MATLAB Code for Assessment of Retinal Vessel Tortuosity in FUNDUS/OPTOS Images

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Summary

This manual provides description on how to use MATLAB tool for vessel tortuosity calculation in retinal fundus or Optos images. The analysis is performed in a circumpapillary region centered on the optic nerve head (ONH). The segmentation/cropping section of the software need to be modified to analyze other retinal regions. The main code is retinal_tortuosity_analysis.m which load the images (one per iteration), segment the vessels and calculates tortuosity for the vessel segments based on user choice of vessel endpoints. Tortuosity measurements are based on **vessel centerline**. Default vessel segmentation setting is tested for OPTOS images acquired by 200TX, California and Daytona machines. Vessel segmentation parameters can be easily adjusted for other fundus or optos images. Also, If the user wants to analysis images acquired by other machines, centerline smoothing parameter may need to be adjusted which is crucial for meaningful tortuosity analysis (see **Appendix II**).
A brief description of tortuosity measures and the procedure for visualizing the parameters that have been extracted from each centerline is provided in the **Appendix I** at the end of the current document.
If you are using this tool or any of the dependencies, please cite the following publications.

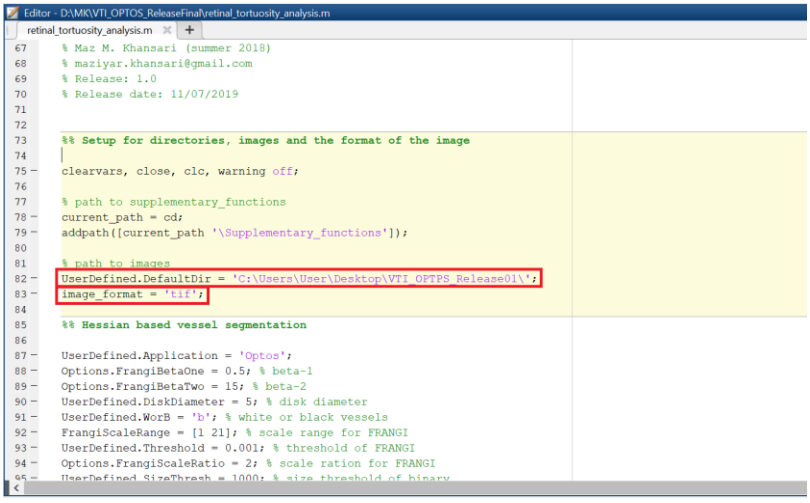
- ***Khansari, Maziyar M., William O'Neill, Jennifer Lim, and Mahnaz Shahidi. "Method for quantitative assessment of retinal vessel tortuosity in optical coherence tomography angiography applied to sickle cell retinopathy." Biomedical optics express 8, no. 8 (2017): 3796-3806.***
- ***Khansari, et al. "Relationship between retinal vessel tortuosity and oxygenation in sickle cell retinopathy" IJRV (Springer Nature), DOI: 10.1186/s40942-019-0198-3***

Dependencies

All the dependencies (MATLAB functions) of the current algorithm are available under *supplementary_functions* folder located in the main directory. This folder will be automatically added to the current MATLAB path.

Preparing data

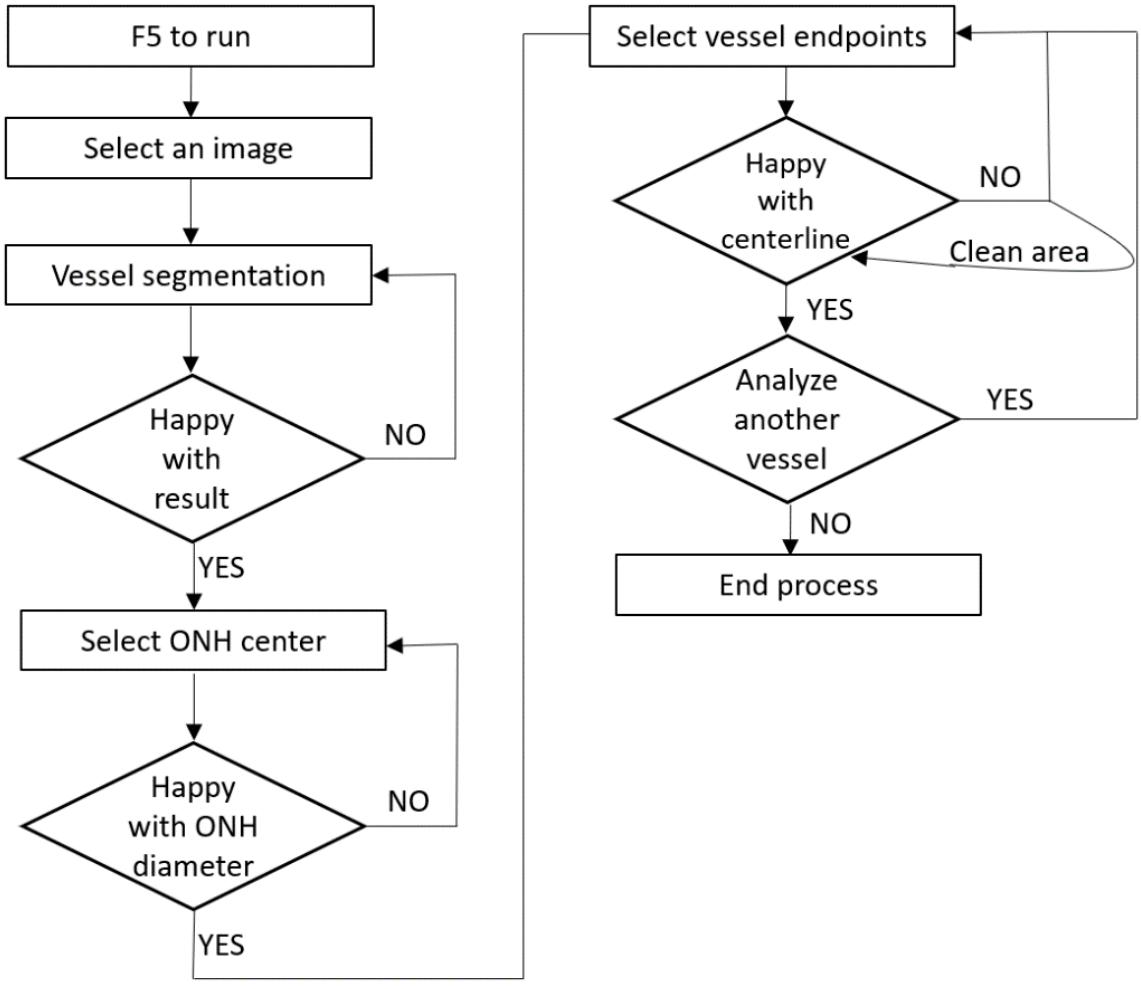
Select the directory where the images are locating by updating **UserDefined.DefaultDir** in retinal_tortuosity_analysis as shown in the following figure. The default path is the main folder. The software will generate a new folder which has the same name as the image to save results. Also, define the format of the image. As can be seen in the following figure, the default format is **tif**.



