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Preliminary measurements of elasticity properties of lung tumor living cells for cancer detection

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***Abstract* — Recently, a change in cell elasticity has been recognized as a marker of cancer. Reliable and reproducible mapping of cell elasticity distribution could be given by a metrological characterized AFM instrument and by a robust statistical cell data analysis. Here, micro and nanoindentation of soft reference materials such as Sylgard were done allowing to test a 10% AFM measurement reproducibility. Contact images and force mapping of A549 living cells were driven. A modal elastic modulus of 0.5 kPa, comparable with literature data, was obtained. A highly spatially resolved elasticity distribution was computed, given a powerful tool for cell mechanics analysis.**

Keywords—AFM; elastic modulus; metrology; tumour cell; diagnosis; sylgard

I. INTRODUCTION

In recent studies it has been shown that a change in cell elasticity, a biophysical properties of cells, can be considered a marker for different diseases, such as for cancer [2][3][4]. Mapping the elastic modulus of cells by AFM has the potentially to investigate on changes in cell elasticity [5]. In order to have reliable and reproducible cell mechanics data, a suitable measurement characterization is necessary and a robust statistical data analysis must be done.

For AFM characterization, measurements over stable and homogeneous elastic modulus material are required to properly set the measurement setup on cells. AFM validation for elastic modulus lower than 5 MPa (biological material range) is not yet available [6]. As investigated in [7] polydimethylsiloxane can be used as reference elastic modulus material in this range.

Reliable cell mechanics data are obtainable only through rigorous statistical analysis over a high number of entities (cells or cell details). A complete mapping of elastic modulus to explore cell heterogeneity need to be accurately designed, taking into account also subtract effects (Fig.1).

In this paper, results of AFM characterization on stable soft materials and a preliminary AFM measurement of elastic modulus behavior of living tumor cells are presented.

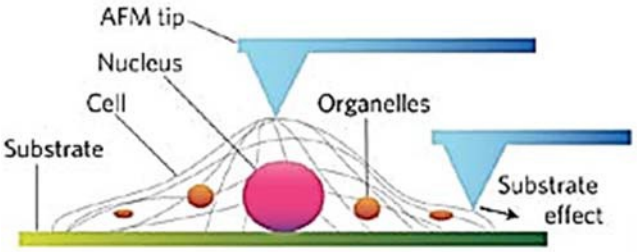

Fig. 1. AFM force spectroscopy measurement of living cell [1].

II. MATERIALS AND METHODS

Sylgard 184 was employed as polydimethylsiloxane materials for stable elastic modulus realization. Two curing/base agents densities were employed, 1:15 and 1:25 to realize thick quadratic samples (1 x 1 cm), as shown in Fig. 2.

A549 lung tumor cell lines were seeded on a 3 cm diameter petri dish. Measurements were done within few hours after get out from the incubator.

AFM measurement were done in liquid, deionized water for Sylgard samples and PBS buffer for living cells, in contact mode. A very low approaching setpoint was employed for cell measurements (0.1 V), indentation load of 0.4 V (relative setpoint) was set realizing indentations lower than 1 um depth.

Rigid cantilevers (40 N/m elastic constant) with a rectangular shape (ACTA) and with spheric or pyramidal indenter were used, to make microscale and nanoscale elastic modulus measurements on Sylgard samples (Fig. 2). Soft cantilever (0.3 N/m elastic constant) with a triangular shape (HYDRA) and with a pyramidal tip was used to make nanoscale measurements of living cells (Fig. 5).

Simple Hertz contact model for a spheric and a pyramidal tip [8] was employed to calculate elastic modulus for both cantilever shapes, without aiming to compare or accurately calculate elastic modulus.

Measurement were done on 50 x 50 um area over both samples, testing more than 2000 surface points.

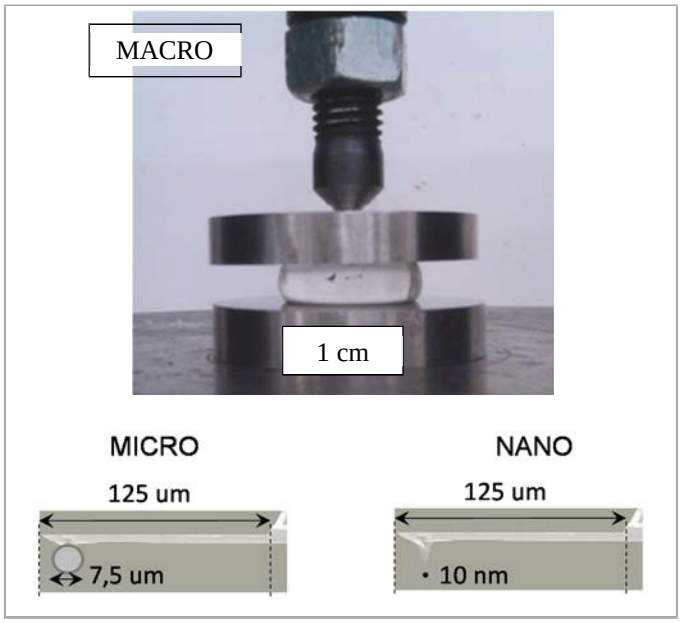

Fig. 2. Characteristic dimension of indenters use to perfom elasticity measurement at macro, micro, nanoscale level over 1 cm Sylgard samples.

