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## ABSTRACT

Gold nanoparticles (AuNPs) have recently attracted considerable attention as promising drug carriers. *In vivo* imaging of AuNPs is required to investigate their pharmacokinetics. However, a direct and highly sensitive method has not yet been established. Therefore, we proposed the activation imaging of AuNPs that radioactivates AuNPs and non-invasively visualizes them using the emitted gamma rays. When the stable isotope of gold, <sup>197</sup>Au, is irradiated with low-energy neutrons, it changes to radioactive <sup>198</sup>Au, which predominantly emits 412-keV gamma rays. In this study, we synthesized [<sup>198</sup>Au]AuNPs through neutron irradiation and intratumorally injected them into tumor-bearing mice. *In vivo* imaging was performed using wideband x rays and gamma rays imager, and the distribution of [<sup>198</sup>Au]AuNPs in the body of mice was observed. Furthermore, we labeled the alpha-emitting therapeutic drug <sup>211</sup>At with [<sup>198</sup>Au]AuNPs. Although <sup>211</sup>At itself can be visualized using its 79-keV x rays, the long-term tracking of <sup>211</sup>At-labeled AuNPs has been challenging due to its relatively short half-life of 7.2 h. The activation of AuNPs enables the long-term tracking of <sup>211</sup>At-labeled AuNPs owing to the 2.7-day half-life of <sup>198</sup>Au. <sup>211</sup>At-labeled [<sup>198</sup>Au]AuNPs were injected intravenously into mice and visualized using a wideband x rays and gamma rays imager. Simultaneous imaging of <sup>211</sup>At and <sup>198</sup>Au was performed on the day of injection and 2 days after injection. Although <sup>211</sup>At could no longer be imaged 2 days after injection, the distribution of the drug was visualized using gamma rays from <sup>198</sup>Au.

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Drug delivery systems (DDSs) that deliver drugs to tumors have been actively researched for effective cancer treatment. Gold nanoparticles (AuNPs) are considered promising drug carriers due to their accumulation in tumors, ease of labeling, and potential biocompatibility, though their metabolism and excretion remain under investigation. It has been reported that the biodistribution of AuNPs varies depending on various factors such as size and shape.<sup>1–4</sup> Hence, *in vivo* imaging of AuNPs is essential to determine the most suitable treatment parameters. Imaging AuNPs by labeling them with specific tracers, such as radioactive nuclides, has been proposed;<sup>5–9</sup> however, the tracers may detach from the AuNPs and accumulate differently. Computed tomography (CT) enables the direct visualization of AuNPs;<sup>10–13</sup> however, its sensitivity is relatively low, often requiring large doses of AuNPs for

effective imaging. Consequently, the CT imaging of AuNPs is generally limited to applications in which they are used as contrast agents. Therefore, we proposed activation imaging of AuNPs as a direct method with high sensitivity. Activation imaging activates materials via neutron irradiation and performs visualization using x-rays and/or gamma rays emitted from the activated atoms.<sup>14,15</sup> Gold has only one stable isotope in nature, <sup>197</sup>Au, which becomes radioactive <sup>198</sup>Au when irradiated with low-energy neutrons. Thermal neutron (0.025 eV) captures cross section of <sup>197</sup>Au is 99 b, with a resonance peak observed in the cross section spectrum at 5 eV.<sup>16,17</sup> <sup>198</sup>Au has a half-life of 2.7 days and predominantly emits 412-keV gamma rays that allow visualization.

In this study, we synthesized [<sup>198</sup>Au] AuNPs using neutron irradiation, injected [<sup>198</sup>Au]AuNPs into tumor-bearing mice, and

performed *in vivo* imaging [Fig. 1(a)]. Moreover, we labeled the therapeutic drug  $^{211}\text{At}$  with  $^{198}\text{Au}$  AuNPs [Fig. 1(b)].  $^{211}\text{At}$  is a promising alpha-emitting therapeutic drug that emits 79-keV x rays during decay, allowing its visualization.<sup>18–21</sup> Furthermore, the labeling of  $^{211}\text{At}$  on AuNPs has been proposed for targeted therapy.<sup>22–24</sup> However, the half-life of  $^{211}\text{At}$  is 7.2 h, which is insufficient for long-term tracking of  $^{211}\text{At}$ -labeled AuNPs. Labeling  $^{211}\text{At}$  with  $^{198}\text{Au}$  AuNPs enables the long-term imaging of  $^{211}\text{At}$ -labeled AuNPs owing to the 2.7-day long half-life of  $^{198}\text{Au}$ . Mice were injected with  $^{211}\text{At}$ -labeled  $^{198}\text{Au}$  AuNPs, and simultaneous imaging of  $^{211}\text{At}$  and  $^{198}\text{Au}$  AuNPs was performed twice, on the day of injection and 2 days after injection, using a wideband x ray and gamma-ray imager. After imaging  $^{198}\text{Au}$  AuNPs and  $^{211}\text{At}$ -labeled  $^{198}\text{Au}$  AuNPs, the radioactivity of each organ in the mice was measured using a gamma counter.

