Graefe's Arch Clin Exp Ophthalmol
(2002) 240:73–78

CLINICAL INVESTIGATION

DOI 10.1007/s00417-001-0413-3

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Analysis of digital scanning laser ophthalmoscopy fundus autofluorescence images of geographic atrophy in advanced age-related macular degeneration

Received: 29 August 2001
Accepted: 14 November 2001
Published online: 26 January 2002

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Presented in part at the ARVO Meeting 2001, Fort Lauderdale, USA and at the 99th annual meeting of the German Society of Ophthalmology (DOG) 2001, Berlin, Germany

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Abstract *Background:* Fundus autofluorescence (AF) imaging using confocal scanning laser ophthalmoscopy (cSLO) has been shown to be superior to fundus photography or angiography for delineating areas of geographic atrophy (GA) in retinal pigment epithelium (RPE) and for recording variation over time. We have evaluated a method for automated computerized detection and quantitation of RPE atrophy. *Methods:* AF images in vivo were recorded with a confocal scanning laser ophthalmoscope (exc. 488 nm, em. >500 nm; Heidelberg Retina Angiograph). The intensity of AF in atrophic areas was markedly decreased. Two independent readers analysed these areas in 24 right eyes manually by outlining GA areas using a mouse-driven arrow (method A) and automatically by image analysis software (Global Lab Image/2) after subjective adjustment of thresholding (method B). Agreement between observers and between methods A and B was assessed by the Bland-Altman design for method-comparison studies. *Results:* Larger areas were measured using method A than B by both readers (agreement A/B: reader 1 mean difference 1.04 mm, 95% CI [0.66,1.42]; reader 2 mean difference 0.62 mm, 95% CI [0.43,0.81]). The agreement between the readers was mean difference 0.39 mm (95% CI [0.02,0.76]) for A and mean difference –0.03 mm (95% CI [–0.23,0.18]) for B. Features making the delineation of borders of GA difficult included large choroidal vessels with autofluorescent properties in the GA area and media opacities. *Conclusions:* Fundus AF cSLO imaging provides a reliable means to delineate areas of GA. The automated image analysis allows more accurate detection and quantitative documentation of atrophic areas than manual outlining. This method will be useful in longitudinal natural history studies and for monitoring effects of future therapeutic interventions to slow down GA progression in patients with advanced atrophic ARMD and other retinal diseases associated with outer retinal atrophy.

Introduction

Age-related macular degeneration (ARMD) is the most common cause of irreversible central vision loss and legal blindness in developed countries [1, 11, 12]. Besides choroidal neovascularization and pigment epithelial detachment, geographic atrophy (GA) of retinal pigment epithelium (RPE) is a frequent cause of severe visual loss in patients with ARMD [7, 13, 20]. The pathophysiological mechanisms underlying the atrophic process, which involves not only the RPE but also the outer neurosensory retina and the choriocapillaris, are poorly understood. GA is not pathognomonic for ARMD, but represents a final common pathogenetic pathway of various

