Multispectral Imaging of the Ocular Fundus using LED Illumination

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ABSTRACT

We present preliminary data from an imaging system based on LED illumination for obtaining sequential multispectral optical images of the human ocular fundus. The system is capable of acquiring images at speeds of up to 20fps and we have demonstrated that the system is fast enough to allow images to be acquired with minimal inter-frame movement. Further improvements have been identified that will improve both imaging speed and image quality. The long-term goal is to use the system in conjunction with novel image analysis algorithms to extract chromophore concentrations from images of the ocular fundus, with a particular emphasis on age-related macular degeneration. The system has also found utility in fluorescence microscopy.

Keywords: multispectral, ocular, retinal, macular, imaging

1. INTRODUCTION

It has long been recognized that imaging of the interior of the human eye (the ocular fundus) is a valuable tool in the diagnosis and monitoring of a number of common medical conditions. Since the fundus allows naked blood vessels to be imaged non-invasively in vivo, it can yield valuable information into the general health of the patient and fundus imaging is widely used as the first line in screening for diabetes[1] , which is known to cause hemorrhages in the retinal blood vessels. Imaging of the fundus can also provide information about many eye-specific diseases such as age-related macular degeneration (AMD)[2] and glaucoma,[3] both of which are major causes of blindness. A variety of imaging methods are used to examine the fundus. The most commonly used technique is conventional RGB color photography, which is used as the principal screening technique for diabetes, and is widely available in optometry clinics. This technique has the advantage of being quick and simple to perform in primary care clinics as no contrast agents are administered to the patient. However, RGB photography acquires images from only three rather broad wavelength bands, and its ability to distinguish between different chromophores is very limited, preventing this technique from providing quantitative information that could be diagnostically useful. The de facto imaging technique for examining blood vessels in the fundus is the fluorescein angiogram. A fluorescent contrast agent is injected into the bloodstream, and time-series images of the fundus taken at a specific wavelength are acquired to image the vasculature. This approach yields extremely high quality images of the vascular network of the fundus, but can only be performed under the supervision of a physician due to the potential for an adverse reaction to the contrast agent and is therefore not suitable for use in the primary care setting. A recent development has been the introduction of the scanning laser ophthalmoscope (SLO), which is gradually becoming more popular in the clinical setting. An SLO acquires images by rapidly scanning a laser across the fundus, and recording the image one pixel at a time. In a typical modern SLO, 2-3 images are acquired using different laser wavelengths that are usually taken from the red, green and (optionally) blue spectral wavebands. The SLO typically obtains images with excellent (20?m) spatial resolution from a very wide field of view, but the resulting images are often significantly distorted. Finally, optical coherence tomography (OCT) is now widely used to identify structural features in the fundus. OCT can provide information about retinal structures up to approximately 1 mm below the surface, but does not provide information about the chromophore concentrations in the tissue. There has been significant interest in recent years in the development

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of multispectral imaging techniques for the fundus. There is no commercial multispectral retinal imaging system available, and various groups have used a variety of different approaches. The most common technique uses a commercial multispectral imaging system attached to a modified ocular microscope[4,5] . These systems typically acquire images sequentially, one wavelength at a time. Some groups have developed sophisticated hardware that allows multiple wavebands to be acquired simultaneously by using different regions of a single CCD to capture each band, but these systems usually restrict the size of the region that can be acquired.[6] The main drawback of the sequential systems is that the acquisition time of a complete set of images is often greater that the natural timescale of the saccadic movements of the eye, with a typical multispectral image stack of 20-25 wavelengths taking at least 5 seconds to acquire. This results in significant movement between frames in the image stack, and is also quite uncomfortable for the subject. Whilst the images can be registered, this is not straightforward. Images acquired from the red part of the visible spectrum tend to lack well-defined features due to the relative transparency of blood at these wavelengths (the vessels are clearly visible below around 600nm, but are effectively transparent at ¿620nm). In addition to this, the curvature of the fundus means that the region of interest is not evenly illuminated, and so any movement of the fundus means that the incident light intensity at a given spatial location may change between images in a sequence. This can cause significant difficulties during the analysis and interpretation of the image data.[5] In response to these issues, we have designed a fast-switching LED-based discrete-wavelength light source that can be used in combination with a high sensitivity CCD camera to obtain movement-free multispectral image sequences of the fundus. The system is capable of acquiring images at least one order of magnitude more quickly than the existing tunable filter system used by our group. We present early images acquired using the new system and identify potential improvements. We conclude by outlining our future program of research with the new system.

