Metasurface-Enhanced Mid-Infrared Microscopy for Imaging Living Cells

CNF Project Number: 2472-16
Principal Investigator(s): Gennady Shvets
User(s): Steven He Huang, Po-Ting Shen

Affiliation(s): Applied and Engineering Physics, Cornell University

Primary Source(s) of Research Funding: National Cancer Institute of the National Institutes of Health award number R21 CA251052. National Institute of General Medical Sciences of NIH award number R21 GM138947

Contact: gs656@cornell.edu, hh623@cornell.edu, ps944@cornell.edu

Research Group Website: http://shvets.aep.cornell.edu

Primary CNF Tools Used: JEOL 9500, SC4500 Evaporator, Zeiss Supra SEM, PDMS Casting Station, Anatech Resist Strip, Glen 1000 Resist Strip, DISCO Dicing Saw

Abstract:

We have developed Metasurface-Enhanced Mid-Infrared Microscopy (MEMIM) as a novel tool for chemical imaging of living cells. In MEMIM, cells are cultured on an array of plasmonic nanoantennas (metasurface), and their near-field interaction alters the scattering cross-section of the nanoantennas, making the cells visible in the far field. The imaging contrast arises from the mid-infrared vibrational absorption of the cells, primarily originating from the major classes of biomolecules including DNA, proteins, and lipids. Our technology is a label-free, non-destructive imaging technique with chemical contrast, holding wide potential applications in biomedical research.

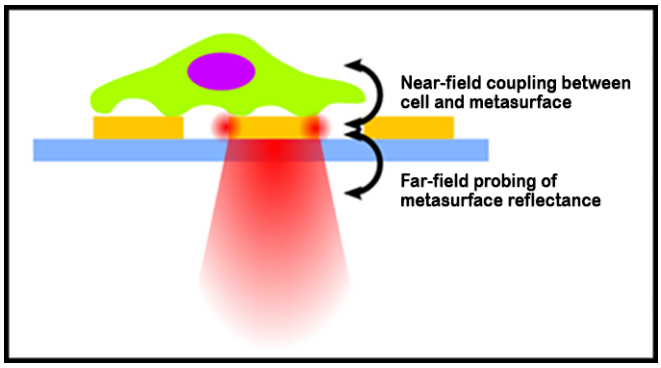

Figure 1: Metasurface-enhanced infrared spectroscopy and chemical microscopy. The near-field interaction of cells with metasurface results in SEIRA, which changes the scattering cross section of the individual nanoantennas. This interaction is probed in the far-field via metasurface reflectance, allowing for non-perturbative infrared spectroscopy of living cells.

Summary of Research:

Infrared (IR) spectroscopy and chemical imaging are widely used to identify chemical compounds through their molecular vibration fingerprints and has found many applications in biological analysis. Yet, the use of IR microscopy for imaging living cells is challenging due to the attenuation of IR light in water, necessitating the use of thin flow cells or attenuated total reflection (ATR) configurations that are difficult to scale to high-throughput measurements. In the past, we have used plasmonic metasurfaces to overcome this challenge, demonstrating the spectroscopic measurement of changes related to cell adhesion and dissociation, cholesterol depletion, and activation of intracellular signaling pathways [1,2]. Recently, we have expanded this technique to a novel chemical imaging technique, called MEMIM, to image living cells in physiological conditions. In MEMIM, cells are grown on an array of resonant plasmonic nanoantennas called metasurfaces. These resonant nanoantennas interact with the cells in the near-field through their plasmonic hotspots, in an effect called surface-enhanced infrared adsorption (SEIRA), leading to a modulation in the far-field scattering cross-section of the nanoantennas. Images of analytes on the metasurface are formed by a mid-infrared laser scanning microscope, focused to the plane of the metasurface (Figure 1).

Our mid-IR metasurface is fabricated as an array of gold nanoantennas on IR transparent CaF2 substrates (0.5 mm thick). The substrate is first cleaned using oxygen plasma etcher (Anatech or Glen 1000 Resist Strip). Metasurface patterns are defined using electron beam lithography with the JEOL 9500 system and poly(methyl methacrylate) (PMMA) as the resist. 5 nm Cr adhesion layer and 70 nm Au are deposited using SC4500 evaporator. 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.

