SECOM

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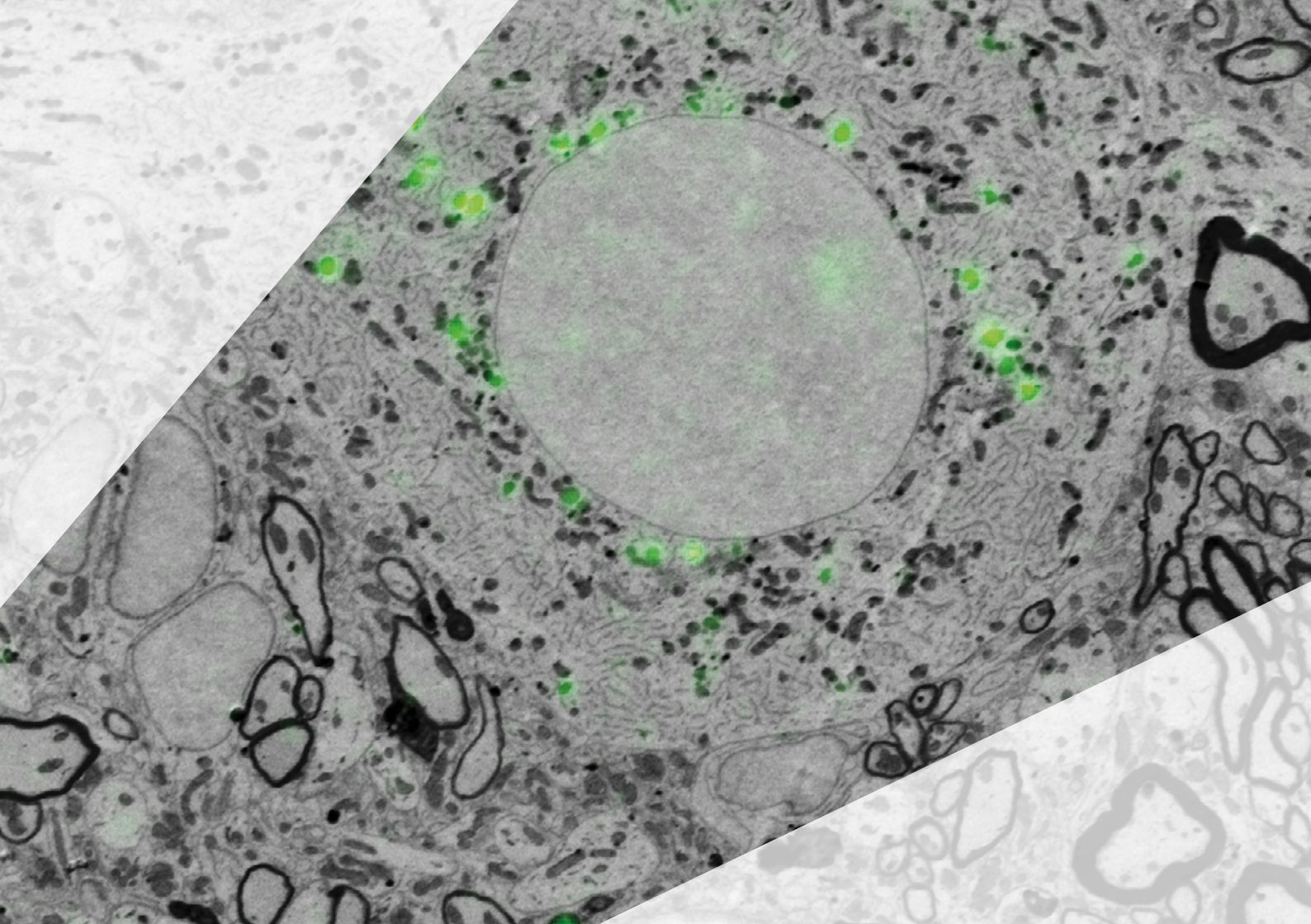
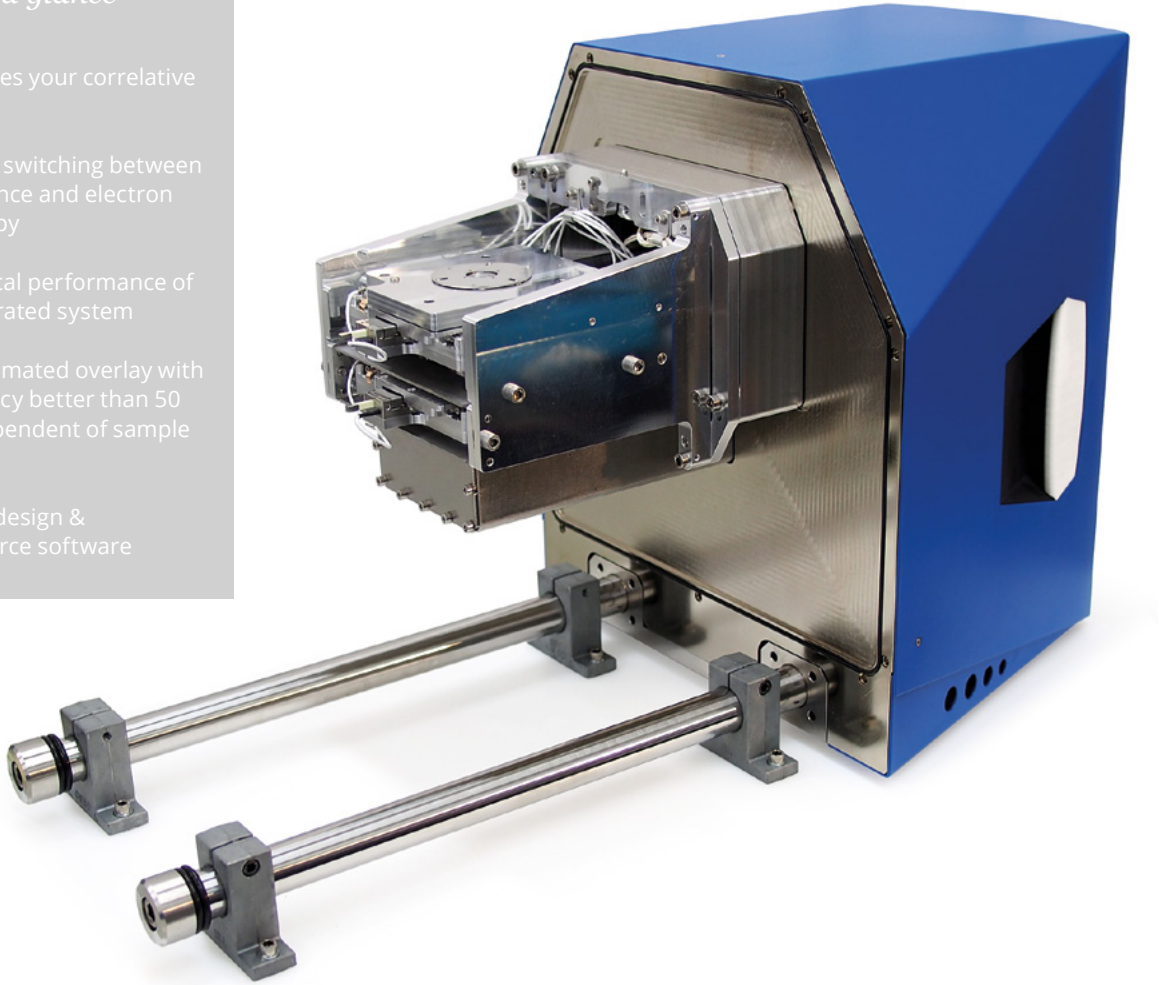
➤
Samples provided by
T. Templier and R.H.R. Hahnloser,
University of Zurich and ETH Zurich.



