AFM characterization of *Pseudomonas aeruginosa* biofilm in a membrane bioreactor

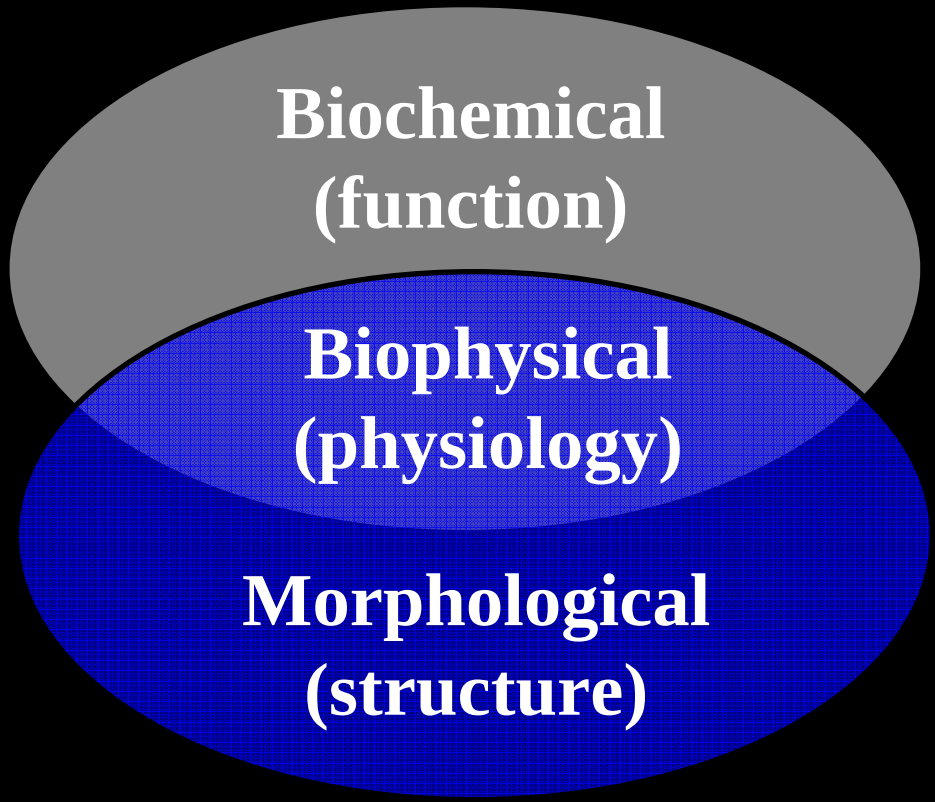
Zhen Huang[1,2], E. S. McLamore[1,2], and M.K. Banks[1]

[1]School of Civil Engineering, Purdue University, West Lafayette, IN

[2]Discovery Park Bindley Bioscience Center, Purdue University, West Lafayette, IN

Biofilm Physiology

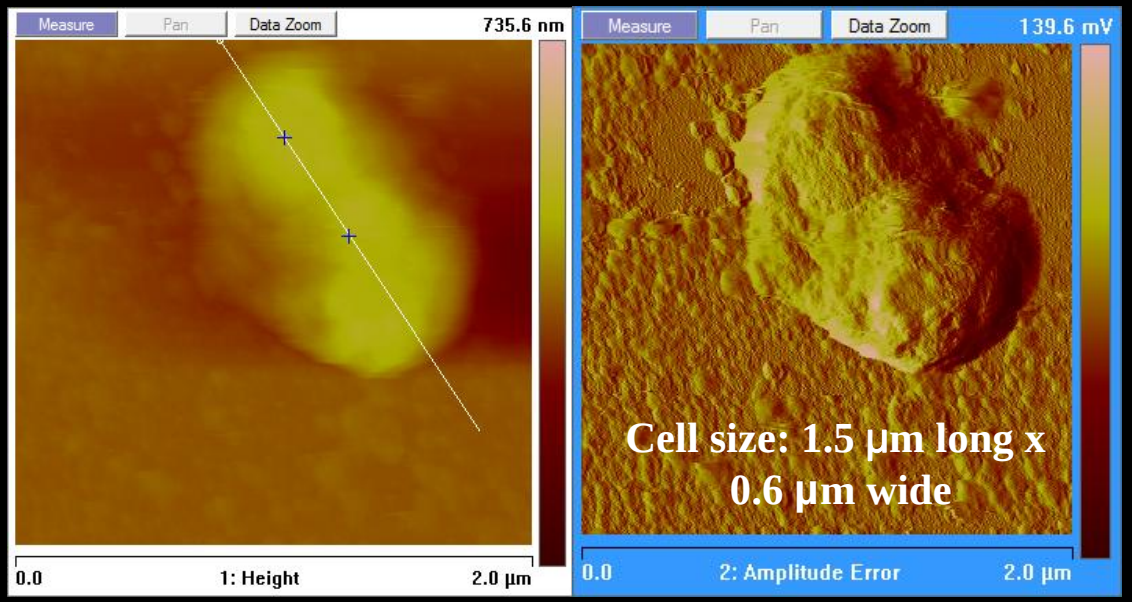
- Structure
  - Atomic force microscopy
    - Limited research on biofilm morphology
- Function
  - Simultaneous analyses
    - McLamore results

