Whole-brain Ca2+ imaging in larval zebrafish

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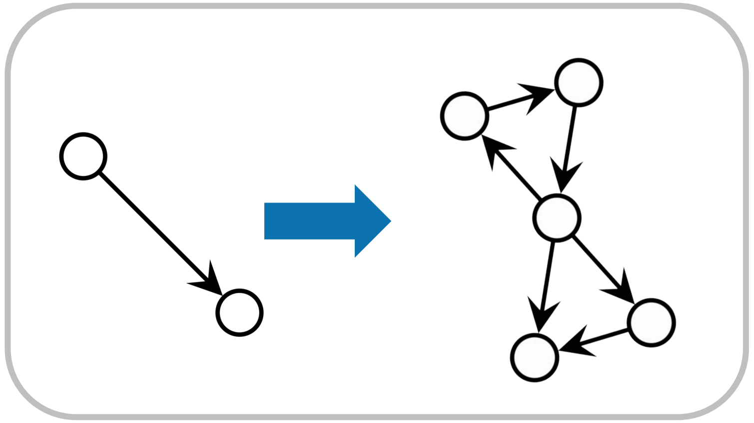
CENTRE DE RECHERCHE CERVO BRAIN RESEARCH CENTRE

UNIVERSITÉ LAVAL

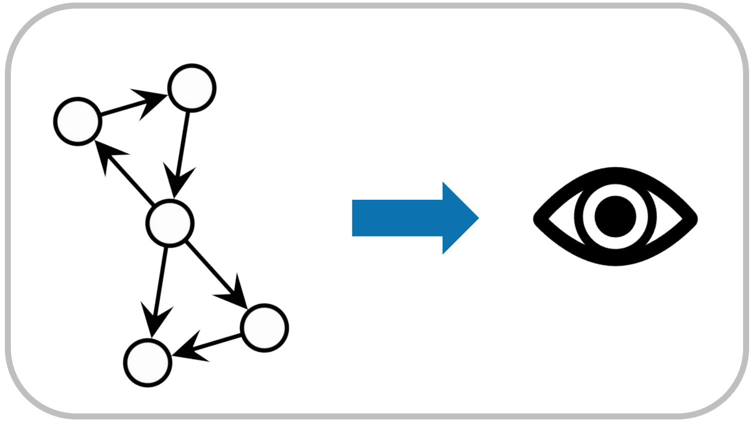
Initial motivations

Interested in developing an experimental model to monitor the early development of neural circuits in the brain, both structurally and functionally in order to understand

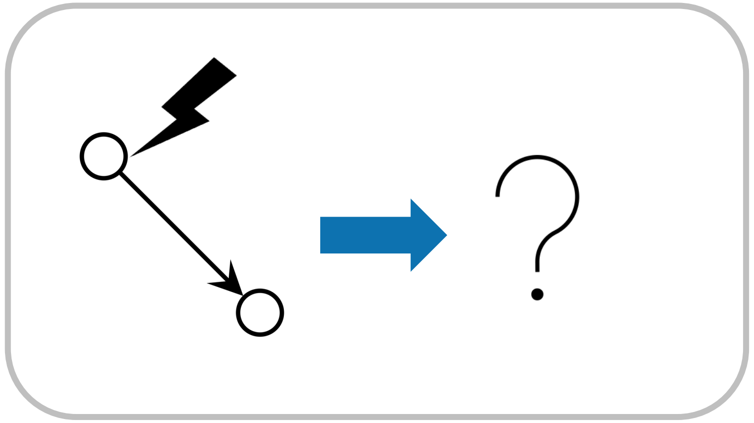
General rules



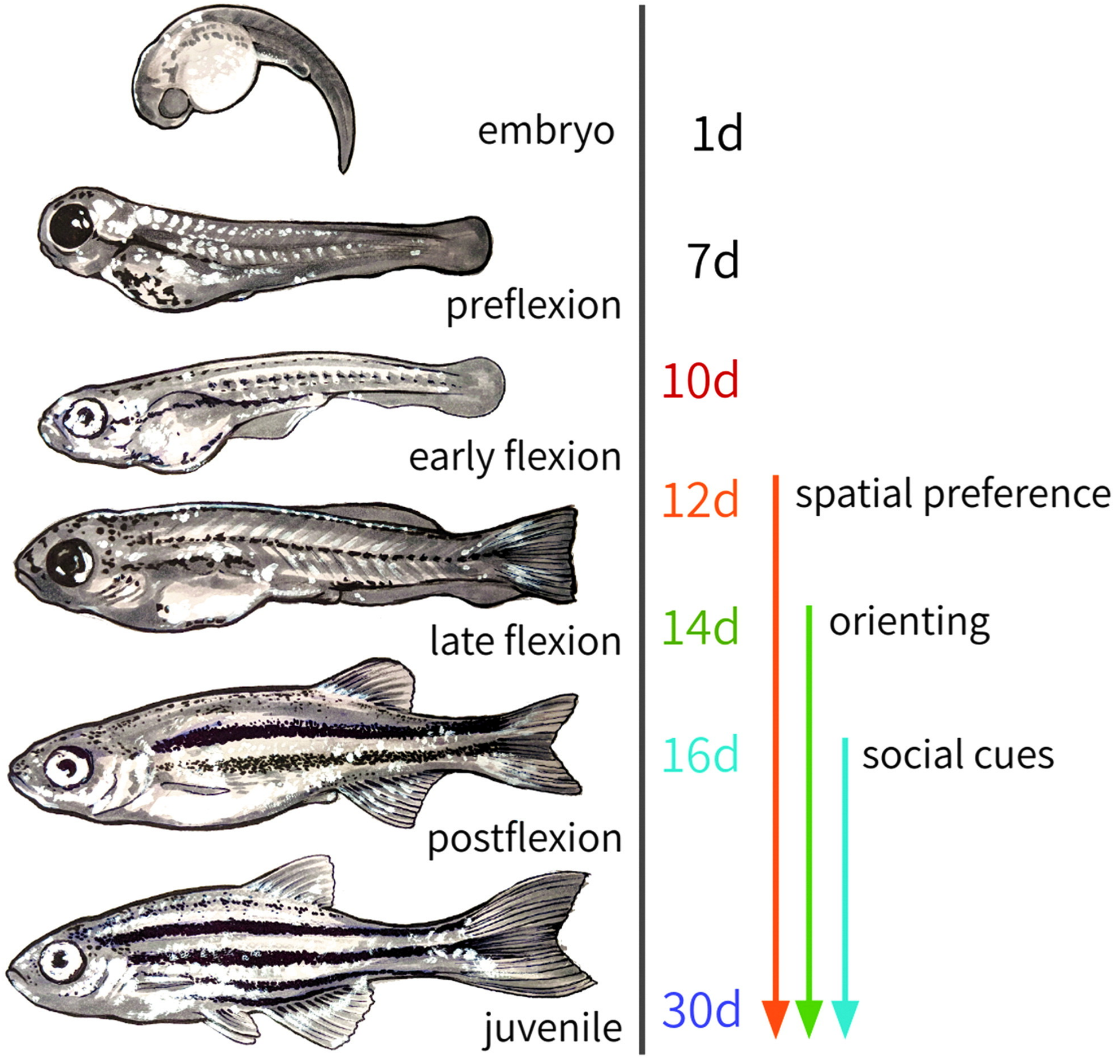
Circuit → Behavior

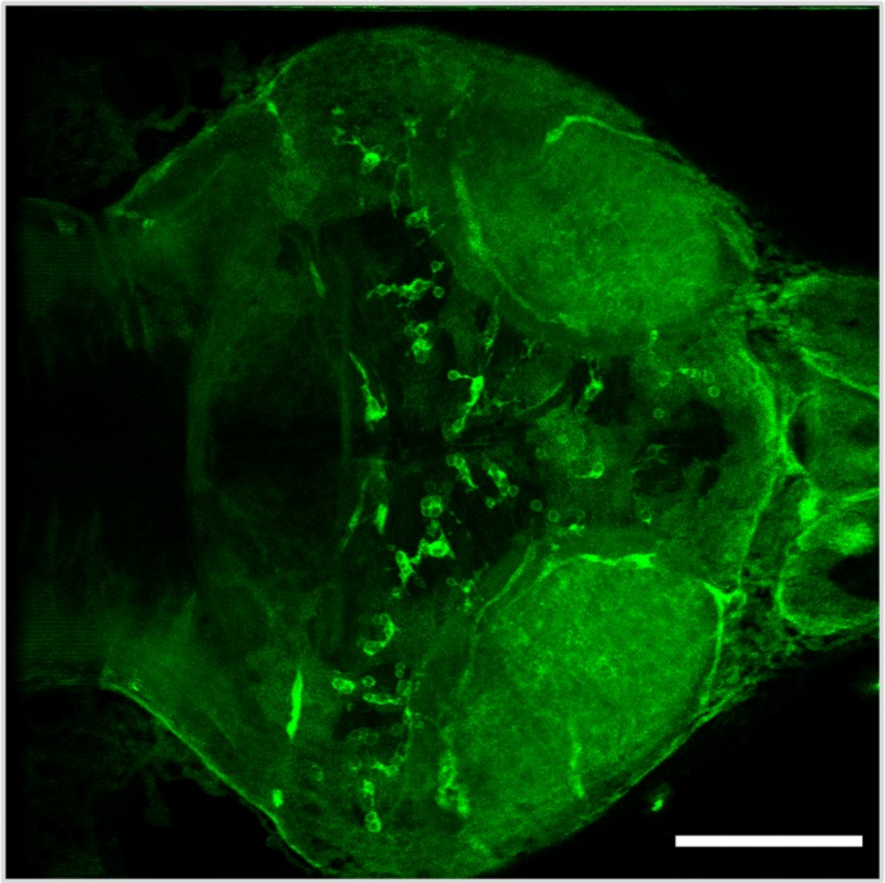


Perturbation



The larval zebrafish

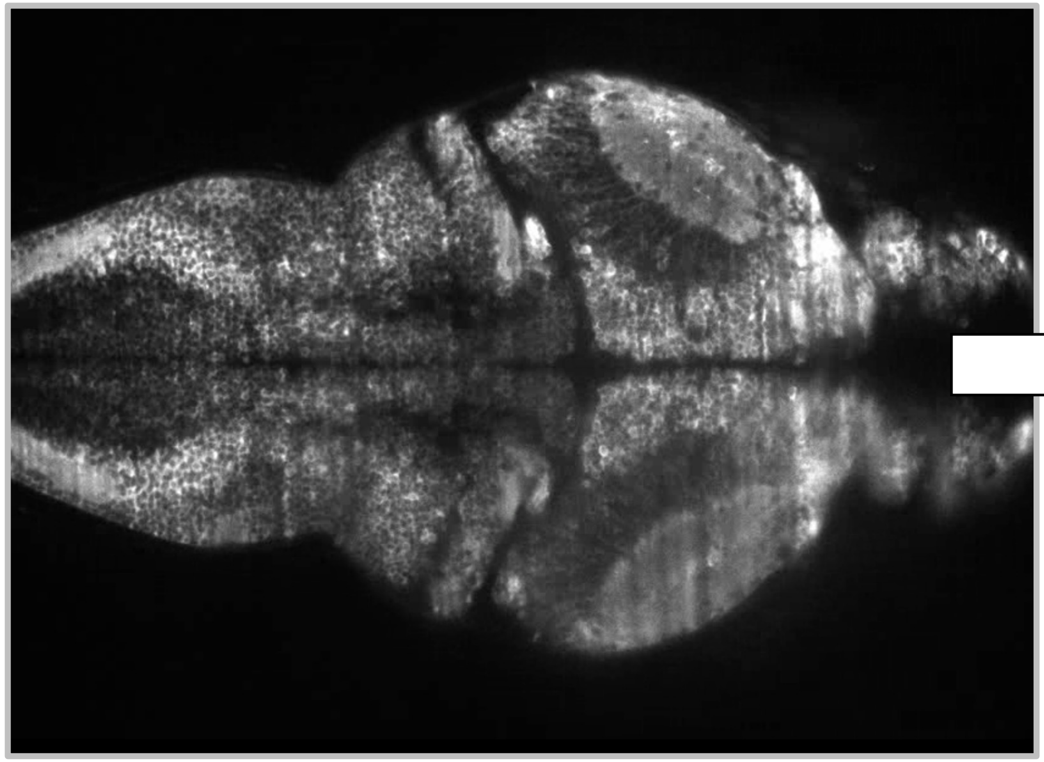




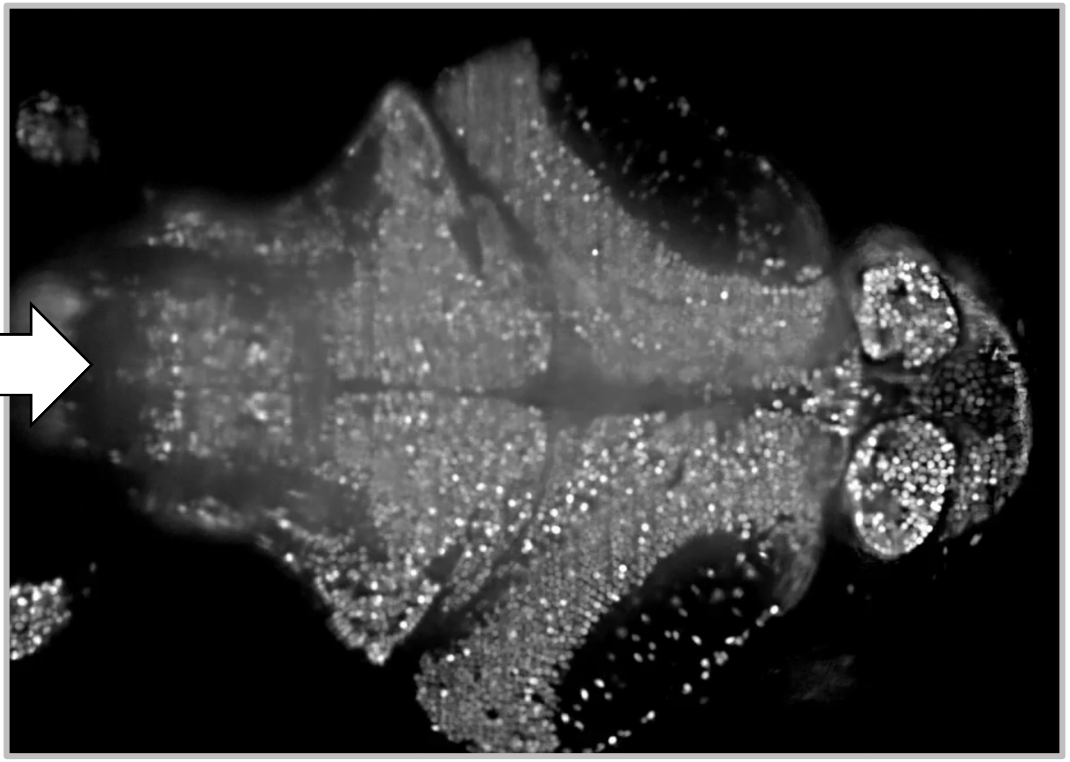
Stednitz & Washbourne (2020) *Zebrafish* **17**

Monitoring neuronal activity

Transgenic fish line from the Ahrens Lab



*elavl3:*GCaMP5G



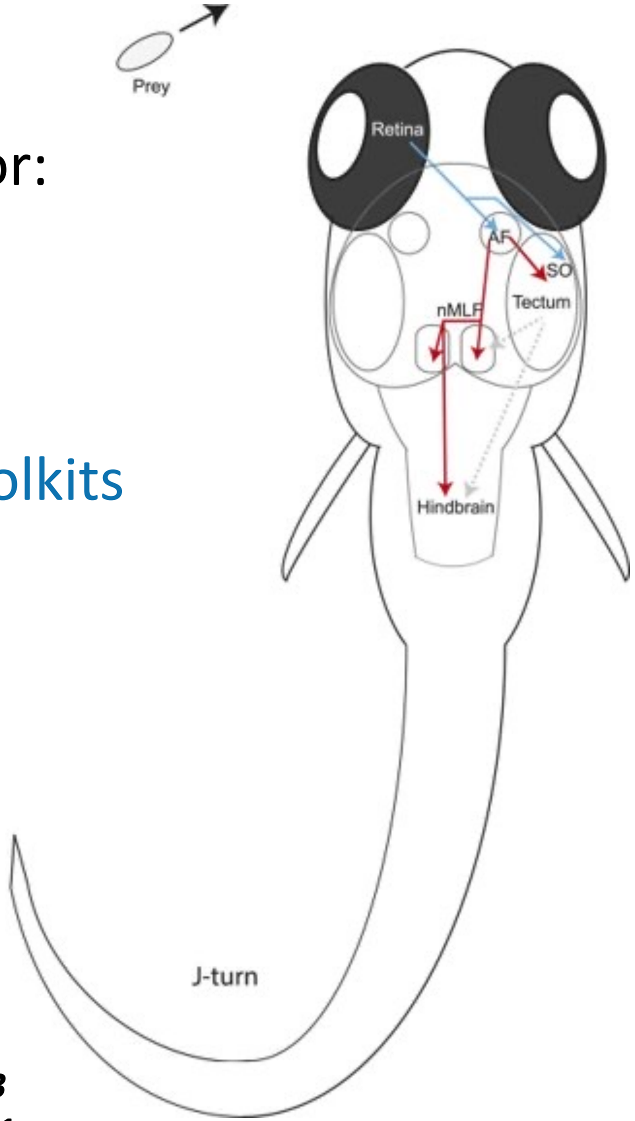
*elavl3:*H2B-GCaMP6s

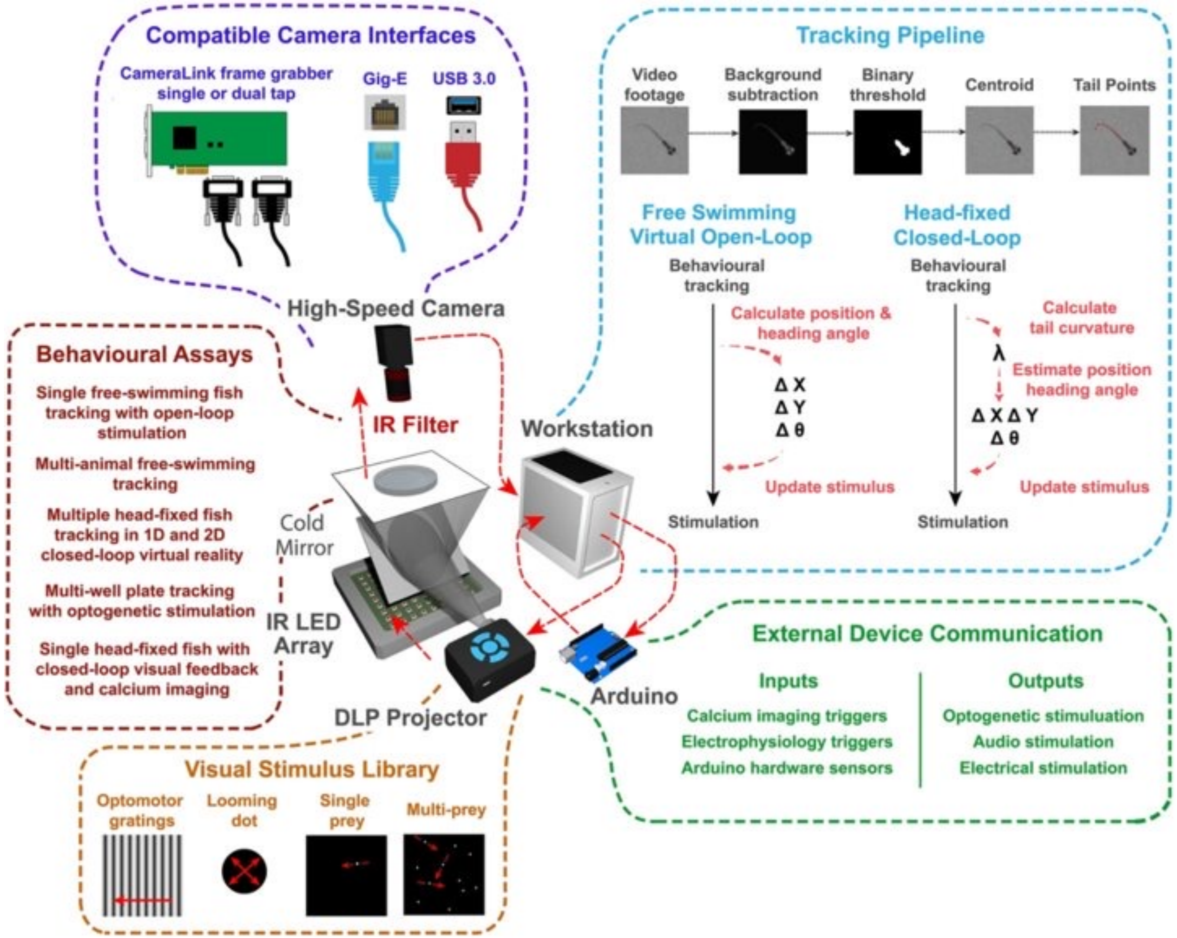
Ahrens *et al.* (2013) *Nat Methods* **10**
Vladimirov *et al.* (2014) *Nat Methods* **11**

