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Development of a CMOS Image Sensor for Real Time In Vivo Imaging of the Protease Activity Inside the Mouse Hippocampus

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1. Introduction

CMOS technology is increasingly being applied into sensors in life sciences. One important application is the imaging of the brain to study its functions [1]. Current technology to image the brain requires expensive equipment which has limitations in terms of image resolution, speed or imaging depth which are essential for the study of the brain. We are developing a CMOS image sensor which is capable of real time in vivo imaging of the intact brain at arbitrary depths [2-3]. In our previous work, we have shown that fluorescence imaging inside the mouse brain was feasible. In this work, we have successfully demonstrated functional imaging inside the intact brain.

2. CMOS Imaging Device

Using standard 0.35 μm CMOS process, we have designed and fabricated a dedicated image sensor chip. The image sensor circuit is shown in Fig. 1. Its specifications is listed in Table 1. The chip is interfaced to a PC for readout of the output signal as well as input of control signals. Fig. 2 shows the schematic of the measurement setup.

In order to enable on-chip fluorescence imaging, we have packaged the sensor chip using a novel packaging technique. Using this technique, an extremely compact device measuring 350 μm in thickness was realized. Furthermore, a filter which has high selectivity for the fluorescence emission of 7-amino-4-methylcoumarin (AMC) is spin-coated onto the surface of the device. This enabled on-chip fluorescence imaging. The fully packaged chip is shown in Fig. 3.

Finally, to demonstrate the CMOS sensor device for imaging the brain activity, it was further developed to include a needle for injection of a fluorophore substrate and an excitation light fiber. Fig. 4 shows the fully developed device. Using this device in conjunction with the fluorophore substrate (PGR-MCA, VPR-MCA), we performed experiments to detect the presence of serine protease inside the mouse hippocampus. In the experiment, kainic acid (KA) was introduced as an agent which causes serine protease to be expressed extracellularly from the postsynaptic neuronal cells.

3. Result and Discussion

In the experiment, serine protease activity was observed in real-time by imaging the AMC fluorescence. The AMC fluorophore is released from the substrate due to the presence of the serine protease. Multiple pixel locations of the image sensor near the outlet of the injection needle were selected and plotted. A plot of the signal level from a single location is shown in Fig. 5. From the result, a significant increase in fluorescence signal is observed at about 1 hr 28 min after KA injection. This increase in signal is the result of the increase in serine protease activity localized near the injection needle. In order to confirm this observation, the mouse brain was extracted at the end of the experiment, sliced and observed under a fluorescence microscope. It was found that the brain slice in front of the sensor chip showed positive indication of AMC fluorescence localized in the hippocampal region where the substrate was injected. This verifies that the signal observed during the in vivo experiment was due to the AMC fluorophore released from the substrate.

From this experiment, several important implications can be made. First, the capability of the CMOS imaging device for detecting functional brain activity in real time is successfully demonstrated for the first time. Second, by using the device for in vivo serine protease imaging, we have independently verified reported findings on the effect of KA on the hippocampus. Finally, it was found that minimal injury was inflicted onto the brain using this method as the brain continued to function and respond normally with the device inside it.

4. Conclusion

We have developed a CMOS imaging device for functional in vivo imaging of the mouse brain. Using the device, we have successfully demonstrated in vivo imaging of serine protease activity inside the brain. Further development work is expected to fully realize the potential of this method.

Acknowledgements

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References

[1] R. D. Frostig, *In Vivo Optical Imaging of Brain Function* (2002) 21.
[2] D. Ng, et al., Extended Abstract SSDM, (2005) 74.
[3] D. Ng, et. al., Proc. SPIE, **6068** (2006) 1.

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

Fig. 1. Block diagram of sensor circuit showing the photosensor pixel and column amplifier circuit.

