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Supervised Texture-Based Classification for 3DEM

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ABSTRACT

Dense 3DEM data recovered by Serial Block Face Imaging (SBFI), Serial Section Transmission Electron Microscopy (ssTEM), and Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) present challenging image segmentation needs. Typically a biologist wants to isolate membranes, organelles, extracellular matrix components, etc. Traditional image processing may struggle to faithfully follow such features. The variance in electron density from blocks of tissue stained with heavy metals provides ample signal for machine learning methods, though. Here, we demonstrate supervised image classification based on image texture as implemented in Amira Software.

INTRODUCTION

Supervised learning in image processing typically requires providing training data from manual segmentation or automated methods. The software then trains a classifier using two statistical categories: features based on co-occurrence matrices[2] and features based on intensity statistics. Features used by the co-occurrence matrix are delimited into textons, or small units of texture[2,5]. The co-occurrence matrix accounts for pairs of pixel values separated by an offset (either directional or rotation invariant). Conversely, intensity statistics are based on the grayscale intensity in the form of first order (mean, variance, etc.) or histogram (quantiles, entropy, etc.) statistics. By providing a quick method for feature rejection and allow accounting for uncertainty in ambiguous regions, one generates a labeled dataset. This could be the result, or input for additional image processing.

MATERIALS AND METHODS

We tested our implementation on a 10 Gb serial block face imaging (SBFI) mouse heart tissue block collected using an Apreo VolumeScope SEM. The 16-bit TIFF data were deconvolved from the BSE input, downsampled to 8-bit, aligned, and cropped. When followed by a small amount of image processing, the texture-supervised classification was able to fully label this dataset with less than 30 minutes of user effort labeling only one layer. We used Thermo Scientific™ Amira™ Software for EM Systems 2019.2 for all image processing of the data.

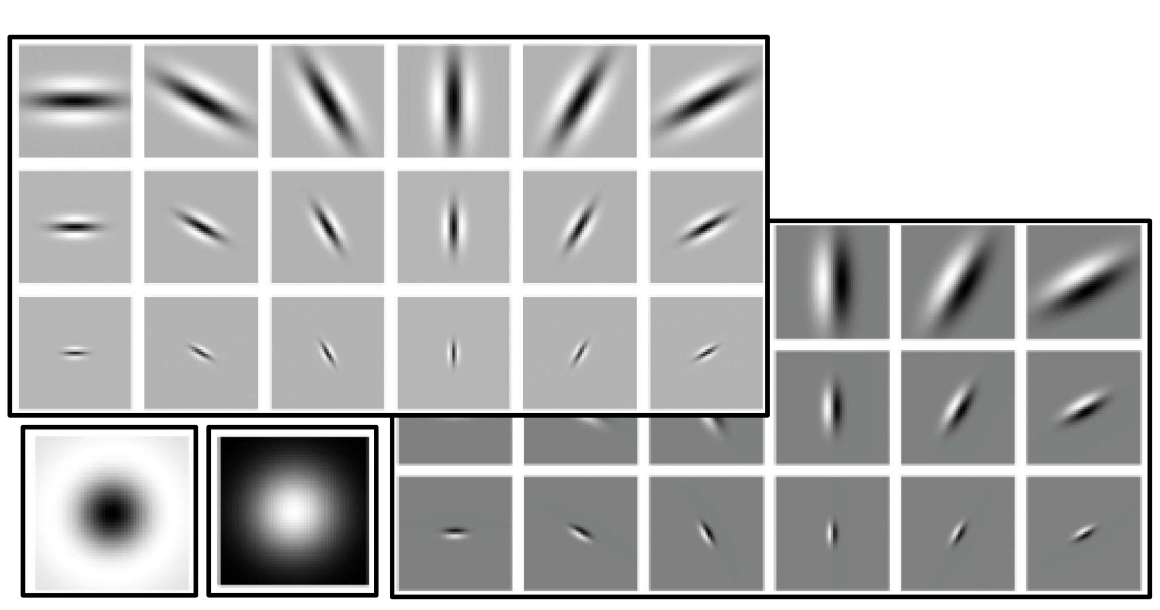

Figure 1 – Textons and Filter Bank

Examples of textons used in a filter bank for image processing (here, the Statistical_MR8 filter bank). Adjusting the size and rotating linear features increases the feature set that can be matched. Adapted from (1,4,5).