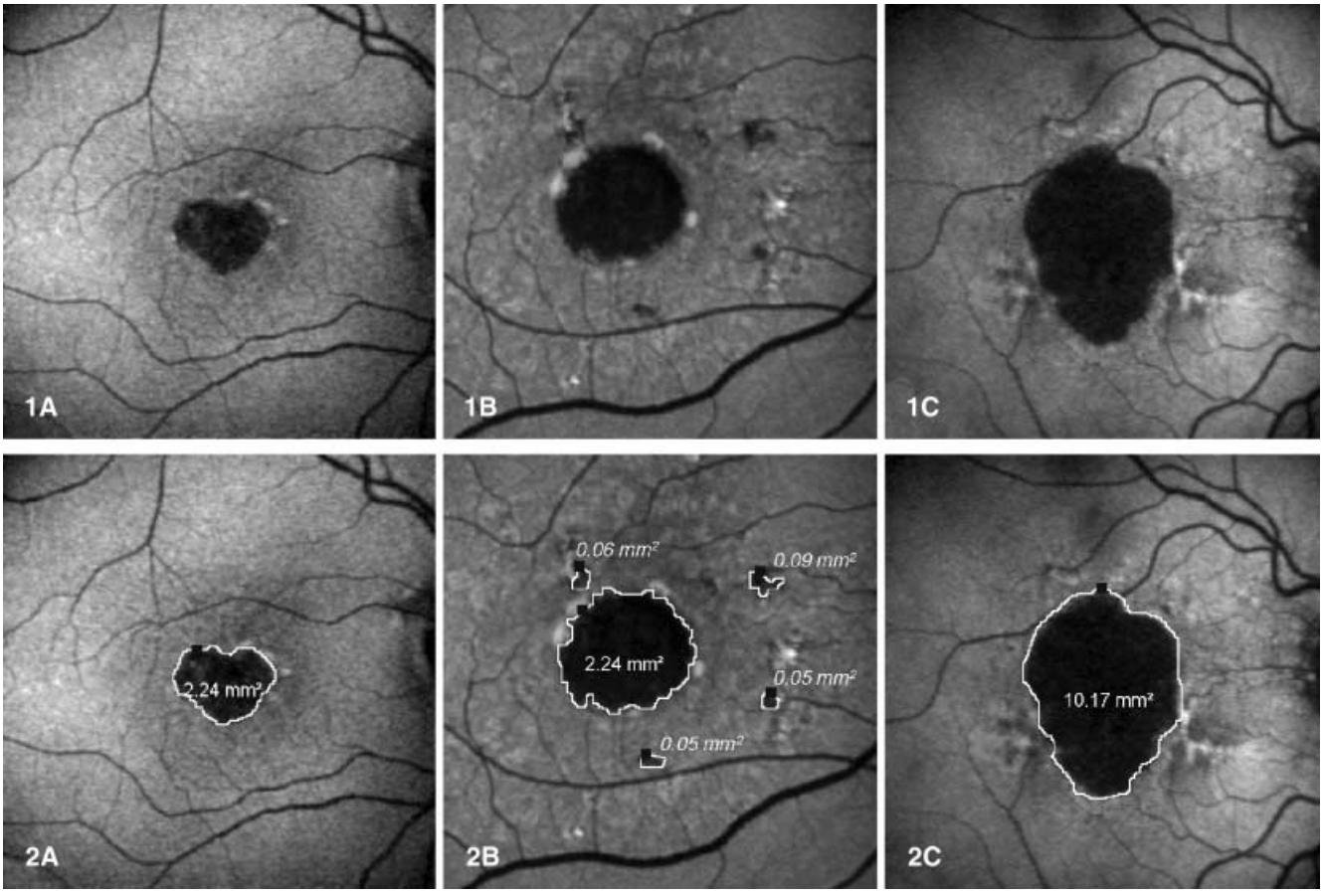


Fig. 1A–C Autofluorescence images of geographic atrophy (GA) secondary to ARMD (cSLO: exc. 488 nm; em: above 500 nm; Heidelberg Retina Angiograph). The same images are used in the following measurements

Fig. 2A–C Method A. Manual detection and quantitation of atrophic areas using the mouse-driven arrow tool of the HEE software. Here, the atrophic areas were outlined according to the assessment of the reader in *white* and their sizes are displayed on the screen

heterogeneous retinal degenerations, including geneticall-ly determined diseases.

With the advent of scanning laser ophthalmoscopy (SLO) it is now possible to image fundus autofluorescence (AF) mediated by RPE lipofuscin accumulations and its spatial distribution over large retinal areas in vivo [2, 14, 16, 18]. Abnormal AF patterns in the junctional zone of GA have recently been demonstrated using confocal SLO (cSLO) in patients with ARMD [3, 9, 16]. Furthermore, we have shown that areas of increased AF precede the development and enlargement of outer retinal atrophy in eyes with ARMD [10].

