Automated Antibody Characterization for Array Tomography

Anish K. Simhal [1*], Belvin Gong [2], James S. Trimmer [2], Richard J. Weinberg [3], Stephen J. Smith [4], Guillermo Sapiro [1], Kristina D. Micheva [5]

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

Challenge

- **How to automatically quantify antibody performance for array tomography?**

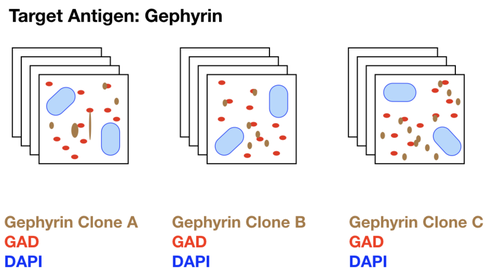

Figure 1: *Large blue blobs represent DAPI. The red dots represent GAD immunostaining, using a previously validated antibody. The brown dots represent the gephyrin antibody clone of interest.*

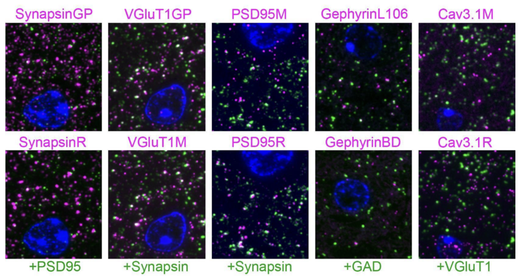

Figure 2: *Pairwise comparison of immunofluorescence signals on single sections from mouse brain. Each column represents an experiment where two antibodies against the same antigen (magenta) are evaluated by co-labeling with a control antibody (green). The sections are also labeled with the nuclear stain DAPI (blue).*

Background

- **Array Tomography Pipeline**

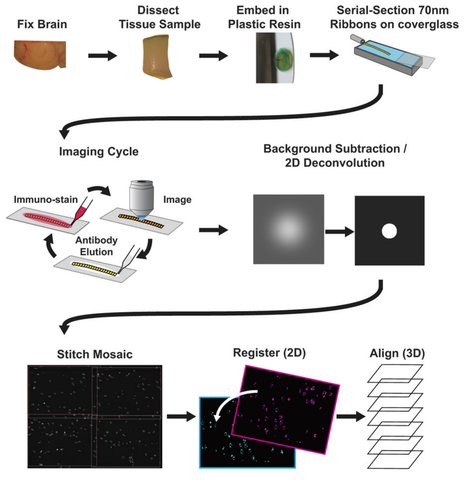

Figure 3: *Array Tomography (AT) methodology used for creating the data [4]*

Action

Method Overview

- Automatically compute quantitative measurements using a probabilistic query-based synapse detector.

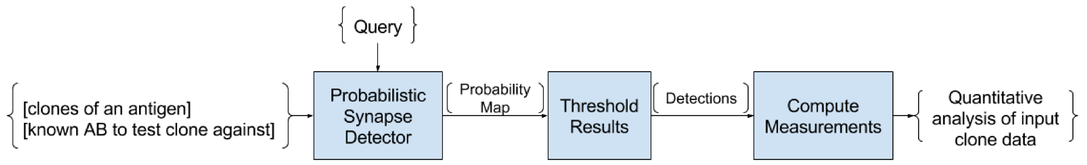

Figure 4: *Pipeline of the proposed automatic antibody characterization and screening method*

Measure 1: Detected Density of Puncta

- Reflects the sensitivity and selectivity of the antibody at the concentration used

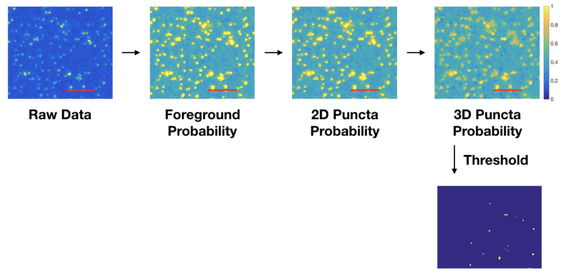

Figure 5: *Automated puncta detection pipeline*

Measure 2: Puncta Volume Size & Standard Deviations

- Reflects the quality and consistency of the antibody staining

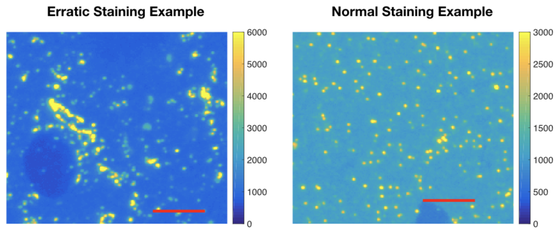

Figure 6: *(Left) Staining for collybistin on a raw IF slice; note the very broad distribution of sizes for puncta. (Right) Relatively 'normal' pattern of staining on a raw IF slice, using a different anti-collybistin clone. This difference is automatically quantified by the proposed framework. Each red scale bar is 5μm.*

Measure 3: Synapse Density

- Useful for evaluating antibodies against targets with a known distribution at synapses, where the density of synapses containing the target protein can be estimated.

