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STSM Topic: Biomolecule detection and atomic force spectroscopy using self sensing piezoresistive cantilevers

Introduction
I spent three months at the Biophysics & NanoScience Centre at the "Universitá della Tuscia" (Viterbo, Italy) under the supervision of the Prof. Salvatore Cannistraro within a Short Term Scientific Mission (STSM).
The purposes of this stay were:

- Learn the atomic force spectroscopy (AFS) methodology for the study of biological complexes undergoing a biorecognition process.
- Mechanical and electronic integration of the piezoresistive cantilever force sensor chips made in IMB-CNM (CSIC) into the PicoLe atomic force microscope (AFM) (Agilent) available in the Biophysics & NanoScience Centre.
- Use of the IMB-CNM force sensors to detect the molecular interaction between a ligand and receptor pair.

Background and purpose
In the Microelectronics Institute of Barcelona (IMB-CNM (CSIC)) I am working on the development and optimization of force sensors based on piezoresistive cantilevers (fig. 1) that can be used in liquid environment for detecting biomolecules (i.e. illnesses markers). Before the STSM we achieved a reliable and high yield production of sensors with high force sensitivity and resolution. We proposed to use them to recognize molecules detecting the intermolecular force between the ligand and the associated receptor. This method is an extension of the well known "molecular force spectroscopy" and also called "Atomic Force Spectroscopy" (AFS). Preliminary experiments to detect the binding force between a couple of ligand-receptor molecules were performed in IMB-CNM but the experimental conditions were not optimal for these measurements and the experiments were not successful. This was due primarily to the lack of knowledge of the AFS technique.
The main purpose of the short stay at the Biophysics & NanoScience Center at the University of Viterbo was therefore the use of the piezoresistive cantilevers to detect biomolecules by measuring the molecular interaction between a ligand-receptor pair using the AFS technique.

Description of the work carried out during the STSM.
During the three months spent in the Biophysics & NanoScience Centre at the "Universitá della Tuscia" I worked in collaboration with the Prof. Cannistraro team on different topics.

As first, I learned the basics of the AFS technique applied to the study of biological complexes. For this purpose we used a well known receptor-ligand pair: biotinylated Bovine Serum Albumin (b-BSA) and neutravidin.
Afterwards we worked on the mechanical integration of the IMB-CNM force sensor chips into the PicoLe AFM. For this purpose we designed a modification of the standard PicoLe nose cone (probe holder) which was made in IMB-CNM by rapid prototyping (fig. 2a). The force sensor chips were bonded to a specifically designed 0.4 mm thick PCB suitable to be inserted into a low profile zero insertion force (ZIF) connector. On the PCB was also glued a 0.1 mm thick iron layer (fig. 2b). This PCB was hold firmly by a neodymium magnet inserted into the nose cone (fig. 2c).
After the mechanical integration we worked on the electronic integration of chip into the AFM electronics. The chip was biased by standard AA Sanyo eneloop rechargeable batteries which give a constant voltage of 5V DC. We choose this option instead than traditional voltage source to decrease as much as possible the input noise. The output signals were amplified (2000 times) by a standard low noise voltage preamplifier (SRS560 Stanford Research) and filtered by a low pass filter (10 kHz) (fig. 1 c). The amplified and filtered signal was then used by the AFM electronics as feedback for approaching the cantilever to the substrate surface and to make AFS curves.
Due to the fact that the developed piezoresistive cantilever has no sharp tip we fabricated substrates with sharp tips (curvature radius between 20 nm and 300 nm) in the cleanroom of the IMB-CNM. The substrate tips and the cantilevers where then aligned by the optical microscope of the AFM. In the fig. 3 is visible the complete set-up.
After the successful mechanical and electronic integration of the chip into the PicoLe AFM we performed AFS experiments in air, water and Phosphate Buffered Saline (PBS) solution. We optimized all the parameters and the set up to obtain the best possible Fz curves in liquid environment.
Finally we used this set up to detect the molecular interaction between b-BSA and neutravidin.

Description of the main results obtained.

