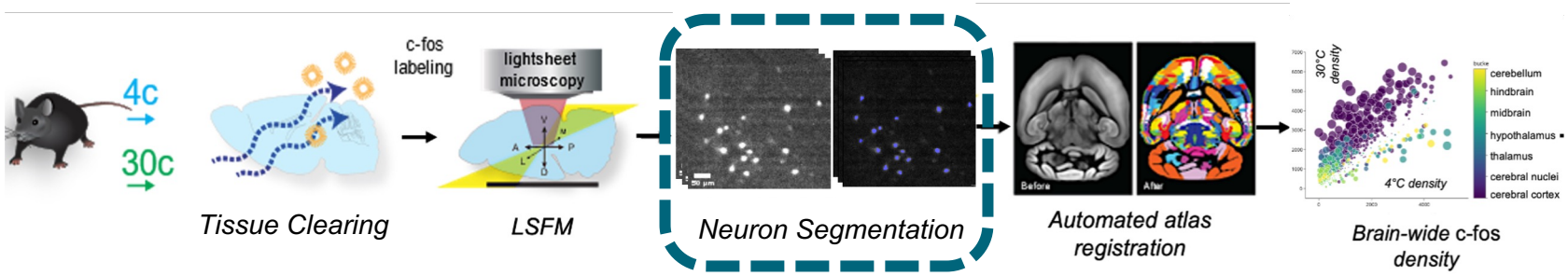
TM ARC PVH VMH DMH LH SCN PO SMN MN ZI

different hypothalamic neuronal subtypes, identified by single cell transcriptomics [1]

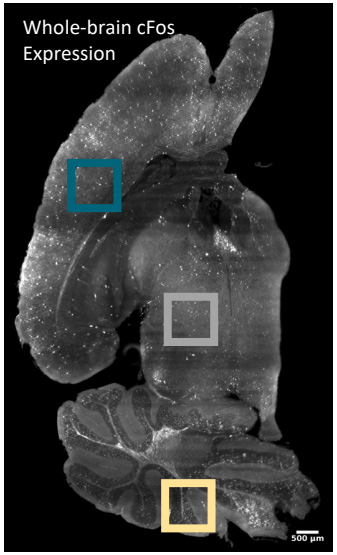
- How do we map local/sub-regional/laminar-specific activity across the brain?

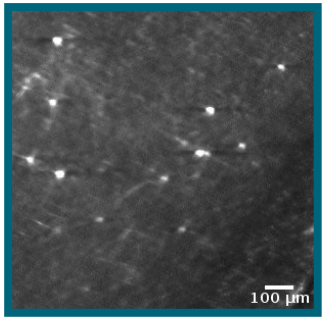
[1] Herb, et al. *Science Advances*, 2023

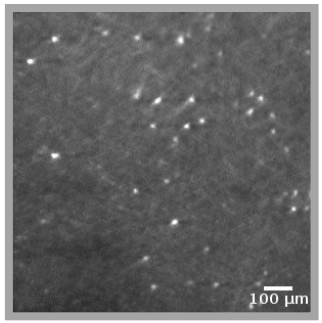
CHALLENGES OF WHOLE-BRAIN MAPPING OF NEURONAL ACTIVITY

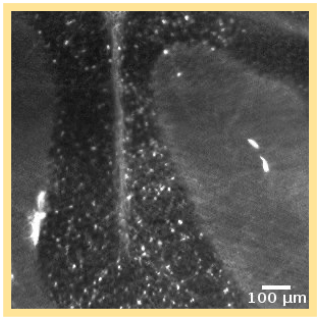


- Current segmentation tools:
  - 2D-based techniques [1, 2]
  - A small area of the brain[3]
  - Conventional processing methods[4] with limited generalizability



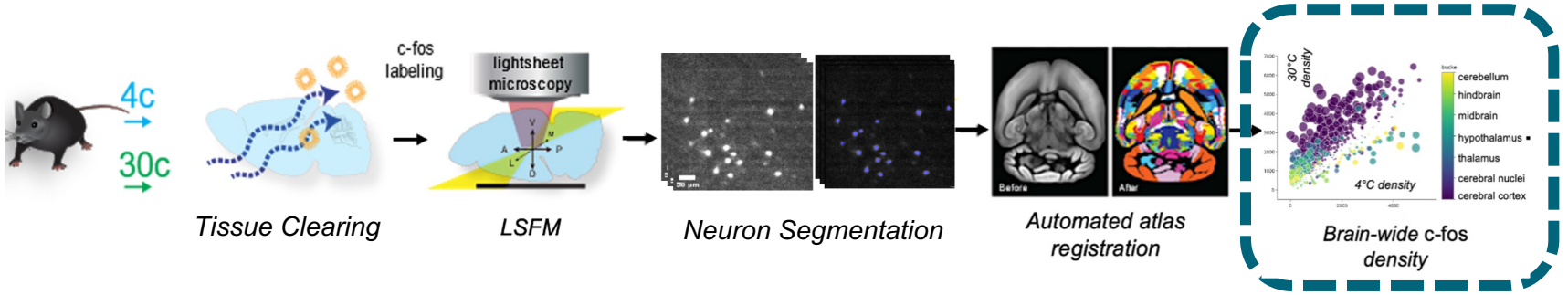






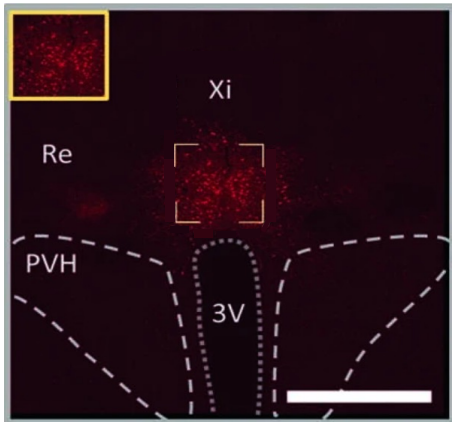
[1] Stringer, et al *Nat. Methods.* 2021 [3] Herbel, et al. *Nat. Methods*, 2018
[2] Schmidt *et al. MICCAI, 2018* [4] Berg, et al. *Nat. Methods*, 2019

CHALLENGES OF WHOLE-BRAIN MAPPING OF NEURONAL ACTIVITY



- Current pipelines (ClearMap[1] and MIRACL[2]) rely on pre-defined atlas regions:
  - Requires a priori knowledge or data-specific expertise to choose ROIs
  - Provides no insight into subregional changes

- In prior work[3], using manual identification of ROIs
  - Xiphoid: a key area for mediating food-seeking behaviors in response to cold stress
  - Xiphoid does not have pre-defined boundaries in the Allen atlas[3]

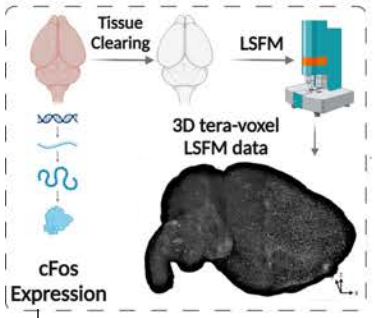


[1] Renier, et al. *Cell*, 216
[2] Goubran *et al. Nat Commun, 2019*
[3] Lal *et al. Nat,* 2023

Aim

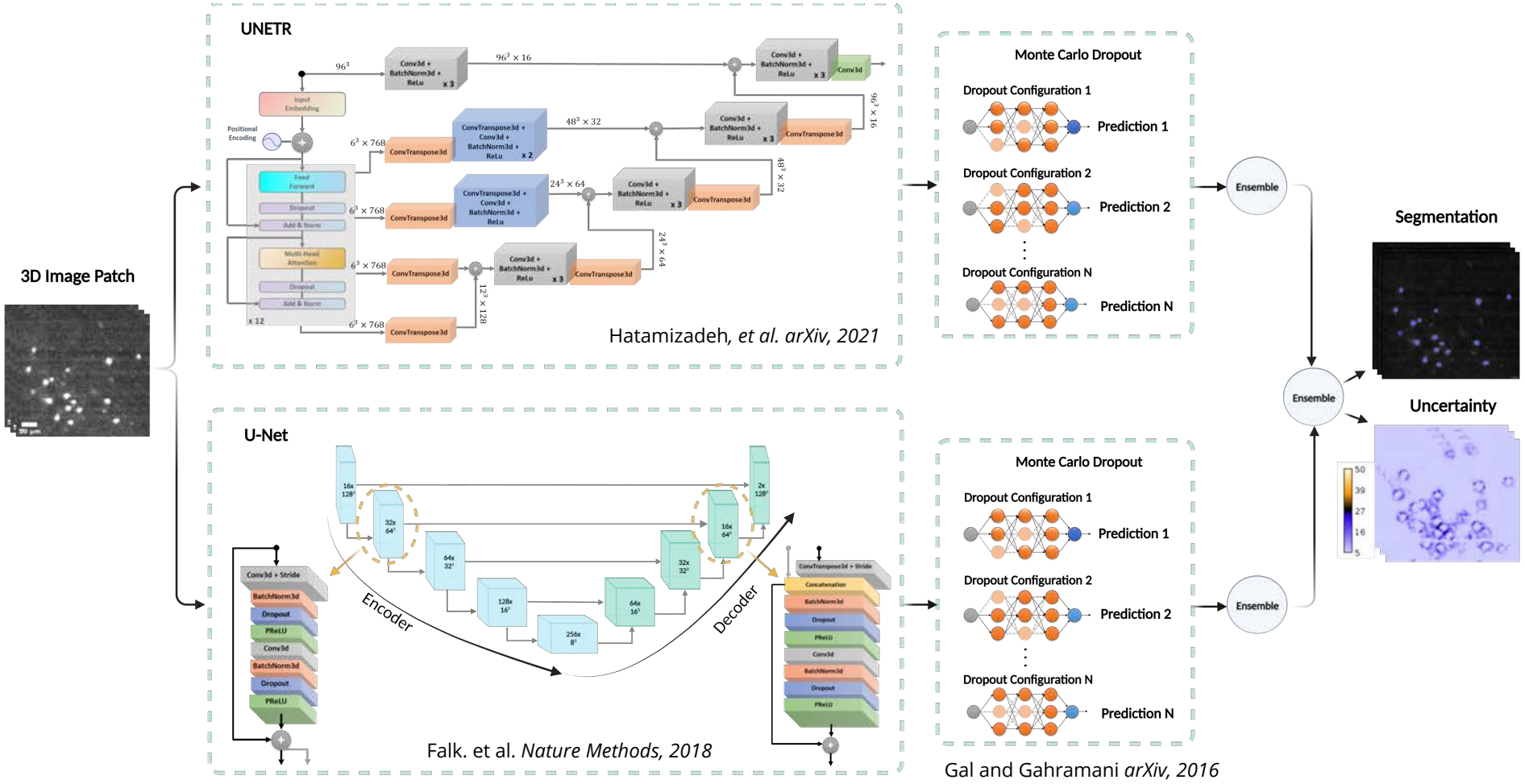
A deep learning pipeline for **3D** mapping of **local** neuronal activity in **tera-voxel** light sheet microscopy data

