LVEM for Pathology

Background

This report will present how LVEM benefits the field of medical pathology. Hospital pathology laboratories have the responsibility to help identify the states of tissue samples collected by doctors in the efforts to understand what the health state of a patient currently is, and thus provide decision-making information for doctors determining a path of care and treatment. Clinical Electron Microscopy (EM) typically uses Transmission Electron Microscopy (TEM) as a tool that enables ultrastructural studies on tissues submitted to pathology labs. The high resolution TEM offers over light microscopy and enables visualization of cellular organelles, intercellular junctions, and extracellular proteins, and can clarify architectural details that aid in the diagnosis of glomerular, neurological, muscular and cutaneous diseases. [1]

Electron Microscopy in Pathology

Pathology employs laboratory examination of samples taken from body tissues for diagnostic or forensic purposes. While it is common for pathologists to use light microscopes and immunohistochemical staining for reasons of lower costs than traditional TEM, it has been stated that "to the pathologist, the transmission electron microscope is like the equivalent of a high-magnification, high-resolution light microscope capable of visualizing small intracellular and extracellular structures in great detail." (Erlandson, 2009). The Pathology Department at the Duke University School of Medicine states that "Clinical EM is a powerful diagnostic tool used to assist in the diagnosis of Kidney Disease, Muscle Disorders, Neurological Disorders, Ciliary Dysfunction, Viral Gastroenteritis, Viral Infections or any disorder that may benefit from the analysis of the fine structures of a biopsy." [2]

Pathology labs also assist in tumor diagnosis. (Ghadially, 2017) Electron microscopy has contributed to the field for over five decades and retains great value for diagnosing peripheral nerve sheath tumors, marker-negative synovial sarcomas, pleomorphic sarcomas, and mesotheliomas; immunohistochemistry has become routinely applied over the last three decades for diagnosing smooth muscle tumors, small round cell tumors, sarcomas with epithelioid morphology, and most synovial sarcomas. (Fisher, 2006) EM remains of particular use when antibodies used for immunohistochemistry face challenges with specificity, such as polyphenotypic tumors or when no specific antigens exist.

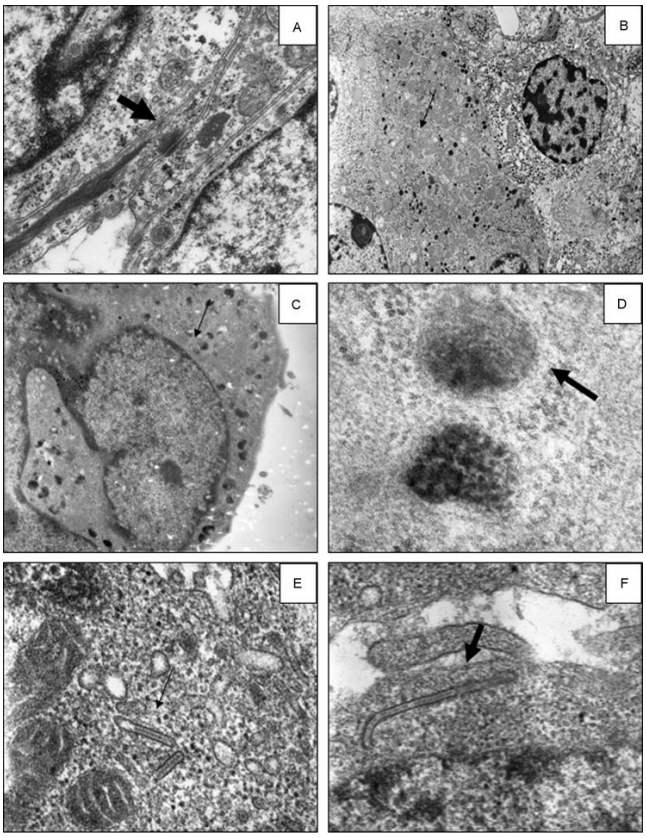


Figure 1. A. Poorly differentiated carcinoma of the thyroid gland in a child. Presence of a desmosome (arrow) and of intermediate filaments (cytokeratine) (arrowhead) (× 4500). B. Poor differentiated neuroendocrine tumour of the larynx. Presence of neurosecretory granules (arrows) (× 1800). C and D. Achromic melanoma of the nasal cavity. Presence of a few melanosoma (arrows) (C × 1800; D × 6700). E and F. Langherans histiocytosis of the thyroid gland. Presence of Birbeck bodies within the cytoplasm (arrows) (E × 3400; F × 7000). Image and figure caption reproduced from (Mari, 2010).

