ORIGINAL ARTICLE

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The Effects of Gain and Noise in Fundus Autofluorescence Imaging

Introduction

Fundus autofluorescence (FAF) is a non-invasive imaging technique for documenting the presence of lipofuscin in the retinal pigment epithelium (RPE). Lipofuscin is a fluorescent pigment that accumulates in the RPE as a metabolic byproduct in the aging eye. When excited with short to medium wavelength visible illumination, lipofuscin granules autofluoresce, exhibiting a broad emission spectrum from 500 to 750 nm with peak emission at about 630 nm.[1] Fundus autofluorescence can be recorded with either a confocal scanning laser ophthalmoscope (cSLO) or a fundus camera equipped with appropriate filters and a monochrome digital sensor.

The original technique for imaging FAF employed a cSLO with the excitation wavelength set at 488 nm and a wide band-pass filter with short wavelength cutoff at 521 nm to act as a barrier to the excitation wavelengths.[2] These are the same excitation and transmission wavelengths used for fluorescein angiography. A confocal aperture placed at a focal plane conjugate with the retina effectively blocks non image-forming light, including autofluorescence that can occur in the crystalline lens at the excitation wavelength of the cSLO.

More recently, digital fundus-camera based systems have been developed which use high-sensitivity monochrome sensors with an excitation filter at 580 nm and a barrier filter at 695 nm (Figure 1). These longer wavelengths are used to avoid confounding autofluorescence from the lens.[3] Despite the disparity in excitation wavelength and barrier filters between the cSLO and fundus camera systems, these two techniques obtain autofluorescent results that are similar in appearance.

Figure 1: Autofluorescence filters. (a) Photograph taken through the standard fluorescein filter set (exciter at 480 nm and barrier at 525 nm) exhibits pseudofluorescence at high flash and sensor gain settings. (b) Photo of the same eye through the FAF filter set (exciter at 580 nm and barrier at 695 nm) from Spectrotech demonstrates autofluorescence from the RPE. Dark areas represent RPE atrophy.

Image Noise

Both cSLO and fundus camera FAF systems are subject to significant amounts of image noise. Noise is false pixel data created during the capture process that can interfere with image detail. It is an inherent characteristic of digital sensors and can be affected by various factors including sensor temperature, pixel size, well capacity, sampling errors, faulty pixels, and signal amplification. Most noise apparent in FAF images is a combination of photon noise and readout noise, both of which occur from poor signal-to-noise ratios and necessary amplification of low luminance levels of fluorescence.

Photon noise is a result of an intrinsic property of light whereby photons strike a photosensitive detector at random intervals, causing statistical fluctuations in their measurement. Photons collected by a sensor exhibit a Poisson distribution, meaning there is a square root relationship between signal and noise.[4,5] As the amount of light incident on a sensor is increased, more photons are available to average out sampling errors and the signal-to-noise ratio improves. Photon noise results in a random, grainy pattern that can obscure fine detail in an image. The negative impact of photon noise is most noticeable with short exposures, dimly lit subjects, and high amplification of the signal.

After photons are collected by a sensor, the resulting charge in each discrete photo-site on the chip must be amplified, measured, and converted to a digital value in order to produce an image. Readout noise, which is sometimes referred to as amplifier noise or bias noise, is introduced by the imperfect nature of signal conversion and the amplification process.[6] It is directly affected by the gain or ISO setting on the sensor. Readout noise is generally consistent from image to image for a given level of amplification, making it more controllable with post-capture image processing techniques. Sensor manufacturers often apply noise reduction algorithms based on a sensor-specific noise profile in the readout process to attenuate the effect of this type of noise.

It is possible to control noise either during capture, or with post-capture image processing techniques. To reduce noise created at capture, the cSLO autofluorescence method utilizes an averaging technique, recording between nine and fifteen frames over two seconds. The frames are then aligned and averaged to produce the final image. Because several frames are required for averaging, eye movement during capture may adversely affect image quality. Image averaging is a very powerful technique,[7] but this noise reduction strategy is not currently available with fundus camera based FAF systems. Future investigation is needed to determine whether this technique could be adapted to fundus camera systems, possibly by averaging fewer individual frames.

