Digital autoradiography using room temperature CCD and CMOS imaging technology

Jorge Cabello¹, Alexis Bailey², Ian Kitchen², Mark Prydderch³, Andy Clark³, Renato Turchetta³ and Kevin Wells¹

¹Centre for Vision, Speech and Signal Processing, School of Electronics and Physical Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK.

²School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, GU2 7XH UK.

³Rutherford Appleton Laboratory, Science and Technology Facilities Council, Chilton, OX11 0QX, UK.

E-mail: j.cabello@surrey.ac.uk

Abstract. CCD and CMOS imaging technologies can be applied to thin tissue autoradiography as potential imaging alternatives to using conventional film. In this work, we compare two particular devices: A CCD operating in slow scan mode and a CMOS-based Active Pixel sensor, operating at near video rates. Both imaging sensors have been operated at room temperature using direct irradiation with images produced from calibrated microscopes and radio-labelled tissue samples. We also compare these digital image sensor technologies with the use of conventional film. We show comparative results obtained with ¹⁴C calibrated microscopes and ³⁵S radiolabelled tissue sections. We also present first results of ³H images produced under direct irradiation of a CCD sensor operating at room temperature. Compared to film, silicon-based imaging technologies exhibit enhanced sensitivity, dynamic range and linearity.

Keywords: Digital Autoradiography, CCD, CMOS, APS, tissue imaging, high resolution.

1. Introduction

Autoradiography (AR) is a widely used technique in biology to map the two dimensional bio-distribution of radiolabelled molecules within thin ($\sim 10 \mu\text{m}$) *ex-vivo* tissue sections. The aim of this technique is to qualitatively observe the relative distribution of the tracer, or, in many cases, to quantify specific areas of uptake (using simultaneous imaging of calibrated microscopes). Traditionally, photographic film has been used as the imaging technology for AR. This exhibits excellent intrinsic spatial resolution ($\sim \text{few } \mu\text{m}$) for low cost ($\sim \3 per sheet for standard film and $\sim \$50$ per sheet for ³H imaging). But this technique presents a number of undesirable qualities, particularly with respect to image quantification. These include poor linearity, limited dynamic range ($\sim 10^2$) and low sensitivity, producing long (typically $\sim \text{days}$, $\sim \text{months}$) exposure times. As a result alternative techniques (described below) based on digital imaging technology have been proposed to replace film. Whilst many proponents of these alternative prototypes have claimed that digital imaging technologies have superior sensitivity compared with film, we are not aware of significant comparative

work (with the exception of the Tribollet *et al* 1991). In this work we attempt to make a first comparison between film and two exemplar imaging sensors.

Some of the more important techniques proposed as alternatives to film in AR include phosphor plates (Johnston *et al* 1990, Reichert *et al* 1992), microchannel plates (Lees *et al* 2002, Lees *et al* 1999) a Beta Camera (Ljunggren and Strand 1990) based on plate technology, multiwire proportional chamber systems (MWPC) (Charpak *et al* 1989, Petegnief *et al* 1998) and solid state detectors. Within solid state detectors several different technologies can be distinguished including the DEPFET pixel Bioscope which has proved its capability to detect ^3H (Neeser *et al* 2000, Ulrici *et al* 2005), single and double-sided silicon strip detectors which have also reported results with ^{32}P (Bertolucci *et al* 1996), ^{14}C and ^{35}S (Overdick *et al* 1997), Charged Coupled Devices (CCD), CMOS hybrid detector technology and CMOS Monolithic Active Pixel Sensors (MAPS) technology. (Barthe *et al* 2004) presented very interesting results with ^3H using a parallel plate avalanche chamber with a CCD (β -Imager 2000TM) and with scintillator sheet and a CCD (μ -ImagerTM) demonstrating an excellent resolution of $50\mu\text{m}$ and $15\mu\text{m}$ for ^3H respectively. The application of CCD technology for AR under cooled conditions using indirect irradiation via a scintillation sheet (Karellas *et al* 1993) and direct irradiation (Ott *et al* 2000) has been demonstrated previously. Preliminary work at room temperature using CCDs under direct irradiation has also been reported (Kokkinou *et al* 2003, Cabello *et al* 2006). The use of hybrid CMOS technology, in which the semiconductor and the readout electronics are separated entities joined usually by bump bonding, in AR has been extensively demonstrated by the Medipix consortium with Medipix1 (Abate *et al* 2001, Bertolucci *et al* 2002) presenting results with ^{32}P and ^{14}C and Medipix2 (Mettivier *et al* 2003, Mettievier *et al* 2004, Mettievier *et al* 2005) presenting results with ^{14}C and ^3H . There are some approaches to CMOS MAPS used for biomedical imaging as CMOS with Silicon on Insulator technology (SoI) (Niemiec *et al* 2005, Marczewski *et al* 2005), hydrogenated amorphous Silicon (a-Si:H) on a CMOS chip (semi-monolithic) (Moraes *et al* 2004), better known as flat panels, extensively used in radiography and CMOS MAPS with charge collection in epitaxial layer. The use of the latter applied to digital AR has been demonstrated in the past by (Deptuch 2005) under cooled conditions and by this same group (Cabello *et al* 2006) at room temperature.

In this work we compare two solid state alternatives to film: a commercial CCD sensor (E2V[†], CCD55-20) and a CMOS Monolithic Active Pixel sensor (Prydderch *et al* 2003), both under direct irradiation from a radiolabelled sample. From an engineering perspective, CCD sensor technology has traditionally provided the "gold standard" in *digital* image performance in terms of high fill factor, quantum efficiency, small pixel size, dynamic range and low noise floor. However, CMOS imaging technology has, in recent years, made great strides in performance (Janesick 2002, Carlson 2002, Vermes 2004), and offers a potentially lower cost alternative additionally providing low power consumption, high level of integration, radiation hardness and high-speed operation. Its main weakness resides in the effective charge generation producing lower quantum efficiency (QE), charge collection, thermal diffusion and lower electric fields. The low QE is due to the often lower fill factor due to transistors that usually partly occupy each pixel to control read-out, although in this device tested here (see section 2) this aspect is not significant (Turchetta *et al* 2000). The charge collection efficiency (CCE) is better in CCD technology because

[†] E2V Technologies Ltd, Chelmsford, Essex, UK

of higher resistivity wafers and concomitantly higher electric fields, used to confine the ionization charge (Janesick 2002, Magnan 2003). This reduces the likelihood of electrons being collected at neighbouring pixels due to thermal diffusion. In this new work we examine the potential of CCD and CMOS solid state imaging technologies operating at room temperature.

