Fundamental studies on radiotracers intended for receptor imaging in dementia

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Citation for published version (APA):
CHAPTER 4

IN-VITRO AND EX-VIVO STORAGE PHOSPHOR IMAGING OF SHORT-LIVING RADIOISOTOPES

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Abstract

Storage phosphor imaging may be of value for biodistribution studies of short-living radiotracers in small animals. Efficiency, sensitivity and resolution of imaging plates for short-living radioisotopes vary considerably but linear response to many radioisotopes was shown previously. However, these properties have not been compared directly for larger series of short-living radioisotopes, and only few studies have directly compared data obtained from phosphor images of tissue slices with results from dissection biodistribution studies. Therefore, we evaluated the properties of imaging plates for 11 short-living radioisotopes (\textsuperscript{18}F, \textsuperscript{32}P, \textsuperscript{67}Ga, \textsuperscript{89}Sr, \textsuperscript{99m}Tc, \textsuperscript{90}Y, \textsuperscript{111}In, \textsuperscript{123}I, \textsuperscript{125}I, \textsuperscript{131}I and \textsuperscript{201}Tl). We also evaluated the biodistribution of \textsuperscript{[123}I\text{]FP-CIT} in rat brain using both the phosphor technique and conventional dissection methods.

The imaging system showed a linear response for all tested radioisotopes over a wide range of radioactive concentrations and the efficiency, sensitivity and resolution varied greatly for the tested radioisotopes. Shielding experiments revealed the contribution of the various emission products of radioisotopes to these properties. However, quantitative biodistribution studies with radiotracers that are labeled with all tested radioisotopes, even \textsuperscript{123}I, are feasible. The results from the ex-vivo biodistribution study, using \textsuperscript{[123}I\text{]FP-CIT} as a radiotracer were similar for the phosphor imaging technique as compared to the dissection technique.

Advantages of phosphor imaging in radiotracer distribution studies in rat brain as compared to dissection experiments may be more precise measurements, possibility to reanalyze imaging data and 3D-reconstruction. In conclusion, phosphor imaging is an attractive alternative for biodistribution studies of short-living radiotracers in small animals.
Introduction

Storage phosphor imaging, also known as radioluminography, is an imaging technique that was introduced in the early 1980’s. It is based on the absorption and storage of radioactive energy by phosphor crystals (e.g. BaFBr:Eu$^{2+}$ or CaSO$_4$:Dy$^{3+}$) that are coated on a plastic plate. After excitation with a laser beam, luminescence light is released from the energy that is stored by these crystals, which is detected by a photomultiplier tube. The latent image on the plate, which results from exposure to the radioactive source, is scanned and the amplified signal is transferred to a computer for further analysis with appropriate software. The plates can then be erased and used again.

Although the major application of storage phosphor imaging is found in the area of X-ray diagnostics, the technique has also been used for research purposes such as analysis of electrophoresis gels, chromatograms and pharmacological studies. Storage phosphor imaging has also been used extensively as a method for whole-body and brain imaging studies of small laboratory animals by exposing radioactive tissue slices to imaging plates (IPs). In this respect the technique has proven to be of value for the development and evaluation of newly synthesized SPECT and PET radiotracers.

Unfortunately, the spatial resolution of storage phosphor imaging systems is limited as compared to the resolution of conventional X-ray film autoradiography. Using X-rays, the resolution of photographic film is typically less than 20µm, while the resolution of an imaging plate (IP) is up to 10 times lower. The most important reason for this may be the granular composition of the phosphor crystals and distortion of laser and luminescence light in these crystals. Moreover, when X-ray films or IPs are exposed to radioisotopes, the resolution seems to be even lower and this may depend on the emission products of the radioisotope.

In spite of that, IPs are known to be much more sensitive than X-ray films. Due to the relatively poor sensitivity of conventional X-ray films for radiation, exposure times for radioactive tissue slices are generally long for autoradiography. By using IPs, exposure times can be reduced to one-tenth of conventional X-ray film exposure times. This allows imaging of a much wider spectrum of radioisotopes including those with a short half-life time such as $^{123}$I, $^{99m}$Tc or even $^{18}$F. These radionuclides are used frequently in SPECT and PET studies in neuropsychiatric disorders. Moreover, the amount of radioactive energy that can be absorbed is high, providing a wide dynamic range which does not lead often to overexposure of the plates.
For quantitative analysis, the detected density on imaging modalities such as X-ray film or phosphor IPs should ideally have a linear relationship with the amount of radiation from the sample that is being analyzed. X-ray film autoradiography is known to be hampered by a non-linear relation between the detected density and the actual radioactive concentrations in the sample, which necessitates comparison with calibration standards or calibrated densitometry\textsuperscript{17}. For storage phosphor imaging, previous studies showed a linear response of the IPs for many radioisotopes over a wide range of radioactive concentrations\textsuperscript{3, 5, 6, 11, 13-16, 18}. However, until now the linearity and efficiency of IPs for large series of different short-living radioisotopes have not been compared directly. Also, data on the influence of short-living radioisotopes and in particular their emission products, on sensitivity and resolution have been lacking. Finally, storage phosphor imaging of tissue slices has only been compared directly with results obtained from classical biodistribution studies in a few studies\textsuperscript{18, 19}.

The aim of this study was therefore to evaluate the linearity, efficiency, sensitivity and resolution of the storage phosphor imaging technique for a number of short-living radioisotopes, most of which are frequently used in routine clinical studies. We also performed additional experiments to evaluate the response of the IPs to the various emission products of the radioisotopes that were used, such as $\gamma$- and $\beta$-radiation. Additionally, we investigated the value of storage phosphor imaging in ex-vivo studies of $[^{123}\text{I}]$FP-CIT binding to dopamine transporters (DATs) in rat brain slices as an alternative for conventional ex-vivo dissection biodistribution and blocking studies. Furthermore, we made a 3-dimensional reconstruction of FP-CIT binding to DATs in a rat brain in order to improve visualization of radiotracer binding.

**Methods**

**In-vitro experiments**

**Standard curves**

Linearity and efficiency of the storage phosphor imaging technique were evaluated for 11 radioisotopes ($^{18}\text{F}, ^{32}\text{P}, ^{67}\text{Ga}, ^{89}\text{Sr}, ^{99m}\text{Tc}, ^{90}\text{Y}, ^{111}\text{In}, ^{123}\text{I}, ^{125}\text{I}, ^{131}\text{I}$ and $^{201}\text{Tl}$). In this study we used a Fuji FLA-3000 Phosphor imager (Fuji Medical Systems, Stamford, CT, USA) and Fuji BAS-MS IPs (BaFBr0.85I0.15; Eu$^{2+}$), which were scanned at a 50$\mu$m pixel size with a 16 bit pixel depth. The linearity between the amount of radioactivity that was exposed to the IP and the detected photostimulated luminescence...
ence (PSL) by the phosphor imaging system was evaluated by a series of standard curves. Dilution samples of the γ-, β- and electron-emitting radioisotopes and the positron emitting radioisotope (8-10 samples between approximately 6.3kBq and 10Bq, dilution factor 2 per sample, n=4-5) were spotted on a silica gel coated TLC chromatography plate (Whatman International, Maidston, England) in 20μL spots. For the 32P dilution samples, 20μL samples (n=5) were spotted on ITLC-SG silica gel impregnated glass fiber sheets (Pall Corporation, Ann Arbor, USA). As the source for the radioisotopes, we used a variety of clinically used radiotracers and radiolabeled laboratory compounds. All radiotracers were diluted using saline. In our experiments, typical diameters of the spots were approximately 12mm. Although this diameter is large, one has to take into account that only the total amount of radioactivity of each spot was measured. The chromatography plates and sheets were covered with Saran wrap (thickness 10μm, density 1.00g/cm³) before exposure to the IPs, in order to protect these from radioactive contamination. Calibration of the γ-emitting radioisotopes was done using a VIK-202 dose calibrator (software version VDC 405), whereas calibration of the positron emitter 18F and the strong β-emitters 89Sr and 90Y was done using another VIK-202 dose calibrator (software version IBC606; Veenstra Instruments, Joure, The Netherlands). For all radioisotopes, the calibration time was set to the start time of exposure to the IP. Amounts of applied radioactivity of the pure β-emitter 32P were determined by counting of each spot in vials with 10mL of scintillation fluid (Ultima Gold, Packard BioScience, Zellik, Belgium) in a liquid scintillation counter (Tri-Carb 2900 TR Liquid Scintillation Analyzer, Packard; Software version: 3100) and the obtained counts were corrected for the efficiency of the counter. This assay was done after exposure to the IP and data were corrected for radioactive decay. All IPs were erased within 2h before exposure to the TLC-plates and the exposure time was precisely 24h. Then the images were scanned using the phosphor imager, approximately 10 minutes after the radioactive samples were removed from the IPs. The densities on the acquired images were read by the AIDA image analysis software (Version 3.20.007, Raytest Isotopenmeßgeräte GmbH, Straubenhardt, Germany) and corrected for background activity (including flare artifacts) by baseline subtraction. Overexposed spots, which were automatically detected by the software, were excluded from further analysis. Linearity was then determined by log-weighted linear regression analysis by the same soft-
ware package. Curve slopes were determined for all tested radioisotopes. Two types of efficiency were distinguished. Efficiency-per-Bq was defined as the detected amount of background corrected PSL per applied Bq of radioisotope after exposure during precisely 24h, irrespective of the half-life of the tested radioisotope. This type of efficiency was obtained directly from the curve slopes that were calculated by linear regression analysis of the standard curves, and is valid only for the linear range of the standard curve of each tested radioisotope (below the concentrations that resulted in overexposure). The relative efficiency-per-Bq of the phosphor imaging system for various radioisotopes as compared to the efficiency-per-Bq of $^{125}$I was then calculated and expressed as a ratio. $^{125}$I was chosen as a reference because of its frequent use in conventional X-ray autoradiography and because of the intermediate efficiency of the phosphor imaging system for this isotope. Efficiency-per-disintegration was defined as the amount of background corrected PSL/occurred disintegration. This variant of efficiency of the phosphor imaging system was calculated for each radioisotope. Finally, the relative efficiency of the various isotopes as compared to the efficiency-per-disintegration of $^{125}$I was calculated.

