PARALLEL CONFOCAL RECORDING OF LOCALIZED RETINAL PHOTORECEPTOR RESPONSES

by

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A THESIS

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IN VITRO AND IN VIVO INTRINSIC OPTICAL SIGNAL IMAGING OF RETINAL ACTIVATION

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BIOMEDICAL ENGINEERING

ABSTRACT

The primary purpose of my thesis research is to validate parallel confocal recording of intrinsic optical signals (IOSs) correlated with localized retinal photoreceptor responses. IOS imaging promises a noninvasive method for retinal disease detection and treatment evaluation. However, IOS identification of localized retinal dysfunction is still challenging due to the complexity of retinal structure. Stimulus-evoked IOSs have been observed in both photoreceptors and inner retinal neurons. IOS pattern of retinal photoreceptor layer may contain cross-talk noise from inner retinal layers. In order to detect localized retinal photoreceptors response without significant contamination from other retinal volumes, we designed a rapid functional imager for the parallel recording of localized IOSs. In this imager, a microlens array (MLA) based illuminator is employed to simultaneously deliver visible stimulus light for retinal stimulation and near-infrared (NIR) recording light for IOS imaging. The parfocal configuration of the stimulus and recording light illumination enables confocal recording of the stimulus-evoked IOSs. In isolated retinas, rapid MLA imaging has revealed robust IOS activities tightly correlated with localized retinal photoreceptor responses. Moreover, we have also recently demonstrated in vivo confocal detection of stimulus-evoked fast IOSs. We anticipate that further development of the IOS imaging technology may provide a new methodology for high resolution identification of localized retinal dysfunctions associated with eye diseases.
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INTRODUCTION

Background

Many eye diseases can cause functional defects to the retina. At the time when pathological changes in retinal morphology can be observed through fundus examination, irreversible damage to the neural retina may have been made. Therefore, retinal function measurement is very important for early detection of eye diseases. It is also well established that different types or stages of eye diseases may damage different retinal cell populations. Therefore, selective evaluation of retinal photoreceptors and post-receptor neurons can improve disease diagnosis and treatment evaluation. Electrophysiological measurements, such as electroretinogram (ERG) and multifocal ERG are well established as objective methods to evaluate retinal neural functions. The changes in the amplitudes and time courses of ERG a- and b-waves are often used signal dysfunction of retinal photoreceptors and inner neurons. However, signal specificities of these ERG waves are relatively low because of the integral effect of the electrophysiological changes over the entire depth of the retina. Therefore, the ERGs may appear normal unless 20% or more of the retina is affected by a disease. Low signal specificity and limited spatial resolution of ERG measurements make it difficult to identify small localized retinal neural dysfunctions, which usually happen in the early stage of several retinal diseases.

Previous research found that light scattering and birefringence could be used to detect rapid structural changes accompanying the action potentials in nerve fibers [1]. In
other words, action potential in some neurons can change the optical properties of the incident light, such as the light intensity, phase and polarization state. It is our belief that the optical properties of the light reflected by neurons before and after their activation carry important information about neurons’ functional capability. Such optical signals are called intrinsic optical signals (IOSs). IOS imaging has been demonstrated in vitro for high resolution detection of stimulus-evoked neuron activities from human brain [2-4]. The application of IOS imaging in the retina provides a noninvasive method for evaluating functional integrity of the retina. IOSs are usually divided into two categories, depending on their time courses. The slow IOSs have a time course that lasts from several minutes up to half an hour. It may be associated with hemodynamic and metabolic changes [5, 6]. The fast IOSs have a time course that is comparable with retinal electrophysiological activities. Previous studies with isolated photoreceptor outer segments and isolated retinas have demonstrated that phototransduction may contribute to observe fast IOSs [7-9].

