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Supporting Material

Stiffness tomography by atomic force microscopy

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Data post processing

The data were post-processed using a home made software written in Matlab®, running under a GNU/Linux environment.

In order to load the force curve, we created two 3D arrays. These arrays contained the trace and retrace curves of all indented pixels. Their size were predefined by the force volume file header values "\force/line" and "\Samps/line" for the dimension of the scan and the number of pixel of each force curve respectively. The data stored in these array were translated in nanometers. The values "\ 4:Z scale: V" and "\@Sens. Deflection: V" from the force volume file header were used to make the conversions.

$$Deflection = data * ZScale * SensDefl$$

The scanner extension was computed with values recovered from the header :

$$ScannerExt = [1 : nbrPoints] * \frac{RampSize * SensZScan}{nbrPoints}$$

where nbrPoints, rampSize and sensZScan correspond respectively to "\Samps/line", "\@4:Ramp size: V" and "\@Sens. Zscan: V" values in the file header.

The scanner extension at the end of the indentation was given as a 2D array that was loaded from the force volume file. Here again, the data were converted in nanometers by using :

$$ScannerHeight = data * \frac{zScale * SensZScan}{2^{16}}$$

where zScale corresponds to "\@2:Z scale:" value in the file header.

The last useful value is the cantilever spring constant contained in the header line "\Spring constant:".

To compute the FI curve, one has first to detect the point of contact between the tip and the sample. The spot where the FD curve "takes off" the linear fit of the “off contact” part of the curve was considered by the software as the contact point. In a next step, a reference force distance (FD) curve recorded on a stiff substrate (the petri dish) was subtracted from the FD curve recorded on the soft sample.

The next step consisted in segmenting the indentation curve. The number and size of each segments is chosen by the user. In the figure S1, we fragmented the indentation curve into 10 segments, beginning from the point of contact I_0. ΔS_i is defined as the segment located between the indentation points I_i and I_{i+1}. The force needed to indent this portion of curve is then ΔF_i.

In the last step, the Young modulus of each segments of the curve was calculated by fitting it to the Hertz model [1] according to equation for the cone :

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

where ν is the Poisson coefficient of the material, δ the indentation depth into the material, E the Young's modulus and α the semi-opening angle of the cone in radian.

By passing E on the right hand side, we obtain the Young modulus :

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

which in our case is :

$$\Delta E = \frac{2}{\pi \tan(\alpha)} * \frac{\Delta F_i}{\Delta S_i^2} * (1 - \nu^2)$$

ΔS_i is the depth of the i^{th} indentation segment and ΔF_i the additional force needed to indent the i^{th} segment. By displaying in false colors the calculated stiffness of each fragment at its corresponding depth, we obtain the “stiffness slice” of the sample, as depicted under the x axes in the figure S1b. The color green depicted zones with a lower Young modulus and red a higher Young modulus.

References

[1] Radmacher, M. (2002). Measuring the elastic properties of living cells by the atomic force microscope. Methods Cell Biol, 68:67–90.

Figure S1

In sub-figure **a**, the indentation curve from the point of contact I_0 to its end, is divided in several segments of defined depth, referred to as ΔS_i . At the depth I_i, the force needed to indent the tip into the segment ΔS_i is ΔF_i .

The sub-figure **b** represents two indentation curves : one into a homogeneous sample (green line) and one into a sample containing a hard inclusion (red line). For each of the segments ΔS_i , a Young's modulus is calculated according to the Hertz model. The resulting value is reported as a pixel colored according to its Young's modulus (Soft = green and Hard = red).

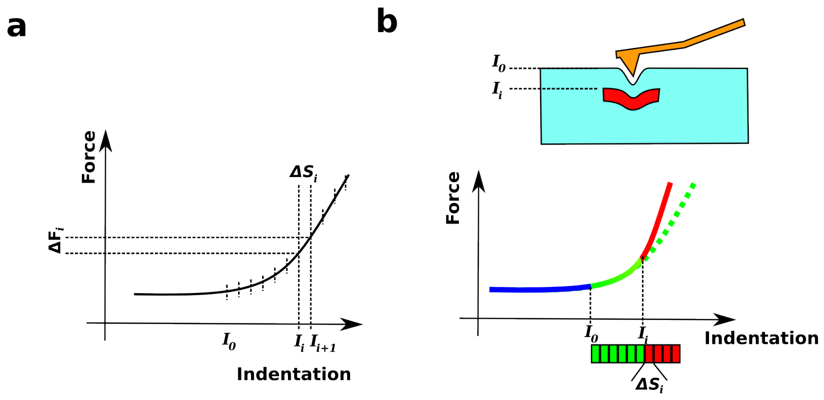

Figure S1

Figure S2

a) Example of a Force Distance (FD), and its corresponding Force Indentation (FI) curve. The FD curve is shown with its point of contact symbolized with a red circle. The contact point is detected once the FD curve (blue) crosses the upper green line which corresponds to the error on the linear fit of the “off contact” domain of the FD curve.

b) The indentation curve of the FD depicted on figure S2a. The limits of the segments are illustrated with the black circles (separation : 10 nm).

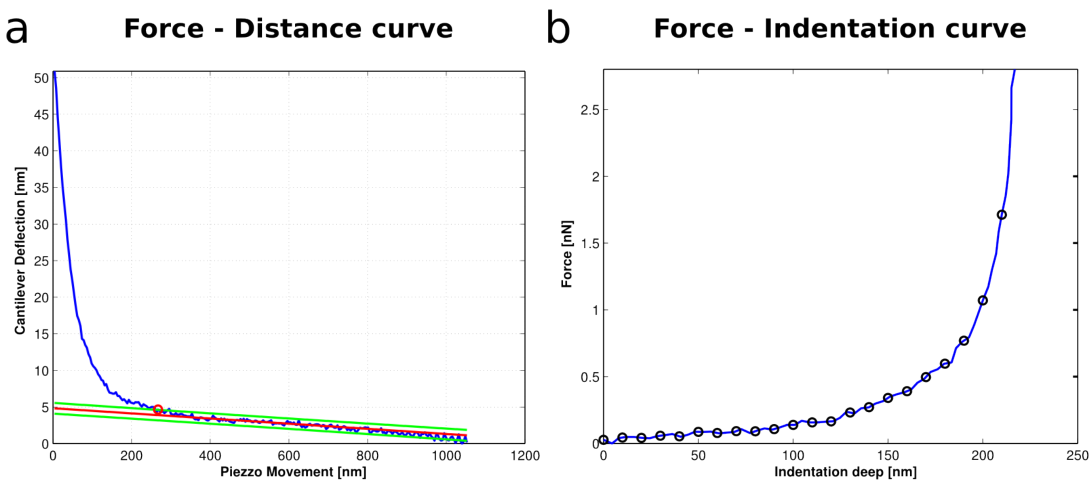

Figure S2

Figure S3

Example of FI curves before (blue) and after (red) the injection of cytochalasin B. The set of blue curves deviates from the set of red curves at a depth varying between 100-200 nm.