Lecture Slides

Jan 22, 2013
 Depth of focus in the electron microscope is such that the final focused image appears the same (except for magnification and rotation changes) over a distance of meters.

A. True

B. False
Because the depth of **focus** in a TEM is so large (meters), the electron microscopist does not have to be too concerned about the precise focus setting of the objective lens.

A. True

B. False
Announcements for Jan 22, 2013

Reading assignment for Thursday: Lecture notes pp.104-end of § I.E

‘Virtual homework’: always check web site for new updates

Answer keys to ‘virtual’ homework problems will be posted on the white board outside NSB 4-105 (usually within 5-7 days AFTER the problems are first available on the course web site)

It is of course HIGHLY RECOMMENDED that you try to answer the questions BEFORE checking the white board !!!

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

TEM facility tour: NEXT WEEK Jan 28,29 (check web site)
TEM Facility Tour

Where: 1510 Bonner Hall basement

When: Mon Jan 28 and Tue Jan 29th

Check class web site for details on dates, times, and directions to facility

Attendance is optional but 5 pts extra credit towards final grade will be awarded

To reserve and guarantee a time slot, email nholson@ucsd.edu

First come, first served.
Class Web Page: Jan 18, 2013

CHEM 165,265 / BIMM 162 / BGGN 262 - 3D Electron Microscopy of Macromolecules
Winter Quarter 2013

Class Tour of the Electron Microscopy Facility
Bonner Hall 1501

Sign-up times:
Monday, January 28 -- 1 pm to 2 pm
Monday, January 28 -- 2 pm to 3 pm
Monday, January 28 -- 3 pm to 4 pm
Tuesday, January 29 -- 10 am to 11 am
Tuesday, January 29 -- 11 am to noon
Tuesday, January 29 -- 1 pm to 2 pm
Tuesday, January 29 -- 2 pm to 3 pm
Tuesday, January 29 -- 3 pm to 4 pm

To reserve a demo session write: Norm Olson
(Only four participants allowed per session - first come-first served)
(Keep an eye on this page: Closed sessions will be in red slanted text)

Directions to the EM Facility

Syllabus (PDF)
Book list (PDF)
Reference list (PDF)

The Bottom Line (PDF) – Key concepts from daily lectures through January 17, 2013

Virtual Homework - Practice Questions for Section 1 (PDF) Updated January 17, 2013 (Password protected)

Lecture Notes
- Sec. IA, Principles of the transmission electron microscope (5.2 MB)
- Sec. IB, Design of the transmission electron microscope (7.8 MB)
- Sec. IC, Contrast and image formation (.5MB)
- Sec. ID, Alignment/adjustment of the microscope (9.6 MB)
- Sec. IE, Operation of the transmission electron microscope (10.1 MB)

Powerpoint® presentations from lecture (PDFs)
- Introduction to the course - January 8, 2013 (48 MB)
- Lecture #1, January 8, 2013 (15.5 MB)
- Lecture #2, January 10, 2013 (4.3 MB)
- Lecture #3, January 15, 2013 (20.8 MB)
- Lecture #4, January 17, 2013 (9.8 MB)
Class Web Page: Jan 16, 2013

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Directions to the EM Facility
I.B DESIGN OF THE TEM

KEY CONCEPTS FROM LECTURE #4

- **Depth of focus** ($D_i$): distance along the optical axis where the IMAGE appears essentially the same.

- **Depth of field** ($D_o$): thickness in the OBJECT that appears to be essentially at the same level of focus at a fixed image plane.

Consequence of large relative depth of field in the TEM:

Images are (to a first approximation) PROJECTIONS of the entire contents of a specimen.
- Resolution in electron images of biological specimens normally limited by contrast [and radiation damage, but we’ll get to that later...], NOT lack of resolving power of the TEM

- Biological specimens have low inherent contrast

- Electron beam scatters from specimen atoms

- Primary types of electron-specimen scatter: Elastic, Inelastic, None

  Elastic scatter: No energy loss (no change in velocity or $\lambda$)

  $\theta_n = \frac{Ze}{Vr_n}$

  Inelastic scatter: ~10-20 eV energy loss (velocity $\downarrow$ $\lambda$ $\uparrow$)

  $\theta_e = \frac{e}{Vr_e}$

  No scatter: No energy loss (no change in velocity or $\lambda$)
- **Aperture/scattering contrast**: loss of amplitude (*i.e.* electrons) from the imaging beam (*particle* nature of electrons)

