III.D FOURIER IMAGE PROCESSING TECHNIQUES

III.D.3 Digital Fourier Analysis of Electron Micrographs

Outline

Optical vs. Computer Image Analysis/Processing

Digital Processing Steps

Hardware / Software

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.a Comparison of Optical and Computer Image Analysis

Advantages of Computer Analysis :

Several advantages to processing images by digital rather than optical Fourier methods

Main advantages derive from the **quantitative** nature and **virtual infinite flexibility** of data manipulation

1. **Example:** In **"pseudo" optical filtering** (digital equivalent of OF), filter masks can be designed with an infinite variety and combination of hole sizes, shapes and "transparencies"

2. **3D reconstruction** and **rotational filtering** are impractical or impossible using the optical Fourier techniques

3. **Quantitative analysis or manipulation** of data **not practical** by optical means, but is the essence of computational processing

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.a Comparison of Optical and Computer Image Analysis

3. **Quantitative analysis or manipulation** of data **not practical** by optical means, but is the essence of computational processing

Examples:

- Removal of **image aberrations** (*e.g.* astigmatism; defocus)

- Removal of **specimen distortions** (*e.g.* filament curvature)

- Averaging of **separate** 2D or 3D reconstructions

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.a Comparison of Optical and Computer Image Analysis

Disadvantages of Computer Analysis:

- Necessity for **discrete sampling** of data

  Introduces **aliasing artifacts** (transform overlap) which can be reduced by judicious choice of scanning conditions, but **never totally removed**

- Cost: may be prohibitive

  No sense developing a system whose main purpose is to provide **qualitative** examination of specimen OD patterns (Optical diffractometers are cheap and operate at speed of light)

- OD still provides best method for **screening images** (quick and inexpensive)

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.a Comparison of Optical and Computer Image Analysis

OPTICAL	**COMPUTER**
Original micrograph used	Micrograph digitized and "floated"
OD bench can be simple and inexpensive	Fast computer needed for “interactive” results
Formation of diffraction pattern instantaneous	Careful digitization normally slow and computation of diffraction patterns may take several seconds
Filtering operations require high quality (*i.e.* expensive) optics	Computers get more powerful and cheaper every day
Accurate filter masks tedious to make	Only limited by quality of software
Filtered image recorded photographically	Reconstructed images displayed and photographed using computer graphics devices
Quantitative information difficult or nearly impossible to obtain	Essence of computing IS to be quantitative
Amplitudes and phases difficult to manipulate	Infinite control over amplitudes and phases

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.a Comparison of Optical and Computer Image Analysis

OPTICAL	**COMPUTER**
Attenuation of zero-order beam to improve contrast in filtered image (may cause frequency doubling)	Control of contrast simple and straightforward
Imposing idealized, **non**-translational symmetries virtually impossible	Any symmetries (even incorrect) can be easily imposed
Correction for lattice distortion virtually impossible	Lattice distortions can be corrected (reinterpo-late original image onto perfect lattice
Data (ODs and filtered images) are continuous (*i.e.* vary smoothly)	Data are discrete (pixels)
Fast for screening and selecting best images for additional analysis	Not until CCD technology gets cheap
Reconstruction of 3D structure essentially impossible	Procedures rather straightforward with "right" software
Impractical to average data from different micrographs	Easy to average data from different micrographs

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

Typical digital processing procedure includes:

1. Selection of images
2. Densitometry
3. Boxing and floating the digital image
4. Fourier transformation
5. Indexing of two-dimensional lattices
6. 2D filtering/3D reconstruction (back-transformation)

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

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III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

1. Image Selection

Micrographs are examined by eye and/or by OD to select a subset of 'best' images for digital processing

- OD pattern from carbon support film provides **rapid check on microscope CTF conditions** at the time the micrograph was recorded (*i.e.* defocus level, astigmatism, drift or vibrations, etc.)

- OD is generally unsuitable for selecting **individual particles** for digital, rotational filtering

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

2. Densitometry

Goal:

Convert **optical densities** in the photographic emulsion to a digitial image (a **numerical array** corresponding to the relative optical densitites in the image)

Each density value in the digitized image is represented as a **pixel** with an intensity ranging between 0 and 255 (an eight bit number) or 4096 (12-bit number) or even higher in some CCD cameras

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

2. Densitometry

Information content in a **single 1024 by 1024** digital image (1,048,576 pixels) is quite staggering: more than the text portion of the lecture notes for both BIO 595R and 595W!

NOTE: at a raster step size of **7 μm** (smallest step size on Zeiss scanner), the area of the micrograph digitized for a 1024 by 1024 array would be ~50 mm^2 or **0.625%** (1/160th) of an 8 x 10 cm micrograph

Hence, the information content of **one** micrograph digitized at 7 μm is about **160 times** the entire course contents!!!

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

2. Densitometry

Rule of Thumb:

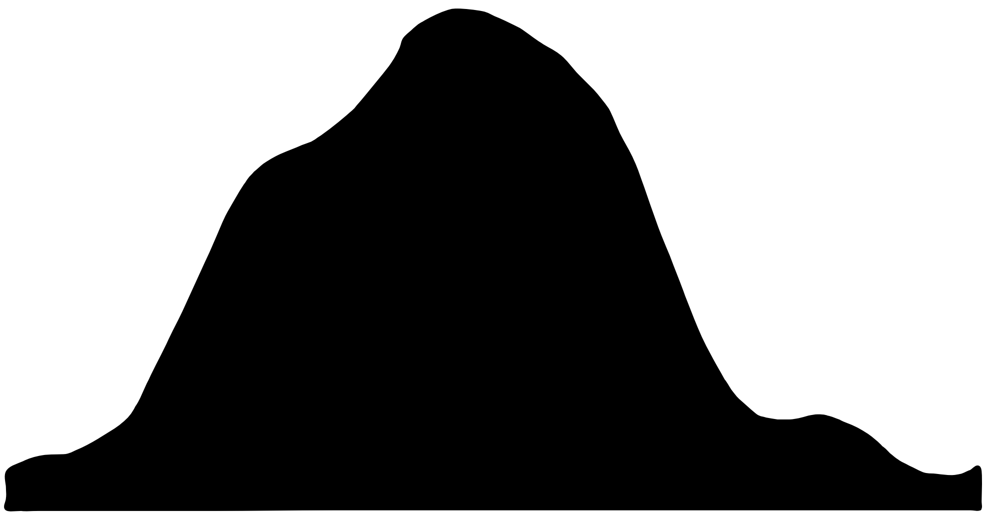
Images should be scanned to give pixels of a size corresponding to **ONE-THIRD OR LESS** than the expected resolution in the image in order to minimize **aliasing** artifacts

This condition is referred to as **over-sampling** the data

Data **under-sampling** leads to **loss** of resolution

III.D.3.b Digital Processing Steps

2. Densitometry



Continuous

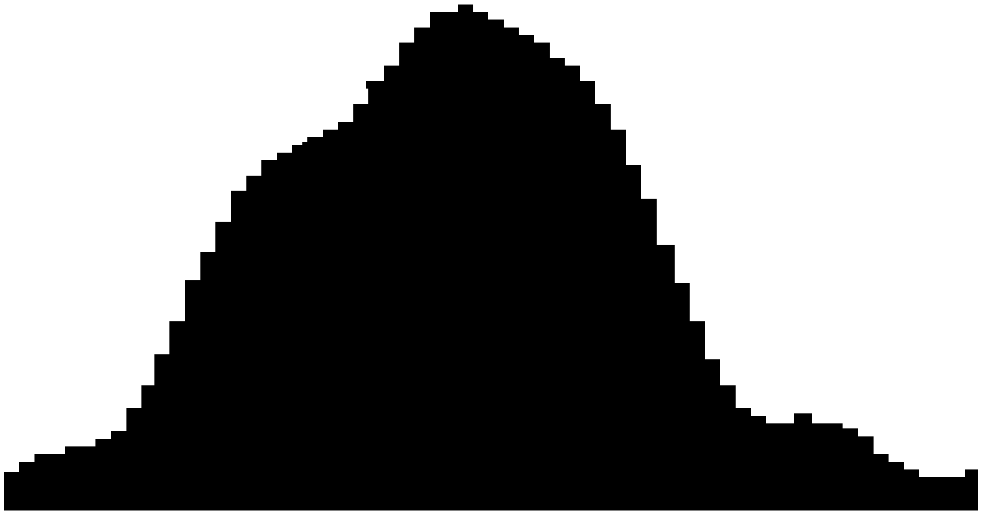
III.D.3.b Digital Processing Steps

2. Densitometry

128

III.D.3.b Digital Processing Steps

2. Densitometry



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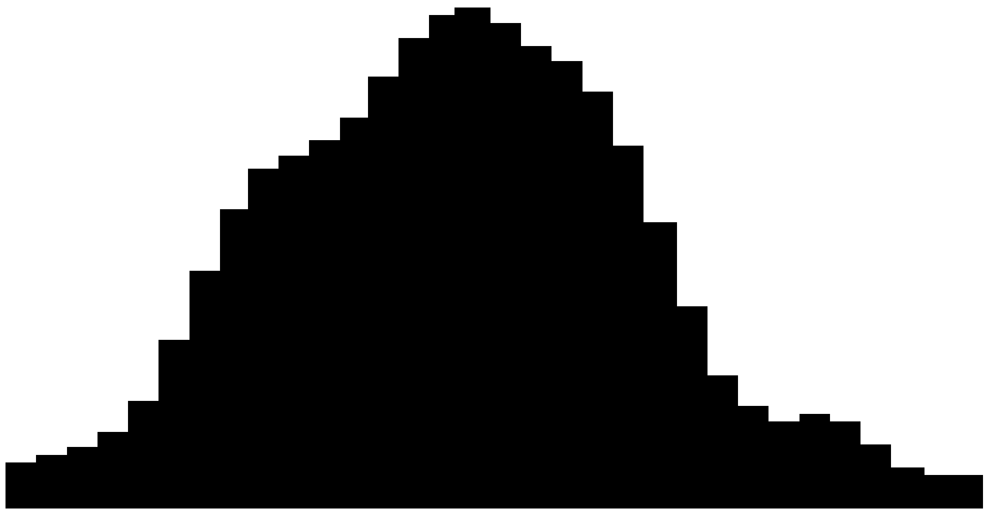
III.D.3.b Digital Processing Steps

