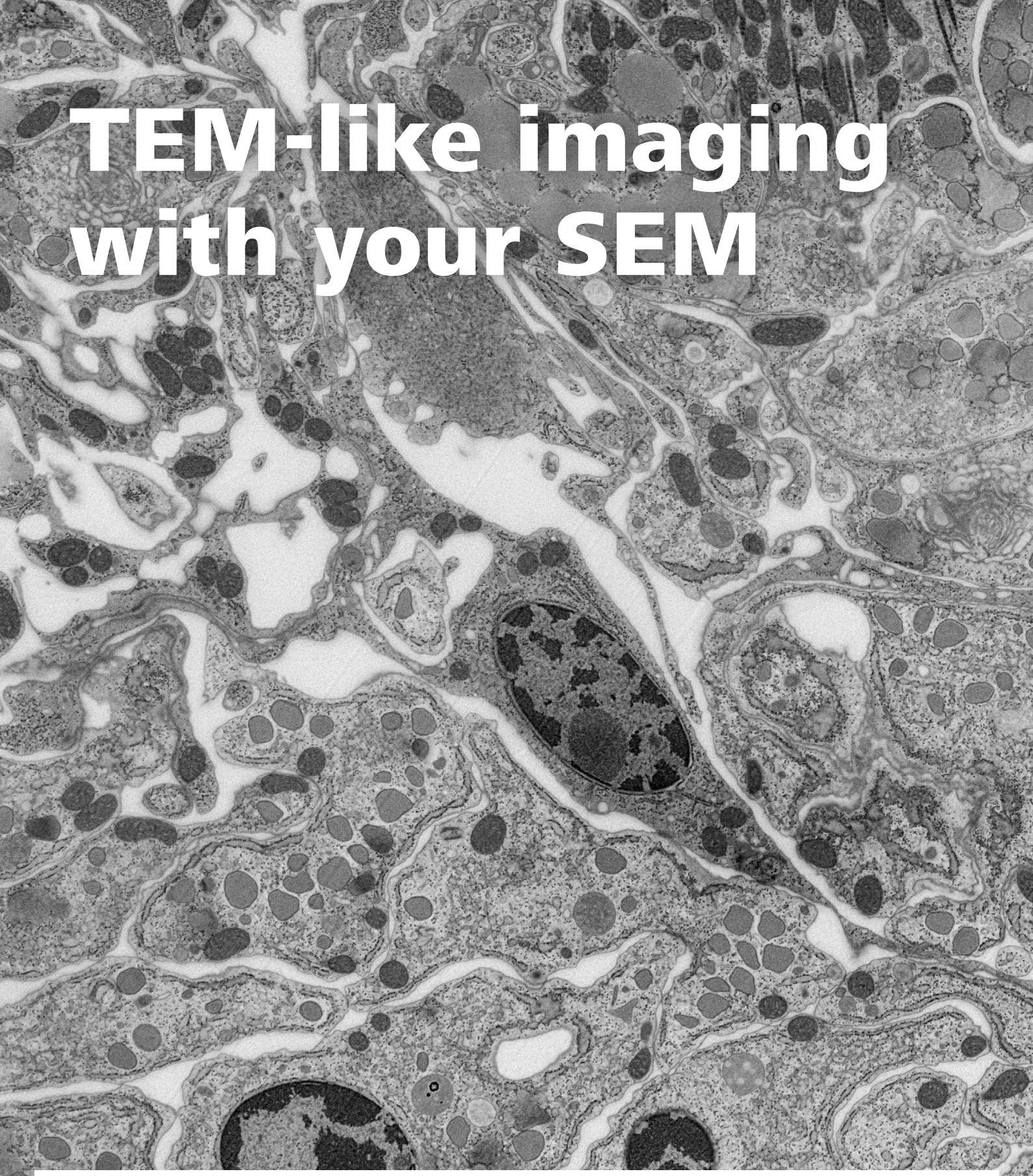


TEM-like imaging with your SEM

ZEISS Sense BSD

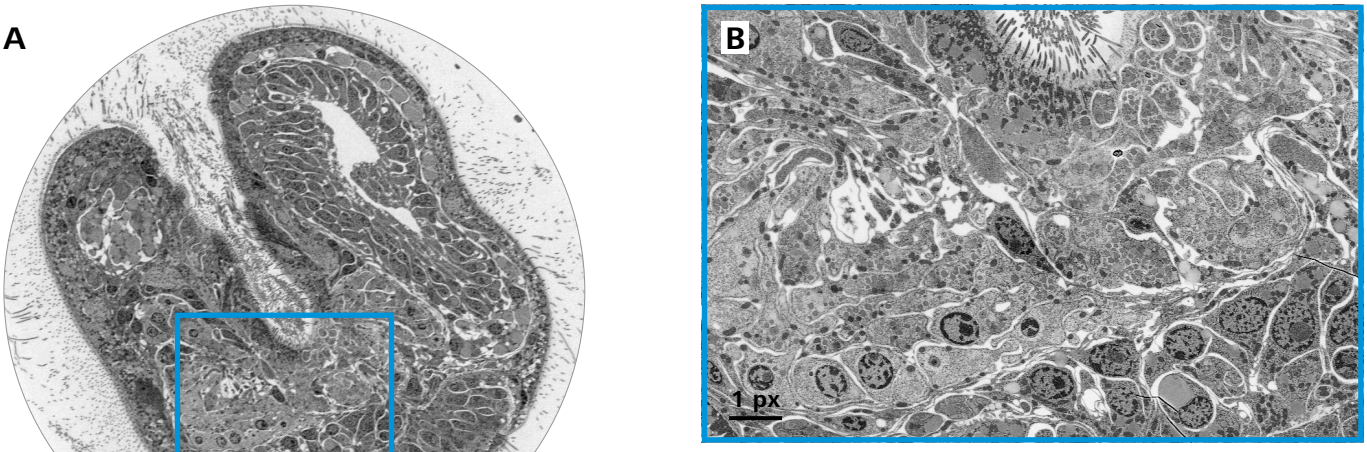
Backscatter Electron Detector for Fast and Gentle Ultrastructural Imaging

ZEISS

Seeing beyond

Imaging Ultrastructure with a New Degree of Speed and Quality

Electron microscopy is traditionally used for high resolution study of the subcellular structures of tissues and cells. Transmission electron microscopes (TEM) have been the preferred choice for ultrastructural imaging, although scanning electron microscopes (SEM) equipped with backscatter electron detectors also enable the acquisition of high-resolution, TEM-like images. However, SEM imaging can present challenges, especially for non-conductive biological samples. Rapid imaging with the desired resolution requires high electron doses and acceleration voltages, which can cause charging effects and sample damage that compromise image quality. ZEISS Sense BSD combines high-resolution ultrastructural imaging with a new degree of efficiency and image quality, making TEM-like imaging possible with your SEM.



(A) Section of the larva of the bryozoan Tricellaria inopinata *acquired with ZEISS Sense BSD at 1.5 kV and 200 nm pixel size. (B) Inset of (A) clearly shows cellular structures such as nuclei or cilia (1 kV, pixel size: 50 nm).*
Sample courtesy by Anna Seybold & Harald Hausen, Sars Centre for Marine Molecular Biology, University of Bergen, Norway

The Solution

With a new diode design and superior detector sensitivity, Sense BSD can detect very small numbers of electrons and convert low signals into high-contrast images. Fast image acquisition with low acceleration voltages and low electron doses becomes possible – your biological sample can be imaged without damage, and deterioration of the image quality induced by charging effects is prevented. The detector is factory-aligned, so no further alignment and calibration is needed. With just a few clicks in the software, you have the detector ready for imaging.

