Panretinal, High-Resolution Color Photography of the Mouse Fundus

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Purpose. To analyze high-resolution color photographs of the mouse fundus.

Methods. A contact fundus camera based on topical endoscopy fundus imaging (TEFI) was built. Fundus photographs of C57 and Balb/c mice obtained by TEFI were qualitatively analyzed.

Results. High-resolution digital imaging of the fundus, including the ciliary body, was routinely obtained. The reflectance and contrast of retinal vessels varied significantly with the amount of incident and reflected light and, thus, with the degree of fundus pigmentation. The combination of chromatic and spherical aberration favored blue light imaging, in term of both field and contrast.

Conclusions. TEFI is a small, low-cost system that allows high-resolution color fundus imaging and fluorescein angiography in conscious mice. Panretinal imaging is facilitated by the presence of the large rounded lens. TEFI significantly improves the quality of in vivo photography of retina and ciliary process of mice. Resolution is, however, affected by chromatic aberration, and should be improved by monochromatic imaging. (*Invest Ophthalmol Vis Sci.* 2007;48:2769–2774) DOI: 10.1167/iovs.06-1099

Imaging the fundus in vivo in experimental models of retinal diseases is of great interest for screening purposes, for characterization of the natural history of retinal diseases, or for monitoring therapeutic effects. Fundus imaging is indeed less time-consuming than histologic examination and is potentially more precise for defining the extent of a retinal lesion. It is likely that funduscopy in mice will gain importance in the near future because of the increasing use of mice as experimental models of eye diseases. An additional interest of funduscopy is the possibility of enhancing the contrast of specific structures by monochromatic filtering, which is related to the presence of absorbers and reflectors—that is, to the retinal and choroidal pigments (of which melanin and hemoglobin are the most important) and to the reflection of light on structures such as the vitreoretinal interface and the nerve fiber layer.[1] Moreover, analysis of vessel reflectance allows evaluation of metabolic parameters such as the arteriovenous saturation difference.[2]

Yet fundus photography remains of limited use in rodents, in part because of the relatively poor resolution of current techniques[3,4] and of their technical difficulties. Contrast of fundus images in mice is often affected by poor contrast from corneal reflections. Moreover, the peripheral retina cannot be documented. High-resolution fundus imaging with viewing of the extreme periphery can be obtained with the scanning laser ophthalmoscope (SLO),[5,6] but it is an expensive technique and allows only monochromatic imaging. Moreover, SLO fundus imaging in albino mice is affected by overexposure, and confocal viewing makes it impractical to image tridimensional structures such as the optic nerve or the ciliary process.

Counteracting corneal refraction by optical applanation allows observation of the fundus of virtually any eye, including the mouse eye, but with a narrow field of view.[7,8] It has been observed that the placement of an illuminating endoscope on the cornea allows wide-field fundus imaging (Paques M, unpublished observation, 1999) given that the diameter of the endoscope was equal or inferior to the diameter of the pupil. In this study, we determined the technical specifications needed to optimize fundus examination by topical endoscopy in the mouse eye and analyzed the resultant images.

Materials and Methods

The principle of topical endoscopy fundus imaging (TEFI) is based on an endoscope with parallel illumination and observation channels being connected to a photographic camera or to a video camera. As a rule, whatever the type of illuminating endoscope, a fundus image can be obtained by merely placing an endoscope on the cornea, as long as the outer diameter does not exceed the diameter of the dilated pupil (i.e., 3–4 mm in the mouse). To optimize the resultant image, we evaluated several combinations of endoscopes and digital cameras. Most endoscopes have a circle-shaped illumination at their tip. Theoretically, this disposition is sufficient to ensure accurate viewing of the fundus. However, we found that the best contrast was obtained with endoscopes that had lateral, crescent-shaped illumination (as shown in Fig. 1C), probably because this disposition minimizes axial reflections. Only digital cameras with manual focus gave satisfactory results because most autofocus systems cannot work with an endoscopic image. To obtain the largest field possible, it was confirmed that the field of view of the endoscope and of the camera were congruent.

