

P410 High Resolution and Quantitative Imaging with a Phosphor Storage System in a Broad Range of Nuclear Medicine Applications

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1 Introduction

The Cyclone Plus Storage Phosphor System is a digital autoradiography system for quantitative imaging of radioactivity distribution in two-dimensional samples in contact with a phosphor screen. Traditionally this technology has been used for contact imaging of gels and chromatograms. The following are four new and diverse uses for this technology demonstrating the versatility of the technique for nuclear medical applications including *in vivo* studies, QC, histology and assessment of radionuclide uptake in cultured cells of a microplate.



2 Application 1: The use of a phosphor imager to study immunotherapy in colorectal mice

Introduction:

The use is shown of digital phosphor imaging as part of a study that demonstrates the utility of the MIN/CEA.Tg mouse as a model for the study of anti-CEA immunotherapy. Furthermore its use is shown in demonstrating the efficiency of tumor localization by PR1A3.

Materials and Methods:

Tissue was fixed overnight in neutral buffered formalin, pinned on to board, and covered with Saran Wrap (DOW). The sample was covered with a multipurpose Cyclone phosphor imaging screen (PerkinElmer) and placed in an x-ray film cassette overnight at room temperature. The latent image generated on the screen was read by using the Cyclone Plus Storage Phosphor System and analyzed by using the proprietary integral OptiQuant software.

Results:

Autoradiography with the PerkinElmer Cyclone of adenomas from mice injected with ¹²⁵I-labeled PR1A3 determined the localization of labeled antibody in the intestine. Quantitative analysis of the autoradiography with the OptiQuant software indicated that PR1A3 uptake in adenomas was up to twice that in surrounding intestinal tissue. No uptake into tumors could be imaged in the intestines of MIN/CEA.Tg injected with the isotope control mAb. Macro-autoradiography finds were refined by a dipped emulsion autoradiography.

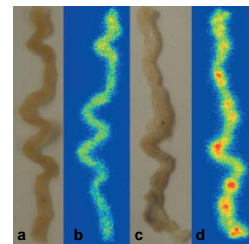


Figure 1 shows that PR1A3 preferentially targets CEA within intestinal adenomas. MIN/CEA.Tg mice were injected *in vivo* with labeled antibodies, and after 48 hours, intestines were removed. Autoradiographs were developed and analyzed. Small intestines are from MIN/CEA.Tg mice injected with either, isotope-matched control mAb (a photograph; b autoradiograph) or PR1A3 (c photograph, d autoradiograph).

Conclusion:

Radiolabeled antibody studies in MIN/CEA.Tg mice using digital phosphor imaging showed that PR1A3 was able to locate specifically the CEA-expressing tissues of the intestine. Analysis of MIN/CEA.Tg intestines also using digital phosphor imaging indicated that PR1A3 was preferentially taken-up and retained within the tumors, compared with the surrounding tissue.

3 Application 2: Simple and reliable chromatographic technique for the quality control of ¹⁷⁷Lu-DOTATATE

Introduction:

A dedicated and reliable quality control procedure (as well as sterility and environmental monitoring) is very important to guarantee the success of therapy trials. ¹⁷⁷Lu-DOTA⁰Tyr³ octreotate (¹⁷⁷Lu-DOTATATE) is successfully used in Peptide Receptor Radionuclide Therapy (PRRT) and its quality assurance should be always carefully determined. Radio-Chemical Purity (RCP), as stated in the European Pharmacopoeia, is an essential parameter ensuring the quality of the radioactive pharmaceutical product. ¹⁷⁷Lu-DOTATATE RCP determination is routinely performed by an analytical technique employing Solid Phase Extraction (SPE). Objective of the current study was to develop another procedure possibly more accurate than SPE. For this reason we set up, in our nuclear medicine lab, a new Thin Layer Chromatographic (TLC) method and compared it to the traditional SPE.

Materials and Methods:

Radiolabeling procedure was carried out at a specific activity ranging between 37-45 MBq ¹⁷⁷Lu/μg of DOTATATE buffered with a solution of sodium acetate and gentisic acid at pH 5.0. Then the mixture was heated for 30 min at 90°C. After labeling, ¹⁷⁷Lu-DOTATATE radiochemical purity was determined by the two analytical techniques employed (TLC and SPE). TLC method involved the use of TLC plates with C18 derivatized Silica-Gel (Whatman) as stationary phase, developed in ammonium acetate (1M, pH 7); methanol (10:90, v/v) mixture. An aliquot of the radiopharmaceutical mixed with an excess of EDTA was spotted on C18 TLC strip. After its development in the solvent system, the strip was air-dried and counted for activity. The radio-chromatographic profile was determined using the Cyclone Storage Phosphor System which provides direct counting of plate without the need for cutting. The Retention factor (Rf) was determined for each radiochemical species (free radioisotope and ¹⁷⁷Lu-DOTATATE). SPE method involved the use of Sep Pak® C18 cartridges (Waters) with methanol and sodium acetate buffer (0.05 M, pH 5.0) as mobile phases. The cartridge was previously conditioned with 2 mL of methanol and 2 mL of sodium acetate; an aliquot of radio-labeled solution mixed with an excess of EDTA was then loaded into the cartridge which was eventually eluted with 2 mL of acetate and 2 mL of methanol, respectively. The first eluted fraction (acetate buffer) contained free ¹⁷⁷Lu and the second (methanol) contained ¹⁷⁷Lu-DOTATATE. To compare the two analytical techniques we assayed forty preparations with high and low RCP.