Researchers around the world have used various methods to detect and study the IOSs. Fundus cameras that are typically used for fundus evaluation were employed to detect IOSs from the cat’s eye[10]. Both positive and negative signals have been recorded and it has been proposed that these IOSs were mainly from outer retina neuron activities. Adaptive optics scanning laser ophthalmoscope (AOSLO) has a high lateral resolution that can image the photoreceptor mosaic in vivo. However, the axial resolution of an AOSLO is low (150 µm). When it is used to record IOSs, the signals from photoreceptors may be contaminated by the activities of neurons in inter neuronal layers of the retina [11]. Optical Coherence Tomography with high axial resolution can provide cross-
sectional pictures of the retina. However, when it was used to detect IOSs from different layers of the isolated rabbit’s retina, the signal was contaminated by adjacent retinal volumes [12].

In common optical imaging systems, when the light scattered or reflected by an examined point in the sample is collected, the signal may be clouded by aberrant rays of scattered light that are deflected from neighboring points both in lateral or vertical directions. Confocal imaging technique can effectively eliminate out-of-focus glare by using point illumination and a spatial pinhole. However, single point light illumination scanning confocal imaging is time consuming and is limited by its low imaging sensitivity and illumination efficiency. The Microlens array (MLA) based optical system we are developing can, to some extent, overcome those problems. With the MLA, a collimated light passing through a square array of lenslets will produce a grid of illumination points at its focal plane. Then we can relay the patterned illumination to the specimen by a telescope system to implement multifocal imaging with discrete sampling volumes. This technique can, in principle, reduce the effect of aberrant rays, increase imaging speed and improve light illumination efficiency.

Significance

Based on our previous studies on isolated retina, we know that robust IOSs can be observed from the photoreceptor layer when the retina is activated[13]. It is important to separate the signals from different retinal layers. However, even within the same retinal neural layer, the IOSs recorded at each location may be contaminated by IOSs from its neighboring locations. In order to detect localized retinal photoreceptor response without
significant contamination from the neighboring retinal volumes, we developed a MLA-based functional imager.

During the experiment, we delivered spatially coincide visible stimulation light spots and near-infrared (NIR) recording light spots on multiple locations on an isolated frog (Rana Pipiens) retina simultaneously. Because the neighboring MLA stimulation/recording locations were widely separated on the retina, and only the photoreceptors at the MLA stimulation and recording locations were stimulated and recorded, the IOS cross-talk effect from adjacent photoreceptors and post-photo receptor neurons were reduced. Besides, the MLA-based imager can evaluate multiple locations over an extended retinal region simultaneously, producing an accurate retinotopic function map in one imaging sequence. This recording method avoids assembling measurements taken from different time frames, and thus is much less affected by inevitable eye movements.

Without pre-retinal optics, blood flow and eye movements, isolated retinas provide the simplest way to record fast IOSs. However, in order to apply IOS imaging to clinical eye care, measurements must be performed in vivo. By effectively rejecting the out of focus background lights, the capacity of a high speed line-scan confocal system was extended to in vivo retina imaging in anesthetized frog retina. We demonstrated the feasibility of in vivo imaging of fast IOSs with comparable time course with ERG. Further investigation of the fast IOSs and technical development toward clinically deployable IOS imagers can provide eye care professionals with a powerful, noninvasive method for concurrent morphological and functional evaluation of the retina.
Scope of this Thesis

There were two objectives for this thesis research, one was to construct a MLA based functional imager for detecting localized retinal photoreceptor responses, and the other was to modify a line-scan confocal imager to in vivo retina imaging. The specific aims of this project were listed as follows.

Aim 1: Design and construction of a MLA based imager that could record localized photoreceptor responses with cellular resolution in both transversal and axial directions. In this recording system, the visible stimulus light and the near infrared recording light were delivered simultaneously to multiple retinal locations through the MLA. During the experiment, the temporal resolution for image acquisition should be in the order of milliseconds to track fast IOSs.

Aim 2: To demonstrate the feasibility of in vivo imaging of fast IOSs in anesthetized frog retina by a line-scan confocal imager. The imager was shown to be able to reveal the spatiotemporal characteristics of fast IOSs in isolated frog retinas. In this study, part of system was modified to resolve individual frog photoreceptors in vivo. Concurrent ERG measurement was conducted during the IOS recording to compare the time courses.

