LOCAL INFRARED MICROSPECTROSCOPY WITH 100NM SPATIAL RESOLUTION AND APPLICATION TO CELL IMAGING

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Abstract

We present a differential method of infrared micro-spectroscopy, which aims at performing "chemical mapping" of various objects with sub-wavelength lateral resolution by using the infrared vibrational signature characterizing different molecular species. Its principle consists in an atomic force microscope tip, probing the local transient deformation induced by an infrared pulsed laser tuned at a sample absorbing wavelength.

METHOD

Our method, called AFMIR, is therefore based on a photothermal effect [1,2,3]: we monitor the deflexion of an AFM (Atomic Force Microscope), with a fast detection (optical recording of the cantilever displacement). We use a short pulse infrared laser, which is lightening the sample.

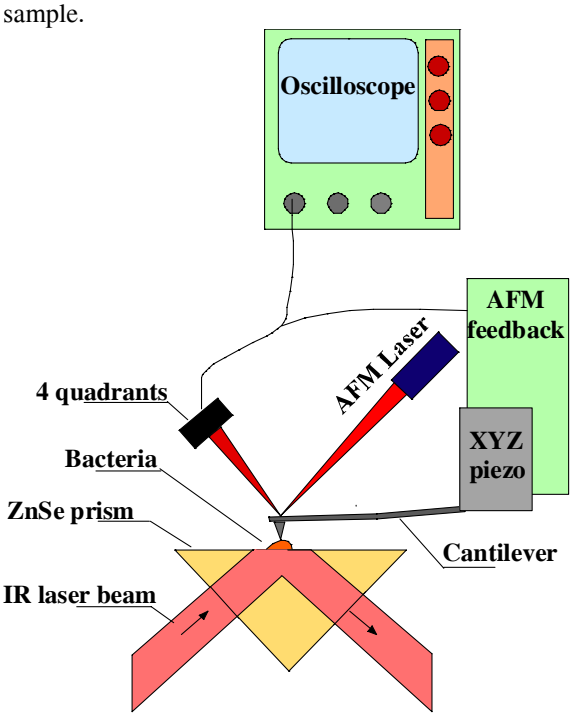

Figure 1: Experimental set-up of the AFMIR photothermal method.

The pulsed irradiation of an absorbing zone of the sample produces a fast local heating that creates an acoustic wave propagating up to the AFM cantilever. The detection of the transient deflection of the cantilever gives a signal corresponding to the surface deformation, i.e. related to the local infrared absorption. Thus this system acts as an amplifier of extremely small motions induced by optical absorption. The cantilever oscillates at its resonant frequencies, recorded simultaneously. Different resonant frequencies correspond to various modes of cantilever vibration and provide different information, leading to a full description of the sample deformation. Indeed, we have to use a short duration excitation and a fast measurement in order to avoid the natural dilution of the heating, which would forbid the measurement or, at least, reduce the spatial resolution.

The laser is either the CLIO infrared FEL (tunable from 3 to 120 µm) or a ns CO_2 laser. In the future, we plan to use OPOs in the 2 to 7 µm range. Images are recorded simultaneously by AFM (topography) and AFMIR (chemical mapping), allowing direct comparison. Also, the tip can be positioned at any desired location in contact with the cell and a spectrum taken by scanning the CLIO wavelength. Fig 2 shows that the fundamental mode of vibration amplitude is readily proportional to the absorption.

