# III.D FOURIER IMAGE PROCESSING TECHNIQUES

## III.D.1 Optical Diffraction

OD the simplest and classically the most widely practiced Fourier image processing technique

- Initial step in many image processing studies

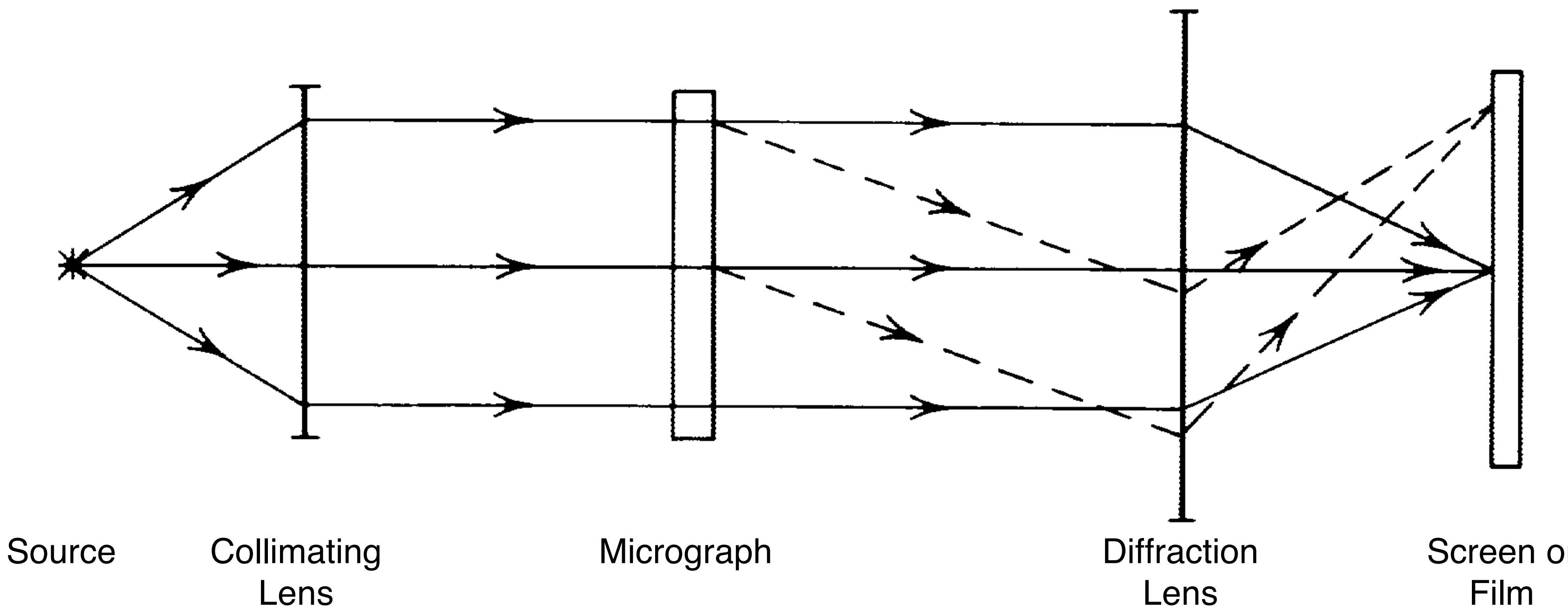
Main advantage:

Objective way to assess and reveal periodic structural information

Klug and Berger (1964) were 1st to use an optical bench to examine diffraction patterns of electron micrographs and analyze biological structure in images of stained specimens

