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DATASHEET

Volumescope 2 SEM for Life Sciences

Highest quality isotropic 3D data from large sample volumes

The Thermo Scientific Volumescope 2 scanning electron microscope (SEM) for life sciences is our state-of-the-art serial block-face imaging system. Keep control of experiments with easy to use technology and protect valuable samples by tried and tested solutions on a wide range of sample types through highest quality in every acquisition step.

Improving large-volume SEM by combining serial block-face imaging with Multi-Energy Deconvolution

Unraveling the complex 3D architecture of cells and tissues in their natural context is crucial for the structure-function correlation in biological systems. In recent years, there have been considerable advances in SEM-based methods for 3D reconstruction of large tissue volumes. Serial Block-Face SEM (SBF-SEM) combines *in situ* sectioning and imaging of plastic-embedded tissue blocks within the SEM vacuum chamber in a fully automated fashion for reconstruction of large tissue volumes. Until now, the axial resolution was limited by the minimal section thickness that can be cut from the block-face; however, with a combination of SBF-SEM and Multi-Energy Deconvolution SEM (MED-SEM), the Thermo Scientific™ Volumescope 2™ SEM now enables large-volume imaging with truly isotropic 3D resolution.

Truly isotropic 3D data

The Volumescope 2 SEM for life sciences offers a novel way to improve the axial resolution: combining mechanical sectioning with optical sectioning, realized by our proprietary MED-SEM. Following *in situ* sectioning of the block-face using a diamond knife, the freshly exposed tissue is imaged several times using increasing accelerating voltages.

These images are subsequently used in a deconvolution algorithm to derive several optical subsurface layers, forming a 3D subset. By repeating this cycle, the Volumescope 2 SEM offers isotropic datasets with 10 nm z-resolution.

Key Benefits

Keep control of experiments with easy to use technology:

- Ability to reuse jobs and system settings
- Create multiple ROI during job acquisition
- Visualize and navigate in Amira Live Tracker during job acquisition to optimize/control outcome
- Ability to perform large 3D volume acquisition unattended and reconstructions in automated manner frees time for researchers
- Easy and fast mounting microtome exchange for normal SEM operation or automated array tomography with optional Thermo Scientific Maps software

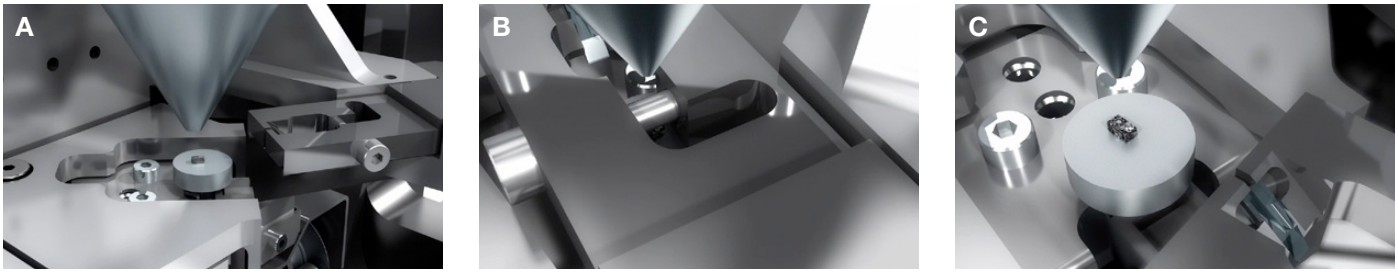
Protect valuable samples with tested solutions on a wide range of sample types reliably at every acquisition step:

- System performance and reliability ensured through intensive testing
- Ultimate sample protection through Debris Trap and Swipe Feature by Debris Recognition
- Reliable acquisition on charging samples with both HiVac and LoVac; the LoVac detector (VSDBS) for highly charged samples provides comparable data quality to the HiVac
- MED assures highest image quality in z-direction (optical slicing at 10 nm for isotropic resolution)



Mouse brain sample. Data acquired by physical slicing at HiVac mode. Imaged in BSE mode at 2 kV accelerating voltage, 0.1 nA beam current, 2 µs dwell time. Acquired pixel size 4 x 4 nm. Fine features as vesicles and synaptic clefts can be observed. *With permission from Dr. Gabor Nyiri, Institute of Experimental Medicine, Budapest, Hungary.*

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Microtome cutting motion. A. Sample is lowered, then knife moves over the sample. B. On the back stroke, sample is raised to the specified section thickness to be cut. C. Ultrathin section is cut on the back stroke.