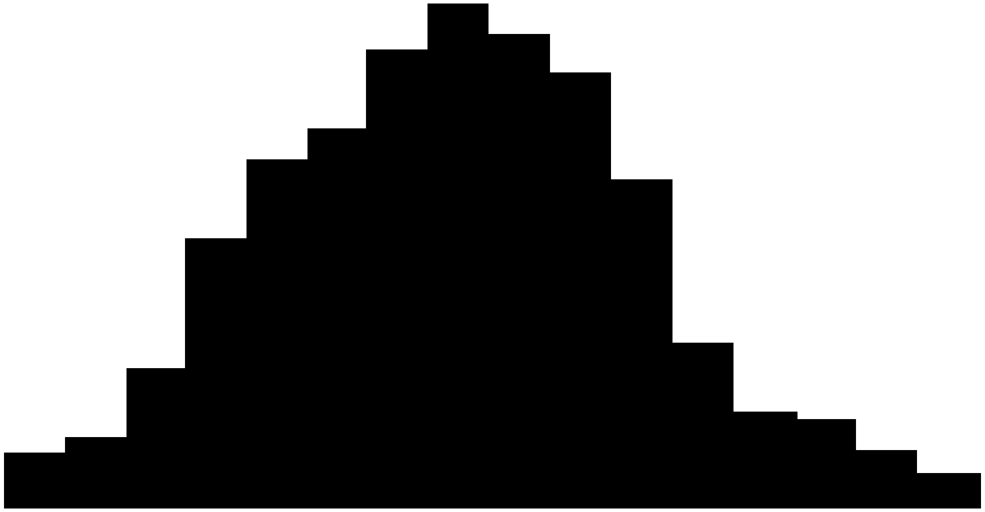
2. Densitometry



32

III.D.3.b Digital Processing Steps

2. Densitometry



16

III.D.3.b Digital Processing Steps

2. Densitometry

8

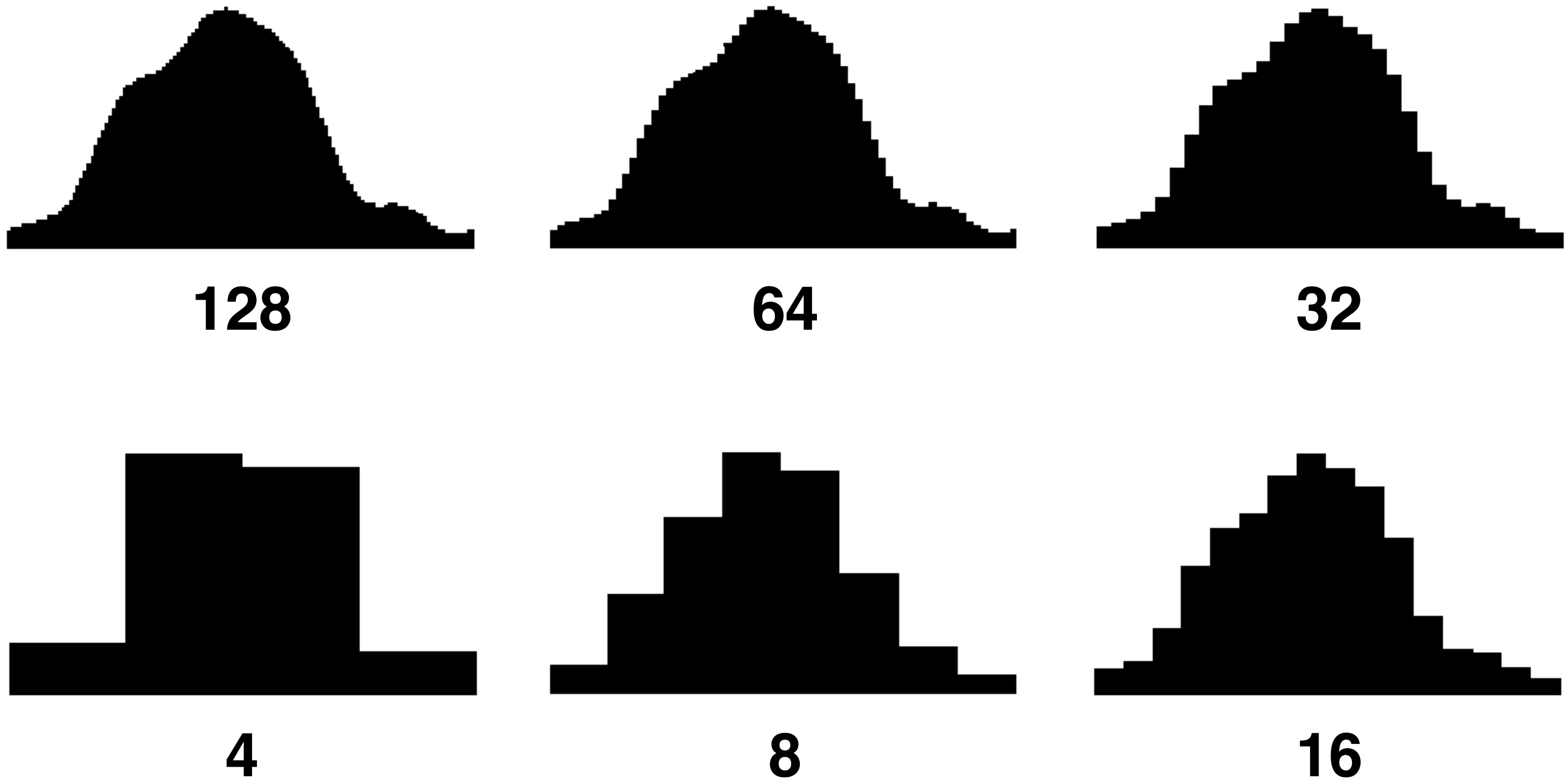
III.D.3.b Digital Processing Steps

2. Densitometry

4

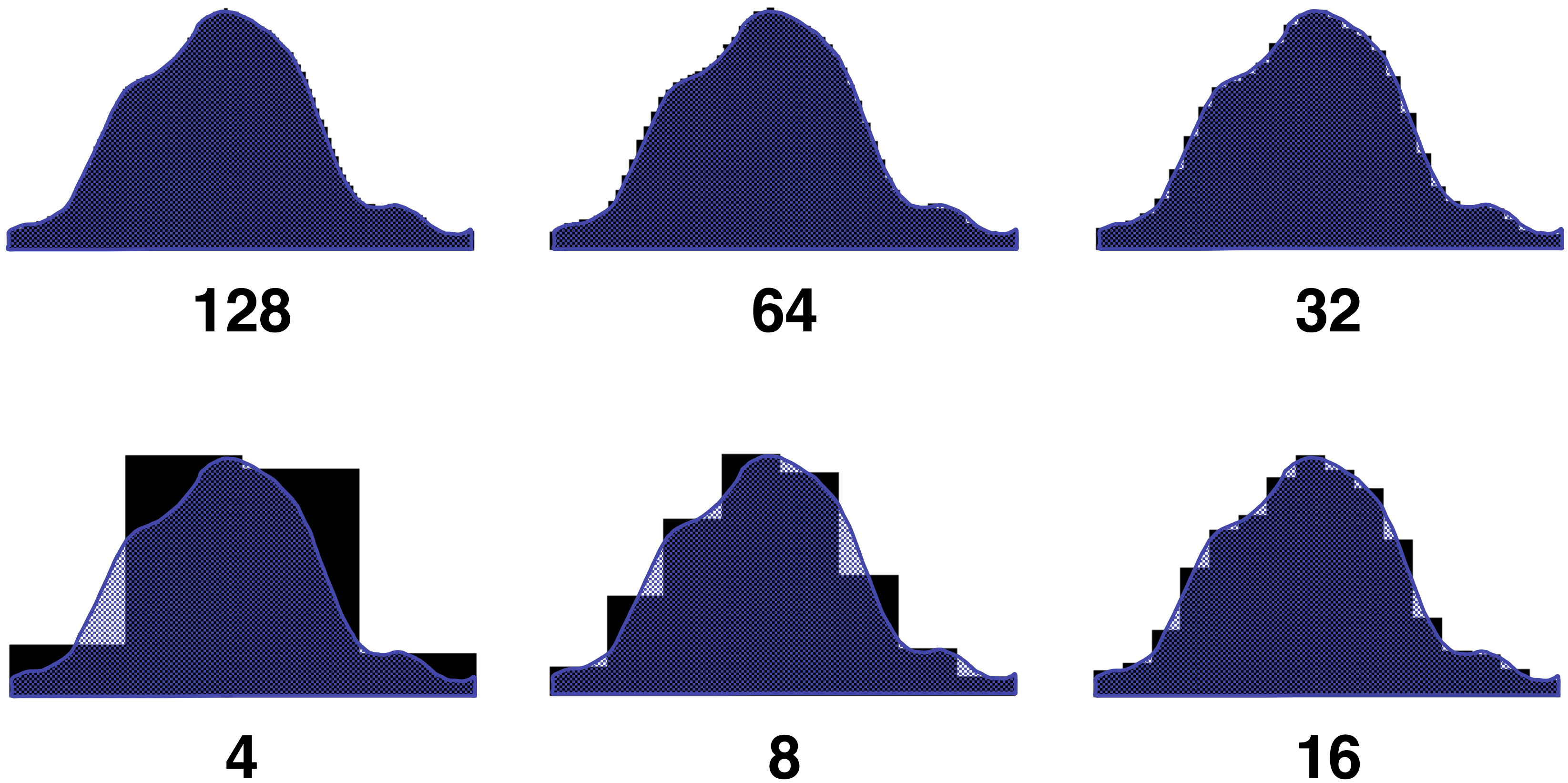
III.D.3.b Digital Processing Steps

2. Densitometry



III.D.3.b Digital Processing Steps

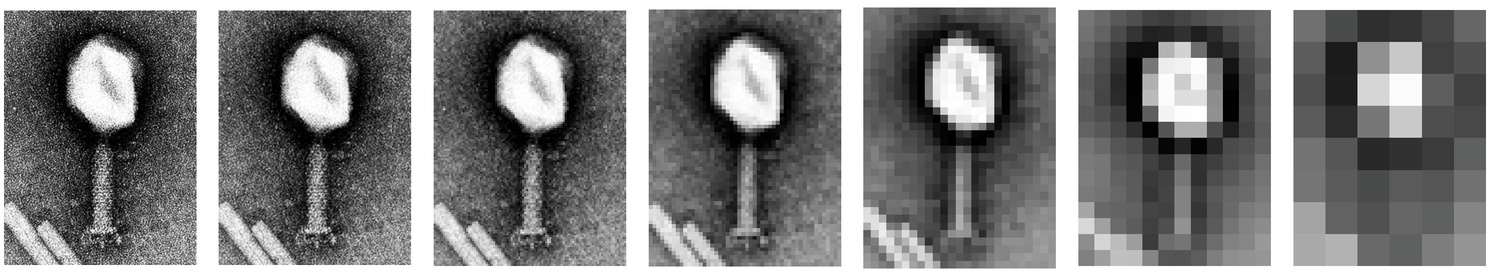
2. Densitometry



III.D.3.b Digital Processing Steps

2. Densitometry

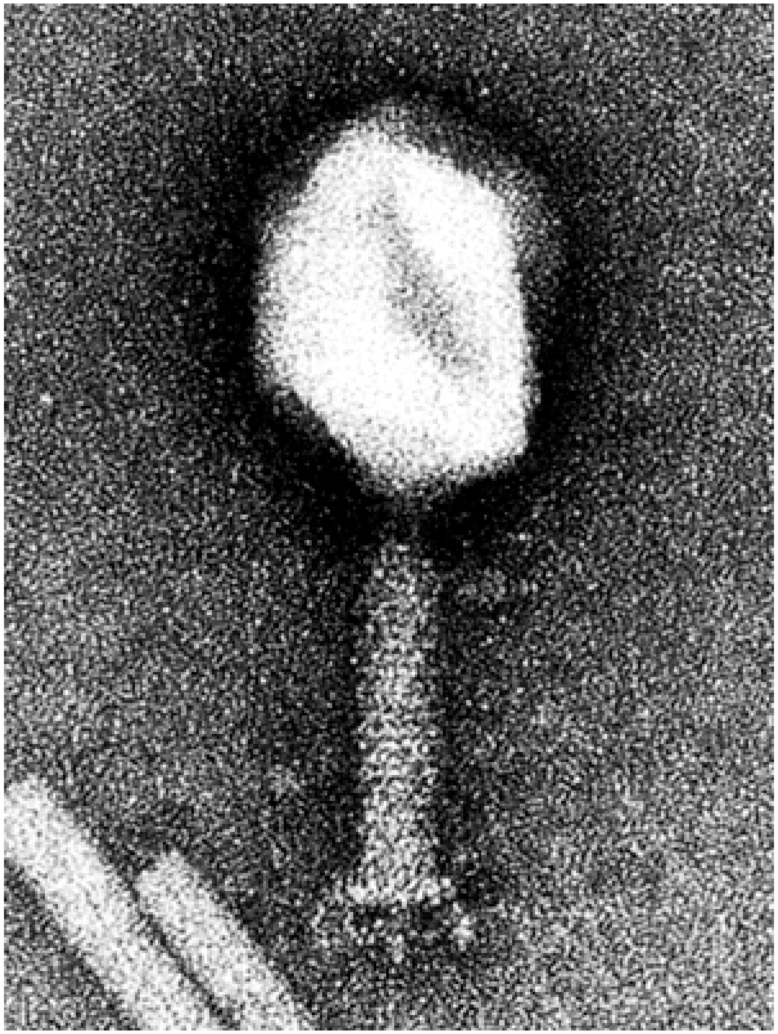
Negatively-stained T4 bacteriophage sampled at different pixel resolutions (in dpi)



