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On-chip biofluorescence imaging inside a brain tissue phantom using a CMOS image sensor for in vivo brain imaging verification

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Abstract

We have developed an on-chip biofluorescence imaging device by packaging a dedicated CMOS image sensor using a simple technique. By using on-chip imaging configuration, we have verified in vivo fluorescence imaging inside a specially developed brain tissue phantom that closely resembles the mammalian brain. In order to implement on-chip imaging, an excitation light filter is applied onto the chip. A transmittance of −44 dB is achieved by multiple coating of the filter. On-chip measurement of the AMC fluorophore shows that detection of concentration of 1 μM is possible. The phantom medium has mechanical rigidity and optical property similar to the mouse brain. Feasibility of imaging the released fluorophore inside the phantom sample is demonstrated. It is shown that light scattering in the phantom medium does not reduce the image resolution considerably, if the imaging depth is kept below 500 μm. The fully packaged chip, specially designed with this technique is about 350 μm thick and 2.7 mm wide. The image sensor pixel size of 7.5 μm × 7.5 μm is close to the size of a single neuron cell. Although the device is designed specially for in vivo imaging of the mouse hippocampus to study its neuronal activity, a wide range of applications are foreseen in the biomedical and pharmaceutical field.
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1. Introduction

Fluorescence technique is a highly specific and sensitive method for detection of chemical and biomolecular events. Because of the high specificity of this method, it is often used to detect the existence of a certain biomolecular species. Biofluorescene refers to fluorescence of biological origin. To date, a great number of fluorescence probes or fluorophores have been developed that target specific cellular ions, DNA, or proteins [1].

Fluorescence detection is possible due to the difference in wavelength of the excitation and emitted fluorescence light. By carefully filtering of the two wavelengths, the emitted fluorescence signal can be detected and measured. Traditionally, fluorescence detection systems are based on an instrument, which consists of a light source, excitation and emission filters and a photodetector. Spectrofluorometers and fluorescence microscopes are examples of these systems that are widely used for fluorescence detection and imaging. However, the size of these instruments excludes their use in high-throughput, portable diagnostic and in vivo sensing applications. Efforts to develop miniaturized systems have resulted in a variety of designs and implementations [2–6]. Recently, CMOS-based devices have shown viability as a new class of emerging micro sensing devices [7–13]. Some of these devices are increasingly being explored to fill a niche application, which involves fluorescence detection [14–16]. Although most of these work reported single pixel structures, one notable recent work involved the use of a CMOS image sensor for luminisecence detection and imaging [17]. In this work, we focus on the development of a dedicated CMOS image device for biofluorescence applications.

The advantages of using a CMOS image sensor for fluorescence imaging are high spatial and temporal resolution imaging at high frame rates. Also, on-chip imaging configuration enables arbitrary depth imaging which is a clear advantage over

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conventional imaging modalities [18–22]. CMOS image sensors have shown comparable sensitivity to conventional CCD image sensors for fluorescence detection [23,24]. Because of its inherent system-on-chip capability, a single chip is all that is required for sensing, signal processing and data interface, resulting in a highly integrated and compact device.

We identified a potentially useful application by using a CMOS image sensor for in vivo imaging of the brain to study synaptic plasticity. Dynamic changes in spines or synapses are important in memory and learning functions [25,26]. The serine protease, neuropsin (NP) is believed to play a major role in synaptic plasticity in the hippocampus [27]. NP is secreted into the extracellular space in an inactivate precursor form and is activated by the processing of a 4-amino acid peptide (QGSK) after its release [28–30]. NP is believed to be activated by an unidentified NP activator (NPA) after theta-burst stimulation in the hippocampus. In this study, a synthetic fluorogenic substrate with the QGSK oligopeptide and a 4-methylcoumarin-7-amide (MCA) fluorogenic derivative, QGSK-MCA, was used as a substitute material to simulate NP activity in vitro. Detection of this substrate was performed by treatment with a peptidase, lysyl endopeptidase (lys-C), which cleaves the peptidal bond, hence releasing the fluorometrically detectable aromatic amine, 7-amino-4-methylcoumarin (AMC) as shown in Fig. 1 [31,32]. In a separate experiment, we found that this substrate had similar response to NP in its activated state whereby both were specifically cleaved by lys-C in vivo. Due to its highly fluorescent product, this substrate was used in combination with lys-C to serve as a convenient enzyme assay whereby imaging and quantitative measurement could be made. A specially prepared brain tissue phantom medium for use as an in vitro surrogate for in vivo brain tissues was developed as the platform for verifying fluorescence from the released AMC can be detected by using the CMOS image sensor.

