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Biological electron microscopy: Workshop report

Subhash C Yadav* & TC Nag

Department of Anatomy, All India Institute of Medical Sciences (AIIMS), New Delhi-110 029, Delhi, India

Research in morphology, cell physiology, chemical biology, and medicine aims for a thorough understanding of cellular processes that are operative at the level of the cell organelles. These are evaluated by understanding the ultrastructural changes under the diseased conditions. For example, to understand whether a biological tissue has suffered from metabolic insult *via* intoxication or exposure to xenobiotics, a physiological stress response is triggered immediately to counteract the adverse effects, which often results in a proliferation of the smooth endoplasmic reticulum. Biological Electron Microscopy is a stand-alone high-resolution imaging technique that can assess the status of this affair inside a cell and its potential for detoxification in a given time-scale. However, the imaging of such cellular event under an electron microscope is not so easy as it appears in the first instance, rather it is a very complex and tedious job that require several steps in tissue processing before they can be imaged under electron microscopes. Biological samples need to be processed in such a way that they must be compatible with either transmission- or scanning electron microscopes. In this 12-day workshop, which was held on November 26 to December 7, 2019, the selected 23 participants (the majority at the mid-level of their Ph.D. career) from all over India experienced hands-on training and lectures on biological electron microscopy in each day by eminent biological and biomedical electron microscopists that spanned from aspects of sample preparation and sectioning to imaging and interpretation of the images.

The workshop was inaugurated in the presence of Prof. TS Roy, Head, Department of Anatomy, Prof. TC Nag (Co-ordinator, Sophisticated Analytical Instrumentation Facility [SAIF], New Delhi) and representatives from the Department of Science and Technology (Govt. of India), who steer the Country's SAIF Program in advance research. Prof. Roy introduced the individual technical staff involved in the hands-on training with the participants. The participants introduced themselves with a short description of their research objectives and the requirement of electron microscopy in their work.

The basic knowledge of transmission electron microscopes with a note on developmental history, theory, and principles, anatomical detail of the equipment, and the possible applications was delivered by Dr. Subhash C. Yadav, AIIMS, New Delhi. He discussed the entire instrumentation to make all participants familiar with the basics of transmission electron microscopes. Later, on the same day, Dr. TC Nag delivered a lecture on the techniques of biological specimen preparation for transmission electron microscopy (TEM). He has given the detailed procedure and principle of primary fixation, secondary fixation, dehydration, infiltration, embedding, and block preparation. He discussed the importance of each step to make the biological samples compatible to TEM by preserving the native structure and strength (fixation), the gradual replacement of water molecule to improve the hydrophobicity (dehydration), the gradual replacement of dehydrants with a resin containing medium (infiltration) and plastic resin block preparation for holding the tissue during ultra-sectioning. In the second half of the day, the participants learned the method of preparation of buffers and preservatives, after which they have demonstrated the method of animal tissue fixation, by vascular perfusion of a euthanised rat with fixative, sampling of different organs and micro-slicing for further fixation, processing and visualisation under transmission- and scanning electron microscopy. Simultaneously, there were technical sessions for fixation and processing of plant tissues, cultured cells, and bacteria under the assistance of the technical officers Mr. Sandeep Arya, Anuraag Singh, Pardeep Vaishnav, and Madan Mohan Sharma.

*Correspondence:
Phone: +91-9868126177 (Mob)
E-mail: subhashmbu@aiims.gov.in

On the second day, the participants learned the methods of secondary tissue fixation for lipids in osmium tetroxide and dehydration of their respective samples. Professor Nag presented a detailed methodology lecture for section cutting using ultramicrotomes (ultramicrotomy), which included the making of glass knives and knife-boats, thick sectioning for light microscopy and staining, ultrathin sectioning and staining using uranyl acetate and lead citrate. These are the important aspect of converting biological samples compatible to TEM so that it can sustain the vacuum condition, sample stability under high energy electron beam (plastic resin), the transmission of the electron through samples (thin sectioning), and contrast of electron micrograph (staining).

Specimen preparation for scanning electron microscopy is entirely different from that of transmission electron microscopy. The detail about the principle and working of scanning electron microscopy was discussed by Dr. Subhash in the workshop. He presented the working flow-chart of scanning electron microscopy and the requirement of different strategies for sample preparation. He discussed the detailed methodology for sample preparation such as air drying, critical point drying, drying in specialised chemicals where critical point drying is unfavourable, freeze-drying, and sputter coating with examples of their application in various facets of biological research. Under hands-on training, the participants learned the steps in the making of glass knives and trimming of resin blocks and methods of critical point drying and sputter coating for scanning electron microscopy, which was demonstrated by Mr. Madan Mohan Sharma and Raj G Mishra, under the supervision of Dr. Subhash.

In the next day, the participants explored the complete steps of infiltration and embedding in resins of their own processed samples for TEM under the assistance of Mr. Raj G Mishra. After this, there was a demonstration of sectioning by Technical officers Mrs. Chanda Pawar and Mr. Meharban Singh under the supervision of Prof. Nag. The method of selection of best quality sections by seeing colour interference from floated sections was shown. The participants invested a lot of their energy on learning the art of cutting of ultrathin sections (60-70 nm thick) and their retrieval on copper grids and by the end of a week, they were able to cut good quality ultrathin sections during training. They also learned the method of coating of grids with formvar, generally used for adsorption of particulate objects on them.

