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Pulse modulation image sensors for in vitro and in vivo on-chip brain imaging

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Abstract: Image sensors with pulse modulation photosensing scheme were designed for *in vitro* (out of a living body) and *in vivo* (in a living body) on-chip brain imaging. The pixel circuitry of the pulse modulation measurement sensor is compatible with a conventional 3-Tr APS pixel. The sensor can be operated in either pulse modulation or APS measurement scheme. A packaging technique for on-chip brain imaging was also developed. Experimental demonstrations of *in vitro* and *in vivo* brain imaging were performed.

I. INTRODUCTION

There is a growing interest in developing LSI-based sensor system for on-chip biosensing/bioimaging applications. Several pioneering works have been done in the field of 2-dimensional voltage sensing of neural activity [1, 2]. We propose CMOS vision-chip-based image sensors specially designed for on-chip bioimaging and biosensing applications. In particular, we aim at the fluorescence measurement applications such as neural activity imaging and DNA microarray measurement [3]. The realization of on-chip fluorescence imaging will be a great breakthrough that realize simple, low-cost and reliable bioassay system available even on-field. Due to the small volume of the target material (fluorescent molecules, such as dyes or proteins), a high sensitivity and wide dynamic range is required for bioimaging applications, For the sake of device cost, it is reasonable to use a standard CMOS process for on-chip bioimaging sensors. We propose to apply pulse modulation detecting scheme for such bioimaging applications. As is discussed in the next section, slight modification in the pixel circuitry enables to implement pulse modulation measurement mode in a conventional 3-Tr APS image sensors. Not only the design of the image sensor circuitry, we develop a packaging technique for on-chip bioimaging applications. *In vitro* and *in vivo* on-chip mouse brain imaging experiments were performed.

II. PULSE MODULATION MEASUREMENT SCHEME FOR IMAGE SENSORS

Figure 1 schematically shows measurement scheme of the pulse modulation image sensor. In pulse modulation measurement, the light intensity is evaluated with the time for the preset voltage drop. The photocarrier accumulation time is adaptively elongated for low incident situations and shortened for high incident situations. The pulse modulation measurement scheme enables to obtain S/N ratio nearly independently from the signal level at a cost of relatively longer measurement time. In a certain part of bioscientific imaging applications, the temporal change of the pixel value is most important. The pulse modulation measurement scheme is advantageous for such bioimaging applications. Of course, a trade off between the dynamic range and longer measurement exists. So the pulse modulation measurement scheme should be implemented with a smallest modification in the sensor circuitry and the sensor must have APS measurement mode.

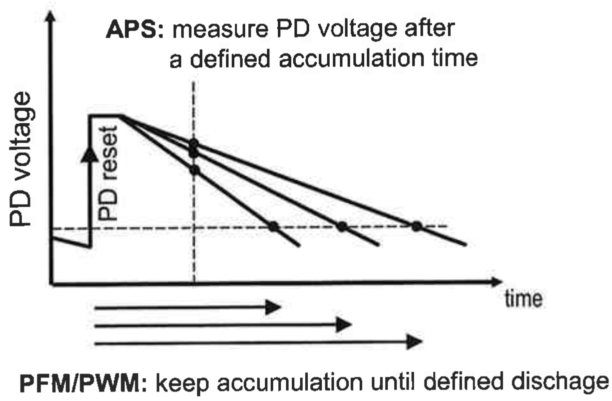

Figure 1: Measurement scheme of the pulse modulation image sensor

Figure 2 show the operation image and the circuitry of the pulse modulation image sensor. The pulse modulation image sensor consists of a pixel-core array, column amplifiers with 1-bit digital logic, and scanners in X- and Y- axis. The pixel circuitry is a modified 3-Tr APS pixel with a column reset line. As shown in Fig. 2-a, each pixel in the selected row is connected to the column amplifier and the column amplifier determines whether the PD level is above / below the threshold level. A frame consists of 1bit/pixel data that represent each pixel has discharged or not. The frame data is serially transferred to a PC and reconstructed into an image. The sensor can be operated in two kinds of pulse modulation imaging. In pulse width modulation (PWM) mode, all the pixels in each row are reset at the same time (rolling reset) and the image is reconstructed with the pulse width as the pixel value. On the other hand, in pulse frequency modulation (PFM) mode, each pixel is reset when the discharge of the PD is detected. Each pixel is reset independently from other pixels in the row and the frequency of the discharge sequence is

