

## Apreo 2 SEM for Life Sciences

Maximize your productivity with flexible, robust, and high-performance 2D/3D life science imaging with the Thermo Scientific Apreo 2 SEM for life sciences.

This highly flexible system boasts many features that can make life science imaging simple. For example, charge-free imaging in combination with simultaneous triple in-column detection ensures the right image at the right time. The Thermo Scientific™ Apreo 2 SEM is the ideal choice for 2D and large-volume analysis.

The Apreo product line is known for versatile, high-quality imaging of difficult and/or delicate life science samples. The Apreo 2 SEM builds on these qualities to provide optimal imaging for an even wider range of critical life science targets. With charge-free, high-resolution, and enhanced-contrast imaging, it is now even easier to find the right conditions for every specimen.

### Lens and Column configurations

The unique Trinity In-Column Detection System of the Apreo 2 SEM allows you to obtain more data from your sample thanks to its final lens design, which includes multiple detectors at different positions in the lens. The system allows a choice in lens presets: the electrostatic final lens (in the Apreo 2 C SEM) is ideal for imaging heavily charging samples, whereas the immersion lens (in the Apreo 2 S SEM) enables increased contrast in life science samples.

### Key Benefits

**All-around high performance for life science samples ranging from tissues to nanoparticles.** Using the compound electrostatic magnetic-immersion lens, ultra-high resolution can be achieved with great depth of field (long working distances).

**Flexible 2D and 3D imaging.** The Apreo 2 SEM can image a wide range of samples in 2D, or, with the addition of our serial block-face imaging system and multi-energy deconvolution software or Array Tomography, it can even unravel complex 3D architectures in their natural context.

**Less time on instrument maintenance.** With Thermo Scientific SmartAlign Technology, optical alignment can be performed automatically so that you can spend more time on data collection and less on routine maintenance.

**Enhanced surface contrast.** Beam deceleration allows for the detection of low-angle backscattered electrons, enhancing the topographical surface contrast of your samples.

**Simultaneous detection of multiple signals.** The Apreo 2 SEM features the unique, in-column Trinity Detection System, which allows simultaneous detection of multiple signals (e.g., composition, topography, and surface information) and is immediately available from the moment the beam is activated.

**Serial block-face imaging system.** Our state-of-the-art serial block-face imaging system enables large 3D volume acquisition without user attendance and volume reconstructions (using Thermo Scientific Amira™ Software) in an automated manner, achieving high-quality isotropic 3D data.

**Array Tomography software.** The Apreo 2 SEM also allows volume imaging with our dedicated Array Tomography software, which enables high-resolution 3D images based on serial ultra-sections of biological tissue blocks.

Within the column, different detectors allow simultaneous information on material contrast (T1 detector; detecting backscatter electrons), topography (T2 detector; detecting secondary electrons), and surface information (T3 detector; detecting low-energy secondary electrons). In all Apreo 2 SEMs, the T1 and T2 detector are available as standard, and the T3 is optional.

Other detector options for the Apreo 2 SEM include the chamber-mounted Thermo Scientific Nav-Cam+™ Camera; the ETD detector, which detects secondary electrons and backscatters; a retractable DBS, which, by its four annular segments, allows discrimination of backscatters based on their angle; and a retractable, segmented STEM detector. The STEM detector allows higher contrast in life science samples in annular dark field. For serial block-face imaging, a specific LoVac lens-mounted backscatter detector can also be configured, providing the best image quality in low-vacuum conditions.

### Chamber and stage options

The Apreo 2 SEM offers a choice of high-resolution HiVac operation or a dedicated LoVac mode. The latter allows chamber pressures up to 500 Pa, which provides a certain hydration level to samples and allows different charge-mitigation strategies to be applied. Mitigation of these charging effects results in excellent resolution in the images. The low-vacuum mode also comes with an automated routine to insert and remove the pressure limiting aperture (PLA). The PLA allows you to choose the proper conditions for imaging instead of manually adjusting the system for LoVac imaging conditions.

The flexible chamber of the Apreo 2 SEM accommodates up to three EDS/WDS ports for fast and sensitive X-ray measurements, which can be analyzed using the revolutionary Thermo Scientific™ ChemiSEM™ Technology. This technology provides elemental information quickly and easily, and, because it is fully integrated into the user interface of the SEM, the elemental information is displayed as color in the live image, meaning that the SEM is no longer a grayscale technique.

The chamber is also compatible with cryogenic stages, cathodoluminescence detectors, integrated Raman, and other analytical techniques that might be required, offering a full package for any application and any research question that needs answering.



