Terahertz polarization sensing based on metasurface microsensor display anti-proliferation of tumor cells with aspirin

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Abstract: The inhibition effects of aspirin on cell proliferation are investigated by both traditional THz resonance sensing and the improved THz polarization sensing method based on a polarization dependent metasurface microsensor. Compared to resonance sensing, the quality factor of polarization sensing is 4–5 times higher than that of resonance sensing, and its figure of merit is at least one order of magnitude higher than that of the resonance sensing with the same metasurface microsensor. Our proposed metasurface-based biosensors may supply a novel viewpoint on cell proliferation from a physical perspective and be a valuable complementary reference for biological study.

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1. Introduction

Several physical apparatuses have been used to identify biological process globally, such as fluorescent confocal microscopy, flow cytometry, mass spectrometer and terahertz (THz) imaging system, elucidating the cellular responses under physiological or pathological conditions. Nowadays, THz technology is growing rapidly as a promising research field owing to the fast development in THz radiation sources and THz time domain spectroscopy (THz-TDS). THz radiation is composed of electromagnetic waves within 0.1–10 THz frequency range [1]. Recently, THz radiation has attracted more attention from biomedical researchers due to its unique spectral characteristics including non-ionizing and non-invasive properties, strong absorption to polar substances, spectral fingerprint [2,3]. Moreover, photon energy of THz can be absorbed by weak intermolecular interactions and organic substances have characteristic spectra in THz region, which makes it suitable for biomedical sensing applications [2,4–6].

Over the past decades, multiple THz biosensors have been developed in biomedical application, such as microfluidic based resonators, waveguides and metamaterials (MMs) [7–13]. As a class of artificial composite materials arranged at subwavelength structures, MMs own extraordinary electromagnetic response that would not be possible with natural materials [11]. By virtue of superior properties of metamaterials, an increasing number of THz metamaterials have been synthesized and applied to the management of THz signals in biomedical research [14–25]. The advantages of the MMs-based biosensors open up a door for the cell detection into low cost, label-free and fast process, being promising method in future biological sensing and
disease diagnosis. For examples, researchers reported a label-free and specific biosensor for streptavidin-agarose (SA) based on THz metamaterial functionalized by octadecanethiols and biotins. A redshift up to 6.76 GHz was measured by the system in the undiluted commercial solution [14]. Then, another metamaterial biosensor obtains a 275 GHz frequency shift for a 3 mmol/L aqueous solution of bovine serum albumin (BSA), with a resolution of 17.7 µmol/L [15]. Recently, a Fano resonance metamaterials (FRMMs)-based biosensor has been employed to monitor the low concentration of HaCaT cells, approximately arrive at 0.2 × 10^5 cells/mL. Although significant improvement has been made in the THz metamaterial sensors, the sensing mechanism based on frequency shift due to the refractive index change is still limited, of which the resonance peak shift is insufficient and the sensitivity remains relatively low.

Cancer is leading causes of death worldwide, the diagnosis of which is critical to initiate therapies [26]. Currently, the methods for examination of cell proliferation could be divided into four categories: metabolic activity determination (e.g., MTT, MTS, and CCK-8 assays), DNA synthesis detection (e.g., cell cycle test, BrdU and EdU assays), cell proliferation-related antigen measurement (e.g., proliferating cell nuclear antigen and Ki67 expression detection) and cell number test (e.g., colony formation assay) [27–30]. Currently the widely-used Flow Cytometry (FC) is labeled, costly, and destructive. Furthermore, the sensitivity of FC is mainly represented by the fluorescence sensitivity of detector [31,32]. Electrochemical biosensors can recognize biochemical signals and convert them into corresponding electrical signals through bio-sensitive element and transducers attached to the electrodes by using biosensor technology combined with electrochemical technology [33,34]. However, the cost of above detections is not cheap, and detections are time consuming due to the irrecoverable consumption of fluorescence-labeled antibody and multiple steps involved. Therefore, label-free, real-time, and in-situ measurement on cell proliferation is highly desirable in cell biology. Compared to the traditional biological methods, THz metamaterial-based cell sensor is simple and more portable, in a label-free as well as self-referenced manner, which is useful in tumor cytopathology [9]. THz metamaterials can couple the incident THz wave to enhance the localized electric field, particularly in the gap area of cells; thus, they provide a highly sensitive sensing platform to detect cells [9,31]. THz sensing based on metasurface microsensor may have potential improvement to explore the influence of drugs on cancer cell biological performance, but the sensing performances of the traditional resonance sensing method need to be improved for better evaluation of cell proliferation. The current methods only obtain the THz amplitude and phase information of the sample, but not polarization information. If the polarization information is obtained and the polarization parameters are used for sensing, the effective information achieved by the measurement system will be greatly improved, and the characteristics of the samples are reflected from different views by multiple sensing physical parameters. However, the MMs-based biosensors combined with the THz polarization sensing have been rarely reported in cell proliferation assay.

