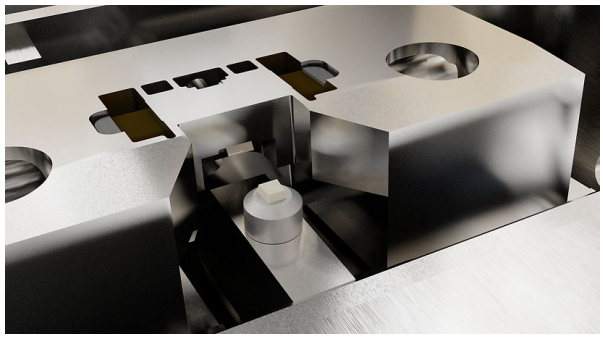
katana microtome

a microtome that transforms a normal SEM to a volume SEM

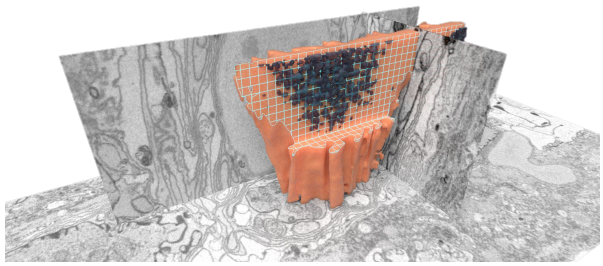
katana microtome and serial block-face SEM

Visualising 3D biological structure on the nanometer scale is vital to furthering our understanding of how our cells, tissues and organs function, enabling groundbreaking steps forward in treatments for disease and disorder. Acquiring large volumes of 3D SEM data has been made possible by imaging using serial block-face electron microscopy (SBEM), where a microtome resides inside the vacuum chamber of an SEM. A diamond knife repeatedly removes a thin layer (as thin as 20 nm) from the surface of a sample block which is stained with heavy metals and fixated in resins. After removing each section, the exposed block surface is imaged by collecting the back scattered electrons. This automated in-situ method can acquire a series of electron micrographs which can be used to reconstruct 3D models.

With its compact size and a height of only 56 mm, katana microtome is designed to fit inside the vacuum chamber of many SEMs. The microtome can be installed and removed from an SEM stage in a matter of seconds, enabling a quick and easy switch of your normal SEM to a serial block-face SEM.



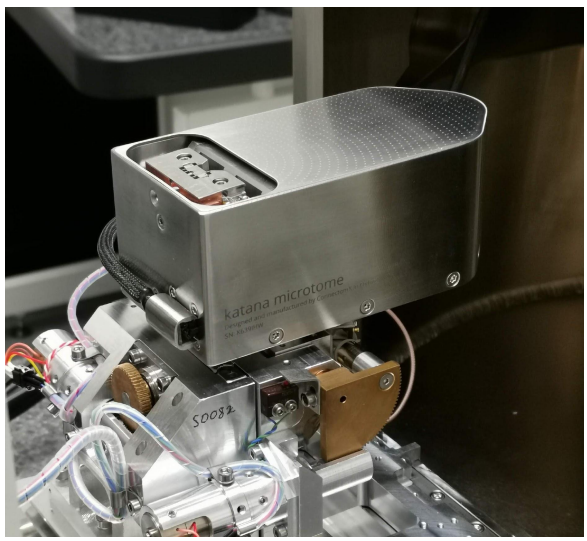
Diamond knife holder and resin-embedded sample on an aluminium sample pin.