### 3D imaging

#### Volumescope System

The addition of our state-of-the-art serial block-face imaging system to your Apreo 2 SEM allows you to move your experiments into 3D, unraveling the complex architecture and structure-function relationships of your sample in its natural context. The Thermo Scientific Volumescope™ System can be quickly and easily mounted onto your Apreo 2 SEM, giving you the ability to perform unattended, large-volume acquisition as well as automatic visualization of the reconstructions.

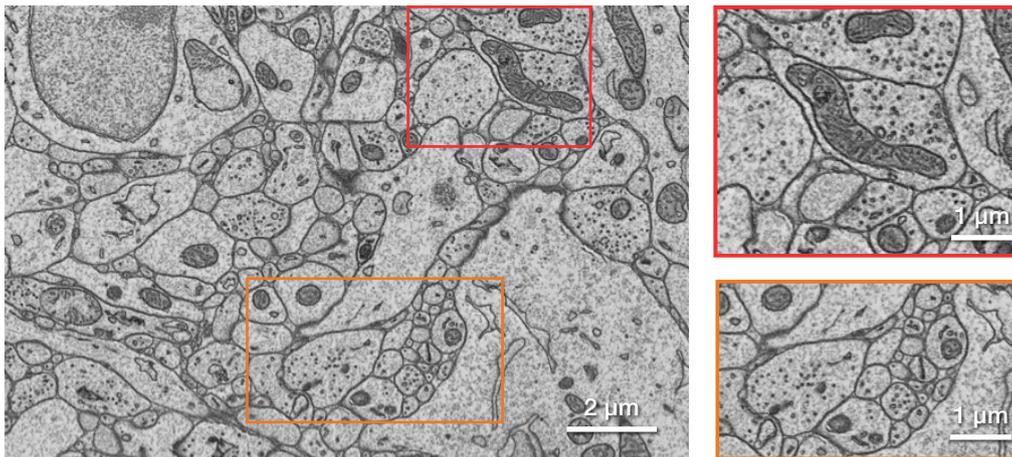
The Volumescope System offers an improved axial resolution by combining the mechanical microtome with optical sectioning. After each section is cut from the block face, the exposed sample is imaged with a series of increasing accelerating voltages. This generates a 3D subset of optical subsurface layers that provide isotropic information with 10 nm Z-resolution. The ability to quickly remove the microtome allows you to switch the system back to normal 2D SEM operation or even to automated tomography through the addition of optional Thermo Scientific Maps™ Software.

#### Array Tomography

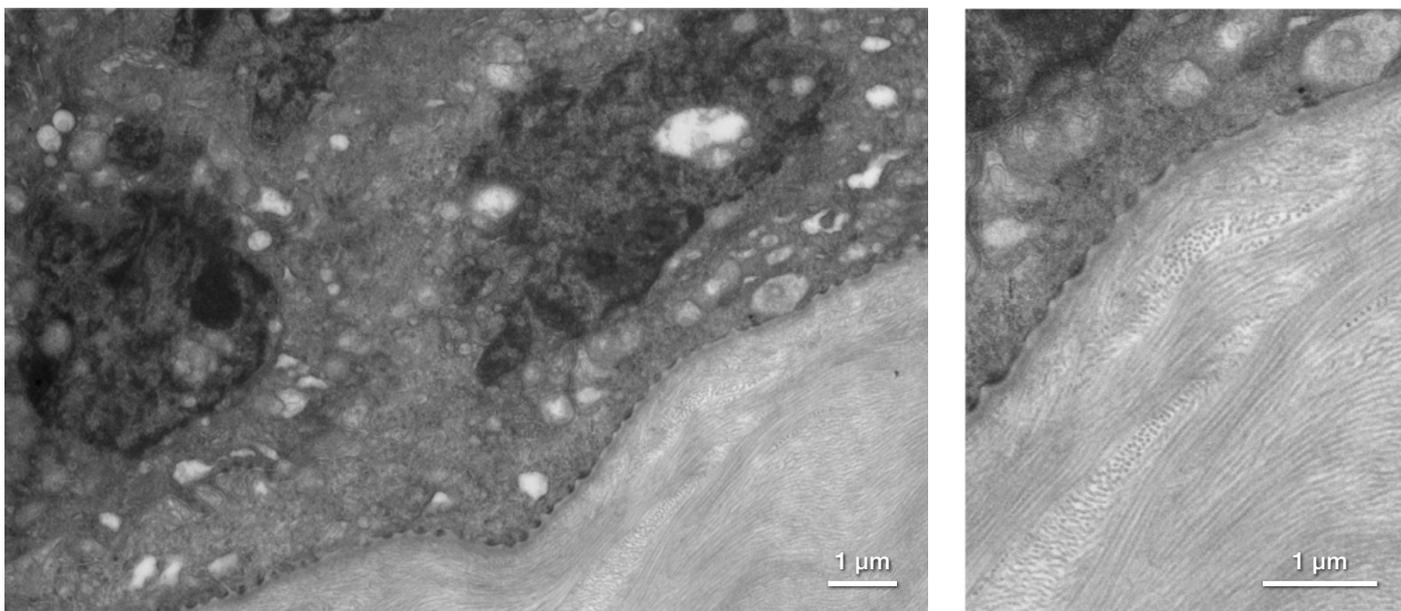
Array Tomography is a software module within Maps Software that enables high-resolution 3D image volumes based on serial ultra-sections of biological tissue blocks. Array Tomography samples are typically carriers that carry hundreds of serial ultra-sections. Until now, the process of creating imaging regions in the same relative location in all sections required hours, or even days, of manual input from a skilled operator. Using the Thermo Scientific Array Tomography workflow, the automatic Section Finder takes over this task.

