An Approach to Identify Circulating Tumor Cell Using Ring Resonator Type of Electrode Using Oscillation Technique at Centimeter Frequency Bands

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SUMMARY   A ring-resonator type of electrode (RRTE) has been proposed to detect the circulating tumor cell (CTC) for evaluation of the current cancer progression and malignancy in clinical applications. Main emphasis is placed on the identification sensitivity for the lossy materials that can be found in biomedical fields. At first, the possibility of the CTC detection was numerically considered to calculate the resonant frequency of the RRTE catching the CTC, and it was evident that the RRTE with the cell has the resonant frequency inherent in the cell featured by its complex permittivity. To confirm the numerical consideration, the BaTiO3 particle, whose size was similar to that of the CTC, was inserted in the RRTE instead of the CTC as a preliminary experiment. Next, the resonant frequencies of the RRTE with internal organs of the beef cattle such as liver, lung, and kidney were measured for evaluation of the lossy materials such as the CTC, and degraded Q curves were observed because the Q-factors inherent in the internal organs were usually low due to the poor loss tangents. To overcome such difficulty, the RRTE, the oscillator circuit consisting of the FET being added, was proposed to improve the identification sensitivity. Comparing the identification sensitivity of the conventional RRTE, it has been improved because the oscillation frequency spectrum inherent in an internal organ could be easily observed thanks to the oscillation condition with negative resistance. Thus, the validity of the proposed technique has been confirmed.
key words: circulating tumor cell, resonant frequency, ring-resonator, primary tumor, oscillator

1.   Introduction

Since the early detection of cancer is important to improve the survival rate, identification of circulating tumor cell, commonly called CTC, has attracted much attention in recent medical fields. The CTC is a kind of cancer cell infiltrating and flowing in blood from the primary and metastatic tumors [1] and the current cancer progression and malignancy can be evaluated to observe the condition of the CTCs. Furthermore, the identification of CTCs originating in the primary tumors of some internal organs is also important because malignant neoplasms from such internal organs as the primary tumors increase the death rate. If we can recognize which CTCs come from which primary tumors, an anticancer drug whose therapeutic effect is high can be provided to cancer patients. However, the detection and evalu-

ation for the CTCs are difficult because the numbers of the CTCs are extremely less comparing with other cells flowing in the blood, and thus, the high technique is necessary.

The Cell Search System (Veridex) is the only detection device approved by FDA (Food and Drug Administration) in the world and is possible to automatically detect the CTCs. It has been however reported that the detection accuracies are at around 70% for the CTC delivered from lung cancer and at 30% for that delivered from the mesothelial tumor, respectively [2], [3].

As another candidate of the CTC detection device, the ring-resonator type of electrode (RRTE) is proposed based on measuring the resonant frequency featured by the complex permittivity of the CTC in this paper.

At first, the possibility of the CTC detection was numerically considered to calculate the resonant frequency of the RRTE catching the CTC, and it was revealed that the RRTE with the cell had the resonant frequency inherent in the cell. To confirm this result, the BaTiO3 particle, whose size was similar to that of the CTC, was inserted in the RRTE instead of the CTC as a preliminary experiment because our laboratory could not get the genuine CTC by the regulation in our institute.

Next, the resonant frequencies of the RRTE with internal organs of the beef cattle such as liver, lung, and kidney were measured for evaluation of the lossy materials such as the CTC. The degraded Q curves were observed because the Q-factors of the internal organs were usually low due to the poor loss tangents.

To overcome such difficulty, the RRTE, the oscillator circuit consisting of the FET being mounted, was proposed to improve the identification sensitivity. Comparing the identification sensitivity of the RRTE without the oscillator circuit, it has been clearly improved because the oscillation frequency spectrums inherent in the internal organs could be easily observed thanks to the oscillation condition with negative resistance. The details are as follows.

