Microscopy and Microanalysis, **29** (Suppl 1), 2023, 1078–1079
https://doi.org/10.1093/micmic/ozad067.554

Microscopy AND Microanalysis

Proceedings

Large-Scale Electron Microscopy to Find Nanoscale Detail in Cancer

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Sadly, cancer will touch every person at some point in time. Unfortunately, this collection of diseases is still not understood enough to provide adequate treatment to all patients. Investigating the structure-function, a.k.a. genotype-phenotype, linkages within cancer cells and the tumor microenvironment is a critical undertaking involving many forms of imaging and computational data. To facilitate this effort, not only do microscopists need to collect large amounts of data, large teams of researchers are needed to provide a complete story of the disease. Here, we will describe the unique challenges and key advantages when trying to apply Scanning Electron Microscopy (SEM)-based imaging to aid in this effort.

The OHSU Serial Measurements of Molecular and Architectural Responses to Therapy (SMMART®) clinical trials aim to find more durable and tolerable treatments for metastatic cancer. Measurements from longitudinal biopsies include biomarker measurements, -omic profiling, and imaging data scaling from whole-body MRI to nanoscale SEM [1]. While not directly impacting clinical decisions, large-format and volume electron microscopy (vEM), including focused ion beam- and serial block face-SEM. has assisted in understanding crucial aspects of breast, pancreas, and prostate cancers, and has identified additional potential therapeutic targets. Next to biomarker monitoring, SEM can identify cellular perturbations with the earliest ultrastructural changes due to the high resolution available over the entire tissue landscape. This level of detail is not found with other imaging modalities and is an important complement to immunohistochemistry (IHC) and cyclic immunofluorescence (cycIF). Our work with the SMMART and Cancer Early Detection Advanced Research (CEDAR) programs has compiled the largest known repository of human cancer data using SEM. At time of submission, 285 samples have been collected and imaged, with at least 50 more expected this year. High-resolution montages at 4nm/pixel have been collected on every specimen, with vEM being collected on targeted areas to gain a deeper understanding of interesting ultrastructural features. All imaging was completed using readily-available standard microscope configurations: FEI/TFS Helios NanoLab™ G3 and UC5 Dualbeams, TFS Apreo Volumescope 2™, and Zeiss Gemini 2 Crossbeam 550. In order to preserve tissue quality, biopsies, and resections are placed into fixative in the operating suite within 5 minutes of collection [2]. Specimens are processed quickly and reproducibly using a Microscopy Innovations, LLC mPrep™ ASP-1000™ automatic specimen processor, enabling raw data generation to keep up with patient treatment schedules [3].

As one can imagine, dealing with this large amount of data can be tricky. Datasets range between 50–75 Gigabytes each, requiring large storage capacity and GPU capabilities. We recognize that imaging each sample at this depth may be considered excessive. However, human tissues are difficult to get and precious, so we collect at the highest resolutions possible, recognizing that down-sampling may be needed and in anticipation of better computational methods available in the future. Efficient and accurate deep learning models to enable automated annotation and segmentation of this data are ongoing development, and are a necessity in order to generate meaningful, clinically-impactful statistics from this large cohort [4–5]. Yet, ground truth must be generated first. Crowd-sourced annotation has been shown to be an effective method of ground truth generation, but takes too much time to be clinically relevant and requires extra quality control due to annotators' inexperience. In addition, HIPAA privacy concerns may not allow data to be shared publicly. For this work, a CloudFactory managed workforce is employed to ensure timely, high-quality, and secure manual annotation and segmentation [6].

Not only is the data large and unruly, but these large projects also require a different way of working than what most electron microscopists are used to. Data described above is also submitted as part of the National Cancer Institute's Human Tumor Atlas Network (HTAN), a national consortium part of the Cancer Moonshot Initiative [7]. These programs require working with large teams including clinicians, making de-identified data publicly available, managing large budgets, and fighting the "light microscopy and omics can do it all" mentality. Accurate metadata reporting is critical for reproducibility and clinical accountability as well as findable, accessible, interoperable, and reusable (FAIR) guiding principles [8–10]. Cross-consortium collaboration for SEM imaging of irreplaceable samples is difficult but can be successfully accomplished. An early-detection and early-stage cancer effort as part of OHSU's CEDAR program is currently being ramped up, and these large-scale considerations are being implemented as we progress. Whether internal or external, these efforts require specific infrastructure needs requiring interdisciplinary assistance. Implementation of something as simple as data access has proven to be time-consuming.

Going forward, this "large-scale microscopy" workflow will be needed to tackle not just cancer, but all diseases requiring a personalized-medicine approach. Additional correlative and vEM techniques, such as Array Tomography, will assist in this effort.

Of course, one cannot overlook the need for skilled human resources to assist in sample collection, imaging, and data post-processing [11].

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11. The authors acknowledge support from the OHSU SMMART Program, National Institutes of Health (NIH), National Cancer Institute (NCI) Human Tumor Atlas Network (HTAN) Research Center (U2CCA233280), the Prospect Creek Foundation, OHSU Brenden-Colson Center for Pancreatic Care, a NIH/NCI Cancer Systems Biology Consortium Center (U54CA209988), the OHSU Knight Cancer Institute-Cancer Early Detection Advanced Research (CEDAR) program, and the Knight Cancer Institute Cancer Center Support Grant (5P30CA69533). Electron microscopy was performed at the OHSU Multiscale Microscopy Core, an OHSU University Shared Resource.