Original Article

High-resolution hyperspectral imaging of the retina with a modified fundus camera

Imagerie hyperspectrale haute résolution de la rétine avec une caméra de fond de l’œil modifiée


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Received 29 May 2010; accepted 28 September 2010
Available online 19 November 2010

Keywords
Hyperspectral;
Multispectral;
Retinal imaging

Summary
Purpose. — To examine the practical feasibility of developing a hyperspectral camera from a Zeiss fundus camera and to illustrate its use in imaging diabetic retinopathy and glaucoma patients.
Methods. — The original light source of the camera was replaced with an external lamp filtered by a fast tunable liquid-crystal filter. The filtered light was then brought into the camera through an optical fiber. The original film camera was replaced by a digital camera. Images were obtained in normals and patients (primary open angle glaucoma, diabetic retinopathy) recruited at the Manchester Royal Eye Hospital.
Results. — A series of eight images were captured across 495- to 720-nm wavelengths, and recording time was less than 1.6 s. The light level at the cornea was below the ANSI limits, and patients judged the measurement to be very comfortable. Images were of high quality and were used to generate a pixel-to-pixel oxygenation map of the optic nerve head. Frame alignment is necessary for frame-to-frame comparison but can be achieved through simple methods.
Conclusions. — We have developed a hyperspectral camera with high spatial and spectral resolution across the whole visible spectrum that can be adapted from a standard fundus camera. The hyperspectral technique allows wavelength-specific visualization of retinal lesions that may be

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doi:10.1016/j.jfo.2010.10.010
Introduction

Retinal fundus imaging has always held a major role in the detection and management of ophthalmic disease. A wide range of fundus imaging techniques hence exist including fundoscopy, confocal imaging and optical coherence tomography. Most of these techniques aim to obtain an image with a very high spatial resolution but usually disregard the spectral information. Some techniques, e.g. fluorescein angiography, use spectral information to isolate specific features but are usually limited to a couple of wavelengths.

Recent developments in optical technology (more sensitive cameras and high speed filters) have made it possible to capture multi-spectral information together with high spatial resolution. This progress opens the door to new studies aimed at increasing our understanding of physiological spatial resolution. This progress opens the door to new studies aimed at increasing our understanding of physiological spatial resolution.

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While several prototype multi-spectral cameras have been reported within the research literature, a majority were research prototypes without the ease of use of standard clinical instruments and difficult to reproduce without technical expertise. Such systems usually separate the spatial and spectral information through different optical path via the use of either scanning motors [11], scanning lasers [5], prisms [12–13], diffraction grating [14–15], a lenslet array [16], or interference filters [17].

Harvey et al. [18] and Hiroara et al. [19] presented an attractive technique based on the use of a liquid crystal wavelength tunable filter inserted within the illumination path of a fundus camera. This method gives increased versatility and flexibility (the wavelengths used and associated exposure times can be adjusted independently), which is well suited for clinical studies. It retains the advantages of a standard fundus camera, i.e. large field of view, simplicity, ease of use and high resolution (snapshot spectral imaging systems have reduced resolution the sensor being divided by the number of spectral images simultaneously recorded). The drawback is the sequential nature of the method. Spectral images are recorded successively, which can potentially lead to long recording time and an increased chance of eye movements artefacts.

In this context, our aim was to assess this technique on a series of eyes with diabetic retinopathy and glaucoma.

Method

Patients

The research followed the tenets of the Declaration of Helsinki. All patients gave their informed consent after receiving an explanation of the nature and possible consequences of the study. The project protocol was approved by the local NHS Research Ethics Committee.

The normal subject, glaucoma patients and diabetics enrolled in the study had no other ophthalmic disease except...
primary open angle glaucoma/diabetic retinopathy (preproliferative or proliferative retinopathy); refractive error was less than $\pm 5.00$ DS equivalent and/or 2.00 DC. Both glaucoma and diabetics patients were recruited from the clinics of Manchester Royal Eye Hospital.

**Modifications of the camera**

We modified a Zeiss Fundus Flash 3 (West Germany) replacing the original light source by an external lamp filtered by a fast tunable liquid-crystal filter. The filtered light is then brought into the camera through an optical fibre. This is in contrast with the set-up of Harvey et al. [18] and Hiroara et al. [19] who maintained the use of the original illumination system. Our approach increases the flexibility of the system by allowing a more powerful lamp or a custom made light source to be used.

