Combined topographic/mechanical colloidal probe imaging for the investigation of cellular interactions

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Introduction

In the last two decades AFM has proved to possess a great potential in the biomedical and regenerative medicine fields as a tool to characterize mechanical and morphological properties of living cells, which were shown to be correlated to the cells' patho-physiological state [1]. Despite the wealth of experimental and technical informations provided by the reports published so far, a robust and commonly accepted methodology, regarding all the steps of the experimental activity, is still missing; this represents an obstacle to the effective exploitation of AFM-based nanomechanical approaches as biomedical tools. We have addressed some major *open issues* in AFM nanomechanics applied to living cells, such as the choice of the best AFM probe and the effect induced by the finite thickness of the cellular specimen on the measured Young modulus (*finite thickness effect*). The proposed solutions have been implemented in a protocol based on the use of micrometer-sized spherical probes for the combined topographic and mechanical imaging of living cells. An example of application of the protocol, regarding the correction for the finite thickness effect, is reported and briefly discussed.

Materials and Methods

Despite the fact that commercial sharp AFM tips have been so far widely employed in the field of cells' nanomechanics, several questions have been raised about the reliability of the mechanical properties measured on such soft and fragile samples using these probes. The critical points can be summarized as follows:

1) The small radii of curvature of commercial AFM tips (typically a few tens of nm) imply the application of high pressures and strains to the cells' surface, potentially leading to the damage of their outer membrane or the underlying cytoskeleton [2];
2) Commercial tips can show significant deviations from the ideal geometrical shape described by the corresponding contact mechanics model, thus introducing a significant source of error in the global statistics;
3) Cells are characterized by a strong structural heterogeneity and dynamical activity at the nanoscale, which could result in large standard deviations in the collected statistics [2,3].

A practical alternative to the commercial AFM tips is represented by spherical *micrometric* probes, also called *colloidal probes*. Their main advantages can be summarized as follows:

1) The applied force can be spread on a much wider area, thus significantly reducing pressures and the risk of cells' damaging;
2) They can be reliably produced and accurately characterized directly in the laboratories, according to an established characterization protocol [4];
3) They smear out nanoscale inhomogeneities, providing mesoscopic robust values of the Young modulus [2,3].

In addition, Dimitriadis and coworkers [2] developed an analytic approximate correction, for the case of spherical probes, to take into account the aforementioned finite thickness effect. This allowed us to characterize mechanical properties also of the thinnest cells' peripheral regions (cytoplasmic protrusions), whose importance is justified by their role in cells' motility and richness in focal adhesions. Here we report on the validation of our topographic/mechanical imaging protocol and of the finite thickness correction procedure, tested on living cells, cultured *in vitro* and then transferred into a custom thermostatic AFM fluid cell (@37°C).

Results and Conclusions

In Figure 1 topographic and mechanical maps acquired on a cell from the line PC12 (pheochromocytoma of the rat adrenal medulla) are shown; maps were acquired by means of a colloidal probe with radius $R \approx 5$ μm. Cells appear more rigid because of their finite thickness and the presence of a rigid substrate underneath.

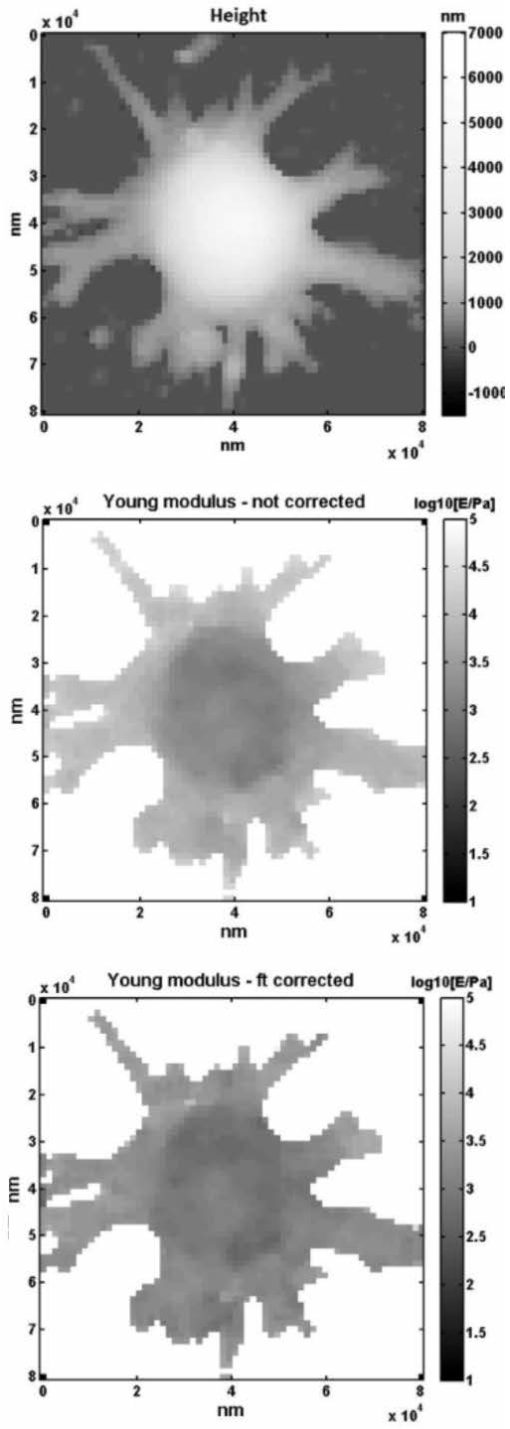

Figure 1. 2D AFM maps of a PC12 cell: height (top), uncorrected Young modulus (center), finite-thickness corrected Young modulus (bottom).

A quantitative comparison between Young moduli with or without finite thickness correction is shown in Figure 2 by means of histograms; when the correction is applied, we notice an overall decrease of the cell's Young modulus, and a relative decrease of the rigidity of the thinner cellular extensions with respect to the higher cell body, according to expectations (the two different contributions are strongly convolved).

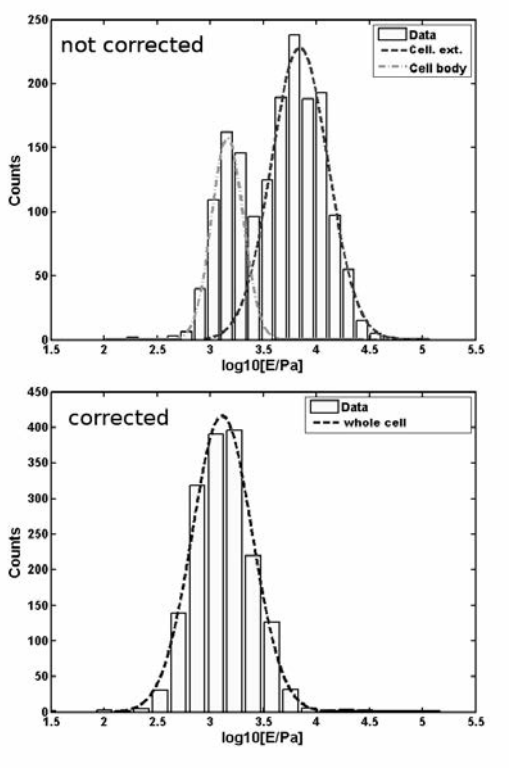

Figure 2. Histograms of Young moduli from maps shown in Figure 1: uncorrected Young modulus (top), finite-thickness corrected Young modulus (bottom).

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