Aalto University
School of Chemical
Technology

Electron microscopy

CHEM-L2000

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Learning objectives

After this lecture, you should be able to

- understand the basic principles behind electron microscopy
- recognize the difference between scanning electron microscopy and and transmission electron microscopy
- be aware of the main practical possibilities and limitations of electron microscopy

Outline

(1) Background

(2) TEM: principles and applications to lignocellulosics

(3) SEM: principles and applications to lignocellulosics

(4) TEM vs. SEM

Background

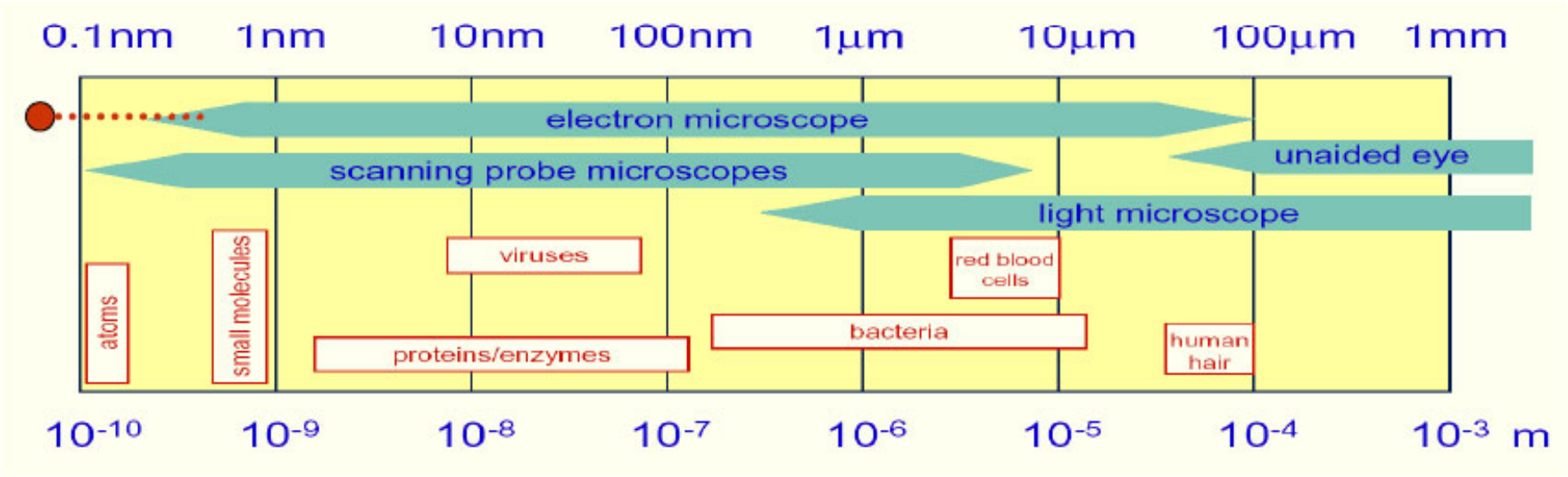
- The resolution in optical microscopy is limited by the wavelength of light, i.e., to 200-300 nm (Abbe 1873)
- Any moving particle or object has an associate wave (de Broglie 1927)
- The wavelength of an electron beam with a voltage of 100 kV is 0.004 nm
- Symmetric electric and magnetic fields can act as lenses for electrons (Busch 1926)

→ Electron emission can be utilized for imaging with very high resolution (in principle)

Background

- Ernst Ruska and Max Knoll (TU Berlin) built the first transmission electron microscope in 1931
- In 1933, Ruska managed to improve the resolution beyond optical microscopy
- First commercial instrument in 1939 (Siemens)
- Ernst Ruska received a Nobel prize in physics in 1986

Note on the resolution



Note on the techniques

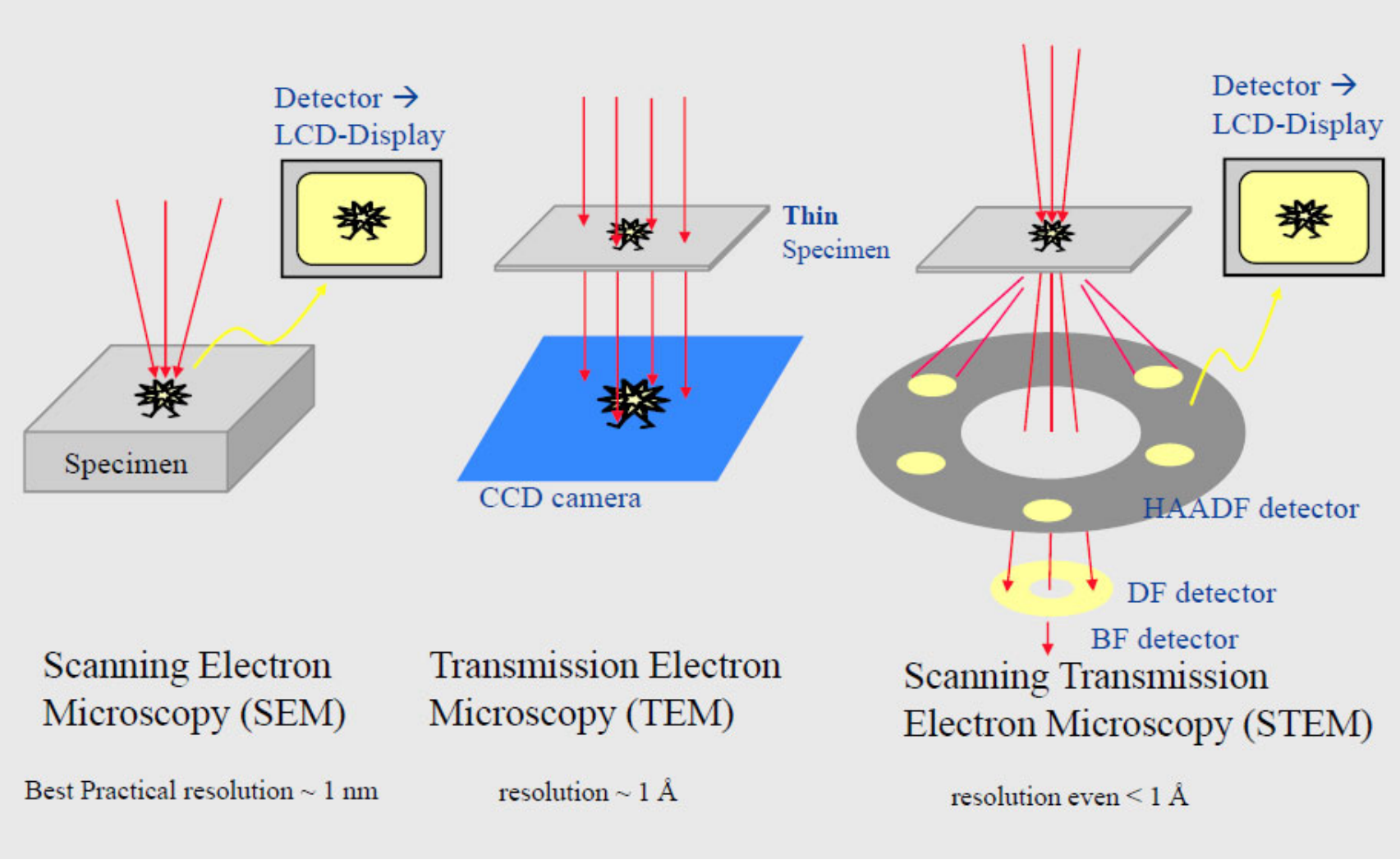
Electron microscopy is a generic term.

Two major techniques fall under the term electron microscopy:

Transmission Electron Microscopy (TEM)

Scanning Electron Microscopy (SEM)

Working principles of SEM and TEM



Working principles of SEM and TEM

SEM
The electrons scatter or cause emission of secondary electrons
→ images the topography

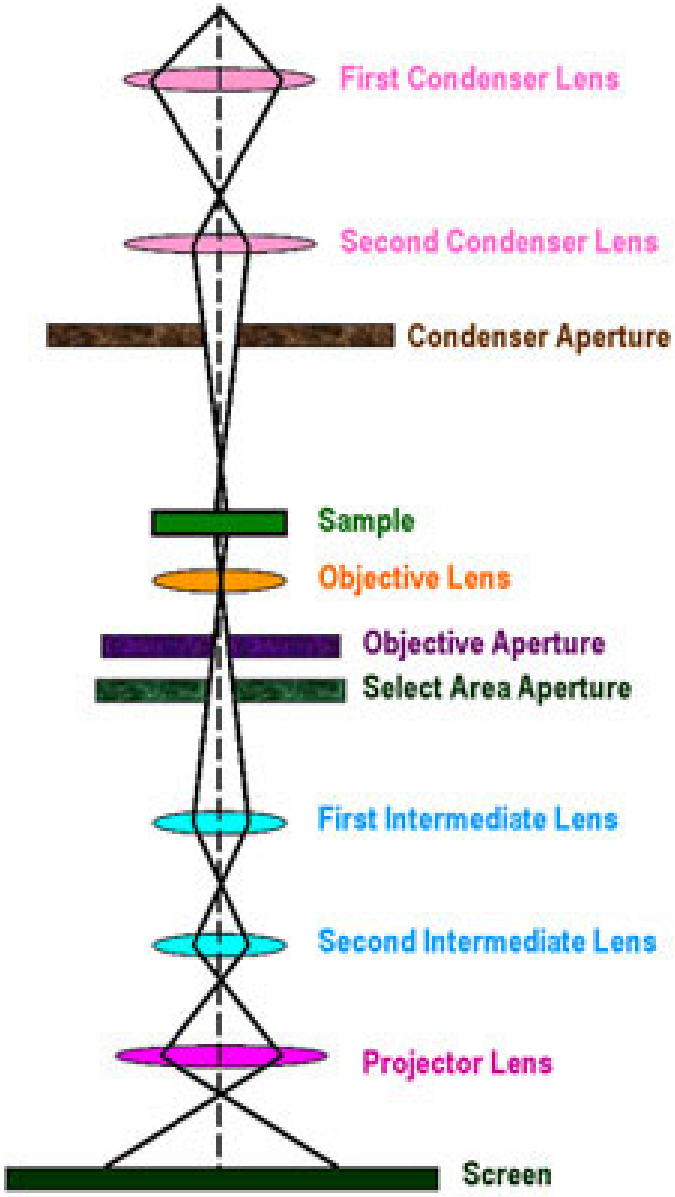
TEM
The electrons go through the sample
→ images the whole sample throughout

TEM

TEM instrument

- Lenses focus the electron beam
- Apertures filter the electrons
- The image is projected on a fluorescent screen

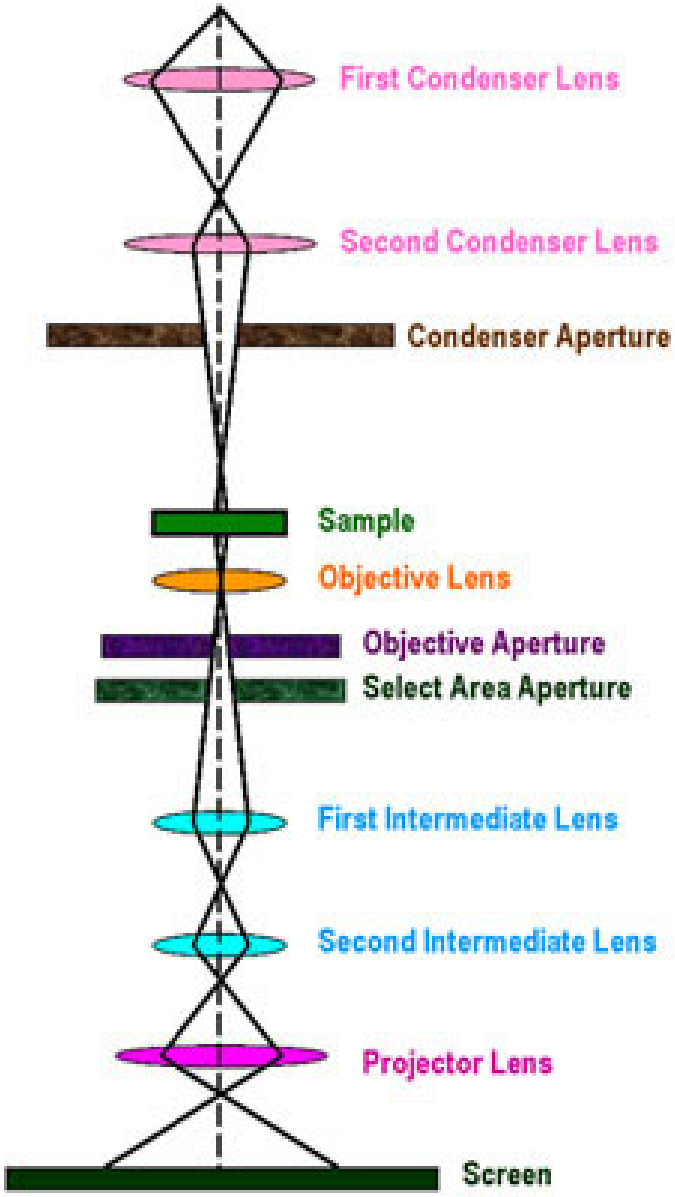
Measuring requires an ultra high vacuum (UHV).