Benefits

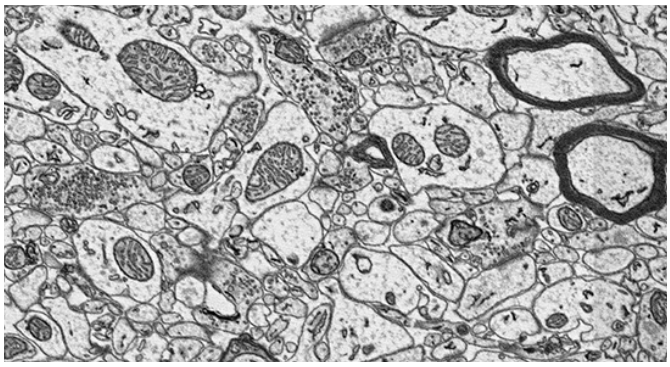
- Well suited for non-conductive, charge-prone biological samples
- Provides the best sample protection and avoids image degradation by allowing the use of low kV imaging.
- Enables high-contrast imaging with its improved detector sensitivity.
- Produces high-quality images in less time.
- Entire sample area accessible for imaging – field of view not restricted by TEM grids.
- Sense BSD is ready to go immediately because of the factory-aligned detector.
- The clear and simple user interface assures easy operability.

Acquisition of Single 2D Images

ZEISS Sense BSD is designed for high-resolution, high-contrast acquisition of 2D images. Low acceleration voltages and low electron doses prevent beam damage to biological samples, and yet still clearly reveal the ultrastructure of tissues or cells. Likewise, gentle excitation conditions prevent the charging effects that are a source of image quality deterioration.

Neuroscience

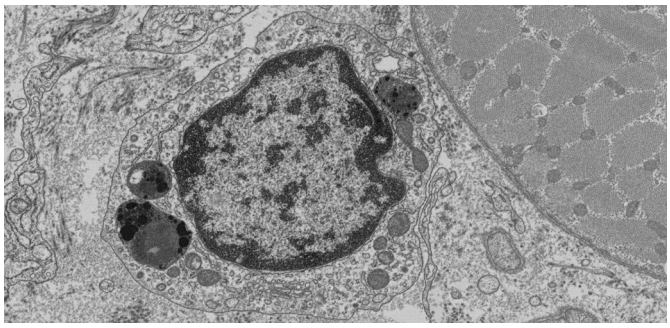
Neurobiological samples, especially brain tissue, are densely packed over a wide area. Visualization and analysis of synapses, with an axon showing synaptic vesicles on one side and the dendrite on the other side, remains challenging, especially when these structures need to be identified within a larger region of the sample. High contrast, high resolution imaging reveals the pathway, structure, and connection of nerve cells.



Brain section imaged with ZEISS GeminiSEM and ZEISS Sense BSD (1.5 kV, pixel size: 1 nm, dwell time: 2 µs). Sample courtesy of Mark H. Ellisman, National Center for Microscopy and Imaging Research, University of California San Diego.

Histology

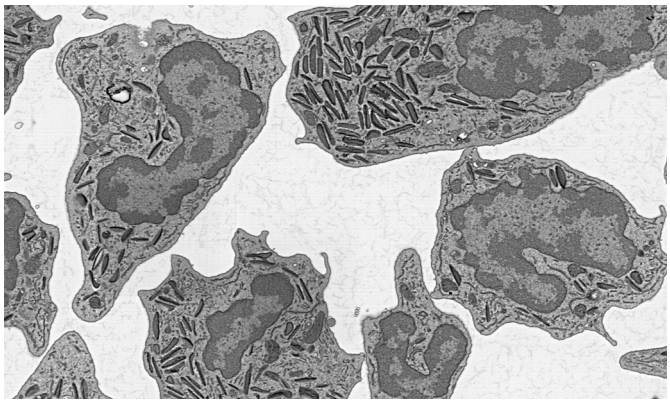
With ZEISS Sense BSD, even the tiniest structures in different tissues can be visualized. The improved sensitivity increases the signal-to-noise ratio, revealing the smallest cellular components. Using low kV for the primary beam prevents sample charging and the resulting degradation of the image quality.



