Scientific Report

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STSM Topic: Standarization in Cell Mechanics Measurements

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Background and purpose

Atomic force microscopy (AFM) allows the assessment of mechanical properties of living cells under physiological conditions. However, mechanical parameters determined with AFM depend on a number of issues including sample manipulation, tip shape, measurement protocol, and computation algorithm. Therefore, accurate and robust determination of cell mechanics requires precise standardization of AFM measurements.

The goal of this STSM is to compare our method to measure the Young's modulus of polyacrilamide (PAA) gels with that of the host lab.

Description of the work carried out and main results obtained

Preparation of PAA gels

The reliable preparation of PAA gels with known indicative stiffness is key to standardize mechanical measurements with AFM. We brought some of our gels to measure in Bremen and shared the protocol to make them. The sample gels were kept in PBS, stored at 4˚C and measured at room temperature after sufficient equilibration time (1 h).

Calibration of the photodiode sensitivity

The first step of any mechanical measurement is the calibration of the photodiode sensitivity to obtain reliable values of cantilever deflection (d). We ramped the cantilever vertically (z, oscillation frequency 1 Hz, travel range 3 µm, velocity 6 µm/s) and recorded the slope of a d-z curve obtained in a hard substrate (glass or plastic).

The maximum deflection obviously depends on the cantilever: a soft (0.01 N/m) cantilever shouldn't bend more than ~1 µm, while a stiffer cantilever (0.1 N/m) shouldn't bend more than ~300 nm. The obtained slope may depend on the part of the curved used. For example, we saw how changing the

range from 100 – 400 nm of deflection to 50 – 100 nm can alter the sensitivity value by 3%. The whole contact region of the d-z curve should be used to obtain more robust sensitivity values.

Using a single d-z curve may introduce statistical variability in the determination of the sensitivity. The average of a reasonable number of curves (10 – 100) should be used to reduce this effect from 5% to 1%.

Calibration of the cantilever spring constant

The calibration of the cantilever spring constant (k) is critical for any mechanical force measurements. We determined k using the thermal noise method, and we saw that it depends greatly on a series of parameters:

- Photodiode sensitivity: it has to be measured accurately.
- Temperature: it has to be measured and make sure that the equipment uses the right value in the computation of k.
- Acquisition time: a long averaging time (1 min) will generate more accurate thermal fluctuations, although some equipment don't allow that.
- Distribution fitting: some equipments offer the possibility of fitting either a Lorentzian distribution or the distribution resulting from the differential equation for a single harmonic oscillator (SHO) to the computed power spectrum. Although the Lorentzian distribution is a very good approximation for any peak, we saw no reason to use it instead of the actual, analytical SHO solution.
- Frequency range: the manual selection of the frequency range to fit obviously alters the resulting k. Knowing the nominal resonance frequency ($f_{0,nom}$) allowed us to set orientate boundaries to the fitting: from $0.5 \times f_{0,nom}$ to $1.5 \times f_{0,nom}$. Despite that, we obtained better fittings to the resonant peak by manually fine tuning these frequencies.

Data acquisition and determination of experimental setting

We acquired d-z curves (or force curves) on top of the gels by ramping a soft (0.01 N/m) cantilever vertically (oscillation frequency 1 Hz, travel range 5 µm, velocity 10 µm/s, maximum deflection 500 nm). A reasonable amount of curves (10 – 100) in a 10 µm × 10 µm area was enough to determine the stiffness of the gel. Since PAA gels are linear and elastic, the obtained stiffness was not dependant on cantilever velocity or indentation: the values above are just indicative.

Tip-sample contact models and measurement of the Young's Modulus (E)

We used MLCT chips (Bruker, Mannheim, Germany), which have soft cantilevers (nominal spring costant of 0.01 N/m) with pyramidal tips. Accordingly, we used the Hertz pyramidal contact model to fit the force curves:

$$d = d_{off} + \frac{3\,E\tan\theta}{4\,(1-\nu^2)k}[(z - z_0) - (d - d_{off})]^2$$

where d_{off} is the deflection offset, z_0 the position of the contact point, E the Young's modulus, ν is the Poisson ratio (assumed to be 0.5) and θ the semi-angle of the pyramidal tip.

There are many ways to implement a non-linear fitting. By processing the same data with both the algorithms used in Bremen and Barcelona, respectively, we located the 2 most important sources of error:

- θ: the Hertz pyramidal contact model assumes a regular pyramid, and new MLCT cantilevers have an asymmetrical pyramidal tip. Moreover, the angles of these pyramids can be 10% off the nominal values. The θ that better emulates the contact surface of the old MLCT pyramidal tips is 18.8º. Considering the high error of these angles, we used θ = 20º to process the curves. This issue could be solved by using old MLCT cantilevers (θ = 35º) or spherical beads.
- Processing range: the region of the curve used to fit the parabola is critical when determining the E, which can be chosen based on a range of deflection or indentation. An indentation range should be set when probing thin samples to avoid the effects of feeling the substrate. For example, setting a maximum indentation of 500 nm when measuring a cell of 5 um of height (10%) should prevent substrate effects and damaging the cell. A deflection range should be set with thicker samples, where indenting in not a problem, just to ensure to remain in the linear deflection regime of the cantilever.

