doi:10.1017/S1431927617000848
Microsc. Microanal. 23 (Suppl 1), 2017

Developments in Reel-to-Reel Electron Microscopy Infrastructure

Christopher S. Own[1], Matthew F. Murfitt[1], Lawrence S. Own[1], Jesse Cushing[1].

[1.] Voxa, Seattle, WA USA

An increasing need for structural imaging at small length scales and simultaneous demand for data from larger volumes in research and commercial applications such as gene sequencing, neuroanatomy, and industrial process monitoring has encouraged new developments in electron microscopy (EM) technology, in particular high throughput tools. We previously reported on construction of a new serial sample imaging tool called the GridStage™ produced by us at Voxa, which enables direct imaging of serial sequential samples loaded onto tape via transmission electron microscopy (TEM), with capacity exceeding 10,000 samples per tape reel [1]. We have used tape delivery in ssDNA sequencing by EM and more recently in 3D serial section reconstruction of tissue, providing neuroanatomy at resolution and detail orders of magnitude greater than ever before.

GridStage and its smaller sibling Sprite™, a simple cartridge-based sample imaging platform able to load and image sixteen 3 mm disc samples at a time (see Fig. 1), are deployed and currently in use at Allen Institute for Brain Sciences. These novel stages are installed onto arrayed JEOL 1200 EX microscopes configured to obtain large montage datasets >1 petabyte in size at speeds exceeding 100 MPixels/s per tool. Others have developed parallel imaging systems based on scanning electron microscopy (SEM) for a similar application [2]. Reel-to-reel (R2R) imaging has the advantages of rapid automated sequential imaging of samples, random access by virtue of sample indexing, and easy storage at air in desiccant chambers or in vacuum chambers ready for future re-imaging.

New powerful imaging capabilities necessitate new methods for sample preparation, which has traditionally been a complex and demanding aspect of high-resolution biological tissue imaging. As development of serial sectioning tools for SEM and TEM [3] opened the path to novel high-throughput serial imaging, experience with serial imaging indicates new types of serial sample post-processing steps that can enhance image acquisition pipeline throughput. We report here on new industrial automation tools we have produced supporting high-throughput continuous automated R2R imaging.

Sample throughput in serial section imaging of cortical tissue is limited in part by the quality of tissue staining protocols. A popular manual protocol for contrast enhancement of structures for EM is double-staining using uranyl acetate in tissue block form followed by post-staining after sectioning with lead citrate. Lead citrate enhances contrast by binding primarily with proteins and glycogens. However, it also forms a water-insoluble toxic lead carbonate white precipitate if exposed to CO_2. This necessitates a controlled environment to prevent unwanted high-Z precipitates that can cause staining artifacts as well as absorb beam energy and catalyze catastrophic damage in suspended tissue sections imaged under high beam currents. While commercial systems are available for applying liquid processes on multiple TEM grids simultaneously using a CO_2-free flow cell or novel TEM carrier systems connected to pipettors [4-5], these do not scale to more than about 50 separate samples per batch; high-throughput R2R based processes require efficiently staining >1000 samples at a time.

We have constructed a new R2R fluid staining system called Strider™ that is equipped to precisely apply stains directly to serial tissue samples mounted on continuous tape (Fig. 2). Strider has room for six customizable computer-controlled modular fluid stations, each capable of applying and draining a fluid without damaging thin supported membranes (Fig. 3). Strider incorporates an environment chamber fillable with purge gas, which when used in conjunction with a solid CO_2 getter material such as NaOH prevents carbonate precipitation. Strider's process rate is similar to that of tissue sectioning machines, thereby lending it to imaging pipeline use for either TEM or SEM tape-based serial imaging.

A second process enhancing tape-based imaging throughput is post-application of electron-transparent conductive coatings, useful at both the sample and tape levels. At the sample level, it may be desirable to passivate surfaces in order to stabilize areas that would otherwise deform or damage under the beam. Coatings also enhance conductivity of the sample and substrate, eliminating local charging of exposed non-conductive surfaces (such as boundaries of electron-transparent window slots), which negatively impact high-resolution imaging. For tapes that accept insertable single-sample carrier substrates, coating may facilitate charge transfer away from the carrier into the tape. To achieve this, we have built an automatic carbon deposition system called Pupa™ that is able to apply a continuous protective coating to tape-mounted samples. It features customizable apertures to apply controlled deposition distributions without damaging samples.

