# Delphi

## Insight Simplified



## Easy and fast
- All-in-one solution for correlative microscopy
- Integration of hardware and software
- Time from sample loading to correlative imaging < 3 min

## Unbiased
- User and sample independent overlay of LM and EM images
- Unique algorithm for perfect results
- Fully automated overlay procedure

## Add context
- Place your fluorescence data in a structural context
- Add functional information to structural EM images
- Seamlessly switch from fluorescence to electron imaging

## Small footprint
- May be placed in any laboratory
- No need for extra infrastructure or equipment
- Plug and play installation; no on site calibration required

•••DELPHI

**Mapping large sets of EM data with the use of CLEM**
Identifying different cell types based on morphological EM contrast requires expert knowledge and can be a time consuming process. With correlative microscopy it is straightforward to localize different cell types based on accurate and reliable immunolabeling techniques. With this approach the user is able to quickly map large areas and zoom in on structures of interest.

5 µm
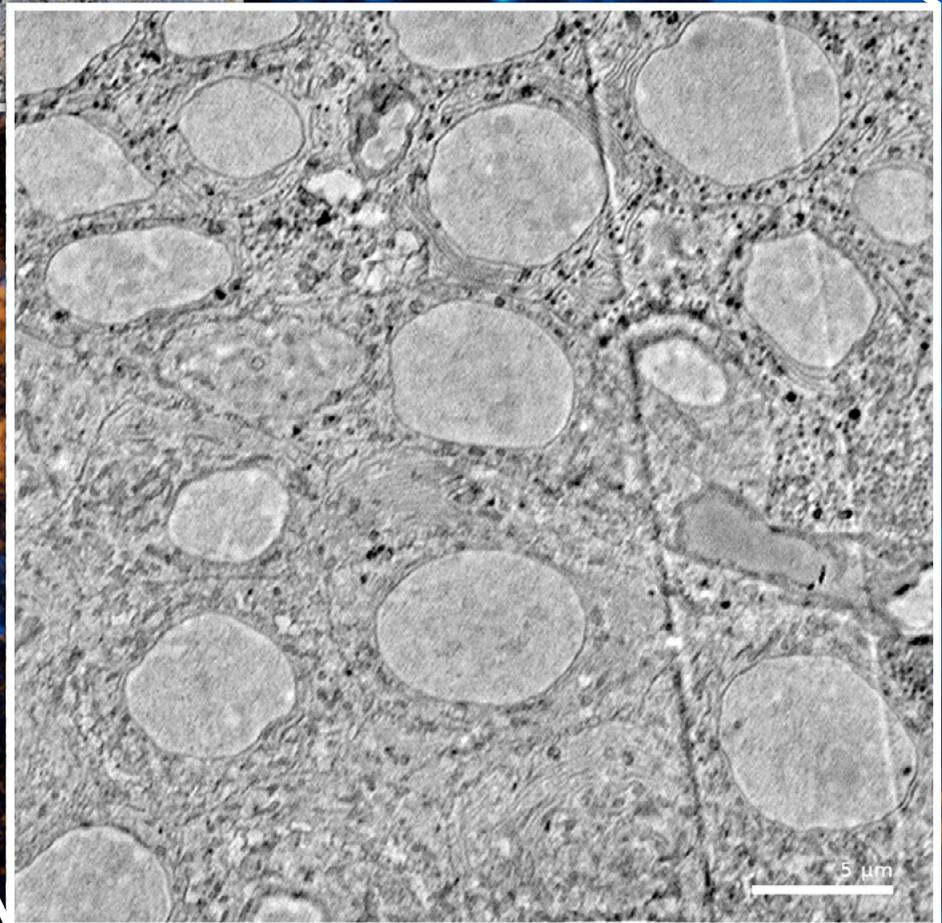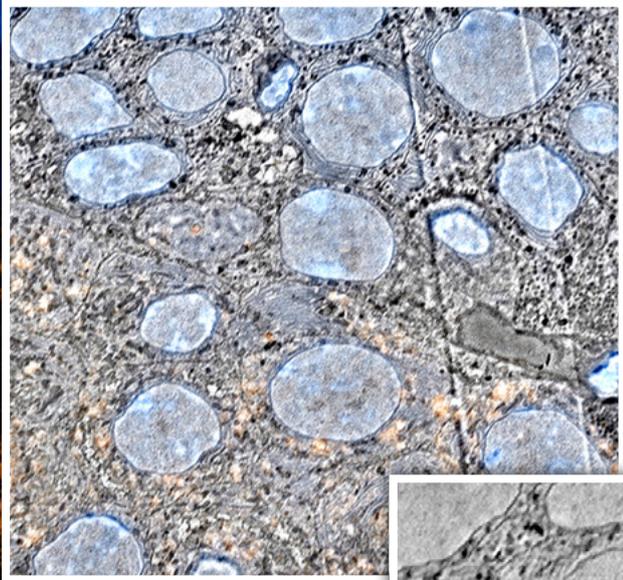
# Advantages of Correlative Light and Electron Microscopy