300 200 100 50 25 12 6

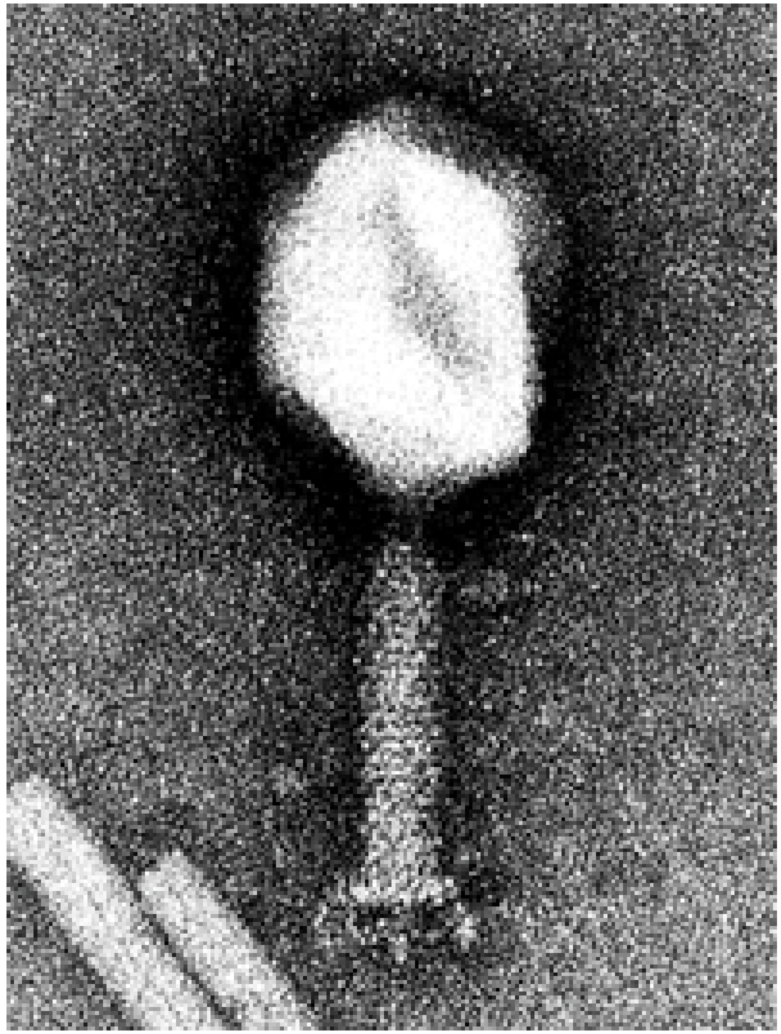
III.D.3.b Digital Processing Steps

2. Densitometry



III.D.3.b Digital Processing Steps

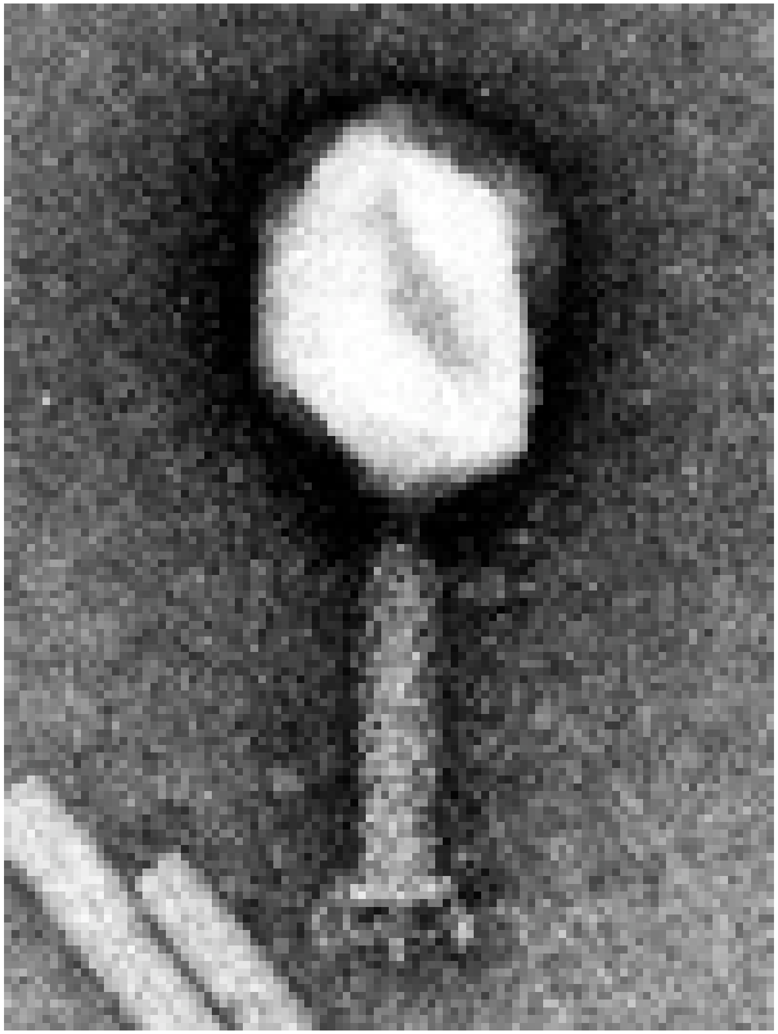
2. Densitometry



200

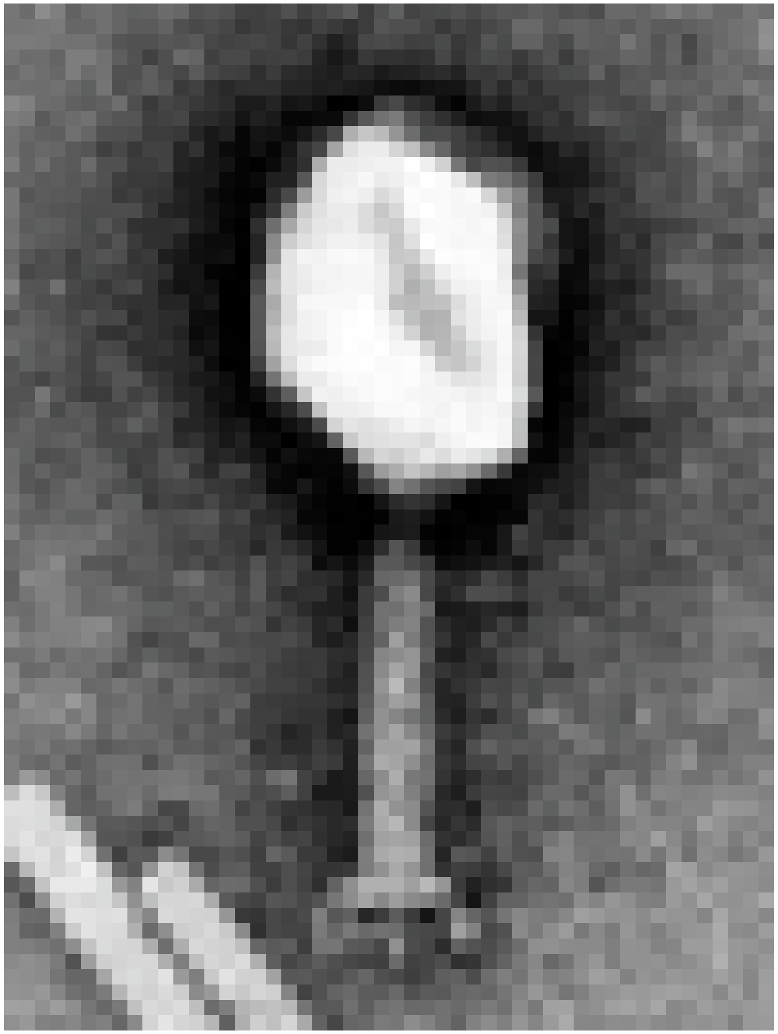
III.D.3.b Digital Processing Steps

2. Densitometry



III.D.3.b Digital Processing Steps

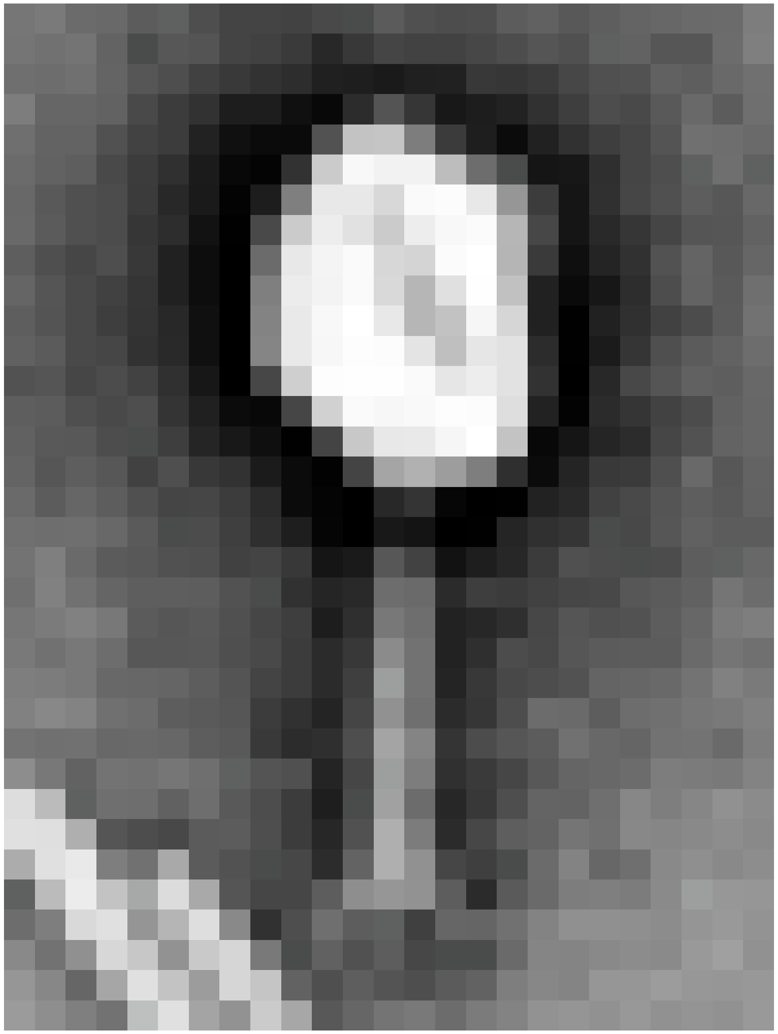
2. Densitometry



50

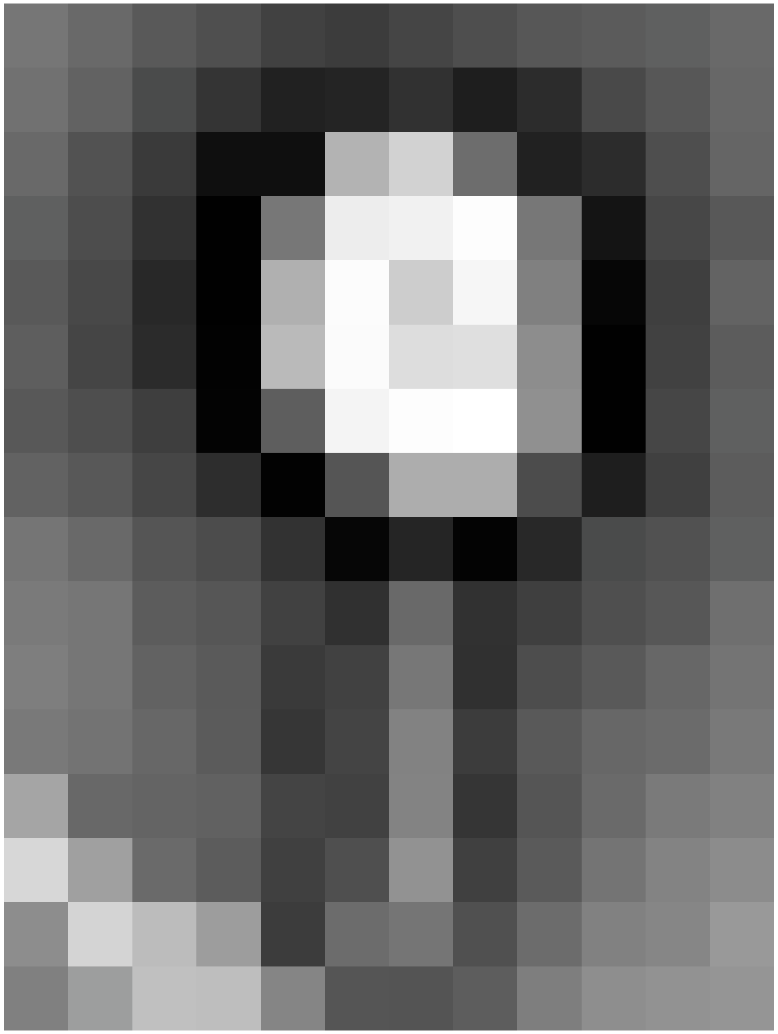
III.D.3.b Digital Processing Steps

2. Densitometry



III.D.3.b Digital Processing Steps

2. Densitometry



III.D.3.b Digital Processing Steps

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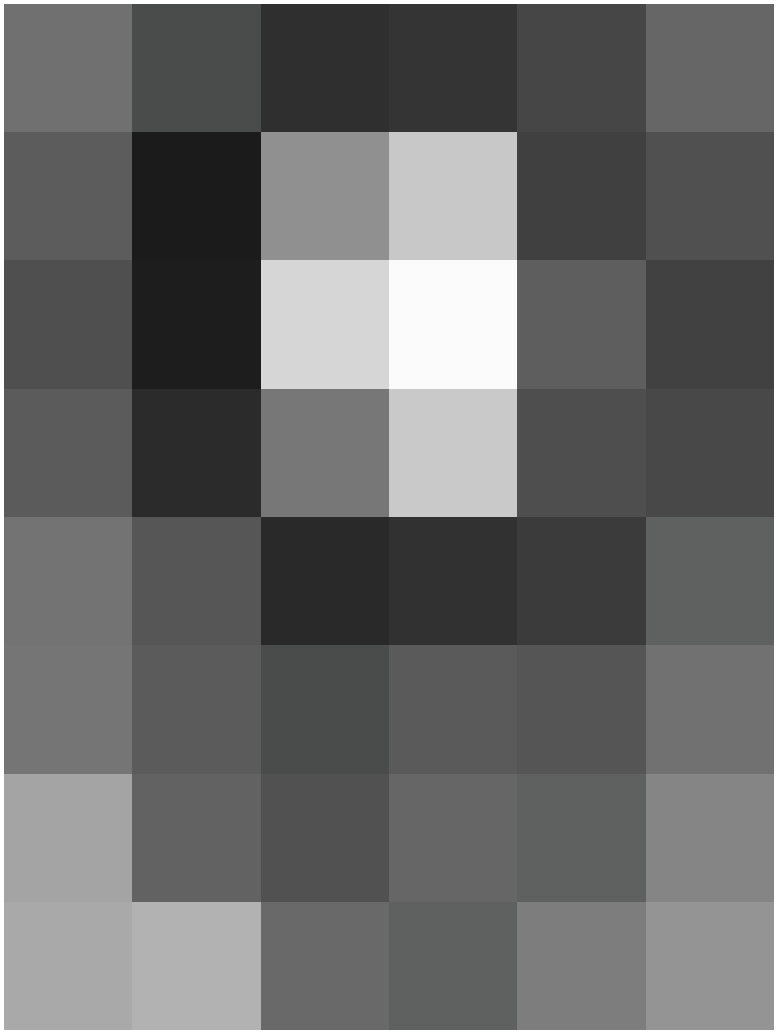
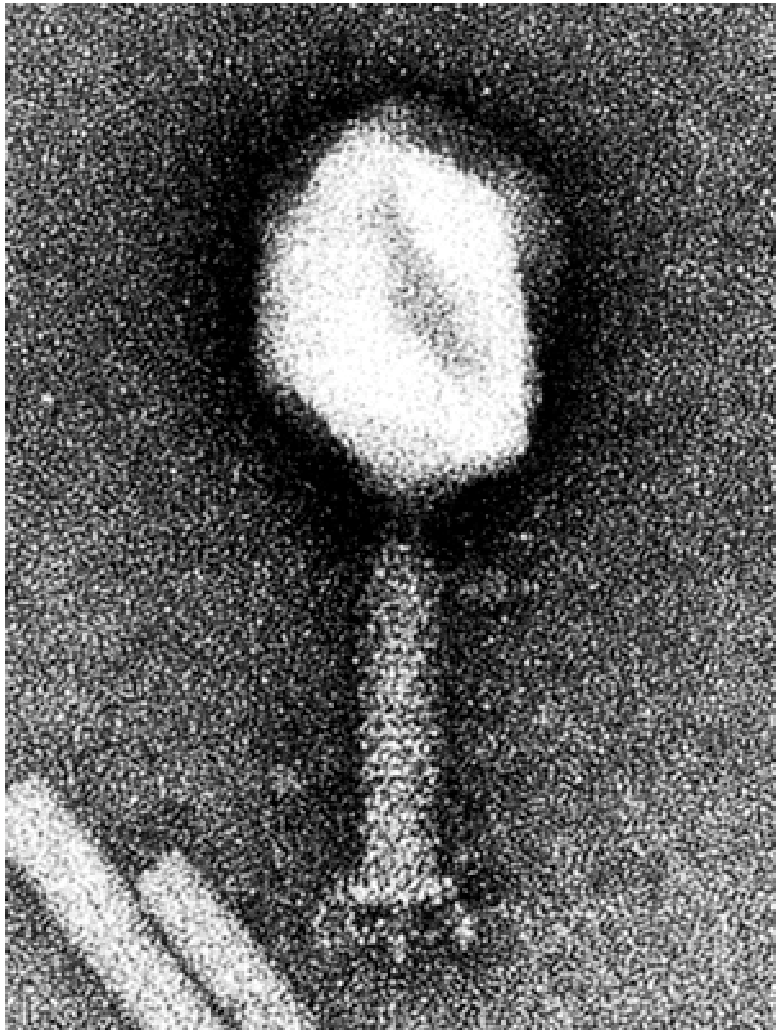


6

III.D.3.b Digital Processing Steps

2. Densitometry



300

III.D.3.b Digital Processing Steps

2. Densitometry

Data Over-Sampling: Good News / Bad News

Good News:

- No loss of resolution recorded in the micrograph

Bad News:

- Increased computation owing to increased data

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

2. Densitometry

Step size (pixel resolution) in the biological specimen depends on magnification of the micrograph scanned

Example: Micrograph magnification = **45,000X**
Scan raster size = **14 μm**
Each pixel corresponds to **0.311 nm** at specimen

Thus, based on the scanning *Rule of Thumb*, at best can only recover information out to ~0.933 nm (= 3 x 0.311)

Note: Calculation assumes the specimen is **preserved** to this resolution and the **electron optical conditions** allow recovery of this information

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

2. Densitometry

Table of **nominal pixel sizes** (in nm) recoverable from a digitized image for different scanner step sizes:

Nominal Pixel Size (nm)

FEI CM300 MICROGRAPH MAG	ZEISS PHODIS SCAN STEP SIZE (μm)			
	7	14	28	56
13,500	0.519	1.037	2.074	4.148
19,500	0.359	0.718	1.436	2.872
24,000	0.292	0.583	1.167	2.333
33,000	0.212	0.424	0.848	1.697
45,000	0.156	0.311	0.622	1.244
61,000	0.115	0.230	0.459	0.918

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

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Note: **Actual** pixel size must be determined from calibrated microscope magnifications

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

2. Densitometry

Scanning Rule of Thumb #1:

Scan images at raster settings corresponding to **ONE-THIRD OR LESS** than the expected resolution in the image in order to minimize aliasing artifacts

Scanning Rule of Thumb #2:

