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

# Lecture Slides

Jan 22, 2013

# *p-Flasher* Question

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

# *p-Flasher* Question

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

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

# 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)

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

# 3D Electron Microscopy of Macromolecules

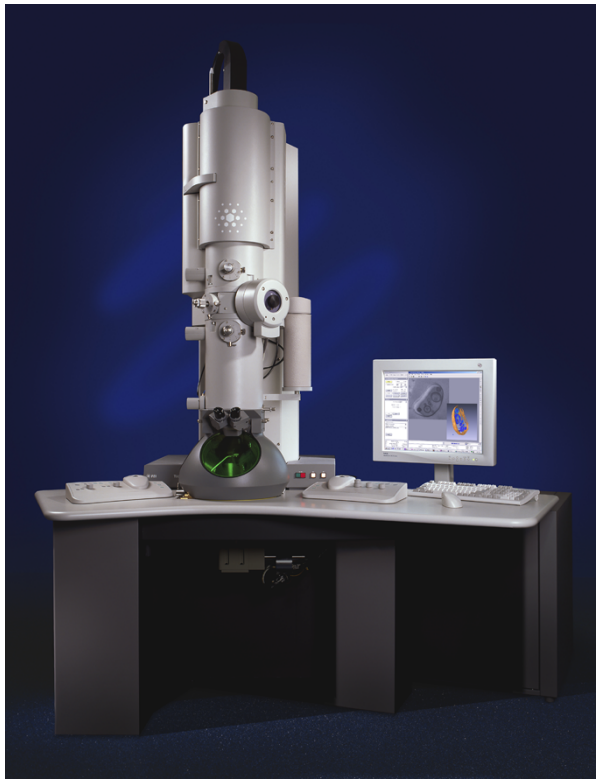
## TEM Facility Tour

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**



FEI Technai Sphera
(200keV; $LaB_6$; $LN_2$)

To reserve and guarantee a time slot, email **nholson@ucsd.edu**
**First come, first served.**

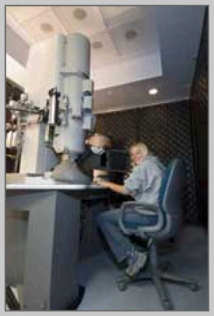
# Class Web Page: Jan 18, 2013

UCSD
UNIVERSITY OF CALIFORNIA, SAN DIEGO

**Dr. Timothy S. Baker**

Intro | Members | Courses | Images | Publications | Software | Documentation and Procedures | Microscope Access | Microscope Facilities

**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 strikeout~~ text)

Directions to the EM Facility

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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 I (PDF) Updated January 17, 2013 (Password protected)

Lecture Notes

- Sec. IA. Principles of the transmission electron microscope (9.2 MB)
- Sec. IB. Design of the transmission electron microscope (3.5 MB)
- Sec. IC. Contrast and image formation (692 KB)
- Sec. ID. Alignment/adjustment of the microscope (963 KB)
- 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 (9.9 MB)

# Class Web Page: Jan 16, 2013

UCSD
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**Dr. Timothy S. Baker**

Intro | Members | Courses | Images | Publications | Software | Documentation and Procedures | Microscope Access | Microscope Facilities

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

# I.C CONTRAST AND IMAGE FORMATION

## MORE CONCEPTS FROM LECTURE #4

- **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 λ) $\theta_n = \frac{Ze}{Vr_n}$

  **Inelastic scatter**: ~10-20 eV **energy loss** (velocity ↓ λ ↑) $\theta_e = \frac{e}{Vr_e}$

  **No scatter**: **No energy loss** (no change in velocity or λ)

# I.C CONTRAST AND IMAGE FORMATION

## MORE CONCEPTS FROM LECTURE #4

- **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)

# I.C CONTRAST AND IMAGE FORMATION

## 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:

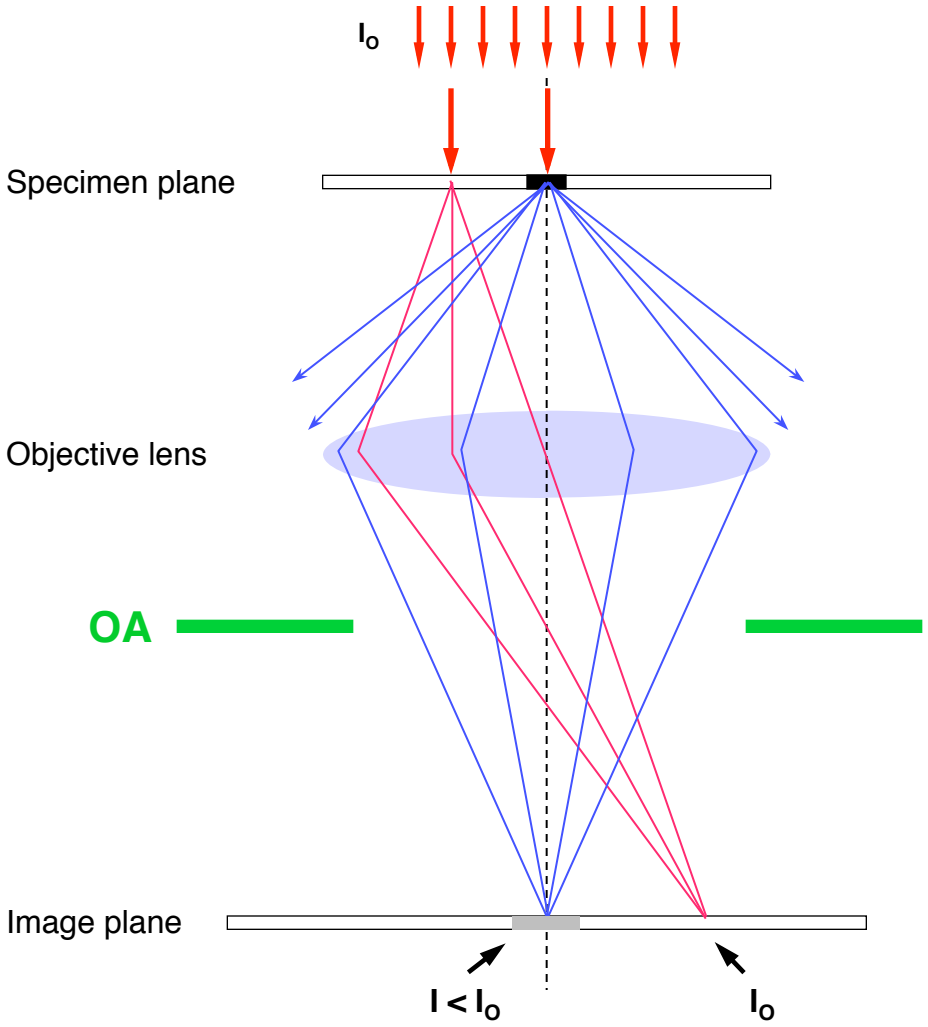
**Defocusing** the **objective lens**

**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:**

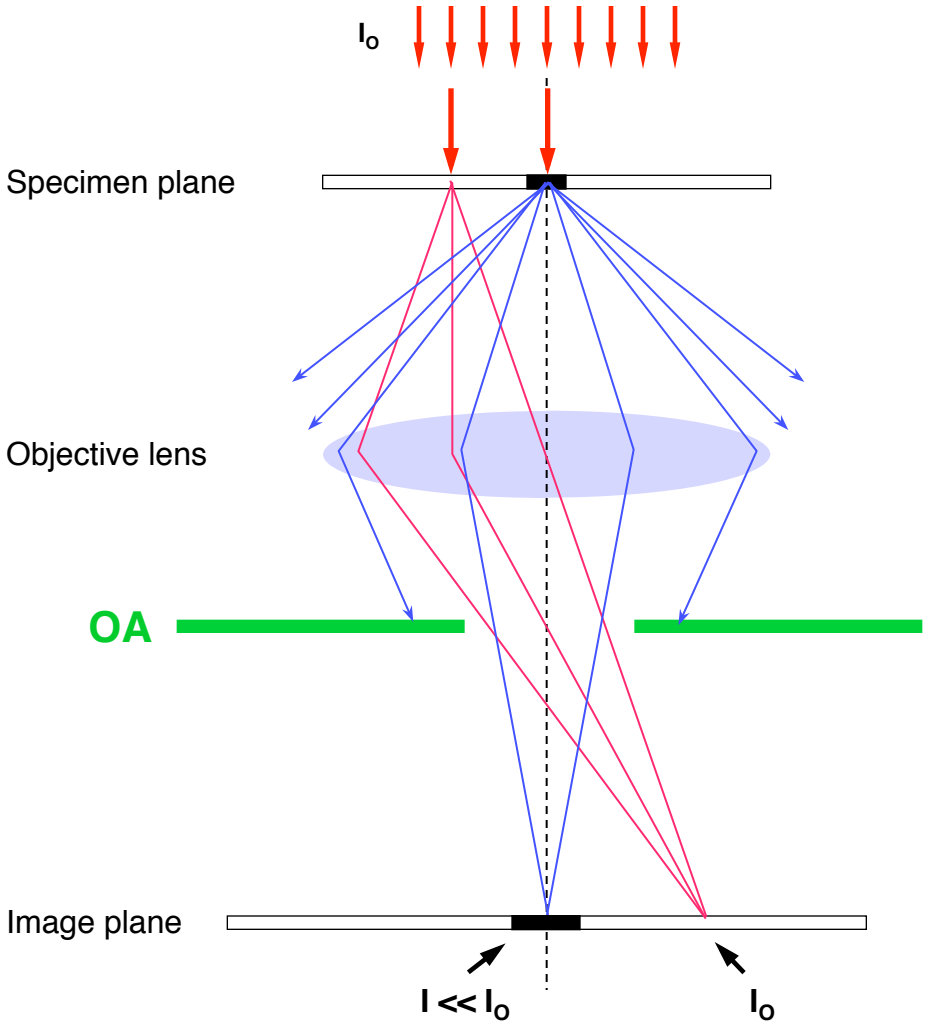
- Choice of accelerating voltage
  (contrast ↑ as V ↓)

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

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

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

# I.C.2.a Scattering (Aperture) Contrast

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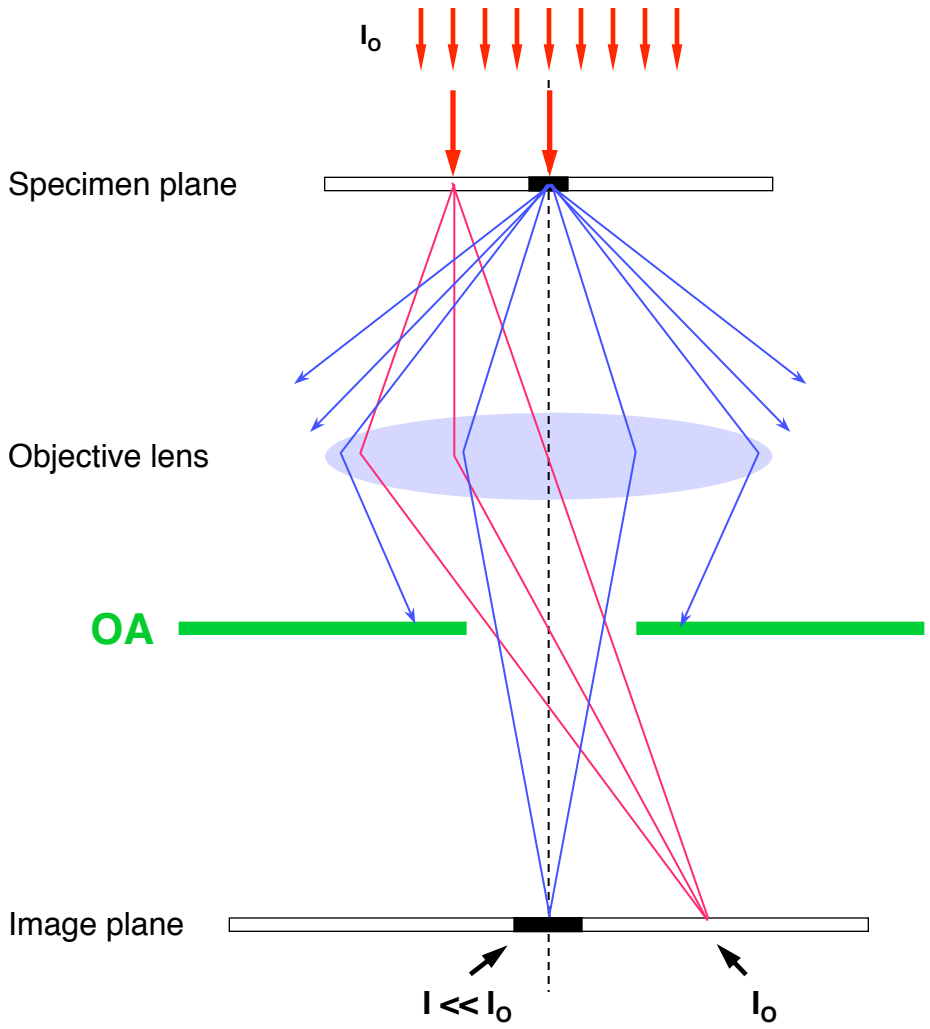
# I.C.2.a Scattering (Aperture) Contrast

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

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

## Warning:

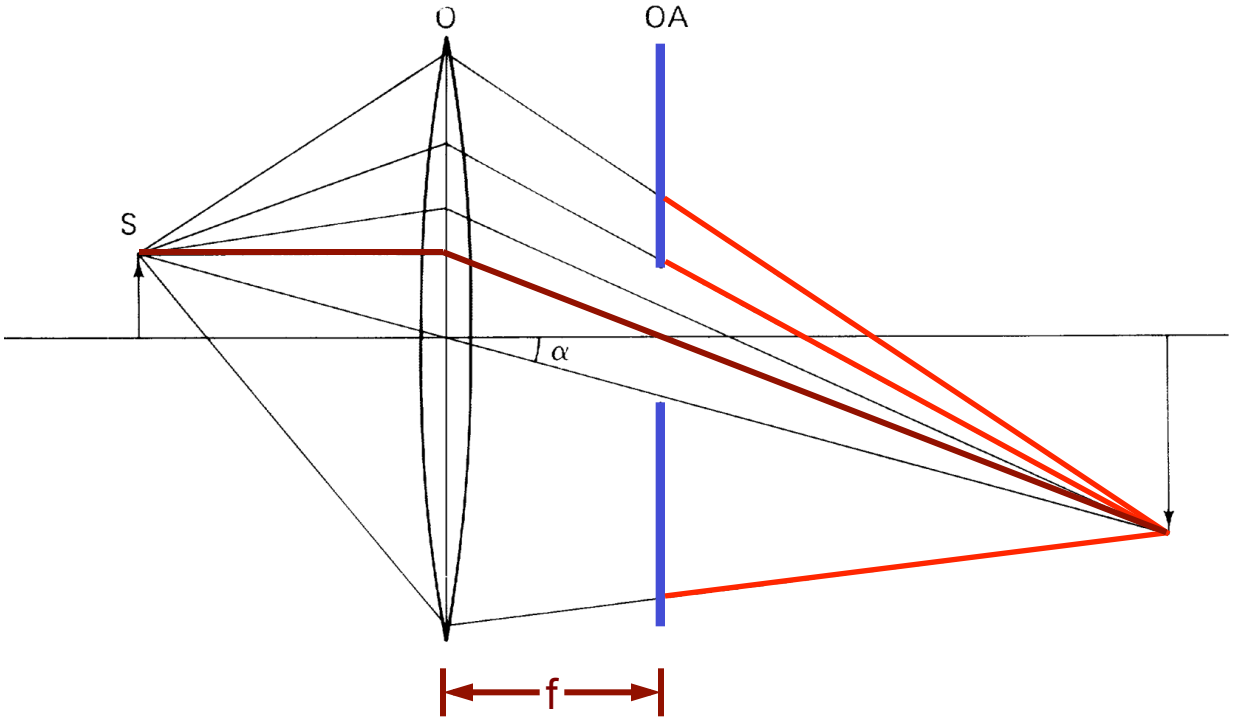
- Lower V leads to **higher chromatic aberration** and **loss of resolution** (esp. for thicker specimens)

Slide not shown in class lecture

# I.C.2.a Scattering (Aperture) Contrast

## Objective Aperture Affects Scattering Contrast

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




From Agar 2nd ed., Fig. 1.21, p.27

## I.C.2.a Scattering (Aperture) Contrast

### Objective Aperture Affects Scattering Contrast

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:

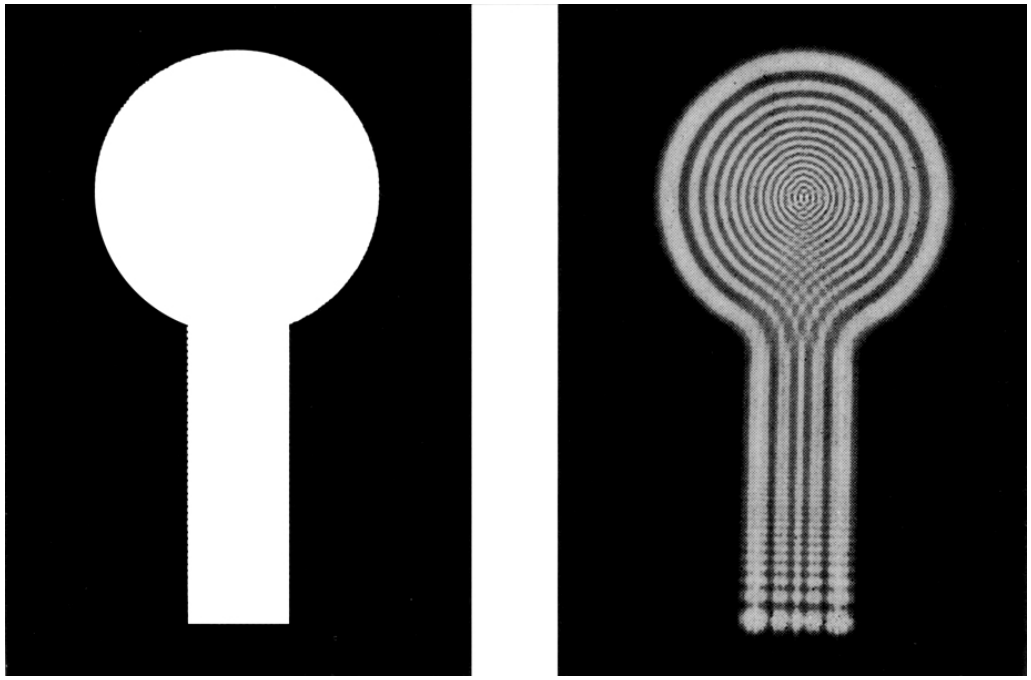
Electrons scattered through large angles are stopped by the OA and never reach the image plane (origin of aperture contrast)

***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"*

# I.C.2.b Interference (Phase) Contrast



From Young., Fig. 3-24, p.95

**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)

# I.C.2.b Interference (Phase) Contrast

## 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**

Slide not shown in class lecture

## I.C.2.b Interference (Phase) Contrast

# Practical Tip

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

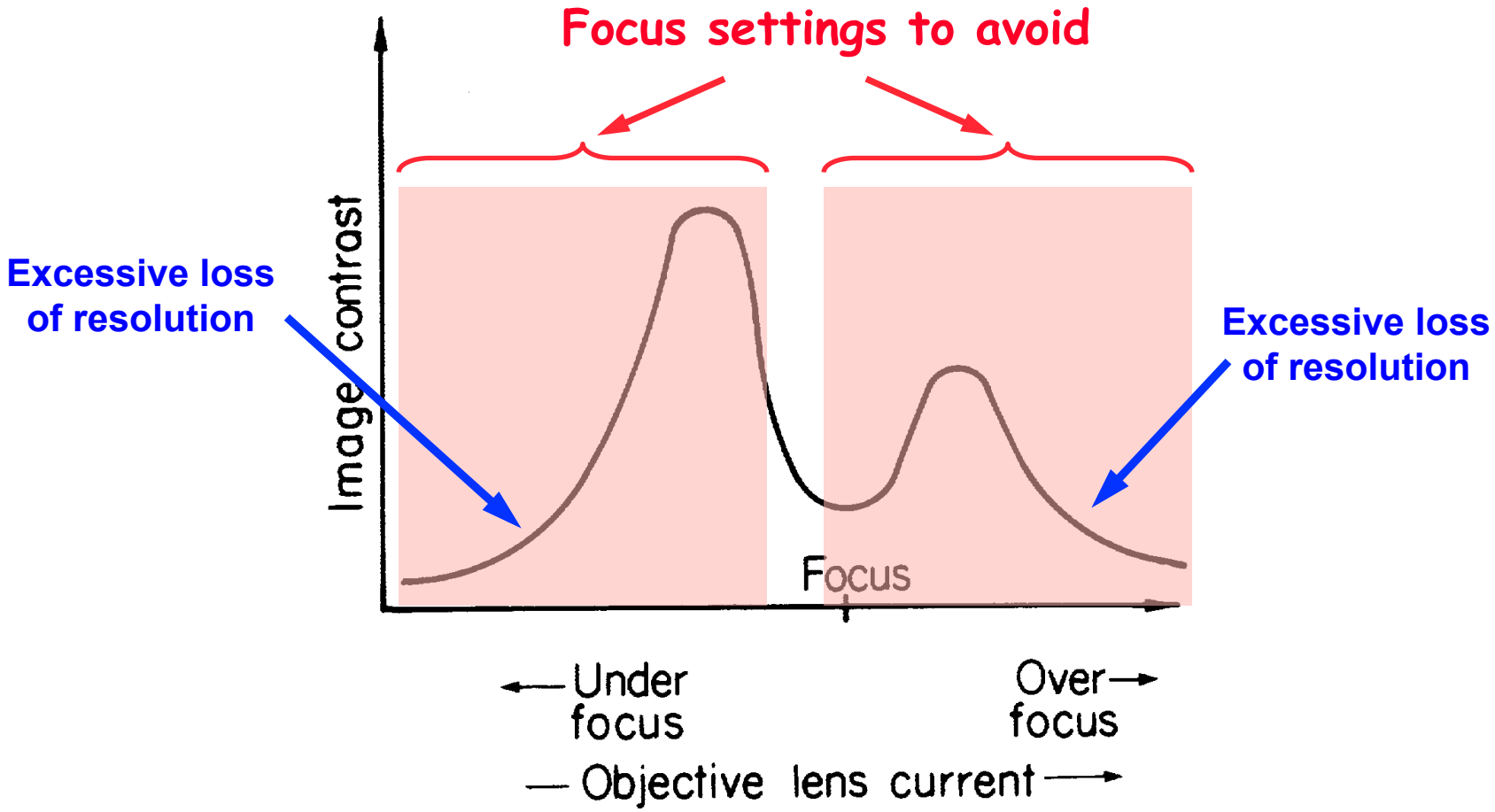
**Image resolution** is **not significantly reduced** if amount of underfocus is not excessive

"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

# I.C CONTRAST AND IMAGE FORMATION

## I.C.3 Contrast Transfer Function

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)

# I.C.3 Contrast Transfer Function

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:

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

where

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

$\nu$ = spatial frequency (in Å$^{-1}$)

$F_{amp}$ = 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

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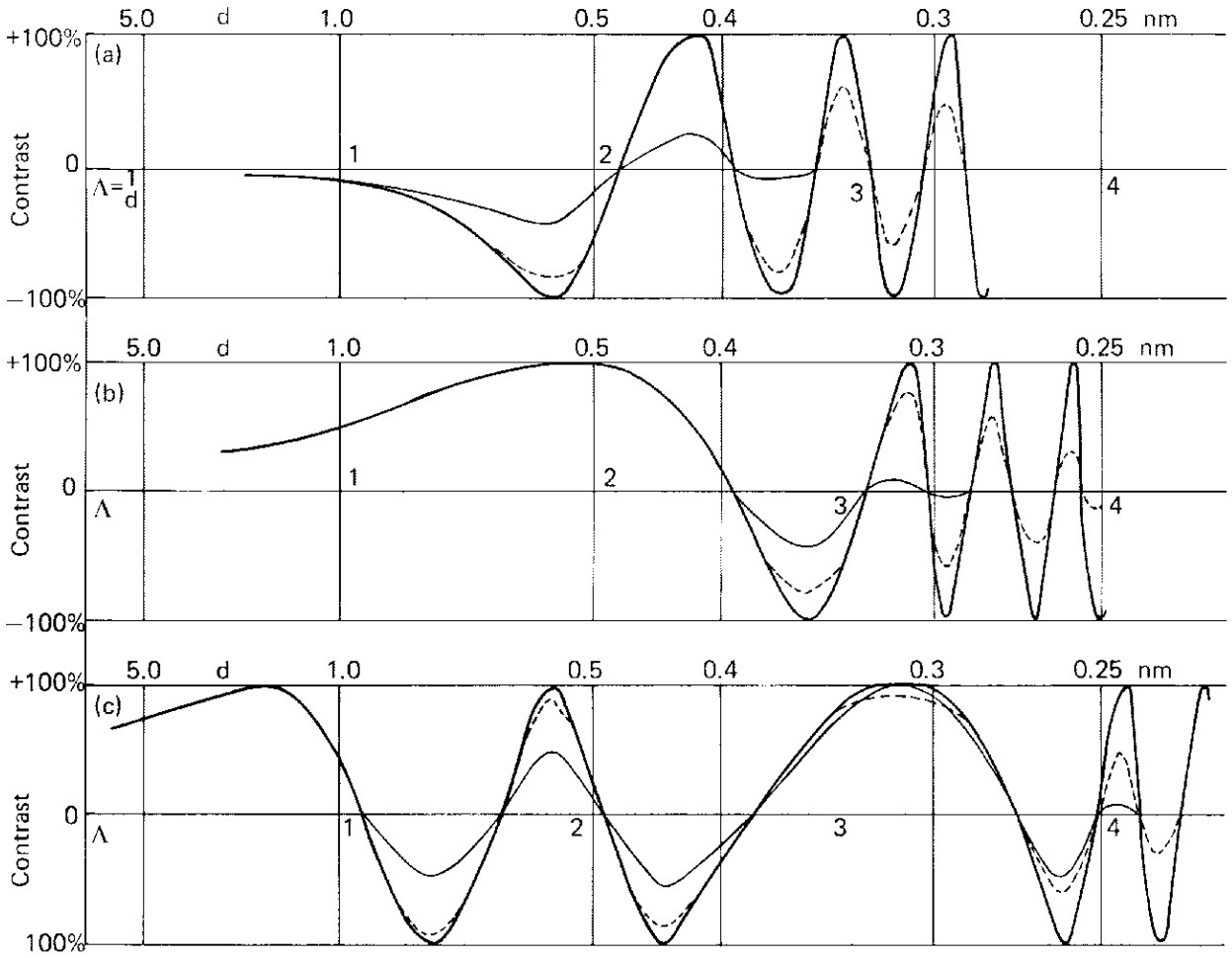
That certainly makes it all look simple and straightforward…

# I.C.3 Phase Contrast Transfer Function

$\Delta f$ = 0 nm

$\Delta f$ = -78 nm

$\Delta f$ = -234 nm




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

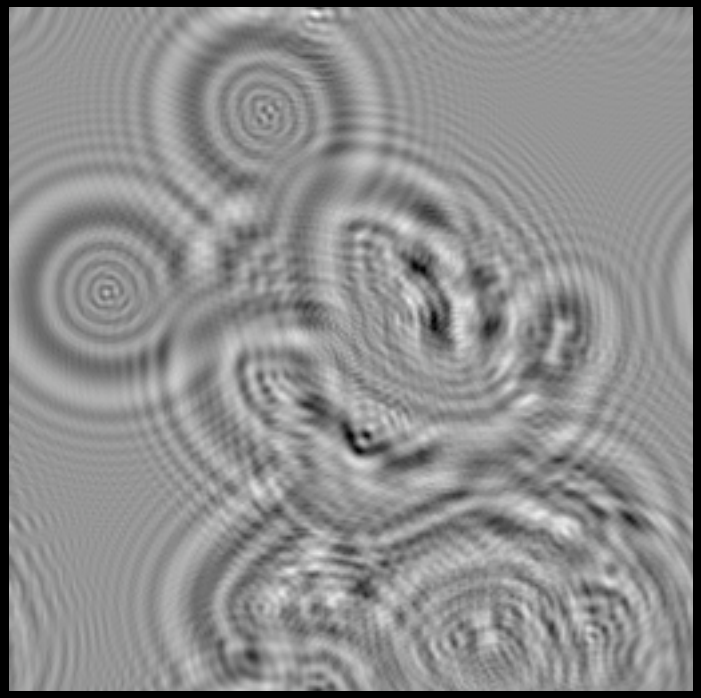
From Agar, Fig 8.3, p. 282

# Virtual TEM

What does the scope CTF do to images?

