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Optical imaging of Tc-99m–based tracers: *in vitro* and *in vivo* results

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Abstract. It has been recently shown that optical imaging (OI) methods can be used to image the *in vivo* biodistribution of several radiopharmaceuticals labeled with beta or alpha emitters. In this work particular attention has been focused on investigating the weaker optical signal induced by an almost pure gamma emitter like Tc-99m. Visible light emission measurements of a water solution containing Tc-99m were performed using a small animal OI system. A sequence of images was acquired for 24 h in order to study the decay of the luminescence signal. The difference between the luminescence decay half life and well-known Tc-99m half life was equal to 1%. *in vivo* imaging was performed by injecting one control nude mice with Tc-99m-MDP. Optical images obtained with equipment designed for bioluminescence imaging showed that a visible light emission was distinguishable and correctly localized in the bladder region where a higher concentration of Tc-99m-MDP was expected. The bladder to background ratio was always greater than 1. We conclude that the experimental data presented in this paper show that it is possible to detect *in vivo* luminescence optical photons induced by Tc-99m. This is important especially considering the large number of Tc-99m–based radiopharmaceutical currently available. [DOI: 10.1117/1.3653963]

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1 Introduction

It has been shown that optical imaging (OI) methods can be used to image the *in vivo* biodistribution of a large number of radiopharmaceuticals labeled with beta plus and beta minus emitters. More precisely, the main goal was the detection of Cerenkov photons emitted by electrons or positrons as they travel into the medium.[1–6] These studies have shown that the emitted optical spectrum follow the predicted $1/\lambda^2$ shape,[7] typical of the Cerenkov radiation within a wavelength range of about 500 to 700 nm.

The use of OI methods with radioisotopes has also been extended to the imaging of alpha emitters such as Ac-225 (Ref. 8) and Am-241 (Ref. 9). In particular, in Ref. 9 we showed that the fluorescence induced by a small alpha particles source of Am-241 can be detected *in vivo*.

With regard to the use of OI approaches for the imaging of gamma emitters in Ref. 1, the authors claim that no optical signal in the visible range was detected when 37 MBq of an almost pure gamma emitter like Tc-99m is imaged and a similar finding, but with a lower activity, was reported in Ref. 5.

In a preliminary work[10] we investigated the *in vivo* imaging of Tc-99m-MDP by using a small animal OI system and covering the animal with and without slabs of bismuth germanate (BGO) scintillating material. More precisely, in Ref. 10 we compared luminescence images considering photons radiance values ranging from 4×10^6 to 20×10^6 (p/s/cm^2/sr), and in this radiance range we did not find any detectable optical signal without the slabs of BGO.

In this work particular attention has been focused on investigating without the use of any scintillating material weaker luminescence signals induced by Tc-99m. In particular, *in vivo* imaging was performed by using nude mice models in order to allow the detection of a smaller number of optical photons.

The paper is organized as follows: in Sec. 2 the experimental methods are described. All the experiments were designed mainly to understand the nature of the detected luminescence signal. In Sec. 3 the main findings of *in vitro* and *in vivo* experiments using Tc-99m-methylene diphosphonate (Tc-99m-MDP) are presented. A discussion and conclusion then follow in Secs. 4 and 5.

In order to distinguish between Cerenkov luminescence imaging and luminescence caused by non-Cerenkov mechanisms, the more general term of radioluminescence imaging (RLI) is used in the rest of the paper.

2 Material and Methods

2.1 *RLI of Tc-99m Water Solution*

Visible light emission measurements of a water solution containing Tc-99m were performed using the IVIS 200 optical imager (Caliper Life Sciences, Alameda, California). The IVIS 200 is equipped with a back-thinned, back-illuminated CCD

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camera cooled at -90°C. The CCD has an active array of 1920 ×1920 pixels with a dimension of 13 μm.

All the images reported in this work were acquired in bioluminescence mode with the following parameters: exposure time of 300 s, the *f*-number of the optics $f = 1$, with a field of view = 12.8 cm. Images were acquired by grouping 16 pixels, analyzed with Living Image 4.0 (Caliper Life Sciences), and corrected for dark measurements.