Chemically fixed and stained mouse muscle tissue, imaged with ZEISS GeminiSEM and ZEISS Sense BSD (2 kV, pixel size: 20 nm). Bundles of muscle fibers and individual myofibrils with myofilaments as well as cellular compartments inside the cells become visible.

Cell Biology / Immunology

Isolated cells prepared for electron microscopy are often surrounded by bare resin, which is non-conductive. Consequently, charging effects occur which then deteriorate the image quality, especially when the acceleration voltage and the electron doses are very high. Therefore, low kV imaging is key to preventing charging effects, and a very sensitive detector is needed to collect the low number of backscattered electrons and convert them into high-contrast images.



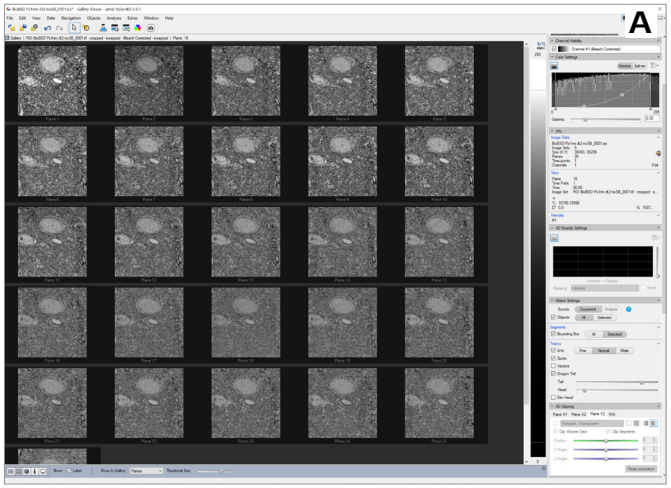
Isolated immune cells from a zebrafish, embedded in epoxy resin and cut into ultrathin sections of 100 nm thickness. The section was imaged with ZEISS GeminiSEM and ZEISS Sense BSD (1.5 kV, 88 pA, pixel size: 3 nm, dwell time: 1.6 µs, stage bias). Elongated, electron-dense, cigar-shaped granules which are typical for neutrophils and other cellular components such as nuclei and mitochondria are clearly visible.

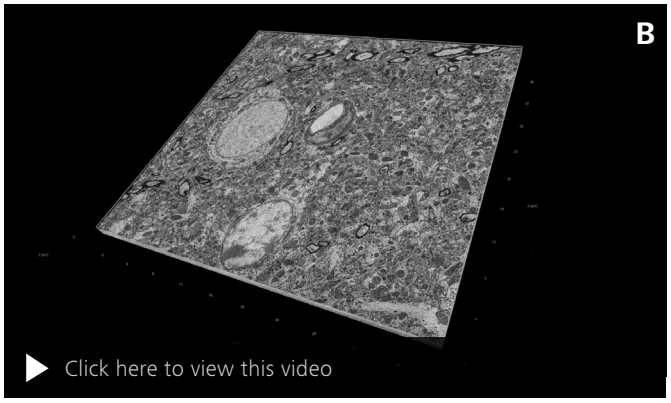
Acquisition of Datasets using Array Tomography

Array Tomography is a method for imaging serial sections of resin-embedded biological samples, then reconstructing a 3D dataset from the image series. ZEISS Sense BSD matches perfectly with the requirements for imaging sections from biological samples: high detector sensitivity enables low kV imaging and prevents sample damage while also allowing fast imaging because fewer backscattered electrons are needed to generate high-contrast images.

