Image Anal Stereol 2026;45:153-166 doi: 10.5566/ias.4011
Review Article

CORRELATIVE LIGHT AND ELECTRON MICROSCOPY IN CELL BIOLOGY: ACCESSIBLE PRE-EMBEDDING AND POST-EMBEDDING STRATEGIES

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(Received February 23, 2026; revised June 23, 2026; accepted June 29, 2026)

ABSTRACT

Correlative light and electron microscopy (CLEM) combines the molecular specificity of fluorescence light microscopy with the ultrastructural resolution of electron microscopy (EM), enabling molecularly defined cellular structures to be localised and interpreted within their cellular ultrastructural context. Although cryo-CLEM, super-resolution CLEM, and volume EM have substantially expanded the spatial, molecular, and three-dimensional capabilities of CLEM, their routine implementation remains constrained by specialised instrumentation, demanding sample-preparation workflows, and the need for expertise in image acquisition, registration, and analysis. This review therefore focuses on conventional CLEM strategies that are accessible to laboratories equipped with standard widefield or confocal fluorescence microscopes and transmission electron microscopes. Pre-embedding and post-embedding approaches are compared, highlighting how the timing of labelling and fluorescence imaging influences key trade-offs among live-cell imaging, correlation accuracy, probe preservation, and ultrastructural integrity. Probes used in pre-embedding workflows are further evaluated, including dual-modality probes such as FluoroNanogold antibodies and quantum dots, fluorescent proteins like GFP (rendered EM-visible through secondary immunolabelling or photoconversion), and luminescent metal complexes, alongside selected protocol examples for both pre-embedding and post-embedding experimental approaches. Overall, successful CLEM depends on careful experimental design, appropriate probe selection, and optimised sample preparation. Conventional workflows remain practical and robust options for routine cell biology studies, particularly when accessibility, reproducibility, and compatibility with existing microscopy infrastructure are priorities.

Keywords: Correlative light and electron microscopy, pre-embedding, post-embedding, correlative imaging, multimodal probes, sample preparation.

INTRODUCTION

Light and electron microscopy have greatly advanced our understanding of the structure and function of specific cellular components, contributing to the resolution of many previously unexplained biological questions. Fluorescence microscopy (FM) remains one of the most widely used imaging techniques in the life sciences to this day. It enables the simultaneous detection of multiple target molecules through multicolour fluorescent labelling. Due to its high specificity and sensitivity, FM is widely used for spatial and temporal tracking of labelled molecules of interest in fixed and live cells (Anderson *et al.*, 2019). While FM allows monitoring the dynamics of individual rare events and recording of live cells in a larger field of view, it is limited to visualising only the labelled molecules. Electron microscopy (EM) on the other hand, enables the observation of the full cell ultrastructure in detail, creating a comprehensive ultrastructural map of the target sample (Spiegelhalter *et al.*, 2014; Anderson *et al.*, 2019). Combining these two techniques in correlative light and electron microscopy (CLEM) enables the correlation of fluorescent signals obtained by FM with the ultrastructural information provided by EM images (Mironov and Beznoussenko, 2013). This approach has become a powerful tool for simultaneously obtaining localisation and ultrastructural information about the same event within the same cell (Djurdjevič *et al.*, 2015).

CLEM was initially introduced more than four decades ago, but its development has accelerated substantially in recent decades (van den Dries *et al.*, 2022). Recent advances in CLEM have introduced innovative approaches that significantly enhance spatial, temporal, and volumetric resolution while preserving molecular specificity. A major development has been the integration of cryogenic techniques (cryo-CLEM), which enable imaging of vitrified specimens to maintain native ultrastructure alongside fluorescence

signals (Tuijtel *et al.*, 2019; Faul *et al.*, 2025). Super-resolution fluorescence methods such as photoactivated localisation microscopy (PALM), stimulated emission depletion microscopy (STED) or stochastic optical reconstruction microscopy (STORM) are being used in tandem with EM to pinpoint molecular locations within complex cellular environments (Jeong and Kim, 2022). Moreover, volume microscopy techniques, such as serial block-face SEM (SBF-SEM) or focused ion beam SEM (FIB-SEM) tomography, are now being correlated with confocal or super-resolution FM in three dimensions to reconstruct large cellular volumes and map molecular distributions within them (Hegermann *et al.*, 2019; Loginov *et al.*, 2022; Hayashi *et al.*, 2023). In parallel, array tomography has emerged as a versatile post-embedding CLEM approach, enabling the integration of FM and EM across serial ultrathin sections to provide three-dimensional visualisation of molecular targets within the ultrastructural context (Lane *et al.*, 2021). Workflows are becoming more automated, with improved image registration, fiducial markers that survive resin embedding, and integrated platforms allowing faster targeting of small volumes, even down to single organelles (Scher *et al.*, 2021; Loginov *et al.*, 2022). Although developments in CLEM approaches have significantly enhanced its potential, several limitations remain. Achieving precise image alignment remains a major challenge, particularly in 3D volume CLEM, where differences in resolution and sample distortion can compromise accurate correlation. Furthermore, these techniques are limited by slow imaging speeds, the small volumes that can be effectively analysed, and the vast amount of generated data, which places significant demands on storage capacity, processing power, and analysis infrastructure. Finally, the high financial cost of specialised equipment, ongoing maintenance, and consumables makes these advanced CLEM approaches inaccessible to many research laboratories.

In this review, we compare conventional straightforward approaches of two CLEM workflows: pre-embedding and post-embedding on-section techniques that are accessible to laboratories with standard EM instrumentation. We discuss the respective advantages of these CLEM approaches as well as their limitations. This review provides practical insights to help researchers choose the most appropriate CLEM strategy for their needs in standard laboratory settings.

CONVENTIONAL CLEM APPROACHES

In CLEM, the microscopist must select compatible imaging methods and understand how sample preparation affects the accuracy of signal correlation. A key component of any CLEM workflow is the precise alignment of FM and EM datasets. The accuracy of correlation is influenced by multiple factors, including the EM protocol, sample stability during processing, and the timing of FM within this sequence. FM can be performed at three main stages in sample preparation: (1) before fixation (live-cell imaging), (2) after fixation but before full EM preparation (post-fixation imaging), or (3) after all EM sample preparation steps (post-EM preparation imaging) (Gibson *et al.*, 2014; Scher and Avinoam, 2021). While these stages are often associated with pre- and post-embedding approaches, the classification of a CLEM workflow as pre- or post-embedding is generally determined by the stage at which labelling is introduced, rather than the timing of FM alone. More precisely, when an extrinsic label (antibody or chemical probe) is used, workflows are commonly classified by the timing of label introduction, as the labelling step is then the distinguishing event. When fluorescence is intrinsic and genetically encoded, the labelling step is not defining, and workflows are often instead classified by the stage at which fluorescence is imaged. This framework can be applied to both immunolabelling-based protocols and the in-resin fluorescence (IRF) variant described below. In general, pre-embedding CLEM involves labelling the sample before resin embedding, and FM is typically performed before or shortly after fixation, although in some workflows the fluorescence is imaged on resin sections after EM preparation. On the other hand, post-embedding CLEM relies on labelling after embedding and sectioning, with FM and EM imaging performed on the same sections (Fig. 1).

Importantly, genetically encoded fluorescent proteins that are expressed before embedding but can retain fluorescence throughout fixation, dehydration, and resin infiltration enable fluorescence imaging directly in resin-embedded samples or ultrathin sections (Peng *et al.*, 2022; Franzkoch *et al.*, 2024). This approach is known as in-resin fluorescence (IRF) CLEM, which refers to the detection of fluorescent signals within resin-embedded specimens (Sanada *et al.*, 2022). Although the fluorescence is present in the sample before embedding, IRF CLEM workflows are typically classified as post-embedding CLEM rather than pre-embedding approaches, and classification in this case depends on when and in which sample state the fluorescence is acquired.

