Endoscopic Device for Functional Imaging of the Retina

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ABSTRACT

Non-invasive imaging of retinal function based on the recording of spatially distributed reflectance changes evoked by visual stimuli has to-date been performed primarily using modified commercial fundus cameras. We have constructed a prototype retinal functional imager, using a commercial endoscope (Storz) for the frontend optics, and a low-cost back-end that includes the needed dichroic beam splitter to separate the stimulus path from the imaging path. This device has been tested to demonstrate its performance for the delivery of adequate near infrared (NIR) illumination, intensity of the visual stimulus and reflectance return in the imaging path. The current device was found to be capable of imaging reflectance changes of 0.1%, similar to that observable using the modified commercial fundus camera approach. The visual stimulus (a 505nm spot of 0.5secs) was used with an interrogation illumination of 780nm, and a sequence of imaged captured. At each pixel, the imaged signal was subtracted and normalized by the baseline reflectance, so that the measurement was $\Delta R/R$. The typical retinal activity signal observed had a $\Delta R/R$ of 0.3-1.0%. The noise levels were measured when no stimulus was applied and found to vary between ± 0.05%.

Functional imaging has been suggested as a means to provide objective information on retina function that may be a preclinical indicator of ocular diseases, such as age-related macular degeneration (AMD), glaucoma, and diabetic retinopathy. The endoscopic approach promises to yield a significantly more economical retinal functional imaging device that would be clinically important.

Keywords: Functional imaging, retina, endoscope.

1. INTRODUCTION

It has long been recognized that the retina's optical properties, when stimulated by visible light, are altered depending on the part of the near infrared (NIR) spectrum that is being used to measure the changes in reflectance [1, 2, 3, 4, 5, 6]. A strategy to capture the signal from the stimulated retina was originally devised by three groups, Kardon et al. [7], Grinvald [8], and Nelson, et al. [9]. Similarly, advanced signal processing techniques for extracting the intrinsic signal were developed by the authors of this paper [10, 11]. A number of proposed sources of this functional signal, including photoreceptors [12], membrane depolarization [13], cell swelling [14], and altered metabolism have been attributed to

the changes in the local optical properties of the retinal tissue that are detectable by measurement of reflected and scattered light signals.

Early signs of most retinal disorders are often manifested by both morphological abnormalities and impaired physiological responses. Some systemic diseases may likewise present early as physiology or functional changes rather than morphological. Detection of these diseases will require new imaging modalities that can detect both morphological and functional changes in the retina. Retina function is measured with devices such as electroretinography (ERG) [15] and multifocal ERG [16]. These instruments have low spatial resolution. At the other extreme of spatial resolution, researchers have developed high-end devices that use adaptive optics to measure functional changes at the photo receptor level. Grieve, Roorda et al. [17], Jonnal, Miller et al. [18], and others have captured videos showing photoreceptors changing reflected intensity as they are stimulated.

In recent papers, Schallek et al. [19, 20] and Tsunoda et al. [4], have established that the intrinsic signal is likely to originate from the outer retina, i.e., the photoreceptors. Schallek et al. performed experiments to pharmacologically suppress activity in the inner retina with intravitreal injections of tetrodotoxin (TTX) and spiking amacrine cells [21]; and injections of amino-phosphonobutyric acid (APB) to block photoreceptor input to the "ON" bipolar cells of the retina [22], while Piperidinedi-carboxylic acid (PDA) to suppress the "OFF" bipolar cell response and cell types down-stream from this pathway [23]. When injected together, these drugs suppress the stimulus-evoked potential at the level of the bipolar cell input, while leaving photoreceptor function intact. The stimulus-evoked intrinsic signal was observed to be as strong under these conditions as under the control, non-pharmacologically altered state. This leads to the conclusion that the intrinsic signal is significantly driven by the photoreceptors stimulation.

In this work we present a new prototype functional retinal imager based on a commercial endoscope that has been integrated into a custom, low-cost optical system. The endoscope-based functional imager of the retina exploits recent technological advances in highly sensitive digital cameras, multispectral imaging, and signal and image processing. This imaging method provides objective, quantitative, and localizing information in the form of a functional image across the retina.

This paper is organized as follows: First, a description of the functional imager is presented. Preliminary results that show the feasibility of this imager are presented next. Finally, conclusions and future work are presented in the last section.