# III.D.1 Optical Diffraction

## III.D.1.a Forming the Diffraction Pattern

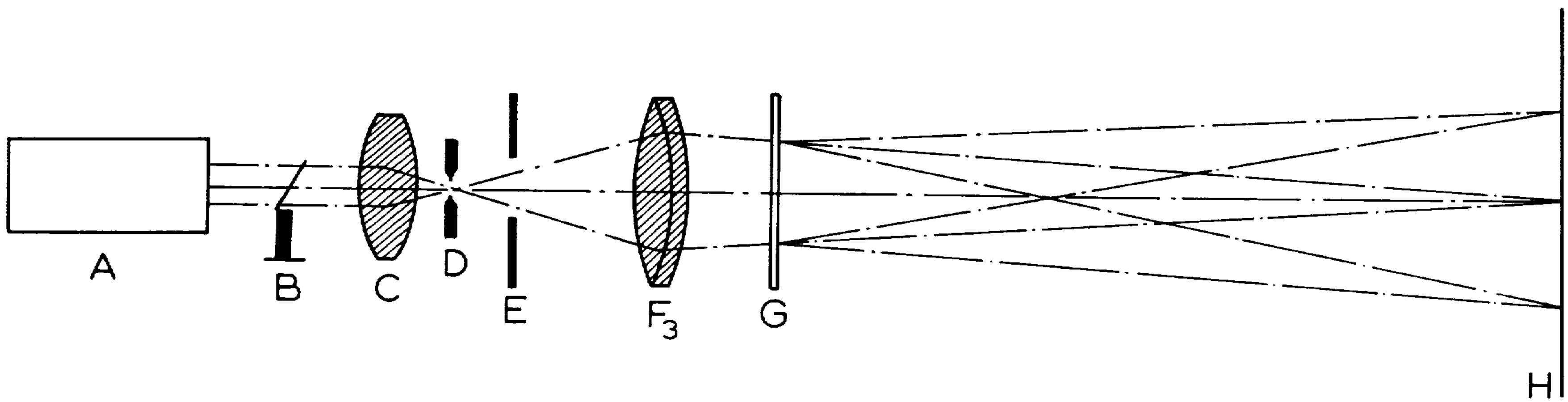



Adapted from Slayter, Fig. 19-12a, p.448

# III.D.1 Optical Diffraction

## III.D.1.b Experimental Apparatus

### Simple (Linear) Optical Diffractometer



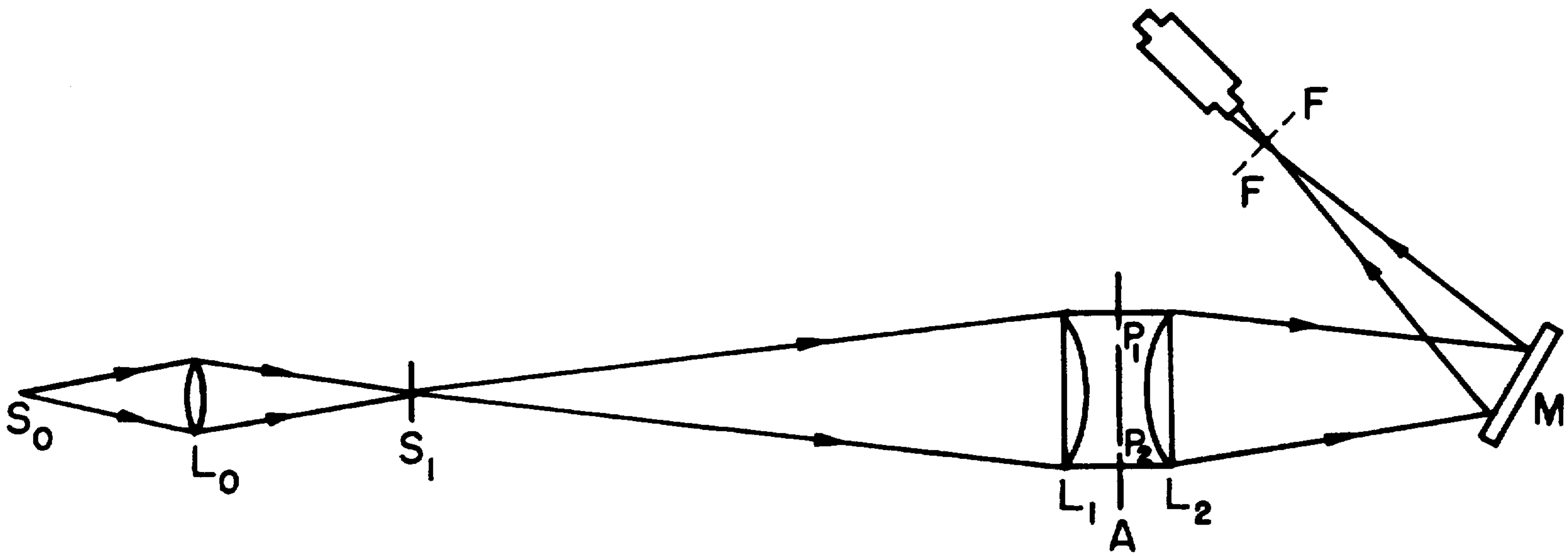

- A. Laser
- B. Shutter
- C. Beam expanding lens
- D. Pinhole
- E. Adjustable diaphragm
- F3. Diffraction lens
- G. Micrograph
- H. Viewing screen or camera

From Horne & Markham, Fig. 1.3, p.336

# III.D.1 Optical Diffraction

## III.D.1.b Experimental Apparatus

## Folded Optical Diffractometer




$S_o$ Radiation source
$L_0$ Beam expanding lens
$S_1$ Pinhole
$L_1$ Collimating lens
A Micrograph
$L_2$ Diffraction lens
M Mirror
F Diffraction pattern focal plane