In this study, a new THz technic integrating THz polarization based on metasurfacemicrosensor with THz polarization spectroscopy is developed in a model of tumor cells. The polarization ellipticity and polarization rotation angle as the key parameters of THz sensing, could be used for better evaluating cell proliferation based on metasurface microsensor. Compared with the traditional resonance sensing with the same sensor device, the quality factor (Q factor) and figure of merit (FoM) of polarization sensing were greatly improved.

2. Material and methods

2.1. Cell experiment

Human embryonic kidney cell line HEK293T (or 293T), mouse melanoma cell line B16 and human hepatocarcinoma cell line HepG2 were purchased from cell bank of Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco’s modified Eagle medium (DMEM) (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS,
Gibco), 100 U/mL penicillin and 100 mg/mL streptomycin (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and kept in incubator containing 5% CO₂ at 37°C.

Aspirin (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA). Given that the aspirin treatment mainly referred to the previous studies in HEK293T cells [35], HepG2 cells [36,37] and B16 cells [38], 2.5 mM aspirin was added into HEK293T, HepG2 and B16 cells for 48 h. After aspirin treatment for 48 h, cells were collected after trypsin digestion and low-speed centrifugation (800 rpm, 5 min). To exclude the effect of residual drugs, cells were washed twice using phosphate buffer saline. Then, the cells were fixed with pre-cold acetone and seeded on the surface of metasurface microsensor maintaining at 37°C.

2.2. MTT assays

Cells were harvested from exponential phase cultures, counted, and plated in 96-well plates. After respectively treatment, the cells were incubated with the MTT substrate (5mg/mL) for 4 h in the dark. After incubation, the culture medium was removed, and DMSO was added. Optical density was measured at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA) to estimate cell proliferative ability.

2.3. Cell cycle detection

Cell cycle distribution pattern was measured using a flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, USA) after Propidium Iodide (PI) staining. Briefly, cells at logarithmic growth phase were plated in 6-well plates (5×10⁵ cells/well). Then, the cells were treated with 2.5 mM aspirin for 48 h and collected. After fixed overnight at 4°C with ice-cold 70% ethanol, cells were stained with PI staining solution containing RNase A. After 30 min incubation at 37°C in the dark, DNA content and cell percentage at different phases were detected at the excitation wavelength of 488 nm and the results were analyzed using a flow cytometer.

2.4. Statistical analysis

All data were obtained from more than three independent experiments and analyzed using SPSS 13.0 software (SPSS, Chicago, Illinois, USA). Results were presented as mean ± standard deviation (SD). Difference analysis between groups was conducted using Student’s t test. P < 0.05 was defined as statistically significant.