Despite the long period for the design and realization of the set up for the mechanical and electronic integration, the proposed solution was very straightforward: the chip was hold firmly onto the nose cone by the neodymium magnet with no detrimental effect during the AFS measurement. Moreover the placing of the chip into the nose cone was quite fast and easy with no risk to damage the chip. About the electronic integration, even if we didn't use a specifically designed on board low noise amplifier placed near the chip, we could obtain signals with low output noise (the noise was approximately 1.5 times higher than the intrinsic input referred noise of the chip).
After the integration of the chip we successfully performed Fz curve in air to measure the sensitivity of the sensor (fig. 4). The measured sensitivity in Viterbo agreed with the measured sensitivity in IMB-CNM measured with a different technique.
When we started to make the measurement in liquid environment we had some problems: the aluminum conductors in some chips were not well passivated and we had high 50 Hz noise (fig. 5). The first problem was solved depositing a thin layer of epoxy resin onto the conductive parts of the chip while we drastically reduced the 50 Hz interference biasing the liquid with the ground potential. We performed successfully Fz curve also in liquid environment. The measured sensitivity agreed with the measured sensitivity in air (fig. 5). One very big problem was represented by the cleaning of the cantilever surface. In fact if too many silicon oxide particles were present onto the cantilever surface, after the micromachining process, we couldn't perform reliable Fz curve in liquid. Therefore we choose the cleanest cantilever to perform AFS experiment.

For biomolecule recognition we choose the b-BSA neutravidin complex. We functionalized the substrate by neutravidin and the cantilever by b-BSA following a previously developed procedure. The cantilevers were functionalized into microcapillaries, using a micromanipulator, while the substrate by immersion. We performed AFS measurements using these cantilever and substrates. Finally in two different experiments it was possible to successfully detect adhesion events that are most probably related to the recognition between b-BSA and neutravidin.

Foreseen publications

We are now in the data analysis and interpretation phase and we hope to have the article ready to be submitted to Apply Physics Letter Journal in March.

Figures

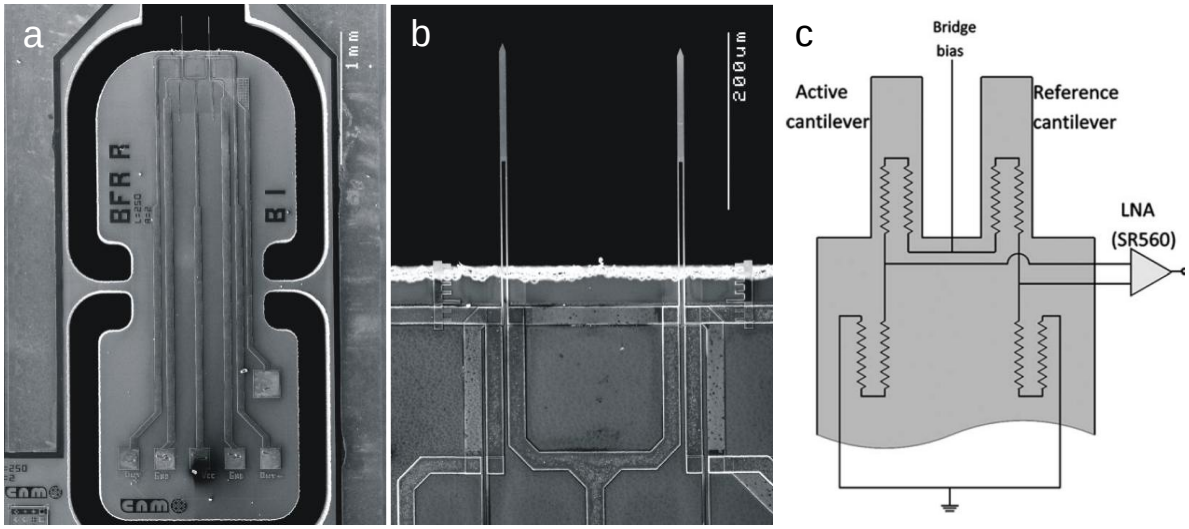

Fig. 1 - (a) SEM image of a piezoresistive cantilever chip: two cantilevers are visible in the top part and the pads for contacting the chip are in the bottom part. (b) SEM image of two piezoresistive cantilevers. In total there are four piezoresistors per chip in a Wheatstone bridge configuration, two in the cantilevers and two in the substrate. (c) The differential voltage of the Wheatstone bridge is amplified by the low noise voltage amplifier. The amplified signal is recorded by the AFM electronics.

