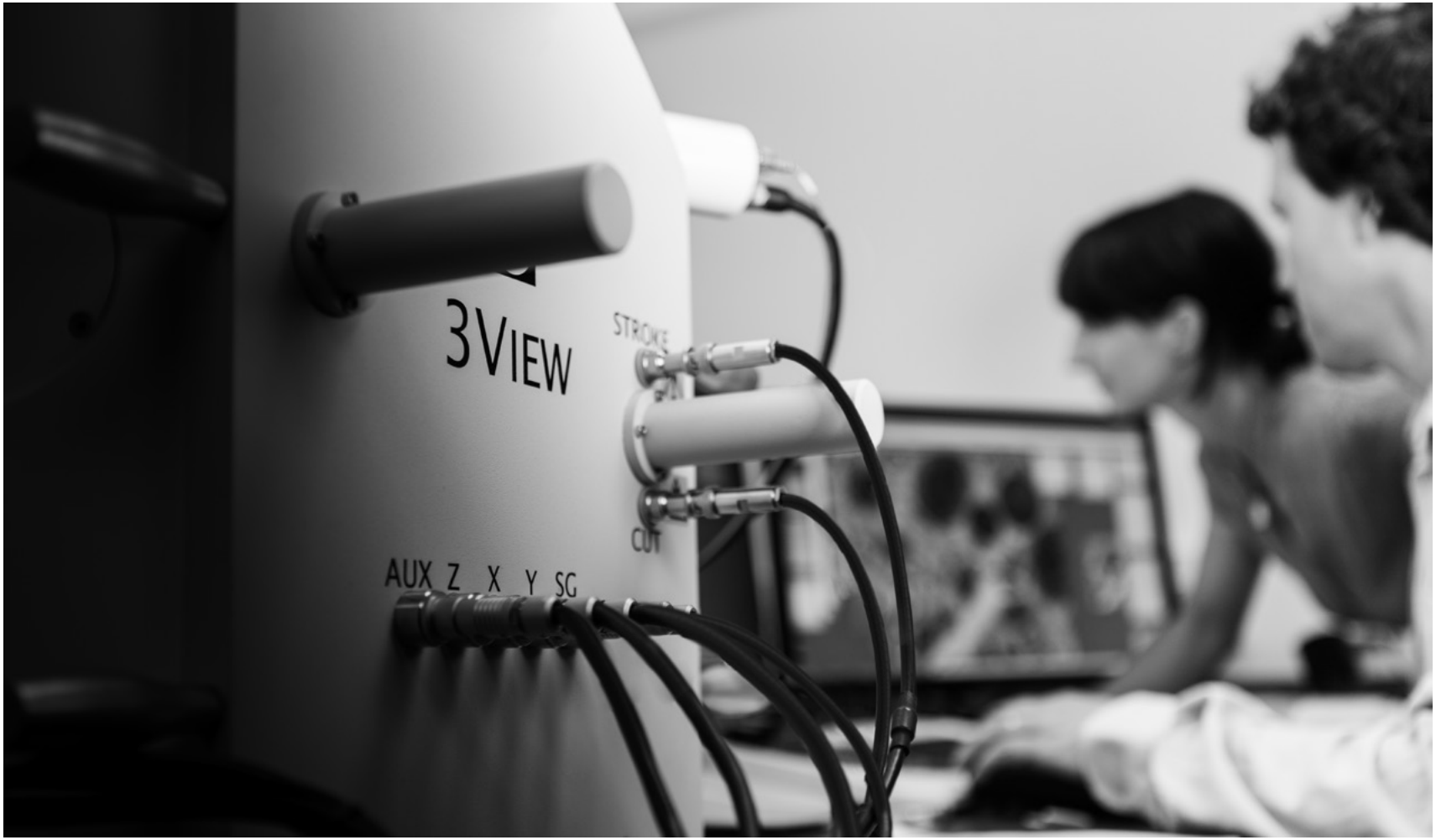


Product Information
Version 2.0

ZEISS Sigma and GeminiSEM with 3View® from Gatan, Inc.

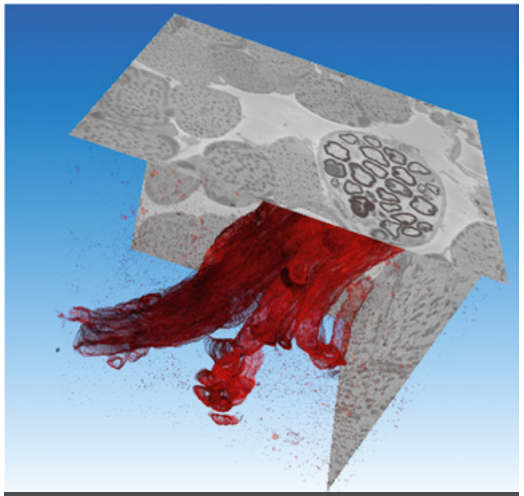
Fast and Convenient 3D Imaging for Biological Samples in the FE-SEM

An Ultramicrotome Turns Your ZEISS FE-SEM into a Fast High Resolution 3D Imaging System

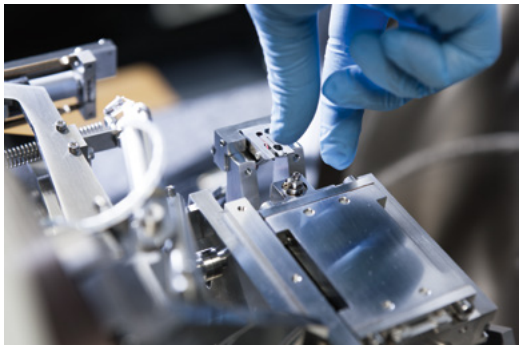
Combine your Sigma and GeminiSEM with 3View® technology from Gatan, Inc. to acquire high resolution 3D data from resin embedded cell and tissue samples. In the shortest possible time and in the most convenient way.

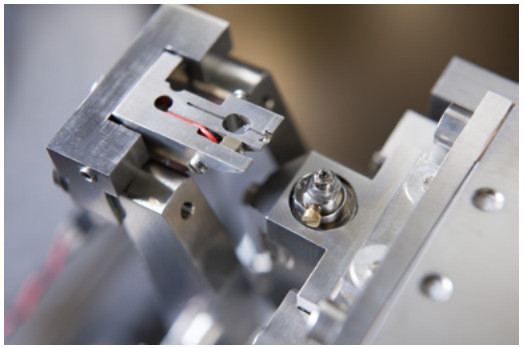
Your 3View® is an ultramicrotome inside the SEM chamber. The sample is continuously cut and imaged so you produce thousands of serial images in a single day – each perfectly aligned because they are all generated from one fixed block.

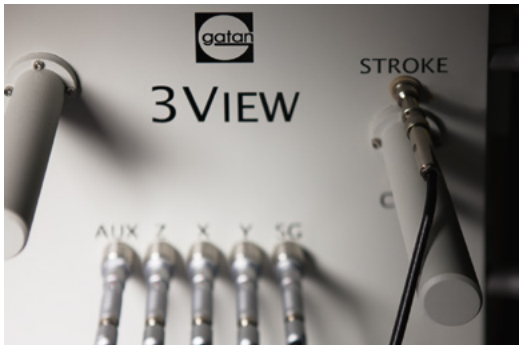
The FE-SEMs Sigma and GeminiSEM from ZEISS are ideally suited to support this application. The unique Gemini column technology delivers images with TEM like quality and allows fields of view of hundreds of microns at nm resolution.



