Amplifying molecular voices: metasurface-enhanced infrared photothermal microscopy and tomography for ultrasensitive chemical analysis

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Infrared (IR) vibrational spectroscopy provides rich molecular fingerprint information for label-free detection of chemical bonds and conformational structures, with broad applications in biochemistry, pharmacology, and materials analysis.[1–3] However, its practical utility in complex, especially aqueous, environments remains constrained by two fundamental limitations: the intrinsically weak vibrational absorption cross-sections of typical biomolecules and the poor spatial resolution dictated by mid-infrared (mid-IR) wavelengths, exacerbated by strong water absorption.[4–6] To address these limitations, surface-enhanced infrared absorption (SEIRA) spectroscopy leverages the strong near-field confinement of plasmonic nanoantennas to amplify local electromagnetic fields by several orders of magnitude, improving the detection sensitivity of trace analytes from millimolar (mM) to micromolar (μM) levels.[6–8] Nevertheless, conventional SEIRA implementations typically rely on ensemble-averaged far-field reflection or transmission measurements collected over large arrays of resonators, which average out nanoscale heterogeneity and obscure distinct thermal and spectral signatures from individual nanoresonators.[8,9]

In an elegant and timely contribution to the field, the collaborative teams led by Ji-Xin Cheng at Boston University and Gennady Shvets at Cornell University recently presented metasurface-enhanced infrared photothermal (MEIP) microscopy,[10] which simultaneously overcomes long-standing sensitivity and spatial resolution barriers in mid-infrared spectroscopy, advancing detection sensitivity to the nanomolar (nM) level. Central to this innovation is the plasmonic enhancement and optical transduction of mid-IR vibrational absorption into a visible-light photothermal signal. A nanoantenna array, spectrally engineered to resonate at specific molecular vibrational bands, concentrates incident mid-IR energy into deeply subwavelength electromagnetic "hot spots." Nonradiative decay within these hot spots gives rise to localized heating, which in turn induces refractive index changes in the surrounding aqueous medium. This perturbation is quantitatively interrogated via multi-angle visible laser illumination, effectively transducing weak IR vibrational absorption into a robust, far-field-detectable signal in the visible regime, where optics and detectors offer superior sensitivity and spatial resolution (Fig. 1).

To retrieve the full three-dimensional (3D) distribution of temperature-induced refractive index perturbations, MEIP incorporates intensity diffraction tomography (IDT)[11–13]—a computational imaging technique that reconstructs the volumetric complex refractive index distributions from multi-angle intensity-only measurements without resorting to interferometry [Fig. 1(a)]. Originally developed for visible-range 3D label-free microscopy,[11–13] IDT has more recently been extended to mid-IR photothermal contexts. By applying IDT to SEIRA signals, MEIP achieves high-resolution volumetric imaging of absorption-induced refractive index changes, with lateral and axial resolutions of ~240 and ~650 nm, respectively. As a result, MEIP enables not only ultrasensitive detection of mid-IR vibrational signatures but also spatially resolved detection at the level of individual nanoantennas, capturing photothermal contrast with spatial resolution fundamentally limited only by the diffraction of the visible probe. In particular, MEIP allows for single-nanoantenna-resolved spectroscopy of protein secondary structures (e.g., α-helices, β-sheets) and nitrile-tagged small molecules, with detection limits down to ~0.24 monolayer surface coverage for proteins and 100 nM concentration for nitrile compounds in aqueous environments, enabling chemical imaging and photothermal mapping with sensitivity and resolution beyond the reach of conventional far-field infrared spectroscopy.

The spatiotemporal dynamics were characterized through complementary depth- and time-resolved photothermal imaging. Intriguingly, the peak temperature rise was not located at the metal–solution interface but rather ~200 nm above the nanoantenna tips, an effect attributed to asymmetric thermal diffusion across the Au-H_2O-CaF_2 tri-interface [Fig. 1(b)]. Time-resolved measurements revealed a thermal decay constant of ~287 ns, reflecting rapid thermoplasmonic relaxation processes. Probe-wavelength tuning further enables polarity inversion of the photothermal signal due to the thermo-optic response of gold, offering additional control over thermal contrast.