In order to investigate *in vitro* the nature of RLI images, a solution of 71 MBq of Tc-99m-MDP was used to fill a 5-mm diameter 6-mm height plastic cylinder in a black plastic 96 well plate. A sequence of images was acquired for over 24 h in order to study the decay of the luminescence signal. A circular region of interest (ROI) was placed on the cylinder and the ROI average photons radiance (p/s/cm^2/sr) was calculated. The ROI values at different time points were used to obtain the luminescence decay curve, such curve was then fitted with a monoexponential function in order compare the luminescence half life with the known 6.02 h half life of Tc-99m.

2.2 *RLI of Tc-99m of Ex Vivo and In Vivo Tissue*

The RLI approach was first investigated *ex vivo* by injecting 20 MBq of Tc-99m-MDP in a 1-cm thick slab of chicken breast. After this preliminary experiment, one control nude mice of 20 g was injected with 100 MBq/0.2 ml of Tc-99m-MDP. Dynamic imaging was then performed in order to investigate the temporal variation of the OI signal *in vivo*. Images were acquired every 6 min for 70 min, and during image acquisition the animal was kept under gaseous anesthesia (2% of isoflurane and 1 l/min of oxygen). All the animal handling procedures were approved by the Institutional Ethical Committee according to the regulations of the Italian Ministry of Health and to the European Communities Council (86/609/EEC) directives.

The tissue time activity curves (TAC) were obtained by placing a circular ROI covering the bladder and a background ROI of similar size was placed in the upper part of the animal. The tissue to background ratio (TBR) at different time points was calculated by dividing the bladder and background ROI mean radiance.

3 Results

3.1 *RLI of Tc-99m-MDP Water Solution*

In this section the main results obtained by imaging a water solution of Tc-99m-MDP are presented. As one can see by looking at Fig. 1(a), the image shows a clear luminescence signal in the central cylinder filled with a solution of Tc-99m-MDP.

Figure 1(b) presents the decay curve of the optical signal and the corresponding fit (dotted line) with an exponential curve. The R^2 value was equal to 0.999 showing good agreement between the theoretical decay curve and the experimental data. The resulting half life determined from the exponential fit was equal to 5.96 h and, thus, the difference with the known Tc-99m half life is equal to 1%. This is clear experimental evidence that the measured luminescence is caused by Tc-99m.

3.2 *RLI of Tc-99m of Ex Vivo and In Vivo Tissue*

In this section the *ex vivo* and *in vivo* RLI obtained by using Tc-99m-MDP are presented. Figure 2 shows an *ex vivo* RLI image obtained by injecting Tc-99m-MDP in the center of a slab of chicken breast. As one can see there is a clear localized light emission in the region where the radiopharmaceutical was injected.

After performing RLI using *ex vivo* tissue, we injected one control mice using Tc-99m-MDP as described in Sec. 2.2. Figures 3(a) and 3(b) present, respectively, RLI images