2.5. THz time domain polarization spectroscopy system

A standard four parabolic mirror THz-TDPS system was used in our experiments to measure the THz properties of the samples. All the experiments were carried out at room temperature with the humidity of about 50%. THz pulses were generated by a low temperature grown GaAs photoconductive antenna, which was excited by a femtosecond laser. The excitation source is a Ti:sapphire laser with 75fs duration of 80 MHz repetition rate working at 800 nm. A ZnTe crystal was used for electro-optic sampling probe. The sample was settled at the focal point of the THz-TDPS system as shown in Fig. 1(e). THz waves excited from the photoconductive antenna were normally incident into the metasurface along z-axis with a y-linear polarized (LP) light. The ZnTe crystal only detects the LP waves strictly along y-axis. We measured the time-domain pulse signals of the samples by THz-TDPS system, of which the reference signal ⃗E_r(t) and the sample signal E_s(t) were obtained. After Fourier transform, their amplitudes E_r(ω), E_s(ω) and phases δ_r(ω), δ_s(ω) of the samples and reference in the frequency domain were obtained, correspondingly. The transmission amplitude is A(ω) = E_s(ω)/E_r(ω), and the phase shift between the sample and the reference Δδ_s = δ_s(ω) − δ_r(ω).

Unlike conventional THz-TDS system, an additional polarizer was placed behind the sample, which could be rotated to obtain the +45° and -45° polarization components that passed through
the sample, as shown in Fig. 1(c). In this method, we measured the $+45^\circ$ and $-45^\circ$ LP signals $E_{+45^\circ}(t)$ and $E_{-45^\circ}(t)$, so we could get $A_{+45^\circ}(\omega)$, $A_{-45^\circ}(\omega)$, and $\Delta \delta(\omega) = \delta_{+45^\circ}(\omega) - \delta_{-45^\circ}(\omega)$. The terminal trajectory equation of electric vector $E$, also called as polarization ellipse, was obtained as follows, so that the output polarization states in the broadband THz spectrum could be completely reconstructed:

$$
\left( \frac{E_x}{A_{-45^\circ}} \right)^2 + \left( \frac{E_y}{A_{+45^\circ}} \right)^2 - \frac{2E_x E_y}{A_{-45^\circ} A_{+45^\circ}} \cos \Delta \delta = \sin^2 \Delta \delta
$$

The polarization conversion characteristics could be further characterized by ellipticity $\varepsilon$ and polarization rotation angle $\psi$, which can be derived as follows:

$$
\tan 2\varepsilon(\omega) = \sin 2\beta(\omega) \sin \Delta \delta(\omega)
$$

$$
\tan 2\psi(\omega) = \tan 2\beta(\omega) \cos \Delta \delta(\omega)
$$

where $\tan \beta(\omega) = A_{+45^\circ}(\omega)/A_{-45^\circ}(\omega)$.

Thus, compared to the amplitude and phase information obtained from the ordinary THz-TDS, three more parameters are available from THz-TDPS: chirality (left-handed or right-handed polarization), ellipticity and polarization rotation angle, providing a more powerful tool for the characterization of the measured sample.

2.6. Device fabrication

The microstructure device with the coated cell samples was processed by photolithography. The photoresist was coated on the SiO$_2$ substrate, photolithography was performed with the
designed mask, and then a layer of Au was deposited by vapor deposition. Finally, the remaining photoresist was stripped along with the metal thereon to obtain the designed device. The structure of the fabricated device was shown in Fig. 1(a) and 1(d), with a substrate of 500 µm-thick SiO₂ and a metasurface of Au structure array with a period of \( P = 160 \, \mu \text{m} \) and a thickness of 0.2 µm. The metal unit structure consists of a regular hexagon, the length of each side was \( L = 60 \, \mu \text{m} \) and the line width was \( w = 6 \, \mu \text{m} \), plus two intersecting diagonal lines, with a hollow ring at each corner and center of the hexagon. The outer radius of the ring was \( R = 15 \, \mu \text{m} \) and the inner radius was \( r = 9 \, \mu \text{m} \).

