Challenges Associated With Ellipsoid Zone Intensity Measurements Using Optical Coherence Tomography

Karen E. Lee¹,*, Heather Heitkotter²,*, and Joseph Carroll²,³

¹ Medical College of Wisconsin, Milwaukee, WI, USA
² Cell Biology, Neurobiology & Anatomy, Medical College of Wisconsin, Milwaukee, WI, USA
³ Ophthalmology & Visual Sciences, Medical College of Wisconsin, Milwaukee, WI, USA

Optical coherence tomography (OCT) allows noninvasive visualization of individual retinal layers and has become a mainstay in the diagnosis and management of a wide range of retinal and systemic diseases. As the number of available treatments increases, there is growing interest in developing sensitive OCT-based biomarkers for assessing therapeutic response. In particular, the hyperreflective outer retinal band just posterior to the external limiting membrane, also known as the ellipsoid zone (EZ), is a widely used biomarker of photoreceptor structure. The integrity of the EZ, EZ lesion size, and width/area of retained EZ are established metrics that have been correlated with visual acuity and other aspects of retinal function (e.g., microperimetry and electroretinography). More recently, EZ reflectivity has emerged as a potentially more sensitive biomarker of photoreceptor structure, as reflectivity has been shown to undergo changes in retinal degenerative conditions prior to more marked changes in EZ integrity. However, multiple challenges exist that prohibit widespread clinical utilization. Interdevice variability can impact OCT image appearance due to differences in hardware, acquisition parameters, and image processing methods. In addition, image analysis practices vary widely across studies—this lack of standardization prevents robust comparison of results between studies and inhibits more widespread adoption of extracted biomarkers. Finally, there is ambiguity as to how well EZ intensity/reflectivity correlates with underlying photoreceptor structure as assessed with adaptive optics-based imaging methods. Here we review these challenges and discuss their impact on the use of EZ reflectivity measurements.

Translational Relevance: Qualitative evaluation of the ellipsoid zone band on optical coherence tomography is a valuable clinical tool for assessing photoreceptor structure, though more quantitative metrics are emerging. Awareness of the challenges involved in interpreting quantitative metrics is important for their clinical translation.
Figure 1. Horizontal line scan through the foveal center of the left eye of a 24-year-old female with normal vision acquired using a Bioptigen SD-OCT device. Scan was acquired using a setting of 1000 A-scans/B-scan, and the image is a registered average of 20 B-scans. OCT line scans enable delineation of the various retinal layers, including the four hyperreflective outer retinal bands. Of particular interest is the second hyperreflective band, also known as the ellipsoid zone (EZ) or inner segment/outer segment (IS/OS) junction (highlighted blue in the inset black box). Scale bar = 200 μm.

There is some controversy with respect to the name and anatomical origin of this hyperreflective band—some suggest it originates from the ellipsoid zone (EZ) of the photoreceptor while others suggest it corresponds to the junction between the photoreceptor inner segment (IS) and outer segment (OS; also known as IS/OS).6–9 Regardless of the exact subcellular origin, changes in the appearance of this band (which we will refer to as the EZ) on OCT are often used as an indicator of photoreceptor pathology and thus may serve as a means to monitor disease progression or therapeutic response. The purpose of this perspective is to review some of the main technical considerations that impact widespread reliable utilization of EZ metrics.

### Imaging Methods

To demonstrate some of the concepts in this review, we utilized retinal images obtained from human subjects. Demographic details of each subject and imaging devices used are provided within their respective figure caption. Images were collected under studies which conformed to the tenets of the Declaration of Helsinki and were reviewed and approved by the Institutional Review Board at the Medical College of Wisconsin. Written informed consent was obtained from all subjects prior to their participation in those imaging studies. The images from those original studies reside in an IRB-approved bank and were extracted for use in this review under an IRB-approved bank access protocol (PRO030741).