Generally best to scan images and store them with the **smallest** nominal pixel size (*e.g.* 7 μm on Zeiss)

Subsequent processing can be performed if desired with larger size pixels by **reinterpolating** or **binning** the original scanned image (the ‘gold’ standard)

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

2. Densitometry

KEY CONCEPT:

Always wise to **carefully plan** out your experiments

- Take a best guess at the resolution you might expect to achieve in your images
- Divide this value by 3 and choose a magnification appropriate for the scanner step size you select
- If radiation damage is a problem (always is!), opt for the **smallest step size and lowest magnification**

FEI CM300 MICROGRAPH	ZEISS PHODIS SCAN STEP SIZE (μm)			
MAG	7	14	28	56
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Nominal Pixel Size (nm)

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

2. Densitometry

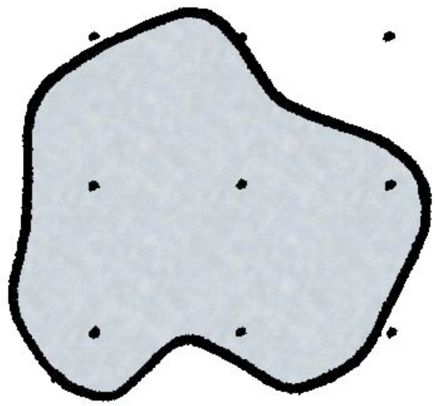
Microdensitometers are computer-driven devices

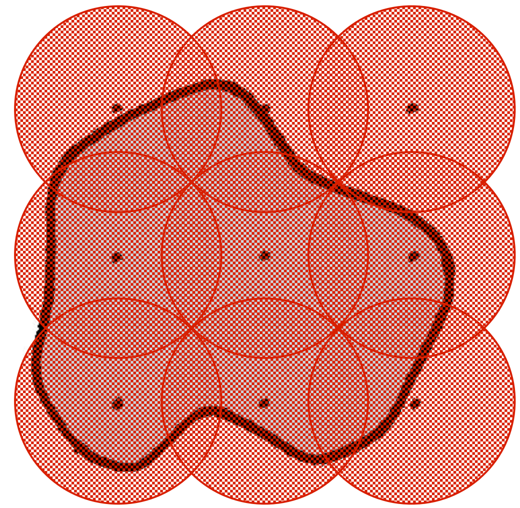
Measure optical densities in micrograph on a **square grid** pattern (*i.e.* at equal step sizes in two mutually perpendicular directions)

Transmission of small beam of light passing through micrograph is measured with a photomultiplier or CCD camera which **converts analog signal** (beam of light) **to a digital signal** (number ranging between 0 and 255 or higher)

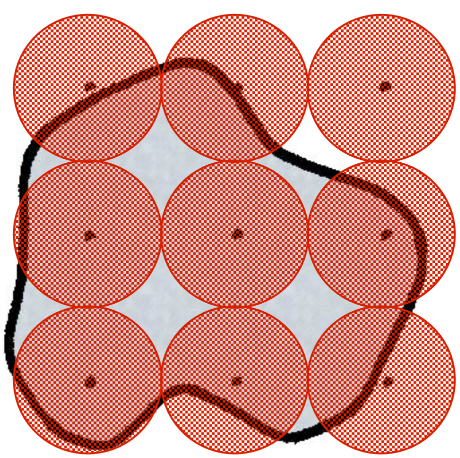
III.D.3.b Digital Processing Steps

2. Densitometry

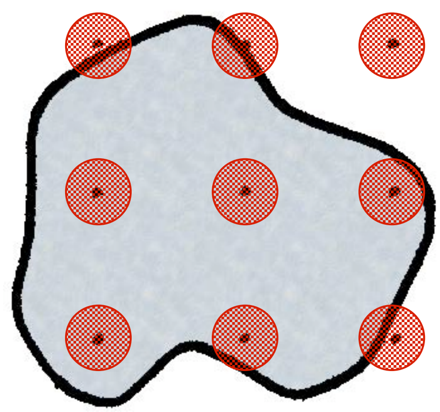




Data smearing



No smearing;
Minimal data loss

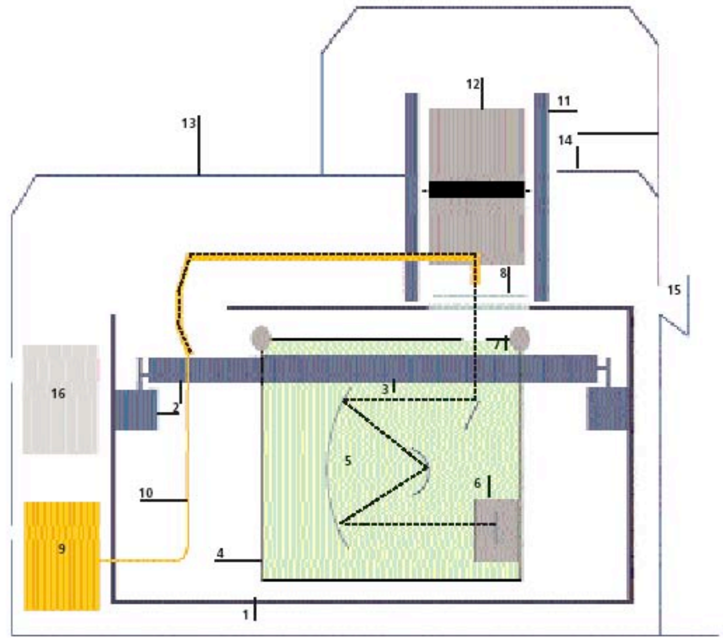


No smearing;
Substantial data loss

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

2. Densitometry



(1) Cast-iron enclosure
(2) Primary guideway and primary carriage with (3) linear encoder
(4) Secondary carriage with (5) mirror lens and (6) CCD module
(7) Photo stage for scan copy and (8) glass cover plate
(9) Lamp module with (10) fiber glass optics
(11) Autowinder with (12) film roll (14) instrument enclosure with cover and (15) control panel
(16) Electronics module

From http://www.ziimaging.com

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

2. Densitometry

Digital images typically displayed on a graphics workstation monitor and stored on magnetic disk or magnetic tape

- Amount of data generated can quickly get quite large

- 1 entire micrograph scanned at **7 μm** step size generates about **163 x 10^6 pixels** which translates into **326 Mb** of data (*i.e.* only 3 micrographs per Gbyte!!!)

