DOI: 10.1017/S1431927605501338
Microsc Microanal 11(Suppl 2), 2005

"Wien-type" Filtered Secondary Electron Imaging of Immunocytochemically Labeled Methacrylate Sections of Tissues in a Field Emission Scanning Electron Microscope (FESEM)

C.A. Ackerley*, A. Tilups* and C. Nielsen**

*Division of Pathology, Hospital for Sick Children, Toronto, ON
**JEOL USA, Peabody, MA

Most immunocytochemical studies rely on labeling using specific antibodies against molecules on tissue sections which are visualized by species specific secondary antibodies bound to an easily detected tag such as peroxidase or gold. Although large fields can be examined using light microscopy (LM), this approach is limited by resolution. Subcellular localization is usually done using transmission electron microscopy (TEM). The complexity associated with thin sectioning and the small size of the samples are limiting factors which have the potential to lead to interpretive biases. Alternatively, resin embedded immunolabeled sections can be viewed using either backscatter electron imaging (BEI) or using a "wien-type" filter in a FESEM at resolutions similar to those obtained in the TEM with ultrathin sections [1,2,3]. This filter consists of a pre aperture control lens anode in a semi-in lens FESEM with an external power supply. Atomic contrast is imaged by applying a negative voltage to the anode. Using immunolabeled methacrylate resins, sections as large as $1cm^2$ and as thin as 2μm can be cut and examined using both light microscopy and either BEI or "wien-type" filtering at near TEM resolutions.

All animals were perfused with a solution of 4% formaldehyde containing 0.1% glutaraldehyde in phosphate buffer and the tissues chopped into pieces approximately 1 x 1x 0.2 cm. Human biopsy specimens were immersion fixed in the same fixative. Following overnight fixation the samples were washed in buffer, dehydrated through a graded series of ethanol to 100% at room temperature and infiltrated and embedded at 4^oC. Tissues were allowed to polymerize at room temperature in embedding molds. Blocks were sectioned on a Sorval JB-4 microtome at a thickness of 2 μm and mounted on glass coverslips. Sections were then incubated with the primary antibody for 1 hour, rinsed thoroughly with PBS containing BSA and then incubated with either a species specific antibody tagged with peroxidase or 10nm colloidal gold. Sections treated with the peroxidase tagged antibodies were reacted a solution containing 25mg of 3,3´-diaminobenzidine tetrahydrochloride (DAB) and 0.1% H_2O_2. After the DAB treatment the sections were treated with 2% OsO_4. The sections treated with the 10nm gold tagged antibodies were either viewed untreated or enhanced with silver. Both peroxidase and silver enhanced gold stained samples were counterstained with haemotoxylin and mounted on glass slides in an aqueous medium for examination under the light microscope prior to examination in the FESEM. Coverslips were removed, washed thoroughly and stained with uranyl acetate and lead citrate along with the gold labeled samples that had not been silver enhanced. Prior to examination in the FESEM samples were rendered conductive with a thin coat of evaporated carbon.

LM of both immunoperoxidase and silver enhanced immunogold labeled methacrylate sections yielded superior results to conventional paraffin embedded material (fig.A,B). Highly specific reaction product was easily discriminated under the LM but the resolution was limited. Immunogold, silver enhanced immunogold and immunoperoxidase staining produced sufficient atomic contrast for

both BEI and “wien-type” filtering. As previously reported, BEI of these specimens requires sufficient beam current to induce extensive electron beam damage [3]. Using BEI ultrastructural detail was somewhat compromised due to atomic contrast generated within the section but immunostaining was easily detected. The ability to do LM prior to examination in the FESEM is possible with immunoperoxidase or silver enhanced immunogold labeled samples (fig.A,B). Filtered images yielded superior ultrastructure and detection of immunocytochemical reaction product (fig.C,D).

Unobscurred low magnification imaging of fields of large immunostained sections in combination with the high resolution of the FESEM filter lends itself well for studies where there is heterogeneous expression throughout the tissue of the material being studied . In this situation, conventional TEM studies are hit and miss and often require multiple preparations. Furthermore this approach will be very useful in correlative studies with the LM.

References

[1] M.D. Mckee et al., J Bone Miner Res 6 (9) (1991) 937.
[2] A. Nanci et al., J Histochem Cytochem 38 (3) (1990) 403.
[3] C.A. Ackerley et al., Microsc Microanal 9 (Suppl 2) (2003) 1196CD
[4] This work was partially supported by an NIH SCOR grant to C.A.A.

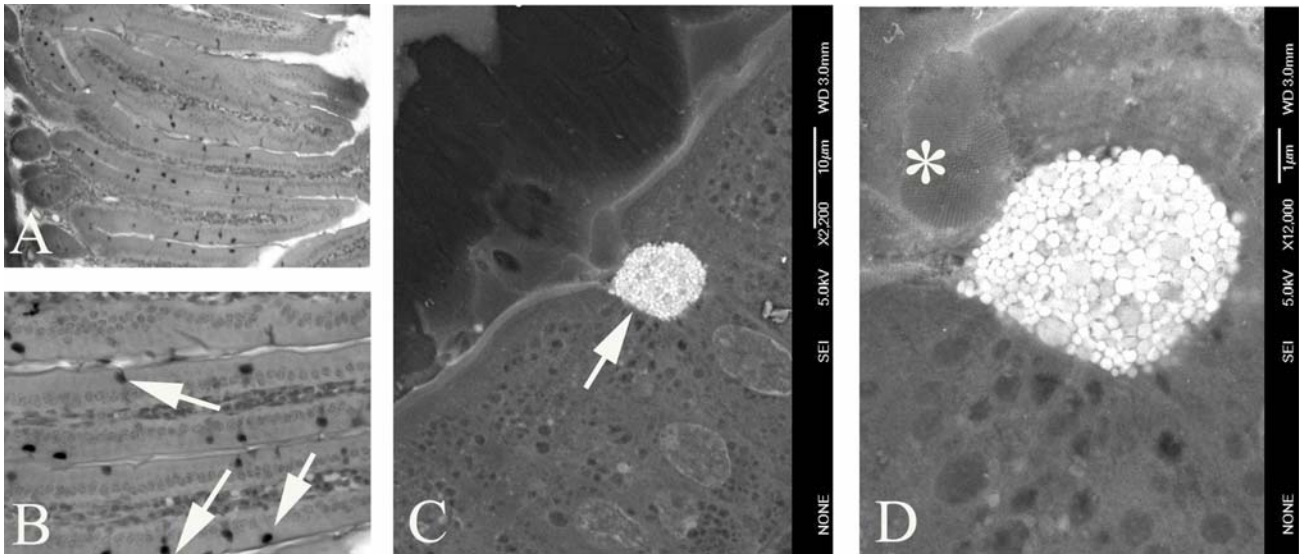

Figure legend

A. Low magnification of a human gut biopsy immunoperoxidase stained with an antibody against the intestinal mucin (MUC3). Picture width is 0.4mm.
B. Higher magnification of A. Note the numerous goblet cells intensely stained with MUC3 (arrows). Picture width is 0.25mm.
C. Low magnification image of the same sample in A and B imaged using the “wien-type” filter in the FESEM. A MUC3 positive goblet cell is seen (arrows). Bar equals 10µm.
D. Higher power of C. Note the intensely stained secretory granules in the cytoplasm of the goblet cell. A field of microvilli cut in cross section is seen in the field of view (asterisk). Bar equals 1µm.

https://doi.org/10.1017/S1431927605501338 Published online by Cambridge University Press