

NeuroSEE: a pipeline for processing and analysing data from multiphoton fluorescence brain imaging experiments

Simon Schultz

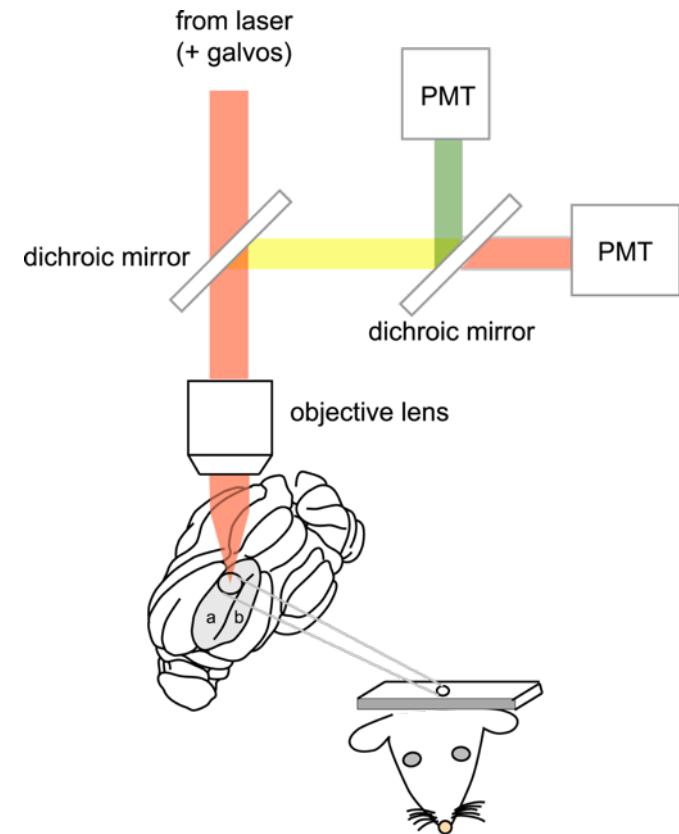
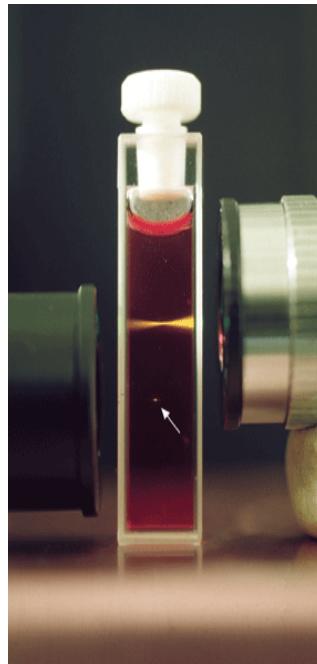
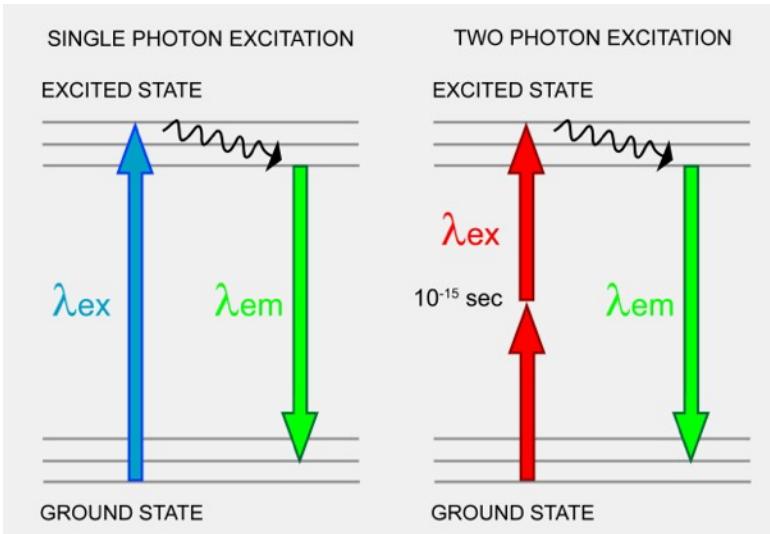
Centre for Neurotechnology & Department of Bioengineering,
Imperial College London



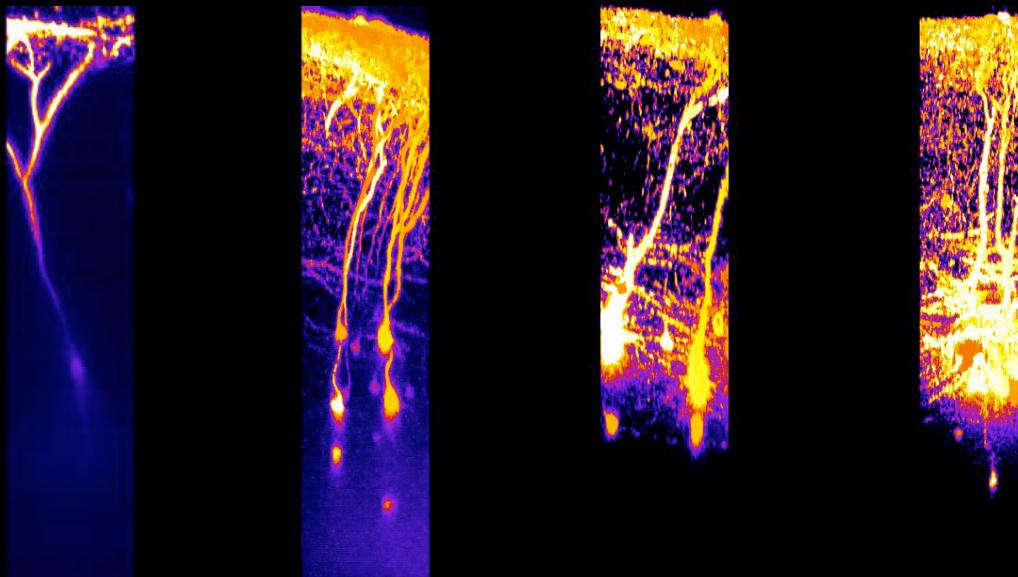
Talk Overview

- Introduction to our data and technologies:
 - 2P-targeted robotically automated *in vivo* patching
 - Imaging neural circuit activity during performance of behavioural tasks
(example given: hippocampal CA1, spatial behaviour)
- NeuroSEE pipeline
 - Neuronal Source Extraction & Exploration
 - Image processing through to data analysis
- Whole-brain, *ex vivo* two photon tomography of labelled circuits

The two photon microscope



In vivo two photon imaging

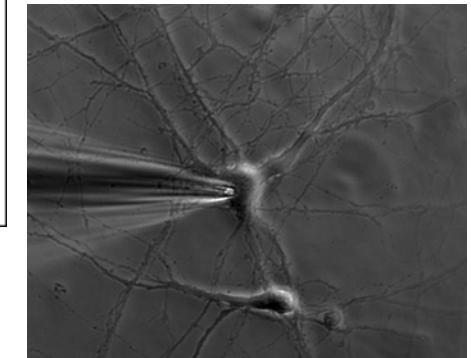
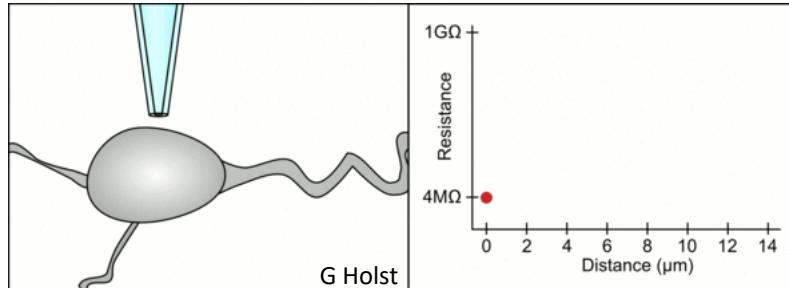
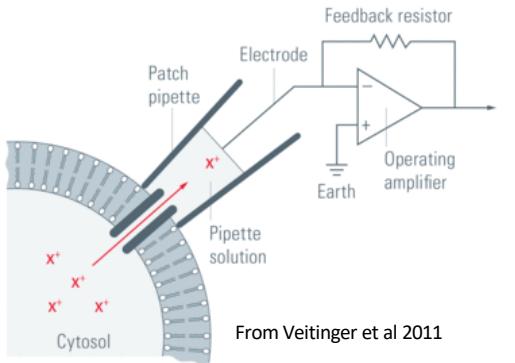


100 μm

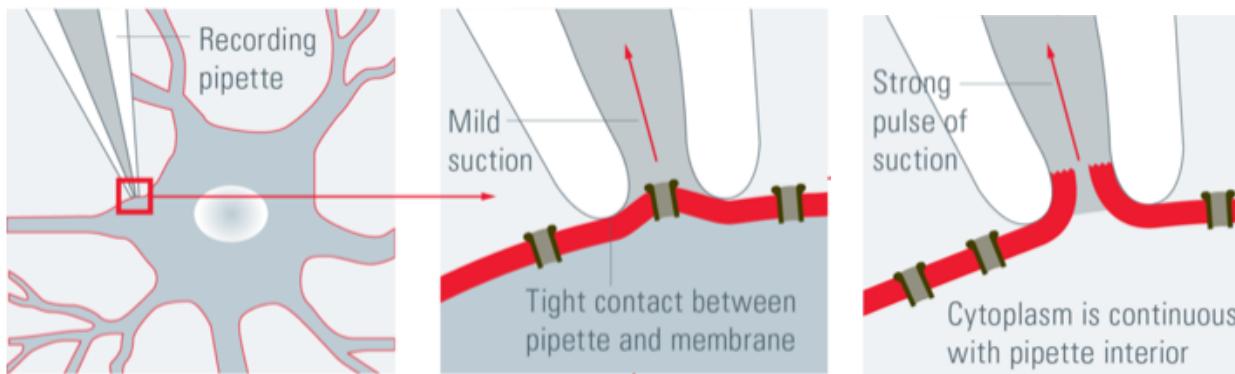
L5 pyramidal cells

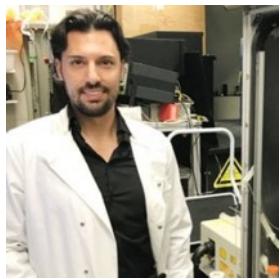
The “gold standard” for monitoring neuronal properties: whole-cell patch clamp

Sakmann & Neher, 1970s; Giga seal: Ernst & Sakmann, 1980.



