METHOD ARTICLE

Improved retinal vessel segmentation using the enhanced pre-processing method for high resolution fundus images

[version 1; peer review: awaiting peer review]

Aziah Ali1, Aini Hussain2, Wan Mimi Diyana Wan Zaki2, Wan Haslina Wan Abdul Halim3, Wan Noorshahida Mohd Isa1, Noramiza Hashim1

1Faculty of Computing & Informatics, Multimedia University, Cyberjaya, Selangor, 63100, Malaysia
2Faculty of Engineering & Built Environment, Universiti Kebangsaan Malaysia, Bangi, Selangor, 43600, Malaysia
3Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Center, Cheras, Kuala Lumpur, 56000, Malaysia

Abstract

Background: By diagnosing using fundus images, ophthalmologists can possibly detect symptoms of retinal diseases such as diabetic retinopathy, age-related macular degeneration, and retinal detachment. A number of studies have also found some links between fundus image analysis data and other underlying systemic diseases such as cardiovascular diseases, including hypertension and kidney dysfunction. Now that imaging technology is advancing further, more fundus cameras are currently equipped with the capability to produce high resolution fundus images. One of the public databases for high-resolution fundus images called High-Resolution Fundus (HRF) is consistently used for validating vessel segmentation algorithms. However, it is noticed that the segmentation outputs from the HRF database normally include noisy pixels near the upper and lower edges of the image. In this study, we propose an enhanced method of pre-processing the images so that these noisy pixels can be eliminated, and thus the overall segmentation performance can be increased. Without eliminating the noisy pixels, the visual segmentation output shows a large number of false positive pixels near the top and bottom edges.

Methods: The proposed method involves adding additional padding to the image before the segmentation procedure is applied. In this study, the Bar-Combination Of Shifted Filter Responses (B-COSFIRE) filter is used for retinal vessel segmentation.

Results: Qualitative assessment of the segmentation results when using the proposed method showed improvement in terms of noisy pixel removal from near the edges. Quantitatively, the additional padding step improves all considered metrics for vessel...
Corresponding author: Aziah Ali (aziah.ali@mmu.edu.my)

Author roles: Ali A: Conceptualization, Formal Analysis, Funding Acquisition, Methodology, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Hussain A: Formal Analysis, Methodology, Writing – Review & Editing; Wan Zaki WMD: Formal Analysis, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; Wan Abdul Halim WH: Formal Analysis, Writing – Review & Editing; Mohd Isa WN: Formal Analysis, Writing – Review & Editing; Hashim N: Formal Analysis, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported in part by a research grant from the Ministry of Higher Education, Malaysia under grant no. FRGS/1/2020/TK0/MMU/03/15.

Copyright: © 2021 Ali A et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Ali A, Hussain A, Wan Zaki WMD et al. Improved retinal vessel segmentation using the enhanced pre-processing method for high resolution fundus images [version 1; peer review: awaiting peer review] F1000Research 2021, 10:1222 https://doi.org/10.12688/f1000research.73397.1

First published: 01 Dec 2021, 10:1222 https://doi.org/10.12688/f1000research.73397.1

segmentation, namely Sensitivity (73.76%), Specificity (97.53%), and Matthew's Correlation Coefficient (MCC) value (71.57%) for the HRF database.

Conclusions: Findings from this study indicate improvement in the overall segmentation performance when using the proposed double-padding method of pre-processing the fundus image prior to segmentation. In the future, more databases with various resolutions and modalities can be included for further validation.

Keywords
Pre-processing, retinal vessel segmentation, fundus image
Introduction
Retinal images play a very important role in ensuring the early detection of symptoms relating to ocular diseases. Early detection will in turn enable timely treatment of eye diseases, which in most cases may significantly decrease the patients’ risk of total vision loss. With the global prevalence of eye diseases being gradually on the rise annually, the World Health Organization has encouraged nations to have routine retinal screening in place. This is intended to diagnose diseases such as diabetic retinopathy (DR), glaucoma and age-related macular degeneration (AMD) early enough so treatment can be administered before worse disease progression. Most hospitals are equipped with fundus cameras that can be used to generate fundus images by imaging a patient’s retina, samples of which are shown in Figure 1.