AI-BASED CARTOGRAPHY OF ENSEMBLES (ACE)



ACE
AI-based
Cartography of
Ensembles

ACE'S SEGMENTATION CORE



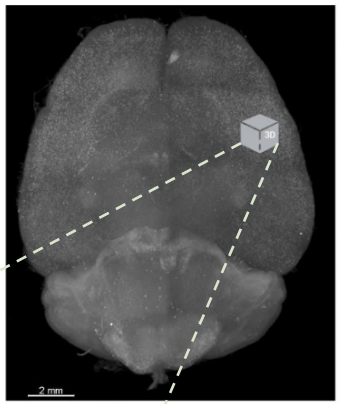
MODEL TRAINING & DATASETS

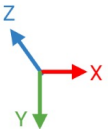
- **LSFM data acquired from 18 TG mice (TRAP2-Ai9 mice: tamoxifen dependent expression of cFos[1])**

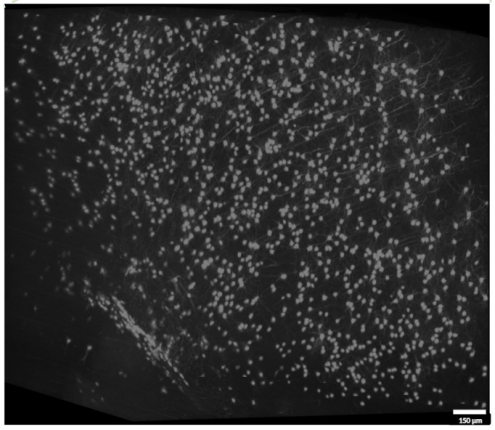
- **Image specifications:**
  - Matrix size: ~ 2800x5500x2100 Voxels
  - File Size: ~ 1 TB per subject
  - Resolution: 3.5x3.5x4 µm
  - 10/3/5 subjects to train/validate/test
  - each patch: 96^3 voxels ~ $0.35x0.35x0.35\ \text{mm}^3$
  - **N=30,400 patches** > 5x existing models

- **Semi-automated ground truth generation of cFos positive neurons (silver standard)**:
  - 3D Watershed Algorithm[2] - precision
  - Ilastik[3] - sensitivity
  - Shape filter[4] – large object removal

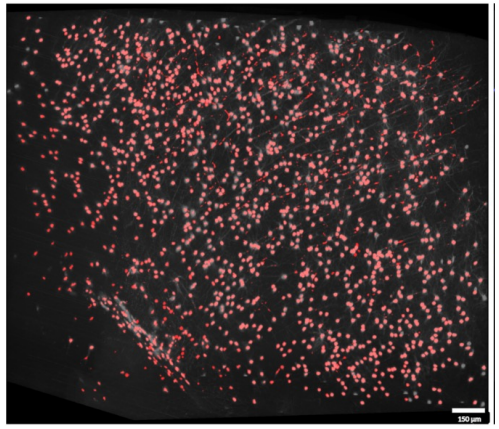
Whole brain cFos expression







3D image patch $\mathbf{1.8{\times}1.8{\times}2\ mm}$



Ground truth (red)

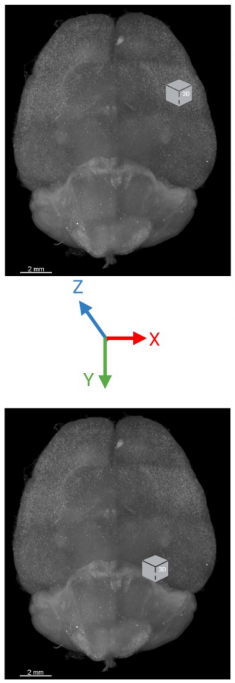
[1] DeNardo *et al. Nat Neuroscience, 2019*
[2] Goubran *et al. Nat Commun, 2019*
[3] Berg, *et al. Nat. Methods, 2019*
[4] Schindelin, et al. *Nat. Methods, 2012*

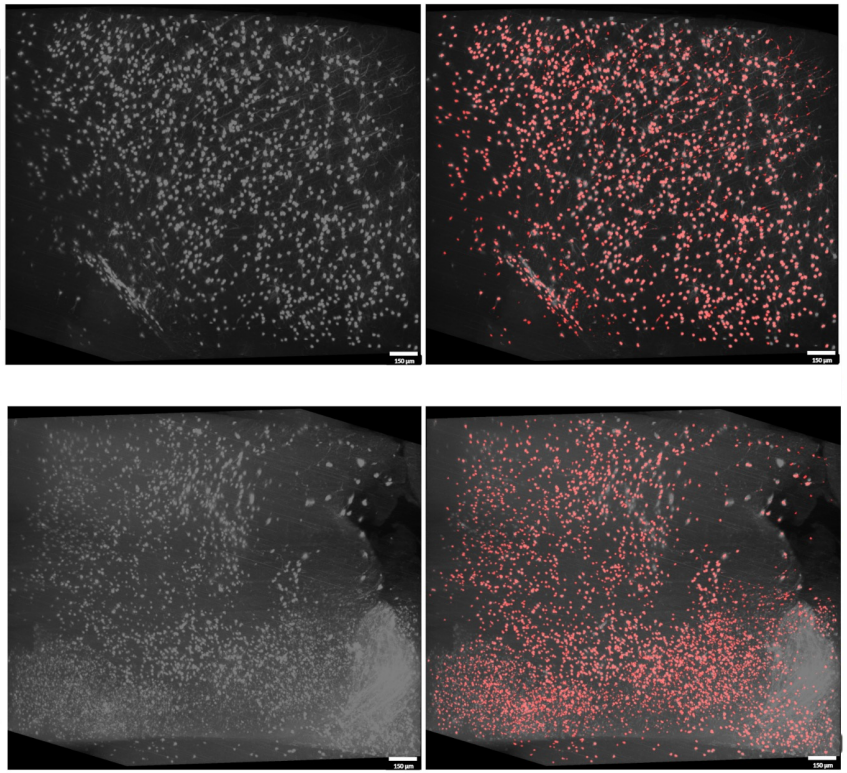
SEGMENTATION RESULTS

Whole-brain cFos expression

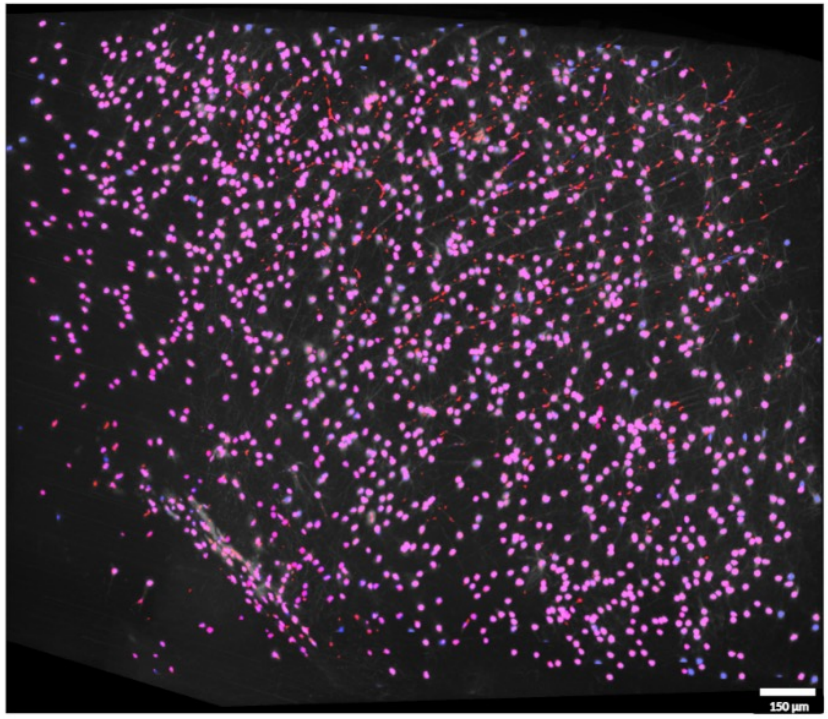
3D image patch 1.8x1.8x2 mm

Ground truth (Red)





Overlay (Purple) / Dice = 0.78



Blue (detected by models; missed in GT)
Red (FP exists in GT)

COMPARISON TO STATE-OF-THE-ART METHODS

$$DSC(GT, P) = 2 \times \frac{|GT \cap P|}{|GT| + |P|}$$

$$HD(X,Y) = max\{sup_{x \in X} inf_{y \in Y} d(x,y), sup_{y \in Y} inf_{x \in X} d(y,x)\}$$

