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Toward the realization of reproducible AFM measurements of elastic modulus in biological samples

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Abstract— The validation of the AFM method for elastic modulus E measurement in soft materials ($E < 5$ MPa) is still missing. The interest of measurements in materials with $E < 5$ MPa is mainly biological, including soft tissues and single cells. For the diagnosis of malignant human tumors, a change in cell elasticity, within tissues, has recently been recognized as a marker of metastatic potential. To measure a cell elasticity difference, reproducible E measurements in biological samples are needed. In this work a robust method for a metrological validation of E measurements in the range 50-5000 kPa was developed, based on the realization of thick E standard samples and on the study of the interactions between the measurement process and the sample at micro and nano scale. E measurement reproducibility limit of 4% has been reached. This allows designing a very sensitive and reproducible measurement of E in biological samples representing thus a powerful diagnostic tool for cancer detection.

Keywords—AFM; elastic modulus; metrology; tumour cell; tumour tissues, cell biology, sylgard

I. INTRODUCTION

Atomic Force Microscopy (AFM) allows high-resolution imaging of biological samples and the characterization of mechanical properties of very soft and non-homogeneous materials, such biological samples, by detecting repulsive and attractive cell surface forces (Cross,2007;Kuznetsova,2007). Young's modulus, or elastic modulus (E) is a measure of materials stiffness; it can be measured by AFM (Kuznetsova,2007;Darling,2007;Costa,2004) and gives information on biological sample (e.g. single cell within a tissue) elasticity.

The validation of AFM method for E measurement in materials with $E < 5$ MPa is still missing (Carrillo,2005). In the low range, the E measurement by AFM is influenced by the interaction between the measurement system and the material of which E is measured. Therefore, a metrological characterization of the system interaction needs to be determined. The interest of E measurements in materials with $E < 5$ MPa is mainly biological: soft tissues and single cells or cell cultures exhibit E in this range (Wenger,2007).

Recently, a change in cell E has been recognized as a marker of disease such as cancer (Cross,2007;Guo,2012;Cross,2008). Changes in the extracellular matrix and cytoskeleton structure has been found translating into cell elasticity changes (Bhadriraju,2002). In 2007, Cross et al. found a difference in E between living human metastatic cancer cells and the corresponding benign cells: they measured by AFM that malignant cells are 70% softer than benign cells. Current and traditional analysis for cancer cell detection (such as cytomorphological and immunohistochemical analysis) (Lekka,2012) are qualitative morphological analysis: they relies on cytoskeleton remodeling leading to cell shape changes. However traditional methods for malignant cells diagnosis have a limitation: frequent morphological overlap between tumor and normal cell types occurs (Cross,2007). Cross et al. also demonstrated that AFM measurements of E well correlate with traditional methods of cancer cell detection. Therefore, AFM mechanical analysis offers the powerful tool to quantitatively distinguish malignant cells from normal cells for cancer detection. To measure a cell elasticity difference, reproducible elasticity measurements of the biological sample are

needed and the target reproducibility must be lower than the expected cell elasticity difference (70%). As a consequence the measurement method, AFM force spectroscopy, must be validated for reproducibility.

Investigation for cancer detection can involve single cells (Lekka,2012 Li,2008) and tissues (Lekka,2012) coming from biopsies. Consequently, investigations should cover measures at macro, micro and nanoscale, respectively for analyzing the extended E in a tissue, the specific E of a single cell and also E of defined cells substructures at nanoscale. It has been shown (Lekka,2012) that E measured at single cell level and tissue level (respectively nano-micro and macro levels) can be different, and the combination of the two AFM measurements offers a precious set of information about cancer detection. To perform reproducible E measurement on different biological samples (tissues, single cell, cells substructures) the AFM method must be validated in different scale ranges. In addition, high indentation speeds must be tested in order to perform measurements in time limits compatible with cellular processes of living cells such as cell mobility (lifespan: seconds) and cell division, apoptosis (lifespan: minutes).

With this work a robust method for a metrological validation of E measurements in the range 500-5000 kPa was developed, based on the realization of thick samples showing an homogeneous E value on macro, micro and nanoscale, and on the study of the interactions between the measurement process and the sample. Sylgard 184 was chosen as modelling material for soft tissues, as also described in our previous work (Demichelis,2013-2014). Sylgard samples in biological elastic range of 50-5000 kPa were prepared. Indentations with the AFM sensor were performed to characterize surface homogeneity and viscoelastic behavior of samples. Its use as multiscale standard was also investigated. Operative measurement settings were obtained for the realization of reproducible elasticity measurements on biological materials.