# I.C.3 Contrast Transfer Function

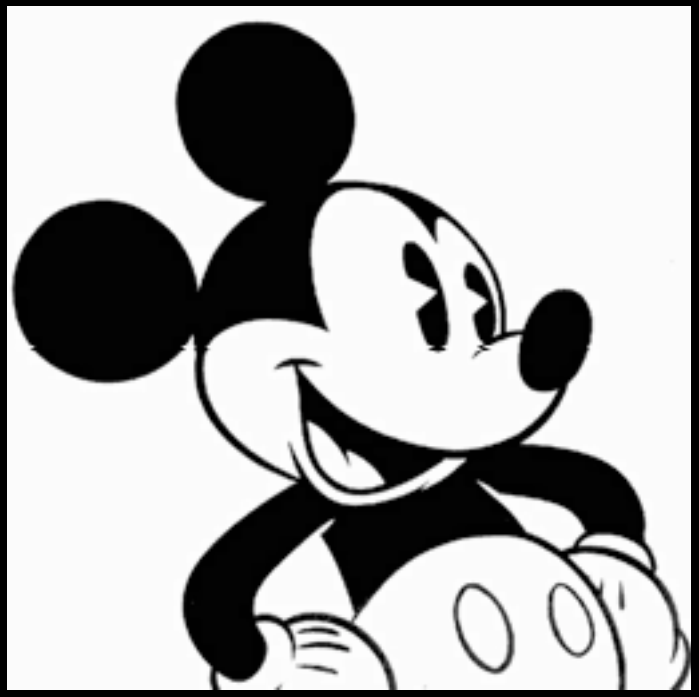
## Simulation of Electron Imaging in a TEM



"Real Image"

# I.C.3 Contrast Transfer Function

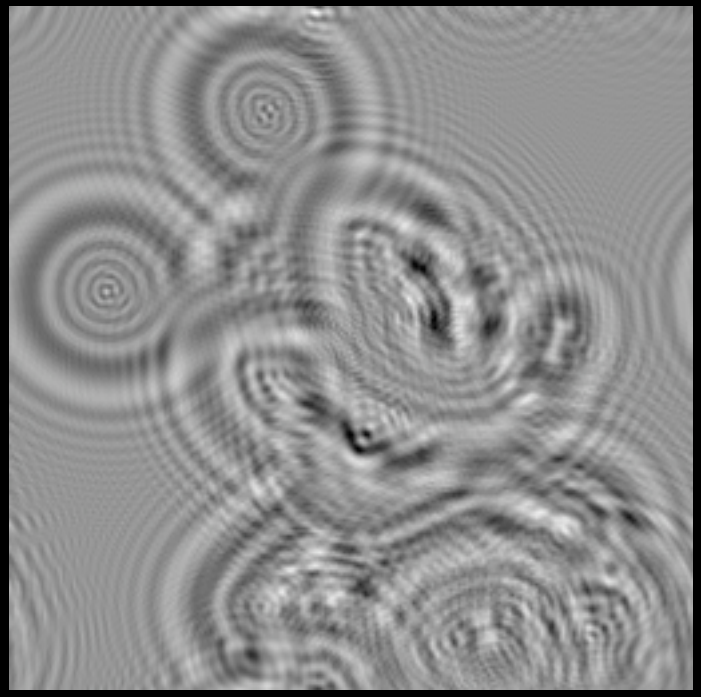
## Simulation of Electron Imaging in a TEM



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

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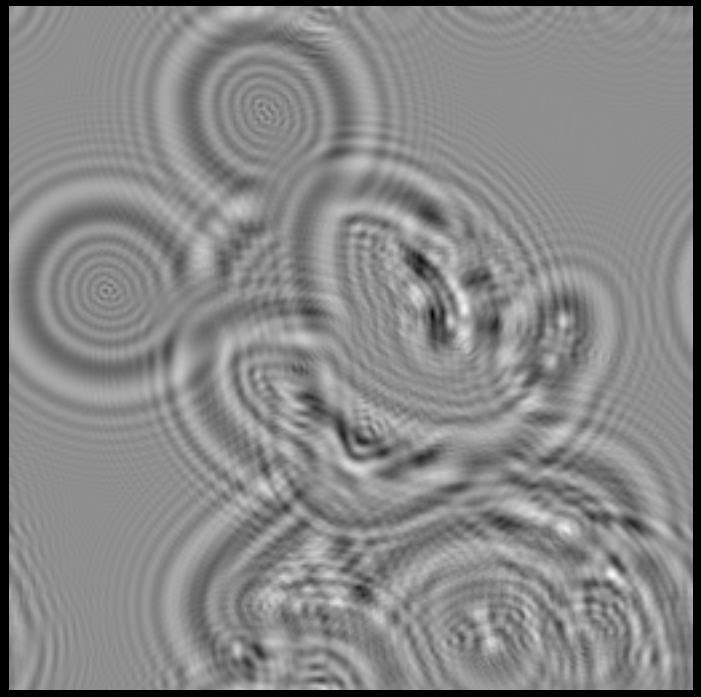

-1.5 μm

underfocus

# I.C.3 Contrast Transfer Function

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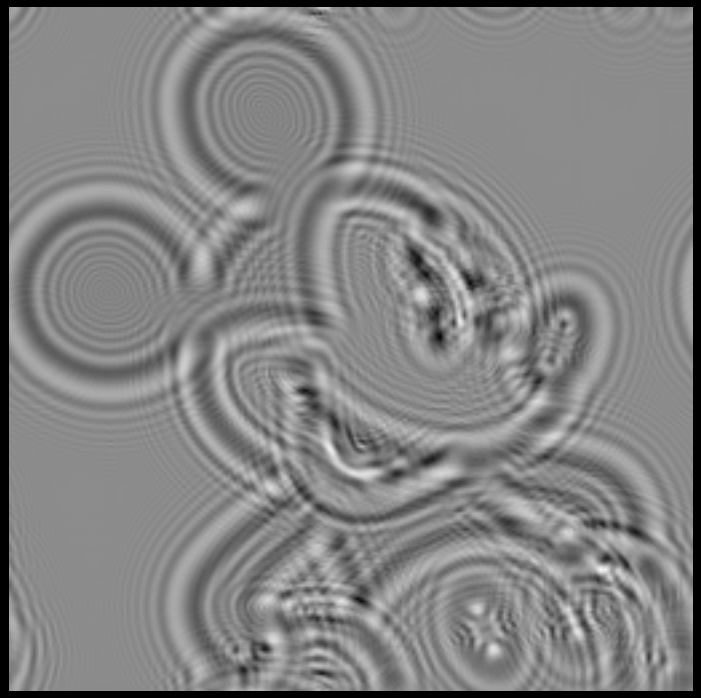

-1.0 μm

underfocus

# I.C.3 Contrast Transfer Function

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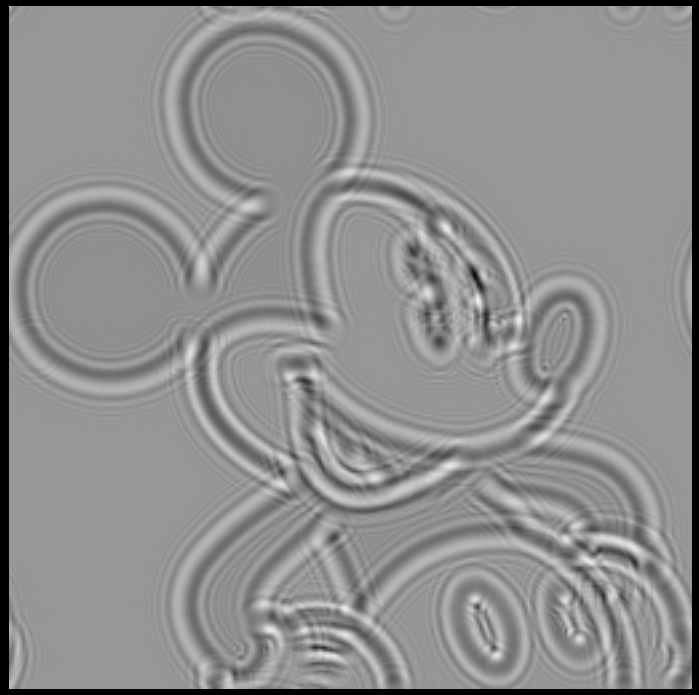


-0.5 μm

underfocus

# I.C.3 Contrast Transfer Function

## Simulation of Electron Imaging in a TEM

-0.25 μm

underfocus

# I.C.3 Contrast Transfer Function

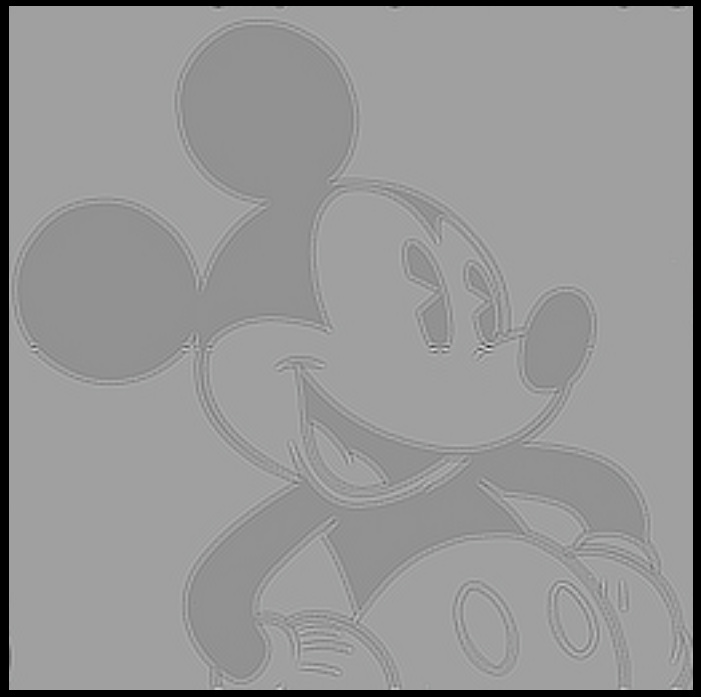
## Simulation of Electron Imaging in a TEM

-0.1 μm

underfocus

# I.C.3 Contrast Transfer Function

## Simulation of Electron Imaging in a TEM

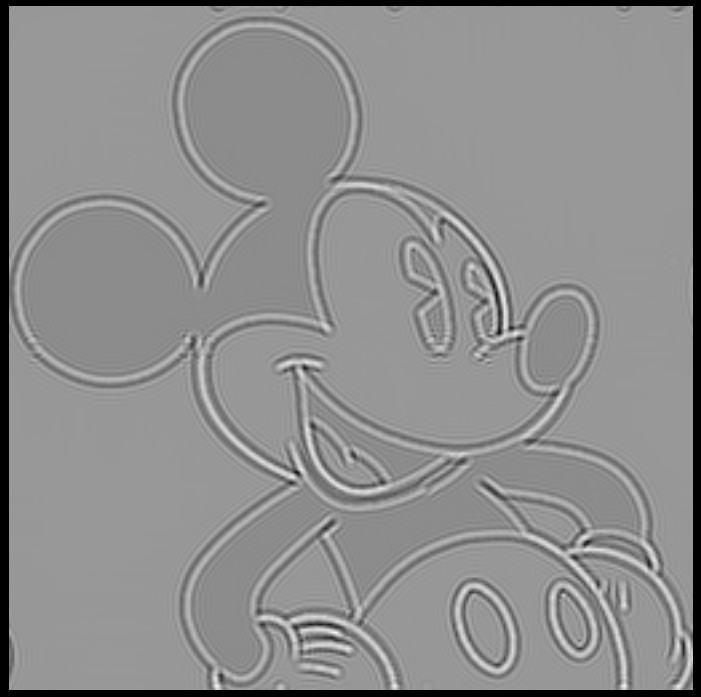


-0.0 μm

near focus

# I.C.3 Contrast Transfer Function

## Simulation of Electron Imaging in a TEM

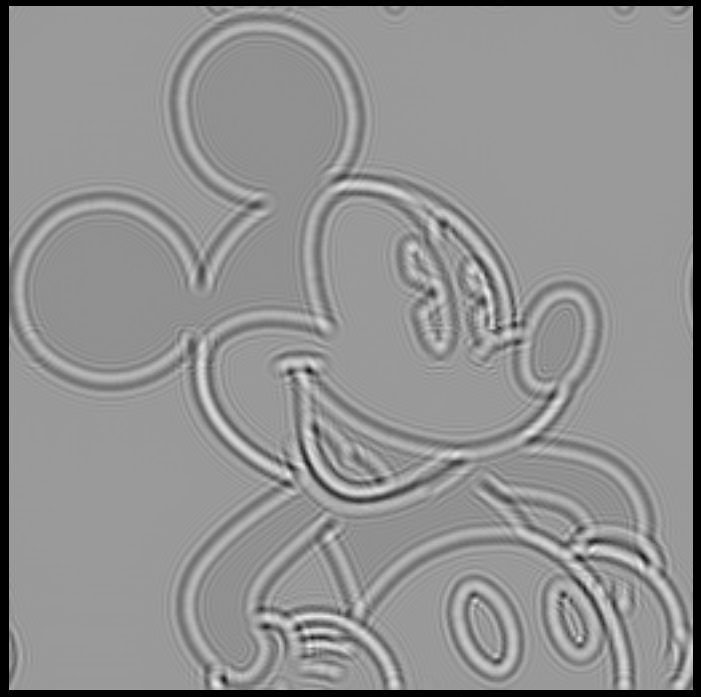


+0.1 μm

overfocus

# I.C.3 Contrast Transfer Function

## Simulation of Electron Imaging in a TEM



+0.25 μm

overfocus

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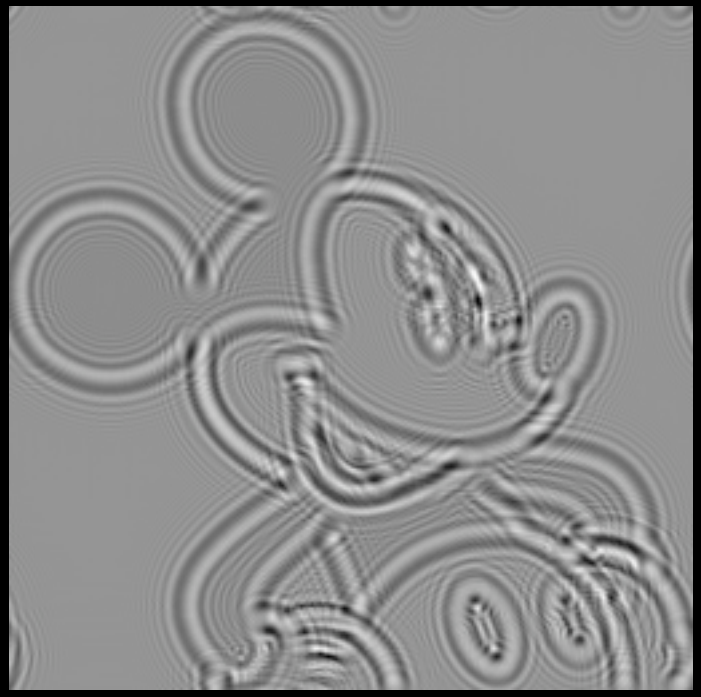
## Simulation of Electron Imaging in a TEM



+0.5 μm

overfocus

# I.C.3 Contrast Transfer Function

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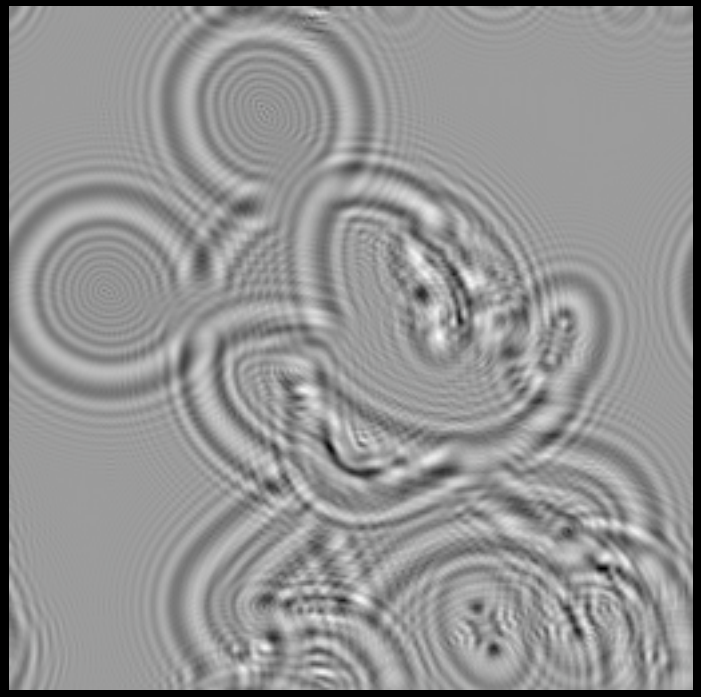
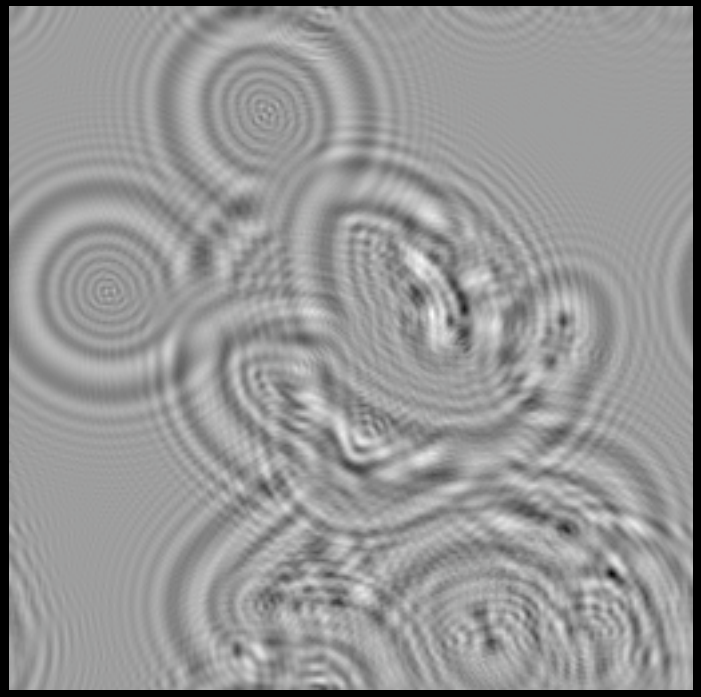


