Evaluating Descriptive Metrics of the Human Cone Mosaic

Robert F Cooper,1,2 Melissa A. Wilk,2 Sergey Tarima,3 and Joseph Carroll1,2,4,5

1Biomedical Engineering, Marquette University, Milwaukee, Wisconsin, United States
2Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin, United States
3Biostatistics, Institute for Health and Society, Medical College of Wisconsin, Milwaukee, Wisconsin, United States
4Biophysics, Medical College of Wisconsin, Milwaukee, Wisconsin, United States
5Ophthalmology, Medical College of Wisconsin, Milwaukee, Wisconsin, United States

Correspondence: Joseph Carroll, Eye Institute, Department of Ophthalmology, Medical College of Wisconsin, 925 N 87th Street, Milwaukee, WI 53226-0509, USA; jcarroll@mcw.edu.


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PURPOSE. To evaluate how metrics used to describe the cone mosaic change in response to simulated photoreceptor undersampling (i.e., cell loss or misidentification).

METHODS. Using an adaptive optics ophthalmoscope, we acquired images of the cone mosaic from the center of fixation to 10° along the temporal, superior, inferior, and nasal meridians in 20 healthy subjects. Regions of interest (n = 1780) were extracted at regular intervals along each meridian. Cone mosaic geometry was assessed using a variety of metrics — density, density recovery profile distance (DRPD), nearest neighbor distance (NND), intercell distance (ICD), farthest neighbor distance (FND), percentage of six-sided Voronoi cells, nearest neighbor regularity (NNR), number of neighbors regularity (NoNR), and Voronoi cell area regularity (VCAR). The “performance” of each metric was evaluated by determining the level of simulated loss necessary to obtain 80% statistical power.

RESULTS. Of the metrics assessed, NND and DRPD were the least sensitive to undersampling, classifying mosaics that lost 50% of their coordinates as indistinguishable from normal. The NoNR was the most sensitive, detecting a significant deviation from normal with only a 10% cell loss.

CONCLUSIONS. The robustness of cone spacing metrics makes them unsuitable for reliably detecting small deviations from normal or for tracking small changes in the mosaic over time. In contrast, regularity metrics are more sensitive to diffuse loss and, therefore, better suited for detecting such changes, provided the fraction of misidentified cells is minimal. Combining metrics with a variety of sensitivities may provide a more complete picture of the integrity of the photoreceptor mosaic.

Keywords: adaptive optics, photoreceptors, modeling, cone mosaic

Adaptive optics (AO) enhanced ophthalmoscopes permit noninvasive visualization of the human retina with cellular resolution. Imaging of the cone1–5 rod6–8 and retinal pigment epithelium (RPE)9–13 mosaics has been demonstrated in healthy and diseased eyes. While pathology can often be quite striking when imaged with single-cell resolution, the ability to use these images to detect subtle changes relies on the ability to extract quantitative information about the mosaic of interest. This process often involves assessing metrics derived from the cell locations within an image. Metrics such as density,14–24 spacing,12,14,15,23,25–31 and regularity19,32–34 are frequently used to characterize the cone mosaic. Despite their broad use, there has been minimal evaluation of the ability of these metrics to detect disruptions of the photoreceptor mosaic. Such testing is needed to objectively assess the strengths and weaknesses of these metrics in evaluating retinal mosaics, especially with the growing demand to image the photoreceptor mosaic over time (either following therapeutic intervention or to monitor disease progression).

