

3View Serial Block-Face Imaging System

Model 3View2.XP

The 3View® system enables life science researchers to obtain 3D data at remarkably fine depth resolution on a field emission scanning electron microscope (FE-SEM). Based on the ground breaking serial block-face scanning electron microscopy (SBFSEM) work developed by MPI Heidelberg¹, the revolutionary 3View system allows automated acquisition of 3D ultrastructure by sequentially imaging a freshly cut, resin embedded block-face. Unlike the 3D imaging techniques in light microscopy, the spatial resolution provided by 3View in the Z direction is comparable to its X,Y resolution and does not degrade with depth.

Sample preparation: Each biological sample is prepared using a method that is similar to a microtome protocol optimized for a transmission electron microscope (TEM). The sample must be fixed, stained, then embedded. These preparation protocols allow the sample to maintain its integrity for hours of imaging in the SEM. The Gatan backscatter detector captures high contrast images from these samples, even at low kV. Please refer to the sample preparation note for further details.

Operation: The 3View system setup and operation is simple. Each system includes its own chamber door that fits into a specific microscope. The standard stage is removed from the FE-SEM by venting the chamber, then is replaced by the dedicated 3View stage to enhance the system's stability and performance during very long sample runs.

You can tailor the experiment to suite the needs of your system. The cut step size can be set from 5 – 200 nm to isolate and collect distinct sample features. You can also adjust the diamond knife cutting speed from 0.05 – 5.0 mm/s to prevent artifacts and accommodate different sample types. The number of images you collect can range from hundreds to thousands in a single run, and will vary based on how much material you want to section through. The 3View system can traverse through 600 µm in depth (Z) to analyze larger samples.

Imaging: The images are acquired from the block-face, instead of the sections. Image capture is done using a Gatan backscatter detector that is optimized for high signal collection at a low accelerating voltage. Because the sample is embedded in a nonconductive resin or epoxy, sample charging is almost inevitable. This is best handled using a variable pressure SEM and low accelerating voltage. Image collection can be customized to the needs of your operator. Options include the ability to choose an image to collect after each cut, obtain one or many images per cut, as well as capture images at several magnifications or in a series of images to create a montage.

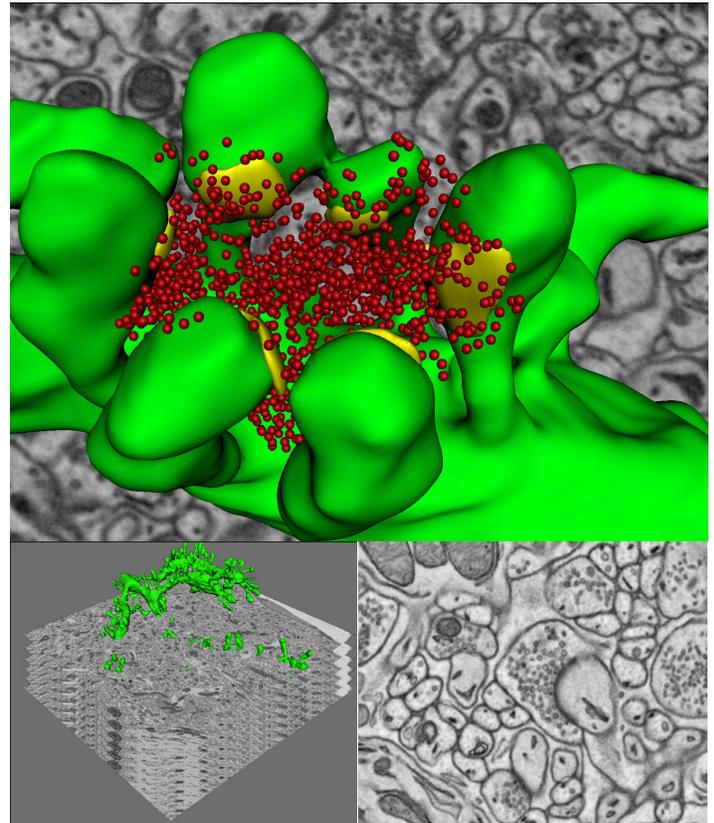


Figure 1. Top: Region of a segmented dendrite in green with synapses in yellow, and synaptic vesicles in red. Lower left: Selected serial images with the reconstructed dendrite in green. Lower right: Single SBFSEM image of a dendritic spine, synapse and synaptic vesicles. Images generated by 3View system. *Sample courtesy of Tom Deerinck and Dr. Mark Ellisman, National Center for Microscopy and Imaging Research, University of California, San Diego.*