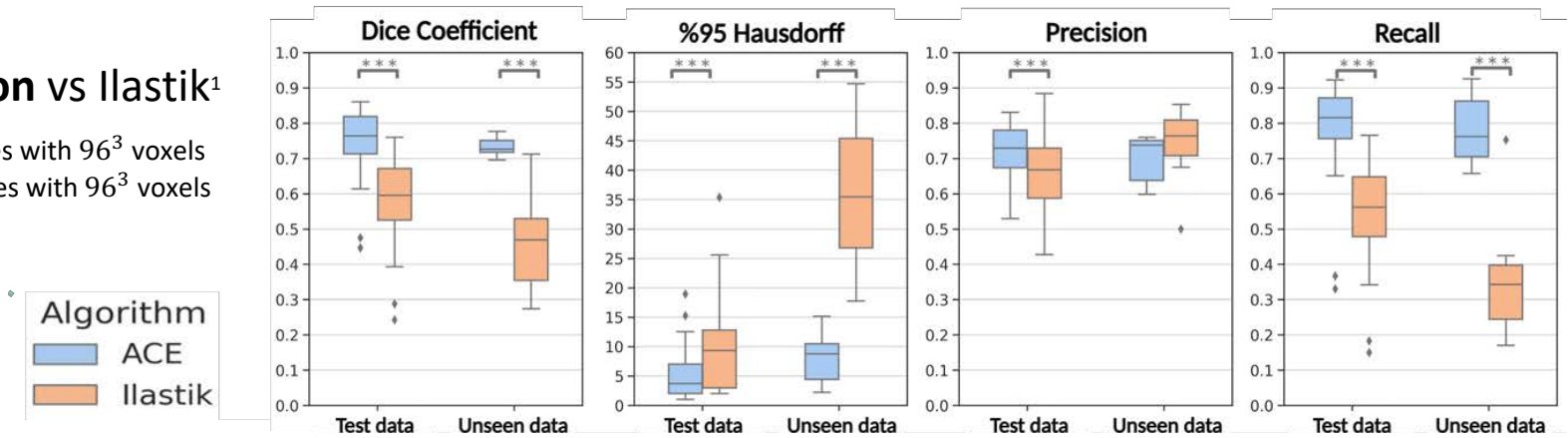
$$Precision = \frac{TP}{TP+FP}$$

$$\text{Recall} = \frac{TP}{TP+FN}$$

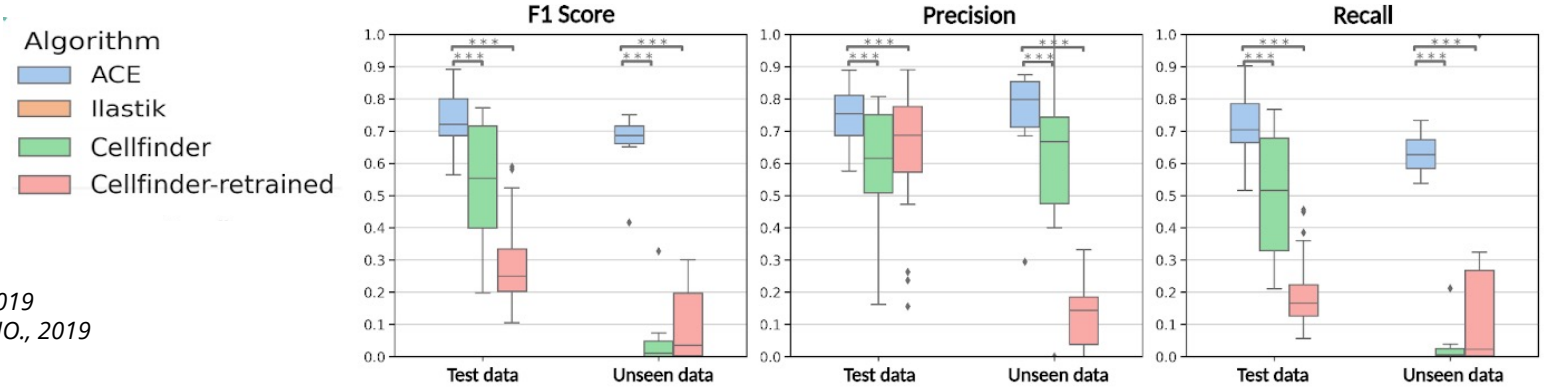
$$\text{F1-score} = \frac{2 \times Precision \times Recall}{Precision + Recall}$$

ACE **Segmentation** vs Ilastik[1]

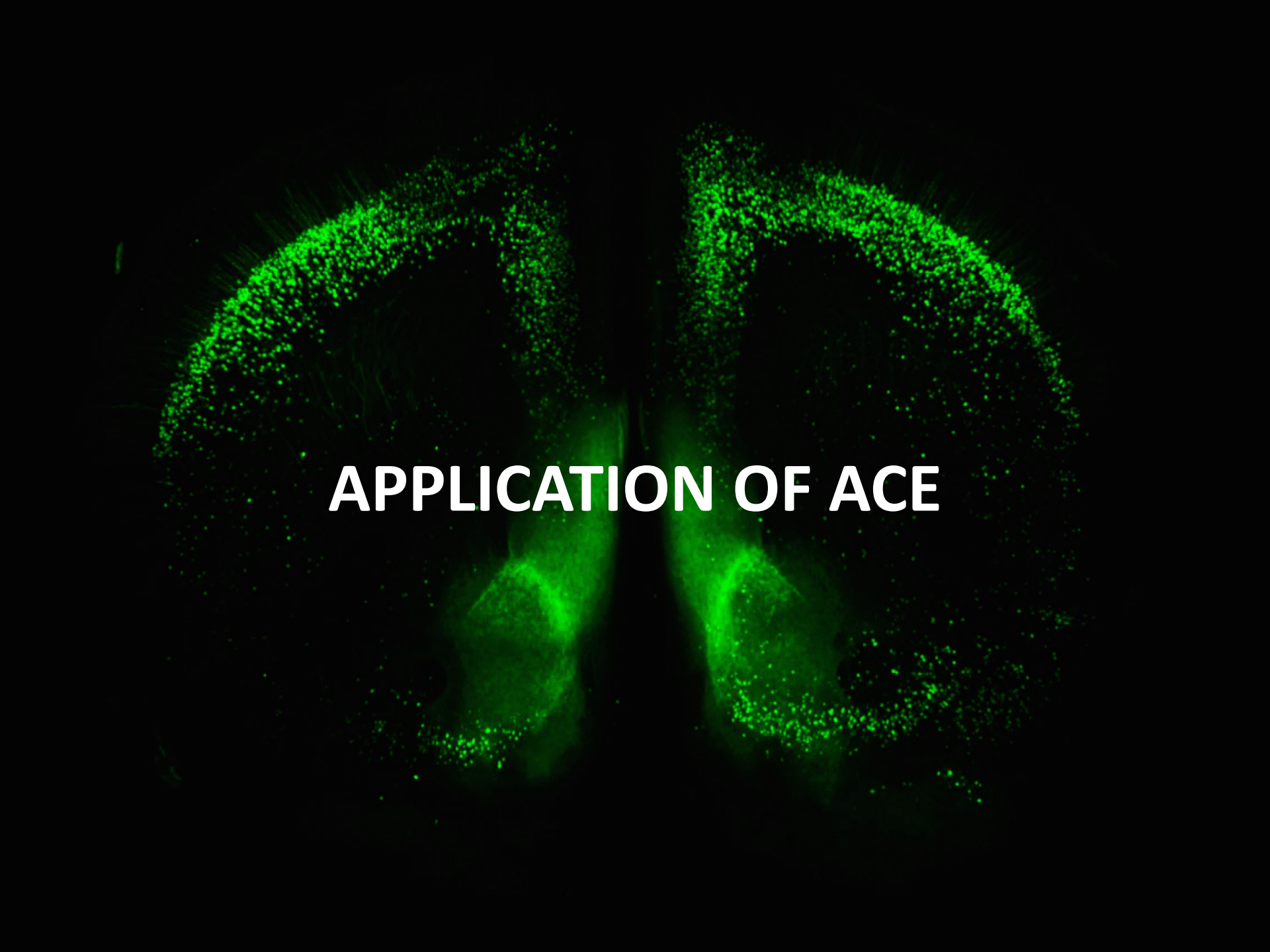
N_{test}: 12,160 unique patches with 96^3 voxels
N_{unseen}:1820 unique patches with 96^3 voxels



ACE **Detection** mode vs Cellfinder[2]



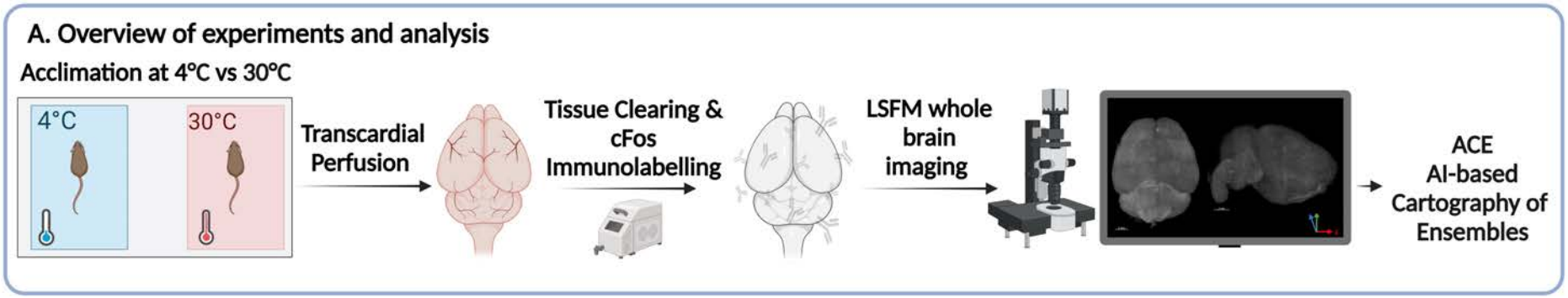
[1] Berg *et al. Nat Method, 2019*
[2] Tyson *et al. PLOS COM. BIO., 2019*


APPLICATION OF ACE

MAPPING OF NEURONAL ENSEMBLES UNDERLYING COLD-INDUCED FOOD SEEKING

- Aim: map neuronal activations underlying food-seeking behavior in response to cold stress[1]

- Wild-type C57BL/J6 mice exposed to either 4°C (cold) or 30°C (thermoneutral) for 6 hrs
- c-*Fos* staining
- N = 4/group
- Image size: ~ 8000x6000x3000 voxels
- Image resolution: 1.75x1.75x4 µm