The SECOM platform
at a glance

+ Streamlines your correlative workflow
+ Seamless switching between fluorescence and electron microscopy
+ Best optical performance of any integrated system
+ Fully automated overlay with an accuracy better than 50 nm, independent of sample and user
+ Modular design & open-source software



SECOM

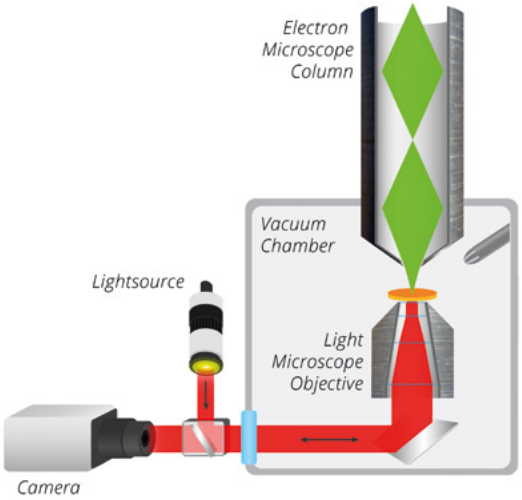
Integrated Correlative Light and Electron Microscope

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

Thanks to its integrated design, switching from fluorescence to electron imaging is seamless and instantaneous. And with the automated alignment procedure, you are directly imaging the right location at a high resolution.

Imaging with the SECOM is just like using a fully equipped high-end optical wide-field microscope, without compromise on either optical or electron performance.

The system is installed as a retrofit by replacing the door to the vacuum chamber and can be fitted to most scanning electron microscopes.



APPLICATION
Imaging thin sections

Using the SECOM for thin sections allows you to use fluorescent markers for pinpointing regions of interest, locating rare events, screening large areas and identifying subcellular structures on a molecular basis. By using multicolor labelling you can have the enormous toolbox of fluorescent markers available in nanometer resolution electron images.