2. METHODS

A. Instrument Description

The methodology for retina functional imaging depends on the characteristic spectral properties of hemoglobin and its dependence on oxygen saturation. Based on measurements by Delori [24], our own spectral measurements of the ocular fundus [25], and absorption spectra by Assendelft [26], it is possible to measure the changes in oxyhemoglobin saturation and blood volume contributions based on reflectance change at various wavelengths. It is clear from these measurements that a strategy can be devised to capture spectral images at selected wavelengths in order to measure this

stimulus-evoked intrinsic signal for any retina. This methodology should not be confused with those attempting to measure retinal oximetry. Our technique does not depend on the difficult and elusive measurement of blood oximetry, rather simply it measures relative changes of state, which do not depend on absolute oximetry calculations.

Our methodology for optical mapping of retinal function consists of three components:

1. Stimulation of the retina in a selected spectral band, green (505nm), in the visible range.
2. Measurement of the reflected intensity from the retina at interrogating spectral bands that reflect the state of hemoglobin saturation before and after stimulation. To maximize the signal-to-noise ratio (SNR) and reduce or eliminate unwanted stimulation of the retina, an interrogation wavelength in the near infrared (750 to 850 nm) is adopted.
3. Mapping of optical changes that result from retina functional activity by analyzing recorded video frames with subtraction of pre-stimulation images from post-stimulation images and signal extraction techniques based on independent component analysis.

The prototype functional endoscope imager is shown in (Figure 1). The imaging device has a field of view of 30 degrees with 1000x1000 pixels per image. This spatial resolution would be superior to the spatial resolution of 1-2 degrees for the multifocal ERG.

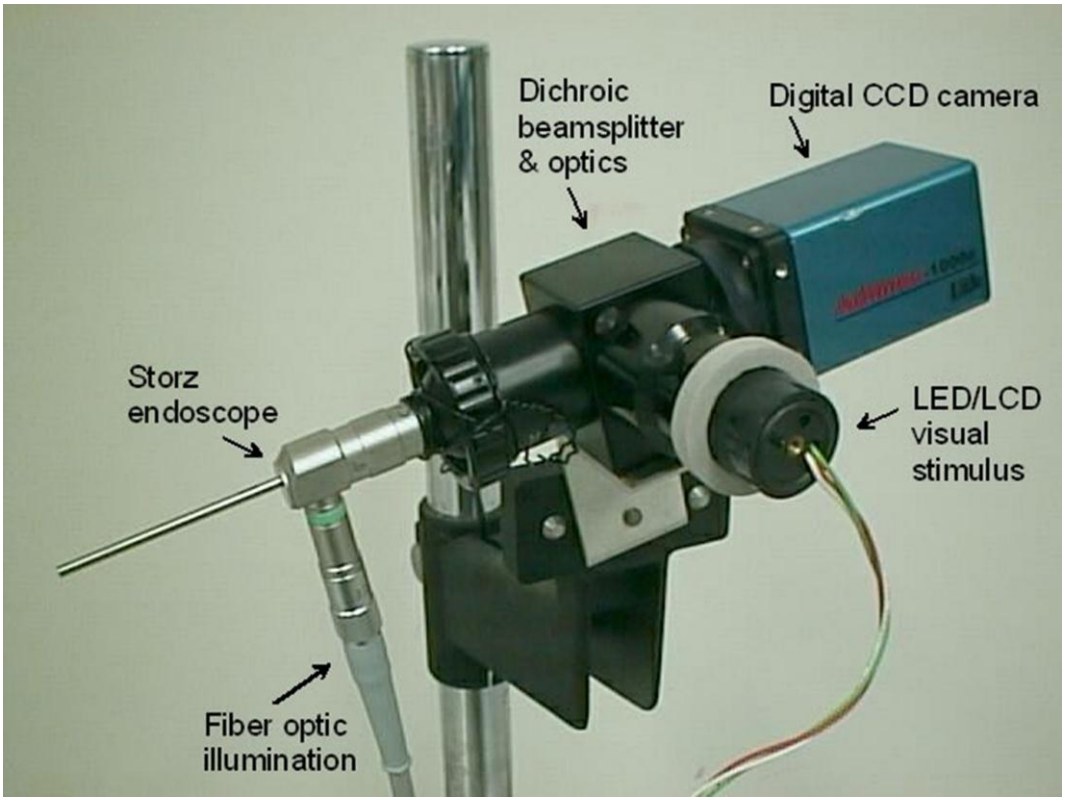

Figure 1 – Endoscope-based retina functional imager prototype developed at the Human Performance Laboratory, Upstate Medical University, Syracuse, NY