**Sensitivity: limit of detection and limit of quantification**

To assess the limit of detection (LOD) for all tested radioisotopes, 10 regions of interest (ROIs) of approximately 1cm$^2$ were drawn on the background of corresponding phosphor images. The images of the resolution study (see Figure 4.5.) were used for these measurements. The ROIs were drawn outside visually perceptible radioactive spill-over and opposite to the direction where flare artifacts$^6,14$ would be expected to occur. For each radioisotope, the LOD was defined as the amount of applied radioactivity per cm$^2$ (Bq/cm$^2$) that is necessary to produce a PSL value of 3 times the standard deviation (SD) of the measured background PSL$^{14,15}$. The PSL-to-Bq/cm$^2$ conversion was based on the curve slope that was obtained from the linear regression analysis of the standard curves for each radioisotope (LOD=$^{(3SDBG)} \cdot$ curve slope) and is valid only for exposure during precisely 24h.

Similarly, the limit of quantification (LOQ) was defined as Bq/cm$^2$ of applied radioisotope that corresponds with a PSL value of 9 times the SD of the measured background activity$^{15}$, and was also calculated using the obtained curve slopes (LOQ=$^{(9SDBG)} \cdot$ curve slope).
Table 4.1a. Properties of the radioisotopes that were tested in the study. Data are expressed as the cumulative yield for the emission products within each category. β-radiation and emitted electrons were taken together. Abbreviations: ND; non-detectable.

<table>
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<th>Isotope</th>
<th>ND (&lt; 50 keV)</th>
<th>Low (50-200 keV)</th>
<th>Medium (100-200 keV)</th>
<th>High (&gt;200 keV)</th>
<th>Very low (&lt; 50 keV)</th>
<th>Low (50-200 keV)</th>
<th>Medium (200-400 keV)</th>
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<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
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<td>-</td>
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<td>-</td>
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<td>0.000</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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**Shielding experiments**

For $^{18}\text{F}$, $^{131}\text{I}$, $^{67}\text{Ga}$, $^{99m}\text{Tc}$ and $^{125}\text{I}$, shielding experiments were performed to assess the contribution of positrons, $\beta$-radiation, electrons and $\gamma$-radiation to the detected PSL. For $^{18}\text{F}$, $^{131}\text{I}$, $^{67}\text{Ga}$ and $^{99m}\text{Tc}$, series of radioactive spots ($n=11$) of 1µL, each containing 100Bq of radioisotope were placed on a silica gel coated TLC plate and shielded by an increasing number of layers of plastic overhead transparencies (0-1.98mm in steps of 180µm). $^{125}\text{I}$ only emits very low energy electrons. Therefore, this isotope was shielded with thinner layers of transparency material (0-0.36mm in steps of 90µm). Since $^{125}\text{I}$ has a half-life of 59 days, we protected the plates in order to prevent contamination by adding a 10µm layer of Saran wrap between the samples and the IPs for these experiments. Also for $^{125}\text{I}$, we spotted 100Bq ($n=11$) samples in 10µL on the plates.

The thickness of each separate transparency was 90µm as measured by a micrometer device and the density ($\rho$) of the transparencies was 1.46g/cm³. The IPs were exposed to the shielded spots during 24h. The PSL of each spot was then measured by the AIDA image analysis software and corrected for background PSL. The maximum range ($R$) in the shielding material using the maximal energy ($E_{\text{max}}$) of the most important emitted particle was calculated for each isotope using the appropriate equation $R = (0.412E_{\text{max}}1.265-0.0954\ln E)/\rho$, which provides a good estimation for the range of electrons and positrons with energies between 0.02-2MeV, and also Feather’s equation for the energy range of 0.6-20MeV (see below).

The contribution of the positrons, $\beta$-radiation spectrum and electrons to the measured PSL was defined as the difference in detected PSL of unshielded spots versus spots that were adequately shielded by transparency material with a thickness that exceeded the range of the most energetic particle of each isotope and was expressed as a percentage. The remaining PSL was attributed to $\gamma$-radiation. Bremsstrahlung was neglected because of the low (mean) Z (atom number) of the shielding material. In contrast to $^{99m}\text{Tc}$ and $^{67}\text{Ga}$, both $^{18}\text{F}$ and $^{131}\text{I}$ emit particles that are able to cross multiple layers of shielding material. These isotopes were therefore used as an internal control. The measured PSL of all spots were corrected by the amount of PSL that was attributed to $\gamma$-radiation and background activity. Transmission for each thickness of shielding material was then calculated by dividing the PSL of all samples by the average PSL of the unshielded samples. Transmission for either the electrons of $^{131}\text{I}$ or the positrons of $^{18}\text{F}$ was plotted against thickness of the transparency material and curve estimation (exponential) was performed by SPSS 11.5. Based on the results of the curve estimation by SPSS, half-
thickness for both the electrons of $^{131}$I and the positrons of $^{18}$F was calculated and compared to the theoretical half-thickness (using Feather’s equation $R = (0.542 \cdot E_{\text{max}} \cdot 0.133)/\rho$, which applies for electrons within the energy range of $0.6 < E_{\text{max}} < 20$MeV).

Resolutions

IP resolution for 10 of the tested radioisotopes (all but $^{90}$Y, due to unavailability at the time of the resolution experiments) was measured by the method of Strome and co-workers. For these experiments, a Hewlett Packard 950C DeskJet printer was used and black inkjet cartridges were emptied and refilled with 15ml of ink and a known amount of radioisotope. The concentration of radioisotope in each cartridge was based on the relative efficiency-per-Bq of the phosphor imaging system for the radioisotope as assessed by the standard curves in order to produce comparable images (concentrations varied between 0.5MBq/15ml for $^{32}$P and 25.5MBq/15ml for $^{99m}$Tc).

Using a printer resolution of 600 DPI, test patterns were printed on glossy photo-paper. Exposure of the IPs by the radioactive test patterns was performed during 24h and images were scanned as described above.
Phosphor images of point sources of 0.1mm in diameter were transferred to HERMES workstation (Hermes Medical Solutions, Stockholm, Sweden) and corrected for background counts using Hermes’ Multimodality 4 software by subtraction of the average background counts. FWHM (n=4-8) was then measured in Hermes’ Quality Control software in rows of 2 pixels in horizontal and vertical direction. The FWHM measurements were based on a pixel size of 50µm (scanning resolution).

**Ex-vivo studies**

[123I]FP-CIT (DaTSCAN), a commercial radiotracer for imaging of DATs, was a generous gift of GE Healthcare, Eindhoven, The Netherlands. Its radiosynthesis was described earlier. [123I]FP-CIT had a specific activity of 185MBq/nmol and a radiochemical purity of >97%.

**Biodistribution studies using phosphor imaging**

In this study, a control group of 3 male Wistar rats (200-300g, obtained from Harlan, Horst, The Netherlands) received 0.4ml sodium acetate buffer (pH 4.75) prior to injection of [123I]FP-CIT. This radiotracer has a high affinity to DATs, but also modest affinity to serotonin transporters (SERTs). In the blocking experiment, 3 rats received the potent DAT blocker methylphenidate (5mg/kg body weight in 0.4ml buffer). After 5 min, both groups received approximately 50MBq [123I]FP-CIT in 0.4ml buffer with 5% ethanol via a tail vein.

All rats were sacrificed by bleeding via heart puncture under ketamine/xylazine (2:1) anesthesia at 2h after injection of the radiotracer. The brains were excised, quickly frozen, and sliced into 25µm horizontal sections using a Jung CM3000 cryomicrotome (Leica Microsystems GmbH, Wetzlar, Germany). Every one in five sections was mounted on a glass plate using standard mounting tape (Scotch, 3M, Minnesota, USA), covered with Saran wrap. The brain sections were exposed to a Fuji BAS-MS IP for approximately 24h. The images were scanned at a 50µm resolution with a 16-bit pixel depth using the Fuji FLA-3000 phosphor imager and analyzed using AIDA image analysis by ROI-analysis.