Methods

Fundus autofluorescent images were captured at different gain settings to demonstrate the relationship between amplifier gain and image noise. Images were taken with a Topcon IMAGEnet fundus autofluorescence system using a Topcon TRC 50EX fundus camera equipped with a Redlake MegaPlus II ES 3200 12-bit monochrome sensor (set for 8 bit capture), and a Spectrotech autofluorescence filter set with peak excitation wavelength of 580 nm and barrier filter at 695 nm, each with a bandwidth of approximately 40 nm (Figure 2). The monochrome sensor was mounted on the upper camera port on a Topcon TL 209 TV Relay Lens. Maximum gain setting on the MegaPlus sensor is 36. Imaging software is IMAGEnet 2000, version 2.55.

Figure 2: Fundus camera FAF system with monochrome sensor and FAF barrier filter mounted on TV relay lens on top camera port.

Exposure

FAF is particularly vulnerable to noise from low light levels because of variability in the amount of lipofuscin present from patient-to-patient depending on age, health of the RPE, and disease process. In the absence of significant accumulation of lipofuscin, underexposure can occur in widely dilated eyes with clear media, eyes that would easily produce well-exposed images at normal settings in conventional fundus photography.

In theory, the best strategy for controlling noise is to deliver more photons to the sensor at capture to improve the signal-to-noise ratio, and use the lowest possible gain setting that will provide adequate exposure. Otherwise, image post-processing techniques will have to be employed to rescue image information. This means setting the fundus camera for maximum flash output and light transmission, which may be difficult for some light-sensitive patients to tolerate. The default camera controls for FAF typically place the sensor's gain near the maximum setting in order to record low-level autofluorescence, so there may be very little room for lowering gain to reduce amplifier noise while still maintaining sufficient exposure to minimize photon noise. At lower gain settings, underexposure can occur, resulting in dark, low-contrast photographs. Enhancement of significantly underexposed images to

