Platform for Live Cells Infrared Chemical Imaging

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Primary CNF Tools Used: JEOL 9500, CVC SC4500 Evaporator, Zeiss Ultra Scanning Electron Microscope, Oxford PECVD, PlasmaTherm 740, Glen Resist Strip, DISCO dicing saw, Heidelberg MLA 150, Schott IR inspector

Abstract:

Our group has been developing infrared spectroscopy and microscopy for live cells analysis by engineering nanostructured antennas on infrared transparent materials and coupling antenna resonances to molecular vibrations. The standard techniques and materials (e.g. e-beam lithography, calcium fluoride substrates, antenna made of gold) used in our device are not scalable due to usage of expensive tools/materials and CMOS incompatible metals. In this report, we focus on replacing our standard substrate, calcium fluoride, used in previous studies with a silicon wafer.

Summary of Research:

Infrared (IR) spectroscopy is a common non-destructive, label-free technique to identify chemical substances. Previously, we have demonstrated devices (MEIRS [1], 3D-ITS [2], 3D-MEIRS [3]) which are based on coupling plasmonic resonances of nanoantennas to molecular vibrations of chemical components. This allows us to monitor live cell activities such as intracellular activities, cholesterol depletion and cell adhesion. Additionally, by using a mid-IR quantum cascade laser (QCL) light source, our group designed a laser-scanning inverted confocal microscope. The QCL emission is focused on a diffraction-limited spot and scanned across the metasurface through movement of a motorized microscope stage. A liquid-nitrogen-cooled mercury-cadmium-telluride (LN-MCT) mid-IR detector collects modulated reflection from the interaction of the analyte with the metasurface near-fields [4]. Recently we utilized this technique to study metabolic cell differentiation [5]. The vibrational contrast from amide II and lipids (Figure 1) clearly show the high surface sensitivity of our device.

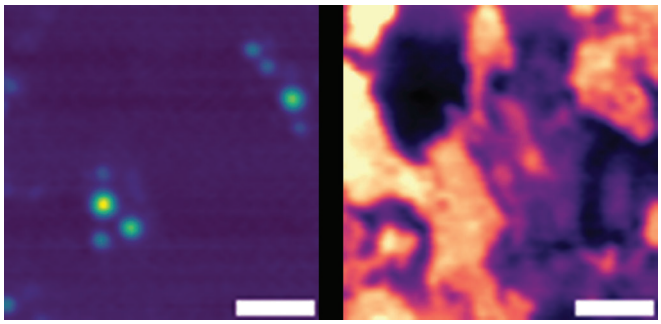

Figure 1: Vibrational contrast of 3T3-L1 at 12C=O ester (left) and amide II (right) bands (scale bar: 40μm).

However, the current substrate (CaF2) is very fragile and poses difficulty if one wants to use larger microplates, typically used for drug discovery studies. This work focuses mostly on replacing CaF2 with a simple silicon wafer with an oxide layer.

The device is made of gold nanoantennas on a double-side polished 4-inch Si wafer with a silicon dioxide spacer. First, the RCA cleaned silicon wafers were deposited by a 2 μm thick silicon dioxide layer. To pattern nanoantennas, a thin layer of PMMA was spin-coated followed by the e-beam exposure with JEOL9500. After developing, SC4500 evaporator was used to deposit a 5nm Ti adhesion layer followed by 70nm layer of gold to form antennas. Since we use the backside of the substrate to focus IR light, back etched marks located exactly under the metasurface arrays were made by backside alignment using Heidelberg MLA 150 for patterning and PT740 for etching. The last step is to lift-off gold by soaking the wafer/pieces into acetone overnight. Schott IR inspector was used to observe both sides of the wafer, top side having metasurfaces and bottom one having marks (Figure 2).

The metasurfaces are then brought to the lab where we culture cancer cells on top of them and acquire images

and spectrum. Figure 3 shows the IR spectra of cells in newly fabricated metasurface. The absorbances magnitudes (vibrational contrast) of amide peaks are comparable to our previous designs, thus making it possible to replace fragile and expensive CaF2 with an unexpensive and easy to make SiO2-on-Si substrates.

Conclusions and Future Steps:

TWe have demonstrated that our plasmonic metasurface-based devices can also be made with simple Si unlike a standard IR-transparent CaF2 which paves the way for scaling up. We plan to replace expensive e-beam lithography with cheaper and more scalable photolithography (e.g. ASML DUV stepper) for metasurface patterning to large-area microplates. Plus, gold is not a compatible CMOS metal, so we also envision to consider different metals like Ti or Al.

References:

[1] 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).

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[5] Huang, S.H., Tulegenov, D., and Shvets, G. Combining quantum cascade lasers and plasmonic metasurfaces to monitor de novo lipogenesis with vibrational contrast microscopy, Nanophotonics (2025).

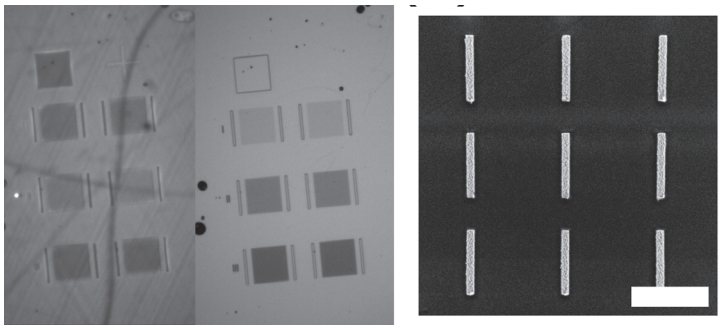

Figure 2: Left and middle figures show the bottom and top side of the wafer, respectively. SEM image of the antennas (right figure, scale bar: 3 μm).

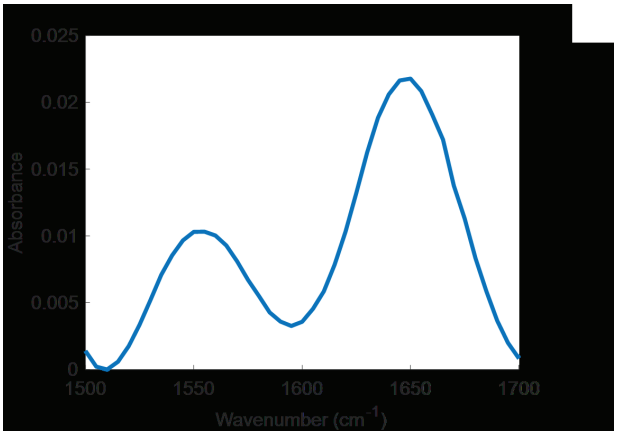

Figure 3: Absorbance spectrum of 3T3-L1 cell measured on the metasurface