The striatum was defined as the area of specific binding to dopaminergic neurons, because of its high levels of DATs. The cerebellum was cho-
osen as the area of non-specific uptake, since in this brain structure the DAT density is negligible. Equally sized circular ROIs were manually placed on the cerebellum, the left and the right striatum in 7 adjacent representative sections of each rat brain. Ratios of specific binding of the left and right striatum to the cerebellum (striatum-cerebellum/cerebellum) were calculated for the control and the methylphenidate-treated groups. These ratios were calculated for each brain sections separately to eliminate calculation errors due to slight variations in sections thickness.

The hypothalamus has a high expression of SERTs, but a low density of DATs. Methylphenidate has no affinity for the SERT, and should therefore not be able to block $[^{123}\text{I}]$FP-CIT binding in this particular brain area. To test this hypothesis, equally sized circular ROIs were also placed on the hypothalamus and cerebellum in 6 adjacent representative brain sections. Then the specific binding ratios of the hypothalamic binding to the cerebellar binding (hypothalamus-cerebellum/cerebellum) were calculated in each brain sections separately, for the methylphenidate-treated and the untreated group.

Additionally, we performed a pilot experiment using another radiotracer, $[^{125}\text{I}]$Iododexetimide. This radiotracer binds non-selectively, but with nanomolar affinity to all muscarinic receptor subtypes. The control rat (n=1) received a placebo injection of 0.4ml saline at 10 minutes prior to injection of 50MBq $[^{125}\text{I}]$Iododexetimide (in 0.4ml acetate buffer) under ketamine/xylazine anesthesia. Another rat was pre-injected with 5mg/kg of the potent muscarinic receptor antagonist scopolamine. Rats were sacrificed at 2h after injection and the brains were sliced and exposed to the IP as stated above.

Dissection biodistribution studies

The obtained data from the phosphor imaging study was compared with data obtained from an earlier performed, yet unpublished, conventional dissection biodistribution study. In short, rats (200-300g) were injected intravenously with either saline (control group, n=5) or methylphenidate (block group, 10mg/kg body weight, n=5) in 0.4ml buffer prior to injection of 3.7MBq $[^{123}\text{I}]$FP-CIT in 0.4ml buffer. The rats were sacrificed at 2h after injection of the radiotracer, by bleeding via heart puncture under ketamine/xylazine anesthesia. Brains were rapidly dissected and striatum, hypothalamus, and cerebellum were separately weighted and counted in a $\gamma$-counter (Minaxi $\gamma$ A5530, Canberra-Packard). The data were corrected for radioactive decay, counter efficiency, brain structure weight and body weight and eventually expressed as the percentage of the injected dose, multiplied by the body weight in grams, per gram tis-
sue (%ID\text*{g/g})\textsuperscript{26}. For comparison with the phosphor imaging study, we used specific binding ratios of striatum-to-cerebellum and hypothalamus-to-cerebellum.

The performed experiments are in agreement with The Dutch Experiments on Animals Act (1977) and were approved by the Animal Ethics Committee (AMC, Amsterdam, The Netherlands).

3D-Mapping of the biodistribution of $[^{123}\text{I}]$FP-CIT in the rat brain
The acquired images of brain sections of one rat were converted, transferred to a Hermes imaging workstation and corrected for rotational errors by motion correction software. Three dimensional reconstruction was subsequently performed by the Multi Modality 4 software package (Hermes Medical Solutions, Stockholm, Sweden).

Statistical analysis
Log weighted linear regression analysis for the standard curves was performed using the AIDA image analysis software. Curve estimation for the transmission data of the shielding experiments was performed by SPSS 11.5. Differences between the FWHM within samples of each radioisotope and between the isotopes were analyzed by SPSS 11.5 using ANOVA with Bonferroni correction for multiple comparisons in the latter. Differences between groups in the ex-vivo blocking experiments were analyzed with the non-parametric Mann-Whitney U test using SPSS 11.5. Probability values $<0.05$ were considered significant.

Results
Properties of the tested radioisotopes
For interpretation of the data, we summarized the properties of the test-
ed radioisotopes in Table 4.1a. This table shows the various radiation types that are emitted by the radioisotopes. To simplify interpretation of the data, we categorized the emission products. For each radioisotope the chance of emission for each radiation type is expressed as a cumulative yield, which is a summation of the yield of the emitted products within the energy range of the category. Electrons and β-radiation were taken together because they are essentially the same. The individual yields and energies were extracted from the computer program Radiation Decay (version 3.6, 2001, Griffith University, Australia) which utilizes ENSDF data from the National Nuclear Data Center (Brookhaven National Laboratory, Upton, USA). The table also shows categorized γ-radiation. The first category in the table shows the cumulative yield of electrons with energies smaller than 50keV and these are considered to be non-detectable due to the composition of the IP and the usage of protective wrap. The plate itself is protected by a cellulose acetate layer (thickness 9µm, density 1.6g/cm³ according to Fuji’s specifications). To protect the plates from contamination, we also used Saran wrap (thickness 10µm, density 1.00g/cm³). The weighted average density of these two layers is 1.30g/cm³. In a medium with a density of 1.30g/cm³ an electron with an
$E_{\text{max}}$ of 50keV would have a range of 62µm (according to the equation $R = (0.412E_{\text{max}}^{1.265} - 0.0954\ln E)/\rho$, which is a good estimation for electrons with an energy between 0.02-2MeV). The half-thickness for such electrons in material of this density is therefore approximately 8.9µm ($R/7$). This means that less than 25% of the initial 50keV electrons are able to cross the protective layers. However, self-absorption of the material on which the isotopes were spotted will also limit the transmission of these low energy electrons. In our experiments we used silica gel coated TLC plates (thickness of silica gel layer: 250µm). Since the distribution of the tested radiotracers in the spots in the silica gel plate is not homogeneous, it is impossible to estimate the amount of self-absorption in this medium using our study design. However, self-absorption has a substantial additional effect on the amount of the 50keV electrons that are able to reach the active layer of the IP, and thus we classified the electrons with energies smaller than 50keV as non-detectable. Furthermore, we arbitrarily discriminated between low energy (50-100keV), medium energy (100-200keV) and high energy (>200keV) electrons. For $\gamma$-radiation, we discriminated between very low energy (<50keV), low energy (50-200keV), medium energy (200-400keV) and high energy (>400keV) photons.

**In-vitro experiments**

**Standard curves**

Figure 4.1. shows the linear relationship between the amount of applied...
radioactivity on the IP and the detected, background corrected PSL by the system for the tested series of radioisotopes ($^{18}$F, $^{32}$P, $^{90}$Y, $^{89}$Sr, $^{131}$I, $^{111}$In, $^{125}$I, $^{201}$Tl, $^{125}$I, $^{99m}$Tc and $^{67}$Ga). A sample of a standard curve is displayed in Figure 4.2a. All isotopes showed a strong linearity ($0.990 < r^2 < 0.999$), Figure 4.2a. Sample of a standard curve of $^{123}$I, dilution factor 2 per step. The highest concentration in this standard curve is approximately 5.5kBq. Photostimulable luminescence (PSL) was measured from these samples as total PSL per spot, and corrected for background activity, including flare artifacts. The circular edge of each spot is the result of the chromatographic properties of the plate on which the radioisotope was spotted (a TLC plate). Spot diameters were approximately 12mm. Figure 4.2b. Shielding of $^{18}$F samples (each containing 100Bq) by an increasing amount of plastic layers (0-1.96mm, 0.18mm per step). The figure shows shielding of positrons of $^{18}$F. There is no substantial contribution to the detected PSL of high energy annihilation photons.

goodness-of-fit in log-weighted linear regression analysis) in the range between 7Bq and 6.3kBq per 20µL spot. At the high end of this range, overexposure was seen for $^{32}$P (above 0.5kBq per spot), $^{90}$Y (above 0.75kBq), $^{89}$Sr (above 1.5kBq) and $^{131}$I (above 2.2kBq). These measurements were excluded from further evaluation, but are displayed in Figure 4.1.

Efficiency
The calculated slope of the curve of each tested radioisotope is depicted in Figure 4.1. and represents the efficiency-per-Bq after 24h of exposure
Figure 3a shows the ratios of the efficiency-per-Bq of each tested radioisotope to the efficiency of $^{125}$I. The figure shows that after 24h of exposure to the IPs by an initially equal amount of radioactivity from different radioisotopes, the detected background corrected PSL varies considerably. As compared to $^{125}$I, exposure to $^{32}$P results in more than a tenfold higher PSL. In contrast, the detected PSL after exposure to $^{99m}$Tc and $^{18}$F is approximately 20% and 60% respectively, of the detected PSL after exposure to $^{125}$I.

Table 4.1b also shows the efficiency-per-disintegration of the phosphor imaging system for the various radioisotopes (range $9.53 \cdot 10^{-4}$ - $2.84 \cdot 10^{-2}$ PSL/disintegration). The relative efficiency-per-disintegration as a ratio to the efficiency of $^{125}$I is depicted in Figure 4.3b. The figure shows a very high efficiency for $^{32}$P, as compared to $^{125}$I. A high efficiency-per-disintegration for $^{18}$F was also revealed, while the efficiency for $^{123}$I, although much lower than the efficiency for $^{32}$P or $^{18}$F, proved to be slightly higher than the efficiency for $^{125}$I.

