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Real time in vivo imaging and measurement of serine protease activity in the mouse hippocampus using a dedicated complementary metal-oxide semiconductor imaging device

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Abstract

The aim of the present study is to demonstrate the application of complementary metal-oxide semiconductor (CMOS) imaging technology for studying the mouse brain. By using a dedicated CMOS image sensor, we have successfully imaged and measured brain serine protease activity in vivo, in real-time, and for an extended period of time. We have developed a biofluorescence imaging device by packaging the CMOS image sensor which enabled on-chip imaging configuration. In this configuration, no optics are required whereby an excitation filter is applied onto the sensor to replace the filter cube block found in conventional fluorescence microscopes. The fully packaged device measures 350 μm thick × 2.7 mm wide, consists of an array of 176 × 144 pixels, and is small enough for measurement inside a single hemisphere of the mouse brain, while still providing sufficient imaging resolution. In the experiment, intraperitoneally injected kainic acid induced upregulation of serine protease activity in the brain. These events were captured in real time by imaging and measuring the fluorescence from a fluorogenic substrate that detected this activity. The entire device, which weighs less than 1% of the body weight of the mouse, holds promise for studying freely moving animals.

Keywords: CMOS image sensor; On-chip imaging; Biofluorescence; In vivo; Hippocampus; Serine protease

1. Introduction

Investigation of brain plasticity is important to elucidate the functions of the brain. The advent of increasingly sophisticated microscopes in combination with advances in fluorescence methods has made possible direct visual identification of structural changes of dendritic spines in the brain. Recently, there has been development in miniaturized microscope systems that are able to provide high-resolution brain images of freely moving animals (Helmchen et al., 2001; Mizrahi et al., 2004). However, these systems are unable to overcome the limitation of photon penetration depth for extremely deep imaging of the brain. Hence, deep structural imaging of the intact brain remains elusive. In this work, we propose an alternative approach to imaging the brain. We have taken the bottom-up approach whereby a single CMOS image sensor element forms the basis of the imaging system.

We propose the use of a cheaper and more widely available CMOS image sensor for optical imaging of the brain due to its many advantages and potential. A CMOS image sensor is capable of high spatial and temporal resolution optical imaging; has potential for multi-parameter sensing which includes gas, temperature, pH, ions, and electrical potential on a single device (Hagleitner et al., 2001; Hammond et al., 2004; Sawada et al., 2005; Tokuda et al., 2006; Zeck and Fromherz, 2001); proven multi-site electrical stimulation capability (Furumiya et al., 2006; Ohta et al., 2005b); and finally potential for wireless operation which will benefit observation of freely moving animals (Chien and Jaw, 2005; Mavoori et al., 2005). We believe that the range of applications for such a device lies somewhere in between established methods for in vitro observations and non-invasive in vivo imaging. Hence, there is a wide range of unexplored, and yet to be identified applications that will benefit from its development once the method has been established.

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The aim of this work is to develop an alternative approach for in vivo biofluorescence imaging of the brain at arbitrary depth. We present the development and verification of a CMOS imaging device for this purpose. Ultimately, this work is expected to lead to a promising new tool where imaging and measurement can be made using a device where power and data are transferred wirelessly. This will lend itself greatly by complementing existing methods for studying freely moving animals.

We previously reported a CMOS imaging device for in vitro and in vivo fluorescence imaging and measurement. It was used to image internally inside the mouse brain (Ng et al., 2006b). Also, using a model reaction inside a brain tissue phantom, we have demonstrated the possibility of biofluorescence measurement in vivo (Ng et al., 2006a). In this work, we describe the use of the device to study serine protease activity in the mouse brain due to the importance of understanding the events that lead to seizures. Early works in this area have linked serine protease induction in the brain with the occurrence of seizures (Chen et al., 1995; Qian et al., 1993). Furthermore, it was determined that the protease actually plays an important role in the onset or facilitation of this event (Momota et al., 1998; Tsirka et al., 1995). In fact, it has been found that serine proteases like tissue-type plasminogen activator (tPA) are induced by kainic acid (KA), which causes epileptic seizures in normal mice (Matsuoka et al., 1998; Nagai et al., 1999). Current methods employed to study the effect of KA induced serine protease activity does not allow the exact time course of the events to be ascertained. That is, the actual onset and propagation of the protease activity leading to seizures are not known. Because of this, detection of serine protease in vivo is used as a model reaction to validate and verify the capability of the device for generating useful results from in vivo imaging. This study will be used as a platform to enable other work where molecular signaling pathways including brain plasticity can be independently verified.

