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Real-time quantitative ex vivo direct autoradiography with 10μm pixel resolution

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***Abstract*–We present three new autoradiography methods to map positron emission rate of a bio-specimen slice with high resolution. One is based on LBNL scientific charge coupled device (CCD) and the other two are based on conventional CCDs. High conversion efficiency (100k e-h pairs / 0.5MeV positron) and low dark current ($1.75x10^{-4}$ e-/pix/sec) can be achieved using the LBNL CCD. The theoretical calculations and preliminary experiments show that an 86μm spatial resolution can be achieved when imaging a 100 μm thick tissue soaked with ^{18}F which produce higher energy positron. The main disadvantage of the LBNL CCD we tested is that a very low operating temperature is required to eliminate dark current. This dramatically increases the system cost. In addition, the integration time of the CCD needs to be short enough to avoid overlapping of the positron trajectories. Conventional CCDs have lower conversion efficiency (2k e-h pairs / 0.5MeV positron) and higher dark current (200 e-/pix/sec), but are more cost-efficient and the requirement for the readout frequency is much lower. The conversion efficiency of the conventional CCD imager can be improved by 17 times by inserting a 100 μm layer of phosphor between the sample and the imager. However, the light emitted from the phosphor screen will be ~100 μm diameter, which severely degrades the spatial resolution. A high readout frequency is also required to avoid the overlapping. The CCD systems designed in this study will be used to map positron emission rate of bio-specimens such as cancerous tissues acquired in regular biopsy procedure. They can also be used to corroborate tracer kinetic modeling at a cellular level.**

***Index Terms*—Charge Coupled Device, Autoradiography, Cancer, Biopsy**

I. Introduction

The goal of this study is to develop a tool to help pathologists identify cancerous cells in prostate cancer biopsies. This study complements our previous research where we merge structural information from transrectal ultrasound or TRUS with functional information from PET[1].

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are sensitive imaging techniques that can identify specific chemical molecules that localize in cancerous tissues in vivo. However, it is not possible for a PET or SPECT system to achieve sub-millimeter spatial resolution in vivo due to some fundamental limitations, such as the distance the positron travels before annihilation and the slight non-collinearity of the annihilation photons in PET.

We present design studies of three autoradiography-based devices to map with high resolution the concentration of a positron emitter in a tissue section. One is based on a 250 μm thick digital charge coupled device (CCD) and the other two are based on conventional CCDs. These devices detect the positrons directly, instead of detecting the 511 keV annihilation photons. We compared the performances (sensitivity, signal-noise ratio and resolution), operating temperature and cost of those methods. The theoretical calculation and preliminary experiments show the potential for an 86 μm spatial resolution imaging system.

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II. Methods

A. Methods to detect positrons

We investigated three methods that can be used to detect the positrons directly:

Method 1: LBNL 250 μm thick CCD [2]

This CCD is originally designed for supernova cosmologic studies. The 250 μm depletion thickness gives it much higher sensitivity than conventional CCDs.

Method 2: Conventional room-temperature CCD or CMOS imager.

Method 3: Conventional room-temperature CCD or CMOS imager with a layer of phosphor [3, 4].

B. Monte Carlo simulation

A Monte Carlo simulation was performed to evaluate the Signal-to-Noise Ratio (SNR), efficiency and spatial resolution of the CCDs. We modeled the tissue sample as a cylindrical shape of soft tissue with a diameter of 1 mm. The thickness of the sample was varied from 50 μm to 300 μm. A ^{18}F point source was placed at the middle of the sample. The trajectories and energy deposits of the positrons are calculated using the Geant4 simulation package[5].

C. Experimental corroboration

An experimental system shown in Fig. 1 was used to corroborate the results of the Monte Carlo simulation. The system consisted of read out circuits, a light tight chamber, a temperature controller and a light source. The system was cooled down to -140°C using liquid nitrogen.

