Validated Filter-Based Photoreceptor Count Algorithm on Retinal Heidelberg High Magnification Module™ Images in Healthy and Pathological Conditions

Timo Mulders 1, Patty Dhooge 1, Ludo van der Zanden 2, Carel B. Hoyng 1 and Thomas Theelen 1,*

1 Department of Ophthalmology, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Centre, 6525 EX Nijmegen, The Netherlands; timo.mulders@radboudumc.nl (T.M.); Patty.Dhooge@radboudumc.nl (P.D.); Carel.Hoyng@radboudumc.nl (C.B.H.)
2 Faculty of Medical Sciences, Radboud University Medical Centre, 6525 EZ Nijmegen, The Netherlands; Ludo.vanderZanden@radboudumc.nl
* Correspondence: Thomas.Theelen@radboudumc.nl; Tel.: +31-02-4361-3138

Abstract: Recently introduced, the Heidelberg Engineering™ high magnification module enables in vivo visualization of cone photoreceptor cells. Currently, a reliable analysis of cone mosaic on high magnification module images is hindered by an unfavorable signal-to-noise ratio. In this paper, we describe how a novel high magnification module high-pass filter may enhance cone signals in healthy participants and patients. We compared the cone counts of our filter-based algorithm to the counts of two human graders. We found a good to excellent intragrader and intergrader correlation in both patients and healthy participants. We identified a good correlation between the average cone counts of both graders and high-pass filter cone counts in patients and healthy participants. We observed no significant difference between manual and filter-based counts via the Bland–Altman analysis. In conclusion, a quantitative cone analysis on high magnification module images is feasible manually by human graders and automatically by a filter-based algorithm. However, larger datasets are needed to improve repeatability and consistency by training human graders.

Keywords: high magnification module™; filter-based algorithm; manual cone counts; healthy subjects; central areolar choroidal dystrophy patients

1. Introduction

Noninvasive in vivo imaging of cone photoreceptors in the human eye has been realized by various adaptive optics (AO) imaging modalities [1–3]. Recently, several studies have claimed to enable the visualization of a photoreceptor mosaic without the use of AO [4–7]. The commercially available Heidelberg Engineering™ high magnification module is an example of such a non-AO imaging device. By equipping the SPECTRALIS™ confocal scanning laser ophthalmoscope (cSLO) with a high magnification lens, the high magnification module is capable of capturing 8 × 8-degree images with a digital lateral resolution of 1,5 micron/pixel in high-resolution mode [8]. This has proven to be sufficient to capture images of cone photoreceptor mosaics in healthy eyes and in various retinal diseases [9–11].

Although operating the high magnification module appears less challenging than AO imaging, capturing images of sufficient quality remains difficult [11]. To improve the signal-to-noise ratio, reducing pupil size, capturing images through corrective glasses or lenses and administration of eye lubricant have been recommended [10,11]. Despite optimization, the nonadaptive nature of high magnification module imaging implies that this novel technique is more prone to noise-induced artifacts compared to AO-based imaging modalities [12].
Further enhancement of the signal-to-noise ratio is important to enable a reliable analysis of high magnification module images. Mendonça et al. failed to demonstrate high repeatability or reproducibility for quantitative assessments using the manual counting method in their study, in which 11 eyes of 8 participants were manually counted by two independent human graders. Konstantinou et al. were able to visualize photoreceptor mosaic or retinal vasculature and landmarks in only 12 of 16 normal and 11 of 16 pathologic retinas [10,11]. To minimalize noise in AO recordings, various imaging filters were successfully employed to ameliorate the detection of photoreceptor cells [13–15]. In this paper, we studied the performance of a novel high-pass cone photoreceptor filter in a quantitative cone analysis on high magnification module images. We evaluated intragrader and intergrader reliability and compared the results of automated, high-pass filtered counts from healthy participants and patients to the manual counts of human graders.

2. Materials and Methods

We obtained ethical approval from the local institutional review board (project number: 2017–3535) and conducted this study according to the tenets of the Declaration of Helsinki. We included both healthy participants and patients with central areolar choroidal dystrophy (CACD) with a genetically confirmed p.Arg142 Trp mutation in the PRPH2 gene. In this specific group of patients, cones located in the central retina are primarily affected [16].