Confirmation and diagnosis of tissue and cellular lesions enables better understanding of the origins of diseases, including emerging infectious diseases. (Mari, 2010) Figures 1 provides examples from the peer reviewed literature of how EM provides clinical evidence of pathologies related to thyroid gland disorders. Figure 2 provides examples of how EM is effective for characterizing and analyzing parasitic infections.

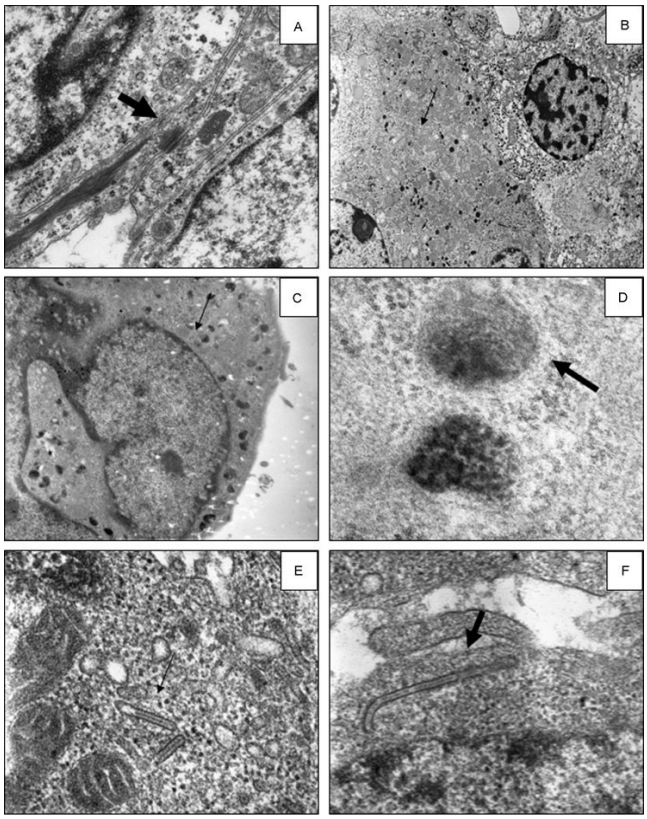


Figure 2. Examples in parasitic infection. A and B. Microsporidium infection (Enterocytozoon bieneusii) showing numerous spores in an intestinal epithelial cell (A × 1400 ; B, × 4500). C and D. Intestinal infection caused by Cryptosporidium parvum (C × 2500; D × 4600). E. Bone marrow infection caused by Toxoplasma gondii. Presence of free trophozoites inside a myelocyte (× 4900). F. Infection of a red blood cell by Plasmodium falciparum (× 2600).

LVEM

For a pathology laboratory manager, there are several well-established operational and business advantages to LVEM compared to traditional TEM instruments to help meet the demands for high quality facilities competing for limited resources, and meet the goals of providing cost-effective approaches.

LVEM Business & Operational Advantages:

- 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. Much as low-cost instruments are ubiquitous in synthesis labs for initial screening characterization, LVEM enables electron microscopy to now become a rapid, affordable and easy microscopy tool, eliminating the need for costly core user facilities often found only at major research universities.

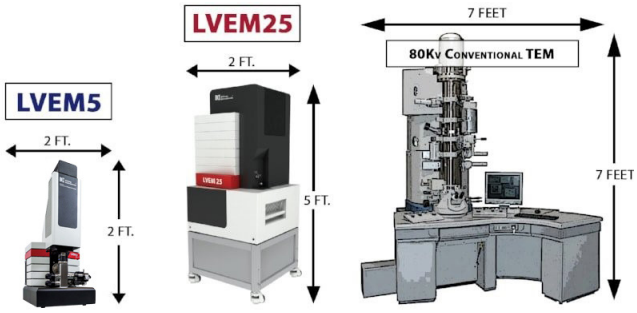


Figure 1. Comparison of the significantly smaller footprint of LVEM instruments vs. traditional TEM instruments

