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A New Neural Imaging Approach Using a CMOS Imaging Device

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Abstract—We have developed and demonstrated the use of a dedicated CMOS device for in vivo functional imaging of the mouse brain. In order to achieve this, a 176 × 144 pixel array image sensor is designed, fabricated and specially packaged using a novel process. By using on-chip fluorescence imaging configuration, we have successfully imaged deep inside the hippocampus of the mouse brain. Functional imaging is verified by using a fluorogenic substrate that detects the presence of serine protease in the brain. Introduction of kainic acid induces the expression of the serine protease. The protease reacts with the substrate which then fluorescence. By imaging and measuring the fluorescence signal, we have successfully measured the brain protease activity and accurately determined its reaction onset. This method represents a novel approach for neural imaging.

I. Introduction

Of all living tissues, the brain must be the most studied due to our fascination with its functions and complexity since antiquity. Efforts to unravel the secrets of the brain have led to the development of myriad of methods from different disciplines. These range from the traditional direct probe electrodes up to highly sophisticated imaging devices like functional magnetic resonance imagers (fMRI).

Recently, semiconductor based devices has been shown to be a viable platform for studying active neuronal networks. [1]. We are exploring ways that CMOS technology can be utilized for neuroimaging applications [2-3]. The CMOS technology is chosen due to its inherent high spatial and temporal resolution imaging capability. Furthermore, the advantages of CMOS sensors extend beyond optical imaging as it has been reported to be able to resolve ions, pH, temperature, pressure, electrical potential and ions [4-7]. Also, multi-site electrical stimulation capability, and the potential for wireless operation makes it a highly attractive platform for further development [8]. By combining these functions onto a single chip device, a multimodal bioimaging and biosensing tool can be realized.

In the present study, we describe the use of a CMOS image sensor for in vivo functional imaging of the mouse brain. By reducing the thickness of the device to approximately that of the diameter of electrodes used in physiological studies, we perform imaging inside the brain. This way, we are able to increase the depth of imaging deep into the hippocampal region of the intact brain beyond the penetration depth of photons, which is a severe limitation for conventional microscopy. We believe that the range of applications for this kind of device lies between established methods for in vitro observations and non-invasive imaging methods. This represents many unexplored applications such as real time physiological and behavioral study of small animals at the cellular response level. Ultimately, this work is expected to lead to a promising neuro-interface device where imaging, stimulation, and recording can be made using a single device, and power and data are transferred wirelessly as shown in Fig. 1. This will revolutionize existing methods for studying the brain of freely moving animals.

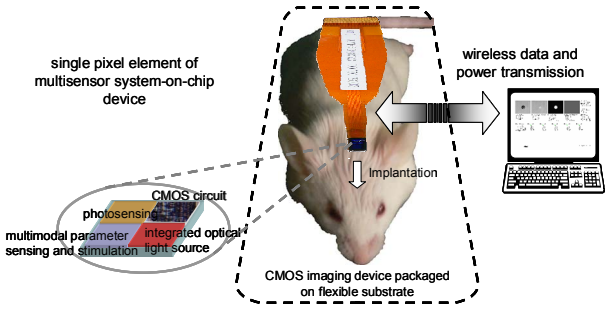

Fig. 1 Schematic of proposed wireless multimodal CMOS imaging system for imaging of freely moving animal.

II. CMOS Imaging Device

A. CMOS Image Sensor

A 176 × 144 array image sensor chip was designed and fabricated using the standard CMOS process. Its specification is shown in Table 1. Each pixel has a size of 7.5 × 7.5 μm^2. The dimension of the chip is 2 × 2.2 mm^2. It was designed to be large enough to image the mouse hippocampus and yet small enough for invasive imaging of each brain hemisphere independently. A photomicrograph of the CMOS image sensor with circuit schematic is shown in Fig. 2. The pixel consists of a modified 3-transistor active pixel sensor circuit. In the design, only a single side of the chip has input-output pads. This helps reduce complexity in wire bonding during the packaging process and helps reduce the final packaged

