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Gigantic Montages with a Fully Automated FE-SEM (Serial Sections of a Mouse Brain Tissue)

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Gigantic montages of ten serial sections of mouse brain tissue, with an area of 0.3 x 0.2 mm^2 used on each section have successfully been acquired. The acquisition consisted of a framework of 20 x 20 frames on each section, frame size of 5,120 x 3,480 pixels (each pixel 3.3 x 3.3 nm^2), using a backscattered electron (BSE) imaging mode in an FE-SEM (JSM-7001F) equipped with a specialized and newly designed fully automated image acquisition system.

Serial sections of a mouse brain tissue with about 50 nm in thickness were prepared by ATLUM (Automatic Tape Collecting Lathe Ultramicrotome) at Harvard University [1]. The tissue was fixed with glutaraldehyde and osmium tetroxide (OsO_4), and embedded in epoxy resin similar to a typical TEM specimen preparation for this type of specimens. The serial sections were automatically mounted one by one on a conductive tape called Capton. They were placed in almost the same direction and at the same distances between neighboring sections. The tape was cut to a certain length, and the individual tapes were stuck on a 4 inch wafer as shown in Fig.1. For imaging with the FE-SEM the surface of the wafer was coated with carbon to avoid specimen charging.

The BSE imaging was employed to obtain contrast of fine intracellular structures, which were heavily stained with OsO_4. The YAG detector was used for the BSE detection. An observation condition of the BSE imaging, which we chose, was 10 kV of accelerating voltage, 1 to 2 nA of probe current, 5,120 x 3,480 pixels/frame of image and 60 s/frame of image acquisition time.

To minimize the number of frames for the montage the minimum magnification of a frame was selected to be 7,000. Because the frame was divided into 5,120 x 3,840 pixels, the size of the pixel corresponded to 3.3 nm^2. This magnification was large enough to observe even the detailed membrane structures of synapses, vesicles, etc. in the cells when the image was digitally zoomed up to 20,000 to 30,000 times. The overlap of neighboring frames was set to be 10 percent in order to be able to stitch neighboring frames into a montage.

This approach of taking multiple images over large number of sections in unattended fashion necessitates the automation of image acquisition. In order to accomplish this task, we have developed an automated image acquisition system. This new system includes auto-focus, auto-astigmatism correction, auto-contrast/brightness control, and auto-stage control. Furthermore, self-check and auto-error correction functions, which are carried out every time following a single frame acquisition, have been added to the automated image acquisition system. These additional functions are extremely important to obtain data from all frames compatible with each other to make a montage. With the help of these functions, montages in ten serial sections were successfully made with the size of 20 x 20 frames of each section. The time of making the ten montages including the image acquisition was 70 h. A part of one montage with four frames is shown in Fig 2. together with digitally zoomed images of two parts of the montage. In the latter images, synapses

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and vesicles are clearly observed.

The analysis of the accuracy of matching in the overlapped area in neighboring frames by a block-matching technique [2] showed that the distortion of the matching excluding translation between neighboring frames was due mainly to the very small fluctuated drift of the electron beam or the mechanical stabilization, and the maximum amount of the displacement was 0.2 percent. This small distortion ensured that the montages could be successfully made to join the corresponding pixels in neighboring frames.

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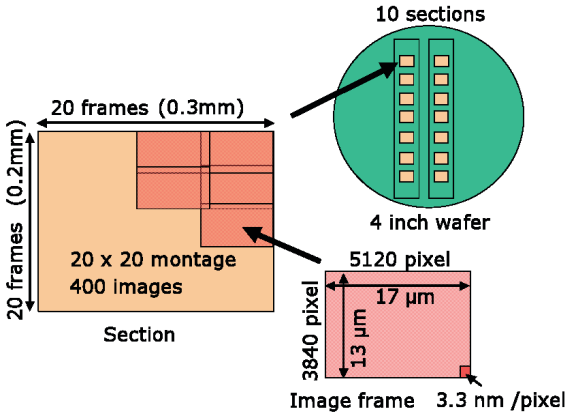

Fig.1 A schematic illustration of serial sections on a wafer

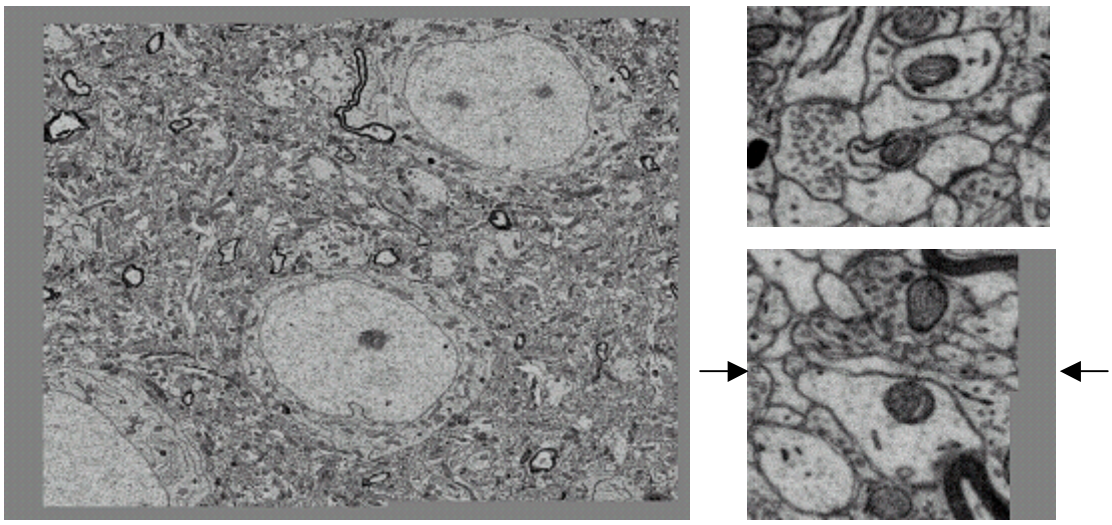

Fig. 2: A part of a montage with zoomed images of two parts of the montage. A horizontal line between two arrows corresponds to a joining line of the upper and the lower frame.

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