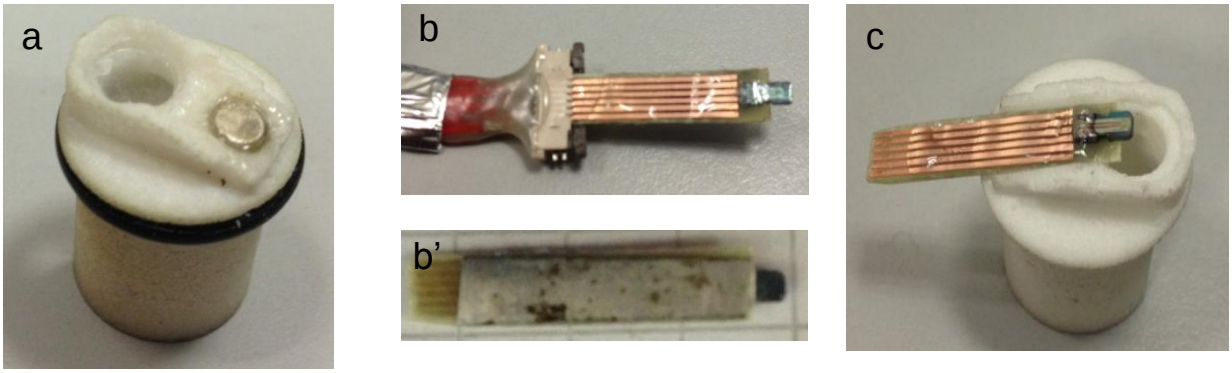

Fig 2 – (a) Modification of the standard nose cone of the PicoLe AFM. (b) Top view and (b') bottom view of the chip bonded onto the PCB wich is inserted into the ZIF profile. (c) The PCB is holded strongly onto the nose cone by the magnet.

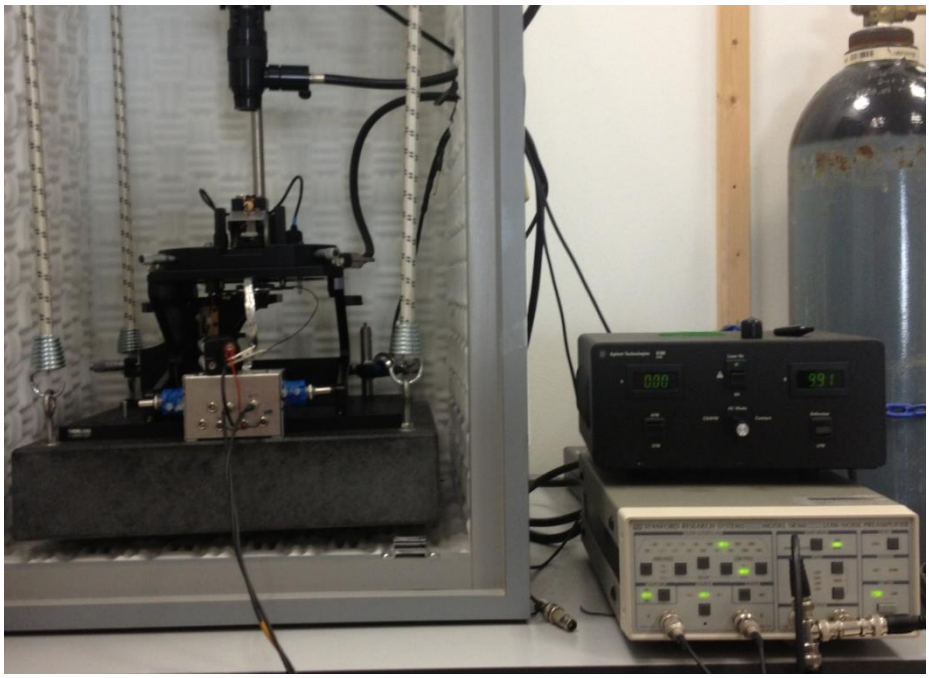

Fig. 3. Final set up for the AFS experiments. On the left is visible the optical microscope, the AFM, the batteries and the cables from the chip. On the right there is the AFM controller and the low noise voltage amplifier.

