



Aalto University
School of Chemical
Technology

Atomic Force Microscopy (AFM) Part I

CHEM-L2000

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Lectures on AFM

Part I

- Principles and practice
- Imaging of native materials, including nanocellulose

Part II

- Surface force measurements on ultrathin films

Part III

- Quantification and special applications

Learning objectives of the current lecture

After this lecture, you will be able to

- Understand the principles behind scanning probe microscopies, especially AFM
- Tell the difference between main operating and imaging modes of AFM
- Possess a clear picture on the instrumentation of tapping mode AFM
- Be aware of the main advantages and limitations of AFM
- Give examples of applying AFM on native (soft) materials, including various nanocellulosic materials

Outline

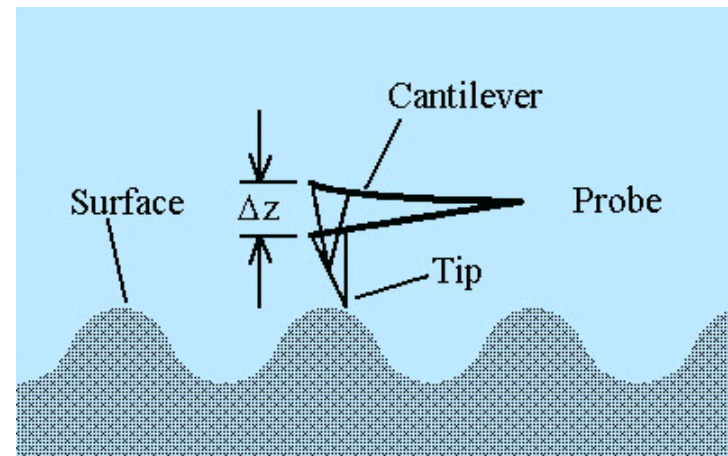
- (1) Scanning probe microscopies in general
- (2) Principle of AFM
- (3) Tapping mode / Peak force tapping
- (4) Experimental aspects of AFM
- (5) Native materials by AFM

Terminology

- AFM belongs to the family of Scanning Probe Microscopies (SPM)
- The first SPM introduced was Scanning Tunnelling Microscopy (STM)
- Other SPM techniques: Scanning Near-field Optical Microscopy (SNOM), Magnetic Force Microscopy (MFM), or Scanning Thermal Microscopy (SThM), among many others
- AFM is often referred to as Scanning Force Microscopy (SFM)
- AFM is by far the most common of the SPM techniques

Basic principle of all SPM techniques

- unlike optical or electron microscopy, SPM is *not* based on electromagnetic waves
- Instead, a probe scans across the surface
- Probe consists of a cantilever and a very sharp tip
- The tip interacts with the surface
- The cantilever deflects
- The deflections in the cantilever are recorded



➔ An image of topography occurs.

Scanning Tunnelling Microscopy (STM)

- Introduced in 1981-1982 by Gerd Binnig and Heinrich Rohrer
→ Nobel Prize in physics in 1986
- Based on the tunnelling current between two objects brought in very close contact

Distance between the sample and the probe increased 1 Å.



Tunnelling current decreases by a factor of ten.

- First images of atomic resolution were published in 1983

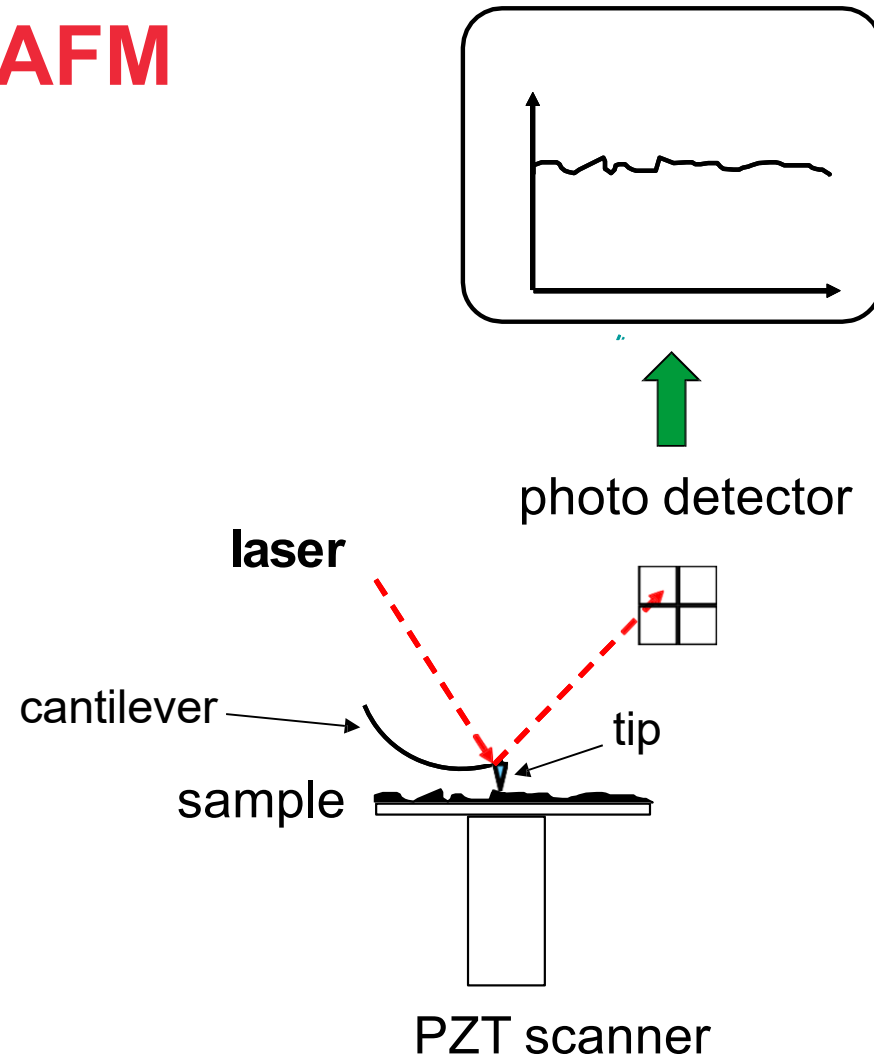
**LIMITATION: STM requires that samples be conductive
And (nearly) atomically smooth.**

AFM – general remarks

- Introduced in 1986 by Binnig et al.
- Does not require conductive samples
→ introduced SPM to most organic materials
- Gained increased popularity in the 1990s
- Has become a routine technique during this decade
- Much of the “revolution” in nanotechnology owes to AFM: otherwise the visualization of nano-objects would not have been feasible (to an extent)

Basic principle of AFM

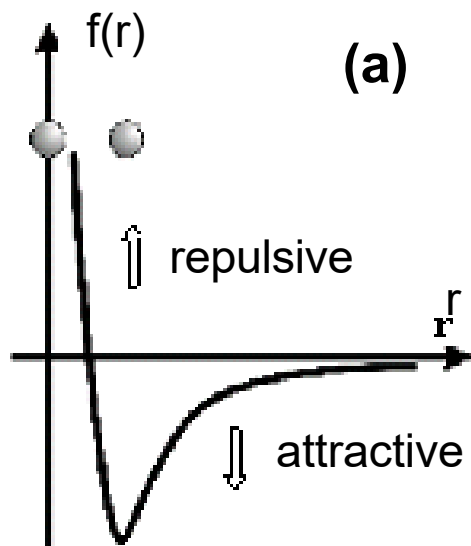
- (1) Surface forces between the tip and the sample deflect cantilever.
- (2) Deflections are recorded by reflecting a laser from the tip of cantilever