LVEM 25 Enhanced Contrast

The LVEM offers enhanced contrast of biological samples compared to traditional TEM, directly on the as-prepared samples. Figure 4 provides direct comparisons of tissue samples imaged by a conventional TEM and a LVEM 25on heart and kidney biopsy samples. "The electron microscope is invaluable for resolving the constituents of the glomerulus, including the glomerular basement membrane." (Erlandsen, 2009). Figure 5 also reveals the LVEM 25 provides excellent contrast for ultrastructural examination. [3]

Clinical Validation of LVEM 25

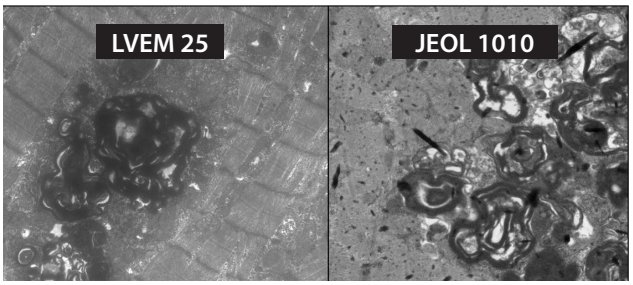
Recently, a thorough clinical validation exercise was performed comparing the diagnostic utility of images obtained on a conventional TEM and compact TEM, in the appropriately titled report "Clinical Validation of Low-Voltage "Compact" Transmission Electron Microscopy for Ultrastructural Evaluation of Kidney and Heart Biopsy Samples" (Lawrence, et al., 2020).

The study design included 90 consecutive clinical biopsies and 10 pre-selected biopsies which were known to have certain ultrastructural features of interest. A parallel testing validation strategy was employed, where three different pathologists captured images in the course of their routine diagnostic work. The data

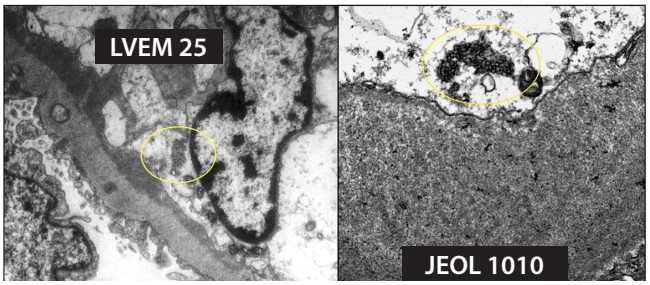
and images were retrospectively compiled, reviewed, and compared for salient ultrastructural features, and are summarized in Figure 4 and Tables 1 and 2. The instruments directly compared samples using a JEOL 1010 TEM at 80 kEV with a Veleta side-mount 4MP camera, and a Delong Instruments LVEM 25 TEM at 25 kEV using its integrated 5.5MP camera. Samples were prepared for TEM using the routine protocols. Side by side comparison results of TEM images obtained by each instrument are shown in Figure 4 and 5. Lawrence and co-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."

Figures: Representative Comparative Case Images

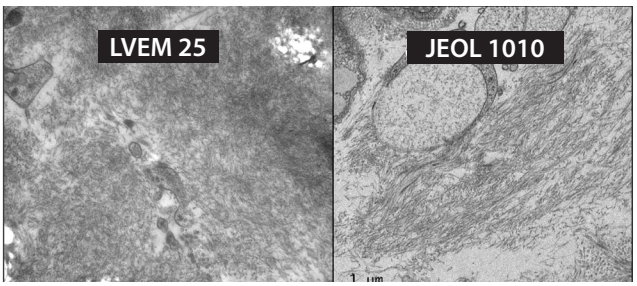
Hydroxychloroquine toxicity



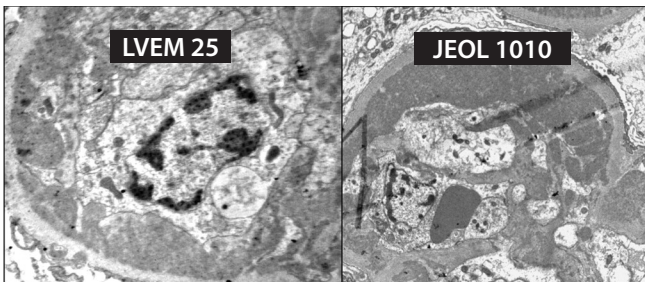
Tubuloreticular Inclusions (Lupus)



