Use of fundus imaging in quantification of age-related macular change

Hannah Bartlett PhD* & Frank Eperjesi PhD

Ophthalmic Research Group, School of Life and Health Sciences, Aston University,

Birmingham, UK

* Corresponding author: H.E.Bartlett@aston.ac.uk

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Abstract

This review will discuss the use of manual grading scales, digital photography, and automated image analysis in the quantification of fundus changes caused by age-related macular disease.

Digital imaging permits processing of images for enhancement, comparison, and feature quantification, and these techniques have been investigated for automated drusen analysis. The accuracy of automated analysis systems has been enhanced by the incorporation of interactive elements, such that the user is able to adjust the sensitivity of the system, or manually add and remove pixels. These methods capitalise on both computer and human image feature recognition and the advantage of computer-based methodologies for quantification.

The histogram-based adaptive local thresholding (HALT) system is able to extract useful information from the image without being affected by the presence of other structures. More recent developments involved compensation for fundus background reflectance, which has most recently been combined with the Otsu method of global thresholding. This method is reported to provide results comparable with manual stereo viewing.

Developments in this area are likely to encourage wider use of automated techniques. This will make grading of photographs easier and cheaper for clinicians and researchers.

Keywords: age-related macular disease, grading, drusen, imaging, quantification

I.   Introduction

The aim of this review is to present the evidence for use of manual grading scales with analogue images and digital images, to discuss the advantages and disadvantages of digital fundus photography, and to review the development of automated image analysis in the quantification of fundus changes caused by age-related macular disease.

Age-related macular disease is the leading cause of visual loss in those over the age of 50 years in the developed world [6, 21, 37, 44]. In the US, the number of people affected by this condition is expected to increase from 1.75 million to almost 3 million by 2020 [85]. There are several classification and grading systems; one of the more commonly used systems [10] describes early stages of the condition as age-related maculopathy (ARM), and later stages as age-related macular degeneration (AMD). ARM refers to the presence of drusen and retinal pigment epithelium (RPE) irregularities, and AMD refers to more advanced stages of the condition such as geographic atrophy (GA and choroidal neovascularisation (CNV). Eyes with ARM may demonstrate excellent visual function but are at risk for the development of AMD, which is associated with greater visual loss [16].

The prevalence of GA and CNV in the US population over 40 years of age has been estimated at 1.47% [95% confidence interval (CI), 1.38% - 1.55%] [85]. The likelihood of visual deterioration in those with exudative AMD may be reduced with laser photocoagulation and photodynamic therapy [4, 12, 41, 42], but the success rate deteriorates with increasing latency of diagnosis as lesions extend towards the foveal avascular zone.

Photographs are commonly used as an outcome measure in clinical trials, and so it is important to be able to quantify features for comparison. This is particularly relevant in the development of treatment and prevention strategies via controlled trials.

II. Manual grading systems

Many studies have employed the use of grading scales or photographic standards for comparison by observers for qualitative or semi-quantitative assessment of fundus photographs [13, 30, 35, 71, 79]. Review of the literature highlights five systems that have been designed for use in age-related macular disease.

A. The Wisconsin Age-related Maculopathy Grading System [35]

A grid of three circles and four radial lines, concentric with the centre of the macula, is placed over fundus photographs, which are mounted on clear plastic sheets, and placed on a fluorescent viewing box and retro-illuminated using light with a Kelvin rating of approximately 6200° (see figure 1). This wavelength is selected because light with a lower Kelvin rating has a yellow hue, and is therefore less likely to allow identification of subtle drusen.

Insert figure 1 about here.

The slides are examined stereoscopically with a total of x 15 magnification. The circles have radii corresponding to 500, 1500, and 3000 µm on the fundus. The grid defines nine subsections of fundus; some characteristics are graded over the whole area, and some according to subsection. Three sets of open circles printed on clear plastic are used to estimate the size of drusen, area covered by drusen, and areas of pigmentation.

The grading system is divided into three sections; drusen characteristics, other lesion typical of age-related macular disease, and other abnormalities.

Independent grading of 857 eyes was used to assess the degree of agreement between two trained graders using the Wisconsin scale. Weighted kappa ($_{K}$) values for each characteristic are shown in table 1, where $_{K}$ 0.41 to 0.60 = moderate agreement, 0.61 to 0.80 = substantial agreement, and ≥ 0.81 = almost perfect agreement (see table 1).

Insert table 1 about here

Within-grader agreement was also assessed using 80 sets of fundus photographs, two years after the original grading. The exact agreement varied from 62.5 % for drusen type to 100 % for geographic atrophy. The weighted and unweighted kappa scores were generally in the moderate to substantial agreement categories.

B. The International Classification and Grading System for Age-related Maculopathy and Age-related Macular Degeneration [10].

Three circles are centred on the foveola, of diameters corresponding to 1000 μm, 3000 μm, and 6000 μm on the retina. Lesions are graded within each of the central, inner, or outer circles. In this system, five open circles printed on clear plastic can be used to estimate the size of drusen, area affected by drusen, and area with increased or decreased pigmentation, or by AMD. Again, photographs are viewed on a fluorescent viewing box furnishing light with a Kelvin rating of 6200 º at a total magnification of 15 X.

The predominant drusen type is classed as the most common type of drusen present within the outer circle. Large drusen (> 63 μm) are counted separately, but small drusen are not. The area covered by the drusen can be estimated within each of the three circles in the grid and expressed as a percentage of the area within the specific subfields defined by the grid.

Geographic atrophy is graded according to presence, location, and area affected. The minimum GA area size is that of the circle corresponding to 175 μm diameter, because it is difficult to detect choroidal vessels and determine the edges of the GA in smaller areas.

Neovascular AMD may be made up of non-rhegmatogenous retinal detachments or serous RPE detachments. These may be difficult to distinguish from each other and are both graded as neovascular AMD. They are graded according to presence, location, and area affected.

Inter- and intra-observer repeatability in grading lesions using this system has been assessed [62] and the κ statistics were interpreted in the same way as they were for the Wisconsin system. The assessment of inter-observer agreement was carried out for each AMD

characteristics in each of the three zones. Three observers took part in the study, and so for each zone three comparisons were made. The average exact agreement and, in most cases, kappa values, for each zone are presented in table 2. Characteristics marked with * have weighted kappa values, and those not marked have unweighted, or exact, kappa values.

Insert table 2 about here.

Intra-observer agreement was also assessed [62], and minimal and maximal agreement and kappa values are reproduced in table 3. Characteristics marked with * have weighted kappa values, and those not marked have unweighted, or exact, kappa values.

Insert table 3 about here.

In summary, the inter- and intra-observer variability was similar for drusen characteristics and pigmentary changes, but slightly more variability was found in the intra-observer grading of features of advanced AMD than inter-observer.

