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ABSTRACT

Purpose: To correlate the appearance of Microaneurysms (MAs) on structural spectral-domain optical coherence tomography (SD-OCT) with their detection on OCT angiography (OCTA) in patients with non-proliferative diabetic retinopathy (NPDR).

Design: Inter-instrument reliability study.

Methods: Sixteen patients with NPDR without macular edema underwent SD-OCT and OCTA. To compare MAs seen on OCTA to those on SD-OCT, we superimposed the OCTA superficial capillary plexus (SCP) vascular landmarks onto those of the near infrared. Two observers blinded to patient groupings evaluated reflectivity of MAs on SD-OCT scans, graded as hypo-, moderate, or hyper-reflective, and their visualization at the level of SCP and deep capillary plexus (DCP) on OCTA.

Results: Among 145 MAs imaged with SD-OCT, 47 (32.4%) appeared as hyperreflective, 71 (49%) as moderately reflective, and 27 (18.6%) as hyporeflective. After excluding 3 eyes (10 MAs) because of poor quality OCTA scans, 135 MAs were evaluated on OCTA; 76 (56.3%) were visible only in the DCP, 9 (6.7%) only in the SCP, 29 (21.5%) were visible in both SCP and DCP; 21 (15.6%) were not visible on OCTA. Compared to MAs with hyper or moderate reflectivity, MAs with hypo reflectivity on structural SD-OCT were significantly less likely to be detected on OCTA (OR: 4.6; 95% CI: 1.5-14.0, $p = 0.008$; and OR: 4.2, 95% CI 1.2-14.2, $p = 0.022$, respectively).

Conclusions: MAs that appear hyporeflective on structural SD-OCT have a lower detection rate on OCTA. The results of this study may help further understand the different blood flow dynamics pattern in MAs.

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4 **Relationship between internal reflectivity of diabetic microaneurysms on SD-OCT**
5 **and detection on OCT Angiography**
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52 **Short Title:** Microaneurysms reflectivity on SD-OCT and OCT Angiography
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INTRODUCTION

Diabetic retinopathy (DR) is the leading cause of blindness among working aged individuals in the developed world and its prevalence increases with increasing duration of the disease. Loss of pericyte cells and proliferation of endothelial cells are early pathologic changes in DR, which lead to weakened vascular walls and the formation of microaneurysms (MAs).¹⁻³ Macular edema, which often arises from leaking MAs, is a common cause of DR related vision loss.^{4,5}

In the past, most knowledge regarding diabetic MA features was obtained from histological and pathological studies.⁶⁻⁸ These studies have shown that diabetic MAs are vascular outpunchings of the retinal capillary bed, primarily arising from the deep part of the inner retinal capillary plexus located in the inner nuclear layer.

Fluorescein angiography (FA) has been widely used to detect early signs of DR^{9,10} including MAs. However, this examination is invasive, costly and time consuming. Recent advances in imaging techniques, such as spectral-domain optical coherence tomography (SD-OCT), have improved image resolution and reduced speckle noise, clearly delineating the individual retinal layers and several lesions of the retinal parenchyma and vasculature.

More recently, optical coherence tomography angiography (OCTA), a fast, noninvasive imaging technique, has allowed for three-dimensional vascular mapping of macular vasculature and differentiation of the superficial and deep vascular plexus.¹¹⁻¹⁴ Several studies have reported new findings of vascular changes in diabetic patients using OCTA such as foveal avascular zone shape changes, retinal non-perfusion, and microaneurysms.¹⁵⁻¹⁸

The purpose of this study was to correlate the appearance of MAs on SD-OCT with their detection on OCTA in patients with non-proliferative DR.

MATERIAL AND METHODS

In this inter-instrument reliability study, MAs from eyes of type 2 diabetic patients with mild, moderate or severe non-proliferative DR were randomly selected and analyzed at the Department of Ophthalmology, G.B. Bietti Eye Foundation–IRCCS, Rome, between March 15, 2016 and July 15, 2016. This study was approved by the Institutional Review Board of the G.B. Bietti Eye Foundation-IRCCS, and followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Inclusion criteria were: Patients with type 2 diabetes mellitus, age > 18 years old, and clinical evidence of DR on ophthalmologic exam, ranging from mild to severe non-proliferative DR.

