LABEL-FREE 3D CLEM

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Emerging correlative light and electron microscopy (CLEM) approaches enable the study of neuronal structure-function relations at unprecedented depth and precision. However methodological hurdles in CLEM are the re-localization of a rather small target volume within a large tissue volume, changing sample orientation during the transition between different microscopy modalities, and structural distortions or preparation artefacts caused by artificial fiducial markers, such as polymer beads and near-infrared branding [1], which might obscure or even disrupt the structure under investigation.

Here we report an efficient, general applicable ”flat embedding” preparation method enabling high-precision overlay of light and electron micrographs using exclusively endogenous landmarks situated throughout the brain: blood vessels, nuclei, and myelinated axons. To demonstrate the applicability and precision of the presented CLEM preparation method, eGFP-expressing dendritic tufts in the somatosensory cortex of Thy1.2-GFP-M mice were imaged by long-term *in vivo* 2-photon microscopy and finally relocated in the corresponding EM specimen using focused ion beam scanning electron microscopy [2].

Summarizing, we introduce a precise and efficient CLEM preparation method, which (1) circumvents the need of artificial fiducials, (2) is compatible with widely accessible optical microscopic techniques and (3) is suitable for various scientific questions.

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