Supporting Information for:

Reliable, standardized measurements for cell mechanical properties

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Supplementary List S1.

lab 1 – Nanowizard 3.0 (Bruker-JPK)

lab 2 – Nanowizard 4.0 (Bruker-JPK)

lab 3 – Nanowizard 4.0 (Bruker-JPK)

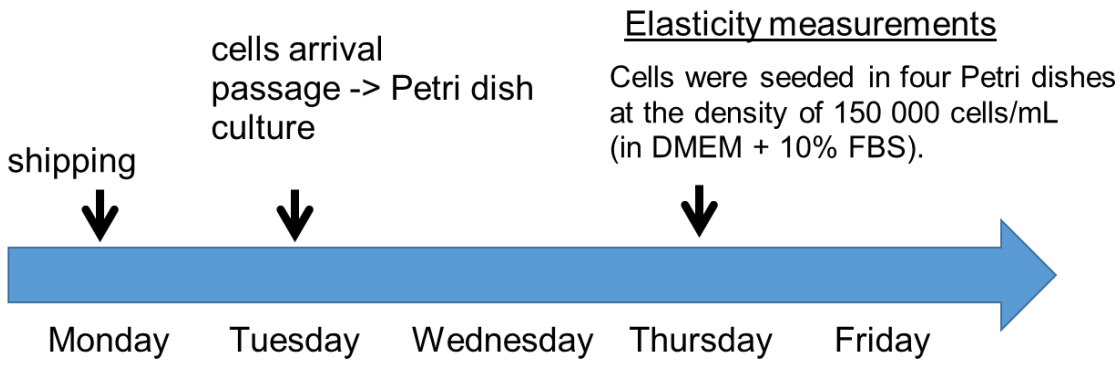
lab 4 – Pavone, Piuma (Optics 11)

lab 5 – MFP3D (Asylum Research, Santa Barbara, CA, USA)

lab 6 – Bioscope Catalyst (Bruker Nano, Santa Barbara/CA, USA)

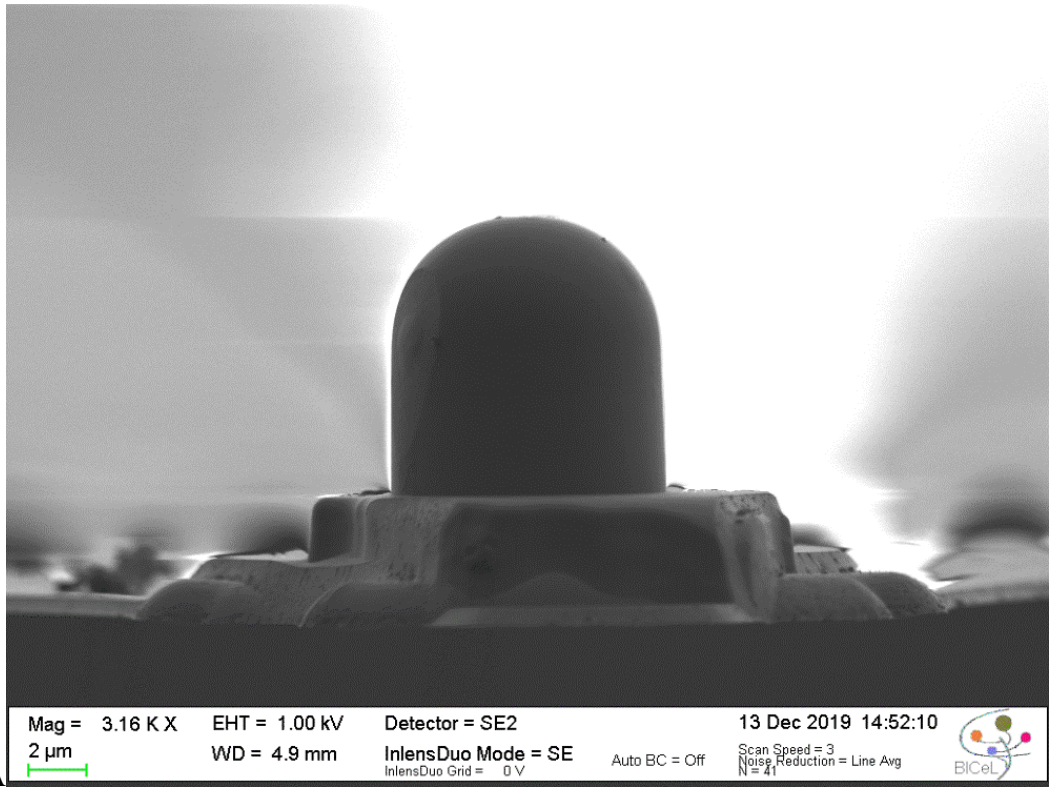
lab 7 – BioScope Resolve (Bruker Nano, Santa Barbara/CA, USA)

