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A CMOS Image Sensor for *in vitro* and *in vivo* Imaging of the Mouse Hippocampus

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1. Introduction

CMOS technology is increasingly being applied into sensors in the biological field. One important application is the imaging of the brain to study its learning and memory functions [1]. Current technology to image the brain requires expensive equipment which has limitations in terms of image resolution and speed or imaging depth which are essential for the study of the brain. We are developing a CMOS image sensor which is capable of both real time *in vitro* and *in vivo* imaging of the brain at arbitrary depths.

2. Image Sensor

Using standard 0.35 μm CMOS process, we have fabricated a prototype of the imaging chip. The image sensor is based on the photosensing circuit as shown in Fig. 1. It consists of a digital and analog output for interface with an external read-out circuit. Image sensing at near video rates is produced using the analog output. The digital output which enables pulse modulation output is suitable where long integration time is required in static imaging [2]. We have developed a novel packaging technique in order to apply the sensor chip for imaging of the brain [3]. The chip is first thinned down to about 200 μm. It is attached to a flexible and biocompatible polyimide substrate and protected with a layer of transparent epoxy. A filter which has high selectivity for the fluorescence emission of the neuronal membrane dye, DiA, is then spin-coated onto the surface of the chip. The fully packaged chip is shown in Fig. 2.

3. Result and Discussion

Photosensitivity

The photosensitivity of the image sensor was measured using a mercury lamp filtered with a 470 nm bandpass filter as the light source. The light intensity is varied using neutral density filters. By varying the frame rates, integration time of the photodetector pixel is changed to cover the input intensity range. The photosensitivity measurement is shown in Fig. 3. The result shows that lower frame rates enable high sensitivity measurement at the low light intensity region. By varying the frame rates, optimum imaging can be obtained during imaging experiments.

In vitro Imaging

A 400 μm mouse DiA-dyed brain slice of the hippocampus was used for *in vitro* imaging experiment. The brain slice was placed on top of the image sensor. Excitation light at 470 nm was used to uniformly illuminate the sample. The light captured by the image sensor originates from the fluorescence emitted from under the slice. As a comparison, the image from above the slice was captured using a CCD camera. Figure 4 shows the captured images. From the static image captured by the image sensor, the morphology of the hippocampus can be observed. In particular, sufficient contrast enabled the stained dentate gyrus layer to be distinguished from the other areas.

In vivo Imaging

The sensor chip is used to demonstrate real time *in vivo* imaging of a live mouse. Excitation light from the mercury lamp, filtered with a 470 nm excitation filter, is used to externally irradiate the exposed mouse brain. Figure 5 (A) shows the images captured by the image sensor during insertion into the brain. Fluorescence emission from the stained region in the hippocampus was clearly observed. The image shows the darker stratum pyramidal layer which is relatively unstained compared to the heavily stained CA1 region. At the end of the experiment, the brain is extracted and the slice facing the image sensor was observed under microscope (Fig. 5 (B)). It is found that only superficial injury is inflicted to the cortical area during the experiment.

4. Conclusion

Using the CMOS image sensor chip, we have successfully demonstrated *in vitro* and *in vivo* imaging of the brain. In this experiment both static and real-time fluorescence images of the dyed-brain were captured. This imaging method alleviates some of the problems related to deep brain imaging encountered by optical tomography and confocal microscopy where imaging is limited to the surface only. Implemented on a wireless platform, potential applications for the image sensor include neuronal imaging of truly-free moving animals.

Acknowledgements

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References

[1] R. D. Frostig, *In Vivo Optical Imaging of Brain Function* (2002) 21.
[2] T. Tokuda, et al., *ITE Technical Report* **28** (2004) 15.
[3] D. Ng, et. al., *Extended Abstracts of the JSAP 52nd. Spring Meeting* **3** (2005) 1466.

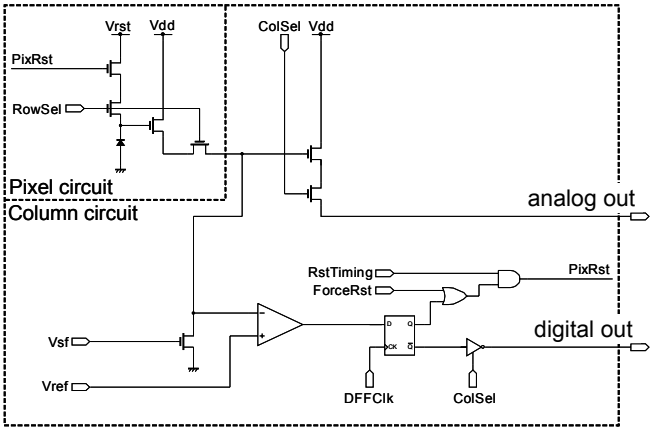

Figure 1. Single pixel photodetection circuit of image sensor.

