AIP Applied Physics Letters

Label-free measurements on cell apoptosis using a terahertz metamaterial-based biosensor

Caihong Zhang, Lanju Liang, Liang Ding, Biaobing Jin, Yayi Hou, Chun Li, Ling Jiang, Weiwei Liu, Wei Hu, Yanqing Lu, Lin Kang, Weiwei Xu, Jian Chen, and Peiheng Wu

Citation: Applied Physics Letters **108**, 241105 (2016); doi: 10.1063/1.4954015
View online: http://dx.doi.org/10.1063/1.4954015
View Table of Contents: http://scitation.aip.org/content/aip/journal/apl/108/24?ver=pdfcov
Published by the AIP Publishing

Label-free measurements on cell apoptosis using a terahertz metamaterial-based biosensor

Caihong Zhang,[1,a)] Lanju Liang,[1,a)] Liang Ding,[2,a)] Biaobing Jin,[1,b)] Yayi Hou,[2,b)] Chun Li,[1] Ling Jiang,[3] Weiwei Liu,[4] Wei Hu,[5] Yanqing Lu,[5] Lin Kang,[1] Weiwei Xu,[1] Jian Chen,[1] and Peiheng Wu[1]

[1]*Research Institute of Superconductor Electronics (RISE), School of Electronic Science and Engineering, Nanjing University, Nanjing 210093, China*
[2]*The State Key Laboratory of Pharmaceutical Biotechnology, Division of Immunology, School of Medicine, Nanjing University, Nanjing 210093, China*
[3]*College of Information Science and Technology, Nanjing Forestry University, Nanjing 210037, China*
[4]*Institute of Modern Optics, Nankai University, Tianjin 300071, China*
[5]*School of Applied Science and Modern Engineering, Nanjing University, Nanjing 210093, China*

(Received 13 March 2016; accepted 2 June 2016; published online 15 June 2016)

Label-free, real-time, and *in-situ* measurement on cell apoptosis is highly desirable in cell biology. We propose here a design of terahertz (THz) metamaterial-based biosensor for meeting this requirement. This metamaterial consists of a planar array of five concentric subwavelength gold ring resonators on a 10 μm-thick polyimide substrate, which can sense the change of dielectric environment above the metamaterial. We employ this sensor to an oral cancer cell (SCC4) with and without cisplatin, a chemotherapy drug for cancer treatment, and find a linear relation between cell apoptosis measured by Flow Cytometry and the relative change of resonant frequencies of the metamaterial measured by THz time-domain spectroscopy. This implies that we can determine the cell apoptosis in a label-free manner. We believe that this metamaterial-based biosensor can be developed into a cheap, label-free, real-time, and *in-situ* detection tool, which is of significant impact on the study of cell biology. *Published by AIP Publishing*. [http://dx.doi.org/10.1063/1.4954015]

Apoptosis plays a pivotal role in shaping of organs in tandem with cell proliferation, regulation, and the removal of defective as well as excessive cells in immune system.[1–3] The evasion of apoptosis is a prominent hallmark of cancer and the defects in apoptosis mechanisms contributing to the carcinogenesis[4,5] apoptosis of tumor is the main goal of modern cancer therapy including radio- and chemotherapy or surgical resection. As the initial platinum analogue, cisplatin was introduced into clinical practice for the chemotherapy of head and neck cancer, mesothelioma, non-small-cell lung cancer, and so on.[6] In recent study of cell biology, Flow Cytometry (FC) in visible band is widely used in the detection of apoptosis. However, the cost of detection is not cheap and detection is time consuming due to the irrecoverable consumption of fluorescence-labeled antibody and multiple steps involved.[7,8] Therefore, it is highly desirable to develop a cheap, *in-situ*, real-time and non-destructive detection approach to quantify cell death.

