AFM

Quantification and special applications

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Engineering

Learning objectives

After this lecture, you will be able to

- Describe the advantages and limitations of simple quantification of morphological data obtained by AFM
- Understand the single-molecular analysis by AFM
- Explain the use of AFM in modern high-tech analytical applications, such as in monitoring enzyme movement on cellulose surface or chemical recognition of individual atoms in an organic molecule

Outline

(1) AFM - recap

(2) Quantification of morphology in AFM images:

- nanosized amorphous cellulose
- polymer blends containing cellulose

(3) Single-molecular analysis

(4) Special imaging setups

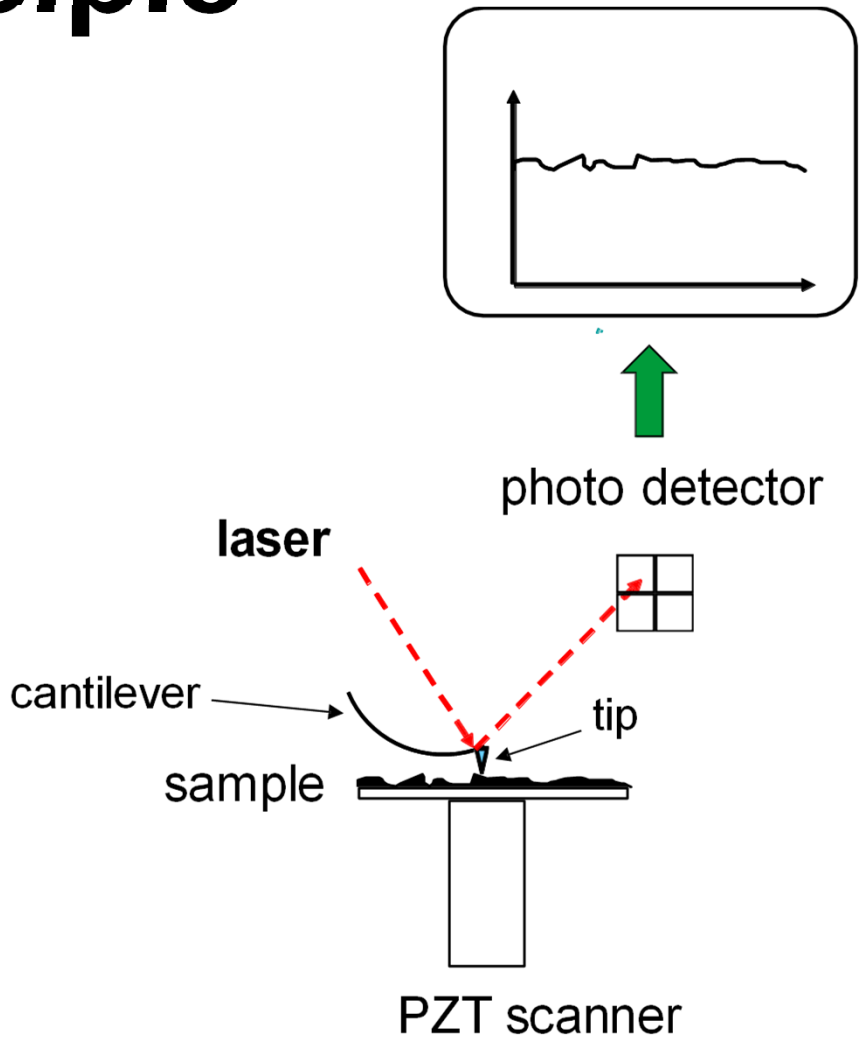
- Tip enhanced Raman spectroscopy
- AFM-IR

(5) Special applications of AFM

AFM recap: principle

(1) Surface forces between the tip and the sample deflect cantilever.

(2) Deflections are recorded by reflecting a laser from the tip of cantilever



AFM recap: height image

Each height adjustment after each amplitude reduction is monitored.
→ Result: **HEIGHT IMAGE**

- height image yields information on the topography of the sample

(a) Height image

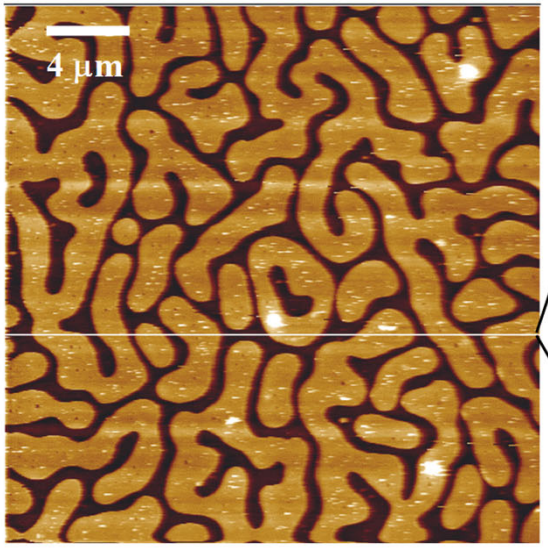
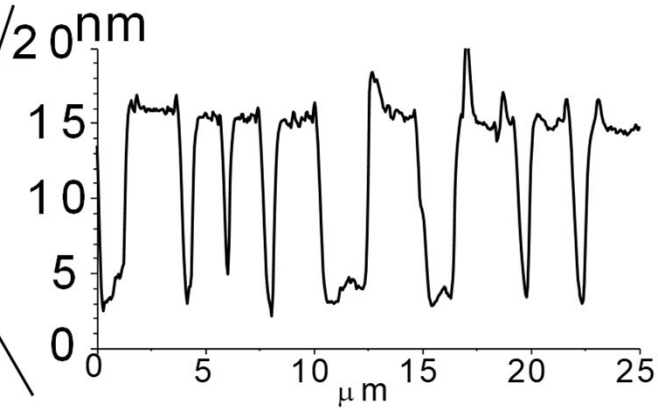


Image: cellulose islands

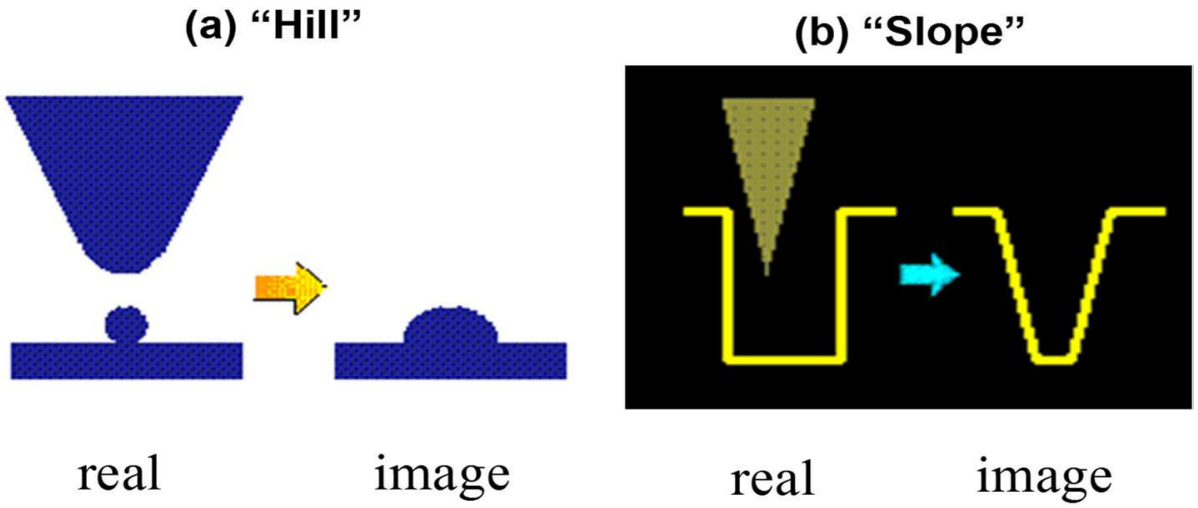
(b) Individual height scan



512 of individual scans make up the height image.

AFM recap: limitation of tip size

- Vertical resolution of AFM is outstanding: < 0.1 nm
- Lateral resolution is limited by the size of the tip



- The lateral exaggeration only concerns features whose size is close to the size of the tip
- Radius of an ordinary AFM tip: 5-10 nm

Quantification of morphology: Amorphous cellulose

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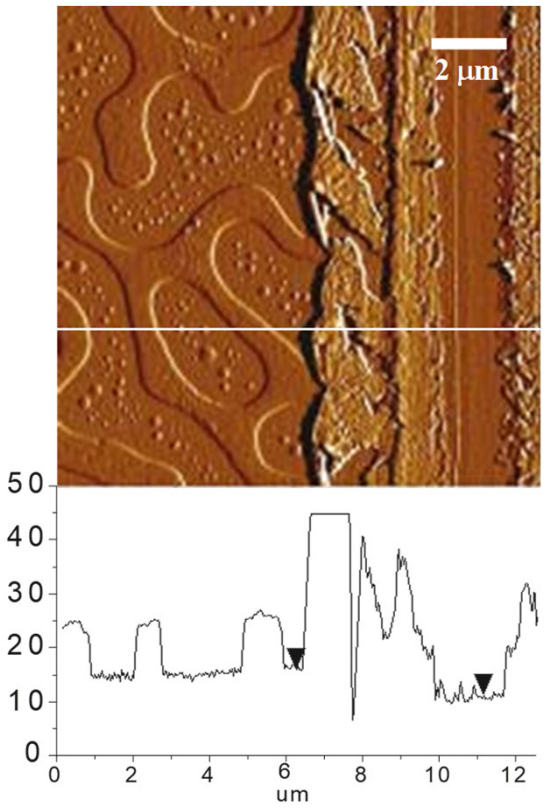
Measuring film thickness



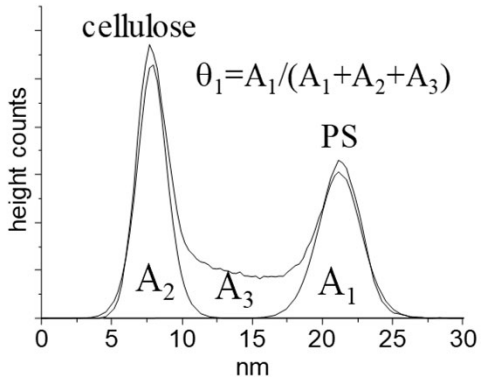
- 3D imaging allows for quantification of film thickness
- The film is scratched with an ordinary surgeon's knife (or something similar)
- Scratching may also reveal interesting qualitative information, e.g., a presence of a sublayer

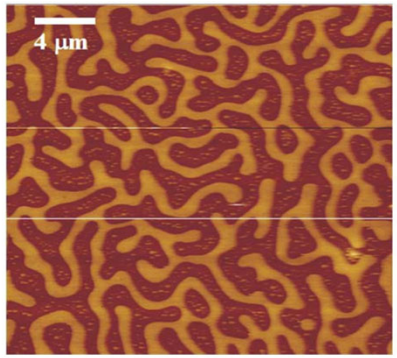
Requirements:

- The substrate has to be reasonably flat
- The substrate has to be stiff enough

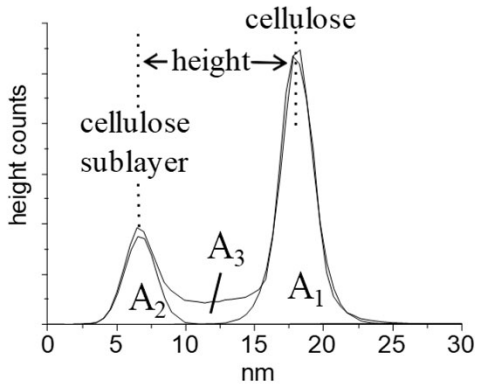
Height distribution histogram

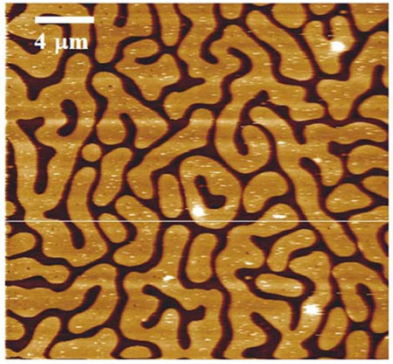
Cellulose/polystyrene





Only cellulose

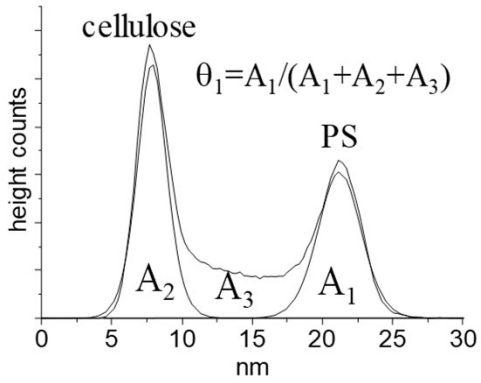


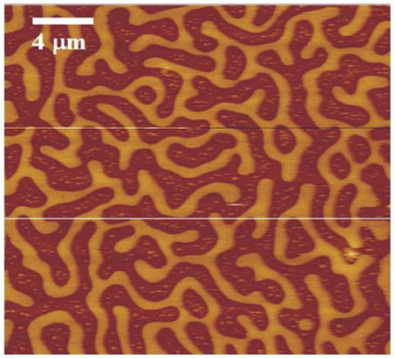


- In an AFM height image, each pixel has a height count
- When height counts are plotted as a function of their actual height values, a *height distribution histogram* results

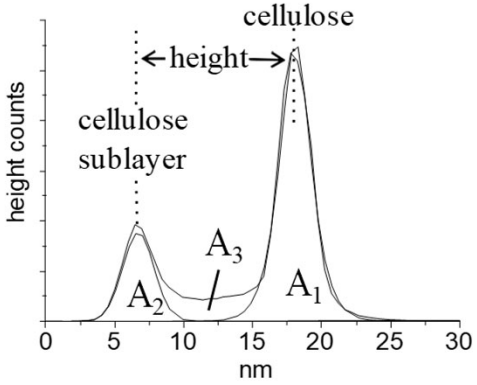
Height distribution histogram

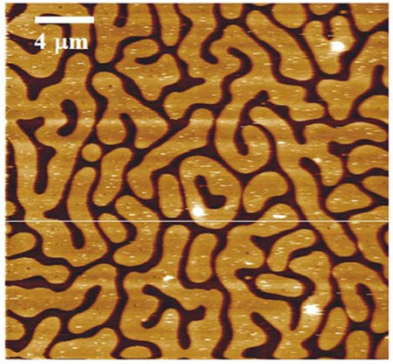
Cellulose/polystyrene





Only cellulose





Height distribution histograms can be used for crude quantification:

