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New approaches to imaging and treatment of ocular melanoma

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Anterior Segment OCTA of Melanocytic Lesions of the Conjunctiva and Iris

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ABSTRACT

PURPOSE: To study the feasibility and diagnostic value of vascular imaging using optical coherence tomography (OCT)-angiography (OCTA) of melanocytic lesions of the conjunctiva and iris.

DESIGN: Cross-sectional study.

METHODS: Twenty-five patients with an untreated conjunctival lesion (5 melanoma, 13 nevus, 7 primary acquired melanosis [PAM]) and 52 patients with an untreated iris lesion (10 melanoma, 42 nevus) were included. Patients were imaged using a commercially available OCTA device, with the addition of an anterior segment lens and manual focussing. Tumor vessel presence, vascular patterns and vascular density were assessed.

RESULTS: Good OCTA images were obtained in 18 of 25 conjunctival lesions and 42 of 52 iris lesions. Failure was caused by lack of patient cooperation, an unfavorable location, or mydriasis. In all imaged conjunctival lesions and 77% of iris lesions, vascular structures were detected. Conjunctival melanoma and nevi demonstrated the same intralesional tortuous patterns, whereas vasculature in eyes with PAM was similar to normal conjunctiva. Both iris melanoma and nevi demonstrated tortuous patterns, distinct from the radially oriented normal iris vasculature.

CONCLUSIONS: Optical coherence tomography angiography (OCTA) allows for noninvasive imaging of the vasculature in melanocytic lesions of the conjunctiva and iris. Good image quality depends highly on patient cooperation and lesion characteristics. Differentiation of benign and malignant lesions was not possible. New software is called for to improve image acquisition and analysis.

INTRODUCTION

Nevi of the conjunctiva or iris are relatively common conditions, requiring no further treatment apart from close observation or excision for cosmetic reasons.^{1,2} Malignant melanoma of the conjunctiva or iris, however, have the potential to metastasize and treatment is generally indicated.^{3,4} Clinical differentiation of these entities can be challenging,^{1,2} despite imaging techniques such as ultrasonography and optical coherence tomography (OCT) of the anterior segment.

A factor to differentiate melanocytic lesions of the eye, is tumor vascularity. Fluorescein angiography (FA) has long been used to differentiate between benign and malignant choroidal lesions.^{5,6} Since the early 1970s, FA was used to study iris lesions as well.^{7,8} Benign iris lesions demonstrated less chaotic patterns than malignant lesions and even showed silencing (masking) of the FA signal,⁹⁻¹² but there has been no full agreement on all FA findings, such as orderly patterns or leakage of dye.^{11,13-15} The usefulness of dye-based angiography of the conjunctiva has long been limited since low-molecular-weight fluorescein easily leaks from vessels,¹⁶ and to these authors' knowledge, no studies of dye-based angiography of the intrinsic vasculature of conjunctival melanoma, nevi or primary acquired melanosis (PAM) exist. As a drawback for FA, the technique is relatively time consuming and requires injection of dye, with potential adverse events.^{17,18}

A new and non-invasive technique to study ocular vasculature is OCT-Angiography (OCTA). This technique has been developed to depict the retinal vasculature.¹⁹ By measuring changes in sequential cross-sectional images (B-scans), flow can be visualized without the need for intravenous dye injection. OCTA has several additional advantages, such as high image quality, visualization of vessels at different depths, and easy operating instructions. Recently it was demonstrated that, apart from imaging retinal vessels, OCTA can image tumor vessels in choroidal melanoma and nevi as well.²⁰⁻²²

With some adjustments to the conventional posterior segment technique (using an anterior segment lens, and (manual) focussing on the ocular surface), OCTA can detect vascular flow in the anterior segment as well.²³ Small series have demonstrated proof-of-principle in iridal, corneal and conjunctival tissue.²⁴⁻²⁶ Most commercially available OCTA devices do not support the full range of image acquisition and analysis modes for the anterior segment that were developed for retinal images; however, and obtaining good image quality can therefore be challenging.²⁷ Using OCTA of the anterior segment, Skalet and associates demonstrated vascular patterns in pigmented lesions of the iris and found a difference in vascular density between three iris nevi and three iris melanomas.²⁸ To the present authors' knowledge, no studies of OCTA of melanocytic lesions of the conjunctiva exist.

The feasibility of OCTA of melanocytic lesions of the conjunctiva and iris were studied, with a second aim of identifying differentiating features between benign and malignant lesions.

METHODS

Patient selection

Patients were recruited at the Leiden University Medical Center (LUMC), a tertiary hospital for ocular oncology in The Netherlands. Included were patients with a treatment-naïve conjunctival melanoma (n=5), conjunctival nevus (n=13), primary acquired melanosis (PAM, n=7), iris melanoma (n=10), or iris nevus (n=42). Patients were seen and diagnosed in the ophthalmology clinic of the Department of Ophthalmology. Standard clinical examinations were performed including slit lamp examination and anterior segment photography.

Approval for this cross-sectional study was obtained from the Institutional Ethics Review Board of the LUMC (approval number P17.134). The tenets of the declaration of Helsinki were followed. All patients signed an informed consent.

