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Beyond the diffraction limit: *In-situ* nanoscale optical imaging and spectroscopy with tip-bound light

Optical microscopy and spectroscopy spanning the visible to terahertz spectral ranges is a foundational scientific tool in modern science, enabling advancements across diverse fields such as biomedical research, materials science, and fundamental physics. However, the spatial resolution of conventional optical techniques is inherently limited to approximately half the wavelength of light due to the diffraction limit of light [1]. This limitation hinders the direct optical investigation of nanoscale structures and their dynamic properties, which increasingly govern technological innovations and material functionalities in everyday life. To overcome this fundamental barrier, various super-resolution methodologies have been developed over the last few decades. Among these techniques, scattering-type scanning near-field optical microscopy (s-SNOM) has emerged as a particularly powerful approach due to its label-free nature [2, 3]. In s-SNOM, a sharp metallic tip, which is typically integrated into an atomic force microscope (AFM) setup, is illuminated by a tightly focused laser beam, generating a highly localized optical near-field at the tip apex. These strongly enhanced fields are created due to the achromatic lightning rod effect present around high curvature nanostructures, shown conceptually in Figure 1a. The near-field interaction between the tip and sample contains the nanoscale optical response of the sample below the tip apex. The near-field response is extracted from the detected light scattered from the tip containing both near-field and far-field response of the sample through interferometric processing on the basis of an asymmetric Michelson interferometry setup as displayed in Figure 1b and higher harmonics signal demodulation nΩ of the AFM tapping frequency Ω [3]. This methodology enables label-free spatially resolved optical probing with exceptional resolution only limited by the tip radius reaching down to 10–20 nm [4], far surpassing the diffraction limit. Furthermore, the optical response is recorded in correlation with the AFM response of the sample obtained through standard AFM-tapping mode operation. S-SNOM has been applied for the investigation of a wide range of materials such as 2D materials, doped semiconductors and phase change materials, as well as their intrinsic properties like charge carrier concentration [5], chemical composition [4] and refractive index [4].

In the last decade, s-SNOM has demonstrated its utility in the field of biology and biophysics by enabling infrared spectroscopic analysis and imaging of single proteins and fibrils [6], lipid monolayers [7], and fixed cellular structures [8] with nanoscale resolution. Yet, until recently, the exploration of dynamic biological and chemical processes and the study of biomolecules and cells in their native aqueous environment remained largely unfeasible. This limitation arose from the technical challenges associated with operating a near-field microscope in liquid, where the strong infrared absorption of the liquid and the mechanical instability of the tip hinder effective signal acquisition and stable measurements over a prolonged period.

Recent advancements, however, have begun to push the boundaries of this frontier, enabling hour-long stable near-field nanoscopy of complex liquid samples. In particular, nanometre thin membranes as shown in Figure 1c can act as a protective capping layer separating the liquid sample compartment from the delicate near-field tip. This method has proven itself to be a successful technique to enable stable operation, easy optical alignment and protection of both the delicate tip and the sample space [9, 10]. Importantly, the technique allows for the detection of both the optical and mechanical properties of the liquid-immersed sample adhering to the membrane [11].

New proof-of-concept studies have demonstrated the significant potential of this technique for the field of biophysics, showing its capability for the investigation of proteins, polymers and living biological samples in the form of E. Coli bacteria and eukaryotic tumour cells with infrared nanoscopy [9]. Furthermore, the technique has been successfully applied to study photoswitchable phospholipid vesicles in their native aqueous environment [12]. These photolipid vesicles are promising candidates for a new form of active targeted drug delivery for cancer therapy [13, 14]. Significantly, it could be shown that the active dynamic morphological modulation of lipid vesicles, driven by the photoisomeric switching of their constituent phospholipid molecules, could be imaged based