## Combine function and structure

In order to understand the **complex nature** of many biological systems it is essential to have a good understanding of both **function and structure**. CLEM has the potential to provide these answers quickly and easily by combining the power of fluorescence and electron microscopy.

## Provide EM context to fluorescence

The combination of the **power and versatility** of fluorescence microscopy with the **structural context** of electron microscopy provides more insight to the images for even better analysis.

## Multicolor EM labeling

Add **flexibility** to electron microscopy by using fluorophores to label structures of interest instead of immunogold. Fluorophores are easier to use, can be genetically encoded and allow the unlimited use of labels.

## Validation of data

**Validate** LM data with EM data to ensure examination of the structure of interest – and **vice versa**.

# Correlative Light and Electron Microscopy

Correlative light and electron microscopy (CLEM) is the combination of fluorescence and electron microscopy. Correlative microscopy is a powerful technique due to its ability to combine **functional** and **structural** information. However, the **complexity** of the correlative microscopy workflow has so far limited the adoption of the technique by the community at large.

Currently CLEM is performed by using two separate microscopes. This makes CLEM challenging, since:
- The Region Of Interest (ROI) is difficult to retrieve
- Transfer may lead to sample alterations
- Shuttling between two microscopes is time consuming
- Correlation accuracy is limited to mechanical precision
- Overlay of images is subjective

Electron microscope column

Vacuum chamber

LED

Light microscope objective

sCMOS
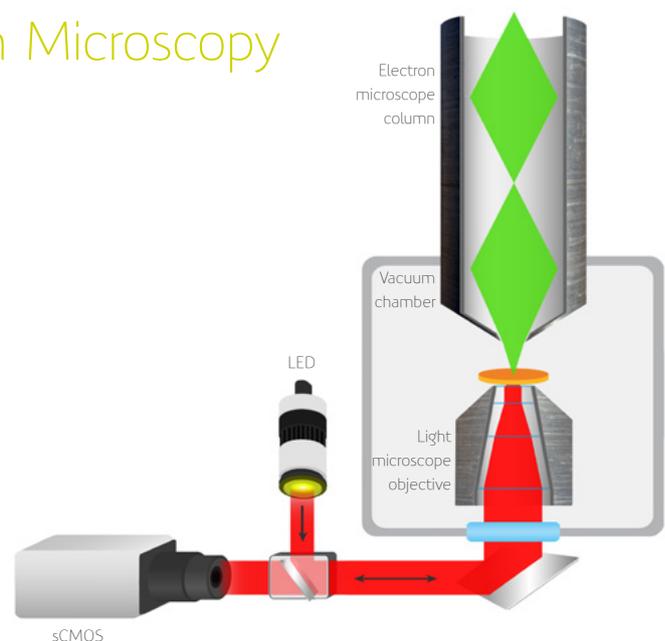
# Advantages of integrated correlative microscopy

## Seamless imaging

With an integrated setup there is no need to retrieve the region of interest. Switching between imaging modalities is **seamless**.