To assist ophthalmologists in performing efficient and accurate fundus image diagnosis, many studies have been conducted to automatically extract important parameters from a fundus image, mainly focusing on automatic retinal blood vessel segmentation and then estimating vessel parameters from the segmentation output. Figure 2 shows a typical flow of blood vessel segmentation procedure.

For validation of the blood vessel segmentation methods, most researches apply their methods to data from two popular benchmark databases, namely Digital Retinal Images for Vessel Extraction (DRIVE) and Structured Analysis of the Retina (STARE). However, it should be noted that these databases consist of images which are at a much lower resolution when compared to the fundus images produced by current modern fundus cameras. The images in the DRIVE database have resolution of 565 by 584 pixels while images in STARE have resolution of 700 by 605 pixels. This is

![Figure 1](image1.png)  
**Figure 1.** Samples of fundus images from a) Digital Retinal Images for Vessel Extraction (DRIVE) database and b) High-Resolution Fundus (HRF) database.

![Figure 2](image2.png)  
**Figure 2.** Processes involved in segmentation of retinal blood vessels from a fundus image (GCI = Green Channel Image, ROI = Region of Interest). The blue-shaded box indicates the pre-processing steps focused in this study.
because the databases date back to the early 2000s, and the fundus cameras of the time did not have the capability to produce high resolution images.

More recent studies have started to include databases with higher resolution fundus images, such as the High-Resolution Fundus (HRF) database. The images in the HRF database have resolution of 3504 by 2336 pixels, markedly higher than images in the DRIVE and STARE databases. Figure 1b shows a sample image from the HRF database.

From Figure 1, it can be seen that the region of interest (ROI) in the DRIVE image is surrounded by dark pixels. However, this is not the case with the image from the HRF database, Figure 1b. The top and bottom edges of the image are not surrounded by the dark area as in Figure 1a. This may result in noisy vessel segmentation output with false positive vessel pixels near the top and bottom images. To the best of our knowledge, this specific problem has not been addressed in the literature.

In this study, we investigated a simple and efficient way to eliminate the noisy pixels in segmentation output for fundus images whose ROIs are not fully surrounded by dark pixels, such as the case with images in HRF database. The proposed method is to be applied in the pre-processing step of retinal blood vessel segmentation workflow, illustrated as the shaded blue box in Figure 2. In order to validate the effectiveness of the proposed pre-processing step, vessel segmentation procedure based on an adapted Bar-Combination Of Shifted Filter REsponses (B-COSFIRE) filter that we previously published is performed on the pre-processed output.

**Methods**

Pre-processing is one of the key steps in retinal blood vessel segmentation techniques, which helps to ensure that the initial fundus image is optimised for the subsequent vessel detection phase. The original red green blue (RGB) format of digital fundus images is not the optimal form for the accurate detection of retinal blood vessels from an image processing point of view due to the natural colours in fundus images that poorly contrast with the retinal background vessels. Issues such as inconsistent illumination across the image, lesser contrast between retinal blood vessels and the retinal background as well as noisy images are other concerns that need to be addressed during the pre-processing step, so that the input image for the vessel segmentation step will be of better clarity in terms of retinal blood vessel structures.

In this study, the pre-processing method employed by Soares is used as the basis since it is considered the established method for this purpose. Figure 3 illustrates the overview of the pre-processing steps where the first step is to extract the green channel image (GCI) from the color fundus image. The GCI displays a noticeably better vessel appearance, while the red channel image shows low vessel-to-background contrast and the blue channel image displays low dynamic range making the vessels appear almost invisible. This decision to use only GCI is supported by most previously established

---

**Figure 3.** Workflow of pre-processing steps applied to a fundus image prior to retinal blood vessel segmentation procedure (GCI = Green Channel Image, ROI = Region of Interest).
methods used for segmenting retinal blood vessels from fundus images.\textsuperscript{18–21} The original code for pre-processing and vessel segmentation using B-COSFIRE can be obtained here.

