A Comparison of En Face Optical Coherence Tomography and Fundus Autofluorescence in Stargardt Disease

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Purpose. To compare morphologic changes on en face images derived from wide-field swept-source optical coherence tomography (ssOCT) to hypo- and hyperautofluorescent (hypoAF, hyperAF) areas on short-wavelength autofluorescence (SW-AF), and near-infrared (NIR)-AF in recessive Stargardt disease (STGD1).

Methods. Wide-field ssOCT cube scans were obtained from 16 patients (16 eyes). Averaged B-scans and SW-AF images were obtained using Spectralis HRA+OCT. NIR-AF images were obtained from 6 eyes. The inner/outer segment (IS/OS), OS/RPE, and RPE/Bruch’s membrane boundaries were segmented, and en face slab images generated. A subRPE slab image was used to measure the abnormal RPE area, and an IS/OS slab image, the IS/OS junction loss area. These were compared to hypo- and abnormal SW-AF areas, and hypo-NIR-AF areas. A preRPE(OS) slab image was used to evaluate the spatial and intraretinal locations of flecks.

Results. For all eyes, RPE atrophy was visualized as a central hyperreflective area on the subRPE slab, and IS/OS junction loss as an abnormal reflective area on the IS/OS slab; the latter was significantly larger (P = 0.04). There was good agreement between the hyperreflective area on the subRPE slab image and hypoSW-AF area, and between the abnormal reflective area on the IS/OS slab and hypo-hyperSW-AF area; the hypoNIR-AF area indicated that the hyperreflective area on the subRPE slab underestimated RPE atrophy. The spatial locations of hyperreflective flecks on the en face preRPE(OS) slab image corresponded to those on the SW-AF images.

Conclusions. Wide-field en face OCT imaging has the potential to be a clinically useful tool for the management of STGD1.

Keywords: wide-field en face optical coherence tomography, Stargardt disease, fundus autofluorescence

Recessive Stargardt disease (STGD1), a form of juvenile macular degeneration, is caused by more than 1000 mutations in the photoreceptor-specific ABCA4 gene, which encodes the ATP-binding cassette transporter in photoreceptors.1,2 The transporter facilitates the clearance of all-trans-retinaldehyde,3 which is generated by photon-mediated cis-trans isomerization of retinaldehyde in the photoreceptor cell. When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4 When the transport activity is reduced or absent, the result is isomerization of retinaldehyde in the photoreceptor cell. Accelerated accumulation of lipofuscin in RPE cells.4
Depending on the imaging modality, it has been suggested that photoreceptor loss occurs before RPE loss,\(^\text{18,19}\) that RPE cell loss and/or dysfunction occurs and then there is secondary photoreceptor layer may occur simultaneously with the development of abnormalities in the RPE layer.\(^\text{20}\) In addition, the intraretinal position of the hyperSW-AF flecks, which often are observed on SW-AF also is of interest. Since the autofluorescent appearance of flecks usually is associated with lipofuscin in the RPE, it has been assumed that they are to be found at the level of the RPE.\(^\text{20–22}\) However, in NIR-AF images, flecks are dark due to loss of RPE, while in horizontal SD-OCT images they correspond to hyperreflective deposits that are anterior to RPE/Bruch’s membrane and traverse photoreceptor-attributable OCT layers.\(^\text{12,23}\) There now is abundant evidence in the posterior pole) and one stage 3 (extensive macular atrophy (presence of numerous yellowish-white flecks throughout the fovea). There is one stage 2 (localized parafoveal or perifoveal flecks), three stage 2 (presence of small atrophic-appearing foveal lesions with thickened retina), and one stage 1 (normal looking retina).”

**Methods**

**Subjects**

We studied 16 patients (16 eyes; 9 male and 7 female patients; mean age, 38.3 ± 17.7 years; range, 11–70 years), with at least one (expected) disease-causing mutation in the ABCA4 gene. Demographic, genetic, and selected clinical data are presented in Table 1. All patients had a complete ocular examination, including best-corrected visual acuity with subjective refraction. Eyes were excluded from the study if there was evidence of significant ocular media opacities, refractive errors greater than ±6 diopter (D) sphere or ±2 D cylinder, elevated IOP > 21 mm Hg, and a history or diagnosis of any other significant ocular comorbidities. The eye with the higher image quality value for the wide-field OCT cube scan was included in the study. Best corrected visual acuity in the tested eye ranged from 0.01 to 1.3 logMAR (20/25–20/400, Snellen acuity).