FIGURE 1. A typical radiochromatogram of ¹⁷⁷Lu-DOTATATE obtained by TLC technique. RCP > 99%.

Analytical procedure employed	¹⁷⁷ Lu-DOTATATE with high RCPs % (mean ± s.d.)
SPE	99.7±0.2
TLC	99.6±0.2

Analytical procedure employed	¹⁷⁷ Lu-DOTATATE with low RCPs % (mean ± s.d.)
SPE	85.2±7
TLC	72.9±2.9

TABLE 2. Radiochemical purities (mean ± s.d.) determination according to the two analytical procedures (TLC and SPE) employed. A) ¹⁷⁷Lu-DOTATATE with high RCPs% (n=30). B) ¹⁷⁷Lu-DOTATATE with low RCPs% (n=10).

Results:

A typical radio-chromatogram of ¹⁷⁷Lu-DOTATATE is shown in Figure 1. TLC profiles showed sharp and narrow peaks for ¹⁷⁷Lu-DOTATATE and free ¹⁷⁷Lu bound to EDTA. The mean RCPs of this radiopharmaceutical, as determined by the TLC and SPE methods were noted. The results were comparable when preparations with high RCPs were tested (Table 2A). However, this similarity was not observed in the samples with low RCPs. TLC technique gave more accurate results compared to SPE. In fact, adding ¹⁷⁷Lu Cl₃ prior to RCP test, only TLC method was able to detect the excess of free radioisotope whereas in the SPE, part of free ¹⁷⁷Lu added was eluted into the methanol fraction. The time required for the two techniques was similar.

Conclusions:

TLC procedure successfully separated ¹⁷⁷Lu-DOTATATE from free ¹⁷⁷Lu when the latter was present in large amount that could be co-eluted in the methanol fraction by SPE, thus originating an inaccurate result. Moreover, the chromatographic method reduces radiation exposure to the operator representing an additional valuable advantage. The new TLC system proved to be a simple, reliable, fast and accurate method to determine ¹⁷⁷Lu-DOTATATE quality control even with low RCP result and, following further investigations, could be adopted to other radiopharmaceuticals such as ¹¹¹In and ⁹⁰Y labeled peptides.

4 Application 3: The use of a phosphor imager for quantitative gamma-only imaging of multi-well culture plates

Introduction:

In order to measure radionuclide uptake in cultured cells in a multi-well plate format it would be useful to be able to image gamma emissions in the wells in order to quantify the radioactivity there. This shows how a collimator was devised to minimize cross-talk between wells, determine the linearity and sensitivity of response of the system for non-contact imaging of gamma emitting radionuclides and produce a calibration curve.

Materials and Methods:

15 mm diameter wells of a 24-well plate were under-laid with customized lead templates having an aperture of 5 mm diameter under the center of each well. In order to vary the thickness of lead in each well the templates were stacked forming a collimator of thickness anywhere from 1 to 8 mm. ¹¹¹In with activity from 0 to 0.8 MBq (0 to 1.6 MBq/ml) was placed in the wells in a constant volume. The plates were then imaged for 2 min using a MS phosphor imaging screen. The screen was subsequently analyzed using a PerkinElmer Cyclone Storage Phosphor System after which regions of interest were analyzed using Cyclone proprietary OptiQuant software.

Results:

For the range 0 MBq/well to least 0.8 MBq/well the response was linear. Cross talk between cells was reduced (to 1.3% in adjacent wells) by a lead thickness of 3 mm or more for 5 mm circular apertures. A thickness of 1 mm was found to be inadequate.

Conclusion:

Where a gamma camera would normally be used the phosphor imaging method is a useful and relatively inexpensive device for quantitative collection and analysis of gamma emitting radionuclide distribution in multi-well plates (not in contact with the phosphor screen).