We used an endoscope with a 5-cm long otoscope with a 3-mm outer diameter (1218AA; Karl Storz, Tuttlingen, Germany) with step index lenses and an angle of view of 0°, a field of view of 80° in air, and a crescent-shaped illuminating tip (Fig. 1). A reflex digital camera with a 6.1-million pixel charge-coupled device (CCD) image sensor (D50 with Nikkor AF 85 /F1.8 D objective; Nikon, Tokyo, Japan) was connected to the endoscope through an adapter containing a +5 lens

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Supported by the Fondation de l'Avenir (Grant RMERJ1009), the INSERM, the University Pierre and Marie Curie-Paris 6, the Fondation Ophthalmologique Adolphe de Rothschild (Paris), the Fédération des Aveugles de France, and the European Economic Community (Grant EVI-GENORET-512036). The endoscope was paid for jointly by Association Sportive de Vialas (Aveyron, France) and Caisse d'Assurance Maladie des Professions Libérales.

Submitted for publication September 14, 2006; revised December 14, 2006; accepted April 10, 2007.

Disclosure: **M. Paques**, None; **J.-L. Guyomard**, None; **M. Simonutti**, None; **M.J. Roux**, None; **S. Picaud**, None; **J.-F. LeGargasson**, None; **J.-A. Sahel**, None

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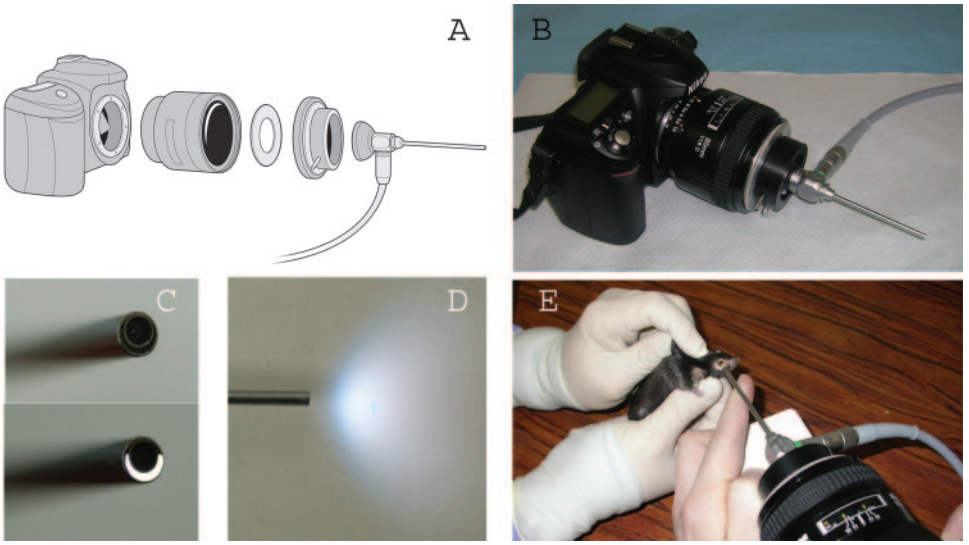

FIGURE 1. (**A**, **B**) Scheme and general view of the TEFI system. (**C**) Tip of the endoscope showing the crescent-shaped illumination. (**D**) Dispersion of light at the tip. Note the chromatic aberration revealed by the *blue-green* halo. (**E**) TEFI procedure.

(approximate value; the distance from the tip to the cornea appeared to have more influence on focus than did the lens power). The preferred settings of the camera are as follows: image format, raw; focus, manual; operating mode, A (priority to opening); diaphragm, 1/1.8; white balance, automatic.

The light source was a xenon lamp (reference 201315-20; Karl Storz) connected through a flexible optic fiber to the endoscope. Light dispersion at 3 mm of the tip revealed a chromatic aberration (i.e., a higher dispersion of short wavelengths; Fig. 1D).