Note: Figure 2 gathers data recorded in 8 separate experiments. One experiment was measured twice by the same lab; therefore, the number of labs does not agree with the number of datasets.

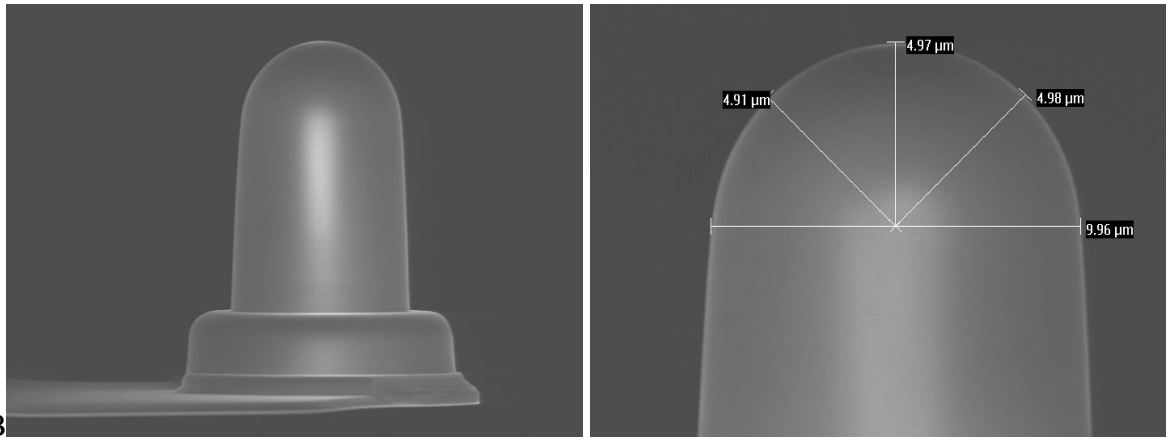


Supplementary Figure S1. The timeline of the experiment on the determination of elastic properties of pancreatic PANC-1 cancer cells.