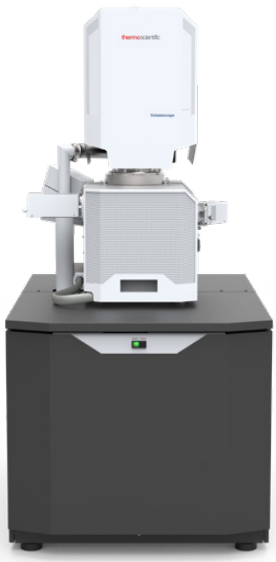
Flexibility and ease-of-use with exceptional performance

The Volumescope 2 was developed on an SEM platform based on the Thermo Scientific™ NiCol™ Electron Column. The system is equipped with a versatile in-lens and in-column detection system providing exceptional contrast. A choice of high-resolution HiVac operation or dedicated LoVac mode makes it ideal for changing sample requirements and challenging samples.

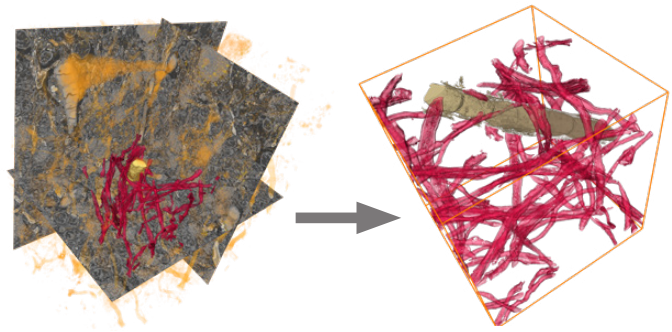
Fully automated column alignments limit the need for highly trained operators to adjust settings, while predefined use cases for common applications give instant productivity for all users. The dedicated serial block-face imaging workflow simplifies system use, and user guidance assists you every step of the way. The Volumescope 2 SEM's versatility extends to alternative use of the SEM by allowing for quick swap of the compact, stage-mounted microtome, enabling fast changeover between serial block-face SEM, routine SEM and array tomography (AT). AT imaging requires optional software module available in Thermo Scientific Maps Software.

Workflow solutions for increased productivity

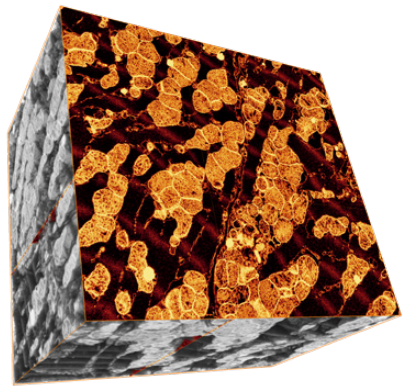
The serial block-face imaging is controlled by a single integrating software interface, Thermo Scientific™ Maps™ Software, that permits convenient importing of images from any light microscope. Direct correlation and targeting of particular regions of interest (e.g. based on fluorescence staining) is straightforward and easy. The final results can be easily visualized and analyzed using the world's leading 3D imaging software, Thermo Scientific™ Amira™ Software for Life Sciences. This powerful software package can be used to directly import the data produced by the Volumescope 2 SEM not only for processing, but more importantly, for visualization and analysis, making it one of the most powerful toolsets available on the market.



