Lecture Slides

Jan 29, 2013

CHM 165,265 / BIMM 162 / BGGN 262 Spring 2013

Announcements for Jan 29, 2013

Reading assignment for Thursday: Lecture notes pp.196-208

Midterm Exam: Tuesday 8:00-9:20 am

Covers material presented through Jan 31 lecture and all lecture notes (pp. 1-208 for graduate students and pp.1-123,146-208 for undergraduate students)

'Virtual' homework: Answers to first 6 sets posted outside NSB 4-105

Recitation session: Friday 5:00-6:00 pm in York 4080A

Last help session before midterm exam on Feb 5

TEM facility tour: Yesterday and today

CHM 165,265 / BIMM 162 / BGGN 262 Winter 2013

3D Electron Microscopy of Macromolecules

Midterm *next* Tuesday, Feb 5, 2013
8:00 - 9:20 AM

Peterson Hall, Room 103
Bring pencils, a ruler, and calculator

Covers material presented through end of ***Thursday's*** (Jan 31st) lecture.

Help session THIS Friday, Feb 1st
York 4080A 5:00-6:00 PM

I.E OPERATION OF THE TEM

KEY CONCEPTS FROM LECTURE #6

- Accelerating voltage

  Usually best to **increase** (reduces damage, improves depth of field and resolution)

- Apertures (Condenser and Objective)

  Smaller CAs give higher coherence e- beam (best for high resolution)
  Smaller OAs improve scattering contrast and reduce spherical and chromatic aberrations
  But, be aware that there are some downsides to smaller apertures (see notes)

- Specimen stage/holder

  Hands off

- Magnification

  Choose carefully based on nature of experiment/specimen
  Use **lowest** magnification consistent with required resolution and the recording medium used

- Focusing

  Small adjustments made to focal length of the objective lens
  Method used depends on magnification
  Use minimum contrast for high resolution imaging
  Slight under focus generally best

I.E OPERATION OF THE TEM

MORE CONCEPTS FROM LECTURE #6

- Magnification Calibration

  Nominal values in TEM not **actual** magnifications
  Need **independent** calibration of magnification to measure specimen dimensions accurately
  Record images of **calibration standards**

- Resolution Tests

  Check TEM performance
  Measure actual resolution in recorded images of well-behaved test specimens (e.g. carbon graphite or gold foil)

- Image intensifiers/TV displays:

  Helpful aids for focusing, astigmatism correction, and working with beam sensitive samples

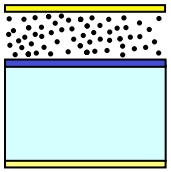
- Microscope maintenance:

  Pay big $$$ for service contract

I.E OPERATION OF THE TEM

MORE CONCEPTS FROM LECTURE #6

- Recording Images Photographically (on film):



Optical Density

Quantitative **measure of blackening** of the photographic emulsion $OD = \log_{10} \frac{I_0}{T}$

Single-Hit Process

Virtually **every** halide crystal hit by an e^- is rendered developable

Electron Diffusion

Electrons scatter sideways as pass through the emulsion
Means resolution in final recorded image is **ALWAYS POORER** than achieved in the electron image

I.E OPERATION OF THE TEM

MORE CONCEPTS FROM LECTURE #6

- Recording Images Digitally (on CCD):

CCD Advantages:

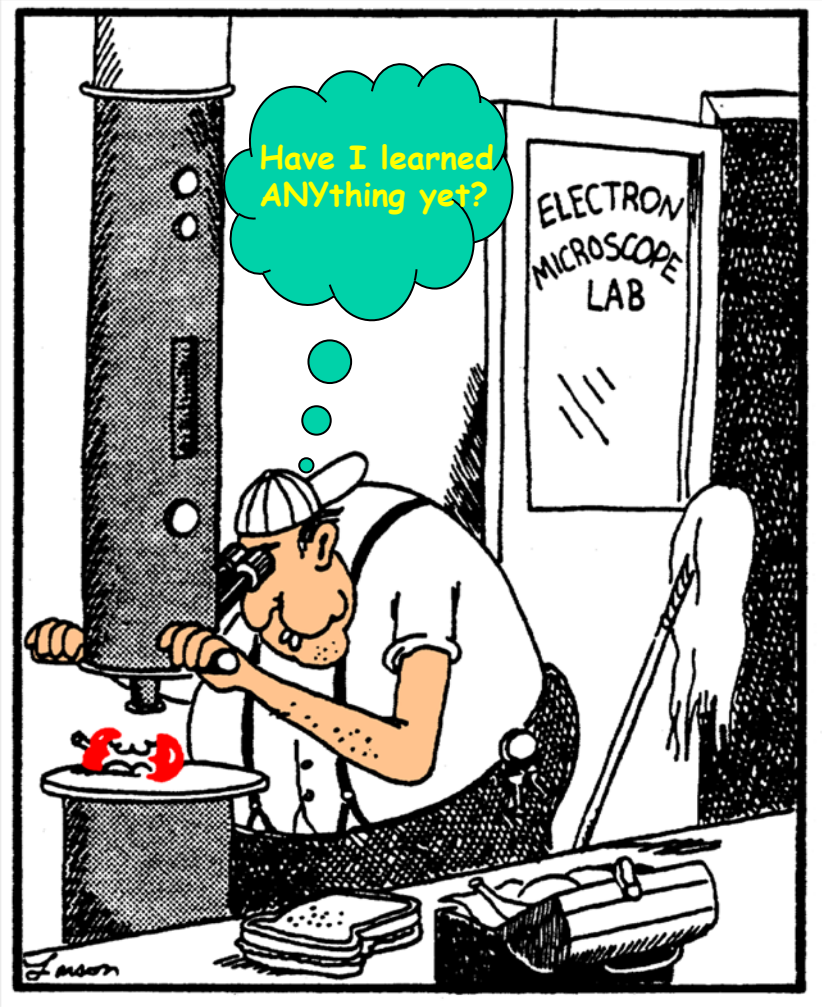
Immediate image access
Large dynamic range
Strict linear response with electron dose
Amenable to numerous automated microscopy tasks (e.g. pixel binning, contrast manipulations, etc.)

CCD Disadvantages:

Poorer pixel resolution than film (15 μm vs. ~ 5-10 μm)
Limited number of pixels (e.g. 4k by 4k vs. ~16k by 20k), hence **small field of view**
High upfront cost

CCD Designs:

Lens-coupled vs. fiber-optic coupled



TOPICS

- Principles of TEM
  - Electrons, lenses and optics
- Design of TEM
  - Components top to bottom
- Contrast and image formation
  - Electron scattering from object
- Optimizing TEM performance
  - Alignment assures ‘best’ images
- Operation of TEM
  - “What do all these buttons do?”
- Other modes of TEM
  - Many ways to ‘observe’ specimens
- Specimen preparation for TEM
  - Getting specimen ready
- Radiation damage
  - Less is better
- 3D reconstruction
  - Specimen 3D structure from 2D images

§ I: The Microscope

I.E Operation of the TEM

I.E.10 Photography (Film)

→ I.E.11 Digital Photography (CCD)

I.E OPERATION OF THE TEM

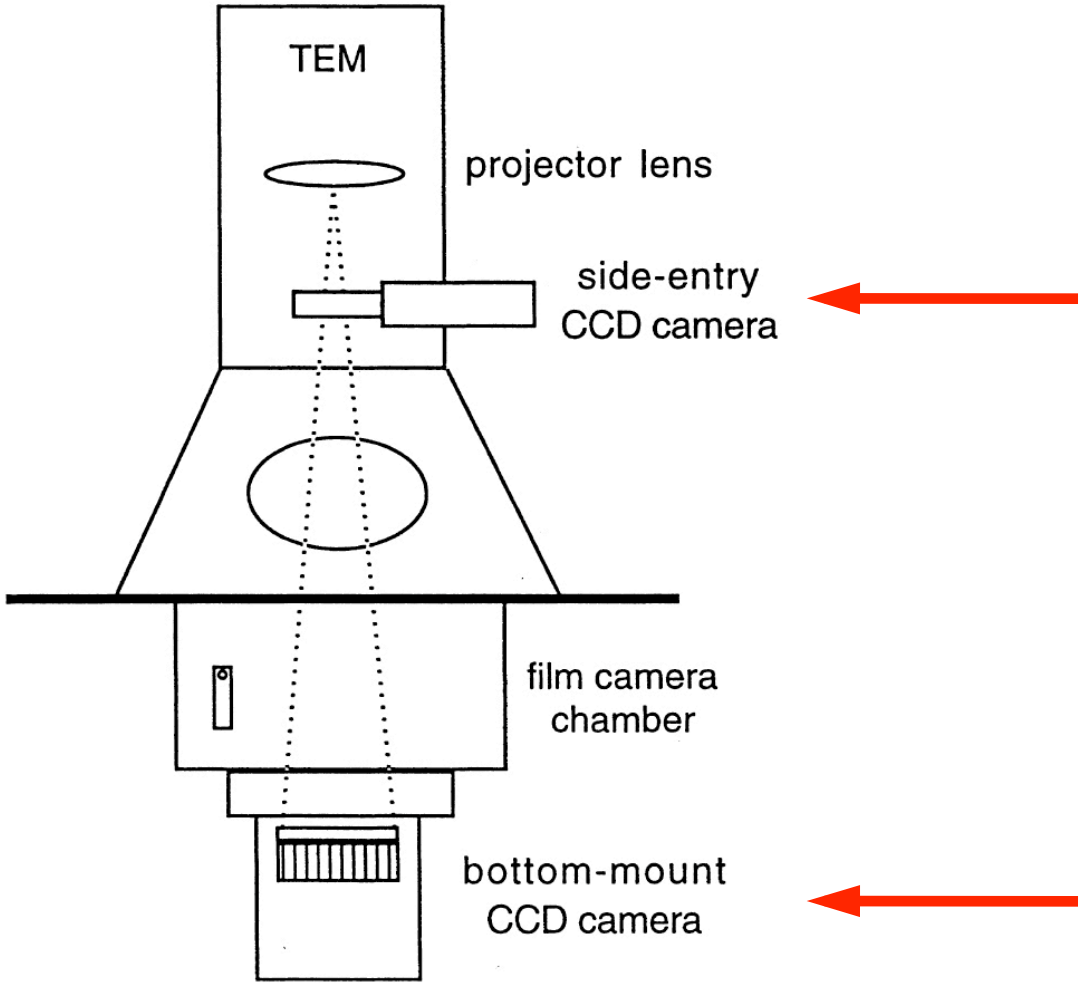
I.E.11 Digital Photography (CCD)

CCD Detectors/Cameras

CCD = Charge Coupled Device

I.E OPERATION OF THE TEM

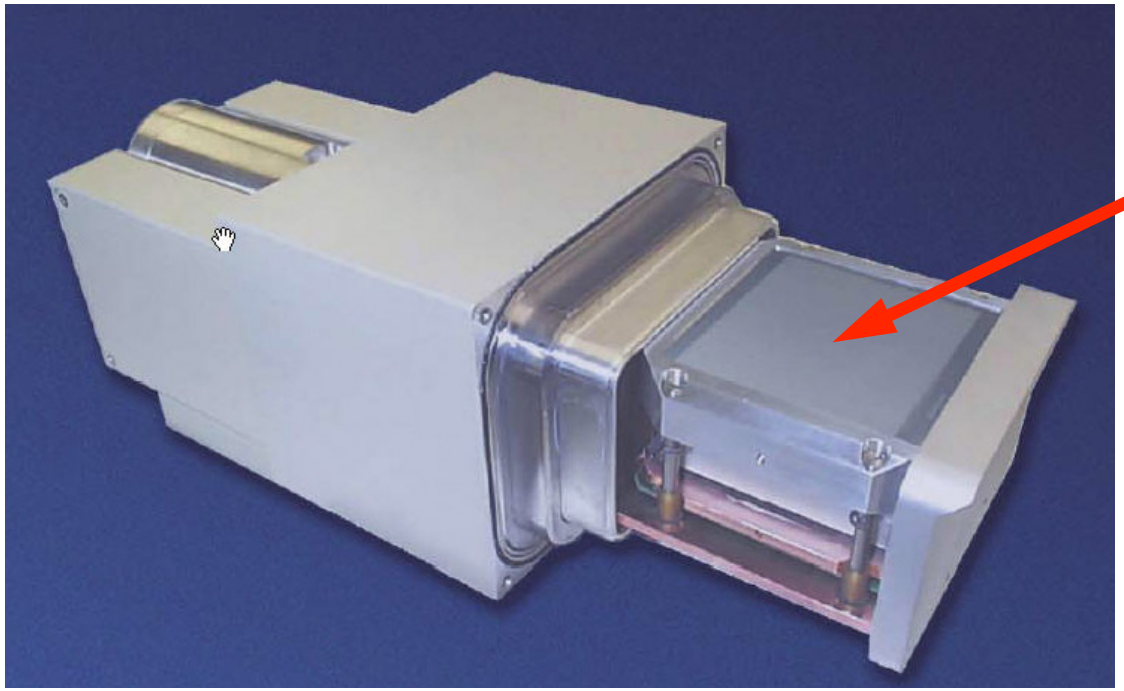
I.E.11 Digital Photography (CCD)



From Fan & Ellisman [2000], p.2

I.E.11 Digital Photography (CCD)

Microscopy with a CCD



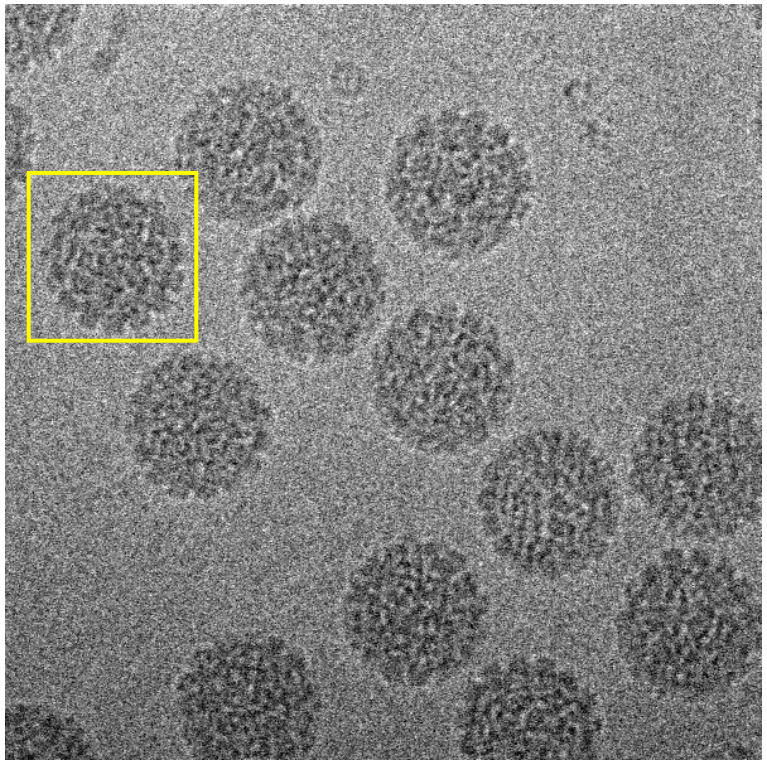
CCDs like the 16 megapixel Gatan Ultrascan™ (4080 x 4080 15 μm pixels) can produce high quality digital images in the TEM

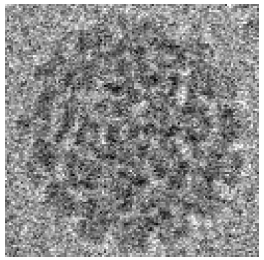
http://www.gatan.com/imaging/ultrascan.html

Digital vs. Film Photography

Information content of film emulsion verses CCD detector

I.E.11 Digital Photography (CCD)

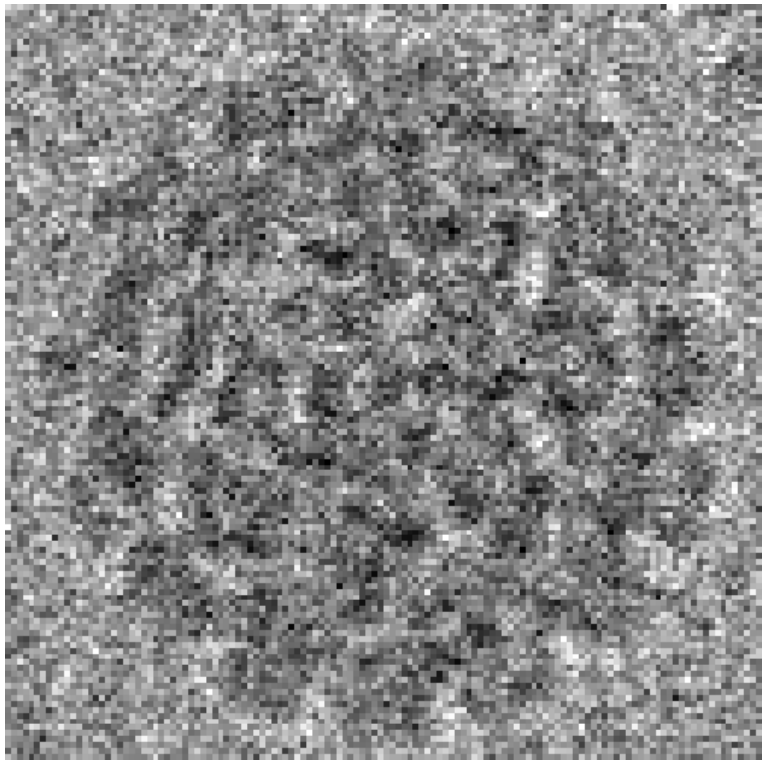


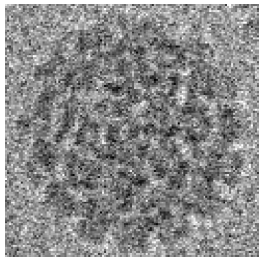


128 x 128

SV40 Virus
Original digital image

I.E.11 Digital Photography (CCD)



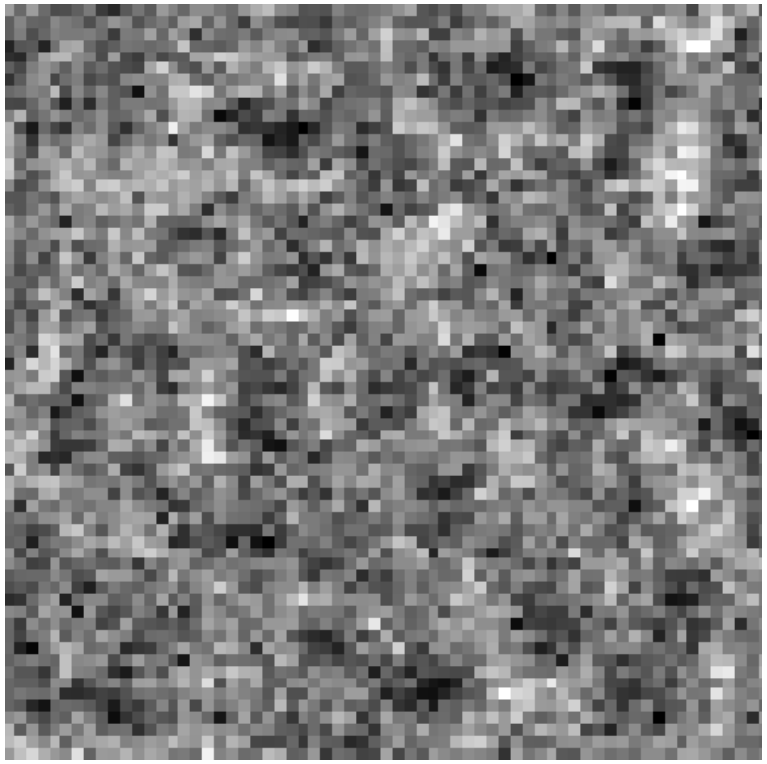


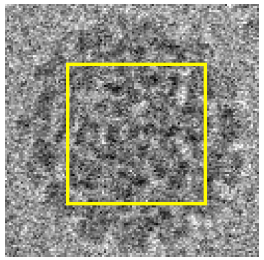
128 x 128

SV40 Virus

Digital image highly magnified to show individual pixels

I.E.11 Digital Photography (CCD)





64 x 64

SV40 Virus

Digital image highly magnified to show individual pixels

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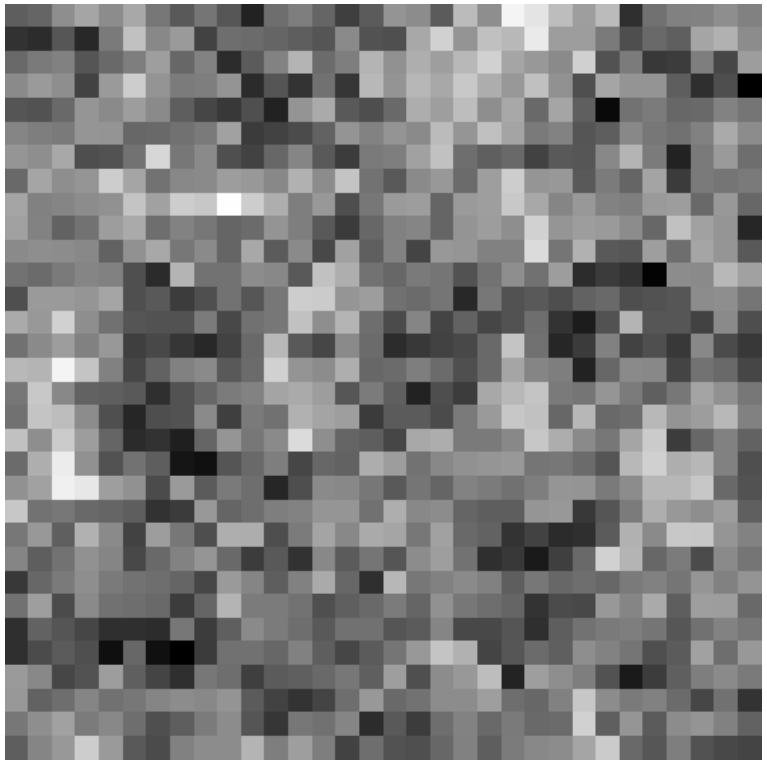
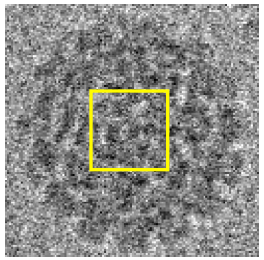




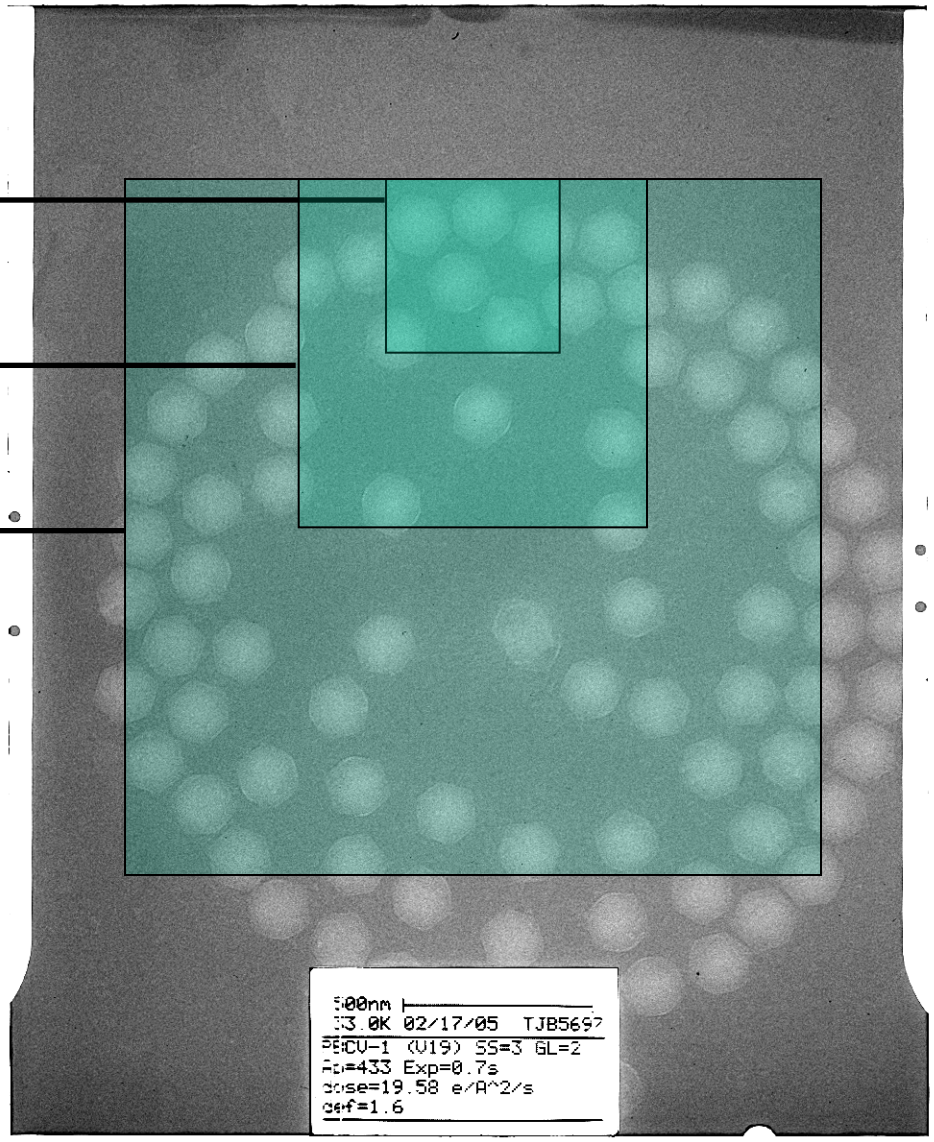
32 x 32

SV40 Virus

Digital image highly magnified to show individual pixels

I.E.11 Digital Photography (CCD)

CCD with
15 μm pixels



	% Area	# Images
1024^2	3	34
2048^2	12	8
4096^2	47	2

Film 8 x 10 cm

I.E.11 Digital Photography (CCD)

Another Advantage of Recording Images Digitally

Can perform **binning** operations

Output from small groups of pixels (*e.g.* 2 x 2 pixels) may be combined into **one** pixel in the digital image

I.E.11 Digital Photography (CCD)

Another Advantage of Recording Images Digitally

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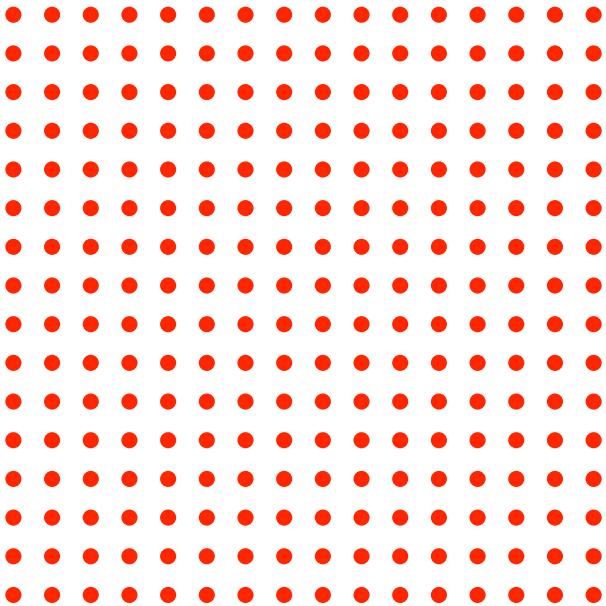

16 x 16 array

I.E.11 Digital Photography (CCD)

Another Advantage of Recording Images Digitally

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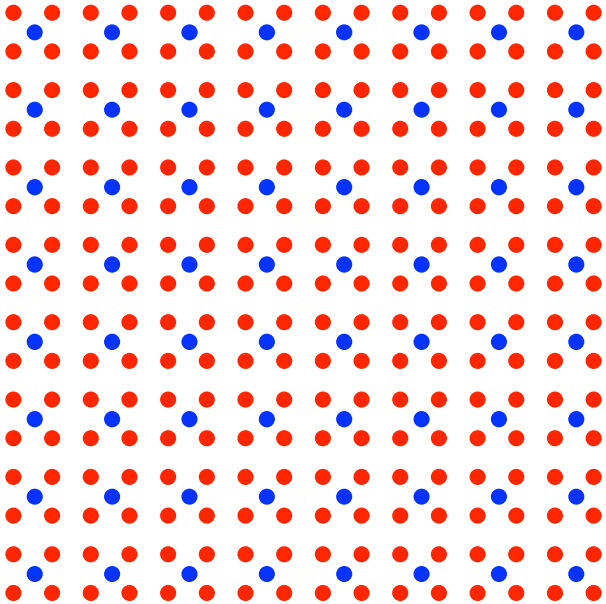

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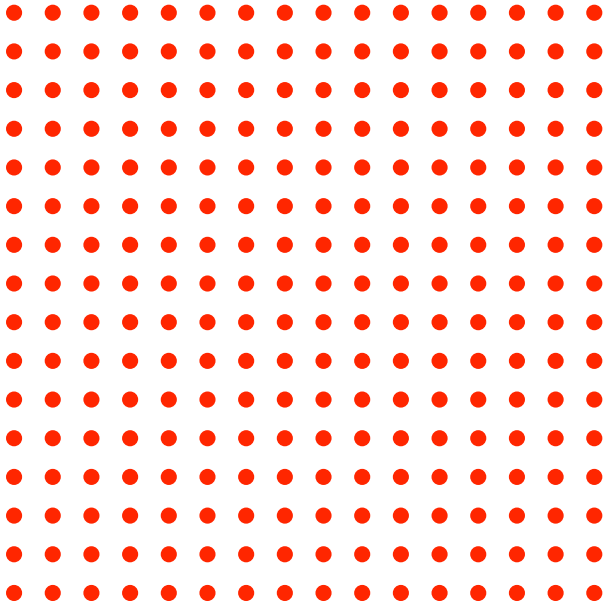
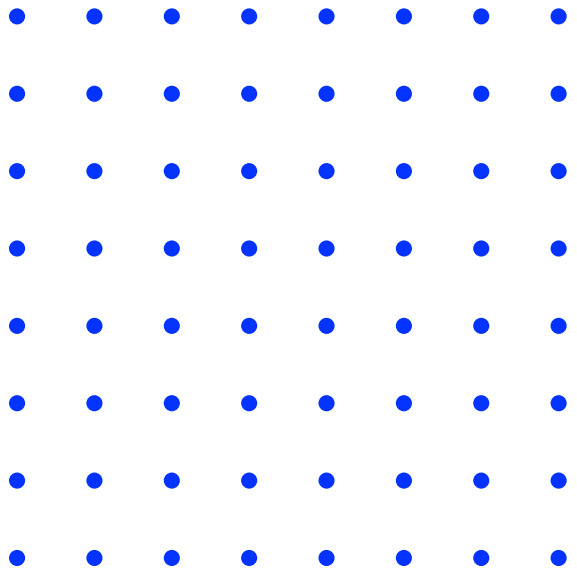


16 x 16 array
256 pixels



8 x 8 array
64 pixels

I.E.11 Digital Photography (CCD)

Another Advantage of Recording Images Digitally

Can perform **binning** operations

Output from small groups of pixels (*e.g.* 2 x 2 pixels) may be combined into **one** pixel in the digital image

Reduces resolution, but increases sensitivity

Allows rapid collection of information from beam sensitive specimens using minimal illumination

For highest resolution digital imaging (*i.e.* to make sure finer specimen details are captured), an **unbinned** image must be recorded

I.E.11 Digital Photography (CCD)

Resolution and Contrast

Compared to images captured on film, **resolution** is generally **lower** in most digital images captured by CCD cameras

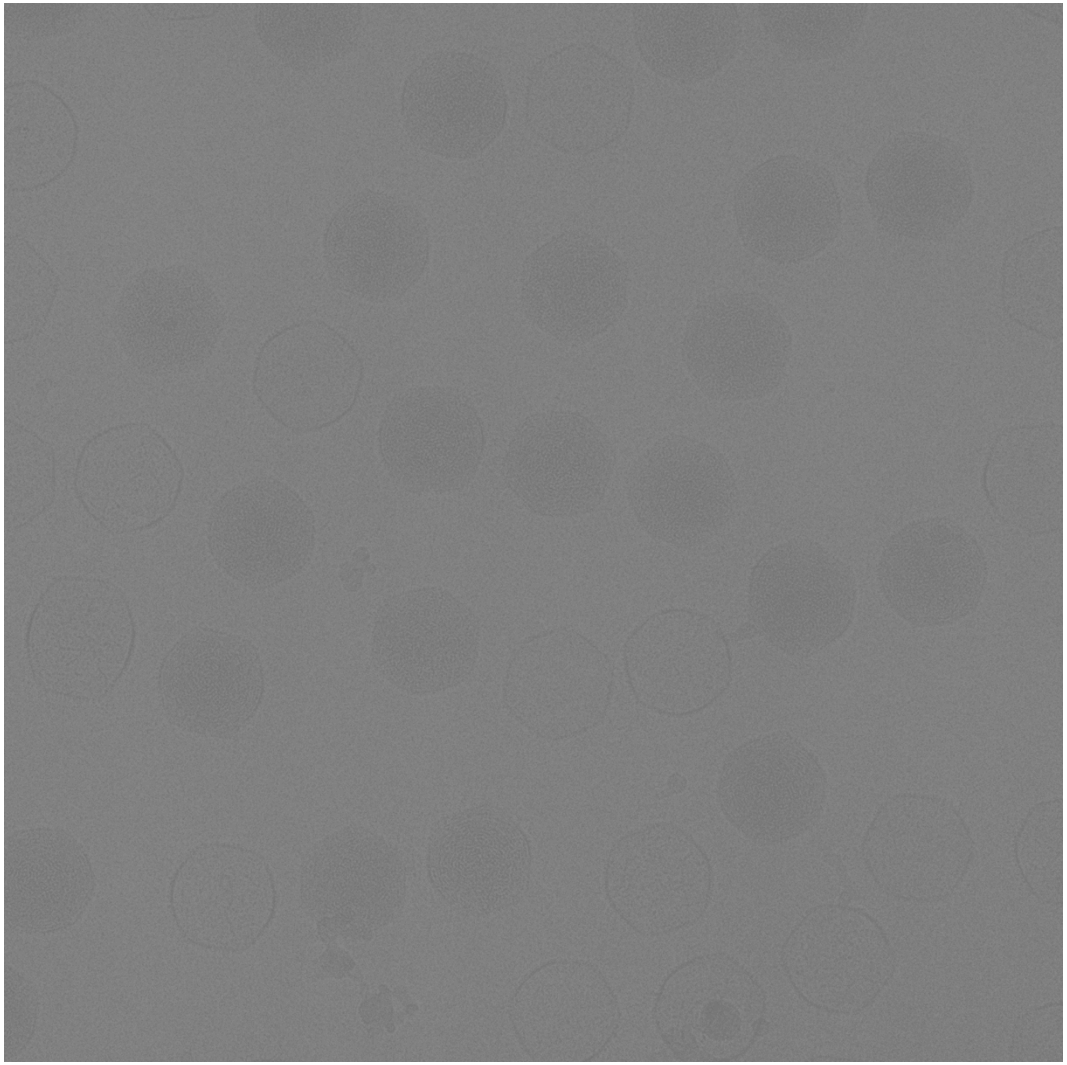
Power of digital imaging:

Pixel intensities and **range of contrast** in a digital image are **easily** and **rapidly manipulated**.

I.E.11 Digital Photography (CCD)

Resolution and Contrast

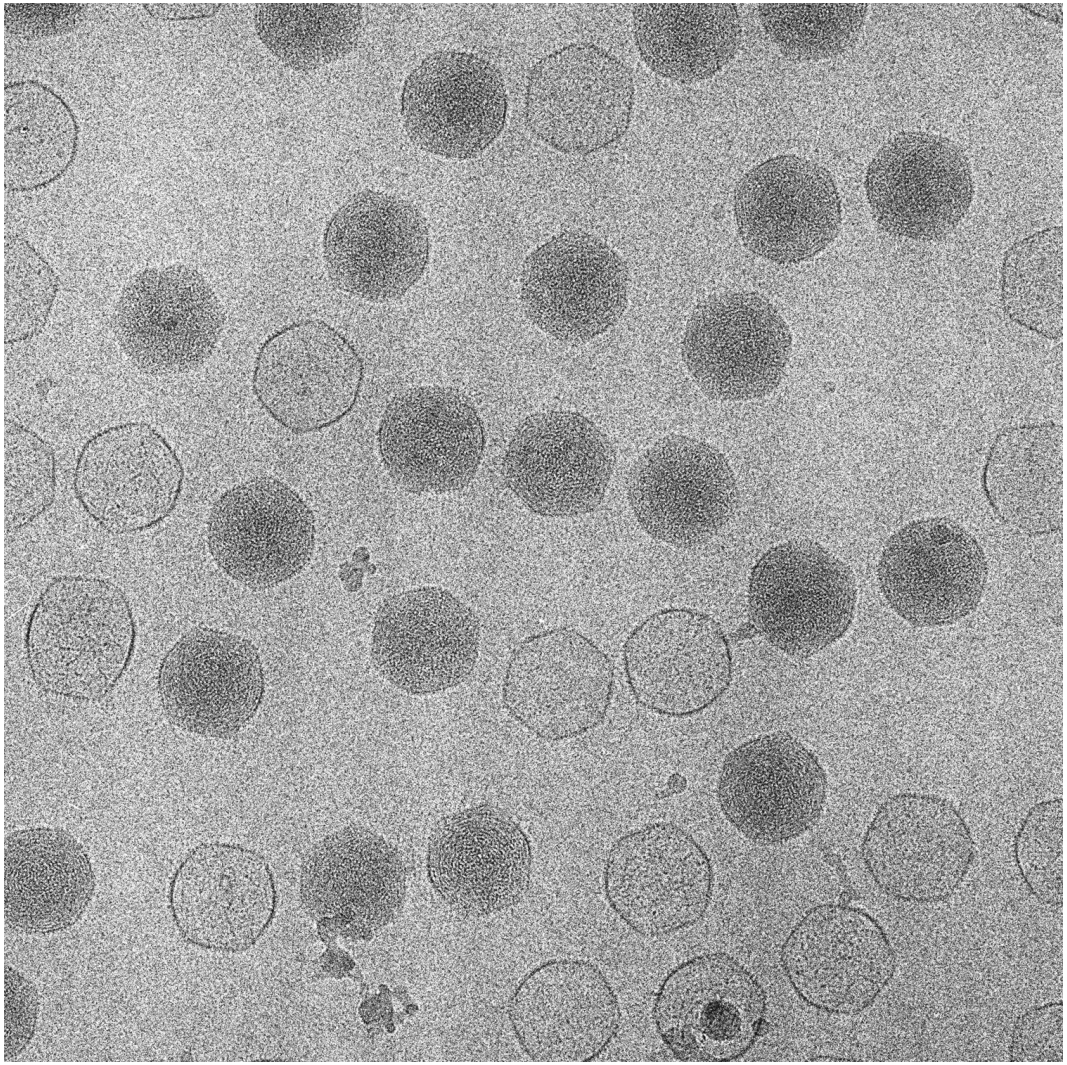
Raw digital image of virus sample



I.E.11 Digital Photography (CCD)

Resolution and Contrast

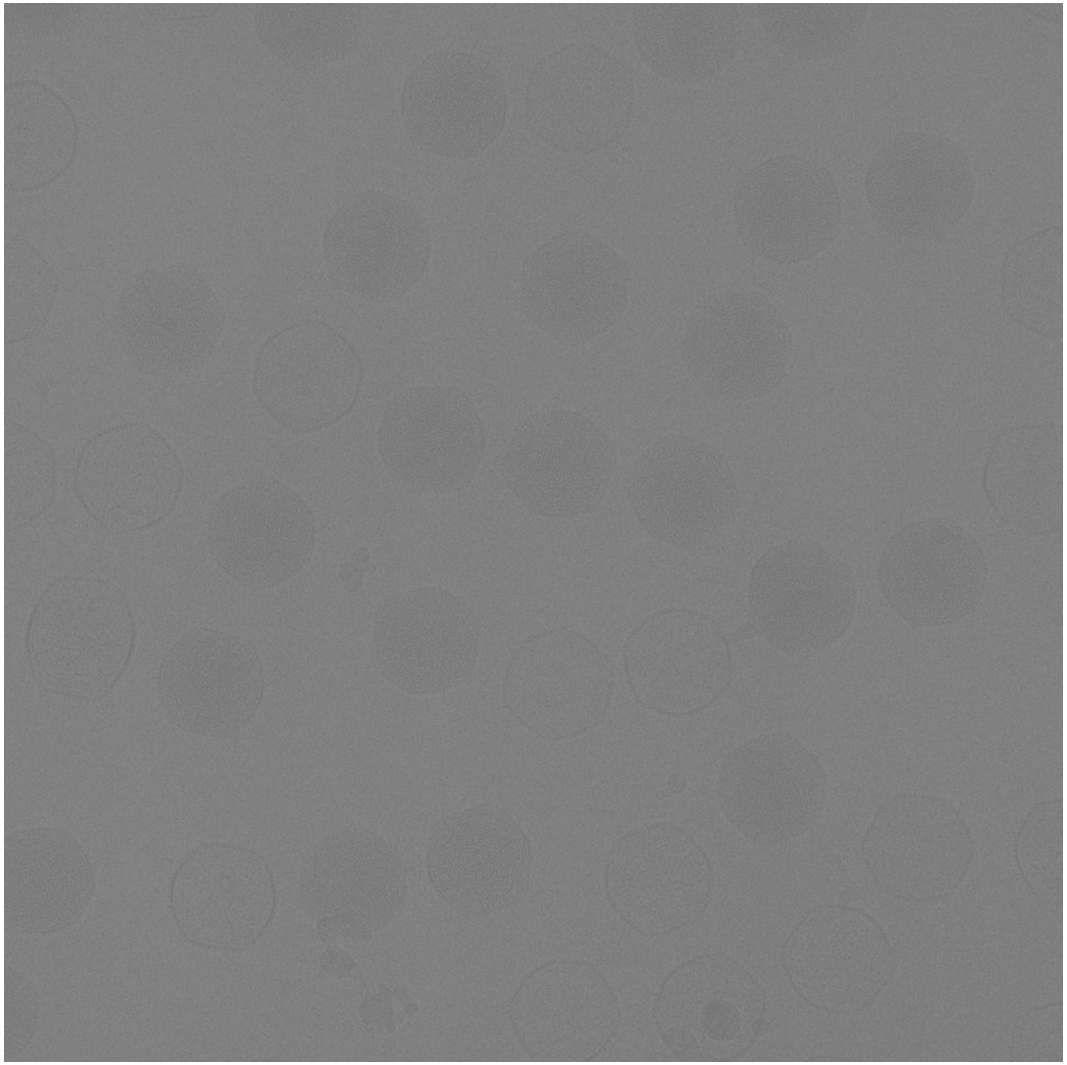
Enhanced (contrast stretched) digital image of virus sample



I.E.11 Digital Photography (CCD)

Resolution and Contrast

Raw digital image of virus sample



I.E.11 Digital Photography (CCD)

How Much Pixel Resolution is Enough?

Goal:

Digitally **preserve** (*i.e.* resolve) detail in the electron image

Nyquist Criterion:

The **finest detail** (*i.e.* highest spatial frequency) we can capture in a digital image is **TWICE** the size of one pixel.

Hence, must sample (digitize) the image at a **step size AT LEAST TWO TIMES SMALLER** than the desired or expected resolution.

For now, just accept this as "**fact**".....this will be a critical issue when we discuss image processing

I.E.11 Digital Photography (CCD)

How Much Pixel Resolution is Enough?

Practical Consideration (for displaying images):

Sampling rate (step size) sometimes reported in **dots per inch** ("dpi")

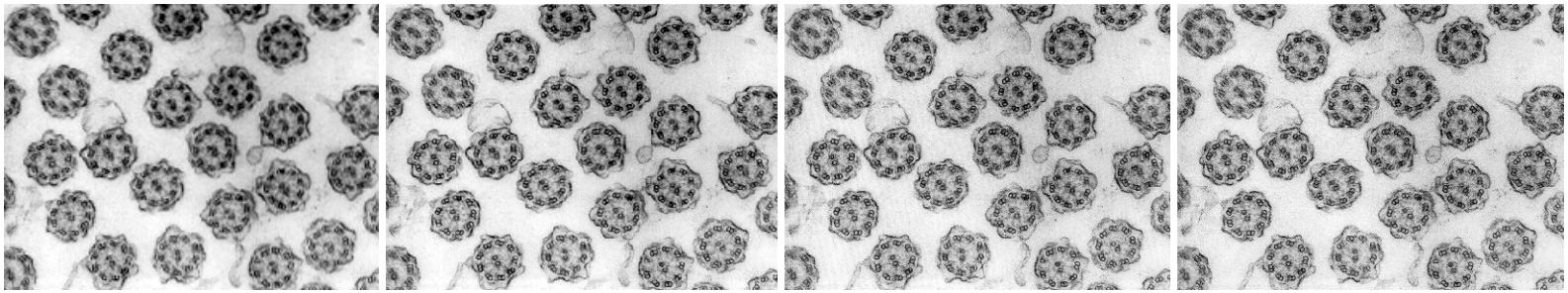
Number of dpi used depends on how the image is to be viewed

Computer screen

Hard copy "print"

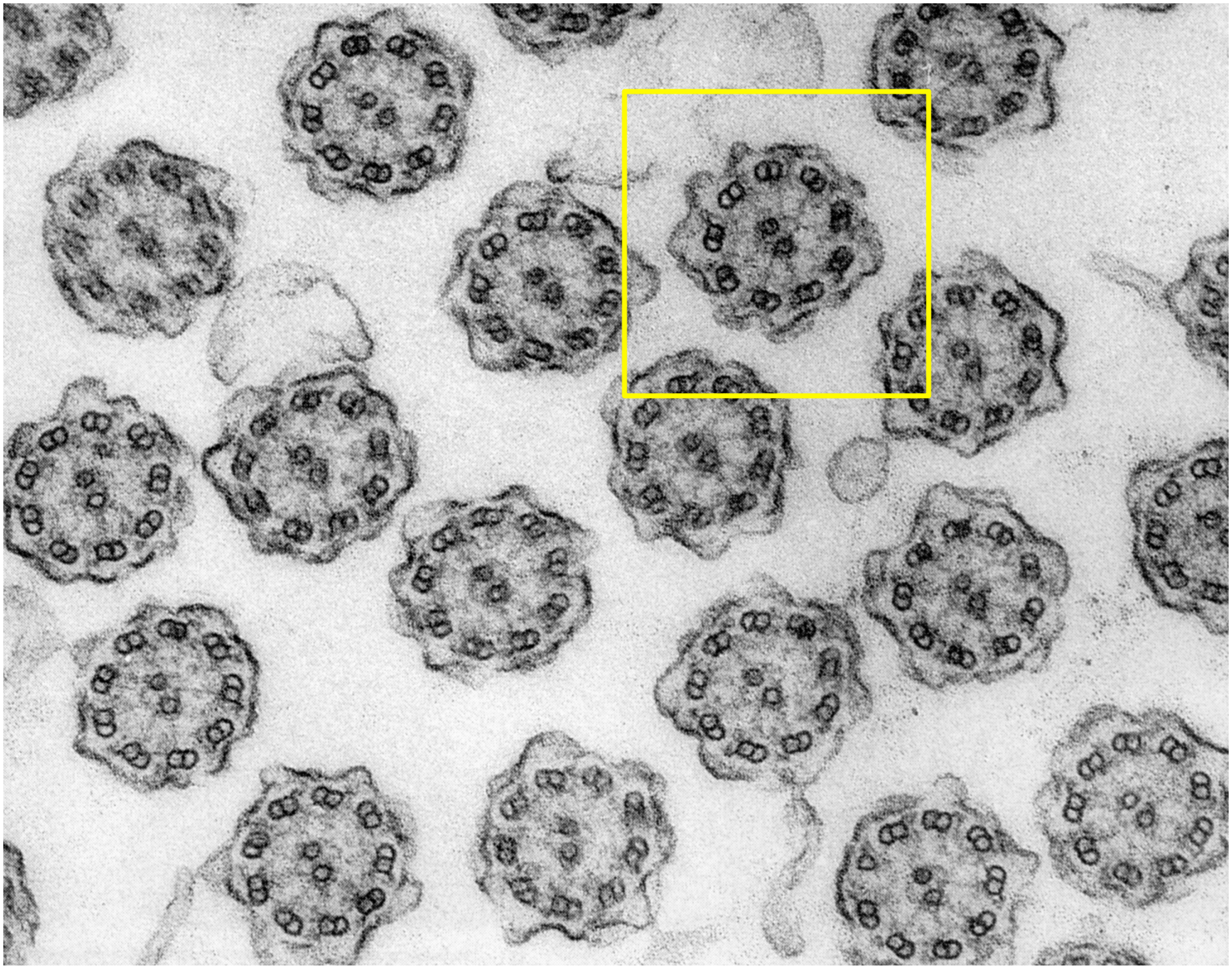
I.E.11 Digital Photography (CCD)

Pixel Resolution



50 dpi 100 dpi 300 dpi 600 dpi

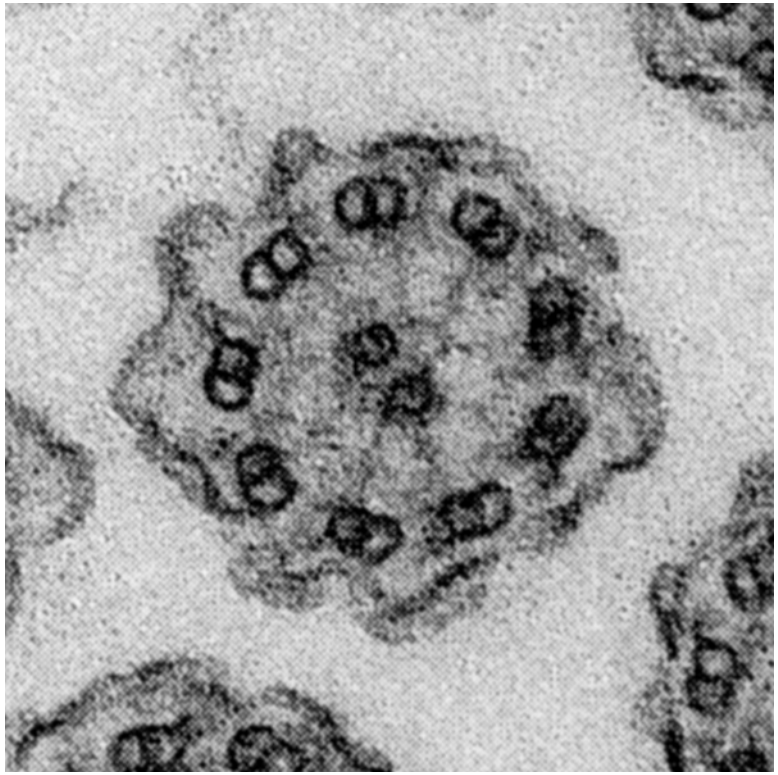
I.E.11 Digital Photography (CCD)



600 dpi

I.E.11 Digital Photography (CCD)

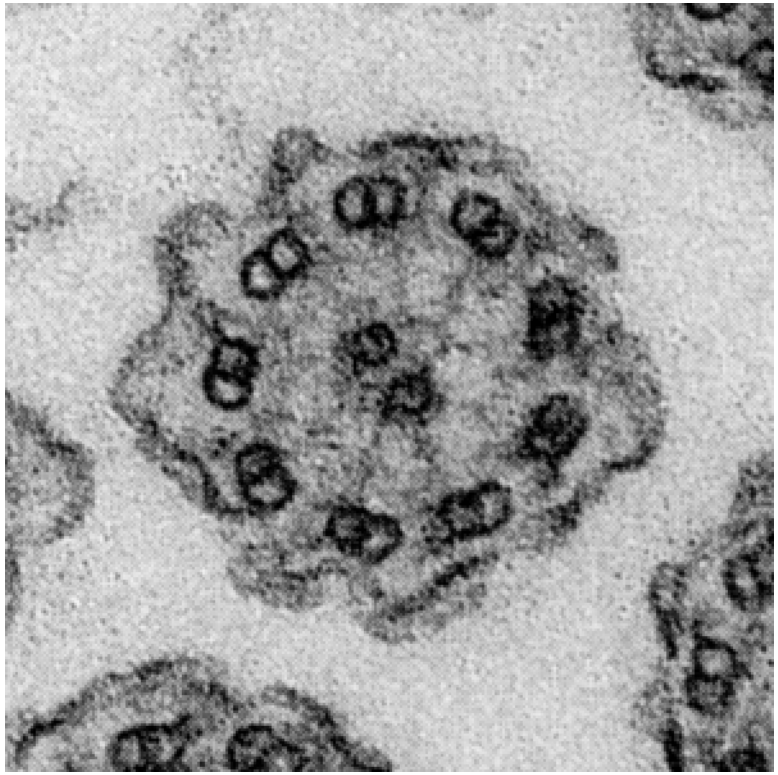
Pixel Resolution



600 dpi

I.E.11 Digital Photography (CCD)

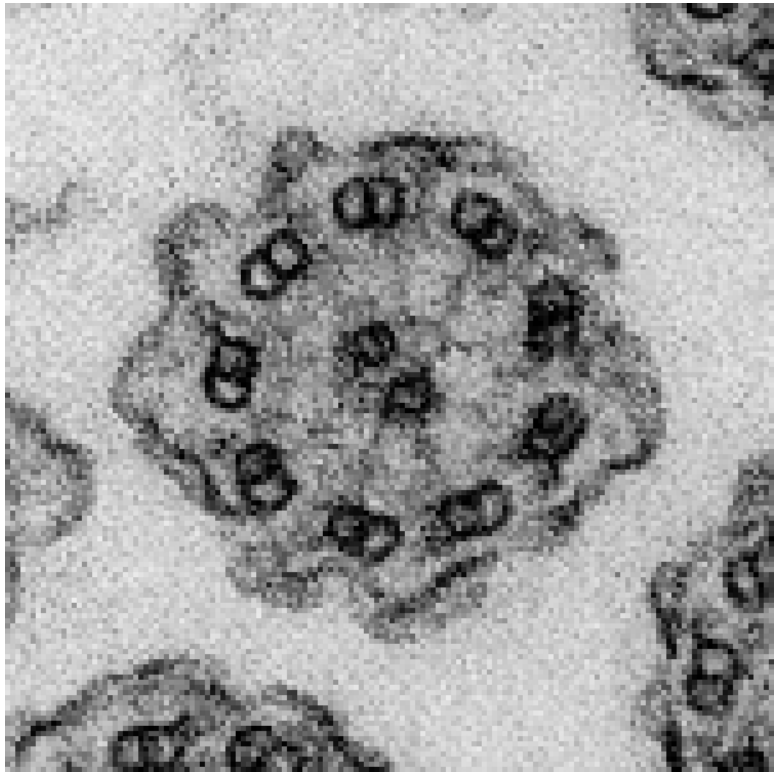
Pixel Resolution



300 dpi

I.E.11 Digital Photography (CCD)