- Average height of features
- Coverage (θ) of features
- Volume of the features
- Works well when the lateral size of the features far exceed the tip size

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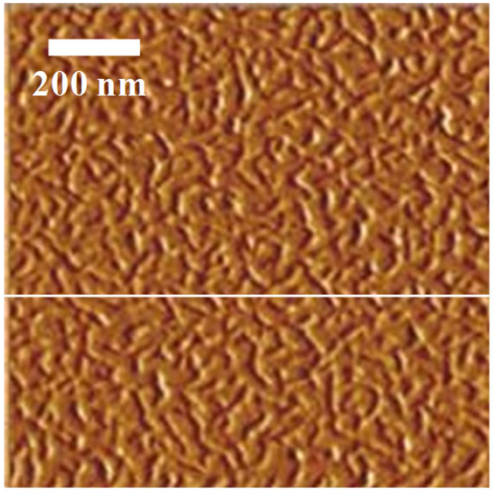
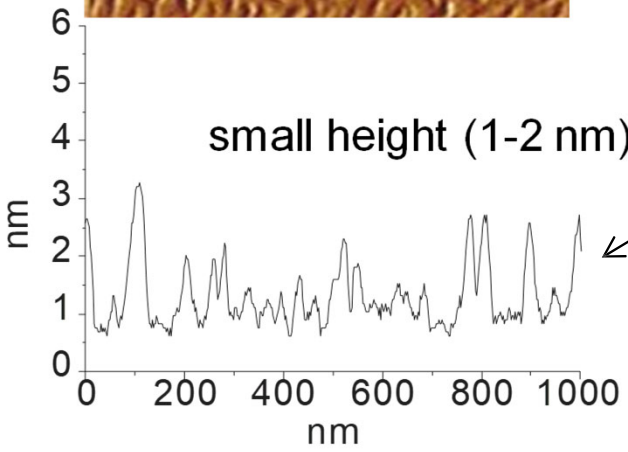
Quantification – small height



Nanosized patches of amorphous cellulose on silica

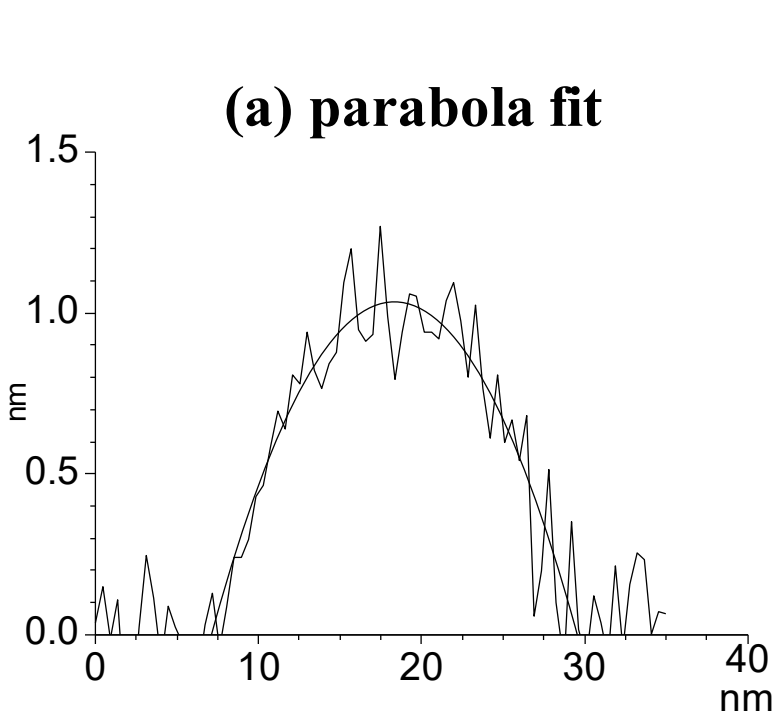
In special cases, histogram analysis works also for very small features, provided that their height is much smaller than the width.



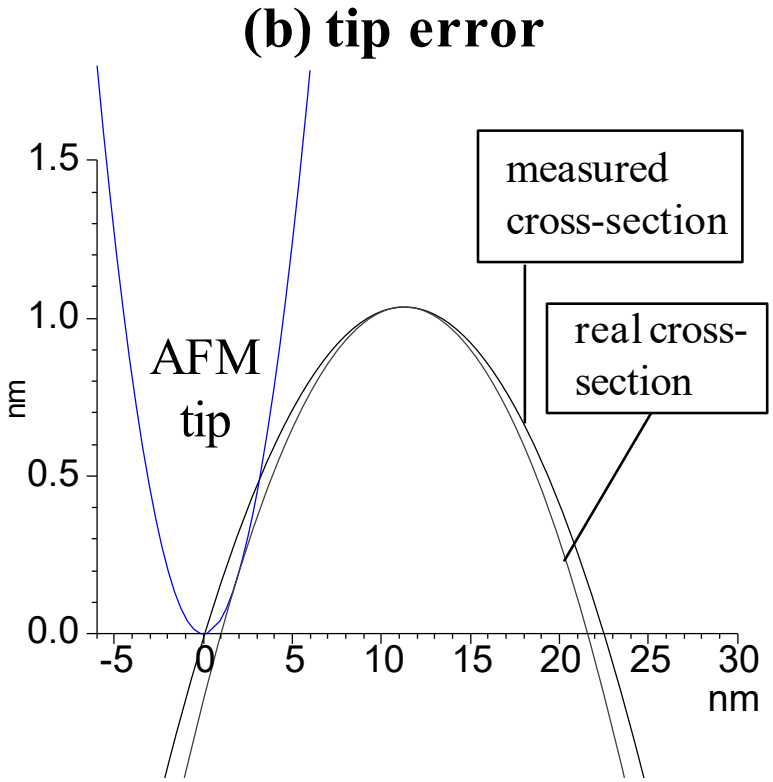
Height profile taken from the location indicated by a white line in the image

Large width (20-30 nm) compared with height (1-2 nm)

Quantification – small height



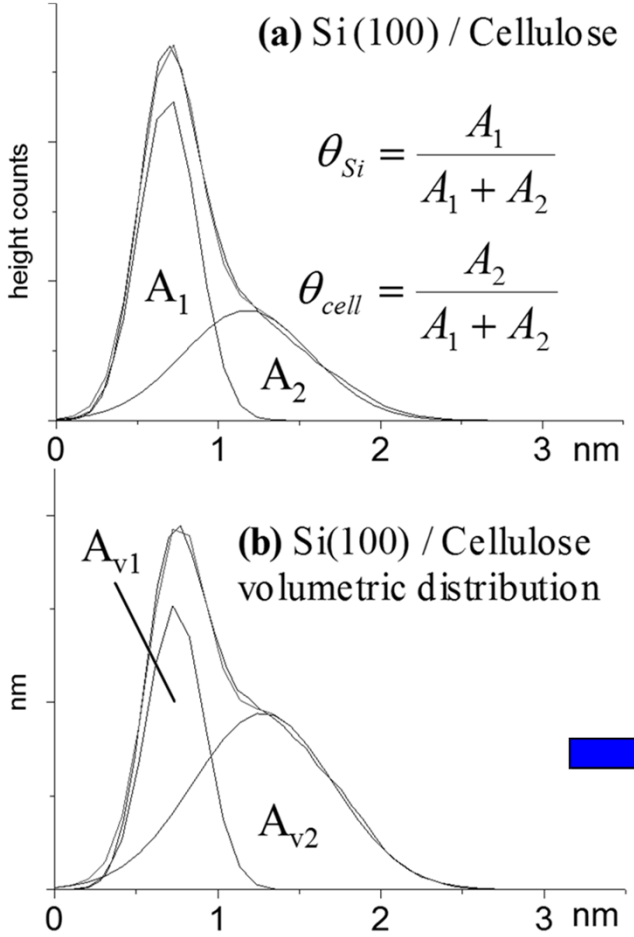
cross section of a cellulose patch



max. radius of curvature for the tip: 10 nm

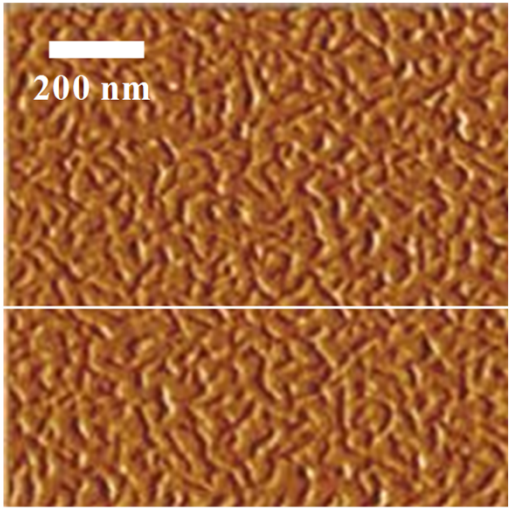
→ error in volume determination ±2%

Quantification: cellulose patches



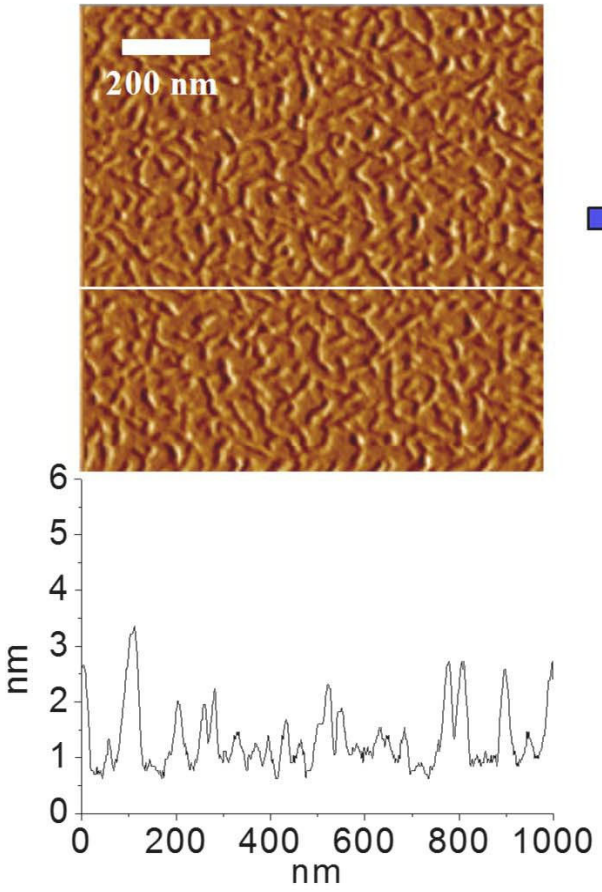
$$\theta_{Si} = \frac{A_1}{A_1 + A_2}$$

$$\theta_{cell} = \frac{A_2}{A_1 + A_2}$$



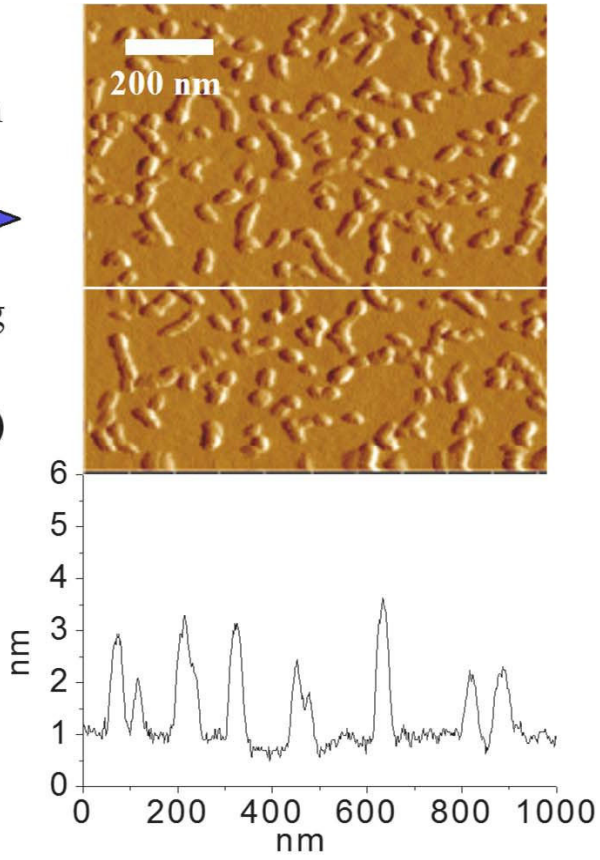
Integration over the volumetric distribution yields volume of cellulose.

Quantification: swelling by water



immersion
15 min

fast drying
80°C
(ca. 3 min)



V=2.8x10^5 nm^3

V=4.4x10^5 nm^3

Nanosized amorphous cellulose is swollen in water and the swelling is quantified by volumetric histogram analysis.

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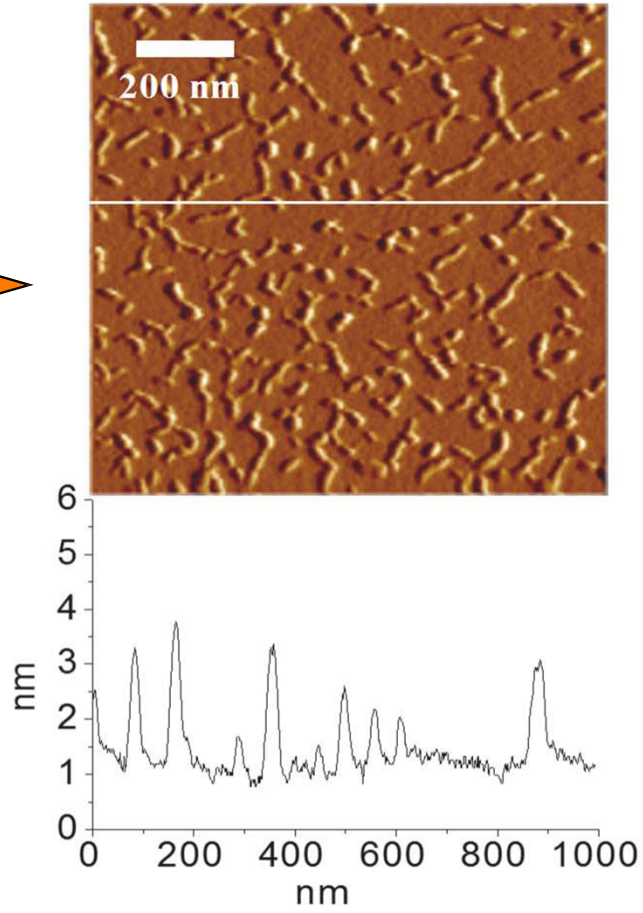
Polymer **2005**, *46*, 3307.