Metamaterial-based terahertz (THz) biosensor has attracted great attention as a promising tool in biological research.[9–17] This is because (1) the photon energy of THz wave is so small that it is unable to damage biomolecules; (2) the vibrational and rotational modes of many complex molecules locate in the THz regime, which results in strong THz wave–biomolecules interaction; (3) very strong confinement of electromagnetic fields in the metamaterial allows us to detect the small changes of the dielectric environment, leading to high sensitivity; (4) the fabrication can be conducted by standard lithography and the procedures are easy and cheap. Up to now many reports have demonstrated the high sensitivity of metamaterial-based terahertz biosensor to the change of dielectric environment, but the practical applications in the cell biology are rarely reported.

Here, we report our metamaterial-based terahertz biosensor to measure cell apoptosis. Our biosensor is formed by a planar array of five concentric subwavelength gold ring resonators on a 10 μm-thick polyimide (PI) substrate with very high sensitivity. Most importantly, when it is employed in apoptosis measurement of a kind of cancer cell, a linear relation is found between cell apoptosis and the relative change of resonant frequencies, which means that our biosensor could be finally developed to be a cheap, label-free, real-time, and *in-situ* detection tool in the research of cell biology. This bridges wonderfully the THz metamaterial and the detection of cell properties, which are the two very active research areas in the current science and technology.

The metamaterial fabrication began with coating 10 μm-thick polyimide films on 1 in. silicon wafer, followed by patterning of periodical concentric golden rings structure by standard photolithography. Finally, the bottom silicon wafer was peeled off using HF solution. The unit cell has a period of 120 μm and consists of five concentric rings with inner radius of 20 μm, 28 μm, 36 μm, 44 μm, and 52 μm, respectively. The line width of each ring and distance between adjacent rings both have a size of 4 μm (shown in the inset of Fig. 1(a)). To obtain the transmitted THz spectrum, transmitted THz time domain spectroscopy (TDS) system (Advantest, TAS7400SP) was utilized to measure the metamaterial under normal incidence. Transmittance spectra of the designed biosensor is

[a)]C. Zhang, L. Liang, and L. Ding contributed equally to this work.
[b)]Electronic addresses: bbjin@nju.edu.cn and yayihou@nju.edu.cn

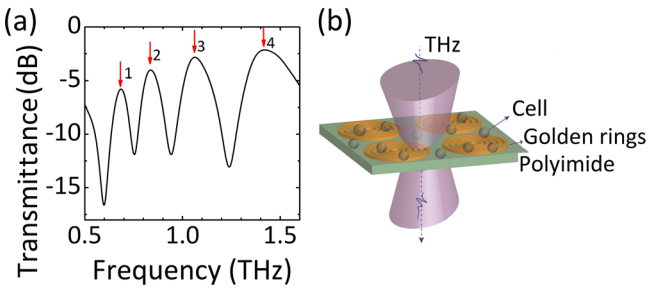

FIG. 1. (a) The transmission spectra of bare metamaterial; (b) The schematic diagram of THz TDS measurement. The unit cell of metamaterial has a period of 120 μm, and the inner radius of five rings is 20 μm, 28 μm, 36 μm, 44 μm, and 52 μm, respectively. The linewidth of each ring and the distance between adjacent rings both have a size of 4 μm.

shown in Fig. 1(a), where four transmission peaks are observed which are due to the resonances by two adjacent rings, like electromagnetically induced transparency (EIT) behavior.[18] Compared with conventional structures, our design has multiple resonant peaks, which are especially useful for dispersive materials, e.g., biological samples, since water is dispersive at THz region and takes a large proportion in most biological samples.

To detect cells, we first choose single layer epithelium-derived oral cancer cell (HSC3) and normal epithelial cell (HaCaT) as analytes. Upon contacting cells one after another, many adherent cancer cells show contact inhibition of locomotion (CIL), i.e., a cell stops migration in a particular direction upon contacting with another cell, leading to the conformation of single-layer structure of cancer cells for growth *in vitro*. Based on this, we studied adherent cells HSC3 and HaCaT. These two kinds of cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Life Technologies, USA) and penicillin 100 μg/ml and streptomycin 10 μg/ml (Invitrogen Life Science, USA) at 37 °C, 5% CO_2 condition. The adherent cells were harvested and washed with phosphate buffered saline (PBS) and then digested with trypsin-EDTA (Invitrogen Life Science) to prepare the single-cell suspensions. For seeding the cells, THz metamaterials were sterilized by 75% ethanol and attached in the bottom of 12-well culture plates. Subsequently, the indicated concentrations of single cell suspensions were seeded as a single-layer structure in the surface of THz metamaterials and cultured at 37 °C, 5% CO_2 condition.