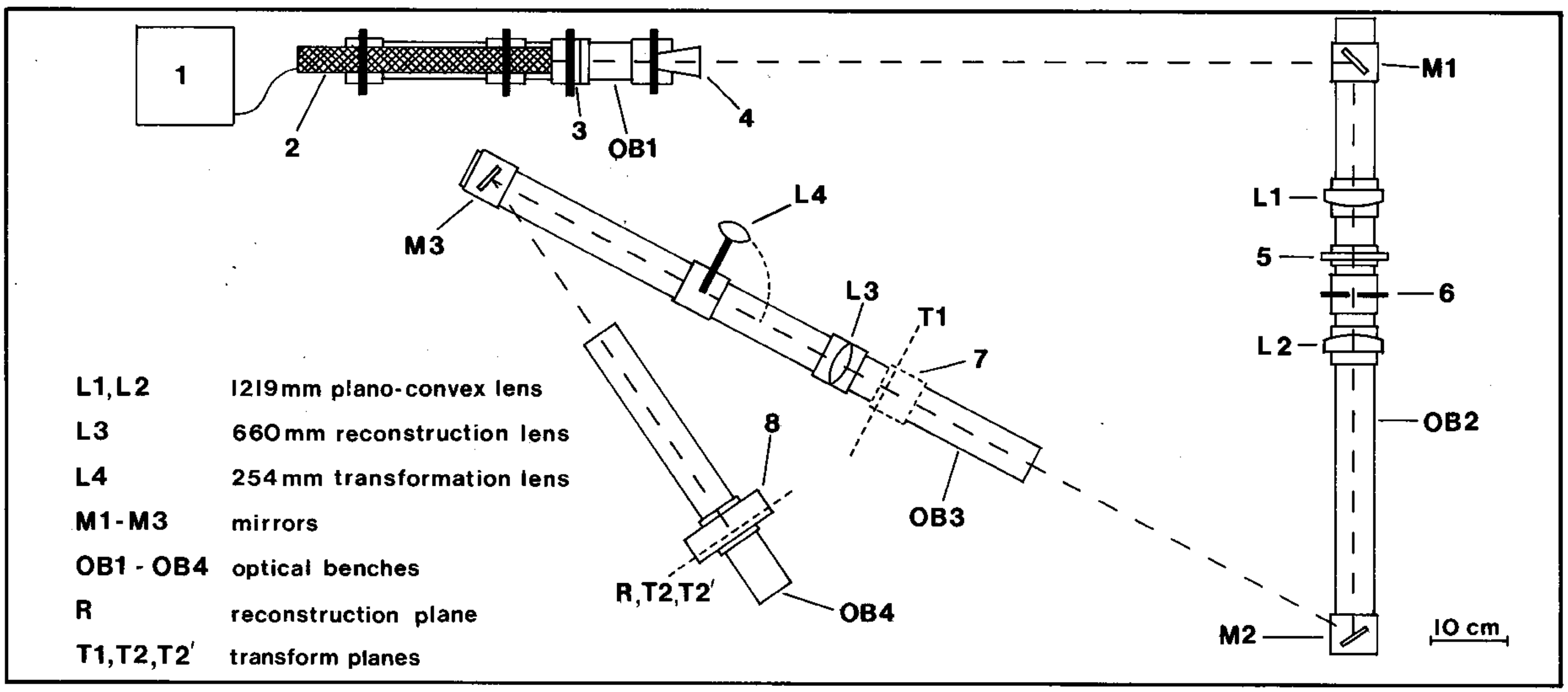
From Thompson, Fig. 12, p.48

# III.D.1 Optical Diffraction

## III.D.1.b Experimental Apparatus

## The UCLA Folded Optical Diffractometer (Circa 1972)

FOLDED OPTICAL DIFFRACTOMETER




1 power supply
2 4.5mW He–Ne laser
3 polarizing filter
4 spatial filter & beam expander
5 EM plate holder
6 limiting aperture
7 camera or filter mask position
8 35mm camera body

From Baker thesis, Fig. C-1, p.162

# III.D.1 Optical Diffraction

## III.D.1.c Applications

- Accurate measurement of **lattice parameters** (unit cell dimensions)

- Detect rotational and translational **symmetry elements**

- Detect and measure specimen **preservation** (distortions, overall resolution, radiation damage) for selecting best images for further image analysis

- Assess short and long range **order** in periodic specimens

- Identify **signal and noise** in images

- Determine **electron optical conditions**, i.*e.* contrast transfer function (focus, drift, astigmatism, etc.) at time micrograph was recorded

- Superb **teaching device** (principles of diffraction, symmetry and Fourier transforms)