Exclusion criteria were: MAs secondary to diseases other than DR (e.g. retinal vascular occlusive diseases), diagnosis of age related macular degeneration, central serous chorioretinopathy, or vitreoretinal interface diseases, diabetic macular edema, diagnosed either clinically or with SD-OCT, that could disrupt the contour of the

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4 segmentation on OCTA, or diffuse edema that could obscure the presence of MAs. We
5 also excluded patients with evidence of significant cataract, graded above NO3 or NC3,
6 in order to avoid optical artifacts that may compromise SD-OCT and OCTA image
7 quality.
8

9 All patients underwent color fundus photograph, SD-OCT, FA and OCTA imaging.
10 For each patient, all analyzed images were obtained on the same day.

11 After acquisition of 45° color fundus photographs (Topcon TRC 50DX, Topcon
12 Corporation, Tokyo, Japan), where the MAs appear as whitish, reddish, or mixed dots⁶,
13 simultaneous FA and SD-OCT (Spectralis HRA+OCT, Heidelberg Engineering,
14 Heidelberg, Germany, version 6.4.7.0) were performed. To match the MAs seen on
15 color fundus photographs to those that appeared as hyperfluorescent dots in the early
16 phase of FA imaging, we superimposed the vascular landmarks of both images;
17 additionally the FA images were overlaid with the near infrared images of the
18 Heidelberg Spectralis HRA+OCT. Color fundus photograph, FA and near infrared were
19 used to verify the identification of MAs detected on SD-OCT. The SD-OCT protocol
20 used included 49 horizontal B-scans per volume scan spanning 20°x20° of the macula
21 volume with either the vertical or horizontal bisecting the center of each microaneurysm
22 (each B-scan including an average of 16 frames).
23

24 To exclude from the selection MAs with fast turnover,^{4, 19} SD-OCT imaging was
25 repeated in all patients, a second time 7 days after the first imaging session. Only MAs
26 present on both dates were included in the analysis. Low-quality SD-OCT images were
27 defined by signal strength below 25 decibels. The internal reflectivity within the lumen of
28 each MA was graded as hypo-, moderate, or hyper-reflective as described previously.²⁰
29 Briefly, the lumen was considered hyperreflective if reflectivity was similar to that of the
30 MA wall, hyporefective if it was similar to that of cystic intraretinal fluid and moderate if
31 the reflectivity was intermediate.
32

33 Finally, to explore the OCTA characteristics of MAs, we used the XR Avanti
34 Optical Coherence Tomography Angiography instrument (Optovue Inc., Fremont,
35 California, USA) with split-spectrum amplitude-decorrelation angiography (SSADA)
36 software.¹² This instrument has an A-scan rate of 70,000 scans per second and uses a
37 light source centered on 840 nm and a bandwidth of 45 nm. A 6 X 6-mm scanning area,
38 centered on the fovea was obtained. Two consecutive B-scans (M-B frames), each
39 containing 304 A scans were captured at each sampling location and SSADA software
40 was used to extract OCTA information. En face OCT angiograms were segmented to
41 define the superficial capillary plexus (SCP) and deep capillary plexus (DCP), using the
42 segmentation algorithm by the built-in software.
43

44 Customized settings were also used to produce thinner slabs that were moved
45 progressively from the outer retina to the inner plexiform layer to look for undetected
46 MAs.
47

48 MAs were identified as focally dilated saccular or fusiform capillaries in the 6x6
49 mm area of the en face OCT angiograms. Low-quality OCTA images defined as OCT
50 angiograms with signal strength below 72 or with motion artifacts, were excluded.
51

52 To compare the MAs seen on OCTA to those seen on Spectralis SD-OCT B-scan,
53 we superimposed the OCTA SCP vascular landmarks onto the vascular landmarks of
54 the near infrared. This allowed point-by-point correlations between both the SCP and
55 DCP and corresponding Spectralis B-scan.
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4 After the characterization of the MA features using OCTA and SD-OCT, the goal
5 of the study was to evaluate if the reflectivity of MAs on SD-OCT scans could influence
6 their visualization at the level either of SCP or DCP on the corresponding OCTA images.
7 With regard to this, the observers determined whether the MAs were visible in the SCP
8 and in the DCP images, both of them or not visible in any scan.
9

