

## Correlative Light and Electron Microscopy (CLEM) Utilizing Hitachi HILEM™ IL1000 Ionic Liquid.

James P. Kilcrease<sup>1</sup>, Osamu Takagi<sup>1</sup>, Gary Bauchan<sup>2</sup>

<sup>1</sup>. Nanotechnology Systems Div., Hitachi High Technologies America, Clarksburg, MD 20871, USA

<sup>2</sup>. Electron and Confocal Microscopy Unit, United States Department of Agriculture – Agricultural Research Service, Beltsville, MD 20705, USA

Observation of delicate, non-conductive samples can present many challenges when attempting to utilize multiple light and electron imaging techniques to generate truly correlative data for a given sample. Traditional aldehyde fixation, sputter coating, and critical point drying techniques are very useful but often require hazardous chemicals and have potential to alter a sample's natural state or structure. Ionic liquids have been proven to help maintain natural structural integrity of biological specimens when exposed to vacuum conditions necessary for high resolution electron microscopy [1]. HILEM IL1000™ is an organic salt that remains in liquid form under normal conditions. This ionic fluid was designed specifically for electron microscopy due to its high ionic conductivity, increased solubility, and non-toxic characteristics with great ability to reduce charging artifacts and preserve delicate samples in their natural form [2,3].

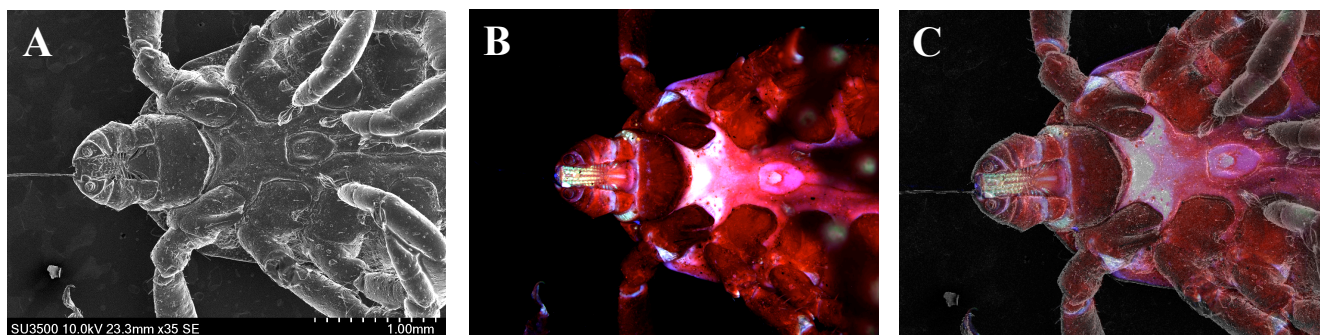
Frozen-hydrated *R. sanguineus* samples were acquired and first imaged with a Zeiss Axiozoom optical fluorescence microscope. Autofluorescence information was collected using a mercury vapor lamp in conjunction with DAPI, eGFP, and mRFP filter sets. Untreated samples were then imaged with a Hitachi SU3500 scanning electron microscope at 10kV. Next, the samples were treated with a 10% HILEM IL1000 solution for 2 hours at room temperature. Excess ionic liquid was removed via filter paper / blow dry and the SEM plus fluorescent imaging techniques repeated utilizing the same parameters. A reduction in charging leading to increased SEM image quality was observed (Figure 1-A), however at high magnification a small amount of residual ionic liquid remained in some areas. Ionic liquid did not have any adverse effects on autofluorescence emission of the sample and fluorescence was maintained (Figure 1-B). The overlay image in Figure 1-C was generated using AZblend (<https://www.astron.co.jp>) software and demonstrated excellent SEM and fluorescent correlative data.

Spectral analysis was also conducted on ionic liquid alone to ensure no artifacts were induced by any autofluorescent properties of the fluid itself. A lambda scan over 415-732nm emission wavelengths was demonstrated with excitation at 405, 488, 561, and 631nm on a Zeiss 710 laser scanning confocal microscope. No emission signal for ionic liquid was detected (Figure 2).

