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# World First for Diamond in Synchrotron-Based IR Photothermal Nanospectroscopy

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# World First for Diamond Light Source in Synchrotron IR Photothermal Nanospectroscopy

For the first time, infrared spectra on the sub-wavelength scale have been delivered by a synchrotron-radiation-induced thermal expansion technique [1]. The novel experimental result was achieved by coupling an atomic force microscope (AFM) to an infrared (IR) beamline at the UK's national synchrotron facility, Diamond Light Source. Via broadband synchrotron illumination and an AFM sub-micron tip, molecular IR spectra were obtained by detecting a resonance-enhanced (RE) photothermal signal with spatial resolution beyond the diffraction limit. Together with results on synchrotron IR nanoscopy in scattering mode from the IR beamline at the Advanced Light Source two years ago, the Diamond photothermal nanoprobe approach moves vibrational analysis beyond the diffraction limit and into nanoscale absorption spectroscopy.

Using state-of-the-art synchrotron-based vibrational microanalysis, it is now possible to observe live cell biochemistry, or microcatalytic reactions *in operando*, as well as the micromolecular changes across ancient painting fragments with a spectral quality and imaging speed conventionally unreachable; until recently, nanoscale spatially resolved IR spectroscopy has been optically prevented.

Two years ago, experiments at the Advanced Light Source demonstrated that light scattering by an AFM tip could overcome this limitation, combining near-field microscopy with synchrotron radiation to achieve IR spectra with nanometer resolution on thin samples [2].

The breakthrough was achieved by the team on Diamond's Multimode InfraRed Imaging and Microspectroscopy (MIRIAM) beamline. MIRIAM, also known as beamline B22, spans the largest IR spectral range—extending from the near-IR up to the far-IR (or THz) region—and is 100–1000 times brighter in the mid-far-IR than any other conventional broadband IR source. Differently from the ALS approach, the nanoIR method used at MIRIAM is not limited to thin samples, and allows direct infrared nanospectroscopy by combining photothermal near-field detection with synchrotron illumination for the first time. While still providing sub-wavelength spatial resolution via an AFM nanotip, the photothermal method gives a truly linear spectrum—signals proportional to sample thickness—in contrast to the scattering technique previously mentioned. The key innovation is using Diamond's broadband IR radiation to measure a direct absorption spectrum at the nanoscale (also known as RE-AFMIR).

Infrared microspectroscopy is a non-destructive, quantitative analytical method of great value across a range of research, including new and composite materials, medical biochemistry and histology, the physical chemistry of surfaces and studies in cultural heritage and archaeology, biomineralogy, and geology. This is particularly relevant in biological and medical studies, where it is routinely used to pinpoint single cell biology. It can show, for example, responses to test drugs for cancer therapy or the differentiation/control of stem cells by chemicals. The optical diffraction limit means that, on its own, this technique cannot clearly resolve sub-cellular structure below the micron scale.

## Experimental Detail

The first experimental results, published in *Optics Express*, report on investigations carried out by the team of Gianfelice Cinque (Principal Beamline Scientist for MIRIAM) into coupling IR spectroscopy with the use of nanoprobes.

The AFM allocates a very fine probe, mounted on a sensitive cantilever arm, to scan the surface of a sample and give accurate measurements of its topography. The MIRIAM team at Diamond have used this well-established tool in a novel way; i.e., using the broadband synchrotron radiation (IR-SR) to excite the IR absorption of the sample and detect its resulting thermal expansion. The IR-SR light is chopped on and off at the mechanical resonance of the AFM cantilever to enhance the AFM signal. In this way, it is pos-

