

Supplementary 4. ImageJ macro for quantifying COL22A1 staining

```
macro "3 level Dab" {
  dir1 = getDirectory("Input Folder images");
  dir2 = getDirectory("Choose a folder to save to");
  list = getFileList(dir1);
  setBatchMode(true);

  for (i=0; i<list.length; i++) {
    showProgress(i+1, list.length);
    filename = dir1 + list[i];
    open(filename);
    Imagename = File.nameWithoutExtension;

//Area_1: Strong intensity (3+)
    Name=getTitle();
    run("8-bit");
    run("Duplicate...", "title=1st");
    rename(Name+"-1st");
    run("Threshold...");
    setThreshold(0, 40); // start at zero, the upper boundary can be changed
    setOption("BlackBackground", false);
    run("Convert to Mask");
    run("Analyze Particles...", "size=5-Infinity show=Masks summarize");

//Area_2: Moderate intensity (2+)
    selectWindow(Name);
    run("Duplicate...", "title=2nd");
    rename(Name+"-2nd");
    run("Threshold...");
    setThreshold(41, 80); // these values can be changed in conjunction with the 2 above
    and 2 below
    setOption("BlackBackground", false);
    run("Convert to Mask");
    run("Analyze Particles...", " show=Masks summarize");

//Area_3: Weak intensity (1+)
    selectWindow(Name);
    run("Duplicate...", "title=3rd");
    rename(Name+"-3rd");
    run("Threshold...");
    setThreshold(81, 120);
    setOption("BlackBackground", false);
    run("Convert to Mask");
    run("Analyze Particles...", " show=Masks summarize");

//Area_4: Whole tissue region
    selectWindow(Name);
    run("Duplicate...", "title=4th");
    rename(Name+"-4th");
    run("Threshold...");
    setThreshold(0, 240); // add
```

```
        setOption("BlackBackground", false);
        run("Convert to Mask");
        run("Analyze Particles...", "  show=Masks summarize");
        saveAs("jpg", dir2+Imagename+"-WholeArea");
        run("Merge Channels...", "c1=[Mask of "+Name+"-1st] c2=[Mask of "+Name+"-3rd]
        c7=[Mask of "+Name+"-2nd] create");
        saveAs("Tif", dir2+Imagename+"-3DABs");
        run("Close All");
    }
    selectWindow("Summary");
    saveAs("Results", dir2+"Summary.csv");
    exit("DAB measured in "+i+" images");
}
```