2.   Circulating Tumor Cell Detection System

In our groups, the CTC detection system is currently being developed as shown in Fig. 1. After a blood sample is collected, the suspected CTCs together with the leucocytes are separated from the blood by using the micro hole because the diameters of the CTC and leucocyte to be several ten mi-
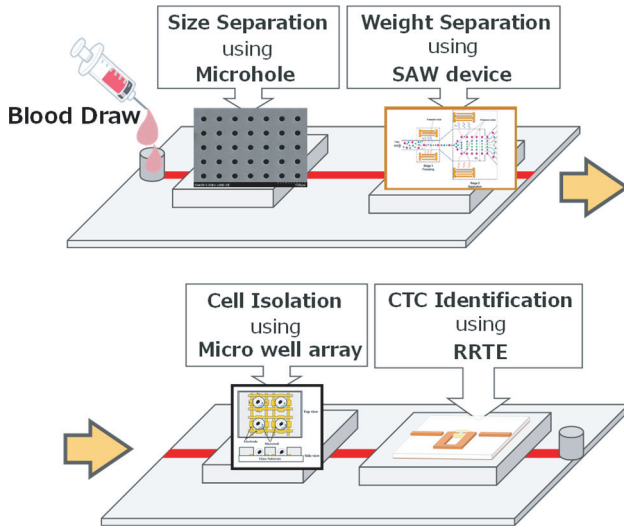
Fig. 1　Schematic view of CTC detection system.

crometer are larger than that of the hematocyte. Secondly, the CTC is separated by using the SAW electrode in saline solution [4] because the weight of the CTC is higher than that of the leucocyte. Since the leucocytes may admix in saline solution, the cells inserted in the microwell array are observed by using the dynamic imaging analysis of rotation speed based on dielectric constant of the cell for electrorotation [5], [6]. Finally, the CTCs are identified by measuring the resonant frequency using the RRTE to increase the sensitivity of our CTCs detection systems. The details of the RRTE are shown as the following subsections.
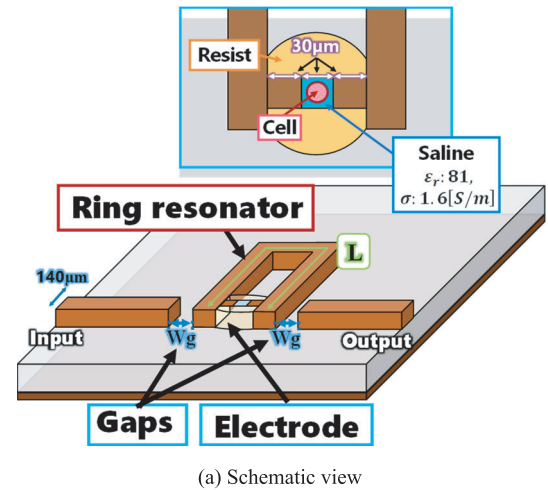
2.1　Structure of Ring-Resonator Type of Electrode

The structure of the RRTE made by the microstrip transmission line is shown in Fig. 2 (a). This is classified into two parts. One is the parallel electrodes, and another is the ring-resonator part. The distance between the parallel electrodes and the width was set at 30 μm assuming the size of CTCs, and the electrodes were surround by the resist to confine a cell in the electrodes. The saline solution is filled between the parallel electrodes as similar material as the blood. The electrode is connected with the ring-resonator [7]. Furthermore, the gaps with a length of Wg are installed between the RRTE and the input and output ports to increase the Q-factor.

　Figure 2 (b) shows the equivalent circuit, where the electrode and gap can be represented by symmetrical T networks consisting of series and parallel capacitive elements and the ring-resonator can be represented by a transmission line having a length L and an effective relative permittivity ε_e of the microstrip transmission line. From the equivalent circuit, the resonant condition of the RRTE is given by

$$\sqrt{\varepsilon_e}k_0 L + \theta_E = 2N\pi \quad (N = 1, 2, 3 \dots) \tag{1}$$

where k_0 and θ_E are the free space wave number and the phase angle of the electrodes. If the cell is inserted in the

(a) Schematic view
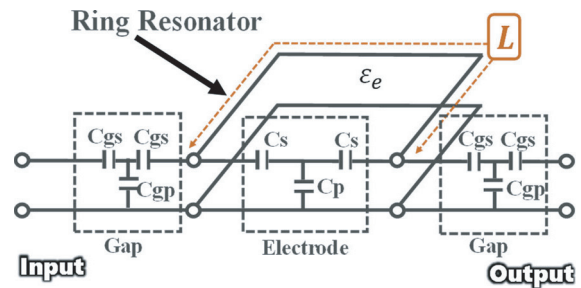
(b) Equivalent circuit

Fig. 2　Structure of ring-resonator type of electrode.

