From Pixels to Minds: Mapping & Understanding the Brain with AI

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Enabled by advances in imaging and computer science, the past two decades have featured dramatic progress in the study of comprehensive synaptic-resolution maps of the nervous system. In this essay, we review these achievements and innovations but also argue that subsequent progress will be substantially driven by artificial intelligence. Specifically, we propose that AI is poised to revolutionize the study of brain wiring by: 1) helping to forge a new, functionally grounded definition of "understanding" the brain; 2) enabling sophisticated simulations and predictive models of neural circuits; and 3) identifying subtle connectomic "fingerprints" of neurological and psychiatric diseases. We envision a future in which the joint contributions of connectomics and AI not only decipher the brain's subtle wiring but also unlock novel avenues for fundamental discovery and therapeutic intervention.

It is no small wonder that brains are poorly understood. In virtually all animals that have nervous systems, the brain exceeds the complexity of all other parts of the body. Because brains both inherit their structure from their ancestors and modify it by virtue of experience, a full description of how they work spans from the role of genetic information and molecular biology and biochemistry all the way to studies of psychology and ethology. If that is not enough, perhaps the deepest obstacle originates from the way brain cells – neurons – carry out their functions. Each cell is part of a neural network connected by biological wires (axons and dendrites) into a vast relay that spans behavior, from sensation to action. Neurons collect information from sensory experiences via their dendrites or from other, farther upstream neurons passing information along. Each neuron conveys what it extracts from its input to downstream neurons via its highly branched axon. The relay finally terminates with the neurons responsible for action by causing the contraction of muscle fibers that let us speak, move, write, and otherwise act.

This network is, even in the simplest organisms, much more complicated than one might have imagined. Neurons are wired not just to a few other cells but, in many cases, thousands of upstream and downstream partners. The partners are

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often far flung and connected by biological wires that extend in many directions over very long distances. The sites of communication are specialized structures called synapses. The human brain has trillions of synapses. Mapping the complete synaptic connectional map, called a *connectome*, is the foundational blueprint of brain function – and this is the quest we and other researchers are on.

Until the late nineteenth century, the study of the brain was constrained by the impossibility of isolating and identifying individual cells in stains of nervous tissue. Then, in 1873, Italian biologist and pathologist Camillo Golgi discovered what would become known as the Golgi stain, a method of staining individual neurons at random, revealing the axon and dendrites of complete nerve cells and thus the structures and connections of the nervous system. Spanish anatomist Santiago Ramón y Cajal improved on this technique and contributed detailed drawings of the branched structure of the brain. Together, Golgi and Cajal showed that neurons are connected to each other in directional pathways, an insight for which they won the Nobel Prize in Physiology or Medicine.[1]

But Cajal's quasidiagrammatic renderings belied a reality that is only now being revealed; his drawings showed only the purported connections that were consistent with the pathway he was trying to illustrate. In contrast, connectomics specifically intends to show *all* the connections. In this sense, connectomics data are not biased, which is a blessing and a curse. On the positive side, the dense labeling of all neurons provides a highly accurate rendering of all the inputs a neuron is receiving and reveals where that information is going via all the downstream neurons its axon establishes synapses with. On the negative side, it is no longer so straightforward to extract a specific functional pathway, given the profound complexity of the overall network.

The journey to map neural circuits has been punctuated by significant technological leaps and landmark discoveries, transforming connectomics from a theoretical aspiration into a data-rich empirical science. In biological fields such as connectomics, genomics, and transcriptomics, as well as physical sciences like astronomy, large amounts of raw data are often required, making data acquisition both a technological challenge and an opportunity for innovative solutions. In the relatively young field of connectomics, data requirements have led to many different approaches to imaging the biological structures underlying the wiring diagram of nerve cells. Because of the miniscule size of the nerve cell wires and their synapses, traditional light microscopy does not have sufficient resolving power. Attempts to overcome this limitation include a technicolor Golgi-like neuron labeling approach called "Brainbow," in which neurons are labeled with many different colors (more than fifty), as well as more recent methods involving physical enlargement of biological tissue.[2] However the most successful method thus far is to avoid light microscopy altogether in favor of using electrons, which have wavelengths, and hence resolutions that are at least ten thousand-fold more detailed than the photons used in light microscopy.

