ZEISS Atlas 5
Large Area Imaging with High Throughput

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ZEISS Atlas 5 combines a 16 bit scan generator and dual super-sampling signal acquisition hardware with image processing and control software for your ZEISS electron microscope. It enables acquisition of images up to 32 k x 32 k pixels (one gigapixel image size), with dwell times from 100 ns to > 100 s, adjustable in 100 ns increments. Images can be saved with eight or sixteen bits of intensity. ZEISS Atlas 5 "Mosaic Tool" is designed to create large image montages and is highly integrated with SmartSEM from ZEISS.

Introduction

The Mosaic Tool enables the automated acquisition of multi-image mosaics at multiple sites on the sample. It automatically moves from image tile to tile, and mosaic site to site, executing autofunctions such as focus, stigmation, brightness, etc. as required. The result is an "Extreme Field of View" image montage that can cover optical microscope scale (or even naked eye scale) areas of your sample, at SEM nanometer scale resolution. Given suitable samples, unattended operation can be performed over a period of days, automatically acquiring terabytes of image data at rates up to 30 gigabytes of image data per hour. This allows the user to collect data from a given region at a resolution approximately seven orders of magnitude smaller than the region.
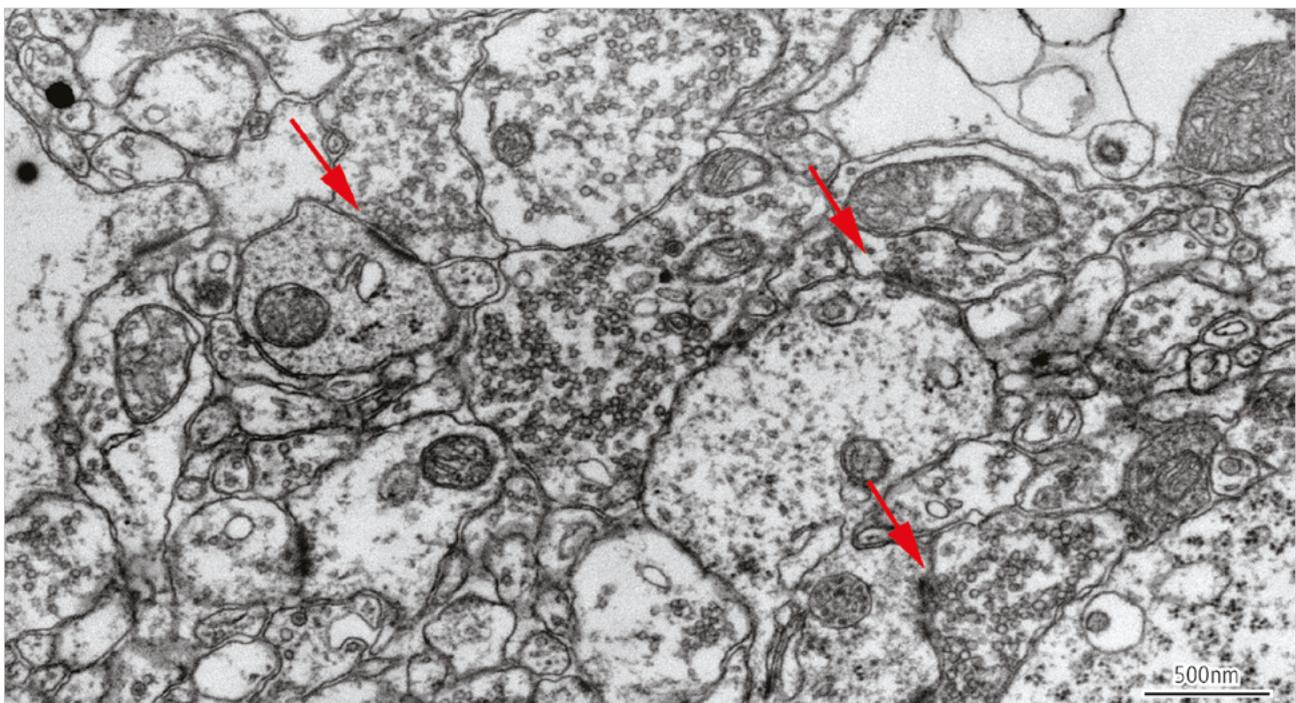

Single 30 nm thick section of mouse brain imaged with backscattered electrons at 8 kV using a ZEISS FE-SEM. Original image resolution was 4 nm per pixel. Red arrows point to areas where one neuron contacts and transfers information to another. Courtesy of: B. Kasthuri, Harvard University Center for Brain Science. USA.

