Author's response to reviews

Title: An Easy Method to Differentiate Retinal Arteries from Veins by Spectral Domain Optical Coherence Tomography: Retrospective, Observational Case Series

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Author's response to reviews: see over
“An Easy Method to Differentiate Retinal Arteries from Veins by Spectral Domain Optical Coherence Tomography: Retrospective, Observational Case Series”

Comments to the reviewers

Reviewer's report (Referee 1):

A well-written article providing a crucial piece of original information in terms of vessel type differentiation on OCT. The methodology is robust and described in great detail and the authors offer a thorough discussion of the current state of knowledge relating to vessel imaging with OCT. The article is fairly long and a degree of down-scaling focusing on the essential new information provided by this work could render the paper more appealing and useful to the scientific audience.

Response: We thank the reviewer for the positive comments. The manuscript has been revised accordingly with more focus on the essential new information.

Reviewer's report (Referee 2):

This is a small retrospective study of 26 eyes (18 patients) to evaluate if certain OCT features can be used to distinguish between arteries and veins. This information is useful particularly for automated detection of vessel types.

Minor essential revisions

I am a bit confused by the group of unclassified vessels. If there is no clear lower border hyperreflective band, wouldn’t the vessel be classified as a vein, rather than unclassified?

Response: Thank you for this comment. The classification of vessels by SD-OCT in our study depends on the reflective feature of the lower border of the vessels. When the vessel was very small or located near hyperreflective layers, sometimes it could not be said with certainty that the lower vessel border reflectivity is hyper- or hypo- compared to the surrounding tissues. It might be highly possible that these ones could be veins as the reviewer suggested. However, our work cannot support this hypothesis. Thus, we prefer to categorize them as “unclassified”. In this way, the sensitivity of the current method to classify vessels might be reduced. But we minimized the possibility to misclassify vessels by SD-OCT, so that the specificity of current method could be high and trustworthy.

A fair number of vessels could not be classified on OCT. Is this related to any underlying factors eg. older age? Hypertension?

Response: Thank you for this valuable idea. In our study, unfortunately, we are dealing with rather small case number as it was a pilot project. While the possibilities that you mention may exist, from our data, we think it is more related to the size of the vessels and location within the retina (i.e. near the IPL or OPL).

Could a worked example of how the sensitivities and specificities arrived at in table 3 be reported? this would be quite useful as it is not very clear from table 2.

Response: A worked example has been added in the method part.
In the discussion it is not always clear if the sens, specs, PPV etc reported are for the unclassified vessels included or excluded groups. Perhaps the unclassified included values may be more accurate, since these would presumably be used in clinical practice?

**Response:** We thank the reviewer for this suggestion. It is true that perhaps the unclassified included values may be more accurate. But different readers may have different perspectives towards this. Thus, we prefer to present our original results in two different ways (both included and excluded) as shown in our Tables. In the discussion part, when sens, specs, PPV etc. was not specifically mentioned, it applied to the results calculated in either way (included or excluded). The readers could use the original result in the tables to compare if needed.

It would be useful to know how this method compares to other methods of identifying aa and vv. Ref 12 is discussed, but it would be useful to know how the method compares to other refs e.g. 8-15 as well?

**Response:** As discussed in the manuscript, our approach is not directly comparable to other publications. One reason is that virtually all of the previous reports used a vessel classification obtained by manual grading of colour images as ground truth (including only median or large vessels that can be recognized by colour images) and compared their (semi-) automatic classification system to this ground truth. To be possibly comparable to these reports, we utilized IR images and FA images as ground truth for vessel type. We also included rather small vessels as ground truth, which potentially resulted in a lower classification rate compared to other reports. The other difficulty for comparison is that it is not clear in these reports if unclassified vessels were also included in their sensitivity/specificity analyses. Thus, we performed analyses by both including and excluding the unclassified vessels in our study.

In Discussion, perhaps some further discussion of the ultrastructural features that may underlie this lower border hyperreflectivity would be useful.

**Response:** We thank for this good suggestion. We're also interested in the ultrastructural features of retinal vessels in histopathology corresponding to our findings. However, we hope to introduce a clinical relevant finding from our study: the lower border hyperreflectivity seen by SD-OCT could be useful to classify retinal vessels. Thus, firstly, the doctors or scientist could potentially use this method to evaluate clinical data. Then, with great interests, more research could be done to find out this ultrastructural correlation. As a result, we hope to only focus on our findings without too much discussion on the hypothesis at the current stage.