Imaging was performed using a hybrid Compton camera (HCC) that can perform imaging using wideband x rays and gamma rays with energies ranging from tens of keV to approximately 1 MeV. The HCC consists of two pixelated Ce-doped  $\text{Gd}_3\text{Al}_2\text{Ga}_3\text{O}_{12}$  (Ce: GAGG) scintillators, a scatterer, and an absorber, and the scatterer has a pinhole at the center [Fig. 2(a)]. The scatterer and absorber consisted of  $45 \times 45$  arrays of scintillator pixels with dimensions of  $1 \times 1 \times 3 \text{ mm}^3$  and  $1 \times 1 \times 5 \text{ mm}^3$ , respectively. The pinhole size of the scatterer was  $3 \times 3 \text{ mm}^2$ . The distance between the scatterer and the absorber was 40 mm. The scintillators were coupled with a multipixel photon counter (MPPC) array to read the signal. For x rays and gamma rays below 200 keV, the HCC scatterer functions as an active pinhole collimator and enables the imaging based on the principles of a pinhole camera (pinhole mode), as shown in Fig. 2(b) (left). For gamma rays over 200 keV, HCC performs imaging based on the principles of a Compton camera (Compton mode). As shown in Fig. 2(b) (right), gamma rays are scattered at the scatterer and absorbed by the absorber. The scattering angle  $\theta$  can be calculated from Compton kinematics as follows:

$$\theta = \arccos \left\{ 1 - m_e c^2 \left( \frac{1}{E_a} - \frac{1}{E_s + E_a} \right) \right\}, \quad (1)$$

where  $m_e c^2$  is the rest mass energy of an electron and  $E_s$  and  $E_a$  are the energy deposits at the scatterer and absorber, respectively. When the image plane is defined, the position of the gamma-ray source is limited to an ellipse. The gamma-ray source is visualized by repeating this process for the detected gamma rays.

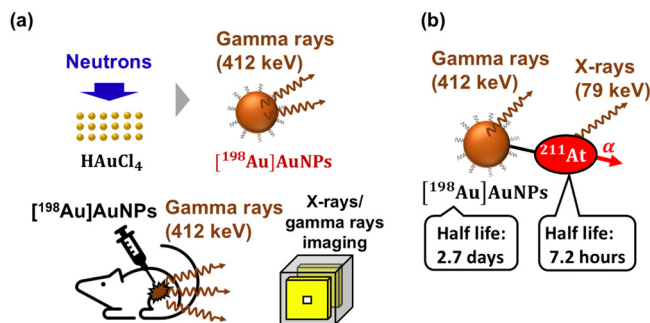


FIG. 1. Illustrations of (a) activation imaging of gold nanoparticles (AuNPs) and (b)  $^{211}\text{At}/^{198}\text{Au}$  AuNPs.

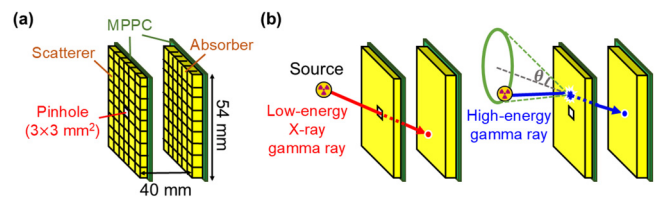


FIG. 2. (a) Configuration of HCC. (b) Imaging in the pinhole mode (left) and the Compton mode (right).