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image

Entire digital image or selected (boxed) areas may used for subsequent processing steps

To use only a portion of the scanned image:

- Area of interest is boxed (**windowed**) in manner similar to masking micrographs for OD or OF

- Thus, **areas outside** the biological specimen (*e.g.* carbon film or vitrified water or other "junk"), which mainly contribute noise to the image, are **selectively removed**

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image

Boxing:

- Operation that **zeroes regions** in the digital image **outside** the area of interest (equivalent to “masking” in OD or OF experiments)

- Performed directly on digital image displayed on a computer graphics monitor

Note: with auto- or semi-automated boxing routines, human intervention is reduced or eliminated and so too the requirement for graphics display of the data

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

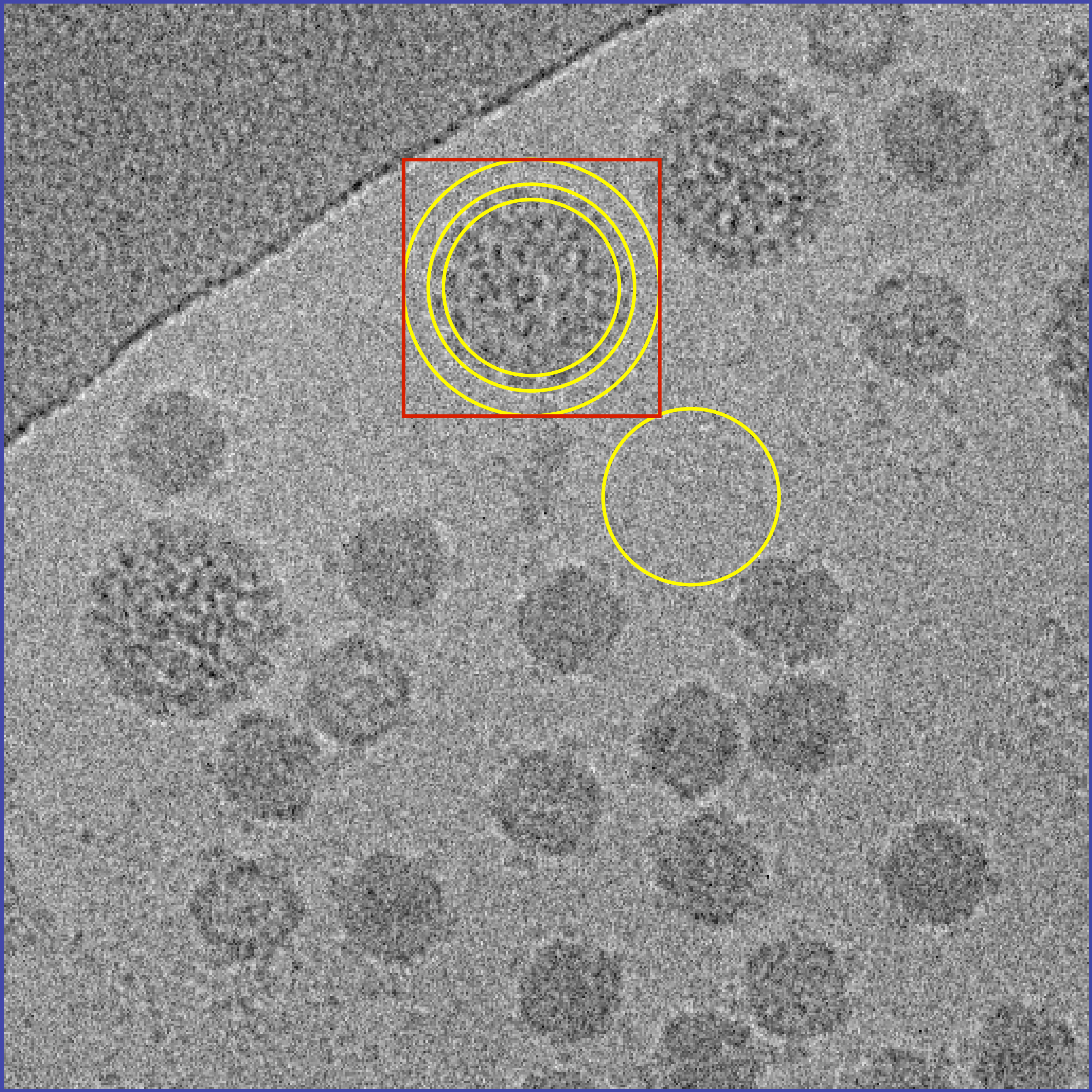
3. Boxing and Floating the Digitized Image

Floating:

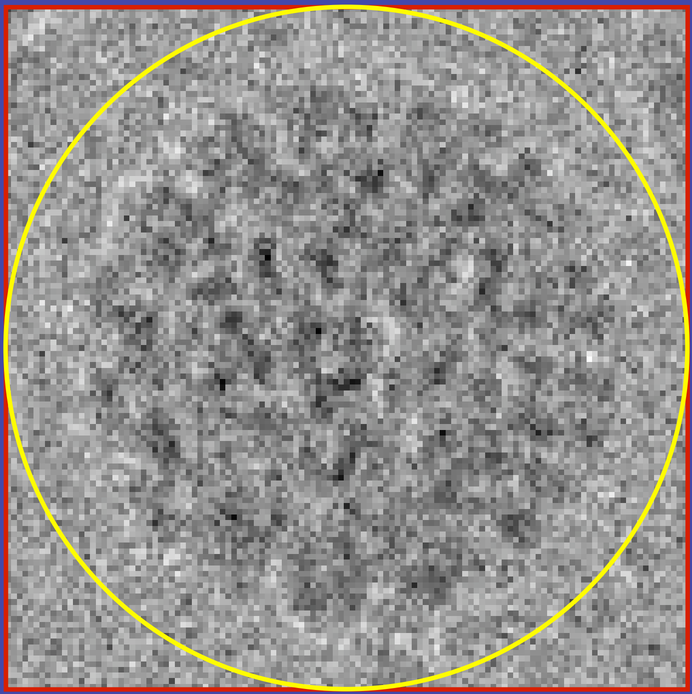
- **Average** image intensity around **box perimeter** is **subtracted** from ***all*** image intensities **within** the masked area

- This suppresses strong diffraction "spikes" which arise from the high-contrast edges of the masked area

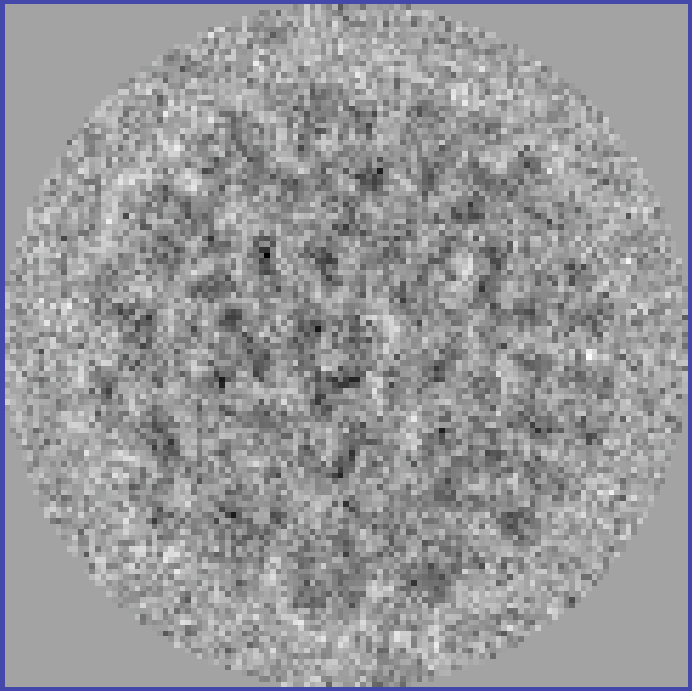
Boxing:



Boxing:

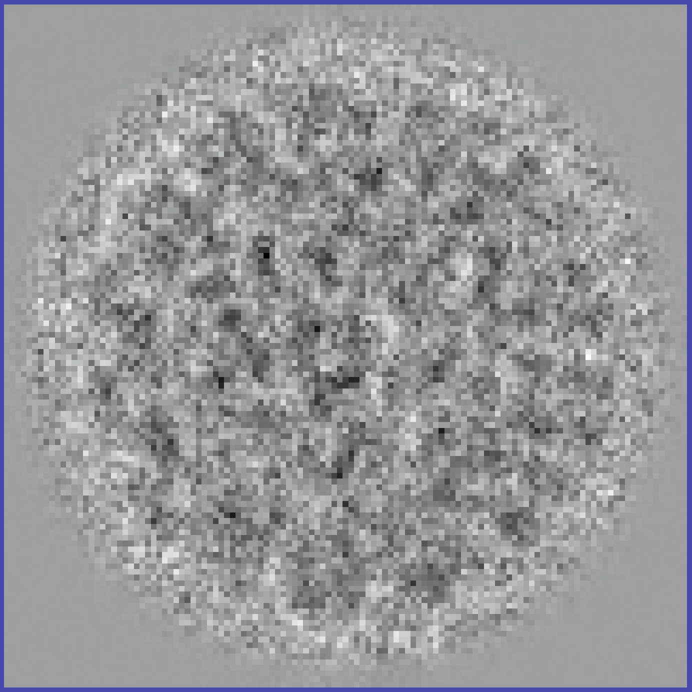


Boxing:



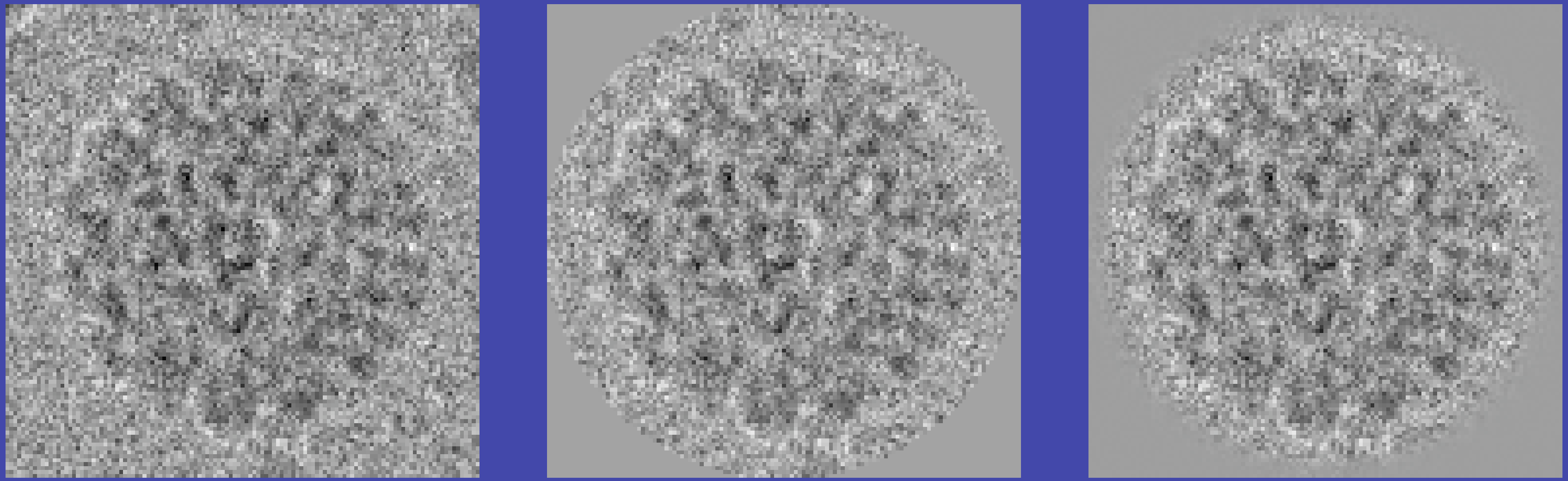
Windowed

Boxing:



Windowed
and Apodized

Boxing:



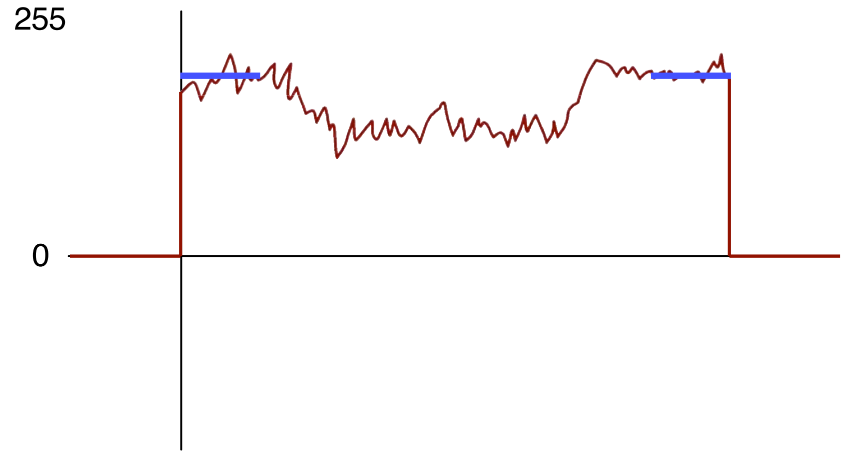
Windowed

Windowed and Apodized

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

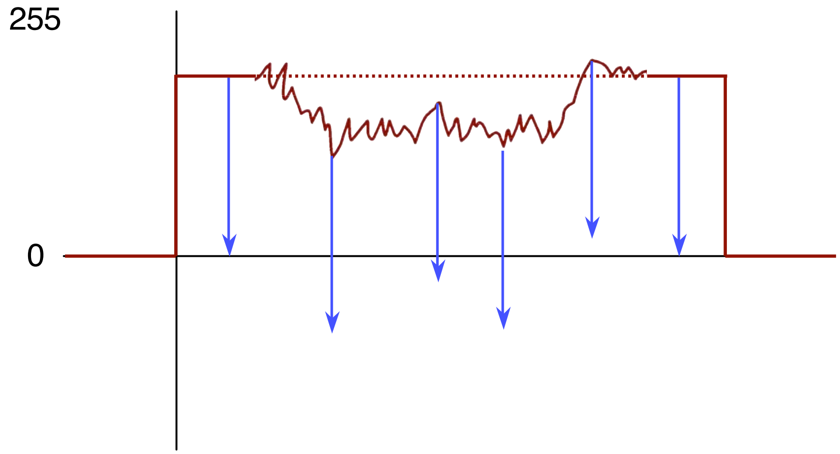
3. Boxing and Floating the Digitized Image