MLA-based imager development

In this MLA-based imager, a near infrared (NIR) (center wavelength: 830 nm; bandwidth: 60 nm) superluminescent laser diode (SLD) (SLD-35-HP, Superlum) was used for IOS imaging. We chose it for several reasons: firstly, the retina is not sensitive to near infrared light. Second, near infrared light is less absorbed before it reaches the retina. The SLD has an additional advantage of having the high power of laser diodes (LD) and
the low coherence of conventional light-emitting diodes (LED). In other words, SLD has higher power than LED and has better signal-to-noise ratio than LD. A red (635 nm) laser diode was used for retinal stimulation and another NIR (850 nm) light-emitting diode (LED) was used to examine the MLA spots on the retina.

A dichroic mirror (DM) could transmit most of NIR light and reflect visible light. Therefore, the recording NIR light and the stimulus light were combined at the DM to illuminate the MLA. After DM, the NIR recording light and the red light shared the same light path. In principle, the MLA would fail to focus light of different wavelengths to the same convergence point due to chromatic aberration. However, because the wavelength of the visible stimulus light we used (635 nm) was close to that of NIR recording light (830 nm), the chromatic aberrations of the NIR and red lights at the specimen (retina) produced by the MLA was negligible, compared with the axial resolution of the system. On the other hand, the red light we used was an effective stimulus for the retina of a leopard frog [14]. The parfocal configuration of the red light stimulus and the NIR recording light enabled confocal recording of the stimulus-evoked IOSs.

At the back focal plane of the MLA, the lights would form a focused light spots array (multifocal pattern). Lens1 and the objective formed a telescope system which relayed the multifocal pattern to the specimen. The light from a NIR LED provided a full field of view of the recording light’s focal plane on the specimen. The polarization-sensitive beam splitter (PBS, G335-599000, Linos) and the quarter wave (QW) plate served as the circular polarizer. The light reflected from the specimen was polarized after a double passing through the quarter wave plate, and thus could transmit without attenuation through the PBS into the imaging path. This design can increase the light collection
efficiency. While fully transmitting the NIR recording light, the filter completely blocked the red light. In this way, NIR light recording of visible light evoked photoreceptors response was accomplished.

A CCD camera (Pike F-032B/C, Allied Vision Technologies) working in a high speed mode (3ms/frame, i.e., 333 Hz) was used for retinal imaging.

**In vivo retina imaging and IOSs recording**

In a previous study, we used a rapid line-scan confocal imager to achieve high spatiotemporal resolution imaging of fast IOSs from isolated animal retinas[15]. In this study, this imager was modified for *in vivo* imaging of fast IOSs in anesthetized frog retinas with cellular resolution. The schematic diagram of the line-scan confocal imager is shown in Fig. 1. The cylindrical lens focused the incident parallel light into one line, which was confocal with the aperture of the linear CCD. Both top view and side view of the light paths were shown. In this system, the same NIR (center wavelength: 830 nm; bandwidth: 60 nm) SLD was used for IOS imaging. The NIR LED was placed besides the eye to illuminate the pupil. The visible light was reflected from the dichroic mirror to serve as a stimulus and was focused at the pupil by lens 3 to form a Maxwellian view [16]. In this way, a large portion of the retina would be stimulated by the visible light.

All the lenses used were achromatic to minimize optical dispersion. Because Lens 2 and Lens 3 were used for both light illumination and light collection, near infrared coating was used to reduce lens surface reflection. A full field CCD camera was employed to monitor the pupil simultaneously.
The frog was anesthetized by tricaine methane sulfocnate (TMS, MS-222) solution (concentration: 500mg/L). The anesthesia was performed in a plastic cage (animal container) with a self-locking lip and vented holes. The depth of anesthesia was tested by the loss of nociceptor response (NR).

During an experiment, a NIR SLD was employed as the recording light. By rejecting the out-of-focus background light, the high resolution confocal imager could reveal individual frog photoreceptors in vivo (Fig. 2a). By selecting a region of interest of 250 x 50 CCD pixels), a 200 Hz frame rate was achieved to record fast IOSs. Green light that is much more sensitive to frog’s photoreceptors than red light was used as stimulus. Each trial lasted 1.5s with a 10ms flash stimulus. ERG recording was conducted under the same condition for comparison. Briefly, ERG was recorded with a silver wire electrode from the corneal surface of the same eye as IOS imaging. Another electrode with a sharp tip was inserted into the frog’s skin to serve as a reference.