A 2 mg of tetrachloroauric(III) acid tetrahydrate ( $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ ) was encapsulated in the evacuated quartz tubes and irradiated with low-energy (primarily thermal) neutrons using a pneumatic irradiation facility (Pn-2) in the Kyoto University Research Reactor (KUR).<sup>27,28</sup> After irradiation, a solution of  $^{198}\text{Au}$ HAuCl<sub>4</sub> (1.82 mg) in  $\text{H}_2\text{O}$  (15.7 ml) was added to aqueous trisodium citrate solution (38.8 mM, 348  $\mu\text{L}$ ) at room temperature. The mixture was stirred for 1 min, and 0.075 w/w%  $\text{NaBH}_4$  in aqueous trisodium citrate (38.8 mM, 174  $\mu\text{L}$ ) was subsequently added at room temperature. After stirring for 10 min at the same temperature, the reaction mixture was purified by ultrafiltration using an Amicon Ultra 15 ml centrifugal filter (50 kDa) (3000 G, 10 min). The ultrafiltration was repeated again with the addition of de-ionized water. The resulting  $^{198}\text{Au}$  AuNPs in  $\text{H}_2\text{O}$  were diluted with  $\text{H}_2\text{O}$  (9.1 ml). The aqueous solution of  $^{198}\text{Au}$  AuNPs was analyzed using ultraviolet-visible absorption spectroscopy (UV-vis). A single peak at 520 nm, corresponding to the surface plasmon resonance peak, was observed, indicating that the AuNPs were spherically shaped without aggregation. The particle size of  $^{198}\text{Au}$  AuNPs was analyzed using dynamic light scattering (DLS) and was determined to be  $6.6 \pm 1.9 \text{ nm}$  (average  $\pm$  standard deviation). DLS measurements were repeated three times, and the results were consistent across all measurements. The synthesized  $^{198}\text{Au}$  AuNPs were coated with methoxy-polyethylene glycol (mPEG) to increase their stability.<sup>29,30</sup> Methoxy polyethylene glycol thiol with an average molecular weight of 5000 (mPEG(5 k)-SH) (1 mM, 190  $\mu\text{L}$ ) was added to the  $^{198}\text{Au}$  AuNPs in  $\text{H}_2\text{O}$  (8.9 ml) at room temperature. After stirring for 3 h at the same temperature, the reaction mixture was purified via ultrafiltration using an Amicon Ultra 15 ml centrifugal filter (50 kDa) (3000 G, 10 min). The ultrafiltration was repeated a few times with the addition of de-ionized water. The wavelength of maximum absorption and particle size of resulting  $^{198}\text{Au}$  AuNPs-S-mPEG(5 k) were

TABLE I. Injected dose, body weight, and tumor volume of the mice.

| Mouse no. | Injected dose (kBq) |                   | Body weight (g) | Tumor volume ( $\text{mm}^3$ ) |
|-----------|---------------------|-------------------|-----------------|--------------------------------|
|           | $^{211}\text{At}$   | $^{198}\text{Au}$ |                 |                                |
| 1         | ...                 | 781               | 23              | 237                            |
| 2         | ...                 | 349               | 23              | 272                            |
| 3         | ...                 | 303               | 22              | 352                            |
| 4         | ...                 | 193               | 21              | 215                            |
| 5         | 690                 | 236               | 23              | 99                             |
| 6         | 682                 | 250               | 22              | 44                             |
| 7         | 1416                | 420               | 21              | 47                             |

