Local nanomechanical properties of HeLa-cell surfaces

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Abstract. Using the Digital Pulsed Force Mode (DPFM) approach, the local mechanical properties of living HeLa cells have been examined. The cells were attached to a favorable glass-like substrate. The AFM used for the experiments was a commercial AFM setup by WITec. At every point of the image, approach and retract curves have been performed. The repetition rate of the cycle was 175 Hz. A total of about 500,000 curves has been recorded and completely evaluated for each experiment. The substrate served as an online reference material for calibration purposes. First, the force trajectories were corrected for the viscous drag force in the liquid environment. Second, the curves within the region of the substrate were phase corrected to compensate for the time lag of the signal in the setup assuming a purely elastic response of the reference material. Finally, all the force traces have been corrected by using this information and evaluated according to common continuum-elastic models. The resulting images allow the assignment of values of Young’s modulus, local adhesion, hysteretic behavior, etc. at a high lateral resolution all over the cell body. In this paper, we describe the procedure of our measurement and the corresponding signal correction strategy of our automated data evaluation.

1. Introduction
The measurement of local mechanical properties of cells utilizing an Atomic Force Microscope (AFM) [1–5] requires the use of small and controlled interaction forces while imaging the cell surface or doing Force-Distance-Curves (FDC) [6, 7]. To obtain images of entire living cells the intermittent contact mode [8–10] has been preferred. The phase lag in resonant intermittent mode imaging can provide information on the dissipative processes on the sample surfaces. On the other hand, the determination of surface force gradients by measuring FDCs while the cantilever is oscillating at its resonance [9] can provide data on the mechanical response of cells. However, the calibration of intermittent contact mode measurements in liquid environment is possible, but quite demanding.

Using an alternative approach to the determination of nanomechanical properties, FDCs and indentation measurements were used to measure cartilage mechanical properties [11]. Non-oscillating FDCs [12] with repetition rates far below the resonant frequency of the cantilever can be performed on a large number of locations on the sample. This imaging mode is known by the name of force volume imaging. The evaluation and calibration of these FDCs is a commonly

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known procedure. Yet, to be practical for measuring living cells, the entire data acquisition has to be quick in order to capture the entire set of data before the cell may move or detach. Thus, the measurements need to be carried out at rather high repetition rates to minimize these unwanted effects. Increasing this rate to reasonable imaging speeds makes it necessary to switch from a triangular modulation of the cantilever position to a sinusoidal trajectory. This is done in Pulsed Force Mode [13] or Digital Pulsed Force Mode (DPFM) [14,15].

2. Materials and methods

2.1. Cells

Over the last couple of years, HeLa cells have been studied into great detail by physiologists working with cancerous cells (For a review see i.e. [16]). For investigation by using an AFM, these cells are well suited due to their maximum height of about 5 µm when attached to a surface. Furthermore, HeLa cells adhere nicely to almost every substrate and are quite robust towards AFM experiments. Thus, this cell line has been chosen as first biological sample for applying our measurement method. To prepare the cells on the substrate, the cells were incubated under physiologic conditions (37 °C, 5 % CO₂) in medium for three days. Before the actual measurement, the medium was rinsed-off with Phosphate Buffered Saline (PBS) which was then also used as liquid environment during the experiments. All experiments have been conducted in liquid and at room temperature.

2.2. Atomic Force Microscope

The measurement setup was an AFM by WITec GmbH (www.witec.de) equipped with both an Digital Pulsed Force Mode and an inverted optical microscope. The maximum scan-range of the system is 100 µm x 100 µm with a maximum z-range of 20 µm. The cells were selected to be in a healthy state for the experiments by visual inspection using the inverted optics. The cantilevers used were type LFMR by Nanosensors (www.nanosensors.de) and had a typical resonant frequency in the range of 19 to 37 kHz and a nominal stiffness of 0.07 to 0.49 N/m. The actual spring constant of the cantilever of each experiment has been calculated from its resonant frequency and known conversion factor (k ∝ ν³ [17]). The amplitude of the DPFM modulation has been determined by a so called Vibrometer by SIOS Messtechnik GmbH (www.sios.de), which follows a Michelson interferometer setup to determine oscillation amplitudes up to 500 kHz and with a nominal accuracy of 0.3 nm. The amplitude used in the following experiments has been set to 375 nm. The repetition rate was chosen to be 175 Hz. The cantilever stiffness for the data presented here was k = 0.18 N/m. During the experiments, the DPFM setup is recording the entire sets of force data at 16 bit resolution and sampling the signal at 5 MS per second. In all, this agglomerates to files of approximately 60 GByte of data for every single experimental run.

