Correlative microscopy using silver-gold intensified DAB staining

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Abstract
Nerve cells have complex structures, ranging from the millimeter to the nano-scale. The study of these complex morphologies requires thus a combination of different microscopic techniques, which has become known as “correlative microscopy”. To this end, neurons are typically filled with a small tracer molecule, usually biocytin, followed by a visualization of this tracer [1]. Detection of biocytin by DAB is known to exhibit a very high contrast and can be visualized with both light and electron microscopes. Fluorescent staining using fluorophores coupled to avidin on the other hand allow visualization using optical sectioning techniques such as confocal or multi-photon microscopy, which is more suitable for thick tissue and automatic segmentation. However, this staining cannot be visualized at the EM level.

We show here that an Ag/Au-intensified DAB staining [2] can be visualized by two-photon induced gold luminescence, thus combining the high contrast offered by the traditional DAB staining with the advantages offered by optical sectioning microscopy. The staining additionally provides improved signal-to-noise ratio for small structures compared to a fluorescent staining of biocytin using Alexa fluorophores coupled to streptavidin.

The same substrate – gold – can also be visualized at the electron microscopic level, which makes it an ideal substrate for correlated light and electron microscopy.

We anticipate that this staining will thus replace the traditional methods, thereby combining the strength of light and electron microscopy in ways not possible so far.

References