TEM instrument

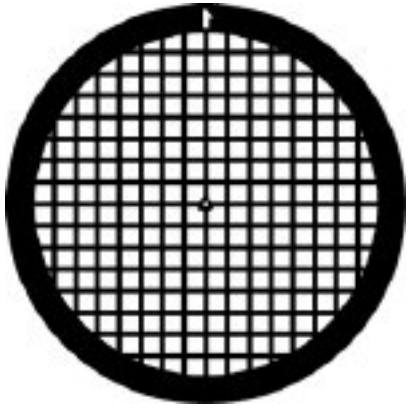
The main limitation of TEM arises from its principle:

The sample must be very thin (< 100 nm) for the electrons to pass through.



TEM instrument

Sample holders are grids with ca. 3 mm diameter and around 100 µm mesh size



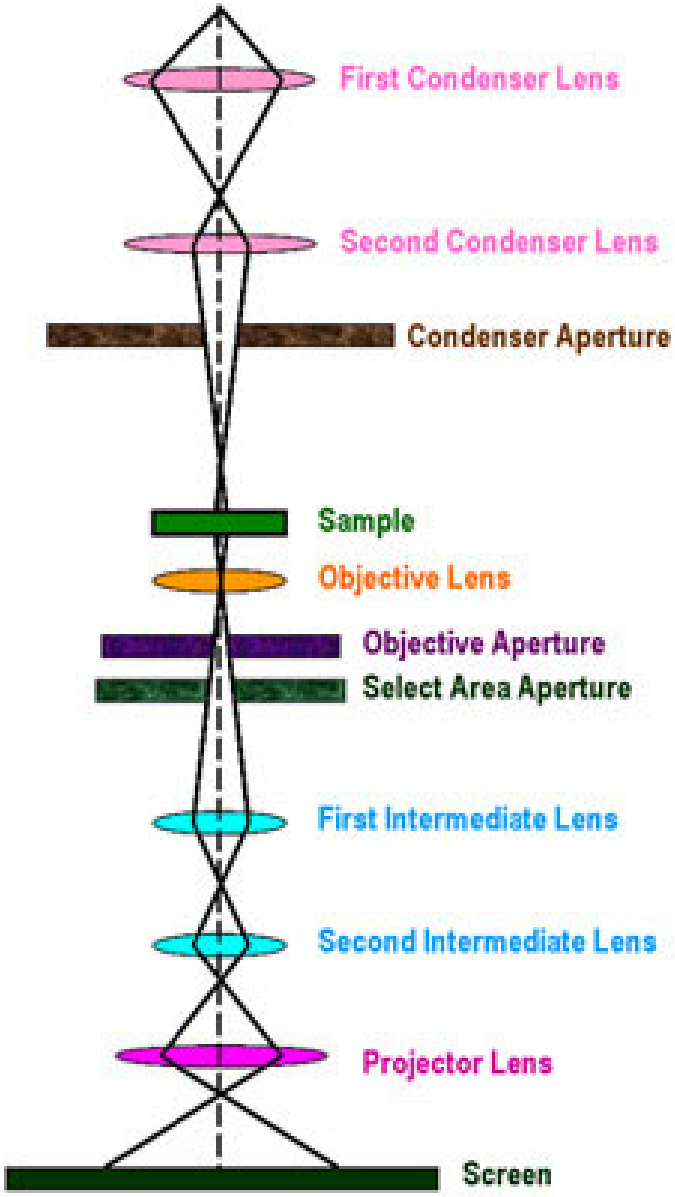


Image formation in TEM

Bright field image

- Contrast due to mass-thickness/ diffraction contrast
- Crystals appear black in the image
- Typical for soft materials or crystals

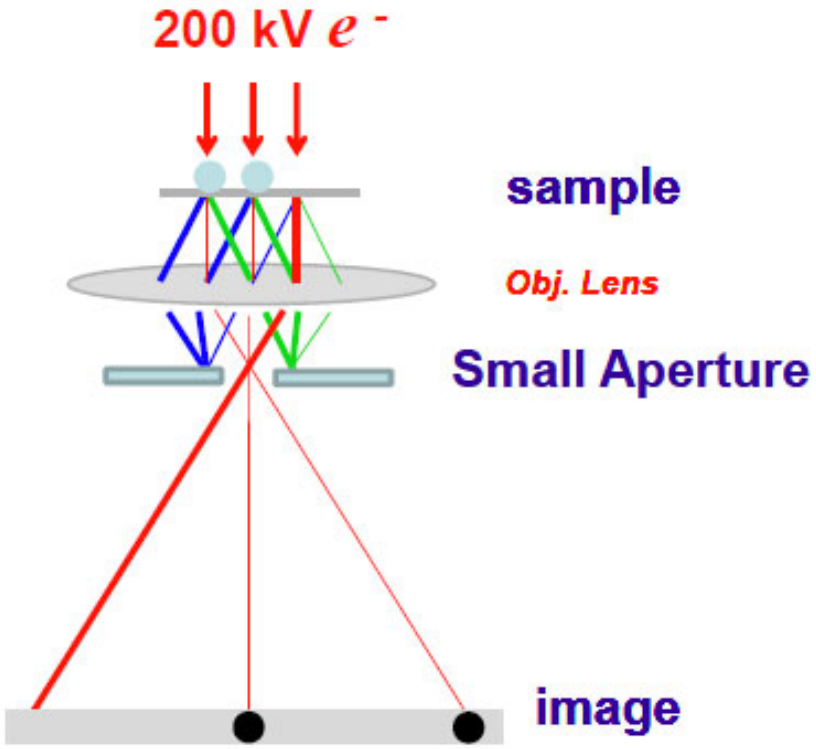


Image formation in TEM

Dark field image

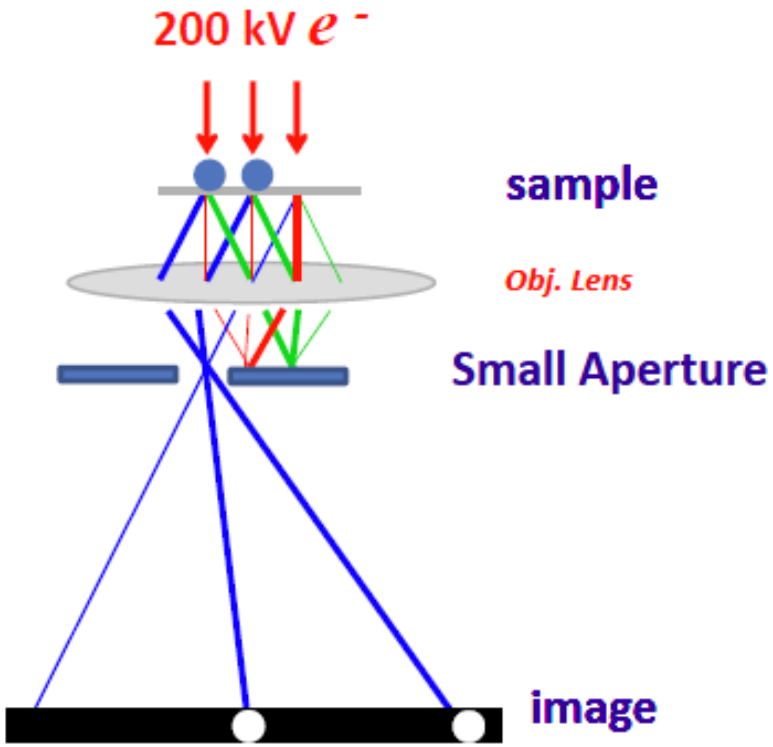
- Contrast due to diffraction

- Unscattered electrons are excluded from the image

→ Locations where there is no material to scatter the electrons, appear dark in the image

→ Crystals appear white in the image

- Typical for crystalline materials



NOTE: The physics behind electron scattering is highly complex.

Sample preparation for TEM

- TEM requires extremely thin samples (< 100 nm)
- Samples can be cut with *an ultramicrotome* (pictured right), usually equipped with a diamond knife
- Cutting is prone to introduce severe artifacts on the sample

- Porous samples are generally embedded in resin before cutting

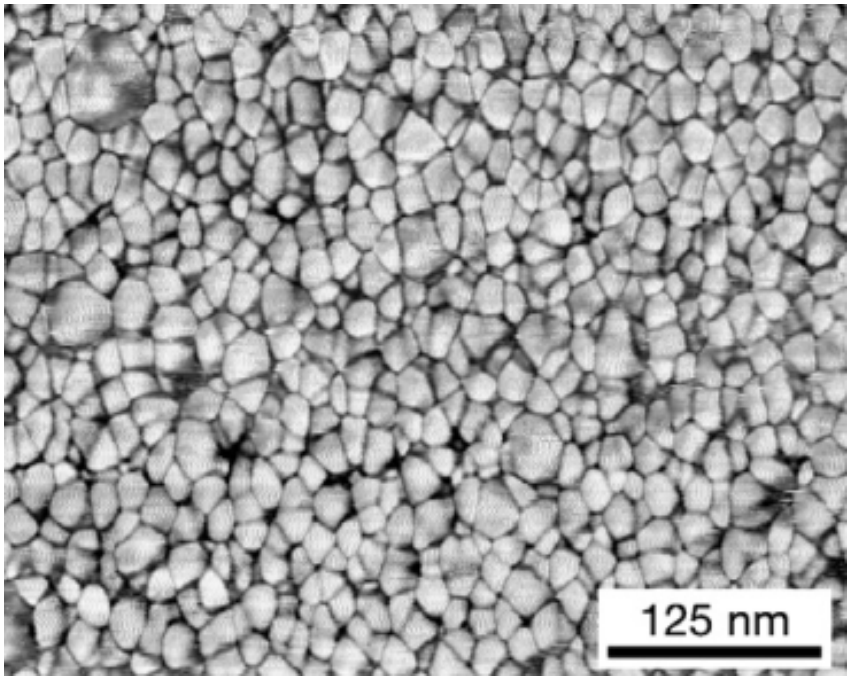
Sample preparation for TEM

- Frozen samples can be cut in a cryo ultramicrotome (pictured right)
- Cryo-cut samples can be further imaged frozen in a cryo TEM

→ Enables imaging of solutions and dispersions

Imaging wood cell wall with TEM

Aggregates: 12-20 nm



TEM image of radial cross-section of wood cell wall.