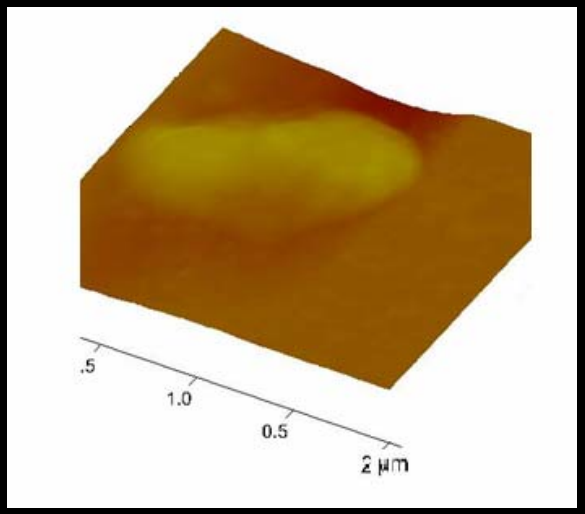


Morphological Characterization

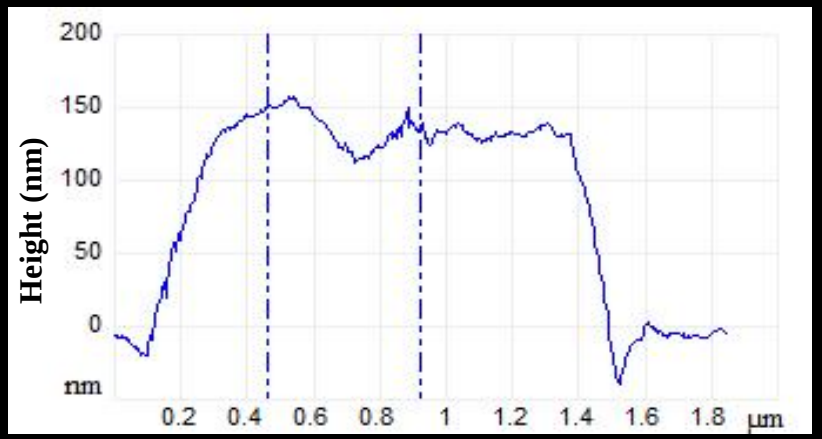
PURDUE UNIVERSITY

Characteristics of individual *P. aeruginosa* cell





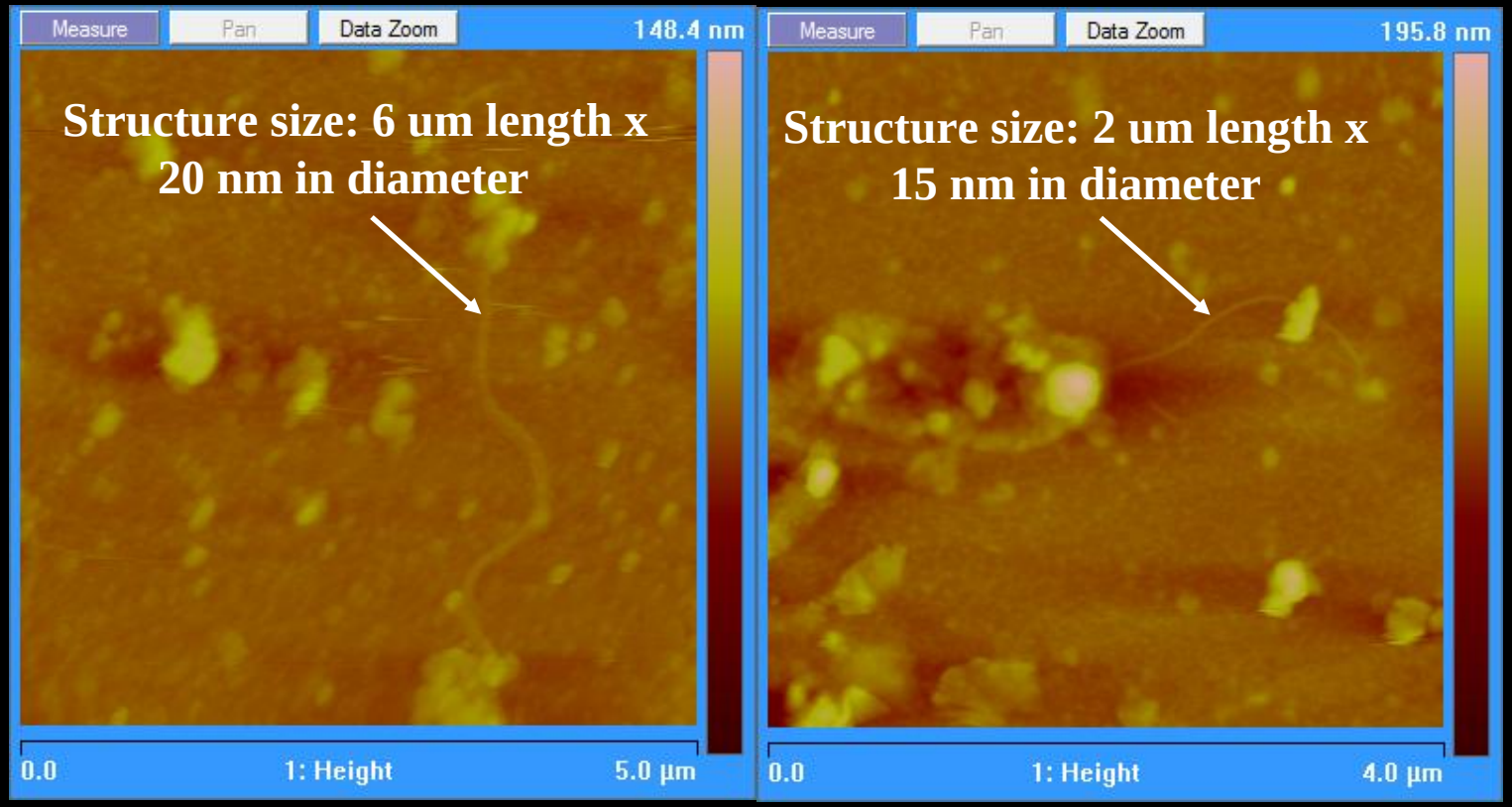