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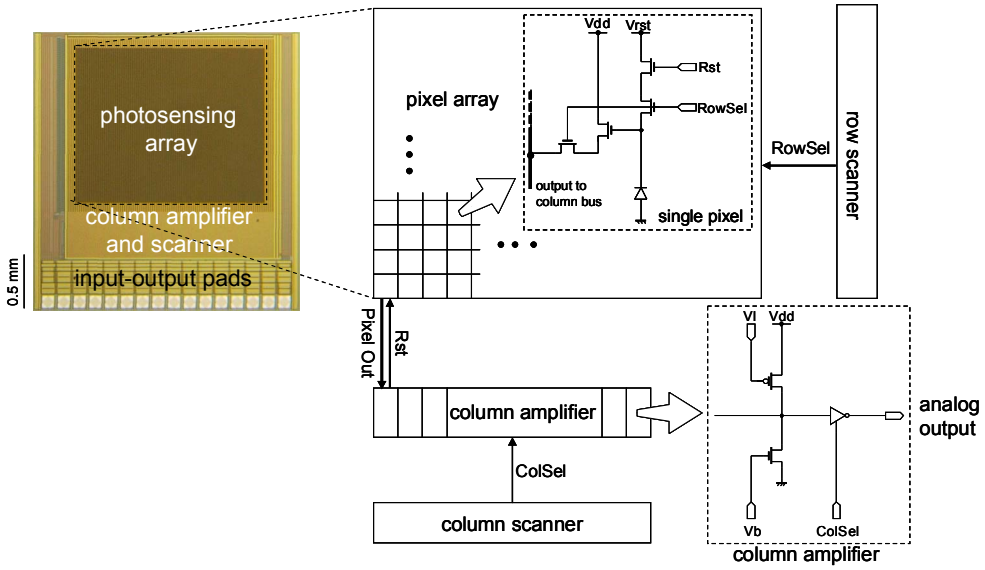

Fig. 2 Photomicrograph of CMOS image sensor chip inlaid with block diagram of sensor circuit showing the photosensor pixel and column amplifier circuit.

TABLE 1 SPECIFICATION OF CMOS IMAGE SENSOR CHIP.

Technology		0.35 µm Std. CMOS 4M2P
Power supply		3.3 V
Chip size		2 mm x 2.2 mm
Pixel	: type	modified 3-transistor APS
	: number	176 x 144 (QCIF)
	: size	7.5 x 7.5 µm^2
Photodiode	: type	Nwell-Psub
	: size	16.2 µm^2
Output		analog current
Frame rate (with external A/D)		0.08 – 16.5 fps

device size. This is an important factor for implementing on-chip in vivo imaging.

The analog output is connected to an off-chip interface circuit board. The measurement setup interface is shown in Fig. 3. The analog voltage output signal from the chip is digitized into 12-bit data using an analog-to-digital (A/D) converter and read into the digital (input-output) I/O board (PCI-2772C Interface Corp., Japan) inside the 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 control signals are supplied by a PC via the I/O board.

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. Image subtraction of a background image captured at the beginning of the program during the program operation enable minute changes to be tracked. Imaging dynamic range is increased by changing the integration time. This way, up to four decades of light intensity change can be faithfully imaged. Further, auto balance operation enabled high contrast image to be displayed. This operation stretches the image grayscales to the maximum 255 so that minute changes can be observed. Finally, real time data plot enable quick monitoring during

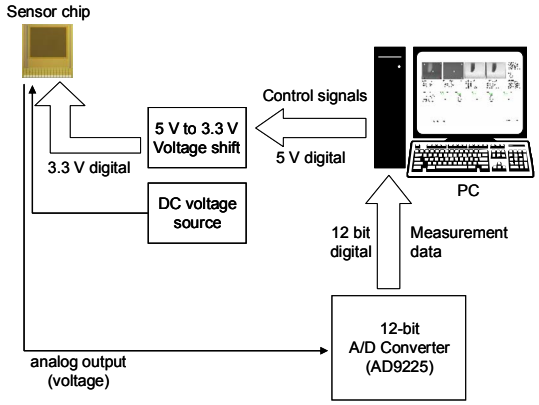

Fig. 3 Schematic of the device measurement setup and PC interface.

the experiment.