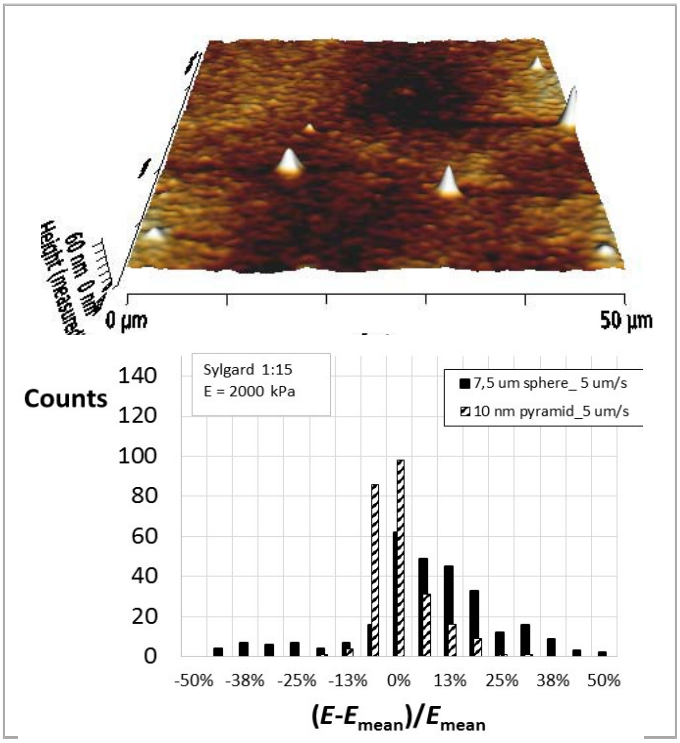

Fig. 3. AFM image of Sylgard 1:15 with elastic modulus distribution measured in 50x50 um area at microscale (spheric indenter) and nanoscale (pyramid indenter)

III. RESULTS AND DISCUSSION:

A. *Characterization of Sylgard samples*

Sylgard samples images obtained with the pyramidal indenter shown a surface average roughness variable in function of sample density (Fig.3-4). In particular roughness at nanoscale was obtained for Sylgard 1:15 (Fig. 3, white pyramids are tip artifacts), roughness at microscale was obtained for Sylgard 1:25 (Fig. 4).

Regarding force mapping of Sylgard samples (Fig 3-4), normal tending distributions were obtained round 2 MPa and 500 kPa elastic modulus values. Data dispersion were contained in 10% over the tested 50x50 um area, lower dispersion was observed at nanoscale level.

By the analysis of elastic modulus along the indentation depth, in [9] was shown an elastic modulus plateau reachable for this kind of material after certain threshold depths. Working in that plateau let to reduce unstable measures of surface elasticity.

Sylgard 184 was thus useful to characterize the AFM measurement system evaluating a limit reproducibility of 10% in a given volume of sample bulk.

With the appropriate contact mechanics model and calibrated nanoindenter, Sylgard 184 could become reference material for soft elastic modulus measurements.

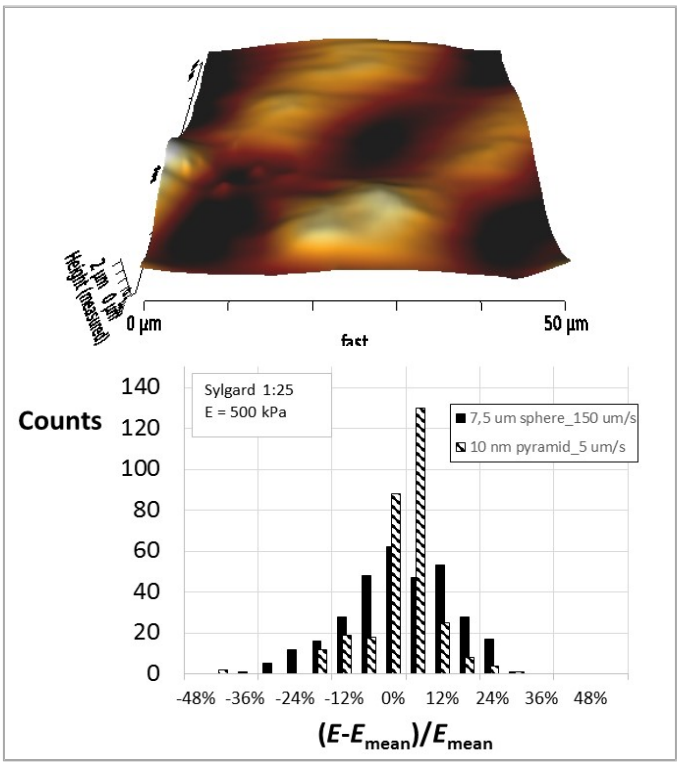

Fig. 4. AFM image of Sylgard 1:25 with elastic modulus distribution measured in 50x50 um area at microscale (spheric indenter) and nanoscale (pyramid indenter)

