Betacam: A Commercial Approach to β - Autoradiography

Cabello J.^a, Holland A.^b, Holland K.^b, Bailey A.^c, Kitchen I.^c and Wells K.^a

^aCentre for Vision, Speech and Signal Processing, Faculty of Engineering and Physical Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK;

^bXCAM Ltd, Northampton, NN3 7TG, UK;

^cFaculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK

ABSTRACT

Autoradiography is a well established imaging modality in Biology and Medicine. This aims to measure the location and concentration of labelled molecules within thin tissue sections. The brain is the most anatomically complex organ and identification of neuroanatomical structures is still a challenge particularly when small animals are used for pre-clinical trials. High spatial resolution and high sensitivity are therefore necessary. This work shows the performance and ability of a prototype commercial system, based on a Charged-Couple Device (CCD), to accurately obtain detailed functional information in brain Autoradiography. The sample is placed in contact with the detector enabling direct detection of β - particles in silicon, and the system is run in a range of quasi-room temperatures (17-22 °C) under stable conditions by using a precision temperature controller. Direct detection of β - particles with low energy down to ~ 5 keV from ^3H is possible using this room temperature approach. The CCD used in this work is an E2V CCD47-20 frame-transfer device which removes the image smear arising in conventional full-frame imaging devices. The temporal stability of the system has been analyzed by exposing a set of ^{14}C calibrated microscales for different periods of time, and measuring the stability of the resultant sensitivity and background noise. The thermal performance of the system has also been analyzed in order to demonstrate its capability of working in other life science applications, where higher working temperatures are required. Once the performance of the system was studied, a set of experiments with biological samples, labelled with typical β - radioisotopes, such as ^3H , has been carried out to demonstrate its application in life sciences.

Keywords: CCD, digital, autoradiography, tritium

1. INTRODUCTION

Autoradiography (AR) is a method used used in life sciences to map and measure the distribution of radiolabelled bio-molecules deposited in a thin tissue specimen. Traditionally Autoradiography film emulsion (Kodak,GE) is placed in direct contact with tissue sections, which have been previously labelled with β radioisotopes, such as ^3H , ^{14}C or ^{35}S , bound to bio-molecule specific ligands.

Film emulsion has been long used as the detecting medium for autoradiography imaging. This offers high spatial resolution due to the small size of the silver halide grains sensitive to ionising radiation (~ 1 nm) contained within the emulsion. On the other hand this also presents some significant drawbacks, such as lack of linearity and poor sensitivity, therefore requiring exposure times as long as several days or weeks for medium energy radioisotopes to months for low energy radioisotopes. To address these issues digital imaging techniques have shown potential as quantitative imaging detectors of low energy electrons, especially solid state detectors made of silicon. Alternatives based on Charge-Coupled Devices (CCD),^{1,2} hybrid detectors³

Further author information: (Send correspondence to J. Cabello)

J. Cabello : E-mail: j.cabello@surrey.ac.uk, Telephone: +44 (0)1483 689856

and detectors based on standard CMOS processes such as DEPFET⁴ or monolithic CMOS detectors^{2,5,6} have been presented as valid alternatives to film in beta AR. One of the outstanding issues impeding widespread adoption of digital technology arises from differences in the AR images with respect to resolution, sensitivity, linearity and noise properties. This, as we will show, leads to significant differences in visual appearance and, potentially, in the quantitative information that is present.

2. METHODOLOGY

The E2V⁷ CCD47-20 detector used here is an inverted mode back-illuminated device comprised of a 1024x1024 array of pixels in the image region on a 13 μm pitch (13.3x13.3 mm² active area). The specific detector used in this work is back-thinned in order to allow the detection of low energy β - particles (~ 5.8 keV). The sensitive region of this detector is 15 μm thick, with half of this depleted and the other half, field-free. Event patterns from the beta particles are analysed and centroiding of event interaction to the sub pixel level is possible. In the case of ³[H] events, the back illumination used here means that the β - electrons will be absorbed in the first 1-2 microns of the field-free layer, and so all events spread by thermal diffusion by a similar amount.

A thin tissue section (~ 20 μm thick) is placed in direct contact with the surface of the detector, within a light tight box. The temperature of the detector is measured and controlled with a Peltier device and a temperature controller, with a resolution of ~ 5 mK, thereby eliminating drift and enabling background subtraction techniques to mitigate the effect of dark current. The temperature used in the experiments shown here was set to 17 °C. The acquisition system has Correlated Double Sampling (CDS) implemented in hardware, therefore reset noise is largely removed. A frame repetition rate of 3 seconds is employed during the process.