A. *Device Packaging*

In order to perform in vivo imaging, the CMOS image sensor chip was packaged to enable on-chip fluorescence imaging. The 150 µm thick image sensor chip was attached to a 100 µm thick flexible polyimide substrate. The input output pads of the chip was wire bonded to the pre-patterned polyimide substrate and the chip was sealed in an optically transparent and epoxy resin to protect it in the biological environment. Finally, a color filter was spin-coated onto the device. The filter offers high selectivity of more than 80 % for the fluorescence emission of 7-amino-4-methylcoumarin (AMC, 3099-v Peptide Institute, Japan) while blocking off excitation light with wavelengths of 460 nm and 370 nm, respectively. By applying multiple coating of the filter, a transmittance below -44 dB, which is close to the discrete filters used in fluorescence microscopes, is achieved. The fully packaged device is shown in Fig. 4. It is about 350 µm thick and 2.7 mm wide. The entire device including the

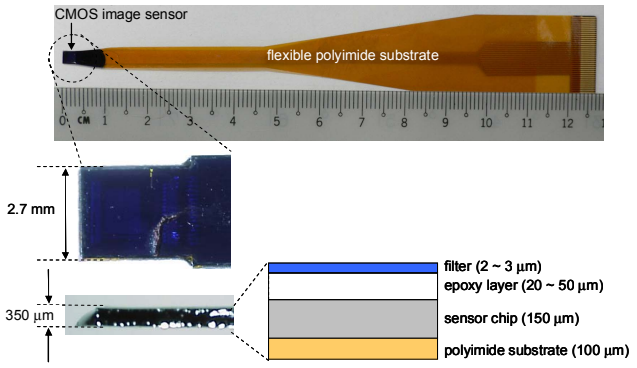

Fig. 4 Top view of fully packaged imaging device. Inset shows top view and side view of CMOS image sensor. The cross sectional view showing the approximate thickness profile is depicted in the diagram on the bottom right.

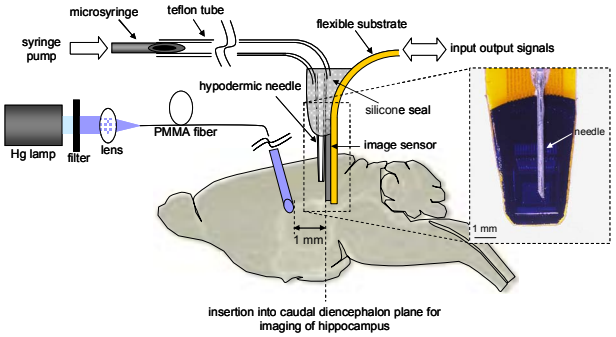

Fig. 5 Schematic showing cross sectional view of the device with electrical and fluidic connections in the mouse brain. The device is inserted along the caudal diencephalon plane for imaging of the hippocampus. Inset shows top view of the hypodermic needle attached to the fully packaged CMOS image sensor.

substrate weighs about 0.3 g, is light enough for experiments involving small laboratory animals such as a mouse.

III. In vivo Functional Neuroimaging

A. Experimental Method

For the in vivo imaging experiments, adult male ddY mice (SLC Co., Hamamatsu, Japan; 9 weeks old) were used. The mice were anesthetized with urethane (1.25 g/kg; i.p.), then placed in a stereotaxic head-holder. The skull of the mouse was exposed and cleaned, and a small rectangular cranial window about 3 mm × 2 mm was made on the right side of the skull.

In order to perform functional imaging, the imaging device was used in conjunction with a fluorophore substrate to detect the activity of serine protease in the brain. Serine protease such as neuropsin and tissue-plasminogen activator can be artificially induced by the presence of kainic acid. Once these serine protease are expressed extracellularly, they react with the substrate to release the AMC fluorophore.

