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Developing and Applying a Correlative Light and Electron Microscopy Technique to Overcome Inherent Transmission Electron Microscopy Shortcomings.

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One challenge with Transmission Electron Microscopy (TEM) of biological samples is the resolution gap compared to data obtained by brightfield or fluorescence microscopy (LM) methods that are typically used in research and pathology. TEM has a limited sample size (1mm^2) and a hindered field of view (grid shadow) and gathering enough relevant data at EM resolution is very time consuming and might not represent the overall pathology of the sample. Serial sectioning of embedded tissue on an ultramicrotome can provide a greater amount of TEM data in the Z plane of the sample, but also has its limitations including the time commitment, which, for many research or clinical labs, would be unreasonable. A way to overcome the challenges of traditional TEM is the development of Correlative light and Electron Microscopy (CLEM). There are a variety of published CLEM techniques, but many require specialized equipment such as a Cryo-TEM or FIB milling [1][2].

We have previously published [3] and now further developed a way to generate CLEM data with equipment many microscopy labs already maintain. We present an approach whereby a sample previously investigated by fluorescence or brightfield LM is processed with an enhanced heavy metal staining protocol, embedded in EPON resin, and then subjected to mechanical polishing with a lapping tool, and backscatter imaging by scanning electron microscope (SEM) [3]. The polishing and SEM imaging is then repeated to create Z-planes. The resulting EM tomogram of the sample can be directly correlated to the regions of interest in the LM data (see Figure 1 and 2). This SEM backscatter imaging technique has the advantage of being able to collect large areas of TEM-like data at multiple depths that can quickly provide high resolution data of samples while overcoming the challenge of small sample size and hindered fields of view.

This CLEM technique offers some great advantages over traditional TEM, however it does have several shortcomings we are working to eliminate. The first of these issues is the time it takes to generate these large-area SEM images. Unlike ultramicrotome serial sectioning and TEM imaging, this technique does not require constant supervision from a technician, but it does take a significant amount of time to take the number of images required. The dwell time on each image area also causes electron beam damage which can hinder subsequent mechanical polishing and imaging. Because of this we are currently limited to an ideal magnification of 10kx to 12kx. To address both time and beam damage issues, we are developing a technique that can utilize machine learning to upscale fast acquired(noisy) images to the quality we are getting from the standard technique. Once fully developed, this CLEM technique has the potential to be applied to a variety of research fields and will greatly improve the throughput of LM and EM labs.

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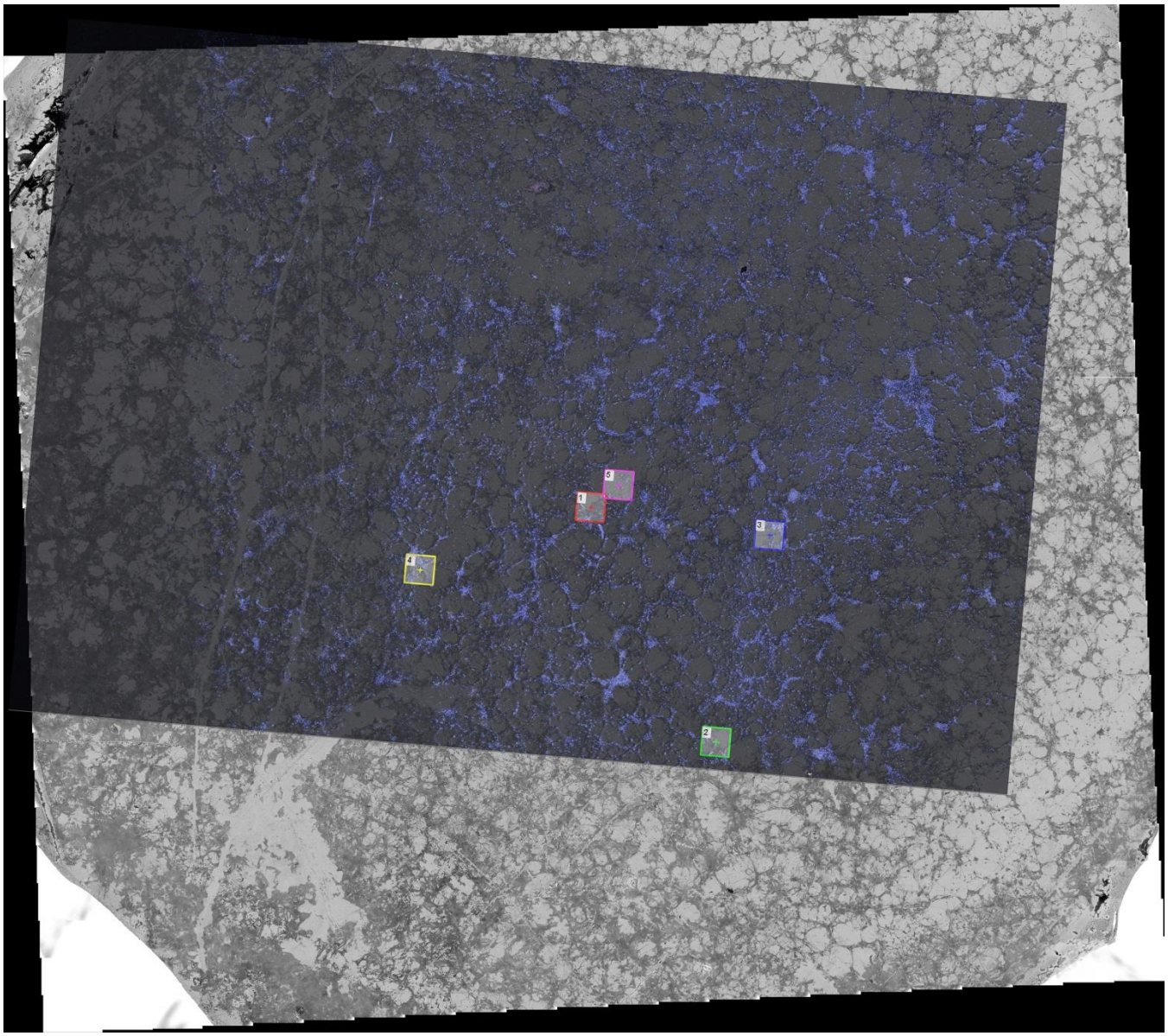

