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Comparison of Fundus Autofluorescence Between Fundus Camera and Confocal Scanning Laser Ophthalmoscope-based Systems

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Abstract

BACKGROUND AND OBJECTIVE—To compare fundus autofluorescence (FAF) imaging via fundus camera (FC) and confocal scanning laser ophthalmoscope (cSLO).

PATIENTS AND METHODS—FAF images were obtained with a digital FC (530 to 580 nm excitation) and a cSLO (488 nm excitation). Two authors evaluated correlation of autofluorescence pattern, atrophic lesion size, and image quality between the two devices.

RESULTS—In 120 eyes, the autofluorescence pattern correlated in 86% of lesions. By lesion subtype, correlation rates were 100% in hemorrhage, 97% in geographic atrophy, 82% in flecks, 75% in drusen, 70% in exudates, 67% in pigment epithelial detachment, 50% in fibrous scars, and 33% in macular hole. The mean lesion size in geographic atrophy was $4.57 \pm 2.3 \text{ mm}^2$ via cSLO and $3.81 \pm 1.94 \text{ mm}^2$ via FC ($P < .0001$). Image quality favored cSLO in 71 eyes.

CONCLUSION—FAF images were highly correlated between the FC and cSLO. Differences between the two devices revealed contrasts. Multiple image capture and confocal optics yielded higher image contrast with the cSLO, although acquisition and exposure time was longer.

INTRODUCTION

Fundus autofluorescence (FAF) imaging at 488 nm excitation noninvasively maps lipofuscin distribution in the retinal pigment epithelium.¹ The age-related and/or pathologic accumulation of lipofuscin, or its conspicuous absence in areas of RPE atrophy, is an indirect means of detecting, quantifying, and monitoring outer retinal disease. FAF is helpful in elucidating pathophysiologic mechanisms of disease, as a diagnostic adjunct, in phenotype-genotype correlation, in identification of predictive markers for disease progression, and in monitoring therapy in retinal diseases.^{1–4}

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Currently available FAF imaging modalities include confocal scanning laser ophthalmoscopy (cSLO), and digital fundus camera-based systems, which we compare herein. At first glance, FAF images produced by the cSLO and fundus camera (FC) appear to be similar. However, each system has unique image acquisition and processing techniques, which lead to qualitative and quantitative image differences. Wide-field autofluorescence imaging recently became available from Optos (Dunfermline, United Kingdom)⁵ but will not be discussed in this study.

The first difference to note between cSLO and FC-based FAF is the excitation wavelength used to induce autofluorescence. Fundus camera systems often utilize longer wavelength (530 to 580 nm) excitation compared to cSLO (488 nm). This has broad imaging implications because although lipofuscin contains the major fluorophores in the ocular fundus, other substances such as melanin exhibit autofluorescence and may be imaged if the appropriate excitation wavelength (in this case, 787 nm) is used.^{2,10} Choice of excitation wavelength also affects the amount of signal captured from non-outer retinal structures during image acquisition. Longer-wavelength FAF excitation with the FC may be less affected by absorption from cataracts and macular pigments (mainly lutein and zeaxanthin) than the shorter-wavelength excitation utilized in cSLO.^{1,6,7}

Image acquisition is an important point of contrast between the two systems. The FC captures a single image with a flash, whereas cSLO records a series of several FAF images using the same short wavelength excitation light for illumination. A mean image is then calculated, and pixel values are normalized to reduce background noise.¹ While this method often improves image resolution and contrast compared with the single image of the FC, poor fixation and excessive eye movement can limit its benefit and sometimes render images unsuitable for processing.^{4,7,8}

Although the cSLO has a relatively high purchase and maintenance cost compared with an FC, it has been the predominant mode of FAF imaging because of its perceived technical advantages. However, Schmitz-Valckenberg et al suggested that geographic atrophy quantification is similar with both imaging methods, and other studies suggest that the FC may detect certain types of lesions better than the cSLO.^{4,6,8–10} Thus, the FC-based FAF imaging system may represent an attractive alternative to the cSLO, considering its relatively low purchase price and incorporation into a color fundus and fluorescein angiography imaging platform.

A barrier to fundus camera-based systems has been the need to make after-market modifications in excitation and emission filters for autofluorescence imaging. This has been described in detail by Spaide,¹⁴ and, although elegant, it may not be as end-user-friendly for the novice compared to the cSLO, which has a factory-set autofluorescence mode.