The entire device was used to perform imaging deep into the hippocampus of the mouse brain as shown in the schematic in Fig. 5. Excitation light was provided using mercury lamp (VB-L10 Keyence, Japan) as the light source. A bandpass filter (OP42311 Keyence, Japan) was used to selectively transmit monochromatic light at 387 nm with a 28 nm band, which was then coupled into a 500 µm diameter PMMA jacketed fiber (SK-20 Mitsubishi Rayon, Japan). It was found that a distance of the fiber tip of about 1 mm from the sensor surface provides the optimum lighting conditions. Due to scattering of light inside the brain, a fairly uniform illumination was obtained. The fiber tip is tapered at 30% from the fiber axis and polished for maximum delivery of light as well as to ease insertion into the brain. The measured output light power was approximately 20 µW at 365 nm. A hypodermic needle (30G × 1" Terumo, Tokyo) was used to inject the fluorogenic substrate close to the surface of the imaging array. It was connected to a microsyringe (1001RN Hamilton, USA) and a syringe pump was used to control the injection flowrate of the substrate into the brain. The fluorogenic substrate consists of an equal mixture of Boc-Val-Pro-Arg-4-methylcoumarin-7-amide (VPR-MCA) and Pyr-Gly-Arg-4-methylcoumarin-7-amide (PGR-MCA), (3093-v and 3145-v respectively Peptide Institute, Japan). Each substrate is specific in detecting the presence of a certain protease species in the brain. VPR-MCA is used to detect activated neuropsin (act NP) which is induced by kainic acid (KA) in the hippocampal region, while PGR-MCA specifically targets tissue-type plasminogen activator (tPA). These substrates are hydrolyzed due to the presence of the specific protease which acts as the catalyst for the process. A 1:1 mixture of 1 mM VPR-MCA and 1 mM PGR-MCA in 45 mM Tris buffer (pH8.0) was prepared. The substrate was pumped into the brain at a rate of 0.08 µl/min for one hour. Fifteen minutes after substrate pump has started, 800 µl (20 mg/kg) of KA at 1 mg/ml was injected intraperitoneally. Serine protease activity was measured continuously for 5 hours after KA injection.

During the initial stage where the imaging device was being inserted into the brain, the image sensor was operated at a high frame rate of 10.8 fps. This enabled visual feedback at near video rate. Once the device was fully inserted into the hippocampal region, illumination was provided by excitation light from the fiber only. Frame rate was then dropped to a slower 3.85 fps to increase measurement sensitivity. At the end of the experiment, the brain was extracted, sliced and observed using a fluorescence microscope.

B. Imaging Results

During the experiment, the images inside the brain was continuously recorded and plotted in real time. In was found that after injection of KA and the substrate, the entire image brightness increased drastically after a certain amount of time. This increase in signal corresponds with the activity inside the brain as AMC fluorescence is released from the substrate. A plot of the signal level from a single pixel location of the captured is shown in Fig. 6. This location is close to the site where the substrate was released from the injection needle. From the plot, the abrupt increase in signal was observed at

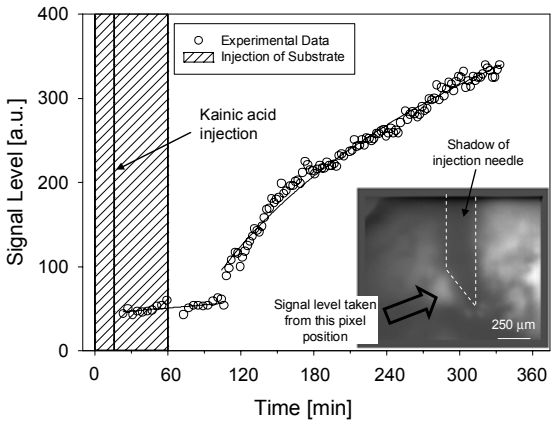

Fig. 6 Measured AMC fluorescence corresponding to the protease activity in the hippocampus. The inset image shows a captured image during in vivo experiment. The brighter region on the right part of the image is due in part to the excitation light from the fiber. The dark shadow in the middle of the image is the shadow of the injection needle. The left portion, which was completely dark initially, gradually brightened.

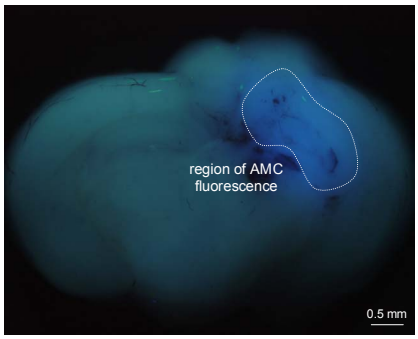

Fig. 7 Image of the brain slice facing the sensor. Note that the AMC fluorescence is localized at the hippocampus where the substrate was injected.

about 1 h 20 min after KA injection. This is the onset of serine protease expression inside the brain. Beyond that, the signal shows a sustained and gradual rise throughout the entire recording period which lasted slightly longer than 5 hours. This indicates that serine protease is somehow activated by KA injection. When the same experiments were repeated, a similar reaction response time was obtained suggesting that KA induced serine protease expression follows a predictable reaction time course.