Minor essential reisions

**figure 2. Are 5, 15 arteries or veins?**

**Response:** Vessel #5 was classified as "uncertain" by IR and/or FA. Vessel #15 had lower border within the hyperreflective layer seen by SD-OCT, thus was classified as "uncertain" by SD-OCT. Figure 2 is to show only the clear grading examples to the readers. Thus, Vessels #5 and #15 were not labeled.

In the abstract and table 3 sens and spec of arteries and veins is exactly the same. could the authors confirm this is not a typo?

**Response:** Thanks for pointing out this interesting result. We use Table 3A from our manuscript as the example to clarify.
Excluding the unclassified vessels, the 2 by 2 tables for arteries and veins are shown below:

<table>
<thead>
<tr>
<th>Original Data</th>
<th>Ground Truth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>artery</td>
</tr>
<tr>
<td>OCT</td>
<td>artery</td>
</tr>
<tr>
<td></td>
<td>vein</td>
</tr>
<tr>
<td></td>
<td>unclassified</td>
</tr>
</tbody>
</table>

**Artery**

sensitivity of classification of artery = \( \frac{148}{148 + 33} \)

specificity of classification of artery = \( \frac{144}{144 + 13} \)

**Vein**

sensitivity of classification of vein = \( \frac{144}{144 + 13} \)

specificity of classification of vein = \( \frac{148}{148 + 33} \)

This means, by using the same ground truth and excluding the unclassified vessels, the sensitivity of classification of arteries equals to the specificity of classification of veins. But except for these two parameters, other classification performance parameters are completely different (as shown in Table 4).

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Response: Revision has been made.

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Response: Revision has been made.

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page 7, what does ART mean?
Response: ART refers to "image averaging via eye tracking".

why only 26 eyes? What happened to the other 10 eyes? (should be 36 eyes from 18 pats)
Response: Since this was a retrospective study, we could not influence patients' selection. The scanning protocol may not have been used in both eyes of the patient for varying reasons. For example, the patients' were not willing to do scans on both eyes. But because we are focusing on a very limited and rather technical detail of the fundus vasculature, this selection bias does not necessarily affect our result and interpretation.

how were vessel diameters measured? from the upper to lower hyperreflective band? this could be described in more detail.
Response: The vessel diameter measurement was the major interest for one of our other studies, which would be published soon. We'll reference the other study once it's published for the current manuscript. For now, description was added to the method section; however, a measuring example is not shown.

Reviewer's report (Referee 3):
Major Compulsory Revisions:
1) Abstract, page 3: "Additional 43 vessels (15 arteries and 28 veins) that IR failed to differentiate were correctly classified by SD-OCT". It remains unclear according to what they were correctly classified. In fact it appears that this refers to those 96 vessels that were determined by FA only. This however means that only 43 of 96 vessels were correctly classified, and in this light the findings are reported in a biased way.
Response: Thank you for this comment. As we know, single imaging modality has limitations to classify vessel types. FA was performed and used as ground truth when IR failed to differentiate the vessel type. A total of 96 vessels were not identifiable by IR. Thus, IR did not classify 96 vessels. However, for these same vessels, 43 were identified correctly by SD-OCT. Thus, 53 vessels were unclassified by SD-OCT. By comparing these two numbers, the readers could find that SD-OCT classifies more vessels correctly than IR. This part of subanalyses showed that OCT outperformed IR in some vessels. We have rephrased the paragraph to make it clearer.

2) The discussion is very lengthy and not well focused and should be compacted and better focused on the main findings.
Response: In accordance with a similar remark from reviewer 1, we have shortened the discussion to make the manuscript more focused.

3) Introduction, page 6, last paragraph: "...(as shown in figure 1 in our preliminary data)." Fig 1 does not show this, but hyperreflective boundaries of the retinal arteries.
Response: Thank you for this remark. In our preliminary data, we did find the hyperreflective boundaries of the retinal arteries different from the ones of veins. These boundaries correspond to the hyperreflective core feature that we see in perpendicular cross sectional vessel scans (as used in our manuscript). However, it can't be certain weather this hyperreflective boundary seen by OCT is the true boundary of retinal artery in regard to histopathology. Thus, to avoid misleading to the readers, we used the descriptive term "hyperreflective core feature" instead of histopathological term "boundary" at this point.
4) Methods, page 7, Data collection, paragraph 1: "Eyes with relatively normal looking retinal structure..." please specify the population more exactly.

Response: Patients were randomly selected from our primary care unit. We have added this information to the manuscript.