10 Two masked examiners (D.D.G, F.S) evaluated all SD-OCT scans and OCTA
11 images independently, and in case of disagreement there was open adjudication until a
12 consensus was established.
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15 **Statistical analysis**

16 Continuous data were described as mean \pm standard deviation and categorical
17 data as frequencies. The association of categorical variables with the groups was
18 calculated using logistic and ordinal logistic regression. Specifically, we investigated the
19 association of increasing reflectivity with MA visibility and position according to OCTA.
20

21 We assessed the inter-grader reliability of the grading of MA reflectivity with SD-
22 OCT using Cohen's weighted kappa for ordered categories. Since MA assessment with
23 OCTA yielded partly unordered categories (MAs non-visible or localized in the
24 superficial layer, deep layer or in both layers) we used an asymptotic symmetry and
25 marginal homogeneity tests.
26

27 Analyses were adjusted for within-subject correlation using mixed models with
28 individuals as a random effect. All statistical analysis was performed using Stata 14.2
29 software (College Station, TX, USA). A $p < 0.05$ was considered statistically significant.
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32 **RESULTS**

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36 One hundred and forty-five randomly selected MAs, identified on color fundus
37 photography, FA, near infrared and B-scan SD-OCT, from 30 eyes of 16 type 2 diabetic
38 patients with mild, moderate or severe non-proliferative DR were analyzed in the study.
39 All 145 MAs selected and analyzed were detectable in the aforementioned techniques.
40 The quality of images was considered as sufficient for qualitative analysis in 100%
41 (30/30) of eyes on SD-OCT and in 90% (27/30) of eyes on OCTA; 10 % (3/30) of eyes
42 imaged with OCTA were not included due to low quality of images. Patients
43 demographics are shown in Table 1.
44

45 Among all 145 diabetic MAs imaged with SD-OCT, 47 (32.4%) was classified as
46 hyperreflective, 71 (49%) as moderate internal reflectivity, and 27 (18.6%) as
47 hyporefective. After excluding 3 eyes with poor quality OCTA scans, of 135 MAs
48 remained, 114 (84.4%) were visible on OCTA, 76 (56.3%) were visible only in the DCP,
49 9 (6.7%) only in the SCP, 29 (21.5%) were visible in both SCP and DCP and 21 (15.6%)
50 were not visible on any OCTA images.
51

52 Considering MAs that appeared hyperreflective on SD-OCT, 40 (88.9%) were
53 visible on OCTA, 28 (62.2%) were visible only in the DCP (Figure 1), 2 (4.4%) only in
54 the SCP, 10 (22.2%) in both the SCP and DCP, and 5 (11.1%) were not visible on any
55 OCTA images.
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4 Among MAs that appeared moderately reflective on SD-OCT 56 (88.9%) were
5 visible on OCTA, 39 (61.9%) were visible only in the DCP, 5 (7.9%) only in the SCP, 12
6 (19%) in both the SCP and DCP, and 7 (11.1%) were not visible on any OCTA images.

7
8 Finally, 18 (66.7%) of MAs that appeared hyporeflective on SD-OCT were visible
9 on OCTA, 9 (33.3%) were visible only in the DCP, 2 (7.4%) only in the SCP, 7 (25.9%)
10 were visible in both the SCP and DCP, and 9 (33.3%) were not visible on any OCTA
11 images (Figure 2) (Table 2). Compared to MAs with hyper reflectivity or moderate
12 reflectivity, MAs with hypo reflectivity on SD-OCT were significantly less likely to be
13 detected on OCTA (OR: 4.6; 95%CI: 1.5-14.0, $p = 0.008$; and OR: 4.2, 95%CI 1.2-14.2,
14 $p = 0.022$, respectively).

15
16 Compared to non-visible MAs, superficial (OR:1.7, 95%CI: 0.4 – 8.4), mixed (OR:
17 2.5; 95%CI: 0.8-7.8) and deep (OR: 3.6; 95%CI: 1.3-9.7) MAs were increasingly more
18 likely to be more reflective (test for trend $p=0.011$), though only the OR of deep MAs
19 reached significance when position was a categorical variable ($p=0.013$).