- **Interference/phase contrast**: originates from shifts in relative phases of portions of imaging beam that combine and contribute to the image (*wave* nature of electrons)
SCATTERING (APERTURE/AMPLITUDE) CONTRAST

Primary source of electron contrast for most biological specimens prepared for TEM using “conventional” methods (i.e. those stained or shadowed with heavy atoms to increase mass thickness)

INTERFERENCE (PHASE) CONTRAST

Arises primarily from two factors:
- **Defocusing** the objective lens
- **Spherical aberration** in the objective lens

Importance of interference contrast increases as the **limit of resolution** in the TEM is approached, and for **small / thin** structures

**Dominant source of contrast** for very small or thin objects of low atomic number
I.C.2.a Scattering (Aperture) Contrast

Aperture contrast controlled to some extent by:

- Choice of accelerating voltage (contrast ↑ as V ↓)

\[ \theta_e = \frac{Ze}{Vr} \]

- Size of objective aperture (contrast ↑ as aperture size ↓)

\[ \theta_o = \frac{e}{Vr} \]
I.C.2.a Scattering (Aperture) Contrast

Aperture contrast controlled to some extent by:

- Choice of accelerating voltage (contrast ↑ as V ↓)

\[ \theta = \frac{Ze}{V_r} \]

- Size of objective aperture (contrast ↑ as aperture size ↓)

\[ \theta = \frac{e}{V_r} \]
I.C.2.a Scattering (Aperture) Contrast

Aperture contrast controlled to some extent by:

- Choice of accelerating voltage (contrast ↑ as V ↓)

- Size of objective aperture (contrast ↑ as aperture size ↓)

Warning:

- Lower V leads to higher chromatic aberration and loss of resolution (esp. for thicker specimens)
Objective Aperture Affects Scattering Contrast

OA placed at **back focal plane** of objective lens

From Agar 2nd ed., Fig. 1.21, p.27
As aperture size is reduced, more scattered electrons are stopped and scattering/aperture contrast improves.

***HOWEVER***

Very small objective apertures (< 20 μm) can limit resolution owing to diffraction effects.

(RECALL: object points are imaged as Airy disks)
I.C.2.a Scattering (Aperture) Contrast

Objective Aperture Affects Scattering Contrast

Practical problems with small objective apertures:

- **Difficult to keep centered exactly** on the optical axis at the back focal plane of the objective lens

- **Susceptible to the effects of contamination** (produces lens asymmetry and astigmatism, thereby reducing resolution)
I.C.2.b Interference (Phase) Contrast

Generated when diffracted and undiffracted waves interfere, and results in intensity variations in the electron image

Also recall:

Electrons scattered through large angles are stopped by the OA and never reach the image plane (origin of aperture contrast)

**IDEALLY**, **ALL other** scattered electrons are focused by a lens at corresponding image points, and arrive there **in phase**

**But what really happens at various image planes?**

**HINT:** Think “Fresnel”
Defocus or phase contrast appears as Fresnel fringes in the specimen (including the support film)

Fringes are especially noticeable wherever there are abrupt changes in mass thickness (keyhole is a very extreme example)
Interference contrast:

Arises from phase differences, and therefore interference between scattered and unscattered electron WAVES in different parts of the image.

Defocusing of objective lens:

- Causes path lengths of scattered rays to change more than for the unscattered rays.
- Enhances phase contrast due to changes in interference.
I.C.2.b Interference (Phase) Contrast

A few facts:

Fresnel fringes *seem to disappear* at “exact” (“near” or “true”) focus because *phase differences are at a minimum*.

Background granularity seen in images of EM support films is simply caused by phase contrast

- *Coarseness of granularity varies with level of defocus*
I.C.2.b Interference (Phase) Contrast

A few facts:

Fresnel fringes seem to disappear at “exact” (“near” or “true”) focus because phase differences are at a minimum.

Background granularity seen in images of EM support films is simply caused by phase contrast:

- Coarseness of granularity varies with level of defocus.
- Granularity must be considered when interpreting high resolution details in images of specimens that sit on top of a support film.

NOTE: Interference contrast is more important than aperture contrast in images of thin or small objects and when working to achieve the highest resolution limit.
I.C.2.b Interference (Phase) Contrast

Practical Tip

Defocus (under focus) the objective lens slightly to enhance interference contrast in the electron image.

Image resolution is not significantly reduced if amount of underfocus is not excessive.