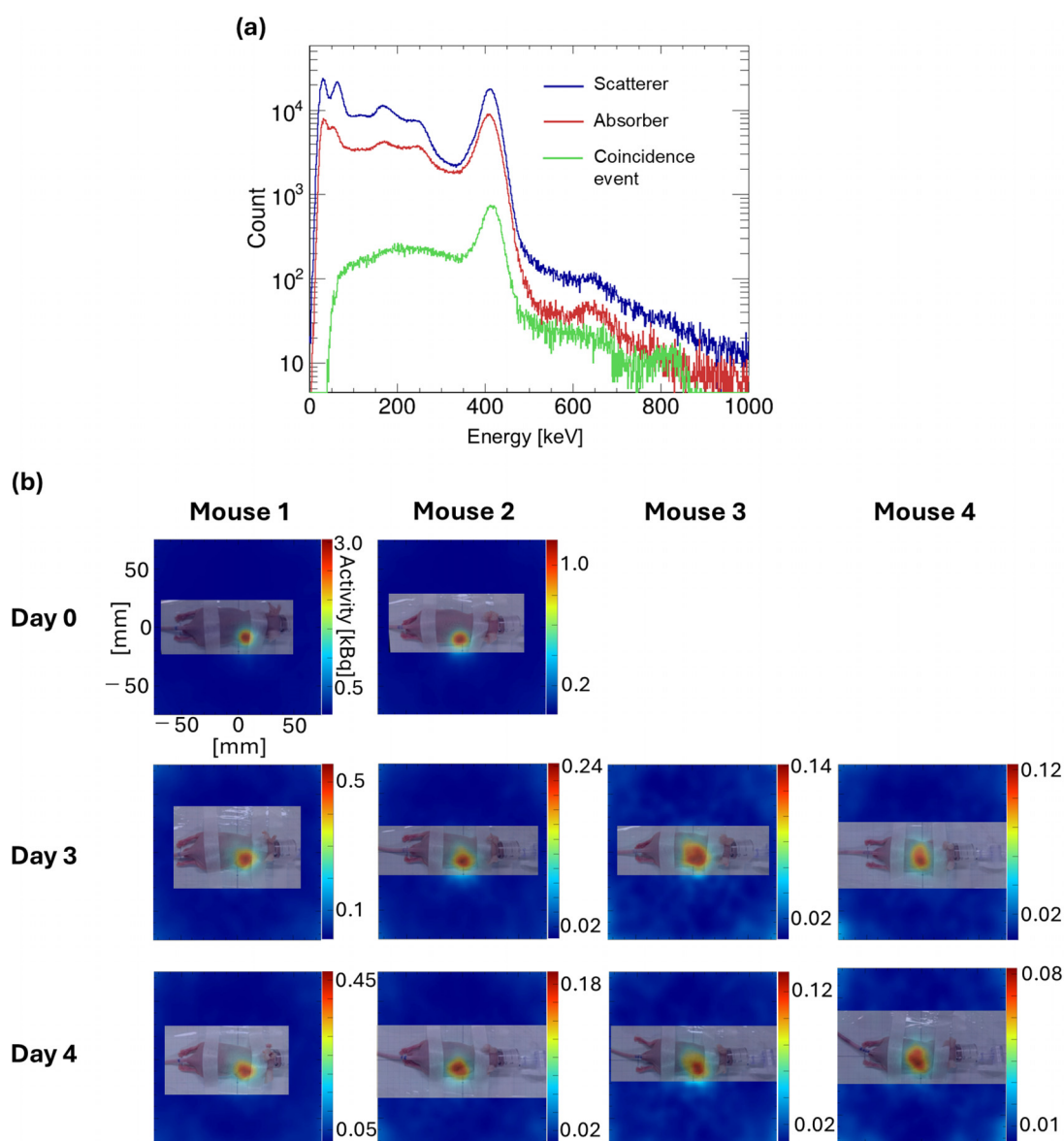
analyzed by UV-vis and DLS and were found to be 518 nm and  $17.4 \pm 4.8$  nm (average  $\pm$  standard deviation), respectively.

Four male nude mice (mice 1–4) aged 5 weeks were subcutaneously transplanted with SAS (human tongue squamous cell carcinoma) cells into the right flank. The mice were intratumorally injected with  $^{198}\text{Au}$ AuNPs-S-mPEG(5 k) 9 days after transplantation. The dose administered to each mouse, tumor volume, and body weight on the day of injection are shown in Table I.

The HCC was positioned 5 cm from the abdomen of the mice, which were placed in the prone position on an acrylic case. Imaging

was performed three times for mice 1 and 2 (on the day of injection, 3 days after injection, and 5 days after injection) and twice for mice 3 and 4 (3 days after injection and 5 days after injection). The spatial resolution of HCC at a distance of 5 cm is 7.9 mm (full width at half maximum, FWHM), which is comparable to the tumor size on the day of injection ( $7.7 \pm 0.5$  mm  $\times$   $11 \pm 1.4$  mm) and 3 days after injection ( $9.8 \pm 0.3$  mm  $\times$   $12 \pm 1.0$  mm).

Figure 3(a) shows an example of the energy spectra of gamma rays obtained with the HCC. The blue and red lines represent the energy spectra of events detected only in the scatterer and absorber,



**FIG. 3.** (a) Energy spectrum of gamma rays obtained with HCC. Blue: events detected only in the scatterer; red: events detected only in the absorber; green: events detected in both the scatterer and absorber. (b) Images of  $^{198}\text{Au}$ AuNPs in the body of mice obtained with HCC. Each column represents a different mouse (mice 1–4), and each row corresponds to days after drug administration (days 0, 3, and 4). The images are overlaid with photographs of mice.