+1.0 μm

overfocus

# I.C.3 Contrast Transfer Function

## Simulation of Electron Imaging in a TEM

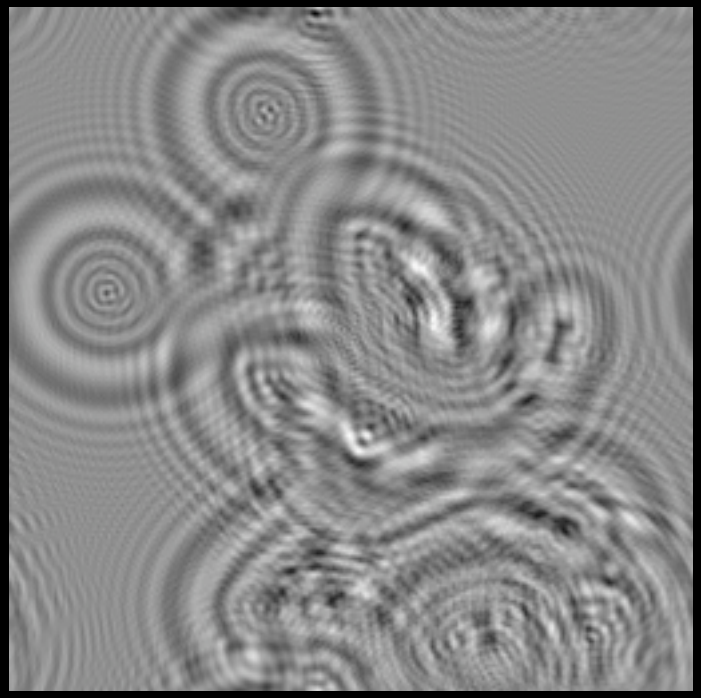


+1.5 μm

overfocus

# I.C.3 Contrast Transfer Function

## Simulation of Electron Imaging in a TEM

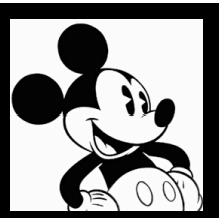


+2.0 μm

overfocus

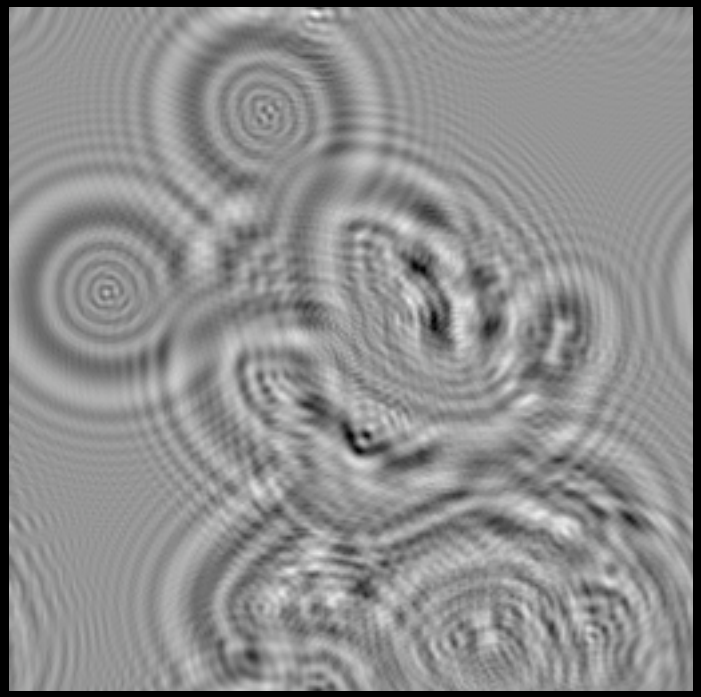
# I.C.3 Contrast Transfer Function

## Simulation of Electron Imaging in a TEM



-0.1 μm

underfocus

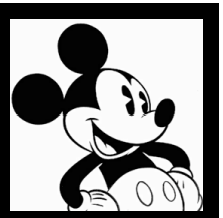


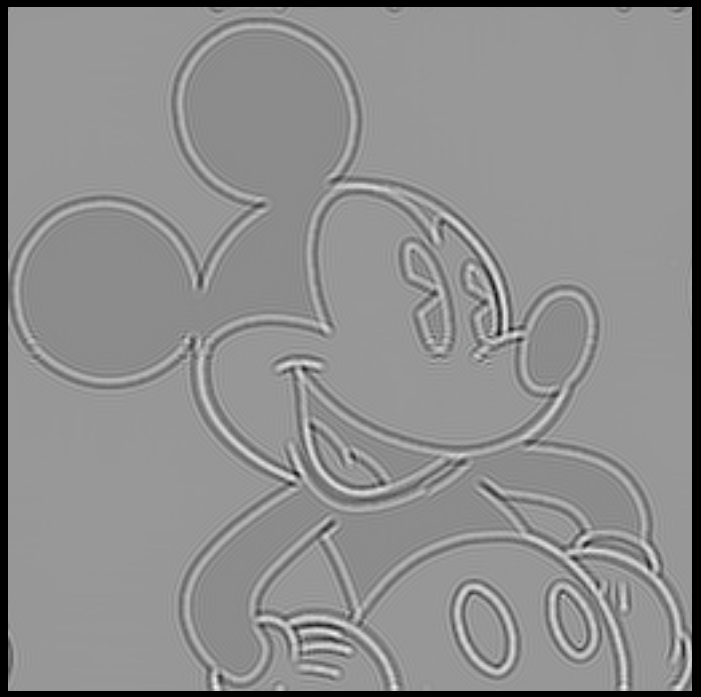
-2.0 μm

underfocus

# I.C.3 Contrast Transfer Function

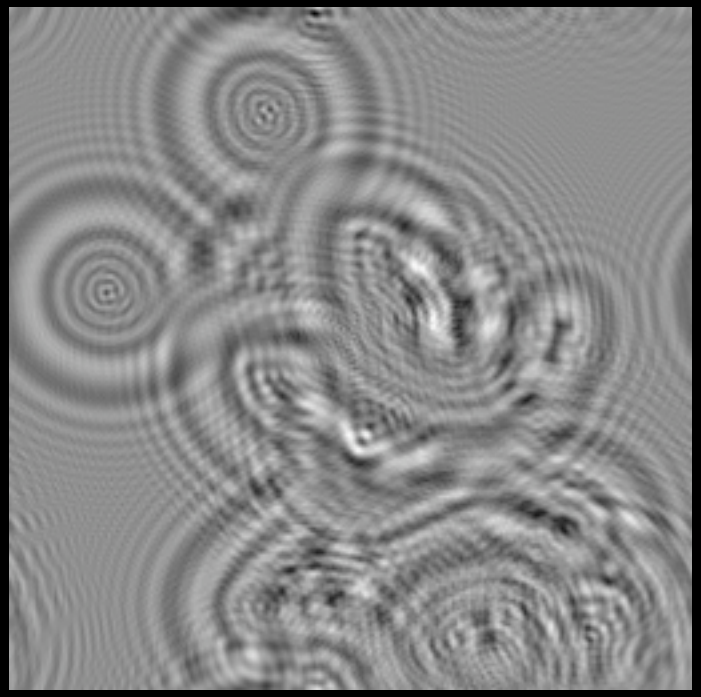
## Simulation of Electron Imaging in a TEM





+0.1 μm

overfocus



+2.0 μm

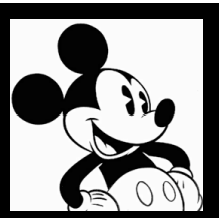
overfocus

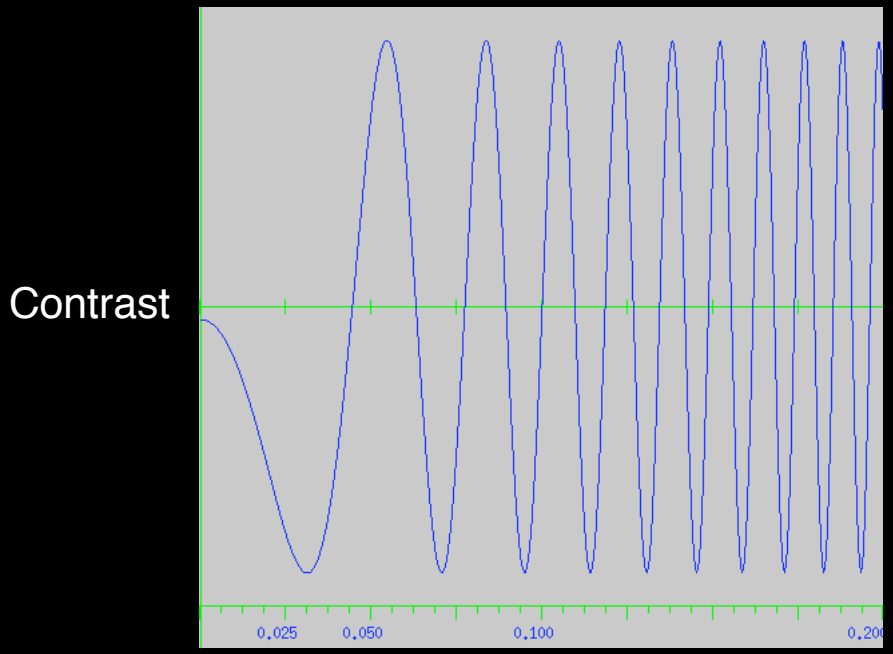
# Virtual TEM

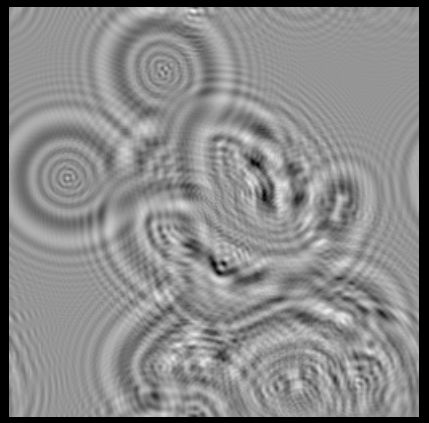
What does the CTF function look like at different defocus settings?

# I.C.3 Contrast Transfer Function

## Simulation of Electron Imaging in a TEM





-2.0 μm

underfocus

# I.C.3 Contrast Transfer Function

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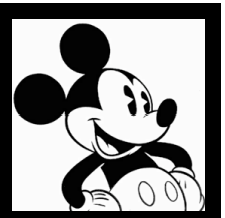

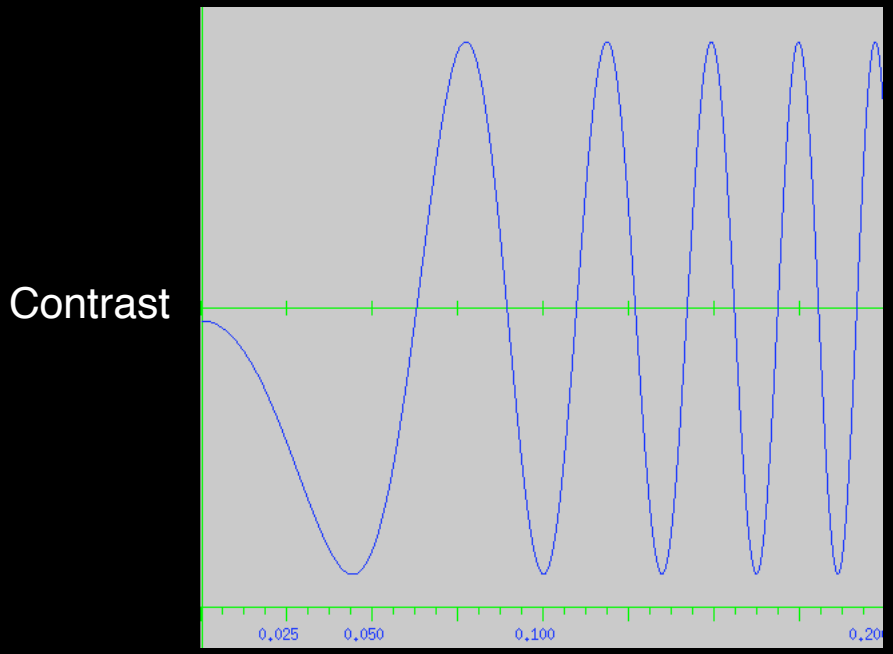


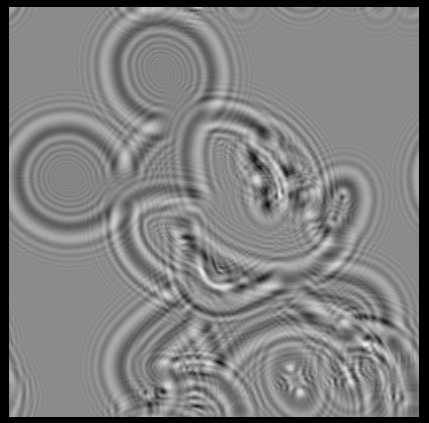

-1.0 μm

underfocus

# I.C.3 Contrast Transfer Function

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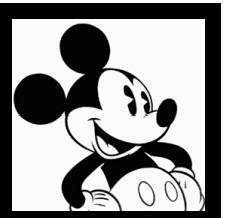
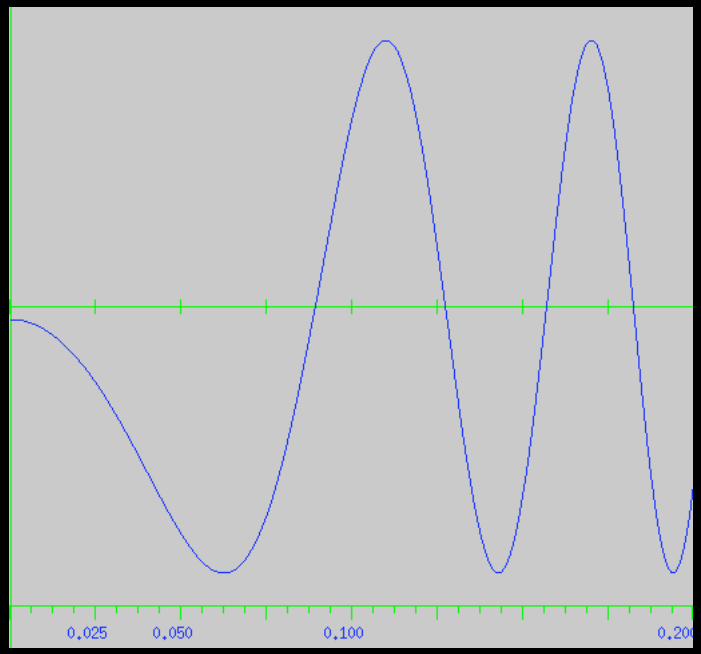
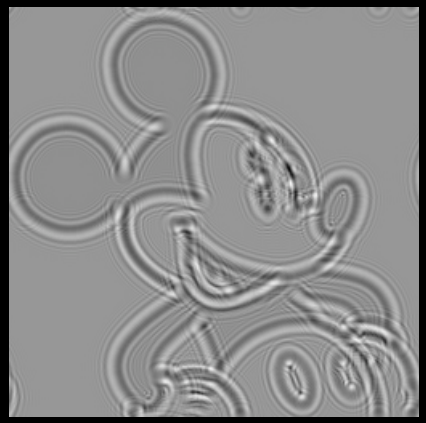


Contrast




Spatial Frequency



-0.5 μm

underfocus

# I.C.3 Contrast Transfer Function

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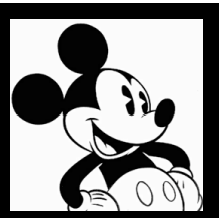
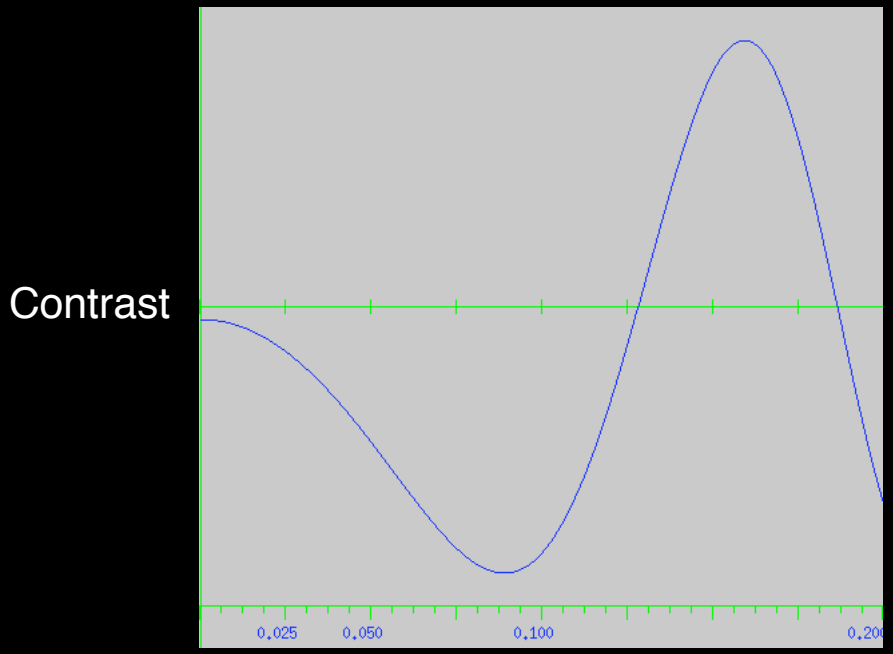


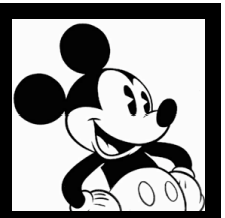


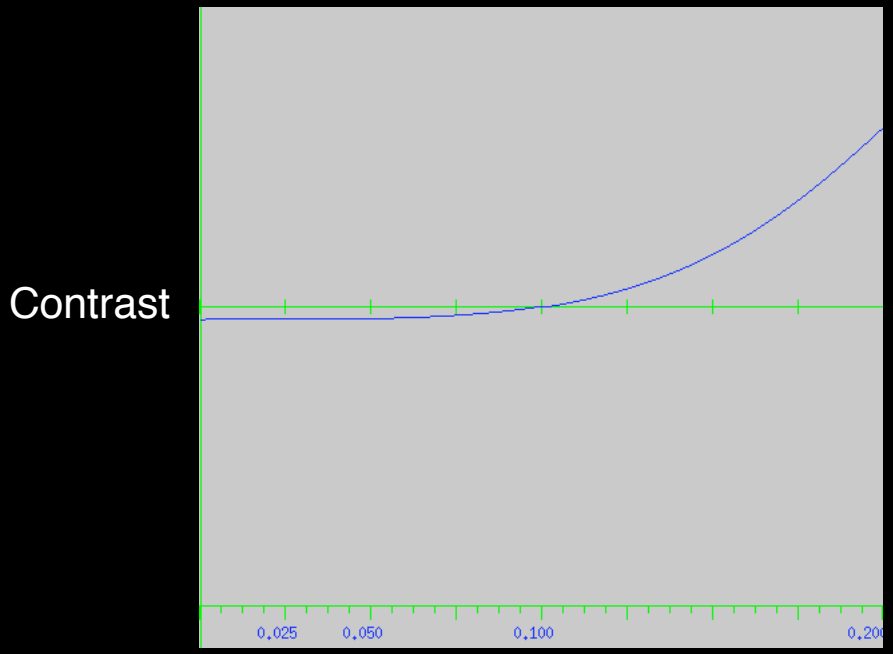

-0.25 μm

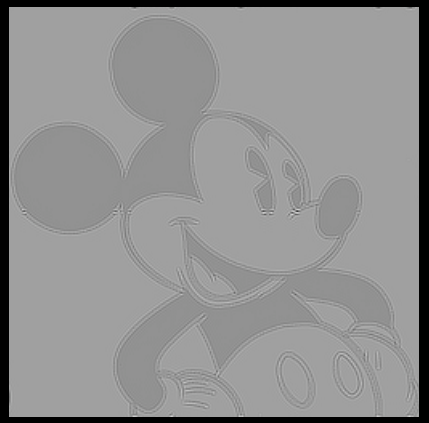
underfocus

# I.C.3 Contrast Transfer Function

## Simulation of Electron Imaging in a TEM





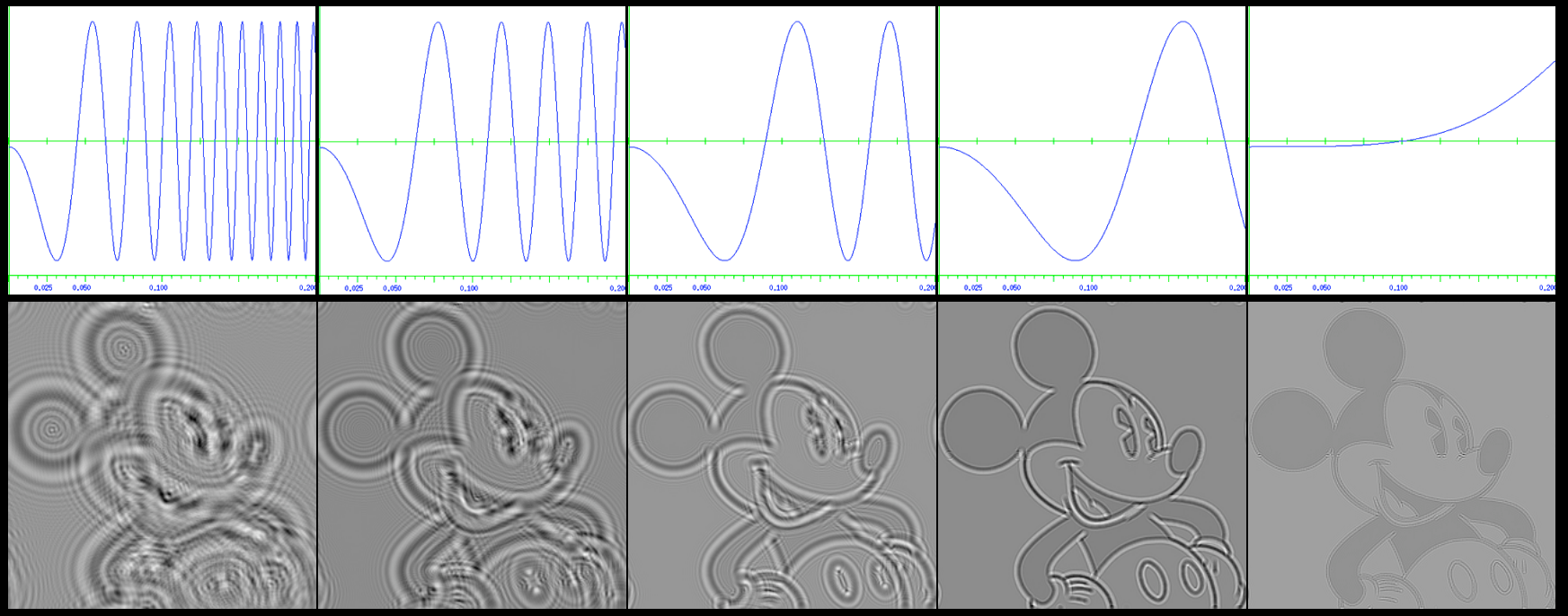



-0.0 μm

near focus

# I.C.3 Contrast Transfer Function

## Simulation of Electron Imaging in a TEM



-2.0 μm -1.0 μm -0.5 μm -0.25 μm -0.0 μm

# I.C CONTRAST AND IMAGE FORMATION

## I.C.5 Other Methods for Enhancing Contrast

**1.** Directly **increase "inherent" specimen contrast** using various preparation procedures (*i.e.* staining, shadowing, etc.)

**Discussed in §II**

**2.** Record images with **longer exposure times**

**Discussed in §I.E**

# § I: The Microscope

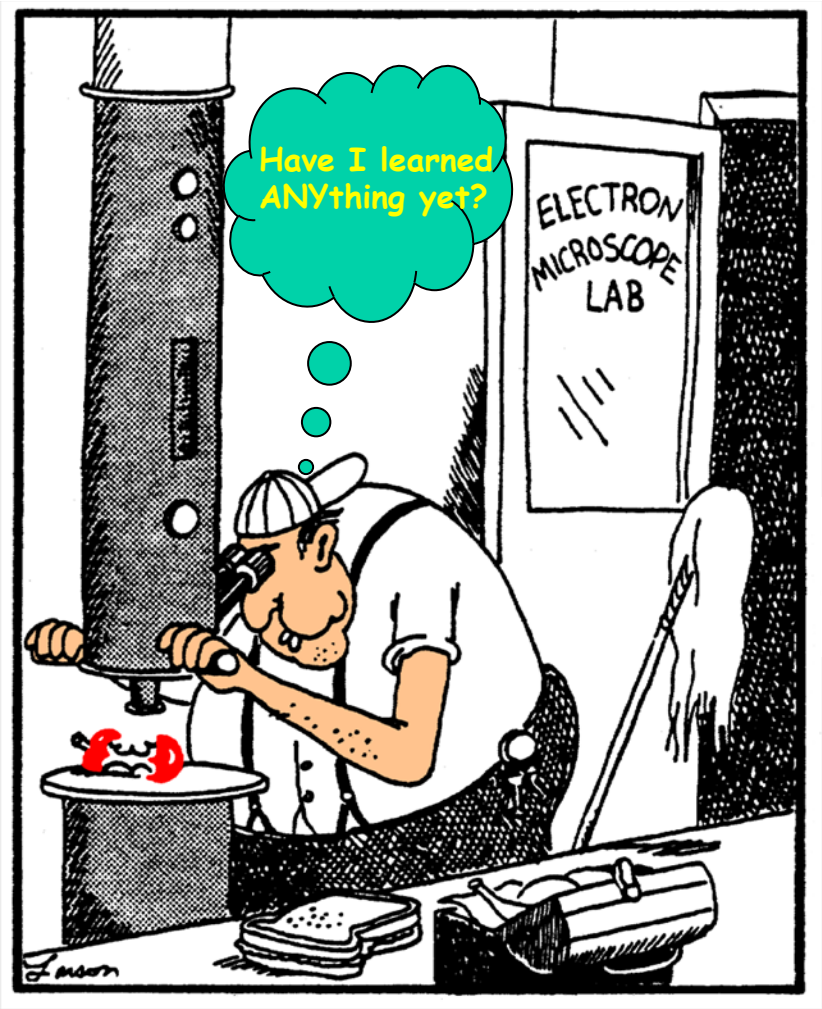
I.A Principles of TEM

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

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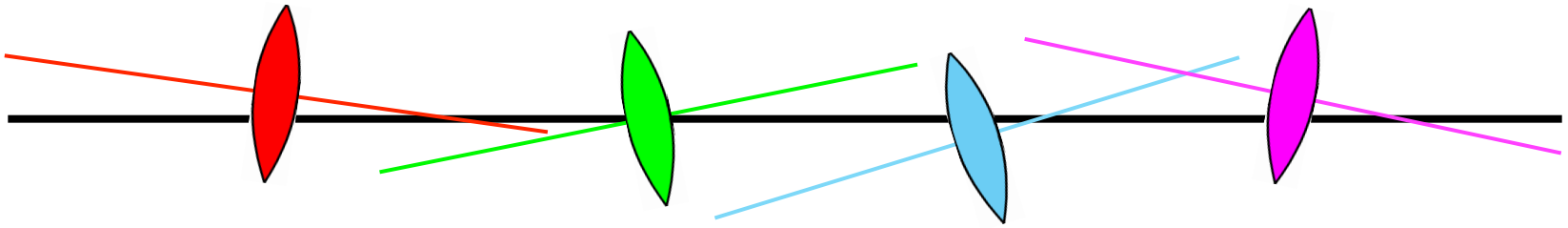

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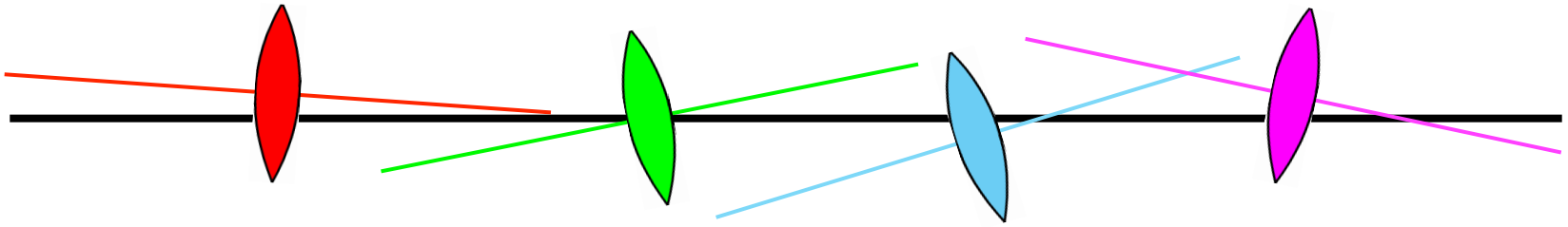

