

## DeepSerialBlockFace: Machine denoising and object segmentation for volume electron microscopy

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Volume electron microscopy (EM) requires the acquisition of three dimensional (3D) data sets with either a transmission electron microscope (TEM) or scanning electron microscope (SEM). Serial block-face (SBF) SEM, focused ion beam (FIB) SEM, and TEM tomography each take different approaches for 3D volumetric EM. However, to extract scientific measurements, all approaches rely on image processing, object segmentation, and volumetric quantification. These image analysis steps are often the rate limiting step of 3D volumetric EM. Significant advances have been made by applying machine learning, and a prime example is the development of the Ilastik open-source tool kit that uses interactive machine learning and random forest classifiers (1). More recently, deep machine learning has been applied for both image processing (2) and image segmentation(3).

We describe a generalized workflow for applying machine learning to denoising and segmentation of SBF-SEM datasets. The approach is based on U-Net (Ronnenberger et al., 2015), which is a deep convolutional neural network architecture that has been applied to a variety of different image analysis tasks. The advantage of U-Net is that it can be trained with a low number of ground truth images, making it a powerful approach for biological datasets in which obtaining ground truth data can be challenging. Our workflow can be easily adapted for a wide variety of biological samples that is typical of an SBF-SEM in a core facility, and it generally consists of two separate U-Nets, one for denoising the data, and the other for object segmentation.

Our workflow implements a U-Net to first denoise the SBF-SEM dataset. SBF-SEM images are backscattered images with high noise. To generate ground truth data for denoising, we take high and low noise image pairs using the same parameters used to acquire the SBF-SEM data set with the only difference: the dwell time of the scan. The low noise images are used as ground truth and the training set is augmented by adding random noise, blurring, rotation, mirroring, and scaling. Training samples are divided into image patches to improve results near image boundaries and to allow for larger training batch sizes. For segmentation, only patches that contain a foreground class above a specific area threshold are used in order to maintain foreground/background class balance when foreground objects are sparse. Adding these low-high imaging pairs to the workflow is relatively simple.

To use U-Net to segment biological structures from SBF-SEM datasets, a subset of one dataset is manually segmented in Seg3D software (freely distributable software that is easy to train novice researchers how to perform manual segmentation). We utilize an iterative training approach where the trained U-Net is first used to predict an initial segmentation of the entire SBF-SEM image stack. We manually correct the worst instances of predicted SBF segmentations, add them back into the original training set, and repeat the training process until the desired segmentation results are obtained. Typically, less than 1% of the SBF-SEM dataset is manually segmented and corrected. Once segmented into objects, various metrics can be obtained, such as: length, volume, shape, displacements, relative distances to other objects, touching different objects, etc. We present the products of our deep learning workflow applied to various collagen-containing samples, and discuss the efficiency and accuracy of this technique in determining the 3D organization of collagen networks.

## References

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