2990
doi:10.1017/S1431927622011205
Microsc. Microanal. 28 (Suppl 1), 2022

A High-throughput Electron Microscopy Workflow and its Applications in Life Sciences.

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Electron microscopy (EM) is increasingly being used in large-scale biological projects, either for volume imaging or large area mapping. In both use cases, a need for high throughput, reliability and automation of electron microscopy highlights the importance of a new approach to large-scale imaging.
In this paper, we present an innovative electron microscopy workflow that increases imaging throughput up to 100 times compared to conventional workflows. Furthermore, we demonstrate its implementation on case studies in life sciences.

The innovative workflow presented here revolves around FAST-EM, a multibeam scanning electron microscope. The imaging speed of a conventional scanning or transmission EM is a limiting factor in many large-scale workflows. At the same time, increasing the throughput of a workflow without negatively impacting image quality can be difficult or impossible. To overcome this challenge, we have developed an automated multibeam scanning EM, which combines simultaneous 64-beam electron imaging with transmission detection [1][2]. This imaging approach presents an enormous improvement for high-throughput transmission EM of biological specimens: FAST-EM increases imaging speed and reduces operator overhead, without compromising image quality.

FAST-EM's high resolution data enables clear identification of the different cell types and organelles, while the high throughput allows for the generation of large-scale overviews at very high speed. Referring to Figure 1, 16 seconds are required to acquire the 102.4 µm x 76.8 µm field of view presented, as opposed to several minutes needed with a conventional SEM system.

FAST-EM, and high-throughput EM in general, will be instrumental in tackling current large-scale projects such as volume-EM of cells and tissues, connectomics, and large-scale 2D nanotomy projects [5]. Looking at an even broader scale, high-throughput EM opens the door for projects of unprecedented scale to be completed within reasonable time frames enabling a shift towards using EM as a fast quantitative analysis tool.

Having eliminated the EM bottleneck from the workflow would not be enough. Sample preparation and collection and then management of the acquired data to extract valuable information from it would still represent relevant barriers for researchers and technicians. Reliable section collection is crucial to maintain high-quality samples for high-throughput EM. Manual serial sectioning is a time-consuming process and mistakes are frequent and costly. Moreover, specimen pickup and placement need to be done in an organized fashion to keep organization of the samples intact.
FAST-EM sample-holding substrates, the scintillators, are compatible with sample collections techniques where multiple ribbons are collected at the same time. They are submerged in the boat during

the sectioning process, and when the desired amount of sections and ribbons are created, then the water of the boat is drained to allow the ribbons to deposit onto the scintillators, see Figure 2.

This technique can be implemented in any existing ultramicrotome provided that the boat is big enough to host an entire scintillator. Alternatively, there are commercially available solutions that enable this process to be completed in full autonomy [4]. That specific system is fully programmable and capable of collecting images on various different substrates, including the FAST-EM scintillators, with flexible block-face sizes (µm to mm). This method allows for sectioning and collecting the sections with a single programmable and automated tool, needing little supervision.

Sectioning and collecting individual sections is a very common approach for array tomography, for various reasons including the dimensions and typology of biological structures and organisms of interest. Recently, a simple and automated technique to handle hundreds of individual sections has been introduced [6]. This approach facilitates convenient bulk staining procedures with liquids and uninterrupted imaging in automated fluorescent light and electron microscopes, including next-generation multibeam electron microscopes such as FAST-EM.

Considering a typically large volume of biological material, for instance $(0.25\ mm)^3$, with slices of 40nm thickness, one would have to produce 6250 sections. Producing and collecting these sections would require roughly 20h with any of the techniques discussed above. Running at the speed of 100 Mpix/s, FAST-EM would image these sections in roughly 76 hours (4 days), in full automation. This biological volume of interest could be imaged in less than 1 week considering sample preparation and imaging. A conventional workflow would require months to complete such an endeavour.

The last element of the innovative workflow we propose here is connected to the storage and management of the data that FAST-EM produces. For the example provided above FAST-EM would produce an amount of data in the order of 60 Tb. Conventional SEM machines don't offer local storage of such a high amount of data. Moving and storing this amount of data is therefore intrinsically complex for conventional SEM users, based on manual processes and extensive use of physical hard drives. Extracting information from such a large quantity of data is even more complex. Parsing the entire dataset to identify the biological elements of interest would not be possible on standard personal computers, and existing software packages that offer tools for data visualization and image filtering would not handle such a large amount of data.