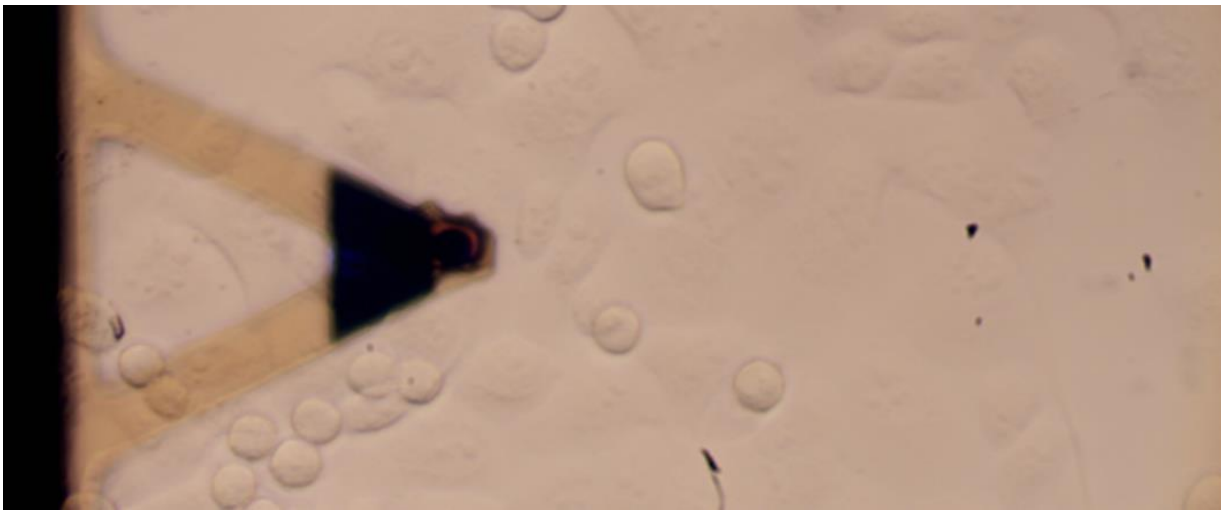
A



B



Supplementary Figure S2. A) SEM (Scanning Electron Microscope) image of the MLCT-SPH-DC silicon nitride cantilevers (Bruker) with a hemispherical tip. The image was collected using SEM available in the BICeL facility (Lille, France). B) SEM images of the currently available cantilevers with SPH tips available for the AFM community (courtesy of A.Dulebo, Bruker).



Supplementary Figure S3. Top view image of the PANC-1 monolayer, the flat area of the monolayer measured in standardization experiments. The image was collected with the top view optics integrated with MFP3D (Asylum Research, Santa Barbara, CA, USA).

Supplementary Note 1 – Details on data analysis (unified approach)

The deflection versus z height (usually called the force curve) is converted to force indentation data, which then can be fitted to the appropriate contact model formula, e.g., the Hertz model. Often, the fit is performed only in a certain range of data since at high forces (indentations), there may be deviations because of non-linearities or inhomogeneities of the sample, at very low forces or indentations, there may also be deviations due to different properties of the cell at its surface (due to the membrane or glycocalyx), due to tip contamination or deviations of the tip shape (e.g. pyramidal tips are often blunted).

The fit range can be determined in terms of force values or indentation values (as used here).

The force is given by the cantilever's force constant k_0 times the deflection d - the deflection offset d_0, i.e., the deflection of the free cantilever.

$$F = k_c (d - d_0)$$

The indentation δ is the difference between the z height and the deflection, taking into account the deflection d_0 offset and the contact point z_0.

$$\delta = (z - z_0) - (d - d_0)$$

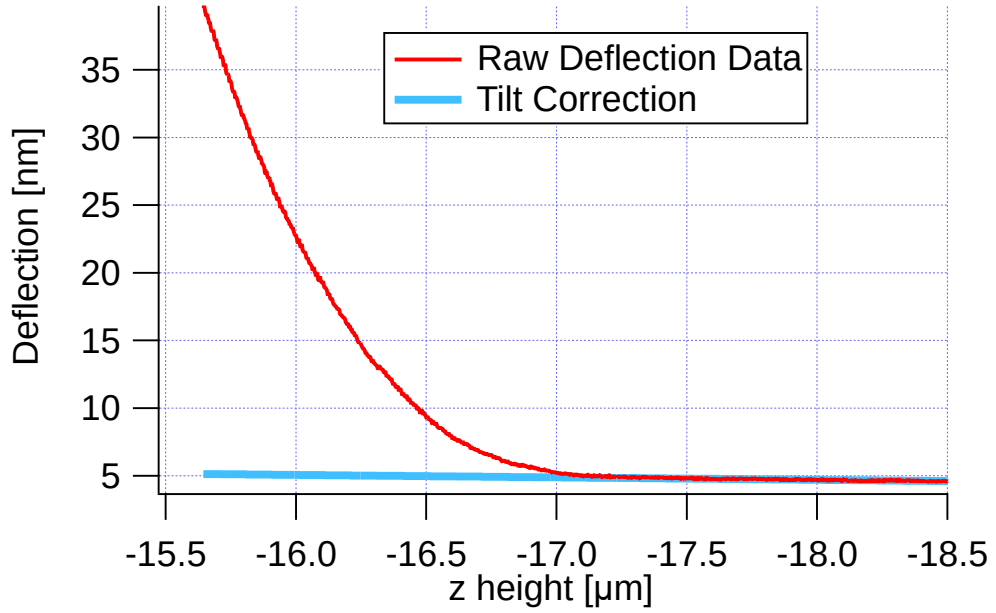
In the case of the spherical probes used here, the appropriate contact mechanics model will be a Hertzian force indentation relation:

$$F = \frac{4}{3} \cdot \frac{E_{cell}}{1 - \nu_{cell}^2} \sqrt{R} \cdot \delta^{3/2}$$