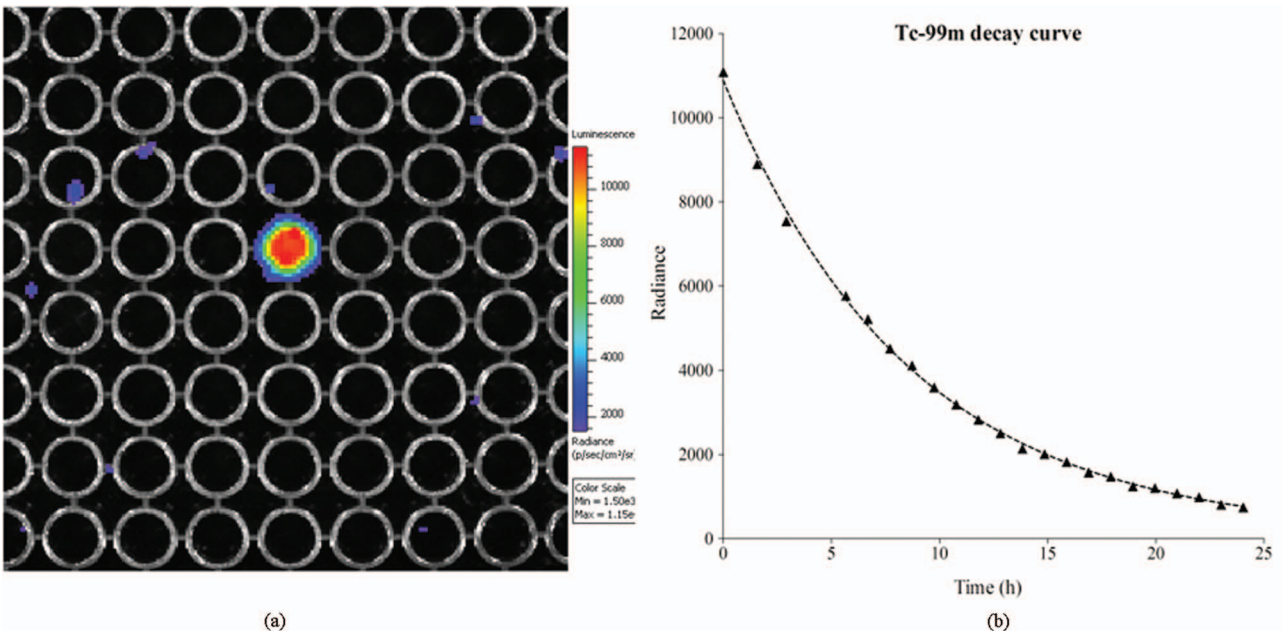

Fig. 1 (a) The thresholded luminescence image of small cylinder in the center of a plastic black plate filled with a Tc-99m water solution. The color scale represents the photons radiance (p/s/cm^2/sr). (b) The decay curve over 24 h of the luminescence signal is presented. The radiance values were obtained by placing a small ROI on the central cylinder and the average photons radiance was calculated. The dotted line corresponds to the fit with an exponential curve giving an R^2 equal to 0.999 and an half life of 5.96 h.

