Appendix A – Computational code for image skeletonization in ImageJ/Fiji

```
print("Select the folder containing images with cancellous bone...");
// ask user to select a folder
dir = getDirectory("Select A folder");
// get the list of files (& folders) in it
fileList = getFileList(dir);
// prepare a folder to output the images
output_dir = dir + File.separator + " output" + File.separator ;
File.makeDirectory(output_dir);

//activate batch mode
setBatchMode(true);

// LOOP to process the list of files
for (i = 0; i < lengthOf(fileList); i++) {
        // define the "path"
        // by concatenation of dir and the i element of the array fileList
        current_imagePath = dir+fileList[i];
        // check that the currentFile is not a directory
        if (!File.isDirectory(current_imagePath)){

                // open the image and split
                open(current_imagePath);
                title = getTitle();

                // Convert to 8-bit
                 run("8-bit");

                // Enhance Contrast with normalization and equalization
                run("Enhance Contrast", "saturated=0.35 normalize equalize");

                // Apply Maximum filter (adjust parameters as needed)
                run("Maximum...", "radius=2");

                // Make Binary
                run("Make Binary");

                // Remove Outliers with a radius of 15 (adjust as needed)
                run("Remove Outliers...", "radius=15 threshold=50 which=Dark");

                // Despeckle to remove dark outliers
                run("Despeckle");

                // Fill Holes
                run("Fill Holes");

                // Apply Median filter with a radius of 11 (adjust as needed)
                run("Median...", "radius=11");
```

```
                // Skeletonize
                run("Skeletonize");

                run("Convert to Mask");

                run("Analyse Skeleton", "prune=shortest branch");

                run("Fractal Box Count...", "box=2,3,4,6,8,12,16,32,64");

// Print the fractal dimension

                selectImage(1);

                title = getTitle();
                len = lengthOf(title);

                output_title = substring(title, lengthOf(title) + '(H&E)');

                print("Image " + output_title + " done!");

                saveAs("tiff", output_dir + output_title);

                run("Close All");
        }
}
print("Images with blue trabeculae saved in: " + dir + "output_blue")
setBatchMode(false);
```