Digital Autoradiography Imaging Using CMOS Technology: First Tritium Autoradiography with a Back-Thinned CMOS Detector and Comparison of CMOS Imaging Performance with Autoradiography Film

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Abstract—It has been shown that CMOS imaging technology can be applied to Digital Autoradiography (AR) as a potential imaging alternative technology to using conventional film emulsion. In this work a thorough investigation on the performance of CMOS technology used in AR is presented. ^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 compared to other beta-emitting radioisotopes. In order to detect β -particles from ^3H beta decay a back-thinned CMOS sensor has been used. Back-thinning is a standard process in CCDs but it is not widely applied to CMOS sensors. In this paper the first results of imaging a calibrated ^3H microscale and the first tritiated autoradiogram obtained with a back-thinned CMOS sensor at room temperature are presented.

Index Terms—Digital Autoradiography, Tritium Autoradiography, CMOS technology, Back-thinned technology.

I. INTRODUCTION

Autoradiography (AR) is a method used to map the distribution of radiolabeled bio-molecules deposited in a thin tissue specimen. Traditionally film emulsion has been used as the detecting medium for autoradiography imaging. However other digital imaging techniques have shown potential as quantitative imaging detectors of low energy electrons, especially with the use of CCD [1], [2] and CMOS [3], [2] silicon imaging sensors 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 in the quantitative information that is present.

Another significant issue when comparing film with digital technology is that any new imaging device should be suitable

for imaging all common β emitters used in AR. However, when using low-cost single-use film cassettes, the user selects a particular film product with respect to choice of β energies, sensitivity and resolution for a particular study. This means a viable digital alternative needs to have good imaging performance across a wide range of β energies, which can be particularly challenging at low energy (<20 keV). We have previously addressed the difficulty of detecting very low energy beta electrons emitted from ^3H with CCD technology [2] at room temperature. Here we present the first results, to our knowledge, of ^3H images obtained with a back-thinned CMOS sensor at room temperature that improves upon previous results reported elsewhere [2], [3], [4]. This represents a flexible low cost solution to imaging the multiplicity of β emitters used in AR.

II. MATERIALS AND METHODS

^3H is the most common radioisotope used in AR because it labels most biomolecules and because it achieves the best resolution due to short range of the emitted β particles. Therefore, any alternative to film must be able to detect and image ^3H efficiently. In this work we use a back-thinned CMOS sensor at room temperature to make this technology a viable option for use in AR.

The CMOS sensor used here, known as VANILLA, to detect ^3H is a backthinned active pixel sensor (APS) comprised of a 512×512 array of pixels on a $25 \mu\text{m}$ pitch. Each APS pixel has a typical 3T architecture designed with added circuitry to be used with hard, soft or flushed reset. The readout is column-parallel with a 12-bit ADC per four columns that is able to read up to 100 frames/sec. The results shown in this paper were obtained from images acquired with hard reset and at a frame rate of 1 frame/sec.

III. ^{14}C AND ^3H IMAGING

Preliminary results were obtained using typical calibrated microscales of ^{14}C and ^3H . These microscales consist of known amounts of radioactivity homogeneously distributed in a plastic tissue equivalent polymer of thickness $120 \mu\text{m}$ for ^{14}C and $50 \mu\text{m}$ for ^3H . Each microscale has eight 3.5×1

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mm² cells, with a 1.5 mm separation, of varying activity with a range of 3.74-588 Bq/mg for ¹⁴C and 0.11-4.04 kBq/mg for ³H [5].

The experiment was set up placing the sensor in a light proof box and placing each microscale in direct contact with the surface of the sensor for 100 minutes for ¹⁴C (fig. 1) and 15 hours for ³H (fig. 2), with images acquired at a frame rate of 1 frame second⁻¹.

Fig. 1. Composite image with ¹⁴C obtained with the back-thinned CMOS sensor after 100 minutes.

Fig. 2. Composite image with ³H obtained with the back-thinned CMOS sensor after 15 hours.

It has been previously shown in [2] that conventional film is unable to obtain an image of these microscales with these lengths of experiments: a ¹⁴C microscale exposed to film for 100 minutes produced a poor quality image with a lack of contrast making the subsequent analysis impossible, and a microscale of ³H exposed for 11 hours to conventional film produced a completely blank image.