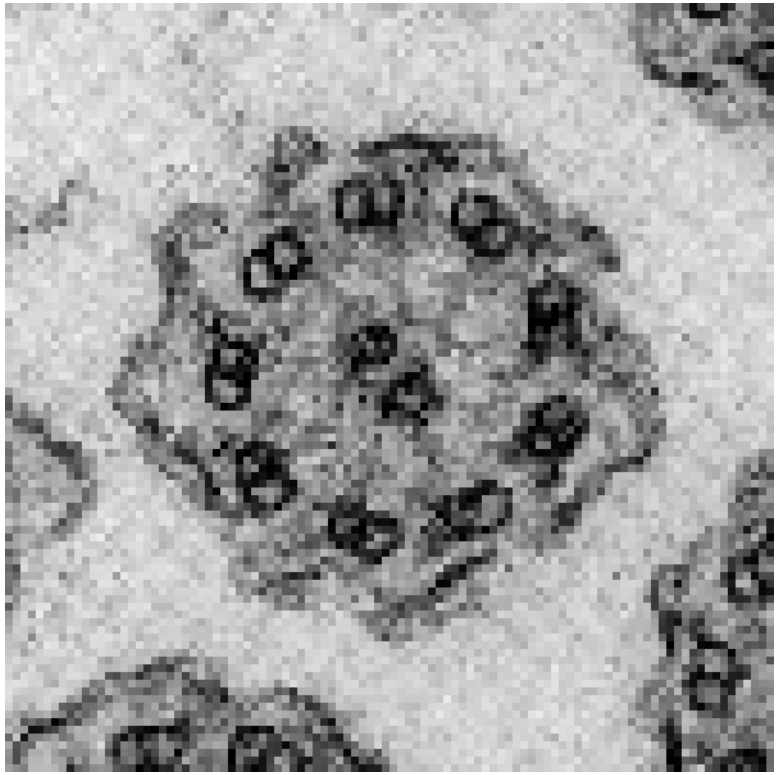
Pixel Resolution



150 dpi

I.E.11 Digital Photography (CCD)

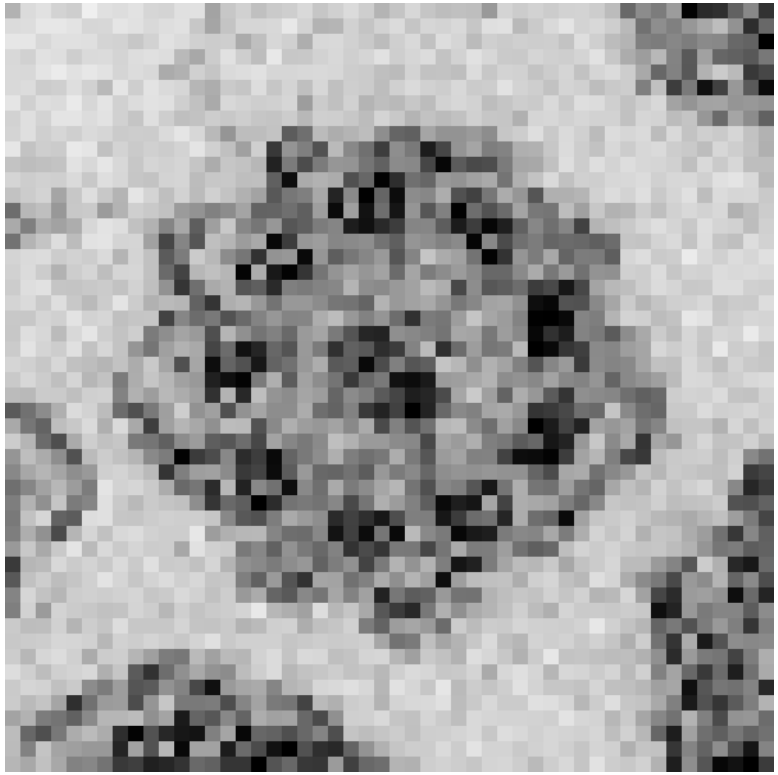
Pixel Resolution



100 dpi

I.E.11 Digital Photography (CCD)

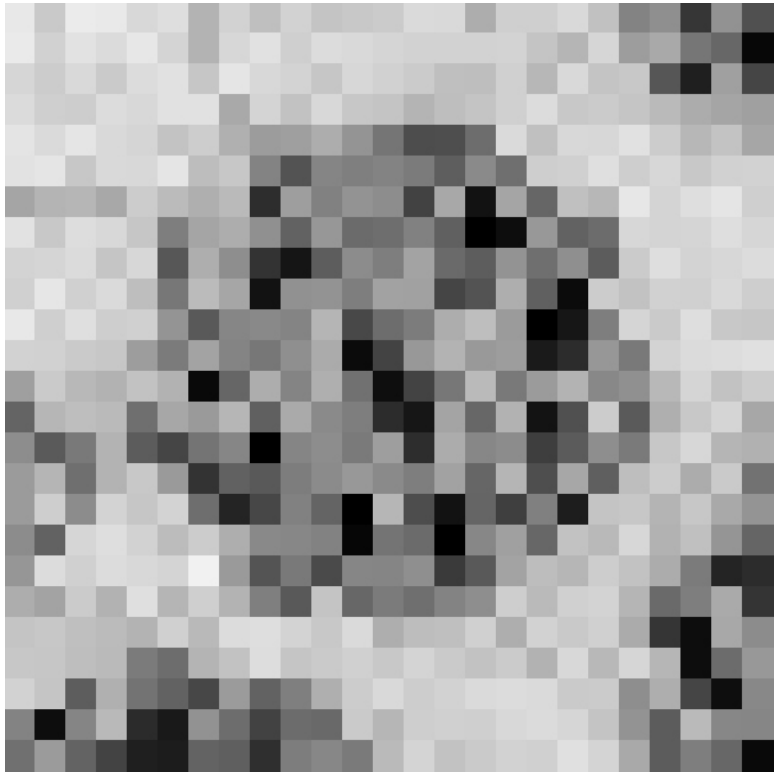
Pixel Resolution



50 dpi

I.E.11 Digital Photography (CCD)

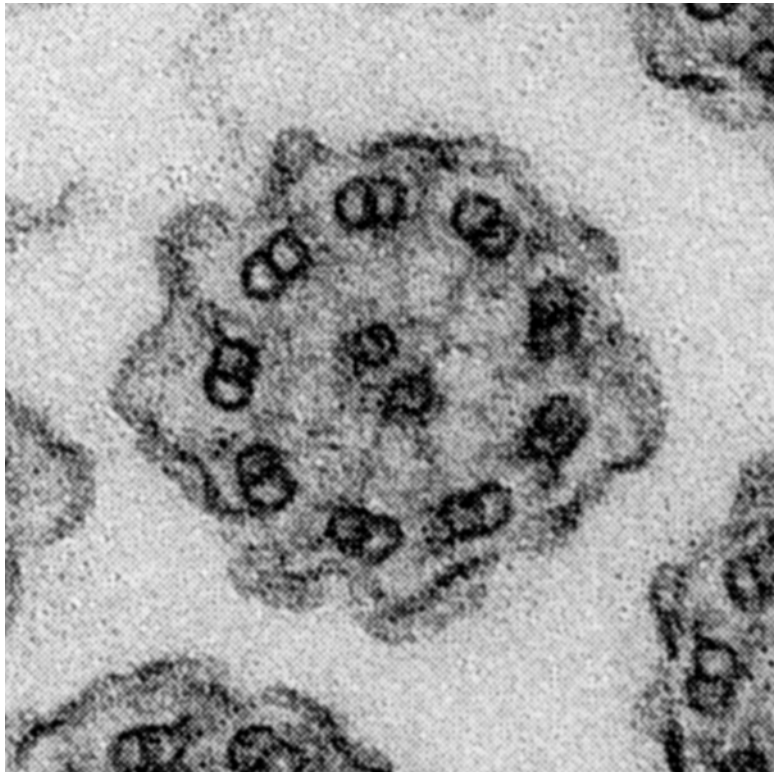
Pixel Resolution



25 dpi

I.E.11 Digital Photography (CCD)

Pixel Resolution



600 dpi

§ I: The Microscope

I.E Operation of the TEM

I.E.10 Photography (Film)

→ I.E.11 Digital Photography (CCD)

→ I.E.12 Digital Photography (DDD)

(pp.119-122 of lecture notes)

I.E OPERATION OF THE TEM

I.E.12 Digital Photography (DDD)

DDD Detectors/Cameras

DDD = Direct Detection Device

CCD cameras rely on indirect detection of electrons

Electron events are converted to photons at a scintillator and these are 'fed' to (*i.e.* imaged by) the CCD

I.E OPERATION OF THE TEM

I.E.12 Digital Photography (DDD)

DDD Detectors/Cameras

DDD = Direct Detection Device

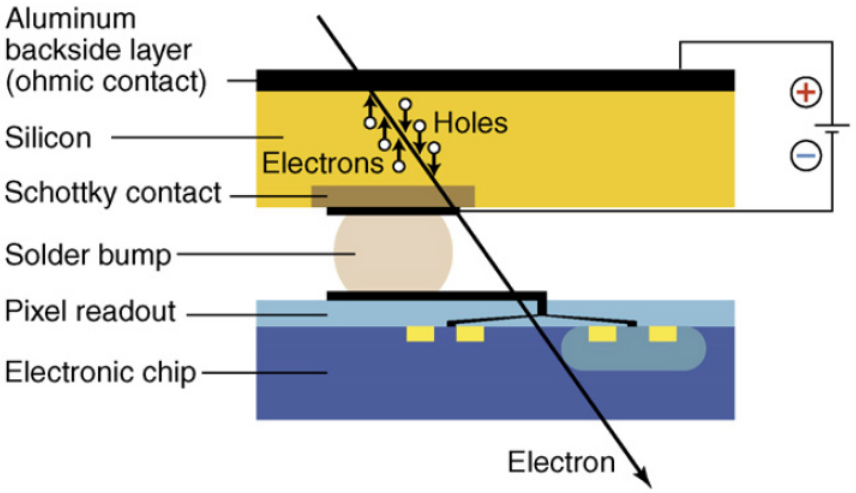
CMOS = Complementary Metal Oxide Semi-conductor

I.E.12 Digital Photography (DDD)

Two Basic DDD Designs

HPDs

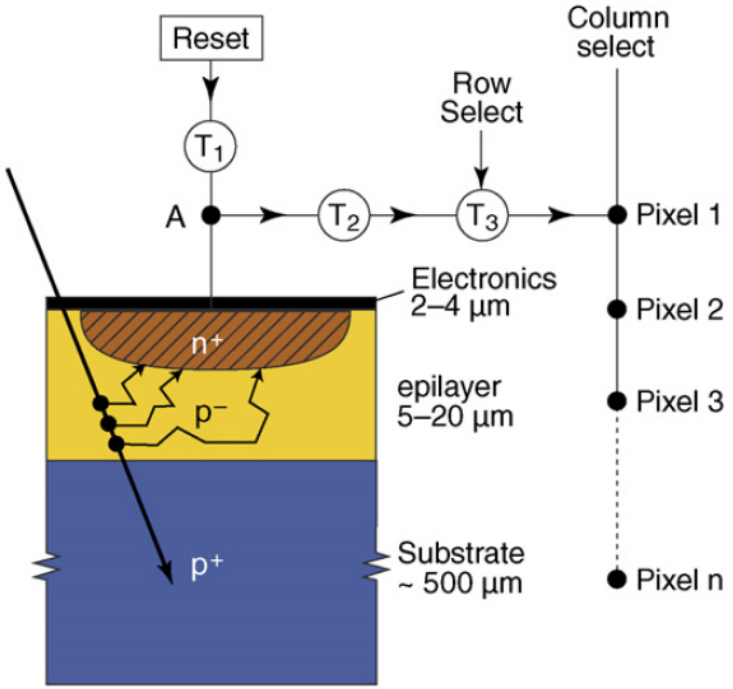
Hybrid Pixel Detectors



From Faruqi & Henderson (2007), p.550

MAPS

Monolithic Active Pixel Sensors

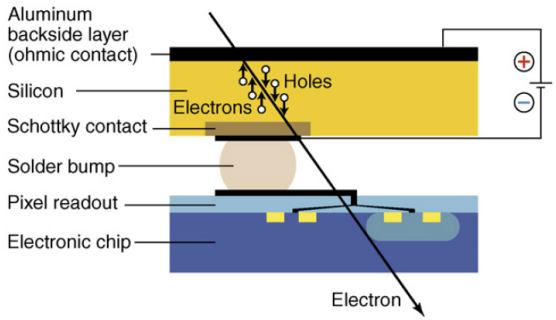


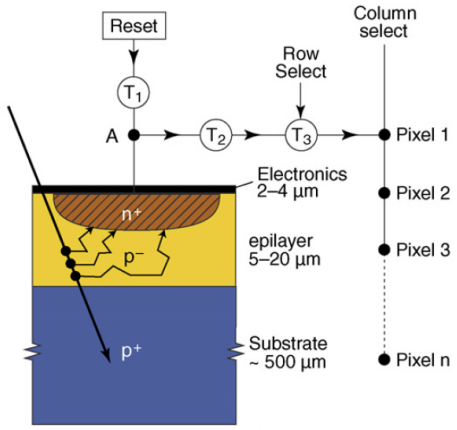
From Faruqi & Henderson (2007), p.550

I.E.12 Digital Photography (DDD)

Two Basic DDD Designs

	HPDs	**MAPS**
	Hybrid Pixel Detectors	Monolithic Active Pixel Sensors
Pixel construction	2 layers	1 layer





I.E.12 Digital Photography (DDD)

Two Basic DDD Designs

	HPDs	MAPS
	Hybrid Pixel Detectors	Monolithic Active Pixel Sensors
Pixel construction	2 layers	1 layer
Pixel size	55 μm	5 μm
# pixels	256 x 256	3840 x 3712
Area covered	~(14 mm)^2	~(19 mm)^2
Signal	33,000 e-hole pairs	280-560 e-hole pairs
Application	Best suited for X-rays	TEM electrons

I.E.12 Digital Photography (DDD)

Two Commercial DDDs

Direct Electron DE12
(4096 x 3072; 6 μm pixels)

Gatan K2
(4096 x 4096; 5 μm pixels)

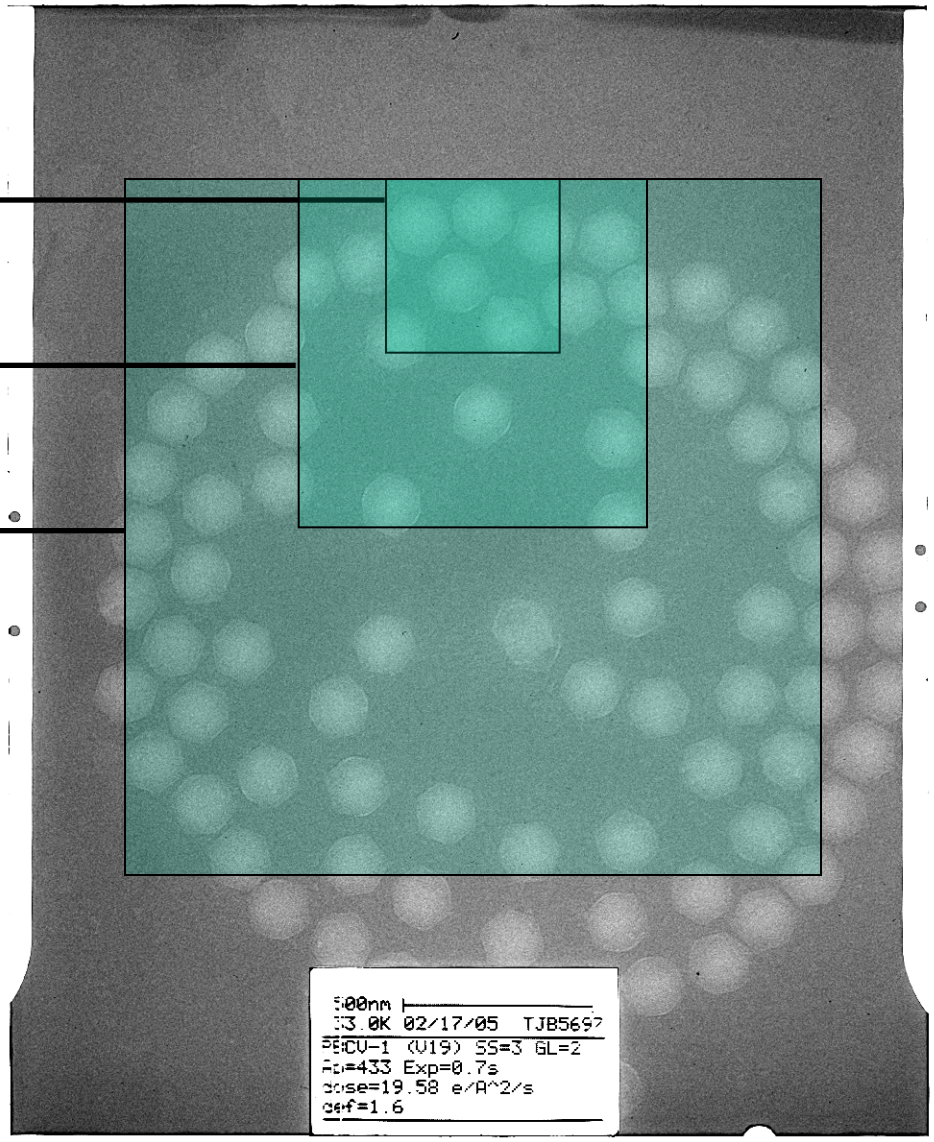
COST: $300,000 – $750,000

www.directelectron.com

www.gatan.com/products/digital_imaging/products/K2/index.php

I.E.11 Digital Photography (CCD)

CCD with
15 μm pixels

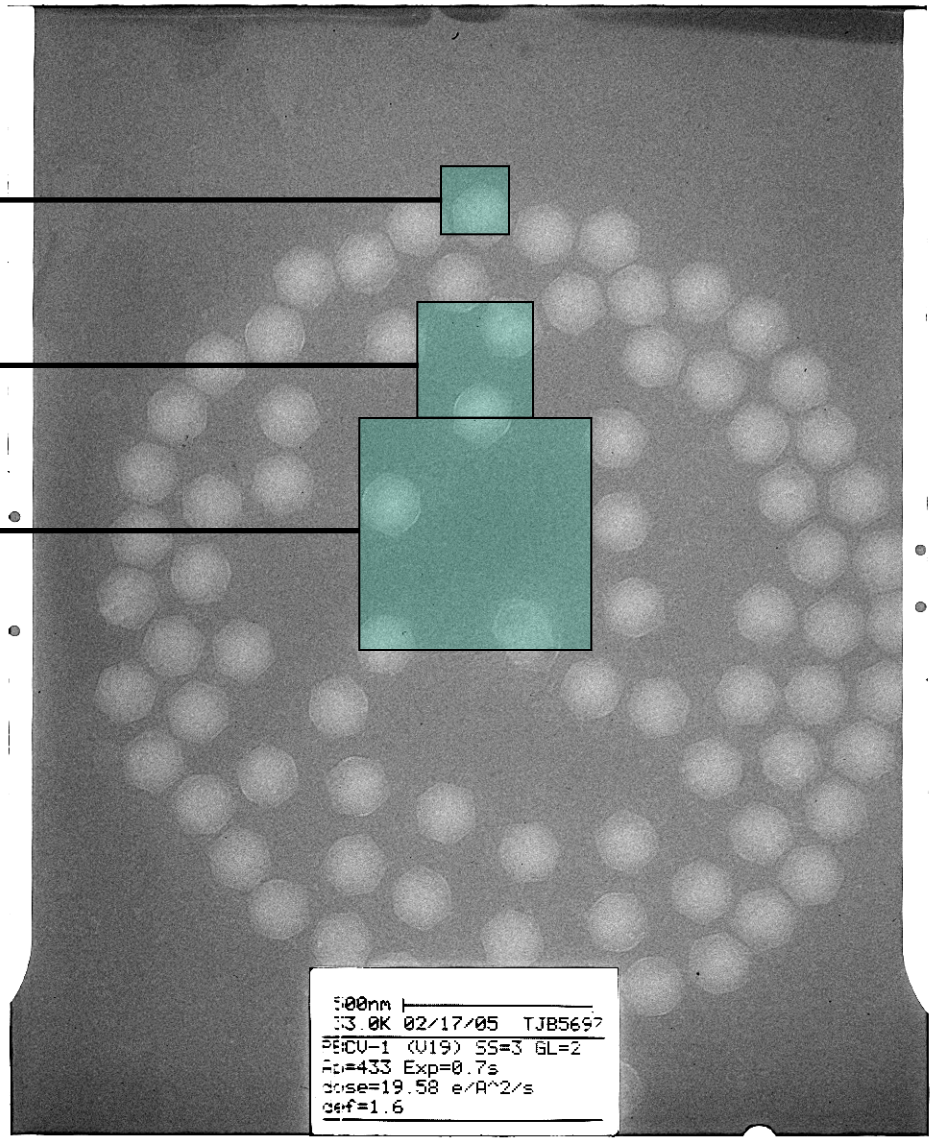


	% Area	# Images
1024^2	3	34
2048^2	12	8
4096^2	47	2

Film 8 x 10 cm

I.E.12 Digital Photography (DDD)

DDD with
5 μm pixels



	% Area	# Images
1024^2	0.4	221
2048^2	1.3	76
4096^2	5.2	19

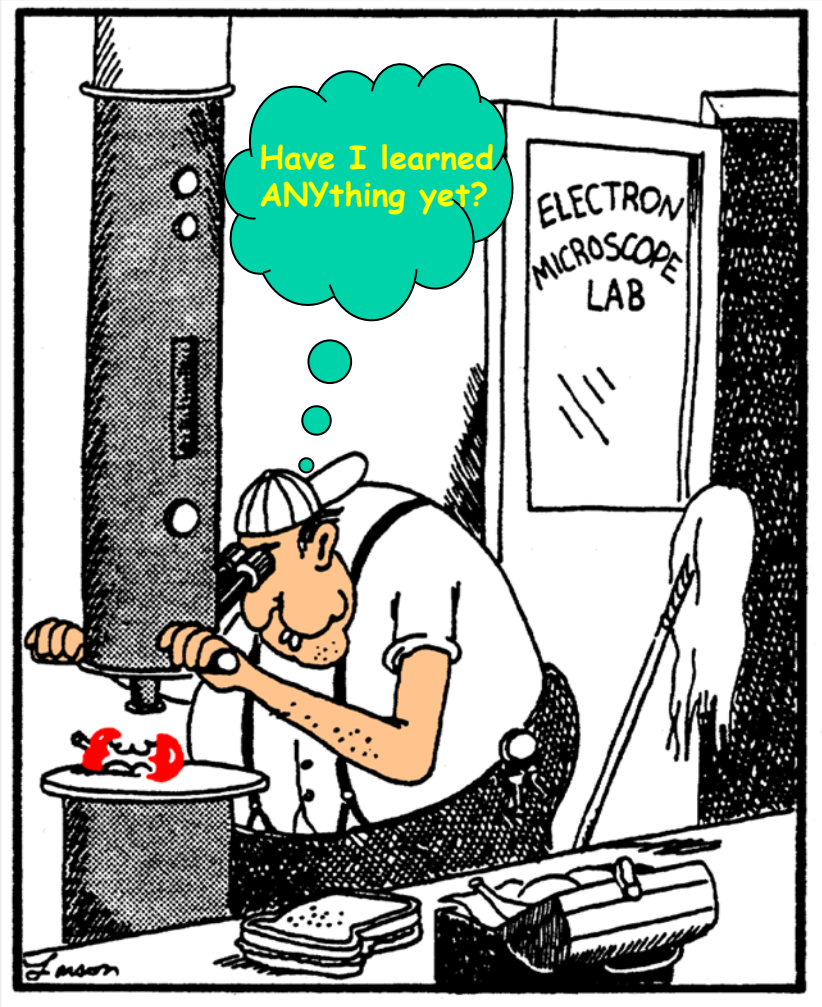
1 piece of film

16,000 x 20,000

320 Megapixels

1.28 Gb data storage

Film 8 x 10 cm



TOPICS

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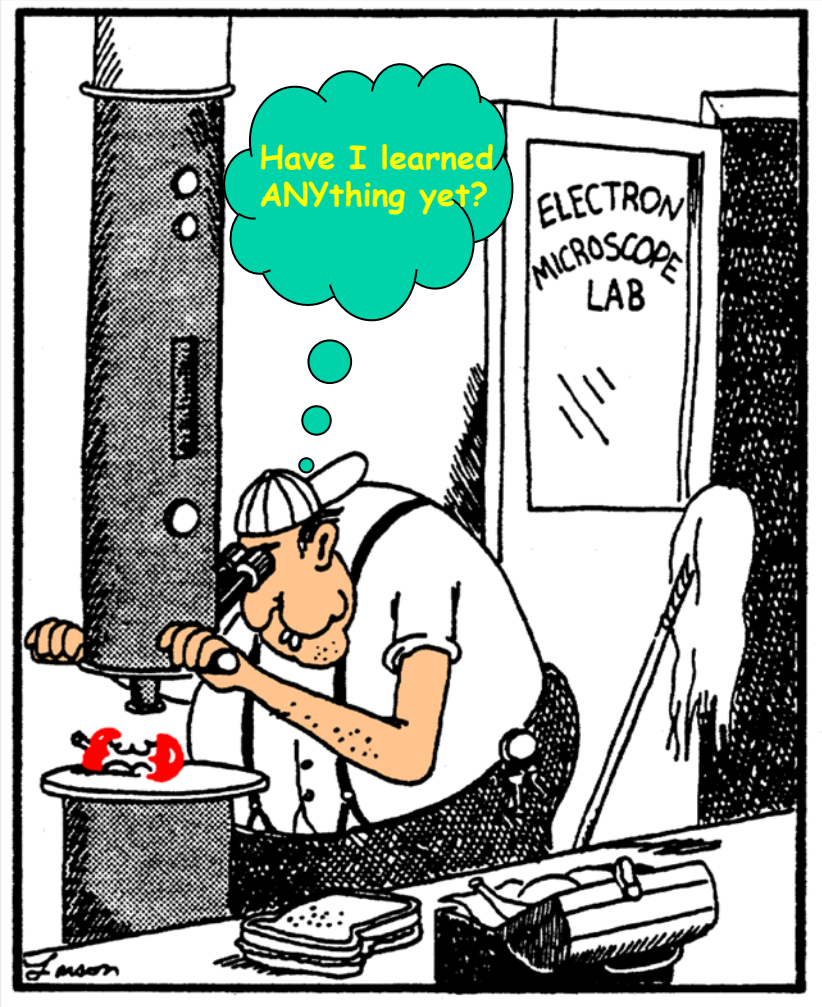
- Design of TEM
  - Components top to bottom
- Contrast and image formation
  - Electron scattering from object
- Optimizing TEM performance
  - Alignment assures ‘best’ images
- Operation of TEM
  - “What do all these buttons do?”
- Other modes of TEM
  - Many ways to ‘observe’ specimens
- Specimen preparation for TEM
  - Getting specimen ready
- Radiation damage
  - Less is better
- 3D reconstruction
  - Specimen 3D structure from 2D images



TOPICS

- Principles of TEM
  - Electrons, lenses and optics
- Design of TEM
  - Components top to bottom
- Contrast and image formation
  - Electron scattering from object
- Optimizing TEM performance
  - Alignment assures ‘best’ images
- Operation of TEM
  - “What do all these buttons do?”

- Other modes of TEM
  - Many ways to ‘observe’ specimens
- Specimen preparation for TEM
  - Getting specimen ready
- Radiation damage
  - Less is better
- 3D reconstruction
  - Specimen 3D structure from 2D images

§ I: The Microscope

I.A Principles of TEM

I.B Design of the TEM

I.C Contrast and Image Formation

I.D Alignment/Adjustment of the TEM

→ I.E Operation of the TEM

→ I.F Other Modes of TEM Operation

(pp.124-145 of lecture notes)

I.F OTHER MODES OF TEM OPERATION

Electron diffraction	- Notes pp.124-130
Dark field microscopy	- Notes pp.131-135
High resolution, high voltage	- Notes pp.135-137
Tilting and stereo microscopy	- Notes pp.137-142
Low temperature microscopy	- Notes p.142
Energy loss spectroscopy	- Notes pp.142-143
X-ray microanalysis	- Notes pp.143-144
Etc., etc., etc.	

I.F OTHER MODES OF TEM OPERATION

Electron diffraction	- Notes pp.124-130
Dark field microscopy	- Notes pp.131-135
High resolution, high voltage	- Notes pp.135-137
Tilting and stereo microscopy	- Notes pp.137-142
Low temperature microscopy	- Notes p.142
Energy loss spectroscopy	- Notes pp.142-143
X-ray microanalysis	- Notes pp.143-144
Etc., etc., etc.	

NOTE: For the purposes of the midterm exam, students in CHM 265 and BGGN 262 may be tested on the basic principles behind these methods, but students taking CHM 165 and BIMM 162 will **NOT**.

§ I: The Microscope

I.F Other Modes of TEM Operation

I.F.1 Electron Diffraction

I.F OTHER MODES OF TEM OPERATION

I.F.1 Electron Diffraction

Great for studying crystalline specimens

Identifying materials (primarily metals - produce strong ED patterns)

Used with crystalline biological specimens (diffract weakly)

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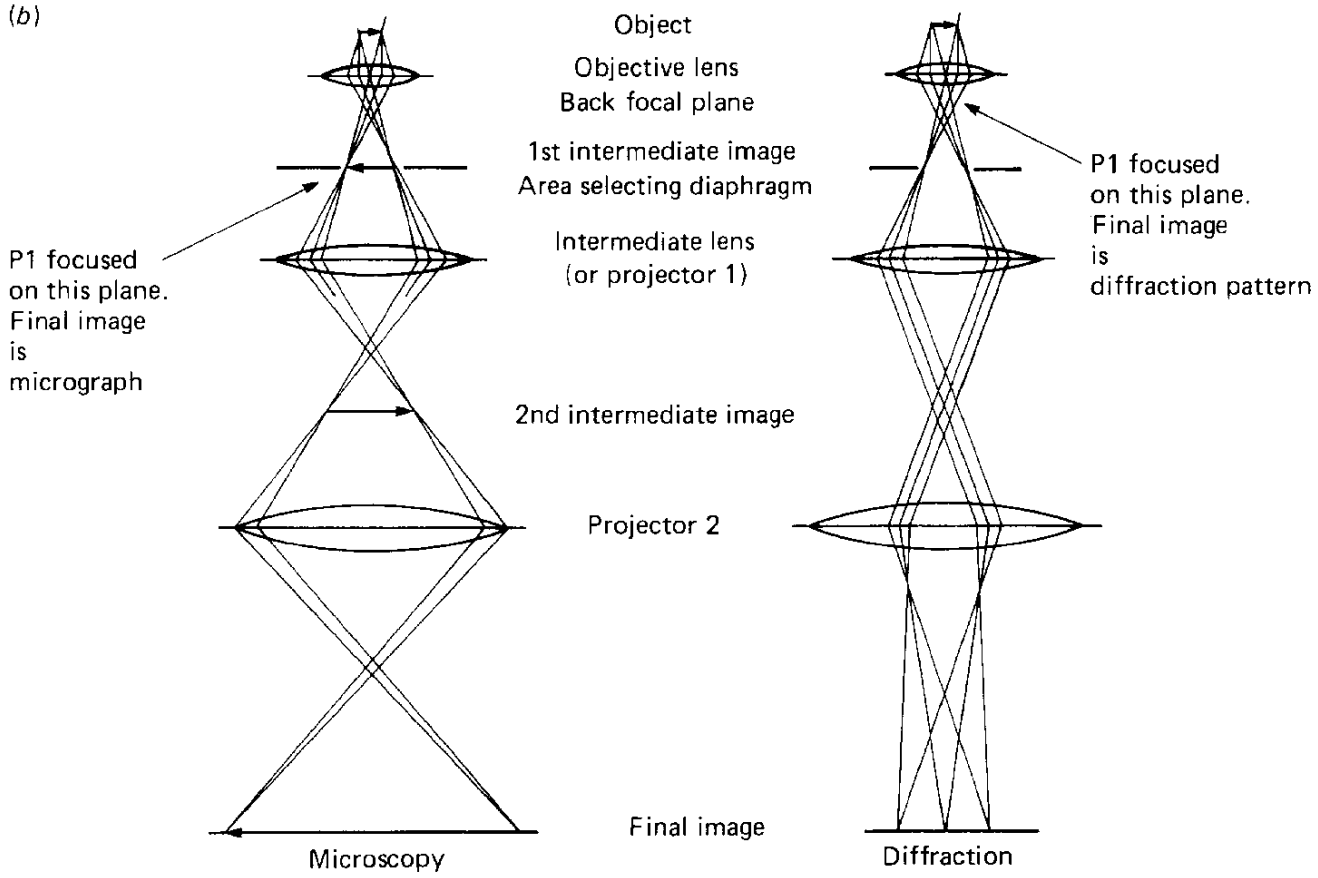
I.F OTHER MODES OF TEM OPERATION

I.F.1 Electron Diffraction

Three Lens Microscope



From Watt, Fig. 4.5b, p. 122

Slide not shown in class lecture

I.F OTHER MODES OF TEM OPERATION

I.F.1 Electron Diffraction

Nature of the ED Pattern

ED pattern usually consists of:

Series of rings (specimens with randomly oriented microcrystals)

Discrete lattice of sharp spots (specimens with a single, crystalline domain)

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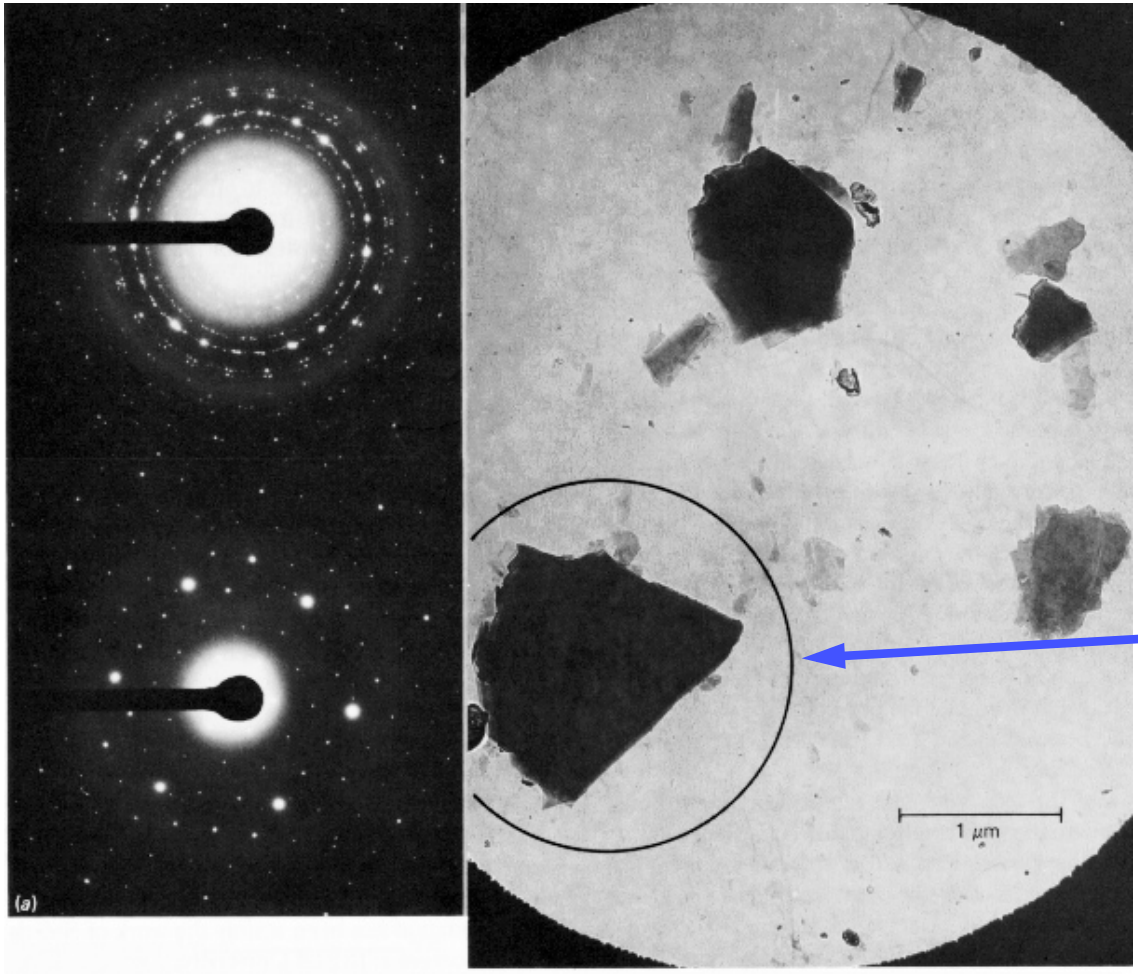
I.F OTHER MODES OF TEM OPERATION

I.F.1 Electron Diffraction

Selected Area Diffraction (S.A.D.)

Field of crystalline particles

'Spotty ring' ED pattern (crystals oriented in many directions)



6 μm aperture

2.3 μm aperture

Single crystal pattern

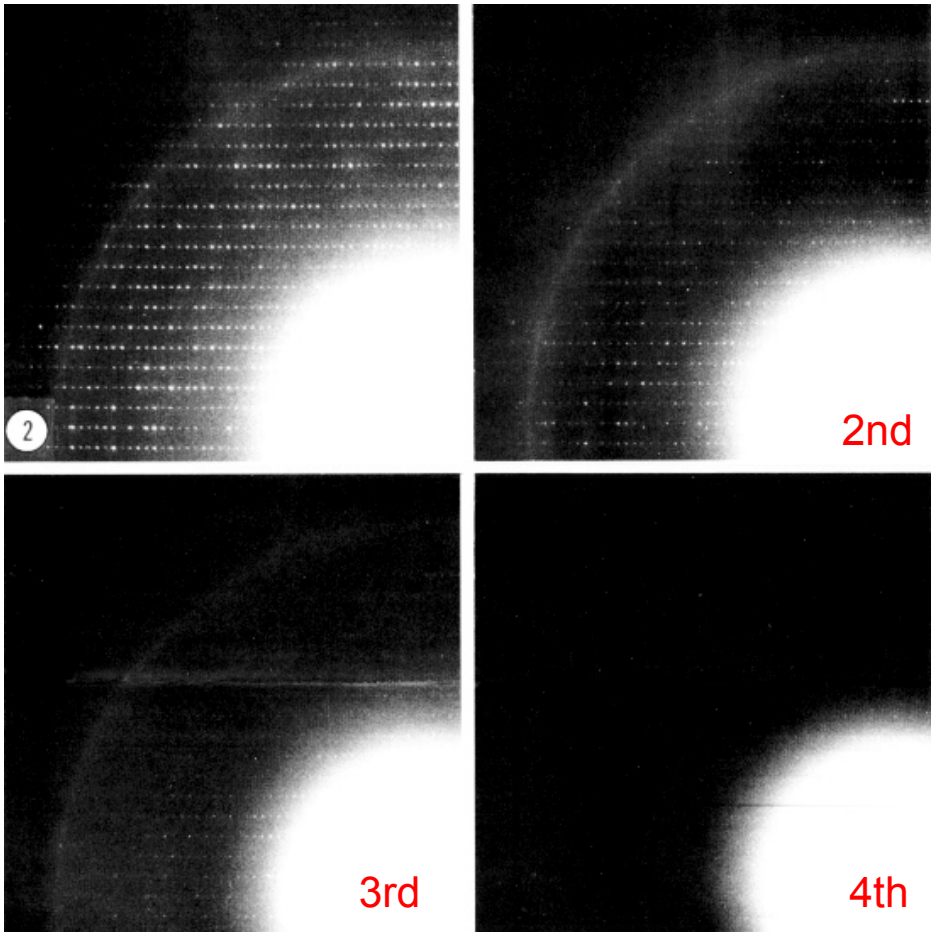
From Watt, Fig. 4.6a, p. 124

Slide not shown in class lecture

I.F OTHER MODES OF TEM OPERATION

I.F.1 Electron Diffraction

Electron diffraction patterns of a single 2D crystal of unstained, frozen-hydrated catalase



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From Taylor and Glaeser, *Science* (1974) **186:1036**

§ I: The Microscope

I.F Other Modes of TEM Operation

I.F.2 Dark Field TEM

I.F OTHER MODES OF TEM OPERATION

I.F.2 Dark Field TEM

Conventional TEM = "Bright field" EM

Dark field EM: images formed **only** from scattered electrons

- Much **higher contrast** than bright field images

  (good for visualizing molecules with very low inherent contrast, like DNA)

- Intensity **very low** (longer exposure time / more radiation damage)

- Difficult to focus and correct for astigmatism (no interference contrast)

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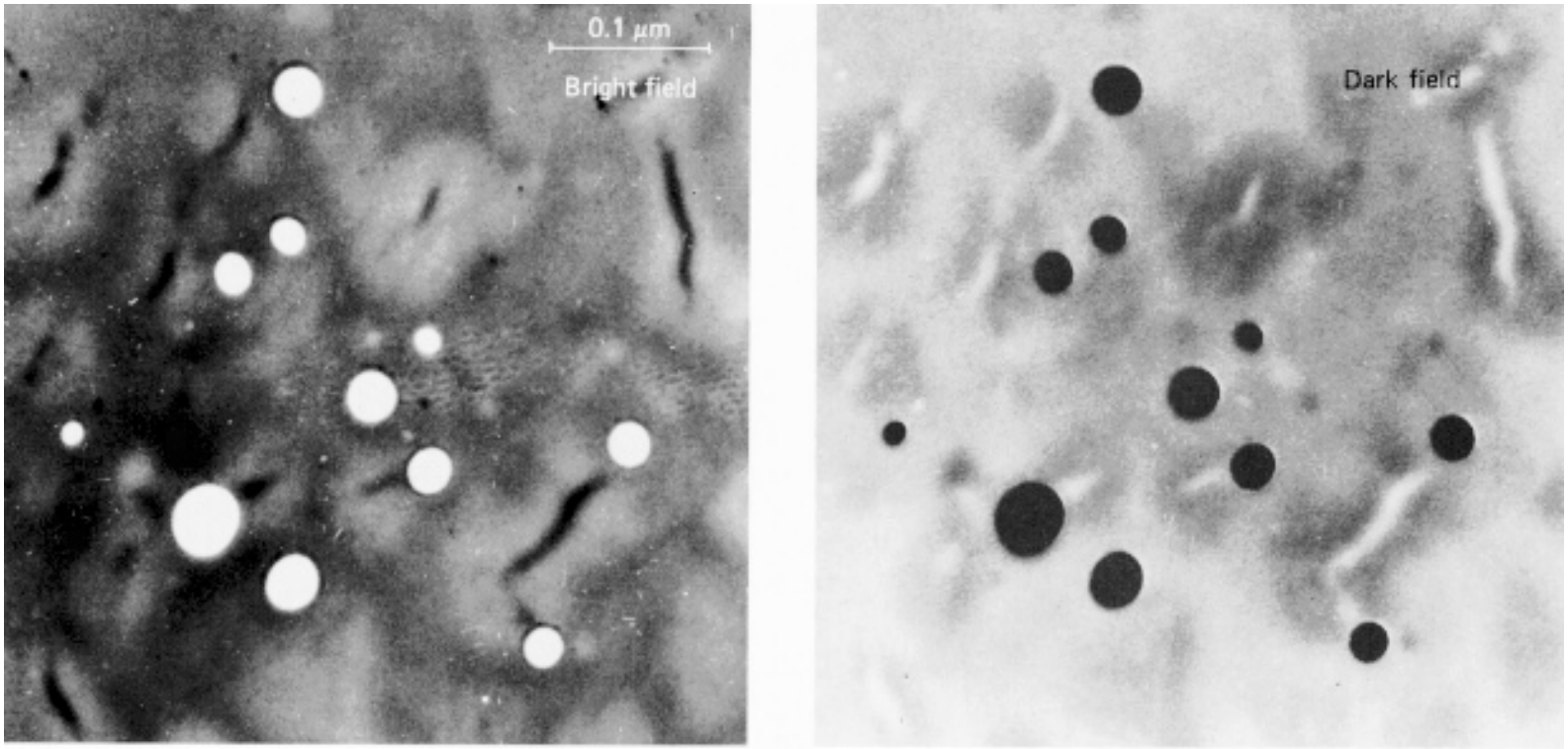
I.F OTHER MODES OF TEM OPERATION

I.F.2 Dark Field TEM

A holey carbon film in bright and dark field.



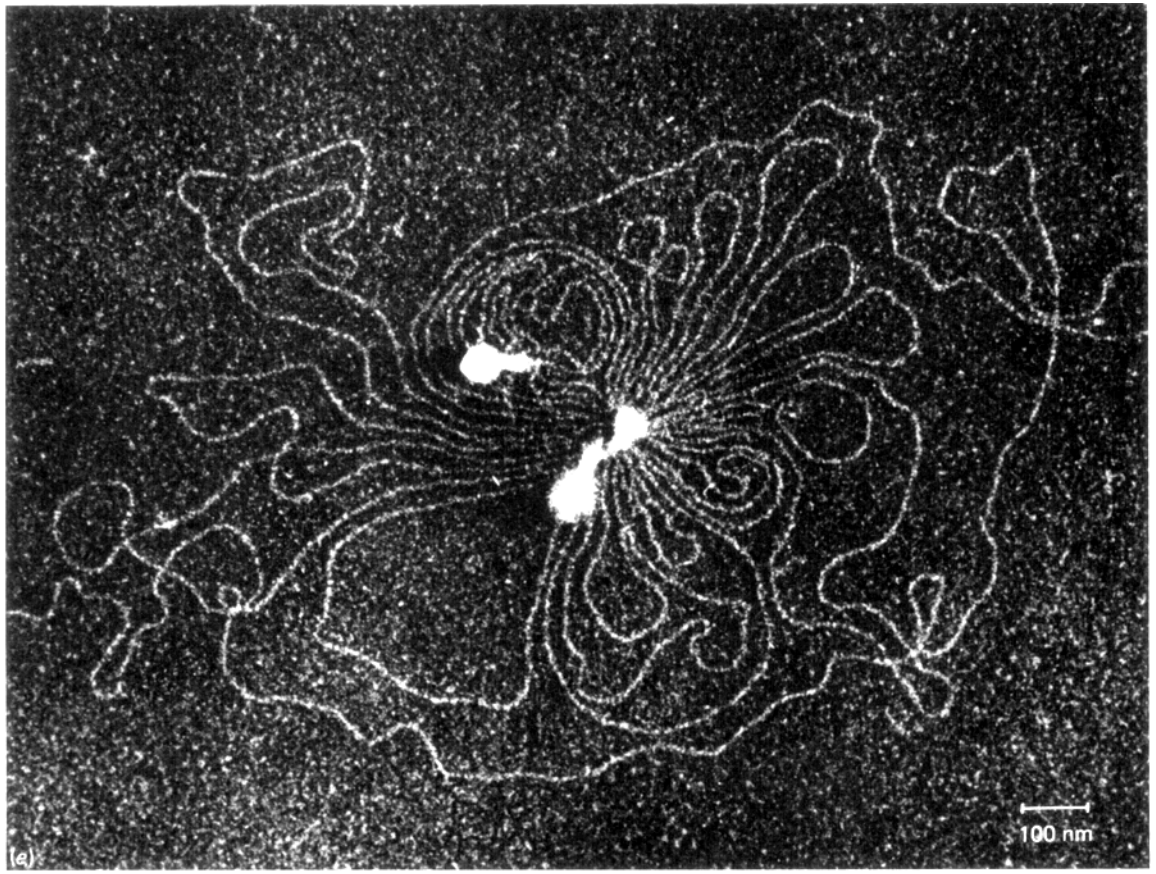
From Watt, Fig. 4.7, p. 126

Slide not shown in class lecture

I.F OTHER MODES OF TEM OPERATION

I.F.2 Dark Field TEM

Strioscopic dark field micrograph of unshadowed, unstained DNA from osmotically-shocked T4 bacteriophage



From Watt, Fig. 4.8e, p. 130

Slide not shown in class lecture

§ I: The Microscope

I.F Other Modes of TEM Operation

I.F.3 High Resolution, High Voltage TEM

I.F OTHER MODES OF TEM OPERATION

I.F.3 High resolution, High Voltage Microscopy

1MeV TEM
Circa 1990
(Boulder, Colorado)

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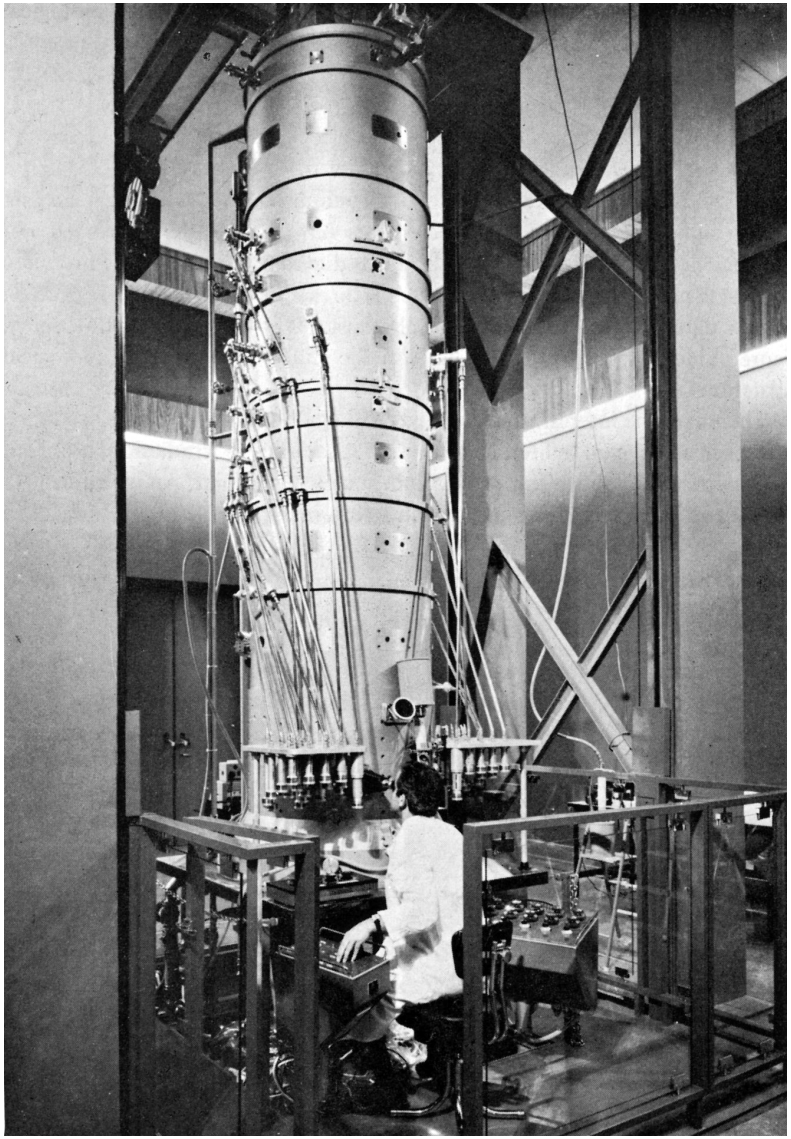
From Bozzola, Fig. 16-1, p. 361

I.F OTHER MODES OF TEM OPERATION

I.F.3 High resolution, High Voltage Microscopy



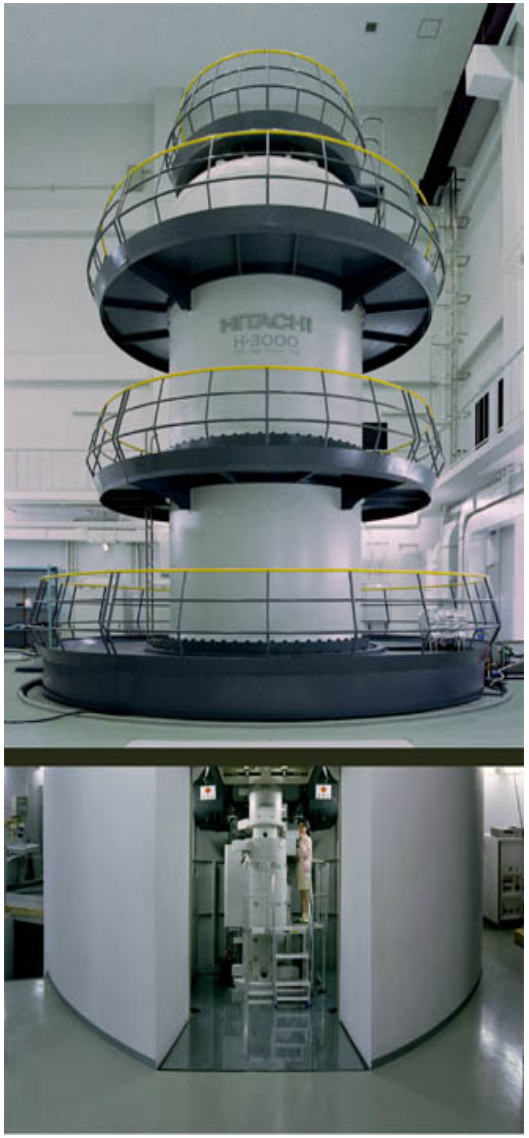
3MeV TEM
Circa 1970
(Toulouse, France)

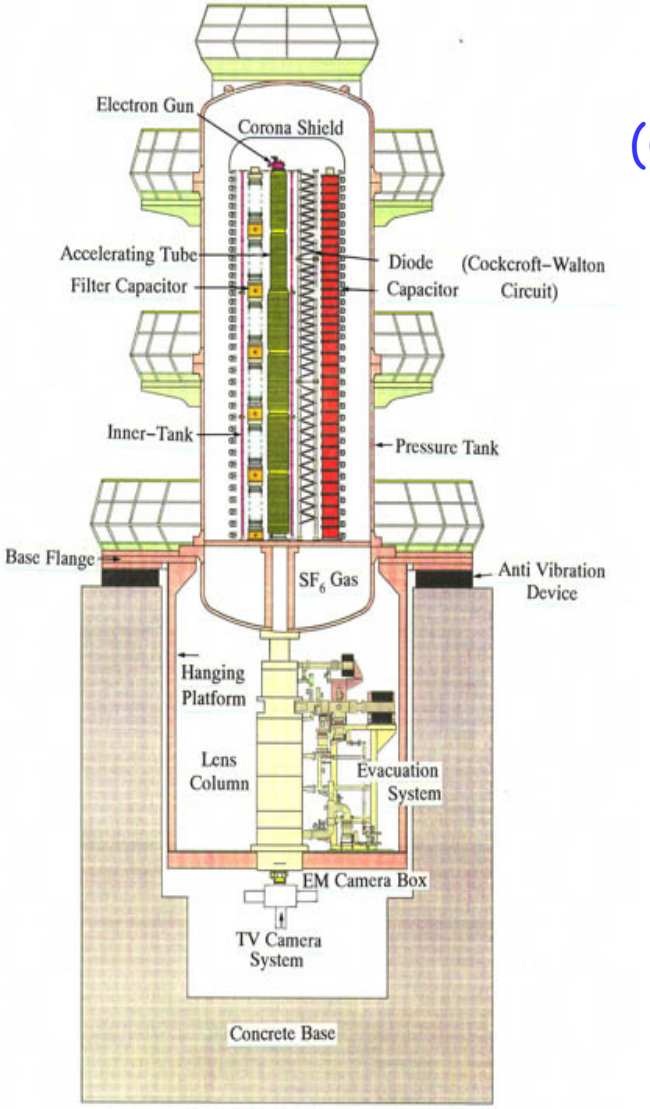
Slide not shown in class lecture

From Agar, Fig. 9-11, p. 314

I.F OTHER MODES OF TEM OPERATION

I.F.3 High resolution, High Voltage Microscopy





3MeV TEM
(Osaka University)