We compared FAF images obtained with an FC and a cSLO in patients with various retinal diseases. We aimed to assess the agreement between the two imaging modalities, infer conclusions about the advantages and disadvantages of each technique, and make recommendations for the most appropriate FAF imaging modality in various disease states.

METHODS

Consecutive patients with any retinal or uveitic disorder, including diabetic retinopathy, age-related macular degeneration, and retinal dystrophies, were included in this study. The clinical diagnosis was established in each patient via comprehensive ophthalmic examination, including patient history, best corrected visual acuity (BCVA), anterior segment evaluation, intraocular pressure (IOP), and detailed fundus examination. Ancillary testing including color fundus photographs, frequency-domain optical coherence tomography, near-infrared photography, and fluorescein angiography was performed.

In each patient, FAF images were obtained by a single examiner after dilation of the pupil using two devices: a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph;² Heidelberg engineering, Heidelberg, Germany) equipped with a 488-nm laser exciter and 500-nm barrier filter and a digital fundus camera (Canon CX-1; Canon Inc., Tokyo, Japan) with a 530- to 580-nm excitation filter and 640-nm barrier filter.

For each eye imaged, color and FAF images from the cSLO and FC were evaluated by two authors and compared with the images that had no detectable abnormalities from both modalities. Agreement between the two FAF images' autofluorescence pattern and lesion size was noted. FAF patterns were analyzed for the following lesions: geographic atrophy, drusen, fibrous scars, retinal pigment epithelial detachment (PED), exudates, hemorrhage, macular hole (MH), and flecks (as seen in Stargardt's and other inherited retinal degenerations).

FAF intensities were classified qualitatively as hyper-, iso-, or hypoautofluorescent compared with the surrounding area. As a result, FAF intensities for studied lesions were classified into seven categories: (1) hyperautofluorescence, (2) isoautofluorescence, (3) hypoautofluorescence, (4) hyperautofluorescence and isoautofluorescence, (5) hypoautofluorescence and isoautofluorescence, (6) hyperautofluorescence and hypoautofluorescence, and (7) hyperautofluorescence, isoautofluorescence, and hypoautofluorescence. Two observers classified fundus lesions using these categories, and the agreement or the disagreement between the cSLO and FC images was assessed.

In cases of GA, the size of the atrophic area imaged on each device was measured by two observers using Matlab software (Mathworks, Natick, MA) (Figure 1, page 534). A new Matlab file was made for each case, with an embedded layer for each image (cSLO and FC). Images were set to the same resolution and size. This Matlab software has an advantage of providing a cropped circular image, which offers better magnification and resolution than the original square image. Thus, circular images from the FC were cropped to square for better comparison to the images from the cSLO. Reproducibility of lesion size measurement between the two observers was assessed using the interclass correlation co-efficient (ICC) and paired *t*-test (Table 1).

GA lesion area as measured on each device was compared by scatter plot (Figure 2) and Bland-Altman plot (Figure 3, page 536), the latter comparing the difference in area between the cSLO and FC as compared to the mean of the two.

Qualitative image superiority was queried in a forced-choice manner (Table 2, page 535), comparing cSLO and FC images for each eye studied. Parameters included clarity of the optic disc, vessels, macula, and posterior pole; ability to discern the pathologic lesion from its surroundings; and contribution to the clinical diagnosis. When the assessment of the two examiners did not agree, the opinion of a third was obtained, and the findings were discussed until a consensus was reached.

We chose not to quantitatively compare the images in this study because ocular media transmission differs between the two imaging devices and because the autofluorescence patterns varied amongst the disease entities studied. Although we chose qualitative analysis, relative autofluorescence intensities between the two modalities were not compared. Due to reasons mentioned above, comparing relative intensity was deemed to be too subjective and therefore inconclusive.

RESULTS

One hundred twenty-two eyes of 64 subjects (including six monocular individuals) were imaged. In each eye, there was a clear diagnosis and no more than two retinal diseases. Thirty-five patients were men and 29 were women; the mean age was 62 ± 10.6 years. Fifty-nine eyes had age-related macular degeneration (AMD), according to inclusion criteria of age 50 years or older and AMD diagnosed according to the international classification and grading system.¹¹ Thirty eyes were diagnosed with retinal dystrophy including recessive Stargardt's disease (STGD1), retinitis pigmentosa, maternally inherited diabetes and deafness, and vitelliform dystrophy. Diabetic retinopathy was present in six eyes (diagnostic classification according to ETDRS report number 10).¹² Other diagnoses included degenerative myopia (five eyes), macular hole (three eyes), and macular pucker (one eye). Sixteen eyes had no detectable abnormal autofluorescence.