One of the more significant factors known to affect metrics used to describe the cone mosaic is undersampling. Undersampling can come from two sources: cell misidentification or cell loss.35,36 First, algorithms used to automatically or semiautomatically identify cells in retinal mosaics have some nonnegligible errors that can vary substantially with image quality.14,15,34 As most metrics rely on cell identification rather than the retinal image itself (though Cooper et al.37 uses a Fourier transform-derived spacing extracted directly from the image), the error introduced by this undersampling is an inherent feature of most current AO analyses. How this source of undersampling affects a given metric provides a direct measure of its “robustness.” Second, various retinal diseases result in the actual loss of cells due in part to the optical waveguiding properties of photoreceptors, the cone mosaic can be imaged with particular ease. In fact, the cone mosaic can be resolved in some individuals even without using AO.38–46 Moreover, cone photoreceptors drive the majority of our visual function and are affected in a variety of retinal diseases. Thus, there is continued interest in the development and validation of metrics for detecting disruptions or changes in the cone mosaic. Following the approach developed by Cook,35 in which he compared versions of the...
of the metric for all cones with bounded Voronoi cells, divided by the
whether a given cone mosaic is normal or abnormal.
represents a strength or a weakness when trying to determine
insensitivity (or robustness) of a metric to diffuse cell loss
and highlight the important
understanding the strengths and limitations of these metrics,
The data presented here provide a useful framework for
undersampling approximates the expected pattern that might
FIGURE 1. A schematic of a hexagonally arranged patch of cones
illustrating the relationship between the distance measurements used
in this study. A single cone (red circle) and its six closest neighbors
(open circles) are highlighted for clarity. The NND is defined as the
distance from a given cone to its closest neighbor (orange dashed line). The FND is defined as the distance from each cone to its most
distant neighbor (blue dashed line), and ICD is defined as the average
distance between a cone and all of its neighbors (dashed lines). In
order to mitigate boundary effects, only cones with bound Voronoi
regions (shaded region) are included when calculating each metric.
The regularity of each of these metrics (M) is defined as the mean (μM)
of the metric for all cones with bounded Voronoi cells, divided by the
metric’s SD (σM).

same mosaic that had different amounts of undersampling, we
examined the performance of a number of metrics by applying
known amounts of diffuse cell loss (i.e., undersampling) to
photoreceptor mosaic coordinates derived from images of the
human cone mosaic. This pattern of cone mosaic disruption has
been observed in conditions such as retinitis pigmentosa,25,41
cone-rod dystrophy,25 red-green color vision deficiency,58 and
glaucoma.25 In addition, this type of undersampling approximates the expected pattern that might
occur as a result of errors in manual or automated cell detection.
The data presented here provide a useful framework for
understanding the strengths and limitations of these metrics,
and highlight the important “philosophical” issue of whether the insensitivity (or robustness) of a metric to diffuse cell loss
represents a strength or a weakness when trying to determine
whether a given cone mosaic is normal or abnormal.

METHODS
Human Subjects
This research followed the tenets of the Declaration of
Helsinki, and was approved by the institutional review boards
at the Medical College of Wisconsin (Milwaukee, WI, USA) and
Marquette University (Milwaukee, WI, USA). Twenty subjects
with normal trichromatic vision were recruited for this study
(median age: 23.5, range, 9–67 years; Supplementary Table S1).
Subjects provided informed consent after the nature and
possible consequences of the study were explained. Individu-
als with high myopia or hyperopia (>10 dipters [D]) were
excluded from this study. Axial length measurements were
obtained on all subjects using an IOL Master (Carl Zeiss
Meditec, Dublin, CA, USA). To convert from image pixels to
retinal distance (μm), we first acquired images of a Ronchi
ruling positioned at the focal plane of a lens with a 19-mm focal
length to determine the conversion between image pixels and
degrees. An adjusted axial length method25 was then used to
approximate the retinal magnification factor (in μm/degree)
and convert to micrometer per pixel.

