Metasurface-Enhanced Infrared Spectroscopy for Real-Time Measurement of 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 for the label-free, nondestructive analysis of biological samples is a rapidly expanding area of research. When combined with multivariate statistical analysis and machine learning techniques, IR spectroscopy has been shown to be a powerful tool that can distinguish different types of tissues and cells. However, current applications of IR spectroscopy for live cells are limited due to the strong attenuation of IR light in water. In our lab, we have developed Metasurface-Enhanced Infrared Spectroscopy (MEIRS) as a novel tool to perform spectral analysis of live cells in standard cell culture conditions. The cells are cultured on plasmonic nanoantennas (metasurface), and the plasmonic hotspots are used to enhance the IR signal. We have used MEIRS to track the changes in the cells *in situ* as they are introduced to different stimuli. We are also investigating the combination of MEIRS with electrical-impedance sensing to create a multi-modal cellular assay.

Summary of Research:

Infrared (IR) spectroscopy is widely used to identify chemical compounds through their molecular vibration fingerprints.

Recently, IR spectroscopy has found applications in biological analysis as a tool for histology and cytopathology, identifying tumor tissues from normal tissues and monitoring the effect of chemotherapeutics on cancer cells. However, IR light is strongly attenuated in water, and thus traditional IR spectroscopy often involves fixing and drying the biological sample, limiting its utility as a cellular assay. We have developed a novel technique that we named metasurface-enhanced infrared spectroscopy (MEIRS) to measure live cells in physiological conditions.

In MEIRS, cells are seeded on a planar array of gold plasmonic nanoantennas called metasurfaces. These resonant nano-antennas support plasmonic hot spots in their vicinity, enhancing the light-matter interaction. The IR absorption signal from cells interacting with these plasmonic hot spots can be measured through the reflected light, overcoming the attenuation in water.

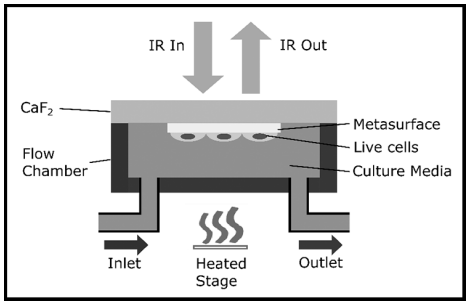

Figure 1: Schematic drawing of the flow-chamber setup for in situ *IR spectroscopy of live cells.*

In the past, we have used this metasurface for the IR spectroscopy of protein monolayers [1] as well as fixed and dried cells [2]. Our current work focus on further extending this technique to the measurement of live cells on the metasurface, monitoring their changes in response to different compounds.

The plasmonic metasurfaces are fabricated in the CNF cleanroom. Metasurfaces are fabricated on IR-transparent CaF_2 substrates. First, patterns are defined 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 the gold nanoantennas.

Once the fabrication is done, Anatech resist strip is used to clean the metasurface and remove any resist residues. To perform *in situ* spectroscopy with live cells, we use a polydimethylsiloxane (PDMS) based flow chamber to maintain physiological conditions, which is also fabricated at the CNF. A schematic drawing of our measurement setup is shown in Figure 1.

Human squamous carcinoma cell line A431 cells were seeded on metasurfaces coated with fibronectin. Scanning electron microscope (SEM) images of the cells on the metasurface (Figure 2) shows that the cells preferentially attach to the gold nanostructures rather than the CaF_2 substrate. The interaction between the cells and the metasurface is still under investigation, but cellular attachment on gold nanoantennas implies that there is a significant spatial overlap between the plasmonic hotspot and the cells, which can lead to a strong enhancement in the detected signal.

