



**HAL**  
open science

## Functional aspects of drusen regression in Age-Related Macular Degeneration

Ferenc B Sallo, Ehud Rechtman, Tunde Peto, Dinu Stanescu-Segall, Gabor Vogt, Alan C Bird, Fred W Fitzke

► **To cite this version:**

Ferenc B Sallo, Ehud Rechtman, Tunde Peto, Dinu Stanescu-Segall, Gabor Vogt, et al.. Functional aspects of drusen regression in Age-Related Macular Degeneration. *British Journal of Ophthalmology*, 2009, 93 (10), pp.1345-n/a. 10.1136/bjo.2008.150334 . hal-00477825

**HAL Id: hal-00477825**

**<https://hal.science/hal-00477825>**

Submitted on 30 Apr 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## FUNCTIONAL ASPECTS OF DRUSEN REGRESSION IN AGE-RELATED MACULAR DEGENERATION

Ferenc B Sallo<sup>1,2</sup>, Ehud Rechtman<sup>1</sup>, Tunde Peto<sup>1</sup>, Dinu Stanescu-Segall<sup>1</sup>, Gabor Vogt<sup>3</sup>,  
Alan C Bird<sup>1,2</sup>, Frederick W Fitzke<sup>2</sup>

<sup>1</sup> Moorfields Eye Hospital, London, UK

<sup>2</sup> UCL Institute of Ophthalmology, London, UK

<sup>3</sup> National Health Centre, Budapest, Hungary

Corresponding author:

Ferenc B Sallo

The Reading Centre

Department of Research and Development

Moorfields Eye Hospital

162 City Road

London

EC1V 2PD

**Keywords:** Macula; Degeneration; Psychophysics; Imaging; AMD; drusen; natural history; regression

**Word count:** 2710 (excluding title page, abstract, references, tables and figures)

## **ABSTRACT**

**Aims:** To investigate the functional implications of macular soft drusen regression in AMD eyes.

**Methods:** Patients were selected from a large ongoing collection of clinical data at Moorfields Eye Hospital. Phenotyping based on standard colour fundus images was performed according to the system defined by the International Classification for ARM, by certified graders masked to the main aim of the study. Fundus Autofluorescence (FA) was recorded using a Heidelberg Retina Angiograph 2. Where drusen regression was confirmed by independent grading, the patient was invited for photopic and scotopic Fine Matrix Mapping (FMM). Phenotype and functional data were analyzed for correlations between fundus appearance, autofluorescence and retinal sensitivity.

**Results:** Fundus and FA images of 960 patients were screened, soft drusen regression was detected in 34 cases, 14 patients agreed to participate in the study, ranging in age from 52 to 84 years (median 72). The mean follow-up period was 5.9 years (range 2.8-14.4 years). FMM showed generalised threshold elevation relative to normal controls both under photopic and scotopic conditions. Scotopic sensitivity loss exceeded photopic loss in all cases. Sensitivity loss over areas with drusen or regressed drusen did not differ significantly from that over non-drusen areas.

**Conclusion:** Macular soft drusen may fade or disappear without detectable ophthalmoscopic, FA or psychophysical signs of local dysfunction. This phenomenon is a potential source of misclassification. The prognosis for cases with true regression of drusen compared with those without needs to be considered in future studies on AMD.

## **INTRODUCTION**

Age-Related Macular Degeneration (AMD) is the leading cause of severe irreversible central visual loss among the elderly in western industrialized countries.[1, 2] The aetiology and pathogenesis of AMD are poorly understood. Risk factors identified include age, certain genetic variants, smoking and the presence of macular soft drusen with high-risk characteristics.[1, 2, 3, 4] Clinically, early AMD is characterized by abnormalities of the retinal pigment epithelium (RPE), soft drusen and mild visual symptoms, predominantly under dark-adapted conditions.[5, 6] Late AMD is dominated by either geographic atrophy (GA) or neovascularisation (NV), typically with severe central function loss.[5] AMD affects primarily the RPE, Bruch's Membrane (BrM) and choriocapillaris (the RPE/BrM complex). In early AMD, a thickening of BrM and an accumulation of focal and diffuse lipid-rich deposits (drusen, basal linear and laminar deposits) under the RPE and within BrM are prominent.[7] The consequential reduction in hydraulic conductivity may impede transport through BrM and thus contribute to the pathogenesis of lesions seen in late disease.[8] The origin and mechanism of debris deposition are little known. Of the sub-RPE deposits, drusen alone are detectable clinically. The natural history of soft drusen may involve an increase in size, area and confluence with subsequent NV, or fading and disappearance which is believed to be associated with atrophy of the overlying RPE and photoreceptors.[3] The functional and prognostic implications of true drusen regression, however, have not been demonstrated. Since the RPE/BrM complex is essential for photoreceptor survival, psychophysical assessment of photoreceptor function may yield information about the significance of clinically observable retinal changes that may not be provided by other methods until later or at all.[9] Imaging the distribution of autofluorescence in the fundus (fundus autofluorescence, FA) represents an additional tool for evaluating RPE health.[10] Visualised by a confocal Scanning Laser Ophthalmoscope (cSLO, excitation  $\lambda=488\text{nm}$ ) FA is mainly derived from lipofuscin (LF),[10, 11] a complex material produced in RPE cells from residues of incomplete lysosomal degradation of photoreceptor outer segment (POS) discs. LF contains components toxic to the RPE (primarily A2-E isomers), its excessive accumulation is believed to precede photoreceptor degeneration in AMD.[11] The aim of this study was to investigate the functional implications of macular soft drusen regression in AMD eyes.