Slide not shown in class lecture

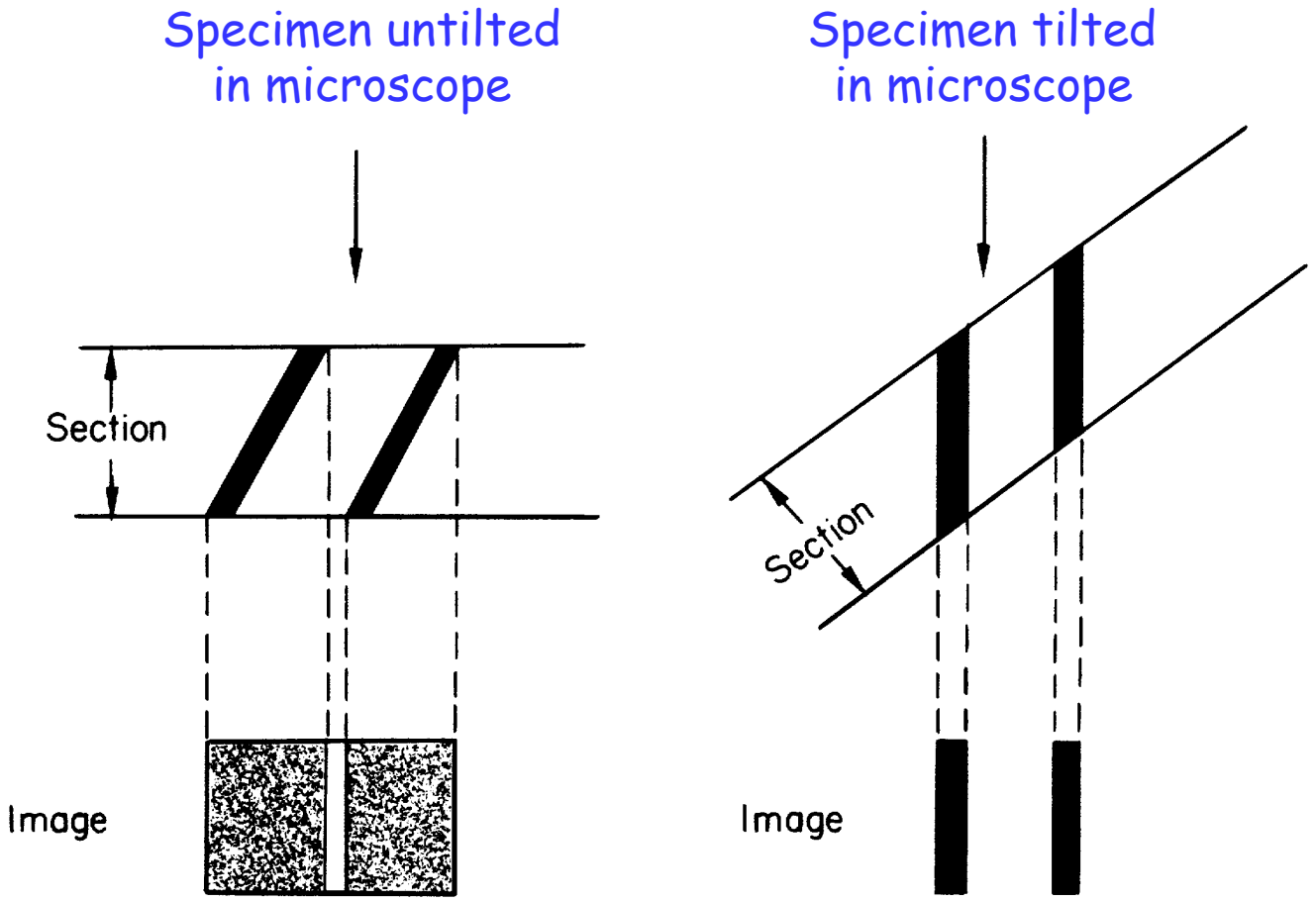
§ I: The Microscope

I.F Other Modes of TEM Operation

I.F.4 Tilt and Stereo TEM

I.F OTHER MODES OF TEM OPERATION

I.F.4 Tilt and Stereo TEM

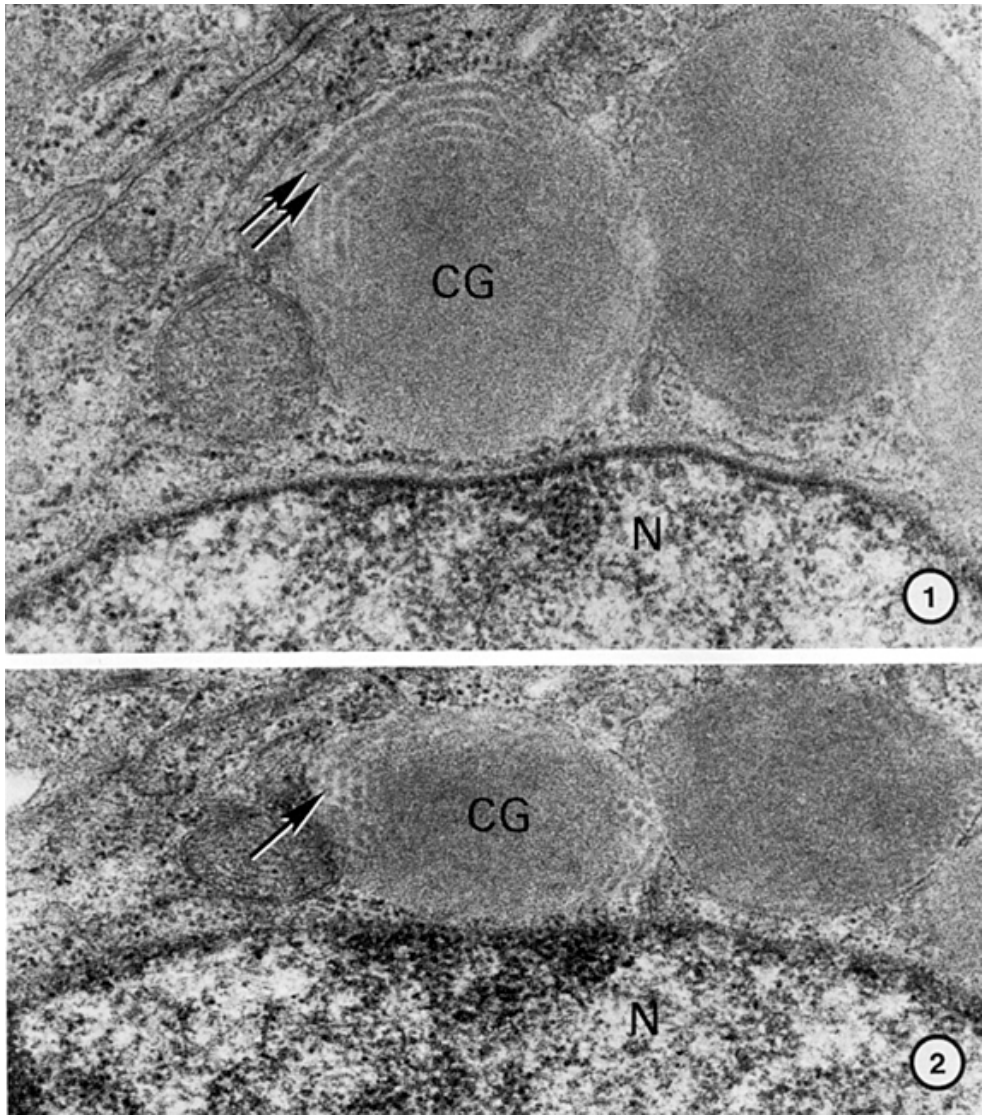


From Meek 1st ed., Fig. 10.11, p. 291

Slide not shown in class lecture

I.F.4 Tilt and Stereo TEM

Untilted and Tilted Cytoplasmic Granules



0° tilt

~45° tilt

From Turek, *Anat. Record* (1982) **203:329**

Slide not shown in class lecture

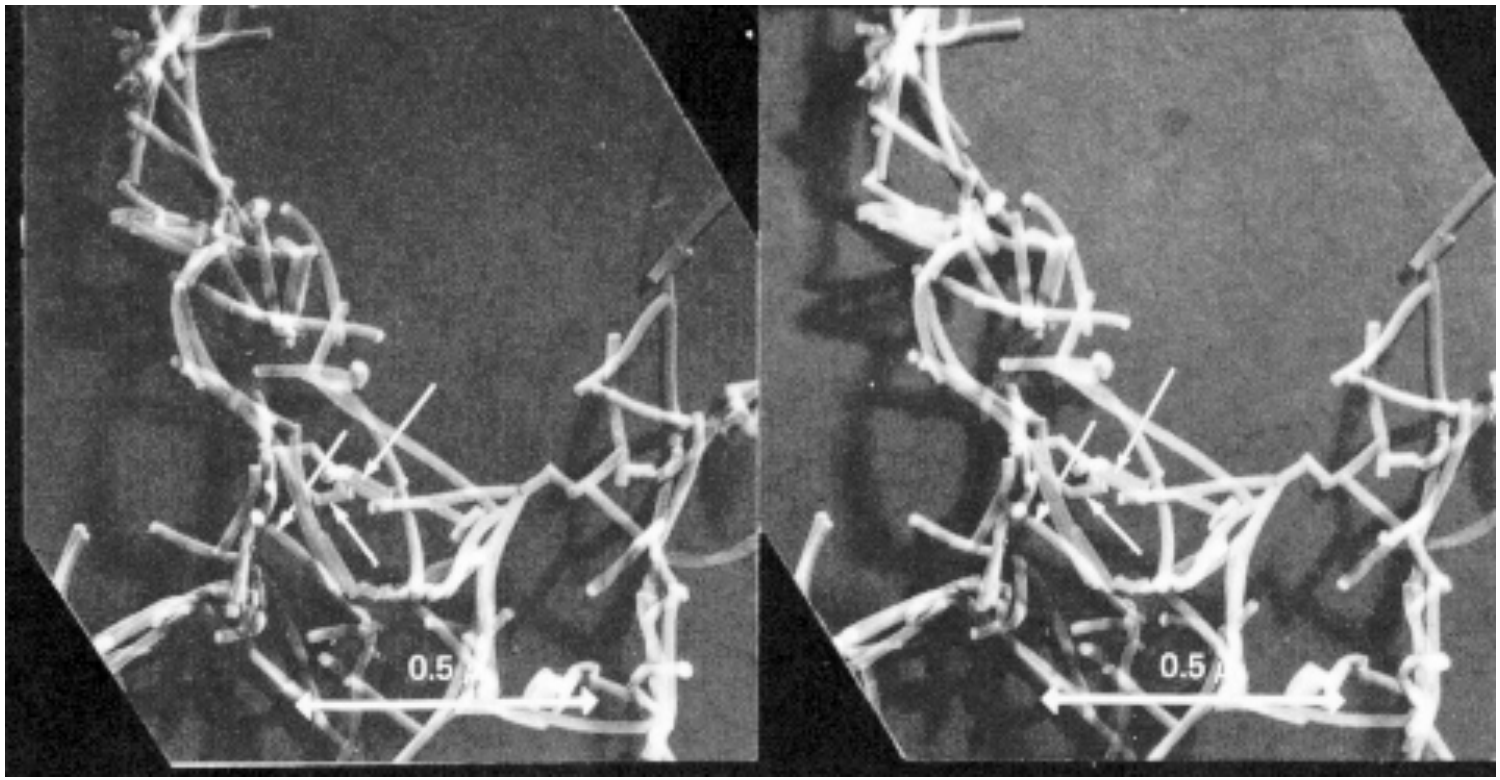
Slide courtesy of John Turek

I.F OTHER MODES OF TEM OPERATION

I.F.4 Tilt and Stereo TEM

Stereoscopic Pair

(freeze-dried, metal-shadowed tobacco mosaic virus particles)

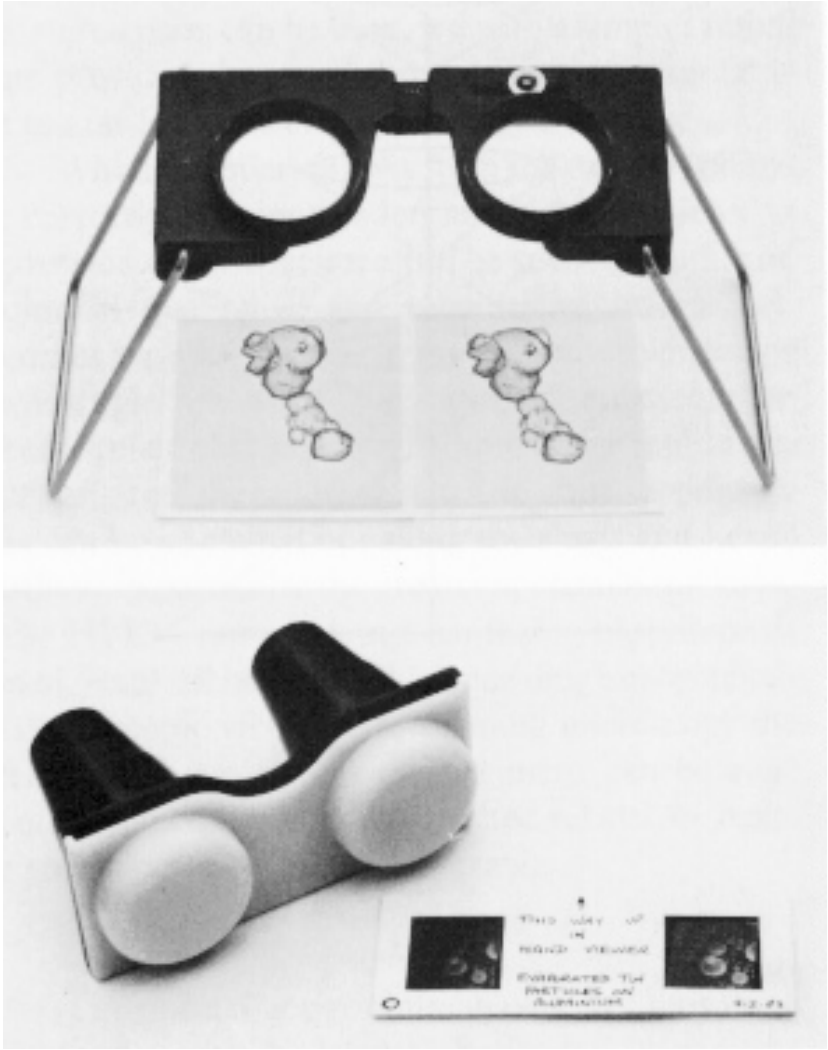


From Watt, Fig. 4.29, p. 157

Slide not shown in class lecture

I.F OTHER MODES OF TEM OPERATION

I.F.4 Tilt and Stereo TEM



Simple viewers for stereoscopic pairs

From Watt, Fig. 4.11, p. 136

Slide not shown in class lecture

§ I: The Microscope

I.F Other Modes of TEM Operation

I.F.5 Low Temperature Microscopy

More about this in §II (Specimen Preparation)

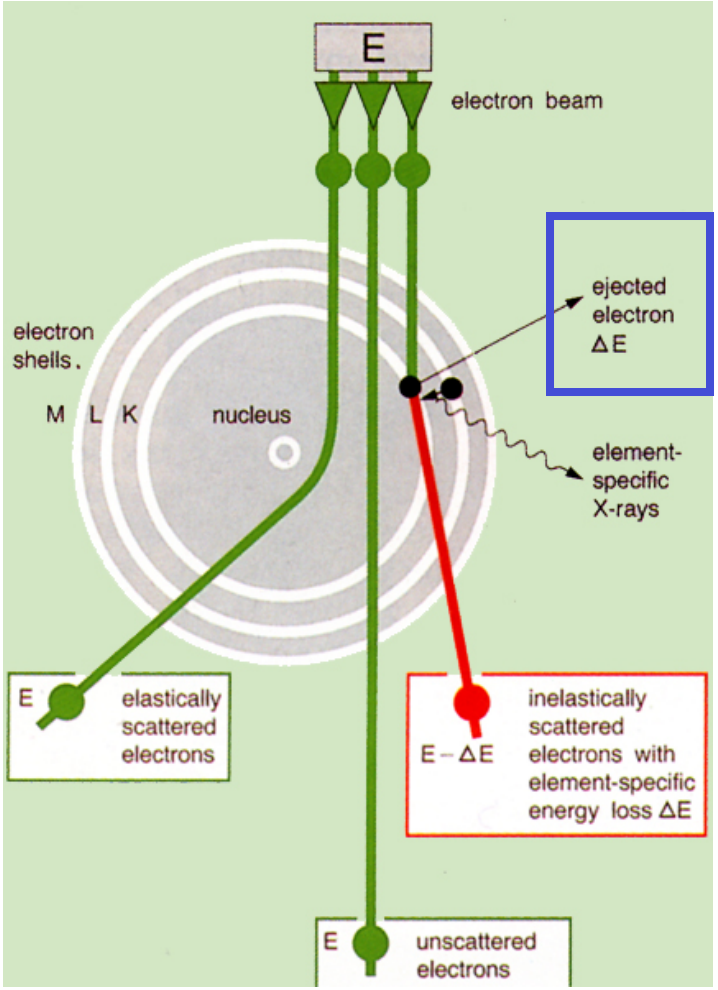
§ I: The Microscope

I.F Other Modes of TEM Operation

I.F.6 Electron Energy Loss Spectroscopy (EELS)

I.F OTHER MODES OF TEM OPERATION

I.F.6 Electron Energy Loss Spectroscopy (EELS)



Elemental analysis based upon element specific X-ray generation or electron energy loss (EELS).

Slide not shown in class lecture

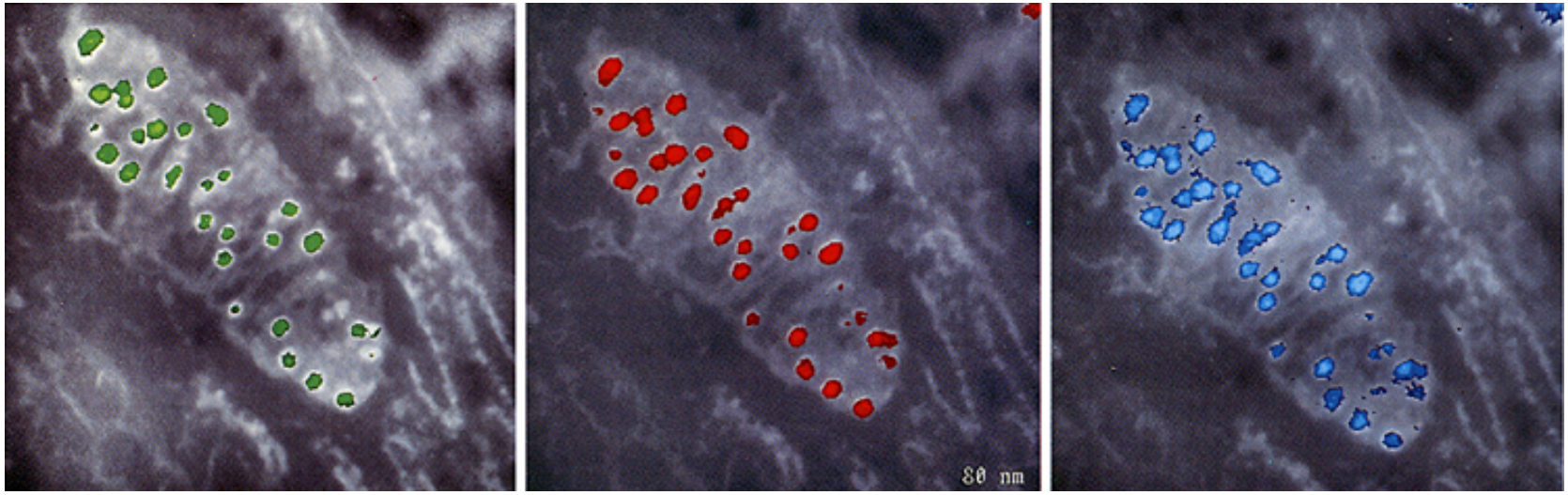
Slide courtesy of John Turek

I.F OTHER MODES OF TEM OPERATION

I.F.6 Electron Energy Loss Spectroscopy (EELS)

Phosphorus, Calcium and Oxygen in Mitochondria

(Zeiss (LEO) -EELS)



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Slide courtesy of John Turek

§ I: The Microscope

I.F Other Modes of TEM Operation

I.F.7 X-ray Microanalysis

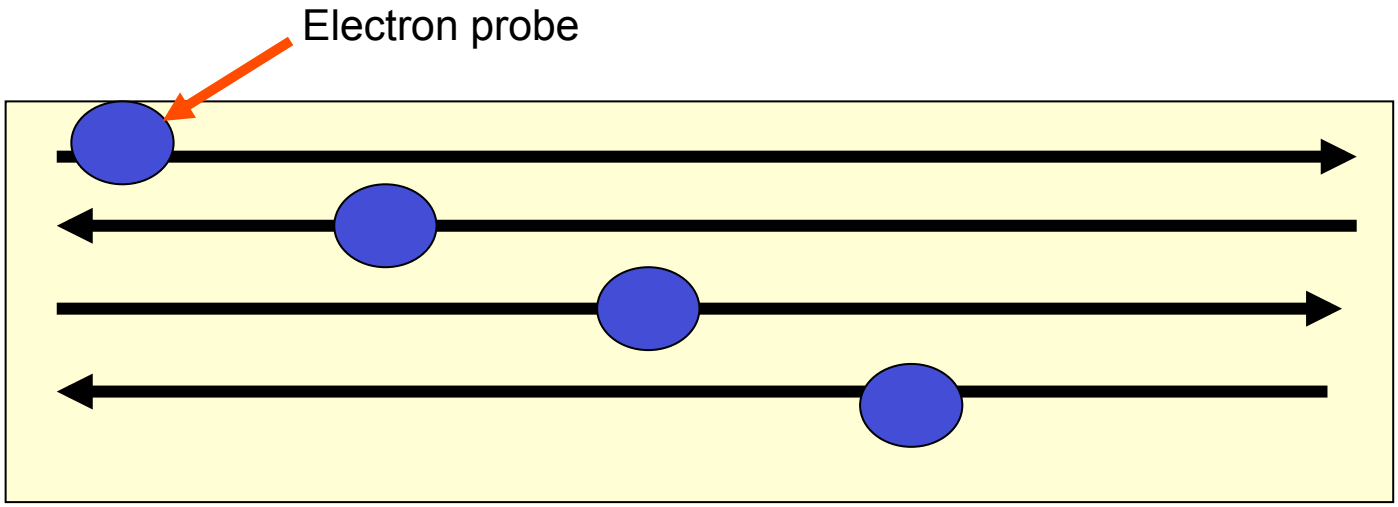
I.F OTHER MODES OF TEM OPERATION

I.F.7 X-Ray Microanalysis

BASIC PRINCIPLE:

When electron beam interacts with a specimen, specimen electrons are boosted to higher energy (orbital) levels

When e^- decay back to a lower orbital they emit some of the energy as X-rays (wavelength is specific for each element and orbital)



Can scan the sample with the probe, or focus the probe on a small area

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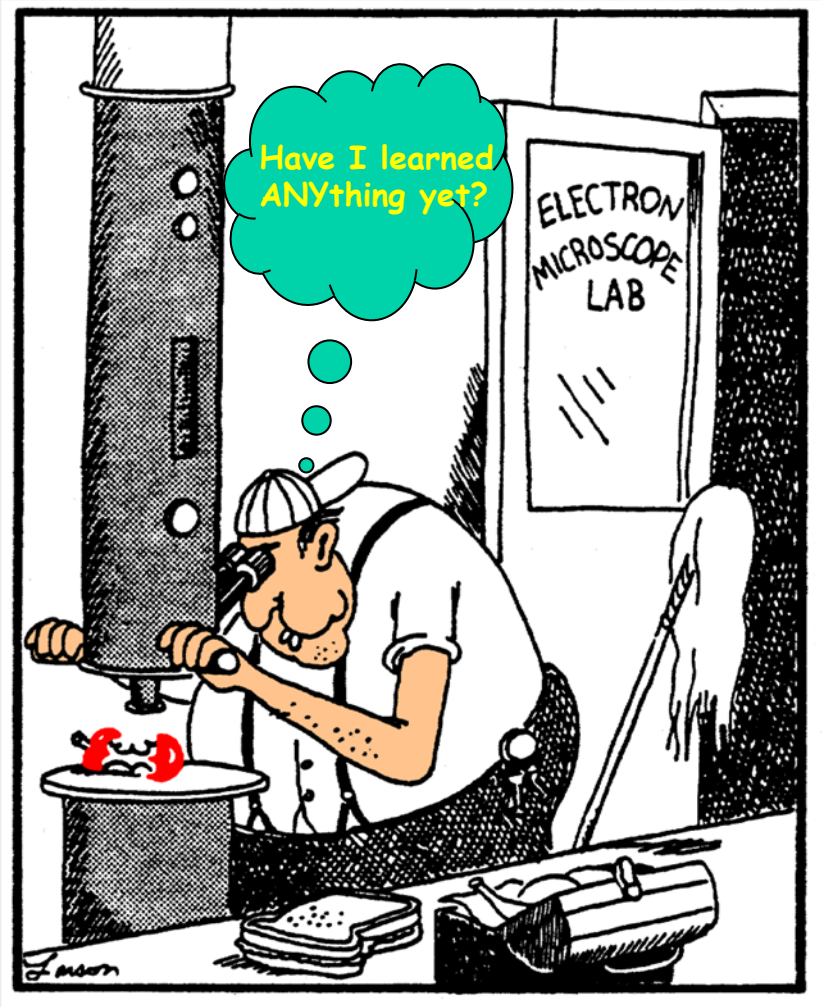
Illustration courtesy of John Turek



TOPICS

- Principles of TEM
  - Electrons, lenses and optics
- Design of TEM
  - Components top to bottom
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  - Electron scattering from object
- Optimizing TEM performance
  - Alignment assures ‘best’ images
- Operation of TEM
  - “What do all these buttons do?”
- Other modes of TEM
  - Many ways to ‘observe’ specimens
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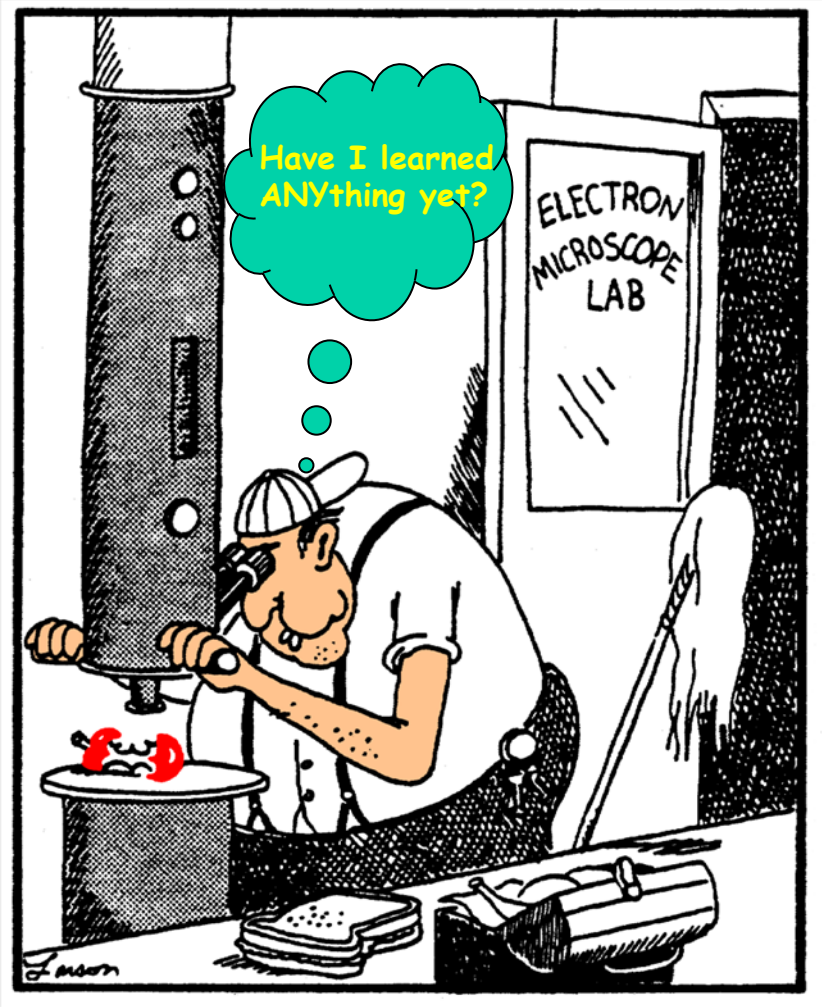
  - Getting specimen ready
- Radiation damage
  - Less is better
- 3D reconstruction
  - Specimen 3D structure from 2D images

§ I: The Microscope



TOPICS

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§ I: The Microscope

§ II: The Specimen

§ I: The Microscope

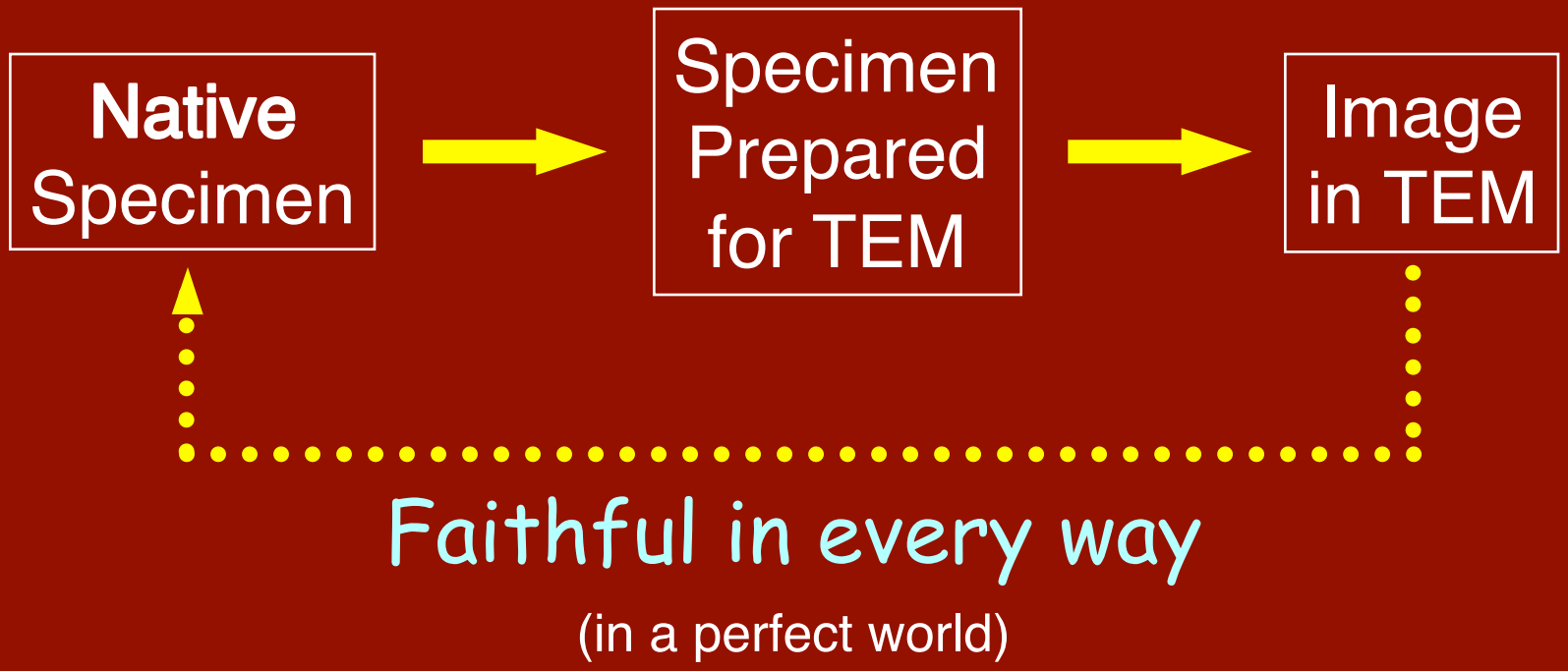
§ II: The Specimen

II.A. Biological Specimen Preparation Techniques

II.B. Radiation Effects

Biological Specimen Preparation

The Goal



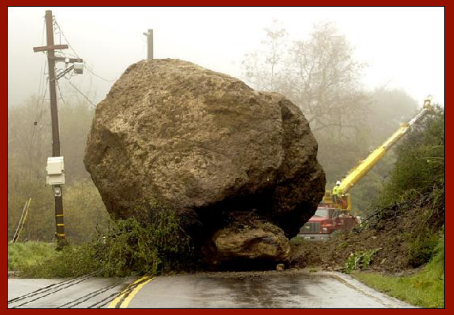
Biological Specimen Preparation

How might one prepare biological specimens for TEM?

- Fix, embed and section
- Metal shadow
- Negative stain
- Positive stain
- Unstained, frozen-hydrated
- Freeze-fracture
- Freeze-etch
- Freeze-dry
- Cryo-section
- Etc., etc., etc.

Biological Specimen Preparation

The Obstacles



Contrast

Thickness

Dehydration

Radiation Damage

Biological Specimen Preparation

The Obstacles

Contrast

Bio. specimens (esp. thin ones) don't have any inherent contrast

Thickness

Electron beam can't penetrate thick specimens

Dehydration

Specimens don't enjoy life in a vacuum

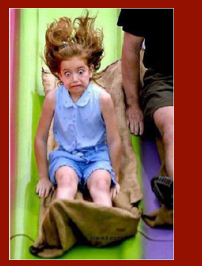
Radiation Damage

Specimens don't enjoy being "cooked" in a high voltage e- beam

§ II: The Specimen

II.A. Biological Specimen Preparation Techniques

- II.A.1 Specimen Support Films
- II.A.2 Thin Sectioning
- II.A.3 Negative Staining
- II.A.4 Metal Shadowing
- II.A.5 Freeze Drying/Etching/Fracture
- II.A.6 Unstained and Frozen-Hydrated



§ II: The Specimen

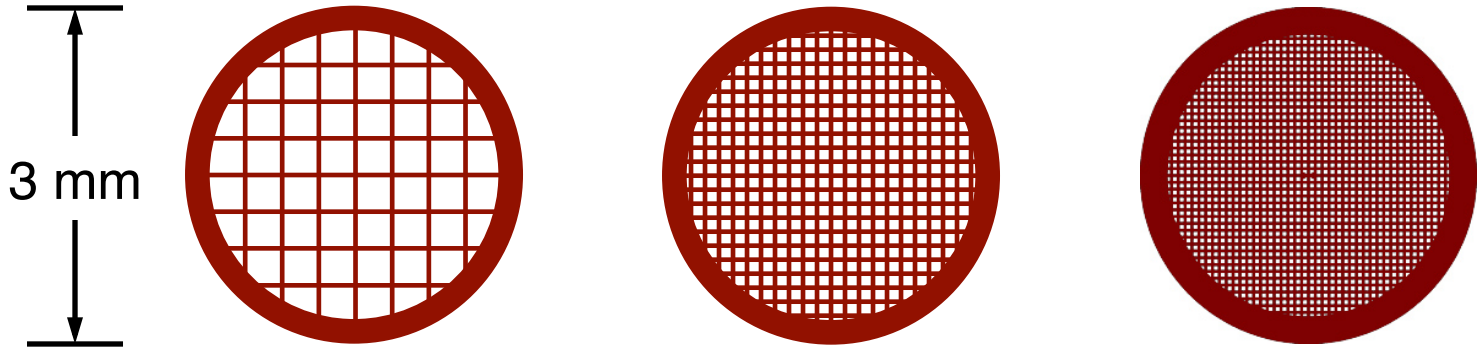
II.A. Biological Specimen Preparation Techniques

- II.A.1 Specimen Support Films
- II.A.2 Thin Sectioning
- II.A.3 Negative Staining
- II.A.4 Metal Shadowing
- II.A.5 Freeze Drying/Etching/Fracture
- II.A.6 Unstained and Frozen-Hydrated

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.1 Specimen Support Films for TEM

It all generally begins with a 3mm copper grid



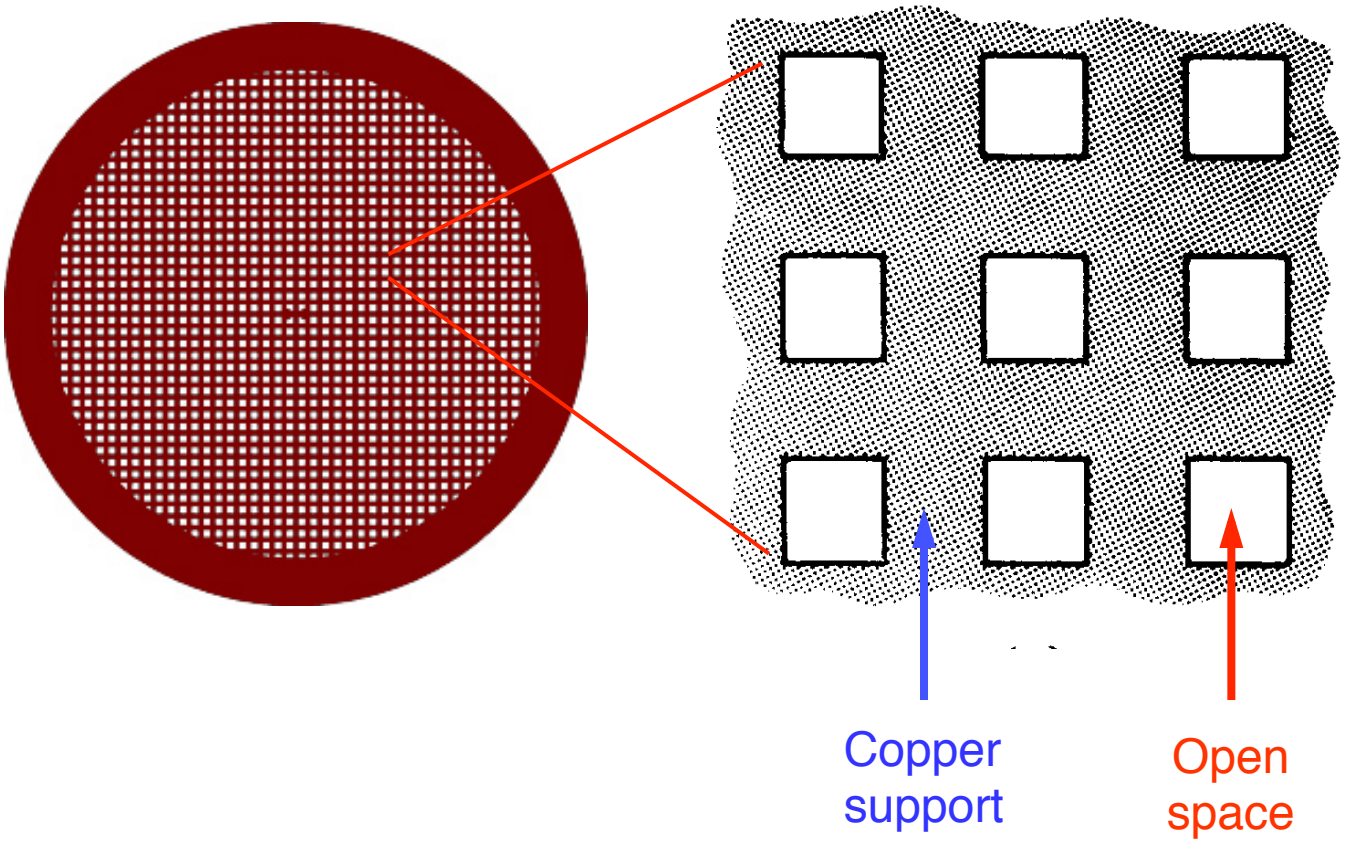
	75 mesh	200 mesh	400 mesh
Open space in 1 square	292 μm	97 μm	42 μm

Mesh size refers to the number of grid bars per inch

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.1 Specimen Support Films for TEM

It all generally begins with a 3mm copper grid



From Slayter (1970), p.391

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.1 Specimen Support Films for TEM

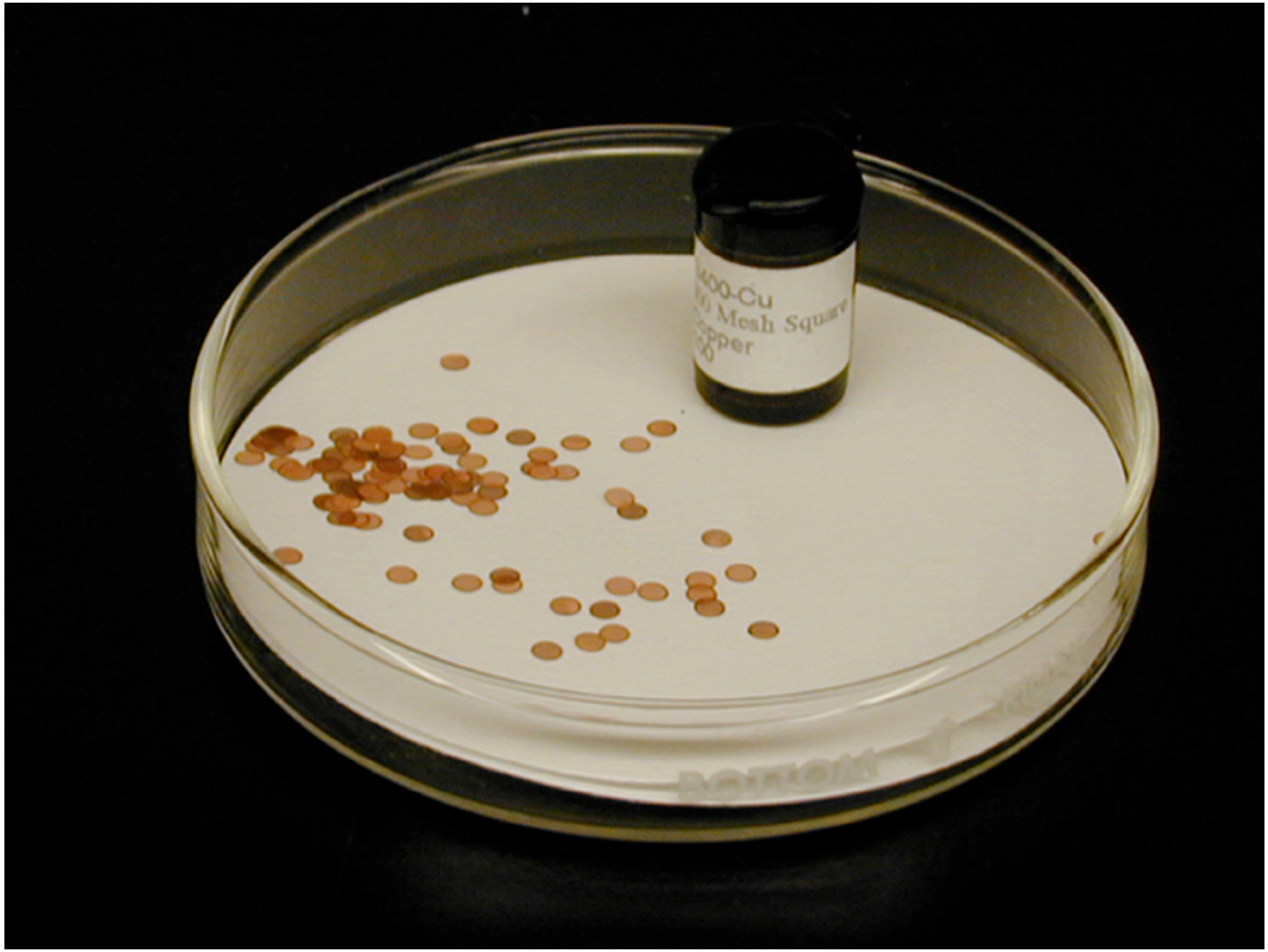
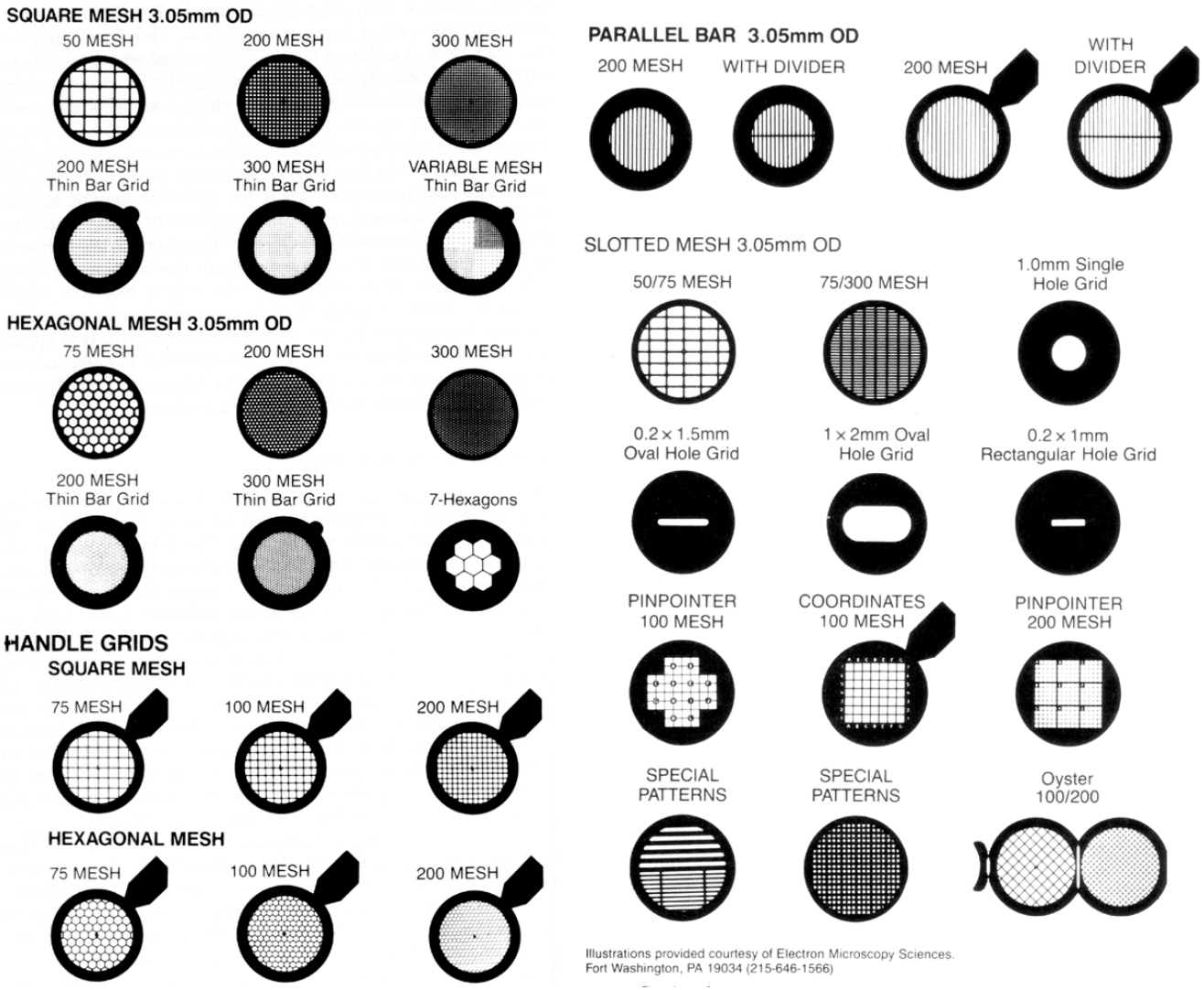


Image courtesy of P. Chipman (2004)

II.A.1 Specimen Support Films for TEM

Commercially Available Grids



From Hayat & Miller (1990) *Negative Staining*, pp.32-33

II.A.1 Specimen Support Films for TEM

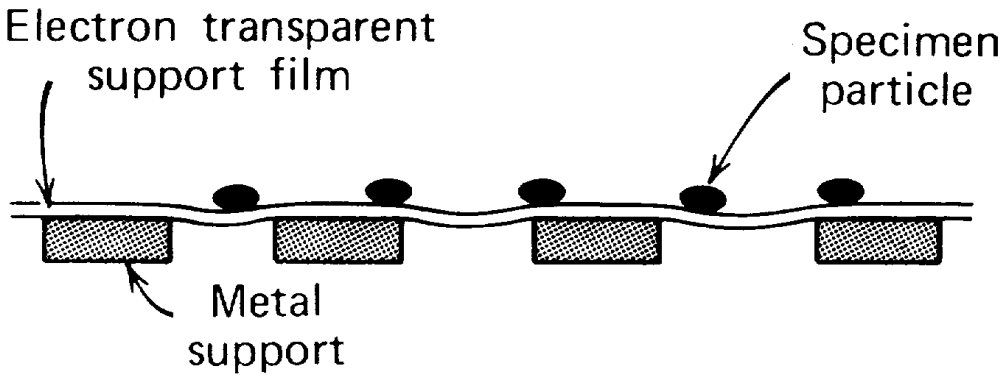
What comes next?

The support film

Why?

Need surface on which to deposit samples

(viruses, macromolecules, thin sections, etc.)



From Slayter (1970), p.391

II.A.1 Specimen Support Films for TEM

What comes next?

The support film

Why?

Need surface on which to deposit samples

(viruses, macromolecules, thin sections, etc.)

Adds physical strength to grid

II.A.1 Specimen Support Films for TEM

What comes next?

The support film

Why?

Need surface on which to deposit samples

(viruses, macromolecules, thin sections, etc.)