### Why acquire "Extremely Large" images?

Extreme field of view imaging:

- Reduces the number of tiles to acquire thus reducing stage motion delay and more importantly reducing the areal fraction of each image "lost" to overlap. For a 4 nm pixel size and a 2 µm stage accuracy, overlap can be 66 % of the image area at 3 k x 2 k (33 % new data), but only 6 % of the image area at 32 k x 32 k (94 % new data / image) thus a 2.5 mm x 2.5 mm mosaic at 4 nm resolution would require ~ 92,000 images with a conventional frame store, but only 400 images when using ZEISS Atlas 5.
- Reduces the number of overlap "seams", leading to less beam damage and degradation of the sample.
- These factors greatly reduce computational complexity – considerably easier to stitch and align when working with 400 images rather than 92,000. This reduction in computational complexity becomes even more critical when three dimensional alignment of serial sections is required.
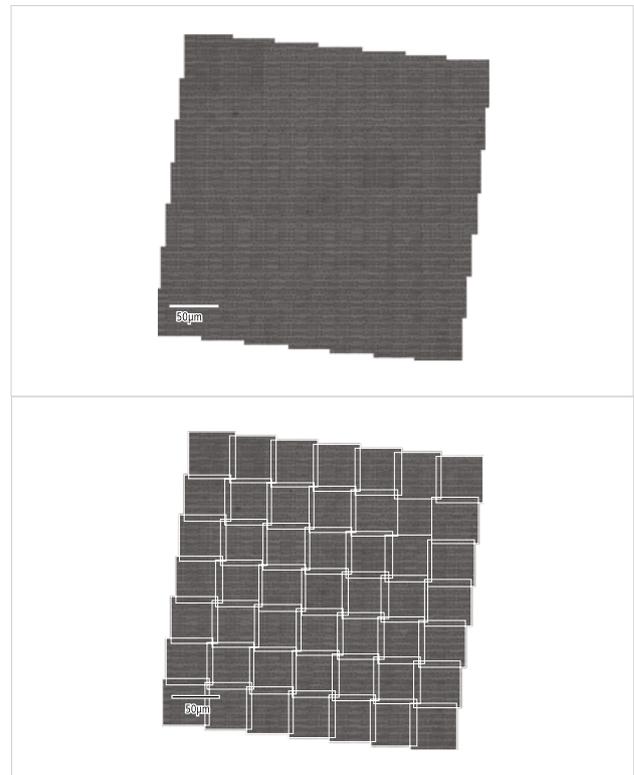
"Extreme Field of View" image montages improve the complete understanding of structures by providing detailed image information from the macroscale to the nanoscale.