Basic principle of AFM

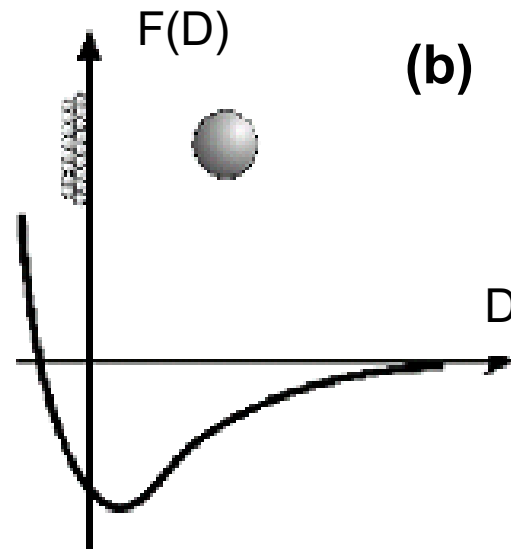
- high force sensitivity

IDEAL



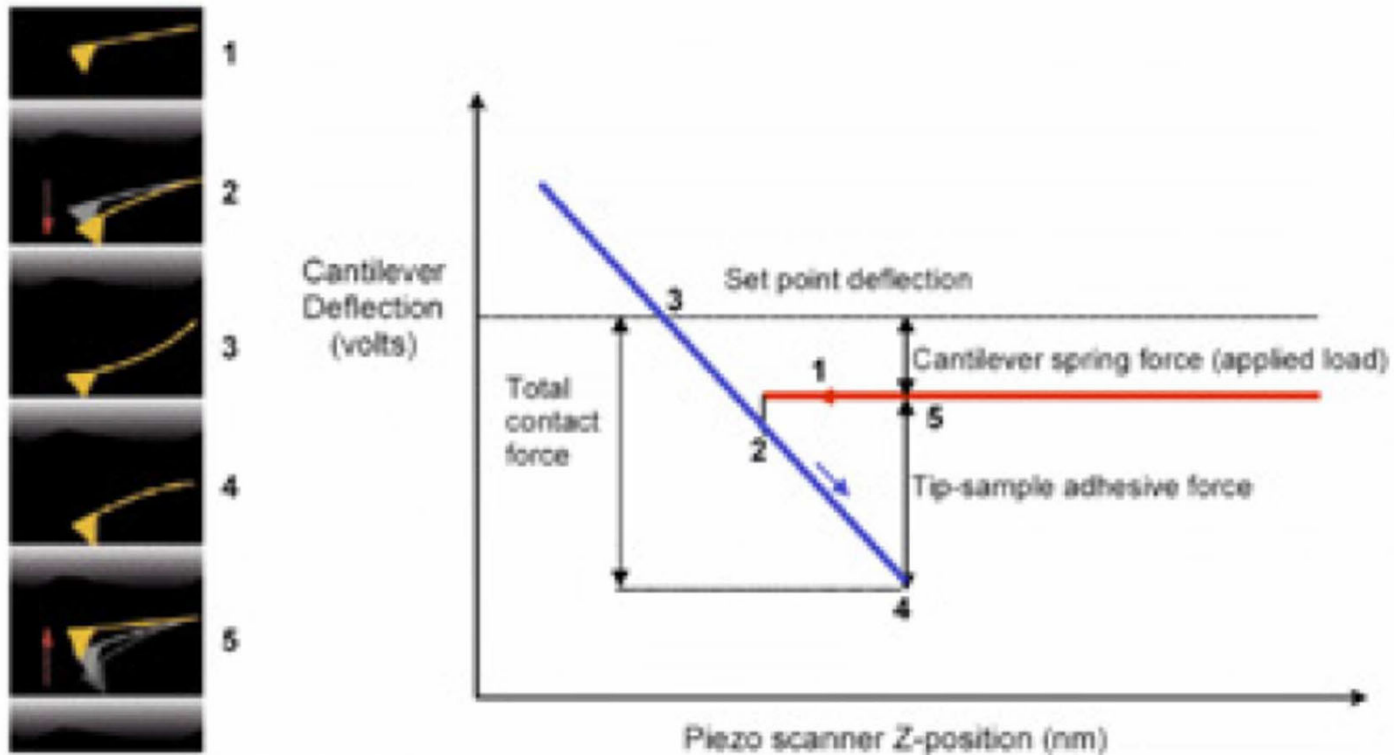
Interaction between
two atoms $F \sim r^{-7}$

REALITY



Interaction between a sphere (tip)
and a surface (sample): $F \sim D^{-2}$

Basic force curve in contact mode AFM



Operating modes of AFM

Contact mode:

- Tip scans in the immediate vicinity (contact) of the sample

Tapping mode (or intermittent contact mode):

- Cantilever is vibrated near its resonance frequency
- Tip-sample interaction causes changes in the amplitude of the vibration
- Changes in the amplitude are monitored

Non-contact mode:

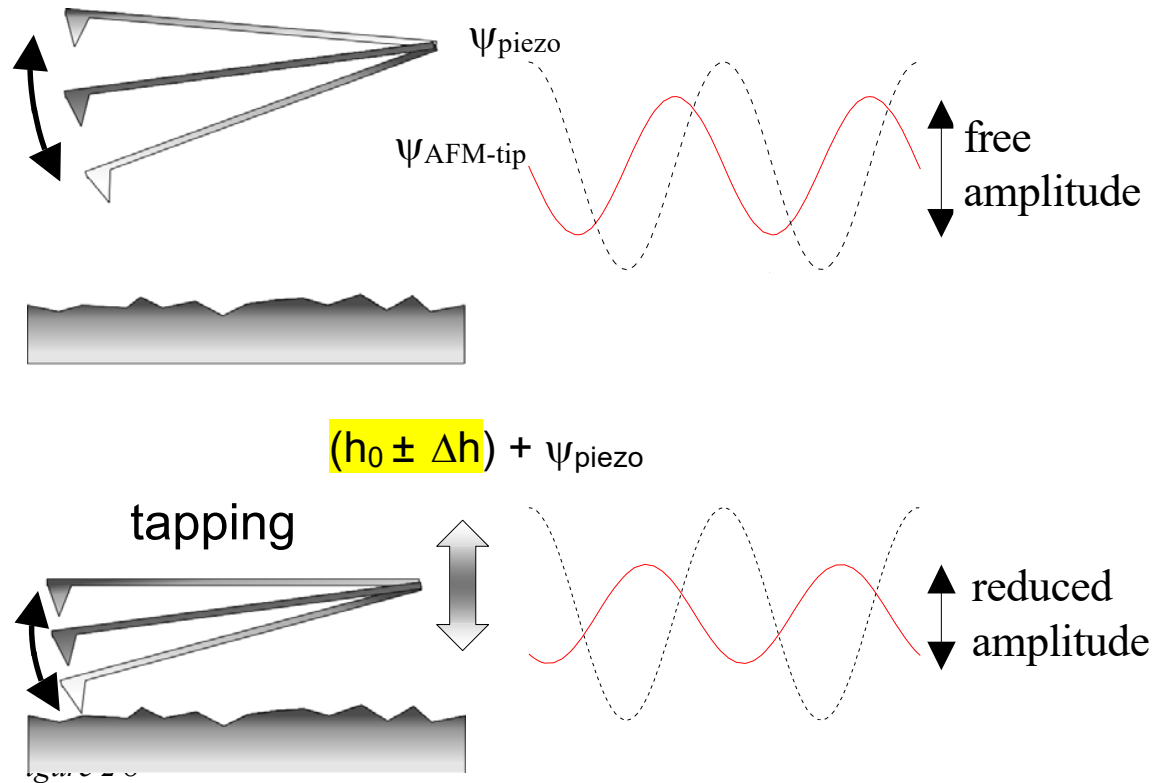
- Same as tapping mode, but changes in the frequency are monitored

NOTE: Tapping mode is mandatory for compliant materials which would be destroyed (scratched) in contact mode imaging, resulting in artefacts in the image.

Practically all polymeric samples are imaged in tapping mode.

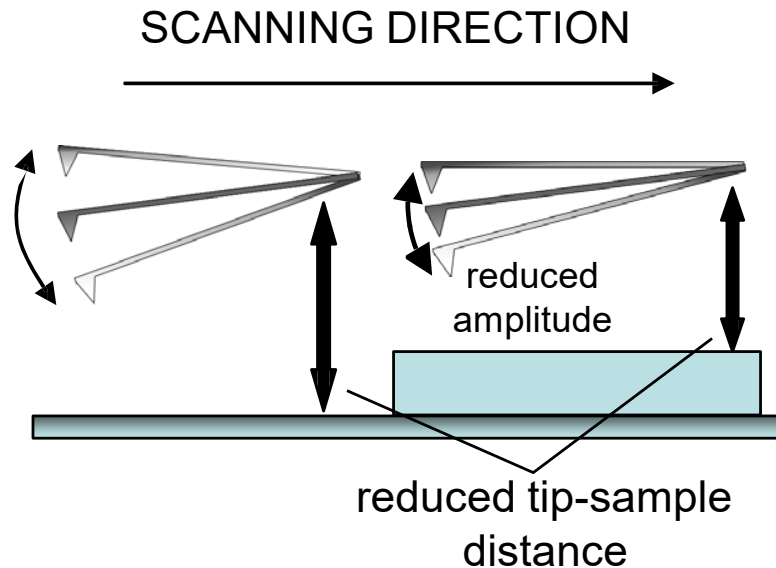
Tapping mode

- interaction with the sample surface causes amplitude displacement



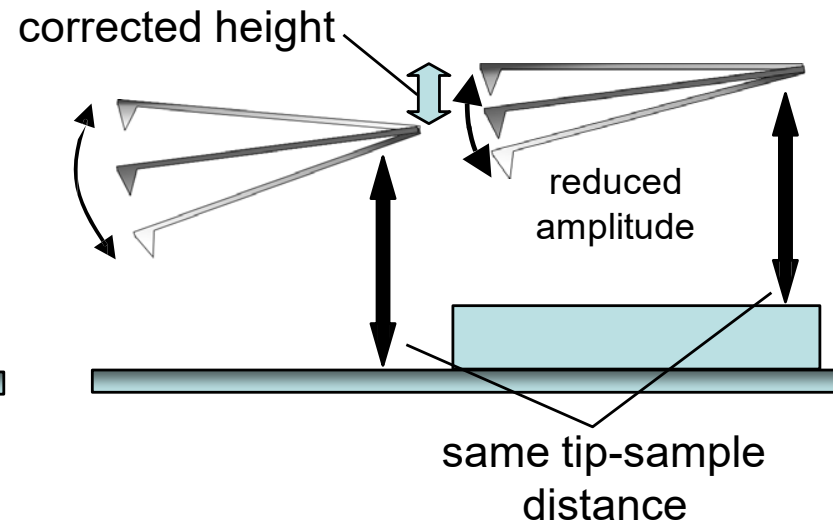
Tapping mode

(a) No tip adjustment



If the height of the vibrating tip is not adjusted, the amplitude reductions will not genuinely map the surface features.

(b) Tip-sample distance adjusted



When the height of the vibrating tip is adjusted always to the same distance from the sample after each amplitude reduction, the imaging is correct.