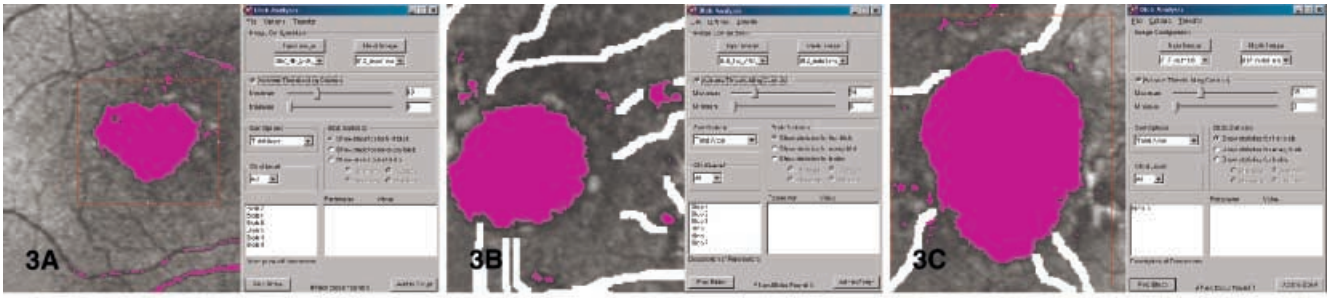
Fundus AF imaging is also useful for accurate detection of areas atrophic. Since the RPE is absent in such areas, and, therefore, dominant fluorophores which are responsible for the in vivo AF phenomenon, the AF signal is markedly reduced as compared to normal background fluorescence. AF imaging has been shown to be superior to fundus photography or angiography for delineating areas of GA [9, 15].

Quantitation of GA has previously been performed by manual techniques [19]. This study indroduces and evaluates a new method for automated detection and quantitation of atrophic areas on the basis of SLO AF images. Accurate recording of GA over time is a prerequisite for the precise determination of longitudinal changes in natural history studies and for the monitoring of variations in future interventional trials in patients with advanced atrophic ARMD.

Methods

A total of 278 patients with GA secondary to ARMD have been recruited to the prospective, multicenter Fundus Autofluorescence in Age-related Macular Degeneration (FAM) Study. Patients underwent routine ophthalmological basic examination, including determination of best-corrected central visual acuity using ETDRS charts. The pupil of the study eye was dilated with 1% tropicamide prior to fundus AF examination. In addition, fundus photographs were obtained.

Fundus AF was recorded using a confocal scanning laser ophthalmoscope [Heidelberg Retina Angiograph, HRA, Heidelberg Engineering, Germany; including Heidelberg Eye Explorer (HEE) software], the optical and technical principles of which we have



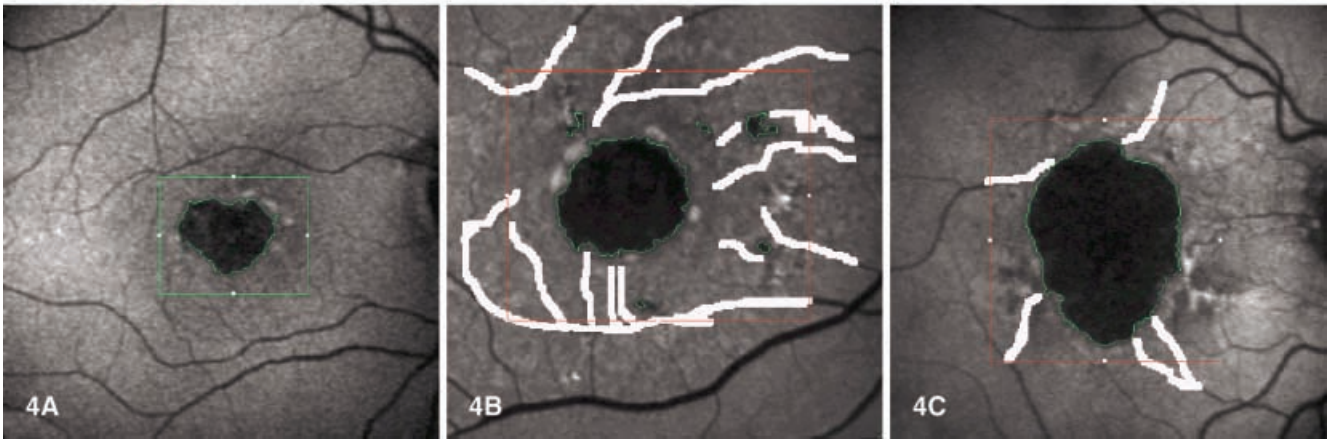


Fig. 3A–C Method B. Thresholding of the modified images by the reader using image analysis software (Global Lab Image/2). AF images had been exported, calibrated and the ROIs had been marked in image analysis software. Here, in the blob analysis tool, minimum (=0) and maximum threshold limits were activated by the reader and are highlighted in *magenta*

