Ultra High Precision, High Resolution and Large Area SEM using Raith E-line Plus

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Large area and volume imaging, with image size beyond a single field of view, has important applications in many research areas such as neurobiology, life science and semiconductors[1,2]. The techniques applied to the imaging and processing of large dataset has also improved in many shape and forms in recently years[3]. While most of the advancements are based on the instrumentation and data analysis, fundamental challenges related to the hardware, such as field distortion, stage accuracy, correlated scanning control, and automatic microscopy are not often addressed.

On the other hand, modern Electron Beam Lithography (EBL) systems that are often used to create nanolithographic patterns beyond a single field of view have most of the apparatus dedicated to overcome these hardware issues. For example, laser interferometric stages and high digital to analogue conversion (DAC) scanning controls are common elements found in EBLs to minimise field distortion and stage stitching error. To compensate for the total thickness variation (TTV), wafer bow and curvature at the substrate surface, EBL systems are equipped with laser height sensors for dynamic focus corrections over large surfaces. Moreover, beam drifting can be accurately compensated with the use of a laser stage, ensuring image pixel accuracy over long hours of operations. Finally, most EBLs have high level of automation that can be easily programmed to acquire images with various conditions (varying acceleration voltage, detectors, and scan directions) while maintaining the image quality. As a result, EBL could be the ideal platform to deliver automated, large area electron microscopy and array tomography for sample analysis and correlated light electron microscopy (CLEM) applications.

In this work we explored the use of Raith e-line Plus, a dedicated high resolution EBL system, for the use in large area and array tomography microscopy. The system is equipped with a laser interferometric stage with accuracy down to less than 2nm. Prior to the image acquisition we used an optical profiler (Zeta-300 from Zeta instruments) to obtain a stitched sample map of the very thin sectioned microtome ribbons of Zebrafish embryo on an ITO coated coverslip (Balzers Optics) with fiducial markers. We were able to correlate the coordinates of these ribbons with the Raith e-Line Plus for an automated imaging workflow. Sixty four high resolution SE images, with field of view of 9.6 m x 9.6 m taken at 13000x were stitched to form a GoogleMap like Zebra fish map with 38400 x 38400 pixels (pixel resolution at 2 nm x 2 nm). The image were stitched together by overlapping just the last 5 pixels (10 nm overlap). The total acquisition time of the entire image was under an hour. The stitched image, and several enlarged views showing the stitch accuracy and image resolution can be found in the figure next page.

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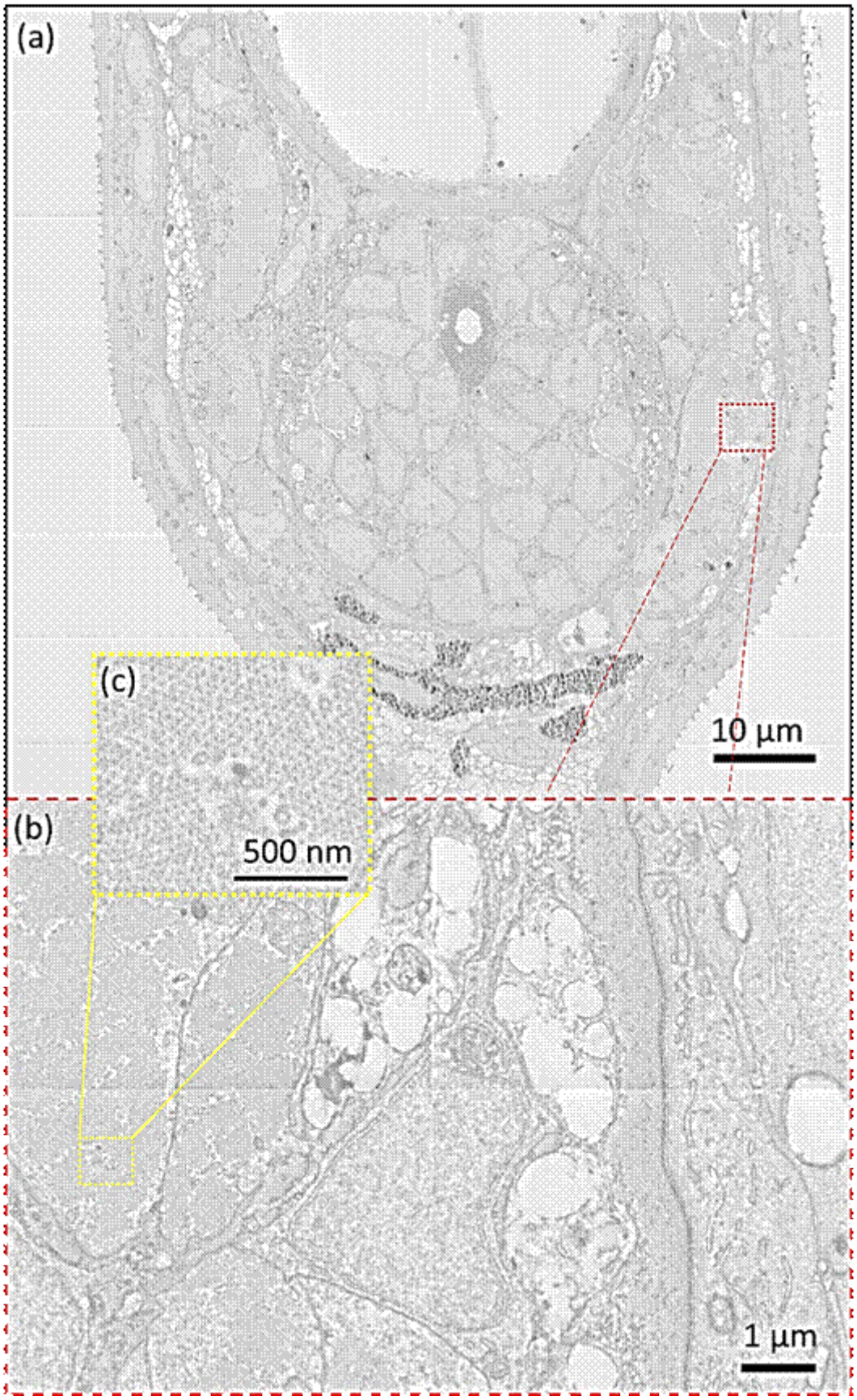

Figure: (a) SEI image of a Zebrafish embryo stitched with 64 SEM micrographs obtained from the Raith e-Line Plus.

(b) Enlarged area from (a) showing the stitched areas between four images.

(c) Enlarged area from (b) showing individual actin muscles of ~ 20 nm in diameter