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Correlating Microscopies From Differing Imaging Modalities: From Experimental Design to Alignment and Overlay of Images.

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Correlated imaging is the process of imaging a single specimen with two (or more) complementary modalities, then registering and overlaying the images to create a composite view. The higher resolution image is colorized using the lower resolution image; for example micro-CT images may be used to colorize LM images [1]; laser scanning confocal (LSC) images may be use to colorize TEM images [2,3]. The lower resolution image is made somewhat transparent, allowing detail in the underlying image to be visible and assisting in the registration of the two images. If correlating images collected by TEM and LSC, the resulting composite image would demonstrate specific ultrastructural features in the high-resolution TEM field colorized by the aligned confocal image. Automated image registration may be facilitated by a variety of sophisticated computer programs and hardware platforms (ie. FEI Corrsight) which are utilized by high-throughput laboratories. This abstract is meant for the more occasional user wishing to align images manually. FIJI is a public domain image processing program developed at the National Institutes of Health. It is available to anyone as a free download and performs marvelously well for the purpose of image registration. Overlaying images is accomplished with ease using FIJI [4].

Sims and Hardin [1] presented a method to overlay LSC images of a GFP construct in C. elegans embryos with TEM images collected from the same ultrathin section and aligned using Adobe Photoshop [2]. We used a similar method to localize mutant cartilage oligomeric protein in human chondrocytes [3]. Unlike the high fluorescence intensity described in C. elegans, the fluorescence intensity in our GFP expressing chondrocytes lacked sufficient brightness to image in ultrathin sections. This required us to correlate confocal images collected from one-micron sections with TEM images collected from the next serial ultrathin section. Given that the sections are taken from close but still different heights (“Z”) within the block face and also that there are differing sectioning artefacts present in each section, alignment is not straight forward. Here one image needs to be non-uniformly and non-linearly stretched and compressed (skewed) during overlay. FIJI allows registration of grossly misaligned images with far greater ease than Photoshop. The “turboreg” plugin in FIJI initially aligns images based on four operator-selected points of reference. Then, if the two images do not register perfectly, many different points of reference may be selected to skew one image so that it aligns perfectly with the other. Step by step keystrokes to perform these alignments have recently been published [4].

The choice of method used to prepare cultures or tissues must balance resolution with the preservation of antigenicity or reactivity of fluorescent constructs. Sims and Hardin [2] found that the GFP construct in C. elegans tolerated mild glutaraldehyde and embedding in HM20; we found that YFP constructs tolerated higher concentrations of glutaraldehyde and embedding in LRWhite; however more recent formulations of LRWhite do not give optimal results. Even so, LRWhite is still our embedding media of choice if the goal is to preserve antigens on the surface of ultrathin sections; here we use the Molecular Probes Alexa 488/10nm secondary antibody conjugate which carries both Alexa 488 and also a 10nm-gold particulate (Figure 1).

SyBr Green is a reagent useful for correlative imaging localizing nucleic acids. SyBr was developed to replace ethidium bromide as a stain for DNA/RNA in electrophoretic gels; however we use this reagent as an enbloc tissue stain to localize nucleic acids in structures as small as viruses. Fluorescent emission of SyBr endures fixation in 1.5% glutaraldehyde and 1% Osmium Tetroxide and embedding in Epon 812 (Figure 2).