Rich behavior

Example behavior:
Prey detection

Software &
experimental toolkits

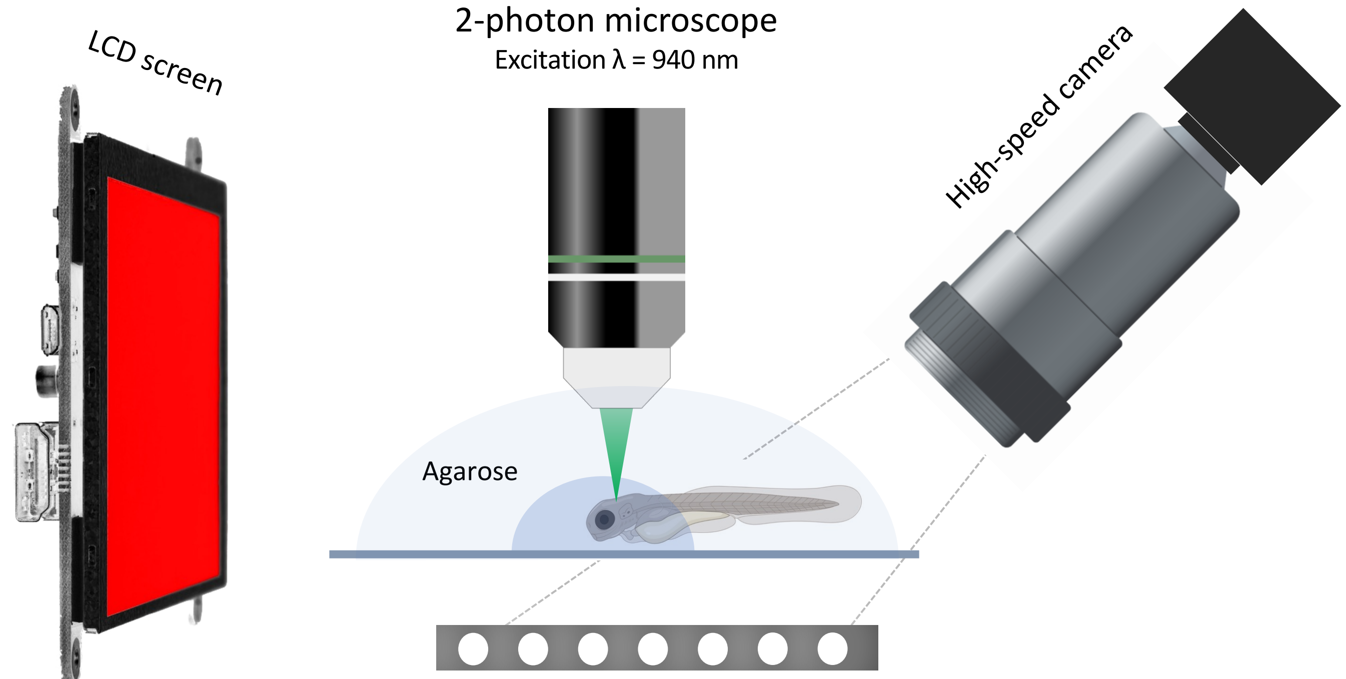


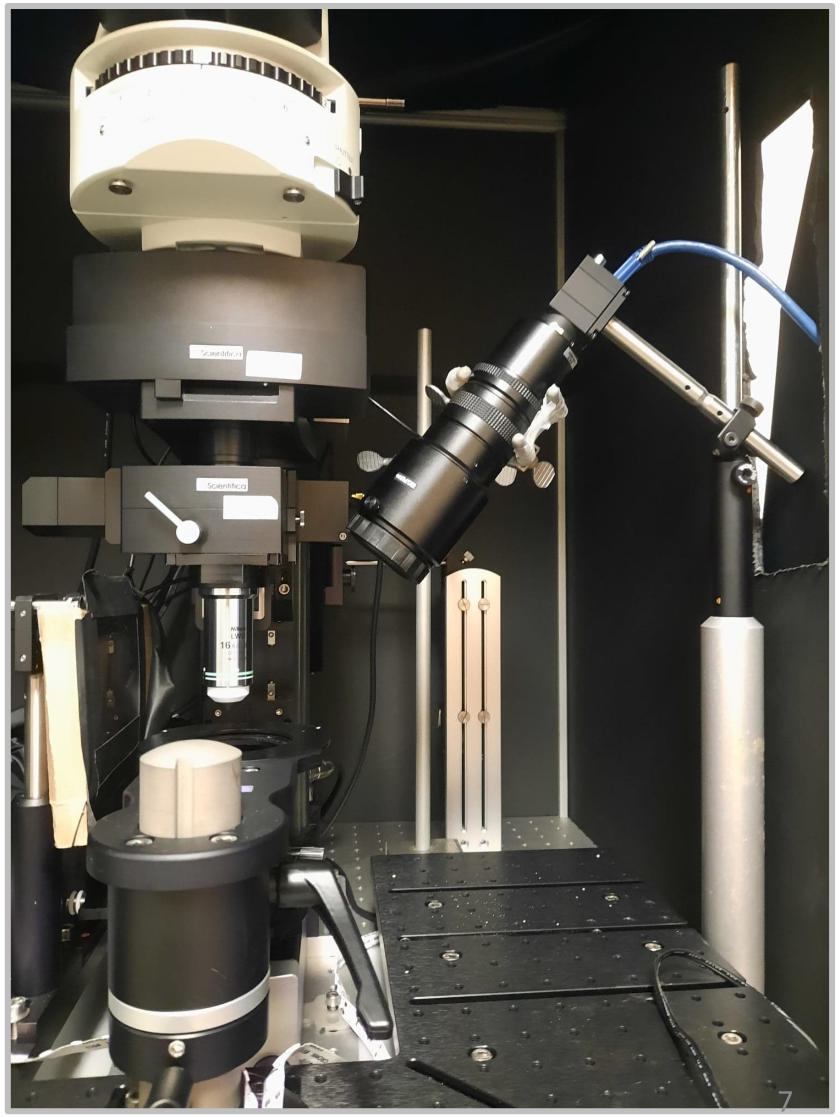


Semmelhack *et al.* (2014). *eLife* **3**
Guilbeault *et al.* (2021) *Sci Rep* **11**

Experimental framework

Experimental setup

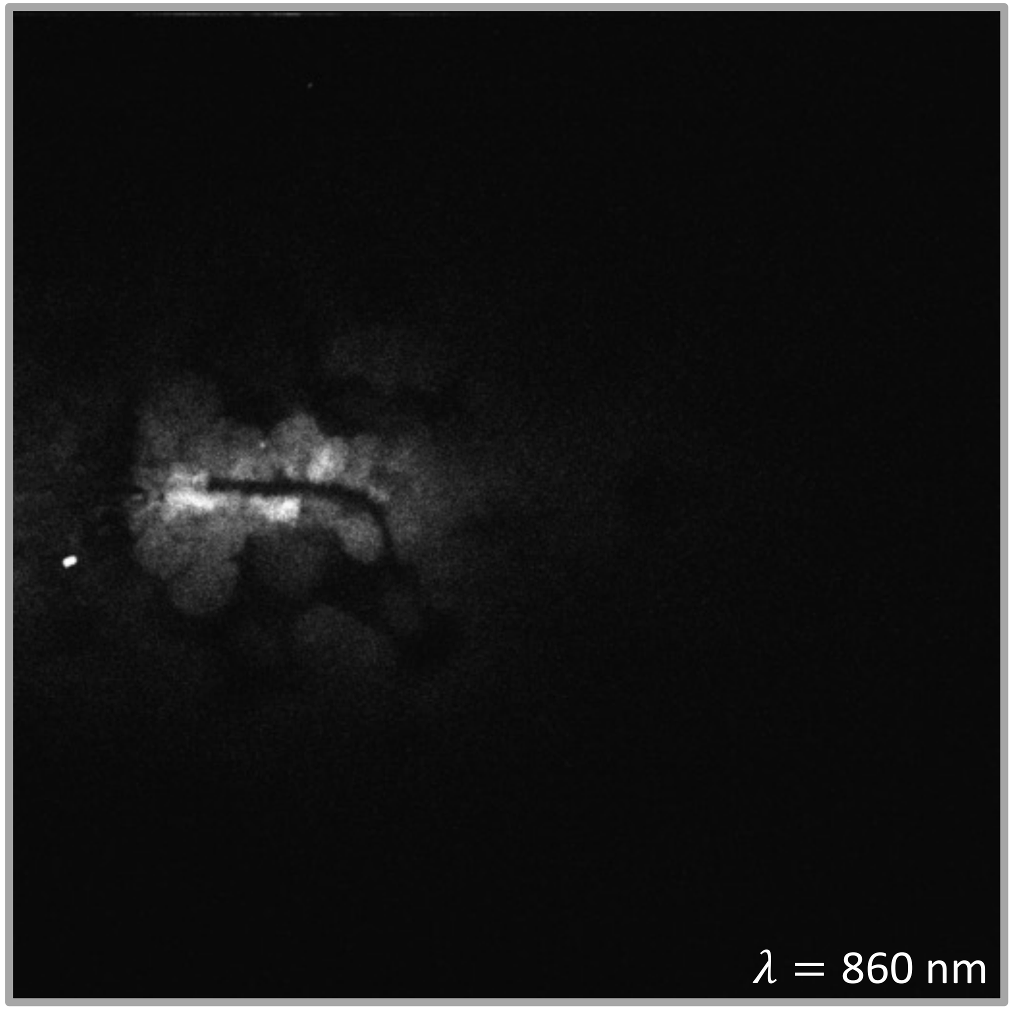




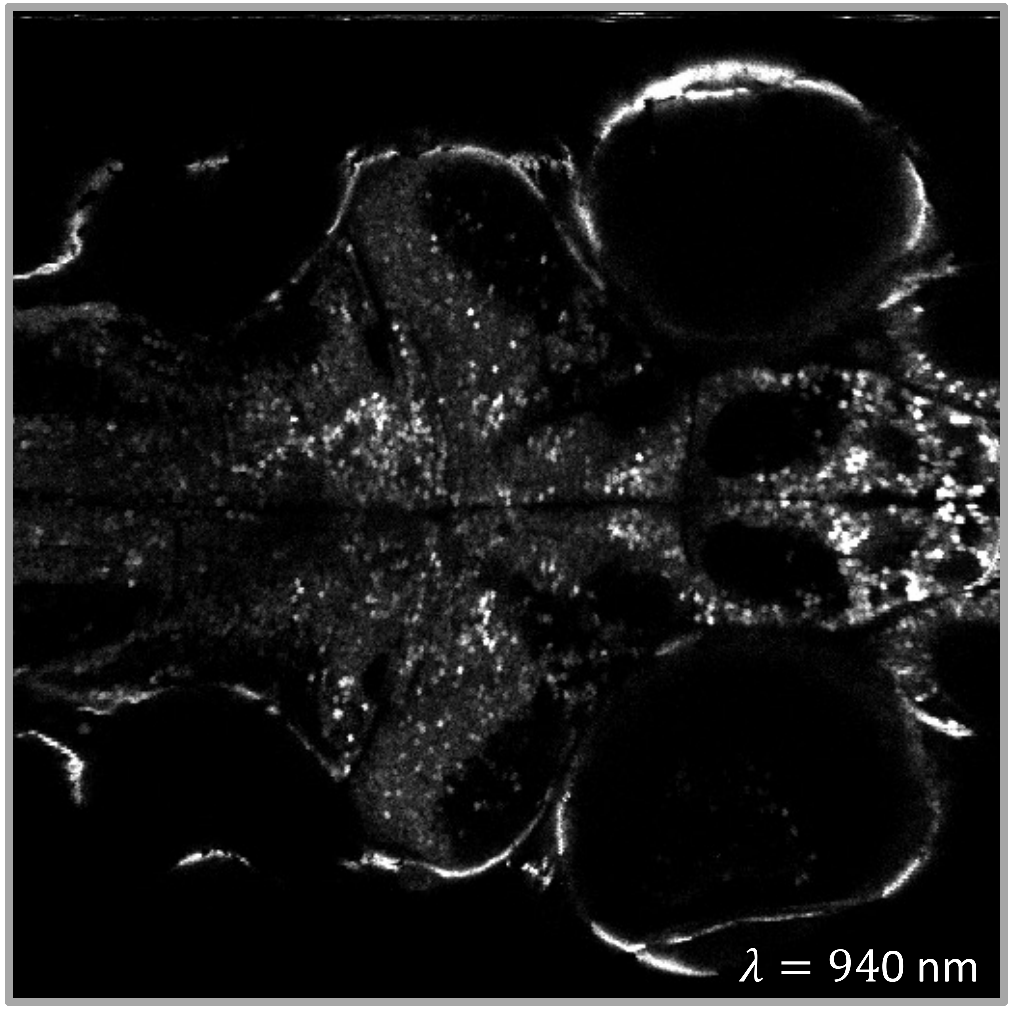
Thanks to Ed Ruthazer and Cynthia Solek for helping us getting started

Neurophotonics Summer School at CERVO

Resonant two-photon imaging

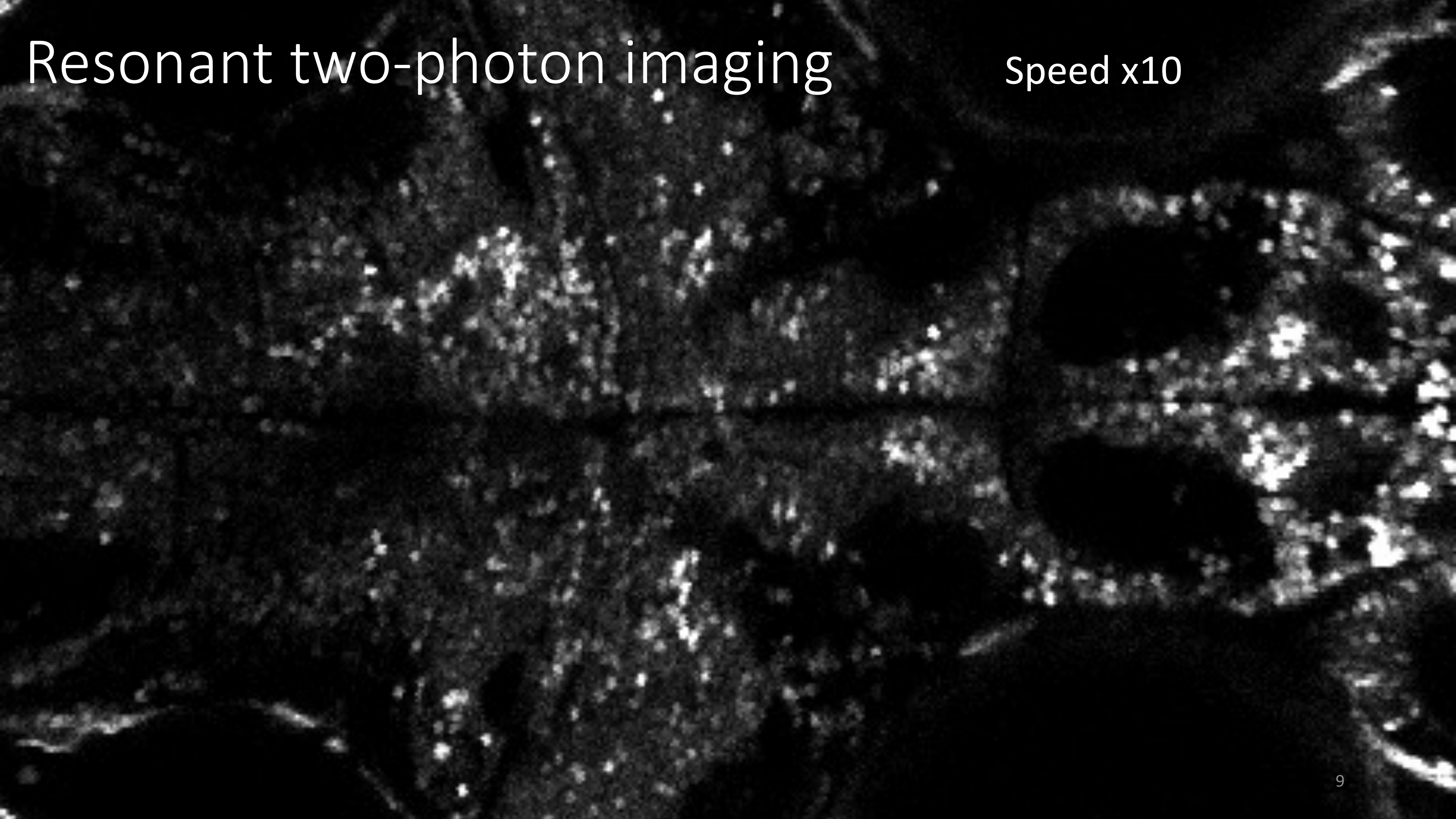


Anatomical stack (~250 planes)



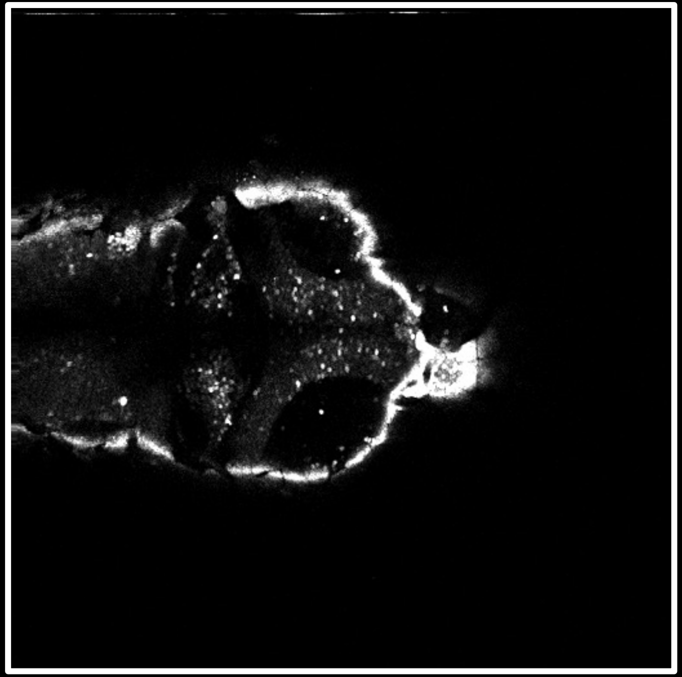
Functional imaging (single plane 30 Hz)

Resonant two-photon imaging



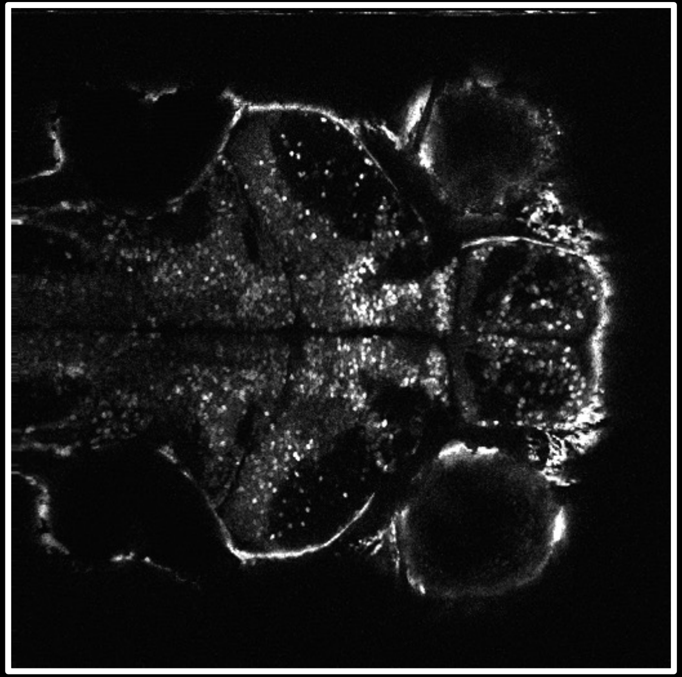
Whole-brain multi-plane imaging

Plane 1



...

Plane 10



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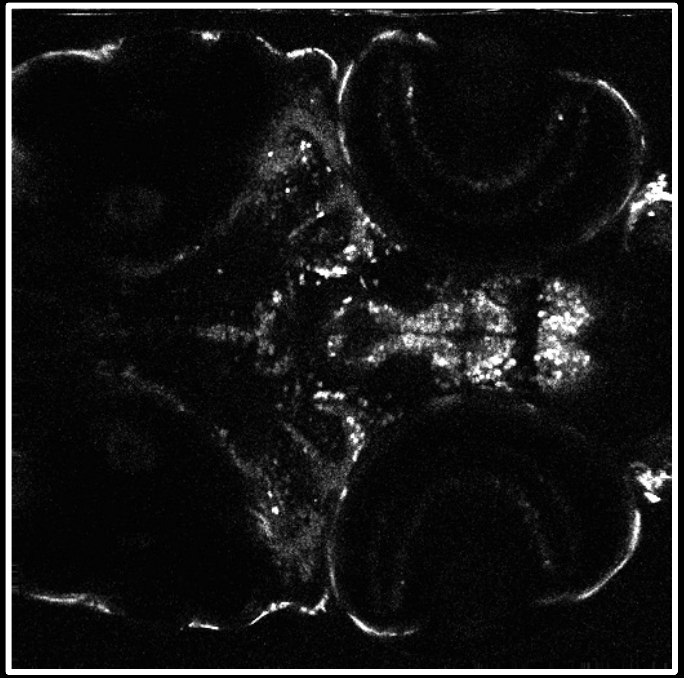
Plane 20