Results obtained in this work will allow designing a very sensitive and reproducible measurement of E in biological samples aimed in measuring elasticity differences below 5%.

II. EXPERIMENTAL

A. *Sylgard as E standard in the range 500-5000 kPa*

The validation of the AFM *force spectroscopy* method on soft materials requires E standards. The standard must have an E defined in all its volume, must present homogeneity properties and stability over time. Procedures for preparation of standards must be defined: they can invalidate the employ of the standard since influence the sample homogeneity in all directions, both xy plane and z direction.

PDMS is a viscoelastic polymer of cross-linked PDMS chains that can be prepared curing short PDMS chains with hydrogenated-PDMS chains. 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 $-\text{CH}_3$ groups. This material, commercialized as Sylgard 184, consists in the base agent (short PDMS chains) and the curing agent (hydrogenated-PDMS chains) that must be blend each other. Sylgard can be a good candidate as standard in this context since presents a tunable E varying the base/curing ration, allows to realize very low E materials (in the range 500-5000 kPa), presents a very homogeneous surface at a microscopic level (Demichelis,2014) and let to construct mechanically stable samples.

B. *Principal influence quantities affecting the interaction between measurement process and sample*

AFM Force spectroscopy method allows obtaining an experimental force – distance curve when indenting a sample, the shape of the force-distance curve reflects the sequence of sample layers with possible different elasticity. E values strongly fluctuate at very low indentation depths (nearly the contact point, i.e., the sample surface). E reaches a plateau by increasing the indentation depth and finally increases when the substrate stiffness is sensed (JPK,2014).

The elasticity measured in each layer depends on indentation speed because of the viscoelastic behavior of the sample (McCrum,2003). When the characteristic time of indentation is smaller than the sample relaxation time (high indentation speed), the outcome is a higher resistance of the sample because interfacing with the PDMS viscous behavior, it results in an apparently higher E . Vice versa, when the indentation time is longer

than the sample relaxation time (low indentation speed), the sample has the possibility to move away from the indenting probe diffuse from the bulk to the sample surface. The outcome is, thus, a lower resistance that results in an apparently lower E . It follows that the indentation speed plays an important role.

The contact mechanics model employed will affect the measured interaction between system and sample. Hertz contact model was chosen for simplicity of calculation, since E value was not concern of this work, just E reproducibility was investigated in function of nominal E values.

Another influence quantity affecting the interaction is the indenter. It is defined by the cantilever elastic constant, the tip radius and shape, and the photodiode sensitivity when hanged to the AFM instrument. In this work indenter was chosen based on previous measures (Demichelis,2013- 2014), its choice is not object of this paper.

III. MATERIALS AND METHODS

A. Preparation of Sylgard samples

Fresh Sylgard 184 (Dow Corning) rectangular samples, 0.5 cm height, were realized in a grid plastic stamp, with nominal base/curing ratio of 15, 25 and 55 by weight. Stirring time of 2 min and curing time of 24h were set. The stamp was put on the AFM stage and each compartment was filled with deionized water, for AFM measurements in liquid. The employed storing method consisted in storing the samples at room temperature, without water on the surface, covering them with a plastic cup, washing the sample surfaces with ultrapure water prior to perform AFM measurements.

B. AFM measurement setup

Force measurements of Sylgard samples were performed in liquid (deionized water) to avoid the jump-to-contact effect (Demichelis,2014). AFM Force Spectroscopy measurements were realized with a JPK Nanowizard II instrument preparing suitable nanoindenters. The nanoindenter for the E measurement at microscale level was realized gluing a SiO₂ sphere (GmbH microparticles, nominal diameter 7.75 μm) on the top of a Silicon tipless cantilever. A bio-compatible adhesive (Dymax OP-29 optical glue) and a rigid tipless cantilever were employed (Nanosensors TL-NCH, nominal elastic constant k 40 N/m, no coating). To

perform E measurement at nanoscale level, a commercial rigid Silicon Nitride cantilever with a pyramidal tip was chosen (AppNano ACTA, nominal elastic constant k 40 N/m, face angle of the quadratic pyramid 31° , radius of the edge tip less than 10 nm, Al coating).

