Application note

TEM-standard imaging on the Phenom Pharos G2 Desktop FEG-STEM

Low-kV STEM imaging of soft tissues for cell biology and pathology

For many decades, transmission electron microscopy (TEM) imaging of ultra-thin sections cut from fixed and embedded blocks has been the modality of choice for fine structural analysis of tissue, changes of the cell interior, organelles, and the surrounding areas.

The ultrastructure revealed by this imaging has not only defined many important cell organelles and their functions but also contributed to pathological diagnoses in disciplines such as anatomical pathology, hematology, and microbiology.

Ultrastructural analysis in these biomedical disciplines is often limited by fixation. When working with competing experimental demands or in clinical settings separate from the electron microscopy facility, fixation can often be suboptimal but still useful for its intended purpose. In these situations, and where lower magnification is required to show cellular context, it is common for transmission electron microscopists to choose a kV lower than the available maximum (say 80 kV) to enhance contrast in the sample and increase perceived detail clarity.

Scanning transmission electron microscopy (STEM) systems have for some time been available as an additional modality for TEM systems, promising increased contrast, particularly from unstained sections. But they have not been widely available outside of specialist comprehensive EM centers. In this application note we discuss the advantages of low-kV STEM imaging using the highly automated Thermo Scientific™ Phenom Pharos™ G2 FEG-SEM with the new scanning transmission electron microscopy (STEM) detector, which can achieve a higher resolution than the BSD detector[1] at only 15 kV.

This instrument features a space-saving desktop design as well as sophisticated stage automation and image acquisition algorithms. It is only the second generation of such an instrument to be fitted with a high-brightness and high-coherence FEG electron source. While STEM systems have been available on TEM instruments for some time, they have not been commonly used for ultrastructural examination in cell biology and pathology, perhaps due to their complexity. Here, we demonstrate the utility and imaging quality attainable with routine preparations of soft tissue samples, including kidney, brain, and pancreas.

Method

The tissues were routinely processed for TEM imaging. Tissue processing for electron microscopy includes fixation with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4. After fixation is complete, the tissue is rinsed in sodium cacodylate buffer for one to two days then processed routinely for electron microscopy. This includes four hours with 2% osmium tetroxide, one hour with 2% uranyl acetate, one hour with sodium acetate, dehydration with graded alcohol and acetone, and finally impregnation with Spurr low-viscosity epoxy resin at the standard formulation. The resin was then cured at 70°C for 15 hours. Sections 70 nm thick were prepared using a PowerTome ultramicrotome (RMC Boeckeler, USA). Sections were mounted on 300 mesh copper grids and lightly carbon coated before being mounted in the grid holder.

Renal, brain, and pancreas samples from animal models were imaged with the Phenom Pharos G2 FEG-SEM. Images were acquired using 15 kV with a working distance of 8.9 mm using the STEM detector.

Results

The ultrastructural features of the normal glomerular tuft within Bowman's capsule are shown in Figure 1. These images are from renal tissue prepared conventionally for TEM observation. The STEM micrographs present a normal glomerular capillary loop and mesangium similar to a TEM micrograph (Moss and Shore, 2002). It is clear that the mesangial matrix is continuous with the glomerular basement membrane (arrows), the mesangial cytoplasm, the detail of podocyte foot processes, and slit pores (arrows) adjacent the glomerular basement membrane. The micrographs show high-resolution ultrastructure features with adequate contrast to quickly detect minimal cellular changes or quantify and analyze features of interest.

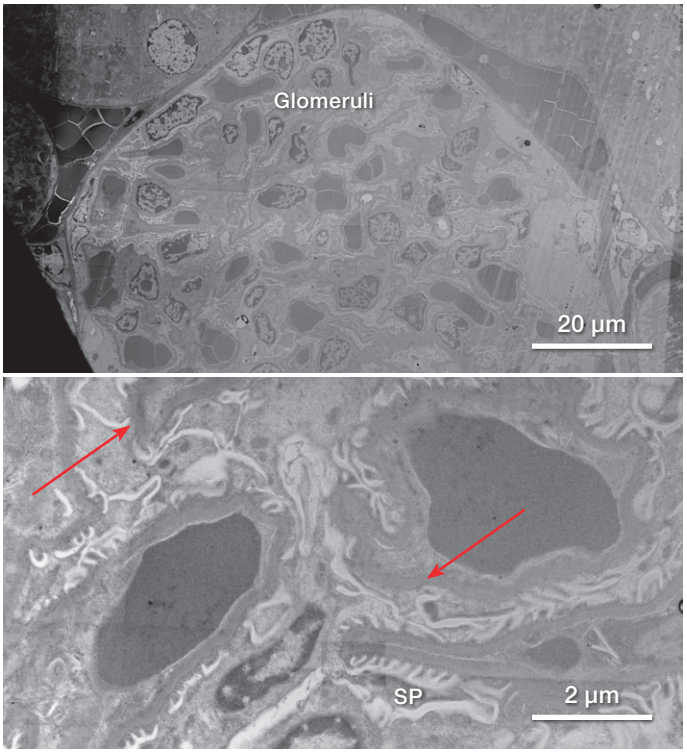

Figure 1. Top: A glomerulus and adjoining tubule from a routinely processed mouse kidney specimen. Bottom: Detail of glomerular capillaries containing red blood cells. The capillaries are surrounded by the glomerular basement membrane and the foot processes of the podocytes (arrow).