Zimmermann et al.
J. Struct. Biol. **2006**, *156*, 363.

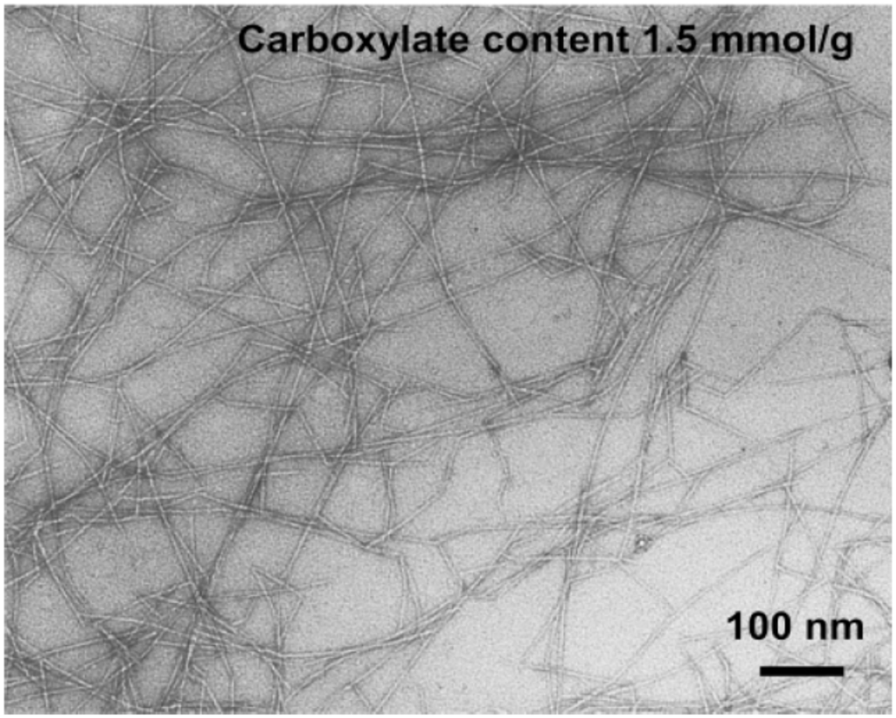
Individual microfibrils: ~3.5 nm

TEM image of longitudinal cross-section of chlorite delignified pine cell wall; freeze-dried and stained.

Heyn *J. Ultrastructure Res.* **1969**, *26*, 52.

Individualization of microfibrils

TEM is able to resolve microfibrils individualized by TEMPO-oxidation



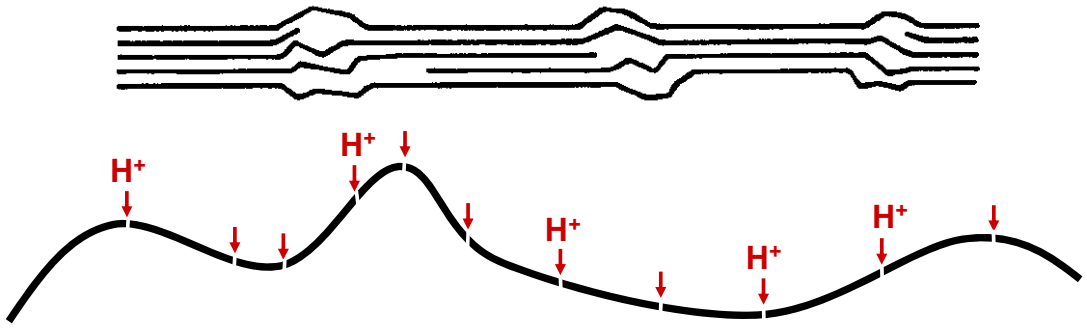
Highly monodisperse individual microfibrils (3-4 nm width) with celluronic acids on the microfibril surface.

Saito et al. *Biomacromolecules* **2007**, *8*, 2485.

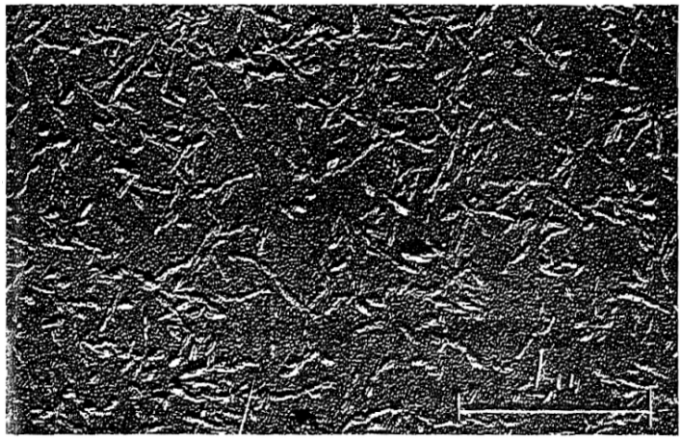
TEM for dimensional analysis

- Cellulose microfibril consists of ordered (crystalline) and disordered regions
- The disordered segments can be selectively targeted with acid hydrolysis

→ RESULT: cellulose nanocrystals



RESULT:
cellulose nanocrystals



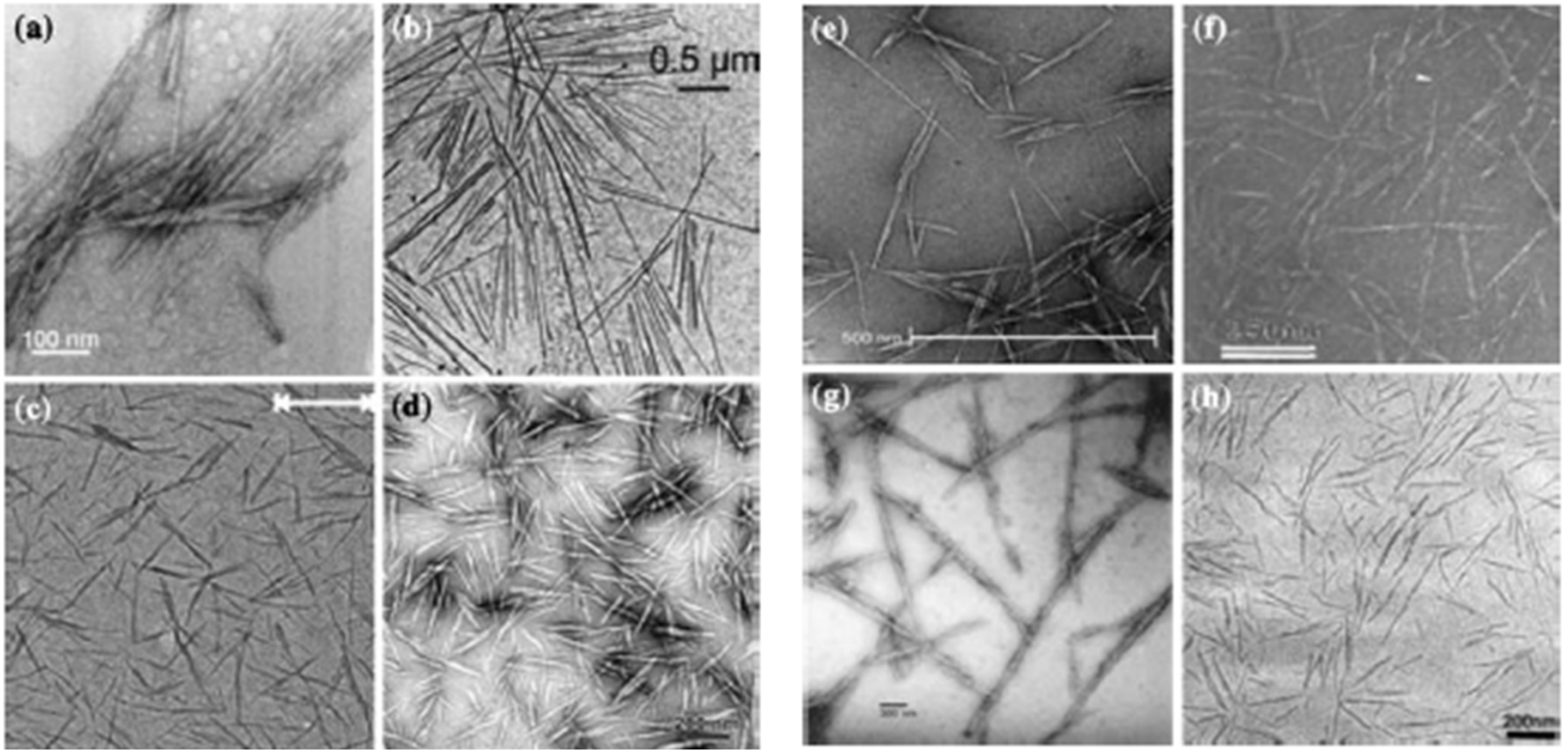
TEM image

Rånby *Discuss. Faraday Soc.* **1951**, *11*, 158.

TEM for dimensional analysis

Nanocrystal dimensions depend on the starting material (botanical source).

Microcrystalline cellulose | Tunicate | Sisal | Straw

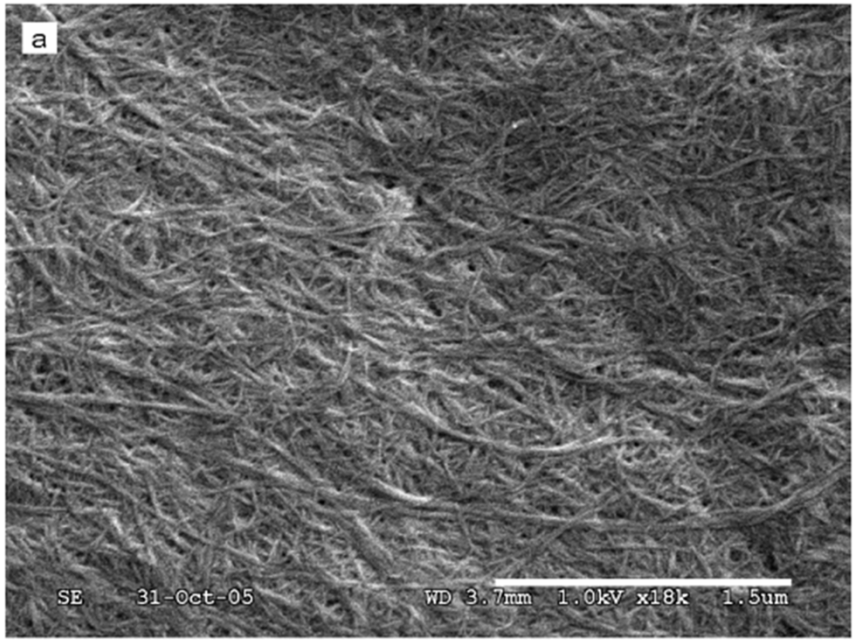


Cotton | Ramie | Bacterial cellulose | Sugarbeet

Eichhorn et al. *J. Mater. Sci.* **2010**, *45*, 1.