For THz measurement, we first took out the metamaterial covered with cells from the culture plate, using deionized water to wash out suspensions and leave one-layer cell on the surface of metamaterial. Then dust-free paper was utilized to remove the suspending water lightly and quickly. THz TDS was finally applied to measure the transmitted spectra for the metamaterial with and without cells (shown in Fig. 1(b)). First, artificial setting of different concentrations of single cell suspensions was prepared for THz TDS measurement. It is found that Δf (the difference of resonant frequency between metamaterial with and without analyte of cells) increases with the cell concentration, but will saturate at high concentration (shown in Figs. 2(a) and 2(b) for HSC3 and HaCaT, respectively). Both cancer cell HSC3

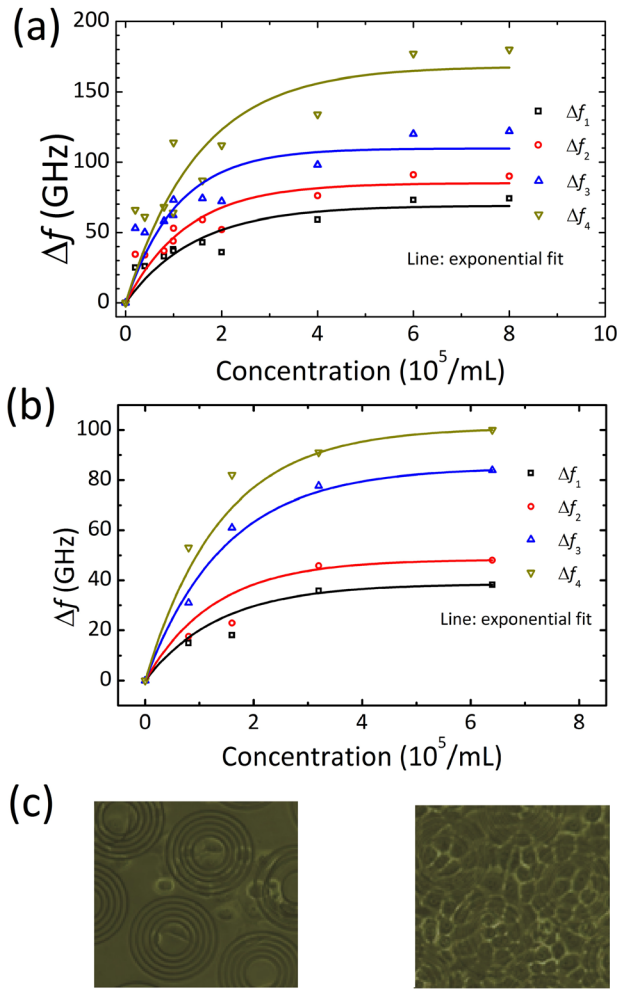

FIG. 2. The frequency shift Δf of metamaterial with and without cell analyte versus concentration for tumor cell (HSC3) (a) and normal cell (HaCaT) (b); symbols are experimental results and lines are exponential fit. (c) Micrograph of our biosensor with cell analyte HSC3 at a concentration of 2×10^5 cells/ml (left) and 6×10^5 cells/ml (right).