20
21 Grading of MA hyperreflectivity proved to be highly reliable, since Cohen's kappa
22 was 0.90 between graders. There was also good agreement on retinal layer location of
23 each MA in the symmetry test since only 7 out of 135 ratings disagreed, 4 of which
24 regarded the 'non-visible MA' category ($p=0.960$).

25 26 27 28 29 **DISCUSSION**

30
31 In this study we imaged diabetic MAs in a heterogeneous group of diabetic
32 patients with SD-OCT and OCTA. We assessed the internal reflectivity of MAs on SD-
33 OCT B-scans and evaluated the relationship between this finding and MA visualization
34 on the OCTA images. Interestingly, MAs with internal hyporeflectivity on SD-OCT B-
35 scan had a significantly lower detection rate on OCTA (66.7%) compared to MAs with
36 internal hyperreflectivity (88.9%) or moderate reflectivity (88.9%).

37
38 One possible explanation for this result could be that MAs that appear
39 hyporeflective on SD-OCT are more likely to have a blood flow rate below the threshold
40 necessary to register as flow in the OCTA system. It has previously been reported that
41 the SSSA algorithm does not allow detecting retinal capillary flow less than 0.3 mm per
42 second.²¹ Other authors have also hypothesized that the blood flow inside MAs is
43 turbulent and may not be shown using OCTA.¹⁶ Another possible explanation that has
44 been previously suggested¹⁷ is that the MAs appear hyporeflective because they
45 contain only plasma without erythrocytes. Previous histologic studies have
46 demonstrated that some microaneurysms are not perfused and have extensive luminal
47 fibrosis and lipid infiltration⁷; it is possible that some of the hyporeflective MAs represent
48 these scleroses, poorly perfused MAs.

49
50 Supporting our data, recently Seidel et al.²² reported that a low retinal blood flow
51 velocity reflects in a visually distinct contrast reduction of the intraluminal pattern of
52 retinal vessels on SD-OCT and that an absent pattern highly correlated with a severely
53 diminished blood flow velocity.

54
55 A previous study¹⁷ has evaluated diabetic MAs using OCTA and FA, but to the
56 best of our knowledge this is the first study to compare SD-OCT reflectivity and OCTA
57 features of MAs. Ishibazawa et al.¹⁶ compared FA and OCTA features of 47 eyes of 25
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4 patients with DR and reported that OCTA can clearly visualize MAs, most of which were
5 located in the DCP, and retinal non perfusion areas. Couturier et al.¹⁷ recently analyzed
6 the clinical features of 20 eyes of 14 patients with DR and demonstrated that FA is more
7 sensitive than OCTA in detecting MAs, while OCTA is more accurate compared to FA in
8 assessing capillary non perfusion. The authors also reported that the number of MAs
9 was significantly higher in the DCP than in the SCP. Similarly, looking at the data
10 regarding MAs localization, we found that the majority of MAs visible on the OCTA
11 (56.3%) were isolated to the DCP. Furthermore, we found a significant correlation (p
12 0.011) between the location of selected MAs (SCP or DCP) and the characteristics of
13 internal reflectivity of the lumen on SD-OCT. In particular, the hyperelective MAs were
14 significantly more likely to be detected on DCP in comparison either with the moderate
15 or the hyporelective MAs (p 0.013). Because the MA formation rate and MA turnover,
16 showed positive correlations with increases in retinal thickness²³, we can speculate that
17 the hyperreflective MAs, characterized by an high blood flow rate, might probably be
18 associated with the extracellular fluid accumulation resulting from alteration of the
19 blood-retinal barrier in the DCP. Further studies are necessary to confirm the
20 associations between the Mas reflectivity and alterations of the inner retinal thickness
21 and DCP in diabetic patients. A recent study²⁴ showed that eyes with diabetic macular
22 edema that are poor responder to anti-vascular endothelial growth factor showed a
23 larger number of MAs along with a significantly lower flow density as well as larger area
24 of the foveal avascular zone in the DCP. With regard to this, we believe that our
25 findings in term of MA characteristics and localization could add some information,
26 which in turn would mean improving the identification of a possible biomarker to the
27 diabetic macular edema treatment response.