Speed x100

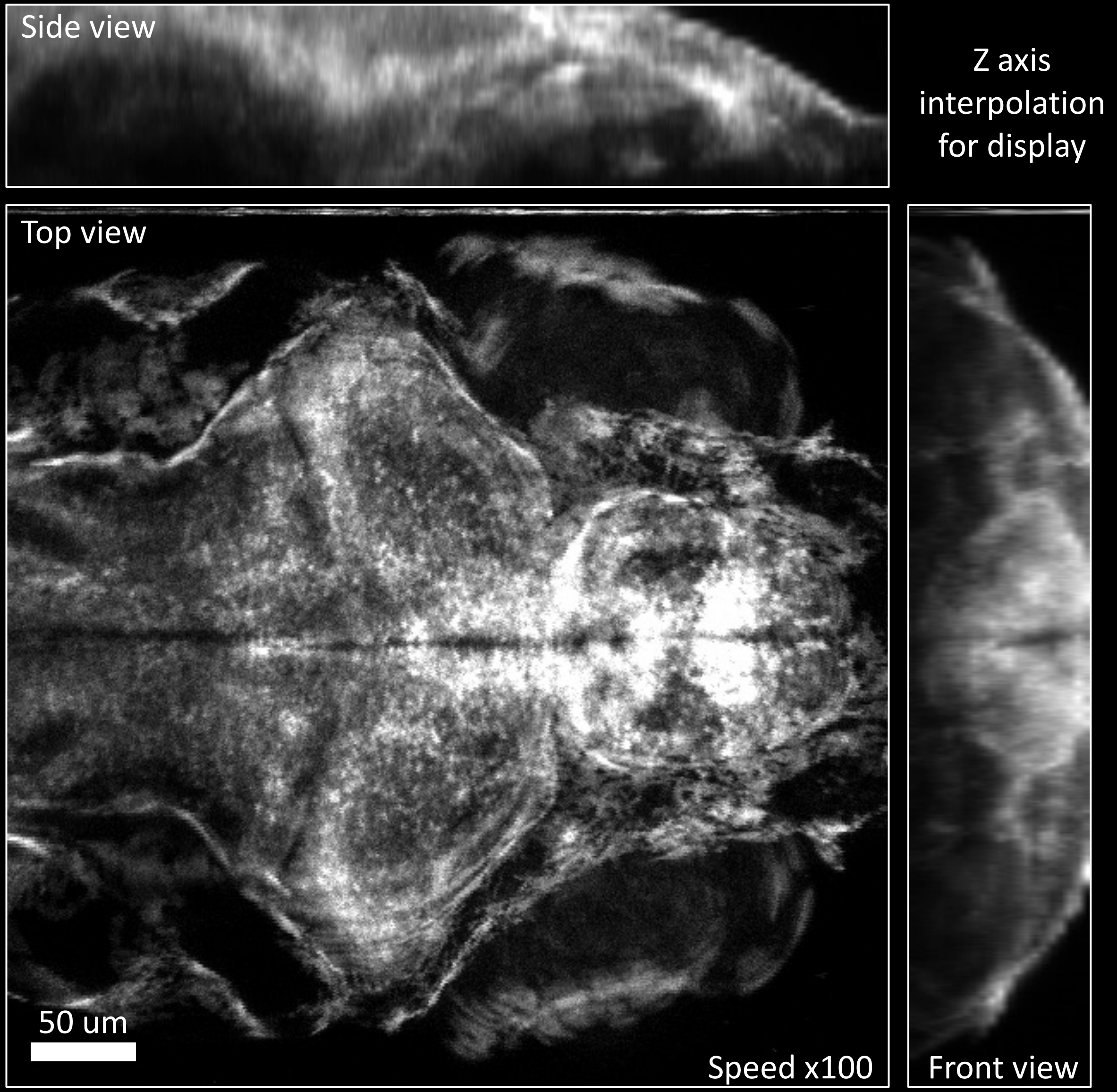
Z

Piezo scanning

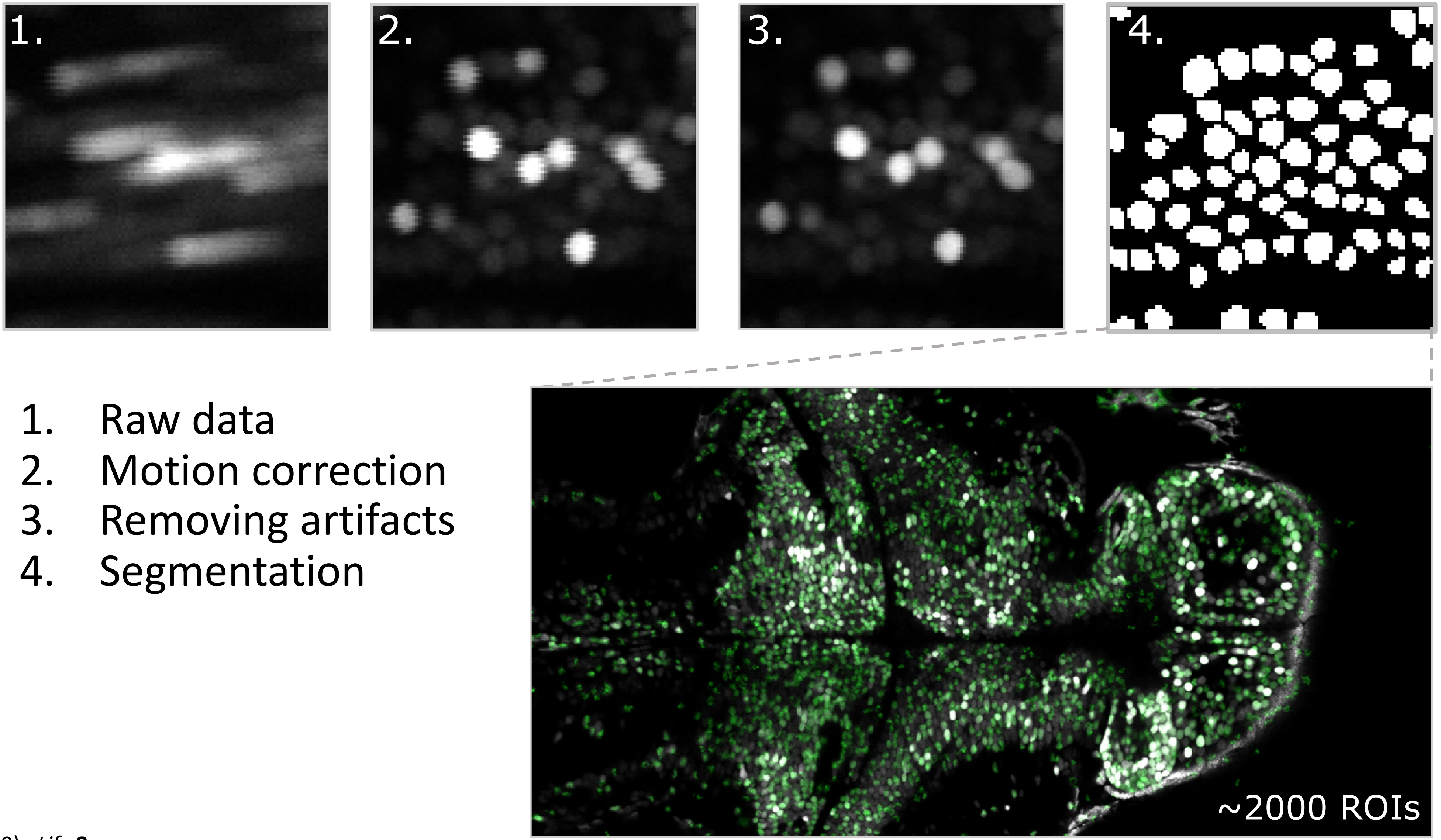
Whole-brain multi-plane imaging

Mean projection

22 planes
@
1.4 Hz



Preprocessing pipeline

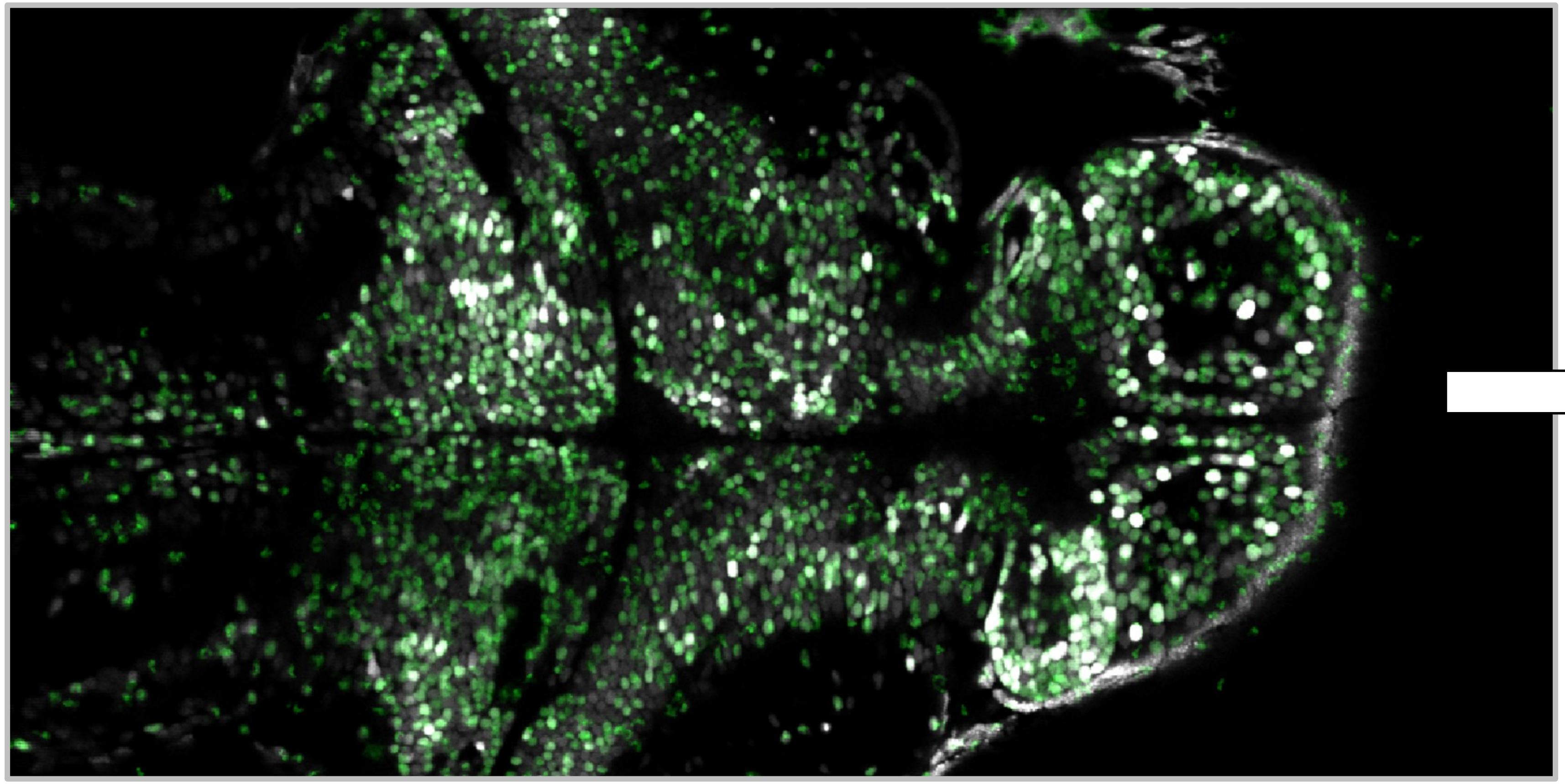


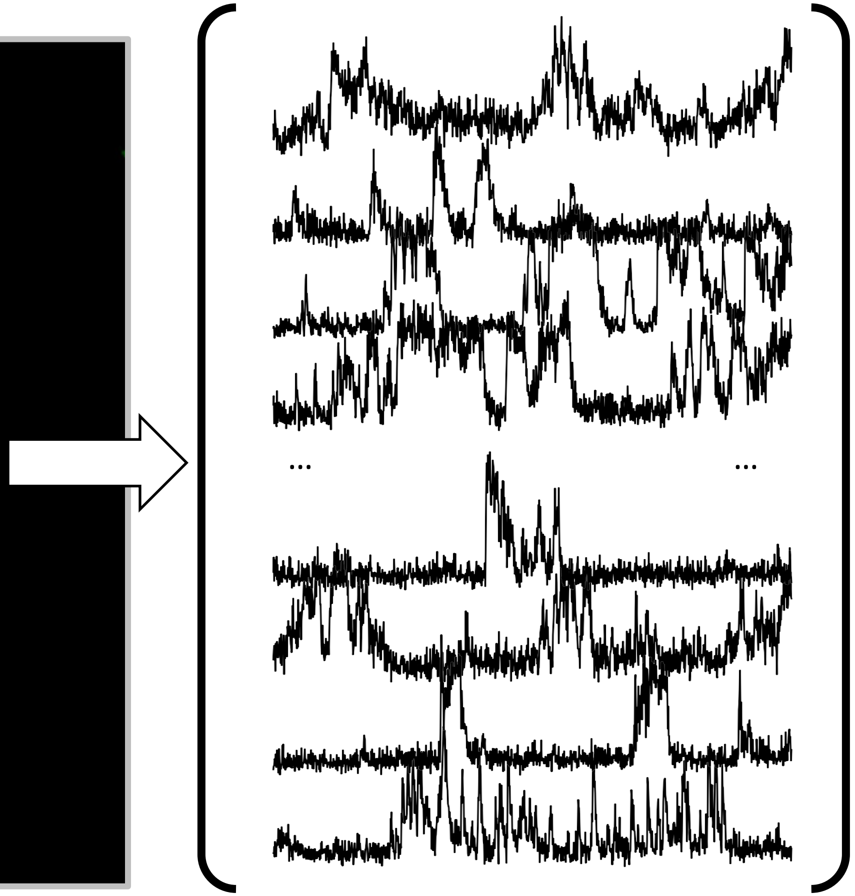
1. Raw data
2. Motion correction
3. Removing artifacts
4. Segmentation

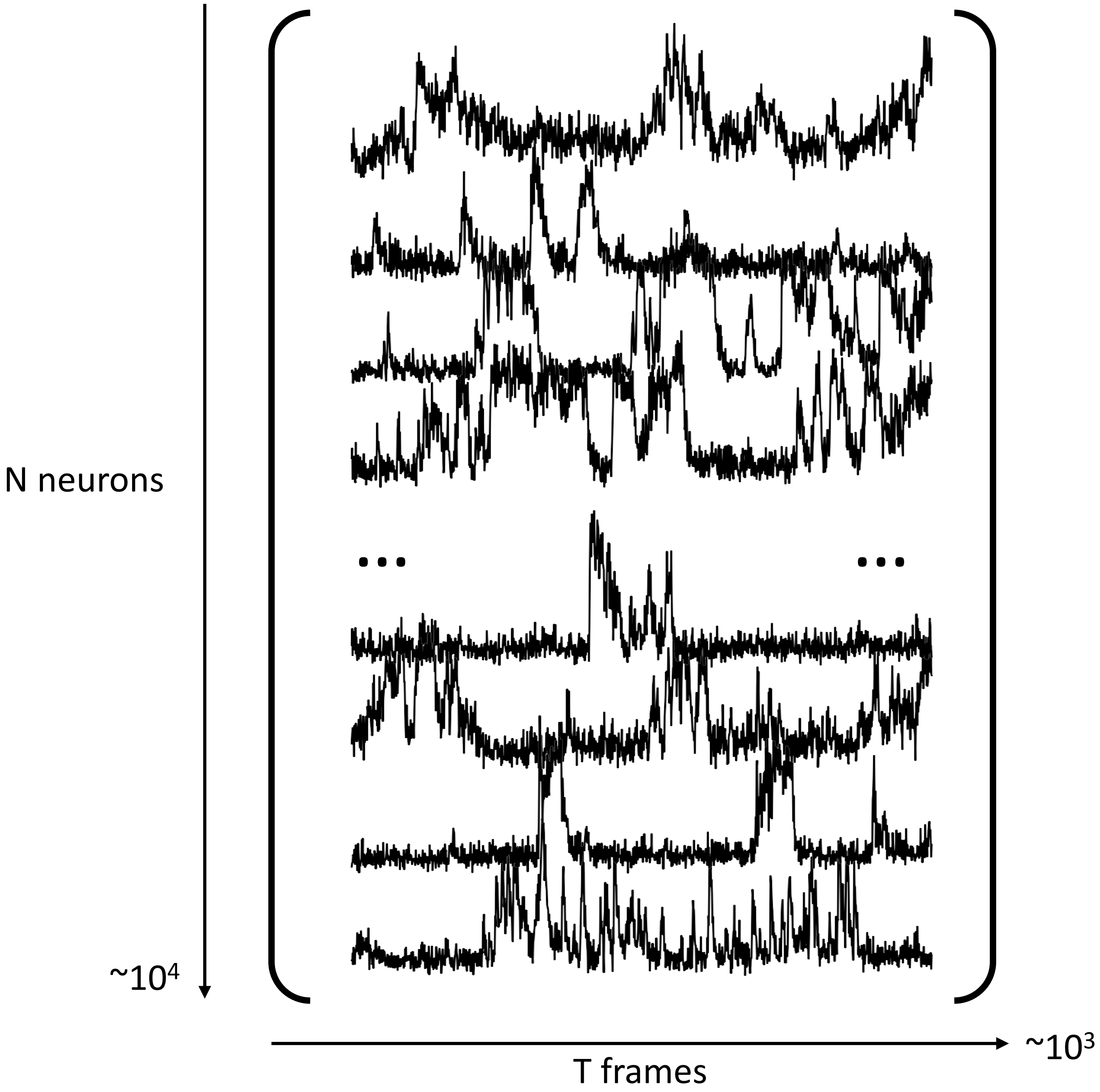
Giovanucci *et al.* (2019) *eLife* **8**

Preprocessing pipeline

Signal extraction for every individual imaging plane

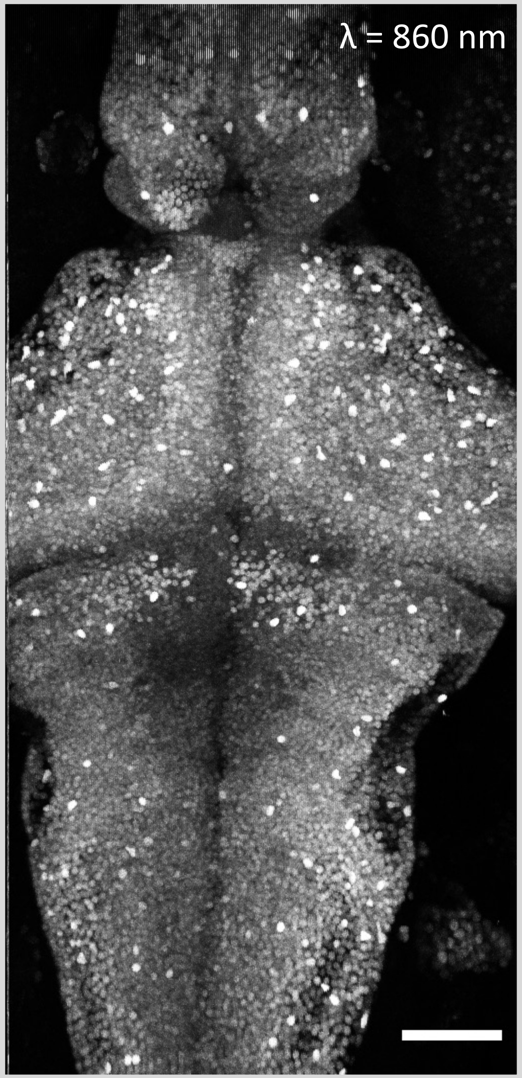





N neurons
~10^4
...
...
~10^3
T frames

Dual registration framework

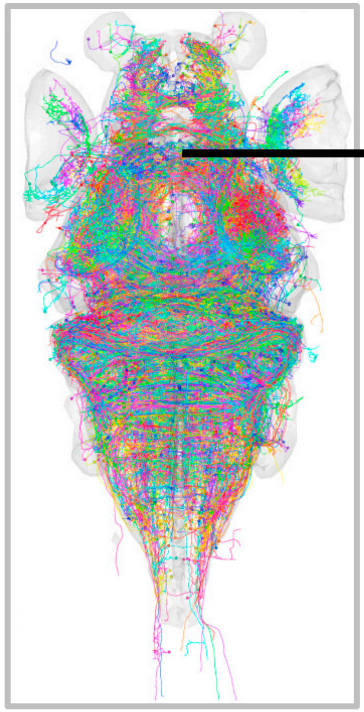
In vivo 2-photon z-stack



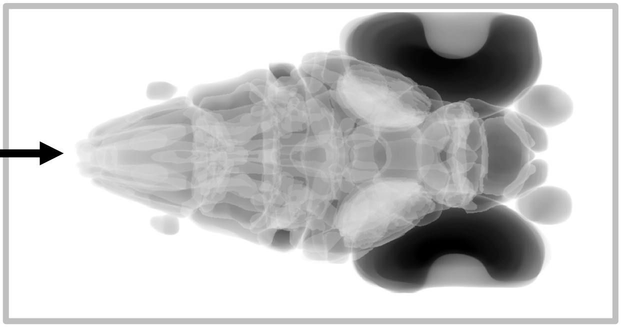
[1] Lovett-Baron *et al.* (2017) *Cell* **171**