Conjunctival lesions

All 5 conjunctival melanoma cases were confirmed by histology. In 9 of 13 cases, the diagnosis of conjunctival nevus was confirmed by histology; 4 cases were diagnosed clinically (with circumscribed lesions and cysts; in 3 of those cases the lesion had not changed in more than 30 years, and in 1 case in more than 9 years).^{2,29} Six cases were diagnosed with PAM, which was confirmed by biopsy (showing 4 cases with atypia, and 2 without), whereas 1 case was diagnosed clinically (with a unilateral diffuse lesion and variable presence [i.e. “waxing and waning”]).² Where possible, the thickness of conjunctival lesions was determined by histology, the largest basal diameter was assessed by clinical measurements.

Iris lesions

Ultrasonography was performed on all iris lesions to determine lesion size and extent. Iris lesions were diagnosed by clinical and ultrasonographic characteristics. Diagnosis of melanoma was based on tumor size, evidence of growth, ultrasonographic structure, visibility of vessels, and secondary symptoms such as elevated intraocular pressure or cataract.³⁰⁻³³ Biopsies were only performed in 2 patients, confirming the clinical diagnosis of 1 iris nevus and 1 iris melanoma.

OCTA acquisition and analysis

Images were taken prior to any surgical procedure. OCTA scans were performed using an “RS 3000 Advance” OCTA device (Nidek, Ltd, Gamagori, JA). A device-specific anterior segment lens was inserted. Patients were positioned in front of the device and instructed to focus on either the external fixator light or gaze in a specific direction. Conjunctival lesions were assessed under dimmed light conditions, and iris lesions were assessed under ambient room lighting to create miosis. First, the anterior segment lens was positioned close to the ocular surface to retrieve a signal from the tissue of

interest. Second, the focus was manually adjusted to obtain the clearest view. Images were acquired in retinal mode (while focussing on the conjunctiva or iris), with an image size setting of 3x3 mm for all patients (for detailed vessel density calculation), and additional scans of up to 9x9 mm were taken for larger lesions (for an overview). Image resolution of all scans (regardless of the size of the depicted area) was 256x256 pixels. Images were acquired in 'skip mode', as the trace function (developed for retinal imaging) proved not suitable to trace the surface of the conjunctiva or iris.

Images were assessed using the device-specific software Navis-Ex version 1.8 (Nidek). As the automated image segmentation (developed to delineate retinal layers) misinterpreted the conjunctival and iridal structures, 2 segmentation lines were drawn manually to include the lesion of interest.

First, it was recorded if an acceptable image was obtained, that is, if a full scan was completed, including the lesion of interest, without major surface-trace issues resulting in total artefacts. Second, the presence of vessel-like segments was noted, in healthy conjunctiva or iris tissue (to assess successful imaging) and at the site of the lesion. When vessels were visible in the lesion, image quality of those vessels was graded subjectively as (grade 1) clear visibility, (grade 2) medium visibility, or (grade 3) nonclear visibility.

Vessel density (VD) was quantified in the en-face images of a subset of patients using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). The lesion of interest was selected manually, binarization was performed with an Otsu threshold.³⁴ In the area of interest, pixels attributed to vessels (those above the threshold) were counted and expressed as a percentage of the total selected area size.

In conjunctival lesions, VD was first calculated in all bulbar lesions (excluding lesions of the caruncle or plica), and a second analysis was performed excluding heavily pigmented and grade 3 quality lesions (to limit the influence of signal masking by heavy pigmentation and noise). In iris lesions, the VD was similarly calculated in those cases with a light or modest tumor pigmentation and grades 1-2 image quality. The en-face area of ectropion uveae was excluded from analysis.

Anterior Segment-OCT imaging

Each lesion was assessed with high-resolution anterior segment (AS)-OCT using the same imaging device that was applied for AS-OCTA. The anterior segment lens was inserted, capturing images in regular anterior segment mode. Images with a width of 6.0 mm were acquired in a radial pattern at 'ultra fine' resolution.

Statistical analysis

Statistical analyses were performed using SPSS version 23 software (IBM, Armonk, New York, USA). For all analyses, p values < 0.05 were considered significant. Differences between continuous data were tested using the Mann-Whitney U or Kruskal-Wallis test. Differences between discrete data were tested with the Fisher exact or linear-by-linear test.

FOV when examining the anterior segment

Due to different optics when examining the anterior segment, the automated scales to measure retinal or choroidal structures were not fully applicable to this study. To determine the field of view (FOV) of the anterior segment, a calliper was placed with a fixed width of 9.0 mm in front of the anterior segment lens and a scan was acquired with a setting of 9 x 9 mm (Supplemental Figure; available at AJO.com). By comparing the ratio between the calliper size and the scanned image size, the true FOV in the settings proved to be 12.3 x 12.3 mm. A similar technique to determine the FOV of anterior segment OCTA was reported by Liu and associates, resulting in comparable (yet device-specific) values.³⁵

RESULTS

Patients

Twenty-five patients with conjunctival lesions were included. Five of the lesions (20%) were diagnosed as conjunctival melanoma, 13 (52%) as conjunctival nevus, and 7 (28%) as PAM. The mean age of patients with a conjunctival lesion was 48.2 year. All lesions were epibulbar or involved the plica or caruncle; all 5 conjunctival melanoma were stage pT1a (TNM staging system, 8th edition).³⁶

Fifty-two patients with an iris lesion were included. Ten of them (19%) received diagnoses of iris melanoma and 42 (81%) of iris nevus. The mean age of patients with an iris lesion was 61.4 years. The TNM stage of the iris melanoma was T1a (n=6), T1b (n=1), T1c (n=1), T2a (n=1); 1 case of predominantly ciliary body melanoma with significant iris involvement was T2b (TNM staging system, 8th edition).³⁷ Further patient and tumor characteristics are provided in Table 1.