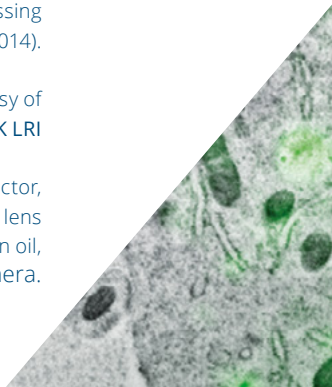
Sectioning biological material that is resin embedded or cryo fixed is the ideal method to reveal subcellular details. This is why most electron microscopists are used to working with sectioned material. For fluorescence microscopy, the axial resolution is greatly enhanced as the sections can be as thin as 50 nm.

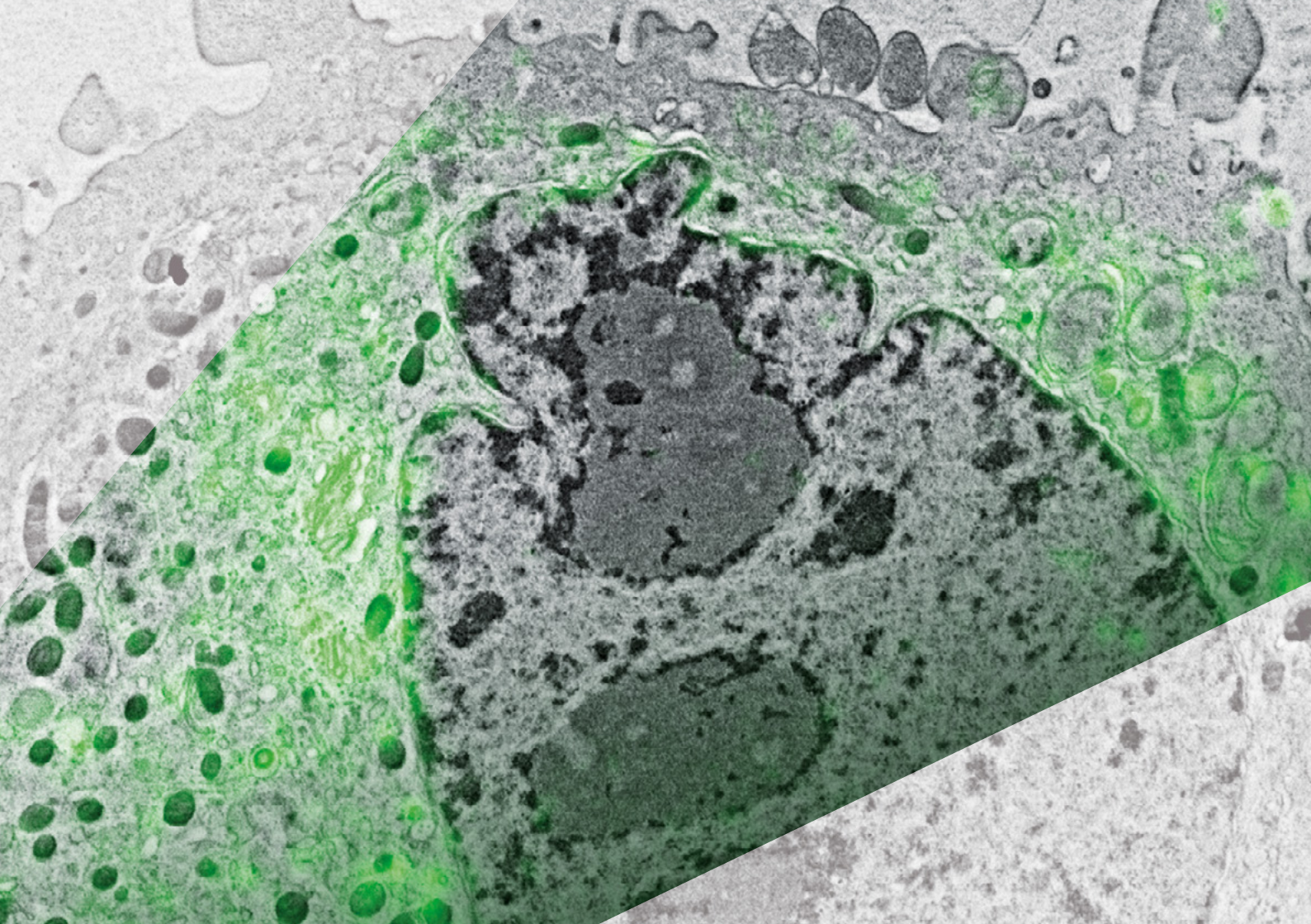
Ultrathin sectioning can be applied to many different biological samples, such as cells in suspension or on a substrate, tissues and animal embryos.