Table 1　Complex permittivity for each cell.

Type of cell	Relative permittivity	Conductivity
T-lymphocyte	103.9	0.65 S/m
B-lymphocyte	154.4	0.73 S/m

electrodes, the series and parallel capacitive elements will be changed, that is to say, θ_E will be changed because the cell has an inherent complex permittivity. Thus, the resonant frequency of the RRTE will be changed by inserting a cell.

2.2　Calculated Frequency Characteristics

The frequency characteristics of the RRTE were simulated in HFSS software when the inserting cell was assumed as a single cell model [8] as the simple model where the dielectric constants of the inserting cell were assumed as those of the B- and T-lymphocytes [9] because the dielectric constant of the CTC was unknown at that time. Table 1 shows the complex permittivities of the B- and T-lymphocytes used this calculation.

　The diameter Φ of each cell was set to be 20 or 30 μm to evaluate the variation of the resonant frequency by the difference of the cell size. Table 2 shows the calculated resonant frequencies in case of inserting the cell having each size in the RRTE, where the whole length of the RRTE was designed to be 8.5 mm. Figure 3 shows the calculated trans-

Table 2 Calculated resonant frequencies in case of inserting a cell having diameter of 20 or 30 μm in RRTE.

|	Resonant frequency [GHz]	
	$\Phi = 20\ \mu$m	$\Phi = 30\ \mu$m
No cell	8.95	
T-lymphocyte	8.92	8.86
B-lymphocyte	8.88	8.71
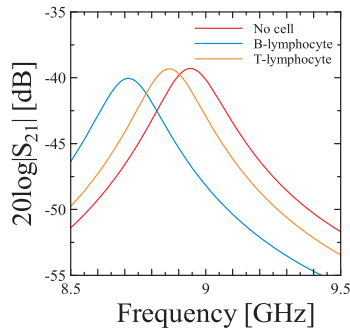
Fig. 3 Calculated S21 versus frequency.
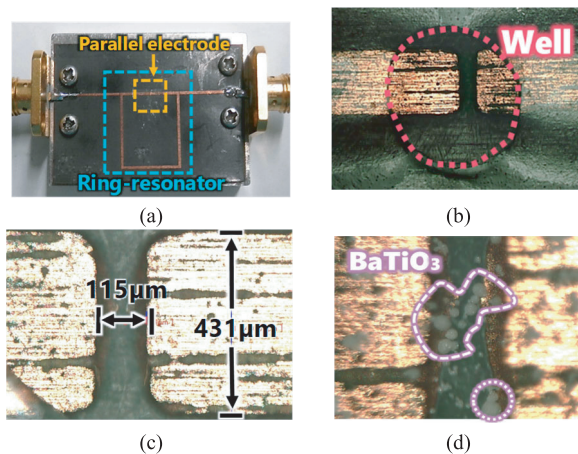
Fig. 4 Photograph of RRTE: (a) whole view, (b) outside of well inserting BiTio$_3$ in saline solution, (c) dimension of parallel electrode, (d) distribution of BaTio$_3$ particles.

mission coefficients versus frequency when the diameter of the cell was set at 30 μm. The calculated results indicate that the resonant frequencies are changed by the different cells.

2.3 Preliminary Experiment Using BaTiO$_3$ Particles

Although the size of the RRTE should be designed to be micrometer-scale, the RRTE was fabricated in several hundred meter scale by using the photolithography as the early investigation by using the Duroid substrate whose relative permittivity and thickness of copper are 2.2 and 36 μm, respectively. Figure 4 shows the fabricated RRTE whose whole length was designed at 45 mm so as to resonate at around 5GHz because of easy observation of the frequency characteristics from a viewpoint of the dynamic range in our equipment. The measured frequency characteristics are shown in Fig. 5, where the parallel electrodes had three con-
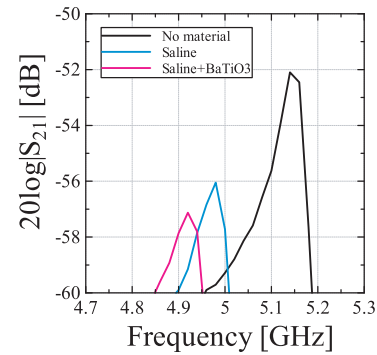
Fig. 5 Measured S21 versus frequency.

ditions. There was no material in the parallel electrodes. The saline solution was only dropped in those using the micropipette, and the barium titanate (BaTiO$_3$) particles were inserted in the saline solution. The reasons why the BaTiO$_3$ particles were selected were that the size was similar to that of the cell and the handling was easy in the experiment. It was confirmed that the resonant frequency was changed when the BaTiO$_3$ particles were inserted in the electrode.