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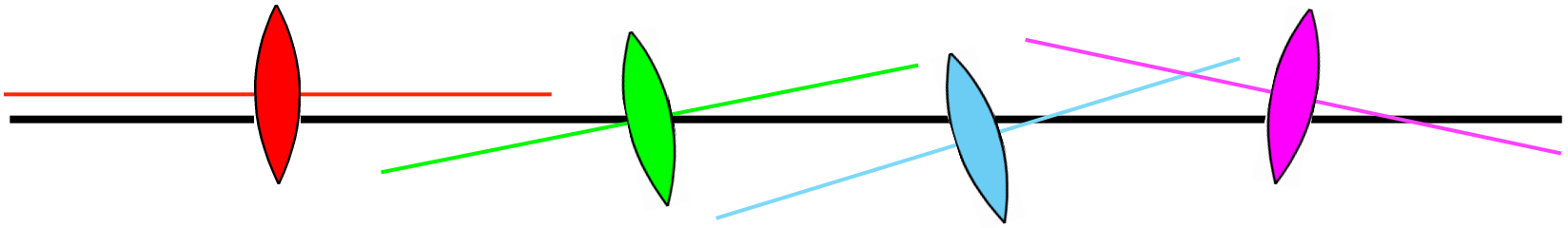

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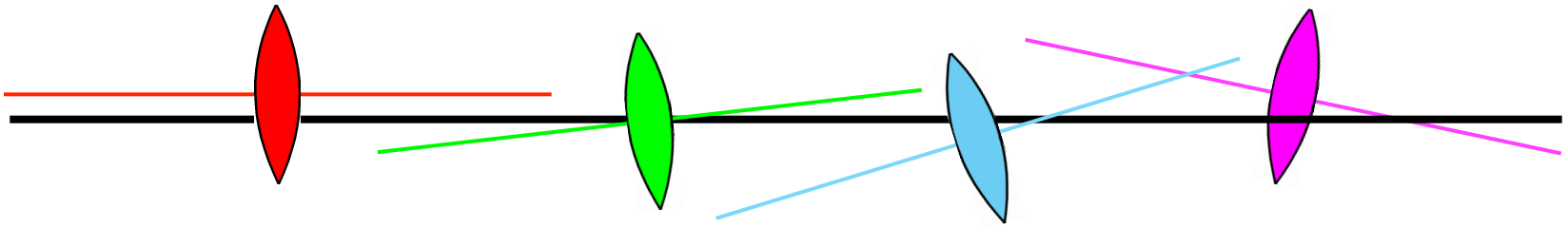

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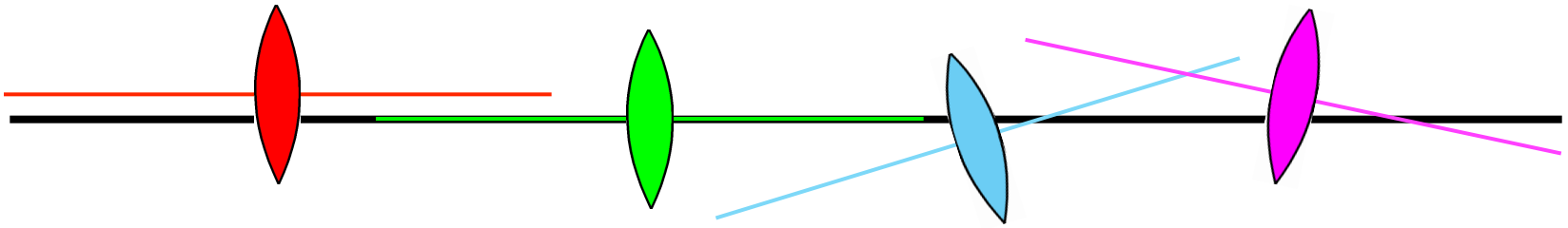

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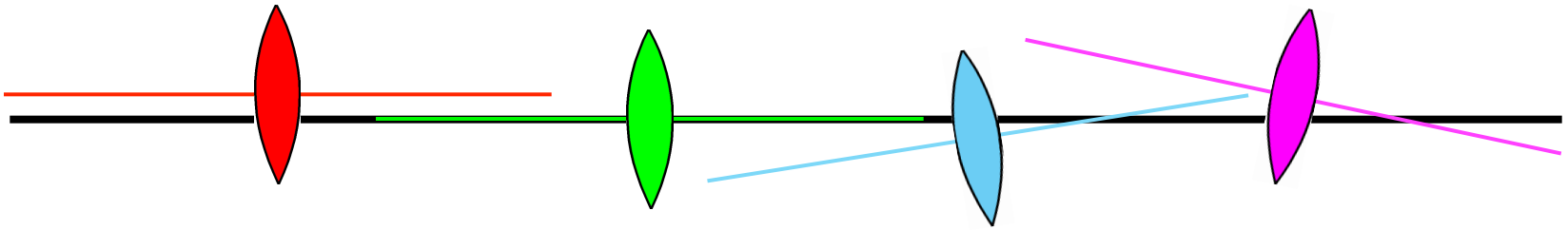

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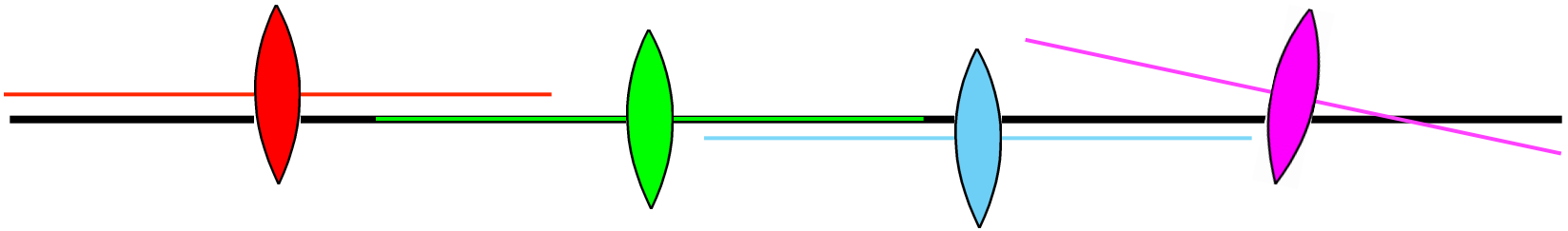

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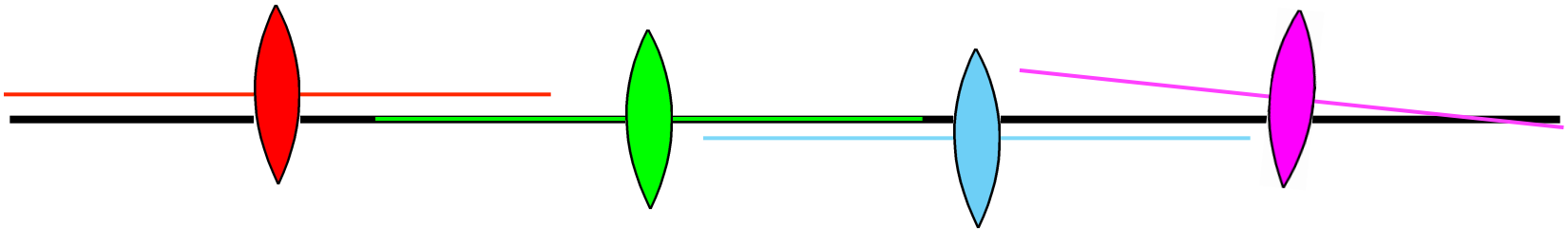

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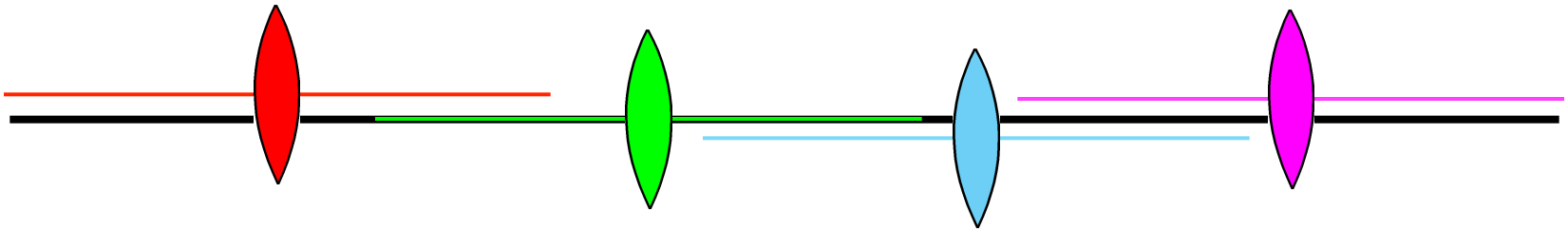

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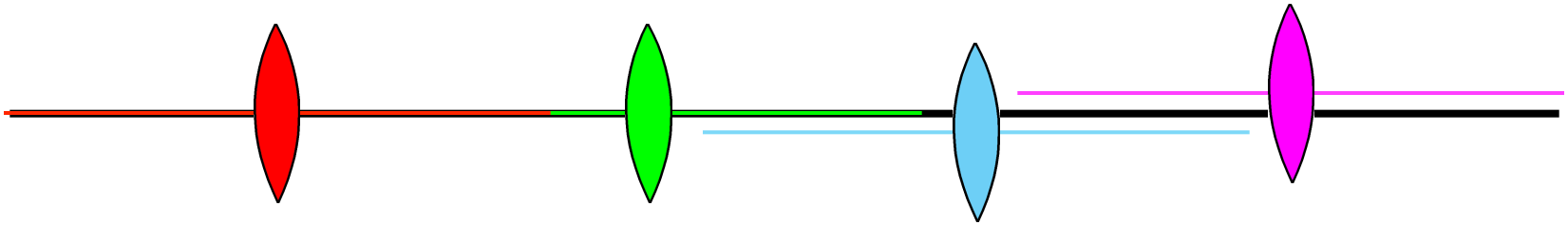

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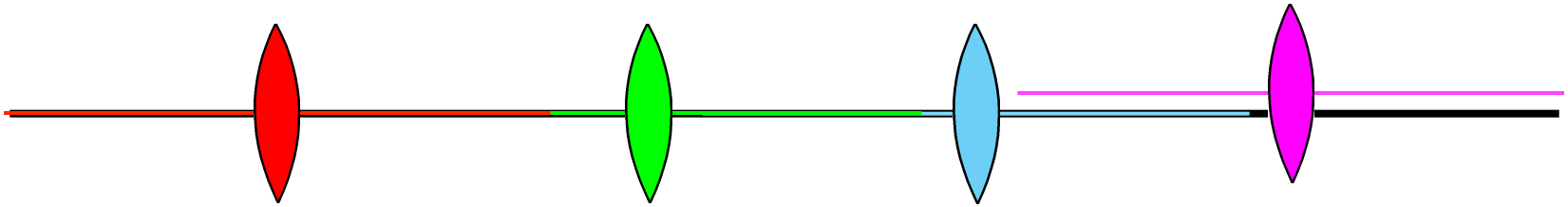

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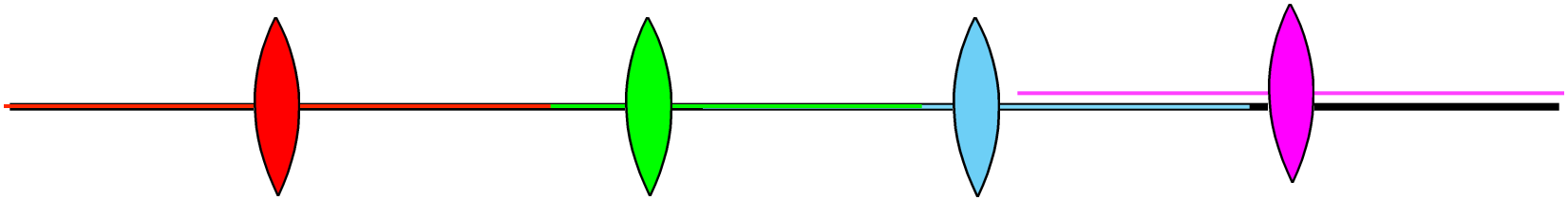

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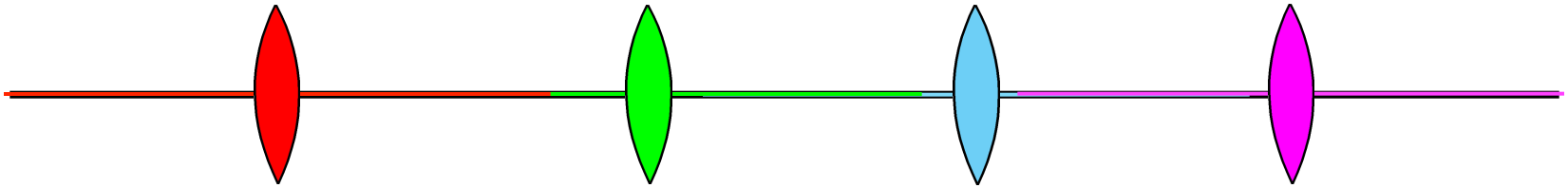

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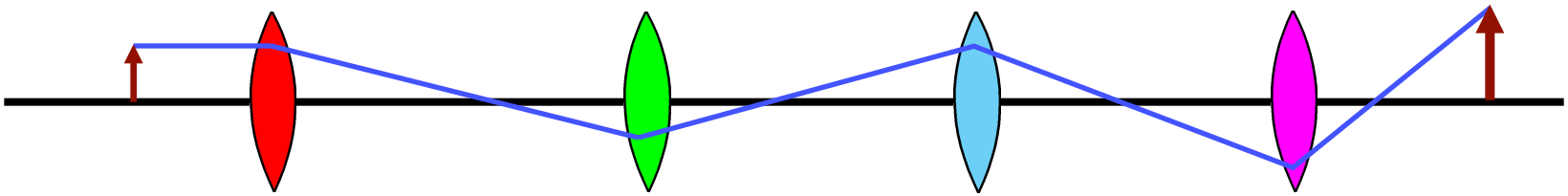

Imagine (*i.e.* I can't think of a good way to illustrate this) what the final image formed by the last (*i.e.* magenta) lens would look like if one or more lenses were misaligned!

# I.D ALIGNMENT/ADJUSTMENT OF THE TEM

## I.D.1 Introduction

### Goal of Alignment Procedure

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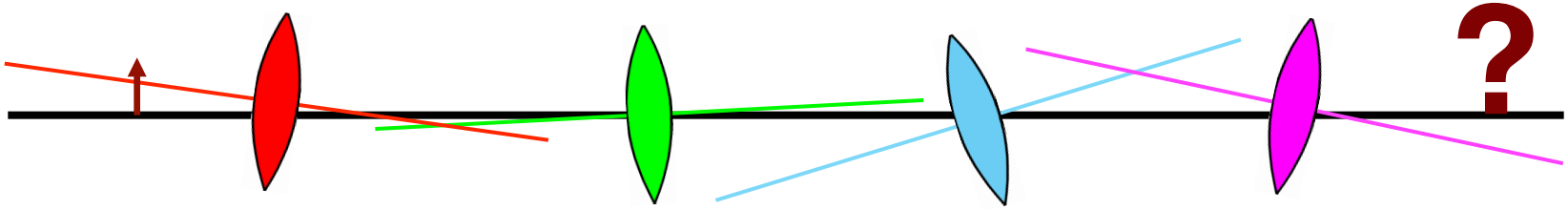
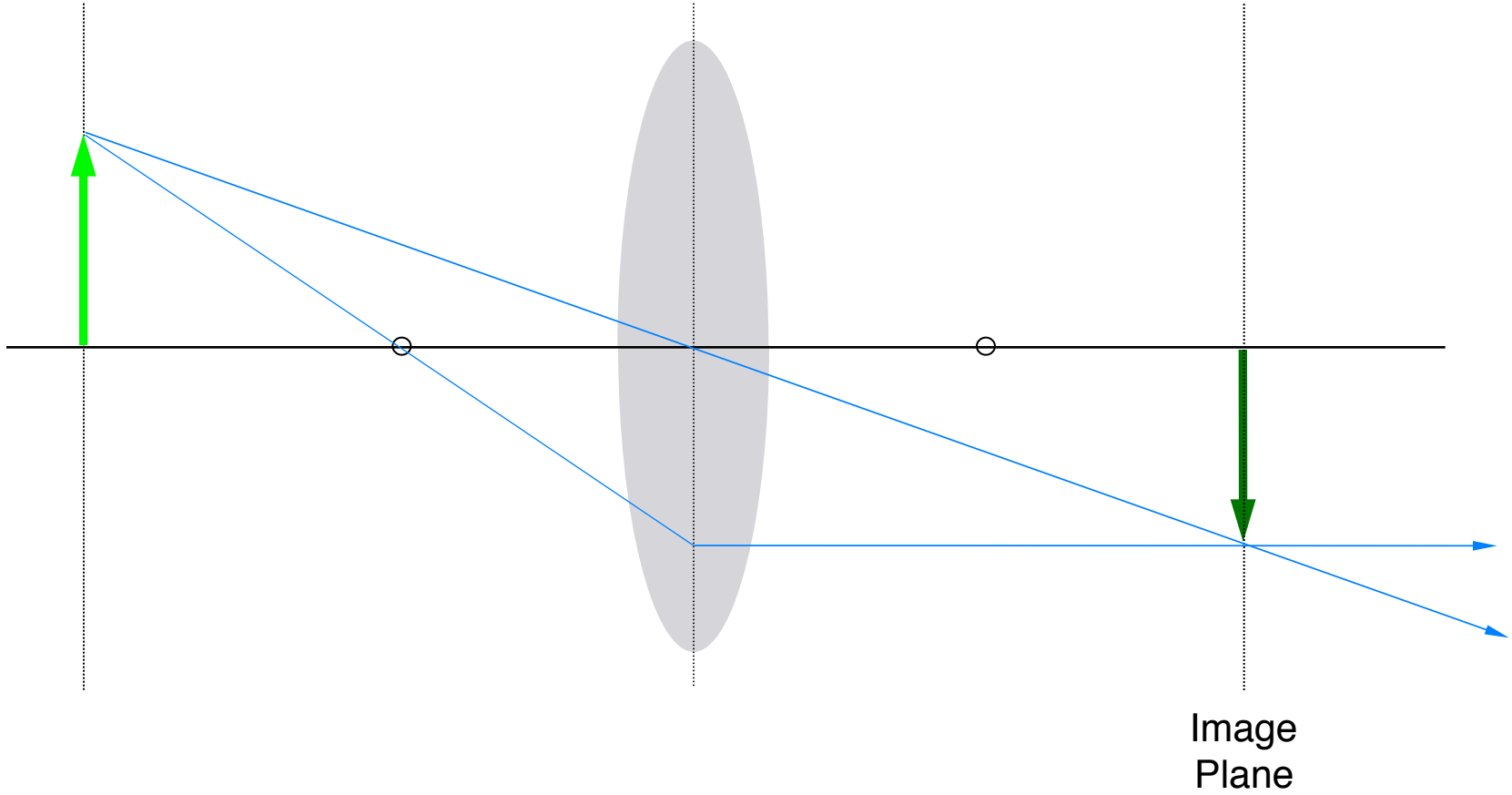



Imagine (*i.e.* I can't think of a good way to illustrate this) what the final image formed by the last (*i.e.* magenta) lens would look like if one or more lenses were misaligned!