A. Sensitivity

To determine sensitivity for ³H and ¹⁴C, ROI analysis was undertaken for each calibrated region observed in the resulting images. Plots of count rate vs specific activity were obtained, shown in figs. 3 and 4. The sensitivity measured with this CMOS sensor for ¹⁴C and ³H, in counts per second per mm² per kBq per gram, was obtained from the slope of the fitted lines

These measurements of sensitivity and background noise level are dependent on the intensity threshold used: to minimize electronic noise a high threshold will decrease the sensitivity and reduce the background noise level and vice-versa. This threshold, applied to each frame, is defined by (1).

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

where T is the threshold applied to a certain pixel, M is the mode dark signal of that pixel, averaged over N blank

Fig. 3. Counts per second per mm² for each band of the ¹⁴C microscale taken with the back-thinned CMOS sensor. The sensitivity is measured from the slope of the fitted lined, $11.47 \cdot 10^{-3}$ cps mm⁻² kBq⁻¹ g. The background noise level for 100 minutes exposure is $3.79 \cdot 10^{-3}$ cps mm⁻².

Fig. 4. Counts per second per mm² for each band of the ³H microscale taken with the back-thinned CMOS sensor. The sensitivity is measured from the slope of the fitted lined, $9.1 \cdot 10^{-6}$ cps mm⁻² kBq⁻¹ g. The background noise level for 15 hours exposure is $4.74 \cdot 10^{-4}$ cps mm⁻².

frames previously acquired, and σ is the dark level standard deviation for that pixel obtained from previously acquired blank frames. The pixel threshold is globally defined in terms of the coefficient k .

To choose an optimal threshold for this application the acquired frame data, in the case of ¹⁴C, have been re-processed several times using different values for the coefficient k . The number of counts detected beneath each band of the microscale and the number of counts in the background have been analyzed for each threshold. The measurement used to define the optimum value for k is defined by the Signal-to-Noise (SNR) ratio defined by (2).

$$SNR = \frac{I_M - I_B}{I_B} \quad (2)$$

where I_M is the number of counts per second per mm² measured in a given band and similarly I_B measured in the background. The evolution of the Signal-to-Noise ratio with coefficient k is shown in fig. 5.

From fig. 5, as a trade-off between sensitivity and background noise level is desired, a value of $k = 6$ was chosen, and this represents the threshold used in fig. 3.

Fig. 5. Evolution of the Signal-to-Noise ratio defined by (2) with coefficient k in the case of ^{14}C .

A comparison with some of the most significant AR systems with published results using ^{14}C is shown in TABLE I. A similar comparison undertaken for ^3H is shown in TABLE II. Note the CMOS sensor called MIMOSA [3] presented results with ^3H acquiring under cooled conditions whereas our work represents room temperature operation. The CCD sensor (CCD55-20) [2] is a back-thinned CCD sensor used at room temperature.

TABLE I
PERFORMANCE OF THE MOST SIGNIFICANT DIGITAL IMAGING SYSTEMS OBTAINED WITH ^{14}C .

System	Sensitivity (cps mm ⁻² kBq ⁻¹ g)	Background noise level (cps mm ⁻²)
BT ¹ CMOS (Vanilla)	11.47 10 ⁻³	3.79 10 ⁻³
CCD55-20 [2]	6.10 10 ⁻³	1.60 10 ⁻³
CMOS (StarTracker) [2]	5.00 10 ⁻³	0.01 10 ⁻³
Medipix2 [6]	3.00 10 ⁻³	2.00 10 ⁻³
Medipix1 [6]	4.70 10 ⁻³	0.03 10 ⁻³
Beta Imager [7]	NA ²	0.16 10 ⁻³
Micro Imager [7]	NA	0.66 10 ⁻³
Micro Channel Plates [8]	~3.43 10 ⁻³	1.20 10 ⁻³

TABLE II
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)
BT CMOS (Vanilla)	9.1 10 ⁻⁶	0.47 10 ⁻³
BT CMOS (MIMOSA) [3]	~0.007 10 ⁻⁶	0.60 10 ⁻³
CCD55-20 [2]	3.39 10 ⁻⁶	0.86 10 ⁻³
Medipix2 [6]	6.20 10 ⁻⁶	5.00 10 ⁻³
Beta Imager	~80.00 10 ⁻⁶ [9]	0.11 10 ⁻³ [10]
Micro Imager [7]	NA	6.66 10 ⁻³

IV. COMPARISON BETWEEN FILM-BASED IMAGES AND DIGITAL-BASED IMAGES

It has been claimed in many previous works that other alternatives/technologies offer better sensitivity than conventional film but there is very little, if any, quantitative comparison work undertaken. In order to quantify the order of magnitude

¹ Back-Thinned

² Data not available

sensitivity improvement offered by digital CMOS technology compared to conventional film the following analysis has been followed.