ROI border padding

As discussed earlier, the ROI for a fundus image is the colored region inside the circular region on the image. The ROI refers to the non-dark area in the middle of the fundus image, which shows the retina. There is a strong contrast between the ROI and the dark area surrounding the ROI from the extracted GCI. Thus, there is a high probability of detecting false vessel pixels for areas just outside the ROI. To minimise this effect on the segmentation output, Soares suggested that the ROI needs to be identified and expanded by padding it with additional interpolated pixels.\textsuperscript{17}

The procedure starts with converting the fundus image from RGB to the CIELab color scheme. CIELab is a way to represent colours using three numerical values, namely L*, a*, and b*.\textsuperscript{22} For this ROI identification step, only the L* component or the luminosity component is used as it shows good contrast between the ROI and the black background. An optimum value for a threshold is then estimated using Otsu’s method to transform the L* image into a mask image, as illustrated in Figure 3. The white pixels (pixel value 1) are all the pixels in the ROI, while the black pixels are all the pixels outside the ROI (pixel value 0).

The mask image is then used to locate the pixels that are located at one pixel distance from the outer border of the ROI in GCI using four-neighbourhood connectivity to define the neighbour pixels. After the set of neighbouring pixels is identified, the ROI is eroded by several pixels to minimise the contrast between the ROI and the artificial ROI region (padding) that is added in the next step. Then, the mean value for each of the pixels in the padding obtained earlier is calculated by considering eight-neighbourhood connectivity. Next, each original neighbouring pixel value is then replaced with the mean pixel value calculated in the previous step. This set of altered pixels is then included as part of the ROI, thus effectively enlarging the ROI by one pixel over the original border. These steps are repeated for a few iterations, where each iteration adds a one-pixel border to the ROI. In this study, the erosion size used is 5 pixels while the number of iteration used is 20 iterations, as applied by Azzopardi et al.\textsuperscript{23} in their B-COSFIRE implementation.

Using this method as proposed by Soares does not add any new pixels to the image, which means the original size is maintained and the top and bottom edges are still not surrounded by the dark pixels. It only converts the grayscale values of pixels surrounding the ROIs with values interpolated from the pixels just inside the ROI border. What we are proposing in this study is the addition of new pixel areas surrounding the original image, thus effectively adding to the resolution of the original image.

Double padding

In our proposed method, prior to changing the values of the pixels just outside the ROI as in Soares’s method, both the GCI and the mask image are padded with an additional 50 layers of zero-valued (black) pixels on all four borders, referred to as double padding. Using the information from these padded images as the input to the Soares’s padding method, a double-padded image is then produced with the resolution increased by 100 pixels in both height and width. This image is then used to produce a contrast-adjusted image in the next step to highlight the vessel structures. This is the only difference from the original Soares method, which we will refer to as single padding.

Contrast adjustment

After the border of the ROI is padded on the fundus image, the next step is to perform image enhancement on the padded fundus image, so that the vessel structures are enhanced in their appearance. A commonly used pre-processing method for fundus image analysis called contrast limited adaptive histogram equalisation or CLAHE\textsuperscript{24} is employed in this study. CLAHE is a variation of the histogram equalisation (HE) method, which is a technique used to transform pixels on an image based on its histogram.

This enhanced pre-processing step is performed on all 45 images in the HRF database before they are processed for segmenting the retinal blood vessels. To ensure validity and reliability, the standard performance metrics for vessel segmentation assessment are adopted to quantify the difference in segmentation performance with and without the proposed enhancement method.

Results & discussion

As described in the introduction section, the modified B-COSFIRE\textsuperscript{16} filter is used to extract the vessel features from the pre-processed output images. Sample outputs of the pre-processed images with their corresponding vessel feature images using the single padding and double padding pre-processing methods are displayed in Table 1. By observing the HRF feature image produced using single padding in Table 1, dark lines can be seen on both the top and bottom borders of the
Table 1. Sample outputs of pre-processing steps applied to High-Resolution Fundus (HRF) images and the corresponding vessel feature images obtained using the Bar-Combination Of Shifted Filter REsponses (B-COSFIRE) filter.

|                | Mask Image | Pre-processed Image | Vessel Feature Image |
|----------------|------------|---------------------|----------------------|
| Single Padding | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| Double Padding | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |

Table 2. Comparison of quantitative segmentation results using single and double padding for pre-processing.

|                | Segmentation output | Zoomed-in |
|----------------|---------------------|-----------|
| Single padding | ![Image](image7.png) | ![Image](image8.png) |
| Double padding | ![Image](image9.png) | ![Image](image10.png) |
vessel feature image. These dark lines will be highly likely to be segmented as false vessel pixels when processed for segmentation. However, the vessel feature image produced using the double padding method does not have these dark lines, thus decreasing the possibility of having a large number of false positive vessel pixels.