Table 4.1b. Summary of experimental results. Properties of the IP for each tested radioisotope are displayed. Abbreviations: Eff/Dis; efficiency-per-disintegration, Eff/Bq; efficiency-per-Bq, PSL; photostimulable luminescence, n; total amount of occurred disintegrations of a radioactive sample in 24h, BG; background, LOD; limit of detection, LOQ; limit of quantification, FWHM; full width at half maximum, SD; standard deviation. Please note that the efficiency-per-Bq is valid only for the linear range of the tested radioisotopes and applies only to an exposure time of 24h. Also, the LOD and LOQ data are valid only for exposure times of 24h. Resolution experiments for $^{90}$Y were not performed.
<table>
<thead>
<tr>
<th>Radioisotope</th>
<th>Sensitivity (limit of detection and limit of quantification)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{125}$I</td>
<td>$2.60 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>$^{99m}$Tc</td>
<td>$1.68 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>$^{67}$Ga</td>
<td>$9.53 \cdot 10^{-4}$</td>
</tr>
</tbody>
</table>

Measurements of background PSL were between 3.2 and 6.3 PSL/mm$^2$ in all samples. Relatively high SD's (Table 4.1b.) were found on plates that were exposed to the $\gamma$-emitters $^{111}$In, $^{201}$Tl, $^{123}$I, $^{67}$Ga and $^{99m}$Tc. The low energy $\gamma$- and low energy electron emitter $^{125}$I showed a low SD. Low SD’s were also calculated for plates that were exposed to typical $\beta$-emitters ($^{32}$P, $^{90}$Y, $^{89}$Sr) and plates that were exposed to isotopes that emit both $\beta$-radiation and high energy $\gamma$-radiation, i.e. $^{131}$I and $^{18}$F.

Estimations of the LOD and LOQ of IPs for each radioisotope after exposure during 24h were expressed as Bq/cm$^2$ and are summarized in Table 4.1. The LOD and LOQ showed higher values for typical $\gamma$-emitters ($^{99m}$Tc, $^{67}$Ga, $^{123}$I, $^{201}$Tl and $^{111}$In) than for typical $\beta$-emitters such as $^{89}$Sr, $^{90}$Y and $^{32}$P. For the mixed $\beta$- and $\gamma$-emitter $^{131}$I, a relatively low LOD and LOQ was calculated. The $\beta^+$ (and annihilation photon) emitter $^{18}$F showed an intermediate LOD and LOQ.
Overall, the sensitivity proved to be high for all tested radioisotopes.

**Shielding experiments**

*Figure 4.2b.* shows an example of shielded $^{18}$F samples by various thicknesses of shielding material (0-1.98mm). As would be expected from Feather’s equation, adequate shielding of positrons was achieved by a thickness of shielding material of 0.90mm, since no further decrease of detected PSL was detected in the spots that were shielded with a thicker layer. The difference between this ‘baseline’ PSL and the PSL of unshielded spots, is considered to be the result of positron emission (and possibly to a very small extent to electron emission). The difference between the ‘baseline’ PSL and the background PSL is considered to be the result of the photons that are released by $^{18}$F.

For unshielded samples of $^{18}$F, we calculated that $93.3 \pm 0.7\%$ (average $\pm$ SD) of the measured background corrected PSL is the result of interaction of positrons with the IP whereas only $6.7\%$ can be attributed to annihilation photons. For unshielded samples of $^{131}$I, we calculated that $95.8 \pm 0.2\%$ of the detected PSL is the result of $\beta$-radiation or other electrons, while only $4.2\%$ can be attributed to $\gamma$-radiation.

*Figure 4.4.* Curve fit of experimental data from shielding experiments on $^{18}$F and $^{131}$I samples. The figure shows the transmission of the radioisotopes as a function of the thickness of the shielding material. Please note that the data were corrected for background activity and photostimulable luminescence that is attributed to $\gamma$-radiation (for $^{131}$I). Error bars represent the standard deviations of the measure-
ments. Curves represent the calculated curve fit by SPSS, for shielding of the samples (equations are shown in the insert). Abbreviations: T; transmission, dl; thickness of shielding material in mm. See color figure appendix for the full color version of this figure.

Figure 4.4. shows the result of the curve estimation based on the experimental data of both $^{18}$F and $^{131}$I in terms of transmission (T). Using the coefficients of these curve estimations, the half-thickness ($T=0.5$) in the shielding material was determined as 0.19 mm for the positrons (disregarding the very low energy electrons) of $^{18}$F and 0.15 mm for the $\beta$-spectrum and other electrons of $^{131}$I. $^{99m}$Tc only emits low energy electrons, which were completely shielded by 0.18 mm of shielding material. For unshielded samples of $^{99m}$Tc, $39.1\pm3.1\%$ of the obtained PSL by the phosphor imaging system was

Figure 4.5. Sample patterns of each tested radioisotope from the resolution experiments. The image on the top left of the figure is a scan of an original print-out of one of the test patterns printed with ink to which $^{123}$I was added. This test pattern has a width of 2 cm, line thickness is 0.1 mm and the distances between the lines are 0.1 or 0.4 mm. The image on the bottom left shows a microscopic photograph of an original print-out of a triangular test pattern of point sources. The points are approximately 0.1 mm in diameter and the distance between the points is 0.4 mm. The total size of each triangular test pattern is 2 mm on each side. Please note that the individual points that were printed by the inkjet printer are not perfectly round. The figure shows the difference in quality of the scan and hence the resolution
between the radioisotopes. The best resolution was achieved with $^{67}$Ga and the worst resolution was calculated for $^{32}$P.

attributed to the emission of these electrons while the remaining 60.9% of the detected PSL was attributed to $\gamma$-radiation. The detected PSL after exposure to unshielded samples of $^{67}$Ga, proved to be the result of $\beta$-radiation for 63.2±1.6% while the remaining 36.8% is the result of $\gamma$-radiation. Shielding of $^{125}$I did not result in a decrease of detected PSL by shielding with increasing layers of transparency material, and the obtained PSL is attributed entirely to $\gamma$-radiation.

**Resolution**

Samples of test patterns of all studied radioisotopes are displayed in Figure 4.5., including a scan and microscopic photograph of an original print-out of a line-source model and point-source triangle. Due to calibration of the radioactive ink-cartridges, based on the calculated efficiency-per-Bq for each tested radioisotope, the obtained images were comparable in terms of PSL density. The line-source model shows horizontal and vertical lines with a thickness of approximately 0.1mm, and the distance between each line is either 0.4mm or 0.8mm. The actual size of each line-source model was 2x2cm (Figure 4.5.). The triangularly ordered point sources are approximately 0.1mm in diameter. The distance between the centers of the points in the sample pattern in Figure 4.5. is 0.4mm. The actual size of the triangles was 2mm on each side.

**Figure 4.6a.** Phosphor image showing a horizontal brain section of a control rat at 2h after injection with $[^{123}]$FP-CIT, which shows high binding of the tracer to dopamine transporters in the striatum.  
**Figure 4.6b.** Brain section of a rat that was injected with methylphenidate prior to injection of $[^{123}]$FP-CIT, which shows a decreased binding of the radiotracer to dopamine transporters in the striatum.  
See color figure appendix for the full color version of this figure.

The figure clearly shows the differences in line thickness or point diameter of the actual print-out as compared to the phosphor images. The point-sources, on which the FWHM measurements were performed, are considered ideal point-sources and therefore, no correction for the size of the original sources was done. In fact, correction for size and shape of
the original sources is not easily done due to the geometric inconsisten-
cies of the printed points as is shown by the microscopic photograph of
the point-source triangle sample in Figure 4.5.
For each tested radioisotope, the FWHM is shown in Table 4.1b. No sta-
tistically significant differences were detected between the FWHM mea-
surements in vertical as compared to horizontal direction of the point
sources and the point spread function was symmetrical for most point
sources. FWHM is therefore presented as the average ± SD for measured
points. Resolution was also measured using horizontal and vertical line
sources, which consistently showed slightly higher FWHMs for each radio-
isotope due to more radioactive spill-over around the lines (data not
shown).
However, differences in resolution between the various isotopes were
shown. The highest resolution was shown for the typical γ-emitting
radioisotopes ⁶⁷Ga, ¹²³I, ⁹⁹mTc, ²⁰¹Tl, except for ¹¹¹In. In general, the β-
emitters ¹⁸F, ⁸⁹Sr, ³²P and ¹³¹I showed higher values of the FWHM and
thus a lower resolution. ¹²⁵I, which only emits low energy γ-radiation
and low energy electrons, also showed a high FWHM.