A tritiated tissue sample section has been exposed to conventional film for 4 weeks (fig. 6(a)) representing the standard exposure time used for this particular study. Subsequently the same section has been exposed to the back-thinned CMOS sensor described in this work for 37 hours (fig. 6(b)).

Fig. 6. ^3H tissue image obtained with hypersensitive film after 4 weeks (a) and with a back-thinned CMOS sensor (b) at room temperature, for 37 hours, demonstrating tritiated ligand binding to D1 receptors in a coronal mouse Striatum section, from the level of the Caudate, Bregma 0.86 mm.

After the two final images were obtained, a quantitative analysis was performed. Several regions of interest (ROIs) were drawn manually in both sections. These sections, indicated in fig. 7, are the Caudate Putamen (CPu), Cingulate Cortex (CgCx), Olfactory Tubercle (Tu), Accumbens Nucleus Shell (AcbSh) and Accumbens Nucleus Core (AcbC). The digital values of such regions were then converted to femtomoles per gram of tissue equivalent ligand concentration, ^3H SCH-23390³ in this specific case, and compared. In the case of the experiment undertaken with film, 6 sections of contiguous bregmas were analyzed to obtain an experimental mean and standard deviation of concentration in the ROIs under study. The values measured with the CMOS sensor, as is shown in fig. 8, were observed to be close to the mean measured with conventional film and within the range (also shown in fig. 8) imposed by one standard deviation of the mean from the film-based ROI analysis.

Fig. 7. Fig. 6(b) with the regions under study labeled.

Subsequently the raw image data acquired with the CMOS sensor were re-processed to determine the image quantitation

³Schering Plough 23390 binding bound with ^3H

Fig. 8. Comparison of ligand concentration in fm/g of the ROIs indicated in fig. 7 with conventional film after 4 weeks and with the CMOS sensor after 37 hours.

of the imaging system as a function of exposure time. Thus an evolution graph of the measured ligand concentration with time was obtained. This allowed us to determine the exact point the ligand concentration measured by the CMOS sensor in each region is quantitatively similar to the conventional film based image. This graph is shown in fig. 9 only for the Caudate Putamen and the Cingulate Cortex as an example. Note the arrows in this graph (fig. 9) showing when the measured ligand concentrations of the analyzed ROIs in the CMOS sensor reach the mean value within one standard deviation. In TABLE III the time at which the radioligand measured in the CMOS-based image reaches the mean concentration measured in film ($t1$) and the mean minus one standard deviation ($t2$) are shown. The average of these two measurements is shown in $t3$. The 5 ROIs under study reached a concentration close to the final concentration, and within the range imposed by $\mu \pm 1\sigma$ obtained with conventional film, in approximately 14 hours. Therefore, we conclude that similar levels of image quantitation were obtained with the back-thinned VANILLA CMOS sensor, but achieved with a factor ~ 50 reduction in exposure time compared to using film.

Fig. 9. Evolution of the radioligand concentration in the Caudate Putamen (blue graph) and Cingulate Cortex (red graph) with time. The arrows indicate where the concentrations measured in the CMOS based image are similar to those measured with conventional film.

TABLE III
EXPOSURE TIME NECESSARY TO OBTAIN
COMPARABLE ROI RADIOLIGAND CONCENTRATION.

ROI	Name	t1 (hrs)	t2 (hrs)	t3 (hrs)
1	Caudate Putamen	19.0	13.7	16.3
2	Cingulate Cortex	12.9	10.4	11.6
3	Olfactory Tubercle	14.7	11.2	12.9
4	Accumbens Nucleus Shell	20.4	12.5	16.4
5	Accumbens Nucleus Core	20.0	12.5	16.3

V. CONCLUSIONS

The first quantitative comparison between film and a CMOS sensor has been presented to the best of our knowledge. This demonstrates highly competitive, and in most cases, superior performance to other digital AR imaging systems. The first image of a tritiated ex-vivo thin tissue sample acquired with a back-thinned CMOS sensor at room temperature has been presented in this paper. A quantitative analysis has been undertaken measuring the ligand concentration in several regions of interest in an image acquired with conventional film and the same image acquired with the CMOS sensor obtaining similar results.

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