**Apreo VS**  
Highest quality isotropic 3D data from large sample volumes

Apreo VS™ is a novel serial block-face imaging solution that combines mechanical and optical sectioning using FEI’s proprietary multi-energy deconvolution technology to facilitate automated acquisition of large sample volumes at isotropic resolution.

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

Unraveling 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 Apreo VS now enables large-volume imaging with truly isotropic 3D resolution.

**Truly isotropic 3D data**

Apreo VS offers a novel solution to improve the axial resolution by combining mechanical sectioning with optical sectioning, realized by FEI’s 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, Apreo VS offers isotropic datasets with less than 10 nm z-resolution.

![Volume reconstruction of a mouse brain acquired with combination of SBF-SEM and MED-SEM, imaged under high vacuum conditions.](image)

**KEY BENEFITS**

| Truly isotropic 3D data from large volumes: | Excellent z-resolution from Multi-Energy Deconvolution SEM combined with the efficiency of *in situ* sectioning. |
| Highest contrast and resolution on all samples: | Highest contrast and optimal SNR with in-lens detector for high vacuum (HiVac) mode, as well as dedicated detector for optimal resolution and charge mitigation in low vacuum mode (LoVac). |
| Simple switch between normal SEM use and serial block face imaging: | Compact, stage-mounted microtome allowing easy exchange. |
| Instant productivity for all operators: | High degree of automation and design for ease-of-use adding to operator efficiency and enabling greater experimentation. |
| Workflow solutions with increased efficiency and accuracy: | Fast identification of regions of interest using CLEM approaches and large volume coverage with automated multi-tile set runs. |

Sample courtesy of P. Laserstein and P. Bastians, Helmstaedter Lab, MPI Brain Research, Germany.
Flexibility and ease-of-use with exceptional performance

Apreo VS is developed on a brand new SEM platform based on the FEI NiCol™ electron column recently introduced with the Scios™ DualBeam™. 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. Apreo VS’s versatility extends to alternative use of the SEM by allowing quick swap of the compact, stage-mounted microtome, enabling fast changeover between routine SEM and serial block-face SEM.

Workflow solutions for increased productivity

Apreo VS is controlled by a single integrating software interface, MAPS, 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, Amira™ for Life Sciences. This powerful SW package can be used to directly import the data produced by Apreo VS not only for processing, but more importantly for visualization and analysis, making it one of the most powerful tool sets available on the market.

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.

Volume reconstruction of a mouse brain acquired with combination of SBF-SEM and MED-SEM, imaged under high vacuum conditions.
Hardware Specifications

**NICol UHR Non-Immersion FESEM Column**
- High-resolution field emission-SEM column, with high-stability Schottky field emission gun
- Source lifetime 12 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
- Landing energy range: 20 eV – 30 keV*
- Accelerating voltage range: 200 V – 30 kV
- User guidance and column presets

**Electron Beam Resolution at Optimum Working Distance**
- High-vacuum imaging
  At optimum WD:
  - 0.8 nm at 30 keV STEM
  - 1.0 nm at 15 keV
  - 1.4 nm at 1 keV

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

**Detectors**
- Serial block face imaging detector; T1 segmented lower in-lens detector
- T2 in-lens detector and ETD (Everhardt-Thornley)
- IR-CCD
- 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

**Sample Holders**
- Standard multi-purpose holder, unique mounting 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 6 S/TEM grids
- Wafer and custom holders*

**STAGE SPECIFICATIONS WITHOUT MICROTOME INSTALLED**

<table>
<thead>
<tr>
<th>Type</th>
<th>Eucentric goniometer stage, 5-axes motorized</th>
</tr>
</thead>
<tbody>
<tr>
<td>XY</td>
<td>110 × 110 mm</td>
</tr>
<tr>
<td>Repeatability</td>
<td>&lt;3.0 μm at 0° tilt</td>
</tr>
<tr>
<td>Motorized Z</td>
<td>65 mm</td>
</tr>
<tr>
<td>Rotation</td>
<td>n × 360°</td>
</tr>
<tr>
<td>Tilt</td>
<td>-15° / +90°</td>
</tr>
<tr>
<td>Max. sample height</td>
<td>Clearance 85 mm to eucentric point</td>
</tr>
<tr>
<td>Max. sample weight</td>
<td>500 g in any stage position (up to 2 kg at 0° tilt)</td>
</tr>
<tr>
<td>Max. sample size</td>
<td>122 mm with full rotation (larger samples possible with limited rotation)</td>
</tr>
</tbody>
</table>

**MICROTOME SPECIFICATIONS**

<table>
<thead>
<tr>
<th>Section thickness</th>
<th>Effective slice thickness using MED ≥ 10 nm</th>
</tr>
</thead>
</table>
| Cutting speed     | User defined: 0.1 – 1 mm/sec
  Recommended speed: 0.1 – 0.5 mm/sec |
| Cutting window    | 2 mm                                        |
| Sample Z travel range | 1.2 mm                                       |

**Supporting Software**
- “Beam per view” graphical user interface concept with up to 4 simultaneously active views
- MAPSTM for automatic acquisition of large volumes
- Image registration
- Navigation montage
- Image analysis software
- Undo / Redo functionality
- User guidance

**Image Processor**
- Dwell time range from 25 ns – 25 ms
- Up to 6144 × 4096 pixels (40k × 40k with MAPS)
- File type: TIFF (8, 16, 24 bit), BMP or JPEG, standard
- Single-frame or 4-view image display
- SmartSCAN™ (256-frame average or integration, line integration and averaging, interlaced scanning)
- DCFI (Drift Compensated Frame Integration)
- Image registration

*Optional
System Control
- 64-bit GUI with Windows 7, keyboard, optical mouse
- “Beam per view” graphical user interface concept, with up to 4 simultaneously active views
- 24-inch LCD display, WUXGA 1920 × 1200 pixels (second monitor optional)
- Optional joystick
- Optional manual user interface (knob board)

Accessories (Optional)
- Sample / chamber cleaning: FEI Integrated Plasma Cleaner

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

Documentation
- Online user guidance
- Operating instructions handbook
- Online help
- Prepared for RAPID™ (remote diagnostic support)
- Free access to FEI for Owners online resources

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 preinstall 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% RH
- 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