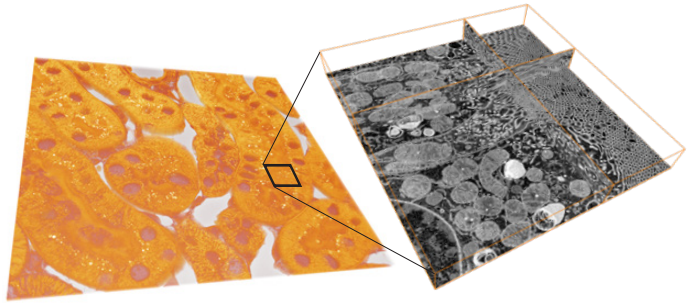
Thermo Scientific Volumescope 2 Scanning Electron Microscope (SEM)



Volume reconstruction of a rat brain sample. Data acquired by physical slicing in LoVac mode (50 Pa) to suppress charging. Imaged in BSE mode at 2.7 kV accelerating voltage, 0.4 nA beam current, 2 µs dwell time. Pixel size of 15 x 15 nm (x, y), 2133 slices @ 40 nm (physical cut), volume of 123 x 123 x 85 µm^3. Representation of complete dataset and processed sub-volume of 36 x 37 x 38 µm^3 to show selected objects.



Volume reconstruction of a mouse heart muscle. Data acquired by a combination of physical and virtual slicing in HiVac mode. The data were acquired in BSE mode with optical sectioning. Nominal resolution of 5 x 5 x 10 nm (x, y, z), 1102 slices @ 40 nm (physical cut), volume of 16 x 15 x 11 µm3. *Specimen courtesy of Dr. Madesh Muniswamy, Temple University, USA.*



Volume reconstruction of a mouse kidney sample. Overview image and detailed view of selected area at higher resolution. Data acquired by a combination of physical and virtual slicing in HiVac mode. The overview image (on left): 1.8 kV, res. 60 x 60 x 30 nm (x, y, z), volume of 162 x 129 x 2.2 µm^3. The detailed view (on right) was acquired in BSE mode with optical sectioning, 0.1 nA beam current, 1 µm dwell time. Nominal isotropic resolution of 10 x 10 x 10 nm (x, y, z), 222 slices (physical + optical ones) @ 30 nm (physical cut), volume of 20 x 17 x 2.2 µm^3. *Specimen courtesy of Jean-Marc Verbavatz, MPI-CBG, Germany.*

Hardware specifications

Electron optics

- High-resolution field emission SEM column
  - High-stability Schottky field emission gun
  - Compound final lens: a combined electrostatic, field-free magnetic and immersion magnetic objective lens
- Source lifetime 24 months
- Auto bakeout, auto start, no mechanical alignments
- Automated heated apertures
- Continuous beam current control and optimized aperture
- Double stage scanning deflection
- Dual objective lens combining electromagnetic and electrostatic lenses
- Beam current range: 1 pA to 400 nA (in serial block-face imaging SEM 50 pA – 3.2 nA)
- Landing energy range: 20 eV – 30 keV*
- Accelerating voltage range: 200 V – 30 kV (in serial block-face imaging SEM 500 V – 6 kV)
- User guidance and column presets

Electron beam resolution at optimum working distance

- Routine SEM High-vacuum imaging
  - 0.8 nm at 30 keV STEM
  - 0.7 nm at 15 keV
  - 1.0 nm at 1 keV

Chamber

- Inside width: 340 mm
- Analytical working distance: 10 mm
- Ports: 12

Detectors

- Serial block-face imaging detectors:
  - T1 segmented lower in-lens detector
  - VS-DBS: LoVac lens-mounted BSED
- T2 in-lens detector
- ETD: Everhart-Thornley SE detector
- IR-CCD
- Low-vacuum SE detector
- STEM 3+ – Retractable segmented detector
- Additional detectors available

Vacuum system

- Complete oil-free vacuum system
- 1 × 220 l/s TMP
- 1 × PVP-scroll
- 2 × IGP
- Chamber vacuum (high-vacuum) <6.3 × 10^{-6} mbar (after 72 hours pumping)
- Low-vacuum mode up to 50 Pa for charge compensation of non-conductive samples
- Evacuation time: ≤3.5 minutes

