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Abstract

This development is related to the design and the integration of a Monolithic Active Pixel Sensor (MAPS) into a photosensitive proximity focusing vacuum-based tube. This EBCMOS project is dedicated to the fluorescent and the bioluminescent high speed imaging. The results of the full characterization of the first prototype are presented. Comparative tests with different fluorescent dyes have been performed in biology laboratories. Preliminary conclusions on the ability of EBCMOS to perform fast single-molecule tracking will be given.

Key words:
EBCMOS, HPD, fluorescence microscopy, single molecule tracking.

1. Introduction

Integrating an electron-bombarded back thinned Monolythic Active Pixel Sensor (MAPS) into a vacuum tube associated with a photocathod (EBCMOS) is one way to develop a new generation of single photon sensitive detector. Furthermore the MAPS, based on CMOS technology, are very promising pixel detectors for ultra-fast readout and real-time signal processing. Combining these advantages, photon counting ability at very high frame-rate could push the EBCMOS detectors beyond the state of the art in multipixels single photon detection [1].

The first EBCMOS demonstrator has been produced by a collaboration consisting of the EBCMOS group (IPNL), the CMOS sensor group (IPHC) and the PHOTONIS company.

We report in the first section on the complete characterization of this prototype. The second one is devoted to tests of the device for imaging of fluorescent dyes. Imagies have been obtained on standard epifluorescence microscopes used in biology laboratories. Finally, the preliminary conclusions on the performances of the demonstrators and the future R&D plans are discussed.

2. Characterization of the EBCMOS demonstrator

2.1. Prototype description

The Minimum Ionizing MOS Active sensor (MIMOSA) chips, developed by the IPHC team, are dedicated to the tracking of charged particles in High Energy Physics experiments. The first mega-pixel sensor (17 μm pitch, 1024x1024 pixels, 3.5 cm^2) of the Mimosa chip family, named MIMOSA5 (the chip design is presented in Refs. [2] and [3]), has been back-thinned and post-processed to be sensitive to low energy electrons [4, 5]. The back-thinned MIMOSA5 chip is mounted in a die cavity of a ceramic carrier. A tunable high voltage (useable range is 5 to 10 kV) is set between the cathode (18 mm diameter) and the sensor over a sub-millimeter gap. The cathode is a standard multi-alkali S20 type

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and provides a quantum efficiency equals to $15 \pm 2\%$ for wavelength of 520 nm.

2.2. Characterization results

The first characterization of the demonstrator is dedicated to single photo-electrons energy measurement. The signal selection is performed on a seed pixel and the energy deposited by the photo-electron is obtained by the sum of the charge over the 5x5 pixels around the seed. The averaged sensitivity to photo-electrons, which is expressed in signal-over-noise-ratio units is equal to 18 on the seed pixel for HV = 8kV (see Figure 1).

The dark count, due to the photocathode emission, has been measured for different temperatures and high voltages. Typical number of dark counts obtained at 6 kV and 10°C is equal to 650 ± 25 photo-electrons per 27 ms per 2.5 cm^2. This corresponds to a dark count rate close to 100 Hz/mm^2. Cooling the EBCMOS window from 20°C to 10°C reduces the dark current by a factor of 2. No real improvement is observed when cooling below 10°C.

The point-spread function (PSF) of the tube is characterized with an optical test bench which projects a 1 μm diameter spot on the photocathode. The pulse duration of the LED source is calibrated to obtain a Poisson distribution with an average of one photo-electron into a selected sub-window (5x5 pixels). The PSF is obtained by computing the distribution of the position of the seed pixel corresponding to a single photo-electron event. A Full Width at Half Maximum equals to $27.1 \pm 0.1 \mu m$ has been measured at HV=8 kV. A variation of 3 μm on the FWHM has been measured between 6 and 8 kV working points.

The photo-electrons counting ability is illustrated on figure 1: the histogram exhibits the Poisson distribution of the detected photo-electrons with peaks corresponding to 0,1, 2 and 3 photo-electrons events.