C. Modified version of the International Classification and Grading System for Age-related Maculopathy and Age-related Macular Degeneration [84]

Subfields are defined by centering a circle with a radius corresponding to 3000 µm on the foveola and bisecting it into semicircles nasal or temporal to the foveola. A circle with radius corresponding to 1500 µm centred on the foveola was superimposed. Thus, inner nasal, inner temporal, outer nasal, and outer temporal sectors were created. Number of drusen greater than 63 µm, size of the largest druse, predominant drusen type, area covered by drusen, confluence of drusen, focal hyperpigmentation, presence of geographic atrophy, and presence of other atrophy are determined for each of these four subfields.

D. Age-Related Macular Disease Study classification system

The Age-Related Eye Disease Study (AREDS) was a randomised controlled trial (RCT) designed to evaluate the effect of a high-dose vitamin C, E, beta-carotene, and zinc

supplement on the progression of age-related macular disease progression and visual acuity [83]. The major age-related macular disease outcomes in this study were the development of GA that involved the centre of the macula or the development of the neovascular form of the disease. The AREDS investigators defined four categories of age-related macular disease, into which participants were allocated at baseline (see table 4).

Insert table 4 about here

Reproducibility was assessed on 1230 eyes; weighted and unweighted kappa values for different disease characteristics have been assessed [82] and range from κ = 0.40 to κ = 0.88 (unweighted) and from κ = 0.51 to κ = 0.88 (weighted). Moderate unweighted agreement between observers was found for fibrovascular/serous pigment epithelial detachment (PED), type of PED, depigmentation within any zone, increased pigment within any zone, presence and maximum size of drusen, drusen within the central zone, within the grid, and outside the grid, reticular drusen and calcified drusen. Moderate weighted agreement was found between observers for depigmentation within any zone and reticular drusen. Substantial unweighted agreement was found between observers for AMD level, type of PED, hard exudates, subretinal pigment epithelial haemorrhage, drusenoid PED, serous sensory retinal detachment, hard exudates, central geographic atrophy, subretinal pigment epithelial haemorrhage, presence and size of drusen, and area covered by drusen within the central subfield. Substantial weighted agreement was found for AMD level, geographic atrophy the central subfield, central zone, and within the grid, increased pigment in all areas, type of drusen, and area occupied by drusen in all areas. Almost perfect agreement was found between observers for presence of advanced AMD and subretinal fibrous tissue (unweighted), and AMD level (weighted).

Assessment of temporal reproducibility based on the sixth annual regarding of images versus baseline found little change except for drusen type κ = 0.32 (unweighted) and κ = 0.53 (weighted).

E. Clinical Age-Related Maculopathy Grading System (CARMS)

CARMS is a modification of the AREDS grading system (Afshari M, Sharma S, Seddon J. Evaluation of the Clinical Age-Related Maculopathy Staging System (CARMS). American Academy of Ophthalmology Annual Meeting. 2000) and has been used in several studies [63-65]. Within this system, macular characteristics are graded within a 3000 μm radius centred on the foveal centre. Eyes with extensive small drusen (≥ 15 small drusen; < 63 μm), non-extensive intermediate drusen (< 20 drusen; ≥ 63 but < 125 μm), or pigment abnormalities associated with AMD are assigned a grade of two. Eyes with extensive intermediate or large drusen (≥ 125 μm) are assigned a grade of three. Eyes with GA receive a grade of four. If evidence of RPE detachment and/or choroidal neovascular membrane was found, a grade of five is assigned. Eyes received a grade of one if none of these signs were present. Advanced AMD is defined as grades four and five.

In order to assess inter-observer agreement, 222 fundus photographs were sent to the Wisconsin Fundus Photographic Reading Center for detailed grading. An algorithm was created to convert the 4-point Wisconsin scale to the CARMS 5-point scale. The level of agreement between one grader and the reading center was determined using the kappa statistic. The comparisons included the grade for the worst eye. The K statistic for inter-observer agreement was 0.77, and the weighted score was 0.84.

D. Summary

The grading scales described were designed for use with stereoscopic fundus photographs. Digital retinal photography is increasingly used in clinical practice and research, prompting investigation of the use of these grading scales with digital images.

III. Use of grading scales with digital images

The advantages of non-mydriatic digital cameras compared to film-based cameras are lower cost, ease of use, good resolution, ability to manipulate the image, provision of immediate feedback regarding the quality of the image for the photographer and the presence of abnormalities for the patient, and no requirement for dilation. Disadvantages include lack of

stereopsis, relative decrease in colour contrast compared to film images in eyes with very red fundi, and a higher frequency of ungradable photographs. Several studies have compared digital photographs and stereoscopic digital photographs with regard to their use in grading of age-related macular change.

Digital stereo images were compared with 35-mm colour transparencies with regard to the quality and reliability of grading age-related macular disease in the context of a multi-centre European epidemiologic study (the EUREYE Study) [86]. The International Classification and Grading System [10] was used to grade 137 digital images and 35-mm slides. The agreement in grading scores between imaging scores was expressed in absolute percentages and was calculated using the weighted κ statistic. Interpretation of the κ statistic employed the following categories [3]: < 0.20, poor; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, good; > 0.81, very good agreement.

The weighted κ value for between-technique agreement ranged from 0.41 for number of drusen < 63 μm to 0.79 for drusen type and total area occupied by drusen. The κ values for atrophic and neovascular end-stage age-related macular disease were 0.87 and 0.94 respectively. The between-technique agreement on stages of age-related macular disease was approximately 0.76. Agreement between graders was similar for both techniques of imaging, and investigators concluded that digital imaging is reliable for the purpose of grading age-related macular disease using this grading system in epidemiological studies.

A more recent study compared 35-mm stereoscopic slide transparencies with digitized non-stereoscopic images for grading abnormalities in age-related maculopathy (ARM) and age-related macular degeneration (AMD) [61] using the International Classification and Grading System [10]. For small hard and intermediate soft drusen, agreement ranged between 77 and 91 % (κ 0.56 – 0.72), and between 83 and 93 % (κ 0.31 – 0.64) for the three macular subfields. Agreement was 12 to 56 % (κ 0.00 – 0.27) for the presence of hyperpigmentation, 94 to 96 % (κ 0.80 – 0.82) for the presence of GA, 93 % (κ 0.78) for the area covered by GA, 94 to 98 % (κ 0.81 – 0.88) for the presence of CNV, and 95 % (κ 0.83) for the area covered by

CNV. The group concluded that digitised non-stereoscopic colour images are useful for grading ARM and AMD.

In 2004, Klein *et al.*, found that gradings from high resolution digital images were comparable with those from film-based images [36]. The images were taken using a 45 º digital camera and standard 30 º fundus camera and graded using the Wisconsin grading system [35]. Exact agreement between gradings of digital and stereoscopic film images taken through pharmacologically dilated pupils was 91 % ($_K$ = 0.85), between digital images taken through dark-adapted pupils and film images taken through pharmacologically dilated pupils was 80 % ($_K$ = 0.69), and between digital images captured through dark adapted and pharmacologically dilated pupils was 86 % ($_K$ = 0.78). Any disagreement for AMD severity levels were attributed to poor quality of digital images. For example, when disagreements were present for specific AMD lesions, drusen ≥ 125 μm were more likely to be graded as present, and increased retinal pigment, RPE depigmentation, and RPE detachment were less likely to be graded as present in manipulated digital images of dark-adapted pupils compared with film images of pharmacologically dilated pupils. Investigators commented that RPE pigmentation was more easily missed by graders on non-stereoscopic digital images.