Two major types of electron microscopy (EM) are used in connectomics.[3] In transmission electron microscopy (TEM), the imaged signal is based on detecting electrons that are transmitted through a sample. Scanning electron microscopy (SEM) approaches, on the other hand, detect electrons that are scattered from the sample's surface. These two approaches also allow for different ways of preparing tissue. It is generally accepted that tracing wires unambiguously requires distinguishing them both from nearby wires in the same plane of focus as well as from wires that lie slightly above or below them. Therefore, the easiest way to avoid blur from tissue above or below a particular neuronal wire is to physically cut brain samples into very thin slices. Automated ways of collecting ultra-thin (one-thousandth the thickness of a hair) sections onto flexible tape are used in both SEM and TEM approaches.[4] This "section then image" approach is something akin to a film strip, with the images of physical brain sections like the frames of a movie. An alternative approach is to "image then section" a thick brain sample (often called a "block"). This technique, known as block-face imaging, uses scanning electron microscopy.[5] The electrons come from the interaction of the scanning electron beam with the top layer of the block. Once an image is generated, the top layer is sectioned away and the block-face is scanned again; this process repeats until a volume of hundreds or thousands of consecutive block-faces have been imaged.

Other novel approaches include trying to use light (photons) rather than electron imaging. One potentially promising avenue is to use short-wavelength light (X-rays) to induce electron backscatter with rapid imaging of osmium stained brains.[6] Visible longer-wavelength light can also be used, despite its lower resolving power, if the specimen is stretched into a larger size – a technique called "expansion microscopy."[7] This approach is especially exciting because it offers a straightforward way of superimposing molecular "labels" that, for example, clearly differentiate cell types in a connectome dataset.[8]

With these new tools in hand, some researchers have begun to transition from developing techniques to obtaining large-volume datasets for neurobiological research. But samples large enough to contain neuronal circuits require automated acquisition techniques. Indeed, using manual techniques, the first connectome of *C. elegans*, the millimeter-long round worm with only about three hundred neurons, took a decade.[9] About forty years later, in 2021, eight worm connectomes were completed in two years, a forty-fold speedup.[10] Beyond *C. elegans*, an intense effort is now underway to create a full description of the nervous system of *Drosophila melanogaster*, the fruit fly.[11] The small size of this animal and its long history as a model organism for genetic studies make it an ideal candidate to merge genetic and structural (circuit) information for a deeper understanding of the underpinnings of behavior. The late biologist and geneticist Seymour Benzer was the first to focus attention on finding the genetic basis of behavior and used the fly as his model organism.[12] At the time, there was little if any circuit information to go on, so his focus was on genes that modify behavior. Today, now that the connectome – the physical substrate of behavior – is becoming accessible, researchers hope this field may see a resurgence. Fly connectomics has used both serial block-face and transmission electron microscopy approaches, carried out in both adults and larval instars.[13] The brains of many other species (for example, the octopus and microwasp) are also being scrutinized.[14] A vertebrate, the larval zebrafish (*Danio rerio*), is a relatively transparent, rapidly developing freshwater fish that is easily bred in captivity and amenable to large-scale genetic screens. It has also been possible to study its behavior and the activity of the neurons related to these behaviors with fluorescence microscopy of cell activity.[15] Mammalian connectomics is moving swiftly. A number of groups are building connectomes of parts of the mouse brain and peripheral nervous system.[16] Many labs are working to devise an approach to obtain the full connectome of a mouse brain. This is a daunting task, as a cubic millimeter is far less than 1 percent of a whole mouse brain, vaulting connectomics from the petascale to the exascale.[17] Meeting this challenge will require both far more automation and ramping up computational and AI tools.

Finally, human connectomics has recently got off the ground with the acquisition and analysis of petascale cortex datasets, revealing novel circuit organization properties (such as much stronger pairwise neuron connections than expected) and some truly unexpected anatomical structures (like axons that tie themselves into knots).[18] Despite the significant imaging and computational achievements associated with such data, these cubic millimeter–scale data represent only one-millionth of an entire human brain. Whatever the future of human connectomics holds, the endeavor is clearly just beginning.

The sheer volume and complexity of connectomic image data render manual analysis an insurmountable bottleneck. AI, particularly neural network–based deep-learning methods, has been crucial in transforming raw imaging data (pixels) into meaningful, reconstructed neural pathways.