Adds physical strength to grid

Increased stability / heat dissipation in e^- beam

Slide not shown in class lecture

II.A.1 Specimen Support Films for TEM

Practical Considerations for Film Selection

Ease of preparation

Ease of handling

Clean smooth film surface

II.A.1 Specimen Support Films for TEM

Commonly Used Types of Films

Plain plastic (collodion or Formvar)

Plastic coated (stabilized) with evaporated carbon

Plain carbon

II.A.1 Specimen Support Films for TEM

Film Properties
(necessary for high resolution work)

Good conductor (transfer heat and charge away from sample)

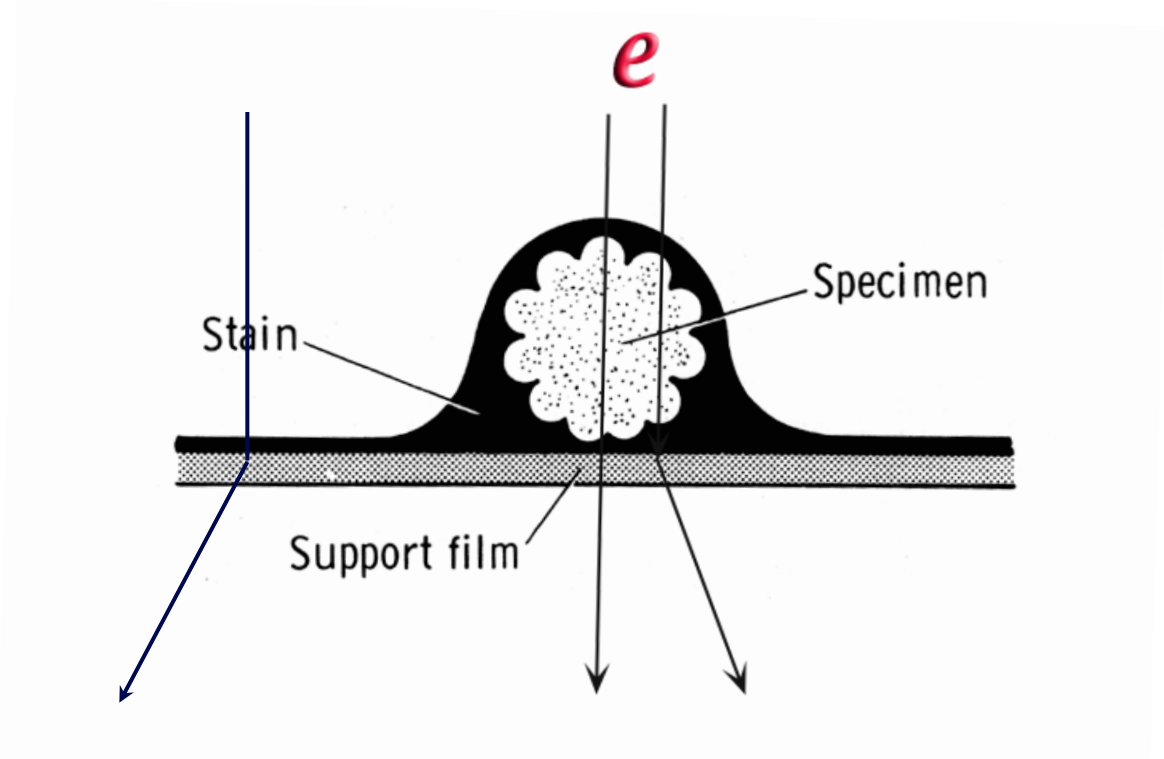
Physical strength (withstand handling and vacuum conditions)

Low electron scattering (should not reduce specimen contrast)

Amorphous (minimize background structure)

II.A.1 Specimen Support Films for TEM

Film "Structure" Superimposed on Sample Structure



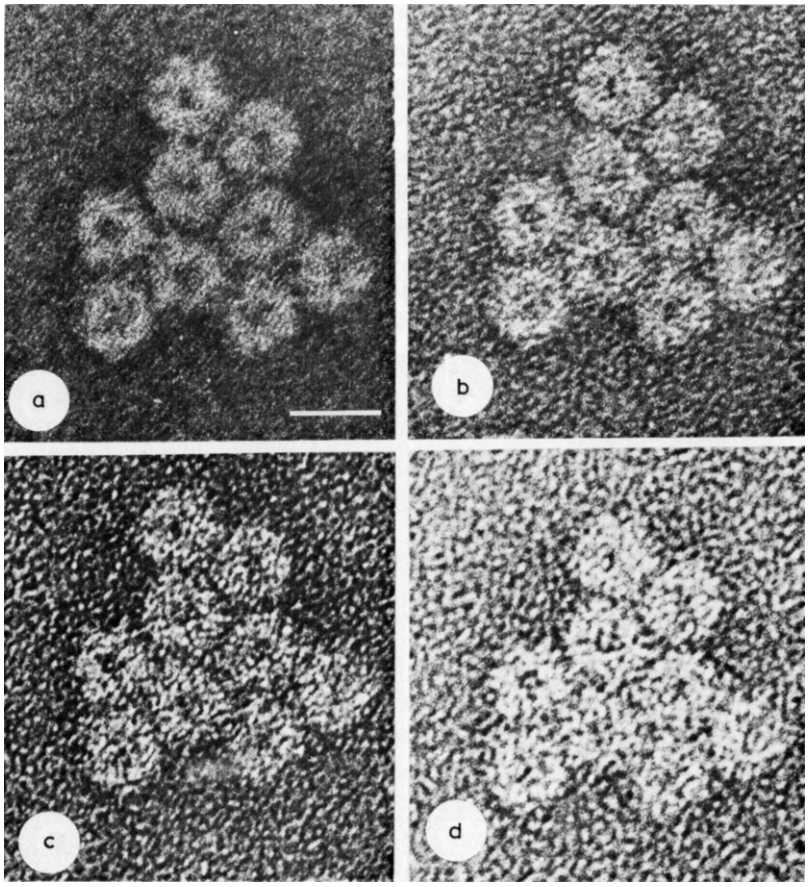
Contrast (both aperture and interference) introduced by a support film ***can be*** comparable to the contrast of the sample, especially thin biological ones

Adapted from Hayat & Miller, p.2

II.A.1 Specimen Support Films for TEM

Film "Structure" Superimposed on Sample Structure

Support film 'phase' granularity superimposed on sample structure



Δf ≅ 0 nm

Δf = 100 nm

Δf = 150 nm

Δf = 200 nm

Focal series of negatively stained adenovirus 'groups of nine' hexons

Misell, Fig.2.4, p.20

II.A.1 Specimen Support Films for TEM

Film Properties
(necessary for high resolution work)

Good conductor (transfer heat and charge away from sample)

Physical strength (withstand handling and vacuum conditions)

Low electron scattering (should not reduce specimen contrast)

Amorphous (minimize background structure)

Evaporated Carbon: has essentially all of these qualities

II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films

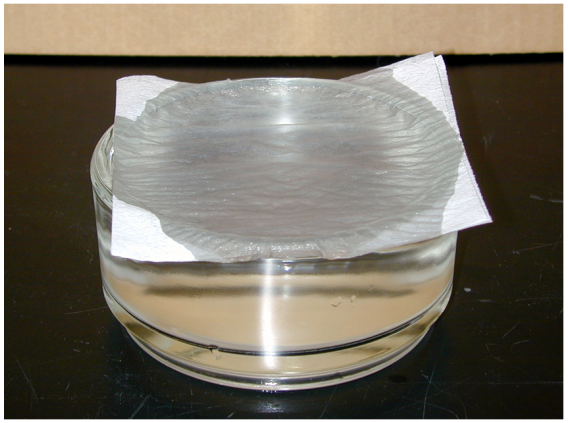
Float method (drop)

Dip method (casting)

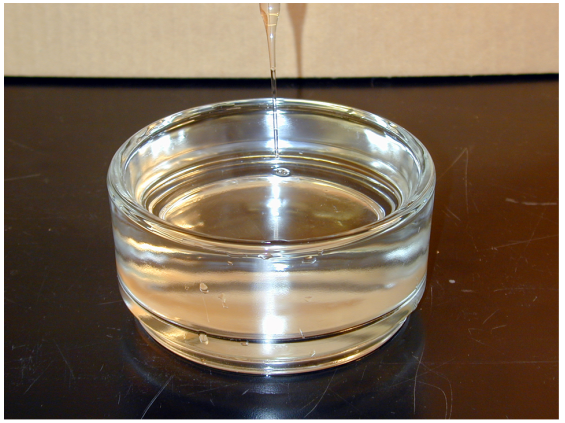
II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films: Drop Method

Clean the water surface



Drop collodion solution onto water surface

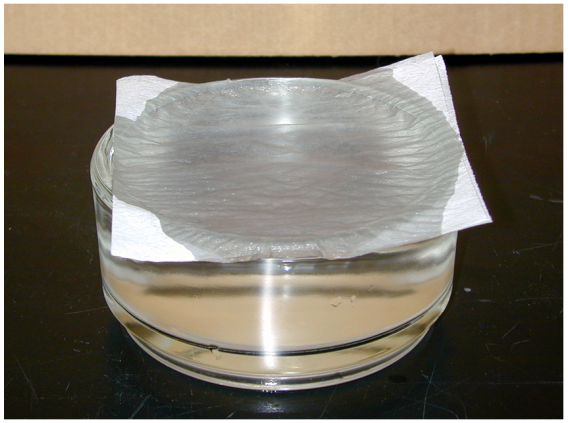


Images courtesy of V. Bowman (2004)

II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films: Drop Method

Clean the water surface



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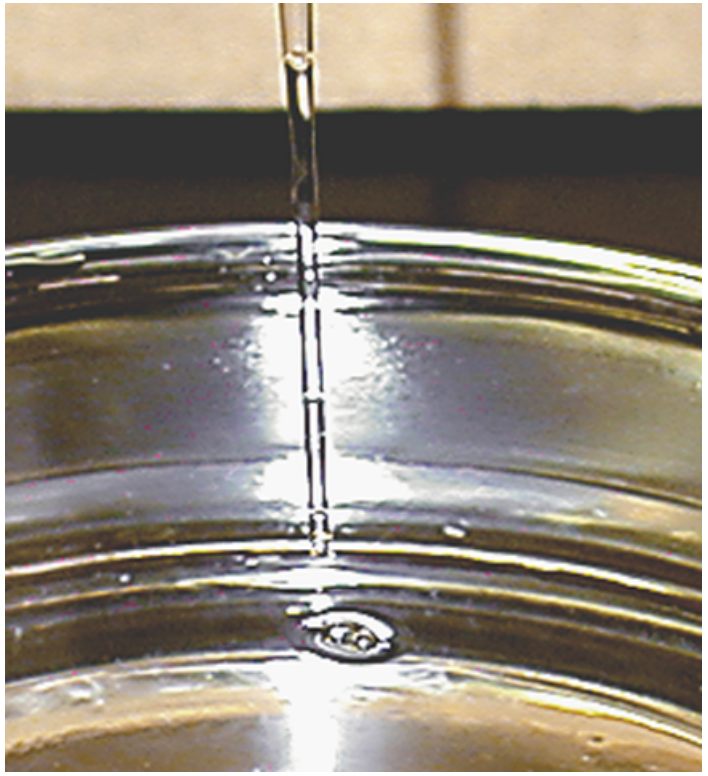
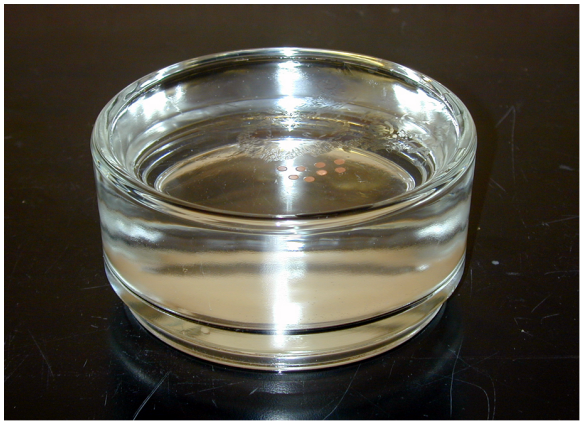


Place grids on dry film

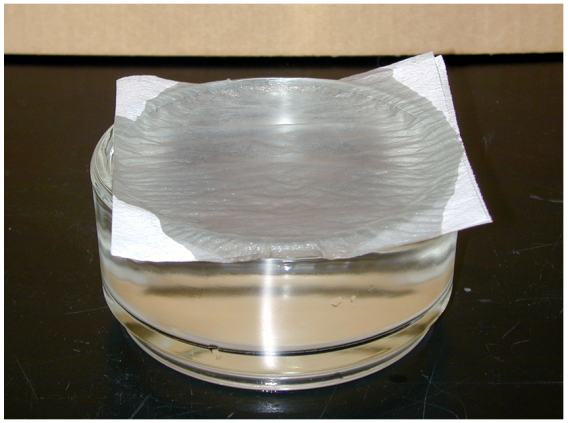


Images courtesy of V. Bowman (2004)

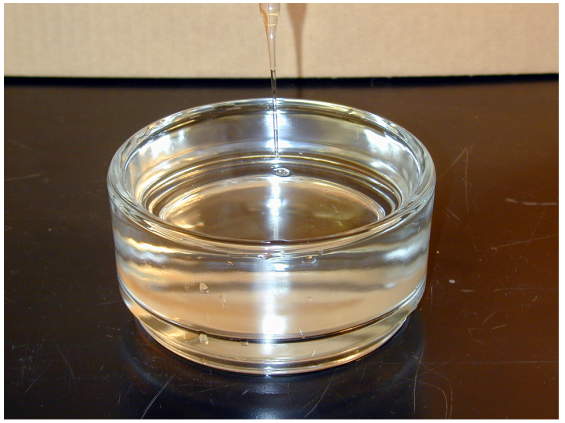
II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films: Drop Method

Clean the water surface

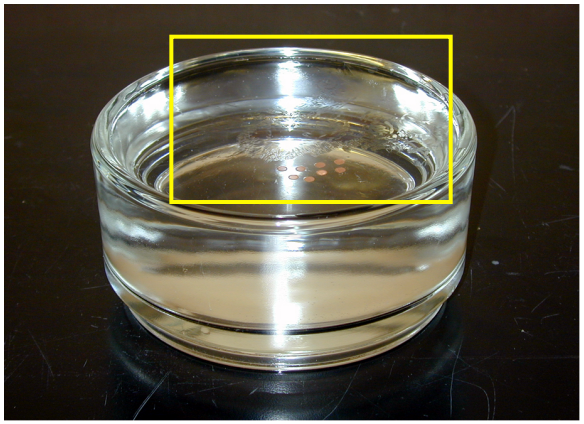


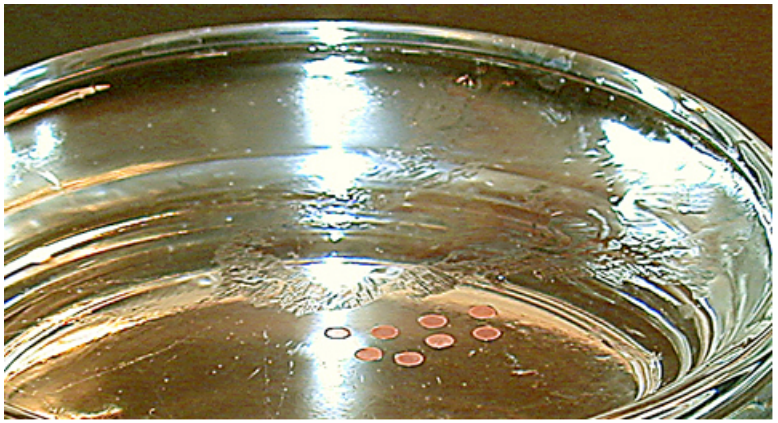
Drop collodion solution onto water surface



Let solvent evaporate

Place grids on dry film





Images courtesy of V. Bowman (2004)

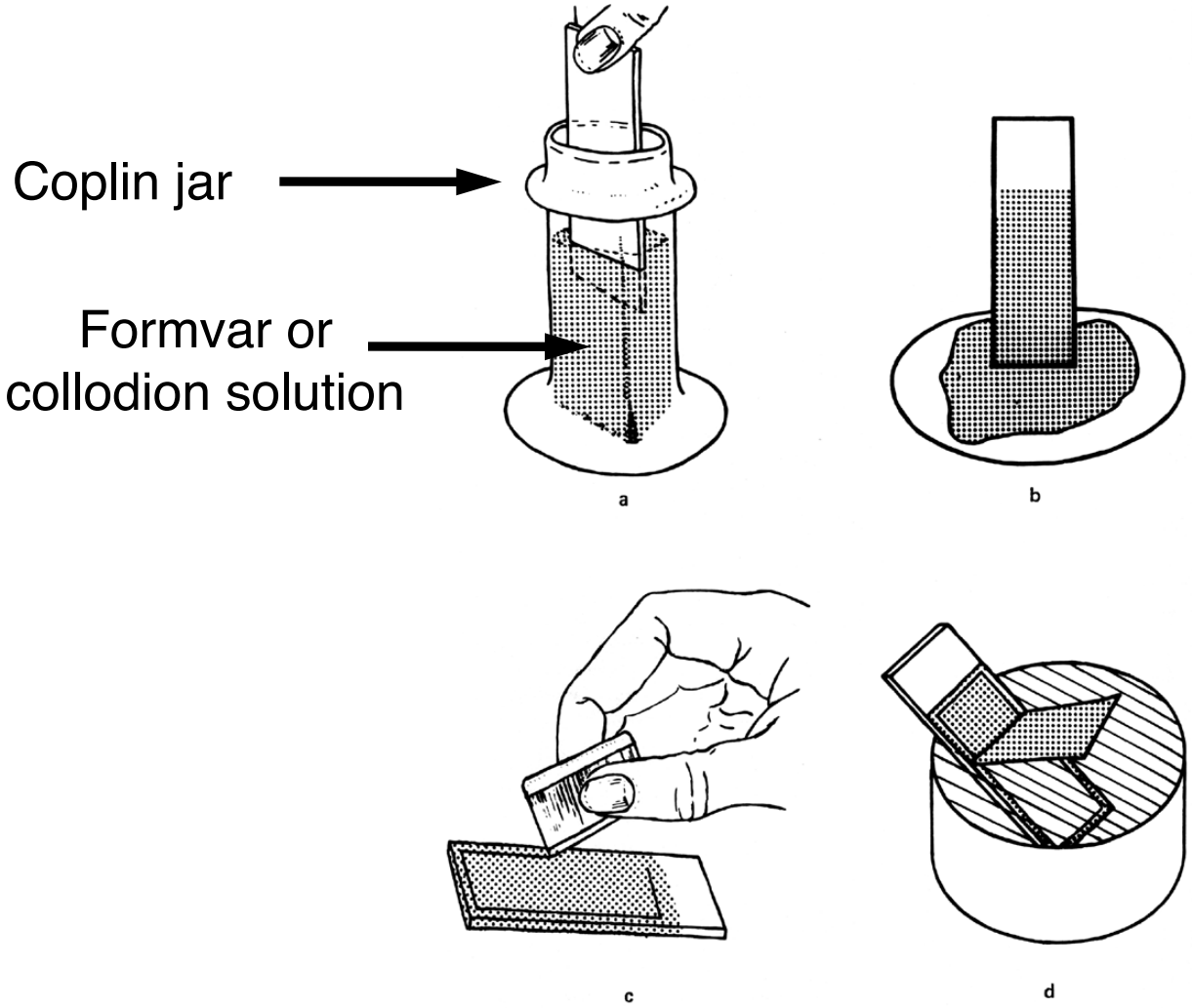
II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films

- Float method (drop)
- Dip method (casting)

II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films: Dip (Casting) Method

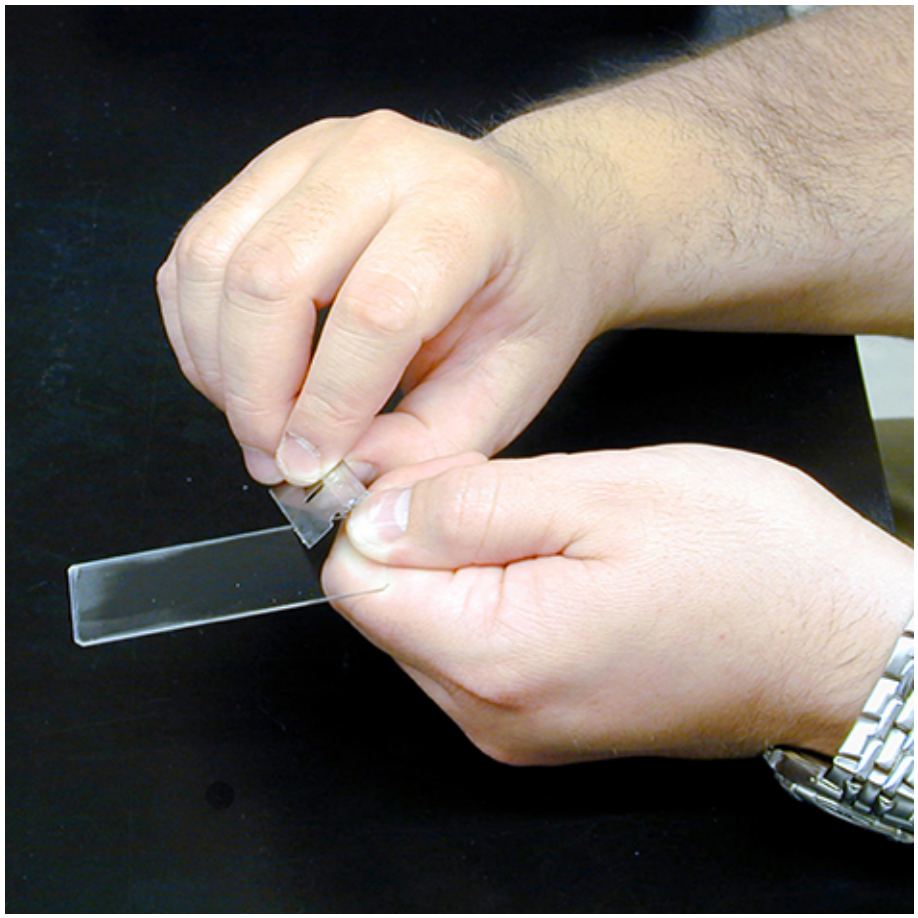


From Hayat and Miller, p.184

II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films: Dip (Casting) Method

Dry Formvar-coated slides
(dust-free environment)

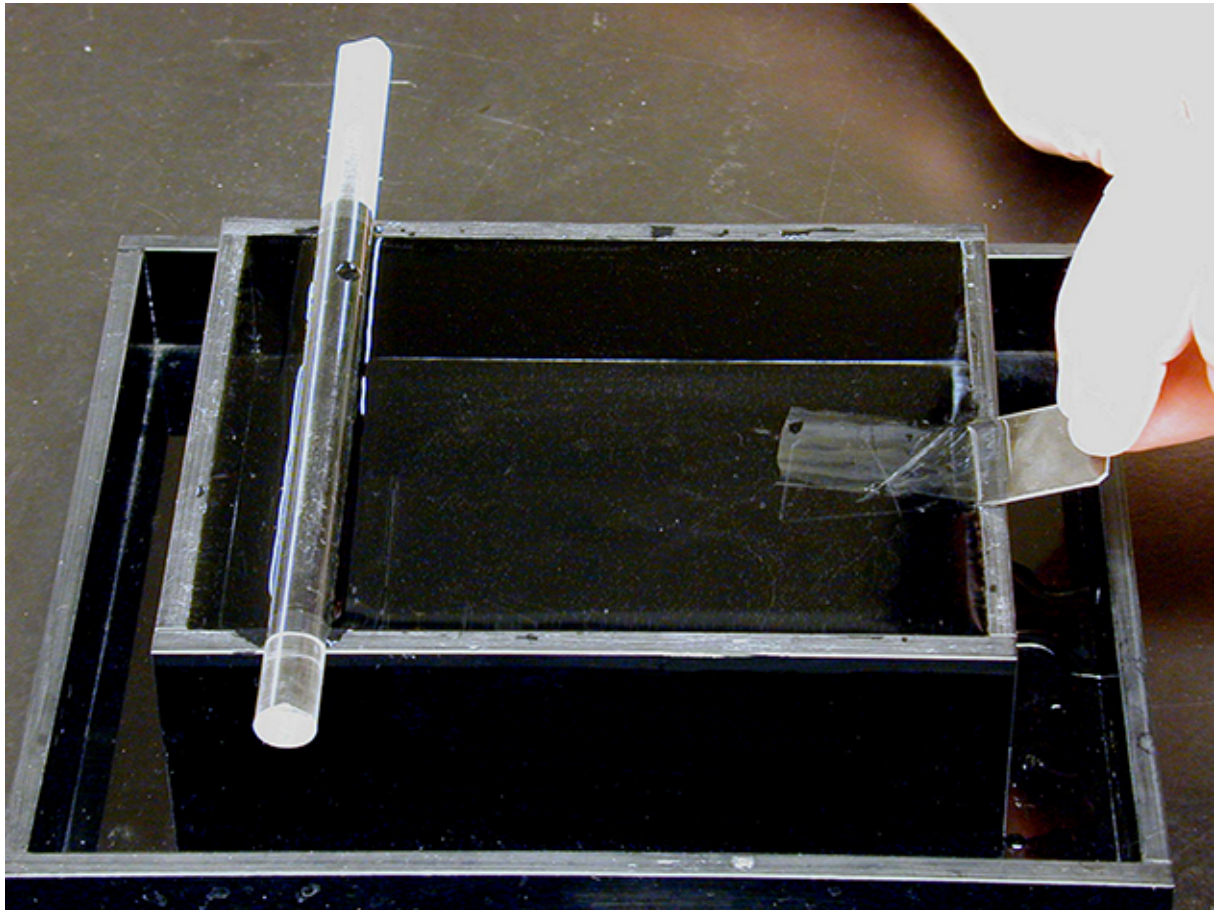


Score edges of film with a clean razor blade

Images courtesy of P. Chipman (2004)

II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films: Dip (Casting) Method

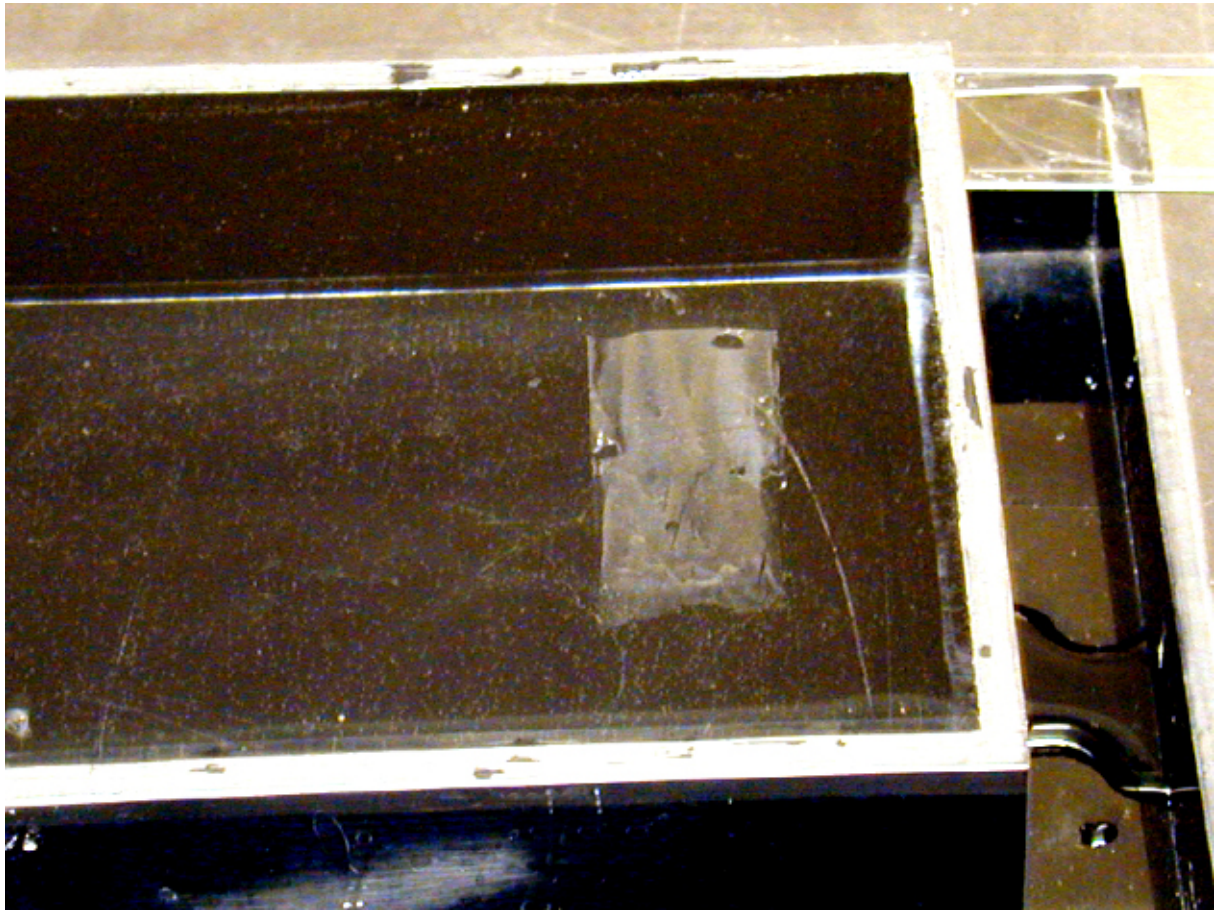


Gently float film onto clean water surface

Image courtesy of P. Chipman (2004)

II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films: Dip (Casting) Method

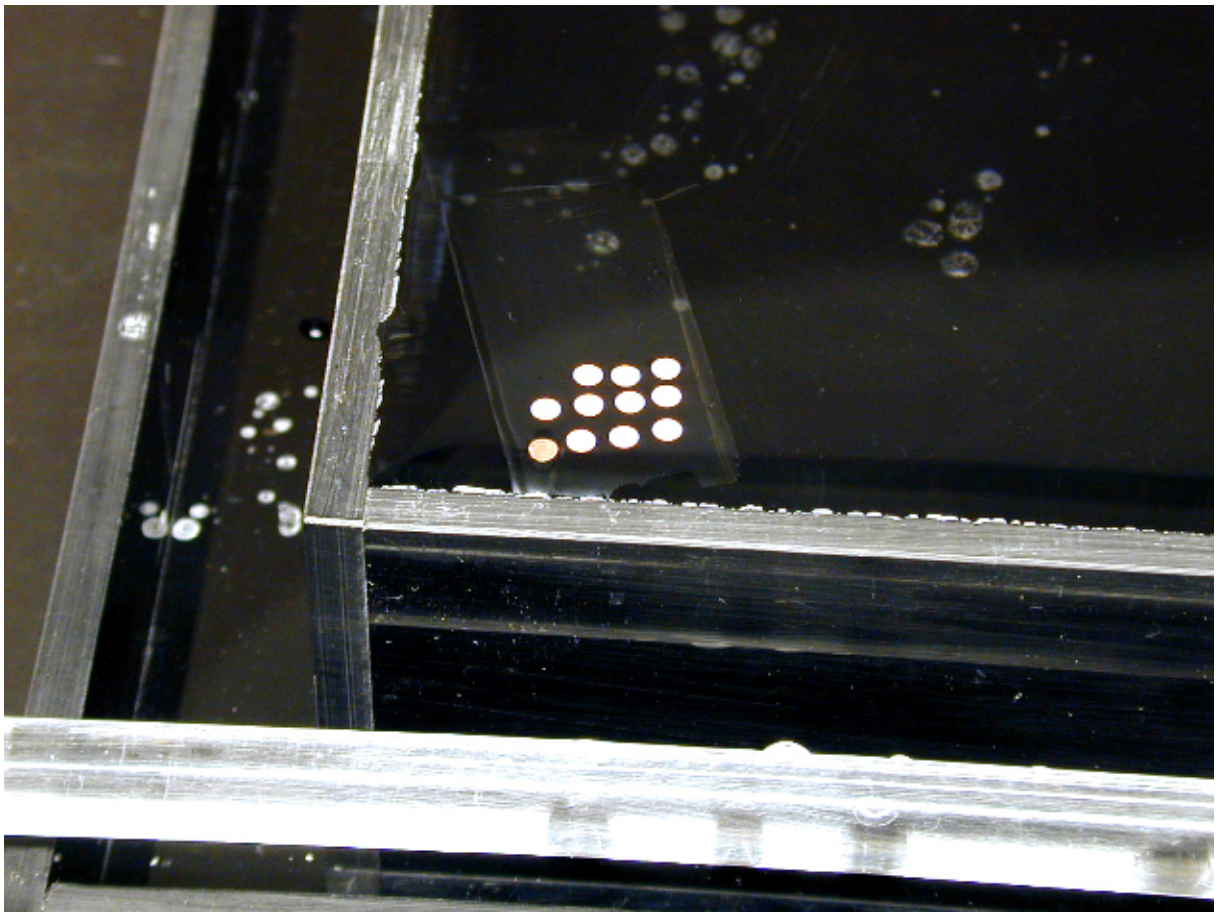


Film on water surface ready for grids

Image courtesy of P. Chipman (2004)

II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films: Dip (Casting) Method

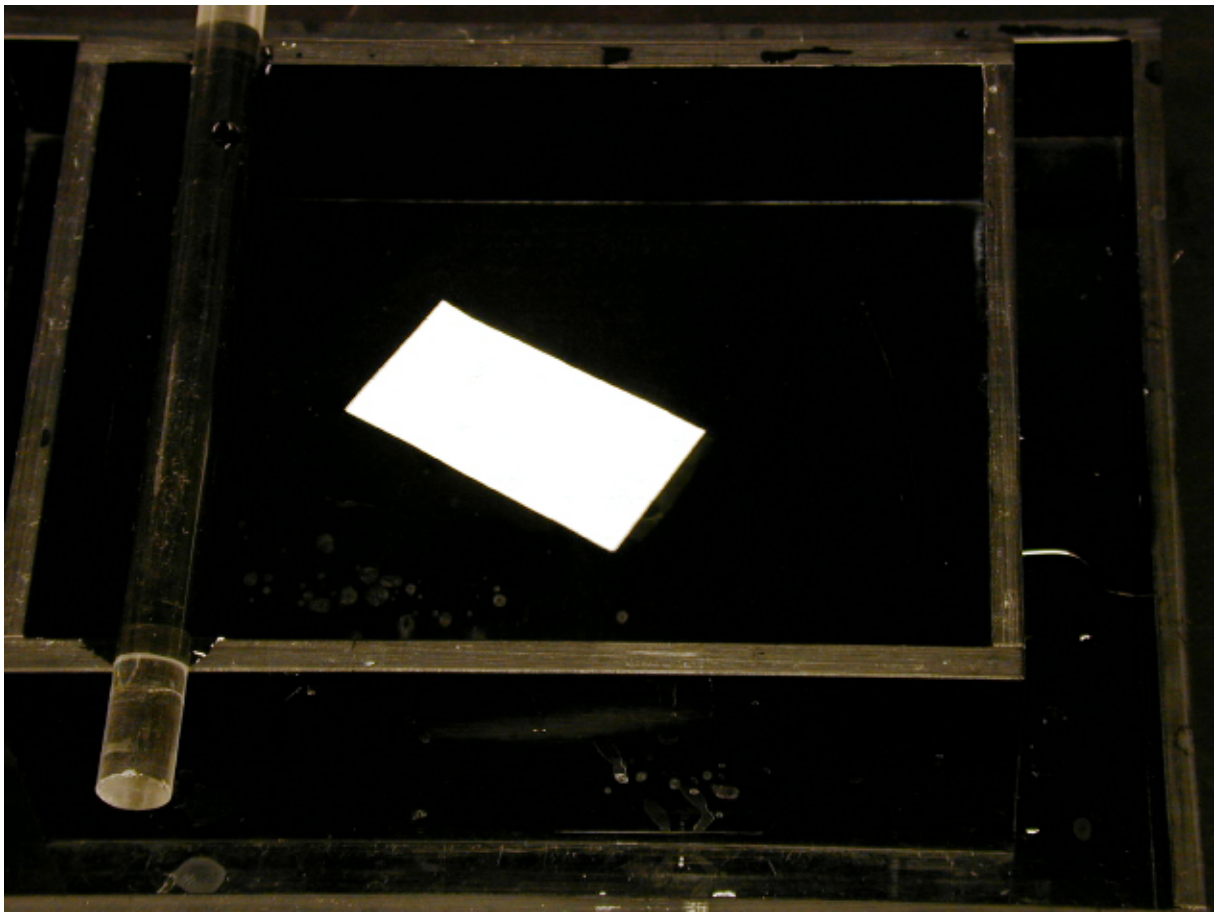


Carefully add grids one by one to film

Image courtesy of P. Chipman (2004)

II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films: Dip (Casting) Method



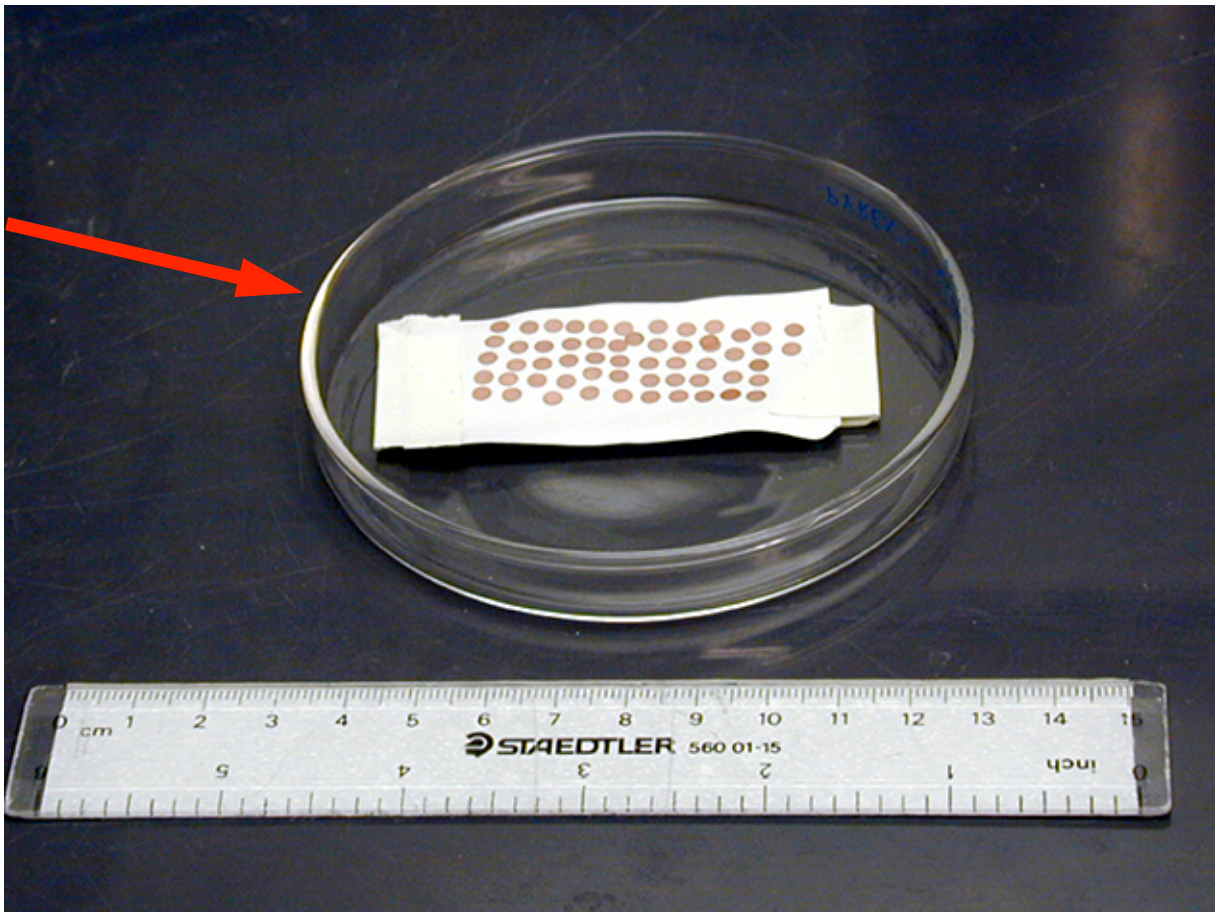
Drop a clean piece of paper on top and use to pick up grids with film

Image courtesy of P. Chipman (2004)

II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films: Dip (Casting) Method

Cover with lid

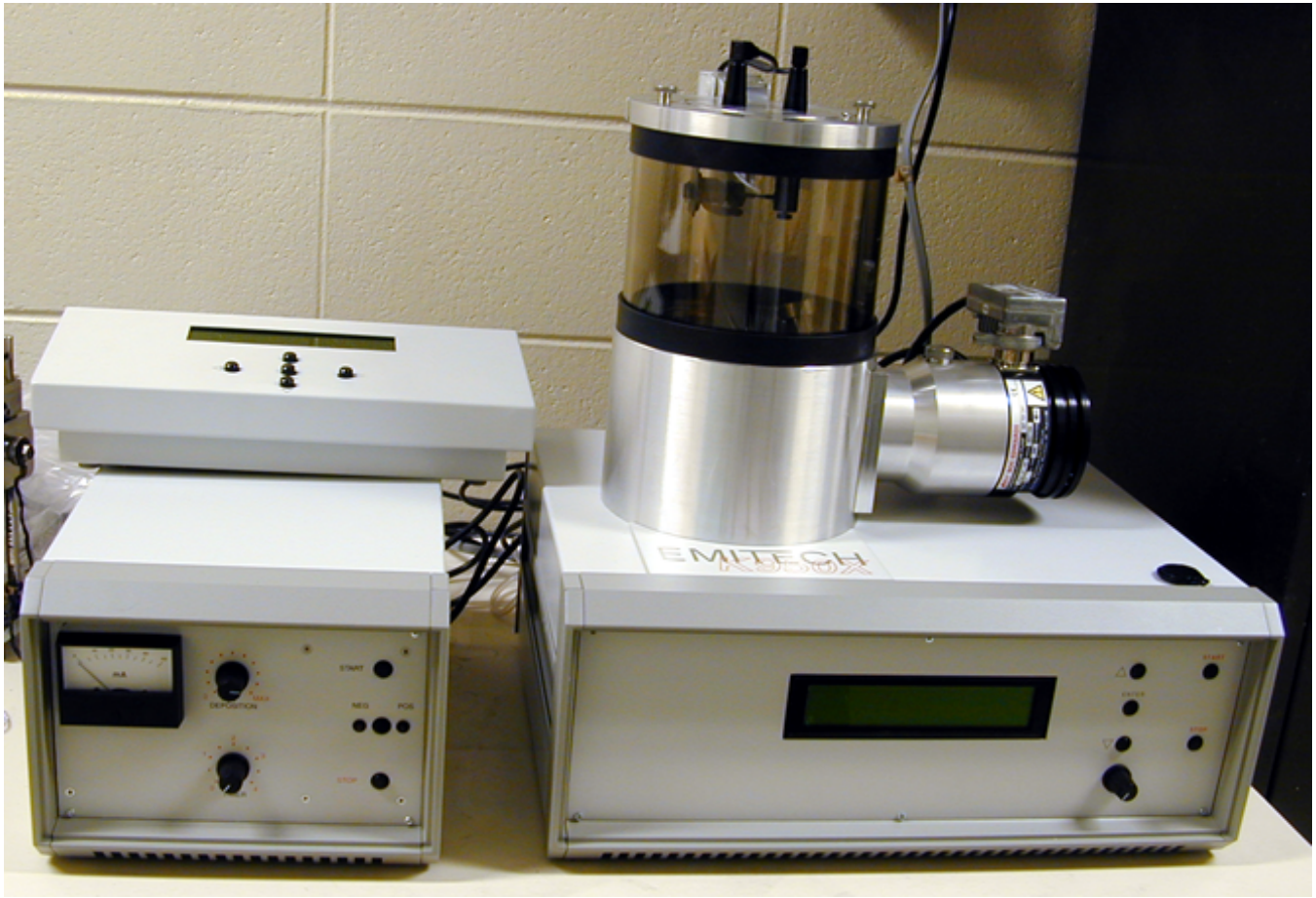


Allow grids to dry before carbon coating

Image courtesy of P. Chipman (2004)

II.A.1 Specimen Support Films for TEM

Stabilizing Plastic Films with Evaporated Carbon



Emitech K950x Turbo Evaporator

Image courtesy of V. Bowman (2004)

II.A.1 Specimen Support Films for TEM

Stabilizing Plastic Films with Evaporated Carbon

Emitech Evaporation Source

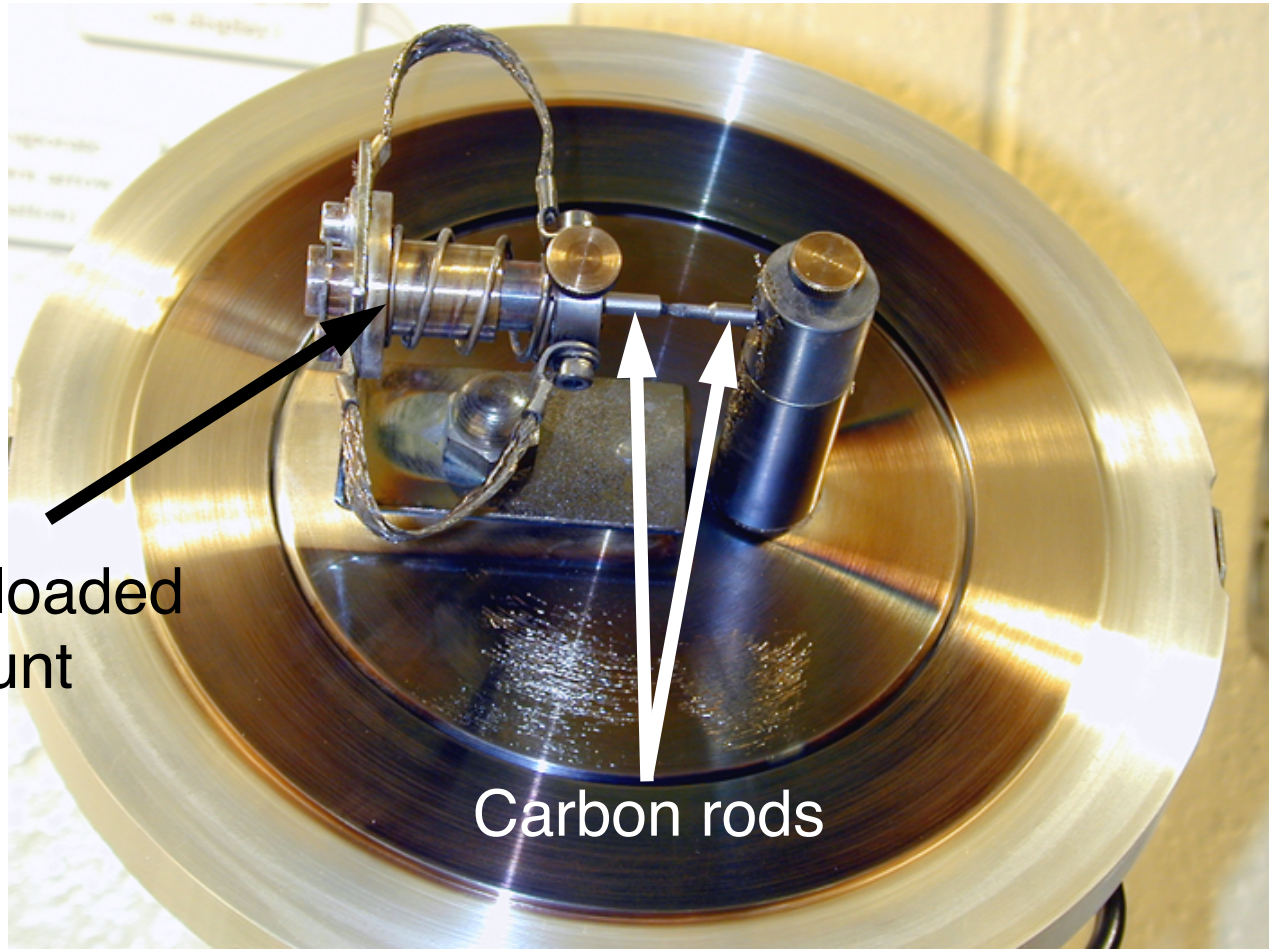
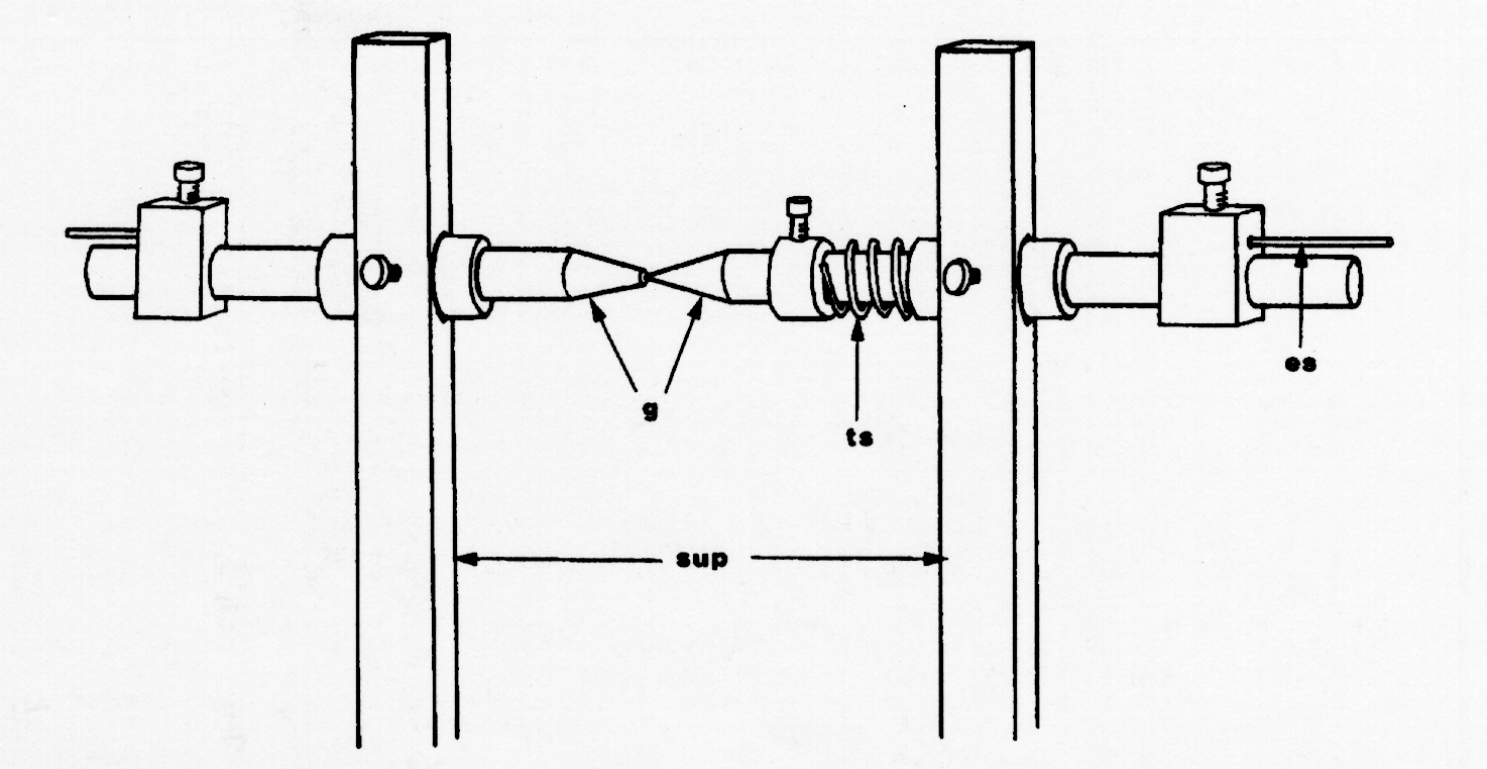


Image courtesy of P. Chipman (2004)

II.A.1 Specimen Support Films for TEM

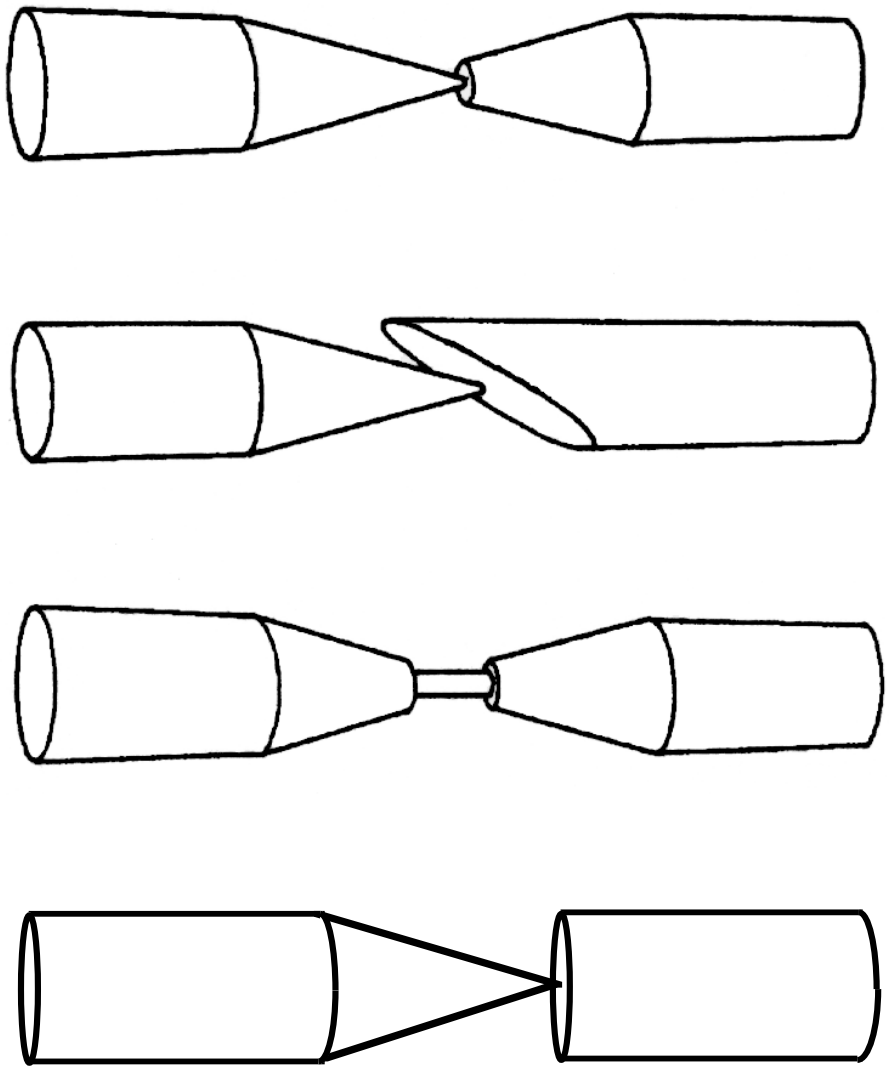
Stabilizing Plastic Films with Evaporated Carbon

Evaporation Source



From Willison and Rowe (1980), p. 34

Carbon Rod Pairs for Evaporation



Slide not shown in class lecture

From Willison and Rowe (1980), p. 34

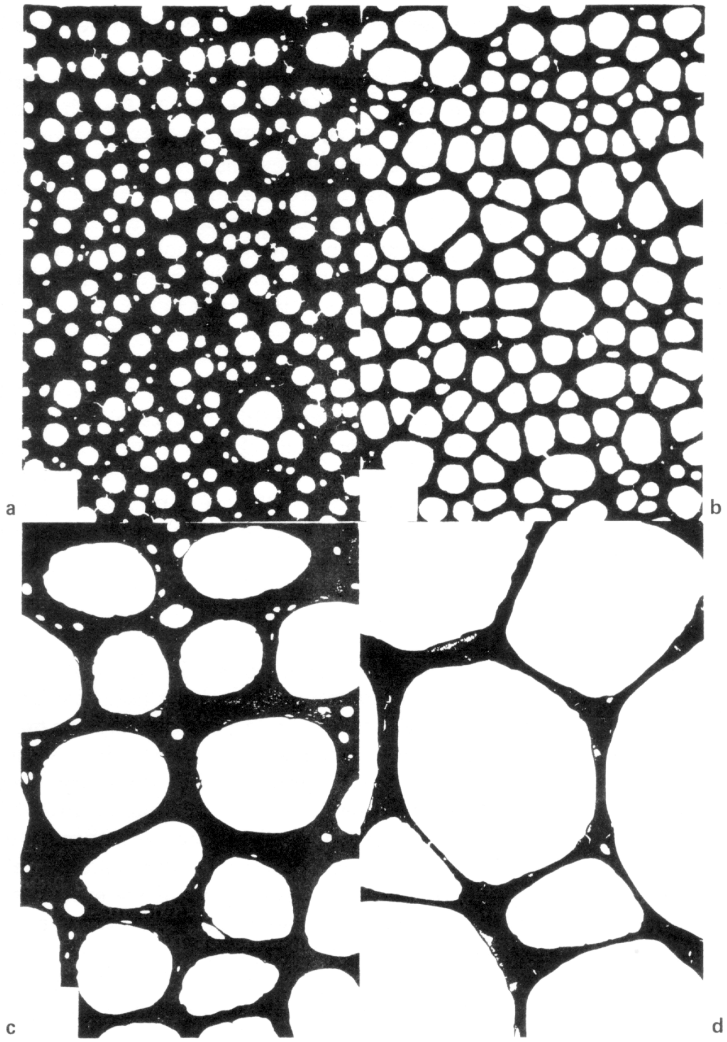
II.A.1 Specimen Support Films for TEM

Producing Continuous Plastic Films

Producing Holey Plastic Films

II.A.1 Specimen Support Films for TEM

Producing Holey Plastic Films



From Hayat & Miller (1990) *Negative Staining*, p.206

II.A.1 Specimen Support Films for TEM

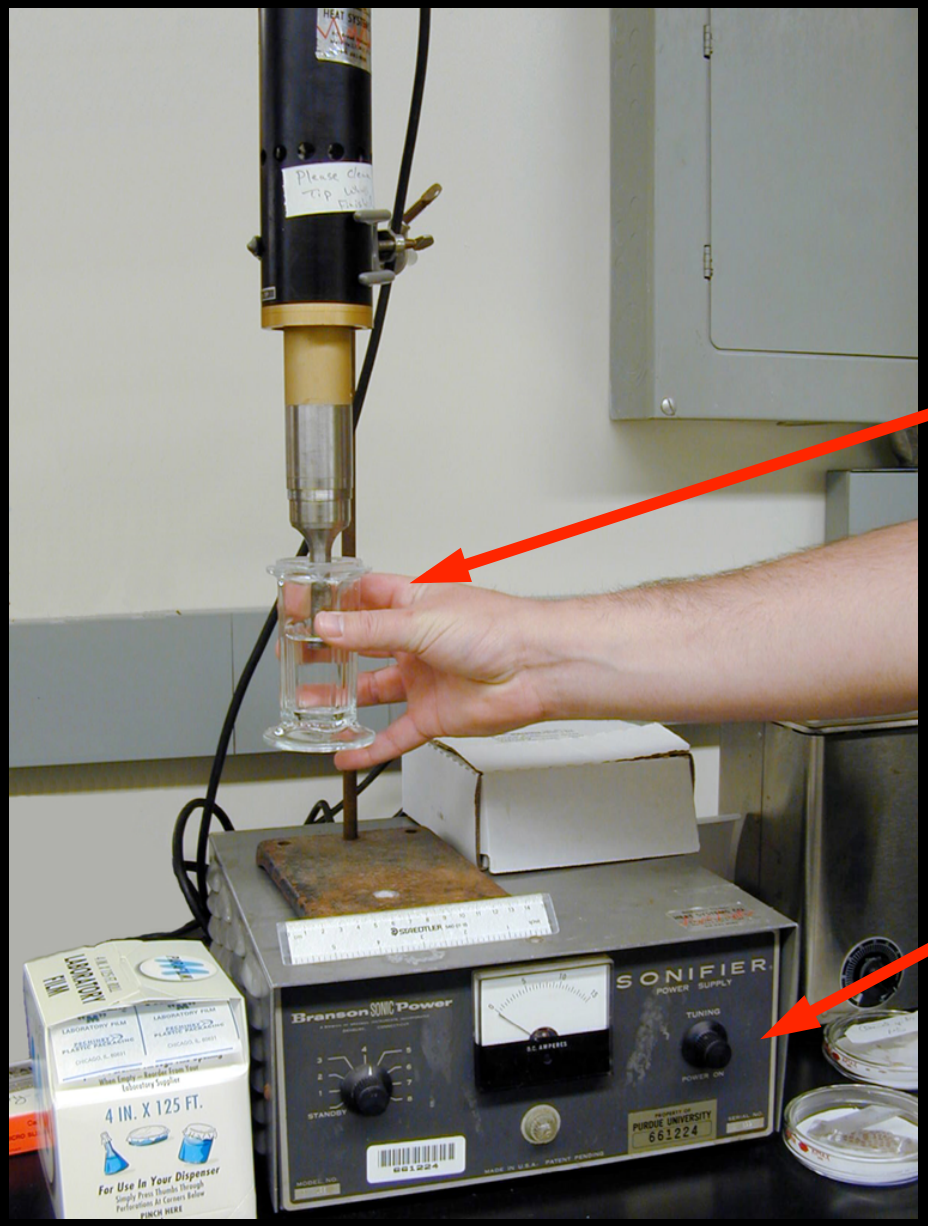
Producing Holey Plastic Films

Glycerol (Sonication)