Another visible improvement is in terms of the brightness level of vessel pixels in the double-padded vessel feature image as opposed to single-padded. To confirm that double padding is better than single padding for the purpose of vessel segmentation, another comparison is performed on segmentation results using the different padding methods.

Table 2 shows segmentation outputs using the different padding methods, together with their zoomed-in versions. Apart from the apparent improvement in much reduced noisy pixels on top and lower border of the ROI, subtle improvements in vessel appearance are also observed. In general, double padding helps in further enhancing the vessel features, with most vessels appearing brighter compared to single padding outputs, including the smaller vessels. It is found that using double padding in pro-processing results in the successful removal of false positive pixels near the top and bottom image borders of all 45 segmentation output images in the HRF database. In order to quantify the improvement of the segmentation performance when using the proposed method, Table 3 summarises the performance metrics for segmentation using the different padding methods. Following the metric selection in our previous study, four metrics are included, namely Sensitivity (Sn), Specificity (Sp), Balanced Accuracy (B-Acc) and Matthew’s Correlation Coefficient (MCC).

As expected, the application of double padding results in improved segmentation performance where performance values are increased across all the four considered metrics. This is attributed to the successful removal of the noisy pixels near the top and bottom borders of all images in HRF database, thus decreasing the number of false positive pixels and improving the overall segmentation performance.

Conclusion
In this study, we proposed a simple but effective method to improve blood vessel segmentation performance for images with the ROI reaching the image borders. The simple method of adding additional layer of dark pixels around the image proves to be effective in removing the noisy pixels at the image borders. Quantitatively, the additional padding step also managed to improve all the four considered metrics for vessel segmentation, namely Sensitivity (73.76%), Specificity (97.53%), Balanced-Accuracy (85.64%) and MCC value (71.57%) for the HRF database. This method has only been validated on a single high-resolution fundus image database for now, so in the future more databases should be included for validation to attest the robustness of the proposed methods on multiple databases. The proposed improvement method, while simplistic in nature, could prove to be very effective in increasing overall vessel segmentation performance, particularly for images that are not fully surrounded by dark pixels such as HRF database images.

Data availability
Source data
The HRF database can be accessed at https://www5.cs.fau.de/research/data/fundus-images/.

Acknowledgments
We would like to thank our collaborator from Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Center, especially Dr Wan Haslina and her team for their valuable inputs for this study.
References

1. Flaxman SR, et al.: Global causes of blindness and distance vision impairment 1990–2020: a systematic review and meta-analysis. Lancet Glob. Health. Dec. 2017; 5(12): e1221–e1234. PubMed Abstract | Publisher Full Text

2. Yau JWY, et al.: Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care. 2012; 35: 556–564. PubMed Abstract | Publisher Full Text | Free Full Text

3. Bernardes R, Serranho P, Lobo C: Digital Ocular Fundus Imaging: A Review. Ophthalmologica. Oct. 2011; 226(4): 161–181. PubMed Abstract | Publisher Full Text

4. Subramanya Jois SP, Harsha S, Harish Kumar JR: Automatic Optic Disc Localization Using Particle Swarm Optimization Technique. IEEE Region 10 Annual International Conference, Proceedings/TENCON. 2019; vol. 2018-Octob: pp. 1718–1722.

5. Fu D, Liu Y, Huang Z: A review of retinal vessel segmentation and artery/vein classification. Lecture Notes in Electrical Engineering. 2018; 459: 727–737. Publisher Full Text

6. Noor NM, Khalid NEA, Ariff NM: Optic cup and disc color channel multi-thresholding segmentation. Proceedings - 2013 IEEE International Conference on Control System, Computing and Engineering, ICCCSE 2013. 2013; pp. 530–534.