Courtesy of P. Munro, School of Ophthalmology, University College London, UK







Simpler. More Intelligent. More Integrated.

Your complete solution from ZEISS

Your FE-SEMs Sigma and GeminiSEM with integrated 3View® allow you to do block face imaging of even large samples with superb image quality. Use your Sigma 3View® with variable pressure for charge neutralization to reduce imaging artifacts. Profit from GeminiSEM 3View® for best low voltage performance and highest flexibility. You can now enhance your GeminiSEM with Focal Charge Compensation to eliminate charging effects. All systems run with excellent long term stability without any user intervention.

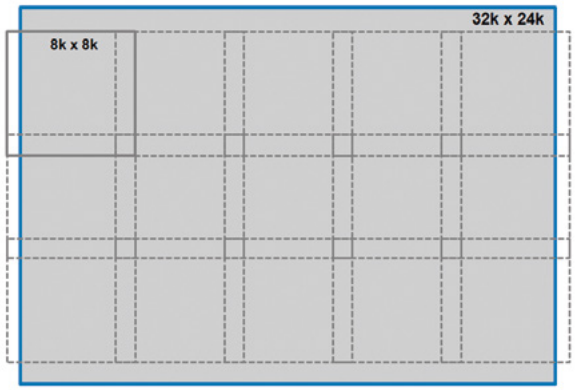
Largest field of view

ZEISS Gemini technology delivers up to 32k × 24k pixels in one scan at nm resolution. With minimized magnetic field at the sample surface you image even large fields of view without blurring at the edges. For most applications that means you won't need to stitch images at all.

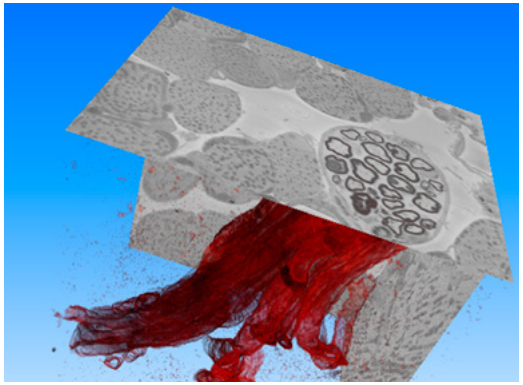
Highest speed

With 3View® you get your 3D results in shortest time. Speed up your application with the unique high current mode of your Sigma. Even faster: the new OnPoint BSE detector and your GeminiSEM deliver highest scan speeds without compromises in resolution. Depending on your application you get your results up to 10 times faster. Overnight instead of over the week!

Your ZEISS FE-SEM with Gemini technology is easy to use. You profit from efficient detection with excellent resolution.



Use your 3View® to image 32k × 24k in one single scan: you have to stitch 15 times less, compared to 8k × 8k images. You save time and avoid double exposures of the necessary stitching overlap.



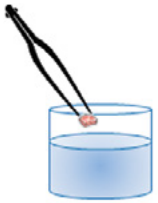
Reconstruction of 100 µm³ of mouse extraocular muscle. Peripheral nerves in red are shown with intricate detail of nerve network, nodes and bends.
Courtesy of P. Munro, School of Ophthalmology, University College London, UK

Your Insight into the Technology Behind It

3View® is an ultramicrotome inside your SEM chamber. The block sample is positioned directly under the SEM column. After imaging the block face, the sample is moved up a very small step (down to 15 nm). The ultramicrotome knife cuts the top of the sample and retracts, exposing a new block face. Then the process repeats. In this way you will cut and image thousands of block faces and reconstruct the data to a complete 3D volume.

Click here to view this video

For preparation of 3View® samples, protocols similar to those for TEM are used.



Fixation and staining

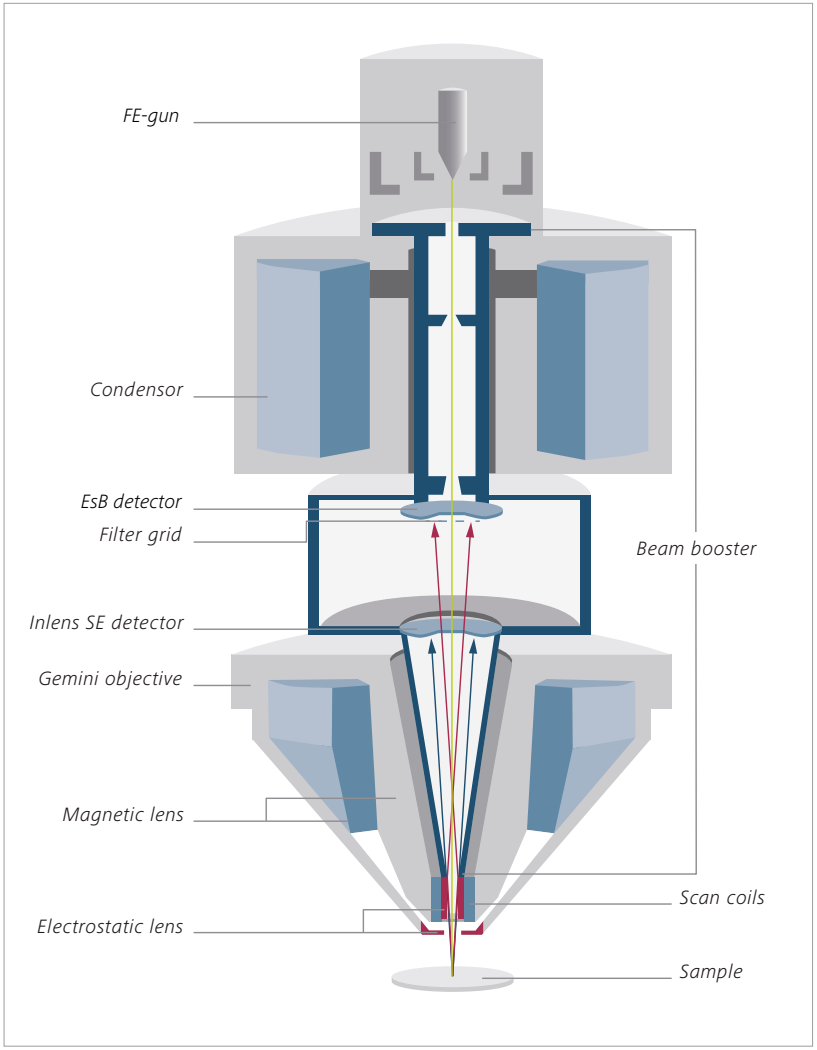
Dehydration

Resin embedding

Curing with heat or UV

Trimming of the block

Your Insight into the Technology Behind It



Schematic cross-section of Gemini column with beam booster, Inlens detectors and Gemini objective.