Using the classification scheme described above, we compared FAF lesion patterns in the each eye as imaged with cSLO and FC-based systems. An overall pattern agreement rate of 86% was observed. Nine percent of FAF images did not agree, and 5% were not comparable because of low image quality (Figure 4). By lesion subtype, the agreement rates were 97% for GA lesions, 75% for drusen, 50% for fibrous lesions, 67% for PED, 70% for exudates, 100% for hemorrhagic lesions, 33% for macular hole, and 82% for flecks (Figure 5). Prototype examples of lesion subtypes with color fundus and dual-modality FAF images are shown in Figures 6 (page 538) and 7 (page 539).

The average calculated area of GA lesions was $4.57 \pm 2.3 \text{ mm}^2$ using the cSLO system and $3.81 \pm 1.94 \text{ mm}^2$ with the FC (Table 1, page 535). The size of the atrophic area was significantly larger with cSLO compared to FC-based imaging, ($P < .0001$, paired t -test). The ICC was 0.99 in both systems. A linear plot of GA area per lesion as imaged with cSLO versus FC devices (Figure 2) suggests that there is strong agreement between the two devices. However, the Bland-Altman plot (Figure 3, page 536) comparing the mean of the two measurements (x axis) versus the difference between the measurements using each device (y axis) and overlaid limits of agreement shows high variance and a bias (average difference) far from the zero line.

Figure 7A (page 539) shows macular retinal pigment epithelium (RPE) mottling in a patient with STGD1. Such RPE mottling was seen in four of 122 eyes (3.3%) with the FC system, yet we could not detect these lesions in the same patients via cSLO (Figure 7B–C, page 539), in which the macula appeared more uniformly hypoautofluorescent. Similar discrete areas of macular hyper-, hypo-, and isoautofluorescence were apparent in other FC-based FAF images in which cSLO images were homogenously hypoautofluorescent.

Choroidal vessel visibility beneath areas of RPE atrophy was observed in seven of 122 eyes (5.8%), only with the FC system (Figure 7E–F, page 539). Macular holes were clearly visualized in three of 122 eyes (2.5%) via cSLO, yet in only one of the same three eyes was the macular hole imaged with the FC.

Results of forced-choice image superiority between cSLO and FC FAF images for each eye revealed a preference for cSLO images in 70.8% of total cases, FC images in 25.8%, with 3% judged to be the same quality. Of 29 eyes with cataract, 20 (69%) of cSLO images and nine (31%) of FC images were judged superior. In 12 eyes with retinitis pigmentosa, six (50%) of cSLO images and four (33.3%) of FC images were judged superior (Table 2, page 535).

DISCUSSION

In this study, we sought to compare FAF images obtained using two commercially available devices to assess for agreement across various retinal pathologies. We studied FAF images obtained with a cSLO equipped with a 488-nm laser exciter as well as a digital FC with longer wavelength excitation between 530 and 580 nm.^{9,10,13} Because of differences in excitation wavelength, image acquisition, and post-acquisition processing, these two techniques do not exhibit 100% agreement. Yet due to lipofuscin's wide emission spectrum between 500 and 750 nm, a majority of image signal with both devices is attributable to the same complement of fluorophores.¹⁴

The cSLO records a series of nine single FAF images; a mean image is then calculated using signal averaging.¹ The FC acquires images with a single flash of nonconfocal light. One result of single-flash, nonconfocal imaging is that reflected or scattered light from sources such as the crystalline lens is captured at the image plane to a greater degree than with confocal imaging.^{1,10} Nonconfocality may also contribute to the higher signal in fibrotic macular lesions, which likely scatter and reflect light via their heterogeneous nature. Both of these artifacts affect image contrast and quality, as confirmed in our study by the forced-choice judgment of image superiority favoring cSLO in 70% of cases.

The potential effect of cataract on FAF image quality is addressed differently by the two devices. Nuclear sclerosis can affect FAF imaging both by exhibiting its own autofluorescence and also by the cataract's diffuse degradation of image quality.¹ The cSLO's confocal optics minimizes autofluorescence from the lens because it emanates from outside the image plane of the fundus. Overall image quality and contrast are maximized with the cSLO's multiple image acquisition and mean image calculation. The FC, while not confocal, uses long-wavelength excitation to minimize autofluorescence from the lens.

