A NOVEL METHOD FOR ANALYSIS OF SHORT-WAVELENGTH AUTOFLUORESCENCE IMAGING IN ROD-CONE DYSTROPHY

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Background and Aims

Short-wavelength fundus autofluorescence (AF) using a confocal scanning laser ophthalmoscope (cSLO) is a non-invasive imaging modality that evaluates retinal disease. Increased autofluorescence is believed to indicate regions of elevated lipofuscin, a product of photoreceptor outer segment disc shedding and phagocytosis, while decreased autofluorescence may signify atrophy of the underlying retinal pigment epithelium[1]. Characteristic patterns of AF have been described in retinitis pigmentosa, a group of inherited rod-cone dystrophies (RCD). A parafoveal hyperfluroescent ring has been well-reported in RCD and shown to correlate with psychophysical, structural and electrodiagnostic deterioration as demonstrated by microperimetry, optical coherence tomography (OCT) and electroretinography respectively[2, 3, 4]. Similar sectoral distributions of AF in RCD include a centrally preserved AF and a peripheral granular loss of AF. The AF patterns have been shown to progress alongside a corresponding functional deterioration[5].

Techniques to quantify AF changes in retinal disease have garnered much attention recently in several retinal dystrophies[6, 7]. However, such methods have not been described in RCD, perhaps due to issues with variable luminance and detector sensitivity between images and the peripheral distribution of AF change. Such a method could facilitate patient prognosis through revealing the rate of AF loss as well as be an adjunct in assessing response to therapeutic intervention. Here we report a novel method for serially evaluating AF in patients with RCD, which neither depends on luminance values, which can be difficult to acquire in routine clinical practice, nor requires the installation of additional equipment to the cSLO.

Methods

The control eye of participants recruited into an interventional open-label clinical trial assessing a novel therapy for RCD (NCT01847365) was used for retrospective AF analysis. All participants were between 18 and 80 years of age, had confirmed RCD and underwent AF imaging at three-monthly intervals for a period of 12 months. AF was obtained using a cSLO (Heidelberg Spectralis HRA/OCT, Heidelberg, Germany) post-mydriasis, which was achieved through administration of 1% tropicamide and 2.5% phenylephrine.

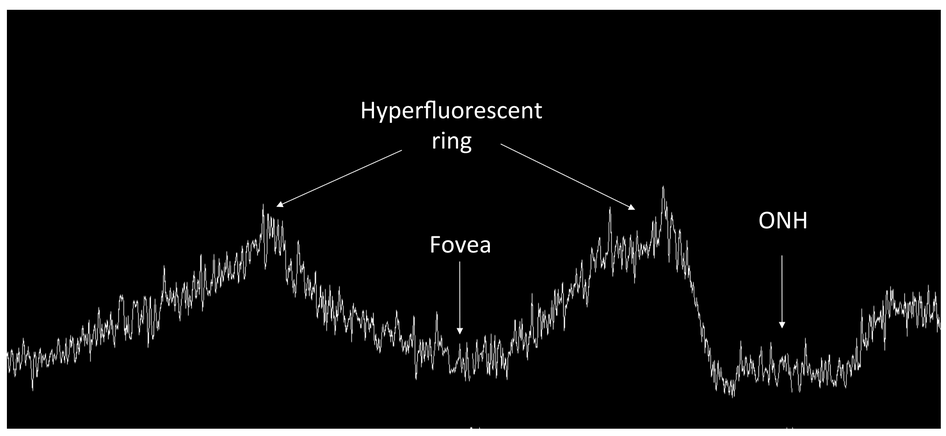

Figure 1: A graph illustrating the changes in fluorescence along the horizontal meridian in one clinical trial participant. The corresponding AF image is shown in Figure 2.