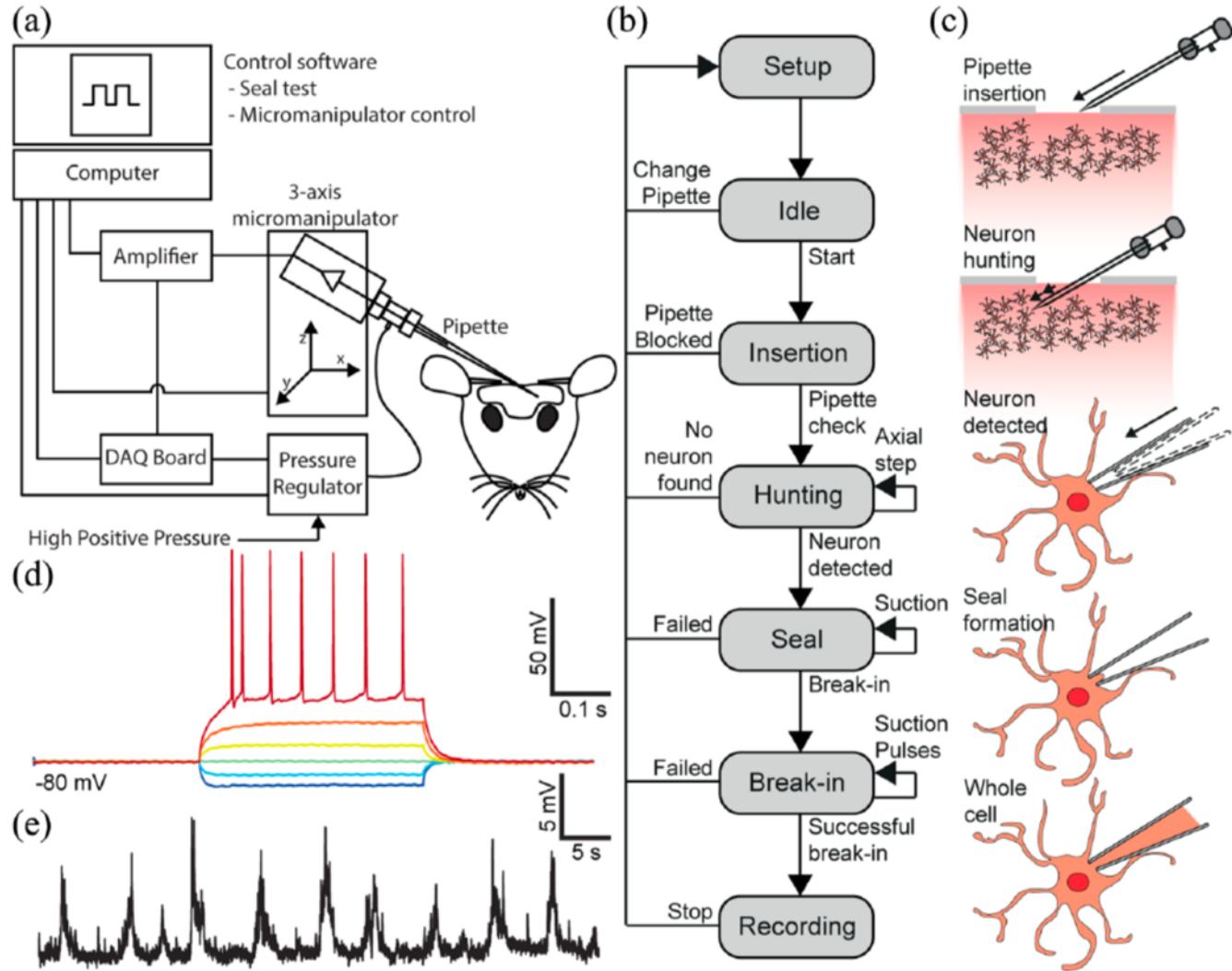
Cell-attached recording





Success rate: 51.4 %.
(Our implementation).
74% for cell-attached.

1st demonstrated by
Kodandaramaiah et. al.
(Success rate 42.7%)



Two photon targeted patching (TPTP)

Even harder than *in vivo* “blind” patching. Why? (Typically, much) fewer targets!

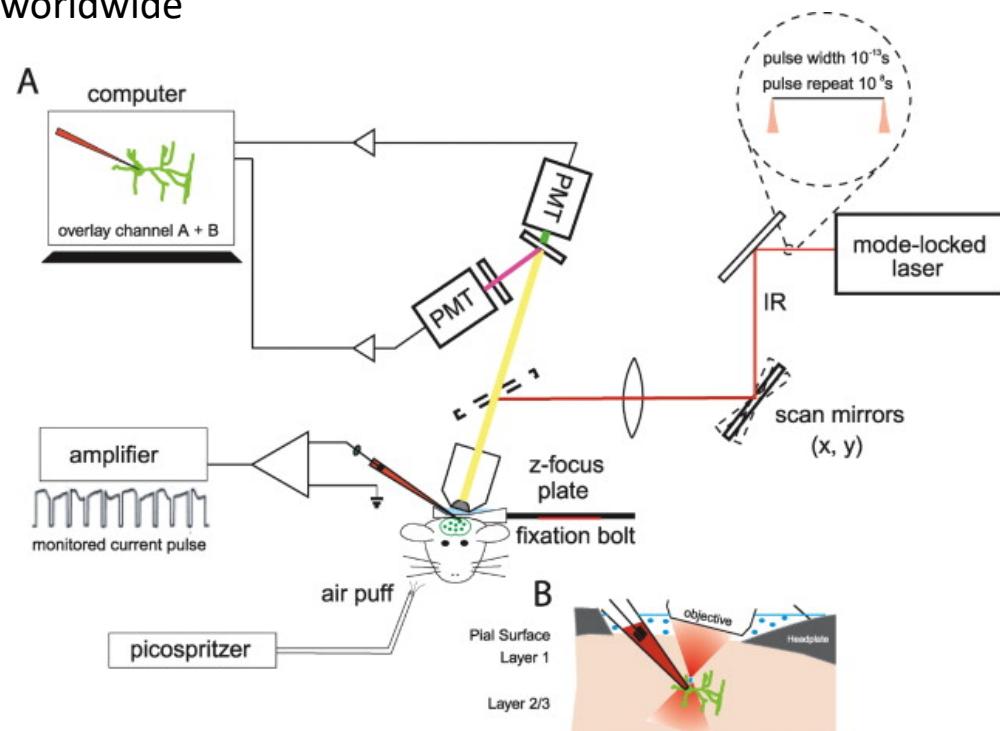
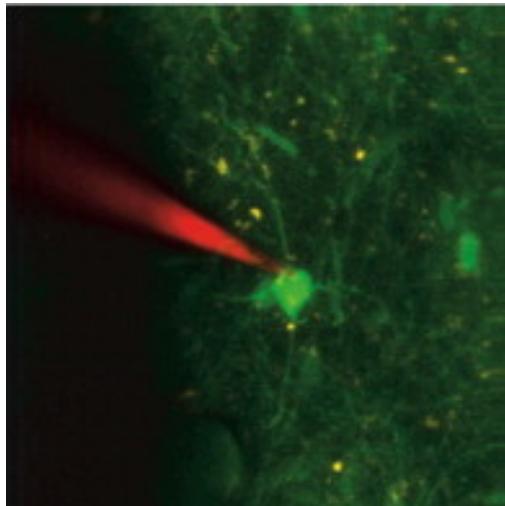
Extremely few experienced TPTPers worldwide

We need to:

Increase throughput & reliability

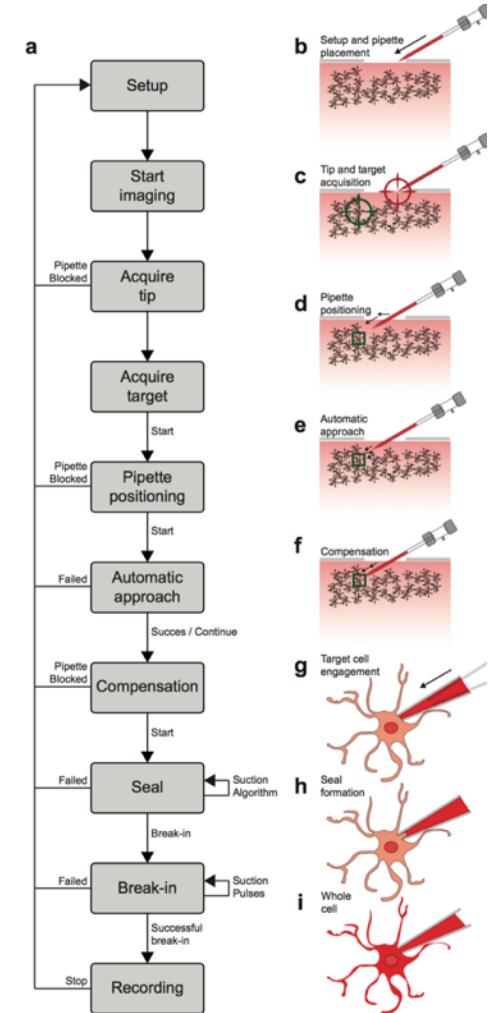
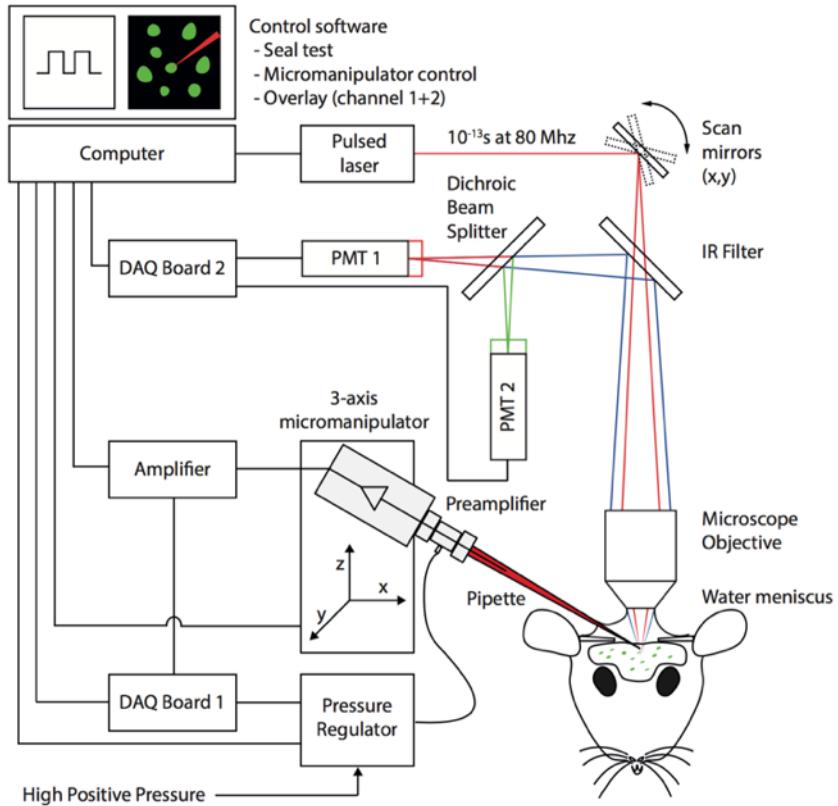
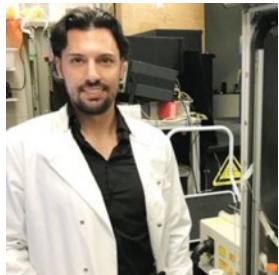
Lower entry barrier

Robotic automation?

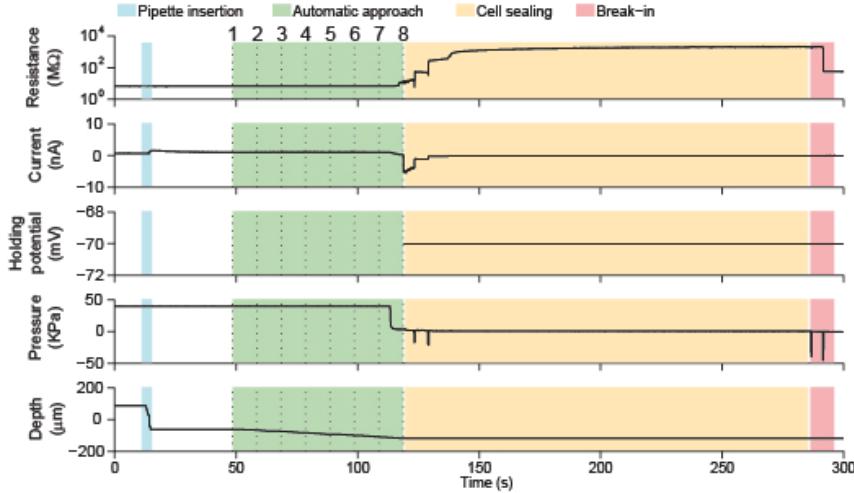
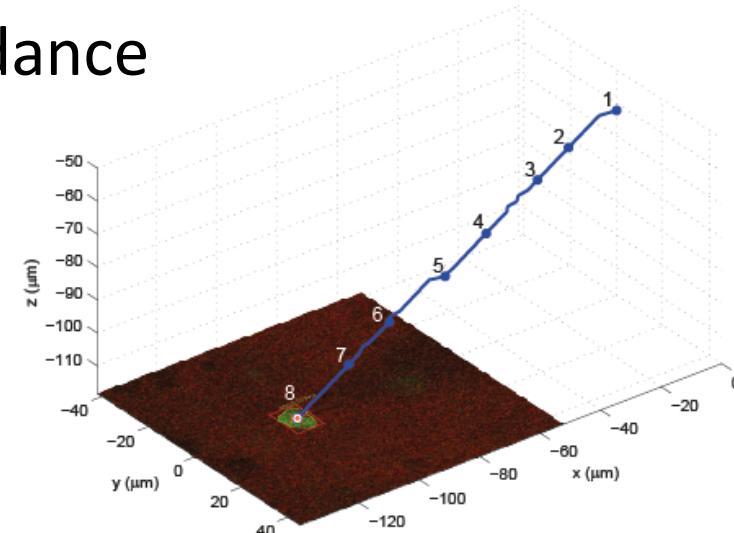
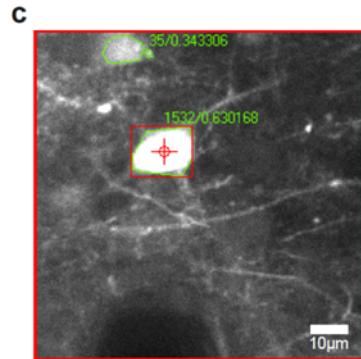
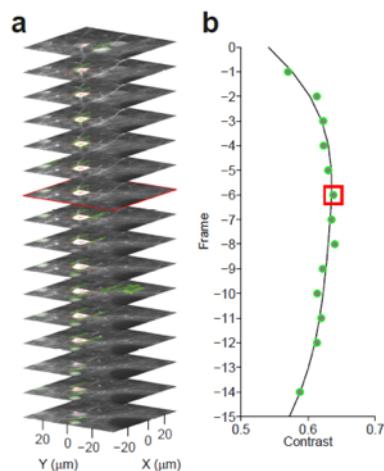
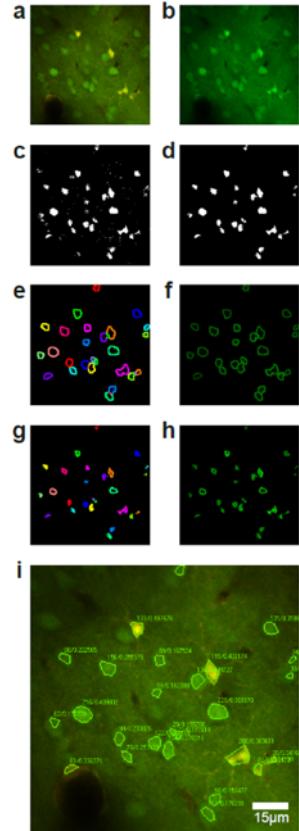