“Goldilocks Rule”
I.C.2.b Interference (Phase) Contrast

BOTTOM LINE:

Image phase contrast varies as function of objective lens defocus

From Meek, 2nd ed., Fig. 5.3, p.100
Unfortunately, as you all ought to be painfully aware by now, TEM images do **NOT** give a completely faithful, direct rendering of the specimen density distribution (i.e. structure)
Unfortunately, as you all ought to be painfully aware by now, TEM images do **NOT** give a completely faithful, direct rendering of the specimen density distribution (i.e. structure).

- Relationship between image and specimen is described by the **contrast transfer function (CTF)**, which is influenced by:
  
  **Specific TEM used**
  **Conditions of imaging**
  **Specimen**

- **Microscope** CTF arises from the objective lens focal setting **AND** from the **spherical aberration** in the lens.
I.C.3 Contrast Transfer Function

Dependence of CTF on resolution, wavelength, defocus, and spherical aberration is given by:

$$ CTF(\nu) = -\left\{ \left(1 - F_{\text{amp}}^2\right)^{\frac{1}{2}} \cdot \sin(\chi(\nu)) + F_{\text{amp}} \cdot \cos(\chi(\nu)) \right\} $$

where

$$ \chi(\nu) = \pi \lambda \nu^2 \left( \Delta f - 0.5C_s \lambda^2 \nu^2 \right) $$

- $\nu$ = spatial frequency (in Å$^{-1}$)
- $F_{\text{amp}}$ = fraction of amplitude contrast
- $\lambda$ = electron wavelength (in Å), where $\lambda = 12.3/\sqrt{V + 0.000000978 \cdot V^2}$ (≈ 0.037, 0.025, and 0.020Å for 100, 200, and 300 keV electrons, respectively)
- $V$ = voltage (in volts)
- $\Delta f$ = underfocus (in Å)
- $C_s$ = spherical aberration coefficient of objective lens of microscope (in Å)

That certainly makes it all look simple and straightforward...
I.C.3 Contrast Transfer Function

Dependence of CTF on resolution, wavelength, defocus, and spherical aberration is given by:

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\( V \) = voltage (in volts)
\( \Delta f \) = underfocus (in Å)
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That certainly makes it all look simple and straightforward...
I.C.3 Phase Contrast Transfer Function

Plot of phase contrast as a function of structure size. Objective lens in focus at top and progressively further underfocus in lower panels.

\( \Delta f = 0 \text{ nm} \)

\( \Delta f = -78 \text{ nm} \)

\( \Delta f = -234 \text{ nm} \)

From Agar, Fig 8.3, p. 282
Virtual TEM

What does the scope CTF do to images?
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

“Real Image”
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

Object
("Perfect Image")
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

-2.0 µm
underfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

-1.5 μm
underfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

-1.0 µm
underfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

-0.5 µm
underfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

-0.25 \( \mu \text{m} \)
underfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

-0.1 μm
underfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

-0.0 \mu m
near focus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

+0.1 µm
overfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

+0.25 μm
overfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

+0.5 µm
overfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

+1.0 µm
overfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

+1.5 μm
overfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

+2.0 µm
overfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

-0.1 μm underfocus

-2.0 μm underfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

+0.1 µm overfocus

+2.0 µm overfocus
Virtual TEM

What does the CTF function look like at different defocus settings?
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

Contrast

Spatial Frequency

-2.0 μm underfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

Contrast

Spatial Frequency

-1.0 µm

underfocus
Simulation of Electron Imaging in a TEM

Contrast Transfer Function

Contrast

Spatial Frequency

-0.5 µm underfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

Contrast vs. Spatial Frequency

-0.25 μm underfocus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM

Contrast

Spatial Frequency

-0.0 µm near focus
I.C.3 Contrast Transfer Function

Simulation of Electron Imaging in a TEM
I.C CONTRAST AND IMAGE FORMATION

I.C.5 Other Methods for Enhancing Contrast

1. Directly \textit{increase} “inherent” specimen contrast using various preparation procedures (\textit{i.e.} staining, shadowing, etc.)

\textbf{Discussed in \S II}

2. Record images with \textit{longer} exposure times

\textbf{Discussed in \S I.E}
§ 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

(pp.81-89 of lecture notes)
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.D Alignment/Adjustment of the TEM

I.D.1 Introduction
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

What’s the Bottom Line?