The images captured by the linear CCD were processed pixel by pixel to obtain relative light intensity changes, i.e. Δ I / I. As shown in fig. 2b, robust IOSs were clearly imaged from the activated frog retina with cellular resolution. High resolution imaging revealed both positive (increasing) and negative (decreasing) signals. The time courses of fast IOSs were comparable to that of the stimulus-evoked retinal ERG dynamics, which may reflect the neurons function in the retina.
Fig. 1 Schematic diagram of \textit{in vivo} line-scan confocal imager, CO: collimator; CL: cylindrical lens; BS: beam splitter; SM: scanning mirror; DM: dichroic mirror. Focal length of the CL is 50 mm. focal lengths of lenses L1-L3 are 50mm, 80mm, 80mm, 25mm, and 160 mm, respectively.
Figure 2. In vivo retinal image of IOSs from anesthetized frog (a) High spatial resolution retina image; (b) Raw image with region of interest: 250 x50 CCD pixels; (c) IOS pattern sequence with 10ms flash stimulus delivered at 0 ms (d) IOS Curves of randomly selected 8 pixels compared with ERG; (e) Representative IOS changes of single pixels. Vertical lines indicate the delivery of stimulus light. 200-ms pre-stimulus baseline and 700-ms post-stimulus IOS recordings are show.
PUBLICATION FROM THIS THESIS

In following part of the thesis, a paper published in the Optics Letters is presented. The first part of this paper was an introduction explaining the importance of developing a rapid functional imager for parallel recording of localized IOSs in the retina. Then detailed information about the system design was presented. A parfocal configuration of the visible light stimulus and the NIR recording light enabled confocal recording of the stimulus-evoked IOSs. In the result section, it was shown that robust IOS activities tightly correlated with localized retinal photoreceptor responses were captured. Finally, the potential of the microlens array technique in recording localized retinal responses was discussed.
MICROLENS ARRAY RECORDING OF LOCALIZED RETINAL RESPONSES

by

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Format adapted [and errata corrected] for thesis
ABSTRACT

We designed a rapid functional imager for the parallel recording of localized intrinsic optical signals (IOSs). This imager used a microlens array (MLA)-based illuminator to deliver visible stimulus light and near-infrared (NIR) recording light simultaneously. The parfocal configuration of the stimulus and recording light illumination enabled confocal recording of the stimulus-evoked IOSs. Because the MLA stimulation/recording spots were widely separated on the retina, and only the photoreceptors within the MLA stimulation/recording spots were stimulated, the potential IOS cross talk effect among neighboring retinal areas was minimized. Our experiments revealed robust IOS activities tightly correlated with localized retinal responses.
INTRODUCTION

Electrophysiological measurements, such as the electroretinogram (ERG), are valuable for objective assessment of retinal dysfunctions. However, ERG integrates stimulus-evoked electrophysiological responses over an extended retinal area; the effects of localized small lesions associated with early retinal diseases may be concealed by spatial integration. Intrinsic optical signal (IOS) imaging holds promise for high-resolution assessment of retinal neural functions. Several imaging techniques, such as fundus cameras [1, 2], adaptive optics ophthalmoscopes [3–5], and optical coherence tomography (OCT) imagers [6–9], have been used to demonstrate the feasibility of IOS imaging of retinal activation. Using isolated amphibian retinas, high-spatial (micrometers) and high-temporal (milliseconds) imaging revealed spatially intermixed positive (increasing) and negative (decreasing) IOS signals [10, 11]. Unless the imaging resolution is high enough, the IOSs of opposite polarities may be integrated spatially and result in poor imaging signal sensitivity. While both confocal [12] and OCT [6–8] systems can provide the high spatial resolution to map IOSs in the retina, signal contamination among adjacent retinal volumes may still exist because stimulus-evoked light dynamics of neighboring volumes, such as transient transmission and scattering changes, may produce dynamic change of the photons collected from the intended sampling volumes. To detect localized retinal response without significant contamination from neighboring retinal volumes, we developed a microlens array (MLA)-based functional imager.
SYSTEM SETUP