T Margrie et al, Neuron 2003

2P-targeted patching robot

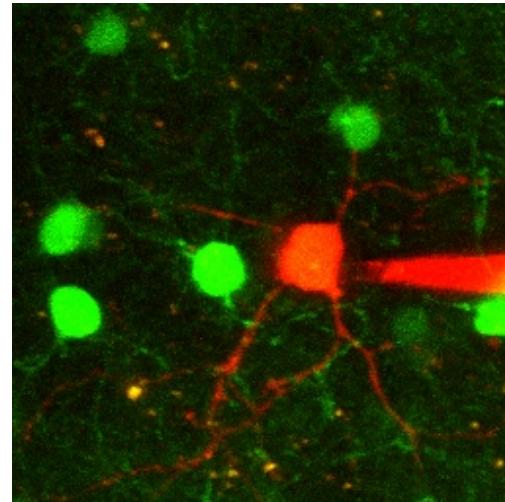
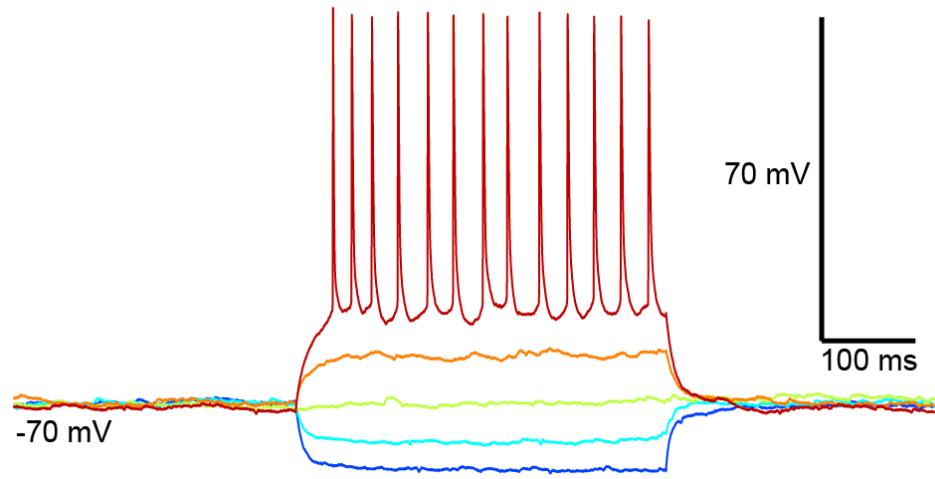


Pipette guidance

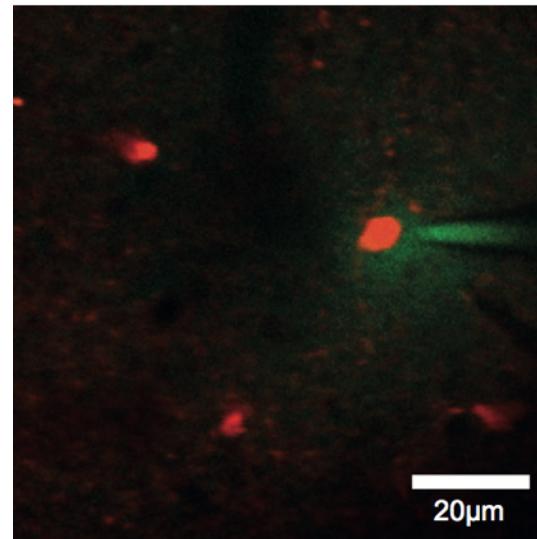
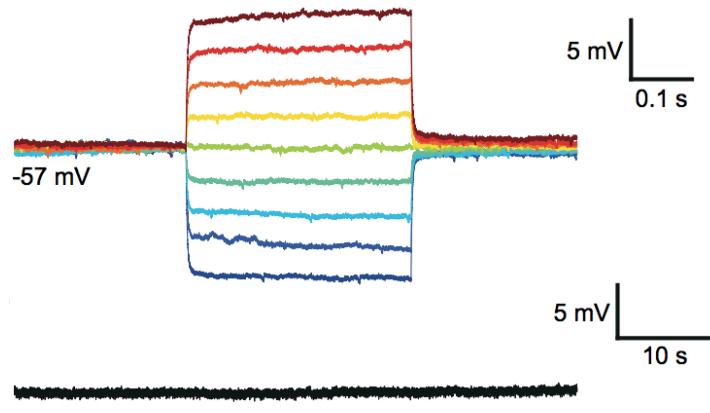


Targeted recording

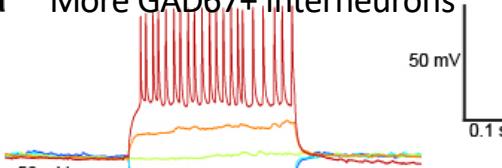
(GAD67-GFP)



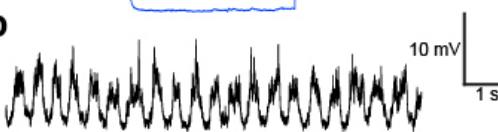
Targeted recording from astrocyte



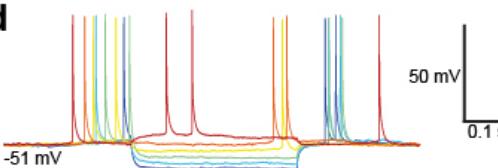
a More GAD67+ interneurons



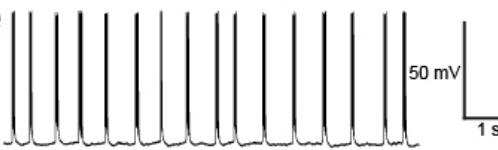
b



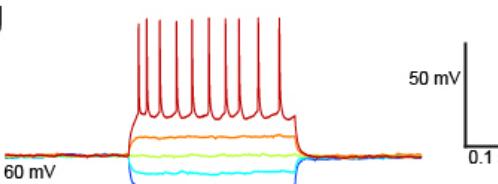
d



e



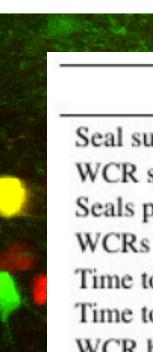
g



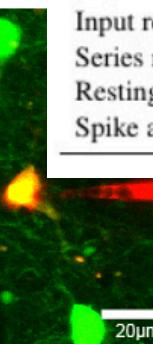
h



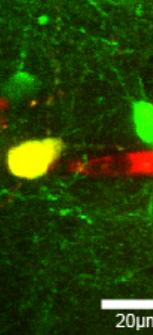
c



f



i



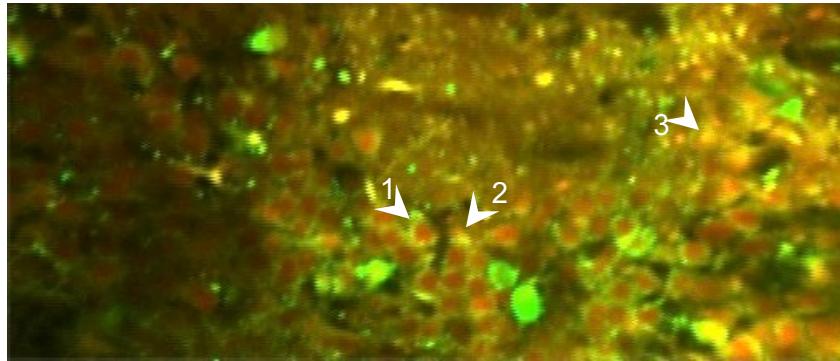
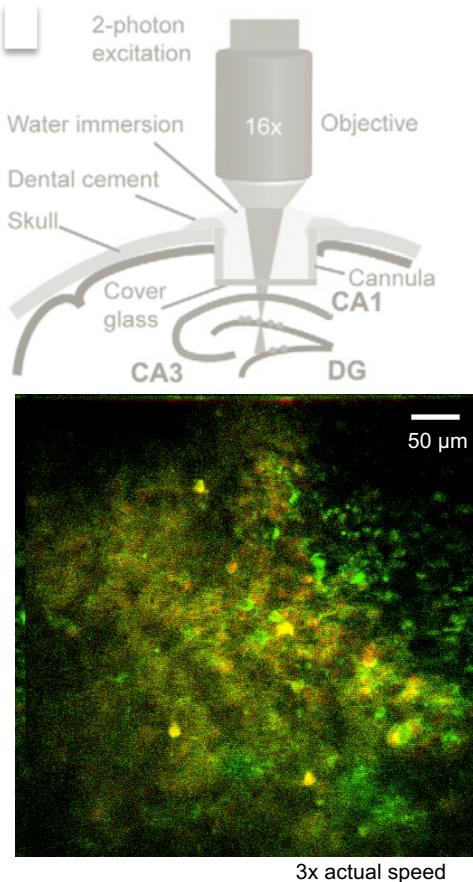
	Robotic	n	Manual	n	Blind	n
Seal success rate (%)	46.6	90	56.4	78	74.2	35
WCR success rate (%)	22.2	90	18.0	78	51.4	35
Seals per animal	2.3 ± 0.3	18	4.9 ± 1.0	9	5.2 ± 0.3	5
WCRs per animal	1.1 ± 0.2	18	1.6 ± 0.3	9	3.6 ± 0.4	5
Time to achieve seal (min)	6.0 ± 0.6	20	9.8 ± 1.4	14	3.3 ± 0.4	26
Time to achieve WCR (min)	6.1 ± 0.6	"	10.0 ± 1.4	"	3.6 ± 0.4	18
WCR holding duration (min)	16.6 ± 3.3	"	10.1 ± 3.6	"	-	-
Input resistance (MΩ)	139.2 ± 11.6	"	183.7 ± 21.0	"	64.1 ± 4.2	18
Series resistance (MΩ)	16.0 ± 0.5	"	16.3 ± 0.3	"	14.9 ± 0.5	"
Resting potential (mV)	-54.1 ± 1.8	"	-54.8 ± 2.0	"	-57.8 ± 4.8	"
Spike amplitude (mV)	42.7 ± 10.7	"	40.2 ± 2.3	"	50.1 ± 5.4	"

Quality of recordings is similar for robots and experienced humans

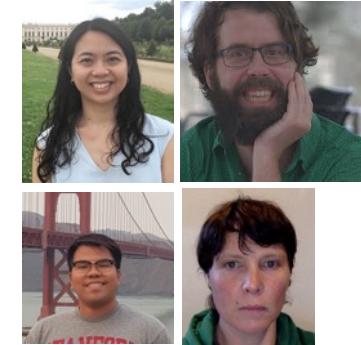
Summary: 2P targeted patching

- Robotic automation of two-photon targeted patch clamp electrophysiology achieved
- Performance comparable to a human operator
- Prospect of improved rollout of TPTP technique across wide range of labs
- Next steps
 - Targeted multipatching, unpatching, etc
 - Applications: e.g. targeted recording from interneurons, cells near plaques etc in mouse models of dementia
 - Dissemination: difficult but we are working on it