3. Results and discussion

3.1. Model of aspirin-mediated cell proliferation

Aspirin as a nonsteroidal anti-inflammatory drug is able to suppress the proliferation of tumor cells *in vitro* and *in vivo* [39,40]. To observe the polarization ellipticity and polarization rotation angle by THz polarization sensing in tumor cells, the model of cancer cell proliferation was developed in HEK293 T, B16 and HepG2 cells with aspirin treatment. The 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay and flow cytometry were used to measure the biological parameters of the three cell lines under 2.5 mM aspirin treatment. The data showed that an approximately 25% reduction in cells concentration from \( 1 \times 10^6 \) cells/mL to \( 7.5 \times 10^5 \) cells/mL was observed in aspirin-treated HEK293 T cells compared to control cells as shown in Fig. 2(a). Furthermore, our data revealed that aspirin treatment led to a significant reduction in cells concentration from \( 1 \times 10^6 \) cells/mL to \( 8.2 \times 10^5 \) cells/mL (about 18%) of B16 cells, and a reduction in cells concentration from \( 1 \times 10^6 \) cells/mL to \( 7.5 \times 10^5 \) cells/mL (about 25%) of HepG2 cells as shown in Fig. 2(b) and 2(c). Aspirin induced an increase of G1 phase cell percentage from 53.56% to 57.95% and a reduction of G2 phase cell percentage from 7.78% to 4.90% in HEK293 T cells, while the cell percentage in S phase was slightly reduced from 38.67% to 37.15% after aspirin treatment as shown in Fig. 2(d), indicating that cell cycle was arrested at G1 phase by aspirin in HEK293 T cells. With respect to the data about cell cycle, the proportion of B16 cells in the G1 phase was reduced from 57.96% to 53.44%, meanwhile, the percentage of B16 cells in the G2 phase was increased from approximately 7.01% to 12.67% after the treatment as shown in Fig. 2(e), indicating that B16 cells were arrested at G2 phase by aspirin. Moreover, the percentage of cells in G1, G2 and S phases was 67.79%, 11.67%, and 20.55% in HepG2 cells, while the corresponding cell proportion converted into 67.51%, 14.18% and 18.31% in aspirin-treated HepG2 cells, respectively shown in Fig. 2(f).

Accordingly, these results suggest that the aspirin inhibits the cell proliferation and induces cell cycle arrest in the cancer cells. At present, THz polarization sensing system has not been applied for characterizing the effect of aspirin in tumor cells yet. Therefore, the model of cancer cell proliferation will be used in this study.

3.2. Anti-proliferation effect of aspirin characterized by terahertz sensing technology

To make an improvement of existing methods, THz spectroscopy is used to facilitate the detection of the effects of aspirin on target cells. Details of the structure and fabrication of THz metasurface microsensor were introduced in Section 2. The representative micrograph of metasurface microsensor coating the cell layer before or after was shown in Fig. 1(a) and 1(b), respectively. In detail, Fig. 1(c) showed the schematic diagram of geometric configuration in detection. THz metasurface microsensor coated with cell layer was placed at the focal plane of the THz-TDPS to detect the THz time domain signal at different rotation angles of THz polarizer as shown in Fig. 1(e).
Fig. 2. A model of aspirin-mediated cell proliferation. (a)–(c) Cells were treated with or without aspirin (2.5 mM) for 48 h. Then, cell proliferative capacity was examined by MTT assays. (a): 293T, (b): B16, (c): HepG2. (d)–(f) Cells were treated with or without aspirin (2.5 mM) for 48 h. Then, cell percentage in G1, G2 and S phases was detected by flow cytometer. Up panel: without aspirin treatment; Down panel: aspirin treatment. (d): 293T, (e): B16, (f): HepG2. All data were obtained from more than three independent experiments.

*P < 0.05.

3.2.1. Terahertz resonance sensing based on metasurface microsensor

Firstly, we used a classical sensing method, in a model of resonance effect of the metasurface microsensor as illustrated in Fig. 3(a), to detect the anti-proliferation effect of aspirin on cells. The THz time-domain signals of samples were measured by the system. The original data of the y-LP time-domain pulses of the microsensor with three cell lines were detected when the THz polarizer is rotated at 0°, as shown in Fig. 4. And the corresponding frequency-domain transmission spectra were obtained after Fourier transform in Fig. 5. The red curve represented the transmission spectrum of the blank metasurface, which showed a distinct resonance peak at 0.787 THz with 32.5 dB intensity. The blue and green curves represented the transmission spectrum of the metasurface coated by cell layer with or without aspirin treatment, respectively. We obtained similar results in three types of cells. After coating the sample of 293 T cell without aspirin, the resonance peak moved to 0.675 THz with a red shift of 112 GHz, and the intensity reduced to 17 dB. After the 293 T cell was treated by aspirin, the peak value increased to 27.5 dB again, and moved back to 0.719 THz with a blue shift of 43.7 GHz as shown in Fig. 5(a).
Fig. 3. Schematic of MMs-based THz microsensor. The incident THz waves propagated through MMs-biosensor. Micrograph of fabricated MMs sample coated by various types of cells. (a) Resonance sensing, (b) Polarization sensing.

