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Volume And Large Field Of View Electron Microscopy As Tools For Rapid And Detailed Cellular Analysis In Preclinical Therapeutic Testing

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Scanning electron microscopy (SEM)-based approaches for imaging tissue sections (also called array tomography) and serially sectioning samples (serial block-face SEM, FIB-SEM) have been available for the past decade, and have greatly impacted biological sciences. For preclinical studies, back-scattered electron-based SEM imaging can be both cost effective and offer valuable subcellular endpoints that may be otherwise unavailable. In heritable disorders that affect axons, for example, genetic defects may impact mitochondria, the cytoskeleton, organelle transport, myelin, and myelinating cells. Using nerve analysis as a context, this presentation will provide some practical approaches to using EM imaging.

2D EM Scanned Sections. Utilizing SEM-based imaging from a background in plastic section histology is not necessarily difficult. Access to a modern SEM with a field emission gun and a back-scattered electron detector is required. Semi-thin sections (0.5–1μm) are used for section scanning because they are easy to cut and handle, and duplicates can be stained for light microscopy (LM) if desired. As an example, aldehyde-fixed peripheral nerve, and other tissue types, are embedded as for LM and transmission EM. For consistency and expedience, we use an ASP1000 staining robot system (Microscopy Innovations). Samples are postfixed in 1% osmium tetroxide, dehydrated in graded ethanols, transferred to 50:50 propylene oxide:resin, and then resin. Sections are cut using a Diatome diamond histoknife and mounted on 12 mm coverslips. Sections are batch stained using methanolic uranyl acetate and lead citrate TEM stains. Only a thin surface layer is impregnated by heavy metals, but that is optimal for this method.

For our studies, imaging is performed using a Sigma VP (Zeiss) or Teneo (ThermoScientific/FEI) scanning electron microscopes equipped with low kV backscattered electron detectors (Gatan, ThermoFisher respectively). Highest resolution is ~4nm/pixel with for 30 nm "optical" sections (imaging at 2kV), although most nerve histology studies use 10–40 nm/pixel. Sections are scanned as 4k or 6k pixel tiles using SEM control software. Acquisition can be scripted (Gatan Digital Micrograph software). Thirty or more samples can be set up for batch imaging, using preset 3-point focusing (Zeiss/Gatan) or automated focusing (ThermoFisher). At 20 nm/pixel resolution, each section of mouse nerve takes about 1 hour to collect. Processing and analysis can be undertaken using many commercial and open-source software packages. Frequent analyses can be expedited by scripting.

Compared with LM results, using EM to study nerve samples facilitates detection and measurement of substantially thinner myelin and smaller axons. In addition, automated determination of axon average density can be used to assess axon integrity: axons contain few organelles and an elevation in average staining density indicates pathology. Axons can then be examined manually for mitochondrial status and cytoskeletal perturbations. Such measures of axon "integrity" are valuable secondary endpoints where dying back neuropathies are under investigation.

Volume EM approaches: Many SEM systems are also equipped with 3View (Gatan) or Volumescope (ThermoScientific) in-chamber utramicrotome systems for serial blockface imaging. Using the same BSE detection as described above, these systems generate sets of serial images. En bloc staining is required for serial blockface EM, and is greatly expedited using the robotic staining system (see reference [1]). Imaging methods have been well described (e.g. ref [2]).

Most volume EM applications require the fine resolutions in x-, y-, and z-dimensions. In nerve studies, high resolution volumes are sampled to reconstruct mitochondrial organization in the axons, and accumulations of organelles that are common to axon dysfunction [2]. For preclinical studies, highest resolution is often not necessary. In nerve samples, for example, skip-serial sectioning of longitudinally mounted specimens can produce low resolution stacks with z-steps of 1 μm or more. Non-linear 3D measures along large diameter motor axons quantifies internodal myelination, which is an important indicator of therapeutic benefit. Benefits of volume EM image acquisition thus are not always derived from higher resolution: sometimes simply not having to manually generate serial sections yourself may make an analysis endpoint viable.

References

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