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Experimental verification of a CMOS imager with block-parallel scanning for focal-plane pinhole effect in multi-beam confocal microscopy

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1. Introduction

A confocal microscopy has become an invaluable tool for investigating molecular dynamics in biology and medical sciences because of enhanced vertical as well as lateral optical resolution [1]. The multi-beam laser scanning method based on the Nipkow disk [2]-[4], which is a mechanical, geometrically operating image scanning device, is suitable for fluorescent observation of living cells. However, it requires a complicated rotating disk composed of precisely aligned microlenses and pinholes to achieve the confocal effect. The authors have reported the possibility of a CMOS imager using peculiar scanning for multi-beam confocal microscopy without the scanning disk [5].

In this paper, the proposed CMOS imager for the compact multi-beam confocal microscopy is experimentally demonstrated. The multiple beams aligned in a two-dimensional grid with the same pitch are scanned in a zig-zag manner and an image with confocal effect has been successfully obtained.

2. Operation principle of the proposed imager

In a conventional microscopy, not only the focused plane light but also the out-of-focus light from the specimen is illuminated the photodetector. This out-of-focus light leads to a reduction in image contrast and a decrease in resolution. In a confocal microscopy, however, all out-of-focus light is suppressed by the pinhole array. The block-parallel scanning of the proposed imager replaces the role of this pinhole array as reading only one pixel in every sub-imager or block, which is at the conjugate position of a light spot. Fig. 1(a) shows a block diagram of the proposed chip for achieving what is called the focal-plane pinhole effect by the block-parallel scanning. The chip consists of the pixel array which is composed of 32×32 blocks each with 8×8 pixels, a two dimensional vertical shift register, horizontal shift register, decoder, multiplexer, and correlated double sampling (CDS) circuit. Every shaded pixel (that is to read by addressing and is at the conjugate position with the light spot) in Fig. 1(a) is moved to the next position along the indicated direction in the pixel array. And all the pixels except the shaded pixels are unread and reset, hence the focal-plane pinhole effect is achieved. For this unique operation, the timing diagram is given as shown in Fig. 1(b). A clock signal (BCK) for the block scanner is given once every 32 clock signals (VCK) for the vertical scanner. The decoder signal (3-bit) is unchanged until the end of scanning of one column in a block. The node capacitance of floating diffusion (FD) of every pixel in the proposed CMOS imager is reset before its selection for eliminating the out-of-focus signal and reducing the random noise.

3. Experimental results and discussion

The prototype chip for the confocal multi-beam scanning microscopy has been fabricated with a 1-poly 4-metal 0.18-μm standard CMOS technology as shown in Fig. 2. The sensor performance and characteristics are summarized in Fig. 3. In the experiment for verifying the confocal effect, a specimen is illuminated by 32×32 405 nm laser beams with the spot pitch of 14 μm and the spot size of 2 μm. The proposed imager has been demonstrated at low frame rate of 0.26 Hz because the scanning rate depends on the limited velocity (x, y scanning rate: 0.26 Hz and 2.08 Hz, respectively) of a two-dimentional piezo stage. Fig. 4 shows the captured images with these conditions as a function of the focal displacement. In confocal mode, a well-focal image is obtained only for the displacement of 0 μm, and images are defocused for the displacement, showing the confocal effect. In the experiment, the extra time (during this time, the beam is awaited to arrive the selected pixel at the start point of each column) is needed to fit the scanning rate between the imager and the multiple beams. This causes the white line on the captured images. The confocal effect is also confirmed by the quantitative analysis as shown in Fig. 5. The depth of focus in the confocal mode by the full width at half maximum (FWHM) is reduced by 48.8% compared with that of the normal mode. Through these results, we can confirm the focal-plane pinhole effect of the proposed imager using the block-parallel scanning.

4. Conclusion

A CMOS image sensor using focal-plane pinhole effect for multi-beam laser scanning confocal microscopy is verified experimentally. For this demonstration, we have measured the axial resolution by moving a surface through the focused plane and plotting the returned signal as a function of the longitudinal displacement. As a result, the depth of focus of confocal mode imaging by the FWHM is reduced by 48.8% compared with that of the normal mode imaging. The proposed imager using the special addressing allows us to realize a compact confocal microscopy without extra scanning disks.

