Retinal Reflectivity Measurement for Cone Impairment Estimation and Visual Assessment After Diabetic Macular Edema Resolution (RECOVER-DME)

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Purpose. Photoreceptor loss has been suspected of being involved in incomplete visual recovery after diabetic macular edema (DME) resolution. Recent studies have shown that cone density in the perifoveal area could be estimated by in vivo measurements of the outer retinal reflectivity on optical coherence tomography (OCT). The main objective of this study was to assess the photoreceptor layer reflectivity after DME resolution and to determine its relationship with final visual acuity (VA).

Methods. In this cross-sectional case–control study, 77 eyes of 58 patients were divided into three groups: a first group ($n = 34$) encompassed eyes with resolved DME (R-DME), a second group ($n = 24$) corresponded to diabetic eyes without DME (no-DME), and a third group ($n = 19$) comprised a control group of nondiabetic healthy eyes. Outer retinal reflectivity was measured on volumetric spectral-domain (SD)-OCT scans acquired 3 months after DME resolution, from the photoreceptor ellipsoid zone (EZ) and the retinal pigment epithelium (RPE).

Results. The mean DME duration was 26.5 ± 13.4 months in the R-DME group. EZ reflectivity was 19.8% lower ($P < 0.0001$) in this group compared to diabetic eyes without DME and 26.5% lower ($P < 0.0001$) than in nondiabetic control eyes. Reflectivity was 7.8% lower in the no-DME group compared to controls ($P < 0.0001$). RPE reflectivity was comparable among the three groups ($P > 0.05$). VA was significantly correlated with EZ reflectivity in diabetic patients ($r^2 = 0.57$; $P < 0.0001$). Reflectivity tended to decrease with prolonged DME duration without reaching statistical significance ($P = 0.10$).

Conclusions. DME significantly impacts the photoreceptor layer. This impairment can be estimated by measuring outer retinal reflectivity on OCT images after edema resorption. We also provide evidence that in diabetic eyes without a history of DME, there is early photoreceptor loss, or at least outer segment (OS) disorganization, in addition to the inner retinal degeneration reported previously. This suggests the neurodegenerative process in diabetes. This quantitative approach may help monitor neuroprotective strategies to rescue photoreceptor cells in diabetic eyes.

Keywords: OCT, diabetic macular edema, reflectivity, photoreceptors

Diabetic macular edema (DME) is one of the leading causes of visual acuity (VA) loss in patients with diabetic retinopathy (DR).[1] Intravitreal therapies are successful in restoring baseline retinal thickness[2–4] and DME resolution can be obtained in up to 75% of cases.[5] Nevertheless, despite a good anatomical response, visual recovery is sometimes disappointing, with only 18% to 45% of patients obtaining a gain of 15 or more letters after 2 years of treatment.[4,6–8] Several studies have reported foveal microstructural defects of the photoreceptor layer occurring after a DME episode.[9–12] Most of these studies examined qualitative parameters such as the integrity of the photoreceptor inner segment–OS (IS/OS) junction band using optical coherence tomography (OCT).[12–15] Recently, we reported a quantitative approach for estimating outer retinal impairment by measuring the reflectivity of the ellipsoid zone (EZ) of the photoreceptor on spectral-domain (SD)-OCT.[16,17] Obtaining a quantitative assessment of photoreceptor layer integrity could be of great help in the management and understanding of DME and its long-term consequences. Therefore, the aim of this study was to estimate photoreceptor impairment after DME resolution by measuring EZ reflectivity on en face SD-OCT images and to compare the results with those of controls. The secondary objective was to investigate the relationship between outer retinal reflectivity and VA in diabetic eyes.