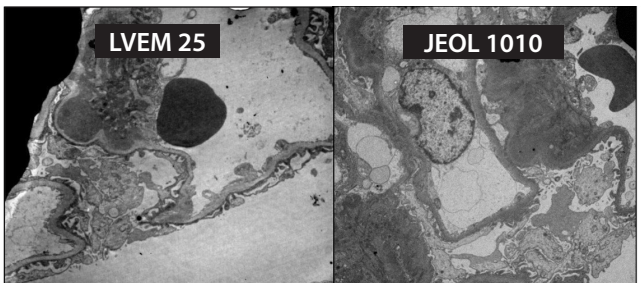
Amyloid



Subendothelial Deposits



IgA Neuropathy



Normal Myocardium

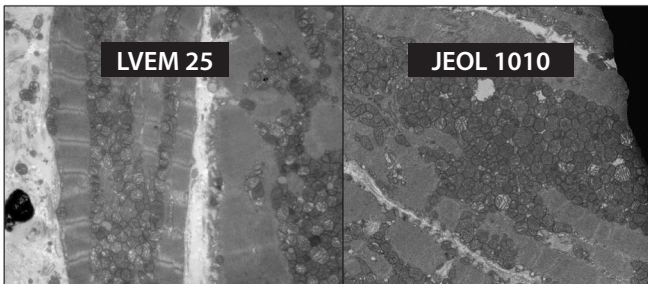


Figure 4. Side by side comparisons of conventional TEM and LVEM 25 images for clinical biopsies of native kidney, transplant kidney, and heart biopsy samples. (Lawrence, 2020)