1 V was set as approaching parameter of indenter (corresponding to a cantilever deflection setpoint of 25 nm when approached to the sample), default feedback parameters for the approach are employed (i-Gain 150 kHz, p-Gain 0.0048). 0.4 V was set as final relative setpoint of cantilever during the force spectroscopy measurements (corresponding to a maximum load of 400 nN for the employed cantilever at the end of the extend process); the maximum experimental z length measurement was set equal to 5 μm .

C. E calculation

Young's modulus E [Pa] of Sylgard sample was calculated from the classical Hertzian model for a spherical indenter (eq.1) (Ladjal,2009), when using the cantilever with the glued sphere, and for a four-sided pyramidal indenter (eq. 2) (Lin,2007), when using the cantilever with the pyramidal tip:

$$F = \frac{4E}{3 \cdot (1 - \nu^2)} \cdot R^{1/2} \cdot \delta^{3/2}$$

eq.1

$$F = \frac{E}{(1 - \nu^2)} \cdot \frac{\tan(\alpha)}{\sqrt{2}} \cdot \delta^2$$

eq.2

In which F [N] is the force that the indenter develops against the sample, ν is the Poisson ratio (in this case 0.5), R [m] is the radius of the spherical indenter, α [$^\circ$] is the face angle of the tip, δ [m] is the indentation depth.

F and δ values were measured by AFM. In Force Spectroscopy mode, the nanoindenter was moved perpendicularly to the sample by a piezoelectric scanner that measures its absolute position z [m]. When the tip comes in contact with the sample (z_0 contact point [m]), a photodiode measures the vertical deflection of the nanoindenter V [Volts]. The measurement output from the AFM sensor is the nanoindenter deflection V

versus position z data, obtained extending and retracting of the cantilever over the sample. F and indentation depth values δ can be calculated as follows:

$$F = k \cdot x \quad \text{eq.3}$$

$$x = S \cdot V \quad \text{eq.4}$$

$$\delta = (z - z_0)_{EXP} - x \quad \text{eq.5}$$

Where k is the nanoindenter elastic constant [N/m], x is the nanoindenter vertical deflection [m], S is the photodiode sensitivity [Volts/m], $(z - z_0)_{EXP}$ is the experimental measurement of cantilever position.

Rearranging eq. 1 with the AFM measured parameters, the E equations for the measurement with the employed micro and nanoindenters become (eq.6 and eq. 7):

$$E_{Micro} = \frac{3(1 - \nu^2)}{4} \cdot \frac{k \cdot S \cdot V}{R^{1/2}[(z - z_0)_{EXP} - S \cdot V]^{3/2}} \quad \text{eq.6}$$

$$E_{Nano} = \sqrt{2}(1 - \nu^2) \cdot \frac{k \cdot S \cdot V}{\tan(\alpha)[(z - z_0)_{EXP} - S \cdot V]^2} \quad \text{eq.7}$$

Sample E were calculated with eq.6 and eq.7 in different fit ranges of the experimental V [Volts] vs. z [m] curve obtained. Experimental curve fitting was done using a specific routine written in Matlab environment. The contact point z_0 [m] was selected in correspondence of 1% of the difference between the maximum and the minimum z obtained in the extend curve. To compute E , voltage data V starting from the z_0 towards the indentation direction were considered subtracting the minimum voltage value in order to taking into account for possible voltage offset. The extend part of the experimental curve is fitted, in order to consider only the linear elastic behavior of PDMS.

D. Force Spectroscopy measurements

Force spectroscopy measurements at different indentation speeds in the range 0.1-1000 $\mu\text{m/s}$ were performed on fresh samples and after 4 months with different indenters (without controlling the amount of glue employed to attach the sphere for the home-made microindenters). E value for each Sylgard sample was calculated with eq.6 and 7 at micro and nanoscale level fitting all the data obtained by the force curve (Fig. 1A-B-C). At the optimal identified speed (5 $\mu\text{m/s}$), 320 E measures were performed on the surface of each Sylgard sample. These measures come from 5 surface maps of $50 \times 50 \mu\text{m}$ with 64 grid points each, taken in different surface position of the sample. The frequency distributions of these elasticity measures on each Sylgard surface are calculated and reported in Fig. 2A-B-C, where E is calculate fitting all the data obtained by the force curves.

