Metasurface-Enhanced Infrared Spectroscopy for the 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, Glen 1000 Resist Strip, Oxford PECVD, Oxford ALD FlexAL, Plasma-Therm 740, DISCO Dicing Saw

Abstract:

We have developed Metasurface-Enhanced Infrared Spectroscopy (MEIRS) as a novel tool to perform spectral analysis of live cells. In MEIRS, cells are cultured on plasmonic nanoantennas (metasurface), which enhances infrared absorption through the plasmonic hotspots. Various cellular responses can be observed from the IR absorption spectra collected in real-time. Our current work focuses on expanding the application of MEIRS through the integration of metasurface with multi-well cell culture chambers for high-throughput measurements, exploring the use of MEIRS to measure cellular response from chemotherapeutics, as well as combining plasmonic metasurfaces with nano-topography to study cell-nanostructure interactions.

Summary of Research:

Infrared (IR) spectroscopy is widely used to identify chemical compounds through their molecular vibration fingerprints and has recently found many applications in biological analysis. We have developed a novel technique called Metasurface-Enhanced Infrared Spectroscopy (MEIRS) to measure live cells in physiological conditions. In MEIRS, cells are grown on an array of plasmonic nanoantennas called metasurfaces. These resonant nanoantennas support plasmonic hot spots, enhancing the light-matter interaction and IR absorption. In the past, we have used MEIRS to detect spectroscopic changes in response to cellular dissociation and cholesterol depletion [1]. Our current work focuses on expanding the application of MEIRS through the integration of metasurface with multi-well cell culture chambers for high-throughput measurement, exploring the use of MEIRS to measure cellular response from chemotherapeutics, as well as combining plasmonic metasurfaces with nano-topography to study cell-nanostructure interactions.

Figure 1 shows a schematic drawing of the MEIRS measurement setup for live cells.

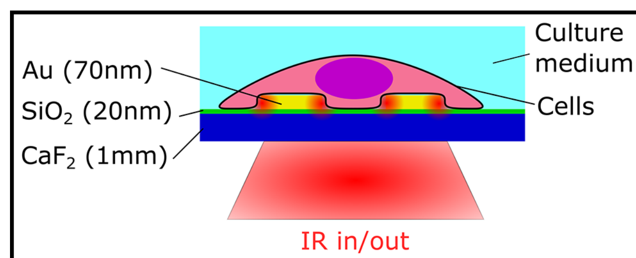

Figure 1: Schematic drawing of the metasurface-enhanced infrared spectroscopy setup for live cell measurement.

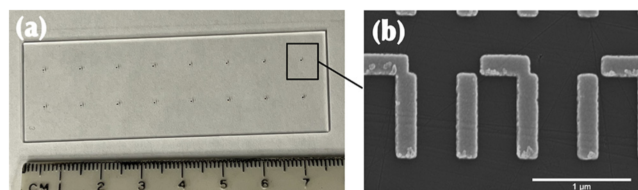

Figure 2: Metasurface integration with multi-well cell culture chamber. (a) 16 metasurface patterns fabricated on 1" × 3" CaF_2 slide, to be attached to a cell culture chamber superstructure (not shown). (b) SEM images of the metasurface. Scale bar: 1 µm.

In order to make metasurface compatible with standard cell culture in multi-well format, metasurface devices are fabricated as repeating patterns on top of a $1^2 \times 3^2$ infrared transparent CaF_2 substrate (Figure 2). The fabrication starts with a 4-inch diameter CaF_2 window, which is coated with 20 nm of SiO_2 using Oxford PECVD as a protection layer.

Metasurface patterns are defined using electron-beam lithography with the JEOL 9500 system and poly(methyl methacrylate) (PMMA) as the resist.

Then, 5 nm Cr and 70 nm Au are deposited using SC4500 evaporator. The 4-inch CaF_2 window is cut into $1^2 \times 3^2$ pieces using the DISCO dicing saw.

As the final step, Anatech or Glen 1000 Resist Strip is used to clean the metasurface sample. Commercial superstructures for multi-well cell culture chambers are attached to this metasurface for cell culture.

