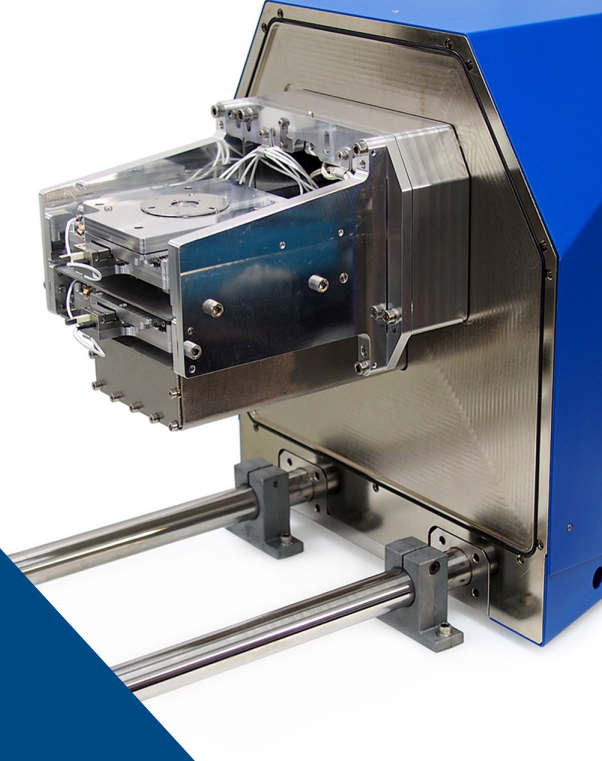

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application note

Cellular Biology: In-Resin Fluorescence Using GFP and mCherry

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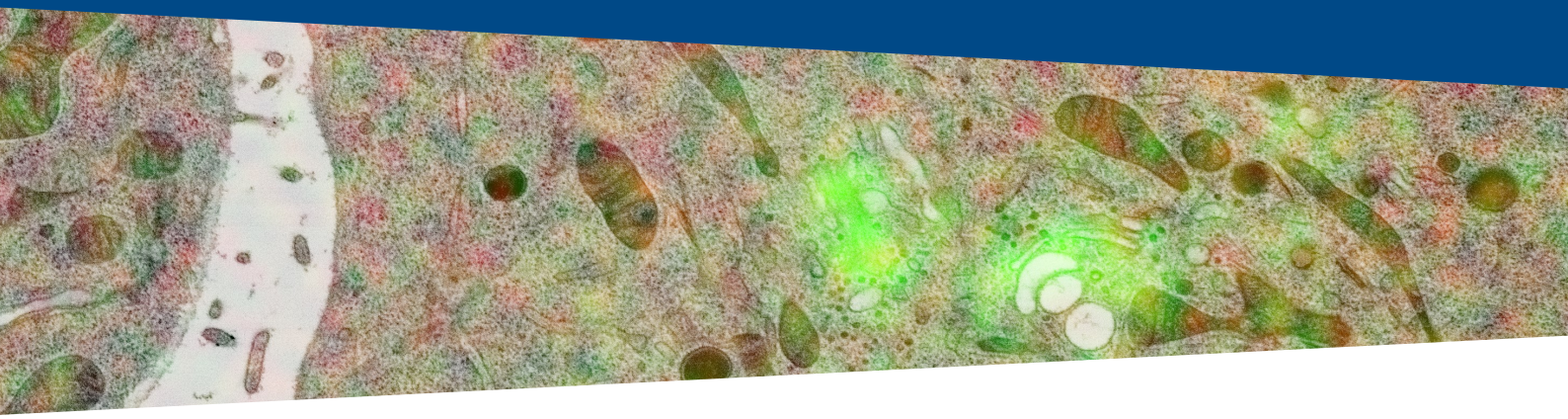


application note

Cellular Biology: In-resin Fluorescence Using GFP and mCherry

Correlative light and electron microscopy (CLEM) is a powerful technique for studying cellular processes on an ultrastructural level. The SECOM platform, a fluorescence microscope designed to be integrated with a scanning electron microscope, can simplify experiments involving CLEM by providing a fully automated overlay procedure and seamless switching between fluorescence and electron microscopy.

