Published in final edited form as:

J Neurosci Res. 2018 July ; 96(7): 1123–1124. doi:10.1002/jnr.24238.

Adding New Dimension to Neuroscience

PS Holcomb^{1,*}

¹Rockefeller Neuroscience Institute, West Virginia University Health Sciences Center, Morgantown, West Virginia

Since the first silver staining by Camillo Golgi, imaging in the nervous system has guided research into how neurons—and, more recently, glial cells—develop, connect, and function (Pannese, 1999). The past century has seen great leaps forward in our ability to image biological systems, with inventions such as immunofluorescence (Coons, 1961), the electron microscope (Ruska, 1993), and the computer. Most recently, three-dimensional imaging techniques providing cellular-level resolution or better (serial block-face scanning electron microscopy (SBEM; Denk & Horstmann, 2004), light sheet microscopy (Huisken, Swoger, Del Bene, Wittbrodt, & Stelzer, 2004), etc.) have provided tremendous insight into the structure and wiring of the brain. However, it now becomes a challenge to the neuroscience community to find ways to visualize these images in a meaningful way, both to extract useful data and to share this data through publication. Leveraging new commercially available virtual reality systems, the company ItoVisio has created one such solution: a virtual reality data visualization and analysis tool called SyGlass (<http://www.syglass.io/>). This software, based on an original CAVE (cave automatic virtual environment) design and compatible with both the Oculus Rift and the HTC Vive, allows the user to import any form of volumetric data for rendering and manipulation in a virtual workspace (Morehead et al., 2014). For those without access to virtual reality hardware, free applications for both smartphones and tablets are available to display data using augmented reality (AR)—displaying a virtual object within the real environment through use of a camera-enabled device. For example, Augment (<http://www.augment.com>) provides both commercial and educational licenses for hosting augmented reality data, along with a free application for viewing this data. To see for yourself, download the free Augment app (available on both iOS and Android devices) and scan the QR code associated with this article to experience the neuron in the cover photo in AR.

The cover image shows a principal neuron in the auditory brainstem of the mouse that was reconstructed from SBEM images. This technique allows for well-aligned images of a tissue block to be captured in series without human handling using an electron microscope with an integrated microtome. As a result, thin structures such as axons can be tracked reliably over lengths of hundreds of microns. This technique has most commonly been used to perform “dense” reconstructions—segmentation and reconstruction of all objects within a small volume of tissue. My work, however, has focused on examining developmental changes in neurons and their associated terminals (axon endings containing multiple synapses) during

*Corresponding Author: Holcomb PS, Rockefeller Neuroscience Institute, West Virginia University Health Sciences Center, Morgantown, West Virginia.

early postnatal development; this research relies on selective segmentation and 3D reconstruction in tissue volumes taken from related animals at a series of ages to answer outstanding questions of neuronal development. For example, the area of the brain that our lab focuses on—the medial nucleus of the trapezoid body (MNTB)—contains one of the largest terminals in the mammalian nervous system: the calyx of Held. Despite its size, this terminal develops rapidly over 48–72 hours; this short developmental window and large size makes the calyx ideal for studying terminal development. However, while most other neural systems go through a predictable developmental cycle—exuberant terminal development, competition between terminals, and finally circuit refinement through terminal removal—it was hypothesized that competition was most likely not occurring in calyx development specifically because of the short developmental time scale. Previous work using electron microscopy and electrophysiology demonstrated the presence of multiple large terminals on these cells during development, suggesting competition between terminals, but whether this was the exception or the rule remained unknown (Hoffpauir, Grimes, Mathers, & Spirou, 2006; Hoffpauir, Kolson, Mathers, & Spirou, 2010). Applying SBEM and hours of painstaking segmentation by hand by a team of over 30 students, I was able to show that separate, large terminals converge on individual principal neurons during development, allowing it to be applied as a model system for neural development (Holcomb et al., 2013). This cover image not only exemplifies this, but also presents several interesting questions of its own: how long does competition last, and what factors separate the “winners” from the “losers” in terminal development?