Fig. 4A–C Method B. Automated detection of atrophic areas using image analysis software (Global Lab Image/2). Only blobs in the ROI (boundary blobs were removed) and with a minimum size of 20 pixel were detected as atrophic areas and their borders are displayed on the screen in *green*

described previously [8, 9]. An argon blue laser (488 nm) was used for excitation, emitted light detected above 500 nm (barrier filter). By using an interference filter, the ratio of the intensities of green and blue light caused by the green share of the argon laser light is below 10^{-7}. Images were immediately digitized and processed using a flexible frame processor and subsequently displayed on a computer screen.

Before recording AF images, individual refraction was corrected by varying the optics of the instrument in the reflection mode. From each individual eye at least 20 AF images were obtained by using the frame grabber (10.2 images per second). Each frame contained 256 (or 512) pixels vertical and 256 (or 512) pixel horizontal. In order to amplify the AF signal, nine images were aligned and a mean image was calculated after automated detection and correction of eye movements using image analysis software [6, 9]. The digital images were saved on disk for further analysis and processing.

Corresponding with funduscopically visible atrophic areas, fundus intensity of AF was markedly decreased (Fig. 1) [9,10]. These dark areas were thresholded for the purpose of computer analysis. Areas were then analysed by two independent readers in 24 right eyes with GA due to advanced atrophic ARMD, applying both method A and method B. Statistical analysis was performed using the Bland–Altman design for method-comparison studies [4].

Method A: manual mouse-driven arrow

Atrophic areas were outlined on the screen using the mouse-driven arrow tool of the HEE software. This was repeated until the most accurate result according to the reader's subjective assessment was achieved (Fig. 2). Data were manually transferred to a Microsoft Excel spreadsheet (Microsoft, Redmont, Wash., USA) for further analysis.

Method B: automated computerized detection and quantitation

Images were exported as bitmap files from the HEE software. If necessary, blood vessels appearing dark (due to blockage of the AF signal) that were continuous with the atrophic areas were overpainted by white colour in Microsoft Paint (Windows 98, Microsoft) at high magnification. Modified images were opened and calibrated in Global Lab Image/2 (Data Translation, Marlboro, Mass., USA) and the regions of interest (ROIs) were marked [17]. Using the blob (*b*inary *l*arge *ob*ject) analysis tool, thresholding controls were determined by the reader (Fig. 3). Automatically, atrophic areas were detected as blobs (minimum area: 20 pixel, boundary blobs removed) and their size was measured (Fig. 4). Data were electronically transferred to a Microsoft Excel spreadsheet for further analysis.

The study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Heidelberg. Informed consent was obtained from the patients prior to recruitment into the study.

Results

Analysis with the Bland–Altman design for method-comparison studies showed that method A measured larger areas (manual mouse driven arrow) than with method B (automated computerized detection and quan-