# III.D.1 Optical Diffraction

## III.D.1.c Applications

**A few more…**

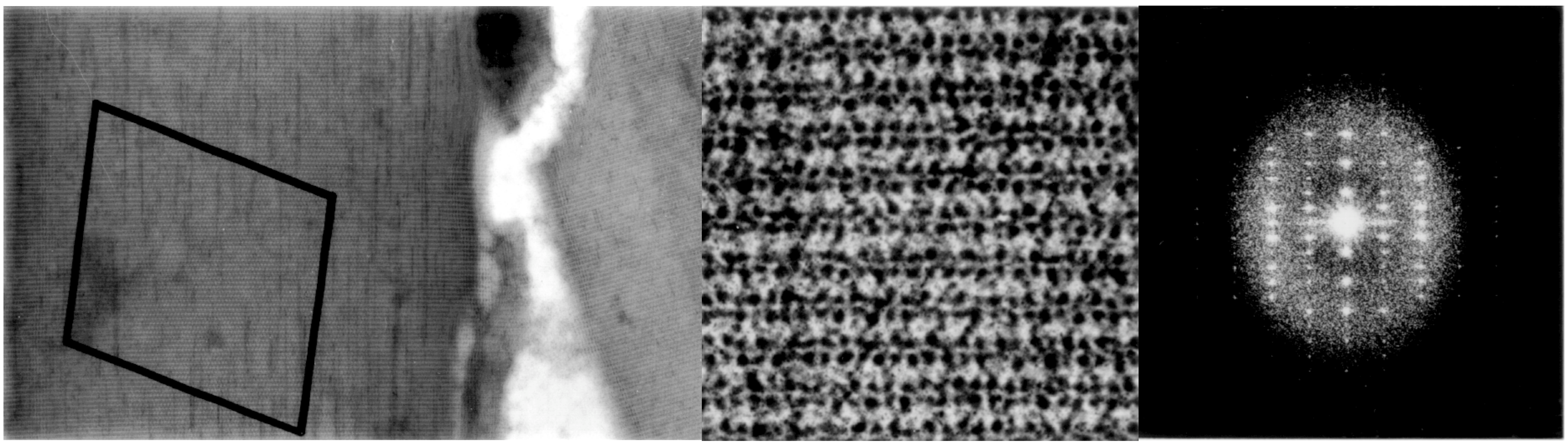
- Able to examine **small regions** of specimen

- Determine **relative orientation** of multilayered specimens (*e.g.* stacked 2D sheets or opposite sides of two-sided structures)

- Determine the **hand** of 3D structures (from metal-shadowed or tilted specimens)

# III.D.1 Optical Diffraction

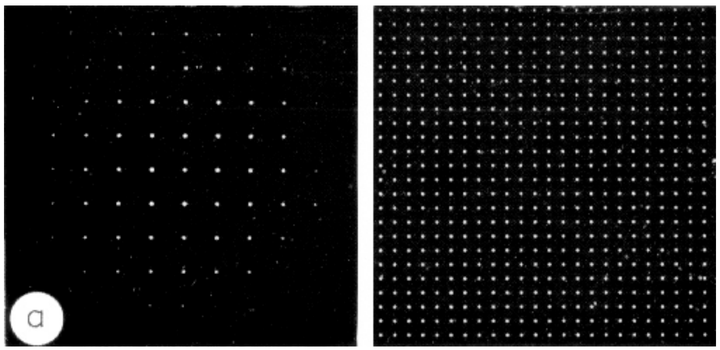
## Optical Diffraction of Negatively Stained Catalase Crystal



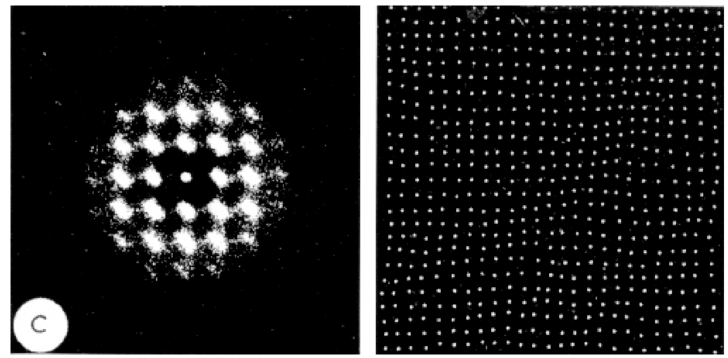
From Baker thesis, Fig. B-5, p.109

# III.D.1 Optical Diffraction

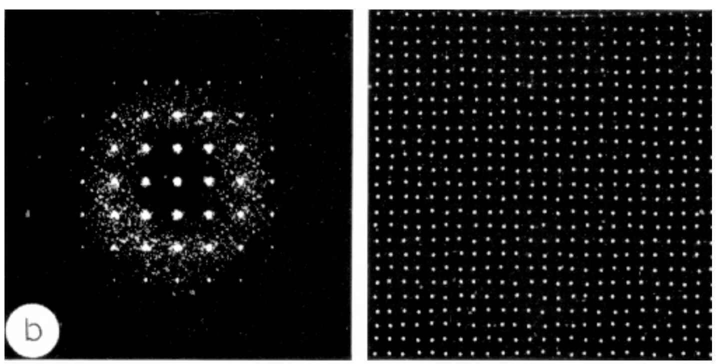
## Effect of Lattice Disorder on Diffraction Pattern