2. Materials and methods

2.1. CMOS image sensor device and interface

The CMOS image sensor consists of a circuit schematic as shown in Fig. 1(A). Each photosensor pixel output is connected to a column bus line. The details of the image sensor have been described previously (Ng et al., 2006a). The analog output is connected to an off-chip interface circuit board, which is shown in Fig. 1(B). The output is amplified and digitized before read into a digital input/output (I/O) board. Control signals are supplied by a personal computer (PC). Because the maximum operating voltage of the chip is limited to 3.3 V, a voltage shift circuit is needed to convert the 5 V signals from the PC. The analog electrical current output signal from the chip is converted to voltage signals via a transimpedance amplifier before being digitized into 12-bit data and read into the digital I/O board inside the PC.

Customized software is developed to control the input and output signals. Digitized data from the image sensor is stored, processed, and displayed onto the screen. A screen capture of the program is shown in Fig. 2. Image 1 shows the reconstructed image from the data acquired from the image sensor chip. Image 2 is the image captured at program initialization when the background signal, i.e. without illumination light, is taken. Image 3 shows the inverted image where Image 2 is subtracted from Image 1. During program operation, the image at any point in time can be captured and used as a reference whereby subsequent images can be compared to. The last image, Image 4 represents the resultant image of the reference image subtracted from Image 1. This enables minute changes to be tracked in real-time. Auto-balancing operation for Image 3 and 4 enables high contrast images to be displayed. This operation essentially stretches the grayscales to the maximum possible 255 so that small changes in the measured values can be seen with the naked eye.

In the program, five pixels can be selected independently and their values can be read and recorded. For data analysis, the measured signal level is the actual recorded signal minus the background level signal. Real time plotting of the data enables quick monitoring of changes in the signal during experiments.

2.2. Device design and packaging

A 176 × 144 array image sensor chip was designed and fabricated using a standard CMOS process. Due to its high photon responsitivity, the *n*-well/*p*-substrate photodiode was utilized. Each pixel has a size of 7.5 μm × 7.5 μm. The dimension of the chip is 2 mm × 2.5 mm. It was designed to be large enough to image the mouse hippocampus yet small enough for invasive imaging of each brain hemisphere independently. The chip was designed in house but was fabricated by an IC foundry using a standard 0.35 μm CMOS process.

The bare chip was returned pre-thinned with a thickness of 150 μm. It was then bonded to a 100 μm thick flexible polyimide substrate. The polyimide substrate has pre-printed wiring for interfacing between the chip and measurement equipment. It is fully flexible and has been shown to be biocompatible for surgical insertion of embedded devices into the living body (Rousche et al., 2001). The bond pads on the chip were then wire-bonded to the polyimide substrate. The chip was then sealed in an optically transparent and water proof epoxy resin to protect it in the biological environment. Finally, a color filter was spin-coated onto the device. The filter is intended to block off excitation light allowing only fluorescent emission to reach the image sensor. A color filter, which has high transmittance for wavelengths in the range from 500 nm and beyond was used. This filter offers high selectivity for the fluorescence emission of 7-amino-4-methylcoumarin (AMC). We achieved a transmittance of −44 dB, which is comparable to discrete filters used in fluorescence microscopes (Ng et al., 2006a).

A hypodermic needle (27G × 3/4 Terumo, Tokyo) was then attached to the device. It was connected to a microsyringe (1001RN Hamilton, USA) via Teflon tubing. A syringe pump (CFV-2100 Nihon Kohden, Japan) was used to control the injection flowrate. Fig. 3 shows the fully fabricated device. The thickness is about 350 μm while the width is about 2.7 mm. A thin device is necessary to minimize damage to the subject during in vivo imaging. The entire device including the substrate weighs about 0.3 g, enabling experiments with small laboratory animals such as a mouse.