Materials and Methods

This cross-sectional observational case–control study was conducted in the Department of Ophthalmology of the University Hospital of Besançon (France) over a 1-year period

(April 2015 to April 2016). Patients with resolved DME (R-DME), defined as a restoration of the foveolar depression with a central macular thickness (CMT) <315 μm[18] for 3 months, were included. A second group of diabetic patients without DME (no-DME) and a third group of nondiabetic healthy patients (controls) were also included. Exclusion criteria encompassed any other nondiabetic maculopathy, history of vitreoretinal surgery, severe macular ischemia (>5 papillary diameters on fluorescein angiography), or any condition that did not allow an eligible imaging acquisition quality (particularly massive hemorrhage, exudates, dense cataract and poor fixation). All patients underwent a comprehensive ophthalmic examination with best-corrected VA (BCVA) measurement on standard Early Treatment Diabetic Retinopathy Study (ETDRS) charts[19] and SD-OCT imaging (Spectralis, Heidelberg Engineering, Heidelberg, Germany). The following data were collected for each patient: sex, age, diabetes type, percent of glycated hemoglobin (HbA1c), lens status (phakic or pseudophakic), previous ophthalmologic treatment such as intravitreal injection, focal laser or panretinal photocoagulation (PRP), and BCVA (ETDRS letters). In the R-DME group, we also collected the duration of the DME (months), the maximum height of the DME during follow-up in the 1-mm central scanned area (maximum CMT = CMT max) and the CMT after edema resolution. All OCT images were reviewed by two experienced readers (MS, BD) in order to detect the existence of macular exudates, residual intraretinal cysts, and epiretinal membrane (ERM).

Quantitative Analysis of the EZ Reflectivity

En face OCT was obtained by generating a high-resolution three-dimensional reconstruction of 131 axial sections, 11 μm apart, in a 15° × 5° rectangle centered on the fovea. Only scans with a signal-to-noise ratio above 25 dB were kept for analysis. A en face view of the retinal layers was generated using the automatic segmentation software of the Heidelberg navigator. Two layers were studied: the photoreceptor EZ and the retinal pigment epithelium (RPE) layer (Supplementary Material S1). Segmentation was checked by two examiners (MS, BG) and then manually adjusted in case of errors using the software's curve-editing tool. The generated en face images were exported with an 8-bit color depth (256 grayscale values) using the export function of the OCT software. OCT contrast and brightness were preset by the OCT software and were not adjusted after exporting. To facilitate reflectivity measurement, the images of the layers studied were exported to in-house software (Matlab, MathWorks, Inc., Natick, MA, USA). This interface enabled more precise and reproducible reflectivity measurement. The user simply has to point to the foveola with a crosswire cursor on the axial section of the OCT, after which a 2-degree radius ring is automatically drawn on the en face image. Then the operator can choose a region of interest where reflectivity was automatically measured and expressed as mean reflectivity in gray level (0–256) The region of interest was located in the inner edge of the ring (which corresponds to a square located between 2 and 2.3° from the fovea) (Supplementary Material S2). This region was chosen in areas free of visible retinal vessels and avoiding the horizontal line artifacts occasionally produced by eye movement on the OCT image. Two squares measuring 0.3 × 0.3° located nasally and temporally and equidistant from the fovea (2°) were studied for each eye. The results presented corresponded to the average of the two measurements. To minimize any shadowing effect produced by an obstacle on the optical path over the photoreceptor layer, EZ relative reflectivity was also calculated as the ratio of EZ reflectivity to RPE reflectivity.

Table 1. Characteristics of Diabetic Eyes Studied

	R-DME Group	No-DME Group	*P*
Number of eyes	34	24	
Age, mean ± SD, y	63.2 ± 11.7	58.8 ± 13.4	0.18
Male/female	19/15	11/13	0.59
Diabetes type, 1/2	2/32	5/19	0.11
Hba1c, mean ± SD, %	7.9 ± 1.9	7.8 ± 1.1	0.81
DME duration, mean ± SD, mo	26.5 ± 13.4	N/A	
PRP, yes/no	23/11	N/A	<0.0001
Focal laser, yes/no	4/30	N/A	
BCVA, ETDRS letters, mean ± SD	70.35 ± 9.3	82.52 ± 3.5	<0.0001
Lens status, phakic/pseudophakic	18/16	15/9	0.43
CMT max,* mean ± SD, μm	498.23 ± 138.84	N/A	
CMT,† mean ± SD, μm	263.32 ± 43.12	268.78 ± 26.01	0.58

N/A, not applicable.

* Maximum height of the DME during follow-up in the 1-mm central scanned area.

† Mean CMT after DME resolution in R-DME group and mean CMT in no-DME group.