Tapping mode: 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

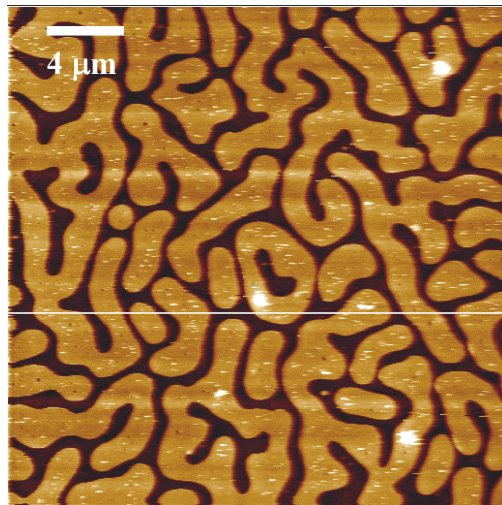
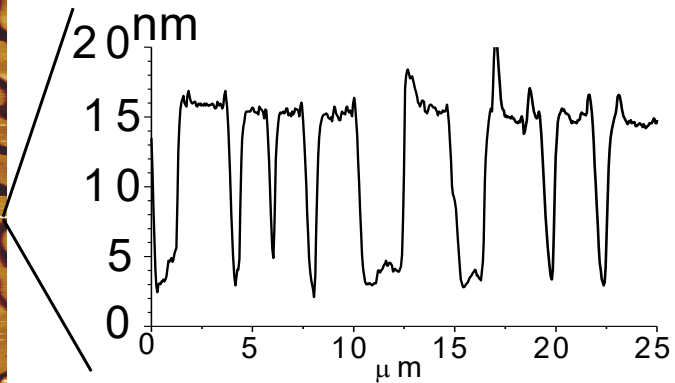


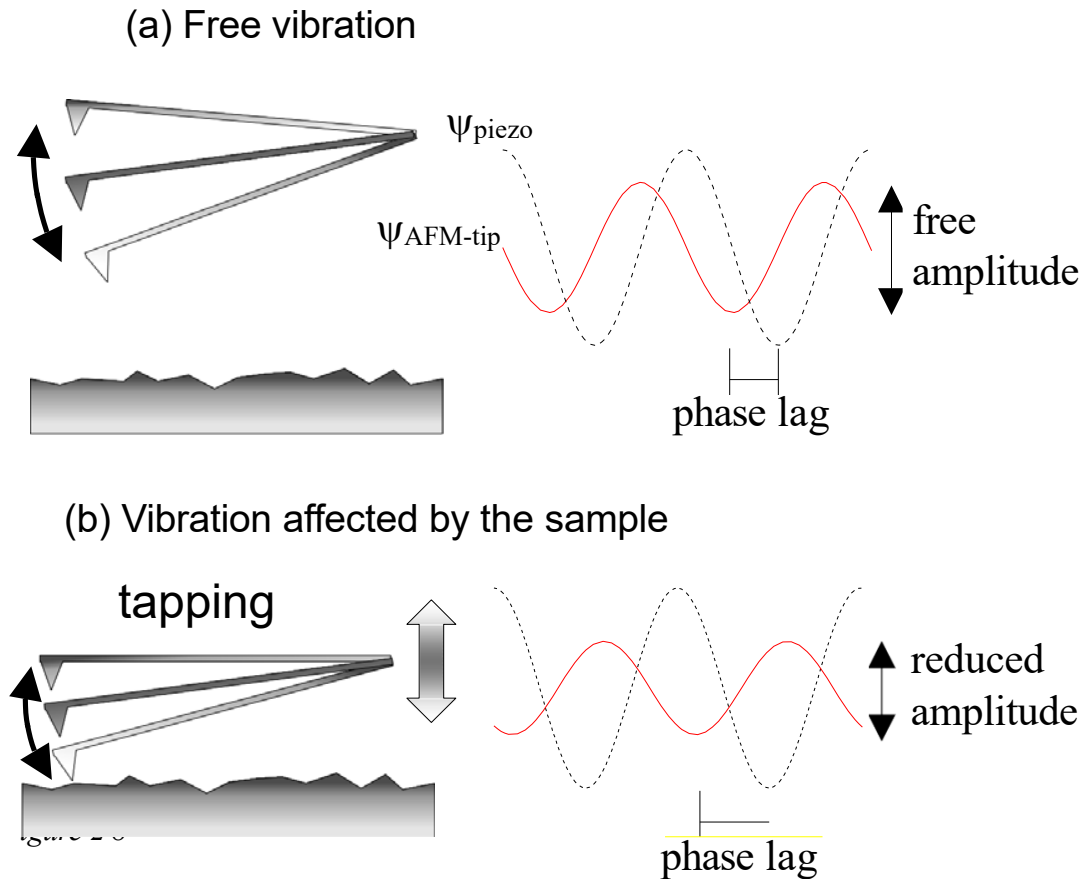
Image: cellulose islands

(b) Individual height scan



512 of individual scans make up the height image.

Tapping mode: phase lag

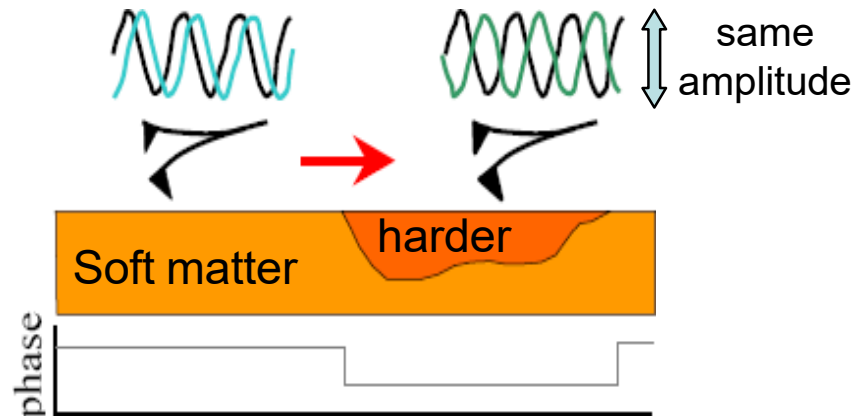


- Tapping mode vibration is a standing wave with a sinusoidal shape

$$y = A \cos(\omega T + \phi)$$

phase of harmonic motion (unit: angle)

Tapping mode: phase image



- Phase lag yields important information on the elasticity of the sample
- If the two materials have the same height (amplitude unaffected), but they differ in elasticity, the phase lag reveals the difference

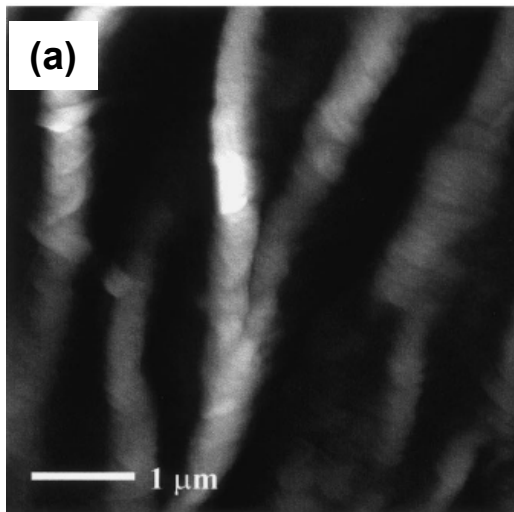


Phase image (based on mapping the phase lag) distinguishes different materials in the sample.

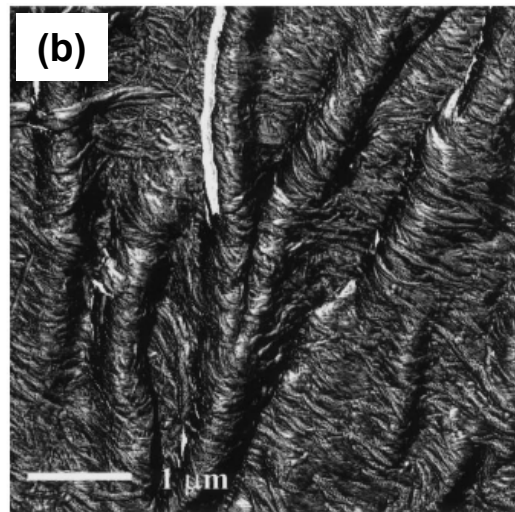
Height image vs. phase image

- Imaging the surface of a single fibre in kraft pulp

Height image



Phase image



Fibre contains: crystalline cellulose microfibrils (hard)
amorphous hemicellulose and lignin (soft)



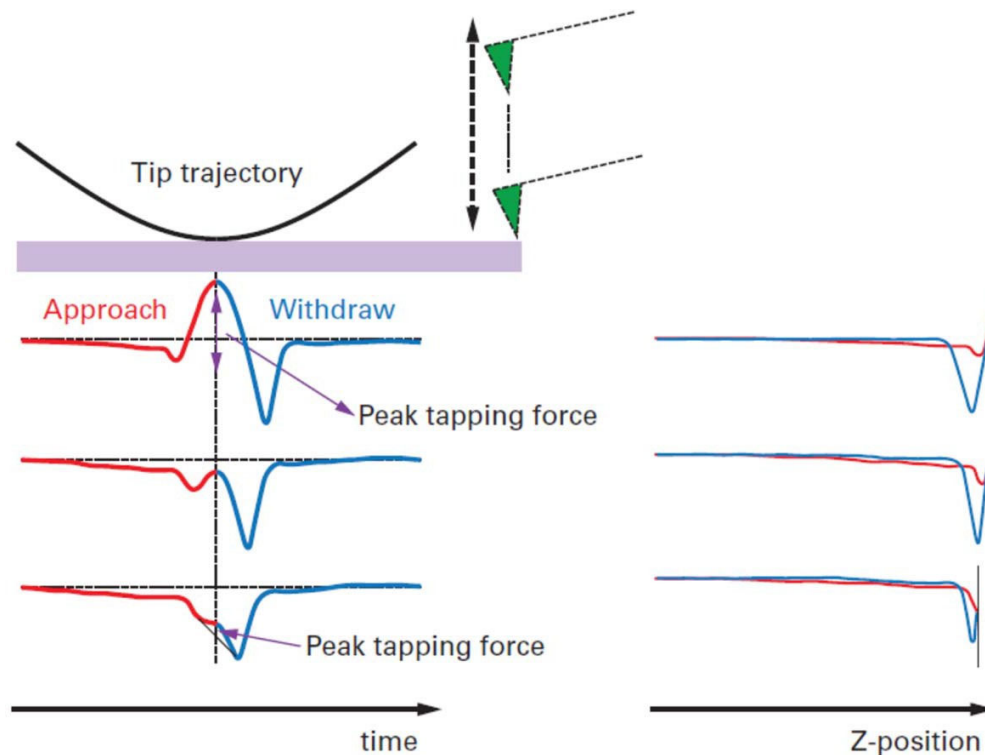
Phase image is able to resolve the microfibrils.

