DEVELOPMENT OF γ-RAY EMISSION IMAGING

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Abstract

Noninvasive imaging, particularly in small animals, has become important for the development of novel drugs and therapies. As a new imaging modality, we tried concurrent multi-probe tomography, using a collimatorless γ-ray emission imaging system consisting of a Compton camera. The camera comprises a pair of planar position-sensitive germanium detectors. As the system can detect a broad range of γ-rays up to 2 MeV with high-energy resolution, it is suitable for the tomographic imaging of a multitracer that contains multiple radioactive nuclides, each of which can tag separate biochemical processes. Here we show an example of biological applications—imaging of a tumor-bearing mouse—that illustrates the essence of concurrent multi-probe tomography.

IMAGING IN BIOLOGY

Multi-probe imaging, which allows the simultaneous visualization of multiple biochemical processes in a biological system, is vital for continued advancements in biomedical science. The method described here has a number of potential applications, such as simultaneous measurement of blood flow and metabolism in the brain, or simultaneous imaging of the pharmacokinetics and the effect of a drug for its development and evaluation. Several biological imaging methods are in use today, such as positron emission tomography (PET), magnetic resonance imaging (MRI), fluorescent protein imaging (FPI), and bioluminescent imaging (BLI). However, each of these methods has following limitations. Simultaneous multi-probe measurement is very difficult with PET because the signal is limited to the annihilation γ-ray of 511 keV for all probes. MRI is relatively insensitive and there are many constraints on the experimental apparatus and manipulations due to the strong magnetic field and small bore size. Although FPI and BLI appear promising, the exact influence of the probes is still unclear, and signals from deep tissue cannot be observed, which may limit the usefulness of these techniques in clinical situations.

We have attempted to apply γ-ray spectroscopy, which is often used in nuclear physics and astrophysics, to biochemical and biomedical imaging. Gamma-ray spectroscopy enables separation of γ-ray signals of different energies and identification of the source distribution. Different radioactive nuclides that emit γ-rays of different energies, possibly in different chemical forms, can be used as independent, simultaneous tracers. We specifically concentrated on metals because of their strong chemical reactivity and flexibility in labeling. Inorganic radioactive metal ions alone can be used as tracers. In addition, complex compounds such as bifunctional chelating-agent conjugates, antibody conjugates, and other types of metal-containing ligands that have high affinity to functional molecules, as well as metal-containing biomolecules, such as receptor-binding metallic proteins or metallic enzymes, can be used to tag various biological substrates such as tumor cells, receptors, enzymes, and nucleic acids. Therefore, by combining γ-ray spectroscopic imaging with radioactive metal tracers, we can conduct *in situ* or even *in vivo* concurrent multi-probe imaging and thereby trace multiple biochemical processes simultaneously. This paper reports examples of concurrent multiple metal probe tomography.

IMAGING MULTITRACER

We first developed the multitracer technique at RIKEN in 1991 as a tool in nuclear and analytical chemistry [1,2]. The irradiation of various metal target foils such as Ti, Fe, Cu, Ge, Ag, Au, or Pb with accelerated ion beams such as ^{12}C, ^{14}N, or ^{16}O produces many radioactive nuclides simultaneously by nuclear fragmentation. After chemical separation of the radioactive nuclides from the target material, they can be used as carrier-free and salt-free radioactive tracers, which are collectively called the multitracer. Because a variety of radioactive nuclides that have various half-lives and emit γ-rays of various energies can be produced, the number of possible independent radioactive signal sources for most of the elements in the periodic table is practically infinite. Recently, a gas-jet-coupled multitarget system [3] was implemented, which increased flexibility and capability of radioisotope production, particularly of short-lived radioactive nuclides.

However, the original multitracer technique could not be used for concurrent multi-probe tomography because no imaging method for the multitracer was available at that time. Since the production and the chemical processing of individual nuclides was difficult as well, we worked on developing techniques for the chemical separation of the multitracer and the production of separated radioactive isotopes called single tracers. These associated techniques are also collectively referred to as the "multitracer technique." In addition, because the primary application of the multitracer technique has been the tracking of trace elements in living organisms, we have been studying uptake, distribution, retention and excretion of specific elements, particularly metals, in specific organs and tissues. One recent finding was that zinc ions accumulate in brain tumors to such a degree that

they produce a higher contrast signal than the conventional [^{14}C] fluorodeoxyglucose in diagnostic imaging [4]. This led us to expect that cancer imaging could be significantly improved with an appropriate method for imaging zinc.

Thus, we have been developing an apparatus that can image multiple γ-ray-emitting nuclides simultaneously and independently using a Compton camera. The second-generation prototype has recently been implemented and is called a γ-ray emission imaging system (GREI) [5,6]. The first-generation prototype was also a Compton camera, consisting of a pair of segmented, planar, high-purity germanium (HPGe) detectors arranged in parallel [7]. Although the first-generation prototype successfully generated a multiple-radionuclide image, it was limited in spatial resolution [8]. Therefore, we developed the second-generation prototype GREI. In the design of the system, energy resolution was important because it is critical for the separation of γ-rays of different energies emitted by various radioactive nuclides and for the estimation of the location of the γ-ray source. In other words, energy resolution was essential for multi-probe imaging at high spatial resolution. Thus, we again used planar germanium detectors. In addition, to improve the spatial resolution further, we implemented a system for the measurement of the depth of the γ-ray interaction point in the detector [9].