Full-field scotopic and photopic electroretinograms (ERGs) were obtained from both eyes of 14 patients according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards.\(^\text{26}\) Based on the full-field ERG results, patients were classified into group 1 if the scotopic and photopic ERG amplitudes were within normal limits (12 patients, 12 eyes), group 2 if the photopic ERG amplitudes were decreased (patients P3 and P6), or group 3 if the scotopic and photopic ERG amplitudes were decreased.\(^\text{27}\) In addition the disease was classified into one of four clinical stages or phenotypes based on those described by Fishman.\(^\text{28}\) A total of 12 patients had stage 1 (characterized by pigmentary changes in the macula ranging from nonspecific pigment motting to the presence of small atrophic-appearing foveal lesions with localized parfoveal or perifoveal flecks), three stage 2 (presence of numerous yellowish-white flecks throughout the posterior pole) and one stage 3 (extensive macular atrophy with diffuse flecks throughout the fundus) disease (Table 1). The study was performed under protocol AAA9906 with the approval of the institutional review board of Columbia University. All study procedures complied with the Health

**Table 1.** Clinical, Demographic, and Genetic Characteristics of Study Patients With Recessive Stargardt Disease

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Decade</th>
<th>Race/Ethnicity</th>
<th>Eye</th>
<th>Snellen</th>
<th>logMAR</th>
<th>Clinical Stage</th>
<th>ABCA4 Mutations</th>
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<td>1</td>
<td>1 p.G1961E</td>
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<tr>
<td>16</td>
<td>F</td>
<td>22</td>
<td>2nd</td>
<td>White</td>
<td>OD</td>
<td>20/100</td>
<td>0.70</td>
<td>1</td>
<td>1 p.G1961E</td>
</tr>
</tbody>
</table>

F, female; M, male; OS, left eye; OD, right eye; ffERG, full-field electroretinogram; N/A, not available.

* Denotes nonsense mutation.
† Lois et al.\(^\text{27}\)
‡ Fishman GA.\(^\text{28}\)
§ Siblings.
following pupil dilation, SW-AF (488 nm excitation) 30° and 55° images were acquired with the Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany) after a 20-second bleach of the photopigments in AF mode.31 Care was taken to obtain high-quality images with maximum field uniformity. In addition, a 9-mm high-resolution horizontal line scan through the fovea was obtained with the Spectralis. The SD-OCT scans were registered automatically to a simultaneous-ly acquired near-infrared reflectance (NIR-R) fundus image. In addition NIR-AF (787 nm excitation) images were acquired from 6 of the 16 eyes with the Heidelberg Retina Angiograph 2 scanning laser ophthalmoscope (HRA2-cSLO, Heidelberg Engineering) using the indocyanine-green angiography mode (without injection of dye) after focus adjustment in infrared reflectance mode.

