

Outer Retinal Bands

I read with interest the recent publication by Jonnal and coworkers¹ evaluating the hypothetical origins of band 2 seen in optical coherence tomography (OCT) images of the retina, which the authors contend originates at the inner segment (IS)/outer segment (OS) border of the photoreceptors. Although the authors mention the article raising question as to this designation,² they do not mention a pertinent editorial concerning interpretation of OCT images.³ In that editorial, the history of how the band in question came to be named the IS/OS boundary in the first place was discussed. In two studies correlating the histology to the OCT appearance, both found the boundary to be relatively nonreflective as compared with surrounding structures.^{4,5} The attribution of the name IS/OS boundary to the reflective band appears to have been a transposition glitch.³ Independent of any other aspect of their paper, for the authors to claim that the IS/OS boundary is reflective they need to perform a histologic correlative study that simultaneously shows that the IS/OS boundary is reflective, and they also need to show how the two previous histologic studies were somehow defective.

In citing our work, the authors claim that we overlaid the OCT B-scans on drawings of cones and found that band 2 overlapped the ellipsoid zone. The authors left out two key pieces of information. One is that we used the longitudinal reflectance profile, and the second is that we found the data for both cones and rods to be consistent.² Jonnal et al.¹ excluded rods, considering only the cones as a class in producing OCT reflections. Rods contribute to the OCT image of band 2 as evidenced by analysis of numerous ocular diseases.

The assumptions made by the authors concerning the Heidelberg images also contained important errors. The authors stated that the linear information was not available for Heidelberg OCT images and that the underlying linear information is shown in logarithmic scale. The linear information from a Heidelberg OCT can be exported as a raw file. Each of the B-scans is separately available, and each point is saved as a 32-bit number. The display image is not based on a simple log transform of the 32-bit number, contrary to what the authors stated.

The authors state that the adaptive optics methodology used imaged individual cones. They do not provide supportive evidence of this claim. In figure 5 they compare a linear image from the Spectralis to an adaptive optics (AO) OCT image. In the AO-OCT image, band 1 is stated to be present, although neither the image nor the longitudinal reflectance profile offers any evidence of its presence. When looking at linear OCT data, the reflection from band 1 is quite dark given the limited range of half-tone printing and the human visual system; however, the lack of precise indication of the location of band 1 precludes any judgment about the relative placement of bands 2 to 4.

Band 2 is shown by a series of bright spots that appear to be 1 to 2 pixels wide by 5 to 6 pixels high. The authors superimposed five colored lines to show representative longitudinal reflectance profiles, thus obscuring part of the image; but the unobscured portion shows that the bright spots in band 2 do not line up with the bright spots found in band 3 or what the authors call band 4. This is demonstrated in the authors' own longitudinal reflectance profiles. I evaluated the unmarked parts of the image between g and h and to the right of j, and created a lateral reflectance profile (Fig. 1). At the retinal eccentricity the authors sampled, cones are separated from each other by rods. If the bright grains each represented

subcellular structures in single cone cells, then they should align and be easily separable, given the stated lateral resolution of the instrument used. However, the lateral reflectance profiles from bands 2 to 4 do not show this in their figure 5. The bright spots in bands 2, 3, and 4 do not show a vertical alignment of the bright spots.

Therefore there are two important problems illustrated here. The authors appear to have confused speckle caused by reflections from cellular substructures of individual cells (and any unaccounted for reflections from the rods) with the substructures themselves. The formation of speckle in thick multilaminar diffusers such as the retina is complex to model, but speckle occurs in response to reflection from the cellular substructures and has a time-varying nature from fluctuations in ocular refraction due to transport phenomena in the eye, perturbations of the tear film, Brownian motion of the structures contributing to the reflection, and other factors. Individual speckle grains occur within a statistical envelope; averaging multiple images together averages, fills in, and smooths over the individual grains. Thus the authors' statement that "averaging over multiple cells leads to an overestimation of layer thickness due to axial displacements of the reflections" (p. 7909) is not necessarily correct. Averaging can produce a band that more accurately represents the underlying source of reflections. The inherent roughness of a segmented representation of band 2 in figure 4 may result from true variation in IS height, and the statistical nature of speckle is also a contributor. The same statistical fluctuation would affect thickness measurements such as in figure 6. Second, the lack of vertical alignment shows, at best, that the individual reflectors imaged by the authors in one layer are not from the same cell as those in adjacent layers. This would seem to preclude conclusions about the intracellular versus intracellular plus extracellular causes of reflection leading to visualization of band 3.

