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

- $S_0$: 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)

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

From Misell, Fig. 3.8, p.60
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)
Dependence of CTF on resolution, wavelength, defocus and spherical aberration is given by:

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

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 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

From Misell, Fig. 3.12, p.65
III.D.1 Optical Diffraction

OD of Negatively-stained T-even Polyheads

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