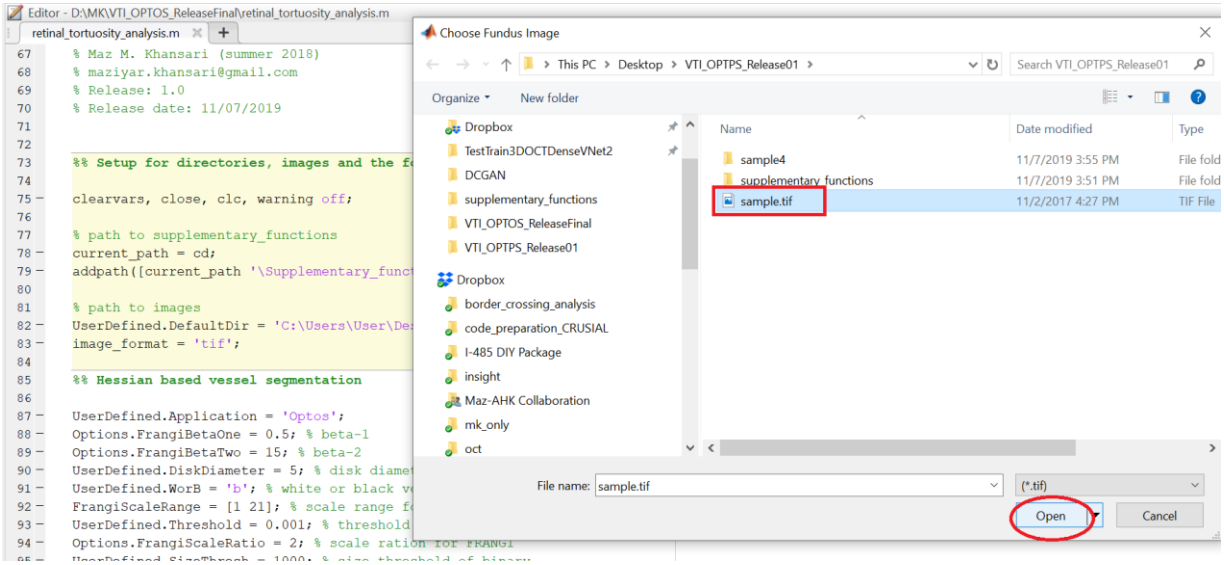
Executing the code for tortuosity analysis

The following are the steps for analysis and each step will be explained in further detail. The flow chart is also providing a demonstration of steps used for the analysis.

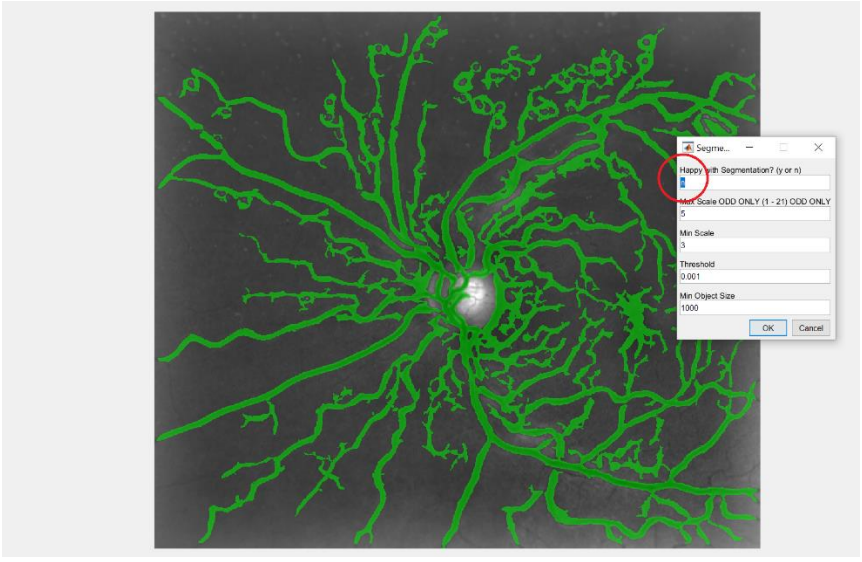
- Run the code (press F5).
- Select an image in the pop-up window to analyze.
- If needed, adjust vessel segmentation parameters in the pop-up window to generate vessel map.
- Select center of optic nerve head (ONH) and adjust the value of radius in the pop-up window.
- Select endpoints of the vessel of an interest in the binary vessel map (one vessel at a time).
- Visualize extracted centerline and make adjustment if necessary.
- Decide to analyze another vessel or end the process.



IMPORTANT NOTE: Make sure all Excel application are closed before executing the main code. Then run the code (click or Run or press F5). A pop-up window will show up as shown in the following figure and let the user select the image. Select the image and then click on open.

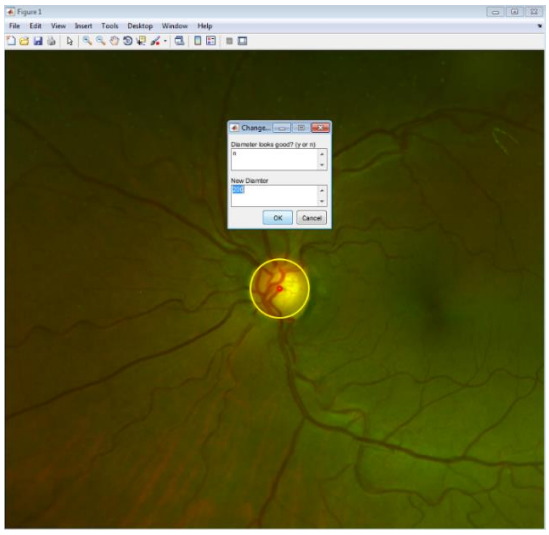


After clicking on **Open**, the algorithm with read the image and start analyzing it for detecting the vessels. An image will be shown with all the detected vessels highlighted by green as demonstrated in the following figure. The pop-up window allows modification of segmentation parameters including Max scale, Min scale, the threshold and minimum object size. These parameters can be adjusted to improve vessel segmentation. Vessel segmentation is based on Frangi Hessian based vesselness filter.