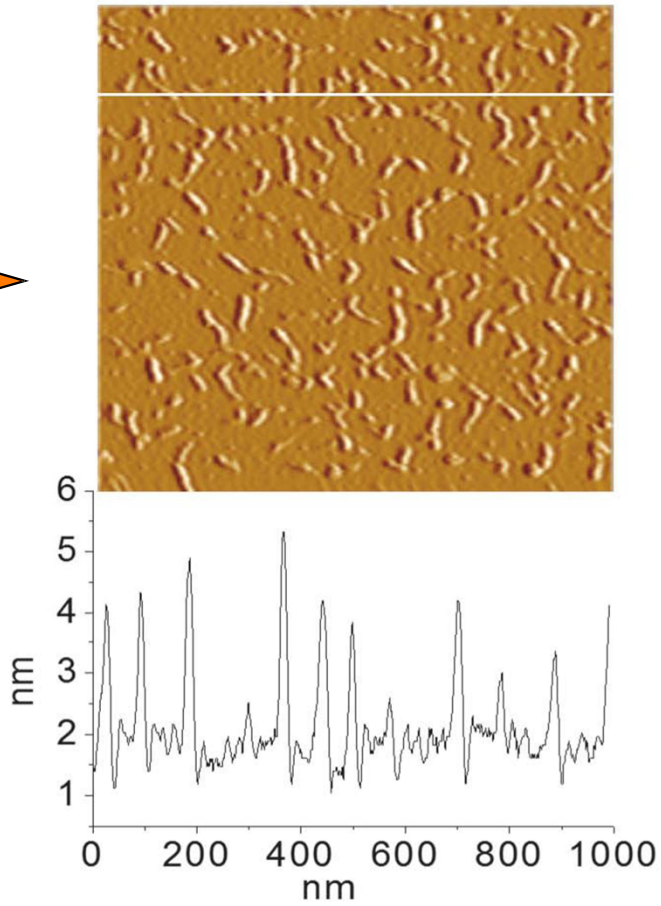
Quantification: shrinking

heating
110°C
+15 min



V=4.0x10^5 nm^3

heating
110°C
+45 min



V=3.6x10^5 nm^3

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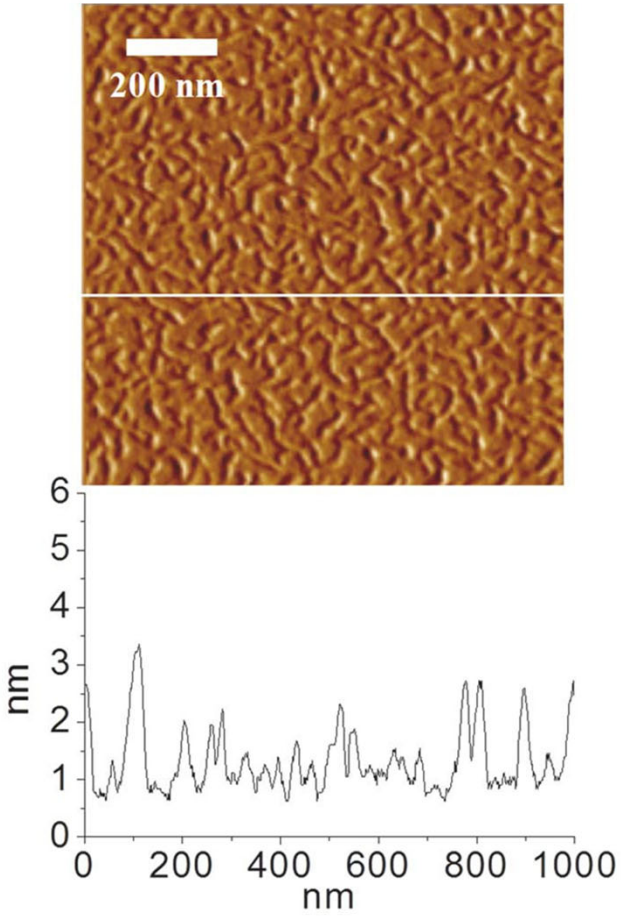
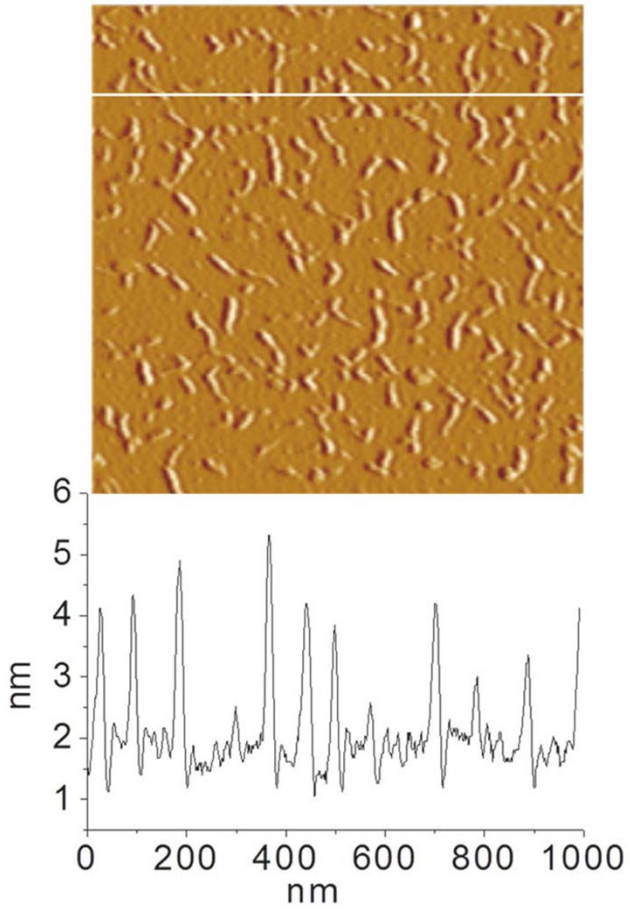
Polymer **2005**, *46*, 3307.

Wetting and drying: final vs. initial

Initial, ambient state



Final state: wetted and dried



Morphology of nanosized amorphous cellulose is not restored upon wetting and subsequent drying.

Quantification of morphology: Polymer blends

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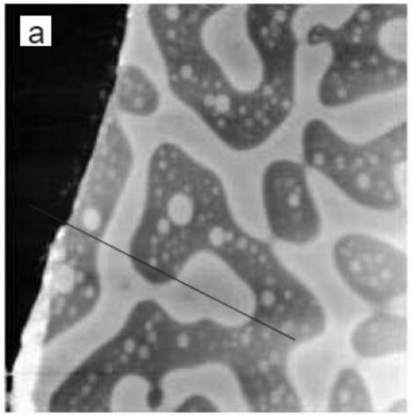
Polymer blends and interfaces

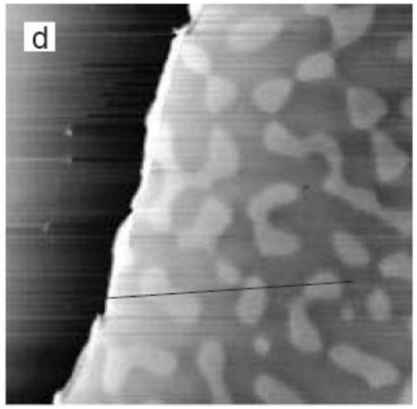
- Ultrathin (<100nm) films of immiscible polymer blends possess highly distinct morphologies

Polystyrene (PS) / poly(methyl methacrylate) (PMMA) blend films spin coated from:

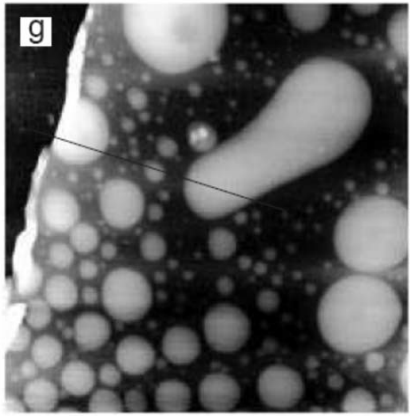
Toluene



Tetrahydrofuran



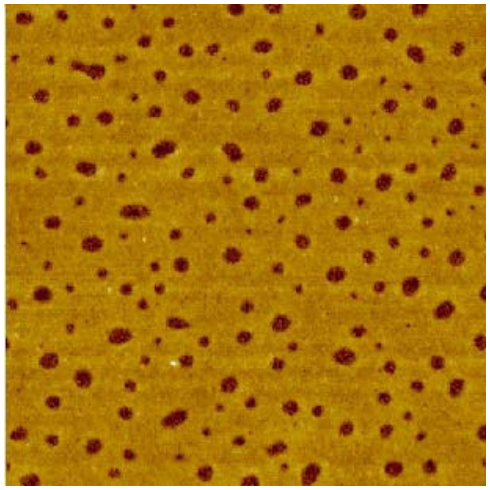
Methylethylketone

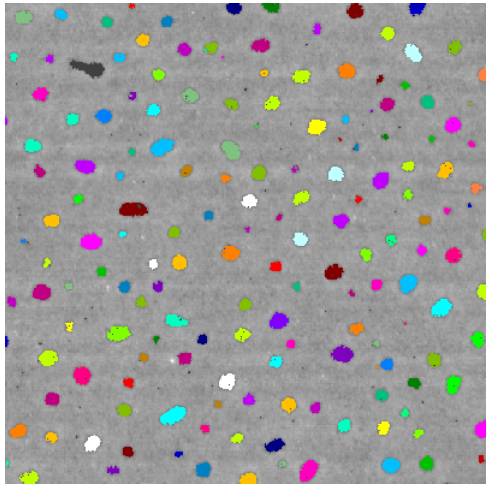


Walheim *et al. Macromolecules* **1997**, *30,* 4995.

Quantification: image analysis

AFM image often consists of features which are higher (grains) or lower (pores) in height than the general “background” of the image.

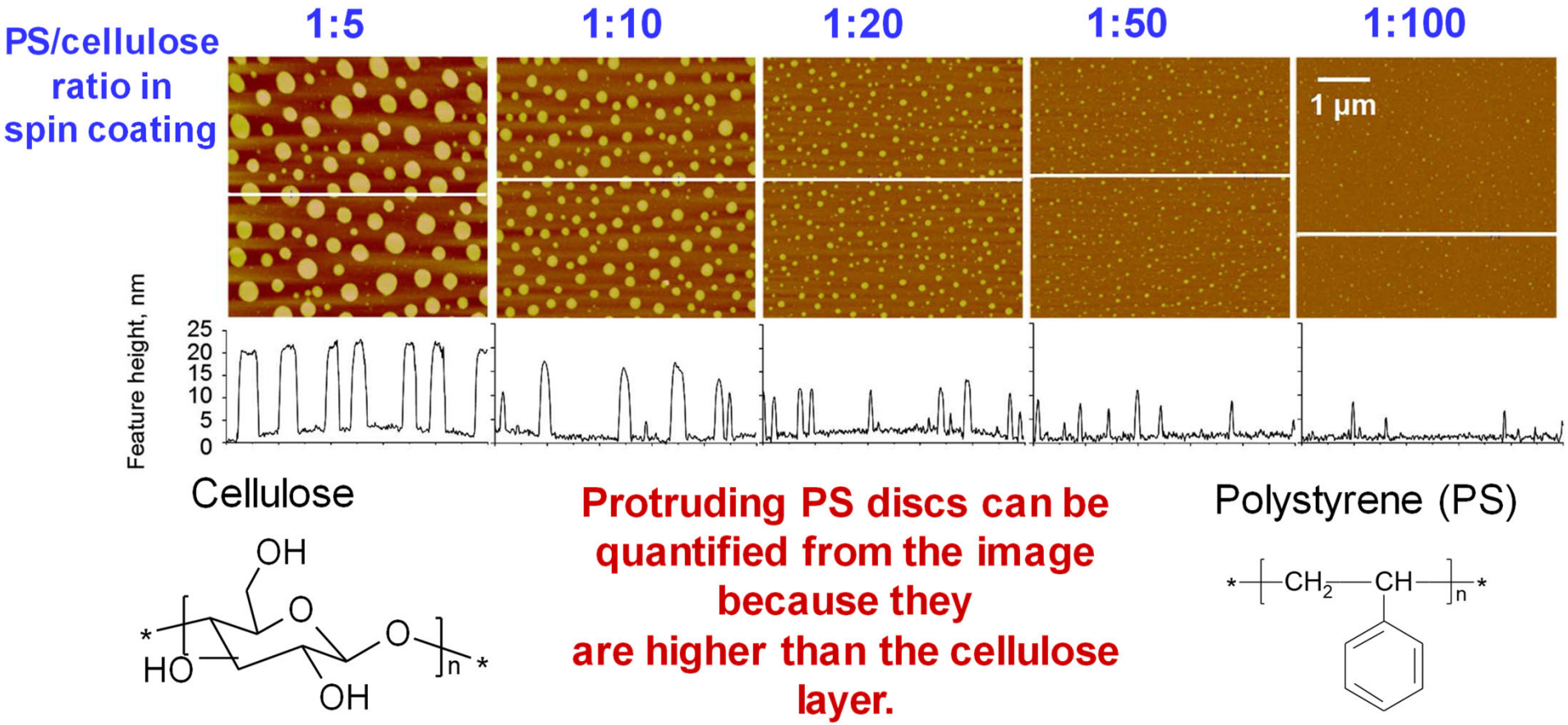




- Commercial image analysis software packages can recognize grains and pores and quantify their number, width, height/depth etc.

Quantification of morphology

Films consist of polystyrene (PS) discs and a cellulose layer under the discs.



Cellulose

Protruding PS discs can be quantified from the image because they are higher than the cellulose layer.

