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Precise Protein Localization in Dissected *Drosophila* Larvae by Correlative Light and Transmission Electron Microscopy

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Bridging light and electron microscopy through correlative light and electron microscopy (CLEM) enables imaging of protein localization within a detailed subcellular context [1]. Certain CLEM approaches, such as array tomography, nanofEM, are compatible with super-resolution imaging to localize the fluorophore with nanometer precision and access the subcellular context through scanning electron microscope (SEM) [2-4]. A recent study of the *Drosophila* central nervous system achieved single-synapse resolution by combining in resin fluorescent imaging and focused-ion beam SEM [5]. However, compared to TEM, high-resolution electron micrographs are difficult to obtain in SEM. Here, we present a CLEM approach that overcomes this obstacle by combining dSTORM [4] of resin-embedded samples and TEM. As detailed below, following staining, super-resolution images were acquired on a slot grid and transmission electron micrographs were collected from the same grid. For proof of concept, we used an anti-actin antibody to detect sarcomere actin in *Drosophila*.

The sarcomere is the basic unit of striated muscle fiber for conducting muscle contraction and relaxation. One sarcomere forms from thin-filaments, thick filaments, and Z-line. The thin-filament attached on the Z-line is made from actin, whereas the thick-filament is composed of myosin. Intriguingly, we discovered that a commercially available mouse monoclonal antibody (Sigma A1978) specifically targets beta-actin in the sarcomere of *Drosophila* larval body-wall muscles. Briefly, dissected 3rd instar larva was fixed in PFA, followed by dehydration in ethanol. The sample was then flat-embedded in LRWhite resin. We collected 70nm ultrathin sections on a nickel slot grid coated with pioloform (Fig1A). Subsequently, the grid was stained with anti-actin antibody similar to immunoEM procedures. For STORM imaging, the grid was mounted on a glass slide and immersed in Vectashield (Fig1B-C). After STORM imaging, the same grid was stained with uranyl acetate and lead citrate, and electron micrographs were captured on an FEI Tecnai T12 equipped with a Gatan OneView camera at 120KeV (Fig1D). Reconstructed STORM images can readily be aligned to the electron micrographs using Adobe Photoshop (Fig1E-F).

In conclusion, we are able to detect sarcomere actin in *Drosophila* larvae with our CLEM-TEM method. By aligning super-resolution images to electron micrographs, we are able to precisely localize sarcomere actin in larval body-wall muscle at nanometer scale. Furthermore, by combining this CLEM-TEM method with other techniques, such as high pressure freezing, one can precisely localize protein of interests at nanometer resolution in near-to-native state.

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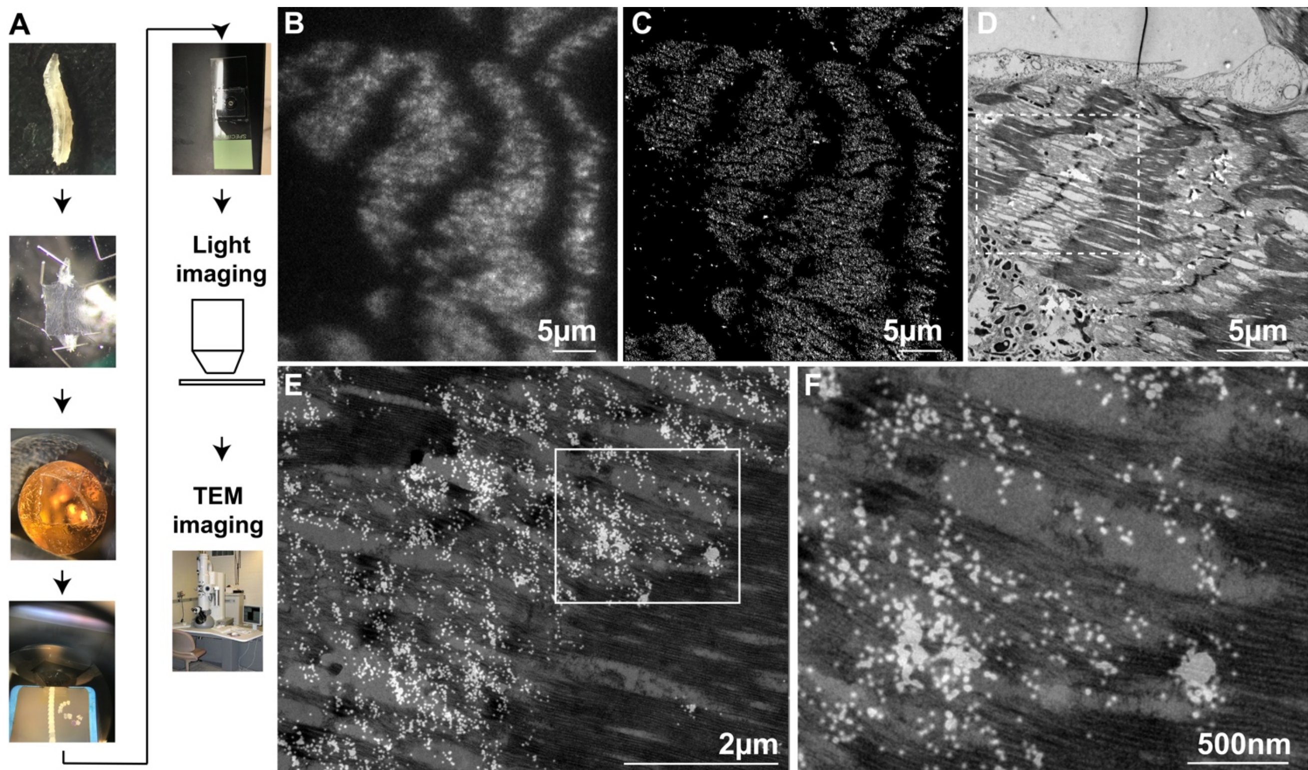

Figure1. Visualization of actin using CLEM-TEM. A, schematic of sample preparation. B, widefield imaging of actin on a TEM slot grid, scale bar: 5µm. C, STORM reconstruction from B, scale bar: 5µm. D, electron micrograph of the muscle imaged by STORM, scale bar: 5µm. E, coarse alignment of STORM and electron micrograph from the dash-line box area in D, scale bar: 2µm. F, zoomed in image from the white box area in E, scale bar: 500nm.

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