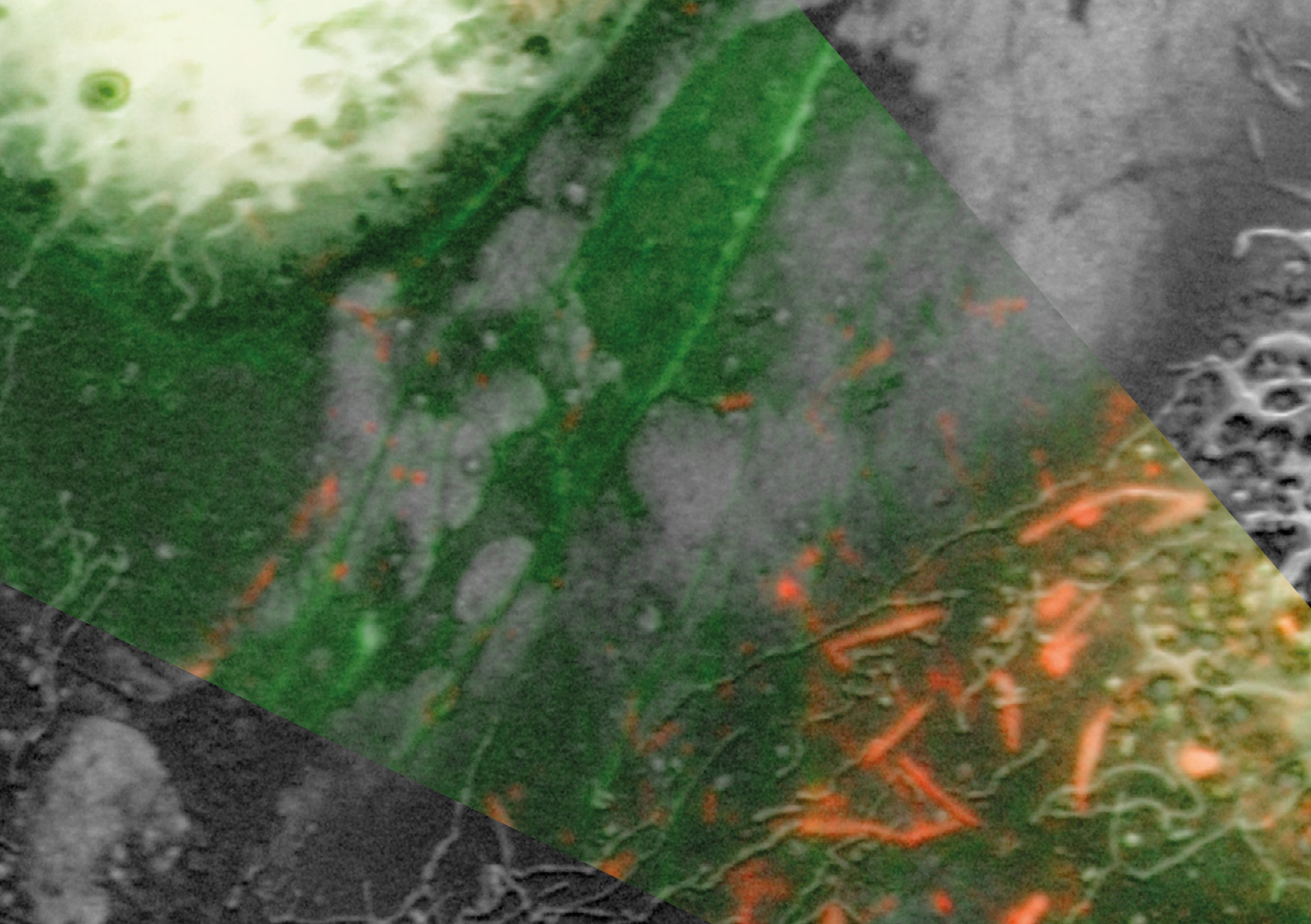
➤ Localization of the lipid diacylglycerol within cellular membranes of HeLa cell expressing GFP-C1 (Peddie et al., 2014).

Image courtesy of
C.J. Peddie and L.M. Collinson, CRUK LRI

Backscatter detector,
100x /1.40 oil immersion lens
using vacuum compatible immersion oil,
laser light source, sCMOS camera.



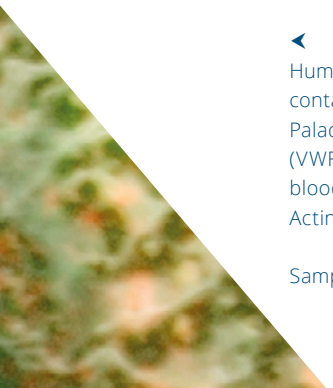




APPLICATION
Imaging cultured cells

The SECOM is the perfect tool to accurately inspect the morphology and surface topology of cultured cells. With the SECOM platform you have the unique opportunity to simultaneously image fluorescent markers together with all the different types of contrast available for SEM. This provides a fast and straightforward method to study cell morphology in correlation with specific proteins of interest.

Growing cells on a substrate is routine work in cell biology. With minor alterations in your sample preparation, you can take advantage of the high resolution and extra contrast provided by electron microscopy.



Human umbilical vein endothelial cells (HUVEC) contain rod-like storage granules called Weibel-Palade bodies which store Von Willebrand factor (VWF). These organells play an important role in blood coagulation.
Actin (Phalloidin Alexa 488) and VWF (Alexa 568).