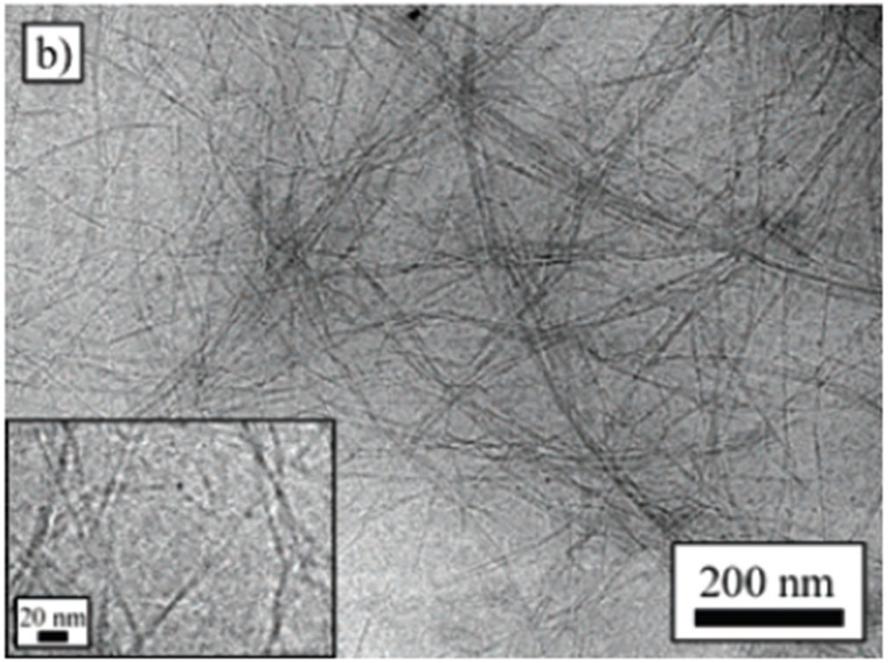
Cryo TEM of cellulose nanofibrils

SEM image of isolated cellulose nanofibrils
→ aggregation during sample preparation



Henriksson et al.
Biomacromolecules **2008**, *9*, 1579

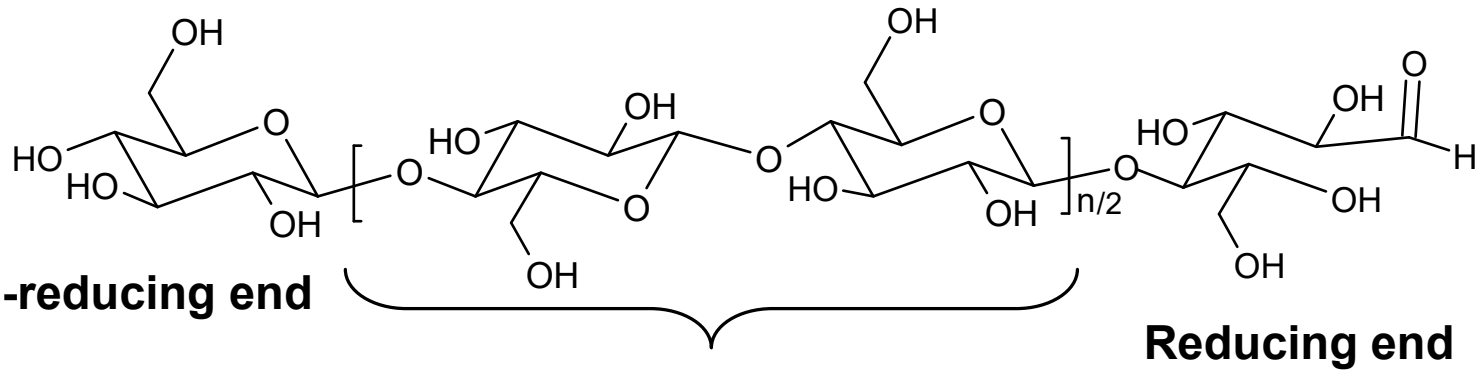
Cryo TEM image of isolated cellulose nanofibrils in aqueous dispersion
→ no aggregation



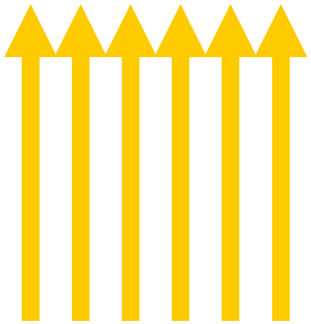
Pääkkö et al.
Biomacromolecules **2007**, *8*, 1934.

Case of cellulose I *vs.* cellulose II

Cellulose chain has a direction:

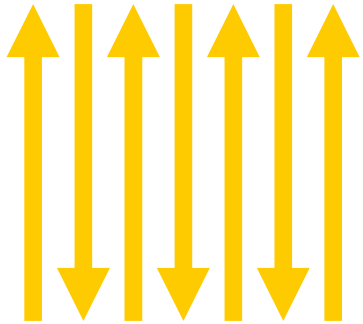


Cellulose I



Parallel

Cellulose II



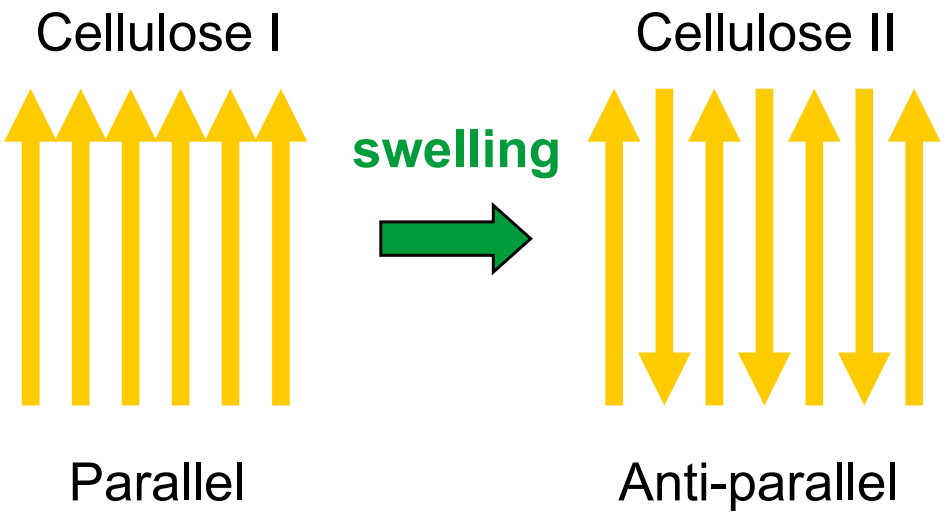
Anti-parallel

Case of cellulose I *vs.* cellulose II

Cellulose II

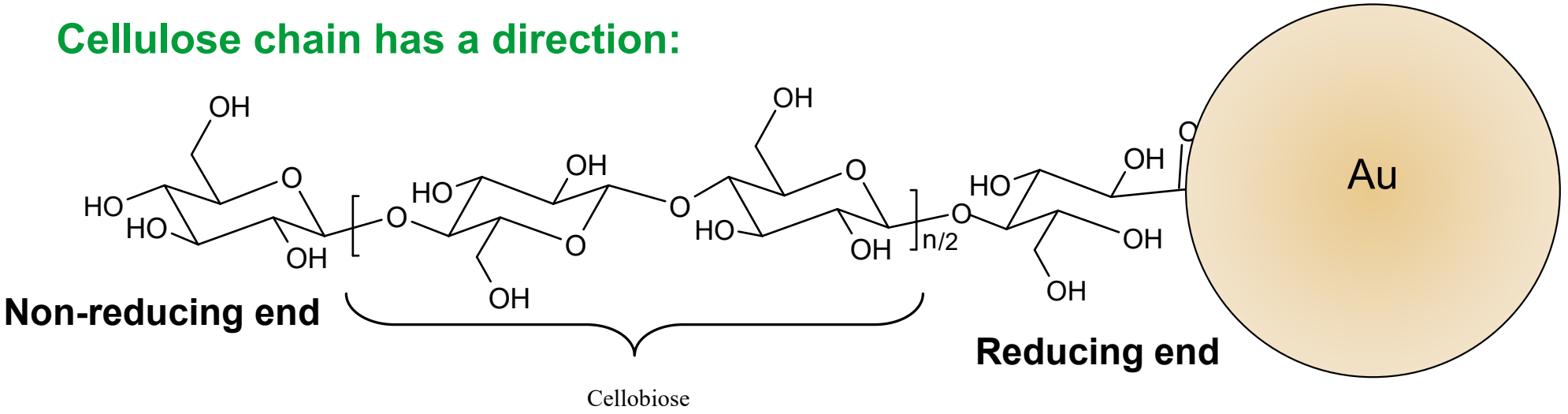
Preparation by: - dissolving the cellulose / regeneration
- swelling in concentrated alkali

How is it possible for the cellulose chains to transform from parallel to anti-parallel without dissolution?



Case of cellulose I *vs.* cellulose II

Cellulose chain has a direction:



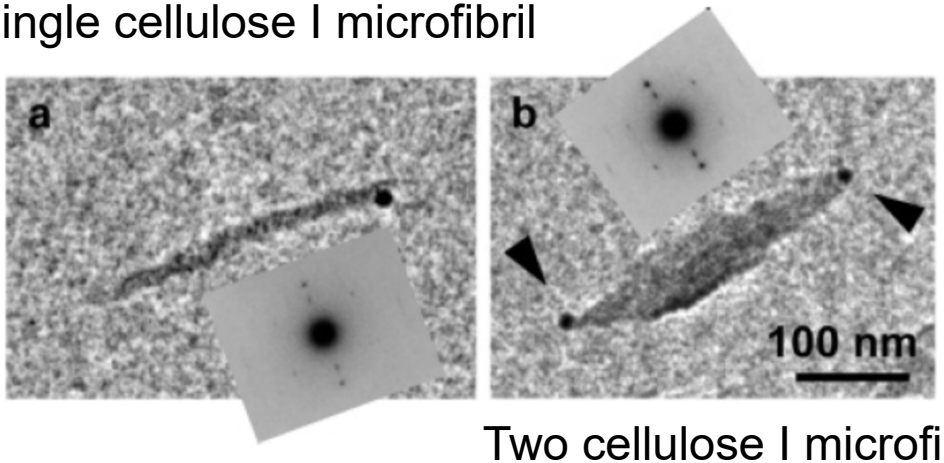
Reducing ends of cellulose chains can be labelled with functionalized gold nanoparticles

→ Heavier elements appear distinct in TEM

Case of cellulose I *vs.* cellulose II

CELLULOSE I

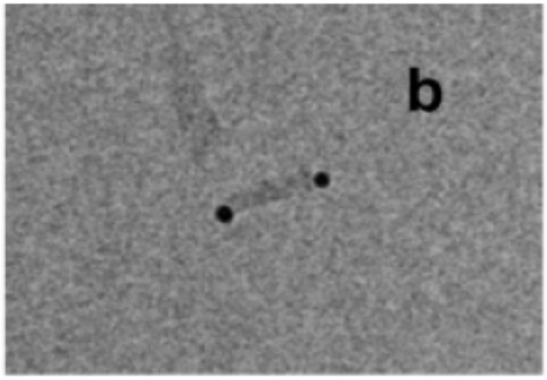
Single cellulose I microfibril



Two cellulose I microfibrils

Swelling in NaOH (aq)
(i.e. mercerization)

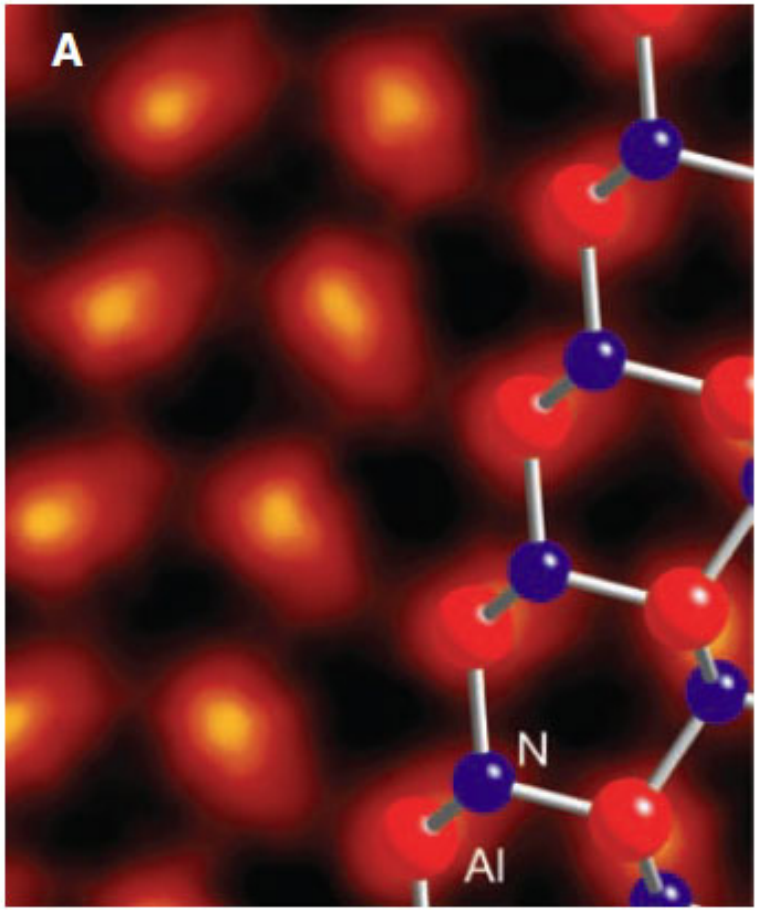
CELLULOSE II



Single cellulose II microfibril

Kim et al. *Biomacromolecules* **2006**, *7*, 274.