Table 1. Characteristics of the included patients.

	Conjunctival Melanoma n=5 Cases (%)	Conjunctival Nevus n=13 Cases (%)	PAM n=7 Cases (%)	Iris Melanoma n=10 Cases (%)	Iris Nevus n=42 Cases (%)
Sex					
Male	2 (40)	4 (31)	2 (29)	5 (50)	23 (55)
Female	3 (60)	9 (69)	5 (71)	5 (50)	19 (45)
Mean age, y	61.0	38.2	57.7	60.4	61.6
Mean thickness, mm ^a	0.77	N.A.	N.A.	2.0	1.1
Mean LBD, mm	6.6	3.3	N.A.	6.4	3.9
Lesion Pigmentation					
Amelanotic/light	3 (60)	5 (38)	3 (43)	2 (20)	15 (36)
Medium	1 (20)	4 (31)	4 (57)	5 (50)	13 (31)
Dark	1 (20)	4 (31)	0 (0)	3 (30)	14 (33)
Location in the conjunctiva					
Bulbar	5 (100)	7 (54)	7 (100)	N.A.	N.A.
Plica/caruncle	0 (0)	6 (46)	0 (0)	N.A.	N.A.
Location in an iris quadrant					
Superior	N.A.	N.A.	N.A.	1 (10)	6 (14)
Inferior	N.A.	N.A.	N.A.	6 (60)	20 (48)
Temporal/Nasal	N.A.	N.A.	N.A.	3 (30)	16 (38)
Mean clock h, size	N.A.	N.A.	N.A.	2.4	1.7
Secondary cataract	N.A.	N.A.	N.A.	0 (0)	1 (2)
IOP > 20 or IOP treatment	N.A.	N.A.	N.A.	3 (30)	7 (17)
Ectropion uveae	N.A.	N.A.	N.A.	6 (60)	20 (48)
Iris Color					
Blue	N.A.	N.A.	N.A.	10 (100)	37 (88)
Green	N.A.	N.A.	N.A.	0 (0)	4 (10)
Brown	N.A.	N.A.	N.A.	0 (0)	1 (2)

IOP = intraocular pressure; LBD = largest basal diameter; N.A. = not applicable; PAM = primary acquired melanosis. Values are n (%).

^a Data for lesion thickness (assessed by histology) were available for all conjunctival melanoma but none of the conjunctival nevi or PAM.

Conjunctival lesions

OCTA scans were acquired successfully in 4 of 5 (80%) conjunctival melanoma patients, and 8 of 13 (62%) conjunctival nevus patients. Causes for failure were patient noncooperation (n=3), lack of focus (n=2 caruncular lesions), or a bulbar location behind the upper eyelid (n=1). Manual lifting of the upper eye lid proved not feasible as this resulted in minor movements that hampered the investigation (Supplemental Table; available at AJO.com).

In all 4 successfully imaged conjunctival melanoma, and all 8 successfully imaged conjunctival nevi, tortuous vascular structures were detected, distinct from the adjacent conjunctiva (Figure 1). Image quality was better in lightly pigmented lesions compared to dark lesions (Table 2). The plica/caruncle proved difficult to image due to its irregular shape (Table 2). In many nevi, cysts were observed that lacked vascularity. No clear differences were seen between vascular patterns of conjunctival nevi and melanoma.

Table 2. Comparison between the Visibility of Vessels (Image Quality) and Lesion Characteristics.

Image quality	Clear n=13 Cases (%)	Medium n=18 Cases (%)	Nonclear n=21 Cases (%)	p-value
Lesion Pigmentation (<i>all</i>)				
Amelanotic/light	9 (69)	7 (39)	5 (24)	0.006 ^a
Medium	3 (23)	9 (50)	9 (43)	
Dark	1 (8)	2 (11)	7 (33)	
Mean patient age, y	44.2	60.5	61.2	0.019 ^{b c}
Location (<i>Conjunctival lesions</i>)	n=4	n=10	n=4	
Bulbar	4 (100)	9 (90)	2 (50)	0.065 ^a
Plica/caruncle	0 (0)	1 (10)	2 (50)	
Location (<i>Iris lesions</i>)	n=9	n=8	n=17	
Superior	0 (0)	0 (0)	2 (12)	0.45 ^a
Inferior	5 (56)	7 (88)	9 (53)	
Temp/Nasal	4 (44)	1 (12)	6 (35)	

Values are n (%).

