



University of Wisconsin
**SCHOOL OF MEDICINE
AND PUBLIC HEALTH**

Fundus Photograph Reading Center

Autofluorescence Using Fundus Cameras (AFF-D)

Effective Date: *06 Jul 2012*

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1. Overview

This document details the Fundus Photograph Reading Center (FPRC) procedure for photographer certification, provides instruction to image autofluorescence using fundus cameras, and offers pointers on imaging technique.

Digital systems must be certified for each study separately. See the *Digital System Certification* document for further details.

Further clarification regarding any information included in this document may be obtained by contacting the FPRC Imaging staff at (608) 410-0619 or by sending an email to Imaging_administrators@rc.opth.wisc.edu.

2. Photographer Certification

2.1. Overview

Photographer certification is specific to each study and photographers taking digital images for studies evaluated by the FPRC must be certified for the relevant procedure(s), before submitting actual subject images. The certification images may be taken on subjects being photographed for clinical purposes or of volunteers. Once a photographer is certified for a specific study, he/she is certified for the duration of that study, provided he/she meets the quality standards set by the FPRC. See section 6 *Evaluation of Image Quality*.

2.2. Certification Requirements

Certification consists of:

- Review of the study synopsis/protocol and imaging procedure(s)
- The ability to perform the imaging procedure(s), demonstrated by the submission of images of acceptable quality

2.2.1. Images Required if Previously Certified

The second certification requirement listed above will be waived if all of the following criteria are met:

- The photographer has prior certification at the FPRC using **an identical procedure**.
- The photographer has been actively taking images during the past 12 months.
- The images are judged to be of good quality by the FPRC.

If a photographer thinks that these criteria have been met or has been certified by the FPRC for a similar procedure, submit a certification request (see section 2.3 *Submission for Certification*) without sending images.

2.2.2. Images Required if Not Previously Certified

Submit a certification request (see section 2.3 *Submission for Certification*), and send autofluorescence images of four eyes (two right eyes and two left eyes, three images

of each eye) taken using this procedure (AFF-D). Two of the eyes should be normal, and two of the eyes should have autofluorescence changes.

2.3. Submission for Certification

Photographers are encouraged to send complete submissions for each procedure for which they are requesting certification (i.e., if four eyes are required for a certification submission, send all four eyes together).

Photographers who meet certification criteria will receive confirmation of certification. Those who do not meet these criteria will receive feedback from the FPRC imaging consultants and may be required to submit additional sets of images. A plan for improving image quality may be necessary after three complete unsuccessful certification submissions.

2.4. Uncertified Photographers

2.4.1. Baseline/Screening Visits

Only FPRC-certified photographers are allowed to take baseline (screening visit) photos, unless an exception to this rule is granted (on a case-by-case basis) by the study sponsor. The baseline images for a subject are critical since all follow-up measurements are compared to this point to determine the study outcome.

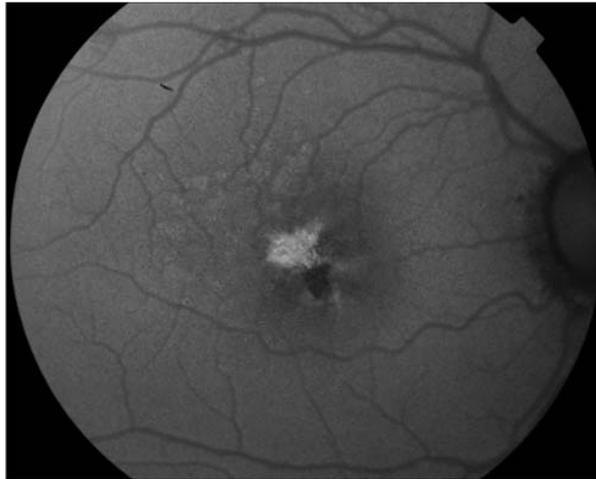
The sponsor may suspend subject enrollment if the site does not have a certified photographer available to take the baseline images.

2.4.2. Follow up Visits

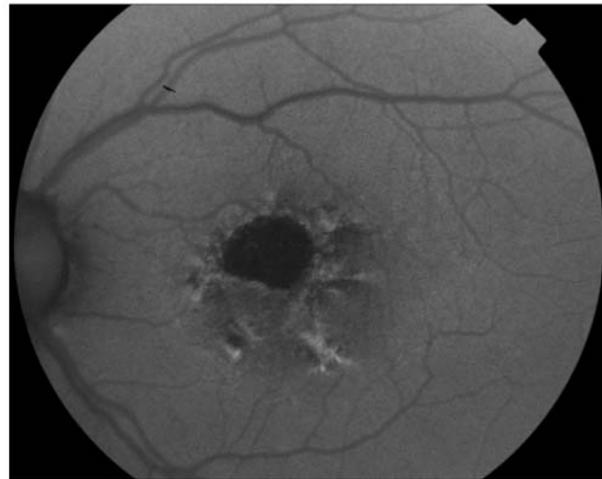
On rare occasions during **follow-up** visits ONLY, when a certified photographer is not available, an uncertified photographer familiar with the procedure(s) may take the images. The uncertified photographer should review the imaging procedure(s) before performing photography to be certain he/she understands the procedure and follows the study requirements. Include a comment with the submission indicating that the photos were taken by an uncertified photographer and the reason why.