## **PATIENTS AND METHODS**

Patients were selected from a large ongoing collection of clinical data at the Reading Centre of Moorfields Eye Hospital. Inclusion criteria were: a clinical diagnosis of AMD, both manifest and resolved soft macular drusen, a level of fixation and general fitness sufficient to perform the full test sequence. Patients with exudative late-stage AMD in the eye studied were excluded.

### **Imaging**

Standard 30° stereo colour images (SCI) of the fundus, centred on the fovea (Field 2) were recorded digitally. FA imaging was performed using a cSLO (Heidelberg Retina Angiograph 2, Heidelberg Engineering GmbH, Dossenheim, Germany). The fundus was illuminated using a solid-state laser (488 nm), induced fluorescence was recorded through a long-pass filter with a short-wavelength cut-off at 500nm. The image of a 30°x30° retinal area centred on the fovea was recorded digitally at 1536x1536 pixel (5µm/pixel) and 256-level grey-scale resolution. To reduce random noise, a master image was produced by averaging 16 individually recorded images for each eye in the study. Lens opacities - where present - were in all cases diffuse and permitted adequate imaging of FA.

### **Phenotyping**

Detailed phenotyping was performed by grading based on Field 2 colour and FA images, according to the system defined in the International Classification for ARM and AMD,<sup>[5]</sup> independently by two certified graders masked to the identity of the patients and the main aim of the study. These gradings were compared for (both inter- and intra-observer) reliability. A final copy was created by adjudication and changes over time in the final copy were analysed. In cases where the simultaneous presence of AMD, disappearance of drusen and absence of exudative disease were confirmed, the patient was invited for functional testing.

### **Psychophysical testing**

For psychophysical testing Fine Matrix Mapping (FMM) was selected due to its superior spatial resolution and its ability to measure wide ranges of sensitivity changes. The full test sequence included a visual acuity test, a standard visual field examination using the 30-2 program of the Humphrey Field Analyzer (HFA, Carl Zeiss Ltd., UK), a photopic FMM test, pupil dilatation (with 1.0% tropicamide and 2.5% phenylephrine), dark adaptation for 45 minutes, a scotopic 30-2 visual field test, scotopic FMM and finally the determination of the location and stability of fixation.

#### *Fine-Matrix Mapping*

FMM was performed using a modified HFA. Test flashes were positioned over the retinal area of interest, including both drusen and areas where the resolution of drusen was confirmed by grading. The coordinates of four interlaced 5x5 grids (25 locations with 2° intervals) were entered in the "Custom Grid" feature of the perimeter. Each grid was offset relative to the other grids by 1° on either the *x*, *y*, or both axes. Data thus obtained were merged into a single 9° x 9° matrix of 100 test locations with 1° intervals. A standard (Goldmann) size 3 stimulus was used. Photopic testing was done using a white stimulus with a background illuminance of 31.5 apostilbs, scotopic measurements were done using a blue stimulus with no background illumination. Fixation stability was monitored through an infrared camera. Detection threshold sensitivity was expressed in decibels and thresholds as

log units. The maximum stimulus illuminance of 10,000 apostilbs was interpreted as 0dB.