respectively. The green line shows the energy spectrum of the events detected in both the scatterer and absorber undergoing Compton scattering. A peak of 412-keV gamma rays from  $^{198}\text{Au}$  was observed. We set the energy window to  $412 \pm 30\text{ keV}$  for the image reconstruction. The time from administration to the start of imaging, measurement time, and number of gamma rays used to reconstruct the images are listed in Table II. Images obtained using the HCC are shown in Fig. 3(b). An algorithm based on list-mode maximum likelihood expectation maximization (MLEM)<sup>26,31,32</sup> was applied during the reconstruction. Images of [ $^{198}\text{Au}$ ]AuNPs were overlaid with photographs of the mice to align the accumulation positions of the AuNPs with their anatomical positions. The images obtained from the abdomen of the mice were inverted to align with the photographs taken from their backs. All the mice were euthanized and dissected, and the radioactivity of each organ was measured with a well-type gamma counter equipped with NaI(Tl) detectors (ALOKA AccuFLEX  $\gamma$  7000) using 412-keV gamma rays 4 days after injection. The activity concentrations of the tumor and liver, which were calculated from the activity of each organ measured using the gamma counter, are shown in Table III. The activities were decay-corrected to the value at the start of imaging conducted 4 days after injection. More detailed results and medical analyses of the measurements of organ activities will be reported by Kato *et al.* (in preparation).

The obtained images and activity concentrations suggested that AuNPs accumulated in both the tumor and liver regions of all mice, with individual variations falling within the expected range of biological and surgical variability. In mice 1 and 2, the activity concentrations in the tumor were higher than those in the liver, as shown in Table III. In contrast, in mice 3 and 4, the liver exhibited higher concentrations than the tumor. This is consistent with the imaging results, where high pixel values were observed primarily in the tumor region in mice 1 and

TABLE II. Time from administration, measurement time, and number of used events during imaging of the mice.

| Mouse no. | Time from administration (h) | Measurement time (min) | Number of used events ( $\times 10^3$ ) |                                    |
|-----------|------------------------------|------------------------|-----------------------------------------|------------------------------------|
|           |                              |                        | Pinhole mode ( $^{211}\text{At}$ )      | Compton mode ( $^{198}\text{Au}$ ) |
| 1         | 0.55                         | 10                     | ...                                     | 21                                 |
|           | 71                           | 20                     | ...                                     | 19                                 |
|           | 92                           | 23                     | ...                                     | 18                                 |
| 2         | 0.15                         | 20                     | ...                                     | 22                                 |
|           | 70                           | 40                     | ...                                     | 20                                 |
|           | 92                           | 24                     | ...                                     | 9.7                                |
| 3         | 71                           | 21                     | ...                                     | 8.1                                |
|           | 92                           | 24                     | ...                                     | 7.4                                |
| 4         | 71                           | 33                     | ...                                     | 8.8                                |
|           | 92                           | 33                     | ...                                     | 6.8                                |
| 5         | 0.083                        | 53                     | 102                                     | 15                                 |
| 6         | 0.13                         | 55                     | 113                                     | 17                                 |
|           | 45                           | 63                     | 49                                      | 12                                 |
| 7         | 0.15                         | 33                     | 119                                     | 16                                 |
|           | 46                           | 53                     | 65                                      | 16                                 |

TABLE III. Activity and activity concentration of the tumor and liver.

| Mouse no. | Days after injection | Nuclide           | Activity (kBq) |       | Activity concentration (kBq/g) |       |
|-----------|----------------------|-------------------|----------------|-------|--------------------------------|-------|
|           |                      |                   | Tumor          | Liver | Tumor                          | Liver |
| 1         | 4                    | $^{198}\text{Au}$ | 16             | 33    | 28                             | 24    |
| 2         | 4                    | $^{198}\text{Au}$ | 25             | 15    | 32                             | 12    |
| 3         | 4                    | $^{198}\text{Au}$ | 8.4            | 22    | 12                             | 17    |
| 4         | 4                    | $^{198}\text{Au}$ | 7.7            | 24    | 12                             | 20    |
| 5         | 1                    | $^{211}\text{At}$ | 8.9            | 63    | 70                             | 60    |
|           |                      | $^{198}\text{Au}$ | 2.2            | 19    | 17                             | 18    |
| 6         | 2                    | $^{198}\text{Au}$ | 3.7            | 83    | 28                             | 74    |
| 7         | 5                    | $^{198}\text{Au}$ | 3.7            | 74    | 49                             | 69    |