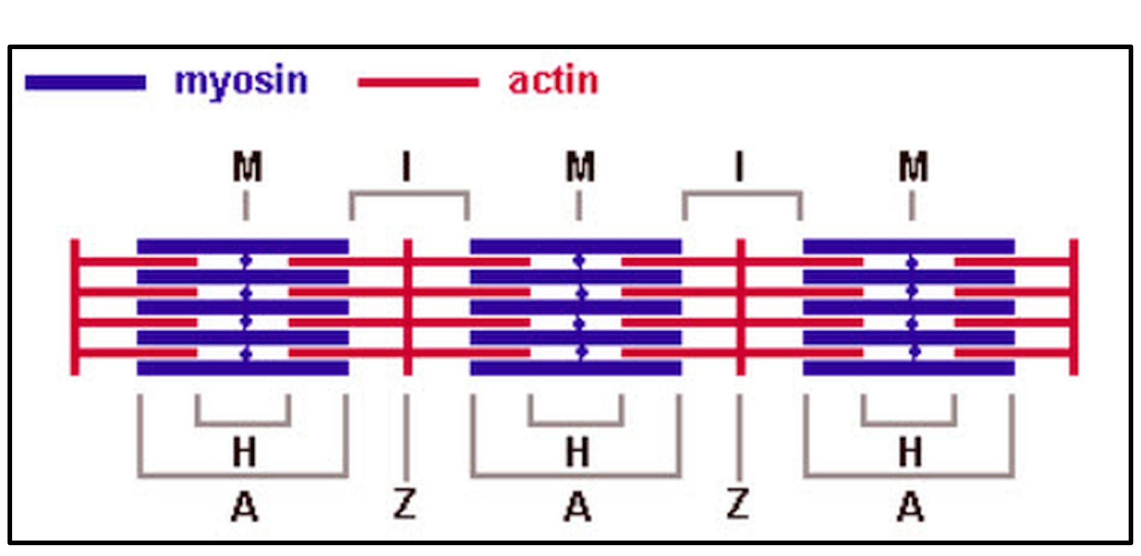

Figure 2 – Sarcomere Overview

M = Myosin, I = I Band, H = H Band, A = A Band, Z = Z Line. Provided by (6).