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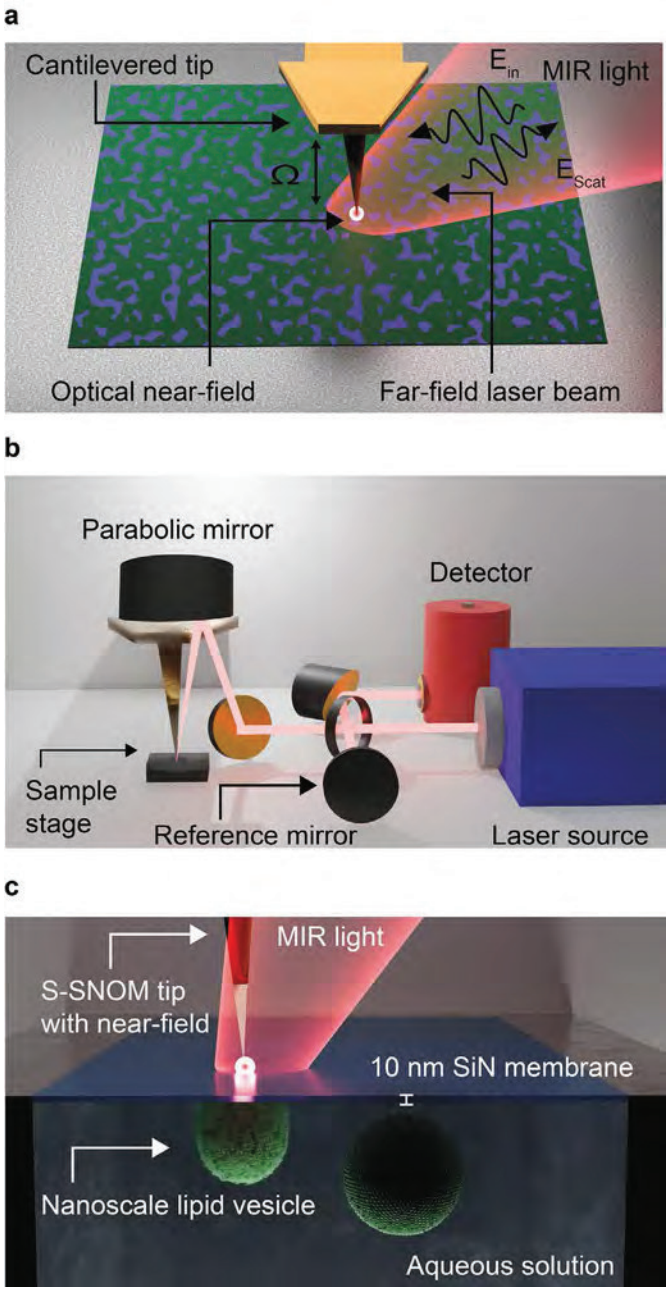

Fig. 1: Scattering scanning near-field optical microscopy (s-SNOM). **a.** s-SNOM cantilever with tip is scanning a sample in tapping mode while being illuminated by a far-field laser spot creating a highly confined near-field at the apex of the tip. The near-field enables the subdiffraction nanoscale resolved optical investigation of the surface of the sample. **b.** Optical setup with the laser beam being focused onto the tip by a parabolic mirror, which recollects the backscattering from the tip. The tip scattered light is modulated by a reference beam, detected by a detector and demodulated based on the tapping frequency Ω to attain the near-field from the far-field background. **c.** *In-situ* liquid s-SNOM method based on an ultra-thin SiN membrane allowing the near-field to penetrate through the membrane into the liquid compartment to investigate nanoscale objects located in close proximity to the membrane.

on their intrinsic IR scattering intensity. *In-situ* infrared near-field spectroscopy has also enabled the differentiation of two distinct photoisomeric states within a single lipid vesicle. Finally, the transient photoswitching dynamics of an individual photolipid vesicle, with dimensions well below the diffraction limit of MIR light, were temporally resolved with millisecond precision by monitoring its MIR response at a wavelength sensitive to the switching-induced perturbation.

