In-Vivo Elemental Analysis by PIXE-μ-CT

V. 2. *In-Vivo* Elemental Analysis by PIXE-μ-CT

Kawamura Y.[1], Ishii K.[1,2], Yamazaki H.[2], Matsuyama S.[1], Kikuchi Y.[1], YamaguchiT.[1], Watanabe Y.[1], Oyama R.[1], Momose G.[1], Ishizaki A.[1], Tsuboi S.[1], Yamanaka K.[1], and Watanabe M.[1]

[1)]Department of Quantum Science and Energy Engineering, Tohoku University,
[2]Cyclotron and Radioisotope Center, Tohoku University

Introduction

PIXE (Particle-induced X-ray emission) has been successfully used as a powerful tool of trace element analysis, because the production cross-section of characteristic X-ray is very large in comparison to those of continuous X-ray. This feature is quite different from the case of electron bombardment, where electron bremsstrahlung contributes predominantly to the X-ray spectrum[1,2)]. X-rays produced by ion microbeams could be used as a monochromatic and low energy m-X-ray source without using monochrometer. Therefore, the 3D micron-CT can be realized by the use of this monochromatic X-ray-point-source, which provides the 3D structure of small object less than 1 mm with a high spatial resolution and high contrast. On the basis of this idea, we have developed a 3D micron-CT using micro-PIXE for *in-vivo* imaging, which is very useful in biological studies[2~4)]. Using a monochromatic X-ray point source produced by bombarding pure metal target by ion microbeam, we could acquire X-ray projection data. 3D images were reconstructed from these 2D projection data by using cone-beam CT reconstruction algorithm[5)]. In the CT system, the target can be readily exchanged. Therefore, the image with excellent contrast could be obtained by using an appropriate target according to the object. Figure 1 shows a 3D image of an ant's head acquired by using this CT system. These images were obtained by Ti-X-rays. A strong intensity which corresponds to the Gnathic Glandular could be seen. It is suspected that the Gnathic Glandular contains heavier elements than the other organs. In the biological studies, it is preferable to know elements that are contained in the organs.

In this study, we applied the dual-energy subtraction imaging method[6-8)] to the 3D micron-CT for *in-vivo* elemental imaging. Since the 3D micron-CT can easily change the X-ray energy by changing the X-ray target, the dual-energy subtraction method is suitable for elemental imaging in the system.

The dual-energy subtraction method is one of the tools which enhance the contrast of the specific elements. The method is based on the sharp change of attenuation coefficient at K-absorption edge of contrast media, which is applied to the X-ray radiography in a human angiography by adding iodinated contrast media. The method uses two different energy X-rays of higher and lower energies of I-K-absorption edge and subtract image obtained by the high energy from the one by the lower energy. Thus the subtract image shows enhanced image of iodine[6,7].

We do not use a contrast media for elemental imaging. By changing the X-ray targets, we obtained projection data corresponding to the X-ray energy. By subtracting the image from the other image obtained by an adjoining X-ray energy, we can obtain elemental image corresponding to the absorption edges. We apply the dual-energy subtraction method for elemental imaging of the Gnathic Glandular, where Mn is known to be accumulated. Figure 2 shows the schema of dual energy subtraction method.

Experiment & Result

The 3D micron-CT comprises a high-speed X-ray CCD camera, a monochromatic X-ray point source and a rotating sample stage[3]. In our system, the X-ray point source and the CCD camera are fixed and the sample is rotated by the stage to acquire the projection data. A monochromatic X-ray is produced by proton microbeam bombardment on the pure metal target. The microbeam system was designed to achieve sub-micrometer beam spot sizes and was developed in collaboration with Tokin Machinery Corp[9]. The system is connected to the 4.5 MV Dynamitron accelerator at Tohoku University. The demagnification factors are 9.2 and 35.4 for horizontal and vertical directions, respectively. A beam spot size of $0.4\times0.4\ \mu m^2$ was obtained using a beam current of several tens of pA. In this study, we adjust the beam spot size to $1.5\times1.5\ \mu m^2$ in order to increase X-ray intensity[2,9]. Beam currents were around 200 pA. An X-ray producing target is set at 30 degrees with respect to the horizontal axis and produces the X-ray cone beam by microbeam bombardment.

A sample was encapsulated in a polycarbonate tube with micrometer dimension (inside diameter of 1000 μm and wall thickness of 25 μm). Because X-ray attenuation in the tube is low and clear projection images could be taken. Since composition of the tube is homogeneous contrary to the majority of biological materials, the tube can be used as a reference in the reconstruction procedure. Inside of the tube was kept in atmospheric pressure. Therefore, we can acquire the CT image *in-vivo*. During acquiring the data of living sample, the sample is encapsulated after anaesthetization. The anaesthetized ant lives around two hours[3]. To reduce the ambiguity of obtained data which depends on the ant's condition, we used the ant which was fixed by formalin. In this application it took more than two hours to obtain the images for two or more X-ray energies and it was difficult to keep an ant living for more than two hours.