The left eye of all study participants was examined via an objective refraction measurement (Nidek Tonoref II, NIDEK Co., Ltd. Gamagori, Japan), 40 degrees color fundus photography centered on the fovea (Topcon, Topcon Corporation, Tokyo, Japan), macular 30 degrees infrared reflectance confocal scanning laser ophthalmoscopy (cSLO) imaging and spectral domain optical coherence tomography (SD-OCT; SPECTRALIST™ Heidelberg Engineering GmbH, Heidelberg, Germany). Our SD-OCT protocol consisted of a 25 × 30 degrees volume of 61 horizontal B-scan lines with 128 µM inter-line distance, covering the macular area and centered on the fovea. Each line scan was obtained by averaging 11 single B-scans online, applying the Automated Real-Time Tracking (ART™) mode of the Heyex™ software (Heidelberg Eye Explorer™ 6.12, Heidelberg Engineering™ GmbH, Heidelberg, Germany).

We used the high magnification module to capture high-resolution ART™ images of the macular region. Five raw frames were automatically averaged by Heyex™ software to obtain a single high magnification module ART™ image. We captured images with an 8 × 8 degrees field of view in the primary position and temporal gaze direction by adjustment of the preinstalled positions of the internal fixation lights of the high magnification module. To ensure the best focus on the photoreceptor layer, we used the spherical equivalent refractive error determined by the objective refraction measurement as a baseline setting and applied a minimal focus correction of no more than ±0.5 diopters by manual adjustment of the focusing knob of the high magnification module. If a subject used corrective glasses or contact lenses in daily life, these were worn during imaging and baseline settings were adjusted to 0.0 diopters. Furthermore, the horizontal orientation of the high magnification module was changed by manually rotating the scanning device in accordance with the primary and temporal ocular gaze direction in such a manner that the scanning direction was always parallel to the retinal surface. The participant was encouraged to maintain a normal blinking rate while we performed the imaging. This helped to prevent tear film irregularities. To ensure reliable distance measurements, we entered corneal diameter measurements acquired from Nidek Tonoref™ in the Heyex™ software before the exam. High magnification module imaging was then performed in non-mydriatic conditions. We used a desk lamp to apply direct illumination to the non-examined eye in order to induce a consensual pupillary constriction in the examined eye, thus reducing the effect of peripheral optical aberrations [17].

To increase the signal-to-noise ratio, we computed compositions of at least five high magnification module ART™ frames in Heyex™ of a single gaze direction. The resulting compositions of each gaze direction were uploaded in the Fiji software (version 15.1 n,
National Institutes of Health, Bethesda, MD, USA) [18]. We adapted pixel size to match the original scale in HEYEX™. We then used the “2D stitching” plugin to merge the central and temporal high magnification module images to a single, continuous image [19].

The fovea was manually identified by a human grader on an OCT volume scan and marked on the accompanying 30 degrees IR image. We used the “Big Warp” plugin to align the corresponding IR and high magnification module images, using retinal vasculature as reference points [20]. Following identification of the fovea on high magnification module images, we used the “Concentric Circle” plugin to generate circles with a radius of 1 to 10 degrees, centered on the fovea on the high magnification module [18]. One degree was assumed to be equal to 291 µM [21]. We manually selected regions of interest (ROIs) of 100 × 100 µM at 3, 6 and 9 degrees in the temporal gaze direction, avoiding blood vessels and imaging artifacts.

Manual counts were performed by two independent human graders using the “Cell Count” plugin [18]. All ROIs were pseudonymized and processed twice by each grader at two different days with a period of at least 7 days in between. This resulted in a total of four counts per ROI. Both graders were instructed to count cellular structures with a round or polygonal shape, regardless of the size. We used examples of manually counted high magnification module images from the study of Mendonça as training images [10]. Cells that crossed ROI boundaries, and were thus not fully visible, were excluded from manual counting.