Each of the above systems are remotely controllable and monitored over network via easy-to-use native tablet and web interfaces, enabling remote operation and monitoring of complex industrial processes. These novel and versatile tools in sample preparation, delivery, and imaging enable scaling of previously tedious manual single-sample or small-batch processes, providing infrastructure to efficiently scale a new paradigm of tailored and pipelined high-resolution imaging processes for next-generation large-scale research and industrial inspection [6].

References:

[1] Own *et al*, Microscopy & Microanalysis (2015), p. 63.

[2] Eberle *et al*, Journal of Microscopy **259** (2015), p.114.

[3] Webster, Bentley, Kearney, Microscopy and Analysis **29(3)** (2015), p.19.

[4] N/A, RMC QG-3100 Automated TEM Stainer, (Boeckeler Instruments, Inc., Tucson, AZ), p.1.

[5] Strader, Goodman, Microscopy and Microanalysis **21(S3)** (2015), p.11.

[6] This work was supported by Voxa, Allen Institute for Brain Sciences, and IARPA. Voxa innovations are patented and patent pending.

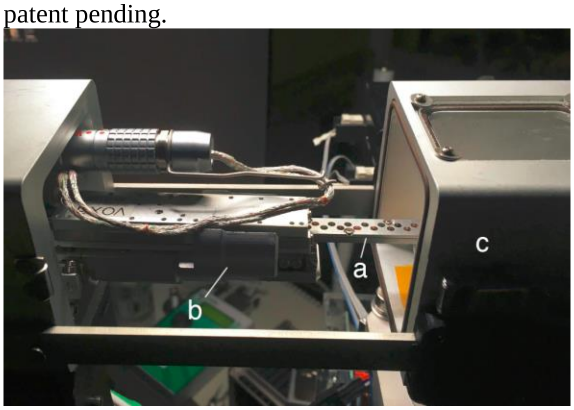

Figure 1. GridStage Sprite™ cartridge-based sample stage. Sample carrier cartridge (a) holds sixteen 3mm grids and is mounted onto (b) stage body comprising multi-axis positioners. Stage body is mounted into a load lock (c) that automatically inserts it into microscope to the right (not shown).

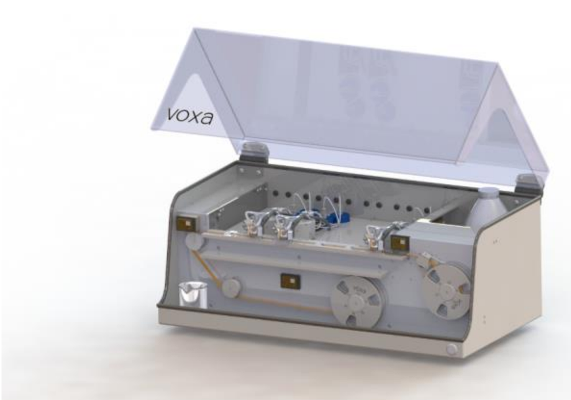

Figure 2. Strider™ tape conveyer TEM sample fluid handling system. Strider incorporates up to 6 active fluid heads to provide controlled fluid delivery, wash, and drying. An atmospherically controlled chamber provides versatile gas and fluid inlets to enable a variety of protocols to be scaled.

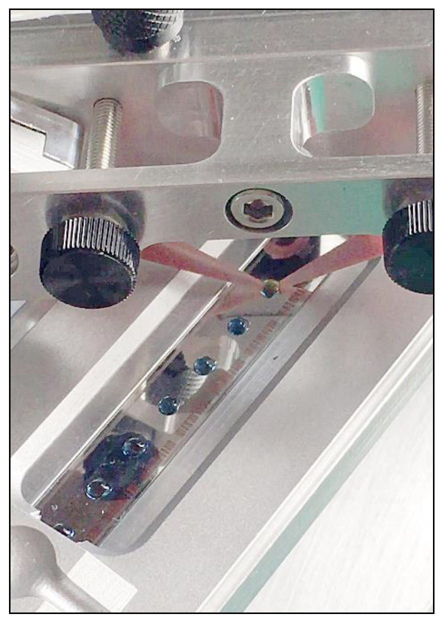

Figure 3. Strider™ fluid head applies measured fluid droplets directly to samples in succession. A subsequent fluid head washes this fluid droplet away, after which the sample air-dries and then respools onto a pickup reel.

https://doi.org/10.1017/S1431927617000848 Published online by Cambridge University Press