Volume Electron Microscopy Workflows for the study of Large-Scale Neural Connectomics

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Volume Electron Microscopy Workflows for the study of Large-Scale Neural Connectomics

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For the past fifteen years, the Lichtman laboratory has developed sample preparation, imaging, and computational tools –along with the associated workflows– for the acquisition of neural tissue volumes ranging from .002 mm3 (c. elegans, ~1 TB) [1] to 1 mm3 (human cortex, > 1.3 PB) [2]. Three distinct workflows developed for SEMs (Zeiss Sigma, Zeiss multiSEM, and Thermofisher Magellan) all rely upon the tape-to-SEM automated tape ultramicrotome (ATUM) technique. In addition, considerable effort has been made in developing correlative light and electron microscopy (CLEM)workflows [3] and using x-ray uCT (Xradia 510) as an intermediate resolution correlation tool. Through collaborative efforts and a resource-sharing U24 grant [4] the lab has provided serial section imaging services for the extended volume electron microscopy community consisting of three user groups: in-lab, external and remote users.

The tape-to-SEM ATUM technology provides workflow flexibility by allowing either secondary electron or backscattered electron imaging strategies and workflow pauses which are not easily available in other serial section imaging technologies. The advantages of the ATUM technology include large sample size, multi-resolution imaging, multi-region imaging, multi-modal imaging, section reimaging strategies (non-destructive), and small penalties for failure. Furthermore, this choice of sectioning technology provides a greater range for tissue staining [5] and post-staining, in addition to section archiving. Sample sizes and types range from human brain biopsy samples to mouse brain tissue to whole animals (zebrafish, hydra, etc.). Typically, projects require 30–65 nm thick serial sections which are collected on carbon-coated Kapton tape with sectioning error rates of 1 section per 500 sections. The number of serial sections range from several hundred to many thousands (largest series cut and collected so far is 33,000 sections). Disadvantages to the ATUM approach, multiple steps, complicated 3D alignment, wrinkle formation –if not collected properly–, and susceptibility to dust particles and scratches.

This presentation gives an overview of the generalized ATUM-based workflow (see Figure 1). Here the entire pipeline from step 1 to step 7 is depicted. Since small block (< 1mm x 1mm x 1 mm) serial imaging can be performed with FIB/SEM or serial block face approaches, this discussion will focus primarily on the sample preparation, section cutting and collecting and the imaging of larger samples. The data in Figure 2 shows serial sections acquired from a newborn (P0) mouse brain (~7 mm x 10 mm), a human retina (~ 2.3 mm x 3 mm), and the whole brain of a zebrafish. The majority of projects use an image pixel size of 4 nm; however, both larger and smaller pixel sizes can be used. The major problems encountered when scaling to larger sample sizes fall into two categories: (1) mechanical limitations imposed by the diamond knife and by the cutting action of the ultramicrotome, and (2) the physical limitations of reagent diffusion and chemical reactions. Examples of approaches to overcome these limitations will be presented [6].

