1038
doi:10.1017/S1431927619005920
Microsc. Microanal. 25 (Suppl 2), 2019

Integrated Array Tomography for High Throughput Electron Microscopy

Ryan Lane[1*], Pascal de Boer[2], Ben N.G. Giepmans[2] and Jacob P. Hoogenboom[1]

[1.] Imaging Physics, Delft University of Technology, Delft, The Netherlands.
[2.] Cell Biology, University Medical Centre Groningen, Groningen, The Netherlands.
* Corresponding author: r.i.lane@tudelft.nl

Volume electron microscopy (EM) is now increasingly looked to for answering complex biological questions, thanks in large part to revolutionary developments in instrumentation and sample preparation techniques. However, due to the slow nature of electron-probe-based scanning techniques, throughput has long remained the primary obstacle in pushing to larger areas at high resolution. An undesirable trade-off is therefore commonly made between imaging resolution, volume, and time. A recent approach that attempts to circumvent this trade-off involves selective imaging of the specimen in multiple iterations at increasing levels of magnification. Between iterations, the EM dataset is reconstructed and inspected to locate sites for higher magnification imaging in the next iteration [1]. While this approach can outpace indiscriminate scanning of the entire volume, overall throughput is limited by the overhead involved in reconstructing and evaluating the intermediate datasets.

An alternative approach for selective imaging is to first survey large areas of the sample using a light microscopy technique. By tagging particular biomolecules or cellular components of interest with e.g. a fluorescent protein, fluorescence microscopy (FM) could then be used to identify regions of interest for subsequent high magnification EM imaging [2]. However, relocating regions of interest (ROI) between imaging modalities is nontrivial when the ROI might be spread out in three dimensions. Moreover, intermediate sample preparation can lead to specimen warpage or shrinkage. One way to overcome these obstacles is by merging separate imaging systems into a single, integrated microscope [3]. To this end, we have begun utilizing an integrated microscope for the development of an automated workflow for correlative array tomography (Figure 1).

Central to the layout of the integrated system is that the fluorescence and electron microscopes share a common optical axis and translation stage. This allows for near-simultaneous imaging of the shared field of view as well as synchronized movements when navigating about the sample. An automated procedure for precisely overlaying the FM and EM fields of view exists which is able to deliver less than 10nm registration accuracy [4]. Following FM-EM registration, the correlative imaging pipeline begins by collecting a series of FM image tiles in a grid-like fashion using a 60x high-NA objective. After FM imaging, we then collect a series of high resolution EM image tiles, thereby avoiding quenching of the fluorescence by the electron beam.

Both the FM and EM image tiles are acquired with a small percentage of overlap. Features common to neighbouring image tiles are found within this overlap region and used to estimate local transformations between neighbouring tiles as well as an optimized global alignment [5]. While the spatial resolution between the two modalities are (by design) vastly different, the axial resolutions are matched by the thickness of the section [6].

Figure 2 shows early results from our array tomography procedure applied to 80nm thin rat pancreas tissue. This clearly shows the vast difference in scale as well as the high overlay precision obtained by the

integrated microscope. Given the scalability of our method, we believe our procedure can pave the way for future large-scale correlative acquisitions—offering a promising approach for the reconstruction of complex biological systems.

References:
[1] DGC Hildebrand et al., Nature **545** (2017), p. 345.
[2] J Delpiano et al., Journal of Microscopy **271** (2018), p. 109.
[3] N Liv et al., PLoS ONE **8** (2013), p. e55707.
[4] MT Haring et al., Scientific Reports **7** (2017).
[5] K Khairy, G Denisov, S Saalfeld, arXiv preprint (2018).
[6] KD Micheva and SJ Smith, Neuron **55** (2007), p. 25.