Dual registration framework

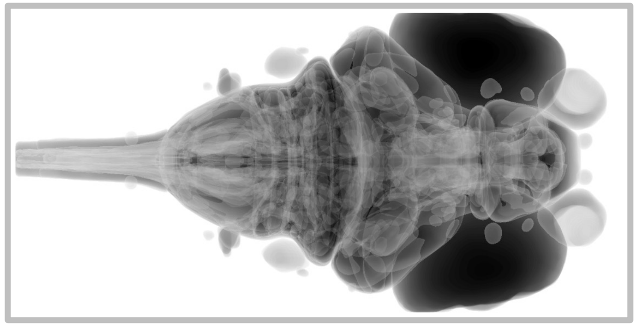
Axonal projections

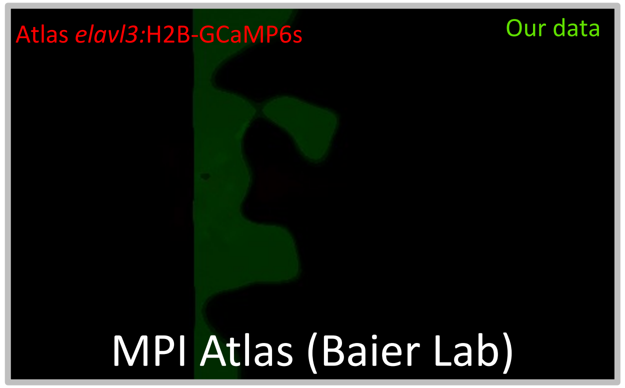


69 brain regions

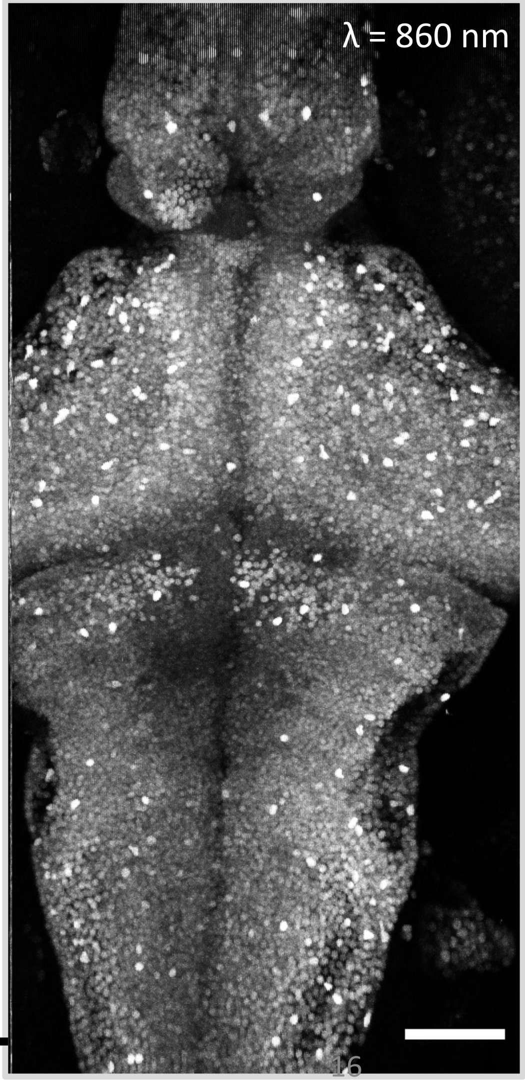


294 brain regions

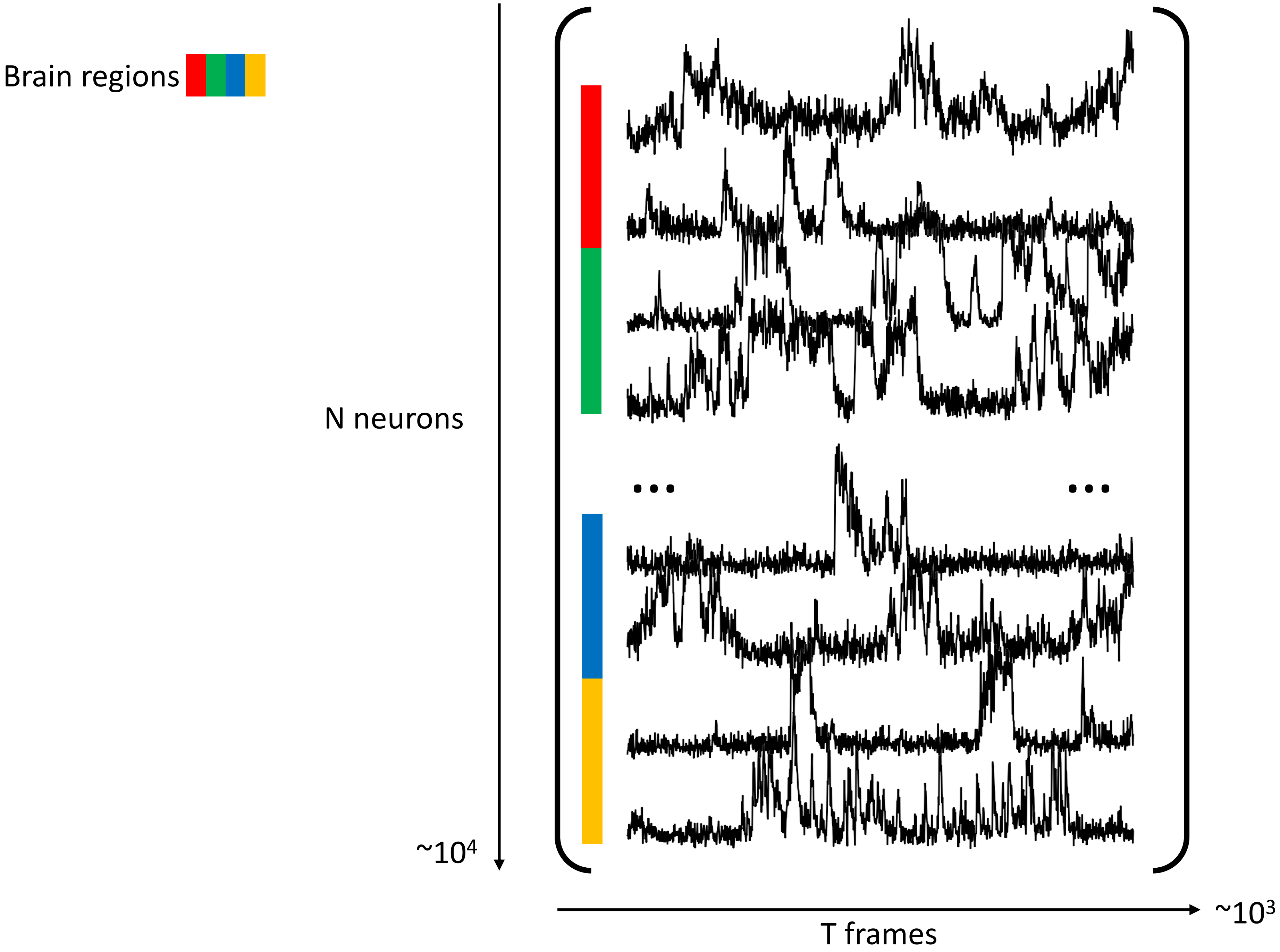




In vivo 2-photon z-stack

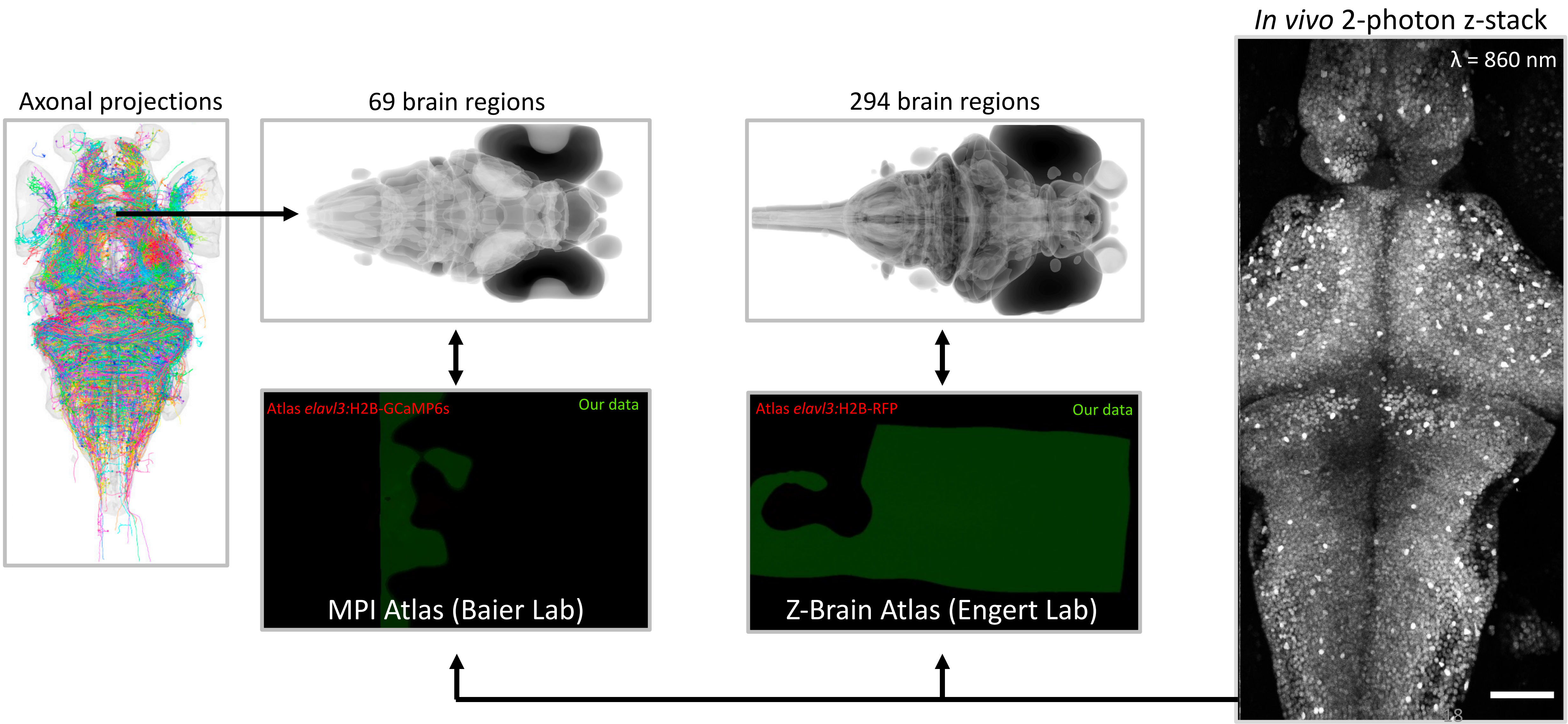


[1] Randlett *et al.* (2015) *Nat Methods* **12**
[2] Kunst *et al.* (2019) *Neuron* **103**

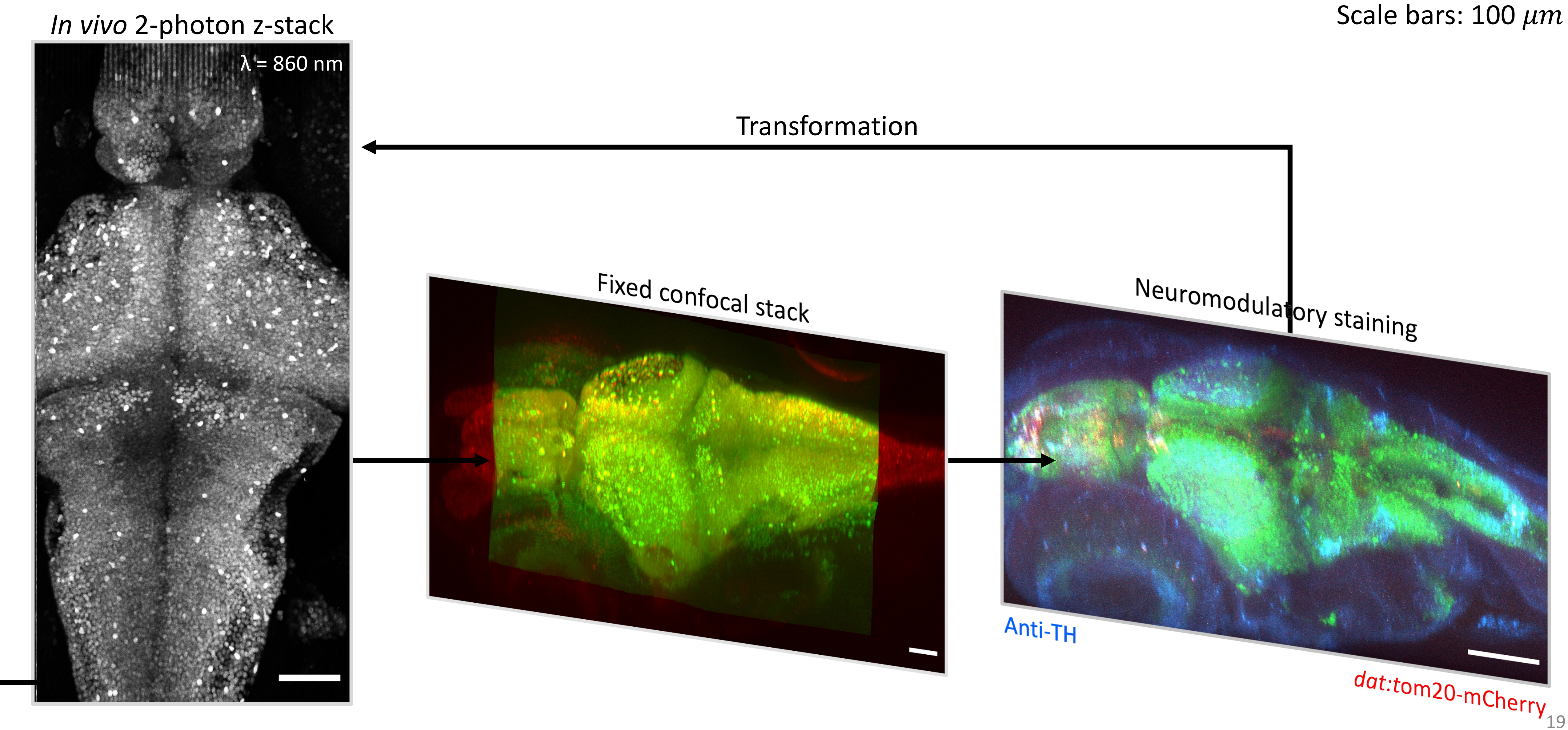


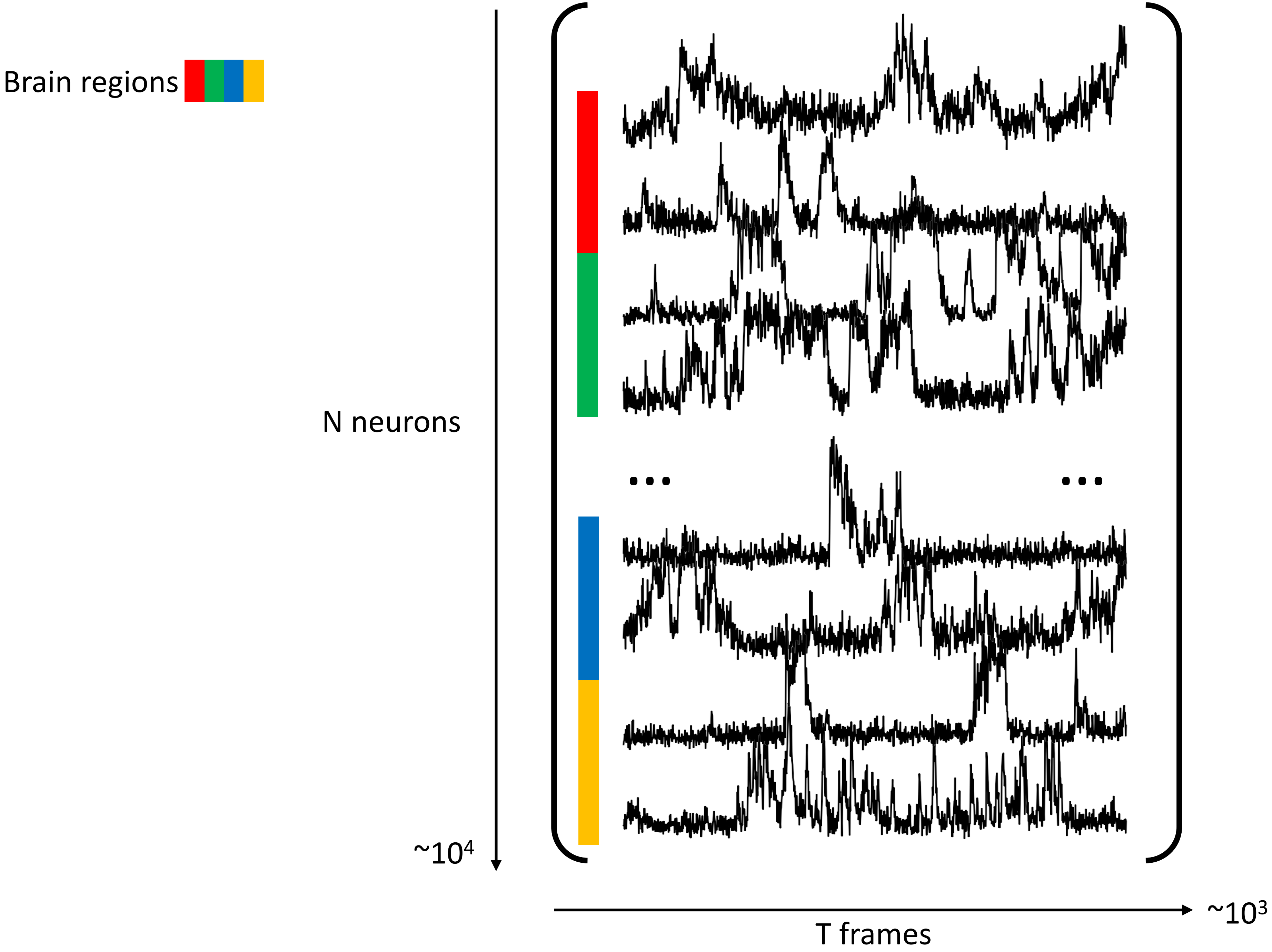
Brain regions
N neurons
~10^4
T frames
~10^3

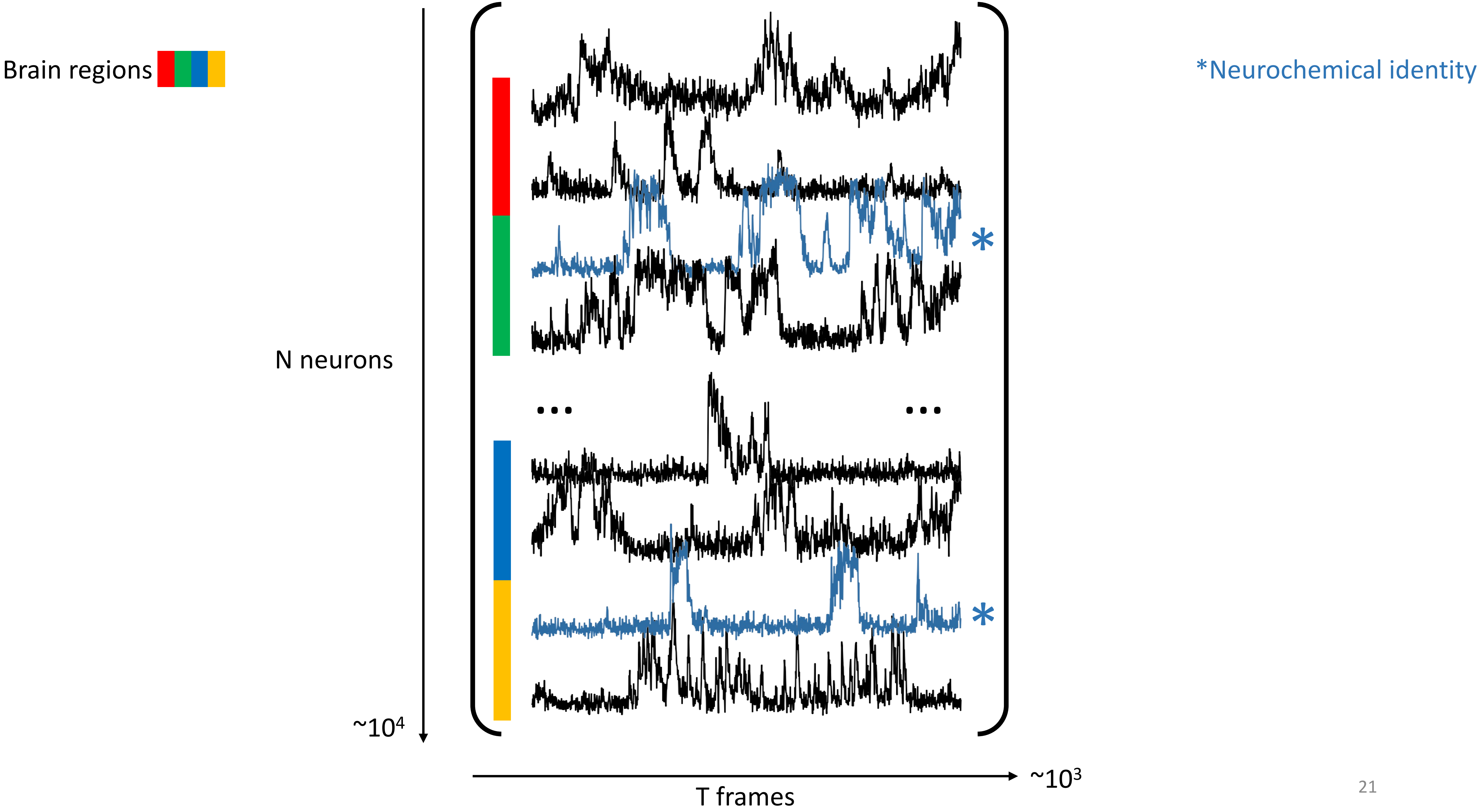
Dual registration framework



Dual registration framework




Brain regions
N neurons
~10^4
T frames
~10^3

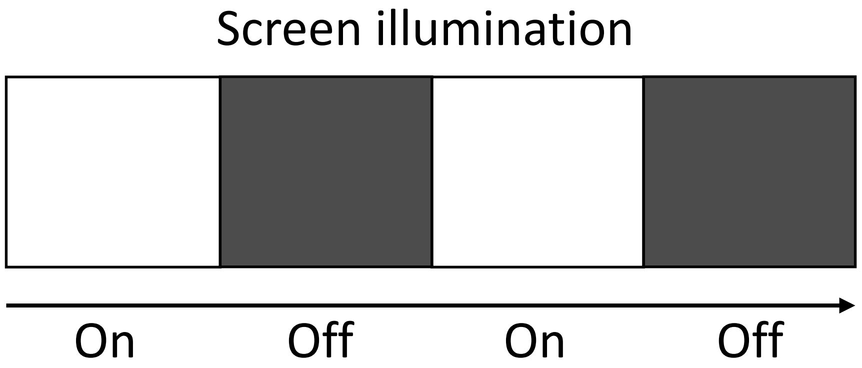


Brain regions
N neurons
~10^4
T frames
~10^3
*Neurochemical identity
*
*
...
...