We then designed a high-pass filter in the Fiji software. We first filtered the original high magnification module image with a Gaussian blur with a predefined sigma radius via the “Gaussian Blur” plugin [18]. We subtracted Gaussian blurred high magnification module images from their original counterparts using the “Image Calculator” plugin [18]:

\[ I'(x_n) = I(x_n) - (I(x_n) \ast G(x, y)) \] (1)

\( I'(x_n) \) is the high-pass filtered high magnification module image, \( I(x_n) \) is the original high magnification module image, \( \ast \) represents the convolution operator and \( G(x, y) \) is the two dimensional Gaussian function. Consequently, only signals above the threshold of the Gaussian blurred image remained in the resulting image. We normalized local contrast in the final image via the “Integral Image Filter” plugin by applying a default X/Y block with a radius of 40 pixels and a standard deviation of 3 [18].

Our Gaussian blur utilizes a two-dimensional Gaussian function, represented by the formula:

\[ G(x, y) = \frac{1}{2\pi\sigma^2} e^{-\frac{x^2 + y^2}{2\sigma^2}} \] (2)

\( x \) and \( y \) are the location indices of the horizontal and vertical axis, respectively, and \( \sigma \) represents the standard deviation of the Gaussian distribution. By increasing the Gaussian sigma radius, a larger image area is blurred. Therefore, adjustment of the sigma radius affects the threshold of the high-pass filter and consequently alters the detection of photoreceptor signals. To optimize the predefined Gaussian sigma radius, we applied a trial-and-error approach and compared the automated cone counts from several distinct sigma radii to the average cone counts of the human graders.

We used the “3D Maxima Finder” plugin to automatically detect cone photoreceptors on high-pass filtered images [22]. This maxima finder enables users to specify the radius of maxima per ROI. The 3D Maxima Finder radii we selected were based upon previously published cone inner segment diameters [23]. Prior to the analysis, all images were converted to 16-bit for analytical purposes. Maxima located on the edge of ROIs were automatically excluded from the analysis. Results were evaluated based on absolute cell counts obtained by the manual and automated counting of cells in ROIs of 0.01 mm². We analyzed intersession and intrasession differences between human graders using an intraclass correlation (ICC) estimate via a Single-Measurements, Absolute-Agreement, 2-Way Random-Effects model in SPSS version 25 (IBM, Armonk, NY, USA). The same
model was used to compare the results of automatically counted, filtered images to the average of manual cone counts. By conducting a Bland–Altman analysis, we studied the intergrader and intragrader differences as well as the difference between human graders and computer-assisted, high-pass filtered counts.

3. Results

We captured central and temporal high magnification module ART™ images of the left eye of 15 healthy participants and 5 CACD patients with a genetically confirmed p.Arg142Trp mutation in the PRPH2 gene. We analyzed a total of 60 ROIs, from which 20 ROIs were located at 3 degrees, 20 ROIs at 6 degrees and 20 ROIs at 9 degrees macular eccentricity. All analyzed ROIs are available as Supplementary Materials.

3.1. Healthy Subjects

Healthy participants had a median age of 29 (range: 21–51) years. We observed no pathological abnormalities on fundus photography and IR OCT imaging of the healthy subjects. On high magnification module imaging, we were able to see cone photoreceptors in every participant. Healthy cones were organized in a homogeneous mosaic, with each cone displaying a variable degree of reflectivity (Figure 1). We evaluated intragrader cone count consistency in grader 1 and found an excellent ICC of 0.980 (0.964–0.989). In grader 2, we calculated a good intragrader ICC of 0.867 (0.674–0.937). We found no significant difference in the number of cones counted by grader 1 in both sessions ($p = 0.775$, one sample $T$-test). However, we found a significant average difference ($p < 0.001$, one sample $T$-test) of 9.38 cones in the number of cones counted by grader 2 in both sessions. We evaluated intergrader agreement by comparing all human grader counts in healthy subjects of grader 1 and 2 and found a good ICC of 0.891 (0.696–0.952). The results of the Bland–Altman analysis of the intergrader and intragrader differences in healthy subjects are depicted in Figure 2. On average, mean counts of grader 1 and grader 2 significantly differed by 19.54 cones ($p < 0.001$, one sample $T$-test) in healthy participants. At 3 degrees, we calculated a median human grader cone count of 158.50 (range: 119–203). At 6 degrees, we found 132.50 cones (range: 97–180), and we identified 108 cones (range: 80–141) at 9 degrees.