This research also has personal significance for me, apart from the interesting scientific questions (and the cool gadgets). Auditory brainstem dysfunction shares a high comorbidity with autism spectrum disorders (ASD; Lynall et al., 2010; Supekar et al., 2013). As a parent of an autistic child, I have experienced first-hand the struggles accompanying this disorder and how vital it is that new treatment pathways be discovered. It is widely held ASD is a disorder of connectivity, with mounting evidence for this even in high-functioning individuals (Joshi et al., 2017). By studying the formation and maturation of the calyx of Held:MNTB connection, it is possible that factors governing its development may be applied more generally to other brain areas and may represent a new avenue of research for ASD.

References

- Coons AH. The beginnings of immunofluorescence. *Journal of Immunology* (Baltimore, Md 3: 1950). 1961; 87:499–503.
- Denk W, Horstmann H. Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. *PLoS Biology*. 2004; 2(11):e329.doi: 10.1371/journal.pbio.0020329 [PubMed: 15514700]
- Hoffpauir BK, Grimes JL, Mathers PH, Spirou Ga. Synaptogenesis of the calyx of Held: rapid onset of function and one-to-one morphological innervation. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2006; 26(20):5511–23. DOI: 10.1523/JNEUROSCI.5525-05.2006 [PubMed: 16707803]
- Hoffpauir BK, Kolson DR, Mathers PH, Spirou GA. Maturation of synaptic partners: functional phenotype and synaptic organization tuned in synchrony. *The Journal of Physiology*. 2010; 588(Pt 22):4365–85. DOI: 10.1113/jphysiol.2010.198564 [PubMed: 20855433]
- Holcomb PS, Hoffpauir BK, Hoyson MC, Jackson DR, Deerinck TJ, Marrs GS, Spirou KGa. Synaptic inputs compete during rapid formation of the calyx of Held: a new model system for neural

development. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2013; 33(32):12954–69. DOI: 10.1523/JNEUROSCI.1087-13.2013 [PubMed: 23926251]

Huisken J, Swoger J, Del Bene F, Wittbrodt J, Stelzer E. Optical Sectioning Deep Inside Live Embryos by Selective Plane.pdf. Science. 2004; 305(5686):1007–1009. [PubMed: 15310904]

Joshi G, Arnold Anteraper S, Patil K, Semwal M, Goldin R, Furtak SK, Whitfield-Gabrieli S. Integration and Segregation of Default Mode Network Resting-state Functional Connectivity in Transition-age Males with High-functioning Autism Spectrum Disorder: A Proof of Concept Study. Brain Connectivity. 2017; 7(9) brain.2016.0483. doi: 10.1089/brain.2016.0483

Lynall MME, Bassett DS, Kerwin R, McKenna PJ, Kitzbichler M, Muller U, Bullmore E. Functional connectivity and brain networks in schizophrenia. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2010; 30(28):9477–87. DOI: 10.1523/JNEUROSCI.0333-10.2010 [PubMed: 20631176]

Morehead M, Jones Q, Blatt J, Holcomb P, Defanti T, Ellisman M, Spirou KG. Poster: BrainTrek - An immersive environment for investigating neuronal tissue. 2014 IEEE Symposium on 3D User Interfaces (3DUI); 2014.

Pannese E. The Golgi Stain: Invention, Diffusion and Impact on Neurosciences. Journal of the History of the Neurosciences. 1999; 8(2):132–140. DOI: 10.1076/jhin.8.2.132.1847 [PubMed: 11624294]

Ruska E. Ernst Ruska - Biographical. In: Frängsmyr T, Ekspång G, editors Nobel Lectures, Physics 1981–1990. Singapore: World Scientific Publishing Co; 1993. Retrieved from https://www.nobelprize.org/nobel_prizes/physics/laureates/1986/ruska-bio.html

Supekar K, Uddin LLQ, Khouzam A, Phillips J, Gaillard WD, Kenworthy LE, Menon KV. Brain hyperconnectivity in children with autism and its links to social deficits. Cell Reports. 2013; 5(3): 738–47. DOI: 10.1016/j.celrep.2013.10.001 [PubMed: 24210821]

Figure 1.
QR code linking to the 3D model of the cover image for use with the Augment app