The contrast and resolution of various organelles can be achieved by proper staining of ultrathin sections. A lecture was presented by Prof. Nag, covering various aspects of staining for biological tissues that show wide variations in structural and chemical compositions. The participants were taught about the preparation of uranyl acetate and lead citrate and double staining method of tissue sections. They also learned the method of negative staining of particulate objects (bacteria, virus and fungi) mounted on formvar-coated grids, using uranyl acetate and phosphotungstic acid. Before the participants attempted to viewing their stained sections, they were taught about the structure of various organelles in plant and animal tissues, their shape and size, their features in normal and pathological conditions, and overall, the basic plan of where to start and what are to be ideally observed in a section under a transmission electron microscope.

The transmission electron microscope is extremely useful for understanding the different aspects of renal and muscular pathology and dermatological lesions. A dedicated lecture by Prof. AK Dinda, Department of Pathology, AIIMS, New Delhi explained the identity of abnormal cellular features in renal and muscular diseases and in certain neoplasms, where electron microscopy is considered a gold standard for accurate diagnosis and staging of the disorders.

Prof. Shashi Wadhwa, Ex-Dean, Academic Affairs, and Ex-Officer-In-Charge, SAIF-New Delhi (AIIMS) delivered two lectures on the application of electron microscopy in neural tissues and stereology, *i.e.*, understanding the 3D-aspects of organelles from serial two-dimensional digitized images and the use and operation of dedicated software for it. Mr. Sandeep Arya, the Senior Technical Officer, showed the steps of viewing under a transmission electron microscope, image acquisition, and interpretation of the images.

Apart from the ultrastructure imaging, TEM, and SEM can be used for the qualitative and semi-quantitative detection of the various elements in the samples. Dr. AK Jain from the ICMR National Institute of Pathology, Safdarjung Hospital, New Delhi discussed in detail the principle and methodology of the elemental detection and their localization in biological specimens using transmission electron microscopy. The detailed steps in the viewing of ultrathin stained sections under a transmission electron microscope and gold-coated bulk samples under a scanning electron microscope were demonstrated. Mr. Sandeep Arya, the Senior Technical Officer,

showed the steps of initialisation of a transmission electron microscope, gun alignment, beam centering and saturation, image acquisition, and interpretation of the images. Mr. Madan Mohan Sharma explained the working principle of a scanning electron microscope, method of acquisition of SEM images, and detection of elements in biological samples using SEM-EDS. Once the participants mastered the basics in microscope operation, they were confident to operate the microscopes without assistance from others.

Under advanced techniques, Dr. Tony G Jacob (Department of Anatomy, AIIMS, New Delhi) delivered a lecture on immunogold electron microscopy (IEM), which is used for the detection of cellular antigens under electron microscopes. The participants learned the specialised methods of tissue fixation for IEM and their embedment in acrylic resins (*e.g.*, LR White resin) and polymerisation. After sections were cut, they performed antibody labelling in those sections (a method called post-embedding IEM), using colloidal gold as a tracer. The participants localised the distribution of glutamine synthetase in neural tissue by this method and additionally, they localised few surface markers of pathogenic bacteria (*Mycobacterium tuberculosis*). Because IEM is not always promising, an alternative method of labelling in ultrathin cryosections (which avoids routine long-term fixation and treatment in dehydrants and polymerisation) was taught in a lecture by Prof. Nag. Cryo-ultramicrotomy, a specialised technique that aims to cut ultrathin frozen sections using the ultramicrotome, was demonstrated to the participants by Ms. Chanda Panwar, Pardeep Vaishnav and Anuraag Singh.

Cryo-electron microscopy, especially for single particle analysis, is an emerging field for the elucidation of complex protein structure. A specialised lecture on cryo-electron microscopy to examine the three-dimensional organisation of virus and protein particles was delivered by Dr. Manidipa Banerjee from the Indian Institute of Technology, New Delhi. She delivered a detailed lecture on the basic principle of cryo-electron microscopy, workflow for sample preparation and imaging in low temperature, and detailed elucidation of an intact virus structure. A demonstration of a Cryo-transmission electron microscope and cryo-grid preparation using a plunge freezing (Vitrobot) system, *i.e.*, liquid ethane preparation, grid loading, etc. was given by Ms. Shikha Chaudhary.

Dr. Yadav delivered a lecture on introductory nanobiology and their application in diagnostics and nanomedicine, preparation of drug-loaded nanoparticles, and characterization of these particulate systems using electron microscopy. He and his group member Ms. Srishty Raman has also shown the preparation of fluorescent quantum dots, their functionalization, and hydrophilicity using the indigenous method of synthesis.

Towards the end of the the workshop, the participants examined and judged the quality of sections cut by them under electron microscopes and remedial measures where there was evidence of errors in their processing of samples by a step-by-step analysis. A lecture on artifacts in electron micrographs by Prof. Nag addressed about how to overcome the various issues with fixative osmolarity, staining, and sectioning, which often result in the appearance of artifacts in biological tissues (*e.g.*, mitochondrial swelling due to hypotonicity and shrinkage of nucleoplasm due to hypertonicity). A written test was conducted to see how far the participants learned about the theory and practical aspects of electron microscopy. The results were unexpectedly praiseworthy.

In the valedictory the session, the participants expressed their gratitude to the staffs of SAIF in making the teaching-learning exercise a complete success. The workshop ended with a note to the participants to explore opportunities in practicing ultrastructural research, besides other facets of research they are interested.