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# Study on the AFM Force Spectroscopy method for elastic modulus measurement of living cells

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**Abstract**. The cell elasticity gives information about its pathological state and metastatic potential. The aim of this paper is to study the AFM Force Spectroscopy technique with the future goal of realizing a reference method for accurate elastic modulus measurement in the elasticity range of living cells. This biological range has not been yet explored with a metrological approach. Practical hints are given for the realization of a Sylgard elasticity scale. Systematic effects given by the sample curing thickness and nanoindenter geometry have been found with regards of the measured elastic modulus. AFM measurement reproducibility better than 20% is obtained in the entire investigated elastic modulus scale of $10^1$ - $10^4$ kPa.

## 1. Introduction

The measurement of mechanical properties of living cells and extracellular components is becoming increasingly important in different field like cancer and developmental biology. The cell elasticity gives information about its pathological state and metastatic potential [1], the extra-cellular matrix elasticity influences cell lineage specification [2]. Accurate cell elasticity measurements can be performed using the AFM Force Spectroscopy method. It allows to quantify the cell Young's modulus $E$ through measures of the cantilever deflection when approached to the cell surface [3].

In a typical force-indentation curve obtained by the AFM measurement the force values on the y-axis are calculated from the measurement of the cantilever vertical deflection $V$ [Volts], the system sensitivity $S$ [nm/V] and the cantilever constant $k$ [N/m]. The indentation values $\delta$ [nm] on the x-axes are calculate from the measurement of the cantilever piezo height $z$ [m]. $S$ depends on the positioning of the cantilever over the cantilever holder. The Young's modulus $E$ of the sample is calculated fitting the extend part of the force-indentation AFM curve with the classical Hertzian model for a spheric indenter [3].

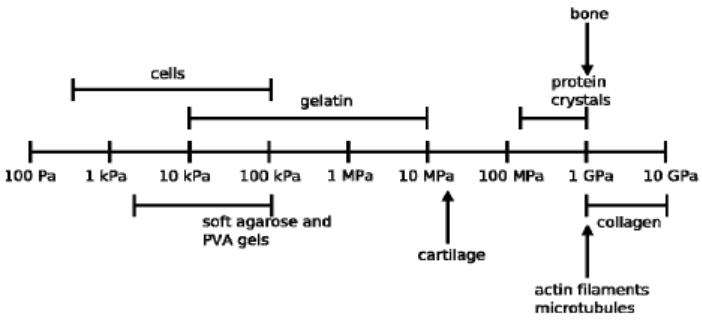


**Figure 1.** Elastic modulus of biological materials [4].

The aim of the present work is to study the AFM nanomechanical analysis in an elasticity range of $10^1$ - $10^4$ kPa. The future goal is to make this method a reference method, in the cell elasticity *E* range of $10^{-1}$ – $10^2$ kPa (figure 1) not yet explored with a metrological approach. To make the AFM method a "reference method" an accuracy budget must be evaluated, with the aid of a stable and homogeneous *E* sample scale. To reach this prefixed objective the following steps have been done in this work: realization of a stable and homogeneous *E* scale in the interesting biological range, realization of the suitable nanoindenter, optimization of the AFM measurement parameters, AFM measurement of the *E* scale, analysis of the AFM measurement uncertainty, comparison with other literature methods.

## 2. Materials and methods

### *2.1. The preparation of the working E scale*

Sylgard 184 has been chosen as materials for the realization of the working *E* scale ($10^1$ - $10^4$ kPa), this because it presents a tunable *E* varying the base/curing ration, it allows to realize very low *E* materials (down to few kPa), it presents at a microscopic level a very homogeneous surface and let to construct mechanically stable samples. Sylgard 184 is a viscoelastic polymer of cross-linked polydimethylsiloxane PDMS chains that can be prepared curing short PDMS chains (the Sylgard 184 base agent) with hydrogenated-PDMS chains (the Sylgard 184 curing agent). The chemical curing reaction (hydrogen addition to the vinyl ends of PDMS chains, catalyzed by Pt and heat) causes the internal re-arranging of the random-distributed PDMS chains that expose to the surface idrofobic – $CH_3$ groups.

Thick Sylgard 184 (Dow Corning) cylinders, 1.5 cm diameter per 1.5 cm height, have been realized weighing in a plastic can the Sylgard components with nominal base/curing ratio of 2.5, 5, 7.5, 15, 25 and 50 by weight and stirring them for 5 minutes. Samples have been realized let curing the Sylgard blend in a 24-well plate.

Thin cylinders, 2.5 cm diameter per 0.1 cm height with nominal base/curing ratio of 5, 15, 30, 60 have been prepared, curing them in rings lean on a glass slides. 20 days curing time at 20°C has been waited leaving the Sylgard cylinders in the well plate submerged by deionized water and leaving the cylinder in the ring exposed to the air.