## Life science samples imaged with the Apreo 2 SEM

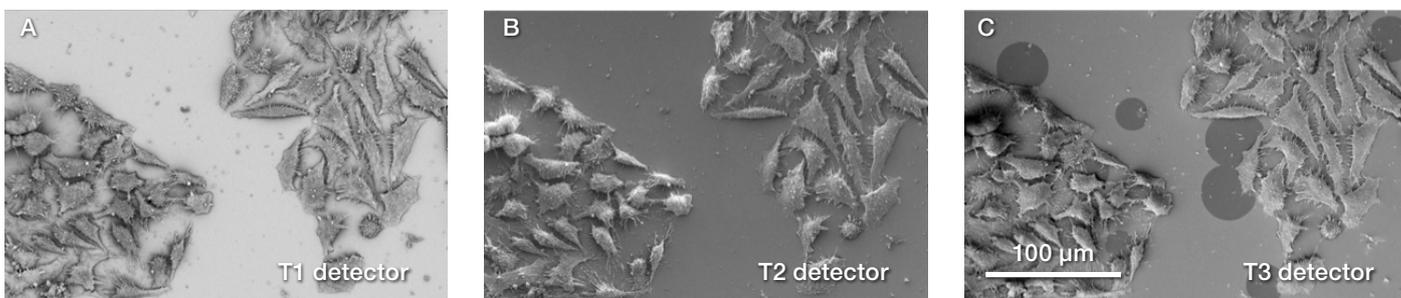
### Cell and tissue biology applications



Resin-embedded, heavy-metal-stained neuronal tissue images in high vacuum using the in-lens T1 backscatter electron detector at 1.5 kV. Left image shows a low-magnification overview. Images on the right represent high-magnification images from the left image, showing contrast differences resulting from backscattered electrons.



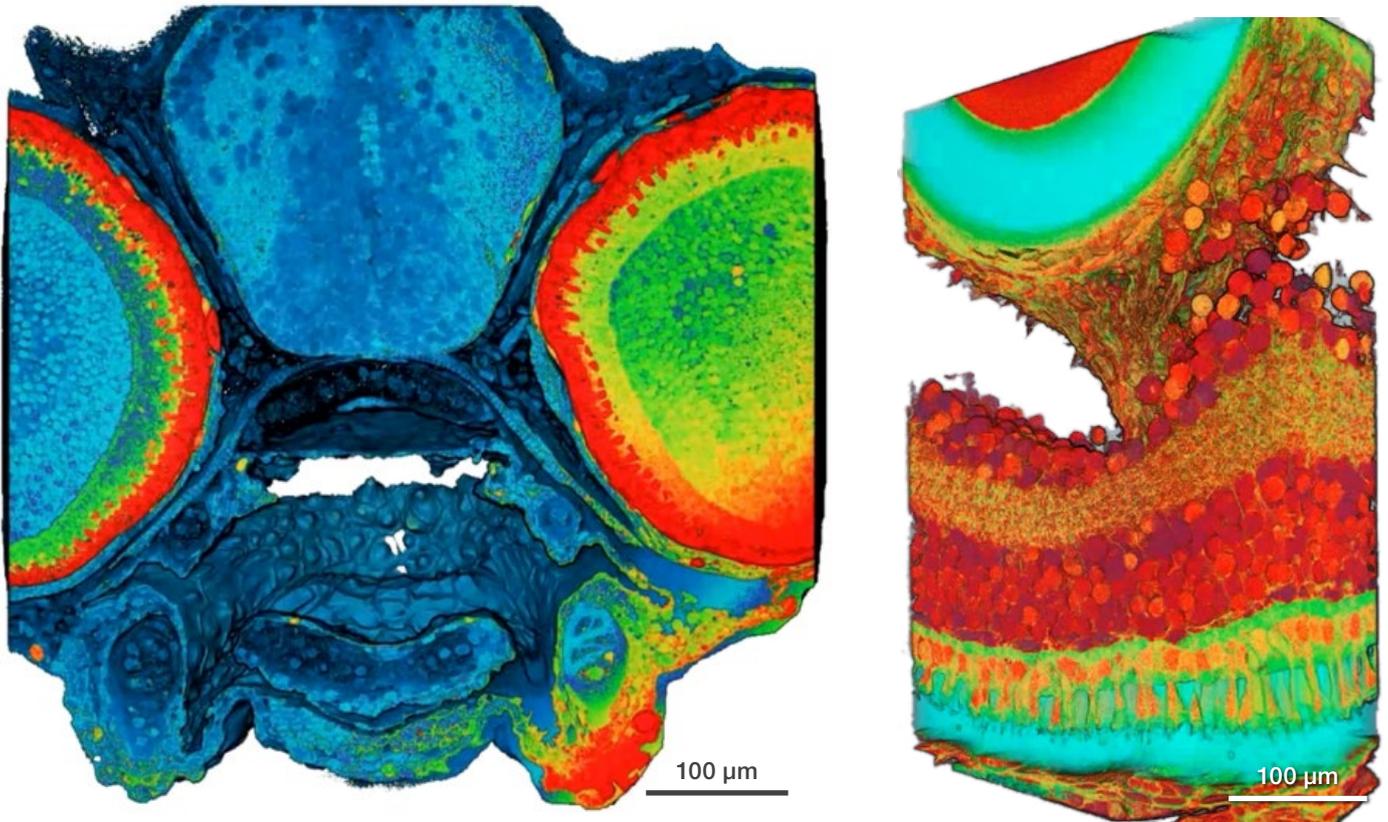
Ultra-thin, resin-embedded section of corneal tissue, imaged at 30 kV using an immersion lens in combination with the retractable STEM detector. Left image shows a low-magnification overview. Image on the right represents a high-magnification image from the left.