Imaging the Human Photoreceptor Mosaic
The photoreceptor mosaic was imaged using an AO scanning
light ophthalmoscope (AOSLO), where both confocal48 and
nonconfocal split-detector imaging modalities were acquired
simultaneously. Imaging was performed along the temporal,
inferior, nasal, and superior meridians using a 790-nm super-
luminescent diode. Using a 1.0° field of view (FOV), each
meridian was sampled every half degree from fixation out to 6°,
and then every degree from 7° to 10°. Using a 1.5° FOV, each
meridian was sampled every degree from fixation out to 10°.
To correct for static intraframe distortion resulting from the
sinusoidal motion of the resonant optical scanner, we
estimated the distortion from images of a stationary Ronchi
ruling and then resampled each frame over a grid of equally
spaced pixels. Then, a reference frame was selected manually
from within each image sequence for subsequent registration
using custom software.59 Montages of overlapping split-
detector and confocal images using both 1.0° and 1.5° FOVs
were created semiautomatically using custom software. To
simplify the process of montaging, custom software was
created in MATLAB (Mathworks, Natick, MA, USA) that allows
the user to rapidly screen which images should be included in
a montage. After screening, the selected images were
automatically placed in a corresponding Photoshop (Adobe,
San Jose, CA, USA) file at a location extracted from the digitized
image acquisition notes. Once the montage was “seeded”
using this software, the user manually positioned the images
within Photoshop to achieve a more accurate alignment.

Analyzing the Cone Photoreceptor Mosaic
Because foveal cones could not be reliably resolved in all
subjects, the location of peak foveal density was determined
using a previously described method.50 First, cone coordinates
were semiautomatically identified from a foveal montage using
a previously described cell identification algorithm.15 Isoden-
sity contour maps were generated from the resulting coordi-
nates. Six contours (at 80%–95% of the peak cone density)
were extracted from each map, and the center (x, y) position
of each contour was averaged to provide an estimate of the
location of peak foveal cone density within the foveal montage.
Regions of interest (ROIs) were then extracted from each
montage, relative to the location of peak foveal cone density,
using custom software (Photoshop and MATLAB). The size
of each ROI varied as a function of eccentricity, using published
AOSLO-derived cone density data31 to estimate the area
necessary to encompass approximately 100 cones at each
ROI as described next. Using the minimum foveal cone density
observed by Wilk et al.51 (84,000 cones/mm²), we set the area
of ROIs at the location of peak foveal cone density to 37 × 37
μm. Due to the minimal change in cone density beyond 10°, we
set the area of ROIs at and beyond 10° to 100 × 100 μm. We
next fit an exponential function to these areas, establishing an
eccentricity-to-ROI area relationship. We obtained ROIs at the
foveal center, every 50 μm from 50- to 600-μm eccentricity,
evory 200 μm from 600- to 1600-μm eccentricity, and every
500 μm from 1600- to 3100-μm eccentricity. Within 500 μm of
peak foveal cone density, ROIs were extracted from the
confocal modality, while beyond 500 μm, ROIs were extracted
from the split-detector modality due to superior cone contrast.
When either blood vessels or seams between overlapping
images occurred at a desired ROI sampling location, we

\[ \text{Regularity} = \frac{\mu_M}{\sigma_M} \]

\begin{align*}
\text{Nearest Neighbor Distance (NND)} & \quad \text{Farthest Neighbor Distance (FND)} \\
\text{Intercone Distance (ICD)} &
\end{align*}
### Table

