Sensing viruses using terahertz nano-gap metamaterials

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Abstract: We demonstrate highly sensitive detection of viruses using terahertz split-ring resonators with various capacitive gap widths. Two types of viruses, with sizes ranging from 60 nm (PRD1) to 30 nm (MS2), were detected at low densities on the metamaterial surface. The dielectric constants of the virus layers in the THz frequency range were first measured using thick films, and the large values found identified them as efficient target substances for dielectric sensing. We observed the resonance-frequency shift of the THz metamaterial following deposition of the viruses on the surface at low-density. The resonance shift was higher for the MS2 virus, which has a relatively large dielectric constant. The frequency shift increases with surface density until saturation and the sensitivity is then obtained from the initial slope. Significantly, the sensitivity increases by about 13 times as the gap width in the metamaterials is decreased from 3 µm to 200 nm. This results from a combination of size-related factors, leading to field enhancement accompanying strong field localization.

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OCIS codes: (160.3918) Metamaterials; (300.6495) Spectroscopy, terahertz; (050.6624) Subwavelength structures; (170.0170) Medical optics and biotechnology.

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https://doi.org/10.1364/BOE.8.003551
Received 19 May 2017; revised 3 Jul 2017; accepted 3 Jul 2017; published 7 Jul 2017
Detection and identification of viruses are of intense interest because of their relation to serious diseases such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), influenza A (virus subtype H1N1), and foodborne illness [1]. In the past few decades, various virus detection techniques including the polymerase chain reaction (PCR) and the branched-chain DNA (bDNA) test have been widely adopted [2–4]. The PCR method is commonly used because it enables accurate identification through the amplification of target substances, but its detection speed is limited [5]. The bDNA test is a method for detecting DNA or RNA using branched-chain DNA [4]; it has the advantage of being time-consuming and labor-intensive; therefore, an alternative is sought that enables fast, in situ, and sensitive detection of nanoscale substances.

Terahertz spectroscopy has been adopted in recent decades for the fast detection and identification of biological samples because it enables label-free, non-contact, and non-destructive sensing [6–9]. However, it is difficult to detect microorganisms such as fungi, bacteria, and viruses because their scattering cross-sections are much smaller than THz wavelengths (\( \lambda = 300 \mu m \) at 1 THz) [10]. Recently, we demonstrated the sensing of microorganisms such as bacteria and fungi using THz metamaterials [10,11]. This is possible because the LC resonant frequency \( f_0 \) of the metamaterials is strongly dependent on the presence of dielectric in capacitive gaps in the structure, and a shift \( \Delta f \) of the resonant frequency occurs when the dielectric constant changes [10–21]. We were able to detect the

\[ \Delta \]
target material with unprecedented sensitivity and could count the number of microorganisms located in the gap area using a tightly defined detection volume of metamaterials. However, current investigations on sensing with THz metamaterials have been restricted to micro-sized microorganisms (~λ/100) because their size corresponds to the typical metamaterial gap dimension. Therefore, the detection of viruses with a typical size of less than 100 nm is still challenging, and has not been addressed before.

Here, we carried out THz-time domain spectroscopy (TDS) measurements to demonstrate detection of the bacteriophage viruses PRD1 (60 nm) and MS2 (30 nm), which are representative double-stranded DNA and single-strand RNA viruses, respectively. Their sizes range from λ/5,000 to λ/10,000 of the incident THz waves. Metamaterials were fabricated with micro- and nano-gaps, and the resonance shifts of the metamaterials were observed as a function of virus surface densities. In addition, we studied the sensitivity of the metamaterials as a function of gap width in order to optimize the detection sensitivity by exploiting size matching.

2. Methods

2.1. Fabrication of metamaterial patterns

Metamaterial patterns for sensing low-density viruses were prepared by e-beam lithography on a quartz substrate of thickness 1 mm. Cr (3 nm) and Au (97 nm) metal films were deposited by e-beam evaporation to define arrays of electrical split-ring resonators with a line width of 4 μm, outer dimensions of 36 μm × 36 μm, and gap sizes of 200 nm, 500 nm, 1 μm, 2 μm, and 3 μm. The periodicity of array patterns was 50 μm. To measure the dielectric constant of a virus layer, we used a different metamaterial device fabricated on a Si substrate (resistivity > 10,000 Ω·cm and thickness 550 μm), employing a conventional photolithography technique, followed by metal evaporation of Cr/Au [22].