2. SYSTEM OVERVIEW

The system consists of an ocular microscope (Zeiss RCM250) to which a number of modifications have been made as shown in Figure 1. The original microscope has four main components: the microscope optics; a halogen light source used for aligning and focusing; a xenon flash unit for image acquisition; a 35mm SLR camera body used for image recording. We have replaced the xenon flash unit with a bespoke LED-based light source, and the 35mm SLR camera back with a back-illuminated electron multiplying CCD camera (Hamamatsu C9100-13) controlled by a frame grabber (Active Silicon PHX-D24CL) installed into a standard PC. Within the ocular microscope, two mirror solenoids that control optical pathways within the microscope have been re-wired so they can be controlled from a modified PC 3 button mouse. The 3rd button on this mouse provides the trigger to begin the image acquisition process (see Section 3). The system is controlled by a National Instruments USB digital controller (type 6501) that is controlled, in turn, by custom software developed with Labwindows running on the PC. This software ensures synchronization between the camera and the light source, so that image acquisition starts and stops precisely in time with each individual light pulse. The camera is also controlled by a separate application, written in Visual C++.

2.1. Light Source Design

The light source we have added to the microscope is based on a previous design that was employed in the UCL Optical Topography System.[7] The system consists of up to twelve LEDs that can be programmed to illuminate in any way that the user chooses, limited only by the speed of the digital electronics. It has a modular design that allows LEDs of different wavelengths to be easily swapped in and out for use in different applications. Each LED is individually addressable, allowing precise control of intensity, illumination time and illumination sequence. The sources are connected to the ocular microscope using 12 fiber optic bundles that are gathered together incoherently into a single bundle to ensure uniform spatial distribution of the different light sources. The bundle is then inserted into the ocular microscope to sit at the focus of the collimating lens of the xenon light source that has been removed.

2.2. User Interface

A simple graphical user interface provides control of the light source. The intensity and duration of each source pulse can be set independently, and sources can be included or excluded from the pulse sequence as needed. The