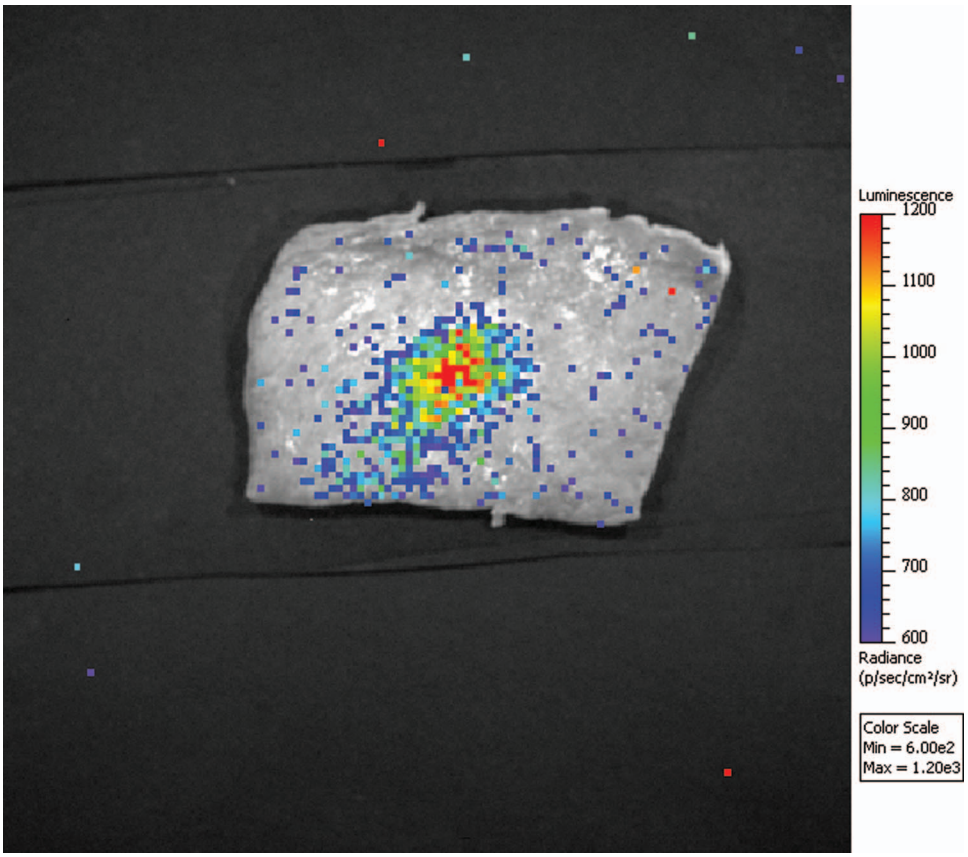

Fig. 2 *Ex vivo* thresholded RLI image obtained by injecting Tc-99m-MDP in the center of a 1-cm thick slice of chicken breast. The color scale represents the photons radiance (p/s/cm^2/sr). The image shows a localized light emission in the central region of the tissue slab where the radiopharmaceutical was injected.

obtained before and 60 min after the injection of Tc-99m-MPD. The luminescence signal can be clearly detected 1 h after the Tc-99m-MPD injection and more importantly is well localized in the bladder region where Tc-99m-MPD is known to accumulate.

In order to further investigate the radiopharmaceutical uptake, a set of RLI dynamic images were acquired as described in Sec. 2.2. Figure 4 presents the corresponding tissue TAC of the bladder and the background region.

The calculated TBR curve can be found in Fig. 5. As one can see by looking at the curve the TBR is always greater than 1. This shows a distinct difference between the bladder and background luminescence signal.

In order to further investigate the differences between the bladder and background, the analysis was also repeated by using five ROIs placed in different positions for both background and bladder at each time point. A t-test was performed considering the five bladder and background mean values at each time point at tracer equilibrium (after 40 min).

The p values calculated for each time point were always < 0.001, showing that the bladder and the background data are significantly statistically different.

4 Discussions

The experimental data presented in Sec. 3.1 show that Tc-99m in a water solution produces a detectable luminescence signal. The very good agreement between the decay of the optical signal (see Fig. 1) and the known Tc-99m half life is clear evidence that the luminescence is caused by Tc-99m.

One possible explanation of the measured luminescence can be the small beta emission[11] of Tc-99m causing Cerenkov light photons; however, we believe this is quite unlikely as will be discussed next. For example, from Ref. 5 we can derive a value of F-18 radiance per activity $\approx$0.11 (p/s/cm^2/sr)/Bq. By rescaling this value with respect to the activity used in our experiments, this should correspond to a radiance of $\approx 8 \times 10^6$ (p/s/cm^2/sr). Now if we multiply this value with the total Tc-99m beta minus intensity per decay[11] equal to 3.6×10^{-5} the resulting radiance is $\approx$280 (p/s/cm^2/sr). This rough calculation is still an overestimation of the contribution of Cerenkov photons to the measured light radiance. More precisely, the beta minus particles emitted by Tc-99m have smaller end point energies with respect to F-18 and, thus, an even smaller light output is expected.[2] This small value of radiance does not match the radiance measurements presented in Fig. 1 and it would be also quite close to the IVIS 200 minimum detectable radiance.

Tc-99m decays for 89% by emitting 140 keV gamma rays. These photons do not have enough energy to produce Compton electrons with energy such that Cerenkov condition is satisfied, since the energy threshold for a beta particle traveling in water is 263 keV.[12]

All these considerations lead us to conclude that the measured luminescence signal is compatible with non-Cerenkov water

