doi:10.1017/S1431927622005967
Microsc. Microanal. 28 (Suppl 1), 2022
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# Comparison of Ultrastructure Determined by Serial Block Face SEM and Focused Ion Beam SEM from Blood Platelets and Thrombi

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With the emergence of 3D electron microscopy techniques of serial block-face scanning electron microscopy (SBF-SEM) [1,2] and focused ion beam scanning electron microscopy (FIB-SEM) [3,4] whereby backscattered electrons are collected from stained embedded biological specimens using 1-keV to 2-keV energy incident probes, it has become feasible to image cellular and tissue ultrastructure at a nanoscale. Each of these two approaches has advantages and limitations, which are illustrated here by an application in the field of hemostasis and thrombosis, where the techniques enable the investigation of blood platelet activation and clotting [5-8].

FIB-SEM provides 3D ultrastructure from volumes ranging in size from 1,000 to 100,000 cubic micrometers, revealing arrangements of alpha-granules and the canalicular system in complete human and mouse platelets at a near-isotropic spatial resolution of about 5 nm (Figure 1). In a mouse model, we have previously been able to visualize decondensed alpha-granules exhibiting fusion pores to the plasma membrane, which permits entry of water and solubilization of granule contents [7].

By using the SBF-SEM, it has been possible to image entire blood clots in a mouse model, where 3D ultrastructure can be obtained from volumes as large as 10 million cubic micrometers, albeit at lower *z*-resolution perpendicular to the block-face. Unexpectedly, we have found that mouse jugular vein puncture wounds result in thrombi that are structured about localized, nucleated platelet aggregates, pedestals and columns, producing a vaulted thrombus capped by extravascular platelet adherence [8]. Despite the lower *z*-resolution in SBF-SEM, the spatial resolution in the block-face *x,y*-plane is sufficient to characterize the platelet activation state based on subcellular ultrastructure (Figure 2).

We have found that the complementary techniques of SBF-SEM and FIB-SEM enable extraction of useful 3D ultrastructural information from blood platelets under different physiological and pathophysiological conditions, including platelets in the unactivated state, in early stages of activation, as well as in late stage activation in thrombi. While FIB-SEM provides details of membrane topology at a resolution of about 5 nm, the technique cannot currently be applied to thrombi, which extend over dimensions of several hundred micrometers, due to slow speed of FIB milling. Acquisition of 3D images is much faster in the SBF-SEM, but the resolution in the *z*-direction is limited to ~ 25 nm due to the minimum slice thickness of *in situ* ultramicrotomy. Importantly, the two techniques can be combined to enable analysis of hybrid regions of interest (ROIs), with the SBF-SEM being used to image the overall structure, and FIB-SEM being used to analyze smaller ROIs at higher spatial resolution in *x*,*y*, and *z*; for

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example, this would enable higher resolution visualization of organelles, such as dense-granules and alpha-granules within platelets contained in thrombi, to determine which type of granule secretes first. Together, the two approaches reveal a new picture of platelet and thrombus ultrastructure in health and disease [9].

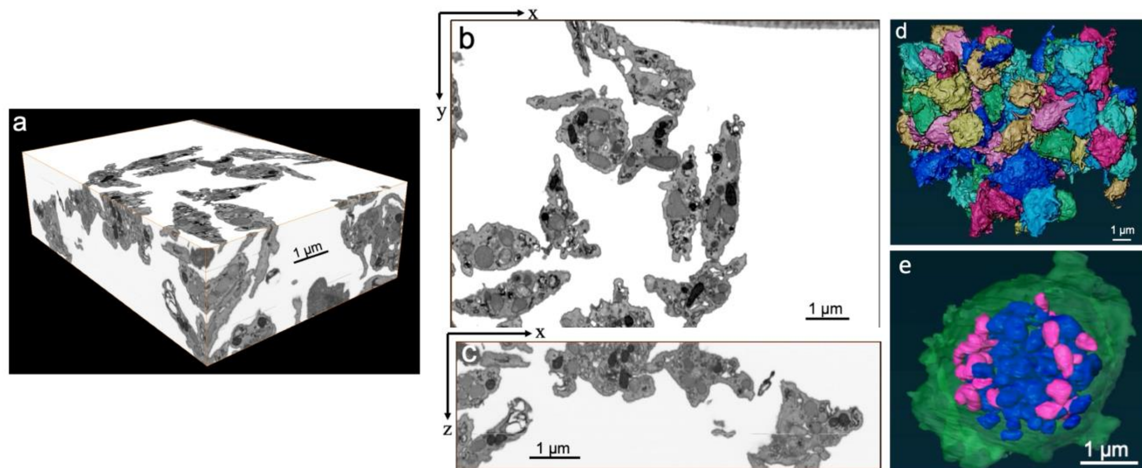


**Figure 1.** FIB-SEM data set from early activation of human blood platelets. (a) data cube showing several cells with a voxel size of 5 nm x 5 nm x 5 nm; (b) x-y slice; (c) x-z slice showing nearly isotropic resolution; (d) aggregate of platelets showing plasma membrane in different colors, and (e) cell at higher magnification in two orientations with alpha granules (blue) and mitochondria (purple).

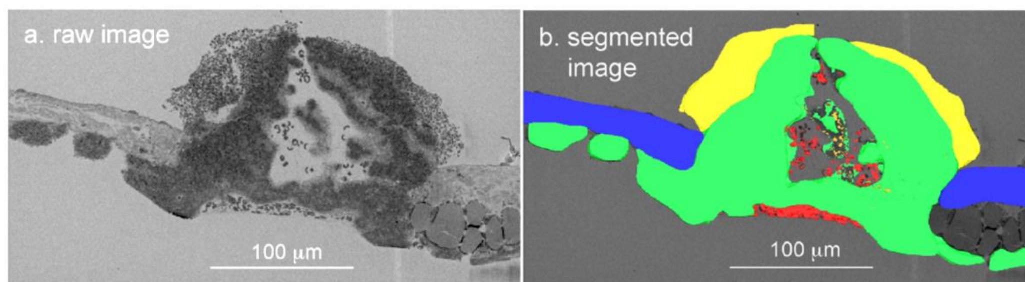


**Figure 2.** Overall 3D structure reveals limited loosely adherent platelet sheathing of 5 min post-puncture wound thrombi. (a) Backscattered electron image showing single slice from a 5-min post-puncture mouse jugular vein thrombus; (b) segmented slice with loosely adherent platelets (yellow), tightly adherent platelets (green), vessel wall (blue), RBCs (red) trapped intravascularly with a vault, and a small RBC patch on the extravascular surface.

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[9] This work was supported by the intramural research program of NIBIB, National Institutes of Health, and by NIH grant R01 HL-119393 and grant R01 HL-155519 to Dr. B. Storrie.

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