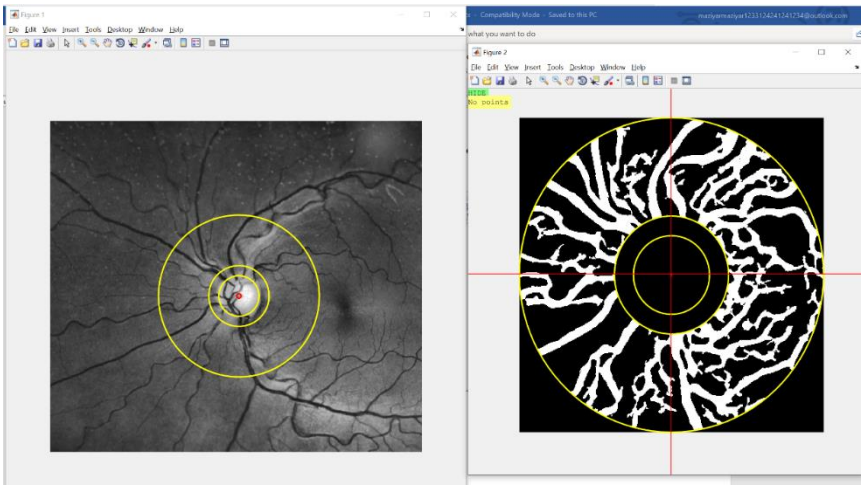


When the parameters are finalized, change “n” to “y” in the first line of the pop-up menu (Happy with Segmentation? (y or n)) and click on OK.

Then a new figure will show up in which the center ONH needs to be selected. Move the mouse cursor to the center of optic nerve head and double left click. A small red circle will be shown on the pixel that was selected and a circumference with radius of 50 pixels centered on the selected point will be overlaid on the image. A pop-up menu will be shown letting the user to adjust diameter of the circumference, as shown in the following figure.

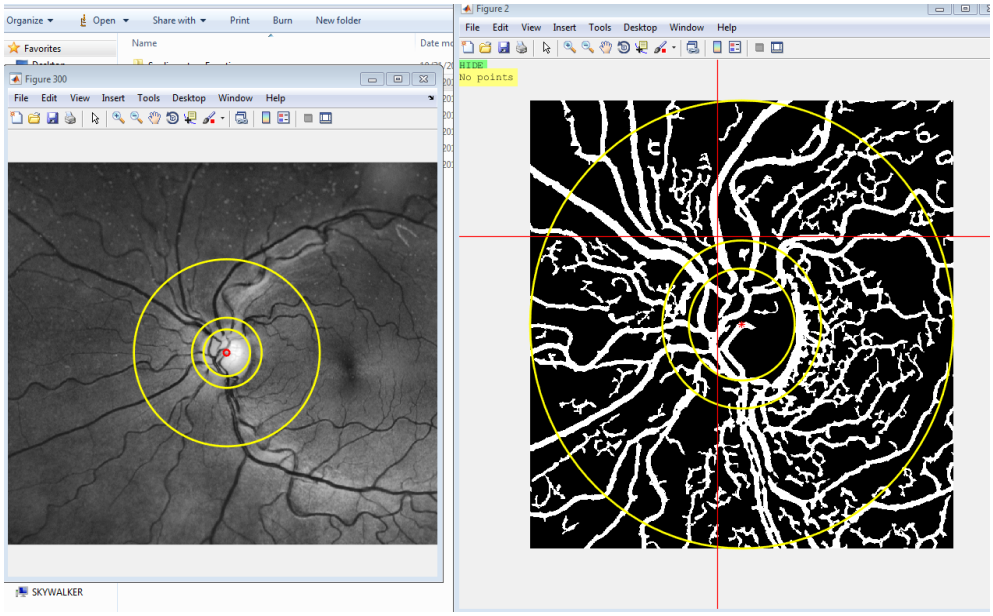


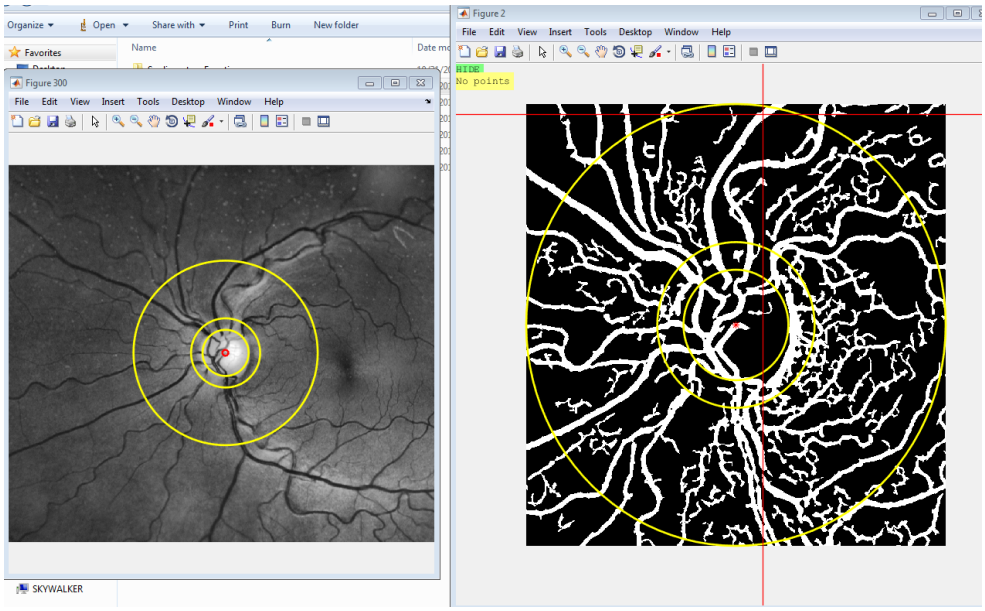
After diameter adjustment, change “n” to “y” in the first line of the pop-up menu (Diameter looks good (y or n)), and then click on ok. Adjust diameter until the yellow circle lies on border of ONH. Afterwards, two figures will be seen in the screen as shown in the following figure. The left figure is green channel of the original image with the circles overlaid (this figure is for visualizing accuracy of extracted centerline). The circumferences are centered on the ONH with radiuses of 1 time, 1.5 times and 4 times the radius of ONH. The binary figure on the right is for selecting endpoints and extracting the centerline. Location of mouse cursor is closely tracked on this figure. The user needs to use the cursor to click on the two endpoints of each vessel. Note that this code is made to analyze one vessel at a time. User can first click on a vessel endpoint close to small circle and then click on the other endpoint which is close to the larger circle.



