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Fluorescence photooxidation with eosin: a method for high resolution immunolocalization and in situ hybridization detection for light and electron microscopy.

T J Deerinck, M E Martone, V Lev-Ram, D P Green, R Y Tsien, D L Spector, S Huang, M H Ellisman

+ Author and Article Information

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A simple method is described for high-resolution light and electron microscopic immunolocalization of proteins in cells and tissues by immunofluorescence and subsequent photooxidation of diaminobenzidine tetrahydrochloride into an insoluble osmiophilic polymer. By using eosin as the fluorescent marker, a substantial improvement in sensitivity is achieved in the photooxidation process over other conventional fluorescent compounds. The technique allows for precise correlative immunolocalization studies on the same sample using fluorescence, transmitted light and electron microscopy. Furthermore, because eosin is smaller in size than other conventional markers, this method results in improved penetration of labeling reagents compared to gold or enzyme based procedures. The improved penetration allows for three-dimensional immunolocalization using high voltage electron microscopy. Fluorescence photooxidation can also be used for high resolution light and electron microscopic localization of specific nucleic acid sequences by in situ hybridization utilizing biotinylated probes followed by an eosin-streptavidin conjugate.

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