and normal cell HaCaT have such similar behavior. This is because cell layer modifies the dielectric environment of the metamaterial. According to perturbation theory, the relative change of the resonant angular frequency $\Delta\omega_0/\omega_0$ is[19]

$$\frac{\Delta\omega}{\omega_0} = \frac{-\int_{v_0}\left(\Delta\varepsilon|\bar{E}_0|^2 + \Delta\mu|\bar{H}_0|^2\right)dv}{\int_{v_0}\left(\varepsilon|\bar{E}_0|^2 + \mu|\bar{H}_0|^2\right)dv} \approx \frac{-\int_{v_0}\left(\Delta\varepsilon|\bar{E}_0|^2\right)dv}{2\int_{v_0}\left(\varepsilon|\bar{E}_0|^2\right)dv}, \quad (1)$$

where E_0 and H_0 are the electric and magnetic fields in the original metamaterial, $\Delta\varepsilon$ is a change of the dielectric constant in the metamaterial, and v_0 is the effective integral volume. We do not consider the change of permeability in the metamaterial since the biomolecules are often non-magnetic materials. According to equivalent medium theory, here the equivalent dielectric constant of single-layer cells ε_{eff} is proportional to the cells number (n_0), which means that the increase of cell numbers will cause an increase of Δf. Fig. 2(c) shows the micrograph of our biosensor with cell analyte HSC3 at a concentration of 2×10^5 cells/ml (left) and 6×10^5 cells/ml (right). It is clear that even only one to a few cells are on each concentric rings unit cell, the Δf can

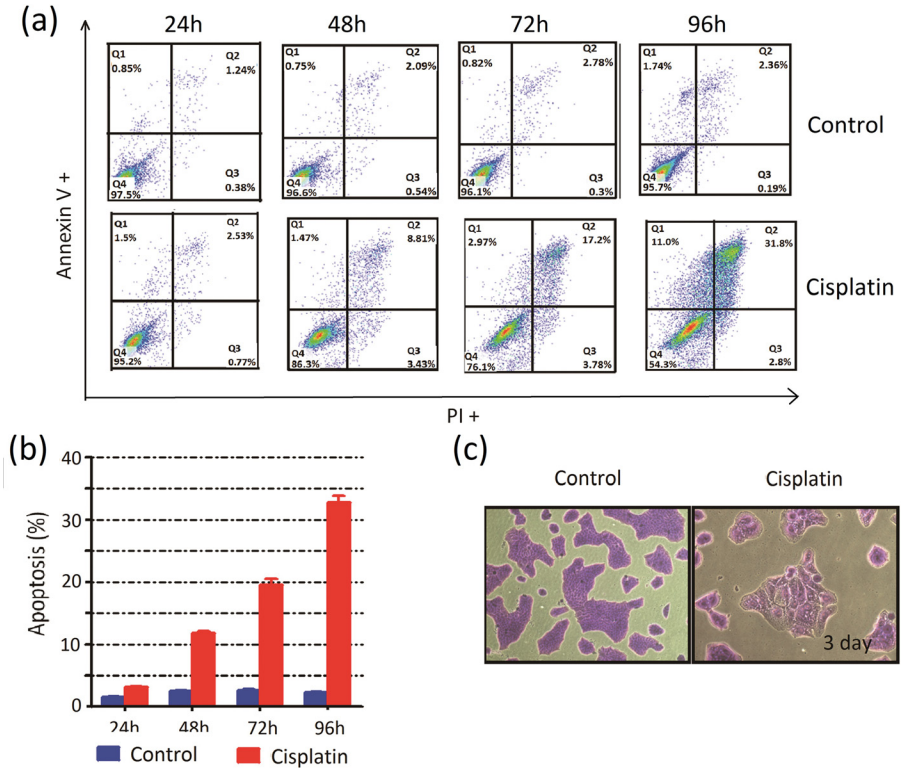

FIG. 3. (a) The apoptosis measured by Flow Cytometry for oral cancer cell SCC4 treated with (Cisplatin group, below row) or without cisplatin (Control group, above row) after 24 h, 48 h, 72 h, and 96 h, respectively. (b) The apoptosis as a function of time. (c) The micrograph of cells by Crystal violet staining.

still be detected. For the saturate behavior, it can be understood that at a concentration of about 6×10^5 cells/ml the cell almost covers the whole surface of metamaterial. Thus larger concentration of adherent cell suspension will not cover more than one-layer on surface of THz metamaterial, and thereby will not alter more of the dielectric environment. Meanwhile we found that Δf caused by the tumor cell is larger than that caused by the normal cell. This may imply the different structures or water content in the tumor cell,[20] which may provide a tumor diagnosis. In addition, higher resonant frequency will bring larger $\Delta\omega_0$ values from Eq. (1), in agreement with the measurements.