Statistical Analysis

All values represent the mean ± standard deviation. Linear regression analysis was performed to study the relationship between reflectivity and VA using the commercially available GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). The three groups were compared using the Fisher's exact *t*-test for nonparametric data, using an α error rate of 0.05. Continuous variables were compared using the Student's *t*-test under the assumption of a normal distribution. The relationship between OCT findings (lens status, ERM, exudates or residual cysts) and reflectivity were investigated using Pearson's χ^2 test.

The study adhered to the tenets of the Declaration of Helsinki and had the approval of the Besançon University Hospital ethics committee. Each patient gave written informed consent to participate in the study.

Results

Seventy-seven eyes of 58 patients were included. Thirty-four eyes ($n = 29$ patients) with resolved edema were included in the R-DME group and 24 eyes ($n = 14$ diabetic patients) without DME (no-DME group) were studied. The characteristics of the diabetic eyes studied are presented in Table 1. The groups were comparable in terms of age ($P = 0.18$), sex ($P = 0.59$), diabetes type ($P = 0.11$), percent of HBA1c ($P = 0.81$), lens status ($P = 0.43$), and CMT ($P = 0.58$). The mean time from diabetes diagnosis was 11.8 ± 4.3 years. In the R-DME group, 23 eyes (67.6%) had previously received PRP, 24 (70.5%) had been treated with ranibizumab. Ten eyes (29.5%) had received a dexamethasone implant. The mean time from DME diagnosis was 26.5 ± 13.4 months and the mean CMT was 263.3 ± 42.4 μm. In the no-DME group, no patients had received previous PRP or intravitreal treatment, and the DR was mild or absent in all eyes. BCVA was lower in the R-DME group than in the no-DME group (70.3 ± 9.3 vs. 82.5 ± 3.5; ETDRS score, $P < 0.0001$). A third group of 19 eyes of 15 nondiabetic healthy patients (control group) was also included. There was no significant difference between the control group and the two diabetic groups in terms of age, sex, and CMT ($P > 0.05$).

Comparison of the Outer Retinal Reflectivity Between Groups

The outer retinal reflectivity measurements are presented in Table 2. Mean EZ reflectivity (expressed in grayscale) was 19.8% lower in the R-DME group compared to the no-DME group (156.3 and 194.8, respectively, $P < 0.0001$). Mean EZ reflectivity in the healthy group (211.7 ± 4.8) was 26.2% and 7.9% higher than in the R-DME and the no-DME group, respectively. There was no statistically significant difference between the three groups in terms of RPE reflectivity ($P > 0.05$). The assessment of EZ relative reflectivity, defined as the ratio of EZ reflectivity to RPE reflectivity, showed similar results: EZ relative reflectivity was 18.6% and 23.9% lower in the R-DME group than in the no-DME and healthy group, respectively ($P < 0.0001$).

A subgroup analysis of the R-DME group (Table 3) showed that there were no statistically significant variations in EZ relative reflectivity in presence of ERM ($P = 1.0$), exudates ($P = 0.6$), or residual retinal cysts ($P = 0.5$). Moreover, there was no significant difference in EZ relative reflectivity between phakic and pseudophakic eyes ($P = 0.1$).

Relationship Between Outer Retinal Reflectivity and Clinical Findings

VA was significantly correlated with EZ reflectivity ($r^2 = 0.38$; $P < 0.0001$) in the R-DME group (Fig. 1). BCVA was also correlated with relative EZ reflectivity calculated to reduce any shadowing effect ($r^2 = 0.38$, $P < 0.0001$) (Fig. 2). In diabetic eyes (R-DME and no-DME groups), BCVA was strongly correlated with absolute and relative EZ reflectivity ($r^2 = 0.57$; $P < 0.0001$ and $r^2 = 0.54$; $P < 0.0001$, respectively) (Supplementary Material S3, S4).

Furthermore, EZ reflectivity was proportional to the CMT after DME resolution ($P < 0.05$) (Supplementary Material S5). However, the maximum height of the macular edema measured during the follow-up (CMT max) was not correlated with the final VA ($P = 0.23$). Finally, the CMT after DME resolution was not correlated with VA ($P = 0.11$). EZ reflectivity tended to decrease as the duration of the DME increased without reaching statistical significance ($P = 0.10$).