#### *Data processing*

FMM thresholds were processed by (3x3) Gaussian filtering to improve repeatability.[12] The attenuation of the original signal and loss of detail inherent in all smoothing methods was in our case insignificant and outweighed by the benefits with respect to noise reduction (repeatability).[13] For each patient, sensitivity data were analyzed intra-individually as well as compared with age-matched normal data (collected under identical conditions) that have been published,[14, 15, 16] through point-by-point subtraction of averaged normal values at corresponding coordinates within the FMM matrix. Filtered data were used to calculate the mean and the maximum threshold elevation from baseline as well as elevation relative to averaged threshold levels of the normal control group. Interpolated threshold values at 0.25° intervals were used to generate a contour plot showing luminance sensitivity gradients across the matrix. Three-dimensional threshold profiles were also generated. Composite images containing overlays of aligned sequential colour fundus and FA images and sensitivity threshold plots were analyzed for correlations between fundus appearance, FA and retinal sensitivity. Total area covered by drusen at baseline and at FMM testing was measured for each zone within the IC grading grid.[5] Areas with disappearing drusen, persistent drusen and drusen-free areas were identified and associated levels of sensitivity loss were compared. Software used include: Adobe Photoshop v7 with the 'FoveaPro' plug-in, ImageJ v1.38x, Microsoft Excel and SPSS v11.

#### *Fixation stability*

A major factor influencing FMM test reliability is the stability of fixation. For the measurement of fixation stability, a customized cSLO was used in a way similar to a technique described previously.[17] Values thus obtained reflect eye movements and are used to calculate the bivariate contour ellipse area (BCEA), which is the area of an ellipse on the retinal surface within which the centre of the target was imaged 68% of the time. BCEA is a standardized measure which provides a means for quantification and comparison of fixation stability. Smaller BCEA values correspond to more precise fixation.

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Before inclusion, written, informed consent was obtained from each participating patient after explanation of the nature of the study. Maximum retinal irradiance of lasers used was well below the limits established by the American National Standards Institute (ANSI Z136.1; 1993) and other international standards.

## **RESULTS**

Sequential colour fundus and FA images of 960 patients were screened for disappearing drusen. Soft drusen regression was detected in 34 cases (25 spontaneous, 9 following prophylactic laser treatment). 19 patients met all inclusion criteria, 14 (10 female, 4 male) agreed to participate in the study, ranging in age from 52 to 84 years (median age was 72 years). The baseline of the study was defined as the earliest date when both clinical data and fundus SCI were available, and the endpoint as the date the patient was last seen clinically. The mean follow-up period was 5.9 years (ranging from 2.8 to 14.4 years).

### *Phenotype*

The predominant phenotype in the study eye at baseline was “soft drusen” in all cases. Disappearance of drusen was followed by NV in one, GA in 3 cases, while in 10 cases no indications of end-stage disease were seen in the colour fundus images. In most cases, parallel to fading drusen, new drusen in other locations appeared and grew in size and confluence. New drusen tended to form at increasingly peripheral locations relative to the fovea (Table 1). Repeated appearance of drusen in the same retinal location was not seen. Representative images are shown in Figure 1.

**Table 1. Change in area covered by drusen.** Columns: (A) Reduction in area of soft drusen present at baseline in respective zone of the IC grading grid. (B) Overall change in area covered by drusen, including drusen appearing after baseline. Negative numbers indicate net decrease from baseline.

patient	Zone 1		Zone 2		Zone 3	
	A	B	A	B	A	B
1	-90%	-31%	-50%	-5%	-13%	0%
2	-100%	-62%	-98%	-59%	-61%	8%
3	-98%	-97%	-69%	-23%	-41%	1%
4	-100%	-5%	-60%	-3%	-48%	1%
5	-5%	19%	-47%	0%	-46%	1%
6	-62%	-2%	-38%	14%	-61%	5%
7	-40%	-4%	-38%	-3%	-64%	-3%
8	-51%	8%	-72%	-16%	-72%	-7%
9	-100%	-46%	-89%	-8%	-48%	3%
10	-100%	-61%	-97%	-40%	-67%	0%
11	-100%	-11%	-66%	-1%	-49%	6%
12	-97%	-90%	-92%	-35%	-61%	-5%
13	-100%	-14%	-84%	-9%	-66%	5%
14	-11%	28%	-10%	23%	-16%	5%

### *Autofluorescence*

FA associated with drusen varied from decreased to increased, no good correspondence was detectable. FA corresponding to areas with disappearing drusen in the absence of pigmentary changes was normal in 7 cases. In two patients increased FA was seen, in one case in an area adjacent to the junctional zone of a GA, in the other adjacent to a large crystalline druse. One other patient showed widely varying levels of FA in connection with regressed drusen (Figure 1, row 3). GA was associated with decreased FA centrally and increased FA along the boundaries. Crystalline drusen showed decreased, granular hyperpigmentation increased FA.