A modified AREDS grading scale [78] was used by another group to compare stereoscopic digital retinal photography through dilated pupils using a 45º non-mydriatic camera with 35 mm slide photography in the identification of AMD. For 203 eyes there was substantial correlation between the two image formats for identifying AREDS grade 3a or greater (κ_ω = 0.64), and excellent correlation for identifying grade 4b or greater (κ_ω = 0.83).

The results of these studies support the use of the Wisconsin grading system, the International Classification system, and the AREDS classification system for use with digital images of age-related macular disease affected fundii. This means that digital imaging can be employed in using fundus photography as an outcome measure in clinical trials. The time and expense involved in grading is, however, only likely to be reduced with the development of automated image analysis systems.

IV. Fluorescein angiography

The previous sections describe the use of manual systems for grading of age-related macular changes, and also discuss the level of agreement between observers using these grading scales. In general, the inter-observer agreement increases with the level of severity of the disease, for example, presence of GA is easier to identify than the number of drusen ≤ 63 µ in size. This information is important with respect to the use of colour fundus photograph grading as an outcome measure in clinical trials. However, in clinical practice, fundus photographic screening for late stages of the disease such as neovascular AMD is not commonly used. The main reason for this is that fluorescein angiography is generally considered necessary for the diagnosis of this form of the disease.

Despite the fact that fluorescein angiography was first described in the 1950s [40], it has only more recently been in general use for studying the retinal and choroidal circulation. It involves the capture of a series of fundus photographs following intravenous injection of fluorescein dye. The information provided by this technique can be divided into three sections:

1. Flow characteristics in the blood vessels as the dye circulates through the retinal and choroid

2. Detection of abnormalities of the retina and choroid by permitting fine detailed assessment of the RPE and retinal circulation. The RPE normally acts as a barrier to leakage of dye from the choriocapillaris, any breach can be indicative of RPE disease

3. Assessment of the functional integrity of the retinal vessels. Normal retinal vessels are impermeable to the dye, and so any leakage is suggestive of an abnormality.

Fluorescein angiography is also used as an outcome measure in various trials listed on the current controlled trials register, including 'Treatment of Patients With Neovascular AMD Using Indocyanine Green-Mediated Photothrombosis (i-MP)' (NCT00331253), 'Celecoxib to Treat Macular Degeneration in Patients Receiving Photodynamic Therapy' (NCT00043680), 'Phase I Study of Corticosteroid Treatment of Ill-Defined Choroidal Neovascularization in Age-Related Macular Degeneration' (NCT00001615), 'Visudyne® in Occult (VIO)' (NCT00121407), 'The impact of indocyanine green (ICG) angiography in multiple diagnostic

imaging for the management of exudative age-related macular degeneration (ARMD): a single blind prospective randomised controlled trial of fluorescein angiography vs FA & ICG' (N0295142266), and 'Study To Determine Safety/Efficacy of Lucentis For Treatment Of Retinal Angiomatous Proliferation Secondary To Age Related Macular Degeneration' (NCT00395707). Fluorescein angiography also features in publications reporting the investigation of age-related macular disease [20, 53].

The University of Wisconsin-Madison Fundus Photograph Reading Center (UW-FPRC) (http://eyephoto.ophth.wisc.edu) and the Digital Angiography Reading Center (DARC) in New York (www.darconline.com) have protocols available for the acquisition of fluorescein angiography images. These protocols are described below.

A.   University of Wisconsin-Madison Fundus Photograph Reading Center (UW-FPRC) Fluorescien Angiography procedure

This protocol is adapted from the Early Treatment Diabetic Retinopathy Study (ETDRS), Macular Photocoagulation Study (MPS) and the AREDS, Manuals of Operations. Photographers taking photographs for studies read by the UW-FRPC must be certified for the relevant procedures before submitting photographs. Suitable cameras include the Zeiss FF4 series and the Topcon TRC-50EX (used at the 35º setting). For angiography, Kodak T-Max or Ilford 400 speed film are recommended, along with the use of Kodak D-11 developer. Pupils should be dilated to at least 4 mm and the cornea should be undisturbed by prior examination with a diagnostic contact lens.

The fluorescein angiogram contains stereoscopic view of two fields at specified times after injection. These fields include the macular (Field 2) of both eyes and the disc (Field 1M) of the study eye. In order to obtain stereopairs that are correctly orientated in the film strip for stereoscopic viewing, the right member of each pair should be taken first, followed by the left member. Stereoscopic red-free photographs are taken of Field 2 in each eye prior to injection of the fluorescein dye.

After the red-free photographs have been taken, the camera is positioned for Field 2 of the study eye. Fluorescein is injected rapidly (less than 5 seconds if possible) into the anticubal, or other convenient vein according to clinical procedure.

1. Early Phase

The first photograph of the early phase is taken at the moment the injection of dye begins. The second is taken at the moment the injection is complete. These two photographs form a stereo pair, can be referred to as the 'control' photographs, and serve to document the integrity of interference filters. The time shown on the second frame documents the rate of injection.

Ideally, 10 to 16 exposures will be taken at one to two second intervals, beginning 15 seconds after the start of fluorescein injection. This usually results in the production of between five and eight stereo pairs following the control pair, typically culminating about 4—45 seconds after the start of injection.

2. Mid-Phase

The photographer then takes stereo pairs of Field 2 and then Field 1M of the study eye at approximately 60 to 90 seconds. The camera is then positioned in front of the fellow eye and a stereo pair of Field 2 is taken at approximately two minutes. The camera is then repositioned back to the study eye and a stereo pair of Field 2 is taken between two and three minutes.

3. Late-Phase

A stereo pair of Field two in the study eye is taken at five minutes. Two final stereo pairs are taken of Field 2 in both eyes at 10 minutes.

The original negatives should be cut into strips of six images per strip and placed in a 10.5 X 9-inch heavy gauge transparent plastic sheet containing six pockets per sheet. Identification labels should be attached to each page of negatives. When cutting the negatives into strips,

care should be taken not to separate stereo- pairs, and clinical centers should retain a copy of each angiogram.