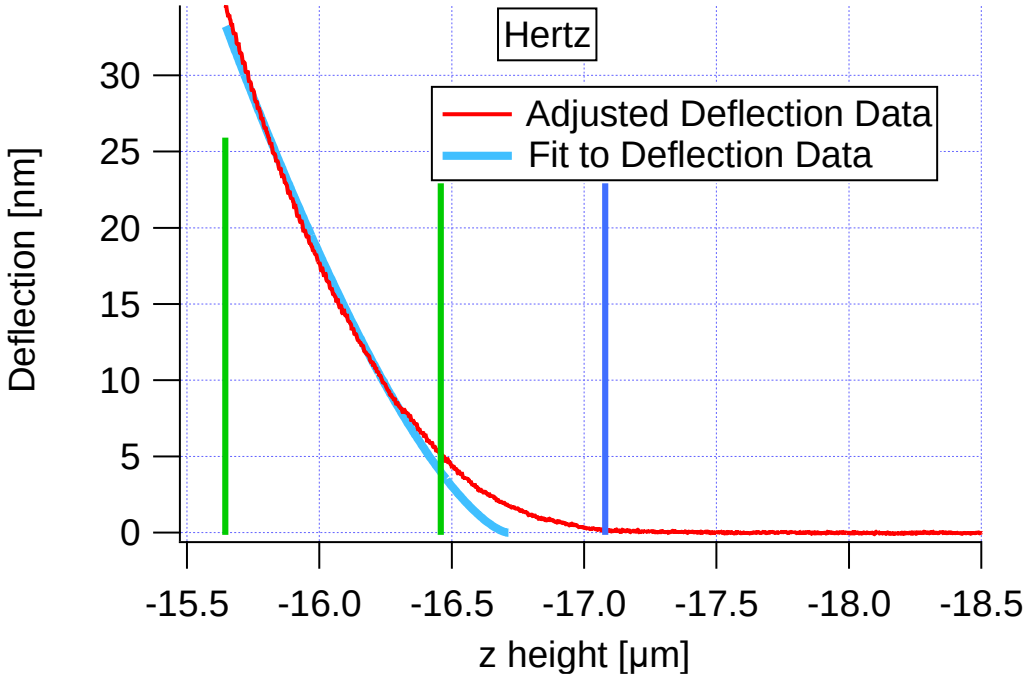
where R is the radius of curvature of the tip, E_{cell} is the cells Young's modulus and ν is the cell's Poisson ratio, usually taken to be 0.5.

1. Tilt correction and deflection offset

Force curves were corrected for tilt, i.e., a line was fitted to the part of the deflection data, which has been taken off the substrate (typically 25% of data points were used), and then this fitted line is subtracted from the entire force curve. This force tilt is most likely due to the warp of the z-piezo while travelling a larger distance. The effect is minor, in our case, often just 1 nm in deflection over a travel of 2 µm in z; however, it affects the subsequent data analysis. This procedure will also subtract the offset from the deflection data since usually the detector is adjusted so that the free cantilever does not generate a zero deflection signal.



Supplementary Figure S4. Raw deflection data (in red) and line fit to the offset surface part in blue, which serves for subtracting the force curve tilt due to piezo errors (movement in *z* will slightly warp the z-piezo and thus result in an artificial deflection signal).