**Ex-vivo studies**

**Biodistribution studies using phosphor imaging**

Figure 4.6. shows an example of a phosphor image obtained from an un-
treated versus a methylphenidate-pretreated rat. In the brain sections of
the rats that were pretreated with methylphenidate, there was a less
pronounced [¹²³I]FP-CIT binding in the striatum than in the control
group (Figure 4.6.).
The ROI analysis showed no significant differences in the specific left
striatum-to-cerebellum ratios (left striatum-cerebellum/cerebellum) as
compared to specific right striatum-to-cerebellum ratios in individual
rats, which indicates a good reproducibility of the ROI-analysis method
in phosphor images (data not shown). For comparison with the
methylphenidate-treated group, the average uptake as measured by the
left and right striatal ROI was used. The data from the ROI-analysis are
summarized in Figure 4.7a. for both groups. The specific striatum-to-
cerebellum ratios were 2.66±0.08 (average ± SD) in the unblocked
group, which matches the known high density of DATs in the striatum.
Injection of methylphenidate prior to injection of [¹²³I]FP-CIT resulted
in an statistically significantly lower binding of the radiotracer in the
striatum, which resulted in specific striatum-to-cerebellum ratios of
1.59±0.21 (P=0.045; Figure 4.7a.).
The specific hypothalamus-to-cerebellum ratios were 0.88±0.03, reflecting the relatively low affinity of FP-CIT for SERTs, which are expressed in this brain area. The hypothalamus-to-cerebellum ratios in the blocked group were 0.87±0.09, which was not significantly different from the unblocked group (Figure 4.6. and 4.7a.).

The pilot study using [123I]Iododexetimide, which was used to visualize muscarinic receptors, resulted in a high uptake of the tracer in brain areas that express a high density of muscarinic receptors such as Figure 4.7a. Effects of pre-administration of methylphenidate on the specific striatum-to-cerebellum ratios and specific hypothalamus-to-cerebellum ratios, as measured by ROI-analysis on storage phosphor images. Figure 4.7b. Effects of pre-administration of methylphenidate on these ratios as measured by the conventional dissection technique. * P=0.045, ** P=0.009. Abbreviations: Struct; brain structure, cer; cerebellum.

Dissection biodistribution studies
Figure 4.7b. shows the results of the dissection biodistribution study using [123I]FP-CIT. Specific striatum-to-cerebellum ratios were 2.63±0.36 for the unblocked group, while pre-injection with methylphenidate resulted in significantly lower ratios of 1.33±0.43 (P=0.009). Specific hypothalamus-to-cerebellum ratios in the unblocked group were 0.68±0.07, whereas after blocking these ratios were 0.76±0.30, which was not significantly different. Figure 4.7. shows similar specific striatum-to-cerebellum ratios for the phosphor imaging study as compared to the dissection study and a significant decrease of specific striatum-to-cerebellum ratios after blocking with methylphenidate. The specific striatum-to-cerebellum ratios of the phosphor imaging study showed smaller variations as compared to the calculated ratios that were obtained from the dissection study.

Figure 4.8. Images of the pilot study using [123I]Iododexetimide binding to muscarinic receptors in the rat brain without (a) and with (b) pre-injection of the...
muscarinic receptor antagonist scopolamine. Please note the decrease of $[^{123}\text{I}]$iododexetimide binding to in the cortex, striatum, and hippocampus after blockade of muscarinic receptors with scopolamine. See color figure appendix for the full color version of this figure.

3D-Mapping of the biodistribution of $[^{123}\text{I}]$FP-CIT in the rat brain
To provide better insight in the $[^{123}\text{I}]$FP-CIT distribution in the rat brain, a 3D-reconstruction was generated from the brain sections of a control rat. Figure 4.9. shows two frames from an animation that was made by 3-dimensional reconstruction of the brain slices of a $[^{123}\text{I}]$FP-CIT injected rat (control rat). The animation (available for download at the publisher’s website) was rendered from brain slices in a 512x512 matrix. For localization purposes, the contrast enhanced $[^{123}\text{I}]$FP-CIT biodistribution data was superimposed in color on a grayscale low-contrast background animation of the same rat brain. The figure shows binding of $[^{123}\text{I}]$FP-CIT in, amongst others, the striatum and the hypothalamus.
Discussion

In this study, we evaluated the linearity, efficiency, sensitivity and resolution of the storage phosphor imaging system for 11 short-living radioisotopes. We also evaluated the value of the technique for ex-vivo [123I]-labeled radiotracer distribution studies in the rat brain, as an alternative for conventional dissection studies.

Linearity of the IP for the tested radioisotopes

All tested radioisotopes (18F, 89Sr, 32P, 67Ga, 99mTc, 90Y, 111In, 123I, 125I, 131I and 201Tl) showed a strong linearity between the amount of applied radioactivity on the TLC plates and detected PSL between approximately 7Bq and 6.3kBq (per 12mm spot), except for the strong β-emitters 32P, 89Sr, 90Y and 131I, since these isotopes showed overexposure of the plate in their highest concentrations. Below these concentrations, a strong linearity was also shown for these radioisotopes. Such linear response has been shown for many isotopes in previous studies3, 6, 16. Our study showed the linearity for commonly used radioisotopes in the clinical practice of Nuclear Medicine (89Sr, 18F, 67Ga, 99mTc, 90Y, 111In, 123I, 125I, 131I and 201Tl), and also 32P, which is frequently used in laboratory studies. The half-life of the tested radioisotopes varies between 109 min (18F) and 59 days (125I). Moreover, to our knowledge, this is the first study that compares a large series of different radioisotopes in this respect. Especially for very short-living radioisotopes, such as 18F, conventional X-ray film autoradiography is not adequate. This is due to the relatively insensitivity of the X-ray film for radioactive energy that is released from many radioisotopes and this generally demands long exposure times. Moreover, X-ray film shows poor linearity when the amounts of radioactivity that are exposed to the film vary greatly16.

Mechanisms of the IP

To be able to explain paradoxical findings in this study, it may be of value to discuss first the mechanisms that underlie the IP’s behavior. In the
past decades these mechanisms have been largely clarified\textsuperscript{28-31} although these are still not completely understood. The active layer of the Fuji-MS IP bears phosphor crystals that are coated as a 115\textmu m dispersion of 1-10\textmu m grains on a polyester support layer. When a roentgen beam or \(\gamma\)-radiation hits the atoms of the phosphor crystals, ionization may occur and free electrons (or electron-hole pairs; excitons) will be formed. By law, the energy that is transferred to a released electron may be equal to or less than the energy of the photon that collided with the electron. When less than the original photon energy is transferred to the electron, the photon will continue to move in a slightly different direction with an energy that is equal to the original energy minus the energy that was transferred to the electron. The degraded photon may then hit another electron and thereby ionize another atom if there is enough energy left.

Some of the photons of the roentgen beam (or \(\gamma\)-emitter) will ionize Eu\textsuperscript{2+}, the most important compound of the phosphor screen. Normally, in Fuji IPs Eu\textsuperscript{3+} is not present\textsuperscript{29, 32}. The majority of X-ray irradiation however, will cause ionizations of other atoms in the IP. The resulting excitons may either recombine directly with primarily ionized Eu\textsuperscript{2+} and emit prompt luminescence\textsuperscript{33} or be trapped in an impurity (defect) in the lattice of the phosphor crystal. The crystal defects are the result of an intentional contamination of the crystal by halogen ions (such as F, Br or I). When an electron is trapped in such a crystal defect, the complex is called a color center\textsuperscript{28, 30}. This produces the latent images on the IP (storage). When the color centers are irradiated by a laser beam, the trapped electron will be thermally released again to the conduction band. This step is performed inside the phosphor imager when the plate is read. The released electrons may then be trapped with electron holes at O-F-sites near Eu\textsuperscript{2+} ions. Simultaneous collapse of these excitons induces excitation of the Eu\textsuperscript{2+} ions, followed by luminescence\textsuperscript{29}. Currently there are some conflicting theories about this process\textsuperscript{31}. The process is accompanied by the emission of visible light (luminescence) which is detected by a photo multiplier tube in the phosphor imager as PSL.

Besides for detection of \(\gamma\)-radiation or roentgen beams, IPs can also be used for the detection of \(\beta\/-\)electron emitting radioisotopes. The emitted electrons may also ionize the atoms of the IP. Part of the energy of the emitted electron (of the radioisotope) will be transferred to the released electron (or exciton) to which it collided, and the originally emitted electron will continue its path by ionizing (or exciting) other atoms or be trapped in a crystal impurity or recombine to form an excited Eu\textsuperscript{2+} ion.
Efficiency of the IP for high energy $\beta$-emitters

Of all tested radioisotopes, the IP showed the highest efficiency for the high energy $\beta$-emitters $^{32}$P, $^{90}$Y and $^{89}$Sr. While $^{32}$P and $^{90}$Y are pure $\beta$-emitters, $^{89}$Sr also emits a small amount of high energy $\gamma$-radiation which is being neglected due to the very low yield. Emission of high energy electrons ($\beta$-particles in this case) provokes more ionizations in the phosphor crystals because these particles are able to transfer their energy to more than one electron, which is less likely for low energy electrons. This explains the higher efficiency of the IP for high energy electrons or $\beta$-radiation, as compared to low energy electron or $\beta$-emitters. Interestingly, the results of our study show a lower efficiency-per-disintegration for $^{90}$Y as compared to $^{32}$P, although the emitted $\beta$-particles of $^{90}$Y have a higher energy. One possible explanation for this phenomenon may be escape of trapped electrons from the color centers due to their high energy, without prior thermal stimulation by a laser. The result would be an increased amount of prompt luminescence and a decreased amount of stored energy, which diminishes the efficiency-per-disintegration of the IP for very high energy $\beta$-emitters.