We next investigated the potential application of our biosensor in the cell apoptosis detection. We choose one kind of the oral cancer cell SCC4 as an example. In the natural growth condition, oral cancer cell SCC4 was separated into two groups, and treated with or without cisplatin having low concentration of 5 μM, which is a chemotherapy drug to induce cell apoptosis for cancer treatment in clinical,[21] for continuously four days. Every day the SCC4 was taken out and the analysis of apoptosis was performed by both the widely-used FC (shown in Fig. 3) and our THz TDS technique (shown in Figs. 4 and 5), respectively, at the same time. We labeled the cancer cell treated without and with cisplatin as Control and Cisplatin samples, respectively. Crystal violet staining showed that the administration of cisplatin triggered the morphological changes and significantly abrogated the proliferation of SCC4 (Fig. 3(c)). For the apoptosis analysis by FC, SCC4 was harvested by trypsin and washed with PBS in centrifuge tube. It is to be noted that the tumor culture supernatant should also be collected into the centrifuge tube due to the suspended dead cells in the culture supernatant; 2 μl of annexin V mixed with 2 μl propidium iodide were added for staining the markers of apoptosis for 30 min. As shown in Fig. 3(a), propidium iodide-positive and annexin V-positive cells were regarded as apoptotic cells. The apoptosis as a function of time is plotted in Fig. 3(b), which shows significant increase of apoptosis after cisplatin treatment.

Meanwhile, the measurement was also performed by THz TDS. Figure 4 gives the THz spectra from Control and Cisplatin samples after 24 h, 48 h, 72 h, and 96 h, respectively. It is obvious that the change in the peak amplitude as well as the frequency shift can be observed. The results shown in Fig. 5(a) give the Δf between the Control samples and bare metamaterials. In the natural growth condition, the cell concentration increases with time, leading to an increasing effective dielectric constant and Δf with time. Fig. 5(b) shows the Δf between Cisplatin samples and bare metamaterials. The

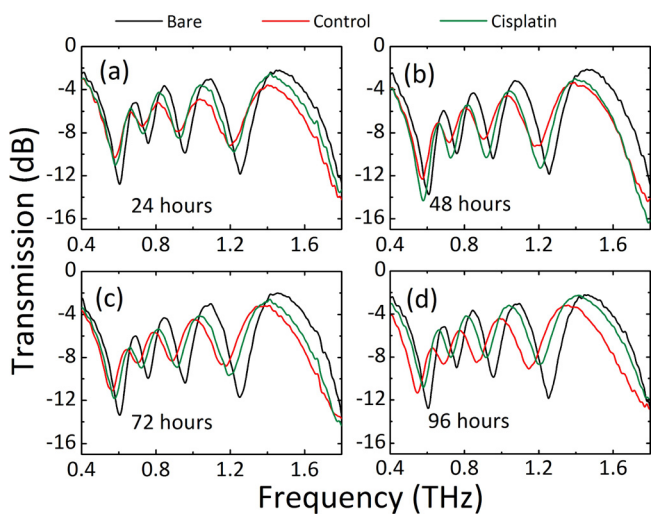

FIG. 4. The THz spectra of oral cancer cell SCC4 treated with (Cisplatin group) or without (Control group) after 24 h, 48 h, 72 h, and 96 h, respectively.