B. Imaging of A549 living cell

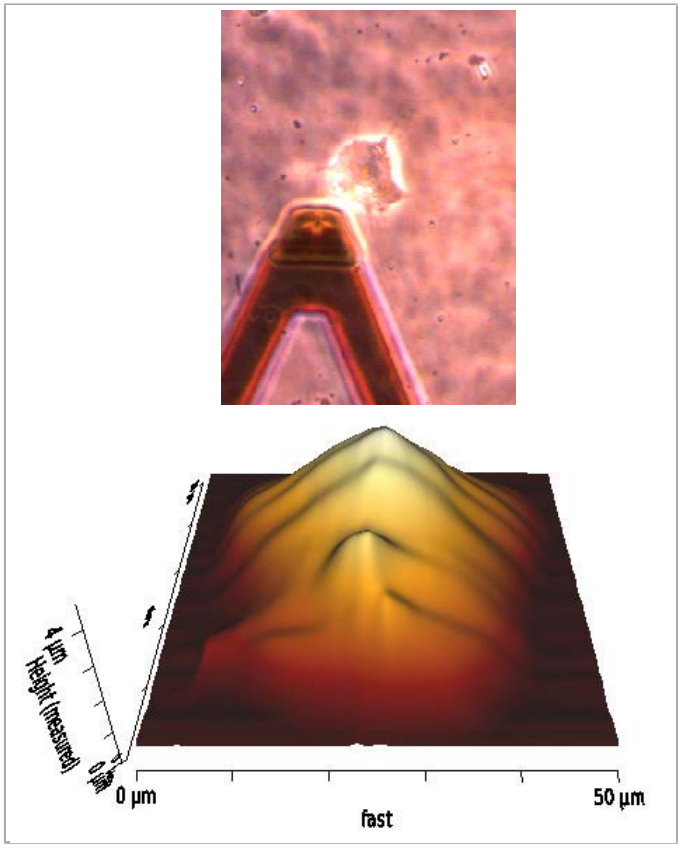

Fig. 5. Measurement of an A549 living cell in contact mode (upper panel), with the resulting 3D image (lower panel).

A549 cells (ATCC® CCL-185™) are a human cell line from a lung carcinoma from donor. A549 were expanded and maintained in F-12K growth medium (catalog no. 30-2004) with FBS 10% v/v (Fetal Bovine Serum, Lonza, Wokingham, UK), L-glutammine 2mM, penicillin 100 U/ml and streptomycin 100 g/ml (Lonza Wokingham, UK) in traditional Petri dishes in incubator at 37°C with 5% CO2. Fresh medium was replaced every 3-4 days until cells reached about the 80% of confluence. For the AFM measurement cells were seeded at very low density (30%) in order to image single cells.

Working on approaching parameters, a contact image of A549 living cell has been obtained (Fig. 5). Optimal measurement setting were found with a not excessively slow scan rate (1 Hz) and a low approaching target height (3 um) in order to explore the complete height of the cell. In addition, varying the scan angle (180°) was possible to obtain the image without moving the cell. A quite repeatable trace and retrace profile was obtained acting on feedback control parameters of the cantilever. 1024x1024 pixels were acquired, with a total measure time of round 30 min.

A cell dimensions of round 50 x 50 um with a maximum height of round 4 um was measured presumably in correspondence of the nucleus.

C. Force mapping of A549 living cell

The imaged living cell was mapped in term of elastic modulus on a 50x50 points grid, represented of fig. 6 with the optimal indentation speed of 5 um/s found on Sylgard. The resulting total mapping time was round 30 min.

The frequency distribution of the calculated elastic modulus were computed and shown in Fig. 6. Four characteristic elastic modulus were observed in the interval of 0.5 – 1 – 1.5 – 2 kPa, in which 0.5 kPa was the modal elastic modulus found. The obtained modal elastic modulus was comparable with literature data [1] of living A549 cell using a triangular shape cantilever with the simple Hertz contact model.