To analyze the E function of z position, E was calculated in different fit range of the extend curve; results are plotted in Fig. 3 in function of the sample z -position, named $z-z_0$ (that numerically correspond to the measured depth δ).

AFM measurement reproducibility was calculated as the E variability measured in a given volume of the Sylgard sample, where Sylgard bulk homogeneity is reached. To calculate the E variability in the given sample volume, ΔE_V , a layer of a certain thickness in the sample was defined. This layer was chosen considering a portion of the experimental curve E vs. $z-z_0$ (Fig.3A) where the E threshold was reached. In order to have comparable data, the same portion, in percentage, of the experimental curve was chosen for each Sylgard sample. It follows that a layer of 50–10 nm was considered respectively for Sylgard 1:25–15. In the identified layer, ΔE_V was calculated composing the elasticity variability along z direction ΔE_Z and along the xy plane ΔE_{XY} quadratically. ΔE_Z data were calculated from the experimental E vs. $z-z_0$ data, at each $z-z_0$ value, considering a uniform distribution of data between maximum and minimum value in the identified layer and reported in Fig. 3B. ΔE_{XY} data were calculated by a selection of the 320 different Sylgard points tested, at each $z-z_0$ value, considering a normal distribution of data at the given cross plane. At each $z-z_0$ ΔE_V was calculated and plotted in Fig 4.

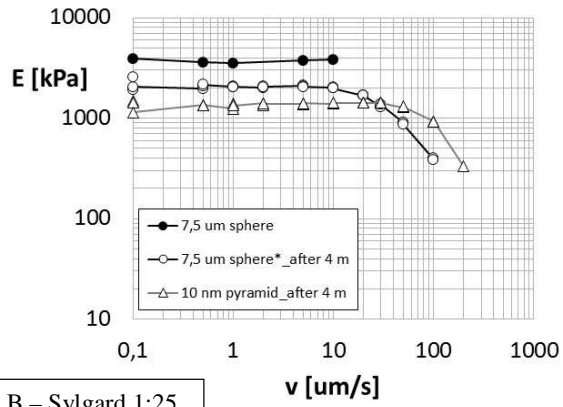
IV. RESULTS:

E function of indentation speed

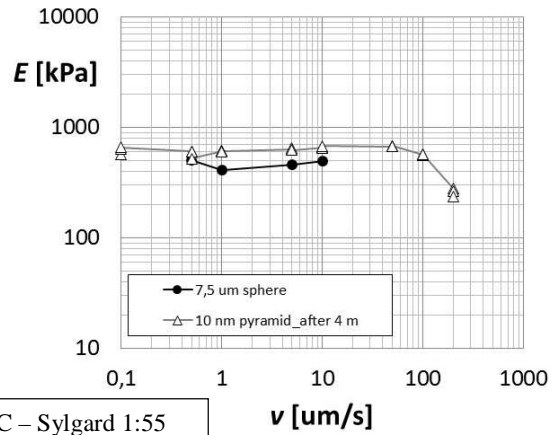
The following results can be derived by Fig. 1:

- Sylgard 1:15-1:25-1:55 presents *E* of round 2000-500-50 kPa, measured with different indenters at different scales.
- For Sylgard 1:15 and 1:25 a 10% variation of *E* is observed in the range 0.1-100 $\mu\text{m/s}$ with a plateau region around 5 $\mu\text{m/s}$. For Sylgard 1:55 the plateau is not easily determined. After 10-100-200 $\mu\text{m/s}$ for respectively Sylgard 1:15, 1:25, 1:55 a significant decrease of *E* is observed.
- The *E* function of indentation speed of Sylgard samples is maintained after 4 months, with different indenters on Sylgard 1:15, and for all samples at micro and nanoscale.
- An indentation speed of 5 $\mu\text{m/s}$ was chosen for all the samples and indenter, except for microindenter on Sylgard 1:25 for which an higher speed was necessary in lieu of the higher tip-sample interaction created by the higher amount of glue employed to attach the spherical tip.