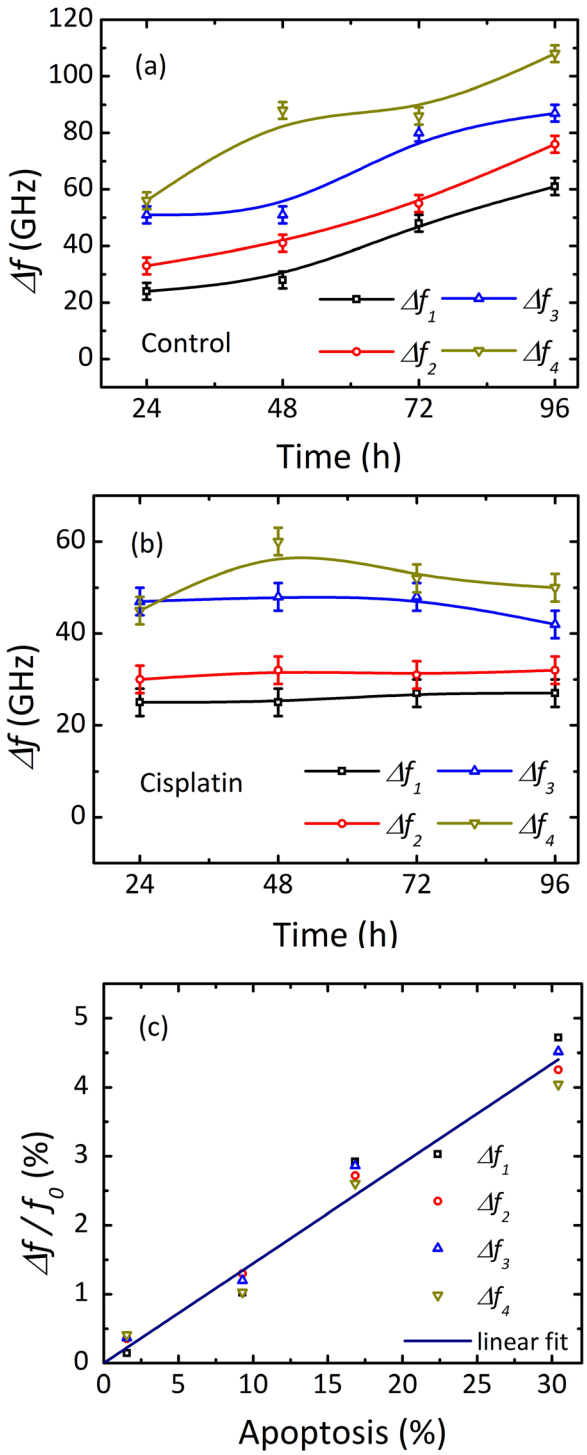

FIG. 5. The frequency shift of Control samples (a) and Cisplatin samples (b) versus time. (c) Relative frequency shift versus the cell apoptosis; the symbol is experimental results, and the lines are linear fit.

difference Δf does not increase obviously with time but has a slight decrease. It means that due to the cell death caused by cisplatin, the number of cells does not increase. As time increases, Δf_4 with highest resolution slightly decreases. It means that the dead cell starts to break away from the THz metamaterial surface and floats in the suspension, and thereby results in a decrease of cell numbers on THz metamaterial. Since the Control group cells already cover the whole surface of THz metamaterial after 96 h, we stop the measurement after 96 h. If we continue the measurement after 96 h, the Δf for Cisplatin group should decrease more. We also plot the relative change of resonance frequencies between Control and Cisplatin samples as a function of apoptosis; a linear relation can be found for all four resonance frequencies (Fig. 5(c)). Although the number of apoptotic cells induced by Cisplatin at day 1 was small and less than 5%, interestingly, remarkable resonant frequency shift by THz TDS could be also noticed. The results can be explained as follows. Because the electric field decays exponentially along the direction normal to the metamaterial, Eq. (1) can be simplified as

$$\frac{\Delta\omega}{\omega_0} \propto \Delta\varepsilon_{cell} \times h_{cell}/l_{field}, \tag{2}$$

where $\Delta\varepsilon_{cell}$ is the equivalent dielectric constant difference between Control and Cisplatin cells, h_{cell} is the equivalent thickness of cell layer; and l_{field} is the penetration depth of THz field. Here we think that the cell sample is approximately non-dispersive in our studied frequency range, and the decay length for each resonance mode is the same. Thus, from Eq. (2), $\Delta\omega_0/\omega_0$ is linear with $\Delta\varepsilon_{cell}$. Moreover, as ε_{eff} is proportional to the number of cells n_0, it is proportional to apoptosis. Therefore, $\Delta\omega_0/\omega_0$ is linear with the apoptosis in agreement with our measurement.