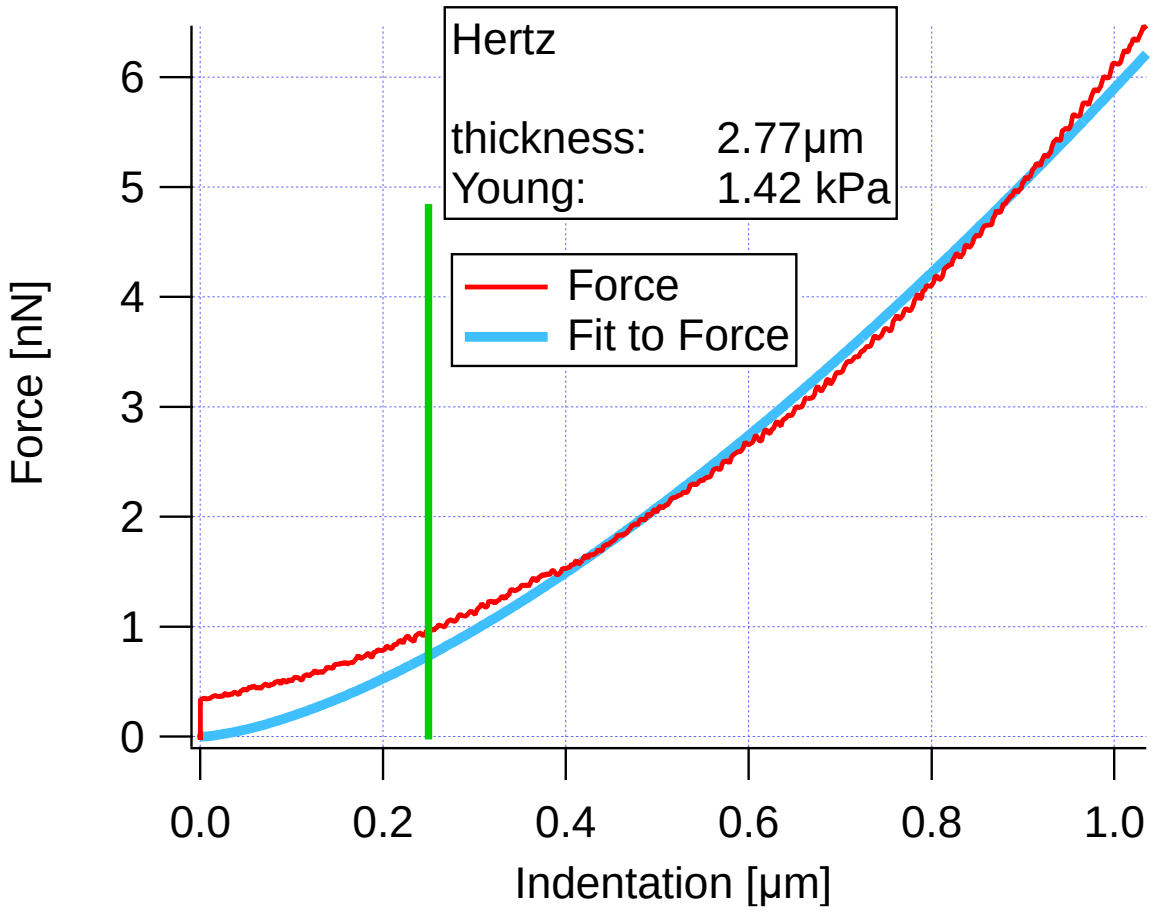
Supplementary Figure S5. Corrected deflection data (after subtracting tilt, the deflection of the free cantilever corresponds to 0 nm). This graph also shows the fit range used by the vertical green lines. Here, we defined the fit range by indentation to be between 250 nm to 1500 nm, based on the initial estimation of the contact point. This indentation range is back-calculated to the corresponding part of the force curve data. The fit result (fitted force versus indentation, see figure below) is also back-calculated in a simulated force curve in light blue. In addition, the contact point (also a result of the fit procedure) is depicted here by a vertical blue line. The large discrepancy between fit and data is due to the bottom effect, which is not corrected here since this force curve has been taken where the cell thickness is just 2.77 µm.

2. Initial guesses for contact point and Young's modulus

The contact point (z offset or z-position of contact) is the subsequent Hertz fit parameter. However, fitting a non-linear function requires a reasonably well-starting point of the fit parameters, such as contact point and Young's modulus. A first guess of the contact point is achieved by locating the z-position at which the deflection signal gets larger than a certain threshold, in our case, we used here 4 nm deflection. Based on this guess for the contact point, and the upper and lower limits of the indentation fit range, we can calculate a new guess of Young's modulus and the contact point.