2.2. THz-TDS measurements

We used a THz-TDS system to record the transmission of THz electromagnetic waves through the metamaterial sensor [9]. A linearly polarized THz pulse was produced by illuminating a photoconductive antenna with a mode-locked femtosecond laser (λ = 800 nm). The THz beam was focused on an area of ~1 mm² of the metamaterial surface, under ambient conditions. The amplitude and phase of the transmitted THz field in the time domain were obtained by adjusting the time delay between the THz beam and the probe beam. We obtained the THz spectrum in the frequency domain by calculating the fast Fourier transform (FFT) of the transmitted time-domain THz field.

2.3. Preparation of virus sample

Bacteriophages were propagated and assayed in their appropriate hosts by the double agar layer plaque technique, as previously described [23]. Briefly, for each bacteriophage, the top agar layer having a confluent lysis of host cells was harvested by scraping into a small volume of phosphate-buffered saline (PBS), and bacteriophages were extracted with an equal volume of chloroform. The supernatant was recovered by low speed (4,000 x g) centrifugation for 30 min at 4°C and stored at −80°C until use [24].

3. Results and discussion

Figure 1(a) shows a schematic of THz nano-gap metamaterials used for sensing viruses. We measured the change in the THz spectrum transmitted through the micro- and nano-gap metamaterials after deposition of the PRD1 and MS2 viruses. The resonant frequency of THz metamaterial is primarily determined by geometrical factors such as the gap width and sidearm length, and also by the substrate refractive index (n_sub) [11,14]. For LC resonance, the resonant frequency is described by

\[ f_0 = \frac{1}{2\pi\sqrt{LC}} \]

where C is the capacitance of the gap
structure and \( L \) is the inductance of the sidearm structure [25]. To account for the dielectric environment of the nanocircuit, an effective refractive index \( n_{\text{eff}} \) is introduced and the resonant frequency varies inversely with \( n_{\text{eff}} \). Here, \( n_{\text{eff}} \) is a linear combination of the refractive indices of substrate and air [26]. Additional dielectric materials such as viruses produce a change in the effective dielectric constant in the metamaterial gap areas, and a shift \( \Delta f \) occurs in the THz transmission function. A scanning electron microscope (SEM) image in Fig. 1(b) shows THz nano-gap metamaterials with a gap width of 200 nm coated with PRD1 viruses. The viruses were deposited in solution onto the metamaterials surface, followed by drying in an 85°C oven for 5 min.

![Fig. 1. (a) Schematic of THz nano-gap metamaterial sensing of viruses. (b) SEM image of viruses deposited on a THz nano-gap metamaterial sensor with a gap width \( w \) of 200 nm.](image)

Information on the dielectric constant \( (\varepsilon_r) \) of the target substances is crucial in dielectric sensing; however, it has not been addressed for viruses, especially in the THz frequency range. We began with dielectric constant measurements of the virus layers using the saturation-thickness behavior of the resonant frequency of THz metamaterials, as schematically shown in Fig. 2(a). This has proven to be an effective technique for obtaining the dielectric constants of polymer films and liquids, which is free from interference effects and does not necessitate the preparation of large quantities of samples [22]. In Fig. 2(b) and 2(c), THz transmission spectra are shown for the two types of virus films; these are for a thickness of 40 \( \mu \)m, which is considerably larger than the saturation thickness of \( \sim 10 \) \( \mu \)m. We used metamaterials fabricated on the Si substrate, and having a resonant frequency at 0.8 THz. Using the relationship between \( \varepsilon_r \) and \( \Delta f_{\text{sat}}/f_0 \) for metamaterials devices with this circuit geometry: \( \varepsilon_r = 33.4 \cdot \left( \Delta f_{\text{sat}}/f_0 \right) + 0.99 \) [22], the dielectric constants of the PRD1 and MS2 virus layers were found from \( \Delta f_{\text{sat}}/f_0 \) to be 3.48 and 3.83 at 0.8 THz, respectively.