HeLa cell (critically point-dried on a coverslip) images with the unique Trinity In-Column Detection System. The left image (A) is taken with the T1 backscatter electron detector, which is positioned inside the tip of the final lens and shows the materials contrast. The middle image (B) is taken with the T2 secondary electron detector, which is positioned just above T1 and shows the topography information of the sample. The right image (C) is taken with the T3 secondary electron detector, which is positioned high up in the column and shows the surface information of the sample.

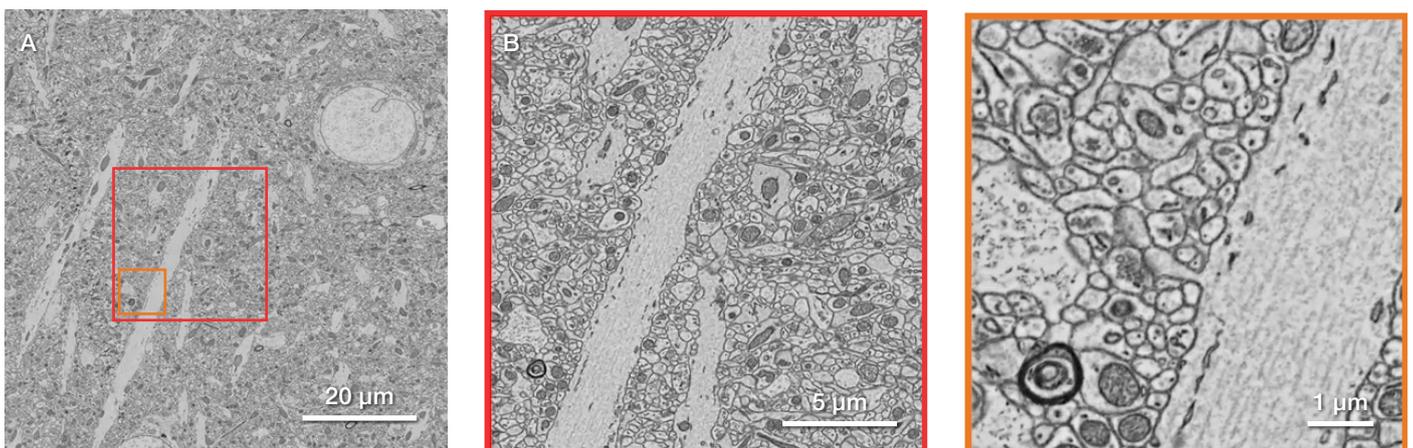
### 3D imaging applications

#### Volume rendering using the Volumescope System

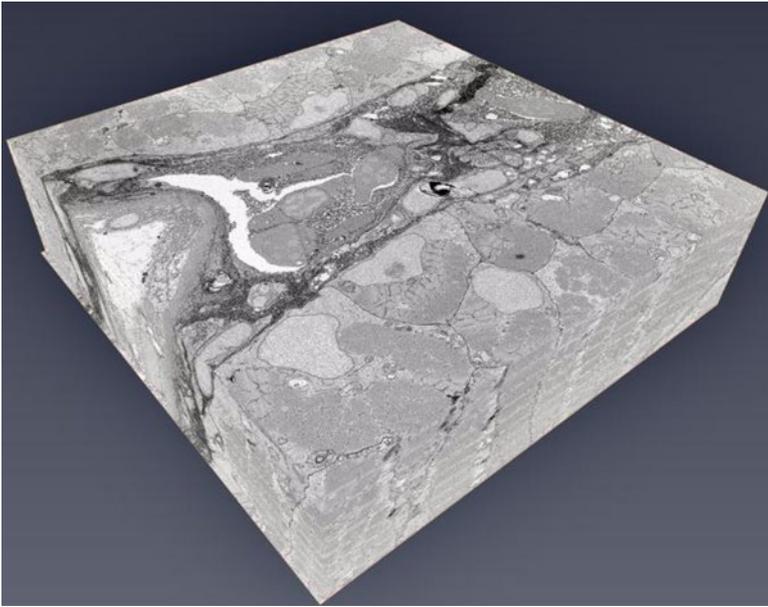


Examples of volume renderings of a zebrafish embryo head (left) and of zebrafish embryo eye tissue (right). Both samples were imaged in low-vacuum mode using the DBS detector. The zebrafish embryo head was imaged