Structured illumination microscopy and automatized image processing as a rapid diagnostic tool for podocyte effacement

Supplemental Information

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Running title: A rapid tool to diagnose MCD

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PAS staining

For PAS staining, the slides were incubated for 15 minutes in 2% sodium metabisulfite, washed in aqua dest followed by 15 min incubation in 1% periodic acid. After rinsing in aqua dest, the slides were incubated for 25 minutes in Schiff reagent, rinsed in tap water and aqua dest. For nuclear counterstaining, the slides were incubated for 15 minutes in hematoxylin, rinsed in tap water followed by an ascending ethanol series, clearing in xylene and mounting in Eukitt (Carl Roth, Karlsruhe, Germany).

Construction of the FIJI macro

The source code of the macro was constructed as follows:

```
run("Grays");
run("Set Scale...", "distance=31.0154 known=1 pixel=1 unit=micron global");
//run("Brightness/Contrast...");
setMinAndMax(0, 4500);
run("Copy");


dir = getDirectory("Image");
  name=getTitle;
  path=dir+name;


      getDateAndTime(year, month, dayOfWeek, dayOfMonth, hour, minute, second,
msec);
     TimeString ="";
     if (hour<10) {TimeString = TimeString+"0";}
     TimeString = TimeString+hour+"_";
     if (minute<10) {TimeString = TimeString+"0";}
     TimeString = TimeString+minute+"_";
     if (second<10) {TimeString = TimeString+"0";}
     TimeString = TimeString+second;
     measurementID = TimeString;
 name = getTitle;
           dotIndex = indexOf(name, ".");
           title = substring(name, 0, dotIndex);
```

```
newImage("name", "8-bit black", 2430, 2430, 1);
run("Paste");
run("Measure");
run("Ridge Detection", "line_width=3.5 high_contrast=230 low_contrast=87
correct_position add_to_manager method_for_overlap_resolution=NONE
sigma=1.51 lower_threshold=3.06 upper_threshold=7.99");
waitForUser("Check for correct Ridge Detection");
saveAs("Jpeg", title + measurementID);
roiManager("Measure");

   name = title + measurementID + ".xls";
   saveAs("Measurements", dir+name);

String.copyResults();
IJ.deleteRows(0, 3000);
roiManager("Delete");
run("Close");
run("Close");
```

The results and a picture of the completed SD detection are automatically saved to the source directory using the same title of the source file accompanied by the system clock time. In the Excel file, cell B2 contains the capillary area (A) and column H (Sum(H:H)) contains the total length of the SD (l_{SD}). Therefore l_{SD}/A can be calculated in Excel as: l_{SD}/A =Sum(H:H)/B2.