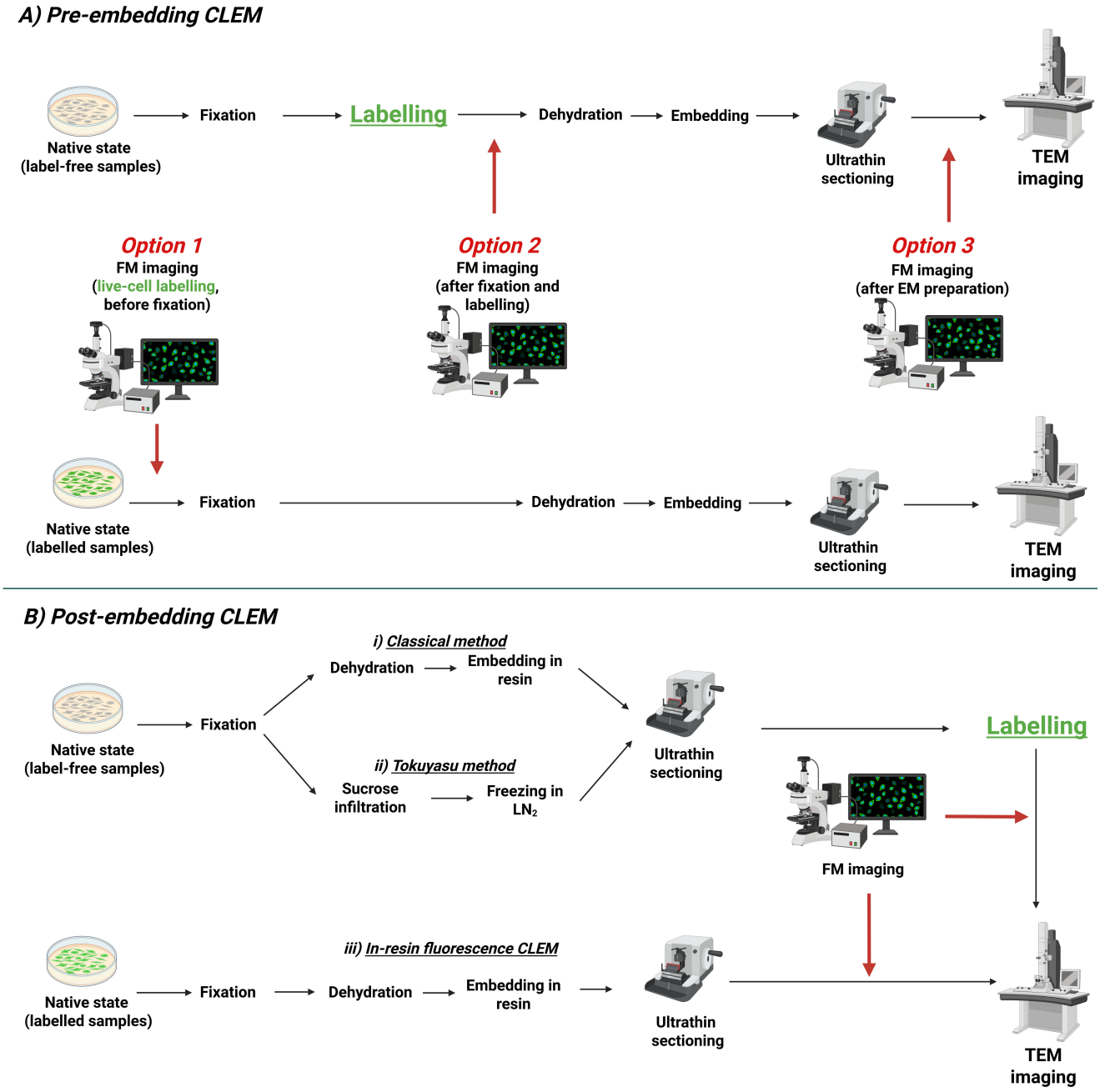

Fig. 1. *Timing of labelling and fluorescence microscopy (FM) within CLEM sample preparation. (A) In the pre-embedding CLEM approach, the sample is labelled before resin embedding, and FM imaging can be performed at three stages: before fixation (live-cell imaging, Option 1), after fixation but before embedding (post-fixation imaging, Option 2), or after ultrathin sectioning (on-section imaging, Option 3). Pre-embedding CLEM therefore refers primarily to the stage at which labelling is introduced, rather than the exact timing of FM acquisition. (B) In the post-embedding approach, the sample is first embedded and sectioned, and fluorescence is detected on ultrathin sections. Three variants are illustrated: (i) classical post-embedding CLEM after conventional dehydration and embedding in epoxy or acrylic resin (e.g. Epon or Lowicryl), with labelling and FM imaging performed on-sections; (ii) the Tokuyasu method, based on sucrose infiltration, freezing and cryosectioning, followed by labelling and FM imaging on cryo-sections; and (iii) in-resin fluorescence CLEM, in which fluorescently tagged proteins expressed before embedding retain fluorescence throughout fixation, dehydration and resin infiltration, enabling FM directly on resin-embedded samples or sections. TEM, transmission electron microscopy. At the final step, the same region is imaged by FM and TEM, and the two datasets are overlaid (FM–EM correlation. Created with BioRender.com.)*

Across the literature, there is some variability in the terminology used to describe pre- and post-embedding CLEM workflows, and although the majority define CLEM approaches by the timing of labelling, there are still studies that define CLEM approaches by the stage of fluorescence imaging or even by a combination of both criteria (for example, a pre-embedding labelling and post-embedding imaging approach). In this review, we apply this classification rule consistently: pre-embedding approaches are those in which labelling is performed before resin embedding, while post-embedding approaches are those in which labelling is introduced after embedding. An exception is made for IRF CLEM, where fluorescent signals are generated before embedding but detected post-embedding, i.e. in resin-embedded specimens. Importantly, to maintain consistency with the primary literature, our classification also follows the original pre- and post-embedding terminology used in the cited studies.

Regardless of this classification, the timing of FM in the workflow determines the balance between temporal resolution, spatial correlation, and experimental throughput. Live cell imaging provides maximal temporal resolution and preserves native cellular dynamics, although accurate correlation with subsequent EM datasets may not always be straightforward, as structural changes can occur between live cell imaging, fixation, and subsequent EM sample preparation. Post-fixation imaging enables high-precision correlation and compatibility with most EM techniques but sacrifices real-time temporal information and risks deformation of the sample during subsequent EM processing. FM imaging after full EM preparation is limited to a preselected EM technique, but it provides the most accurate spatial correlation, as both FM and EM image the sample after it has been fully processed and stabilised (Scher and Avinoam, 2021).

In many laboratories, however, the available equipment determines which CLEM approach can be used. As a result, researchers focus on understanding the method's strengths and limitations while optimizing the protocol as effectively as possible (Fig. 2).

PRE-EMBEDDING CLEM APPROACHES

The pre-embedding approach involves labelling biological samples with fluorescent probes or antibodies before embedding the sample in resin for EM. It enables fluorescence imaging of the specimen in a near-native, hydrated state, allowing precise localisation of target molecules (Boassa, 2015). After fluorescence imaging, the sample undergoes processing and resin embedding for EM. The present review evaluates and compares several straightforward approaches to the pre-embedding CLEM method that can be performed in standard laboratory settings. Specifically, we selected three representative methodological studies that provide detailed and experimentally validated pre-embedding CLEM protocols. Their respective advantages and limitations are summarised in Table 1, which highlights the technical complexity of each approach and its suitability for different experimental questions. A broader discussion of CLEM labelling and imaging strategies is provided in the main text.