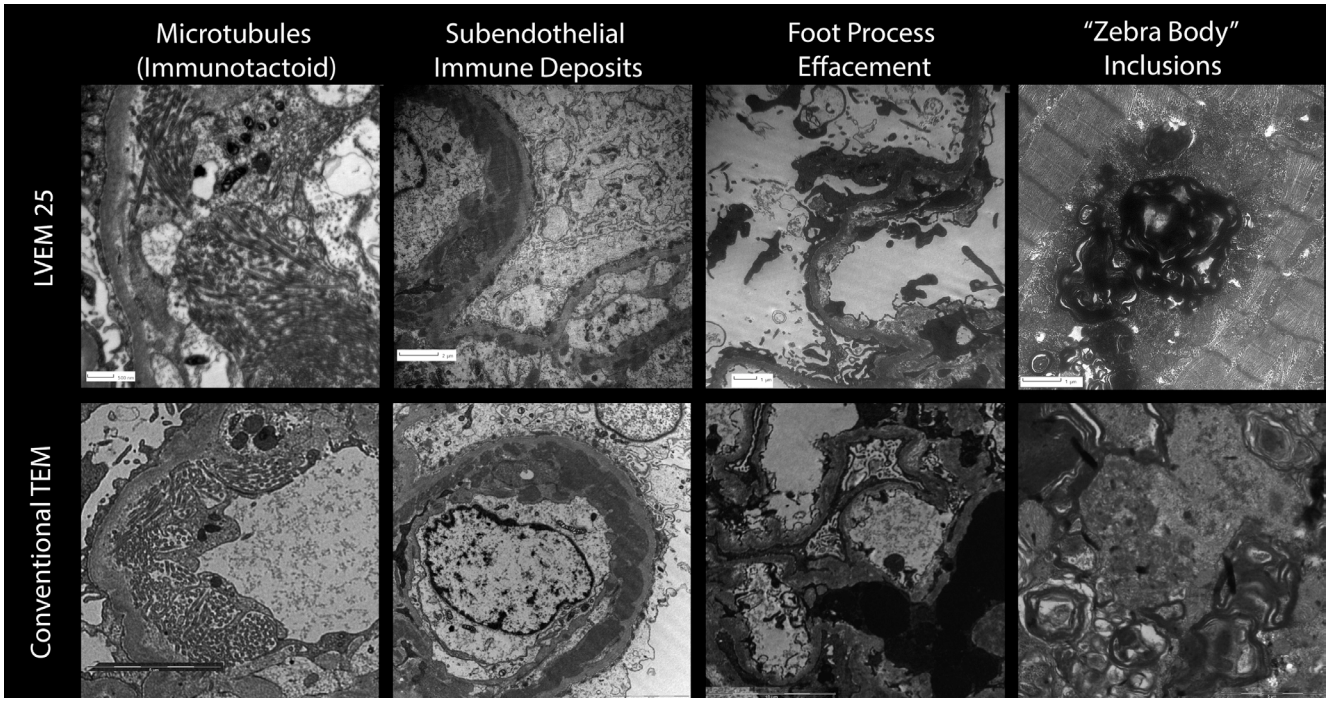


Figure 5. Side by side comparison of conventional TEM and compact LVEM images for pathology analysis. [3]

Table 1. Examples of Morphology Characterization Techniques

MICROSCOPIC DISEASE PROCESS	n=
Mesangial Immune Deposits	26
Subepithelial Immune Deposits	13
Subendothelial Immune Deposits	9
Tubuloreticular Inclusions	1
Thickened Glomerular Basement Membranes	16
Thin Glomerular Basement Membranes	3
Podoctye Foot Process Effacement	15
Fibrillary Deposits	4
Organized Substructure	2

Table 2. Cases by Disease Process

DIAGNOSIS	n=
AIN	8
Amyloid	4
ANCA GN	4
ATN	5
Cast Nephropathy	1
Chloroquine cardiotoxicity	1
Chronic TMA	1
Cortical necrosis	1
Diabetes	14
DM + immune complex	1
FSGS	9
HTN	7
IDCM	2
Idiopathic nodular sclerosis	1
IgA	9
IgA + Diabetes	1
IgG-K MPGN w crescents (same pt)	2
Immune complex GN in transplant	1
Immunotactoid (same pt)	2
Lupus	9
Membranous	1
Minimal change disease	4
Minimal change + IgA	1
Myocarditis	2
Negative heart biopsy	3
Postinfectious GN	2
Recurrent IgA in transplant	1
Thin GBM	2
Transplant TCMR	1

Conclusion

Electron microscopy "is still an essential tool for the surgical pathologist." (Mari, 2010). LVEM is an enabling technology for the widespread deployment of EM for pathology. Compared to traditional high voltage TEM, LVEM offers benefits including lower costs, easier operation, and rapid results. Clinical validation of pathology samples has demonstrated the LVEM 25 is strongly suited as a compact and affordable TEM option for pathology laboratories.

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

References:

[1] https://www.pathology.med.umich.edu/anatomic-pathology/electron-microscopy accessed 2/11/21.

[2] https://pathology.duke.edu/patient-care/anatomic-pathology/specialty-laboratories/electron-microscopy-diagnostic-viral accessed 2/11/21.

[3] https://www.lv-em.com/clinical-validation-of-lvem-for-kidney-and-heart-biopsy-samples accessed 2/22/21.

Erlandson RA. Role of electron microscopy in modern diagnostic surgical pathology. Modern Surgical Pathology. 2009:71.

Fisher C. The comparative roles of electron microscopy and immunohistochemistry in the diagnosis of soft tissue tumours. Histopathology. 2006 Jan;48(1):32–41.

Ghadially FN. Diagnostic electron microscopy of tumours. Butterworth-Heinemann; 2017 May 17.

Lawrence R, Isaac J, Lloyd I, Miller D. Clinical Validation of Low-Voltage" Compact" Transmission Electron Microscopy for Ultrastructural Evaluation of Kidney and Heart Biopsy Samples. In LABORATORY INVESTIGATION 2020 Mar 1 (Vol. 100, No. SUPPL 1, pp. 1701–1702). 75 Varick St, 9th Flr, New York, NY 10013–1917 USA: Nature Publishing Group.

Mari M, Hofman V, Butori C, Ilie M, Lassalle S, Grier P, Sadoulet D, Scoazec JY, Hofman P. What is new in 2010 for electron microscopy in surgical pathology? Annales de pathologie. 2010 Jul 31 (Vol. 30, No. 4, pp. 263–272).

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.