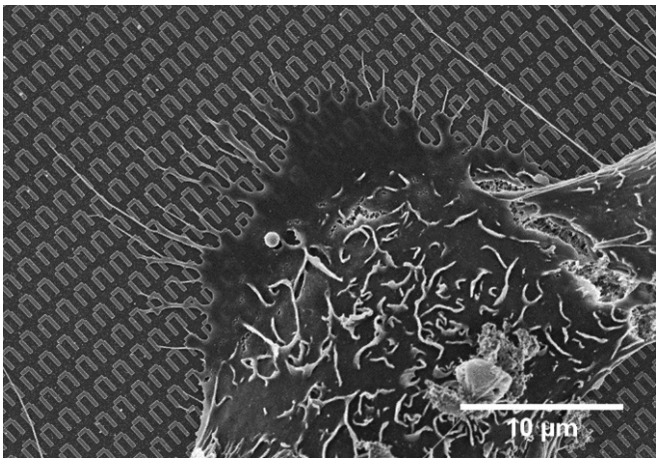

Figure 2: Scanning electron microscope image of a A431 human skin cancer cell on the metasurface. Scale bar: 10 µm.

Figure 2 shows a scanning electron microscope image of a cell on the metasurface.

Our group recently designed and built an inverted point-scanning confocal microscope using a mid-IR quantum cascade laser (QCL) light source. The emission from a QCL is focused on a diffraction-limited spot on the metasurface and scanned across it by moving the sample with a motorized microscope stage. The reflection, modulated by the metasurface's near-field interaction with the analyte, is collected by a liquid-nitrogen-cooled mercury-cadmium-telluride (MCT) mid-IR detector. The lateral resolution of our imaging system is diffraction-limited to about 5 μm.

Using this setup, we have demonstrated the efficacy of MEMIM for label-free imaging of both fixed and living cells on the metasurface. Images of fixed 3T3-L1 fibroblasts (pre-adipocytes), shown in Figure 3, reveal clear chemical contrast from proteins (amide I/II bands, 1500-1700 cm-1) and DNA (PO2- phosphate bands, 1085 cm-1). Comparison with fluorescent images shows good correlation, with phosphate band IR images aligning well with nucleus-stained fluorescence images. The protein IR image also matches the actin-stained fluorescence image, capturing the cell's outer morphology, although differences arise because the protein IR image includes all proteins, not just actin. Lipid droplets in differentiated 3T3-L1 adipocytes can also be imaged through the C=O ester carbonyl vibration of the lipids at 1740 cm-1 (image not shown). We also performed time-lapse imaging of living cells (data not shown) to monitor cell adhesion and motility on the metasurface. These live cell imaging results confirm that living cells can be imaged using our technique without affecting cell viability and behavior under mid-IR light.

Conclusions and Future Steps:

We have developed a plasmonic metasurface-based mid-infrared chemical imaging technology for the label-free, non-destructive imaging of living cells with chemical contrast. Imaging cells with contrast arising from proteins, DNAs, and lipids have been demonstrated. This technique could allow for the non-perturbative metabolic imaging of living cells over several days, which is currently not possible using other similar techniques. We plan to expand this technology to the metabolic imaging of cells using small IR-active metabolic labels, such as azides, deuterium, and 13C. This could help in characterizing heterogeneity in metabolic rates within the cell population and how they are changed through drug treatment or other environmental cues, with implications in metabolic diseases such as diabete and fatty liver diseases.

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] 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).

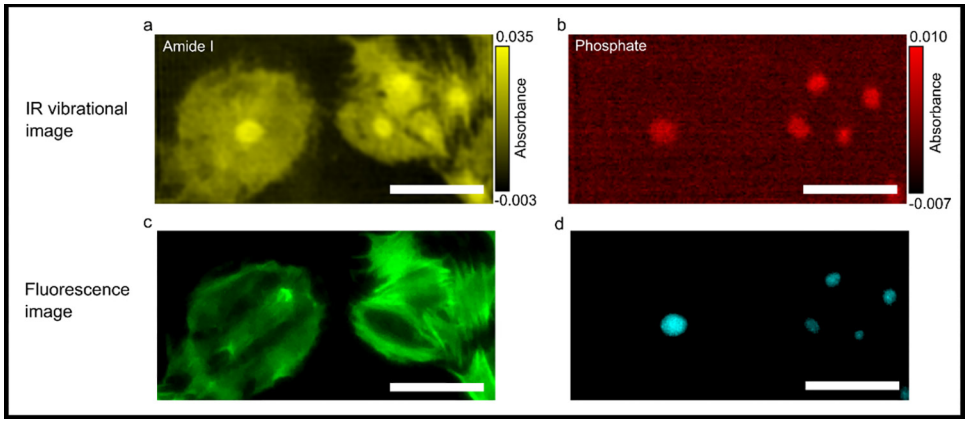

Figure 3: Metasurface-enhanced IR chemical microscopy of fibroblasts. (a): IR vibrational image at amide I band (1655 cm^{-1}, contrast from total proteins). (b): IR vibrational image at phosphate band (1085 cm^{-1}, contrast from DNA). (c): Actin-stained fluorescent image. (d): Nucleus-stained fluorescent image. Scale bar: 100 µm.