Polystyrene (PS)

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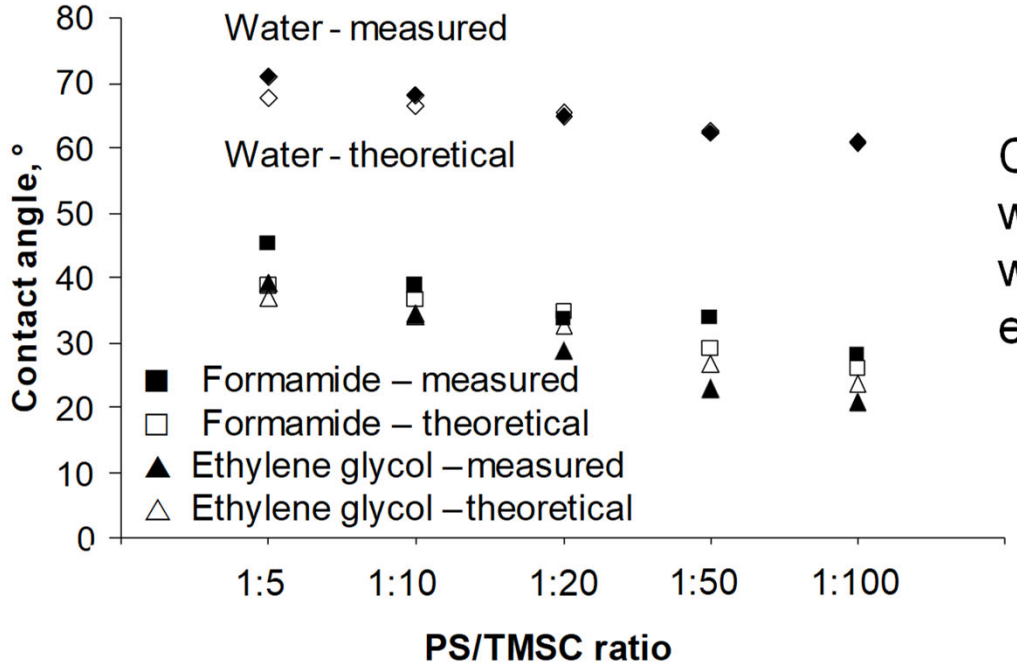
Biomacromolecules **2009**, *10*, 1276.

Utilizing quantitative data



Contact angles measured with three different solvents: water, formamide, and ethylene glycol

Coverage deduced from AFM image analysis

Cassie-Baxter equation (theoretical)

$$\cos\theta_c = \phi_A \cos\theta_A + \phi_B \cos\theta_B$$

θ_c is the contact angle of the blend,
φ_A and φ_B are the coverage of the components A and B and
θ_A and θ_B are the contact angles of the pure components

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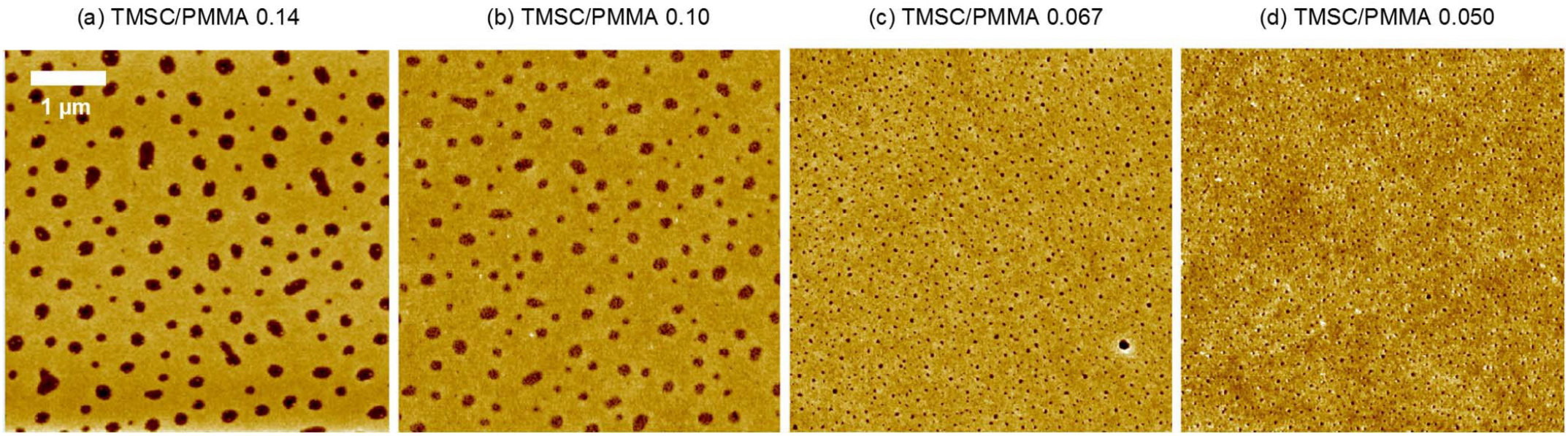
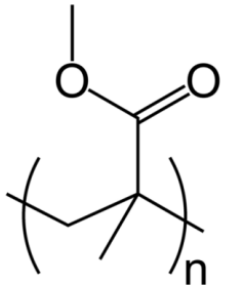
Biomacromolecules **2009**, *10*, 1276.

Pore analysis from polymer blends



Films consist of PMMA layer with cavities (pores) which have cellulose embedded on the bottom.

PMMA



→ Cavities can be quantified because they are on a lower level than the PMMA layer.

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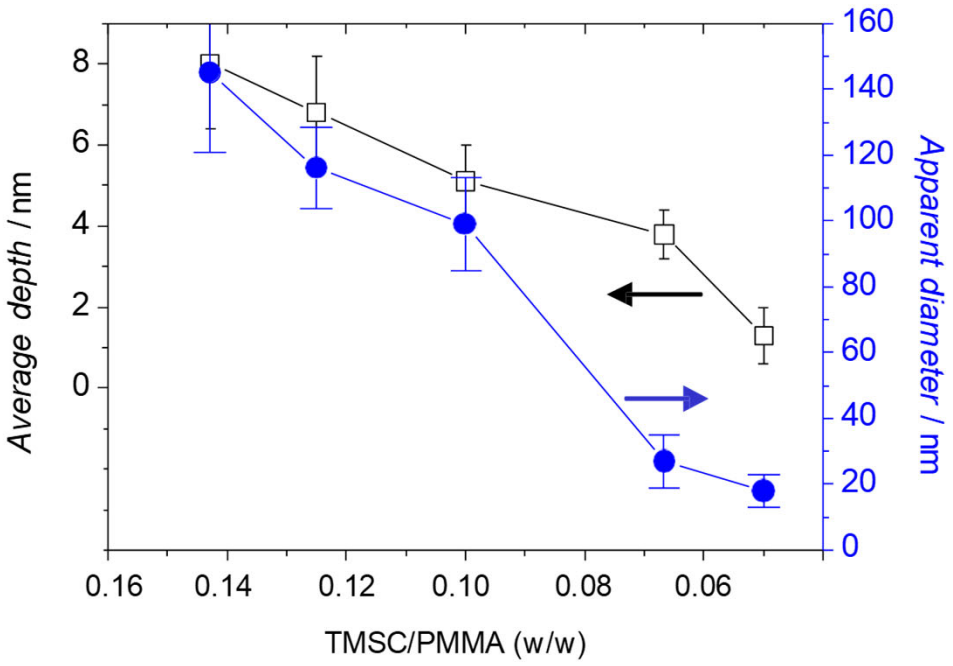
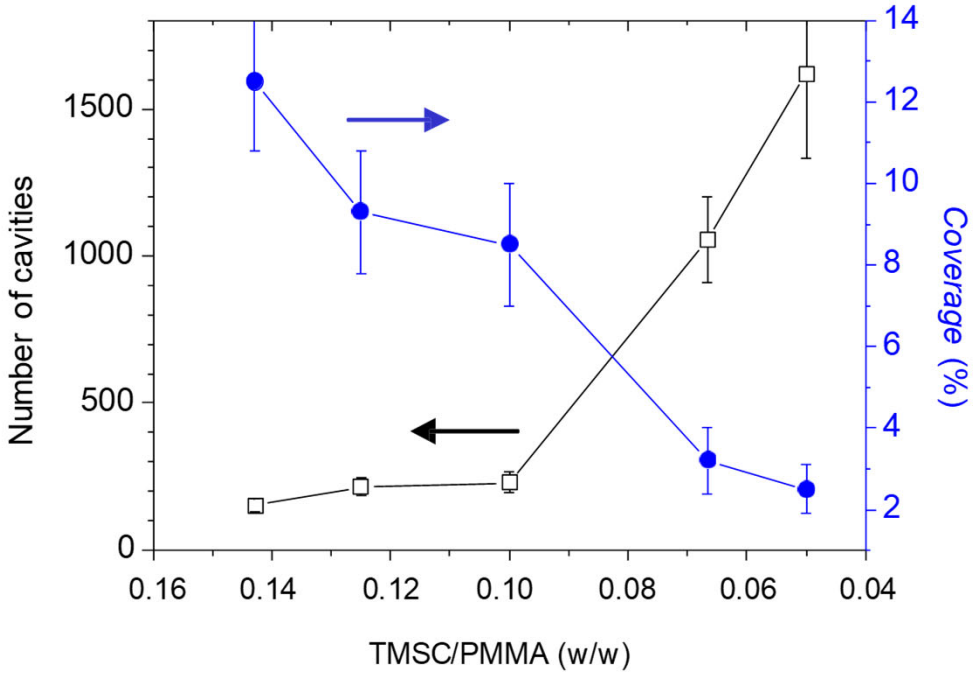
Soft Matter **2009**, *5*, 1786.

Pore analysis from polymer blends





Quantified qualities from the pores: - depth
- diameter
- number
- coverage

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Soft Matter **2009**, *5*, 1786.

AFM on individual polymers

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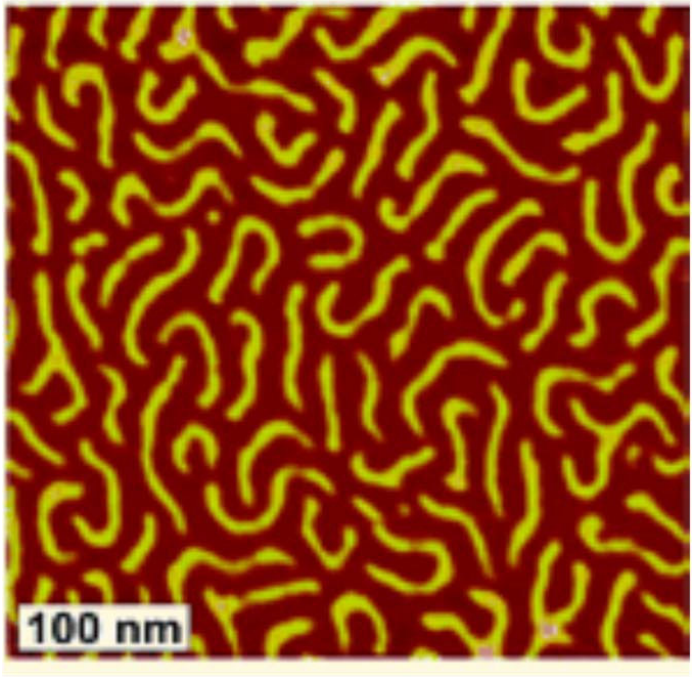
Molecular weight determination

- Very smooth substrates enable distinction of individual polymers with open films



Notice the vast tip exaggeration of lateral dimensions (width of polymers).

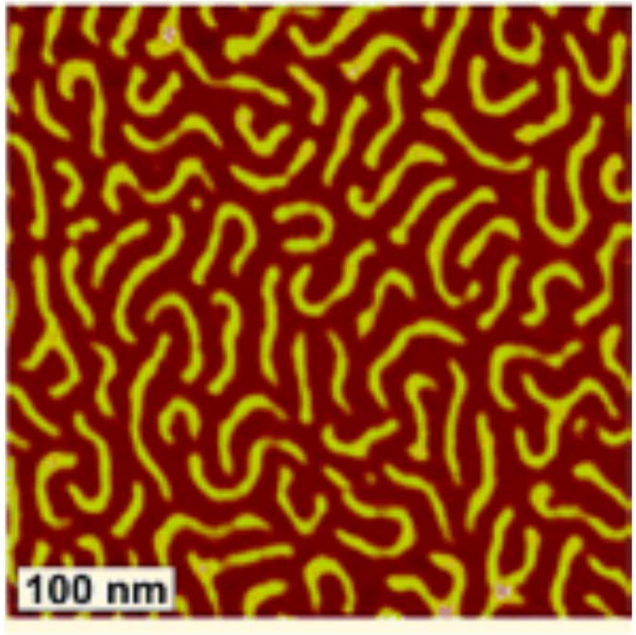
The length, however, is hardly affected by the tip exaggeration.

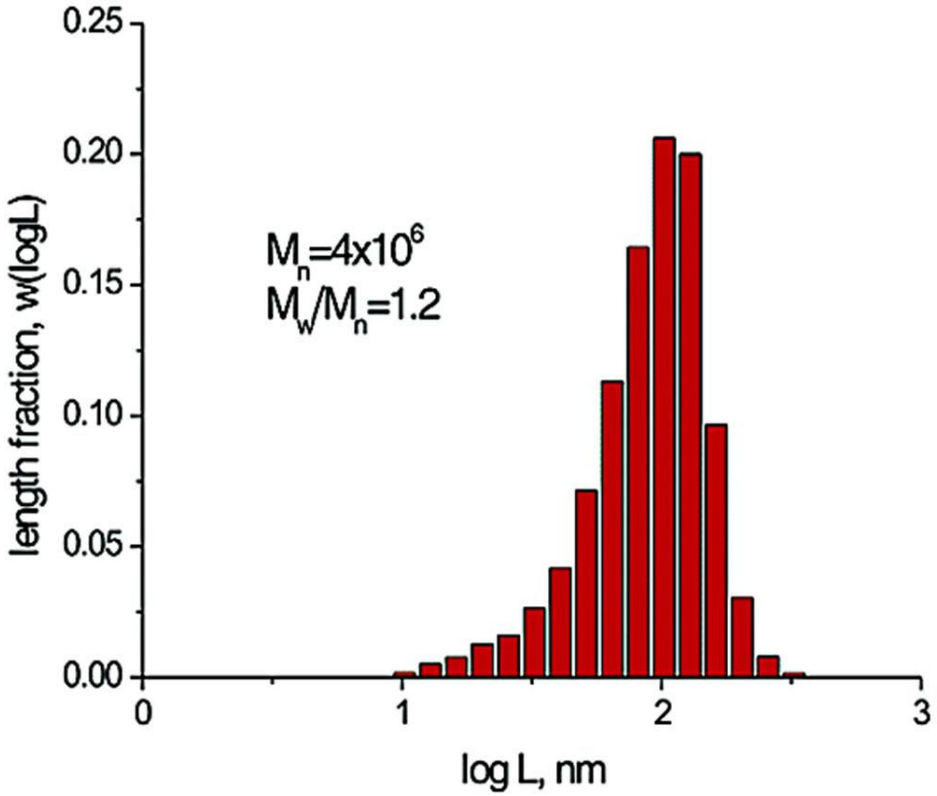
Open film of single poly(butylacrylate) molecules on mica

J. Am. Chem. Soc. **2003**, *125*, 6725.

Molecular weight determination

- Very smooth substrates enable distinction of individual polymers with open films





When the length of the repeating unit in a polymer is known: AFM can be used to determine the molecular weight distribution.