Misalignment of TEM causes:

Loss of resolving power

Inconveniences in operation

- Center of field of view moves when magnification is changed
- Beam illumination becomes uneven or moves off-center as C2 is changed
- Image moves across screen while focusing
- Loss of illumination can occur if switch from one mode of operation to another (e.g. from normal imaging to electron diffraction mode and back)
- Etc. etc. etc.
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

Goal of Alignment Procedure

Arrange all optical elements of microscope to be coaxial
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

Goal of Alignment Procedure

Arrange all optical elements of microscope to be **coaxial**
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

**Goal of Alignment Procedure**

Arrange all optical elements of microscope to be **coaxial**
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

*Goal of Alignment Procedure*

Arrange all optical elements of microscope to be *coaxial*
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

Goal of Alignment Procedure

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I.D ALIGNMENT/ADJUSTMENT OF THE TEM

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I.D ALIGNMENT/ADJUSTMENT OF THE TEM

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I.D ALIGNMENT/ADJUSTMENT OF THE TEM

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I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

Goal of Alignment Procedure

Arrange all optical elements of microscope to be *coaxial*
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

Goal of Alignment Procedure

Arrange all optical elements of microscope to be **coaxial**
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

Goal of Alignment Procedure

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I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

**Goal of Alignment Procedure**

Arrange all optical elements of microscope to be *coaxial*
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

Goal of Alignment Procedure

Arrange all optical elements of microscope to be \textit{coaxial}
I.D.1 Introduction

**Goal of Alignment Procedure**

Arrange all optical elements of microscope to be **coaxial**

Imagine (*i.e. I can’t think of a good way to illustrate this*) what the final image formed by the last (*i.e. magenta*) lens would look like if one or more lenses were misaligned!
Arrange all optical elements of microscope to be **coaxial**

Imagine (*i.e.* I can’t think of a good way to illustrate this) what the final image formed by the last (*i.e.* magenta) lens would look like if one or more lenses were misaligned!
I.D.1 Introduction

Lens Misalignment
I.D.1 Introduction

Lens Misalignment
I.D.1 Introduction

Lens Misalignment
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

Optic Axis

Defined by two points: **objective lens center** and **viewing screen center**
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.1 Introduction

Optic Axis
Defined by two points: objective lens center and viewing screen center

- Axis of symmetry of every lens must exactly coincide with optic axis
- Alignment performed with respect to electron gun, condenser, and imaging lenses

RESULT: optical center of image coincides with center of viewing screen
§ I: The Microscope

I.D Alignment/Adjustment of the TEM

I.D.1 Introduction

I.D.2 Alignment of TEM Components
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.2 Alignment of TEM Components

**Bottom Line:**

*The devil is ALWAYS in the details!*

- Concentrate on understanding the *basic* principles as presented in your notes

- Detailed TEM column alignment procedures differ for each microscope
I.D.2 Alignment of TEM Components

I.D.2.b Condenser System

Simulation of Aligned Condenser System

TEM fluorescent viewing screen
I.D.2 Alignment of TEM Components

I.D.2.b Condenser System

Simulation #1 of Misaligned Condenser System
(Tilt and translation off)
I.D.2 Alignment of TEM Components

I.D.2.b Condenser System

Simulation #2 of Misaligned Condenser System
(Tilt aligned; Translation off)
Astigmatism in the 2nd condenser lens leads to elongated, asymmetrical spot shape on either side of focus

From Agar, Fig. 4.6, p.129
I.D.2 Alignment of TEM Components

I.D.2.b Condenser System

Simulation of **Astigmatic** Condenser System
I.D.2 Alignment of TEM Components

I.D.2.b Condenser System

Simulation of Astigmatic Condenser System
I.D.2 Alignment of TEM Components

I.D.2.b Condenser System

Simulation of Astigmatic Condenser System
Simulation of *Astigmatic* Condenser System
I.D.2 Alignment of TEM Components

I.D.2.b Condenser System

Simulation of Astigmatic Condenser System
I.D.2 Alignment of TEM Components

I.D.2.b Condenser System

Simulation of Astigmatic Condenser System
I.D.2 Alignment of TEM Components

I.D.2.b Condenser System

Simulation of *Astigmatic* Condenser System
Simulation of *Astigmatic* Condenser System
I.D.2 Alignment of TEM Components

I.D.2.b Condenser System

Simulation of Astigmatic Condenser System
I.D.2 Alignment of TEM Components

I.D.2.c Imaging System

Alignment procedure:

Make axes of all imaging lenses coincide with instrument mechanical (optical) axis

When all imaging lenses are in line:

Object point on the objective lens axis will be imaged at the viewing screen center WHATEVER the magnification setting
I.D.2 Alignment of TEM Components

I.D.2.c Imaging System

Voltage Center

Effects of misalignment and high voltage ripple:

Ideal, aligned image (no high voltage fluctuations)  Aligned, but with voltage fluctuations  Misalignment and voltage fluctuations
Fluctuations in lens currents also give rise to superimposed images of different magnifications and hence blurring.