at 2 kV (100 pA) with 3 μs dwelltime. The total volume is 350 μm x 350 μm x 83 μm using a total of 829 images (with a slice thickness of 100 nm and voxel size of 42 nm x 42 nm x 100 nm). The images required 7 days of continuous imaging. Scale bar: 100 μm.

The zebrafish embryo eye tissue was imaged at 3 kV (100 pA) with 3 μs dwelltime. The total volume is 136 μm x 94 μm x 31 μm using a total of 772 images (with a slice thickness of 40 nm and voxel size of 48 nm x 48 nm x 40 nm). Courtesy for both samples: Robbert Creton, Brown University. Scale bar: 20 μm.

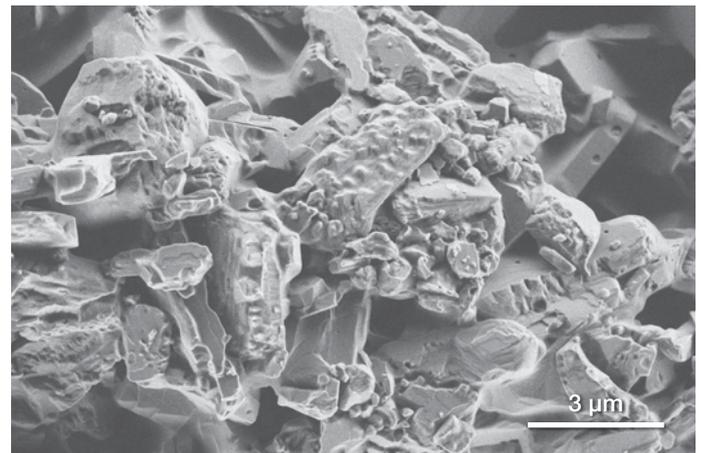
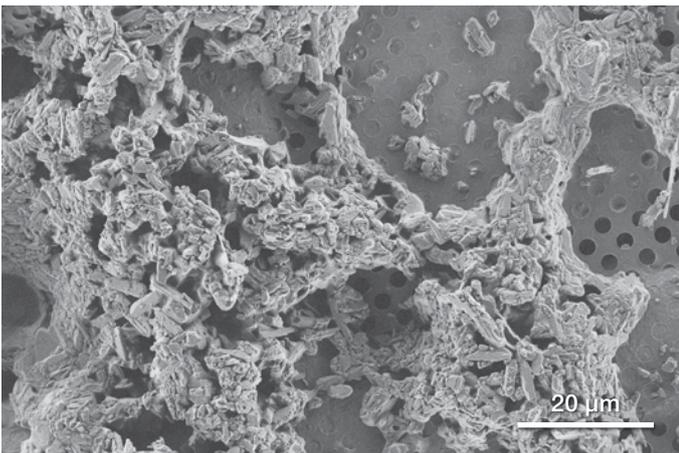


Example of Array Tomography with a mouse brain tissue sample. Large overview images were recorded at a resolution sufficient for detection of synapses in multiple sections; synapses were mapped with pan & zoom in the Maps Software, and high-resolution imaging was performed in sub-areas of multiple sections.