A high-speed X-ray CCD camera (Hamamatsu, C8800X) was used to detect transmitted X-rays. The pixel size of the CCD is $8\times8\mu m^2$ and the number of pixels is 1000x1000. The CCD is cooled to –50°C by a Peltier cooler to suppress dark currents. The CCD starts to exposure by an outside trigger and it stops when the beam charge has been accumulated to a constant value; readout of data finishes while the sample rotates. A 100 μm Mylar film is placed in front to prevent recoil protons into the CCD[1].

The spatial resolution of this CT system depends strongly on the geometrical condition such as X-ray source to sample and sample to the CCD distances. We set the source-to-sample distance was set to 2100 μm and the sample-to-the CCD was set to 3900 μm. In this condition, the spatial resolution was estimated to be 6 μm[4].

The micron-CT changes the X-ray energy by changing the metal targets. Figure 3 shows the transmittance of X-ray through water and the measured energy spectra of X-rays, which were detected by a Si(Li)-detector for comparison. Transmittance of X-ray for energies higher than 10 keV is too high and not appropriate for biological application. Considering the detection efficiency of the CCD, we use Ti, Fe and Co K-X-rays.

To determine the elements that are contained in the Gnathic Glandular, the dual energy subtraction method was applied. After obtaining the 3D images for Ti-, Fe- and Co-K-X-rays, the CT values are compared. The ML-EM reconstruction method was used for 3D imaging to reduce the background. However, Fe- and Co-K-X-rays are not appropriate for imaging since transmittance is too high compared to Ti-K-X-ray imaging for a small object. Figure 4 shows the cross sectional views obtained by Fe- and Co-K-X-rays. Co-K-X-rays are strongly absorbed in the Gnathic Glandular.

In applying the dual energy subtraction method using these two different data (i.e. Fe and Co), we normalize the data by using the calculated CT values of polycarbonate tube. Because the X-ray energy of Fe and Co are close, the tube's CT values of Fe and Co are almost equivalent. By this normalization, we could observe the distribution of Mn with less interference of other organs. Figure 4 shows the result of dual energy subtraction using 3D data set. The distribution of Mn clearly observable and we could confirm that the position in which Mn has concentrated corresponds to the position of Gnathic Glandular in the ant's head.

Conclusions

We have developed the micron-CT using micro-PIXE for *in-vivo* imaging. The ion microbeam system is used as a monochromatic m-X-ray source by bombarding a pure metal target. The sample was placed in a tube of a small diameter, and rotated by a stepping motor. The 3D images were reconstructed from the obtained projection data by using cone-beam CT reconstruction algorithm. X-ray spectra produced by heavy charged particle bombardment exhibits a much smaller continuous background compared to that of electron bombardment. Therefore, X-rays produced by ion beam can be used as a monochromatic and low energy X-ray source. The feature is very effective to investigate small insects in micrometer dimension. Moreover we can get elemental distribution image of object by choosing appropriate characteristic X-rays corresponding to the absorption edge. Using this system, we were able to get 3D images of a living ant's head with 6 μm spatial resolution. The Dual energy subtraction method was applied for 3D elemental analysis. By using Fe-K-X-rays (6.40 keV) and Co-K-X-rays (6.93 keV), we can investigate the 3D distribution of Mn (K-absorption edge=6.54 keV) in an ant's head. The 3D micron-CT is useful for 3D elemental imaging of small insect *in-vivo* as well as electron density imaging and will be a powerful tool for biological studies.

Acknowledgements

This study was supported by 21 COE Program "Future Medical Engineering based on Bio-nanotechnology" and a Grant-in-Aid for Scientific Research (S) No. 13852017 (K. Ishii) of the Ministry of Education, Culture, Science, Sports and Technology.

References

1) Ishii K., Morita S., *Int. J. PIXE*, **1** (1990) 1.
2) Ishii K., Matsuyama S., Yamazaki H., Watanabe Y., Yamaguchi T., Momose G., Amarttaivan Ts., Suzuki A., Kikuchi Y., Galster W., *Int. J. PIXE*, **15** (2005) 111.
3) Ishii K., Matsuyama S., Watanabe Y., Kawamura Y., Yamaguchi T., Oyama R., Momose G., Ishizaki A., Yamazaki H., Kikuchi Y., *Nucl. Instr. and Meth.*. **A571** (2007) 64.
4) Ishii K., Matsuyama S., Yamazaki H., Watanabe Y., Kawamura Y., Yamaguchi T., Momose G., Kikuchi Y., Terakawa A., Galster W., *Nucl. Instr. and Meth.*. **B249** (2006) 726.