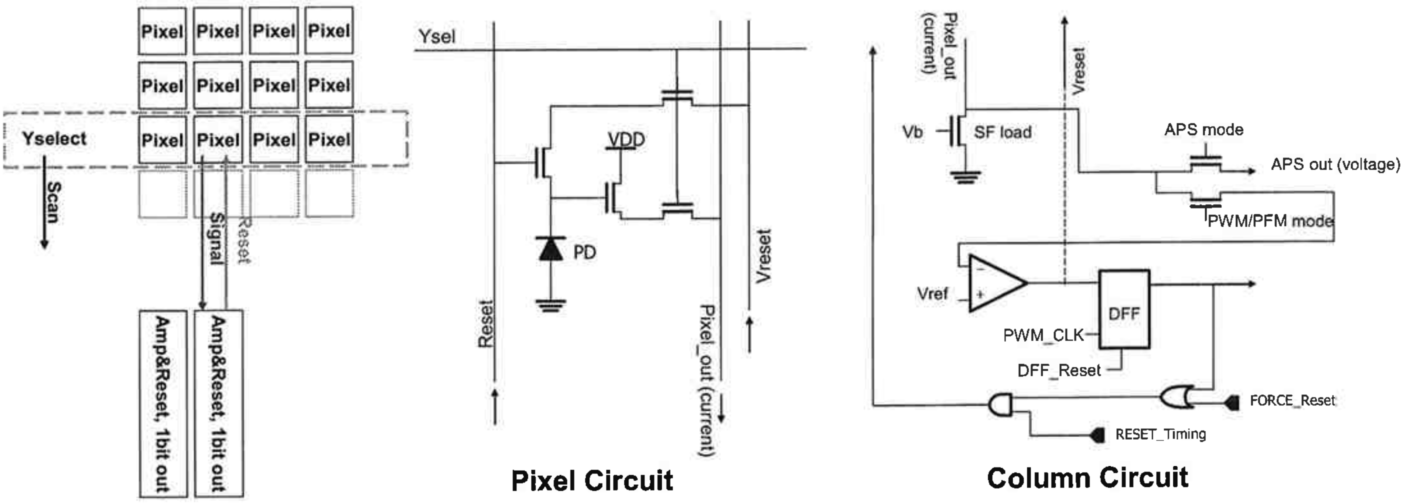

Figure 2: Operation image and the circuitry of the pulse modulation image sensor

Table 1: Specifications of the fabricated image sensors

sensor for "*in vitro*" imaging

Process	2poly 3metal 0.6μm Standard CMOS
Pixel number	64 × 64
Pixel size	15μm × 15μm
Pixel array size	1150μm × 1500μm
Operation voltage	5V

sensor for "*in vivo*" imaging

Process	2poly 4metal 0.35μm Standard CMOS
Pixel number	176 × 144
Pixel size	7.5μm × 7.5μm
Sensor chip size	2000μm × 2500μm
Operation voltage	3.3V

interpreted as the pixel value. The sensor circuitry includes a conventional APS image sensor. So the sensor can be operated as a conventional APS image sensor, too.

III. DESIGN AND PACKAGING OF PULSE MODULATION IMAGE SENSORS FOR ON-CHIP BRAIN IMAGING

We designed two image sensors to demonstrate feasibility of the pulse modulation measurement scheme for on-chip bioimaging applications. The one is a 64×64-pixels sensor for only *in vitro* brain slice imaging, and the other is a 176×144-pixels (QCIF) sensor for both *in vitro* and *in vivo* brain imaging. Table 1 shows the specifications of the image sensors. The difference between two sensors is on size and number of the pixel, and dye size. The QCIF sensor for *in vivo* imaging has I/O pads only on one of four edges of the dye and the chip size is as small as 2x2.5mm.