The first approach describes the pre-embedding CLEM method, which involves transfecting cells with constructs encoding proteins of interest fused to fluorescent proteins (e.g., GFP and its spectral variants CFP and YFP, as well as red fluorescent proteins such as DsRed or mCherry). This enables live fluorescence imaging to identify protein localisation and monitor dynamic processes (Polishchuk and Polishchuk, 2013). To visualise the same GFP-tagged proteins at the EM level following fluorescence imaging and fixation but prior to resin embedding, nanogold-conjugated secondary antibodies are used to label the fluorescently tagged proteins, providing electron-dense markers after silver or gold enhancement. This technique allows precise correlation between the fluorescent and the corresponding electron-dense signal, ensuring good spatial accuracy (Polishchuk *et al.*, 2000; Polishchuk and Polishchuk, 2013). For example, this approach was used to resolve the fragmented Golgi architecture in differentiated uroepithelial cells (Kreft *et al.*, 2010) and to dissect the trafficking pathways responsible for this remodelling (Višnjar *et al.*, 2017).

A variation of the pre-embedding immunolabelling approach, instead of transfection, uses antibodies conjugated simultaneously with a fluorophore and nanogold particles known as FluoroNanogold antibodies (Table 2). They consist of a 1.4 nm gold particle and a fluorescent molecule coupled to an antibody Fab' fragment, IgG, or streptavidin and have high labelling efficacy due to their small size (Takizawa *et al.*, 2015; Peddie and Schieber, 2019). These dual probes enable the same antibody to be visualised by FM, via the fluorochrome signal, and by EM, after silver or gold enhancement of the nanogold particles. Using FluoroNanogold antibodies again allows precise correlation of fluorescent and electron-dense signals, but without the need for separate labelling steps. As with other pre-embedding immunolabelling strategies, FluoroNanogold labelling is performed on fixed and

Table 1. *Comparison of different protocols for the pre-embedding CLEM approach with advantages and limitations.*

Pre-embedding CLEM approach	Timing and protocol	Advantages	Disadvantages and reference
Fluorescent protein-based pre-embedding CLEM with immunogold labelling	**Timing:** FM imaging before EM processing. **FM:** Transfection of cells with chimeric constructs of the target protein fused with GFP; stable cell lines expressing the GFP-fusion protein can also be used. Live-cell imaging is performed on gridded imaging dishes with a confocal microscope. **EM:** Cell fixation; primary antibody against GFP; secondary antibody coupled to 1.4 nm gold particles; gold enhancement reaction; post-fixation; embedding in Epon; ultrathin sectioning; transmission EM.	FM reveals dynamic and functional data (e.g., protein trafficking), while EM shows precise membrane organization and organelle morphology in the same sample.	During processing of the sample for EM, physical distortions (e.g., shrinkage, sectioning artefacts) can complicate correlation between FM and EM datasets. **Ref.:** (Polishchuk and Polishchuk, 2013)
Luminescent metal complex-based dual-modality pre-embedding CLEM	**Timing:** FM imaging before EM processing. **FM:** Incubation of live cells with *Iridium complex 1*. Live-cell imaging is performed on gridded imaging dishes with a confocal microscope. **EM:** Cell fixation; embedding in Epon; ultrathin sectioning; transmission EM.	The same metal complex provides both luminescence (for FM) and electron scattering contrast (for EM), which improves alignment accuracy. Metal complexes can be designed for targeting organelles or even specific proteins.	Metal complexes are synthetic compounds that often require multistep synthesis and purification and are not widely commercially available. Iridium- and ruthenium-based complexes provide weaker contrast than osmium tetroxide or uranyl acetate staining. **Ref.:** (Shewring *et al.*, 2021)
Antibody-based pre-embedding CLEM with resin-compatible fluorescence detection	**Timing:** FM imaging after EM processing. **FM/EM:** Cell fixation; preparation of frozen sections; immunolabelling with primary and secondary antibodies, followed by streptavidin-conjugated Fluolid NS Orange for fluorescence detection; post-fixation for EM; Durcupan™ ACM epoxy resin embedding; ultrathin sectioning. The same regions of sections on a finder grid with navigation markers are observed first with a fluorescent microscope and then in a transmission electron microscope.	Usage of a fluorescent probe that does not exhibit fluorescence quenching due to post-fixation and resin embedding, and an electron beam-stable resin. The fluorescent signal marks the exact ultrastructural location of the antigen, improving correlation accuracy between LM and EM.	Loss of live imaging capability. No specific labelling on the ultrastructural level. **Ref.:** (Kanemaru *et al.*, 2021)

CLEM, correlative light and electron microscopy; FM, fluorescence microscopy; EM, electron microscopy; LM, light microscopy; GFP, green fluorescent protein; CFP, cyan fluorescent protein; YFP, yellow fluorescent protein.

Table 2. *Comparison of dual probes frequently used in CLEM.*

	FluoroNanogold	Quantum dots	Luminescent metal complexes
Size	Very small gold core (∼1.4 nm); hydrodynamic size ∼3–5 nm	Large (∼10–25 nm including shell and surface coatings)	Very small (∼1–2 nm)
Fluorescence brightness	Moderate	Excellent	Moderate, depending on the complex
Photostability	Moderate to good	Excellent	Good
EM contrast without enhancement	Poor	Poor to moderate	Poor
EM contrast with enhancement	Excellent	Not required	Not required
Silver/gold enhancement	Yes	No	No
Epitope accessibility	High	Low	Excellent

EM, electron microscopy.

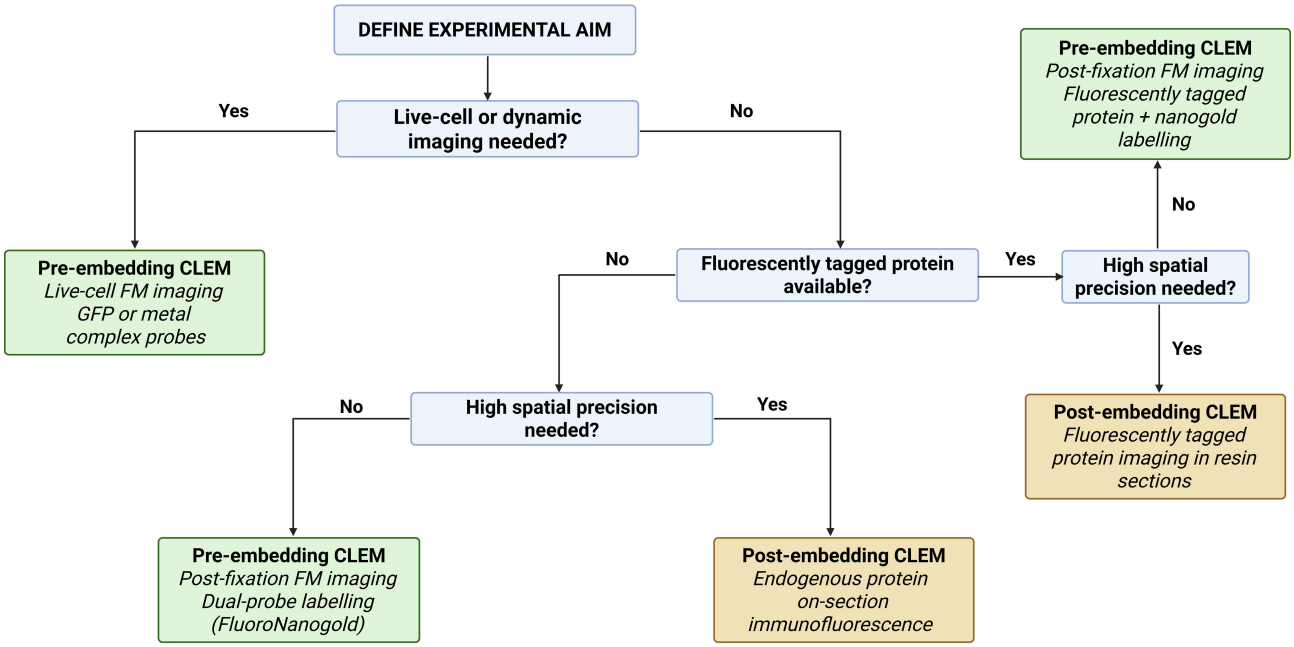

Fig. 2. *Decision flowchart for selecting a conventional CLEM workflow based on experimental priorities. The first decision distinguishes between live-cell and fixed-cell approaches. Subsequent decisions consider the availability of fluorescently tagged protein and the requirement for spatial precision. Green boxes indicate pre-embedding CLEM approaches; amber boxes indicate post-embedding CLEM approaches. Note that when a fluorescently tagged protein is available, the choice between pre- and post-embedding depends on whether the fluorophore retains sufficient signal after resin processing. FM, fluorescence microscopy; GFP, green fluorescent protein. Created with BioRender.com.*

