Wide-area correlative light and electron microscopy (CLEM) applied to routinely prepared human pathology samples

Killingsworth, M.[1,2,3,4], Cohen Hyams, T.[2,3] and Ng, Y.F.[5,6]

[1] NSW Health Pathology, Australia, [2] Ingham Institute Correlative Microscopy Facility, Australia, [3] University of NSW, Australia, [4] Western Sydney University, Australia, [5] Singapore General Hospital, Singapore, Australia, [6] Western Sydney University, Australia

Correlative light and electron microscopy (CLEM) is a powerful approach for obtaining structural and functional information from single cell or tissue samples at nanometre resolution. The technique holds great promise for application in pathology to obtain maximum data from a single sample. At present it is common practice to split samples and route different portions to separate benches with a resultant loss of correlation. Added to this is the possibility of gaining valuable research insights from rare clinical cases through retrospective studies of archival tissue. However, room temperature CLEM requires the use of heavy metal enhanced, plastic embedded electron microscopy (EM) material and archival EM tissue has traditionally been considered suboptimal for immunocytochemistry. Fortunately advances in antigen retrieval techniques, the use of nanoparticle labelling for fluorescence overlay data and wide-area field emission scanning electron microscopy (FESEM) for ultrastructural imaging have opened up this approach for use with a larger range of antibodies and have provided more contextual correlation.

The work to be presented will illustrate several studies where CLEM data has been obtained from routinely prepared human pathology tissue. We have successfully immunolabelled tissue and cell samples, in some cases up to 20 years old. Primary antibodies used were polyclonal with detection based on a biotinylated secondary antibody followed by streptavidin conjugated quantum dots, either green 525 nm or red 655 nm (Invitrogen, USA). To demonstrate the technique we have focused on amylase localisation in pancreatic acinar cells and Dapi nucleic acid staining to highlight steps taken in the method development process.

In all the cases presented the tissue was routinely fixed in 2.5% glutaraldehyde in sodium cacodylate buffer pH 7.4 and processed into Spurr low viscosity epoxy resin. En bloc staining with 2% osmium tetroxide and 2% uranyl acetate was carried out. Ultrathin sections 90 nm thick, or semithin sections 300 nm thick, were immunolabelled for TEM or FESEM respectively. TEM sections were mounted on nickel grids, incubated over reagent droplets and viewed at either 80 kV in a 268D Morgagni TEM (Thermo Scientific, The Netherlands) or 30 kV in aSTEM HAADF mode in a GeminiSEM 300 FESEM (Carl Zeiss, Germany). Sections for FESEM imaging were mounted on Superfrost Plus coated glass slides (Thermo Scientific, Germany), immunolabelled with droplets over the sections, carbon coated and viewed at 7 kV using the backscattered detector of a GeminiSEM 300 FESEM (Carl Zeiss, Germany) with contrast inversion to obtain "TEM-like" images. Wide area FESEM background imaging and overlay of fluorescence data was carried out using an Atlas 5.1 scan generator system integrated with the GeminiSEM 300 FESEM. Fluorescence light microscopy images were acquired from quantum dot (Invitrogen, USA) nanoparticle immunolabelled sections at x20 and x40 using and AxioScopeA.1 microscope fitted with an appropriate filter set (02) and an Axiocam 506 color camera (Carl Zeiss, Germany). These images were then overlaid and pinned to FESEM tiled images of the corresponding field of view. Resolution of up to 2 nm could be obtained from the FESEM images with ultrastructural features correlated to fluorescence data.