

Chromophore Mapping via Low-Energy Loss Imaging in a Modified EM902: Formation of the Immune Synapse

C.W. Hong, A.B. Fernandes, J.A. Davis, J.M. Penninger and F. P. Ottensmeyer

Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, 610 University Ave., Toronto, Ontario, M5G 2M9, Canada

We have recently modified our Zeiss EM902 energy filter transmission electron microscope by designing, building, and installing an improved electron energy filter that permits an energy resolution of 1 eV (10 mradian acceptance angle) over the entire normal photo-format of the microscope [1,2,3]. Such an energy filter should make it possible to select electrons that in traversing the specimen have lost energies of only a few electron volts, corresponding to absorption energies of visible colours or UV wavelengths. If the specimen can withstand the electron dose to produce an image before the corresponding molecule is damaged (fading of the signal) then this approach is in some respect the electron optical equivalent of light microscopy of colours, but potentially with electron optical spatial resolution. We have tentatively called the technique HIMEX, for High resolution Imaging using Molecular orbital EXcitations.

Early control experiments using the red colour of hematin, the blue of Alcian Blue, the orange of Mercury Orange, the red of chlorin e6, and the blue-green absorption of FITC, indicated that all of these chromophores survived the electron bombardment sufficiently long to obtain electron micrographs before fading of the signal [3]. Using the sharp edges of embedded and sectioned crystals of hematin a measure of 16 Å was obtained for the spatial resolution in the image at an energy loss of about 3 eV [2].

Each of these trials involved only a single colour, or orbital excitation, that was used to produce the image. In principle with a 1 eV spectrometer it should be possible to image two colours sequentially, e.g. one for absorption corresponding to a loss of 1.5 eV (red region) and one at about 3 eV (blue region). However, a voltage ripple of about 1.5 eV in the energy loss spectrum precluded such attempts. After fruitless investigations of the prism power supply, bucking of magnetic fields from nearby building circuits, examination of potential ground loops, etc., the cause was found to be principally in the ripple of a low voltage power supply to the high tension of the microscope. This has now been corrected (Fig. 1). Thus two-colour imaging is now worth attempting.

As a more general approach to the use of colour in identifying biological structures and processes we have begun investigating the application of green fluorescent protein (GFP) as a general and as a specific marker within sections of cells. Experimental success has been obtained with single GFP chromophores both as a general cytoplasmic marker freely diffusing in HepG2, a hepatocyte cell line, and as a GFP fusion protein linked to protein HBx of hepatitis virus B in compartments in transfected Chang liver cells [4]. Both cell types were embedded in Durcupan.

As an extension of this approach, experiments are underway to fuse two GFPs of sufficiently separated absorption wavelengths to different proteins participating in the formation of the immune synapse (Fig. 2, [5]). It is expected that the application of HIMEX to this important aspect of T-cell

activation will do much to elucidate the relationship of proteins in the signalling pathways involved in the immune response.

References

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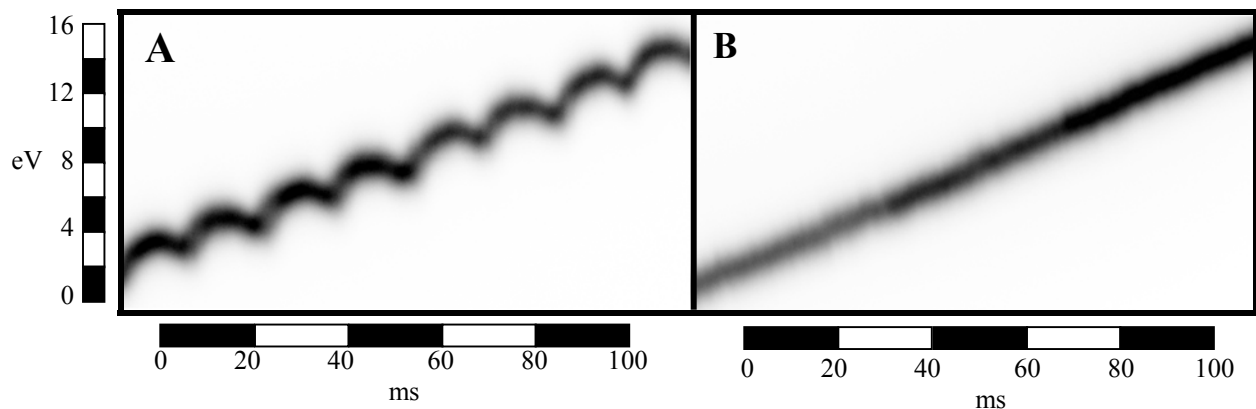


FIG. 1 Comparison of voltage stability measured with the imaging electron spectrometer before (A) and after correction (B). The horizontal scale represents time in milliseconds (ms) and the vertical scale represents energy in electron volts (eV) measured along the trace and at right angles to it, respectively.

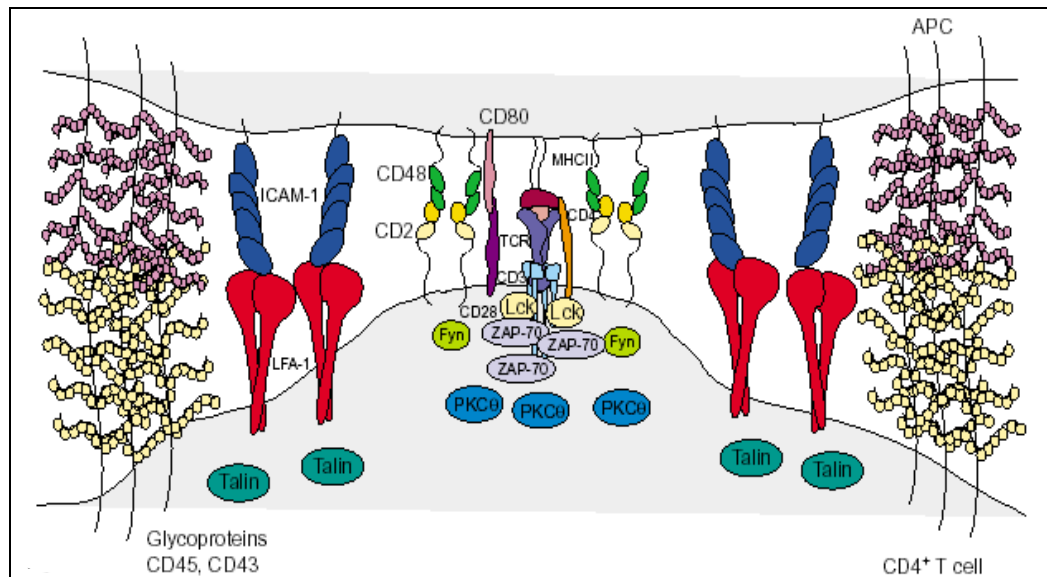


FIG. 2 Schematic diagram of the Immune Synapse.