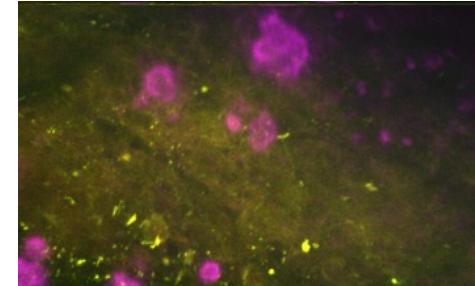
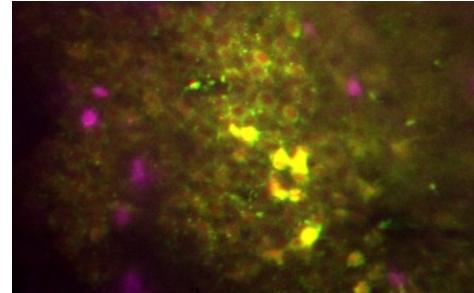
Imaging neural activity during a memory task



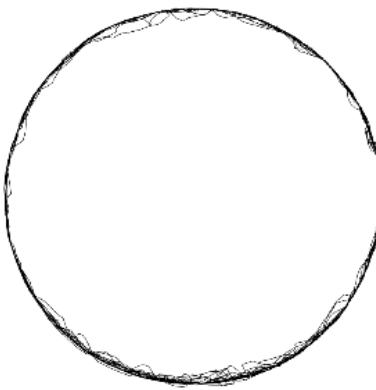
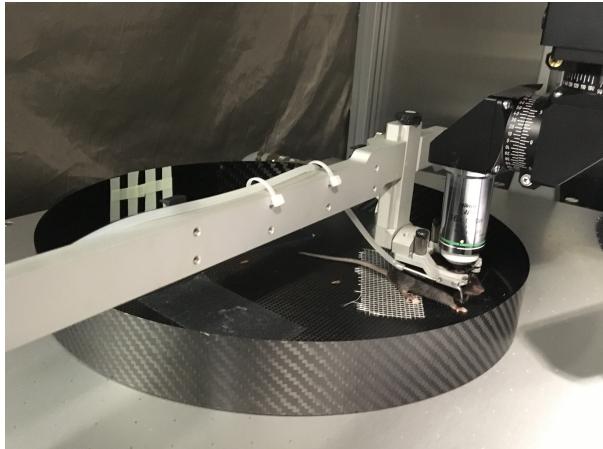
AAV delivery of GCaMP6s-mRuby
(T Rose, MPI München)



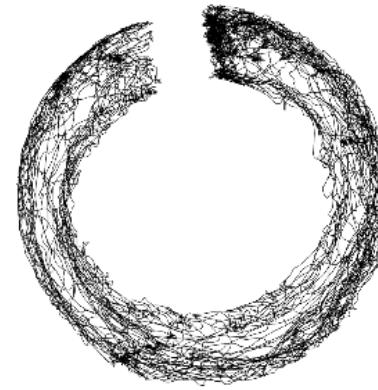
Methoxy-04



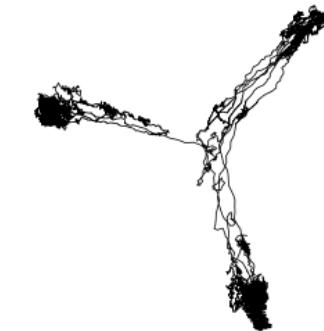
How does Alzheimer's Disease disrupt information processing and memory encoding/recall?



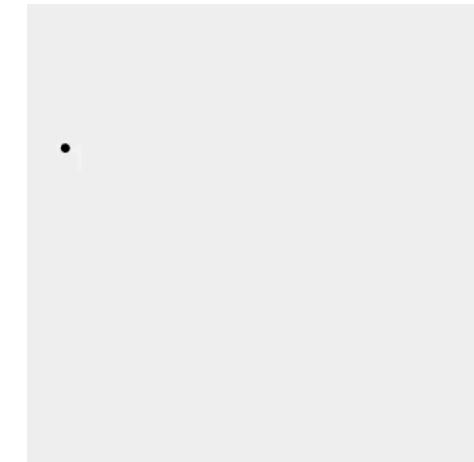
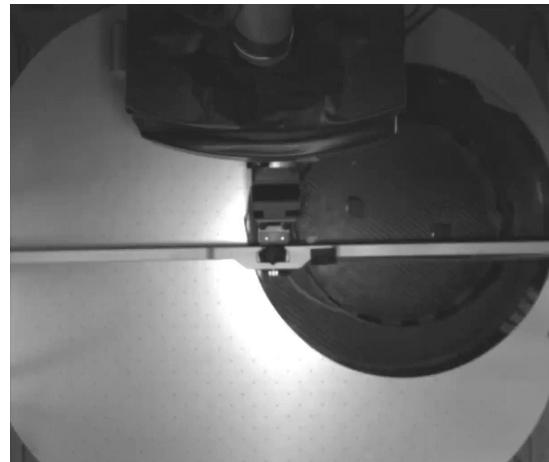
Circular linear track



Circular linear track
with stop

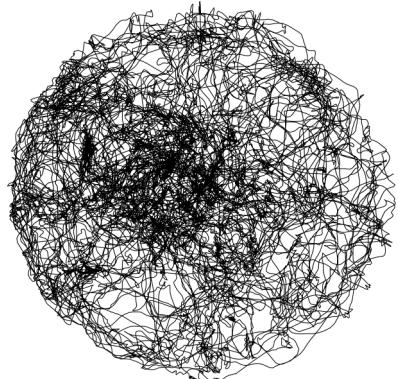


Y-maze

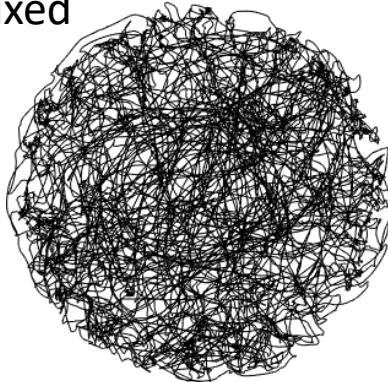


Open Field

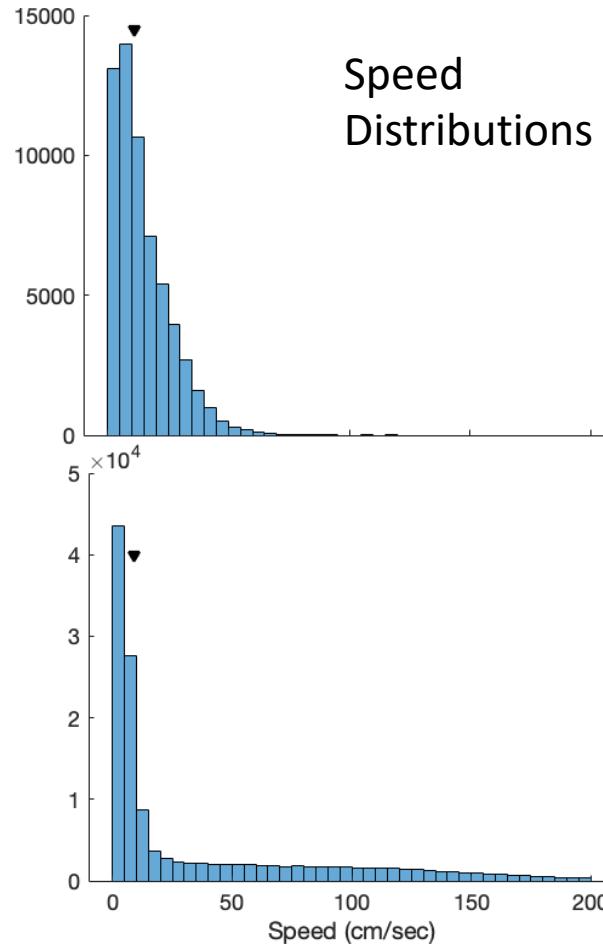
Tethered (Dupret lab)

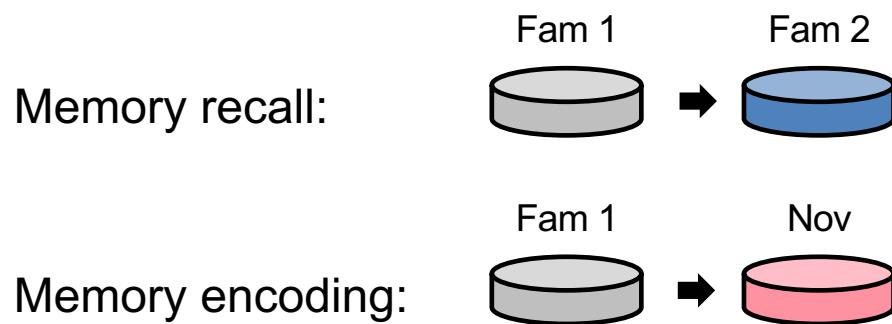
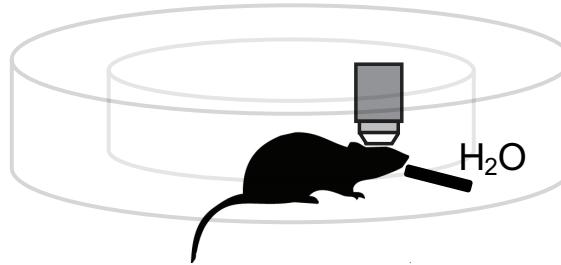
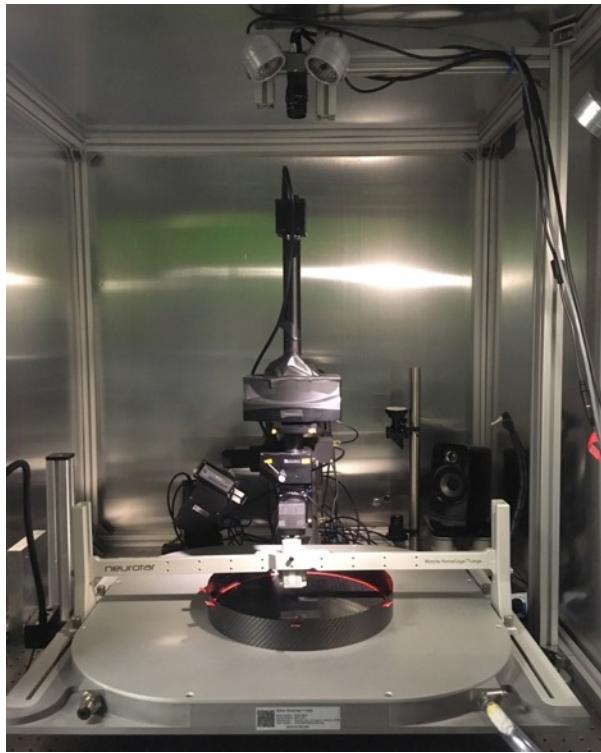