![Figure 1. Example of a healthy cone mosaic on high magnification module imaging in the central and temporal gaze direction (A). The green cross marks the location of the fovea. The red lines indicate the corresponding retinal eccentricities. The yellow squares indicate selected ROIs, depicted magnified at approximately 3 degrees (B), 6 degrees (C) and 9 degrees (D) eccentricity. A typical artifact of high magnification module images is visible as a large bright spot (asterisk). Scale bar: 20 µM.](image-url)
3.2. Patients

The median age of the CACD patients was 59 (range 43–71) years. We were able to capture high magnification module images displaying cone photoreceptors in each CACD patient. Contrary to the healthy participants, we observed apparent disruptions in the cone mosaic of all CACD patients (Figure 3). The intragrader cone count consistency of human graders in our patient group revealed an excellent ICC of 0.983 (0.945–0.995) in grader 1 and an excellent ICC of 0.914 (0.744–0.971) in grader 2. We identified no significant differences in the number of cones counted by grader 1 in grading session 1 and 2 ($p = 0.071$, one sample $T$-test), and no significant differences ($p = 0.115$, one sample $T$-test) in the number of cones counted by grader 2 in both grading sessions as well (Figure 2A). We found a good intergrader agreement of cone counts in CACD patients, with an ICC of 0.757 (0.483–0.907). The Bland–Altman analysis revealed no significant difference ($p = 0.131$, one sample $T$-test) in the average cone counts of grader 1 and average cone count of grader 2 (Figure 2C). We calculated the human grader cone count in CACD patients and identified a median of 128 (range: 93–160), 135 (range: 94–190) and 112.50 (range: 92–185) cones at 3.6 and 9 degrees, respectively.

3.3. High-Pass Filter

We tested our high-pass filter algorithm based on the manual cone counts. A sigma radius of 1.5 μM yielded close resemblance with the average human grader counts in healthy participants. The high-pass filtered automated counts revealed a median of 154 cones (range: 133–194) at 3 degrees, 123 cones (range: 109–132) at 6 degrees and 115 cones (range: 98–154) at 9 degrees. We calculated a good ICC of 0.865 (range: 0.755–0.926), and the Bland–Altman analysis (Figure 2D) revealed no significant difference ($p = 0.137$, one sample $T$-test) between average manual counts and computer-assisted high-pass filter counts in our healthy subjects. We also excluded the presence of proportional bias via a linear regression analysis ($p = 0.185$). In 6 out of 45 counts, the difference between manual and
computer-assisted counts was larger than the computed limits of agreement. In our CACD patient group, a high-pass filter sigma radius of 1.5 µM resulted in a mean overestimation of 52.82 cones (standard deviation: 19.37) of computer-assisted cone counts versus human graders and we found a corresponding poor ICC of 0.467 (range: 0.075–0.765). Increasing the Gaussian sigma radius via trial-and-error to 2.5 µM resulted in a higher level of agreement (Figure 2D). We identified a good ICC of 0.816 (0.462–0.938) when using a sigma radius of 2.5 µM, and the Bland–Altman analysis showed no statistically significant difference \(p = 0.085\), one sample \(T\)-test) in average manual counts and computer-assisted high-pass filtered counts in CACD patients. We used a linear regression analysis to exclude the presence of proportional bias \(p = 0.435\). In 2 out of 15 counts, the difference between manual and computer-assisted counts was larger than the computed limits of agreement. At 3, 6 and 9 degrees, we found a median count of 136 (range: 117–149), 135 (range: 110–167) and 131 (range: 108–155) cones, respectively. Although the cone counts of patients and healthy subjects partially appeared similar, in CACD patients, there was no progressive decrease in cone count with increasing eccentricity, as was characteristic in our healthy participants (Figure 4).