^a Linear-by-linear test;

^b Kruskal-Wallis test;

^c P value comparing age in groups 'clear' vs 'medium' quality is p=0.022 (Mann-Whitney U test)

In 6 of 7 patients (86%) with PAM, OCTA scans were acquired successfully. In 1 case, the lesion proved small and was misidentified during scanning. Vascular structures were detected in all images, both when the full conjunctiva and sclera were selected, and when only a superficial layer of tissue (approximating the epithelium) was selected for analysis (Figure 2). The vasculature at the area of PAM appeared similar to the normal conjunctival vessels of the contralateral eye, although in PAM the vasculature appeared to be somewhat finer (Figure 2).

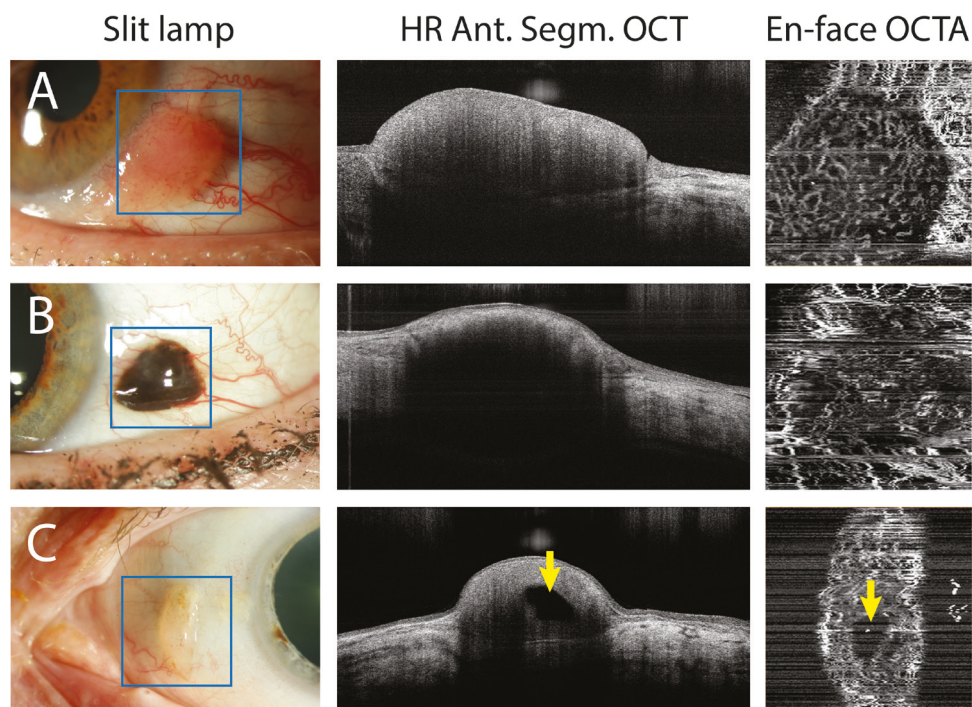


Figure 1. Conjunctival melanoma and nevi. Vascular structures were seen in all conjunctival lesions. Image quality was higher in lightly pigmented lesions (Patient A, conjunctival melanoma) compared to those with dark lesions (Patient B, conjunctival melanoma), and had better signal penetrance. In conjunctival nevi (Patient C), cysts were often seen as areas with no apparent vasculature. (All OCTA scans were acquired in a 3x3mm setting.)

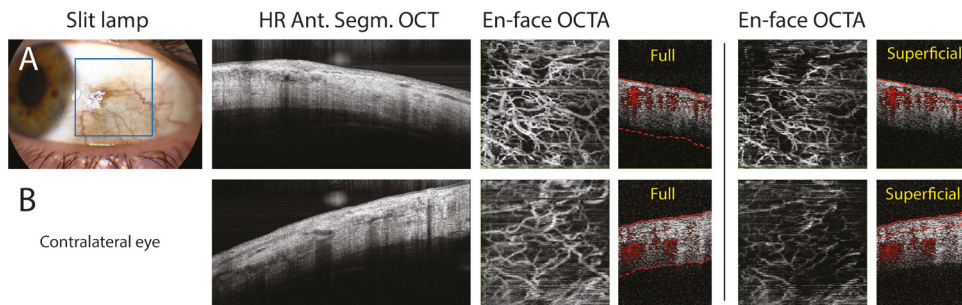


Figure 2. PAM and healthy conjunctiva. The vascular pattern in eyes with PAM (A, top row) was similar to the pattern in the contralateral eye with unaffected conjunctiva (B, bottom row), although the meshwork might have been somewhat finer. Both a full-thickness selection of tissue (including sclera and conjunctiva) and a superficial layer selection (approximating the conjunctival epithelium) are presented. (All OCTA scans were acquired in a 3x3mm setting.) PAM = primary acquired melanosis.

The median vascular density (VD) of quantified bulbar lesions was 35.5% in conjunctival melanoma (n=4), and 32.5% in conjunctival nevi (n=5) ($p=0.62$). In a further selection of light and medium pigmented cases only, this was 38.3% (melanoma, n=3) and 37.0% (nevi, n=3) ($p=0.51$) (Table 3). The median VD of PAM was 40.3% (n=5), whereas that of paired conjunctiva tissue of contralateral eyes was 41.1% ($p=0.14$). The VD was decreased in heavily pigmented conjunctival lesions compared to lightly pigmented lesions (median VD 29.1% n=3, and 38.4% n=12, $p=0.014$) (Figure 3). The presence of cysts was noticed: cysts were seen on scans in 0 of 6 melanoma, 4 of 8 nevi and 0 of 6 PAM, $p=0.046$.