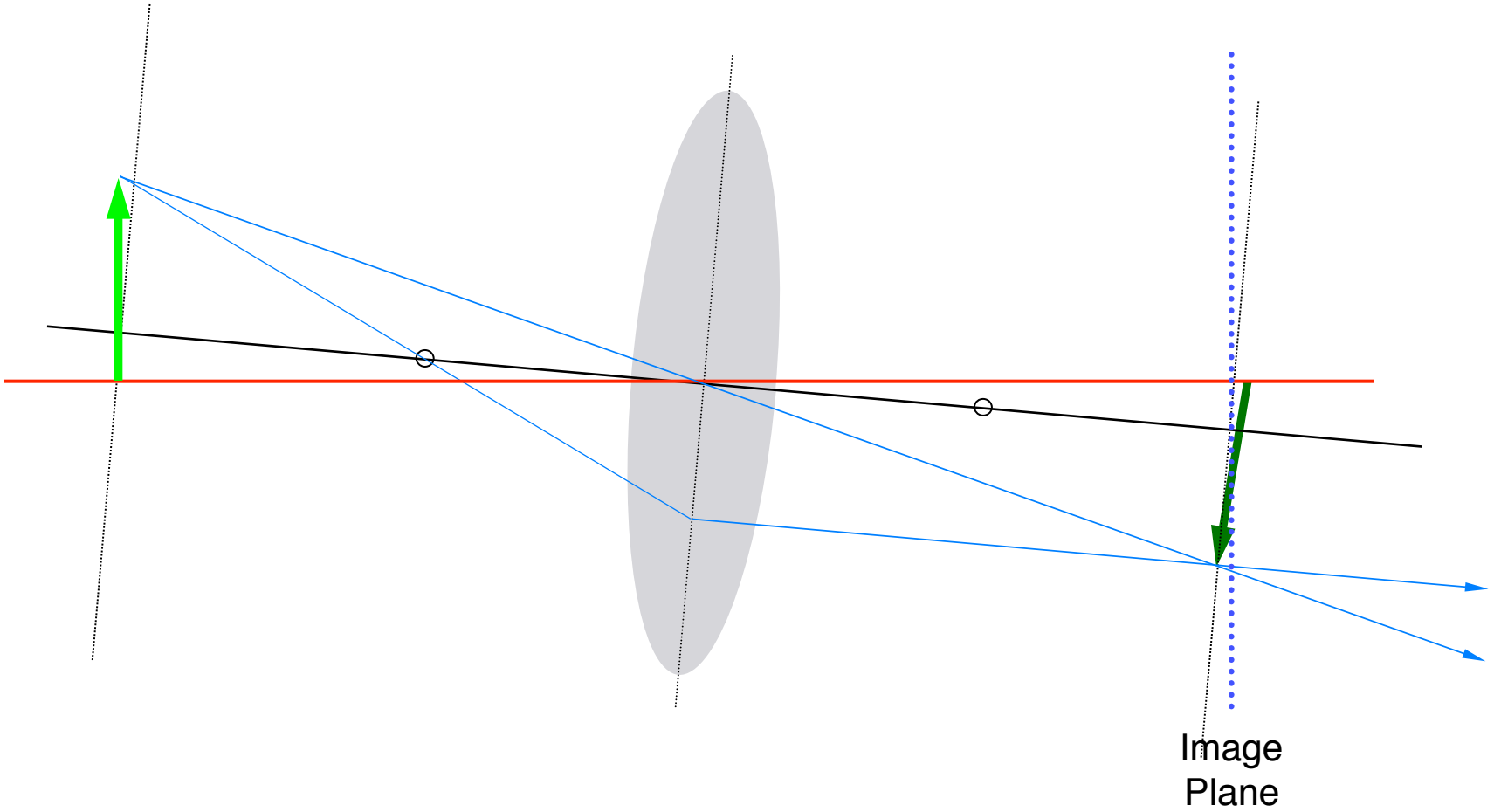
# I.D.1 Introduction

## Lens Misalignment



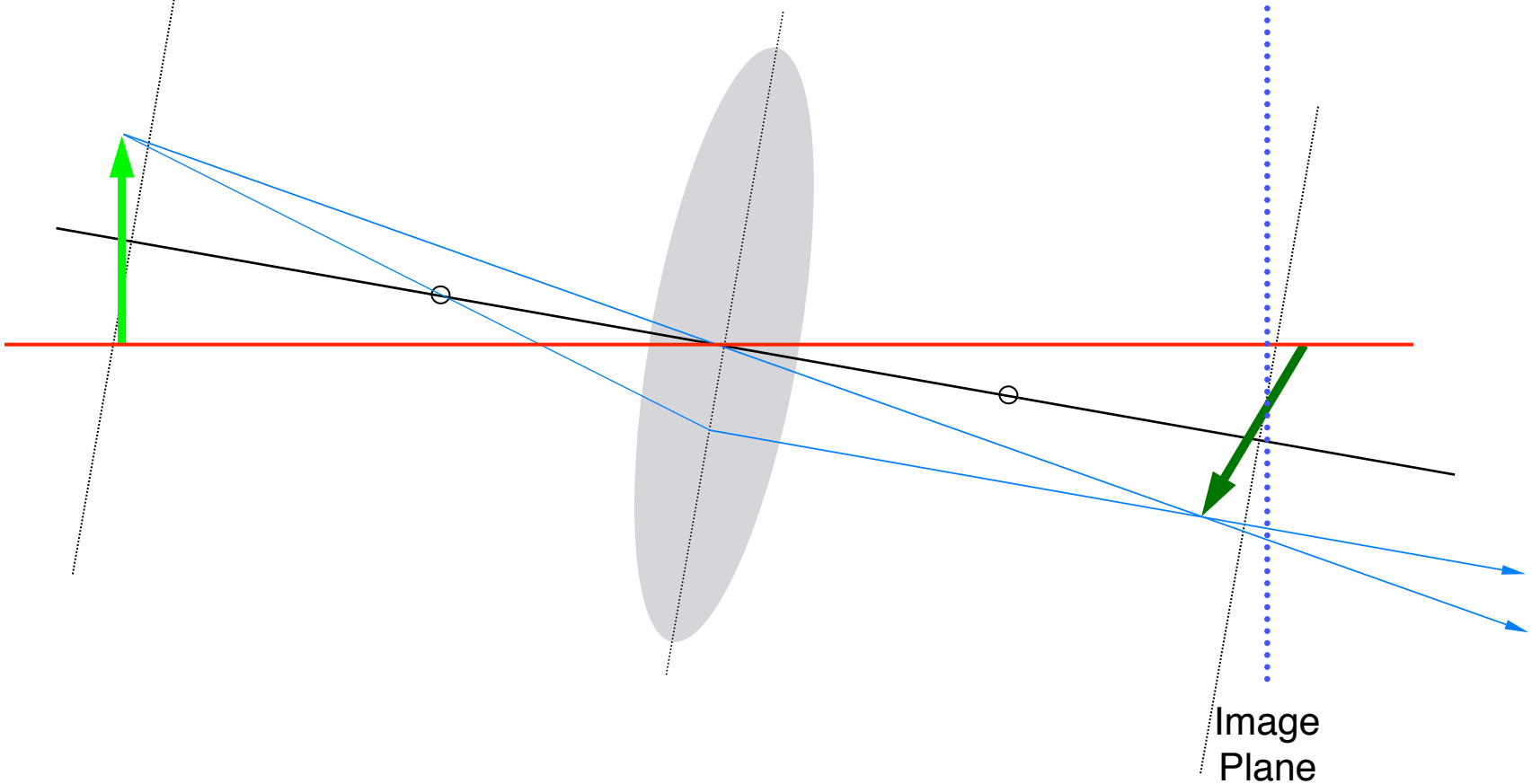
# I.D.1 Introduction

## Lens Misalignment



# I.D.1 Introduction

## Lens Misalignment

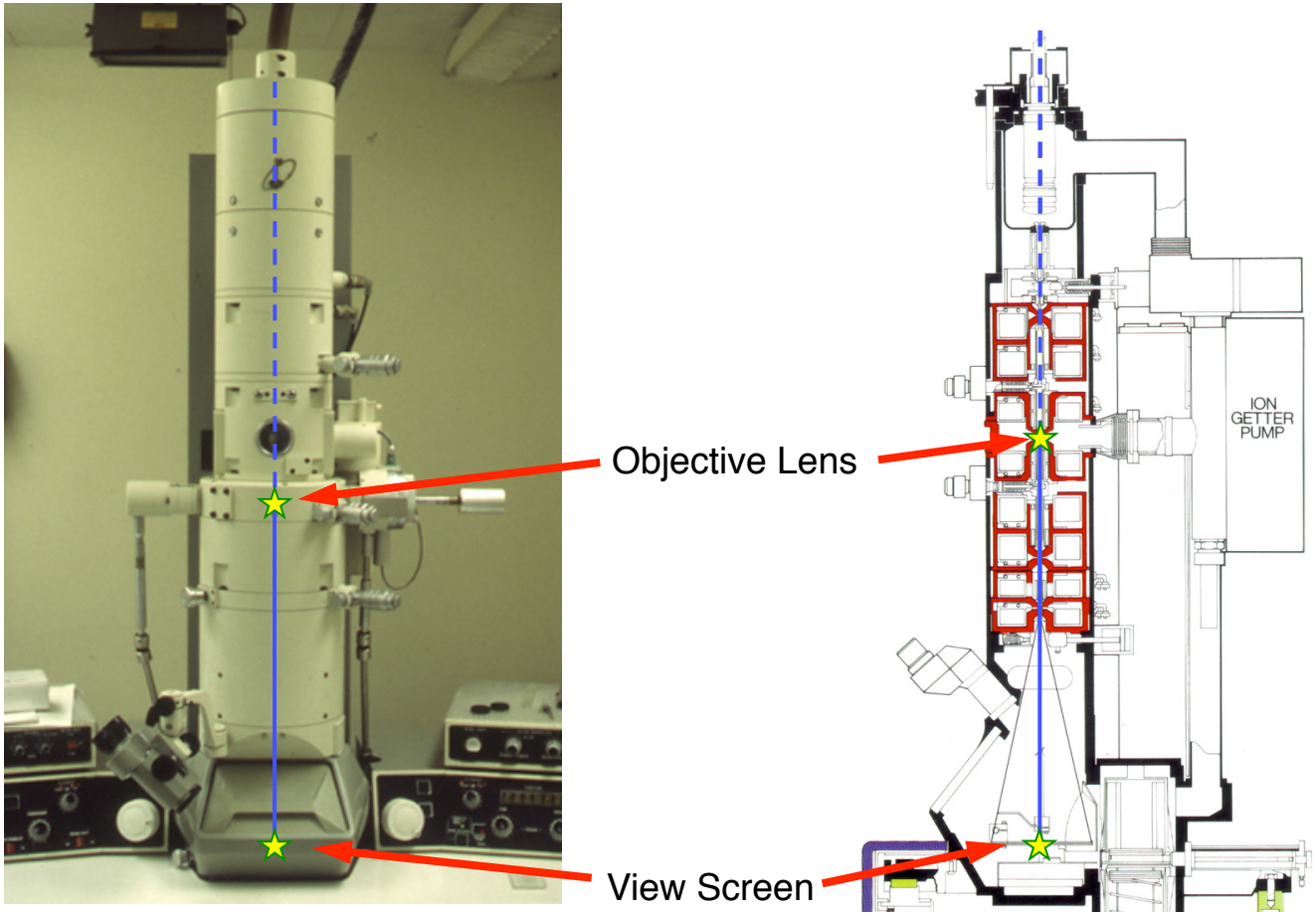


# I.D ALIGNMENT/ADJUSTMENT OF THE TEM

## 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:**

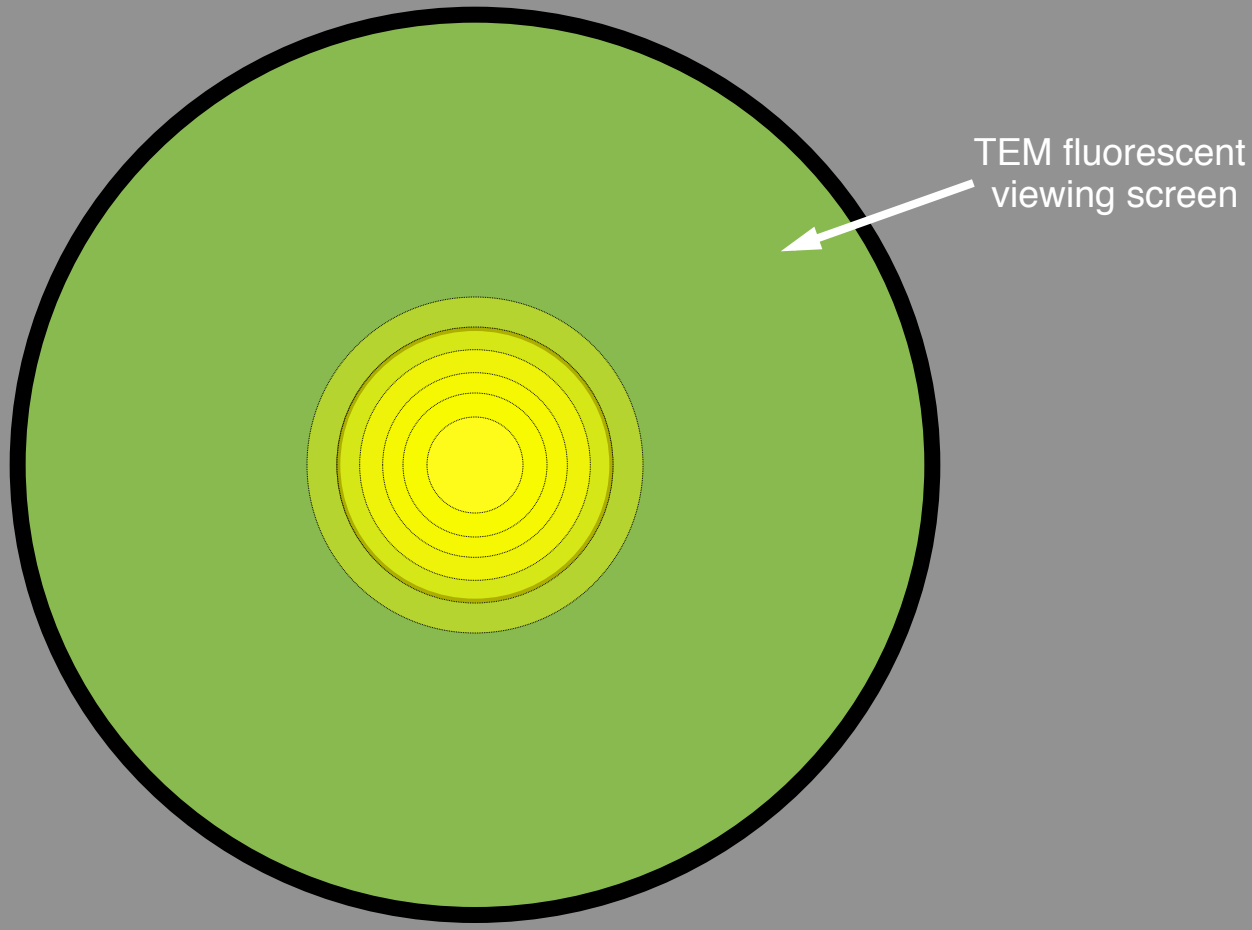
***The devil is ALWAYS in the details!***

- 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

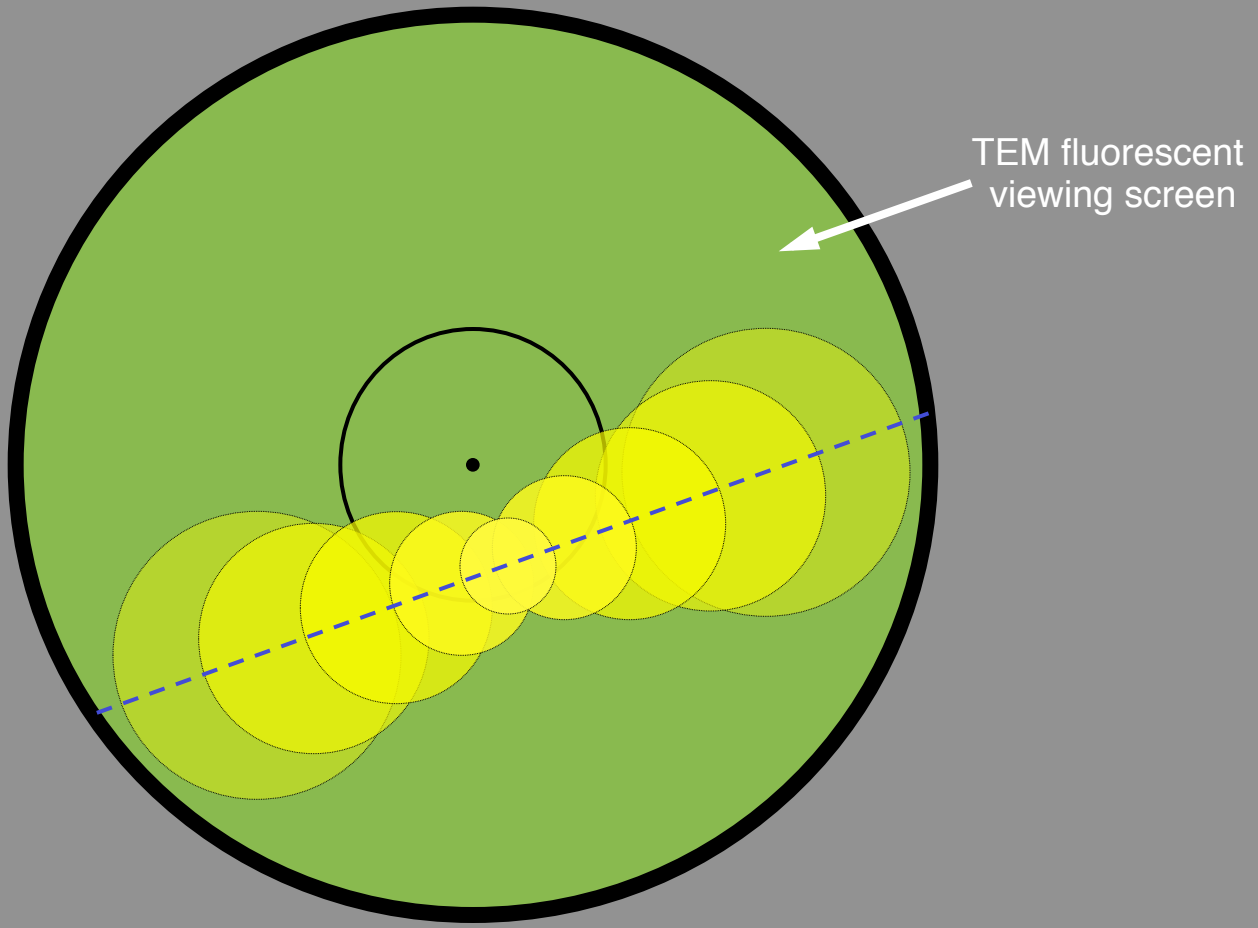
## I.D.2.b Condenser System




Simulation of Aligned Condenser System

# I.D.2 Alignment of TEM Components

## I.D.2.b Condenser System

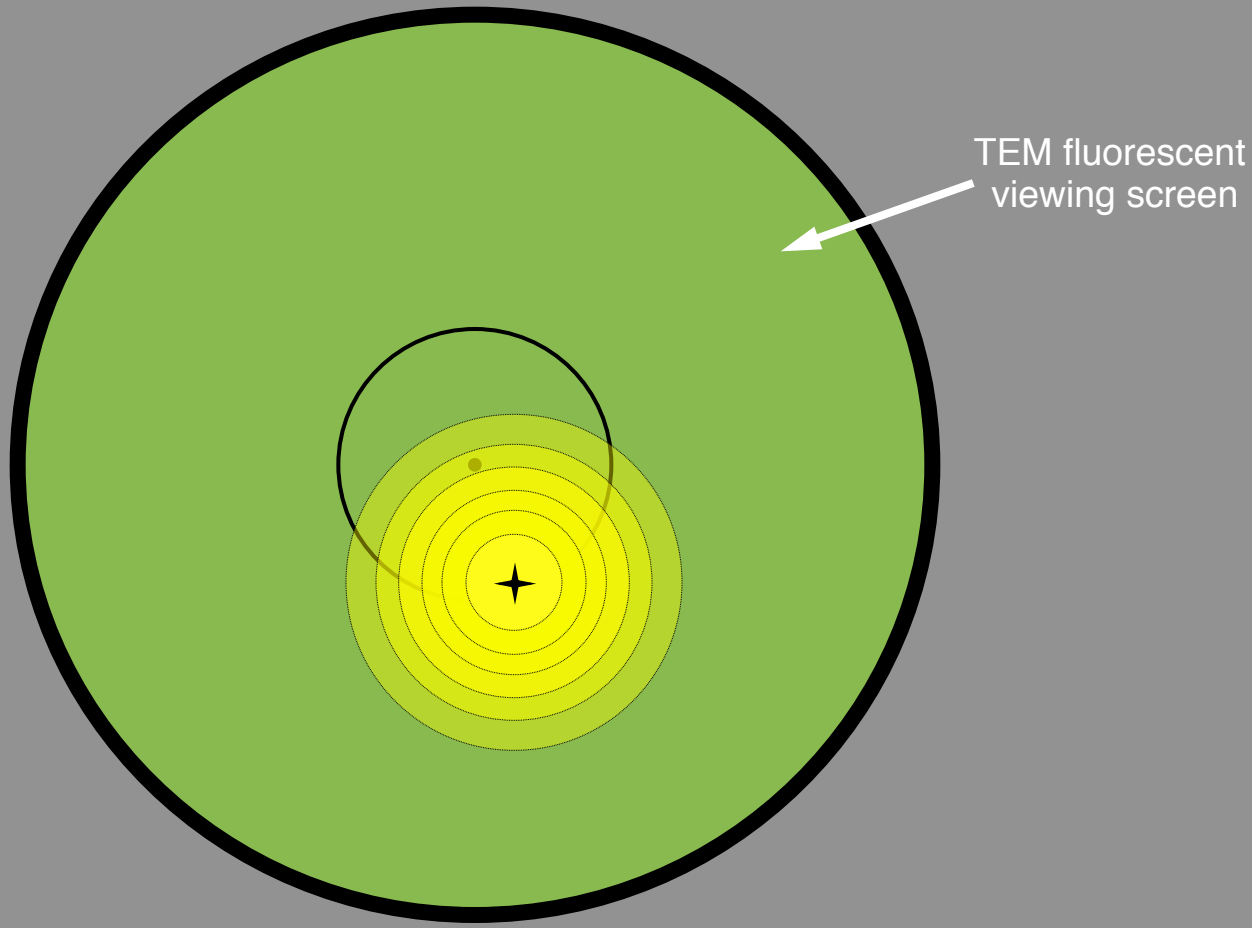



Simulation #1 of Misaligned Condenser System

(Tilt and translation off)

# I.D.2 Alignment of TEM Components

## I.D.2.b Condenser System



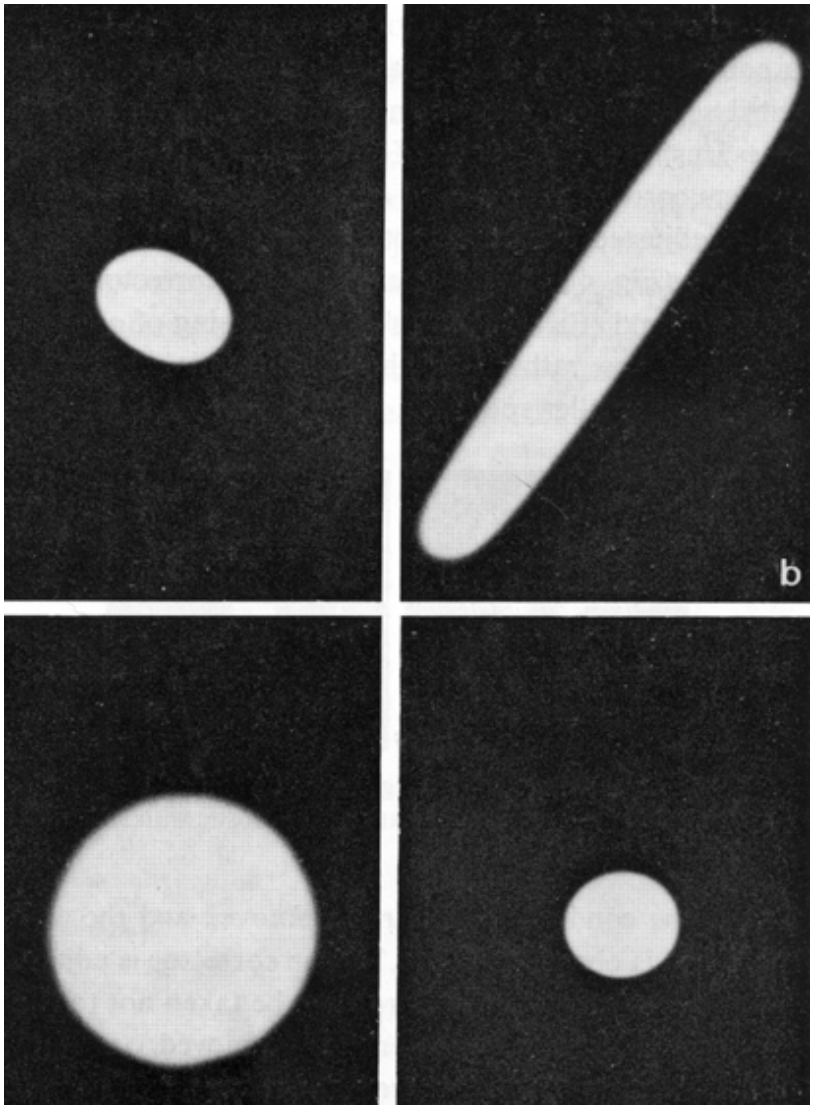

Simulation #2 of Misaligned Condenser System

(Tilt aligned; Translation off)

# I.D.2 Alignment of TEM Components

## I.D.2.b Condenser System

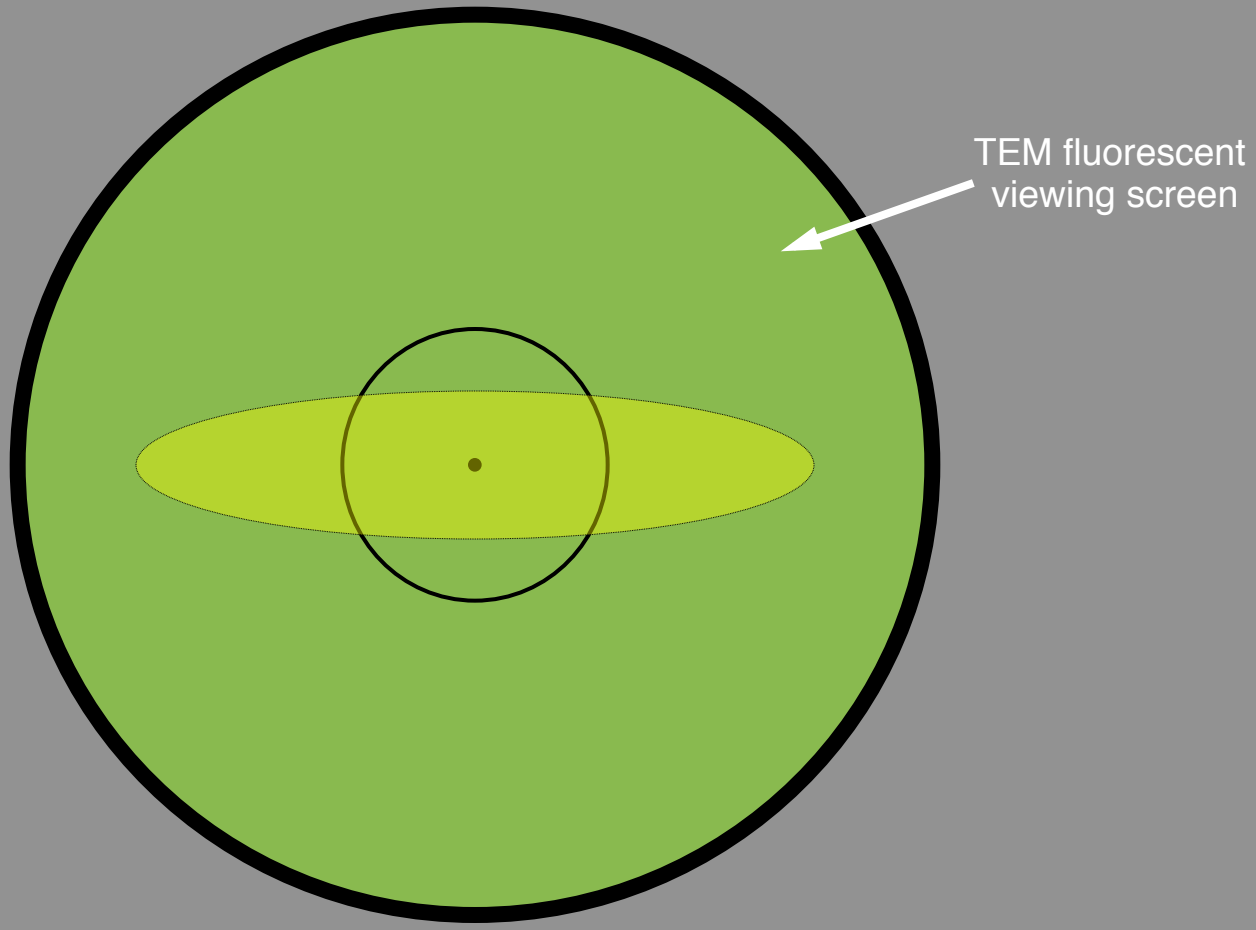
**Astigmatism** in the 2nd condenser lens leads to elongated, asymmetrical spot shape on either side of focus