The brain slices after the imaging experiment is shown in Fig. 7. From this, we obtained visual confirmation of the AMC fluorescence which was localized in the hippocampal region as expected. Furthermore, we observed that there was no blood coagulation inside the brain and injury to the brain was minimal. This suggests that the device inflicted minimal injury to the brain because the increase in fluorescence signal during in vivo imaging suggests that the brain cells continue to function and respond normally. In a separate experiment, stimulus and recording electrodes were inserted into the brain during the imaging experiment to confirm that the cell in the hippocampus continues to respond normally.

The result obtained in this experiment is important in a number of ways. First, we have demonstrated the capability of the imaging device for detecting brain activity in real time. We believe that this is the first reported simultaneous high resolution imaging and fluorometric measurement using a CMOS image sensor. Second, we have independently verified findings which reported the increase in protease activity due to KA which was achieved by in vitro methods [9]. Third, minimal injury was inflicted onto the brain using this imaging method. This was verified during the experiment, whereby images inside the brain show that the unaffected part continued to function normally and expressed the serine protease. Also, direct visual observation of the brain slice served as additional evidence to support this result.

IV. Conclusion

We have designed and developed a CMOS device for neuroimaging applications. A dedicated CMOS image sensor was developed and by packaging the image sensor chip for in vivo fluorescence imaging, we have successfully demonstrated functional in vivo imaging of the mouse hippocampus. This paves the way for further development of a CMOS device with multifunctional capability for the study of small animal brain.

References

[1] G. Zeck and P. Fromherz, "Noninvasive neuroelectronic interfacing with synaptically connected snail neurons immobilized on a semiconductor chip," *Proc. Natl. Acad. Soc.*, vol. 98, pp. 10457-10462, 2001.

[2] D. C. Ng, T. Tokuda, A. Yamamoto, M. Matsuo M. Nunoshita, H. Tamura, Y. Ishikawa, S. Shiosaka, and J. Ohta, " On-chip biofluorescence imaging inside a brain tissue phantom using a CMOS image sensor for in vivo brain imaging verification," *Sens. Actuators B,* 2006; in press.

[3] D. C. Ng, T. Tokuda, A. Yamamoto, M. Matsuo M. Nunoshita, H. Tamura, Y. Ishikawa, S. Shiosaka, and J. Ohta, "A complementary metal-oxide semiconductor image sensor for on-chip in vitro and in vivo imaging of the mouse hippocampus," *Jpn. J. Appl. Phys.*, 2006; in press.

[4] C. Hagleitner, A. Hierlemann, D. Lange, A. Kummer, N. Kerness, O. Brand, and H. Baltes, "Smart single-chip gas sensor microsystem," *Nature*, vol. 414, pp. 293-196, 2001.

[5] P. A. Hammond, D. Ali, and D. R. S. Cumming, "A System-on-Chip Digital pH Meter for Use in a Wireless Diagnostic Capsule," *IEEE Sensors J.*, vol. 4, pp. 706-712, 2005.

[6] T. Tokuda, A. Yamamoto, K. Kagawa, M. Nunoshita, and J. Ohta, A CMOS image sensor with optical and potential dual imaging function for on-chip bioscientific applications, *Sens. Actuators A*, vol. 125, pp. 273-280, 2006.

[7] Sawada K, Ohshina T, Hizawa T, Takao H, Ishida M., "A novel fused sensor for photo- and ion-sensing," *Sens. Actuators B*, vol. 106, pp. 614-618, 2005.

[8] M. Ghovanloo, and K. Najafi, "A modular 32-site wireless neural stimulation microsystem," *IEEE J. Solid-State Circuits*, vol. 39, pp. 2457-2466, 2004.

[9] N. Nagai, T. Urano, E. Endo, H. Takahashi, Y. Takada, and A. Takada, "Neuronal degeneration and a decrease in laminin-like immunoreactivity is associated with elevated tissue-type plasminogen activator in the rat hippocampus after kainic acid injection," *Neurosci. Res.*, vol. 33, pp. 147-154, 1999.