III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

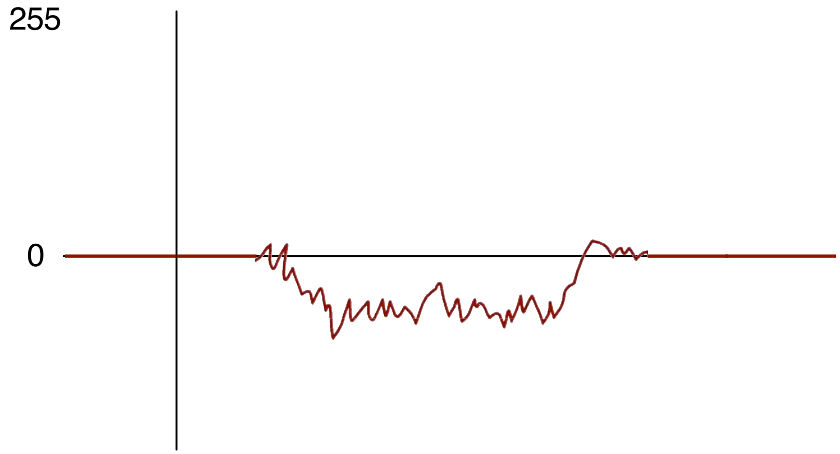
3. Boxing and Floating the Digitized Image



III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

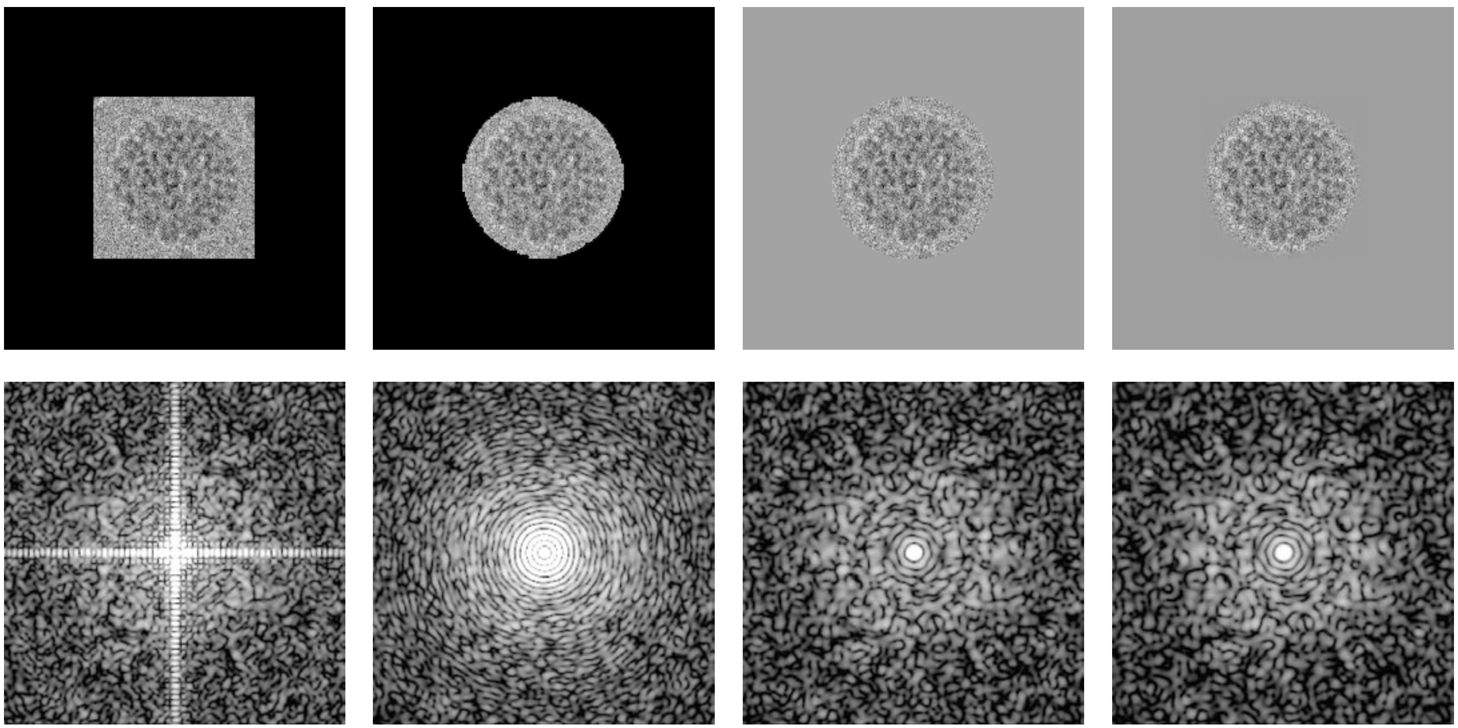
3. Boxing and Floating the Digitized Image



III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

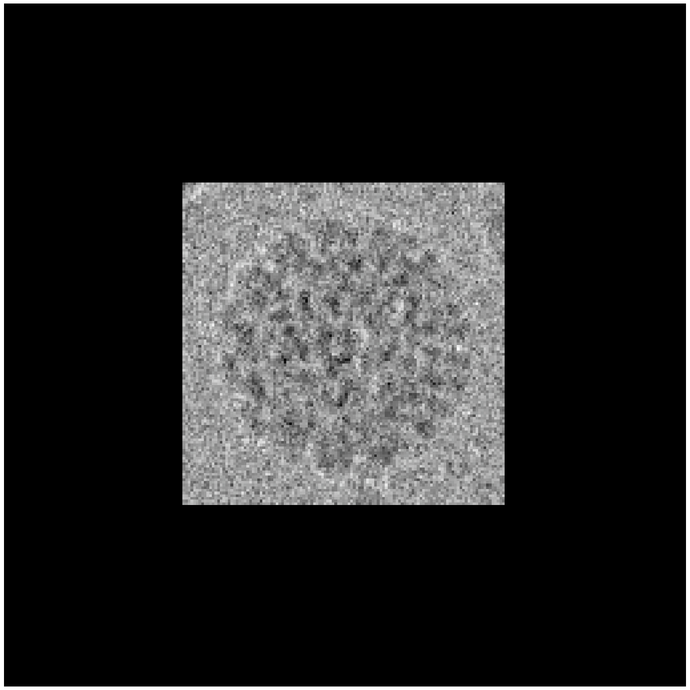
3. Boxing and Floating the Digitized Image