### *Functional characteristics*

Best corrected visual acuities in the study eye assessed at the time of FMM testing ranged from 6/12 to 6/5 (median=6/6). All patients had less than two lines loss in BCVA compared to the baseline value. Fixation Stability was good in all cases (Table 2). Two patients (2 and 4), both with end-stage disease, showed significant (more than 2 lines) deterioration in BCVA at the endpoint as compared to the baseline value. Fine Matrix Mapping showed generalised threshold elevation relative to normal controls both under photopic and scotopic conditions (Table 2). Scotopic sensitivity loss exceeded photopic loss in all cases (Figure 2). Scotopic loss over areas with drusen or regressed drusen did not differ significantly from that over non-drusen areas ( $p=0.289$  and  $p=0.989$  respectively, ANOVA, Figure 3). Elevated scotopic thresholds were seen associated with GA, crystalline drusen and coarse granular hyperpigmentation, all in connection with abnormal FA. Photopic thresholds showed little topographic variation except in areas with GA.

**Table 2. Functional characteristics.** FMM Global estimates of sensitivity loss are based on Gaussian filtered data and are expressed in log units.

Patient	FMM Photopic loss		FMM Scotopic loss		Fixation stability (BCEA, arc min <sup>2</sup> )	BCVA (study eye)
	Background (mean)	Discrete (max)	Background (mean)	Discrete (max)		
1	0.34	0.91	1.04	1.62	74.41	6/6+1
2	0.77	3.22	1.15	2.32	16.60	6/12
3	0.75	1.01	0.78	1.27	88.20	6/6-1
4	0.87	1.25	1.48	1.81	26.50	6/12-1
5	0.53	0.70	0.69	1.06	46.51	6/6-4
6	0.58	0.87	1.26	2.13	42.08	6/6-2
7	1.00	1.40	1.11	1.83	50.90	6/6-1
8	0.69	1.02	1.03	1.93	116.15	6/5
9	0.26	0.44	1.11	1.55	133.10	6/6
10	0.82	1.93	1.38	2.19	63.22	6/6+2
11	0.64	0.75	0.90	1.50	90.83	6/12-2
12	0.52	0.75	1.60	2.36	80.99	6/12
13	0.52	1.08	1.69	2.40	42.59	6/5
14	0.63	1.00	1.45	2.13	50.55	6/9

## DISCUSSION

Regression of macular soft drusen has been described in clinical,[2, 4, 18] as well as in histopathological studies.[3, 19] Gass noted that drusen may fade and disappear, leaving only an irregular mottling of the RPE, while visual acuity may not be affected.[3] Gass also observed that most cases GA follow the fading of drusen or the collapse of a serous detachment of the RPE.[3] Drusen disappearance is thus believed to be associated with subsequent degeneration and atrophy of the RPE and photoreceptors.[3] It was however noted in large population-based studies that in some patients, drusen may also regress without residual signs.[2, 4, 18] The prognostic implications of true drusen regression is unknown. From the clinical aspect, it raises the possibility that arrested progression or even regression of the disease process may exist naturally. In this study, ten out of 14 patients showed no ophthalmoscopic indications of manifest or incipient end-stage disease in the fundus following drusen regression. However, in nine, parallel to regression, new drusen in other locations appeared and grew in size and confluence, signifying the continued activity of the disease, with a tendency toward the periphery. Mean retinal sensitivity relative to normal controls was reduced in all patients tested, both in the light and dark adapted states, with significantly higher loss under scotopic conditions. This observation confirms that in AMD, rods are at increased risk for degeneration and function loss occurs before progression to the late clinical stage.[9, 20] Earlier psychophysical studies found that rod-mediated sensitivity declines faster with age than photopic sensitivity and patients with early AMD have significantly lower mean central scotopic sensitivity than age-matched controls. Also, in most AMD patients, mean scotopic sensitivity loss exceeded mean photopic sensitivity loss and the peak deficit in scotopic sensitivity was within 9° of fixation, corresponding to the parafovea.[20] Histopathologic studies show direct correlates to these findings. In early AMD, a preferential loss of macular rod photoreceptors was demonstrated, with the greatest loss occurring in the parafovea. In late AMD this leads to a reversal of the macular (9:1) rod predominance seen in the young.[9] The rod system also shows altered kinetics with aging and in AMD.[6] One possible explanation for this preferential vulnerability of rods is provided by the retinoid deficiency hypothesis.[9] RPE cells are responsible for the transport of nutrients from the choriocapillary circulation to the photoreceptors, as well as for the recycling of the end-products of POS disc degradation. 11-cis retinal (a derivative of Vitamin A) is essential for the regeneration of photoreceptor pigment after bleaching by light as well as for photoreceptor survival. In AMD, diffuse sub-RPE deposits may act as a diffusion barrier between the choriocapillaris and the RPE,[8] thereby disrupting transport across BrM and leading to a local scarcity of 11-cis-retinal. Vitamin A deprivation is a known cause of outer segment degeneration and photoreceptor death. Cones have an additional retinoid delivery pathway involving Müller cells and possibly the neurosensory retina and may thus be less vulnerable to reduced transport across Bruch's membrane.[9, 21] Although generalised sensitivity loss was measured in all our patients, topographic variation in scotopic (and photopic) sensitivity loss over drusen relative to areas with normal appearance was not significant. This confirms earlier observations that the presence of macular soft drusen seems to have little effect on the local sensitivity of affected retinal areas,[22, 23] with the exception of large, soft foveal drusen. These may be regarded as small RPE detachments and show mildly reduced photopic and considerably reduced scotopic sensitivity as well as increased FA.[23] It appears reasonable, that the impact of focal barriers such as drusen on RPE metabolism and photoreceptor function may be more limited than that of diffuse deposits. We also found that scotopic sensitivity over areas with regressed drusen was not substantially different from that over unaffected retinal areas, thus we did not find functional evidence for manifest or incipient photoreceptor atrophy. In diseases, where the RPE is primarily affected,

