Summary

Adaptive optics (AO) images afford direct in vivo visualization of the photoreceptor mosaic. Owing to their clinical application for detection and tracking of photoreceptor degeneration, a common metric used to analyze these images is cell density. Density can be computed by directly counting individual cells or by using estimates of global cell spacing (DFT-based), each of which has advantages for clinical applications. Here we sought to analyze the efficacy of DFT techniques in deriving estimates of density in a variety of AO images.

1 DFT Analysis and “Yellott’s Ring”

“Yellott’s ring” first described the annular power spectrum of the cone mosaic, using histological samples published by Petryk. The modal frequency depicted by “Yellott’s ring” can be elucidated by taking the radial average of the image’s power spectrum. This modal frequency is inversely related to the modal spacing of the mosaic. In both the histology and in vivo images, a rod and cone peak is visible in the radial average of the corresponding DFT. These DFT-derived estimates of rod density overestimate the direct count density by over 25%. Scale bar is 20 µm.

2 Imaging The Foveal Cone Mosaic

Shown below are foveal images from 12 subjects, acquired using a newly constructed AOSO. Images were obtained at 0.9° (A) or 0.75° (B–C) from the center of fixation. Each image is an average of 50 individual AOSO frames that were registered using a “striping” registration method, in which the images are divided into strips, aligning each one to the location in a reference frame that maximizes the normalized cross correlation. Scale bar is 20 µm.

3 Equivalent of Direct Counting and DFT-based Cone Density Estimates

We compared direct counting and DFT-based density estimates for each of the 12 images in Figure 2. Density estimates from the two methods are positively correlated (A), and a Blend-Allen plot reveals good agreement between the two techniques (B), with no systematic bias. Solid line is the mean difference, dashed lines are 95% confidence limits.

4 Disruptions in the Rod Mosaic Degrade the Accuracy of DFT-based Rod Density Estimates

We have recently developed the ability to image the complete rod mosaic using an AOSO. A natural extension is to apply the DFT analysis to these rod mosaic. The extraction of rod density from estimates of modal rod spacing is complicated by the interference cone mosaic (interacting the crystalline rod mosaic). In both the histology and in vivo images, a rod and cone peak is visible in the radial average of the corresponding DFT. These DFT-derived estimates of rod density overestimate the direct count density by over 25%. Scale bar is 20 µm.

5 Estimating Photoreceptor Density in Retinal Imagery

Just as the presence of cones can disrupt DFT-based density estimates of the peripheral rod mosaic, the presence of photoreceptor pathology will similarly disrupt cone and rod density estimates. Shown below are images from individuals with red-green color vision deficiency (A), congenital stationary night blindness (B), and blue cone monochromacy (C). DFT-based density estimates were in error by an average of 19% (range = 3.8% - 43.9%). In some cases, as cones degenerate, the remaining rod mosaic may fill the space, resulting in a more reliable DFT-based estimate. In these conditions, local estimates of rod and cone spacing may be more appropriate to capture the behavior of the photoreceptor mosaic. Scale bar is 20 µm.

6 Estimating DFT-based and Direct-count Estimates of Photoreceptor Density in Adaptive Optics Retinal Imaging

DFT-based and direct-count estimates of density diverge as the mosaic of interest becomes disrupted, either due to the presence of small-cell type or retinal degeneration. This is in part due to the implied periodicity of a foveal origin of measurement: the assumption that is a cycle occurs without interruption through a spatial or time series. Thus, DFT-based estimates of photoreceptor density will be inherently wrong without an adjustment for area. As AO systems can now image rod and cone mosaics simultaneously, it is important to develop methods to reliably estimate density estimates of both mosaics, without relying to counting every cell (as 10% of the cells may be photoreceptor). If the spatial organization of the mosaic is to be studied, individual cell identification is required. The clinical utility of alternative measures of spatial organization remains to be determined.

Conclusions

DFT-based and direct-count estimates of density diverge as the mosaic of interest becomes disrupted, either due to the presence of small-cell type or retinal degeneration. This is in part due to the implied periodicity of a foveal origin of measurement: the assumption that a cycle occurs without interruption through a spatial or time series. Thus, DFT-based estimates of photoreceptor density will be inherently wrong without an adjustment for area. As AO systems can now image rod and cone mosaics simultaneously, it is important to develop methods to reliably estimate density estimates of both mosaics, without relying to counting every cell (as 10% of the cells may be photoreceptor). If the spatial organization of the mosaic is to be studied, individual cell identification is required. The clinical utility of alternative measures of spatial organization remains to be determined.

References

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