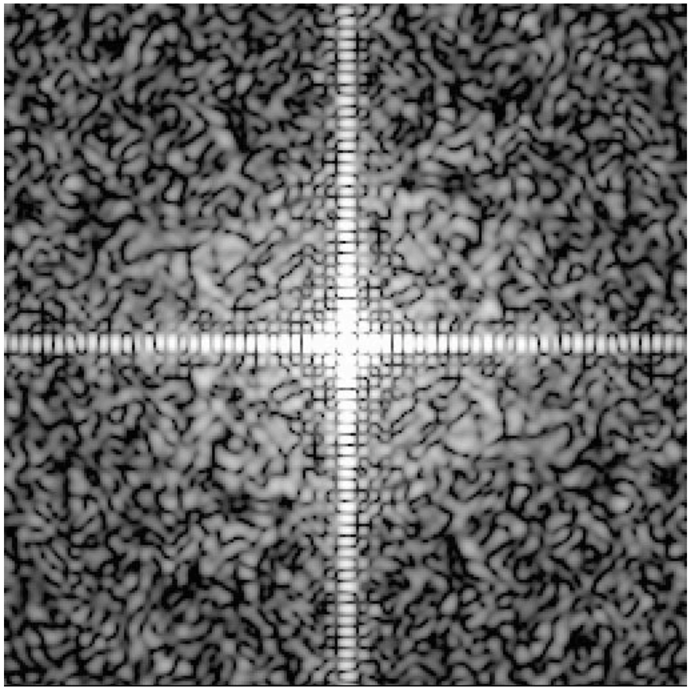


III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image



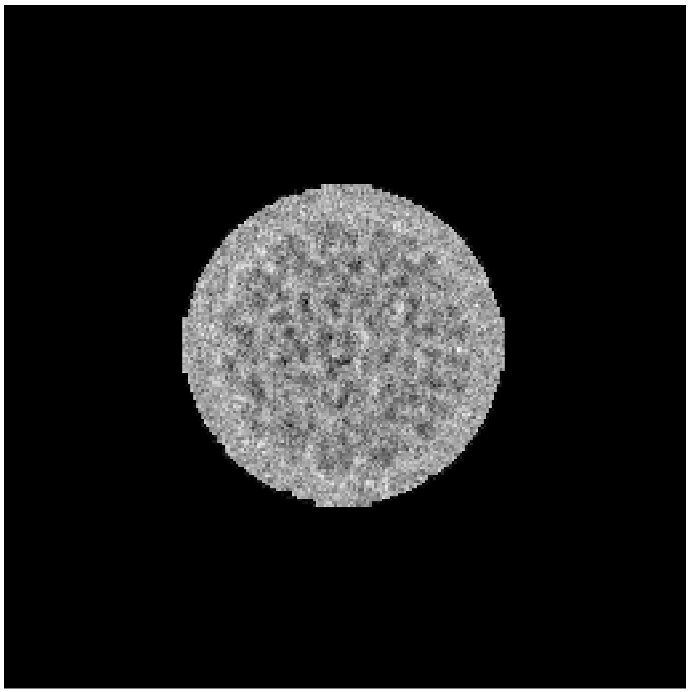


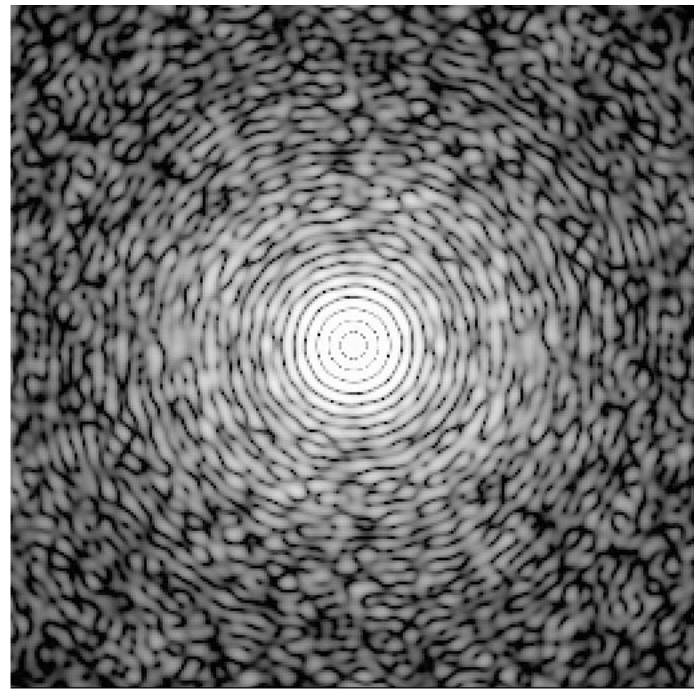
Square window; **unfloated**

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image



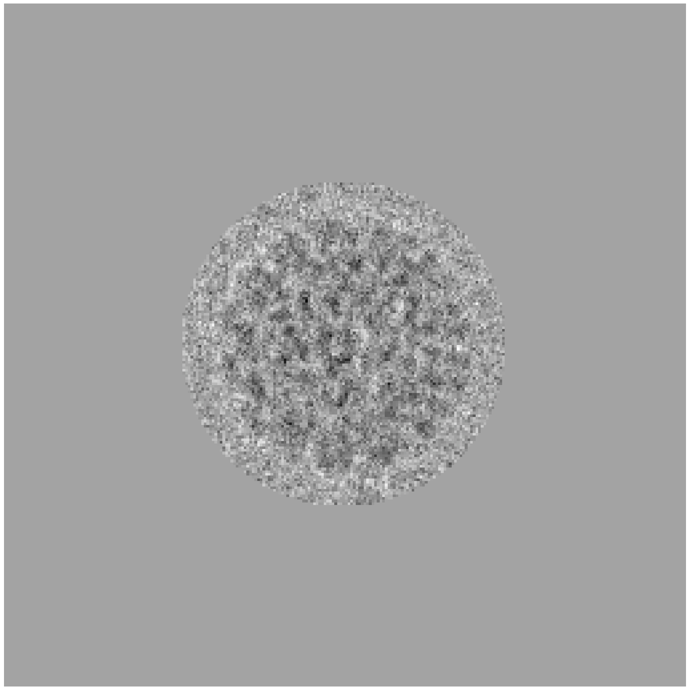


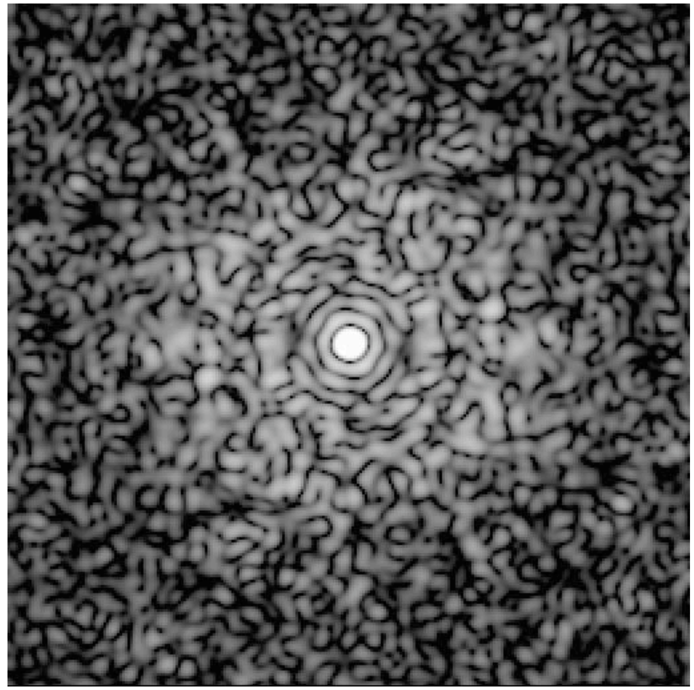
Circular window; **<u>un</u>**floated

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image



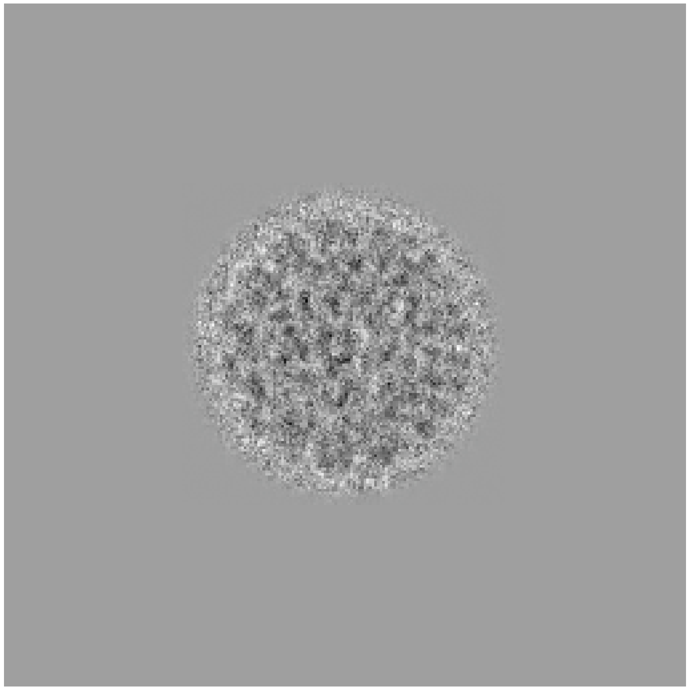


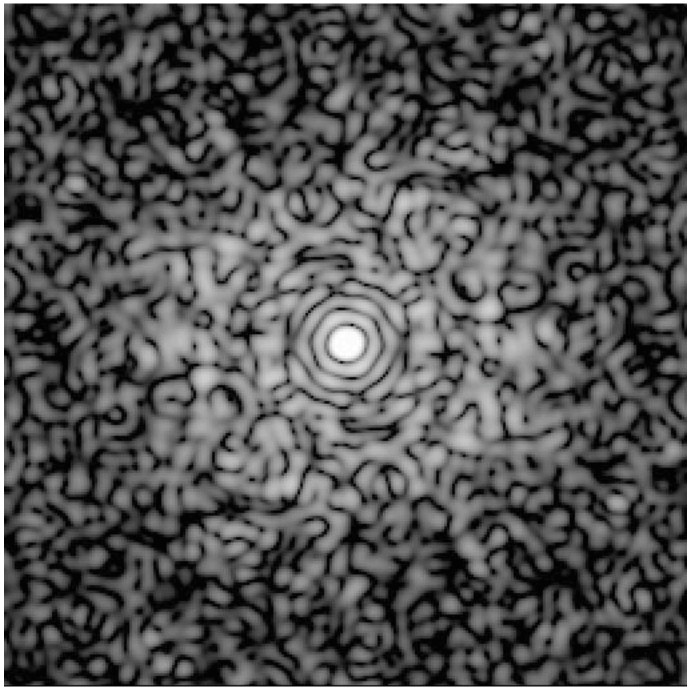
Circular window; floated

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image



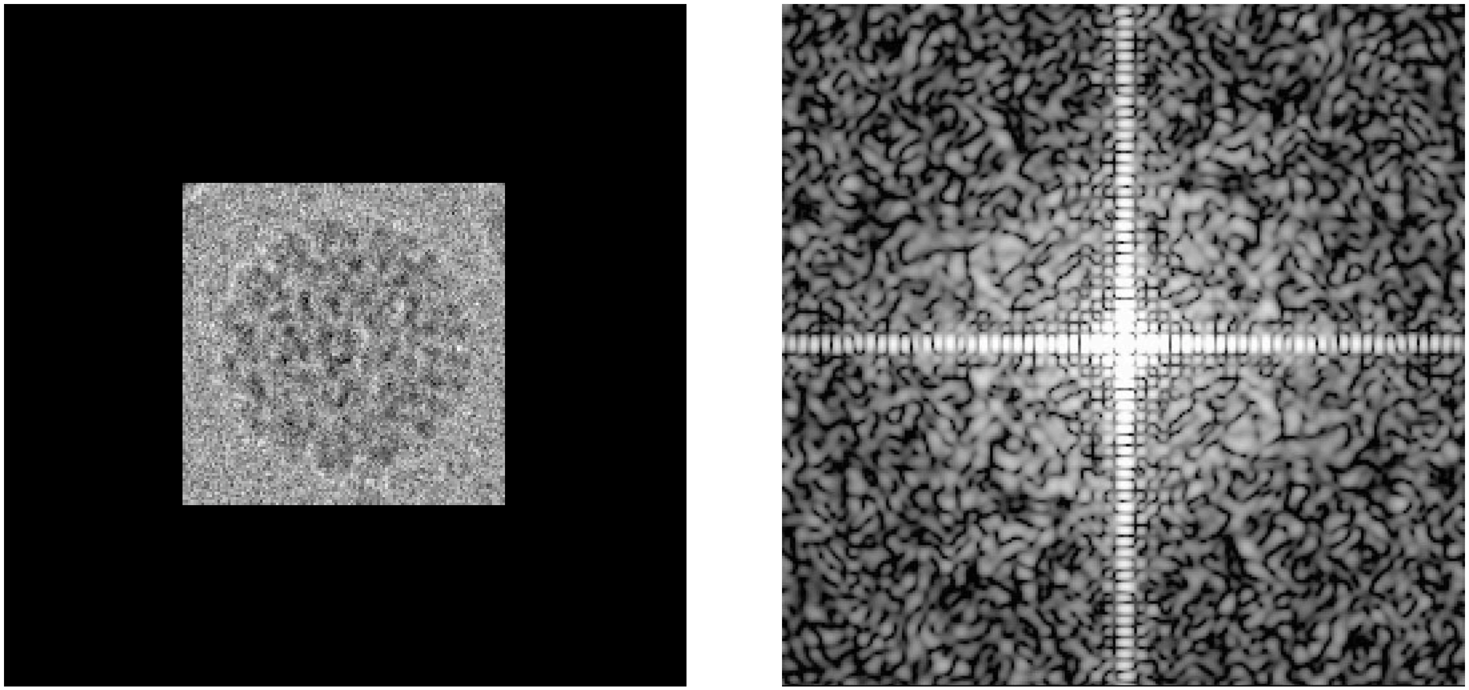


Circular window; apodized & floated

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image

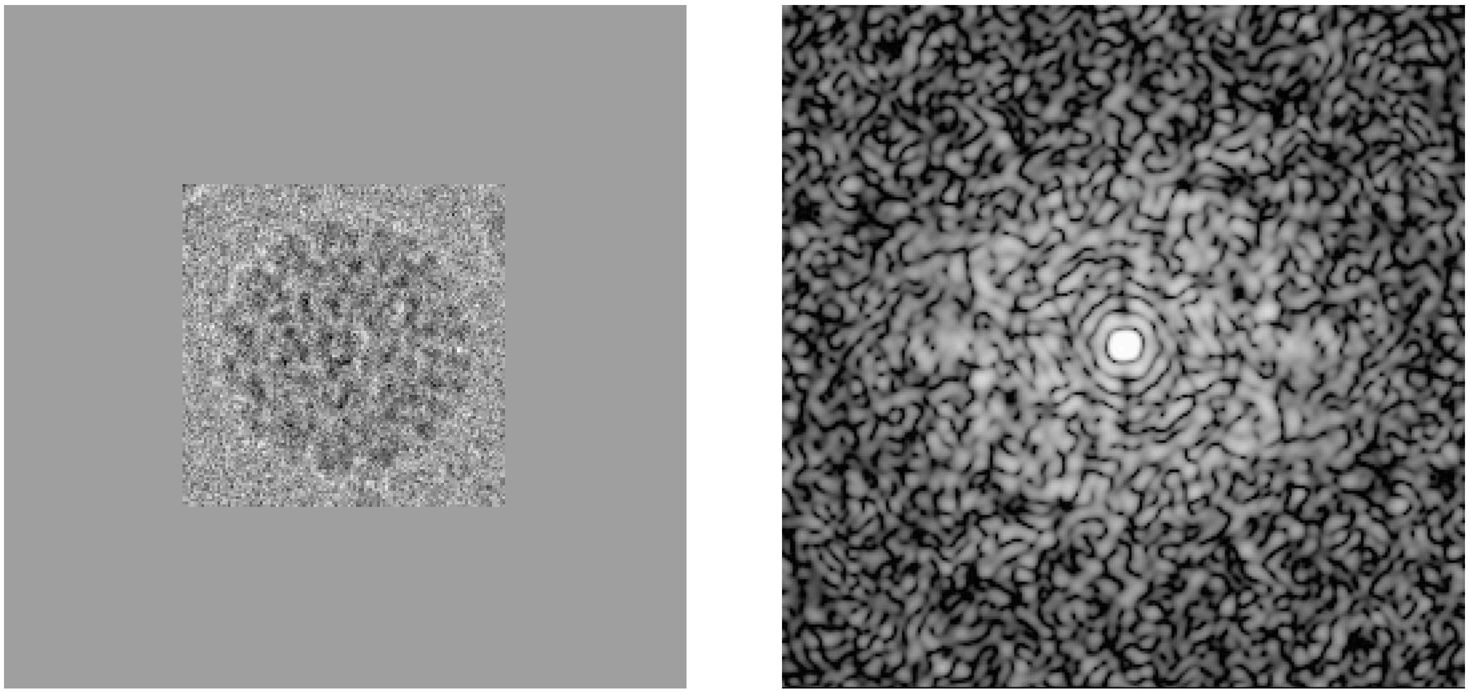


Square window; **<u>un</u>**floated

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image

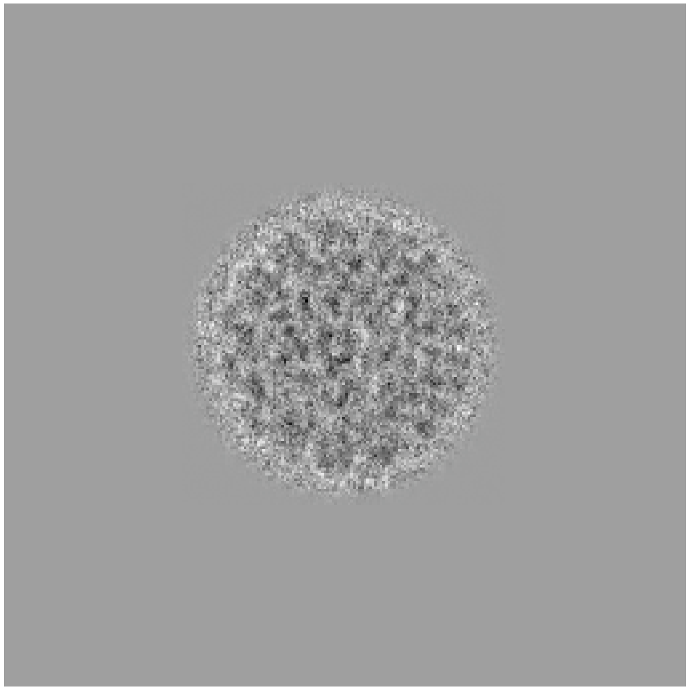


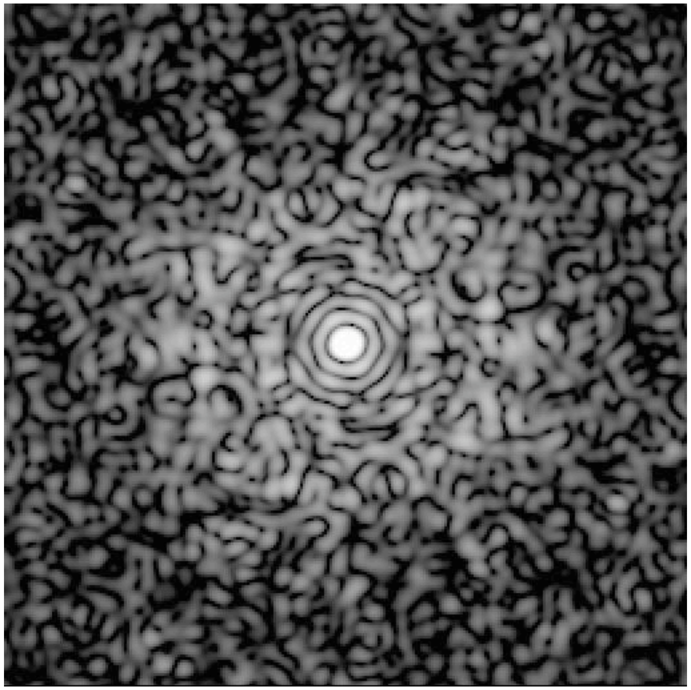
Square window; floated

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image





Circular window; apodized & floated

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

4. Fourier Transformation

Fourier transform of numerical array computed by fast-Fourier (**FFT**) methods

- Nothing magical or mystical to FFT routines

- Have been readily available for decades and are well tested

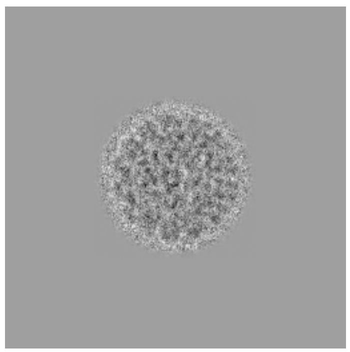
III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

