LVEM in Cell Biology

Background

Cellular biology research has benefited tremendously by the use of electron microscopy (EM). The high magnification power of EM enables visualization of cellular structures smaller than those visible by light microscopy. For example, the intracellular organelles, individual viruses, and macromolecular protein complexes can be imaged with significant detail, allowing ultrastructural determinations. When used in comparative studies, the ability to elucidate healthy vs. diseased states of cells, as well as the role of expression of specific regulator genes on cellular microstructure morphologies allows visual insights that improve our scientific knowledge and enhances the communication of those learnings.

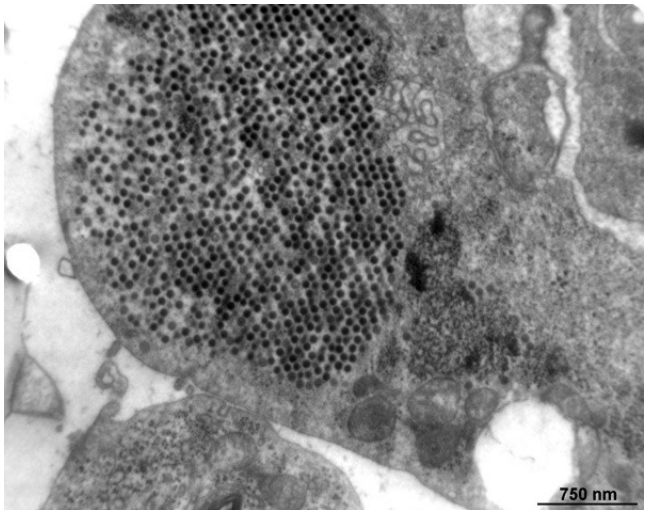
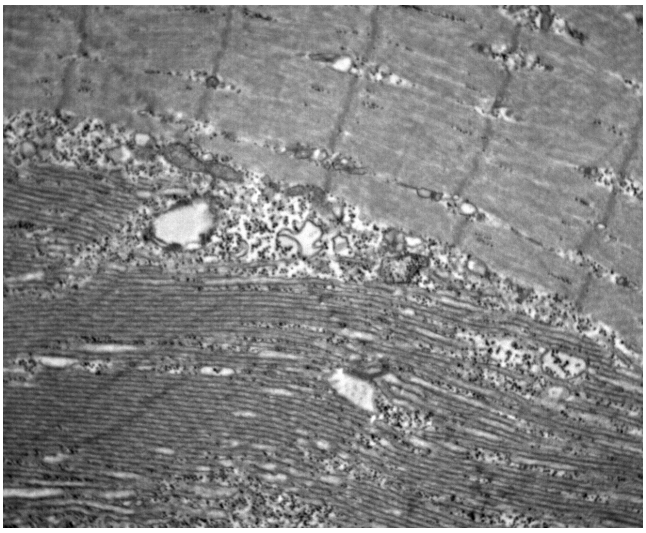


Figure 1. LVEM image of unstained adenovirus.

EM Sample Prep

EM is considered the gold standard technique for characterizing intracellular microstructures. Sample preparation techniques include tissue sectioning and sample fixation procedures that allow preservation of the structure. Collection of the tissue samples in a cacodylate buffer followed by fixation with glutaraldehyde and paraformaldehyde allows samples to be cut into small cubes and dehydrates by a series of alcohol or acetone solutions. The block is then embedded with EM grade epoxy resin, and cut into ultrathin sections before mounting on uncoated 300 or 400 mesh TEM grids.



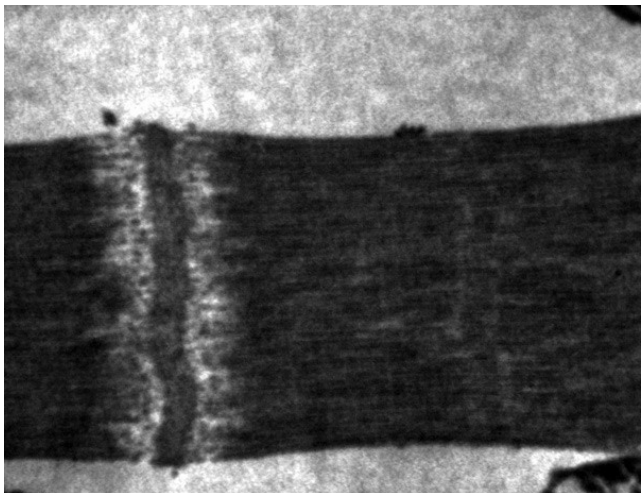


Figure 2. LVEM images of muscle tissue and muscle fibers.