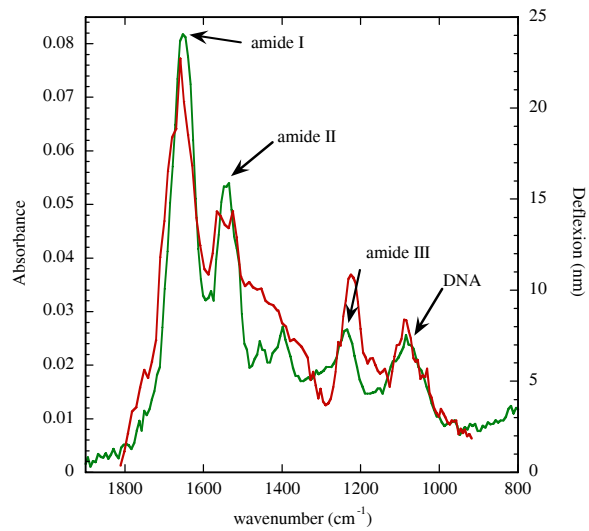

Figure 2: Comparison of the FT-IR and the AFMIR spectra. The red curves are the AFMIR amplitude of the vibration of the cantilever (spectrum of a part of a single bacterium). The green curve is the FT-IR spectrum of an assembly of bacteria.

TEST ON CELLS

Image at a given wavelength is recorded by scanning the probe. For a spectrally homogeneous object, the image reproduces the topography, with a spatial resolution much better than 1 µm. This is true only for

sufficiently small samples, such that the incident light distribution is uniform (Fig.4).

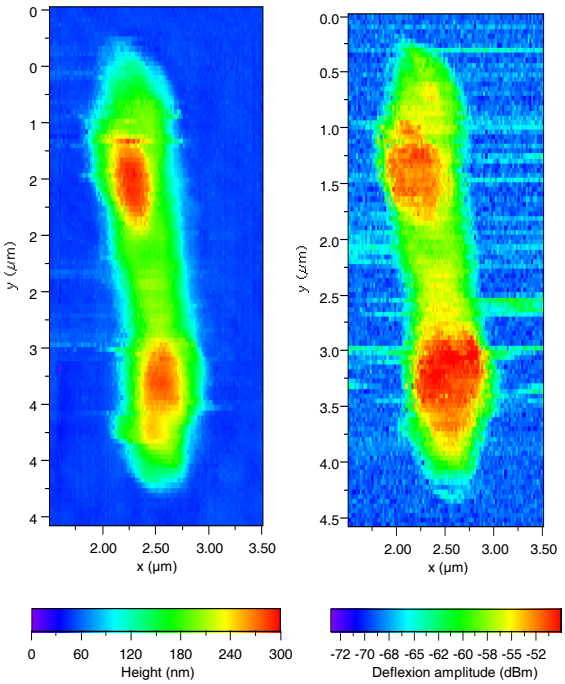

Figure 3: Mapping of an E. Coli. **Left** : AFM topography of the bacterium. **Right** : Amide I chemical mapping corresponding to the fundamental mode of cantilever deflection (normal expansion)

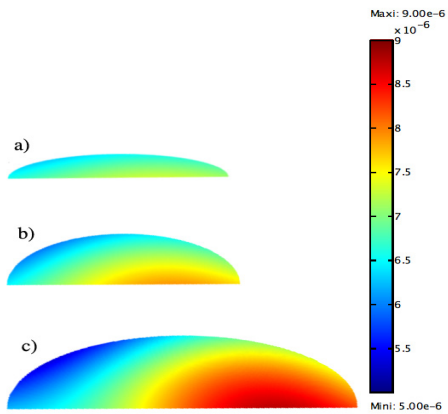

Figure 4: Calculation of light distribution for 3 homogeneous samples of different sizes:

a) Height=100 nm width =1.5µm
b) Height=210 nm width =1.5µm
c) Height=450 nm width =2µm

TEST ON VIRUSES

With AFMIR, we can also observe objects as small as viruses (Fig.5). In some cases, it is even possible to observe viruses located inside bacteria [4].