Head-fixed

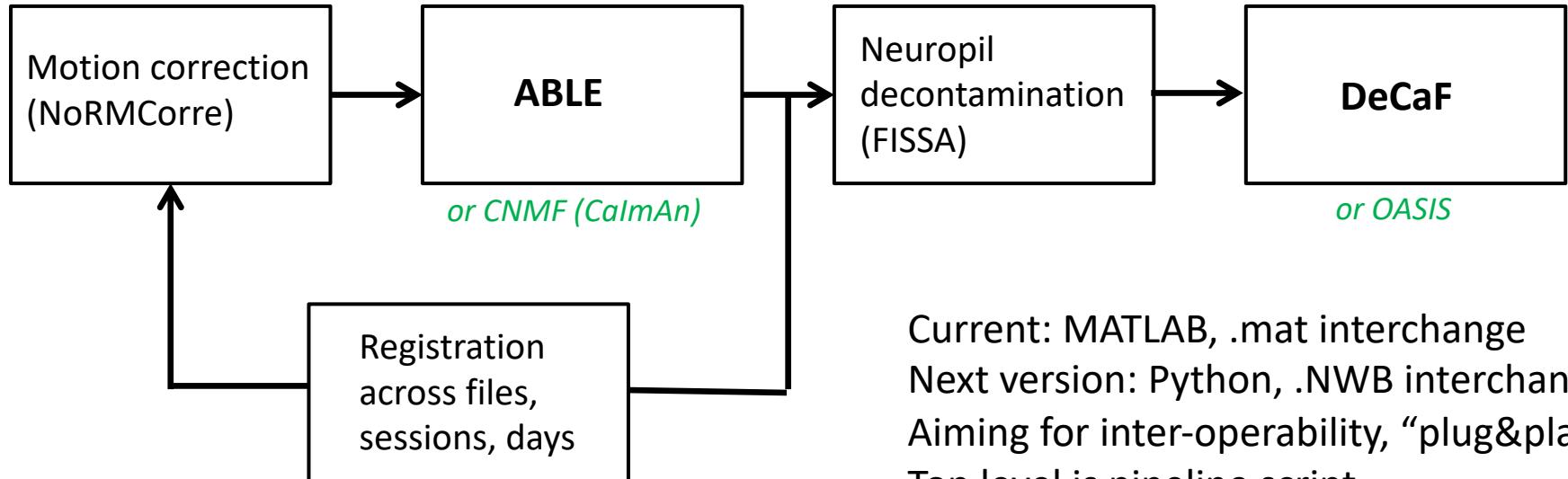


Speed
Distributions





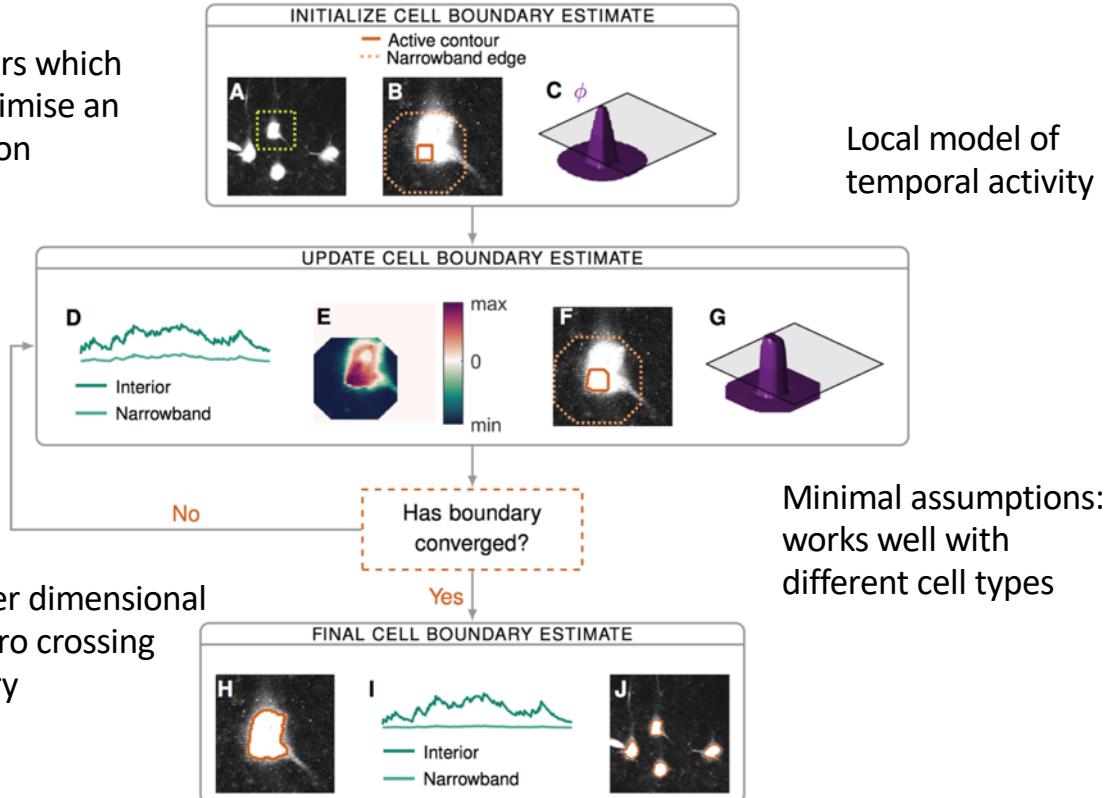
NeuroSEE



Current: MATLAB, .mat interchange
Next version: Python, .NWB interchange
Aiming for inter-operability, “plug&play”
Top level is pipeline script
Levels below modular, reusable
Manages use of HPC

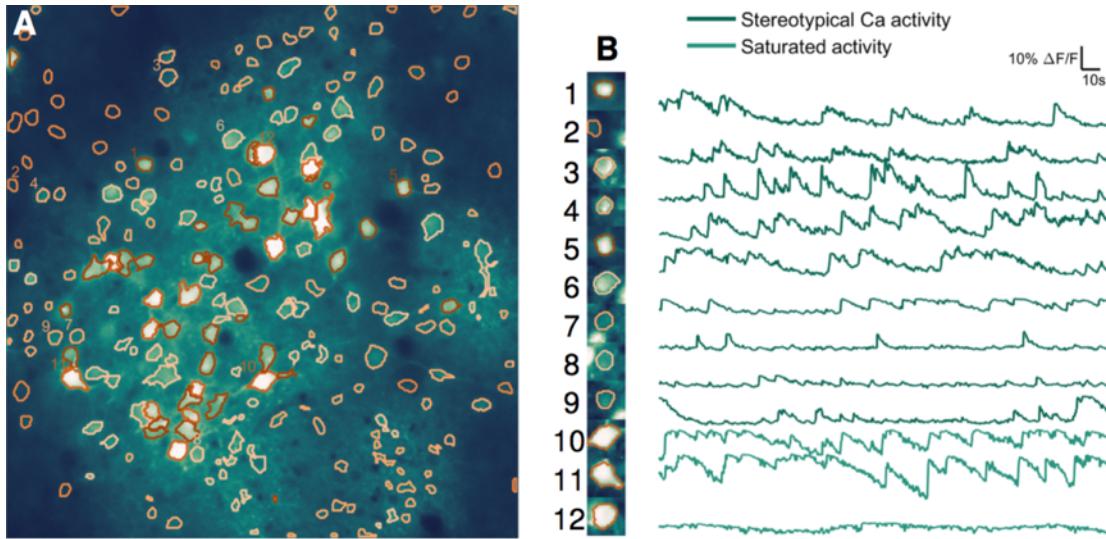
Improved ROI detection: ABLE (Activity Based Level Set Segmentation)

Active contours which evolve to minimise an energy function

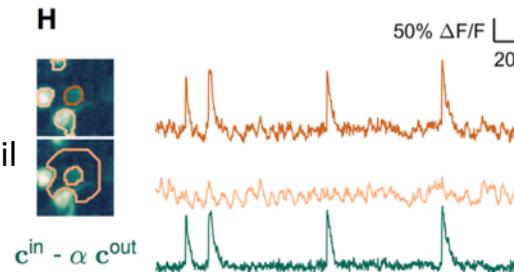


ABLE in action

Recovers cell types often missed due to temporal profile

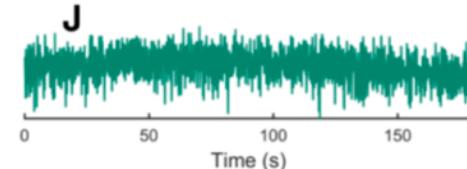
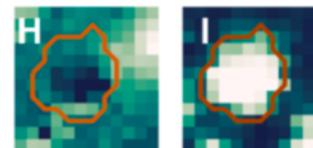
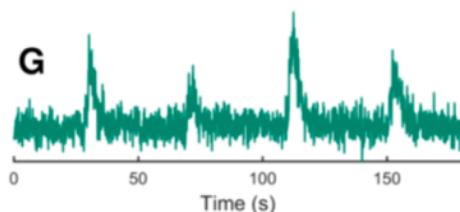
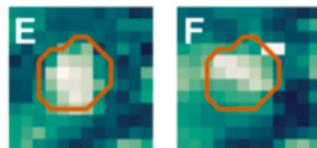
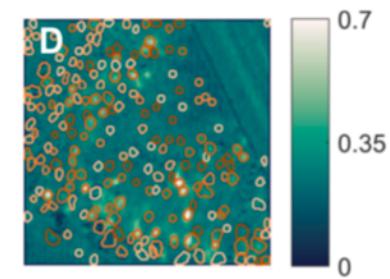
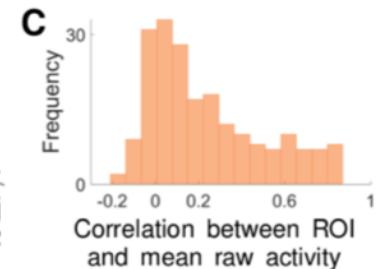
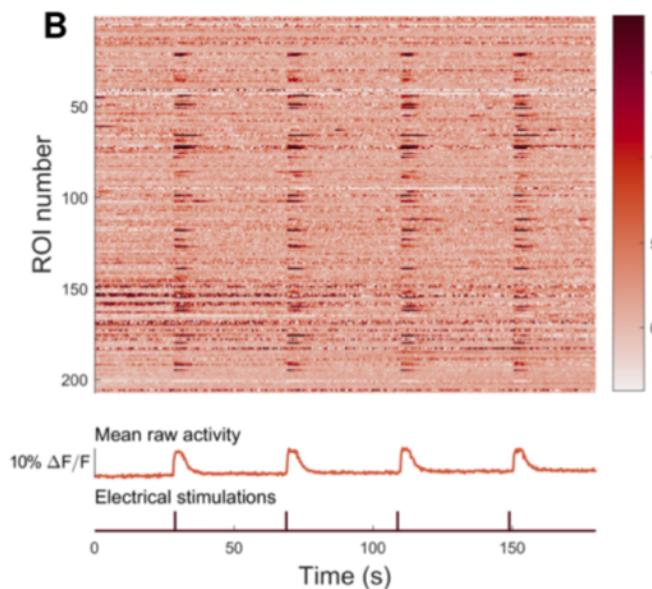
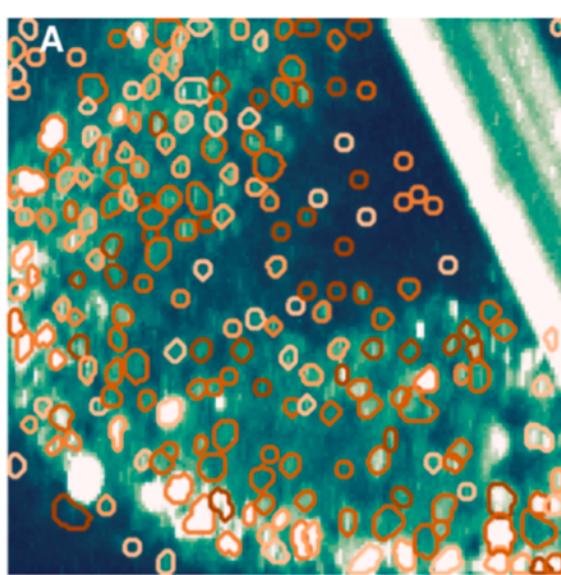