2. Materials and methods

The most common radioisotopes used in AR are ^{32}P , ^{33}P , ^{35}S , ^{14}C and ^3H , shown in table 1. Particular AR applications favour the use of different radioisotopes. For high spatial resolution, lower energy emitters are used that have, concomitantly, smaller β pathlengths. For comparison, the maximum ranges of the emitted beta-particles in silicon and in film emulsion gel for these radioisotopes are shown in table 2. These ranges were obtained from the NIST ESTAR (Stopping-Power and Range tables for electrons) database (NIST 2000). This database provides the CSDA range (g/cm²) depending on the energy from 10 keV up to 1 GeV at fixed steps. As the maximum energies of the radioisotopes considered in this work are not included in this database, to find out the range at these energies a linear interpolation was done between the two closest energies in the CSDA table.

Table 1. Common radioisotopes used in AR.

Radioisotope	Average energy	Maximum energy	Half life
^{32}P	0.7 MeV	1.71 MeV	14.3 days
^{33}P	76 keV	249 keV	25.3 days
^{35}S	48-53 keV	167 keV	87.4 days
^{14}C	49 keV	156 keV	5730 years
^3H	5.7 keV	18.6 keV	12.26 years

The densities used here are 2.33 g/cm³ for silicon and 1.2914 g/cm³ for photographic gel emulsion (NIST 2000). As can be seen in Table 2 the range in film emulsion gel is longer due to lower density. However, this simple calculation ignores range-loss effects: in film this is due to the overlying anti-scratch coating, and in silicon image sensors due to overlying electrode structures or antireflection coatings.

Table 2. Range of beta-electrons in silicon and gel.

Radioisotope	Maximum range in silicon	Maximum range in gel
^{32}P	4296 μm	6584 μm
^{33}P	341 μm	504 μm
^{35}S	182 μm	268 μm
^{14}C	163 μm	239 μm
^3H	4.33 μm	6.04 μm

Typical autoradiography film sheet contains a sensitive emulsion layer placed between a plastic base and an anti-scratch coating. Silver bromide crystals are suspended approximately uniformly in the emulsion gel, the ionization of which forms the basis of the image formation process. In the specific case of ^3H sensitive Hyperfilm, there is no anti-scratch layer that would otherwise impede direct detection of soft ^3H beta-electrons.

Tritium, or ^3H , is a particularly important radioisotope used in AR because it can label many sites on biomolecules and provides the highest resolution images due to its low energy and hence low particle range (see Table 2) compared to other beta-emitting radioisotopes. It is therefore one of the most common labels in molecular AR imaging studies.

2.1. Imaging System Description

We have used, in our initial experiments, an E2V CCD55-20 inverted mode sensor and a StarTracker CMOS active pixel sensor (APS) (Prydderch *et al* 2003) to detect ^{14}C and ^{35}S . A back-illuminated CCD55-20 sensor has also been used to make the first demonstration of ^3H imaging at room temperature using a CCD under direct irradiation.

The 55-20 CCD comprises a 770×1152 array of pixels on a $22.5 \mu\text{m}$ pitch with a depletion region of $7 \mu\text{m}$ and a sensitive field free region of $13 \mu\text{m}$. The charge to voltage conversion is $3 \mu\text{V}/\text{electron}$, as quoted by the manufacturer (E2V Technologies). The CCD has a 3-phase structure and is read out in full frame slow scan mode at a rate of $0.1 \text{ frames s}^{-1}$ using correlated double sampling. This corresponds to a clock speed of 1 MHz, which is the highest clock speed available with the driver electronics described below. The passivation layers of the front-illuminated device comprise electrode layers and oxide layers totalling $\sim 1.5 \mu\text{m}$ thickness. For ^3H imaging, a back-thinned 55-20 sensor was used, with the same pixel array geometry as the front-illuminated device, but with an anti-reflection layer added (of a few 100 nm thickness), which in this case only serves to protect the surface. Being back-illuminated, the ionized particles first encounter the field-free region rather than the depletion region.

In comparison to (Kokkinou *et al* 2003) where a CCD05-20 was used, the CCD55-20 is the natural upgrade of the latter. Among the most important improvements in this sensor are the lower noise output amplifier, from 6 e-rms for the CCD05-20 to 3 e-rms for the CCD55-20, the pixel full well capacity has been enhanced from 100,000 electrons to 450,000 electrons, the responsivity has been increased from $2 \mu\text{V}/\text{e}^-$ to $3 \mu\text{V}/\text{e}^-$ for the low noise output and the new design is now compatible with the standard backthinning process. With these features, better performance in terms of sensitivity and background noise is expected.

The CCD acquisition system used is a CDB01-X CCD Driver Assembly previously manufactured by E2V. This comprises an analogue read-out board, with several amplification stages, with Correlated Double Sampling (to remove reset noise), and a digital control board controlled by an ERA60100PBA FPGA that generates all the clock pulse waveforms to control the CCD. The resultant video signal is applied to a 12 bit ADC integrated in a National Instruments[†] Data Acquisition card within the PC under the control of a bespoke LabView application which saves each image frame to file. These images are subsequently processed off-line using in-house Matlab code.

The CMOS StarTracker image sensor (Prydderch *et al* 2003) is a 525×525 array of pixels on a $25 \mu\text{m}$ pitch with $5 \mu\text{m}$ of overlying passivation layers and a depletion region of $4 \mu\text{m}$. Each CMOS pixel has a structure made of 3 transistors (a reset MOSFET, a source follower MOSFET, which converts signal charge to voltage, and a row-select MOSFET) and four n-well/p-substrate photodiodes placed on a $12.5 \mu\text{m}$ pitch. This architecture optimizes the charge collection having, at the same time, low

[†] National Instruments UK & Ireland, Newbury, Berkshire, UK

node capacitance (~ 18 fF) yielding a charge to voltage conversion of $\sim 8.9 \mu\text{V}/\text{electron}$. The readout is column-parallel with an adjustable gain amplifier and a 10-bit ramp ADC per column that reads out at a fixed rate of 10 frames s^{-1} .

