ULTRASTRUCTURAL MAPPING OF NEURAL CIRCUITRY: A COMPUTATIONAL FRAMEWORK

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ABSTRACT:
Complete mapping of neuronal networks requires data acquisition at synaptic resolution with canonical coverage of tissues and robust neuronal classification. Transmission electron microscopy (TEM) remains the optimal tool for network mapping. However, capturing high resolution, large, serial section TEM (ssTEM) image volumes is complicated by the need to precisely mosaic distorted image tiles and subsequently register distorted mosaics. Moreover, most cell or tissue class markers are not optimized for TEM imaging.

We present a complete framework for neuronal reconstruction at ultrastructural resolution, allowing the elucidation of complete neuronal circuits. This workflow combines TEM-compliant small molecule profiling with automated image tile mosaicking, automated slice-to-slice image registration and terabyte-scale image browsing for volume annotation. Networks that previously would require decades of assembly can now be completed in months, enabling large-scale connectivity analyses of both new and legacy data. Additionally, these approaches can be extended to other tissue or biological network systems.

INTRODUCTION:
Deciphering and reconstructing complete neuronal networks and is one of the grand challenge in neuroscience, particularly in vision science. Defining connectomes [1, 2] or complete network (CN) maps for canonical regions of any metazoan tissue requires robust cataloguing of classes [3-7], mapping of statistically distinct patterns [8-11] and tracing of characteristic connections [12-14]. Anatomic and physiologic methods for network analysis particularly in neural systems has not kept pace with the demands for phenotyping an ever expanding genetic library of neurologic disorders in general [15] and retinal disorders in particular [16]. Historically, anatomy has shown far more power to define neural network identity than either modeling or physiological strategies and, in practice, ssTEM has remained the gold standard for validating existing network maps. expanding these current state of the art beyond a select, few laboratories in a critical need and tools that resolve these problems will open a new era of exploration and understanding. To this end, we have developed a complete suite of software tools and strategies that leverage the current installed base of ultrastructural resources. Commercial [17] and academic [18, 19] software solutions for small-scale, user-guided mosaicking, combined with multimodal registration have been available, but are not viable for large datasets representing CN assemblies or high throughput approaches. By providing tools capable of precisely and automatically tiling many images (≈ 1000-4000) into large mosaics, precisely registering serial mosaics (including multimodal frames) with the capability to browse and annotate gigabyte to terabyte sized image sets and volumes, we hope to enable expanded analysis of connectivity patterns not only in existing but also in new image databases.

Elucidating connective patterns in complex neural tissue and the characterization of pathological circuitry in disease states requires sampling scales that have been until recently, impossible. Indeed, some neural reconstruction tasks are so large that they transcend grant cycles or even investigator lifetimes using current resources [20]. The volume that must be constructed to approach sampling completeness in the inner plexiform layer of the mammalian retina is three orders of magnitude larger than most typical ssTEM volumes used in CNS [18, 21]. One approach is to address these challenges by developing novel platforms to acquire pre-aligned serial electron microscopy images [22-25]. However these platforms alone are not the sole nor optimal strategies for ssTEM volume assembly due to their destructive methods employed to capture data. Furthermore, approaches other than ssTEM, are limited in resolution and speed, and most of the platforms are developmental or highly restricted in availability. Conversely, ssTEM has the ability to capture high resolution data with tremendous flexibility in staining and immunocytochemical options, very fast acquisition times and the potential for parallelization with other TEM facilities working to acquire data concurrently. Data assembly of projects working in parallel could be readily done if tools to harmonize the effort were widely available.

We present the essential software tools, specifically those for assembling large-scale mosaics and achieving slice-to-slice image registration for use not only in neural systems, but also allowing the fusion of molecular profiling, image acquisition, volume assembly and data viewers/annotators to be used as tools for discovery in non-neural systems. The potential for practical genetic screening and diagnostics can not be overlooked.

