

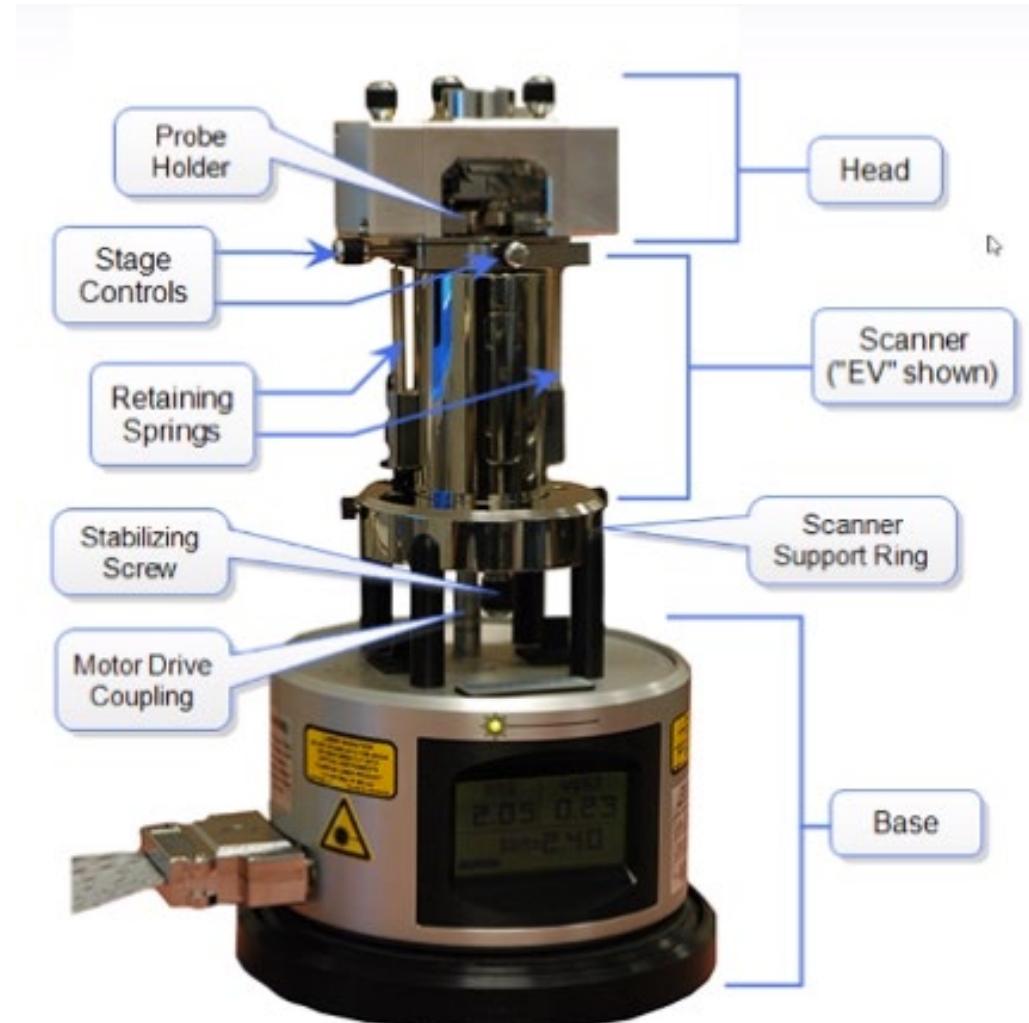
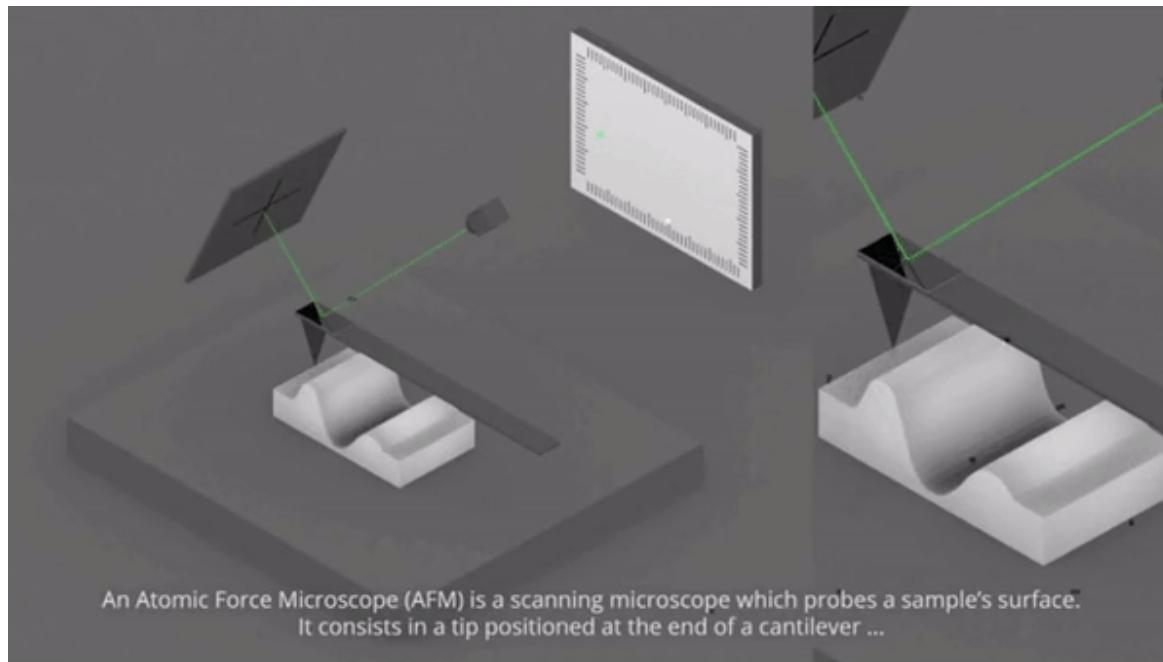
MICRO429-Metrology Practicals

