**Note.** This code is designed for analysis of 10x spleen H&E sections on the Windows 10 operating system, using(Fiji Is Just) ImageJ 2.3.0, Java 1.8.0\_172 [64-bit], R version 4.2.2, and RStudio version 2023.03.0. Troubleshooting and reformatting may be required if this code is used with different tissues, operating systems, or versions of any of the associated programs. R “cleanup” code requires installation of the tidyverse package.

**Folder Setup and Image Naming.** 10x images of spleen sections to be analyzed must be the only files in the folder upon initiation of the ImageJ batch program. The image file names must end with “\_O” or the ImageJ macro will not analyze them (this can be changed in line 14). R code assumes all files are named “Spleen (number) 10x (number)\_O”, if this is not the case, changes will need to be made in line 30 to properly extract the sample numbers.

**ImageJ Batch Analysis.** Open the ImageJ macro file (IJM) using (Fiji Is Just) ImageJ and click “run”. A pop-up will allow you to select the folder containing the images you want to analyze. ImageJ will then cycle through the images in the folder collecting the measurements and saving the masks for white pulp (WP), acellular area (AC), and faded eosin score (FE). When the script is finished it will save a .csv file named “HE\_Results\_RAW” containing the measured values. If you change the name of this output file, you will also need to change the R “cleanup” code on line 11. While not required, you should manually save the “Log” window data as a text file, as it will print calculated measurement values for each image, which may be useful.

**Cleaning Up the ImageJ Output Using R.** Once you have the output file from the ImageJ macro, copy and paste the RStudio “cleanup” file into the folder with the images. R will re-format the output, calculate the area ratios, average the replicate images, and save a “Happy\_Data” .csv file. You can graph this output directly in RStudio, or transfer the output to another program.