Peak Force Tapping Technology

- Like tapping mode, based on oscillating the cantilever
 - Unlike tapping mode, PeakForce Tapping operates in *non-resonant* mode
 - Tapping oscillation is performed at frequencies well below the cantilever resonance
- Dynamics of a resonating system are avoided
- Direct force control is enabled

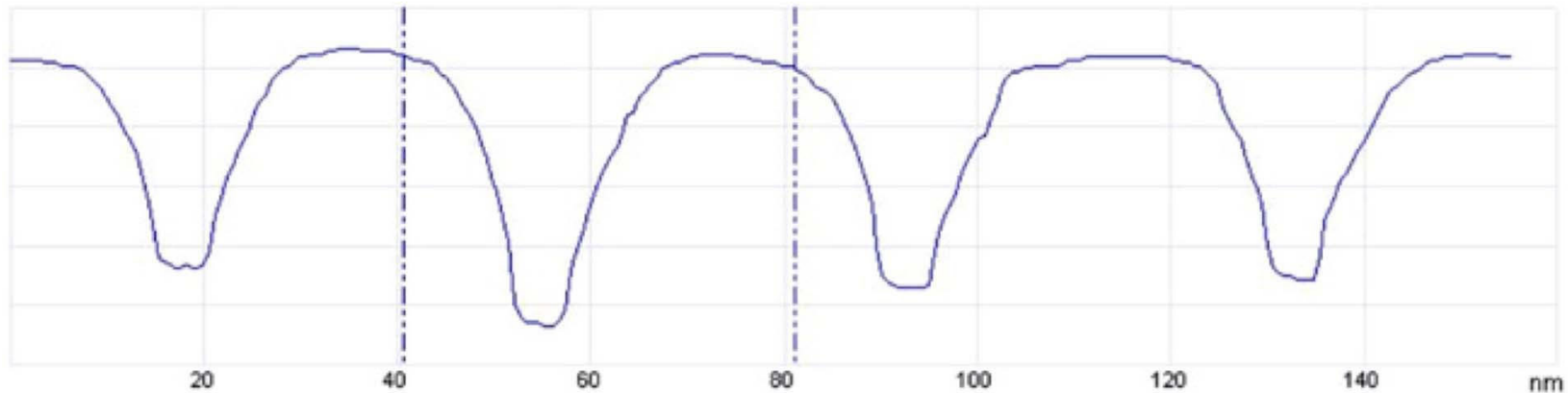
Peak Force Tapping Technology

- Position of the tip is modulated by a sine wave
→ Unwanted resonances at turn points are avoided



Peak Force Tapping Technology

Example: Peak Force Tapping line scan of narrow and steep, frequently recurring trenches in nanoscale

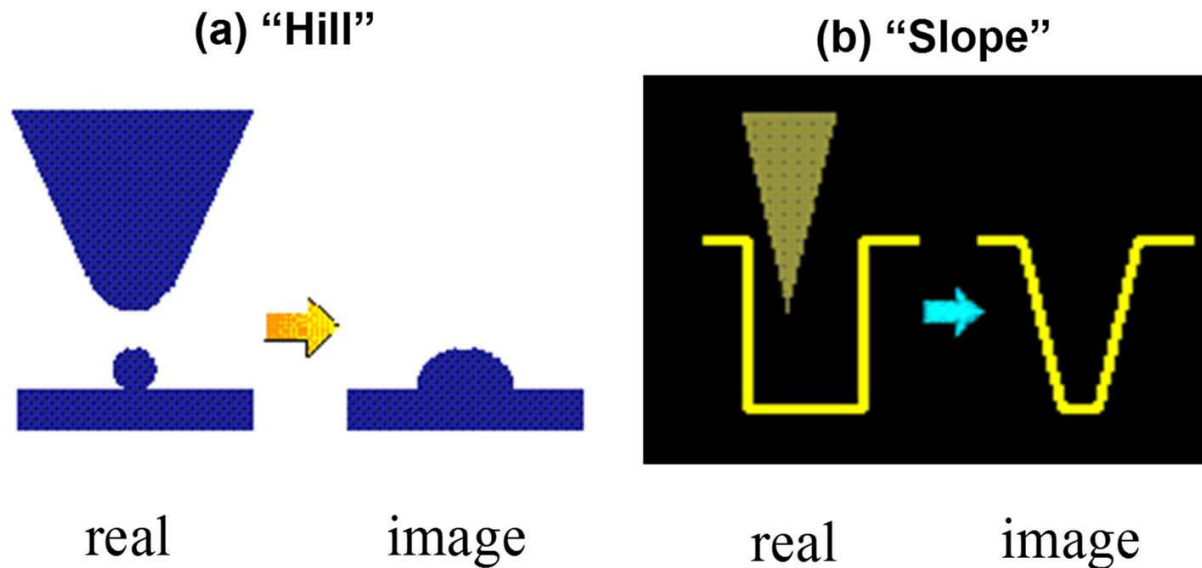


- In tapping mode the high frequency oscillation would cause the tip to “stick” to the sidewalls; flat bottoms would not be visible
- In Peak Force Tapping, there are fewer artefacts due to tip-surface interactions and cantilever dynamics

EXPERIMENTAL ASPECTS

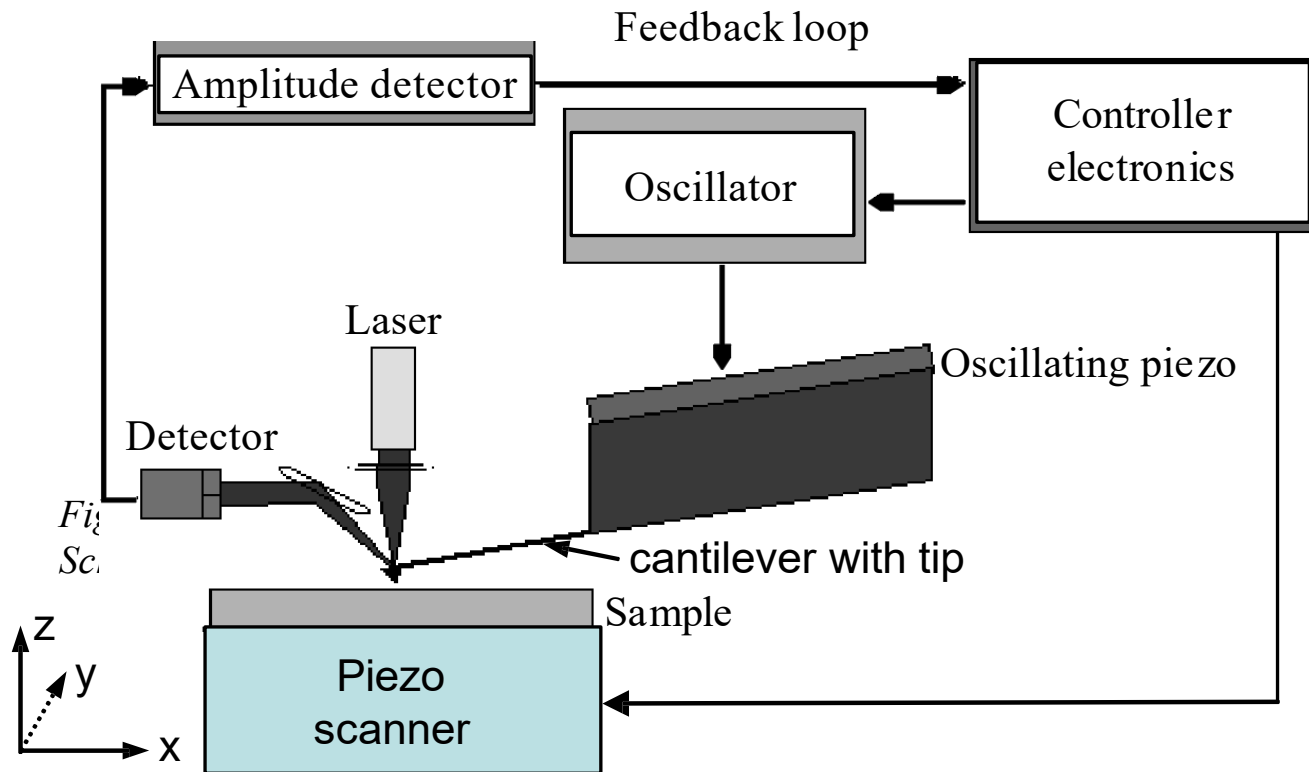
Limitation by the tip size

- 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

AFM instrumentation



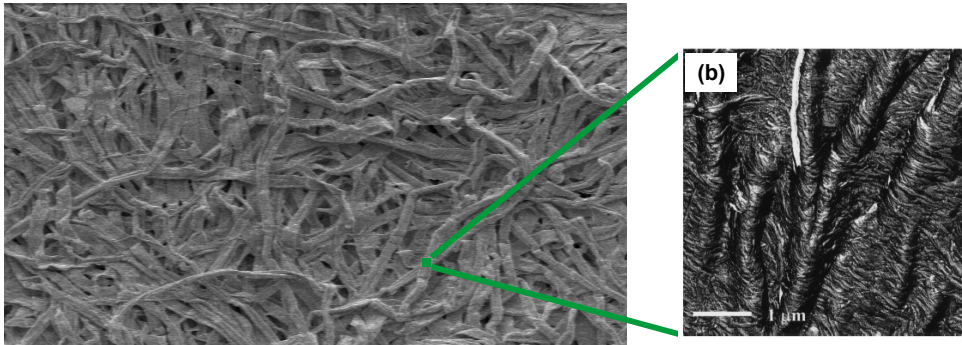
Sample preparation and limitations

- AFM requires no sample preparation
- Roughness of the sample sets limitation
- Rule of thumb: roughness $>1\mu\text{m}$ cannot be imaged (piezo scanner is too sensitive for “high” roughness)