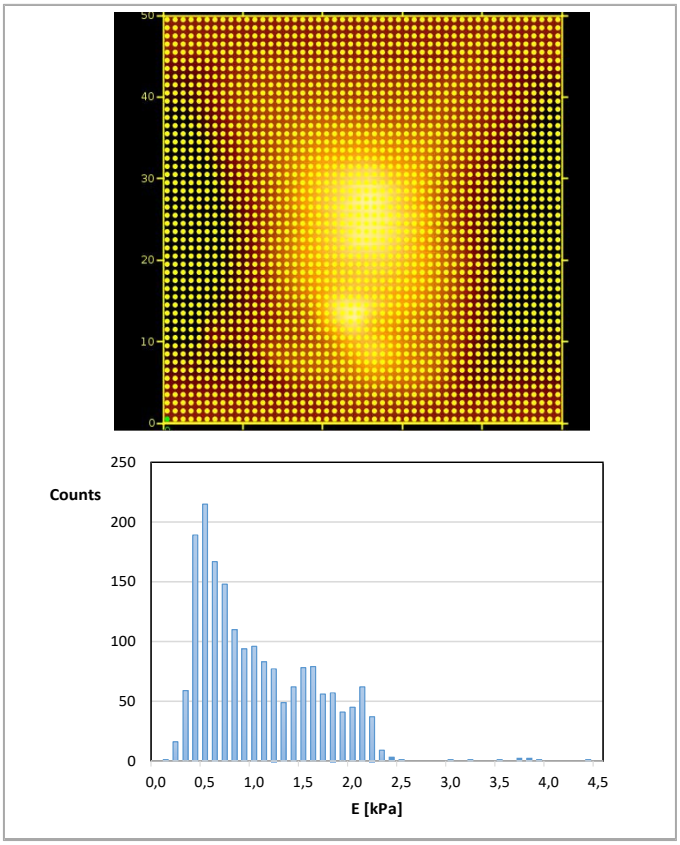

Fig. 6. 2D image of A549 living cell with overlapped grid used to perform force mapping; below is shown the resulting elastic modulus dstribution.

Elastic modulus frequency distribution was converted into spatial distribution in order to obtain the real elasticity map of the living cell (Fig. 7). The modal elastic modulus of 0.5 kPa is represented by the white region, presumably covered mainly by the cell nucleus. The frequent elastic modulus of 1 kPa is represented by the yellow region distributed in the cell cytoplasm, 1.5 and 2 kPa (red and green regions) are located around the central region (presumably the nucleus) describing more rigid cell components. The alteration of cell elasticity in the upper part of Fig. 7 could indicate a probable cell movement toward the end of the measurement.

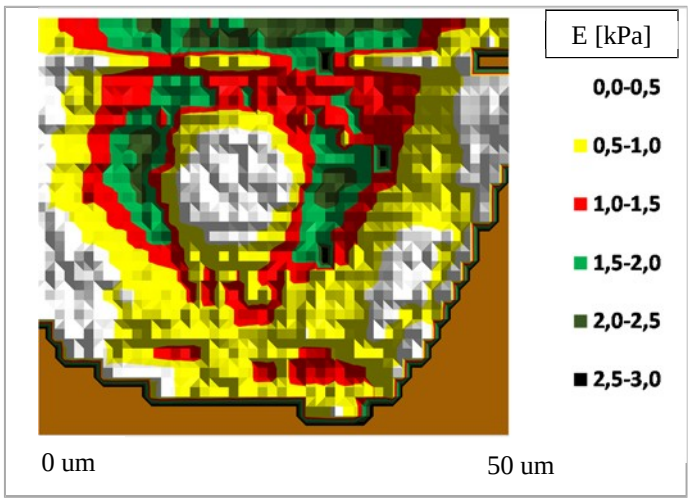

Fig. 7. Elastic modulus mapping of tested A549 living cell.

IV. Conclusion

A metrological characterization of the AFM measurement system for elastic modulus measurements of living cell was given.

Sylgard 184 was demonstrated to be a feasible material for testing a 10% AFM measurement reproducibility in the soft range (below 5 MPa). A more complete Sylgard and measurement characterization could allow Sylgard to become a reference materials for soft elastic modulus measurements.

Contact images of living A549 cells were obtained opportunely setting the measurement system, on the base of Sylgard measurements. High resolution force mapping were undertaken obtaining elastic modulus distribution. A modal elastic modulus of 0.5 kPa was measured according to literature value for the same triangular cantilever and contact model employed. The characteristic elastic modulus were mapped on the cell image in order to identify cell compartment mechanics. The lowest elastic modulus was measured on the nucleus region.

By statistical analysis over a high number of cells, frequency and spatial distribution of elastic modulus can be confirmed giving a reliable pattern of cell mechanics. By traditional fluorescence images of the different cellular compartments these results can be furtherly confirmed. This experimental setup allows exploring changes in biophysical properties of cell, such as cancer detection, in a reliable and reproducible way.

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