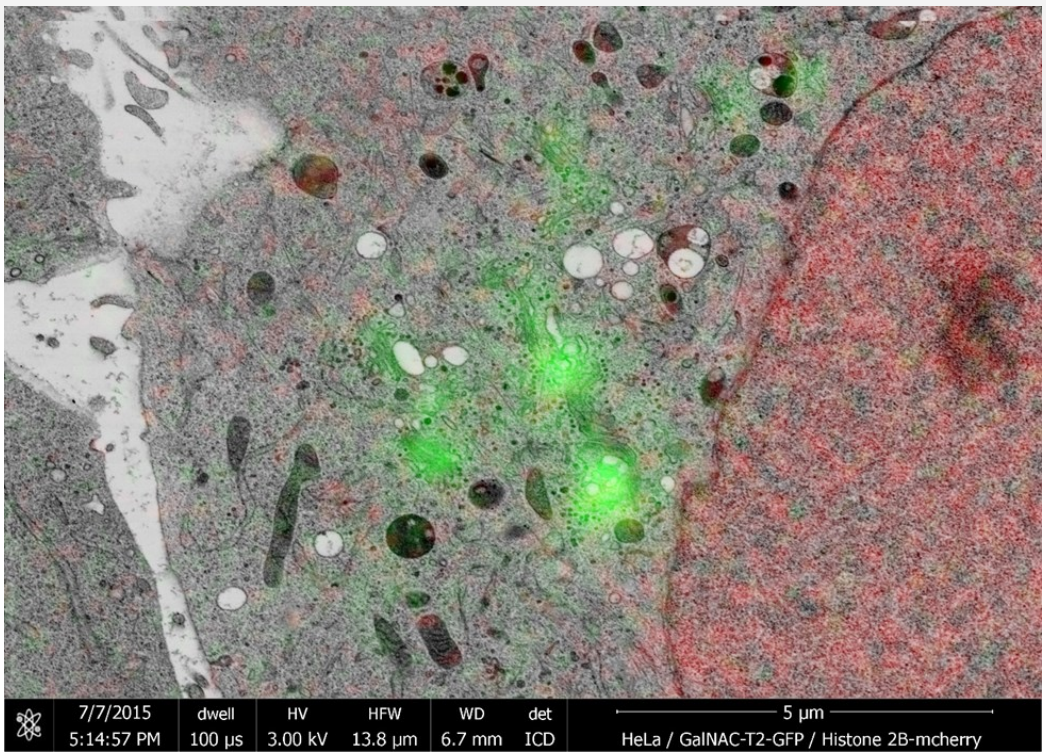
Sample preparation, however, remains the crux of any experiment. To achieve the best overlay accuracy, one ideally acquires both the fluorescence and electron images on the same resin-embedded section [1,2]. This does pose a challenge with regards to sample preparation. Fortunately, recent advances in sample preparation have led to a range of protocols that demonstrate in-resin fluorescence of fluorescent proteins [1-5].

Two studies report that imaging GFP and YFP in vacuum has a large influence on the fluorescent intensity. From [3]: "*First, we observed a drop in fluorescence intensity as the vacuum pressure decreases due to extraction of water from the sample, and which can be reversed by re-introducing water into the system at partial vacuum pressure or atmospheric pressure. Second, we show that although fluorescence intensity is reduced at a partial pressure of 200 Pa (created using water vapour), the FP intensity is remarkably stable and resistant to photobleaching during imaging. Finally, we show that holding IRF sections in vacuum leads to very minor losses in fluorescence over time.*"

Here we used Hela Kyoto cells stably expressing GalNAC-T2-GFP and Histone 2B-mcherry. The cells were grown on carbon coated sapphire disks and high pressure frozen. The cells were then freeze substituted with 0.1% UA in glass distilled acetone (slightly modified from [2] and [6]) and infiltrated in Lowicryl HM20.

Imaging was performed using the SECOM platform with a 40x/0.95 NA objective, mounted on a Verios 460L SEM (FEI). Fluorescence excitation was performed using a 'Pinkel' configuration multiband filter set and LED excitation of 474 nm and 554 nm for GFP and mCherry respectively. Images were collected using an sCMOS camera. SEM imaging was performed with a 3 kV acceleration voltage and using the in-column detector (ICD).

Figure 1 *Results*



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DELMIC B.V. is a company based in Delft, the Netherlands that produces correlative light and electron microscopy solutions. DELMIC's systems cater to a broad range of researchers in fields ranging from nanophotonics to cell biology.

The SECOM platform is a fluorescence microscope made to be integrated with a scanning electron microscope produced by DELMIC, that enables extremely fast correlative microscopy, with the highest optical quality and overlay accuracy.

For questions regarding this note, contact our SECOM Application Specialist at: voortman@delmic.com

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