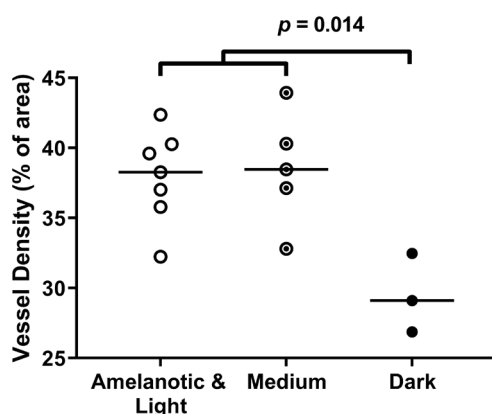


Figure 3. Vascular density and pigmentation of conjunctival lesions. The VD was higher in lightly pigmented lesions compared to dark lesions ($p=0.014$), suggesting a masking effect due to pigment. VD = vessel density.

Iris lesions

OCTA scans were acquired successfully in 9 of 10 patients (90%) with iris melanoma and 35 of 42 patients (83%) with iris nevi. Unsuccessful acquisition was due to patient noncooperation (n=4), pharmacological mydriasis (n=2), a lesion located too far in the ciliary body (n=1), or a location behind the upper eyelid (n=1). Similar to the investigation of conjunctival lesions, manual lifting of the eyelid proved not feasible as this induced movement (Supplemental Table; available at AJO.com).

Vascular structures were seen in the area of the lesion in all 9 (100%) successfully imaged iris melanoma, and 25 of 35 iris nevi (71%) (p=0.09) (Table 3). Of the 10 cases with no visible lesion vessels, 8 cases (80%) did demonstrate vessels outside the lesion, indicating that the OCTA technique was feasible, but lesion characteristics (such as significant masking, or absence of vessels) caused a reduced vascular signal.

Table 3. Comparing OCTA Features among Various Lesions.^f

	Conjunctival Melanoma n=4 Cases (%)	Conjunctival Nevi n=8 Cases (%)	PAM n=6 Cases (%)	P value	Iris Melanoma n=9 Cases (%)	Iris Nevi n=35 Cases (%)	P value
Vessels in lesion							
Present	4 (100)	8 (100)	6 (100)	N.A.	9 (100)	25 (71)	0.09 ^a
Absent	0	0	0		0 (0)	10 (29)	
Vessel visibility							
1 (clear)	1 (25)	2 (25)	1 (17)	1.00 ^b	2 (22)	7 (20)	0.071 ^b
2 (medium)	2 (50)	4 (50)	4 (67)		2 (22)	6 (17)	
3 (nonclear)	1 (25)	2 (25)	1 (17)		5 (56)	12 (34)	
Vessels absent					0 (0)	10 (29)	
VD (overall)	n=4	n=5	n=6		n=4	n=13	
Median (%)	35.5	32.5	39.4	0.14 ^c	31.8	30.5	0.82 ^d
VD (selection ^e)	n=3	n=3	n=5		n=4	n=12	
Median (%)	38.3	37.0	40.3	0.26 ^c	31.8	29.8	1.00 ^d

NA = not applicable; PAM = primary acquired melanosis; VD = vessel density.

^a Fisher Exact test. ^b Linear-by-linear test. ^c Kruskal-Wallis test. ^d Mann-Whitney U test. ^e in the selection of lesions with a light/medium pigmentation and grades 1-2 image quality. ^f Reported are patients whose imaging scans were 'acceptable'. P-values refer to the comparison of all conjunctival lesions, and all iris lesions, respectively.

In healthy iris tissue, radially oriented vessels were seen (Figure 4). The vasculature was remarkably more pronounced in the posterior layers of iris stroma than in the anterior layers (Figure 4). This is consistent with findings from FA studies, describing that iris veins (which are larger and more tortuous than arteries) are located in the posterior stroma.³⁸

Tortuous patterns were visible in iris melanoma as well as nevi (Figure 5). Image quality was graded as good or medium in 4 cases (44%) of melanoma, and 13 cases (37%) of nevi. Image quality was better in amelanotic or lightly pigmented lesions than in those with dark pigmentation, and in those with a younger age than those who were older (Figure 5, Table 2). In cases with ectropion uveae, it was seen that the OCTA signal was blocked, causing a shadow on the cross-sectional B-scan, and an apparent avascular area on the en-face OCTA (Figure 5). We did not see clear differences in vascular patterns between iris nevi and melanoma. The signal tended to be more often absent or of low quality in nevi; however, that could have been due either to masking or because of a truly reduced vascular density ($p=0.07$).