Wide-field (12 × 9 mm) cube scans consisting of 256 B-scans, each with 512 A-scans, were obtained from all 16 eyes with ssOCT (DRI-OCT; Topcon, Inc., Tokyo, Japan). Following manual correction of the automated segmentation of the IS/OS junction, OS/RPE, and RPE/BM boundaries,32 special purpose software (ATL 3D-Suite; Fortune B, et al. JOVS 2014;55:ARVO Eabstract 2644) was used to generate en face slab images based on the average reflectance intensity for slabs of constant thickness.33 To obtain optimal visualization of the morphologic changes, we chose the following en face slabs: A subRPE slab with a thickness of 208 μm below the RPE/BM boundary; an IS/OS slab positioned on the IS/OS junction with a thickness of 13 μm; and a preRPE(OS) slab positioned just above the proximal RPE border and protruding into the IS/OS region with a thickness ranging from 13 μm. Examples of the three slabs together with their en face images for a healthy control are shown in Figures 1A to 1C. The thicknesses of the slabs were chosen based on a pilot study and on those used by Sodi et al.18 The subRPE slab image was used to measure the central RPE lesion (i.e., on the subRPE slab image the atrophic RPE area was visualized indirectly as a “hyperreflective area”; the increased OCT signal resulted from the loss of the RPE and choriocapillaris); the IS/OS slab image was used to measure the area of IS/OS junction loss; and the preRPE(OS) slab image was used to evaluate the spatial and intraretinal location of flecks. The areas of RPE atrophy and IS/OS junction loss were measured in mm² by two independent observers using ImageJ64 (National Institutes of Health [NIH], Bethesda, MD, USA; available in the public domain at imagej.nih.gov/ij/). The quality of some of the en face IS/OS slab images affected the clarity of the boundaries of the area of IS/OS junction loss. For these cases, the locations chosen to mark the boundary were checked by comparing them to the locations of IS/OS junction loss on the corresponding individual B-scans. The areas of RPE atrophy and IS/OS junction loss then were compared respectively to the area of hypoAF (i.e., absent AF on the SW-AF image and central darkened area on the NIRAf image) and
to the area of abnormal AF (i.e., the hypo- and surrounding hyperAF areas on the SW-AF image). The latter also were measured using ImageJ64. Lastly the spatial and intraretinal locations of flecks observed on the preRPE(OS) slab image were compared to those on the SW-AF image. All en face, SW-AF, and NIR-AF images were aligned to each other using i2kRetina software (DualAlign LLC, Clifton Park, NY, USA) and Adobe Photoshop CS5 (Adobe, Mountain View, CA, USA).

**Statistical Analyses**

Statistical analysis was performed using PRISM 5 (GraphPad Software, Inc., La Jolla, CA, USA). Bland-Altman plots were generated to assess the agreement between the en face and SW-AF measurements. The statistical significance of the differences between the subRPE and IS/OS areas, and between the hypoAF and total abnormal AF areas was tested with paired-samples t-tests using SPSS Statistics 20.0 for Mac (SPSS IBM, Chicago, IL, USA). In addition, to assess the agreement between the measurements made by the two observers, interclass correlation coefficients (ICC) were calculated in SPSS.

**RESULTS**

**Changes on SW-AF**

All 16 eyes exhibited changes on SW-AF. The SW-AF images for 11 of the 16 eyes showed a central area of hypoAF (yellow arrow) surrounded by a well-defined ring of hyperAF (red arrow). The central hypoAF area on the SW-AF image for P8 and the central area of abnormal AF are outlined in the insets. (B) The central area is surrounded by a mottled pattern of flecks (red arrow). (C, D) The fovea is encircled by hyper- and hypoAF flecks in the perifovea of varying size and shape. (E) Mottling on the central fovea. (F) Widespread hypo- and hyperAF changes.

**TABLE 2. Summary of SW-AF and En Face OCT Measurements**

<table>
<thead>
<tr>
<th></th>
<th>Short Wavelength-Autofluorescence (SW-AF)</th>
<th>SubRPE Slab</th>
<th>IS/OS Slab</th>
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<tr>
<td></td>
<td>HypoAF</td>
<td>Abnormal AF</td>
<td>Central Hyperreflective Area</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Mean</td>
<td>4.66 mm$^2$</td>
<td>14.37 mm$^2$</td>
<td>5.26 mm$^2$</td>
</tr>
<tr>
<td>SD</td>
<td>4.57 mm$^2$</td>
<td>13.18 mm$^2$</td>
<td>4.06 mm$^2$</td>
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</table>

* The abnormal SW-AF area was significantly larger than the hypoAF area ($P = 0.01$).
† The IS/OS area was significantly larger than the subRPE area ($P = 0.04$).
FIGURE 3. NIR and SW-AF images for P11 and P14.

