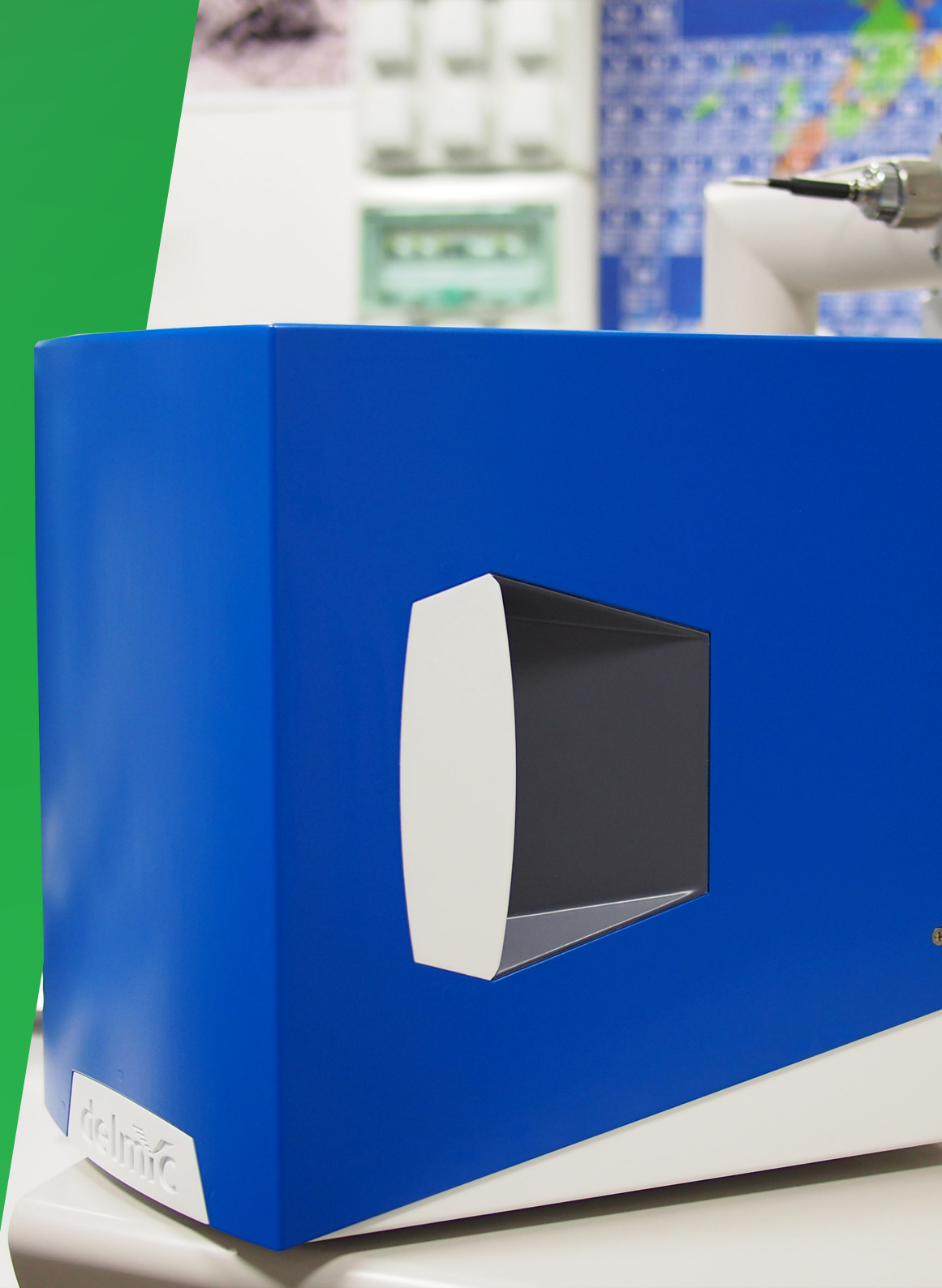
secom sr

Correlative Microscopy by Delmic

The integrated super-resolution CLEM system

delmic



SECOM

Integrated super-resolution and electron microscopy

Correlative light and electron microscopy (CLEM) is the combination of fluorescence microscopy (FM) and electron microscopy. The combination of the labelling power of fluorescence imaging and the highresolution structural information provided by electron microscopy makes correlative microscopy the ideal tool for studying the complex relation between form and function in biology. Combining the highest resolution fluorescence microscopy within its cellular context is the key to understanding the intricate details of life.