3. Fitting of the contact mechanics model to force versus indentation data

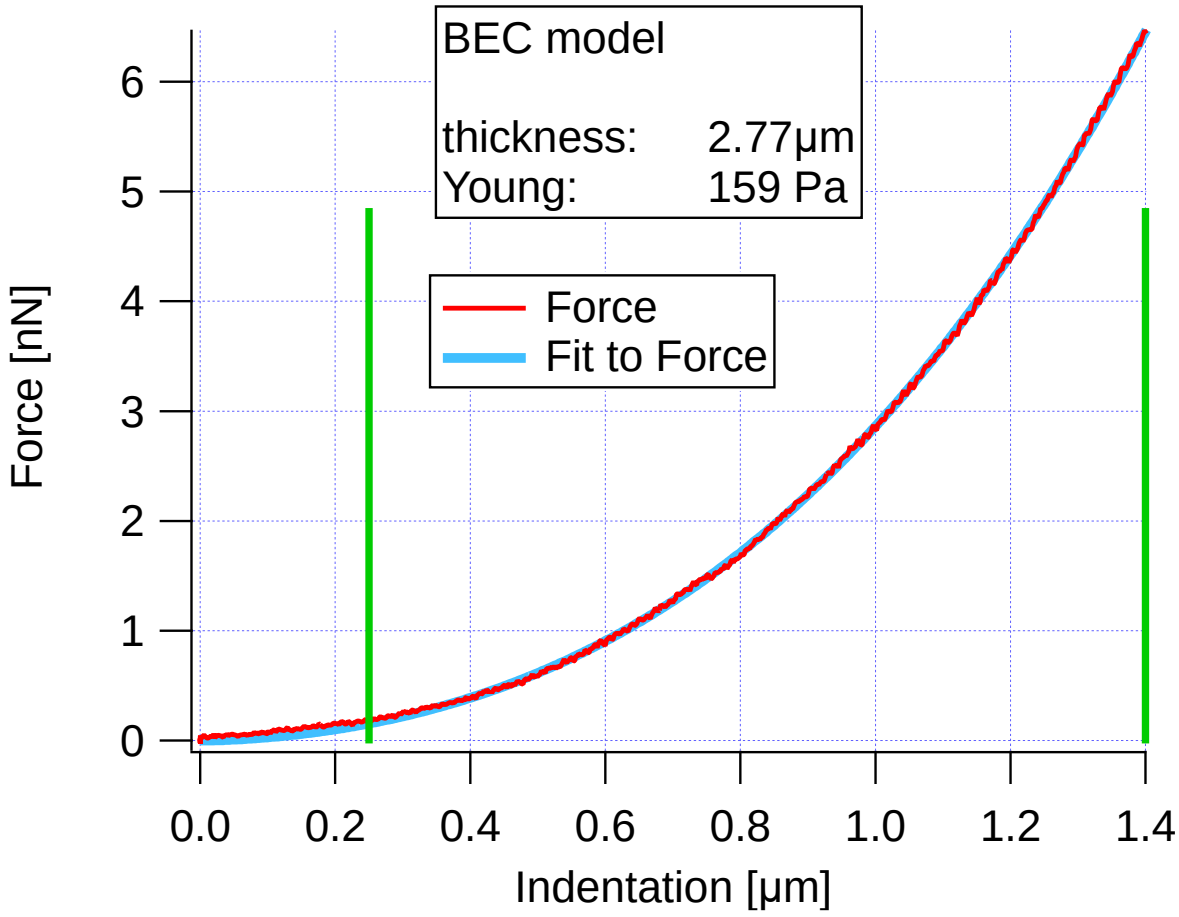
Based on this contact point, the force versus indentation data are calculated, and a first fit of the contact model is performed. This process is iterated three times to finally get a stable value for the contact point and Young's modulus.



Supplementary Figure S6. Force versus indentation data (in red) with the fitted data (in light blue). The fit follows only poorly the data in the fit range (green lines at indentation 250 nm and 1500 nm, respectively) largely due to the bottom effect.