γ-RAY EMISSION IMAGING (GREI)

The GREI system consists of two planar HPGe detectors (Eurisys Mesures) that are arranged in parallel, front and rear. For each γ-ray, the front detector is for detecting the Compton scattering and the rear detector is for detecting the coincident photoelectric effect. The source nuclide is determined by the sum of the energies observed by the two detectors. The possible incident angles of the γ-ray are estimated by applying the Compton kinematics to the energy distribution between the detectors and the three-dimensional positions of the detected γ-ray interactions. Because HPGe is used as the detector material, a broad range of γ-rays, including the multitracer γ-rays ranging from 200 keV to 2500 keV, can be detected with high resolution. To determine the positions of interactions in the detectors, we placed 13 strips on the front and rear sides of the detectors (13 x 2 x 2 = 52 strips total). The strips on the front side are orthogonal to those on the rear side. The depth of the γ-ray interaction point in the detector is determined by measuring the signal time difference between the front and rear strips. The transverse position of a γ-ray interaction point in a detector is determined by the combination of the front and rear strips that detected the signal, with the accuracy being determined by the width of the strip. The image is reconstructed in two steps. First, a simple back-projection is generated. Then, the simple back-projection is deconvoluted with an estimated point-spread function (PSF), assuming shift invariance. The PSF is computed by a simulation. A Butterworth filter is applied to remove the high-frequency noise. A complete description of the system is reported elsewhere [10].

IMAGING EXPERIMENTS

Figure 1 shows the results of the first animal imaging experiment. The experiment had been approved by the Wako Animal Experiment Committee of RIKEN. A male C57BL6 mouse (6 weeks old) was implanted with 0.2 mL of Ehrlich's ascites carcinoma cells (2 x 10^7 cells) in its hind thigh, 8 days before the intraperitoneal administration of a multitracer, containing 60 kBq ^{65}Zn, 30 kBq ^{59}Fe, and 10 kBq ^{88}Y, and six other radioactive nuclides. Twenty-four hours after administration, the mouse was sacrificed under deep anesthesia, and the carcinoma was measured. The γ-ray spectrum (Figure 1a) and the deconvoluted images of ^{65}Zn, ^{59}Fe, and ^{88}Y (Figure 1b-d) are presented. Zinc-65 uptake was high in the carcinoma and the liver, as indicated with a red arrow and a yellow arrow, respectively. The bright white spots in the ^{59}Fe and ^{88}Y images depict the carcinoma. These results are consistent with our previous reports [4, 11,12].

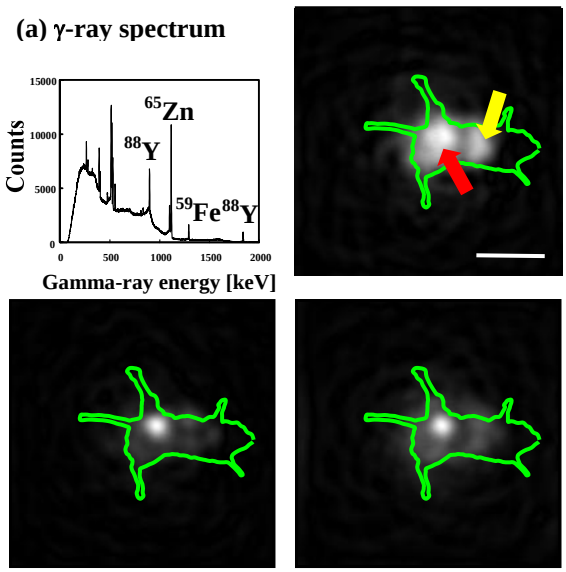

Figure 1: The results of animal imaging.

As another potential application, a young soybean plant (*Glycine max*), a few days after sprouting, was administered with 630 kBq ^{137}Cs, 140 kBq ^{59}Fe, and 50 kBq ^{65}Zn in a hydroponic pot. After 4 days, the plant was harvested, separated into the shoot and the root, and dried before being measured. Cesium-137, which is a potassium analogue, was widely distributed throughout the organism, including in the leaves. Iron-59 remained in the stem. Zinc-65 was found in the stem and the apical growing points, congruously with the plant physiology.

Thus, the distribution of several elements in biological samples was observed nondestructively under an identical experimental condition. Concurrent multi-probe tomography using multitracer was successful.

The significance of concurrent multiple metal probe tomography using GREI is as follows. First, metals themselves can be used as tracers. Therefore, GREI can be used for the study of metallic biotrace elements, which often play important roles in oxidation, antioxidative and redox reactions. Second, metals can form complex compounds that may tag receptors as ligands, enzymes, other proteomes and transcriptomes. Since the activity or the binding property of the complex compounds is not highly dependent on the metal, it is possible to design an experiment in which several metals compose several probes which tag several biochemical processes. GREI can be used to measure all of them simultaneously under an identical experimental condition, in real-time. Quantitative comparisons between probes can be done easily without any complex image processing such as registration.

CURRENT DIRECTIONS

As a final note, we describe our current direction. In the field of nuclear medicine, the Compton camera is often considered to be a collimatorless PET [14,15]. However, we envision it as a different modality because concurrent multiple metal multiple probe tomography provides novel types of information. The exposure to high-energy γ-rays in clinical application may raise some concern. However, it should be noted that metals do not always emit high-energy γ-rays and there is an argument that the biological effect of γ-ray emitters can be smaller that that of the corresponding positron emitters [14]. In that sense, GREI may be safer than PET. With respect to the apparatus, we have been developing a technique for the detection of the transverse position of a γ-ray interaction point in a detector. With respect to the probe, we are continuing to develop chemical separation techniques for more elements in the periodic table, including the rare earth elements, which can label nucleic acids. Thus, concurrent multiple metal probe tomography may enable simultaneous imaging of multiple metabolomes, proteomes, and transcriptomes, possibly *in vivo*, which would improve the process of drug development and evaluation in the future. In fact, we have already initiated related studies such as investigation of the *in vivo* distribution of metal-conjugated immunoglobulin [16], and the development of techniques for *in vivo* fixationless imaging [17].

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