Imaging of lignocellulosic fibres

- Width of individual fibres is $> 10 \mu\text{m}$
→ Fibre joints and “holes” between fibres are too rough for AFM

Paper or pulp



- (1) AFM is usually combined with optical microscope
- (2) Individual fibre is chosen
- (3) Surface of individual fibre is imaged with AFM

AFM in practice

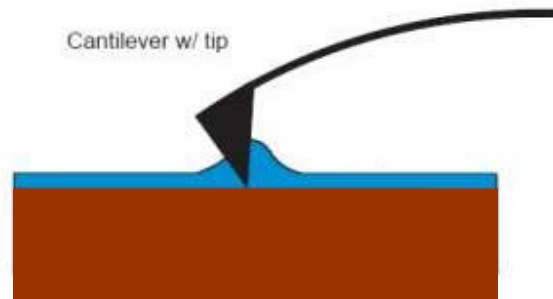
- Scan size is usually between $1 \times 1 \mu\text{m}^2$ and $5 \times 5 \mu\text{m}^2$
- If roughness of the sample allows, $50 \times 50 \mu\text{m}^2$ images are feasible (some instruments allow even $100 \times 100 \mu\text{m}^2$ scans)
- Acquiring (scanning) one image takes about 15 minutes
- Modern equipment can scan multiple (adjacent) images at one go
- Acquiring a decent image can take several hours or days, depending on how challenging the material is

AFM in practice

Difficulties on AFM imaging often depend on the material:

- **hard material: easy to image**
- **soft material: difficult to image**

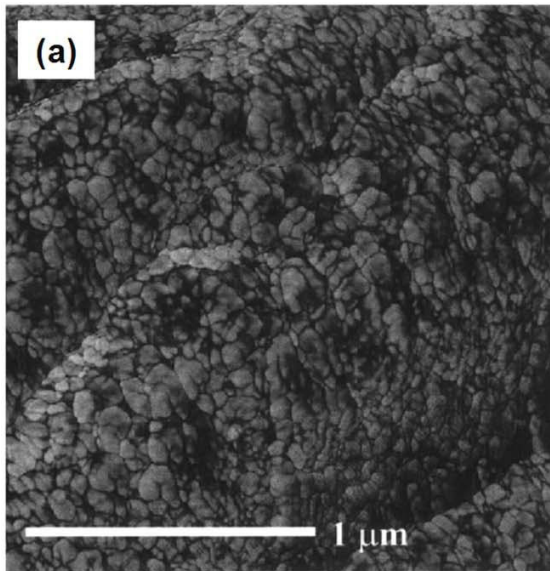
Soft materials may deform during tapping.
Soft materials may also get stuck on the tip.
→ Image is distorted.



APPLICATIONS WITH NATIVE MATERIALS

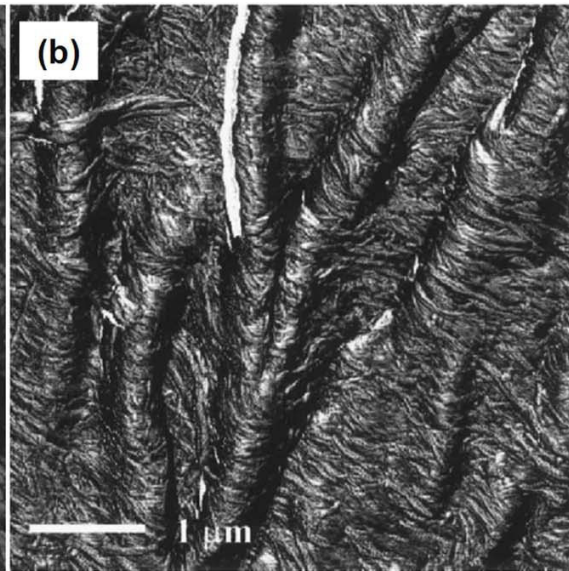
Visual changes during chemical pulping

**Kraft pulp
cooked for 10 min**



Low delignification

**Kraft pulp
cooked for 220 min
and oxygen delignified**

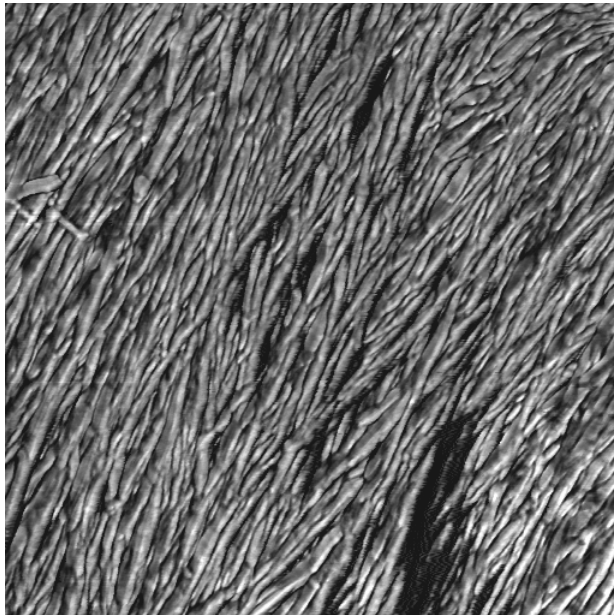


High delignification

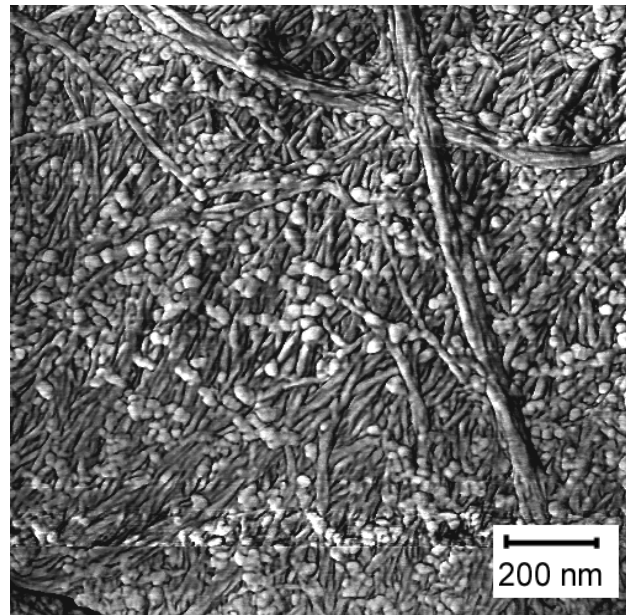
➔ **Lignin enriched on surface as granular structures (~10-100 nm width)**

Deliberate adsorption of lignin

Bleached pulp,
no lignin added

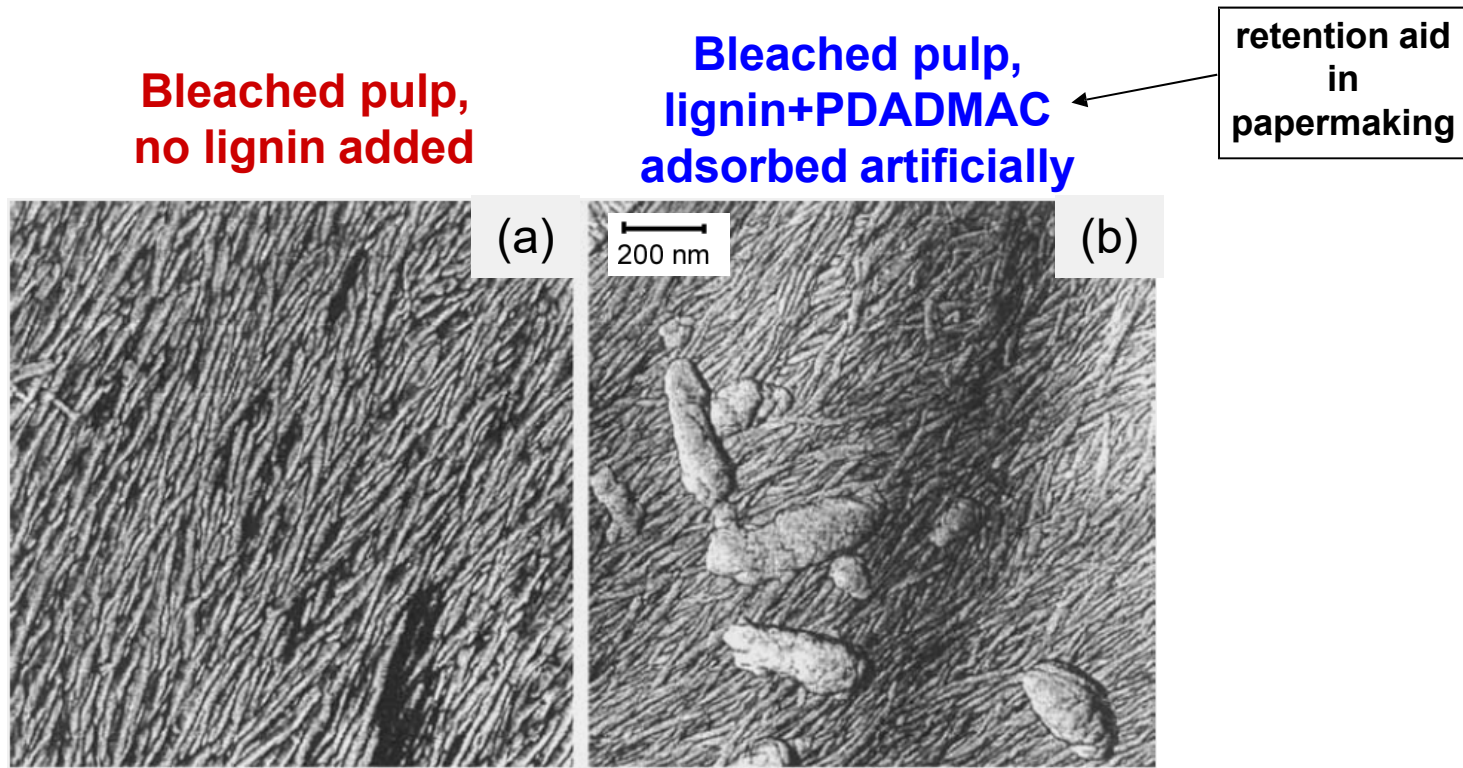


Bleached pulp,
lignin adsorbed artificially



➔ Hard evidence for the appearance of lignin.
(AFM is only an imaging technique; it yields **no** chemical information.)