FAST-EM is equipped with a storage and data platform that allows for locally offloading data starting from 250 TB and provides an open API for visualization tools, such as CATMAID, and for connecting a data stream to custom made scientific code, for instance using python as a programming language. Every region of interest is automatically visualised in CATMAID after all the fields of view that compose the region of interest have been stitched in 2D. This offers the possibility to immediately navigate the acquired data with both nanometer level of detail and ultrastructural context. Stitching the various fields of view is done automatically, saving time and effort. The connection to custom-developed scientific code allows for rapid retrieval of biological data, with the required field of view and zoom level, enabling rapid and effective use of routines for automated interpretation of EM images.

Concluding, the workflow presented here, Figure 3, is capable of providing high throughput consistently from sample preparation to insight generation. Scientists will no longer have to compromise between the level of detail and amount of imaged material, they will now be able to go seamlessly from sections to

https://doi.org/10.1017/S1431927622011205 Published online by Cambridge University Press

information unlocking the potential of EM as a quantitative tool for faster advancements in life sciences research [7].

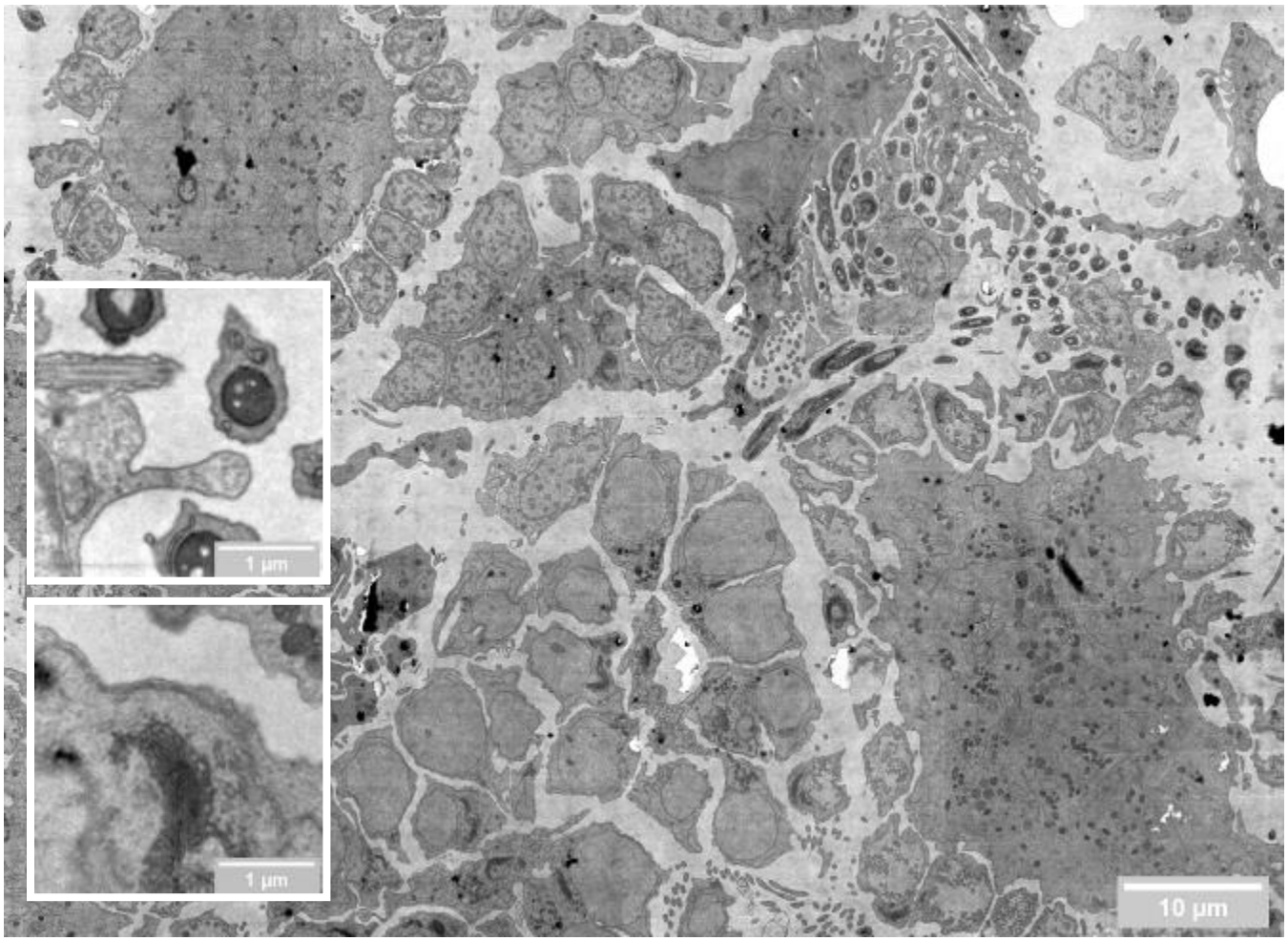

Figure 1. Annelid worm tissue – 80 nm sections of OTO stained samples. FOV 102.4 x 76.8 um, 16 seconds acquisition time. Samples courtesy of Karol Małota, university of Silesia. Zoom 1 (top): Longitudinal cross section view of microtubules in a cilium of cells undergoing spermatogenesis. Zoom 2 (bottom): Visualization of the golgi complex in Annelid spermatid cells

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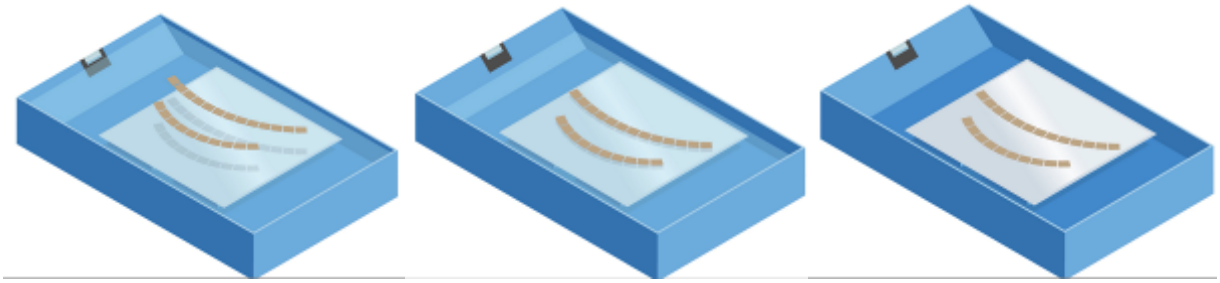

Figure 2. The scintillator is positioned in the microtome boat. (Left) Ribbons after sectioning floating in the boat. (Center) water being drained. (Right) ribbons being deposited onto the scintillator.

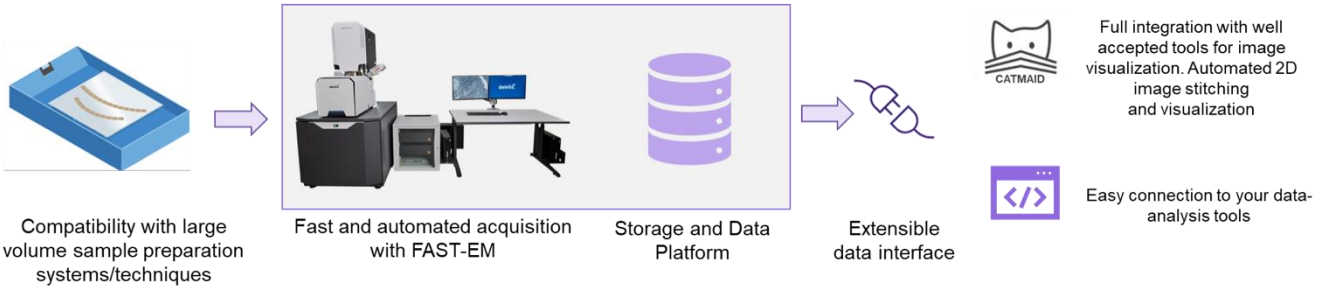

Figure 3. Innovative high throughput and ultra automated workflow for electron microscopy based on the multibeam SEM, FAST-EM.

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https://doi.org/10.1017/S1431927622011205 Published online by Cambridge University Press