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 conventional film emulsion. In this work a thorough investigation on the performance of CMOS technology used in AR is presented. $^3$H 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 $\beta$-particles from $^3$H 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 $^3$H 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 $\beta$ 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 $\beta$ 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 $\beta$ 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 $^3$H with CCD technology [2] at room temperature. Here we present the first results, to our knowledge, of $^3$H 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 $\beta$ emitters used in AR.

II. MATERIALS AND METHODS

$^3$H 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 $\beta$ particles. Therefore, any alternative to film must be able to detect and image $^3$H 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 $^3$H is a backthinned active pixel sensor (APS) comprised of a 512 x 512 array of pixels on a 25 $\mu$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 $^3$H IMAGING

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

![Composite image with 14C obtained with the back-thinned CMOS sensor after 100 minutes.](image1)

![Composite image with 3H obtained with the back-thinned CMOS sensor after 15 hours.](image2)

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).

\[ T = M + k\sigma \]  

(1)

where \( T \) is the threshold applied to a certain pixel, \( M \) is the mode dark signal of that pixel, averaged over \( N \) blank frames previously acquired, and \( \sigma \) 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).

\[ SNR = \frac{I_M - I_B}{I_B} \]  

(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.
A comparison with some of the most significant AR systems with published results using 14C 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 14C.**

<table>
<thead>
<tr>
<th>System</th>
<th>Sensitivity (cps mm⁻² kg⁻¹ g⁻¹)</th>
<th>Background noise level (cps mm⁻² g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT ¹ CMOS (Vanilla)</td>
<td>11.47 × 10⁻³</td>
<td>3.79 × 10⁻³</td>
</tr>
<tr>
<td>CCD55-20 [2]</td>
<td>6.10 × 10⁻³</td>
<td>1.60 × 10⁻³</td>
</tr>
<tr>
<td>CMOS (StarTracker) [2]</td>
<td>5.00 × 10⁻³</td>
<td>0.01 × 10⁻³</td>
</tr>
<tr>
<td>Medipix2 [6]</td>
<td>3.00 × 10⁻³</td>
<td>2.00 × 10⁻³</td>
</tr>
<tr>
<td>Medipix1 [6]</td>
<td>4.70 × 10⁻³</td>
<td>0.03 × 10⁻³</td>
</tr>
<tr>
<td>Beta Imager [7]</td>
<td>NA</td>
<td>0.16 × 10⁻³</td>
</tr>
<tr>
<td>Micro Imager [7]</td>
<td>NA</td>
<td>0.66 × 10⁻³</td>
</tr>
<tr>
<td>Micro Channel Plates [8]</td>
<td>∼3.43 × 10⁻³</td>
<td>1.20 × 10⁻³</td>
</tr>
</tbody>
</table>

**TABLE II**

**PERFORMANCE OF THE MOST SIGNIFICANT DIGITAL IMAGING SYSTEMS OBTAINED WITH 3H.**

<table>
<thead>
<tr>
<th>System</th>
<th>Sensitivity (cps mm⁻² kg⁻¹ g⁻¹)</th>
<th>Background noise level (cps mm⁻² g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT CMOS (Vanilla)</td>
<td>9.1 × 10⁻⁶</td>
<td>0.47 × 10⁻⁵</td>
</tr>
<tr>
<td>BT CMOS (MIMOSA) [3]</td>
<td>∼0.007 × 10⁻⁶</td>
<td>0.06 × 10⁻⁵</td>
</tr>
<tr>
<td>CCD55-20 [2]</td>
<td>3.39 × 10⁻⁶</td>
<td>0.86 × 10⁻⁵</td>
</tr>
<tr>
<td>Medipix2 [6]</td>
<td>6.20 × 10⁻⁶</td>
<td>5.00 × 10⁻⁵</td>
</tr>
<tr>
<td>Beta Imager [7]</td>
<td>∼0.00 × 10⁻⁶ [9]</td>
<td>0.11 × 10⁻⁵ [10]</td>
</tr>
<tr>
<td>Micro Imager [7]</td>
<td>NA</td>
<td>0.66 × 10⁻³</td>
</tr>
</tbody>
</table>

**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 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)).

![Image](image1)

**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 initiated 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, ³[H]SCH-23390 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.

![Image](image2)

**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

[1] Back-Thinned
[2] Data not available
[3] Schering Plough 23390 binding with 3H
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.

VI. ACKNOWLEDGMENT

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REFERENCES