To correct for the dark current and the fixed pattern noise present in the CCD detector, pre-processing the raw images is necessary to detect the true β events emitted by the sample. This correction is based on an average set of N frames acquired under the same temperature conditions. By applying this correction dark current and fixed pattern noise are mitigated, and by thresholding, the remaining noise is removed. Some residual pixel effects still occur due to phenomenon of *flickering pixels*, however the majority of these can be removed easily via post-acquisition post-processing.

The threshold used here is calculated based on the system noise. This is obtained from the intensity histogram of the resulting image, after averaging the set of N frames previously acquired. From this intensity histogram, that represents the noise distribution and can be approximated to a Gaussian distribution, the upper and lower intensity levels are manually selected at the two Full-Width-Half-at-Maximum points. From these two points the noise system in electrons rms is computed. After using the histogram to determine the one sigma value for the Gaussian noise distribution, a threshold at k sigma above the image mean is applied, which ensures only a few true noisy pixels per frame will remain. These are single pixels *isolated* events and can be easily rejected by software.

3. RESULTS

A number of experiments with a set of ¹⁴[C] and ³[H] calibrated microscopes has been carried out, to measure the linearity, sensitivity and background noise of the detector under evaluation, and to evaluate the temporal stability of the system, by exposing different sources for different periods of time, and analyzing the aforementioned parameters for each experiment.

3.1 Sensitivity

A calibrated set of ¹⁴[C] microscopes was placed directly in top of the CCD detector and exposed for 12 hours. The system noise measured was 3.3 electrons rms and a value of $k=3$ was used for this experiment. Figure 1 shows an example of the recorded count density (cps mm⁻²) measured within each band of the microscope

plotted against the known activity (kBq g^{-1}). It is observed in the inset the composite image obtained from the microscale. The data points are fitted to a straight line using the least squares method. The sensitivity measured from the slope of this fitted line is $24.6 \cdot 10^{-3} \text{ cps mm}^{-2} \text{ kBq g}^{-1}$ with a measure of the background noise of $2.0 \cdot 10^{-3} \text{ cps mm}^{-2}$. This is comparable to the best sensitivity available with solid state devices.²

Figure 1: Counts per second per mm^2 for each band of the microscale of ^{14}C acquired after 12 hours of exposure. The sensitivity is measured from the slope of the fitted line, $24.6 \cdot 10^{-3} \text{ cps mm}^{-2} \text{ kBq g}^{-1}$. The background noise level is $2.0 \cdot 10^{-3} \text{ cps mm}^{-2}$. It can be observed in the inset the resulting composite image of the ^{14}C microscale source.

Similarly to the previous experiment undertaken with ^{14}C , a ^3H microscale was placed in direct contact with the surface of the detector for 48 hours. The same system noise was measured (3.3 electrons rms), given that the operation temperature was not changed, and a value of $k=3$ was used for this experiment. Figure 2 demonstrates the linear response of the detector, showing the count rate (cps mm^{-2}) measured beneath each band of the ^3H microscale plotted against the activity (kBq mg^{-1}). It is observed in the inset the composite image obtained from the microscale. The data points are fitted to a straight line, to obtain the sensitivity from the slope of this fitted line, demonstrating a sensitivity of $1.2 \cdot 10^{-5} \text{ cps mm}^{-2} \text{ Bq mg}^{-1}$ with a measure of the background noise of $2.9 \cdot 10^{-4} \text{ cps mm}^{-2}$, after exposing for 48 hours. This slightly outperforms the sensitivity measurement previously published for the Vanilla CMOS detector⁶ ($0.9 \cdot 10^{-5} \text{ cps mm}^{-2} \text{ Bq mg}^{-1}$) after an exposure time of 15 hours, and lies between those measurements published recently with MedipixII ($1.0 \cdot 10^{-5} \text{ cps mm}^{-2} \text{ Bq mg}^{-1}$) and MIMOSAV ($2.0 \cdot 10^{-5} \text{ cps mm}^{-2} \text{ Bq mg}^{-1}$).⁸ Regarding the background noise, the count rate measured in the background is slightly higher than MedipixII ($1.3 \cdot 10^{-4} \text{ cps mm}^{-2}$) but lower than those measured for the CMOS detectors Vanilla ($4.7 \cdot 10^{-4} \text{ cps mm}^{-2}$) and MIMOSAV ($6.6 \cdot 10^{-4} \text{ cps mm}^{-2}$). All these measurements are summarized in Table 1. It is worth noting that the exposure time used in these experiments⁸ was only 1 hour for MedipixII and 2 hours for MIMOSAV. However, we have observed that for the device we have studied at least, sensitivity hits a maximum value within the first couple of hours of exposure time, and then decreases until it settles down after >10 hours.⁶

3.2 Temporal Stability

The temporal stability of the system has also been analyzed, by measuring the sensitivity and the background noise for ^{14}C and ^3H for different periods of time. A value of $k=3$ was used for all the experiments. In Figure 3 the sensitivity and the background noise obtained, after exposing a ^{14}C microscale, are plotted against time for a set of experiments that were carried out under the same temperature conditions, at 17°C .