![Figure 3](image_url)

**Figure 3.** Example of the appearance of the cone mosaic on high magnification module imaging in a CACD patient (A). The green cross marks the location of the fovea. The red lines indicate corresponding retinal eccentricities. The yellow squares represent selected ROIs, depicted magnified at approximately 3 degrees (B), 6 degrees (C) and 9 degrees (D) from the fovea. An imaging artifact is marked by the asterisk. Scale bar: 20 µM.
to exclude the presence of proportional bias ($p = 0.435$). In 2 out of 15 counts, the difference between manual and computer-assisted counts was larger than the computed limits of agreement. At 3, 6 and 9 degrees, we found a median count of 136 (range: 117–149), 135 (range: 110–167) and 131 (range: 108–155) cones, respectively. Although the cone counts of patients and healthy subjects partially appeared similar, in CACD patients, there was no progressive decrease in cone count with increasing eccentricity, as was characteristic in our healthy participants (Figure 4).

Figure 4. Median cone counts at 3, 6 and 9 degrees in healthy subjects (A) and CACD patients (B), according to human graders and automated high-pass filtered counts. Gray punctate areas indicate the range of cone counts according to human graders, and red upper and lower segmented lines represent the range of cone counts according to high-pass filtered cone counts.

4. Discussion

In the present study, we compared computer-assisted cone counts on high-pass filtered images to the average counts of two independent human graders and consequently identified optimal high-pass filter settings in CACD patients and healthy subjects. Photoreceptor cells were clearly visible in every high magnification module image acquired in our study. The visibility of photoreceptors was sufficient to grade the corresponding images as "good quality", according to the high magnification module grading system proposed by Konstantinou et al. [11]. Nonetheless, after utilizing a Gaussian sigma radius of 1.5 µM, the analysis of 6 ROIs of healthy participants resulted in automated cone counts outside of the limits of agreement on the corresponding Bland–Altman plot. In CACD patients analyzed via a Gaussian sigma radius of 2.5 µM, the results within 2 ROIs were outside of the limits of agreement on the corresponding Bland–Altman plots. In all eight cases, the automated cone counts were higher than the manual counts. Plausibly, the subtle qualitative differences in high magnification module images might require adjusted high-pass filter
settings or the application of additional filters to constrain distinct noise-induced artifacts in order to prevent false-positive “cone” identification by a computer [24,25]. Uncertain variation in qualitative characteristics of high magnification module images might require fuzzy logic-based preprocessing techniques to further ameliorate cone detection prior to the improvement of image quality grading parameters [26,27].

The development of refined, objective high magnification module image quality grading parameters should result in a table of the figure where operators can easily identify the filter settings best used under predefined image and instrument characteristics [28].

Expected cone count should be incorporated as one of the image parameters for the applied Gaussian blur filter. In this scenario, the expected cone count represents the amount of signal present in an image, and other retinal structures represent noise. Until large high magnification module databases are available, the expected cone count may be derived from AO and histological studies or by manually counting a selection of representative ROIs.

Various image parameters influence the expected cone count. In the healthy retina, the number of cones per square millimeter decreases with increasing distance from the macula [29]. Therefore, if a constant cone signal is warranted, the size of analyzed ROIs should be correspondingly adjusted to the distance from the macula. Inversely to cone density, the average cone spacing increases in the periphery [21]. While approximate cone spacing at the macula may be 5 microns, it increases up to roughly 8 microns at 5 degrees eccentricity, which is about 1.6 times the macular value [30]. Therefore, it is important to accordingly adapt the span of the sigma radius in a Gaussian blur filter to avoid positive signals that may be falsely recorded as cone signals [24]. The identified Gaussian sigma radius of 1.5 µM should, for this reason, presumably be adapted if our high-pass filter is utilized to identify cones located closer than ~3 degrees or beyond ~9 degrees from the fovea in healthy eyes.