FIGURE 4. SubRPE, IS/OS en face, and SW-AF images for one patient, P4. The yellow arrows on the subRPE, IS/OS, and SW-AF images indicate foveal sparing. The subRPE slab image shows a central hyperreflective area. The corresponding en face IS/OS slab image shows a central relatively hyporeflective area surrounded by a hyperreflective border that appears to be larger than the area on the subRPE slab image. The SW-AF image shows a large central hypoAF area. The lower panels show horizontal ssOCT B-scans and respective subRPE slab and IS/OS slab.
and mottling on the foveal center with no other visible areas of hypo- or hyperAF was observed in another eye (Fig. 2E). The areas of abnormal AF (i.e., the hypo- and surrounding hyperAF areas) and the areas of central hypoAF or absent AF were measured (see examples in Figs. 2A, 2B inserts). Unsurprisingly, for the 11 eyes with a central hypoAF area, the abnormal AF area was significantly larger (mean $= 14.37$ mm$^2$, SD $= 13.18$ mm$^2$) than the hypoAF area (mean $= 4.66$ mm$^2$, SD $= 4.57$ mm$^2$; $P = 0.01$; Table 2). For the six eyes with NIR-AF, the darkened areas in the NIR-AF images, indicating a reduction or absence of the NIR-AF signal, exhibited good correspondence to the areas of abnormal AF in the SW-AF images (see examples P11 and P14, Fig. 3).

OCT En Face Slabs

The subRPE slab images for the 11 eyes with central hypoAF on SW-AF showed a central hyperreflective area (see Figs. 4, 5A-D). The hyperreflectivity of this zone is attributable to scleral reflectance due to penetration of light into the choroid due to loss of the RPE and atrophy of the choriocapillaris as visualized in the ssOCT B-scan (Fig. 4, bottom left). As can be seen in Figures 4 and 5, the hyperreflective areas were similar in size and shape to the central hypoAF areas on SW-AF. For the subgroup of eyes with NIR-AF, these hyperreflective areas were smaller than the hypoNIR-AF areas.

Foveal sparing, present in four eyes (P1, P2, P4, and P7), also was an identifiable feature on the en face image. It was discernable as a hyporeflective area in the fovea, and as a relatively hyperAF area in the SW-AF image (Fig. 4, yellow arrows). Of interest, the subRPE slab image for the eye with widespread hypo- and hyperSW-AF changes (P6, Fig. 2F) showed a well-defined central hyperreflective area surrounded by hypo- and hyperreflective patterning (Fig. 5A), while the eye with foveal mottling on SW-AF (P5, Fig. 2E) exhibited an ill-defined central hyporeflective area on the subRPE slab image.

Figure 5. SubRPE, SW-AF, and IS/OS en face images for patients P6, P8, P3, and P5.
subRPE area (mean ± SD = 5.26 ± 9.77 mm², P = 0.04 (see Figure 5D). The corresponding en face IS/OS slab images (Fig. 4, center column and Figs. 5A–D, right column) for these patients showed either a central hyperreflective area or a relatively hyporeflective area surrounded by a hyperreflective border. This central abnormal reflective area appeared to be larger than the area on the subRPE slab image (see Figs. 4 and 5A–D). To assess this quantitatively, these areas were measured on the IS/OS and subRPE slab images (see examples of areas in Fig. 5C). The en face IS/OS areas were larger than the subRPE areas. There was a significant difference between the measurements for the IS/OS area (mean = 9.77 ± 8.90 mm²) and the subRPE area (mean = 5.26 ± 4.06 mm²), P = 0.04 (see Table 2).

Comparison of SW-AF and OCT En Face Slab Images
To assess the agreement between the two techniques used to quantify the extent of RPE atrophy and IS/OS junction loss, measurements of the central hypoAF area on the SW-AF image were compared to those of the central hyperreflective area on the subRPE en face image, and measurements of the abnormal AF area on the SW-AF image were compared to the central abnormal reflective (central hyperreflective or hyporeflective area and surrounding hyperreflective border) area on the IS/OS en face image. The Bland-Altman plot in Figure 6A illustrates the similarity of these measurements for the two techniques. There was no evidence of bias regarding the comparison between measurements of the central hypoAF area on SW-AF and the subRPE en face images. The Bland-Altman plot in Figure 6B comparing the abnormal AF area on the SW-AF image to the central abnormal reflective area on the IS/OS en face image also showed similarity of measurements for the two techniques; however, on average, the SW-AF measurements of the abnormal AF area were slightly larger than the en face IS/OS measurements (1.8 mm²). Average ICCs revealed excellent agreement between the two independent observers for all area measurements (range, 0.93–0.98).