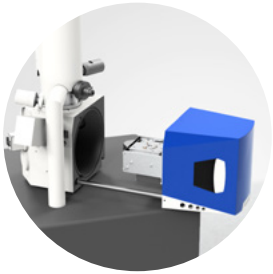
Samples courtesy of **M.J. Mourik, LUMC**

Secondary electron detector,
60x /0.95 lens, multicolor LED light engine,
CCD camera.

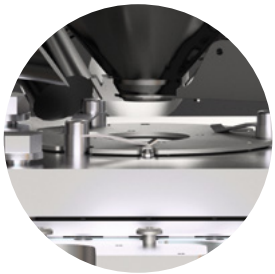
FEATURES

Motorized precision stage
The SECOM sample stage is equipped with precision piezoelectric stepping motors and optical linear encoders, enabling easy and accurate sample navigation.

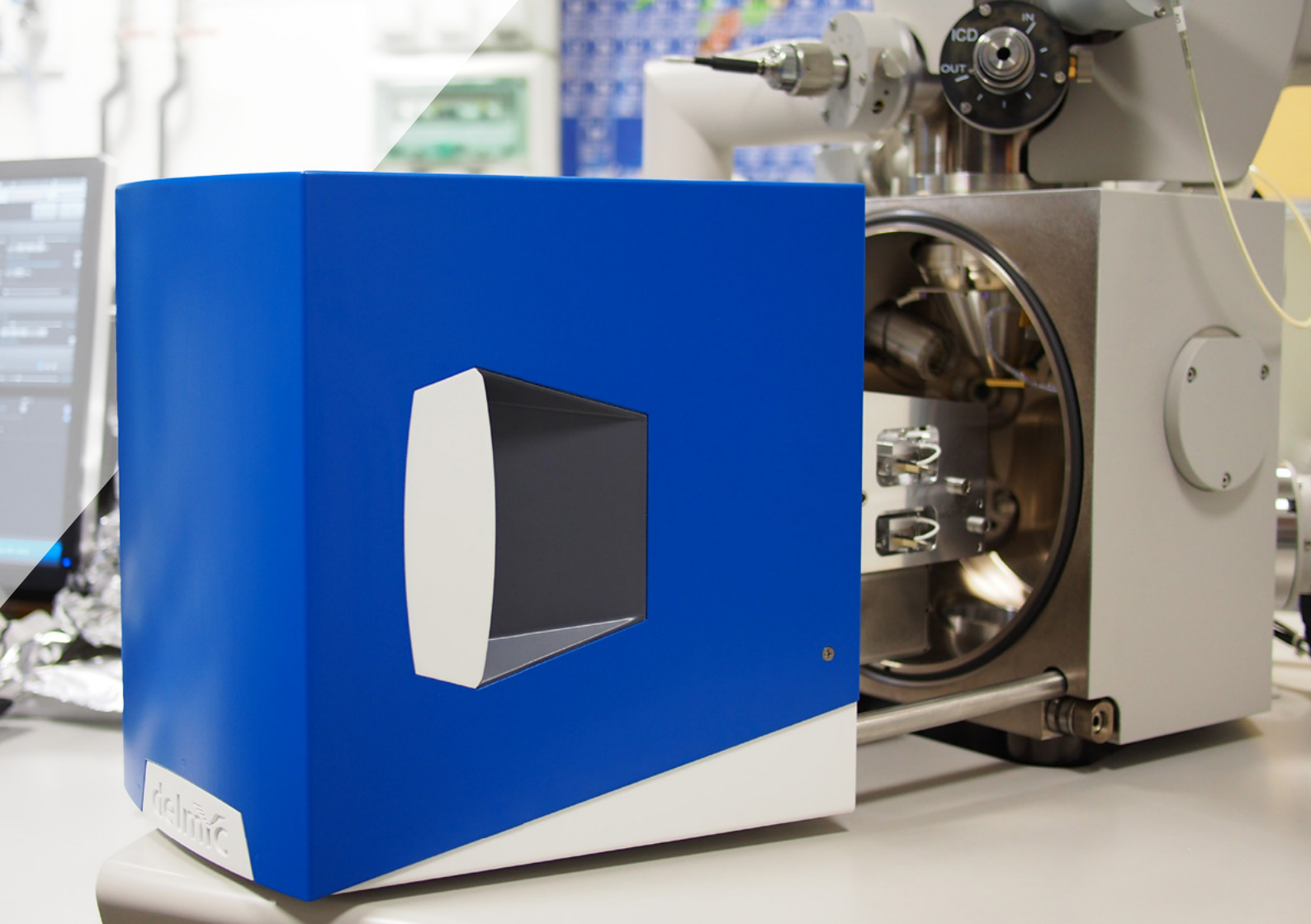
Retrofit
The SECOM is installed as a retrofit to an existing SEM by replacing the vacuum chamber door. As a result, it is easy to switch between the SECOM platform and the original door.



Compatibility
The space above the sample stage in the SEM is kept free; thus allowing access to all the standard detectors. The system is fully compatible with a broad range of SEM detector including: ETD, BSD, EDX and others.