In diseases affecting the macula, the pathological reduction in cones can induce a spatial increase between residual macular cones [31]. Thus, a corresponding adaptation of the Gaussian sigma in pathological conditions such as CACD may be required to obtain a reliable cone count. As mentioned, the reduction in cones hypothetically affects the amount of signal in an image. Consequently, care should be taken in diseased retinas containing areas of atrophy. In atrophic areas, the expected cone count is extremely low, and therefore signal-to-noise ratio is unfavorable. To avoid selecting ROIs in regions of atrophy, operators can utilize the SPECTRALIS™ multimodal imaging platform and acquire an (en-face) OCT of the corresponding retinal area in addition to the high magnification module image.

A drawback of our study is the current lack of a golden standard regarding cone identification. Only a limited high magnification module training set was available to train human graders. This may be one reason why the Bland–Altman analysis revealed statistically significant differences in agreement between human graders. On the other hand, we found a good intergrader correlation, and we were able to quantify cones in a plausible manner automatically. Both graders had at least four months of experience in high magnification module imaging and achieved good to excellent intragrader and intergrader consistency. Nevertheless, the validation of our results by large image samples with test and validation sets is indispensable.

In conclusion, a regular quantitative cone analysis on high magnification module images is feasible manually by human graders and reliable automatically by a filter-based algorithm. Larger datasets are needed to improve repeatability and consistency by training human graders and validating spatial and disease-related automated cone analyses. We hope that our presented methodology facilitates and encourages researchers to further improve automated cone analyses on high magnification module imaging.
Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app1125347/s1.

Author Contributions: Conceptualization, T.T. and C.B.H.; Methodology, T.M. and T.T.; Software, T.M.; Validation, T.M., P.D. and T.T.; Formal Analysis, T.M.; Investigation, T.M. and L.v.d.Z.; Resources, C.B.H. and T.T. Data Curation, T.M.; Writing—Original Draft Preparation, T.M.; Writing—Review and Editing, T.T., P.D., L.v.d.Z. and C.B.H.; Visualization, T.M. and P.D.; Supervision, T.T. and C.B.H.; Project Administration, T.T.; Funding Acquisition, P.D., T.T. and C.B.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Stichting A.F. Deutman Oogheelkunde Researchfonds (SAF-DOR), Nijmegen, The Netherlands; Landelijke Stichting voor Blinden en Slechtzienden (LSBS), Ede, The Netherlands; Algemene Nederlandse Vereniging ter voorkoming van blindheid (ANVVB), Doorn, The Netherlands; Oogfonds, Utrecht, The Netherlands; Stichting Beheer Het Schild, Wolfheze, The Netherlands.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Radboudumc (protocol code 2017–3353, approval data: 28 January 2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

| Acronym | Definition                                      |
|---------|------------------------------------------------|
| AO      | Adaptive optics                                |
| ART™    | Automated real-time tracking                   |
| CACD    | Central areolar choroidal dystrophy            |
| cSLO    | Confocal scanning laser ophthalmoscope         |
| ICC     | Intraclass correlation coefficient             |
| IR      | Infrared                                       |
| OCT     | Optical coherence tomography                   |
| PRPH2   | Peripherin 2                                   |
| ROI     | Region of interest                             |
| SD-OCT  | Spectral domain optical coherence tomography  |

