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Comparison of Techniques for Fine Alignment of Image Stacks in Serial Block-Face Electron Microscopy

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Serial block-face electron microscopy (SBEM) provides nanoscale 3D ultrastructure of eukaryotic cells and tissue extending over volumes ranging from ~10^3 to > 10^6 μm^3. In principle, SBEM enables automated acquisition of thousands of sequential images from a rigid block face to yield image stacks that are in perfect registration. However, in practice, misalignments occur within the image stack due to scan distortions, mechanical instabilities and build-up of surface charge [1]. Such imaging registration errors pose challenges to the processing of the 3D data because single pixel precision in image stack alignment is often essential for computer-aided image segmentation and volume rendering. We have therefore investigated effective means of improving fine-alignment in three dimensions to facilitate 3D image data reconstruction, visualization and quantification.

The recent availability of a focal charge compensation device for the Zeiss Sigma VP/Gatan 3View SBEM system greatly reduces image distortion due to electrical charging of the specimen block while maintaining high vacuum within the electron optical column of the scanning electron microscope without resorting to operation in the variable pressure mode. Nevertheless, some subtle misalignments remain, especially when backscattered electron imaging is performed at multiple primary energies for the extraction of sub-slice depth information, which requires image stack alignment with single voxel precision for alternating incident electron energies. For this fine alignment, we have tested three available programs: scale-invariant feature transform (SIFT) [2], patch-based cross correlation in IMOD [3], and TeraSticher [4], which can be applied to very large datasets.

Comparisons of alignments using the SIFT and IMOD programs are shown in Figure 1. Data were taken from a mouse brain sample using Zeiss Sigma VP/Gatan 3View SBEM system. For illustration purposes, here we have selected a small stack of 65 slices. Fig. 1A shows the x-y views of slice 32 of the stack before and after fine alignment. It is noticed that compared to the coarse aligned data, the IMOD alignment tends to pad right and bottom of the image with the mean pixel value of the image, while SIFT tends to only pad top of the image with zero-value pixels. From this example, it is observed that IMOD is better at retaining the original aspect ratio and size since IMOD tends to adjust both x and y to pursue the desired alignment, whereas the adjustments in SIFT are heavily focused on the y-axis. From the x-z views (Fig.1B), it is concluded that both the SIFT and IMOD program greatly improve image quality, see the red circle. However, the myelin structure indicated by the red circle in the IMOD aligned image seems closer to its original appearance compared to the one in the SIFT aligned image, which shows some distortion in the x direction [5].

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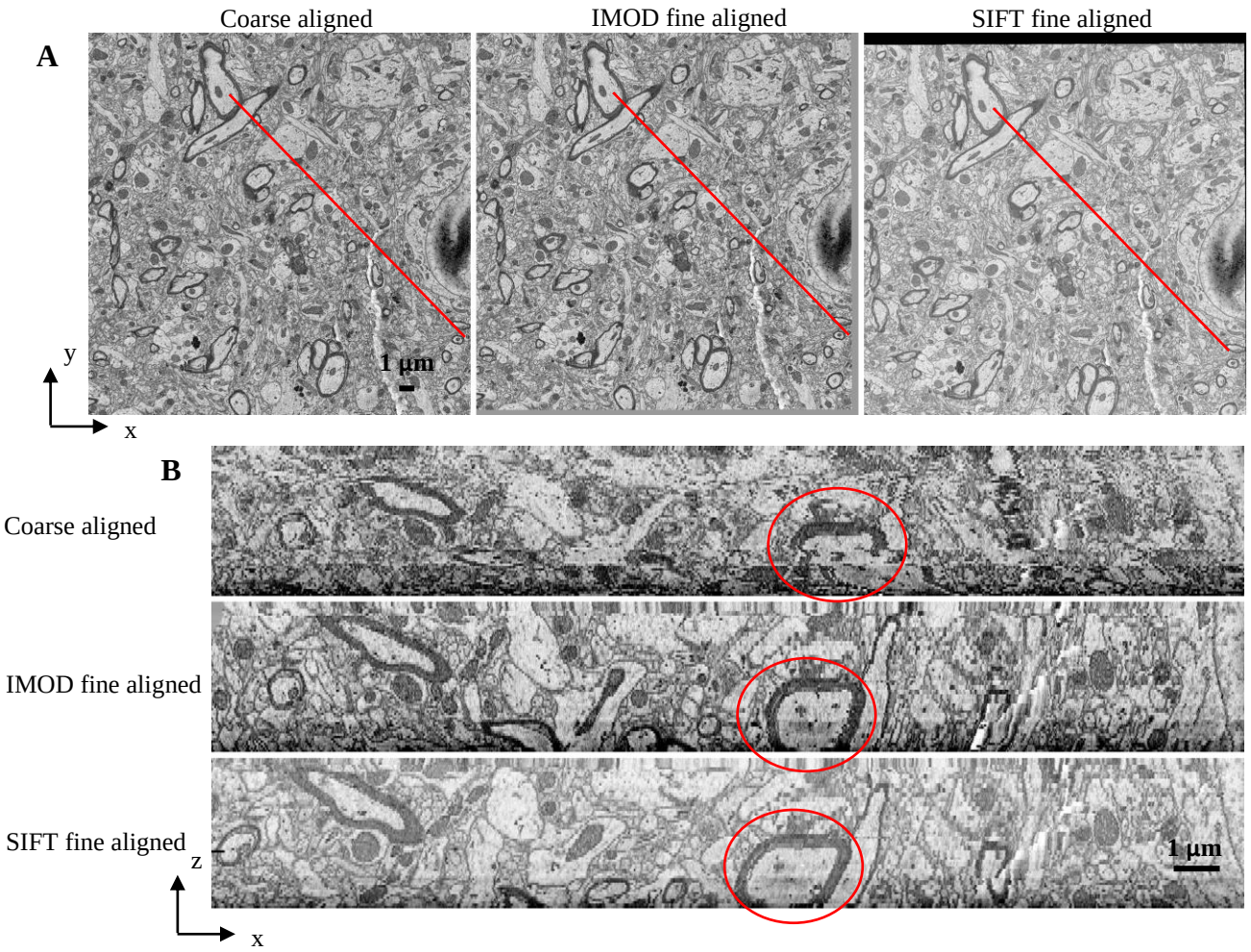

Figure 1. Comparisons of alignments using the SIFT and IMOD programs based on x-y views (Fig.1A) and x-z views (Fig.1B). Images labeled 'Coarse aligned' shows the stack before applying SIFT or IMOD fine alignment. Red line in Fig.1A serves as a length reference against the relevant distance between certain features in x-y views. Circle in Fig. 1B highlights the same feature in the x-z view. Pixel size in Fig.1B has a rectangular shape, corresponding to the pixel size difference in x (12.5 nm) and z (50 nm).

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