The CMOS acquisition system is based around a board using a Virtex-II ProTM 20FF1152 FPGA which drives and controls the CMOS sensor. The digitized image is then sent to the acquisition PC via optical connection. The image acquisition protocol is accessible using Matlab through a dynamic link library which interacts directly with the board.

In order to correct for dark current and inter-pixel non-uniformities (Fixed Pattern Noise), every image acquired on each system is processed using in-house software. First a set of blank frames is acquired to compute the mean dark current offset of each pixel. For the case of the CCD sensor, this set of blank frames is used to apply a dynamic Fixed Pattern Noise (FPN) correction (Wells and Kokkinou 2006). For the case of the CMOS sensor, a static correction is applied, subtracting the mean offset of each blank pixel from the observed pixel intensity in the acquired image. After this first step, the subsequent processing is the same for both sensors.

A statistical threshold is then applied to each pixel defined by (1).

$$T = M + k\sigma \quad (1)$$

where T is the threshold applied, M is the mode dark signal of the pixel and σ is the dark level standard deviation for each pixel obtained from the aforementioned blank frames. The pixel threshold is then globally defined in terms of the coefficient k .

The resulting binary images are then labelled using 8-connectivity analysis and the location (x and y coordinates of each pixel belonging to the cluster), size (in pixels) and intensity (sum of all pixels within the cluster in post-correction ADC units) of each event cluster is saved. In order to obtain a useful composite image, the thresholded images, described above, were accumulated and summed. The result of this process applied to the image data from the CCD is shown in figure 1. Note the scale of each image.

In the case of the CMOS sensor the most important sources of noise are reset noise (kTC noise) and residual inter-pixel FPN. In the case of FPN, due to the short integration time used in this experiment (100 ms), is not as significant as with the CCD system used here (10 s), and furthermore the FPN correction helps mitigate this effect. The effect of this post processing applied to CMOS sensor images is shown in figure 2.

2.2. Calibration

In order to compare sensor performance it was necessary to calibrate the energy response of both imaging systems, so that a comparative event threshold in calibrated keV units could be used. The CCD sensor was exposed to a point source of ^{241}Am (activity of 41 kBq) situated around 30 mm from the CCD with a layer of insulating tape to absorb the associated α particles. The geometry was set up such that the chances of obtaining overlapping event clusters within a single image frame was considered negligible.

Peaks corresponding to the ^{241}Am photon energies of 11.9 keV, 13.9 keV, 17.5 keV, 22 keV and 26 keV were observed in the reconstructed energy spectrum.

Figure 1. Raw (left), corrected (middle) and thresholded (right) images of the front illuminated CCD sensor, showing the stages used to correct for pattern noise. (b) and (c) represent the image data prior and post application of equation 1. This shows a single frame from a ^{14}C exposure for 10 secs integration time.

Figure 2. Raw (left), corrected (middle) and thresholded (right) images of the front illuminated CMOS sensor. (b) and (c) represent the image data prior and post application of equation 1. This shows a single frame from a ^{14}C exposure for 0.1 secs integration time.

Exposing the CCD sensor to a ^{14}C source also provided a reference energy of 49 keV from the associated peak (mode) in the beta deposited energy spectrum. From these data, a fitted calibration line is shown in figure 3 with a slope of 0.36 keV/ADC unit and an intersection point of -2.5 keV, from which 1 keV corresponds to 9.72 ADC units. Assuming 3.65 eV is required to liberate 1 electron in silicon, then this yields a slope of 98 electrons/ADC units and an intersection of -684 electrons.

Having calibrated the CCD sensor energy response, we can now quantify equation

Figure 3. Energy calibration curve for CCD sensor. X axis represents the signal in ADC units and Y axis represents the signal in electrons.

1 in keV. Thus, expressing the threshold, T , (see equation 1) applied to the corrected images in keV, a mean pixel threshold of 6.7 keV ($k=8$) is obtained with an approximately Gaussian inter-pixel variation of width $\sigma=1.9$ keV. This width represents the variation in effective pixel threshold, due to inter-pixel variations in performance.

In the case of the CMOS sensor, it was not possible to calibrate with a beta or x-ray source because of the thin epitaxial layer of the sensor ($4 \mu\text{m}$). Therefore calibration data obtained from the Photon Transfer Technique taken from (Arvanitis *et al* 2006) was used, shown in figure 4. In this technique the sensor was exposed to known quantities of light emission from a calibrated photodiode, from which the mean signal and variance were then plotted. From these data the gain (slope of the fitted line) obtained is 247 electrons/ADC units and the read-out noise obtained is 159 electrons. These parameters expressed in keV are 0.89 keV/ADC units and 0.57 keV respectively.

Expressing the threshold (see equation 1) applied to the corrected images in keV, in the case of the CMOS device, a mean threshold of 3.5 keV ($k=2$) with an approximately Gaussian variation of width $\sigma=0.6$ keV was obtained.

The above thresholds were empirically selected for each device. In the case of the CCD device, different thresholds over an interval of $1 < k < 8$ were selected to determine an optimal threshold maximizing true events and minimizing noise event detection. In the case of the CMOS device, k was varied between 1 and 3 in steps of 0.2 using the same criterion as before. It was found that a threshold of $k=2$ maximizes the the number of true events minimizing the counts in the background.

2.3. Image Acquisition

Results obtained using calibrated microscopes of ^{14}C and ^3H and authentic radiolabelled tissue sections are presented below. The microscopes consist of known amounts of radioactivity homogeneously distributed in a plastic tissue equivalent polymer of $50(^3\text{H})$ - $120(^{14}\text{C}) \mu\text{m}$ thickness. Each microscale has 8 cells of varying activity; for ^3H the range is 0.11-4.04 kBq/mg and for ^{14}C , this is 1.11-31.89 kBq/g

Figure 4. Photon Transfer Technique curve for CMOS sensor. X axis represents the signal (mean) in ADC units and Y axis represents the noise (variance) in ADC units, taken from (Arvanitis *et al* 2006).