Benefits

- High precision ultra microtome, capable of cutting slices of <30 nm with diamond knife on a Gatan standard specimen
- Ultra high performance BSED, speed and sensitivity optimized for low kV imaging conditions
- High stability manual X,Y stage (movements sufficient to cover 1 x 1 mm specimen block)
- SEM beam control and image acquisition; allows recording of a Z-stack of images.
- Optical microscope and lighting available to enable coarse specimen approach

Applications

- **Life science:** cell biology, neuroscience, tissue biology/histology
- **Material science:** soft materials, alloys, plastics

Specifications

Accelerating voltage (kV)	<1 – 5
Cutting speed (mm/s)	
Available	0.1 – 1.2
Typical	0.5 – 1
Cut thickness (nm)	
Availability	15 – 200
Typical for biological specimens	25 – 50
Knife cutting travel distance (mm)	1.2
Knife size (mm)	≤2
Approximate Z travel in X and Y (μm)	±700
Approximate working distance when used with Gatan BSED (mm)	6
Pixel density supported (pixel)	24k x 32k
<i>Actual image size is SEM dependent</i>	
Imaging modes	Single frame Multiple fields of view and magnifications Stage montage for large fields of view
Image throughput (T/wk.)	
Theoretical max.	2.22
Sustained operation	1.70 – 2.21
Gatan Microscopy Suite® software	Included
SEM port requirements	One small port required for BSED feed through

Specifications are subject to change.

Ordering

Model	Description
3View2	Second generation system for automated 3D microscopy in the SEM
3View2.XP	Advanced second generation system for automated 3D microscopy in the SEM
3VRBSS	Replacement BSED sensor
3VDKN	New diamond knife and piezo oscillator mounted and aligned on new knife holder
3VDKE	Replacement diamond knife, mounted and aligned on knife holder
3VDKRE	Resharpener diamond knife, mounted and aligned on knife holder

¹ 3View is a product based on work performed by W. Denk and H. Horstmann, Max-Planck Institute for Medical Research, Heidelberg, Germany, "Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure." Plos Biology, 2004.2(11):p.1900 – 1909

Cutting thin

The 3View system has the ability to cut biological and material samples thinner than 15 nm. This is made possible by precise Z stage control, stabilization of the microtome, and a custom diamond knife. The 3View Z movement is controlled by a piezoelectric device, allowing precise movements at the nanometer scale. Adjusting the cutting thickness is controlled by DigitalMicrograph® software. In addition, the 3View stage is stabilized to minimize vibrations reducing cutting discrepancy

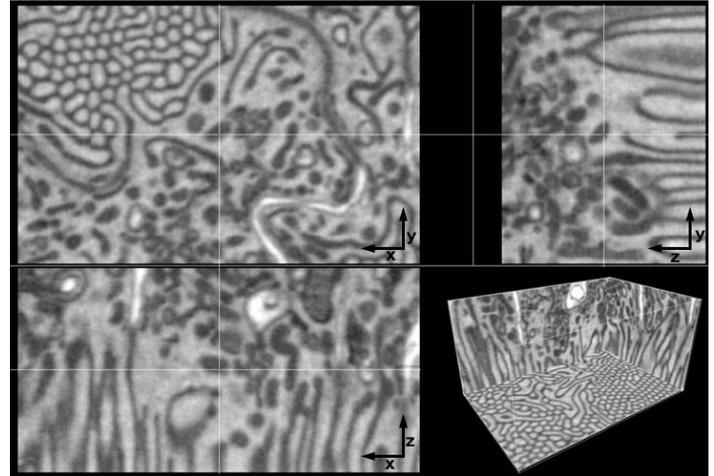


Figure 2. Mouse kidney prepared with the National Center for Microscopy and Imaging Research (NCMIR) 3View specimen preparation protocol embedded with Durcupan resin imaged with 7 nm pixels, and cut with 7 nm cuts creating 7 nm isotropic voxels.

Simplifying electron microscopy

The 3View setup and operation is intuitive and simple. Removing the most difficult step, cutting and collecting ultrathin sections, the 3View system can turn an embedded sample into thousands of images overnight. In addition 3View can also cut thinner than a conventional ultramicrotome, cutting a sample from <15 – 200 nm with a total traverse of 600 μm.