Plays nicely with removal of neuropil contamination

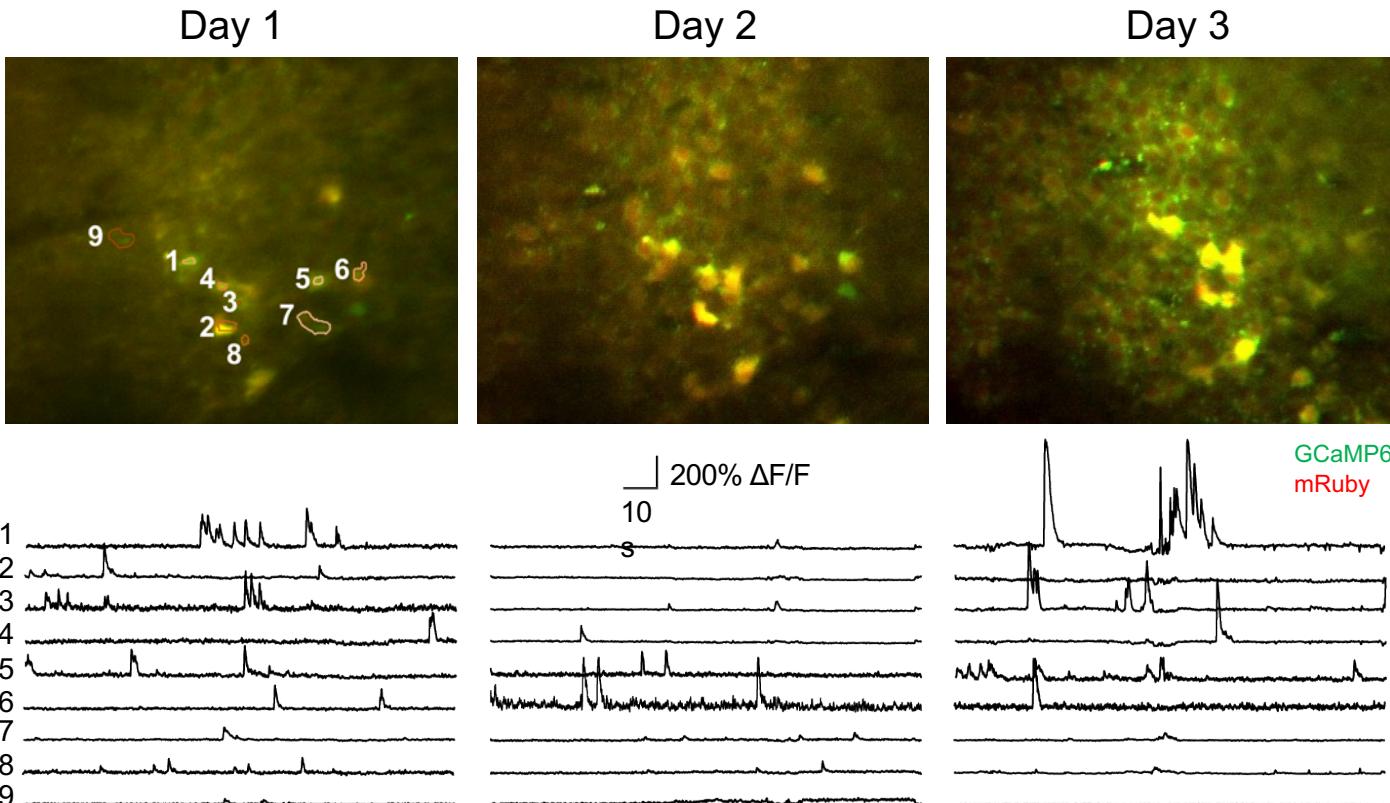


- Exceeds performance of Suite2P, CNMF (Neurofinder)
- Improved by seeding with morphological filter
- Even better: red channel

Can deal with dense, synchronous spiking

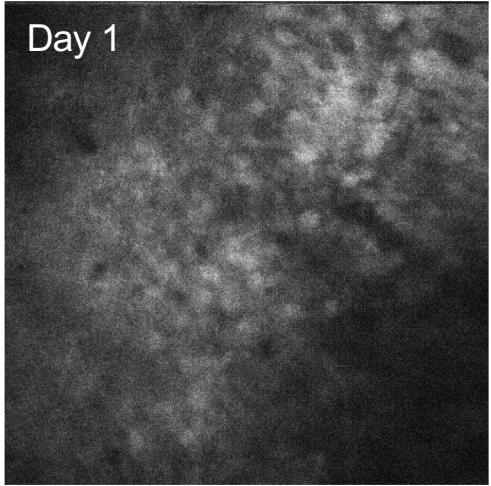


Electrical stimulation of dentate gyrus in slice

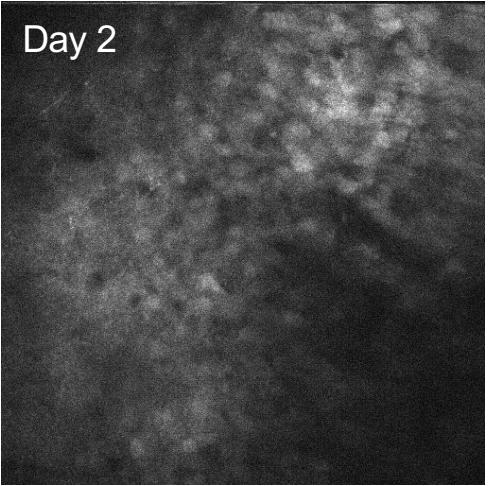


- Data stored in .nwb (Neurodata Without Borders) format
- In-house analysis pipeline – NEUROSEE (designed to be modular)

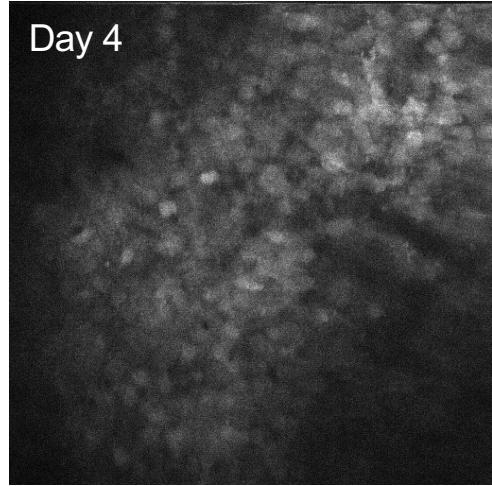
Day 1



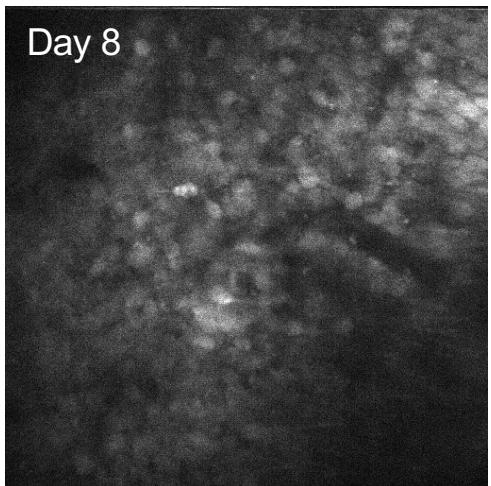
Day 2



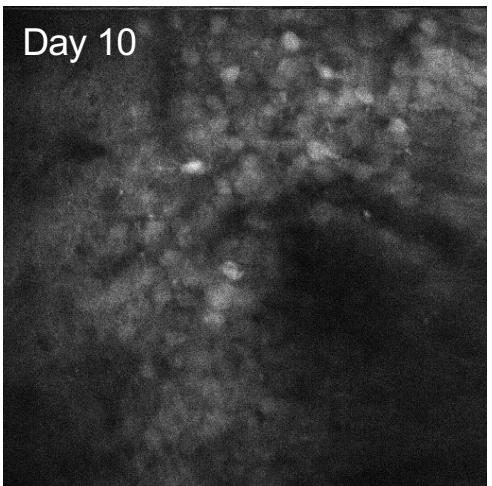
Day 4



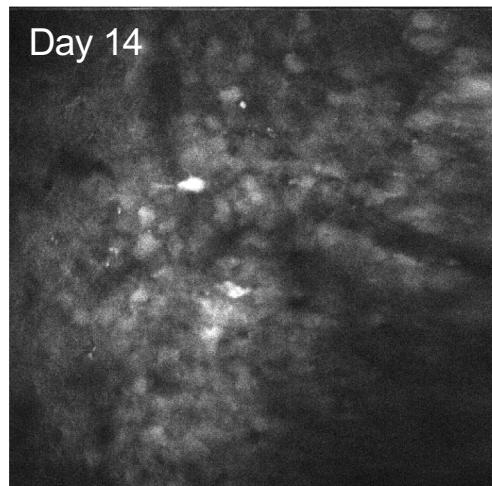
Day 8

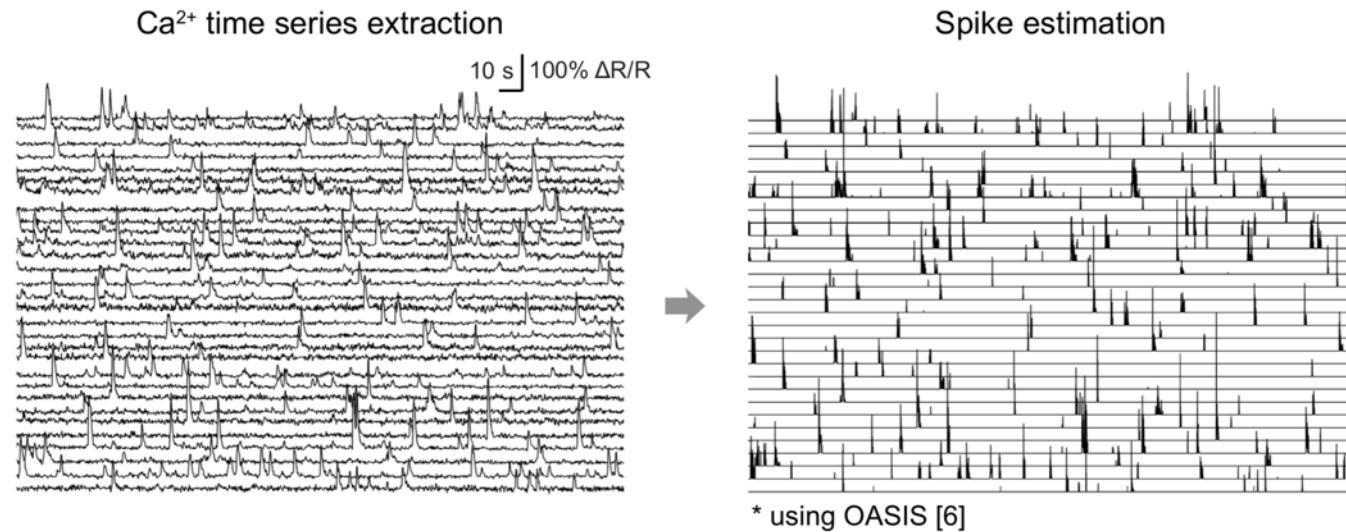
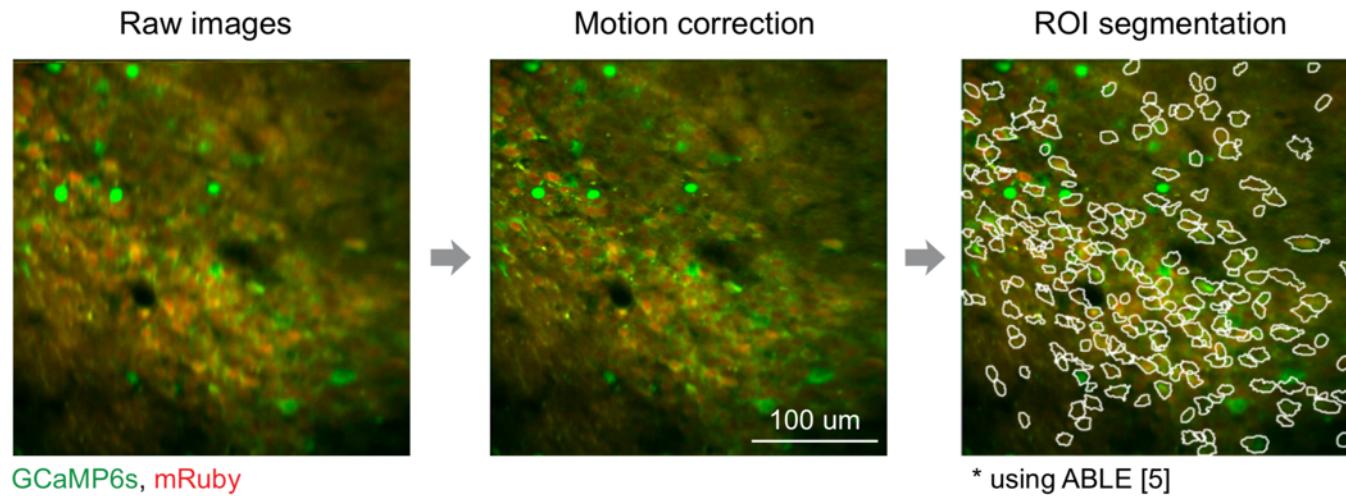


Day 10



Day 14

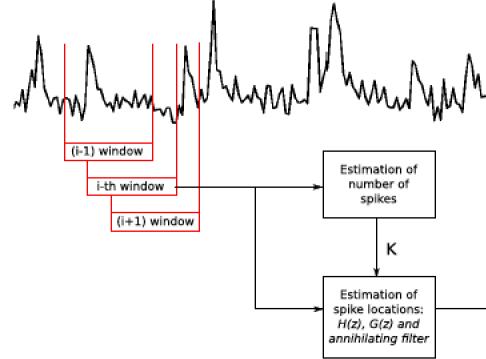




DeCaF (Detection of Calcium Fluorescence signals)

Based on Finite Rate of Innovation theory (Vetterli)

Collaboration with PL Dragotti



Run algorithm twice:

