Metasurface-Enhanced Infrared Spectroscopy for the Measurement of Live Cells

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

Abstract:

Infrared (IR) spectroscopy for the label-free, nondestructive analysis of biological samples is a rapidly expanding area of research. 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 spectral changes in the cells *in situ* as they are being treated with different chemical compounds. We are also investigating the effect of surface functionalization on the metasurface, with the aim of enhancing cellular signal by increasing the overlap between the cells and the infrared optical field.

Summary of Research:

Infrared (IR) spectroscopy is widely used to identify chemical compounds through their molecular vibration fingerprints and has recently found applications in the biological analysis as a tool for histology and cytopathology, identifying tumor tissues from normal tissues and monitoring the effect of chemotherapeutics on cancer cells. We have developed a novel technique called 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 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 further extending the application of this technique to the measurement of cellular response from chemotherapeutics, as well as exploring different chemical functionalization to improve the sensing capability of the device.

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

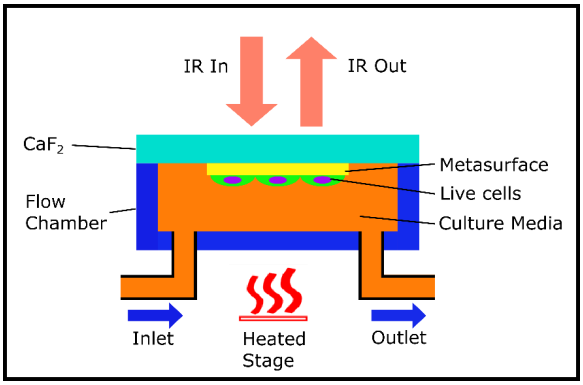

Figure 1: Schematic drawing of the flow-chamber setup for in situ *IR spectroscopy of live cells.*

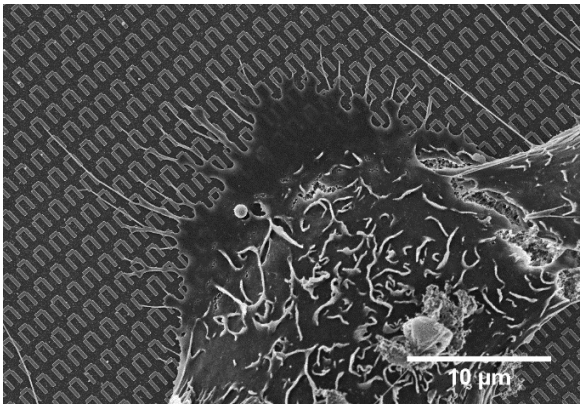

Figure 2: SEM of A431 cells grown on the plasmonic metasurface.

maintain physiological conditions, which is also fabricated at the CNF. A schematic drawing of our measurement setup is shown in Figure 1. We use human squamous carcinoma cell line A431 cells as a model system to investigate the cellular response. 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.

Using MEIRS, we have investigated the action of tricarbonyl rhenium isonitrile polypyridyl (TRIP) complex [2], a novel chemo-therapeutic developed by our collaborator (Wilson group, Cornell University), on A431 cells. TRIP has been previously shown to induced endoplasmic reticulum (ER) stress that eventually leads to apoptosis. Figure 3 shows the detection of protein absorbance signal with MEIRS in real-time while the cells are being treated by TRIP at different concentrations. The control group showed a slight increase in protein signal, while TRIP-treated cells showed clear reduction in protein signal. The protein signal reduction is larger for a higher dosage of TRIP and this is in line with what we expect, given that TRIP can induce unfolded protein response through ER stress.

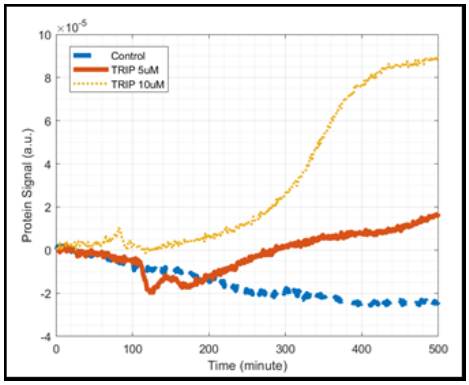

Figure 3: Protein signal from A431 cells in response to TRIP treatment. Infrared spectra collected in real-time are analyzed with principal component analysis (PCA). The spectral features at amide I and amide II absorption peaks are captured by the first principal component, the score from which is shown as the protein signal. The +y direction corresponds to a decrease in protein signal from cells.

To improve the sensitivity of the metasurface we use chemical functionalization to achieve cell blocking on the IR transparent substrate while enhancing their attachment to the metallic antennas. The interaction between the evanescent fields of the metasurface and the cells depends principally on the placement of their focal adhesion on the metasurface. This functionalization process aims to increase the overlap of the cells with the metasurface hotspot, due to which we expect a significant increase in the spectroscopic intensity.

To controllably block cell adhesion on the CaF_2 substrate, it is first coated with a thin layer of silica (~100 nm) using the Oxford PECVD tool, followed by deposition of a self-assembled monolayer of silane conjugated polyethylene glycol (PEG) [3]. The gold nano-antennas are functionalized using n-alkanethiols [3]. This ensures the cells preferentially attach to the nanoantennas. Figure 4 (left) shows A431 cell attachment on an un-functionalized metasurface. We can see how the cells cover the metasurface as well as the substrate. Figure 4 (right) shows A431 cell attachment after the metasurface was functionalized. We can see the cells cover only the metasurface area and are blocked from the substrate region.

References:

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[2] A. P. King, S. C. Marker, R. V. Swanda, J. J. Woods, S. B. Qian and J. J. Wilson, Chem. - A Eur. J., 2019, 25, 9206-9210.

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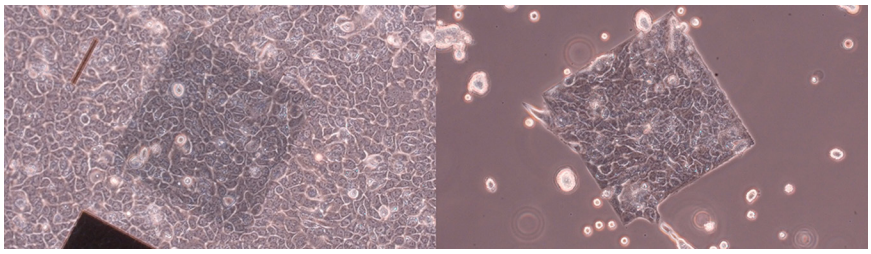

Figure 4: (left) A431 cell attachment on an unfunctionalized metasurface sample. (right) A431 cell attachment on metasurface sample after functionalization.