Metasurface Enhanced Infrared Spectroscopy for Live Cells

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Primary CNF Tools Used: JEOL 9500, SC4500 evaporator, Zeiss Supra SEM, PDMS casting station, Anatech resist strip

Abstract:

Infrared (IR) spectroscopy is a powerful tool for biological analyses since it is a label-free, non-invasive technique that provides information on molecular composition. IR spectroscopy of live cells, however, remains challenging due to the strong attenuation of mid-IR light in water. In our lab, we are investigating the use of metasurface-enhanced infrared spectroscopy (MEIRS) to measure live cells in cell culture. The cells are grown on a nanoplasmonic metasurface and we utilize the strong near-field hotspot of the plasmonic nanoantennas to collect IR spectra from the cells. We have demonstrated the spectral imaging of cells adhered on the metasurface as well as spectroscopically probing the response of the cells to different drugs.

Summary of Research:

Infrared (IR) spectroscopy, in which materials are identified through their molecular vibration fingerprints, has a wide range of applications in chemistry, geology, and material sciences. Applied to biological tissues, IR spectroscopy can be used as a histology or cytopathology tool, identifying tumor tissues from normal tissues and looking at the effect of chemotherapeutics on cancer cells. We have developed a new technique, using surface-enhanced infrared absorption (SEIRA) from plasmonic metasurfaces to probe the IR absorption of biomolecules and cells. This technique, which we named metasurface-enhanced infrared spectroscopy (MEIRS), had been used to measure protein monolayers [1], as well as fixed cancer cells [2]. Our current work involves further extending this technique to the measurement of live cells grown on the metasurface, in particular focusing on the spectroscopic investigation of different drugs and stimuli on these cells.

We fabricate our plasmonic metasurface in the CNF cleanroom. Starting from an IR-transparent CaF_2 substrate, we define the patterns on poly(methyl methacrylate) (PMMA) using electron-beam lithography with the JEOL 9500 system. This is followed by gold evaporation and lift off in acetone to create a working metasurface device. Scanning electron microscopy (SEM) image of our plasmonic metasurface is shown in Figure 1. In order to grow the cells on this metasurface and deliver different drugs as needed, we use a polydimethylsiloxane (PDMS) microfluidic cell culture, which is also fabricated at the CNF.

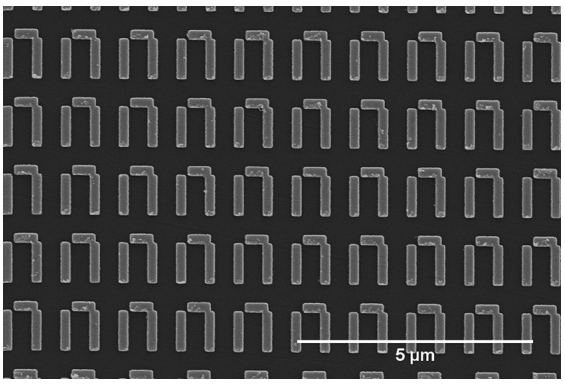

Figure 1: SEM micrograph of the plasmonic metasurface. The metasurface nanoantennas are fabricated in an array covering approximately 250 µm × 250 µm. These nanoantennas are designed to have specific optical resonances, matching the molecular vibrations of the biomolecules of interest (primarily lipids and proteins). Scale bar: 5 µm.

To measure live cells, the metasurface is treated with fibronectin and the cells are initially seeded on the metasurface in a multi-well dish. After the cells are adhered on the metasurface, the metasurface is assembled on a PDMS microfluidic cell culture chamber and measured in reflection mode with an IR microscope coupled to a Fourier Transform IR (FTIR) spectrometer. Our plasmonic metasurface has optical resonances in the

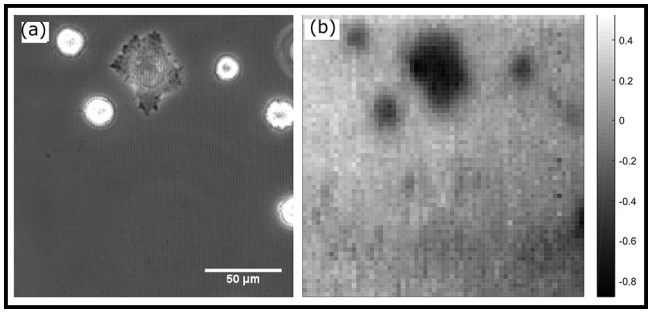

Figure 2: IR spectroscopic imaging using MEIRS combined with a focal plane array detector. (a) Phase contrast microscopy of HeLa cells grown on a metasurface. (b) The same metasurface imaged through MEIRS. The score of a principal component from PCA is plotted here. The MEIRS image reflects the cell's adhesion to the metasurface. Scale bar: 50 µm.

mid-IR, and when probed by IR light, it generates intense plasmonic "hotspots" in the vicinity of these plasmonic nanoantennas, with a penetration depth of 50-100 nm into the surrounding. Sensing cells with our metasurface relies on the spatial overlap between the cells and these plasmonic hotspots, and thus our signal is very sensitive to the degree of cell adhesion on the metasurface. Figure 2 shows a comparison between an image of HeLa cells on the metasurface obtained with phase contrast microscopy and the same cells imaged through MEIRS using an IR focal plane array detector. The MEIRS image was obtained by processing the spectra using principal component analysis (PCA). The distribution and morphology of the cells seen with the two techniques agree well, and this demonstrates that we can clearly see cells adhered on the metasurface through MEIRS.