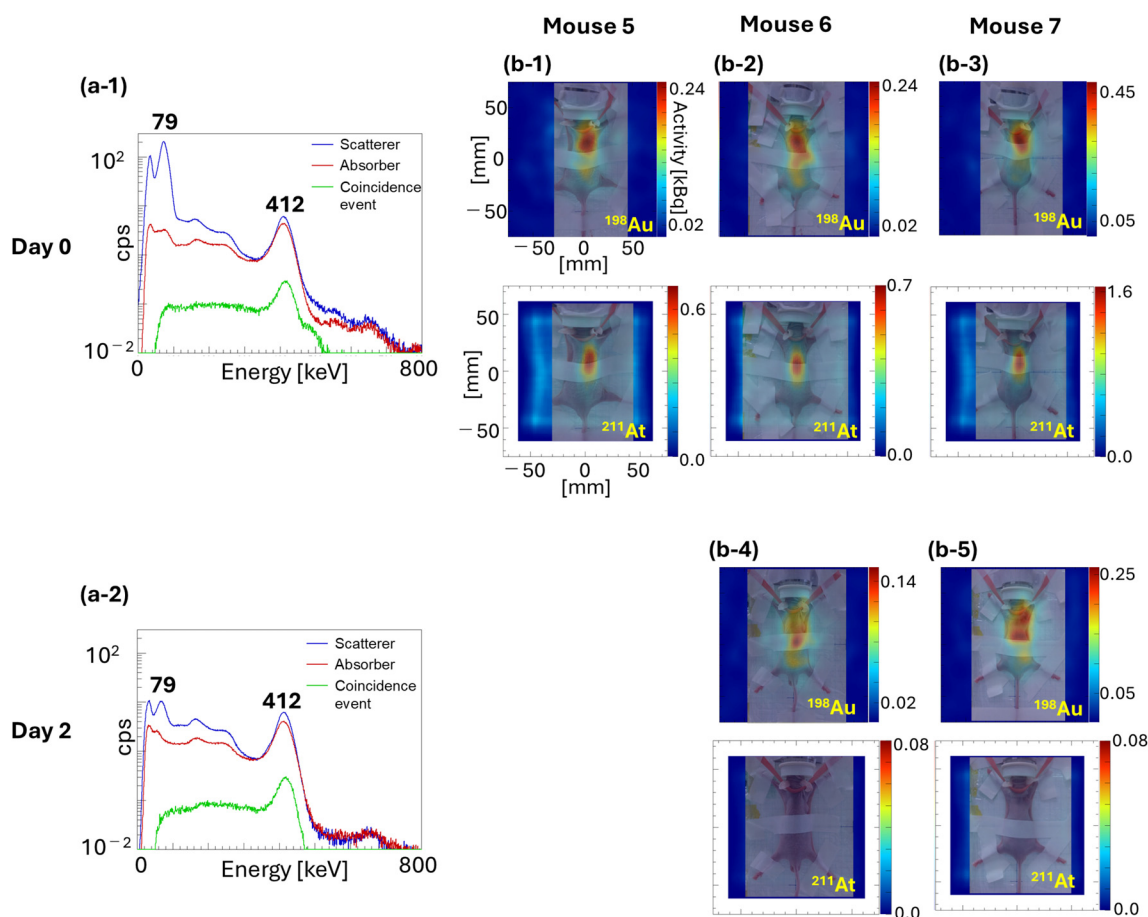
2, whereas the liver region also showed high pixel values for mice 3 and 4 [Fig. 3(b)].

To perform long-term tracking of  $^{211}\text{At}$ -labeled AuNPs, we synthesized  $^{211}\text{At}$ -labeled [ $^{198}\text{Au}$ ]AuNPs.  $^{211}\text{At}$  in  $\text{H}_2\text{O}$  (8.2 MBq) was added to a solution of [ $^{198}\text{Au}$ ]AuNPs-S-mPEG(5 k) (2.4 MBq) in  $\text{H}_2\text{O}$ , and the reaction mixture was stirred for 15 min at room temperature. These procedures for  $^{211}\text{At}$  labeling were based on those described by Kato *et al.*<sup>22</sup> The reaction solution was added to the Amicon Ultra 0.5 ml centrifugal filter (50 kDa) and centrifuged to separate free  $^{211}\text{At}$  from  $^{211}\text{At}$ -[ $^{198}\text{Au}$ ]AuNP-S-mPEG. The radioactivity of  $^{211}\text{At}$  in the filtrates was measured using a high-purity germanium detector, and the results were below the detection limit. This indicated that nearly all the  $^{211}\text{At}$  was labeled onto the [ $^{198}\text{Au}$ ]AuNPs, resulting in a quantitative yield.

Three male nude mice (mice 5–7) aged 5 weeks were subcutaneously transplanted with PANC-1 cells in the right flank. The mice were intravenously injected with  $^{211}\text{At}$ -labeled [ $^{198}\text{Au}$ ]AuNPs-S-mPEG(5 k) through the tail vein 14 days after transplantation. The dose administered to each mouse, tumor volume, and body weight on the day of injection are shown in Table I.