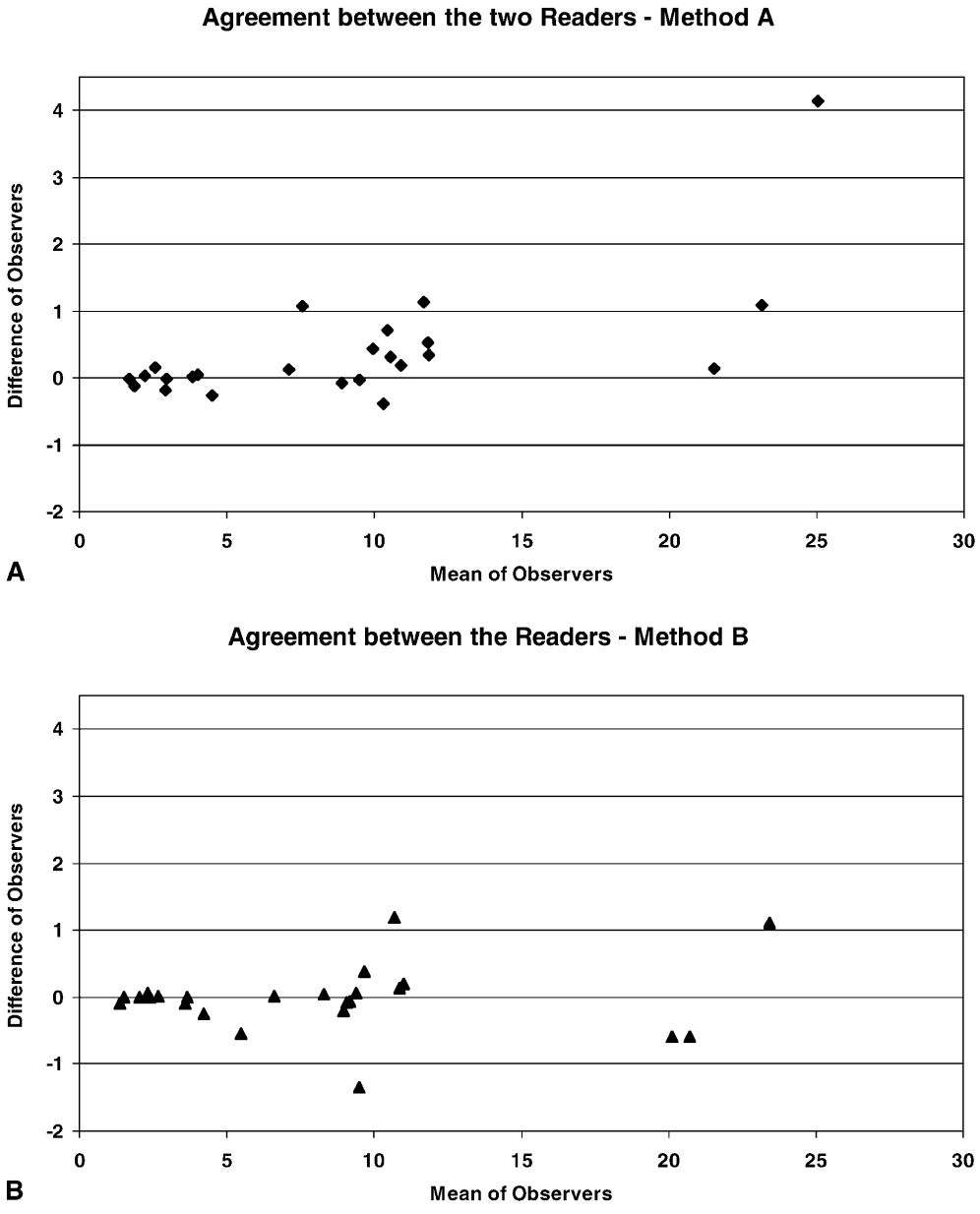


Fig. 5A, B Results of statistical analysis (Bland–Altman design for method-comparison studies) for evaluation of agreement between the readers for method A and method B

titation) by both readers (agreement A/B: reader 1 mean difference 1.04 mm, 95% CI [0.66, 1.42], 95% REF (limits of agreement) [–0.83, 2.91]; reader 2 mean difference 0.62 mm, 95% CI [0.43, 0.81], 95% REF [–0.31, 1.55]). The agreement between readers had a mean difference of 0.39 mm (95% CI [0.02,0.76], 95% REF [–1.41, 2.20]) for method A and a mean difference of –0.03 mm (95% CI [–0.23;0.18], 95% REF [–1.03, 0.97]) for method B, respectively (Fig. 5).

In addition to poorer interobserver agreement, method A required time-consuming processing. Even at higher magnifications it was obvious that it is sometimes difficult to precisely delineate the border between dark-appearing atrophy and the surrounding retina with an increased AF signal due to insufficient accuracy of the hand–mouse interface. In contrast, delineation using method B was performed automatically after a few PC commands. Figures 2 and 4 illustrate examples of areas of atrophy delineated and quantified using both methods.

Various features have been observed which make the delineation of GA borders more difficult. In the presence of larger choroidal vessels with prominent autofluorescent properties in the GA area and AF signals above the preset threshold, method B did not recognize these areas as part of the GA region. In this case, manual correction was mandatory. Both methods require high image quali-

ty, which may be impaired due to opacities at the level of the cornea, the anterior chamber, the lens or the vitreous. Furthermore, uneven distribution secondary to incorrect axial orientation of laser scanner during examination may lead to incorrect measurements.