3. Identification Sensitivity Enhancement Using Oscillator Circuit

3.1 Resonant Characteristics of Ring-Resonator Type of Electrode Using Cattle Organ Tissues

Since it is predicted that the loss tangent of the CTC may be poor [10], the resonant curves of the RRTE were observed by using the internal organs of the beef cattle such as liver, lung, and kidney to know how much identification sensitivity will degrade. To investigate the individual difference of the organ and to confirm the reliability in this experiment, the same experiments were repeated at 5 times in each tissue.

Figures 7 and 8 show the measured frequency response for each tissue and the distributions of measured resonant frequencies versus organ tissue, respectively. In Fig. 8, the maximum displacement rate of the resonant frequency was about 2%. Focusing on the resonant curve, the high Q-factor is needed to well identify the organ tissue because observation of the resonant frequencies among the frequency responses were difficult because of high losses of the beef cattle organs.

3.2 Ring-Resonator Type of Electrode with Oscillator Circuit

Because identifying the organ tissue was difficult, the oscillator circuit consisting of the FET including a capacitance as the feedback circuit was introduced in the RRTE. Figure 9 (a) shows the equivalent circuit of the RRTE connected with the oscillator circuit via transmission lines with a length of l. Figure 9 (b) shows the fabricated RRTE. The
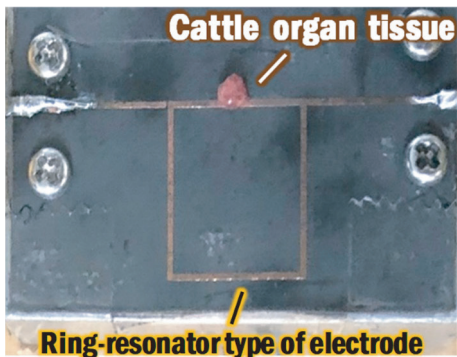
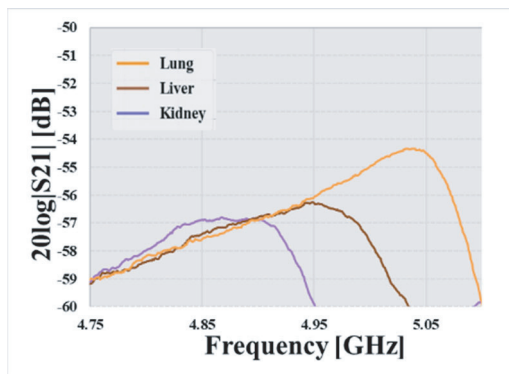**Fig. 6** Cattle organ tissue on RRTE.
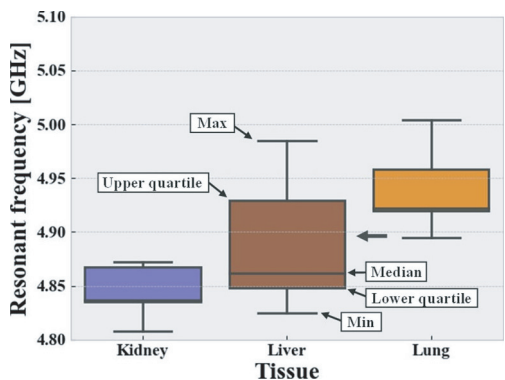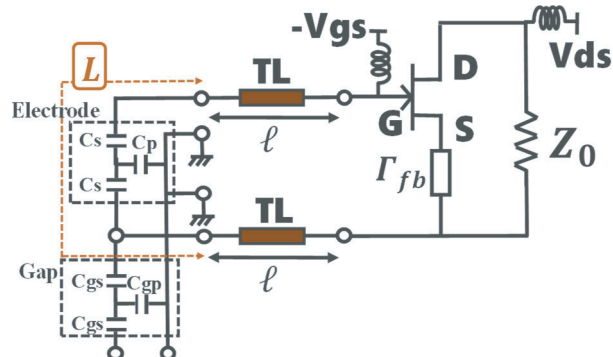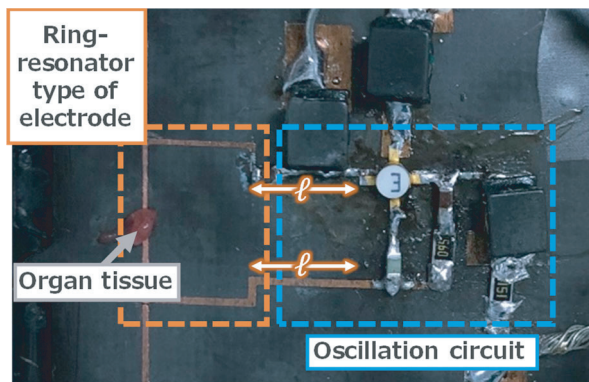