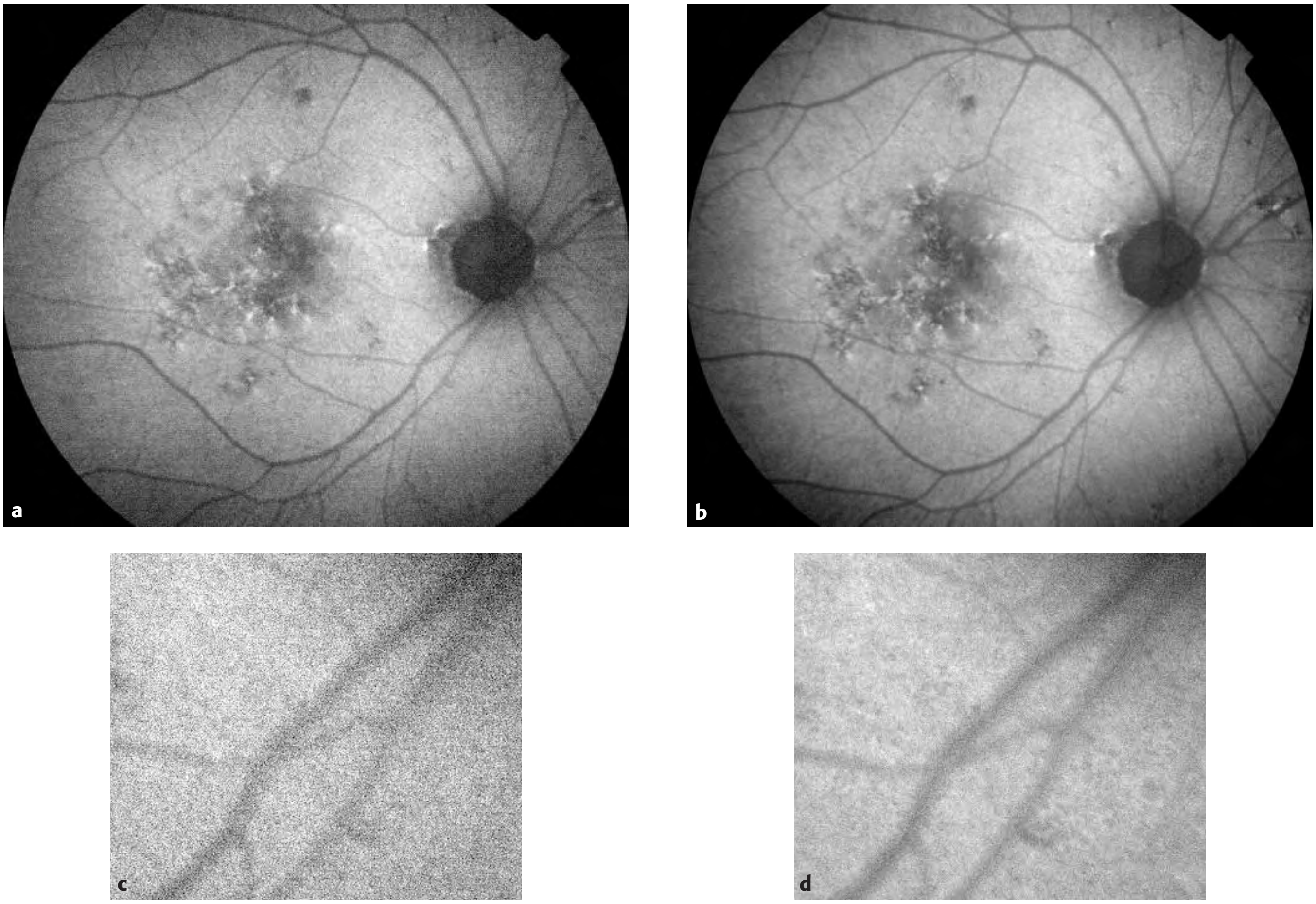

Figure 3: Gain comparison. (a) FAF image taken with the camera gain at default setting of 30. (b) Same eye photographed at reduced gain setting of 20 demonstrates reduction in apparent noise and an increase in detail. (c) Cropped section of (a) demonstrating significant noise at a gain setting of 30. (d) Cropped section of (b) showing noise reduction and improved detail at the lower gain setting.

improve brightness and contrast will increase noise in a manner similar to increasing gain.

All controls should be set for maximum light transmission, including flash settings and illumination diaphragm. Light transmission may be best at the widest-angle setting in some variable angle fundus cameras. If the fundus camera is equipped with an illumination diaphragm, it should be set to the largest aperture. When light transmission is maximized in this manner, eyes with significant accumulation of lipofuscin can be imaged with reduced gain settings while still maintaining adequate exposure (Figure 3).

Post Capture Image Processing

Post-capture image processing techniques may reduce the appearance of noise in FAF images, but care must be taken to avoid destroying important detail in the process. Software manufacturers use various image processing algorithms to average neighboring pixels to reduce brightness differences. The primary flaw in this approach is that it does not discriminate between brightness differences due to noise, or actual subject detail. Most commercial ophthalmic digital systems provide some basic image editing tools. The Smooth tool in IMAGEnet's capture software is an averaging filter with three preset strength levels.[8] The Smooth tool may reduce apparent noise but will also blur image details (Figure 4). Attempts to subsequently Sharpen the image will usually result in further destruction of detail.

Adobe® Photoshop® or other secondary imaging programs may provide more robust image processing capabilities to combat noise, but any such noise reduction strategy is a compromise between suppressing noise and destroying detail.[9] Photoshop filters that can be used to suppress noise include: Despeckle, Median, Average, Box Blur, Gaussian Blur, Smart Blur, and Reduce Noise. These noise reduction tools generally work best with noise of a similar spatial frequency and uniform pattern that can be reliably predicted, such as readout noise. Random noise such as photon noise or isolated pixel noise is more difficult to correct with smooth, median, or blur tools in the capture software or Photoshop. Compression artifacts, which are common in JPEG image files, can compound the problem by adding an additional flavor of noise to an already compromised image.

