3D CORRELATIVE SUPERRESOLUTION LIGHT AND ELECTRON MICROSCOPY 
BASED ON SERIAL SECTIONS

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Light (LM) and electron microscopy (EM) are highly successful technologies when used independently. New insights into the functionality and the associated ultra-structure of biological specimens can be gained by combining both methods. One popular approach to image three-dimensional objects is to slice them into ordered arrays of ultrathin, resin-embedded sections and reconstruct the 3D volume from the recorded images [1].

Here we applied this method to Neuromuscular Junctions (NMJ) in muscle tissue, which was specifically prepared for correlative light and electron microscopy [2]. We employed Shuttle & Find [3] to image the same regions of interest (ROIs) with superresolution fluorescence microscopy (Elyra PS.1, Carl Zeiss Microscopy GmbH) and with scanning electron microscopy (Neon 40, Carl Zeiss Microscopy GmbH).

Under intense laser illumination the fluorophores showed a specific blinking behavior, which enables localization microscopy techniques [4]. The high density of the active fluorophores per image frame in the resin embedded tissue didn’t allow analysis of single, well separated fluorophores. Therefore we applied suitable algorithms accounting for several fluorophores within one resolution limited spot [5] and estimated an localization accuracy in the range of 50 nm. EM images of corresponding ROIs were recorded with a pixel resolution of few nanometers.

To achieve a correct registration of both imaging modalities within a few nanometers, we investigated Q-dots and dye doped polymer particles to serve as fiducials in the nanometer range. Both could be specifically visualized with optimized imaging parameters in the SEM.

In combination with 3D alignment of the sections we were able to obtain high resolution correlative datasets of lager volumes which enables the investigation of functional and morphological features on the nanometer scale.


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