LVEM Sample Prep

LVEM is widely beneficial for cellular biology studies. It has been stated that "low-voltage EM (LVEM) in addition to conventional TEM or SEM provide unprecedented visual evidences to give basic data a whole new dimension." (Chatterjee, 2012)

In general, LVEM provides exceptional contrast for carbon, nitrogen, oxygen and lower atomic number (Z) elements. This impacts LVEM sample prep in two direct ways: 1) negative staining with heavy metals is typically not required, and 2) thinner carbon support films are needed on TEMs. One study found that carbon support films of <10 nm thickness provided better image quality than carbon support films of ~30 nm. (Sintorn, 2013)

LVEM Enables Direct Imaging, No Negative Staining Required

The lower accelerating voltage provides a darker contrast with lower Z elements. This is a strong advantage for carbon-based microstructures found inside of cells. TEM often utilized higher accelerating voltages over 100 kV, necessitating negative staining techniques with heavy metals in order to enhance the contrast of the carbonaceous structures. (Laue, 2016; Chatterjee, 2012) The LVEM provides significantly higher contrast for carbon-based materials. This ability of LVEM to directly image biological structures eliminates the need for negative staining reduced the use of toxic heavy metal reagents such as uranyl acetate or lead acetate.

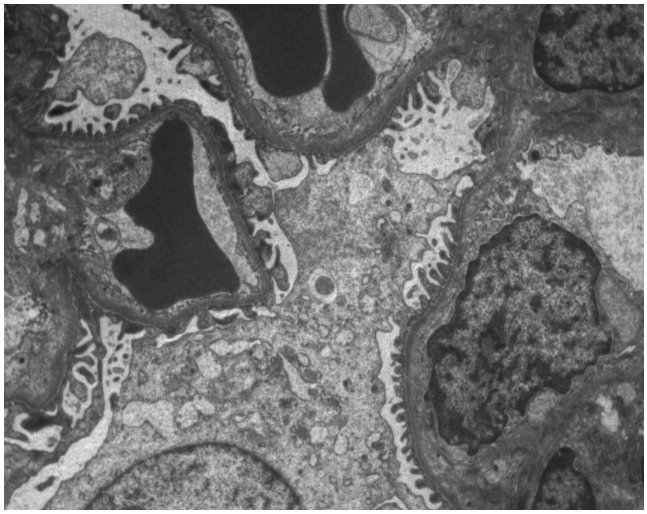


Figure 3. LVEM image of a kidney glomerulus microstructure.

The use of low accelerating voltages for EM provides a number of advantages. For example, the authors of a recent study on the microstructural morphology of coconut and palm kernel shells stated "Low voltage was operated at relatively low electron accelerating voltage between 5 and 25 kV. This was particularly useful to increase image contrast and especially important for our biological samples." (Ntenga, 2019)

Large fields of view are also capable in the SEM mode of an LVEM, enabling the imaging of the morphology of large bacterial multicellular formations grown on supporting surfaces. (Undabarrena, 2017)

Viruses

The imaging of virus structures by electron microscopy has also led to important advances. For example, during the initial phases of research on the SARS-CoV-2 virus, TEM images of the spike proteins provided researchers with critical insights into understanding the virus and designing better candidates for vaccines and therapeutic candidates.

Viruses can be diagnosed and identified by their morphology. Researchers in Florida were able to identify a plant virus that newly arrived to the state in the article "First Report of Frangipani Mosaic Virus Infecting Frangipani (Plumeria spp.) in the USA" (Dey et al, 2020). The LVEM 5 was used to examine the sap from a set of plants exhibiting unknown disease symptoms when initial genetic analysis failed to identify a cause. The rod-like shape and dimensions of the viruses found in the sap led to confirmation with additional genetic analysis.

Immunostaining

In order to identify protein expression locations on intracellular microstructures, antibodies tagged with metal nanoparticles are often used. Typically monoclonal antibodies will provide the most specificity for the target protein epitope, while polyclonal antibodies can recognize more "faces" of the structures and provide more binding events.

LVEM Operational Advantages

It is becoming more widely known that there are several well-established operational and business advantages to LVEM compared to traditional TEM instruments. (Savas, 2019).

- Lower initial cost
- Lower operating cost
- Easier operation
- Easier maintenance
- Smaller laboratory footprint
- No specialized site prep required

The significantly lower initial cost of a new LVEM instrument compared to even a used TEM is

a tremendous advantage, allowing routine access to electron microscopy images when otherwise unobtainable and freeing up larger budgets for other critical tasks.