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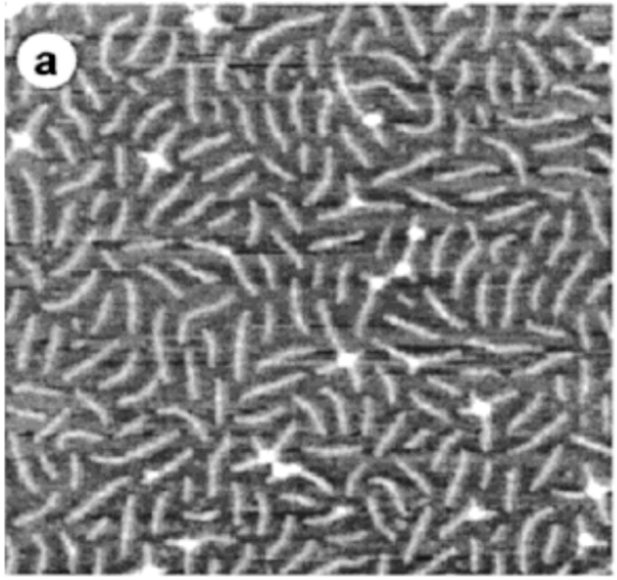
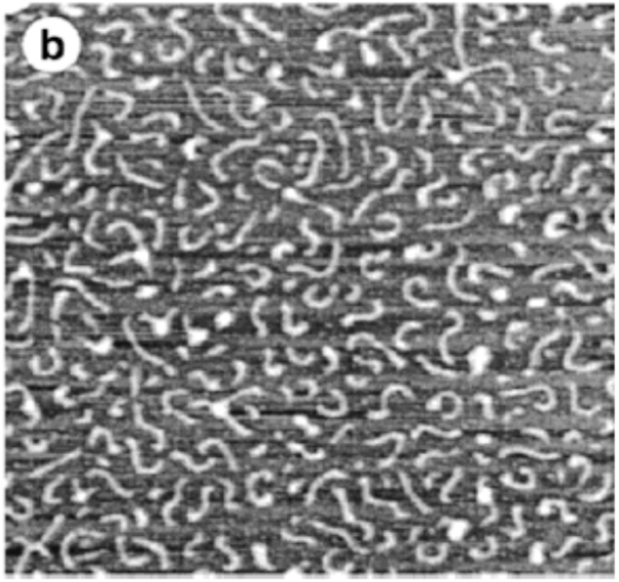
J. Am. Chem. Soc. **2003**, *125*, 6725.

Conformational changes upon annealing



poly(*n*-butylacrylate)
brush molecules



poly(*n*-butylacrylate)
brush molecules,
annealed at 120^{O}C for 48h

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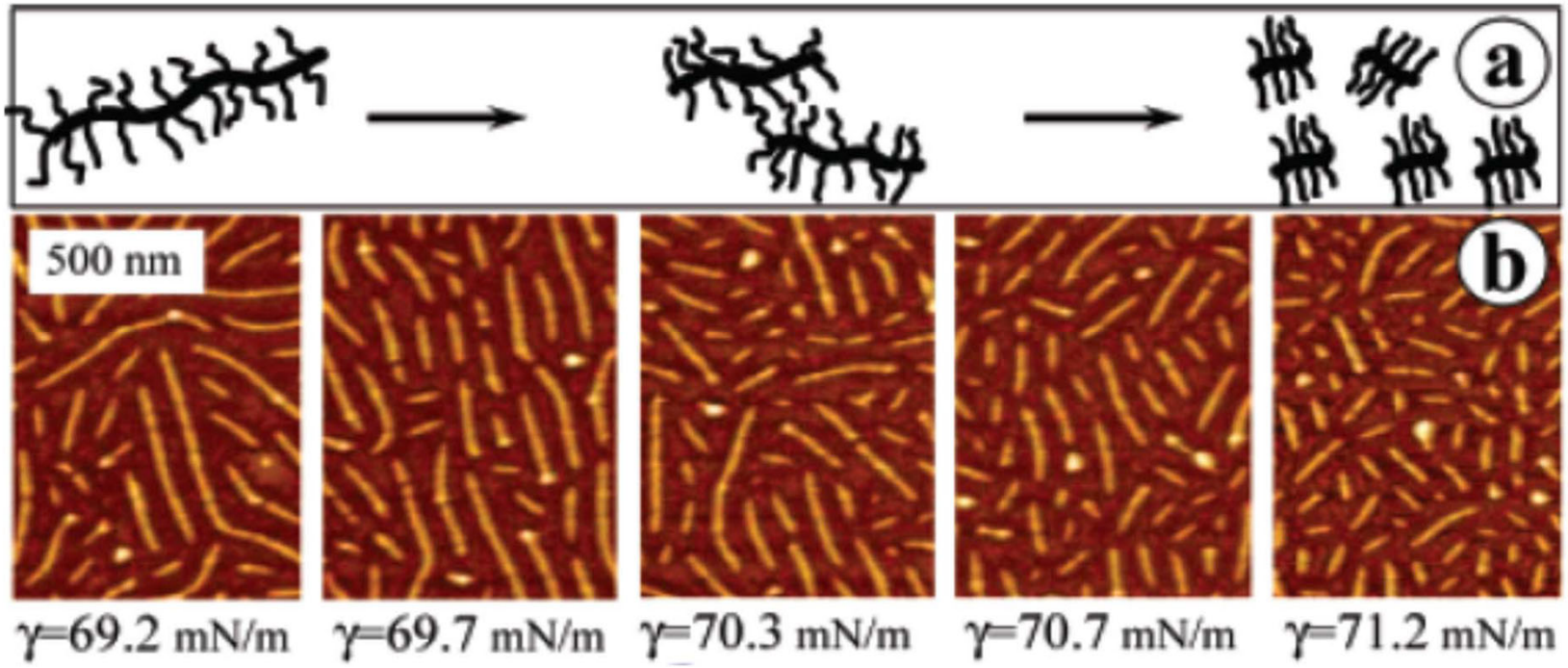
Chem. Rev. **2001**, *101*, 4099.

Fatal adsorption



- LB deposition on mica (adsorption occurs at air/liquid interface)
- Subphase in LB deposition: water-isopropanol mixtures with different surface energy
- Adsorption on water/liquid interface causes strain and C-C bond scission

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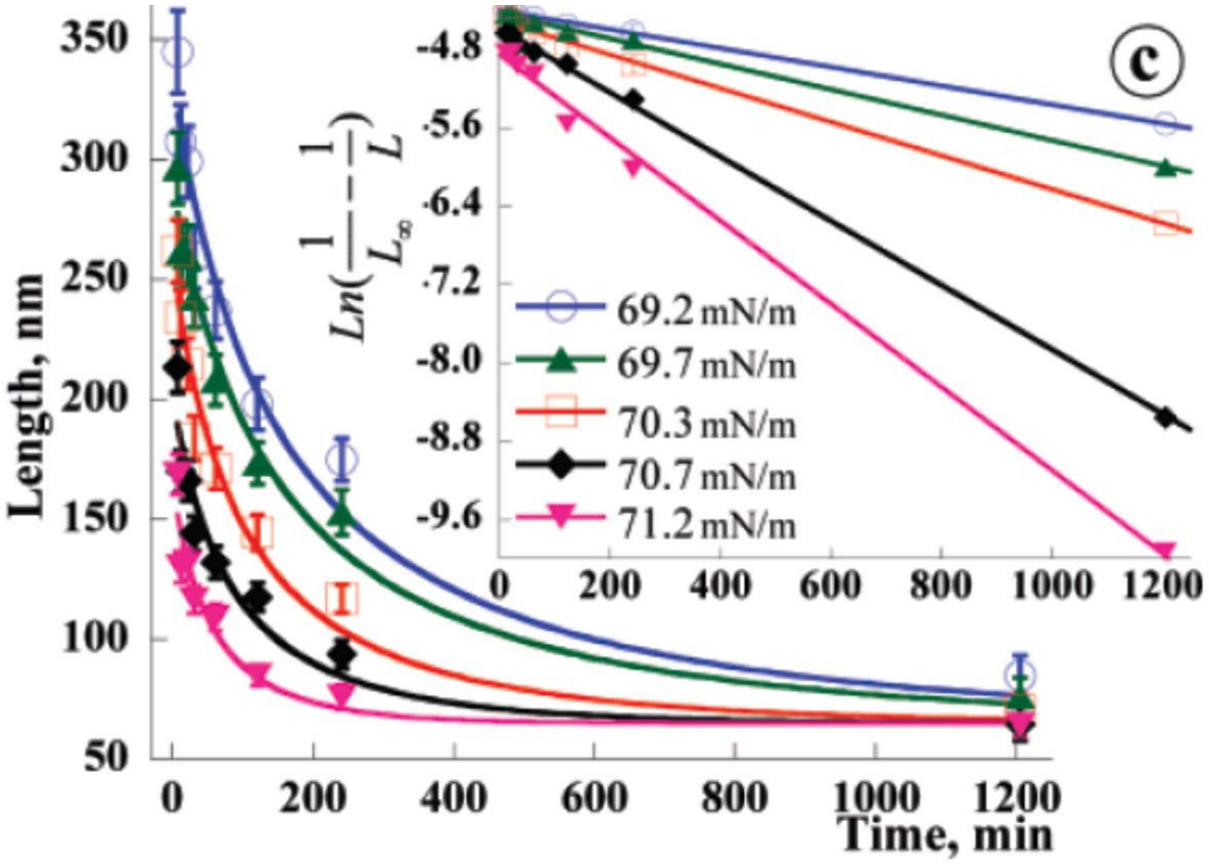
J. Am. Chem. Soc. **2008**, *130*, 4228.

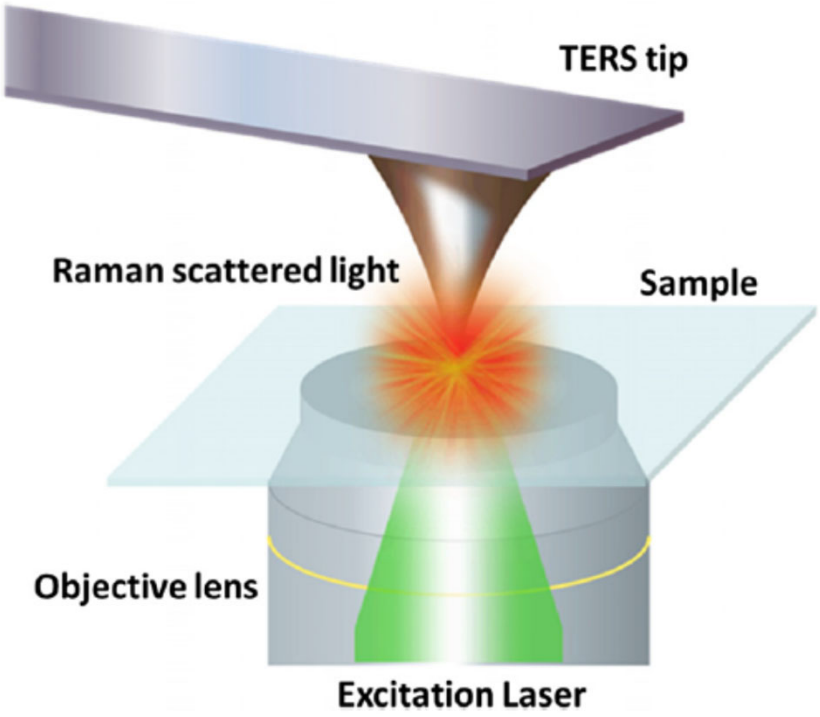
Fatal adsorption



- LB deposition on mica (adsorption occurs at air/liquid interface)
- Time of adsorption makes a significant contribution to C-C scission

Special imaging setups

Tip enhanced Raman Spectroscopy (TERS)



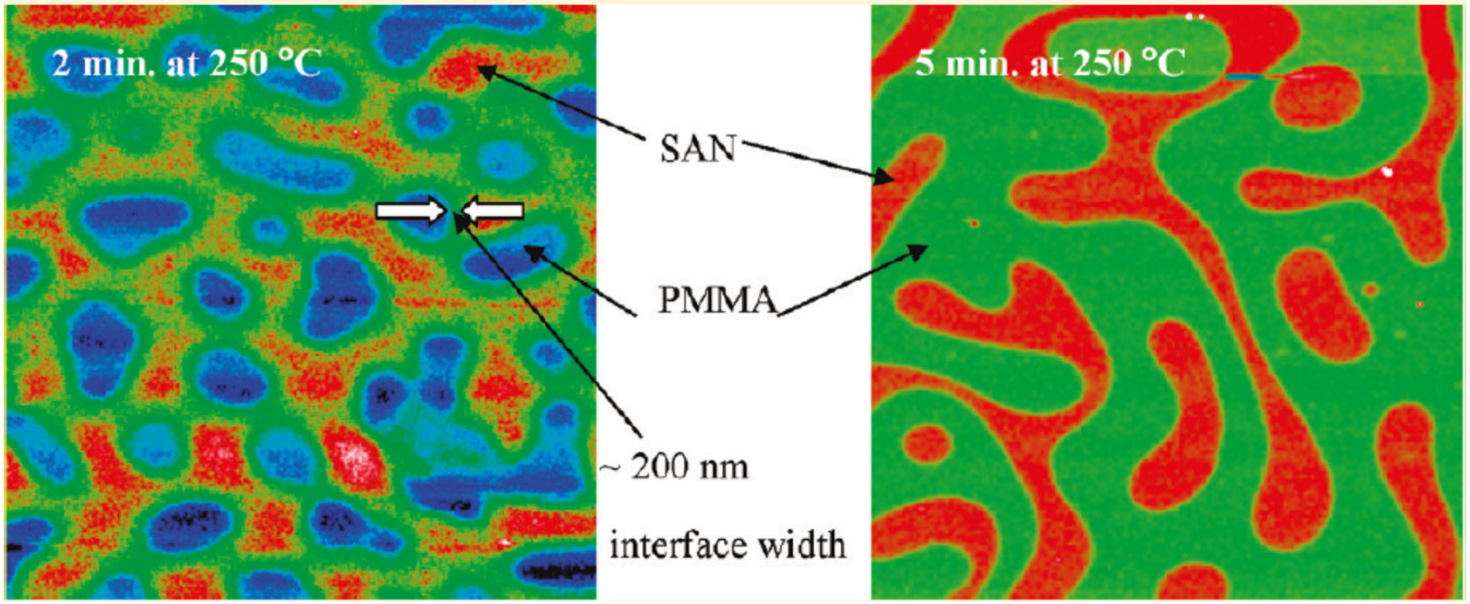
- An AFM tip is positioned at the centre of focus of an excitation laser
- Electromagnetic field is confined and enhanced at the tip apex