4. Cell height determination

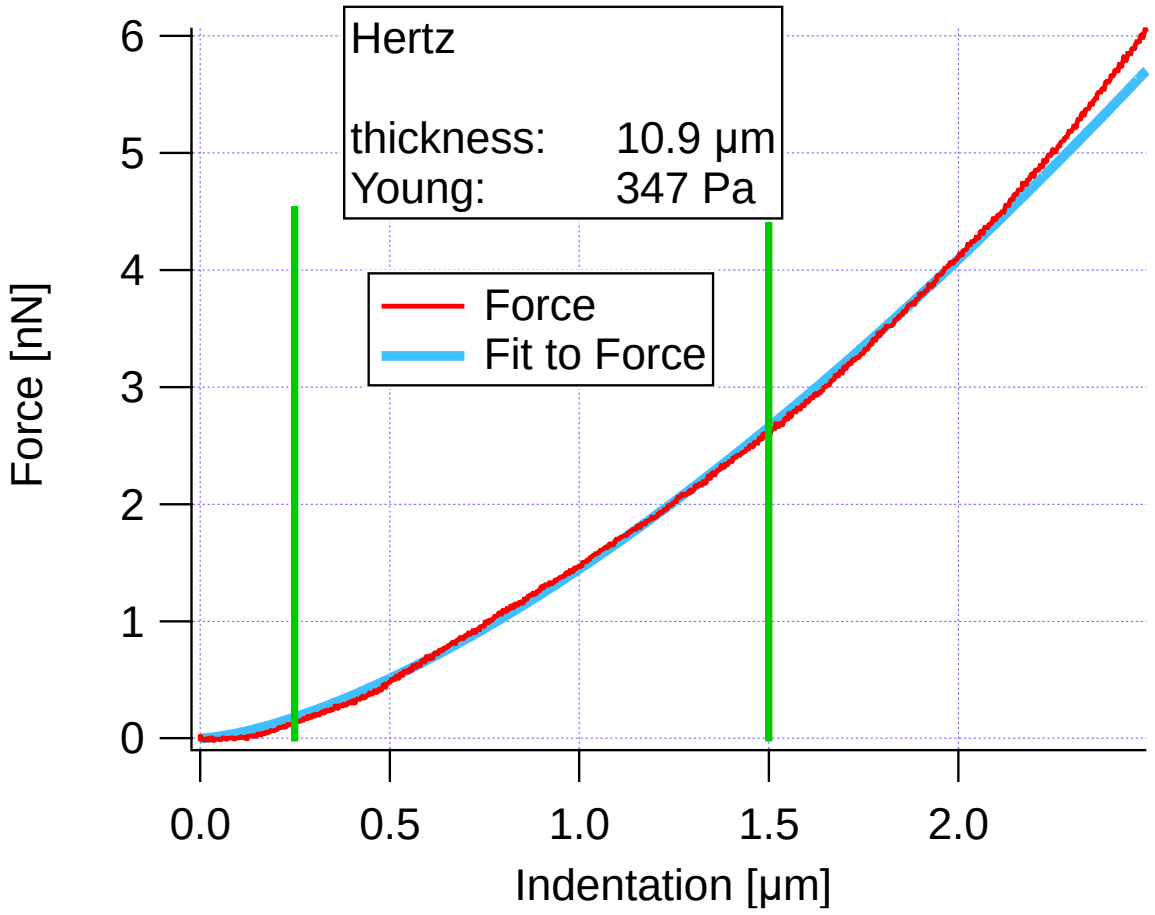
After fitting the Hertz model to all data of a force volume, the cell height can be determined. The contact point values (i.e., the z height at point contact as determined by the Hertzian fit) define the substrate profile or the cell at zero force. All data points where the slope of the force curve is larger than a certain threshold (theoretically, all slope values on the substrate should be 1, but it is safer to use a smaller threshold here. Often 0.2 works well), define the plane of the substrate, which is subtracted from all contact point values, resulting in the thickness of the sample, i.e., the height of the cell. This information is then used in a second round of fitting, where a bottom effect correction is applied to the Hertz model.