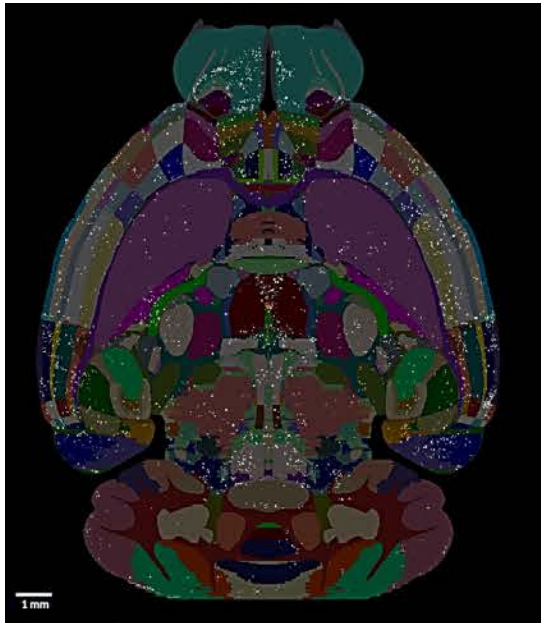


[1] Lal, *et al. Nature, 2023*

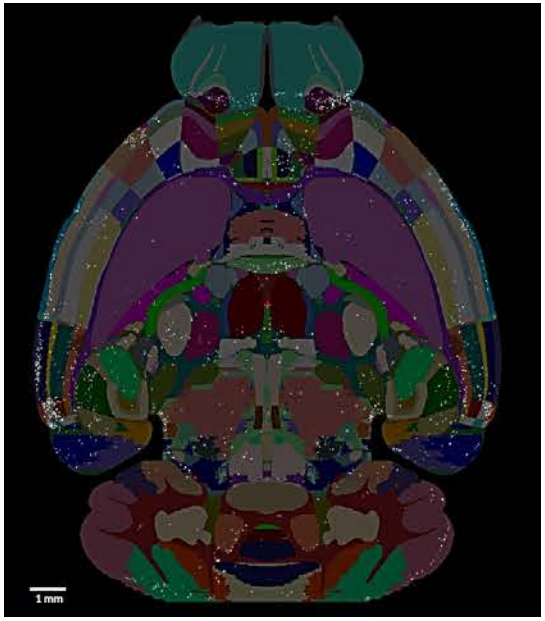
MAPPING OF NEURONAL ENSEMBLES UNDERLYING COLD-INDUCED FOOD SEEKING

- segment:
  - ACE UNET/UNETR ensemble of ensembles
- register:
  - Interface to MIRACL[1]
- voxelize & warp to the Allen[2] atlas (10 um)
- Generate group density heat maps

4c Subject

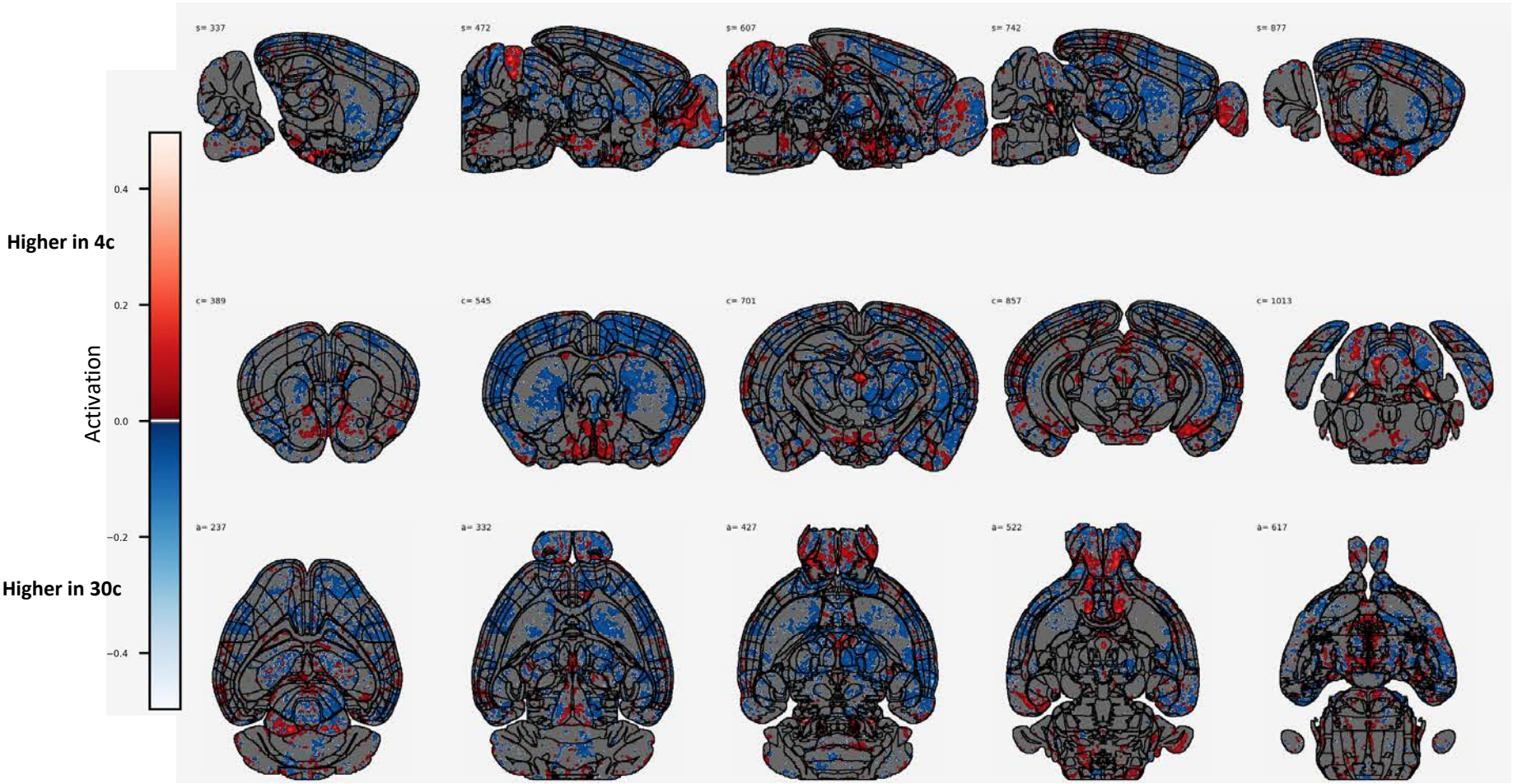


30c Subject



[1]Goubran *et al. Nat Commun, 2019*
[2] Wang, et al. *Cell*, 2020

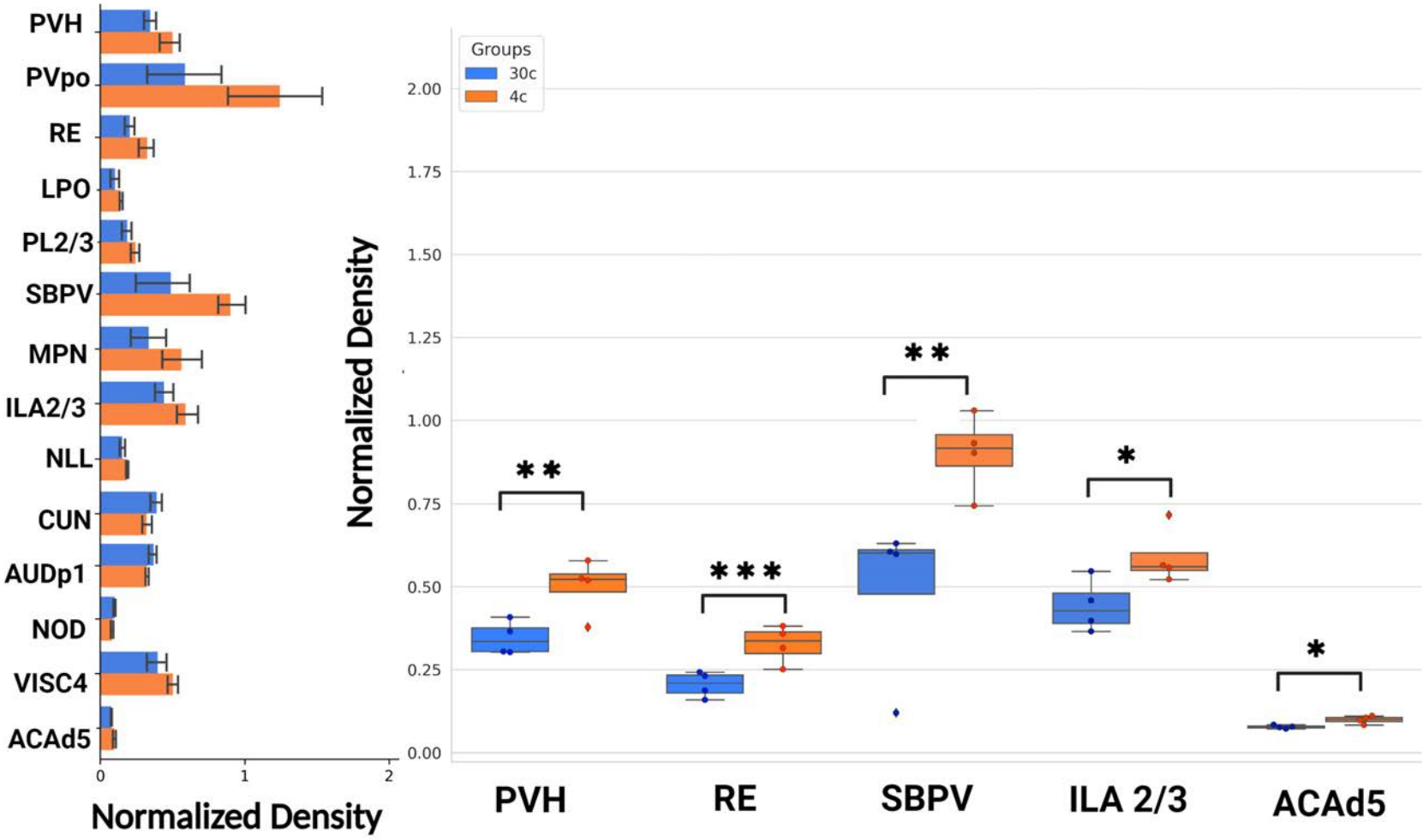
GROUP NEURONAL (C-FOS) DENSITY HEATMAPS



ROI-BASED STATISTICAL ANALYSIS (USING ARA LABELS)