Fig. 4. Detected $y$-LP time-domain pulses of the microsensor and with the three cell lines when the THz polarizer is rotated at $0^\circ$. (a) HEK293T, (b) B16, (c) HepG2.

The key sensing parameters (i.e. the frequency and intensity of resonance peak) were extracted and plotted in Fig. 5(d), where we could see that the cell layer made that the resonance frequency moved to lower frequency with damping resonance intensity. The frequency shift and the intensity of various sample cells were different, whereas, the general trend was similar, suggesting that the higher number of proliferating cells is corresponding to the larger frequency shift and the lower resonance intensity. After aspirin treatment, the resonance frequency moved back and the intensity increased, which indicated that the number of aspirin-treated cells were fewer than that control cell sample without aspirin, so that aspirin inhibited cell proliferation. The results using the THz technology were highly consistent with the observation by biological methods.

The parameters that evaluate the performance of sensor include as follows:

1) Sensitivity ($S$) reflects the value of sensor physical quantity (i.e. the changes in frequency or polarization parameter here) that can be detected under the unit target quantity (i.e. Number of cells here).

2) Quality factor ($Q$) reflects the sensitivity of the sensor itself and is no relationship with the performance of the measuring system and the type of sample being tested.
Fig. 5. The corresponding y-LP transmission spectra of three cell lines. (a)–(c) Frequency-domain transmission spectra of the microsensor with the three cell lines. (a): 293T, (b): B16, (c): HepG2. (d) Absorption peak and corresponding frequency in different groups, where # denoted that cells were treated with aspirin.

3) Figure of merit (FoM) indicates the comprehensive performance of the sensor, the detected sample and the measuring system.

For resonance sensing, these parameters are defined as:

\[ S = \frac{\Delta f}{\Delta n} \]  \hspace{1cm} (4)

\[ Q = f_0 / \text{FWHM} \]  \hspace{1cm} (5)

\[ \text{FoM} = \frac{S}{\Delta f'} = \frac{\Delta f}{(\Delta n \cdot \Delta f')} \]  \hspace{1cm} (6)

where \( \Delta f \) is the frequency shift of the resonance peak, \( \Delta n \) is the change in the number of cells per unit volume, \( f_0 \) is the resonance center frequency of the transmission spectrum of the sensor, FWHM is the full width at half maximum of the resonance peak and \( \Delta f' \) is the minimum resolvable frequency of the spectral system. The accuracy of the detected cell concentration depends not only on the sensing measurement system, but also on the sensitivity of the sensor and the characteristics of the detected cells. The detection accuracy \( (A) \), that is the minimum change of the cell concentration that can be resolved, can be determined as follows

\[ A = \frac{1}{\text{FoM}} \]  \hspace{1cm} (7)

The performance parameters of THz resonance sensing were calculated in Table 1. We could see that, although the resonance intensity of the resonance sensor exceeds 30 dB, the Q value was not high, only 7.4. This also resulted in a low sensitivity and FoM. Compared with the results of
the three types of cells, the sensitivity and FoM of 293 T reached 17.5 GHz·mL/10^5 cells and 2.8 mL/10^5 cells, respectively, but those of HepG2 were only 5 GHz·mL/10^5 cells and 0.8 mL/10^5 cells, respectively. Therefore, because of the limitations of the FWHM, frequency shift value of the resonance sensor and the frequency sampling resolution of the measurement system, the sensing performances of the traditional resonance sensing method remain to be improved.