(GE Healthcare[†]). The experiments were set up placing each sensor in a light proof box and placing the microscale in direct contact with the surface of the sensor. The exposure time of the experiments with ^{14}C for the CCD sensor and the CMOS sensor was 1 hour and 40 minutes. For ^3H experiments using the back-thinned CCD, the exposure time was 11 hours for the microscales and the tissue specimen, and for ^{35}S experiments, the exposure time for the tissue sections was 16 hours.

For comparison, we have also exposed a Biomax MR film to the same calibrated source for 100 minutes. The film was developed after exposure, dried and finally digitized. To digitize the sheet of film, a dedicated analog camera system (SONY model XC-77CE SONY[‡]) was used. The sheets of film were externally illuminated from below the samples and placed in the focal plane of the camera. Illumination was varied to optimise the level of contrast. Different types of film were used depending on the radioactive source to be used: Biomax MR film (Kodak) was used for ^{14}C and ^{35}S while a special tritiated hypersensitive hyperfilm (GE), with no anti-scratch layer, was used to detect ^3H .

To examine the digital imaging systems, performance under realistic conditions, various coronal brain tissue sections were prepared as part of wider pharmacology investigations in our laboratory into the activation of μ -opioid receptors with the use of μ -opioid receptor stimulated ^{35}S [S]GTP γ S autoradiography and D_1 dopamine receptor binding with the use of ^3H [H]SCH-23390 autoradiography. Several mouse tissue sections were preincubated with 1 mM GDP § and then incubated for 2 hours with ^{35}S [S]GTP γ S (0.04 nM) and 1 mM GDP (Bailey *et al* 2004). After this preparation the sections were placed initially within a film cassette, in direct contact with film, for ~ 4 days in the case of ^{35}S and ~ 5 weeks in the case of ^3H , and then placed in contact with each digital image sensor.

[†] GE Healthcare UK Limited, Pollards Wood, Nightingales Lane, Chalfont St.Giles, Bucks, UK, HP8 4SP

[‡] SONY Corporation, Melton Mowbray, Leicestershire, UK

[§] Guanosine 5'-Diphosphate

3. Results & Discussion

3.1. Sensitivity

In figure 5 we present a composite image (accumulation of thresholded images) of calibrated ^{14}C microscales exposed for 100 minutes acquired at a frame rate of 0.1 frames s^{-1} using a mean threshold of 6.7 keV with the front illuminated CCD sensor, obtained using the methodology described in section 2.3. Figure 6 demonstrates the corresponding image obtained from the CMOS sensor with ^{14}C for the same length of time acquired at a frame rate of 10 frames s^{-1} using a mean threshold of 3.5 keV, representing the first use of this particular CMOS imaging device for AR. Figure 7 shows the corresponding image obtained with film with the same reference source and for the same exposure period. Clearly, the level of contrast against background fogging makes image quantification with film impossible in such a short exposure time.

To analyse the sensitivity of the CCD and the CMOS sensors, from the composite images (figures 5 and 6), the number of counts, defined as isolated clusters of pixels, per second per band (cps mm^{-2}) are plotted in figure 8 against the specific activity per band (kBq g^{-1}). From this image the sensitivity of each sensor is extracted from the slope of the fitting line for each set of data points. In the case of the CCD sensor, the sensitivity is $6.1 \cdot 10^{-3} \text{ cps mm}^{-2} \text{ kBq}^{-1} \text{ g}$ and in the case of the CMOS sensor the sensitivity is $5 \cdot 10^{-3} \text{ cps mm}^{-2} \text{ kBq}^{-1} \text{ g}$. The background noise level for each sensor is also shown in the figure 8. In the case of the CCD sensor this is $160 \cdot 10^{-5} \text{ cps mm}^{-2}$ and in the case of the CMOS sensor this is $1.11 \cdot 10^{-5} \text{ cps mm}^{-2}$. Although both images appear visually similar, this demonstrates the lower sensitivity of the CMOS sensor, which is to be expected due to its thinner epitaxial layer of this CMOS sensor compared to the CCD sensor. It is worth noting the exceptional low level of background noise in the CMOS sensor, even below the typical level of cosmic rays at sea level ($1.7\text{--}3.4 \cdot 10^{-3} \text{ cps mm}^{-2}$), due to the removal of event clusters over 20 pixels size by means of the correction algorithm applied to the processed images. This plot also shows the excellent linear response for both sensors. To place these results in perspective, these represent a slightly better performance compared to that of Medipix2 and BETAvue, based on Medipix1 (Mettivier *et al* 2005) ($3 \cdot 10^{-3} \text{ cps mm}^{-2} \text{ kBq}^{-1} \text{ g}$ for Medipix2 and $4.7 \cdot 10^{-3} \text{ cps mm}^{-2} \text{ kBq}^{-1} \text{ g}$ for BETAvue). In table 3 a comparison of some of the most significant systems is shown presenting sensitivity, background noise level, minimum detectable activity and exposure time of each experiment. The minimum detectable activity is measured from the absolute activity of the last visible band taking into account the self-absorption effect (Puertolas *et al* 1996, Lees *et al* 1998, Mettievier *et al* 2003). In our case the minimum detectable activity with the CCD and the CMOS sensors is estimated at 0.09 Bq in both cases because both sensors are able to distinguish the last band. Nevertheless we will demonstrate in a future study that, at least with the CMOS sensor, the minimum detectable activity is lower than that presented in this paper.

3.2. Energy Spectra

In figure 9 we show the deposited energy spectrum of ^{14}C obtained with the 55-20 CCD and the StarTracker CMOS sensors. These spectra were obtained by computing the histogram of the intensity of every event cluster from the data mentioned in section 2.3.

Figure 5. Composite image with ^{14}C obtained with the CCD sensor after 100 minutes exposure

Figure 6. Composite image with ^{14}C obtained with the CMOS sensor after 100 minutes exposure

Figure 7. Exemplar image produced using ^{14}C microscale after exposure to Biomax MR film for 100 minutes.