B Digital Angiography Reading Center (DARC)

The DARC website includes details of their preferred fluorescein angiography photography sequence in tabular form. This has been reproduced below:

1. Red-free stereo pair of the fellow eye
2. Red-free stereo pair of the study eye
3. Control photograph of the study eye at the start of dye injection
4. Control photograph of the study eye at the end of dye injection
5. Stereo pair of the study eye at 30 seconds
6. Stereo pair of the study eye at 60 seconds
7. Stereo pair of the fellow eye at 60 seconds
8. Stereo pair of the fellow eye at two minutes
9. Stereo pair of the study eye at two minutes
10. Stereo pair of the study eye at five minutes
11. Photograph of the optic disc of the study eye at five minutes
12. Photograph of the optic disc of the fellow eye at five minutes
13. Stereo pair of the fellow eye at five minutes
14. Stereo pair of the fellow eye at ten minutes
15. Stereo pair of the study eye at ten minutes.

All images should be taken at 35 degrees and extra photographs can be taken if needed.

C Grading of fluorescein angiography images

Neither the UW-FPRC or the DARC have published protocols for grading of fluorescein angiography images. However, guidelines for the interpretation of fluorescein angiograms of subfoveal neovascular lesions have been published [7]. Photodynamic therapy with verteporfin (Visudyne; Novartis Ophthalmics AG, Basel, Switzerland) has been shown to reduce the risk

of moderate to severe vision loss in selected patients with subfoveal choroidal neovascularization due to age-related macular degeneration [5, 12, 14]. However, it has been shown that the magnitude of the treatment effect varies according to the baseline composition and size of the choroidal neovascular lesion [15]. This means that it is important to identify lesion components and proportions in the selection of eyes for treatment with this therapy.

The photographic eligibility criteria for patients who benefited from the therapy in the Treatment of Age-Related macular degeneration With Photodynamic Therapy (TAP) Investigation [5] and the Verteporfin in Photodynamic Therapy (VIP) Trial [7] are summarised below [7]:

1. Evidence of AMD in either eye, with the absence of any other fundus disease known to be associated with CNV in the eye to receive the therapy

2. A subfoveal lesion in which either new or recurrent classic or occult choroidal neovascularization (CNV) underlies the foveal center (except for lesions that are judged to have subfoveal CNV based on the 360º rule)

3. A lesion with predominantly classic CNV (TAP Investigation [5] only)

4. A lesion with a greatest linear dimension ≤ 5400 µm on the retina

5. If classic CNV is present, approximate Snellen equivalent from letter score better than 20/40 (VIP Trial [7] only)

6. If occult CNV with no classic CNV, then presumed recent disease progression (VIP Trial only) as evidences by blood associated with the lesion or at least five-letter (approximate one line) loss within the previous three months or at least 10 % increase in the greatest linear dimension of the lesion on fluorescein angiography within the previous three months.

Reliability of grading classification by the VIP and TAP study groups was assessed using the kappa statistic on 180 fluorescein angiograms. It varied between 0.70 and 0.85 for percentage of lesion with classic CNV, presence of occult CNV, lesion size, and greatest linear dimension.

D Comparison of photographic and fluorescein angiographic screening for neovascular AMD

The potential for identification of subjects with potentially treatable neovascular AMD using colour fundus photographs has been investigated. Seventy-four stereo pairs of Kodachrome colour slides were evaluated a) nonstereoscopically, b) stereoscopically, and c) stereoscopically with visual acuity and visual symptoms by two retinal specialists. The aim was to identify active exudative lesions. Treatable lesions were considered present if there was fluorescein angiographic evidence of classic or occult CNV within Field 2 (the macula). A consensus between two retinal specialists identified that neovascular AMD was present in 46 % of cases. Agreement between this finding and that of the two readers using colour fundus photographs was excellent; 0.92 (95 % CI: 0.83-1.00) for non-stereo images, 0.87 (95 % CI: 0.75-0.98) for stereo pairs, and 0.86 (95 % CI: 0.75-0.98) for stereo pairs with visual acuity and presenting complaint data.

The investigators concluded that colour fundus image evaluation using either stereo pairs or single images was adequate to identify a high percentage of subjects with active neovascular AMD. False positives were most often associated with misinterpretation of drusen and pigment clumps as lipid or subretinal fluid. Access to visual acuity and visual symptom information yielded the highest sensitivity but the lowest screening specificity. From a clinical viewpoint, the results indicate that evaluation of colour fundus images can be diagnostic for neovascular AMD and may result in timelier referral for angiography and treatment.

V. Automated analysis of digital images

Advantages of digital imaging include the ability to process images for enhancement, comparison, and feature quantification. Computer storage and transmission of digital images has implications in screening of those at risk of age-related macular disease, as well as in clinical research. Digital image analysis techniques have the potential to improve on subjective grading scales in the use of fundus images as an outcome measure.

There are advantages of analogue photographs, including lower cost of equipment and higher resolution. Analogue systems are particularly economical when it comes to stereoscopic imaging, although digital images can be stored locally on disk, or centrally if the computer is networked. Digital images take up a lot of space, for example, if an image is n x n pixels in size, with b bytes per pixel, then the image will occupy at least bn^2 bytes. Hence, a 512 x 512 pixel image of 1-byte pixels will occupy 0.25 MB [23].

A. Image compression

Image compression allows storage of images in a smaller amount of storage space. It can occur with (lossy compression) or without (lossless compression) loss of information. In TIFF (Tagged Information File) compression, the full information can be retrieved but file sizes are large. In JPEG compression (Joint Photographic Experts Group) information is permanently deleted, although different compression levels can be selected. The highest quality JPEG image reduces the file size by 90.2 % compared with a TIFF image [51].

The amount of redundancy in the original data set can be described using the compression ratio (C_R). If n_1 and n_2 represent the number of information-carrying units in two sets of data, the relative data redundancy (R_D) of the first set of data can be calculated from:

$$R_D = 1 - 1/C_R$$

where C_R (the compression ratio) is

$$C_R = n_1/n_2$$

If $n_1 = n_2$, $C_R = 1$ and $R_D = 0$, indicating that the first representation of the data (n_1) contains no redundant data compared to the second representation (n_2). When $n_2 \ll n_1$, $C_R \rightarrow \infty$ and $R_D \rightarrow 1$, implying significant compression and highly redundant data in the first set [28]. Original, TIFF, and low-compression JPEG (30:1) are reported to be indistinguishable in terms of manual grading and digital image analysis, although high-compression JPEG images had noticeably reduced image quality [39].

Another consideration when using digital fundus images for grading is the resolution of the image. A reduction in resolution will also reduce the file size, for example, a 767 x 569 pixel image has a 88 % reduced file size compared with a 2048 x 1360 pixel image, with no perceivable loss of image quality [51]. However, comparison will be hindered if images are of different resolution. This is important when selecting a display medium; if the monitor size is less than the resolution of the image, then data will be lost. Therefore, it is important to ensure that the image resolution is equal to or less than the monitor resolution.

B. Image processing

The objective of ocular image processing is to minimize noise, maximise contrast, and correct for uneven illumination, lens edge artefact, and natural background pigment heterogeneity. Image analysis can generally be divided into five stages [26]:

1. Display of an array of pixels on a computer screen or on paper.

2. Filtering the image by applying transformations based on groups of pixels. This reduces the 'noise' within the image and emphasizes edges (noise is a term used to describe disturbances in data that are either uninterpretable or not of interest). The moving-median filter smoothes flat region of images, but preserves edges by replacing each pixel value with the median of the values in a specified local region.