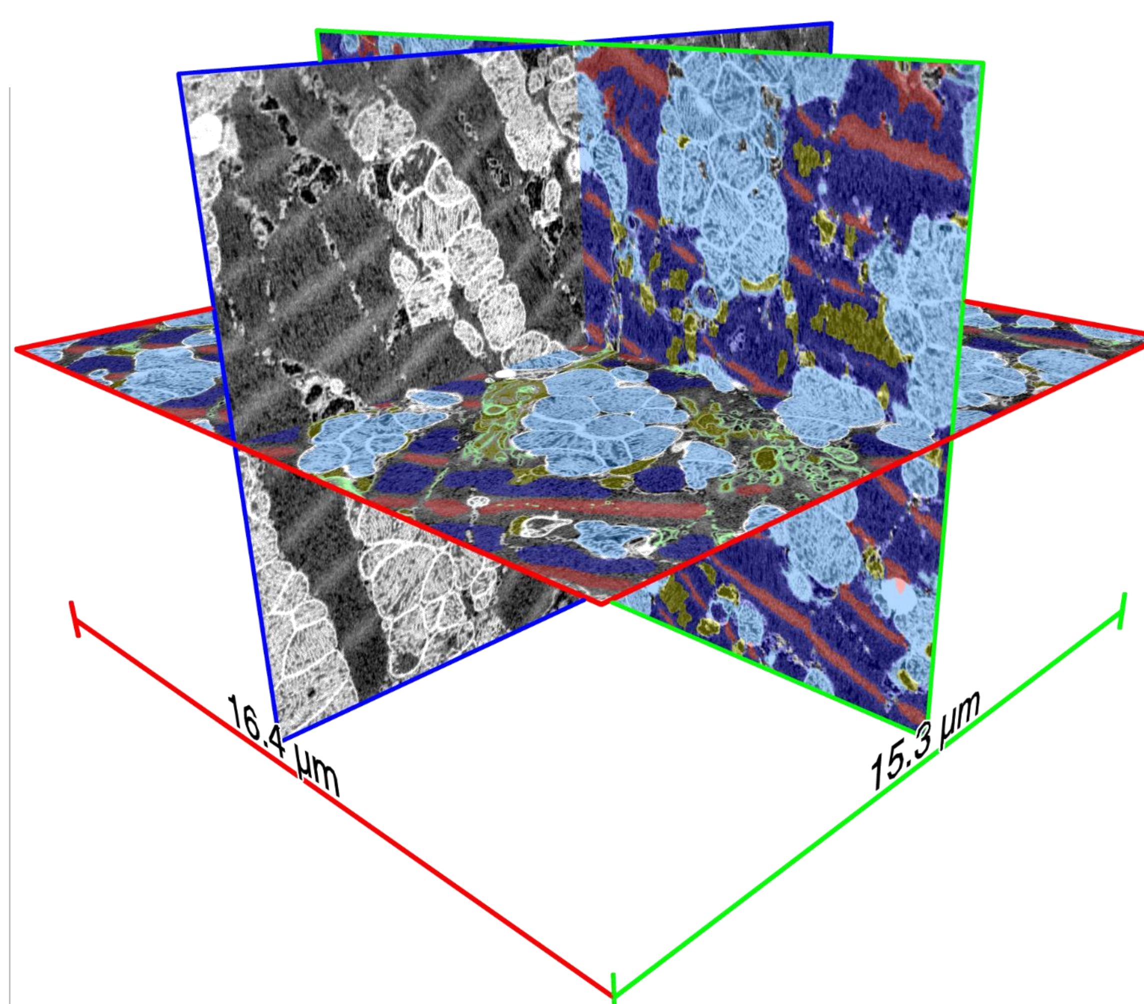

Figure 3 – 3DEM Data Segmented by Texture Classification

Serial Block Face (SBFI) SEM of mouse heart tissue. Original data are in YZ (Blue outline), single slice of training input are in XY (red outline), and fully labeled data are in XZ (green outline). Mitochondria are labeled in light blue, myosin in red, actin in dark blue, and a combination of sarcoplasmeric reticulum and t tubules in yellow.