### Current EZ Metrics

Common metrics for evaluating the EZ include band integrity, EZ lesion area, and width/area of retained EZ (see Fig. 2). One of the more basic measures of EZ integrity is a subjective assessment of whether the band is intact, disrupted, or absent.10–12 Longitudinal reflectivity profiles (LRPs), which evaluate the gray value intensity axially through the OCT image,13 can be used to facilitate this assessment, though this is really only practical for focal assessment of EZ integrity.14,15 Categorical grading schemes capture regional properties of the EZ and have been developed to describe EZ band disruption at the fovea in certain retinal conditions such as diabetic macular edema,16 retinitis pigmentosa (RP),17 and epiretinal membrane.11 In multiple studies EZ integrity is categorically graded (present, absent, attenuated) to correlate with visual acuity, either related to disease severity or recovery posttreatment.11,16,18–20 Another grading scheme has been developed specifically for patients with achromatopsia, where grade 1 indicates an intact foveal EZ, grade 2 shows a small focal disruption or mottled appearance, grade 3 indicates absence of the EZ with a collapsed ELM and normal retinal pigment epithelium (RPE) appearance, grade 4 denotes a hyporeflective zone or foveal cavitation, while grade 5 indicates an absence of the EZ with complete macular atrophy.21 Regardless of the method used to assess EZ integrity, there have been numerous studies across a wide range of diseases examining how EZ integrity correlates to measures of visual function, either to better understand disease pathophysiology or to develop a prognostic indicator of functional outcomes.16,18,20,22–28

EZ lesion size is a quantitative metric defined as the extent of EZ absence/disruption and is commonly reported as total lesion area (px² or mm²). Typically, EZ lesion size is used in populations where breaks in EZ reflectance occur near the fovea, while the peripheral EZ remains intact. EZ lesion size has been correlated with disease severity and progression, with previous studies demonstrating that EZ lesions show associated loss of retinal function and decreased visual acuity in patients with Best vitelliform macular dystrophy,29 solar maculopathies,30 macular telangiectasia (MacTel) type 2,31 and retinal vein occlusion.32 En face OCT has been used to cross-sectionally quantify the attenuation of macular EZ lesion area in Stargardt disease,33 and the rate of EZ loss exhibited high intra- and intergrader reliability,34 suggesting its potential use as a valuable structural outcome measure in clinical trials.
Conversely, retained EZ width or retained EZ area is used to characterize the central region of preserved EZ. In conditions such as RP, the rate of decline in EZ width correlates with the rate of change for the equivalent area of viable retina, and has been used as a surrogate for deterioration of the visual field. Excellent repeatability and reproducibility of EZ width measurements have been demonstrated, supporting its use as a reliable metric to monitor disease progression over time in clinical trials of RP. Despite these strengths, EZ width is measured on a single OCT line scan, which samples only a small portion of the region of preserved photoreceptors and may not capture nonuniformities in the pattern of EZ constriction. On the other hand, measuring the area of preserved EZ with volumetric OCT scans provides a more complete assessment of the retained EZ structure. Sampling the entire EZ rather than a single B-scan can reduce the risk of error, and may more accurately reflect the extent of a functional visual field. Consequently, preserved EZ area has been suggested as a potential anatomic outcome measure for choroideremia and RP clinical trials—as a slower rate of change in EZ retained area could indicate positive treatment response.

Despite their widespread use, the above EZ metrics have some important limitations. For EZ integrity, many categorical grading schemes are subjective, which can result in ambiguity between graders when assessing characteristics of the EZ on OCT scans. Such ambiguity can limit comparison of data between studies. In addition, EZ integrity on its own cannot be used to discriminate between rod and cone photoreceptor structure. While quantitative, EZ width and area metrics (whether representing EZ lesion(s) or the retained EZ) require proper lateral scaling of the OCT image, which necessitates knowledge of the retinal magnification factor for a given eye. Retinal magnification varies between patients due primarily to differences in axial length, but also varies between devices due to differences in the optical design and optical model used to derive the nominal image scale. Proper scaling of OCT and OCT-angiography images is not widespread in the literature, which limits the ability to compare lateral measurements (such as EZ lesion size or retained EZ width/area) across studies. This may not affect longitudinal assessment of EZ structure on a patient level, assuming the patient’s axial length remains constant. However, as trials for inherited retinal degenerations expand to pediatric populations, this will become a major limitation in monitoring disease progression and therapeutic response in individuals where the eye is still growing.
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spectrometer depth (Fig. 4). As such, it is critical to utilize the same acquisition mode (EDI or non-EDI) and control for spectrometer depth, especially if tracking EZ reflectivity over time in the same patient.