→ Raman signal from the vicinity of the tip-apex is enhanced

→ Diffraction limit is overcome

→ Nanoscale chemical imaging is enabled

TERS in characterization of polymer blends



- Spin coated films of poly(methyl methacrylate) (PMMA) and poly(styrene-*co*-acrylonitrile)
- Annealing reveals energy relaxation according to glass transition, as revealed by TERS

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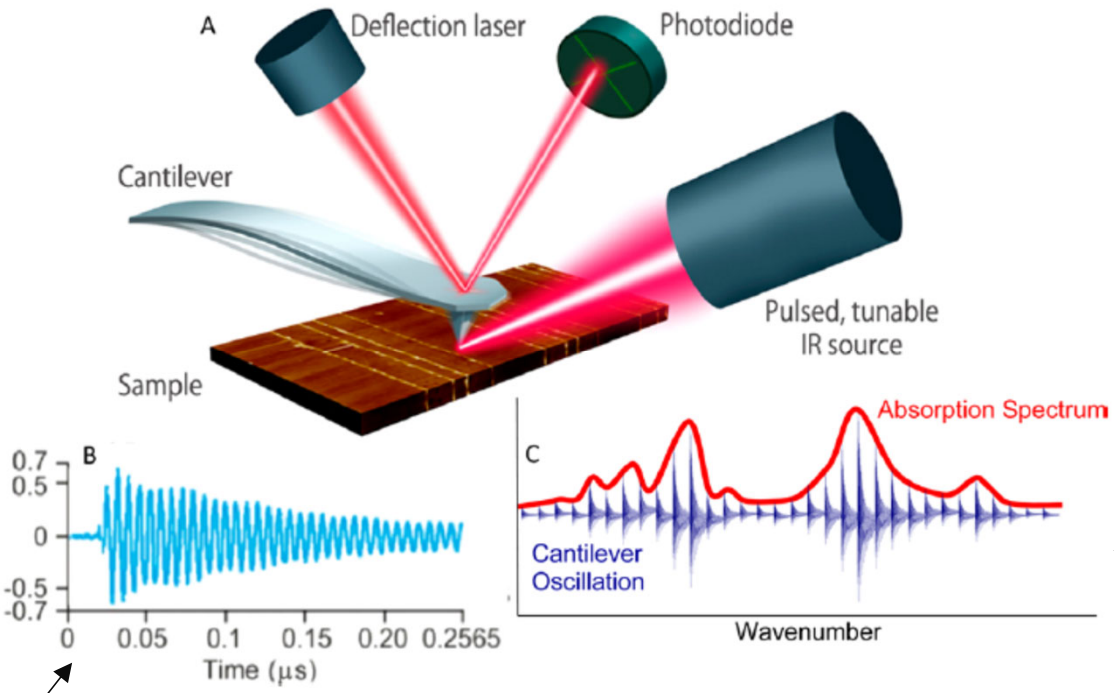
Macromolecules **2011**, *44*, 2852.

AFM-IR



- Pulsed tunable laser is focused near the AFM tip
- Absorbed light results in photothermal expansion of absorbing regions of the sample
- AFM tip is used as a local detector for IR absorption

Photothermal expansion induces a transient cantilever oscillation, proportional to IR absorption

Measuring oscillation amplitude as a function of IR wavenumber results in a local absorption spectrum with nanoscale spatial resolution.

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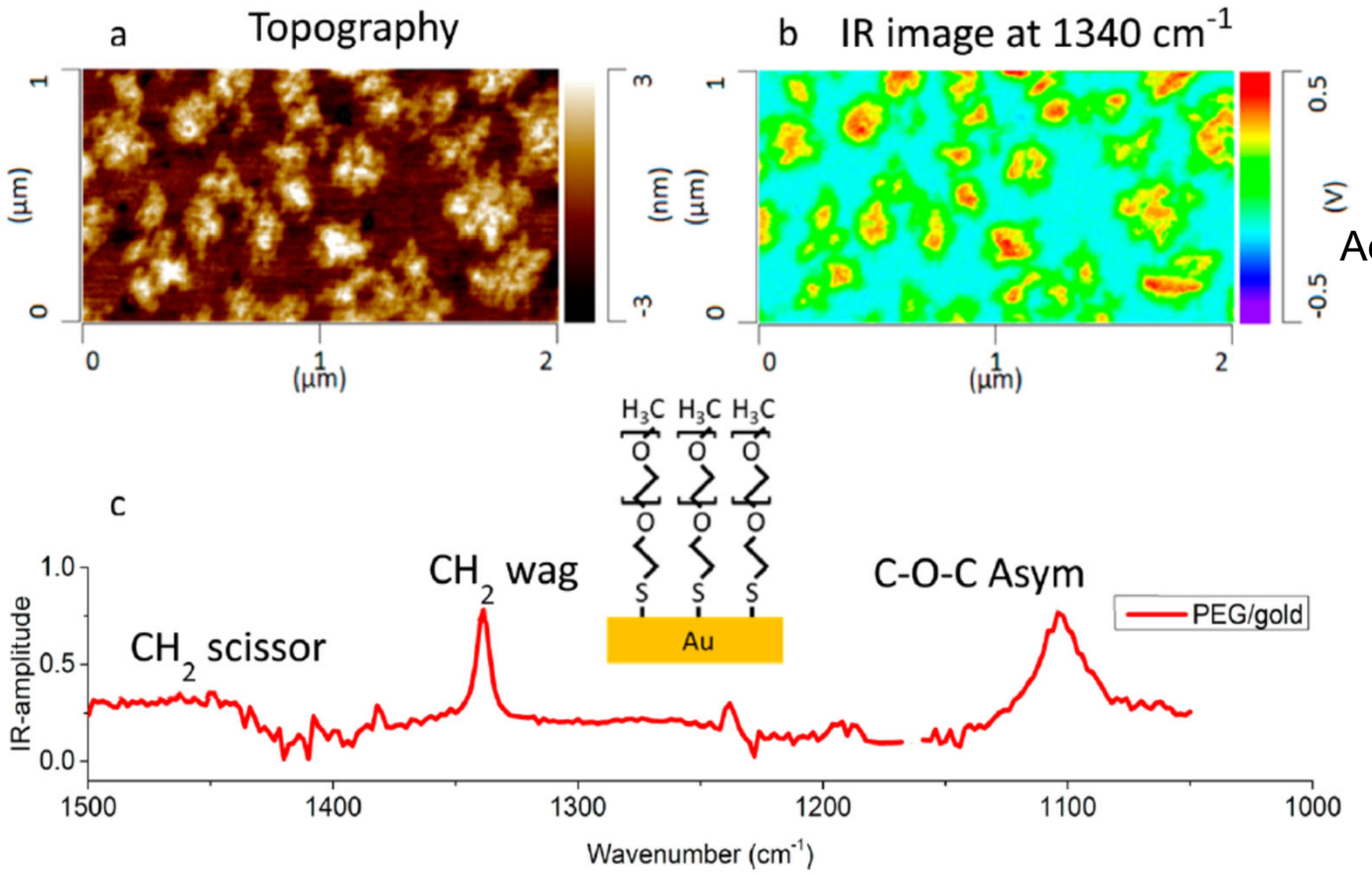
Chem. Rev. **2017**, *117*, 5146.

PEG monolayer imaged by AFM-IR



According to CH_2 wag

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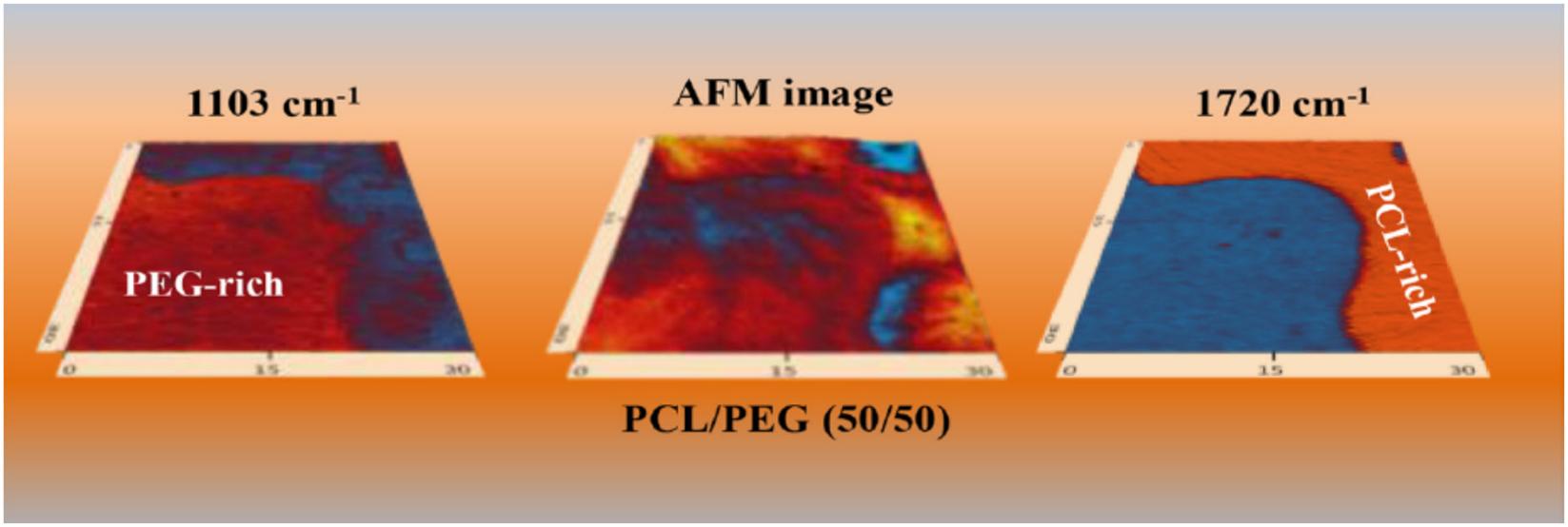
Chem. Rev. **2017**, *117*, 5146.

Polymer blends in AFM-IR



- Drop casted poly(ethylene glycol) (PEG) and poly(caprolactone) (PCL) blends
- IR functionality is able to distinguish the different phases

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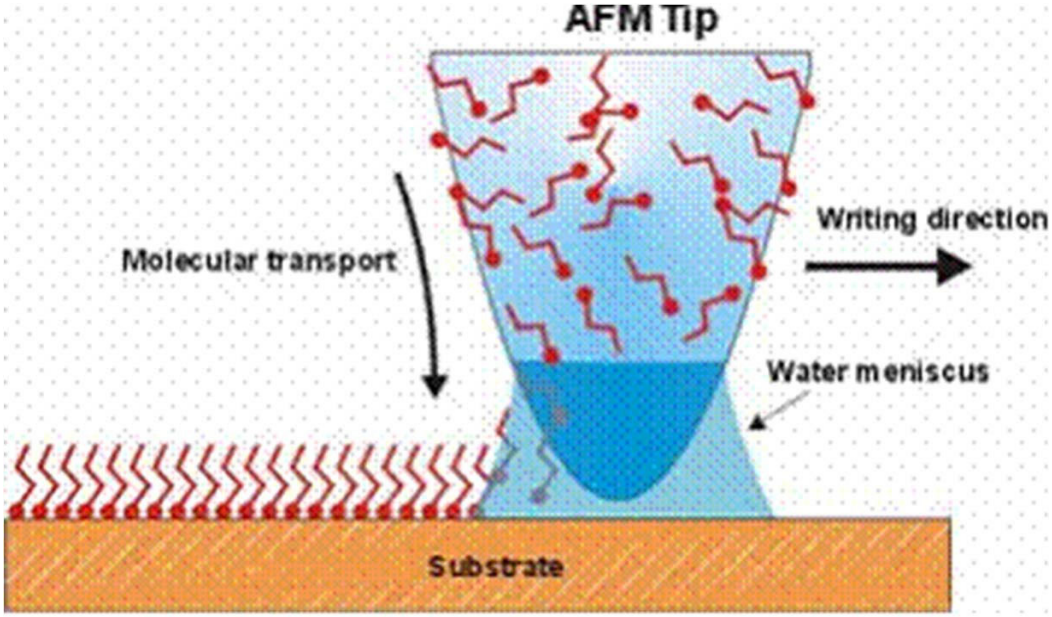
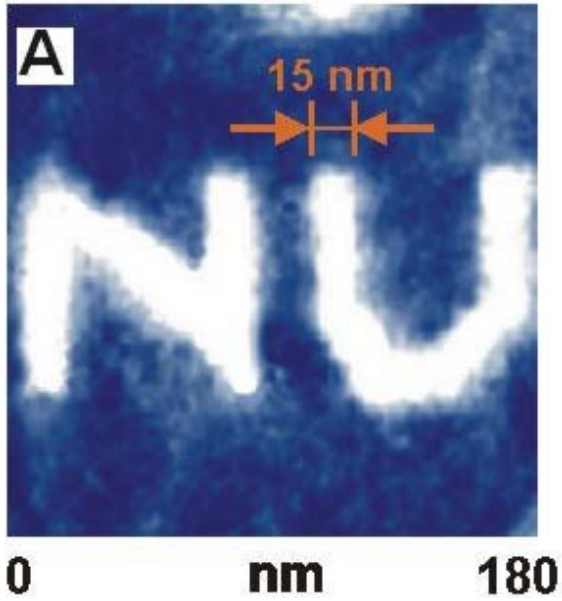
Macromolecules **2018**, *51*, 7266.