3. Imaging of fluorescent dyes with microscopes

Tests on fluorescence Imaging have been performed at IGBMC [6] on a Leica DMRXA2 microscope and at ENS-LKB [7] on a Nikon Eclipse Ti microscope. Epi-fluorescence microscopy was tried varying the excitation wavelength as well as the intensity. Fluorescent dyes emitting in different wavelength from red to blue (GFP, mitotracker , Hoescht, and Sytogreen) were fixed into cells to be used for our sensitivity tests. Resolution has been studied with standard fluorescent beads deposited on glass. Imaging of CdSe Quantum Dots (QDs) fixed on the membrane of Hela Cells have been performed to give some hints on single molecule detection capabilities. The well known blinking effect of a single QD has been observed.
The main goals of the fluorescence imaging tests were:

- evaluate the sensitivity and the spatial resolution of the EBMI5 compared to Standard CCD and Electron Multiplying (EM) CCD (sensitivity, resolution, dark count) [8];

- evaluate EBMI5 under single photon experiment condition and extrapolate the signal processing strategy to work at least at 1 kHz frame rate (dynamics, occupancy rate, temporal filtering).

Our prototype EBMI5 has been compared to a standard cooled CCD (CoolSNAP HQ2) and EMCCD (Cascade 2) from Roper Scientist at IGBMC and with EMCCD Ixon DU 897 BI from ANDOR at ENS Biology laboratory.
The two camera (EBMI5 and CCD or EMCCD) were put on the same microscope with the possibility to switch from one to the other. Therefore the imaging light condition was identical for the same field of view. The EMCCDs were used with different Electron Multiplication gain (EMGain) up to the maximum for single photon condition imaging. Almost the same integration time than EBMI5 (27 ms) was set on EMCCD (25 ms).

We summarize the results obtained from the data analysis in what follows:

Sensitivity and dark count:

- The EBMI5 has a better sensitivity than standard cooled CCD in the whole VIS spectrum.

- The EBMI5 is competitive to EMCCD in the green spectrum where the QE is optimized. EBMI5 is less sensitive than EMCCD for CdSe emission at 605 nm and 8% of cathode QE. The next cathode production for EBCMOS prototype should be optimized between the red and green part of the VIS spectrum.

- The background due to the dark count of the device is lower than 10% of the total background (mainly the photon noise coming from the microscopes and from off-focus fluorescent dyes).

- EBMI5 device has a equivalent (and for some cases better) contrast than EMCCD.
- In more intense light condition the HV can be lowered and keeping a good image quality. Since the CMOS is back illuminated with the photons which pass the cathode a image composed of photons and photo-electrons is obtained. We discover during our tests that this configuration could be used in biological experiment were high light condition should be used continuously with ultra low light or single molecule tracking.
- As can be expected, single photon imaging is very sensitive to photon background coming from off-focus dyes. Algorithm such as Kalman filter [9] has been implemented off-line to reduce dark count which is spatially and temporally randomly distributed on the CMOS.
- Ion feedback, a well-known effect of HPD[10] has been characterized and should be filtered in our next acquisition software.
- The limited dynamical range of the pixels (1 to 30 photo-electron at 8 kV) has to be be compensated by the increase of the frame rate. Furthermore the incoming number of photons has to be compatible with single molecule detection, therefore we expect, for the next device, a occupancy rate of the pixels smaller than 10%.

Resolution:

- The resolution of the EBCMOS is related to the convolution of two Point Spread Functions: the photo-electron PSF of the tube itself and the PSF of the CMOS sensor due to charge sharing between pixels. The first one is dominant (see section 2.1) because some clustering algorithm (Center of Gravity of pixel charges) can reduce the second down to few microns [2]. We tested deconvolution method with standard software [11] to improve the resolution. An example of the results obtained on GFP imaging of root of lily cells is shown in Figure 2.
- The imaging of latex beads at the diffraction limit of the microscope show a resolution on the beads: $\sigma = 245 \pm 30$ nm. This result has to be compared to the very good resolution of the coolSNAP HQ2, $\sigma = 130 \pm 15$ nm, due to a pitch of 6.5 μm.

Single molecule imaging and on-line processing:

- The blinking of a QD has been observed. The effect of the implemented filtering algorithm [9] is shown in Figure 3. This first off-line results on QDs imaging is a good starting point to implement on-line QDs tracking into the future acquisition system based on FPGAs.

As a general conclusion we can say that EBCMOS is a device with very good single photon sensitivity-low noise- and fast readout. It gives the possibility to improve the performances of target tracking on a large field of view by taking advantages from his intrinsic qualities by online signal processing. An improvement of background rejection and spatial resolution is expected from on-line software developments keeping in mind that ultra fast frame rate allow more processing to be performed at the level of the quantum basic element of an image: the single photon.

4. Future plans

To overcome the limitation of the MIMOSA5 for the considered applications, we will produce in 2009 a Large-scale Ultra-fast SIngle PHoton recordER (LUSIPHER) with a dedicated back-thinned CMOS chip (medium-scale 400x800 pixels, 10 μm pitch, 8 analogue outputs and 40 MHz clock frequency). A new acquisition ethernet board is currently developed to achieve 1000 frames per second (the equivalent data flow is equal to 3.6 Gb/s). LUSIPHER will be applied to single molecule tracking in fluorescence and bioluminescence experiments.

