**Guide to Image Processing and Analysis of 3D Micro-computed Tomography Images using Fiji ImageJ (plugins Trainable WEKA Segmentation & MorphoLibJ)**

Getting Started:

Follow this link to download Fiji ImageJ: (https://imagej.net/software/fiji/downloads)

Trainable WEKA Segmentation usually comes along with the default installation of Fiji ImageJ.

To install MorpholibJ:
Help → Update → Click on "Manage Update Site" → Select IJPB-plugin → Apply and Close → Apply Changes → Restart Fiji ImageJ

Processing of images involves three (3) major steps which include: Stacking, Segmentation and Labelling

**a. Stacking of two-dimensional high-resolution images into three-dimensional images.**

- Open ImageJ and import your images (File > Import > Image Sequence). The number of images and types to be imported can be determined here.
- Convert the image into the desired type. (Image → Type → 8-bit).
- Select ROI (region of interest). For example, if you are interested in a cylindrical column, choose the Oval tool in the toolbar and manually draw the circle. Also, can do Edit → Selection → Specify if you know the exact dimensions of your ROI. Once it is selected, do Image → Crop and then Edit → Clear Outside.
- Save stacked images (File → Save As → Tiff)

**b. Segmentation (Binarization)**

- Select Plugin → Segmentation → Trainable WEKA Segmentation 3D (or Trainable WEKA Segmentation for single image).
- You can label each of the phases (eg. pore and grain) - In the segmentation window choose Settings. Name the phases (e.g., "grain" and "pore"). As for training features, you may want to play around with them to determine which is most effective for your dataset. I have found Gaussian Blur to work well, with the default minimum and maximum sigma values.
- Now, use the freehand selection tool and/or the freehand line tool to manually "draw in" a few grains and pores (or whatever your phases are). Add the selection to its respective phase when you are done with each one. Try to capture the full range of grey values that are present in each phase. Zoom in if necessary to be as precise as possible. When done, click "Train Classifier". The algorithm will try to separate the image volume into distinct

phases based on what you've given it. If you are not satisfied, simply keep manually adding selections to the phases, or remove previous selections if you think they were no good. Choose "Train classifier" when you want to try again. Once you are satisfied with the binarization, choose "Create result".

- Classifiers can be saved and applied to similar images when segmenting a large volume of images. To save the classifier (Click "Save classifier" on the TWS interface after training the dataset.
- To apply saved classifier (Plugin → Segmentation → Trainable WEKA Segmentation 3D (or Trainable WEKA Segmentation for single image) → Load Classifier → Apply Classifier → Select the image to be classified
- Once you have a binary image volume, do Image → Type → 8-bit to make it greyscale
- Do Image → Adjust → Threshold and adjust the threshold bars to your liking (e.g. the grain voxels white (255) and the pore voxels black (0)). Important: do not click "Apply". Simply close the Threshold window. Then, do Process → Binary → Make Binary and make sure to deselect "Calculate threshold for each image" before you hit OK.
- At this point, you can determine the ratio of the two phases (i.e. the porosity) if desired. Make sure you have the ROI selected. Instead of grey values of 0 and 255, you will need values of 0 and 1. Do Process → Math → Divide and enter 255. Now do Analyze → Histogram, which will tell you the mean grey value of all the images. This mean, or 1 - mean, is the porosity (depending on if the pore space is ones or zeros).
- If your binary image volume is in 0s and 1s, convert back to 0s and 255s: do Process → Math → Multiply and enter 255.

**c. Labeling of grains**

- Labeling of the grains is done using the MorphoLibJ plugin. Now, a watershed segmentation will separate and label each grain. Do Plugins → MorphoLibJ → Binary Images → Distance Transform Watershed 3D. Again, you may need to play around with the settings to see what gives the best result. The following parameters work well: Distance map = Borgefors, 16bit output, dynamic = 10 (high dynamic value closes the grains), connectivity = 6.

- At this point, you are done with segmentation, and can now extract measurements of the particles. If desired, MorphoLibJ can provide some measurements (e.g., grain size, bounding box, sphericity): do Plugins → MorphoLibJ → Analyze → Analyze Regions 3D. Otherwise, save the segmentation for use in different software.
- Saved labeled image is further analyzed using the "Sand Analysis" program.