<table>
<thead>
<tr>
<th>Eccentricity Bin, μm</th>
<th>Density, Cones/mm²</th>
<th>NND, μm</th>
<th>DRPD, μm</th>
<th>ICD, μm</th>
<th>FND, μm</th>
<th>% 6-Sided</th>
<th>VCAR</th>
<th>NoNR</th>
<th>NNR</th>
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<td>0</td>
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<td>2.43 ± 0.24</td>
<td>3.10 ± 0.37</td>
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<td>8.37 ± 1.38</td>
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<td>50</td>
<td>114,000 ± 20,200</td>
<td>2.56 ± 0.23</td>
<td>3.28 ± 0.34</td>
<td>3.27 ± 0.32</td>
<td>4.06 ± 0.45</td>
<td>62.9 ± 12.2</td>
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<td>8.35 ± 1.14</td>
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<td>3.41 ± 0.31</td>
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<td>3.71 ± 0.31</td>
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<tr>
<td>300</td>
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<td>9.55 ± 1.65</td>
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<td>350</td>
<td>52,200 ± 9,900</td>
<td>3.78 ± 0.33</td>
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<td>42,700 ± 10,100</td>
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<td>5.01 ± 0.38</td>
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<tr>
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<td>7.35 ± 0.83</td>
<td>52.9 ± 6.08</td>
<td>6.92 ± 1.69</td>
<td>7.92 ± 0.88</td>
<td>8.59 ± 2.05</td>
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<tr>
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<td>4.72 ± 0.46</td>
<td>5.50 ± 0.55</td>
<td>6.15 ± 0.64</td>
<td>7.72 ± 0.83</td>
<td>52.5 ± 6.10</td>
<td>7.00 ± 1.79</td>
<td>7.95 ± 0.84</td>
<td>8.55 ± 2.22</td>
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<tr>
<td>600</td>
<td>24,200 ± 5,860</td>
<td>5.46 ± 0.71</td>
<td>6.40 ± 0.96</td>
<td>7.18 ± 0.90</td>
<td>9.01 ± 1.21</td>
<td>51.8 ± 6.55</td>
<td>6.93 ± 1.60</td>
<td>7.85 ± 0.82</td>
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<tr>
<td>1000</td>
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<td>5.98 ± 0.78</td>
<td>7.15 ± 1.21</td>
<td>7.85 ± 0.93</td>
<td>9.85 ± 1.22</td>
<td>52.6 ± 6.56</td>
<td>6.96 ± 1.45</td>
<td>7.92 ± 0.95</td>
<td>7.59 ± 1.21</td>
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<td>7.82 ± 1.27</td>
<td>8.61 ± 0.78</td>
<td>10.8 ± 1.02</td>
<td>54.5 ± 6.09</td>
<td>7.18 ± 1.44</td>
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<td>7.86 ± 1.32</td>
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<td>8.95 ± 0.76</td>
<td>11.2 ± 1.09</td>
<td>54.1 ± 5.85</td>
<td>7.45 ± 1.43</td>
<td>8.11 ± 0.84</td>
<td>7.87 ± 1.23</td>
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<td>13,100 ± 2,080</td>
<td>7.35 ± 0.56</td>
<td>9.09 ± 0.86</td>
<td>9.56 ± 0.78</td>
<td>12.0 ± 1.14</td>
<td>55.2 ± 6.64</td>
<td>7.52 ± 1.56</td>
<td>7.96 ± 0.84</td>
<td>8.33 ± 1.27</td>
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<tr>
<td>1900</td>
<td>11,900 ± 1,960</td>
<td>7.73 ± 0.60</td>
<td>9.38 ± 0.89</td>
<td>10.1 ± 0.88</td>
<td>12.6 ± 1.40</td>
<td>53.4 ± 7.10</td>
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<td>8.05 ± 0.89</td>
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<tr>
<td>2500</td>
<td>9,570 ± 1,390</td>
<td>8.48 ± 0.60</td>
<td>10.5 ± 1.11</td>
<td>11.2 ± 0.80</td>
<td>14.0 ± 1.11</td>
<td>50.3 ± 7.19</td>
<td>7.46 ± 1.50</td>
<td>7.56 ± 0.89</td>
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<td>11.0 ± 1.12</td>
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<td>3100</td>
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<td>9.21 ± 0.68</td>
<td>11.4 ± 1.39</td>
<td>12.2 ± 1.02</td>
<td>15.4 ± 1.59</td>
<td>48.6 ± 7.65</td>
<td>7.02 ± 1.50</td>
<td>7.42 ± 0.92</td>
<td>7.64 ± 1.10</td>
</tr>
</tbody>
</table>