From Agar, Fig. 4.6, p.129

# I.D.2 Alignment of TEM Components

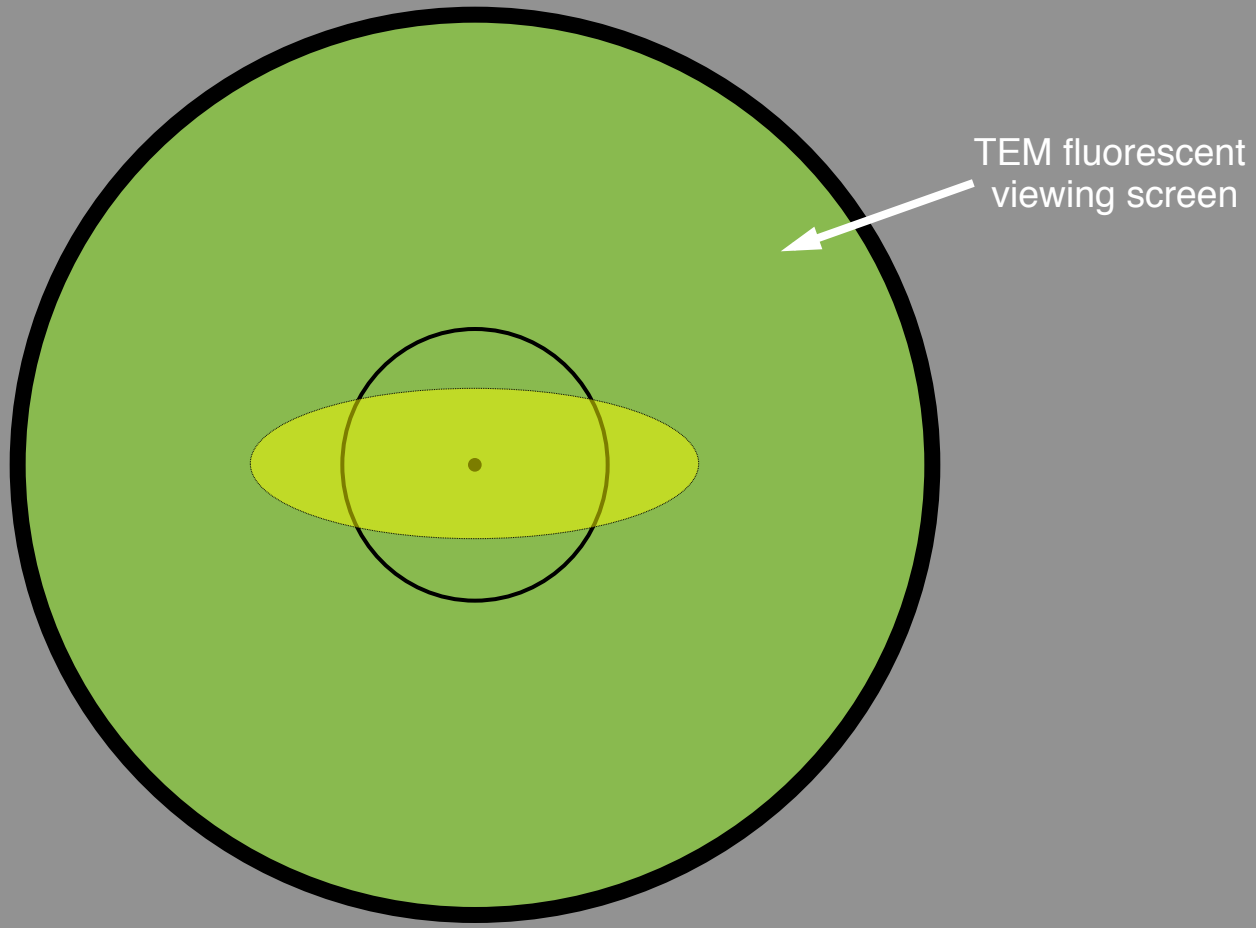
## I.D.2.b Condenser System




Simulation of Astigmatic Condenser System

# I.D.2 Alignment of TEM Components

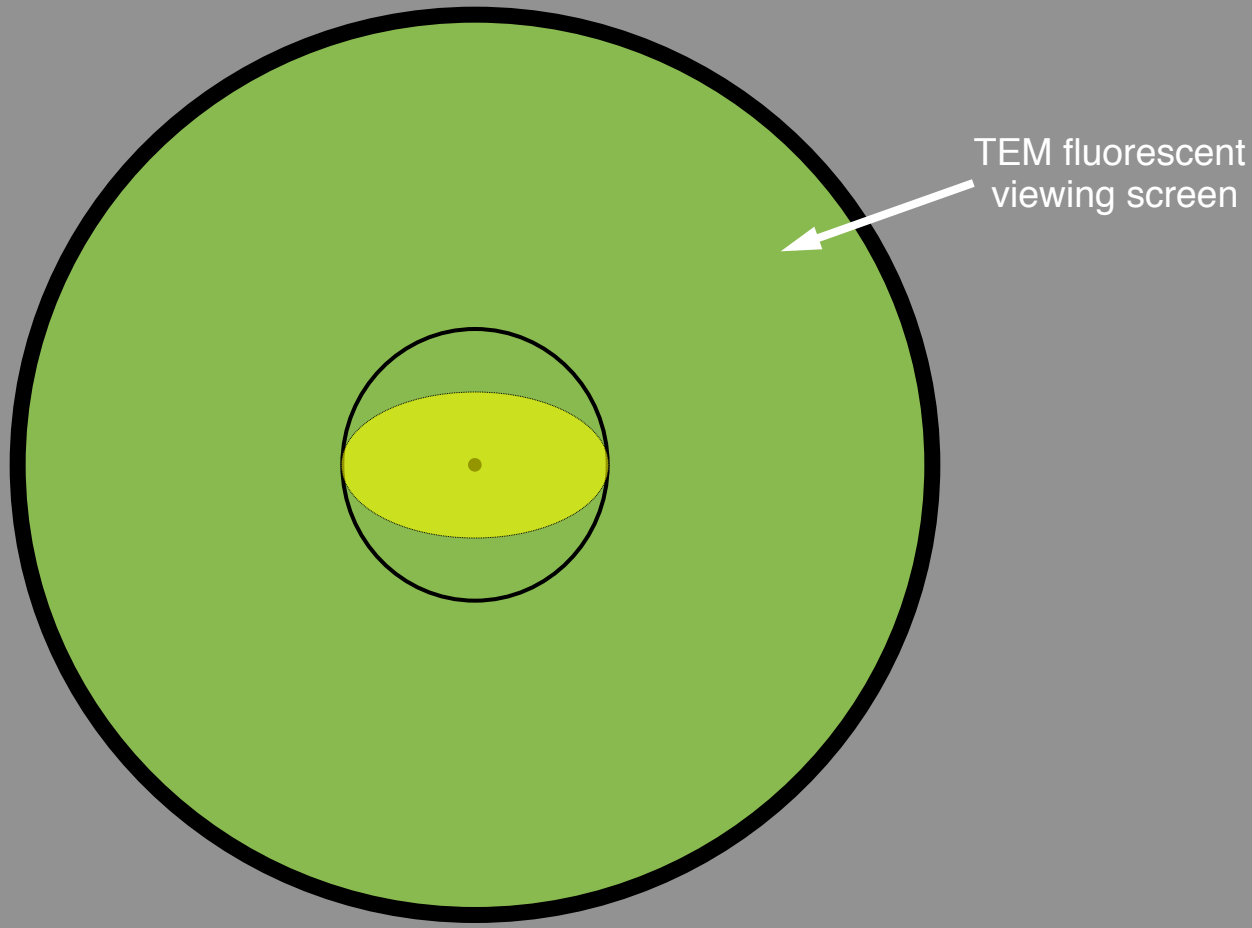
## I.D.2.b Condenser System




Simulation of Astigmatic Condenser System

# I.D.2 Alignment of TEM Components

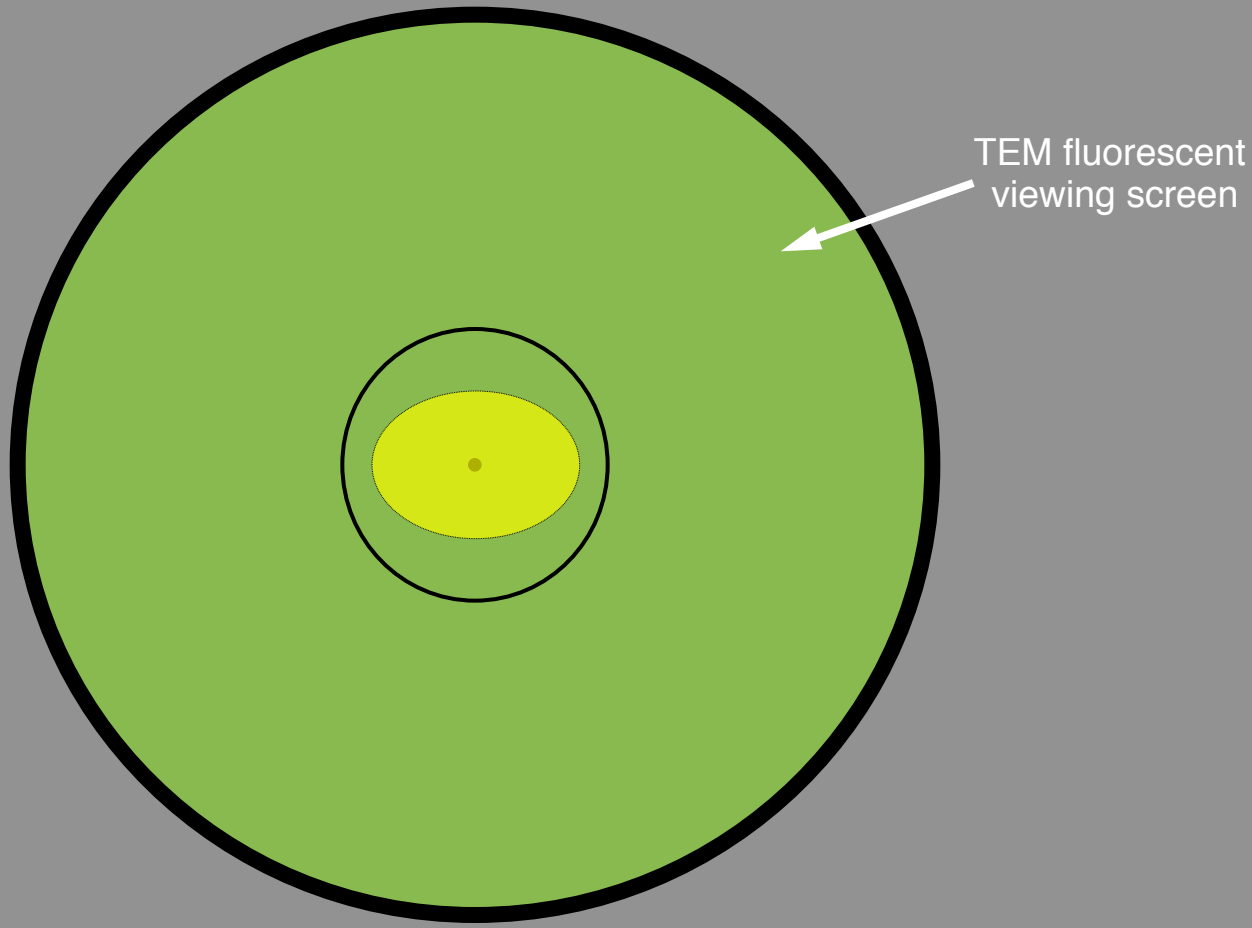
## I.D.2.b Condenser System




Simulation of Astigmatic Condenser System

# I.D.2 Alignment of TEM Components

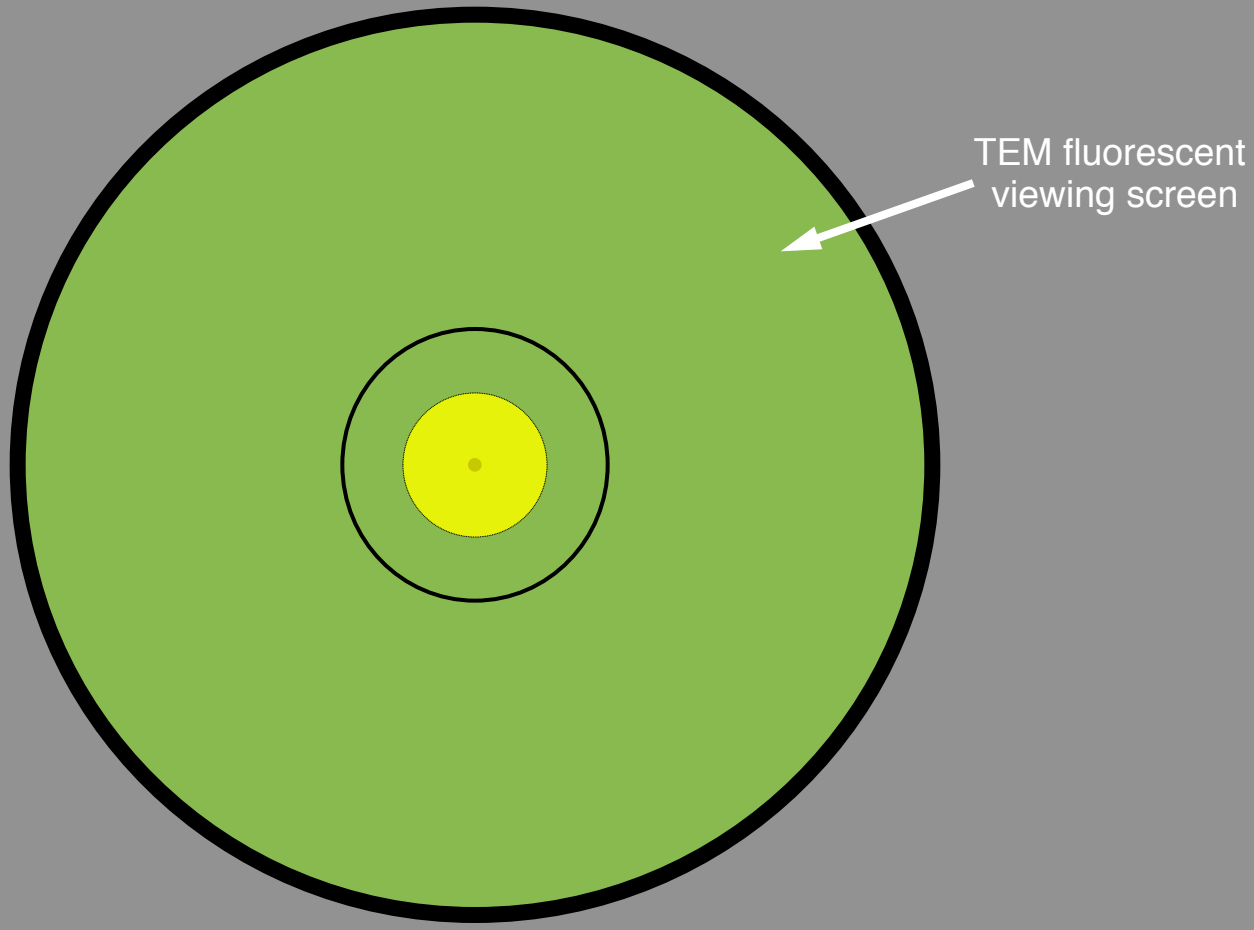
## I.D.2.b Condenser System




Simulation of Astigmatic Condenser System

# I.D.2 Alignment of TEM Components

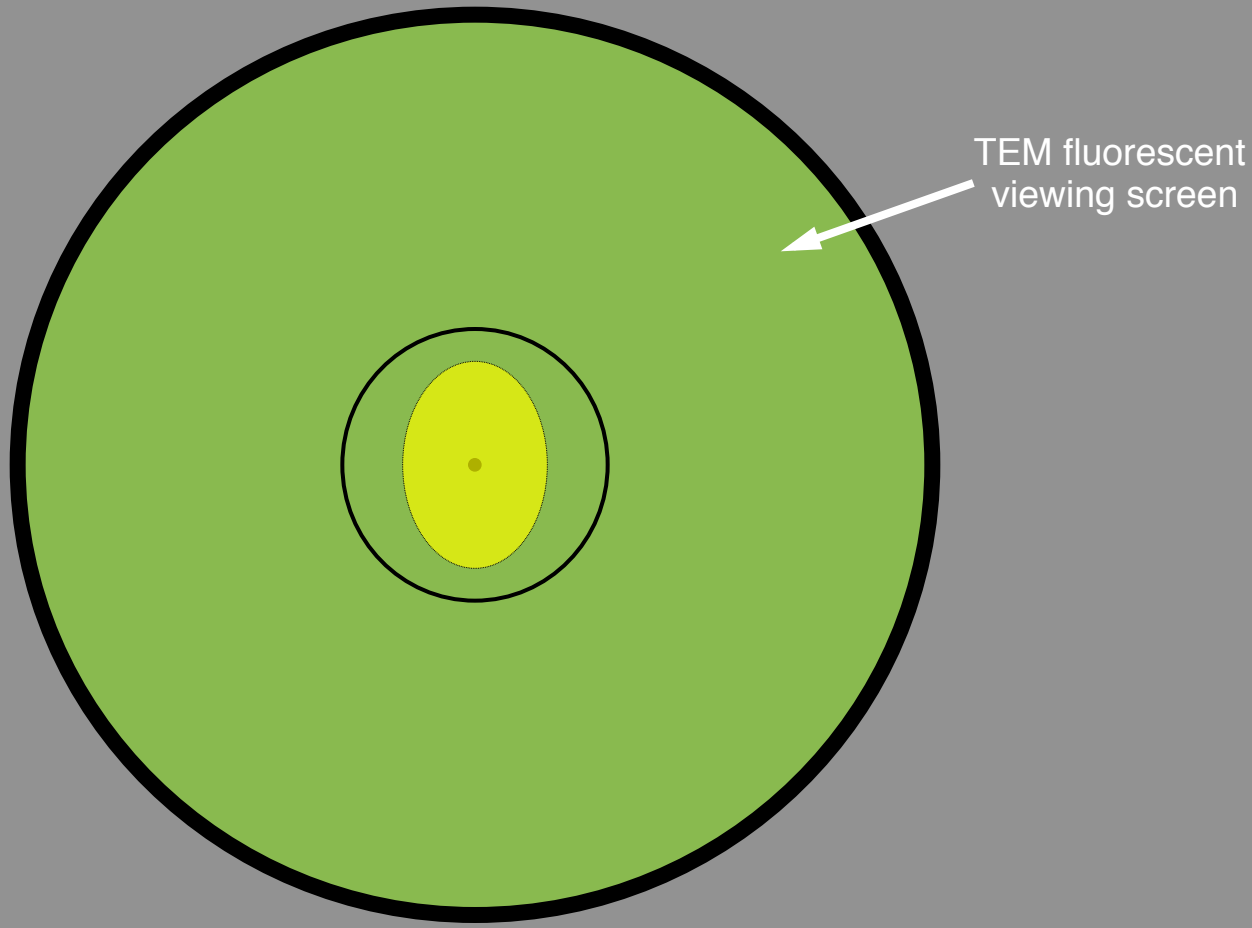
## I.D.2.b Condenser System




Simulation of Astigmatic Condenser System

# I.D.2 Alignment of TEM Components

## I.D.2.b Condenser System




Simulation of Astigmatic Condenser System

# I.D.2 Alignment of TEM Components

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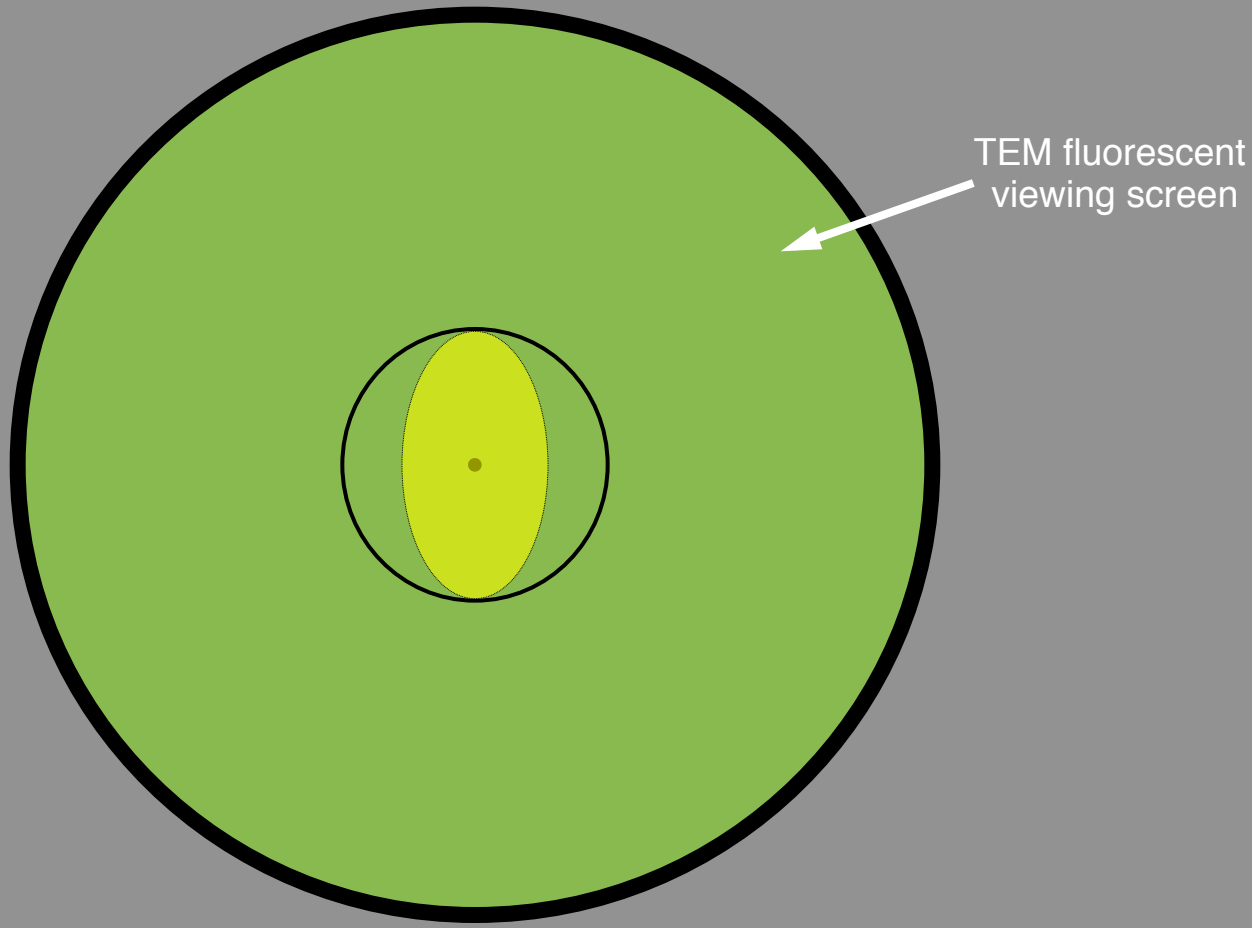
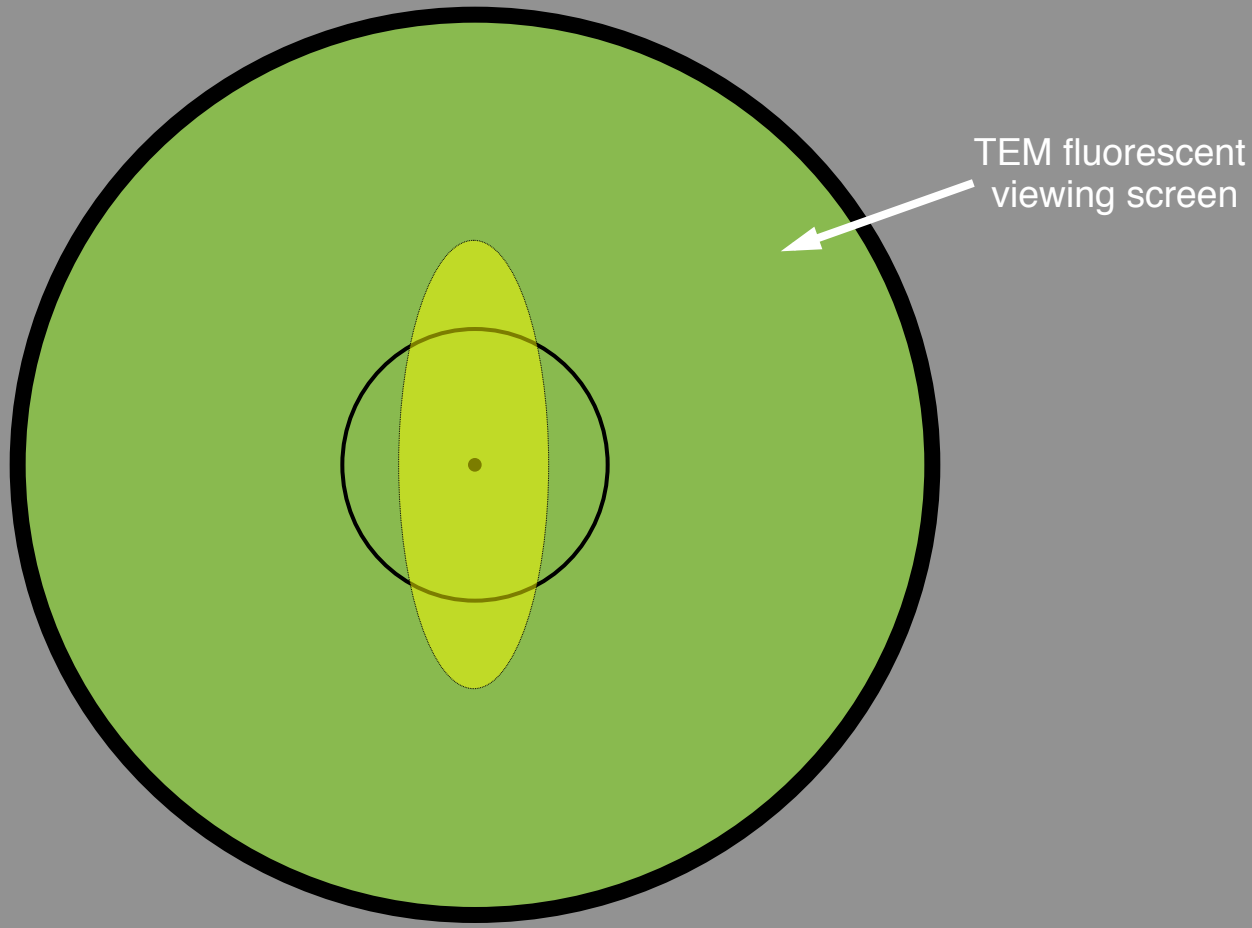