We have monitored the response of A431 cells to 10 mM methyl-beta-cyclodextrin (MBCD), a compound known to extract cholesterol from the cellular membrane. The result was analyzed by principal component analysis (PCA) followed by promax rotation (Figure 3). Two different cellular processes, with different spectral features as well as temporal dynamics, can be seen. We attribute component 1 to the detachment of adhesion sites from the metasurface, while component 2 is thought to reflect changes in IR light scattered by the cells due to changes in cell morphology. We also found significant changes in the IR absorption from lipids right after MBCD was added to the flow chamber, which is attributed to the cholesterol extraction from the cellular membrane.

We are also investigating the combination of electrical impedance-based measurement with our metasurface-enhanced spectroscopy. The cell electrical impedance sensing (ECIS) is a proven technique to monitor *in situ* cell behaviors. The technique involves growing the cells on a surface with patterned microelectrodes and monitoring the changes in electrical impedance as the cells undergo certain changes.

Figure 4 shows A431 cells on the microelectrodes in our prototype device. The electrodes are connected to an impedance analyzer (we use an oscilloscope for preliminary tests) that uses AC voltage to measure the voltage and current across the attached cells. The electrodes of ECIS must be patterned in a specific way such that the attached cells block the current between electrodes. Different phases of cell attachment, spreading, migration, and proliferation can be distinguished through the change in impedance. We believe that although both ECIS and MEIRS detect phenotypical changes in the cells, there is a subtle difference in their signal, and this could provide more insight into the biological processes occurring in the cells.

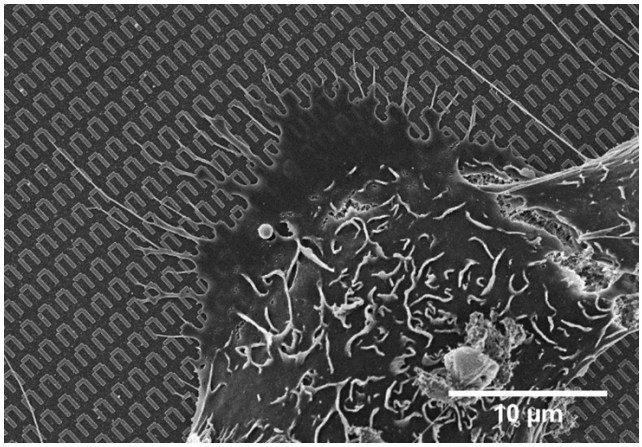

Figure 2: SEM image of A431 cells grown on the plasmonic metasurface.

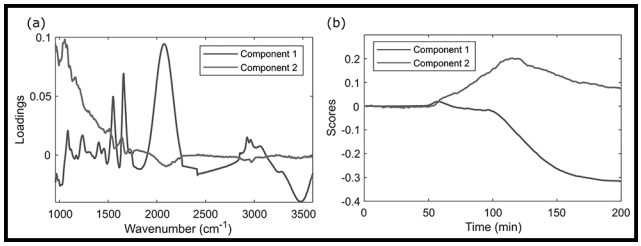

Figure 3: The spectral changes of A431 cell in response to MBCD. The results were analyzed using PCA, followed by promax rotation. (a) Component loadings and (b) the associated component scores. MBCD arrives at the flow chamber at approximately t = 45 min.

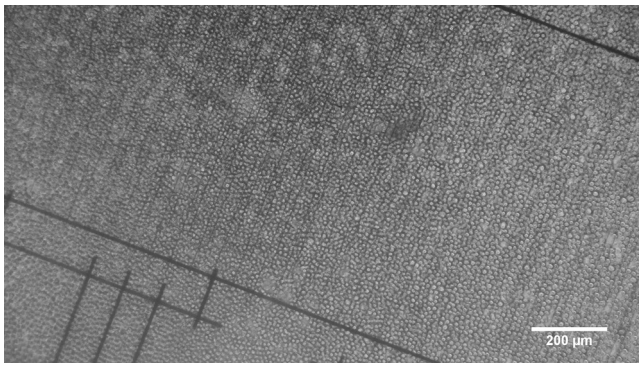

Figure 4: A431 cells on a microelectrode-patterned surface for electrical impedance measurement.

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).