Processing zebrafish for correlated light and electron microscopy studies

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Correlative light and electron microscopy (CLEM) is defined by the combination of functional information provided by light microscopy and high-resolution ultrastructural information collected through electron microscopy. The success in acquiring quality CLEM image data for high throughput analysis relies on good sample preparation. Fluorescence retention and ultrastructure preservation are often incompatible when using classical sample preparation approaches. Cryotechniques, however, serve as good alternatives for structural and fluorescence preservation, - but cryogenic sample preparation techniques are tedious, technical demanding and require highly specialized and expensive equipment.

Therefore, we present herein a simple and alternative sample preparation protocol utilizing only uranyl acetate as contrasting agent and LR White resin as embedding medium. This protocol allowed us to locate albumin conjugated to TexasRed within the zebrafish larvae across different microscopy modalities. In brief, we have successfully retained *en bloc* fluorescence while preserving the ultrastructure at high-resolution. Sections as thin as 90 nm, from blocks as old as 6 months post-polymerization, still presented intense fluorescence under wide-field fluorescent microscope and fine structural detail at the subcellular level that could be easily discerned using scanning & transmission electron microscopy.

This method allowed us to collect fluorescent data on the uptake and distribution of albumin in the different microanatomical compartments of the zebrafish liver that could then be correlated with high-resolution electron microscopy imaging down to the subcellular level. We will present quantitative analysis studies performed on the zebrafish liver albumin uptake and discuss the strengths, applications as well as challenges of this CLEM approach.