Penetration Monitoring of Fixation Solution into Tissues Using Millimeter Waves

Maya Mizuno, Kaori Fukunaga, and Iwao Hosako
National Institute of Information and Communications technology, Koganei, Tokyo 184-8795 Japan

Abstract— **We developed a system for characterizing the dielectric properties of various materials by transmission imaging using wavelengths in the millimeter range from 25 GHz to 65 GHz. By using this system, we confirmed that the difference in the transmission between commercial pork containing only water and pork containing ethanol was significant and that we could monitor and image the penetration of ethanol into pork. These experiments suggest the possibility of applying millimeter waves to the inspection of tissue specimens in medicine and biology.**

I. Introduction and Background

Tissue specimens are used in pathological diagnosis and medicinal and biological studies. In the fixation protocol, the penetration of fixation solutions such as ethanol is important in determining how well cell shape and biological information are maintained [1]. However, the time required for complete penetration must be determined experimentally for each tissue. Therefore, an easy, universal method of imaging the penetration is necessary to verify fixation inside of the sample. This method is also useful in the cryopreservation of tissues [2]. In the case of imaging living tissues, which have various dielectric properties, the frequencies suitable for distinguishing different substances may vary from tissue to tissue. For imaging tissues at suitable frequencies under every condition, a frequency-tunable imaging system is required. Given these characteristics and requirements, our research group constructed a frequency-tunable millimeter-wave imaging system that covers the frequency range from 25 GHz to 65 GHz. In this paper, we describe the constitution of the developed millimeter-wave transmission imaging system and report on the results of discrimination between pork with and without ethanol.

II. Millimeter-Wave Imaging System

Figure 1 shows a schematic diagram of the millimeter-wave imaging system. This imaging system used a network analyzer (Agilent E8363B, 10 MHz–70 GHz) as the generator and detector. A coaxial cable with a 2.4-mm male connector (Agilent 85133E) was attached to the network analyzer, and a semispherical silicon lens (refractive index: 3.4; diameter: 30 mm) with a focal length of 6.3 mm was used to limit the spread of the millimeter waves irradiated onto the sample. The distance between the connector and the lens was 8 mm, and a wire grid was placed in this space to obtain linear polarization. We measured the near-field pattern of the beam at 25, 35, 45, 55, and 65 GHz at a position approximately 1 mm from the end face of the semispherical silicon lens (Figure 2). We confirmed that the beam emitted from the 2.4-mm connector (inner diameter: ~6.2 mm, length of center conductor: 1.4 mm) had a circular interference pattern. The diameters of the zero-order diffraction waves were estimated at 9, 7.8, 12.2, 7.2, and 8 mm for transmission imaging frequencies of 25, 35, 45, 55, and 65 GHz, respectively. As shown in Figure 2, the dark zones of the interference pattern were not clear for the 45-GHz beam; hence the diameter of the zero-order diffraction wave of that beam may have been overestimated. By using the lens effect, the size of each circular interference pattern could be maintained constant until approximately 10 mm from the end face of the semispherical silicon lens. On the detector side, only the zero-order diffraction waves of those transmitted through the sample were collected by using another semispherical silicon lens; the signals were then input to the network analyzer. Here, the 2.4-mm male connector of the coaxial cable was placed 1 mm from the silicon semispherical lens. In this study, the sample was scanned three-dimensionally using an XYZ stage; the transmittance data were then processed by a PC to produce the final two-dimensional (XZ) image. In the Y direction, reflected wave from the sample surface interfered with waves from the transmitter, so an average of the wave was used to produce the image and remove the effects of the interference.

Since the sample was irradiated at a power of less than 300 nW, the thermal effects in the sample were ignored. The signal-to-noise (S/N) ratio of the transmittance measurement was approximately 10 dB, and the intensity fluctuation was less than 0.4 dB at approximately 20 percent of maximum power. It is possible to increase the S/N ratio (imaging sensitivity) by narrowing the IF bandwidth of the network analyzer. However, we confirmed that to achieve this, the sweep time must be increased; this also results in an increase in the time required to acquire the data.

III. Penetration Monitoring

We prepared a 1-mm-thick sample of commercial pork and measured its transmittance after ethanol was allowed to penetrate into it for different lengths of time at 25 ºC (Figure 3). We confirmed by spectroscopy that there was a difference between the transmittance of the pork containing a large amount of water and that of the pork containing 99.5 % ethanol;

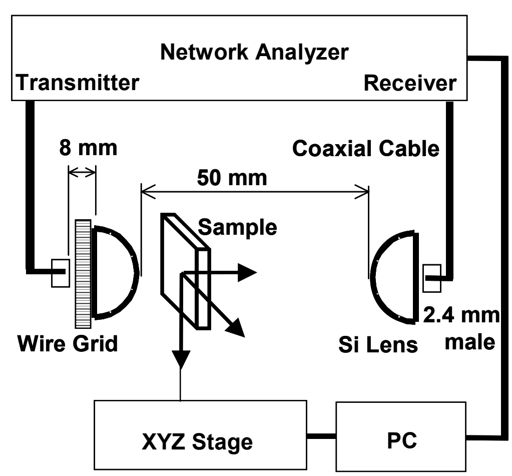

Fig. 1 Experimental setup for millimeter-wave imaging.