LOD and LOQ on the IP for high energy $\beta$-emitters

Due to the limited range of (high energy) $\beta$-particles (or electrons) in the IP, ionizations only occur near the source of radiation (i.e. the sample). Therefore, macroscopically there isn’t much radioactive spill-over, and hence, the amount of background PSL on a larger distance is low for these radioisotopes. In our measurements, the standard deviations of the background PSL on the plates of these high energy $\beta$-emitters proved to be very low, and this is probably the result of the homogeneous character of normal background radiation. The result is a low LOD and LOQ for all high energy $\beta$-emitters. Because of the high efficiency of the IP for these radioisotopes, only a small amount of radioactivity is necessary to induce a higher PSL than the normal background PSL.

Resolution on the IP of high energy $\beta$-emitters

The range of high energy $\beta$-particles is also reflected in the FWHM. Although there are no statistically significant differences between the FWHM of the high energy $\beta$-emitters, there is a relation between the $E_{\text{max}}$ and the measured FWHM. A higher $E_{\text{max}}$ leads to a worse resolution, which may be due to the larger range of high energy $\beta$-particles as compared to low energy $\beta$-particles. Therefore, low energy $\beta$-emitters...
such as $^3$H should produce the best resolution possible, however the low energy particles of $^3$H do not penetrate the protective layers of the phosphor screens used in this study.

The limited resolution of the high energy $\beta$-emitters in our experiments is not likely to be the result of a flare effect; a scanner artifact that may occur perpendicular to the scan direction due to the granular composition of the active layer of the IP. There are three reasons for this. First of all, we measured that PSL as a result of a flare artifact only contributes to the total detected PSL for approximately 3-4% for intense spots in our imaging system and even less for spots that resulted in a medium intensity PSL (data not shown). Second, the concentrations of radioactivity in the ink of the inkjet cartridges were chosen such that one printed pixel (5-8pL of ink) would produce such a spot of medium intensity on the scanned images. Moreover, cartridges were calibrated to each other in terms of radioactive concentrations, by using the efficiency-per-Bq unit, in order to produce comparable images of the test patterns with respect to PSL density. Third, a flare effect would lead to differences between measurements of FWHM in either horizontal or vertical direction. On the scanned images of the radioactive point sources, no statistically significant differences between the horizontal and vertical FWHM were seen for any of the tested radioisotopes.
Efficiency, LOD, LOQ and resolution of mixed high energy β/high energy γ emitters

The shielding experiments on samples that contained $^{18}$F showed that 93% of the detected PSL can be attributed to the emission of the 634keV positron ($E_{\text{max}}$), because this is the percentage of activity that can be shielded by layers of plastic (Figure 4.2b.). From the curve estimation that was calculated based on the results of the experimental data (Figure 4.4.), a half-thickness of 0.19mm in shielding material was calculated. Theoretically, according the equation of Feather for electrons within the energy range of 0.6-20MeV ($R = (0.542 \cdot E_{\text{max}} - 0.133)/\rho$, with $R$ as cm, $E_{\text{max}}$ as MeV and $\rho$ as g/cm$^3$) the range for the positrons of $^{18}$F should be 1.44mm. The half-thickness is estimated by dividing the maximal range by 7 and is therefore approximately 0.21mm, which is close to the experimental findings. The small difference may be explained by experimental error or annihilation of the positrons before the shielding material is crossed.

For $^{121}$I, 96% of the PSL on the phosphor screen can be attributed to several high and medium energy β-particles and electrons (predominantly the 606keV β-particle). The calculated half-thickness based on the curve estimation (Figure 4.4.) was 0.15mm. Theoretically, the maximal range in the shielding material for the most important β-particles that are emitted by $^{121}$I ($E_{\text{max}} = 606$keV, $y = 0.894$), should be approximately 1.34mm and half-thickness should therefore be approximately 0.19mm. The results of our shielding experiment show a slightly lower half-thickness for $^{121}$I. This discrepancy is explained by the energy spectrum of emitted particles of $^{121}$I, which also include lower energy electrons that are shielded more efficiently by the shielding material.

For $^{18}$F and $^{121}$I respectively, the remaining 7% and 4% of the detected PSL which is not the result of high energy β-particles, should be the result of the emitted high energy γ-radiation. Although the yield for this γ-radiation is high for both $^{18}$F and $^{121}$I, the contribution to the detected PSL is very small. The emitted high energy γ-radiation also does not lead to an increased amount of background-PSL. This may be explained by the low mass attenuation coefficient of any material for high energetic γ-radiation, which decreases the chance of interaction (photo-electric of Compton-effect) as compared to low energy γ-radiation.

Although positrons will not be trapped by a color center of the phosphor crystals, the efficiency-per-disintegration proved to be higher for $^{18}$F than for $^{121}$I. The higher $E_{\text{max}}$ and higher yield of the positron of $^{18}$F as compared to the most important β-particle of $^{121}$I therefore explains the higher efficiency-per-disintegration of $^{18}$F. As expected, the efficiency of
$^{18}$F proved to be less than the efficiency of the tested higher energy $\beta$-emitters. Eventually, the positrons annihilate with an electron and release two 511keV annihilation photons. Due to the high energy of these photons (and therefore low mass attenuation coefficient in material), the chance of interaction with the IP is low so these photons are not likely to increase the absolute background PSL. However, the efficiency-per-Bq is low for $^{18}$F, which is the result of the short half-life of the radioisotope and the relatively long exposure time (24h). To calculate the LOD and LOQ, the measured background PSL is corrected by the efficiency-per-Bq (in order to express LOD and LOQ as Bq/cm$^2$). Therefore, de SD of the background measurements was rather high and as a result, the LOD and LOQ were also high as compared to the other high energy $\beta$-emitters. On the other hand, these LOD and LOQ estimations are actually valid, because due to the long exposure time the LOD and LOQ will be compromised. Long exposure (such as 24h) of $^{18}$F samples will lead to an unnecessary relatively high amount of background PSL. To avoid this, one may choose for a shorter exposure time. 3x half-life should be optimal and exposure of the IP in a Perspex and lead shielded cassette may also optimize the results.

The low resolution of $^{18}$F, which is reflected in the high FWHM, is most probably the result of the high energy positrons which are able to induce ionizations at relatively large distances from their source, due to their high energy (as compared to low energy $\beta$-emitters). As expected, the measured FWHM of $^{18}$F therefore follows the FWHM of the higher energetic $\beta$-emitters $^{32}$P and $^{89}$Sr. The FWHM of $^{18}$F and $^{131}$I are not statistically significantly different, but visually and by average, the resolution of $^{131}$I is slightly better. This may be due to the fact that $^{131}$I also emits 300-500keV electrons (cumulative yield approximately 10%, Table 4.1a.), which will travel less far from their source than higher energy particles and therefore may result in a better resolution. The 960keV positron emitter $^{11}$C is used frequently in experimental PET-radiotracers but has not been evaluated in the present experiments. The properties of the IP for this radioisotope should comparable to the properties for $^{18}$F. Based on the data of the presently tested high energy beta emitters, the efficiency-per-disintegration is expected to be somewhat higher than that of $^{18}$F. Due to the very short half-life of $^{11}$C, the efficiency-per-Bq is probably relatively low for this radioisotope when the exposure duration is 24h. The short half-life should also have effects on the LOD and LOQ, which will probably be slightly higher for this high energy positron emitter as compared to $^{18}$F. The resolution of
$^{11}$C on the IP should be in the same range as the resolution of $^{89}$Sr and $^{18}$F.

**Efficiency of the IP for mixed electron/$\gamma$-emitters**

For isotopes that do not emit high energy, but medium energy electrons and a variable amount of medium energy $\gamma$-radiation, interpretation of our results is slightly more complicated. First, we consider the contribution to the calculated IP efficiency that is the result of $\gamma$-radiation. The results of the shielding experiments by using layers of plastic between the $^{18}$F or $^{131}$I samples and the IP indicate that high energy $\gamma$-radiation hardly contributes to the amount of detected PSL. Similarly, high energy $\gamma$-radiation also contributes to only a small extent to the efficiency of IPs for radioisotopes that emit such products. This is shown by the absolute background PSL which is not increased on plates that were exposed to high energy $\gamma$-emitters such as $^{18}$F (annihilation photons, 511keV, yield 1.94) or $^{131}$I (multiple $\gamma$'s; cumulative yield 0.91; Table 4.1). Again, this is in agreement with the fact that, in comparison with lower energy $\gamma$-radiation, there is a smaller chance of interaction with material due to a lower mass attenuation coefficient. Moreover, the efficiency calculations are based on background corrected measurements. This means that the PSL of each sample is corrected by the mean background PSL which also may be the result of interaction of photons on a large distance from their source. However, in the area near to the source, the photon flux is higher (inverse-square law), which increases the chance of interactions. This may explain the 7% and 4% remaining PSL after adequate shielding of the $^{18}$F and $^{131}$I, respectively.