3. Segmentation refers to the extraction from the image of features of interest, for example, drusen [1, 19, 31].

4. Mathematical morphology uses a collection of operations to study the shapes of objects.

5. Measurement is the final stage of the analysis and involves the extraction of quantitative information from the image.

C. Image pre-processing

Image pre-processing, or filtering, reduces 'noise' and permits improved performance of segmentation algorithms. For example, when a 5 x 5 moving average filter is applied to an image, each pixel is replaced by the average of pixel values in a 5 x 5 square, centred on that pixel. The result is to reduce noise within the image, but also to blur the edges. Application of

a Laplacian filter gives an output consisting of the original image minus the output from the moving average filter. A clearer image is obtained by adding the output from the Laplacian filter to the original image because transitions at edges are magnified. This effect is known as unsharp masking. These filters are all linear. Non-linear filters are more difficult to categorise, but have the capacity to reduce noise without blurring edges [24]. Figure 2 shows an example of pre-processing of a fundus image for drusen quantification.

Insert figure 2 about here.

D. Segmentation

In fundus analysis the segmentation process is used for separation and identification of objects of interest. Objects can be separated from background using pixel intensity, a process known as thresholding. The simplest thresholding algorithm involves selecting a single intensity value above which pixels belong to the object set, and below which pixels belong to the background set. However, it would be unusual to find a situation in which a single intensity value that allows discrimination between objects of interest and the background. For this reason, threshold intensities are calculated from intensity distributions within the local region of interest [67].

In edge-based segmentation, an edge filter is applied to the image and pixels are classified as either edge or non-edge depending on the filter output. Pixels that are not separated by an edge are allocated to the same category. Region-based segmentation algorithms operate by grouping together neighbouring pixels that have similar values, and splitting groups of pixels that have dissimilar values [25].

Objects can also be discriminated according to shape using morphologic algorithms. An example is the segmentation of retinal vessels using an algorithm that identifies long, thin objects [67]. Shape discrimination has also been achieved using matched filters (filters that match the shape of the object to segment) for retinal vessel segmentation [18].

VI. Review of automated digital image analysis for age-related macular disease

It is difficult for the human observer to make accurate quantitative judgements from fundus photographs. For this reason, computer analysis of fundus photograph parameters has been investigated, and is reported to be a highly reliable method of quantifying features such as optic disc pallor, optic disc cupping, and identification of microaneurysms from fluorescein angiograms [2, 38, 46]. Another application is the detection of retinal blood vessels [17], which along with detection of microaneurysms, hemorrhages [69] and hard exudates [8] is of use in diabetic screening. A review of digital image analysis of these features has been published [49].

It would be expected that computer identification and quantification of drusen may be more difficult and less reliable than that of other features such as vessels, exudates, and microaneurysms. Drusen are irregular in shape, and may appear, disappear, or become confluent. Automated segmentation and quantification of macular drusen has, however, been investigated by several groups.

A. Thresholding techniques

In early attempts to use automated image analysis for objective detection and measurement of drusen, an adaptive thresholding technique was used to account for illumination changes and pigmentation variation across the image [50]. The image was divided into overlapping windows of 8 x 8 pixels. It is assumed that if the 8 x 8 pixel window contains both drusen and background, then a grey level histogram of the 64 pixels within the window should have two distinct lobes and peaks. One lobe includes the grey levels of the pixels included in the bright drusen and the other lobe contains those pixels found within the dark background. The valley between the two lobes serves as the proper threshold for this 8 x 8 area. When all the areas had been assigned a threshold value, the thresholds over the entire image were interpolated for all windows using two dimensional linear interpolation. A threshold value was then calculated for each point within the windows and each point was designated as either drusen or background.

The reproducibility was assessed using repeat photographs of the same eye during one sitting. It was calculated as 6.1 % (range: 4.2 – 10.4 %) based on three repeat readings of four eyes. The lowest boundary of reproducibility was assessed via the processing of a photographic duplicate. The reproducibility of four pairs of duplicates was 2.3 % (range: 1.3 – 3.3 %).

The Structured Analysis of the Retina (STARE) project aimed to develop algorithms and software to analyse digitized images of the ocular fundus for the automated diagnosis of disease with ophthalmologic manifestations [27]. Investigators concluded that colour can be used to distinguish objects with markedly different colours such as hemorrhages, exudates and melanomas, but other features such as size, shape, edge sharpness, and texture are required for discrimination when objects are similar in colour. They also describe a method of sequentially adding features selected for their ability to enhance classification [29, 80]. For example, suppose that 95 % of cotton-wool spots, 10 % of drusen, and no exudates have fuzzy edges. If after further colour analysis, an unknown object has a 70 % probability of being a cotton-wool spot and is then found to have fuzzy edges, then its probability (using Baye's formula) of being a cotton wool spot is 87.5 %. Additional features can be added until a threshold level of acceptability is reached [27].

Further attempts at automated extraction and quantification of drusen [45] involved thresholding digitized images, 256 x 192 pixels in size, using an algorithm devised by Otsu [47]. The algorithm depended on the formation of a histogram of gray levels versus their frequency of occurrence, and presumed that there would be two normal distributions of gray levels, separated by a relative reduction in gray level frequencies. In other words, darker levels, corresponding to vessels for example, would be normally distributed, and brighter levels, corresponding to drusen, for example, would be normally distributed. Graphs presented two distinct normal distributions and the point which maximally separated the two distributions was be used as a threshold value. To account for varying retinal reflectance, the image was divided into 16 x 16 pixel blocks for calculations. The final threshold for a particular pixel was calculated by using the mean value of its block, as well as the mean value of adjacent blocks.

A threshold map is formed and subtracted from the original image, leaving the significantly brighter pixels.

Retinal images including drusen were divided into brighter and darker halves. In a threshold map of the darker half, pixels whose values were greater than threshold would theoretically be background or drusen. Thresholding again should have isolated the drusen. This final threshold was increased by 16 grey levels because of the presence of brighter noise and to achieve a balance between false-positive and false-negative results. The final result was a binary image with pixels shown as drusen (white) or not drusen (black).

The number of drusen counted by this automated technique differed significantly from manual counts for 24 images (χ^2 = 10.54; df = 3, p = 0.05). Concordance was high for hard drusen (> 0.12 x 0.12 mm), but soft drusen tended to be under-detected by the automated technique.

VII. Review of semi-automated digital image analysis for age-related macular disease

Automated drusen quantification was taken a step further with the development of an interactive element to image processing. A software package was designed to quantify the area subtended by drusen in colour fundus photographs [68]. Drusen were found to be most easily seen on monochromatic red-free (green) images compared to colour fundus photographs.