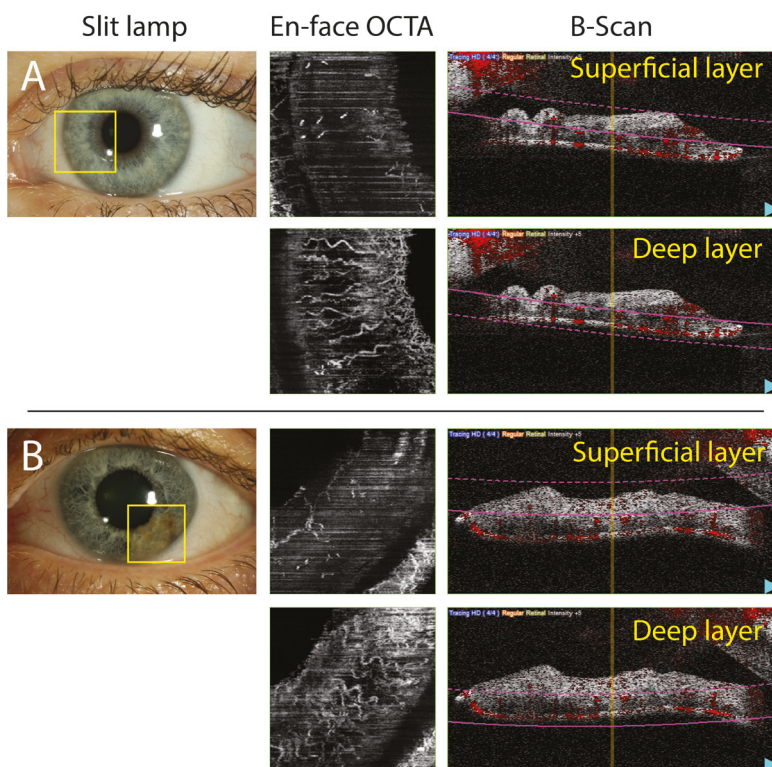


Figure 4. Iris vessels at different depths of tissue. (A) In a healthy iris, the radial pattern of iris vessels was clearly visible with OCTA. The vasculature was often more pronounced in the deep (posterior) layers of the iris than in the superficial (anterior) layers. (B) The vessels (iris nevus) may be intrinsic to the tumor or derived from normal vessels. (All OCTA Scans were acquired in a 3x3mm setting.) OCTA = optical coherence tomography angiography.

The median VD of light or medium pigmented iris melanoma was 31.8% (n=4); for iris nevi this was 29.8% (n=12) (p=0.99). The median VD of all iris lesions combined was 30.5%, this was significantly less than the median VD of paired healthy iris tissue of contralateral eyes (n=15) (35.6%, p=0.012).