We have applied MEIRS to the study of chemotherapeutic drugs on cancer models. Here, we measured the spectroscopic response of feline carcinoma cells to four drug combinations, control (no drug), salinomycin, doxorubicin, and a combination of both salinomycin and doxorubicin. Salinomycin is a known inhibitor of multidrug resistance protein 1 in cancer cells, and although it does not lead to high cytotoxicity alone, it increases the potency of doxorubicin when administered in combination. Figure 3 shows the MEIRS spectra measured from these cells after 2-days drug treatment. Cells treated with the combination treatment had much lower IR absorption due to proteins and lipids, and this is attributed to the cell detachment from the metasurface caused by doxorubicin-induced apoptosis. This result agrees with what we expect from salinomycin acting as a sensitizer for doxorubicin. The spectra were further analyzed by PCA and the score plot for the 1st and 2nd principal components (PCs) is shown in Figure 4. From the PCA score plot, clear separation between the cells with different treatment can be seen, even for treatments that do not significantly affect cell viability (control vs. salinomycin).

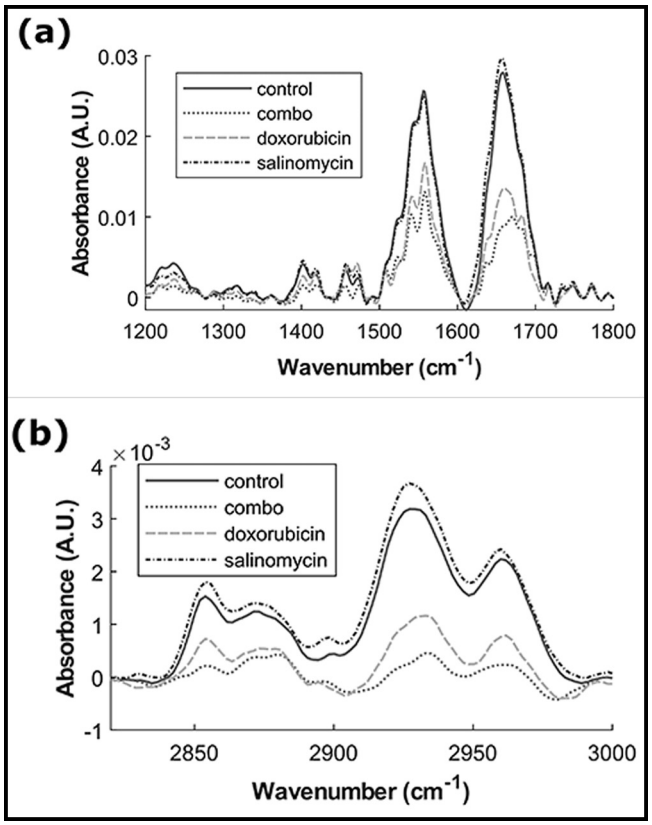

Figure 3: MEIRS spectra for feline carcinoma cells with different drug treatments. (a) Fingerprint region and (b) Lipid absorption region.

References:

[1] Wu, C., et al. Fano-resonant asymmetric metamaterials for ultrasensitive spectroscopy and identification of molecular monolayers. Nat. Mater. 11, 69-75 (2011).

[2] Kelp, G., et al. Application of metasurface-enhanced infra-red spectroscopy to distinguish between normal and cancerous cell types. Analyst 144, 1115-1127 (2019).

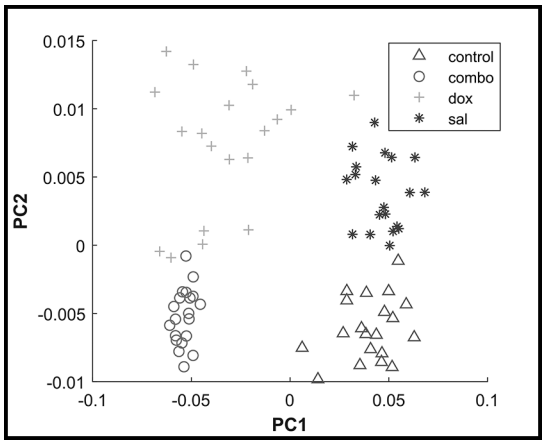

Figure 4: PCA score plot of the IR absorbance spectra from cells with different drug treatments. Data points for cells with the same treatment cluster among themselves, and cells with different drug treatment can be clearly distinguished from each other.