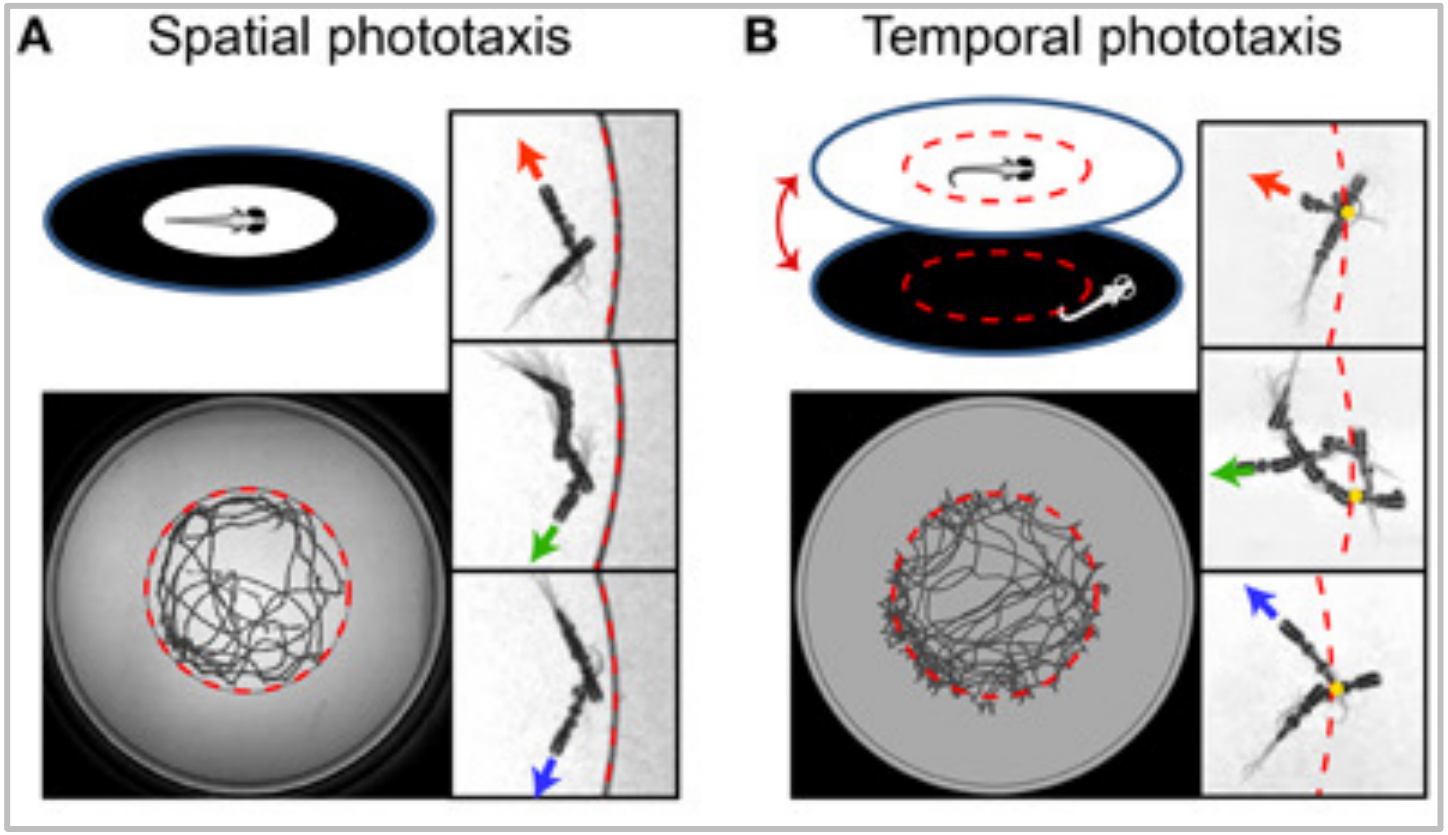
Visual Stimulation

Visual stimulation to probe neural circuits

Dark-flash stimulation paradigm



- Triggers locomotion
- Navigational strategy

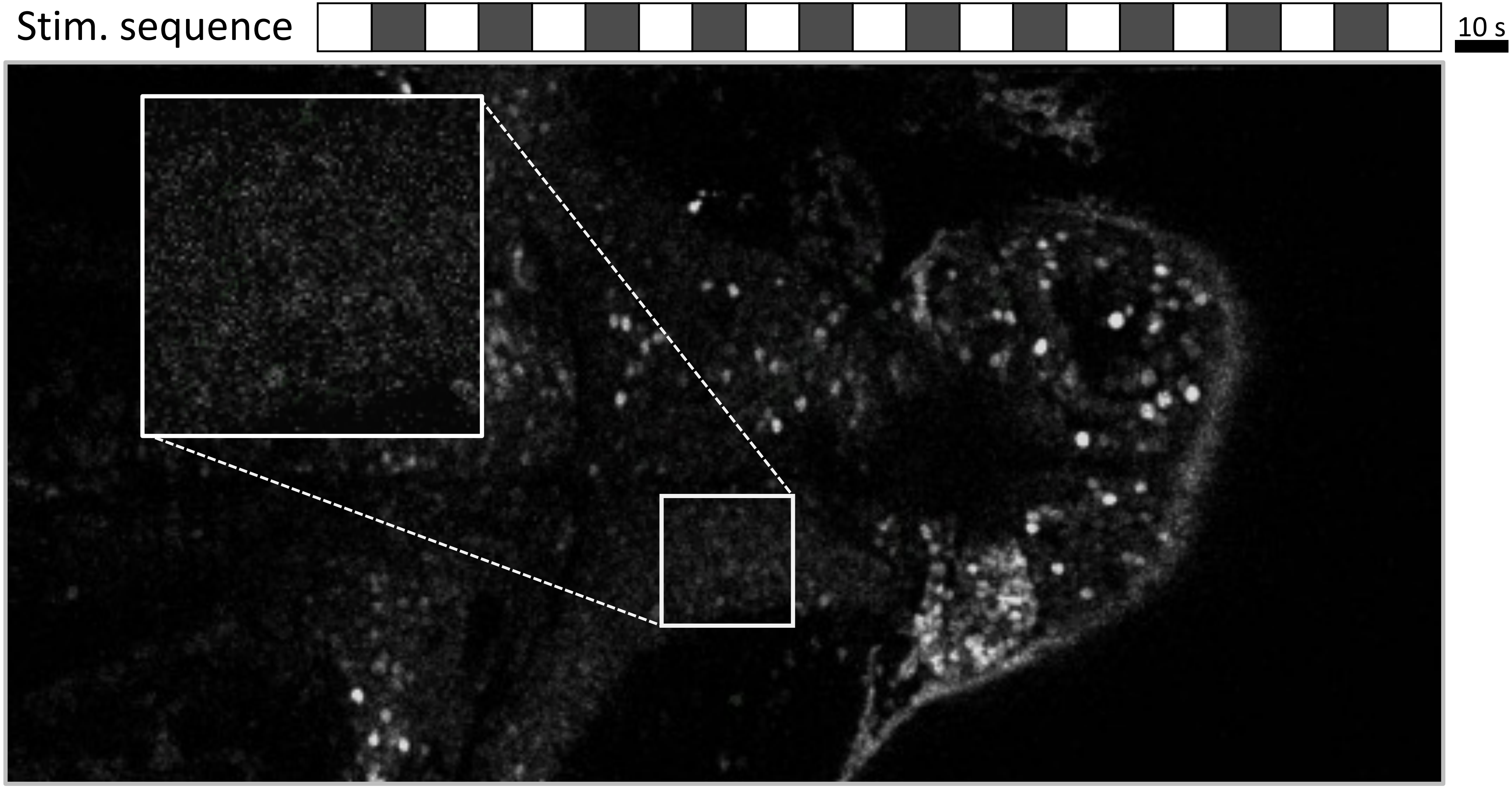


By turning on and off the light, the fish is constrained to a *virtual circle*

Chen & Engert (2014) *Front Syst Neurosci* **8**

Visual stimulation to probe neural circuits

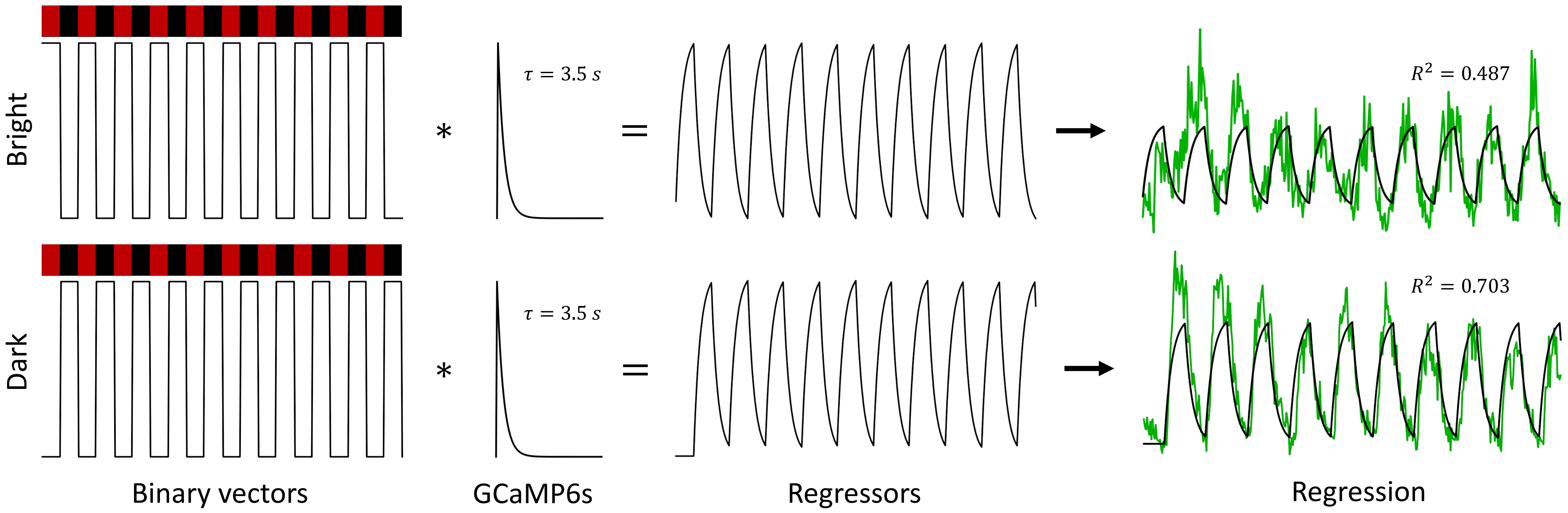
Stim. sequence



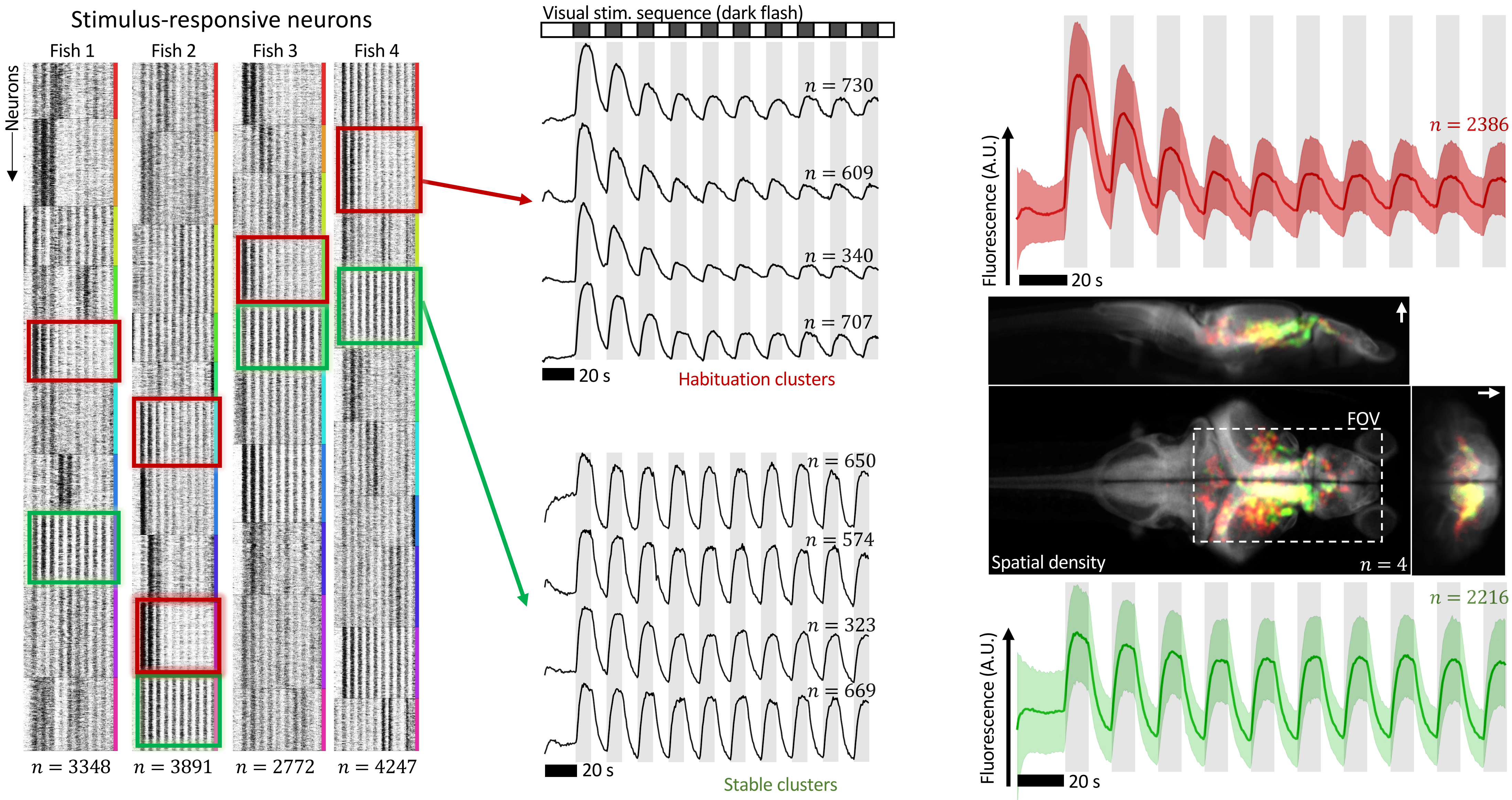
Green: Stable response Red: Habituation

Visual stimulation to probe neural circuits

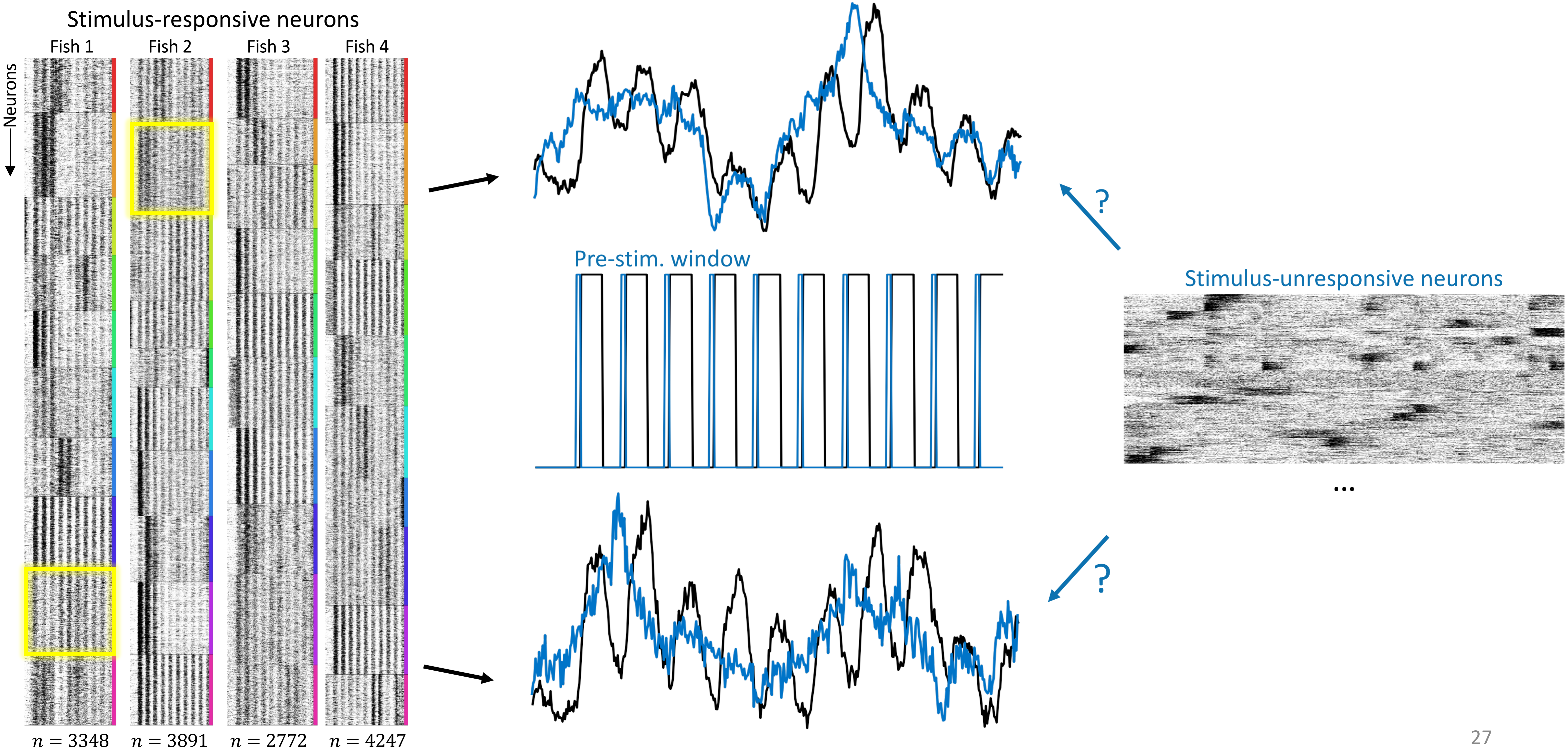
How to identify stimulus-responsive neurons?



Different response clusters to darkflash



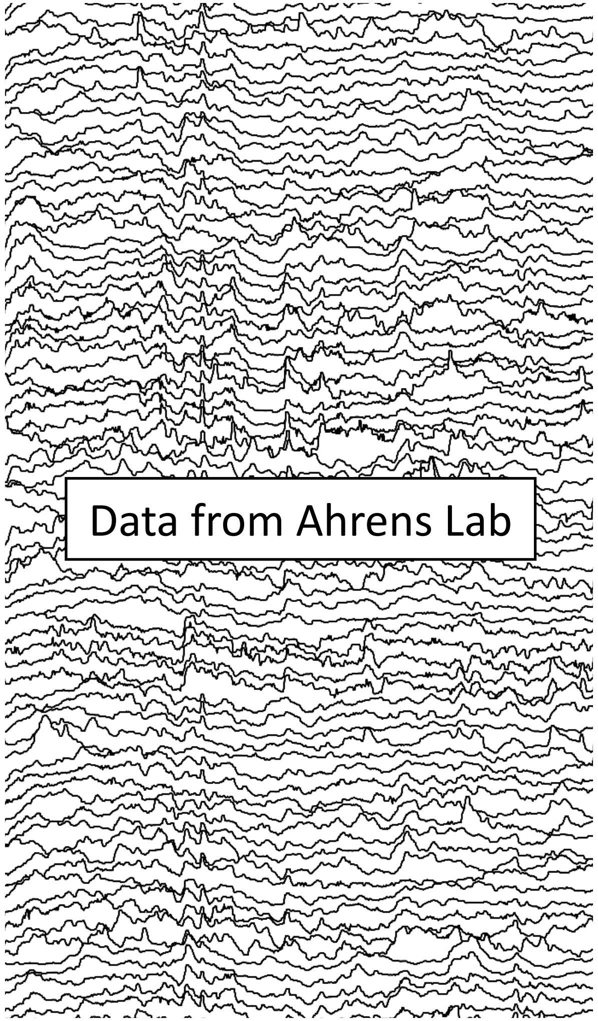
Different response clusters to darkflash



