# 3D CORRELATIVE LIGHT AND ELECTRON MICROSCOPY BASED ON NON-CONTRASTED TISSUE SECTIONS

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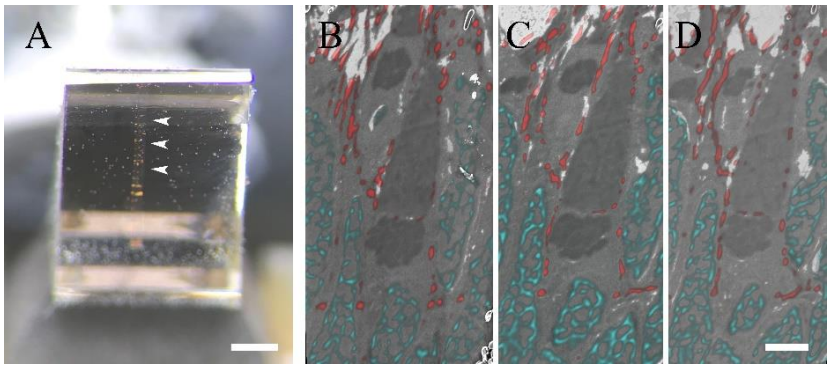
The subcellular localization of a protein provides key information to understand its function. Such studies present additional challenges when applied to tissues because of the inherent 3D complexity and required Z-resolution. Protocols combining light and electron microscopy provide subcellular resolution and context information for complex samples in 3D. We present here a method to obtain serial sections from any tissue, and apply light microscopy followed by correlation with scanning electron microscopy data. Briefly, the tissue is cut frozen at a thickness of 110 nm [1] and serial sections are collected on silicon wafers [2]. No resin embedding, neither contrast agents are required providing a high antigenicity preservation. Although any fluorescent microscopy method could be applied, we propose here the use of Super Resolution Radial Fluctuations (SRRF, [3]) because of the high performance in 2D sections and simplicity. SRRF provides super-resolution data in XY (~ 70 nm) and the use of these serial thin sections (110 nm) improves the resolution in Z of the analyzed volume. The correlation with scanning electron microscopy adds the ultrastructural context information to the fluorescent protein location into the volume.

We have applied SRRF to zebrafish retina serial sections (20-30 sections), reconstructing complete photoreceptors or similar subcellular structures.




A. Serial Tokuyasu cryo-sections (arrowheads) on a diamond knife ready to be collected and transferred to a silicon wafer. B-D. Three serial sections from a zebrafish retina imaged by super-resolution fluorescent and electron microscopy. Complete photoreceptors are analyzed in 3D with Z-resolution of 110 nm. Scale bars: A: 500 µm; D: 2 µm.

References;

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