The most labor-intensive step in early connectomics was the manual tracing of neuronal processes (segmentation) and the identification of synapses across thousands of electron microscopy images. AI has revolutionized this domain. Early computational methods provided minimal assistance, but the advent of deep learning, particularly convolutional neural networks, convolutional outgrowths like U-Nets, and more specialized architectures like flood-filling networks, has enabled highly accurate automated neurite segmentation and synapse detection.[19] These algorithms can learn the complex visual patterns associated with cell membranes and organelles like mitochondria and synaptic vesicles directly from human-annotated training data, achieving performance levels that, while not yet perfect, significantly reduce the need for manual intervention, thus speeding the segmentation process profoundly.

Beyond reconstruction, AI is also proving vital for the initial analysis of connectomic data. For instance, machine-learning algorithms, including self-supervised learning methods, are being used for the automated classification of cell types based on their intricate morphology and detailed connectivity patterns.[20] This allows for a more objective and scalable approach to cataloging the brain's cellular constituents, moving beyond traditional, often subjective, classification schemes.

This work has produced a number of insights that challenge our existing views of the ways brains work. For example, the diversity of neuronal structures and connectivity in individual animals is astonishing; in *Drosophila*, there may be more than a thousand different types of neurons, each of which has its own structure, gene expression profile, and connectivity rules.

Therefore, while mapping the structural connectome – the graph of synaptic connections – is a monumental and critical step forward, it is becoming clear that a purely structural map, however detailed, provides an incomplete picture of brain function. Neurons are not just nodes in a graph; they are dynamic, molecularly diverse entities.

A critical limitation of traditional connectomics is the absence of information about the molecular and physiological diversity inherent in neural circuits. The brain utilizes a vast set of neurotransmitters, each acting on a similarly diverse array of receptors, leading to varied effects on neural activity. Ion channels, with their distinct biophysical properties, shape neuronal excitability and signaling. Furthermore, neuromodulatory systems that release substances like dopamine, serotonin, or acetylcholine can globally reconfigure circuit states and synaptic efficacy, often without overt structural changes detectable by standard microscopy.[21] These molecular dimensions are largely invisible in purely anatomical reconstructions.

To address these limitations, the field is moving toward "multimodal" connectomics. This involves integrating data from various "omics" disciplines – genomics, transcriptomics, and proteomics – with structural connectomic maps to create a "molecular connectome."[22] Pioneering imaging approaches are being developed to generate such multimodal data. Correlative light and electron microscopy (CLEM) allows researchers to link functional or molecular information obtained with light microscopy to ultrastructural details from EM.[23] Techniques like light microscopy–based connectomics (LICONN) use expansion microscopy and light microscopy to visualize synaptic connectivity and molecular components in the same tissue.[24] AI is set to play a crucial role in fusing these disparate data types. Early successes are emerging in using AI for cross-modal understanding and prediction, such as inferring molecular features or cell types directly from EM image data, leveraging the subtle textural and morphological cues that AI models can learn.[25] This AI-enabled integration will vastly improve the interpretability of connectomic data.

The accumulation of vast connectomic datasets, while a triumph of modern neuroscience and engineering, presents a new, even more formidable challenge: interpretation. How do we move from petabytes of anatomical data to a genuine understanding of how neural circuits compute, learn, and generate behavior?

Consider the history of *C. elegans* research. Although it might seem straightforward to relate neural circuitry to behavior in an organism that has only three hundred brain cells, this ambition has been much more challenging than expected. Indeed, the original *C. elegans* connectome project was part of an ambitious plan to relate genetics, the nervous system, and behavior. This effort led to several landmark discoveries concerning the lineage of the cells that make up the nervous system, the molecules that underlie the naturally occurring death of cells, and, of course, the first whole organism connectome. But a grand synthesis of the crucial strands of patterns of gene expression, neural development, neural wiring, and behavior has been elusive. The problem is complexity. All the aspects of an animal's physiology are honed by millions of years of evolution. Over time, "accidents" due to random mutations become ingenious tweaks, or sometimes less elegant kludges, embedded in the genetic makeup of an animal. Biological systems are thus relatively difficult to reverse engineer from the point of view of a simple, human-interpretable model or explanation derived by manual analysis. We therefore argue that AI is essential not only for managing and reconstructing data but for unlocking its meaning.

The very notion of "understanding" a system as complex as the brain is multifaceted and has evolved with our scientific capabilities. Historically, understanding might have meant a detailed anatomical description or a qualitative model of information flow. However, in the context of connectomics and AI, we propose a more rigorous and functional definition of understanding.