7. Khan MAU, Carmichael JN, Sarirete A, et al.: Thin Vessel Detection and Thick Vessel Edge Enhancement to Boost Performance of Retinal Vessel Extraction Methods. Procedia Computer Science. 2019; 163: 618–638. Publisher Full Text

8. Abramoff MD, Garvin MK, Sonka M: Retinal imaging and image analysis. IEEE Rev. Biomed. Eng. 2010; 3: 169–208. PubMed Abstract | Publisher Full Text | Free Full Text

9. Nunley KA, et al.: Long-term changes in retinal vascular diameter and cognitive impairment in type 1 diabetes. Diab. Vasc. Dis. Res. May 2018; 15(3): 223–232. PubMed Abstract | Publisher Full Text

10. Chalakkal RJ, Abdulla WH, Hong SC: Fundus retinal image analyses for screening and diagnosing diabetic retinopathy, macular edema, and glaucoma disorders. Diabetes and Fundus OCT. Elsevier; 2020, pp. 59–111.

11. Mittal K, Rajam VM: Computerized retinal image analysis - a survey. Multimed. Tools Appl. Aug. 2020; 79(31–32): 22389–22421. Publisher Full Text

12. Garg M, Gupta S: Retinal blood vessel segmentation algorithms: A comparative survey. Int. J. Bio-Science Bio-Technology. 2016; 8: 63–76. Publisher Full Text

13. Staal J, Abràmoff MD, Niemeijer M, et al.: Ridge-based vessel segmentation in color images of the retina. IEEE Trans. Med. Imaging. 2004; 23(4): 501–509. PubMed Abstract | Publisher Full Text

14. Hoover A: Locating blood vessels in retinal images by piecewise threshold probing of a matched filter response. IEEE Trans. Med. Imaging. 2000; 19(3): 203–210. PubMed Abstract | Publisher Full Text

15. Budai A, Bock R, Maier A, et al.: Robust Vessel Segmentation in Fundus Images. Int. J. Biomed. Imaging. Dec. 2013; 2013: 1–11. Publisher Full Text

16. Ali A, Zaki WMDW, Hussain A: Retinal blood vessel segmentation from retinal image using B-COSFIRE and adaptive thresholding. Indones. J. Electr. Comput. Sci. Mar. 2019; 19(3): 1199–1207. Publisher Full Text

17. Soares JVB, Leandro JIG, Cesar RM Jr, et al.: Retinal vessel segmentation using the 2-D Gabor wavelet and supervised classification. Medical Imaging. 2006; 25(9): 1214–1222. PubMed Abstract | Publisher Full Text

18. Moccia S, De Momi E, El Hadji S, et al.: Blood vessel segmentation algorithms — Review of methods, datasets and evaluation metrics. Comput. Methods Prog. Biomed. May 2018; 158: 71–91. PubMed Abstract | Publisher Full Text

19. Fraz MM, et al.: Blood vessel segmentation methodologies in retinal images – A survey. Comput. Methods Prog. Biomed. 2012; 108(1): 407–433. PubMed Abstract | Publisher Full Text

20. Roseline RH, Priyadarsini RJ: Survey on Ocular Blood Vessel Segmentation. Int. J. Adv. Res. Comput. Sci. Softw. Eng. 2017; 7: 318. Publisher Full Text

21. Badar M, Hario M, Fatima A: Application of deep learning for retinal image analysis: A review. Computer Science Review. 01-Feb-2020; vol. 35: pp. 100203. Elsevier Ireland Ltd. Publisher Full Text

22. Zhang X, Wandell BA: A spatial extension of CIELAB for digital color-image reproduction. J. Soc. Inf. Disp. 1997; 5: 61. Publisher Full Text

23. Azzopardi G, Strisciuglio N, Vento M, et al.: Trainable COSFIRE filters for vessel delineation with application to retinal images. Med. Image Anal. 2015; 19(1): 46–57. PubMed Abstract | Publisher Full Text

24. Pizer SM, et al.: Adaptive Histogram Equalization and its Variations. Comput. Vision, Graph. Image Process. Sep. 1987; 39(3): 355–368. Publisher Full Text
The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com