Note that the spectrum obtained with the CCD sensor is similar to the expected continuous spectrum of ^{14}C (Pohm *et al* 1955, Cross *et al* 1983), in contrast to that exhibited by the CMOS sensor. This difference is mainly attributed to the different thickness of the epitaxial layers: in the CCD this thickness is $20\ \mu\text{m}$ whereas in the CMOS sensor, it is $4\ \mu\text{m}$. Calculating the range of mean energy beta particles from ^{14}C in silicon as $\sim 23\ \mu\text{m}$ (NIST 2000) means that because of the thinner epitaxial layer of the CMOS device, the sensor mainly behaves as a thin dE/dx detector, partially sampling the particle energy for all but the lowest energy betas, or those with highly oblique incidence. In contrast the CCD provides a relatively high chance of observing the full beta energy deposition, with a combined sensitive thickness of $20\ \mu\text{m}$, thus able to capture all β trajectories that perpendicularly traverse the detector with energies up to, and over, $50\ \text{keV}$.

Figure 8. Counts per second per mm^2 for each band of the microscale of ^{14}C taken with the CCD sensor (circles) and the CMOS sensor (squares). The sensitivity is measured from the slope of each fitted lined, $6.1 \cdot 10^{-3} \text{ cps mm}^{-2} \text{ kBq}^{-1} \text{ g}$ for the CCD and $5 \cdot 10^{-3} \text{ cps mm}^{-2} \text{ kBq}^{-1} \text{ g}$ for the CMOS sensor. The background noise level for 100 minute exposure, of each sensor is $160 \cdot 10^{-5} \text{ cps mm}^{-2}$ for the CCD and $1.11 \cdot 10^{-5} \text{ cps mm}^{-2}$ for the CMOS sensor.

Table 3. Performance of the most significant digital imaging systems obtained with ^{14}C .

System	Sensitivity (cps $\text{mm}^{-2} \text{ kBq}^{-1} \text{ g}$)	Background noise level (cps mm^{-2})	Minimum detectable activity	Exposure time
CCD55-20	$6.10 \cdot 10^{-3}$	$1.60 \cdot 10^{-3}$	0.09 Bq	100 mins
StarTracker (CMOS)	$5.00 \cdot 10^{-3}$	$0.01 \cdot 10^{-3}$	0.09 Bq	100 mins
Medipix2†	$3.00 \cdot 10^{-3}$	$2.00 \cdot 10^{-3}$	0.012 Bq	10 hours
Medipix1†	$4.70 \cdot 10^{-3}$	$0.03 \cdot 10^{-3}$	NA	7 hours
Beta Imager§	NA	$0.16 \cdot 10^{-3}$	NA	NA
Micro Imager§	NA	$0.66 \cdot 10^{-3}$	NA	NA
Micro Channel Plates	$\sim 3.43 \cdot 10^{-3}$	$1.20 \cdot 10^{-3}$	0.031 Bq	20 hours

NA: Data not available

† Mettivier *et al* 2005

§ Barthe *et al* 2004

|| Computed from Lees *et al* 1998

3.3. Size of Events

In figure 10 we show the histograms of detected event cluster sizes from ^{14}C . This was obtained computing the histograms of the size of every event from the data mentioned in subsection 2.3. This shows that, in both sensors, most of the detected events are 1-2 pixels in size with a mean cluster size of 1.76 pixels for the CCD device and 1.57 pixels for the CMOS device. Events over 5 pixels are not normally observed due to the maximum beta range (see Table 2) for ^{14}C , unless these have highly oblique trajectories. Note the CCD, with its thicker sensitive region, produces relatively more of the larger (>2 pixels) events. This is attributed to greater charge diffusion and enhanced oblique angle sampling compared to the CMOS sensor.

Figure 9. Deposited energy spectrum of ^{14}C obtained with the CCD (figure 9(a)) and the CMOS (figure 9(b)) sensors. 9(b) compared to 9(a) suggests the the CMOS sensor only partially samples the incident energy.

Figure 10. Size of events with ^{14}C obtained with the CCD (left) and with the CMOS (right), is calculated after thresholding using equation 1.

3.4. ^3H Imaging

As mentioned above, the most common radioisotope in AR is ^3H . The energy of the beta emissions from this isotope are so low that in a front-illuminated device, these would be stopped in the overlying passivation layers. Therefore a back-thinned CCD55-20 device was substituted for ^3H imaging to demonstrate the potential of pixelated detectors operating at room temperature and using direct detection.

Using the same calibration procedure as undertaken for the front-illuminated CCD sensor, the back-thinned device utilized a mean threshold, T , of 3.46 keV with an inter-pixel threshold variation of $\sigma=0.3$ keV. The differing architectures of the front vs back-illuminated devices allows a lower threshold to be used in the post acquisition image processing. In figure 11 we show the first ^3H image obtained with a CCD at

room temperature under direct irradiation using a set of calibrated ^3H microscapes with an 11 hour exposure period acquired at a frame rate of $0.1 \text{ frames s}^{-1}$. To make a comparative assessment against using tritium sensitive film we repeated this experiment for exposure periods of 11 hours, one, two and four weeks, and applied a similar ROI analysis as described previously on the digitized film images.

Figure 11. Composite image with ^3H obtained with the CCD after 11 hours.