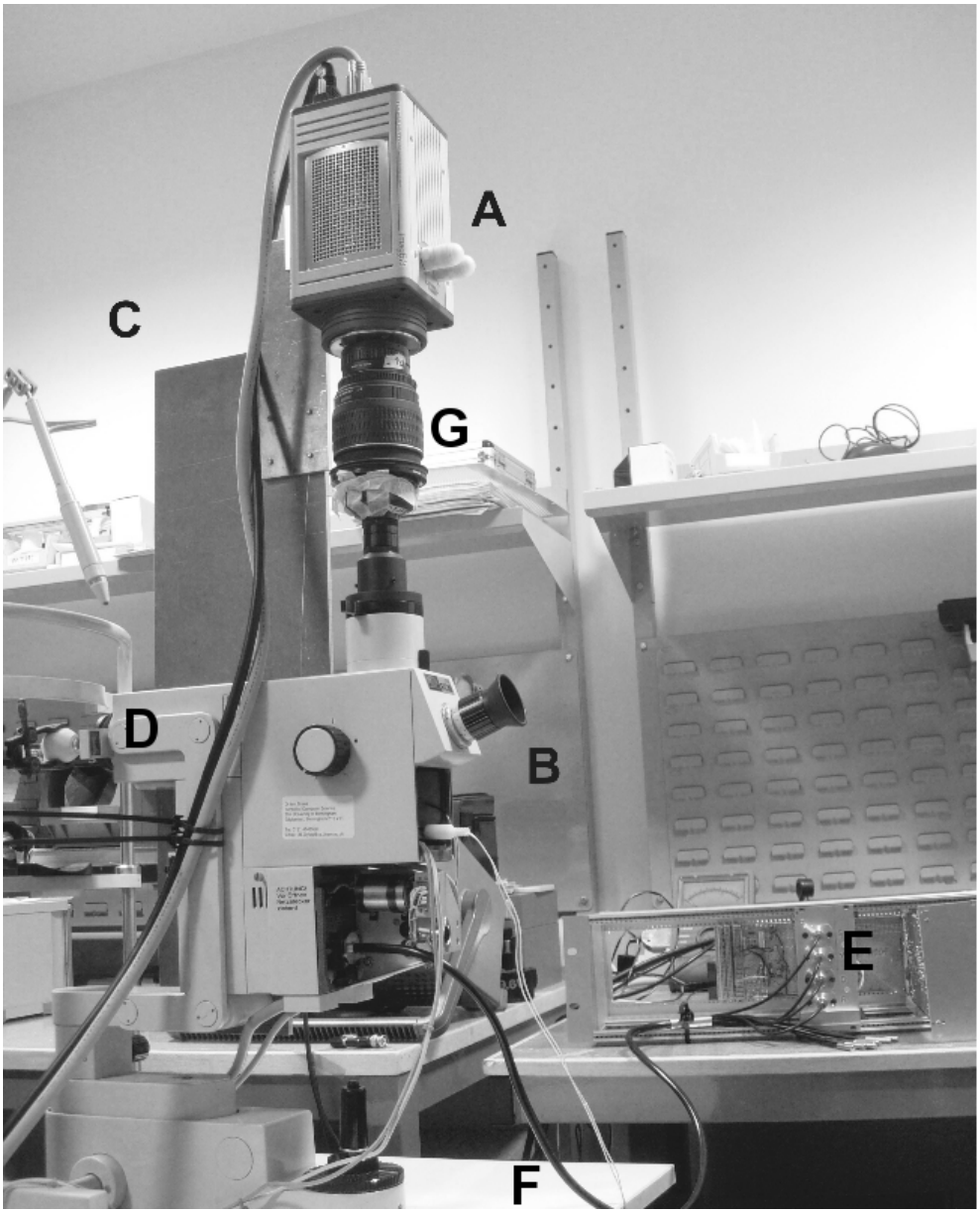


Figure 1. Photograph of the imaging system. The main components are: (A) Hamamatsu C9100-13 back-thinned electron multiplying CCD camera; (B) Viewing Eyepiece; (C) Support arm for CCD camera; (D) Microscope objective; (E) LED source; (F) Optic fibre; (G) Corrective optics.

number of repetitions of the pulse sequence can be set, or the system can be set to run continuously. The pulse sequence can be started from the user interface or from the mouse trigger attached to the ocular microscope.

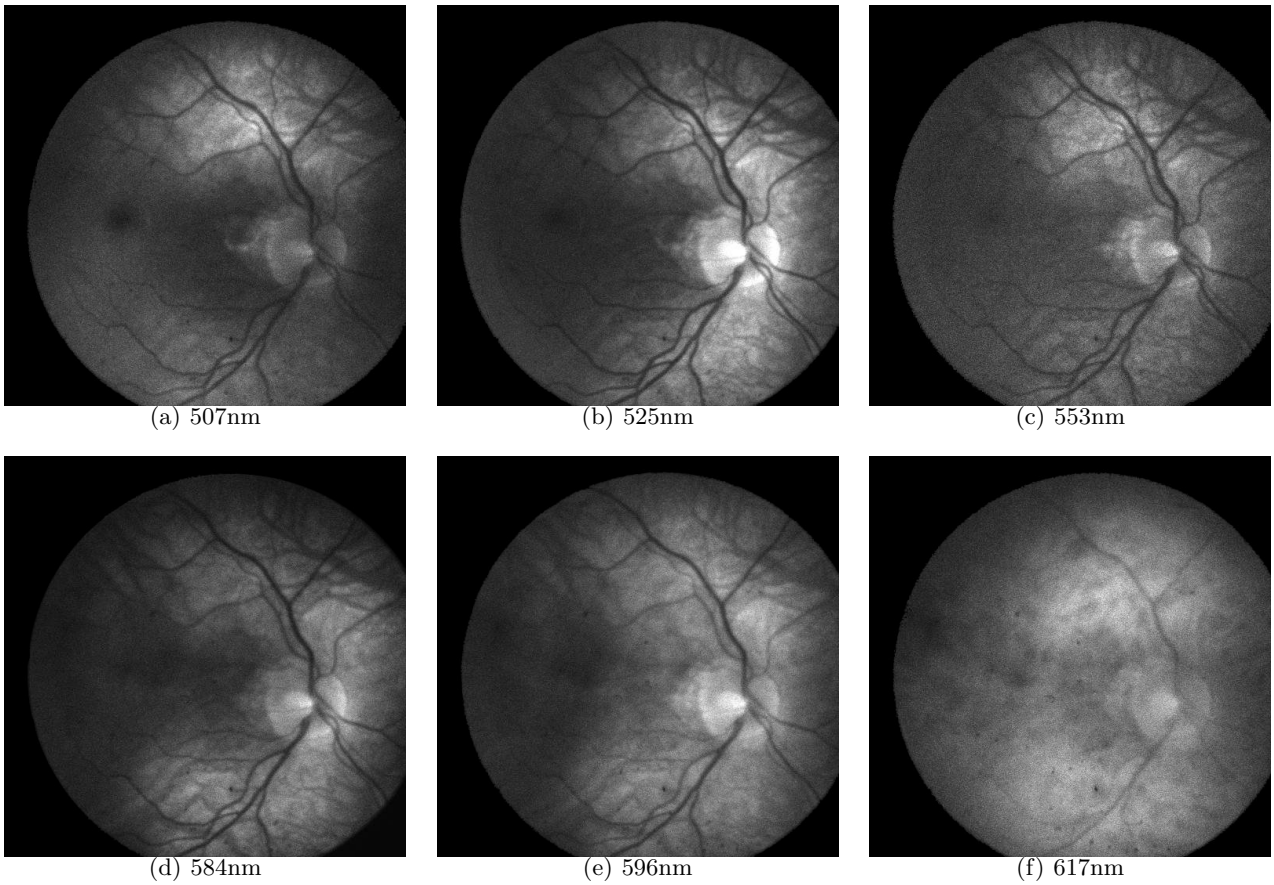
2.3. System Operation

The imaging process proceeds as follows: the light source parameters are set using the graphical user interface. The camera parameters are set similarly. The operator adjusts the microscope using the existing tungsten filament illumination to obtain the best image of the fundus. The 3 buttons of the microscope mouse are then depressed simultaneously. This actuates the two mirrors of the microscope - one to switch the light source

