Questions to answer:

Which area of brain getting data from? Mouse cortex!

Are we detecting resin in our data? Check intro

Check Harvard bookmarks on remarks about resin used to fix the solution

How do they achieve this voxel to voxel accuracy? See intro

Catching mitochondria in DAPI channel? Autofluoresence of mitochondria a problem? It binds mitochondrial DNA!

Write up of Colleman Paper:

List of immunohistochemistry:

1. DAPI: fluorescent stain for the nucleus, stains DNA and mitochondria.
2. Synapsin 1: a **synaptic vesicle protein**, present on the cytoplasmic side of synaptic vesicles. There are two isoforms (check if difference between them). Also loosely associated vesicular membrane, easily disturbed with detergents, etc (does that affect protein localization during methods?). **Presynaptic**, near cloud of synaptic vesicles (check methods relating to GABA in paper).
3. Synaptophysin: **synaptic vesicle** glycoprotein. In neuroendocrine + most neurons in brain/spine (check to make sure only neurons and not glial cells).
4. VGlut1: vesicular glutamate transporter 1. READ: (<http://www.pnas.org/content/101/18/7158.full)> Glutamate is excitatory. Vglut intakes glutamate. On **glutamatergic neurons- mostly cerebral, cerebellum, hippocampus. Presynaptic (b/c intakes glutamate- check)**
5. PSD-95 (postsynaptic density protein 95) aka DLG4: kinase, **postsynaptic, associated with NMDA** receptor (**glutamate receptor**), most likely **excitatory.** In hippocampal neuron (check with other sources??), in **presynaptic** regions of **inhibitory synapses in cerebellar** basket cells synapsing to Purkinje cells (of interest? Our data from cortex, correct?). Check: <https://www.phosphosite.org/proteinAction.action?id=7100>
6. GluN1 (Glutamate (NMDA) receptor subunit zeta-1) aka GRIN1: **postsynaptic cell membrance, excitatory.** Requires glutamate and glycine agonists. Common excitatory receptor in CNS- hippocampus (should be in other regions, check specifically which areas)
7. GABA: **inhibitory neurotransmitter** in CNS. **Both in presynaptic and postsynaptic** neurons- check the papers methods of how the identified per vs post!
8. GAD2 (glutamate decarboxylase 2): makes GABA from glutamic acid. Most likely **presynaptic and inhibitory** (see: http://www.uniprot.org/uniprot/Q05329) . Most likely inside cell compartments- ex: golgi.. in cytoplasm (see above). Check to make sure.
9. Gephryin: neuronal assembly protein, anchors **inhibitory** neurotransmitter receptor, so **postsynaptic.** So helps recruitment of postsynaptic and anchoring of GABA + glycine receptor (GLYR). (see: <http://www.sciencedirect.com/topics/neuroscience/gephyrin)>. (See: <http://www.uniprot.org/uniprot/Q9NQX3)>
10. α-Tublin: part of microtubules- cytoskeleton of cell. Important in intracellular transport and structural support. Both **post-and presynaptic, excitatory and inhibitory**. Most likely used to image d**endrite**s (check (maybe axon imaged too?) and see Colleman Fig 2).
11. acetylated α-Tublin: same a alpha tubulin, acetylation helps polymerize microtubules. This paper showed acetylated MTs in **dendrites of CNS:** <https://www.ncbi.nlm.nih.gov/pubmed/1786591>
12. BetaIII tubulin: component of MTs, exclusively in neurons. Important in axon guidance and maintenance (<http://www.uniprot.org/uniprot/Q13509> ). This protein exculsively in neurons, so helps separate neurons from glial cells in CNS (Wiki).
13. γ-actin: part of cell cytoskeleton. In all mammalian cells, so post and presynaptic and excitatory and inhibitory.
14. Glutamine synthetase: takes glutamate (neurotransmitter) and makes glutamine (amino acid). Used to recycle glutamate neurotransmitter after nerve impulse. Found **mostly in astrocyte**s <https://www.ncbi.nlm.nih.gov/pubmed/12020613> , so neither excitatory/inhibitory and neither post/pre. But GS high in astrocytes located in glutamatergic areas.
15. Prohibitin: present in many cellular compartments (mitochondria, cytoplasm, etc), but of importance could be its importance in myelination (https://www.nature.com/articles/ncomms9303 ). Important in interaction between axons and glial cells but for myelin sheath, which is in PNS, what about CNS?? It’s function in mitochondria protects cells from oxidative stress? (<http://stroke.ahajournals.org/content/strokeaha/45/4/1131.full.pdf> )
16. MBP (myelin basic protein): component of myelin sheath of oligodendrocytes and Schwann cells. **So axons!!**