Heavy breathing

Deep pockets

Sonicating Glycerol/Formvar Solution



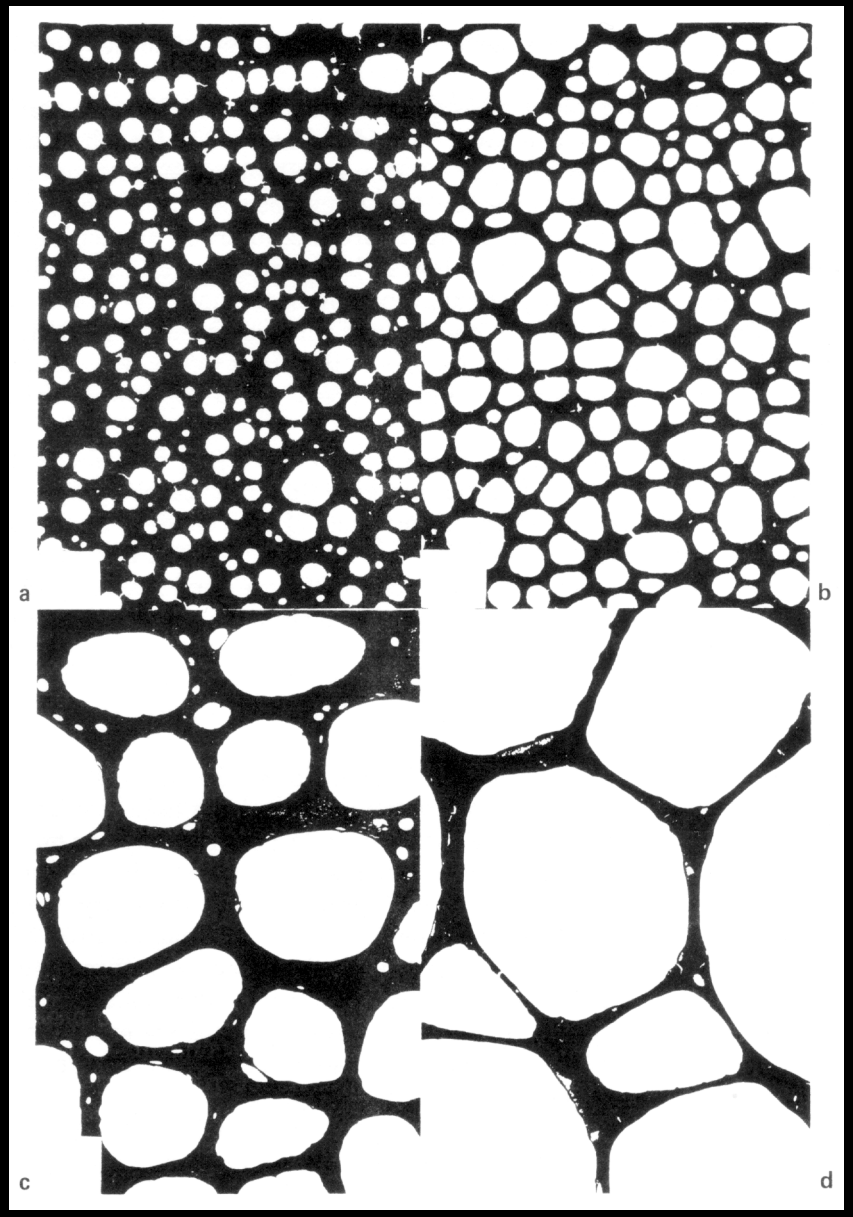
Probe generates glycerol bubbles in formvar solution

Varying intensity and duration of sonication controls size of bubbles (& holes in film)

Image courtesy of P. Chipman (2004)

Hole Size vs. Glycerol Concentration

0.1% glycerol



0.3% glycerol

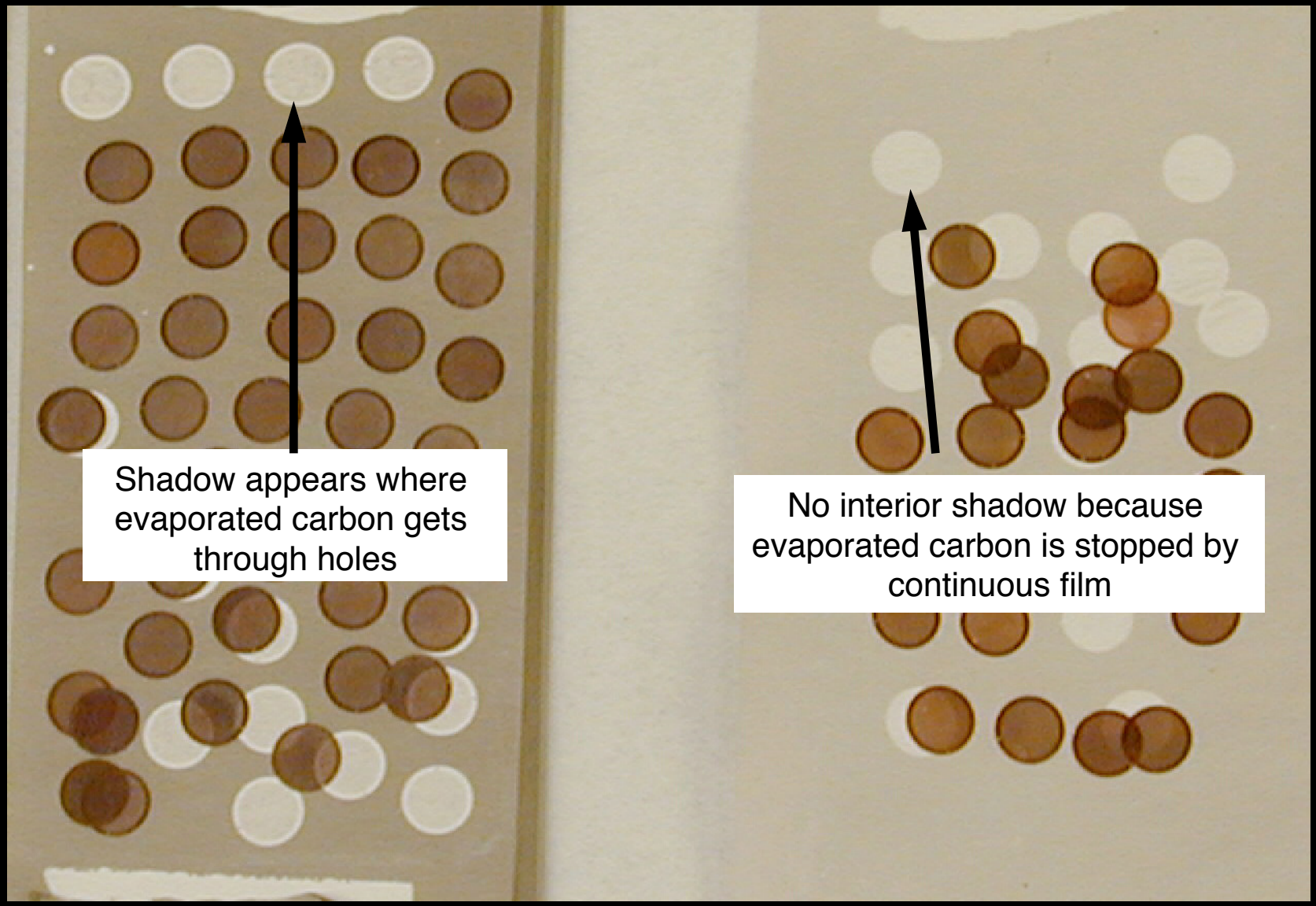
1% glycerol

3% glycerol

From Hayat & Miller (1990) *Negative Staining*, p.206

Carbon Coated Holey Grids

Carbon Coated, Continuous Film Grids



Slide not shown in class lecture

Image courtesy of P. Chipman (2004)

II.A.1 Specimen Support Films for TEM

Producing Holey Plastic Films

Glycerol (Sonication)

Heavy breathing

Deep pockets

II.A.1 Specimen Support Films for TEM

Producing Holey Plastic Films

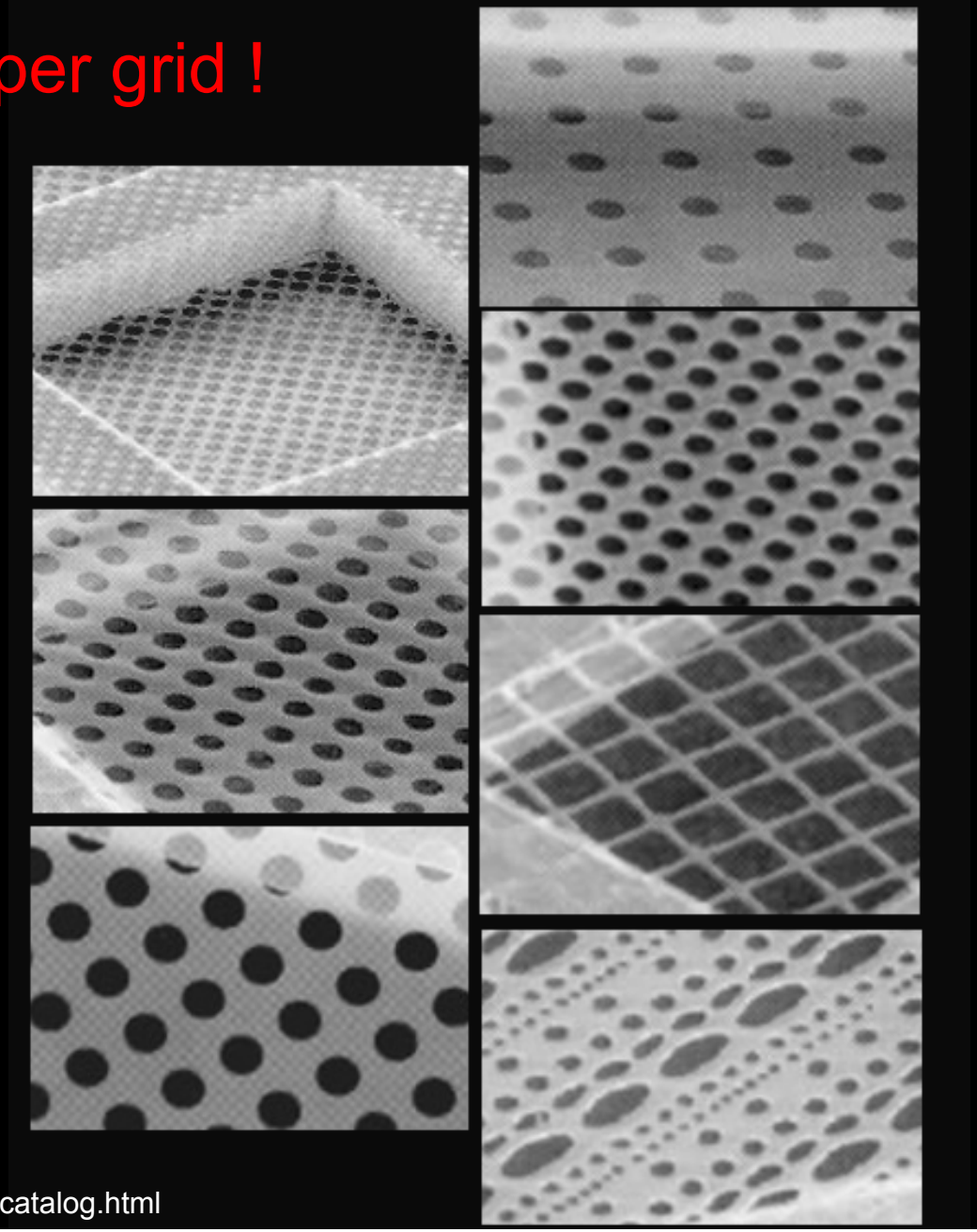
Glycerol (Sonication)

Heavy breathing

Deep pockets

Quantifoil® Holey Carbon Films

@ $7-8 per grid !



1.2μ/1.2μ

2μ/2μ

2μ/2μ

2μ/4μ

2μ/1μ

S7μ/2μ

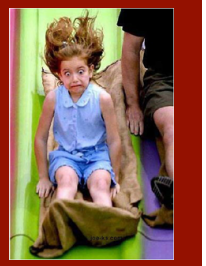
Multi

www.emsdiasum.com/ems/catalog.html

§ II: The Specimen

II.A. Biological Specimen Preparation Techniques

- II.A.1 Specimen Support Films
- II.A.2 Thin Sectioning
- II.A.3 Negative Staining
- II.A.4 Metal Shadowing
- II.A.5 Freeze Drying/Etching/Fracture
- II.A.6 Unstained and Frozen-Hydrated



§ II: The Specimen

II.A. Biological Specimen Preparation Techniques

- II.A.1 Specimen Support Films
- II.A.2 Thin Sectioning (pp.154-168)
- II.A.3 Negative Staining
- II.A.4 Metal Shadowing
- II.A.5 Freeze Drying/Etching/Fracture
- II.A.6 Unstained and Frozen-Hydrated

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.2 Thin Sectioning

What is it used for?

Mostly tissue samples

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

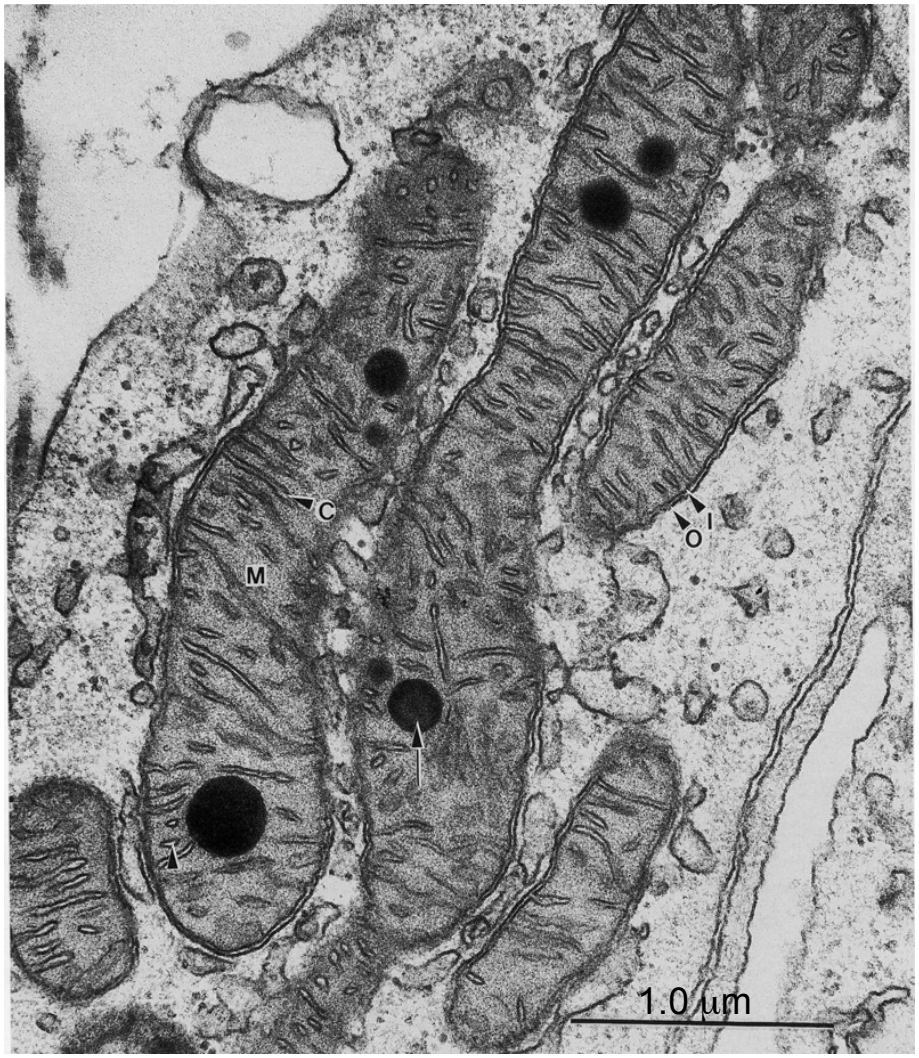
II.A.2 Thin Sectioning

Why sectioning?

Most “tissue” samples are too thick

II.A.2 Thin Sectioning (Examples)

Animal cell mitochondria

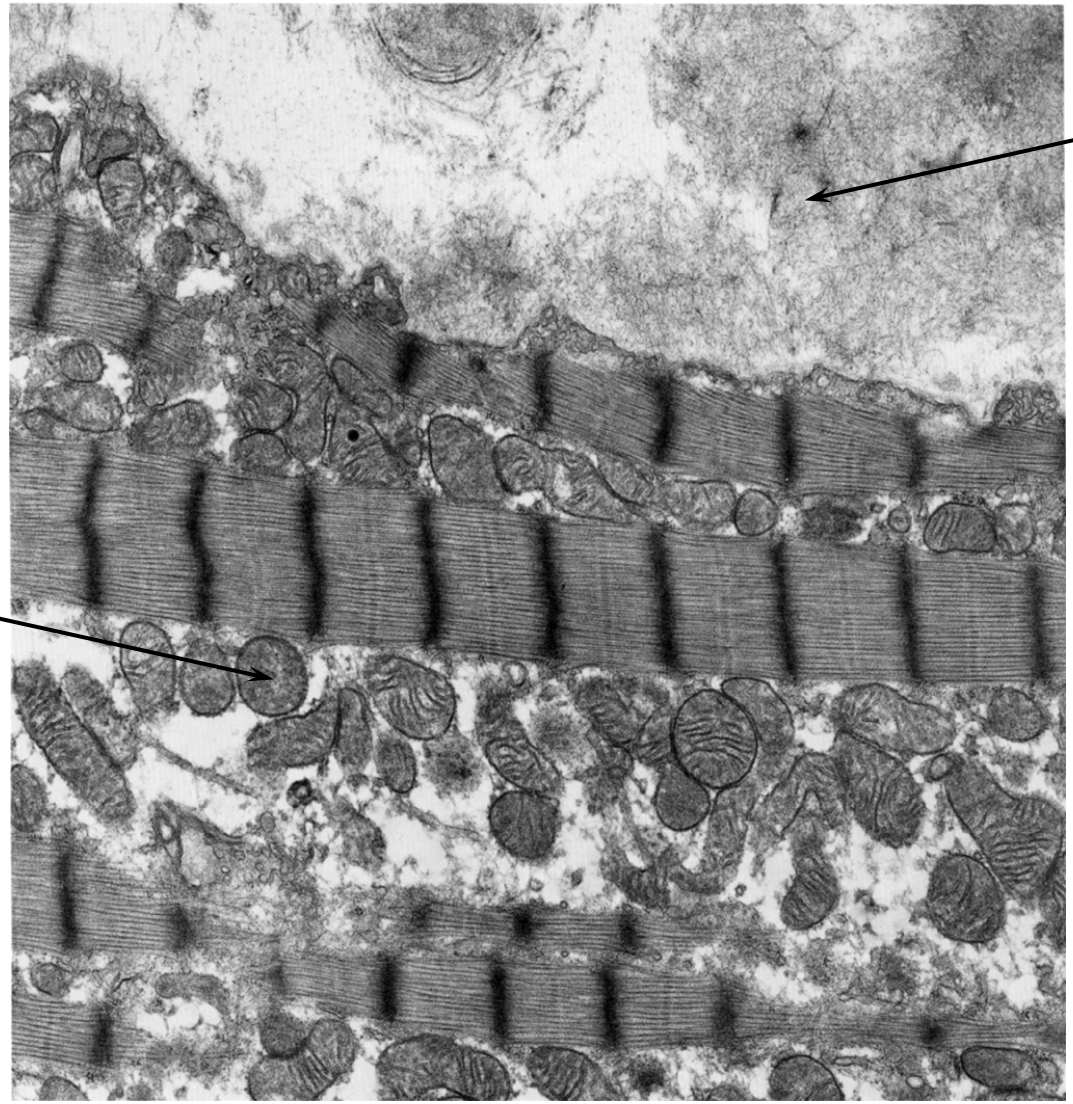


C = cristae
O= outer mito membrane
I=inner mito membrane

From Bozzolla and Russell, Fig. 19-52, p.441

II.A.2 Thin Sectioning (Examples)

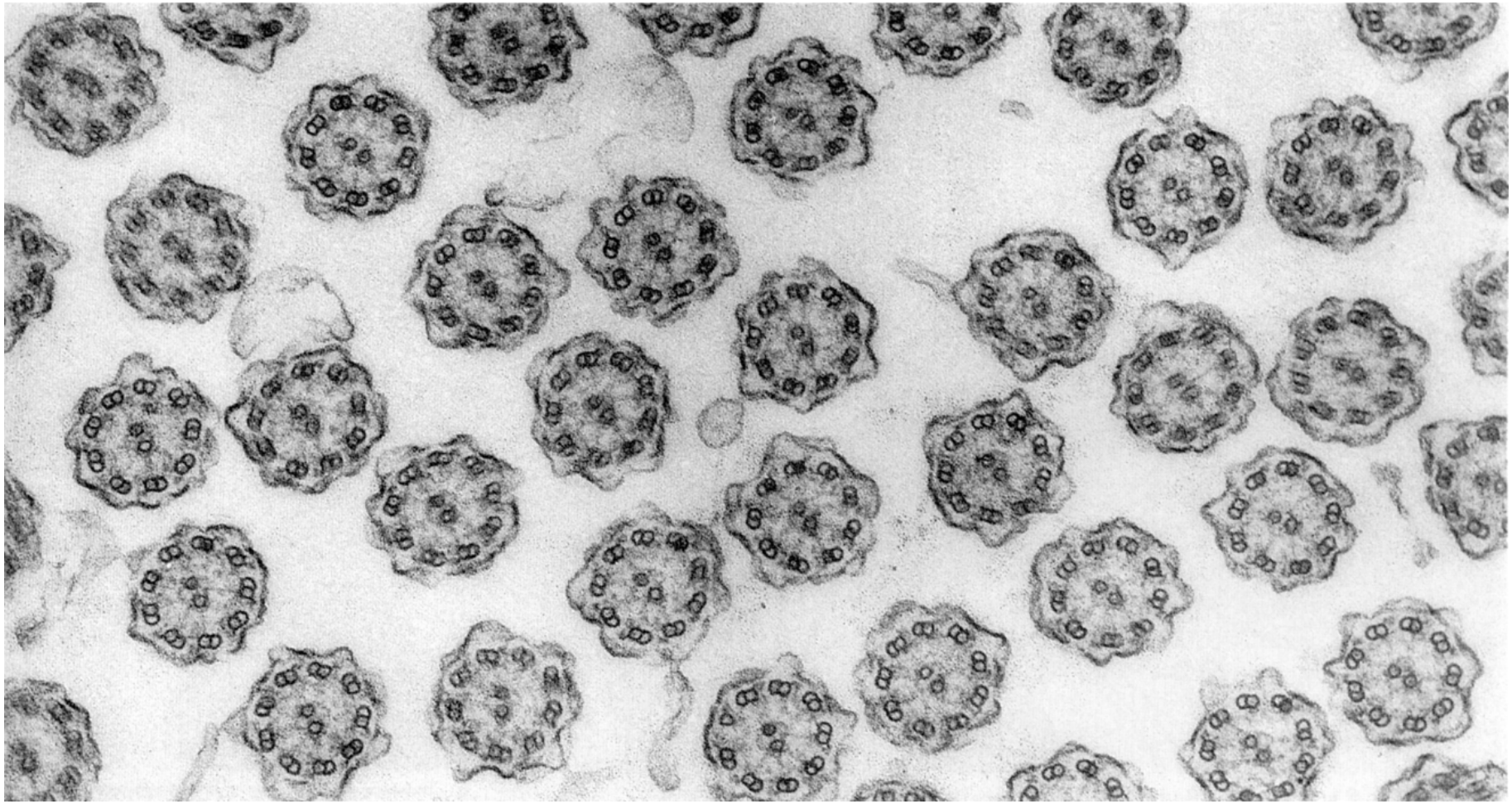
Cardiac muscle fibers



From Dvorak and Monahan-Earley, Fig. 2, p.4

II.A.2 Thin Sectioning (Examples)

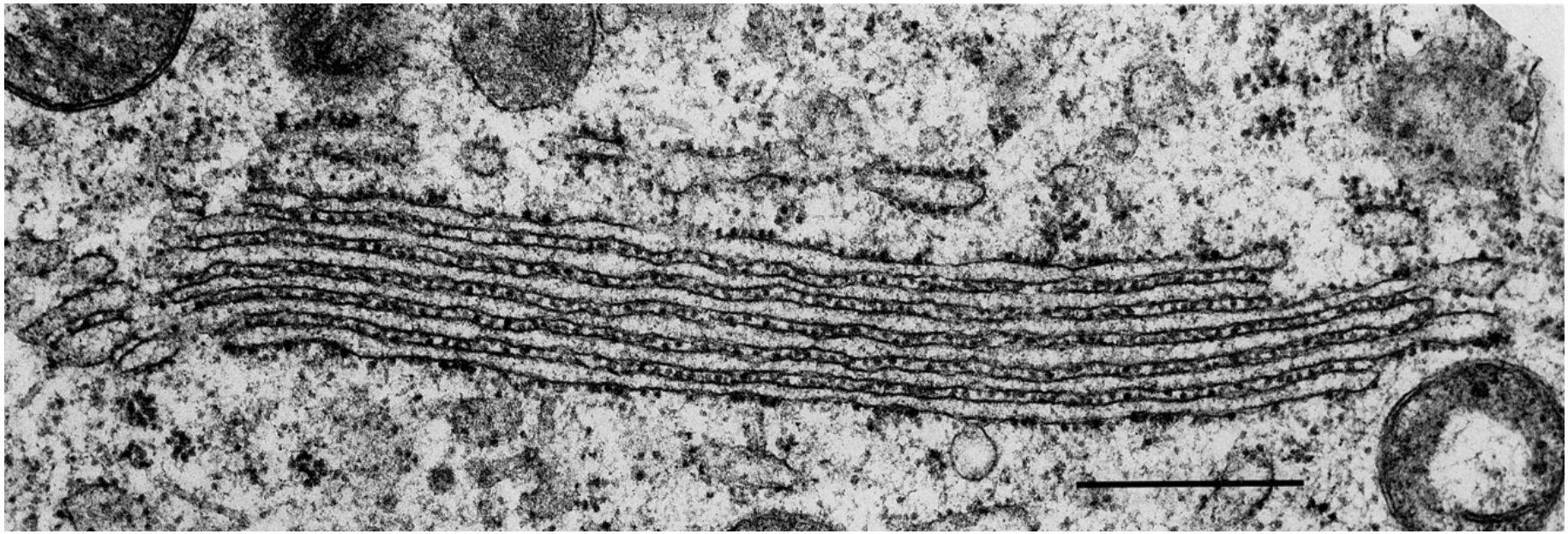
Ciliary axonemes from respiratory epithelium



From Bozzolla and Russell, Fig. 19-79, p.457

II.A.2 Thin Sectioning (Examples)

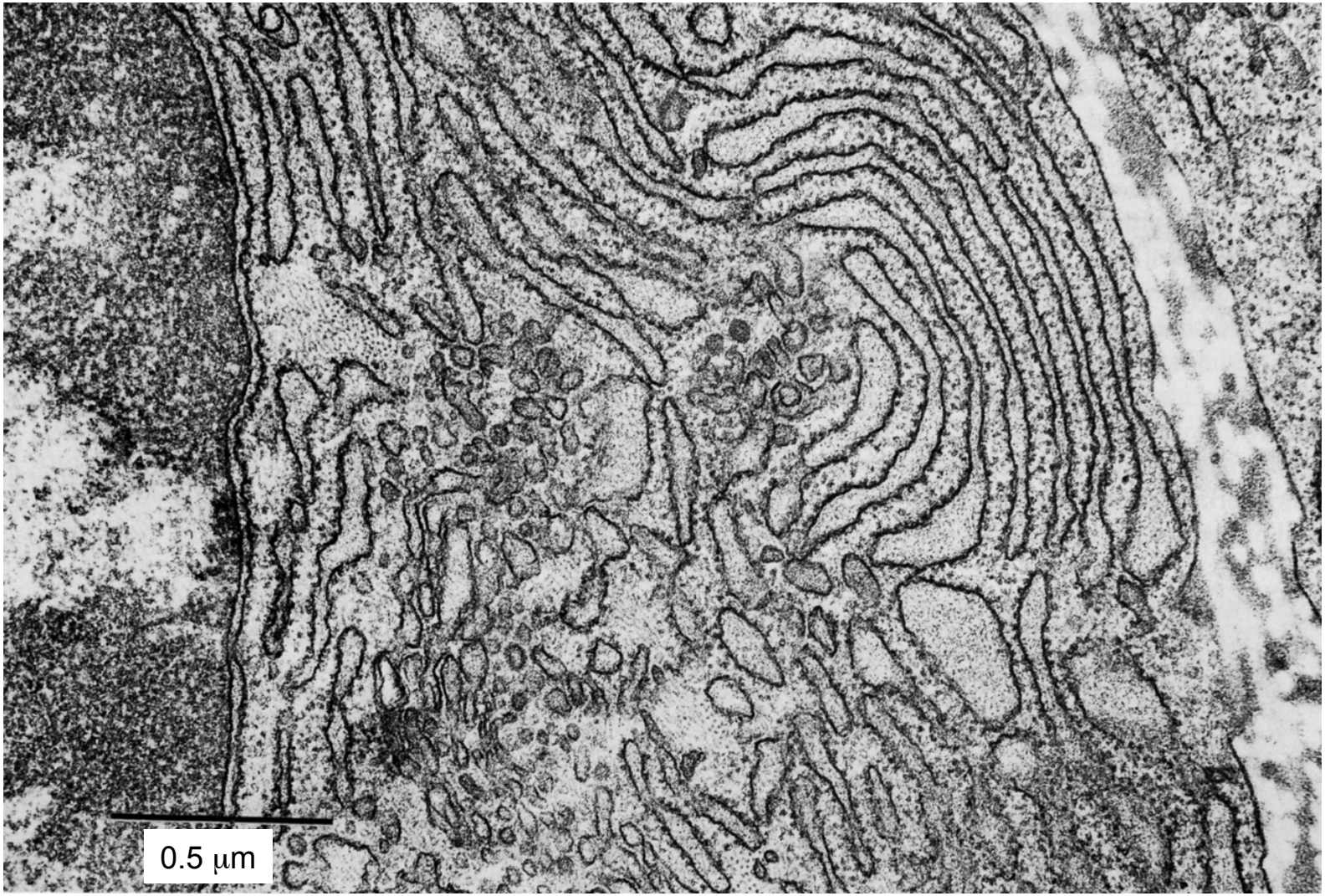
Rough endoplasmic reticulum forming stack of parallel cisternae



From Bozzolla and Russell, Fig. 19-67, p.449

II.A.2 Thin Sectioning (Examples)

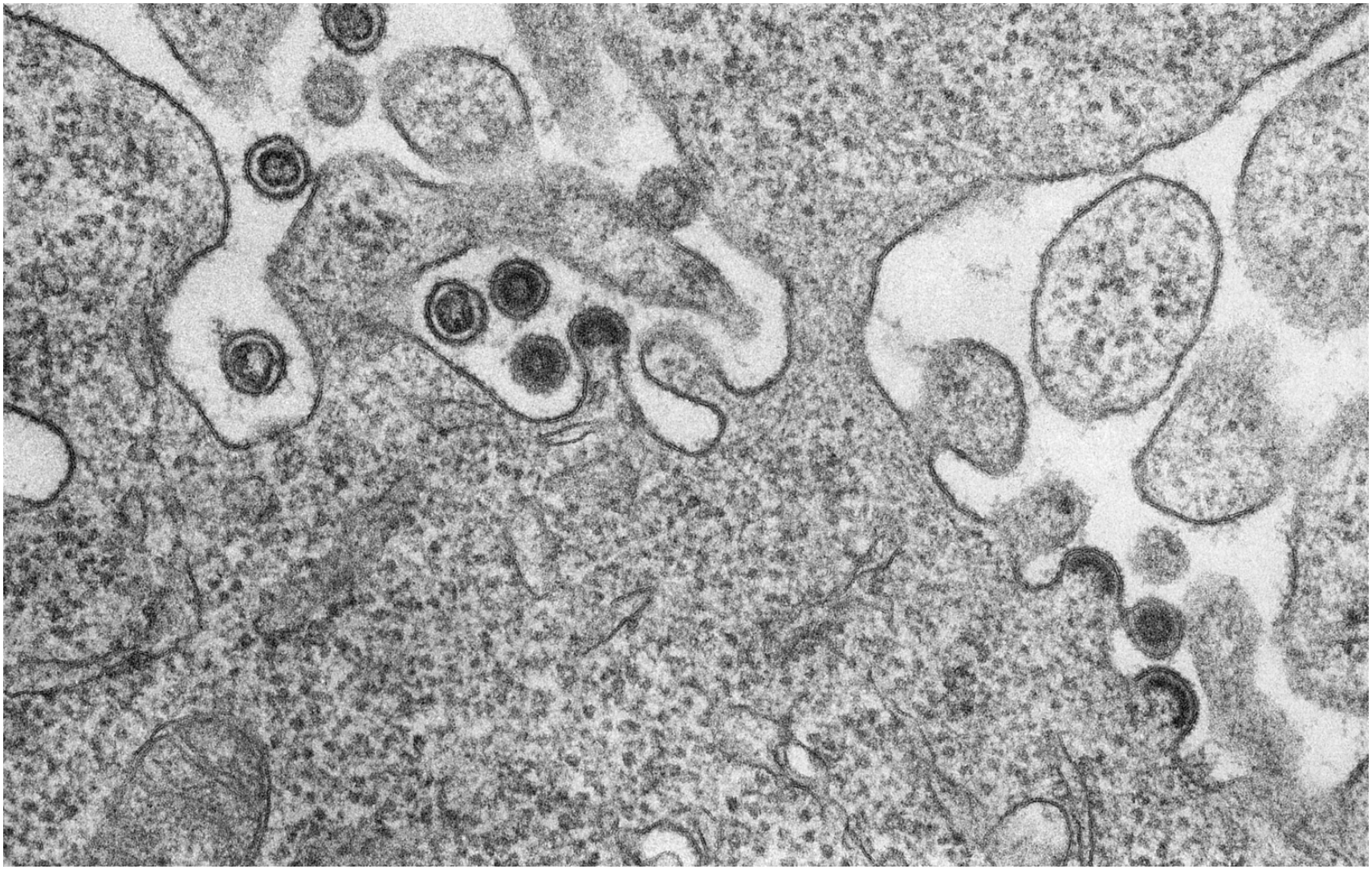
Rough endoplasmic reticulum heavily populated with ribosomes



From Bozzolla and Russell, Fig. 19-66, p.449

II.A.2 Thin Sectioning (Examples)

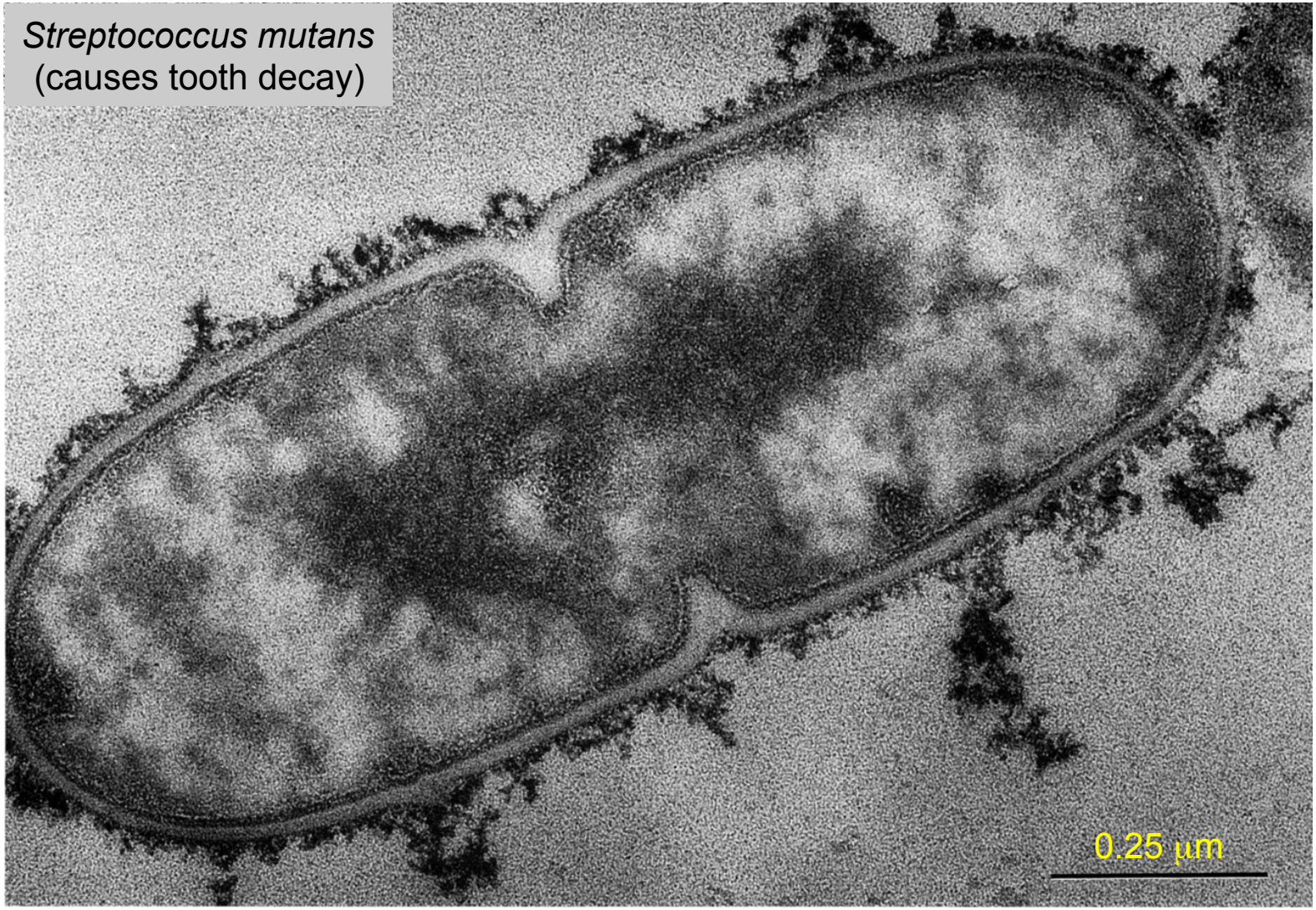
Budding retroviruses from leukemia cell



From Bozzolla and Russell, Fig. 19-129, p.488

II.A.2 Thin Sectioning (Examples)

Ultra thin section of gram positive bacterium



From Bozzolla and Russell, Fig. 19-119, p.482

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.2 Thin Sectioning

Primary Steps

Fixation

Dehydration and Embedding

Sectioning (Microtomy)

Staining

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.2 Thin Sectioning

Goal of Each Step

Fixation

Stabilize "normal" ultrastructure of specimen via chemical or physical preservation

Dehydration and Embedding

Replace all water in the specimen with stiff plastic

Sectioning (Microtomy)

Cut VERY thin slice of embedded sample (usually ~50-100nm thick)

Staining

Increase mass thickness of specimen to enhance aperture contrast

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.2 Thin Sectioning

See "hidden slides" and read lecture notes (pp.154-168)

1. Fixation

Goal: stabilize "normal" ultrastructure of specimen via chemical or physical preservation

II.A.2 Thin Sectioning (Fixation)

Primary Goal of Fixation:

Stabilize "normal" ultrastructure of specimen via chemical or physical preservation

- Minimally perturb specimen from living state

- Protect against disruption when embedding and sectioning

- Minimize shrinkage or swelling

- Rapid penetration

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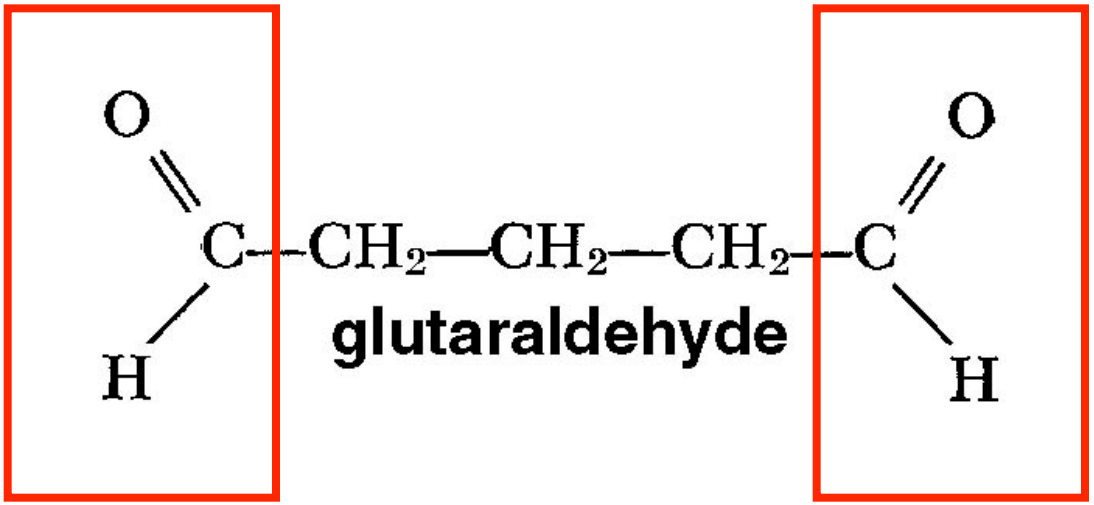
II.A.2 Thin Sectioning (Fixation)

Standard fixative: Glutaralydehyde ($C_5H_8O_2$)



Cross-links protein molecules

(intra- and inter-molecular)

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II.A.2 Thin Sectioning (Fixation)

What affects fixation quality?

Buffer

pH

Osmolarity of buffer solution

Concentration of fixative

Temperature and time of fixation

Size of sample (< 1mm^3 best)

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2. Dehydration and Embedding

Goal: replace all water in the specimen with stiff plastic

II.A.2 Thin Sectioning (Dehydration/Embedding)

Goal of dehydration:

Remove H_2O from fixed specimen to allow non-water-soluble embedding medium to infiltrate specimen

Generally use **acetone** or **alcohol**

Goal of embedding:

Infiltrate tissue with liquid polymer (*e.g.* epoxy resin) that is hardened after infiltration is complete

Hardened polymer gives specimen necessary **firmness** to permit cutting thin sections

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II.A.2 Thin Sectioning (Dehydration/Embedding)

"Standard" embedding protocol (after glutaraldehyde fixation)

1. Millonig's phosphate buffer (0.15M, pH 7.2-7.4).	10 min
2. Phosphate buffer (**Rinsing**)	10 min
3. Phosphate buffer	10 min
4. 1% OsO_4 in phosphate buffer (**Post fixation**)	1 hr
5. Buffer	10 min
6. Buffer	10 min
7. 30% acetone (**Dehydration**)	10 min
8. 60% acetone	10 min
9. 90% acetone	10 min
10. 100% acetone	10 min
11. 100% acetone	10 min
12. 100% acetone	10 min
13. 100% acetone	10 min
14. 3:1 acetone:resin (**Infiltration**)	≥ 1 hr
15. 1:1 acetone:resin	≥ 1 hr
16. 1:3 acetone:resin	≥ 1 hr
17. Undiluted resin	overnight
18. **Polymerize** resin at 60°C	18-24 hours

Tissue may be placed under vacuum for steps 15-18 to enhance resin penetration.

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II.A.2 Thin Sectioning (Dehydration/Embedding)

Resins (many to choose from):

- Epoxy (*e.g.* "Epon" - Poly/Bed 812)

- Spurr's low viscocity embedding medium

- Araldite

- Acrylic resins (LR white and LR gold)

- Polyester resins (*e.g.* Vestopal)

- Water miscible resins (*e.g.* Durcupan, Nanoplast)

- Removable embedding medium (*e.g.* Diethylene glycol disterate)

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II.A.2 Thin Sectioning (Dehydration/Embedding)

Resin handling:

~10 mm

Polyethylene embedding capsules

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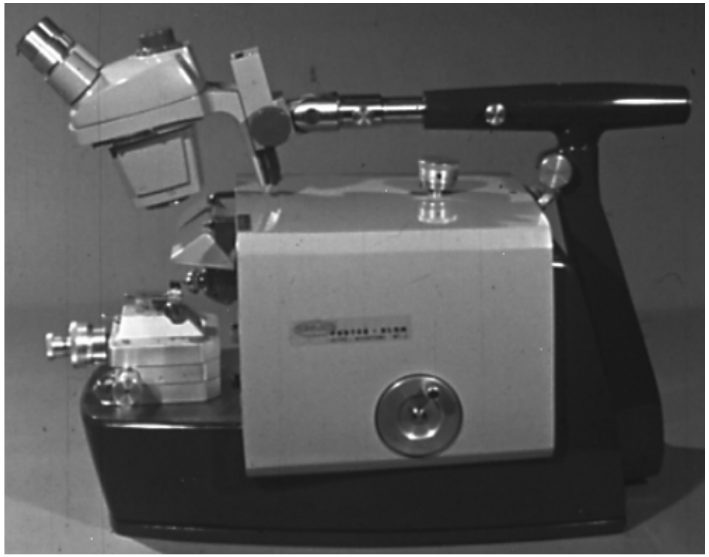
3. Sectioning (Microtomy)

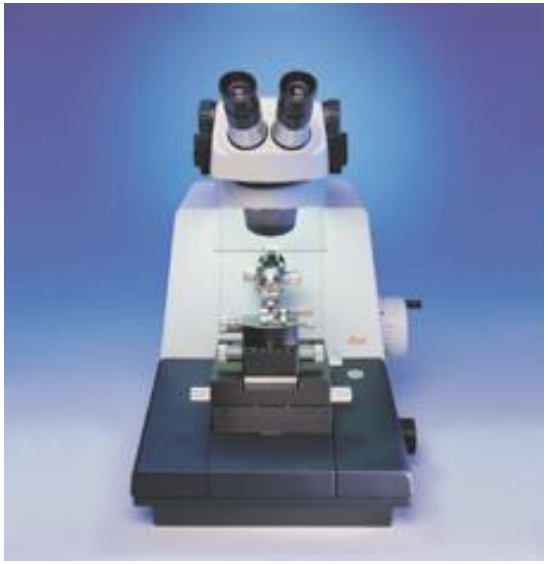
Just cut me a VERY thin slice please…

II.A.2 Thin Sectioning (Microtomy)



Sorvall MT-2B ultramicrotome

RMC MT-X



Leica Ultracut

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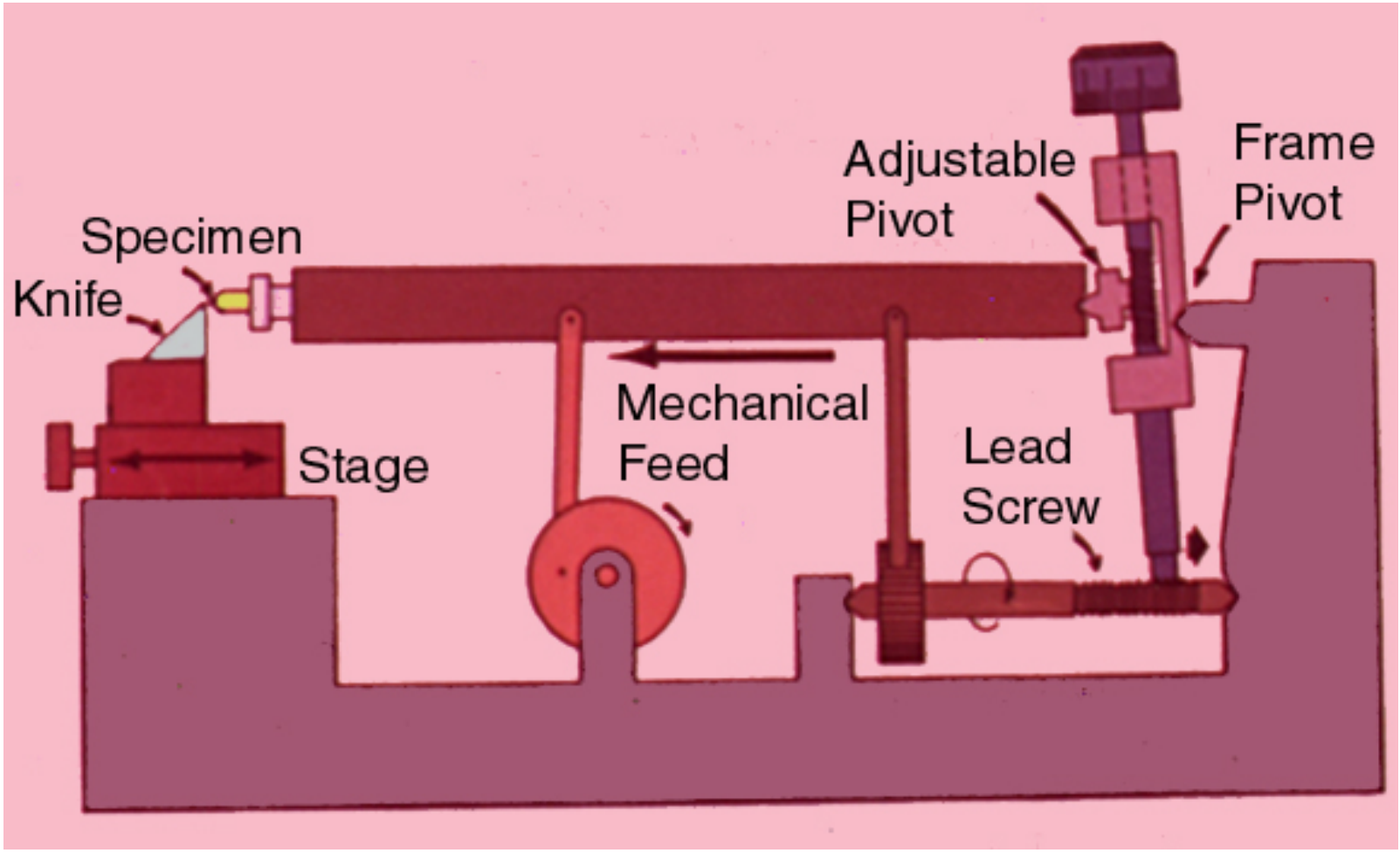
II.A.2 Thin Sectioning (Microtomy)



Schematic diagram of manual ultramicrotome

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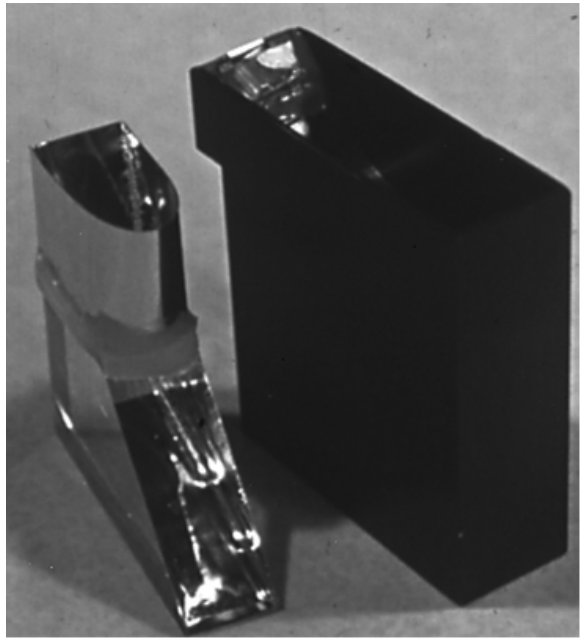
II.A.2 Thin Sectioning (Microtomy)



