

ILLUMINATING THE MICROSCOPIC REALM: APPLICATION OF RESIN R221 FOR CLEM IN MICROBES AND PLANT TISSUES

Andrea Cheradil¹, Dr Julia Buchner¹, Dr Matthias Ostermeier², Isabella Gantner¹, Jennifer Grünert¹, Prof. Dr. Andreas Kling¹

¹Plant Development & Electron Microscopy, Planegg-Martinsried, Munich, Germany, ²Department of Molecular Plant Science, Planegg-Martinsried, Munich, Germany

Background incl. aims

Correlative light and electron microscopy (CLEM) is a powerful imaging tool that combines the advantages of light/fluorescence (LM/FM) and electron microscopy (EM). In CLEM, FM images can be used to observe the localization of one or more molecules of interest indicating where the region of interest (ROI) the needle in the haystack is located whereas EM provides the ultrastructural information. Samples used for investigation of ultrastructure by EM are subjected to chemical fixation and are treated with heavy metal salts and contrasting agents such as osmium tetroxide and uranyl acetate to improve imaging contrast and later embedded in resin as it stabilizes the samples so that it withstands the vacuum condition of the EM and enables long storage. However, chemical fixation and resin embedding process which is essential for good quality of the ultrastructure is impaired by the contrasting agents or the fluorescence protein tag in the samples lose their ability to fluoresce due to protein denaturation which possess a major challenge for CLEM. The discovery that fluorescence can be retained in resin-embedded specimens following moderate heavy metal staining revolutionised CLEM. R221 (CryoCapCell) is a methacrylate based acrylic resin with promising results allowing fluorescence preservation facilitating CLEM approaches to identify the ROI and obtain high resolution ultrastructural images of the targeted ROI. We aim to use Resin R221 to identify the interface of plant microbe interaction which is ROI under fluorescence microscope with the help of fluorescent tags before thin sectioning for EM ultrastructural analysis. This immensely reduces the time required to locate the ROI in plant samples. For instance, symbiotic bacteria present in root cells can be easily identified if the bacteria are fluorescently tagged allowing efficient identification of the bacteria infected cells for EM analysis.

Method:

In the initial phase cyanobacterial and algal strains are subjected to high pressure freezing followed by freeze substitution (FS) in a cocktail containing uranyl acetate, glutaraldehyde, H₂O and acetone (at -90 to -30°C) followed by R221 resin infiltration and polymerization in low oxygen level and exposure to UV light to ensure proper polymerization. After polymerization the specimen blocks are stored at room temperature protected from light to preserve fluorescence. Thin sections of 0.7 µm/70 nm are placed on copper

grids coated with collodium which are then mounted on glass slides with cover slips sealed with paraffin wax for CLEM analysis.

Results:

Three different fluorescent tags could be detected in sections of samples prepared in the resin R221 so far. This facilitates the application of CLEM analysis to identify the cyanobacterial and algal strains as well as plants associated with microbes under the TEM. The R221 resin paves a way for multimodal analysis without having to follow different sample preparation for fluorescence and electron microscopy.

Conclusion:

While resin already gave us very promising results, there's still work to do in fine tuning the workflow for plant materials to facilitate the identification of ROI and target rare events. Additionally, it is also essential to explore chemical fixation methods as it is a more convenient method especially for plant materials. Subsequent steps will involve application of this optimized workflow to various This will be followed by applications of different plant material, plant-microbe associations and implementation of other plant material and with other fluorescent tags.

Keywords:

R221, Fluorescence microscopy, CLEM, TEM

Reference:

Heiligenstein, X., & Lucas, M. S. (2022). One for all, all for one: a close look at in-resin fluorescence protocols for CLEM. *Frontiers in Cell and Developmental Biology*, *10*, 866472.
<https://doi.org/10.3389/fcell.2022.866472>

Tanida, I., Yamaguchi, J., Kakuta, S., Uchiyama, Y. (2023). Osmium-Resistant Fluorescent Proteins and In-Resin Correlative Light-Electron Microscopy of Epon-Embedded Mammalian Cultured Cells. In: Sharma, M. (eds) *Fluorescent Proteins. Methods in Molecular Biology*, vol 2564. Humana, New York, NY.
<https://doi.org/10.3389/fcell.2022.866472>

Tanida, I., Yamaguchi, J., Kakuta, S., & Uchiyama, Y. (2022). Osmium-resistant fluorescent proteins and in-resin correlative light-electron microscopy of Epon-embedded mammalian cultured cells. In *Fluorescent Proteins: Methods and Protocols* (pp. 287-297). New York, NY: Springer US. doi: 10.1007/978-1-0716-2667-2_15. PMID: 36107349.