A – Sylgard 1:15



B – Sylgard 1:25



C – Sylgard 1:55

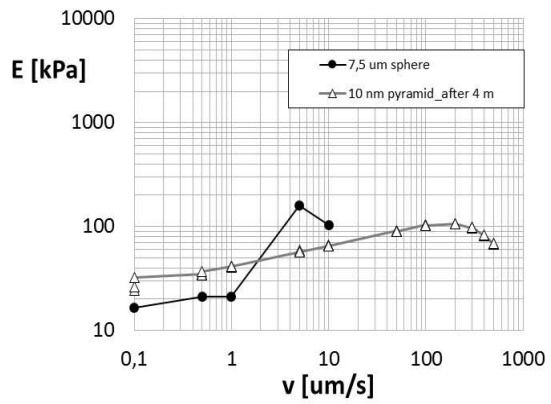
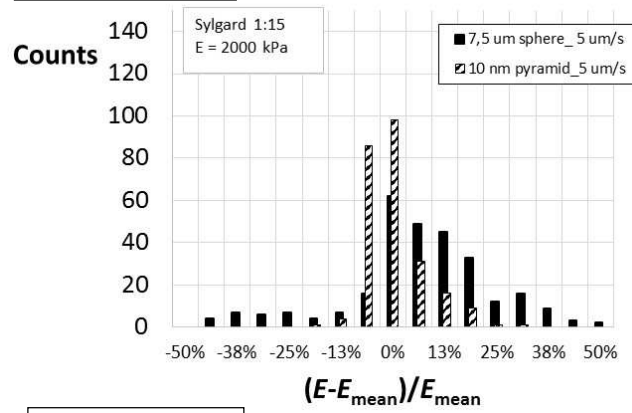


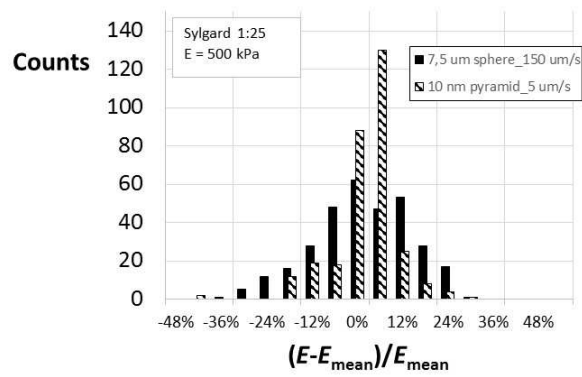
Fig. 1: E measurements of Sylgard and different densities, indenting with micro and nanoindenter within a 4 months period.

E distribution in the xy plane

A – Sylgard 1:15



B – Sylgard 1:25



C – Sylgard 1:55

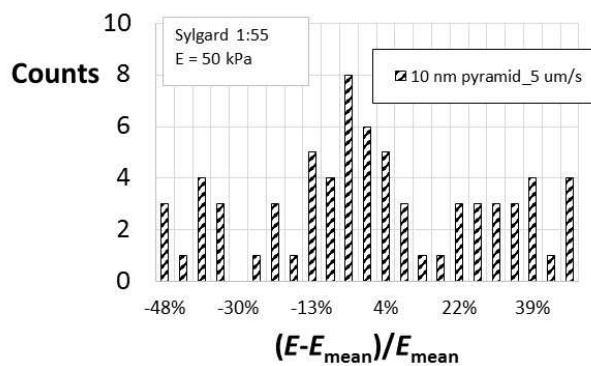


Fig. 2: Distribution of E measures in 320 points of Sylgard area.

The following results can be derived by Fig. 2:

- The distributions of elasticity data were calculated over all the force curve measured in the xy plane of the sample ($n = 320$), are thus representative of an high sample surface
- The distribution tends to a normal shape at 2 MPa and 500 kPa level with a standard deviation of round 20% at micro scale and 10% at nano scale.
- Narrower and higher distributions were observed for measures with nanoindenters than microindenters
- The distribution of elasticity data on very soft samples (50 kPa) shows a big spread of values tending to an uniform distribution. Measures were done with an unstable cantilever approach, probably due to the fluidic surface of the sample. Sylgard 1:55 data were not further treated in this paper.