## Reliable results

With integrated CLEM the transfer step between the two microscopes is eliminated. This greatly **reduces the risk of sample damage and alteration**.

## Perfect overlay

Integrated systems are uniquely capable of harnessing the power of the combination to provide a **perfectly unbiased overlay**.

## Higher throughput

An integrated setup reduces correlative workflow time and allows the user to perform more experiments for **quantitative results**.

# Integrated correlative microscopy: the Delphi

The Delphi is an **all-in-one solution** for correlative microscopy. The Delphi integrates a tabletop scanning electron microscope with an inverted fluorescence microscope. This **integration** enables scientists to do correlative microscopy without the challenges typically associated with the technique.

*"The Delphi is a fast and convenient imaging solution, allowing us to move between light and electrons in one compact microscope."*

Lucy Collinson, Francis Crick Institute, London, United Kingdom

## Integrated design

The Delphi is not only integrated by hardware but also makes use of a **single software package** that allows the user to control both imaging modalities as a single microscope. Integrated design means that the correlative workflow is greatly simplified. The sample only needs to be **loaded once** in the microscope to obtain both fluorescence and electron images. The intuitive software interface means that any user can do **correlative microscopy with minimal training**. Integration allows immediate focus on analyzing results.

Many different types of samples can be inspected in the Delphi. Different sample carriers can be mounted in the Delphi, the standard sample carrier is a 14x14mm coverslip. This coverslip is easily mounted on the Delphi sample holder by the use of carbon tape corners which are already there. Whether looking at cultured cells, tissue sections or thin sections: **sample mounting is straightforward and fast**.

EM pole piece

coverslip

LM objective
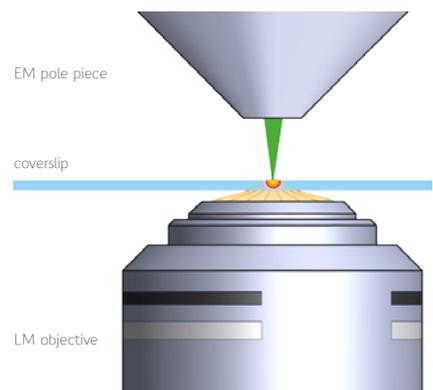
## Automated overlay

The Delphi greatly **enhances reliability** of the correlative workflow by providing an accurate and unbiased overlay. The Delphi uses an alignment procedure that is fully automated and **independent of both user and sample**.