A crucial aspect of this redefinition involves moving beyond purely descriptive cataloging of connections and cell types. While such maps are foundational, a more powerful form of understanding implies the ability to make predictions about the system's behavior and ultimately to generate or simulate aspects of its function. This shifts the benchmark for understanding toward the development of causal, predictive, and generative models of neural circuits.

Under this framework, "understanding" a neural circuit could be benchmarked by the ability to:

1. *Accurately simulate circuit activity.* Given a structural connectome and relevant physiological parameters (some of which might be inferred by AI), can we simulate the patterns of neural activity that would arise under diverse physiological inputs or pathological conditions?[26]
2. *Predict the functional consequences of perturbations.* Can we predict how the circuit's output or internal dynamics would change if specific neurons

were silenced, connections were severed (as in a lesion study), or synaptic strengths were altered?

3. *Generate realistic synthetic connectomes.* Can AI models, trained on existing connectomic data, learn the underlying "design principles" or "grammatical rules" of neural wiring to the point at which they can generate novel, synthetic connectomes that are statistically and perhaps even functionally indistinguishable from biological ones?

One of the most exciting prospects of integrating AI with connectomics is the ability to create large-scale, biologically constrained simulations of neural circuits. These *in silico* experiments can complement and guide *in vivo* and *in vitro* studies, allowing for rapid hypothesis testing and exploration of parameter spaces that would be intractable experimentally.

AI models can be trained to forecast network activity, information flow, and emergent properties based on structural connectomes. For instance, given the wiring diagram of a cortical column, an AI model might predict how patterns of sensory input propagate through the layers, how different cell types contribute to a computation, or how network oscillations arise. Benchmarks for evaluating such predictive models will be crucial for driving progress and comparing different modeling approaches.[27] These models can be employed for *in silico* hypothesis testing regarding circuit mechanisms, such as by simulating the effect of blocking a particular receptor type or altering the firing properties of a specific interneuron class. A significant challenge is to develop models that can robustly predict functional connectivity (patterns of correlated activity) from detailed structural connectomes, bridging the gap between anatomical maps and dynamic brain states.

Connectomic data provide an unprecedented causal foundation and set of modeling constraints for comprehensive neural simulations.[28] However, structure alone is insufficient; physiological parameters such as synaptic weights, channel conductances, and cell-intrinsic properties are also needed. AI methodologies offer a path to inferring these missing parameters. For example, machine learning models could be trained on datasets combining sparse functional recordings (for instance, multi-electrode arrays or calcium imaging) with dense connectomic reconstructions from the same tissue volume. Computational models could learn to estimate synaptic strengths or neuronal excitability profiles that best reproduce the observed functional data, given the anatomical constraints. This AI-driven fusion of multimodal data, integrating electrophysiology, activity imaging, and even molecular profiles (such as from spatial transcriptomics correlated with connectomic data), is a key area for future development.

The brain's wiring is shaped by evolution, development, and experience to perform sophisticated computations. AI can help discover hidden principles, motifs, and rules that govern this "neural syntax."

The connectome is fundamentally a graph, with neurons as nodes and synapses as edges. Traditional graph theory has provided initial insights, but the sheer scale and complexity of mammalian connectomes demand more powerful analytical tools. Graph neural networks and other geometric deep-learning approaches are well suited for analyzing such complex relational data. These AI models can learn to identify higher-order connectivity motifs (patterns involving multiple neurons and synapses), circuit invariants (recurring structural features across different brain regions or even species), and fundamental organizational principles that might not be apparent through manual inspection or simpler statistical analyses. Furthermore, unsupervised and self-supervised learning techniques can be applied to discover novel structural and functional features within connectomic data without requiring *a priori* hypotheses or explicit labels, potentially revealing entirely new ways of categorizing neurons or understanding circuit organization.

Evolution has sculpted nervous systems to meet diverse behavioral demands. Comparing connectomes across different species, developmental stages, learning paradigms, or even between sexes can reveal conserved computational solutions and species-specific adaptations. AI can be instrumental in this comparative analysis. For instance, researchers may develop and employ AI tools for the robust alignment and comparison of massive, complex connectomic datasets, even when there isn't a one-to-one correspondence between neurons. This can help quantify interindividual variability in connectomes and its functional implications, a key research area for understanding both normal brain function and the basis of individual differences.[29] Identifying conserved circuit motifs that appear across different phyla might point to fundamental computational building blocks, while divergent features could explain unique cognitive abilities.