The schematic diagram of the MLA-based imager is shown in Fig. 1a. In this system, a NIR (center wavelength: 830 nm; bandwidth: 60 nm) superluminescent laser diode (SLD) (SLD-35-HP, Superlum) was used for IOS imaging, a NIR (850 nm) light-emitting diode (LED) was used to examine the MLA spots on the retina, and a red (635 nm) laser diode was used for retinal stimulation. At the dichroic mirror (DM), collimated NIR light and visible light were combined to illuminate the MLA (MLA-0200-1.0-S, Adaptive Optics Associates), and thus simultaneously produced the MLA visible stimulation light and NIR recording light patterns on the back focal plane of the MLA. The MLA spot pattern was coincided with the front focal plane of the lens L1 (f = 80mm), and thus the MLA patterns could be relayed to the specimen (retina) at the back focal plane of the objective (f = 18mm, 10X/0.3), with a demagnification of 0.225 (i.e., 18 mm/80 mm). The pitch of the MLA was 200 μm, and the spacing of the spots on the retinal sample was 45 μm. The back focal plane of the lens L1 coincided with the incident aperture of the objective, and thus the numeric aperture (NA) of the objective could be fully used to produce optimized (i.e., sharpest and minimized) light foci. Using a 10X/0.3 objective, this MLA-based system, in theory, could achieve a transversal resolution (0.61λ/NA) of ~1.7 μm and axial resolution (λ/NA^2) of ~9.2 μm. In order to minimize the optical dispersions between the NIR illumination light and visible stimulus light, achromatic lenses were used in the system shown in Fig. 1(a) (i.e. L1, L2). The MLA associated dispersions should also be considered. In theory, the chromatic aberration of the NIR (830nm) and red light at the specimen (retina) produced by the MLA is ~2.1 μm, which is negligible compared to the axial resolution (9.2 μm) of the system. Therefore, the confocal configuration of the NIR
illumination and red stimulation light foci was feasible to realize localized multiple spots measurement of stimulus-evoked IOSs with cellular resolution in both transversal and axial directions.

A polarization-sensitive beam splitter (PBS, G335-599000, Linos) was employed to ensure the high efficiency of the NIR imaging light. Linear s-polarized NIR light was reflected into the specimen (retina) by the PBS. Between the PBS and the objective, a quarter-wave (λ/4) retarder, with fast axis at 45° relative to the polarization plane of the illumination light, converted the linear-polarized (s-state) light to circular-polarized. For the reflected light from the specimen, it would be p-polarized after a double-passing through the quarter-wave plate, and thus could fully transmit through the PBS into the imaging path. A CCD camera (Pike F-032B/C, Allied Vision Technologies) working in a high speed mode (3ms/frame, i.e., 333 Hz) was used for retinal imaging.
EXPERIMENT RESULTS

Flat-mounted, isolated leopard frog (Rana Pipiens) retinas were used in the experiments. Briefly, retinal dissection was conducted in a dark room with dim red illumination [13]. After dark adaptation, the frog was euthanized by rapid decapitation and double pithing before the eyes were enucleated. The eyeball was hemisected below the equator with a pair of fine scissors to remove the lens and anterior structures before separating the retina from the retinal pigment epithelium. In principle, the MLA recording system can simultaneously monitor 13 x 9 retinal foci (i.e., 640 x 480 CCD pixels) with a focal distance of 45 µm at the specimen. However, in order to improve the IOS imaging speed, a reduced image area (300 x 300 CCD pixels) was used to monitor 6 x 6 retinal locations. Fig.1b shows the MLA pattern by placing a mirror at the specimen plane. When the retina was imaged, the MLA pattern was a little blurred due to the light scattering in the retina. However, the MLA spots were still distinguishable (Fig. 1c), and the cross-talk noise among adjacent spots were negligible. Before IOS recording, the NIR LED was turned on to confirm that the MLA was focused at the photoreceptor layer (Fig. 1d). During the IOS recording, the NIR LED for transmission imaging was turned off. In each imaging session, pre-stimulus baseline recording was collected for 1.2 s (i.e., 400 frames) before the 1-s visible red light stimulus was delivered.