In contrast, medium energy and low energy photons seem to be able to interact with the IP at larger distances from their source, which is in agreement with the higher mass attenuation coefficient for material for lower energy photons. The result is an increase of absolute background PSL at large distances from the original sources. The measurements were performed on the plates that were used for the resolution measurements and due to the inhomogeneous distribution of the test patterns, there is a relatively high SD of the background measurements. This will also occur when $\gamma$-emitting tissue slices are being exposed.

Medium and low energy photons only contribute to the calculated efficiency of the IP to a small extent because, similar to the IPs of the high energy photon emitters, the efficiency calculations are based on background corrected data. However, PSL as a result of the higher photon flux close to the radioactive sample, to some extent, does contribute to the calculated efficiency.
In the shielding experiments of $^{125}$I, no decrease of detected PSL was shown with increasing layers of transparency material. This means that all low energy electrons are filtered out by the protection layers of the IP and by self absorption in the silica gel layer of the TLC plate. Therefore, it is assumed that the detected PSL from the IP is the result of very low energy $\gamma$-radiation only.

Very low energy $\gamma$-radiation has a relatively high probability to interact with material due to a relatively high mass attenuation coefficient, and is therefore attenuated easily. Our experimental results show that such very low energy photons do not lead to a higher amount of absolute background PSL. These photons therefore do contribute to the calculated efficiency. The obtained PSL in $^{125}$I is caused exclusively by these photons.

To summarize, the contribution of low, medium and high energy photons to the efficiency of IPs is not nihil, but rather unimportant for radioisotopes that also emit electrons that are able to cross the protective layer(s) of the IP in a sufficient quantity. In contrast, very low energy photons are a more important contributor to the IP efficiency, especially when there are few or no co-emitted electrons.

Of the very low and low energy $\gamma$-emitters, the efficiency proved to be best for $^{111}$In, which is the only radioisotope of these $\gamma$-emitters that emits a high energy electron. $^{123}$I performs slightly less. Although $^{123}$I emits more medium energy electrons than $^{111}$In, the sum of yields of the medium and high energy electrons of $^{111}$In equals the yield of the medium energy electrons of $^{123}$I (Table 4.1a.). Since the IP is more efficient for high energy electrons, and the amount of very low and low energy $\gamma$-radiation for $^{123}$I and $^{111}$In is comparable and since medium and high energy $\gamma$-radiation does not have large effects on plate efficiency, the efficiency-per-disintegration for $^{111}$In is higher than for $^{123}$I. The efficiency-per-Bq for $^{123}$I is lower than that for $^{201}$Tl due to the short half-life of $^{123}$I.

The efficiency-per-disintegration for $^{201}$Tl is lower than for $^{123}$I, due to the lower amount of emitted medium energy electrons. $^{125}$I shows a higher efficiency than both $^{99m}$Tc and $^{67}$Ga due to its very high yield for very low energy photons. Finally, $^{99m}$Tc shows a higher efficiency-per-disintegration than $^{67}$Ga due to the amount of medium energy that is emitted from $^{99m}$Tc, in spite of the higher yield for low energy electrons and very low energy photons that are emitted by $^{67}$Ga. Again, the lower efficiency-per-Bq for $^{99m}$Tc as compared to $^{67}$Ga is the result of differences in half-life.

Theoretically, the results of the $^{99m}$Tc experiments may be influenced by
the strong $\beta$-radiation of its parent radioisotope $^{99}$Mo (most important $\beta$: 1.2MeV, $\gamma$=0.83), which inevitably is present in the sample (for clinical practice less than 0.1% is accepted). However, in our experiments the presence of $^{99}$Mo was not an issue. We proved this by comparing a TLC of the radiotracer (tetrofosmin was used as the carrier for $^{99m}$Tc) that was scanned using a Minigitta flatbed scanner (energy range set to 15-200keV; Canberra, Zellik, Belgium) with a TLC strip that was exposed to the IP. The fraction of radioactivity in the mobile phase proved to be comparable. If present, $^{99}$Mo would have been found in this mobile phase, and would not be detected by the Minigitta scanner in contrast to the IP (data not shown).

**LOD and LOQ of the IP for mixed electron/$\gamma$-emitters**

As shown in Table 1b., all plates that were exposed to mixed medium and low and medium energy $\gamma$-emitters showed an increased absolute background PSL (4.3-5.6 PSL/mm$^2$) as compared to the pure $\beta$-emitters (2.9-3.8 PSL/mm$^2$). The SD of 10 background samples for each isotope proved to be relatively more increased (about 3-7x the standard deviation of the pure $\beta$-emitters) which is most likely due to the abovementioned inhomogeneous distribution of the radioactive sources on the plate. This results in a larger LOD and LOQ for low and medium energy $\gamma$-emitters. Moreover, by correction with the efficiency-per-Bq term, which is necessary to express the LOD and LOQ as Bq/cm$^2$, these increases in LOD and LOQ are enhanced, especially for very short living radioisotopes. A high LOD and LOQ is realistic for short-living radioisotopes such as $^{99m}$Tc and $^{123}$I, because the relative contribution of background PSL to the obtained images becomes larger when time passes. For such short-living radioisotopes, a short exposure of no more than 3x the half-life of the radioisotopes is advised (18h for $^{99m}$Tc). The LOD and LOQ may also be higher for IPs that are exposed to radioisotopes for which the plates have a low efficiency-per-disintegration. Shielding of the IP cassettes with lead during the exposure will enhance the sensitivity particularly for these radioisotopes.

The $\gamma$-emitter $^{125}$I showed a low absolute background PSL and also a very low standard deviation of the background measurements, comparable to those of the high energy $\beta$-emitters. This is explained by the limited ‘range’ (or high attenuation) of the very low energy photons that are emitted by this radioisotope, and is also reflected in the low LOD and LOQ.
Resolution on the IP of mixed electron/$\gamma$-emitters

The resolution of the tested $\gamma$-emitters seems to be the resultant of the co-emitted electrons but also, to a lesser extent, of the emission of very low energy photons (which is actually due to ionization of IP atoms by these photons). We conclude, from the resolution of $^{125}$I, that exposure to very low energy $\gamma$-radiation results in a low resolution on IPs. The achievable resolution of $^{125}$I on the protected IP is comparable to the resolution of high energy $\beta$-emitters.

$^{111}$In, $^{123}$I, $^{201}$Tl, $^{99m}$Tc and $^{67}$Ga all emit substantial amounts of very low energy $\gamma$-radiation, however these radioisotopes show the best resolution. This seems to be due to the co-emitted low and (to a lesser extent) medium energy electrons. Due to the high efficiency of the IP for these electrons, the beneficial effects on the resolution of the electrons outweigh the negative effects of the very low and low energy $\gamma$-radiation. This may be the reason why the resolution for $^{111}$In, which only emits a small amount of medium energy electrons but a relatively large amount of low and very low energy photons, is moderate (Table 4.1b., Figure 4.5.). The resolution of $^{125}$I and $^{99m}$Tc is comparable (Table 4.1b., Figure 4.5.). The reason for this may be not only the higher cumulative yield, but also the higher average energy of electrons within the medium energy category of $^{125}$I as compared to $^{99m}$Tc. This apparently outweighs the effect of the higher quantity of very low energy photons that are emitted by $^{125}$I. The favorable resolution of $^{99m}$Tc is the result of medium energy electrons which, as we showed in the shielding experiments, contribute to the total PSL for approximately 40%, in combination with the low cumulative yield for very low energy $\gamma$-radiation. The remaining 60% of the detected PSL in $^{99m}$Tc, resulting from low energy photons, do not have a major adverse effect on the resolution for this radioisotope. The resolution of $^{201}$Tl is moderate, although the measurement is somewhat uncertain (high SD). The moderate resolution may be due to low cumulative yield of low energy electrons, so the effects of the very low energy photons are relatively important.

The good resolution (Table 4.1b., Figure 4.5.) of $^{67}$Ga is probably due to the favorable characteristics of the electrons in the low energy category. The vast majority of these electrons have an $E_{\text{max}}$ between 82-93keV and these are therefore theoretically capable of penetrating both protective layers of the phosphor screen. From the shielding experiments, it has become clear that the detected PSL can be attributed to these electrons for 63%. This percentage was based on the difference between measurements without saran wrap (unshielded samples), so in theory the differ-
ence could also be the result of shielding of otherwise undetectable electrons (that are normally shielded by the saran wrap). However, this is not the case, since none of the otherwise undetected electrons (i.e. when using saran wrap) is able to cross the intrinsic protective layer of the phosphor screen (this also applies to the shielding experiment results for $^{99m}$Tc). The contribution of 63% of low energy electrons to the PSL, explains the good resolution of $^{67}$Ga samples on the IPs, in spite of its high cumulative yield for very low and low energy photons.