E function of z-position

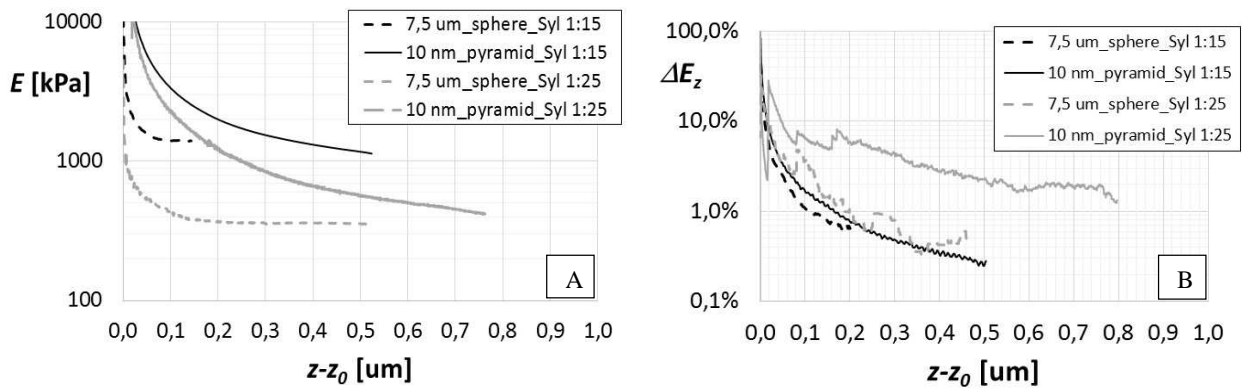


Fig. 3: Calculation of E of Sylgard (A) and its variation (B) in function of z-position for micro and nanoindenters on Sylgard 1:15 and 1:25.

The following results can be derived by Fig. 3:

- Nanoindenters allows exploring a higher amount of sample with respect to microindenters with the same maximum load on the same sample.

- The indenter load of 400 nN allows to indent, with the microindenter, 150 – 500 nm of sample and, with the nanoindenter, 500 – 800 nm of sample at respectively 2000-500 kPa nominal E
- After 100 and 200 nm, at nominal 2000 and 500 kPa, the Sylgard isotropic layer in the bulk can be considered reached, for a statistic sample of force curves ($n > 100$). When indenting with nanoindenter a further indentation is required to reach the isotropic layer.
- Close to the sample surface (left part of the figure) the variability of E is resulted higher in respect of the bulk (right part of the figure)
- ΔE_Z considers the average variability among the selected force curves for each sample
- ΔE_Z results lower than 1% can be reached in a region deeper than 150–800 nm for Sylgard 1:15–25 respectively indented with nanoindenter and in a region deeper than 100–200 nm for Sylgard 1:15-25 respectively indented with microindenter. Sylgard 1:15 results more homogeneous than Sylgard 1:25 at nanoscale, along z direction in the tested region.

Reproducibility of elasticity measurements

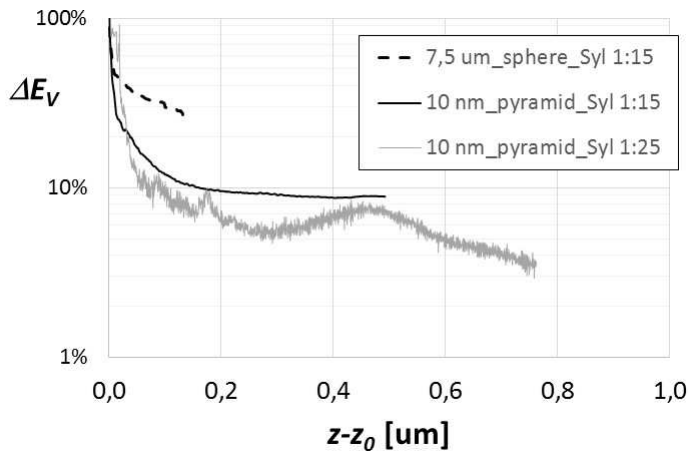


Fig. 4: Calculation of AFM measurement reproducibility of E in a volume of Sylgard samples at 2000 and 500 kPa level.