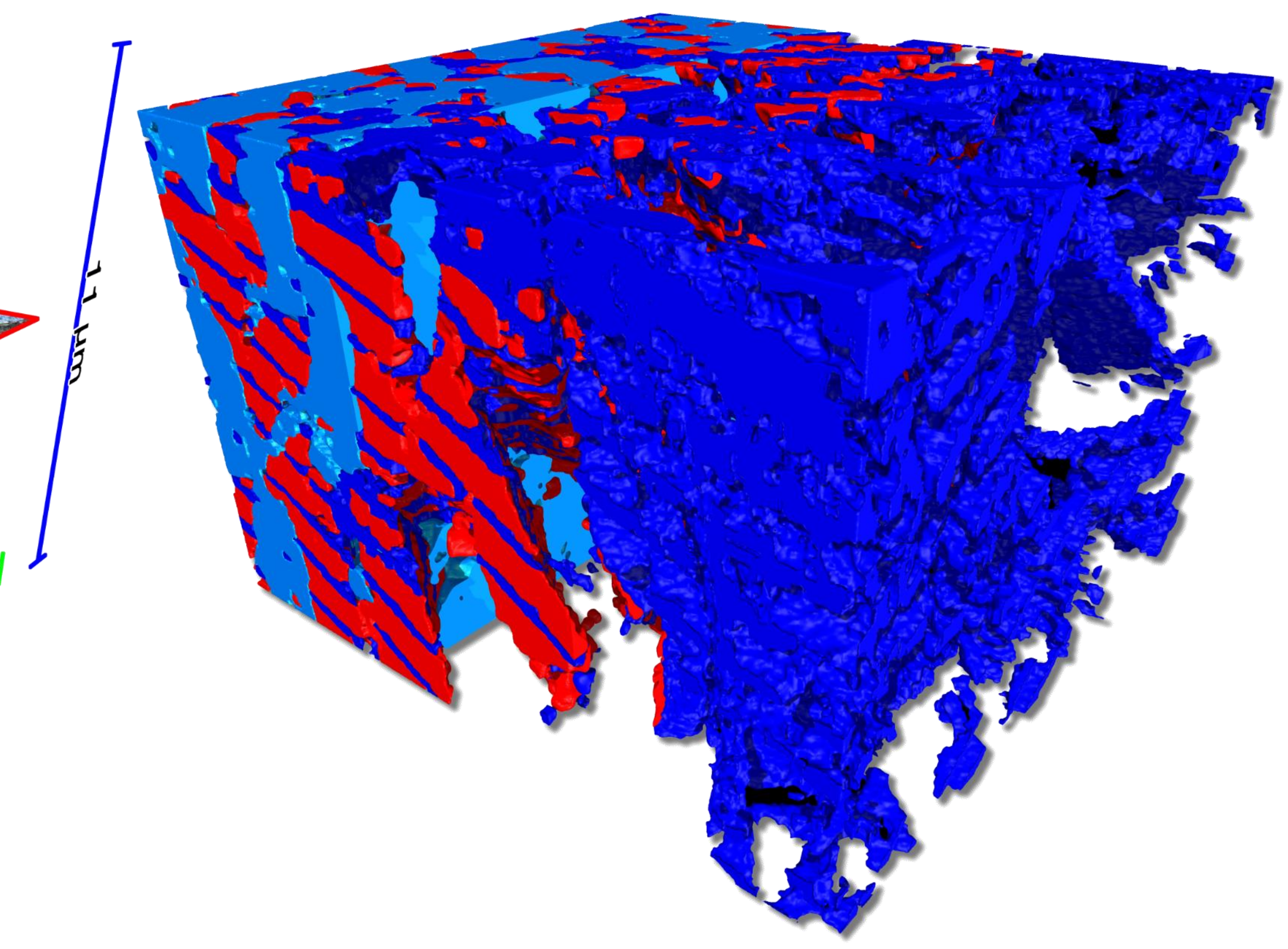

Figure 4 – Fully Labeled 1102-Slice 3DEM Dataset

The entire 3DEM dataset was labeled using a combination of supervised classification followed by morphological image processing. This figure combines surfaces generated from three of the tissue's largest features by volume: mitochondria are labeled in light blue, myosin in red, and actin in dark blue. The mitochondria and myosin have been clipped to reveal further ultrastructure.

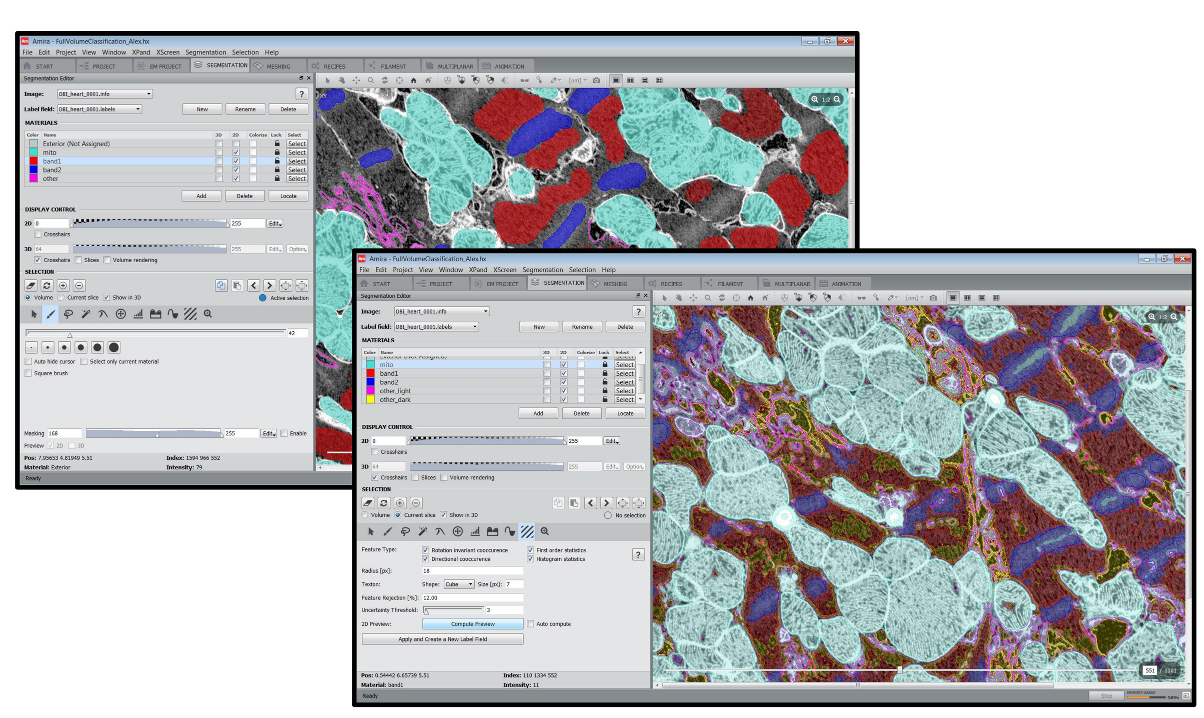

Figure 5 – Manually Segmenting Training Data with a Preview of Texture-Based Classification