Left - disposable glass knife

Right - diamond knife

Diatome diamond knives

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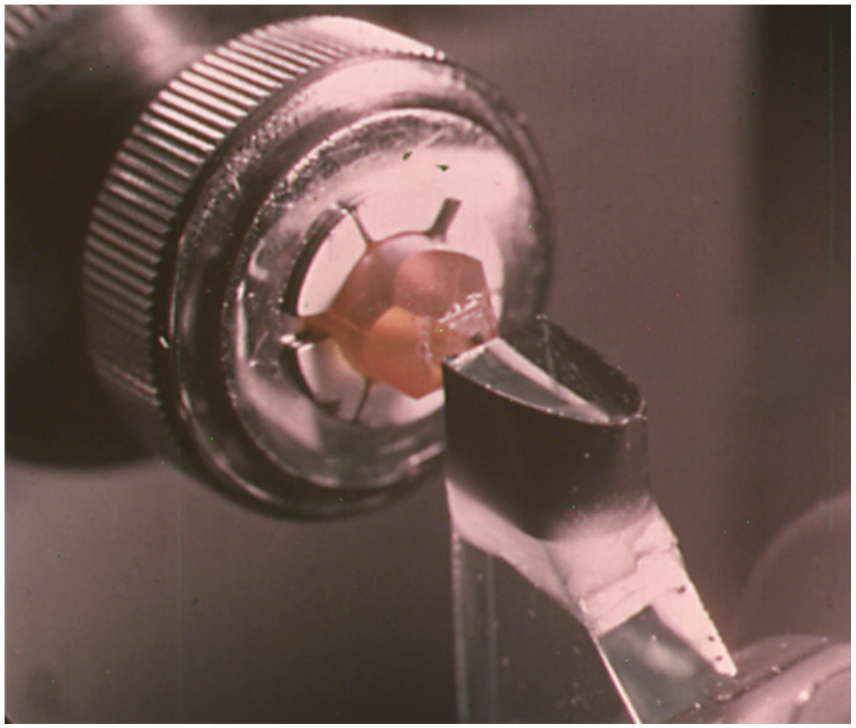
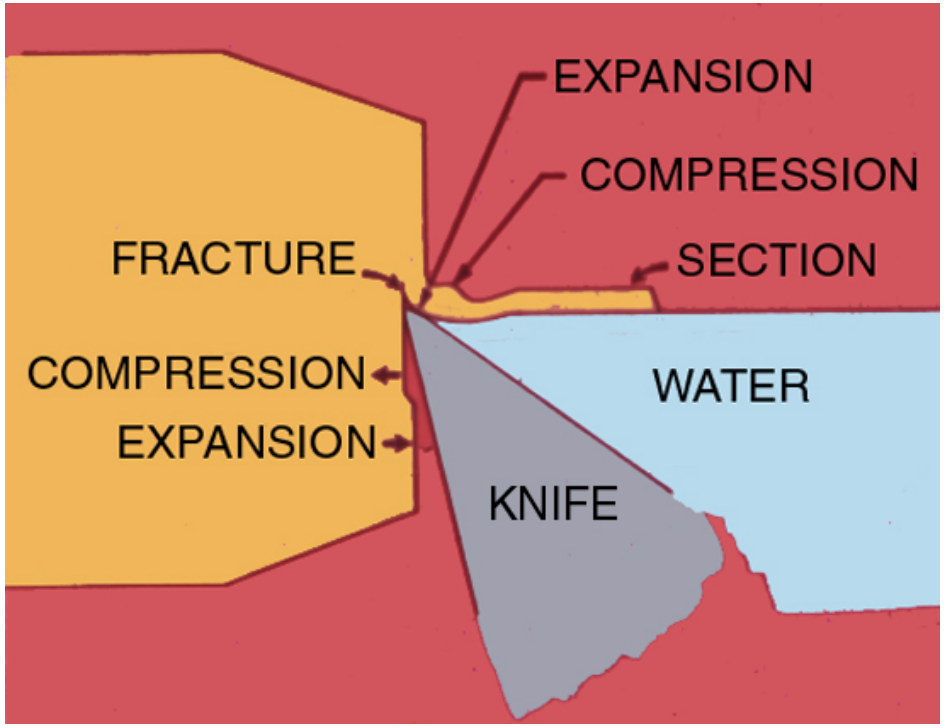
II.A.2 Thin Sectioning (Microtomy)





Cut sections float off onto the water surface

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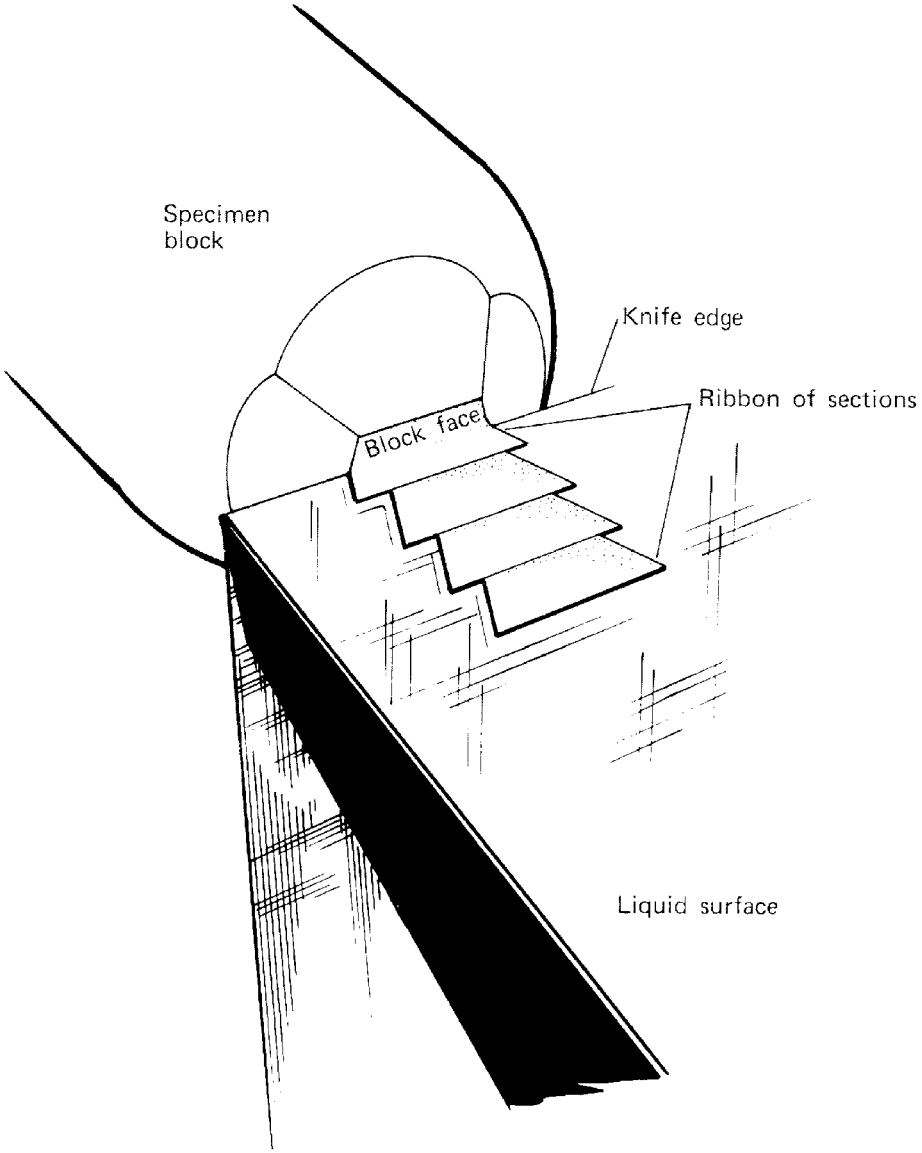
II.A.2 Thin Sectioning (Microtomy)



Ribbon of thin sections

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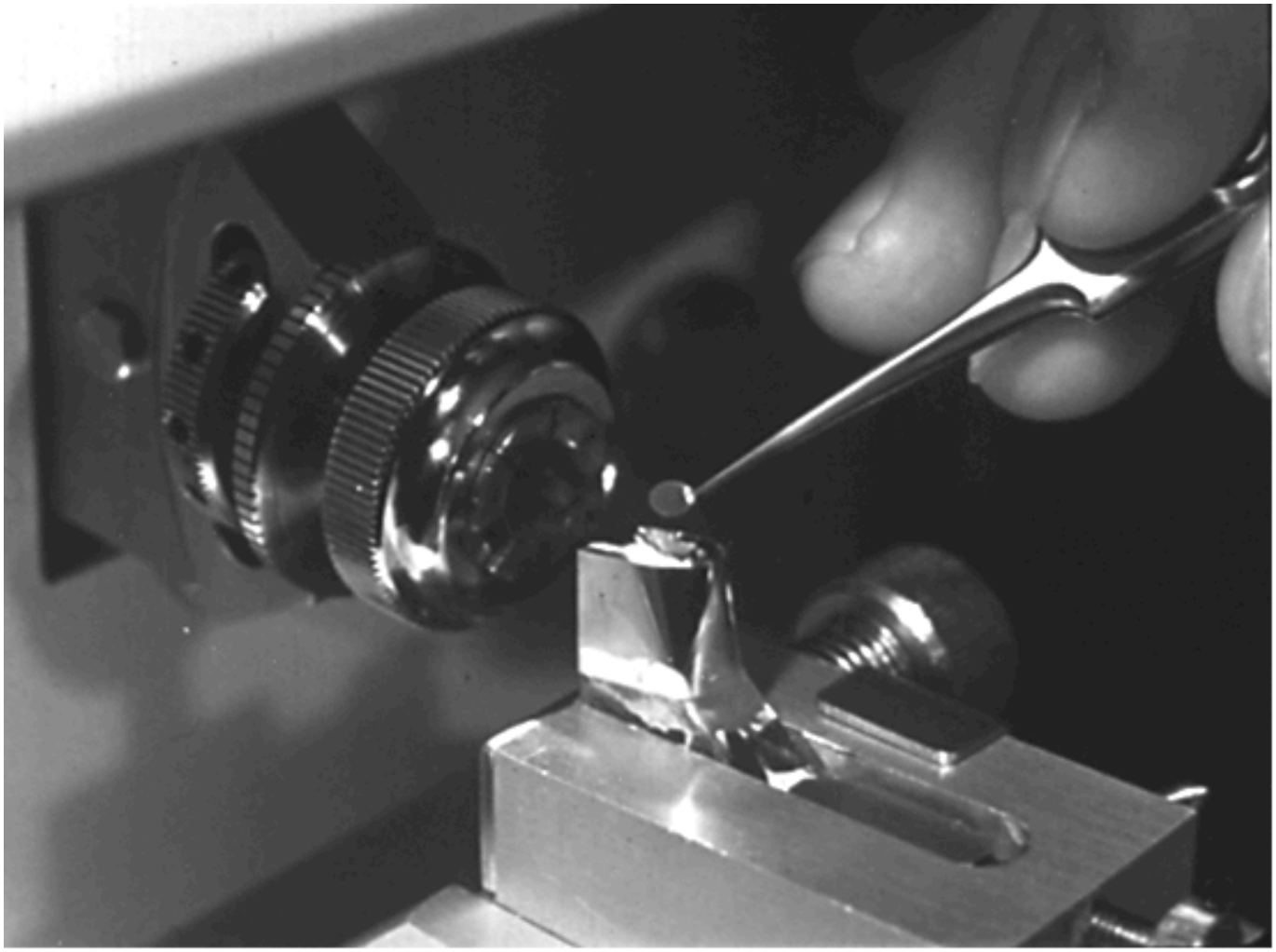
From Reid, Fig. 1.4, p.222

II.A.2 Thin Sectioning (Microtomy)



Touch grid to sections to pick them up

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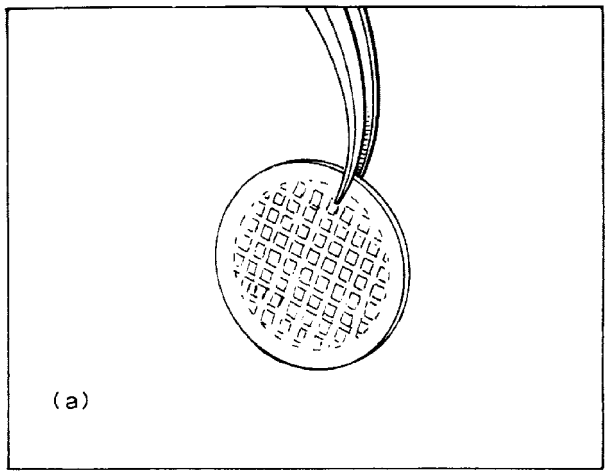
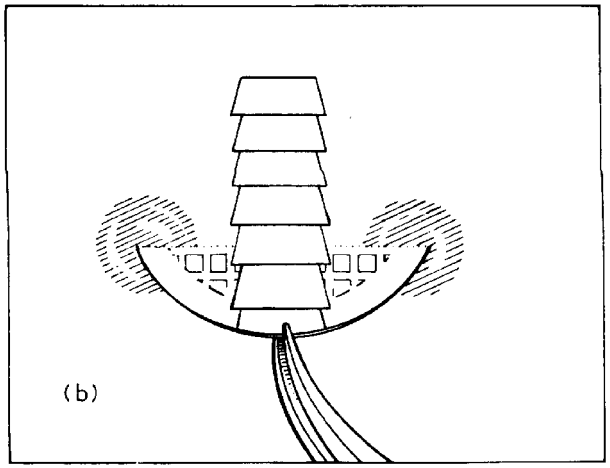
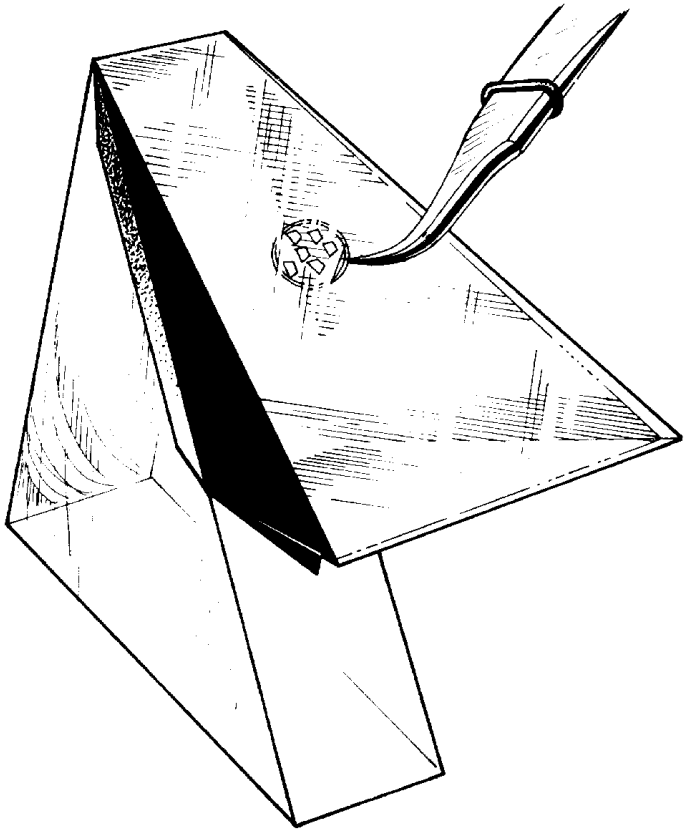
II.A.2 Thin Sectioning (Microtomy)





From Reid, Fig. 8.3, p.315

Sections can be picked up by touching the top of the section ribbon or coming up from beneath



From Reid, Fig. 8.4, p.316

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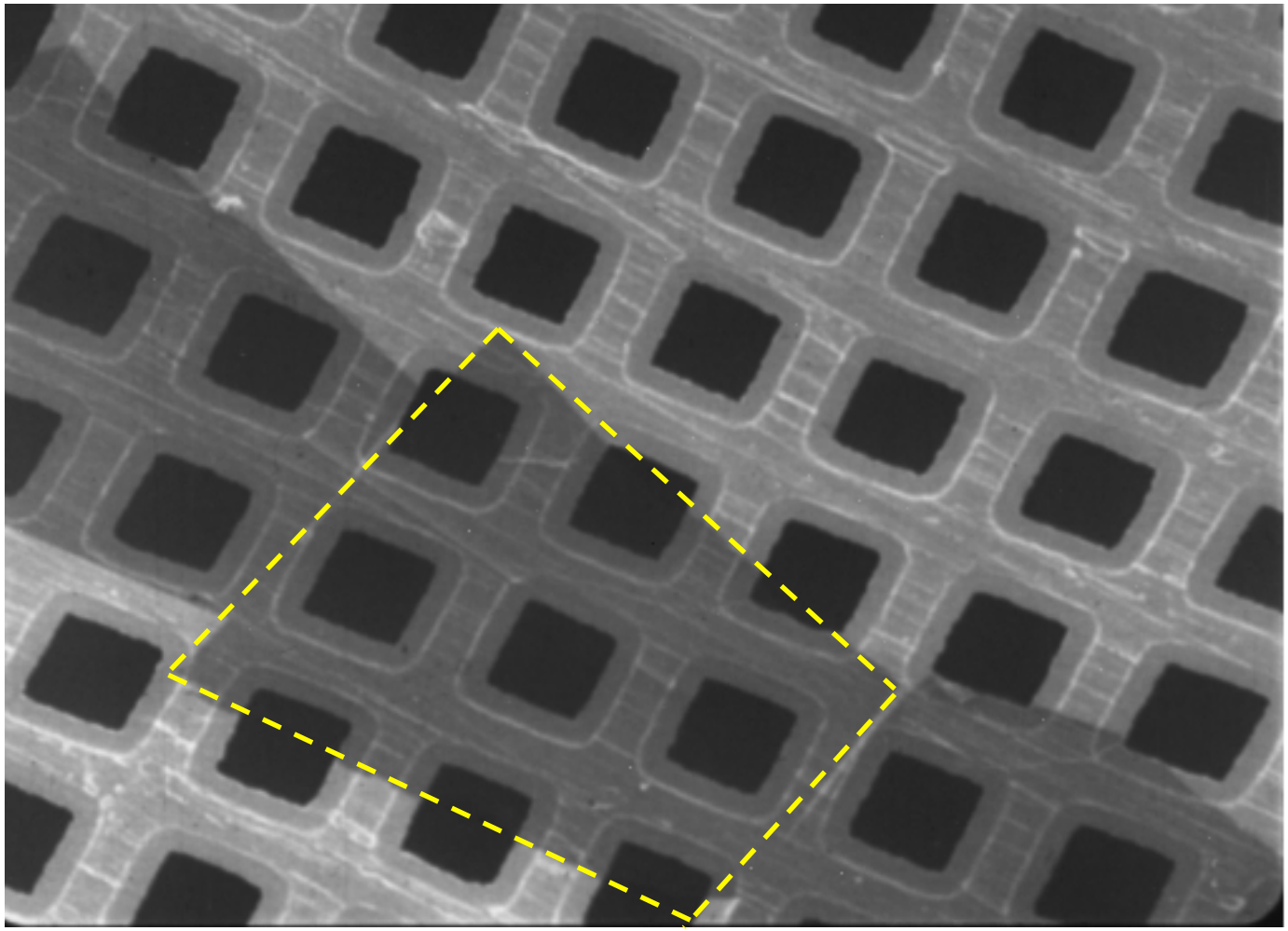
II.A.2 Thin Sectioning (Microtomy)



Section ribbon mounted on specimen grid

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4. Staining

Goal: increase mass thickness of specimen to enhance aperture contrast

II.A.2 Thin Sectioning (Staining)

Typical Protocol

1. Uranyl acetate (10-40 min)
2. Reynolds lead citrate (1-4 min)

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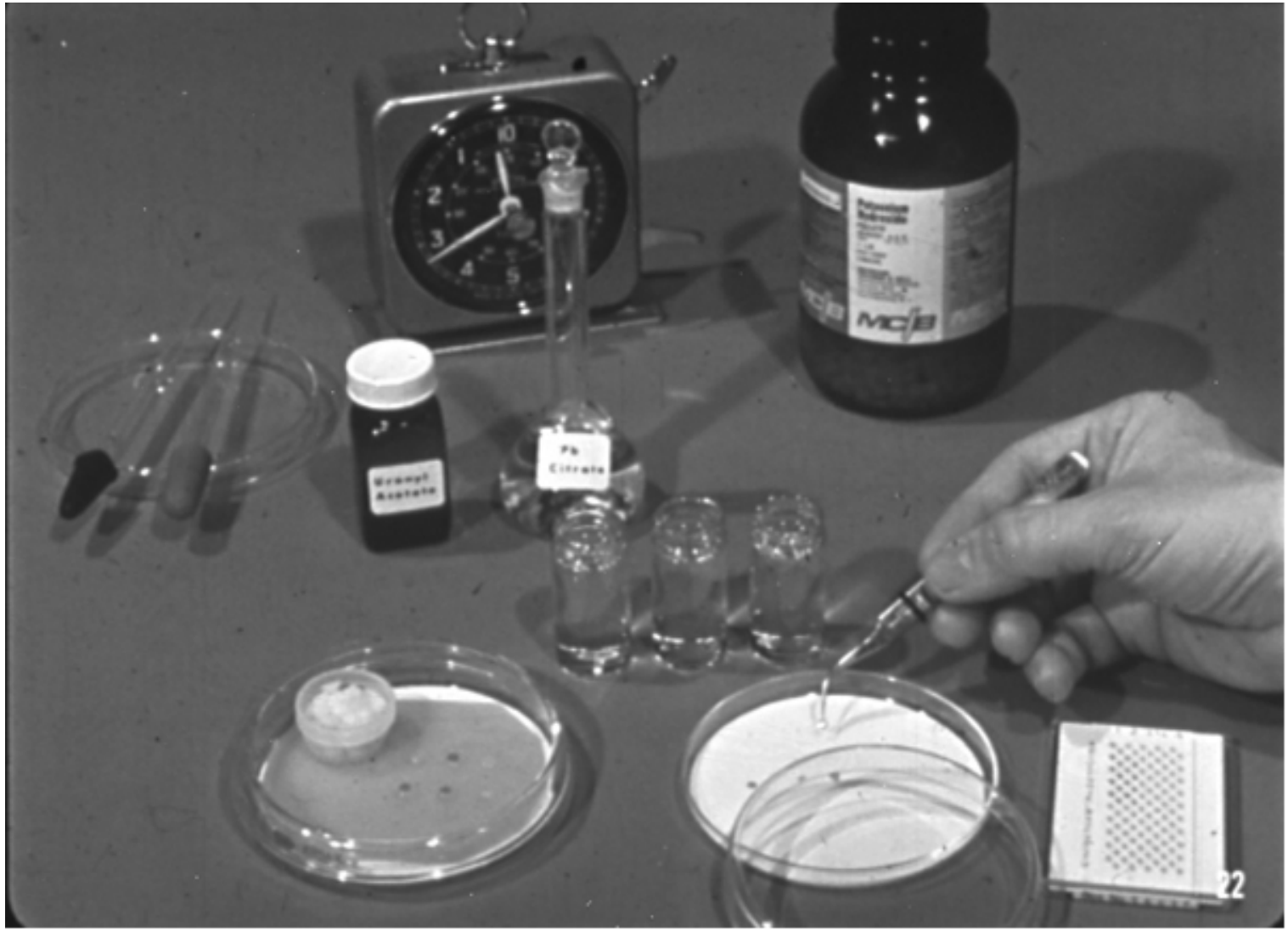
II.A.2 Thin Sectioning (Staining)



Grids placed face down on 50-100μl droplets of stain solutions

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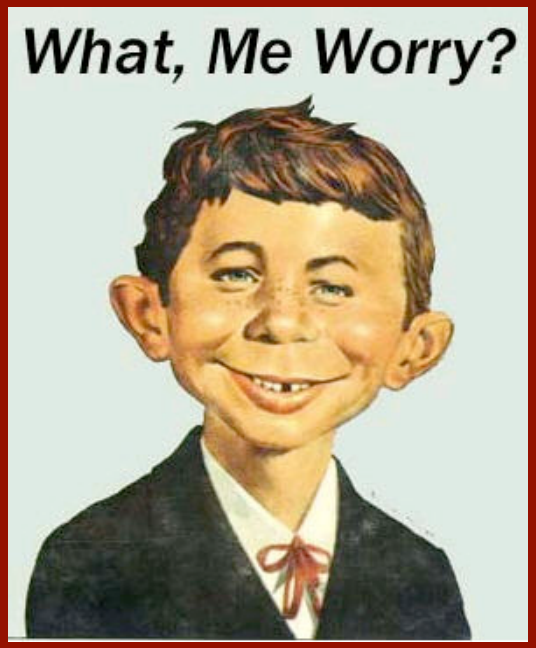
Artifacts of microtomy

OK, so what could possibly go wrong?



Answer: Just about everything!!!

II.A.2 Thin Sectioning (Artifacts)

Chatter

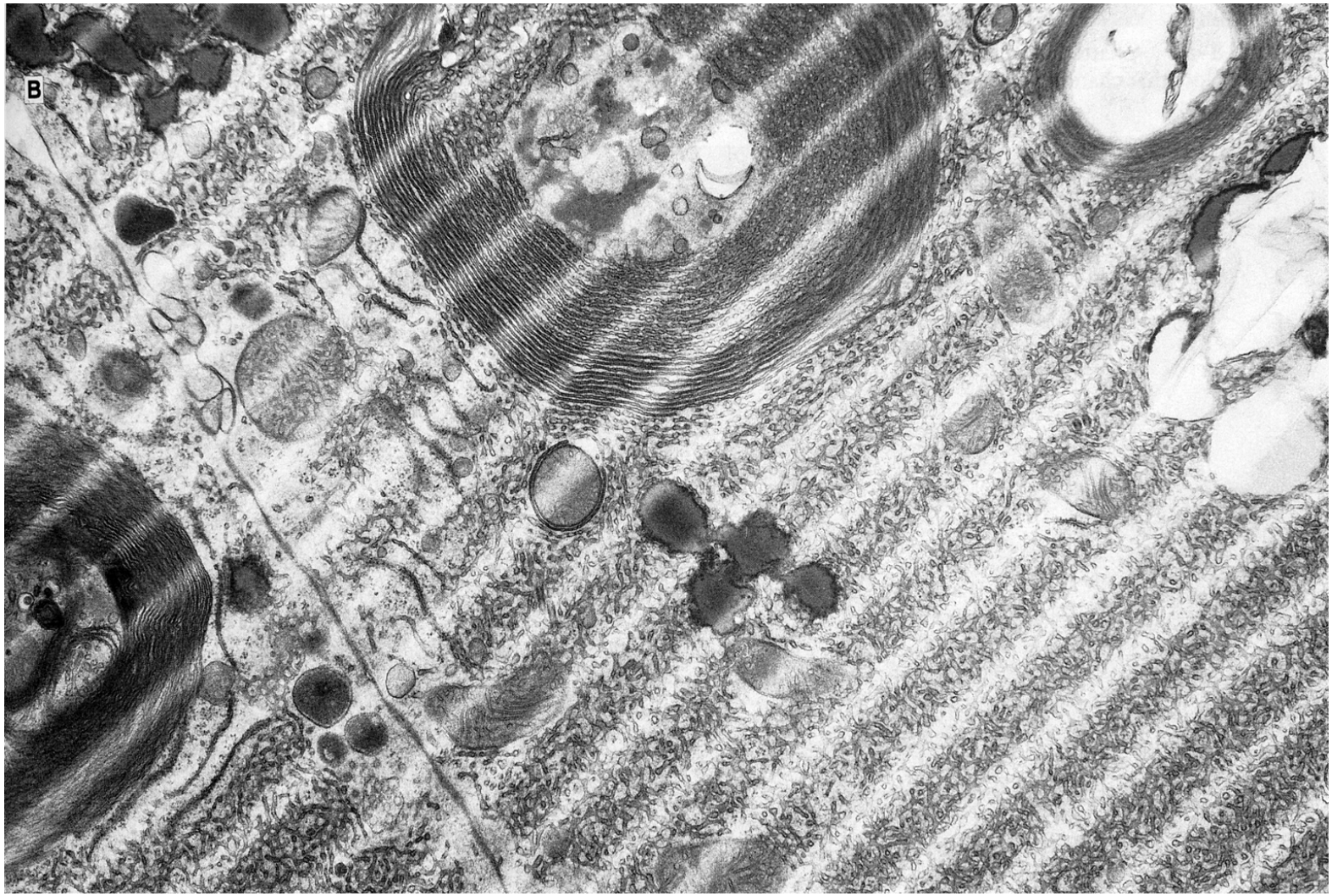
Knife marks

Compression

Slide not shown in class lecture

II.A.2 Thin Sectioning (Artifacts)

Section Chatter

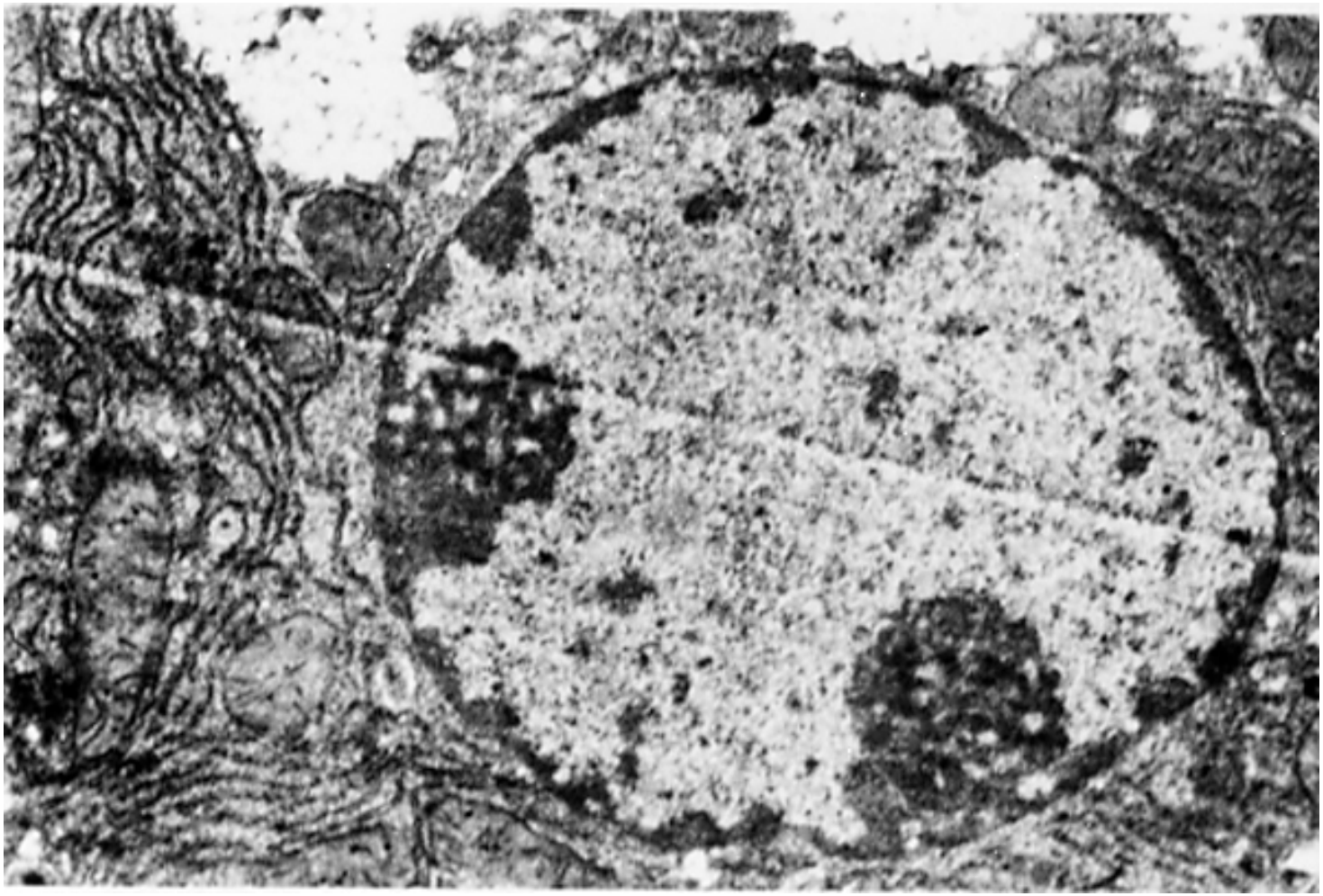


Slide not shown in class lecture

From Bozzolla and Russell, Fig. 18-17B, p.393

II.A.2 Thin Sectioning (Artifacts)

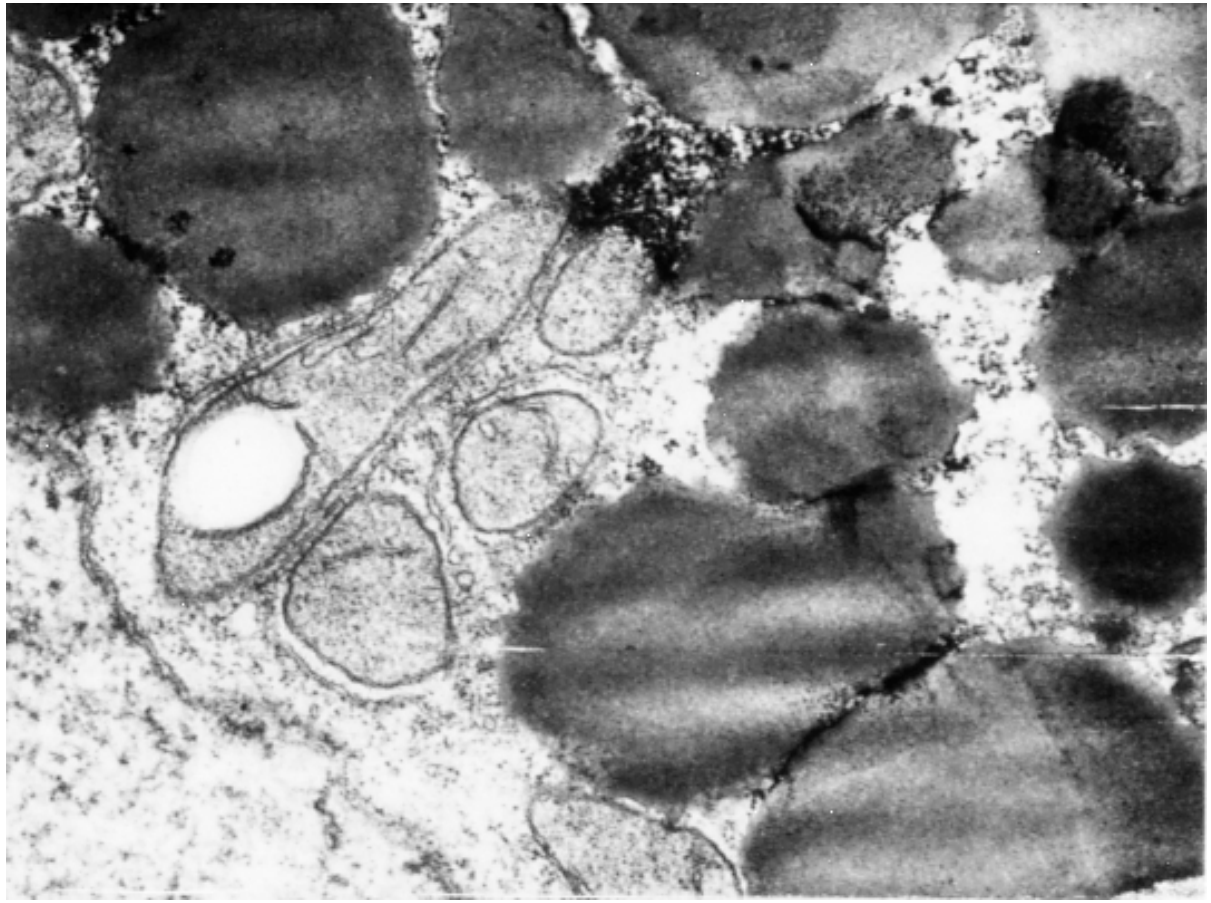
Knife Marks

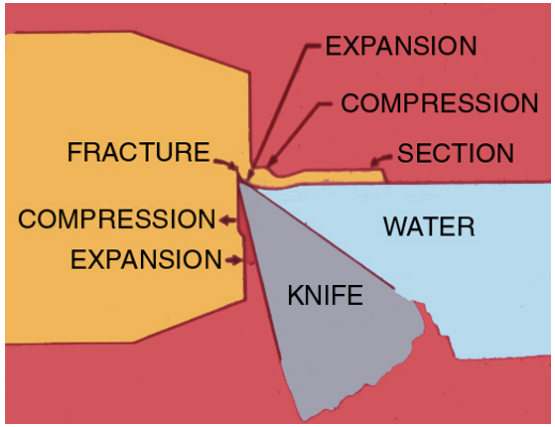


Slide not shown in class lecture

II.A.2 Thin Sectioning (Artifacts)

Compression





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§ II: The Specimen

II.A. Biological Specimen Preparation Techniques

- II.A.1 Specimen Support Films
- II.A.2 Thin Sectioning (pp.154-168)
- II.A.3 Negative Staining
- II.A.4 Metal Shadowing
- II.A.5 Freeze Drying/Etching/Fracture
- II.A.6 Unstained and Frozen-Hydrated

§ II: The Specimen

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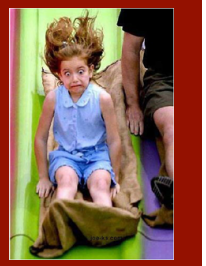


§ II: The Specimen

II.A. Biological Specimen Preparation Techniques

- II.A.1 Specimen Support Films
- II.A.2 Thin Sectioning
- II.A.3 Negative Staining
- II.A.4 Metal Shadowing
- II.A.5 Freeze-Drying, Etching, Fracture
- II.A.6 Unstained and Frozen-Hydrated

Folks, it doesn't get much easier than negative staining

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.3 Negative Staining

What is it used for?

Mostly particulate samples

Macromolecules and macromolecular complexes

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.3 Negative Staining

Why negative staining?

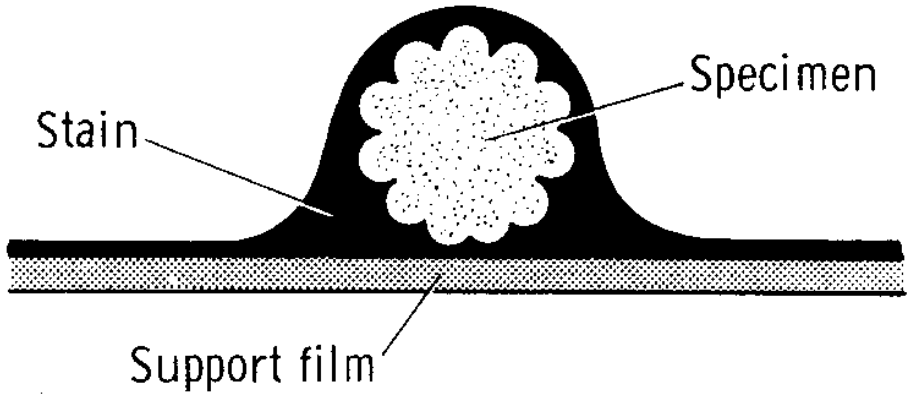
Quick and easy

Excellent aperture contrast

Fairly good resolution: 15-25 Å

Specimen preservation: OK

II.A.3 Negative Staining



Embed sample in a uniform layer of a heavy metal salt

- High contrast at stain-particle boundaries (stain-excluding regions)
- Replace water
- Be inert

Increases mass thickness (scattering/aperture contrast)

"Negative Staining": sample appears light on a dark background

Protects specimen from dehydration-induced effects (e.g. collapse)

From Hayat & Miller, Fig. 1, p.2

II.A.3 Negative Staining

Frequently Used Negative Stains

Negative Stain	Chemical Formula	pH for use
Uranyl acetate	$UO_2(CH_3COO)_2$	2 – 4.5
Sodium phosphotungstate	$Na_3PO_412WO_3$	5 - 8
Sodium silicotungstate	$Na_3SiO_212WO_3$	5 - 8
Ammonium molybdate	$(NH_4)_6Mo_7O_{26}$	5 - 8

Page 173 of lecture notes gives more examples

From R. Harris & R. Horn

II.A.3 Negative Staining

Frequently Used Negative Stains

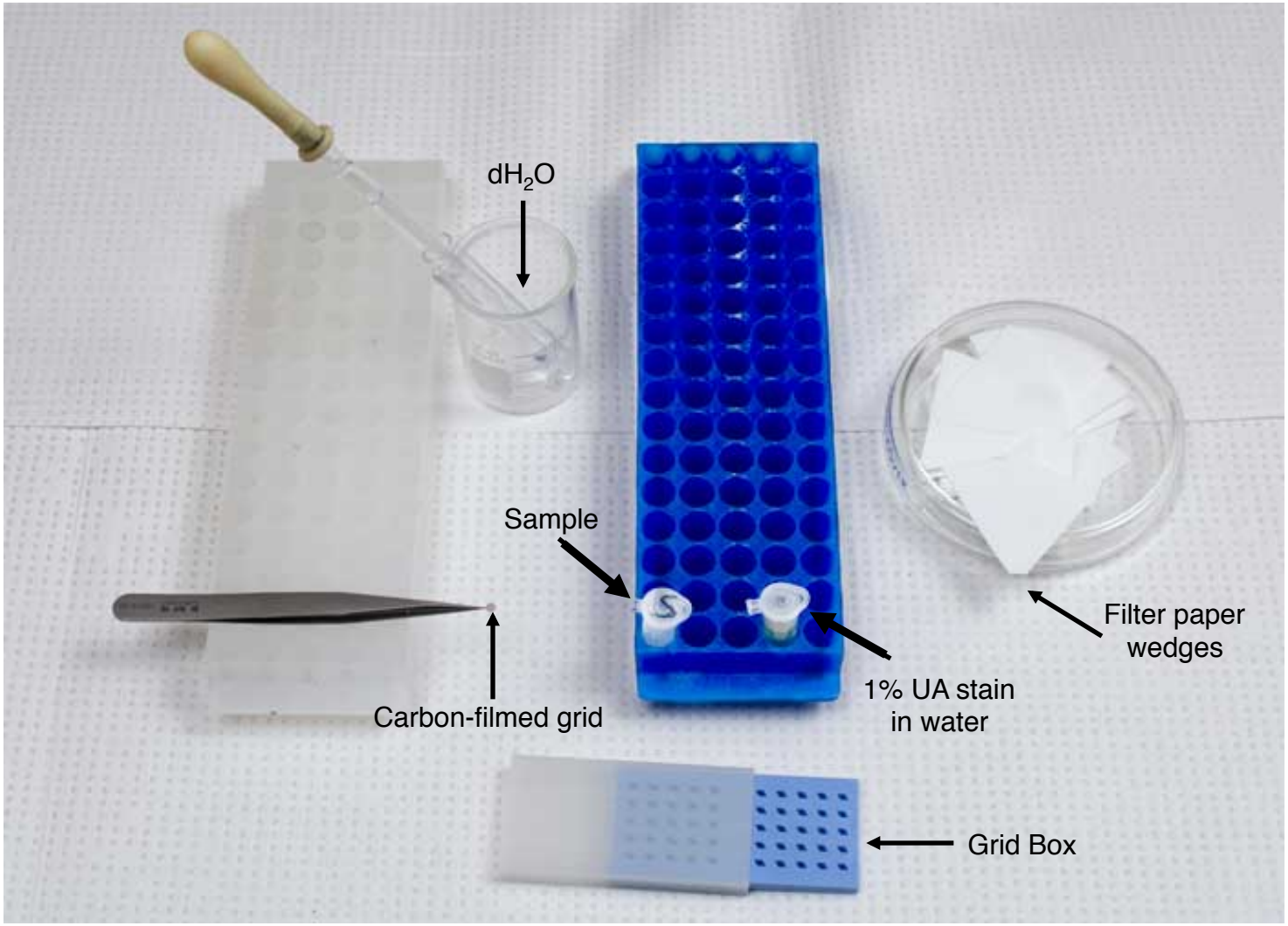
	Negative Stain	Chemical Formula	pH for use
Cationic	Uranyl acetate	$\mathbf{U}O_2(CH_3COO)_2$	2 – 4.5
Anionic	Sodium phosphotungstate	$Na_3PO_4 12\mathbf{W}O_3$	5 - 8
Anionic	Sodium silicotungstate	$Na_3SiO_2 12\mathbf{W}O_3$	5 - 8
Anionic	Ammonium molybdate	$(NH_4)_6\mathbf{Mo}_7O_{26}$	5 - 8