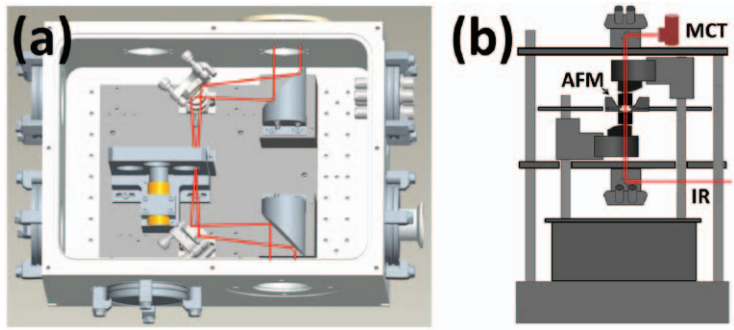


*Figure 1: Optical path schematic for the RE-AFMIR set-up. (a) Optics used to focus the synchrotron radiation through the slot of a high-speed chopper. Chopping the synchrotron radiation at the resonance frequency of the AFM cantilever causes the signal to increase by several orders of magnitude. This is the basis of the resonance-enhanced method of RE-AFMIR. (b) Optical path through the dual microscope. The synchrotron radiation is brought in through the bottom objective to focus under the AFM tip. An MCT detector is mounted on the top as an alignment aid. It is possible to bring the synchrotron radiation in from the top for experiments where this geometry would be advantageous.* 

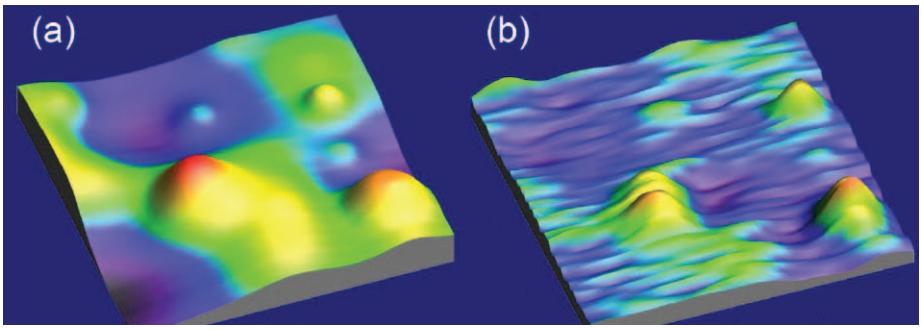


*Figure 2: (a) AFM topography image of PMMA (700 nm diameter) and PS (1 μm) beads embedded in a protein (Bovine Serum Albumin) film; (b) RE AFMIR map of the same 5 × 5 μm² area obtained via broadband synchrotron IR illumination at the MIRIAM beamline of Diamond. The IR signal is the full absorbance integrated across the spectral range 4000–1000 cm⁻¹ (cut off of $CaF_2$ substrate), in circa 20 minutes per full image. With the protein layer contributing to the IR signal, it demonstrates the photothermal IR sensitivity to bulk properties by measuring IR spectra of the beads from beneath the surface of a micron thick sample, a major advantage of RE-AFMIR for biologically significant specimens over existing light-scattering-based techniques, which are inherently surface limited.*

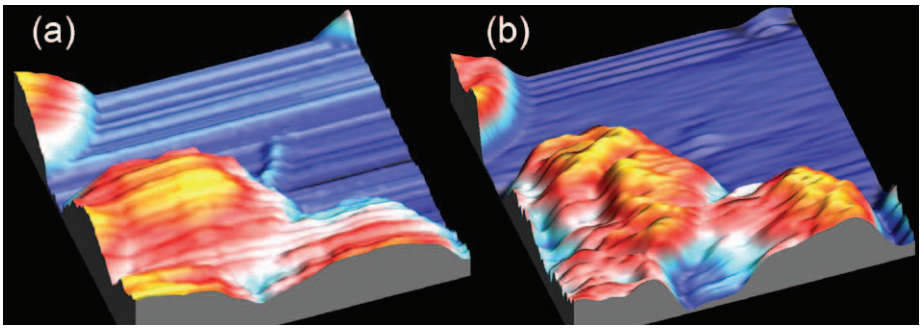


*Figure 3: (a) AFM topography of H. Pylori infected cells; (b) RE-AFMIR map of the same area obtained with a QCL tuned to 1650 cm⁻¹ protein signal peak. This image confirms the capacity to image biomedical specimens via photothermal nanoIR.*

amplitude results in an absorption spectrum, showing which molecular species (so-called IR fingerprint) are present quantitatively (via their absorption intensity).

Where the sample is a soft organic material, such as biological tissue or a composite plastic, the experiment is set up so that the IR beam enters from below and illuminates the whole microsample. Absorption of an IR photon by a molecule causes the molecule to immediately vibrate, and then de-excite thermally, creating a localized heat bump. As heat diffuses across the sample, this local information is lost in a few microseconds. The AFM probe, in contact with the sample surface, must rapidly measure the tiny expansion (less than the size of an atom) that results from a tiny temperature change (less than a hundredth of a degree).