permeabilised cells before resin embedding, with the silver or gold enhancement step also carried out prior to embedding. Nevertheless, due to their small size, nanogold particles in both described approaches cannot be directly visualised with EM and require silver or gold enhancement to increase their size and electron density (Brown and Verkade, 2010; de Boer *et al.*, 2015). This enhancement step can introduce artefacts, such as uneven particle growth, non-specific background deposition, or loss of spatial resolution, which may compromise the accuracy of protein localisation (Robinson *et al.*, 2000; Slot and Geuze, 2007). Additionally, the chemical conditions required for silver or gold enhancement can alter tissue morphology or quench fluorescence signals if not carefully optimized (Takizawa *et al.*, 2015). Besides nanogold, antibodies can also be conjugated with horseradish peroxidase (HRP) for immunoperoxidase labelling. HRP catalyses diaminobenzidine (DAB) polymerisation into a precipitate that becomes electron-dense after osmium tetroxide treatment. Unlike FluoroNanogold, however, HRP-conjugated antibodies typically lack a fluorescent counterpart and must therefore be paired with a separate fluorescent label for correlation. A further limitation is that diffusion of the DAB product from the reaction site can compromise the detection of soluble cytosolic targets, whereas labelling within membrane-bound compartments remains efficient because the membrane confines the precipitate (Polishchuk and Polishchuk, 2013).

Quantum dots (QDs) are another widely used dual probe (Table 2). These are semiconductor nanocrystals that exhibit bright and highly photostable fluorescence with narrow emission spectra, while their inorganic cores provide some intrinsic electron density, allowing direct EM detection (Byers and Hitchman, 2011). Nevertheless, EM detectability is a limitation of these probes, as their inorganic cores are typically weakly electron dense and can be difficult to distinguish from the background. In addition, variability in quantum dot size can further complicate interpretation (Walther, 2015; Le *et al.*, 2020). QDs are larger than gold

nanoparticles used in FluoroNanogold antibodies and can be conjugated to antibodies or other biomolecules for targeting. Due to their coatings, QDs can reach an effective hydrodynamic size of 10–25 nm, which may hinder epitope accessibility and compromise the precision of protein localisation at the ultrastructural level (Francis *et al.*, 2017). Taken together, QDs are best suited for CLEM applications primarily driven by light microscopy, where EM serves mainly a confirmatory role, or when fluorescence imaging is used to preselect regions of interest within large specimens.

An alternative approach describes luminescent metal complexes as an alternative solution for use as dual probes in CLEM (Table 2). These complexes contain an electron-dense transition-metal centre, most commonly from iridium, ruthenium, or rhenium ions, coordinated by organic ligands that define the luminescence properties and influence organelle or protein targeting (Shewring *et al.*, 2021; Lee and Lo, 2022). These probes are small and well-defined in size, highly photostable, and resistant to EM sample preparation conditions. However, their application may be limited by restricted commercial availability, potential cytotoxicity, and the need for complex chemical synthesis or functionalisation to achieve specific targeting (Holden *et al.*, 2021; Lee and Lo, 2024). In protocols of this type, osmium tetroxide post-fixation is often reduced or omitted, because the metal complex itself provides substantial electron contrast (Shewring *et al.*, 2021) and conventional heavy-metal contrasting is well known to impair fluorescence signals (van den Dries *et al.*, 2022). Overall, a major advantage of dual probes compared to fluorescently tagged proteins combined with post-embedding nanogold immunolabelling is the better correlation accuracy between FM and EM signals. Since the same probe delivers both fluorescence and electron contrast, spatial registration errors introduced by separate labelling steps are minimized.

However, independent of the probe used, a drawback of the pre-embedding CLEM approach is that the sample may undergo structural and molecular changes during the embedding step (Perkovic *et al.*, 2014; Bykov *et al.*, 2016), which can compromise the correlation accuracy between the light and EM images. Many fluorescent probes are incompatible with EM protocols, often resulting in substantial fluorescence quenching or complete signal loss. Nevertheless, certain fluorophores have been shown to withstand osmication and resin infiltration, retaining sufficient fluorescence to allow imaging after embedding. Kanemaru et al. describe a pre-embedding CLEM protocol in which the sample is first labelled with the fluorophore Fluolid NS Orange, processed for EM and finally imaged by both FM and EM (Kanemaru *et al.*, 2021). The Fluolid NS Orange is characterized by high chemical stability and resistance to osmium tetroxide–induced quenching, which is a limitation of many conventional dyes. This allows the fluorescent signal to be preserved after fixation, osmication, and embedding in the epoxy resin Durcupan, allowing FM to be performed after completion of all EM processing steps, directly on ultrathin resin sections. In this workflow, antibody labelling is carried out on sectioned tissue prior to resin embedding rather than on intact specimens, which facilitates more uniform antibody penetration and mitigates the limited access that antibodies typically have in thicker samples. By imaging fluorescence and ultrastructure on the same physical sections, the method minimises errors in spatial alignment and eliminates the need for post-embedding immunolabelling or external fiducial markers. Furthermore, in this protocol the antigen itself is not directly labelled with an electron-dense marker; instead, its ultrastructural position is inferred by correlating the preserved Fluolid NS Orange fluorescence with the osmium-stained ultrastructure in the same resin section, so that localisation at the EM level is correlative rather than direct. Importantly, the approach maintains high-quality ultrastructural preservation while remaining experimentally simple, making it a practical solution for the CLEM workflow.

Taken together, among these pre-embedding approaches, live-cell fluorescence imaging is feasible with genetically encoded fluorescent proteins and cell-permeant luminescent metal complexes, whereas antibody-based labels such as FluoroNanogold or HRP require prior fixation and permeabilisation. In the resin-stable Fluolid NS Orange protocol, labelling is performed on frozen sections before embedding, but the fluorescence is imaged only after EM processing, on ultrathin resin sections.

POST-EMBEDDING CLEM APPROACHES

In the post-embedding CLEM approach, labelling is performed after the sample has been fully processed, either embedded in resin or frozen and prepared according to the Tokuyasu method, with both imaging modalities carried out on the same ultrathin sections (Figure 1B). This sequential on-section workflow eliminates the structural distortions introduced during embedding that can compromise correlation in pre-embedding protocols and offers the highest spatial precision of all conventional CLEM

strategies (Bykov *et al.*, 2016). A critical determinant of success in post-embedding CLEM is the choice of embedding resin, which must balance ultrastructural preservation with retention of both fluorescence and antigenicity. Acrylic resins such as LR White, valued for their hydrophilic nature, and methacrylate resins such as Lowicryl HM20, favoured for their low-temperature polymerisation properties, are the preferred choices for post-embedding immunolabelling and fluorescence imaging due to their compatibility with fluorescent proteins and preservation of epitope accessibility (Tanida *et al.*, 2020; Heiligenstein and Lucas, 2022). By contrast, epoxy resins, though widely used in conventional EM for their excellent ultrastructural preservation, are not recommended for post-embedding CLEM. Their hydrophobic character promotes cross-linking with biological material, which quenches fluorescence signals and severely reduces antigen accessibility (Koistinen *et al.*, 2016; Heiligenstein and Lucas, 2022). However, interest in epoxy-based post-embedding CLEM has recently been renewed, particularly through IRF CLEM approaches. In these approaches, fluorescent signal is not introduced after embedding, but are preserved through osmium tetroxide staining, room-temperature dehydration and resin embedding, enabling fluorescence imaging directly in resin-embedded samples. Franzkoch et al. demonstrated that certain synthetic dyes, including Janelia Fluor JFX549 and JFX554 conjugated to self-labelling enzyme tags such as HaloTag, retain fluorescence after conventional Epon embedding, osmium tetroxide staining, and room-temperature dehydration. The fluorescent protein StayGold was also found to resist conventional Epon embedding in this system, enabling dual-fluorescence in-resin CLEM without the need for specialised low-temperature resins (Franzkoch *et al.*, 2024).