DISCUSSION:
There are three major barriers to large scale ssTEM reconstruction: mosaicking, registration and viewing. While mathematically robust tools have long existed for analyst-guided non-linear mosaicking and registration (e.g. PCI Geomatica ®); see Marc and Cameron 2002 [26], and many solid efforts have been made to provide tools that work on small volumes

[19].However, the scale of ssTEM canonical volume reconstruction precludes a user-guided software solution and demands some automation. The ability of our software tools, ir-fft / ir-grid-refine to automatically mosaic individual tiles and ir-stos-brute / ir-stos-grid to automatically register mosaics enables any laboratory to build high-performance ssTEM volumes from new data or existing legacy data in film formats. Many extremely high quality ssTEM datasets have been produced in the past three decades [21, 27-32], but their analyses have been restricted to one-time manual tabulations, drawings and representative halftone imagery.. And despite the development of early far-sighted reconstruction frameworks [33] and subsequent enhancements, the code, platforms and throughput of those schemata reached neither the performance nor availability required for canonical field reconstructions, orphaning those datasets from further exploration and participation. By contrast, our tools provide the framework for global access of legacy datasets via a central repository

CMP and ssTEM.
The importance of molecular classification of neural data cannot is fundamental to understanding the participants in networks. Without even partial classification, ssTEM reconstructions remain of limited value. This remains true even with the ability to nominally identify individual cells by stochastic, ad-hoc or even multivariate protein expression [1]. In contrast, small molecule computational molecular phenotyping (CMP) allows the categorization of participants in networks before the network is built from ssTEM. Classification by post-hoc unraveling of connectivity is an impractical and statistically difficult way to identify synaptic connectivities.

THE RETINAL CN MAPPING FRAMEWORK.

Our objective in developing these tools is retinal CN mapping. We have begun implementation of this process by developing a rabbit retinal preparation with strong image segmentation. As shown previously, [34-36] augmenting CMP libraries with the activity marker 1-amino-4-guanidobutane (AGB) generates a nearly complete neuronal classification. These signals are also fully compatible with ssTEM rendering a functional assessment combined with traditional anatomical approaches. We have prepared a single retinal preparation with sixteen regions, each defined as a canonical field for CN mapping. These regions are being sectioned, stained and captured with an estimated completion within a year. The strategy uses horizontal serial sections (sections in the plane of the retina) beginning from either the AC or GC side of the inner plexiform layer. Those cellular layers are first classified as CMP bounding layers registered to the ssTEM set of >400 sections, with each section captured in mosaics of 950-1100 tiles. Upon completion,each volumewill be made available for our own and community browsing and annotation, described as follows.

A PROPOSAL FOR MULTI-TEM PROJECTS.

Most of the example ssTEM volumes our group has produced so far have been collected with a single high-performance microscope. We can capture ~4000 tiles in 24hrs. However, the install base of manual TEM systems or film-based systems with montaging stages far exceeds those with high-resolution digital cameras. Further, the performance of film is still superior to any digital system and the potential for capturing high bit-depth scanned images manually augmented with positional metadata makes our ultrastructural framework even more practical. By fragmenting large projects into packets of grids that can be captured in parallel, it is possible to process and scan data in parallel followed by distribution of tiles to a central resource for volume builds. The analysis phase of CN mapping includes building descriptions of connectivity through identification of cells and processes followed by markup of synaptic connections.. We are currently developing tools to automate synapse and gap junction recognition. However, our experience is that trained analysts perform tagging and synapse markup with high precision and speed. Furthermore, large datasets can be analyzed in parallel by large groups as shown by those in the astronomy fields withthe www.galaxyzoo.org project to classify millions of galaxies imaged by various platforms such as the Sloan Digital Sky Survey (www.sdss.org). Given the importance of mammalian CNS circuitry analysis in neurological disorders, and the scope of the tasks, the feasibility of a single labs performing entire reconstrcutions is becoming increasingly impractical, as is the notion that computational pattern recognition can adequately screen data without missing important observations. Human eyes remain the best pattern recognition systems for ssTEM data. The value of our strategy to develop a scalable, web-compliant viewer for community markup lies in the fact that new, powerful acquisition platforms [23-25] and their descendants will soon create an additional deluge of high-quality data.

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