Many neurological and psychiatric disorders are increasingly thought to have underpinnings in altered neural circuitry, a concept often termed "connectopathies."[30] AI-supported synapse-level connectomics offers the potential to identify subtle structural "fingerprints" of these conditions, paving the way for new diagnostic tools and a deeper understanding of disease mechanisms.

AI algorithms can be trained to detect subtle, disease-associated alterations in synaptic density, the morphology of dendritic spines or axons, or specific patterns of miswiring in animal models of disease or, where available, in human pre- or postmortem tissue.[31] For example, an AI system could learn to distinguish the synaptic architecture in a mouse model of autism from that of wild-type control (unmodified) mice, even if the differences are too subtle or distributed for a human pathologist to reliably identify. This enables high-throughput screening of connectomic changes, potentially leading to the discovery of novel biomarkers for early diagnosis, disease stratification, or monitoring treatment response.

Beyond biomarker discovery, AI-driven simulations based on connectomes from disease models can help investigate how specific connectomic aberrations lead to functional deficits. For instance, if a particular interneuron type is found to have reduced synaptic input in a schizophrenia model, simulations could explore how this local change impacts broader network dynamics and information processing, potentially linking the connectomic alteration to cognitive symptoms. Such models could also be used to develop predictive tools for disease progression or to simulate the effects of potential therapeutic interventions that aim to correct the underlying circuit pathology.

A significant hurdle in applying connectomics to human disease is the limited availability of synapse-level human brain tissue suitable for EM, especially from living patients or for psychiatric conditions with which postmortem changes can be confounding. The recent H01 dataset from a human cortical sample – a 1.4 petabyte reconstruction of one cubic millimeter of human brain tissue – while from a patient with epilepsy, represents a step forward, but continued efforts to acquire and analyze such samples, combined with AI's ability to extract maximal information from them, will be crucial.[32]

The brain is the only existing proof that general, robust, and energy-efficient intelligence is possible. As connectomics unveils the detailed circuit diagrams of biological brains, it will provide a rich source of inspiration for new AI architectures and algorithms. Insights into how neurons are wired, the types of circuit motifs employed, principles of sparsity and wiring economy, and the structural basis of learning rules could directly inform the design of next-generation AI systems that are more powerful, energy-efficient, data-efficient, and biologically plausible.[33] For instance, understanding the specific connectivity patterns in brain regions known for particular cognitive functions (like navigation, language, or planning) might lead to AI models that excel at these tasks by mimicking biological solutions.

The synergy also flows in the reverse direction. As AI research develops increasingly powerful architectures, such as transformers, the fascinating question arises : Do biological neural circuits implement analogous computational principles ?[34] Large-scale connectomics provides the data to investigate this. By analyzing detailed wiring diagrams, neuroscientists can search for structural motifs or patterns of connectivity that might correspond to components of successful AI models. For example, are there patterns of long-range connections and local inhibitory circuits in the cortex that could implement something akin to the artificially engineered attention mechanisms in transformers ? Discovering such homologies would not only deepen our understanding of brain function but could also help refine AI concepts.

The prospect of acquiring and analyzing highly detailed brain data, potentially including synapse-level connectomes from humans, raises significant ethical considerations that must be proactively addressed.

Human connectomic data are arguably among the most personal and sensitive information imaginable, especially if and when such data are generated at significant scale. Robust frameworks for data privacy, secure storage, and controlled access are essential. Questions of data ownership – whether by the individual, the research institution, or the funding agency – need careful consideration, especially as datasets become more widely shared for collaborative research.

As AI tools identify potential connectomic biomarkers for complex traits or disorders, there is also a risk of misinterpretation or oversimplification. Brain function and behavior are highly complex, arising from interactions across multiple scales. Attributing a complex psychiatric condition solely to a specific wiring abnormality identified by AI would be a gross oversimplification and could lead to stigmatization or flawed therapeutic approaches. Clear communication of the limitations and probabilistic nature of such findings is crucial.

We must also consider the profound philosophical and societal implications of the ability to deeply understand, simulate, and potentially, in the distant future, modify brain circuits. What does it mean for our concepts of self, free will, and consciousness if we can create detailed computational replicas of brain activity? While therapeutic applications for devastating brain disorders are a primary motivator, the potential for nontherapeutic uses or misuse of such powerful technologies cannot be ignored. Open discussion involving scientists, ethicists, policymakers, and the public will be necessary to navigate these complex issues responsibly.