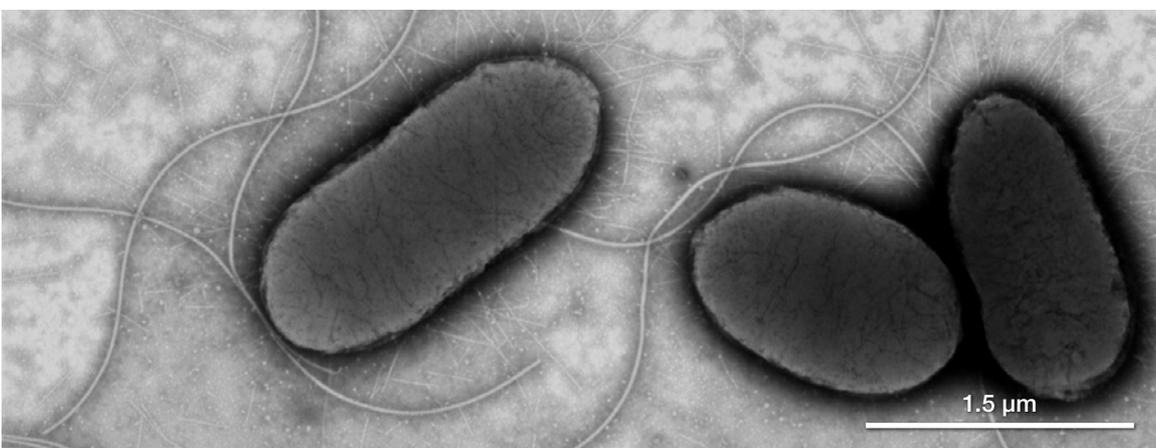


Example of Array Tomography in a zebrafish embryo. The sample comprised 12 pieces of tape with 20 sections per tape. A  $40\ \mu\text{m} \times 40\ \mu\text{m} \times 20\ \mu\text{m}$  volume was recorded at a resolution of  $5 \times 5 \times 80\ \text{nm}$  in the aorta region of the 48h PF embryo.

### Pharmaceutical and Biotechnology applications

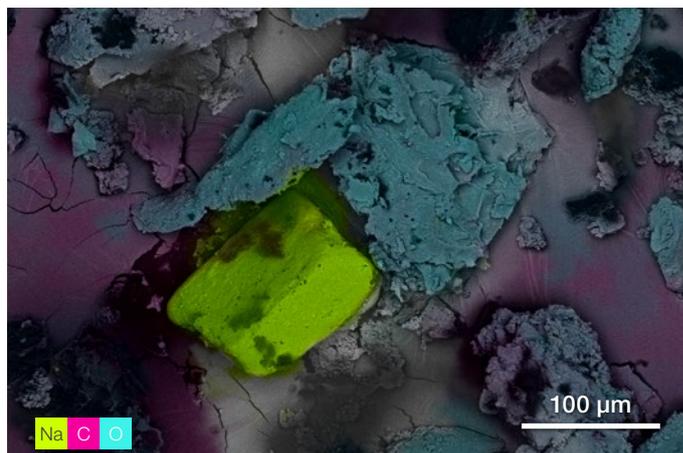
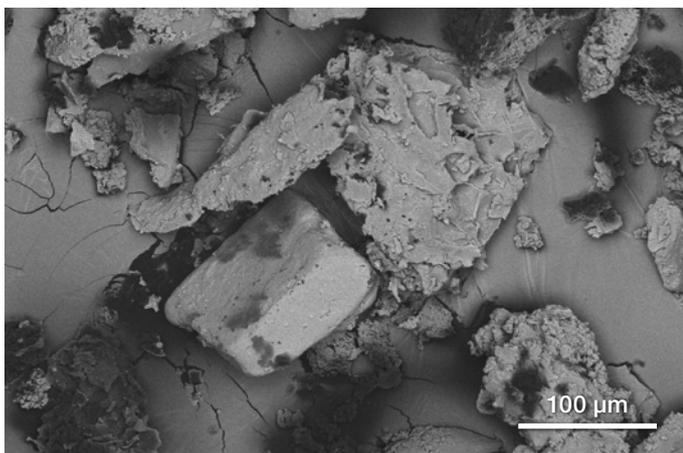


Images of an anti-asthmatic drug deposited from a compressed-gas delivery inhaler onto a TEM grid. In the left image at low magnification, you can see the particles deposited on the surface. The second is a close-up of the particles at a higher magnification showing fine surface details. Taken using the immersion lens at 1 kV accelerating voltage with the in-lens backscatter detector (T1), without metal coating. This shows how high contrast and surface detail can be obtained without complex sample preparation on low-atomic-weight materials using the Trinity and Immersion modes.