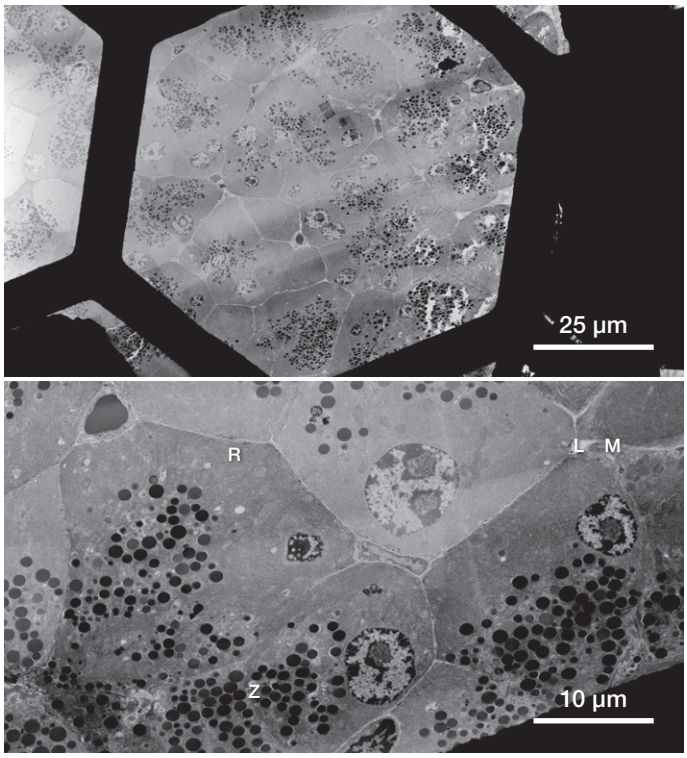

Figure 2. Normal architecture of pancreatic acinar cells in low- and high-power images of pancreas tissue from a mouse model. The micrographs detail zymogen granules (Z), vacuole, mitochondria (M), acinar lumen (L), and rough endoplasmic reticulum (R). Top: A pancreatic satellite cell. Bottom: The fine structure of the endoplasmic reticulum.

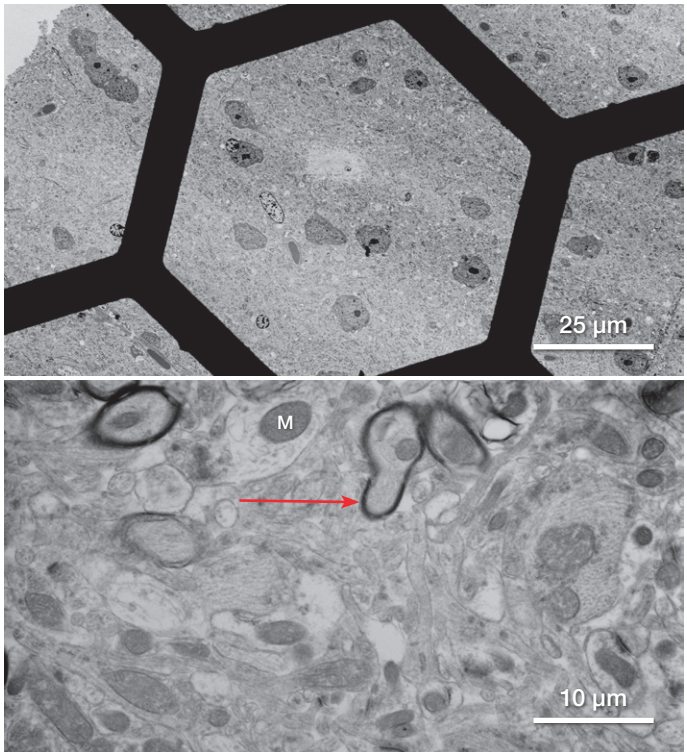

Figure 3. Human brain tumor tissue. Top: The micrographs clearly present valuable ultrastructural features like myelin sheathed axons (arrowhead), mitochondria (M) including the cristae layout, and astrocyte-like cells (arrow) containing prominent cytoplasmic filament bundles and vesicles. Bottom: The relations to other organelles and cellular structures are also observed.