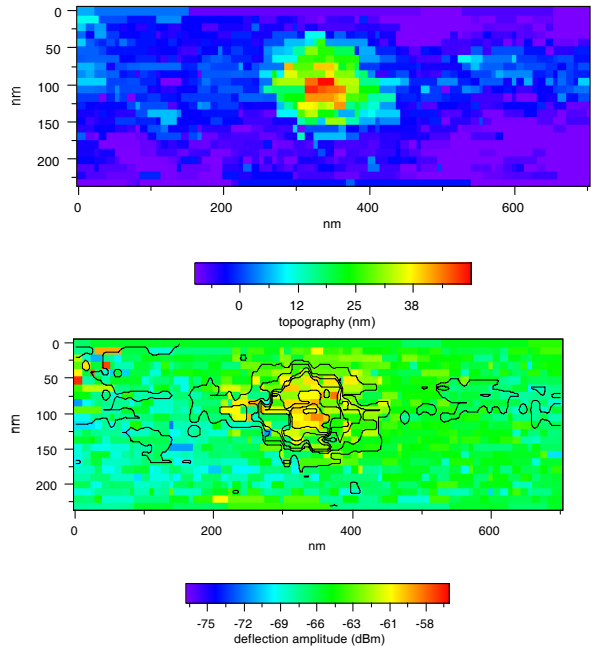

Figure 5: Topography and AFMIR mapping of a T5 isolated virus. It is mapped in the amide band since it has lost its DNA. Therefore only the capside appears and the signal is weak.

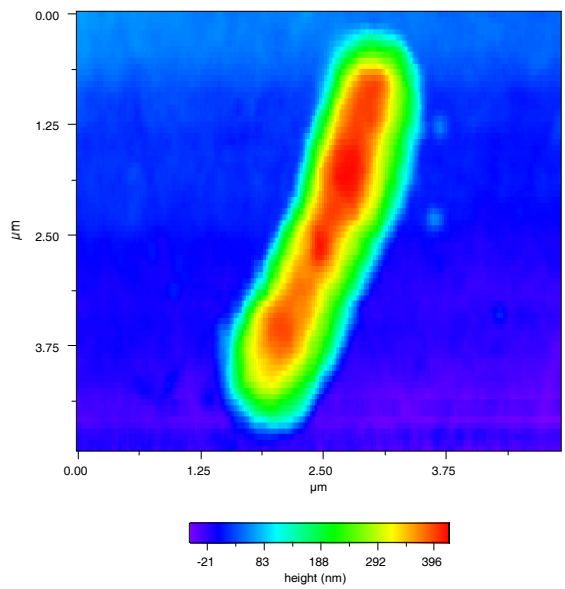

Figure 6: Topography of an E. Coli cell infected by a virus: No particular signal appears.

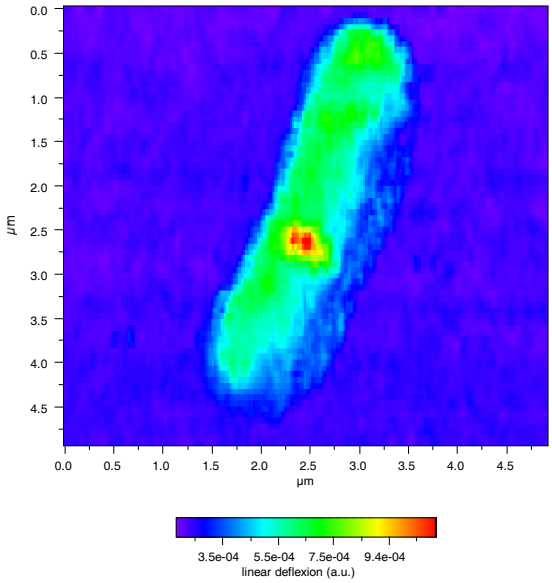

Figure 7: AFMIR mapping in the DNA band of the same bacterium: the T5 virus appears clearly.