Discussion

DME is a major cause of visual loss in the working-age population. It is estimated that worldwide, 20.6 million adults have DME.[20] Despite significant progress in DME treatment[4,8,21,22] providing DME resolution in up to 75% of cases,[5] visual recovery is not guaranteed, with only 18% to 45% of patients obtaining a gain of 15 or more letters after 2 years of treatment.[4,6–8] The relationship between DME and visual function has been extensively studied and several causes of functional impairment have been suspected, including neural apoptosis, ischemia, glial reactivity, and reduction in the thickness of the inner retinal layers.[23–25] For instance, it has been shown that VA is poorly correlated with retinal thickness.[26,27] The involvement of the outer retina in the visual loss observed after DME and more precisely the relationship between photoreceptor integrity on OCT and visual function have also been explored in depth (Table 4). However, only qualitative or semiquantitative analysis of photoreceptor layer impairment on OCT has been reported in DME.[9–15] For instance, photoreceptor outer-segment length was shown to be slightly correlated with VA, but the repeatability of the measurements was limited.[28]

Table 2. Outer Retinal Reflectivity Measurement and Comparison Between the Three Groups

	R-DME Group	No-DME Group	Δ % P	R-DME Group	Healthy Group	Δ % P	No-DME Group	Healthy Group	Δ % P
Number of eyes	34	24		34	19		24	19	
EZ reflectivity	156.3 ± 27.4	194.8 ± 6.7	Δ = 19.8% $P < 0.0001$	156.3 ± 27.4	211.7 ± 4.8	Δ = 26.2% $P < 0.0001$	194.8 ± 6.7	211.7 ± 4.8	Δ = 7.9% $P < 0.0001$
RPE reflectivity	223.8 ± 15.9	224.9 ± 6.9	$P = 0.74$	223.8 ± 15.9	228.4 ± 5.7	$P = 0.32$	224.9 ± 6.9	228.4 ± 5.7	$P = 0.13$
EZ Relative reflectivity	0.70 ± 0.14	0.86 ± 0.03	Δ = 18.6% $P < 0.0001$	0.70 ± 0.14	0.92 ± 0.02	Δ = 23.9% $P < 0.0001$	0.86 ± 0.03	0.92 ± 0.02	Δ = 6.5% $P < 0.0001$

Values are presented as the mean ± SD.

Table 3. Relationship Between EZ Relative Reflectivity in the R-DME Group and the Other OCT Findings

	Hard Exudates		Residual Cysts		ERM		Lens Status	
	Yes	No	Yes	No	Yes	No	Pseudo-phakic	Phakic
Eyes	25	9	27	7	8	26	16	18
EZ relative reflectivity	0.67 ± 0.14	0.70 ± 0.14	0.68 ± 0.14	0.77 ± 0.10	0.70 ± 0.17	0.70 ± 0.13	0.69 ± 0.14	0.72 ± 0.11
Pearson χ^2								
P	0.58		0.12		1.0		0.48	

Values are presented as the mean ± SD.

An association between percent disruption of the photoreceptor IS/OS, the external limiting membrane, and VA in DME was also observed.[29,30] The term IS/OS employed in these studies is no longer appropriate. In a recent comparative study between OCT and histology, Spaide et al.[31] showed that this band better correlates with the ellipsoid component of the cones. This area is rich in mitochondria, which scatter light, resulting in high reflectivity potential.[32] In an attempt to reach a consensus on the terminology for retinal layers and the bands that appear on SD-OCT imaging, a panel of retinal specialists proposed a nomenclature for the posterior segment, choosing to call this band the EZ.[33] However, controversy continues to surround this terminology and recent reports with adaptive optics (AO) OCT concluded that the IS/OS junction was colocalized with the second hyper reflective band.[34] For practical reason we will use the EZ denomination.

