Conjugate IF-SEM: A tool to provide ground truth for synapse detection and analysis

328.09

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Introduction

By enabling imaging of individual synaptic compartments with both highly multiplexed immunofluorescence and high-resolution electron microscopy, ***conjugate fluorescence/electron array tomography (cAT)*** offers unique advantages for the study of synaptic structure and molecular composition[1-3]. In these two posters, we describe early results from a multi-institution collaboration aiming to explore cohorts of six different postsynaptic glutamate-gated ion channel subunits present in synapses from mouse neocortex. We have begun the work of generating systematic synapse taxonomies to quantitatively summarize the distinct molecular compositions, ultrastructure and tissue context of synaptic receptor cohorts present at synapses connecting diverse cell type in mouse cortex.

1. Smith SJ: **Q&A: Array tomography**. *BMC Biol* 2018, **16**:98. PMID: **30189863**

2. Micheva KD, Busse B, Weiler NC, O'Rourke N, Smith SJ: **Single-synapse analysis of a diverse synapse population: proteomic imaging methods and markers**. *Neuron* 2010, **68**:639-653. PMID: **21092855**

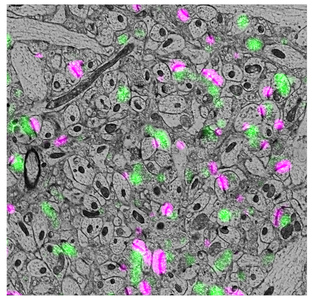
3. Micheva KD, Smith SJ: **Array tomography: a new tool for imaging the molecular architecture and ultrastructure of neural circuits**. *Neuron* 2007, **55**:25-36. PMID: **17610815**

Data Overview

Fixed tissue from adult mouse neocortex (temporal association (TeA) area) was immunolabeled with antibodies against general synaptic markers and ionotropic glutamate receptor subunits.

Dataset 1 (3 channels):
- Synaptophysin (presynaptic)
- VGluT1 (excitatory presynaptic)
- PSD-95 (excitatory postsynaptic)

Dataset 2 (12 channels):
- Synapsin 1/2 (presynaptic)
- VGluT1 (excitatory presynaptic)
- PSD-95 (excitatory postsynaptic)
- GluA1, GluA2, GluA3, GluA4 (AMPA receptors: excitatory postsynaptic);
- GluN1, GluN2B (NMDA receptors: excitatory postsynaptic)
- Gephyrin (inhibitory postsynaptic)
- GABA (inhibitory neurotransmitter)
- Myelin basic protein (myelin)



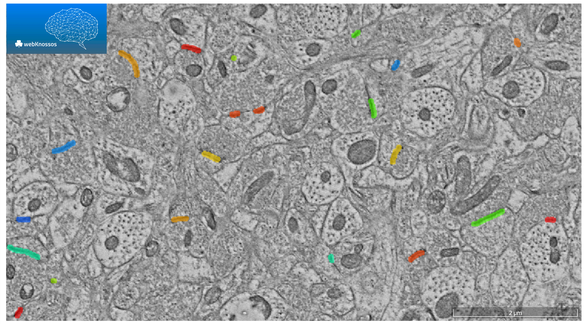
Dataset 1: PSD-95 (magenta) and synaptophysin (green) IF overlaid SEM data

For antibody info see adjacent poster 328.08

Manually labeled EM annotations

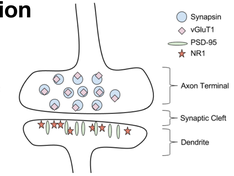
All excitatory synapses were annotated in both datasets using **WebKnossos**[4], which enables very efficient annotation and collaborative exploration and analysis of volume data, including both EM and multiple immunofluorescent channels.

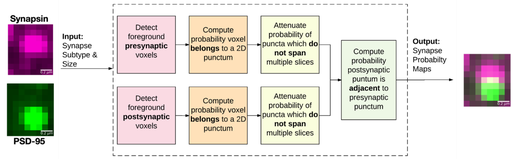
4. Boergens KM, Berning M, Bocklisch T, Bräunlein D, Drawitsch F, Frohnhofen J, Herold T, Otto P, Rzepka N, Werkmeister T, Werner D, Wiese G, Wissler H, Helmstaedter M. webKnossos: efficient online 3D data annotation for connectomics. Nat Methods. 2017 Jul;14(7):691-694. PMID: 28604722.



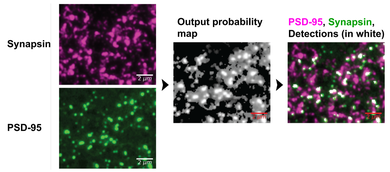
Probabilistic Synapse detection

Using immunofluorescence data, one can define a synapse based on the juxtaposition of postsynaptic and presynaptic proteins.





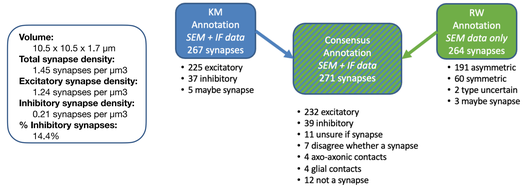
Overview of the probabilistic synapse detection pipeline. The only parameters that need to be adjusted are at the first and last steps.



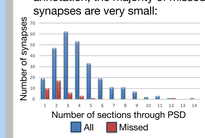
Example input to and output from the procedure. The left panel shows the input IF channels. The middle panel is the output of the probabilistic synapse detection method. The right panel shows the detections, overlaid with the original IF channels.