**Challenge 2: Logarithmic Versus Linear Display**

OCT captures a large dynamic range of backscattered light to render an image, and these images are regularly presented in a logarithmic scale for easier perception of retinal layers compared to raw/linear data. While this transform enhances perception of contrast toward the lower end of the dynamic range, it results in misrepresentation of real differences in reflectivity and a loss of information. Furthermore, by distorting gray values, hyperreflective outer retinal bands are broadened and their vertical position can be altered within the scan. Measurements of EZ intensity made from logarithmic scale images should therefore be evaluated with caution.

Some studies have utilized linear data for EZ reflectivity analyses. One such study showed a reduction in EZ reflectivity in AMD subjects compared to controls and validated an automated method for extraction of EZ reflectivity to ultimately use on volumetric SD-OCT images. A study of Oguchi disease demonstrated that in light-adapted OCT images acquired with linear scale, the OS layer exhibits reduced Michelson contrast likely due to increased scattering of the EZ. It has also been shown that contrast-enhanced reflectivity obtained from logarithmic transformed images systemically overestimated band thicknesses and altered their position. Although this transformation can be mathematically converted into linear raw data using device specifications provided by the OCT manufacturer, the exact transform is not always disclosed, so even studies that attempt to convert their logarithmic images to a linear scale may be introducing additional errors in layer reflectivity.

**Challenge 3: Normalization Technique**

As discussed above, interdevice variation in EZ intensity has been demonstrated. To correct for this, it has been shown that normalizing EZ reflectivity as
a ratio of the intensity of the EZ band to a retinal layer that exhibits relative constancy through disease states is necessary.71 This normalization allows for comparison across subjects, devices, time points, and can also compensate for differences in spectrometer depth discussed above. However, this can be a complicating factor because each OCT device has proprietary methods of image acquisition and thus different ways to optimize parameters including working distance, reference arm, and spectrometer depth. This must be considered when comparing reflectivity measurements longitudinally, especially for disease states where pathological changes occur gradually over time.

Despite the need for EZ reflectivity normalization for data analysis, there is currently no consensus on a standardized method. For example, some studies have utilized the ELM,66,69,79 RPE,1 or a combination of the retinal nerve fiber layer (RNFL) and vitreous80—each with justification for the chosen layer for normalization. One group normalized to the local area around the specific EZ segment defined as extending 275 μm to either side of the segment and extending axially between the Bruch’s membrane/choroid interface and posterior border of the RNFL.56 Similarly, another group normalized to the mean intensity of the whole retina at the same position for which EZ intensity measurements were taken.57 One study evaluating achromatopsia normalized to a local region of the retinal ganglion cell inner plexiform layer.21 This study demonstrated a significant difference in mean EZ, but not ELM, intensity between achromatopsia subjects and controls, suggesting this normalization method was effective. Within-scan normalization has also been used,81 and while this is an effective way to address the issue of spectrometer depth and EDI/non-EDI mode, it would not correctly account for EZ reflectivity differences due to scan angle. Across these methods for normalizing EZ intensity, some may be better than others due to physiological differences in layer reflectivity. For example, the RNFL has the highest degree of variance in optical intensity and RNFL intensity has been shown to decrease with age, suggesting it as a poor choice for normalization.82

The ONL may be suggested as a possible candidate as it exhibits the least variance in optical intensity,82 however, the Henle fiber layer can increase the apparent ONL intensity (Fig. 3) and can be altered by disruption in cone structure.75 Additionally, the ELM may serve as a reliable layer for calculating relative EZ reflectivity, as it exhibits minimal intensity variation across eccentricity.64 Regardless, variability in normalization technique may preclude comparison of EZ reflectance across studies.

Challenge 4: Relationship of the EZ to Photoreceptor Structure

Beyond the above issues surrounding image acquisition and analysis, perhaps the biggest hurdle impeding the clinical utility of EZ metrics (including reflectance) is their correlation with underlying photoreceptor structure. Cellular-resolution imaging of rod and cone structure is possible with the use of adaptive optics
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(AO) retinal imaging, which correct for the monochromatic aberrations of the eye. Such images enable extraction of information about photoreceptor density and topography in healthy and diseased retinae. In particular, AO scanning-light ophthalmoscopy (AOSLO) has been used to image photoreceptor structure in a wide range of retinal degenerative conditions.