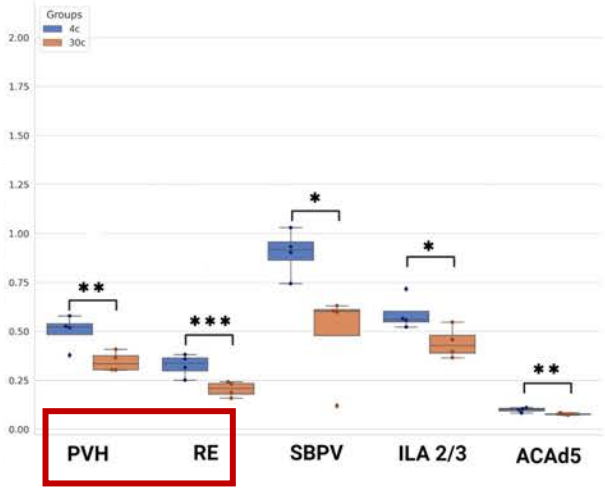
Trending regions

Significant regions

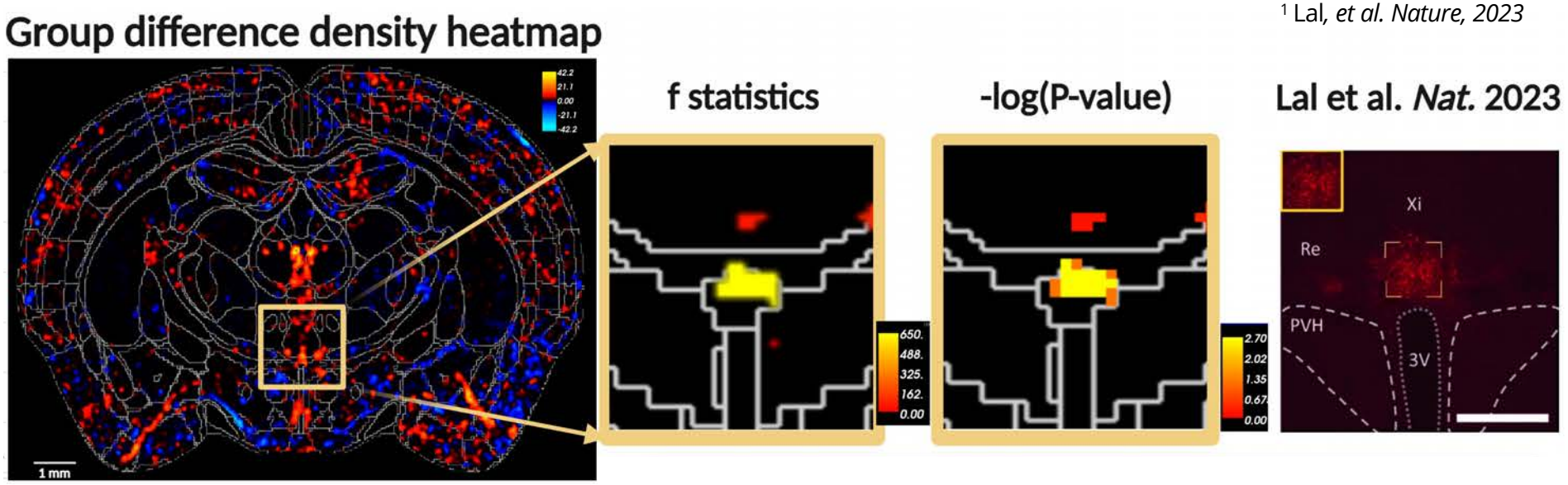


CLUSTER-WISE ANALYSIS DETECTS *LOCAL* NEURONAL ENSEMBLES UNDERLYING FOOD-SEEKING BEHAVIOR DURING COLD STRESS

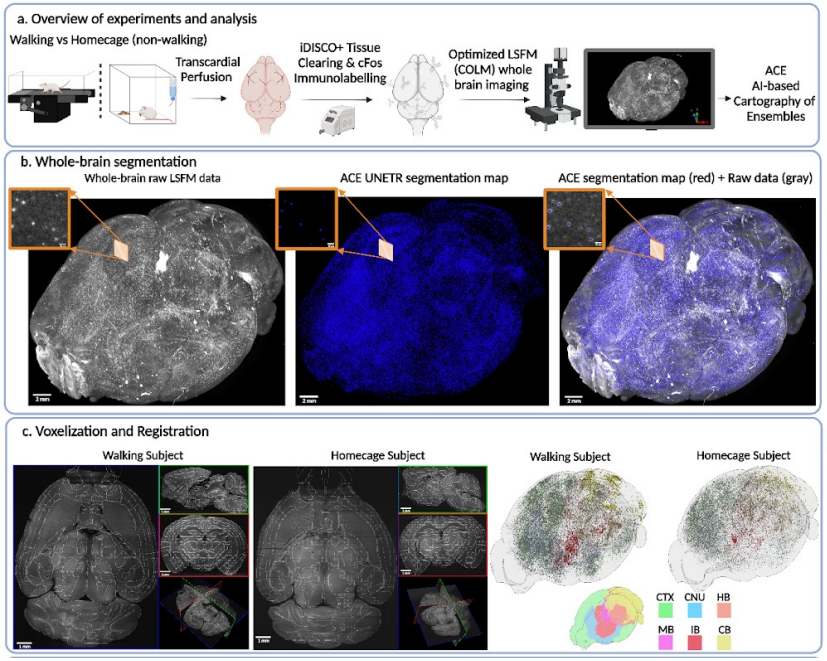
- map *localized* differences (w excellent sensitivity) in c-Fos in an atlas-agnostic manner by a cluster-wise threshold-free cluster enhancement permutation test using a group-wise ANOVA
- detect a cluster in Xiphoid - a small nucleus in midline thalamus:
  - no pre-defined boundary in Allen Atlas
  - proximal to paraventricular hypothal nuc (PVH) & nuc of reunions (RE)
  - selectively activated by prolonged cold exposure[1]