FAF images exhibiting obvious noise were post-processed

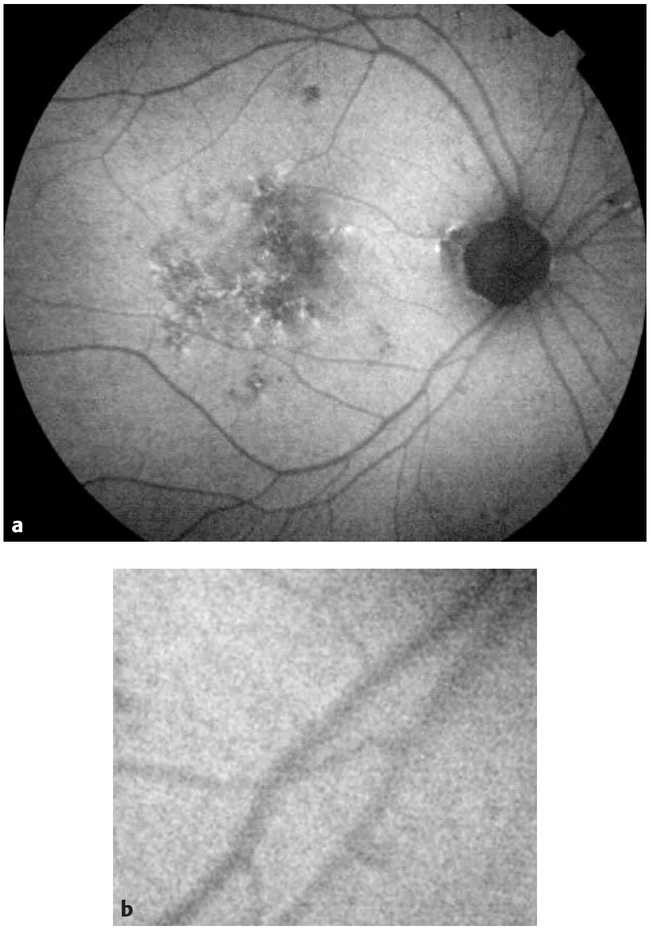

Figure 4: Topcon Smooth tool. (a,b) Original image shot at gain setting of 30 after enhancement with the IMAGEnet Smooth tool at the highest setting. Note softening of noise, but slight blurring of detail.

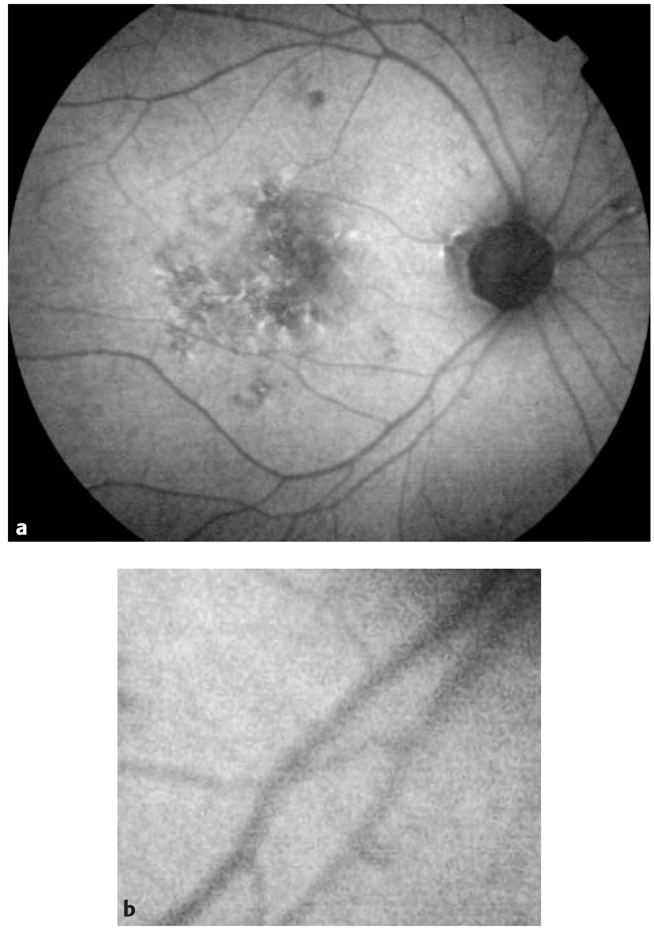

Figure 5: Photoshop workflow. (a,b) Original image shot at gain setting of 30 after enhancement using a workflow in Photoshop CS (Reduce Noise filter strength 10, Sharpen Detail 100, Gaussian Blur 1.0). Note improvement over the Topcon Smooth Tool.