Your Gemini column: ideally suited for block face imaging

Highly stable thermal FEG

- Extreme long term stability for constant imaging conditions

Beam booster

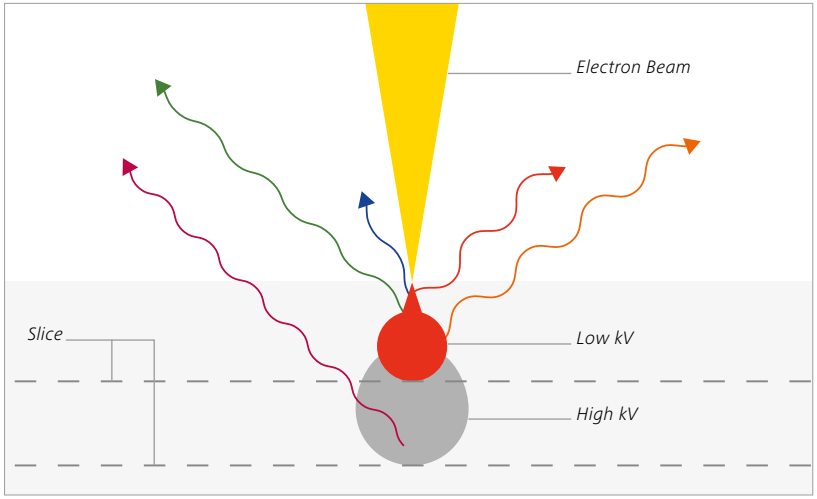
- Superb image resolution at low voltages

Minimized magnetic field at the specimen

- Large fields of view at nm resolution without blurring at the edges

Why low voltage?

Your Gemini column from Zeiss has excellent low voltage performance.
You get the BSE signal only from a thin surface layer after each milling step.
No unwanted signal from deep inside the sample influences your image quality.



With low-voltage operation the signal comes only from the top surface layer.

Your Insight into the Technology Behind It

Charging effects compromise image quality

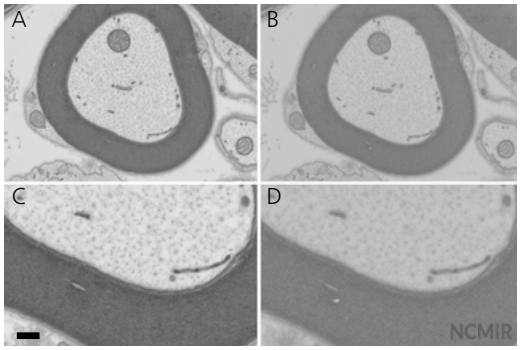
Quality of serial images obtained by block face SEM is limited by signal to noise. Specimen charging, particularly in samples containing large regions of bare resin (e.g. cell culture monolayers or highly vascularized tissues) results in a significant degradation in image quality and distortion. Typically, charging is mitigated by variable pressure SEM, however this is at the expense of signal to noise and resolution.

Charging effects can be prevented by Focal Charge Compensation

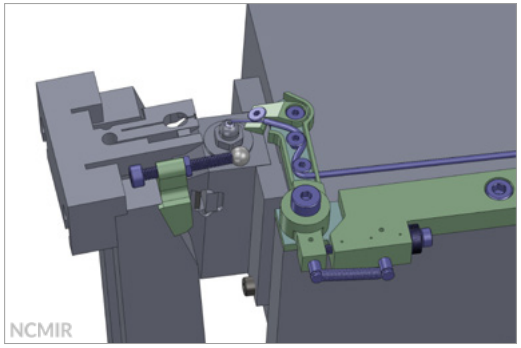
In collaboration with the National Center for Microscopy and Imaging Research (NCMIR), ZEISS has released Focal Charge Compensation. This extension of the 3View® system eliminates specimen charging. A gas injection system consisting of a tiny capillary needle is precisely located above the sample. Nitrogen is guided through this needle directly onto the block face surface while the chamber is maintained under high vacuum. This eliminates charging without degrading image quality. The needle retracts automatically during the cutting cycle so the workflow is uninterrupted and high acquisition rates are maintained.

Charging effects are now a thing of the past

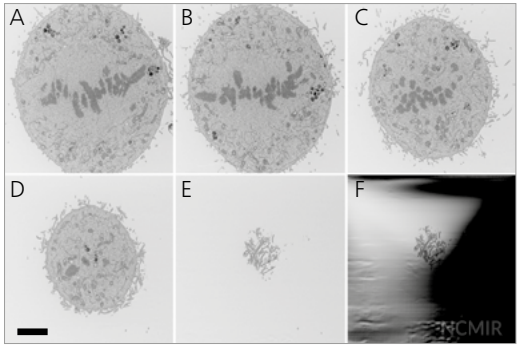
Using Focal Charge Compensation, the result is spectacular image quality without the need for long acquisition times or repeated imaging of the same position. Not only does this enable easy imaging of the most charge-prone samples, but it also significantly reduces the pixel dwell time. Reducing beam exposure time not only ensures fast acquisition rates but also guards against sample damage, which is key to ensuring a reliable and reproducible 3D dataset. High Resolution 3D imaging of all samples has never been so easy or so quick.



Cross-section of rat spinal root showing myelinated axons imaged with Focal Charge Compensation (A+C) and without Focal Charge Compensation but under variable pressure (VP) (B+D). VP imaging affects image quality by reducing signal-to-noise and resolution. Focal Charge Compensation allows for resolving both major and minor dense lines in myelin, which are not resolved using VP. Scale bar: 200 nm. Images courtesy of NCMIR.



Focal Charge Compensation fits conveniently on the 3View® unit without modifications and does not affect the imaging and cutting cycles. The image shows a schematic drawing of the 3View® with Focal Charge Compensation. Image courtesy of NCMIR.



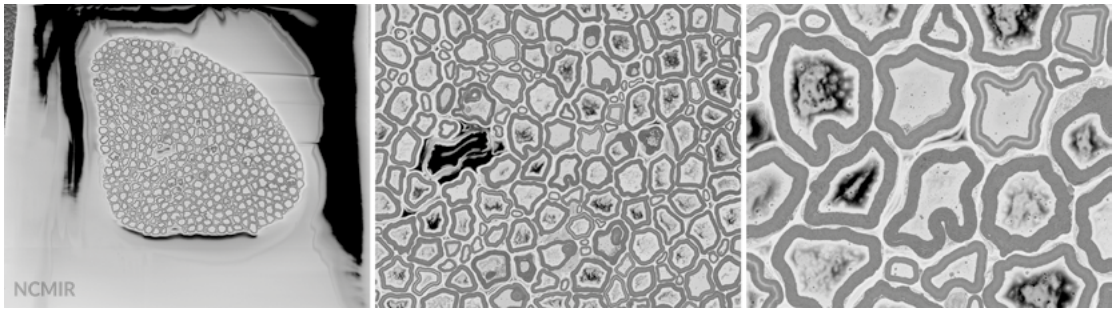
Z-slices of single mitotic HeLa cell with DNA staining. Images A – E acquired with, and F acquired without Focal Charge Compensation. Charging effects – due to the large amount of resin – only occur after switching Focal Charge Compensation off. Imaged at 2.5 keV and 1 µs dwell time. Scale bar: 2 microns. Images courtesy of NCMIR.