3-D image of individual *P. aeruginosa* cell on surface of biofilm after 8 hours



Distance in X direction

Height image taken on individual *P. aeruginosa* cell near surface of a 8 hours attached biofilm

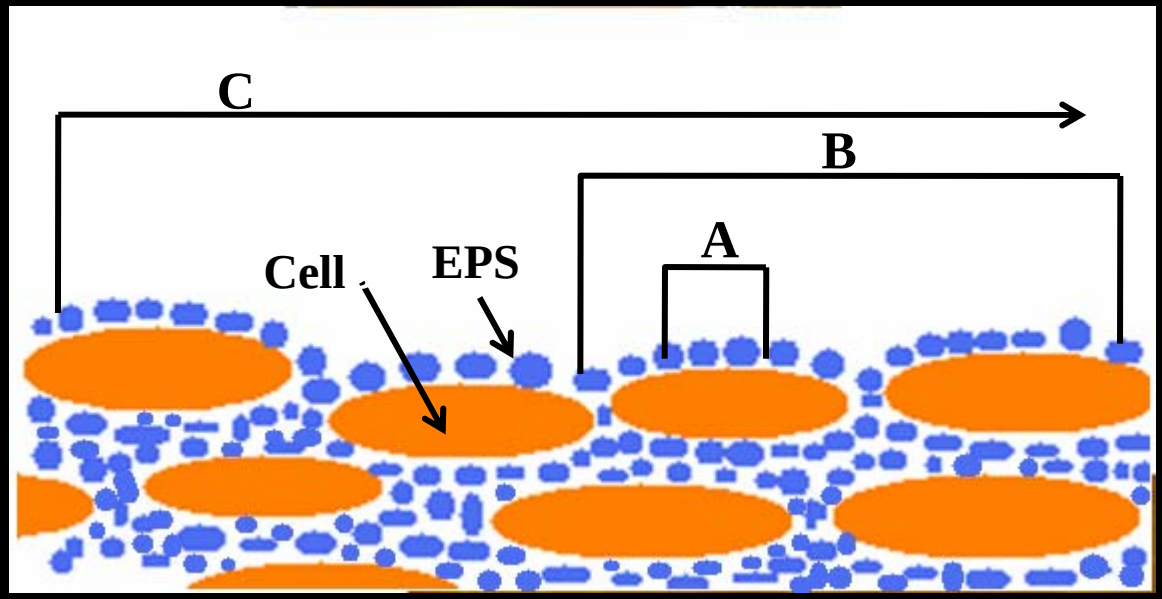
Morphological Characterization (cont.)