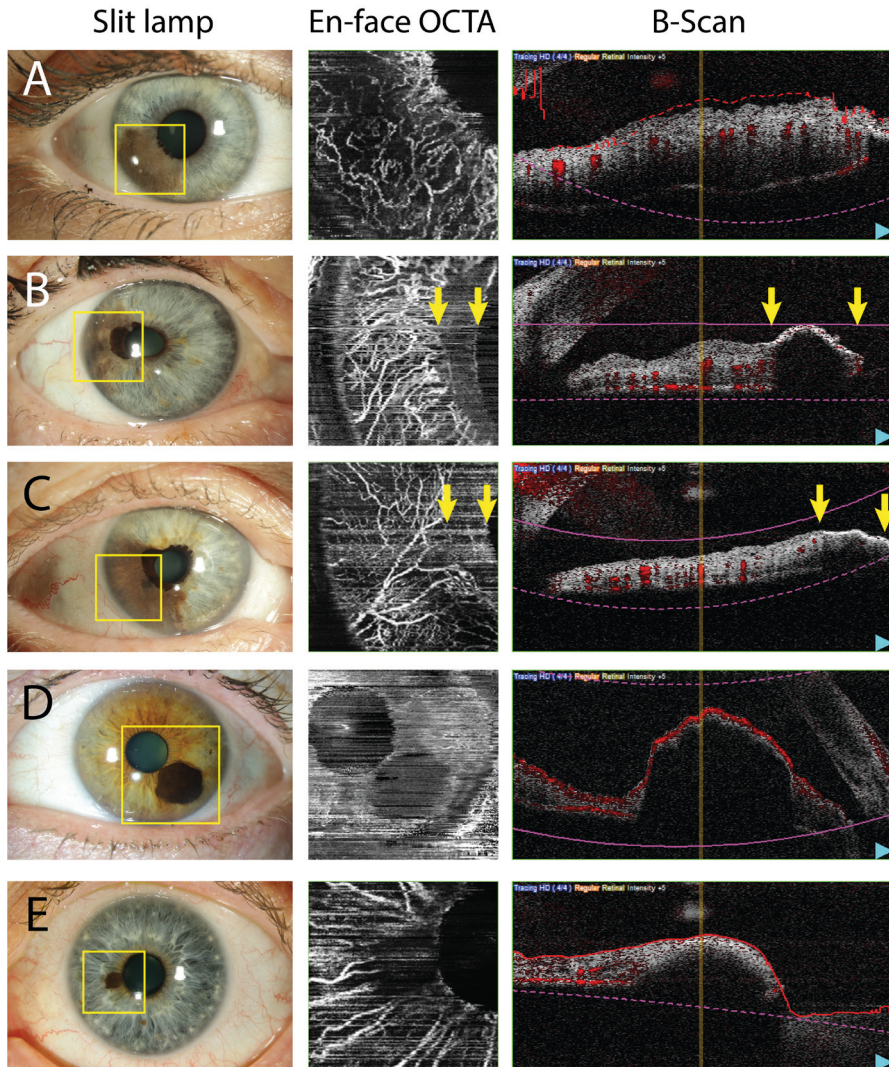


Figure 5. Iris melanoma and nevi. Three examples of iris lesions with visible tortuous vascular patterns are shown. (A) melanoma, (B) melanoma and (C) nevus. The dark pigmented lesion of Patient D (nevus) and E (nevus) blocked the OCT signal, resulting in an apparent avascular area on OCTA. Similarly, in Patient B and C, the blocking effect of an ectropion uveae was seen. (Scans A, B, C, and E were acquired in a 3x3mm setting; scan D was acquired in a 6x6mm setting.)

DISCUSSION

Various melanocytic lesions of the anterior segment were studied using OCTA. To the authors' knowledge, this is the first report of OCTA in melanocytic lesions of the conjunctiva, and the largest study of OCTA in melanocytic lesions of the iris. Vessels were depicted in all conjunctival and most iris lesions. Obtaining good quality images depended largely on patient cooperation, lesion location, and tumor pigmentation. Significantly better imaging was seen in lightly pigmented lesions and younger patients. Although vascular patterns of the melanocytic lesions were distinct from healthy tissue, no differentiating OCTA features were found between nevi and melanoma of either the conjunctiva or iris.

Tortuous vascular patterns were detected in both conjunctival nevi and melanoma, which was different from PAM, which apparently lacked an intrinsic vasculature. No vascular patterns or VD measurements were observed that discriminated between conjunctival nevi and melanoma, apart from avascular areas in nevi due to cysts. The absence of distinct vessels in PAM might have been due to confinement to epithelium, not requiring internal vessels as with thicker nodular lesions; the apparent finer meshwork compared to normal conjunctiva may be due to masking by pigment.

Few studies of OCTA of the conjunctiva exist. Healthy conjunctiva has been studied using OCTA resulting in a better visibility of vessels compared to biomicroscopy.^{25,35} In a single conjunctival haemangioma, OCTA depicted vasculature better than conventional FA.³⁹ Recently, OCTA of the conjunctiva and cornea was reported to be better at detecting ischemia compared to clinical examination in patients with chemical injury,⁴⁰ and OCTA proved feasible in pinguecula and pterygia,⁴¹ and ocular surface squamous neoplasia.⁴²

Some authors have studied vasculature in conjunctival melanoma using immunohistochemistry. Tuomaala and associates quantified vessels in 56 samples by using endothelial marker CD34 and noted no relationship with tumor thickness or survival.⁴³ Subjectively, in that work, the microvascular density was comparable to uveal melanoma. A later report found less CD34 expression in conjunctival melanoma than in surrounding noncancerous stroma, suggesting it to be a hypovascular tumor, despite VEGF production.⁴⁴ Heindl and associates studied lymphatic vessels using immunohistochemistry in 109 samples and found a relationship between the presence of vessels and larger lesions and a worse recurrence and survival rate.⁴⁵ In the present study, however, the authors could not differentiate between blood vessels and lymphatic vessels.

OCTA was used to detect vessels in healthy iris tissue of all but 2 cases. Interestingly, those 2 irises were brown or green, unlike most of the patients, who had blue irises. This was consistent with earlier studies that found that darker iris pigmentation related to worse detection of vessels using OCTA.⁴⁶ Iris lesions displayed tortuous patterns, which were clearly distinct from healthy radial

vessels (Figure 4 and 5). Different patterns between iris nevi and melanoma were not observed, but vessels tended to be absent more frequently in iris nevi. This is consistent with earlier reports of FA in iris tumors. Masking has been related to benign lesions, whereas melanoma demonstrated more chaotic patterns.⁹⁻¹² The value of these patterns is debated, as geometric patterns were observed in benign lesions as well, and pigment is a known masking factor when using FA.^{10,12,13}

The VD in this study did not differ between lightly pigmented iris melanoma (n=4) and nevi (n=12). This may not be surprising, considering the small sample size, but is remarkably different from earlier observations that described a higher VD in 3 iris melanoma compared to 3 iris nevi.²⁸ In that work, the presence of darkly pigmented, or cyst-containing lesions was not reported, however, while that may have influenced measurements. Alternatively, the present technique might have been less sensitive to changes.

The OCTA device applied in this study (Nidek) uses a spectral domain (SD) technique with an 880 nm wavelength. Present commercially available OCTA devices usually apply either a SD or swept source (SS) technique, with some differences in the underlying technique. SS systems use light of a higher wavelength, allowing visualization of deeper layers, but at a lower resolution.²⁷ Skalet and associates reported that pigmented lesions of the iris were better imaged with a 1050 nm technique than with 840 nm,²⁸ mainly due to tissue penetrance. The present study, in line with others,^{35,39} demonstrated that images can be acquired using the SD technique as well but that tissue penetrance is an important limitation, possibly favouring techniques with longer wavelengths.

