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Correlative light and electron microscopy technique using commercially available reagents to facilitate immunolocalization via epi-fluorescence and TEM

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In order to examine the localization of proteins to particular biological structures, it is common to use immunofluorescence labelling with primary antibodies against the protein of interest, followed by staining with fluorophore-coupled secondary antibodies, with subsequent image acquisition via fluorescent microscopy. In order to observe the morphology of cellular structures at high resolution, transmission electron microscopy (TEM) can be employed. However, there often is a need to investigate the same cell or cellular structure in fluorescence microscopy and TEM to create overlays of protein localization with high-resolution morphology, which can be achieved by Correlative Light and Electron Microscopy (CLEM). We chose to develop our approach to this correlation using existing commercially available reagents, standard epi-fluorescent, and standard TEM technology in order to make the technique universally applicable and available to a large group of researchers.

Previously, we have employed CLEM techniques of samples imaged first by fluorescent microscopy, followed by scanning electron microscopy using shuttle and find technology in order to locate identical fields of view in both modalities [1]. While this works well for identifying surface structures such as cilia, it is not an approach that can be used for labelling and visualizing intracellular protein structures. Therefore, in order to broaden our CLEM approach, we pursued a combination of fluorescence with TEM.

The goal of this study was to develop a method to prepare/fix samples stained with primary and secondary antibody, which is conjugated to both an Alexa fluorophore and 10nm gold, which allows for observation of identical fields of view in both fluorescence as well as TEM with a single staining procedure.

Our first model system to which we applied this technique was a monolayer of fibroblasts infected with *Toxoplasma gondii* [2]. Cells were seeded on photo etched, gridded coverslips, which have coordinates to facilitate relocating the same cells of interest. We used a primary antibody to stain the MAG2 protein in the toxoplasma cyst matrix. Cells were fixed in standard 4% paraformaldehyde fixation commonly used for immunofluorescence. Cells were subsequently incubated with primary antibodies, followed by staining with the commercially available secondary antibody coupled to both an Alexa488 fluorophore and 10nm gold. Fluorescent and phase images were acquired on a standard epi-fluorescence microscope. After viewing on the light microscope, the cells were fixed with glutaraldehyde, post fixed with osmium tetroxide, processed for electron microscopy using a standard protocol, then sectioned on a Leica UC7 ultramicrotome using a Diatome diamond knife [3]. Due to the presence of the alpha-numeric grid, we were subsequently able to relocate the same cells on the TEM (Figure 1) to acquire images revealing the

gold labelling as shown in Figure 2 [4].

Figure 1:

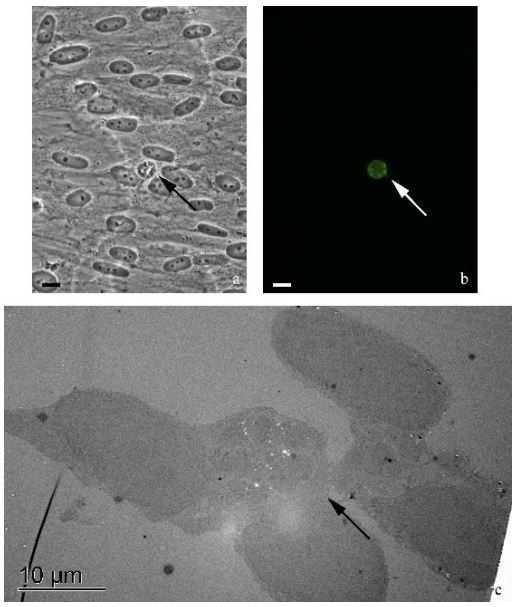

Figure 1: Corresponding light and TEM images of the Fibroblast of interest infected with *Toxoplasma gondii*.
A. Phase image to show orientation of cells on coverslip.
B. Fluorescent image showing the Alexa 488
C. TEM image showing the matching orientation of the cells and the cyst (arrow) of interest. Scale bars = 10 um

Figure 2:

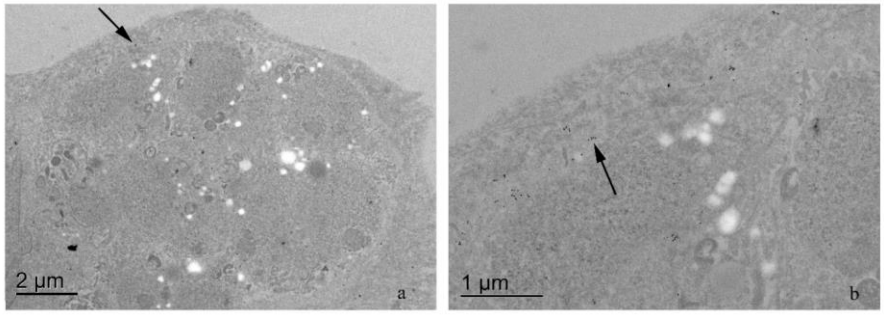

Figure 2: TEM images of a Fibroblast infected with *Toxoplasma gondii* after incubation with dual-coupled secondary antibody. A. Low magnification to show preserved morphology. B. High magnification indicates gold labelling (arrows).

References:

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[3] Bozzola, JJ . Electron Microscopy. 3rd ed. London, UK: Jones and Bartlett; 2012.
[4] All imaging was conducted in the Analytical Imaging Facility (AIF) (funded by NCI Cancer Grant P30CA013330). TEM Imaging was conducted on a JEOL 1400Plus funded by SIG (1S10OD016214-01A1). The authors would also like to thank Dr. Vera DesMarais for help in editing this manuscript and Xheni Nishku for help with the figures.

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