Connectomics has advanced from its highly conceptual origins to its current state as a data-rich, technologically advanced field. The initial, painstaking efforts to map the nervous system of *C. elegans* laid a crucial foundation, demonstrating the feasibility and potential value of complete wiring diagrams. Over the past two decades, driven by exponential improvements in electron microscopy, automated sectioning, and computational power, the scale and scope of connectomic projects have expanded dramatically, culminating in recent landmark datasets from *Drosophila*, mice, and even fragments of the human brain. Throughout this evolution, machine learning and AI have transitioned from a peripheral aid to a central and indispensable partner. Initially applied to the formidable challenge of segmenting neurons and synapses from vast image volumes, AI's role has broadened to encompass more detailed forms of data annotation.

Now, however, AI is poised to play an even more fundamental role in scientific interpretation of brain data. Specifically, AI offers the tools to redefine what it means to "understand" the brain, moving toward predictive and generative models that can be rigorously tested and iteratively refined. It allows us to perform *in silico* experiments at scales and with precision previously unimaginable, to uncover hidden organizational principles within the enormous complexity of neural wiring, and to identify the subtle connectomic fingerprints that may unlock the mysteries of devastating brain disorders.

Looking forward, the potential for paradigm-shifting breakthroughs is immense. The coevolution of connectomics and AI promises not only to accelerate discoveries in fundamental neuroscience but also to inspire new generations of artificial intelligence, potentially leading to AI systems that are more robust and efficient. In the realm of medicine, the ability to link specific connectomic alterations to disease states could substantially accelerate diagnostics and pave the way for novel therapeutic strategies targeted at the circuit level.

However, realizing this vision requires a sustained and intensified commitment to interdisciplinary collaboration. The challenges ahead are too vast and complex for any single discipline or entity to tackle alone. Neuroscientists, AI researchers, computer scientists, engineers, physicists, mathematicians, and ethicists must work in concert, sharing data, tools, and insights. Initiatives that foster such "big team science," alongside continued investment in foundational technologies and computational infrastructure, will be critical.

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ENDNOTES

1 Santiago Ramón y Cajal, "The Structure and Connexions of Neurons: Nobel Lecture, December 12, 1906," The Nobel Prize, https://www.nobelprize.org/uploads/2018/06/cajal-lecture.pdf.

2 Fred Y. Shen, Margaret M. Harrington, Logan A. Walker, et al., "Light Microscopy Based Approach for Mapping Connectivity with Molecular Specificity," *Nature Communications* 11 (1) (2020); and Mojtaba R. Tavakoli, Julia Lyudchik, Michał Januszewski, et al., "Light-Microscopy-Based Connectomic Reconstruction of Mammalian Brain Tissue," *Nature* 642 (8067) (2025): 398–410.

3 Kevin L. Briggman and Davi D. Bock, "Volume Electron Microscopy for Neuronal Circuit Reconstruction," *Current Opinion in Neurobiology* 22 (1) (2012): 154–161.

4 Narayanan Kasthuri, Kenneth Jeffrey Hayworth, Daniel Raimund Berger, et al., "Saturated Reconstruction of a Volume of Neocortex," *Cell* 162 (3) (2015): 648–661; and Jasper S. Phelps, David Grant Colburn Hildebrand, Brett J. Graham, et al., "Reconstruction of Motor Control Circuits in Adult *Drosophila* Using Automated Transmission Electron Microscopy," *Cell* 184 (3) (2021): 759–774.

5 Winfried Denk and Heinz Horstmann, "Serial Block-Face Scanning Electron Microscopy to Reconstruct Three-Dimensional Tissue Nanostructure," *PLOS Biology* 2 (11) (2004), https://doi.org/10.1371/journal.pbio.0020329.

6 Kevin M. Boergens, Gregg Wildenberg, Ruiyu Li, et al., "Photoemission Electron Microscopy for Connectomics," bioRxiv (2024), https://doi.org/10.1101/2023.09.05.556423.

7 Asmamaw T. Wassie, Yongxin Zhao, and Edward S. Boyden, "Expansion Microscopy: Principles and Uses in Biological Research," *Nature Methods* 16 (1) (2019): 33–41.

8 Tavakoli, Lyudchik, Januszewski, et al., "Light-Microscopy-Based Connectomic Reconstruction of Mammalian Brain Tissue."