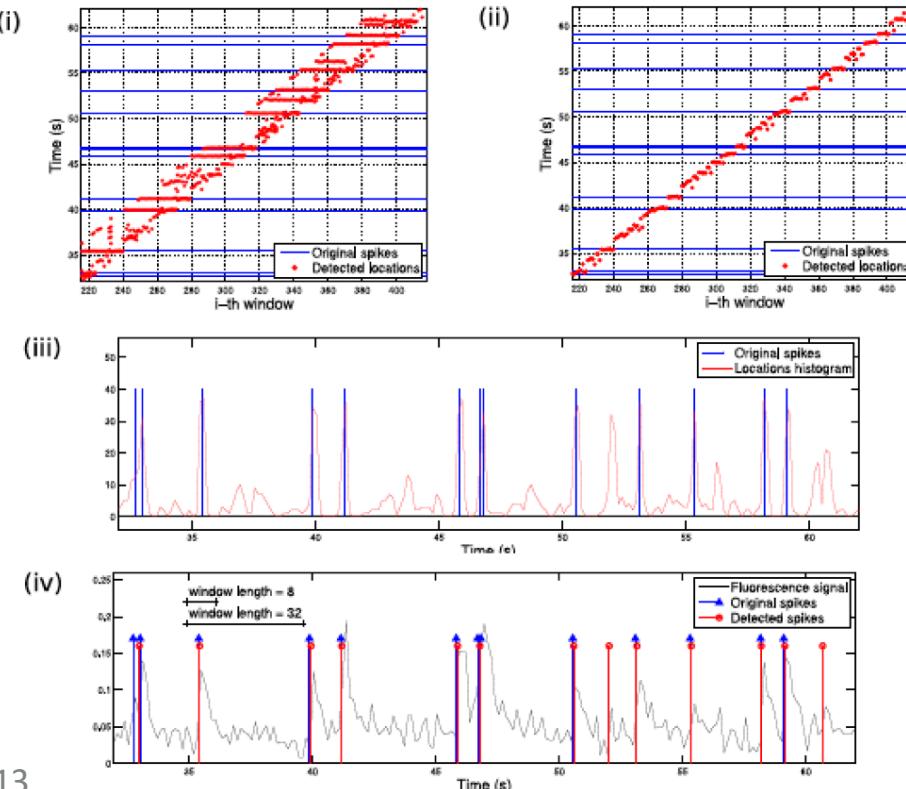
- first with wide window

(capture #spikes)

- 2nd with narrow window

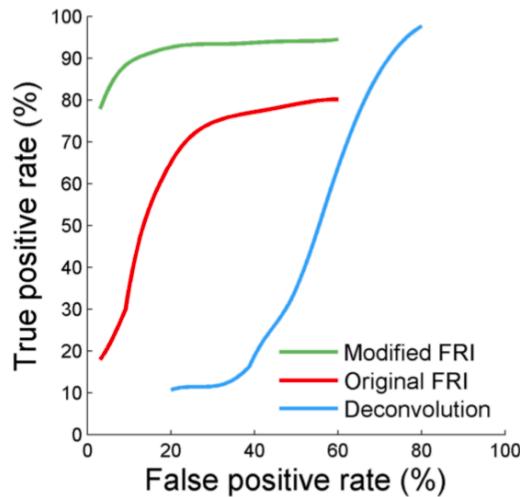
(~ single spike)

Real spike locations are **stable**,
noise is **unstable** wrt window position

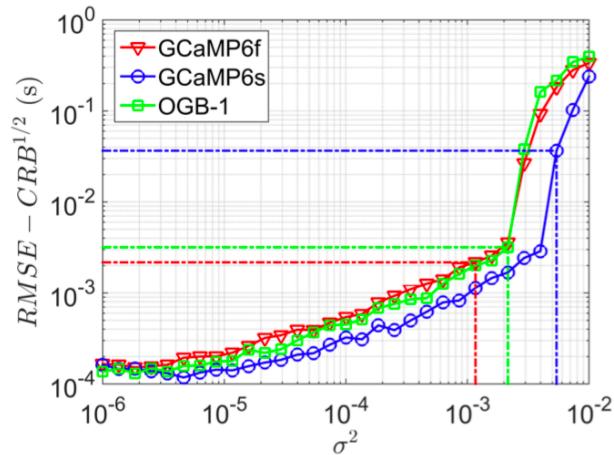
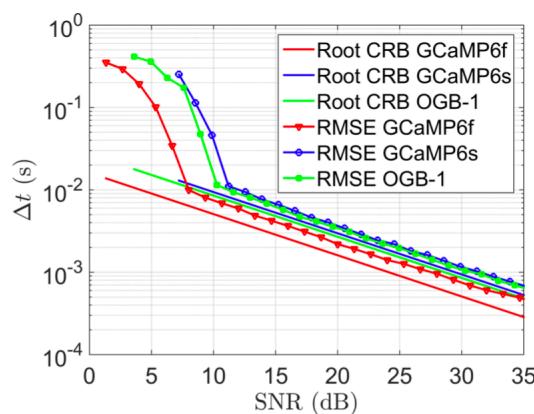


DeCaF (Detection of Calcium Fluorescence signals)

Outperforms OASIS on ground-truth data

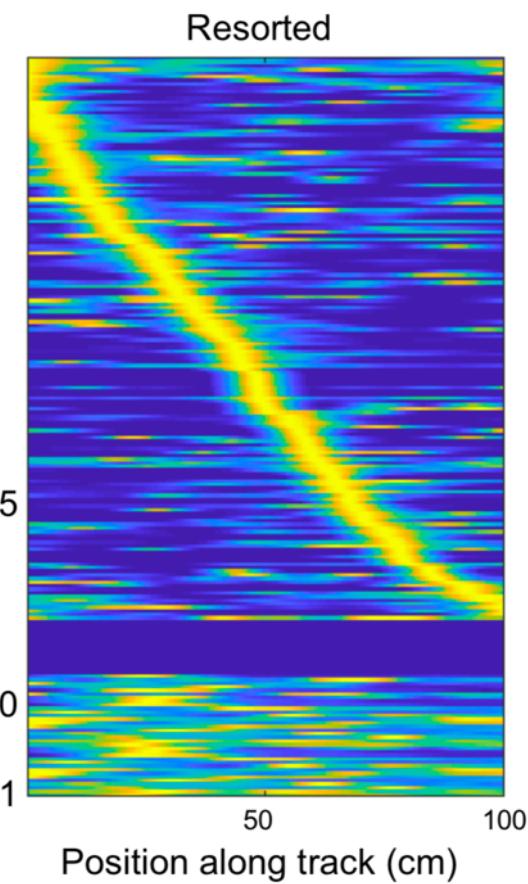
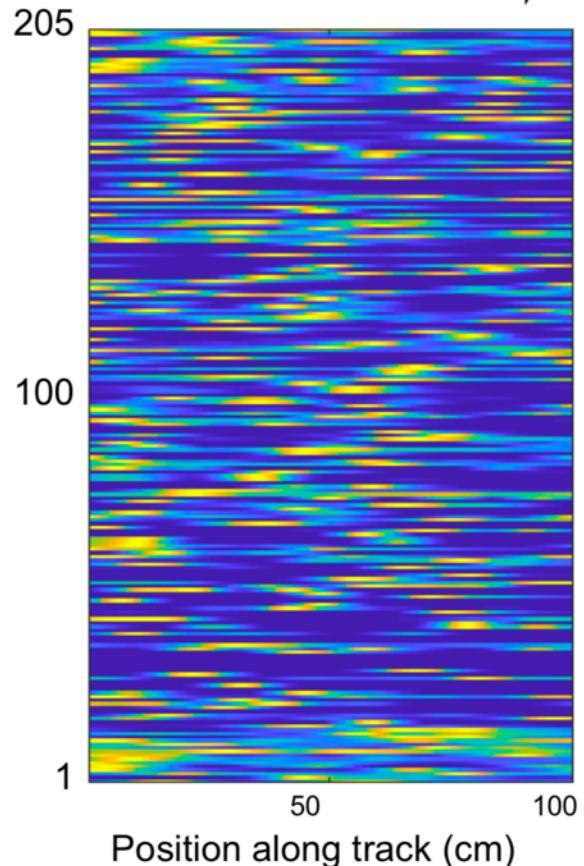
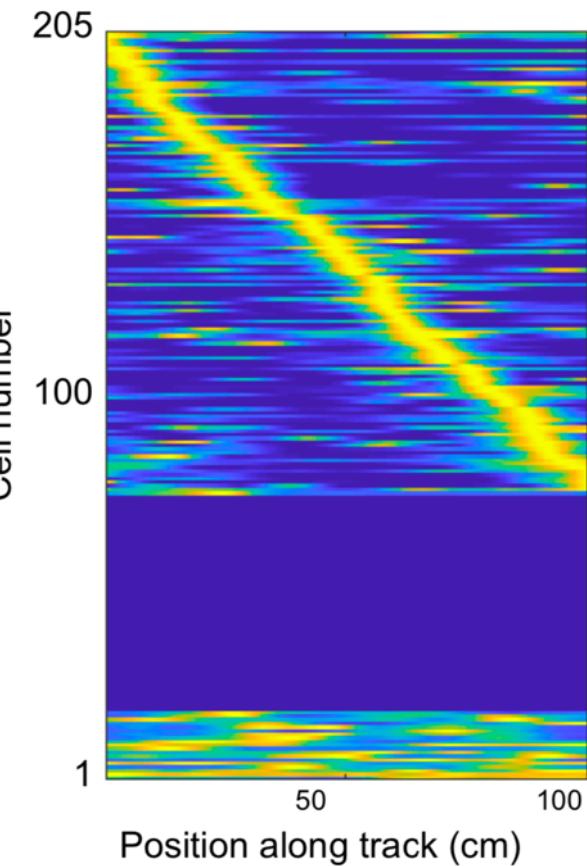
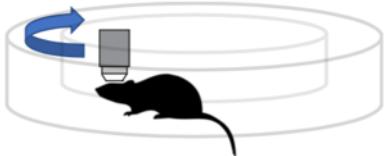


Enhancements to optimize for the kinetics of specific GCaMP variants



An example (hard detection scenario)

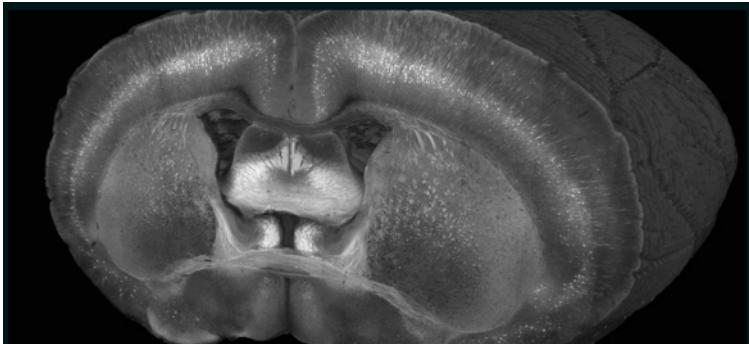
Now updating algorithm again, bundling together in new “DeCaF” python package



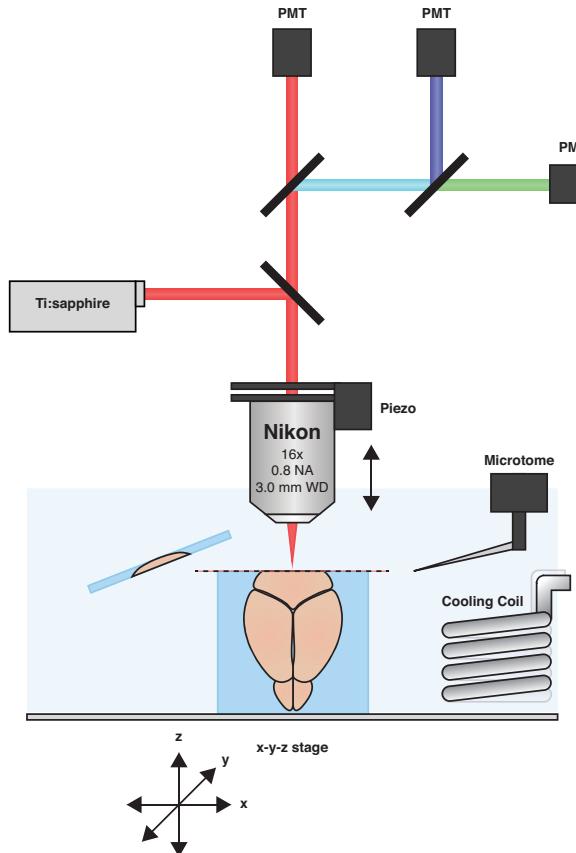
Summary – in vivo imaging

- We have developed a pipeline for imaging calcium signals in populations of neurons during behavior
 - Applied to spatial memory (circular linear track and open field) task
 - Working memory tasks also possible with Y-maze version
 - Contribution of neurons to behavior can be studied as a function of their individual amyloid load profile
- Able to observe recall of old, and formation of new, spatial memories
- Can track the same neurons over several weeks