Adsorption of lignin and polyelectrolyte

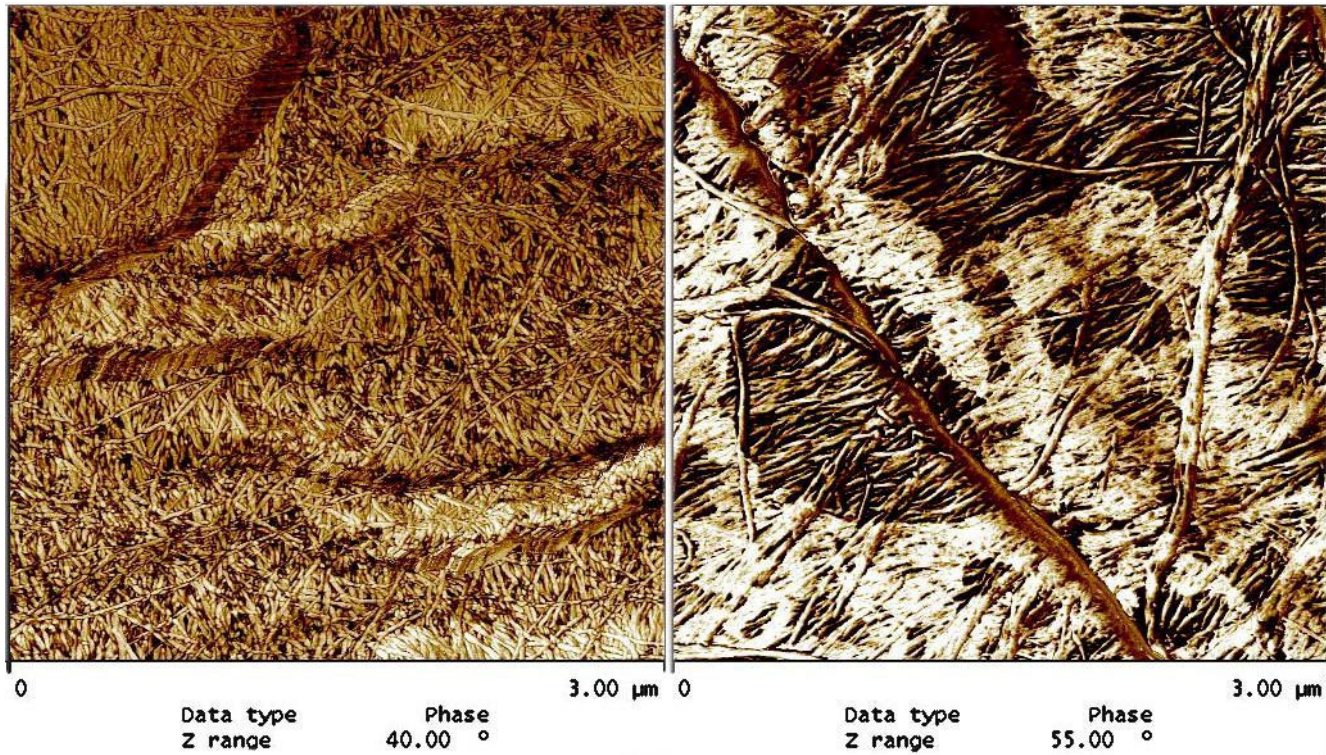


➔ Lignin and adsorbed cationic polyelectrolyte form a gel-like complex.

Adsorption of extractives

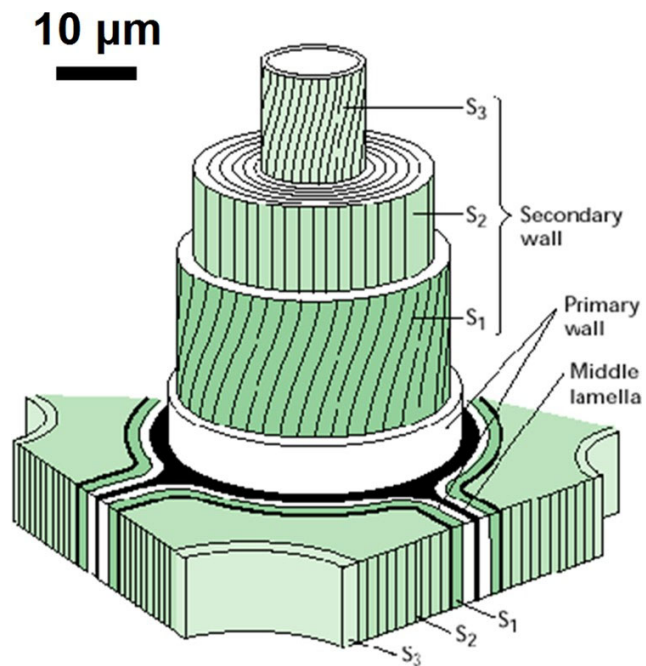
**Bleached pulp,
no extractives added**

**Bleached pulp,
extractives adsorbed artificially**

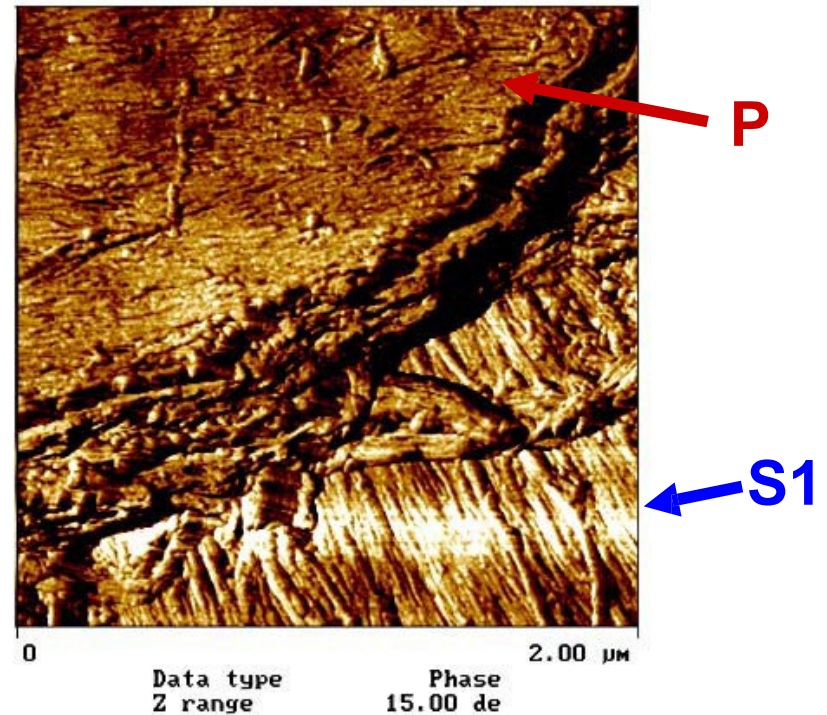


Imaging a wood cell

Cell wall layers – schematic view



Surface of a TMP fibre*
(unbleached) by AFM



Easy to detect the cell wall layers
by microfibril orientation.

*) Isolated wood cell

Microfibril aggregates

- Individual microfibril* width in wood: 3-4 nm
- Microfibrils tend to form aggregates of ca. 10-20 nm

Cross-sections of different cell wall layers

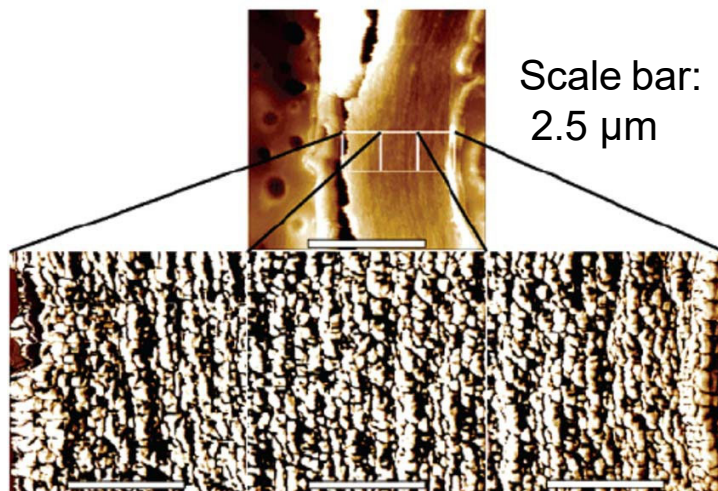
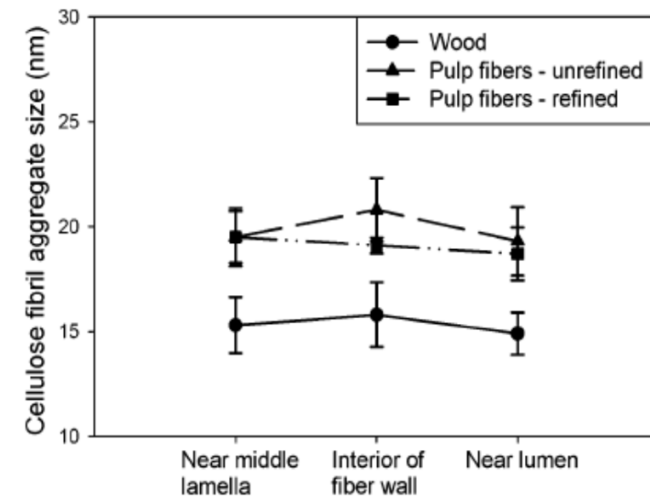