The key to this alignment procedure is the physical principal of **cathodoluminescence**. Light is generated where the electron beam hits the glass coverslip. This light is detected by the camera of the fluorescence microscope and acts as a **temporary fiducial marker**. A grid of these temporary fiducial markers can be used to produce a perfect overlay. **The unbiased overlay means that reliable results can be obtained by any user.**
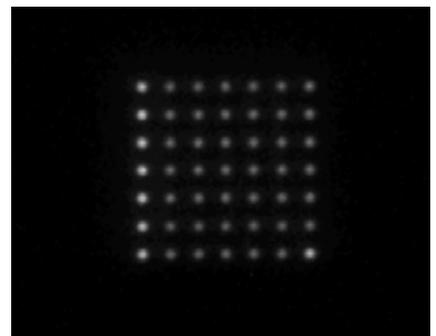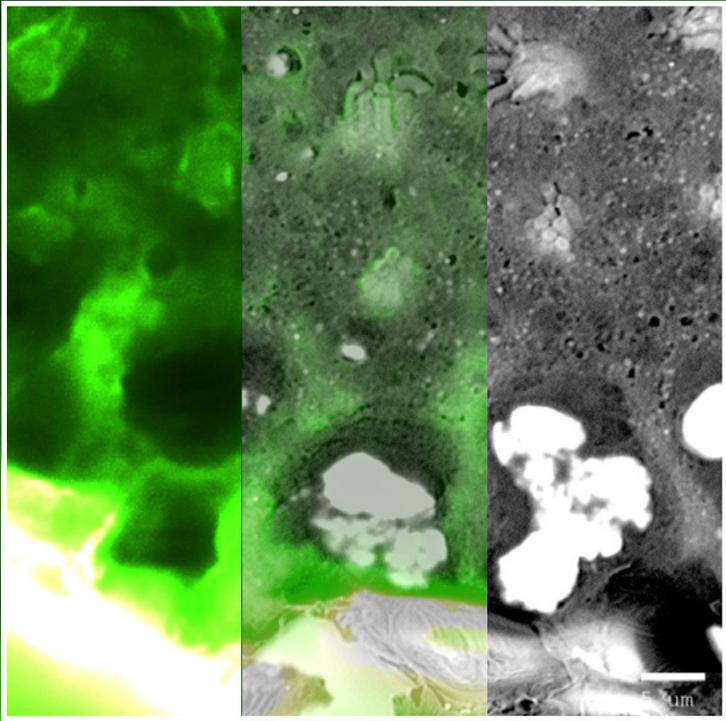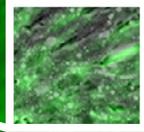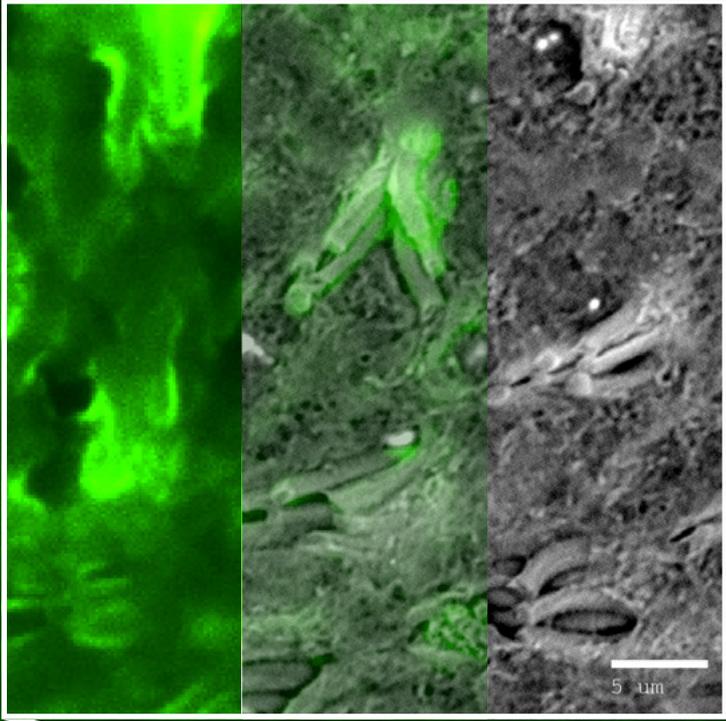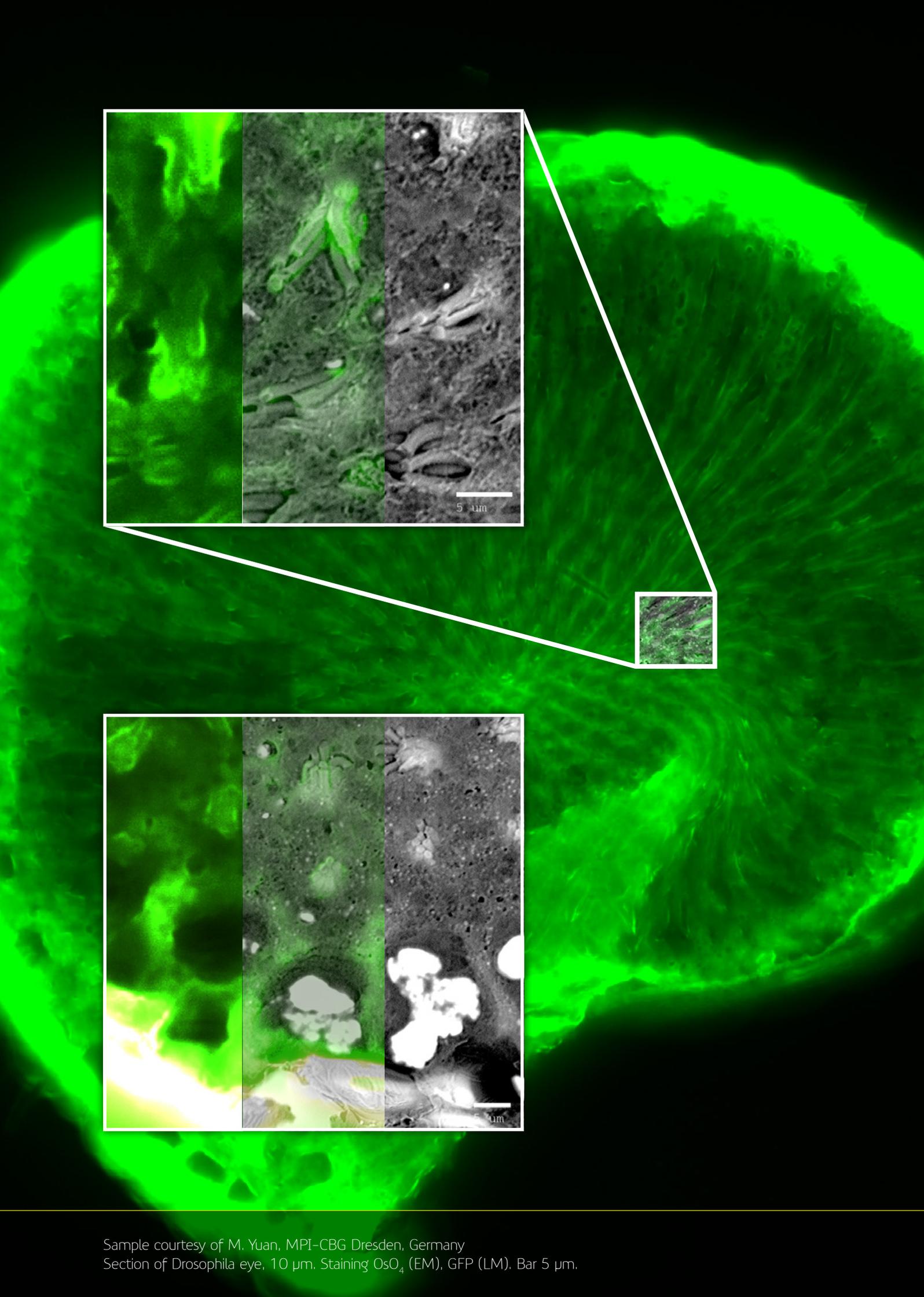
Sample courtesy of M. Yuan, MPI-CBG Dresden, Germany
Section of Drosophila eye, 10 μm. Staining $OsO_4$ (EM), GFP (LM). Bar 5 μm.