Figure 1. An example image of how regions of interest (ROI) are located and correlated. This image of primary neuron cells was created by overlaying a low magnification (10x) fluorescence large-area stitched image on a low magnification (50x) back-scatter SEM large-area stitched image. The numbered boxes are indicating ROI where 60x stacked confocal images were taken.

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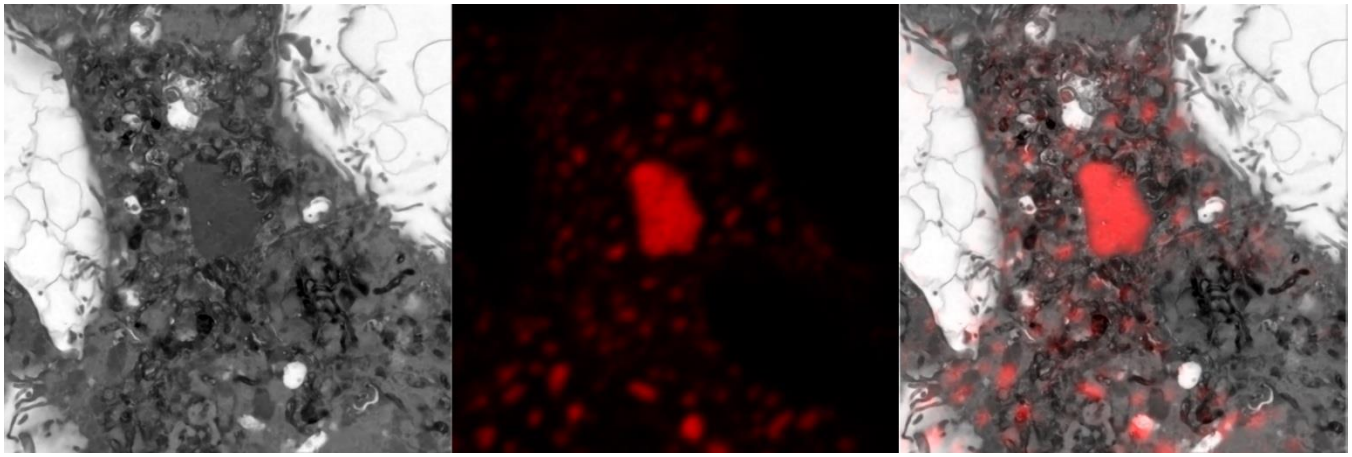

Figure 2. An example of the final overlay of ROI. This image is able to highlight what fluorescent cellular condensates (red) look like under EM conditions. This is an example of applying this CLEM technique to an ongoing condensate biology study.

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