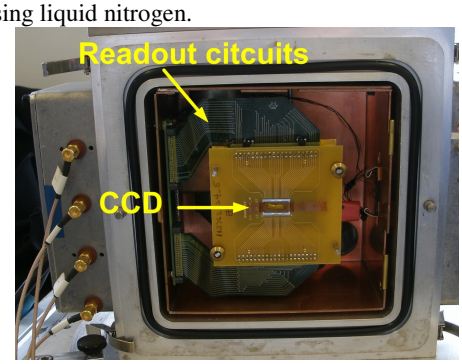


Fig 1 A picture of the experimental system. A LBNL 250 μm thick CCD (1450 x 752, 10.5 μm pixel resolution) was used in this study. A 2 mm x 10 mm sewing thread phantom (3 mg in weight) was soaked with about 18 nCi (about 6 μCi/cc) of ^{18}F radiotracer. The thread phantom wasfixed by tape on the surface of the CCD that was protected by another piece of low static polyimide tape (40 μm in

thickness). The integration time of the CCD was 20 seconds, and the readout time was 5 seconds. The substrate bias voltage was 20V.

A 2 mm x 10 mm sewing thread phantom (3 mg in weight), shown in Fig. 2, was soaked with about 18 nCi (about 6 µCi/cc) of ^{18}F radiotracer and imaged using LBNL 250 µm thick CCD with a 10µm pixel resolution.

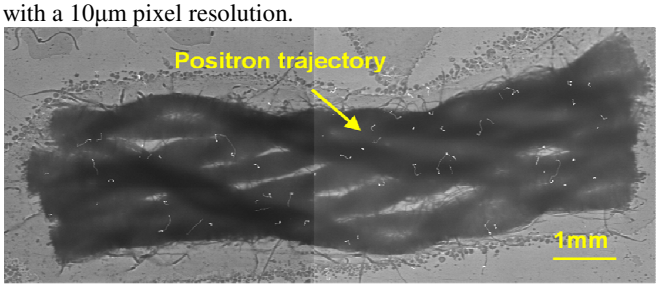


Fig 2 A conventional optical image of the thread phantom taken by the CCD imager. The positron trajectories are also visible in the image.

III. Results

A. *Efficiency Estimation*

Method 1: LBNL 250 µm thick CCD.

For ^{18}F, the average kinetic energy of positrons is 0.202 MeV. As the energy required to create one electron-hole pair in silicon is ~3.6 eV and the CCD is thick enough to completely absorb most of the emitted positrons, one positron will produce 56,111 electron-hole pairs.

Method 2: Conventional room-temperature CCD or CMOS imager.

For a 0.1 to 0.5 keV positron, dE/dx is about 0.5 keV/µm in silicon, implying that it produces ~2,000 electron-hole pairs when it passes through a conventional 20µm thick CCD. The dark current and readout noise in a conventional room temperature CCD imager is usually much less than 2,000 e-. For example, CCD 3041 [6](Fairchild Imaging Inc.) has very low dark current (200e-/pix/sec). But the 2000 e- are along a trajectory path that does not project to one pixel, thus 2000 e- might not be enough to overcome the dark current.

Method 3: Conventional room-temperature CCD or CMOS imager with a layer of phosphor.

The efficiency can be improved by inserting a layer of phosphor between the sample and the imager. We calculate the conversion efficiency for a front side illuminated CCD with a 100 µm phosphor layer as 36eV/pair. Therefore, one 0.5 MeV positron will produce 34,500 electron-hole pairs. This is 17 times higher than that without a phosphor layer.

B. *Simulation results*

The simulation shows that all of the energy for a positron entering the CCD is completely deposited for a CCD of 100 µm thickness. Fig. 3 shows the relation between the CCD thickness and efficiency (blue curve) and the number of electron-hole pairs (green curve).

The simulation also shows that the tissue samples must also be thin enough for the positrons to exit into the CCD. About 76% of the positrons are able to travel out of a 50 µm thick sample. By contrast, only 52% of the positrons are able to travel out of a 300 µm thick sample. Fig. 4 shows the relation between tissue thickness and the efficiency (blue curve) and the number of electron-hole pairs (green curve).

