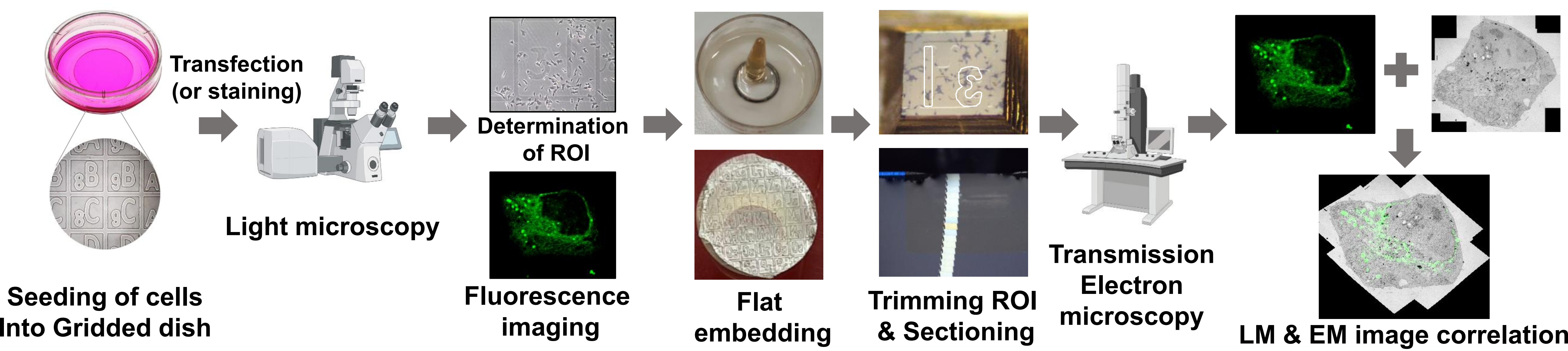


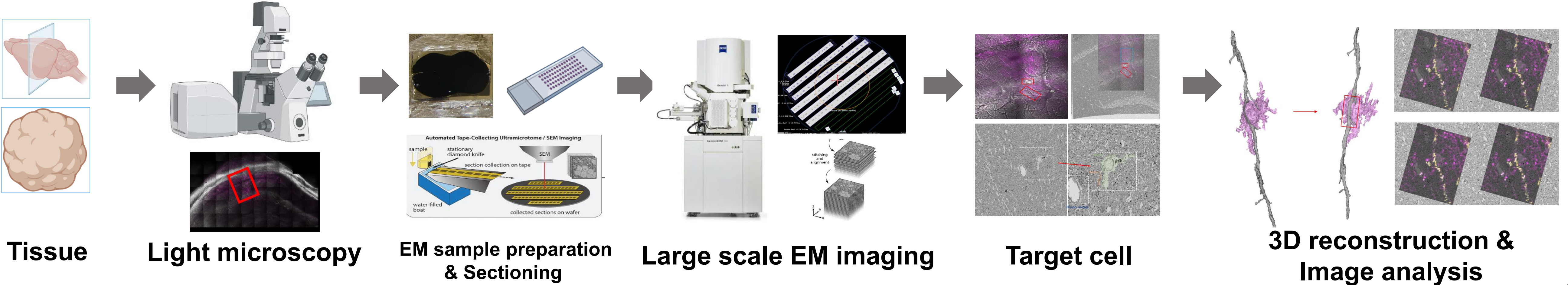
Abstracts

Correlative Light and Electron Microscopy (CLEM) has emerged as a key imaging technique that enables simultaneous acquisition of molecular and ultrastructural information from the same biological specimen. Fluorescence microscopy (FM) offers molecular specificity by visualizing proteins of interest (POIs) in multiple colors, while electron microscopy (EM) provides nanometer-scale resolution of subcellular structures. These complementary features make CLEM a powerful tool for accurately elucidating the structure-function relationship within cells and tissues. A variety of CLEM approaches have been developed, and their biological applications continue to expand. In this study, we present a systematic comparison of CLEM methodologies based on scanning electron microscopy (SEM) and transmission electron microscopy (TEM), using real imaging data acquired from biological samples. This study evaluates the key aspects of each technique, including imaging depth, spatial resolution, the complexity of sample preparation, and the preservation of the fluorescence signal. We propose optimized CLEM strategies tailored for addressing various neurobiological questions. Ultimately, this study offers a novel framework for high-resolution multimodal imaging, enabling more precise and reliable analysis of complex neuronal and brain tissue structures.