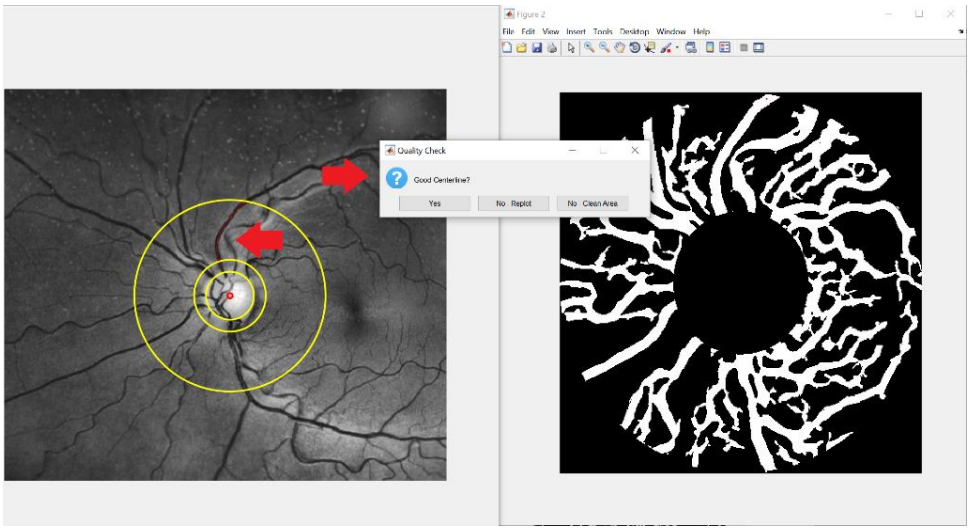
Note both these images are needed for quality analysis. Their position and size must be adjusted based on the screen size and availability of a second monitor. Please see **Appendix IV** for instruction on adjusting appearance of these figures.

Now the user can start selecting end points of each vessel that lies between the 2 outermost circumferences. Start by selecting the first endpoint of a vessel segment closer to the center of the image as shown in the following. Then select the other endpoint which is further from the center of the image and is close to the image boarder, as shown in the following figure.

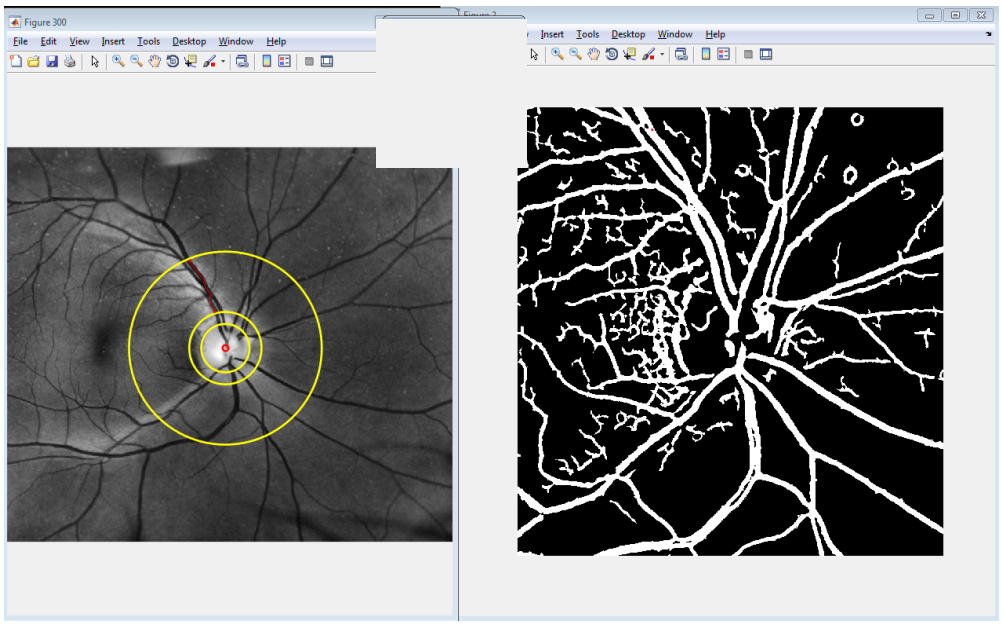




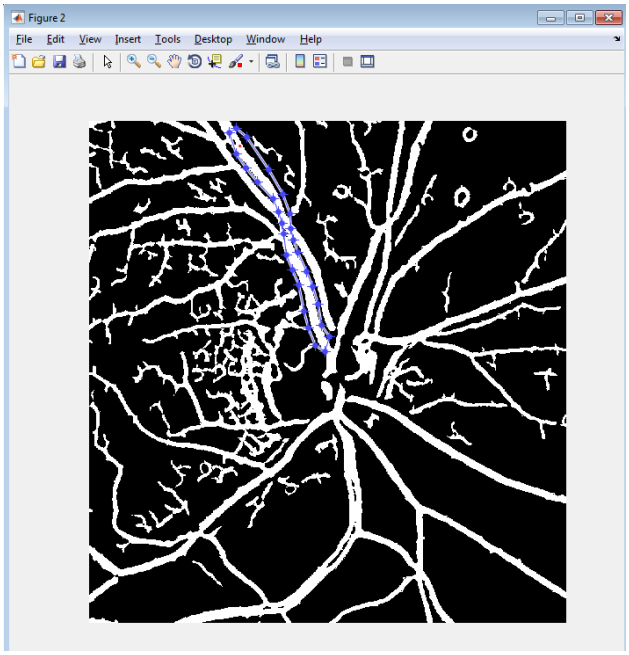
After selecting the second endpoint, the algorithm will find the vessel centerline between the 2 selected endpoints and will over lay the centerline on the vessel on Figure 1 (i.e. the green channel image) using red line. Further, a pop-up menu will become available with 3 options described in the following.



