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Retina

High-resolution/High-Speed Gap Can Distinguish Different Intraretinal Perfusion Signals by Optical Coherence Tomography Angiography

Alessandro Arrigo[1], Michel Teussink[2], Lorenzo Bianco[1], Alessio Antropoli[1], Emanuela Aragona[1], Giuseppe Cappuccio[1], Francesco Bandello[1], and Maurizio Battaglia Parodi[1]

[1] Department of Ophthalmology, IRCCS San Raffaele Scientific Institute, via Olgettina 60, Milan, Italy
[2] Heidelberg Engineering GmbH, Heidelberg, Germany

Correspondence: Alessandro Arrigo, Department of Ophthalmology, IRCCS Ospedale San Raffaele, University Vita-Salute, via Olgettina 60, Milan, 20132, Italy. e-mail: alessandro.arrigo@hotmail.com

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Purpose: The development of optical coherence tomography angiography (OCTA) has radically changed the diagnostic assessment of the intraretinal vascular network. Two different OCTA acquisition modalities have recently been introduced in clinical practice, namely high-resolution (HR) and high-speed (HS) scans. HR OCTA requires more acquisition time and provides higher quality data, whereas HS OCTA is faster but furnishes lower quality data. The main aim of the present study is to gauge how much extra blood flow perfusion information can be obtained through the combined use of HR and HS OCTA.

Methods: We compared HR and HS OCTA acquisitions to assess the reliability of both techniques, also putting forward a new set of quantitative metrics to measure the HR/HS OCTA gap and to highlight different perfusion information.

Results: In essence, both HR and HS OCTA acquisitions proved highly feasible in detecting the intraretinal vascular flow signal, as confirmed by the stability of quantitative OCTA metrics, thus displaying their suitability for use in clinical practice. We detected an HR/HS overlapping gap of 21.6 $\pm$ 6.5% for intraretinal capillaries, and 4.3 $\pm$ 1.2% for choriocapillaris, highlighting the greater information obtained by HR OCTA.

Conclusions: This novel HR/HS OCTA gap assessment might pave the way for the development of new quantitative metrics for retinal diseases that would focus on the earlier detection of perfusion impairment and relate it to the stage of the disease and its progression.

Translational Relevance: This study proposes a new quantitative way to detect different perfusion signals based on OCTA. The findings presented in this paper can lay the foundations for the development of new quantitative metrics focused on the separate analysis of high flow and low flow signals, enabling very early changes in intraretinal perfusion to be detected.

Introduction

Optical coherence tomography angiography (OCTA) still represents a milestone in noninvasive diagnostics in retinal diseases. The development of even more advanced quantitative metrics has increased the information OCTA can yield radically, bringing new insights into the pathogenesis of retinal diseases and introducing new method of classification.[1–7] However, the fact that only 1 acquisition setup is available is a major drawback of current OCTA approaches, because this means that only a part of the entire intraretinal perfusion signal can be captured. Indeed, it is well-known that OCTA output mainly depends on the range of sensitivity in detecting the motion signal, which is the purpose of the acquisition parameters, including the interslice gap and the

number of A-scans per single line.[8] The Heidelberg Spectralis device (Heidelberg Engineering, Heidelberg, Germany) includes a full-spectrum probabilistic algorithm for detecting the OCTA motion signal. High resolution (HR) (approximately 5.7 µm lateral pixel spacing) OCTA acquisition has been developed to provide very high-quality data; however, it is certainly more time consuming. In contrast, high-speed (HS) (approximately 11.4 µm lateral pixel spacing) OCTA acquisition is faster, but in this case individual retinal capillaries can be less clearly discernible. To date, only one paper has investigated the differences between HR and HS OCTA scans, covering highly repeatable and stable measurements for both types of acquisitions.[9]

It was the aim of this study to perform a deep quantitative assessment of HR–HS OCTA differences, including more OCTA quantitative metrics, to establish the reliability of both acquisitions in clinical practice. We have also introduced a new OCTA quantitative assessment, referred to hereafter as the HR/HS OCTA gap, which combines the foveal avascular zone (FAZ) area, mean vessel diameter, and HR/HS overlapping gap percentage, so as to distinguish and selectively analyze perfusion signals possibly caused by different intravascular flow speeds.