Brain Networks

Functional brain networks

R



$\sim 10^2$

$\sim 10^3$

T

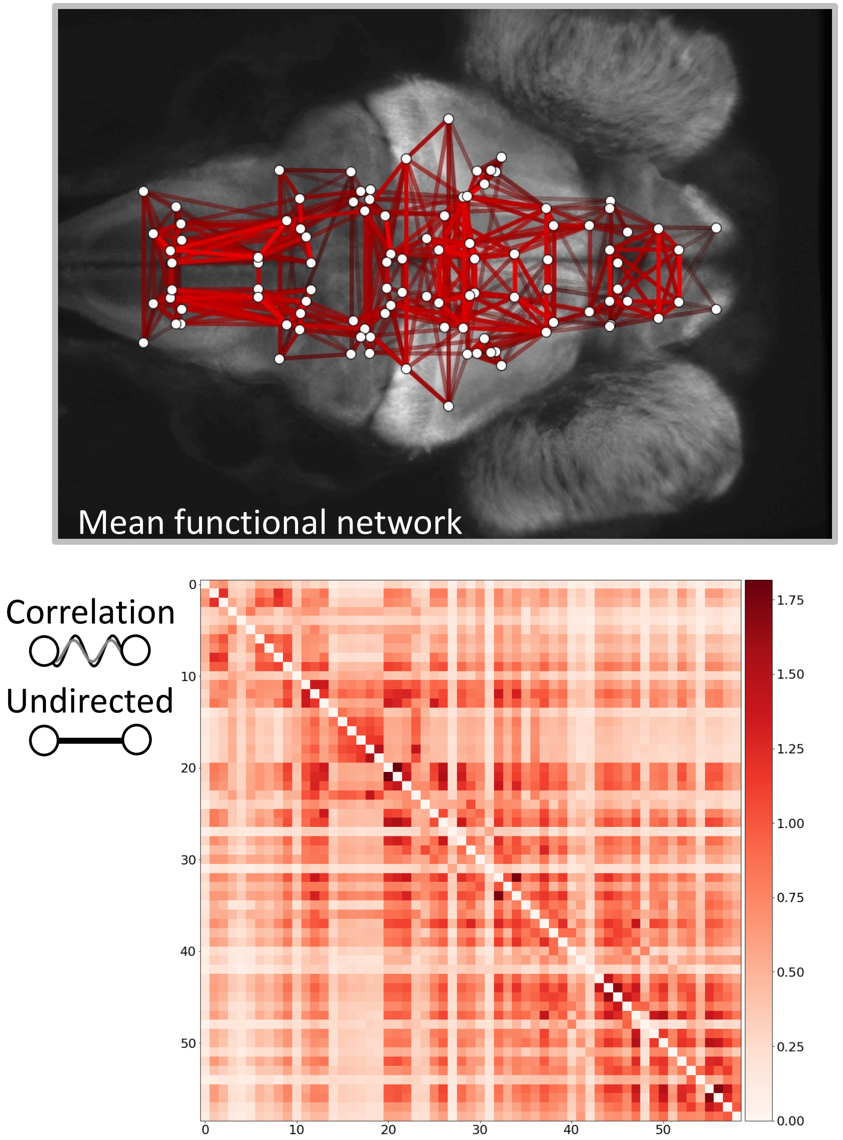
Correlation matrix

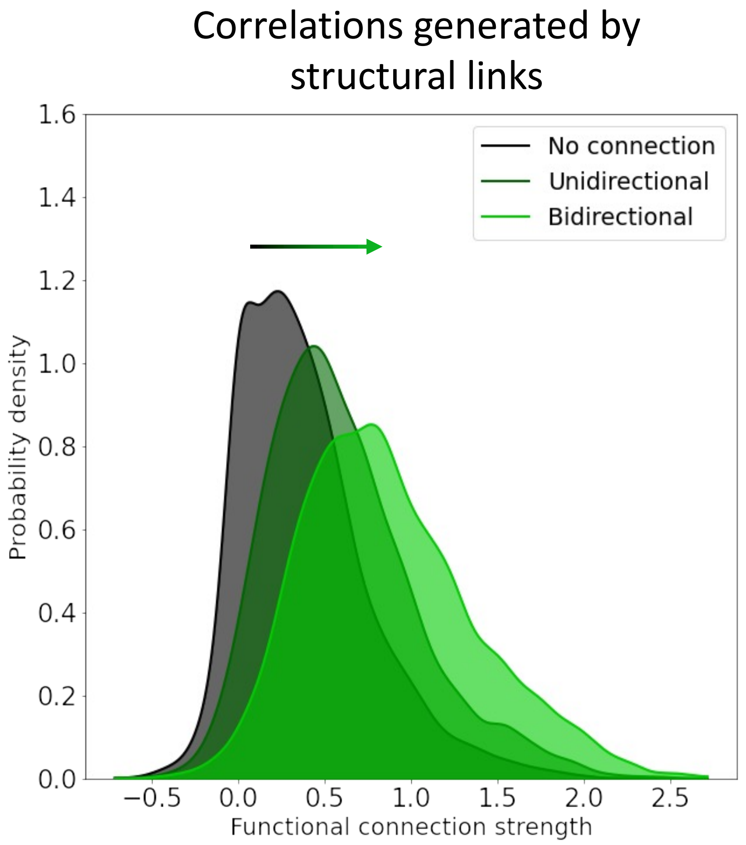
$N \times N$

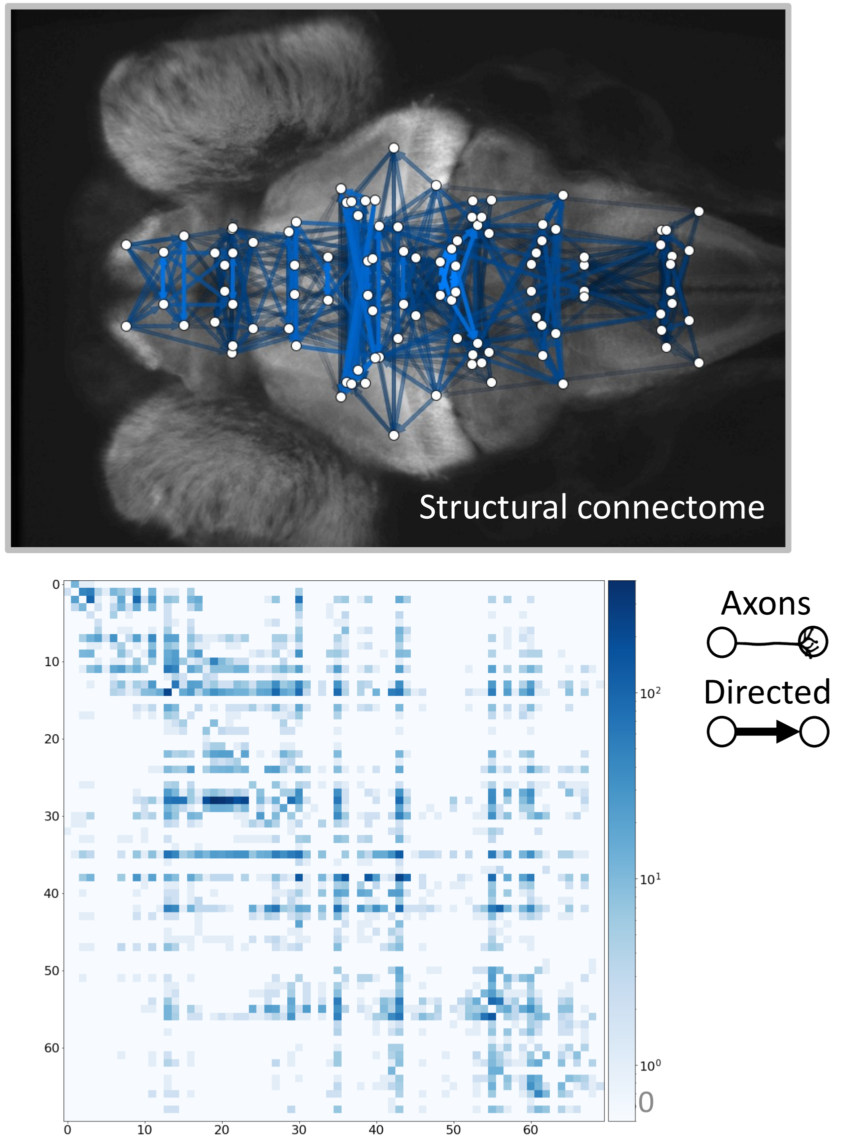
A_{ij} : Pearson correlation between region i and region j

Chen *et al.* (2018) *Neuron* **100**

Structural *vs* functional networks

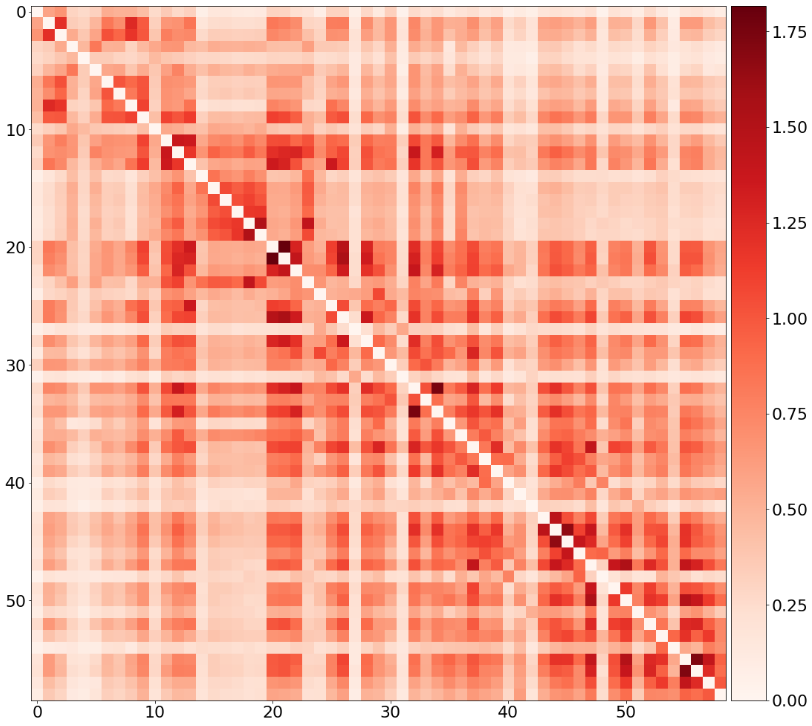




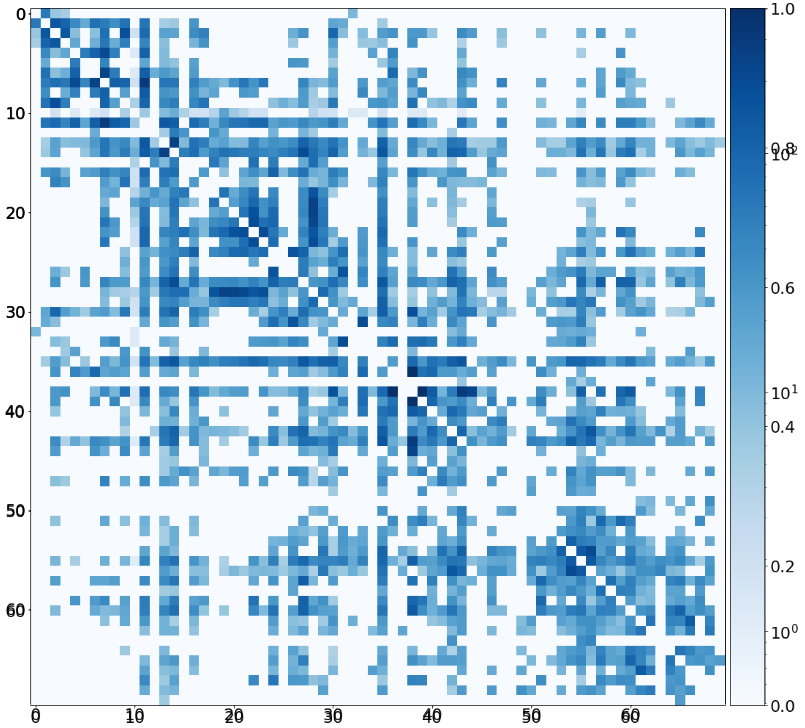


Structural *vs* functional networks

Mean functional network



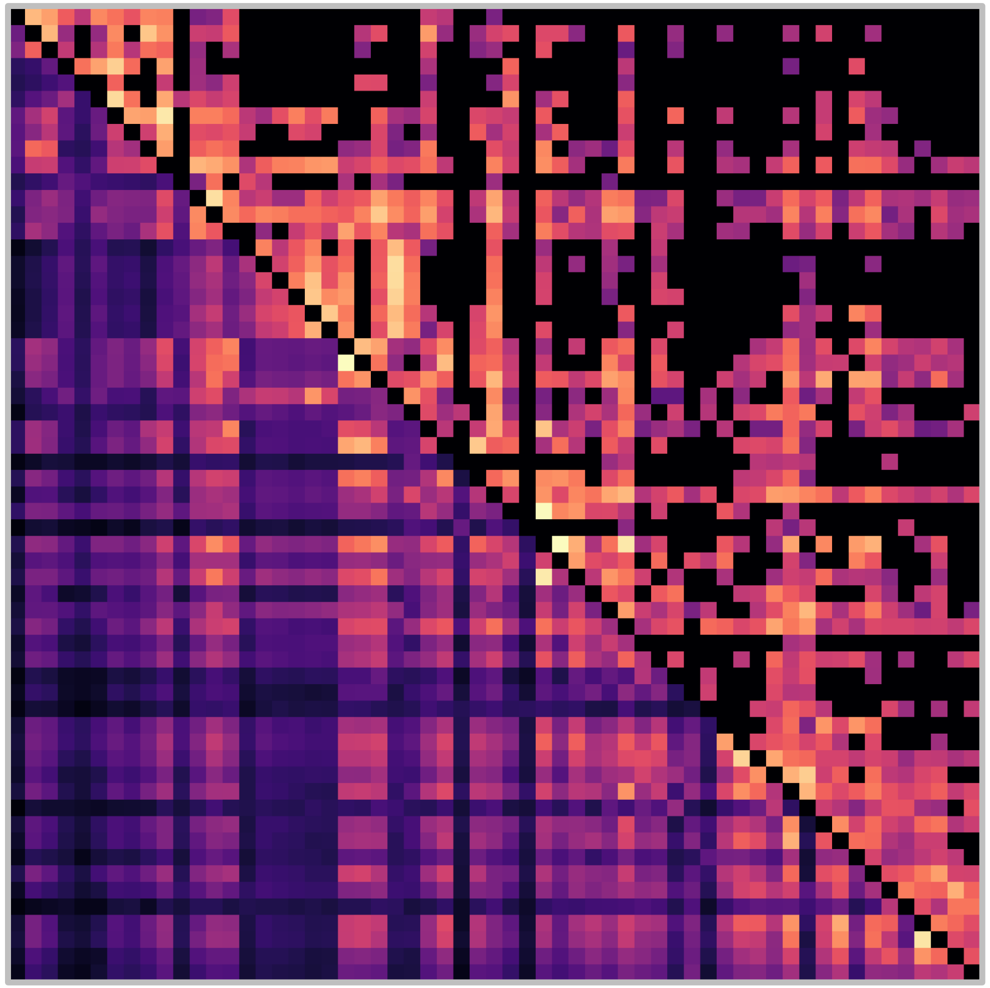
Structural connectome



Mirrored + Normalized

Structural *vs* functional networks

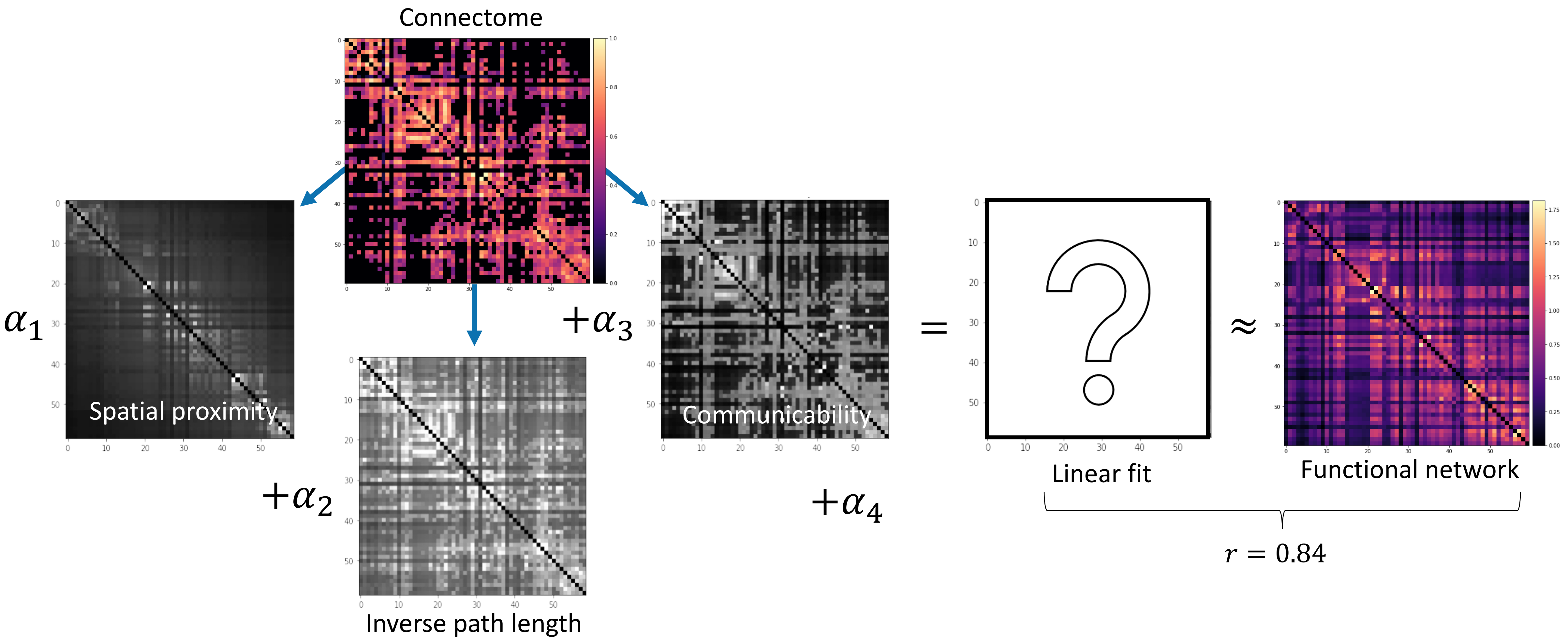
Mean functional network



Structural connectome

$r = 0.554$

Indirect pathways explain functional connectivity

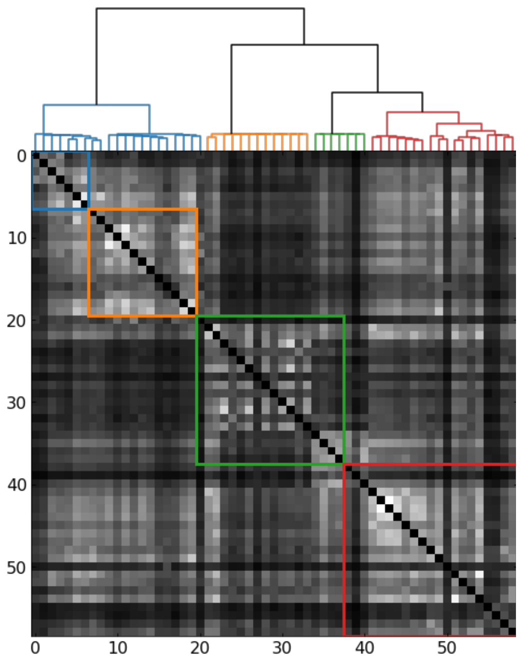


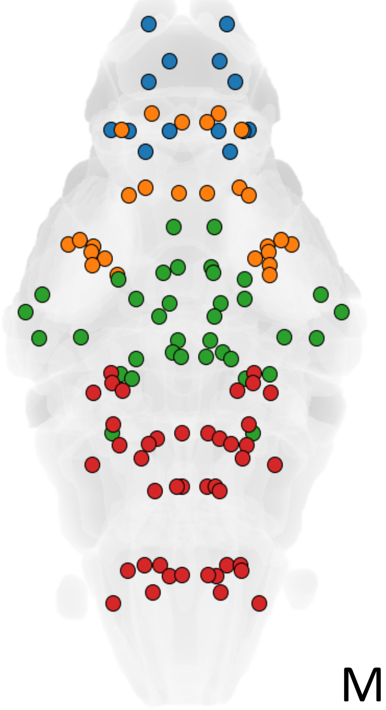
Spatial proximity: Distance separating regions
Inverse path length: Synapses separating regions
Communicability: Random diffusion over all possible pathways

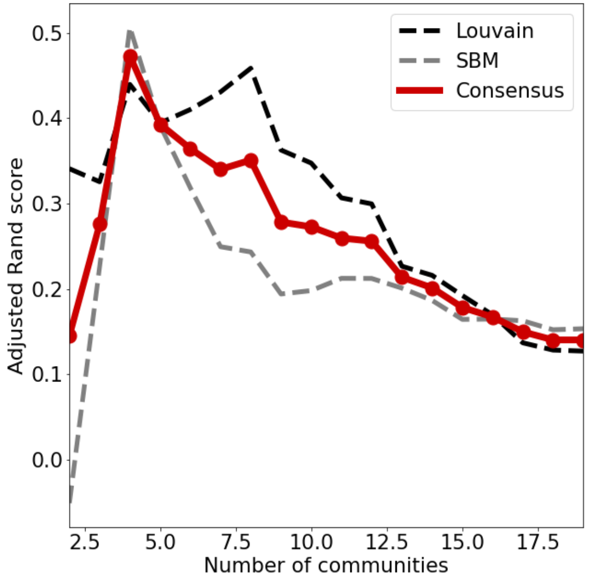
Vazquez-Rodr ıguez *et al.* (2019) *PNAS* **116**

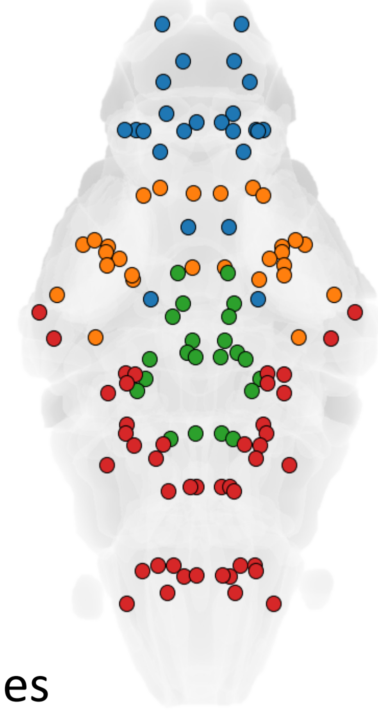
Modular structure of brain networks

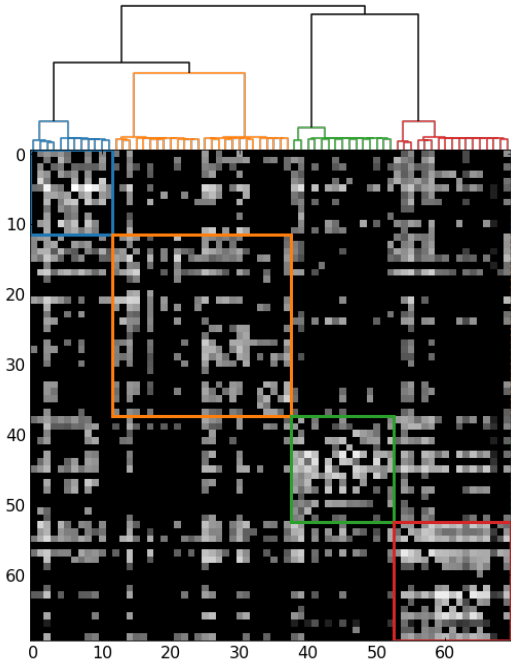
Communities/Modules: groups of brain regions with dense internal connections, and sparser connections between groups.