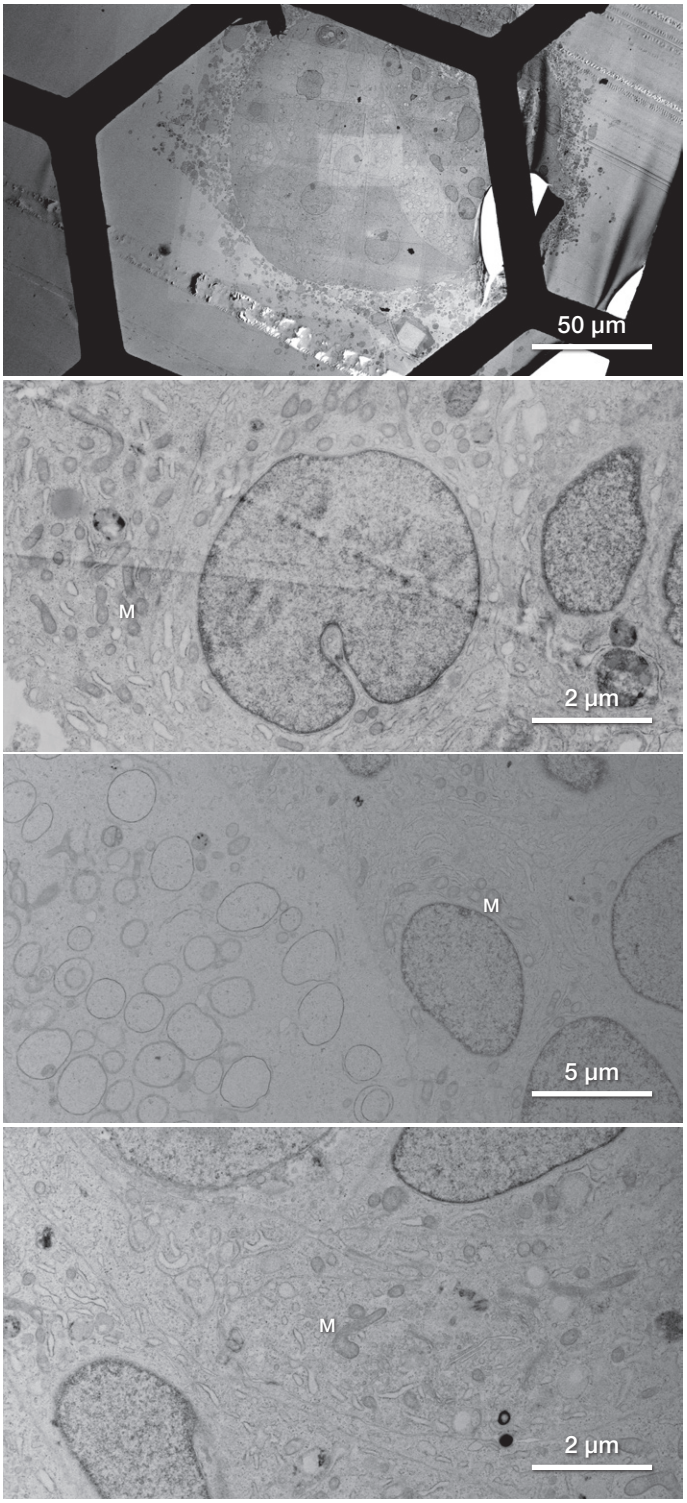

Figure 4. Details of microlens-like structures harvested from pluripotent stem cell cultures. The lens epithelial cells show numerous cytoplasmic organelles such as mitochondria and ovoid nuclei. The uniform cytoplasmic appearance is reminiscent of an early phase of differentiation[2]. Electron microscopy data from micro-lenses derived from ROR1e LECs. The micro-lenses contained LEC-like cells near the periphery of the tissue that were adjacent to immature lens fiber-like cells with rod-shaped nuclei. Cells with circular nuclei reminiscent of lens fiber cells.

Conclusion

Desktop FEG-SEM systems deliver a space-saving form that, when equipped with a low-kV FEG STEM, may be used for fine high-resolution ultrastructural characterization of cell biology. These images can be acquired quickly and easily, are suitable for advanced research, and deliver resolution close to a TEM with a larger field of view.

References

1. Cohen Hyams T; Mam K; Killingsworth MC, 2020, 'Scanning electron microscopy as a new tool for diagnostic pathology and cell biology', Micron, vol. 130, pp. 102797 - 102797, http://dx.doi.org/10.1016/j.micron.2019.102797.
2. C. U., Devi, M. Masona, T. Cohen Hyams, M. C. Killingsworth, D. G. Harmana V. Gnanasambandapillai, L. Liyanage and M. D. O'Connor, 'A simplified method for producing human lens epithelial cells and light-focusing micro-lenses from pluripotent stem cells', Experimental Eye Research (2020) https://doi.org/10.1016/j.exer.2020.108317

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