Manual Synapse Detection:

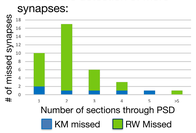
Comparison between 2 experts using different modalities



Compared to the consensus annotation, the majority of missed synapses are very small:

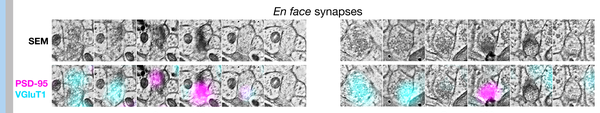


Use of conjugate IF-SEM data enables the detection of more synapses:



Conjugate IF-SEM is needed for the detection of most *en face* synapses:

En face synapses

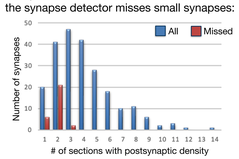


Synapse 138 Synapse 266

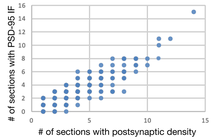
Ground truth comparison:

Manual synapse detection vs. probabilistic synapse detector

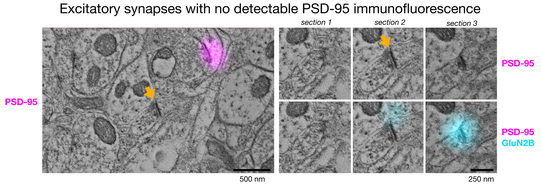
Compared to the consensus manual annotation the synapse detector misses small synapses:



4% of excitatory synapses have no PSD-95 immunofluorescence associated with them:



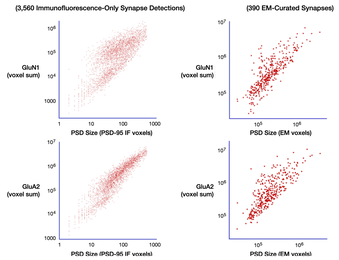
Excitatory synapses with no detectable PSD-95 immunofluorescence



Our approach permits robust estimation of detection efficiency. While highly effective, automated synapse detection is most likely to miss tiny synapses. This is mainly due to their low PSD-95 immunofluorescence signal. Manual synapse detection using SEM data, supplemented with IF data, is much more efficient in detecting small synapses.

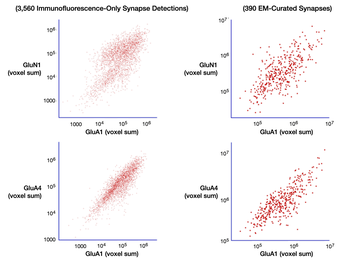
Quantification Examples

Single-Synapse GluN1 and GluA2 Immunofluorescence Vs. PSD Size



NMDAR patterns suggests two distinct synapse populations (not apparent for AMPARs)

Single-Synapse GluN1 and GluA4 Immunofluorescence Vs. GluA1 Immunofluorescence



GluN1 appears only weakly correlated with GluA1 (with a hint of multiple subpopulations), in contrast to the tight relationship with GluA4. Generally similar patterns are seen for the IF only dataset (left) and the cAT set (right), but note the higher density of tiny synapses on the right.

Observations

The automated synapse detector, using immunofluorescence data only, reliably identifies the large majority of excitatory synapses, but approximately 10% of synapses observed in the electron micrographs are too small and with insufficient immunofluorescent signal to be detected.

Manual synapse detection using only SEM data is most likely to miss *en face* synapses (synapses sectioned parallel to the postsynaptic density). Including conjugate IF data enables the detection of such synapses.

Automated immunofluorescence-based synapse detection effectively measures overall synapse densities and the relative prevalence of different synapse types, and is well suited to investigate correlations between synapse size or type and receptor composition.

Electron microscopic information is indispensable for the detection of very small synapses, or multi-synapse arrangements such as dually innervated spines (see adjacent poster 328.08).

Conclusions

It is now well established that synapses – even within constrained neurotransmitter classes such as "glutamateric" – are highly diverse in molecular, structural and functional characteristics[5-7]. Here we have demonstrated the collaborative use of conjugate fluorescence/electron array tomography (cAT) to quantify synapse-by-synapse variations in the relative abundance of six different glutamate receptor subunits. These pilot studies demonstrate that single-synapse detections and receptor antigen measurements made by high-throughput immunofluorescence-microscopy-only imaging give results closely comparable to those validated by labor-intensive electron microscopy. Building upon this validation, we have begun the work of generating systematic synapse taxonomies to quantitatively summarize the distinct molecular compositions, ultrastructure, and tissue context of diverse synaptic receptor cohorts present at synapses connecting diverse cell type in mouse cortex.

5. Bulovaite E, Qiu Z, Kratschke M, Zgraj A, Fricker DG, Tuck EJ, Gokhale R, Koniaris B, Jami SA, Merino-Serrais P, et al.: **A brain atlas of synapse protein lifetime across the mouse lifespan**. *Neuron* 2022. PMID: **36202095**

6. Zhu F, Cizeron M, Qiu Z, Benavides-Piccione R, Kopanitsa MV, Skene NG, Koniaris B, DeFelipe J, Fransen E, Komiyama NH, et al.: **Architecture of the Mouse Brain Synaptome**. *Neuron* 2018, **99**:781-799 e710. PMID: **30078578**

7. O'Rourke NA, Weiler NC, Micheva KD, Smith SJ: **Deep molecular diversity of mammalian synapses: why it matters and how to measure it**. *Nat Rev Neurosci* 2012, **13**:365-379. PMID: **22573027**

Where to find the software?

- Jupyter notebooks explaining how to use this code are available to download: github.com/aksimhal/synapse-detection-examples
- Code is available in both MATLAB and Python

Acknowledgements

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