A 65 nm technology node graphics processor integrated circuit, stripped to its silicon substrate with HF acid etching. The mosaic consists of 49 images, each ~ 500 Megapixels, automatically stitched by the VE-Viewer into a ~ 1/3 mm x 1/3 mm mosaic. This mosaic was acquired stepping at an angle, with scan rotation used to align image features to the scan axes, in order to accentuate the stitching for illustrative purposes. Top: Image tile boundaries are hidden. Bottom: The position of image tile boundaries are displayed (white lines). Sample courtesy of: D. Unrau, Fibics Incorporated, Ottawa, Canada.
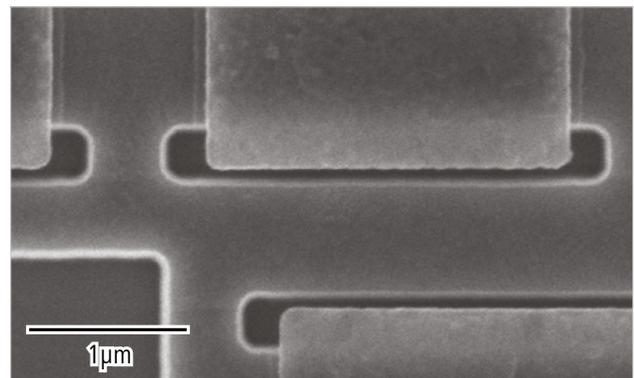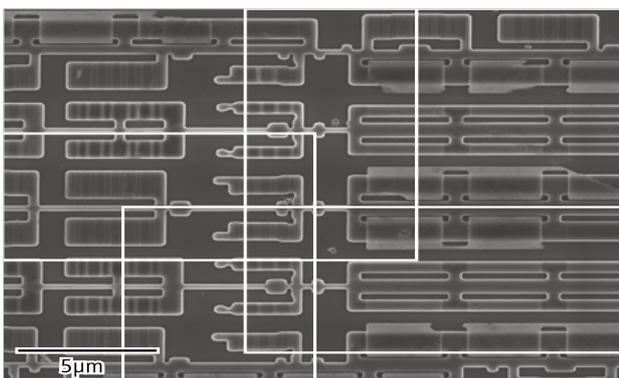

Left: A magnified view of the junction between four tiles as shown above, with tile boundaries displayed in white, as otherwise the boundaries between tiles are virtually seamless.
Right: A sample of the high resolution detail visible across the entire Extreme Field of View mosaic.

ZEISS Atlas 5 and STEM-in-SEM

By using a detection system for transmitted electrons the information limit of ZEISS FE-SEMs can be extended beyond the nanometer range. The annular STEM detector (aSTEM) delivers brightfield, darkfield and annular darkfield images. The images of ultrathin biological sections obtained by the aSTEM detector allow to investigate sub-cellular details.
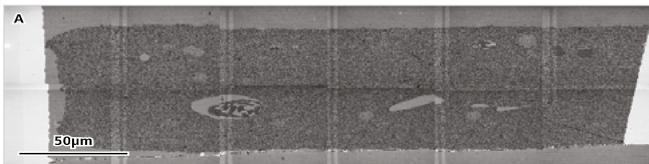
STEM-in-SEM images of rat hippocampus acquired on a ZEISS FE-SEM equipped with ZEISS Atlas 5.