AFM

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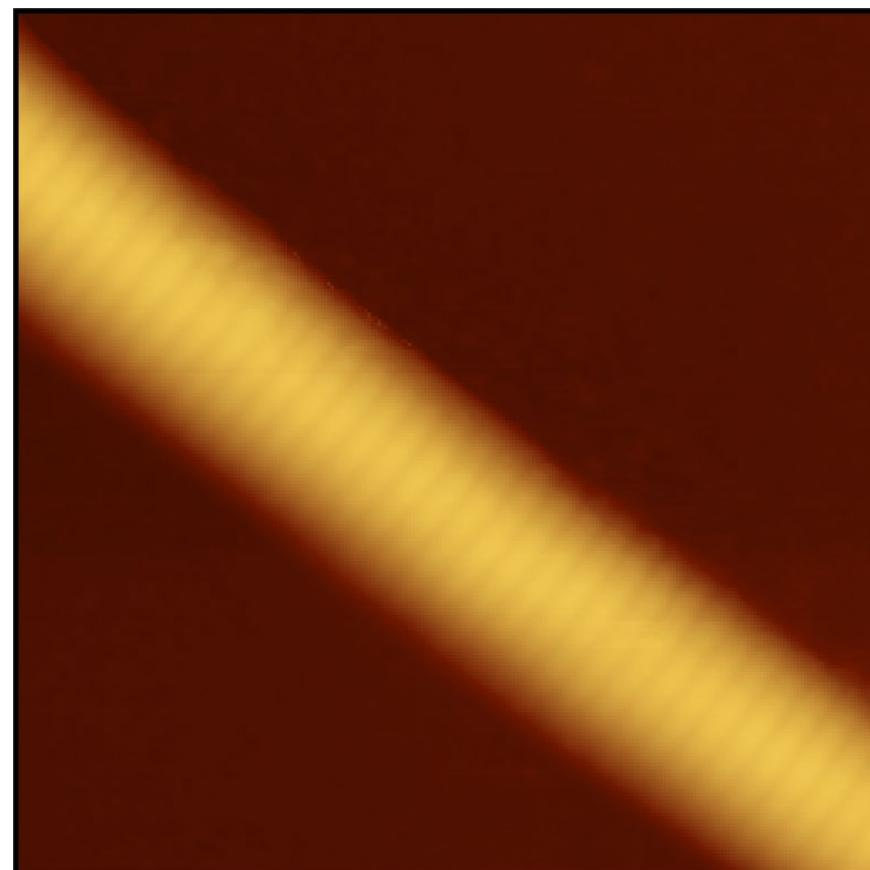
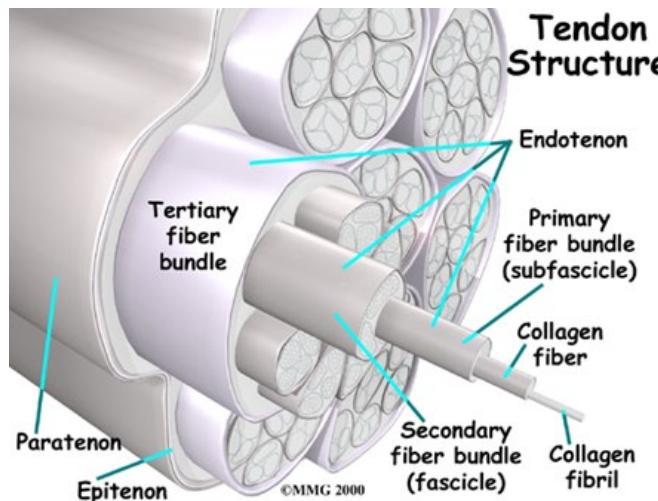