ZEISS GeminiSEM 300 with 3View® at Work

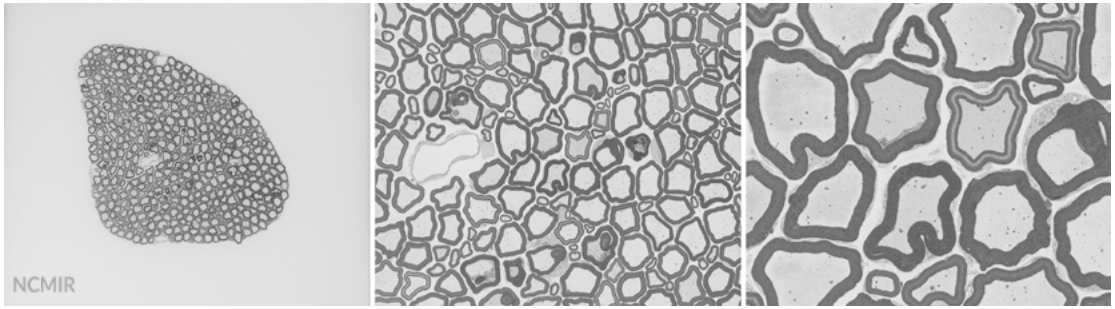
Neuroscience: myelinated axons

Axon myelenization is altered in diseases such as Multiple Sclerosis and Parkinson's Disease. Electron micrographs provide high resolution information sufficient to count the number of single myelin lamellae and measure overall sheath thickness. The sparse nature of structures in these samples leads to significant charging effects. Using Focal Charge Compensation eliminates these effects – you can now image with highest resolution in all three dimensions.

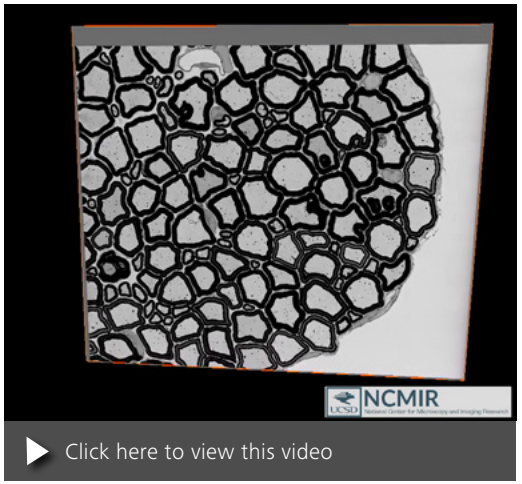
Rat Spinal root imaged under high vacuum



Rat Spinal root imaged with Focal Charge Compensation



Charging effects are clearly visible under high vacuum, in contrast to images taken with Focal Charge Compensation, which show no charging effects even in large expanses of bare resin. The images show a ~300 micron diameter axon bundle at different magnifications. Images courtesy NCMIR.



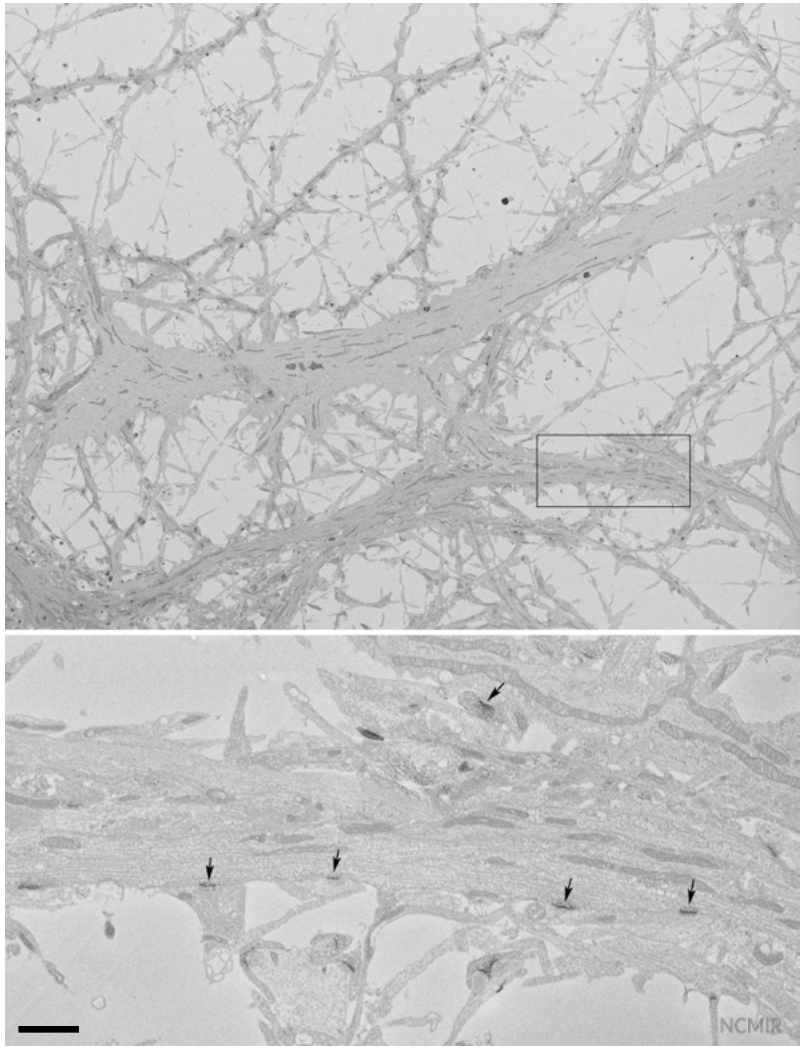
The animation shows a run through single slices (x-y) of rat spinal cord using 3View® and Focal Charge Compensation. Single lamellae within the myelin sheaths of the axons are clearly visible as well as microtubules and other cellular organelles in the original data set. Courtesy of NCMIR.

ZEISS Merlin with 3View® at Work

Neuroscience: cultured neurons

High resolution imaging of features such as thin dendrites, axons, cell protrusions and connections between single neurons is key for fundamental understanding of neuronal morphologies and networks.

With a high proportion of resin, neuronal network samples are challenging to image with high resolution due to charging. Using 3View® with Focal Charge Compensation, this charging is eliminated. You now see fine details which were previously impossible to visualize under high vacuum conditions.