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34 Taken together, OCTA and SD-OCT MAs findings provide not only static but also
35 dynamic information allowing for a better understanding of the pathogenetic
36 mechanisms of DR. In addition, this study also demonstrate that OCTA is a very useful
37 device to image and study the MA features in diabetic patients, although it may
38 underestimate the presence of MAs that appear hyporelective on SD-OCT.

39
40 There are some limitations of this study mainly related to the inclusion of patients
41 with different disease stages and duration, and to the relatively small numbers of MAs
42 evaluated.

43
44 In conclusion, we demonstrated that MAs that appear hyporelective on SD-OCT
45 have a lower detection rate on OCTA images. This relationship between the internal
46 reflectivity of MAs on SD-OCT and their visualization on the OCTA images may help
47 further understand the different pattern of blood flow dynamics in MAs, and improve our
48 interpretation of MA detection on OCTA.

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4 **Figure Captions**
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9 **Figure 1**

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11 The Spectralis B-scan showing an hyporeflective microaneurysms (yellow arrow) (Top
12 left) and the infrared image (Top right) with the green line with arrow passing through
13 the microaneurysm. This exactly corresponds to the focally dilated microanerysm
14 highlighted by red circle at the level of DCP in the OCTA imaging (6x6 scanning area)
15 (Bottom right); the inset shows the characteristics of hyporeflective microaneurysms in
16 details. *En face* imaging with red and green lines indicating the location of B-scans
17 (Optovue). The DCP segmentation boundaries (green lines) passing through the
18 microanerisysm are visible (Bottom left).
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24 **Figure 2**

25
26 The Spectralis B-scan showing an hyporeflective microaneurysms (yellow arrow) (Top
27 right) and the infrared image (Top left) with the green line with arrow passing through
28 the microaneurysm. The microanerysm (red circle) cannot be detected at the level of
29 the deep capillary plexus (DCP) by means OCT Angiography (OCTA) imaging (6x6
30 scanning area) (Bottom left); the inset (green box) shows the area of interest with more
31 details. *En face* imaging with red and green lines indicating the location of B-scans
32 (Optovue). The DCP segmentation boundaries (green lines) passing through the
33 microanerisysm are visible (Bottom right).
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TABLE 1 - Demographics and characteristics of patients

Patients/Eyes, n	16/30
Male/Female, n	9/7
Age, Years Mean	
Mean±SD	64.3±8.16
Range	52-77
Duration of diabetes, years	
Mean±SD	9.3±5.2
Range	4-20
HbA1c (%) Mean ± SD	8.1±1.6
Range	6.4-11.4
DR stage, n eyes (%)	
Mild nonproliferative	8 (26.7)
Moderate nonproliferative	12 (40)
Severe nonproliferative	10 (33.3)

n, number; SD, standard deviation; DR, diabetic retinopathy; HbA1c, glycated hemoglobin;

Table 2 – SD-OCT internal reflectivity and OCTA visualization of diabetic microaneurysms

Internal reflectivity		All MAs (N=145) N (%)	OCTA Visualization				
			SCP N (%)	DCP N (%)	SCP+DCP N (%)	Total Not Visible N (%)	N/A
S D - O C T	Hyper	47 (32.4)	Total visible N (%)			5 (11.1)	2
			40 (88.9)				
				2 (4.4)	28 (62.2)	10 (22.2)	
	Moderate	71 (49)	Total visible N (%)			7 (11.1)	8
			56 (88.9)				
				5 (7.9)	39 (61.9)	12 (19)	
Hypo	27 (18.6)	Total visible N (%)			9 (33.3)	-	
		18 (66.7)					
			2 (7.4)	9 (33.3)	7 (25.9)		

N, number; MAs, Microaneurysms; SCP, Superficial capillary plexus; DCP, Deep capillary plexus; N/A, Not Applicable

