¹⁴C autoradiography with a novel wafer scale CMOS Active Pixel Sensor

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ABSTRACT: ¹⁴C autoradiography is a well established technique for structural and metabolic analysis of cells and tissues. The most common detection medium for this application is film emulsion, which offers unbeatable spatial resolution due to its fine granularity but at the same time has some limiting drawbacks such as poor linearity and rapid saturation. In recent years several digital detectors have been developed, following the technological transition from analog to digital-based detection systems in the medical and biological field. Even so such digital systems have been greatly limited by the size of their active area (a few square centimeters), which have made them unsuitable for routine use in many biological applications where sample areas are typically $\sim 10 - 100 \text{ cm}^2$. The Multidimensional Integrated Intelligent Imaging (MI3-Plus) consortium has recently developed a new large area CMOS Active Pixel Sensor ($12.8 \text{ cm} \times 13.1 \text{ cm}$). This detector, based on the use of two different pixel resolutions, is capable of providing simultaneously low noise and high dynamic range on a wafer scale. In this paper we will demonstrate the suitability of this detector for routine beta autoradiography in a comparative approach with widely used film emulsion.

KEYWORDS: Detectors for Beta autoradiography, Multi-modality systems, Solid state detectors, Pixelated detectors and associated VLSI electronics; Radiation-hard detectors.

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1. Introduction

^{14}C is a radionuclide commonly used in autoradiography (AR) for structural, functional and metabolic study of tissues. The long half-life of this beta-emitter (5568 years) allows prolonged measurements at almost constant activity, which is of particular importance when film emulsion is used for detection. Film emulsion is the detection medium of choice for AR. It offers an unbeatable spatial resolution ($1\text{--}5\mu\text{m}$ for low energy beta-emitters) due to its fine granularity, but on the other side it offers significant drawbacks which are strongly limiting for imaging performance. In fact film emulsion suffers limited dynamic range and non-linear response, which does not allow high and low activity regions of the same sample to be simultaneously images. Moreover film emulsion is characterized by a low sensitivity which requires lengthy exposures, up to several months for low energy emitters (^3H): a significant bottleneck in the routine experimental work-flow.

A range of alternative digital technologies have been proposed to address these limitations, mirroring the general transition from analogue-based to digital-based systems, generally observed in medical imaging. Indirect detection in AR has been investigated through storage phosphors and scintillators, offering a 10 to 100 fold increase in sensitivity compared to film emulsion [1] together with a relative high spatial resolution ($20\mu\text{m}$ [2]). Multi-wire proportional chambers (MWPCs) have been used for this application exhibiting a relatively low spatial resolution ($400\mu\text{m}$ [3]), whereas a better resolution is offered by Micro-Channel Plates (MCPs): $26\mu\text{m}$ for small area devices and $60\mu\text{m}$ for larger areas [4, 5]. Alternative approaches have been attempted with micro-strip detectors [7, 6] and gaseous detectors [8].

Silicon based pixel detectors have been studied at length, as a suitable digital alternative to film emulsion, ranging from Charge Coupled Devices (CCDs) [9], CMOS Active Pixel Sensors (APSs)[9] to CMOS Hybrid pixel sensors [10, 11]. Even where these silicon based systems have demonstrated suitability for AR applications, because of a good sensitivity, low background and acceptable resolution, they are not yet used routinely because of their of their modest active area (a few square

centimeters) compared with the typical sample sizes (between 10 and 100 cm^2). Their limited active area places an upper bound on throughput, off-setting the benefits of any performance gain, and so limiting application beyond proof-of-concept.

Recent advances in photolithographic techniques [12] have made available *reticle-stitching process* to scale up CMOS APSs up to wafer scale (13 cm $\times$ 13 cm). Wafer scale CMOS APSs can therefore present a valuable alternative to overcome the performance limitation of film emulsion whilst offering a sensitive area that meets the needs of most AR imaging applications. In this paper we propose a new wafer scale CMOS APSs as a suitable detection system for digital AR. First exemplar results using beta-labelled ex-vivo tissue sections (using ^{14}C -sulphur mustard) are presented in a comparative approach with film emulsion.