For the AFM measurements the thick cylinders are indented on both surfaces: for the upper surface the nanoindenter has been immerged in the corresponding well, for the botton surface (flat and not-water and air contaminated) the cylinders are extracted from the plastic plate, turned upside down and fixed with Vaseline on a petri dish filled with deionized water. The thin cylinders (attached at the botton to the glass slide) instead are analyzed immerging the entire system in the petri dish filled with deionized water.

### *2.2. The preparation of the nanoindenter*

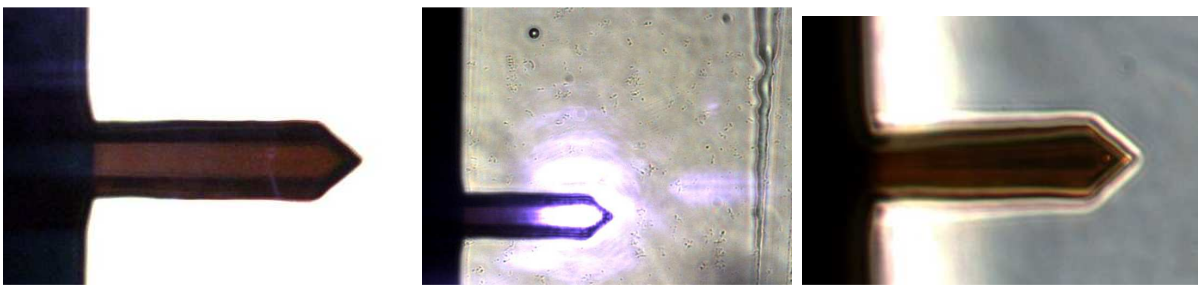

**Figure 3.** Nanoindenter construction procedure. From left to right: the clean and new tipless cantilever; cantilever with the laser spot on it, the glue at the right and the $SiO_2$ sphere in the top; the realized indenter with the attached sphere.

As nanoindenter geometry a $SiO_2$ sphere with a known diameter has been attached to the top of a tipless rigid AFM cantilever. A very small quantity of glue (Dymax OP-29), a drop with a diameter similar to the $SiO_2$ sphere diameter (GmbH microparticles o.d. 7.75 ± 0.29 μm), has been load on the top of a tipless cantilever (Nanosensors TL-NCH) acting on the setpoint parameters of the approach and force curve measurement of the AFM instrument (JPK Nanowizard II). In a second moment the sphere has been attached performing a force curve on its top. In figure 3 is reported the optical images step of the construction procedure of the nanoindenter.
A 40 N/m value has been used in this work for the elasticity constant of the nanoindenter (nominal manufacturer value). A 25 nm/V value has been used in this work for the sensitivity of the piezo-positioning of the AFM instrument.
Two geometries of nanoindenter are realized, following named indenter 1 and 2.

## 3. Results

*3.1. Optimization of the AFM indentation speed*
To optimize the AFM measurement the *E* value has been analyzed in function of the indentation speed. A petri dish with a thin Sylgard sample is placed on the AFM stage and force curve measurements are acquired in liquid, in order to avoid the "jump-to-contact" effect, in an indention speed range from 0.1 to 10 μm/s. 1 V has been set as preload setpoint of cantilever during the approach and force spectroscopy measurement, 5 μm is set as total measurement z length.
As a result (figure 2), a speed such that the obtained *E* lies in the plateau between low indentations (where the *E* is underestimated because of the closeness to the contact point) and high indentations (where the *E* is overestimated because of the sensing of the substrate stiffness or sample viscous effects) has been chosen. Moreover in the *E* plateau the speed that maximize the work of adhesion of the sample to the indenter (in correspondence of the maximum of the adhesion forces $F_{AD}$) is chosen, obtaining the optimum value of 1 μm/s (measurement z length of 5 μm in 5 seconds extend time).