Functional modules

Maximal similarity at 4 modules

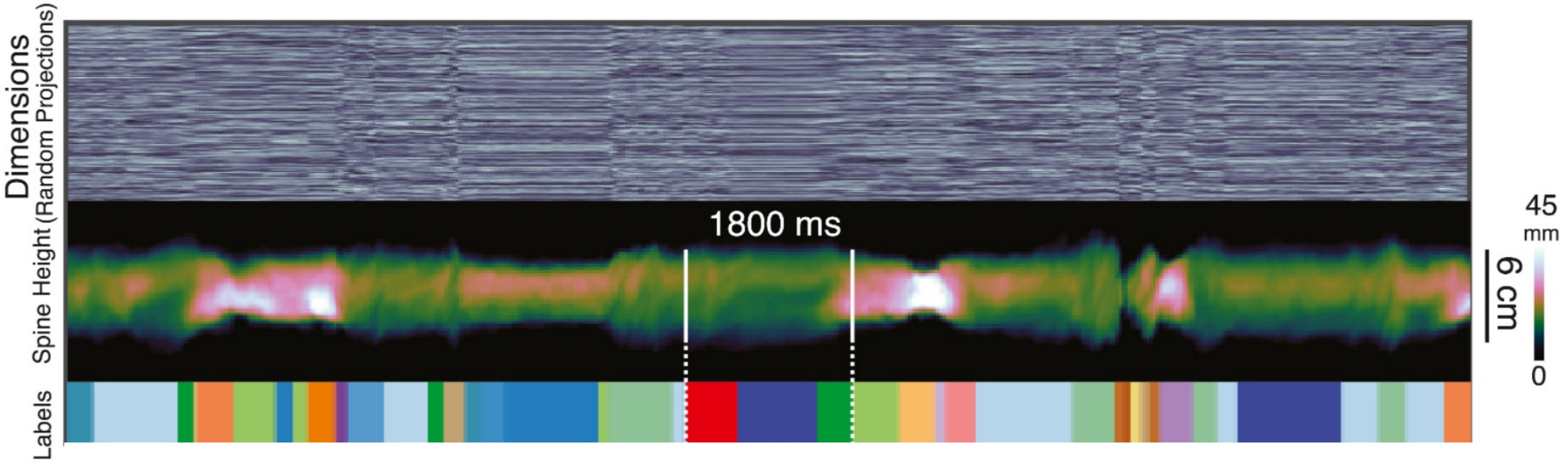
Structural modules

Brain States

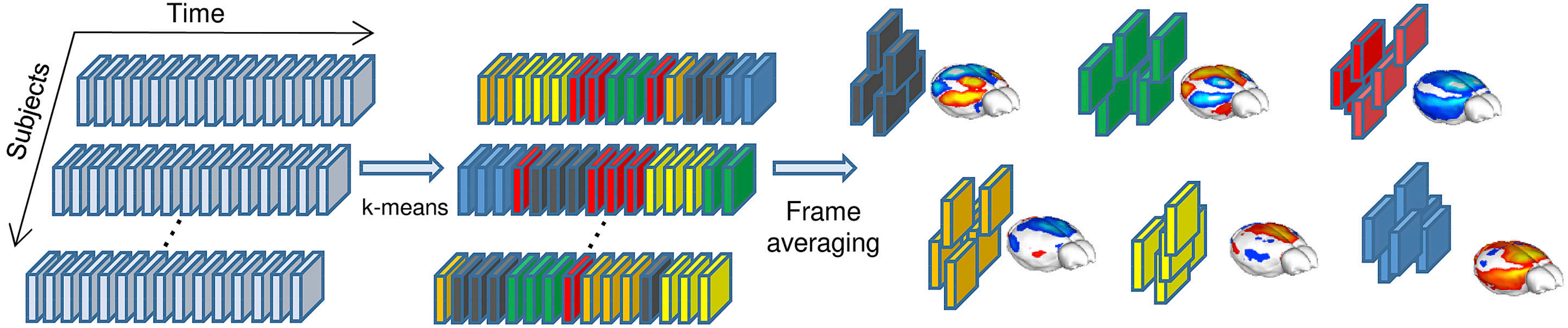
Finding recurring brain states

Thanks to Alex McGirr for pointing us towards this approach.

2015: decomposing mouse behavior into a sequence of discrete behavioral states

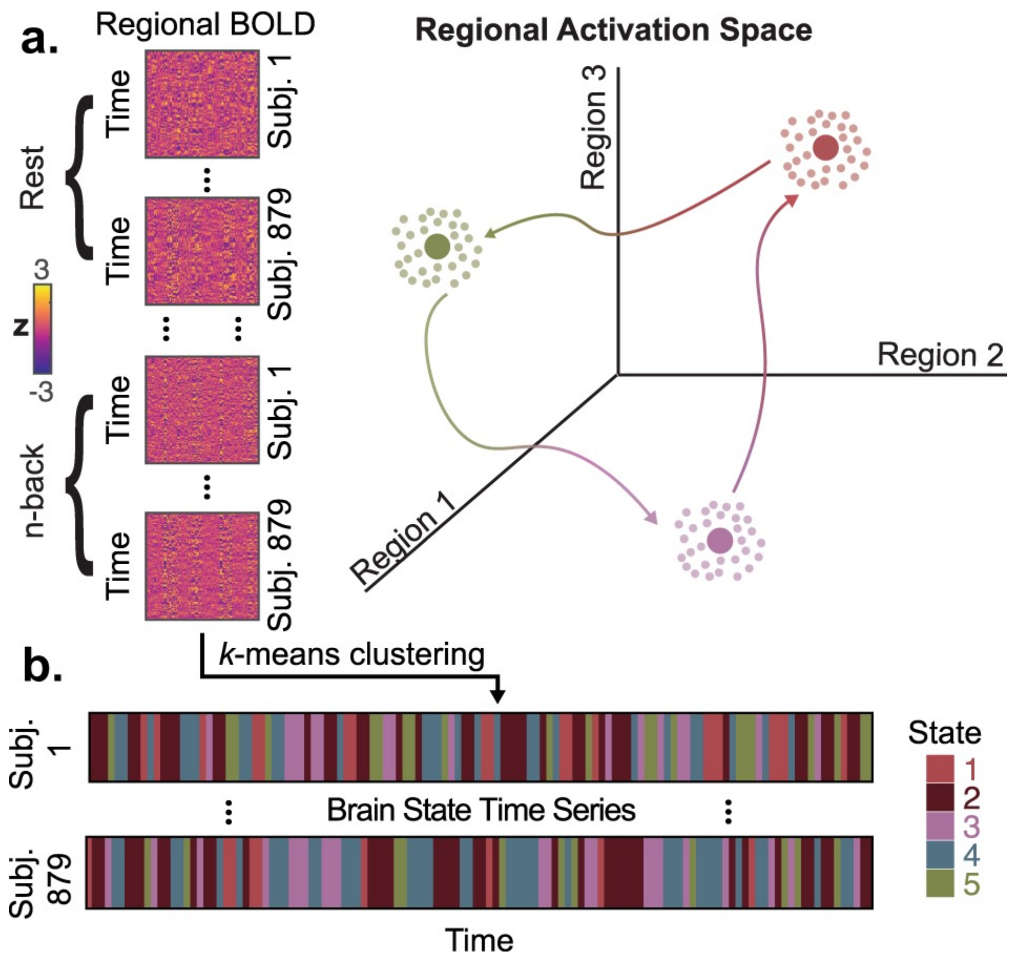


2019: decomposing mouse fMRI data into discrete brain states

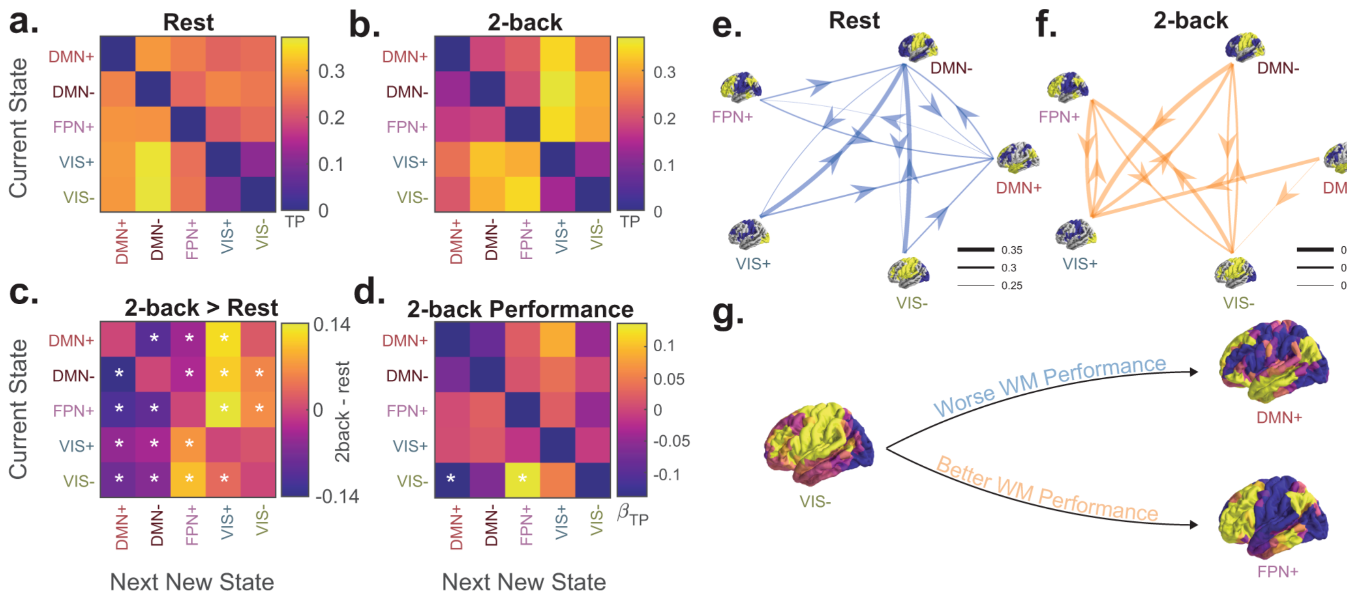


Wiltschko *et al.* (2015) *Neuron* **88**
Gutierrez-Barragan *et al.* (2019) *Current Biology* **29**

Finding recurring brain states



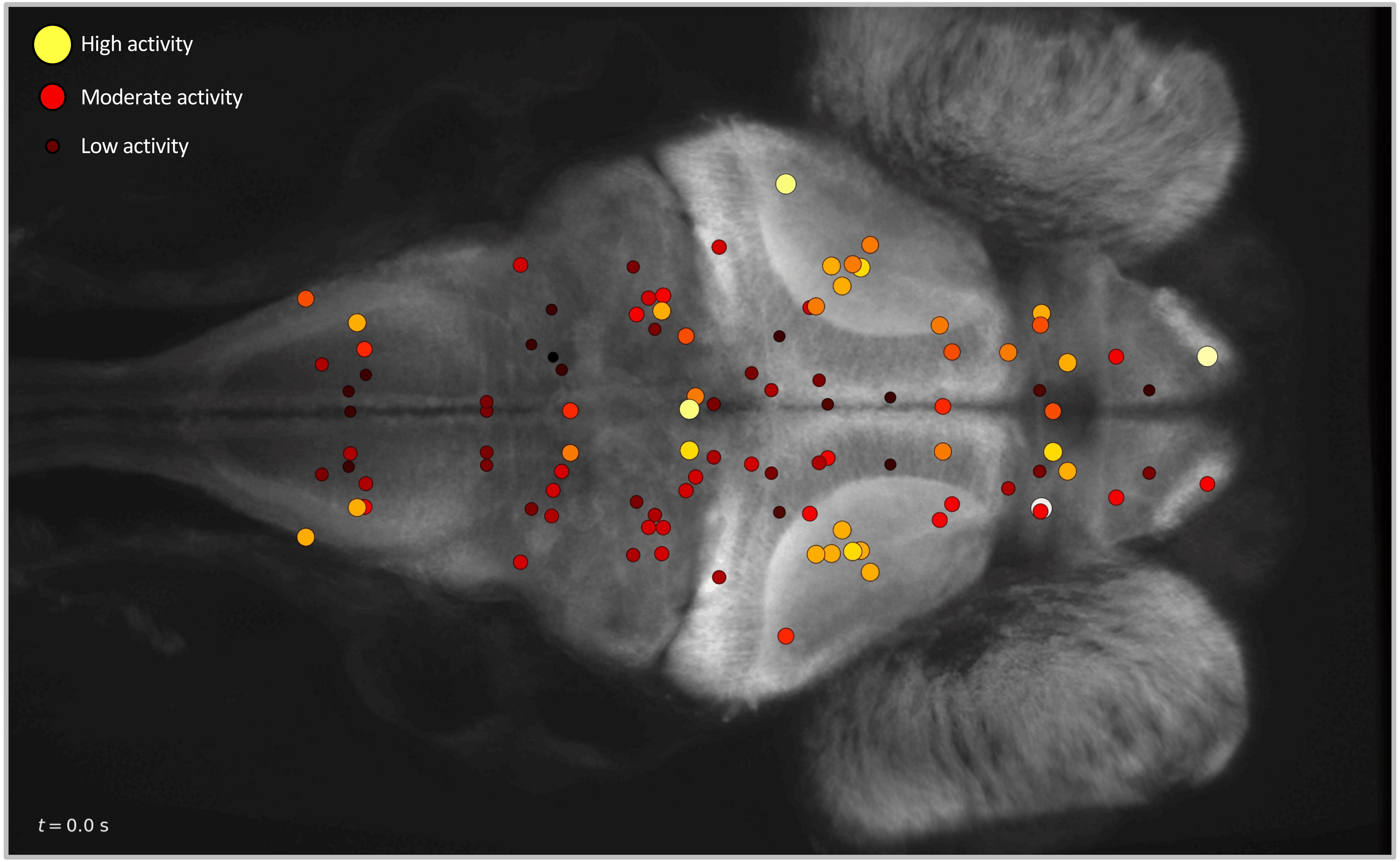
2020: decomposing human fMRI data into discrete brain states and inferring transitions probabilities between states.



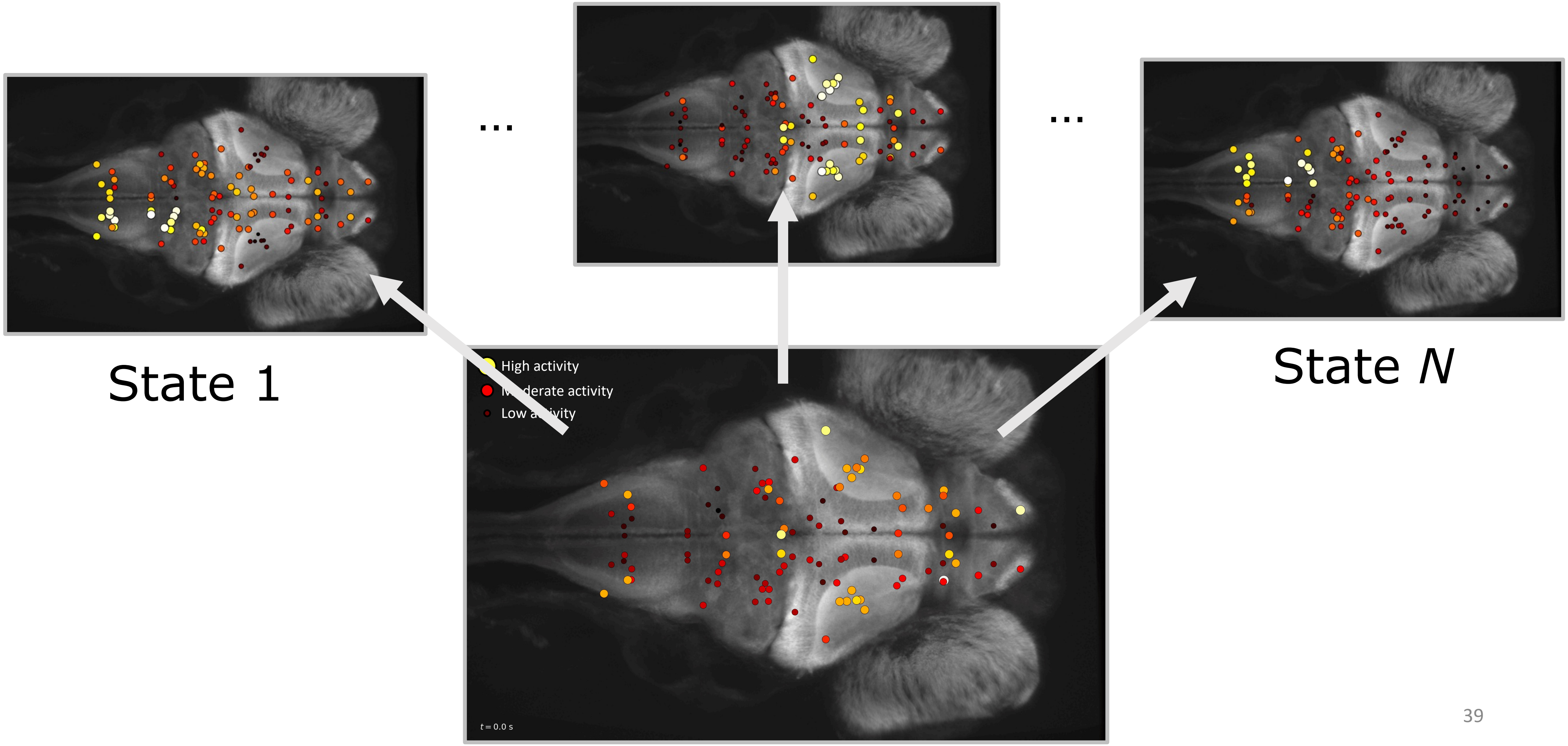
Cornblath *et al.*. (2020) *Commun Biol* **3**