Numerous studies have compared EZ structure on OCT with photoreceptor metrics from AO imaging. Many studies relate photoreceptor metrics from OCT, such as EZ reflectivity, with AO-derived metrics, finding good concordance between modalities in patients with maculopathies, acute macular neuroretinopathy, macular hole, and central serous chorioretinopathy. However, there are some important examples of disconnects, including studies in patients with Usher syndrome, Stargardt disease, and MacTel Type 2 that revealed an intact EZ on OCT even in areas where cone number was reduced and/or cones were damaged in corresponding AO images (Fig. 5). Moreover, some AO imaging studies have shown that loss of EZ integrity may not necessarily indicate an absence of underlying cone structure. For example, studies utilizing split detector AOSLO suggest the presence of remnant inner segment structure within foveal EZ lesions not visible with standard ophthalmic imaging in conditions such as MacTel Type 2, macular hole, cone-rod dystrophy, Best vitelliform macular dystrophy, and achromatopsia. Likewise, the presence of the EZ is not necessarily indicative of completely normal cone structure. For example, in some patients with ocular trauma, distinct cone loss is observed in areas with an intact and normally reflective EZ (though with an altered IZ band). In patients with Bornholm eye disease, there can be pronounced disruption in cone waveguiding despite completely normal EZ structure on OCT.

Furthermore, subjects with albinism and dramatically reduced foveal cone density do not show overt attenuation or reduction of EZ reflectivity. Newer methods of quantifying EZ reflectivity may be worth examining in cases with specific amounts of cone and/or rod photoreceptor degeneration on AOSLO.

These disconnects between underlying photoreceptor and EZ metrics suggest there may be important limitations on the sensitivity of EZ measures for quantifying photoreceptor degeneration across patients or over time within individual patients. Furthermore, it is important to note that existing EZ metrics (including reflectivity measures) from clinical OCT images cannot disambiguate the relative contribution of rods versus cones to the EZ band. These limitations may be overcome with future studies utilizing AO-OCT, which has demonstrated the ability to resolve separate bands associated with the rod and cone outer segments. Additional studies using clinical OCT, split detector and confocal AOSLO (for precise quantification of remnant rod and cone structure), and AO-OCT in populations with variable levels of photoreceptor degeneration could be key to elucidating the limits of EZ metrics extracted from clinical OCT imagery.

**Implications for Clinical Practice and Research**

The challenges reviewed above suggest that standardized methods to evaluate EZ reflectivity...
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It is critical to establish a standardized practice for measuring retinal layer reflectance, particularly for the evaluation of photoreceptor biomarkers. Various EZ metrics serve different purposes, but many rely on the use of the retinal reflectance profile. For example, segmentation software programs often use LRPCs to delineate the individual layers of the retina. Similarly, there are metrics derived from the EZ band using LRPCs, such as measuring outer segment length, that are used as biomarkers of photoreceptor density and spacing. The clinical utility of these metrics requires automation for the processing and analysis involved, like that seen with commercially available segmentation software, or databases available on commercial OCT devices that are used to assess retinal thinning. This is an area of rapid expansion, with new machine-learning and artificial-intelligence based algorithms emerging on an almost daily basis. Many studies have advanced automated methods for classifying various EZ metrics, though determining the extent to which these approaches accurately represent underlying photoreceptor structure (assessed with AO imagery) will be central to defining their clinical value.

A final point to consider is that static assessment of EZ reflectance only relates to structure while dynamic measures of reflectance may inform photoreceptor function. Emerging functional imaging techniques (e.g., dubbed intrinsic optical signal imaging, optophysiology, or optoretinography) capture structural changes in the photoreceptor in response to light. These manifest as changes in the appearance of the EZ and other outer retinal bands in AO-OCT images, or changes in photoreceptor reflectance in AOSLO images. This technique provides the opportunity to better understand biophysical changes to the retinal related to phototransduction, to classify specific photoreceptor classes, and to assess photoreceptor physiology in targeted regions of healthy and diseased retinae. While work is needed to understand how these functional imaging techniques relate to standard structural measures from clinical OCT imaging, optoretinography seems certain to become a valuable tool for improving the diagnosis, management, and treatment of retinal disease.

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* KEL and HH contributed equality to this work and should be regarded as equivalent authors.

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