Operator focus and single-flash acquisition are the primary methods used to overcome overall image degradation from cataract.

The observed effect of cataract between cSLO- and FC-based devices has been reported with varying results. In some comparative studies, cSLO FAF imaging has been reported to have a better signal-to-noise ratio and higher contrast than the FC.¹⁰ Yet Yamamoto et al reported a higher rate of successful image acquisition with the FC than the cSLO. In their study, image degradation from cataract was less with the FC, despite nonconfocality and single image acquisition.⁸

In our study, image quality in eyes with cataract was judged superior with the cSLO in 69% of patients, essentially the same percentage as the total patient population (70.8%). In patients with retinitis pigmentosa, the percentage of FC images judged superior was slightly higher (33.3%) than the percentage of total and cataract patients (25.8% and 31%, respectively). However, because cSLO is the most commonly used imaging technique for FAF, it is likely that our observers familiar with its images may be biased toward its use. Thus, the subjective superiority of cSLO over FC may be affected by this inherent bias and should be considered.

With regards to normal subjects, FAF images exhibit differences with the cSLO and FC, largely because of differences from excitation wavelength and image acquisition and processing techniques noted above.¹ The optic disc is imaged uniformly dark in the cSLO system because of a lack of lipofuscin, but it exhibits autofluorescence signal with the FC, likely attributable to reflectance, with a possible contribution from intrinsic collagen autofluorescence.² In addition, images generated with the FC's long-wavelength excitation are less affected by macular pigments (which absorb at shorter wavelengths) than are images with the cSLO, thus leading to a smaller hypofluorescent area in the macula.^{1,7,10}

Because of decreased absorption in the macula, perifoveal RPE mottling was observed only in the FC system. Increased ability to visualize heterogeneity in foveal autofluorescence with the FC may provide an opportunity to detect progression in foveal outer retinal disease. The FC-based system was better able to image the choroidal vasculature in patients with outer retinal atrophy, either due to reflectance or perhaps intrinsic autofluorescence via long-wavelength excitation of the choroidal vasculature's collagenous/elastin wall.¹⁵

Retinal vessels are less hypoautofluorescent with the FC. This observation could be due to several reasons, including decreased hemoglobin absorbance at higher wavelengths, aforementioned signal from vessel wall collagen and elastin imaged selectively with long-wavelength excitation, or decreased contrast between hypoautofluorescent blood and isoautofluorescent retina with the FC technique.^{2,4,7} Despite differences in imaging vessels, hemorrhagic lesions found in patients with diabetic retinopathy and/or AMD had 100% pattern agreement (hypoautofluorescence) with both 488- and 530- to 580-nm excitation.

The calculated area of hypoautofluorescent GA lesions was slightly smaller in the FC than the cSLO. Schmitz-Valckenberg et al also calculated the size of atrophic lesions in patients with AMD using cSLO and FC, and our findings are in agreement with this study.⁴ We found that the difference in GA area between the two devices was evident not only in

patients with AMD but also in patients with retinal dystrophy and diabetic retinopathy. Differences in autofluorescence contrast at the junction of atrophic RPE and surrounding retina may influence where lesion borders are interpreted and thus outlined, although increased signal at this junction due to an accumulation of bisretinoids in the RPE or photoreceptors could play a role as well.¹⁶

Regardless of the cause of the smaller GA area measured with the fundus camera, the Bland-Altman plot is significant to the degree that these devices should not be used interchangeably when calculating GA lesion size. In a research setting, imaging and measuring GA lesion area across multiple patients should be performed with one device or the other. The clinical implication is that baseline and follow-up FAF imaging to discern GA progression should be done with the same methodology each time. Which device is better suited to document GA progression remains to be determined. However, given dependency on operator focus using the FC, serial image-to-image quality would appear to be more variable than the averaged images from the cSLO, implying that operator error could make serial measurements difficult using the FC.

Our data showed that alterations of fundus autofluorescence are reliably detected with both imaging systems in patients with various retinal disorders. Although our study demonstrated a qualitative difference in image quality between the two devices in the majority of cases, FAF imaging is feasible with both methods, with most lesions exhibiting a similar autofluorescence pattern when comparing cSLO and FC systems, as shown by the overall autofluorescence agreement rate of 86%. Patterns seen in GA, hemorrhage, flecks, PED, and drusen correlated fairly well between the two imaging modalities. Calculated atrophic lesion area, though slightly smaller with the FC, was of similar shape and magnitude in both systems.