Materials and Methods

This study was designed as an observational, cross-sectional case series of healthy adults consecutively recruited at the Department of Ophthalmology of IRCCS San Raffaele Scientific Institute, Milan, Italy. Written informed consent was obtained from all subjects and the study adhered to the tenets of the Declaration of Helsinki. The study was approved by the Ethical Committee of Scientific Institute San Raffaele, Milan (MIRD).

Inclusion criteria were (1) healthy systemic and ocular conditions, (2) no history of ocular surgery and a refractive error of less than ±2D (3), with perfectly clear optical media.

All subjects underwent a complete ophthalmologic examination, including best-corrected visual acuity testing, slit-lamp biomicroscopy, Goldmann applanation tonometry, and funduscopic examination.

Imaging

The OCTA acquisition protocol (Spectralis HRA2+OCT, Heidelberg Engineering) comprised an HR OCTA scan (OCT scan angle 10°, lateral pixel spacing approximately 5.73 µm/pixel, digital depth resolution 3.87 µm/pixel, ART 5 images, 512 B-scans, and inter B-scan distance 12 µm) and an HS OCTA scan (scan angle 10°, lateral pixel spacing approximately 11.46 µm/pixel, ART 5 images, number of images 256, inter B-scans distance 12 µm). Retinal boundary segmentations of superficial capillary (SCP), deep capillary (DCP), and choriocapillaris (CC) plexuses were performed by the on-board software.

Image Analysis

Vessel density (VD) was calculated for all the vascular plexuses following the previously verified methods explained in Arrigo et al (2022).[7] Images were binarized by using an Otsu threshold to highlight capillaries against the background. Then, the percentage of capillaries was calculated and considered as VD. For SCP and DCP, we manually segmented the FAZ to exclude it from VD calculation. Moreover, considering SCP and DCP, we used in-house scripts to calculate the FAZ, vessel tortuosity (VT), vessel dispersion (Vdisp), and vessel rarefaction (VR) metrics.[7] VT and VR required the binarized images to be skeletonized, which was performed using the "skeletonize" function included in the ImageJ platform.[10] We then used the "analyze skeleton" tool to extract the VT metric, and we adopted the "directionality pipeline" to measure Vdisp. In addition, we used in-house scripts to calculate the CC porosity metric, intended as a quantitative measurement of CC flow voids. The entire postprocessing operation was performed using the ImageJ platform.[10] The pipeline is shown in Figure 1.

Furthermore, based on the SCP, DCP, and CC en face images, we assessed the differences between HR and HS OCTA acquisitions, by calculating the mean vessel diameter of 10 different samples of ten different retinal vessels. This procedure was done at the same retinal locations between HR and HS acquisition, considering the binarized OCTA image, by using an Otsu threshold and by applying the "edge finder" function provided in ImageJ to enhance the edges of the vessels.[11] In addition, we started by calculating the HR/HS images overlap, which is the overlap of the two vascular network reconstructions divided by the smaller, and we used in-house scripts to calculate the HR/HS overlapping gap percentage, which is the percentage overlap in terms of total number of pixels between HR and HS reconstructions. To make the analyses reliable and decrease possible segmentation-related issues, we preferred, bearing in mind the feasibility of the study, to calculate all the parameters considering the SCP and DCP segmentations combined.