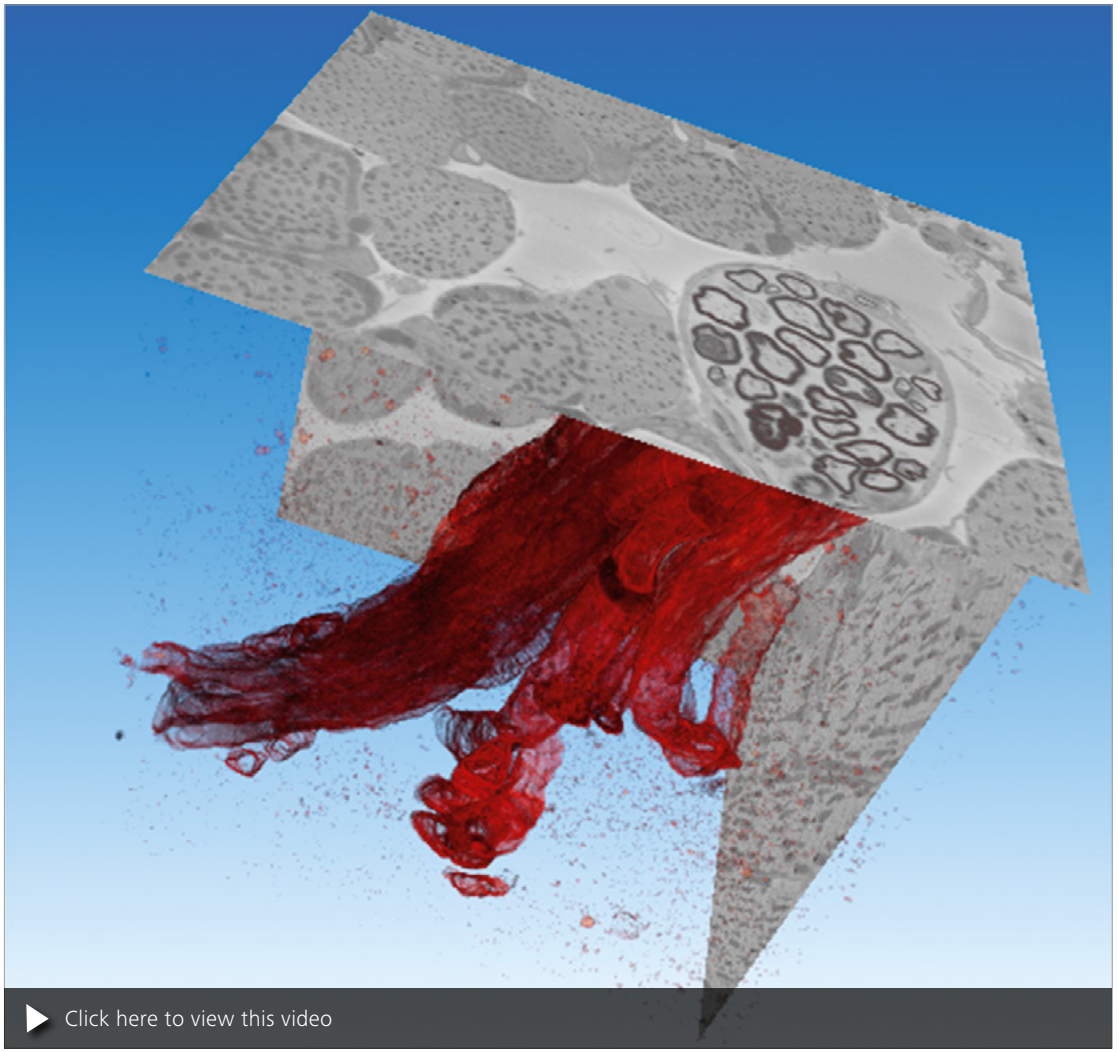


The images show a single slice from a 3D dataset of cultured hippocampal neurons expressing PSD95-APEX2 to stain post-synaptic densities (arrows). Image was acquired using a Merlin SEM with 3View® and Focal Charge Compensation. Ultrastructure such as thin dendrites and connections are visible with high resolution due to the removal of charging effects. Block face sample imaged at 2.5 keV, 1 µs pixel dwell time and high vacuum using Focal Charge Compensation device. Scale bar: 1 micron. Images courtesy NCMIR.

ZEISS Sigma with 3View® at Work

Neuroscience: Mouse extraocular muscle with reconstructed peripheral nerves
Extraocular muscles ensure precise and fast movement of the eye to keep targets in view. These muscles are controlled by abducent nerves. The study of the network of these nerve cells is of great interest, since incorrect nerve connections can cause problems such as displopia (double vision).

The animation shows 1000 images stacked and rendered into a 3D volume image. The extraocular muscles are the large grey cells with small islands of mitochondria. The dark rings are the myelin sheaths that encapsulate the peripheral nerve cells. Any feature inside the volume can be reconstructed to display its 3D shape. The myelin sheaths are shown in red. The 3D nerve network, nodes and bends are clearly shown within the matrix of extraocular muscle cells.



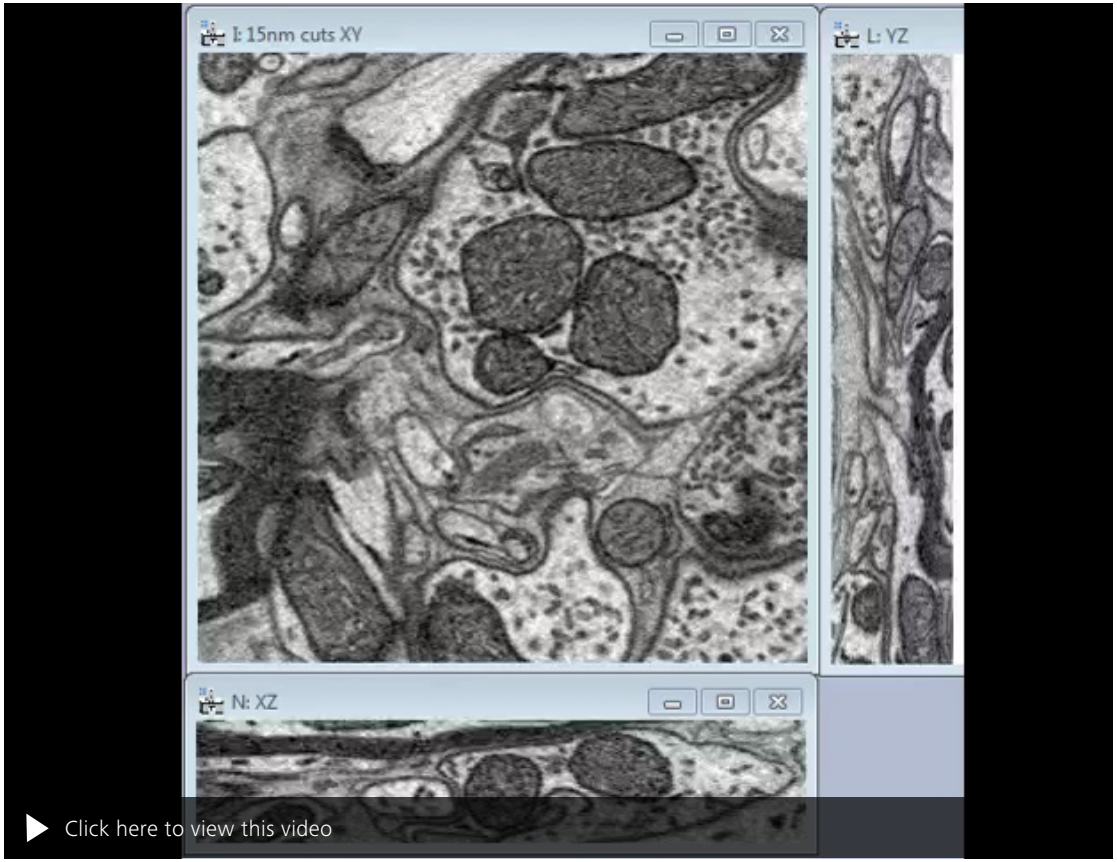
Reconstruction of 100 µm³ of mouse extraocular muscle. Peripheral nerves in red are shown with intricate detail of nerve network, nodes and bends. 1,000 slices were automatically taken over night with a voxel size of 100 nm.
Courtesy of P. Munro, School of Ophthalmology, University College London, UK

ZEISS Sigma with 3View® at Work

Neuroscience: mouse brain

The high current mode of your Sigma doubles the probe current and gives a strong backscattered electron signal to always deliver high contrast images.