Adult mice (C57/BL6 and Balb/c) were provided by Janvier (Saint-Ile le Genest, France) or Charles Rivers (L'Arbresle, France). All experiments were performed in accordance with the ARVO Statement for the Care and Use of Animals in Ophthalmic and Vision Research. All procedures were performed in conscious mice. The pupil was dilated with topical 1% tropicamide and 1% atropine sulfate. Topical oxybropucaine was applied for corneal anesthesia (all eyedrops were from Novartis Ophthalmics, Rueil Malmaison, France).

The imaging procedure was as follows (Fig. 1E): the camera was placed on a platform, and the endoscope was slowly moved toward the mouse. Once contact with the gel covering the cornea was obtained, the photographer adjusted the position of the endoscope by horizontally displacing the tip. Focus and illumination were adjusted during examination of the fundus through the camera. Bursts of four images were repeatedly captured. Images were then transferred to a computer, and, if necessary, contrast and luminosity were adjusted. As a rule, each pixel of a CCD codes for three color channels (RGB mode)—red (peak, approximately 610 nm), green (peak, 550 nm), and blue (peak, 470nm)—with a certain degree of overlap between channels. There are two green pixels per blue or red pixel. From the trichromatic RGB images, monochromatic gray-level images, reflecting luminance in the red, green, or blue channels,

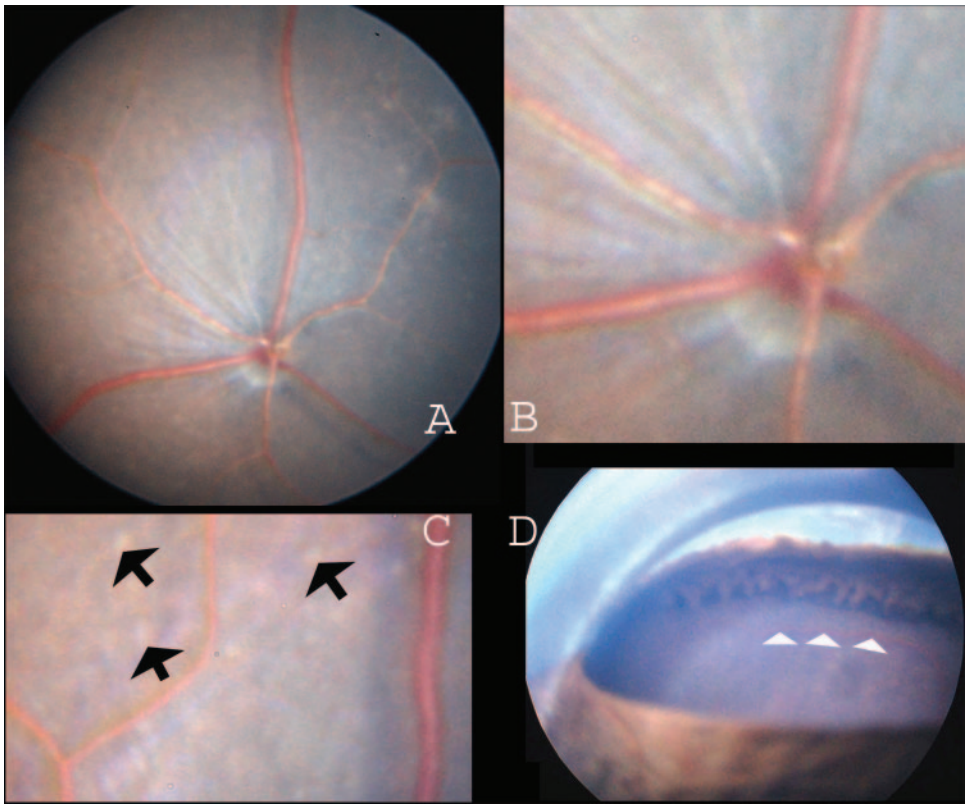

FIGURE 2. TEFI in a C57 mouse. (**A**) Posterior pole. The scale is suggested by the optic nerve, which is approximately 220 μm in diameter.[5] (**B**) Magnification of optic nerve and vessels in (**A**). Note the irregularity of the light reflex of the arteriole, the clear delineation of the contour of the optic nerve, and the nerve fiber layer. (**C**) Magnification of drusenlike dots (*arrows*) at the level of the RPE. (**D**) Peripheral retina and ciliary process. Note the presence of the peripheral longitudinal vein (*arrowheads*).

