Tip-enhanced infrared nanospectroscopy of single and double biomolecular layers

Michele Ortolani[1], Michela Badioli[1], Leonetta Baldassarre[1,2], Valeria Giliberti[1,2],
Paolo Calvani[1], Alessandro Nucara[1], Ulrich Schade[3], Peter Hegemann[4], Eglof Ritter[4]

1. Dipartimento di Fisica, Sapienza Università di Roma, Rome, Italy
2. Istituto Italiano di Tecnologia, Centre for Life Nano Science, Rome, Italy
3. BESSY-II, Helmoltz-Zentrum Berlin, Berlin, Germany
4. Institut für Biologie, Humboldt Universität, Berlin, Germany

Mid-infrared (IR) spectroscopy is commonly regarded as the basic tool to identify a wide variety of molecules through their vibrational spectrum. Chemical identification at the nanoscale, which is crucial for the study of inhomogeneous materials as well as biological matter, can be achieved by combining tuneable or broadband mid-IR sources to atomic force microscopes (AFM) that provide deeply-subwavelength resolution. Scattering-type scanning near-field optical microscopy (s-SNOM), where radiation backscattered by the tip is detected in the far-field, is already an established technique for mid-IR nanospectroscopy. Recently, a novel resonant AFM-IR technique that allows a direct measurement of the absorption coefficient of few molecular layers has been introduced. Using a mid-IR pulsed laser with repetition rate matching the frequency of one of the AFM cantilever mechanical bending modes, resonant mechanical detection of the modulation of the tip-sample contact interaction is obtained [1]. Moreover, high electromagnetic field concentration is obtained through the formation of a nanogap between the gold-coated tip in contact-mode and ultraflat gold substrates where single molecular layers are dispersed [2]. Such expedients have recently allowed to enhance the sensitivity of the Photo-thermal Induced Resonance (PTIR) spectroscopy, making it possible to measure the force exerted by the anharmonic vibrations of single molecular layers on the tip, the phenomenon which is at the origin of thermal expansion at the macroscopic scale.

Here, we apply the resonant AFM-IR technique to vibrational contrast imaging of cell membranes, unraveling its potential for applications to life sciences. Our samples are flakes of purple membrane deposited on ultraflat gold. Purple membrane consists of a 4 nm thick lipid bilayer densely filled with single bacteriorhodopsin molecules (bR), a protein acting as a proton pump across the cell membrane of Halobacteria. Our experiments are performed using the NanoIR-2 system by Anasys Instruments, equipped with a Quantum Cascade Laser tuneable in the 1575-1725 cm^{-1} spectral range covering the amide-I vibrational band of proteins. In Fig. 1 we show the topography and AFM-IR maps of purple membrane flakes. Combining topography and AFM-IR maps at different IR frequencies, we can clearly identify a single membrane (SM) layer, a region with two overlapping membranes or a double-membrane (DM), and a lipid droplet (LD), as indicated by the arrows in Fig. 1b. The map in Fig. 1c taken at the amide-I peak frequency of 1660 cm^{-1} shows strong AFM-IR signal in the areas where proteins are present, and does not show any signal in the thicker lipid droplet where no protein is present. In the maps taken at IR frequency away from the amide-I vibration (1724 cm^{-1}), the membrane flakes are barely distinguishable from the gold substrate. Considering our AFM tip radius of 20 nm and the bR density in the purple membrane, we calculate that we probe the photo-expansion of about 16 bR molecules per pixel of the map. The signal-to-noise ratio is 5 in a typical map taking 15 minutes per square micron at a 50 nm resolution, therefore the resonant AFM-IR technique is sensitive to as few as ~700 peptidic bonds, corresponding to about 3 bR molecules, which is comparable to what reported in s-SNOM experiments [3].

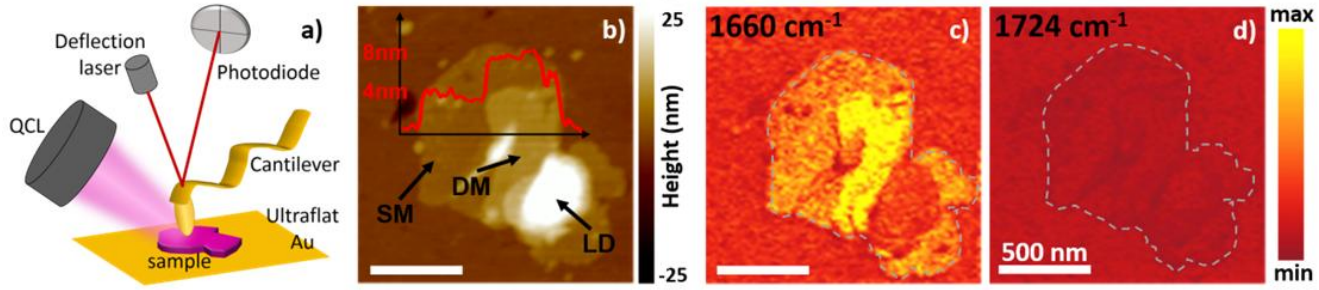

Fig. 1. a) Sketch of the AFM-IR setup. b) Sample topography: the arrows indicate the single membrane (SM), double membrane (DM) and lipid droplet (LD). The inset in red shows a profile across a SM-DM step. c), d) AFM-IR map taken at 1660 cm^{-1} and 1724 cm^{-1} respectively (at the amide-I peak IR frequency and away from it). The grey dashed line is the sample cotour taken from the topography.

References

[1]. F. Lu, M. A. Belkin, "Infrared absorption nano-spectroscopy using sample photoexpansion induced by tunable quantum cascade lasers", Opt. Expr., **19**, 21 (2011).
[2]. F. Lu, M. Jin, M. A. Belkin, "Tip-enhanced infrared nanospectroscopy via molecular expansion force detection", Nat. Photonics, **8**, (2014).
[3]. S. Berweger, D. M. Nguyen, E. A. Muller, H. A. Bechtel, T.T. Perkins, M.B. Raschke, "Nano-chemical infrared imaging of membrane proteins in lipid bilayers", J.A.C.S., **135**, 49 (2013).