Image noise was reduced by application of a 3- x 3-pixel mean filter followed by a 5- x 5-pixel median filter. The images were corrected for uneven illumination and pigmentation by dividing them with the image convolved with an 85 - x 85 -pixel mean filter. This provided an image without sharp detail but with local brightness. A large mean filter was then used to normalise each pixel in the original image with respect to the local brightness image. The size of this filter was chosen such that it averaged high-frequency intensity fluctuations that may be associated with vessels and drusen, but captured some degree of low-frequency brightness variation.

Drusen segmentation was performed on the pre-processed green channel image. The user could specify a region of 1500 μm or 3000 μm radius centred by a mouse click on the fovea. The region of interest was divided into smaller square areas ranging from 20 to 1100 pixels on each side and changing in increments of 10 pixels. It was determined that drusen were present if the skewness of the set of pixel intensities was greater than - 0.5. Drusen are represented by high intensity outliers, and a positive skew reflects a high proportion of these. The areas with skewness greater than – 0.5 were analysed, with the threshold set as the mean pixel value across the region of interest plus a term proportional to the product of the local area pixel brightness standard deviation.

The interactive element to this analysis system involved two levels of supervision. Firstly, the user is able to adjust the sensitivity of the system, where low sensitivity increases the threshold value and fewer pixels are identified. Secondly, for more complex images, the user may add or remove erroneously segmented drusen or add or remove pixels by mouse. An intensity-based region-growing algorithm was also implemented to expand incompletely identified drusen.

Validity was assessed by comparing the computer-assisted method with the current gold standard – manual grading by an expert observer. There was close agreement between the manual and computer-assisted methods and between the two supervisors. The investigators concluded that this method capitalises on both computer and human image feature recognition and the advantages of computer based methods for quantification.

A. Histogram-based adaptive local thresholding (HALT)

Histogram-based adaptive local thresholding was developed to extract useful information from an image without being affected by the presence of other structures [52]. Drusen visibility in various colour spaces was investigated, and found to agree with previous investigation [68] that the green band is most informative and least affected by the overall variation of illumination. A simple technique such as homomorphic filtering [57] was used to compensate for the shape irregularity of the retina causing variable shading across the image [68]. Histogram equalisation

was used for contrast enhancement with development of the multilevel histogram equalisation (MLE) technique. In the MLE operation, the first 'pass' is responsible for enhancing the brightest parts of the image, including small, bright drusen, and the central parts of larger drusen. The darker areas belonging to larger drusen need to be enhanced further. The second stage involves generating more distance between the 'hidden' anomalies and their surrounding areas [52].

Two methods have been employed for drusen segmentation: stochastic classification of pixels into object classes [48], and histogram thresholding for clustering similar intensity pixels into compact objects. The aim of this group was to separate the drusen without being affected by intensity variations caused by vessels, noise, and uncompensated non-uniform illumination.

The HALT operator consists of the following steps: 1) compensation for illumination irregularities using a homomorphic filter, 2) an enhancement operation that stretches intensity differences characterising drusen and background. Thresholding techniques can now be used to detect intense drusen. Global thresholding [47, 58] can be used to remove the background areas. A two-stage histogram-thresholding approach is proposed because a single threshold is not able to identify small intensity differences. The first stage applies to global Otsu threshold [47] to provide an initial segmentation map. This threshold only detects areas of evident abnormalities that are crisply separated from their background. The second stage of thresholding refines the segmentation map by operating on a local level and defining a different threshold for each local region of interest. For this stage, the novel HALT operator was employed. The HALT operator checks the local histogram for general symmetry or asymmetry and uses shape tendency indicators for assessing regions as drusen or background.

The HALT operator is preceded by a morphological dilation operator, which expands the areas that are not removed by global thresholding. The main advantage of this is seen in regions that contain only one or two large drusen without background. In these areas, direct

application of any local threshold would eliminate the drusen area. The morphological dilation operator allows better distinction between bright area and their darker surrounds.

The HALT operator applies different thresholds to regions of the image, depending on the properties of the corresponding histogram. The image is split into 9 windows, which can be split into nine sub-windows if more detailed segmentation is required. Within each window, the HALT operator checks the statistics of the local histogram and assigns the appropriate threshold. A positively skewed distribution indicates that drusen are influencing the higher part of the intensity distribution.

The performance of this operator was assessed by comparing the algorithm's classification (drusen versus background) classification by two experts. Using 23 images, the sensitivity was found to be 98.85 % and the specificity was 99.32 %. False negative detection was 1.15 %, suggesting that the algorithm underestimated drusen area in this case. Investigators concluded that the algorithm provides a diagnosis aid for indicating drusen presence for further examination by a doctor.

VIII. Compensating for fundus background reflectance

Normal macular reflectance increases from the centre outwards towards the arcades. This variation is partly due to luteal pigment in the central macula[11, 76]. A semi-automated digital technique was developed to level the macular background [74], in which the intensity of the green channel image was gradually raised within user-defined oval regions. Using this technique, the background becomes uniform while drusen are simultaneously brightened for segmentation. The technique is reported to be reproducible and to correlate well with standard fundus grading [74]. In a validation study, this technique was successfully used by two institutions where digital drusen quantification was possible in 79 % of the images analysed. There was good correlation between graders (ICC, 0.83; 95 % CI: 67 to 95 for the central subfield and ICC, 0.84; 95 % CI: 69 to 99) for the middle subfield, and also with manual stereo grading [70].

This technique was improved upon [72] by replacing the interactive element with a mathematical model [75]. This 'dots' method firstly requires application of a partial luteal correction to the green channel image. The user then manually selects 25 µm squares (dots) of the remaining normal macular background, from this image. A geometric model of the macular background is then computed using the selected dots, and the luteal corrected image. The model is displayed as an elliptical contour map of grey scale intensity levels. In order to level the background and brighten the drusen deposits, this model is subtracted from the luteal corrected fundus image. The drusen were then segmented by global thresholding and superimposed onto the contrast enhanced image. The threshold was adjusted such that the boundaries of the segmented drusen objects and those of the contrast enhanced objects corresponded optimally upon subjective examination. The final step was to quantify drusen as a percentage of the subfield. This technique compared well with manual drawings (95 % LoA: - 8.3 – 2.8 %) and with a semi-automated method (95 % LoA: - 8.3 – 2.8 %), and was reproducible.

The most recent development in this area involved automating the Otsu method of global thresholding [73]. Background variability has previously limited the success of histogram-based methods, but they could now be combined with the analytic model for macular background to give a completely automated measurement of drusen area. This method involved combining the stereo viewing method with manual tracing on a graphic tablet and an automated method with automatic threshold selection. The technique is reported to provide results that are comparable with stereo viewing [73]. The sensitivity (median, 0.70) of the automated method was less than the specificity (median, 0.81) with respect to the stereo viewing method. This was explained by the fact that lowering the threshold for drusen identification past critical levels resulted in an increased number of false positives. Investigators reported, however, that they found stereo drawing measurements of the same macula by two retinal experts could vary comparably.