2.1.1 Collagen

Material

Rattails, scissors, petri dish, mica on a metal disc, tweezers, scotch tape

- How to prepare a sample on mica
- How to set up the AFM for imaging
- How tapping mode works



T2.1 - Sample preparation and imaging

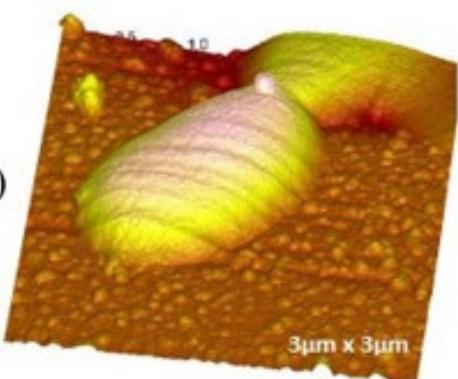
2.1.2 E.coli

Material

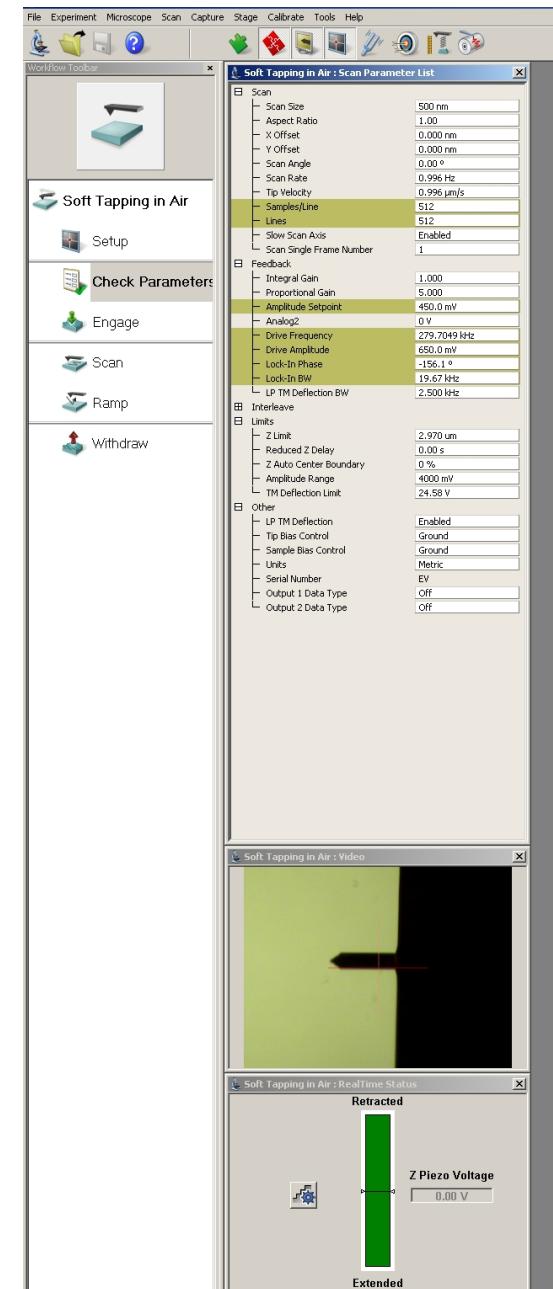
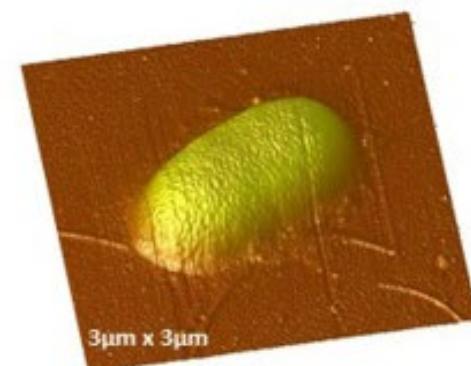
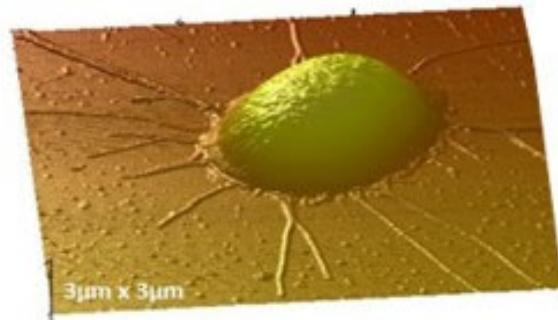
Centrifuge, pipette, fresh E.coli, mica