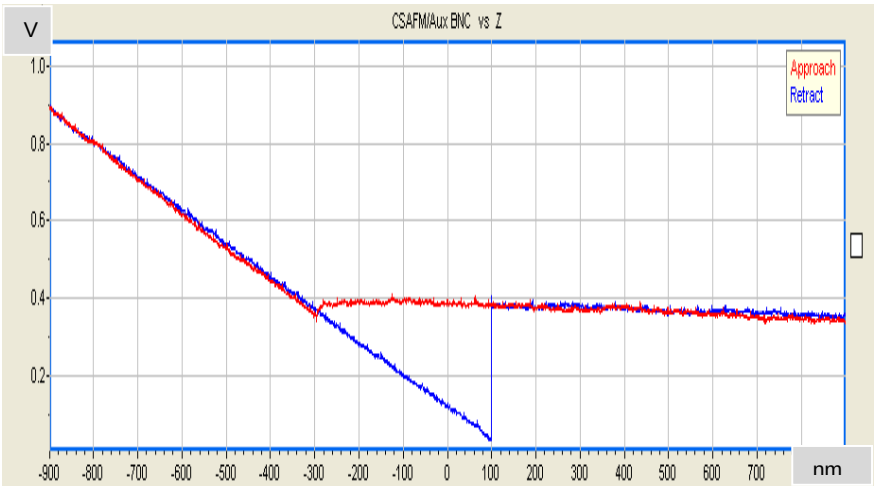

Fig 4 – Fz measurement in air. On the y-axis is reported the amplified output differential voltage which can be converted in force knowing the sensitivity (450 μV/μm with no amplification) and the sping constant (1 nN/um) of the cantilever. In x-axis is reported the displacement of the piezoactuator of the AFM. The adhesion force due to the water meniscus is visible in the retract curve.

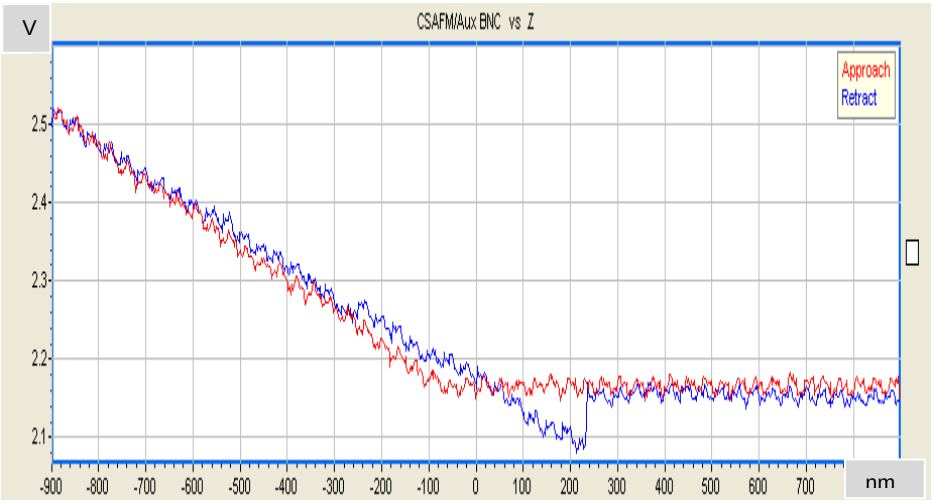

Fig 5 – Fz measurement in liquid. It is particularly visible the interference at 50 Hz. Also in this case it is visible a small adhesion. The sensitivity is 400 μV/μm (with no amplification) and the spring constant 1 nN/um