Our data set was large considering the rarity of the studied diseases, but small for statistical analysis. Motion artefacts influenced imaging significantly, resulting in some uninterpretable images and possible underestimation of effects. Motion caused increased 'flow', whereas masking or nonpenetration of the OCT signal caused decreased 'flow' (which may explain the decreased VD in iris lesions compared to healthy tissue). The authors, therefore, call for the development of new software to help in image acquisition and analysis (eg, for stabilizing images as in assessment of the retina).

We regard it as a strength that almost all conjunctival lesions in this project were diagnosed by histology. In 3 cases of conjunctival nevus and 1 case of PAM, no tissue was obtained as the clinical diagnosis was clear and not suspicious for malignancy. This is common in clinical management of conjunctival lesions,^{2,29,47} and these authors do not believe that obtaining tissue would have influenced results. Even so, a limitation of this study was that iris lesions were usually diagnosed by clinical investigation and ultrasonography only.³⁰⁻³² Although there is debate about the exact clinical features of benign and malignant iris lesions,⁴⁸ it is common to treat malignant lesions without obtaining histology, and to manage unsuspecting iris lesions by observation.^{30,31,33}

Our study shows that OCTA can be used in anterior segment ocular oncology, but better software and enhanced imaging techniques are needed before conclusions about its clinical utility can be drawn. Imaging techniques that are not dependent on light (such as ultrasound biomicroscopy, using sound waves) may be more suitable to depict tumor size,⁴⁹ but AS-OCT⁵⁰ and AS-OCTA may provide an additional parameter for differentiating disease (eg, providing reassurance when no abnormal vessels are seen). With better techniques, a prognostic value of angiography may be established. For now, these authors propose that OCTA is most suitable for superficial and nonpigmented disease (eg, lymphoma, ocular surface squamous neoplasia, or basal cell carcinoma) or lightly pigmented melanoma or nevi. As OCTA requires no intravenous dye, with its potential adverse events,^{17,18} the use of OCTA may become more widespread than fluorescein angiography or indocyanine green angiography has been up to now.

We conclude that it is feasible to obtain OCTA images of melanocytic lesions of the anterior segment. Good image quality, however, depends highly on patient cooperation and lesion characteristics such as location and pigmentation. New software is called for to improve image acquisition and analysis, and to further develop OCTA analysis of anterior segment lesions. While promising by the noninvasive nature and clinical ease, the role of OCTA in clinical and investigational anterior segment ocular oncology is yet to be established.

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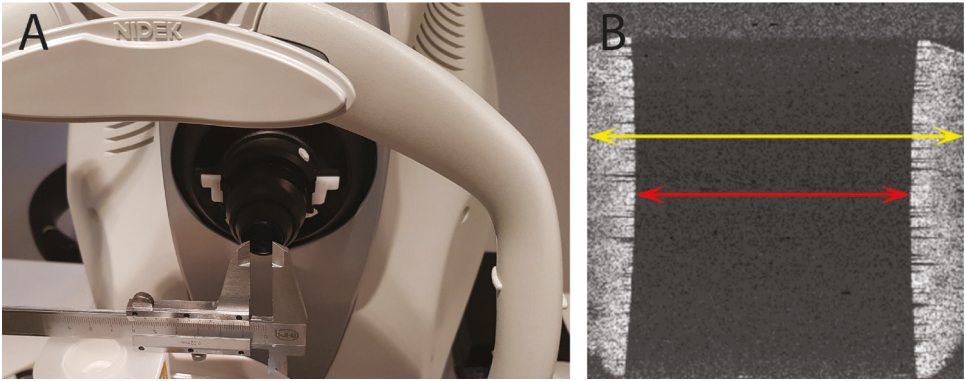
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SUPPLEMENTARY MATERIAL



Supplemental Figure. Imaging of calliper with an anterior segment lens. (A) a calliper was positioned in front of the OCTA device at some distance from the anterior-segment lens, similar to the situation of true ocular imaging. (B) The image was acquired in a 9.0 mm setting (yellow arrow), but due to different optics when using the anterior segment lens, the calliper distance with a width of 9.0 mm (red arrow), appears smaller. Using the ratio of these arrows, the true imaging size was determined.

Supplemental Table. Flow Chart of Patient Numbers, Explaining the Numbers of Analysed Cases.

	Conjunctival Melanoma (n, %)	Conjunctival Nevus (n, %)	PAM (n, %)	Iris Melanoma (n, %)	Iris Nevus (n, %)
Included patients:	5 (100)	13 (100)	7 (100)	10 (100)	42 (100)
Loss due to:	<i>Behind eye lid 1</i>	<i>Non-coop 3 No focus 2</i>	<i>Wrong area 1</i>	<i>Hidden in ciliary body 1</i>	<i>Non-coop 4 Behind eye lid 1 Mydriasis 2</i>
Acceptable OCTA:	4 (80)	8 (62)	6 (86)	9 (90)	35 (83)
Vessels outside lesion					
Present	4 (100)	8 (100)	5 (100) ^a	9 (100)	33 (94)
Absent	0	0	0	0 (0)	2 (6)

Non-coop = non-cooperation; OCTA = optical coherence tomography angiography; PAM = primary acquired melanosis.
^a *in 1 case of PAM, no nonaffected tissue could be analyzed.*