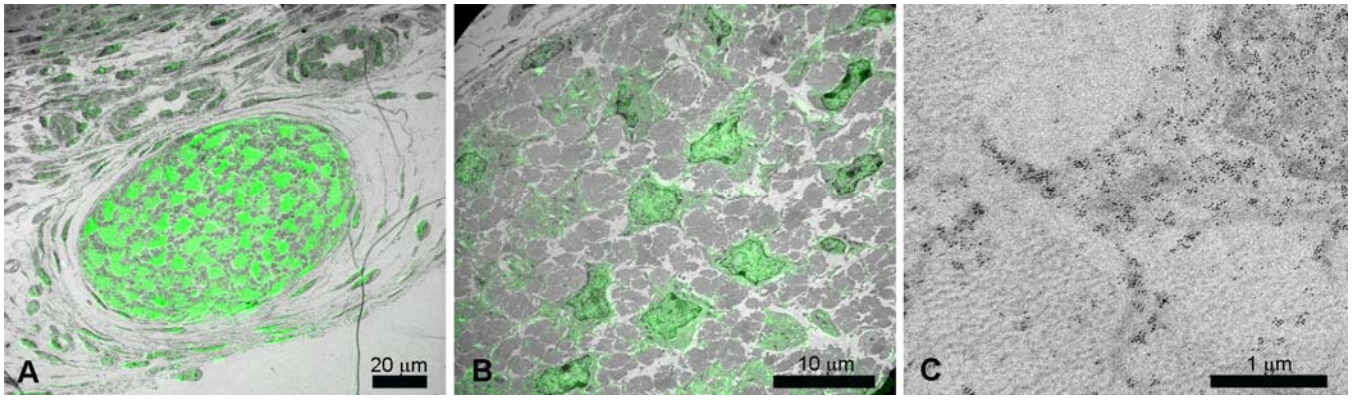

Figure 1. GFP expression could not be detected by LSC within ultrathin or one-micron sections of GFP expressing tenocytes. Therefore antibody to GFP was applied to the surface of LRWhite sections followed by Molecular Probes Alexa 488/10nm-gold, resulting in strong labeling specific to GFP expressing cells (A). Higher magnification demonstrates that all tenocytes within the tendon are labeled (B). Higher resolution immunolabeling of cell extensions is afforded by -10nm gold particles (C).

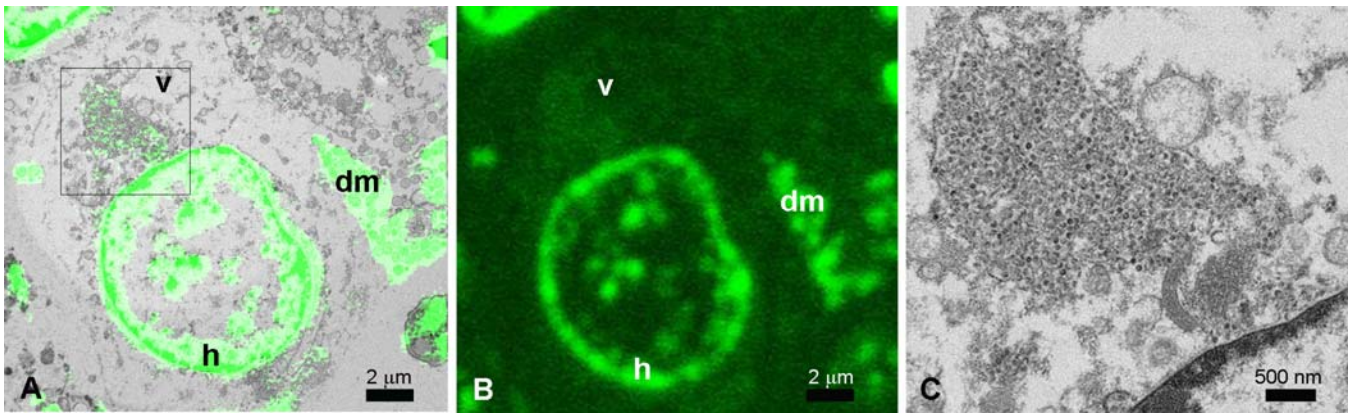

Figure 2. Cells infected with hepatitis virus were fixed in 1% glutaraldehyde, and then exposed to Life Technology SyBr (1:1000 in Tris-HCl). Heterochromatin (h) and nucleoli label intensely as does viroplasm within double membrane vesicles (dm) (A,B). The presence of viruses within fluorescing regions (v) (inset, A) were confirmed by higher magnification TEM (C).

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