An important consideration regarding these studies is how EZ layer disruption was evaluated since they do not define a clear cutoff to distinguish disrupted from undisrupted layers. It appeared that the results may be consistent and objective only on condition that trained observers are enrolled and adequate scores defined.[14] However, these constraints prevent comparing. For all these reasons, a quantitative tool, automatized and easy to use, could significantly progress the analysis of the photoreceptor layer. Advances in OCT imaging enabled the reconstruction of a en face view of the photoreceptor layer[35–38] at different depths. A strong relationship was recently established between EZ reflectivity and cone density measured with a high-resolution adaptive optics camera in the same perifoveal area.[16,17] In the current study, in diabetic eyes with resolved DME, we observed a 20% decrease in EZ reflectivity compared to diabetic eyes with no history of DME. Furthermore, reflectivity in diabetic eyes without a history of DME was also lower than in control eyes. Interestingly, Lombardo et al.[39] found a subtle decrease of parafoveal cone density in type 1 diabetic patients without maculopathy compared with age-matched control subjects via high-resolution adaptive optics retinal imaging. This decrease reached approximately 10% of the cone density, a result comparable with the 7.9% difference in reflectivity that we observed. It should be noted that decrease in reflectivity does not necessary imply a photoreceptor loss since modified adaptive optics scanning laser ophthalmoscopy (AO/SLO) techniques, allowing to image the ISs regardless of the status of OSs, have shown that enlarged photoreceptor bodies are still present in the dark areas even though it is not known if these remnant photoreceptor are functional.[40] The origin of this decrease was not clearly elucidated. There is an ongoing debate to determine whether diabetic retinal neuropathy is the effect of vascular DR or is primarily caused by direct neurologic damage from chronic hyperglycemia. For instance, Van Dijk et al.[41] demonstrated that there is retinal neurodegeneration and neuroglial cell apoptosis even in the early stages of DR. Furthermore, a retinal neurodegeneration has been reported to occur before any microcirculatory abnormalities. Increased rate of photoreceptor apoptosis and activation of glial cells are major factors identified in retinal neurodegeneration.[42] A vascular cause can also be suspected since abnormal deep

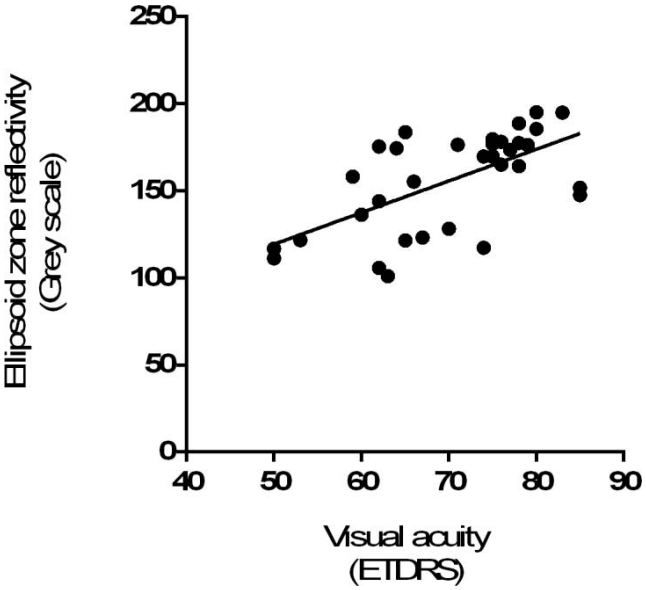

Figure 1. Relationship between EZ reflectivity and BCVA in the R-DME group ($r^2 = 0.38$; $P < 0.0001$).

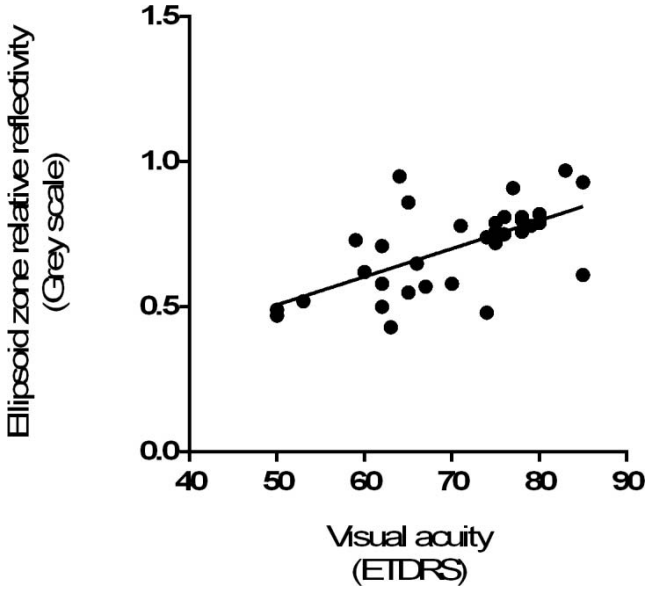

Figure 2. Relationship between EZ relative reflectivity and BCVA in the R-DME group ($r^2 = 0.38$; $P < 0.0001$).

