The capability of detecting nanomaterials (NMs) in biological samples represents one of the main challenges in bionanoscience, as it would allow the monitoring of cell-NMs interactions at the nanoscale, which is of primary importance in several fields of application, from drug delivery to nanotoxicology. To this aim, Atomic Force Microscopy (AFM) has been proposed as a versatile platform for the detection of NMs in biological matrices. By detecting the inhomogeneity of the mechanical, electrical or magnetic properties of the host-guest systems, the presence of NMs can be revealed [1].

In this work, different AFM-based techniques are employed to investigate the interactions between Magnetic Nanoparticles (MNPs) and cells. We show the possibility to reveal the presence of MNPs in biological system by detecting both the magnetic and the mechanical properties of the samples. First of all Magnetic Force Microscopy (MFM), a two-pass AFM-based technique which requires a tip coated with a magnetic film to obtain images reflecting the local magnetic properties of the samples, is employed for the imaging of MNPs internalized in cells. In addition to this, buried MNPs in soft biological matrices are visualized using three different AFM-based techniques in which the contrast reflects the non homogeneous mechanical properties of the host and guest systems. In particular, images of the local sample stiffness are obtained using the AFM force-volume imaging mode allowing the detection of force-distance curves. Moreover, the Contact Resonance Frequencies (CRFs) of the cantilever in contact with the sample surface, which are related to the local elastic modulus of the sample, are recorded employing the Atomic Force Acoustic Microscopy (AFAM). Following an offline procedure, the semi-quantitative CRFs maps are then converted into quantitative indentation modulus maps by assuming a proper model for the cantilever-tip-sample system. Furthermore, online maps of the local indentation modulus of the samples are recorded using Torsional Harmonics AFM (TH-AFM) which allows the evaluation of the local sample stiffness by acquiring force-distance curves in tapping mode. Finally, the mechanical properties evaluated with these three different techniques are compared and the influence of the penetration depth of each technique on the results is discussed and rationalized.

A careful comparison between the images obtained using all these techniques based both on the magnetic and the mechanical contrast allows to detect NMs incorporated in biological matrices and represents a clear indication of the AFM powerfulness in the field of nanobiotechnology.