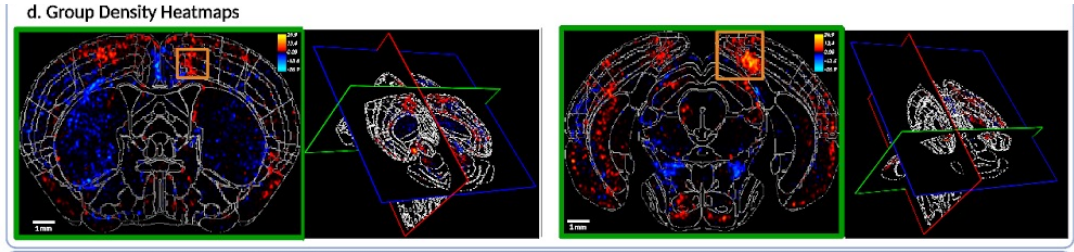


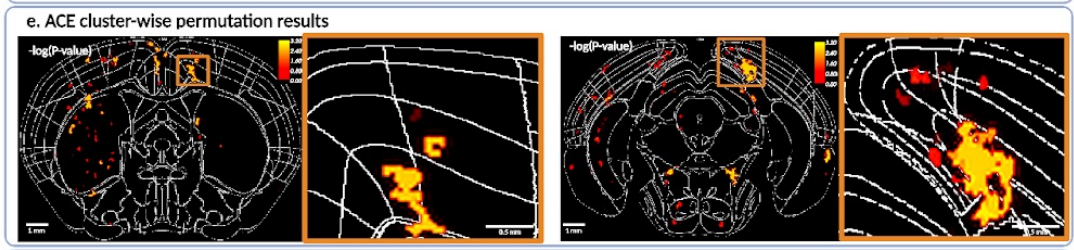
[1] Lal, *et al. Nature, 2023*

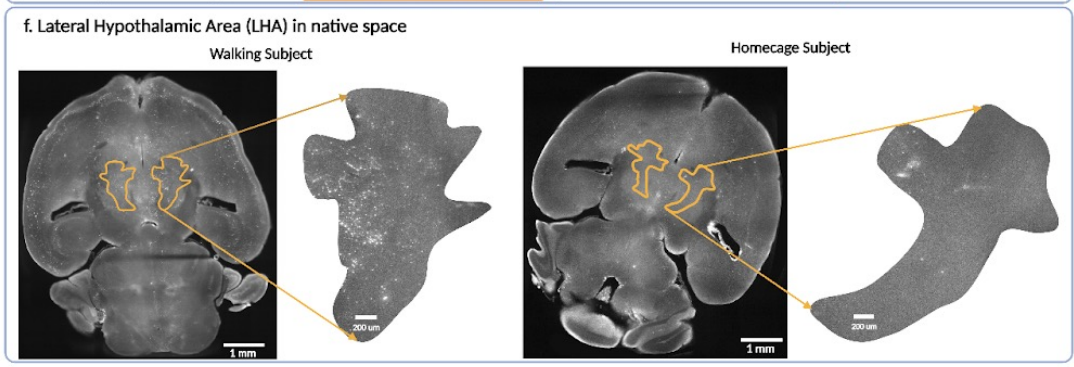


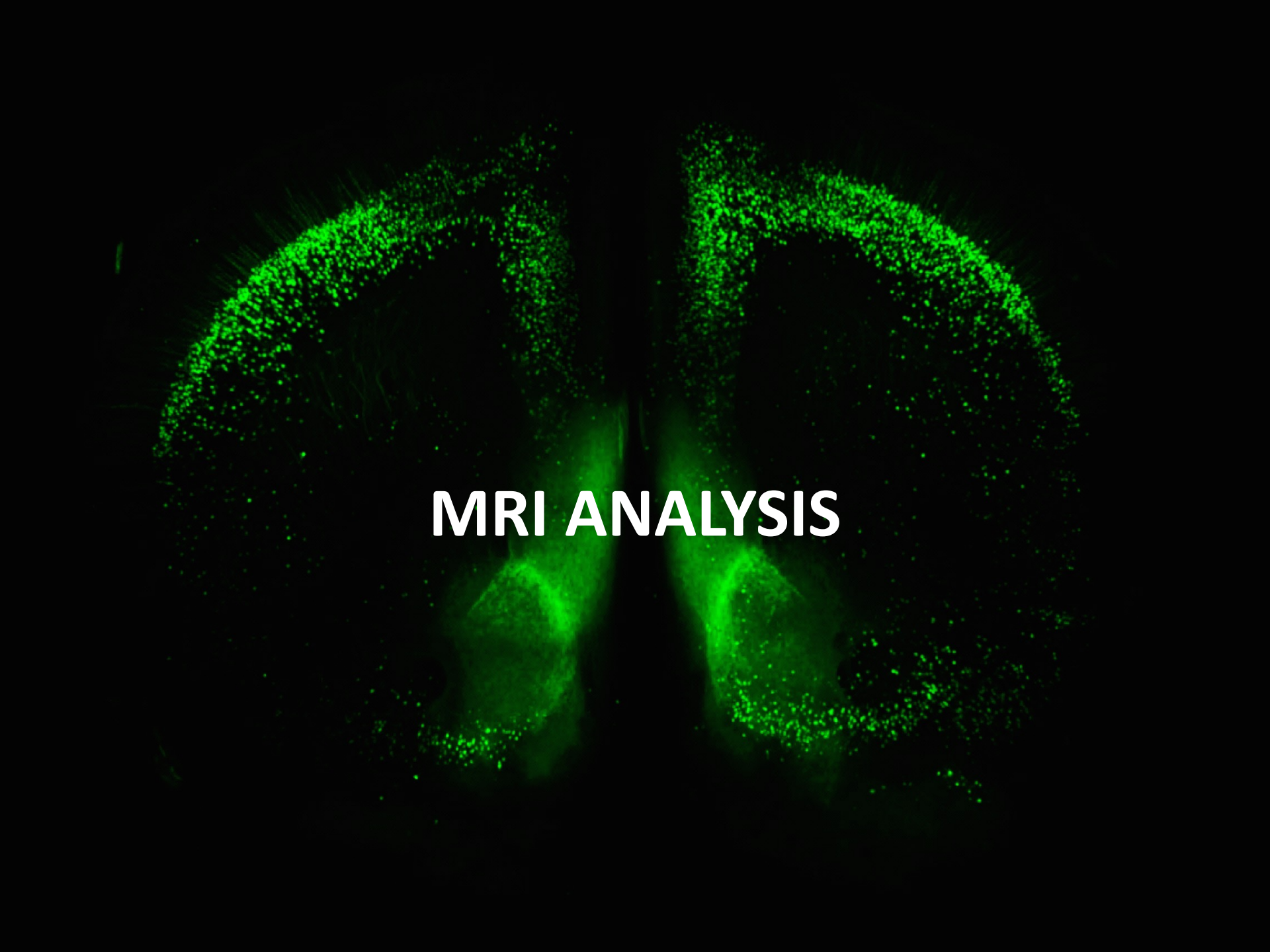
APPLICATION OF ACE: WALKING VS HOMECAGE



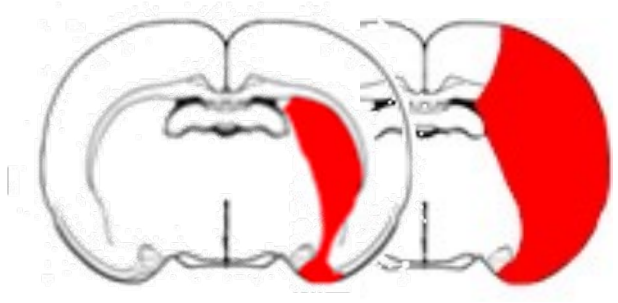







MRI ANALYSIS

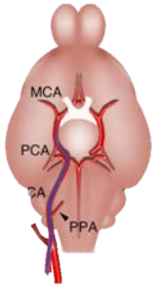
STROKE MODEL & STUDY DESIGN



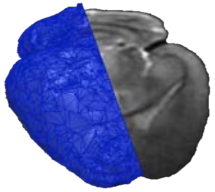
10

10

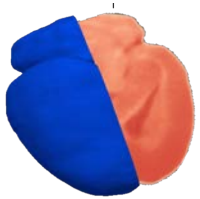
Thy1-YFP mice



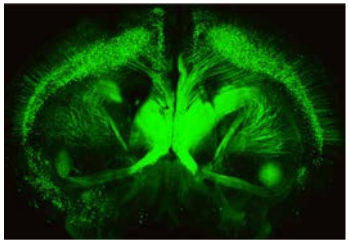
MCAO model



In-vivo MRI



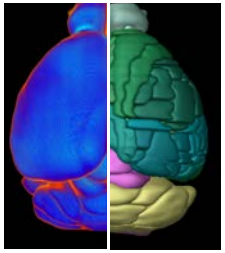
Ex-vivo CT



Clarity

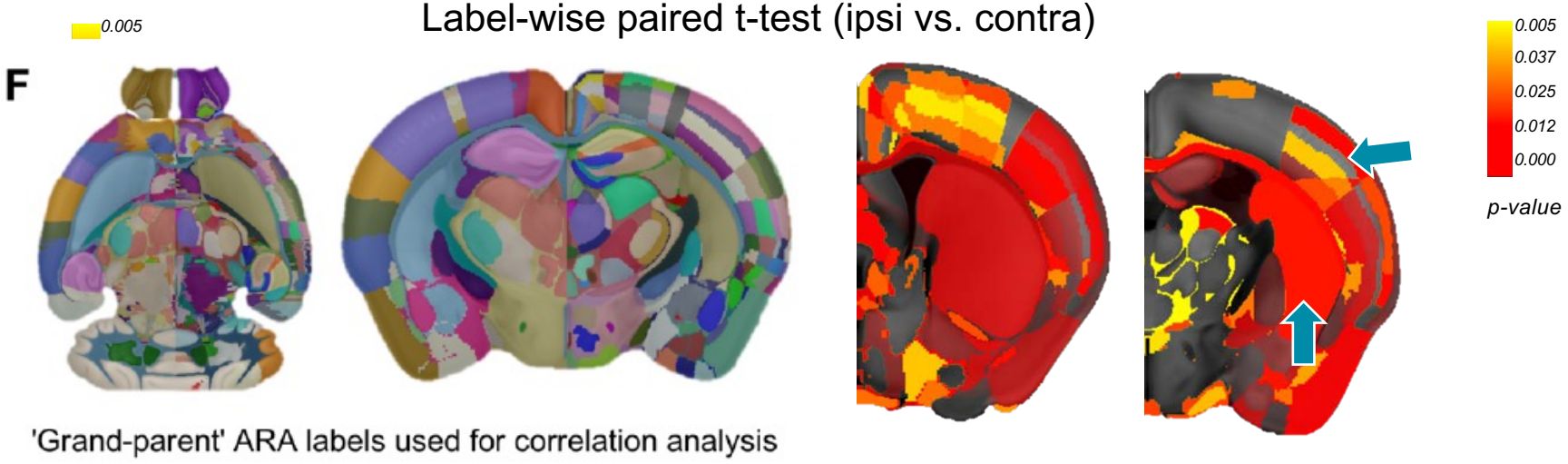
T1 & T2 maps