Our results demonstrate that there is significant difference of resonance frequency between Control and Cisplatin samples, which mainly comes from the change of the dielectric constant in the metamaterial $\Delta\varepsilon$. Here, $\Delta\varepsilon$ originates from both water content and structure in the cell.[20] Thus, our biosensor could determine the resonance frequency, and thereby the absolute value of cell apoptosis. This behavior could be expanded to many kinds of biological samples. On the other hand, the THz metamaterial is sterilized in the same condition with cells during cells growth and treatment. So it has almost no influence to the cells. This is a very important step to develop it as a label-free, cheap, non-destructive and practical biosensor for *in-situ* detection. In contrast, currently the widely-used FC is labeled, costly, and destructive. Furthermore, the sensitivity of FC is mainly represented by the fluorescence sensitivity of detector. On some level, the sensitivity depends on the amount of used fluorescence. In most circumstances, detected positively stained cells rate by FC detector should be larger than 50 cells per second, and the number of cells used for the analysis of FC are huge, e.g., the number is usually at least 10 000. In contrast, the sensitivity of our biosensor mainly depends on the cell numbers on the surface of THz metamaterials. It is clear that one to a few cell numbers on each concentric rings unit cell can still be detected (Fig. 2(c), left). THz TDS system normally has a THz beam size of around 5 mm; there are around 1360 units of our metamaterial structures in such area. Hence, thousands of cells are enough to be detected. Therefore, our biosensor is enough for most cases of cell detection.

In practice, it is very essential and helpful for us to monitor the cell status during natural growth or in any wanted time after some treatment in the germfree culture plate, hereby our biosensor, which is non-destructive to the cell, is a very promising method. For example, we could attach the metamaterial at the bottom of 12-well culture plates which is transparent in the THz regime and measure it with reflection TDS system. We could get the change of resonant frequency

from the reflective waves, obtaining the condition of cells, such as cell apoptosis in real time.

We have demonstrated here a THz metamaterial-based biosensor for label-free measurement on cell apoptosis. A linear relation is found between the relative change of resonant frequency and the cell apoptosis by Flow Cytometry. This relation is explained by the change of cell numbers above the metamaterial, leading to the change of effective dielectric constant of the dielectric environment above the biosensor. This work makes us to believe fully that our biosensor can be developed as a cheap, label-free, real-time, and *in-situ* detection tool. We think the work is a key step toward a real application of THz biosensor, which could make significant impact on the study of cell biology.

This work was partly supported by the MOST973 (No. 2014CB339800), National Instrumentation Program under Grant No. 2012YQ14005, the National Natural Science Foundation (Nos. 61371035, 11173015, 11227904, 61521001, and 61501219), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), and Jiangsu Provincial Key Laboratory of Advanced Manipulating Technique of Electromagnetic Wave. Dr. Liang with Zaozhuang University was also supported by Technological Development project of Shandong Province (Grant No. 2013GGA04021). B. B. Jin and J. Chen also appreciate the support from Cooperative Innovation Centre of Terahertz Science, University of Electronic Science and Technology, Chengdu 611731, China.