High-end optics
Because only the highest quality optical components are used, the SECOM ensures the best optical performance of any integrated system. It is even possible to use special immersion oils in vacuum. Multiband imaging is part of the standard configuration.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INTEGRATED WORKFLOW

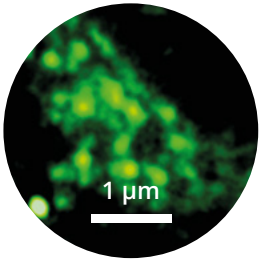
Fast and precise

Correlative imaging with an integrated system is very different from traditional correlative procedures. With the SECOM platform you acquire your fluorescence and electron images in one microscope, allowing you to quickly switch between both modalities. This drastically reduces the time from sample preparation to correlative imaging. After loading your sample, you immediately get your results: in one acquisition you get your fluorescence and high resolution electron images combined with an accurate overlay. Moreover, by easily switching between imaging modes you can quickly assess the quality of your sample preparation and adapt your imaging parameters accordingly. Thanks to the automated alignment procedure of the SECOM, you can accurately correlate subcellular structures, without the risk of introducing a user-bias. The use of an integrated sample preparation protocol prevents unpredictable sample shrinkage and deformation.

Since the sample is not moved from ambient to vacuum conditions, it is guaranteed that your sample is in the same conformation for both light and electron microscopy.

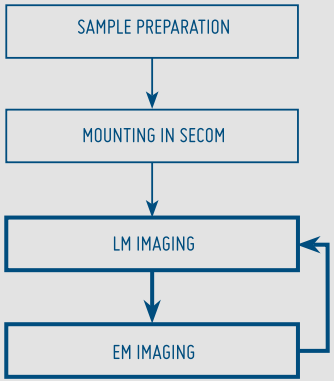
High Numerical Aperture

Because of the excellent optical performance of the SECOM, there is no need to switch to another optical microscope.



SECOM workflow

Thanks to the integration of the fluorescence microscope within the vacuum of the electron microscope, switching between fluorescence and electron imaging can be done over and over again.



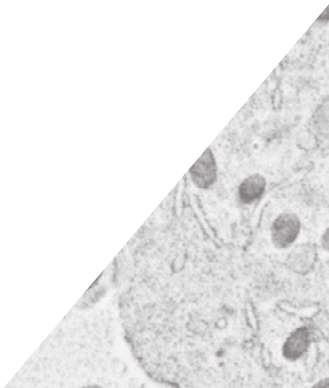
AUTOMATED OVERLAY

High accuracy, independent of user and sample

Alignment of fluorescence and electron images is a crucial step in correlative microscopy. The 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. With the SECOM platform you therefore always look at exactly the same location with both the fluorescence and the electron microscope.

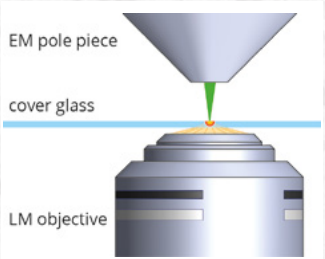
The key to this alignment procedure is the physical principle of cathodoluminescence. Light is generated at the position where the electron beam hits the glass. This light can be detected by the camera of the fluorescence microscope and acts as a temporary fiducial marker. By positioning the electron beam such that not one, but many spots are created, a grid of such temporary fiducial markers is generated. Using this procedure, the electron and fluorescence images are exactly aligned, correcting for translation, scaling and rotation; unbiased and independent of the specimen.