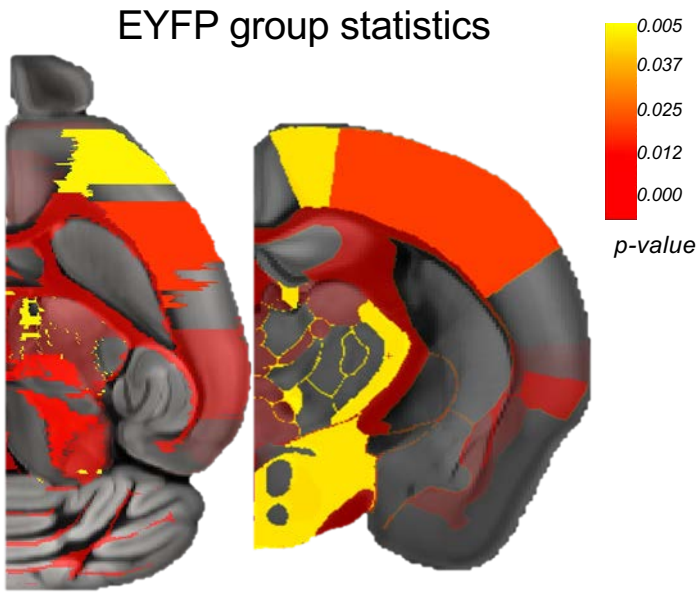
DTI

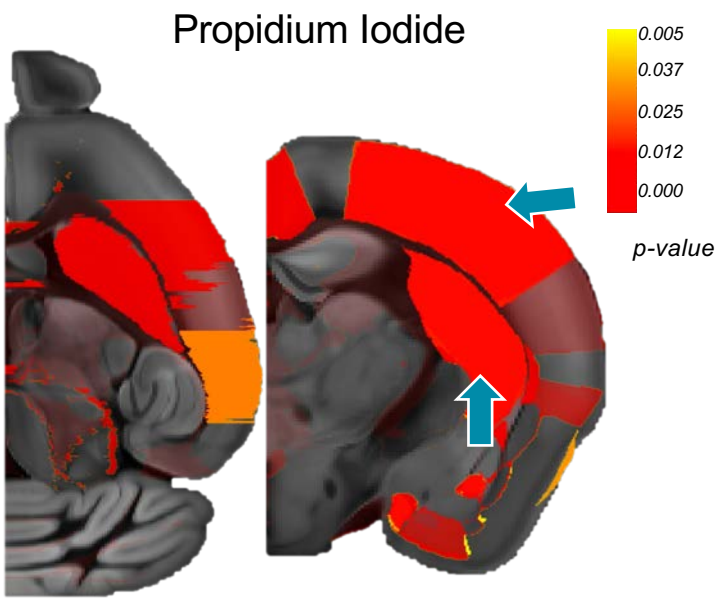


Allen Brain Atlas

IPSILESIONAL VS. CONTRALESIONAL STATISTICS



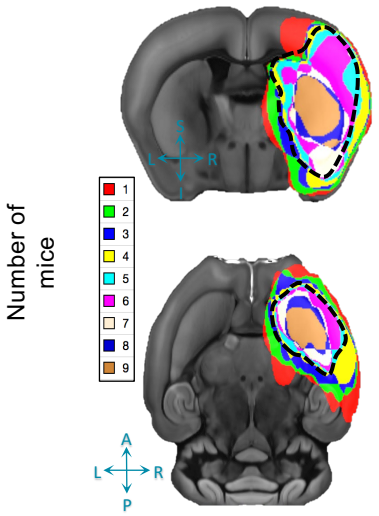




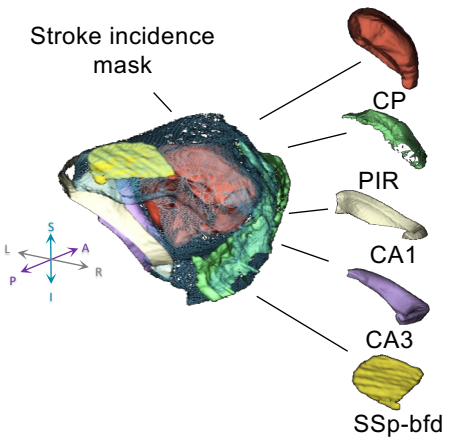
Quantitative T2

Mean Diffusivity

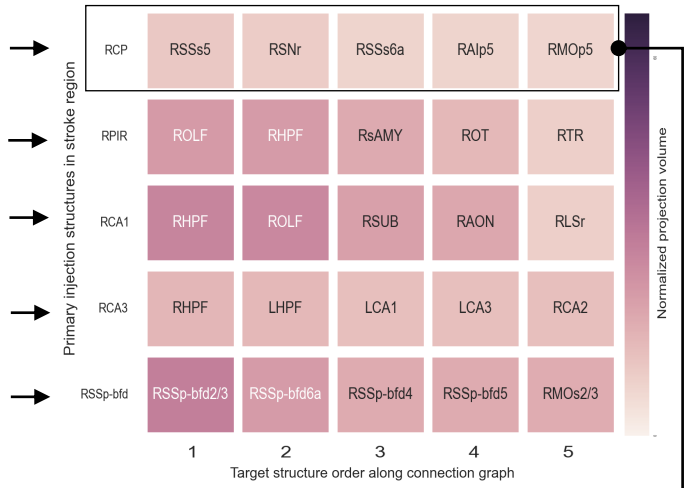
CELL DENSITY IN CONNECTED REGIONS TO THE STROKE



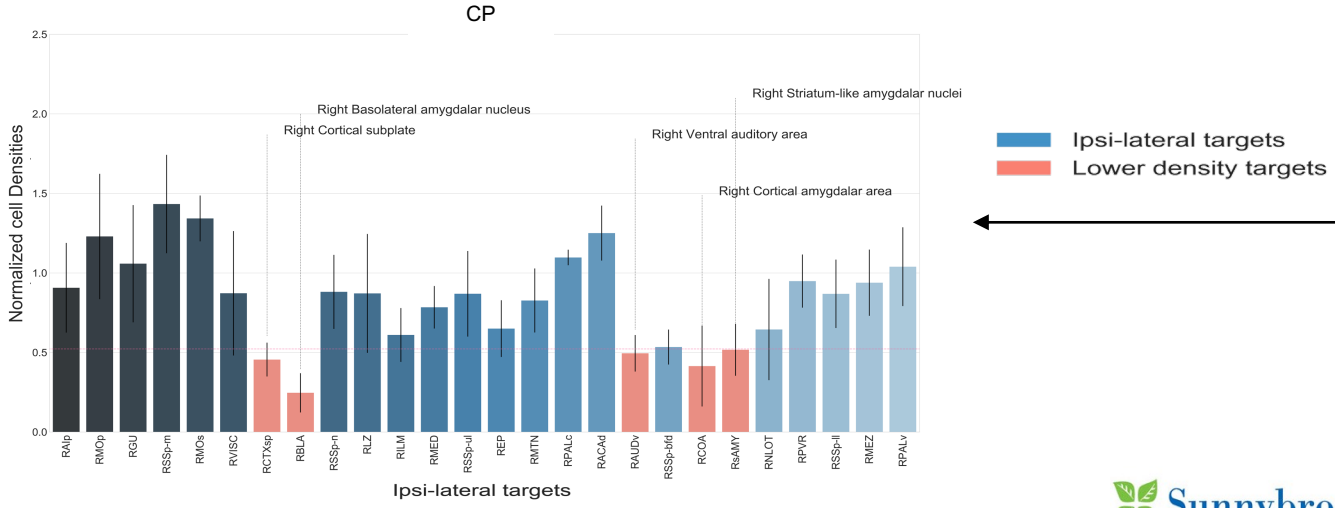
Stroke incidence atlas



Extraction of labels in stroke region

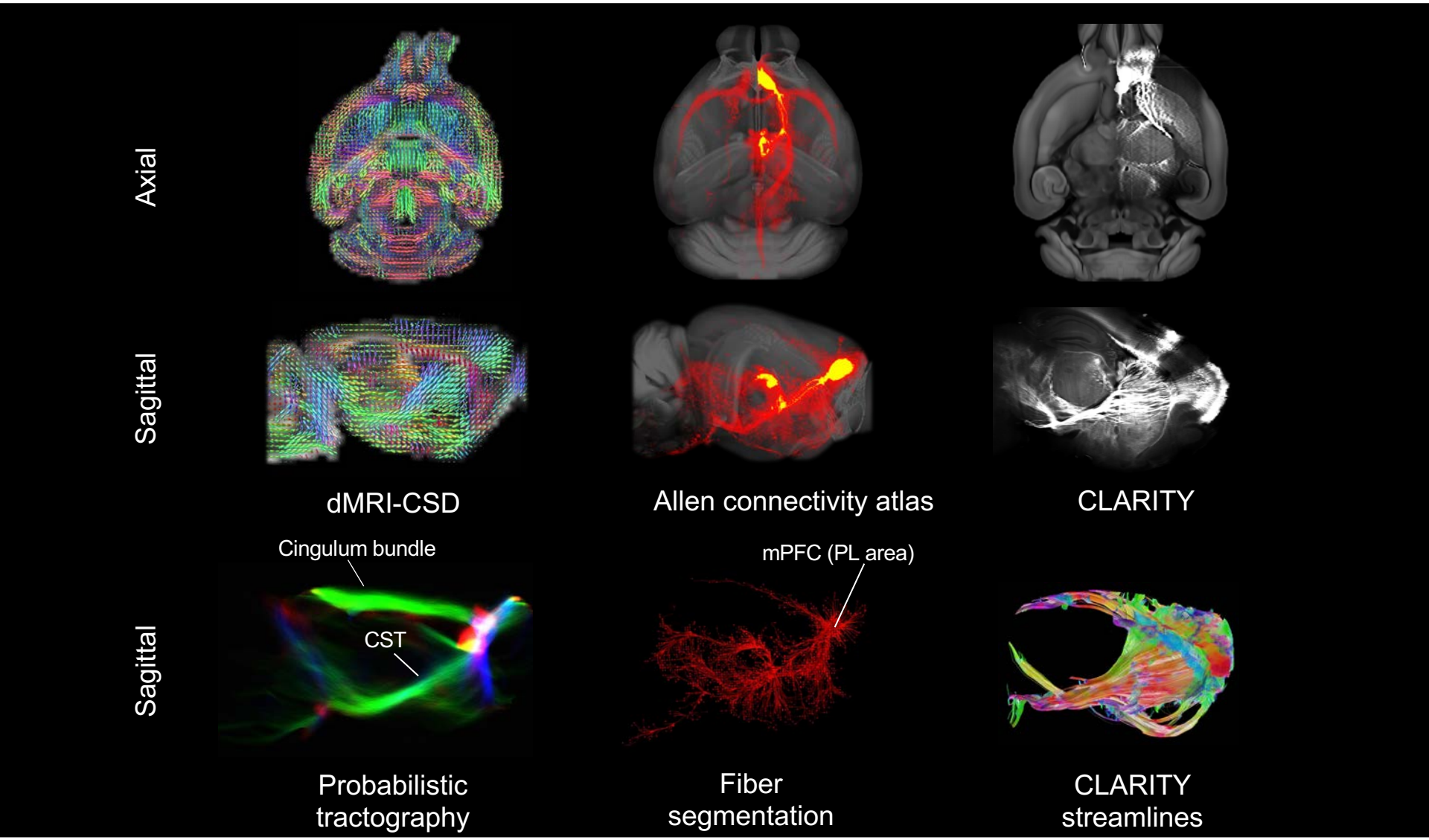


Connectivity projection map of largest labels in stroke region



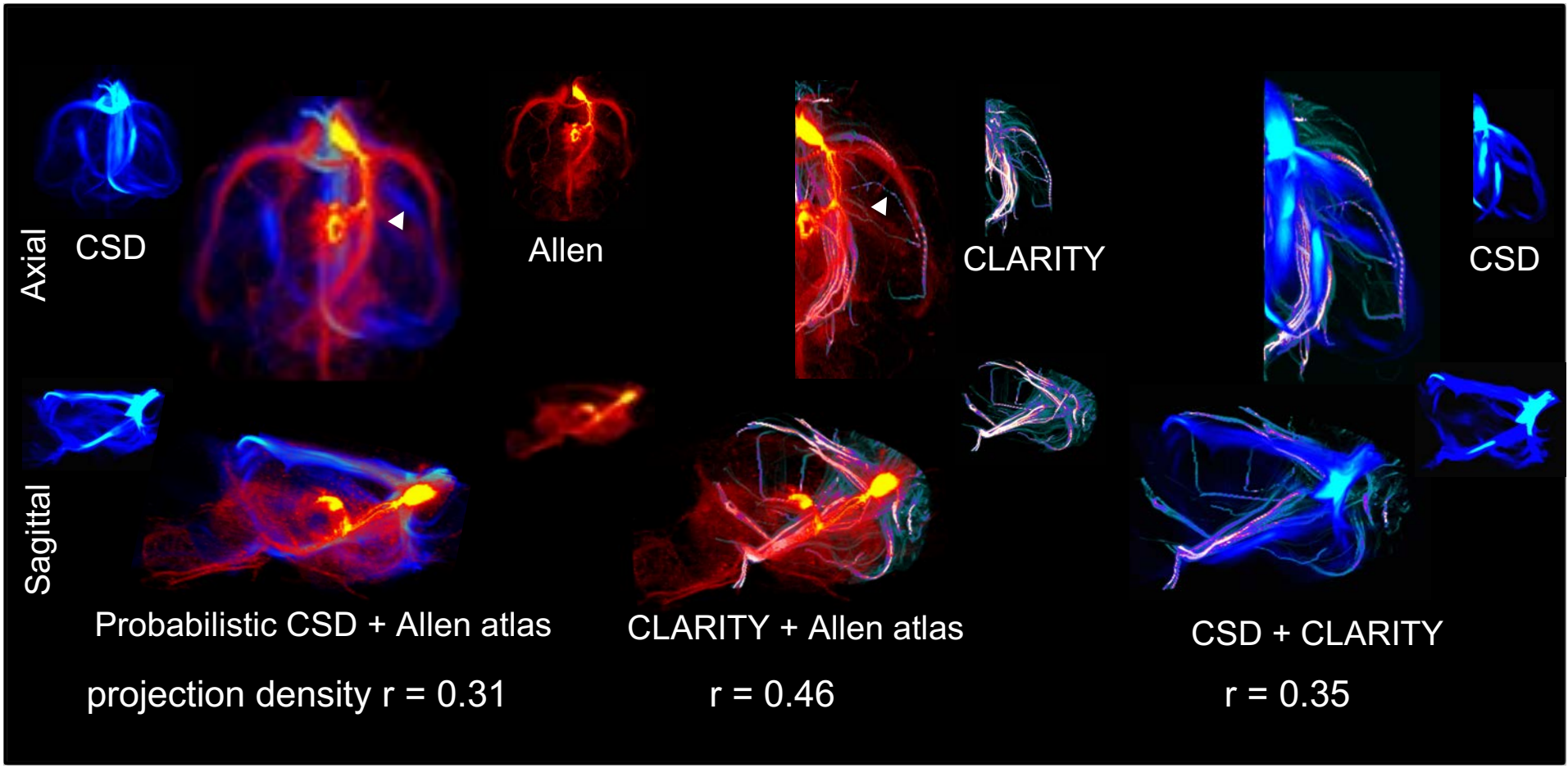
Normalized cell densities in ipsilateral targets connected to the right CP