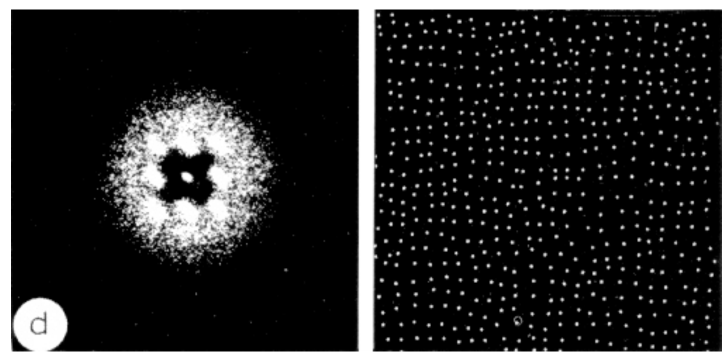

Ordered lattice




2-D disorder of ±10%




Vertical disorder of ±10%

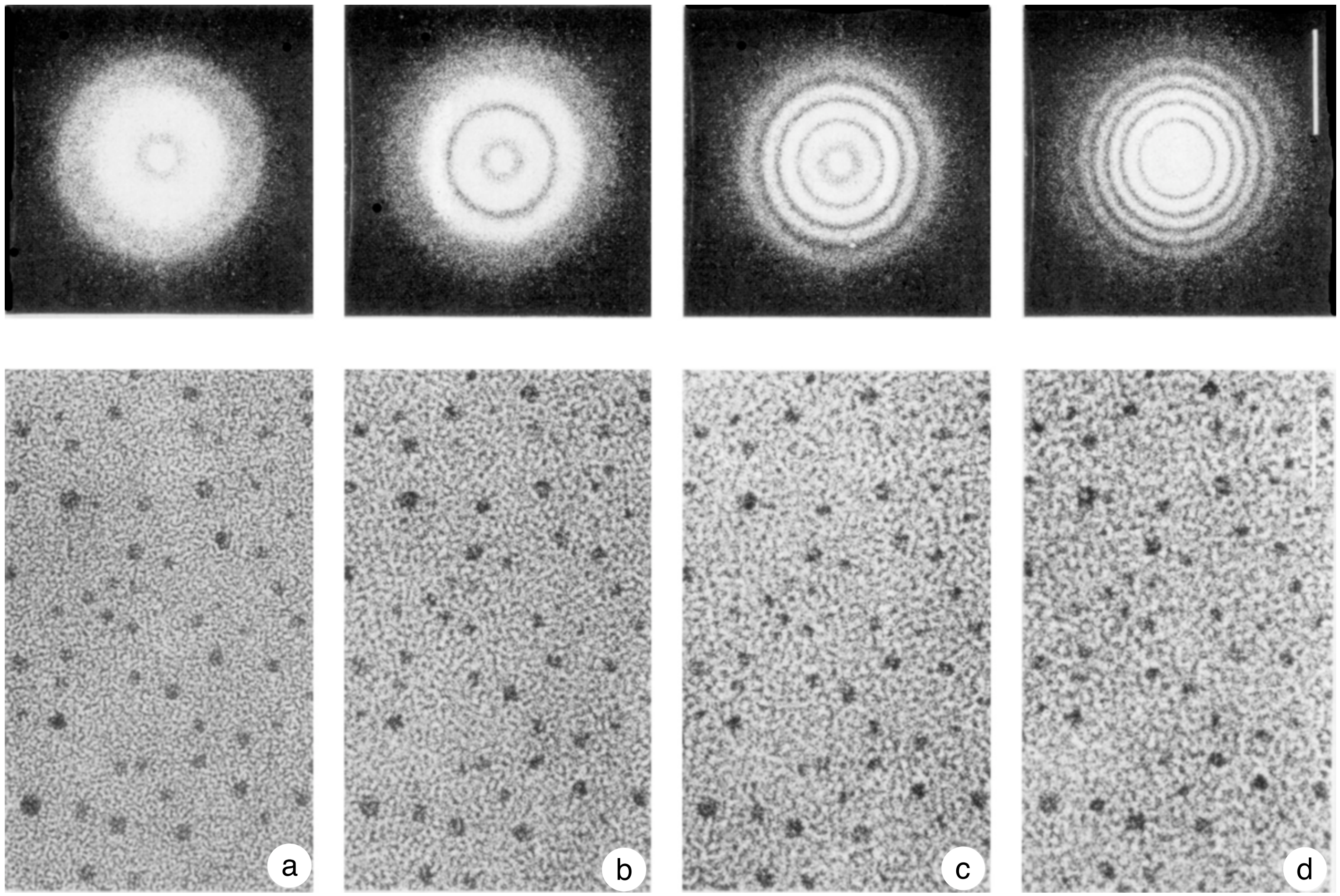



2-D disorder of ±25%

From Misell, Fig. 3.17, p.72

# III.D.1 Optical Diffraction

## Focus Series of Thin Carbon Film with Gold Atoms




Optimum defocus | -150 nm | -210 nm | -250 nm

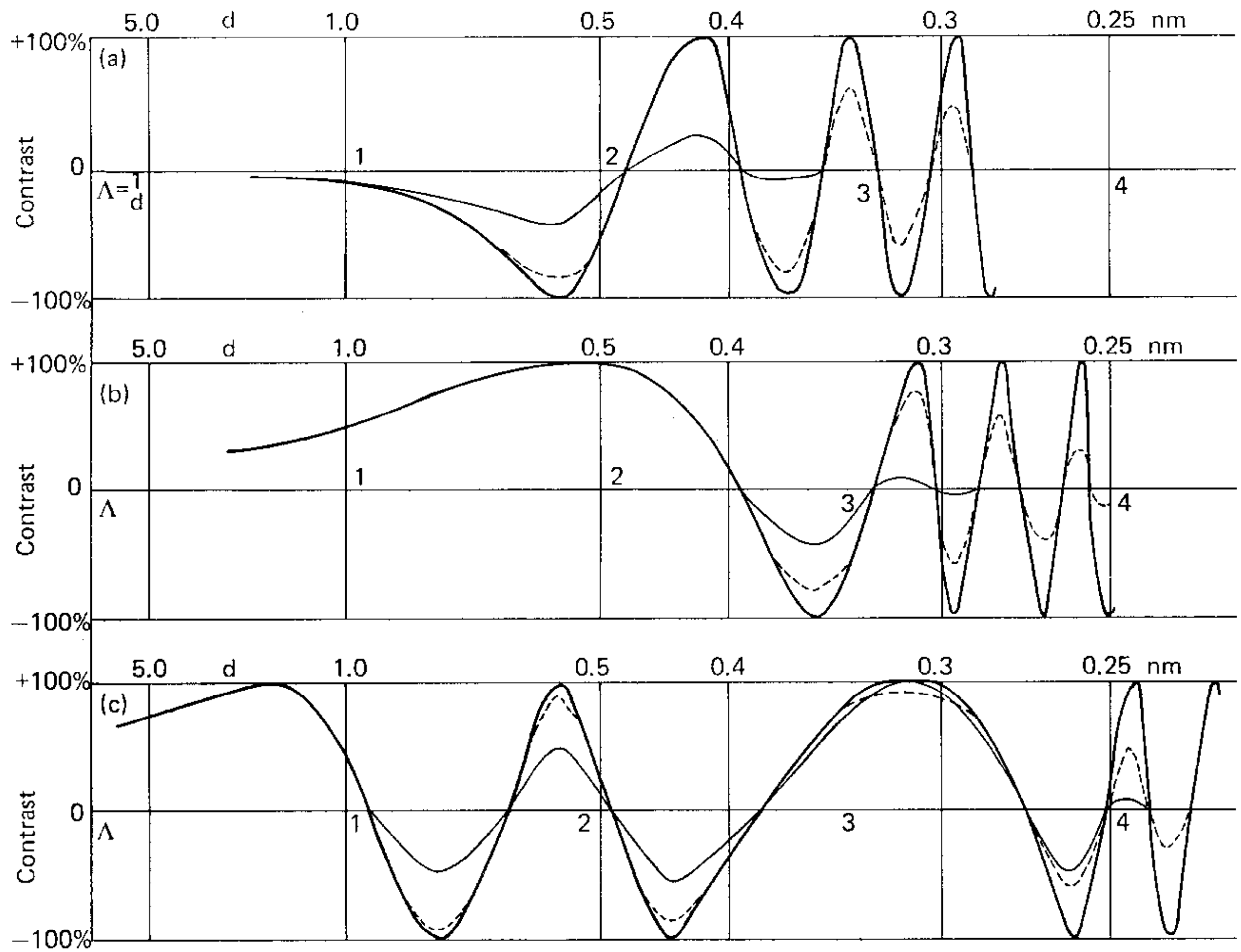
From Misell, Fig. 3.8, p.60

# 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. (a) Objective lens in focus. (b) Objective lens 78 nm underfocus. (c) Objective lens 234 nm underfocus

(Agar, p. 282)

BIO 595R

# I.C.3 Phase Contrast Transfer Function

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

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

where

$$\chi(\nu) = \pi \lambda \nu^{2}\left(\Delta f - 0.5 C_{s} \lambda^{2} \nu^{2}\right)$$

$\nu$ = 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)

