Advanced Infrared Nanospectroscopy: Improvements and applications.

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Infrared spectroscopy is a powerful tool to specifically probe molecular vibrations of organic components without exogenous labelling. From fundamental physic to biomedical field, environmental science and agronomy, it allows analysis, chemical identification and diagnosis and becomes even appealing to cellular and tissue biology. The coupling with microscopy and all the improvements done (aberration correction, confocal system, FPA) in the last decade have reinforced this trend. The main drawback is still remaining: the resolution limited by the diffraction. Yet in many cases, the chemical identification of smaller and smaller objects with sub micrometric size becomes crucial such as isolated bacteria, virus or proteic assemblies. The solution to the problem was in the side of the near-field techniques.

Indeed near-field techniques play a fundamental role in Nanoscience microscopy. Two different ways exist to make infrared studies with near-field techniques: optical techniques measuring the transmitted signal coming from the nano-object and photo thermal approaches using thermometer to link temperature to absorption measurements. After several years of research in the field and considering all the previous methods limitations, we have developed at our lab an innovative infrared nanospectromicroscopy : AFMIR (Alexandre DAZZI, patent 2007).

The main idea was to rule out optical detector and keep the spectral capacity. In 1880, Alexander Graham Bell proposed an ingenious way to determine the absorption of a gas without measuring the absorbed: by measuring the acoustic waves generated after the absorption. Indeed for whatsoever wavelength, the energy absorbed by an object is converted into heat, resulting in a dilatation of this object and photo-acoustic spectroscopy is possible. This concept was only exploited in the ‘70s for infrared (IR) spectroscopy. Spectra of diffusing or highly heterogeneous objects, such as clouds, small particles in suspension or gases, cannot be measured by conventional IR spectroscopy. Photo-acoustic spectroscopy was thus applied and developed even more. However, images with a resolution below 100 microns were impossible by acoustic microscopy (ultrasound type). As a consequence, the atomic force microscope (AFM), a scanning probe technique widely spread for surface analysis at the nanoscale, emerged as an effective solution to overcome the resolution criteria of optical or acoustic microscopes. AFM-IR was born.

AFMIR is a cutting-edge near-field technique using a setup in which an atomic force microscope (AFM) is coupled with a tunable pulsed IR laser to record spatially resolved absorption measurements.

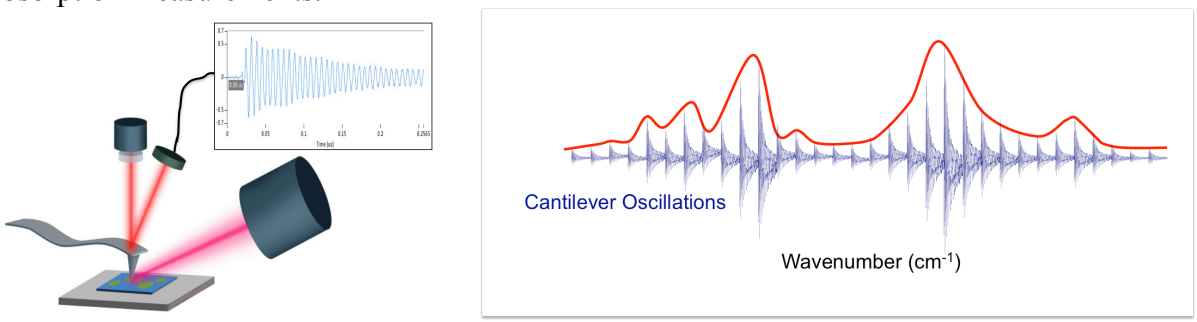

Figure *: (left side) The pulsed IR laser highlights the sample. If the laser is tuned on an absorption band of the sample, the resulting dilatation induced an impact in the cantilever via the tip. (right side) The dilatation is quicker than the dynamic response of the cantilever (ns), the oscillations measured*

with the 4-quadrant correspond to the vibration of the cantilever. The amplitude of the oscillations is reported as a function of the wavenumber. The local IR spectrum is thus reconstructed.

The principle of this technique is to illuminate the sample with a monochromatic source tuned on a specific absorption band of the sample. The absorbed energy leads to a local expansion through an increase in temperature via the photothermal effect. The cantilever tip of an AFM detects this expansion. For each expansion, generated by the absorbed pulsed light, the cantilever will oscillate. By analysis of the spectrum of tip oscillations, the absorption spectrum of the sample at the position of the tip can be reconstructed. This allows IR mapping of molecules of interest within a sample (polymer, tissue, cells) with a sensitivity of ten of nanometer.
We will present the experimental set-up and some of the critical technical sides. Then to illustrate technological advances we will show applications on biomaterial.

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