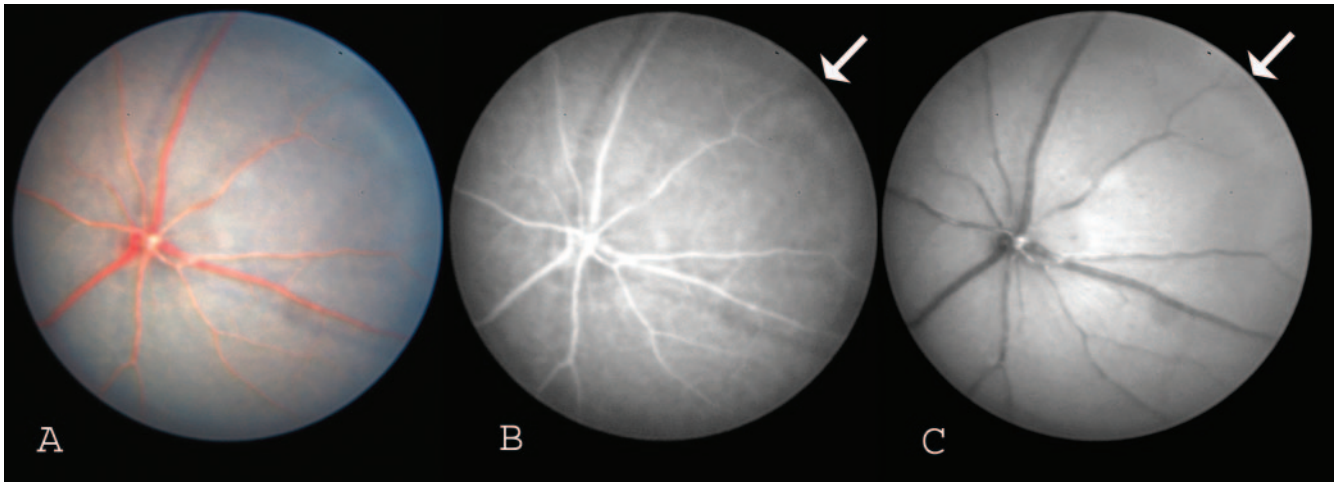

FIGURE 3. Illustration of optical aberration on TEFI. (**A**) Color TEFI. (**B**, **C**) *Red* and *blue* monochromatic images, respectively. Note the larger field of view in blue light, illustrated by the visibility of peripheral vessels (*arrows*).

were extracted from the raw images (Adobe Photoshop; Adobe Corporation, Mountain View, CA). For fluorescein angiography, 50 μL of 10% sodium fluorescein (Novartis Ophthalmics) was injected intraperitoneally, and appropriate excitation and barrier filters (provided by the manufacturer) were placed on the light path.

RESULTS

Examples of fundus images are shown in Figure 2. Given that it covered 31.1% of the field of the camera, the estimated definition of a fundus image was 1.9 million pixels. Retinal vessels created shadows on the retinal pigment epithelium (RPE), as a result of the lateral disposition of the illumination channel. Magnification of Figure 2A (Fig. 2B) shows the blue-green reflex of the nerve fiber layer and the yellowish central reflex over arterioles and venules. Over arterioles, this reflex was discontinuous. At the level of the RPE, small drusenlike dots were present in most mice, especially in older ones (Fig. 2C). Changing the orientation of the endoscope permitted image capture of the entire retina on composite imaging. For instance, placing the endoscope perpendicularly to the limbus allowed imaging of the peripheral retina and the ciliary process (Fig. 2D).

Comparison of blue, green, and red monochromatic images is shown in Figures 3 and 4. Chromatic or spherical aberration was suggested by the larger field of view of shorter wavelengths; blue light images had a larger field than red light images (Fig. 3). This matched the stronger dispersion of blue light at the tip (Fig. 1C).