3. Fundus Camera and Filters

A digital retinal camera system, equipped with suitable autofluorescence filters and a nominal 30°-35° magnification setting is required. Suitable combinations of excitation and barrier filters produce good quality, bright, contrasty images, capturing the autofluorescence of retinal lipofuscin. The ideal filter pair allows some signal penetration through macular hyperpigmentation, yet minimizes the impact of confounding autofluorescence induced by lens sclerosis (resulting in contrast degradation). The image below exhibits acceptable image quality.



OD (RE)



OS (LE)

Many different retinal camera and filter combinations are available for digital fundus autofluorescence imaging. While no one combination of excitation and barrier filters has been shown to be optimal for all retinal cameras, the FPRC recommends that you contact your camera system vendor to obtain their most current filter combination for autofluorescence imaging. Some filter sets do not produce images with adequate signal-to-noise ratio (resulting in loss of detail) or inadequate contrast and may not be suitable for autofluorescence imaging.

A one mega-pixel or slightly higher resolution monochrome image capture system (lower resolution sensors have bigger pixel wells for greater dynamic range), similar to those for fluorescein and ICG angiography are preferable. Higher resolution sensors (around 5-6 mega-pixels), like those for color fundus photography, may not provide adequate sensitivity and signal-to-noise ratio and are less suitable for autofluorescence imaging. The gain setting should be adjusted to produce good exposure without excessive noise (grainy images). For details about how to certify a system refer to the *Digital System Certification* document. Contact the FPRC if you have questions about the system or filters you intend to use.

4. Acquiring Autofluorescence Images

To increase the retinal exposure to light and ensure photoreceptor photopigment bleaching color retinal images should always be taken just before the autofluorescence images. Large pupil dilation will provide maximum light delivery to the retina. Three non-stereoscopic, 30°-35° images, centered on the macula, are taken of each eye. Images are saved using no compression (TIFF or BMP) or lossless (PNG) compression. Lossy compressed (standard JPEG) images may be acceptable but will need to be evaluated by the FPRC on a case-by-case basis.

The camera should be adjusted to the optimal combination of flash and gain settings to produce well exposed images with good contrast. Focus should be adjusted to produce crisp images, capturing areas of autofluorescence changes such as geographic atrophy. This is accomplished by initially focusing on the retinal vessels using red-free light and then adjusting the plane of focus of the autofluorescence image to the level of the retinal pigment epithelium.

5. Format for Study Images Submitted to the FPRC

Images should be saved and sent at full resolution, using no compression or lossless compression (PNG). For additional information on specific image handling procedures, see the *Digital System Certification* document.

6. Evaluation of Image Quality

6.1. At the Study Site

The autofluorescence images should be evaluated for quality by the principal investigator and/or photographer (unless prohibited by Study Protocol) before submission to the FPRC. If quality is not adequate for assessment of key features, and if no irremediable cause of inadequate quality is present (such as lens opacities or a pupil that will not dilate adequately), the images should be retaken before submission to the FPRC.

6.2. At the FPRC

Autofluorescence images of each eye are reviewed and assigned a grade for overall quality. Feedback will be provided to the photographers as needed to help with resolution of any problems. Special attention will be given to photographers having difficulty meeting study photo quality standards. If a certified photographer consistently fails to meet study standards, certification may be suspended.

7. Pointers on Imaging Technique

7.1. General

Photography of the photophobic subject can be challenging for the photographer and uncomfortable for the subject. Minimizing the number of flashes and the length of time the eye is exposed to a bright viewing lamp are two things that can help make the photography procedure more comfortable. Additionally, keeping the view lamp as low as possible (maybe even dimming the room lights) can help make the photography procedure more tolerable. Patients should be asked to blink frequently to keep the cornea clear.

If the subject has great difficulty tolerating the screening visit photography procedure and the photographer thinks this will lead to a problem at follow-up visits, the situation should be discussed with the principal investigator and/or coordinator. Consideration should be given to not enrolling the subject in the study.

7.2. Focus/Clarity

The best image quality is obtained if corneas are not disturbed by prior examination with a diagnostic contact lens.

Focusing the image in red-free light does not guarantee crisp focus of changes appearing at the level of the RPE. Review the images on the computer monitor, adjusting focus as necessary, to obtain the best image sharpness.

7.3. Exposure

Autofluorescent areas emit very little light. Imaging this weak autofluorescence requires a careful balance between camera alignment, flash intensity, and the digital system gain setting. Since the fluorescence occurs in the 695nm range it is most desirable to use a monochrome (B&W) sensor. High-resolution color sensors are not as sensitive to light and are generally inadequate for autofluorescence imaging.

Position the camera carefully to direct as much of the light through the pupil as possible. No stereo images are taken.

To increase image brightness, engage the small pupil setting on the fundus camera, if available. In some retinal cameras this can increase the amount of light delivered to the retina.

Always adjust the camera flash setting before increasing the gain setting. Increasing the gain too much increases the amount of “noise” in the image. It is important to have a good balance of flash output vs. gain for good autofluorescence photos.

Autofluorescence images should be taken before fluorescein or indocyanine green angiography is performed since the presence of these dyes can interfere with image quality.