the presence of abnormal FA may be an early sign of progression. Areas with increased FA were found to precede the development of new or the enlargement of existing atrophic patches.[24] Decreased FA over the drusen overall may also reflect incipient atrophy.[25] In eyes with soft drusen but without apparent GA, if focal decreased FA is present, patches with increased FA in areas adjacent to rather than corresponding to drusen may be markers for progression to atrophy.[26] In this study, autofluorescence of retinal areas with normal appearance in the colour image following drusen regression was in most cases normal and none of the above FA patterns associated with impending atrophy was seen. Thus, although the generalised disease persists, retinal sensitivity and FA, two direct and sensitive measures of retinal health do not indicate that disappearance of drusen is necessarily followed by local function loss and atrophy.

In summary, although the limitations of this study in terms of subject numbers and follow-up time as well as the lack of psychophysical testing before the drusen disappeared may not permit definitive conclusions, our results do indicate that macular soft drusen may fade or disappear without detectable ophthalmoscopic, FA or psychophysical signs of local dysfunction or incipient atrophy. This phenomenon suggests that some normal-appearing eyes at the present time may have had some features of AMD in the past and is thus a potential source of misclassification and needs to be remembered in epidemiologic studies investigating the natural history of the disease as well as in clinical trials that evaluate the efficacy of possible therapies.[18] It is not known to what extent focal and diffuse sub-RPE deposits coincide and whether the regression of drusen is accompanied by the regression of diffuse deposits. The prognosis for cases with true regression of drusen compared with those without needs to be considered in future studies on AMD.

**ACKNOWLEDGEMENTS**

The authors thank Mr Vy Luong for his technical support and Mr Tibor Fermon and Mr Istvan Toth for useful discussions and general support.

**COMPETING INTERESTS:** None declared.

(All authors declare that the answer to the questions on your competing interest form are all No and therefore have nothing to declare.)

**FUNDING:** NONE

**LICENCE FOR PUBLICATION**

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd to permit this article (if accepted) to be published in BJO and any other BMJPGJL products and sublicences such use and exploit all subsidiary rights, as set out in our licence

## **REFERENCES**

1

Klein R, Peto T, Bird A, Vannewkirk MR. The epidemiology of age-related macular degeneration. *Am J Ophthalmol.* 2004 Mar;137(3):486-95. Review. PMID: 15013873

2

Klein R, Klein BE, Tomany SC, Meuer SM, Huang GH. Ten-year incidence and progression of age-related maculopathy: The Beaver Dam eye study. *Ophthalmology.* 2002 Oct;109(10):1767-79. PMID: 12359593

3

Gass JD. Drusen and disciform macular detachment and degeneration. *Arch Ophthalmol.* 1973 Sep;90(3):206-17. PMID: 4738143 Also in: *Retina.* 2003 Dec;23(6 Suppl):409-36. PMID: 15035389

4

Sparrow JM, Dickinson AJ, Duke AM, Thompson JR, Gibson JM, Rosenthal AR. Seven year follow-up of age-related maculopathy in an elderly British population. *Eye.* 1997;11 ( Pt 3):315-24. PMID: 9373468