Simulation of Astigmatic Condenser System

# I.D.2 Alignment of TEM Components

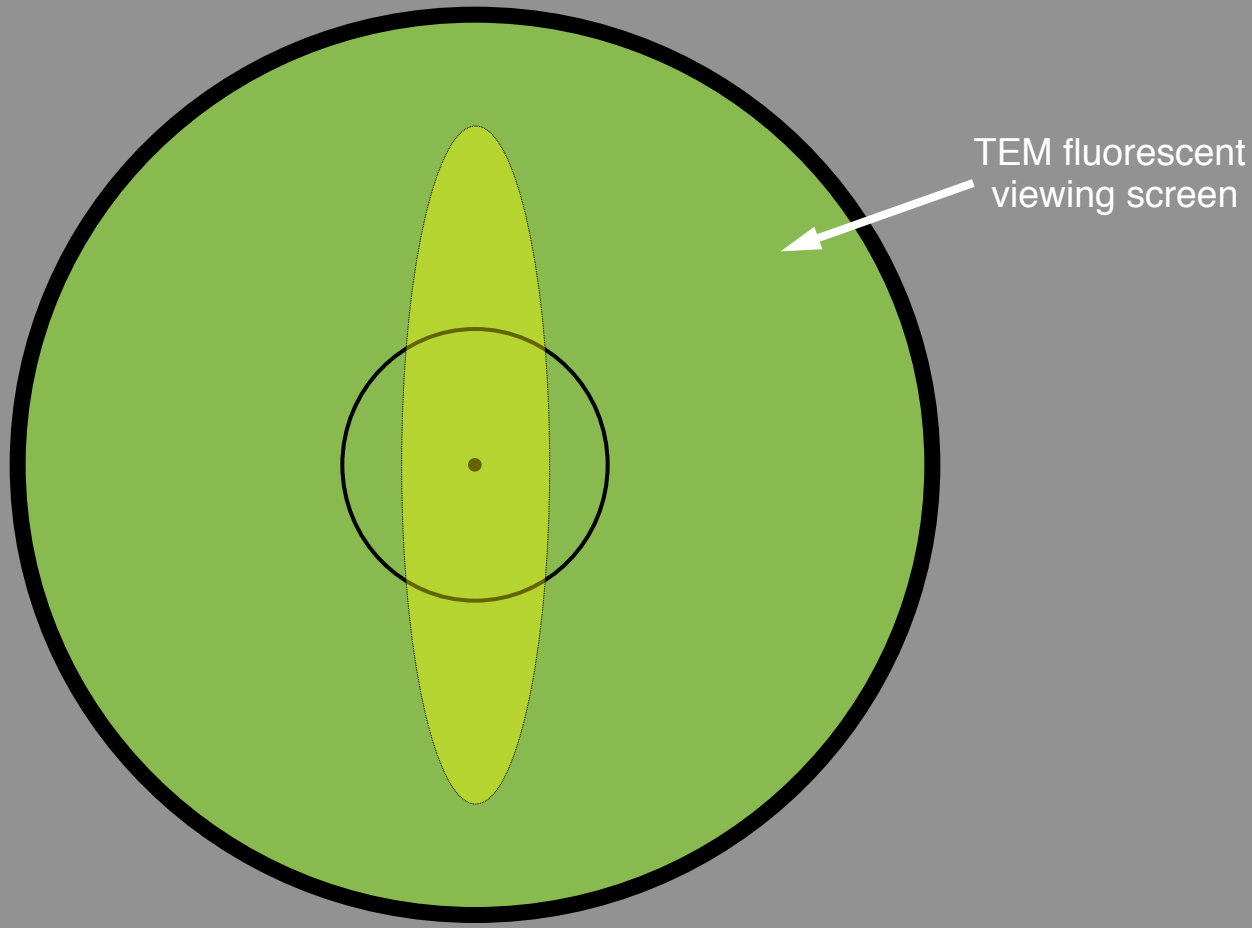
## I.D.2.b Condenser System




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.c Imaging System

### Alignment procedure:

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

### When all imaging lenses are in line:

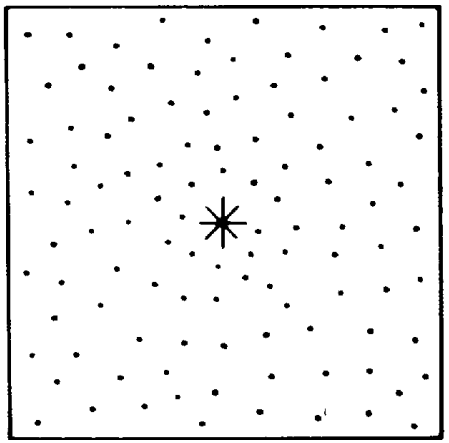
Object point **on** the objective lens axis will be imaged at the viewing screen center **WHATEVER** the magnification setting

# I.D.2 Alignment of TEM Components

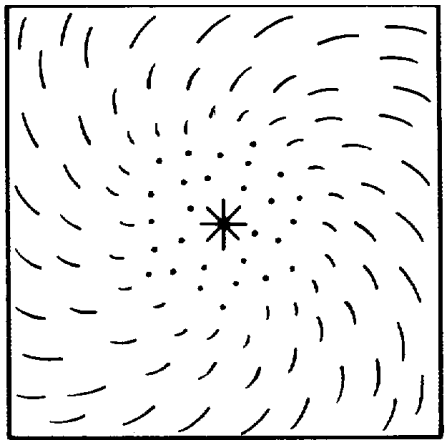
## I.D.2.c Imaging System

## Voltage Center

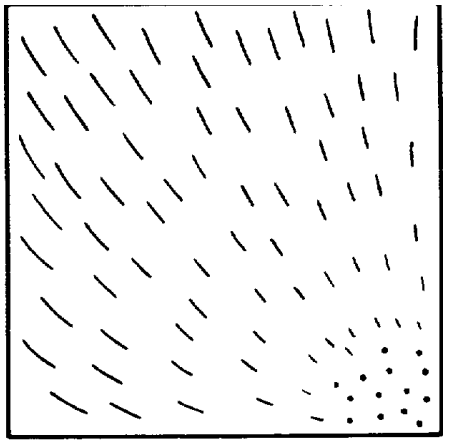
Effects of misalignment and high voltage ripple:



Ideal, aligned image (no high voltage fluctuations)



Aligned, but with voltage fluctuations



Misalignment and voltage fluctuations

*

From Slayter, Fig. 18-16, p.401

I.D.2 Alignment of TEM Components

I.D.2.c Imaging System

## Current Center

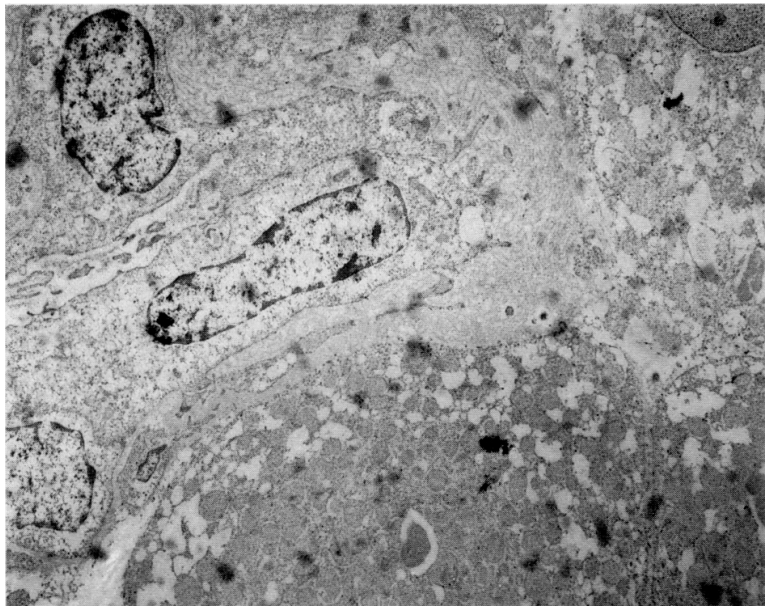
**Fluctuations in lens currents** also give rise to **superimposed images** of different magnifications and hence blurring

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

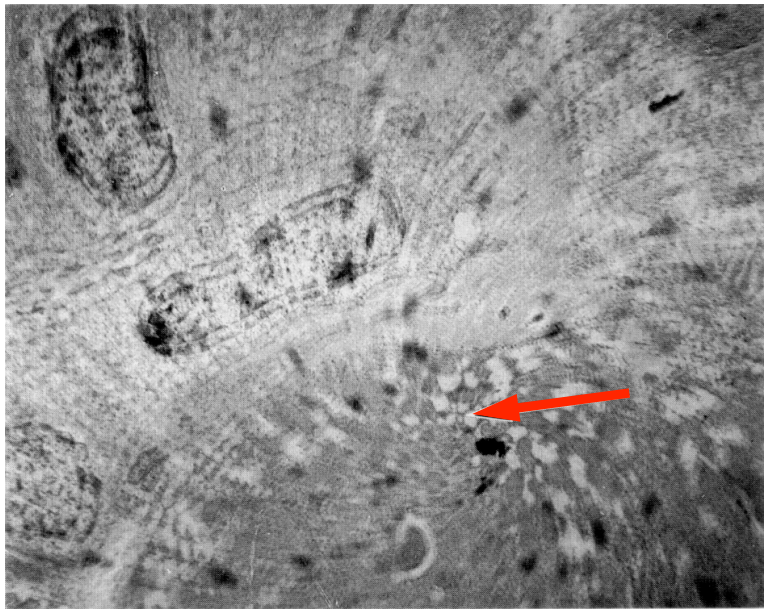


Image rotation caused by varying ("wobbling") the objective lens current. Rotation center marked by arrow identifies the optic axis of the lens

Slide not shown in class lecture

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

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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

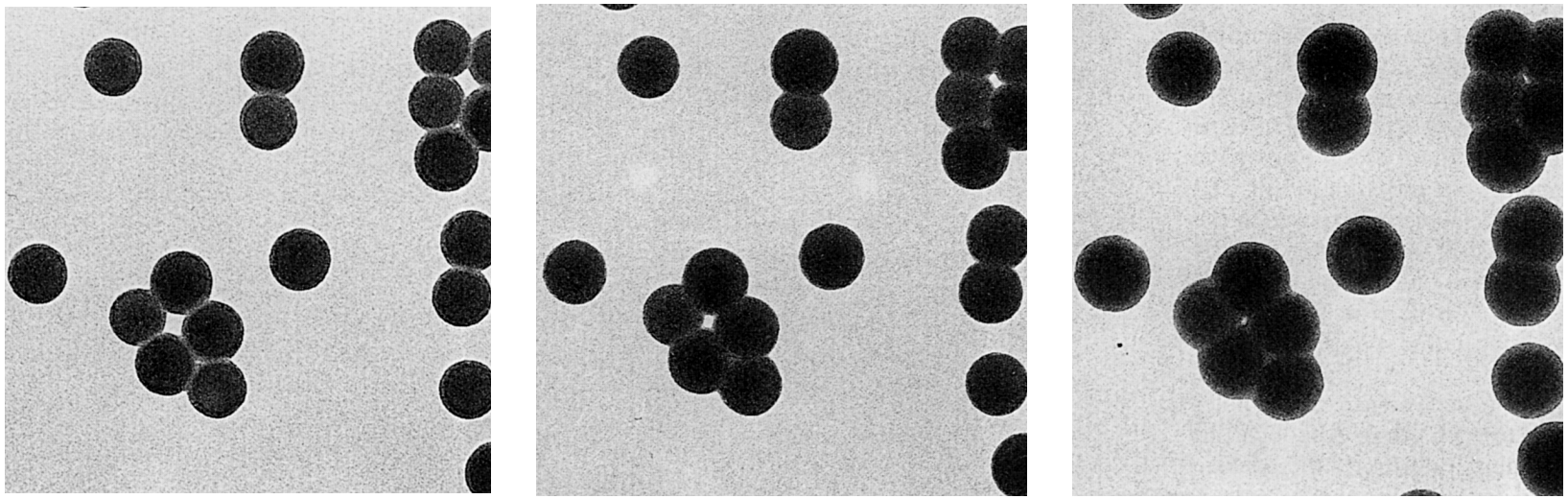
Deposition of a uniform layer of contamination on specimen **reduces** its **contrast**

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)**



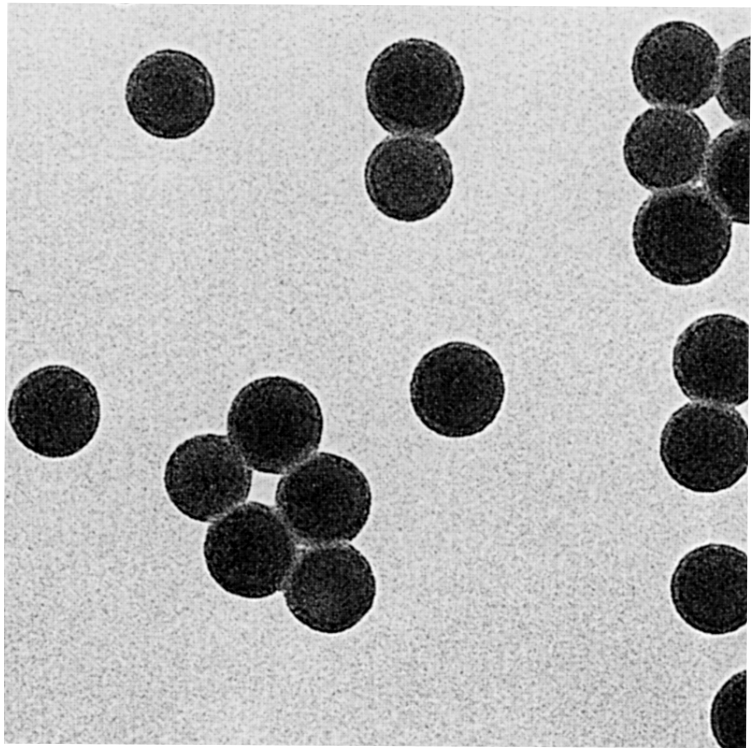
< 1 min. ~25 min. ~50 min.

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)**



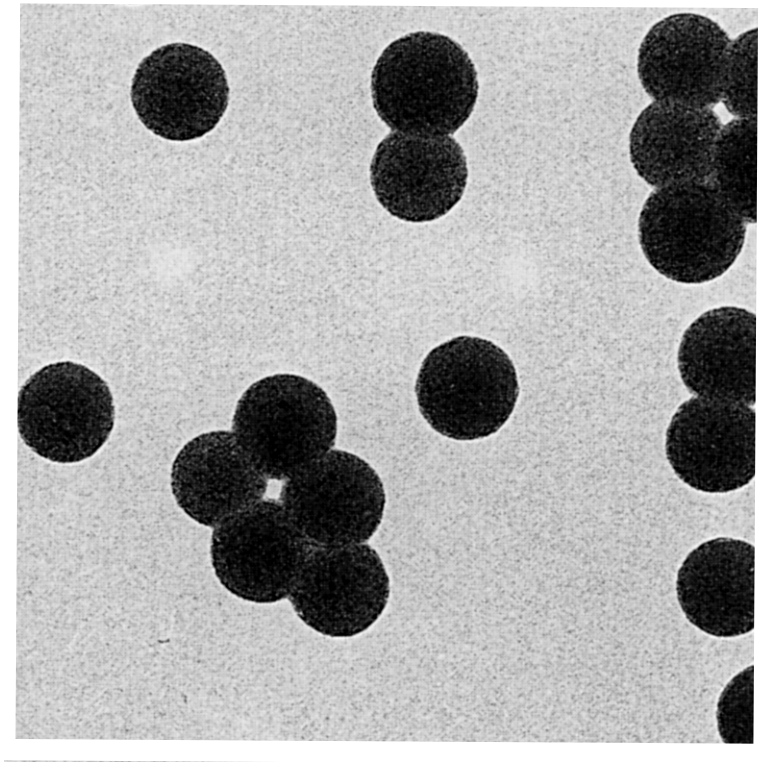
< 1 min.

From Maunsbach, Fig. 10-19A, p.267

# I.D.3 Disturbances to Microscope Performance

## I.D.3.a Contamination

**Latex Spheres (0.088 μm diameter)**



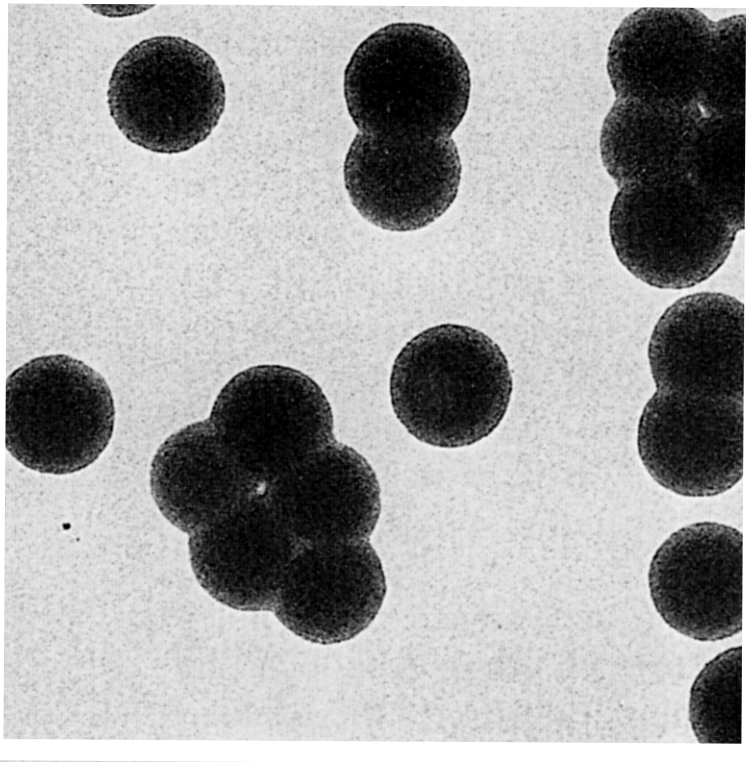
~25 min.

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)**



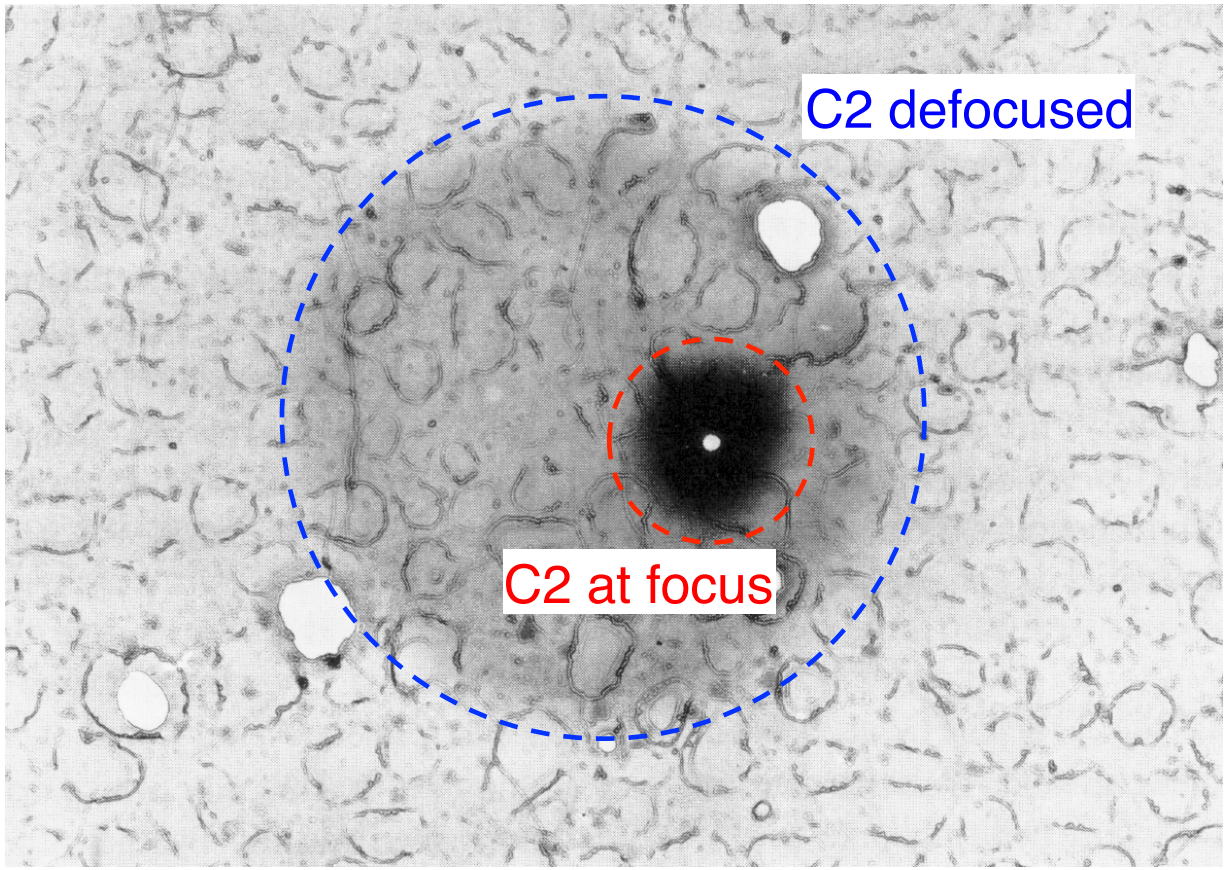
~50 min.

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.