Fig. 7 Measured S21 versus frequency.

Fig. 8 Distributions of measured resonant frequencies versus organ tissues.

(a) Equivalent circuit.

(b) Photograph.

Fig. 9 RRTE connecting oscillator circuit.

Fig. 10 Measured oscillation frequency spectrums.

oscillation frequency is decided by whole length of the ring-resonator and the complex permittivity of the organ tissues. The dissipation loss of the organ tissue will be compensated by the oscillator circuit because the oscillator acts in the negative resistance condition [11]–[13], and thus, well-identification of the organ can be expected by observing the oscillation frequency spectrums. The oscillator circuit was constructed by the FET(NE76084) with the capacitance (C=3pF) in the source terminal. To investigate the individual difference of the organ and to confirm the reliability in this experiment, the same experiments were also repeated 5 times for each tissue, where the gate to source voltage and

drain to source voltage were fixed to be −0.5V and 4.0V, respectively. Figures 10 and 11 show the measured oscillation frequency spectrums in each beef cattle tissue and the distributions of the measured oscillation frequencies versus organ tissues.

It was confirmed that the oscillation frequency spectrums were clearly separated in each tissue and the maximum displacement rate of the oscillation frequency was about 1.1%. The distribution of the oscillation frequency for the organ tissue of the kidney was close to that of the liver. The reason is considered that the relative permittivity of the kidney is close to that of the liver in this frequency band [10].
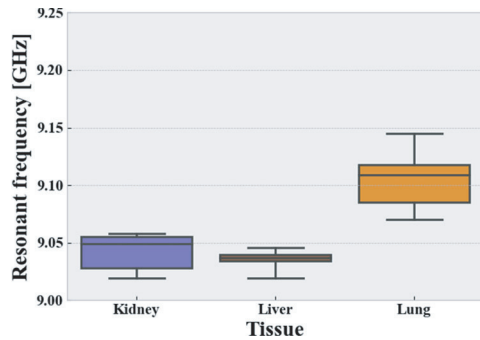
Fig. 11 Distribution of measured oscillation frequency versus organ tissue.

From these results, the identification sensitivity enhancement can be performed by using the RRTE connecting the oscillation circuit.

4. Conclusions

A ring-resonator type of electrode (RRTE) was proposed to detect the circulating tumor cell (CTC) for evaluation of the current cancer progression and malignancy in clinical applications.

At first, the possibility of the CTC detection was numerically considered to calculate the resonant frequency of the RRTE catching the CTC, and it was evident that the RRTE with the cell has the resonant frequency inherent in the cell featured by its complex permittivity. To confirm the numerical consideration, the BaTiO3 particle, whose size was similar to that of the CTC, was inserted in the RRTE instead of the CTC as a preliminary experiment.

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To overcome such difficulty, the RRTE, the oscillator circuit consisting of the FET being added, was proposed to improve the identification sensitivity. Comparing the identification sensitivity of the conventional RRTE, it has been improved because the oscillation frequency spectrum inherent in an internal organ could be easily observed thanks to the oscillation condition with negative resistance. Thus, the validity of the proposed technique has been confirmed.

The next step of this research will be to investigate the RRTE having genuine CTC in saline solution in the medical fields.

Acknowledgments

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