Various application examples

Dip pen nanolithography

- Sub-species of soft lithography
- Tip is coated with the “molecular ink”
- Water condensing from the environment forms a capillary between the tip and the substrate which aids the transport of the ink molecules onto the surface





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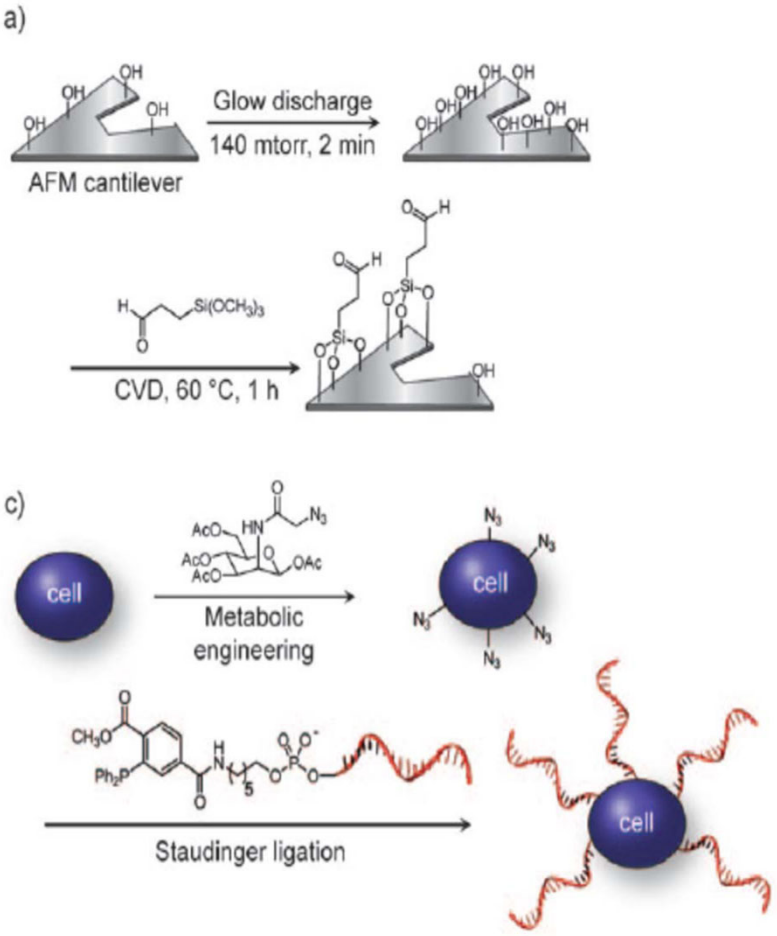
Science **1999**, *283*, 661.

Dip pen lithography for cell engineering

- Aldehyde groups are attached directly to the cantilever by silylation
- Amine-functionalized DNA strands are attached to the aldehydes through reductive amination
- DNA can be attached on a specific location on a cell surface by several reaction steps that detach it from the cantilever



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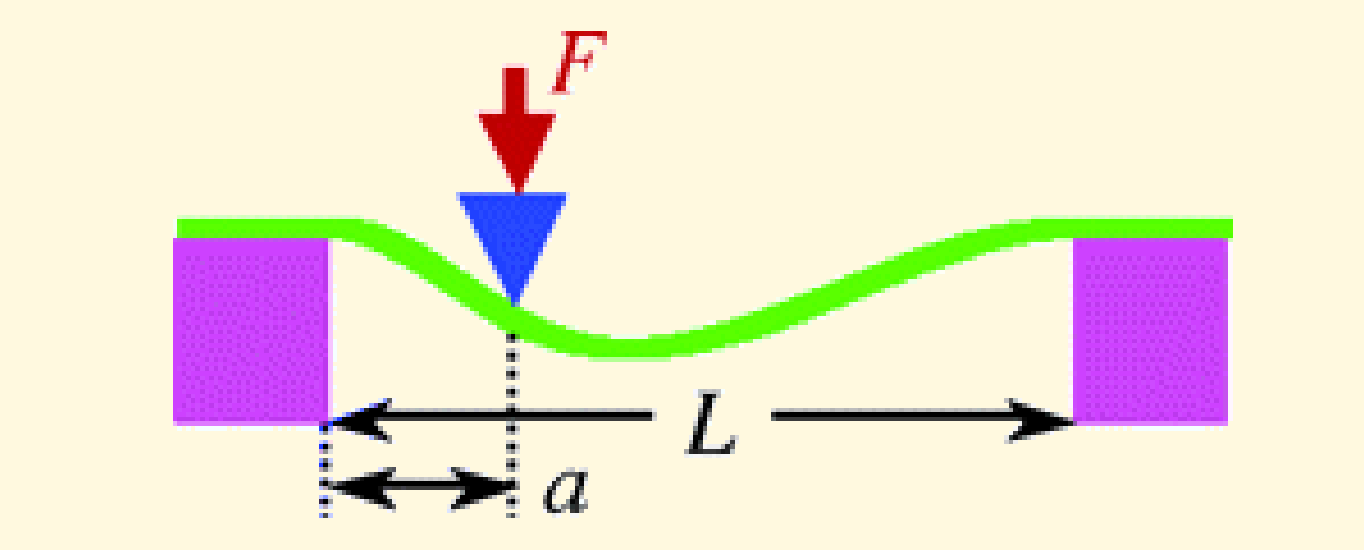
Angew. Chem. Int. Ed. **2008**, *47*, 8473.

Measuring elastic modulus



- The force (*F*) exerted at location *a* by the cantilever (through the tip) causes deformation in the sample
- The deformation can be monitored through cantilever deflection
- The deformation at a known force is proportional to the elastic modulus

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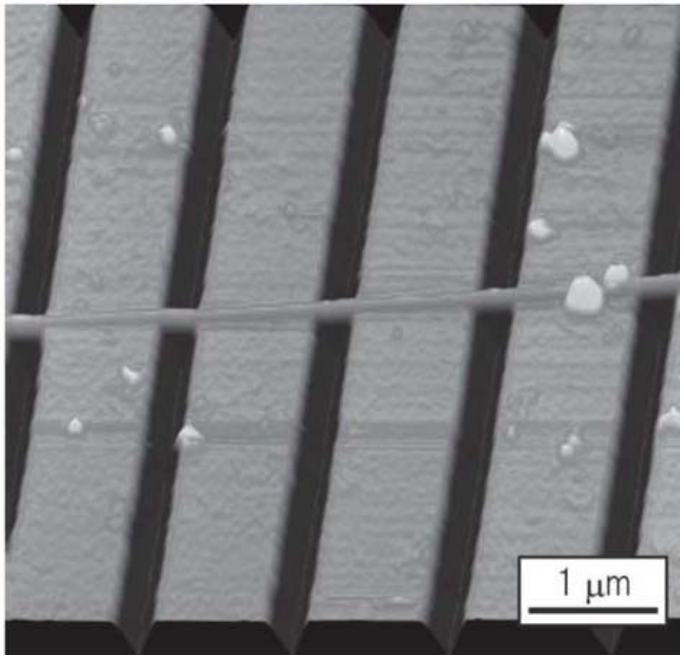
Langmuir **2005**, *21*, 6642.

Measuring elastic modulus of a single cellulose fibril



- Cellulose microfibril is isolated from a native fibre with TEMPO oxidation
- The individualized nanofibril is placed on microfabricated silica support
- Elastic modulus is measured by bending the fibril with the AFM tip

→ 150 GPa

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Biomacromolecules **2009**, *10*, 2571.

High speed AFM

Visualising the movement of a cellulase enzyme on a cellulose microfibril

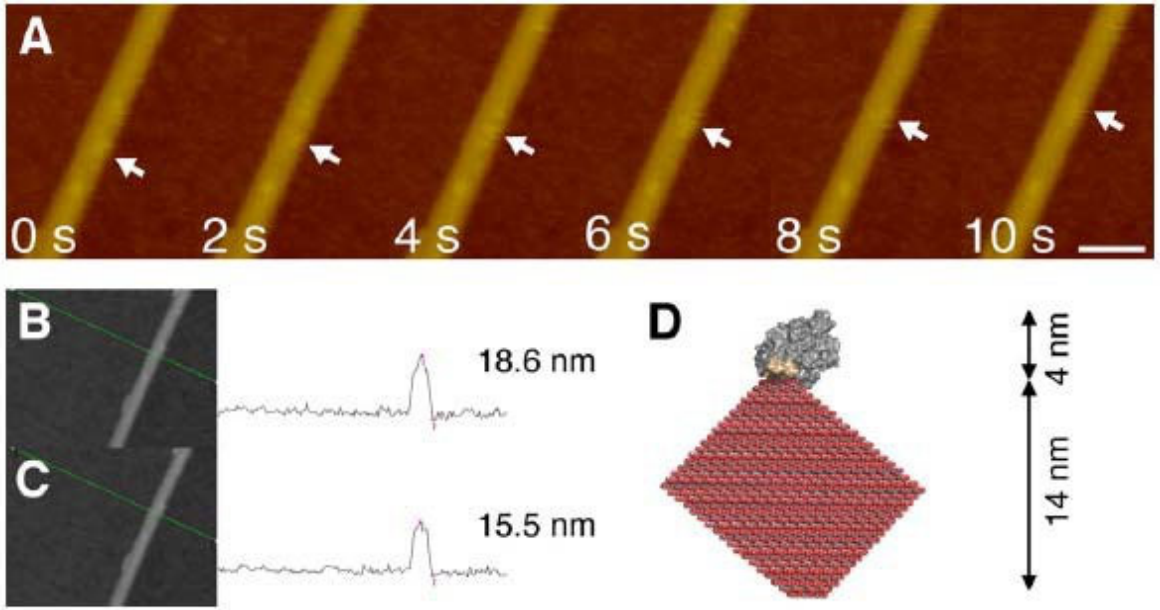

FIGURE 2. **Real time observation of *Tr*Cel7A molecules on a highly crystalline cellulose.** *A*, time lapse images of *Tr*Cel7A (*white arrows*) sliding on the substrate. *Scale bar*, 50 nm. *B* and *C*, height analysis of crystalline cellulose with (*B*) and without (*C*) enzyme molecule. *D*, three-dimensional structure of the *Tr*Cel7A CD (Protein Data Bank (PDB) code 8CEL) (13) and the CBD (PDB code 1CBH) (37), and cellulose I_α (3). For better recognition of *Tr*Cel7A sliding on crystalline cellulose, see supplemental Video S1.

The movement of a cellulose-degrading enzyme on cellulose microifbril can be visualized after the enzyme has bound. Velocity: 3.5 nm/s

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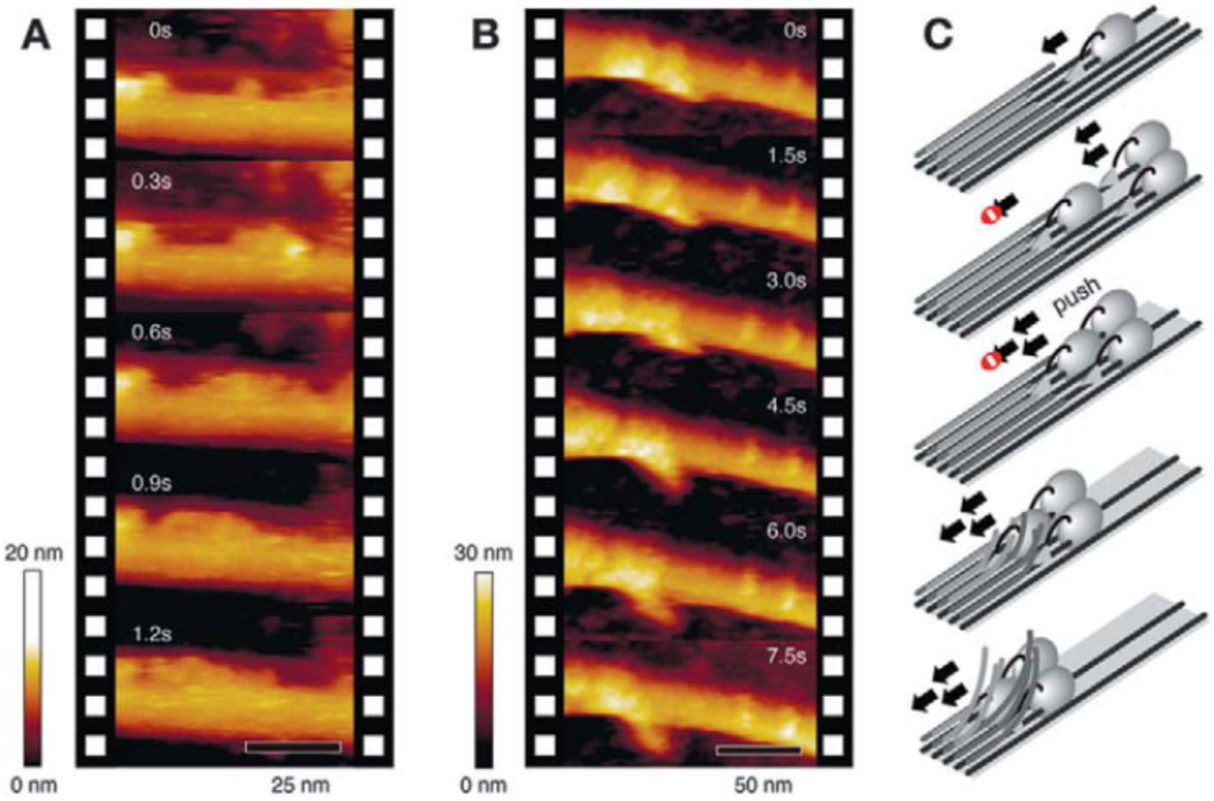
J. Biol. Chem. **2009**, *284*, 36186.

High speed AFM



Traffic jams:

- Overcrowding of cellulase enzymes on a cellulose fibril cause collective halting

→ Analogous to traffic jams

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Science **2011**, *333*, 1279.