Typical alignment scheme uses current variations (much easier to vary than voltage)
I.D.2 Alignment of TEM Components

I.D.2.c Imaging System

Current Center

Normal image: No lens current instabilities

Image rotation caused by varying ("wobbling") the objective lens current. Rotation center marked by arrow identifies the optic axis of the lens

From Bozzola, 1st Ed., Fig. 6-45, p.171
§ I: The Microscope

I.D Alignment/Adjustment of the TEM

I.D.1 Introduction
I.D.2 Alignment of TEM Components
I.D.3 Disturbances to Microscope Performance

(pp.86-89 of lecture notes)
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.3 Disturbances to Microscope Performance

a. Contamination

b. Image drift and mechanical instabilities

c. Electrical and magnetic instabilities

d. Image astigmatism

e. Focal drift
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.3 Disturbances to Microscope Performance

a. Contamination

b. Image drift and mechanical instabilities

c. Electrical and magnetic instabilities

d. Image astigmatism

e. Focal drift
I.D.3 Disturbances to Microscope Performance
   I.D.3.a Contamination

Contamination of TEM column, apertures, and specimen lead to:
   - Astigmatism
   - Drift
   - Decreased contrast

Deposition of a uniform layer of contamination on specimen reduces its contrast

Contamination layer obscures fine details in specimen structures
I.D.3 Disturbances to Microscope Performance

I.D.3.a Contamination

Latex Spheres (0.088 µm diameter)

< 1 min.  ~25 min.  ~50 min.

From Maunsbach, Fig. 10-19, p.267
I.D.3 Disturbances to Microscope Performance

I.D.3.a Contamination

**Latex Spheres (0.088 µm diameter)**

From Maunsbach, Fig. 10-19A, p.267
I.D.3 Disturbances to Microscope Performance

I.D.3.a Contamination

Latex Spheres (0.088 \(\mu\)m diameter)

\(~25\) min.

From Maunsbach, Fig. 10-19B, p.267
I.D.3 Disturbances to Microscope Performance
I.D.3.a Contamination

Latex Spheres (0.088 µm diameter)

~50 min.

From Maunsbach, Fig. 10-19C, p.267
I.D.3 Disturbances to Microscope Performance

I.D.3.a Contamination

Effect of contamination on a thin carbon film

Two contaminated areas, each irradiated for 3 min.

From Meek, 1st Ed., Fig. 6.6, p.150
I.D.3 Disturbances to Microscope Performance

I.D.3.a Contamination

Two simple solutions:

1. Keep your grimy hands off parts exposed to vacuum!!
2. Use an anticontaminator

Cooled surface placed close to the specimen preferentially condenses potential contaminants (*i.e.* residual gases) on its surface and protects the specimen.

Most anticontaminators are cooled with liquid nitrogen.

From www.gatan.com/holders/index.html
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.3 Disturbances to Microscope Performance

a. Contamination

b. **Image drift and mechanical instabilities**

c. Electrical and magnetic instabilities

d. Image astigmatism

e. Focal drift
I.D.3 Disturbances to Microscope Performance
I.D.3.b Image Drift and Mechanical Instabilities

**Important consideration:**
Exposure times of \( \sim 1-2 \text{ seconds} \) or more required for recording electron images on photographic film

**Consequence:**
Specimen *movements* of a few Å/sec can *limit resolution*

*Movements caused by instabilities in:*
- Specimen holder
- Stage assembly
- Specimen
I.D.3 Disturbances to Microscope Performance
I.D.3.b Image Drift and Mechanical Instabilities

Thermal Drift of Specimen

Measuring Drift Rate

- Record **double exposure** of any “simple” object

- Shows magnitude of specimen drift between exposures

From Agar, Fig. 5.7, p.154
Measuring Drift Rate

- Record **double exposure** of any “simple” object

- Shows magnitude of specimen drift between exposures

- Also allows measurement of TEM **contamination rate** (deposit of contamination layer shows up as a reduction in hole diameter)
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.3 Disturbances to Microscope Performance

a. Contamination

b. Image drift and mechanical instabilities

c. Electrical and magnetic instabilities
   TEM shielding from stray, external fields; TEM location

d. Image astigmatism

e. Focal drift
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.3 Disturbances to Microscope Performance

a. Contamination
b. Image drift and mechanical instabilities
c. Electrical and magnetic instabilities
d. Image astigmatism
e. Focal drift
I.D.3 Disturbances to Microscope Performance

I.D.3.d Image Astigmatism

Image astigmatism can, and often does limit the resolution achieved in TEM images.