**Table 1. THz resonance sensing performance**

|       | An (10^5 cells/mL) | Af (GHz) | Q  | S (GHz·mL/10^5 cells) | FoM (mL/10^5 cells) |
|-------|-------------------|----------|----|-----------------------|---------------------|
| 293T  | 2.5               | 43.75    |    | 17.5                  | 2.8                 |
| B16   | 1.8               | 18.75    | 7.4 | 10.4                  | 1.7                 |
| HepG2 | 2.5               | 12.5     |    | 5                     | 0.8                 |

f_0 = 787.5 GHz, FWHM = 106.25 GHz, Δf/τ = 6.25 GHz.

### 3.2.2. Terahertz polarization sensing based on metasurface microsensor

Next, we adopted a new polarization sensing method as shown in Fig. 1(c) and 3(b), based on the same metasurface microsensor but in different ways of getting sample information. Here, the time-domain signals of the output ±45° LP polarization components were measured by the THz-TDPS system through rotating THz polarizer to ±45°. From the view of information acquisition, the information obtained by this method was at least twice as much as the former one discussed in Section 3.2.1, and in fact, all electromagnetic information of output signal was completely extracted, including the amplitude, phase and polarization state. The original time domain data of ±45° LP polarization components through the metasurface sample are shown in Fig. 6(a)–6(c). And we obtained the phase difference of transmitted ±45° LP polarization components Δδ(ω) = δ_45°(ω) – δ_{–45°}(ω) of the three cell lines, as shown in Fig. 6(d)–(f), which indicate the dramatic changes of the phase shift in the THz regime due to the strong THz anisotropy of the sensor device.

![Fig. 6. Time-domain pulses and frequency-domain phase difference of ±45° LP polarization components.](image)
Here, the amplitude and phase of two orthogonal LP signals were processed to obtain two polarization parameters, i.e., the polarization ellipticity and polarization rotation angle. The signs of polarization ellipticity reflected the chirality of the output light, that positive values mean right-handed rotation and negative values mean left-handed rotation. The ellipticity ranged from -45° to 45°, where 0° corresponded to LP waves, 45° corresponded to right-handed circularly polarized (RCP) waves, and -45° corresponded to left-handed circularly polarized (LCP) waves. In fact, the spectrum of polarization ellipticity is equivalent to the circular dichroism (CD) spectrum. The polarization rotation angle ranged from -90° to 90°, at which the polarization direction of the output wave was rotated with respect to the polarization direction of the incident wave, displaying the optical rotation dispersion (ORD) spectrum. The clockwise direction of the polarization rotation angle was positive, while the counterclockwise direction was negative. The data processing of ellipticity and polarization rotation angle was described in details in the Section 2.2.

The polarization ellipticity and polarization rotation angle spectral curves of three cell layers coating on the metasurface were shown in Fig. 7. There was a sudden change in both ellipticity and rotation angle near the resonance frequency of 0.78 THz for blank metasurface, indicating that the polarization state of the emitted light varies sharply with the frequency. For polarization sensing, the quality factor Q is also defined by Eq. (5). The Q factors of the ellipticity and rotation angle could be obtained as 31 and 39.4 as shown in Table 2 and 3. The ellipticity changes from -15.5° to 26° and the rotation angle ranged from -39° to 4°, providing a high sensitivity for detecting based on the variation of polarization state. Compared with the resonance sensing, we could see that the Q factor of polarization sensing was 4~5 times higher than that of resonance sensing with the same sensor device but the different sensing method.