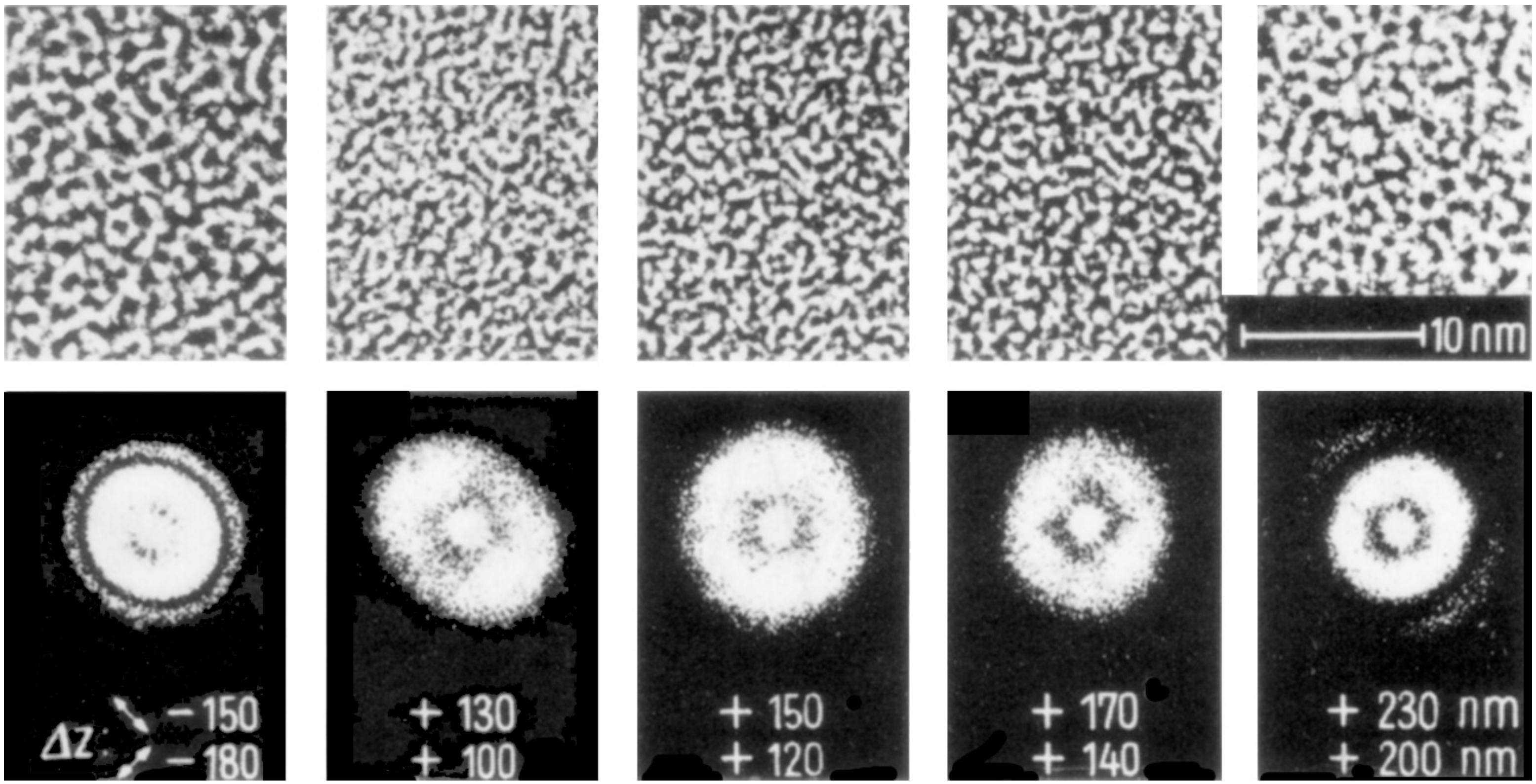
$V$ = voltage (in volts)

$\Delta f$ = underfocus (in Å)

$C_s$ = spherical aberration of objective lens of microscope (in Å)

# III.D.1 Optical Diffraction

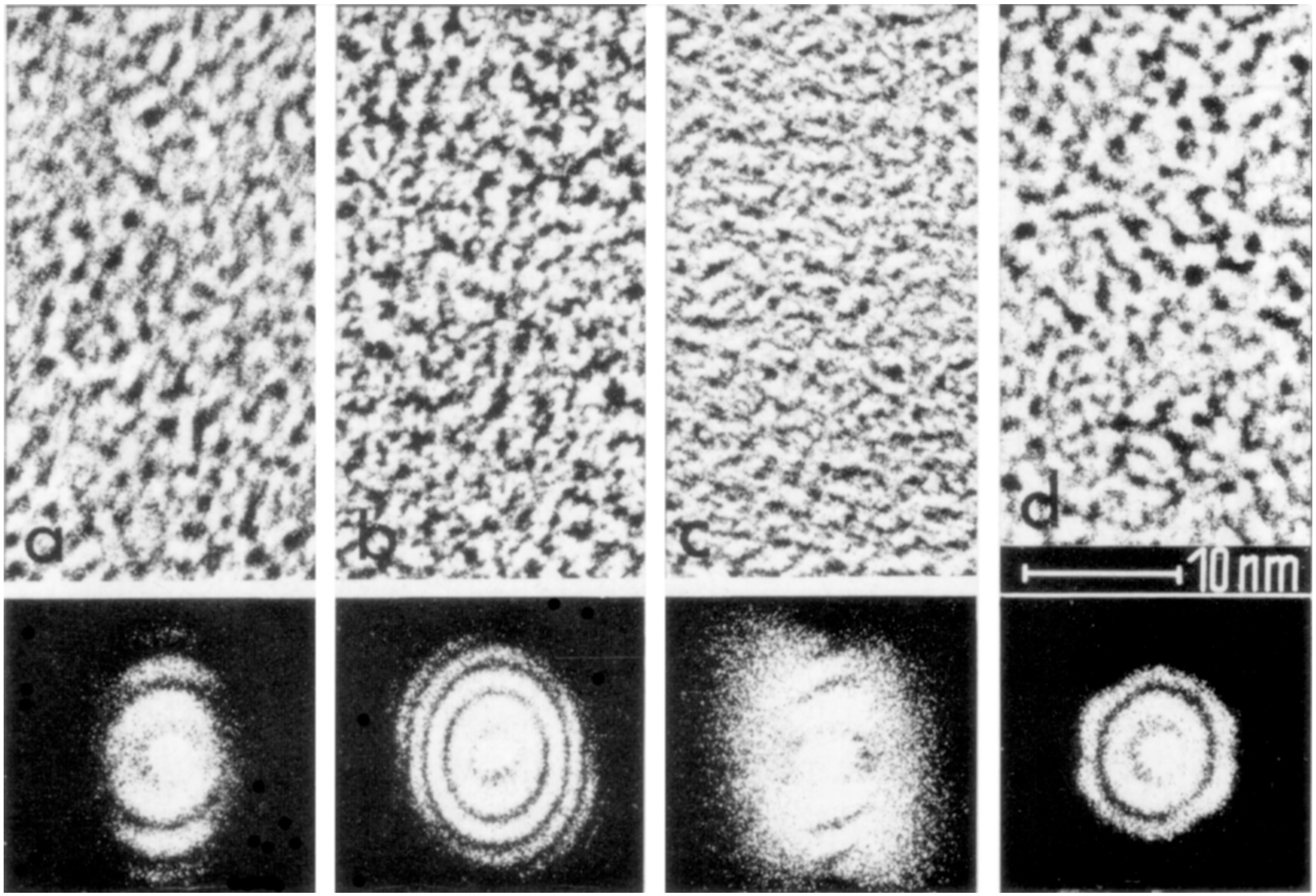
## Axial Astigmatism in Images of Thin Carbon Film




