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Chemical mapping of the distribution of viruses into infected bacteria with a photothermal method

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Abstract

We show that an infrared spectromicroscopy method based on a photo-thermal effect, is able to localize single viruses as well when they are isolated and when they are located inside the bacteria they have infected. In this latter case, although the topography performed by an AFM cannot image the viruses, the AFMIR is able to do so. In addition, we are able to determine different stages of the bacteria infection.

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1. Introduction

The combination of infrared spectroscopy and imaging is a powerful tool [1] to identify and localize chemical species by their "fingerprints" (i.e., infrared spectra). However, in most cases, such as cell imaging, a high lateral resolution is needed. This cannot be obtained with usual, far-field, optical microscopy. Infrared near-field microscopy has been studied by several authors [2–5]. However, in this case it is extremely difficult to separate the information due to sample topography and inhomogeneities (real part of the index of refraction) from the information of interest: infrared absorption (imaginary part of the index of refraction). Recently, we have proposed a photothermal method, AFMIR [6–8] that possesses the advantage of being sensitive only to the sample absorption with a lateral resolution <100 nm. In this paper, we show that the AFMIR is able to detect object as small as viruses and, moreover, to discriminate them from the rest of the biological material.

2. Set up and sample description

The AFMIR technique is based on the coupling between a pulsed infrared laser (in our case a free-electron laser) and an atomic force microscope. When a sample is illuminated by the laser at the wavelength corresponding to one of its absorption bands, it absorbs one part of the incident energy and is heated almost instantaneously compared to the AFM response time (typically $<1\,\mu$s). The increase of temperature creates a fast expansion of the object that displaces the AFM tip (Fig. 1). The AFM cantilever starts then to oscillate. By measuring the amplitude of oscillation we get a signal, which is proportional to the absorption. By recording this signal as function of wavelength of the laser, we are able to make ultralocal infrared spectroscopy [6]. By fixing wavelength, we can image the surface giving us corresponding chemical mapping at nanometric scale [7,8].

The increase of temperature induces a stress inside the object that creates a displacement of matter. The stress can be written in a simple way for an isotropic object:

$$\sigma = \alpha E \Delta T,$$

where α is the thermal expansion coefficient and E is the Young modulus of the object.

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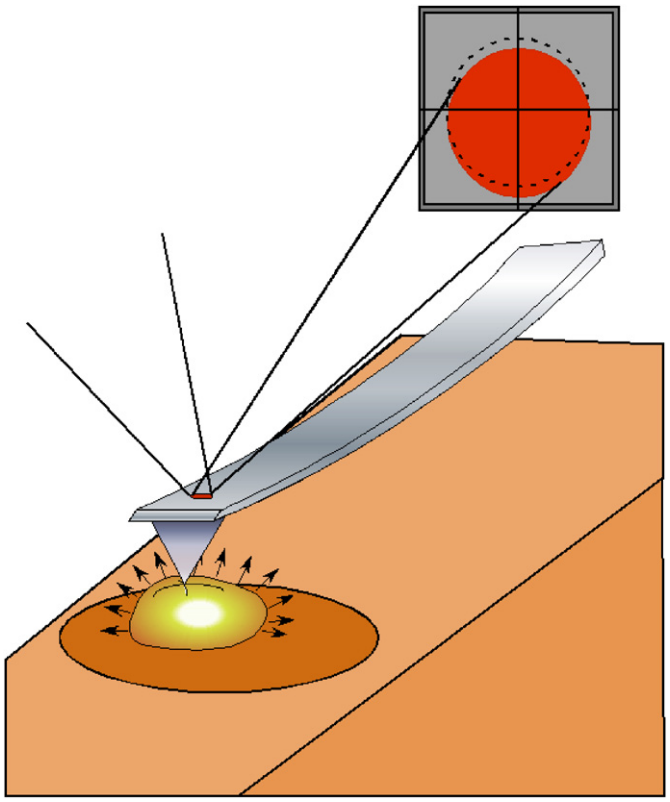

Fig. 1. Experimental set-up. The thermal expansion of a sample induces a displacement of the tip of an AFM cantilever, which is monitored by an HeNe laser and a 4-quadrant detector.

