Fluorescence Detection of Fluorescence-labeled Organelles In-resin Section using Antifade Reagents in Correlative Light and Electron Microscopy

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Correlative light and electron microscopy (CLEM) enables ultrastructural-level visualization of fluorescence-labeled molecules by combining images obtained from both fluorescence and electron microscopes. A technical challenge with the CLEM technique is the effective detection of fluorescence from biological samples embedded in resins, which generally cause fluorescence decay. To overcome this problem, we developed a method for fluorescence recovery of green fluorescent protein (GFP) and the variants in resin-embedded sections, using commercially available antifade reagents. By applying this method, we successfully visualized the GFP-labeled organelles in plastic resin by using a combination of confocal laser scanning microscopy (LSM) and field-emission scanning electron microscopy (FE-SEM) or transmission electron microscopy (TEM).

*Arabidopsis thaliana* seedlings expressing GFP-labeled organelles, including peroxisomes and ER, were fixed with a formaldehyde-glutaraldehyde solution and then embedded in acrylic resin, LR White. The resin blocks were sectioned with an ultra-microtome and semi-thin sections (1 µm) for FE-SEM were placed on a round coverslip with an embossed grid pattern. And thin sections (200 nm) for TEM were placed on formvar-coated copper TEM grids with an alphanumeric character. To detect the fluorescence of GFP in plant organelles, the sections of acryl-resin embedded samples were observed with LSM. GFP fluorescence signal dissipates due to low pH of the resin, but recovery of these fluorophores for tissue embedded in acryl-resin has been achieved by utilizing alkaline solutions such as Na₂CO₃ and NaOH [3]. This method is especially useful regarding fluorescence detection in resin-embedded material for two-photon microscopy because these microscopic observation techniques are unsuitable for live imaging. To recover fluorescence in this experiment, a Na₂CO₃ solution was applied to the thick section and the GFP signal of peroxisomes observed with LSM. Moreover, the effective fluorescence recovery of GFP in embedded thick sections using commercially antifade reagents was also tested. Although antifade reagents have been used to maintain the fluorescence of fluorochemicals, such as FITC and rhodamine, newly available commercial antifade reagents preserve fluorescent proteins. The sections were treated using antifade reagents such as SlowFade Diamond (Thermo Fisher Scientific), and Fluoro-KEEPER (Nacalai Tesque Inc.) and fluorescence recovery compared via LSM. The GFP fluorescence treated by antifade reagents was observed to be brighter than that of Na₂CO₃ treatment [4].

After fluorescence imaging was conducted, the same sections on coverslips were stained with uranyl acetate plus lead citrate. The sections on coverslips were observed with FE-SEM (Hitachi SU8220) and the images were acquired using YAGBSE detector at 5 kV, high current mode. And the sections on grids were observed with TEM (Hitachi HT7800) at 100kV. The regions of interest expressing GFP fluorescence were acquired and merged by CLEM system “MirrorCLEM”[5]. Most of the fluorescent dots in plants expressing GFP tagged-ER body and peroxisome markers were clearly localized in spindle-shaped ER body structures and round peroxisome structures, respectively, indicating that the treatment with antifade reagents was actually effective in recovering the GFP fluorescence emitted from each organelle.
The GFP fluorescence recovery methods for CLEM demonstrated in this study provide an easy and straightforward solution for technical challenges associated with accurate understanding of the behavior of GFP-labeled proteins in a vast variety of organisms, not only plants.


Figure 1. CLEM images obtained using antifade solution. (A-C) The LSM images of GFP-labeled peroxisome were taken after treatment with Fluoro-KEEPER. (A) FE-SEM images at the same position were acquired using a YAG-BSE detector. LSM (left) and FE-SEM images (right) were merged (lower). (B) TEM images at the same position were acquired. LSM (left) and TEM images (right) were merged (lower). (C) Higher magnification image of (B).