PREFACE

Introduction to Correlative Light and Electron Microscopy

Correlative microscopy, as an approach to combine methods of different modalities, has become increasingly important over the past years. With the advent of GFP and the accompanying improvements in light as well as in electron microscopy technology, it is the combination of both techniques in **Correlative Light and Electron Microscopy (CLEM)** that has generated the most attention. The rise in the importance of CLEM is reflected by a steady increase in publications related to the fusion of these imaging techniques. In 2002 approximately 10 studies were published that employed CLEM, whereas over 60 were published in 2011. Today, the term CLEM is applied to a number of approaches, all having in common that the imaging and analysis of the same sample employs both methods. Earlier studies, however, did not apply this combination of methods in the strict sense of using the same specimen, but these approaches are still very valuable as highlighted in some chapters.

The oldest set of papers on "correlative microscopy" that can be found in PubMed was published in 1960 (Godman et al., 1960a and b). In these studies light level histochemical staining patterns, published in the first paper, were correlated with electron microscopic observations and presented in the accompanying second. Again, the authors did not examine the same structures with both types of microscopes. The earliest example of CLEM on the same specimen was published 15 years later (Abandowitz and Geissinger, 1975). Interestingly, the sample was first imaged by scanning electron microscopy, followed then by light microscopy interferometry to ascertain dry mass. The approach of examining the same samples and/or objects has been carried forward to the present day in a number of variations. For a more complete historical perspective the reader is referred to the reference lists of the individual chapters in this volume as well as those in the earlier book by Hayat (1987).

One of the most common approaches of CLEM involves the use of fluorescence light followed by electron microscopy on the same cells. Webster et al., (1978) examined microtubules using immunofluorescence microscopy and subsequent transmission electron microscopy in whole mount, detergent extracted PtK2 cells. Another early example of CLEM on single specimens utilized correlative immunofluorescence and electron microscopy on Epon sections (Rieder and Bowser, 1985). In this study, virally infected AC-20 cells were labeled with antibodies prior to Epon embedding and then sections were cut for subsequent fluorescence, phase contrast, and electron microscopy. Imaging of the same exact structures by fluorescence and electron microscopy was further explored later when the enzyme myeloperoxidase was detected via immunofluorescence using the bi-functional reagent FluoroNanogold (Takizawa et al., 1998). Another important advancement in the field of CLEM involved combinating live GFP imaging with subsequent electron microscopy of transport carriers inside the cell

(Polishchuk et al., 2000). Since then a number of groups have succeeded in adopting this strict application of CLEM.

Additionally, the use of alternating semi-thick and thin sections for parallel LM and EM analysis was developed further by Schwarz and colleagues (Schwarz and Humbel, 2007). Ultrathin cryosections were collected on Formvar-coated EM grids. Fluorescence images were generated and then the section was subjected to a silver enhancement reaction for EM-level Nanogold visualization. Later, the thin-section approach was modified by embedding tissue in LR White resin. Immunofluorescence labeling was carried out directly on collected thin sections, which could then be examined by scanning electron microscopy to provide ultrastructural detail (Micheva and Smith, 2007).

The motivation to perform CLEM can be compared when one contrasts the pros and cons of previous versus current approaches. Independent from the methodologies of these specific techniques, one wishes to bridge the gaps, thus merging the advantages of both microscopy 'worlds' to optimize the information gained. The combination should ideally enhance both quantity and quality of information over applying either technique separately.

Alternatively, rather than overlaying fluorescence signal on an EM image of the same sample post fixation/embedding, many current CLEM approaches try to capture the dynamics of cells by live-cell light microscopy and then process the sample for ultrastrucural analysis. As previously summarized (McDonald, 2009), the rational here is:

I, to combine contextual information from light microscopy with the resolution of EM;

II, to increase EM sample size and throughput;

III, to locate a rare event and/or structure; and

IV, to observe a dynamic process within a known region of interest.

Along these lines, we have employed CLEM to visualize specific processes during intracellular traffic (Verkade, 2008; van Weering et al., 2010), or to analyze intermediate stages of both centriole duplication and cell division (Pelletier et al., 2006; Guizetti et al., 2011). Our own research motivated us to present a number of different CLEM approaches within a single volume of *Methods in Cell Biology*.

Clearly, not all current CLEM approaches could be described within this volume, however, we attempted to discuss the most applicable and interesting combinations of techniques/imaging modes. These approaches include the following: the use of either plastic and/or Tokuyasu cryo-sections for CLEM (Kobayashi et al., Polishchuk et al., Takizawa and Robinson, Lousset et al., Fabig et al., Cortese et al.); the application of multifunctional marker molecules for both light and electron microscopy (Grabenbauer, Ellisman et al., Sjollema et al.,); the use of cryo-fixation as a starting point for CLEM (Brown et al., Kolotuev et al., Woog et al., Kukulski et al.); the combination of high-end light and/or electron microscopy, such as FIB-SEM for specimen preparation (Rigort et al., Lucas et al.) or imaging (Bushby et al); the advantages of super-resolution light microscopy for structural studies (Watanabe and Jorgensen); and lastly, the integration of light and electron microscopy into one instrument (Morrison et al.).

In parallel to this volume of *Methods in Cell Biology*, we have designed an annual practical EMBO course on CLEM, where students can gain hands-on experience in applying combinatorial approaches to their specific research questions. It is our hope that this practical course, in concert with this MCB volume, will stimulate further crosstalk between light and electron microscopy leading to the development of new correlative approaches in cell biology.

Thomas Müller-Reichert, Paul Verkade

Dresden/Bristol

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