To confirm these values, we prepared thick films containing a high density of virus, and measured the dielectric constants. The films were prepared by stacking virus layers on a cellulose membrane whose dielectric constant at THz frequencies is close to unity, making the films virtually freestanding. By measuring the amplitudes and phases of transmitted THz pulses, we extracted the complex dielectric constants of films consisting of closely packed PRD1 (thickness 300 \( \mu \)m) or MS2 (150 \( \mu \)m) virus; these are shown in Fig. 2(d) (PRD1) and 2(e) (MS2). The data at 0.8 THz show excellent agreement with the values obtained by metamaterial sensing techniques, as depicted by blue squares [10]. These values are relatively large compared to those of molds and bacteria layers such as Penicillium and E. coli, which range from 1.20 to 1.53 [10]. However, they are close to that of a yeast layer showing a value of 3.60 [10]. Without doubt, the large dielectric constant of typical viruses will be beneficial for their sensitive detection. From the values found here, the sensitivity may be higher for the MS2 virus than for PRD1; however, we also have to consider that MS2 is relatively smaller in size than PRD1.
Fig. 2. (a) Schematic of dielectric constant measurements of virus layers using THz metamaterials. THz transmission amplitude versus frequency, before and after deposition of a layer of (b) PRD1 and (c) MS2. A dielectric constant of 3.48 (PRD1) and 3.83 (MS2) is observed from the resonant frequency shift. Plots of frequency-dependent, complex dielectric constants of the (d) PRD1 and (e) MS2 layers, obtained from THz transmission through thick virus films. The thicknesses of the PRD1 and MS2 layers were 300 µm and 150 µm, respectively.

Although we obtained the dielectric constant information from the thick virus films, our primary goal in this work is to achieve detection at the extreme limit of a single particle per µm² in surface density. THz transmission amplitudes for a THz metamaterial sensor with a gap width (w) of 2 µm are shown in Fig. 3 for low surface densities of PRD1 and MS2 viruses. Here, we used metamaterials fabricated on quartz substrates with a resonant frequency at 1.26 THz, because the sensitivity is higher for substrates with a lower dielectric constant [11,27]. The surface density of the viruses was controlled by manipulating the number of coats; a virus solution with a density of 10⁹/ml was used. We deposited 10 µl of the virus solution in a 10 mm² coating area at a time. The volume density of virus solution was converted to surface density from the specification of the coating area. Because there is a fluctuation in the local surface density of viruses, we observe the ensemble average of Δf from more than ~300 eSRR elements located in the focused THz spot. Obviously, Δf occurs with the deposition of viruses with surface number density of 4/µm² for PRD1 (Fig. 3(a)), resulting from a change in the effective dielectric constant in the gap area. The shift in resonant frequency can be explained by considering the relationship $\Delta f \propto f_0 N(\varepsilon_r - \varepsilon_{\text{air}})/\varepsilon_{\text{eff}}$, where $N$ is the number of viruses; $\varepsilon_r$ is the dielectric constant of the individual virus, $\varepsilon_{\text{air}}$ is the dielectric constant of air, and $\varepsilon_{\text{eff}} = n_{\text{eff}}^2$ is the effective dielectric constant in the gap area without viruses [10].

We also found a similar but larger frequency shift for MS2 viruses (Fig. 3(b)). Interestingly, $\Delta f$ was consistently higher for MS2 by about two times for various surface densities we tested. The reason for the higher sensitivity with MS2 is not apparent, because the dielectric constant difference between PRD1 and MS2 is relatively small in terms of $(\varepsilon_r - \varepsilon_{\text{air}})/\varepsilon_{\text{eff}}$. 
Furthermore, the size of MS2 is only half that of PRD1, and hence, the MS2 will occupy less gap area for a given number density. One plausible reason is that the sensitivity is higher because the smaller object is located closer to the substrate in the gap area, enhancing the sensitivity. In addition, as we have reported previously, the sensitivity depends also on the shape of the individual substances [14]; specifically, sensitivity is higher for objects with aspherical shapes and with more detailed structure. The enhanced sensitivity for MS2, which is higher than that expected from the dielectric constant, presents an interesting issue that requires future investigations to fully understand the underlying mechanisms and predict the sensitivity for different types of viruses.

As mentioned previously, the size compatibility between the substances and the gap-size is one key to understanding the enhanced sensitivity of metamaterial sensors. In consequence, THz metamaterials in which micro-gaps provide circuit capacitance are best suited for sensing fungi and bacteria with their typical sizes of a few micrometers. In contrast, for viruses with sizes ranging 30–60 nm, we need to reduce the metamaterial gap to optimize the sensitivity as a result of the field enhancement that accompanies strong field localization. In Fig. 4, we show the sensitivity to PRD1 for metamaterials fabricated with various gap widths from 3 μm to 200 nm. We first demonstrate the normalized THz transmission amplitudes for THz metamaterial sensors with gap widths of 3 μm (Fig. 4(a)) and 200 nm (Fig. 4(b)) when we deposit low surface densities of PRD1 viruses. Obviously, Δf increases until it saturates with number density from \( N_{av} = 1/\mu\text{m}^2 \) to 4/μm². They are summarized in Fig. 4(c) which shows a plot of Δf as a function of the surface density and gap width; the frequency shift is larger and more rapidly saturated as the gap width decreases. This result can be understood by the enhancement of the electric field in the gap region as the gap width decreases [11,28–30].