Brain states in zebrafish

Spontaneous calcium dynamics of 104 brain regions

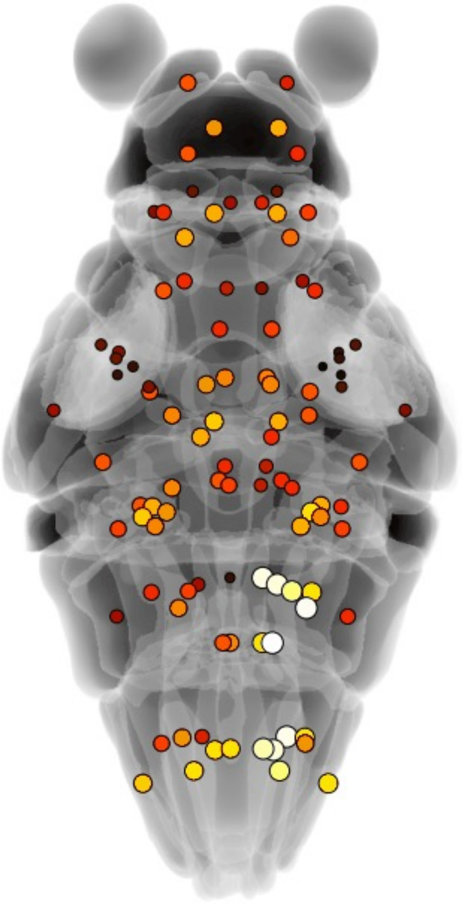


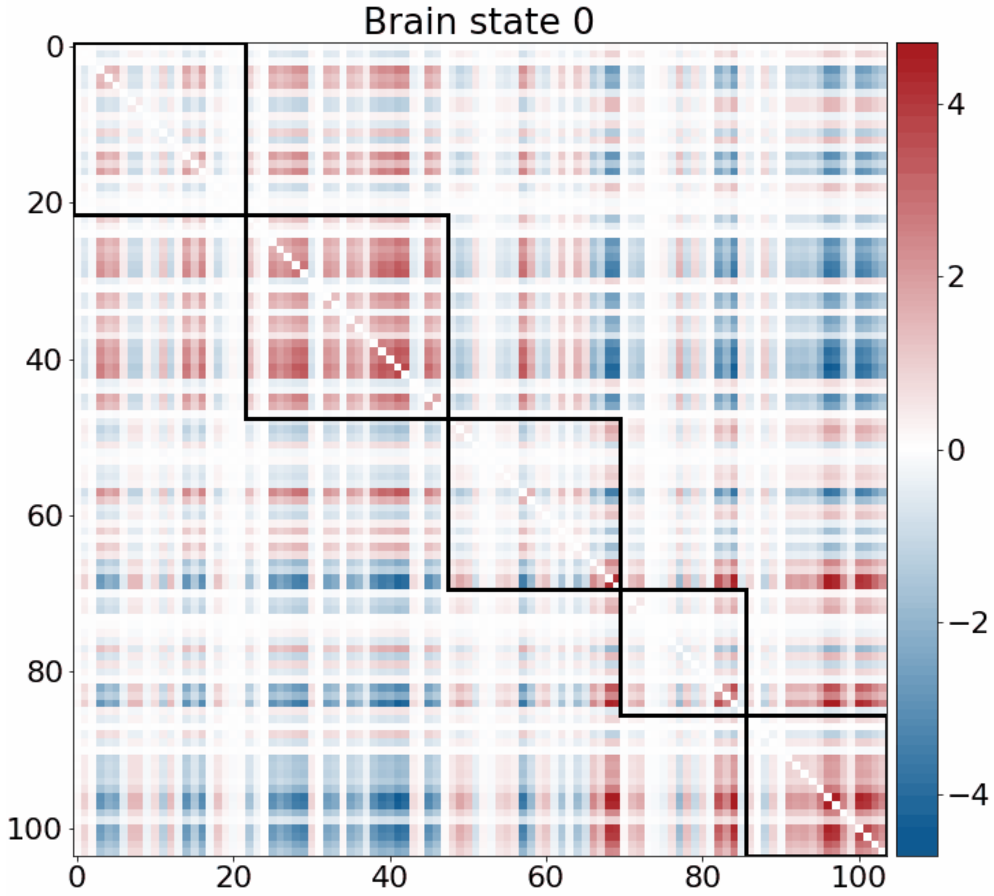
Brain states in zebrafish



Brain states emerge from structural modules

Cluster averages

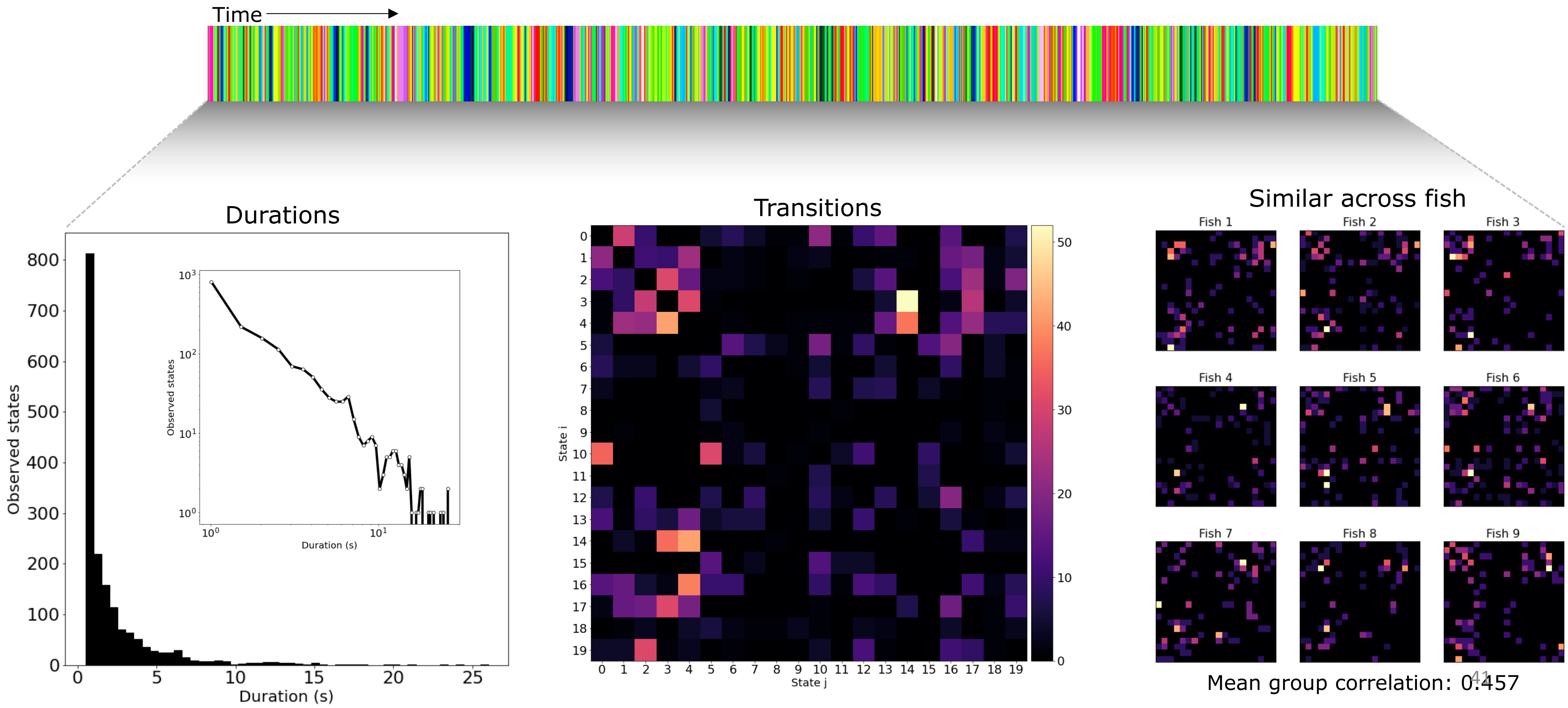




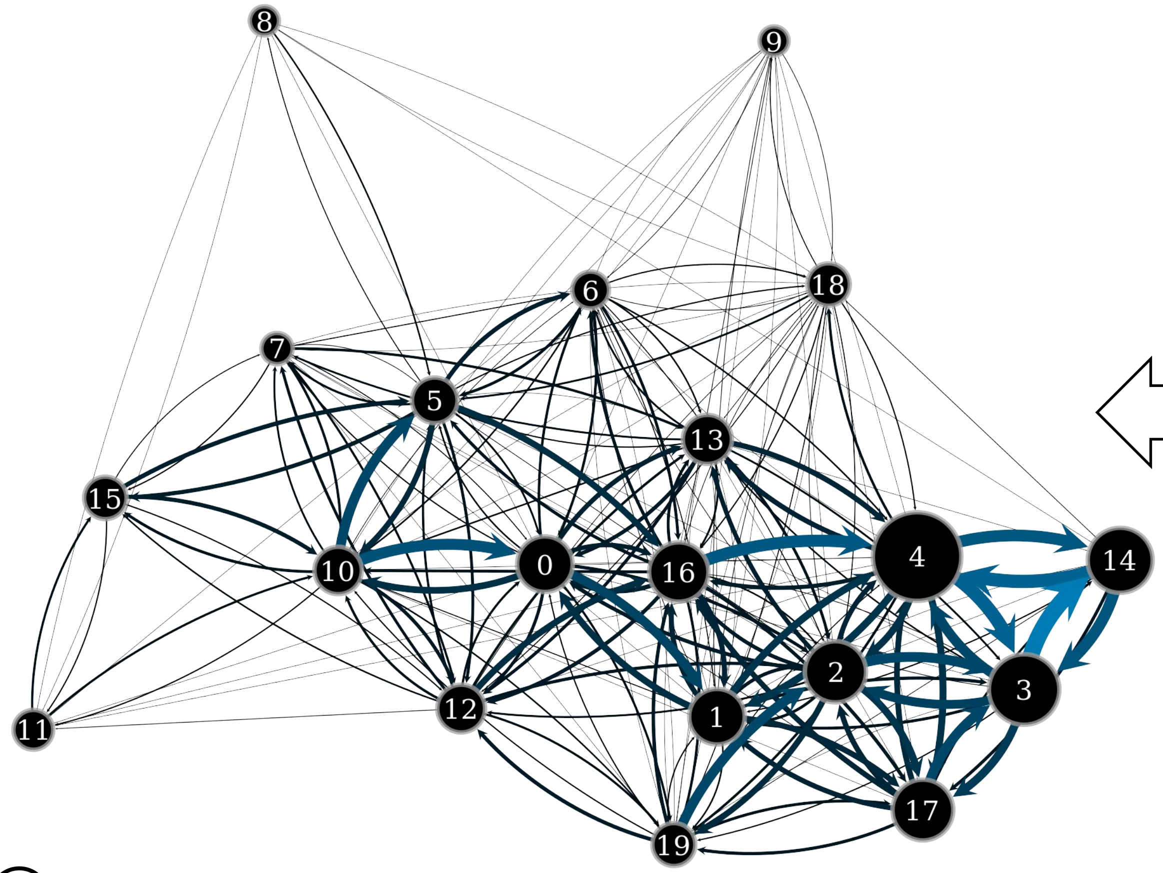
Instant correlations

Black boxes represent $N = 5$ structural modules

Brain states have distinct temporal properties



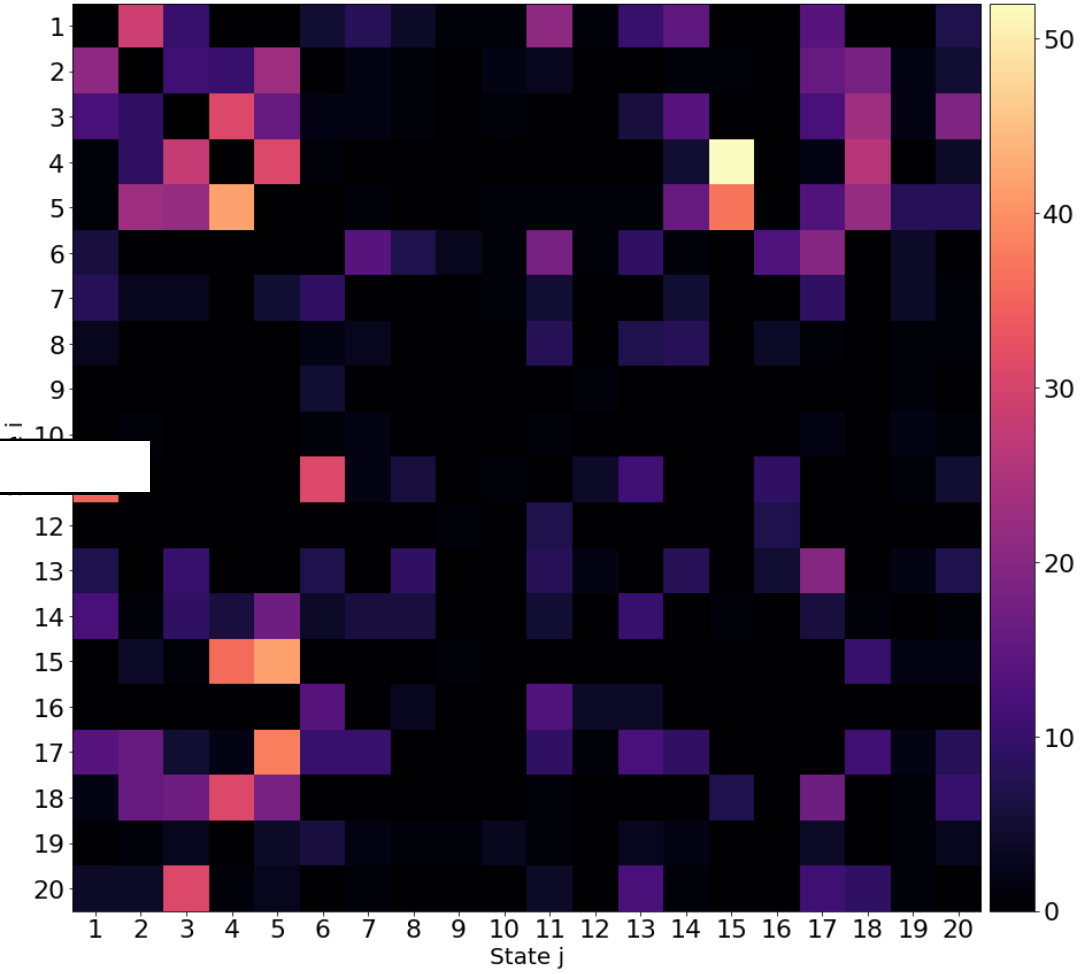
Brain states network



Node size: Time spent in state

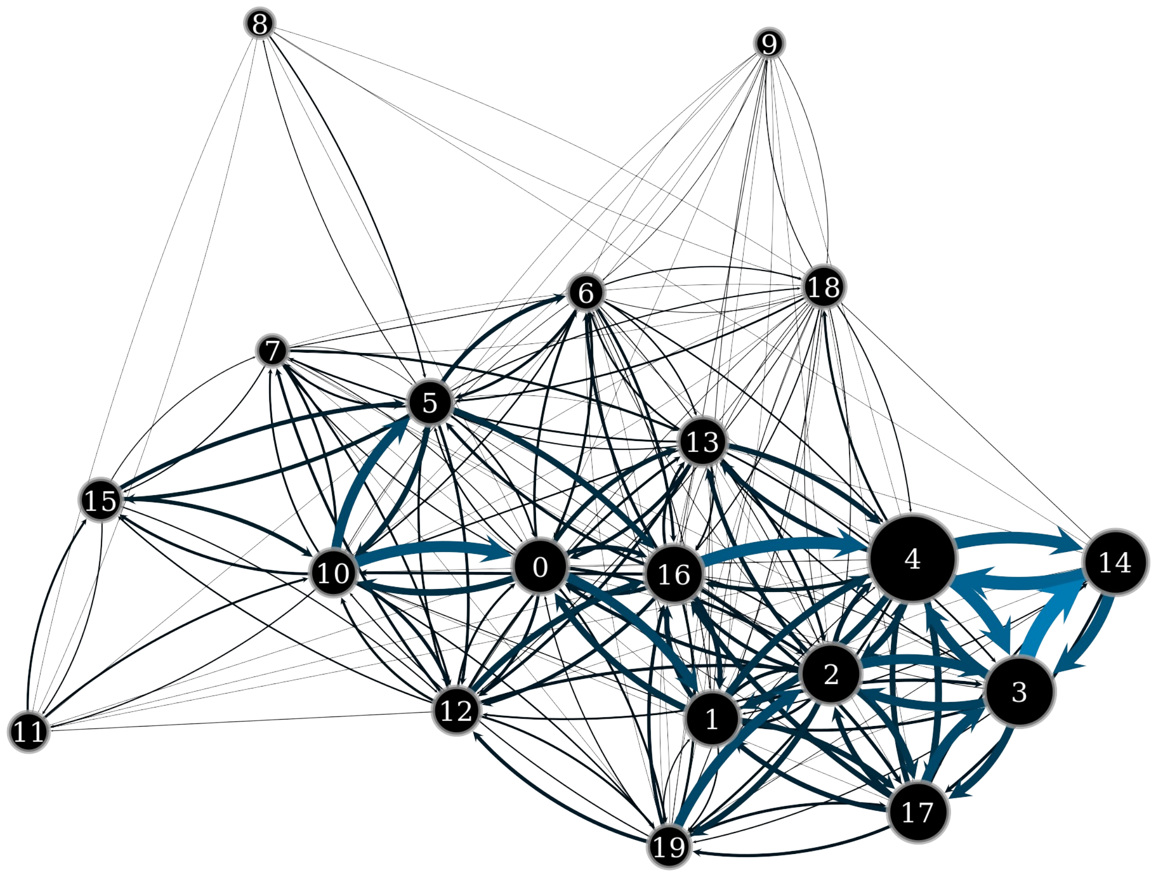
Arrow size: Number of observed transitions

Adjacency matrix

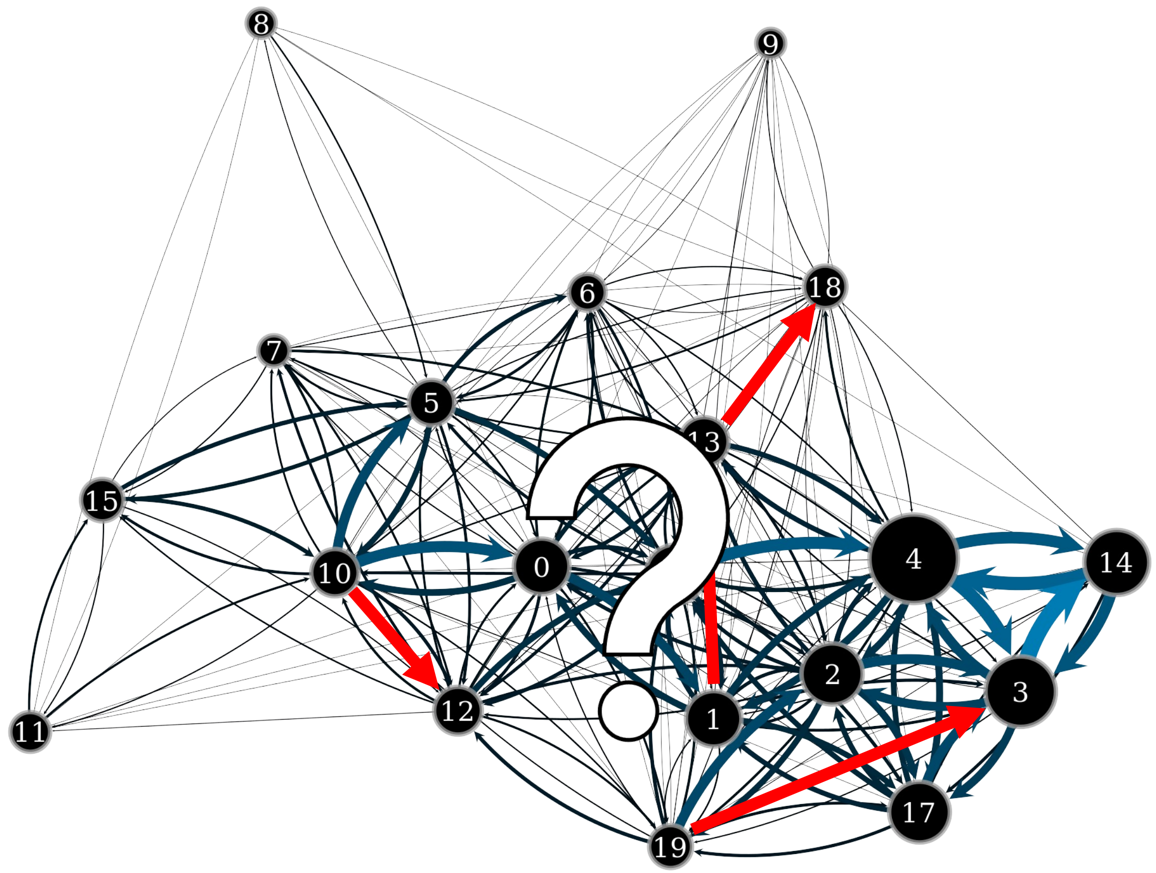


Brain states are organized into a core-periphery structure

Brain state transitions to describe the healthy brain



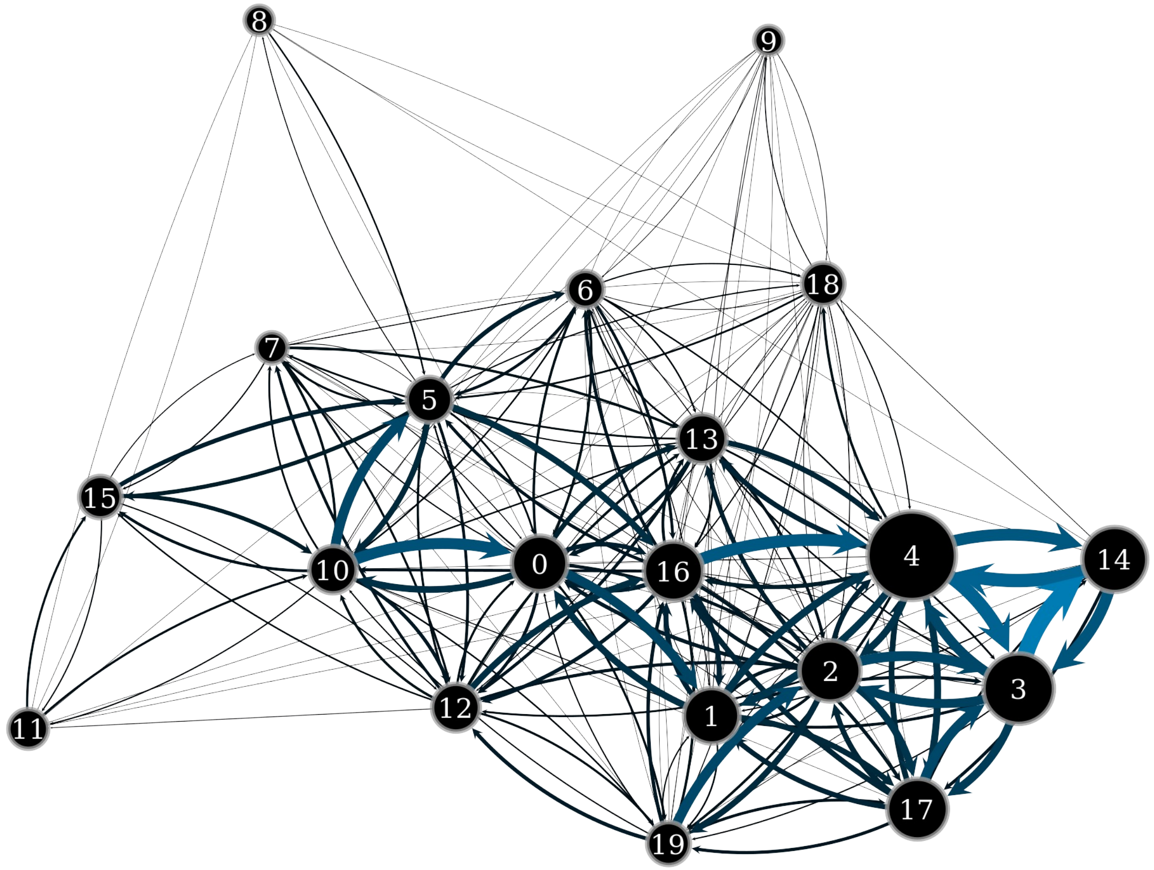
Healthy brain dynamics



Diseased/perturbed state

Outlook

Summary (work in progress)



- Neuronal correlates of trial to trial sensory response variability
- Strong structure/function relationship in zebrafish brain networks
- Discrete non-overlapping brain states for characterizing spontaneous brain activity

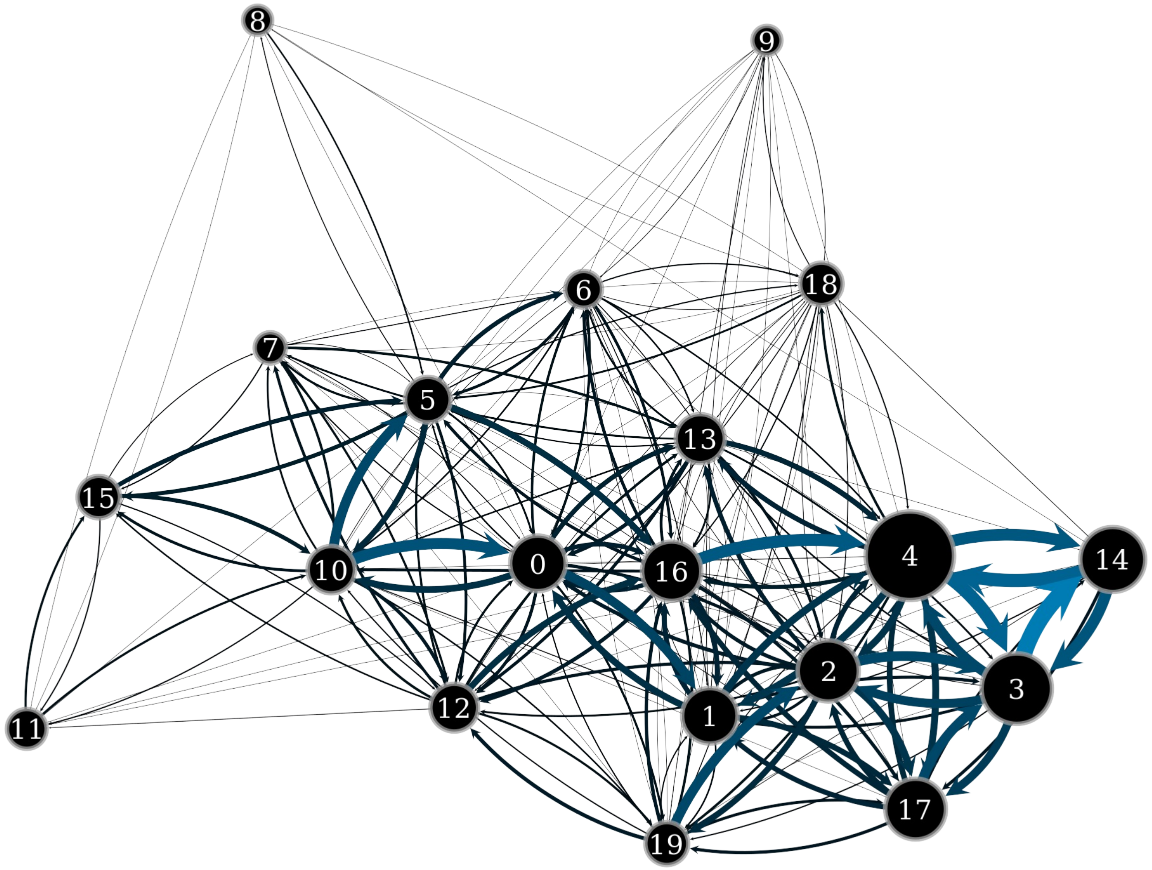
Future outlooks

How brain states are shaped by

- Neuromodulation
- Different conditions (stress, gut microbiota, sleep deprivation, etc)
- Learning

Optogenetics

- Inhibit or trigger transitions between global states



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