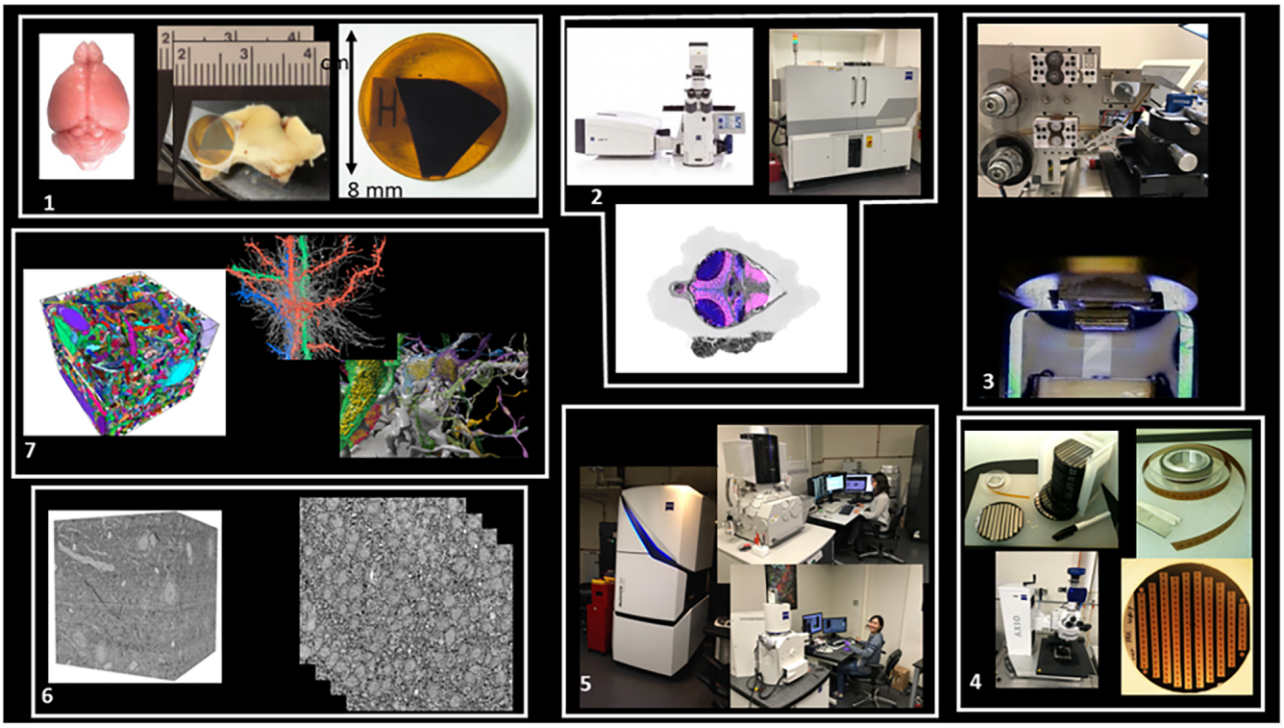

Fig. 1. Overview of the essential workflow steps. The workflow can be broken down into 7 distinct phases. Each phase stands alone and provides a stopping/starting point.

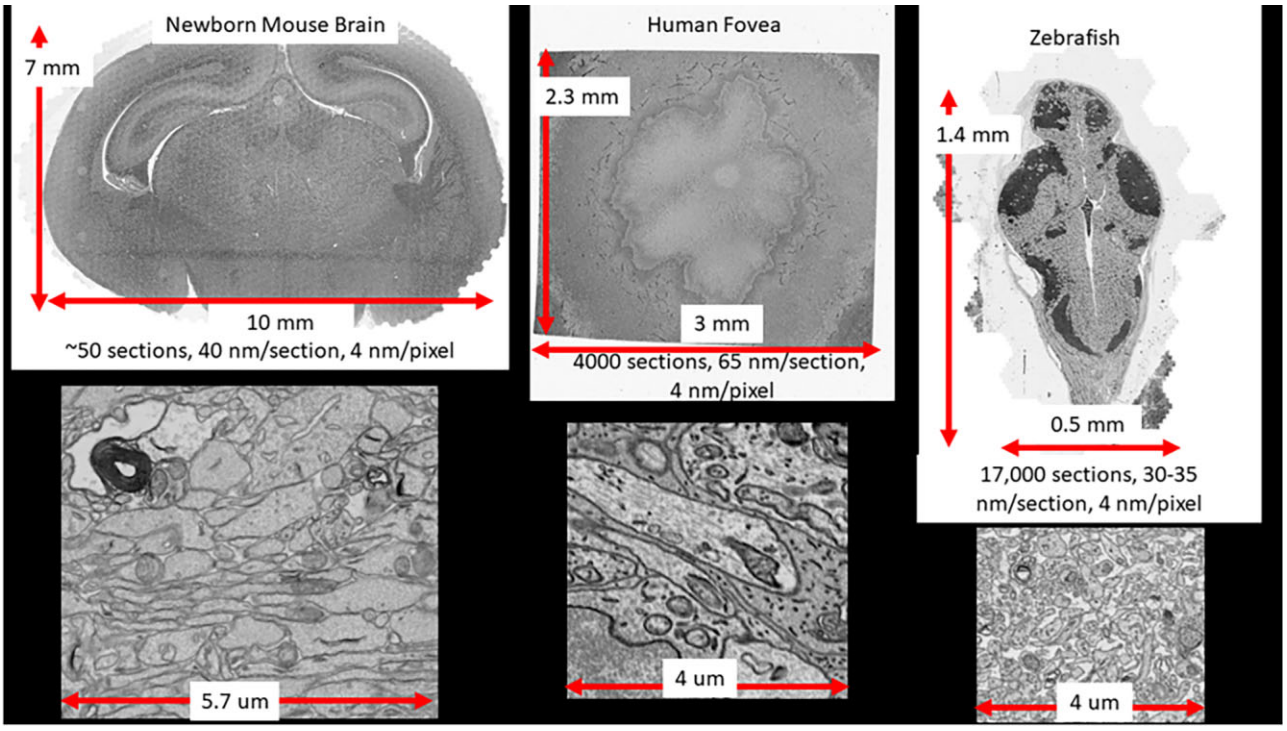

Fig. 2. Examples illustrating the scale of the sample preparation, section cutting and collecting, and imaging among three different user groups.

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