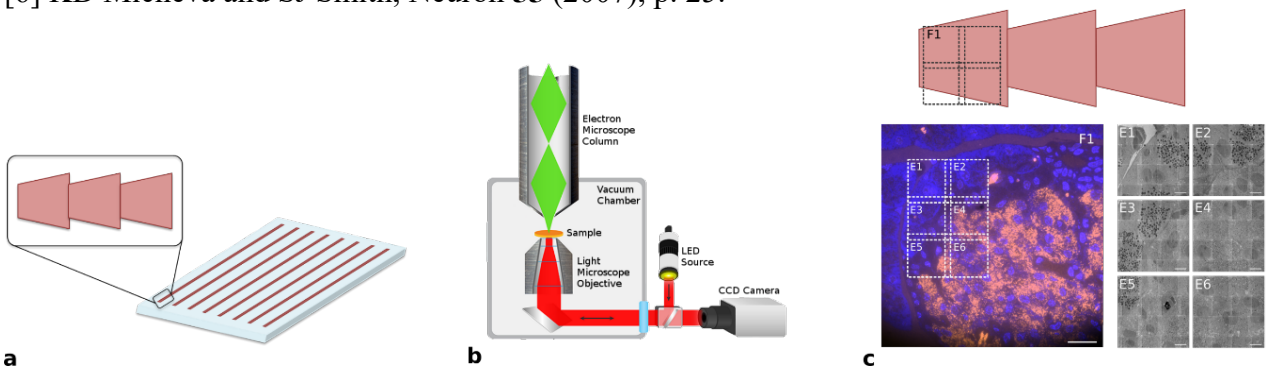

Figure 1. Conceptual overview of integrated array tomography workflow. (**a**) Serial tissue sections are cut and placed into ribbons for insertion into (**b**) the integrated microscope. (**c**) Registered FM and EM images are collected for subsequent alignment and reconstruction.

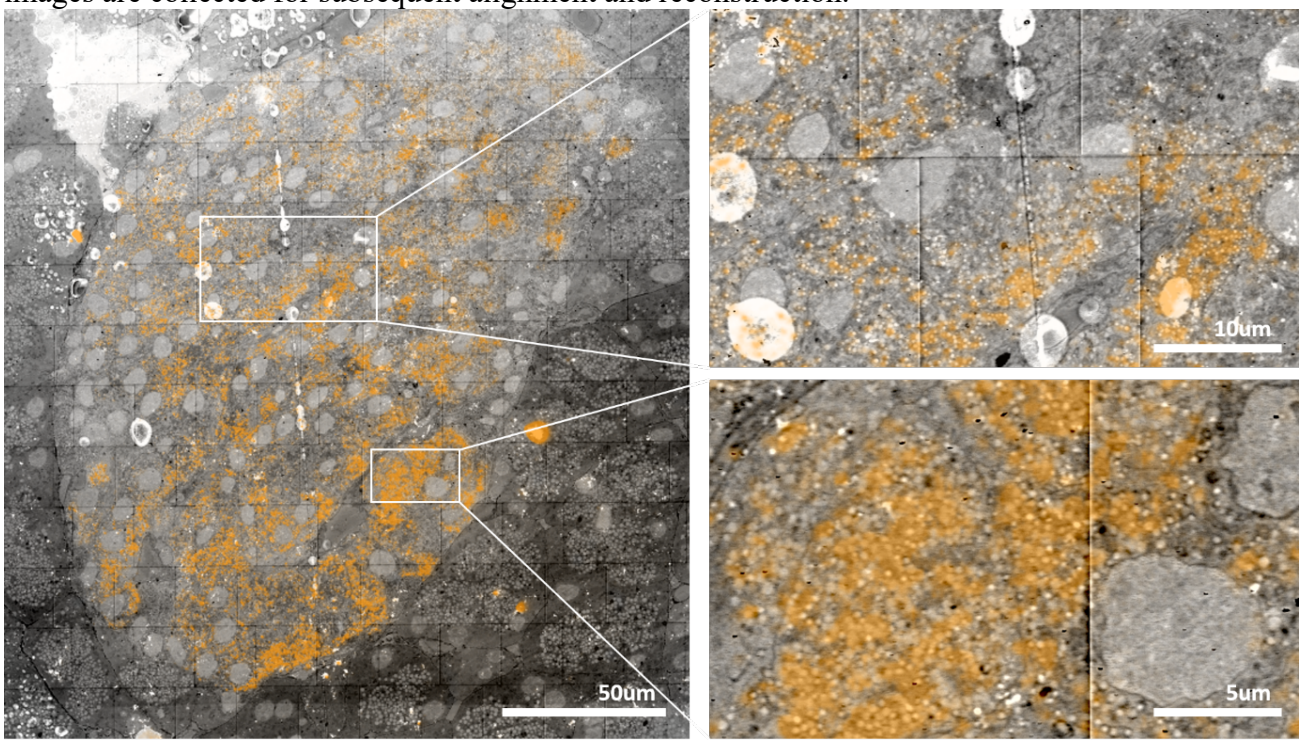

Figure 2. Aligned, 2D mosaic images of rat pancreas tissue with FM (orange) overlaid on ultrastructure EM (grey). (**Left**) Zoomed out, correlative mosaic images. (**Right**) Select cut-outs from overview image at intermediate (above) and high (below) resolution scales, highlighting structural detail and FM-EM overlay precision.

https://doi.org/10.1017/S1431927619005920 Published online by Cambridge University Press