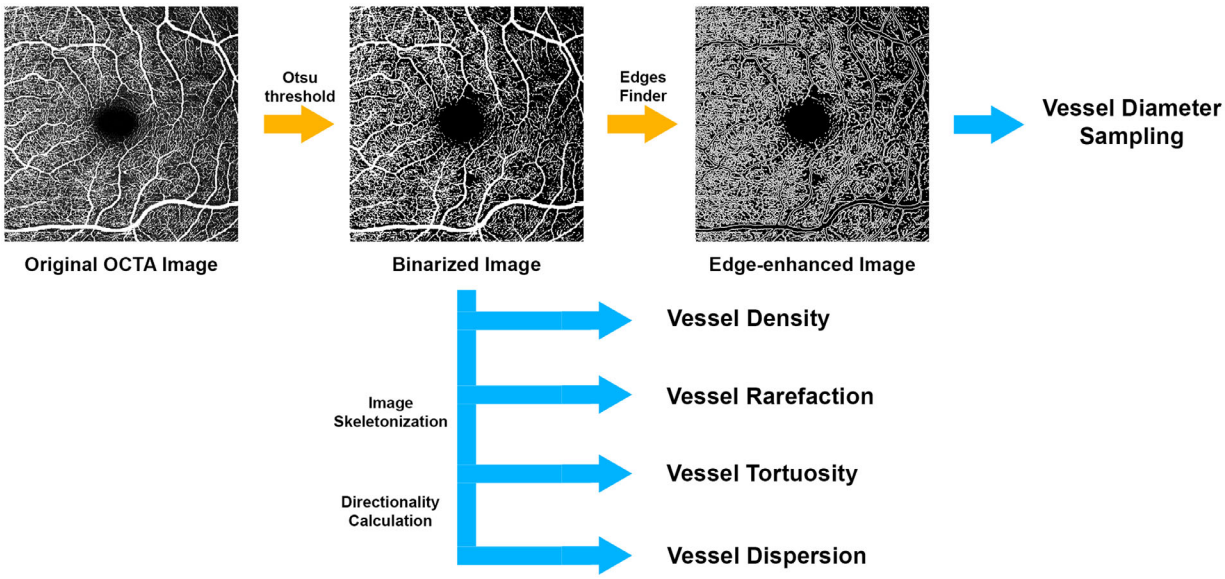

Figure 1. Postprocessing pipeline for OCTA quantitative analyses.

Statistical Analyses

All the collected metrics were statistically analyzed to assess the agreement between HR and HS OCTA acquisitions. Results for descriptive analyses are expressed as mean ± standard deviation for quantitative values and as frequency and percentages for categorical ones. Differences between sample means were statistically assessed by two-tailed *t* tests. Results were considered statistically significant for a *P* value of less than 0.05, with all the analyses being performed using the SPSS Statistics Version 21.0 software package (IBM, Armonk, NY).

Table 1. OCTA Quantitative Analysis

Parameter	OCTA Acquisition	Mean ± Standard Deviation	*P* Value
SCP + DCP VD	HR OCTA	0.42 ± 0.01	>0.05
	HS OCTA	0.41 ± 0.01	
CC VD	HR OCTA	0.50 ± 0.01	>0.05
	HS OCTA	0.50 ± 0.02	
SCP + DCP VT	HR OCTA	7.4 ± 0.3	<0.001
	HS OCTA	6.5 ± 0.4	
SCP + DCP Vdisp	HR OCTA	10.6 ± 4.2	>0.05
	HS OCTA	11.2 ± 3.9	
SCP + DCP VR	HR OCTA	0.38 ± 0.01	>0.05
	HS OCTA	0.40 ± 0.01	
CC porosity	HR OCTA	3.95 ± 0.48	<0.001
	HS OCTA	5.52 ± 0.53	

Results

This study is based on 30 eyes of 30 healthy controls (15 males; mean age, 25 ± 5 years; logarithm of the minimum angle of resolution best-corrected visual acuity 0.0 ± 0.0). The ophthalmologic and multimodal imaging assessments confirmed the healthy conditions of the included eyes. The entire quantitative analysis is extensively reported in Table 1.

HR OCTA and HS OCTA showed no significant differences in terms of VD values for both SCP + DCP and CC plexus (all $P > 0.05$). Similarly, Vdisp and VR values were similar in both OCTA acquisitions (all $P > 0.05$). In contrast, we found significantly higher VT values for HR OCTA than HS OCTA ($P < 0.05$). HR OCTA detected considerably more capillaries in skeletonized reconstructions than HS OCTA (Fig. 2).

At the same time, CC porosity was significantly lower in HR OCTA than HS OCTA ($P < 0.05$). The results of the HR/HS OCTA gap calculation are recorded in Table 2. The FAZ area was not significantly different in the HR and HS OCTA acquisitions (Fig. 3). In contrast, we found statistically significant differences in the mean vessel diameter measured by HR and HS OCTA acquisitions, being significantly greater in HR OCTA compared with HS OCTA images ($P < 0.05$). This finding is consistent with a significant HR/HS overlapping gap of 21.6 ± 6.5% for SCP + DCP