5

Bird AC, Bressler NM, Bressler SB, Chisholm IH, Coscas G, Davis MD, de Jong PT, Klaver CC, Klein BE, Klein R, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol.* 1995 Mar-Apr;39(5):367-74. Review. PMID: 7604360

6

Steinmetz RL, Haimovici R, Jubb C, Fitzke FW, Bird AC. Symptomatic abnormalities of dark adaptation in patients with age-related Bruch's membrane change. *Br J Ophthalmol.* 1993 Sep;77(9):549-54. PMID: 8218049

7

Sarks SH. Ageing and degeneration in the macular region: a clinico-pathological study. *Br J Ophthalmol.* 1976 May;60(5):324-41. PMID: 952802

8

Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. *Arch Ophthalmol.* 2004 Apr;122(4):598-614. Review. PMID: 15078679

9

Curcio CA, Owsley C, Jackson GR. Spare the rods, save the cones in aging and age-related maculopathy. *Invest Ophthalmol Vis Sci.* 2000 Jul;41(8):2015-8. Review. PMID: 10892836

10

von Rückmann A, Fitzke FW, Bird AC. Fundus autofluorescence in age-related macular disease imaged with a laser scanning ophthalmoscope. *Invest Ophthalmol Vis Sci.* 1997 Feb;38(2):478-86. PMID: 9040481

11

Dorey CK, Wu G, Ebenstein D, Garsd A, Weiter JJ. Cell loss in the aging retina. Relationship to lipofuscin accumulation and macular degeneration. *Invest Ophthalmol Vis Sci*. 1989 Aug;30(8):1691-9. PMID: 2759786

12

Fitzke FW, Crabb DP, McNaught AI, Edgar DF, Hitchings RA. Image processing of computerised visual field data. *Br J Ophthalmol*. 1995 Mar;79(3):207-12. PMID: 7703195

13

Fitzke FW, Kemp CM. Probing visual function with psychophysics and photochemistry. *Eye*. 1989;3:84-89.

14

Westcott MC, McNaught AI, Crabb DP, Fitzke FW, Hitchings RA. High spatial resolution automated perimetry in glaucoma. *Br J Ophthalmol*. 1997 Jun;81(6):452-9. PMID: 9274408

15

Guymmer RH, Gross-Jendroska M, Owens SL, Bird AC, Fitzke FW. Laser treatment in subjects with high-risk clinical features of age-related macular degeneration. Posterior pole appearance and retinal function. *Arch Ophthalmol*. 1997 May;115(5):595-603. PMID: 9152126

16

Wu D, Bird AC, Mcnaught A, Buckland MS, Fitzke FW. Fine matrix mapping of the macular region in normal subjects. *Zhonghua Yan Ke Za Zhi*. 1995 Jul;31(4):243-9. PMID: 8745513

17

Culham LE, Fitzke FW, Timberlake GT, Marshall J. Assessment of fixation stability in normal subjects and patients using a scanning laser ophthalmoscope. *Clin Vision Sci*. 1993;8:551-561.

18

Bressler NM, Munoz B, Maguire MG, Vitale SE, Schein OD, Taylor HR, West SK. Five-year incidence and disappearance of drusen and retinal pigment epithelial abnormalities. Waterman study. *Arch Ophthalmol*. 1995 Mar;113(3):301-8. PMID: 7534060

19

Sarks JP, Sarks SH, Killingsworth MC. Evolution of geographic atrophy of the retinal pigment epithelium. *Eye*. 1988;2 (Pt 5):552-77. PMID: 2476333

20

Owsley C, Jackson GR, Cideciyan AV, Huang Y, Fine SL, Ho AC, Maguire MG, Lolley V, Jacobson SG. Psychophysical evidence for rod vulnerability in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2000 Jan;41(1):267-73. PMID: 10634630

21

Mata NL, Radu RA, Clemmons RC, Travis GH. Isomerization and oxidation of vitamin a in cone-dominant retinas: a novel pathway for visual-pigment regeneration in daylight. *Neuron*. 2002 Sep 26;36(1):69-80. PMID: 12367507

22

Sunness JS, Johnson MA, Massof RW, Marcus S. Retinal sensitivity over drusen and nondrusen areas. A study using fundus perimetry. *Arch Ophthalmol*. 1988 Aug;106(8):1081-4. PMID: 3401135

23

Scholl HP, Bellmann C, Dandekar SS, Bird AC, Fitzke FW. Photopic and scotopic fine matrix mapping of retinal areas of increased fundus autofluorescence in patients with age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2004 Feb;45(2):574-83. PMID: 14744901

