An Array Tomography Exploration Tool:
Exploring Synapses from FMR1 Knockout Mice

Anish K. Simhal [1*], Kristina D. Micheva [2] Yi Zuo [3], Richard J. Weinberg [4],
Stephen J. Smith [5], Guillermo Sapiro [1]

[1]Duke University [2] Stanford University School of Medicine [3] University of California, Santa Cruz
[4] University of North Carolina, Chapel Hill [5] Allen Institute for Brain Sciences

Overview

- Array tomography allows collection of data sets containing synaptic protein markers and markers for dendrites, axons, glial cells, nuclei, myelin, and mitochondria.
- We here introduce the *Array Tomography Exploration Tool (ATET)*, a suite of computer vision methods that facilitate the exploration of array tomography data.
- We use array tomography and ATET to compare the relationship between astrocytes and synapses in the barrel cortex of a knockout mouse versus a wild-type mouse.

Fragile X syndrome (FXS) is a genetic condition that causes a range of developmental problems including learning disabilities and cognitive impairment. It is caused by the transcriptional silencing of the FMR1 gene that encodes the fragile X mental retardation protein. In this study, we used 4-month-old FMR1 knock out mice (a mouse model of FXS) and characterized synapses in the barrel cortex between FMR1$^{+/y}$ and FMR1$^{-/y}$ males.

Array Tomography Pipeline

Figure 1: *Pipeline of the array tomography (AT) approach used for creating the data*

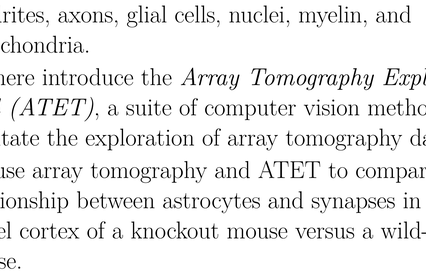

Figure 2: *Cartoon showing the relative spatial arrangement of the different parts of a tripartite synapse*

References

[1] Micheva KD, Smith SJ. Array tomography: a new tool for imaging the molecular architecture and ultrastructure of neural circuits. Neuron. 2007 Jul 5;55(1):25-36.

[2] Simhal, Anish K., et al. Probabilistic Fluorescence-Based Synapse Detection. PLoS Computational Biology, May 2017.

[3] Simhal, Anish K., et al. A Computational Synaptic Antibody Characterization Tool for Array Tomography. Frontiers in Neuroanatomy. 2018;12.

Array Tomography Exploration Tool

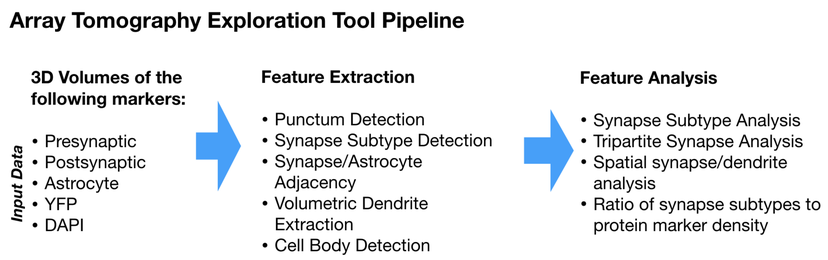

Figure 3: *Pipeline of the ATET process. Individual parts are highlighted below*

Synapse Detection

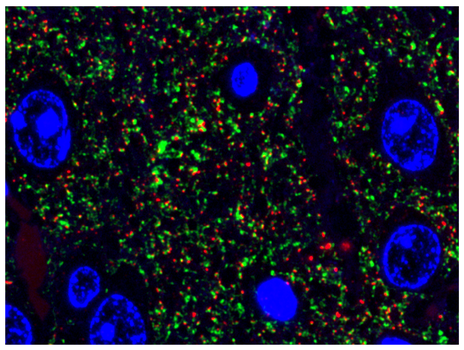

Figure 4: *Example cutout of a single slice showing three channels: red, PSD-95; green, synapsin; blue, DAPI*

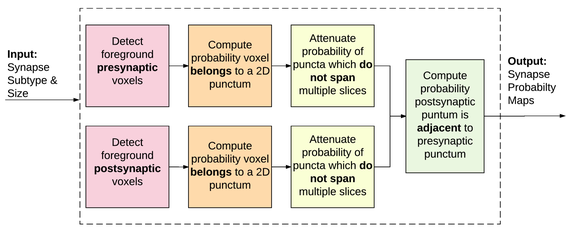

Figure 5: *Probabilistic synapse detection pipeline*

Dendrite Segmentation

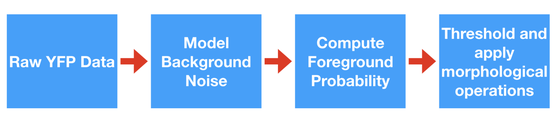

Figure 6: *Pipeline for dendrite segmentation*

A probabilistic model for the background noise, p_B, is

$$p_B(x,y,z) = \frac{1}{\sigma_B\sqrt{2\pi}} \int_{v(x,y,z)}^{\infty} e^{\frac{-(t-\mu_B)^2}{2\sigma_B^2}} dt. \quad (1)$$