Page 173 of lecture notes gives more examples

From R. Harris & R. Horn

II.A.3 Negative Staining

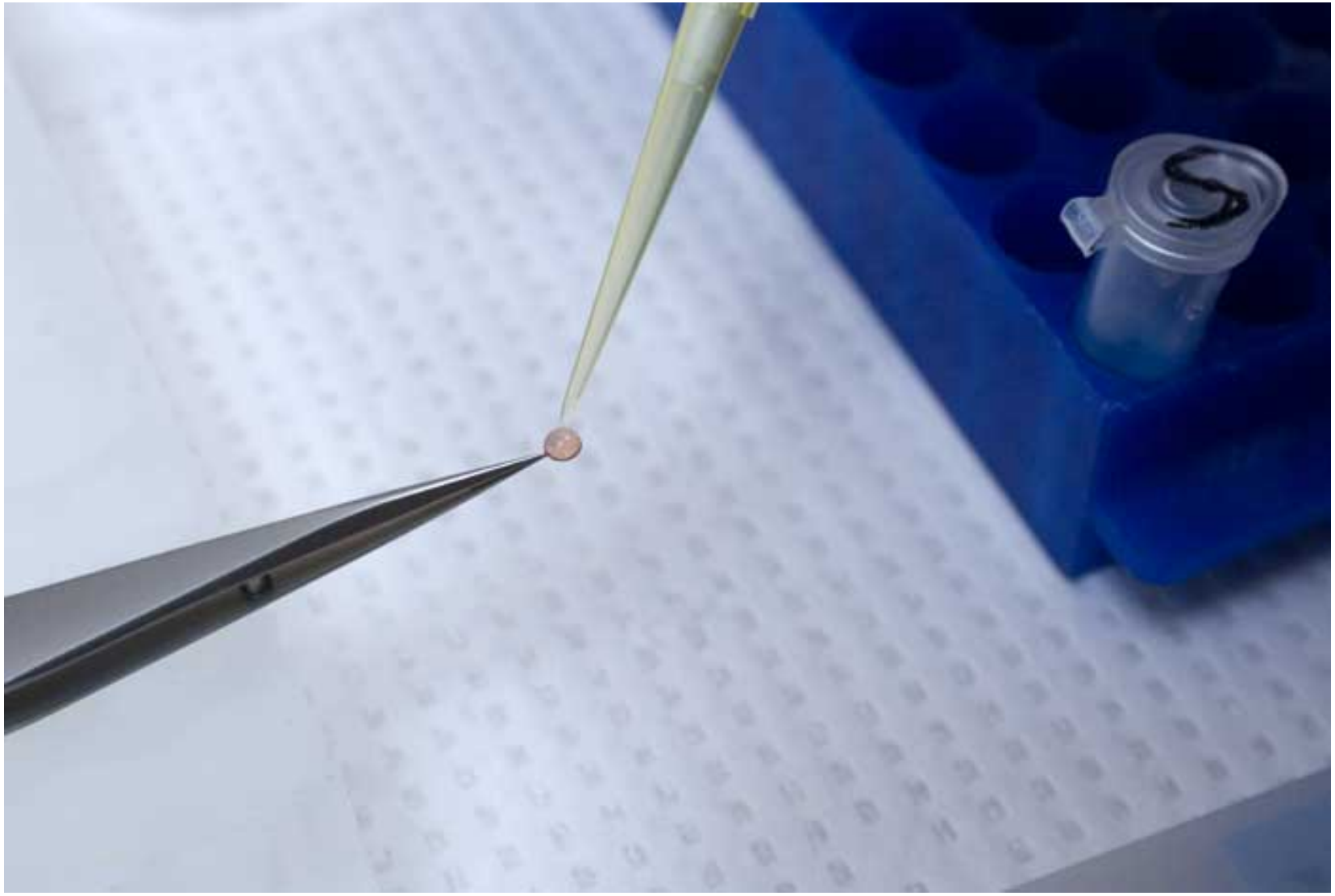
Procedure: Setup



Slide courtesy of N. Olson

II.A.3 Negative Staining

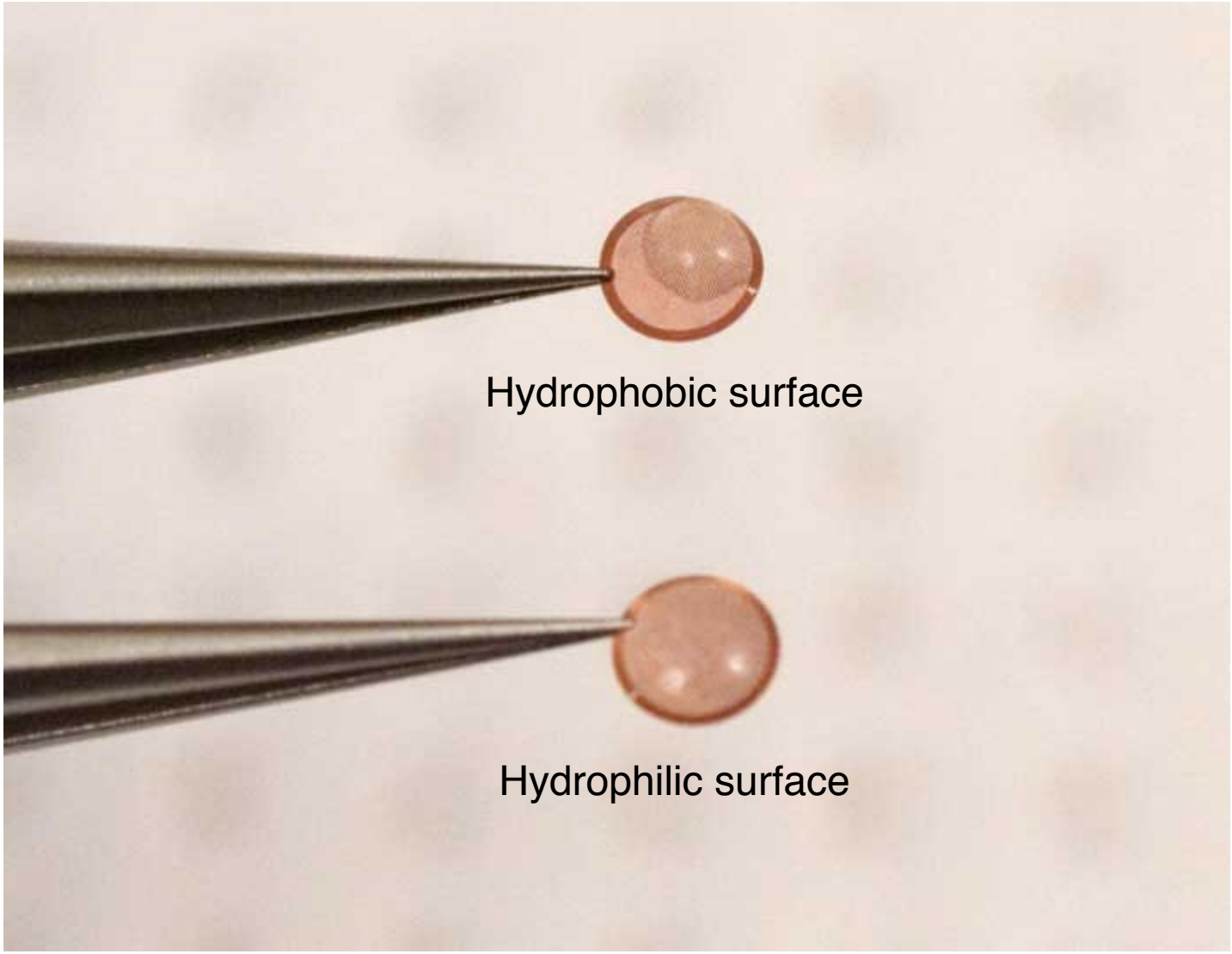
Procedure: Apply 5-10 μl sample to grid



Slide courtesy of N. Olson

II.A.3 Negative Staining

Procedure: Wetting of carbon surface



Slide courtesy of N. Olson

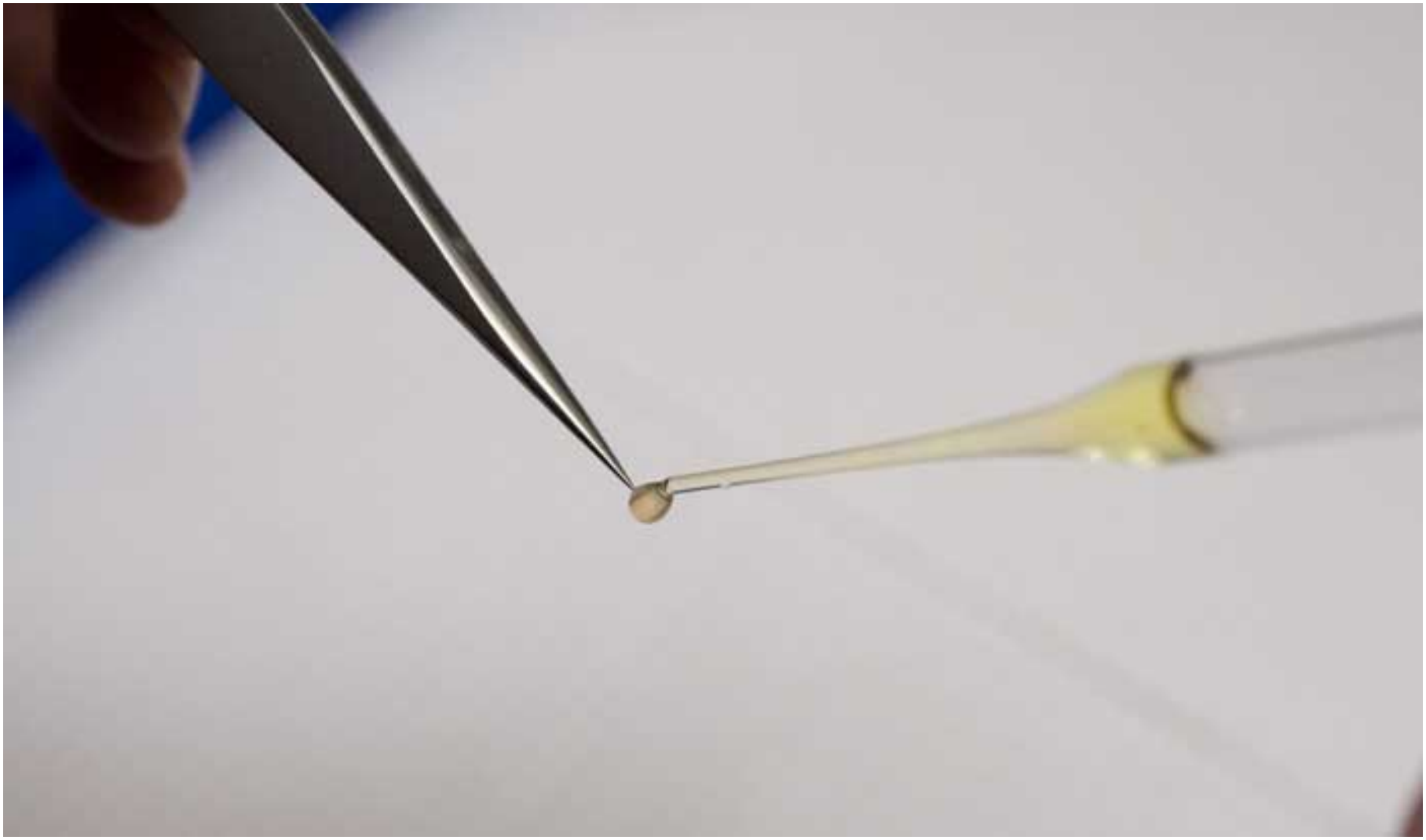
II.A.3 Negative Staining

Procedure: Wash with distilled H_2O

Slide courtesy of N. Olson

II.A.3 Negative Staining

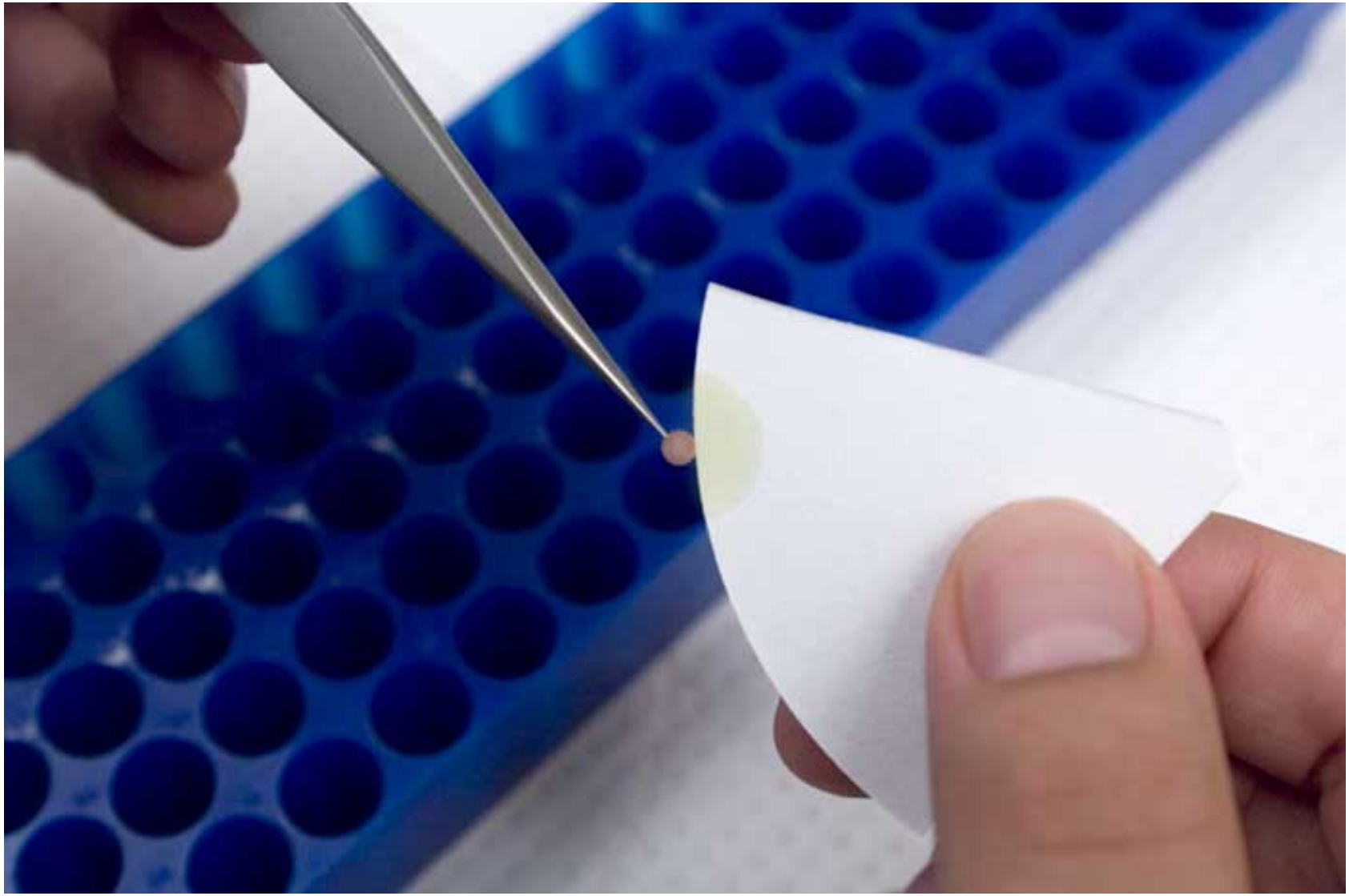
Procedure: Apply Stain



Slide courtesy of N. Olson

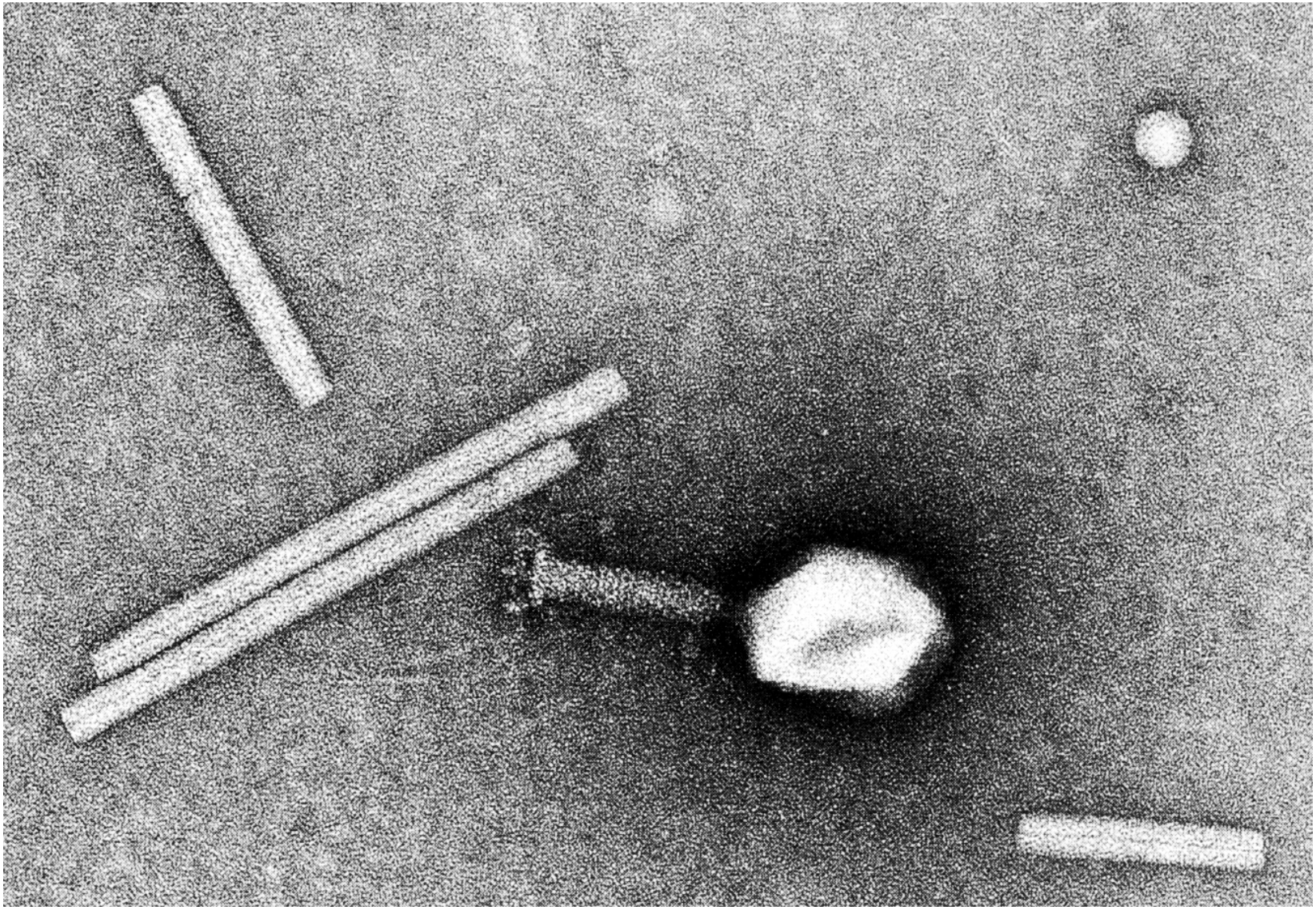
II.A.3 Negative Staining

Procedure: Wait, then blot with filter paper



Slide courtesy of N. Olson

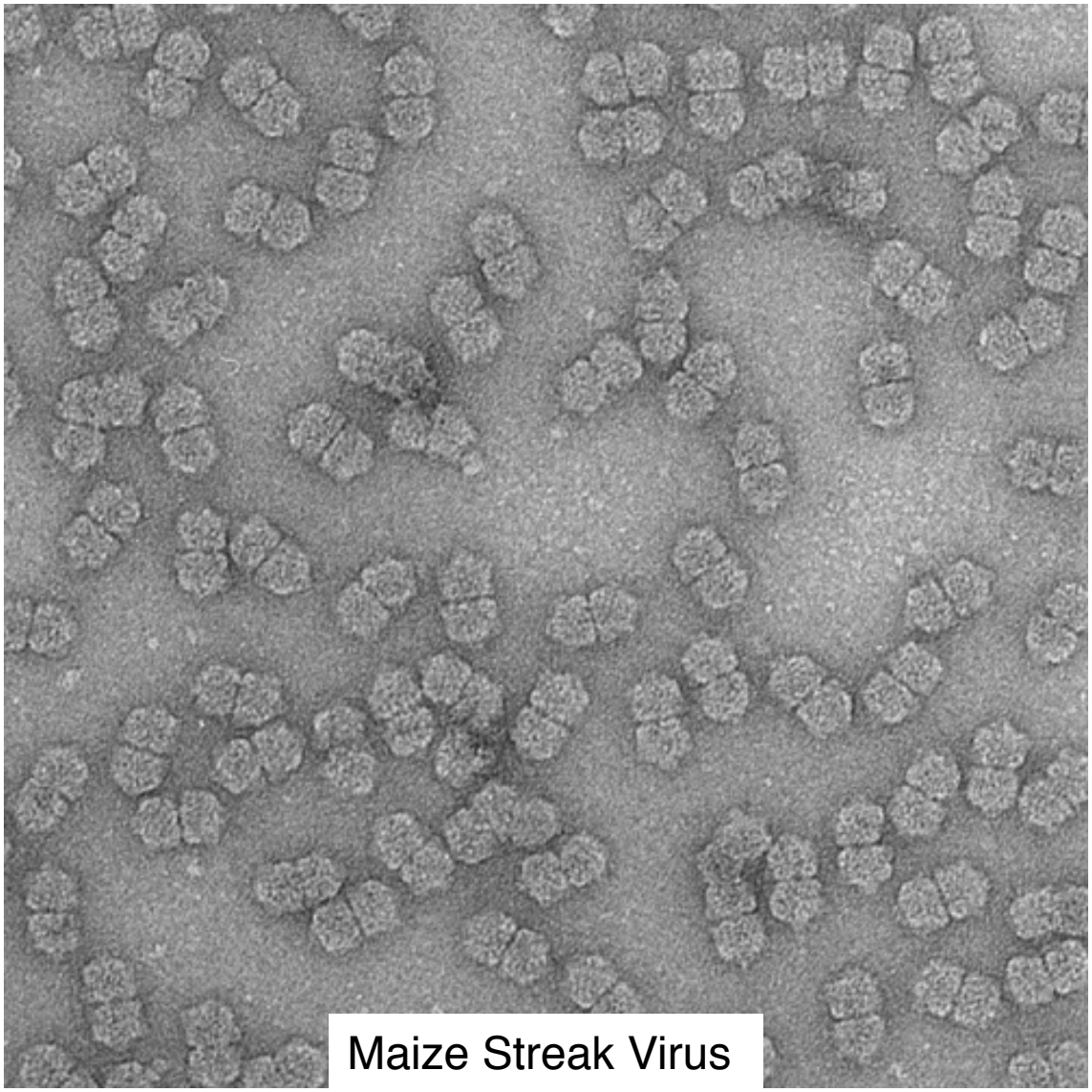
II.A.3 Negative Staining (Examples)



TMV and bacteriophages T4 and ϕX174

Image taken by F. Eiserling

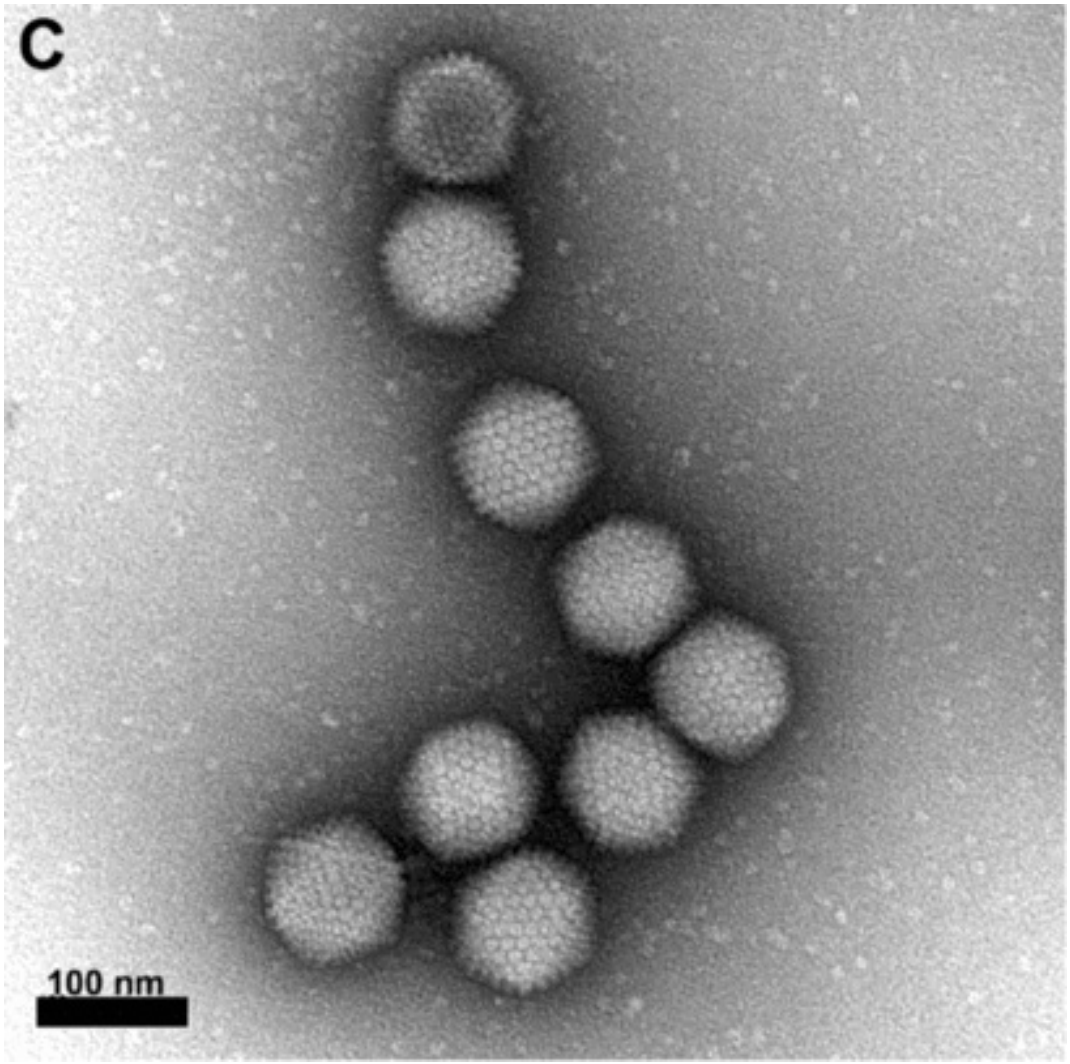
II.A.3 Negative Staining (Examples)



Maize Streak Virus

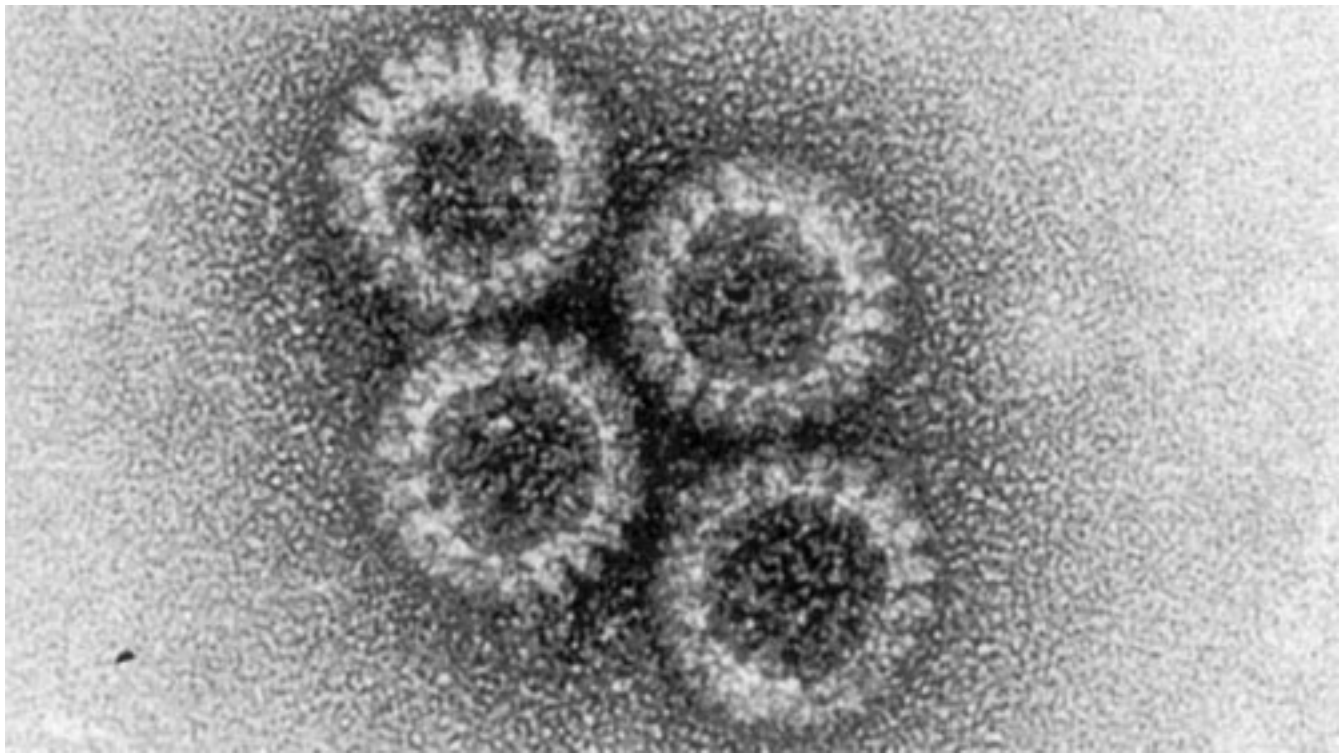
Image courtesy of W. Zhang

II.A.3 Negative Staining (Examples)



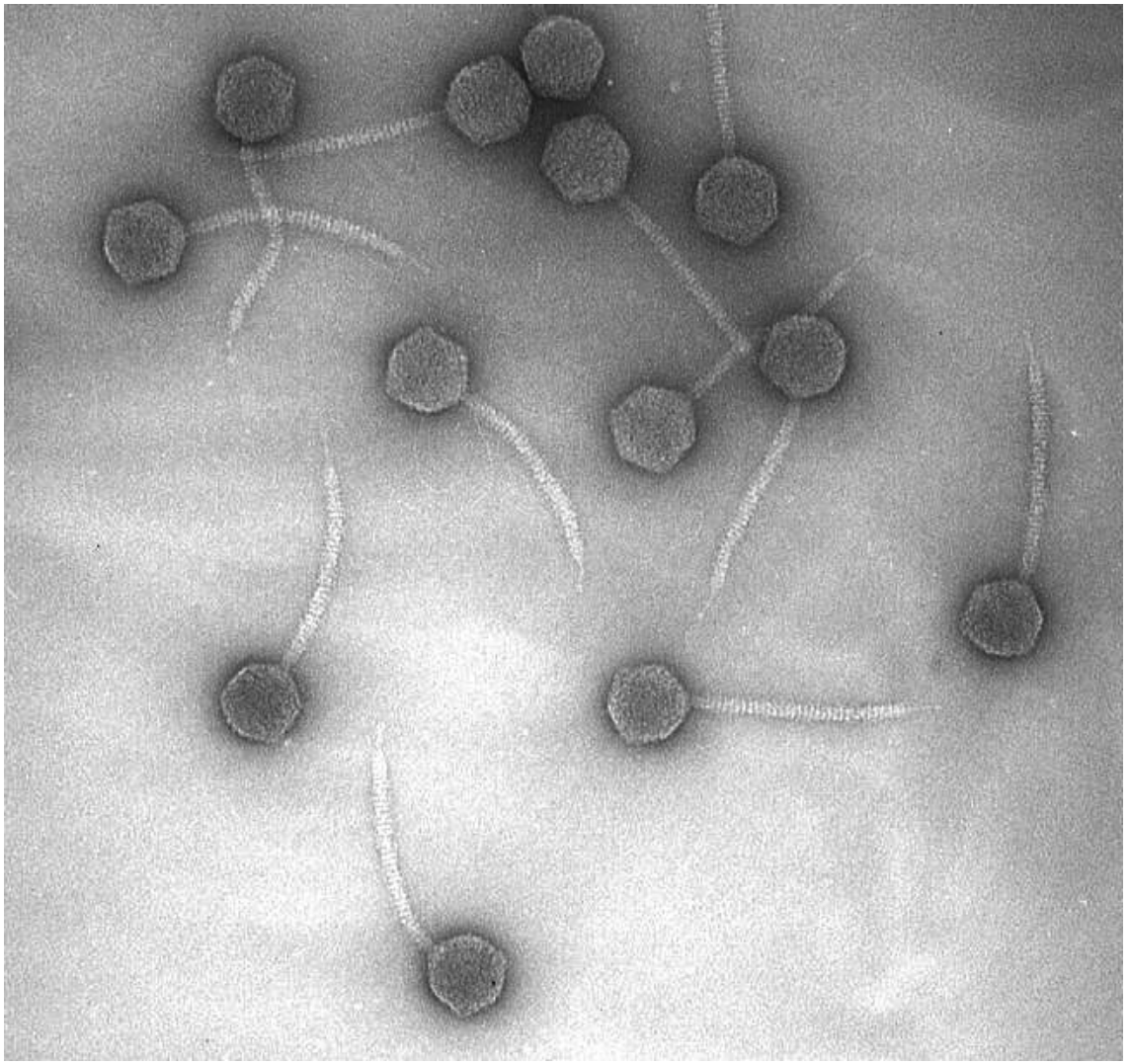
Adenovirus

II.A.3 Negative Staining (Examples)



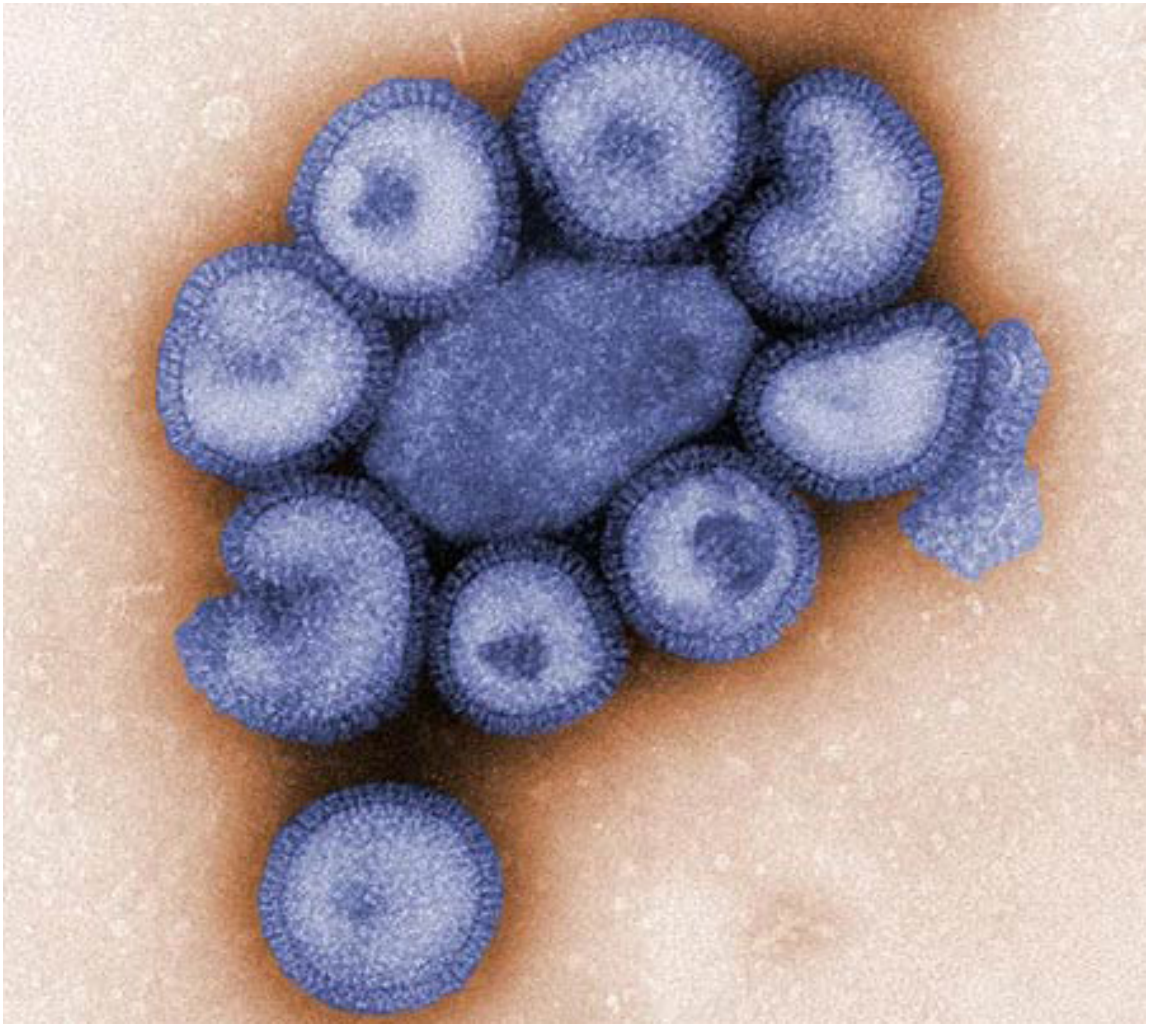
Rotavirus

II.A.3 Negative Staining (Examples)



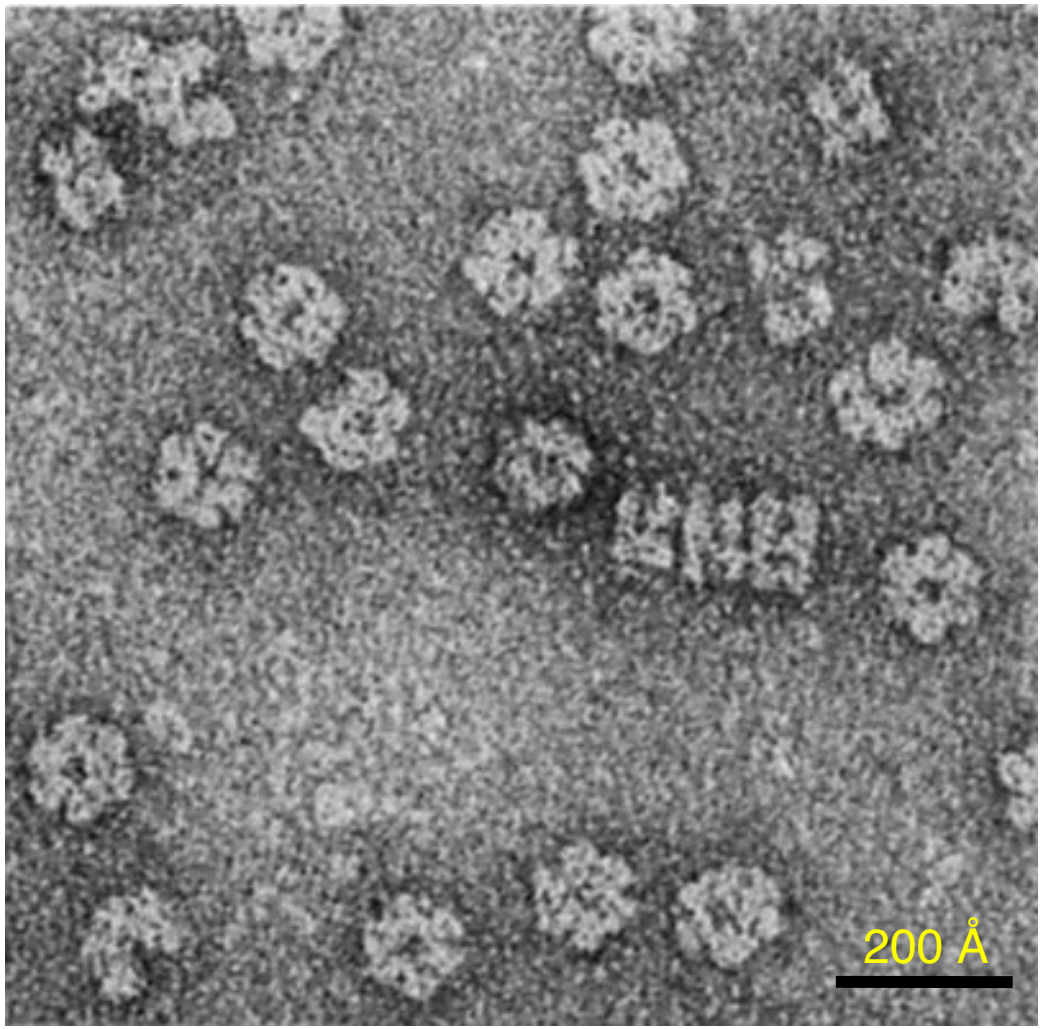
Bacteriophage λ

II.A.3 Negative Staining (Examples)



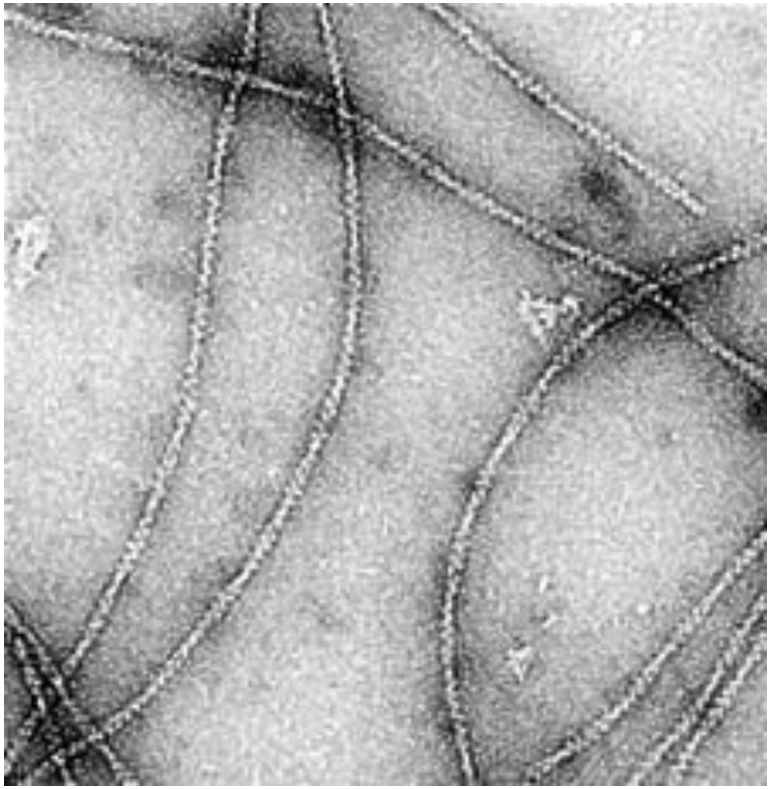
Swine flu

II.A.3 Negative Staining (Examples)

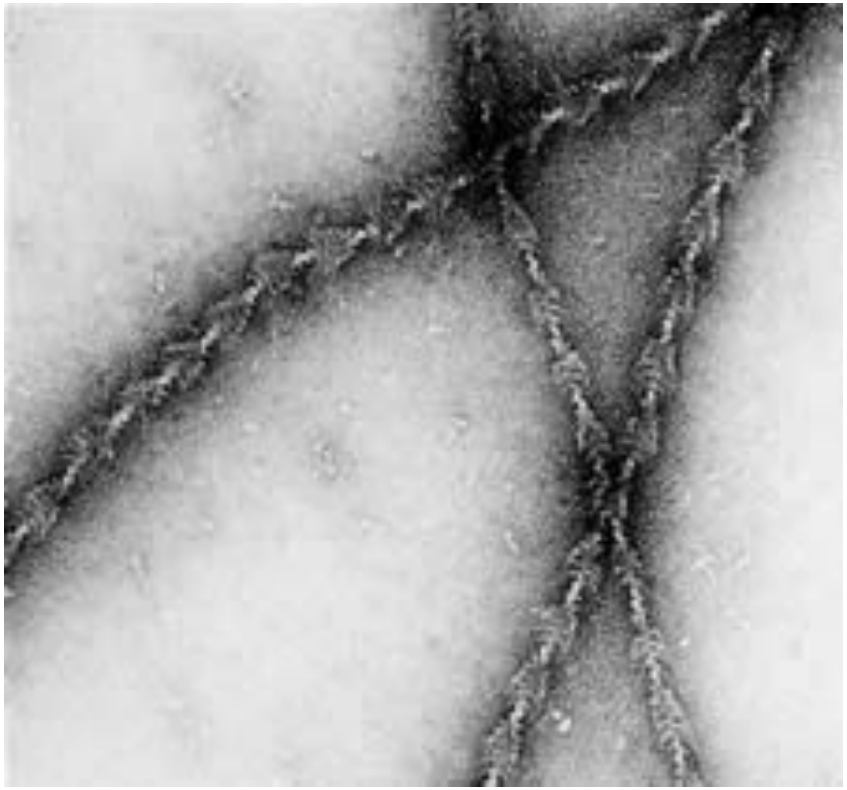


Glutamine synthetase

II.A.3 Negative Staining (Examples)



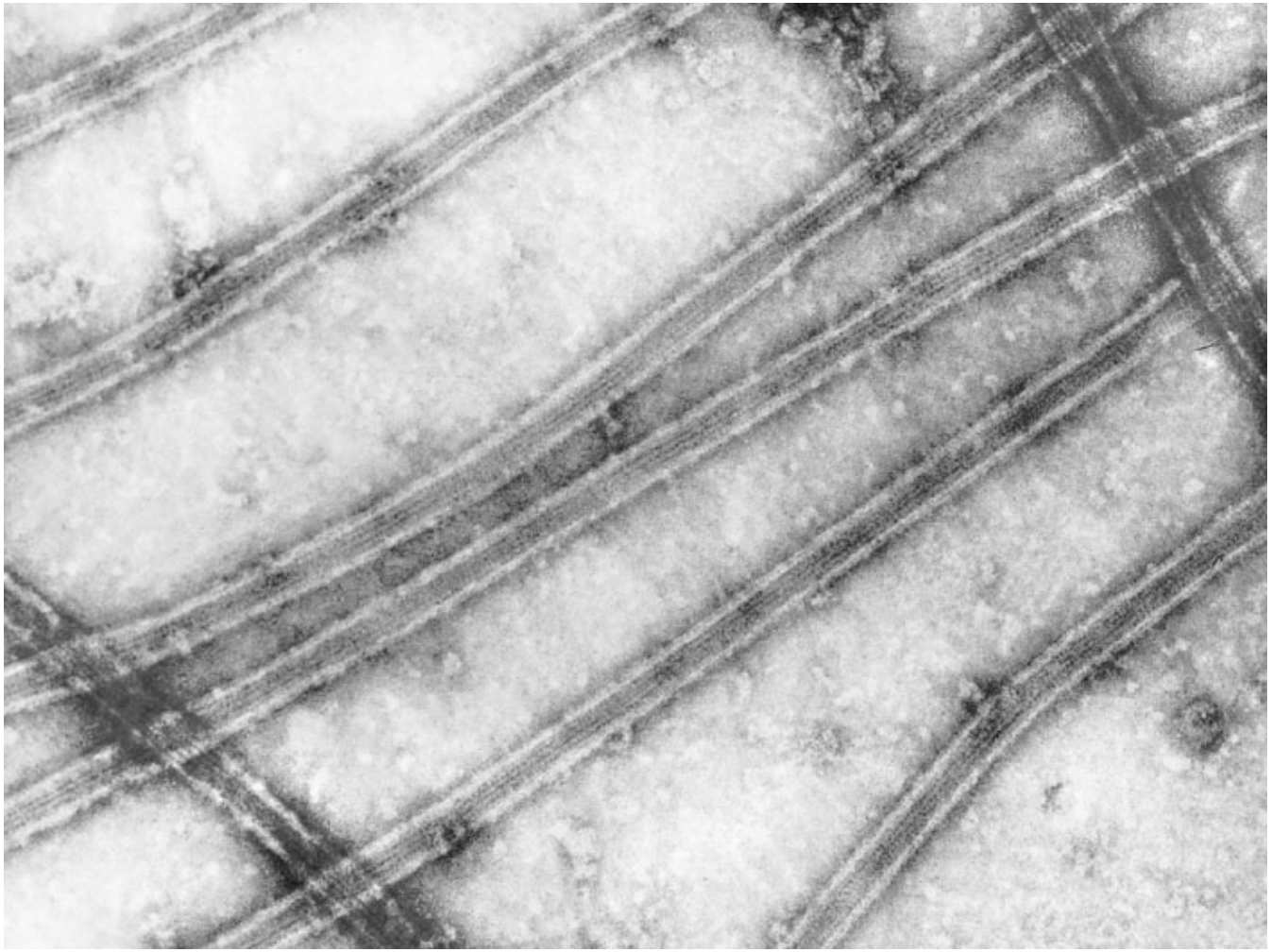
Actin filament



Actomyosin filament

Images taken by R. Graig (see www.umassmed.edu/cemf/negstain.aspx)

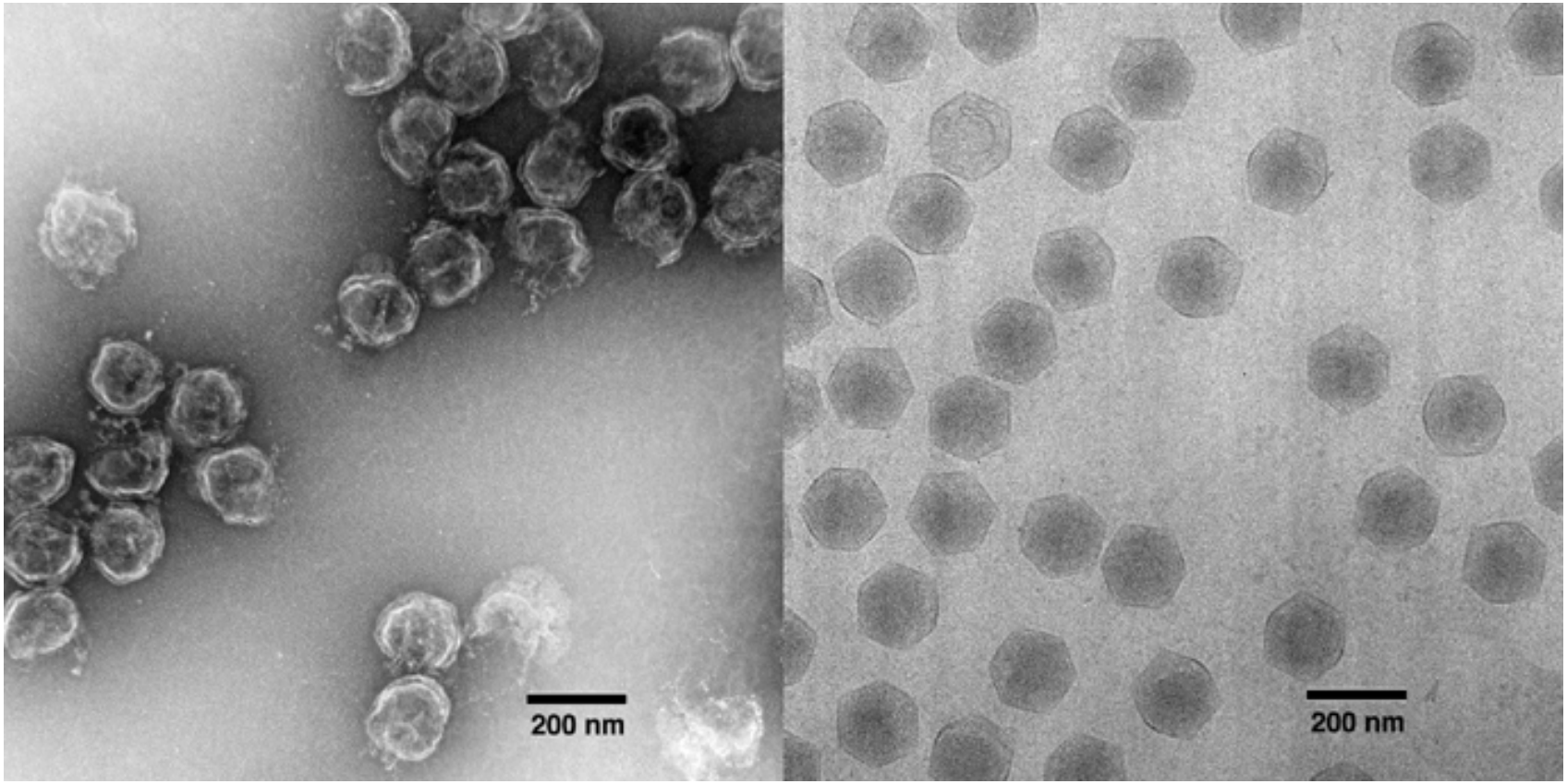
II.A.3 Negative Staining (Examples)



Microtubules

II.A.3 Negative Staining

Potential Problems with Negative Staining Procedures



UA-stained

Frozen-hydrated

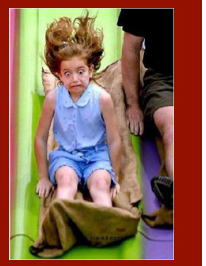
Parmecium bursaria Chlorella virus-1

Images taken by N. Olson

§ II: The Specimen

II.A. Biological Specimen Preparation Techniques

- II.A.1 Specimen Support Films
- II.A.2 Thin Sectioning
- II.A.3 Negative Staining
- II.A.4 Metal Shadowing
- II.A.5 Freeze Drying/Etching/Fracture
- II.A.6 Unstained and Frozen-Hydrated



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II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.4 Metal Shadowing

What is it used for?

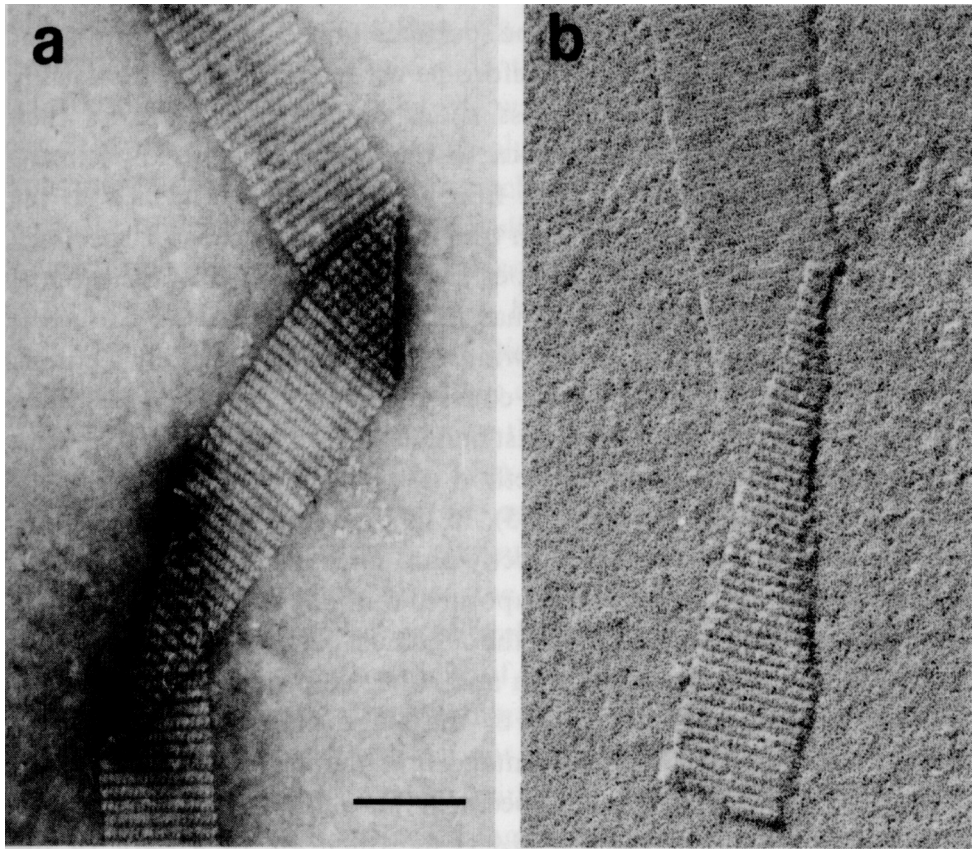
View specimen surfaces

- Particulate samples
- Freeze-fractured/etched cells

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.4 Metal Shadowing

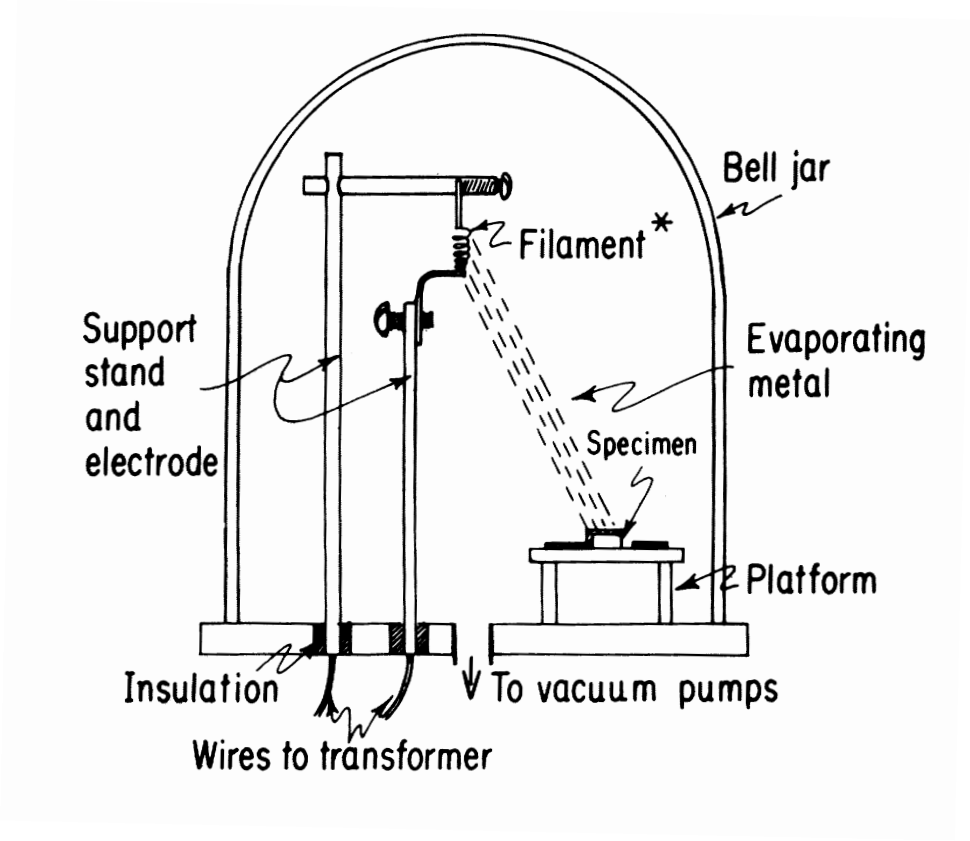
Bacteria Surface 'Spinae'



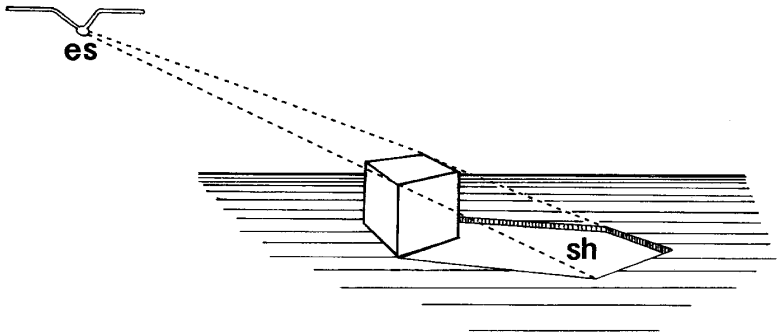
Negative Stain Metal Shadow

From Willison and Rowe, Fig. 3.2, p.61

II.A.4 Metal Shadowing (Unidirectional)

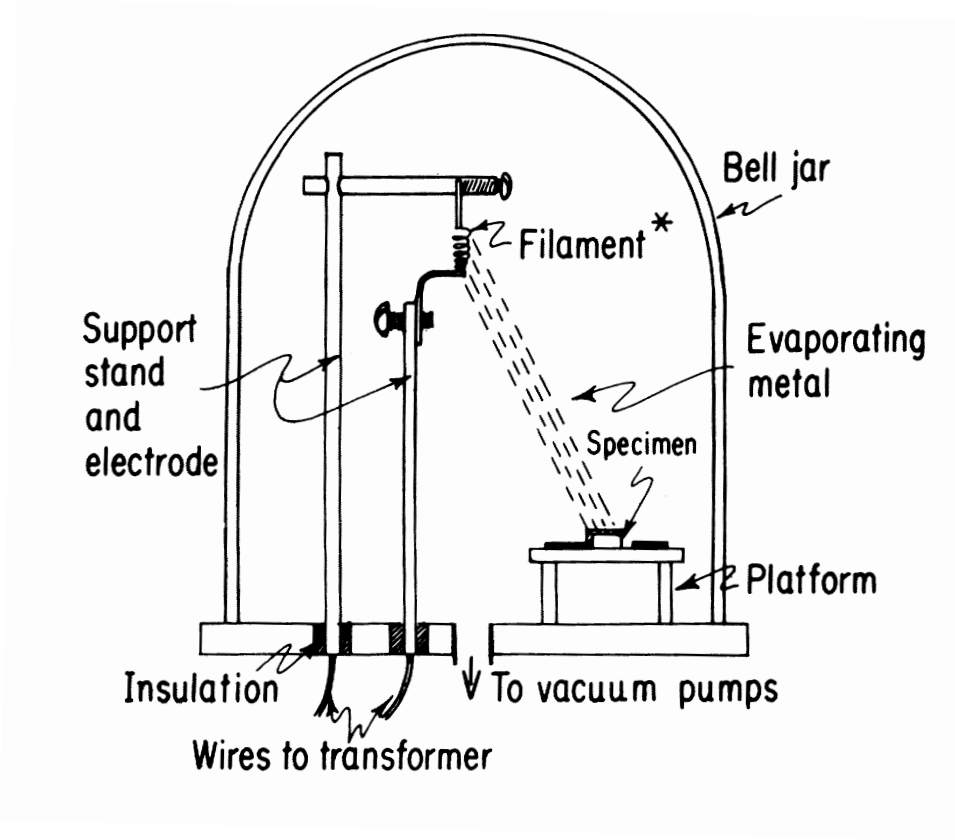


From Wischnitzer, 2nd Ed., Fig. L1, p.244

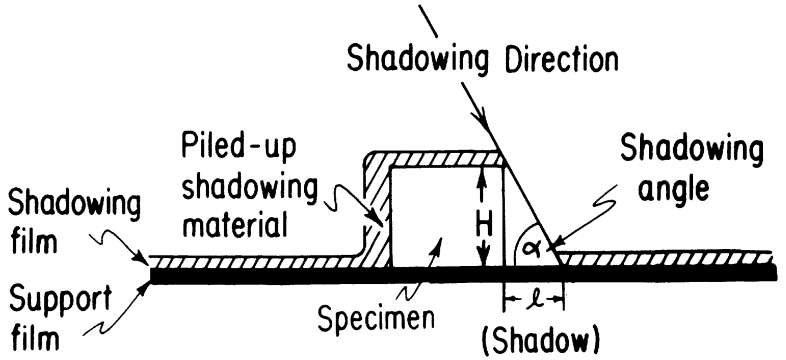


From Willison and Rowe, Fig. 1.1, p.5

II.A.4 Metal Shadowing (Unidirectional)



From Wischnitzer, 2nd Ed., Fig. L1, p.244

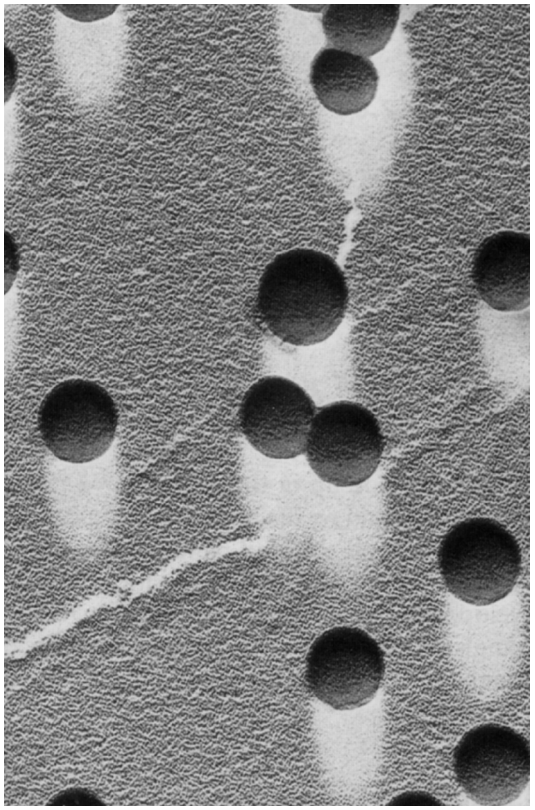


From Wischnitzer, 2nd Ed., Fig. L1, p.244

Shadow length directly related to the shadowing angle and specimen height

Slide not shown in class lecture

II.A.4 Metal Shadowing (Unidirectional)