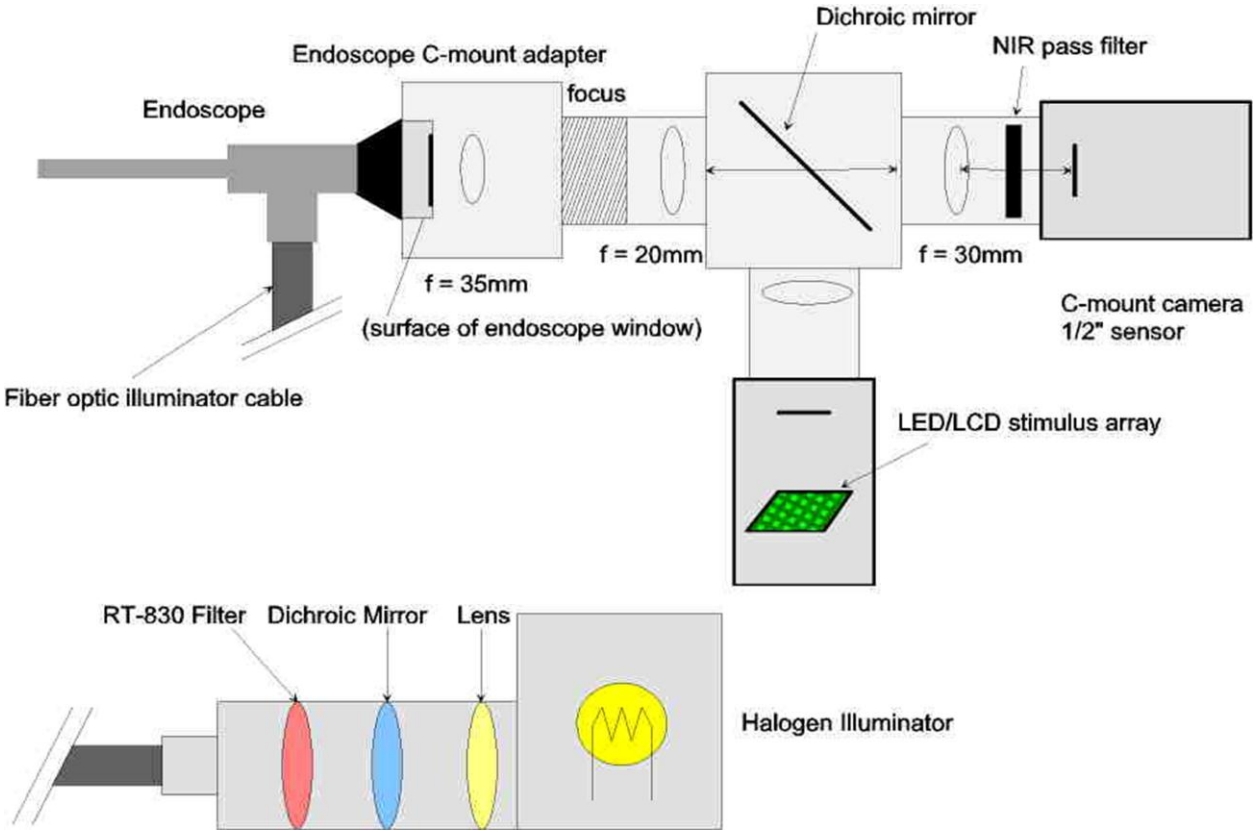

Figure 2 – Optical design of endoscope for functional imaging of the retina. An infrared filter and dichroic mirror block visible light and allow infrared light to pass through. Another dichroic mirror within the imager reflects the visible light from the stimulus towards the eye and allows infrared light to pass through towards the camera.

Figure 2 shows the optical layout of the endoscope-based retina functional imager, which was used in the pilot study. In the optical diagram, the key components are the stimulus source which operates at 505 nm, the dichroic beam splitter, and the interrogation light source, which operates in the NIR. The stimulus source is in a region of the spectrum where retinal cones are most sensitive. The stimulus source (LED/LCD in Figure 2) produces a stimulus pattern that is applied to the retina. The stimulus is automatically started at 4 seconds after an initial period of baseline (4 seconds of pre-stimulus) image collection. The stimulus may be programmed for any duration as a steady illumination, or as a pre-determined on/off cycle from 1 to 5 Hz. Our protocol was set to stimulate the retina for 0.5 seconds. Our experience has been that this duration produces the best response. The existing endoscope imaging illumination was replaced with a NIR source that operates at 780 nm broad band. At this wavelength, the reflectance is sensitive to changes in blood oxygenation saturation and volume. The digital camera scans the field of view at 2 Hz.