The HCC was positioned 10 cm from the abdomen of the mouse, which was in an upright position. All the mice were imaged on the day of injection. Mouse 5 was euthanized and dissected, and the activity of its organs was measured 1 day after injection. Mice 6 and 7 were imaged again 2 days after injection, and the activity of the organs was measured 2 days and 5 days after injection, respectively. The spatial resolution of HCC in pinhole and Compton modes at a distance of 10 cm is 16 mm (FWHM), and the tumor size in the mice on the day of injection was  $5.2 \pm 0.5\text{ mm} \times 6.7 \pm 1.1\text{ mm}$ .

Figures 4(a-1) and 4(a-2) show examples of the energy spectra of gamma rays obtained with the HCC on the day of injection and 2 days after injection, respectively. The blue line represents the energy spectrum of events detected only in the scatterer. The red line represents the energy spectrum of the events detected only in the absorber, which was used for the pinhole mode image reconstruction. The green line shows the energy spectrum of the events detected in both the scatterer and absorber undergoing Compton scattering. Figure 4(a-1) shows the peaks at 79 and 412 keV, corresponding to  $^{211}\text{At}$  and  $^{198}\text{Au}$ , respectively. Figure 4(a-2) shows a significant decrease in the height of the



**FIG. 4.** (a) Energy spectrum of gamma rays obtained with HCC. Blue: events detected only in the scatterer; red: events detected only in the absorber; green: events detected in both the scatterer and absorber. (b) Images of  $^{211}\text{At}$ -labeled [ $^{198}\text{Au}$ ]AuNPs in the body of mice obtained with HCC. (b-1)–(b-3) are the images of mice 5–7 obtained on the day of injection. (b-4) and (b-5) are the images of mice 6 and 7 obtained 2 days after injection, respectively. The top and bottom images show the distribution of  $^{198}\text{Au}$  and  $^{211}\text{At}$ , respectively. The images are overlaid with photographs of mice.

79 keV peak owing to the decay of  $^{211}\text{At}$ , while the peak at 412 keV maintains its height. The energy window was set to  $79 \pm 10$  keV and  $412 \pm 30$  keV for the pinhole mode and Compton mode image reconstruction, respectively. Although  $^{211}\text{At}$  emits high-energy gamma rays, such as those at 570 keV, during its decay, the peak intensity at 570 keV is approximately two orders of magnitude smaller than that at 412 keV [Fig. 4(a-1)]. Therefore, we consider that high-energy gamma rays from  $^{211}\text{At}$  do not significantly affect the imaging results of AuNPs. The time from administration to the start of imaging, measurement time, and number of gamma rays used to reconstruct the images are listed in Table II.

Images obtained with the HCC are shown in Fig. 4(b). The images of  $^{211}\text{At}$ -labeled [ $^{198}\text{Au}$ ]AuNPs are overlaid with photographs of mice. MLEM was applied during the reconstruction. The images obtained from the abdomen of the mice were inverted to align with the photographs taken from their backs. Here, the spatial resolution of HCC [16 mm (FWHM)] was insufficient to clearly distinguish the mouse's tumors or small organs because of the distance between the mouse and HCC. Nevertheless, a rough distribution of the drug was

still observable. Figures 4(b-1) and 4(b-3) show the images obtained on the day of injection. For all mice,  $^{198}\text{Au}$  and  $^{211}\text{At}$  were visualized in the Compton mode and the pinhole mode, respectively. Figures 4(b-4) and 4(b-5) show the images of mice 6 and 7 obtained 2 days after injection. Imaging of  $^{211}\text{At}$  was no longer possible because of its decay. The faint grid-like structure observed in the images of  $^{211}\text{At}$  was considered background noise originating from the tungsten case of the HCC, which becomes dominant when signal values are low. Most of the events used for the imaging conducted 2 days after injection are likely such background events. In contrast, the distribution of the drug was imaged using 412-keV gamma rays from [ $^{198}\text{Au}$ ]AuNPs. The activity and activity concentration of  $^{198}\text{Au}$  in the tumors and livers of the mice are shown in Table III. The activities were decay-corrected to the value at the start of imaging conducted on the day of injection for mouse 5, and 2 days after injection for mice 6 and 7. Additionally, the activity and activity concentration of  $^{211}\text{At}$  in the tumor and liver of mouse 5 are shown in Table III. These measurements could not be obtained for mice 6 and 7 due to the decay of  $^{211}\text{At}$ .