Additionally, placement of an LVEM is possible in many laboratories, making for much more efficient collection of routine characterization data. LVEM enables electron microscopy to now become a rapid, affordable and easy screening tool, eliminating the need for costly core user facilities.

LVEM Cellular Biology Adjacencies

The LVEM is useful in areas that support cellular biology research as well. For example, when understanding how materials in the local cellular environment will impact the biological functions of the cell, it is important to gather detailed information on the microstructures and chemical compositions of those materials coming into contact with the outer cell walls or materials. For example, this conceptually allows understanding of "what the cell sees in bionanoscience." (Walczyk, 2010) Aside from characterizing drug delivery nanoparticles or other theranostics (materials with diagnostic, therapeutic, and reporting moieties) themselves, capturing their fate and transformations inside of cells provides significant research insights into the development of novel therapies.

Clinical Sample Analysis

Transmission electron microscopy (TEM) is an integral part of the pathologic of many medical specimens, especially renal and cardiac biopsies.

A thorough clinical validation exercise was performed comparing the diagnostic utility of images obtained on a conventional TEM and compact TEM, in the appropriately titled report "Clinical Validation of Low-Voltage "Compact" Transmission Electron Microscopy for Ultrastructural Evaluation of Kidney and Heart Biopsy Samples" (Lawrence, et al., 2020). The Delong Instruments LVEM 25 was selected, and deemed by the authors a new "compact" TEM instrument, which circumvents some of the aforementioned drawbacks posed by conventional TEM. The study's authors concluded that "Compact TEM is a clinically valid means of ultrastructural evaluation of renal and cardiac biopsy specimens," and "Compact TEM maintains comparable ability to discriminate key diagnostic findings in these samples."

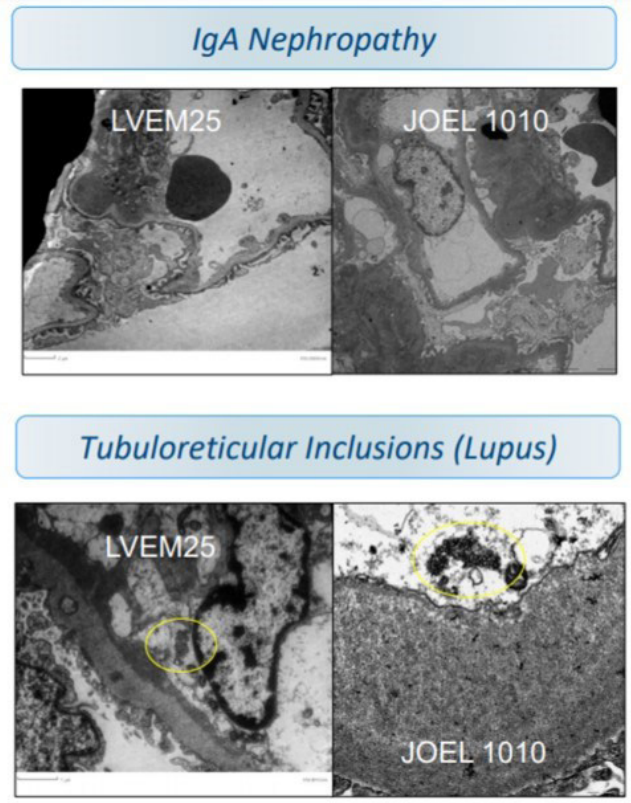


Figure 4. Comparison of LVEM 25 and TEM data used for clinical tissue specimen diagnostics. (Lawrence, et al., 2020)

Conclusion

Electron microscopy is a long-standing and powerful tool for cellular biology research. The LVEM 5 and LVEM 25 offer affordable ways to achieve modern low voltage electron microscopy imaging, without the need for complex negative staining procedures, and with an efficient laboratory footprint and operating budget.

The world's best low voltage electron microscopes, the Delong LVEM 5 and LVEM 25, continue to contribute to many scientific disciplines beyond cell biology, including materials science, higher education, environmental toxicology, and energy research.

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About the author:

Robert I. MacCuspie, Ph.D., has over twenty years of experience in nanotechnology and materials characterization. Career highlights include leading the team that developed the silver nanoparticle reference materials at the National Institute of Standards and Technology, the first faculty and Director of Nanotechnology and Multifunctional Materials Program at Florida Polytechnic University, and over five years of consulting at the business-science interface from MacCuspie Innovations, helping companies commercialize and educate on technologies to improve human health.