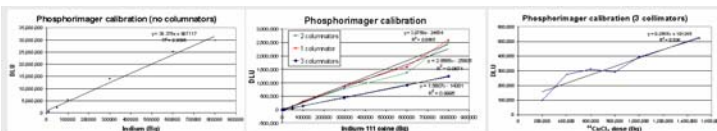


Figure 1. Calibration of the phosphor imager with no collimators using ¹¹¹In oxinate.

Figure 2. Calibration of the phosphor imager with 1-3 collimators using ¹¹¹In oxinate, showing that 3 collimators gives the best linearity.

Figure 3. Calibration of the phosphor imager with 3 collimators using ⁶⁴Cu dichloride.

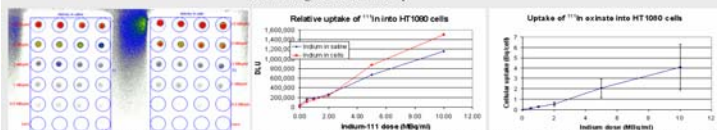


Figure 4. Phosphor image acquired from exposure of HT1080 cells (right) treated with increasing doses of ¹¹¹In oxinate in saline. Cells were incubated for 1 hour with ¹¹¹In oxinate at doses of 0-10 MBq/ml in quadruplicate, before removal of saline to a fresh 24 well plate (left).

Figure 5. Relative uptake of ¹¹¹In oxinate into HT1080 cells. The phosphor image shown in Figure 4 was quantified using OptiQuant software and the data was plotted versus indium dose.

Figure 6. Uptake of ¹¹¹In oxinate into HT1080 cells. The ¹¹¹In phosphor image calibration was used along with cell counts to convert uptake data to uptake per cell. Data points show averages from quadruplicate wells from two independent experiments.

5 Application 4: The use of a phosphor imager for histological analysis of rat tissue

Introduction:

A method is shown for the autoradiography of rat brain sections demonstrating the use of the technique in a histological context. We further show how screen choice can be optimized either for high sensitivity or resolution depending on the needs of the experiment.

Materials and Methods:

Tissue sections (10 μm) are mounted on Superfrost plus slides and stored at -20°C. Before use, sections are air-dried, pre-incubated for 10 min at room temperature (RT) in 170 mM Tris-HCl buffer, pH 7.6, with 5 mM MgCl₂ (Tris-buffer) and 0.25% (w/v) BSA and then incubated for 60 min at RT with 10⁻¹⁰ M radiolabeled compound in Tris-buffer containing 1% BSA and 1 mg bacitracin. Specific activity of this compound should be high; 10⁻¹⁰ M dilution should contain 5-20 kBq/ml. After incubation, the sections were rinsed twice for 5 min in cold Tris-buffer with 0.25% BSA, subsequently 5 min in cold Tris-buffer without BSA and a short rinse with cold MilliQ. The sections were air-dried and exposed to phosphor imaging screens in X-ray cassettes for several hours to several days dependent on radionuclide and dose. The screens were read using a Cyclone Storage Phosphor System and a computer-assisted OptiQuant 03.00 image processing system. OptiQuant software was used to quantify the intensity of radioactivity, expressed as digital light units (DLU) per mm².

Results:

Good linear binding response is shown between the DLU/mm² results and the level of added radioactive ¹¹¹In-DTPA-Octreotide (up to 7.5 x 10⁻¹⁰ M) and clear autoradiographical images are obtained with rat brain sections. Use of a SR phosphor imaging screen results in less DLUs, but higher resolution quality of the image, compared to a MS screen.

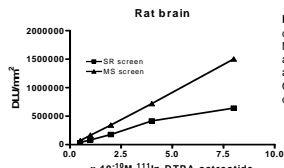


Figure 1 shows quantification of detected radioactivity, both with MS and SR screens, after *in vitro* autoradiography with increasing amounts of ¹¹¹In-DTPA-Octreotide. OptiQuant software was used to determine DLU per mm².

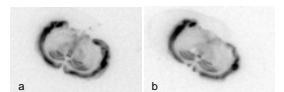


Figure 2 shows *in vitro* autoradiography on sections of rat brain, incubated with 10⁻¹⁰ M ¹¹¹In-DTPA-Octreotide on SR screen, range 3-1000 (a) or MS screen (b), range 3-1000.

Conclusions:

In vitro phosphor imaging can be used enabling ¹¹¹In (and ⁹⁹Tc, ¹²⁵I, ¹⁷⁷Lu, etc) labeled tissue to be imaged and furthermore optimized via screen selection for sensitivity and resolution.

6 General Conclusions

Although traditionally considered to be a technology used for contact imaging of gels and chromatograms, the poster demonstrates four new and diverse uses for phosphor imaging technology particularly in nuclear medical applications including *in vivo* studies, QC, histology and assessment of radionuclide uptake in cultured cells of a microplate.

References

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