In figure 2, the authors show en face images purported to be band 2 and band 3, stating that since both levels reveal regular mosaics of bright spots, the light is confined laterally to the interiors of the photoreceptor cells. The authors provide two small images to support this claim. I extracted and thresholded these images to create a color overlay (Fig. 2). Many of the spots from one band do not overlap those of the other layer. Therefore the limited information presented in figure 2 does not support the claim of intracellular origin of the reflections, and it is not

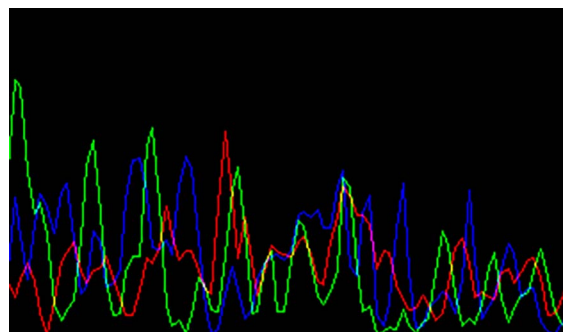


FIGURE 1. The intervals between the color lines used to show the longitudinal reflectance profiles were used to generate lateral reflectance profiles shown above. Band 4 is shown in red. The averaged brightness of vertical columns of pixels across the image is shown; brighter areas would be shown as peaks. The same was done for band 3 (green) and band 2 (blue). Note that the peaks in one layer do not correspond to peaks in either of the other layers.

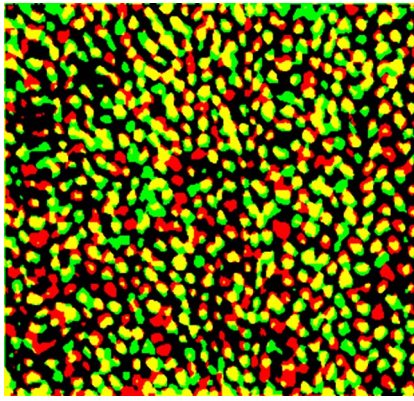


FIGURE 2. Data shown as en face images of band 2 and band 3 from Figure 2 of Jonnal et al. are compared. Bright spots in band 2 are shown in *red* and bright spots in band 3 are *green*. The overlap of the two would produce yellow. Note that there are bright spots in band 2 that don't necessarily overlie the bright spots in band 3 and therefore do not necessarily arise from the same cells.

clear how parts of cells could be imaged in one layer but not in subjacent layers.

An overarching aspect that is difficult to put into perspective is the nature of the sampling strategy used in the study. Although rods undoubtedly contribute to OCT images, these were not included in the authors' images or model, and it is not known if the technique to remove the rod contribution adversely affected the images obtained from the cones. In the images provided, and by their own admission, only some of the cones in any field were imaged. No explanation was given as to how imaged cones were included, what processes caused other cones not to be imaged, or biases introduced by these processes. If some cones aren't even imaged, how can it be possible to assume the cones that are imaged are an unbiased sample? The problematical nature of the sampling is evident in the lack of correspondence between the levels imaged (figs. 2, 5) indicating that even parts of the same cell were not imaged. Thus the authors' data do not support their claims.

The anatomical IS/OS junction is not abrupt; there are multiple smaller discs at the basal evaginations, extension and overlap of the inner segment and the outer segments in the form of the cilium and the calycal processes.² Thus there is no sharp transition of refractive index. Estimates of the refractive index change are greater between the myoid and ellipsoid sections than between the ellipsoid and the outer segments.² The origin of reflections can be made more difficult to discern when waveguiding exists. While the photoreceptors have been modeled as waveguides, it is clear from the anatomy of photoreceptor cells that they are functionally composed of several serial waveguides, each with the potential to have some amount of backscatter within and between the waveguiding segments. This makes deducing the exact origin of reflections difficult. At the junction of the ellipsoid with the outer segments, the calculated reflectance according to the authors' calculations requires a sensitivity of 57 dB, certainly within the realm of possibilities for spectral domain OCT, but also outside of the 43-dB range the authors claim for the Heidelberg spectral-domain OCT instrument.

These difficulties highlight the uncertainty of the origin of the reflections. In the deliberations concerning the standardization of nomenclature, these difficulties were recognized.⁶ There is an uncertainty as to what exactly is the origin of band 2, and this was openly stated in our paper² and in the lexicon paper.⁶ The location overlaps the ellipsoid region of the photoreceptors, and as such the band was called the ellipsoid zone instead of actually being named the ellipsoid layer itself. The authors state that changing this name would necessitate rethinking past assumptions. However, this is the basis of scientific advance and is not a negative. Consider that band 3 was under contention when our paper was published.² The Davis Eye Group claimed that this was Verhoeff's membrane, which is actually the aggregate of tight junctions between retinal pigment epithelial cells.⁷ Clearly the tight junction between cells cannot exist as a separate structure above the same cells, as we pointed out when we suggested that the reflection actually arose in the zone of overlap between the apical processes of the retinal pigment epithelial cells and the cone outer segments.² This change was not catastrophic and in fact was not even mentioned by the authors.

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