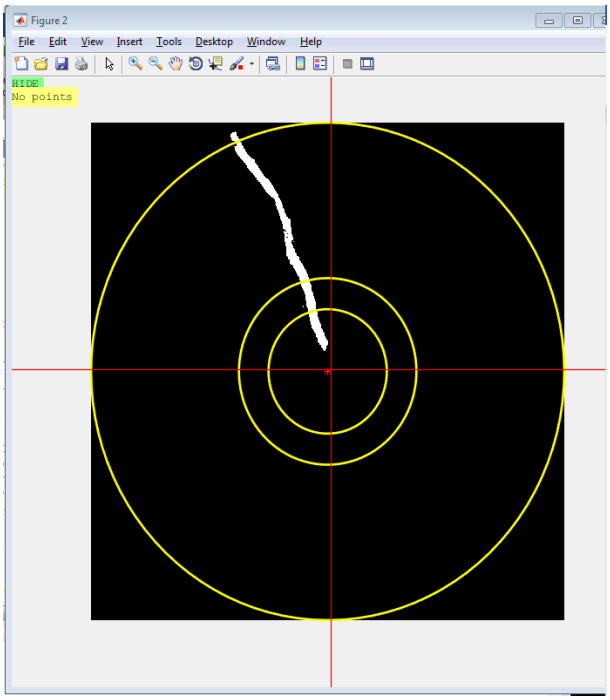
YSE: Centerline looks good, calculate its tortuosity.
NO – Replot: Centerline is not good. Remove the centerline and let user select vessel endpoints again.
No – Clean Area: Centerline is not good. Let the user to select a region in the binary image before selecting the endpoints. This option is designed for critical adjustment and can be used to correct the extracted centerlines in cross-over points. It can be used when the centerline is good but not perfect due to cross overs. This option allows drawing a region of interest along the vessel of interest to eliminate the effect of other close vessels and cross overs on the centerline extraction. By way of illustrations, consider the following example in which the centerline was not accurately extracted as can be seen on the left figure.



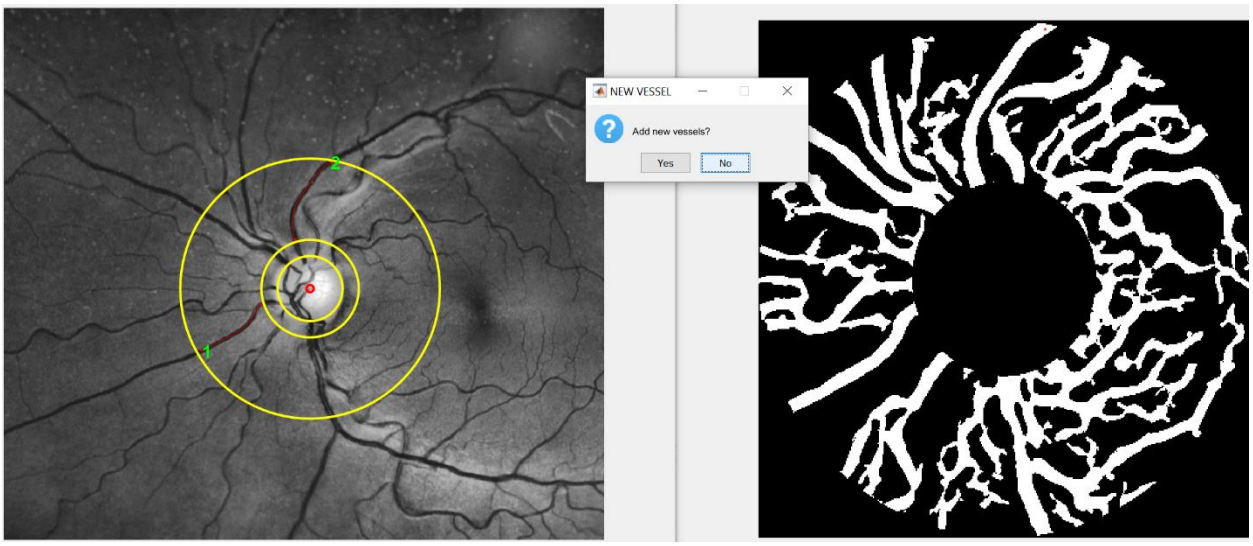
To solve the issue, click on **No – Clean Area**, then draw a region of interest along the vessel of interest as shown in the following figure.



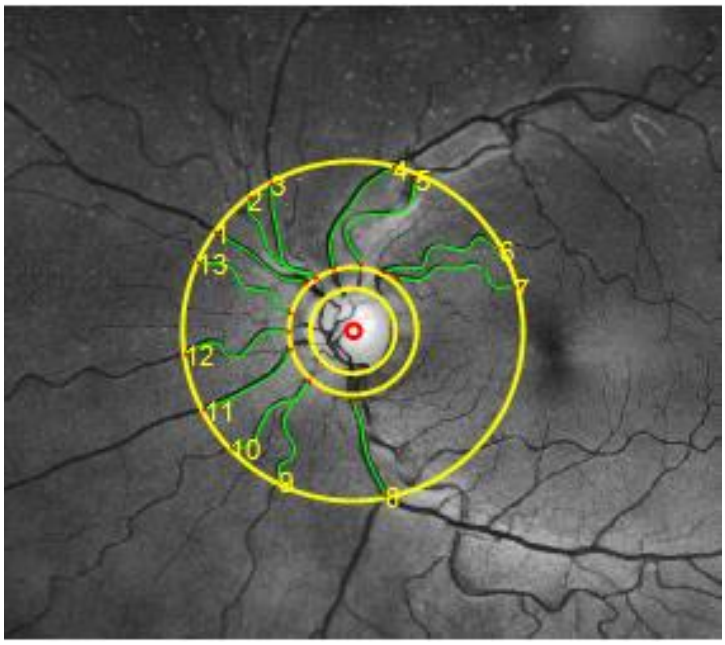
The algorithm then allows the user to keel a binary image only with the selected ROI or re-select ROI based on original binary image (this will be done through a pop-up menu of **Keep** or **Clean**). If the ROI looks fine, click on **Keep** bottom in the pop-up menu to get the following figure in which the user can select the endpoints of the vessel segment. Using the option all other vessels will be eliminated and would not affect centerline extraction.



After selecting the endpoints of the vessel of interest, the new centerline will be extracted and overlaid on the green channel figure (Figure 1) for user verification. If the centerline looks good, the algorithm will calculate VTI and other tortuosity measurements for the vessel segment and save the result.
After tortuosity calculation, the algorithm will add a number for the vessel segment close to the second selected endpoint as can be seen on the following figure. It will also ask if the user wants to analyze more vessels from the current image. This will happen through a pop-up menu (**Add new vessel?**) which asks if the user wants to analyze another vessel (**YES**) or end the process (**NO**). The whole process will be repeated to the point when the user selects **NO** in this menu.