IX. Newer examination methods for macular disorders: use in clinical diagnosis and grading?

A. Retinal thickness analyser (RTA)

The RTA is an in vivo imaging device that combines a digital fundus camera with a scanning retinal thickness analyser to create a three - dimensional image of the retina and disc overlaying a colour or red-free digital photograph of the posterior pole. It uses a 543 nm slit beam from a green helium-neon laser to scan the posterior pole and create a false colour image of retinal thickness and disc topography.

The ability of the RTA to create cross-sectional images of the central macula with axial resolution of 50 μm and reproducibility of 13 μm [88] supports its use in the diagnosis and monitoring of age-related macular disease [22]. Its performance is limited however, in those with moderate nuclear or sclerotic lens opacities, or posterior capsular opacification.

B. Heidelberg Retina Tomograph (HRT)

In scanning laser ophthalmoscopy (SLO), a laser beam from a light-emitting diode (LED) is used to create a topographic view of the posterior pole. Confocal SLO (cSLO) permits the generation of a 3-D topographical image [87]. The original HRT scans 32 sections deep with 256 x 256 pixels per coronal section that can be set to 10º x 10º, 15º x 15º, or 20º x 20º. The HRT II creates a 15º x 15º 3-D composite of axial scan images between 16 and 64 sections deep with 384 x 384 pixels per coronal section. The relative positions of the different pixels with the highest reflectivity are used to develop false colour 3-D topographic map of the disc or macula.

The advantages of cSLO over fundus photography include improved image contrast by the selection of the appropriate wavelength for the laser [66], reduced power levels, and confocal images. Colour cSLO images can be obtained with a system that uses three primary colour lasers. The resultant images are combined to produce a true colour image [43]. Confocal scanning laser ophthalmoscopy imaging may aid more effective computer analysis of retinal images. The contrast of pathological features such as macular oedema, exudates, and

drusen can be increased by varying the laser wavelength. This should enable improved accuracy for computer algorithms.

Automated detection of drusen from colour fundus photographs and SLO images has been compared with manual counting of drusen [34]. For a specificity of 90 %, the sensitivity of the colour photographs was higher (60 %) than the SLO (35 %) when compared to manual counting. The colour photograph techniques also showed superior reproducibility compared with the SLO. However, use of the cSLO may be more suitable due to superior image contrast of these images [34].

Scanning laser ophthalmoscopy also enables imaging of fundus autofluorescence (AF), which is mediated by RPE lipofuscin accumulation [54, 55, 77]. Confocal scanning laser ophthalmoscopy has been used to demonstrate abnormal AF patterns in the junctional zone of GA [32, 55] (Bellman C, Schutt F, Unnebrink K, et al.: Symmetric manifestation of geographic atrophy of the retinal pigment epithelium in patients with age-related macular degeneration (ARVO abstract 1667). Invest Ophthalmol Vis Sci 40: S315, 1999). Areas of increased AF have been shown to precede the development and enlargement of outer retinal atrophy in eyes with AMD [33], and positive correlation has been reported between areas of increased AF around areas of GA, and the enlargement of GA over time ($r = 0.06$, $p = 0.0002$) [59]. Imaging of AF is reported to be superior to fundus photography for outlining areas of GA [32, 56].

An automated method of image analysis has been shown to be more accurate than manual outlining of areas of GA [60]. Manual outlining measured larger areas than quantification (reader 1: mean difference = 1.04 mm, 95 % CI [0.66, 1.42], 95 % LOA [-0.83, 2.91], reader 2: mean difference = 0.62 mm, 95 % CI [0.43, 0.81], 95 % LOA [- 0.31, 1.55]. The agreement between readers had a mean difference of 0.39 mm (95 % CI [0.02, 0.76], 95 % LOA [- 1.41, 2.20]) for manual outlining and a mean difference of – 0.03 mm (95 % CI [- 0.23, 0.18], 95 % LOA – 1.03, 0.97]) for automated quantification. Furthermore, a classification system has been developed to distinguish phenotypic patterns of AF alterations in the junctional zone of GA [9], with a view to identification of prognostic determinants for the spread of GA and visual loss.

Investigation of AF in choroidal neovascularization (CNV) patients found preservation of AF in those who developed CNV within the previous six months [61]. As evidence suggests that a continuous area of AF represents surviving RPE [32], this apparent initial conservation of RPE may have implications for treatment interventions [61].

X. Conclusion

Grading scales and digital image analysis have been investigated with regard to anterior eye pathology, largely because of the necessity to track contact-lens induced changes in clinical practice. In the retina, diabetic screening has prompted the same investigations. It could be argued that grading of age-related macular change is of little consequence in the clinical setting. The only treatment option reported for those with early stages of the condition is a high-dose vitamin and mineral formulation, reported to slow the progression of the condition by 25 % [83]. However, quantification of change in this condition is vital for use as an outcome measure in clinical trials investigating new treatments and prevention strategies.

Analysis of inter-observer agreement for the four main grading systems included in this review suggest moderate to substantial agreement for most features of age-related macular disease. The general trend is for an increase in the level of agreement with increasing severity of disease. For example, for the Wisconsin grading system, the kappa value for inter-observer agreement is 0.68 for drusen size, and 0.87 for the presence of GA. Similarly, for the International Classification and Grading System, the kappa value for inter-observer agreement is 0.45 for hard drusen (0.63 µm) and 0.80 for presence of GA.

Evidence suggests that commonly used grading systems can be used with digital retinal photographs. When assessing the intra-observer agreement between grading of colour fundus photographs and digital images using the same grading system, there is a similar trend as was found for inter-observer agreement using the same grading system. For example, one group found weighted kappa values of 0.41 for number of drusen < 63 µm and 0.87 for presence of GA [86] using the International Classification and Grading System [10].

Digital photography allows automated analysis of images. This has been successful in quantifying features such as vessels, microaneurysms, and haemorrhages. Drusen segmentation proves more difficult. It could be argued that measurement of the area affected by drusen is more relevant than the number of drusen, as this may more realistically represent the extent of RPE damage.

Review of the literature shows great advancement in digital image analysis over the past 20 years. The development of the HALT operator by Rapantzikos *et al.* [52], extends the work of others in the development of robust, unsupervised detection and reliable quantitative mapping of drusen abnormalities. Most recent work has involved compensation for fundus background reflectance. These automated techniques are not yet widely used in clinical or research settings. However, the use of digital photography is more commonly used, and investigation into the use of established grading scales with digital, as opposed to analogue images, has shown that it is a convenient and versatile way of imaging the fundus.

Method of literature search

We identified pertinent articles on grading and imaging of age-related macular disease published in peer-reviewed journals, through a multi-staged, systematic approach. In the first stage, a computerized search of the PubMed database (National Library of Medicine) and the Web of Science database was performed to identify all relevant articles published between 1980 and August 2005. Terms and words used for the search included age-related macular degeneration, age-related macular disease, maculopathy, macula, drusen, fundus, imaging, photograph, digital imaging, and quantification. In the second stage all abstracts were examined to identify articles that described the use of imaging and grading in age-related macular disease. Copies of the entire articles were obtained. Bibliographies of the retrieved articles were manually searched with use of the same search guidelines. In the third stage, articles were reviewed and information relating to the quantification of age-related macular change was incorporated in to the manuscript. The literature search was not limited to the English language, although no translation was required.