The following results can be derived by Fig. 4:

- The evaluated reproducibility of E measurement is lower than 30% at microscale level and lower than 10% at nanoscale level when indenting at least 100 nm of both Sylgard samples.
- The limit measurement reproducibility reached with the adopted measurement configuration (400 nN as cantilever load) is lower than 4% at nanoscale level at nominal 500 kPa.
- The main contribution to measurement reproducibility for all samples is given by ΔE_{XY} , calculated over the selected force curves that realize the bulk E of Sylgard. The contribution of ΔE_Z decreases in the z direction down to a not relevant contribution in the surrounding of the limit z -position measured. For lower density Sylgard 1:25 instead, a relevant ΔE_Z contribution to measurement reproducibility is obtained

V. DISCUSSION:

The E realized from Sylgard 15, 25 and 55 are consistent with the target range below 5 MPa, are stable over time and maintained at micro and nanoscale with different indenters. It follows that Sylgard 184 can be considered a suitable material to realize elasticity standards.

The variation of elasticity in a range 0.1-100 $\mu\text{m/s}$ is contained in the measurement reproducibility, where an indentation speed of 5 $\mu\text{m/s}$ can be considered optimal to measure the elasticity behavior of PDMS sample in the range between 2000-50 kPa. 10-100-200 $\mu\text{m/s}$ can be considered as upper speed limits respectively at 2000-500-50 kPa since the tip is no more able to sense the sample, giving a lower E . E measurements faster than 1 second are feasible, thus fast cellular processes could be measured with the present AFM method.

The closer distribution of elasticity data at nanoscale, instead of microscale, may be explainable by different motivations. The tested microindenter was home-made realized attaching a sphere to the cantilever edge using a not controlled amount of glue, the tested nanoindenter instead was commercially manufactured with a solid pyramidal tip. Probably the home-made realized indenter does not assure enough mechanical stability of the tip, which can move in the glue, maybe not perfectly UV cured.

The limitation to make elasticity measures on Sylgard 1:55 maybe depends on the impossibility of the few hydrogenated-PDMS chains to react with the random distributed PDMS chains. This realizes very few cross-links, with the result of a dense fluid instead of a solid material. Therefore Sylgard 184 may not be recommended to realize E standard at 50 kPa level, other materials should be employed.

Close to the surface, the interaction between measurement system and sample was relevant and it was not possible to discriminate between measurement artifacts and surface properties. However, it was possible for the microindenter to define a region in which the interaction was no more relevant, the plateau of Fig.3 at high $z-z_0$, called isotropic region. Here is possible to give a more realistic estimation of measurement reproducibility. Higher indenter load can be recommended to better reach the Sylgard isotropic layer with nanoindenters.

The measurement of elasticity variation in a given volume of the isotropic region of Sylgard allows defining the measurement reproducibility of the AFM method, that considers measurement repeatability and sample homogeneity. On the other hand it is possible to say that when the AFM measurement reproducibility want to be characterize, Sylgard sample could be used, but suitable indentation depths must be employed to reach the sample isotropic layer, in which measurement artifacts are minimized.

Since the main contribution to the measurement reproducibility was found to be given by the elasticity distribution in the xy plan, measurements with nanoindenters allow to reach better measurement reproducibility (lower than 10%).

In summary, we could propose the following actions to make reproducible E measures with AFM in the biological range 500-5000 kPa:

- realization of E standard at the biological level required, using Sylgard 184 material
- definition of the isotropic region of the standard
- identify the best AFM measurement settings (nanoindenter, indentation speed) to reach the isotropic region
- evaluation of measurement reproducibility, in a layer of the isotropic region of the standard
- maintain the same measurement settings for the biological material under test

The least elasticity difference measurable in the biological sample, or between two biological samples, will be given by the reproducibility of the tested AFM measurement.

When thin biological layers want to be measured, the interaction region between measurement system and sample must be carefully characterized: the region of the biological sample in which reproducible E measurements can be obtained will correspond to the region in which interaction will be not relevant.

Comments on measurement accuracy

To measure the elasticity value of a sample with AFM, calibrated indenters and accurate contact models are needed. In this way the E measured in the isotropic region of Sylgard can be assigned as the E of the standard. For the calibration of indenters, accurate measurement of their geometry, elastic constant and photodiode sensitivity must be given. To realize accurate contact models, the shape of the cantilever, the geometry of the tip and the interaction with the sample must be considered.

To make measurement of elasticity difference on biological samples, measurement reproducibility is required, measurement accuracy not necessarily.