Undertaking this same ROI analysis, in figures 12 and 13 we show the deposited energy spectrum of ^3H and the number of counts per second per mm^2 in each band of this radioisotope with the back-illuminated CCD for 11 hours exposure. From figure 13, the sensitivity measured is $3.39 \cdot 10^{-6} \text{ cps mm}^{-2} \text{ kBq}^{-1} \text{ g}$ with a background noise level of $8.65 \cdot 10^{-4} \text{ cps mm}^{-2}$. Compared to the measurements reported for the front-illuminated CCD, this represents an apparent $\sim 10^{-3}$ loss in sensitivity for ^3H compared to ^{14}C . However, this apparent loss in sensitivity is a consequence of two processes: the first is the challenge of detecting low energy events in the presence of noise. Higher performance read-out electronics and more sophisticated pattern noise correction methods should produce improvements in this area. The second of these is due to self-absorption in the source. Using the beta ranges from the NIST database and a density of 1.2914 g/cm^3 for the tissue equivalent plastic microscapes, self absorption for β -electrons in ^3H microscapes ($\sim 50 \mu\text{m}$ thickness) is estimated to be $\sim 99\%$ and $\sim 86\%$ for β -electrons in the ^{14}C microscapes ($\sim 120 \mu\text{m}$ thickness) (Puertolas *et al* 1996, Mettievier *et al* 2005). These results compared to those presented with Medipix2 (Mettievier *et al* 2005) (sensitivity of $6.2 \cdot 10^{-6} \text{ cps mm}^{-2} \text{ kBq}^{-1} \text{ g}$ and background noise level of $5.0 \cdot 10^{-3} \text{ cps mm}^{-2}$) show a worse figure of sensitivity but better figure of background noise. This can be explained by a sub-optimized acquisition system. This acquisition system was designed to control a certain family of CCD sensors. The back-thinned CCD55-20 sensor was designed later, and since the timing of the control signals is different to those of the front illuminated CCD55-20 sensor, the read-out of the images is therefore defective and the final performance below optimum. In table 4 a comparison of this sensor with other sensors found in the literature for use in ^3H autoradiography is shown. The minimum detectable activity has been obtained using the same procedure as explained for ^{14}C .

For comparison with film, in figure 14, we present an image obtained from digitising a sheet of tritiated hypersensitive hyperfilm exposed to a ^3H microscapes, but in this case exposed for two weeks (the standard exposure time for ^3H imaging in our laboratory) instead of 11 hours. For the 11 hour film exposure, the result was a completely blank image, discarding the typical background fogging, and therefore further analysis was impossible. A similar ROI analysis to that shown with the digital sensors, was undertaken with the tritium-sensitive film. However, in this case the

Figure 12. Deposited energy spectrum of ³H obtained with the back-thinned CCD.

Figure 13. Counts per second per mm² for each band of the microscale of ³H taken with the CCD sensor after 11 hour exposure. The sensitivity measured from the slope of the fitted lined is 3.39×10^{-6} cps mm⁻² kBq⁻¹ g. The background noise level is also shown as dashed line (8.65×10^{-4} cps mm⁻²).

mean pixel value (optical density value) of each band was computed and corrected by subtraction of the mean pixel value measured in the background, for each sheet of film, corresponding to each exposure. As can be seen in figure 15 the resulting data tends to a log-like response rather than the straight line seen with the digital sensors. Note that for the four weeks exposure, the weakest of the eight bands from the calibrated microscale is not detected; for the two weeks exposure, the two weakest bands are not detected, and for the experiment, taken for one week exposure, the five weakest bands are not detected. The background fog level measured in each exposure was 62, 61 and 73 (arbitrary) ADC units respectively. Given that the CCD sensor could detect five of the eight bands above the CCD background noise level in 11 hours, this appears to represent an equivalent performance to film in the interval of 1-2 weeks

Table 4. Performance of the most significant digital imaging systems obtained with ^3H .

System	Sensitivity (cps mm $^{-2}$ kBq $^{-1}$ g)	Background noise level (cps mm $^{-2}$)	Minimum detectable activity	Exposure time
CCD55-20 (back-thinned)	$3.39 \cdot 10^{-6}$	$0.86 \cdot 10^{-3}$	2.43 Bq	11 hours
Medipix2 †	$6.20 \cdot 10^{-6}$	$5.00 \cdot 10^{-3}$	0.32 Bq	14 hours
MIMOSA ‡	$\sim 0.007 \cdot 10^{-6}$	$0.60 \cdot 10^{-3}$	~ 75.46 Bq	32 hours
Beta Imager	$\sim 80.00 \cdot 10^{-6}$ §	$0.11 \cdot 10^{-3}$	NA	NA
Micro Imager ¶	NA	$6.66 \cdot 10^{-3}$	NA	NA

NA: Data not available

 † Mettievier *et al* 2005

‡ Computed from Deptuch 2005

 § Computed from Tribollet *et al* 1991

 || Computed from Barthe *et al* 1999

 ¶ Barthe *et al* 2004

exposure. Therefore an estimated factor of 14-28 improvement in sensitivity of the back-illuminated CCD compared to film is estimated.

Figure 14. Exemplar image produced using ^3H microscale after exposure to tritiated hypersensitive hyperfilm for two weeks.

3.5. Tissue Imaging using ^3H and ^{35}S

Having assessed the quantitative performance of the CMOS and CCD image sensors compared to film, experiments were undertaken to examine the performance under authentic tissue imaging conditions. Initially mouse brain sections using ^{35}S [S]GTP γ S AR were prepared (see section 2.3) and exposed to film. The autoradiograms obtained following ~ 4 days exposure using film are shown from the level of the Caudate (Bregma 0.38mm) (figure 16 left) and from the level of the Hippocampus (Bregma 1.58 mm) (figure 16 right). The physical size of these sections was measured as 10.2 mm x 7.0 mm.

To demonstrate the comparative biological imaging potential of CCD and CMOS technology compared to film, exemplar images produced using direct irradiation of the same radiolabelled biological mouse samples are presented in figures 17 and 18 acquired at a frame rate of 0.1 frames $^{-1}$ and 10 frames $^{-1}$ respectively. This shows a comparison of images taken with film (figure 16) for four days (5760 minutes, the usual exposure time used in our laboratory for such studies), and with the CCD (figure 17) and CMOS sensors (figure 18) each exposed for 16 hours ($\sim 10^3$ minutes,

Figure 15. Calibration plots for Hypersensitive Hyperfilm using ^3H microscales. Experiment undertaken with ^3H exposed to film for one (stars), two (circles) and four weeks (squares) on separate sheets of film.

~6 times shorter) using in both cases the same thresholds used for the microscales stated at 6.7 keV for the CCD sensor and 3.5 keV for the CMOS sensor. These images were then smoothed with a 3x3 block filter applied twice. All tissue images obtained from the digital image sensors have been reverse grayscaled to demonstrate better visual similarity with the original film AR imagery. Note the apparent mottle in the CCD and CMOS image data is more noticeable because of the linear response of the CCD/CMOS sensors and the lack of background fogging.