This aim of this work is to develop a new approach for in vivo biofluorescence imaging. We present the development and verification of a CMOS imaging device for in vivo biofluorescence imaging. This work is expected to lead to a promising new tool for in vivo imaging of freely moving animals.

2. CMOS image sensor

2.1. Sensor design and interface

The heart of a CMOS photosensor is a photodiode, which converts light into electrical current. The photodiode structure is available in standard CMOS fabrication process and can be integrated with other electronic components. Monolithic connections from the photodiode to transistor switches enable the signal output of the photodiode to be read-out. Fig. 2(a) shows the schematic of the photosensing circuit. It is a modified 3-transistor active pixel sensor circuit, which consists of a photodiode, and select and reset transistor switches. Photons that impinge onto the photodiode are converted into electrical charges. A constant light intensity, Φ is converted into a steady photocurrent, i_L. The photocurrent discharges the photodiode and the photodiode voltage decreases with time. The slope of the discharge curve is related to the intensity of the incident light. This relationship can be used to measure the incident light intensity. The relationship between photocurrent, i_L and discharge time, t_{dis} can be expressed by

$$t_{dis} = \frac{C_{PD}\Delta V}{i_L^\gamma}, \quad (1)$$

where C_{PD} is the photodiode capacitance, and ΔV, the voltage drop between the duration time t_{dis}. The denominator term, i_L has a power correction factor γ. This term is closely tied to the experimental measurement condition, and is equal to 1 for the ideal case. Meanwhile, the photocurrent is related to the incident light intensity as follows

$$i_L = R \cdot A \cdot \Phi + i_D, \quad (2)$$

where R is the photodiode responsivity, A, the photodiode area and i_D, the photodiode dark current. By substituting Eq. (2) for Eq. (1), a modeling equation with the expression

$$y = \frac{a}{(1 + x/b)^\gamma}, \quad (3)$$

can be used to describe the photosensor sensitivity, where y is the output and x is the input corresponding to t_{dis} and i_L, respectively. Here, a, b, and γ are fitting constants.

The image sensor comprises of an array of photosensors or pixels, each being addressed while its output is read-out consecutively. The output from the photosensors are connected to a vertical column bus and selected using the row select signal, RowSel. The block diagram of the image sensor circuit is shown in Fig. 2(b). The pixel reset signal, Rst controls the integration or frame time of the image sensor. A row and column scanner circuit is used to generate the row and column select signals RowSel and ColSel, respectively. The image sensor output is connected to a buffer for output to an external current-to-voltage (I/V) converter followed by a 12-bit analog-to-digital (A/D) converter.

Serial interface is used for the readout in order to reduce the number of interconnections for the device. In this

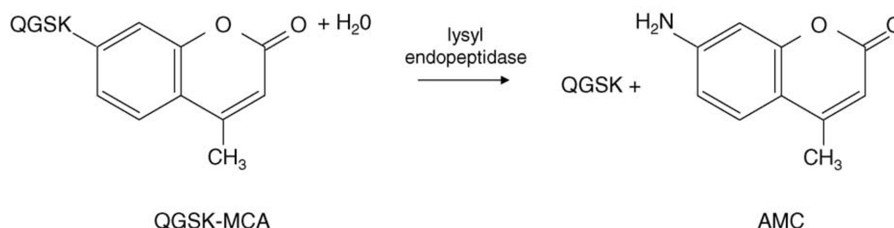

Fig. 1. Cleaving of synthetic substrate, QGSK-MCA by lysyl endopeptidase releases AMC, which can be detected fluorometrically.

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