Both positive and negative IOS signals were observed around each MLA spot (Fig. 2). Rapid IOS responses occurred almost immediately after the stimulus onset (Fig. 2g). The amplitude peaks of localized positive (such as the signal represented by trace 3 in Fig. 2(c)) and negative (such as the signal represented by trace 8) IOSs could be as large as 40% \( \Delta I/I \), where \( \Delta I \) was the dynamic optical changes and I was the average pre-
stimulus background pixel value (light intensity). Previous studies with isolated photoreceptor outer segments and isolated retinas have demonstrated transient IOSs associated with phototransduction [14-16]. Both binding and release of G-proteins to photoexcited rhodopsin might contribute to the IOSs [15]. Localized IOSs at different MLA recording spots had slightly different signal distributions, time courses and signal amplitudes (Fig. 2b and 2c), but the averages of overall positive and negative signals appeared to have similar time courses (Fig. 2d). Because of the comparable time courses of the overall positive and negative signals, the IOS magnitude, i.e., absolute value |IOS| can be used as the single parameter to quantify the strength of localized photoreceptor activity (Fig. 2e). In order to minimize the effect of spatial variations, including MLA spot locations relative to localized photoreceptors, on each MLA recording location, we recorded additional 5 image sequences on the same specimen by shifting the sample platform in the same direction for 2, 4, 6, 8, 10 μm, respectively, in transversal direction; with the MLA illumination consistently focused on the photoreceptor layer. Fig. 2f represents the averaged IOS magnitude pattern of these 6 recording trials. In Fig. 2f, a relatively homogenous IOS pattern was observed (compared to single-trial recording shown in Fig. 2e), and all MLA recording spots revealed robust IOSs with a magnitude up to 15% ΔI/I. Fig. 2g shows temporal dynamics of the averaged IOS activity, and confirms that rapid IOS occurred almost immediately after the stimulus onset.
CONCLUSION

In summary, we demonstrated that a high-speed MLA-based imager can simultaneously record stimulus-evoked IOSs from multiple retinal locations in flat-mounted frog retinas that were associated with localized neural responses to visible light stimulation. In this recording system, the MLA provided the visible stimulus light and the near infrared recording light simultaneously. Because only the photoreceptors within the stimulus/recording locations were activated, the IOS cross-talk effect from adjacent photoreceptors and post-photoreceptor neurons were reduced (compared to full-field illumination/recording system). Localized IOS responses with magnitude peaks up to 40% ΔI/I were consistently observed (Fig. 2). Because both the visible stimulus and the NIR recording light were focused at the photoreceptor layer, and because the very short IOS delay after stimulus onset, we believe that the observed IOSs were resulted primarily from the activities of the photoreceptors. Moreover, the MLA-based imager can evaluate multiple locations over an extended retinal region simultaneously, producing an accurate retinotopic function map in one image sequence. This recording method avoids assembling measurements taken from different time frames, and thus is much less vulnerable to inevitable eye movements. In the future development of IOS-based clinical ophthalmic devices, the ability of parallel recording of multiple retinal loci can be a great advantage over other retinal function evaluation modalities, such as visual field or multi-focal ERG. We anticipate that further experimentation on the properties of the visible light stimulus, such as its temporal modulation, spatial layout, color and intensity will allow selective evaluation of the rod and cone systems. Accurate assessment of localized retinal rod and/or cone photoreceptor dysfunctions will lead to early detection and improved treat-
ment evaluation of the eye diseases such as cone-rod dystrophy, AMD, RP, that are known to produce pathological changes in the retinal photoreceptors in their early stage.