9 John Graham White, Eileen Southgate, J. N. Thomson, and Sydney Brenner, "The Structure of the Nervous System of the Nematode *Caenorhabditis elegans*," *Philosophical Transactions of the Royal Society B: Biological Sciences* 314 (1165) (1986): 1–340.

10 Daniel Witvliet, Ben Mulcahy, James K. Mitchell, et al., "Connectomes across Development Reveal Principles of Brain Maturation," *Nature* 596 (7871) (2021): 257–261.

11 Louis K. Scheffer, C. Shan Xu, Michal Januszewski, et al., "A Connectome and Analysis of the Adult Central Brain," *Elife* 9 (2020), https://doi.org/10.7554/elife.57443; and Sven Dorkenwald, Arie Matsliah, Amy R. Sterling, et al., "Neuronal Wiring Diagram of an Adult Brain," *Nature* 634 (8032) (2024): 124–138.

12 Seymour Benzer, "From the Gene to Behavior," *JAMA* 218 (7) (1971): 1015–1022.

13 Zhihao Zheng, J. Scott Lauritzen, Eric Perlman, et al., "A Complete Electron Microscopy Volume of the Brain of Adult *Drosophila melanogaster*," *Cell* 174 (3) (2018): 730–743, https://doi.org/10.1016/j.cell.2018.06.019; Zhiyuan Lu, C. Shan Xu, Kenneth J. Hayworth, et al., "*En bloc* Preparation of *Drosophila* Brains Enables High-Throughput FIB-SEM Connectomics," *Frontiers in Neural Circuits* 16 (2022): 917251; and Michael Winding, Benjamin D. Pedigo, Christopher L. Barnes, et al., "The Connectome of an Insect Brain," *Science* 379 (6636) (2023), https://doi.org/10.1126/science.add9330.

14 Flavie Bidel, Yaron Meirovitch, Richard Lee Schalek, et al., "Connectomics of the *Octopus vulgaris* Vertical Lobe Provides Insight into Conserved and Novel Principles of a Memory Acquisition Network," *eLife* 12 (2023), https://doi.org/10.7554/eLife.84257; and Nicholas J. Chua, Anastasia A. Makarova, Pat Gunn, et al., "A Complete Reconstruction of the Early Visual System of an Adult Insect," *Current Biology* 33 (21) (2023): 4611–4623.

15 Misha B. Ahrens, Michael B. Orger, Drew N. Robson, et al., "Whole-Brain Functional Imaging at Cellular Resolution Using Light-Sheet Microscopy," *Nature Methods* 10 (5) (2013): 413–420; and Jan-Matthis Lueckmann, Alexander Immer, Alex Bo-Yuan Chen, et al., "ZAPBench: A Benchmark for Whole-Brain Activity Prediction in Zebrafish," in *The 13th International Conference on Learning Representations* (ICLR, 2024).

[16] Alessandro Motta, Manuel Berning, Kevin M. Boergens, et al., "Dense Connectomic Reconstruction in Layer 4 of the Somatosensory Cortex," *Science* 366 (6469) (2019), https://doi.org/10.1126/science.aay3134; Davi D. Bock, Wei-Chung Allen Lee, Aaron M. Kerlin, et al., "Network Anatomy and *in vivo* Physiology of Visual Cortical Neurons," *Nature* 471 (2011): 177–182; and Ju Lu, Juan Carlos Tapia, Olivia L. White, and Jeff W. Lichtman, "The Interscutularis Muscle Connectome," *PLOS Biology* 7 (2) (2009), https://doi.org/10.1371/journal.pbio.1000032.

[17] Larry F. Abbott, Davi D. Bock, Edward M. Callaway, et al., "The Mind of a Mouse," *Cell* 182 (6) (2020): 1372–1376.

[18] Alexander Shapson-Coe, Michał Januszewski, Daniel R. Berger, et al., "A Petavoxel Fragment of Human Cerebral Cortex Reconstructed at Nanoscale Resolution," *Science* 384 (6696) (2024), https://doi.org/10.1126/science.adk4858.