![Fig. 7. Transmission wave polarization information of three cell lines. (a)–(c) Polarization ellipticity spectral curves of the three cell lines coated on the microsensor. (a): 293T, (b): B16, (c): HepG2. (d)–(f) Polarization rotation angle spectral curves of the three cell lines coated on the microsensor. (d): 293T, (e): B16, (f): HepG2.](image)

The variation trends of above two polarization parameters of three cells were similar. After coating the cell layer without aspirin treatment, the peaks of two polarization parameter curves of the sensor were reduced to almost disappear. However, the peaks reappeared after the aspirin treatment, but both red-shift by 100 GHz compared with blank metasurface sensor. Taking B16 as an example, the maximum value of ellipticity became 24.5°, which was reduced to 1.5°, moreover, the peak value of rotation angle decreased from 5.5° to -33.5° compared with the
Table 2. THz polarization sensing performance with ellipticity

|       | ∆n (10^5 cells/mL) | ∆θ (°) | Q   | S (°-mL/10^5 cells) | FoM (mL/10^5 cells) |
|-------|-------------------|--------|-----|---------------------|---------------------|
| 293T  | 2.5               | 16.9   | 6.8 | 13.6                |
| B16   | 1.8               | 21.3   | 31  | 11.8                | 23.6                |
| HepG2 | 2.5               | 10.7   | 4.3 | 8.6                 |

f₀ = 775 GHz, FWHM = 25 GHz, ∆θ' = 0.5°.

Blank metasurface as shown in Fig. 7(b) and 7(e), implying that the changes in two polarization parameters reflect the changes in cell’s number. It suggests that the changes in the number of cells are associated with the frequency shifts in polarization spectral peaks, so that it leads to the changes in polarization parameters (i.e. polarization ellipticity and polarization rotation angle). The significant changes of polarization sensing spectral lines indicate the inhibitory effect of aspirin on cell proliferation.

Next, the effects of aspirin were analyzed for the polarization states of some special frequency points. The frequency points in Fig. 8(a)–(c) corresponded to the peaks of the ellipticity spectra, and the frequency points in Fig. 8(d)–(f) corresponded to the peaks of the rotation angle spectra after aspirin treatment. Figure 8(a)–(c) showed that the polarization ellipticity was significantly increased in the cells treated with aspirin. HepG2 displayed a small ellipticity of about 18°, while 293T and B16 demonstrated an ellipticity of more than 20°. In Fig. 8(d)–(f), after the blank metasurface was coated with different cell layers, the polarization direction rotated counterclockwise. And after adding aspirin, the polarization direction was rotated counterclockwise by a larger angle. Compared with the other two cells, the rotation angle of aspirin-treated B16 cells was larger, reaching to -33.5°.

Fig. 8. THz polarization ellipse of the output waves through the different samples. (a)–(c) THz polarization state of three cell lines at the peak frequencies of polarization ellipticity spectrums. (a): 293T, (b): B16, (c): HepG2. (d)–(f) THz polarization state of three cell lines at the peak frequencies of respective polarization rotation angle spectrums. (d): 293T, (e): B16, (f): HepG2.
For polarization sensing, the sensitivity $S$ and figure of merit FoM can be calculated by the following formulas:

$$S = \frac{\Delta \theta}{\Delta n}$$

(8)

$$\text{FoM} = \frac{S}{\Delta \theta'} = \frac{\Delta \theta}{(\Delta n \cdot \Delta \theta')}$$

(9)

where $\Delta \theta$ is the change of polarization ellipticity or rotation angle, $f_0$ is the resonance center frequency of the sensor’s ellipticity spectrum or optical rotation spectrum, FWHM is the full width at half maximum of the resonance peak and $\Delta \theta' = 0.5^\circ$ is the minimum resolvable polarization angle of the spectral system.

The performance parameters of THz polarization sensing were summarized in Table 2 and 3. Compared with the resonance sensing, the physical parameter of the polarization sensing became the changing value of ellipticity or rotation angle, instead of frequency shift value as shown in Section 3.2.1. The results showed that the sensitivity and FoM of ellipticity were slightly larger than those of rotation angle for 293T cells, but smaller for B16 cells and HepG2 cells, which indicated the complementarity of the two sensing parameters. For the different cells, the sensing performances were quite different. The sensitivity and FoM of B16 reached 16.6 $^\circ\cdot$mL/10$^5$ cells and 33.2 mL/10$^5$ cells in the rotation angle, respectively, but those of 293T was only 5.8 $^\circ\cdot$mL/10$^5$ cells and 11.6 mL/10$^5$ cells, respectively. Notably, the sensitivity of the two methods (resonance sensing v.s. polarization sensing) cannot be directly compared due to the different physical parameters in sensing, but the comparison of Q factor and FoM is available. Because Q factor is not related to the type of cells, only reflecting the performance of device itself, we find that the Q factor of polarization sensing is 4~5 times higher than that of resonance sensing. Although FoM is related to the type of sample, combining the results of the three cell types, we can conclude that FoM of the polarization sensing is at least one order of magnitude higher than that of the resonance sensing.