From Misell, Fig. 3.11, p.64

# III.D.1 Optical Diffraction

## Defects in Images of Thin Carbon Film




Specimen drift

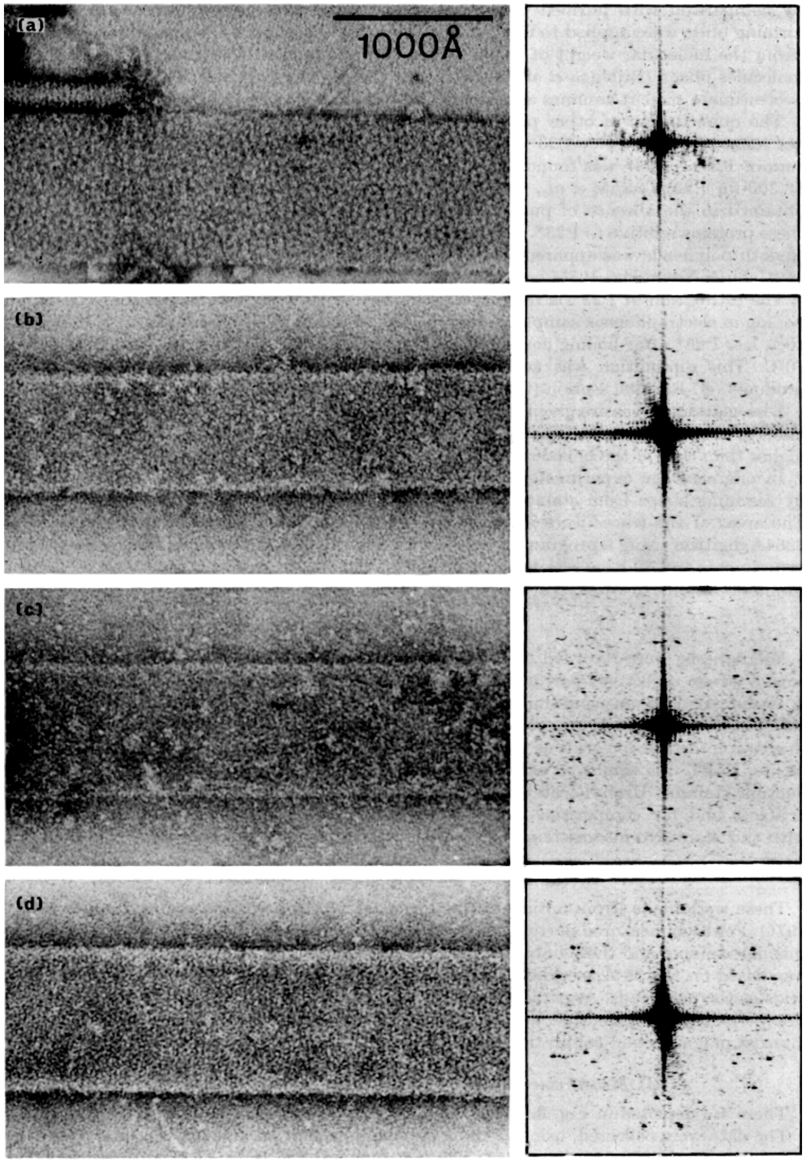
Miscentered objective aperture

Electrical charging of objective aperture

From Misell, Fig. 3.12, p.65

# III.D.1 Optical Diffraction

## OD of Negatively-stained T-even Polyheads




Coarse polyhead

A-type polyhead

B-type polyhead

C-type polyhead

Differences in OD patterns reflect differences in the crystal lattice structures (can't be seen by eye)

From Steven et al., (1976) Fig. 1, p.192

End of Sec.III.D.1