Exercise: Mapping Mechanical Properties of Soft Material – AFM

Objectives:

1.) Prepare samples of various materials with different compositions.
2.) Perform indentation tests in a 16x16 square pattern on the sample using AFM at different temperatures.
3.) Evaluate their elastic modulus using the Hertz-Sneddon model at each indentation point.
4.) Create a map of elastic moduli and analyze it
5.) Compare the Young's modulus depending on the material.
6.) Discuss the results.

Keywords: Elastic modulus, AFM, Indentation test, Hertz-Sneddon model, modulus mapping, force mapping.

Introduction:

Atomic Force Microscopy (AFM) was invented in 1986 by Prof. Binnig for imaging objects where the resolution of optical microscopes is insufficient. The AFM principle is based on scanning the object with a very small tip. During this scanning, the deformation of the cantilever, on which the tip is placed, is detected. This deformation is measured using an optical lever, where a laser beam is directed onto the cantilever, and its reflection is detected by a photodetector (quadratic photodiode). This principle allows us to measure the deflection of the cantilever in both lateral and vertical directions. This method enables the measurement of the topography of the scanned sample.

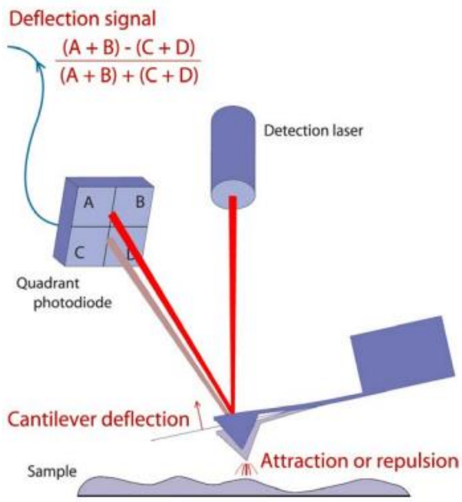
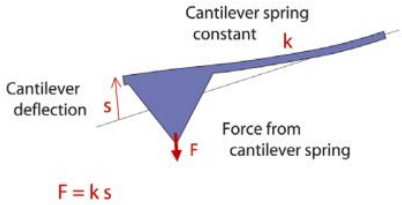


Figure 1 AFM principle - JPK Instruments NanoWizard® Handbook Version 2.2a

One of the currently most widely used techniques for measuring the mechanical properties of small microscopic objects, such as cells, is the so-called indentation test (according to the nomenclature of the JPK Force Spectroscopy company). Due to the basic principle of AFM, this test can be performed using AFM. The key element in studying the mechanical properties of materials is to monitor their response to deformation or loading. In the case of the indentation test, an object (probe) is pressed into the material and the force and depth of the indentation are recorded. In the case of AFM, the force is determined by the deformation of the cantilever on which the probe is placed. Each cantilever has its own spring constant, which allows us to calculate the force required for its deformation.

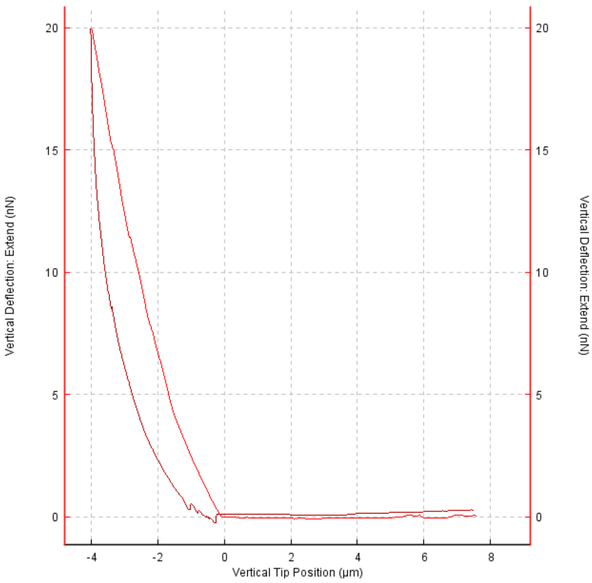
$$F = k \cdot s$$

The depth of indentation can then be obtained directly from the measured displacement of the head and the deflection of the cantilever.



