**Analysis of the confocal microscopy picture using the MATLAB script (above):**

1.        The first thing to do is to open each picture in Image J and split the channels, save the green channel (FMA) in grayscale mods a PNG file.

2.        Make a central folder (call it MATLAB) to import into MATLAB with the following files:  the script called “QuantUnsort”, area.csv file, perimeter.csv file, and all the pictures you are going to analyze (all these files are available as online supplements).

[the “QuantUnsort“script will give you data for all measured vessels i.e. including large arteries and veins to get only capillary data use the “QuantFinalR“ script (online supplements) instead of the “QuantUnsort” script, this will exclude all areas smaller than 4.9µm2 and larger than 100µm2.

3.        Open MATLAB

4.        Import the folder into MATLAB.  You might be prompted to set this folder as the MATLAB “path”.  Say yes to this.

5.        Type “QuantUnsort” into the command window.

6.        A window will pop up and ask you which folder you want to open.  Click on the MATLAB folder and press enter.  The MATLAB folder may also be pre-selected.  If this is the case, just press “open”.

7.        Another window will pop up and ask you to choose a file.  This will be asking you which image you want to analyze.  If you can’t see your file in the window, make sure the file type is set to “All Files”.  A common problem is that by default MATLAB will only look for matlab files and will not “see” any .png files.  Chose your image and press enter.  The script will run and analyze it.

8.        A series of 4 images will pop up.  The first is the original image, the second is the same image but with different colors assigned to different capillaries.  These are there to make sure that the right image was analyzed and to track progress.  The next two images are histograms of the area data and perimeter data for the analyzed image.  By this point, the data has been exported to the respective AreaQuant1.csv or PerimeterQuant1.csv files.

9.        Open up Excel and open the AreaQuant1.csv and/or PerimeterQuant1.csv file.  All of the measurements should be in one column.  The area is in µm2 and the perimeter is in µm.  Copy and paste this into another excel file where you can compile your data.  After you do that, make sure you close the area.csv or perimter.csv files or else when you analyze the next image, MATLAB won’t be able to export the new data to these files.

10.     Return to matlab and type “clc” then press enter into the command window.  This resets the program and allows you to analyze another image.  Make sure you do this after each picture!

11.     Re-do steps 1-9 for the rest of the images.

Hier das script für MATLAB:

**MATLAB script for the automated high-throughput analysis of the microvasculature:**

1.) This script generates all raw data of capillary area (in µm2) and perimeter (in µm) without any cut off value (cut off values can be chosen within the excel sheet or by analyzing the pictures with script #2:

folder\_name = uigetdir; %Prompts user to select folder

filename = uigetfile; %Prompts user to select file to be analyzed

uiimport = (filename); %Imports selected file name

I = imread(filename); %Reads imported file

background = imopen(I,strel('disk', 15)); %Standardizes background and threshold

figure, surf(double(background(1:8:end,1:8:end))),zlim([0 255]);

set(gca,'ydir','reverse');

I2 = I - background; %Removes excess noise

imshow(I2);

level = graythresh(I2);

bw = im2bw(I2, level);

bw = bwareaopen(bw,50); %States capillary area

cc = bwconncomp(bw,4);

cc.NumObjects;

labeled = labelmatrix(cc);

whos labeled;

RGB\_label = label2rgb(labeled, @spring, 'c', 'shuffle'); %colors individual capillaries

figure, imshow(RGB\_label);

capillarydata = regionprops(cc,'all'); %reads all perimeter data of the capillaries

capillary\_peri = [capillarydata.Perimeter];

capillary\_area = [capillarydata.Area];

[min\_perim, idx] = min(capillary\_peri);

capillary = false(size(bw));

capillary(cc.PixelIdxList{idx}) = true;

%Converts perimeter data to micrometers

PDataInMicrons =capillary\_peri\*0.30120';

%Insert conversion factor here in microns per pixel

%Converts Area data to Micrometers

ADataInMicrons =capillary\_area\*0.0907';

%Insert conversion factor here in microns-squared per pixel-squared

nbins = 50;

figure, hist(ADataInMicrons, nbins) %Generates capillary Area histogram

title('Histogram of Capillary Area Data')

figure, hist(PDataInMicrons, nbins) %Generates capillary Perimeter histogram

title('Histogram of Capillary Perimeter Data')

SA = ADataInMicrons';

SP = PDataInMicrons';

csvwrite('AreaQuant1.csv', SA) %Writes data to area excel sheet

csvwrite('PerimQuant1.csv', SP) %Writes data to perimeter excel sheet

B) This script generates data of capillary area (in µm2) and perimeter (in µm) with a cut off value of individual capillary area (>4.9µm2 and < 100µm2) to automatically exclude arterioles, arteries, veins, venules and glomerular capillary convolutes:

folder\_name = uigetdir; %Prompts user to select folder

filename = uigetfile; %Prompts user to select file to be analyzed

uiimport = (filename); %Imports selected file name

I = imread(filename); %Reads imported file

background = imopen(I,strel('disk', 15)); %Standardizes background and threshold

figure, surf(double(background(1:8:end,1:8:end))),zlim([0 255]);

set(gca,'ydir','reverse');

I2 = I - background; %Removes excess noise

imshow(I2);

level = graythresh(I2);

bw = im2bw(I2, level);

bw = bwareaopen(bw,50); %States capillary area

cc = bwconncomp(bw,4);

cc.NumObjects;

labeled = labelmatrix(cc);

whos labeled;

RGB\_label = label2rgb(labeled, @spring, 'c', 'shuffle'); %colors individual capillaries

figure, imshow(RGB\_label);

capillarydata = regionprops(cc,'all'); %reads all perimeter data of the capillaries

capillary\_peri = [capillarydata.Perimeter];

capillary\_area = [capillarydata.Area];

[min\_perim, idx] = min(capillary\_peri);

capillary = false(size(bw));

capillary(cc.PixelIdxList{idx}) = true;

%Converts perimeter data to micrometers

PDataInMicrons =capillary\_peri\*0.30120';

%Converts Area data to Micrometers

ADataInMicrons =capillary\_area\*0.0907';

%counter variable

n = 1;

%number of entries in the data set you are looking for

arraysz = length(ADataInMicrons);

%while loop: look at each point, it it is greater than 10, put in into new vector

while n < (arraysz+1)

 if ADataInMicrons(n) > 4.9

 if ADataInMicrons(n) < 100

   Data1Sorted(n) = ADataInMicrons(n);

 end

 end

  n = n + 1;

end

%reset your counter

n = 1;

nbins = 50;

figure, hist(Data1Sorted, nbins) %Generates capillary Area histogram

title('Histogram of Capillary Area Data')

figure, hist(PDataInMicrons, nbins) %Generates capillary Perimeter histogram

title('Histogram of Capillary Perimeter Data')

SA = Data1Sorted';

SP = PDataInMicrons';

csvwrite('AreaQuant1.csv', SA) %Writes data to area excel sheet

csvwrite('PerimQuant1.csv', SP) %Writes data to perimeter excel sheet