From a practical standpoint, the cSLO has a relatively high purchase and maintenance cost compared with the FC. Excluding focus time, the acquisition rate is much faster with the FC (1/60 s) than the cSLO (2.5 s). The cSLO uses the same short-wavelength, high-intensity light for focusing as it does to capture the image, thus prolonging overall exposure time. Exposure to high levels of confocal short-wavelength light can potentially cause patient discomfort and untoward effects on retinal function.^{17,18} The lower cost and faster rate of acquisition with the CX-1 should be balanced against an often higher-contrast image with the HRA2, but both systems deliver images suitably clear for evaluation in the vast majority of cases.

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Figure 1.

Calculation of geographic atrophy lesion area using Matlab software (Mathworks, Natick, MA). FAF images acquired using (A) confocal scanning laser ophthalmoscope (cSLO) and (C) fundus camera. Lesion borders were manually delineated. Atrophic lesion area in the same patient as calculated using (B) cSLO and (D) fundus camera images.

Figure 2. Geographic atrophy lesion size (mm^2) for confocal scanning laser ophthalmoscope versus fundus camera.

Figure 3. Bland-Altman plot of geographic atrophy lesion area (mm^2), measured with confocal scanning laser ophthalmoscope (cSLO) and fundus camera (FC) techniques, with the average lesion size for the two techniques on the x axis and the difference between cSLO and FC measurements for each lesion on the y axis.

Figure 4.

Agreement of fundus autofluorescence (FAF) pattern between confocal scanning laser ophthalmoscope and fundus camera-based images of the same eye. FAF images were the same pattern in 86% of cases, with disagreement in 9% and inability to compare in 5%.

Figure 5. Fundus autofluorescence pattern agreement rates with respect to lesion subtypes: hemorrhage, 100%; geographic atrophy, 96.5%; flecks, 81.8%; drusen, 75%; exudates, 70%; pigment epithelial detachment, 66.7%; fibrous lesions, 50%; and macular hole, 33.5%

Figure 6.

Imaging in age-related macular degeneration (AMD). (A–C) Color fundus, fundus camera (FC) fundus autofluorescence (FAF), and confocal scanning laser ophthalmoscope (cSLO) FAF images of drusen in nonexudative AMD. (D–F) Fundus color, FC FAF, and cSLO FAF images of a fibrovascular lesion in exudative AMD. The foveal aspect of the fibrous tissue lesion shows hyper and hypoautofluorescence with the FC (E) and hypoautofluorescence with the cSLO (F). In the peripheral aspect of the lesion, the fibrous tissue exhibits hyper- and hypoautofluorescence with both the FC and cSLO techniques (E–F).

Figure 7.

Fundus images in a patient with STGD1 (A–C) and one with retinitis pigmentosa (D–F). (A, D) Color fundus images. (B, E) Fundus camera (FC) fundus autofluorescence (FAF). (C, F) Confocal scanning laser ophthalmoscope (cSLO) FAF. Both systems detect hypoautofluorescence indicative of atrophic regions and flecks (B–C). Foveal RPE mottling is imaged as a speckled pattern of isoautofluorescence and hypoautofluorescence with the FC, with uniform hypoautofluorescence with the cSLO (B–C, red circles). Choroidal vessels are visible in the FC but not cSLO FAF images (E–F, white arrows).

TABLE 1

Average Geographic Atrophy Lesion Area (mm²) Calculated by Each Observer With Confocal Scanning Laser Ophthalmoscope and Fundus Camera Images

	cSLO		FC		P Value [*]
	Observer 1	Observer 2	Observer 1	Observer 2	
Mean (± SD)	4.56 ± 2.53	4.59 ± 2.3	3.82 ± 1.94	3.8 ± 1.94	
Combined mean (± SD)	4.57 ± 2.3		3.81 ± 1.94		< .001
ICC	0.99		0.99		

^{*} Paired t-test.

cSLO = confocal scanning laser ophthalmoscope; FC = fundus camera; ICC = interclass correlation coefficient.

TABLE 2

Forced-Choice Query of Image Superiority for Confocal Scanning Laser Ophthalmoscope and Fundus Camera

	cSLO	FC	Equal Quality
All eyes	70.8%	25.8%	3.3%
Cataract	69%	31%	0%
RP	50%	33.3%	16.7%

cSLO = confocal scanning laser ophthalmoscope; FC = fundus camera; RP = retinitis pigmentosa.