using a noise reduction workflow in Adobe Photoshop and compared with the original image (Figure 5). Multi-step Photoshop noise reduction workflows typically involve edge-detection, blurring, and then sharpening, using masks and adjustment layers. These strategies work well in full-color images with sharp edge details, but aren't as helpful for grayscale FAF images. The Reduce Noise filter is probably the most practical and flexible control in Photoshop for noise suppression in FAF images. If enhancements are necessary to increase contrast and brightness of the image, these adjustments should be made after any simple smooth or blur filters are applied. This way, the brightness values of neighboring pixels will be relatively close and the blur algorithms will more easily detect them at a given threshold setting. Tools like Photoshop's Smart Blur allow you to adjust threshold settings to adapt to increased contrast levels, but many tools have preset threshold and radius settings that cannot be adjusted. It should be noted that while very elaborate non-destructive workflows utilizing multiple channels, layer masks, and multi-pass filtering are certainly possible, the random patterns of noise and information inherent to ophthalmic images make such processes questionable for routine clinical application.

In addition to the tools found in the capture software or Photoshop, several dedicated noise reduction programs are available either as stand-alone programs or as "plug-ins" to Photoshop. Software such as Imagenomic Noiseware™, MediaChance PureImage, Picture Cooler, and others use sophisticated algorithms to profile and adjust to different noise types or noise frequencies within the same image. For example, they may apply higher noise suppression in areas of little detail while sparing obvious edge detail, or they may more aggressively target shadow areas where photon noise is more apparent. They use different strategies to suppress noise and offer a variety of controls including separate tools for luminance and chrominance noise, controls for noise of different sizes or spatial frequencies, controls for noise in highlights, mid-tones, or shadows, and blending of the processed image with the original (Figure 6).

Even with this level of sophistication, the results may be less than desirable for diagnostic imaging. Noise reduction and subsequent sharpening can often improve

Figure 6: (a,b) Noise reduction post-processing with Picture Cooler version 2.45 at default noise reduction, with a 50% blend of original image and processed image. Changing the blend allows for very fine adjustment of the noise to blur ratio.

Figure 7: (a,b) Noise reduction post-processing with NoiseWare Professional version 2.6 at the default setting with additional 200% Noise Level Adjustment in the high frequency channel. Image noise is almost completely removed without excessive blurring of the blood vessels, but some fine drusen (that were apparent in varying degrees with all other methods) disappear.

the appearance of the image, but the use of extensive post-capture processing alters data to a degree that may not be acceptable for diagnosis or accurate documentation (Figure 7). Over-processing can also introduce a different type of artifact that may be worse than the original noise pattern, resulting in an artificial or "plastic" look. Use of one of these noise reduction programs is best reserved for preparing illustrative FAF images for publication.

Discussion/Conclusions

Fundus camera based FAF has proven itself to be a viable and practical diagnostic imaging modality; but for it to become a widely accepted alternative to the cSLO technique, every effort must be made to maximize light transmission to obtain consistent high quality images (Figure 8). Controlling noise at capture is preferable to post-capture image processing. Although various combinations of illumination settings and gain can be used to achieve sufficient exposure, noise can be significantly reduced if the lowest possible gain setting is used. The challenge for the imager is trying to achieve a balance between exposure and noise. With proper exposure, little image enhancement is necessary to produce quality FAF images with fundus camera based systems. We recommend setting the flash and illumination diaphragm to the maximum settings and then controlling exposure through gain adjustment.

If post-capture image processing tools are used, they should be applied judiciously to avoid over-processing and potential loss of detail. The JPEG image file format should be avoided to prevent compounding existing noise with compression artifacts. If the JPEG format is used, then the lowest possible compression setting should be selected to reduce introduction of additional artifacts. As with any image processing of diagnostic images, an original unaltered image file should be retained along with the enhanced version.

Suggestions for improving light transmission in future FAF systems would be mounting the digital sensor directly on the primary camera mount, rather than a relay lens or tube where light efficiency is lost. Development of new high-transmission filter sets with wider bandwidth or more

Figure 8: (a) FAF image of hyperautofluorescent optic nerve drusen and hypoautofluorescent area of RPE atrophy. (b) FAF image of a patient with adult foveomacular vitellifrom dystrophy with scattered hypoautofluorescent RPE changes and a central area of hyperautofluorescence corresponding to the vitelliform lesion. Both images were processed for publication using NoiseWare Professional.

efficient excitation wavelengths may also be possible. Future engineering might include utilization of pixel binning with higher resolution sensors, optimizing capture integration timing, and exploring novel illumination sources.

The authors have no proprietary interest in any of the products discussed in this article.

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