NND, nearest neighbor distance; DRPD, density recovery profile distance; ICD, intercell distance; FND, farthest neighbor distance; VCAR, Voronoi cell area regularity; NoNR, number of neighbors regularity; NNR, nearest neighbor regularity.
lost.

abnormal cone mosaic until greater than 24% of the cells have been undersampling, implying that density cannot reliably detect an abnormal cone mosaic until greater than 24% of the cells have been lost.

mitigate this effect, we used the Voronoi tessellation to establish which cell locations should be included for analysis. To enable easier comparison, each ROI was binned based on the nearest sample location. Regions of interest within an eccentricity bin were then compared across all subjects. On average, the ROIs deviated from their bin location by 4.7 μm within 600 μm of the foveal center, and 67.4 μm beyond 600 μm from the foveal center. Cone coordinates were then semi-automatically identified within each confocal ROI,15 and manually identified within each split-detector ROI using custom software (Java 1.8; Oracle, Redwood City, CA, USA) by a single observer (RFC).

Mitigation of Boundary Effects
All geometrical descriptors extracted from a discrete set of coordinates are subject to boundary effects at the ROI edges. The edge cells do not necessarily contribute all of their connected neighbors to a spacing measurement, or even all of the area that they encompass to a density measurement. To mitigate this effect, we used the Voronoi tessellation to establish which cell locations should be included for analysis. Only cones with their corresponding Voronoi cell fully contained within the ROI (i.e., “bound”) were considered for the metric calculations.

Descriptive Metrics of the Cone Mosaic
The cone coordinates for each ROI were analyzed using the following spacing and regularity metrics (regularity metrics, as the name implies, capture the variation of a particular metric over an ROI):

Density. As mentioned above, Voronoi tessellation of the cone coordinates was used to define the bound Voronoi cells in a given ROI. Density was defined as the ratio of the number of bound Voronoi cells in an ROI to the summed area of the bound Voronoi cells. The shaded Voronoi polygons in Figure 1 represent bound Voronoi cells.

Percent Six-Sided Voronoi Cells. The number of sides of each bound Voronoi cell was determined, and the number of Voronoi cells with six sides was divided by the total number of bound Voronoi cells within an ROI.

Density Recovery Profile Distance (DRPD). The density recovery profile (DRP) is a method based on a two-dimensional autocorrelogram that is an expression of the spatial density of cells as a function of the distance of each cell from all other cells.26,36 To automatically determine spacing from the DRP, we first determined the width of each bin as defined by equation 16 in Rodieck et al.,36 assuming a reliability of two. After calculating the DRP, we interpolated between each bin using splines. We then found the first local maximum within the spline that was greater than the DRP density mean. The x-axis location (distance) of the maximum was taken as the DRPD.

Nearest Neighbor Distance (NND). The distance between a given cone and its closest neighbor, where the neighbors of a given cone are comprised of all cones with adjacent Voronoi cells. The NND reported for each ROI is the average NND for all of the cones with bound Voronoi cells in that ROI (Fig. 1, orange dashed line).

Intercell Distance (ICD). The average distance between a given cone and each of its neighbors, where the neighbors of a given cone are comprised of all cones with adjacent Voronoi cells. The ICD reported for each ROI is the average ICD for all of the cones with bound Voronoi cells in that ROI (Fig. 1, black dashed lines).

Farthest Neighbor Distance (FND). The distance between a given cone and its farthest neighbor, where the neighbors of a given cone are comprised of all cones with adjacent Voronoi cells. The FND reported for each ROI is the average FND for all of the cones with bound Voronoi cells in that ROI (Fig. 1, blue dashed line).

Nearest Neighbor Regularity (NNR). The mean nearest neighbor distance (NND) for all of the cones with bound Voronoi cells in an ROI divided by the standard deviation (SD) of the NND for all of the cones with bound Voronoi cells in that ROI.