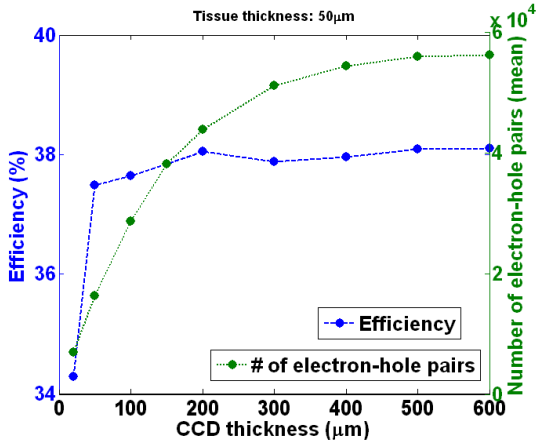


Fig 3 Relation between the CCD thickness and efficiency (blue curve) and the number of electron-hole pairs (green curve). The thickness of the tissue is 50 µm. A ^{18}F point source was used in the simulation.

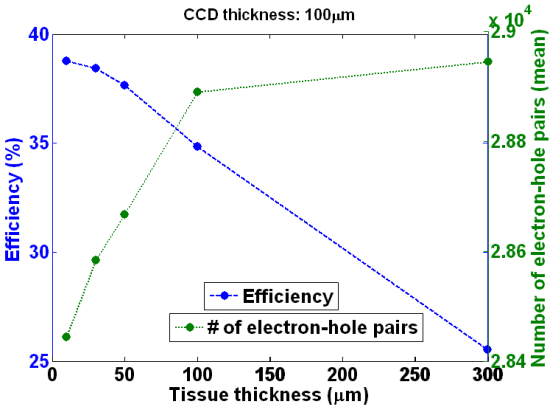


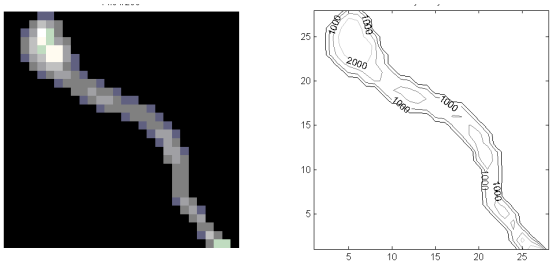
Fig 4 Relation between tissue thickness and the efficiency (blue curve) and the number of electron-hole pairs (green curve).

C. *Experimental results*

The efficiency we achieved in the experiment was 38.5%, in excellent agreement with our predicted value of 35–38% as shown in Fig. 3 and Fig. 4.

We could not accurately determine the spatial resolution, because the thread that we imaged was too large. In addition, the individual positron could travel up to several hundred microns before coming to rest, which degrades the resolution. However, by analyzing the intensity of the electron-hole pairs along the trajectory (Fig. 5), we were able to locate the incident point with a single pixel resolution (10.5 µm).

Fig 6 shows the registered optical image and positron image.



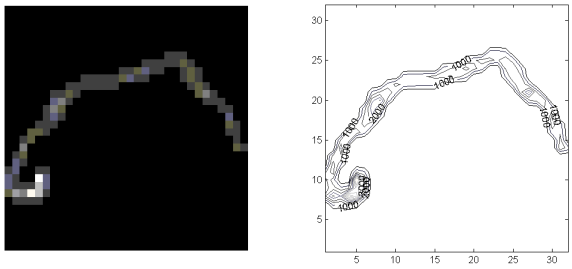


Fig 5 The trajectory of incident positrons. Left: gray level plot; right: contour plot.

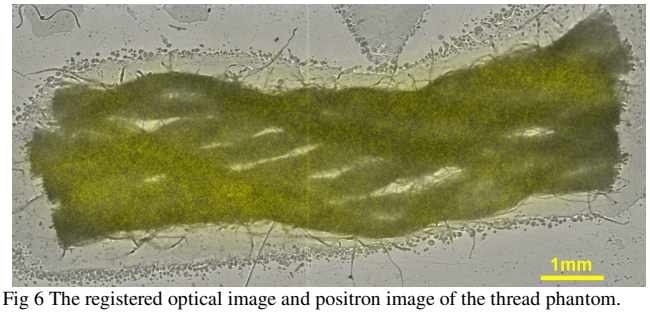


Fig 6 The registered optical image and positron image of the thread phantom. The total integration time was 33 minutes.