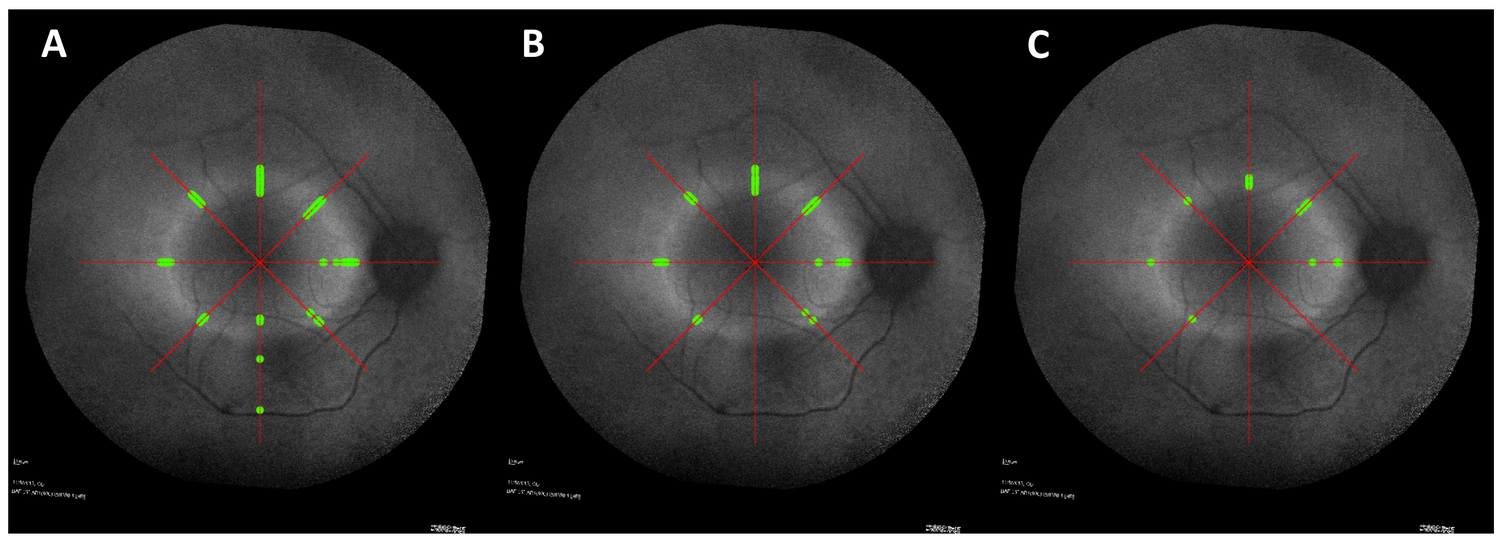

Figure 2: Baseline AF images of the right eye from one participant. The green points indicate instances of fluorescence that correspond to the 80th (A), 85th (B) and 90th percentile (C) of the maximum luminance. Whilst margins were narrower in the 90th percentile image (C) and excluded anomalous points, some regions, such as the inferonasal aspect of the hyperfluorescent annulus would be excluded.

The centre of the optic nerve head (ONH) and fovea were marked on acquired 55 degree normalised AF images. Where the foveal location was not clear, accompanying OCT images were examined to identify the foveal depression. A demarcation line passing through the fovea and ONH centre acted as the horziontal meridian with additional lines positioned at 45, 90 and 135 degrees to this. FME software (Safe Software, Canada) was used to spatially align and match magnification of the marked images. A graph of luminance changes was created (Figure 1). Additionally, FME was programmed to indicate regions which corresponded to the 80th, 85th and 90th percentile of maximum AF (Figure 2). The distance between fovea and ONH centre was set to 3.0mm in all patients in order to convert from arbitrary units.