The displacement of the object is directly proportional to the thermal expansion coefficient:

$$\alpha \Delta T = \frac{u}{R},$$

where u is the displacement and R is the size of the object.

For example, for a bacterium of 1 μm radius and a ΔT increase of $10\,K^{-1}$, the displacement u is 1 nm (taking the thermal expansion coefficient to 10^{-4}). This value is well within the vertical sensitivity of an AFM (a few Å). In our case, due to the resonance of the cantilever, the observed displacement and thus the sensitivity are greatly enhanced. Calculation of this effect is in progress. In practice, these experiments were conducted by attenuating by a factor of 10 the incident laser power below the damage threshold of the sample. We may assume that damage arises when the temperature reaches 100 °C, since there is always some water remaining inside dried samples. Therefore, the temperature rise inside the sample can be estimated to be of the order of 10 K and the measured displacement of a $\cong$100 nm wide virus of about 0.1 nm.

The sample is deposited on a ZnSe prism that is transparent in the mid infrared. The laser light is incident with an angle to be propagative inside the sample and evanescent in the air, allowing to protect the AFM tip from the direct illumination.

The laser is the free-electron laser "CLIO" [9]. Its pulse length is 9 μs, composed of about 600 micropulses separated by 16 ns. It is continuously tunable between 3000 and 80 cm^{-1}. Spectra can be taken within a factor of two in wavelength within a few minutes, or more in case of averaging. Access to another spectral range requires a tuning of the machine, which takes typically an hour.

The sample was composed of *Escherichia coli* bacteria and T5 phage (bacteria virus). Bacteriophages T5 st(0) were produced from *E. coli F* and purified as described in Ref. [10]. The final concentration of the phage stock was evaluated to 1.8×10^{13} infecting phages/ml. Bacteria *E. coli F* were grown in LB medium to the exponential growth phase (3×10^8 cells/ml) and infected by phages with an average multiplicity of 60. The infection was stopped 20 min after the beginning by adding chloramphenicol at a final concentration of 50 μg/ml. Chloramphenicol is an antibiotic inhibiting bacterial protein synthesis and consequently, blocking the phage multiplication. Infected bacteria were centrifuged and the pellet was washed and suspended three times in pure water to a final concentration of approximately 1.5×10^8 cells/ml. We have studied two different types of samples: phages alone and bacteria infected by bacteriophages. A drop of the solution was deposited on the ZnSe prism and dried at the room temperature. For the infected bacteria, as the infection was stopped by chloramphenicol addition after only 20 min, various stages of the virus development can be found inside the cells [11].

3. Localization of isolated viruses

When the droplet of phage solution has evaporated, one expects the viruses to have preserved their structure and their DNA inside their protein envelope (capsid). To verify this, we have studied the surface of the prism at two different wave numbers: 1650 cm^{-1} (amide I) characterizing the proteins of the capsid and 1080 cm^{-1}, which is the maximum of the DNA band.

Fig. 2(a) and (b) show the topography and the corresponding chemical mapping of a single virus, recorded for the wavelength of 1650 cm^{-1} (amide I). This wavelength is situated in the absorption band of proteins. We can see that the absorption signal of the phage (Fig. 2(b)) corresponds to its topography. There is no real detectable lateral expansion due to the heating. Preliminary calculations indicate that this expansion should not be larger than one nanometer. The signal magnitude is weak, because proteins constitute only a small fraction of the phage head, which is mainly constituted of DNA (about 70% of the phage mass). However, the contrast with the background (+6 dB) is sufficient to identify unambiguously the virus, showing that this technique is really sensitive even for such a small entity.

Topography and chemical mapping at 1080 cm^{-1} (DNA band) of several isolated viruses are represented in Fig. 3(a) and (b). In this case, the AFMIR image is blurred compared to the topography. These results indicate that part of the phages have certainly been damaged and have