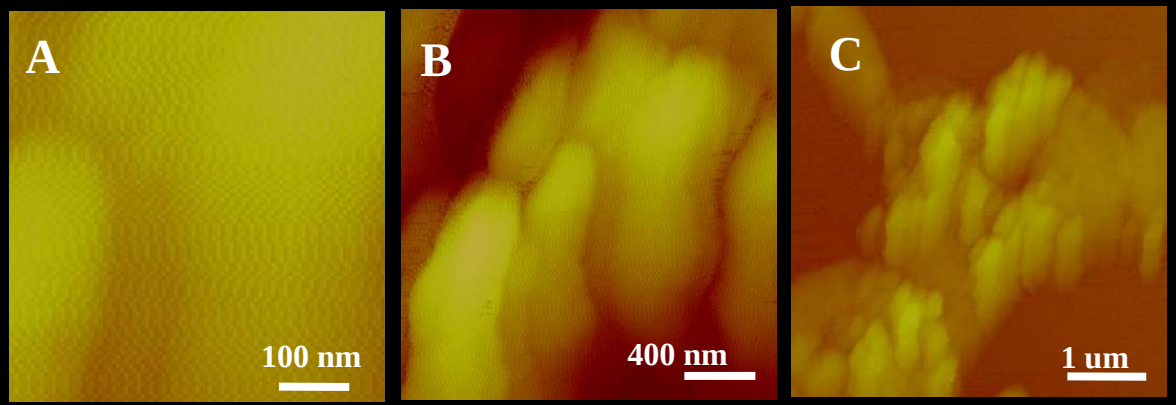


Surface roughness image taken of individual *P. aeruginosa* cell near surface of a 6 hour-biofilm

Surface Roughness vs. Scale

PURDUE UNIVERSITY



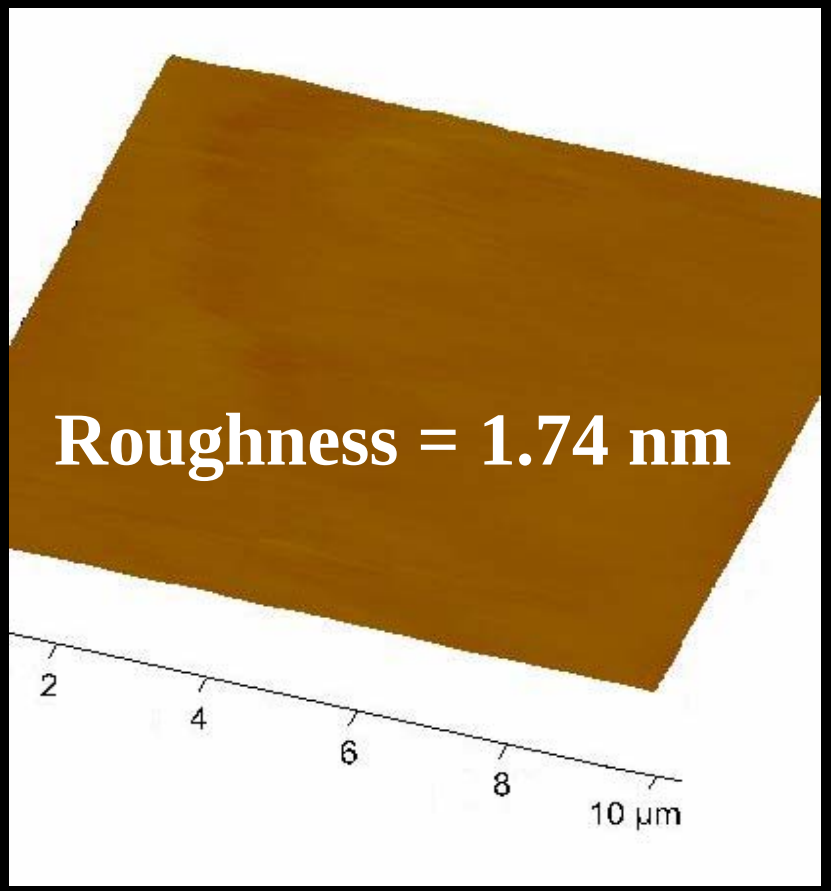


(Adapted from Auerbach et al. 2000)

