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Parsing the Relationship Between Sample Preparation and SBF-SEM Performance

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Serial block-face scanning electron microscopy via the Gatan 3View system allows for the generation of image volumes by means of serial electron micrographs. Samples that are traditionally processed for thin-sectioning and imaging with transmission electron microscopy are viable candidates for SBF-SEM. When planning an SBF-SEM imaging experiment there is the need to account for how different samples and their preparation methods affect the achievable imaging parameters of the resulting dataset. Defining the image parameters - the pixel size and slice thickness of the dataset – poses a challenge as each sample is different in its electron dose sensitivity. As a research driven Microscopy Laboratory, we work with myriad sample types from our broad cell biology research community. There are myriad sample varieties but they typically fall into one of three categories which we investigate in this experiment: *in vitro* cultured cell pellets, en-face embedded cell monolayers and animal tissue. For the purposes of this experiment each sample type is imaged with varying beam accelerations, beam apertures and beam dwell times to examine sample sensitivity to electron dose. Pixel size and cut thickness are constant, resulting in final voxel dimensions of 4nm x 4nm x 50nm which we deem sufficient for reconstruction of most cellular ultrastructure. The performance is assessed by determining whether the sample was sectioned successfully by the ultramicrotome. A successfully imaged sample should exhibit no cutting artifacts from the diamond knife nor image distortion from electron buildup in the sample resin or tissue. Accumulated electrons distort the imaged face of the sample block, which reduces the efficacy of the microtome. This typically causes incomplete cuts of the imaged area of the block-face that are visible as a banding or patchy appearance in the scanned image. A sample with more electron charge tolerance is more able to ground any accumulated electrons and exhibit fewer charging artifacts. Datasets with successful imaging conditions should be sufficient for later semi-automated segmentation of cellular ultrastructure. We find that the sample types have varying ratios of embedding resin to tissue which affects the sensitivity to electron dose. Imaging conditions can be adjusted per sample type to overcome dose sensitivity while avoiding compromises to pixel size or slice thickness.

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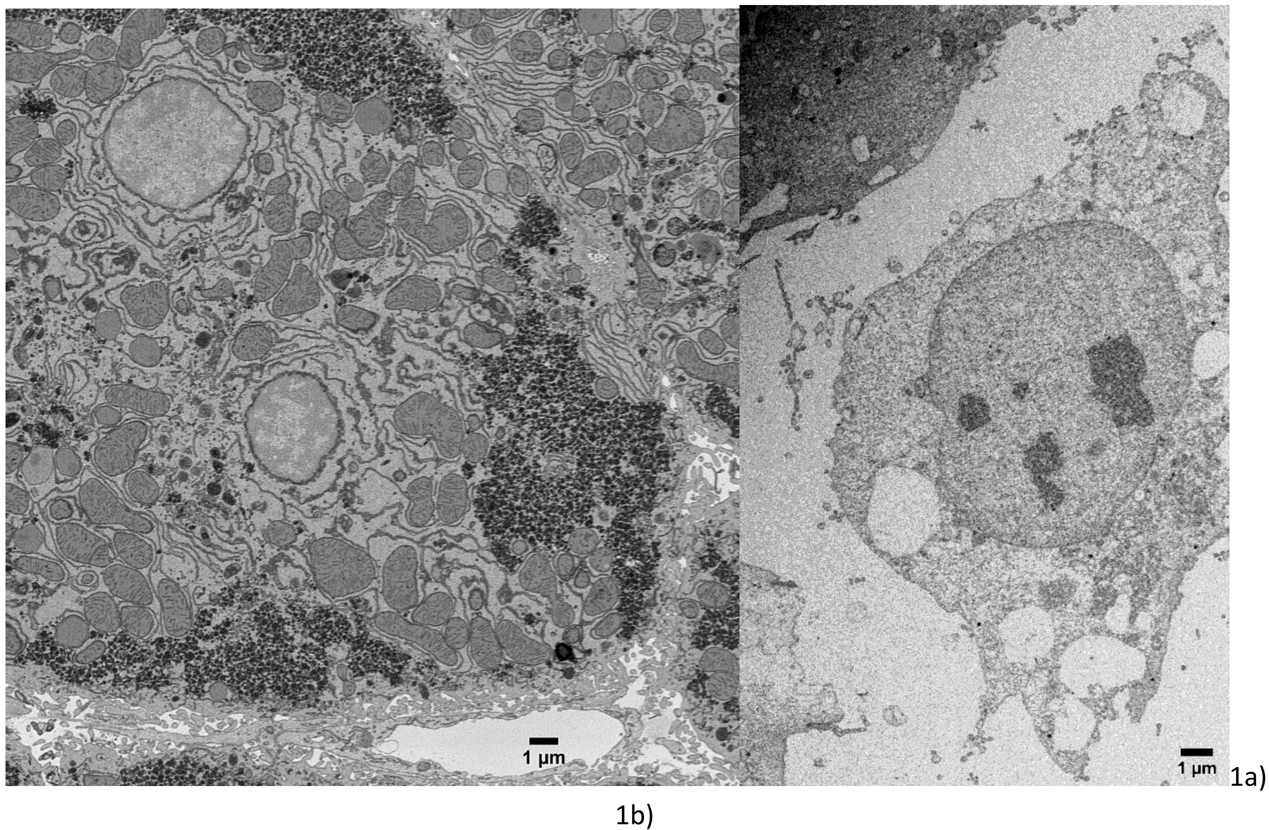