# Working with the Delphi
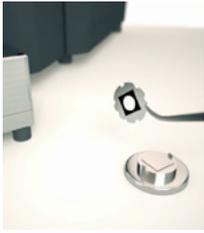
### 1. Mount sample

Sample mounting for the Delphi is designed around 14x14 mm **transparent conductive coverslips**. To mount a sample, the coverslip is attached to a metal carrier ring using adhesive conductive carbon tape.

### 2. Place in sample holder

The coverslip and the carrier ring are then placed on the sample holder. **The orientation of the sample is uniquely defined** which ensures it is easy to relocate a structure of interest.
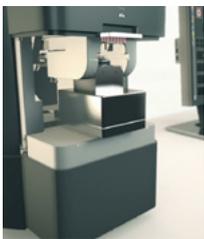
### 3. Load the sample holder

The final step is to slide the sample holder into place and to lower the loading door. This commences the loading procedure which starts with the acquisition of a color navigation image.

### 4. Correlative Imaging

After a **loading procedure of less than 3 minutes**, the system is ready to start imaging.
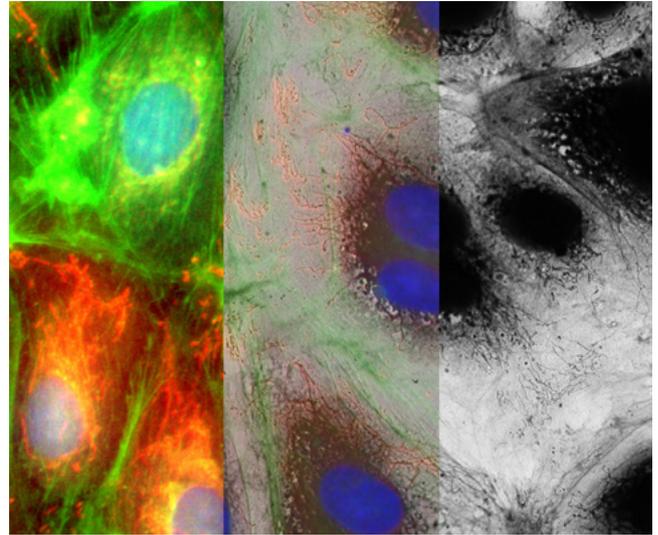


Sample courtesy of M.J. Mourik, LUMC, Leiden, The Netherlands
Cultured HUVEC cells grown on ITO coated coverslip. Staining DAPI (blue), Phalloidin (green), MitoTracker (red). No EM staining.

# Bridging the gap

As a stand-alone microscope, the Delphi is an extremely powerful and **easy to use system**. At the heart of the system is a unique overlay procedure which gives an automatic and unbiased correlation.

The Delphi is also a powerful solution to **easily bridge the gap between different types of fluorescence and electron microscopes**.

The Delphi enables the **combination of correlative images of the Delphi with images acquired on any other fluorescence or electron microscope**. The main idea of this approach is that the comparison of two fluorescence images, or alternatively two electron microscopy images, can be done easily and accurately because the type of contrast is identical.

Automatic LM-EM overlay
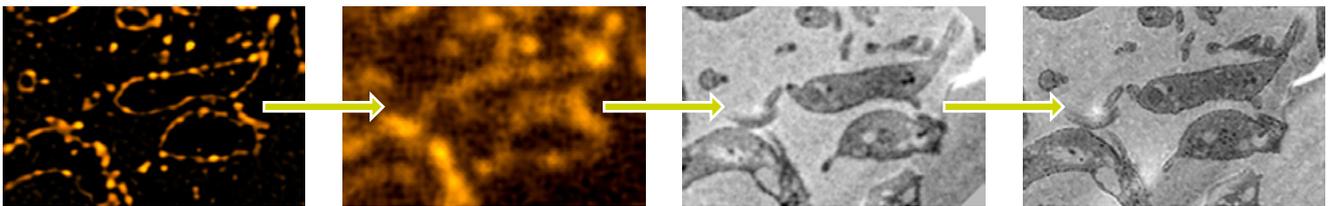


Any fluorescence microscope

Any electron microscope
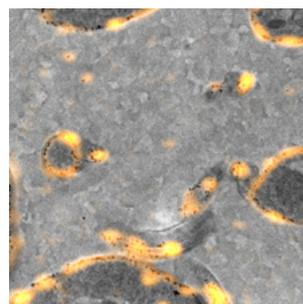


Automatic overlay using Delphi microscope,
LM: BioAxial CODIM100,
EM: FEI Verios 460

Sample courtesy of H. Schwarz, MPI Tübingen. Trypanosoma Brucei labeled for α-tubulin with Cy3 and 10 nm immuno-gold.