Obrázek 2 Cantilever deformation - JPK Instruments NanoWizard® Handbook Version 2.2a

Using the indentation test, we obtain the final relationship between the depth of indentation and the detected force, both with the loading and unloading curves, as shown in Figure 3, where the left curve is the unloading curve and the right curve is the loading curve. For our purposes, the loading curve is particularly important.



Obrázek 3 Example of force – indentation depth

From the basic theory of elasticity we can say that the modulus of elasticity is the slope of the deformation curve. However, in the case of the indentation test, this cannot be utilized because the contact area changes due to the geometry of the indenter. Therefore, it is necessary to use one of the models. There are many such models, but the Hertz model, or alternatively, the Hertz/Sneddon model, is probably the most commonly used. For a spherical indenter, the model looks as follows:

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

where E[Pa] is the modulus of elasticity, ν[-] is Poisson's ration, R[m] is the radius of the tip, and δ[m] is depth of indent.

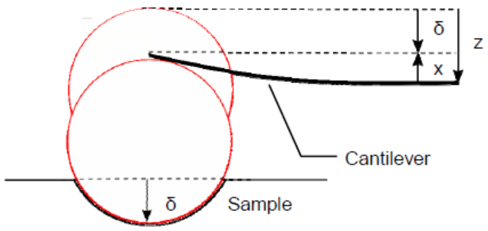


Figure 4 Indentation depth - JPK instruments aplication note (©JPK instruments AG)

The modulus of elasticity from this equation is then obtained by fitting the model to the curve. The problem is that at first glance it is evident that there are two unknowns, E and ν, in the equation. Unfortunately, it is not easy to obtain the Poisson's ratio for each measured material, so the value of this ratio is estimated to be 0.5 for biological materials.

Modulus mapping, also known as force mapping, is a method in AFM (atomic force microscopy) used to create maps of the mechanical properties of a material. Unlike force spectroscopy, which records a force curve at a single point, force mapping enables the creation of maps of interactions across a larger area of the sample. It automatically performs a series of indents based on the pattern you choose (square or rectangular).

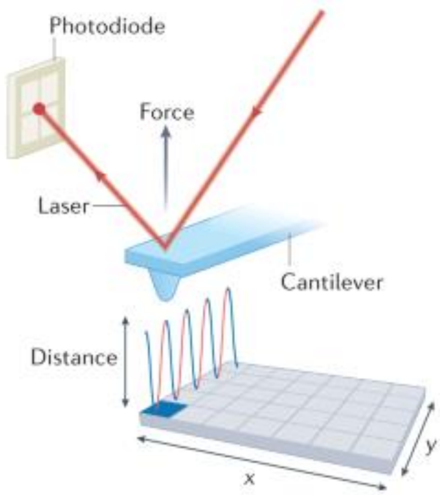


*Figure 5 Force mapping mmod - (VILJOEN, Albertus, Marion MATHELIÉ-GUINLET, Ankita RAY et al. Force spectroscopy of single cells using atomic force microscopy. Nature Reviews Methods Primers. 2021, **1**(1). ISSN 2662-8449. Dostupné z: doi:10.1038/s43586-021-00062-x)*

The evaluation of such a map is similar to the evaluation of an individual indentation, as described earlier, with the difference that it is applied repeatedly to each curve in the map. The result of the analysis using force mapping is the distribution of mechanical properties of the material, such as the modulus of elasticity, stiffness, adhesion, or the topography of the material.

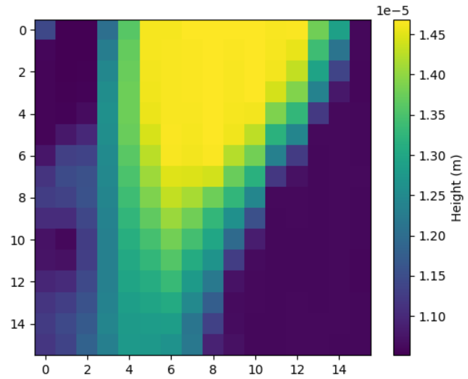


Figure 6 Topography of cell using force mapping techique - Staňková R.: Analýza mechanických vlastností buněk, FBMI ČVUT, 2023

