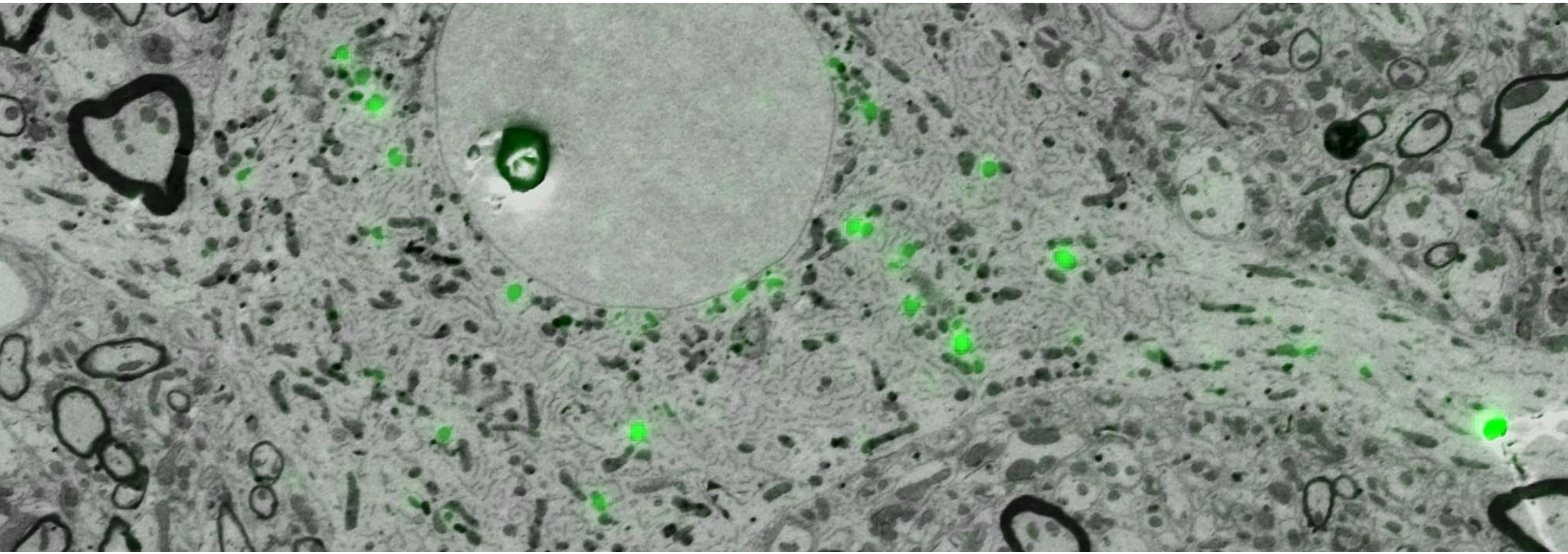

SAMPLE PREPARATION

for Integrated Correlative Light and Electron Microscopy

delmic

Integration without compromise

Index

Sample Preparation for Integrated Correlative Light and Electron Microscopy

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Integrated correlative light and electron microscopy (CLEM) offers the possibility to study the same area on a sample using both fluorescence microscopy and electron microscopy. One of the challenges associated with integrated CLEM (iCLEM) is the preparation of samples suitable for both FM and EM. Here, we discuss several methods suitable for an integrated imaging workflow, and present results obtained using a variety of techniques and samples, including: cultured cells and tissue sections, chemical fixation or cryo-fixation, genetic labels and immunolabeled samples. While particularly suited for iCLEM, these protocols may also improve correlation in non-integrated approaches or a combination of both. This is not intended as an extensive list of sample preparation methods, which are many and varied. Furthermore, different types of specimens typically require specifically optimized protocols. This short review provides insights in the possibilities offered by integrated imaging workflows and represents a useful starting point for exploring these techniques.

INTRODUCTION

During the past few years, correlative light and electron microscopy (CLEM) has gained popularity as a research tool. This growing interest is because CLEM combines the strengths of fluorescence microscopy (FM) and electron microscopy (EM): FM is the ideal tool to collect functional information about specific components inside tissues, cells and organelles; EM offers substantially higher resolution and can provide detailed contextual structural information. FM can thus be used to pinpoint regions of interest for subsequent higher resolution EM.

Until recently, however, CLEM has been challenging, costly, time consuming and thus requiring high levels of expertise. Correlative methods normally require two distinctly different imaging setups which are traditionally located in separate facilities, and the sample preparation methods for each tend to be incompatible. Due to the fundamental differences between microscopes, extra sample preparation steps are also usually necessary when switching from FM to EM. This often distorts the sample, hampering accurate correlation. In addition, it can be extremely challenging to relocate a region of interest originally identified with FM in EM since the information used to navigate in FM is not visible in EM, and this problem becomes more significant as the size of specimen increases.

Integrated CLEM (iCLEM) overcomes most of these difficulties. By integrating fluorescence and scanning electron microscopy, the need to transfer between two different microscopes is eliminated. Finding back a region of interest becomes much simpler as the same area of the sample is observable with both microscopes. Furthermore, since the sample is not subjected to intermediate preparation steps, its conformation is guaranteed to be identical.

Sample preparation for iCLEM is also a new research area, and a limited number of protocols have been published to date. For non-integrated CLEM, on the other hand, excellent overviews of sample preparation methods exist [1-3]. One of the difficulties of integrating sample preparation for FM and EM, is that EM sample preparation protocols typically use heavy metal stains to introduce electron contrast. It is well known that these heavy metals can quench nearby fluorescence. Furthermore, EM requires vacuum compatible samples. As such, samples need to be dried, which can influence the amount of fluorescence for hydration-sensitive dyes [4]. A recent publication has also shown that in SEM the amount of fluorescence can be influenced by variation of the vacuum pressure [5]. As iCLEM develops further as a powerful research tool, we will see a corresponding increase in the number of published iCLEM specific sample preparation protocols.

Here we present four different protocols for iCLEM. We have deliberately chosen to use examples with