By fitting the data with the relation \( \Delta f = \Delta f_{sat}(1 - \exp(-N_{av}/N_{sat})) \), we plotted the sensitivity of the metamaterial sensors as a function of the gap width in Fig. 4(d). Here, the sensitivity (S) is defined by the initial slope of the curves in Fig. 4(c), leading to \( S = \Delta f / N_{av} \).

For a dielectric sensing, GHz/RIU is a common unit to represent the sensitivity. For example, the sensitivity of our metamaterial sensors on Si substrate shown in Fig. 2 corresponds to 70 GHz/RIU. However, the sensitivity of the low-density virus detection can be better represented by the frequency shift for a given surface density. The sensitivity of the metamaterials in terms of the number of virus particles increases from 6 GHz·μm²/particle (w = 3 μm) to 80 GHz·μm²/particle (w = 200 nm), which is an increase of 13 times. Because \( f_0 \) is decreased for smaller w, the sensitivity enhancement with respect to \( \Delta f / f_0 \) reaches as large as 17 times. We also note that the sensitivity reaches up to 40 GHz/particle in terms of the number of viruses located within the gap area, considering the size of the gap area (2 μm²) for \( w = 200 \) nm. In other words, we will be able to develop highly sensitive virus sensors enabling single virus detection, potentially by reducing the number of metamaterial elements. The sensitivity can be further enhanced by optimizing the geometry of the pattern and
substrate configuration [11], and selective detection of viruses in water environments can also be achieved by immobilizing their antibody to the gap structure [10]. In addition, because the shape of the individual substances is one of the key elements in the metamaterials sensitivity [14], numerical simulation is needed to fully understand our observation of the relatively large sensitivity especially for the MS2 viruses. However, this is very challenging because the size of the viruses is extremely small relative to the THz waves, requiring future investigation. Our work also points to future studies on selective identification of viruses through surface functionalization, and label-free detection based on multichannel platforms exploiting the dispersive nature of viruses.

![Image](image.png)

**Fig. 4.** Normalized THz transmission amplitudes of THz metamaterials after deposition of PRD1 at various surface densities for gap width of (a) \( w = 3 \mu m \) (b) \( w = 200 \) nm. (c) Resonant frequency shift (\( \Delta f \)) as a function of surface density for various gap widths in the range 0.2–3.0 \( \mu m \). (d) Sensitivity of THz metamaterials for sensing PRD1 as a function of gap width, fitted to a \( 1/w \) function.

4. Conclusions

We demonstrated THz metamaterials as an effective sensing platform for detecting low-density viruses such as PRD1 and MS2, which are representative double-stranded DNA and single-strand RNA viruses, respectively. Because THz metamaterials are used as the platform for dielectric sensing, we first measured the dielectric constants of the virus layers at THz frequencies, which has not been addressed before. Using thick films, dielectric constants of 3.48 and 3.87 are obtained for PRD1 and MS2, respectively. To test the potential application in low-concentration virus detection, we observed the resonant frequency shift of the metamaterials following the deposition of viruses. The resonant frequency shift is higher for the MS2 virus, partly because of its larger dielectric constant relative to PRD1. We found that the sensitivity for PRD1 increases as gap width decreases, from 6 GHz-\( \mu m^2/\text{particle} \) (\( w = 3 \mu m \)) to 80 GHz-\( \mu m^2/\text{particle} \) (\( w = 200 \) nm). In other words, the sensitivity is about 13 times higher with metamaterials with a 200 nm gap than with conventional micro-gaps. The sensitivity could be further enhanced by reducing the size of gap further and by optimizing other parameters of the metamaterial pattern specific to the virus detection. Our work will contribute in the development of effective sensors for rapid, accurate, and in situ detection of viruses in diverse environments.
**Funding**

This work was supported by Midcareer Researcher Programs (2014R1A2A1A11052108 and 2017R1A2B4009177) through National Research Foundation grant funded by the Korea Government (MSIP) and by Human Resources Program in Energy Technology (20164030201380) of the Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant funded by Korea Government (MOTIE).

**Disclosures**

The authors declare that there are no conflicts of interest related to this article.