[1]A. Kaczmarek, P. Vandenabeele, and D. V. Krysko, Immunity **38**, 209–223 (2013).
[2]A. Kamal, S. Faazil, and M. S. Malik, "Apoptosis-inducing agents: A patent review (2010–2013)," Expert Opin. Ther. Pat. **24**, 339–354 (2014).
[3]B. Levine and J. Y. Yuan, "Autophagy in cell death: An innocent convict?" J. Clin. Invest. **115**, 2679–2688 (2005).
[4]D. Hanahan and R. A. Weinberg, "The hallmarks of cancer," Cell **100**, 57–70 (2000).
[5]M. Hassan, H. Watari, A. AbuAlmaaty, Y. Ohba, and N. Sakuragi, "Apoptosis and molecular targeting therapy in cancer," BioMed Res. Int. **2014**, 150845.
[6]J. Lokich and N. Anderson, "Carboplatin versus cisplatin in solid tumors: An analysis of the literature," Ann. Oncol. **9**, 13–21 (1998).
[7]R. Sgonc and G. Wick, "Methods for the detection of apoptosis," Int. Arch. Allergy Immunol. **105**, 327–332 (1994).
[8]A. V. Tkach, L. A. Ivanova, and V. StatsenkoIu, "Methods of detection and quantitative evaluation of apoptosis (review of literature)," Med. Tr. Prom. Ekol. **12**, 28–35 (2008).
[9]T. Driscoll, G. O. Andreev, D. N. Basov, S. Palit, S. Y. Cho, N. M. Jokerst, and D. R. Smith, "Tunned permeability in terahertz split-ring resonator for devices and sensors," Appl. Phys. Lett. **91**, 062511 (2007).
[10]H. Tao, A. C. Strikwerda, M. K. Liu, J. P. Mondia, E. Ekmekci, K. B. Fan, D. L. Kaplan, W. J. Padilla, X. Zhang, R. D. Averitt, and F. G. Omenetto, "Performance enhancement of terahertz metamaterials on ultrathin substrates for sensing application," Appl. Phys. Lett. **97**, 261909 (2010).
[11]H. Tao, E. A. Kadlec, A. C. Strikwerda, K. B. Fan, W. J. Padilla, R. D. Averitt, E. A. Shaner, and X. Zhang, "Microwave and terahertz wave sensing with metamaterials," Opt. Express **19**, 21620–21626 (2011).
[12]X. J. Wu, B. G. Quan, X. C. Pan, X. L. Xu, X. C. Lu, C. Z. Gu, and L. Wang, "Alkanethiol-functionalized terahertz metamaterial as label-free, highly-sensitive and specific biosensor," Biosens. Bioelectron. **42**, 626–631 (2013).
[13]R. Singh, W. Cao, I. Al-Naib, L. Q. Cong, W. Withayachumnankul, and W. L. Zhang, "Ultrasensitive terahertz sensing with high-Q Fano resonances in metasurfaces," Appl. Phys. Lett. **105**, 171101 (2014).
[14]L. Q. Cong, S. Y. Tan, R. Yahiaoui, F. P. Yan, W. L. Zhang, and R. Singh, "Experimental demonstration of ultrasensitive sensing with terahertz metamaterial absorbers: A comparison with the metasurfaces," Appl. Phys. Lett. **106**, 031107 (2015).
[15]S. H. Wu, S. Y. Hsieh, K. L. Lee, R. H. Weng, A. Chiou, and P. K. Wei, "Cell viability monitoring using Fano resonance in gold nanoslit array," Appl. Phys. Lett. **103**, 133702 (2013).
[16]S. J. Park, J. T. Hong, S. J. Choi, H. S. Kim, W. K. Park, S. T. Han, J. Y. Park, S. Lee, D. S. Kim, and Y. H. Ahn, "Detection of microorganisms using terahertz metamaterials," Sci. Rep. **4**, 4988 (2014).
[17]L. J. Xie, W. L. Gao, J. Shu, Y. B. Ying, and J. C. Kono, "Extraordinary sensitivity enhancement by metasurfaces in terahertz detection of antibiotics," Sci. Rep. **5**, 8671 (2015).
[18]N. Papasimakis, Y. H. Fu, V. A. Fedotov, S. L. Prosvirnin, D. P. Tsai, and N. I. Zheludev, "Metamaterial with polarization and direction insensitive resonant transmission response mimicking electromagnetically induced transparency," Appl. Phys. Lett. **94**, 211902 (2009).
[19]M. David, *Microwave Engineering* (John Wily & Sons, Inc., 1998), p. 341.
[20]S. Sy, S. Huang, Y. X. J. Wang, J. Yu, A. T. Ahuja, Y. T. Zhang, and E. Pickwell-MacPherson, "Terahertz spectroscopy of liver cirrhosis: Investigating the origin of contrast," Phys. Med. Biol. **55**, 7587–7596 (2010).
[21]W. Lieberthal, V. Triaca, and J. Levine, "Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: Apoptosis vs. necrosis," Am. J. Physiol. **270**, F700–F708 (1996).