Figure 4 addresses to the reflectance of specific fundus structures. Red monochromatic imaging enhanced the contrast of pigment alterations and the axial reflex from vessels. Green filtering enhanced the contrast of veins, whereas arteries remained relatively isoreflective to the surrounding background. The blue channel provided the best contrast for arteries and veins. Reflectance of the nerve fiber layer was best evidenced in blue or green monochromatic images.

This pattern of absorbance and reflectance was compared to that of a fundus photograph from a human subject (Fig. 5). To compensate for differences in fundus pigmentation in illumination intensity and in vessel size, several fundus images of a human subject were acquired with a conventional fundus camera (50 IA; Topcon, Tokyo, Japan) at different flash intensities, and magnified to visualize small retinal vessels (magnitude, 40–60 μm). This showed that, while in low-flash intensity, the best contrast of arterioles was obtained in green light increasing the flash intensity increased the axial reflection from arterioles and the amount of light backscattered from the choroid/RPE, thus altering the contrast of arterioles.

For fundus imaging in albino mice (Fig. 6), decreasing incident light intensity and duration of exposure was necessary. In addition, avoiding pressure on the globe was critical because even slight compression decreased choroidal perfusion, thus altering the fundus aspect. Indeed, in albino fundi, the choroidal vasculature and the reflected light from the sclera accounted for most of the image and overwhelmed light reflection from the nerve fiber layer. Retinal vessels did not have a yellowish reflex. Green and blue filtering increased the contrast of choroidal vessels (Fig. 6A). Vessels appeared slightly larger in blue than in green light (Fig. 6C), probably because of the combination of the reflected light from the sclera, which blurred the vessel borders, and of chromatic aberration. The latter was suggested by the yellowish smear at vessel borders (Fig. 6C, arrowhead).

The peripheral retina and the ciliary process of an albino mouse are shown in Figure 7. The overall organization of the ciliary body venous drainage into the vortex veins was discernible. At high magnification, the venous drainage of individual ciliary processes was clearly visible (Fig. 7B).

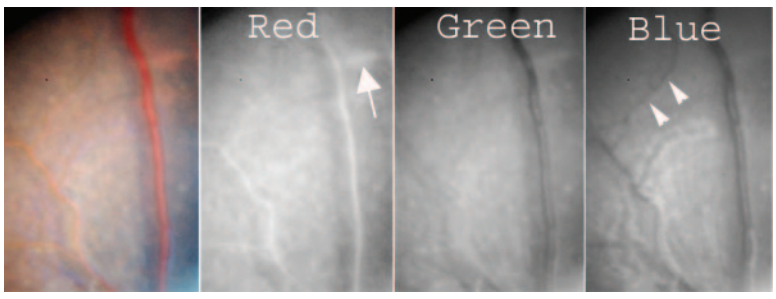

FIGURE 4. Reflectance of retinal vessels of pigmented mice. In the red light channel image, note the axial reflex over arterioles and venules and the visualization of a pigment defect (*arrow*). The best contrast of retinal vessels over the choroid was obtained in blue light (*arrowheads*).