**General remarks**

In general, the efficiency of the IPs for high energy electron emitters is high, but high electron energies compromise the resolution of the plates for the radioisotope. Low energy electrons that are able to escape the silica gel and able to cross the protective layer(s) of the IP are beneficial to the resolution, but the efficiency of the IP is less for low energy than for high energy electron emitters. Electrons don’t have any effects on the absolute background PSL, but they may have an effect on LOD and LOQ (when expressed as Bq/cm$^2$ per timeframe) because they are of importance for the efficiency. IPs are rather insensitive for high energy photons. These photons almost show no effects on absolute background PSL, LOD, LOQ or resolution. Medium energy photons show a very small positive effect on IP efficiency for the radioisotope, but also a negative effect on the absolute background PSL, LOD and LOQ. Low energy photons have a small positive effect on plate efficiency. However, these photons will also increase the absolute background activity and have negative effects on the LOD and LOQ. Finally, very low energy photons have a positive effect on IP efficiency but do not increase the absolute background PSL. They, in analogy to electrons, have an effect on the LOD and LOQ. The resolution however, seems to be negatively affected by very low energy photons.

**Clinical appliances of the tested short-living radioisotopes with respect to neuroimaging**

Many of the tested radioisotopes are currently used for clinical diagnostic PET or SPECT imaging of neurological or neuro-endocrine diseases. For SPECT-imaging of the human brain $^{99m}$Tc (in e.g. $[^{99m}$Tc]HMPAO brain diffusion studies or $[^{99m}$Tc]TRODAT for DAT-imaging) and $^{123}$I (in e.g. $[^{123}$I]FP-CIT or $[^{123}$I]IBZM for DAT-imaging or D$_2$-receptor imaging, respectively) are used frequently. $^{123}$I in newly developed radiotracers is commonly replaced by $^{125}$I during evaluation and characterization experi-
ments, because the longer half-life of $^{125}\text{I}$ is logistically more favorable. $^{201}\text{Tl}$ is used frequently to diagnose and differentiate brain tumors. $^{111}\text{In}$ is being used for the detection of neuroendocrine tumors ([$^{111}\text{In}$]Pentetreotide). For PET imaging $^{18}\text{F}$ is used widely as [$^{18}\text{F}$]FDG, which is a marker for enhanced metabolism in tumors or infection (also in brain). Although not evaluated in the present experiments, the positron emitter $^{11}\text{C}$ is used often in PET-radiotracers that are used for neuroimaging, for instance as [$^{11}\text{C}$]Raclopride. Earlier studies have evaluated the storage phosphor imaging for this particular radioisotope and the imaging technique was used successfully in several preclinical studies using this isotope. In the future $^{68}\text{Ga}$ may also be used as the positron emitting radioisotope in tracers for PET-studies. Such tracers may be tested first using $^{67}\text{Ga}$ as the radioisotope, since the half-life of the latter is much longer and therefore also its availability.

$^{131}\text{I}$ is currently used therapeutically as [$^{131}\text{I}$]MIBG, to irradiate neuroblastomas in children. $^{90}\text{Y}$ is used clinically in some institutes as [$^{90}\text{Y}$]DOTATOC, for local irradiation of somatostatine receptor bearing neuroendocrine tumors. We included $^{89}\text{Sr}$ in our experiments to compensate for $^{90}\text{Y}$, which was not available at the time of the resolution experiments. $^{32}\text{P}$ was included as a strong $\beta$-emitter, for interpretation purposes. However, all radioisotopes that are used for diagnostic purposes are short-living radioisotopes that emit (predominantly) $\gamma$-radiation. These are chosen in order to lower the radioactive burden as much as possible for the patient. Many more radiotracers are currently under development, in particular positron emitting tracers for PET-imaging, but also $\gamma$-emitting tracers for SPECT-imaging. In the past few years, the use of phosphor imaging has become increasingly important in the development of these new tracers.

**In-vivo experiments**

Despite the lower efficiency of the IPs for short-living $\gamma$-emitting isotopes, imaging of these isotopes in e.g. biodistribution studies is feasible. In this study we performed a simple blocking experiment using [$^{123}\text{I}$]FP-CIT, a commercially available radiotracer with high affinity for the DAT, but also some affinity for the SERT. Indeed, we were able to visualize the high density of DATs in the rat striatum as compared to other brain structures using [$^{123}\text{I}$]FP-CIT and ROI-analysis on phosphor images of rat brain sections (Figure 4.6.). The rat brains were sliced in the horizontal orientation so striatum, hypothalamus and cerebellum were visible on the same brain section. By calculation of the specific striatum-to-cere-
bellum and hypothalamus-to-cerebellum ratio on a single brain section, variations in total radioactivity in the brain sections due to slight variations in section thickness were eliminated. By using this approach, also no correction was necessary for bodyweight of the rat and the amount of injected tracer. The calculated specific striatum-to-cerebellum ratios in multiple sections showed good agreement within individual rats (left vs. right striatum-to-cerebellum) and between the rats within a group. As expected, the specific hypothalamus-to-cerebellum ratios were low. The results of the phosphor imaging study were compared with a conventional dissection study (Figure 4.7.) and the specific striatum-to-cerebellum ratios were similar for both study protocols. The variation in the ratios was smaller in the phosphor study in spite of the smaller groups that were used. We suggest that the smaller variations in the measurements, may allow the use of smaller groups of rats for this type of biodistribution experiments. Other important advantages of the phosphor imaging technique as compared to dissection biodistribution studies of the rat brain include the higher sensitivity which allows detection of minute amounts of radioactivity. Also, when using phosphor imaging techniques, one has the possibility to reanalyze imaging data and the possibility to study tracer distribution in smaller or partial brain structures (such as specific layers of the cortex) which are not easily dissected. Although resolution of the phosphor imaging may be poor as compared to X-ray autoradiography, as compared to classical dissection experiments, the measurements are more precise.

As expected, in the phosphor imaging study, intravenous pre-treatment with methylphenidate resulted in blockage of binding to DAT in the striatum (Figure 4.6., Figure 4.7.). In the dissection study, the protocol was slightly different. In that experiment, 10mg/kg methylphenidate was administered intravenously to the rats prior to injection of the radiotracer, whereas in the phosphor imaging study 5mg/kg was given. This may explain the lower ratios of the blocked group in the dissection study as compared to the phosphor imaging study. Pre-administration of methylphenidate did not affect the binding of $[^{123}\text{I}]$FP-CIT in the hypothalamus in both experiments, which indicates the lack of specific binding to the DAT in this brain structure.

In the ex-vivo studies, ketamine/xylazine 2:1 was used as the anesthetic agent. Although there may be effects of ketamine on the dopaminergic system, we consider such possible effects as systematic, since the same anesthesia was used for all study groups. Moreover, anesthetics were used only from the moment of the first injection. Also, pretreated rats
that were given methylphenidate did show the expected decline of FP-CIT binding, which indicates the feasibility of both models to detect changes in DAT availability.

To test the value of the phosphor imaging technique for biodistribution studies using other $^{123}$I-radiotracers, we also performed a pilot experiment in two rats that were injected with $[^{123}$I]Iododexetimide, a radiotracer that binds to muscarinic receptors. Blocking of the muscarinic receptors was achieved by pre-injection of the high-affinity muscarinic antagonist scopolamine (Figure 4.8.). A striking effect of the blocking medication was seen in this pilot experiment.

Although planar phosphor images alone improve insight in the biodistribution of radiotracers in the rat brain as compared to dissection biodistribution studies, an even better understanding of tracer distribution can be obtained by viewing 3D-reconstructed images of the brain slices. The distribution of $[^{123}$I]FP-CIT in the rat brain that is depicted in Figure 4.9. is not only the result of binding to the DAT, but also to a lower extent to SERT. 3D reconstruction of the rat brain slices provides a rapid overview of the tracer distribution.

**Conclusion**

In conclusion, the storage phosphor imaging system shows a linear response over a wide dynamic range for all tested short-living radioisotopes. Although the efficiency, sensitivity and resolution of the phosphor imaging technique varies greatly for different radioisotopes and emitted particles and their radiation energies, we showed that this technique allows quantitative imaging of distribution of radiotracers that are labeled with all tested radioisotopes, even short-living $\gamma$-emitters such as $^{99m}$Tc or $^{123}$I. We also showed that $^{123}$I-labeled radiotracer-distribution studies in the rat brain using the phosphor imaging technique are an attractive alternative for conventional dissection studies. The main advantages of the phosphor imaging technique in radiotracer distribution studies in the rat brain include smaller variations in the measurements and the possibility to reanalyze radiotracer distribution retrospectively. Due to the high sensitivity of the IPs, minute amounts of radioactivity should be detectable in tissue sections. Also, phosphor imaging provides a more detailed insight in the distribution of radiotracers in the rat brain, which can be further improved by 3D-reconstruction of the rat brain sections.
Acknowledgments

The authors wish to thank Arjen van der Ree for technical assistance on the FWHM-measurements and the 3D-reconstruction using Hermes’ software, Jan Habraken and Joop Deeterink for advice on the radiation calculations and helpful discussions and Henk Westenbrink for logistical assistance.

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