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REFERENCE


Fig.1. (a) Schematic diagram of the MLA recording system: CO, collimator; DM, dichroic mirror; MLA, microlens array; PBS, polarization-sensitive beam splitter; QW, quarter-wave plate. Focal lengths of the lenses L1 and L2 are 80 and 150 mm, respectively. NIR SLD was used for IOS imaging. Red laser diode (LD) was used for retinal stimulation. NIR LED was used for transmission imaging of the retina. At the dichroic mirror (DM), collimated NIR recording light and visible stimulus light were combined together to illuminate microlens array (MLA) simultaneously. (b) Representative MLA pattern by placing a mirror at the specimen plane. (c) Representative MLA imaging pattern of an isolated frog retina. (d) Simultaneous MLA recording and transmission imaging of the retina.
Fig. 2. (a) MLA recording of retinal activation. Each illustrated IOS image is an average over 600 ms interval (i.e., 200 frames, 3 ms/frame). (b) Enlargement of the 4th frame in (a). (c) Black traces 1-8 show IOSs of randomly selected 8 pixels pointed by the arrowheads in (b). (d) Black traces 1 and 4 show the average of all the positive and negative IOSs within the MLA spot areas. Gray traces 2 and 3 show the positive and negative IOSs without stimulus. (e) IOS magnitude map of the single-trial MLA recording shown in (b). (f) Averaged IOS magnitude map of 6 MLA recordings. (g) Temporal dynamics of overall IOS magnitude shown in (f).
CONCLUSIONS AND FUTURE DIRECTIONS

A rapid microlens array (MLA) imager was developed for parallel recording of intrinsic optical signals (IOSs) correlated with localized retinal photoreceptor responses. In this imager, a microlens array MLA based illuminator is employed to simultaneously deliver visible stimulus light for retinal stimulation and near-infrared (NIR) recording light for IOS imaging. The parfocal configuration of the stimulus and recording light illumination enables confocal recording of the stimulus-evoked IOSs. Because the multiple stimulation/recording light spots are widely separated on the retina, and only the photoreceptors within the light spots are stimulated, the potential IOS cross talk effect among neighboring retinal areas can be minimized. Using freshly isolated animal retinas, our preliminary experiments revealed robust IOS activities tightly correlated with localized retinal photoreceptor responses. The microlens array (MLA) imager was integrated with optical coherence tomography to further decrease the cross talk effect.

Isolated retina is an easier way to detect and measure the IOSs from photoreceptors. To step towards the development of IOS based clinical ophthalmic device, line scan confocal imager was employed to achieve high spatiotemporal resolution imaging of fast IOSs in anesthetized frog retina. By rejecting out-of-focus background light, individual frog photoreceptor was resolved in vivo. With hemodynamic changes and intact nerve system, fast IOSs were still detected with comparable time course with ERG, which further confirmed the clinical application of fast IOSs in the field of retinal functional measurement.

With continuous oxygen and nutrition supply, in vivo IOS imaging would be conducted in more stable conditions, such as high retinal neuron viability, recovery from
photoreceptor bleaching during the experiment. Further investigation in different visible light stimulus conditions, such as the change of stimulus light length, light wavelength, and light intensity may be able to differentiate the IOS responses between rod and cone systems. Accurate assessment of localized retinal rod or cone photoreceptor dysfunctions will lead to early detection and improved treatment evaluation of eye diseases, such as cone-rod dystrophy, age-related macular degeneration, and retinitis pigmentosa.
GENERAL LIST OF REFERENCE


APPENDIX A

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Animal Care and Use Committee (IACUC)

NOTICE OF APPROVAL

DATE: July 20, 2010
TO: Yao, Xincheng
     Shelby 805
     934-7459

FROM: Judith A. Kapp, Ph.D., Chair
       Institutional Animal Care and Use Committee

SUBJECT: Title: Development of a Microlens Array Confocal Ophthalmoscope
         Sponsor: Eyesight Foundation of Alabama
         Animal Project Number: 100708546

On July 20, 2010, the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) reviewed the animal use proposed in the above referenced application. It approved the use of the following species and numbers of animals:

<table>
<thead>
<tr>
<th>Species</th>
<th>Use Category</th>
<th>Number in Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frogs</td>
<td>A</td>
<td>96</td>
</tr>
<tr>
<td>Rabbits</td>
<td>B</td>
<td>24</td>
</tr>
</tbody>
</table>

Animal use is scheduled for review one year from July 2010. Approval from the IACUC must be obtained before implementing any changes or modifications in the approved animal use.

Please keep this record for your files, and forward the attached letter to the appropriate granting agency.

Refer to Animal Protocol Number (APN) 100708546 when ordering animals or in any correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at 934-7692.