*The OCTA percentages are considered in relation to the total of evaluable MAs

Figure 1
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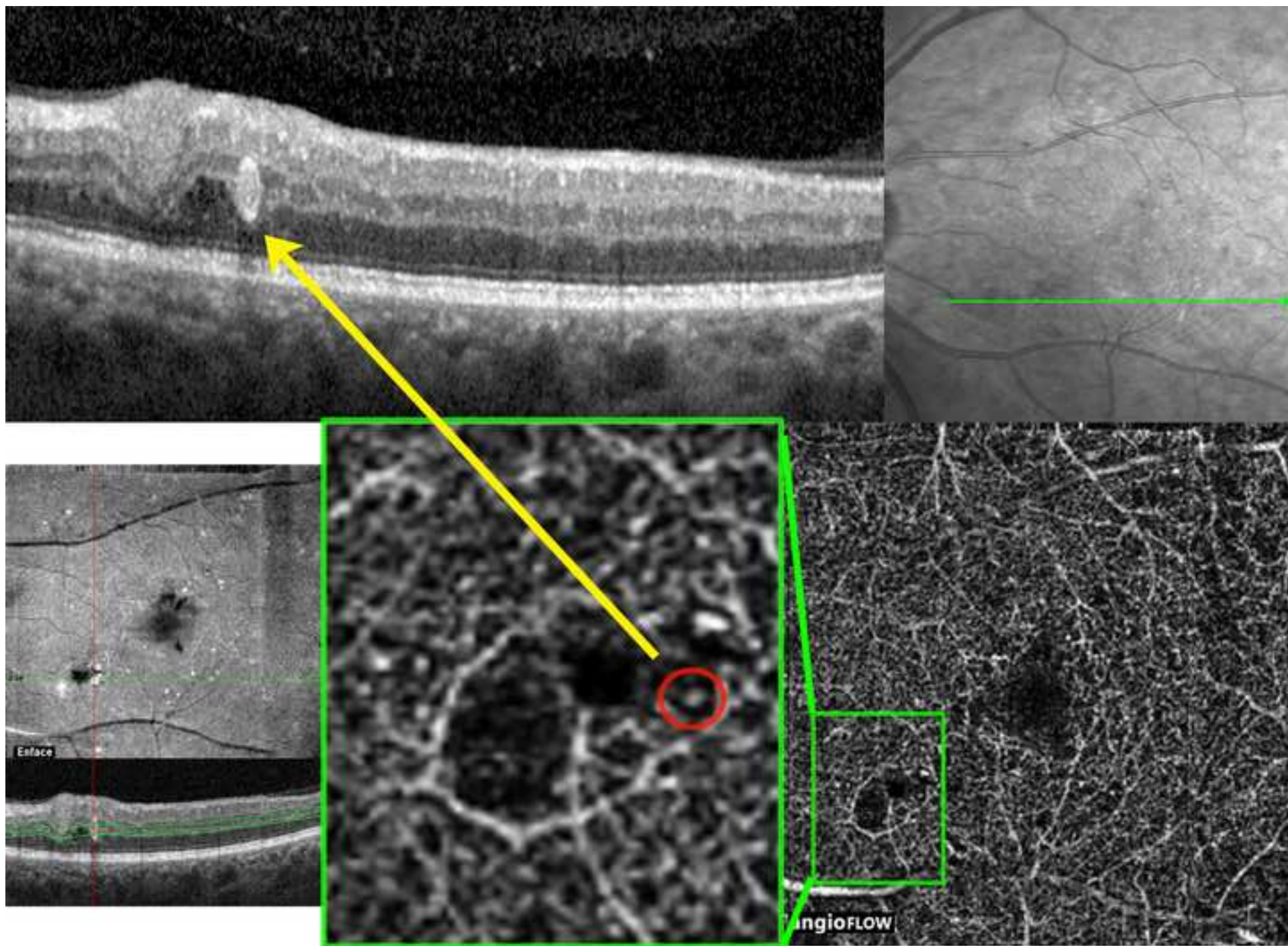
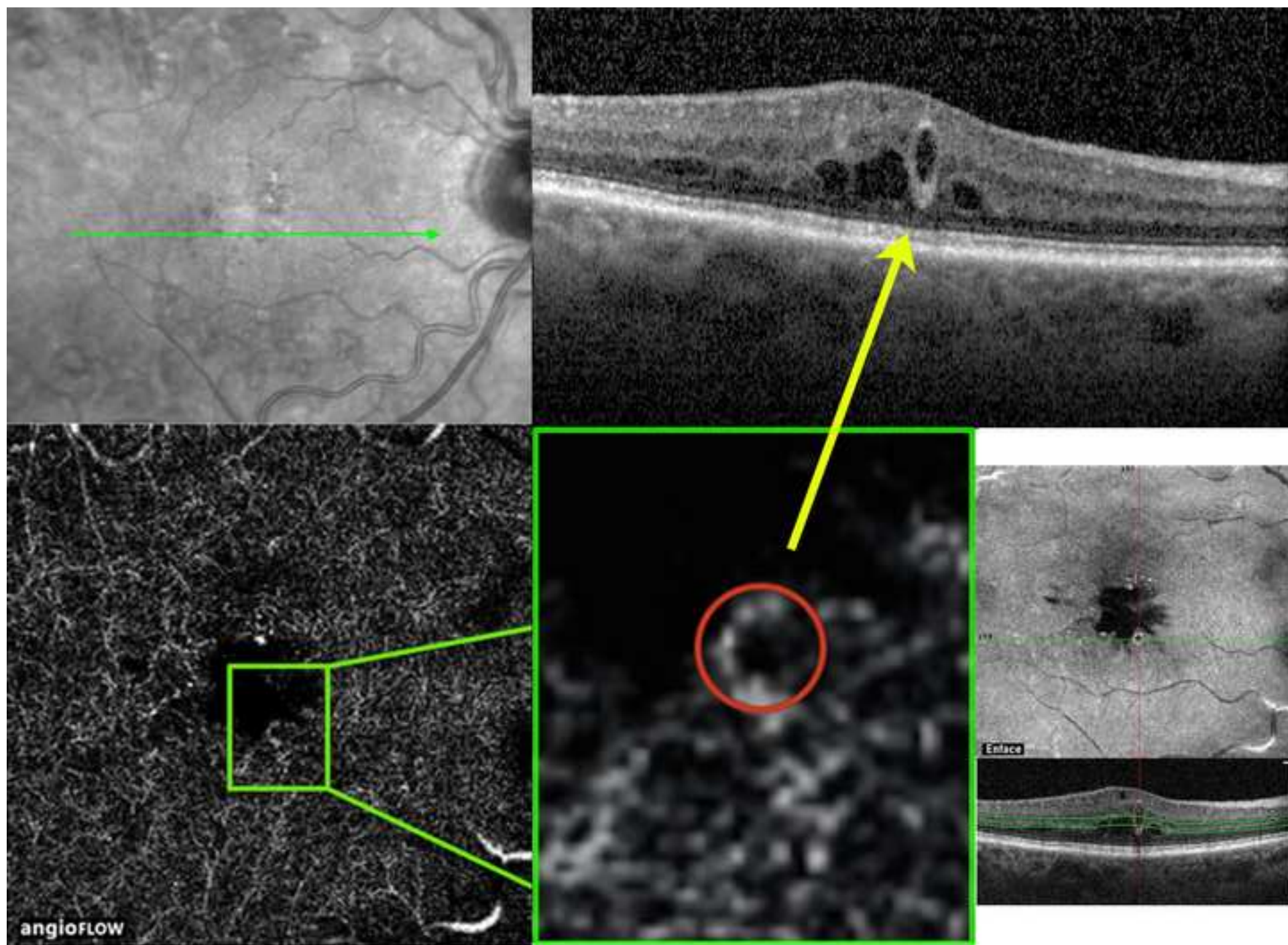


Figure 2
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Relationship between internal reflectivity of diabetic microaneurysms on SD-OCT and detection on OCT Angiography

Manuscript number: AJO-17-69

This study shows a lower detection rate on optical coherence tomography angiography of microaneurysms that appear hyporeflective on spectral domain optical coherence tomography. On the contrary, the majorities of hyperreflective microaneurysms are visible on the optical coherence tomography angiography and are isolated at the level of the deep capillary plexus. Because hyperreflective microaneurysms are characterized by a high blood flow rate, this finding could suggest an important role in the pathophysiology of diabetic macular edema.

Biosketch Dr. Parravano

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Biosketch Photo: File name must be authors name.
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