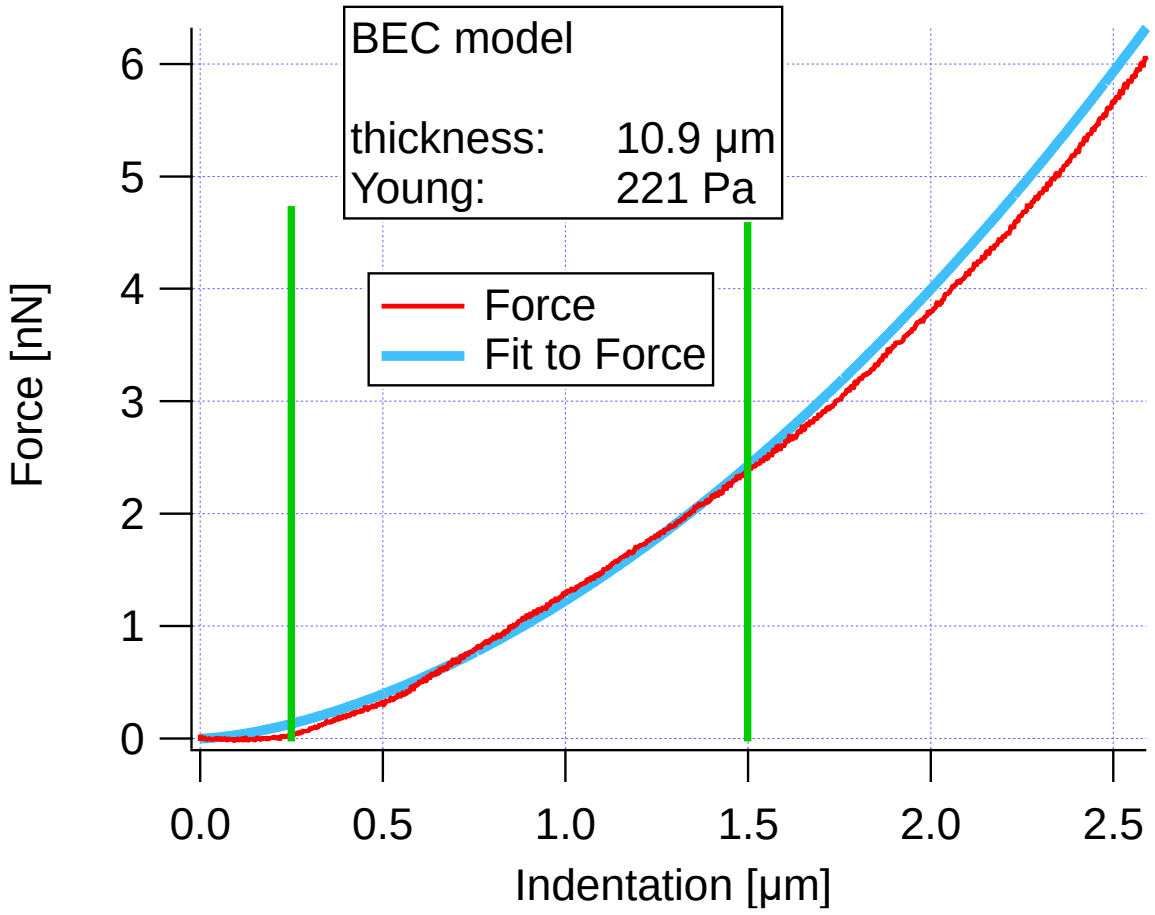
Supplementary Figure S7. Force versus indentation data (in red) with the fitted data (in light blue). The same data as in Supplementary Figure S6, now fitted with bottom effected corrected Hertz model (BEC). The cell height at this location is 2.77 µm, which is of the same order as the maximum indentation (1.4 µm). The tip radius of 5.5 µm is comparable to both indentation and cell thickness. Please note the huge difference between the calculated Young's modulus values: 159 Pa for BEC and 1250 Pa for Hertz without bottom effect correction.

5. Bottom effect correction (BEC)

The above example of a force curve on a thin - relative to the radius of the tip - part of the cell showed a large deviation between the standard Hertz fit and the BEC fit. Supplementary Figures S8&S9 present the force indentation data taken on a thicker part of the same cell as the above data with a much larger thickness (10.76 µm), where both fits seem to match the data very well. Nevertheless, Young's modulus values still show a notable difference: 269 Pa vs 181 Pa.



Supplementary Figure S8. Force versus indentation data (in red) with the fitted data (in light blue) taken on a thicker part of the same cell.



Supplementary Figure S9. Force versus indentation data (in red) with the fitted data (in light blue) taken on a thicker part of the same cell.