References

1. Liang, J.; Grimm, B.; Goelz, S.; Bille, J.F. Objective measurement of wave aberrations of the human eye with the use of a Hartmann–Shack wave-front sensor. *Opt. Soc. Am. A Opt. Image Sci. Vis.* **1994**, *11*, 1949–1957. [CrossRef]
2. Roorda, A.; Romero-Borca, F.; Donnelly, W.J., III; Queener, H.; Hebert, T.J.; Campbell, M.C.W. Adaptive optics scanning laser ophthalmoscopy. *Opt. Express* **2002**, *10*, 405–412. [CrossRef] [PubMed]
3. Merino, D.; Dainty, C.; Brada, A.; Podoleanu, A.G. Adaptive optics enhanced simultaneous en-face optical coherence tomography and scanning laser ophthalmoscopy. *Opt. Express* **2006**, *14*, 3345–3353. [CrossRef]
4. LaRocca, F.; Dhalla, A.-H.; Kelly, M.P.; Farsiu, F.; Izatt, J.A. Optimization of confocal scanning laser ophthalmoscope design. *J. Biomed. Opt.* **2013**, *18*. [CrossRef]
5. Pircher, M.; Baumann, B.; Götzinger, E.; Hitzenberger, C.K. Retinal cone mosaic imaged with transverse scanning optical coherence tomography. *Opt. Lett.* **2006**, *31*, 1821–1823. [CrossRef]
6. Sheehy, C.K.; Yang, Q.; Arathorn, D.W.; Tiruveedhula, P.; de Boer, J.F.; Roorda, A. High-speed, image-based eye tracking with a scanning laser ophthalmoscope. *Biomed. Opt. Express* **2012**, *3*, 2611–2622. [CrossRef]
7. Okada, M.; Heeren, T.F.C.; Mulholland, P.J.; Maloca, P.M.; Cilкова, M.; Rocco, V.; Fruttiger, M.; Egan, C.A.; Anderson, R.S.; Tufail, A. High-Resolution In Vivo Fundus Angiography using a Nonadaptive Optics Imaging System. *Transl. Vis. Sci. Technol.* **2019**, *8*, 54. [CrossRef]
8. Jayabalan, G.S.; Kessler, R.; Fischer, J.; Bille, J.F. Compact Adaptive Optics Scanning Laser Ophthalmoscope with Phase Plates. In *High Resolution Imaging in Microscopy and Ophthalmoscopy*; Bille, J.F., Ed.; Springer: Cham, Switzerland, 2019; pp. 377–394.
9. Vasseur, V.; Arej, N.; Alonso, A.-S.; Lafolie, J.; Philibert, M.; Vignal-Clermont, C.; Mauguet-Faïsse, M. Spectralis High Magnification Module imaging in a case of Multiple Evanescent White Dot Syndrome. Am. J. Ophthalmal. Case Rep. 2020, 19, 100727. [CrossRef] [PubMed]

10. Mendonça, L.S.M.; Braun, P.X.; Martin, S.M.; Hübner, A.; Mehta, N.; Zhao, Y.; Abu-Qamar, O.; Konstantinou, E.K.; Regatieri, C.V.S.; Witkin, A.J. Repeatability and Reproducibility of Photoreceptor Density Measurement in the Macula Using the Spectralis High Magnification Module. Ophthalmol. Retina 2020. [CrossRef] [PubMed]

11. Konstantinou, E.K.; Mendonça, L.S.M.; Braun, P.; Monahan, K.M.; Mehta, N.; Gendelman, I.; Levine, E.S.; Baumal, C.R.; Witkin, A.J.; Duker, J.S.; et al. Retinal Imaging using a confocal scanning laser ophthalmoscopy-based high magnification module. Ophthalmol. Retina 2020. [CrossRef] [PubMed]

12. Zhong, J.; Luo, Y.H.-L.; Sim, D.A.; Da Cruz, L.; Anderson, R.; Keane, P.A.; Egan, C.A.; Tufail, A. Non-adaptive Optics Cone Imaging: A comparative study with the Imagine Eyes RTX1. Invest. Ophthalmol. Vis. Sci. 2014, 55, 1596.

13. Muthiah, M.N.; Gias, C.; Chen, F.K.; Zhong, J.; McClelland, Z.; Sallo, F.B.; Peto, T.; Coffey, P.; da Cruz, L. Cone photoreceptor definition on adaptive optics retinal imaging. Br. J. Ophthalmol. 2014, 98, 1073–1079. [CrossRef] [PubMed]

14. Cunefare, D.; Cooper, R.F.; Higgins, B.; Katz, D.F.; Dubra, A.; Carroll, J.; Farsiu, S. Automatic detection of cone photoreceptors in split detector adaptive optics scanning light ophthalmoscope images. Biomed. Opt. Express 2016, 7, 2036–2050. [CrossRef] [PubMed]