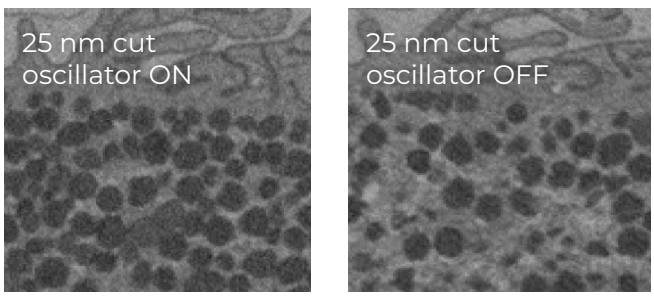


Volume rendering of a pigment sac containing pigment granules of a squid skin chromatophore sample. Imaged on a Zeiss Sigma at 1.9 kV in variable pressure mode using katana. Voxel size is 16 x 16 x 25 nm (XYZ) with a total volume of 5800 μm^3. *Sample courtesy of Dr Alan Kuzirian at Marine Biology Laboratory.*

Ultrasonic knife to improve ultra-thin cutting performance

Think about the sawing motion used when slicing a loaf of bread. The cutting force is greatly reduced and we get a clean cut. In the same way by oscillating the diamond knife we can vastly improve the surface texture of the sample after each cut in SBEM. The FEA optimised knife design enables a piezo driven in-plane oscillation at an amplitude up to 150 nm at tens of kHz. The oscillation amplitude is constantly measured by a sensing piezo. The dual-piezo (driving and feedback) design of the knife holder provides constant monitoring of the knife oscillation and allows fine tuning to optimise cutting performance across a wide range of samples.





Images above showing two adjacent slices to compare the effects of knife oscillation on ultra-thin cutting. When the oscillator is turned off, the pigment granules (black dots) are missing in the image which indicates they are being pulled out by the knife during cutting. This behaviour is not seen when oscillator is turned on.