Figure 16. Film-based AR images from coronal mouse sections, from the level of the Caudate (Bregma 0.38mm) (left) and from the level of the Hippocampus (Bregma 1.58 mm) (right), bound with ^{35}S GTP γ S exposed to film for four days, with key anatomical regions indicated.

To demonstrate the potential of digital technology for direct detection with ^3H in tissue samples, several ex-vivo brain tissue sections labelled with the selective D_1 dopamine receptor radioligand ^3H SCH-23390† (4 nM) (Lena *et al* 2004) were prepared. These sections shown are from the level of the Caudate, Bregma 0.62mm (top) and 0.02mm (bottom) (figure 19), and were exposed to the back-thinned CCD sensor for 11 hours, using a threshold of 3.46 keV as for the microscale, in comparison

† Schering Plough 23990 binding bound with ^3H

Figure 17. Coronal mouse sections, from the level of the Caudate (Bregma 0.38mm) (left) and from the level of the Hippocampus (Bregma 1.58 mm) (right), bound with $^{35}\text{S}[\text{GTP}\gamma\text{S}]$ exposed to CCD sensor for 16 hours. Note the enhanced Hypothalamus and Amygdalas regions (right) and Lateral Septum and Medial Preoptic Area (left) regions barely discerned from background in the film images.

Figure 18. Coronal mouse sections, from the level of the Caudate (Bregma 0.38mm) (left) and from the level of the Hippocampus (Bregma 1.58 mm) (right), bound with $^{35}\text{S}[\text{GTP}\gamma\text{S}]$ exposed to CMOS sensor for 16 hours. As with fig. 17, this shows similar enhanced regions of uptake compared to corresponding barely discernable areas of the digitized 4 day exposure film images.

to the typical length of these experiments (~ 5 weeks) when using film. This first demonstration of ^3H tissue imaging with a room temperature CCD has reproduced the main target areas of uptake in a fraction of the usual 5 week exposure time required with film.

4. Conclusions

We have demonstrated thus far that it is possible to use CCD and CMOS sensors operating at room temperature for AR.

Both of the image sensors used in this work have demonstrated similar levels of qualitative imaging performance, and similar levels of tissue imaging performance. Although some further development is required to produce film-like tissue image quality, all the major areas of uptake have been represented in both the CMOS and CCD AR images in a fraction of the time that may be needed when using conventional film imaging methods. Thus silicon imaging technology has the potential to reduce exposure time, improve sensitivity and linearity, and produce enhanced image quantification compared to using traditional film. We have also demonstrated that it is possible to detect and image ^3H radiation with a back-thinned CCD operating at room temperature. This work has shown that direct detection techniques can be used for all major low-medium energy β -emitting radioisotopes used in AR. Further development to improve the read-out scheme and post-acquisition image processing is expected to yield further improvements in sensitivity. This first demonstration

Figure 19. ^3H tissue image obtained with a CCD at room temperature, for 11 hours, demonstrating tritiated ligand binding to D_1 receptors in a coronal mouse Striatum section, from the level of the Caudate, Bregma 0.62 mm (top) and 0.02mm (bottom)

of room temperature CMOS Monolithic Active Pixel Sensors (MAPS) imaging for AR has shown that despite the slower read-out speed used for the CCD used here (and associated higher susceptibility to dark-current induced pattern noise), the CCD technology still retains higher sensitivity than the CMOS device. However, CMOS technology also represents a serious choice to be considered for future digital AR development.

Acknowledgments

This work is supported by the RC-UK Basic Technology Multidimensional Integrated Intelligent Imaging (MI^3) programme (GR/S85733/01). We would like to thank Natural Environment Research Council (NERC) for allowing the MI^3 consortium to re-manufacture the StarTracker sensor. This sensor was originally designed and first manufactured under NERC funding. The CCD component of this work was funded by a University of Surrey Proof of Concept Award.

References

- Abate L, Bertolucci E, Conti M, Mettievier G, Montesi M C and Russo P 2001 GaAs Pixel Arrays for β Imaging in Medicine and Biology *Nucl. Instrum. Methods* **A460** 97–106
- Arvanitis C D *et al* 2006 A Novel Active Pixel Sensor with On-Pixel Analog-to-Digital Converter for Mammography *IEEE Nucl. Sci. Symp. Medical Imaging Conf., San Diego, USA, October 29 - November 4* **6** 3576–80
- Bailey A, Lesscher H M B, Kelly M, Ledent C, Davis L, Hourani S M O and Kitchen I 2004 Enhanced morphine withdrawal and μ -opioid receptor G-protein coupling in $\text{A}_{2\text{A}}$ adenosine receptor knockout mice *J. of Neurochemistry* **88** 827–34
- Barthe N, Chatti K, Coulon P, Maitrejean S and Basse-Cathalinat B 2004 Recent technologic developments on high-resolution beta imaging for quantitative autoradiography and double labeling applications *Nucl. Instrum. Methods* **A527** 41–5
- Barthe N, Coulon P, Hennion C, Ducassou D, Basse-Cathalinat B and Charpak G 1999 Optimization of a New Scintillation Gas Detector Used to Localize Electrons Emitted by $^{99\text{m}}\text{Tc}$ *The Journal of Nuclear Medicine* **40** 868–75