5) Methods, page 7, Data collection, paragraph 1: "Written consent was given by the patients for their information to be stored in the hospital database and used for research." How was this done retrospectively? Were all patients contacted and asked to consent?

Response: The written consent is requested from every patient seen in our unit at the time of image acquisition in case we want to use images for teaching purposes or research. Thus, a second retrospective consent is not needed.

6) Methods, page 7, Grading methodology: "Vessels that did not share a common root with any other vessel at the intersection with the circle..." This is difficult to understand. Please describe more clearly.

Response: Thank you for pointing this out, we have rephrased this part.

7) Methods, page 8, paragraph 2: had all patients also FA? If yes: why were they not used in all cases? If no: please specify exactly how many patients/vessels had both techniques, and how many had only IR or only FA.

Response: As shown in the result, all eyes were imaged with IR, and OCT (26 eyes). Additional 22 eyes had FA.

8) Methods, page 8, paragraph 2: "Vessel types were labelled as "Artery", "Vein" or "uncertain" by the maximum findings from IR and FA..." what does this mean?

Response: As we know, single imaging modality has limitations to classify vessel types. Thus, to use maximum findings from two modalities could increase the number of classified vessels as ground truth. For example, we first performed vessel classification independently on FA and IR for one single vessel. When the classification is “uncertain” by IR and “Artery” by FA, then the combined maximum finding is “Artery” for this vessel. When the classification is “uncertain” by IR and “uncertain” by FA, then the combined maximum finding is “uncertain” for this vessel.

9) Methods, page 9 and 10: terms like sensitivity, specificity, PPV, NPV, and the likelihood ratios are standard terms and need not to be explained within this manuscript. Please skip this part.

Response: We agree with the reviewer that terms like sensitivity are standard and should not be simply listed in the manuscript. However since our study used complicated analysis (e.g. arteries or veins, unclassified vessels included or excluded), Reviewer #2 did ask for more information how the sensitivity and specificity were calculated. Thus, instead of using the standard formula, a worked example was shown to clarify our analyses.

10) Methods, page 11, last paragraph: "...43 vessels (15 arteries and 28 veins) that IR failed to differentiate were correctly recognized by SD-OCT. " was this the 96 vessels which were only identifiable by FA, as described above? If so, a correct classification of 43 vessels out of 96 implies that possibly 53 were wrongly classified (maybe this number is lower, since some of the vessels my not have been classified by the SD-OCT.

Response: Thanks for pointing out this potential confusion. This point is the same as Point 1. As we mentioned before, single imaging modality has limitations to classify vessel types. A total of 96 vessels were not identifiable by IR. Thus, 96 vessels were "wrongly classified" by IR. However, for these same vessels, 43 were identified correctly by SD-OCT. Thus, 53 vessels were "wrongly classified" by SD-OCT. By comparing these two numbers, the readers could find that SD-OCT classifies more vessels correctly than IR. This part of subanalyses showed that OCT outperformed IR in some vessels.
11) Table 3 is not referenced within the manuscript. This has to be changed. However within this table there is redundant information which should be omitted: “Specificity” and “False positive rate”, as well as “Classification Accuracy Rate” and “Classification Error Rate”. These redundant parts should be omitted.

Response: Thank you for the comment. Table 3 is now referenced. However, we would like to keep the full list of calculated statistical parameters in there so that a broad spectrum of readers can relate to the statistics in this manuscript.

12) Discussion page 16, last paragraph: “Because arteries carry blood rich in oxygen, their inner part is brighter than their walls compared to veins, ...” This is reported as a proven fact, but is it so? At least the arguments should be presented.

Response: We are sorry for any kind of confusion. This is not our original hypothesis but was mentioned by Mirsharif et.al. (Reference No. 3). We have revised the paragraph to specify this is only a hypothesis but not a proven fact.

13) Table 4, page 26: it would be interesting to see scatterplots of the vessel widths comparing OCT with IR or with IR+FA.

Response: Thanks for the good suggestion. However, this part of the analyses will be shown in one of our other publications soon.

Minor Essential Revisions:
1) Reference 22 is incomplete and should be completed.
2) Figure legends, page 22, figure 1: “liner scanning”, correct “line scanning”

Response: Both changes were made. Thank you for the comment.

Discretionary Revisions:
1) Page 6, paragraph 1: “For example, retinal vessel blood flow, instead of retinal vessel diameter or AVR, could be obtained with this method, which makes the result not easily comparable with many other publications. In addition, both Doppler FD-OCT system and the trained expert to interpret and validate the results are needed; which limits its application.” This part of the introduction should be omitted.

Response: This paragraph was removed from the introduction.