Similarly, the sensitivity and background noise obtained after exposing a ^3H microscale for 12-48 hours are plotted against time. A value of $k=3$ was used for all the experiments. The background noise shown in

Figure 2: Counts per second per mm^2 for each band of the microscale of $^3\text{[H]}$ acquired after 48 hours of exposure. The sensitivity is measured from the slope of the fitted lined, $1.2 \cdot 10^{-5} \text{ cps mm}^{-2} \text{ Bq mg}^{-1}$. The background noise level is $2.9 \cdot 10^{-4} \text{ cps mm}^{-2}$. It can be observed in the inset the resulting composite image of the $^3\text{[H]}$ microscale.

Table 1: Sensitivity and background noise measured in silicon-based detectors

Detector	Radioisotope	Sensitivity (cps $\text{mm}^{-2} \text{ Bq mg}^{-1}$)	Background noise (cps mm^{-2})	Experiment length
E2V CCD47-20	$^3\text{[H]}$ ¹	$1.2 \cdot 10^{-5}$	$2.9 \cdot 10^{-4}$	48 hours
E2V CCD47-20	$^{14}\text{[C]}$ ²	$24.6 \cdot 10^{-3}$	$2.0 \cdot 10^{-3}$	12 hours
CMOS Vanilla	$^3\text{[H]}$ ¹	$0.9 \cdot 10^{-5}$	$4.7 \cdot 10^{-4}$	15 hours
CMOS Vanilla	$^{14}\text{[C]}$ ²	$11.4 \cdot 10^{-3}$	$3.79 \cdot 10^{-3}$	100 minutes
CMOS MIMOSAV	$^3\text{[H]}$ ¹	$2.0 \cdot 10^{-5}$	$6.6 \cdot 10^{-4}$	2 hours
Hybrid MedipixII	$^3\text{[H]}$ ¹	$1.0 \cdot 10^{-5}$	$1.4 \cdot 10^{-4}$	1 hour

¹ The background recorded with the $^3\text{[H]}$ source represents the true laboratory background arising from ground based cosmic rays and local radioactivity in the building.

² The background recorded with the $^{14}\text{[C]}$ source results to the effect that the medium energy β electrons create their own background (through Compton process) resulting in higher measures background.

Figure 3: Sensitivity (cps $\text{mm}^{-2} \text{ kBq g}^{-1}$) and background noise (cps mm^{-2}) measured with a $^{14}\text{[C]}$ microscale for 1, 6, 9, 12 and 18 hours demonstrating high temporal stability of the performance of the detector.

Figure 4(b) shows lower values than those shown in Figure 3(b) due to the higher gain used for the acquisition system for $^3\text{[H]}$, and also due to the different k value used for both radioisotopes.

Figure 4: Sensitivity (cps mm⁻² Bq mg⁻¹) and background noise (cps mm⁻²) measured with a $^3\text{[H]}$ microscale for 12, 24, 36 and 48 hours demonstrating high temporal stability of the performance of the detector.

These plots demonstrate that the system offers temporal stability, necessary for the typical long experiments carried out in β - Autoradiography (~ 48 hours for $^3\text{[H]}$). It has to be noted that the reference set of blank frames previously acquired for the noise correction of the images, was only acquired in the first experiment, and thus ignores any temporal change in fixed pattern noise. This further demonstrates the temporal stability of this system.

3.3 Performance as a Function of Temperature

A study of the dependency of the performance, mainly comprised by the sensitivity and the background noise, of the detector and the temperature has also been undertaken. Given that the temperature of the detector can be accurately controlled with a resolution of ~ 5 mK, experiments exposing a $^{14}\text{[C]}$ microscale for 5 hours in a range from 17°C up to 30°C have been undertaken. It has been observed how as the operation temperature increased, the dark current noise and the fixed pattern noise increased according to the temperature. The N frames necessary for the dark current and fixed pattern noise correction is acquired every time the temperature is modified. This effect had a negative impact on the noise system, and given that the threshold is set according to the noise system, the higher the temperature the higher the system noise. Using $k=5$ for all the experiments, the sensitivity measured for 17°C , 20°C , 25°C and 30°C is shown in Figure 5. The system noise measured for each temperature was 3.65 e- rms, 2.69 e- rms, 4.84 e- rms and 9.40 e- rms respectively. The system noise was also measured for 15°C and 35°C , exhibiting 1.69 e- rms and 18.54 e- rms respectively. No results at 15°C are presented because they do not represent any improvement compared to those at 17°C , and no results at 35°C are presented either because the system noise was too high to produce useful results. However we believe that by enhanced post-processing we would be able to image biological samples at such temperature. This graph shows how, as the temperature increases the sensitivity is negatively affected, produced by a higher system noise. This effect could be counteracted by reducing the parameter k (reducing the threshold), concomitantly producing a higher count rate in the background, i.e. higher background noise.