IV. Conclusions and Discussion

A. *Point Spread Function (PSF)*

The spatial resolution depends on the thickness of the tissue samples. For a 100 µm thick tissue, the full width at half maximum (FWHM) of the PSF is 130 µm.

The FWHM of the PSF was improved by 34% to 86 µm (red curve in Fig. 7) by an algorithm which estimates the incident direction by analyzing the positron trajectories. Further improvements might be achieved by incorporation of an artificial neural network model.

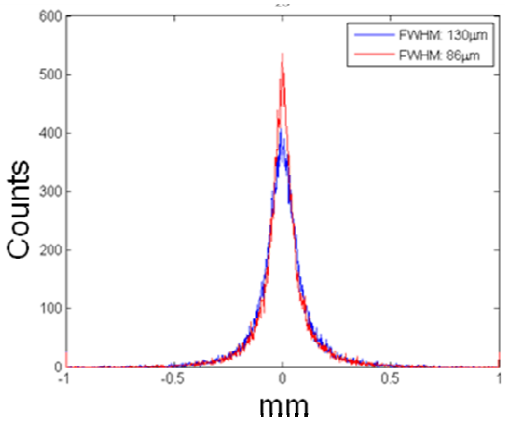


Fig. 7 PSF improved 34% by an algorithm which estimates the incident direction by analyzing the positron trajectories. The blue curve and the red curved show the original PSF and the improved PSF respectively.

The spatial resolution of the experimental system might be evaluated using a glass or plastic capillary tube (e.g. Vitrocom CV0508, inner diameter: 50 µm, outer diameter: 80 µm, length: 20 mm) filled with radiotracer.

B. *Radioactivity in Tissue Samples*

Tissue specimens taken by biopsy after systemic injection of a radiopharmaceutical do not have sufficient activity for conventional autoradiography because tissues are not expected to have activity much greater than 0.15 µCi/cc. A typical biopsy core is only 1 mm diameter by 6 mm long, thus the disintegration rate for the entire sample is only 26 events/s.

One feasible method to increase the radioactivity of the samples is to incubate the live samples in solutions with radio-tracers [7]. We believe that we can achieve up to 150µCi/cc activity in prostate samples with 30 minutes of incubation. The disintegration rate of a 30µm thick sample with an activity of 150 µCi/cc is about 1000 events/s. Therefore, there will be 600,000 events (about 25 events per a cell volume of 5µm radius) in 10 minutes, which is enough for 2D mapping of the sample with a 10µm pixel resolution (600x100 pixels).

C. *Comparison of three methods*

In this paper, we presented three methods to image the positron concentration in biopsy specimens with high resolution using CCD technologies. The performances of those methods are summarized in Table 1.

Table. 1. Comparison of three methods

	Method 1	Method 2	Method 3
Expected resolution (µm)	10	10	100
Conversion efficiency (e-h pairs / 0.5MeV positron)	100k	2k	34.5k
Dark current(e-/pix/sec)	1.75×10^{-4}	200	200
Signal-noise ratio (dB)	135	20	36.7
Readout frequency	High	Low	High
Operating temperature	Low	Room	Room
Cost	High	Low	Low

Method 1: LBNL CCD
Method 2: Room-temperature CCD
Method 2: Room-temperature CCD with a phosphor layer

We'd like to point out that we tested LBNL CCD with an extremely low operating temperature (-140°C) and achieved an extremely high SNR (135dB). The CCD dark current increases by a factor of 10 when the operating temperature increases by 10°C. Therefore, a 55dB SNR can be achieved when the operating temperature is -100°C. In addition, the operating temperature can be further increased by adapting new technologies in the CCD design. For example, Peter Denes and his group have developed CCDs based on the LBNL CCD that have many output amplifiers for high frame rates and can operate at much higher temperatures[8].

Similarly, the SNR of the conventional room temperature CCD can also be improved by decreasing the ambient temperature. For example, by lowering the ambient temperature by 20 degrees to about 5°C, the dark current of the Fairchild CCD3041 can be lowered by a factor of 100 to 2e-/pixel/sec.

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V. REFERENCES

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