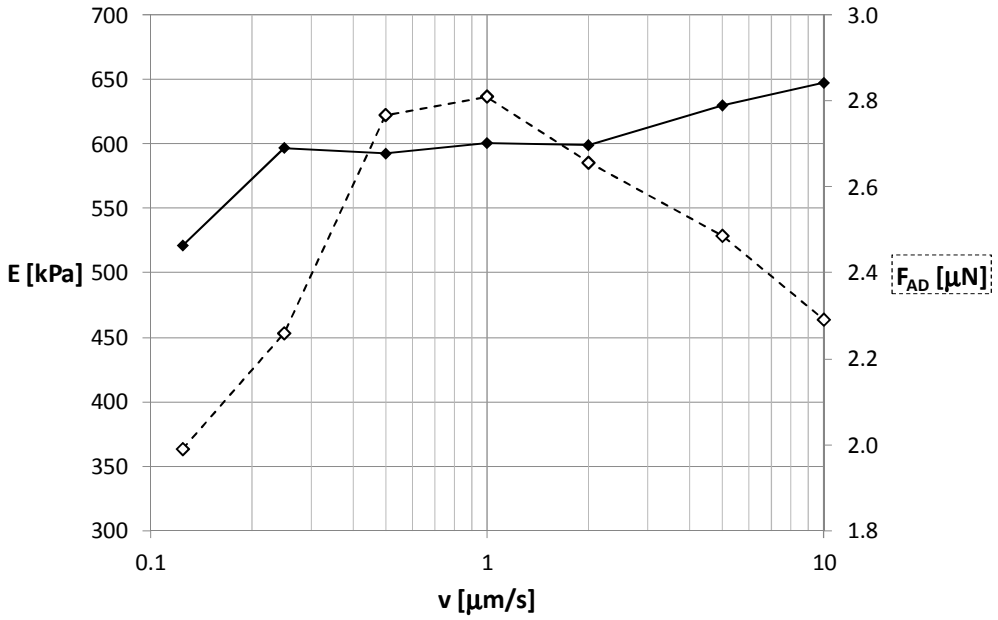


**Figure 4.** Indentification of the optimum indentation speed for Sylgard samples.

*3.2. Study of the AFM measurement uncertainty*

In figure 5 are reported the *E* measurements done on the Sylgard reference thick cylinders (contaminated and uncontaminated surface), on the thin cylinders and on the thick cylinders tested with nanoindenter 2. The error bars represents the obtained 20% reproducibility of the thick cylinders measurements with indenter 1.

In figure 6 are reported the AFM measurement of this work in comparison with the unconfined compression measurement done over the same thick Sylgard cylinders. In addition AFM measurement done by Carillo et al. [5] with a sample curing thickness similar to our thick samples, and microfluidic measurements done by Gutierrez et al. [6] are reported.

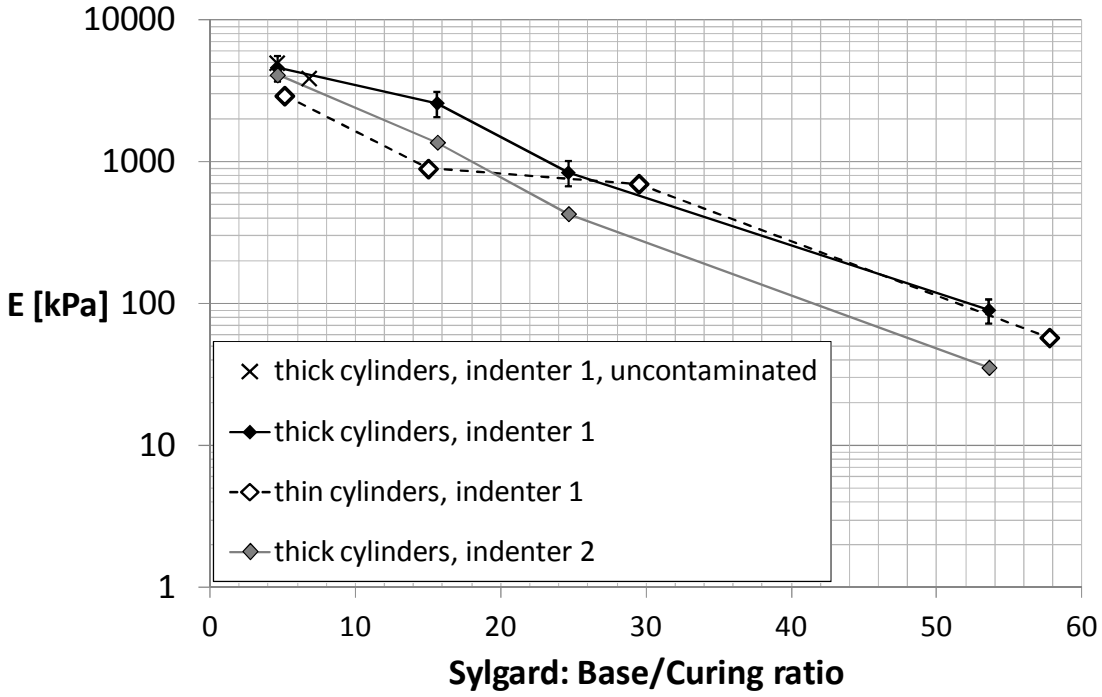


**Figure 5.** Analysis of the AFM results in function of the nanoindenter geometry and sample thickness.

**4. Discussion and Conclusion**

In this work has been metrologically analyzed the AFM method for Young' modulus measurements on a wide *E* range comprising the cellular elasticity range. A Sylgard working *E* scale in this range has been prepared in different curing and contamination conditions. AFM reproducibility parameters have been optimized allowing to obtain a total measurement reproducibility of 20% along all the tested *E* measure range. This reproducibility value considers a 10% homogeneity of the Sylgard surface elasticity and a 10% measurement repeatability of the approach and nanoindentation process.