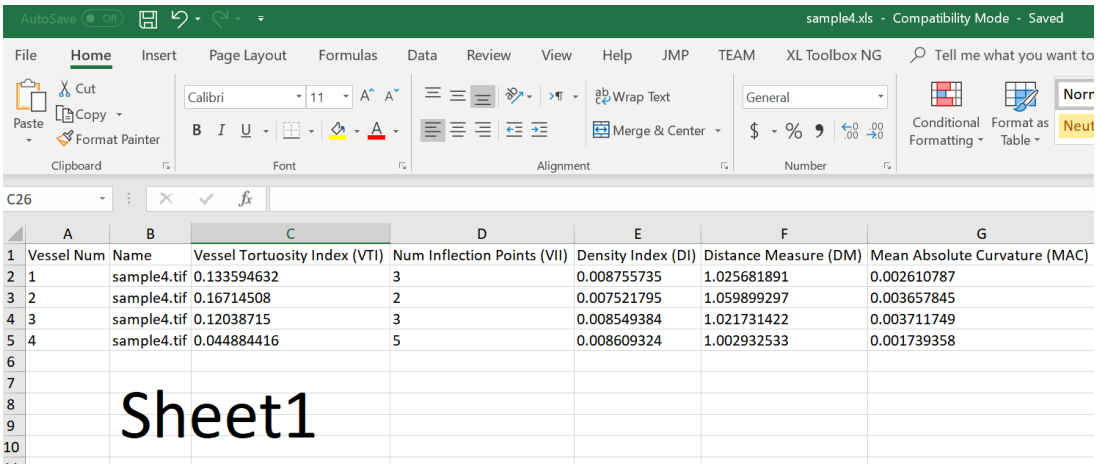


An example of a retinal image with all the selected centerlines are shown in the following image. As can be seen, all the vessel centerlines that extended between the two outers most circles have been extracted.



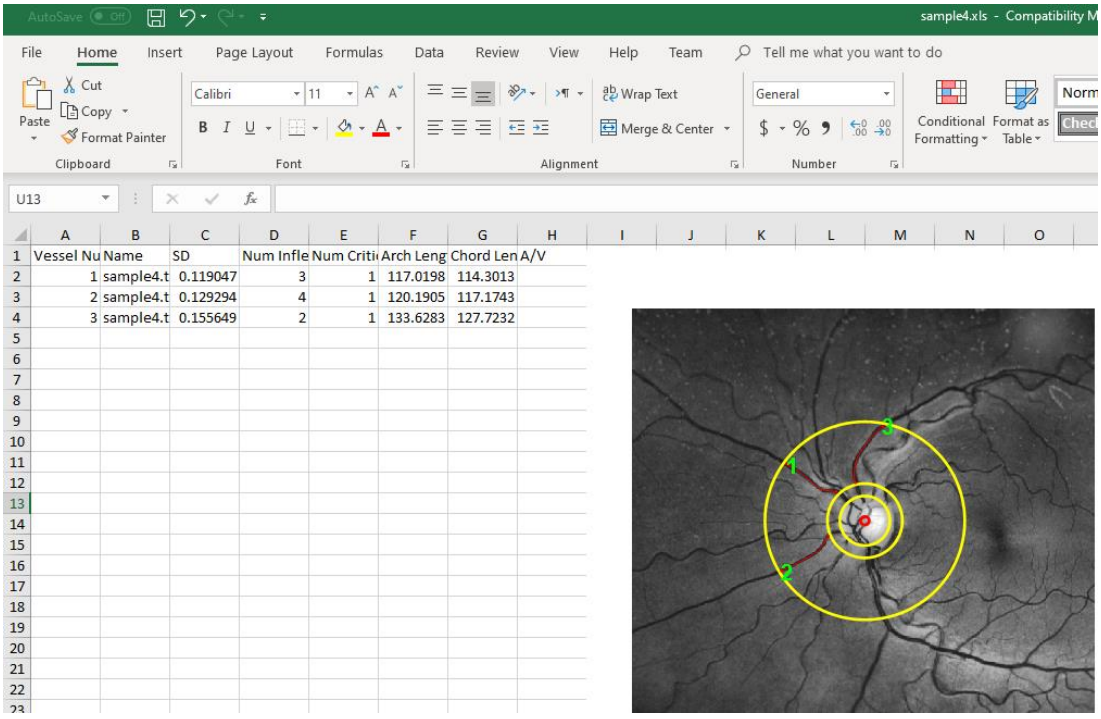
Outputs:

The algorithm automatically saves data in an excel files with the same name as the original image. This file will be in a new folder (same name as the image) in the main directory. The datasheet will also have the same name as the image. Additionally, coordinates of extracted vessel centerlines will be saved in the same folder. Also, the coordinates of the center of ONH will be saved in a text file. This information will become useful if the user wants to replot the centerlines and validate the result **(Appendix III)**. Results are saves in Sheet1 and Sheet2 of the datasheet as shown in the following figure. Description of the parameters is presented in the following page.



	A	B	C	D	E	F	G
1	Vessel Num	Name	Vessel Tortuosity Index (VTI)	Num Inflection Points (VII)	Density Index (DI)	Distance Measure (DM)	Mean Absolute Curvature (MAC)
2	1	sample4.tif	0.133594632	3	0.008755735	1.025681891	0.002610787
3	2	sample4.tif	0.16714508	2	0.007521795	1.059899297	0.003657845
4	3	sample4.tif	0.12038715	3	0.008549384	1.021731422	0.003711749
5	4	sample4.tif	0.044884416	5	0.008609324	1.002932533	0.001739358

Sheet1



	A	B	C	D	E	F	G	H
1	Vessel Nu	Name	SD	Num Infle	Num Criti	Arch Leng	Chord Len	A/V
2	1	sample4.t	0.119047	3	1	117.0198	114.3013	
3	2	sample4.t	0.129294	4	1	120.1905	117.1743	
4	3	sample4.t	0.155649	2	1	133.6283	127.7232	

Sheet 1 contains following information:

- **Vessel Num:** Number of the vessel.
- **Name:** Name of the image (this become useful for integrating result from different images).
- **Vessel Tortuosity Index (VTI):** main measure of tortuosity.
- **Num Inflection Points (VII):** Number of inflection points along the centerline.
- **Density Index (DI):** Mean distance measure between inflection points, normalized by vessel length.
- **Distance Measure (DM):** Ratio of vessel length to its chord length.
- **Mean Absolute Curvature (MAC):** Absolute value of average curvature along the centerline.