Using Amira, we labeled five materials (four shown in these images). We used the brush tool and brush tool with masking. Segmentation was performed with a mouse and on only one slice of data for <0.1% of total data coverage in reference to the complete 3DEM dataset. In this interface, one can view a preview of segmentation via texture-based classification on one XY slice of data. Here, we can see that this combination of parameters nicely isolates the mitochondria, actin, and myosin. The sarcoplasmeric reticulum, t tubules, and gap junction structures (in pink) are still ambiguous, likely due to their overlapping parameter space on this slice of data.

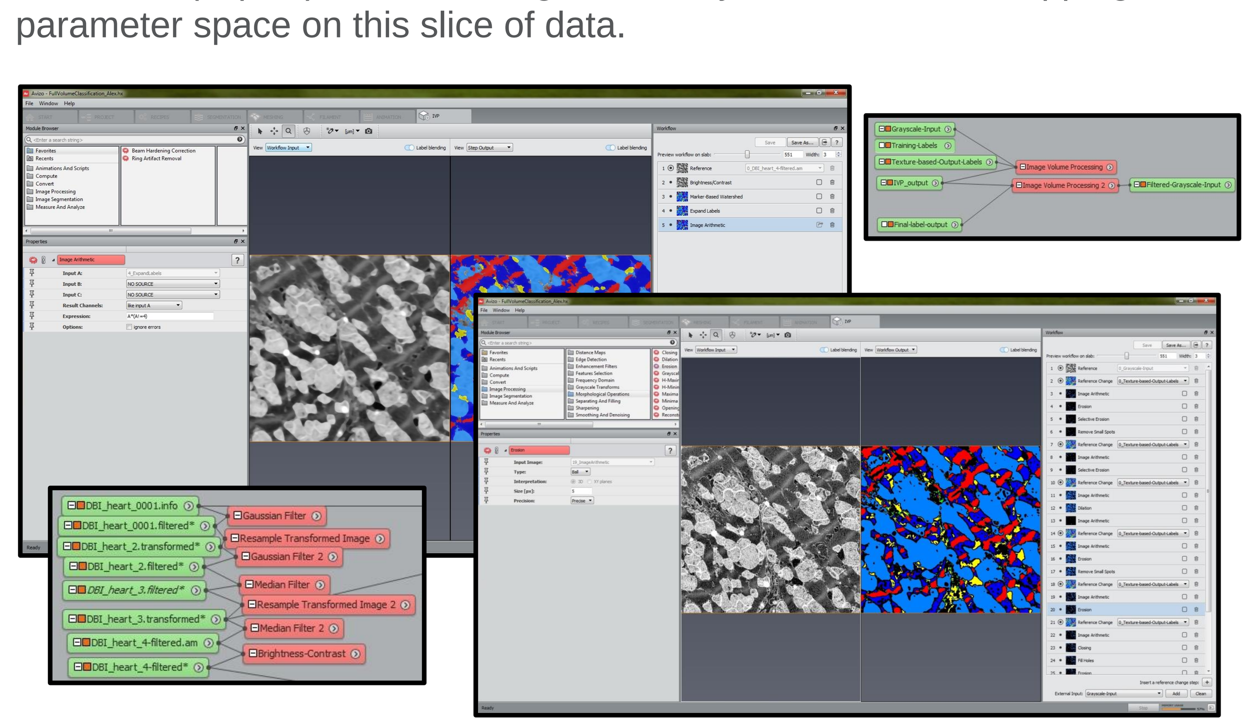

Figure 6 – Morphological Image Processing Improved Label Accuracy and Precision

Since the texture-based classification carries no knowledge of the underlying biology or features of interest, it is often necessary to adjust the raw labeled output with morphological image processing. Typically, this involves eroding small labels and sharp edges followed by marker-based watershed. This can fill in ambiguous or missing unlabeled areas as long as the image gradient contains information about the edges of the features of interest. Since the Z-bands and myosin have a gradual transition, additional steps were used in Amira to distinguish these labels in particular.