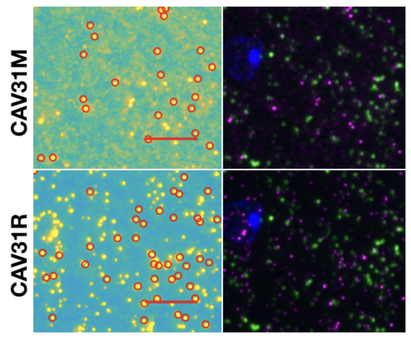

Figure 7: *Pairwise comparison of CAV31 IF signals on single sections from mouse brain. The images in the left column show color scaled IF images with synapse detections overlaid in red. The right column shows the 3 IF channels collated - CAV31 (purple), VGluT1 (green), & DAPI (blue)*

Measure 4: Antibody Staining Precision

- Estimates the magnitude of 'extra' (nonsynaptic) staining. When comparing two antibodies against the same target, differences in precision are likely to reflect differences in their specificity.

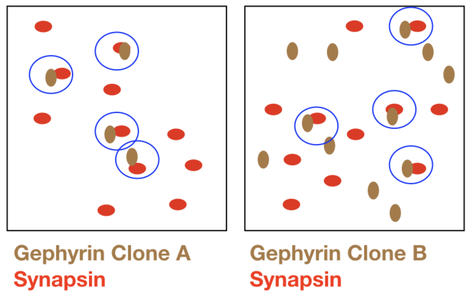

Figure 8: *In the left panel, each gephyrin punctum lies adjacent to a synapsin puncta, indicating a synapse. The right panel shows multiple gephyrin punctum unassociated with synapsin puncta, indicating possible poor specificity.*

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] Saper CB. A guide to the perplexed on the specificity of antibodies. Journal of Histochemistry & Cytochemistry. 2009;57(1):1-5.

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

[4] Weiler NC, Collman F, Vogelstein JT, Burns R, Smith SJ. Synaptic molecular imaging in spared and deprived columns of mouse barrel cortex with array tomography. Scientific Data. 2014 Dec 23;1.

Resolution

Pairwise Antibody Comparisons

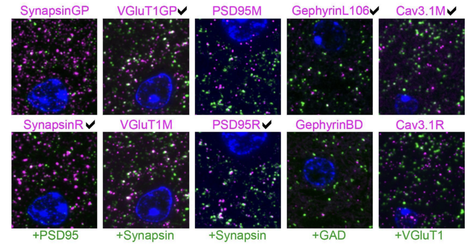

Figure 9: *Image from Figure 2 with checkmarks indicating the superior antibody. The better antibody is judged to be the one that has more puncta associated with the control antibody and fewer puncta not associated with the control antibody. Each image is* $16 \times 18 \mu m$.

Target Antigen	Clones	Density of synapses / µm³	Precision
Synapsin	Synapsin GP	0.547	0.272
	Synapsin R	0.493	**0.452 ✓**
VGluT1	**VGluT1GP**	**0.433 ✓**	**0.280 ✓**
	VGluT1M	0.324	0.254
PSD-95	PSD-95m	0.534	0.563
	PSD-95r	**0.788 ✓**	**0.689 ✓**
Gephyrin	**GephyrinL106**	**0.129 ✓**	**0.179 ✓**
	GephyrinBD	0.079	0.171
Cav 3.1	**Cav3.1M**	0.326	**0.564 ✓**
	Cav3.1R	0.327	0.268

Figure 10: *Results from pairwise antibody comparisons, as shown in Figure 9. The name in bold represents the antibody preferred by an expert based on visual examination. Check marks are placed next to the measurements used to determine the optimal antibody.*

Comparing Multiple Antibody Clones

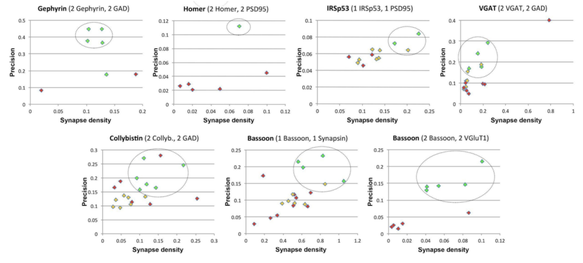

Figure 11: *Synapse density and precision scores of multiple clones of the same antibody, with the best ranking clones circled. Expert ranking is color coded: green - best, orange - unclear, red - fail. The specific query in each case is indicated in the plot title, where the number indicates the minimum punctum size for each label.*

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.