Experienced microscopists can minimize astigmatism and therefore reduce the possibility that resolution will be limited by this.
I.D.3.d **Image Astigmatism**

**Correcting Astigmatism**
(Holey Film Method)

Image of small hole in carbon support film

- a. Astigmatic image with asymmetric Fresnel fringe
- b. Astigmatism corrector switched on, full strength, arbitrary direction
- c. Corrector oriented to oppose the astigmatism seen in (a)
- d. Corrector strength reduced to stigmathe the image (uniform fringe)

From Agar, Fig. 4.12, p.139
I.D.3.d  **Image Astigmatism**

**Correcting Astigmatism**  
(Holey Film Method)

Image of small hole in carbon support film

a. Astigmatic image with asymmetric Fresnel fringe

b. Astigmatism corrector switched on, full strength, arbitrary direction

c. Corrector oriented to oppose the astigmatism seen in (a)

d. Corrector strength reduced to stigmatize the image (uniform fringe)

From Agar, Fig. 4.12, p.139
I.D.3 Disturbances to Microscope Performance

I.D.3.d **Image Astigmatism**

**Correcting Astigmatism** (Holey Film Method)

Stigmated Image

Astigmatic Image

From Agar, Fig. 4.12, p.139
I.D.3 Disturbances to Microscope Performance

I.D.3.d Image Astigmatism

Correcting Astigmatism (Holey Film Method)
I.D.3 Disturbances to Microscope Performance
I.D.3.d Image Astigmatism

Correcting Astigmatism

Minimize phase contrast method
(for “experts”)

See pp.87-88 of lecture notes
I.D ALIGNMENT/ADJUSTMENT OF THE TEM

I.D.3 Disturbances to Microscope Performance

a. Contamination

b. Image drift and mechanical instabilities

c. Electrical and magnetic instabilities

d. Image astigmatism

e. Focal drift  (Small changes in focus occur while observing the image)

Likely cause: micro-discharges in the electron gun
§ 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

(pp.89-119 of lecture notes)
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

Have I learned ANYthing yet?
I.E OPERATION OF THE TEM

* Choice of accelerating voltage
  Choice of apertures
Specimen stage/holder
Choice of magnification
Focusing
Magnification calibration
Resolution tests
Image intensifiers/TV displays
Microscope maintenance
Photography (analog and digital)
§ I: The Microscope

I.E Operation of the TEM

I.E.1 Choice of Accelerating Voltage
I.E OPERATION OF THE TEM

I.E.1 Choice of Accelerating Voltage

Most **routine** biological microscopy: **100-120kV**

Most **high resolution** biological microscopy: **200-300kV**

Voltage affects many things.....
I.E.1 Choice of Accelerating Voltage

What happens as $V$ is increased?

1. **Specimen penetration increases**: thicker specimens can be studied

2. **Amplitude contrast decreases** (fewer elastically and inelastically scattered electrons stopped by objective aperture)

3. **Resolution limit** due to diffraction effects **improves** (electron $\lambda$ decreases)

   Abbe equation (diffraction limit): $d = \frac{0.612\lambda}{n \cdot \sin \alpha}$

4. **Inelastic scattering decreases**: thus chromatic aberration effects **decrease** (get better resolution) AND radiation damage **decreases** (longer specimen lifetime)

5. **Efficiency** of most photographic emulsions **decreases**

6. **Quality of image** capture by CCD **decreases**
I.E.1 Choice of Accelerating Voltage

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7. **Electron gun more sensitive** to vacuum quality - high voltage stability **decreases**

8. **Electron gun brightness increases**

9. **Efficiency** of screen phosphor **increases**
I.E.1 Choice of Accelerating Voltage

What happens as V is increased?

1. Specimen penetration increases: thicker specimens can be studied

2. Amplitude contrast decreases (fewer elastically and inelastically scattered electrons stopped by objective aperture)

3. Resolution limit due to diffraction effects improves (electron λ decreases)

   Abbe equation (diffraction limit): $d = \frac{0.612\lambda}{n \cdot \sin \alpha}$

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