- ☐ How to prepare E.coli sample (wet) on mica
- ☐ How to optimize Imaging parameters

b)



c)



The screenshot shows the Nanoscope software interface with the following windows:

- Workflow Toolbar:** Includes icons for File, Experiment, Microscope, Scan, Capture, Stage, Calibrate, Tools, and Help.
- Soft Tapping in Air : Scan Parameter List:** A list of scan parameters:
 - Scan: Scan Size (500 nm), Aspect Ratio (1.00), X Offset (0.000 nm), Y Offset (0.000 nm), Scan Angle (0.00 °), Scan Rate (0.996 Hz), Tip Velocity (0.996 μm/s), Samples/Line (S12), Lines (S12), Slow Scan Axis (Enabled), Scan Single Frame Number (1).
 - Feedback: Integral Gain (1.000), Proportional Gain (5.000), Amplitude Setpoint (450.0 mV), Analog2 (0 V), Drive Frequency (279.7049 kHz), Drive Amplitude (650.0 mV), Lock-In Phase (-156.1 °), Lock-In BW (19.67 kHz), LP TM Deflection BW (2.500 kHz).
 - Interleave: Z Limit (2.970 μm), Reduced Z Delay (0.00 s), Z Auto Center Boundary (0 %), Amplitude Range (4000 mV), TM Deflection Limit (24.58 V).
 - Other: LP TM Deflection (Enabled), Tip Bias Control (Ground), Sample Bias Control (Ground), Units (Metric), Serial Number (EV), Output 1 Data Type (Off), Output 2 Data Type (Off).
- Soft Tapping in Air : Video:** A video preview window showing a bright, elongated cell on a mica surface.
- Soft Tapping in Air : RealTime Status:** A window showing the Z Piezo Voltage status, with a green bar indicating 'Extended' and a red bar indicating 'Retracted'.

Images to collect:

Collagen

- 30um x 30um scan at 1024 (samples per line) x 256 (lines)
- 10um x 10um scan at 1024 x 256
- 2-4um x 2-4um scan on single collagen fiber 1024 x 256

E.coli

- 30um x 30um scan at 1024 x 256
- 1 single E.coli ~5um x 5um at 1024x256

Software: Gwyddion

Image corrections

- Line median matching
- 1st order plane leveling
- Three-point fitting
- Scar correction
- Higher order leveling
- ...

Image representation

- Color palettes
- 3D Data representation

Data analysis

- Extracting profiles
- 2D FFT Analysis
- Tip diameter estimation
- ...

Task:

- Process all the images you obtained with AFM, print them and put them into your laboratory notebook.
- Analyzing AFM image of collagen fibrils and E.coli bacteria.

Location: BM5114