Note: atomic resolution



Atomic resolution is feasible with TEM. However, only when coupled with quantum mechanical calculations.

Individual atoms do not absorb electrons like bulk material does.

In principle, the resolution of a modern aberration-corrected TEM is < 0.1 nm.

Science **2008**, *321*, 506.

SEM

Background

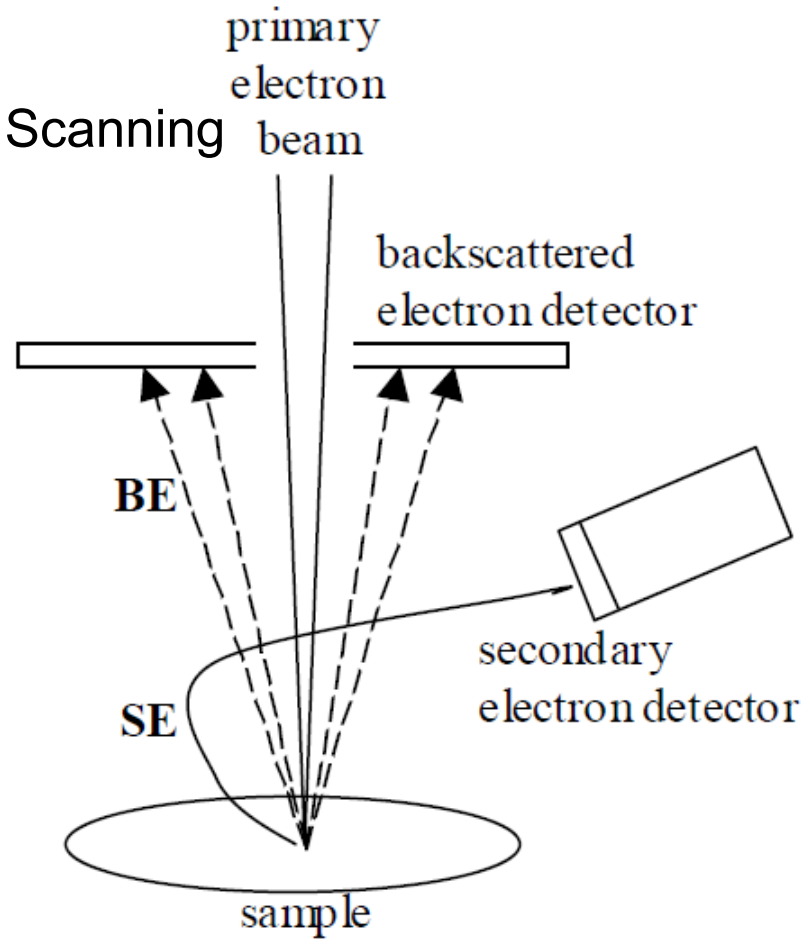
- The most obvious limitation of TEM is the requirement of ultrathin samples
- To circumvent this requirement, Scanning Electron Microscopy (SEM) has been developed
- SEM is based on scattering or emission of electrons from the sample surface

→ Surface of bulk samples can be imaged

Background

- Physical principles of electron beam/material –interactions were laid down already in 1937 by Manfred von Ardenne
- Group of Sir Charles Oatley in Cambridge (UK) developed SEM throughout 1940s and 1950s
- The first commercial instrument was manufactured in 1965

Principle


Scanning
primary
electron
beam
backscattered
electron detector
BE
SE
secondary
electron detector
sample

Secondary electrons

- Most popular imaging mode
- Secondary electrons are released from the atoms on the sample surface (depth of just a few nanometers)
- Contrast is based on orientation: points on the surface facing the detector appear brighter than the ones pointing away from the detector

→ 3-dimensional appearance for the image (although the quantitative information from the image is 2-dimensional)

Backscattered electrons

- Backscattered electrons are primary electrons that elastically scatter from the sample

- Heavier elements scatter more electrons

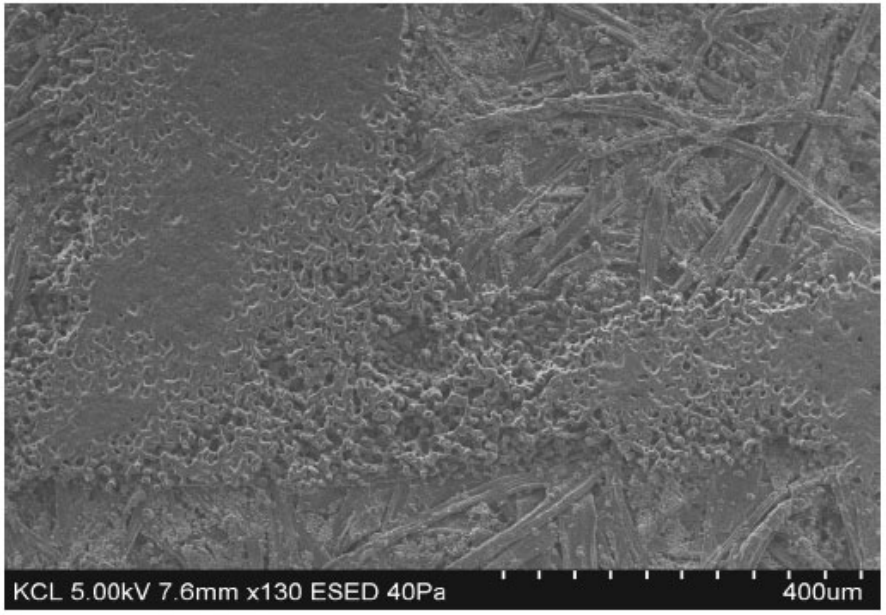
→ heavier elements appear darker in the image

- Electrons are backscattered from a larger area than secondary electrons are generated

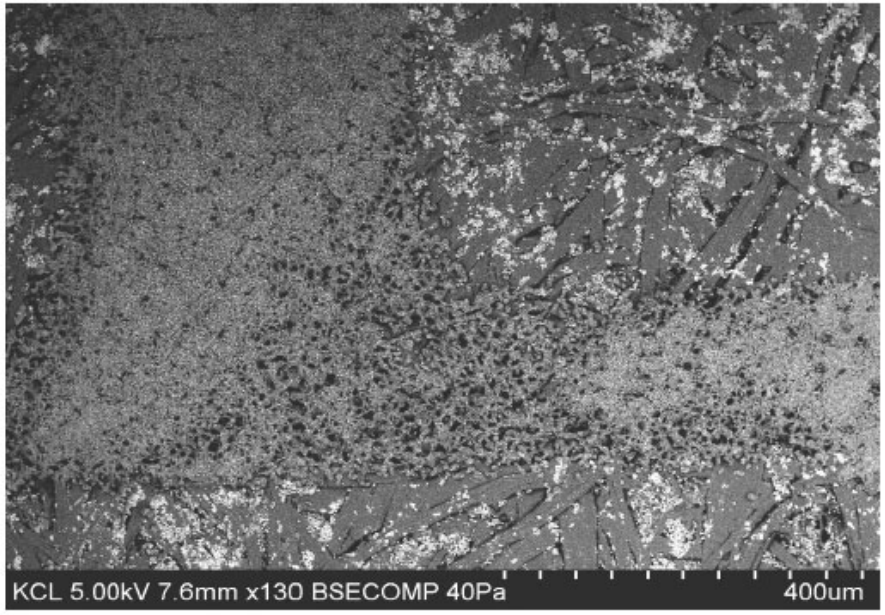
→ Images from backscattered electrons possess a poorer spatial resolution than the images from secondary electrons

Secondary *vs.* backscattered electrons

Images of ink on a paper surface



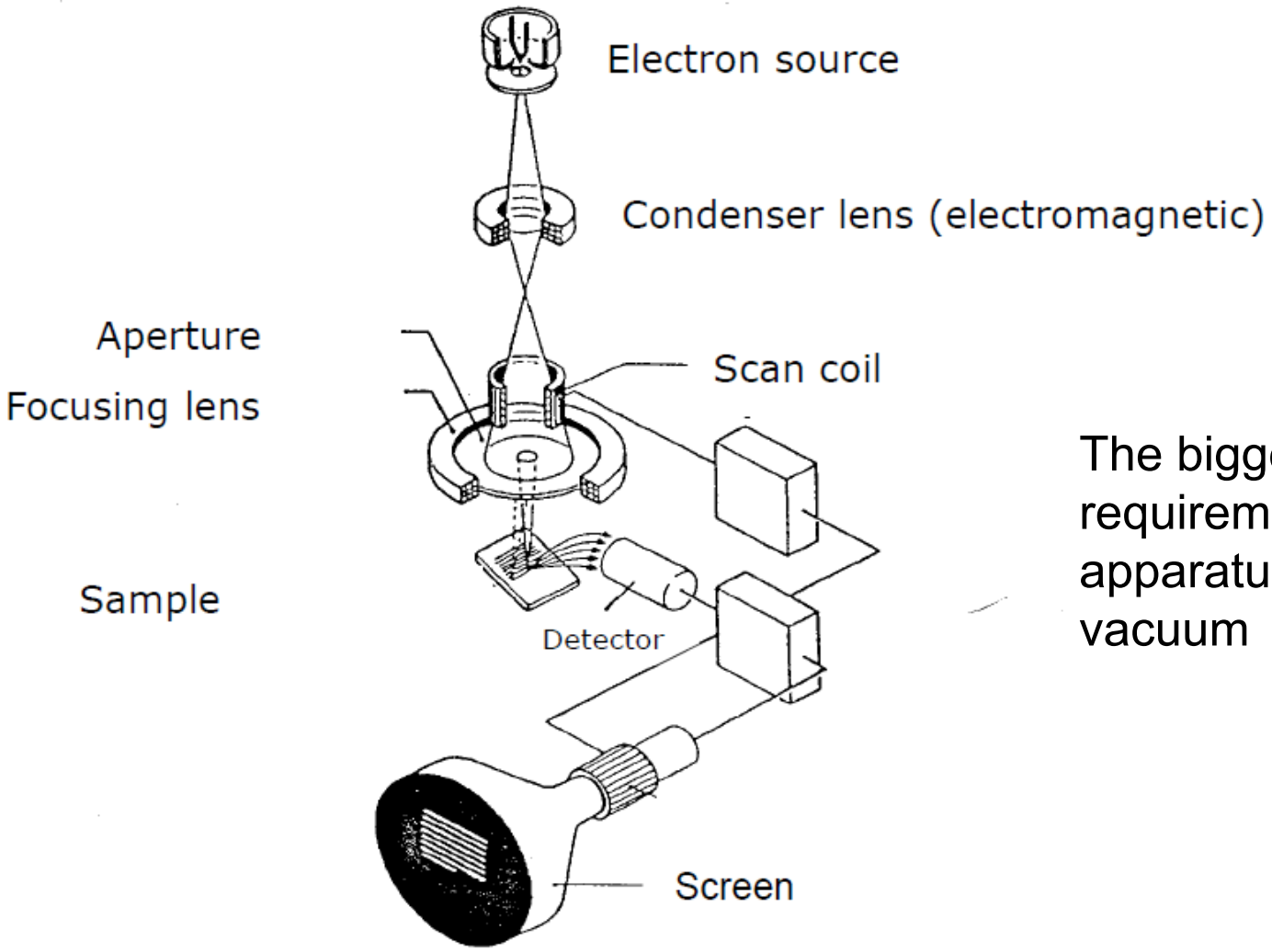
Secondary electron image



Back-scattered electron image

Images courtesy of Tiina Pöhler (VTT)