Staining with heavy metals, routinely used to enhance contrast in EM presents an additional challenge, as it can further compromise both epitope presentation and fluorescence signal (Kandela and Albrecht, 2007; Heiligenstein and Lucas, 2022). High-pressure freezing (HPF) followed by freeze substitution (FS) can help mitigate these effects, improving antigen preservation and allowing post-embedding labelling while still providing sufficient EM contrast (Lonsdale *et al.*, 1999; de Boer *et al.*, 2015). Similarly, the post-embedding CLEM approach can be applied to Tokuyasu ultrathin cryo-sections, which are prepared from aldehyde-fixed, sucrose-infiltrated specimens and sectioned at cryogenic temperatures. This alternative offers superior preservation of antigenicity and is well suited to post-embedding immunolabelling, though it requires access to specialised cryo-ultramicrotomy equipment (de Boer *et al.*, 2015; Möbius and Posthuma, 2019).

In line with the pre-embedding approaches, we also evaluate and compare three representative and experimentally validated post-embedding CLEM protocols that can be implemented in standard laboratory settings. Each is described in a methodological study providing detailed protocols, and their respective advantages and limitations are summarised in Table 3.

The first post-embedding approach, described by Kukulski et al., combines HPF and FS with Lowicryl HM20 low-temperature embedding and uses fluorescent fiducial markers, specifically TetraSpeck multicolour beads deposited on the surface of ultrathin sections, to enable precise co-registration of FM and EM images (Kukulski *et al.*, 2012). The fiducials are visible in both modalities and serve as spatial reference points for the image overlay algorithm, achieving <100 nm registration accuracy. Sections are first imaged by a confocal microscope to record fluorescent protein signals and fiducial positions, and the same grid is subsequently transferred to the TEM for ultrastructural imaging, with the fiducials enabling accurate transformation of one dataset onto the other. This method is particularly well suited to the localisation of endogenously expressed or fluorescently tagged proteins within their ultrastructural context and is compatible with standard confocal and transmission electron microscopes.

A second approach, described by Peng et al., describes IRF CLEM approach. It identifies novel genetically encoded fluorescent protein variants that are expressed by the cells intrinsically. These proteins retain sufficient brightness after mild osmication, dehydration, and Lowicryl HM20 embedding, permitting dual-colour post-embedding CLEM without immunolabelling (Peng *et al.*, 2022). The fluorescent proteins selected for this approach, including mEos4b and mMaple3, were originally developed as fixation-resistant photoactivatable variants specifically designed to withstand aldehyde fixation and resin embedding (Paez-Segala *et al.*, 2015). Since the fluorescence is preserved on ultrathin resin sections, FM can be performed after embedding, so the workflow is considered post-embedding CLEM, despite the signal being intrinsic. For this approach ultrathin sections are imaged directly by widefield FM, followed by TEM of the same sections, with grid squares used as navigational landmarks for region identification. This approach is advantageous in that it does not require optimisation of antibody-based labelling on-sections and the fluorescent signal directly reports on the protein of interest. However, it is limited to genetically encoded proteins and depends on the

Table 3. *Comparison of different protocols for the post-embedding CLEM approach with advantages and limitations.*

Post-embedding CLEM approach	FM and EM protocol	Advantages	Disadvantages and reference
Fluorescent protein–based post-embedding CLEM on resin sections with fiducial marker registration	Cells expressing classic fluorescently tagged proteins (GFP/RFP). HPF + FS and embedding in Lowicryl HM20 at low temperature. Ultrathin sections on finder grids. FM of GFP/RFP on Lowicryl ultrathin sections. TetraSpeck fiducial beads for image registration. Transmission EM.	Nanometre-scale correlation precision. Fiducials visible in both FM and EM. Compatible with standard confocal and transmission EM.	Requires HPF equipment. No live imaging. Limited to tagged or endogenous proteins detectable by FM. **Ref.:** (Kukulski *et al.*, 2012)
Fluorescent protein–based post-embedding CLEM on resin sections (in resin fluorescence CLEM)	Cells expressing improved fluorescently tagged constructs (mEos4b, mMaple3). Chemical fixation, mild osmication and embedding in Lowicryl HM20. Ultrathin sections on grids. FM of improved fluorescent proteins directly on Lowicryl ultrathin sections. Grid squares as navigation landmarks. Transmission EM.	Dual-colour CLEM without immunolabelling. Direct FP signal - no antibody optimisation required.	Limited to fluorescently tagged proteins. Not all FPs survive EM processing. Requires stable cell lines. No specific labelling on the ultrastructural level. **Ref.:** (Peng *et al.*, 2022)
Antibody-based post-embedding CLEM on Tokuyasu cryo-sections	Mild chemical fixation. The sample is infiltrated with sucrose and frozen in liquid nitrogen. Ultrathin sections are cut at cryogenic temperature. Immunofluorescence on Tokuyasu ultrathin cryo-sections using primary antibodies and fluorophore-conjugated secondary antibodies for FM; optional immunogold labelling using Protein A–gold for EM, or dual labelling with FluoroNanogold. Standard FM. Transmission EM.	High preservation of antigenicity. Endogenous protein detection. Near-native structural preservation. Compatible with dual labelling (FM+EM).	Need for cryo-ultramicrotome, which is not standard laboratory equipment. **Ref.:** (van der Beek *et al.*, 2023)

CLEM, correlative light and electron microscopy; FM, fluorescence microscopy; EM, electron microscopy; HPF, high-pressure freezing; FS, freeze substitution; FP, fluorescent protein; mEos4b, monomeric Eos fluorescent protein 4b; mMaple3, monomeric Maple fluorescent protein 3.

availability of cell lines or organisms expressing the appropriate constructs.

Although the protocol described by Peng et al. (Table 3) seems functionally analogous to that described by Kanemaru et al. (Table 1) in the context of CLEM workflow, they differ in how the fluorescent signal is introduced, comparing genetically encoded fluorescence signal versus classical immunolabelling (Kanemaru *et al.*, 2021; Peng *et al.*, 2022). Peng et al. use novel genetically encoded fluorescence, preserved through embedding in an IRF CLEM approach, representing a post-embedding workflow, while the protocol of Kanemaru requires introduction of fluorescently labelled antibodies before embedding, making it consistent with a pre-embedding workflow, despite the fluorescence remaining detectable after embedding (Kanemaru *et al.*, 2021). In both workflows, however, the fluorescence signal is correlated with ultrastructural features in EM without the use of electron-dense markers such as immunogold labelling. Consequently, the resulting datasets provide indirect, correlative localization rather than definitive molecular identification at the EM level.