B. Stimulus Light Source

The optical requirements on the stimulus, shown in Figure 2, are to provide a visible moving signal directed via beam splitters to the retina. The wavelength chosen for the stimulus of 505 nm, having served its stimulation function, is then blocked from entering the measurement CCD camera. The second requirement of the stimulus is for alignment of the system, which is made possible by a second broadband source at 780nm that is imaged by the CCD camera. Design light levels, typically near 1.2mW (505 nm), as shown above, are substantially below the maximum safety value, when considering losses of 0.1 mW in the optical train leading to the eye.

Several interacting issues influence the decision on the optimal wavelengths of the interrogation light. The primate/human retina is responsive to light past 820nm, though at greatly reduced sensitivity. Since these functional optical signals are small (0.1-1.0%), a minimum number of photons must be captured by the CCD camera in order to yield an adequate signal-to-noise ratio to reveal the functional signals. This constraint dictates the use of an interrogation light of high intensity. At 780nm, the deep red background is still appreciably dimmer than the visual (505nm) stimuli that we have been using.

C. Preparation of Animals and Test Protocol

The endoscopic functional imager device was tested at the Institute for Human Performance, Upstate Medical University, Syracuse, NY. Approval for animal testing was provided by the University IAUCC. The purpose of the animal testing was to assess the sensitivity of the endoscopic functional imager on mice with no known retinal pathology. The mice were sedated and anesthetized using ketamine/xylazine and paralyzed for imaging stability. Pupils were dilated and accommodation was inhibited with atropine and phenylephrine. Hypromellose was applied to lubricate the eye and prevent corneal drying. Mice were imaged in a stereotaxic frame and physiologically monitored throughout the experiment.

The stimulus applied for this pilot experiment was a round pattern. Our protocol was set to stimulate the retina for 0.5 seconds, a flash. Images were acquired at a rate of one frame per second, and for a period of 20-40 seconds. 16 or 8 trials were performed, with each trial consisting of two stimulus and two blank conditions. This is to allow complete recovery of the photoreceptors to their baseline state, and to generate reference non-stimulated images to be compared with the videos of the stimulated retina.

3. RESULTS

Figure 3 shows two images captured using the device. The image on the left is one where no stimulation was applied, while the one on the right was captured after visual stimulation. It is clear that there is a change in the reflectance due to visual stimulation.

A region of interest (ROI) was selected and the data was analyzed for fractional change in reflectance using a custom written Matlab program and other custom software. Figure 4 shows change in reflectance ($\Delta R/R$) within an ROI from mouse retinal imaging over 20 seconds, stimulated and non-stimulated. The 0.5 second stimulus pulse (green LED) is presented at seven seconds (arrow) and leads to a negative signal (a reflectance decrease, a darkening).

Finally, in Figure 6 we show averaged images for different blank (bottom two rows) and stimulated (top two rows) conditions. The stimulus presence is signaled by the arrow on the top. It can be clearly seen that the visual stimulation produces changes in reflectance on the averaged images. According to the scale on the top right of the figure, those changes are less than 0.5% of the baseline.

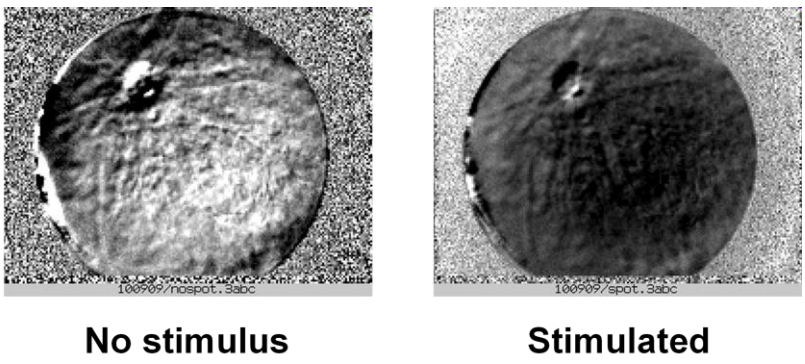

Figure 3 – Differential images of retina showing decrease in reflectance in response to visual stimulation (darkened retina). A region of interest analyzed for average fractional change in reflectance, plotted in Figure 4.