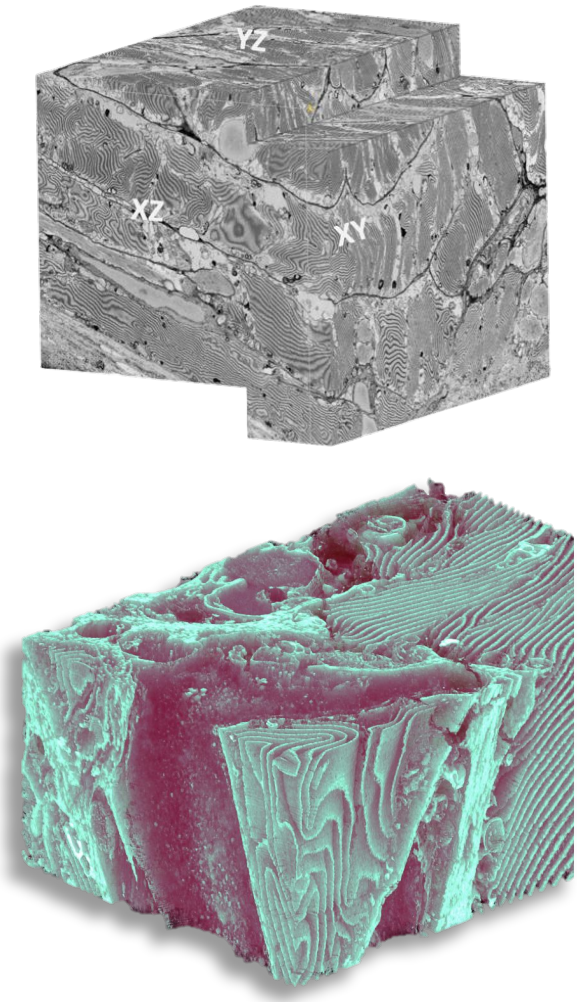


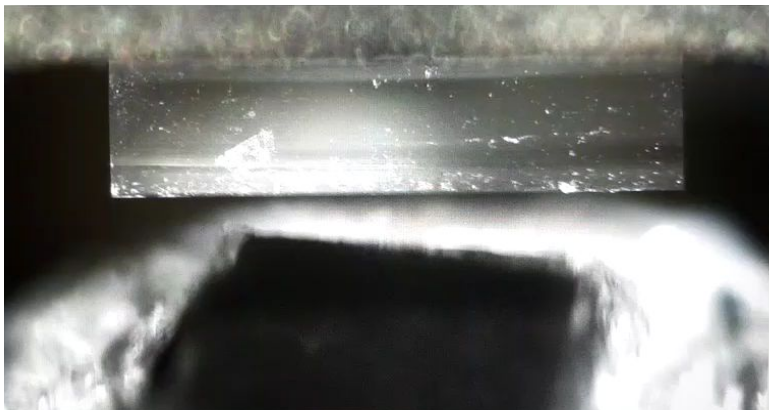
Image cube of lamprey spinal cord tissue showing XY, YZ and XZ planes after reconstruction from serial 2D images. A high precision sample stage is important to achieve accurate Z scaling in 3D reconstructions. Image acquired on a Zeiss Sigma in variable pressure mode at 3 kV. Cut thickness is 40 nm. *Sample courtesy of Dr Alan Kuzirian at Marine Biology Laboratory.*

Nanometer precision

A precise and repeatable stepping motion in Z-direction is crucial to produce accurate Z scaling in final 3D volume data. The Z position of the sample stage - driven by an ultra-fast motor - is measured constantly by a nanometre resolution encoder so that any drift can be instantly corrected. Additional benefits include: 1) debris mitigation: before the knife retracts, the sample can be lowered by hundreds of microns, reducing the chance of debris being attracted to the sample block; 2) reduced working distance: the sample can be raised by several hundred microns during imaging, improving the collection efficiency of the detector and therefore a higher signal-to-noise ratio.

Finding cutting plane made simple

For someone who is new to ultramicrotomy, finding the cutting plane is often a daunting experience. Our Digital Viewer is designed to assist the user slowly and precisely bring the sample up towards the cutting plane without damaging the diamond knife. The digital viewer sits over the microtome so that the approach process can be done on an SEM stage without having to take off the microtome. A high frame rate CMOS camera coupled with a high resolution lens creates a crisp and low latency view of the knife and sample. A series of internal mirrors tracks the movement of the knife so that the blade of the diamond always stays focused and stationary on the screen.



View from the digital viewer. Even though the diamond knife is the moving mechanism, a series of mirrors allow the blade always stay in focus in the live view. This makes it easier to judge the distance between the blade and the block face.

Software integration

katana microtome is controlled via a familiar multi-touch user interface, communicating with our SEM Control software via a simple USB connection. The SEM software is designed to be compatible with all SEM software user interfaces and provides simple and straightforward automation and synchronisation between cutting and image acquisition.

katana microtome is fully integrated with SBEMImage[1], an open source SBFSEM acquisition software that has many advanced functionalities such as debris detection, autofocus, real-time image inspection, adaptive tile selection etc. As of the end of 2022, SBEMImage can be used on a selection of Zeiss and TESCAN SEMs. Integration with more SEM manufacturers are in the pipeline.

JEOL has fully integrated katana with their own SEM Supporter software providing automated image acquisition. Advanced features include dynamic tile selection, multiple ROIs and overlay SEM images onto optical images.

[1] Titze B., Genoud C. and Friedrich R. W. (2018). SBEMimage: Versatile Acquisition Control Software for Serial Block-Face Electron Microscopy. Front. Neural Circuits 12:54. doi: 10.3389/fncir.2018.00054

Sample preparation

Preparing samples in a form that can be imaged by scanning electron beams in vacuum whilst preserving the biological features as close to their pristine states as possible is crucial to successful acquisitions of SBEM data. A typical sample preparation protocol for SBEM begins with chemical fixation of the sample via either perfusion or immersion. This is followed by staining with osmium tetroxide, combining with potassium ferrocyanide and thiocarbohydrazide. Many samples requires a secondary staining using uranyl acetate and lead aspartate. The sample is then dehydrated by a solvent that is compatible with the subsequent liquid resin which will infiltrate the voids. The resin is then hardened by heating or UV, ready to be trimmed to a size that can be glued on a sample. A thin layer of Au/Pt coating is usually applied to improve the conductivity.