Serial Two-Photon Tomography

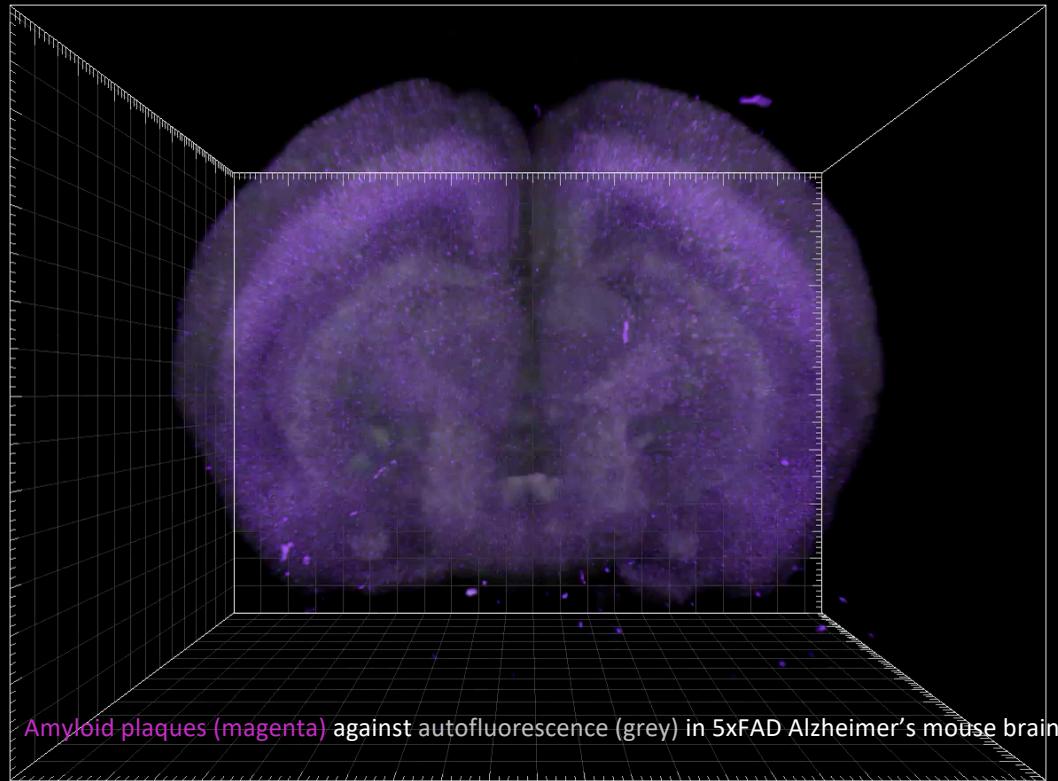


- Serial-two Photon Tomography system (TissueCyte 1000, TissueVision) performs serial sectioning and imaging across entire tissue volumes
- Tissue sample is embedded in agarose and submerged in phosphate buffer
- A motorized stage translates the sample between a microtome and Nikon 16x, 0.8 NA, 3.0 mm WD, objective
- Sectioned slices remain free-floating in phosphate buffer which is cooled with the addition of a cooling coil to 4°C to preserve slices for immunohistochemistry
- A piezo control enables the objective to move in the z-axis to achieve a z-sampling resolution as small as 1 μm
- Two-photon laser excitation with a Ti:sapphire laser (Coherent Chameleon)
- Emission is collected across three PMTs for red, green and blue channel acquisition

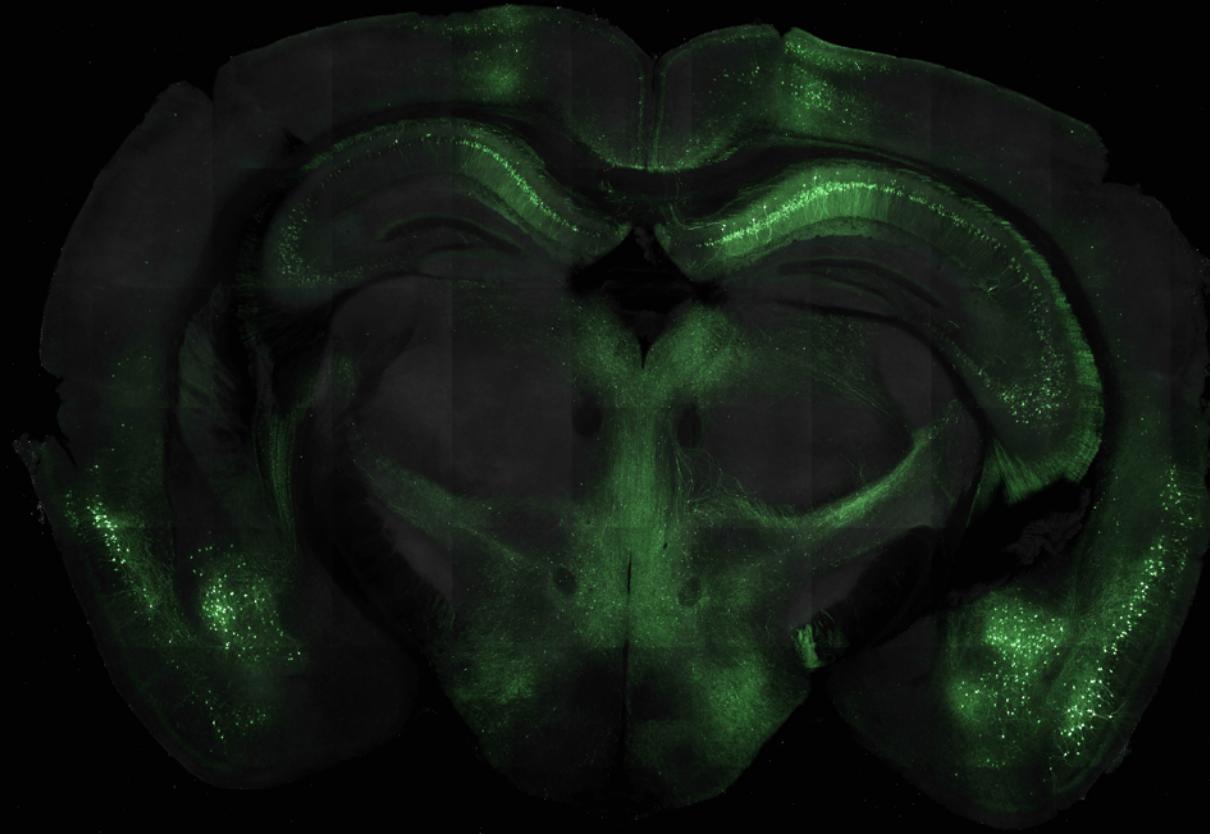


In collaboration with Brickley group





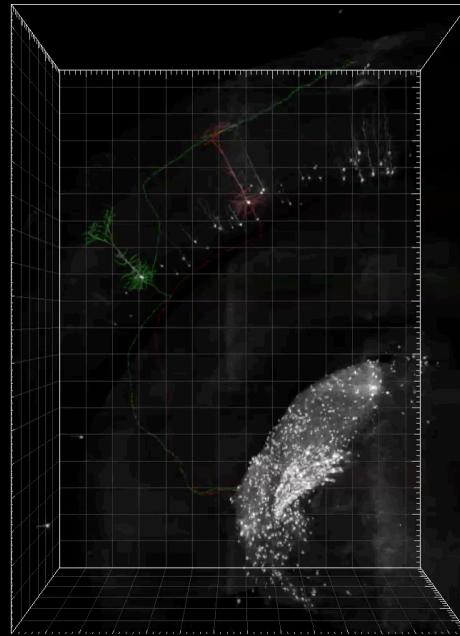
Amyloid plaques (magenta) against autofluorescence (grey) in 5xFAD Alzheimer's mouse brain



Retro-AAV injection: infra-limbic cortex
Resolution: 0.5 μ m (x,y), 5 μ m (z)
Green: GFP, Grey: autofluorescence



Monosynaptic Tracing with Rabies Virus



Axonal tracings of two layer 5 pyramidal neurons in mouse visual cortex projecting to lateral geniculate nucleus

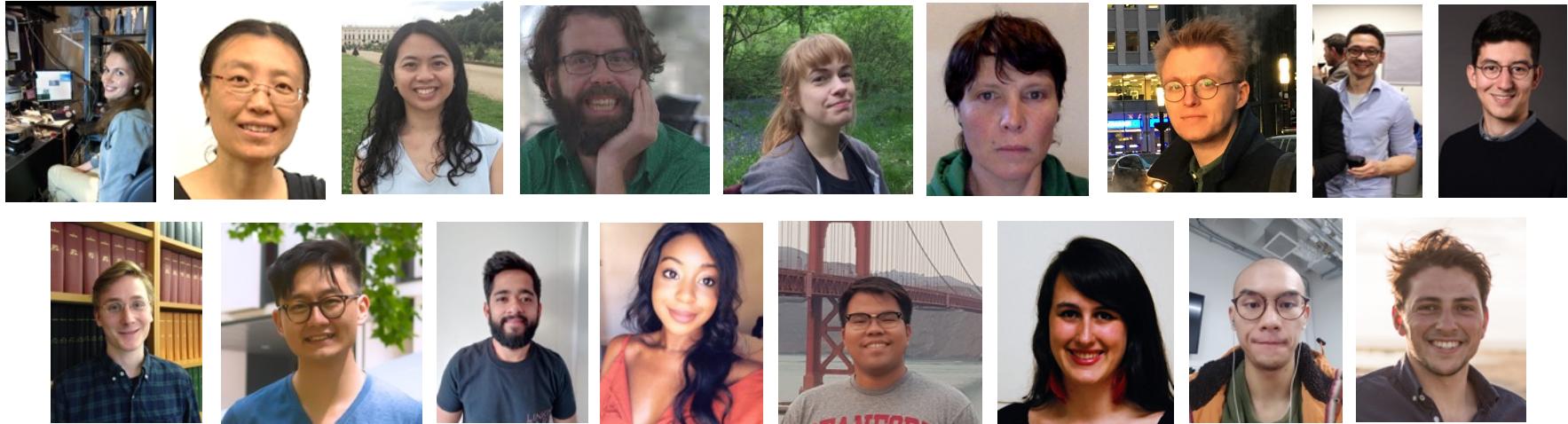
Requirements for useful data sharing

- Considerable heterogeneity in the data descriptions needed, even for the experimental data from a single mouse
- Moving towards much higher content per mouse
- Big files (~ 5 TB per mouse)
 - Normally likely to share only processed form, but useful to be able to pull up full data

Learning from CARMEN (my 2 cents)

- Project about repository of “workflows” (analysis tools)
 - Not sure driven enough by experimental demand
- Insufficient focus on common data format
 - With common data interchange format, easy to use your own computational resources
- Ontology rabbit warren ... and too much focused on “old hat” data types, have to recognize fast changing nature of field
- Overhead to get data into standard format and onto online databases *in a useful way**. This needs to be funded to make it happen.
 - * Journal requirements have not really been effective. Too easy to cop out.

Acknowledgements



Plus many **alumni** and **collaborators**

Luca Annecchino, Caroline Copeland, Steph Reynolds, Paul Chadderton, Pier Luigi Dragotti, David Dupret, Stephen Brickley, Alessio Delogu, Richard Morris, Leo Khiroug

Neuron

NeuroResource

Robotic Automation of In Vivo Two-Photon Targeted Whole-Cell Patch-Clamp Electrophysiology

Authors

Luca A. Annecchino,
Alexander R. Morris,
Caroline S. Copeland,
Oshiorenoya E. Agabi,
Paul Chadderton, Simon R. Schultz

Neuron

NeuroResource

Closed-Loop Real-Time Imaging Enables Fully Automated Cell-Targeted Patch-Clamp Neural Recording *In Vivo*

Authors

Ho-Jun Suk, Ingrid van Welie,
Suhasa B. Kodandaramaiah,
Brian Allen, Craig R. Forest,
Edward S. Boyden

Differences in the techniques