Table 4. Summary Table of the Main Studies on the Relationship Between Photoreceptor Integrity on OCT and Visual Function in DME, Using a Qualitative or Semiquantitative Analysis of the EZ Layer Impairment

	The Association Between Percent Disruption of the Photoreceptor IS/OS Junction and VA in DME (Maheshwary et al. 2010)[14]	Association Between Foveal Photoreceptor Status and VA After Resolution of DME by Pars-Plana Vitrectomy (Sakamoto et al. 2009)[10]	Correlation Between Visual Function and Photoreceptor Integrity in DME: SDOCT (Shen et al. 2016)[12]	Association Between Hyperreflective Foci in the Outer Retina, Status of Photoreceptor Layer, and VA in DME. (Uji et al. 2012)[11]	Correlation Between VA and Foveal Microstructural Changes in DME (Otani et al. 2010)[9]
EZ layer analysis technique	EZ integrity gradation on SD-OCT, generating a percent of disruption	EZ integrity gradation on SD-OCT	EZ line integrity gradation on SD-OCT	EZ line integrity gradation on SD-OCT	EZ length of disruption gradation on SD-OCT
	Grade 0: intact layer				
	Grade 1: focal disruption <200 μm	Grade 1: intact	+ = complete	+ = complete	Grade 1: >1.4 mm
	Grade 2: disruption >200 μm	Grade 2: partially visible or absent	± = incomplete	± = incomplete	Grade 2: >0.4 mm but <1.4 mm
			− = undetectable	− = undetectable	Grade 3: <0.4 mm
Number of eyes	62	37	31	108	154
Results	Correlation between EZ percent disruption and VA in DME ($P = 0.031$)	After DME resolution, final VA without intact EZ was significantly poorer ($P = 0.004$)	EZ integrity correlated positively with visual function in DME patients ($P < 0.001$)	Disruption of the EZ was correlated with poor VA ($P < 0.0001$)	The EZ score was strongly correlated with the BCVA ($P < 0.001$)

retinal capillary networking and nonperfusion were observed in the outer nuclear layer in diabetic eyes.[43,44] Such outer retinal impairment has functional consequences: we observed a relationship between reflectivity and VA (Supplementary Material S6). Others have observed that disruption of the IS/OS junction was correlated with a significant decrease in retinal sensitivity in DME eyes.[45]

This cross-sectional study has limitations. EZ reflectivity seems to decrease with increased DME duration. These results are in accordance with recent studies that demonstrated that delayed treatment leads to lower visual recovery.[21,46,47] The lack of statistical significance may stem from the impossibility of accurately determining DME onset, since the patient does not always seek medical advice immediately. We focused on the EZ but other structures could have been of interest such as the ELM or the interdigitation zone, which are also impaired in DME.[29,48] However, due to their reflectivity and thickness, these layers are harder to segment accurately and the analysis could have been more cumbersome and less reliable. Another important limitation to consider is that, from the A-scan to the en face image, multiple image-processing steps, such as sampling density normalization or speckle noise reduction, can be performed automatically by the OCT software. Thus, B scans in the same volume can receive different processing treatment resulting in significant reflectivity changes that we were not able to quantify since we did not have access to the raw OCT signal recorded by the device. In addition, photoreceptor degeneration is not the only cause of visual loss after DME. Several structural changes of the individual retinal layers take place in DME,[25,41,49–51] in particular in the inner retina, and the impact of these changes on VA are established.[52] Finally, reflectivity may change with longer follow-up.[53]

Conclusions

Our conclusion suggests that DME causes damage to the photoreceptor cells, which limits visual recovery after resolution. This impairment can be accurately measured based on reflectivity analysis. We also provide evidence that in diabetic eyes without a history of DME, in addition to the inner retinal degeneration reported previously, there was early photoreceptor loss or at least outer-segment disorganization taking place in these eyes. This suggests the importance of the neurodegenerative process in diabetes. The preservation of these photoreceptors is a major challenge in the struggle to protect the vision of diabetic patients. In the future, this objective approach could be used to monitor neuroprotective strategies precisely.

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Investigative Ophthalmology & Visual Science

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