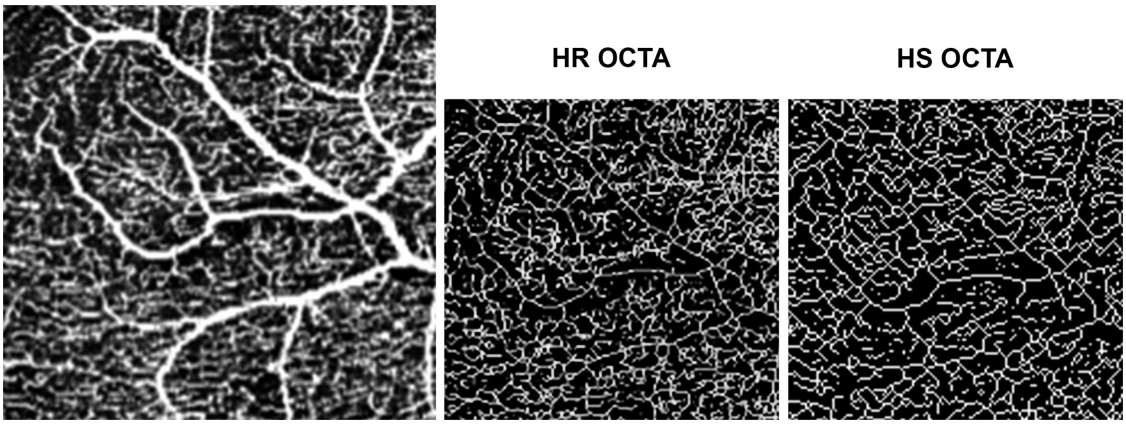

Figure 2. VT differences between HR and HS OCTA acquisitions. S tarting from the details in the original image **(A)**, we observe a higher percentage of vessels detected after the skeletonization process in HR OCTA **(B)**, compared with HS OCTA **(C)**.

Table 2. HR/HS OCTA Gap

Parameter	Reference	Mean ± Standard Deviation	*P* Value
FAZ area (mm^2)	HR OCTA	0.40 ± 0.12	>0.05
	HS OCTA	0.42 ± 0.24	
Vessel diameter (micron)	HR OCTA	57 ± 8	<0.001
	HS OCTA	42 ± 10	
HR/HS overlapping gap (%)	SCP+DCP	21.6 ± 6.5	
	CC	4.3 ± 1.2	

reconstructions (Fig. 4). Interestingly, the visual inspection of HR/HS overlapped images highlighted vessel bifurcations as the most evident sites of the HR/HS overlapping gap. Considering an arbitrary HR/HS overlapping gap threshold of 20%, we calculated that 90% (standard deviation of ±2%) of bifurcations showed an HR/HS overlapping gap of 20% or more. In contrast, the HR/HS overlapping gap for CC reconstruction was of 4.3 ± 1.2% (Table 2). The overlapped images (Fig. 5) revealed clear differences that appeared to coincide with a greater presence of CC signal voids in HS OCTA images.