Beyond thermal profiling, MEIP demonstrates exceptional molecular specificity through label-free identification of protein secondary structures. By tuning the plasmonic resonance of the metasurface to match the amide-I vibrational mode (~1650 cm^{-1}), MEIP distinguishes α-helical BSA from β-sheet-rich avidin, providing single-resonator vibrational fingerprints of protein secondary structures—a longstanding goal in mid-IR microspectroscopy.[14] The concentration-dependent photothermal responses exhibit saturation behavior and adsorption kinetics that are well described by Langmuir isotherms, consistent with prior studies on protein-gold interactions.[15] Notably, despite the localized photothermal excitation, no detectable protein denaturation was observed, highlighting MEIP's biocompatibility.

To extend the MEIP's scalability, the authors also implemented a widefield, reflection-mode MEIP system, enabling high-throughput detection in a simplified configuration. This variant leverages temperature-dependent changes in the visible reflectance of gold nanoantennas (rather than the surrounding water), enabling real-time sensing of photothermal responses across large fields of view. Using a metasurface tuned to the nitrile stretching mode in the "silent window" of water (~2100 cm^{-1}), the system achieves label-free detection of 2-hydroxybenzonitrile, a nitrile-tagged pharmaceutical analogue,[14] with a detection limit of 100 nM in aqueous solution. These results underscore MEIP's potential for label-free, high-sensitivity detection of bioactive molecules and therapeutic compounds under physiological conditions.

Looking forward, MEIP establishes a versatile and generalizable platform for label-free chemical sensing and imaging at the nanoscale,

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 [DOI: 10.1117/1.AP.7.5.050501]

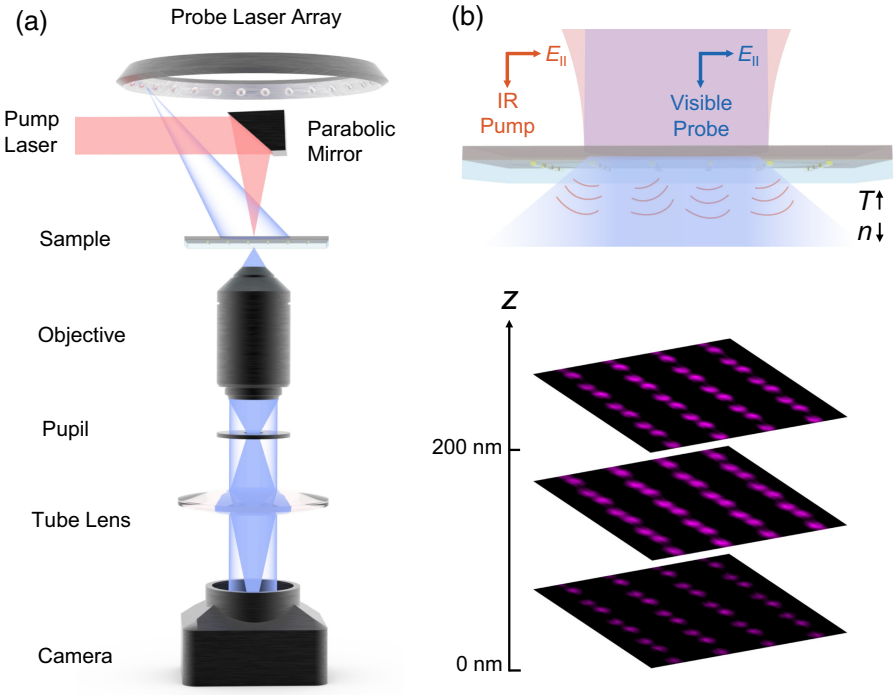

Fig. 1 Metasurface-enhanced infrared photothermal microscopy integrated with intensity diffraction tomography. (a) Conceptual 3D schematic of the MEIP system incorporating multi-angle visible laser probe illumination and IDT for volumetric reconstruction of photothermal signals. (b) Zoomed view of the sample region, highlighting the interaction of the IR pump and visible probe beams with the nanoantenna metasurface, along with the corresponding layered tomographic reconstruction of photothermal absorption-induced refractive index changes.

with broad implications across molecular biology, pharmaceutical development, and surface chemistry. By synergistically integrating plasmonic field enhancement, photothermal transduction, and computational tomography, MEIP amplifies the otherwise elusive vibrational "voices" of molecules and extends the reach of infrared spectroscopy into a new regime, enabling volumetric, far-field mapping of molecular vibrational absorption with unprecedented sensitivity and spatial resolution. Future developments may incorporate spectrally multiplexed metasurfaces for simultaneous multi-bond detection,[16] ultrafast light sources for capturing transient thermoplasmonic dynamics,[17] and super-resolution strategies to further push the spatial resolution beyond the diffraction limit.[18]

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