Instrumentation



The biggest practical requirement in a SEM apparatus is ultra-high vacuum

Image courtesy of Tiina Pöhler (VTT)

Sample preparation

- Samples should be conductive

- Most bio-based samples are organic and not conductive

→ Non-conductive samples must be coated with a very thin metal layer such as Au, Pt or Pd (*sputtering*)

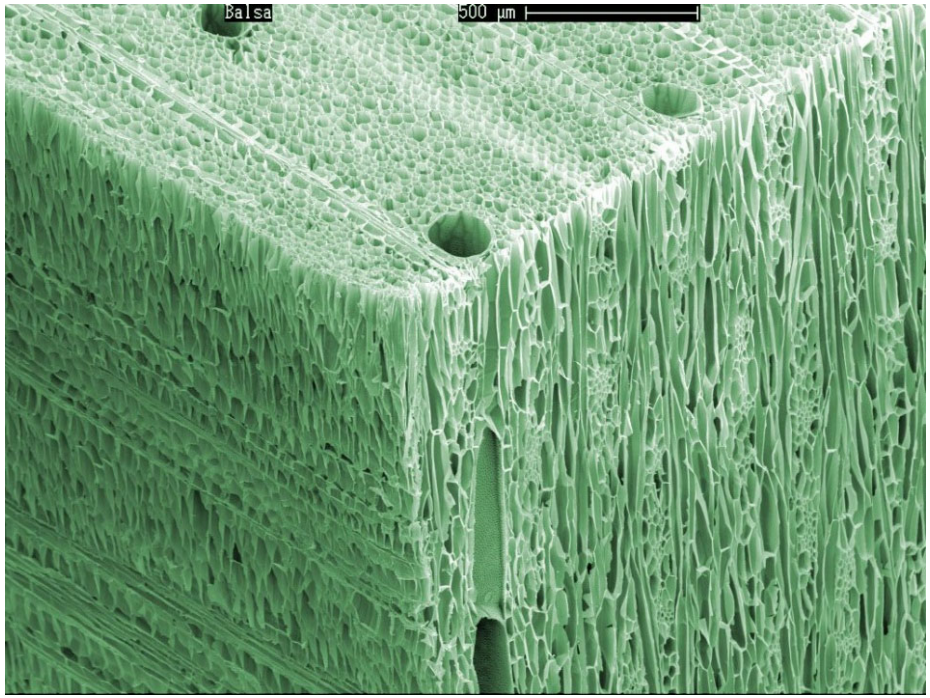
Sample preparation

- Samples should be dry (because of ultra high vacuum)
- Biological samples often contain water

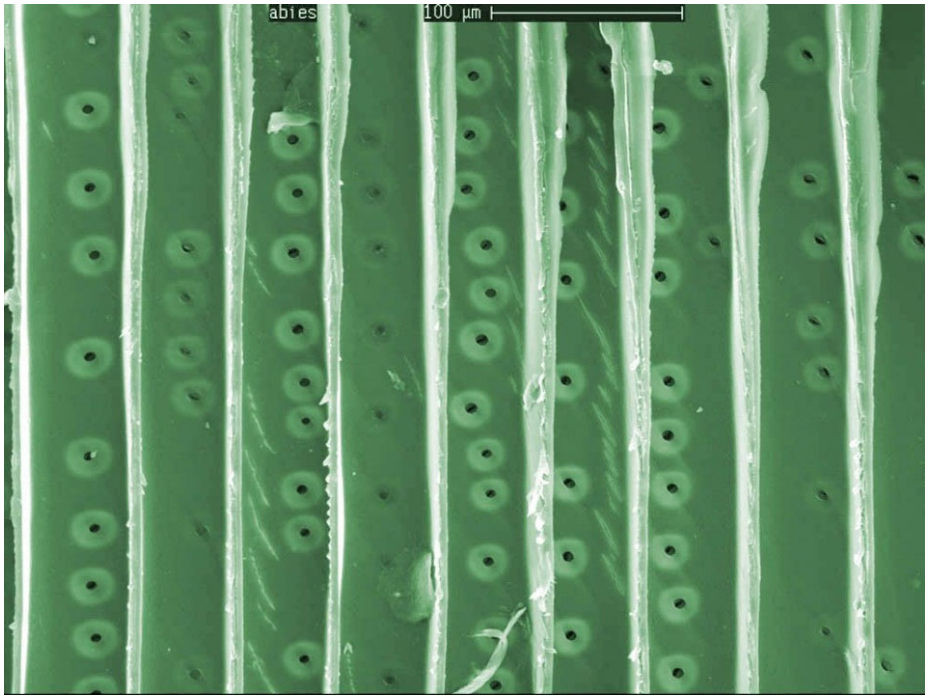
Drying methods:

- simple oven drying (elevated temperature)
- freeze drying (sublimation)
- critical point drying (solvent exchange)