2. Materials and Methods

2.1 Detector

The detector used in this work is a CMOS Active Pixel Sensor (APS) called Dynamic Range Adjustable for Medical Imaging Technology (DynAMITe) and developed by the Multidimensional Integrated Intelligent Imaging (MI3-Plus) consortium. A picture showing the sensor together with a microscope slide (as commonly used to mount samples in AR) is shown in fig. 1 *a*. Manufactured by means of the reticle stitching technique [12, 13], the sensor covers an active image area of 12.8 cm $\times$ 13.1 cm and is designed to be two-sides buttable so that the active area can be further increased up to 25.6 cm $\times$ 26.2 cm. The concept underlying the development of this detector is the use of different well capacity diodes on the same pixel matrix, in order to gain simultaneously high dynamic range and low noise. In fact, high well capacity diodes can offer high dynamic range, whereas low well capacity diodes offer low noise. The sensor consists of two pixel grids geometrically superimposed: 1280 $\times$ 1312 large capacitance pixels placed at 100 μm pitch and 2560 $\times$ 2624 low capacitance pixels placed at 50 μm pitch. The combined use of both pixel grids allows the low capacitance diode to collect low intensity signals, so offering low noise, and the large capacitance diodes to collect high intensity signals, so offering an extended dynamic range whilst simultaneously preserving the Signal to Noise Ratio performance of the system. Moreover the sensor has been designed to be radiation hard by design [14] for ionizing applications. A more detailed description of the sensor pixel design, readout architecture and electro-optical performance can be found in [15, 16].

2.2 ^{14}C tissue samples

^{14}C tissue samples used in this work were generated as part of a series of in vivo experiments investigating the role of showering on skin decontamination following a radiological incident (ORCHIDS, U.K. Health Protection Agency [17]). In vivo experiments on porcine tissues were designed to compare a putative optimized showering protocol against the current UK protocol employed by the Fire and Rescue Service (FRS protocol), as well as compare the penetration of ^{14}C -sulphur mustard at the snout to the flank in an animal model.

Table 1 reports all the 8 tissue types used for this experiment. Animals (*Sus scrofa*) were dosed with ^{14}C -sulphur mustard (10 μl , 185 KBq) as a 2 cm line (Sites C, D and E) or as a droplet (10

Figure 1. A picture showing the DynAMITe detector together with a ruler and a microscope slide (7.5 cm $\times$ 2.5 cm), a commonly used sample holder in AR.

Label	Site	Dosed	Notes
A	Skin Flank	No	Negative control
B	Skin Flank	No	Showered Negative control
C	Skin Flank	Yes	Positive control
D	Skin Flank	Yes	FRS Protocol
E	Skin Flank	Yes	ORCHIDS Protocol
F	Bridge of snout	Yes	–
G	Planum rostrale	Yes	–
H	Rump	Yes	–

Table 1. Summary of samples used in this work.

μ l, 185 KBq, sites F, G and H). The animals were euthanized 6 hours after dosing by barbiturate overdose. Portions of the dosing sites ($\sim$ 0.5 cm thick) were immediately excised and snap-frozen by dipping in liquid nitrogen before immediate storing at -70° C. These portions were then allowed to thaw to approximately -15° C, mounted on stubs, and sectioned by cryostat at a thickness of 8 μ m. The sections were transferred to polysine glass slides and allowed to air dry.

3. Results

3.1 ^{14}C tissue imaging

Fig. 2 shows the first images of ^{14}C labeled tissue sections imaged with a wafer scale CMOS APS at room temperature. Porcine ex-vivo tissue sections, together with a RPS 504 micro-scale, were imaged with the DynAMITe detector and images, acquired at 1 frame per second, integrated over 8 hours.

A magnified view of tissue sections F and G is displayed in fig. 3. Insets labeled as *a*, *b* and *c* represent the digital images obtained with 3, 6 and 8 h integration time respectively. Insets *d* show an image obtained when the same sample is exposed to film emulsion for one week.

Figure 2. ^{14}C labeled skin sections (samples A-G) and RPA 504 micro-scale images with the DynAMITe detector for 8 h.

Figure 3. ^{14}C labeled skin section (sample F inset *a* and sample G inset *b*) imaged for 3 h (a), 6 h (b), 8 h (c) represented on the same false color scale shown in counts/pixel. The same sample imaged with film emulsion after 1 week exposure (d). The red box highlights the section of the original sample exposed to the digital detector.

From images in fig. 3, all of the details contained in the film image (inset *d*) are recognizable in the digital images with only 3 h exposure time (sample F, inset *a*) and in 6 h (sample G, inset *b*).

