HOW DEEP IS “TOO DEEP”: EXPERIMENTAL EVALUATION OF DIFFERENT OBJECTIVES IN THE CASE OF HIGHLY MISMATCHED MEDIA

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Correlative Light and Electron Microscopy (CLEM) is getting more and more popular in the recent years. While integrated CLEM approach undoubtedly offers many exciting possibilities, it also invariably leads to substantial new design challenges. In our group the 2D integrated CLEM (scanning fluorescent microscope plus TEM, – iLEM) was realized. Now 3D integrated CLEM (Confocal Laser Scanning Microscope (CLSM) plus Focused Ion Beam / Scanning Electron Microscope (FIB/SEM)) is being developed.

FIB/SEM requires fixed samples. For room temperature FIB/SEM samples are dehydrated and resin embedded (refractive index approximately of 1.5). However standard commercial available microscopic objectives are designed to work with biological samples with refractive index approximately 1.4. This mismatch raises interesting questions: which type of objective shall be chosen; what is the most preferable NA (for different applications); what actual focal plane position is; what the behavior of the Point Spread Function (PSF) is; etc. The questions above can be answered numerically, but due to the lack of explicit information on the optical design of many objectives experimental approach was chosen.

In this poster the experimental study of optical performance of different objectives types are presented. The results of this study help to make inexplicit choice of the objective for the integrated Correlative Light and Electron Microscopy (CLEM).