from tungsten to LED, the other to switch the viewing path from the operator's eyepiece to the camera. At the same time the third button sends a digital trigger to the PC that starts the light source sequence and the camera imaging sequence simultaneously. In this way many short exposure images of the fundus can be obtained consecutively, limited only by patient tolerance to the light source.

3. RESULTS

Initial tests of the system were carried out using a set of six LEDs with central wavelengths of 507nm, 525nm, 553nm, 584nm, 596nm and 617nm. The images were obtained using an exposure times of 0.05s with the electron-multiplying gain of the camera set to 610, with the exception of the 553nm LED, which required an exposure time of 0.2s. This was because this particular LED was supplied with a different casing and was much harder to couple to the optic fibre resulting in significant light loss. The sequence of six images was therefore obtained in 0.45s. A set of sample images obtained using these settings is shown in Figure 2.



(a) 507nm (b) 525nm (c) 553nm

(d) 584nm (e) 596nm (f) 617nm

Figure 2. Sample Images

We repeated the imaging sequence six times on the same subject and performed an analysis of the movement of the eye during the sequence. This was done using the output of the generalized dual bootstrap-ICP image registration algorithm.[8] Across the six sets of six images acquired, the inter-frame movement within a set was found to be up to two pixels. We performed the same test on a stationary artificial eye with similar results, demonstrating that this is as good a result as could be expected. We have also measured the spatial resolution of the system by imaging a resolution target embedded in an artificial eye and found that we could clearly resolve features of 50 microns. This is sufficient to show small retinal blood vessels and microhaemorrhages. In

addition to the high quality of the images obtained, the use of the narrow-band LED illumination (as opposed to a broad-band illuminant) greatly reduces the total exposure of the subject's fundus to light. Subjects reported that the imaging procedure itself was considerably more comfortable than the focusing and alignment process performed using the ocular microscope's built-in halogen source.

4. CONCLUSIONS AND FUTURE WORK

We have demonstrated that LED illumination combined with a high-sensitivity CCD camera is capable of acquiring high-quality multispectral images of the human fundus. Initial results have demonstrated that the system is fast enough to be able to sequentially acquire a set of six wavelengths with minimal inter-frame movement (no more than two pixels within the set); and produces images of sufficient quality to allow important features such as small retinal blood vessels and microhaemorrhages to be resolved. Subject feedback indicates that the imaging process is comfortable.

These preliminary results show great promise for this system, but also suggest a number of possible improvements that could lead to significant further improvements in both image quality and system usability. We first note that the subject perception of the imaging procedure is that it is very tolerable, and indeed the power of the LEDs used is measured, at the microscope objective, to be only a fraction of that of the system's halogen source. The LEDs used in the light source are rated at 1W which was calculated to be well within recognised safety margins.[9] However, the degree of light loss due to LED-fibre coupling and in the internal optics of the microscope is much greater than expected. We are currently working to understand where these losses occur and how they can be reduced. This explains the subject perception that the LEDs are not very bright. Despite the lack of optical power, the system has still produced good quality images and if we can increase the power significantly (whilst remaining well within the safety limits) we should be able to obtain further improvements in quality and also reduce the image acquisition time. The lower limit for the acquisition time is approx 25ms and is dictated by the read-out time of the camera, and we currently believe that we should be able to make full use of this speed.

In addition to ocular imaging, the light source and camera have also found application in fluorescence microscopy where it can be used to replace existing light source and filter combinations. We have demonstrated that both fluorescent beads and live cells tagged with YFP and GFP can be imaged with acquisition times of 10ms for the beads and 100ms for the live cells. In this application, low power is desirable to prevent damage to the cells, and the narrow bandwidth of the LEDs is a distinct advantage for this application.

The long-term aim of this work is to develop an imaging system that can be used to quantify chromophore concentrations in the ocular fundus. In previous work[5] we have identified a set of wavelengths and an image analysis algorithm that can be used to quantify chromophores. The progress of this earlier work was limited by the performance of the imaging system used. The results presented here have demonstrated that the performance of the new system is sufficient to allow continued further progress. We aim to investigate the use of the system, in conjunction with the image analysis algorithms previously developed to image and characterize a variety of ocular diseases with a particular focus on AMD, where our approach has already shown promise.

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