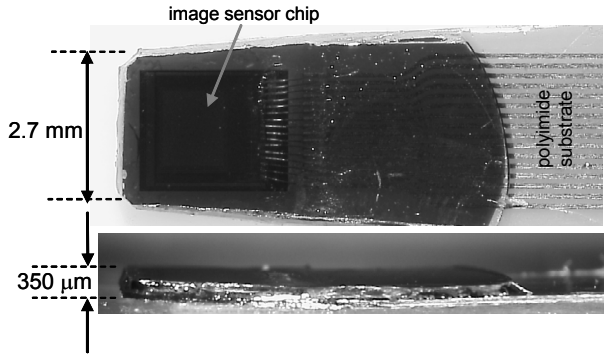

Figure 2. Packaged chip for *in vitro* and *in vivo* imaging. Darkened area show spin-coated emission light filter coating.

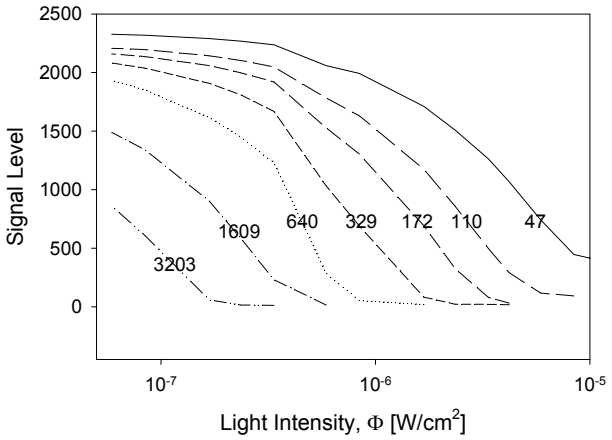

Figure 3. Light detection sensitivity of image sensor. Measurement is done using excitation light at 470 nm at varying intensities. The integration time (in ms) is varied over the range of incident light intensities.

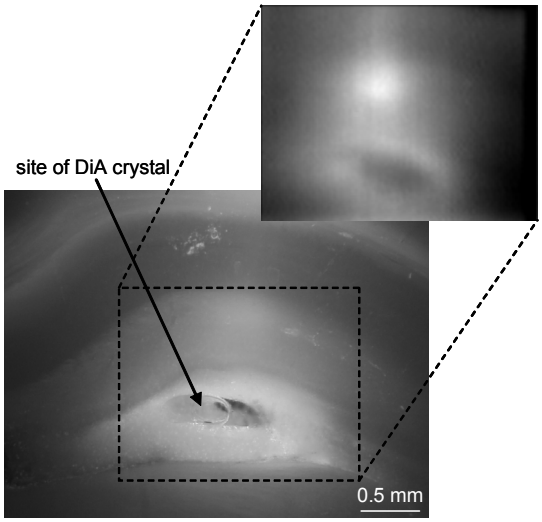

Figure 4. Fluorescence image of a 400 μm thick DiA-dyed mouse brain slice showing the hippocampus structure captured by image sensor (top) compared to image captured by CCD camera (bottom).

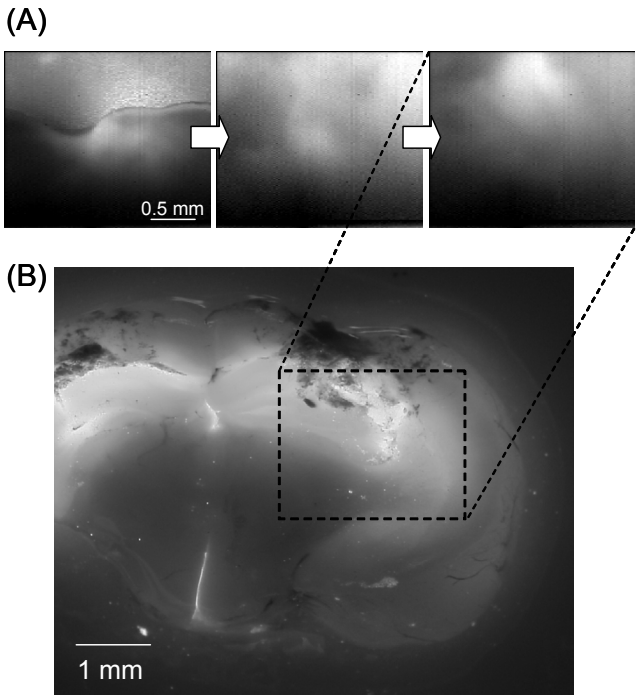

Figure 5. (A) Successive images from real-time video captured during *in vivo* imaging experiment. The final image shows fluorescence emission from the stained region in the hippocampus. (B) Comparison made with the brain slice after the experiment shows fluorescence from the same stained region as detected by the image sensor.