A third post-embedding approach, described by van der Beek et al. demonstrates on-section CLEM for endogenous proteins using standard immunofluorescence labelling of Tokuyasu ultrathin cryo-sections (van der Beek *et al.*, 2023). Following mild chemical fixation, samples are cryoprotected by sucrose infiltration and frozen in liquid nitrogen, and ultrathin sections are cut at cryogenic temperature. Ultrathin cryo-sections are collected on grids and incubated with primary antibodies, followed by fluorophore-conjugated secondary antibodies for fluorescence imaging. In purely fluorescence-based CLEM, no EM labelling is required, as fluorescence signals can be directly correlated with ultrastructural features such as specific organelles. If EM localisation is required, immunogold labelling can be incorporated in the same workflow after FM labelling using Fc binding probes, such as colloidal gold particles conjugated to protein A (van der Beek *et al.*, 2023). Protein A–gold binds to the Fc region of IgG antibodies already bound to the antigen and therefore does not interfere with antigen–antibody binding. However, in densely labelled areas, steric hindrance from fluorophore-conjugated secondary antibodies may restrict access to antibody Fc regions, potentially reducing the efficiency of protein A–gold binding (Roth and Heitz, 1989). Alternatively, dual fluorophore/gold secondary antibodies (e.g., FluoroNanogold) can be used, avoiding the need for an additional Fc-binding step and providing a single probe for both FM and EM. FluoroNanogold, however, requires silver or gold enhancement to achieve sufficient EM contrast,

which can occasionally introduce non-specific metal deposition artefacts (Joshi *et al.*, 2010). QDs may likewise be applied on-sections, offering bright, photostable fluorescence together with direct electron density (Nisman *et al.*, 2004), although their larger size can restrict epitope accessibility and their EM contrast is often weaker than that of protein A–gold. Protein A–gold therefore remains particularly advantageous on Tokuyasu sections, where its defined particle size and the pale-membrane background result in clear EM signal. After FM and EM labelling, the ultrathin sections are first imaged by FM. Following contrast staining of the grids, the corresponding areas are relocated using cellular landmarks, such as cell nuclei, and subsequently imaged by TEM (van der Beek *et al.*, 2023). Importantly, this workflow does not require genetic manipulation of the biological sample, making it broadly applicable in standard research settings without the need for stable cell lines or transfection protocols. A limitation of this approach is that it requires access to a cryoultramicrotome, which may not be available in all laboratories.

The EM detection step in post-embedding CLEM does not necessarily require additional protein-specific labelling, and the choice of detection strategy depends on the experimental question. In the approaches described by Kukulski et al., and Peng et al., the ultrastructure of the resin section itself serves as the EM readout and conventional TEM imaging provides the ultrastructural context into which the fluorescent signal is correlated, without the use of additional EM-specific labels (Kukulski *et al.*, 2012; Peng *et al.*, 2022). This is one of the strengths of post-embedding CLEM: when the fluorescent signal is sufficiently preserved in the resin, the correlation between molecular localisation and ultrastructure can be achieved directly. When detection of a specific protein at the ultrastructural level is required, on-section immunogold labelling can be added, as demonstrated by van der Beek et al. They used colloidal gold particles of defined size, typically 5–15 nm, conjugated to secondary antibodies or protein A and applied them directly to the section surface (van der Beek *et al.*, 2023). In contrast to smaller probes such as FluoroNanogold, which require silver or gold enhancement, these larger particles are inherently electron-dense and do not require additional enhancement steps. Multiplexed labelling of two or more targets can be achieved by using gold particles of different sizes in combination, though the ability to resolve closely spaced labels decreases as section thickness increases (Mayhew and Lucocq, 2011).

Image registration in post-embedding CLEM is inherently more straightforward than in pre-embedding workflows, because both FM and EM are performed on the same physical section, so no additional sample-preparation steps occur between the two imaging modalities. This reduces the geometric distortions that complicate correlation, although some deformation (for example, beam-induced shrinkage during EM) can still occur. Spatial correlation can be achieved using fluorescent fiducial beads, the holes or alphanumeric patterns of the EM support grid, or dedicated finder grids as common reference landmarks visible in both modalities (Kukulski *et al.*, 2012; Peng *et al.*, 2022; van der Beek *et al.*, 2023). After image acquisition, semi-automated algorithms use these reference points to transform and overlay the two datasets. Because the resin section is rigid, a simple affine transformation, which preserves straight lines and relative distances, is generally sufficient for accurate alignment (Paul-Gilloteaux *et al.*, 2017; Krentzel *et al.*, 2025).

Overall, the principal advantage of the post-embedding CLEM approach over pre-embedding strategies is the high correlation accuracy achievable when both FM and EM are performed on identical physical sections, minimising registration errors arising from sample deformation or volume changes (Bykov *et al.*, 2016; Scher and Avinoam, 2021). However, this approach sacrifices live-imaging capability entirely, and the fluorescence signal available after full resin EM sample preparation is invariably weaker than what can be obtained in pre-embedding workflows (Gibson *et al.*, 2014; Scher and Avinoam, 2021). The relatively small number of fluorescent proteins and synthetic dyes that can withstand Lowicryl embedding and mild heavy-metal contrasting remains a practical constraint, and careful optimisation of fixation and staining conditions is required for each new target and sample type (Heiligenstein and Lucas, 2022). Furthermore, the fluorescence signal in post-embedding CLEM is generally weaker because imaging is performed on ultrathin sections (typically 60–80 nm), representing only a fraction of the cell volume, rather than on entire cells as in pre-embedding approaches. Notably, the Tokuyasu method is often considered the preferable approach, as it preserves samples in a near-native state through mild chemical fixation and cryogenic processing following freezing. In contrast to conventional resin-embedded samples, which undergo dehydration and heavy metal staining that can compromise antigenicity and alter cellular ultrastructure, this approach preserves membrane morphology and cytoplasmic architecture while maintaining epitope accessibility (Möbius and Posthuma, 2019). It also enhances the visibility of electron-dense gold particles, as membranes in Tokuyasu ultrathin cryo-sections appear pale in TEM,

unlike the darkly stained membranes of osmicated resin-embedded samples.

COMMON TROUBLESHOOTING ISSUES IN CLEM

Despite careful optimisation, CLEM workflows frequently encounter technical challenges arising from the conflicting requirements of FM and EM sample preparation. Table 4 summarises the most common problems, their likely causes, and practical solutions applicable in standard laboratory settings.

CONCLUSIONS

Despite recent advances in cryo-EM (Guaita *et al.*, 2022), super-resolution microscopy (Bond *et al.*, 2022), and volume EM (McCafferty *et al.*, 2024), conventional pre-embedding and post-embedding CLEM workflows remain highly relevant, as biologically meaningful questions can be effectively addressed using optimized strategies compatible with standard FM and transmission EM (Polishchuk and Polishchuk, 2019; van den Dries *et al.*, 2022).

The choice between pre-embedding and post-embedding CLEM ultimately depends on experimental priorities. Pre-embedding approaches offer greater flexibility and compatibility with dynamic or near-native imaging conditions but require careful control of fluorescence preservation and embedding-induced distortions (Polishchuk and Polishchuk, 2019; Heiligenstein *et al.*, 2021). Post-embedding approaches offer superior spatial precision and structural stability by imaging both modalities on the same sections, though at the expense of temporal resolution and, in some cases, antigen accessibility (Kukulski *et al.*, 2011; Heiligenstein and Lucas, 2022).

Table 4. *Technical challenges and solutions in correlative light and electron microscopy.*

Problem	Possible cause	Practical solution	CLEM approach
Loss of sections	Poor grid adhesion; harsh washes	Use formvar/carbon-coated grids, apply glow-discharge to the coated grids before use, and handle with minimal agitation.	Pre- and post-embedding
High background in FM	Non-specific antibody binding; high antibody concentration	Increase the concentration of blocking solution (1–5% BSA/serum), increase blocking time (60 min), include detergent (i.e. 0.05% Tween), and optimize antibody dilution.	Pre- and post-embedding
Grid contamination	Dust, dirty solutions, poor handling	Work in a clean environment, use filtered solutions, and use clean tools and covered containers.	Pre- and post-embedding
Fluorescence loss	Photobleaching; resin quenching	Minimize light exposure, use anti-fade reagents, and choose robust fluorophores.	Pre- and post-embedding
Poor correlation LM–EM	Sample drift; shrinkage	Use fiducial markers and minimize delay between LM and EM imaging.	Pre- and post-embedding
Weak labelling in EM	Epitope masking due to fixation	Optimize fixation by reducing glutaraldehyde concentration or fixation time, and consider antigen retrieval.	Pre- and post-embedding
Section compression or wrinkling	Suboptimal knife angle and/or cutting speed; inappropriate temperature; static charge build-up due to insufficient air ionisation in cryo-ultrathin sectioning	Optimize ultramicrotomy settings by adjusting cutting speed and angle, maintain stable temperature, use a clean and undamaged diamond knife, and use an ioniser during cryo-ultrathin sectioning.	Pre- and post-embedding
Limited antibody penetration into resin sections	Resin hardness; section thickness	Switch to more permeable resins (Lowicryl vs. Epon), reduce section thickness (50–70 nm), or use Tokuyasu cryo-ultrathin sections.	Post-embedding
Autofluorescence interference	Fixation- or resin-induced signal	Choose low-autofluorescence resins (LR White or Lowicryl), reduce glutaraldehyde, and include negative controls.	Pre- and post-embedding (fixation-related); post-embedding (resin-related)
Poor contrast in EM	Weak, uneven, or contaminated staining	Use freshly prepared uranyl acetate and lead citrate, control staining time, and rinse well to avoid artefacts.	Pre- and post-embedding

BSA, bovine serum albumin; CLEM, correlative light and electron microscopy; EM, electron microscopy; FM, fluorescence microscopy; LM, light microscopy.