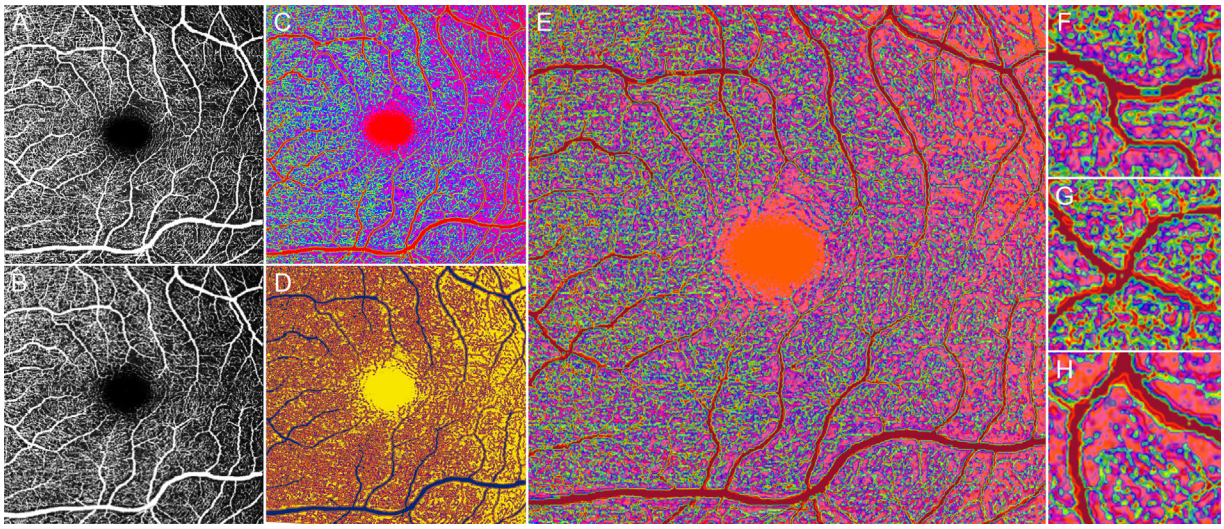

Figure 3. FAZ area differences between HR and HS OCTA acquisitions. Starting from the original HR OCTA **(A)** and HS OCTA **(B)** acquisitions, we obtained the co-registered reconstructions (**C** and **D**, respectively), suitably color mapped to highlight differences better. Considering the larger vessels in this image, the overlapped image shows no obvious preponderance of red or yellow color **(E)**. The microvasculature immediately surrounding the FAZ is detected/visualized better in the HR image.

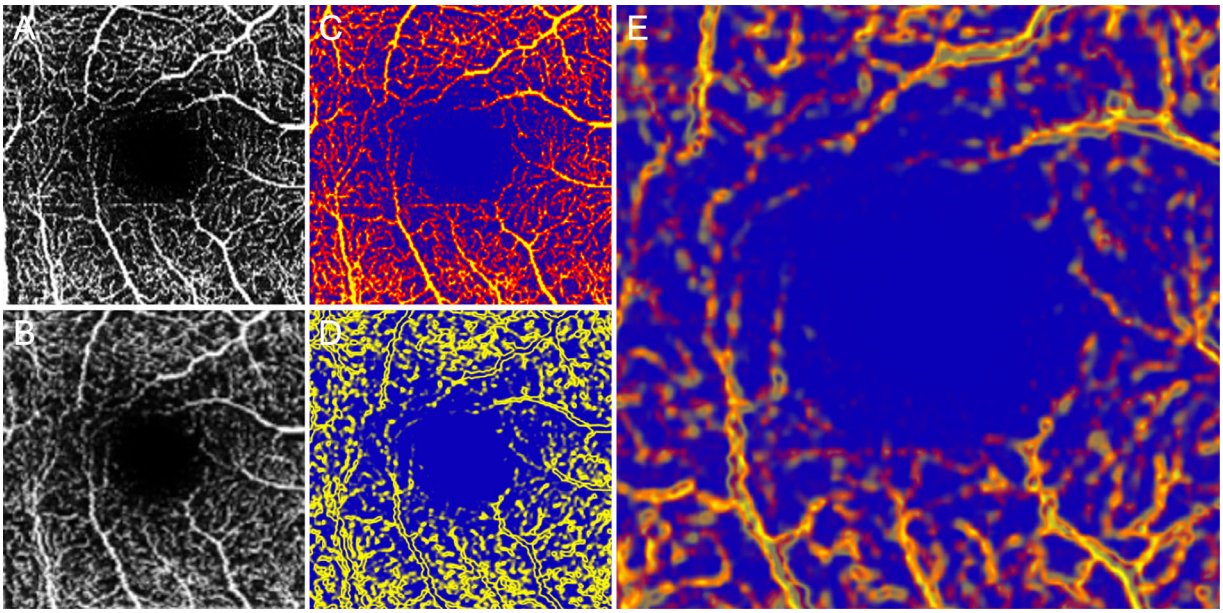

Figure 4. HR/HS OCTA overlapping gap for SCP+DCP reconstruction. Starting from the original HR OCTA **(A)** and HS OCTA **(B)** acquisitions, we obtained the co-registered reconstructions (**C** and **D**, respectively), suitably color mapped to highlight differences better. The overlapped image shows a remarkable overlapping gap, which can be easily highlighted especially upon inspection of the bigger vessels **(E)**: the magnified images (**F**–**H**) show three examples, where red indicates a broader vessel visualized by HR OCTA, compared with the brown of HS OCTA.