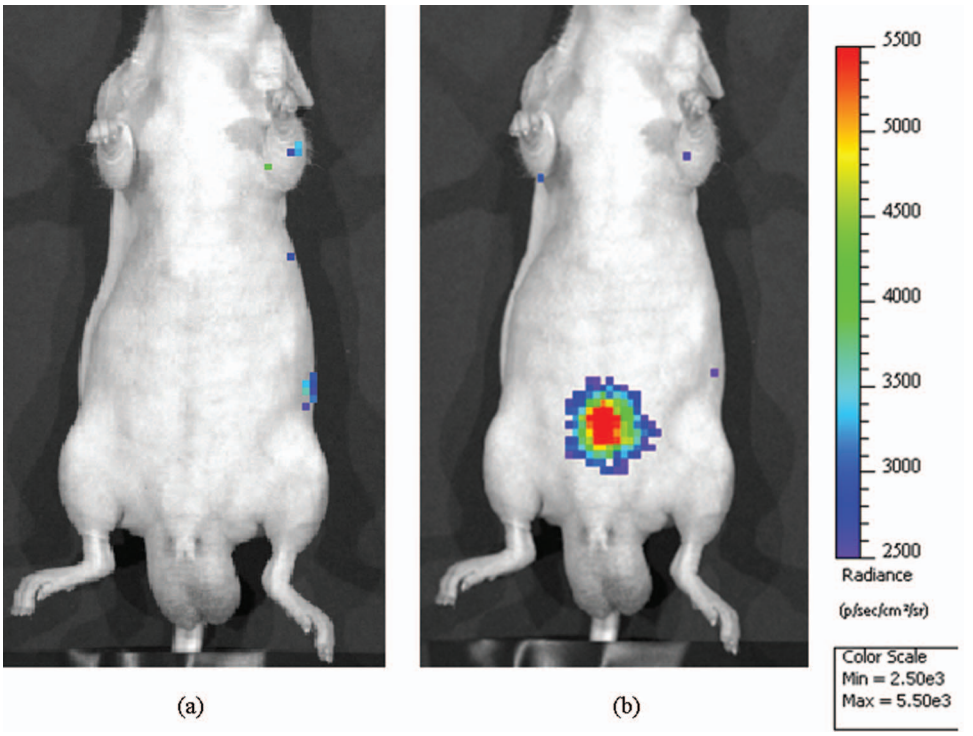

Fig. 3 *in vivo* thresholded RLI image acquired respectively before (a) and 60 min after the injection of Tc99m-MDP (b). As one can see by looking at the luminescence image in (b) the signal is localized in the bladder region where the Tc-99m-MPD is known to accumulate.

fluorescence induced by ionizing radiations.[13,14] In Refs. 13 and 14, the authors find both Cerenkov and non-Cerenkov components in the fluorescence spectra for water exposed to ambient ionizing radiations or gamma rays from a Co-60 source. The large number of low energy Compton electrons generated by the 140 keV gamma rays produce ionized and excited water molecules leading to several possible reactions[13] including, for example, the transition of excited OH radicals.[14]

The *ex vivo* and *in vivo* results further confirm the findings obtained by imaging Tc-99m in a water solution. More precisely in both cases a luminescence signal was distinguishable and correctly localized in regions where a higher concentration of Tc-99m was expected. In particular, the results of RLI *in vivo* imaging showed that the luminescence signal agrees with the well-known bladder accumulation commonly found with SPECT imaging.

As one can see by looking at the bladder TAC and TBR curves in Figs. 4 and 5, there is a distinct difference between the bladder and background signal. More precisely, the TBR increases and is always greater than 1.

Generally speaking, the amount of tracer was chosen depending on the type of experiment to be performed. The value of 71 MBq/0.3 ml in the well plates was chosen in order to study the Tc-99m decay in an adequate range (71 to 4.4 MBq). The slightly higher value of 100 MBq/0.2 ml was chosen to compensate for the tissue absorption and tracer distribution in the mouse volume. The smaller 20 MBq value for *ex vivo* imaging was chosen since in this case the tracer is concentrated and localized near the injection point.