In recent years, superresolution (SR) microscopy has proven itself to be an extremely powerful technique in biological research. With the invention of SR microscopy, it is now possible to precisely localize biomolecules at length scales that were previously only accessible using electron microscopy. Especially at this length scale, it is important to visualize and understand the micro-environment of these biomolecules; resolution only becomes truly valuable with contextual information.

SECOM SR at glance

Sub-diffraction limited imaging

Down to 80 nm optical resolution demonstrated with 200 nm thin sections with HeLa cells.

Optimized workflow for SR-CLEM in vacuo

Optimal sample preparation that preserves fluorescence while providing sufficient EM contrast. Workflow involving the collection of sequences of ~30000 images

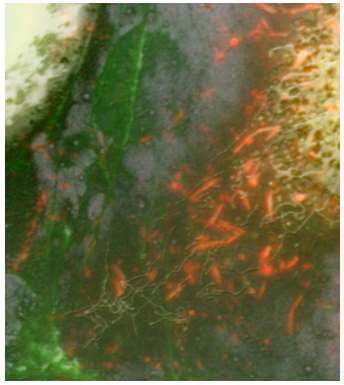
High signal collection efficiency

Optical imaging with high NA objective lens using vacuum-compatible immersion oil

Imaging with standard fluorescent proteins

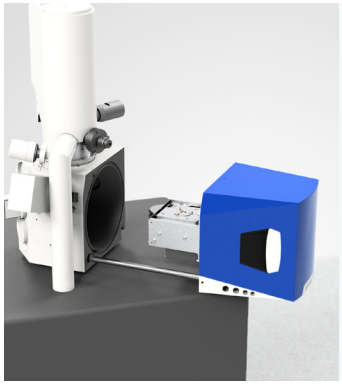
Optical imaging of in-resin fluorescence from widely used fluorescence proteins GFP and RFP, with EM providing ground truth data

Key features



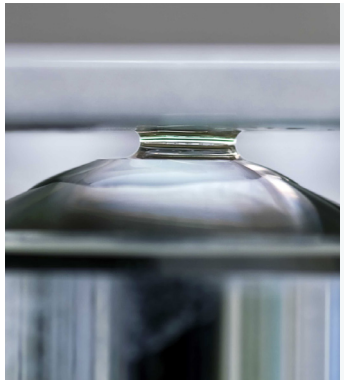
Unrivaled overlay accuracy

With the automated overlay procedure of the SECOM SR platform, EM and FM images are always aligned perfectly. Using the automated overlay, time can be spent acquiring rather than aligning data and since the overlay procedure is unbiased it truly strengthens your scientific discoveries.



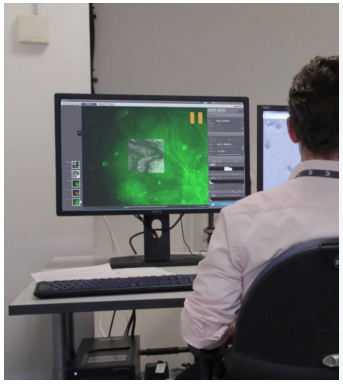
It's a match!

The SECOM SR is fully compatible with all the standard detectors, including ETD, BSD, EDX, and others, allowing you to use the full potential of your electron microscope. Furthermore, special features such as beam deceleration and immersion mode are also supported. Like all DELMIC products, the SECOM SR is compatible with all major SEM manufacturers.



Leading optical performance

Because only the highest quality optical components are used, the SECOM SR ensures the best optical performance of any integrated system. One of the unique features is the possibility to use vacuum compatible immersion oil.



User-friendly

The modular approach in combination with the open-source controlling software ODEMIS ensures a user-friendly solution that can serve a broad user base as well as delivering a system that is a truly unique and an ultimately versatile research instrument.