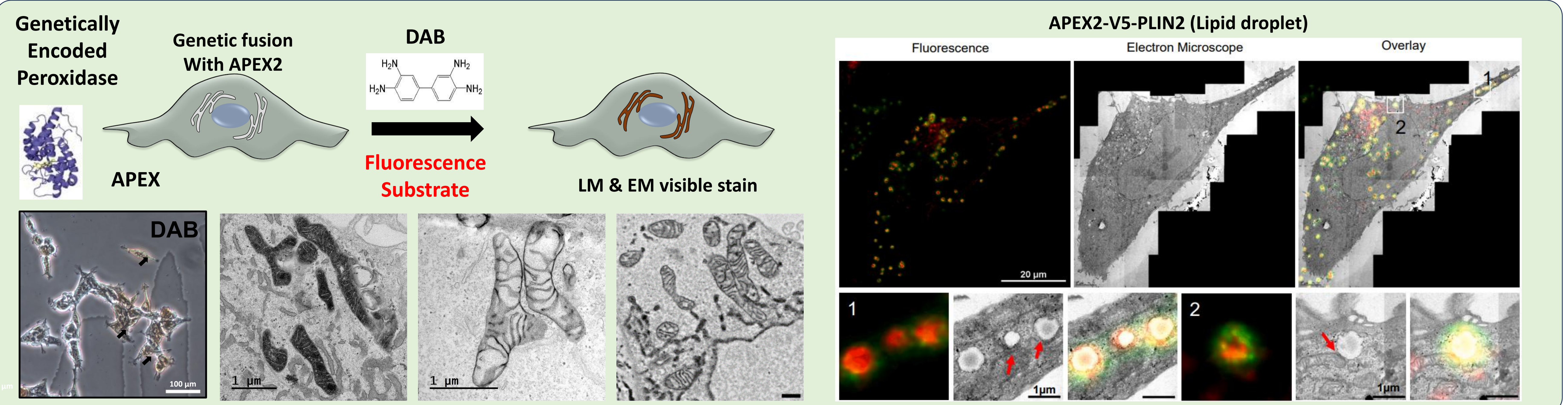
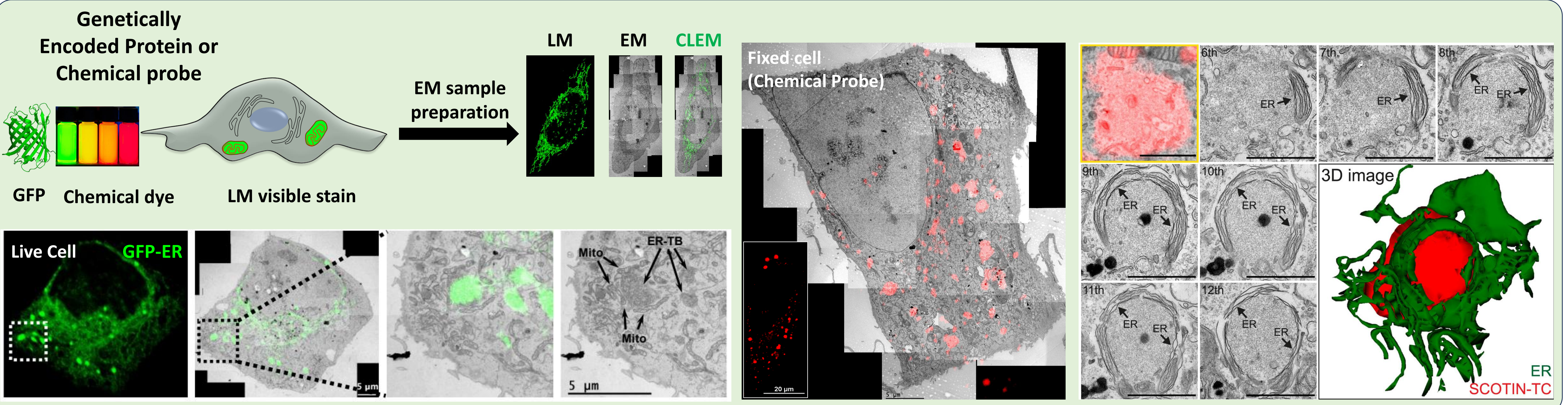
TEM_CLEM methods