Despite the fact that the RLI *in vivo* imaging results presented in Sec. 3.2 show a clear luminescence signal related to the radiopharmaceuticals uptake, the measured optical radiance has a lower magnitude in comparison with Cerenkov luminescence obtained when using radiopharmaceuticals labeled, for example, with beta plus[2–4] or pure beta minus[6]

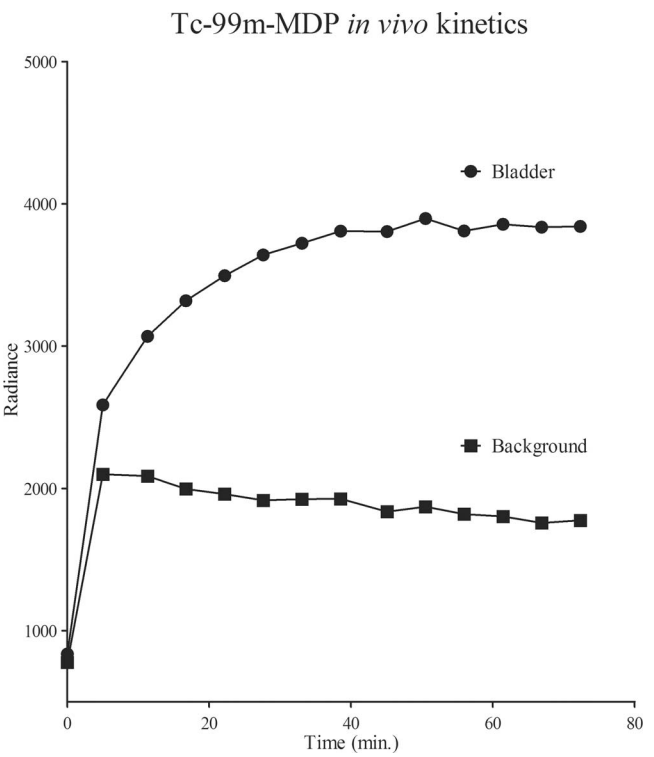

Fig. 4 The plot shows the tissue TAC of the bladder (circles) and the background (squares) region. The *y* axis represents the measured photons radiance (p/s/cm^2/sr).

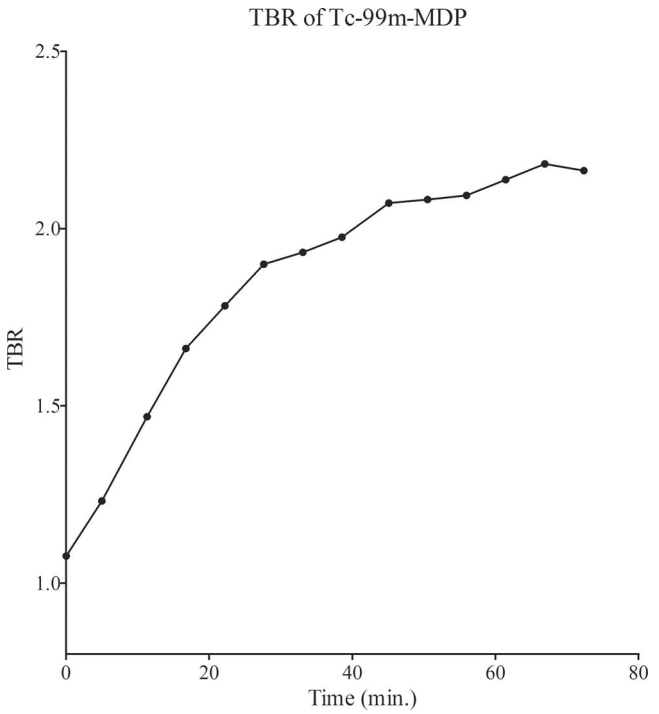

Fig. 5 The plot shows the TBR calculated by dividing the bladder and background ROI mean radiances. As one can see by looking at the curve the TBR increases and is always greater than 1, showing a distinct difference between the bladder and background luminescence signal.

radioisotopes. This lower light output can, to some extent, limit the detectability of visible photons coming from inner and smaller structures (such as bones for example) and/or in regions with a modest radiopharmaceutical uptake.

5 Conclusions

The experimental data presented in this paper show that it is possible to detect *in vivo* luminescence optical photons induced by Tc-99m decay. This is important especially considering the large number of Tc-99m–based radiopharmaceuticals that are currently available.

The main advantage of using the RLI optical imaging approach described here is the possibility of using commercial OI systems instead of more expensive dedicated small animal SPECT scanners. The use of OI systems allows more animals to be acquired at the same time and a shorter acquisition time with respect to conventional SPECT imaging.

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