5) Yamaguchi T., Ishii K., Yamazaki H., Matsuyama S., Watanabe Y., Abe S., Inomata M., Ishizaki A., Oyama O., Kawamura Y., *Int. J. PIXE*, **15** (2005) 195.
6) Oguri Y., Hasegawa J., Ogawa M., *Proceedings of 23rd PIXE Symposium*
7) Umetani K., Ueki H., Ueda K., Hirai T., Takeda T., Doi T., Wu J., Itai Y., Akisada M., *J. Synchrotron Rad.* **3** (1996) 136.
8) Rubenstein E., Hughes E.B., Campbell L.E., Hostadter R., Kirk R.L., Krolicki T.J., Stone J.P., Wilson S., Zeman H.D., Brody W.R., Macovski A., Thompson A.C., *SPIE.* **314** (1981) 42.
9) Matsuyama S., Ishii K., Yamazaki H., Sakamoto R., Fujisawa M., Amarttaivan Ts., Oishi Y., Rodriguez M., Suzuki A., Kamiya T., Oikawa M., Arakawa K.,Matsumoto N., *Nucl. Instr. and Meth.*. **B210** (2003) 59.

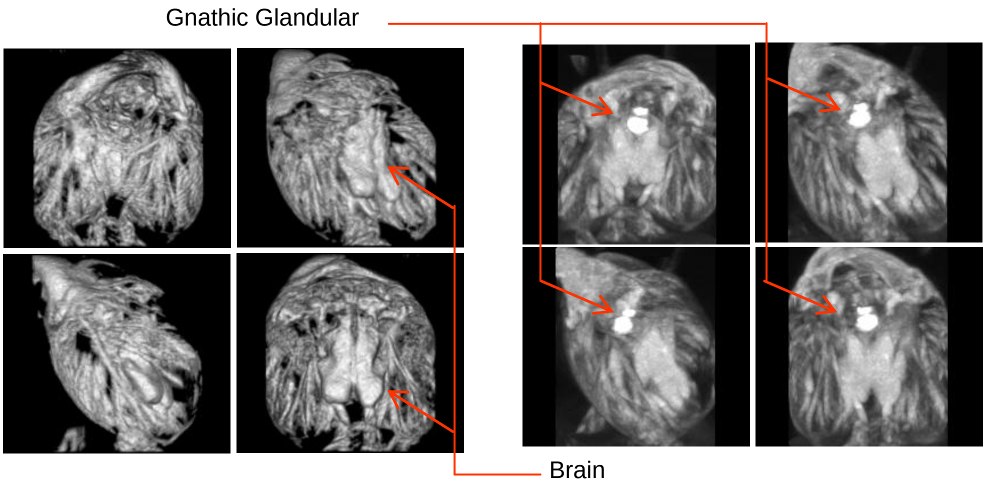

Figure 1. 3D images of ant's head (Volume rendering: left, MIP : right).

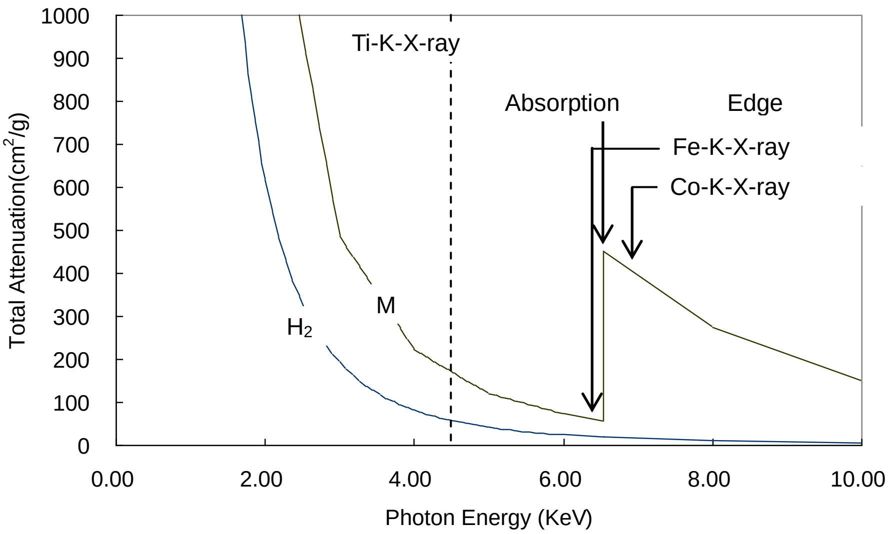

Figure 2. Dual Energy subtraction method.