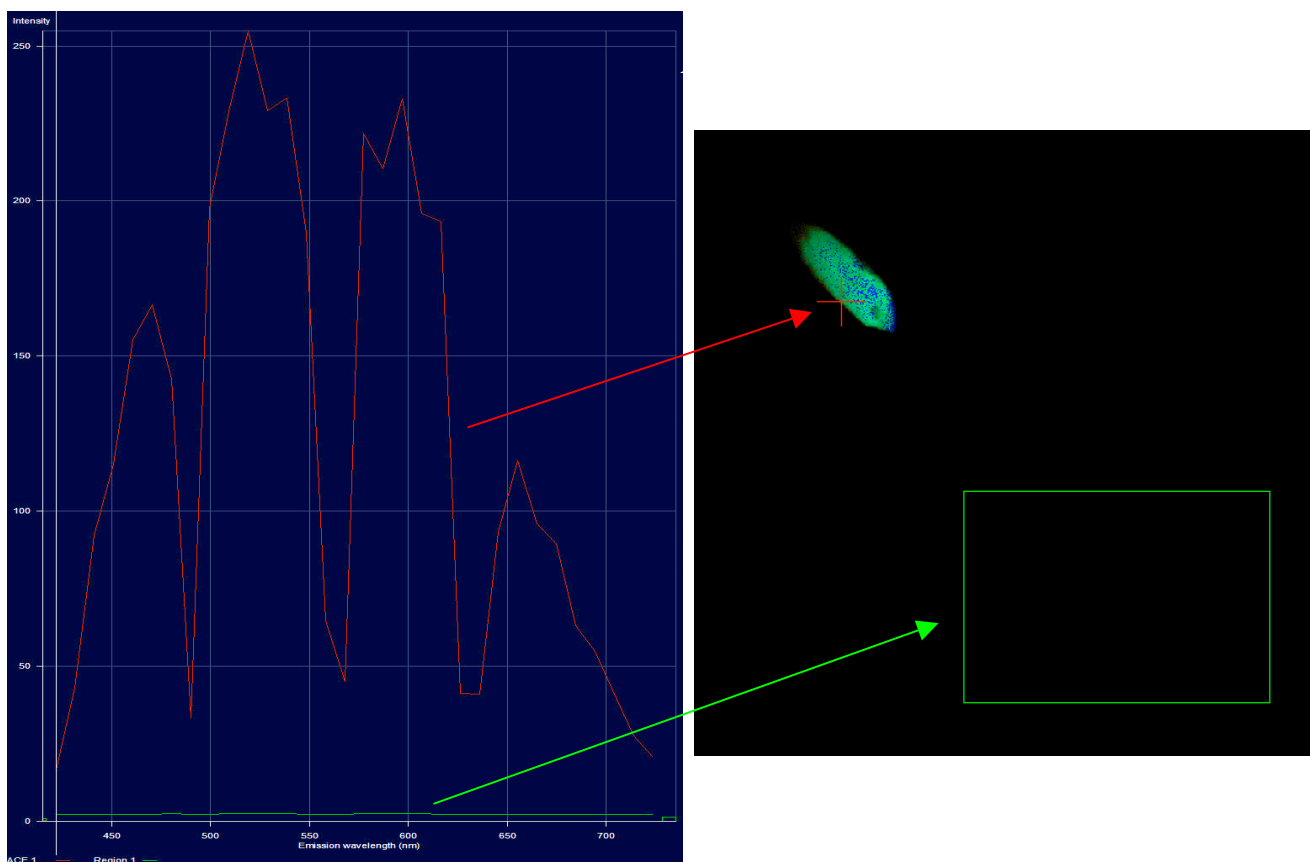
The results of this data demonstrate that a pretreatment with ionic liquid can help minimize or even eliminate many of the difficulties encountered when conducting electron microscopy correlation with fluorescent or light-optical techniques on biological specimens. Including but not limited to; reducing charging by increasing surface conductivity, increasing stability under vacuum conditions, and helping to maintain structural integrity without inducing any additional fluorescence artifacts. This leads to an overall reduction in processing time with better quality image generation and the benefit of performing multiple correlative techniques on a single sample.

References:

- [1] Kawai, K., et al. Langmuir Letters 27 (2011) p. 9671-9675.
- [2] Sakaue et al. Microsc. Microanal. 20, Suppl 3 (2014), p. 1012..
- [3] Shiono et al. Microsc. Microanal. 20, Suppl 3 (2014), p. 1016.



**Figure 1.** CLEM micrograph series of *R. sanguineus* pretreated with HILEM IL1000. Figure A, SEM micrograph. Figure B, optical autofluorescence image. Figure C, CLEM overlay image.



**Figure 2.** Confocal lambda analysis of HILEM IL1000 droplet plus debris placed in flat bottom petri dish. Excitation; 405, 488, 561, 631nm. Emission 416 - 732nm. Signal profile on left colored in red corresponds to area at red cross on right (debris). Signal profile on left colored in green corresponds to green boxed area on right containing only ionic liquid.