Primary fixation → **Secondary fixation** → **Post-fixation staining**
osmium tetroxide
potassium ferrocyanide
thiocarbohydrazide
→ **En bloc stain**
uranyl acetate
lead aspartate →

Dehydration → **Resin infiltration**
Durcupan/Epon
→ **Embedding**
Durcupan/Epon
→ **Trimming** →

Mounting on sample pins → **Serial block-face Imaging**

Microtome specification	
Dimension	135 x 66 x 56 (H) mm
Z stage resolution	1 nm
Z stage travel range	1.3 mm
Diamond blade width	1.5 mm
Knife oscillation frequency	0 - 70 kHz
Knife oscillation amplitude	0 - 150 nm
Minimum working distance (sample to detector)	3.0 mm
Maximum sample size (XYZ) **Recommended*	1.0 x 1.0 x 1.0 mm
Digital viewer camera	40 fps 2.3MP CMOS USB3.0
Controller	ARM Cortex Cortex®-M7+M4 dual core
Accessories	
Included	Aluminium sample pins, sample storage vials, carry case
Optional	Touchscreen laptop, sample measurement tool

Frequently asked questions

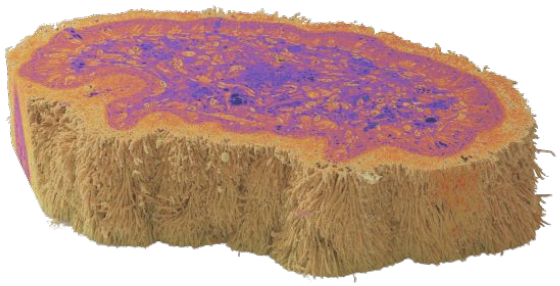
- *Would katana microtome fit my SEM?*

In most cases, yes. katana microtome is merely 56 mm in height. As long as the distance between the stage and the pole piece is greater than 56 mm plus the thickness of the detector, katana can be used in your SEM to acquire volume EM data.

- *How thin can katana microtome cut?*

For typical well stained resin-embedded biological samples, you can expect to hit a lower limit of approx. 20 nm. There is a complex tradeoff between the electron dose on the sample (and hence image quality) and cutting performance.

- If you want to cut thinner cleanly, you will need to use a low electron dose as this causes less damage to the surface resin. However, this can potentially result in lower resolution.
- If you increase the electron dose by increasing your beam current, taking multiple images or using very small pixel size, you can get high signal-to-noise ratio images, however, you may need to cut thicker to maintain clean and uniform cutting.
- If you have a very sensitive detector, you can get the same SNR from lower electron dose, so you can cut thinner.
- A low accelerating voltage may be required to achieve very thin cutting. Higher accelerating voltages will generally increase the interaction volume and limit ultimate Z-resolution.
- From our experience, typical imaging conditions of 2 KV acceleration voltage and an electron dose of 15 e/nm^2 will produce good results with ~4:1 SNR and 10 nm x 10 nm x 30 nm voxel size.



3D volume reconstruction of resin-embedded flatworm (Platyhelminthes) sample. Dataset collected by TESCAN MIRA at 4 keV, 150 pA. Low energy BSE detector, inverted contrast. Low vacuum mode, 20 Pa, Pixel size 16 nm, Number of slices: 300, Volume acquired: 130 x 130 x 25 µm. Sample courtesy of Dr P. Ladurner and W. Salvenmoser, Department of Zoology, University of Innsbruck.

Accessories

- Cabin size storage case
- 50x aluminium sample pins
- Sample storage vials
- Diamond cleaning rods
- Tweezers
- SEM flange
- SEM stage adapter

Accessories (Optional)

- Spare diamond knife
- Touchscreen laptop
- Sample measurement tool

Consumables

- Diamond knife
- Aluminium sample pin

Warranty and training

- 2 year warranty
- Extended warranty up to 3 years
- Choice of on-site/remote training
- Choice of image processing training

Find out more about katana microtome and all our products at **connectomx.com**

Get in touch: **hello@connectomx.com**

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