Successful CLEM relies not solely on technological sophistication but also on thoughtful experimental design, appropriate probe selection, careful sample preparation, and proper image acquisition (de Boer *et al.*, 2015; Tanner *et al.*, 2023). By prioritising methodological clarity and accessibility, conventional CLEM workflows continue to provide robust correlations between molecular localisation and ultrastructural context, making them a practical foundation for laboratories entering the CLEM field or addressing routine cell biology questions (Mäntylä and Verkade, 2024; Jiang and Dickson, 2025).

FUNDING

The authors acknowledge the financial support of the Slovenian Research and Innovation Agency (Research Core Funding No. P3-0108 and MRIC UL IP-0510 Infrastructure Program).

DATA AVAILABILITY STATEMENT

No original research data were generated in this review article. The manuscript synthesizes information from previously published studies, all of which are cited in the reference list. Data sharing is not applicable.

REFERENCES

Anderson K, Nilsson T, Fernandez-Rodriguez J (2019). Challenges for clem from a light microscopy perspective. In: Verkade P, Collinson L (eds). Correlative imaging: Focusing on the future. Chichester, West Sussex, United Kingdom: John Wiley & Sons Ltd. 23-35.

Boassa D (2015). Correlative microscopy for localization of proteins in situ: Pre-embedding immunoelectron microscopy using fluoronanogold, gold enhancement, and low-temperature resin. Methods Mol Biol 1318:173-80.

Bond C, Santiago-Ruiz AN, Tang Q, Lakadamyali M (2022). Technological advances in super-resolution microscopy to study cellular processes. Mol Cell 82:315-32.

Brown E, Verkade P (2010). The use of markers for correlative light electron microscopy. Protoplasma 244:91-7.

Byers RJ, Hitchman ER (2011). Quantum dots brighten biological imaging. Prog Histochem Cytochem 45:201-37.

Bykov YS, Cortese M, Briggs JA, Bartenschlager R (2016). Correlative light and electron microscopy methods for the study of virus-cell interactions. FEBS Lett 590:1877-95.

de Boer P, Hoogenboom JP, Giepmans BN (2015). Correlated light and electron microscopy: Ultrastructure lights up! Nat Methods 12:503-13.

Djurdjevič I, Kreft ME, Sušnik Bajec S (2015). Comparison of pigment cell ultrastructure and organisation in the dermis of marble trout and brown trout, and first description of erythrophore ultrastructure in salmonids. J Anat 227:583-95.

Faul N, Chen S-Y, Lamberz C, Bruckner M, Dienemann C, Burg TP (2025). Cryo-iclem: Cryo correlative light and electron microscopy with immersion objectives. Journal of Structural Biology 217:108179.

Francis JE, Mason D, Lévy R (2017). Evaluation of quantum dot conjugated antibodies for immunofluorescent labelling of cellular targets. Beilstein Journal of Nanotechnology 8:1238-49.

Franzkoch R, Wilkening S, Liss V, Holtmannspötter M, Kurre R, Psathaki OE, Hensel M (2024). Rapid in-epon clem: Combining fast and efficient labeling of self-labeling enzyme tags with em-resistant janelia fluor dyes and staygold. Heliyon 10:e28055.

Gibson KH, Vorkel D, Meissner J, Verbavatz JM (2014). Fluorescing the electron: Strategies in correlative experimental design. Methods Cell Biol 124:23-54.

Guaita M, Watters SC, Loerch S (2022). Recent advances and current trends in cryo-electron microscopy. Curr Opin Struct Biol 77:102484.

Hayashi S, Ohno N, Knott G, Molnár Z (2023). Correlative light and volume electron microscopy to study brain development. Microscopy (Oxf) 72:279-86.

Hegermann J, Wrede C, Fassbender S, Schliep R, Ochs M, Knudsen L, Mühlfeld C (2019). Volume-clem: A method for correlative light and electron microscopy in three dimensions. Am J Physiol Lung Cell Mol Physiol 317:L778-l84.

Heiligenstein X, de Beer M, Heiligenstein J, Eyraud F, Manet L, Schmitt F, Lamers E, Lindenau J, Kea-Te Lindert M, Salamero J, Raposo G, Sommerdijk N, Belle M, Akiva A (2021). Hpm live µ for a full clem workflow. Methods Cell Biol 162:115-49.

Heiligenstein X, Lucas MS (2022). One for all, all for one: A close look at in-resin fluorescence protocols for clem. Front Cell Dev Biol 10:866472.

Holden L, Burke CS, Cullinane D, Keyes TE (2021). Strategies to promote permeation and vectoriza-

tion, and reduce cytotoxicity of metal complex luminophores for bioimaging and intracellular sensing. RSC Chem Biol 2:1021-49.

Jeong D, Kim D (2022). Recent developments in correlative super-resolution fluorescence microscopy and electron microscopy. Molecules and Cells 45:41-50.

Jiang P, Dickson DW (2025). Correlative light and electron microscopy imaging of proteinaceous deposits in cell cultures and brain tissues. Acta Neuropathol Commun 13:53.

Joshi VN, Mitra D, England MD, Furuya FR, Powell RD, Hainfeld JF (2010). Large covalently linked fluorescent and gold nanoparticle immunoprobes. Microsc Microanal 16:966-7.

Kandela IK, Albrecht RM (2007). Fluorescence quenching by colloidal heavy metals nanoparticles: Implications for correlative fluorescence and electron microscopy studies. Scanning 29:152-61.

Kanemaru T, Kondo T, Nakamura KI, Morimoto H, Nishi K, Isobe SI (2021). A simple preparation method for clem using pre-embedding immunohistochemistry with a novel fluorescent probe and stable embedding resin. Microscopy (Oxf) 70:368-74.

Koistinen KA, Blancett CD, Sun MG (2016). Correlative light and electron microscopy (clem) and its applications in infectious disease. Retrieved May 04, 2026, from https://api.semanticscholar.org/CorpusID:27609748

Kreft ME, Di Giandomenico D, Beznoussenko GV, Resnik N, Mironov AA, Jezernik K (2010). Golgi apparatus fragmentation as a mechanism responsible for uniform delivery of uroplakins to the apical plasma membrane of uroepithelial cells. Biol Cell 102:593-607.

Krentzel D, Elphick M, Domart M-C, Peddie CJ, Laine RF, Shand C, Henriques R, Collinson LM, Jones ML (2025). Clem-reg: An automated point cloud-based registration algorithm for volume correlative light and electron microscopy. Nature Methods 22:1923-34.

Kukulski W, Schorb M, Welsch S, Picco A, Kaksonen M, Briggs JA (2011). Correlated fluorescence and 3d electron microscopy with high sensitivity and spatial precision. J Cell Biol 192:111-9.

