Multiscale 3-D Imaging Techniques Based on Focused Electron Probes with Applications to Biological Systems

Leapman, R.[1], Aronova, M.[1], McBride, E.[1], Rao, A.[1], Zhang, G.[1], Guay, M.[1], He, Q.[1], Desai, R.[1], Tobin, M.[1], Sousa, A.[2], Prince, A.[3], Pokrovskaya, I.[3] and Storrie, B.[3]

[1] National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, United States, [2] Department of Biochemistry, Federal University of Sao Paulo, Brazil, [3] Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, United States

Microscopies based on focused electron probes allow the cell biologist to image the 3D ultrastructure of eukaryotic cells and tissues extending over large volumes, thus providing new insight into the relationship between cellular architecture and function of organelles. Here we consider two such techniques: electron tomography in conjunction with axial bright-field scanning transmission electron microscopy (BF-STEM) [1], and serial block face scanning electron microscopy (SBF-SEM) [2].

The advantages and limitations of each technique are illustrated by their application to determining the 3D ultrastructure of human blood platelets and other biological systems, in terms of specimen geometry and spatial resolution [3]. For example, many features of the complex membranes composing the platelet organelles can be determined from both approaches, although STEM tomography offers a higher ~ 3 nm isotropic pixel size, compared with ~5 nm for SBF-SEM in the plane of the block face and ~25 nm in the perpendicular direction. We demonstrate that STEM tomography is advantageous for visualizing the platelet canalicular system (CS), which consists of an interconnected network of narrow (~50 to 100 nm) membranous cisternae [4]. However, SBF-SEM enables visualization of complete platelets, each of which extends ~2 micrometers in the minimum dimension, whereas BF-STEM tomography can typically only image approximately half of the platelet volume due to a rapid non-linear loss of signal in specimens of thickness greater than ~1.5 micrometers.

Despite the difficulty in using SBF-SEM to visualize the small organelles of CS in blood platelets, the technique can still provide quantitative results by applying stereological methods to randomly selected slices through the 3-D data [3]. Currently, the performance of SBF-SEM is limited by radiation damage, which reduces the depth information perpendicular to the block face due to shrinkage of the embedding material. New approaches to 3D image segmentation based on deep learning techniques are likely to be available soon, which promise to extend greatly the usefulness of both methods [5, 6].

1. Hohmann-Marriott M. *et al.* Nat. Methods (2009) 6(10): 729-731.
2. Denk W. and Horstmann H. PLoS Biol. (2004) 2(11): e329.
3. McBride E.L. *et al.* J. Struct. Biol. (2018) (in press), https://doi.org/10.1016/j.jsb.2018.01.012
4. Pokrovskaya I. *et al.* J. Thromb. Haem. (2015) 14: 572-584.
5. Guay M. *et al.* Biophys. J. (2018) 114(3) Suppl. 1: 343a.
6. This work was supported by the intramural research program of NIBIB, NIH, Bethesda, Maryland. The research performed in the Storrie laboratory at the University of Arkansas for Medical Sciences in Little Rock, Arkansas was funded in part by NIH grant R01 HL119393.

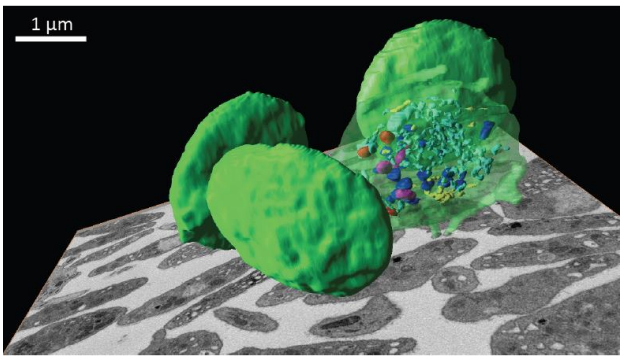

Fig. 1. Morphology of unactivated human blood platelets visualized by serial block-face scanning electron microscopy; transparent plasma membrane in one of the cells reveals internal structure of canalicular system (cyan and yellow), mitochondria (magenta), alpha granules (blue), and dense core vesicles (red).