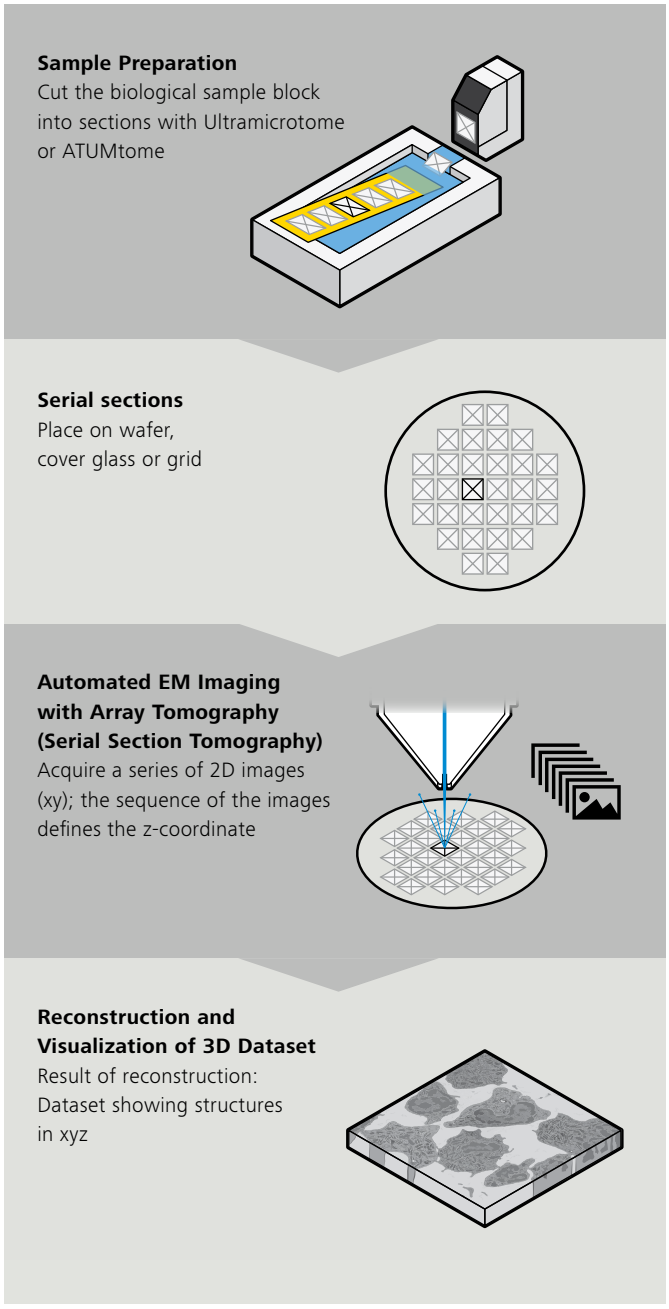
Application Example: Neuroscience

Imaging brain samples requires the challenging combination of high resolution and 3D acquisition to enable visualization of cellular structures at the same time as tracing neurons through whole volumes. Array Tomography in combination with ZEISS Sense BSD enables high-contrast imaging of serial sections and the subsequent reconstruction of 3D datasets with high-resolution in x, y and z.





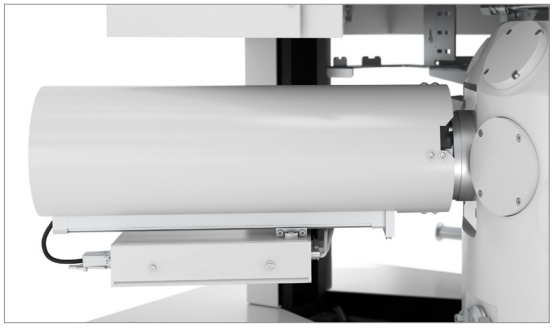
Mouse Hippocampus prepared with NCMIR's staining protocol for Serial Blockface Scanning EM. (A) A series of 2D images in the Gallery View of arivis Vision4D gives detailed insight into the ultrastructure. (B) An animated dataset of the series shows detailed structures in a broader context in 3D. Acquired with GeminiSEM and Sense BSD (1.5 kV, pixel size: 1 nm, dwell time: 2 µs). Sample courtesy of Mark H. Ellisman, National Center for Microscopy and Imaging Research, University of California San Diego.



The array tomography workflow begins with sectioning the biological sample that has been prepared for electron microscopy. Once the serial sections are collected, either on a cover glass or on a tape attached to a wafer, the sample is imaged with an SEM. The resulting 2D images are then reconstructed into a 3D data set.