## Results and Conclusions

Using IR-SR as the light source, rather than a benchtop laser, takes advantage of the bright and broadband nature of IR-SR to enhance the sample vibrational spectra quality on small samples, and dramatically speed up data acquisition by exploring the whole IR range. Diamond's IR radiation beam extends from the near-IR up to the far-IR (THz) simultaneously, and the illuminated area is of the order of 10 to 100 μm, respectively.

The team at MIRIAM has achieved an experimental demonstration of the use of IR-SR as a broadband source for photothermal near-field IR spectroscopy. Comparing cantilever resonant thermal expansion against scanning

sible to examine, for example, polymer nanostructure or organelles inside a cell.

For this experiment at Diamond, the IR-SR was relayed into an interferometer using ellipsoidal and toroidal mirrors. The IR light exiting the interferometer was focused through an ultrafast chopper that spins at around 20,000 rpm in vacuum. This challenging addition that has been implemented on MIRIAM allows modulation of the illumination beam and setting up the timescale required to keep thermal diffusion at the sub-micron level to preserve the spatial resolution. With the chopper tuned to work in resonance with the AFM cantilever, larger oscillations and signals are produced. Correlating the infrared wavelength to the oscillation

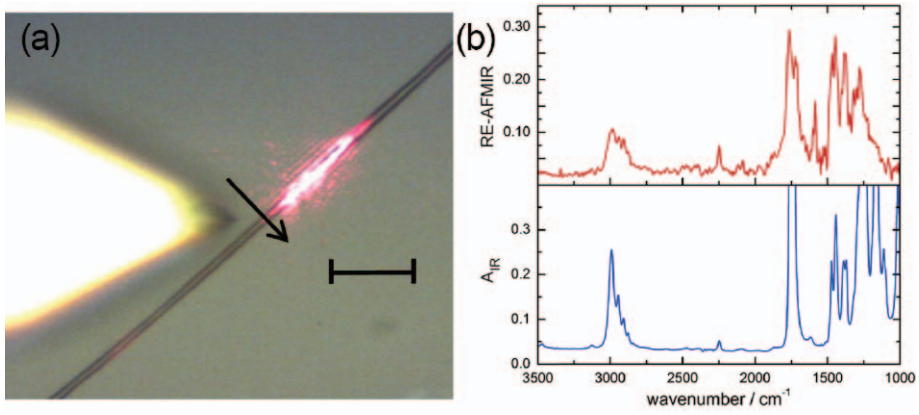


*Figure 4: (a) Cyanoacrylate ridge and AFM cantilever; arrow showing scan direction, scale bar 15 μm; (b) near-field IR spectrum by synchrotron IR obtained in 10 min (red) versus conventional optical IR absorbance (blue).*

thermal microscopy, the scientists at MIRIAM confirmed that this new method of signal transduction produces measurements below the diffraction limit, achieving a spatial resolution in the 100 nm scale. This is more than one order of magnitude better than IR microscopy on micrometric thick specimens, with directly comparable vibrational absorption spectra of the soft/organic matter of the sample matrices.

## Future

At present, the typical acquisition times are in the range 5 to 15 minutes per near-field IR-SR spectrum, which is acceptable for point-spectromicroscopy and line mapping. The collection of an image—requiring hundreds of points—will only be practical when this time can be reduced by a factor of ten. This reduction should be achievable via improvements in delivery optics, an increase in AFM resonance, signal recovery, and stabilization of the contact mode resonance frequency.

It is anticipated that the IR photo-thermal nanoprobe will evolve to the point where it could be used to examine the interior of single cells, and cell machinery at the organelle level—highlighting, for example, the effects of bacterial and viral infections, and *ex vivo* cellular response to trial drug compounds.

Another potential application for IR nanoscopy is to explore the local behavior of nanoscale heterogenous or nanocomposite materials, which is not yet fully understood. By coupling measurements of molecular chemistry and physical morphology, this new technique can show how a sample is locally different at the molecular level, or locally changing with alterations, for example, of thermodynamic parameters such as pressure and temperature. ■

## References


1. P.M. Donaldson et al., *Optics Express* **24**(3), 1852–1864 (2016).
2. H.A. Bechtel et al., *PNAS* **111**, 7191–7196 (2014).



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