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Architecture of Pancreatic Islets Imaged by Serial Block Face SEM

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The serial block-face scanning electron microscope (SBF-SEM) comprises an automated ultramicrotome built into the specimen chamber, a high-brightness field-emission electron gun, and a high-efficiency backscattered electron detector, enabling high-resolution 3D imaging of heavy-atom stained and embedded tissues [1]. The technique provides ultrastructural images of tissue volumes as large as 10^7 μm^3, with a spatial resolution of 10-nm in the plane of the block face and ~25-nm in the cutting direction. The technique complements conventional transmission electron tomography and scanning transmission electron tomography [2].

We have applied SBF-SEM to image entire mouse islets of Langerhans, which are microscopic endocrine organs distributed throughout the pancreas whose main function is to regulate blood glucose levels. Our data provide quantitative information about the distributions of glucagon-secreting alpha cells and insulin-secreting beta cells, as well as the nuclear, mitochondrial and total cell volumes, and the numbers of secretory granules in each cell type (Fig. 1) [3].

By segmenting randomly selected areas, we have determined the cellular volume fraction of the dense cores composed of crystalline insulin in beta cell secretory granules, as illustrated in Fig. 2, from which it was found that 13.2% ±2.7% (std. dev.) of the granule-rich image areas consisted of dense-cores. By considering the fraction of the beta cell volume excluded by the nucleus and mitochondria and the dry density of crystalline insulin, it was estimated that beta cells contained 0.045 g ± 0.010 g (std. dev.) of insulin per gram of cells [4]. Thus, for a typical beta cell volume of 930 μm^3, we can estimate that each cell contains approximately 42 pg of insulin, which is consistent with biochemical measurements reported in the literature.

We have also determined the organization of the microvasculature including the blood vessels and surrounding pericapillary space, which accounted for 9.0 ± 0.3% of the islet's volume. Each beta cell was found to be in contact with the pericapillary space, with a mean contact area 9 ± 5% of the cell's surface area. The role of the pericapillary space as a zone of mediation between blood and endocrine tissue cells fulfills the islet's crucial need for efficient perfusion [5].

References
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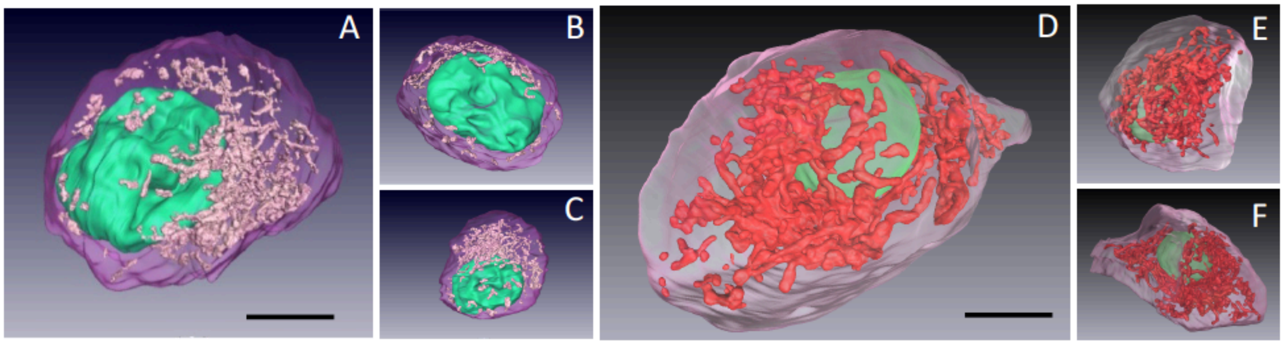

Fig. 1. Full 3-D surface rendering of typical alpha and beta cells: (A–C) Different views of alpha cell displaying plasma membrane (purple), nucleus (green), and mitochondria (pink). This cell had total volume of 570±14 μm^3 and nuclear volume of 126±3 μm^3. Bar = 5 μm. (D–F) Different views of beta cell displaying plasma membrane (pink), nucleus (green), and mitochondria (red). This cell had total volume of 1,010±30 μm^3 and nuclear volume of 115±3 μm^3. Bar = 5 μm. Adapted from reference [3].

