Time-Lapse Mid-Infrared Spectroscopy of Live Cells Using High-Aspect-Ratio Metal-on-Dielectric Nanostructures

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Primary CNF Tools Used: JEOL 9500, SC4500 Evaporator, Zeiss Ultra Scanning Electron Microscope, Oxford Plasma Enhanced Chemical Vapor Deposition, Plasma-Therm 740, Anatech Resist Strip, DISCO Dicing Saw

Abstract:

Our group previously introduced Metasurface-Enhanced Infrared Spectroscopy (MEIRS) for spectral analysis and chemical imaging of live cells. MEIRS uses plasmonic nanoantenna arrays (metasurfaces) to enhance infrared signals by coupling molecular vibrations to plasmonic resonances. However, flat plasmonic metasurfaces (2D nanoantennas) have a limited probing volume near the plasma membrane. Inspired by high-aspect-ratio nanostructures, we demonstrate that integrating plasmonic metasurfaces with tall dielectric nanostructures significantly improves FTIR spectroscopy sensing capabilities.

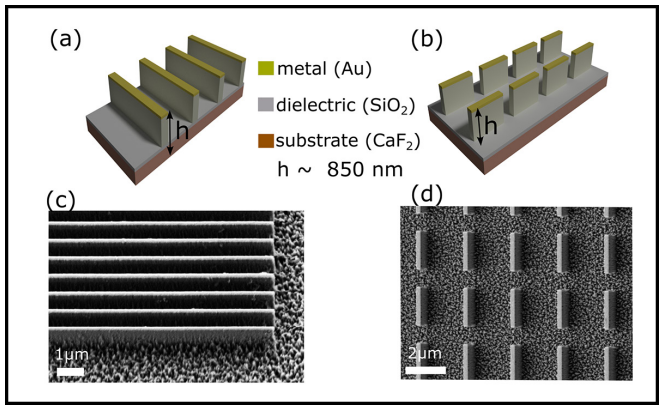

Figure 1: High-aspect-ratio metal-on-dielectric metasurfaces. (a) and (b) show schematic of 3D nanograting device and 3D nanoantenna metasurface respectively. (c) and (d) are SEM images of the fabricated 3D nanograting device and 3D nanoantenna metasurface respectively (Scale bar: 1 μm and 2 μm, respectively).

Summary of Research:

Infrared (IR) spectroscopy is widely employed to identify chemical compounds and has numerous biological applications. We developed Metasurface-Enhanced Infrared Spectroscopy (MEIRS) to measure live cell activity under physiological conditions. In MEIRS, cells are cultured on plasmonic nanoantenna arrays (metasurfaces) that enhance IR absorption. We have utilized MEIRS to detect changes in cell adhesion, cholesterol levels, and intracellular signaling [1,2]. This work improves on MEIRS by combining plasmonic metasurfaces with nano-topography to enhance FTIR sensitivity and pave the way for an optical platform to study cell-nanostructure interactions.

Figures 1(a) and 1(b) illustrate schematics of different device designs we fabricated. Figure 1(a) shows a nanoantenna array atop dielectric pillars (referred to as 3D nanoantennas), while Figure 1(b) displays an array of gratings atop dielectric pillars (referred to as 3D nanogratings). These high-aspect-ratio metasurfaces consist of gold nanoantennas on silica nanopillars deposited on IR-transparent CaF2 substrates. The CaF2 substrate is first cleaned using an oxygen plasma etcher (Anatech Resist Strip) and then coated with a 1 μm layer of SiO2 using plasma-enhanced chemical vapor deposition (Oxford PECVD) to form the dielectric thin film. Metasurface patterns are defined using electron beam lithography with the JEOL 9500 system and poly(methyl methacrylate) (PMMA) as the resist. Layers of 5 nm Cr, 70 nm Au, and 20 nm Cr (as a hard mask for the subsequent etch step) are deposited using the SC4500 evaporator. If necessary, metasurfaces fabricated on large substrates (up to 4" diameter) are diced into smaller pieces using a DISCO dicing saw. The final step involves cleaning the metasurface sample with an oxygen plasma etcher (Anatech Resist Strip).