we also confirmed that transmittance increased as a result of ethanol penetration into the pork. Here, a smoothing of nine experimental points was applied. This result shows that we can discriminate between normal pork and pork containing ethanol on the basis of transmittance; It also shows that we can effectively discriminate between them at higher frequencies. By using this transmittance property, we attempted to monitor the ethanol penetration. We acquired transmittance images of the pork sample at penetration times of 0, 90, and 1140 minutes, and produced the corresponding differential images by using a PC (Figure 4). We confirmed that we could distinguish the samples at 25 GHz, 35 GHz, 45 GHz, 55 GHz, and 65 GHz and that we could discriminate between the penetration times of ethanol into the pork at each frequency. Moreover, we verified that the image at 65 GHz was clearest among all the images because of the water content. Figure 5 shows differential images at 65 GHz for various penetration times. This confirms that the difference in transmittance depends on penetration time; we can also see that the ethanol penetrated from the outer layers of the pork to the inner layers. At 1140 minutes, which is enough time for complete penetration, the transmittance difference outside the pork was lowest due to coupling loop interference. This experimental results show that it is possible to verify whether ethanol completely penetrates into the tissue by monitoring the increase in the transmittance at the center of the tissue.

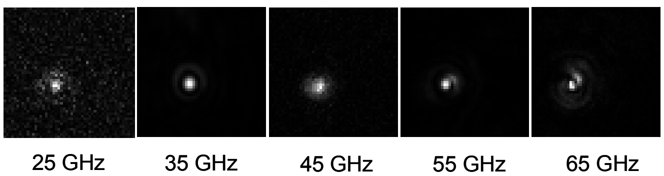

Fig. 2 Millimeter-wave beam pattern irradiated on the sample.

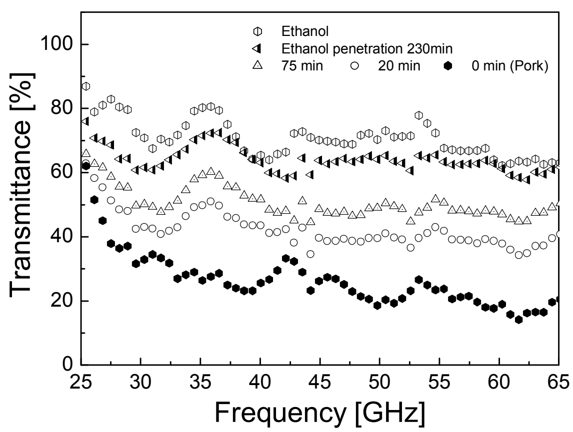

Fig. 3 Spectra of ethanol and of pork with ethanol.

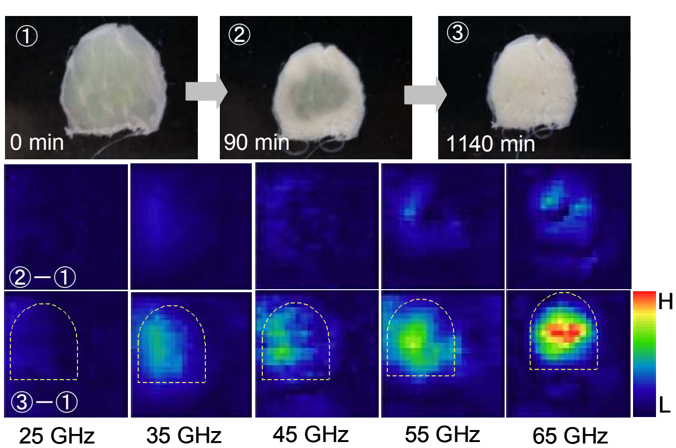

Fig. 4 Images and results of differential imaging of pork with ethanol at 25 GHz, 35 GHz, 45 GHz, 55 GHz, and 65 GHz.

IV. CONCLUSION

We developed an imaging system for clarifying the dielectric properties of various materials using transmission imaging in the millimeter range from 25 GHz to 65 GHz. This system enabled us to discriminate among degrees of ethanol penetration into pork at various frequencies. Our experiment demonstrated that it is possible to monitor ethanol penetration in tissue specimens using millimeter waves.

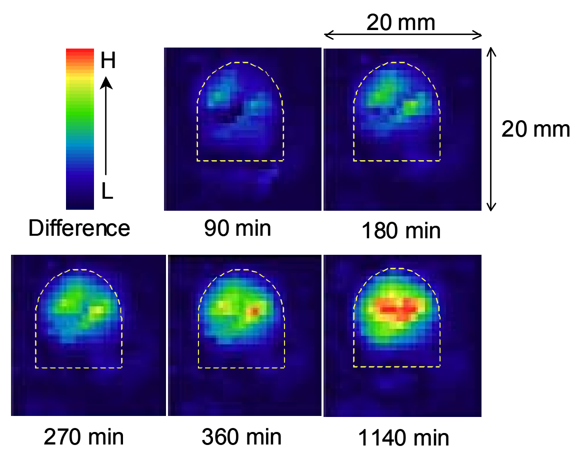

Fig. 5 Results of differential imaging at 65 GHz of pork containing ethanol.

REFERENCES

[1] Sainte-Marie G., "A paraffin embedding technique for studies employing immunofluorescence," J. Histochem. Cytochem. vol. 10, 1962, pp. 250–256.

[2] Stephanie A. Isbell, Colin A. Fyfe, Richard L. M. Ammons, and Betty Pearson, "Measurement of cryoprotective solvent penetration into intact organ tissues using high-field NMR microimaging," Cryobiology vol. 35, 1997, pp. 165–172.

978-1-4244-2120-6/08/$25.00©IEEE.