Exercise: Measurement of Mechanical Properties of Cells/Liposomes – AFM

Objectives:

1.) Prepare a sample of a cell/liposome model.
2.) Conduct an indentation test on liposomes using AFM at various temperatures.
3.) Measure their geometric properties.
4.) Evaluate their elastic modulus using the Hertz-Sneddon model.
5.) Compare the Young's modulus of elasticity, depending on the temperature of the environment in which the sample is measured.
6.) Compare the Young's modulus of elasticity depending on the size of the liposome.
7.) Discuss the results.

Keywords: Liposome, Elastic Modulus, AFM, Indentation test, Hertz-Sneddon Model

Introduction:

As cells often do not have uniform dimensions, liposomes are commonly used as a cell model. Uniform geometry is often helpful, especially when trying to find a model to describe a particular problem, such as, in our case, describing the mechanics of a cell. By using liposomes as a model, we can examine the influence of the measured subject's size on its mechanical properties. Therefore, liposomes will be used as a cell model in this task.

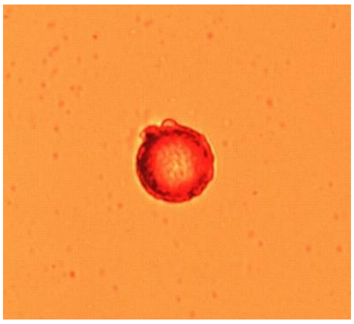
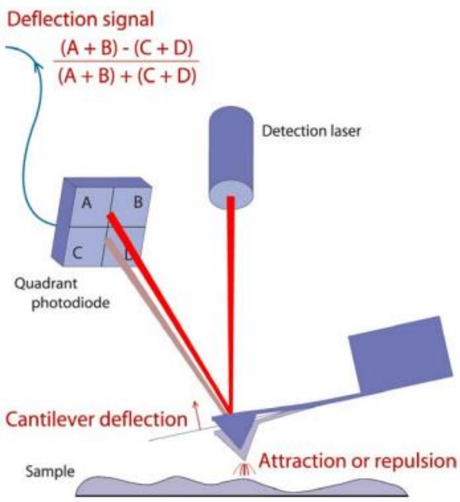


Figure 1 Liposome

Atomic force microscopy (AFM) was invented in 1986 by Prof. Binnig for imaging objects that surpass the resolution of optical microscopes. The principle of AFM 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 the so-called optical lever, where a laser beam hits the cantilever, and its reflection is detected by a detector (quadratic photodiode). This principle allows for measuring the deflection of the cantilever in both the lateral and vertical directions. This method enables us to measure the topography of the scanned sample.



Fugure 2: Schematic of the AFM principle - taken from the 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 JPK Force spectroscopy). Because of the fundamental principle of AFM, this test can be conducted using AFM. The basis of any investigation into mechanical properties is monitoring the material's response to its deformation or loading. In the case of an indentation test, the object (tip) is pressed into the material, and the force and depth of the indent are recorded. In the case of AFM, the force is determined by the deformation of the cantilever on which the tip is placed. Each cantilever has its stiffness constant, allowing us to obtain the force required for its deformation.

$$F = k \cdot s$$

The depth of the indent itself is then obtained directly from the measured displacement of the head and the deflection of the cantilever.

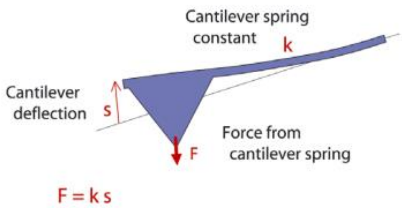


Figure 3 Cantilever deformation diagram - taken from JPK Instruments NanoWizard® Handbook Version 2.2a

Through the indentation test, we obtain the final relationship between the depth of the indentation and the detected force, both for the loading and unloading curves, as shown in Figure 3, where the left curve is unloading and the right one is loading. For our purposes, the loading curve is of particular importance.

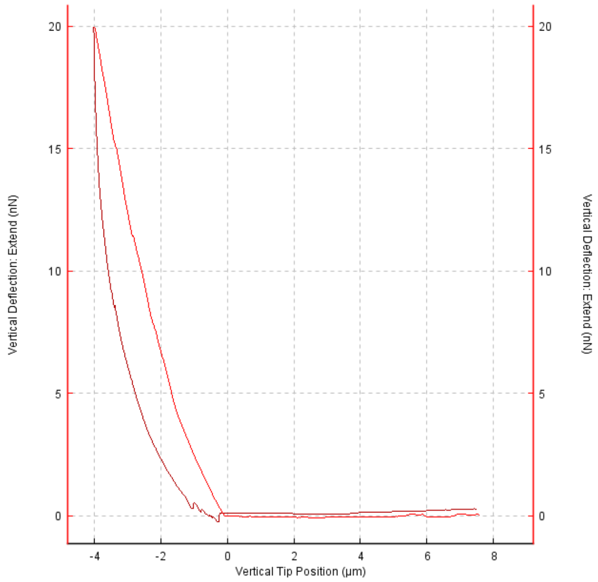


Figure 4 Example of the measured dependence force - indentation depth

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 tip. Therefore, it is necessary to use one of the models. There are many such models, but perhaps the most used is the Hertz model or the Hertz/Sneddon model. For a spherical tip, the model looks like this:

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

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 the depth of the indent.

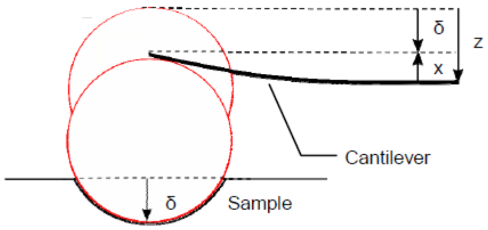


Figure 5 Indent diagram - taken from the JPK instrument aplication note (©JPK instruments AG)

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.

Required equipment: Pipette, Petri dish, AFM, inverted microscope, CCD camera, PBS (Phosphate-Buffered Saline), and measurement cell.

Instructions:

Determining the Mechanical Properties of Cell Models Using 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 into 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 the sensitivity and the 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 liposome.
    - Use the microscope to measure the diameter of the liposome and take a photo.
    - Approach the tip close to the liposome so that the tip is in the centre of the sample (from above).
    - After an automatic approach, measure the sample.
        - Settings: Setpoint – 10nN, z-length - 5um, constant speed – 1μm/s.
        - After measurement, move to a safe distance.
        - Save the data.
    - Repeat the procedure for at least 5 liposomes.
    - Gradually decrease the temperature by 3 degrees to 28°C, repeating the procedure each time on the same liposomes.
9. Remove the measurement head, measurement cell, and holder; clean up the sample and measurement space.
10. Evaluate the measured curves
    - Use the JPK Data Processing software on the measurement PC for evaluation.
        - Follow the software instructions.
        - Find the parameters of the probe on the respective box
11. Turn off the PC and light sources before leaving
12. Developing a protocol.
    - Formulate a hypothesis regarding how the mechanical properties of liposomes might differ at different temperatures and how the size of the liposome might affect its mechanical properties.
    - Outline the methodology, including sample identification, sample preparation, measurement procedures, loading regime, equipment used, and data processing.
    - Summarise the results.
    - Discuss and interpret the results, noting differences between individual samples and possible influences on the results.
    - Conclude by summarising the entire experiment.