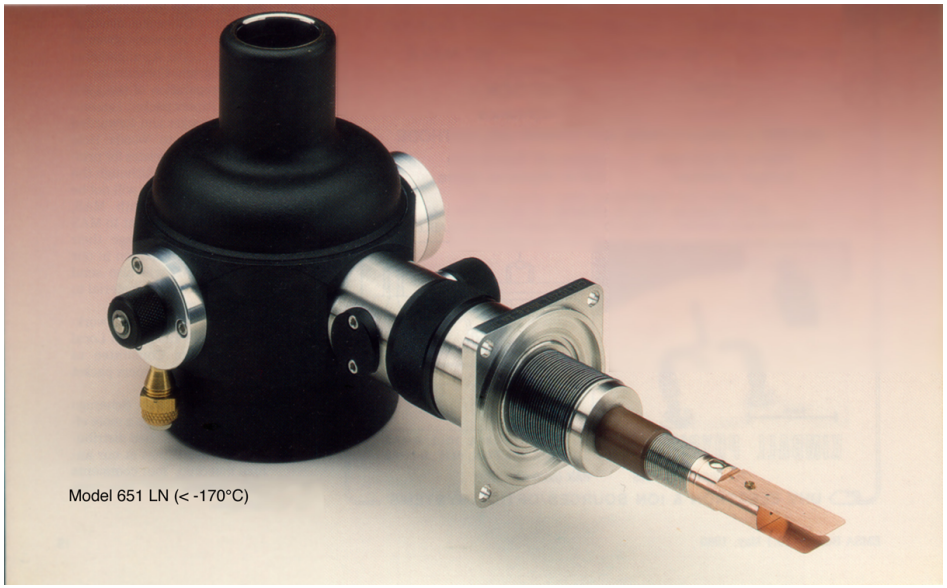
From Meek, 1st Ed., Fig. 6.6, p.150

# 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**




From www.gatan.com/holders/index.html

**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**

# 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 ~ **1-2 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

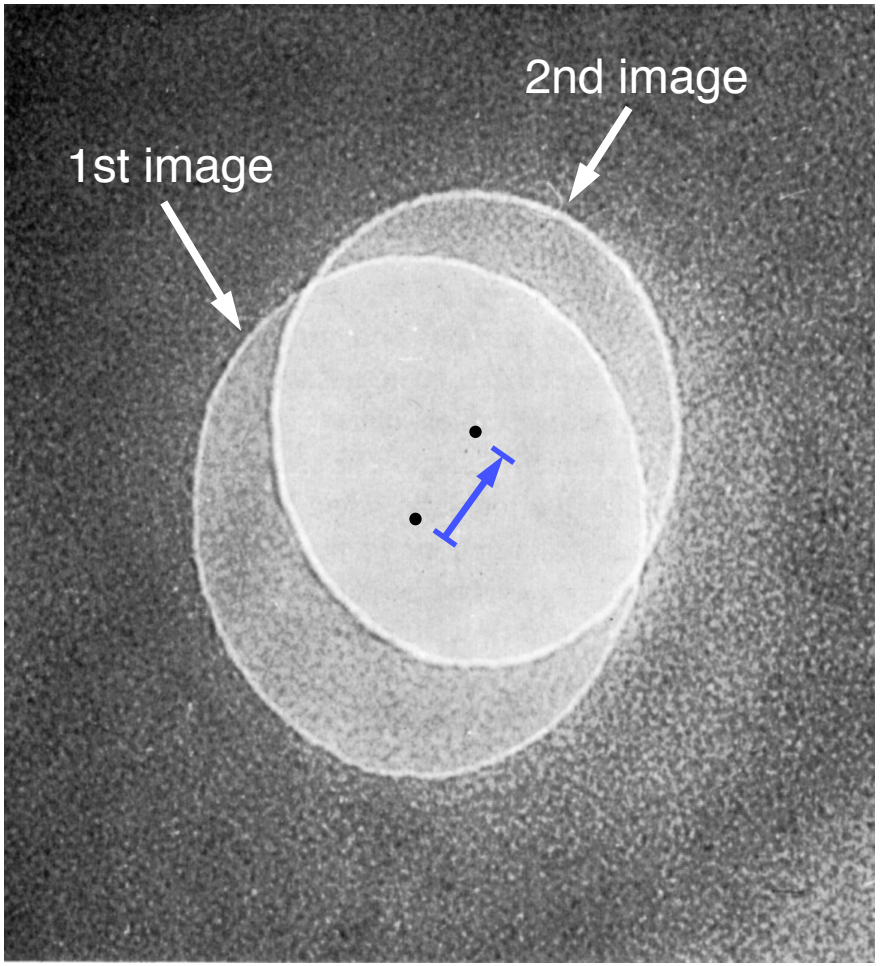
## I.D.3.b Image Drift and Mechanical Instabilities

## 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

# I.D.3 Disturbances to Microscope Performance

## I.D.3.b Image Drift and Mechanical Instabilities

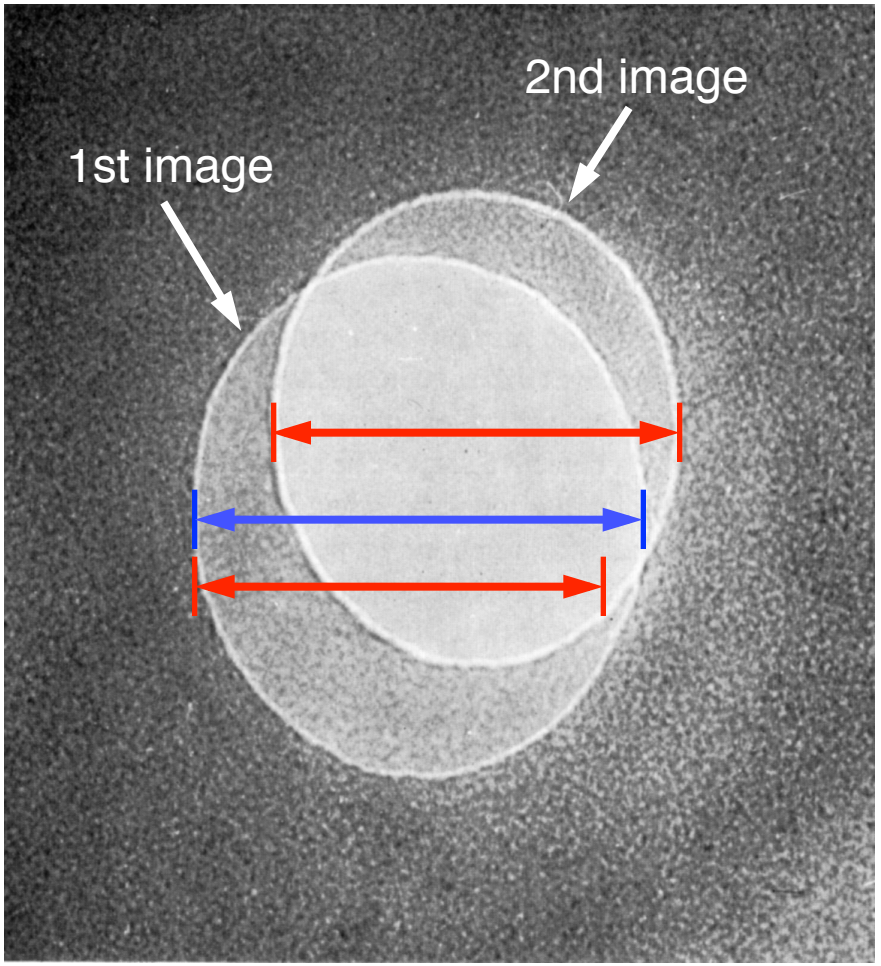
## Thermal Drift of Specimen

### Measuring Drift Rate

- Record **double exposure** of any "simple" object

- Shows magnitude of specimen drift between exposures

- Also allows measurement of TEM **contamination rate** (deposit of contamination layer shows up as a reduction in hole diameter)




From Agar, Fig. 5.7, p.154

# 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)

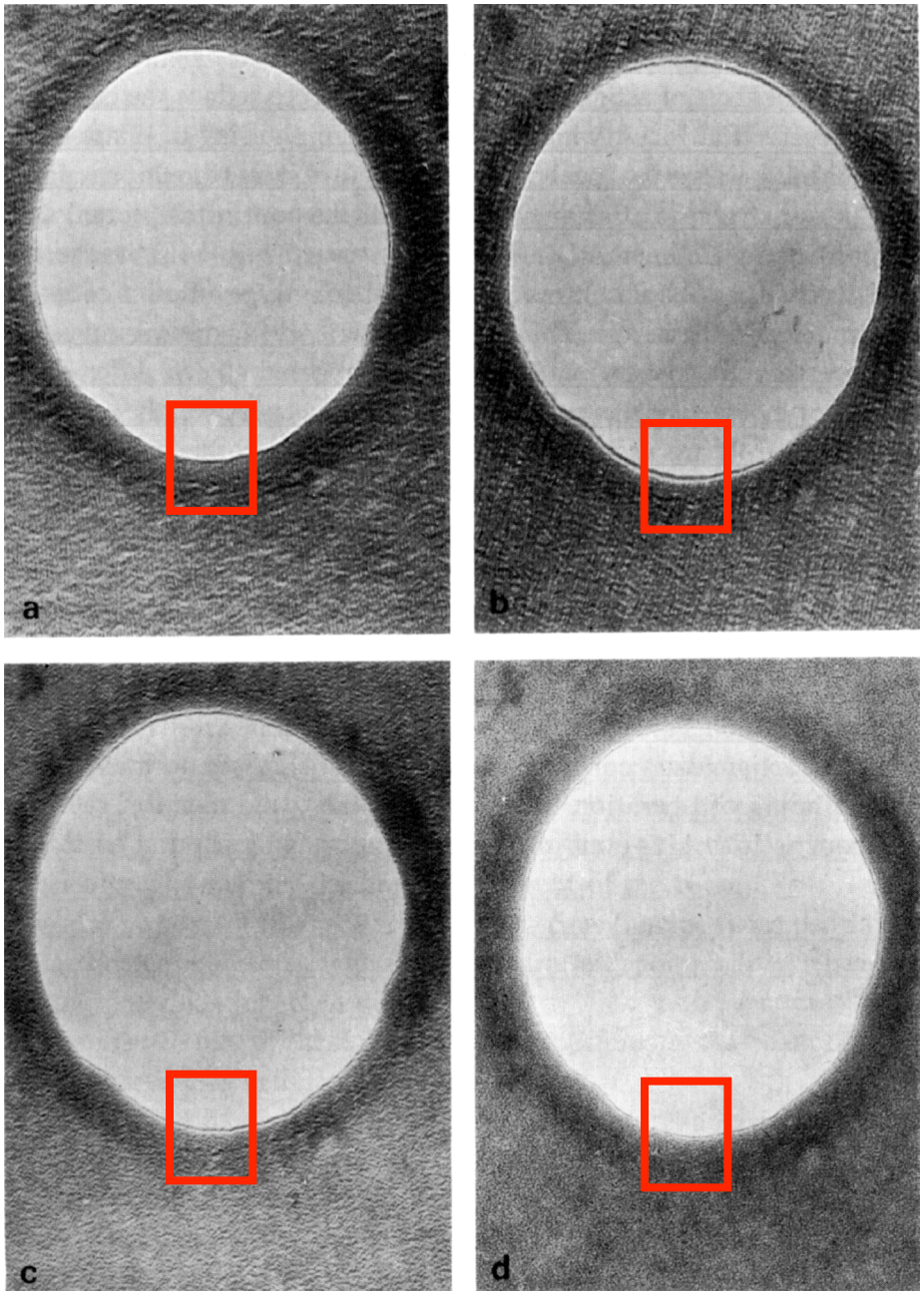
Image of small hole in carbon support film

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 stigmate the image (uniform fringe)




From Agar, Fig. 4.12, p.139

# I.D.3.d Image Astigmatism

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### (Holey Film Method)

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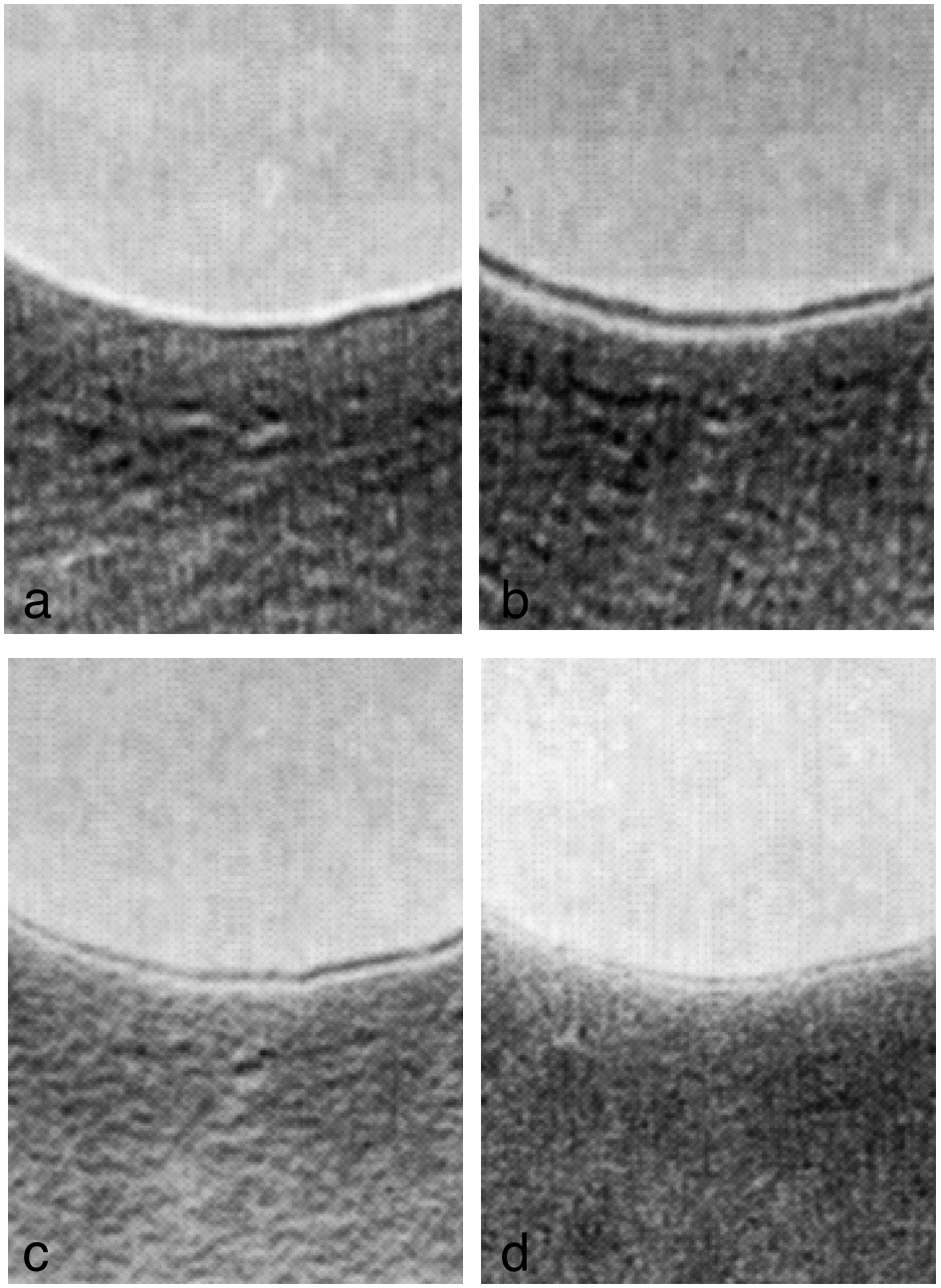
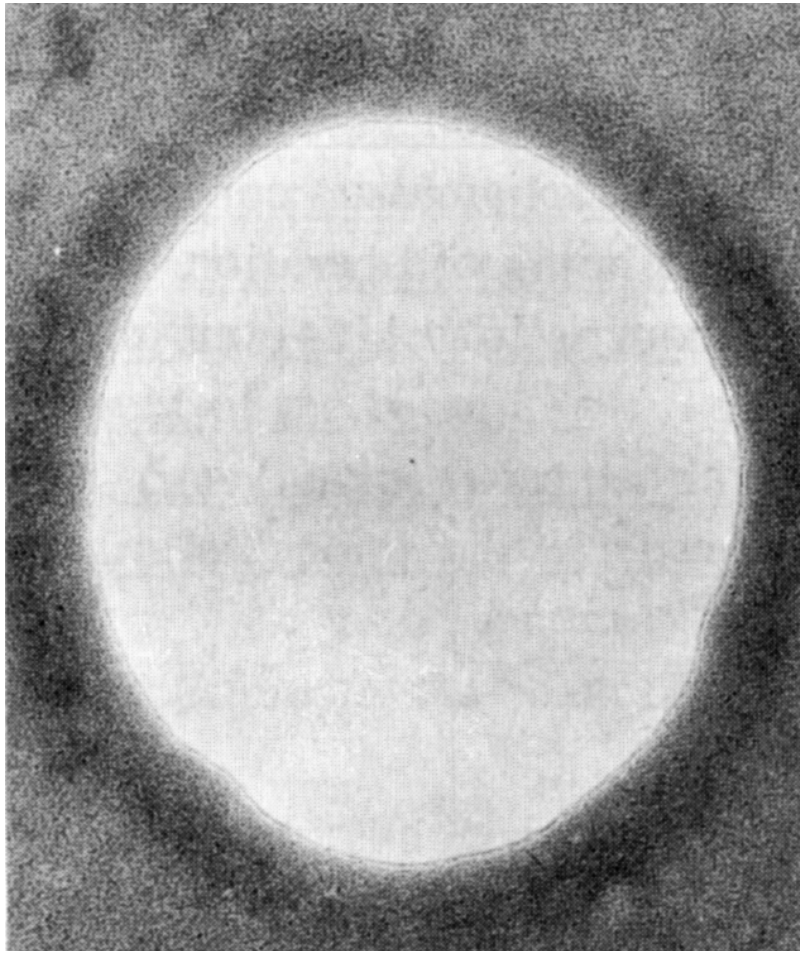



From Agar, Fig. 4.12, p.139

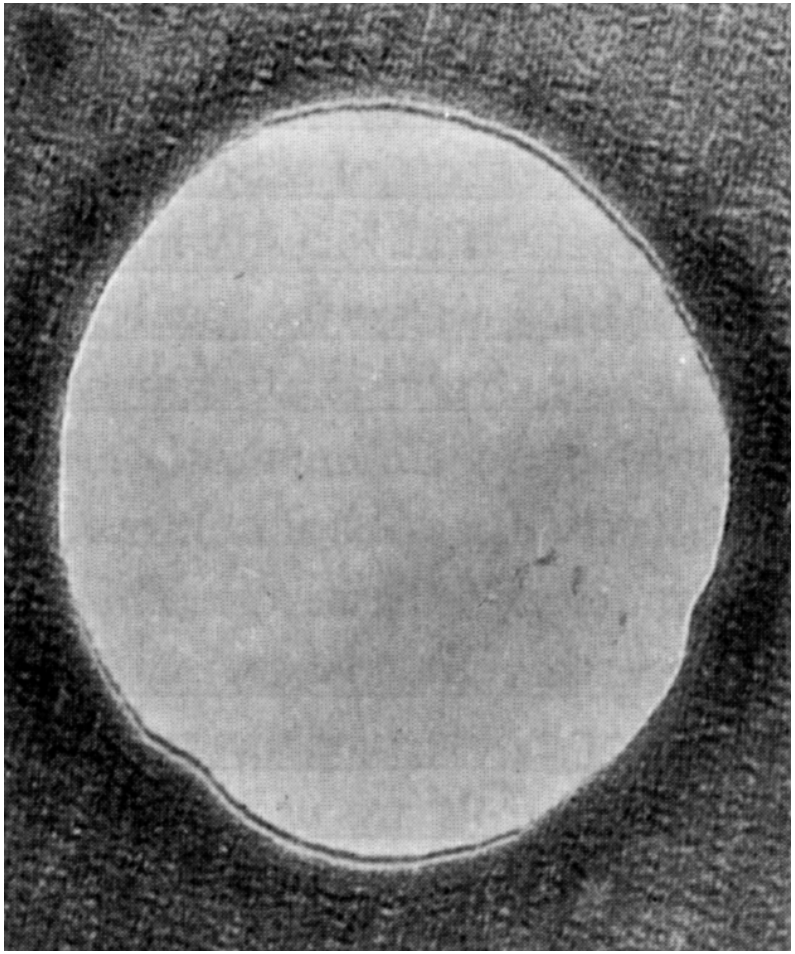
# I.D.3 Disturbances to Microscope Performance

## I.D.3.d Image Astigmatism

### Correcting Astigmatism (Holey Film Method)



Stigmated Image



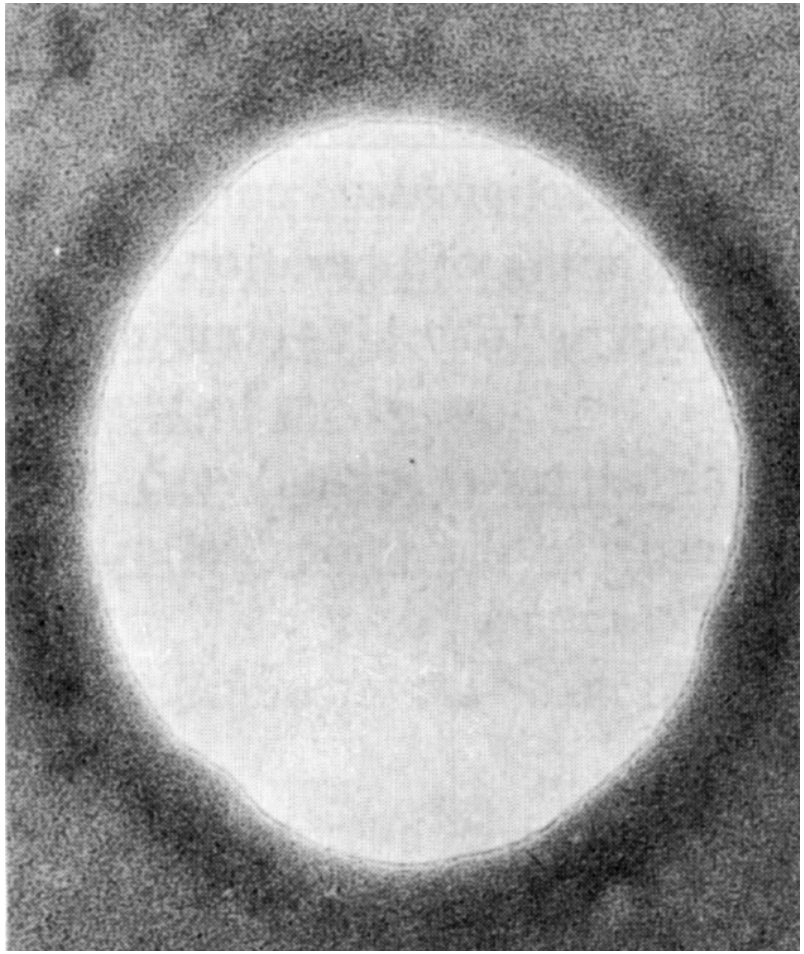
Astigmatic Image

From Agar, Fig. 4.12, p.139

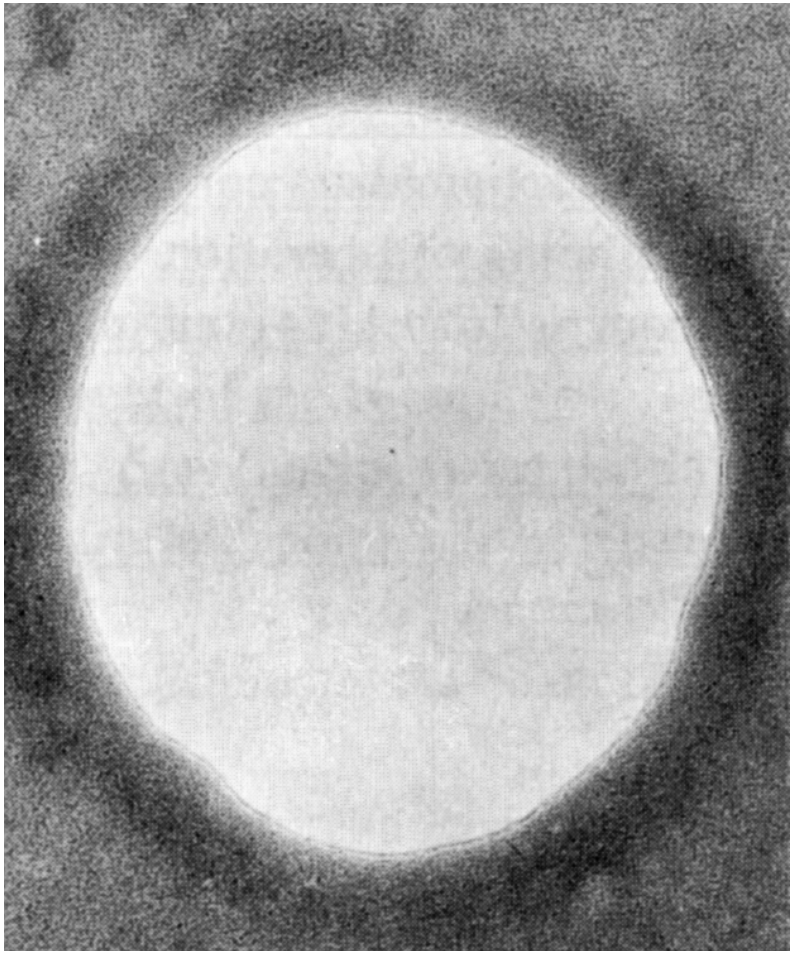
# I.D.3 Disturbances to Microscope Performance

## I.D.3.d Image Astigmatism

### Correcting Astigmatism (Holey Film Method)



Stigmated Image



Stigmated Image

From Agar, Fig. 4.12, p.139

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

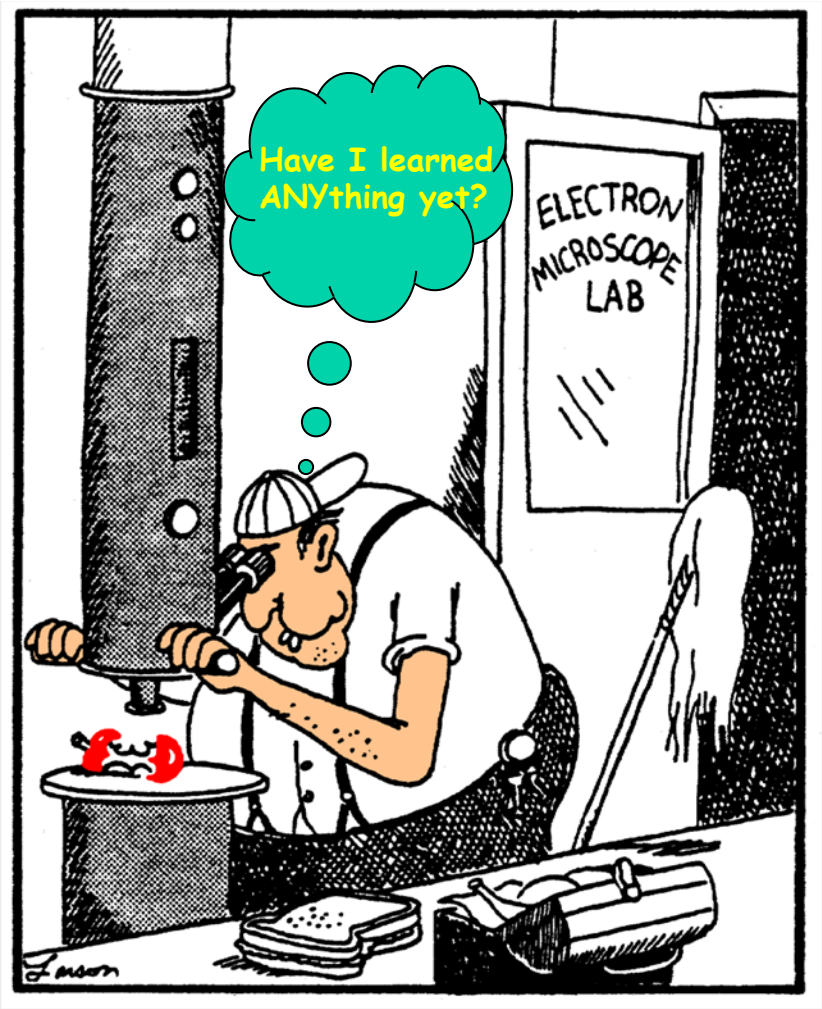
I.B Design of the TEM

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

# 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 λ **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**

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6. Quality of image capture by CCD **decreases**
7. Electron gun **more sensitive** to vacuum quality - high voltage stability **decreases**
8. Electron gun brightness **increases**
9. Efficiency of screen phosphor **increases**

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