Number of Neighbors Regularity (NoNR). The mean number of sides of all bound Voronoi cells in an ROI divided by the SD of the number of sides of all bound Voronoi cells in that ROI.

Voronoi Cell Area Regularity (VCAR). The mean area of the bound Voronoi cells in an ROI divided by the SD of the area of the bound Voronoi cells in that ROI.

Examining the Sensitivity of Metrics to Undersampling
After calculating each metric from each normal ROI, we used a statistical classifier to determine the threshold at which a metric could sensitively detect diffuse loss. To create the
classifier, both the eccentricity of each ROI and the normal metric values from each subject were transformed to conform to statistical assumptions for linear models. Each ROI's eccentricity was transformed as follows:

\[ E_t = \frac{1}{1 + E_{\text{lm}}} \]  

where \( E_{\text{lm}} \) is eccentricity in \( \mu \)m, and \( E_t \) is the transformed eccentricity value. The metric values were transformed using the natural log. These transformed data were then fit to a polynomial (orders 1–4). The (1–4) polynomial coefficients from each fit were used to create a 95% prediction ellipsoid, which defined the plausible values for each coefficient.

We used this classifier to assess the sensitivity of each metric to undersampling with the following process: First, we randomly selected a subject and removed between 5% and 80% of the cone coordinates from each of their ROI's (again, representing diffuse cell loss due to disease, or cells missed during the identification step). Cones were removed by first permuting the cone coordinate list according to a uniform random distribution using the randperm MATLAB function. After permuting the cone coordinate list, the number of coordinates defined by the percent loss was removed from the beginning of the list. Next, the remaining (now undersampled) cone coordinates were analyzed using the metrics described above. We then transformed the resultant metric and eccentricity data and performed a polynomial fit as described above on these undersampled mosaics. Finally, we determined if the set of fit coefficients were significantly different from normal by comparing them with the prediction ellipsoid using Hotelling's \( t^2 \)-squared statistic with a 95% significance cutoff. We used this process to calculate an “abnormal mosaic detection rate.”

Using this process, we assessed the detection rate of abnormalities (or statistical power) for each metric at different percent loss values. A metric was considered sensitive to loss at a given percent cone loss when it correctly identified abnormal mosaics in 80% of trials. Finally, at each eccentricity, we constructed 95% pointwise prediction intervals (PIs) for each of the above metrics to describe pointwise uncertainty.

**RESULTS**

We were able to obtain images from all 20 subjects across each eccentricity. The numerical results are summarized in the Table (for meridian-specific values, refer to Supplementary Table S2). Figure 2A illustrates the expected exponential decrease of cone density with eccentricity as reported in previous studies, and the 95% PI for our population. The PI appears larger near the foveal center due to the increased normal variability in foveal cone density. In contrast, the cone spacing metrics increased monotonically as a function of eccentricity (Fig. 3), with the 95% PI being smaller near the fovea (\(< 500 \mu \)m). The three regularity metrics and percent six-sided cells followed previously observed patterns, peaking at about 250 \( \mu \)m (Fig. 4).

To characterize how each metric was affected by undersampling, we first applied undersampling to a single ROI that exhibited average metric values (JC_10145, 200-\( \mu \)m eccentricity). Figure 5 illustrates the effect of 40% and 80% undersampling on this particular ROI. Qualitatively, the mosaic appears less regular with fewer cells remaining. However, without a priori eccentricity information, the ROI could simply be from a location more distant to the fovea. The histograms of each type of spacing each appear different; NND remains tightly clustered about the mean, whereas the mean and spread of ICD and FND measurements dramatically change as
increasing amounts of loss are applied. In the DRP, the mean only slightly changes; in fact, the estimated spacing decreased, though this is likely an artifact due to the bin size selection algorithm. All measurements of regularity and percent six-sided cells for this ROI decreased in response to undersampling (Fig. 6). In this single ROI, the percentage of six-sided cells decreased by a similar amount (by 39% between 0%–40% undersampling, by 40% between 40% and 80% undersampling) between each percent undersampling. Number of neighbors regularity decreased by 47% between 0% and 40% undersampling, and 32% between 40% and 80% undersampling. Interestingly, VCAR decreased by 75% between 0% and 40% undersampling, and roughly half that (31%) between 40% and 80% undersampling, implying that the metric changes more with lower amounts of loss. NNR was the opposite, decreasing only 27% between 0% and 40% undersampling, but substantially more (79%) between 40% and 80%.