A systematic *E* effect due to the sample curing thickness has been observed for the less elastic cylinders (*E* decreases for thin 5 and 15 cylinders). It follows that the shape of the curing container plays an important role on the surface elasticity, at constant curing time and temperature and base/curing ratio. This can be explain considering that the reducing of the confinement space of the polymer (i.e. the well-plate in respect to the open flat ring) increase the curing reaction efficiency leading to stiffer sample. In fact in more confined space the conformational entropy of the polymeric chains decreases, the chains free-energy increases, the thermal stress energy increase and the chains are more attracted between each other.

Another systematic *E* effect due to the geometry of the nanoindenter has been observed for the more elastic cylinders (*E* decreases for 15, 25, 50 cylinders tested with nanoindenter 2). This can be due to the significant variation of the cantilever elasticity constant once realizing a new nanoindenter.
No effects of the Sylgard surface contamination (by water and air) are found on the surface elasticity, therefore seems not to be necessary to preserve the Sylgard cylinders surface during storage.

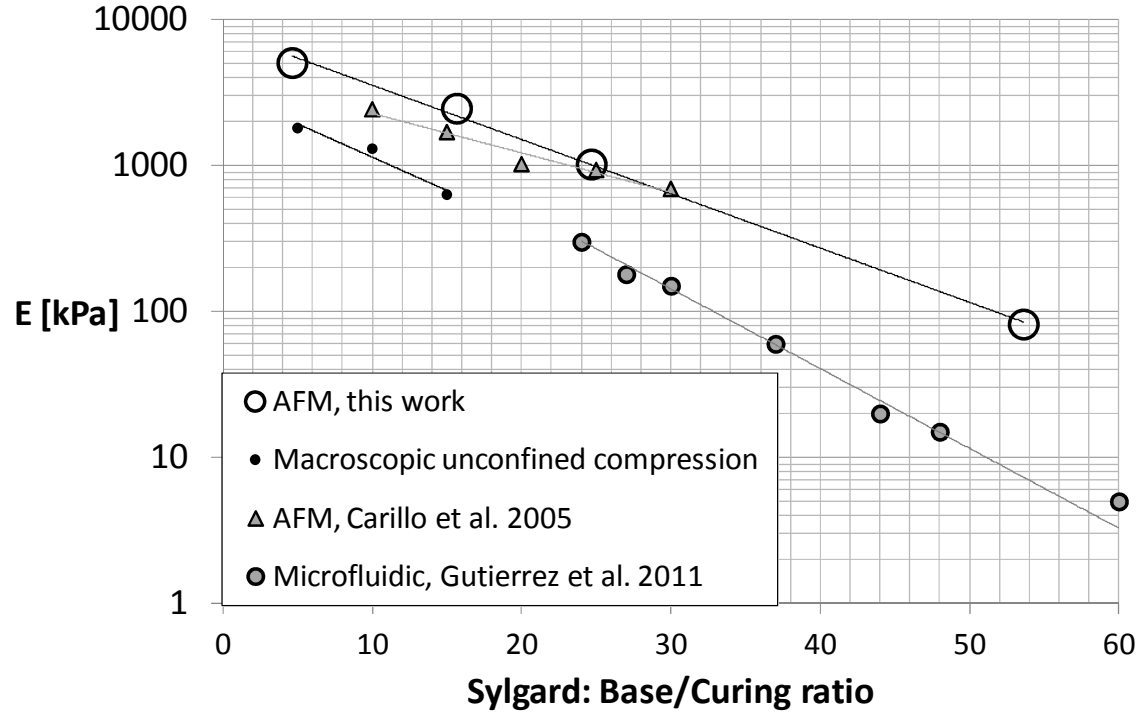


**Figure 6.** Comparison of the Sylgard *E* measures of the present work with literature values.

The *E* measures of this work are resulted in accord with the literature AFM values done by Carillo et al. (figure 6), but are resulted different from the same measurements done with other techniques (macroscopic compression and microfluidic). This stress the fact that an accuracy budget of the reported methods are needed in order to let a comparison.
In our opinion accurate AFM measures can be realized quantifying the uncertainty of the following influence quantities: the sensitivity of the AFM piezo-positioning, the elasticity constant of the nanoindenter, the diameter of the indentation sphere and considering the adhesion forces of the Sylgard surface in the contact mechanics model (the Hertzian model underestimated the effective contact area). Moreover if Sylgard cylinders are intended to be used for the metrological characterization of the AFM instrument the sample curing thickness must to be kept constant in order to realize stable measuring samples.
Exploring these metrological issues will give a right way of performing accurate elastic modulus measurement on living cells, where further expedients should be then taken with regards to the inhomogeneous and instable softer surface.

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