Retinal Flecks
Retinal flecks of varying shape and size were identified on the SW-AF images of 11 of the 16 eyes. The hyper- and hypoAF flecks were located in the perifoveal region and/or diffusely throughout the fundus (Figs. 2B–D, 2F). The preRPE(OS) slab provided the best means for detecting flecks. On the en face preRPE(OS) image (Figs. 7A–D, upper left), they were apparent as small hyperreflective structures either surrounding the central hyporeflective area or widely distributed throughout the image; a hyporeflective border surrounded some of the flecks. The spatial locations of the hyperreflective flecks corresponded to those on the SW-AF images (Figs. 7A–D, lower right). The flecks appear as hyperreflective regions that project anterior to RPE/Bruch’s membrane disrupting the IS/OS junction band. In some cases they extend toward the outer limiting membrane (Figs. 7C, 7D, lower right).

DISCUSSION
The morphologic changes on en face images derived from wide-field ssOCT scans were compared qualitatively and quantitatively to changes visible on SW-AF. In agreement with previous reports, it is a central hyperreflective area attributable to RPE atrophy was observed on the en face subRPE slab image (Figs. 4, 5). The area was similar in size and shape to the hypoAF area in the central macula in the SW-AF image. Consistent with observations by Sodi et al., measurements of the hyperreflective area on the en face subRPE slab image (i.e., the area of RPE atrophy) revealed that this lesion area was significantly smaller than the area of abnormal reflectance on the IS/OS slab image (i.e., the area related to IS/OS junction loss). However, it also was smaller than the area of RPE cell loss identified by NIR-AF.

Our findings provided some insight into the possible sequence of outer retinal degenerative changes in STGD1. First, we assume that IS/OS junction loss is correlated with photoreceptor loss and/or dysfunction; it is hypo–SW-AF or absent SW-AF in STGD1 represents RPE loss and/or atrophy; that hyper–SW-AF can be attributed to accelerated bisretinoid production from degenerating photoreceptor cells; and that it is associated with RPE cells being absent or with thinning and dysfunctional RPE. Second, our SW-AF results indicated that the area of abnormal AF was significantly larger than the hypo- or absent AF area, and that it included the area of IS/OS junction loss. However, these observations cannot be interpreted as evidence that photoreceptor degeneration precedes RPE atrophy in STGD1. Additionally, the tested eye of P5 at the initial visit showed IS/OS junction loss in the fovea on ssOCT and SD-OCT, and central mottling in the SW-AF image that
would be indicative of RPE atrophy (see Figs. 2D, 5D). This interpretation raises questions about the results of previous studies comparing NIR- and SW-AF in patients with STGD1. In these studies, the zone of decreased NIR-AF was reported to be larger than the zone of reduced or abnormal SW-AF and more closely related to the extent of the IS/OS junction loss seen on SD-OCT. As the main source of NIR-AF is melanin in the RPE and choroid, it was suggested that the results were consistent with RPE cell loss preceding photoreceptor cell degeneration. In the current study, NIR-AF images were obtained from six of the 16 patients and in agreement with previous studies the central area of absent or hypoAF was larger on NIR than SW-AF. In addition, the lesion area, indicated by darkening in the NIR-AF image, was larger than the en face subRPE area. When the surrounding clusters of hypoAF, that is, patchy hypoAF areas on NIR-AF, were included in the measurement, the total area was greater than either the en face IS/OS or abnormal SW-AF areas (see Fig. 3). Since the NIR-AF signal is produced, for the most part, from RPE melanin, absence of NIR-AF is indicative of loss of RPE cells. Residual levels of NIR-AF could be accounted for readily by melanin-containing RPE debris from the degenerating cells. If the latter is assumed, the combined NIR-, SW-AF and en face results suggested that photoreceptor cell degeneration has occurred in the presence of dead and/or dying dysfunctional RPE cells.