4. Conclusions

A new wafer scale CMOS APS has been presented as a suitable alternative to film emulsion in AR. The first images of ^{14}C labeled tissue sections imaged with a wafer scale CMOS APS have been shown and compared with corresponding film emulsion images. From this comparison it emerged that the DynAMITe imaging system is able to offer images of similar quality to those from film emulsion with an improvement in exposure time between 56 and 38 fold depending on the sample activity.

Combining the performance demonstrated in AR with previous results in chemiluminescence de-

tection for western blotting electrophoresis [19], the DynAMite system appears to be a viable and performant single platform technology for multi-modality imaging in life sciences, suitable for use across a broad spectrum of pre-clinical ionizing and non-ionizing imaging applications ranging from imaging of protein and DNA sequences to functional analysis of labeled tissue sections.

Acknowledgments

This work was supported by the EPSRC Multidimensional Integrated Intelligent Imaging Plus (MI-3 Plus) programme (EP/G037671/1)

References

- [1] S. Kanekal et al. *Storage-phosphor autoradiography: a rapid and highly sensitive method for spatial imaging and quantisation of radioisotopes*, *J. Pharm. Toxicol. Meth.* **33** (1995) 171-8.
- [2] N. Barthe et al. *Recent technological developments on high-resolution beta imaging systems for quantitative autoradiography and double labeling applications*, *Nucl. Instr. Meth. A*(**527**) (2004) 41-5.
- [3] Y. Petegnief et al. *Quantitative autoradiography using a radio-imager based on a multiwire proportional chamber*, *Phys. Med. Biol.* **43** (1988) 3629-38.
- [4] J. P. Lees et al. *Direct beta autoradiography using MCP detectors*, *Nucl. Instr. Meth. A*(**392**) (1997) 349-53.
- [5] J. P. Lees et al. *Microchannel plate detectors for ^{14}C autoradiography*, *IEEE Trans. Nucl. Sc.* **45** (1998) 1288-92
- [6] M. Overdick et al. *A bioscope system using double-sided silicon strip detectors and self-triggering read-out chips*, *Nucl. Instr. Meth. A*(**392**) (1997) 173-7.
- [7] B. Sanghera et al. *Digital beta autoradiography using silicon microstrip detectors*, *Nucl. Instr. Meth. A*(**310**) (1997) 455-9.
- [8] J. Donnard et al. *The micro-pattern gas detector PIM: a multi-modality solution for novel investigations in functional imaging*, *Nucl. Instr. Meth. A*(**610**) (2009) 158-60.
- [9] J. Cabello et al. *Digital autoradiography using room temperature CCD and CMOS imaging technology*, *Phys. Med. Biol.* **52** (2007) 4993-5011.
- [10] G. Mettivier et al. *First images of a digital autoradiography system based on a Medipix2 hybrid silicon pixel detector*, *Phys. Med. Biol.* **48** (2003) 173-81.
- [11] M. Esposito et al. ^{14}C autoradiography with an energy-sensitive silicon pixel detector, *Phys. Med. Biol.* **56** (2011) 1947-65.
- [12] D. Scheffer et al., *Wafer scale active pixel CMOS image sensor for generic X-ray radiology*, *Proc. SPIE*, **6510** (2007) 65100O
- [13] R. Turchetta et al., *Large area CMOS image sensors*, 2011 *JINST* **6** C01099
- [14] R. C. Lacoe et al., *Improving integrated circuit performance through the application of hardness-by-design methodology*, *IEEE Trans. Nucl. Sc.*, **55**(4) (2009).
- [15] M. Esposito et al., *DynAMite: a wafer scale sensor for biomedical applications*, 2011 *JINST* **6** C12064

- [16] M. Esposito et al., *DynAMITE: a Large Area Sensor for Biomedical Applications with Bimodal Dynamic Range and Resolution*, presented at *IEEE. Nucl. Sci. Symp.* , 23rd-29th October 2011, Valencia, Spain.
- [17] ORCHIDS project, U.K. Health Protection Agency
<http://www.orchidsproject.eu/>
- [18] J. R. Janesick ,*Photon Transfer $DN \rightarrow \lambda$* , SPIE Press, Bellingham, WA 2007.
- [19] M. Esposito et al. *Using a large area CMOS APS for direct chemiluminescence detection in Western blotting electrophoresis*, *Proc. SPIE* **8317** (2012) 831710.