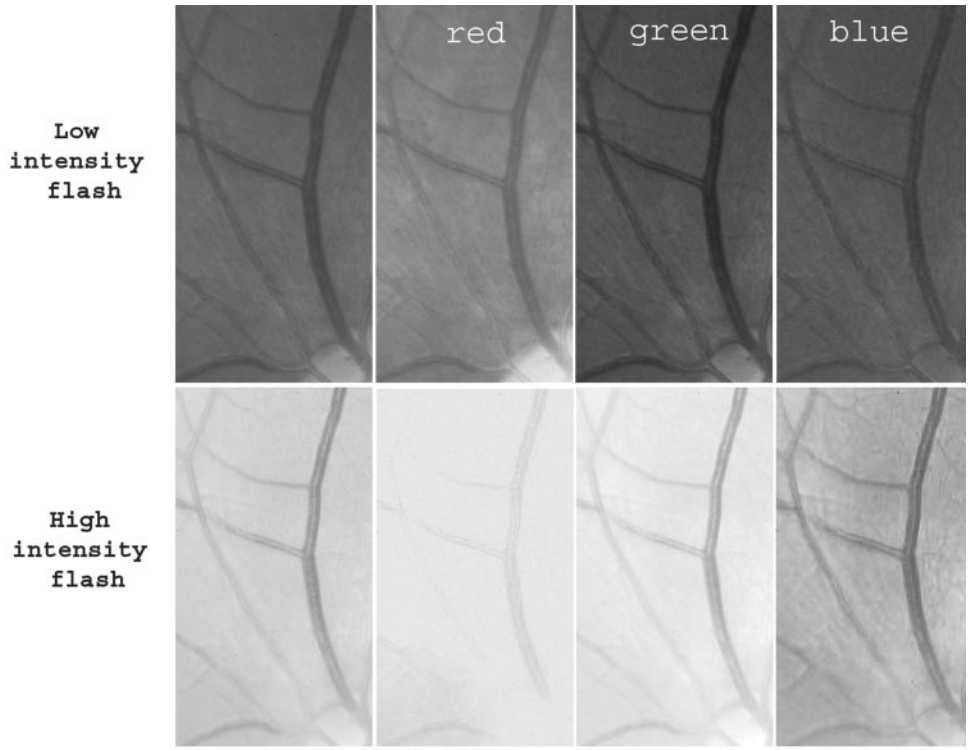

FIGURE 5. Illustration of the variation of contrast of human retinal vessels with increased intensity of incident light. Conventional color fundus photography in a pigmented subject has been taken at normal flash intensity (*top*) and with high-intensity flash (*bottom*). The contrast of arterioles over the choroid/RPE is higher in green light with the low intensity flash, but is better in blue light with the high intensity flash. Note also that the axial reflex of veins is faintly visible with a low-intensity flash but is clearly visible with a high-intensity flash.

Figure 8 shows a typical fluorescein angiogram obtained by TEFI after the interposition of excitation and barrier filters.

DISCUSSION

We report here that TEFI allows high-resolution, wide-field digital fundus imaging in the mouse eye. Additionally, the TEFI system is compact and relatively inexpensive. Compared with current fundus photographic techniques, specific advantages of TEFI are the high contrast resulting from the absence of corneal reflections and from lateral illumination, the possibility of imaging the extreme periphery, and the short training needed to perform the examination. Additionally, TEFI allows in vivo imaging of the ciliary process in mice, which to our knowledge has not yet been reported.

This technique provided us with the opportunity to analyze the reflectance of fundus structures of mice. In C57 mice, there was a strong axial reflex on vessels and a contrast pattern for