Figure 5 shows that this system works very well as a *room temperature* detector, but above 20°C , the leakage current integrated over the frame time yields an increased noise, hence detection threshold, leading to a reduced count rate. The effect of poor sensitivity (by increased threshold) could be compensated in the future by reducing the frame time through faster readout if operating at temperatures $> 20^\circ\text{C}$.

Figure 5: Sensitivity (cps mm⁻² kBq g⁻¹) measured after exposing a ¹⁴[C] microscale for 5 hours for a range of different temperatures. Clearly above room temperature, thermal noise degrades sensitivity.

3.4 Tissue Imaging

Figure 6(c) represents a comparison of a coronal mouse section labelled with ³[H]SCH-23390*, exposed to conventional autoradiography film (Figure 6(a)), to a scientific CMOS sensor⁶ (Figure 6(b)) and to the CCD sensor (Figure 6(c)). This image exhibits detailed areas with high level of uptake corresponding with brain regions with a significant amount of D1 neuroreceptors, subject of this experiment.

The image shown in Figure 6(a) has been exposed to conventional autoradiography film for five weeks, following the typical protocol in autoradiography experiments in our life sciences laboratory. Similarly, the image shown in Figure 6(b) has been exposed for 40 hours to an off-the-shelf back-thinned CMOS detector⁶ for comparison purposes. Finally the image shown in Figure 6(c) has been exposed to the CCD detector used in this work, for 48 hours at 17 °C.

Preliminary observations of this image by experts demonstrated that the results obtained by the CCD and the CMOS detectors show that the key structures are clearly visible and easily measured. The image shown in Figure 6(b) shows an evident less capability of resolving small structures, compared to that one obtained with the CCD detector, shown in Figure 6(c), due to its intrinsic worse spatial resolution. This effect is attributed to the larger pixel size of the CMOS detector (25 μm) compared to the CCD detector (13 μm). Figure 6(b) also shows a visible noisier background, mainly due to the higher dark current noise typically exhibited by CMOS detectors, and aggravated by the absence of control over the detector temperature. The equivalent image produced using film, exposed to conventional autoradiography film for the same 48 hours, produced no discernable structural detail.

4. CONCLUSIONS

In this paper we describe the first demonstration of a commercial prototype system for room temperature direct detection using CCDs for β- Autoradiography. Detection at room temperature and in ambient air avoids the need for device cooling and associated complications, and the system efficiently detects β- particles with energies down to ~5 keV from ³[H] with high detection efficiency. This work has evaluated a prototype system 1kx1k pixels, however the same system and techniques can be applied to 2kx2k image formats allowing imaging of larger samples, or enabling several experiments to be conducted in parallel.

The system demonstrated temporal stability of the performance, measuring consistent sensitivity and noise floor for a range of experiments going from 1 hour up to 48 hours. The system also demonstrated consistent

*Schering Plough 23990 binding bound with ³[H]

Figure 6: Coronal mouse Striatum section, from the level of Caudate, Bregma 0.86 mm, labelled with $^3\text{[H]}$ exposed for 5 weeks to conventional autoradiography film (a), for 37 hours to the Vanilla back-thinned CMOS detector at room temperature (b) and for 48 hours to the CCD detector at 17°C (c), demonstrating $^3\text{[H]}$ SCH-23390 ligand binding to D1 neuroreceptors in the areas of the Caudate Putamen, Acumbens Nucleus Core and Olfactory Tubercle.

performance at different working temperatures, demonstrating the viability of this system for other life science applications requiring higher temperature working conditions.

We have demonstrated a digital imaging system using direct coupling of the samples to a silicon CCD. The detector was operated at room temperature in the laboratory ambient, and produced high resolution images, with high sensitivity to both $^{14}\text{[C]}$ and $^3\text{[H]}$ radioisotopes. This system outperforms traditional film, reducing significantly the exposure time of biological samples for 4-5 weeks to 1-2 days.

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