24

Holz FG, Bellman C, Staudt S, Schütt F, Völcker HE. Fundus autofluorescence and development of geographic atrophy in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2001 Apr;42(5):1051-6. PMID: 11274085

25

Sunness JS, Ziegler MD, Applegate CA. Issues in quantifying atrophic macular disease using retinal autofluorescence. *Retina*. 2006 Jul-Aug;26(6):666-72. PMID: 16829810

26

Smith RT, Chan JK, Busuioic M, Sivagnanavel V, Bird AC, Chong NV. Autofluorescence characteristics of early, atrophic, and high-risk fellow eyes in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2006 Dec;47(12):5495-504. PMID: 17122141

## **APPENDICES**

### **Figure legends:**

#### **Figure 1.**

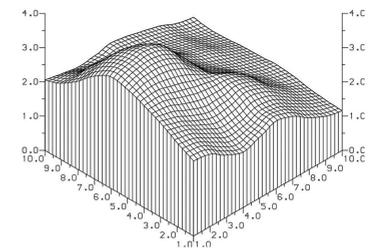
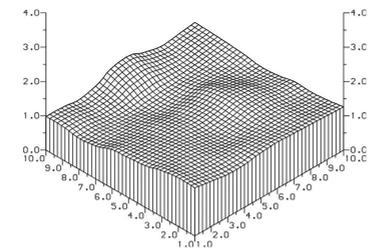
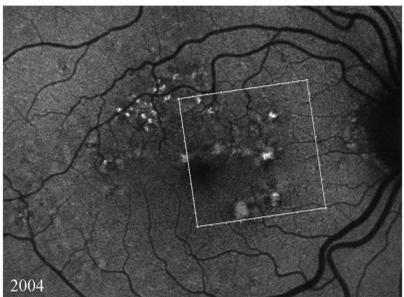
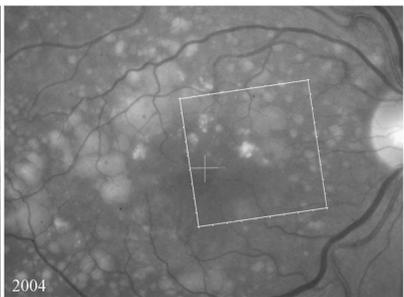
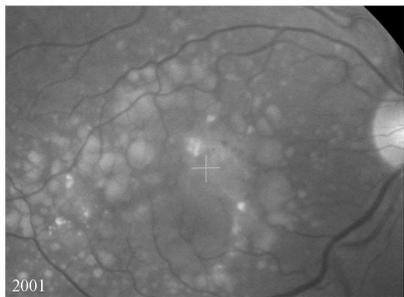
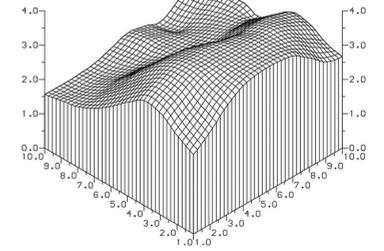
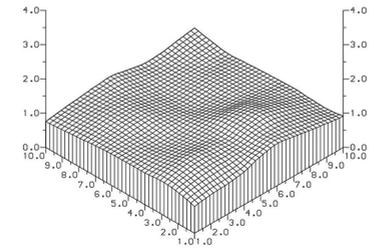
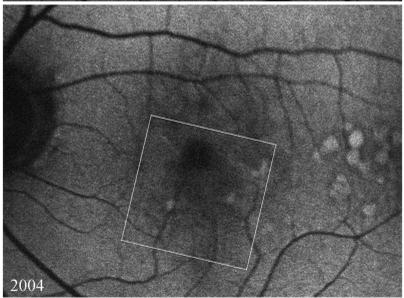
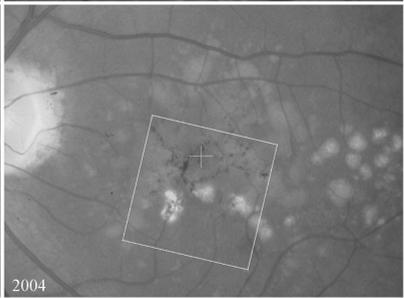
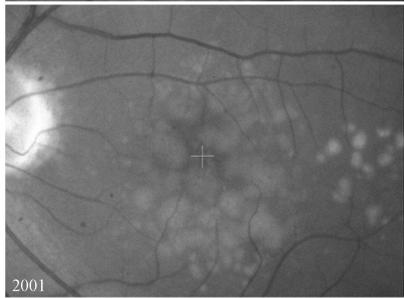
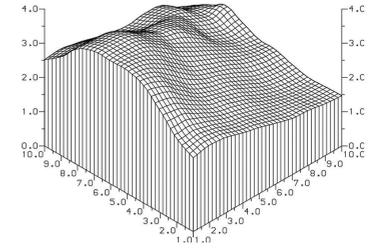
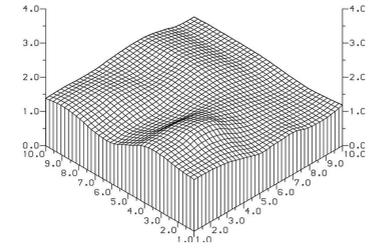