Results

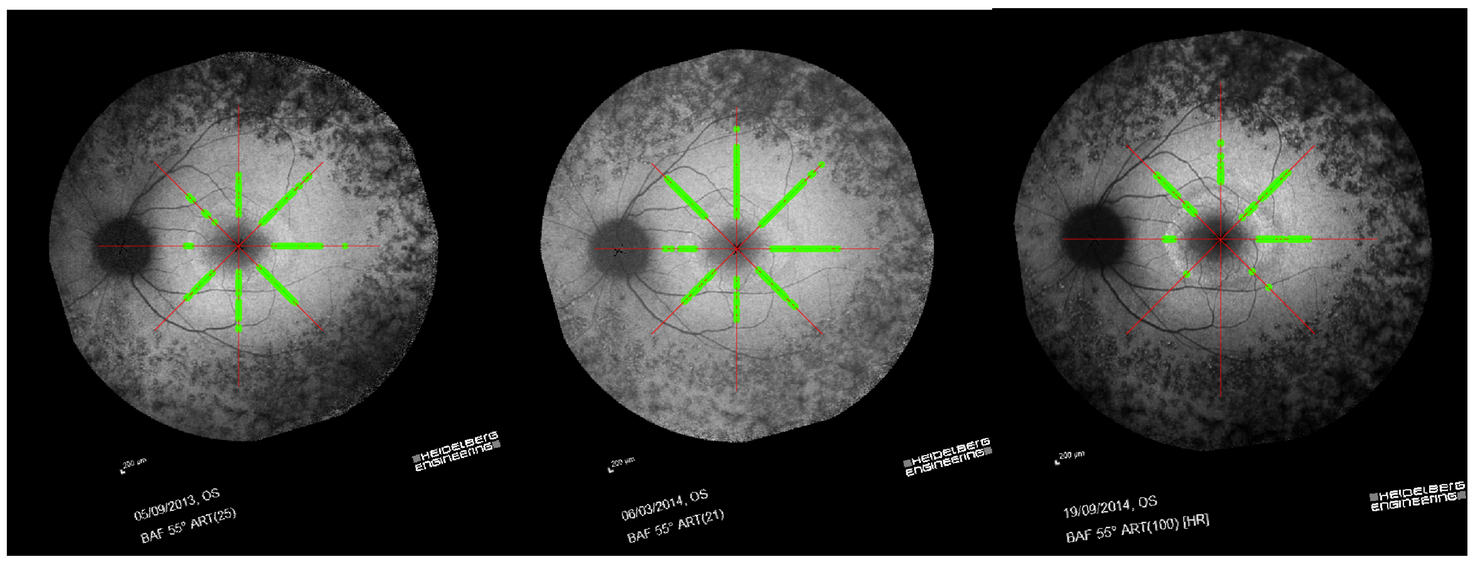

Figure 3: 55 degree AF images from one participant at baseline (A), 6 months (B) and 12 months (C). Corresponding figures for the fluorescent markers are shown in the adjacent table.

Serial AF at baseline, 6 months and 12 months with 85th percentile fluorescence markers for one trial participant is shown in Figure 3. The mean distance between the fovea and each meridian is shown in Table 1 in microns. There was a general constriction in the size of the ring after 12 months with a mean reduction in annulus radius of 230 microns.

Meridian	Baseline (V1)	6 months (V3)	12 months (V5)	V5 – V1
1A	1918.818885	1873.052407	1488.388534	-430.4303515
1B	1346.487237	1421.862847	1184.878793	-161.6084442
2A	1675.669777	1994.023449	1647.779028	-27.89074899
2B	1569.228182	1494.112144	Not detected	N/A
3A	1957.256455	1893.117787	1524.519868	-44.70831422
3B	1477.327298	1407.558262	1058.973654	-898.2828016
4A	1451.547556	1825.768229	1519.943738	42.61644047
4B	1586.226445	1654.699398	1364.539068	-87.00848818

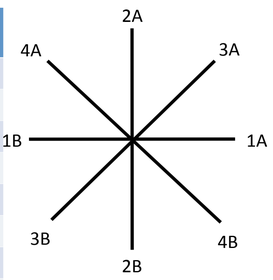

Table 1: Distance from fovea to the green indicators along each meridian are shown in microns. Note that there is a general trend for constriction of the ring apart from in 4A where there was a slight increase. Meridian 2B could not be detected in the 12 month image. Mean reduction in the radius of the annulus was 230 microns. Labeling of the meridians is shown in the diagram on the right.

Conclusions

The methodology illustrated identifies regions of increased AF through a pixel-by-pixel percentile grading system. Selection of an appropriate percentile, such as the 85th, can identify the hyperfluorescent annulus, which is often a hallmark of RCD. Quantitative serial analysis within the same patient can thus be achieved with a relatively straightforward algorithm and without the need for an internal fluorescent reference on the cSLO. In principle, the method may also be extrapolated to measure the peripheral granular loss of AF by utilising an alternative feature. The technique is most informative in patients with a well-defined annulus and may be of limited value in those with primary cone involvement or sectoral RCD, although future work may redress this.

References

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