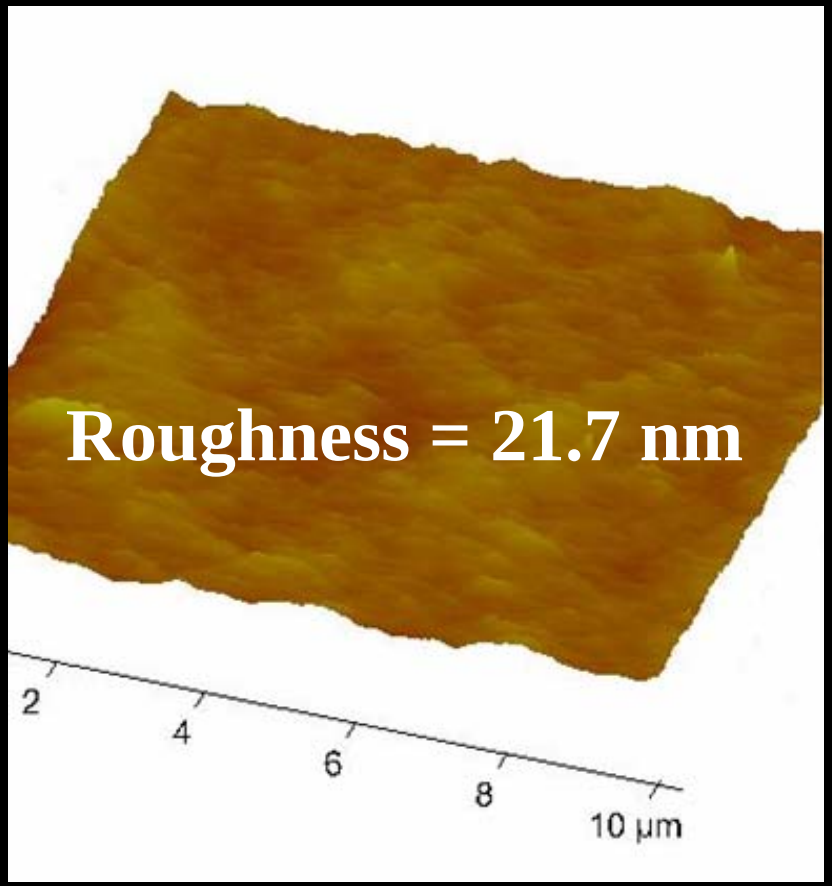
Biofilm Surface Roughness

PURDUE UNIVERSITY

$$R_{rms} = \sqrt{\frac{\sum_i (h_i - \langle h \rangle)^2}{n}}$$

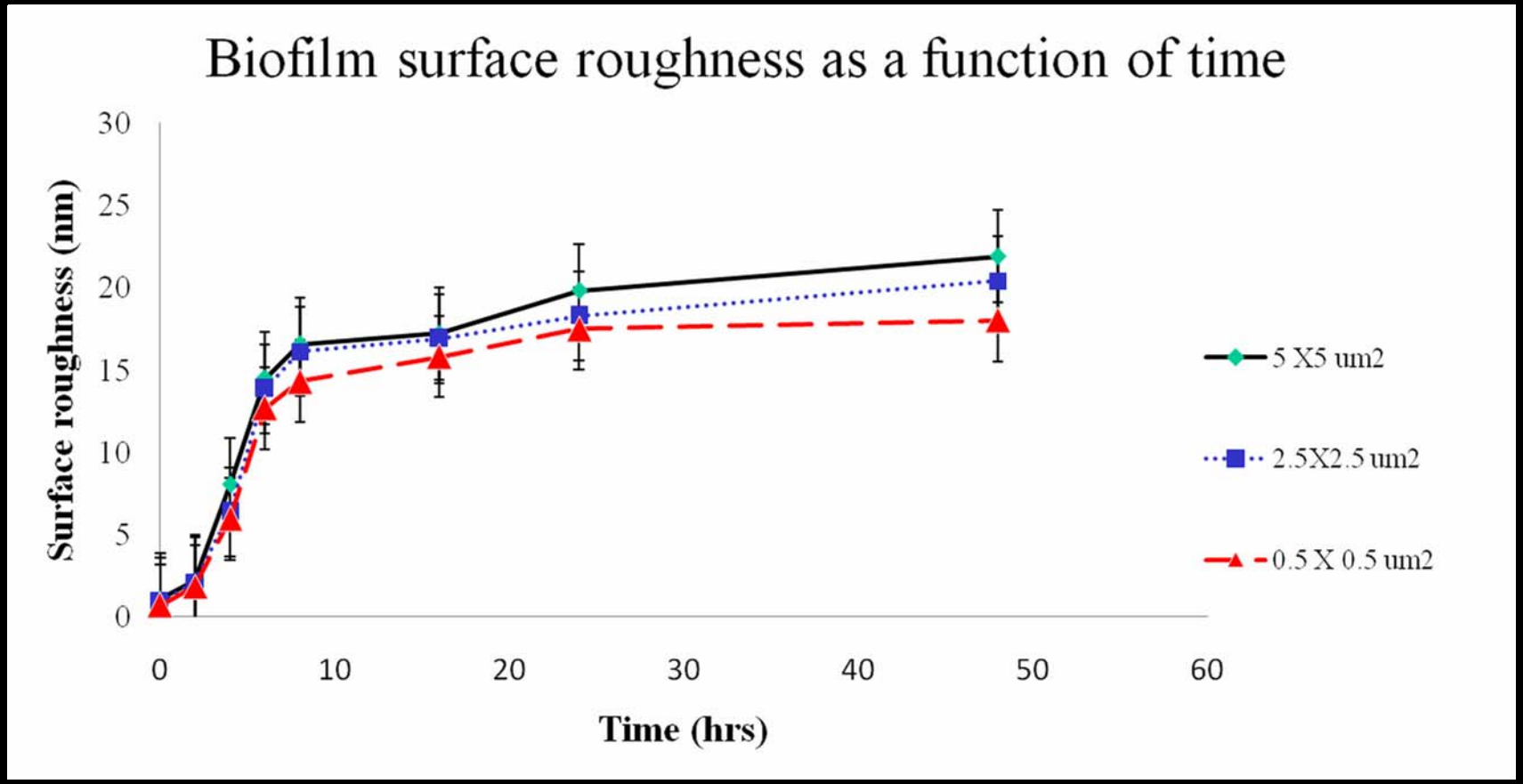


Bare silicon membrane used as a control



Mature *P. aeruginosa* biofilm grown on silicon membrane

PURDUE UNIVERSITY



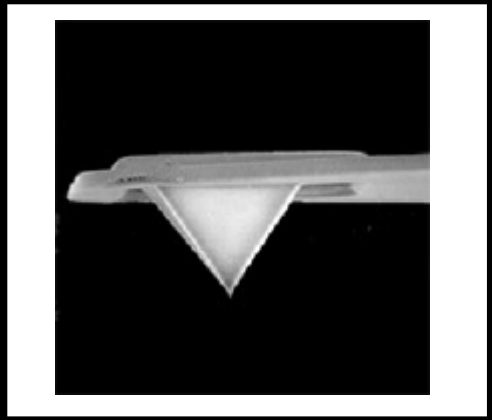