Courtesy of: J. Mendenhall, Center for Learning and Memory, University of Texas at Austin.
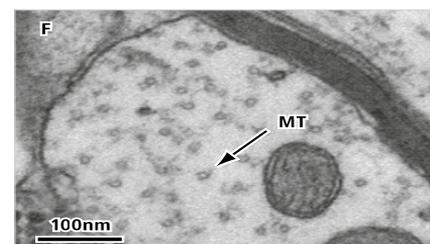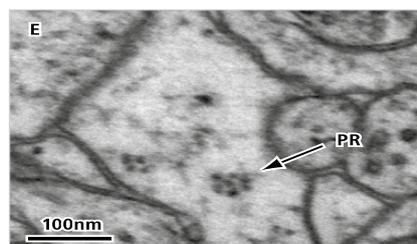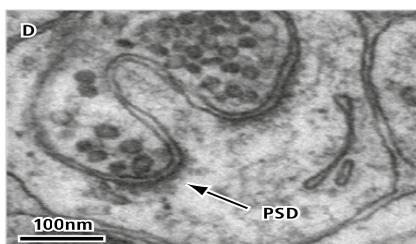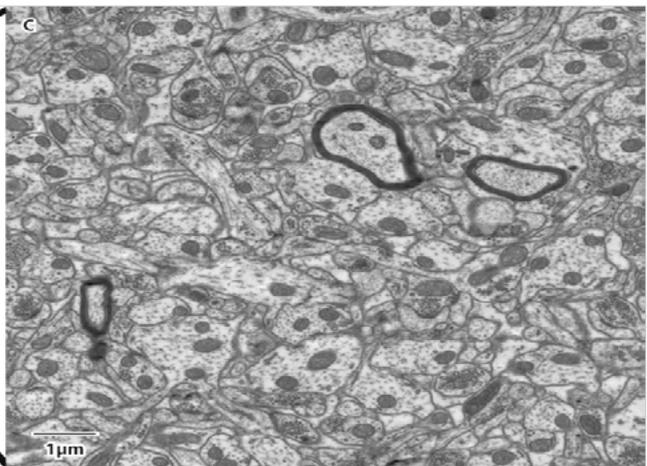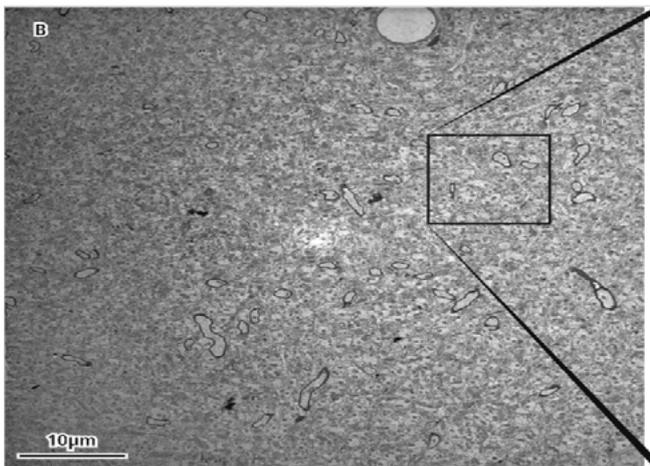
After an electrophysiological experiment, the rat was glutaraldehyde perfused and the hippocampus was sectioned with a vibrating knife to 70 µm thickness. The section was osmicated with potassium ferrocyanide en bloc, dehydrated in ethanol, embedded in Ladd LX112 epoxy via propylene oxide, ultrasectioned to 45 nm thickness, post-section stained with uranyl acetate and lead citrate.

(A) Low magnification overview image of a single mosaic site consisting of 6 x 2 tiles, each 49 µm x 49 µm, acquired using the multi-mode STEM detector in a ZEISS FE-SEM with Gemini optic. Each image tile was acquired at 2 nm per pixel.

(B) A single 49 µm SEM-in-STEM image, acquired at 2 nm per pixel. The inset square illustrates the comparative size of a TEM image acquired on a 4 k x 4 k camera, also at 2 nm per pixel.

(C) Detail from an approximately 9 µm x 9 µm region of Image B. Note, the image quality is comparable to results obtained by conventional TEM.

At full resolution, key organelles are readily resolved including

(D) post synaptic densities at synapses,

(E) polyribosomes and

(F) cross-sectioned microtubules suitable for serial tracing and dense reconstruction.

Conclusions

- Available for all SEMs, FE-SEMs and FIB-SEMs,
  also as field upgrade
- Acquire images up to 1 gigapixel in a single scan,
  increased from previous ~7 megapixel maximum
- Continuously selectable scan size from 1 x 1
  to 32 k x 32 k pixels
- Continuously definable field of view and pixel resolution
- Scan rates selectable from 100 ns to > 100 s dwell
  per pixel, in 100 ns increments
- Save image data as 8 or 16 bit TIFF files
- Automation enables unattended operation, with
  automatic stage motion, focus, stigmation,
  brightness and contrast adjustment as required
- Acquire and stitch high resolution image Mosaics
  at optical fields of view with nanometer resolution
- Includes the Viewer, an image viewer tailored to
  efficiently handle the multi-gigabyte mosaics
  produced by ZEISS Atlas 5.
  The Viewer allows the user to open, stitch, navigate
  and intelligently re-render the large 2D datasets
  produced by ZEISS Atlas 5.

Bottom: Overview image of semiconductor device showing the capability of ZEISS Atlas 5 to also acquire non-square images.
Top: Detail of semiconductor structure.

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