Probability of a voxel associated with the foreground, p_F, is

$$p_F(x,y,z) = 1 - p_B(x,y,z). \quad (2)$$

Dendrite + Synapses

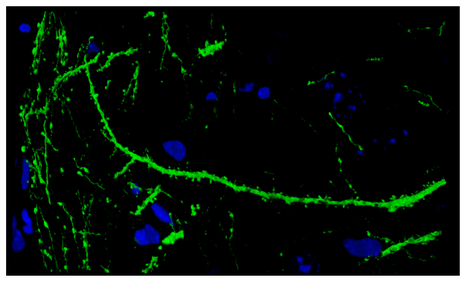

Figure 7: *Maximum intensity projection of a portion of the YFP channel*

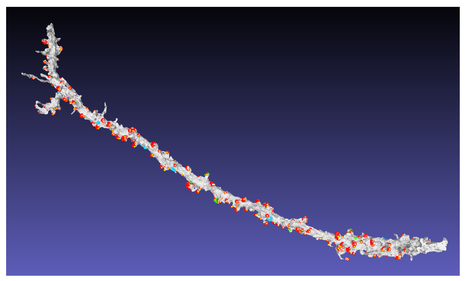

Figure 8: *Segmented dendrite with various synapse subtypes marked*

Wild-type Synapse Distribution

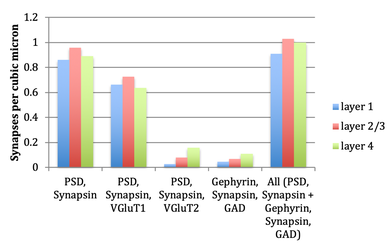

Figure 9: *Density distribution of different synapse subtypes in the upper layers of mouse barrel cortex*

Acknowledgments

This work was supported by the National Institutes of Health (NIH-TRA 1R01NS092474), the Allen Institute for Brain Sciences (AIBS), National Science Foundation, Department of Defense, and the Information Initiative at Duke University.

FMR1 Knockout Mice Analysis

Animals

Mouse specimen #	DOB	Age	KO or WT
2SS	6/27/17	4 months	WT
3SS	6/27/17	4 months	KO
4SS	7/04/17	4 months	WT
6SS	7/04/17	4 months	KO
5SS	7/04/17	4 months	WT
7SS	7/04/17	4 months	KO
2SS	6/27/17	4 months	WT
1SS	6/27/17	4 months	KO

Table 1: *Mice used for this experiment.*

Antibodies

Antigen	Host	Antibody Source	Dilution
Synapsin	Rabbit	Cell Signaling 5297	1:100
PSD95	Rabbit	Cell Signaling 3450	1:100
VGluT1	Guinea pig	Millipore AB5905	1:5000
VGluT2	Guinea pig	Millipore AB2251	1:5000
GAD2	Rabbit	Cell Signaling 5843	1:100
VGAT	Mouse	Synaptic Systems 131 011	1:300
Gephyrin	Mouse	NeuroMab 75-443	1:100
Glutamine synthetase	Mouse	BD Biosciences 610517	1:25

Table 2: *Anitbodies used for this experiment.*

Density changes were calculated as $((KO - WT)/WT) * 100$. 'Small synapse' refers to synapses whose puncta only span a single slice of data.

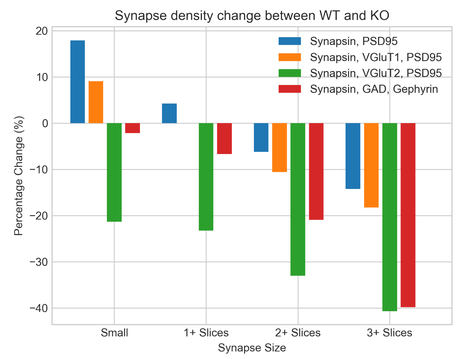

Figure 10: *Synapse density change.* Plot showing the percent change in synapse density between the wild type and knockout mice

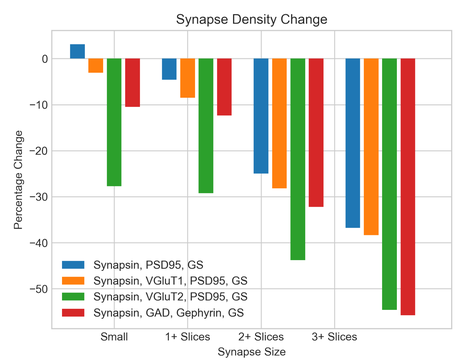

Figure 11: *Glial coverage change* This plot shows the percentage change in synapses that have an associated astrocytic marker between the wild type and knockout mice, for four different synapse subtypes