Sheet 2 contains following information which are intermediate parameters used for tortuosity measurements. ***A copy of the final retinal image with centerlines overlaid and numbered is also included in this sheet for reference.***

- **SD:** Standard deviation of angels between lines tangent to every pixel along the centerline.
- **Num Inflection Points:** Number of inflection points along the centerline.
- **Num Critical Points:** Number of critical points along the centerline.
- **Arch Length:** Centerline length (pixel).
- **Chord Length:** Centerline chord length (pixel).
- **A/V:** An empty cell for indicating if the vessel is an artery (**A**) or a vein (**V**).

Appendixes

Appendix I: Mathematical Definition of Tortuosity

Tortuosity is an important geometric vessel parameter which has been considered as a risk factor of multiple retinal pathologies such as diabetic retinopathy and sickle cell retinopathy. Also, increased vessel tortuosity is known to be among the first microvascular alterations due to many retinal diseases. There is no widely accepted mathematical definition for tortuosity. In retina, tortuosity evaluation has been mainly performed by clinicians which is subjective and not repeatable. Therefore, there have been attempts to quantify vessel tortuosity to present it with a number that can match visual perception of tortuosity. The current tool provides four commonly used measures of tortuosity for retinal vessels. These measures are: Vessel tortuosity index (VTI), distance measure (DM), tortusity density index (DI) and mean absolute curvature. Mathematical definition of VTI is as following.

$$VTI = \frac{0.1 SD_{\theta}.N.M.L_A}{L_C} \qquad M = \frac{1}{Ip+2}\sum_{i=1}^{Ip+2}\frac{L_{Ai}}{L_{Ci}}$$

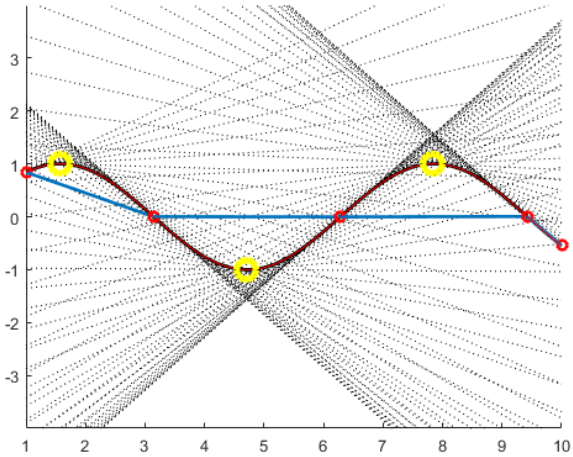
where SD_{ϑ} is standard deviation of angle differences between lines tangent to each centerline pixel and the x-axis. N is number of critical points where the first derivative of the centerline vanishes. M is average ratio of centerline length to its chord length between pairs of inflection points including centerline endpoints. Finally, L_A and L_C are the length of centerline and its chord, respectively. VTI is invariant to rigid transformation and provides good correspondence with visual perception of tortuosity by human observers (Khansari et al, 2017).

Distance measure (DM) is the ratio of vessel length to its chord length (L_A/ L_C). Tortuosity density index (DI) is mean distance measure between inflection points, normalized by vessel length. Finally, the

curvature-based method is obtained by taking mean absolute value of curvature which is calculated as following. All the measurements are based on centerline of the vessel.

$$k(l)=\frac{\frac{dx(l)}{dl}\frac{d^2y(l)}{dl^2}-\frac{d^2x(l)}{dl^2}\frac{dy(l)}{dl}}{\left(\left(\frac{dx(l)}{dl}\right)^2+\left(\frac{dy(l)}{dl}\right)^2\right)^{\frac{3}{2}}}$$

The VTI is made to calculate tortuosity of vasculature based on local and global parameters extracted from the centerline. The main MATLAB function for VTI calculation is vessel_tortousity_index.m. This code calculates VTI for a set of given centerline coordinates (i.e. x and y). Parameters that were used for DM, DI and curvature are included in VTI calculation and can be extracted from the same function. The function has 3 entries of *x*, *y* and *isshow*. x and *y* are the coordinates of vessel centerline. The *isshow* is to provide demonstration of parameters extracted from the centerline and can take value of either 0 for no demonstration and 1 for showing the extracted parameters. The following figure shows example of extracted parameters. For the simulated centerline, tangent lines to the centerline are shown by black dashed line (the code shows tangent lines for every 10 pixels on the centerline to provide a clear demonstration, however, it calculates it for all the pixels on the centerline). The yellow circles are location of critical points and the red circles are location of inflection points. Blue lines connect consecutive inflection points including the end points of the centerline and the ratio of centerline length to the cord length (i.e. blue line) between these points were used in VTI calculation. **Whenever using images with different dimension and resolution, its useful to set *isshow* to 1 for a few vessel segments to visually confirm that all the parameters have been extracted correctly.**



Appendix II: Adjusting Centerline Smoothing Parameter

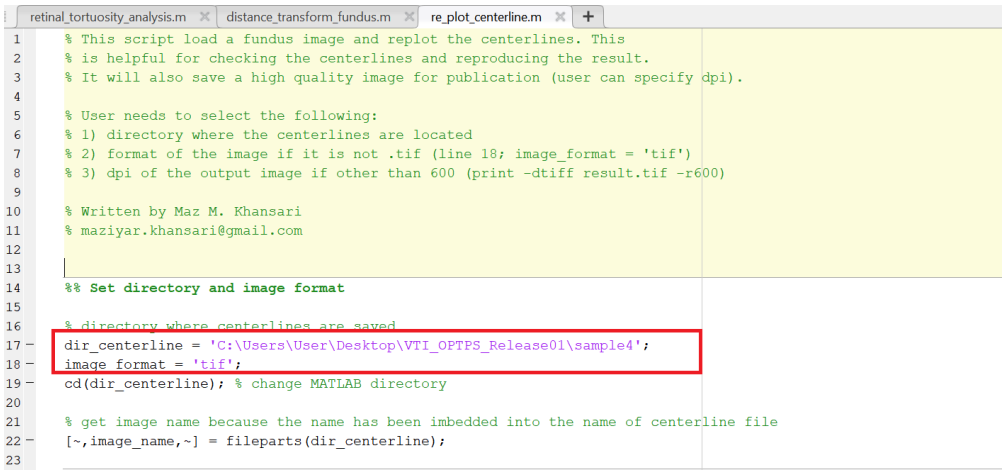
Tortuosity measurements in the current software and majority of previous works are based on centerline of the vessel. Therefore, a reliable centerline extraction technique is needed for a meaningful tortuosity analysis. In the current software, centerlines are extracted by distance transformation on a binary vessel map based on user choice of vessel endpoints. Extracted centerline is smoothed by fitting a cubic B-spline and the smoothing parameter depends on the resolution of the image.