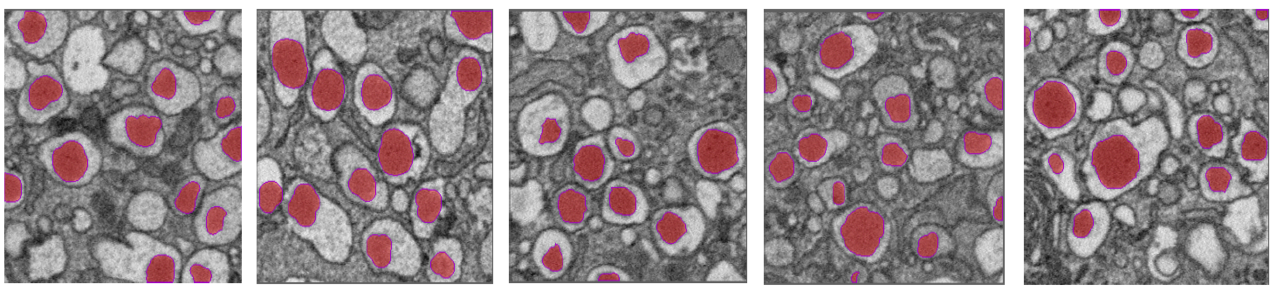

Fig. 2. Determination of total volume of secretory granule dense-cores in beta cell. Sub-images of size 1.5 μm x 1.5 μm within beta cell are manually segmented. Adapted from reference [4].

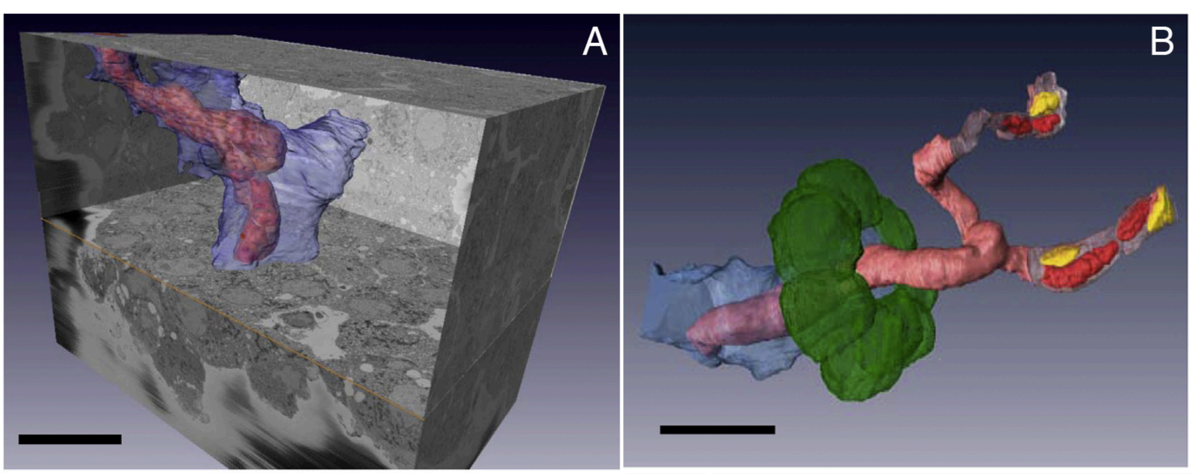

Fig. 3. Islet's microvasculature: (A) rendered blood vessel (pink) and surrounding pericapillary space (blue). Bar = 20 μm; (B) Different layers of blood vessel are shown including representative ring of beta cells (green) surrounding pericapillary space (blue), endothelial-cell lining of capillary (pink), erythrocytes (red) and endothelial nuclei (yellow) inside capillary. Bar = 20 μm. Adapted from reference [3].