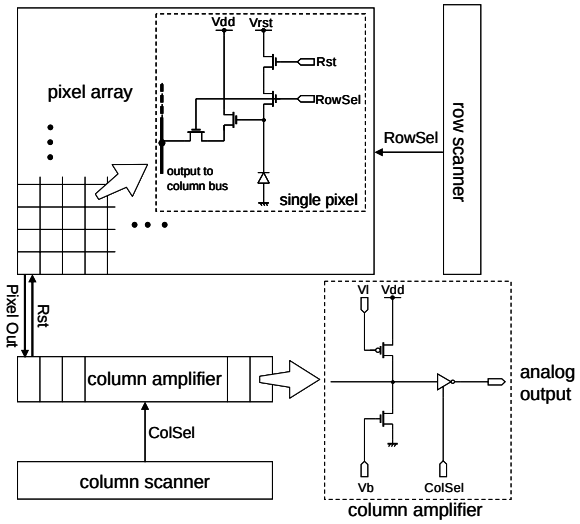

Fig. 2. Schematic of the device measurement setup and PC interface.

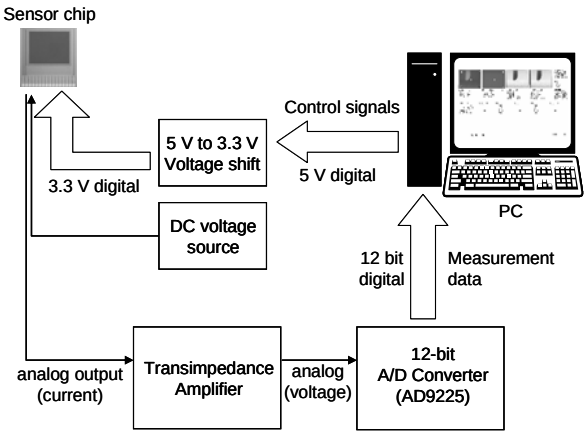

Fig. 3. Top view of fully packaged imaging device. Inset shows top view and side view of CMOS image sensor. The cross sectional view showing the approximate thickness profile is depicted in the diagram on the bottom right.

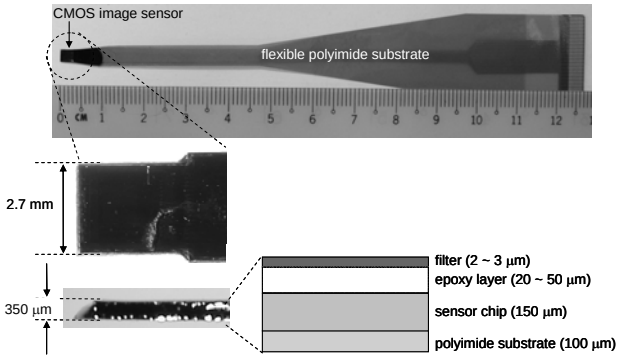

Fig. 4. Schematic showing cross sectional view of the device with electrical and fluidic connections in the mouse brain. The device is inserted along the caudal diencephalon plane for imaging of the hippocampus. Inset shows top view of the hypodermic needle attached to the fully packaged CMOS image sensor.

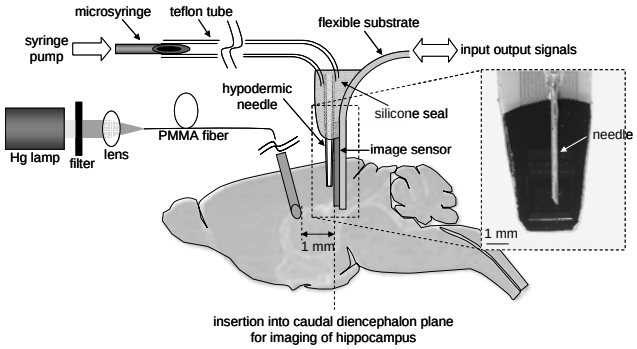

Fig. 5. Measured AMC fluorescence corresponding to the protease activity in the hippocampus.