2.3. Calibration and correction procedures

To compensate the viscous drag force (Fig. 1 (a)) in the liquid surrounding, the sinusoidal modulation in the off-contact region of the force traces needs to be removed. In a first-order correction approach, we subtract this systematic force signal as a simple sine-function (Fig. 1 (a), red curve) from each force trace (Fig. 1 (b)).

Furthermore, the phase-lag due to signal run-times in the experimental setup (Fig. 1 (c)) is compensated by introducing a phase shift into the conversion function from the time-domain (where the forces are recorded) to the position of the base of the cantilever. On a reference surface (hard, elastic), the optimized phase shift is reached when the trace and retrace signals of a FDC are not intersecting each other and when the area enclosed by them during the repulsive contact is minimized (Fig. 1 (d)). A detailed description is given in the PhD thesis by A. Gigler [18].
Figure 1. (a) Temporal response of the cantilever in the liquid environment (black curve). The red curve indicates the viscous drag force that has to be subtracted from the data. (b) Corrected force trace in the time domain. (c) Force trace (black) and position modulation (red) of the cantilever. (d) Phase-corrected force trace on the reference material. The phase angle is determined from the optimization assuming a hard reference surface.

3. Results and discussion

Figure 2 (a) shows the topographic information of a HeLa cell adhering to the substrate. On top of the nucleus of the cell, a height of 1.6 µm has been measured. This is the height above the substrate at which the maximum force of 10 nN had been reached. Since, for each pixel of the image at least one force curve has been acquired, the indentation of the tip of the cantilever into the cell body can be investigated. Four exemplary curves are shown in figure 2 (b) and the positions are marked in Fig. 2 (a). One can easily see the differences in the force versus indentation-depth plots. On the very flat regions of the cell (labeled tail and edge, Fig. 2 (b)) it is obvious that for excessive forces, i.e. too large indentations, the cell body is penetrated all the way to the substrate and that these force curves thus have two important regions. On the thicker parts of the cell, the curves are almost only dominated by the compliant behavior of the cell itself. Since a second cell happened to lie attached to the substrate within the scan-field of the AFM image, it was also possible to compare the results between two independent cells imaged at the same time. Figure 2 (b) shows that the second cell (labeled cell II) behaves very similar to the first one (labeled cell I) for a comparable thickness of or position on the cell. It is negligent to only evaluate single traces and then claim to know the exact mechanical behavior of the cells. However, it is also quite tedious to evaluate the entire indentation data in a curve by curve manner except when using an automated procedure. Thus, such an automation has been developed for the evaluation of DPFM acquired data.

The resulting maps of the mechanical properties of the materials in the scan-field are shown in figure 3. The local adhesion map (Fig. 3 (a)) corresponds to the stickiness of the sample towards the tip of the cantilever. The cell is less adhesive than the underlying substrate. The tip-sample-contact area depends on the topography, influencing the adhesion measurement too. Figure 3 (b) shows the effective modulus of the sample. This quantity is obtained by fitting a
Figure 2. (a) Topography of the HeLa cell. The marks show the positions of the exemplary curves which are presented in (b). Tail: Thin tail region of the cell. The cell is penetrated by the tip onto the substrate; Edge: Even at a thicker region of the cell, the tip still reaches the substrate. Cell I & II: At the same thickness of two different cells, the elastic and viscoelastic behavior is comparable.

JKR-based model [19] to the indentation data. We obtain a range of about 50 to 500 kPa for the cell and a range of 100 to 300 MPa for the substrate. The map shows the lateral distribution of the Young’s modulus. The data analysis process allows to quantify the energy-losses due to the dissipative behavior of the viscous liquids (Hysteretic loss shown in figure 3 (c)). Since the indentation depth of the tip of the cantilever into the sample is known after the conversion of the deflection signals into real forces, one is able to determine the deformation of the sample when applying the maximum load $F_{\text{max}}$. The maximum deformation is more or less the same throughout the entire cell body (Fig. 3 (d)) except for the regions where the tip is penetrating all the way to the substrate as in Fig. 3 (b).

We have shown that rapid FDC measurements at a repetition rate of over 170 Hz gives meaningful data on mechanical properties of cells. Those properties at contact times of about 1 ms are available with a good spatial resolution. The key to obtain the data was the procedure to remove the influence of the viscous drag forces.

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Figure 3. (a) Local adhesion serves as a measure for the stickiness of the material. In liquids this is mainly governed by the affinity of the material towards the silicon tip, since a water meniscus cannot appear. (b) Log-scaled map of the Young’s modulus of the cell and the substrate calculated by a JKR-based model. (c) The energy lost during a cycle gives a measure for the hysteresis that occurs during one loop. (d) Indentation at $F_{\text{max}}$ shows that the thin regions of the cell are easily penetrated by the tip and that the substrate is sensed.

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