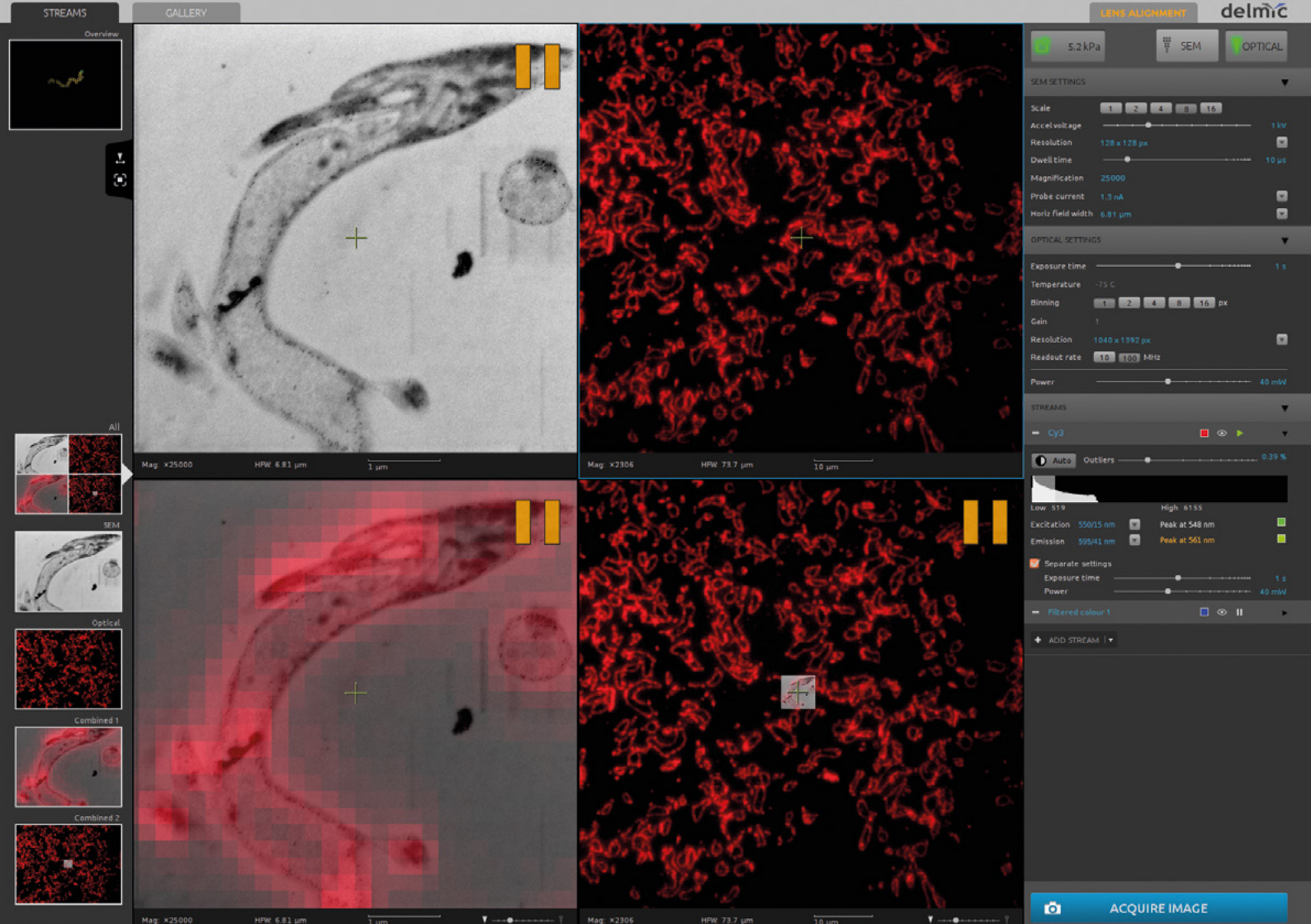
Because the procedure uses the cathodoluminescence of the cover glass substrate, the procedure is sample independent and works without any additional fiducial markers or other landmarks in your sample preparation.



Grid of temporary fiducial markers
A grid of spots used for the alignment procedure. The spots are generated using the electron beam, and detected using the camera of the fluorescence microscope.

Cathodoluminescence
When electrons hit a luminescent material, photons are produced that can be detected using the optical microscope.




STREAMS
GALLERY
LENS ALIGNMENT
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Overview
All
SEM
Optical
Combined 1
Combined 2
Mag: ×25000
HFW: 6.81 µm
1 µm
Mag: ×2306
HFW: 73.7 µm
10 µm
5.2 kPa
SEM
OPTICAL
SEM SETTINGS
Scale
Accel voltage
Resolution
Dwell time
Magnification
Probe current
Horiz field width
OPTICAL SETTINGS
Exposure time
Temperature
Binning
Gain
Resolution
Readout rate
Power
STREAMS
Cy3
Auto
Outliers
Low 519
High 6155
Excitation
Emission
Peak at 548 nm
Peak at 561 nm
Separate settings
Exposure time
Power
Filtered colour 1
ADD STREAM
ACQUIRE IMAGE

ODEMIS
Integrated software

Combining different types of contrast at very different length scales makes correlative microscopy a challenging task. The software package ODEMIS greatly simplifies your correlative imaging workflow. You no longer have to worry about overlay accuracy and stage coordinates, giving you time to focus on your imaging.

ODEMIS is a user-friendly program that gives you control over both the fluorescence and electron microscope. Navigating the sample is easy with either the fluorescence or SEM channel and you can use multiple color channels with individual settings per channel. For expert users, a scripting interface in Python gives you full control of the hardware and the imaging algorithms.
To simplify correlative microscopy, ODEMIS features a fully automated alignment procedure resulting in accurate, unbiased overlays on every specimen. This overlay is directly visualized in the acquisition software, allowing you to quickly adapt your imaging conditions. Other powerful tools that improve your imaging workflow include auto-focus and a history trail that records your previous stage coordinates.

ODEMIS is open-source and makes use of the open file formats OME-TIFF and HDF5.

SPECIFICATIONS

Excitation

- **Multiband setup** in Pinkel configuration optimized for DAPI, FITC, TRITC, & Cy5 and other like fluorophores.
- Four channel solid-state (LED) excitation source with digital On/Off and intensity control.
- Default excitation wavelengths at 387/11, 485/20, 560/25 and 650/13nm.

Others **available on request.**

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.

