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The Past, Present, and Future of Single Neuron Reconstruction

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More than one hundred years ago, Ramon y Cajal found that neurons are polarized cells with distinct input and output sides, the dendrites and axons respectively. These discoveries were based on optical microscopy and single neuron reconstructions. The Golgi technique allowed Cajal to visualize individual neurons embedded in dense, complex networks and to extract the structure of individual neurons with unprecendented clarity. Cajal's principles, the neuron doctrine and the law of dynamic polarization, were the results of a synthesis of his observations on neuronal structure.

Neuronal structure is intimately related to function. For example, neuronal geometry shapes dendritic integration and cellular excitation, and predicts the neuron's position within the circuit. Even though synapses had not yet been discovered, Cajal intuited this second point, and used the overlap of axons and dendrites to predict neuronal connections. Applying this trick to many types of neural tissue, Cajal elucidated the circuit logic of the retina, the hippocampus and the cerebellum. Used implicitly or explicitly, similar approaches based on neuronal structure underlie the majority of what we know about the circuit organization of complex brains.

Despite these early successes, neuroanatomy is still a nascent field. Even in well-studied regions of the brain such as the rodent barrel cortex or the hippocampus, the exact number and prevalence of distinct cell types remains unknown. In other areas even a rough guess as to the number of cell types is lacking. This is a huge, somewhat embarrassing hole in our understanding of the brain. After all, 'cell types' are the nodes, the key modules, in any description of neural circuits.

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How can we enumerate cell types? One challenge is the very definition of cell type. A logical definition would be functional: neurons that perform the same function within the circuit belong to the same cell type. But this definition is useless, since the function of most neurons in the brain remains to be discovered. Instead cell types have been operationally defined by one or a combination of several parameters, including location of the soma, gene expression, electrophysiological properties and neuronal structure. Among these parameters neuronal structure is most closely linked to function. Neuronal structure is also related to the other parameters: gene expression patterns underlie neuronal structure, and neuronal structure in turn shapes the cell's electrophysiological properties. A catalog of neuronal structures, reconstructed in their entirety, would thus likely yield a list of cell types.

The second challenge lies in the numbers involved. The mammalian brain contains approximately 1,000 brain areas, each likely with more than ten cell types, in some cases considerably more. Each cell type would have to be sampled multiple times. A complete catalogue of cell types would require reconstructions of more than 100,000 neurons.

Over the last forty years, major technical barriers to reconstructing single neurons have been overcome. Labeling neurons with intracellular pipettes has allowed high contrast imaging and reconstructions of large parts of individual neurons. These methods revealed that axonal arbors are larger and more complex than expected based on the Golgi method. Moreover, intracellular labeling has allowed linking the structure of individual neurons with activity patterns *in vivo*.

Genetic labeling methods based on fluorescent proteins have started another revolution in neuroanatomy. Relative to traditional methods they provide experimental control and reproducibility. Morphological analyses can be focused

on relatively uniform, molecularly identified cell populations. Genetic methods help with targeting rare cell-types, which would be missed with sparse but random labeling. Most importantly, genetic labeling methods are typically much simpler than the classical techniques and can be achieved with high throughput and consistent results.

Digital imaging has also advanced rapidly. New microscopy methods, such as slide scanners, block-face two-photon microscopes, array tomography and others promise high-resolution imaging of entire mammalian brains. The limitations on archiving terabyte data sets have disappeared with the falling costs of hard disk drives. As a result of these developments, several anatomy projects are now attempting large scale imaging of brains labeled sparsely using genetic methods and fluorescent proteins. However, none of these projects are attempting single neurons reconstructions.

The critical remaining bottleneck in single neurons reconstructions from light microscopy is data mining. Manual reconstructions are labor intensive: reconstructing the full axonal arbor of a single mammalian projection neuron requires months of labor. In fact, not even a single cortical projection neuron has been reconstructed in its entirety. Manual reconstructions are simply not practical for projects on scales of thousands of neurons. Computer scientists often express the opinion that full automation should be achievable, but despite efforts by commercial entities and academic labs, the vast majority of data are still analyzed manually.

The DIADEM Challenge—short for Digital Reconstruction of Axonal and Dendritic Morphology—was designed to focus efforts in the computer science community to develop a new generation of computer tools for single neuron reconstructions. It attracted numerous talented teams and investigators who attacked the problem in multiple creative ways. DIADEM did not solve the problem: 10-fold increases in speed compared to manual reconstructions were clocked on most of the data sets, whereas 100,000-fold increases may ultimately be required. However, the clever new tools emerging from the challenge triggered great optimism by experimentalists and computer scientists alike. It may be that DIADEM signaled the beginning of the end for manual reconstructions, ringing in a new era in the study of neuronal structure and neural circuits.