This parameter can be found under **supplementary_functions** folder in distance_transform_fundus.m line 75 and 76 as shown in the following figure. The orange square shows location of line for smoothing centerline and the red square shows cubic B-spline smoothing parameter (3e-5). If you are using a retinal image with higher or lower resolution, this parameter must be adjusted accordingly to obtain a smooth centerline.

Appendix III: Replotting Extracted Centerlines

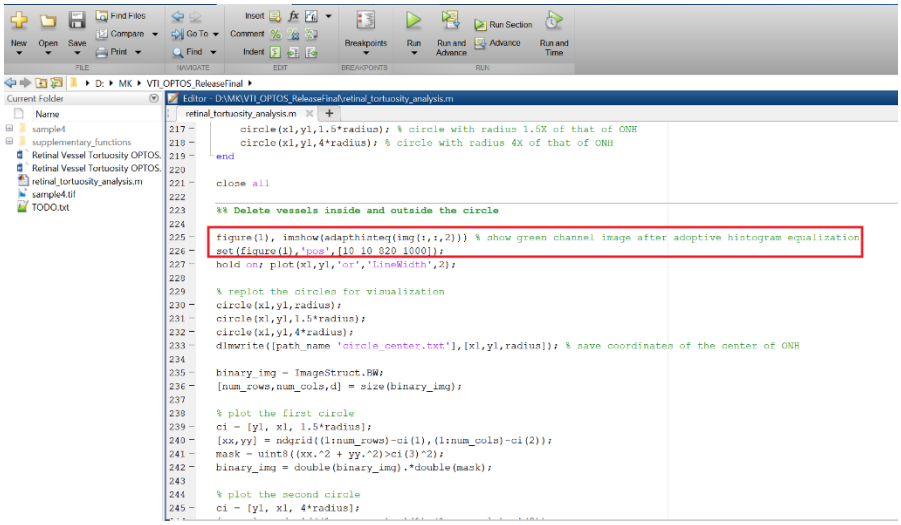
There is a function called re_plot_centerline.m under **supplementary_functions** folder for replotting extracted centerlines. This function will become useful for verifying centerline extraction and creating figure for publication.

The user needs to set directory and format of the image as shown in the following figure. The directory is path to the folder which contains result of tortuosity analysis of a retinal image.

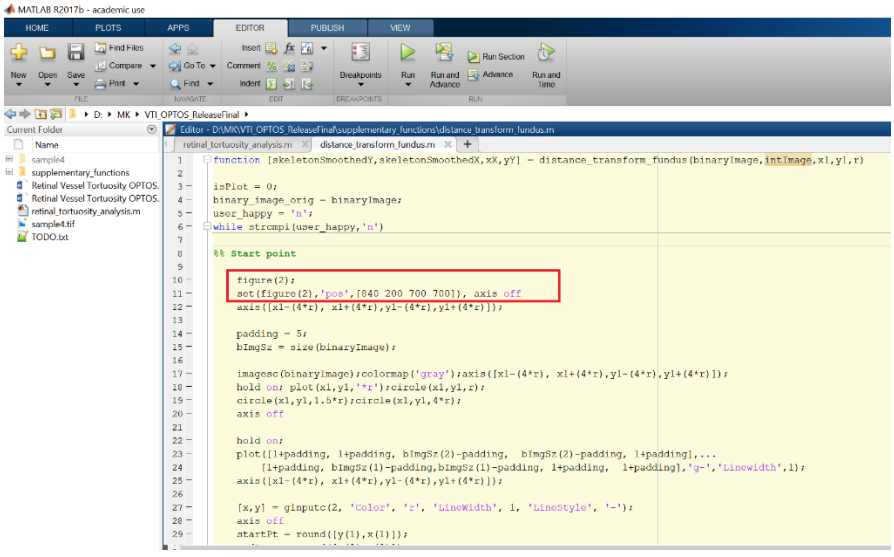


Appendix IV: Adjusting Appearance of Figures

During the analysis two figure will show up on the screen. One is green channel image of the retina which is used for overlaying centerlines and the other is a binary vessel map for selecting vessel endpoints. These two figures are needed for quality analysis. The size of the image on the left can be adjusted using line **232** of the main code (retinal_tortuosity_analysis.m) as shown in the following figure. The 4 digits that are currently set to [10 10 820 1000] can be updated. The format is **[x start, y start, width, height]**. Note that this needs to be done before running the code. Also, note that this step needs to be done only once when using the code on a different monitor.



The position and size of the figure on the right can be adjusted in a function called distance_transform_fundus.m in line 11 as shown in the following figure.



If you have limited space, it is recommended to keep Figure 2 as large as possible and make Figure 1 small because vessel endpoints should be selected on Figure 2.

References

- Khansari, Maziyar M., William O'Neill, Jennifer Lim, and Mahnaz Shahidi. "Method for quantitative assessment of retinal vessel tortuosity in optical coherence tomography angiography applied to sickle cell retinopathy." *Biomedical optics express* 8, no. 8 (2017): 3796-3806.
- Khansari, Maziyar M., Sarah L. Garvey, Shayan Farzad, Yonggang Shi, and Mahnaz Shahidi. "Relationship between retinal vessel tortuosity and oxygenation in sickle cell retinopathy." *International Journal of Retina and Vitreous* 5, no. 1 (2019): 47.
- Cano, Jennifer, Shayan Farzad, Maziyar M. Khansari, Ou Tan, David Huang, Jennifer I. Lim, and Mahnaz Shahidi. "Relating retinal blood flow and vessel morphology in sickle cell retinopathy." *Eye* (2019): 1-6.
- Frangi, Alejandro F., Wiro J. Niessen, Koen L. Vincken, and Max A. Viergever. "Multiscale vessel enhancement filtering." In *International conference on medical image computing and computer-assisted intervention*, pp. 130-137. Springer, Berlin, Heidelberg, 1998.