Figure 1 1a shows a single scan from a liver tissue block that has been processed for SBF-SEM. The sample consists mainly of tissue with very few empty fields of embedding resin. This represents the type of sample that is most resistant to charge and can more easily be sectioned and imaged for higher resolution datasets. Figure 1b shows a single scan from an en-face embedded cell monolayer that has been processed for SBF-SEM. Depending on the confluence of the cell layer, the sample can consist mostly of resin. This would represent a sample that is difficult to image at the same resolution and slice thickness without making considerations to image parameters and reducing the amount of resin when mounting the sample for imaging.

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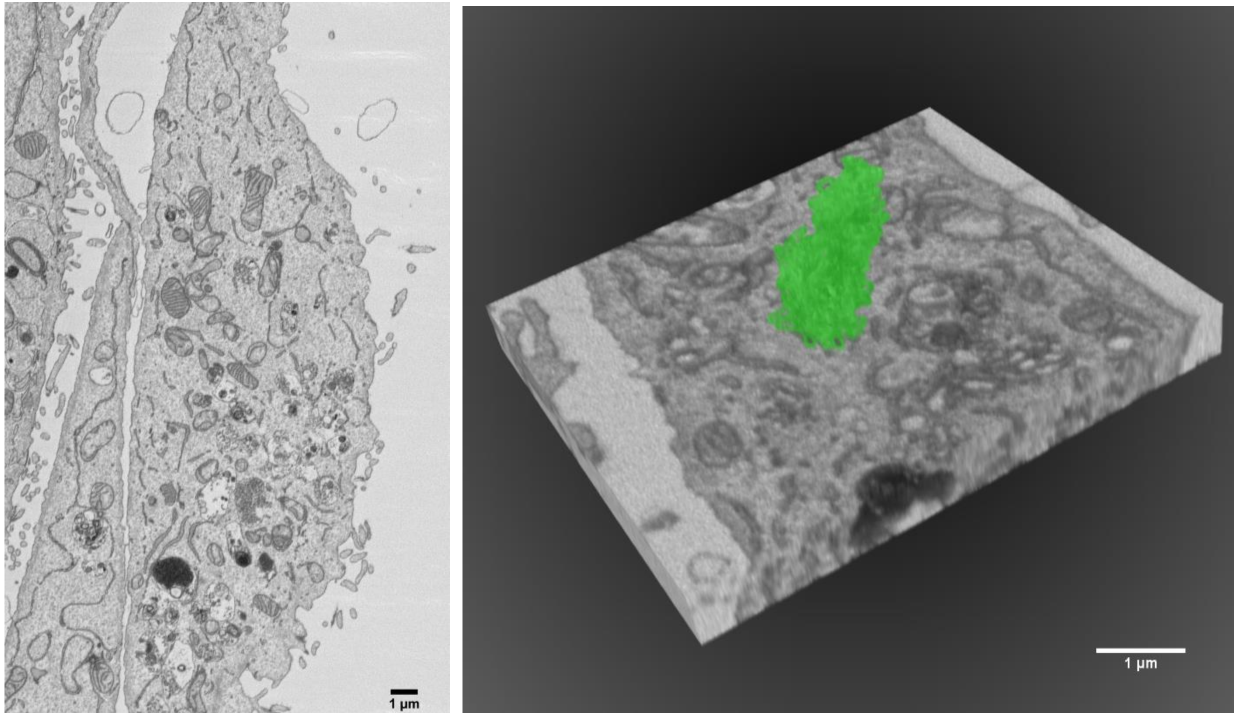

2a) 2b)

Figure 2 2a is a single scan from a cell pellet processed and embedded for SBF-SEM. The structure of a a cell pellet can be described as porous, where there are interconnected touching cells that provide a path to ground for accumulated electrons. However this is still susceptible to charging, and considerations should be made to reduce as much resin as possible. Figure 2a shows a 3D reconstruction of segmented golgi body in green, with the volume of the dataset partially cut away to reveal the highlighted shape of the organelle. This is a classic application of SBF-SEM, but it is only achievable with datasets that have minimal ultramicrotome cutting artifacts introduced by electron charge.

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