Your Insights into the Technology Behind it

The components of ZEISS Sense BSD are engineered to treat your biological samples gently, guarantee fast acquisition, and deliver high-quality images.

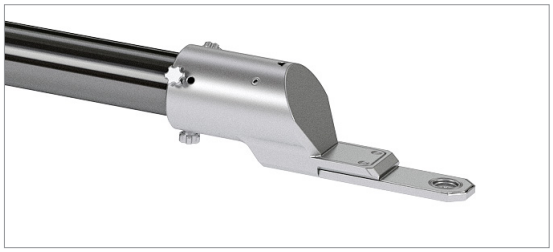


Housing

The design of the housing protects the detector from acoustic and mechanical noise, ensuring stable imaging conditions. As a result, you get the highest quality high-resolution images.

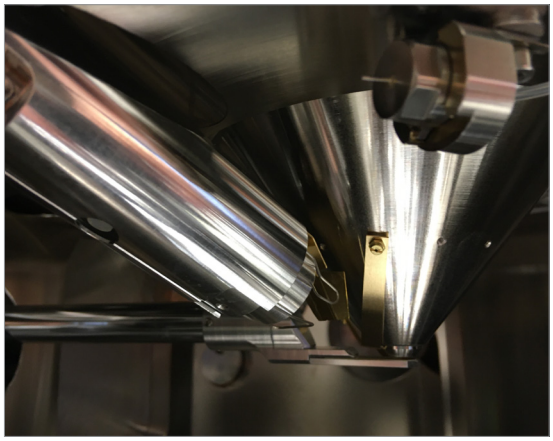
Amplification

The newly designed amplifier improves the signal-to-noise ratio to allow high-speed imaging.



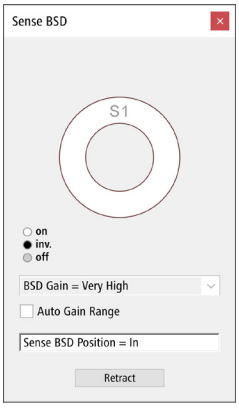
Diode

The silicon-type diode is optimized for imaging at low kV. The detection area is enlarged by reducing the central hole for the primary electron beam without affecting the beam negatively. This allows most of the backscattered electrons to be detected.



Adjustment

The diode is directly located under the objective lens. The factory-set detector does not require any additional user interaction. Ease of use is further enhanced by automatic adaption of the objective lens in combination with working distance and magnification adjustment.



Software and User Interface

The control window is integrated into SmartSEM, the operating software for the SEM. The user interface is clear, and the user interaction is reduced to a few steps, such as setting contrast, selecting gain, and use of the push and pull buttons to insert and retract the detector with a single click!

Technical Data

Parameter	Specification
Type of Diode	Silicon based diode for direct detection of backscattered electrons, one segment
Image Polarity	Configurable. Default: "TEM" like contrast
Optimum Working Distance	4 – 6 mm
Energy Range	< = 7 keV
Optimum Primary Beam Current	50 pA – 1nA
Mechanical Module	Highly stable mechanical module with acoustic dampening cover
Easy Servicability	Diode exchange is plug & play
Protection	Protective cover to avoid charging and damage to the diode.
	Integrated protection during plasma cleaning of sample/chamber.
Lifetime	Expected lifespan of detector diode: 2 years under average usage conditions
System Integration	Collision control with ZEISS hardware implemented.
	Acceleration voltage limited to <7 kV.
	EO table correction is applied.
User Interface	Default settings optimized for ease of use.
Insertion and Retraction	Pneumatic

ZEISS Sense BSD can be used with

- Sigma 300, 500
- GeminiSEM 360, 460, 560
- Crossbeam 350, 550 (not for 3D Tomography)

Software:

- Smart SEM (7.1 and higher)

Retrofit possible on following systems:

- GeminiSEM 300, 450, 500
- Crossbeam 540
- Merlin

* Front page image shows the ultrastructure of *Tricellaria inopinata*. Sample courtesy: Harald Hausen, Sars Centre for Marine Molecular Biology, University of Bergen, Norway

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