Acknowledgements

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References

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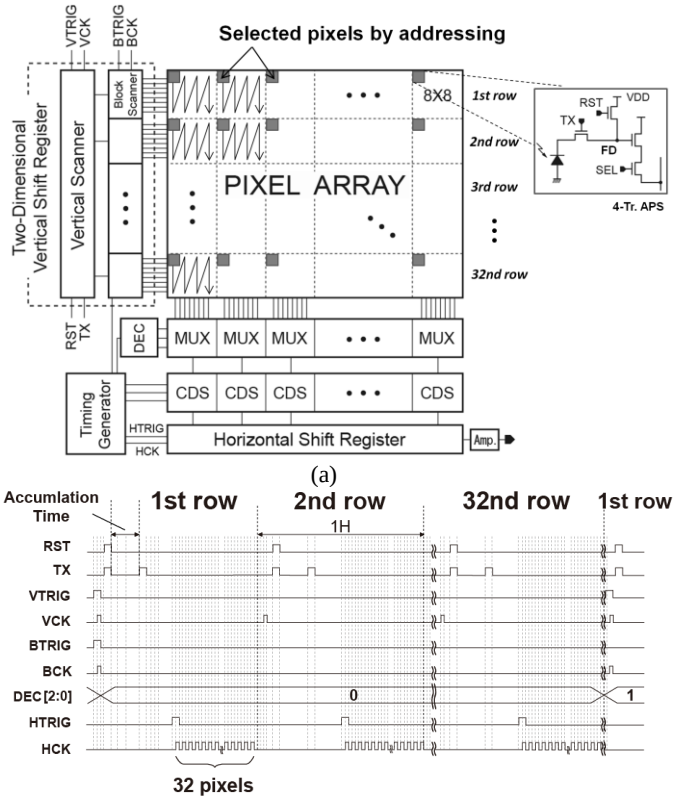

Fig. 1 (a) Block diagram of the chip. (b) Timing diagram.

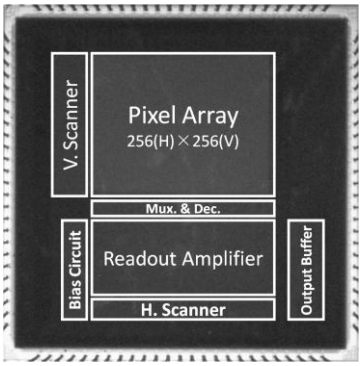

Fig. 2 Implemented chip photomicrograph.

Parameter	Value
Technology	0.18 μm 1P4M CIS process
Total area	5.0 (H) mm X 5.0 (V) mm
Power supplies	1.8V (Digtal), 3.3V (Analog)
Number of pixels	256 (H) X 256 (V)
Pixel type	4-TR (Pinned Photodiode)
Pixel size	7.5 μm X 7.5 μm
Fill factor	45 %
Sensitivity	25.9 ke-/lx·s (@ 3740K light source with IR cut filter)
ADC resolution	12b
Conversion gain	70 μV/e^-_{rms}
Noise	11.8 e^-_{rms} (@ Gain=1x, mean)
	4.9 e^-_{rms} (@ Gain=32x, mean)
Frame rate	30 fps
Power consumption	124.5 mW

Fig. 3 Specifications and characteristics of implemented prototype chip.

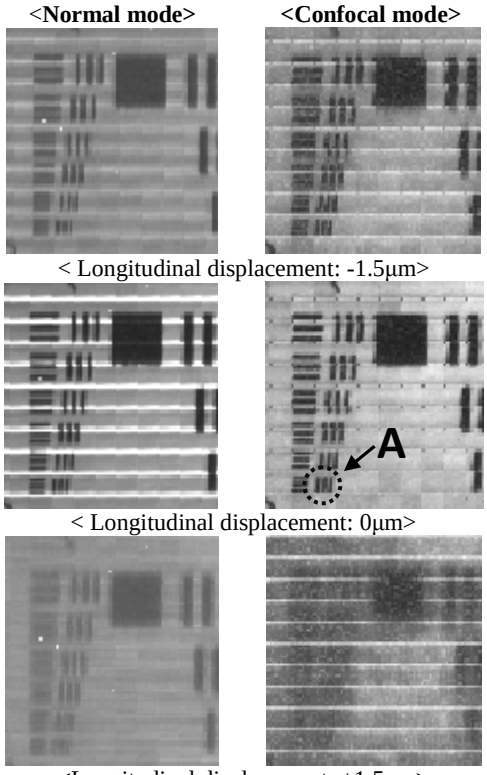

Fig. 4 Captured images as a function of the focal displacement (@ line pair of mark A=4.39μm).

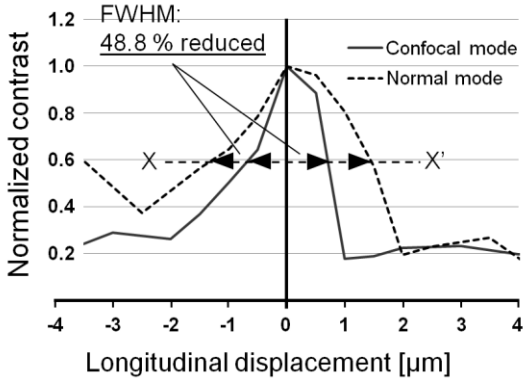

Fig. 5 Comparison of the normalized contrast at mark A between the confocal mode and normal mode of the proposed imager.