SEM imaging: wood cells

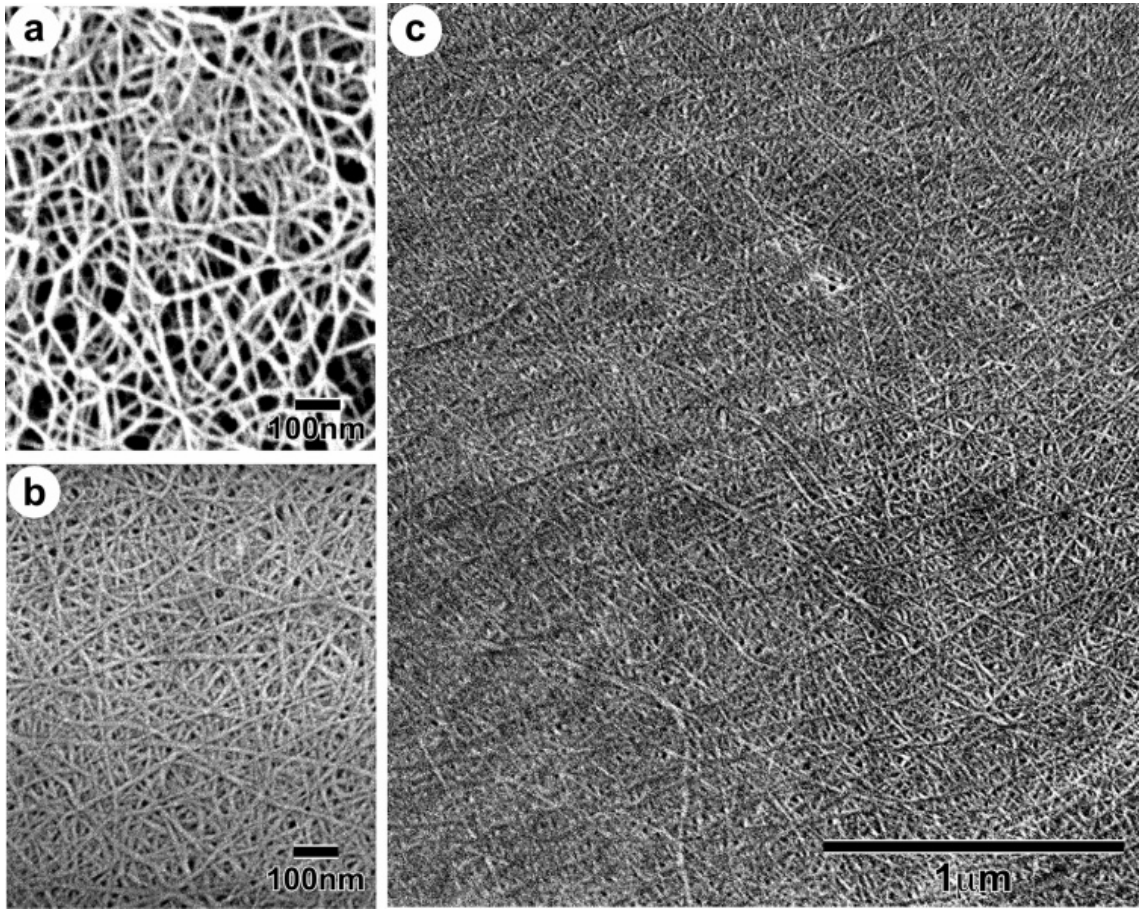


Wood xylem



Tracheids with bordered pits

SEM imaging: nanofibrillar cellulose

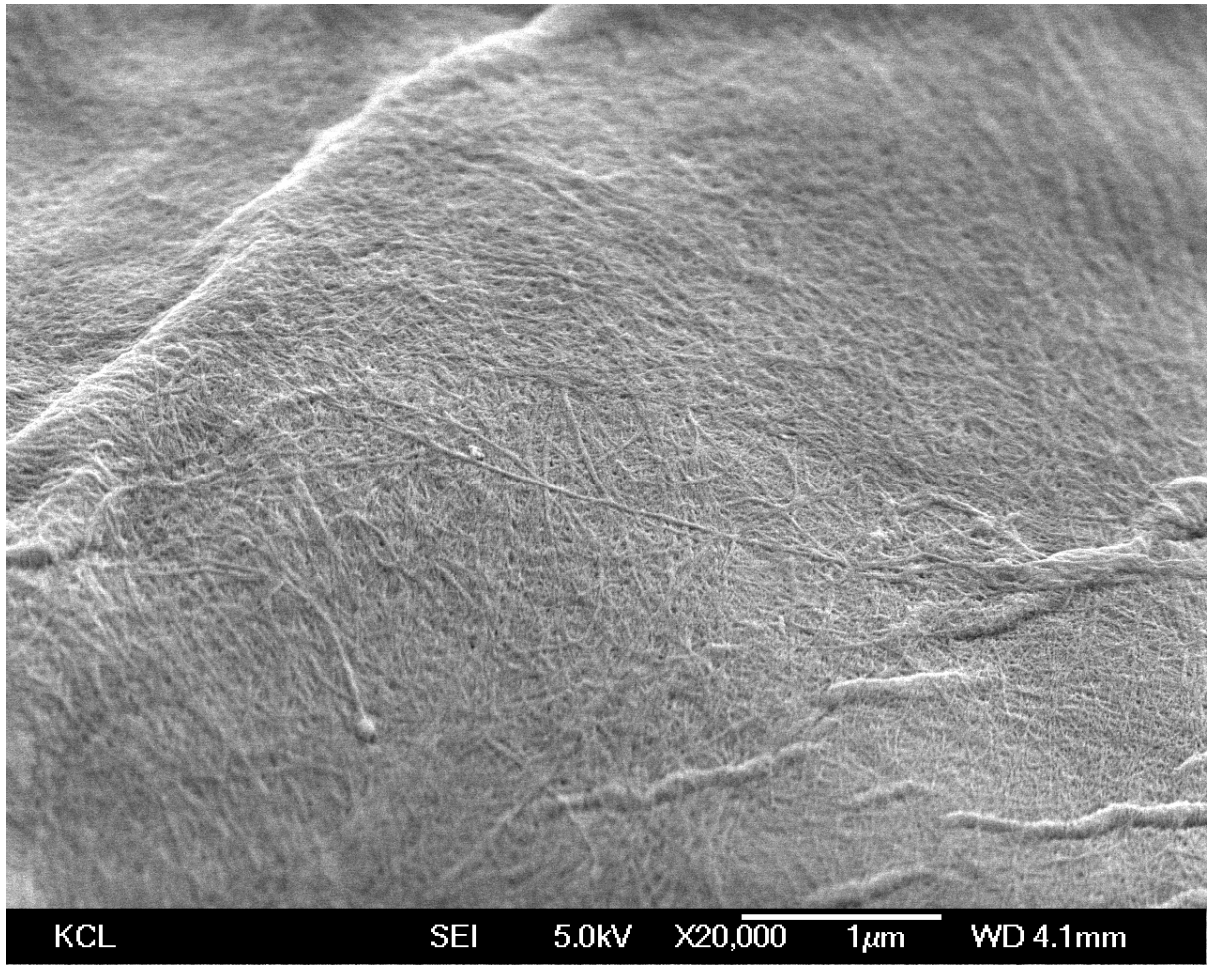


Nanofibrillar cellulose extracted from delignified wood.

Width: 15 nm

Abe et al. *Biomacromolecules* **2007**, *8*, 3276.

SEM imaging: fiber surface



Surface of bleached softwood kraft pulp fibre

Microfibrils are visible but only just

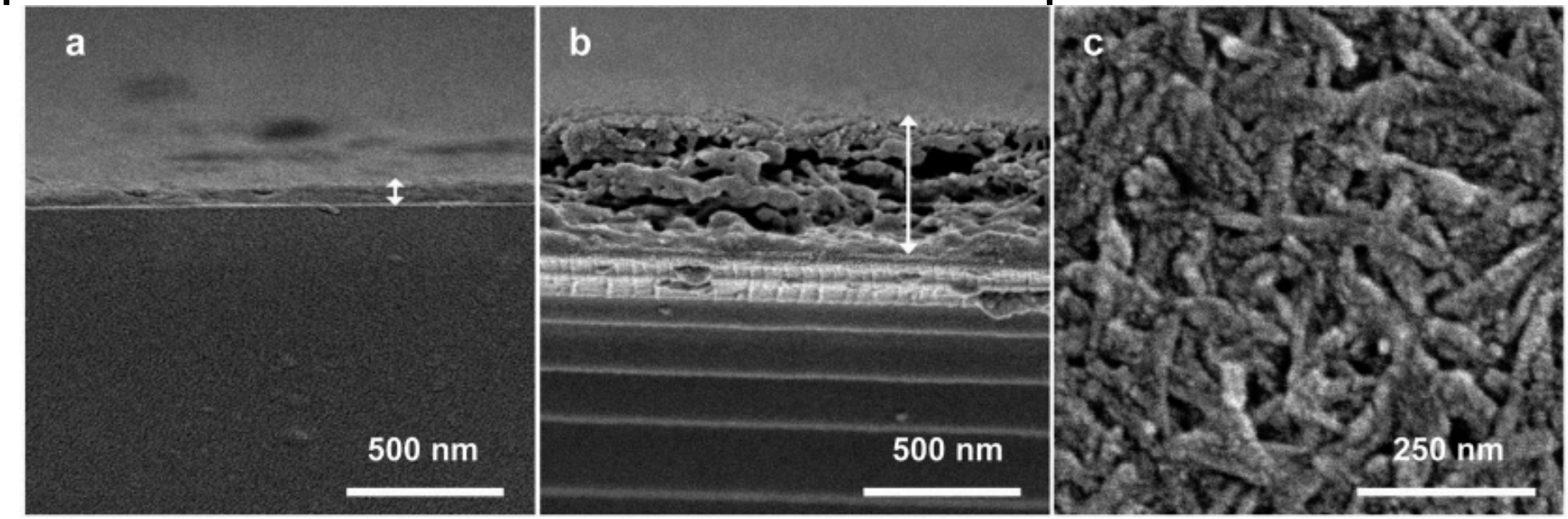
More detailed analysis requires TEM and cross-sectioning

Thin films cross-sections with SEM

Supported films from cellulose nanocrystals and a cationic polyelectrolyte

Cross sections

Top view

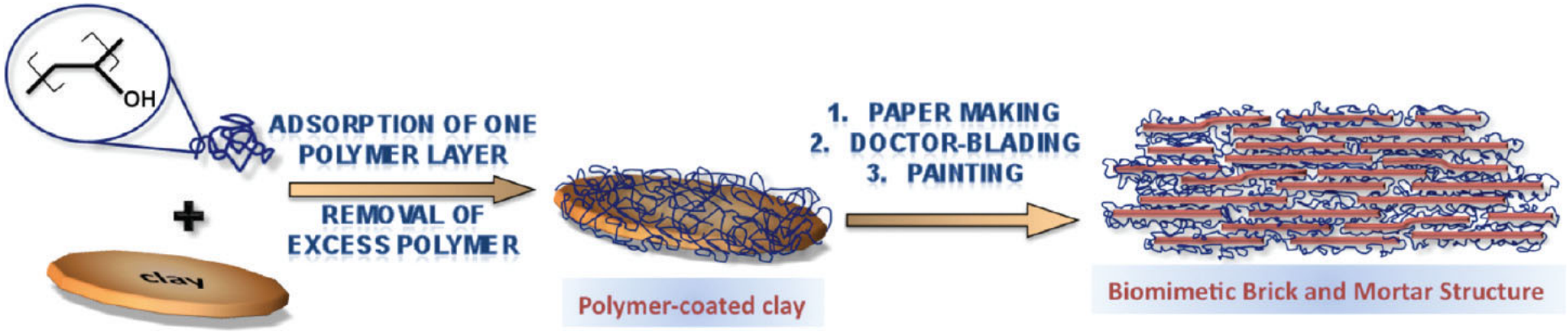


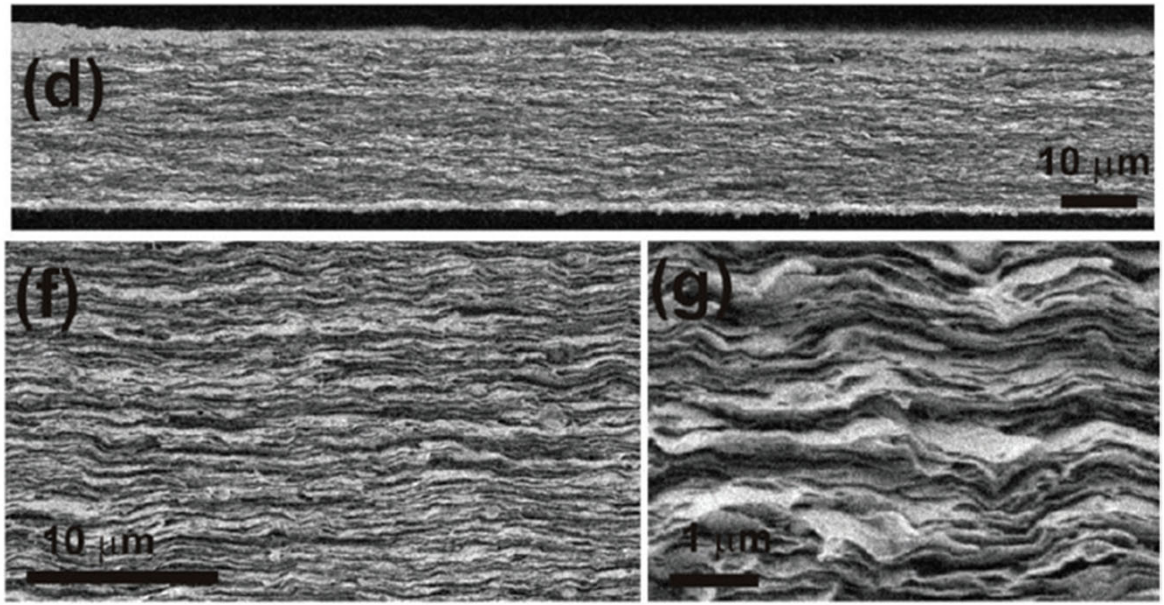
Preparation:
layer-by-layer
deposition

Preparation:
spin coating

Biomacromolecules **2006**, *7*, 2522.

Thin films cross-sections with SEM

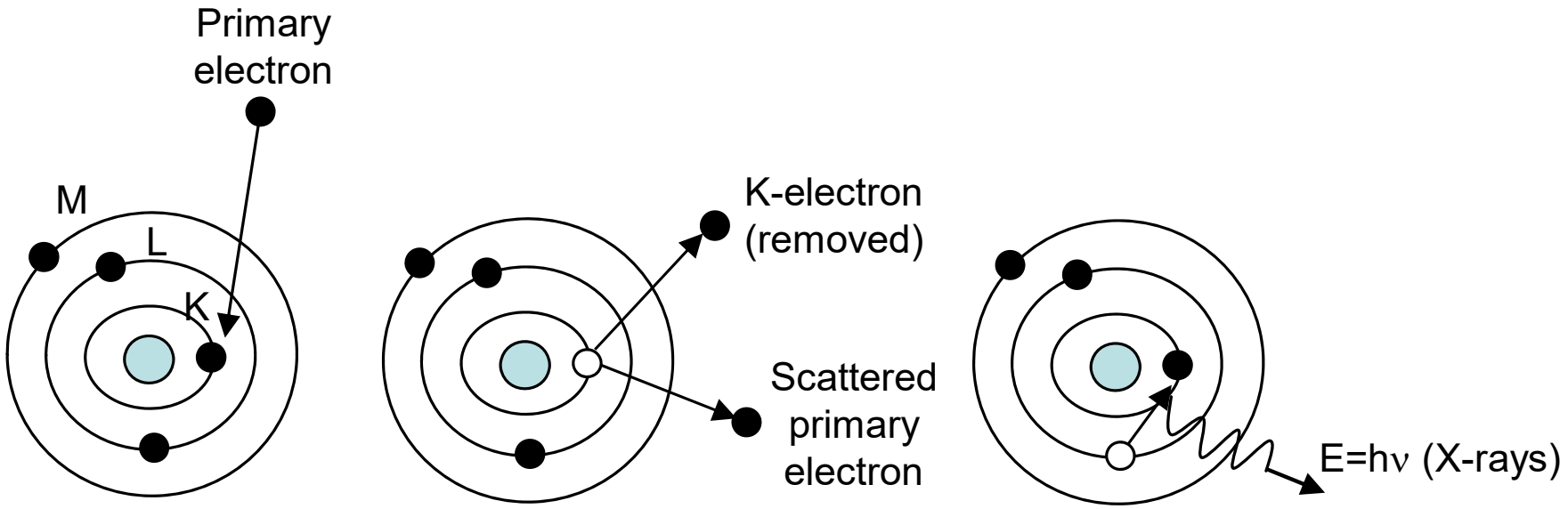




SEM images of cross sections expose the homogeneous, layered structure.

