

# *Infrared Nanoscopy Applied to Microbiology*

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We have developed an innovative infrared microspectroscopy technique, called AFM-IR, based on the coupling between a tunable infrared laser and an AFM (Atomic Force Microscope). This coupling allows us to perform ultra-local infrared spectroscopy and chemical mapping at the nanometer scale. The principle<sup>1</sup> is based on detecting the local thermal expansion of the sample, irradiated at the wavelength of its absorption bands. This expansion is detected by the AFM tip in contact mode. As the duration of expansion and relaxation of the sample is always shorter than the response time of the cantilever in contact, the excitation transmitted to the cantilever acts as an impulse function, exciting oscillations at resonant frequencies of the cantilever. The technique can create nanoscale IR absorption spectra by recording the amplitude of these oscillations as a function of wavelength and chemical maps by measuring the oscillation amplitude as a function of position. We have validated this technique by comparing the infrared spectrum of a single *E.coli* bacterium and the corresponding FTIR spectrum, and showing the possibility to perform chemical mapping with sub-wavelength spatial resolution (50 nm)<sup>2</sup>. Later, similar outcomes have been obtained in nanophotonics (20 nm resolution)<sup>3</sup>.

Our work is now mainly focused on microbiology<sup>4</sup> systems and cell imaging<sup>5</sup>. For example, we are now interested by the production optimization of bio-polymer (PolyHydroxyButyrate) done by a photosynthetic bacteria, *Rhodobacter sphaeroides*. The AFMIR technique allows us to easily detect the polymer (PHB) vesicles inside the bacterium due its specific absorption band (ester carbonyl at 1740 cm<sup>-1</sup>) that is different from those of the bacterium. Similar studies are also provided on different bacteria like *Streptomyces* to optimize the production of bio-fuel precursor (triacylglycerols).

[1] A.Dazzi et al, *Opt. Lett.* **30**, Issue 18, 2388-2390 (2005).

[2] C. Mayet et al, *Analyst* **135**, 2540-2545 (2010).

[3] J.Houel et al, *Phys. Rev. Lett.* **99**, 217404 (2007).

[4] C. Mayet et al, *Biothechnology advances*, Vol.**31**, issue 3, 369-374 (2013).

[5] C. Policar et al, *Angewandte Chemie International Edition*, Vol. **50**, Issue 4, 860–864 (2011).