Kukulski W, Schorb M, Welsch S, Picco A, Kaksonen M, Briggs JA (2012). Precise, correlated fluorescence microscopy and electron tomography of lowicryl sections using fluorescent fiducial markers. Methods Cell Biol 111:235-57.

Lane R, Wolters AHG, Giepmans BNG, Hoogenboom JP (2021). Integrated array tomography for 3d correlative light and electron microscopy. Front Mol Biosci 8:822232.

Le P, Vaidya R, Smith LD, Han Z, Zahid MU, Winter J, Sarkar S, Chung HJ, Perez-Pinera P, Selvin PR, Smith AM (2020). Optimizing quantum dot probe size for single-receptor imaging. ACS Nano 14:8343-58.

Lee LC, Lo KK (2022). Luminescent and photofunctional transition metal complexes: From molecular design to diagnostic and therapeutic applications. J Am Chem Soc 144:14420-40.

Lee LC, Lo KK (2024). Shining new light on biological systems: Luminescent transition metal complexes for bioimaging and biosensing applications. Chem Rev 124:8825-9014.

Loginov SV, Fermie J, Fokkema J, Agronskaia AV, De Heus C, Blab GA, Klumperman J, Gerritsen HC, Liv N (2022). Correlative organelle microscopy: Fluorescence guided volume electron microscopy of intracellular processes. Front Cell Dev Biol 10:829545.

Lonsdale JE, McDonald KL, Jones RL (1999). High pressure freezing and freeze substitution reveal new aspects of fine structure and maintain protein antigenicity in barley aleurone cells. The Plant Journal 17:221-9.

Mäntylä E, Verkade P (2024). Chapter two - some tips and tricks for a correlative light electron microscopy workflow using stable expression of fluorescent proteins. In: Müller-Reichert T, Verkade P (eds). Methods in cell biology. Amsterdam: Academic Press. 43-56.

Mayhew TM, Lucocq JM (2011). Multiple-labelling immunoem using different sizes of colloidal gold: Alternative approaches to test for differential distribution and colocalization in subcellular structures. Histochemistry and Cell Biology 135:317-26.

McCafferty CL, Klumpe S, Amaro RE, Kukulski W, Collinson L, Engel BD (2024). Integrating cellular electron microscopy with multimodal data to explore biology across space and time. Cell 187:563-84.

Mironov AA, Beznoussenko GV (2013). Correlative microscopy. Methods Cell Biol 113:209-55.

Möbius W, Posthuma G (2019). Sugar and ice: Immunoelectron microscopy using cryosections according to the tokuyasu method. Tissue Cell 57:90-102.

Nisman R, Dellaire G, Ren Y, Li R, Bazett-Jones DP (2004). Application of quantum dots as probes

for correlative fluorescence, conventional, and energy-filtered transmission electron microscopy. J Histochem Cytochem 52:13-8.

Paez-Segala MG, Sun MG, Shtengel G, Viswanathan S, Baird MA, Macklin JJ, Patel R, Allen JR, Howe ES, Piszczek G, Hess HF, Davidson MW, Wang Y, Looger LL (2015). Fixation-resistant photoactivatable fluorescent proteins for clem. Nat Methods 12:215-8, 4 p following 8.

Paul-Gilloteaux P, Heiligenstein X, Belle M, Domart M-C, Larijani B, Collinson L, Raposo G, Salamero J (2017). Ec-clem: Flexible multidimensional registration software for correlative microscopies. Nature Methods 14:102-3.

Peddie CJ, Schieber NL (2019). The importance of sample processing for correlative imaging (or, rubbish in, rubbish out). In: Verkade P, Collinson L (eds). Correlative Imaging. Chichester: Wiley. 37-66.

Peng D, Li N, He W, Drasbek KR, Xu T, Zhang M, Xu P (2022). Improved fluorescent proteins for dual-colour post-embedding clem. Cells 11:1077.

Perkovic M, Kunz M, Endesfelder U, Bunse S, Wigge C, Yu Z, Hodirnau VV, Scheffer MP, Seybert A, Malkusch S, Schuman EM, Heilemann M, Frangakis AS (2014). Correlative light- and electron microscopy with chemical tags. J Struct Biol 186:205-13.

Polishchuk EV, Polishchuk RS (2013). Chapter 15 - analysis of golgi complex function using correlative light-electron microscopy. In: Perez F, Stephens DJ (eds). Methods in cell biology. Amsterdam: Academic Press. 243-58.

Polishchuk EV, Polishchuk RS (2019). Pre-embedding labeling for subcellular detection of molecules with electron microscopy. Tissue and Cell 57:103-10.

Polishchuk RS, Polishchuk EV, Marra P, Alberti S, Buccione R, Luini A, Mironov AA (2000). Correlative light-electron microscopy reveals the tubular-saccular ultrastructure of carriers operating between golgi apparatus and plasma membrane. J Cell Biol 148:45-58.

Robinson JM, Takizawa T, Vandré DD (2000). Enhanced labeling efficiency using ultrasmall immunogold probes: Immunocytochemistry. J Histochem Cytochem 48:487-92.

Roth J, Heitz PU (1989). Immunolabeling with the protein a-gold technique: An overview. Ultrastruct Pathol 13:467-84.

Sanada T, Yamaguchi J, Furuta Y, Kakuta S, Tanida I, Uchiyama Y (2022). In-resin clem of epon-embedded cells using proximity labeling. Sci Rep-Uk 12:11130.

Scher N, Avinoam O (2021). 50 shades of clem: How to choose the right approach for you. Methods Cell Biol 162:1-11.

Scher N, Rechav K, Paul-Gilloteaux P, Avinoam O (2021). In situ fiducial markers for 3d correlative cryo-fluorescence and fib-sem imaging. iScience 24:102714.

Shewring JR, Hodgson L, Bryant HL, Bullough PA, Weinstein JA, Verkade P (2021). Chapter 4 - refining a correlative light electron microscopy workflow using luminescent metal complexes. In: Müller-Reichert T, Verkade P (eds). Methods in cell biology. Amsterdam: Academic Press. 69-87.

Slot JW, Geuze HJ (2007). Cryosectioning and immunolabeling. Nat Protoc 2:2480-91.

Spiegelhalter C, Laporte JF, Schwab Y (2014). Correlative light and electron microscopy: From live cell dynamic to 3d ultrastructure. Methods Mol Biol 1117:485-501.

Takizawa T, Powell RD, Hainfeld JF, Robinson JM (2015). Fluoronanogold: An important probe for correlative microscopy. J Chem Biol 8:129-42.

Tanida I, Furuta Y, Yamaguchi J, Kakuta S, Oliva Trejo JA, Uchiyama Y (2020). Two-color in-resin clem of epon-embedded cells using osmium resistant green and red fluorescent proteins. Scientific Reports 10:21871.

Tanner H, Sherwin O, Verkade P (2023). Labelling strategies for correlative light electron microscopy. Microscopy Research and Technique 86:901-10.

Tuijtel MW, Koster AJ, Jakobs S, Faas FGA, Sharp TH (2019). Correlative cryo super-resolution light and electron microscopy on mammalian cells using fluorescent proteins. Sci Rep-Uk 9:1369.

van den Dries K, Fransen J, Cambi A (2022). Fluorescence clem in biology: Historic developments and current super-resolution applications. FEBS Letters 596:2486-96.

van der Beek J, Veenendaal T, de Heus C, van Dijk S, ten Brink C, Liv N, Klumperman J (2023). Ultrastructural localization of endogenous lc3 by on-section correlative light-electron microscopy. J Vis Exp 193:1-17.

Višnjar T, Chesi G, Iacobacci S, Polishchuk E, Resnik N, Robenek H, Kreft M, Romih R, Polishchuk R, Kreft ME (2017). Uroplakin traffic through the golgi apparatus induces its fragmentation: New insights from novel in vitro models. Sci Rep 7:12842.

Walther T (2015). Electron microscopy of quantum dots. J Microsc 257:171-8.