For a relatively smooth biofilm (e.g., pure culture *P. aeruginosa*), there is no significant difference between roughness at different scales.

PURDUE UNIVERSITY

AFM Tips

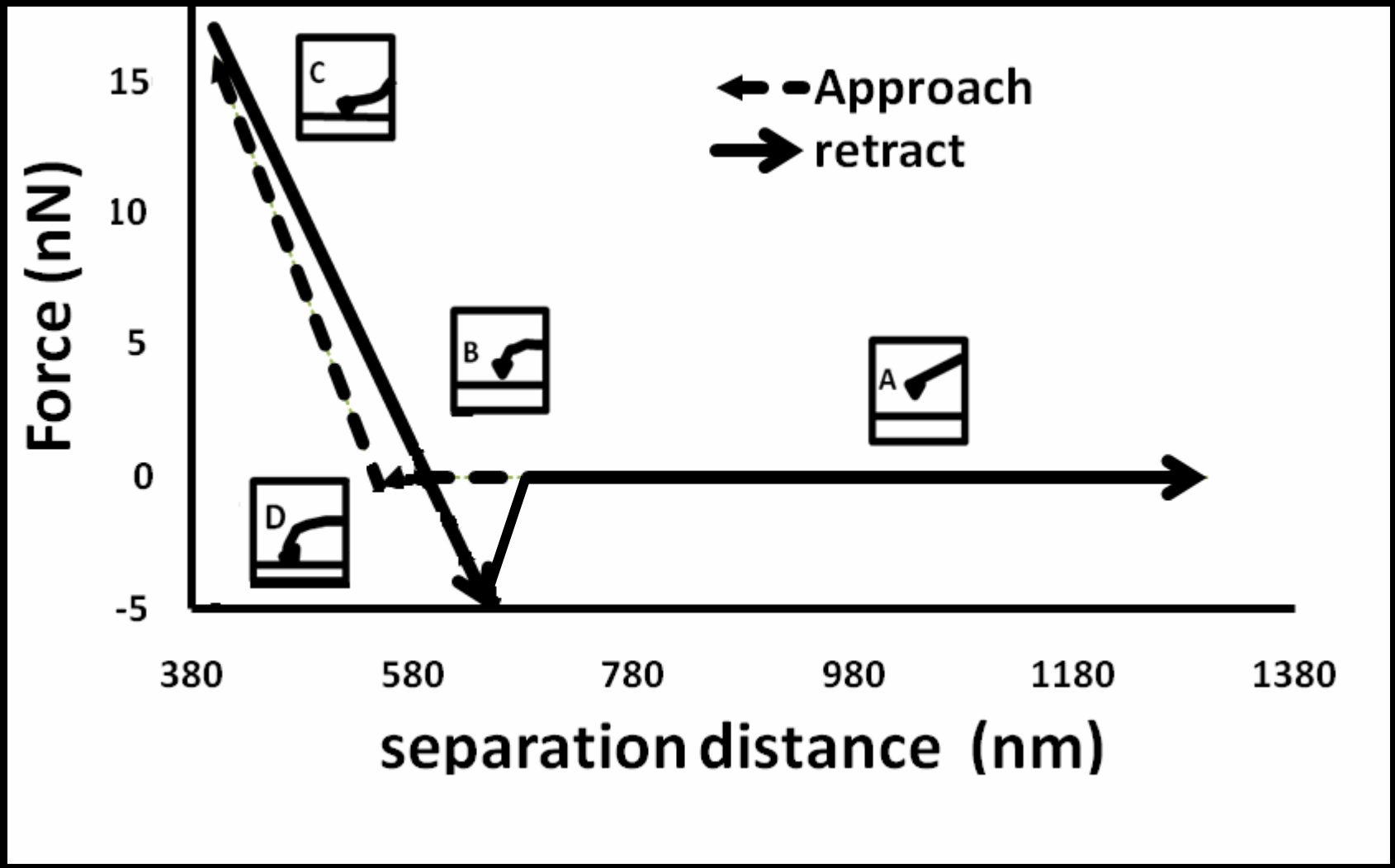
- Silicon nitride
  - Charge neutral over pH range from 6 to 8.5
  - Equal numbers of silanol (-SiOH) and silylamine (-$SiNH_2$ and possibly –$SiNH_3$) surface groups.

