

Supplementary Code 1: The script used for the analysis of Fig. 5b, d and Supplementary Fig. 12b. Please use the image of KI-stained pollen observed under a microscope as input to extract pollen regions and quantify their brightness.

```
/*
 * An imagej (fiji Version:2.0.0-rc-59/1.51n) script to quantify the degree of pollen
 staining.
 * 2020/10/06
 * Yosuke Toda.
 * ITbM, Nagoya Univ.
 * tyosuke@aquaseerser.com
 * https://totti0223.github.io/
 * https://www.phytometrics.jp/
 */

//GUI
#@ File (label = "Input directory", style = "directory") input

//Clear the Results Window and ROI info if present prior to analysis.
if (nResults > 0)
{
    run("Clear Results");
}

if (roiManager("count") > 0)
{
    roiManager("Deselect");
    roiManager("Delete");
}

//Create a subfolder named "outputs" in the same directory where the files to be analyzed
are present.
output = input + File.separator+ "outputs";
File.makeDirectory(output);

//Main function
```

```
processFolder(input);
```

```
//Save csv to output directory using the Results window
```

```
selectWindow("Results");
```

```
saveAs("Measurements", output + File.separator + "Results.csv");
```

```
close("");
```

```
//Scan image files and parse by processFile function
```

```
function processFolder(input) {
```

```
    list = getFileList(input);
```

```
    list = Array.sort(list);
```

```
    for (i = 0; i < list.length; i++) {
```

```
        s = list[i];
```

```
        dot = indexOf(s, ".");
```

```
        if (dot >= 0)
```

```
            s = substring(s, dot, lengthOf(list[i]));
```

```
        else
```

```
            s = 0;
```

```
        print(s);
```

```
        if (indexOf("..jpeg.jpg.JPEG.JPG.tiff.TIFF",s) > 0)
```

```
            processFile(input, output, list[i]);
```

```
    }
```

```
}
```

```
//Image analysis module
```

```
function processFile(input, output, file) {
```

```
    //Delete all roi first that are from the n-1 image. do not delete results table.
```

```
    if (roiManager("count") > 0)
```

```
    {
```

```
        roiManager("Deselect");
```

```
        roiManager("Delete");
```

```
    }
```

```

//open image
input_path = input + File.separator + file;
print("Processing: " + input_path);
open(input_path);
original=getTitle();

//get mask preprocessing
run("Duplicate...", " ");
mask=getTitle();
run("Gaussian Blur...", "sigma=2");
run("8-bit");
run("Subtract Background...", "rolling=40 light sliding");
run("Auto Threshold", "method=Default");

//depending on the image appearance, the below watershed process can be
ignored by commenting out.
//run("Watershed");

//get candidate region
run("Analyze Particles...", "size=0-Infinity circularity=0.80-1.00 show=Overlay
add");
roiManager("Set Color", "red");
roiManager("Set Line Width", 1);
run("Labels...", "color=blue font=12 show draw");

//save mask image
selectWindow(mask);
run("Hide Overlay");
maskpath = output + File.separator + file + "_1.jpg";
saveAs("Jpeg", maskpath);
close();

//get the b of hsb(hsv) for the degree of pollen staining.
selectWindow(original);

```

```
run("Show Overlay");
run("Duplicate...", " ");
hsb = getTitle();
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue");
close();
selectWindow("Saturation");
close();
selectWindow("Brightness");
brightness = getTitle();
run("Invert");
run("Show Overlay");
```

```
//append to results table with image file name column and pollen region id.
```

```
if (roiManager("count") > 0)
```

```
{
```

```
    prevn = nResults;
```

```
    roiManager("Measure");
```

```
    print(prevn,nResults);
```

```
    print("____");
```

```
    //add filename to result table
```

```
    //pollen id per image
```

```
    k=0;
```

```
    for (i = prevn; i < nResults; i++)
```

```
    {
```

```
        setResult("filename", i, file);
```

```
        setResult("pollen_id", i, k);
```

```
        k+=1;
```

```
    }
```

```
}
```

```
selectWindow(brightness);
```

```
close();
```

```
selectWindow(original);
```

```
run("Show Overlay");  
run("Flatten");  
annpath = output + File.separator + file + "_2.jpg";  
saveAs("Jpeg", annpath);  
close();  
selectWindow(original);  
close();  
}
```