We also were interested in determining whether the wide-field en face technique could be used to corroborate our previous findings related to the intraretinal location of flecks in patients with STGD1. These retinal flecks are an important clinical feature of STGD1. They were evident as small hyperreflective structures either surrounding the central hyporeflective area or widely distributed throughout the image. A hyporeflective border surrounded some of the structures. They corresponded spatially to hyperAF flecks identified on SW-AF images (see examples, Figs. 7A, 7C, 7D) and to darkened foci in NIR-AF images as has been described previously. With regard to their intraretinal location, it had been assumed that the AF of flecks in SW-AF images originated from RPE cells since SW-AF is associated with lipofuscin in the RPE. However, horizontal ss- and SD-OCT B-scans through the fovea (Figs. 7A–D, lower), revealed that, in some cases, the flecks extended anteriorly through photoreceptor attributable bands and interrupted the IS/OS junction band, and even the band attributable to the outer limiting membrane (Fig. 7D). These cases are in agreement with those of Sparrow et al., who reported that flecks identified in SW-AF images corresponded to hyperreflective deposits traversing photoreceptor-attributable bands in SD-OCT scans. The latter lesions are interpreted as being indicative of degenerating photoreceptor cells.

Our results using the wide-field en face approach have implications for the clinician. They suggested that en face wide-field ssOCT has the potential to be a clinically useful tool in the management of patients with STGD1. In agreement with recent studies, we have shown that the subRPE slab enhances visualization of RPE atrophy. We also have shown that the IS/OS slab provides the best means for visualizing IS/OS junction loss, and the preRPE(OS) slab for flecks. In addition, when we compared the en face sub-RPE measurements to the central hypoAF area measurements on SW-AF, and the en face

**Figure 7.** Examples of the en face preRPE(OS) slab images with corresponding SW-AF foveal ssOCT, and SD-OCT scan images for four patients. The horizontal arrows in (A) and (C) indicate that the spatial locations of the hyperreflective flecks correspond to those on the SW-AF images. The vertical arrows in (C) and (D) connecting the SW-AF images to the horizontal foveal SD-OCT scan indicate the intraretinal position of the flecks.
IS/OS measurements to the abnormal AF measurements, we found good agreement. The results of the Bland-Altman plots suggested that the en face wide-field ssOCT technique can be used as a complementary test to SW-AF, the current clinical gold standard, for measuring and monitoring changes in RPE atrophy. Moreover, the en face approach we used has the added advantage that the clinician can visualize and measure the area of IS/OS junction loss, a marker of photoreceptor loss, inspect the cross-sectional scans to obtain information regarding the status of the RPE and photoreceptor layers, and determine the intraretinal location of flecks. The use of the wide-field ssOCT technique can serve as a complimentary test to SW-AF for detecting and monitoring changes at various stages of the disease whether SW-AF changes present as moderately defined mottling as in Figure 2E, or are widespread as in Figure 2F. In both cases, we were able to observe, and measure, a central hyperreflective area indicating RPE atrophy on the en face sub-RPE slab image.

One of the limitations of the en face approach is that it is dependent on reliable segmentation of the IS/OS junction band and RPE/BM boundary. We found that we had to correct some of the automated segmentation of these boundaries manually, particularly in the region of the lesion. In addition, the quality of some of the en face IS/OS slab images affected the clarity of the boundaries of IS/OS junction loss. For these cases, we checked the accuracy of our en face IS/OS area measurements by comparing them to the extent of IS/OS junction loss on the corresponding individual B-scans. Another limitation of the current study was that comparison with NIR-AF imaging was possible only in a small subgroup of patients. Despite these limitations and the small sample size used in this study, we demonstrated that the wide-field en face slab approach has the potential to be a useful clinical tool for detecting and monitoring changes in RPE atrophy, IS/OS junction loss, and fleck distribution in patients with STGD1. In addition, a comparison between en face and SW-AF results has contributed to our understanding of the spatial and intraretinal location of flecks, a prominent clinical feature of STGD1.

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