Sample Stage

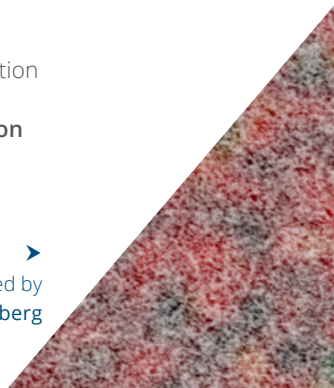
- Total stroke of **18x18mm** in XY.
- Equipped with **precision piezoelectric** stepping motors and **optical linear encoders.**
- Minimum incremental motion of 300 nm, repeatability of 500 nm.

Camera

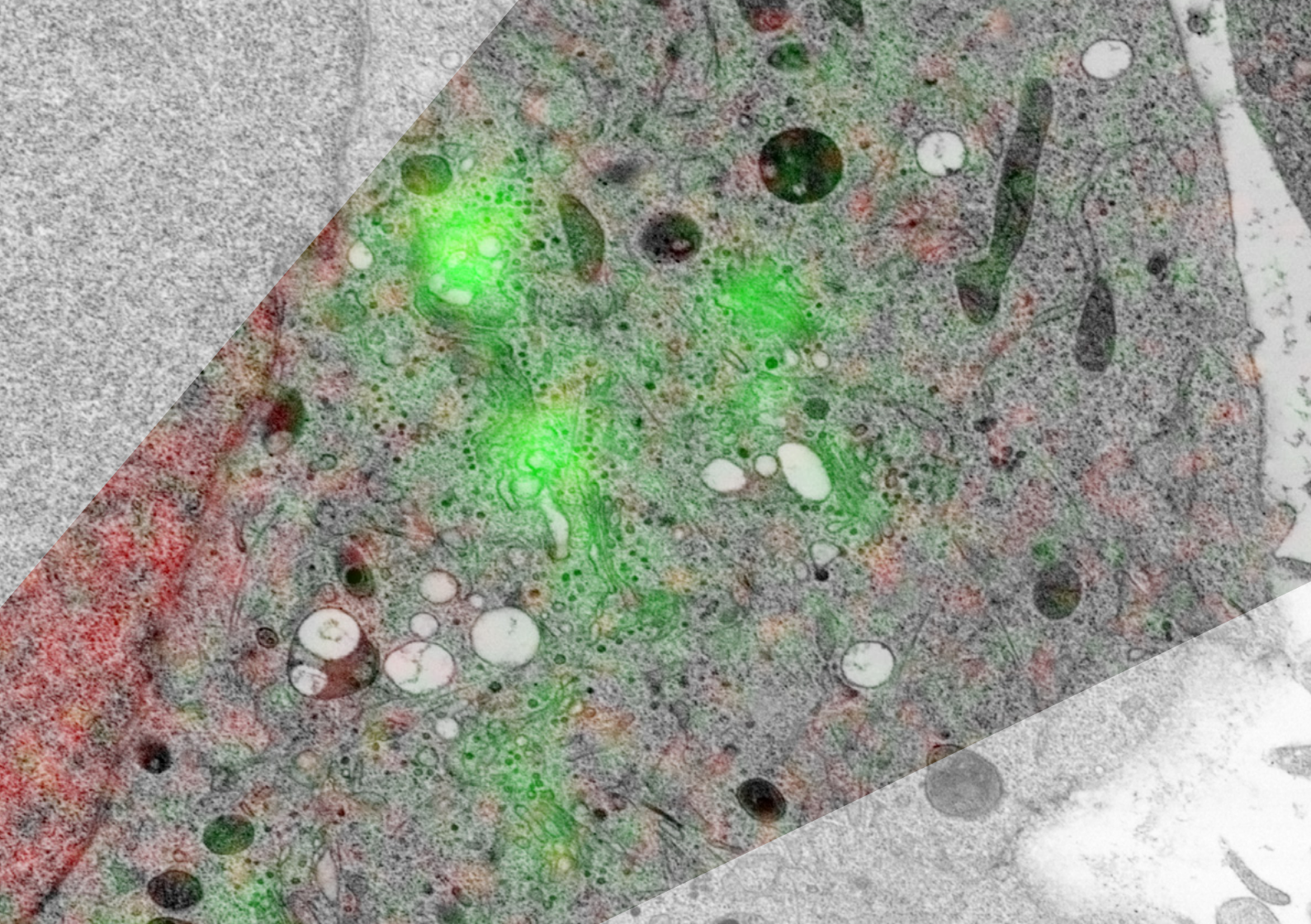
- Scientific CMOS camera allows imaging with **low noise** and **large field of view**.
- 2048x2048 pixels with a pixel size of 6.5 µm yields
- 330x330 µm field of view at 40x magnification

Objective lens

- Plan Apochromat objective lens, magnification 40x, **numerical aperture 0.95.**
  Other objective lenses, including **immersion lenses** (up to NA 1.4), available on request.
- User exchangeable



Samples provided by
P. Ronchi and Y. Schwab, EMBL, Heidelberg



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Integration without compromise

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