The animation shows the ultrastructure of a mouse brain sample in 3D. Cellular components can easily be identified in all z-positions of the reconstructed 3D dataset.



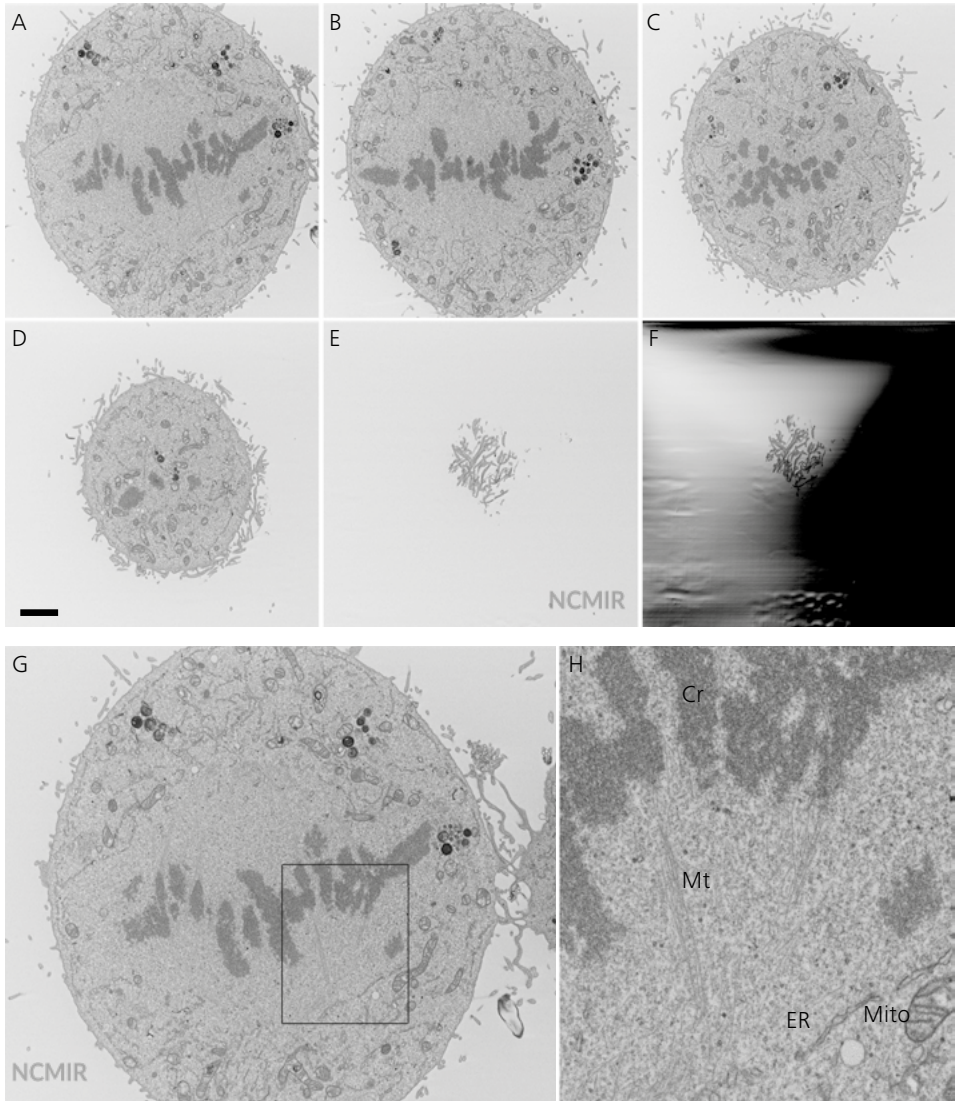
The animation shows a run through single slices (x-y). As the 3D volume is completely reconstructed it allows also the visualization of planes which were not imaged directly (x-z, y-z). Courtesy of N. Kamasawa, Max Planck Florida Institute, USA and R. Shigemoto, National Institute for Physiological Sciences, Okazaki, Japan.

ZEISS GeminiSEM 300 with 3View® at Work

Cell biology: single cells

In order to study cellular structure, function and phenotype, you often need high resolution imaging of cultured monolayers of cells.

A high ratio of resin to sample means that cell culture monolayers are extremely difficult to image with block face SEM due to charging effects. Now, Focal Charge Compensation eliminates these imaging artifacts and enables high signal-to-noise 3D imaging of cellular ultra-structure.



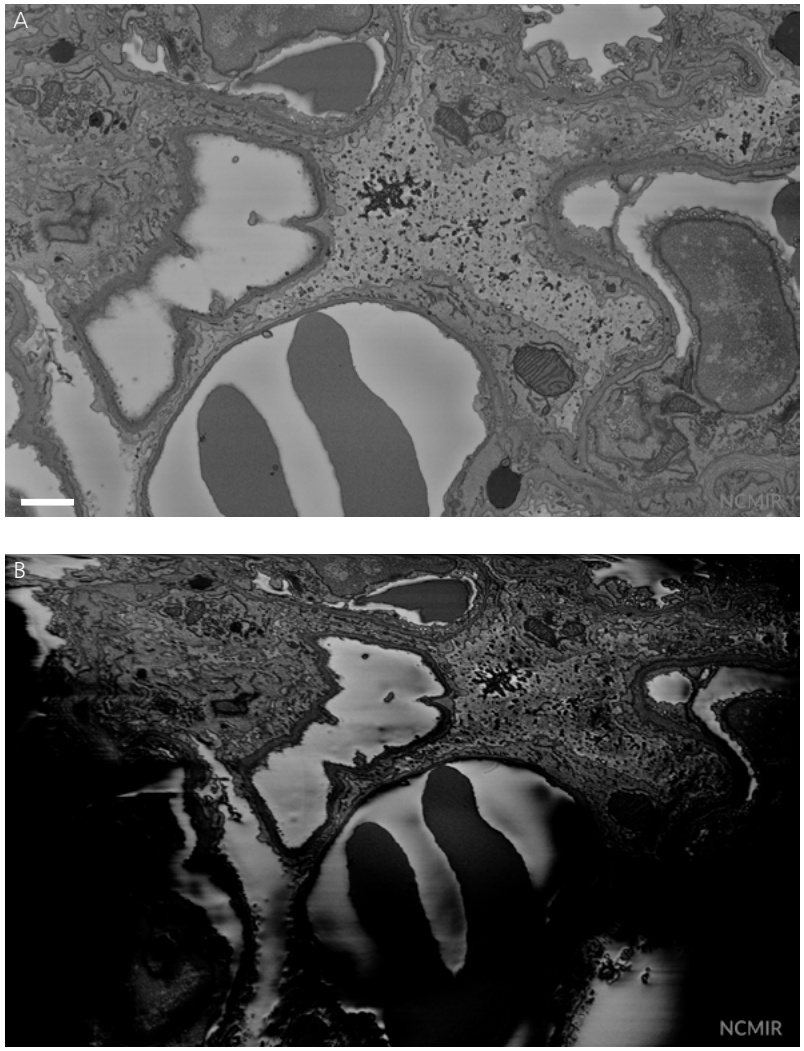
Single mitotic HeLa cell with the DNA stained and embedded in Durcupan resin imaged using 3View®. Different z-positions are shown. Images A – E were acquired using Focal Charge Compensation. Charging effects due to the large amount of resin in the field of view are not visible when Focal Charge Compensation is in use. (F) single section acquired without Focal Charge Compensation and (H) zoomed region from (G). Chromosomes (Cr), Microtubules (Mt), Mitochondria (Mito) and ER are clearly visible. Images were taken with 2.5 keV, 1 µs pixel dwell time. Scale bar: 2 microns. Images courtesy of NCMIR.