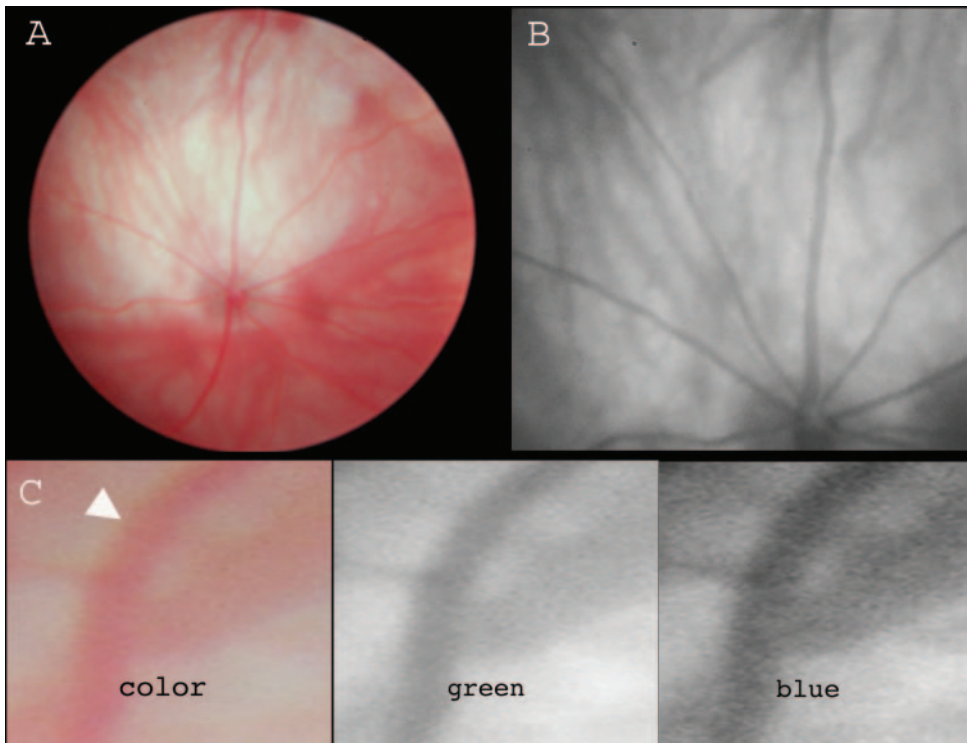

FIGURE 6. TEFI of a normal albino mice. (**A**) Posterior pole. (**B**) Blue light filtering of (**A**). (**C**) Magnification of an arteriole in (**A**), illustrating the chromatic aberration (images have been horizontally stretched). Note the *yellowish* smear at one border of the vessel (*arrowhead*) and the focus mismatch between green and blue channel images.

vessels that made them more visible in blue light. This is different from what is noted in human fundus photographs, in which retinal vessels have better contrast in green light.[9] This difference was probably the result of the high incident light intensity necessary for TEFI, which induced an increased reflex of arterioles and a higher reflection from the choroid/RPE, thus a loss of contrast of arterioles. Indeed, such shifting toward a better contrast in blue light was reproduced in a human fundus when high flash intensity was used. Few data have been published on the mechanism underlying the axial reflection of vessels; therefore, it remains to be determined at which level of the vessel this reflection occurs. It is likely that because most reflected light is shifted toward red, the red blood cells themselves reflect incident light. The implications, if any, of other elements, such as the plasma or the vessel adventitia, remains to be determined. The reason why axial reflexion is more important in arterioles than in venules is uncertain. It may be related to the peak sensitivity of the green CCD channel at 550 nm, which is the wavelength at which the reflectance of oxyhemoglobin is higher than that of carbhemoglobin.[1]

TEFI images were affected by chromatic and spherical aberrations that resulted, for instance, in a larger field of view in blue light and a yellowish smear along vessels. Two potential sources of chromatic aberration in TEFI images were the light dispersion at the tip of the endoscope and the ocular media. The mouse eye is indeed known to have a high chromatic aberration[10]; the refractive error in red light has been estimated to be 5 to 20 D superior to the refractive error in blue light. In a previous study, we also found that chromatic aberration affected fundus imaging by cSLO.[5] Our TEFI setup favored blue light imaging in terms of contrast and field of view. However, the blue pixels of a CCD array account for only 25% of pixels. Thus, fundus imaging with monochromatic incident light and monochromatic CCD (without Bayer filter) to overcome chromatic aberration and obtaining high-definition fluorescein angiograms may be of interest.

A specific interest of TEFI is the possibility of imaging the ciliary body. Several anatomic factors may contribute to the viewing of the peripheral retina, among them the large angle between the peripheral retina and the iris (i.e., the narrow iridocorneal angle) due to the presence of the lens; the posterior position of the ciliary process compared with the human eye; and the direct contact between the lens and the ciliary process. In addition, the rounded lens of the mouse induces fewer peripheral aberrations than the flat-oval lens of humans. Moreover, spherical aberration is in fact an advantage for peripheral imaging because it tends to compensate for the curvature of the ciliary body, thus increasing the field of view. Accordingly, in our experience, ciliary process imaging with TEFI can only be performed in eyes with large, rounded lenses, such as rodent eyes.