Nano Lett. **2010**, *10*, 2742.

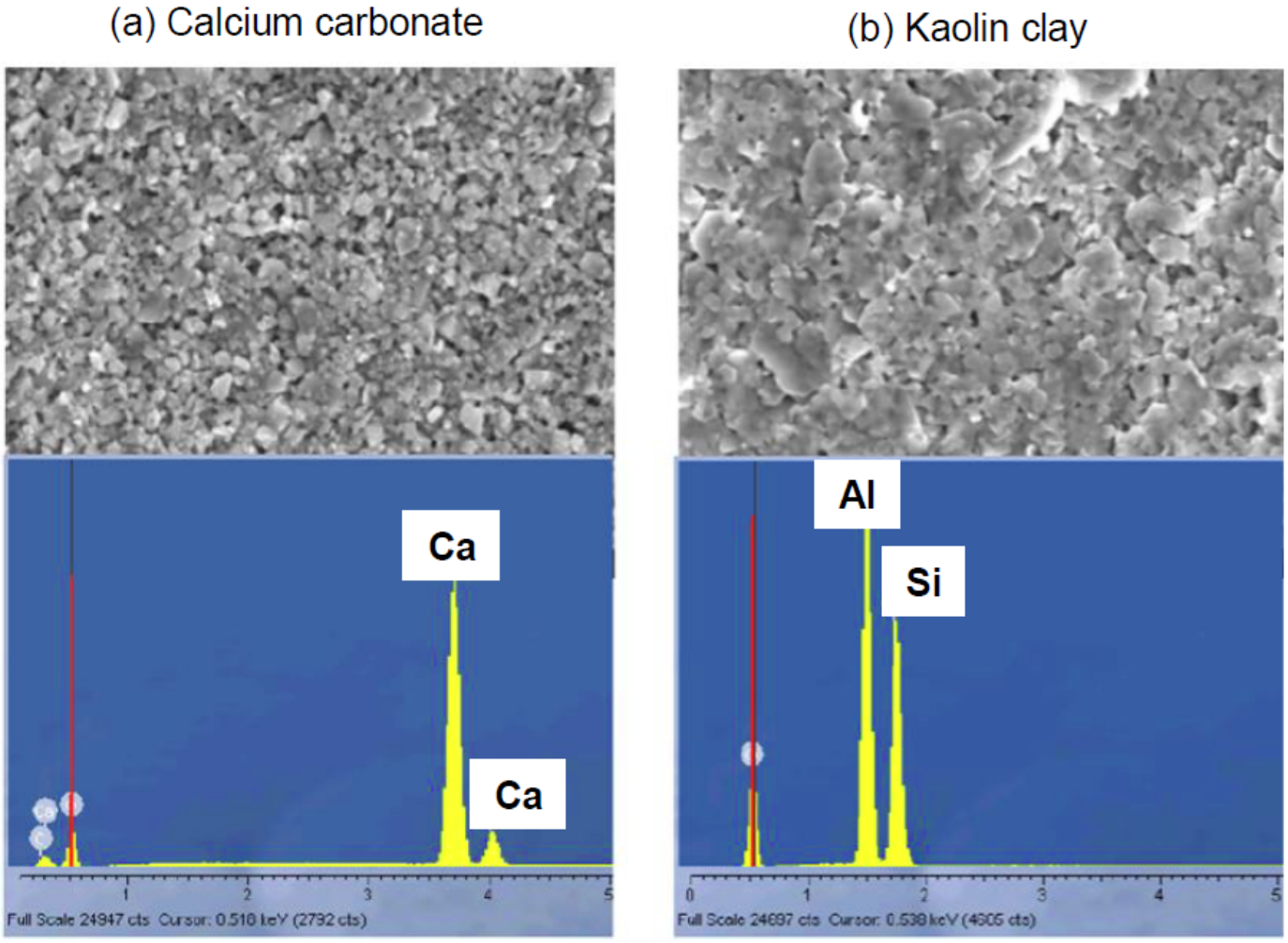
Further accessory with SEM: Energy Dispersive X-ray analysis (EDX)



- Electrons induce emission of X-rays from the sample material
- X-rays are element-specific

→ analytical tool

SEM accompanied with EDX



SEM accompanied with EDX

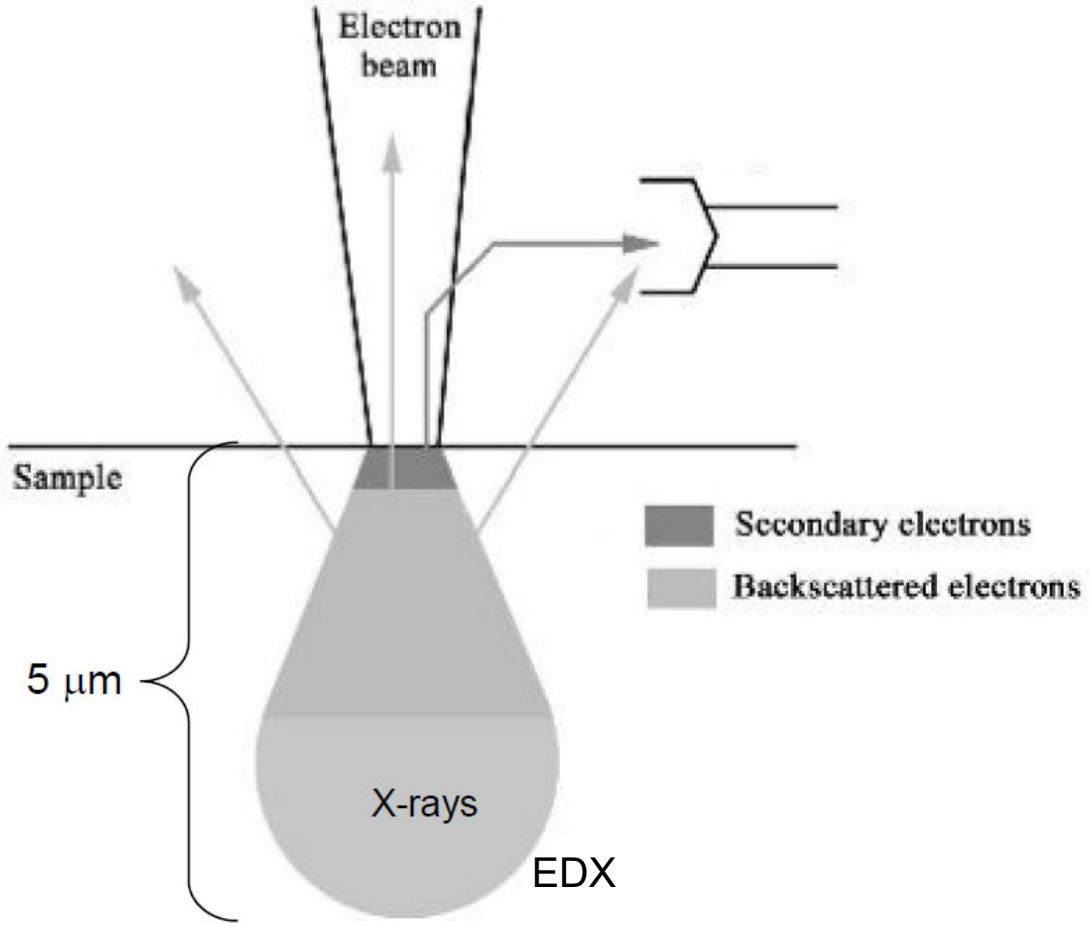
- Nearly all modern SEM instruments contain an X-ray detector for EDX

- It is feasible to choose specific, interesting features from a SEM image and focus an EDX analysis on that particular spot

→ elemental composition of certain features

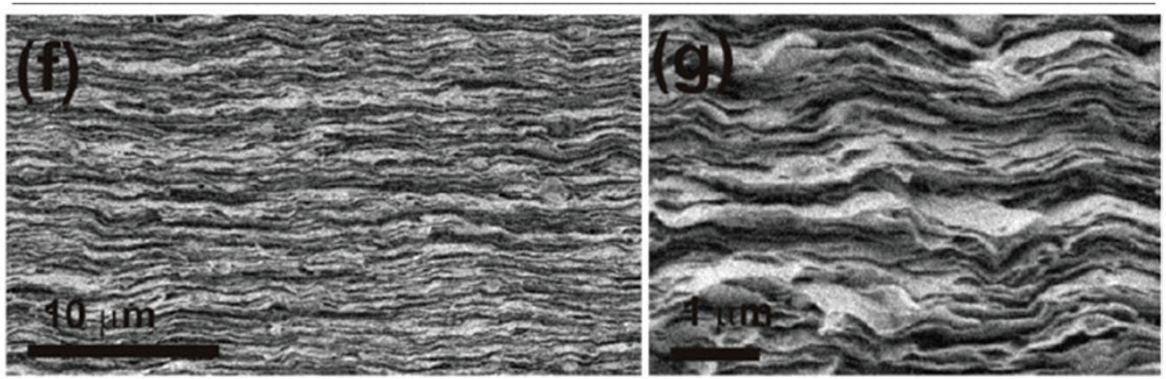
- Mapping of elemental composition is also possible but it is usually worthwhile with inorganic samples

Analytical depth

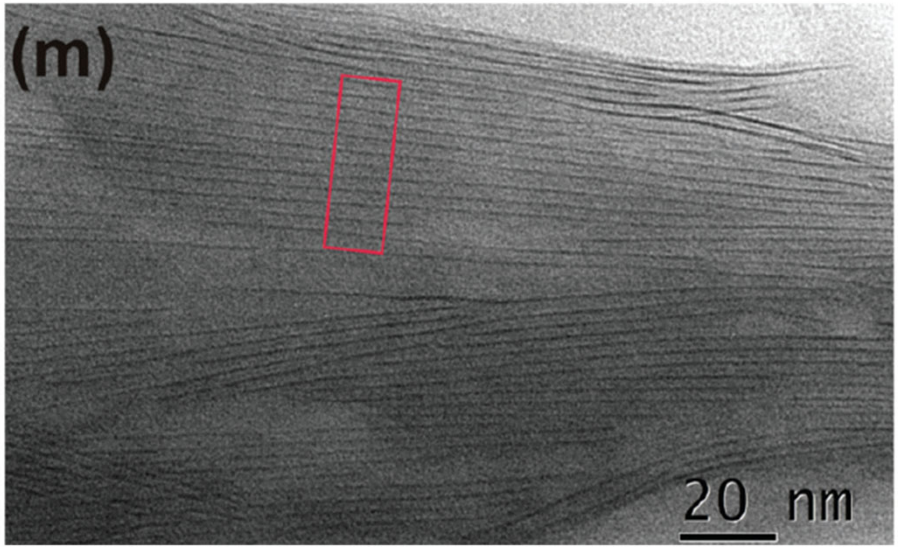


SEM vs. TEM

Cross sections of poly(vinyl alcohol) / clay nanocomposites



SEM
good overview of morphology



TEM
detailed supramolecular (and molecular) order

Nano Lett. **2010**, *10*, 2742.

SEM vs. TEM

- TEM is superior in resolution; however, the extreme resolutions are rarely applied in neither TEM nor SEM

- Usual resolution applied:
  - TEM: tens of nanometers
  - SEM: micrometers, hundreds of nanometers

- SEM is relatively fast and easy to use; it is possible to ”surf” along the sample surface and search for interesting spots where one can zoom at

SEM vs. TEM

	SEM	TEM
Resolution	1-2 nm	< 0.1 nm
Sample preparation	Drying, sputtering (in case of non-conductive samples)	Ultrathin cuts with a microtome
Ease of analysis	Good	Poor