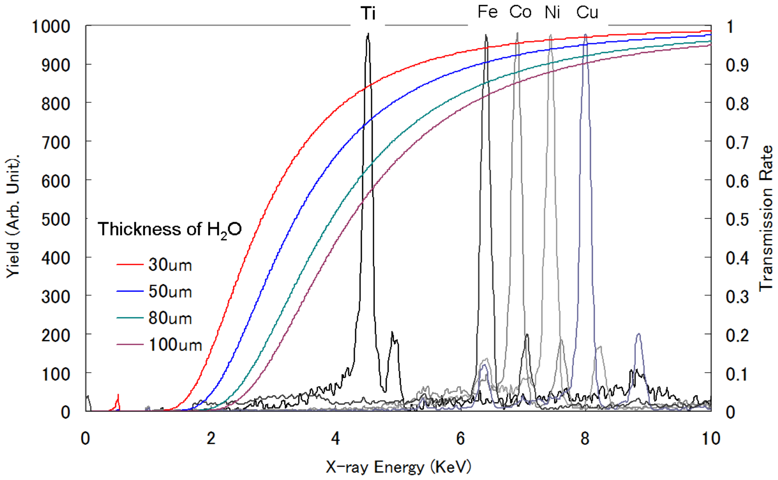

Figure 3. Transmittance of X-ray through water.

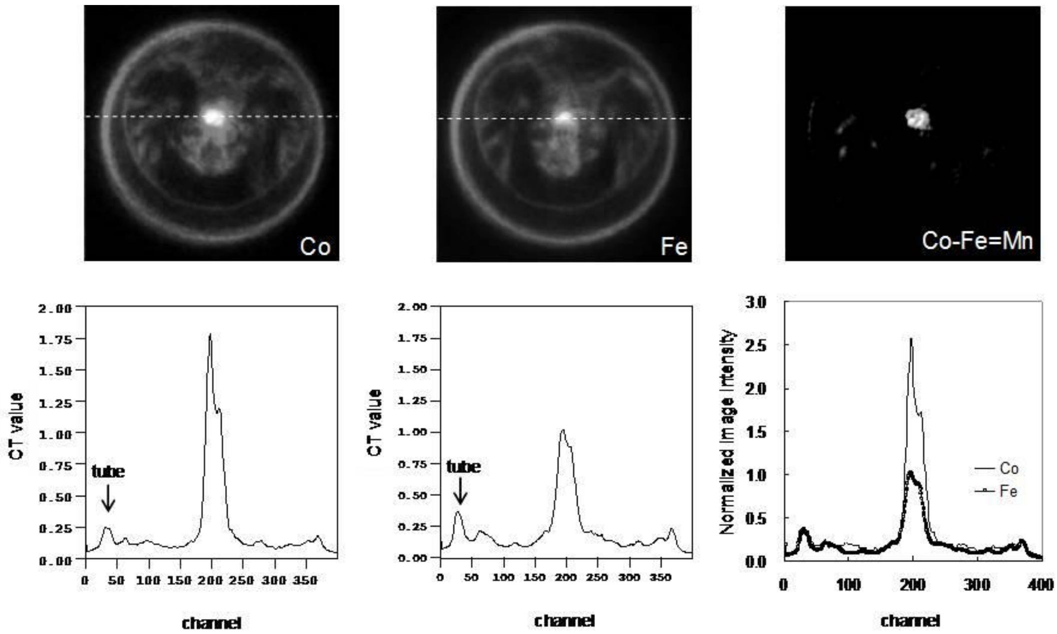

Figure 4. CT images of Gnathic Glandular and line profile of CT value along red broken line.

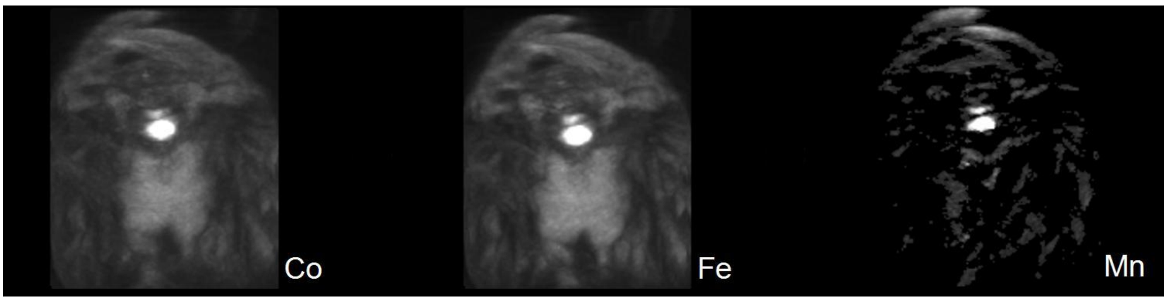

Figure 5. Dual Energy subtraction 3D MIP image (3D mapping of Mn).