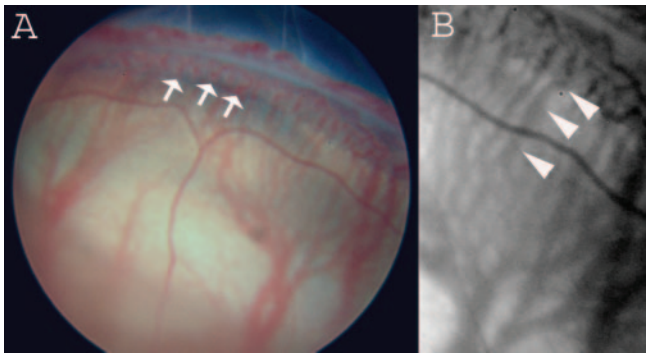

FIGURE 7. Peripheral retina of an albino mouse. (**A**) Color photography (*arrows*; ciliary process). (**B**) Monochromatic imaging. Note the venous drainage of ciliary process into choroidal veins (*arrowheads*).

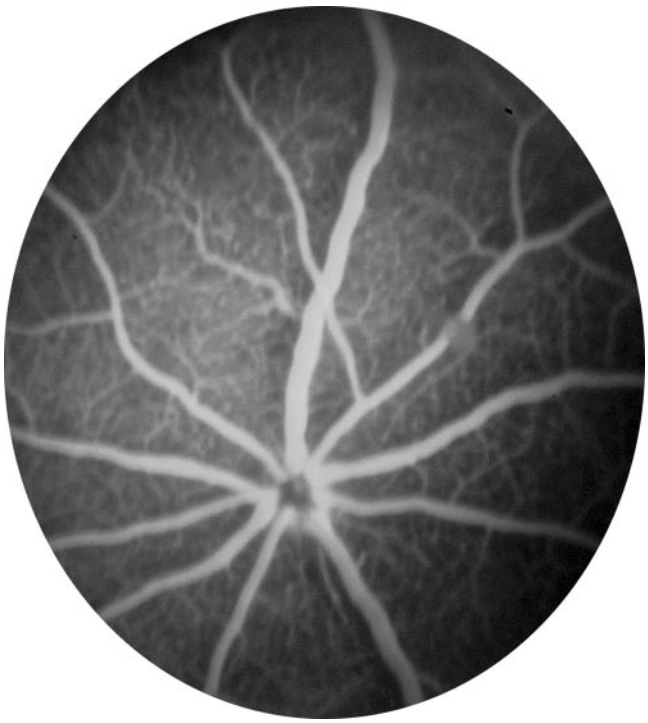

FIGURE 8. Fluorescein angiogram obtained with TEFI in a pigmented mouse.

In conclusion, high-resolution fundus imaging in conscious mice can be performed by TEFI, a compact, low-cost system. TEFI thus represents significant progress in retinal imaging in the mouse and may contribute to a better analysis of retinal and uveal physiology and pathology. TEFI may indeed be of interest for the noninvasive evaluation and follow-up of various experimental models of eye disease. Metabolic parameters such as arteriovenous saturation can theoretically be measured. TEFI may be useful for determining the progression of diseases affecting the RPE, such as retinal and choroidal dystrophies, especially if they affect the peripheral retina as in vigabatrin-induced photoreceptor toxicity.[11] Additionally, ciliary process imaging may be useful for observing the breakdown of the anterior blood–ocular barrier after experimental uveitis, monitoring pharmacologic effects on ciliary process vascularization,[12] or evaluating cyclodestructive surgery. Choroidal vascular diseases may be observed in albino animals with the use of blue light imaging.

Technical modifications are likely to increase the resolution of TEFI fundus images. For instance, resolution may be improved by decreasing chromatic aberration. Endoscopes with graded index of refraction (GRIN) lenses have larger depth of focus and reduced chromatic aberration, which should increase the resolution of fundus images; however, this was not confirmed by a recent study.[13] Correction of the light source chromatic aberration should also improve the quality of the images. Making use of monochromatic CCD sensors is another way of suppressing chromatic aberration and may be considered in specific applications, such as vascular or nerve fiber layer imaging.

Acknowledgments

The authors thank Serge Rosolen, Olivier Goureau, Vincent Borderie, and the staff at the Institut Mutualiste Montsouris Recherche (Paris, France) for technical help.

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