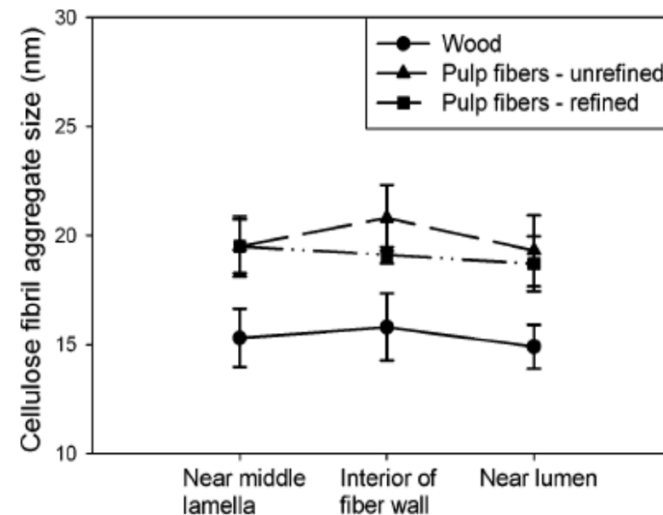
Image analysis



→ Width of microfibril aggregates change during pulping, not during beating

Microfibril aggregates

**CAREFUL OF THE
TIP ERROR!**

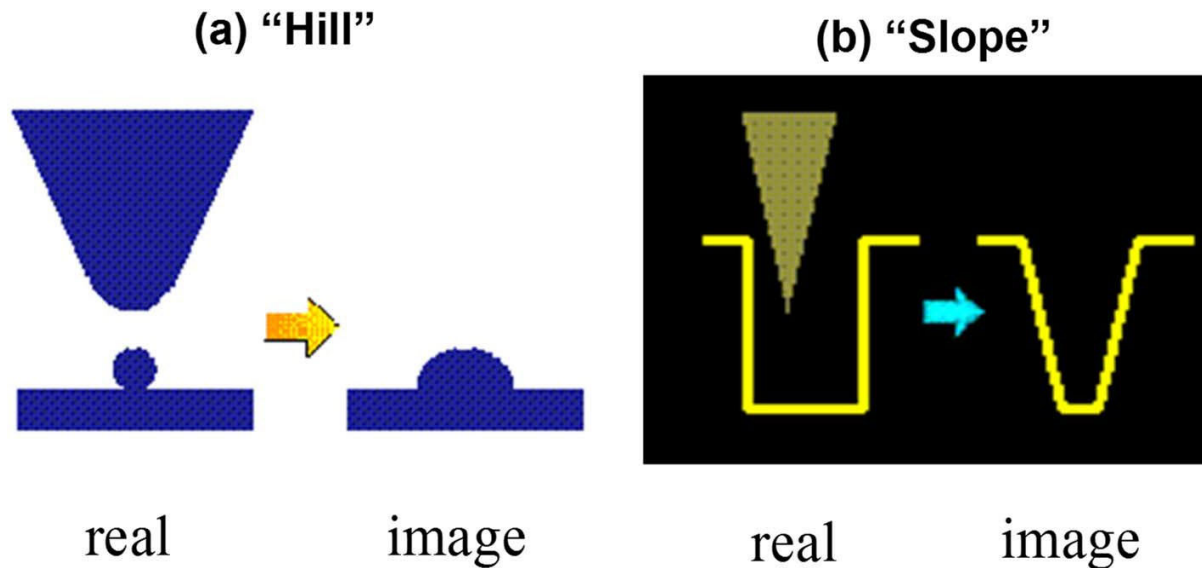


Tip error can be neglected in several cases but it always has to be considered.

In this case, the microfibril cross-sections are close to each other (no significant height differences) that the error can be neglected.

Reminder: limitation by the tip size

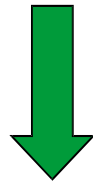
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 - Radius of an ordinary AFM tip: 5-10 nm
-

Dimensional analysis of nanosized cellulose

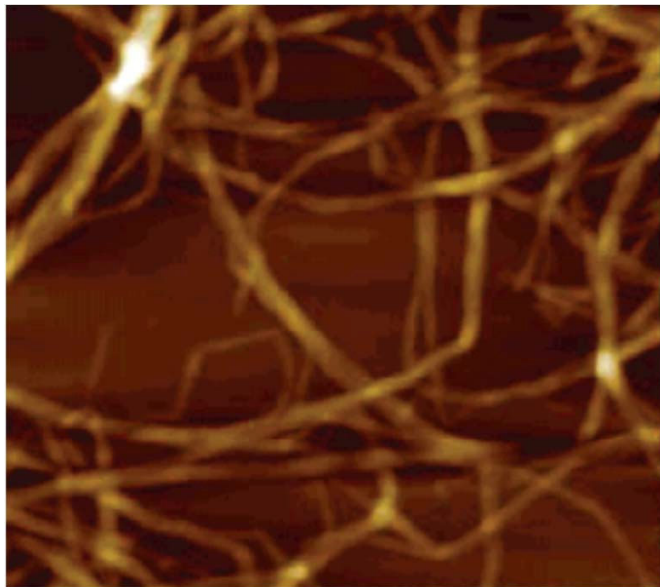
Spread individual crystals on a flat substrate



Atomic Force Microscopy (AFM)

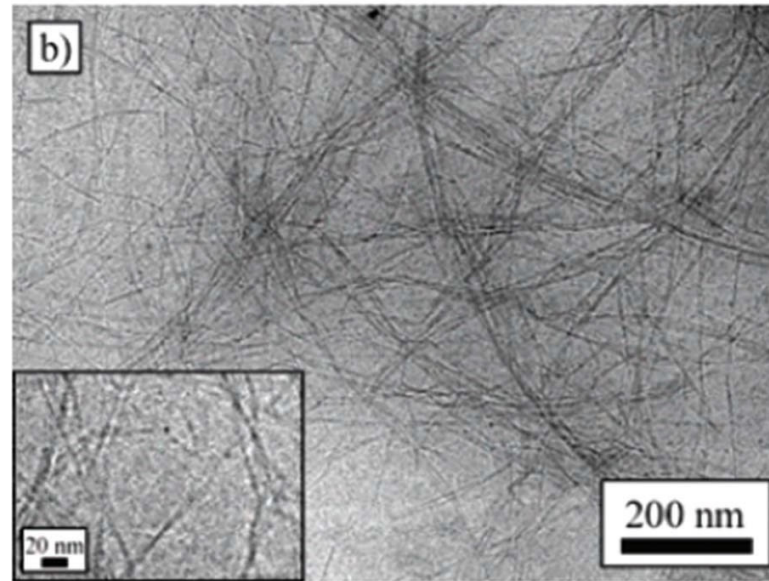
Nanofibrillar cellulose: AFM vs. TEM

AFM image of
nanofibrillar cellulose (NFC)



Fibril size: 10-30 nm

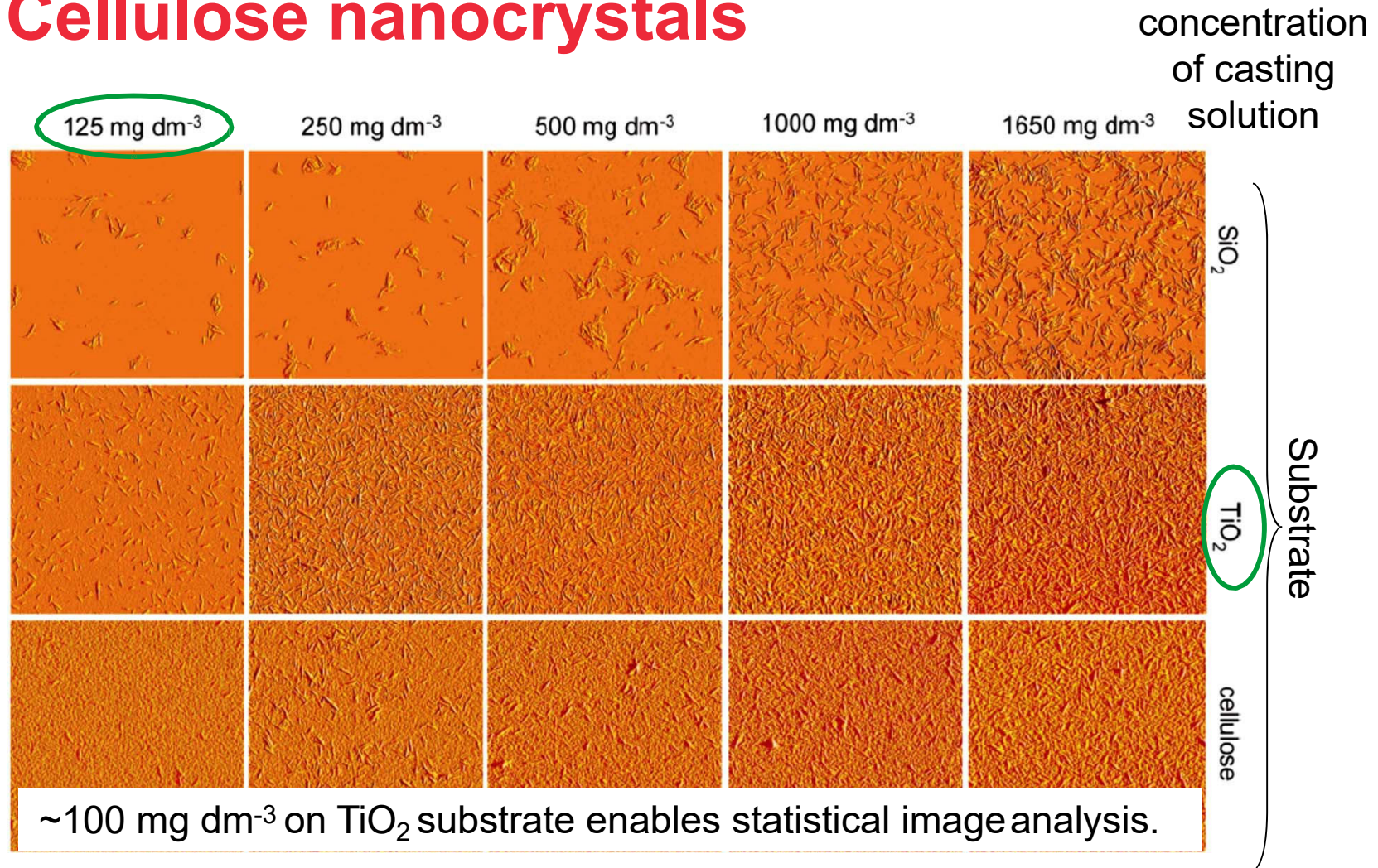
Cryo-TEM image of
nanofibrillar cellulose (NFC)