Tip radius = 20 nm
Tip spring constant = 0.3 N/m



Characterization of Biofilm Surface Properties

PURDUE UNIVERSITY



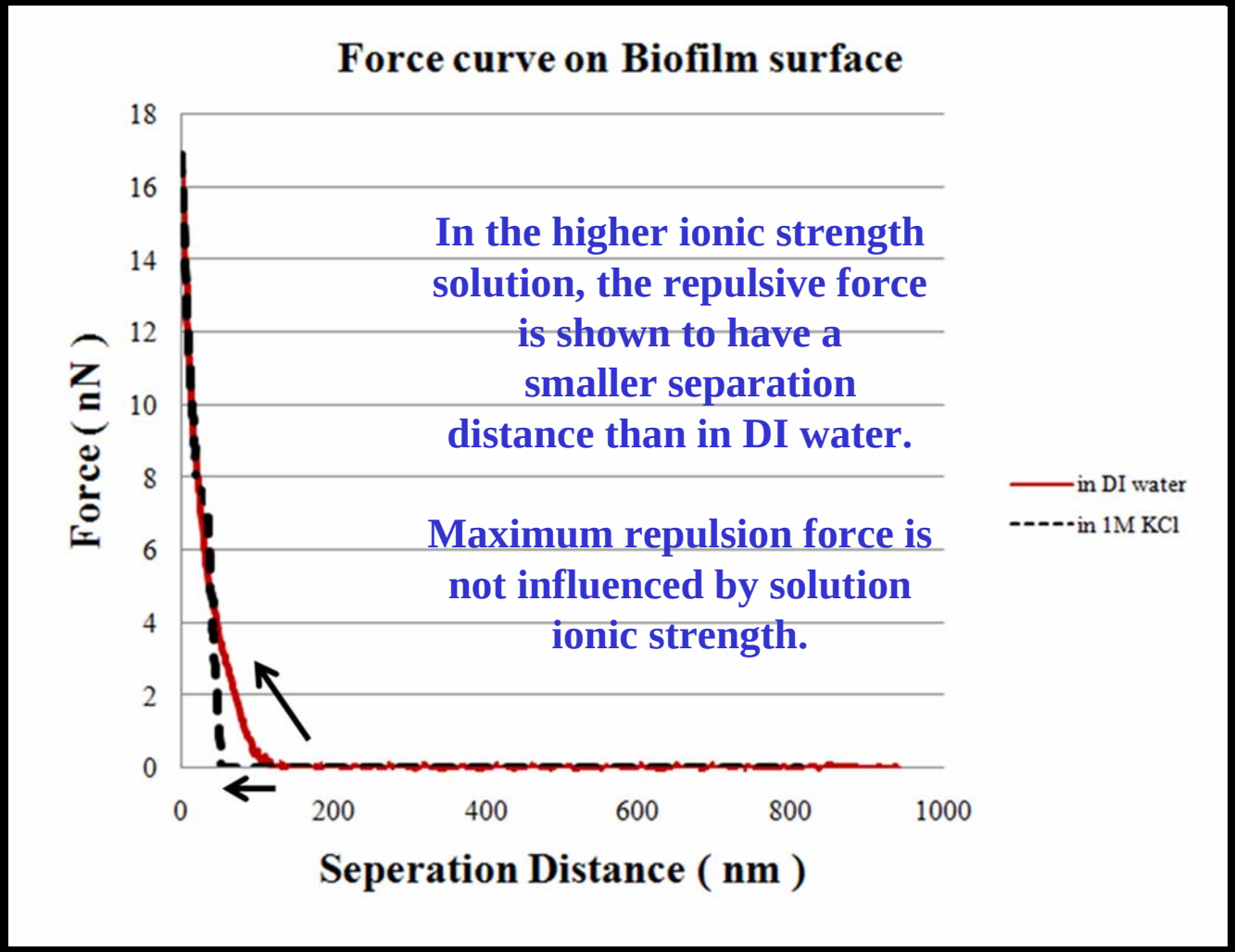
Typical force curve plot from AFM analysis