We then used the prediction ellipse method described above to examine each metric’s ability to detect undersampling in simulations from all 20 subjects. Density did not reliably detect an abnormality until 24% of the cones had been removed across all eccentricities (Fig. 2B). The NND and DRPD were remarkably insensitive to undersampling; an abnormal mosaic was unable to be detected for either metric until 53% and 55% of cone coordinates were removed, respectively (Fig. 7). In contrast, ICD and FND were able to detect an abnormal mosaic at 29% and 23% undersampling, respectively (Fig. 7). Of the regularity metrics, NNR was the least sensitive, and detected abnormality with above 35% undersampling (Fig. 7). The VCAR and percentage of six-sided Voronoi cells were similarly sensitive and were able to consistently detect a deviation from normal beyond 17% and 14% undersampling, respectively (Fig. 7). Of the regularity metrics, NoNR was the most sensitive, and was able to detect an abnormal mosaic after only 10% of the cone coordinates had been removed (Fig. 7).

**DISCUSSION**

We characterized the normal cone mosaic as a function of eccentricity using both new and previously described geometrical metrics. The metrics examined here had different 95% PI widths, suggesting each metric had different variance. While we examined a wide variety of metrics describing the cone mosaic, this is not an exhaustive list; new metrics may be derived as other retinal cell types are imaged, or as disease processes are better understood. Additionally, metrics can be derived directly from the retinal image; approaches based on analysis of the Fourier spectrum of the image (“Yellot’s Ring”) are already in use,12,25,26,29,30,57,42,54 and others have been published to assess beam direction in the lamina cribrosa.55 Nevertheless, different metrics respond more sensitively to undersampling than others. NND, DRPD, and NNR were the least sensitive to cone undersampling, whereas percentage of six-sided Voronoi cells, VCAR, and NoNR were the most sensitive. Intuitively, one might think that the most sensitive metrics should always be used; however, there are some important points that should be reviewed to provide context to these results.

The pointwise PIs constructed here represent the range that individual metric values will fall, within 95% likelihood. The PIs are constructed from 20 subjects; assuming our 20 healthy subjects are representative of the variance in the population, our estimate of the PI is more conservative than it would be had we included a larger population. For each metric’s PI, we aggregated the results from all meridians to construct the PI. In our population, metrics measured along each meridian (temporal, inferior, nasal, and superior) behaved similarly, which may not always hold.52,56–58 In contrast, the

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**Figure 4.** Mean population regularity measurements. Three different regularity measurements: (A) NNR; (B) VCAR; (C) Number of neighbors regularity and percent six-sided cells (D) are plotted as a function of eccentricity (solid lines) with their respective 95% prediction intervals (dashed lines). All three regularity metrics and percent six-sided cells increased in the parafoveal region and decreased near the foveal center.
classifier tools were constructed for each metric to classify all ROIs from a single subject as either abnormal or normal. However, these classifiers were based on multiple regressions of our data. While density and spacing metrics fit lower order polynomial models closely ($R^2$ goodness-of-fit > 0.95), the unusual shape of the regularity metrics required higher-order (fourth) polynomials to fit well ($R^2$ > 0.8). While on average regularity metrics had $R^2$ goodness-of-fit values above 0.8, without a closer fit ($R^2$ > 0.95), our classifier may underestimate the true amount of variability in regularity metrics across all subjects.