Neuronal progenitors in the developing mouse brain are electroporated with a plasmid for expression of a fluorescent (mCherry-tagged) protein. In order to understand the full effect on the cell morphology, the resolution of the SEM is essential in order to compare the ultrastructure of the fluorescent cells and their untransfected neighbors.

2 um
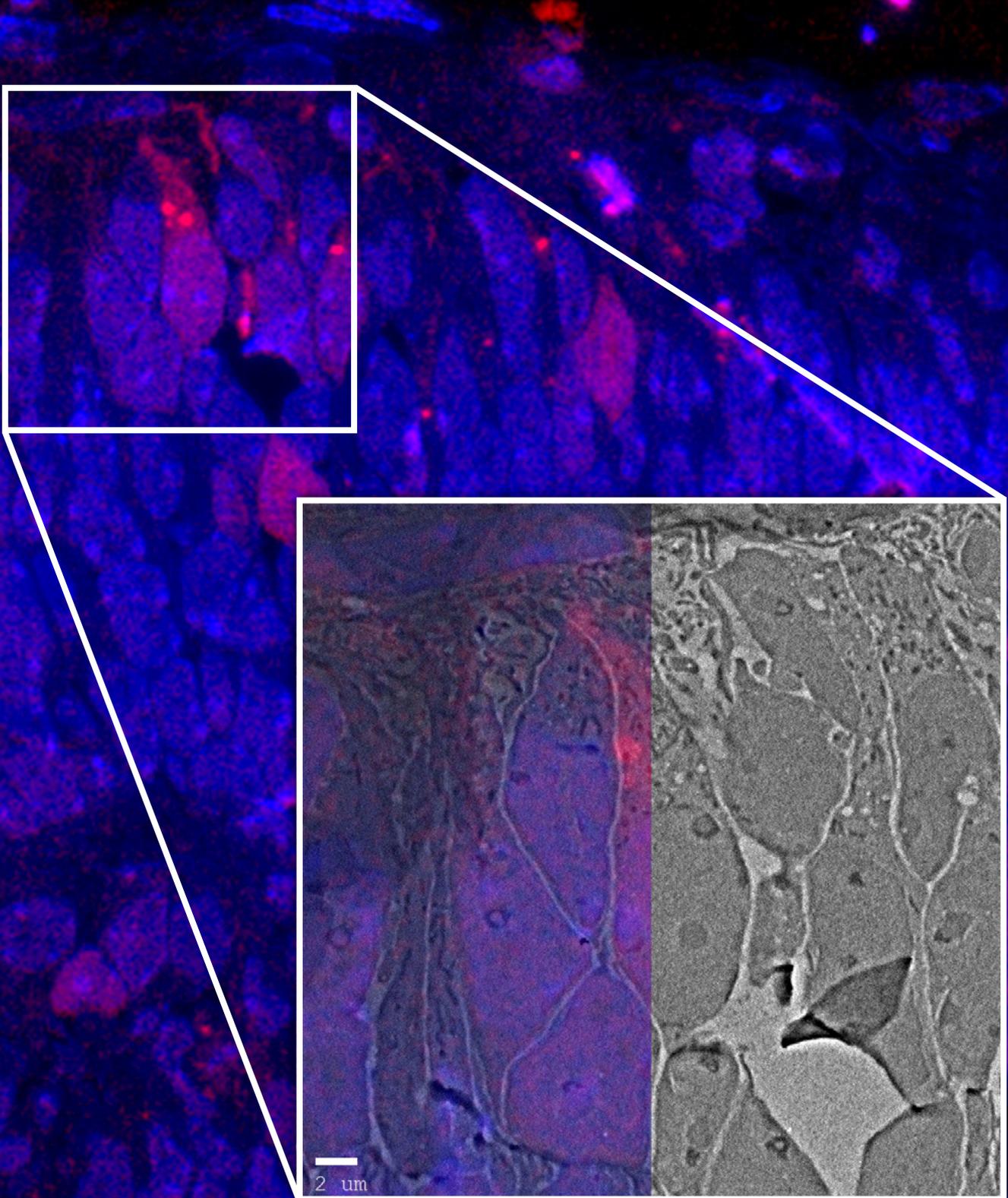
# Delphi Specifications

## General

| | |
|---|---|
| Sample loading time | · To navigation camera in less than 5 s |
| | · To SEM/fluorescence microscope in less than 60 s |
| Sample size | Up to 8 mm diameter |
| Stage | Computer-controlled motorized X and Y and focus |
| Power usage | 700 W at maximum power |

## Navigation

| | |
|---|---|
| Camera | · Full color navigation camera with bright field and dark field modes |
| | · 20 – 120x magnification |

## Fluorescence

| | |
|---|---|
| Objective lens | 40x, 0.95NA, Plan Apochromatic |
| Resolution | 290 nm at 550 nm emission wavelength |
| Illumination | · Four channel solid-state (LED) excitation source with digital On/Off and intensity control. |
| | · Excitation at 392, 474, 554, and 635 nm |
| Filter cube | Multiband Pinkel configuration optimized for DAPI, FITC, TRITC, Cy5 and other like fluorophores |
| Camera | Scientific CMOS camera with 2048 x 2048 pixels (6.5 µm pixel size) and peak quantum efficiency of 60% at 600 nm with at least 40% QE over the range 430 – 750 nm |

## Electron optics

| | |
|---|---|