To apply image sensor chip for on-chip bioimaging, the sensor chip and wires should be covered with a waterproof molding layer. Not only molding layer, but also an optical filter layer is required to reduce ghost signal caused by excitation light. We adopted an epoxy resin as the molding material. Figure 3 shows the assembled image sensors for *in vitro* imaging. The sensor with Al wires was molded with an epoxy resin and a filter resist layer was spun on. For in vitro slice imaging, one can use both UV-excited and visible-exited dyes, because the thickness of the sample is smaller than 1mm and sufficient excitation light can penetrate into the brain slice sample. We chose DAPI (4', 6-Diamidino-2-phenylindole) as the staining dye. DAPI stains DNAs in cells. DAPI is excited with UV with wavelength of 300-400 nm, and the peak wavelength of the fluorescence is 460nm. A commercially available blue filter resist for image sensors can be used as the filter layer for on-chip DAPI imaging. A mouse's brain slice sample was stained with DAPI and mounted on the assembled sensor, as shown in Fig. 3. A hippocampus structure in the slice was successfully observed in on-chip configuration, as shown in Fig. 4. The imaging was performed in PWM imaging mode. A top image of the slice observed with a conventional fluorescence microscope is also shown in Fig. 4. A slight structural difference between the top and the bottom of the brain slice can be observed between two images.

For *in vivo* brain imaging, situation is different from *in vitro* imaging in two aspects. One is allowed size of the sensor module and the other is excitation configuration. To insert into a mouse's brain, the thickness and width of the sensor module must be as small as possible. In this work, we thinned the sensor chip to 150-200um and assembled. The sensor is bonded onto a polyimide flexible substrate and molded in the same manner with the *in vitro* sensor.

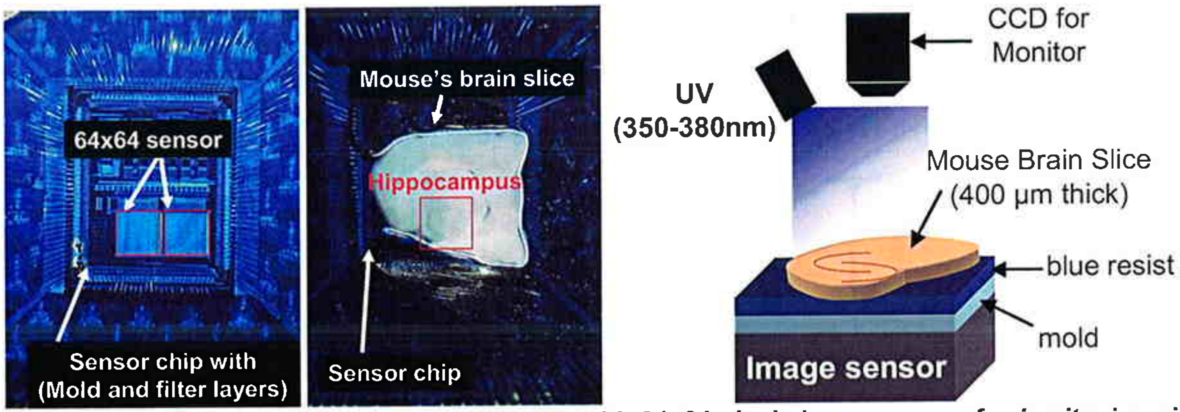

Figure 3: Chip packaging and experimental setup with 64x64-pixels image sensor for *in vitro* imaging.

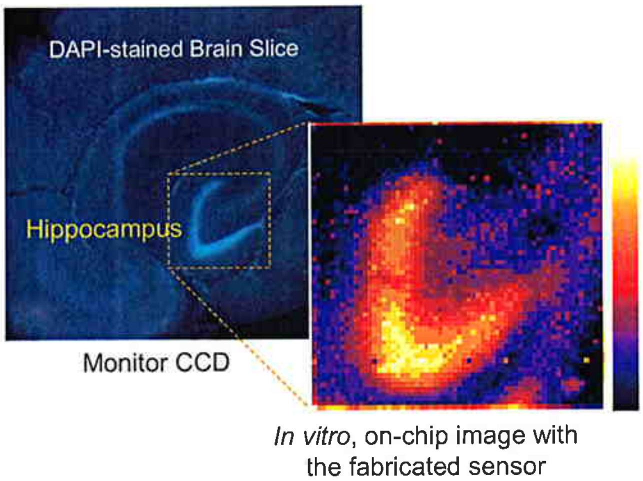

Figure 4.: A on-chip image of mouse's hippocampus with a top image of the slice observed with a conventional fluorescence microscope