- Bertolucci E *et al* 1996 Autoradiography with Silicon Strip Detectors *Nucl. Instrum. Methods* **A381** 527–30
- Bertolucci E, Conti M, Mettievier G and Montesi M C 2002 BETAvue: a Digital β -Imaging System for Dynamic Studies of Biological Phenomena *Nucl. Instrum. Methods* **A478** 109–13
- Cabello J *et al* 2006 Digital Autoradiography Using CCD and CMOS Imaging Technology *IEEE Nucl. Sci. Symp. Medical Imaging Conf., San Diego, USA, October 29 - November 4* **4** 2607–12
- Carlson B S 2002 Comparison of modern CCD and CMOS image sensor technologies and systems for low resolution imaging *Proceedings of IEEE Sensors* **1** 171–6
- Charpak G, Dominik W, Zagandis N 1989 Optical Imaging of the Spatial Distribution of β -particles Emerging from Surfaces *Proceedings of the National Academy of Sciences of the United States of America* **86**(6) 1741–5
- Cross W G, Ing H and Freedman N 1983 A short atlas of beta-ray spectra *Phys. Med. Biol.* **28** 1251–60
- Deptuch G 2005 Tritium Autoradiography with Thinned and Back-side Illuminated Monolithic Active Pixel Sensor Device *Nucl. Instrum. Methods* **A543** 537–48
- Janesick J 2002 Lux transfer: CMOS versus CCD *Proc. SPIE (San Jose, CA)* **4669A** 232–49
- Johnston R F, Pickett S C and Barker D L 1990 Autoradiography Using Storage Phosphor Technology *Electrophoresis* **11** 355–60
- Karellas A, Liu H, Reindhart C, Harris L J and Brill A B 1993 Imaging of Radionuclide Emissions with a Low-noise Charge-Coupled Device *IEEE Trans. on Nuclear Science* **40** 979–82
- Kokkinou E, Wells K, Petrou M and Bailey A 2003 Digital Autoradiography Imaging Using Direct Irradiation of a CCD Between 278–309 K *IEEE Trans. on Nuclear Science* **50** 1702–7
- Lees J E, Murray A, Perkins A C and Fraser G W 2002 Autoradiography of High-Energy Radionuclides Using a Microchannel Plate Detector *IEEE Trans. on Nuclear Science* **49** 153–5
- Lees J E, Pearson J F, Fraser G W, Hales J M and Richards P G 1999 An MCP-based system for beta autoradiography *IEEE Trans. on Nuclear Science* **46** 636–8
- Lees J E and Fraser G W 1998 Microchannel plate detectors for C^{14} autoradiography *IEEE Trans. on Nuclear Science* **45** 1288–92
- Lena I, Kieffer B and Kitchen I 2004 Quantitative autoradiography of dopamine receptors in the brains of μ -opioid receptor knockout mice *Neuroscience Letters* **356** 220–4
- Ljunggren K and Strand S E 1990 Beta camera for static and dynamic imaging of charged-particle emitting radionuclides in biologic samples *J. Nucl. Med* **31** 2058–63
- Magnan P 2003 Detection of visible photons in CCD and CMOS: A comparative view *Nucl. Instrum. Methods* **504** 199–212
- Marczewski J *et al* 2005 Monolithic silicon pixel detectors in SOI technology *Nucl. Instrum. Methods* **A549** 112–6
- Mettievier G, Montesi M C and Russo P 2005 Digital Autoradiography with a Medipix2 Hybrid Silicon Pixel Detector *IEEE Trans. on Nuclear Science* **52** 46–50
- Mettievier G, Montesi M C and Russo P 2004 Tritium Digital Autoradiography with a Medipix2 Hybrid Silicon Pixel Detector *Nucl. Instrum. Methods* **516** 554–63
- Mettievier G, Montesi M C and Russo P 2003 First images of a digital autoradiography system based on a Medipix2 hybrid silicon pixel detector *Phys. Med. Biol.* **48** 173–81
- Moraes D *et al* 2004 A novel low noise hydrogenated amorphous silicon pixel detector *Journal of Non-Crystalline Solids* **338–340** 729–31
- Neeser W *et al* 2000 The DEPFET pixel BIOSCOPE *IEEE Trans. on Nuclear Science* **47** 1246–50
- Niemiec H *et al* 2005 Monolithic active pixel sensor realized in SOI technology concept and verification *Microelectronics Reliability* **45** 1202–7
- NIST 2000 *Physical Reference Data, E-STAR*, <http://physics.nist.gov/PhysRefData/Star/Text/>
- Ott R J, MacDonald J and Wells K 2000 The performance of a CCD digital autoradiography imaging system *Phys. Med. Biol.* **45** 2011–27
- Overdick M *et al* 1997 A "Bioscope" system using double-sided silicon strip detectors and self-triggering read-out chips *Nucl. Instrum. Methods* **A392** 173–7
- Petegnief Y, Petiet A, Peker M C, Bonnin F, Meulemans A and Guludec D 1998 Quantitative autoradiography using a radioimager based on a multiwire proportional chamber *Phys. Med. Biol.* **43** 3629–39
- Pohm A V, Waddell R C, Powers J P and Jensen E N 1955 Beta Spectrum of ^{14}C *Physical Review* **97** 432–3
- Prydderch M L *et al* 2003 A 512 x 512 CMOS Monolithic Active Pixel Sensor with integrated ADCs for space science *Nucl. Instrum. Methods* **512** 358–67
- Puertolas D, Piedigrossi D, Leutz H, Gys T and D'Ambrosio C 1996 An ISPA Camera for Beta Radiography *IEEE Trans. on Nuclear Science* **43** 2477–87

- Reichert W L, Stein J E, French B, Goodwin P and Varanasi U 1992 Storage phosphor imaging technique for detection and quantitation of DNA adducts measured by the ^{32}P -postlabeling assay *Electrophoresis* **13** 1475–9
- Tribollet E, Dreiffus J J, Charpak G, Dominik W and Zaganidis N 1991 Localization and quantitation of tritiated compounds in tissue sections with a gaseous detector of β particles: Comparison with film autoradiography *Proc. Natl. Acad. Sci.* **88** 1466–8
- Turchetta R *et al* 2001 A monolithic active pixel sensor for charged particle tracking and imaging using standard VLSI CMOS technology *Nucl. Instrum. Methods* **458** 677–89
- Ulrici J, Fischer P, Klein P, Lutz G, Neeser W, Richter R, Strüder L, Trimpl M and Vermes N 2005 Imaging Performance of a DEPFET Pixel Bioscope System in Tritium Autoradiography *Nucl. Instrum. Methods* **A547** 424–36
- Wells K and Kokkinou E 2006 UK Patent GB2397959: Imaging system for use in Autoradiography
- Vermes N 2004 Trends in Pixel Detectors: Tracking and Imaging *IEEE Trans. on Nuclear Science* **51** 1006–15