Characterization of Biofilm Surface Properties

PURDUE UNIVERSITY

- Slope of force curve is a function of:
  - Elastic modulus of sample and AFM tip
  - Tip geometry
- Type of interaction forces between AFM tip and biofilm surface:
  - Steric forces
  - Electrostatic interactions
  - Van der Waals forces

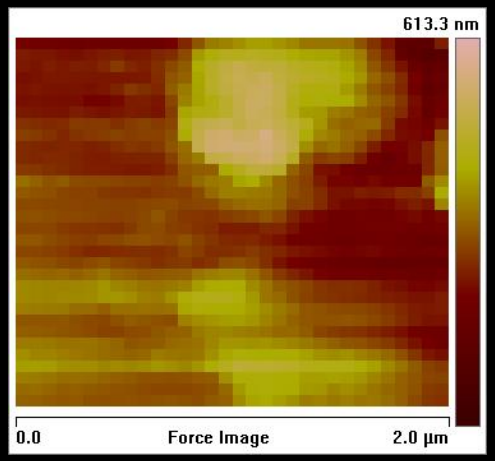
PURDUE UNIVERSITY



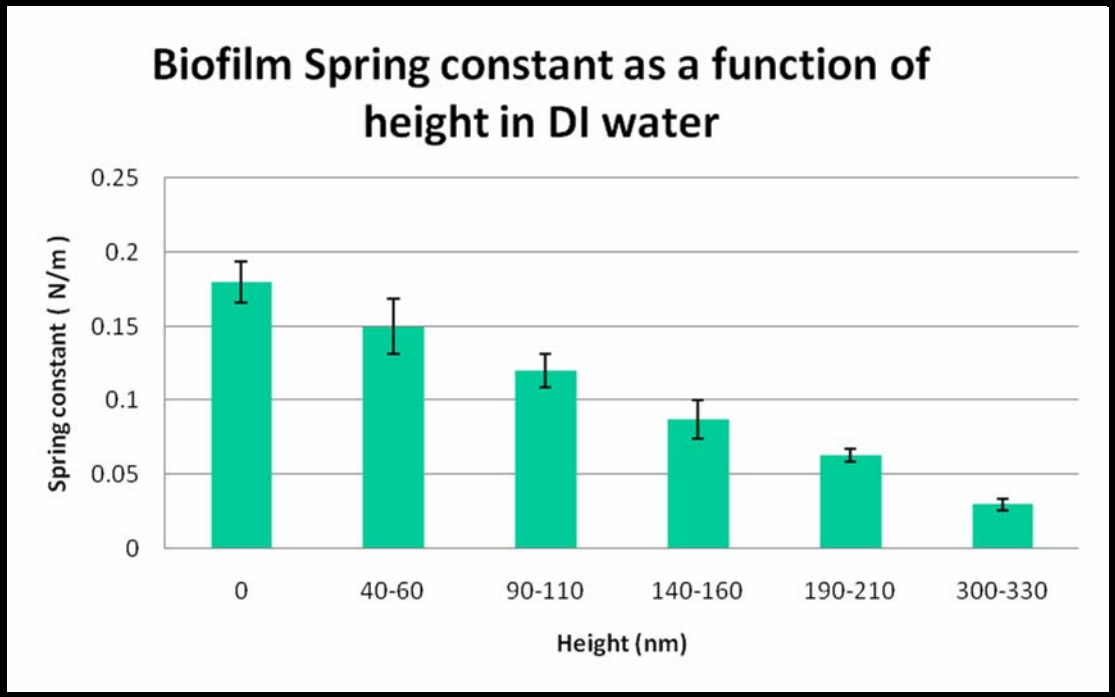
Same results were obtained in more than 10 measurements made in different regions of the sample with different tips.

PURDUE UNIVERSITY

Biofilm Spring Constant



Biofilm stiffness (rigidity) may be inversely related to biofilm thickness.



PURDUE UNIVERSITY

Conclusions

- Individual cell morphology can be distinguished within early stages of biofilm growth.
- No significant difference was noted when characterizing roughness at different scales.
  - Allows characterization of surface area in a shorter period of time
- Biofilm spring constant was shown to decrease with an increase in biofilm surface height (or biofilm thickness).

Ongoing Research

- Correlate biofilm surface friction coefficient and surface roughness
- Correlate biofilm density and spring constant obtained by the AFM.
- Treat silicon nitride cantilever with hydrophobic silane. Measure hydrophobic interactions between tip and biofilm surface