Automated overlay

High accuracy, independent of user and sample

The unique alignment procedure of the SECOM is fully automated and achieves an accuracy of 50 nm or better, independent of the sample. This accuracy is achieved using a patented alignment procedure. The key to this alignment procedure is the use of cathodoluminescence. The electrons that hit the sample generate backscattered and secondary electrons, but also photons. This light can be detected by the camera of the fluorescence microscope and acts as a temporary fiducial marker.

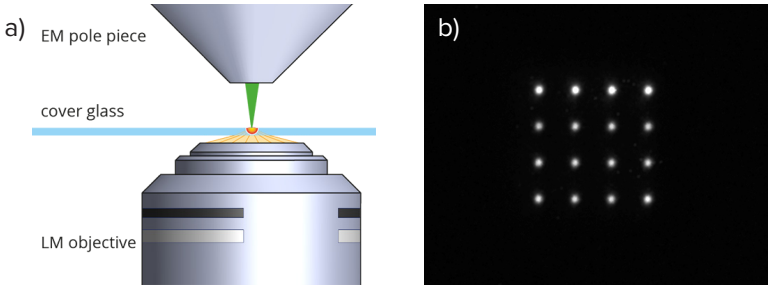


Figure 1: (a) When the e-beam (in green) hits the glass, CL emission is detected using the LM objective. (b) Using a grid of CL pointers, the alignment procedure corrects for translation, scaling and rotation.

Customer story

At our EM Science Technology Platform, we image samples from macromolecules to cells and tissues, using a wide range of sample preparation techniques, in a variety of advanced microscopes. Most of our experiments are correlative – linking functional information from the light microscope with structural information from the electron microscope. We use the SECOM SR platform to perform high accuracy CLEM experiments on resin -embedded cells and tissues containing fluorescent proteins. Implementing superresolution localisation microscopy in the SECOM SR using the blinking of standard fluorescent proteins in -resin takes us to the next level. We expect to achieve at least 50 nm isotropic voxel resolution in the reconstructed fluorescence images, enabling us to localise GFP –tagged molecules to structures as small as virus particles, trafficking vesicles and membrane subdomains. With the new SECOM SR workflow, we can deliver highly accurate correlation of superresolution fluorescence to electron microscopy at an unprecedented pace.

Dr. Lucy M. Collinson, Francis Crick Institute

System specifications

Excitation

- Compatible lasers 405 nm, 457 nm, 488 nm, 561 nm, 647 nm.
- Multi-color imaging

*Others **available on request.***

Sample stage

- Total Stroke of 18 x 18 mm in XY.
- Equipped with precision piezoelectric stepping motors and optical linear encoders.
- Minimum incremental motion of 300 nm, repeatability of 500 nm.

Objective stage

- Stage uses piezoelectric stepping motors that remain full blocking force when not actuated, resulting in small thermal load and thus low drift.
- Minimum incremental motion in XY less than 500 nm.
- Repeatability of Z-axis (focusing) 50 nm.
- Use of optical linear encoder for closed loop driving.

Compatible cameras

- Andor Zyla 4.2 PLUS sCMOS camera.
- Andor iXon Ultra 888 EMCCD camera.

Compatible objective

- Plan Apochromat oil immersion objective lens, magnification 100x, numerical aperture 1.4
- Plan Apochromat water immersion objective lens, magnification 60x, numerical aperture 1.25
- Plan Apochromat objective lens, magnification 60x, numerical aperture 0.95

ODEMIS integrated software

- Open source software suite under the license GPL 2
- ODEMIS outputs files in either PNG or OME-TIFF
- Obtaining fluorescence images (multi - channel) and electron images simultaneously
- Automatic high-precision overlay of fluorescence and electron images using DELMIC CL markers concept
- Software is fully compatible with the camera and excitations source, ensuring that the system works out of the box
- Software based auto focus
- History trail that makes it easy to see your previous images and track how you have navigated the sample

Interested?

For more information on this topic visit www.delmic.com

About

Delmic is a passionate high-tech company based in Delft, the Netherlands that develops powerful and user-friendly solutions for light and electron microscopy. Our systems are used by researchers and companies all over the world in fields ranging from life sciences, geology, material sciences to nanophotonics.

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