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References

[1] R. Barbier, et al., Proceedings of the ICATTP conference, 8-12 October 2007, Como, Italy.
[2] R. Turchetta, et al., *Nucl. Instr. and Meth. A* **458** 677 (2001).
[3] G. Deptuch et al. , *Nucl. Instr. and Meth. A* **511** 240 (2003); Yu. Gornushkin, et al., *Nucl. Instr. and Meth. A* **513** 291 (2003).
[4] W. Dulinski, *Nucl. Instr. and Meth. A* **546** 274 (2005).
[5] M. Caccia, et al., *Nucl. Phys. B (Poc. Suppl.)* **125** 133 (2003).
[6] IGBMC stands for Institut de Génomique et de Biologie Moléculaire et Cellulaire, Unit Mixte de Recherches CNRS/Inserm/Universit Louis Pasteur Strasbourg.
[7] ENS stands for Ecole Normale Suprieure, LKB stands for Laboratoire Kastler Brossel, Paris.

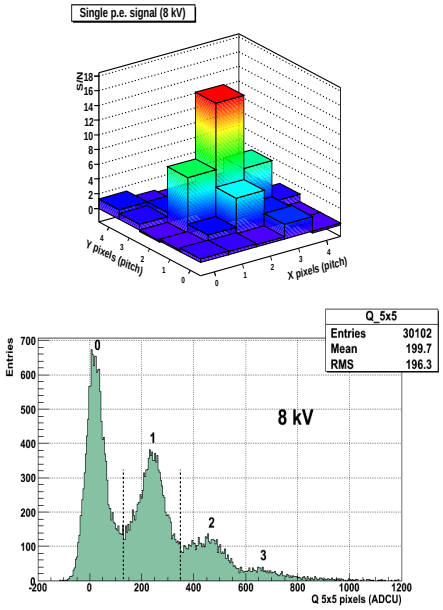

Figure 1: 2D Signal over Noise distribution of a photo-electron event (top) and the multi-photo-electron spectrum in a 5x5 pixel cluster (bottom).

[8] EMCCD from Roper can be found on *roperscientific.fr* and from Andor on *www.andor.com*
[9] R. E. Kalman, *Journal of Basic Engineering* **82 (1)** 35 (1960).
[10] T. Gys et al., *Nucl. Instr. and Meth. A* **355** 386 (1995).
[11] ImageJ software plugin for deconvolution, *http://piotr.wendykier.googlepages.com/spectraldeconvolution*

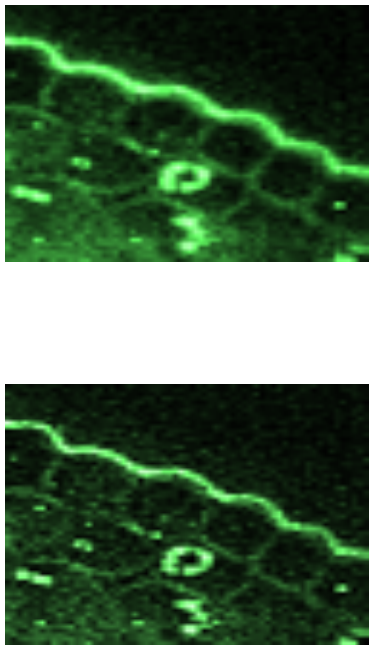

Figure 2: Top: Zoom (200x150 pixels) on a raw image from lily cells tagged with GFP . Bottom: same image after PSF deconvolution. Magnification of the microscope was set at x40.

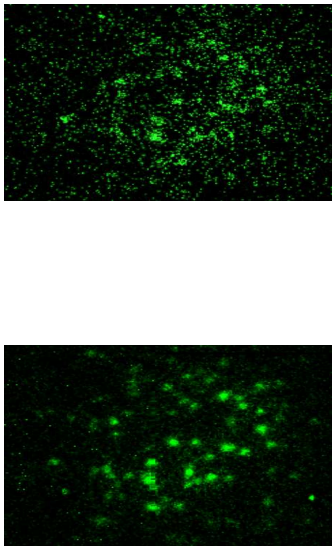

Figure 3: Left: Image of QDs fixed on Hela cell. Right: Same image with pixel-by-pixel Kalman filter. The two images corresponds to a window of 200x200 pixels. Magnification of the microscope was set at X100.