Discussion

Fundus AF imaging provides a reliable means superior to fundus photography or fluorescence angiography to delineate areas of GA in patients with advanced atrophic ARMD. In this study, it was shown that automated computerized image analysis (method B) was more accurate than manual outlining (method A) in the detection and quantitation of GA areas in the digital images obtained by cSLO fundus AF imaging. Furthermore, manual outlining of atrophic areas is time-consuming and less precise due to the insufficient hand–mouse interface and the relatively low contrast sensitivity of the reader's own eyes. In contrast, automated detection and quantitation of atrophic areas based on the grey value of each pixel allows more accurate measurements.

Atrophic areas are associated with a markedly reduced AF signal due to the absence of RPE and, therefore, the dominant fluorophores which are responsible for the AF signal [5]. A limitation of the automated method of analysis is that it cannot distinguish between a reduced AF signal due to GA and other causes of signal attenuation, as it is based on measurements of grey levels. These include the optic disc or large retinal vessels of the inner neurosensory retina which absorb emitted fluorescent light from posterior planes. Therefore, it is necessary to mark the ROI, so that only blobs (minimum area: 20 pixels) in the ROI are detected. Blobs outside the ROI as well as boundary blobs (blobs touching the boundaries of the ROI) are excluded. As a result, retinal vessels and the optic disc outside the ROI are ignored.

However, it may occur that some larger retinal vessels are in contact with the atrophic area and, therefore, within the ROI. In order to avoid their misinterpretation as atrophic areas, the reader must overpaint such dark-appearing retinal vessels. Although this can be achieved at higher magnifications, a subjective modification of the image is introduced into the quantitations by the reader. Despite this, these manual modifications are a much smaller source of inaccuracy than pure manual outlining of the atrophic areas.

While it was not possible to save outlined images using method A (with the presently available HEE software), the automated detection and measurement (method B) of the atrophic areas could be easily repeated by knowing the threshold value and using the same modified image. Instead of method A, the results of method B are easily reproducible.

In contrast to method B, method A can be performed with no additional software if the HRA is used for AF imaging, since the HEE software that comes with the HRA encompasses the possibility for manual outlining. In addition, while using method B, images had to be exported and calibrated due to different sizes of the images. Future improvements of the HEE software, including the possibility to export all images in one defined size, would be helpful. Further advantages of method B include the electronic data transfer, in contrast to the manual data transfer of method A.

Sunness et al. [19] have reported an alternative method for measuring GA in advanced ARMD based on conventional fundus photographs instead of digital AF images. Their approach is to project the fundus photo onto a sheet of white paper taped to the viewing surface of a microfilm reader. Retinal landmarks, atrophic areas and spared areas within the atrophic are traced by a pencil on the sheet. In a second step, the reader traces the outline of the areas of atrophy on the sheet once again using a Summagraphics digitizing tablet. The areas are measured in square millimetres on the drawing itself by the computer. This measurement is then converted to Macular Photocoagulation Study standard disc areas (DA; 1 DA is equivalent to 2.54 mm^2 on the retina). Compared with the automated analysis presented herein, this method is also more time-consuming and prone to additional factors, negatively influencing area measurements, e.g. no correction of refractive errors, outlining of areas twice at different magnifications and inaccurate identification of disc area due to peripapillary atrophy.

In summary, automated computerized image analysis of digital fundus AF images facilitates detection and measurement of the size of atrophic areas. In addition, a higher degree of agreement between different readers is achieved with automated quantitation than with manual methods. Automated detection and quantitation will be useful in prospective natural history studies of retinal diseases associated with atrophy and for monitoring the effects of future therapeutic interventions to slow down progression in patients with advanced atrophic ARMD.

Acknowledgements Supported by the German Research Council (Deutsche Forschungsgemeinschaft, DFG), Bonn, Germany, grant Ho 1926/2-1, DFG Priority Program AMD SPP 1088, and by the Research Program of the University of Heidelberg, 500/2000.

Appendix: Fundus Autofluorescence in Age-Related Macular Degeneration (FAM) Study Group

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