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, Oxford PECVD, Oxford ALD FlexAL, Plasma-Therm 720/740, DISCO dicing saw

Abstract:

We have developed Metasurface-Enhanced Infrared Spectroscopy (MEIRS) as a novel tool to perform spectral analysis and chemical imaging of live cells. In MEIRS, cells are cultured on an array of plasmonic nanoantennas (metasurface), which enhances infrared vibrational signal through the coupling of molecular vibrations to plasmonic resonances. Various cellular responses can be observed from the infrared spectra collected in real-time. Our current work focuses on expanding the application of MEIRS to the chemical imaging of live cells 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 nano-antennas support plasmonic hot spots, enhancing the light-matter interaction and IR absorption. We have used MEIRS to detect spectroscopic changes related to cell adhesion and dissociation, cholesterol depletion, response to chemotherapeutics, and activation of intracellular signaling pathways [1-3]. Our current work focuses on expanding the application of MEIRS to mid-infrared chemical imaging, 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.

Our mid-IR metasurface is fabricated as an array of gold nanoantennas on IR transparent CaF_2 substrates. The substrate is first cleaned using oxygen plasma etcher (Anatech or Glen 1000 Resist Strip) and optionally coated with 20 nm of SiO_2 using plasma enhanced chemical vapor deposition (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. 5 nm Cr and 70 nm Au are deposited using SC4500 evaporator. If needed, metasurfaces fabricated on large substrates (up to 4" diameter) are diced to smaller pieces using DISCO dicing saw. As the final step, oxygen plasma etcher (Anatech or Glen 1000 Resist Strip) is used to clean the metasurface sample. The metasurface is then attached to superstructures for cell culture chambers and cells are grown on top of the metasurface for analysis. Figure 2 shows a scanning electron microscope image of a cell on the metasurface.

We are currently investigating the mid-IR chemical imaging of cells on the metasurface using a quantum cascade laser (QCL). An inverted laser point-scanning confocal microscope operating in the mid-IR is designed and built, providing a spatial resolution around 5 μm in the protein absorption bands (amide I and II, 1500 cm^{-1} to 1700 cm^{-1}). A hyperspectral image of formalin-fixed cells on the metasurface, with contrast from protein vibrational absorption, is presented in Figure 3. Although the spatial resolution of mid-IR microscopy is lower than visible microscopy, MEIRS microscopy provides chemical contrast in a label-free manner and is suitable for the long-term monitoring of live cells. We plan to apply this MEIRS microscopy to study the localization of different chemical species in live cells and the variation in spectral signature from a heterogeneous population of cancer cells.

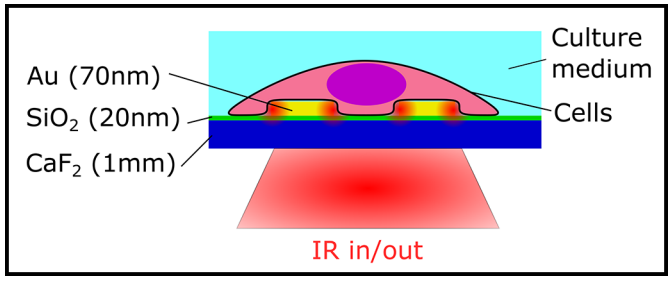

Figure 1: Schematic drawing of the metasurface-enhanced infrared spectroscopy setup for live cell measurement.

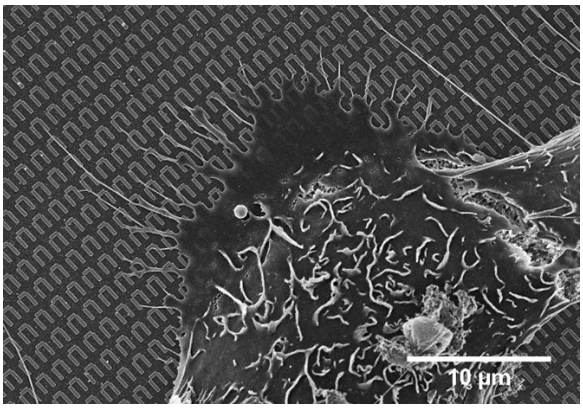

Figure 2: Scanning electron microscope image of a human skin cancer cell on the metasurface. Scale bar: 10 µm.

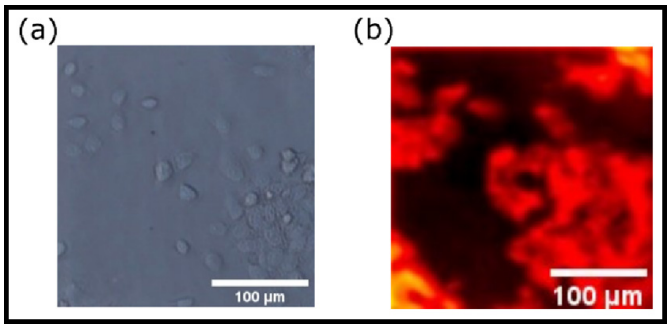

Figure 3: Formalin-fixed human skin cancer cells on the metasurface. (a) Phase contrast image. (b) MEIRS microscopy image with contrast from protein absorption. Scale bar: 100 µm.

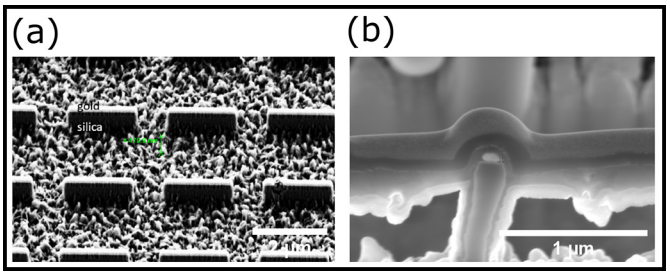

Figure 4: Nanoantenna-on-nanopillar structure. (a) SEM image of the nanoantenna-on-nanopillar structure without cells. Scale bar: 2 µm. (b) Cross section SEM image of one nanoantenna, with a cell adhered on top. Cell membrane can be seen curved around the nanoantenna-on-nanopillar. Scale bar: 1 µm.

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., et al. 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).

[3] Huang, S. H., et al. Metasurface-enhanced infrared spectroscopy in multiwell format for real-time assaying of live cells. Lab Chip 23, 2228-2240 (2023).