| Table 3. THz polarization rotation sensing performance with rotation angle |
|-------------------------|--------|-------|-------|-------|
| $\Delta n$ (10$^5$cells/mL) | $\Delta \theta$ ($^\circ$) | $Q$ | $S$ (°·mL/10$^5$ cells) | FoM (mL/10$^5$ cells) |
| 293T | 2.5 | 14.6 | 5.8 | 11.6 |
| B16 | 1.8 | 29.9 | 39.4 | 16.6 | 33.2 |
| HepG2 | 2.5 | 15.3 | 6.1 | 12.2 |

$f_0 = 787.5$ GHz, FWHM = 20 GHz, $\Delta \theta' = 0.5^\circ$.

From the Eqs. (6)–(8), we know that FoM also reflects the detection accuracy ($A$). According to Tables 1–3, the detection accuracy of three sensing methods can be obtained for each cell line. Take B16 cells for example, $A = 5.88\times10^4$ cells/mL for resonance sensing, $4.2\times10^3$ cells/mL for polarization sensing with ellipticity and $3.0\times10^3$ cells/mL for polarization sensing with rotation angle. Recently, Zhang et. al reported a cell biosensing work based on an anisotropic resonant metasurface [41]. The dependences of resonant frequency and the phase slope on the cancer cell concentration at different polarization angles are used for THz sensing parameters, which get the minimum cell concentration of $1\times10^4$ cells/mL. Although the polarization-dependent information is introduced into the sensing, which greatly increases the effective information of the sensor and improves the detection accuracy, this work still belongs to resonance sensing. In our work, the polarization parameters are directly detected using THz sensing, and a higher FoM has been obtained. The minimum cell concentration reduces to $3.0\times10^3$ cells/mL.

4. Conclusions

Taken together, we adopt a newly polarization sensing method based on metasurface microsensor. Here, we can summarize three important characteristics of the polarization dependent metasurface
for THz polarization sensing method as follows: 1. the geometry of the metasurface unit cell should be anisotropic, that is polarization dependent; 2. this metasurface should have a high Q resonance in the THz regime; 3. the device also needs to have strong polarization conversion for THz waves, so that the polarization state of the beam can change dramatically with the frequency. These three conditions are associated with each other, resulting in the polarization state of the output waves through the metasurface is very sensitive to the changes of the surface environment. Since the surface of the sensor is coated with cell layer, the changes of THz optical properties (i.e. the refractive index, absorption and possible optical activity) caused by cell numbers lead to resonance frequency shift and the changes of polarization state. By detecting the change of polarization state through the sensor, we can deduce the change of cell number on the sensor. This is the basic principle of THz polarization sensing.

By using THz-TDPS system, all electromagnetic information of the output signal can be completely extracted, including the amplitude, phase and polarization state. The frequency of resonance peak, polarization ellipticity and polarization rotation angle are used as sensing parameters to assess the cell proliferation. The ellipticity changes from -15.5° to 26° and the rotation angle range from -39° to 4°, providing a high sensitivity approach based on the variation of polarization state. The quality factor of polarization sensing is 4–5 times higher than that of the traditional resonance sensing, and its figure of merit is at least one order of magnitude higher than that of the resonance sensing with the same metasurface microsensor. The minimum cell concentration reduces to 3.0×10^3 cells/mL for polarization sensing with rotation angle, compared to 5.88×10^4 cells/mL for resonance sensing. Our finding provides new insights into the application of THz polarization sensing in monitoring cell proliferation from physical perspective, which is a promising approach for biological studies.

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Disclosures

The authors declare no conflicts of interest.

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