A schematic diagram of the original Zeiss camera is represented in Fig. 1 (additional details are available from [20]). Light from the Xenon flash source is collected by the condenser L1 and directed through a series of lenses and mirrors to the eye of the patient. According to Gullstrand’s principle, the mirror M2 is annular so that the incoming and viewing paths are separated. In this configuration, the mirror is conjugated with the plane of the pupil. Once the light is reflected by the retina, it goes through the centre of the mirror and forms an image on the photographic film. To monitor the image in real time, an auxiliary viewing system (M3, M4, L7) with its own light source (S1) is available.

The schematic diagram of the modified camera is represented in Fig. 2. In order to design an approach adaptable to other systems, and to benefit from the excellent design and ease of use of the camera, we tried to minimise the number of modifications. The original film camera was replaced with a low-noise Peltier-cooled digital camera (Orca C4742-80-12AG, Hamamatsu Photonics, Hamamatsu, Japan) with a spatial resolution of $336 \times 256$ pixels (with $4 \times 4$ binning). The use of the CCD camera enables us to perform the observation (e.g. selecting the area of interest and focusing) and acquisition with the same light source so the auxiliary viewing system was removed.

The external light source is depicted in Fig. 3. It consists in a white light source filtered by a liquid crystal tunable filter (F) (7 nm half width at half maximum, Varispec VIS 07-20 STD, Cambridge Research Instrumentation, UK). We initially used a Xenon lamp (150 W, Woltan), to which we added a heat filter (Calflex CTM, Linos) to protect the Varispec filter, and a collimating optics (condensing lens + telescopic beam expander) to optimize the transmission through the filter. We eventually replaced this system with a 250 W slide lamp projector (halogen) where we made use of the in built heat filter and projecting lens. The advantage of the second system is its reduced cost and lower emission profile in the UV. An aspheric condenser lens (L2)(F$\#0.85$, Linos) injects the light transmitted by the Varispec filter into an optical fibre (liquid light guide, Lot-Oriel, core diameter of 5 mm, NA = 0.59). The end of this fibre is positioned in place of the Xenon lamp within the camera (Fig. 2).

**Results**

Modifying the cameras was straightforward due to its relatively simple design. The fibre was inserted through a hole drilled in the casing of the camera and a three-axis translation stage was fixed in place of the Xenon bulb (Fig. 4) to control the positioning of the fibre. This positioning is deli-
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Figure 4. Left image: photo of the collimating optics in use with the Xenon lamp, filter, coupling optics and liquid light guide. Central image: the modified Zeiss camera. Right image: a three-axis translation stage is used to control the positioning of the optical fibre.

Table 1  Corneal irradiance using the halogen lamp.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>496</th>
<th>550</th>
<th>570</th>
<th>575</th>
<th>580</th>
<th>586</th>
<th>610</th>
<th>700</th>
<th>720</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiance (µW/cm²)</td>
<td>15.5</td>
<td>45.5</td>
<td>57.9</td>
<td>60.3</td>
<td>62.7</td>
<td>65.6</td>
<td>74.3</td>
<td>75.7</td>
<td>72.3</td>
</tr>
</tbody>
</table>

cate but once the optimum position has been found, the fibre can be fixed permanently. The modified camera is depicted in Fig. 4.

With this configuration, the light in the plane of the pupil is below the maximum permissible exposure (MPE) set by the ANSI limits [21] (Table 1) and considered very comfortable by patients, thus increasing the chance of recording good quality images (i.e. less blinking and eye movements). Using 4× binning and a gain set to 50, we can record seven images at 496, 550, 570, 575, 580, 586, 610, 720 nm in less than 1.6 s (i.e. an acquisition time similar to other instruments such as the Heidelberg HRT). Exposure times below 460 nm are comparatively long as the transmission coefficient of the filter decreases. Shorter wavelengths are, however, of limited clinical use since, in most subjects, scattering and possibly lens absorption would strongly degrade the image quality.

Although the focusing of the image was achieved when illuminating the retina at midrange of our spectral interval, images obtained at the extreme wavelength were not significantly degraded by chromatic aberrations. This is in agreement with our tests in an artificial eye and can be explained by the limited spectral interval we considered (≈220 nm) and the inevitable image degradation associated with scattering at very short wavelengths or in the deep red.

Figure 5. Images recorded with (a) white light (with a scanning laser ophthalmoscope [Optos PLC, Dunfermline, Fife, Scotland, UK]) and at (b) 496 nm, (c) 586 nm and (d) 610 nm in a patient with diabetic retinopathy. The image (a) was cropped to correspond to the same field of view as illustrated in images (b-d).
The sensor of the CCD being approximately four times smaller than the original photographic film, the field of view of the modified camera is approximately $15^\circ$ large.