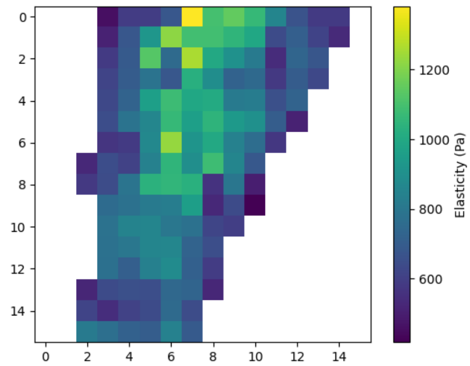


Figure 7 Map of elastic muduli of the cell on figure 6. - Staňková R.: Analýza mechanických vlastností buněk, FBMI ČVUT, 2023

Required equipment: Pipette, Petri dish, AFM, Inverted microscope, CCD camera, PBS, measurement chamber.

Instructions:

Mapping mechanical properties of soft material – AFM:

1. Prepare the sample.
    - Transfer the sample from the prepared test tube with liposomes to a Petri dish and cover it with PBS.
    - Place the Petri dish in the measurement cell and heat it to 40 ° C.
2. Prepare the AFM
    - Launch the SPM software on the measurement PC and QuickPHOTO on the microscope PC
    - Install the cantilever with the tip into the holder.
        - Insert the holder from the cantilever installation kit, rotate it 90 degrees so that the upper surface of the holder is horizontal, and secure the holder.
        - Use tweezers to remove the cantilever and place it in the holder, secure it with a cross screw.
        - Proceed carefully following the instructions and guidance provided by the instructors.
        - Ensure that the cantilever does not fall out and that you do not touch it with anything, eliminating the risk of damage.
        - Before this procedure, practice the process using the damaged cantilever provided. Surface properties measurement using AFM.

3. Insert the holder into the measuring head.
    - Connect all cables.
    - Exercise caution to avoid damaging the tip.
4. Perform cantilever calibration.
    - Place a glass slide into the measurement space.
    - Insert the AFM measuring head.
    - Adjust the laser beam to aim at the cantilever in the tip area.
        - Follow the manual.
        - Monitor the beam on the microscope PC screen.
    - Set the contact mode and force spectroscopy mode.
    - Slowly lower the tip to the glass.
        - Be careful not to hit the glass with the tip.
        - Use the automatic approach for the final approach.
    - Perform the indentation of the glass.
        - Settings: Setpoint - 0.5mV, z-length - 5um, constant speed – $1\mu m/s$.
    - Raise the probe to a safe distance.
    - In the SPM software, launch the calibration manager, find, and set the parameters for sensitivity and spring constant.
        - Follow the instructions.
5. Insert the sample into the measurement cell.
6. Lower the measurement head to be submerged in PBS.
7. Adjust the settings of the diodes and mirrors to compensate for the light refraction on the surface.
8. Measure the selected material.
    - Approach the sample with the cantilever.
        - Note: In this case, you won't see the cantilever on the microscope, as it will be shadowed. Approach the sample with caution.
        - Observe the approach of the tip from the side and approach the tip in shorter steps.
        - Preferably, leave the automatic approach at a longer distance.
    - After the automatic approach, measure the sample.
        - Change the module setting to Force Mapping,
        - Settings: Setpoint – 10nN, z-length - 5µm, constant speed – 1µm/s, Grid 16x16, measurement area 17x17µm
        - After the measurement, retract to a safe distance.
        - Save the data.
    - Repeat the procedure for another sample.
9. Remove the measuring head, measuring chamber, holder, and cantilever. Remove the sample and clean up the measurement area.

10. Evaluate the measured curves.
    - Use the JPK Data Processing software on the measurement PC.
        - Open curves using "Open Batch of force spectroscopy curves"
        - Evaluate each curve one by one.
        - Follow the software instructions.
        - Find the parameters of the tip in the respective box
11. Before leaving, turn off the PC and light sources
12. Write the protocol.
    - -Hypothesis (how you expect the mechanical properties of samples of different materials to differ, whether you expect homogeneous behaviour of mechanical properties of individual samples, etc.)
    - Methodology (identification and description of samples, sample preparation procedure, measurement procedure, chosen loading regime, instruments used, data processing method).
    - Results (Summary of experimental results, including modulus maps for individual samples).
    - Discussion and interpretation of results (Discuss how the results of individual samples differ from each other, any potential impact on results, etc.)
    - Conclusion (Summarise the entire experiment).