doi:10.1017/S1431927617007231
Microsc. Microanal. 23 (Suppl 1), 2017

3D Virtual Histology and its Potential Contributions to Science

Keith C. Cheng[1]

[1.] Department of Pathology and Jake Gittlen Laboratories for Cancer Research, Penn State College of Medicine, Hershey, PA 17033; Penn State Consortium for Interdisciplinary Image Informatics and Visualization, USA.

> "… *die Zellen, die eigentlichen Herde des Lebens und demnach auch der Krankheit, die wahren Träger der lebendigen, pflanzlichen oder tierischen Funktion sind, an deren Existenz das Leben gebunden.*"
>
> "...cells, the fundamental units of life, and consequently, of disease, are true representations of plant and animal function, whose existence binds all of life. "
>
> -R. Virchow

Two of the most profound insights in the history of biology and medicine include the *cell theory* – all living things are made up of cells, and *cellular pathology* – insight into disease processes can be gained through the histological study of the cells of diseased tissues. Both insights were made possible in the 1800s by the development of the compound microscope, cellular stains, and tissue sectioning. Reaching "cellular" resolution was critical, which amounts to the ability to discern subcellular structures such as nuclei and vacuoles (reviewed by Cheng et al., 2016[1]), and requires pixels of about 1 micron in dimension. Histology is broadly used in biology for the detailed study of cells in the context of organismal tissue, and in medicine as the foundation for understanding disease and for tissue diagnosis. Here, we review the basis of this power, and then consider what will become possible as soon as we are able to accomplish a 3-dimensional "virtual histology" in which sectioning is done *in silico*.

Whence comes the power of histology? Histology allows us to visualize tissue architecture despite sample opacity and pigmentation. It provides breadth of scale, allowing us to examine millimeters to centimeters of tissue at cellular resolution (less than about 1 micron pixel resolution[1]). Its power is also dependent upon the staining of universal cellular components. Most commonly, nucleic acid is stained by hematoxylin and protein stained by eosin. The staining of all cell types has striking power in both biology and medicine. Histology has made us become aware of the similarities between the same types of tissue across phylogeny. For example, histology makes it instantly apparent that the muscle of an insect has the same basic structure as the muscle of a human. In the practice of medicine, histology allows the pathologist to recognize cellular patterns characteristic of disease across virtually all cell types, tissues, and multicellular organisms. Molecular probes such as antibodies, visualized using color, precipitate, fluorescence, and other means, provide molecular specificity when needed. Given this power, why is it that histology has yet to be fully tapped for the high-throughput phenotypic analysis of plants and animals?

A new bottleneck that needs to be addressed in our age of systems biology and facile genome editing and chemical screens, is the rate and depth of phenotypic analysis. Today we know that vertebrate genomes contain more than 20,000 genes and that tens of thousands of chemicals are commonly screened for candidate therapeutics, and/or found in our environment as potential toxicants; each can cause phenotypes across organ systems in multicellular organisms[1]. Due to limitations in organ-specific

expertise, it has become important for complete, raw data to be made available from large-scale projects. To study hundreds of thousands of samples across tens of thousands of variables, high-throughput methods are needed for phenotyping that is quantitative, reproducible, statistically significant, and amenable to asynchronous crowd-sourced analysis. In sum, histology has superior resolution, pan-cellular staining, scale, and the availability of molecular specificity, but is poorly suited for high-throughput, full volume tissue analysis. Histology's weaknesses include the analog nature of physical samples, tissue distortion, sampling error, sample destruction by sectioning, inability to section the same sample in multiple orientations, limitations of volumetric analysis, and poor insight into structures such as nerve tracts or vessels that cross multiple sections. The analog nature of histology makes it difficult to measure quantitative features of tissue such as cell number, shape, and cellular state. For example, histology cannot resolve whether a smaller nuclear profile is a portion of a nucleus or a smaller nucleus. Cells and structures such as vessels and nerve tracts larger than 5 microns can only be partially represented. Block face imaging of cut surfaces is one way of gaining 3-dimensional data at high resolution, and has been used in the mouse phenome project[2]. The voxels, however, may be non-isotropic. Resolution or field of view is usually limited, and throughput is limited by the need for physical sectioning. In consideration of the needs of modern phenomics[1] it would be optimal to have a method of imaging that retains histology's strengths and addresses its weaknesses, and has high-throughput potential.

Micron-scale tomography, or microCT, is based on the computational reconstruction of 3-dimensional structure from rotational x-ray projections. While most often used for the imaging of bone, soft tissue can be fixed and stained intact, potentially enabling histology at histological resolutions when optimized[3]. The resultant 3D data sets would allow the creation of full sets of virtual "sections" in any plane, at thicknesses that are any multiple of voxel resolution, and of animations of 3-dimensional structures of mm scale. With adequate resolution, accurate cell counts can be generated without histology's problems of object distortion caused by physical sectioning or handling artefact. We anticipate that optimization and automation of sample preparation, loading, imaging optics, accuracy of sample movement during imaging, image processing, and image analysis will increase throughput. File sizes for even mm scale samples are in the range of 100 Gb per file, too large for typical PCs. The computational power required to generate visualizations currently makes precomputation of cloud-based images necessary for broad data access. Logistical and administrative challenges suggest a need for increased access to synchrotron sources to customize imaging for biological samples in higher throughput. Soft tissue microCT seems to have brought us near the tipping point for high-throughput virtual 3D histology for phenomics. The breadth of problems addressed in genetic, chemical, and environmental phenomics suggests that such a new tool has the potential for powerful impact on society.

We thank the NIH and Penn State for the support of work towards these goals[4].

References:

[1] KC Cheng, et al. Advances in Genetics **95**, (2016), pp. 89-115.
[2] WJ Weninger, et al. Disease Models & Mechanisms, (2014), pp. 1143-1152.
[3] PJ La Riviere et al. in "Developments in X-ray Tomography VII, ed. SR Stock, Proc. of SPIE **7804** (2010), pp.1-8.
[4] The author is grateful for supported from NIH grants 2R24 RR017441 and 1R24OD18559 to KCC, and the Jake Gittlen Memorial Golf Tournament at the Penn State College of Medicine. The author also thanks members of our laboratory for discussion and editorial assistance.