Table III showed that the activity concentrations in the tumor and liver were approximately at similar levels for mouse 5, which was consistent with the spread accumulation of  $^{198}\text{Au}$  and  $^{211}\text{At}$  observed in the images [Fig. 3(b-1)]. Mouse 6 showed a 2.6 times higher activity concentration of  $^{198}\text{Au}$  in the liver than in the tumors. The obtained image of  $^{198}\text{Au}$  [Fig. 3(b-4) (top)] shows the dominant concentration in the region corresponding to the liver, which supports the results of the activity measurement. The accumulation concentration in the liver was 1.4 times higher than that in the tumor for mouse 7, and the image of  $^{198}\text{Au}$  [Fig. 3(b-5) (top)] shows a high concentration in the liver region, with accumulation also seen in the tumor region. Nevertheless, note that the image and activity measurement of mouse 7 do not necessarily match because the activity was measured 3 days after imaging, allowing potential changes in accumulation over the days between imaging and activity measurement. The images of  $^{211}\text{At}$  obtained 2 days after injection [Figs. 3(b-4) and 3(b-5) (bottom)] indicated that the imaging of drug distribution using  $^{211}\text{At}$  over several days was challenging. Neutron-activated AuNPs enabled the imaging of  $^{211}\text{At}$ -labeled AuNPs 2 days after injection owing to the long half-life of  $^{198}\text{Au}$ , as shown in Figs. 3(b-4) and 3(b-5) (top).

In this study, we demonstrated the *in vivo* activation imaging of AuNPs. [ $^{198}\text{Au}$ ]AuNPs were synthesized by neutron irradiation and injected into tumor-bearing mice. Imaging was performed using HCC, a wideband x-ray/gamma-ray imaging device covering from a few tens of keV to nearly 1 MeV. [ $^{198}\text{Au}$ ]AuNPs were imaged using its 412-keV gamma rays. After imaging, the mice were euthanized and dissected, and the radioactivity of each organ was measured. The imaging results were generally consistent with the organ activity. Additionally, we labeled  $^{211}\text{At}$ , a promising alpha-emitting drug, onto [ $^{198}\text{Au}$ ]AuNPs. Although the 7.2-h half-life of  $^{211}\text{At}$  is insufficient to track the pharmacokinetics of  $^{211}\text{At}$ -labeled AuNPs for several days, the 2.7-day half-life of  $^{198}\text{Au}$  enables long-term tracking. Simultaneous imaging of  $^{211}\text{At}$  and  $^{198}\text{Au}$  was performed with HCC using 79-keV x rays and 412-keV gamma rays, respectively. The drugs were visualized using both  $^{211}\text{At}$  and  $^{198}\text{Au}$  on the day of injection. Imaging of  $^{211}\text{At}$  was no longer possible 2 days after injection; however, drug distribution was imaged using 412 keV gamma rays from  $^{198}\text{Au}$ , owing to its 2.7-day half-life. Although this demonstration indicates the feasibility of activation imaging of AuNPs, a higher spatial resolution is required for a more detailed investigation of their pharmacokinetics. In the future, we will perform imaging using the developed high-contrast Compton camera,<sup>33</sup> which is a wideband imaging device capable of distinguishing organ accumulation more clearly. Additionally, we are developing a SPECT system specifically designed for imaging high-energy gamma rays to achieve a higher spatial resolution.

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This study was conducted according to the guidelines of the ARRIVE (Animal Research: Reporting In Vivo Experiments) and the Osaka University Animal Experiment Regulations and approved by the Osaka University Animal Experiment Committee (protocol code 30-103-006 and date of approval 2019/02/20).

## AUTHOR DECLARATIONS

### Conflict of Interest

The authors have no conflicts to disclose.

## Author Contributions

**N. Koshikawa:** Conceptualization (lead); Formal analysis (lead); Investigation (lead); Methodology (equal); Writing – original draft (lead). **Y. Kikuchi:** Investigation (supporting). **K. S. Tanaka:** Investigation (supporting); Methodology (equal); Supervision (equal). **K. Tokoi:** Investigation (supporting); Methodology (equal). **A. Mitsukai:** Investigation (supporting). **H. Aoto:** Investigation (supporting); Methodology (equal). **Y. Kadonaga:** Investigation (supporting); Methodology (lead); Writing – review & editing (equal). **A. Toyoshima:** Investigation (supporting); Methodology (equal); Writing – review & editing (equal). **H. Kato:** Investigation (supporting); Methodology (equal); Supervision (equal); Validation (equal); Writing – review & editing (equal). **K. Ooe:** Investigation (supporting); Writing – review & editing (equal). **K. Takamiya:** Investigation (supporting); Methodology (equal); Writing – review & editing (equal). **J. Kataoka:** Conceptualization (lead); Investigation (supporting); Methodology (equal); Supervision (lead); Writing – review & editing (lead).

## DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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