ZEISS GeminiSEM 300 with 3View® at Work

3D nano-histology: lung tissue

Electron microscopic investigation of tissue samples such as liver, kidney and lung by block face imaging is extremely valuable for pathological research. The addition of 3View® opens up the possibility of performing fast and easy 3D histological studies. However, many embedded tissue samples are prone to charging effects and the resulting compromises in image quality.

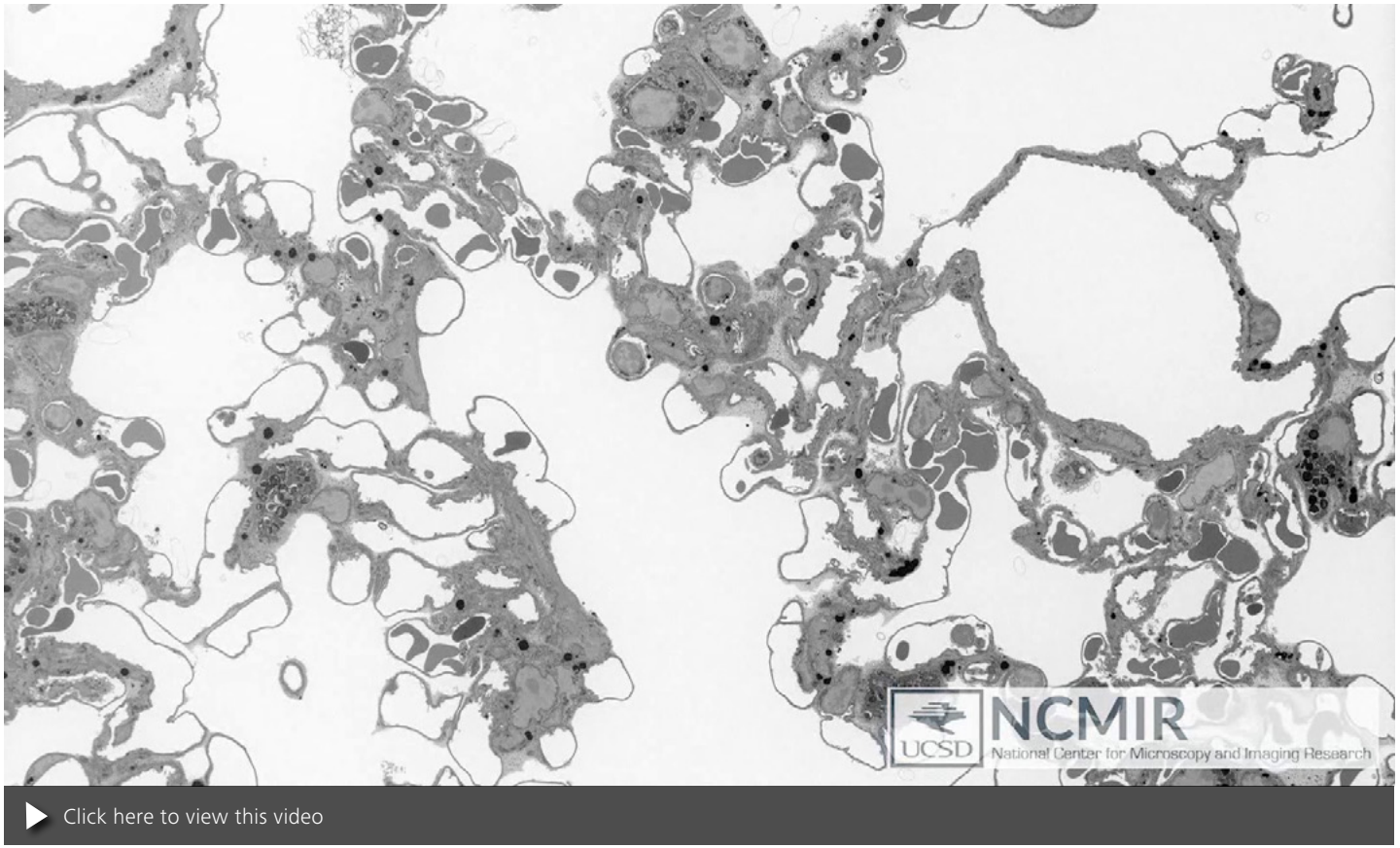
With Focal Charge Compensation, these tissue samples can be imaged with high resolution and speed in three dimensions due to the elimination of charging.



Block face images of mouse lung tissue (A) with Focal Charge Compensation and (B) without Focal Charge Compensation. Scale bar: 1 micron. Images courtesy of NCMIR.

ZEISS GeminiSEM 300 with 3View® at Work

3D nano-histology: lung tissue



The animation shows a run through single z-slices of a piece of mouse lung imaged with 3View®. The first slices were imaged with Focal Charge Compensation. The last slices were acquired without Focal Charge Compensation. You clearly see that – even in this highly charge-prone sample – charging artifacts are completely eliminated as long as Focal Charge Compensation is switched on. Imaged with 2.5 keV and 1 µs pixel dwell time. Courtesy of NCMIR.

3View® integrated in ZEISS FE-SEMs: Your Flexible Choice of Components

Product	SEM system	Microtome	VP	Focal Charge Compensation	x/y motors	Multi ROI/ montage	Cut thickness	In-lens BSE detector (EsB®)	Speed factor
Sigma 300 with 3View2®	Sigma 300 VP	3View2®	Yes	No	manual	No	30–200 nm	No	1
Sigma with 300 3View2XP®	Sigma 300 VP	3View2XP®	Yes	No	automated	Yes	15–200 nm	No	1
GeminiSEM with 3View2XP®	GeminiSEM 300	3View2XP®	Yes	Yes	automated	Yes	15–200 nm	Yes	up to 10
Merlin with 3View2XP®	Merlin	3View2XP®	No	Yes	automated	Yes	15–200 nm	Yes	up to 10

Technical Specifications

Sigma 300 VP	
Resolution	1.2 nm at 15 kV 2.2 nm at 1 kV Variable pressure (VP) mode: 2.0 nm at 30 kV
Magnification	10× – 1,000,000×
Electron Emitter	Thermal field emission type
Standard Detectors	In-lens SE detector, ETSE detector, VPSE detector (in VP mode)
Image Processing	7 integration and averaging modes
System Control	Windows based SmartSEM

Merlin	
Resolution (optimal WD) All resolution specifications are dependent on the system configuration.	0.8 nm at 15 kV 1.4 nm at 1 kV
Acceleration Voltage	0.02 – 30 kV
Probe Current	10 pA up to 300 nA (depending on system configuration)
Magnification	12× – 2,000,000×
Electron Emitter	Thermal field emission type, stability > 0.2 %/ h
Detectors	In-lens SE detector, ETSE detector, EsB detector with filtering grid, filtering voltage 0 – 1,500 V (upgrade)
System Control	Windows based SmartSEM