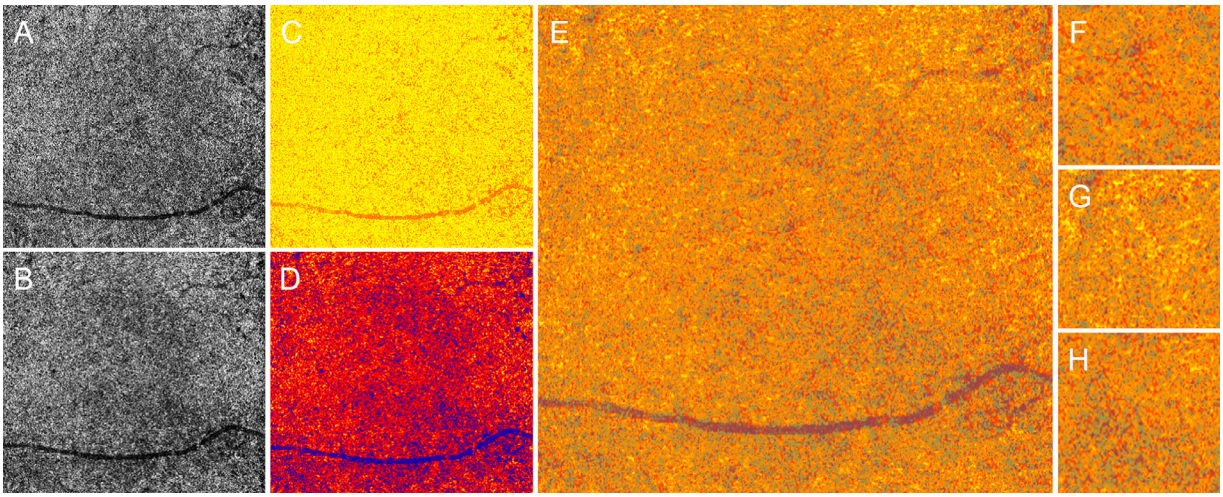

Figure 5. HR/HS OCTA overlapping gap for CC reconstruction. Starting from the original HR OCTA **(A)** and HS OCTA **(B)** acquisitions, we obtained the co-registered reconstructions (**C** and **D**, respectively), suitably color mapped to highlight differences better. The overlapped image shows a small overlapping gap **(E)**. The magnified images (**F**–**H**) show three examples, where red highlights the greater flow detected by HR OCTA, with respect to HS OCTA. Interestingly, the higher concentration of red corresponds to regions of flow signal voids on HS OCTA.

Discussion

This study assesses differences concerning HR and HS OCTA acquisitions quantitatively in healthy subjects. Taking an HR OCTA scan as the benchmark, HS OCTA proved highly reliable in detecting SCP, DCP, and CC plexuses, as confirmed by the similar values for the VD and FAZ. The fact that the FAZ area was similar in HR and HS OCTA provides further interesting information regarding the physiology of the FAZ. Indeed, the lack of a significant HR/HS difference may be interpreted as a sign that peri-FAZ vessels typically display a highly perfused network in

healthy eyes. This factor is important in ensuring that oxygen and nutrients are passively diffused through the foveal region. The important role of FAZ area assessment in retinal diseases is well-recognized as a sensitive sign of macular perfusion defects.[12–14] However, to the best of our knowledge, this noninvasive report is the first supporting the high perfusion nature of perifoveal capillaries and paving the way for intriguing new FAZ-focused analyses of retinal diseases based on the combined use of HR and HS OCTA scans. The values of Vdisp and VR were not significantly different, suggesting that that HR and HS OCTA acquisitions do not differ in terms of the geometric properties and spatial disorganization of intraretinal capillary networks.[3,4,7] From this point of view, based on VD, FAZ, Vdisp, and VR, we can assert that as regards quantitative vascular characterization, HS OCTA seems to be as reliable as HR OCTA and that it can be considered a valuable scan option, especially when patients are reluctant to collaborate or in the event of fixation loss.

Conversely, HR and HS OCTA showed significant differences in the VT metric, which correlates with intraretinal perfusion and can be considered an indirect measure thereof.[3,4,7] The higher resolution of HR OCTA acquisition likely improves its ability to distinguish intraretinal capillaries, especially the smaller vessels, as well as their geometry, which would seem to lend itself to a more precise VT assessment. This process leads to the detection of higher VT values for HR OCTA than HS OCTA, and we might hypothesize that this effect is an indirect sign of a more stable measurement of intraretinal perfusion. Similar considerations might apply to the HR/HS differences regarding CC porosity. Indeed, HR OCTA turned out to detect significantly more perfusion signal than HS OCTA, proving more reliable in reconstructing the sponge-like structure of the CC. Once again, in view of the growing interest in the diagnostic role of CC flow void measurement,[15–19] studying the HR/HS OCTA CC gap might provide useful new diagnostic assessments and improve the detection of chorioretinal perfusion impairment in retinal diseases.