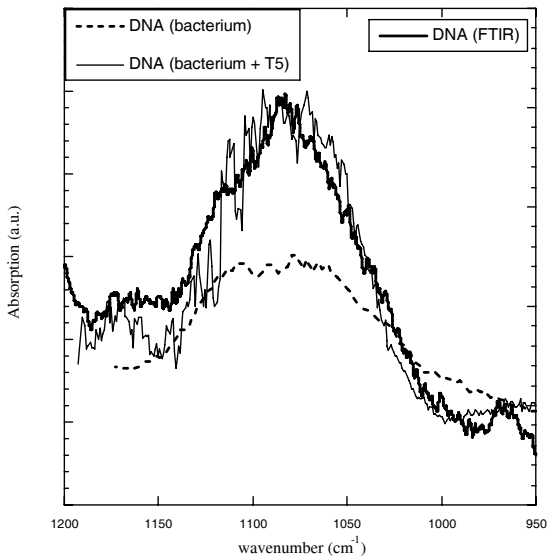

Figure 8: Local AFMIR spectroscopy on the same bacterium. The contrast appears to be due to the high DNA concentration inside the virus, which has kept its genetic material.

STUDY IN WATER

Many studies in biology are relevant only if they are made "live", i.e. with the sample ins water. Preliminary studies have been performed on hyphae of candida albicans cells [5]. On Fig. 9 and 10 is displayed a comparison of the Fourier analysis of the AFM cantilever response at 1080 cm^{-1} for dried and living cell. Despite the perturbation of the cantilever vibration induced by water, selecting the peak at 31,6 kHz, characteristic of the hyphae in water, allows to map selectively the DNA absorption. This is shown in the figures below.

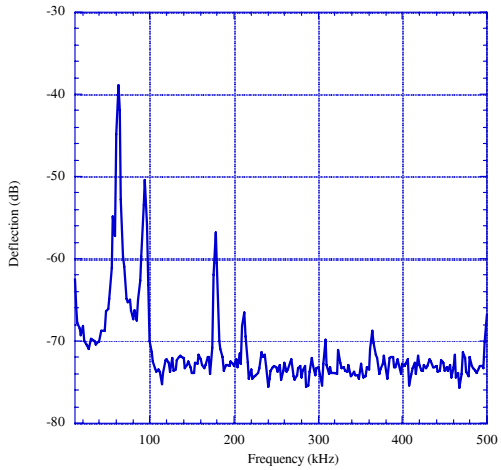

Figure 9: Cantilever spectrum for a dried hyphae deposited on the substrate.

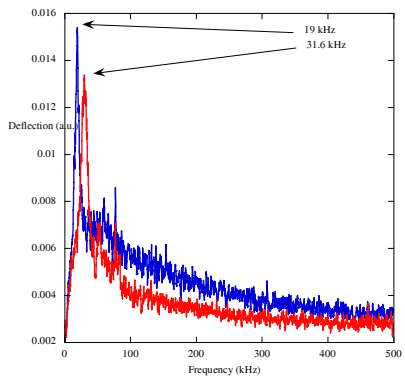

Figure 10: Cantilever spectrum of a cell in water (red) and for water only (blue).

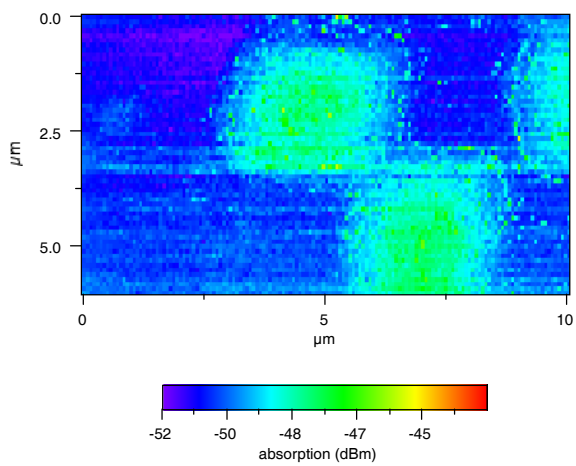

Figure 11: The AFMIR image, taken at 31,6 kHz reproduces exactly the topography (Fig.12), since the sample is homogeneous with respect to its DNA content.