In addition to affecting the size of the PI, the sample size can cause artifacts when constructing the statistical power curves. The prediction ellipse-based classifier used to generate the power curves is constructed from the normal data with no loss; thus, the classifier should correctly identify normal mosaics at a rate similar to the significance level of 95%, or statistical power of 5%.

A different issue relates to the type of cone loss that was adopted for these analyses. Photoreceptor loss is a dynamic process; when cones or rods die, their neighbors can move and fill the gaps, albeit to varying degrees. This is seen in part in the image in Figure 8, which is from a subject with significant cone mosaic disruption (evidenced by the interleaved dark regions throughout the image). The image has density and ICD values that correspond to only 48% of the normal mean at that eccentricity. However, the NND and DRPD values are

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**Figure 5.** An illustration of the effect of cone undersampling on histograms of cell distances (NND, ICD, FND) and the DRPD from a single subject (JC_10145, image acquired 200 μm from the fovea). In each plot, the blue dashed line is the mean of the histogram from the complete mosaic, while the orange dashed line is the mean of the histograms from the 40% (middle row) and the 80% undersampled mosaics (bottom row). On all plots, the y-axis is the number of cells within each histogram bin. The NND histogram is only marginally affected (indicated by the similarity in the blue and orange dashed lines), even with an 80% loss. Similarly, the DRPD is largely unaffected by cell loss; its estimated spacing is only affected when the bin size increases (bottom right) due to a decrease in density. In contrast, the mean (indicated by further separation of the blue and orange dashed lines) and spread of both ICD and FND increase substantially with cell loss.

**Figure 6.** The effect of cone undersampling on measurements of regularity and percent six-sided cells in the same ROI shown in Figure 5. The measured value (normal) is represented by white bars. Undersampling the mosaic by 40% (gray bars) and 80% (black bars) results in a reduction in all four metrics, though each metric decreases at a different rate. Note: Percent six-sided cells has been divided by 10 to fit the scale.
Figure 7. Statistical power curves (solid lines) for each metric as a function of the amount of undersampling (i.e., cell loss). The sensitivity of a given measurement was defined as the point at which the statistical power curve crossed 80% power (horizontal dashed line). Mean NND and DRPD are insensitive to cone undersampling and are only able to reliably detect cell loss when 53% and 55% of cone coordinates were removed, respectively. Mean ICD can reliably detect that the cone mosaic is abnormal after 29% of the cones were removed. Mean FND is the most sensitive of the four spacing metrics and is able to detect an abnormal mosaic after 25% of the cones were removed. Mean NNR was relatively insensitive and was unable to detect an abnormal mosaic until 40% of cones were removed. Percent six-sided are consistent with only a 20% cell loss for this retinal location. Given that each of these metrics describe a different aspect of the mosaic, and that there is such a large disparity between the actual value of each metric and the value predicted by simulated undersampling, the inconsistency of these metrics is likely indicative of an alternative type of loss (such as photoreceptor remodeling). Regardless, exploring the relationship between different metrics and examining how each responds to both simulated and real loss could enable a more quantitative description of the type of cone loss in different retinal degenerations/loss types.

A major concern with the translation of AO imaging to the clinical arena (specifically clinical trials) is that image quality may not always be sufficient to visualize the entire photoreceptor mosaic. In addition to differences in hardware capabilities, pathologies such as AMD and RP are linked with poor image quality due to age or secondary effects of the disease (e.g., cataracts or cystoid macular edema).6,24 In these situations, the use of a metric that is insensitive (i.e., robust) to undersampling (DRPD, NND, NNR) should be used. However, as shown here, these same metrics would be poorly suited for use in longitudinal studies, due to this very same insensitivity. Thus, one has to be very explicit with what it is they are trying to measure when choosing which metric to use. In the end, the most sensitive metric cannot be assumed to be the “best” metric.

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References