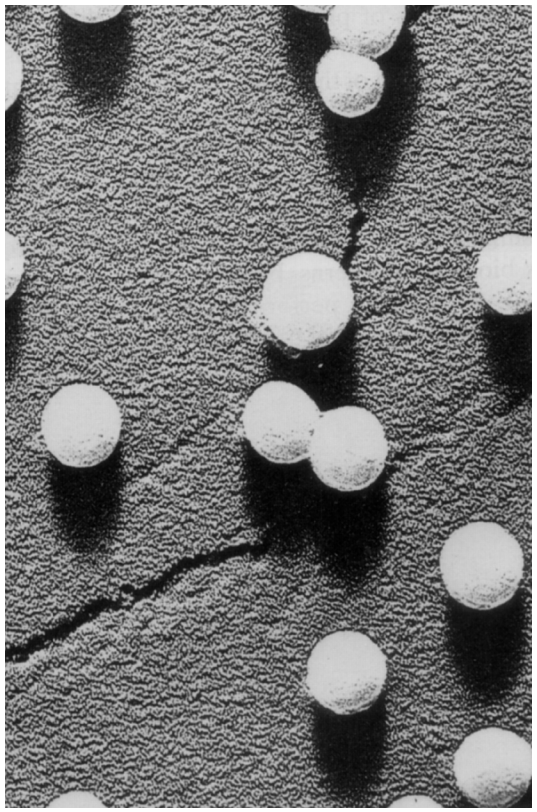


Appearance of image on fluorescent screen
(print 'positive')

Slide not shown in class lecture

From Maunsbach & Afzelius in *Biomedical Elec. Micros.* (1999) Fig.12.7, p.327

II.A.4 Metal Shadowing (Unidirectional)



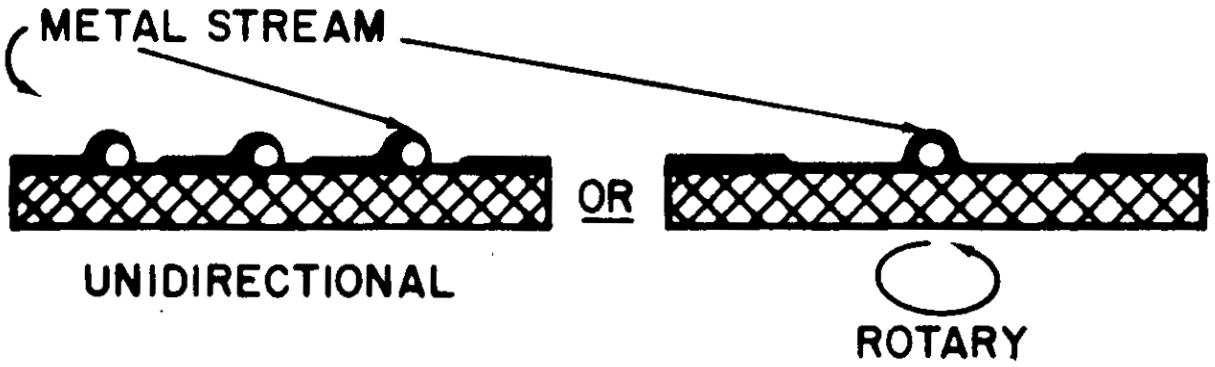
Appearance of actual micrograph
(film 'negative')

Slide not shown in class lecture

From Maunsbach & Afzelius in *Biomedical Elec. Micros.* (1999) Fig.12.7, p.327

II.A.4 Metal Shadowing

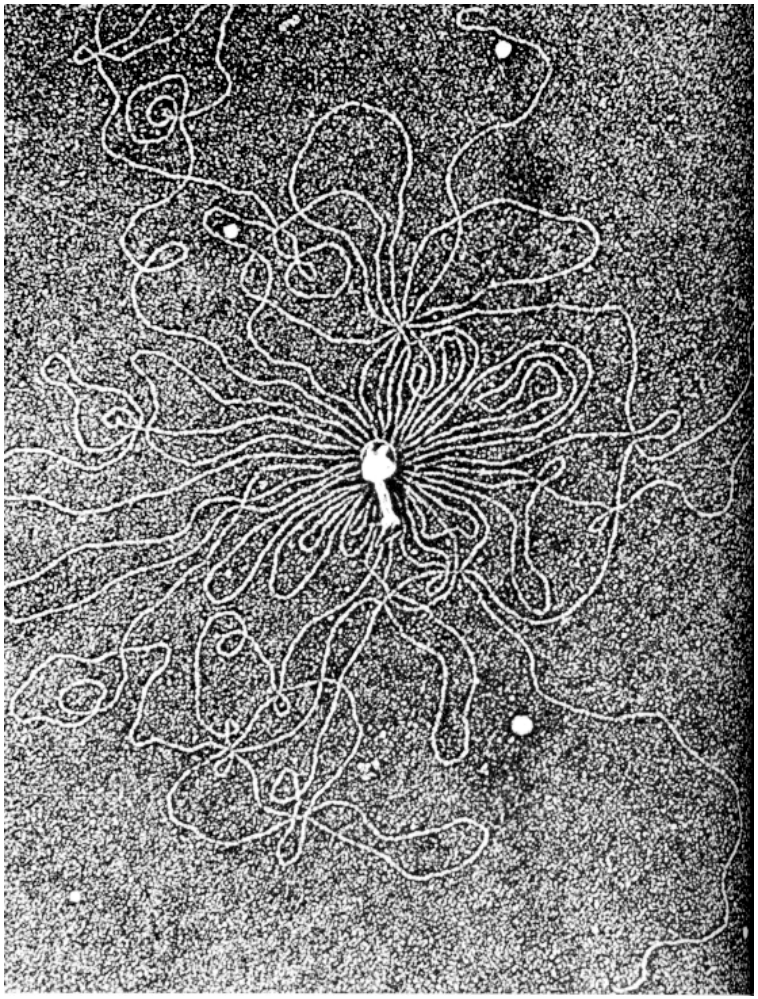
Specimen Unidirectional or Rotary Shadowed



Specimen rotated during shadowing

From H. S. Slayter (1976) Fig.6

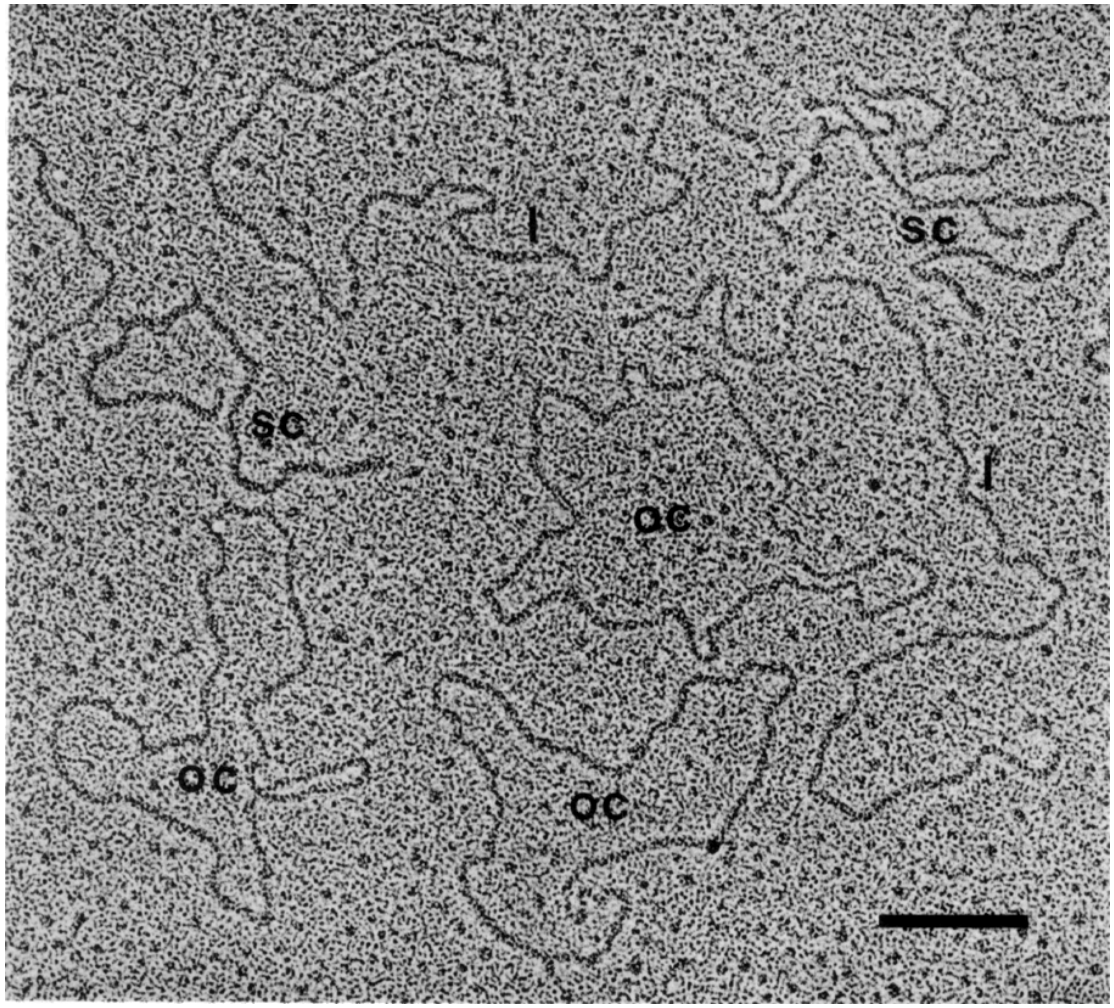
II.A.4 Metal Shadowing (Examples)



Rotary shadowed, osmotically-shocked T2 bacteriophage
('negative' image)

From Wischnitzer, 3rd Ed., Fig. 105, p.206

II.A.4 Metal Shadowing (Examples)

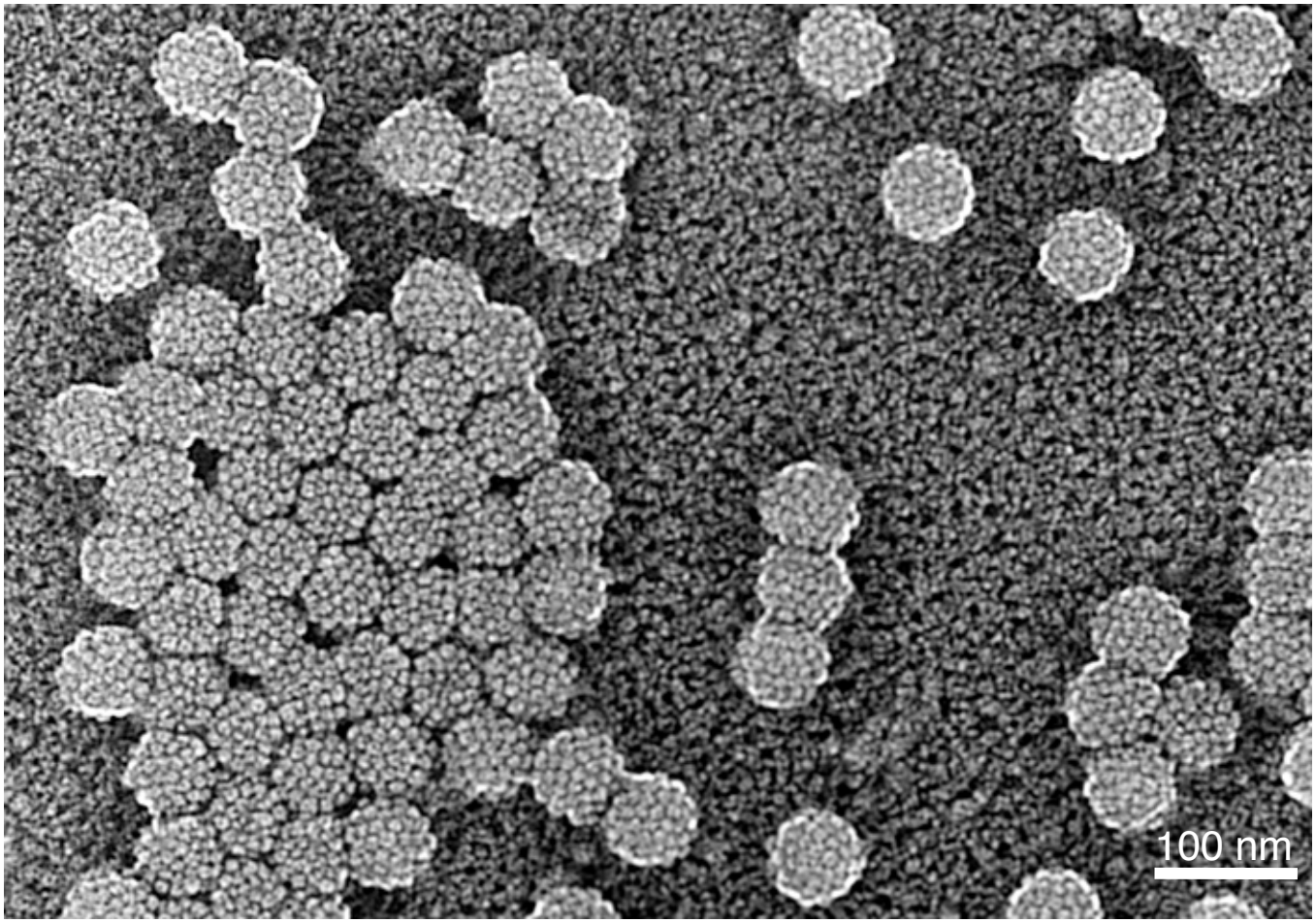


Rotary shadowed plasmid DNA spread in ammonium acetate
('positive' image)

From Sommerville and Scheer, Fig. 1.6, p.12

II.A.4 Metal Shadowing (Examples)

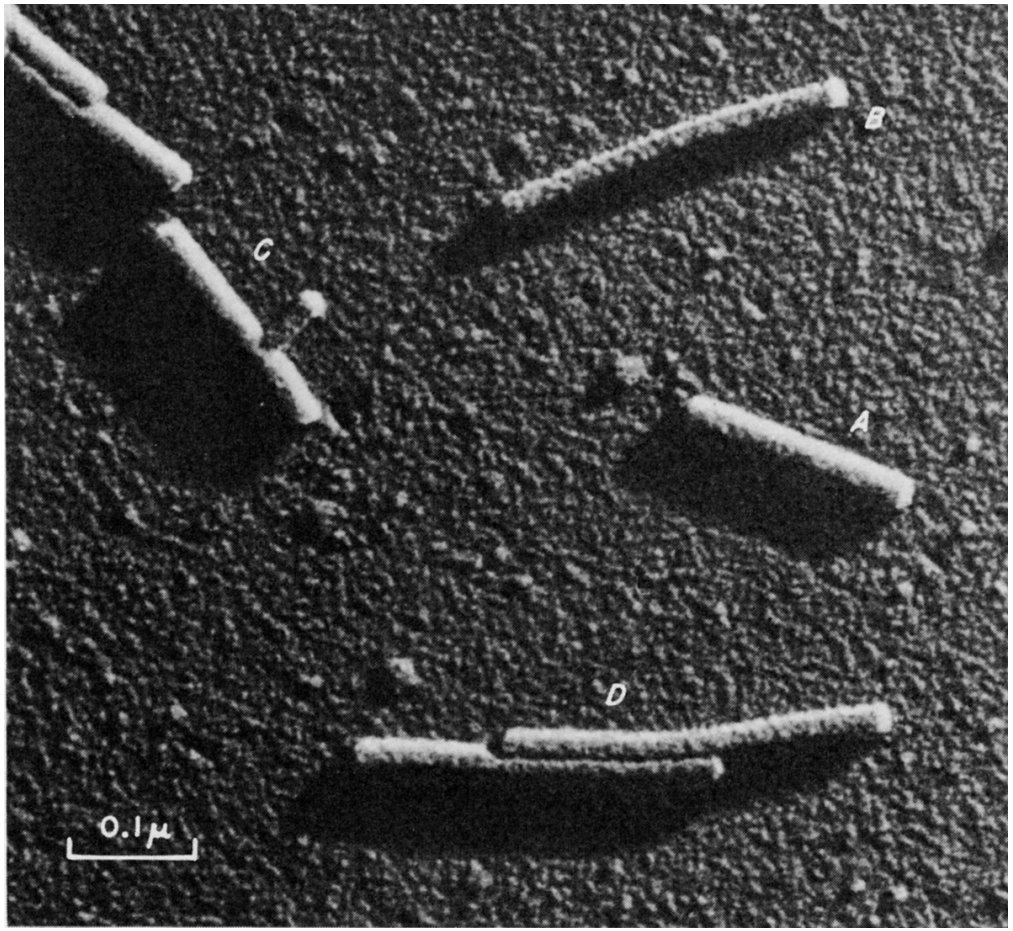
Specimen Rotary Shadowed



Rotary shadowed bovine papilloma virus
('negative' image)

II.A.4 Metal Shadowing (Examples)

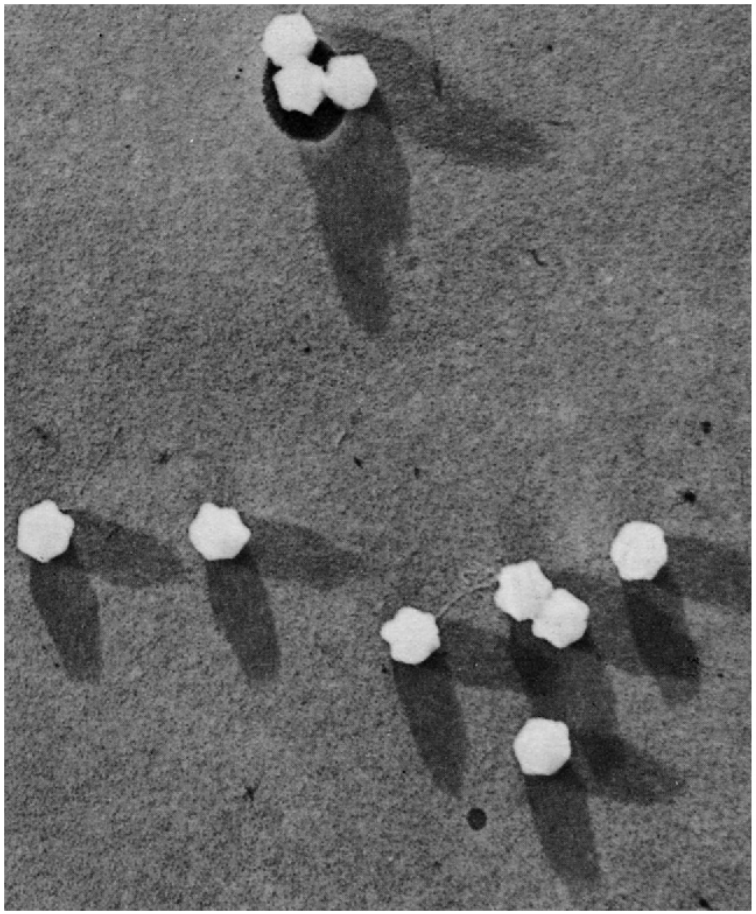
Specimen Unidirectional Shadowed



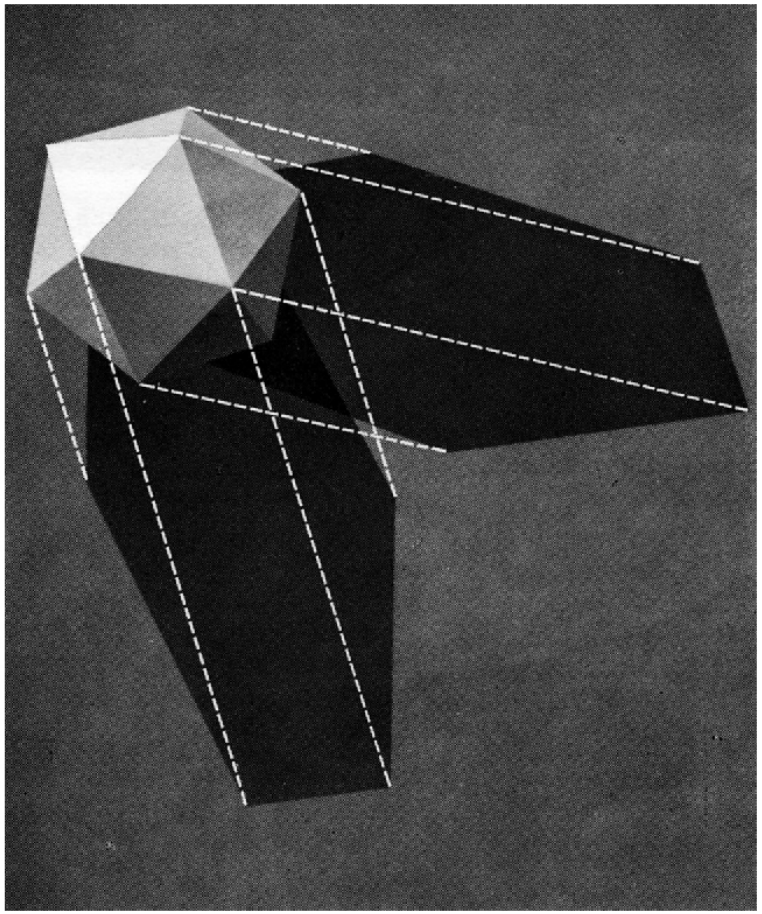
Tobacco mosaic virus
('negative' image)

II.A.4 Metal Shadowing (Examples)

Specimen Bi-directional Shadowed



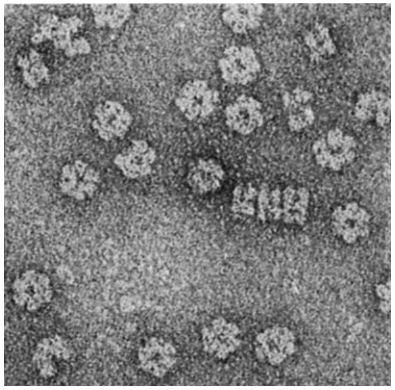
Tipula iridescent virus
('negative' image)



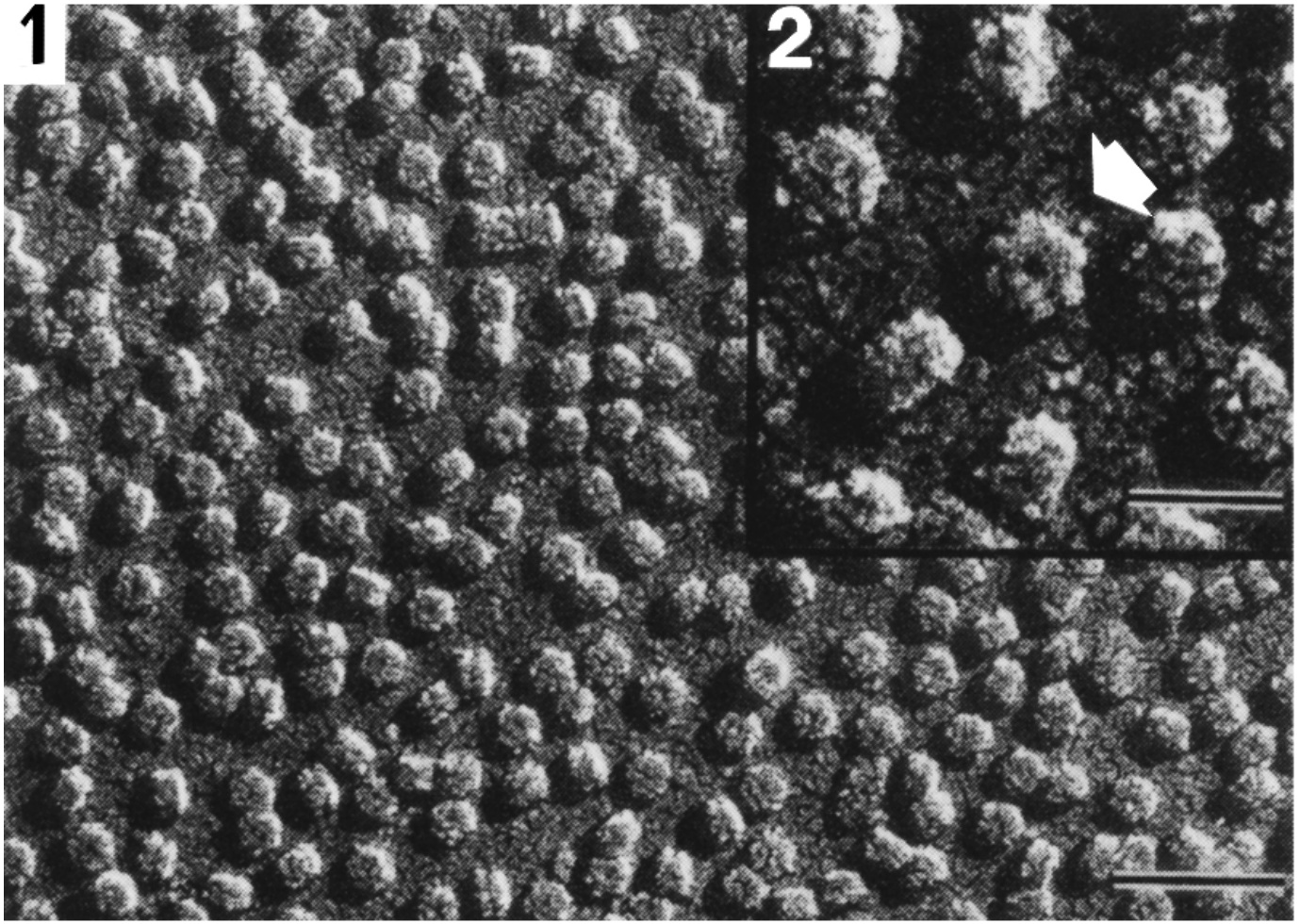
Model

II.A.4 Metal Shadowing (Examples)

Specimen Unidirectional Shadowed



Negatively stained GS



Glutamine synthetase
('negative' image)

§ II: The Specimen

II.A. Biological Specimen Preparation Techniques

- II.A.1 Specimen Support Films
- II.A.2 Thin Sectioning
- II.A.3 Negative Staining
- II.A.4 Metal Shadowing
- II.A.5 Freeze Drying/Etching/Fracture
- II.A.6 Unstained and Frozen-Hydrated

§ II: The Specimen

II.A. Biological Specimen Preparation Techniques

- II.A.1 Specimen Support Films
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II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

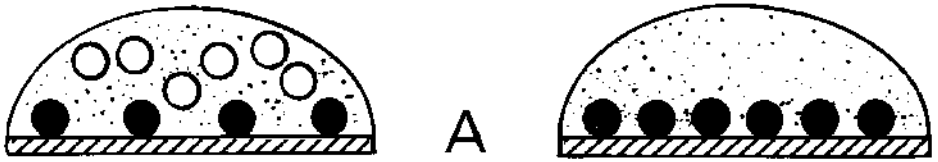
II.A.5 Freeze Drying/Etching/Fracture

See "hidden slides" and read lecture notes (pp.183-187)

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.5 Freeze Drying/Etching/Fracture

Air-Drying Freeze-Drying



A

A. Air-drying: adsorbed and unadsorbed particles present

Freeze-drying: only adsorbed particles

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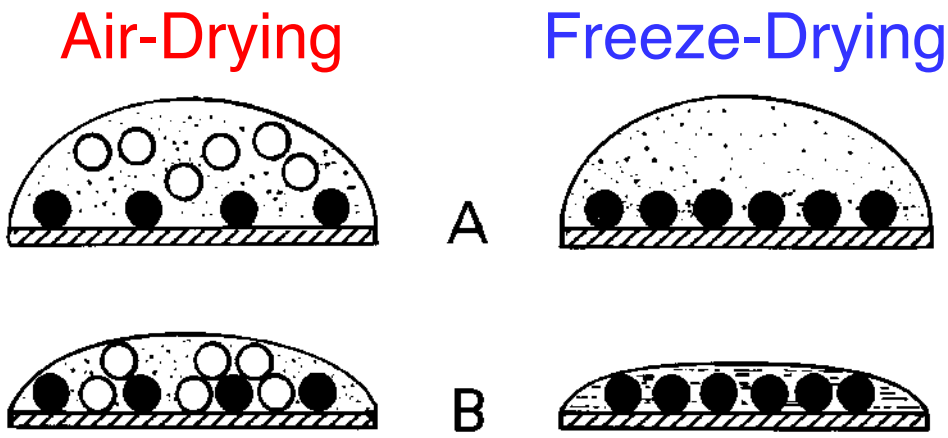
From Nermut, Fig. 2.1, p.80

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.5 Freeze Drying/Etching/Fracture



A. Air-drying: adsorbed and unadsorbed particles present

Freeze-drying: only adsorbed particles

B. Air-drying: unadsorbed particles aggregate / overlap

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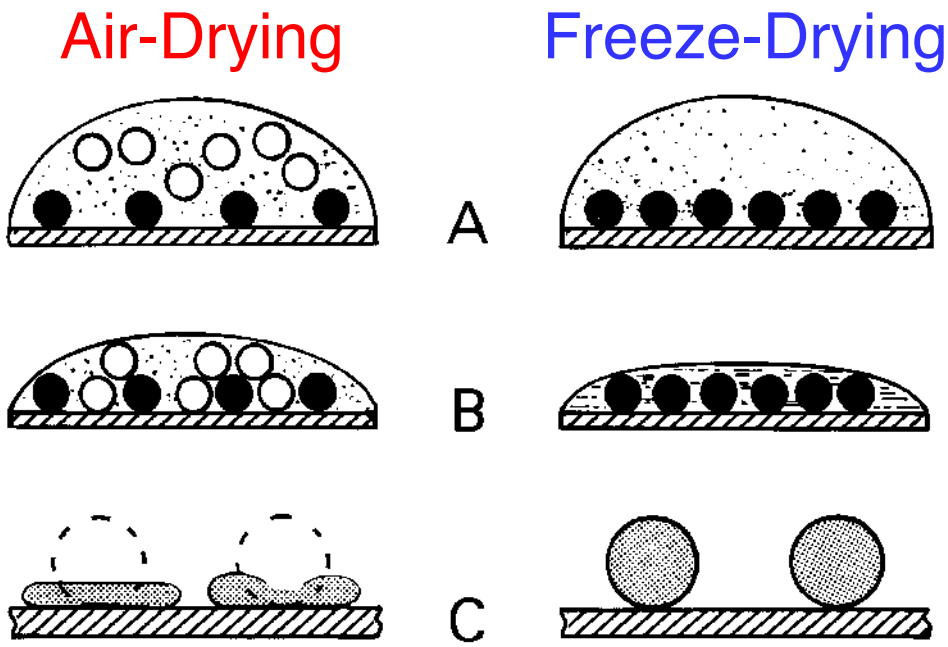
From Nermut, Fig. 2.1, p.80

II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.5 Freeze Drying/Etching/Fracture



A. Air-drying: adsorbed and unadsorbed particles present
   Freeze-drying: only adsorbed particles

B. Air-drying: unadsorbed particles aggregate / overlap

C. Air-drying: particles collapse (surface tension)

From Nermut, Fig. 2.1, p.80

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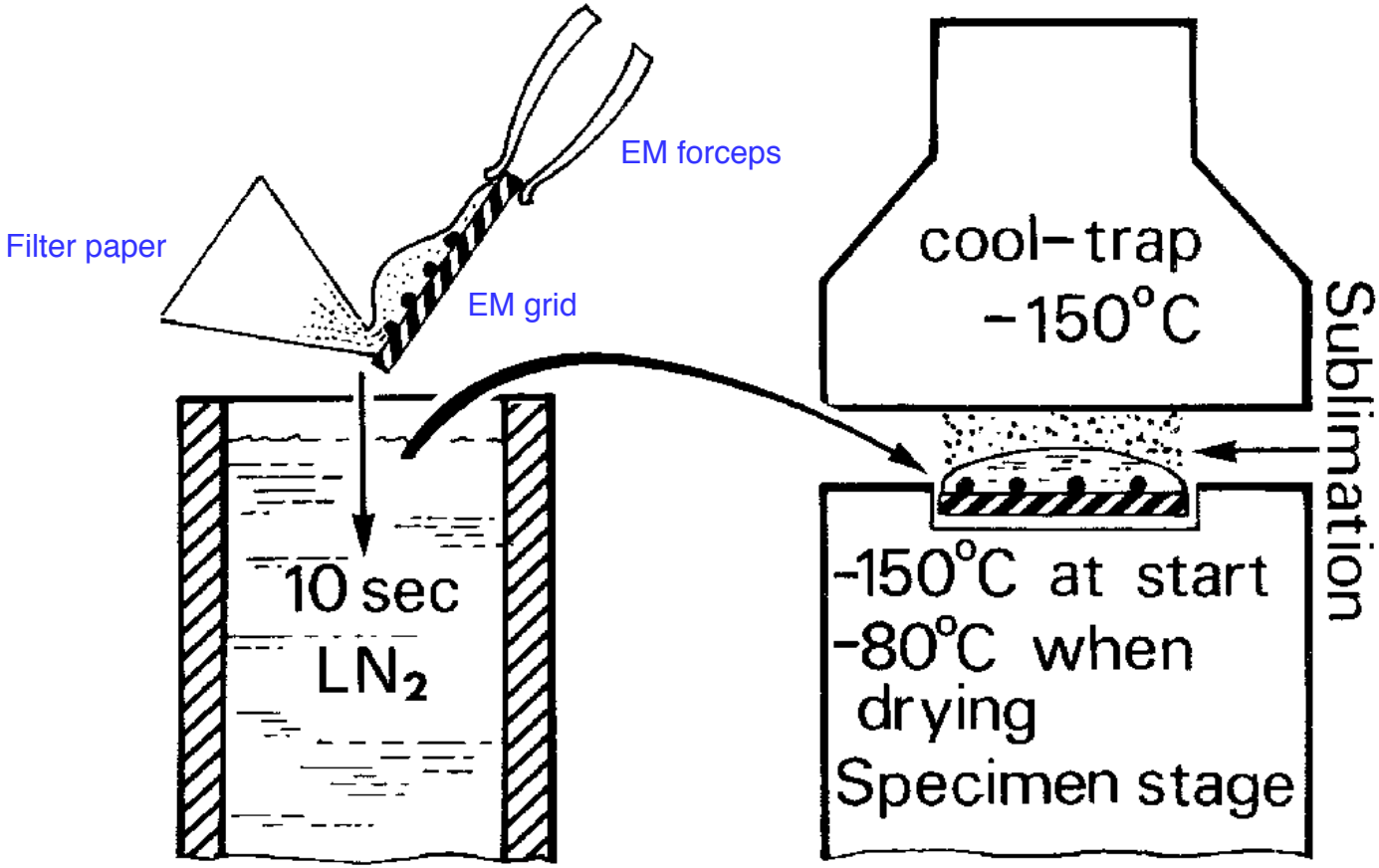
II.A BIOLOGICAL SPECIMEN PREPARATION TECHNIQUES

II.A.5 Freeze Drying/Etching/Fracture

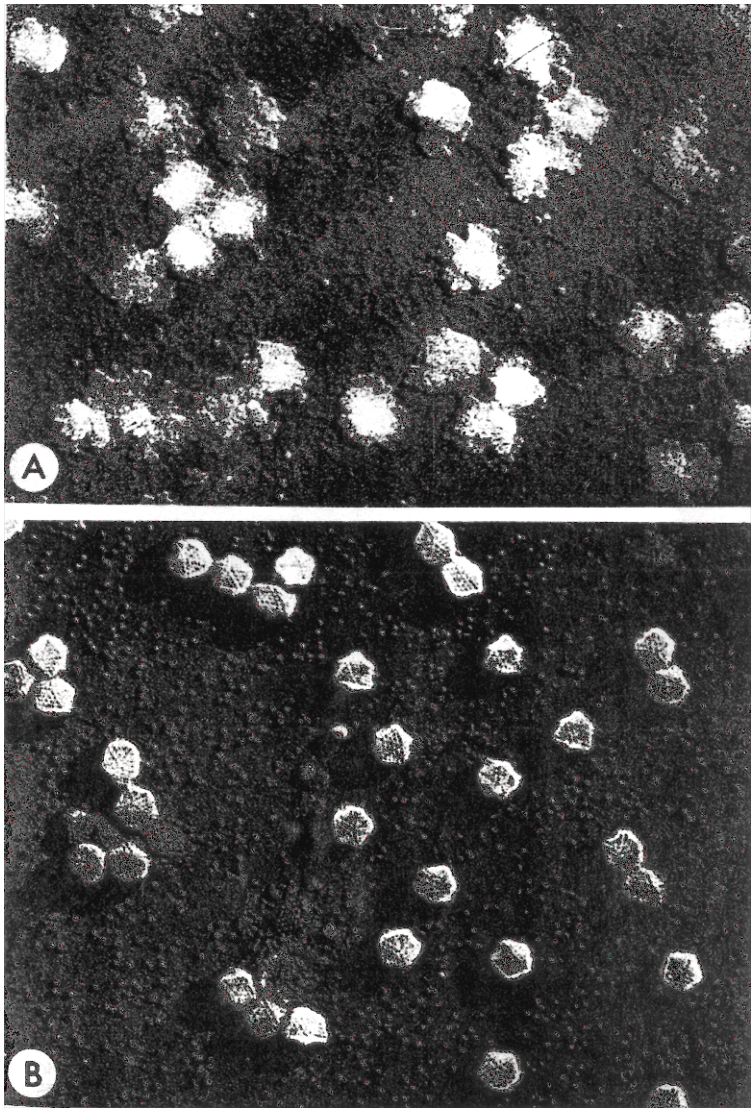


From Nermut, Fig. 2.5, p.87

Slide not shown in class lecture

II.A.5 Freeze Drying/Etching/Fracture

Adenovirus Type 5 Shadowed with Pt-C



Air-dried

Freeze-dried

From Nermut, Fig. 2.16, p.101

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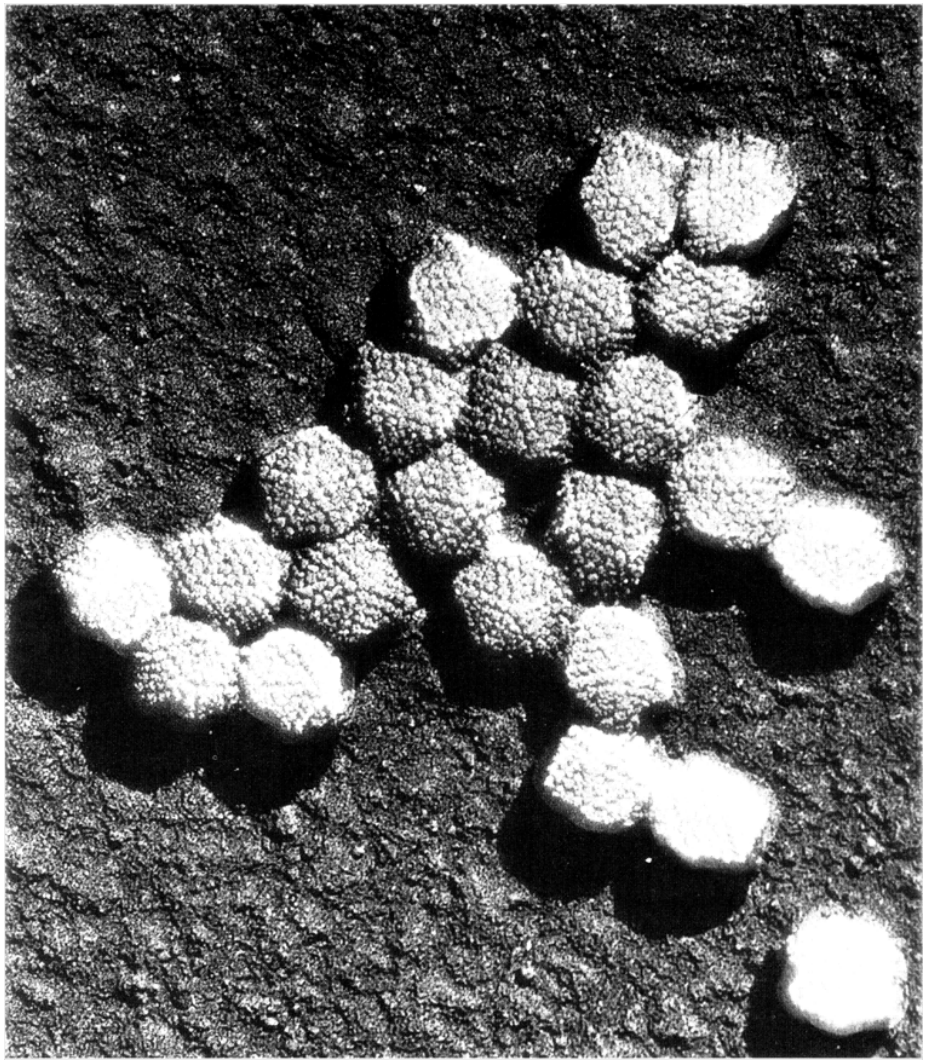
II.A.5 Freeze Drying/Etching/Fracture

Avian Adenovirus Freeze-Dried and Shadowed with Pt-C



From M. V. Nermut (1977) in *Princ. Tech. Elec. Microsc.* **7:102**

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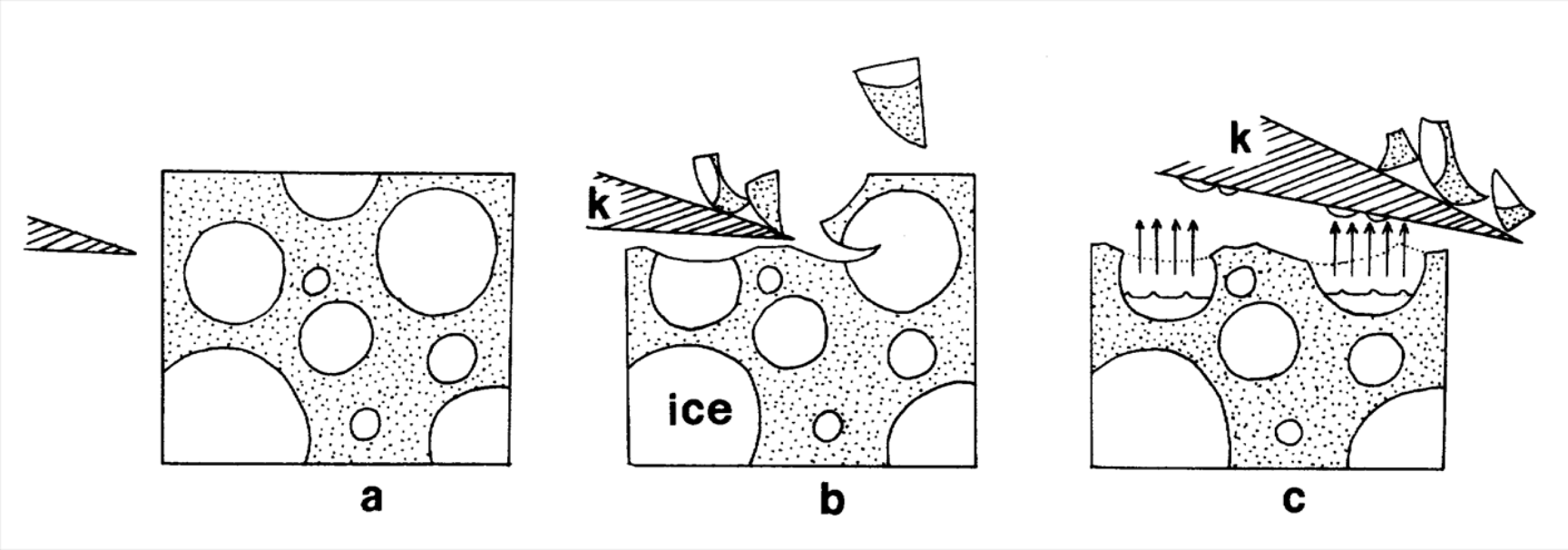
II.A.5 Freeze Drying/Etching/Fracture

Freeze-Fracture / Freeze-Etch



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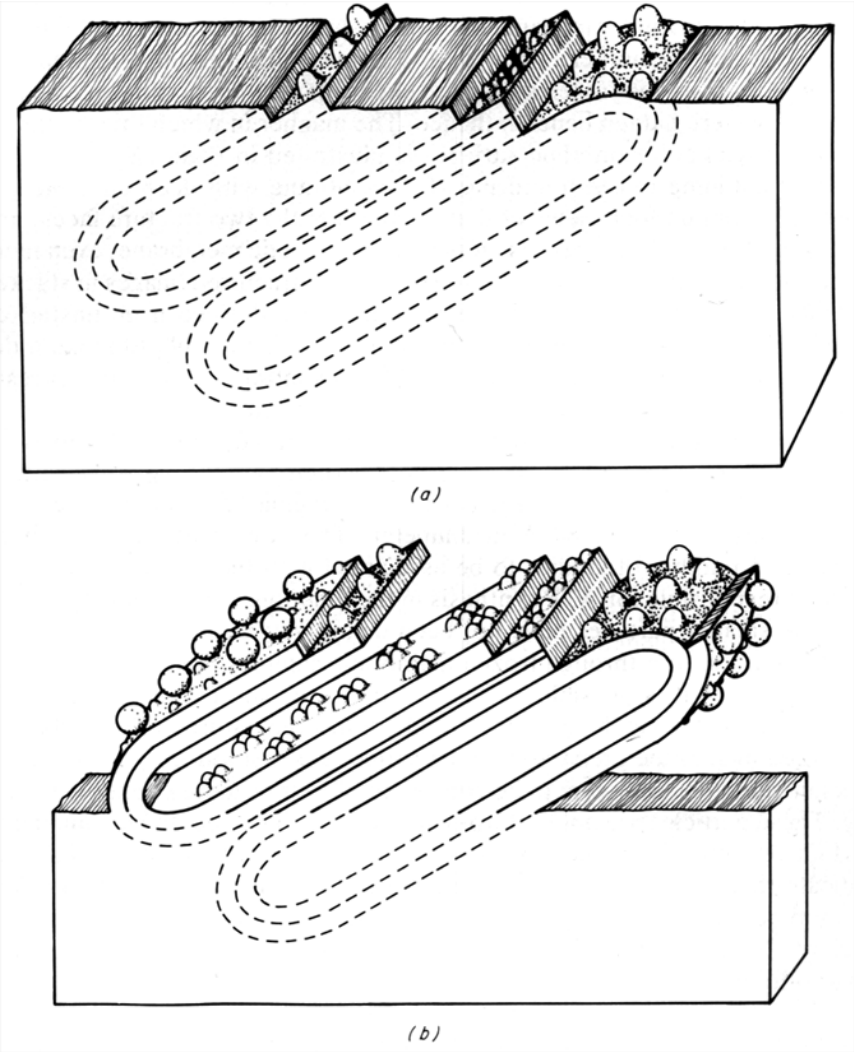
From Willison and Rowe, Fig. 7.30, p.216

II.A.5 Freeze Drying/Etching/Fracture

Frozen samples often fracture near membranes

Fractured frozen thylakoid membranes

Deep etching exposes surfaces previously covered in ice.



From K. R. Miller (1977) in *Princ. Tech. Elec. Microsc.* **7**

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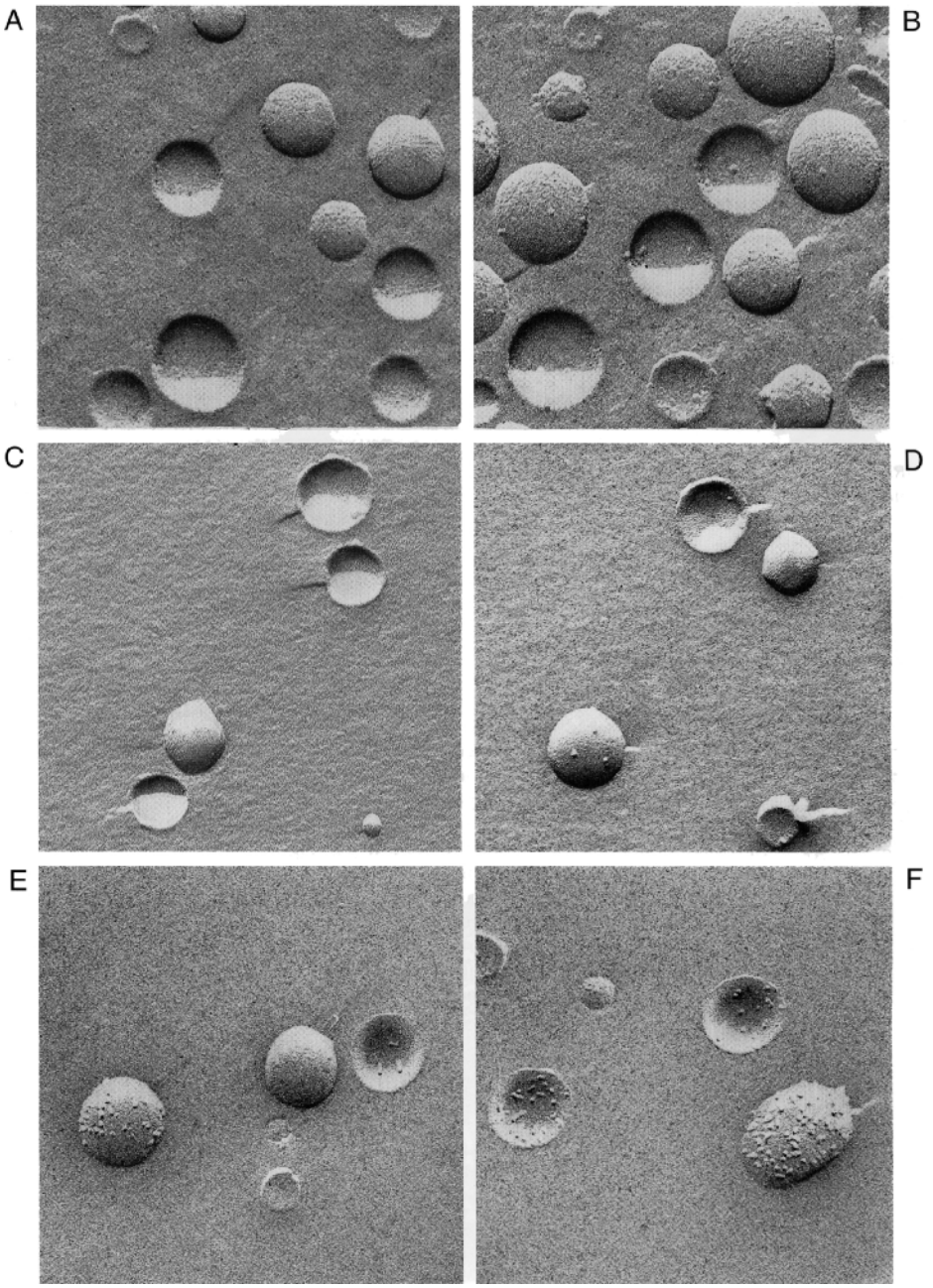
Freeze-Fracture Replica

A) Pure liposomes

B) Liposomes with Na,K-ATPase

C) Liposomes

D-F) Liposomes as in (C) with increasing [aquaporin]

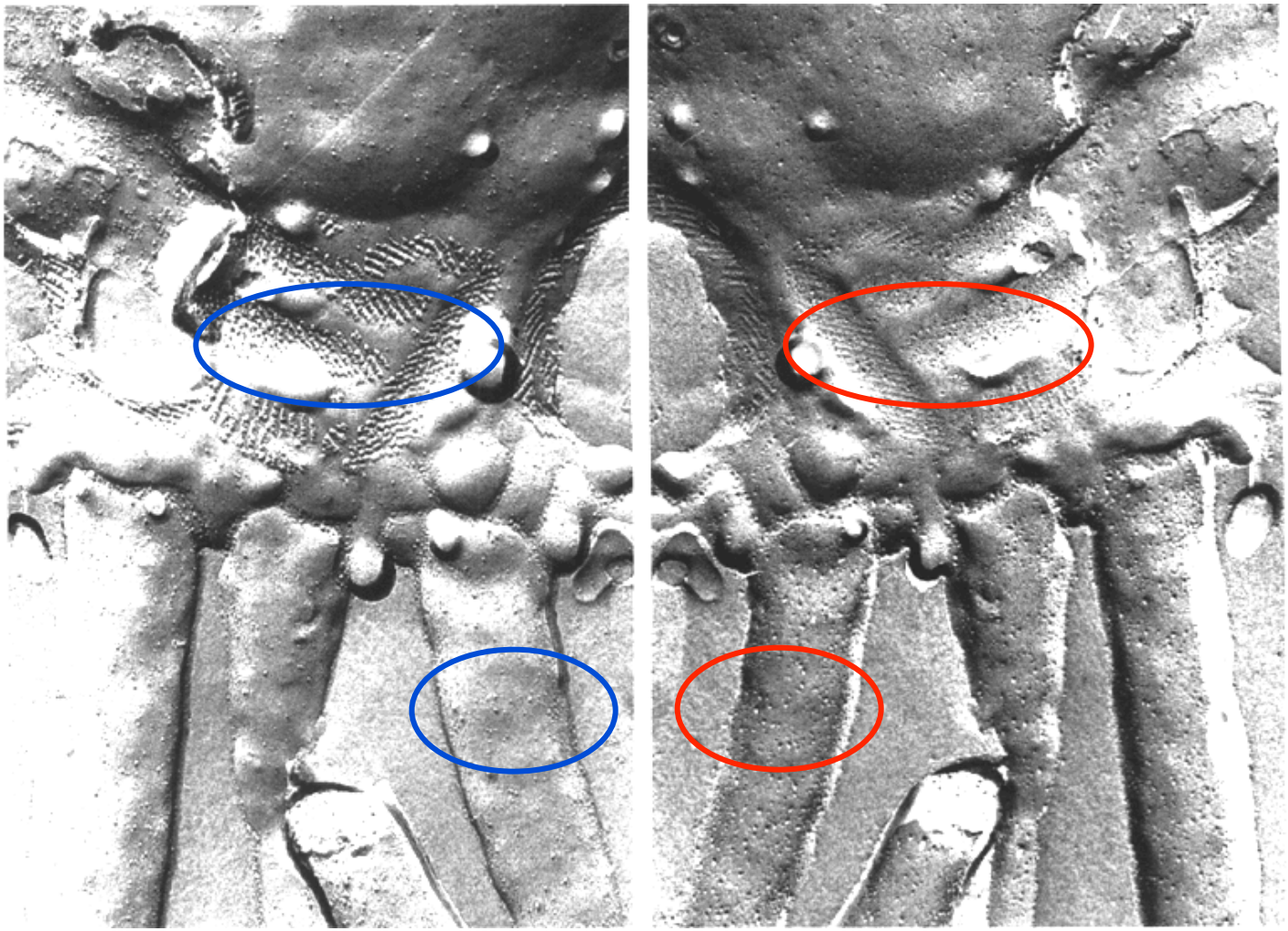


From Maunsbach & Afzelius in *Biomedical Elec. Micros.* (1999) Fig.17.10, p. 447

Slide not shown in class lecture

Complimentary Freeze-Fracture Replicas

Apyrene Snail Spermatozoon



From Maunsbach & Afzelius in *Biomedical Elec. Micros.* (1999) Fig.17.7, p. 441

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§ II: The Specimen

II.A. Biological Specimen Preparation Techniques

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- II.A.4 Metal Shadowing
- II.A.5 Freeze Drying/Etching/Fracture (183-187)
- II.A.6 Unstained and Frozen-Hydrated