VI. CONCLUSION

In this work, a robust method for the validation of E measurements in the biological range between 500-5000 kPa was proposed. It is based on the realization of thick E standards, identification of conditions for reproducible measures of standards and maintaining of the same measurement setting on biological samples. A method for the calculation with AFM of the E variability in a given sample layer is given. Standards respectively at 2000-500-50 kPa were realized in a range not currently available. For the first time it was demonstrated that Sylgard 184 could be used as a standard for E in the range 500-5000 kPa . It presents a mechanical stability over time, and E maintained at micro and nanoscale level for which statistically relevant data was given. Moreover, it shows an isotropic layer, which allows characterizing the AFM measurements reproducibility. For the realization of E standard at a level lower than 500 kPa other materials should be recommended.

With the realized standards, a limit of 4% reproducibility of the AFM method was measured with indentation speeds lower than 100 $\mu\text{m/s}$ and with nanoindenters working on low density Sylgard. This allows realizing E measurements on biological samples with a reproducibility of 4% within the same sample or an elasticity difference of 4% between two biological samples. Making elasticity difference measurement in soft material with high reproducibility is fundamental to discriminate between normal and tumor cells.

AFM needs to be validated for very soft materials and this work is a robust starting point to obtain future reliable and accurate results.

REFERENCES

- McCrum, Buckley, and Bucknell (2003): "Principles of Polymer Engineering," 117-176
- Bhadriraju K. and L.K.Hansen, *Exp.Cell.Res.*, vol.278, pp.92–100, 2002.
- Carrillo F., S.Gupta, M.Balooch, S.J.Marshall, G.W.Marshall, L.Pruitt, C.M.Puttlitza, *J.Mater.Res.*, vol.20, no.10, 2005.
- Costa K.D., *Disease Markers*, vol.19, pp.139-154, 2004.
- Cross S.E., Y.Jin, J.Tondre, R.Wong, J.Y.Rao and J.K.Gimzewski, *Nanotechnology*, vol.19, 384003(8pp), 2008.
- Cross S.E., Y.S.Jin, J.Rao, J.K.Gimzewski, *Nature Nanotech.*, vol.2, pp.780–783, 2007.
- Darling E.M., S.Zauscher, J.A.Block, F.Guilak, *Biophysical Journal*, vol.92, pp.1784-1791, 2007.
- Demichelis A., C.Divieto, L.Mortati, S.Pavarelli, G.Sassi, M.Sassi, *Proceedings of IEEE/MeMeA International Symposium*, 2014.
- Demichelis A., *NIS colloquium IBAT06*, 2013.
- Demichelis A., S.Pavarelli, L.Mortati, G.Sassi, M.Sassi. *J.Phys.:Conf.Ser.*, vol.459, 012050, 2013 doi:10.1088/1742-6596/459/1/012050.
- Guo Q., Y.Xia, M.Sandig, J.Yang, *J.Biomech.*, vol.45, pp.304-309, 2012.

- JPK application note <http://www.jpk.com/cellular-adhesion-cytomechanics.234.en.html>
- Kuznetsova T.G., M.N.Starodubtseva, N.I.Yegorenkov, S.A.Chizhik, R.I.Zhdanov, *Micron*, vol.38, pp.824-833, 2007.
- Ladjal H., J.Hanus, A.Pillarisetti, C.Keefer, A.Ferreira, J.P.Desai, *Proceedings of IEEE/RSJ International Conference*, 2009. doi:10.1109/IROS.2009.5354351.
- Lekka M., Pogoda K., Gostek J., Klymenko O., Prauzner-Bechcicki S., Wiltowska-Zubera J., Jaczewska J., Lekki J., Stachura Z., *Micron*, vol.43, pp.1259-1266, 2012.
- Lekka M., G. Dorota, Pogoda K. , Dulin´ ska-Litewka J. , Jach R., Gostek J., Klymenko O., Prauzner-Bechcicki S., Stachura Z., Wiltowska-Zuber J., Okon´ K. , Laidler P., *Archives of Biochemistry and Biophysics* 518 pp.151–156, 2012
- Li Q.S., Lee G.Y.H., Ong C.N., Lim C.T., *Biochemical and Biophysical Research Communications* 374 609–613, 2008
- Lin D.C., Dimitridas E.K., Horkay F, *ASME* 129:430-440, June 2007
- Wenger M.P.E., L.Bozec, M.A.Horton, P.Mesquida, *Biophys.J.*, vol.93 pp.1255-1263, 2007.