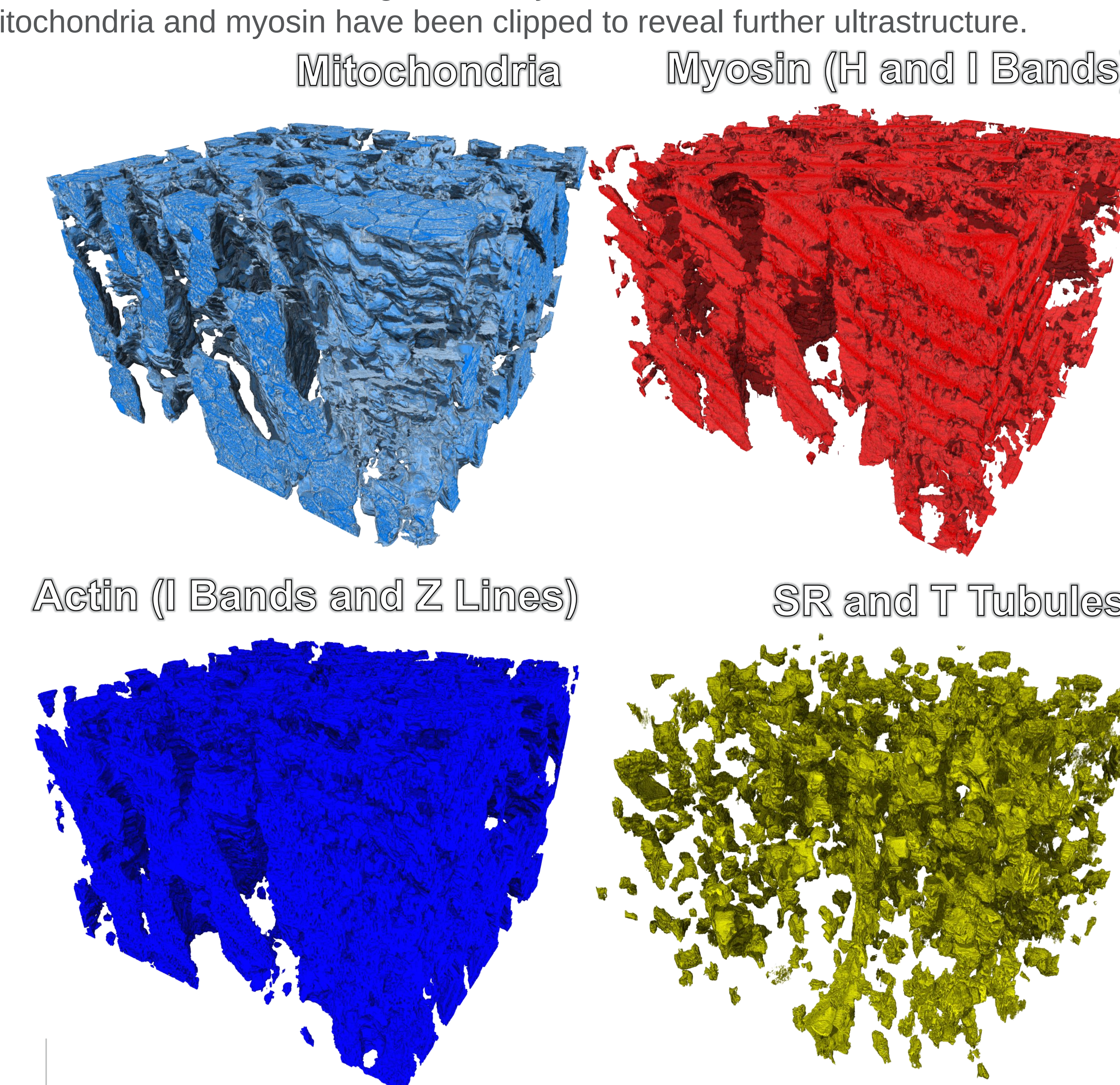

Figure 8 – Individual Components of Heart Muscle Tissue

Voxelized rendering of each label from the workflow described in this study. SR = Sarcoplasmeric Reticulum

CONCLUSION

When ample texture is available in a dense dataset, texture-based classification offers a compelling alternative to traditional morphological image processing for segmentation. By expanding Amira's automated segmentation capabilities to include texture-based classification, we aim to reduce segmentation efforts for orthogonal image data such as 3DEM.

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