The road ahead for *in-situ* optical nanoscopy

Based on these initial studies, *in-situ* near-field nanoscopy emerges as a highly promising technique for investigating various biological and chemical phenomena, such as the nature of lipid domains, drug release processes associated with lipid nanoparticles, cellular membrane chemistry, and degradation of heterogeneous catalysts. Furthermore, the recent development of a microfluidic cell module for *in-situ* s-SNOM by Attocube Systems AG and Norcada Inc. will help the broader scientific s-SNOM community to conduct liquid-phase experiments through dynamic exchange of the surrounding medium. This module enables nanoscopic investigations of the sample while simultaneously inducing precise variations in pH, osmolarity, solvent composition, or introducing specific chemical compounds. This capability could unlock entirely new experimental possibilities previously unattainable like the optical investigation of crystallisation and self-assembly processes on the nanoscale or the pH-induced change in secondary structure of single neurotoxic protein fibrils. Further progress in environmental control, such as temperature modulation (heating, cooling) or electrical biasing, as well as the integration of complementary measurement modalities like correlative fluorescence or Raman microscopy, could further expand the potential of *in-situ* s-SNOM. These correlative measurements would allow to compare conventional imaging modalities, such as fluorescence microscopy of cellular samples, with the enhanced spatial resolution and novel contrast mechanisms afforded by near-field microscopy. This integrated approach will provide deeper insights into the sample's nanoscale architecture and molecular organization, surpassing the information content accessible through standard techniques alone. In conclusion, the advancements outlined above underscore the growing applicability of optical nanoscopy to liquid-phase systems and highlight its transformative power for enabling nanoscale optical imaging of samples with spatial resolution well beyond the diffraction limit.

References

[1] E. Abbe, *Archiv f. mikrosk. Anatomie* 1873, **9**, 413.

[2] X. Chen, D. Hu, R. Mescall, G. You, D. N. Basov, Q. Dai, M. Liu, *Advanced materials (Deerfield Beach, Fla.)* 2019, **31**, e1804774.

[3] R. Hillenbrand, Y. Abate, M. Liu, X. Chen, D. N. Basov, *Nat Rev Mater* 2025.

[4] F. Huth, A. Govyadinov, S. Amarie, W. Nuansing, F. Keilmann, R. Hillenbrand, *Nano letters* 2012, **12**, 3973.

[5] A. J. Huber, F. Keilmann, J. Wittborn, J. Aizpurua, R. Hillenbrand, *Nano letters* 2008, **8**, 3766.

[6] I. Amenabar, S. Poly, W. Nuansing, E. H. Hubrich, A. A. Govyadinov, F. Huth, R. Krutokhvostov, L. Zhang, M. Knez, J. Heberle, A. M. Bittner, R. Hillenbrand, *Nature communications* 2013, **4**, 2890.

[7] A. Cernescu, M. Szuwarzyński, U. Kwolek, P. Wydro, M. Kepczynski, S. Zapotoczny, M. Nowakowska, L. Quaroni, *Analytical chemistry* 2018, **90**, 10179.

[8] K. Kanevche, D. J. Burr, D. J. Nürnberg, P. K. Hass, A. Elsaesser, J. Heberle, *Communications biology* 2021, **4**, 1341.

[9] K. J. Kaltenecker, T. Gölz, E. Bau, F. Keilmann, *Scientific reports* 2021, **11**, 21860. Doi: https://doi.org/10.1038/s41598-021-01425-w

[10] Y. Shan, X. Zhao, M. Fonseca Guzman, A. Jana, S. Chen, S. Yu, K. C. Ng, I. Roh, H. Chen, V. Altoe, S. N. Gilbert Corder, H. A. Bechtel, J. Qian, M. B. Salmeron, P. Yang, *Nat Catal* 2024, **7**, 422.

[11] E. Baù, T. Gölz, M. Benoit, A. Tittl, F. Keilmann, *Small* (Weinheim an der Bergstraße, Germany) 2024, **20**, e2402568. Doi: https://doi.org/10.1002/smll.202402568)

[12] T. Gölz, E. Baù, J. Zhang, K. Kaltenecker, D. Trauner, S. A. Maier, F. Keilmann, T. Lohmüller, A. Tittl, Transient infrared nanoscopy resolves the millisecond photoswitching dynamics of single lipid vesicles in water 2024. Doi: https://doi.org/10.48550/arXiv.2406.02513.

[13] S. D. Pritzl, P. Urban, A. Prasselsperger, D. B. Konrad, J. A. Frank, D. Trauner, T. Lohmüller, *Langmuir the ACS journal of surfaces and colloids* 2020, **36**, 13509. Doi: https://doi.org/10.1021/acs.langmuir.0c02229

[14] N. Chander, J. Morstein, J. S. Bolten, A. Shemet, P. R. Cullis, D. Trauner, D. Witzigmann, *Small (Weinheim an der Bergstraße, Germany)* 2021, **17**, e2008198. Doi: https://doi.org/10.1002/smll.202008198

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