We used MEIRS to measure the response of live cancer cells to a novel chemotherapeutic metal complex: tricarbonyl rhenium isonitrile polypyridyl (TRIP) [2]. This drug is an endoplasmic reticulum (ER) stress inducer and regulates important biological functions such as protein synthesis. By analyzing the measured infrared spectra using linear regression, we extract the temporal changes in proteins and lipids IR absorption and local refractive index changes from the shift of metasurface plasmonic resonance. In addition, the local concentration of TRIP can be observed through its intrinsic IR absorption at the carbonyl stretching modes and is useful in monitoring the precise drug delivery time. Figure 3 shows the protein IR absorption signal of the cells in response to TRIP treatment.

We have observed an increase in protein signal in the short-term (tens of minutes) and a reduction in protein signal in the long-term (several hours). This result demonstrates that MEIRS is an effective label-free real-time cellular assay capable of detecting and interpreting the early phenotypic responses of cells to chemotherapeutics.

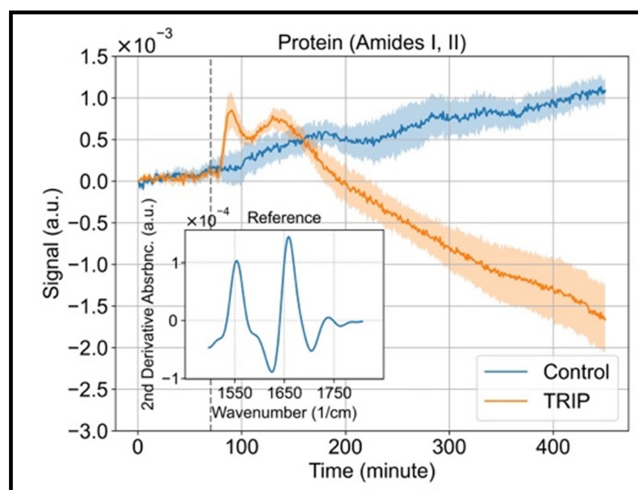

Figure 3: Cellular response to TRIP treatment. Inset: reference spectra of amide I and amide II bands attributed to proteins, used for linear regression. Dashed black line shows the timing of TRIP arrival.

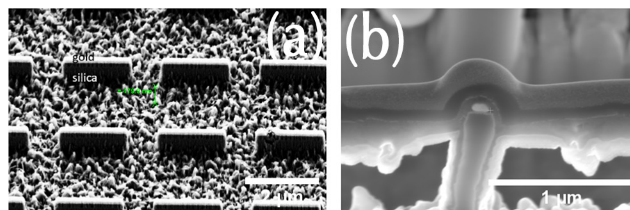

Figure 4: Nanoantenna-on-nanopillar structure. (a) SEM image of the nanoantenna-on-nanopillar structure without cells. Scale bar: 2 µm. (b) Cross section SEM image of one nanoantenna, with a cell adhered on top. Cell membrane can be seen curved around the nanoantenna-on-nanopillar. Scale bar: 1 µm.

Another direction in this project is the combination of metasurfaces with nano-topography to study cell-nanostructure interactions. Current research in surface nano-projections has shown that they can be used as effective tools to manipulate cellular attachment. We use nanopillars to incite physical and chemical responses in cells, which are then monitored through MEIRS.

We have fabricated gold nanoantennas on top of silica nanopillars (Figure 4(a)). The fabrication process starts with growing a layer of silica (~1µm) on top of CaF_2 substrate using the Oxford PECVD. Next, metasurface patterns are defined using electron-beam lithography (JEOL 9500). Gold is deposited in the patterned region using the CVC SC4500 even/odd hour evaporator. We also deposit a thin layer of chromium above the gold nanoantenna and use it as a mask to chemically etch the silica using the Plasma-Therm 740. When cells attach to such nanopillar structures, cell membrane curves around these nanopillars (Figure 4(b)), increasing the overlap between the metasurface hotspots and the cells and also increasing the concentration of certain proteins (actin, clathrin) in the metasurface hotspots.

Spectroscopically, we have observed that IR absorption from these cells on the nano-contoured metasurfaces is enhanced and shows different spectral features compared with cells on flat metasurfaces, likely related to protein secondary structures.

References:

[1] Huang, S. H., Li, J., Fan, Z., Delgado, R. and Shvets, G. Monitoring the effects of chemical stimuli on live cells with metasurface-enhanced infrared reflection spectroscopy. Lab Chip 21, 3991-4004 (2021).

[2] Shen, P.-T., et al. Probing the Drug Dynamics of Chemotherapeutics Using Metasurface-Enhanced Infrared Reflection Spectroscopy of Live Cells. Cells 11, (2022).