Example of microorganisms imaged using the retractable STEM detector, allowing for better contrast and resolution in life science samples.

## Analytical applications



ChemiSEM image of “Salt and Pepper” imaged at 15 kV with the T1 in-lens backscatter detector and integrated EDS ChemiSEM EDS detector. The image on the left shows backscatter contrast of the particles on a carbon sticky tab. The image on the right shows the live, automatically applied elemental image for sodium (Na), representing the salt and oxygen-rich pepper in blue (O), and (C) showing the carbon sticky tape. Note the similarity of the phases in backscatter, yet the elemental analysis clearly shows the phase separation. This extra color can help you distinguish between phases that could otherwise be confused.

### Electron optics

High-resolution FEG column with	High-stability Schottky field emission gun to provide stable high-resolution analytical currents
	Compound final lens: a combined electrostatic, field-free magnetic and immersion magnetic objective lens
	60° objective lens geometry allows for tilting of larger samples
	Automated heated apertures to ensure cleanliness and touch-free aperture changes
	SmartAlign Technology for user-free alignment
	Through-the-lens differential pumping for low vacuum* reduces beam skirting for accurate analysis and high resolution
	Beam deceleration with stage bias from -4,000 V to +600 V
	Continuous beam current control and optimized aperture angle
	Double stage scanning deflection
	Easy gun installation and maintenance: auto bake-out, auto start, no mechanical alignments
	PivotBeam Mode for selected area electron channeling, also known as rocking beam mode (Apreo 2 S SEM only)
	Guaranteed minimum source lifetime: 24 months

### Electron beam resolution

Apreo 2 SEM	Apreo 2 C SEM	Apreo 2 S SEM
Final lens	Electrostatic	Compound
High-vacuum resolution		
30 kV (STEM)	0.7 nm	0.7 nm
15 kV (BD)	0.9 nm	0.5 nm
15 kV (6.4 nA, WD 10 mm)		
1 kV	1.2 nm	0.9 nm
1 kV (BD)	1.0 nm	0.8 nm
1 kV (BV, WD 10 mm)		
500 V (BD)	1.2 nm	0.8 nm
200 V (BD)		
Low-vacuum resolution*		
3 kV (30 Pa)	1.8 nm	1.8 nm
15 kV (30 Pa)	1.2 nm	1.2 nm

## Electron beam parameter space

Beam current range	1 pA–50 nA (400 nA configuration also available)
Accelerating voltage range	200 V–30 kV
Landing energy range	20 eV–30 keV (in serial block-face imaging SEM 500 V–6 kV)
Max. horizontal field width	3 mm at 10 mm WD (corresponds to 29x min magnification)

## Chamber

Inner width	340 mm
Analytical working distance	10 mm
Ports	12
EDS take-off angle	35°
Options	Three simultaneous EDS detectors possible; two at 180° Coplanar EDS/EBSD orthogonal to the tilt axis of the stage
Detectors	The Apreo 2 SEM detects up to four signals simultaneously from any combination of the available detectors or detector segments*: Trinity Detection System (in-lens and in-column) T1 segmented lower in-lens detector T2 upper in-lens detector T3 in-column detector* VS-DBS: LoVac lens mounted BSED (for Volumescope System) ETD—Everhart-Thornley SE detector DBS—Retractable segmented under-the-lens BSED* Low-vacuum SE detector* DBS-GAD—Lens-mounted gaseous analytical BSED* STEM 3+—Retractable segmented detector (BF, DF, HADF, HAADF)* IR-CCD Nav-Cam+ Camera (chamber-mounted)
Vacuum system	Complete oil-free vacuum system 1 × 240 l/s TMP 1 × PVP-scroll 2 × IGP Chamber vacuum (high vacuum) <math>6.3 \times 10^{-6}</math> mbar (after 12 hours pumping without Volumescope System)* (after 72 hours pumping with Volumescope System) Evacuation time: ≤3.5 minute Optional low-vacuum mode up to 50 Pa for charge compensation of non-conductive samples 10–500 Pa chamber pressure Automatic Pressure Limiting Aperture (PLA) Loader
Sample holders	Standard multi-purpose holder uniquely mounts directly onto the stage, hosts up to 18 standard stubs (Ø12 mm), three pre-tilted stubs, cross-section samples, and two pre-tilted row-bar holders* (38° and 90°). Tools are not required to mount a sample. Each optional row-bar accommodates 6 S/TEM grids Wafer and custom holders*

## 3D imaging

### Volumescope System

### Array Tomography

Resolution	High image quality in Z direction @ 10 nm isotropic resolution (using MED-SEM)	The achievable resolution is dependent of the SEM column that is used.
Control software	Maps Software	Maps Software
Visualization software	Amira Software for Life Sciences	Amira Software for Life Sciences
Post processing software	Multi-Energy Deconvolution SEM (MED-SEM)	