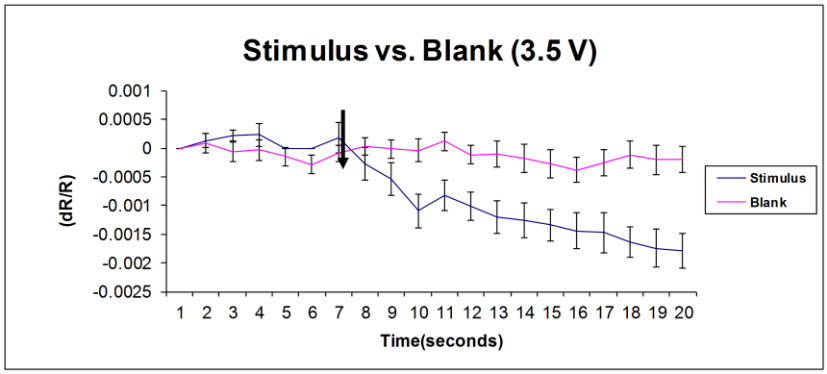

Figure 4 – Temporal responses of ROIs from images in Figure 3.

3. CONCLUSIONS AND FUTURE WORK

We have presented a novel functional imaging technique in mouse retina that has demonstrated stimulus-driven reflectance change signals similar to those observed in the macaque monkey and cat retina. A change in reflectance due to visual stimulation can be clearly noticed in the experiments. This change in reflectance has been observed to be between 0.2% and 0.5% of the baseline reflectance, which is in agreement with previous imaging experiments in monkey and cat retinas [5, 19, 20].

Future studies using particular knock mouse models promises to help answer the specific anatomic and biophysical origins of these optical signals. Studies using knockout mice models may also yield important results relevant to human diseases of the retina.

This device reflects the trend in the next generation medical imaging devices that reveal not only structural but also functional information. Our functional imager of the retina is aimed at providing a new approach for clinicians to evaluate diseases of the optic nerve and retina. This research aims to design a low-cost device for functional imaging of the retina that can be used in clinical environments for screening, diagnosis, and clinical trials.

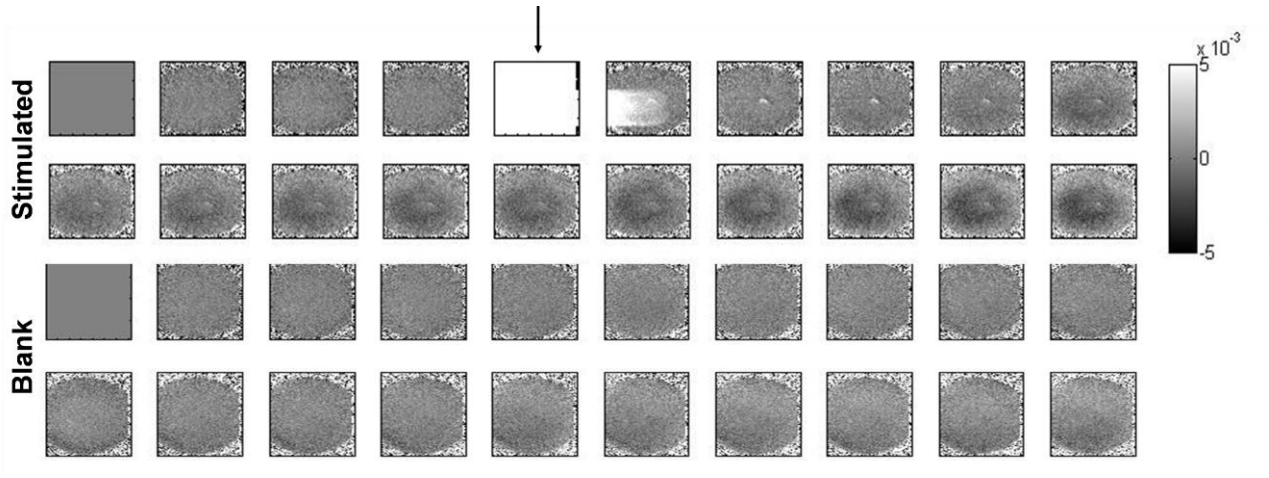

Figure 6 – Averaged images for blank and stimulus conditions (green LED). After a 200 ms stimulus pulse (arrow), images were acquired at a rate of one per second. Notice response after stimulus presentation vs. blank condition.

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