Detection of atoms in a molecule

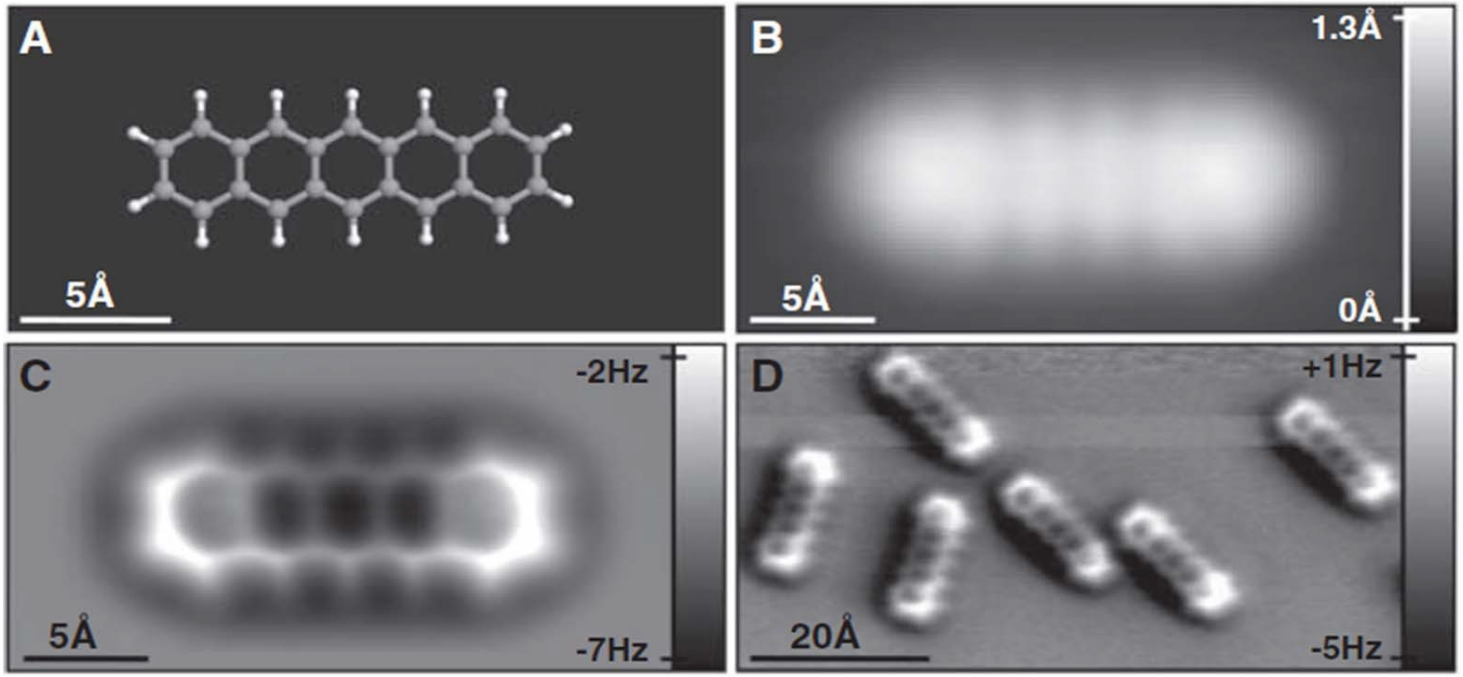

Fig. 1. STM and AFM imaging of pentacene on Cu(111). (**A**) Ball-and-stick model of the pentacene molecule. (**B**) Constant-current STM and (**C** and **D**) constant-height AFM images of pentacene acquired with a CO-modified tip. Imaging parameters are as follows: (B) set point $I = 110$ pA, $V = 170$ mV; (C) tip height $z = -0.1$ Å [with respect to the STM set point above Cu(111)], oscillation amplitude $A = 0.2$ Å; and (D) $z = 0.0$ Å, $A = 0.8$ Å. The asymmetry in the molecular imaging in (D) (showing a "shadow" only on the left side of the molecules) is probably caused by asymmetric adsorption geometry of the CO molecule at the tip apex.

Tip modified with adsorbed CO molecule is sensitive towards different atoms in a pentacene molecule

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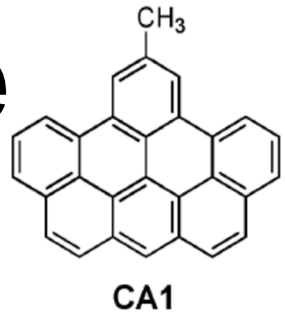
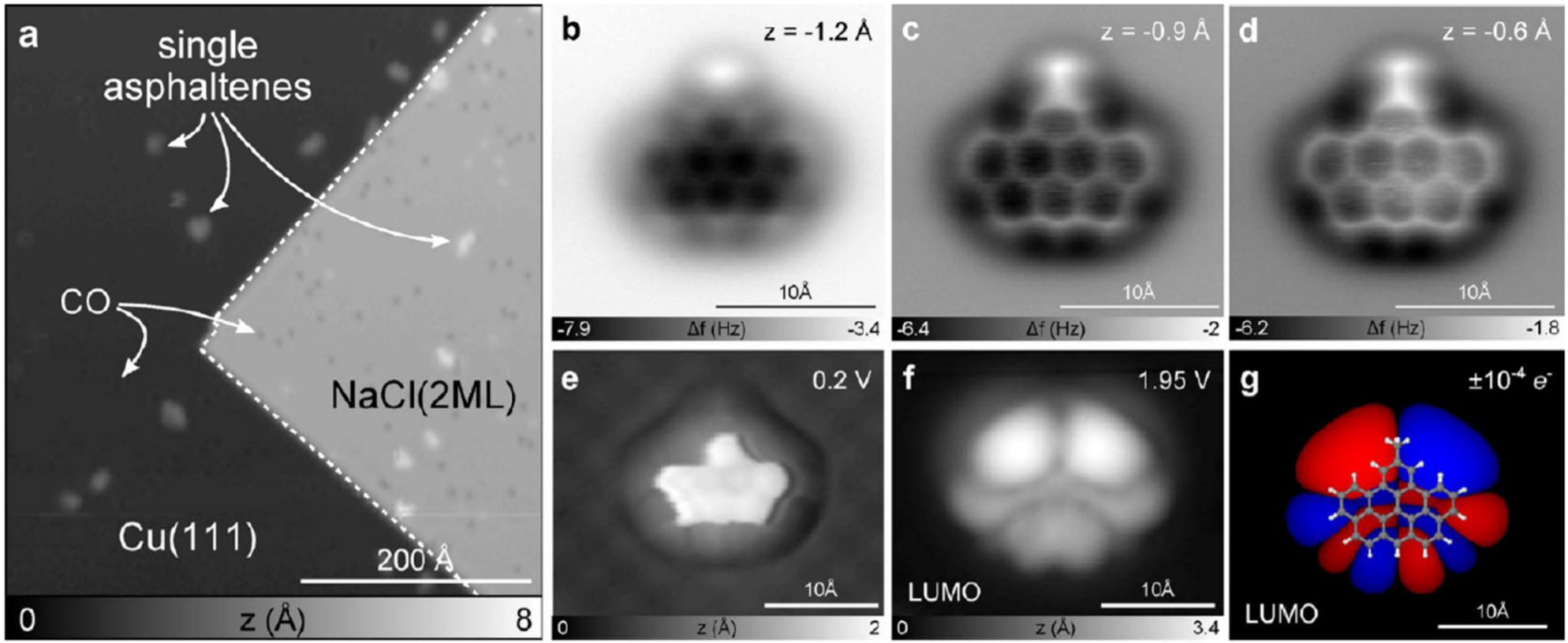
Science **2009**, *325*, 1110.

Analysis of molecular structure



Asphaltene molecules: solid components in curde oil



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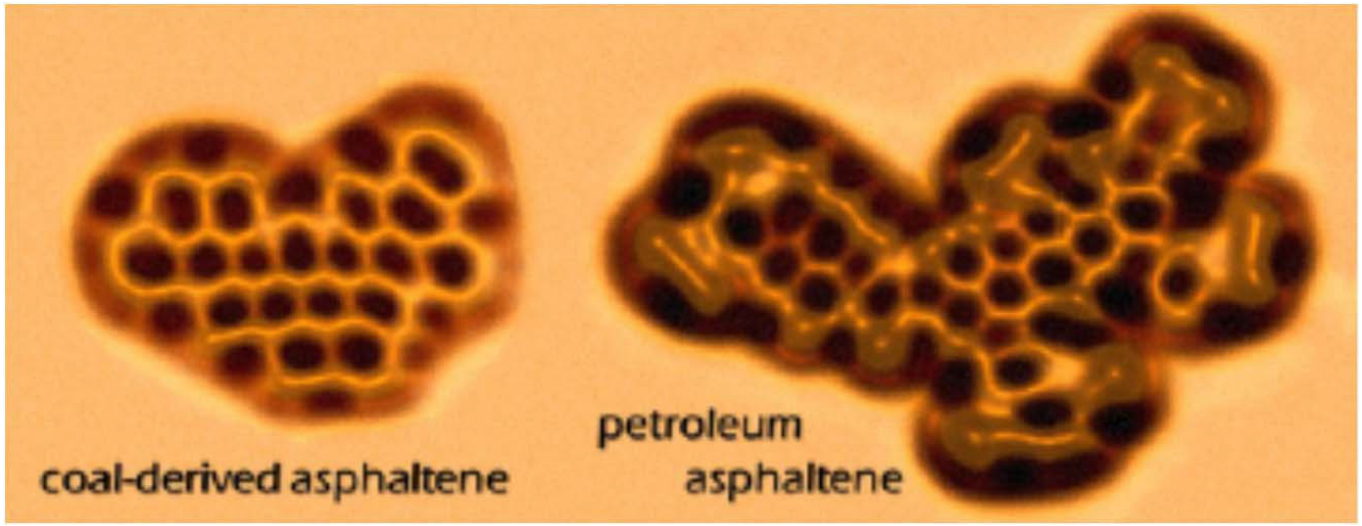
J. Am. Chem. Soc. **2015**, *137*, 9870.

Analysis of molecular structure

Asphaltene molecules: solid components in curde oil



- The authors were able to determine molecular the structures in asphaltene molecules from genuine natural sources
- Because asphaltences are always complex mixtures, AFM is *the only* way to analyse the exact chemical structures of the molecules

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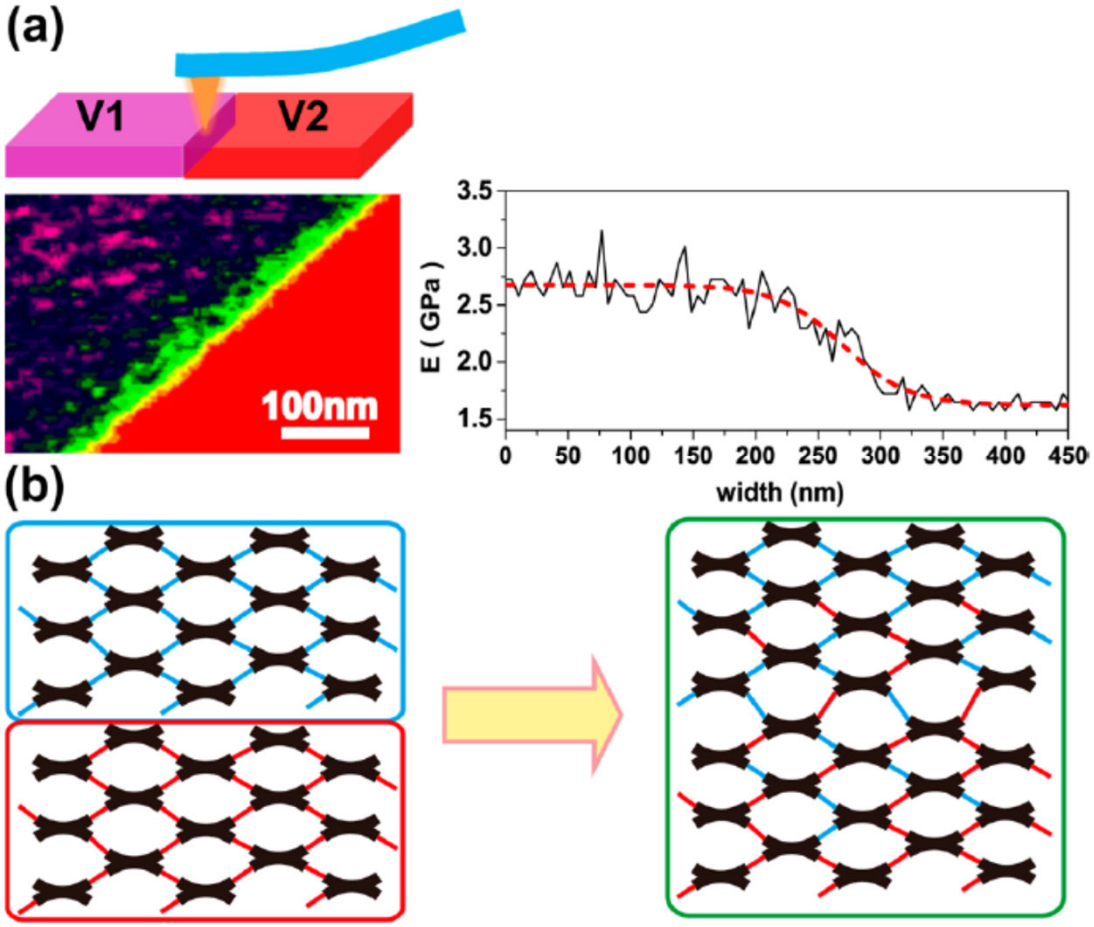
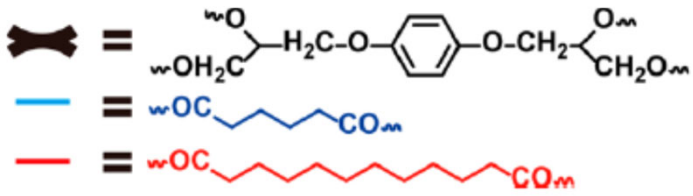
J. Am. Chem. Soc. **2015**, *137*, 9870.

Nanomechanical mapping



Vitrimers: polymer networks that can reconfigure by activated associative bond-exchange reactions of junction points

AFM PeakForce mapping is able to reveal different areas of two adjacent networks (interfacial broadening)



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J. Am. Chem. Soc. **2018**, *140*, 6793.

Summary

- Quantification of AFM data always requires consideration of the tip exaggeration
- Height distribution histograms can be utilized to quantify coverage, height and volume of features
- Image analysis can be used to quantify features that are higher (grains) or lower (pores) than the main image background
- Quantified data revealing the composition of the surface can be applied to theoretical predictions of that surface
- Imaging of individual polymers with AFM can reveal, e.g., information on their conformation upon adsorption or annealing
- AFM-IR and TERS have enabled chemical characterization for AFM-based techniques