| Source | Long-lifetime high-brightness source ($CeB_6$) |
| Acceleration voltages | Adjustable range between 4,8 kV and 10 kV |
| Magnification | 20 – 130,000x |
| Resolution | ≤ 14 nm |
| Detector | High sensitivity backscattered electron detector |

# ODEMIS Integrated Software

## General
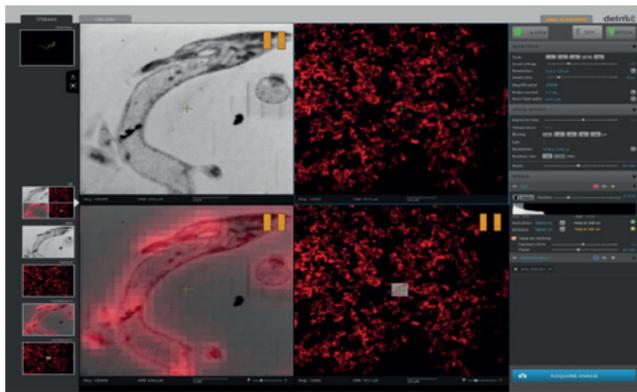


- Control of all optical settings
- Control of all SEM settings
- Fully automated alignment procedure resulting in accurate, unbiased overlays on every specimen
- Overlaid images directly visualized
- Control of the sample stage
- Auto-focus for both SEM and optical microscope
- History trail to track previous imaging locations

| | |
|---|---|
| Image formats | OME-TIFF, HDF5 |
| Data storage | Local, USB flash drive or network |
| License | Open-source GPLv.2 |

## System specifications

### Dimensions

| | |
|---|---|
| Imaging module | 350(w) x 600(d) x 650(h) mm, excluding screen and pump |
| Monitor | 24" monitor with PC and network router mounted |

Delphi is a result of a unique collaboration between two Dutch companies, DELMIC (Delft) and Phenom-World (Eindhoven). *"Almost 70 years ago Philips built its first Electron Microscopes in Eindhoven. Already back then, Delft University played a key role"*, according to Emile Asselbergs, CEO of Phenom-World. *"The Delphi is another example that fits in the Eindhoven-Delft tradition of jointly developing high-quality electron microscopy systems."*

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