Large area SEM_Volume CLEM methods

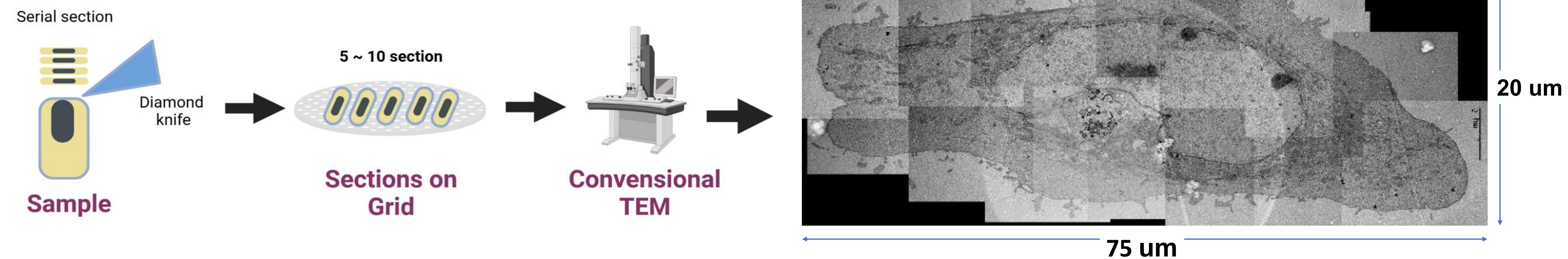


CLEM Probe

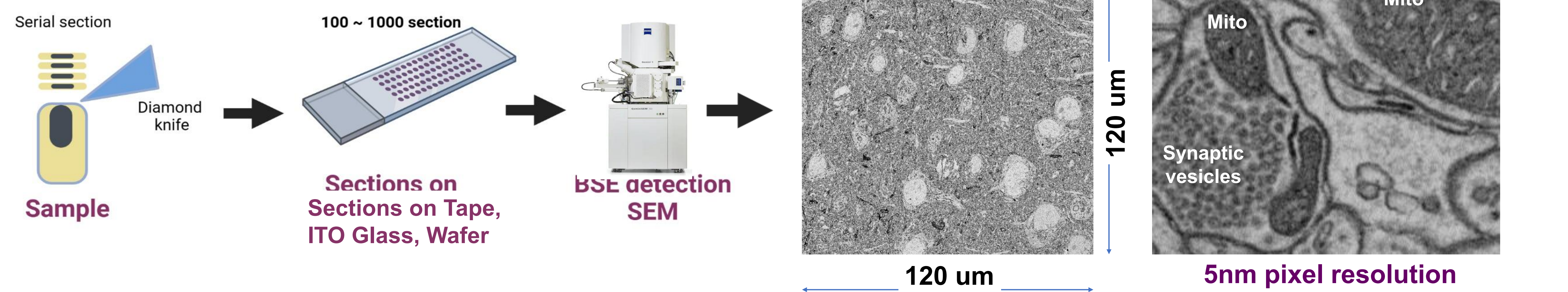


TEM vs SEM

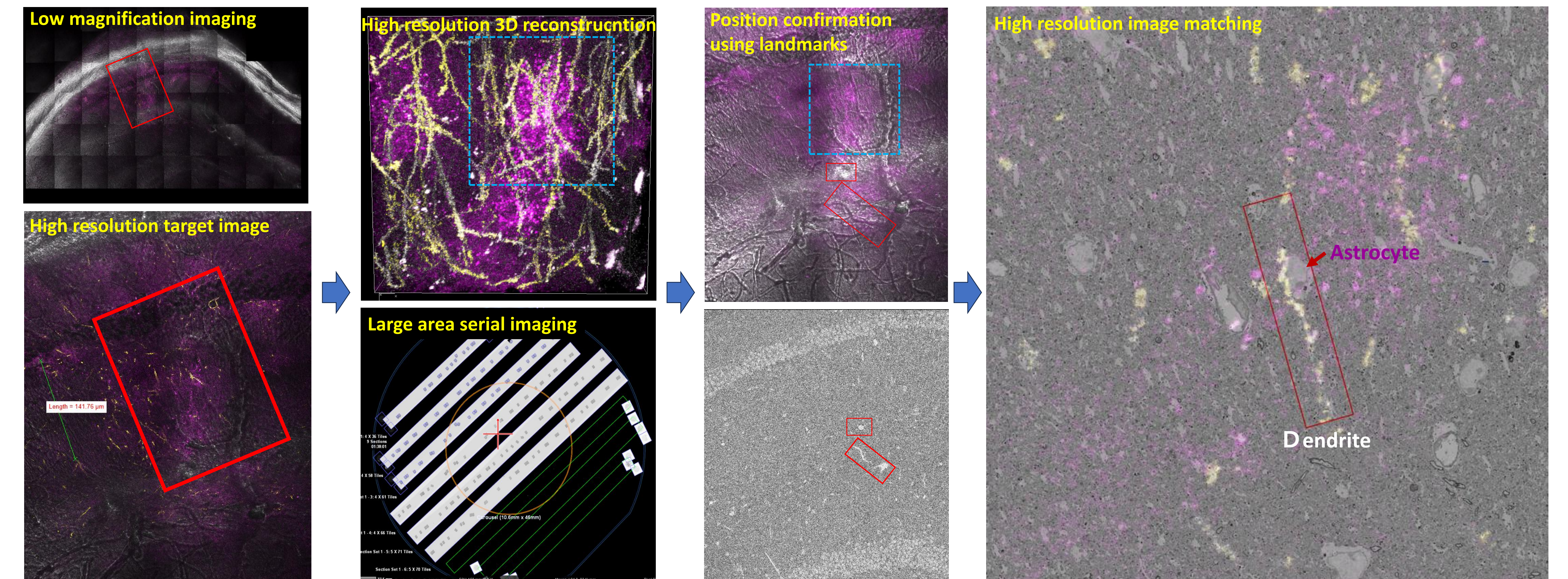
Conventional TEM method



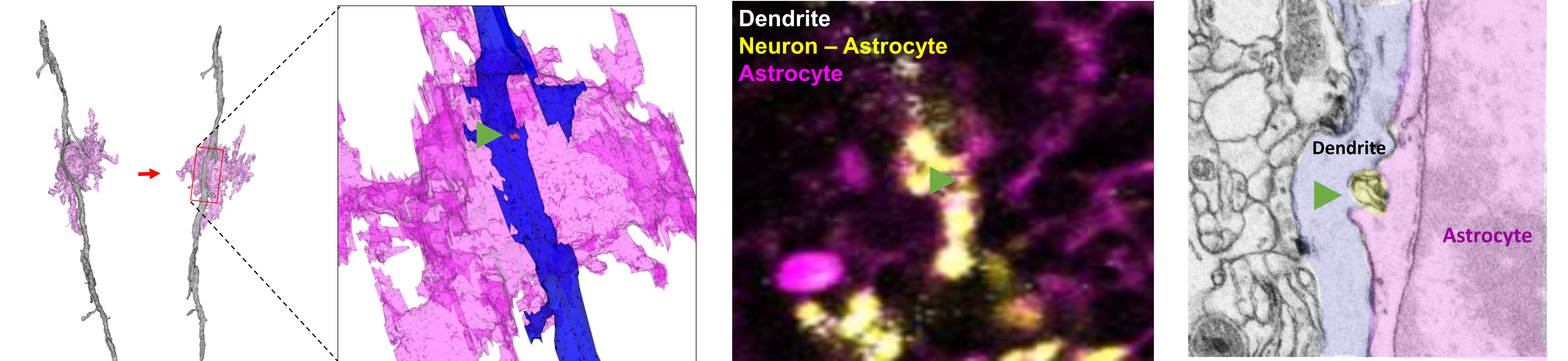
Large scale SEM image



Astrocyte_Neuron contact



3D Reconstruction



Discussion

- CLEM is a very useful method to study structural changes of cellular organelles or protein localization.
- CLEM-compatible probes have distinct strengths and limitations, so it is important to choose a probe that best aligns with research objective.
- SEM-based volume CLEM allows us to obtain a larger volume of 3D data on cellular or subcellular ultrastructure.
- Volume CLEM enables accurate 3D tracing of extended and complex structures, such as neuron-astrocyte contact and synaptic networks.