Another feature typifying HR OCTA is its significantly greater vessel diameter compared with HS OCTA, which tallies with the observations already made in a previous investigation.[9] This aspect may be explained by the laminar nature of the intracapillary blood flow. Indeed, if we see each vessel as a tube, the flow is faster at the center and gradually slows down closer to the wall owing to the effect of friction. Moreover, the visual inspection of the images highlighted the fact that the most evident HR/HS difference is to be found at the vessel bifurcations, where the blood flow becomes turbulent. Our main hypothesis is that HR OCTA's higher resolution of might enable it to detect a wider range of perfusion signal than HS OCTA, and this process might include detecting a perfusion signal even in the presence of turbulence. We measured this difference with the newly introduced HR/HS overlapping gap metric, which has been developed to quantify the amount of additional perfusion signal detected by HR OCTA, corresponding with 22% for SCP + DCP and 4% for CC in healthy samples. The small HR/HS CC overlapping gap might be explained by the fact that the sponge-like organization of the CC is less affected by blood flow speed-related issues than the more strictly the tubular SCP and DCP. In addition, the lobular organization of the CC, as described by histology,[20] might decrease the range of blood flow speeds, thus rendering the sensitivity of HR and HS OCTA acquisitions comparable. These findings further support a recent investigation that emphasized the high reliability and repeatability of HR and HS OCTA modalities, although highlighting differences in terms of vessel detection.[11] However, one of the novelties of the present investigation regards the exceptional detailing provided in assessing the HR/HS differences in SCP-DCP or CC networks. Moreover, our analyses stressed the important contribution of retinal vessel bifurcation to the different levels of perfusion detection shown by HR and HS OCTA acquisitions. More important, this study introduces a completely new quantitative metric, namely the HR/HS overlapping gap. This metric represents the first step in the development of new quantitative approaches focused on detecting different perfusion signals characterizing a vascular network. This factor has potentially interesting clinical applications, because we might hypothesize that the decreased perfusion typical of retinal diseases leads to a much greater HR/HS overlapping gap. In this scenario, the HR/HS overlapping gap might provide ways of classifying retinal disease subtypes better and of stratifying the risk of disease progression and the onset of complications.

We are aware that this study is burdened by a number of limitations. The cross-sectional design of the study prevented us from measuring possible interscan variability and performing reliability assessments. These must be left to possible future studies. In contrast, we are confident of the high reliability of HR and HS OCTA, as already demonstrated by Corvi et al. (2020),[11] and we considered additional reliability analyses not essential to achieve the main goals of our study. The number of eyes included is

relatively small, although the exploratory nature of the investigation and the low variability of the collected metrics might suffice to consider the paper's findings reliable. Even so, further larger scale investigations are warranted to support our data. We must acknowledge that using an ART 7 setting instead of ART 5 could have provided higher quality images. However, the very long acquisition time required for HR OCTA with ART 7 irremediably increases the number of artifacts and the subjects' distress, and also makes this approach less suitable for patients affected by retinal diseases who show poor fixation. Hence, we chose ART 5 both for HR and HS OCTA acquisitions, considering it a good compromise in terms of image quality and, more important, an acquisition protocol that can be applied to retinal diseases. Moreover, our theoretical and clinical speculations are merely based on the interpretation of the current findings and would benefit from validation obtained from other angiographic techniques and histology. Because of the investigation's nature as a feasibility study, we included a limited number of samples. And of course all imaging-based approaches are prone to artifacts, undermining the reliability of the findings.[7,21] We included only very high-quality data to restrict potential biases, but further studies are warranted to reproduce our study and to provide support for our findings.

In conclusion, this study provides quantitative data supporting the reliability of both HR and HS OCTA acquisitions. HR OCTA can provide much more information regarding the perfusion status of SCP, DCP and CC than HS OCTA. The latter can be considered a valid choice for patients with poor fixation. We put forward a new OCTA quantitative assessment based on the combined evaluation of FAZ, vessel diameter, and HR/HS overlapping gap, under the umbrella term HR/HS OCTA gap. The result is a fresh range of possibilities regarding the detection of different blood flow speeds, paving the way for a new generation of OCTA quantitative assessments in retinal diseases.

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