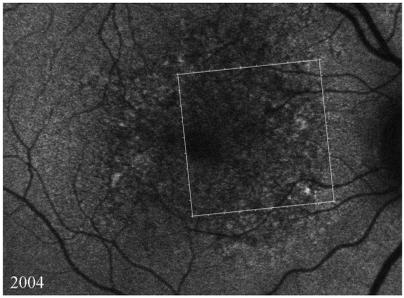
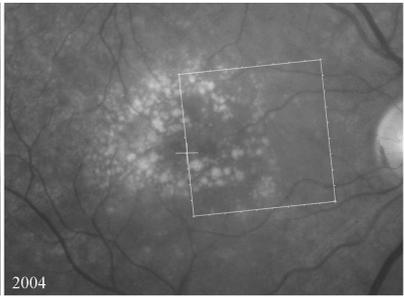
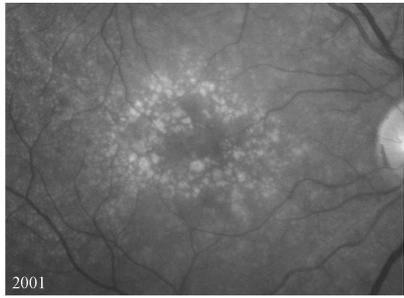
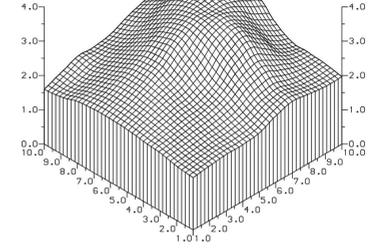
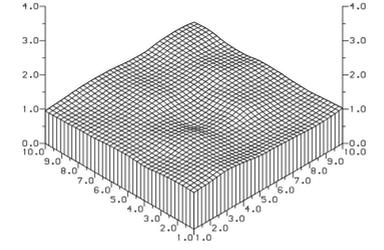
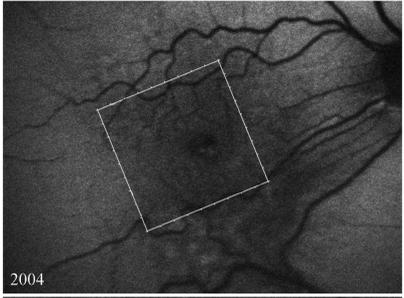
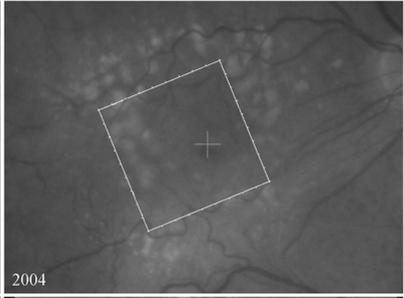
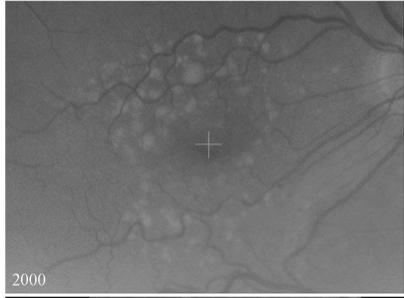
Representative images from selected cases. Columns: (A) the fundus at baseline, (B-C) colour and FA images taken at FMM testing, white rectangles mark the placement of respective FMM test grid, (D-E) surface plots of photopic and scotopic FMM thresholds. Rows 1-2 and 4 (patients 3, 6 and 11) illustrate the typical picture seen in the majority of patients. Row 3 shows a case (patient 7) where areas with regressed drusen appear normal in the colour fundus image but show a speckled variation in FA. Row 5 shows a case where progression to atrophy was noted (patient 2).

#### **Figure 2.**

Photopic versus scotopic sensitivity loss of the 14 patients in the study.

#### **Figure 3.**

Regional differences in scotopic sensitivity loss at retinal locations with (C) drusen, (B) regressed drusen and (A) areas with normal appearance.

**A****B****C****D****E****1****2****3****4****5**