15. Bao, H.; Rao, C.; Zhang, Y.; Dai, Y.; Rao, X.; Fan, Y. Hybrid filtering and enhancement of high-resolution adaptive-optics retinal images. Opt. Lett. 2009, 34, 3484–3486. [CrossRef] [PubMed]

16. Boon, C.J.F.; Klevering, B.J.; Cremers, F.P.M.; Zonneveld-Vrieling, M.N.; Theelen, T.; Den Hollander, A.I.; Hoyng, C.B. Central Areolar Choroidal Dystrophy. Ophthalmology 2009, 116, 771–782.e771. [CrossRef] [PubMed]

17. Campbell, F.W.; Gubisch, R.W. Optical quality of the human eye. J. Physiol. 1966, 186, 558–578. [CrossRef] [PubMed]

18. Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; et al. Fiji: An open-source platform for biological-image analysis. Nat. Methods 2012, 9, 676–682. [CrossRef]

19. Preibisch, S.; Saalfeld, S.; Tomancak, P. Globally optimal stitching of tiled 3D microscopic image acquisitions. Bioinformatics 2009, 25, 1463–1465. [CrossRef] [PubMed]

20. Bogovic, J.A.; Hanslovsky, P.; Wong, A.; Saalfeld, S. Robust registration of calcium images by learned contrast synthesis. In Proceedings of the ISBI, Prague, Czech Republic, 13–16 April 2016; pp. 1123–1126.

21. Hirsch, J.; Curcio, C.A. The spatial resolution capacity of human foveal retina. Vis. Res. 1989, 29, 1095–1101. [CrossRef]

22. Ollion, J.; Cochenne, J.; Loll, F.; Escudé, C.; Boudier, T. TANGO: A generic tool for high-throughput 3D image analysis for studying nuclear organization. Bioinformatics 2013, 29, 1840–1841. [CrossRef]

23. Scoles, D.; Sulai, Y.N.; Langlo, C.S.; Fishman, G.A.; Curcio, C.A.; Carroll, J.; Dubra, A. In Vivo Imaging of Human Cone Photoreceptor Inner Segments. Invest. Ophthalmol. Vis. Sci. 2014, 55, 2442–2451. [CrossRef]

24. Li, K.Y.; Roorda, A. Automated identification of cone photoreceptors in adaptive optics retinal images. J. Opt. Soc. Am. A 2007, 24, 1358–1363. [CrossRef] [PubMed]

25. Mariotti, L.; Devaney, N. Performance analysis of cone detection algorithms. J. Opt. Soc. Am. A 2015, 32, 497–506. [CrossRef]

26. Versaci, M.; Morabito, F.C.; Angiulli, G. Adaptive Image Contrast Enhancement by Computing Distances into a 4-Dimensional Fuzzy Unit Hypercube. IEEE Access 2017, 5, 26922–26931. [CrossRef]

27. Oruvov, F.; Maskeliūnas, R.; Damaševičius, R.; Wei, W. Fuzzy based image edge detection algorithm for blood vessel detection in retinal images. Appl. Soft Comput. 2020, 94, 106452. [CrossRef]

28. Oberholzer, M.; Ostreicher, M.; Christen, H.; Brühlmann, M. Methods in quantitative image analysis. Histochem. Cell Biol. 1996, 105, 333–355. [CrossRef] [PubMed]

29. Curcio, C.; Sloan, S.; Kalina, R.E.; Hendrickson, A.E. Human photoreceptor topography. J. Comp. Neurol. 1990. [CrossRef]

30. Sawides, L.; de Castro, A.; Burns, S.A. The organization of the cone photoreceptor mosaic measured in the living human retina. Vis. Res. 2017, 132, 34–44. [CrossRef]

31. Georgiou, M.; Lits, K.M.; Kalitzeos, A.; Langlo, C.S.; Kane, T.; Singh, N.; Kassilian, M.; Hirji, N.; Kumaran, N.; Dubra, A.; et al. Adaptive Optics Retinal Imaging in CNGA3-Associated Achromatopsia: Retinal Characterization, Interocular Symmetry, and Intrafamilial Variability. Invest. Ophthalmol. Vis. Sci. 2019, 60, 383–396. [CrossRef]