[19] Yann LeCun, Bernhard Boser, John S. Denker, et al., "Backpropagation Applied to Handwritten Zip Code Recognition," *Neural Computation* 1 (4) (1989): 541–551; Viren Jain, Joseph F. Murray, Fabian Roth, et al., "Supervised Learning of Image Restoration with Convolutional Networks," in *2007 IEEE 11th International Conference on Computer Vision* (IEEE, 2007), https://ieeexplore.ieee.org/document/4408909; Olaf Ronneberger, Philipp Fischer, and Thomas Brox, "U-Net: Convolutional Networks for Biomedical Image Segmentation," in *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015*, ed. Nassir Navab, Joachim Hornegger, William M. Wells, and Alejandro F. Frangi (Springer, 2015), 234–241; Kisuk Lee, Jonathan Zung, Peter Li, et al., "Superhuman Accuracy on the SNEMI3D Connectomics Challenge," arXiv (2017), https://doi.org/10.48550/arXiv.1706.00120; and Michał Januszewski, Jörgen Kornfeld, Peter H. Li, et al., "High-Precision Automated Reconstruction of Neurons with Flood-Filling Networks," *Nature Methods* 15 (8) (2018): 605–610.

[20] Sven Dorkenwald, Peter H. Li, Michał Januszewski, et al., "Multi-Layered Maps of Neuropil with Segmentation-Guided Contrastive Learning," *Nature Methods* 20 (12) (2023): 2011–2020.

[21] Cornelia I. Bargmann and Eve Marder, "From the Connectome to Brain Function," *Nature Methods* 10 (6) (2013): 483–490.

[22] Jeff W. Lichtman and Joshua R. Sanes, "Ome Sweet Ome: What Can the Genome Tell Us about the Connectome?" *Current Opinion in Neurobiology* 18 (3) (2008): 346–353.

[23] Tao Fang, Xiaotang Lu, Daniel Berger, et al., "Nanobody Immunostaining for Correlated Light and Electron Microscopy with Preservation of Ultrastructure," *Nature Methods* 15 (12) (2018): 1029–1032; and Xiaomeng Han, Xiaotang Lu, Peter H. Li, et al., "Multiplexed Volumetric CLEM Enabled by scFvs Provides Insights into the Cytology of Cerebellar Cortex," *Nature Communications* 15 (1) (2024): 6648.

[24] Tavakoli, Lyudchik, Januszewski, et al., "Light-Microscopy-Based Connectomic Reconstruction of Mammalian Brain Tissue."

[25] Dorkenwald, Li, Januszewski, et al., "Multi-Layered Maps of Neuropil with Segmentation-Guided Contrastive Learning"; and Nils Eckstein, Alexander Shakeel Bates, Andrew Champion, et al., "Neurotransmitter Classification from Electron Microscopy Images at Synaptic Sites in *Drosophila melanogaster*," *Cell* 187 (10) (2024): 2574–2594, https://doi.org/10.1016/j.cell.2024.03.016.

[26] Lueckmann, Immer, Chen, et al., "ZAPBench: A Benchmark for Whole-Brain Activity Prediction in Zebrafish"; and Viren Jain, "How AI Could Lead to a Better Understanding

of the Brain," *Nature* 623 (7986) (2023): 247–250, http://dx.doi.org/10.1038/d41586-023-03426-3.

[27] Lueckmann, Immer, Chen, et al., "ZAPBench: A Benchmark for Whole-Brain Activity Prediction in Zebrafish."

[28] Lappalainen Janne, Tschopp Fabian, Prakhya Sridhama, et al., "Connectome-Constrained Networks Predict Neural Activity Across the Fly Visual System," *Nature* 634 (2024): 1132–1140.

[29] Joshua L. Morgan and Jeff W. Lichtman, "Why Not Connectomics?" *Nature Methods* 10 (6) (2013): 494–500.

[30] Mikail Rubinov and Ed Bullmore, "Fledgling Pathoconnectomics of Psychiatric Disorders," *Trends in Cognitive Sciences* 17 (12) (2013): 641–647.

[31] Shapson-Coe, Januszewski, Berger, et al., "A Petavoxel Fragment of Human Cerebral Cortex Reconstructed at Nanoscale Resolution."

[32] Ibid.

[33] Abbott, Bock, Callaway, et al., "The Mind of a Mouse."

[34] Ashish Vaswani, Noam Shazeer, Niki Parmar, et al., "Attention Is All You Need," in *NIPS '17: Proceedings of the 31st International Conference on Neural Information Processing Systems,* ed. Ulrike von Luxburg, Isabelle Guyon, Samy Bengio, et al. (Curran Associates, Inc., 2017).