Session 8

ATOMIC FORCE MICROSCOPY
The Approach

- G. Binning (Stanford) in 1986
- Deflection of a cantilever spring
- Main parts
  - Cantilever tip
  - Laser Diode
  - Photodiode
  - Stage

![Diagram of the approach with a cantilever spring, laser diode, photodiode, and stage.](image-url)
The System

- Configured with optical microscopes
- Cells need softest cantilevers
- Range: 100 μm scan, 10 μm tall

\[
F = K \cdot d = \left( \frac{4Ewt^3}{L^3} \right) \cdot d
\]

- \( F \) Loading Force
- \( K \) Bending Stiffness (35 nN/μm)
- \( d \) Displacement
- \( E \) Modulus of Elasticity (100 GPa)
- \( w \) Cantilever width (30 μm)
- \( t \) Cantilever thickness (1 μm)
- \( L \) Cantilever length (150 μm)
Imaging Mode

- **Constant deflection mode** (constant force)
- **Distance Feedback**
  - **Height signal**: output of the feedback
    - Specimen height (3-4 μm)
  - **Deflection signal**: residual, uncompensated deflections
    - Provides fine details (100 nm)
  - **True Height**: A + B
- **DC vs. AC mode**
**Force Measurement**

- **Force Curve**
  - Out of contact, no deflection
  - In contact, deflection proportion to sample height

![Diagram of force curve with symbols for tip deflection (d) and sample height (z), showing stages of contact and non-contact]
Force Measurement

- Indentation comes from sample compression
  - \( d = (z-z_0) - \delta \)
  - Hertzian contact for a “conical punch”

\[
F = \frac{2}{\pi} \tan(\alpha) \left( \frac{E}{1-\nu^2} \right) \delta^2
\]
Force Measurement

- Sample Stiffness

$$z - z_0 = d + \sqrt{\frac{2dK(1-\nu^2)}{\pi\tan(\alpha)E}}$$

- Measurement for sample height ($z_0$) not easy
- Two fit-parameters: $E$ and $z_0$
Regional difference in cell stiffness
Line Scan

- Stacked force curves
- Shows height of cell along scan
- Cell body: more compressible
- Periphery: less compressible
2D Scan

- Graph a shows varying height with lateral position.
- Graph c has height and lateral position with 0.4 nN and 3.6 nN annotations.
- Graph b displays similar height variations.
- Graph d also shows height variations with lateral position.
Constant Force Topography

a 0.4 nN
b 1.2 nN
c 2.0 nN
d 2.8 nN
Feel the substrate

- Low loads – surface
- High loads – CSK
Elastic Tomography
Stiffness Matching

- Glass
  (1 GPa)

- Gel
  (5 kPa)
Stiffness regimes

- For 1-5 kPa, cell stiffness matches substrate
- For >5 kPa, cells are softer than substrate
The softness of cancer

- Method to distinguish between metastatic cancer and normal cells
- Cancer needs to be flexible to migrate through tissue to get into blood stream

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Force of protrusion

- Sideways mounted AFM
- Stall force detected
  - \( F = 1.18 \pm 0.35 \text{ nN} \)
- Elastic Brownian Ratchet
  - 3 \( \mu \text{m} \times 0.2 \mu \text{m} \) AFM contact area
  - 4 pN force per filament
  - 100 actin filaments pushing
  - 2 kPa lamellipodia pressure
The Future?

- Nanoink’s 2D nano PrintArray for nanoscale patterns

- High throughput cell measurements?
Adhesion Force Measurement

- If adhesive, tip sticks during retraction
- Van der waals forces hold onto the tip
Integrin Adhesion Forces

- Fibronectin and $\alpha_5\beta_1$-integrin on vascular smooth muscle cells
- Individual bond force is $39 \pm 8$ pN
- Cells have multiple bonds at each focal adhesion due to integrin clustering