Stage specifications without microtome installed	
Type	Eucentric goniometer stage, 5-axes motorized
XY	110 × 110 mm
Repeatability	<3.0 µm (at 0° tilt)
Motorized Z	65 mm
Rotation	n × 360°
Tilt	-15° / +90°
Max. sample height	Clearance 85 mm to eucentric point
Max. sample weight	500 g in any stage position (up to 2 kg at 0° tilt)
Max. sample size	122 mm with full rotation (larger samples possible with limited rotation)

Microtome specifications	
Section thickness	Effective section thickness using MED ≥10 nm
Cutting thickness	Guaranteed 40 nm Achievable 25 nm
Cutting speed	User defined: 0.1 – 1 mm/sec
Cutting window	2 mm
Sample Z travel range	1.2 mm
Sample size	600 x 600 µm

Sample holders

- Standard multi-purpose holder; uniquely mounts directly onto the stage; hosts up to 18 standard stubs (Ø12 mm), three pre-tilted stubs, two vertical and two pre-tilted row-bar holders* (38 degrees and 90 degrees)
- Each optional row-bar accommodates six S/TEM grids
- Wafer and custom holders

Supporting Software

- “Beam per view” graphical user interface concept with up to four simultaneously active views
- Maps Software for automatic acquisition of large volumes including optional module for automated array tomography
- Image registration
- Navigation montage
- Image analysis software
- Undo / Redo functionality
- User guidance

Image processor

- Dwell time range from 25 ns – 25 ms (in Volumescope 2 SEM 50 ns – 1 ms)
- Up to 6144 × 4096 pixels (40k × 40k with Maps Software)
- File type: TIFF (8-, 16-, 24-bit), BMP or JPEG (in serial block-face imaging 8-, 16-bit)
- Single-frame or 4-view image display
- Thermo Scientific™ SmartSCAN™ Mode (256-frame average or integration, line integration and averaging, interlaced scanning)
- DCFI (Drift Compensated Frame Integration)

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System control
- 64-bit GUI with Windows® 7, keyboard, optical mouse
- “Beam per view” graphical user interface concept, with up to four simultaneously active views
- 24-inch LCD display, WUXGA 1920 × 1200 pixels (second monitor optional)
- Undo / Redo functionality
- User guidance for basic operations and applications
- Optional joystick
- Optional manual user interface (knob board)

Accessories (optional)
Sample / chamber cleaning: Integrated Plasma Cleaner

Software options
- Maps Software correlative workflow
- 3D reconstruction and image analysis software; Amira Software for Life Sciences
- Web-enabled data archive software

Documentation
- Online user guidance
- Operating instructions handbook
- Online help
- Prepared for Thermo Scientific™ RAPID™ Support (remote diagnostic support)

Warranty and training
- 1 year warranty
- Choice of service maintenance contracts
- Choice of operation / application training contracts

Consumables (partial list)
- Replacement Schottky electron source module
- Aperture strips for electron columns
- Diamond knife from external supplier (Diatome)

Installation requirements
Refer to preinstallation guide for detailed data.

Power
- Voltage: 100 – 240 V AC (-6%, +10%)
- Frequency: 50 or 60 Hz (±1%)
- Consumption: <3.0 kVA for basic microscope
- Earth resistance: <0.1Ω

Environment
- Temperature: 20°C ±3°C
- Relative humidity below 80%
- Stray AC magnetic fields <40 nT asynchronous, <100 nT synchronous for line times, 20 ms (50 Hz mains) or 17 ms (60 Hz mains)
- Minimum door size: 0.9 m wide × 1.9 m high
- Weight: column console 980 kg
- Dry nitrogen
- Compressed air 4–6 bar — clean, dry and oil-free
- System chiller
- Acoustics: site survey required, as acoustic spectrum relevant
- Floor vibrations: site survey required, as floor spectrum relevant
- Optional vibration isolation table

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