Once these two parameters were set equal, we reached great accordance (differences < 0.5%) in the calculation of E, since both data processes fit E, z_0 and d_{off} based on the same non-linear least squares fitting algorithm. The processing of a force curve in a gel is illustrated in Fig. 1.

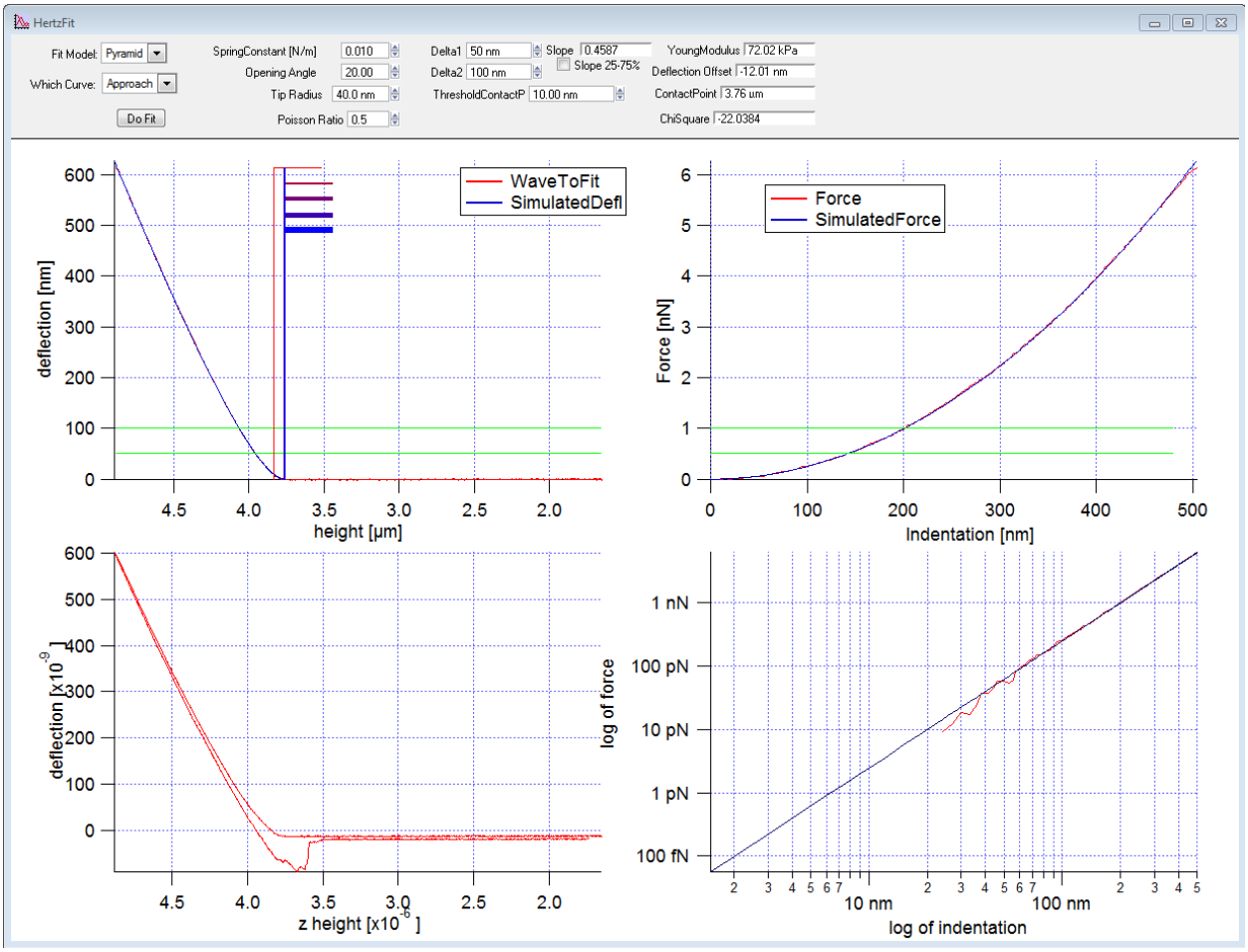

Figure 1. Processing of a force curves acquired on a gel by the host lab.

Future collaboration with host institution

The present STSM was the initial step of a series of collaborations between the laboratories of Prof. Radmacher (Bremen), Prof. Navajas (Barcelona) and several other laboratories. This is an ongoing collaboration with the aim of standardizing mechanical measurements on cells, and it's developing in several branches, such as PAA gel preparation or accurate tip geometry determination.