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Reprint address

Dr Hannah Bartlett

Ophthalmic Research Group

School of Life and Health Sciences

Aston University

Birmingham

B4 7ET

UK

Figure legends

Figure 1: Grading grids used with the Wisconsin Age-related Maculopathy Grading System

Figure 2: Example of pre-processing of a fundus image for drusen quantification

Tables

Characteristic	Weighted kappa (SE of kappa)
Drusen size	0.68 (0.02)
Drusen type	0.65 (0.02)
Drusen area	0.71 (0.02)
Drusen confluence	0.60 (0.03)
Retinal pigment epithelial degeneration	0.75 (0.03)
Increased pigment	0.81 (0.03)
Subretinal fibrous scar	0.48 (0.21)
Geographic atrophy	0.87 (0.09)

Table 1: Weighted kappa values for inter-observer agreement using the Wisconsin grading scale [35]

Characteristic	Zone 1 [% agreement (kappa)]	Zone 2 [% agreement (kappa)]	Zone 3 [% agreement (kappa)]
Hard drusen (0.63 µm)	83.7 (0.45)	76.0 (0.55)	81.3 (0.62)
Intermediate soft drusen (63 - 125 µm)*	93.0 (0.37)	87.3 (0.60)	79.0 (0.49)
Large semi-solid drusen (125-250 µm)*	96.7	94.1	92.9
Large semi-solid drusen (250-500 µm)*	98.2	95.6	95.8
Large semi-solid drusen (>500 µm)*	99.3	97.9	98.4
Crystalline drusen*	98.7 (0.65)	95.7 (0.51)	98.0 (0.59)
Serogranular drusen*	98	96	98
Hyperpigmentation (presence)	63.3 (0.37)	73.3 (0.51)	86.7 (0.45)
Hyperpigmentation (type)	73.3 (0.40)	58.7 (0.28)	83.3 (0.37)
Hypopigmentation (presence)	85.3	85.3	92.0
Geographic atrophy (presence)	93.3 (0.80)	92.0 (0.78)	94.7 (0.74)
Geographic atrophy (area covered)*	95.0 (0.85)		
Neovascular AMD (presence)	89.3 (0.75)	89.3 (0.75)	97.3 (0.85)
Neovascular AMD (features)	82.7 (0.40)	80.7 (0.33)	94.7 (0.51)
Neovascular AMD (scar/fibrous)	93.3 (0.76)	96.0 (0.83)	97.3 (0.82)
Neovascular AMD (retinal haemorrhage)	97.3	97.3	98.7
Neovascular AMD (area covered)*	90.7 (0.78)		

Table 2: Inter-observer variability for grading of AMD characteristics

Characteristic	Zone 1 [% agreement (kappa)]	Zone 2 [% agreement (kappa)]	Zone 3 [% agreement (kappa)]
Hard drusen (0.63 µm)*	82.0 (0.45) 96.0 (0.83)	72.0 (0.43) 76.0 (0.55)	78.0 (0.60) 81.0 (0.64)
Intermediate soft drusen (63 - 125 µm)*	88.0 (0.13) 98.0 (0.75)	87.0 (0.51) 96.0 (0.81)	81.0 (0.50) 93.0 (0.78)
Large semi-solid drusen (> 125 µm)*	84.0 100.0	92.0 100.0	90.0 100.0
Crystalline drusen*	92.0 (0.46) 100.0 (0.94)	92.0 (0.67) 99.0 (0.85)	90.0 (0.23) 100.0 (0.95)
Serogranular drusen*	96.0 100.0	-	-
Hyperpigmentation (presence)	62.0 (0.30) 82.0 (0.68)	70.0 (0.39) 82.0 (0.69)	84.0 (0.36) 86.0 (0.54)
Hyperpigmentation (type)	54.0 (0.21) 68.0 (0.46)	64.0 (0.30) 76.0 (0.61)	78.0 (0.27) 88.0 (0.46)
Hypopigmentation (presence)	88.0 100.0	86.0 98.0	82.0 90.0
Geographic atrophy (presence)	92.0 (0.52) 95.0 (0.78)	88.0 (0.65) 97.0 (0.77)	94.0 (0.69) 96.0 (0.81)
Geographic atrophy (area covered)*	90.0 (0.61) 96.0 (0.88)	-	-
Neovascular AMD (presence)	82.0 (0.42) 90.0 (0.72)	88.0 (0.46) 96.0 (0.82)	94.0 (0.63) 99.0 (0.90)
Neovascular AMD (features)	80.0 (0.36) 94.0 (0.76)	86.0 (0.49) 96.0 (0.84)	92.0 (0.18) 98.0 (0.66)
Neovascular AMD (scar/fibrous)	86.0 (0.46) 96.0 (0.87)	88.0 (0.50) 98.0 (0.92)	94.0 (0.54) 96.0 (0.78)

Neovascular AMD (retinal haemorrhage)	96.0 96.0	98.0 100.0	96.0 100.0
Neovascular AMD (area covered)	87.0 (0.65) 92.0 (0.77)	-	-

Table 3: Intra-observer variability for grading of AMD characteristics

	First eye[a]			Second eye
AMD category	Drusen size[b]	Drusen area[b]	Pigment abnormalities[c]	
1	None or small (< 63 μm)	< 125 μm diameter circle (≈ 5 – 15 small drusen)	None	Same as first
2	Small (< 63 μm) Or Intermediate (≥ 63, > 125 μm) None required if pigment abnormalities present	≥ 125 μm diameter circle At least one druse	Absent or present, but GA absent	Same as first or category 1
3a	Intermediate (≥ 63, < 125 μm) Or Large (≥ 125 μm) None required if non-central GA present	≥ 360 μm diameter circle if soft indistinct drusen are present (≈ 20 intermediate drusen. ≥ 656 μm diameter circle if soft indistinct drusen are absent. At least one druse	Absent or present but GA absent	Same as first or category 1 or 2
3b	First eye same category as 3a			VA < 20.32 due to AMD; or uniocular disqualifying disorder present
4a	First eye same category as 1, 2, or 3a			Advanced AMD[d]
4b	First eye same category as 1, 2, or 3a			VA < 20/32 due to AMD, but advanced AMD not present.

Table4: The AREDS AMD categories [81].

[a] Must have VA ≥ 20/32, no advanced AMD, and no disqualifying lesion.

[b] Drusen and GA are assessed within two disc diameters of the centre of the macula.

[c] Pigment abnormalities within one disc diameter of the centre of the macula.

[d] GA involving centre of macula or signs of choroidal neovascularisation (presence beneath the RPE or sensory retina of fluid, blood, or fibrovascular or fibrous tissue).