Due to its original design (Zeiss camera), the system is easy to use in clinics and we recorded high quality images in 28 normals and patients, examples of which are given in Figs. 5–7. These figures also allow to illustrate the influence of the main absorbers on the recorded images and are in agreement with results previously reported [21,22].

Fig. 5 represents series of four images recorded with white light (Optos scanning laser ophthalmoscope) and at 496, 586 and 610 nm in a patient with diabetic retinopathy. In the image recorded at 496 nm, the retinal nerve fibre layer is visible as well-demarcated striations. The striations become less visible at 586 nm and completely absent for higher wavelengths as the contribution from the nerve fibre layer decrease and the one from deeper layers increase. Abnormal blood vessels, aneurysms and haemorrhages (arrows) are best appreciated around 580 nm where haemoglobin absorption is high and superficial layers relatively transparent. This relationship reverses for longer wavelengths and the image at 610 nm is dramatically different. There is little reflection from retinal layers, blood absorption is low and hence the choroidal vasculature can be visualized in positive contrast.

Fig. 6 (series of three images recorded at 496, 575 and 610 nm in a glaucoma patient) also illustrates that the appearance of the optic nerve head varies with the wavelength used, becoming paler at long wavelengths where the interaction with the nerve fibres is minimum. The cup is observed with maximum contrast around 575 nm.

Fig. 7 represents the macula of a diabetic patient. Microaneurysms are visible at 550 and 570 nm as well as a vitreoretinal adhesion at the level of the internal limiting membrane (confirmed by OCT). At 610 nm and 650 nm, these structures are not visible but choroidal vessels can be visualized and there are bright signals arising presumably from melanin within the choroidal layer. This may provide non-invasive imaging in subretinal disorders such as poypoidal choroidal vasculopathy. This condition is associated with nodular hyperfluorescence from polyps, and spectral imaging may allow diagnostic visualization of branching vascular networks and nodularity of polyp lesions. In diabetic macular disease, the diagnostic intraretinal features may be

![Figure 6. Images recorded at (a) 496 nm, (b) 575 nm and (c) 610 nm in a glaucoma patient.](image)

![Figure 7. Images recorded at (a) 550 nm, (b) 570 nm, (c) 610 nm and (d) 650 nm in a patient with diabetic retinopathy.](image)
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Discussion

This paper gives information on how to convert a standard fundus camera into a hyperspectral camera with off the shelf elements (CCD camera, liquid crystal filter, optical fibre and slide lamp projector). The system is versatile (i.e. can be used to image at any wavelength), it retains the ease of use of the original clinical instrument and provides important spectral information while retaining high spatial resolution. Technically, its main limitation is the low transmission of the filter (20% max for unpolarized light below 650 nm), which limits imaging below 460 nm.

Clinically, it allows imaging with optimum contrast the structures of interest (e.g. blood vessels in diabetic retinopathy) hence possibly facilitating the detection (automated or not) of pathological structures. The technique may prove particularly useful in developing an automatic screening system for diabetic retinopathy. Automatic detection of diabetic retinopathy features (e.g. micro-aneurysms, new tortuous blood vessels) in colour fundus images is a difficult problem. As most segmentation algorithms use thresholding and region growing techniques, the higher contrast offered by images recorded around 580 nm (Fig. 5) may provide important benefits.

The system may also aid in the diagnosis and management of several diseases by providing information on the oxygenation of retinal tissues. In a pilot study we conducted, oxygenation maps corresponded well to structural measures (HRT3 topography) [9] and we have now initiated a study aiming at assessing the relation between oxygenation defects at the level of the optic disc and field loss in glaucoma patients.

In addition, the technique presents also a strong potential for detecting ischemia. Fluorescein angiography is currently a standard method to detect ischaemic regions but is invasive, unpleasant for the patient, time consuming and may trigger adverse reactions. Our method may provide a low-cost, rapid and non-invasive alternative to fluorescein angiography, which would be highly beneficial in the management and early detection of retinal diseases.

Detection of pathological structures and oxygenation mapping are only two usage of a vast amount of information relatively untapped. The real potential of this system is in the associated software. Due to the digital natures of the images, sophisticated analysis can be carried out to explore the spectral information and analyse the role of different absorbers (e.g. oxygenation, macular pigment) in relation to various pathologies.

Support

This study was supported by Manchester Academic Health Sciences Centre and NIHR Manchester Biomedical Research Centre.

Conflicts of interest statement

The authors do not have any conflict of interest related to this manuscript.

References