## Stage specifications

Stage and sample WITHOUT Volumescape microtome	Type	Eucentric goniometer stage, 5-axes motorized
	XY	110x110 mm
	Repeatability	<3.0 $\mu\text{m}$ (at 0° tilt)
	Motorized Z	65 mm
	Rotation	n $\times$ 360°
	Tilt	-15° / +90°
	Max. sample height	Clearance 85 mm to eucentric point
	Max. sample weight	<ul style="list-style-type: none"> <li>• 500 g in any stage position</li> <li>• Up to 5 kg at 0° tilt (some restrictions apply)</li> </ul>
Stage and sample WITH Volumescape microtome	Section thickness	Effective section thickness using MED $\geq$ 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 $\mu\text{m}$
System control	64-bit GUI with Windows 10, keyboard, optical mouse	
	24-inch LCD display, WUXGA 1920 $\times$ 1200 (second monitor optional)	
	Customizable graphical user interface, with up to 4 simultaneously active views	
	FLASH automated image tuning for focus, lens align, and stigmator	
	Image registration	
	Navigation montage	
	Image analysis software	
	Undo / Redo functionality	
	User guidance for basic operations / applications	
	Optional joystick	
Optional manual user interface (knob board)		
Image processor	Dwelltime range from 25 ns to 25 ms/pixel (in Volumescape 2 SEM: 50 ns–1 ms)	
	Up to 6144 $\times$ 4096 pixels (40k $\times$ 40k with Maps Software)	
	File type: TIFF (8-, 16-, 24-bit), JPEG, or BMP (in serial block-face imaging: 8-, 16-bit)	
	Single-frame or 4-view image display	
	SmartScan Mode (256-frame average or integration, line integration and averaging, interlaced scanning)	
	DCFI Mode (drift-compensated frame integration)	
Digital image improvement and noise reduction filter		
Accessories (optional)	Sample / chamber cleaning: CryoCleaner, Integrated Plasma Cleaner	
	Analysis: EDS, EBSD, WDS, CL, Raman	
	Thermo Scientific QuickLoader™ Load Lock for fast sample transfer	
	Navigation: correlative navigation, Maps Software tiling and stitching	
	Gas injection: up to 2 units (other accessories may limit number of GIS available) for beam-induced deposition of platinum, tungsten, carbon	
	Manipulators	
	Cryo-stage	
	Electrical probing / multi-probing stations	
	Electrostatic beam blanker	

Software options	<p>Maps Software for automatic large-area acquisition using tiling and stitching; correlative work</p> <p>3D reconstruction and image analysis software; Amira Software for Life Sciences</p> <p>Thermo Scientific AutoScript™ 4 Software: Python-based application programming interface</p> <p>TopoMaps for image colorization, image analysis, and 3D surface reconstruction</p> <p>Advanced image analysis software</p> <p>Remote control software</p>
Documentation	<p>Online user guidance</p> <p>Operating instructions handbook</p> <p>Online help</p> <p>Prepared for RAPID (remote diagnostic support)</p> <p>Free access to online resources for owners</p>
Warranty and training	<p>1-year warranty</p> <p>Choice of service maintenance contracts</p> <p>Choice of operation / application training contracts</p>
Consumables	<p>Replacement Schottky electron source module</p> <p>Aperture strips for electron columns</p> <p>Diamond knife from external supplier (Diatome) for Volumescope System</p>
Installation requirements	<p>Power:</p> <ul style="list-style-type: none"> <li>• Voltage 100–240 V AC (-6%, +10%)</li> <li>• Frequency 50 or 60 Hz (+1%)</li> <li>• Consumption: &lt;3.0 kVA for basic microscope</li> </ul> <p>Earth resistance &lt;0.1 Ω</p> <p>Environment:</p> <ul style="list-style-type: none"> <li>• Temperature (20+ 3)°C</li> <li>• Relative humidity below 80%</li> <li>• Stray AC magnetic fields &lt;40 nT asynchronous, &lt;100 nT synchronous for line times, 20 ms (50 Hz mains) or 17 ms (60 Hz mains)</li> </ul> <p>Minimum door size: 0.9 m wide × 1.9 m high</p> <p>Weight: column console 980 kg</p> <p>Dry nitrogen recommended for venting</p> <p>Compressed air 4–6 bar, clean, dry and oil-free</p> <p>System chiller</p> <p>Acoustics: site survey required, as acoustic spectrum relevant</p> <p>Floor vibrations: site survey required, as floor spectrum relevant</p> <p>Optional active vibration isolation table</p>

 Learn more at [thermofisher.com/apreo](https://thermofisher.com/apreo)