GeminiSEM 300	
Resolution	0.8 nm at 15 kV 1.4 nm at 1 kV
Acceleration Voltage	0.02 – 30 kV
Probe Current	3pA – 20nA
Magnification	12× – 2,000,000×
Electron Emitter	Thermal field emission type, stability > 0.2 %/h
Detectors	In-lens SE, ETSE detector, EsB detector with filtering grid, filtering voltage 0 – 1,500 V (upgrade)
System Control	Windows based SmartSEM

Technical Specifications

	3View2®	3View2XP®
Charge Neutralization	Variable pressure SEM required; 5–40 Pa is typical at 2 kV	High vacuum compatible. Variable pressure optional.
Accelerating Voltage	1 kV – 5 kV	< 1 kV – 5 kV
Cutting Speed	User defined: 0.1 – 1.2 mm/sec Recommended speed: 0.6 – 1 mm/sec	User defined: 0.1 – 1.2 mm/sec Recommended speed: 0.5 – 1 mm/sec
Cut Thickness	Microtome can cut from 30 to 200 nm. 30 to 50 nm is typical with biological specimens.	Microtome can cut from 15 to 200 nm. 25 to 50 nm is typical with biological specimens.
Knife Cutting Travel Distance	1.2 mm	1.2 mm
Z Travel Distance	Maximum of 600 µm	Maximum of 600 µm
3View® Stage Travel Distance	Traverses approximately ± 700 µm in X and Y	Traverses approximately ± 700 µm in X and Y
SEM Stage	3View2® replaces SEM stage. High Stability Manual x-y-stage (movement sufficient to cover 1 mm × 1 mm specimen block).	3View2XP® replaces SEM stage. High Vacuum Compatible automated x-y-stage (movement sufficient to cover 1 mm × 1 mm specimen block).
Working Distance	Approximately 6 mm when used with Gatan BSED	Approximately 6 mm when used with Gatan BSED
Low Magnification Field of View	Depends on specific electron optics. Gatan OnPoint BSE detector has a 1 mm aperture and FOV is typically 1.2 mm × 1.2 mm.	Depends on specific electron optics. Gatan OnPoint BSE detector has a 1 mm aperture and FOV is typically 1.2 mm × 1.2 mm.
Image Acquisition	Gatan DigiScan® uses SEM external scan control input. 2 – 16 bit analog inputs can work simultaneously. 3View2® acquisition is typically BSED only. Gatan recommends its own BSE detector optimized for low kV image collection.	Gatan DigiScan® uses SEM external scan control input. 2 – 16 bit analog inputs can work simultaneously. 3View2XP® acquisition is typically BSED only. Gatan recommends its own BSE detector optimized for low kV image collection.
DigiScan® Pixel Density	3View2® supports images up to 24k × 32k pixels	3View2XP® supports images up to 24k × 32k pixels
Pixel Dwell Time	Microscope and sample dependant. 1 – 20 µs is typical	Microscope and sample dependant. 1 – 20 µs is typical
Image Scanning	NA	Choice of single pass or configurable multiple frames
Image Calibration	DigiScan® calibration is kV and magnification specific	DigiScan® calibration is kV and magnification specific
SEM-PC Communication	Follows SEM protocol. Communication for kV, FOV, Magnification, Vacuum, Beam Blanking.	Follows SEM protocol. Communication for kV, FOV, Magnification, Vacuum, Beam Blanking.
3View® Setup	Defined protocol for approach sequence protects diamond knife. Utilizes optical zoom microscope with chamber door at air.	Defined protocol for approach sequence protects diamond knife. Utilizes optical zoom microscope with chamber door at air.

Technical Specifications

	3View2®				3View2XP®			
Operation	Unattended, once setup. May be set up to send notification e-mail if collected images show an image collection problem has occurred. Allows confidence that unattended data collection is occurring as desired. Maximum length of experiment determined by specimen thickness, image capture time and number of images collected. Recently acquired data can be viewed without pausing acquisition.				Unattended, once setup. May be set up to send notification e-mail if collected images show an image collection problem has occurred. Allows confidence that unattended data collection is occurring as desired. Maximum length of experiment determined by specimen thickness, image capture time and number of images collected. Recently acquired data can be viewed without pausing acquisition.			
Typical Specimen Size and Requirements	Typical block face size: 600 µm × 600 µm Embedding resin: Epon, Durcupan, or Araldite Contrast: en-bloc staining (heavy metals)				Typical block face size: 600 µm × 600 µm Embedding resin: Epon, Durcupan, or Araldite Contrast: en-bloc staining (heavy metals)			
Imaging Modes	Single frame per cut				Single frame per cut Multiple fields of view and magnifications per cut Stage montage for large fields of view			
Image Throughput	Theoretical maximum: 316 GB/day or 2.22 TB/week Sustained operation: 10 – 315 GB/day or 0.70 – 2.21 TB/week				Theoretical maximum: 316 GB/day or 2.22 TB/week Sustained operation: 10 – 315 GB/day or 0.70 – 2.21 TB/week			
Sample Throughput	Acquisition Cycle	Isotropic Voxel Size (nm)	1 Day (µm)	1 Week (µm)	Acquisition Cycle	Isotropic Voxel Size (nm)	1 Day (µm)	1 Week (µm)
	1 min	30	43×43×43	302×302×302	1 min	15	21×21×21	302×302×302
		50	72×72×72	525×525×525		50	72×72×72	525×525×525
		200	288×288×288	600×600×600 (3 Days)		200	288×288×288	600×600×600 (3 Days)
	20 sec	30	130×130×130	600×600×600 (6 Days)	20 sec	15	64×64×64	453×453×453
		50	216×216×216	600×600×600 (4 Days)		50	216×216×216	600×600×600 (4 Days)
		200	864×864×864	600×600×600 (1 Day)		200	864×864×864	600×600×600 (1 Day)
SEM Port Requirements	One small port required for BSED feed through				One small port required for BSED feed through			

Count on Service in the True Sense of the Word

Because the ZEISS microscope system is one of your most important tools, we make sure it is always ready to perform. What's more, we'll see to it that you are employing all the options that get the best from your microscope. You can choose from a range of service products, each delivered by highly qualified ZEISS specialists who will support you long beyond the purchase of your system. Our aim is to enable you to experience those special moments that inspire your work.

Repair. Maintain. Optimize.
Attain maximum uptime with your microscope. A ZEISS Protect Service Agreement lets you budget for operating costs, all the while reducing costly downtime and achieving the best results through the improved performance of your system. Choose from service agreements designed to give you a range of options and control levels. We'll work with you to select the service program that addresses your system needs and usage requirements, in line with your organization's standard practices.

Our service on-demand also brings you distinct advantages. ZEISS service staff will analyze issues at hand and resolve them – whether using remote maintenance software or working on site.

Enhance Your Microscope System.
Your ZEISS microscope system is designed for a variety of updates: open interfaces allow you to maintain a high technological level at all times. As a result you'll work more efficiently now, while extending the productive lifetime of your microscope as new update possibilities come on stream.

Profit from the optimized performance of your microscope system with services from ZEISS – now and for years to come.

>> www.zeiss.com/microservice

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