Figure 5 shows the fabricated image sensor module for *in vivo* applications. For *in vivo* imaging, there is a difficulty in using UV-excited dye, because of large UV absorption by brain tissue. We chose a visible-excited dye DiA (molecular probes D3883) for *in vivo* imaging. The DiA can be excited with Ar^+ ion laser (488nm) and emits green - red fluorescence. A conventional red filter resist can be applied for excitation filter. An in vivo imaging experiment was performed. Figure 6 shows the experimental configuration. Figure 7 shows the images taken with the fabricated imaging module during the insertion into the mouse's brain. An image of the stained hippocampus structure was obtained in the last image.

IV. CONCLUSION

Image sensors with pulse modulation photosensing scheme were designed for *in vitro* and *in vivo* on-chip brain imaging. The sensor is capable to take image in either APS or pulse modulation imaging mode. A packaging technique for on-chip brain imaging was developed. Experimental demonstrations of *in vitro* and *in vivo* brain imaging were performed. We successfully demonstrated in vitro brain slice imaging with an UV-excited dye and in vivo brain imaging with a visible-excited dye.

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Figure 5: 176x144-pixels (QCIF) image sensor assembled for *in vivo* applications

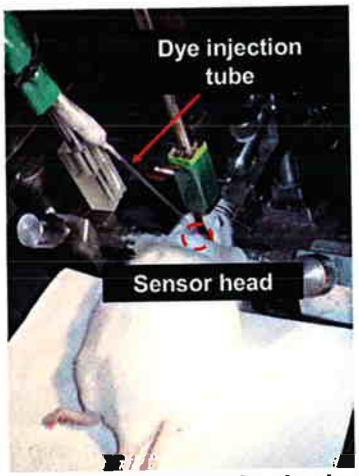

Figure 6: Experimental setup for *in vivo* brain imaging

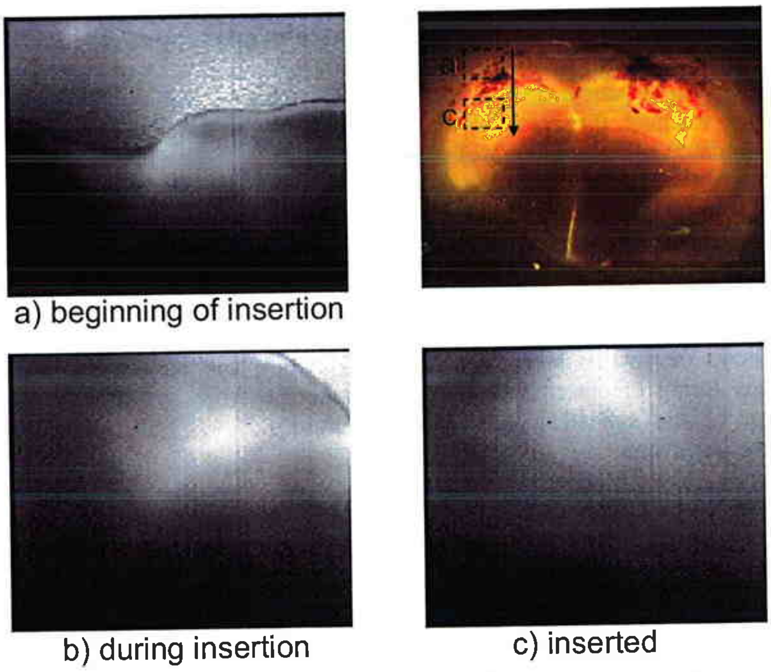

Figure 7: Images observed in *in vivo* brain imaging