Fibril size: 5-10 nm

TIP EXAGGERATION YIELDS UNRELIABLE WIDTHS FOR NFC

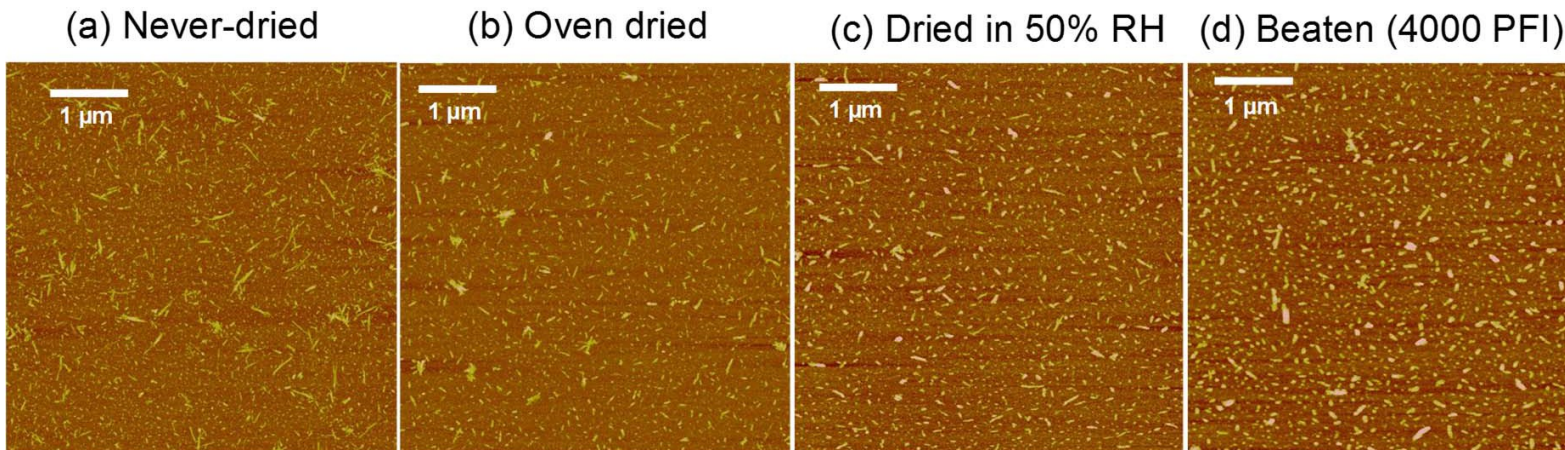
Cellulose nanocrystals



Langmuir **2007**, 23, 9674.

Cellulose nanocrystals

Nanocrystals from bleached softwood kraft pulp with different pretreatments

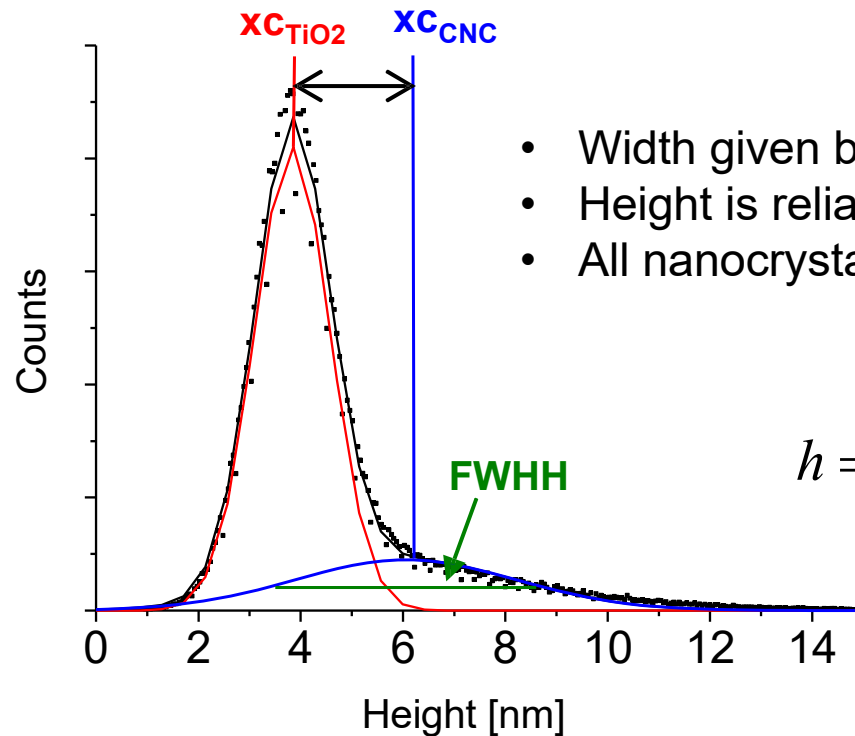


- Most of the nanocrystals in all samples are very small (<100 nm)
- Nanocrystals from never-dried pulp contains visibly nanocrystals that are visibly larger than in the other pulps
- Width (height) of the nanocrystals is constant in all samples (~ 4 nm)

Cellulose nanocrystals – width

Remember that AFM yields 3D information.

Height distribution histogram of cellulose nanocrystals (CNC) on TiO₂ substrate



- Width given by AFM is not reliable (tip exaggeration)
- Height is reliable
- All nanocrystals in the sample had a height of ~4 nm

$$h = (x_{C_{CNC}} - x_{C_{TiO_2}}) + \frac{FWHH}{2}$$

Cellulose nanocrystals – length

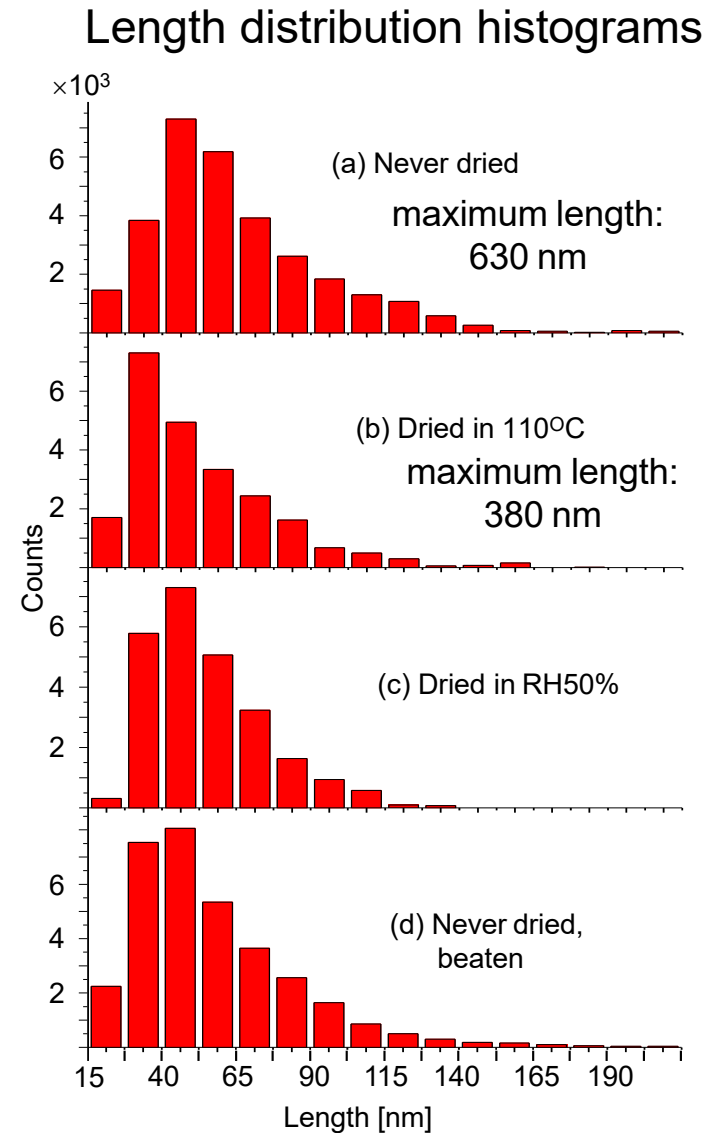
Average length is roughly similar in all samples (~60 nm)

- higher number of longer nanocrystals in never dried samples

- higher amount of shorter nanocrystals in dried samples



ACID HYDROLYSIS IS MORE EFFECTIVE ON DRIED FIBRES



Summary

- AFM is based on a tip that scans across the sample surface
- Surface forces interact between the tip and the sample → image
- Extremely high resolution (down to < 1 nm, **limited by tip error**)

- Height image shows topographical contrasts
- Phase image can distinguish different materials

- AFM can resolve individual cellulose microfibrils (or microfibril aggregates)
- AFM can detect different cell wall layers (fibril angle)
- Morphological appearance of e.g. residual lignin on surface can be visualized by AFM
- AFM enables characterising the distribution and size of pigment in paper coatings
- AFM enables the (statistical) dimensional analysis of cellulose nanocrystals

NOTE: AFM yields morphological information, not chemical

→ combination with other techniques like XPS yields added value