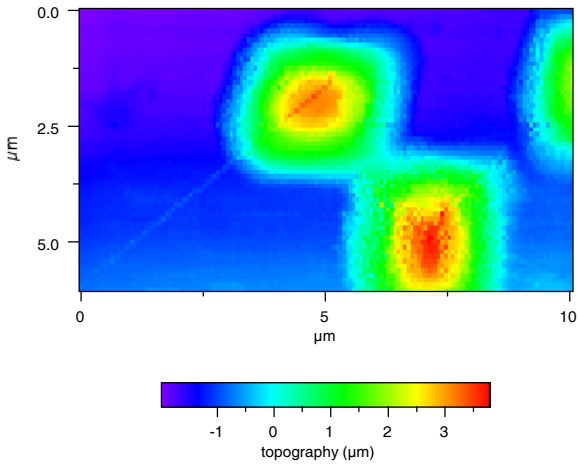

Figure 12: AFM image of the hyphaes.

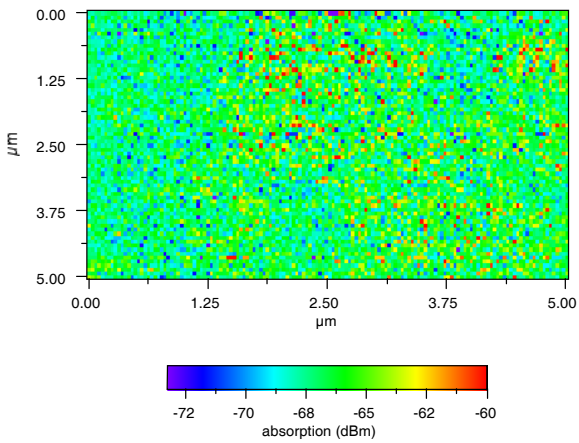

Figure 13: The AFMIR image, taken at 80 kHz displays only noise, showing the selectivity of the method.

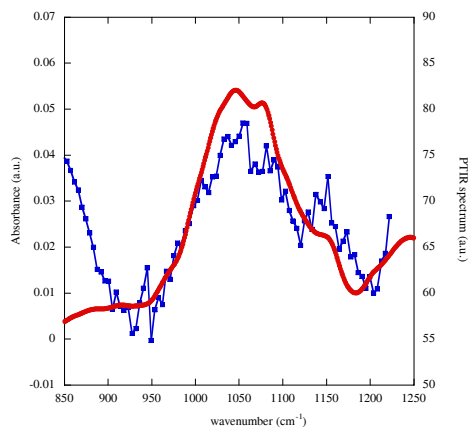

Figure 14: The AFMIR spectrum (blue) of a small zone of a single cell, taken at 31,6 kHz reproduces the FTIR spectrum (red) of a layer (comprising millions of hyphens).

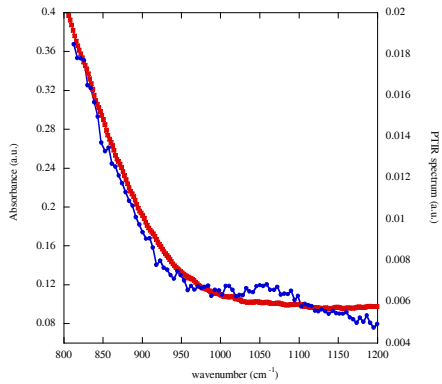

Figure 15: AFMIR spectrum (blue) of water on the prism, taken at 19kHz, reproduces the FTIR spectrum (red) of water.

CONCLUSION

These results demonstrate the unprecedented possibilities of the AFMIR method for chemical mapping. This method allows overcoming the problem encountered with optical near-field methods since the AFMIR is sensitive only to the imaginary part and not to the variations of the real part of the index of refraction (i.e. topography and inhomogeneities). Moreover, it can measure very small absorptions, as it is a differential method : there is no signal in the absence of absorption, contrary to full optical methods. The spatial resolution has been demonstrated to be better than 100 nm i.e. λ/100. Also, chemical mapping of living cells has been demonstrated. Various biological problems are now under study with this method at the CLIO infrared FEL facility.

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