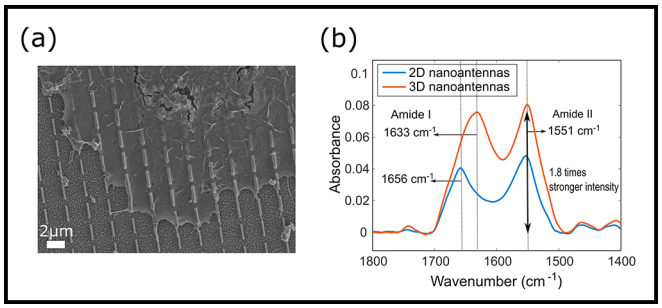

Figure 2: (a) SEM image of a human skin cancer cell on the 3D nanoantenna metasurface. (Scale bar: 2 µm) (b) Comparison of Fourier Transform Infrared Absorbance spectra of human skin cancer cells grown on 2D and 3D nanoantenna metasurfaces.

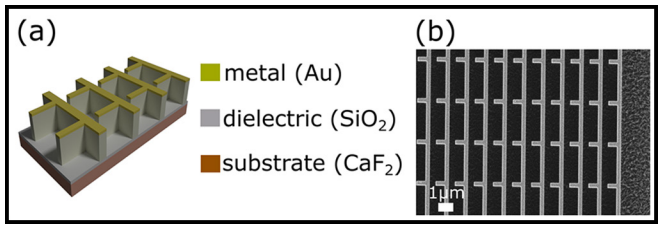

Figure 3: Grantenna — a device which can function as both 3D nanoantenna and 3D nanograting depending on the incident light polarization. (a) Schematic of device. (b) SEM image of fabricated device. (Scale bar: 1 µm).

The metasurface is then attached to superstructures for cell culture chambers, and cells are grown on top of the metasurface for analysis. Figure 1(c) shows the fabricated 3D nanoantennas device, and Figure 1(d) depicts the fabricated 3D nanograting device.

When cells attach to high-aspect-ratio metasurfaces, they exhibit cellular responses not observed with flat 2D nanoantennas. One notable response is the induction of plasma membrane curvature, leading to cell deformation and wrapping around the vertical structures in accordance with the surface nanotopography. This phenomenon of cell wrapping addresses the issue of shallow field penetration seen with 2D metasurfaces, utilizing an optical process called transflection.

Figure 2(a) shows an SEM image of fixed and dried cells grown on these 3D nanoantennas. The nanoantennas, which are 1.8 μm long, reflect light in the spectral region of 1500 cm.. to 1700 cm.., suitable for analyzing proteins (amides) in cells. Figure 2(b) compares the infrared absorbance spectra obtained from cells grown on 2D and 3D nanoantenna metasurfaces. Spectra from the 3D nanoantenna metasurfaces (about 850 nm tall) exhibit a 1.8-fold improvement in spectral intensity in the Amide II region. A signature of unique cellular responses induced by the vertical nanostructures is also evident from the peak shift in the Amide I region observed in Figure 2(b).

While the 3D nanoantennas are resonant structures that reflect light only in specific spectral ranges based on antenna design, the 3D nanograting device shown in Figures 1(b) and 1(d) is a broadband optical device utilizing non-resonant plasmonic structures [3]. This device functions similarly to a wire grid polarizer under normal incidence, reflecting light with an electric field polarized parallel to the gratings when the grating periodicity is much smaller than the incident wavelength. This broadband reflectance device can be used for biological sensing of cells or other analytes in the region between the dielectric pillars. For the 3D nanoantennas, the plasmonic near fields are strongest near the gold nanoantenna, while for the 3D nanograting, the fields are strongest near the substrate (away from the gold layer) due to the standing wave effect. This makes the two devices complementary for studying live cells. The 3D nanoantennas are particularly sensitive to the cellular region around the metallic nanoantenna (the plasma membrane), whereas the 3D nanograting is more sensitive to the cell body in the trenches separating the tall dielectric gratings.

The device shown in Figure 3 combines the functionality of 3D nanoantennas and 3D nanograting into one, which we refer to as Grantennas. Depending on the polarization of the incident light, this device functions as either a 3D nanoantenna metasurface or a 3D nanograting. In the future, in addition to microwell-based cellular spectroscopy, we plan to employ these devices for rapid chemical imaging of live cells with sub-cellular resolution.

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).

[3] Mahalanabish, A.; Huang, S.; Shvets, G. Inverted Transflection Spectroscopy of Live Cells Using Metallic Grating on Elevated Nanopillars. ACS Sens 9 (3), 1218-1226 (2024).