DMRI VS CLARITY VS ALLEN ATLAS



Leuze*, Goubran* et al. *Neuroimage*. 2021

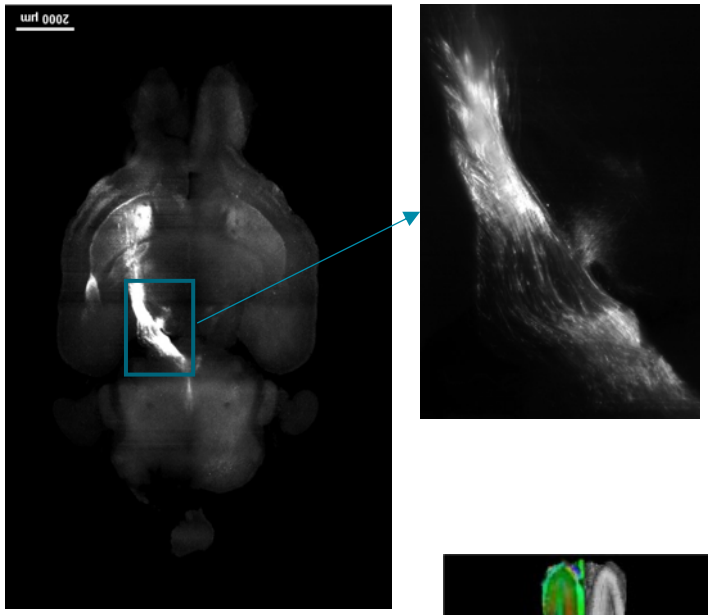
DMRI VS CLARITY VS ALLEN ATLAS

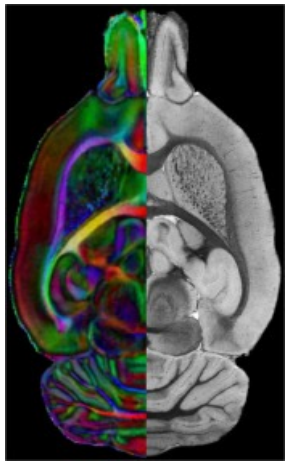


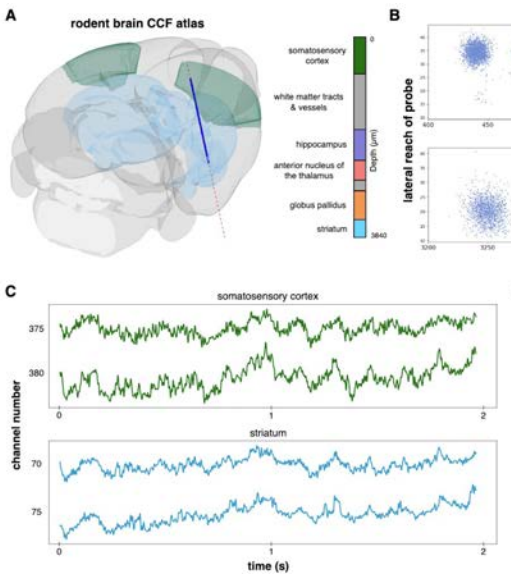
FUTURE WORK

- AI models for quantification of single-axon projections
- AI-assisted registration
- More atlases
- Integration of fMRI & Neuropixels (high-density Ephys)

Virus tracing in the rat

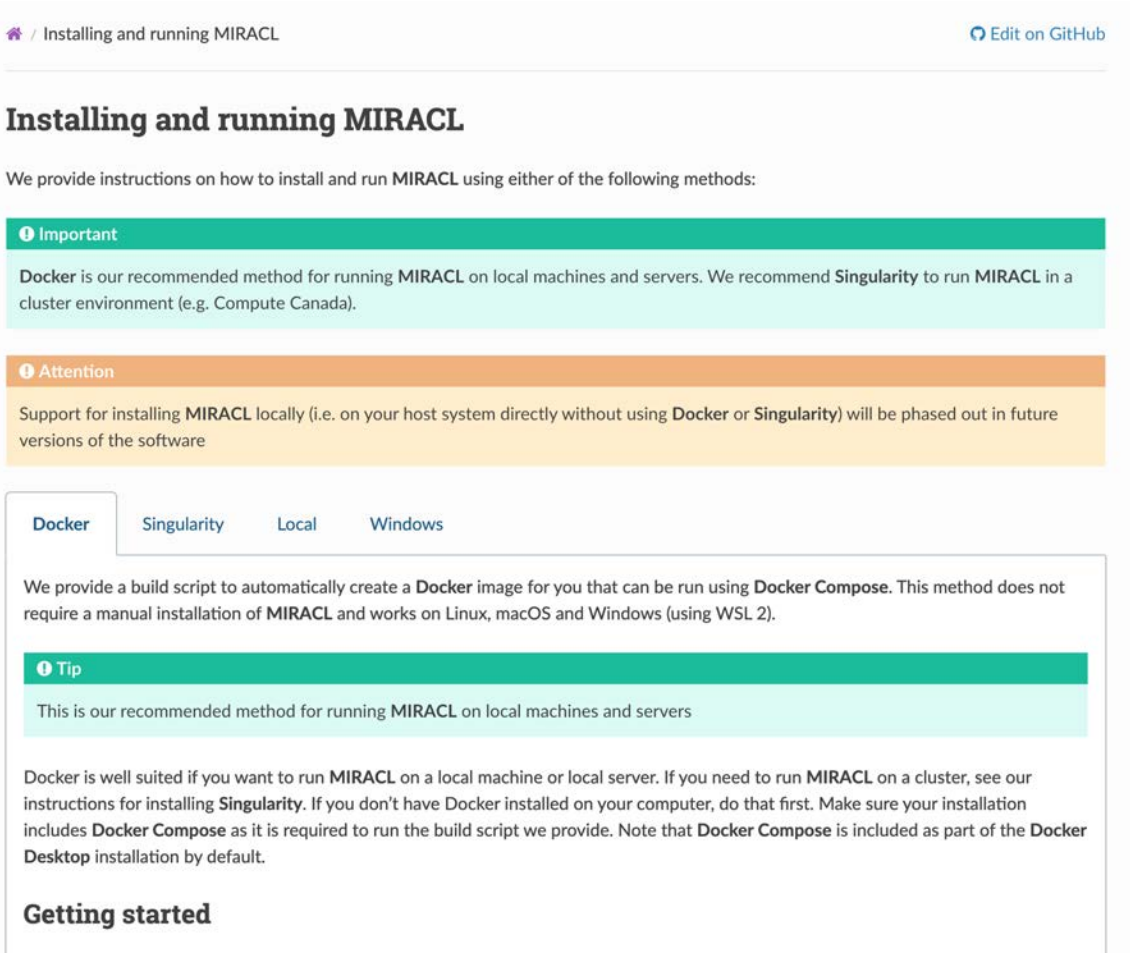






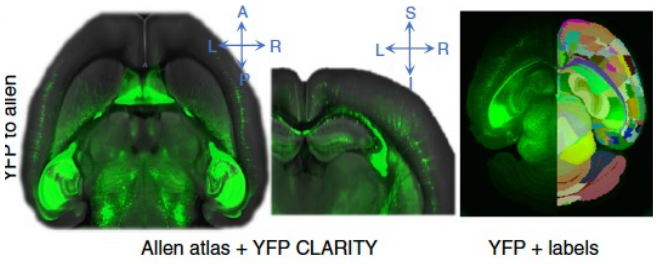
WEBSITE

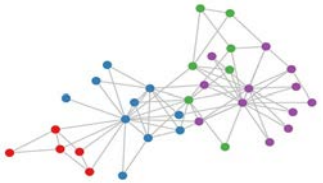
- https://miracl.readthedocs.io/
- Installation
  - Docker
  - Singularity
  - Sherlock
- Tutorials
- Troubleshooting
- Example data

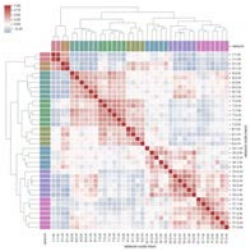


SUMMARY

1. Registration of cleared and imaging data to atlases
2. 3D Segmentation and feature extraction of cleared data
3. Tract-specific or network-level connectivity analysis
4. Statistical analysis of cleared and imaging data
5. Comparison of dMRI/tractography, virus tracing, & connectivity atlases
6. Atlas generation and Label manipulation







THANK YOU

https://aiconslab.github.io/

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