4. Fourier Transformation

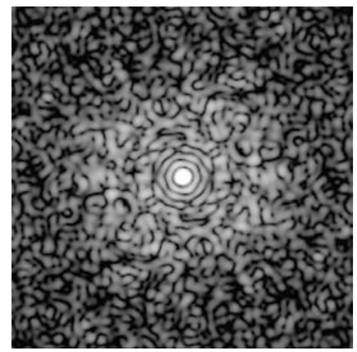
Fourier transform of an *n* by *m* pixel image results in an *n* by *m* **complex** array of numbers (structure factors)

512^2 pixels



$\rho(x,y)$

FT →



$F_{h,l}$

512^2 SFs

Recall:

$$F_{h,l} = \sum_{y} \sum_{x} \rho(x, y) e^{2\pi i (h x + k y)}$$

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

4. Fourier Transformation

Fourier transform of an *n* by *m* pixel image results in an *n* by *m* **complex** array of numbers (structure factors)

Each structure factor is stored in computer memory either as an **amplitude and phase** or as a **real** (A-part) **and imaginary** (B-part) part

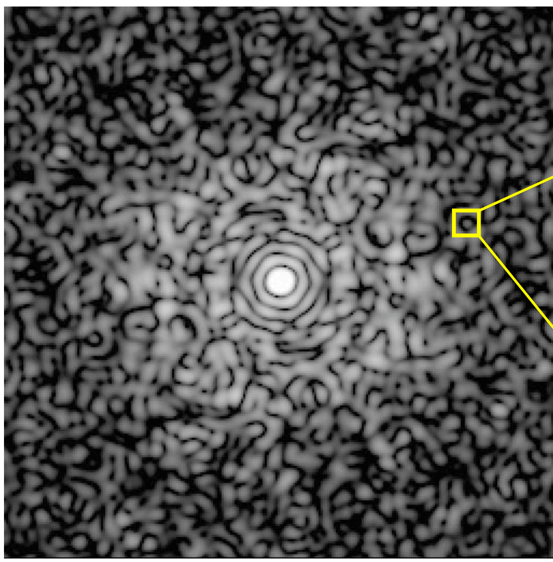
Transforms or diffraction patterns generally displayed on a graphics monitor (*e.g.* in RobEM)

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

4. Fourier Transformation

Fourier transform of an *n* by *m* pixel image results in an *n* by *m* **complex** array of numbers (structure factors)



$F_{h,k}$

AMPLITUDES

5	10	7	11	5	4	10	5	6
6	6	21	9	3	8	1	13	9
4	14	8	8	5	3	1	5	1
9	16	15	7	8	11	7	4	5
9	14	19	10	35	18	25	14	10
10	10	13	15	40	27	15	18	7
8	6	7	7	12	6	14	10	6
9	5	6	6	21	16	8	3	6
6	3	5	7	15	13	4	13	5

$|F_{h,k}|$

PHASES

3	30	24	31	36	31	31	6	23
33	18	10	9	31	7	9	9	10
29	3	5	7	5	29	14	16	25
28	1	4	1	13	14	2	21	20
7	26	3	17	23	25	4	24	16
1	20	30	15	23	31	9	25	33
30	34	35	7	7	18	20	32	2
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32

$\alpha_{h,k} / 10$

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

5. Indexing of 2-D Lattices

Correct indexing of diffraction pattern **ESSENTIAL** for successful image reconstruction analysis

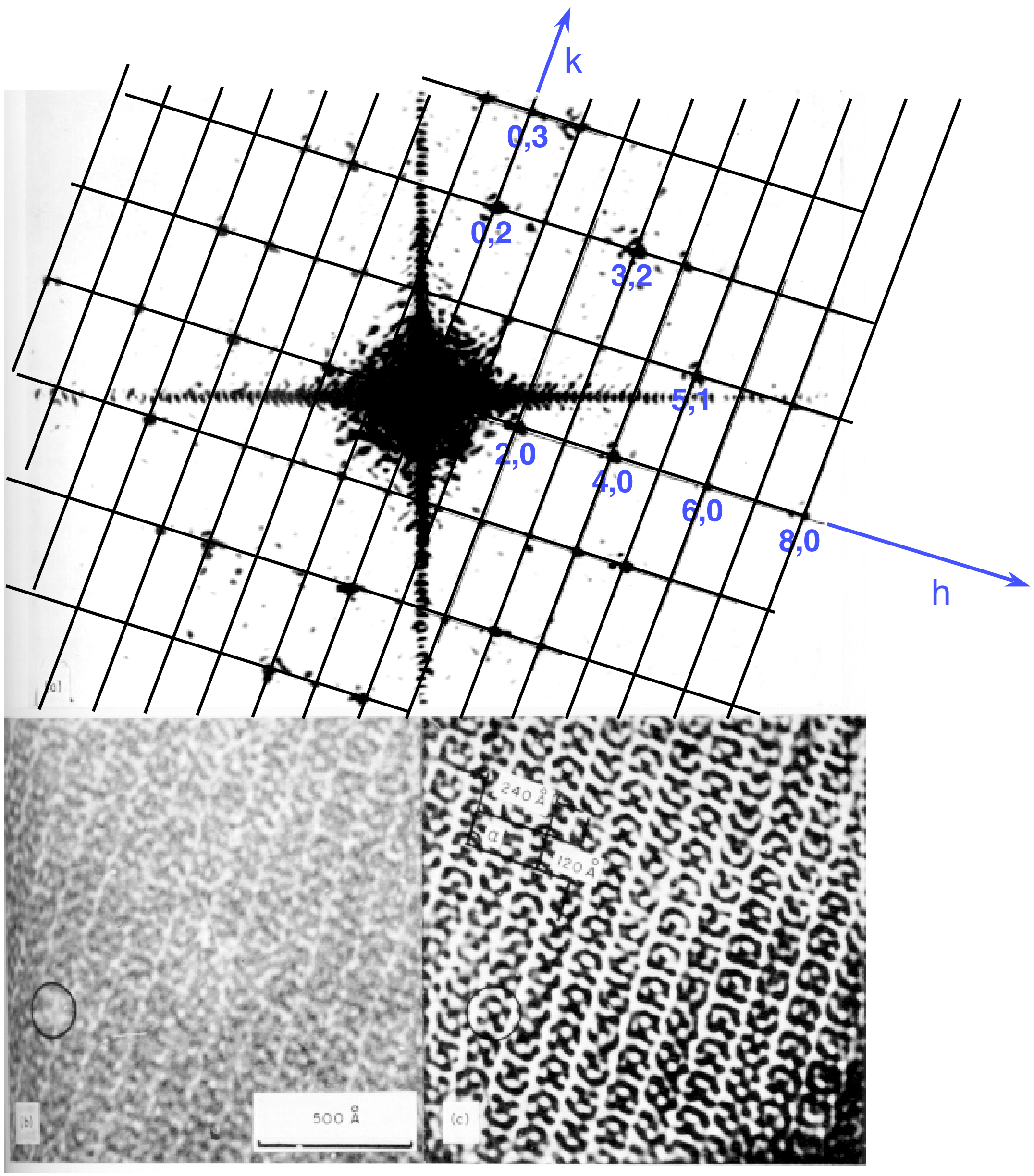
For a well-ordered biological specimen (*e.g.* 2D crystal):

- Diffraction pattern is a series of discrete, sharp spots (Bragg reflections) on a reciprocal lattice

- Such patterns usually fairly easy to index (*i.e.* define reciprocal lattice parameters and assign a Miller index to each spot)

Example: Phosphorylase b crystal

III.D.2.a Indexing the Optical Diffraction Pattern



Unfiltered

Filtered

OD and Filtration of Negatively-stained Phosphorylase b Crystal

From Kiselev et al., (1971) Plate III

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

5. Indexing of 2-D Lattices

For multilayered or two-sided structures (*e.g.* biological aggregates with helical symmetry):

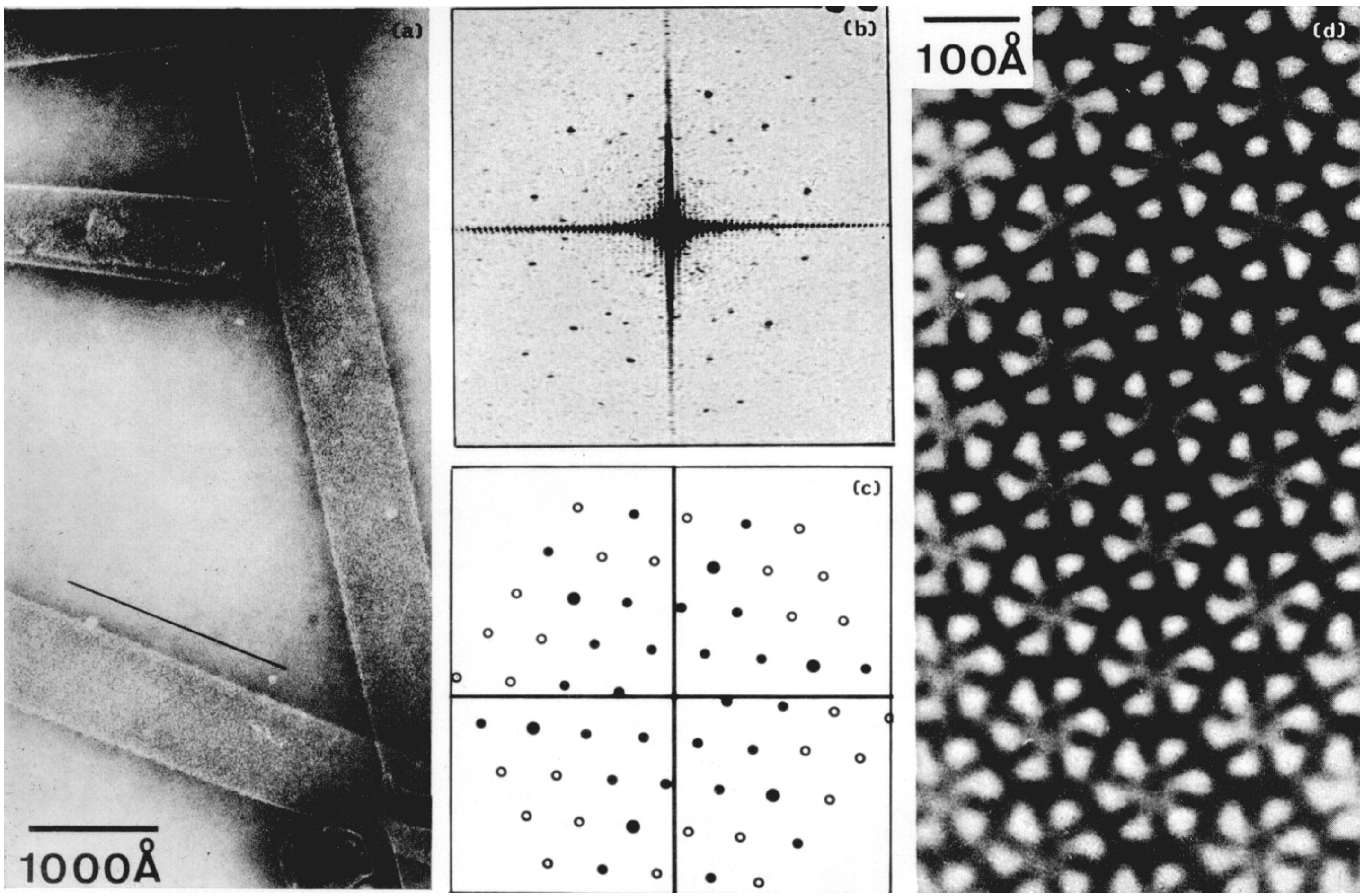
- Indexing can be quite tricky

Example: T4 Polyhead

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

5. Indexing of 2-D Lattices

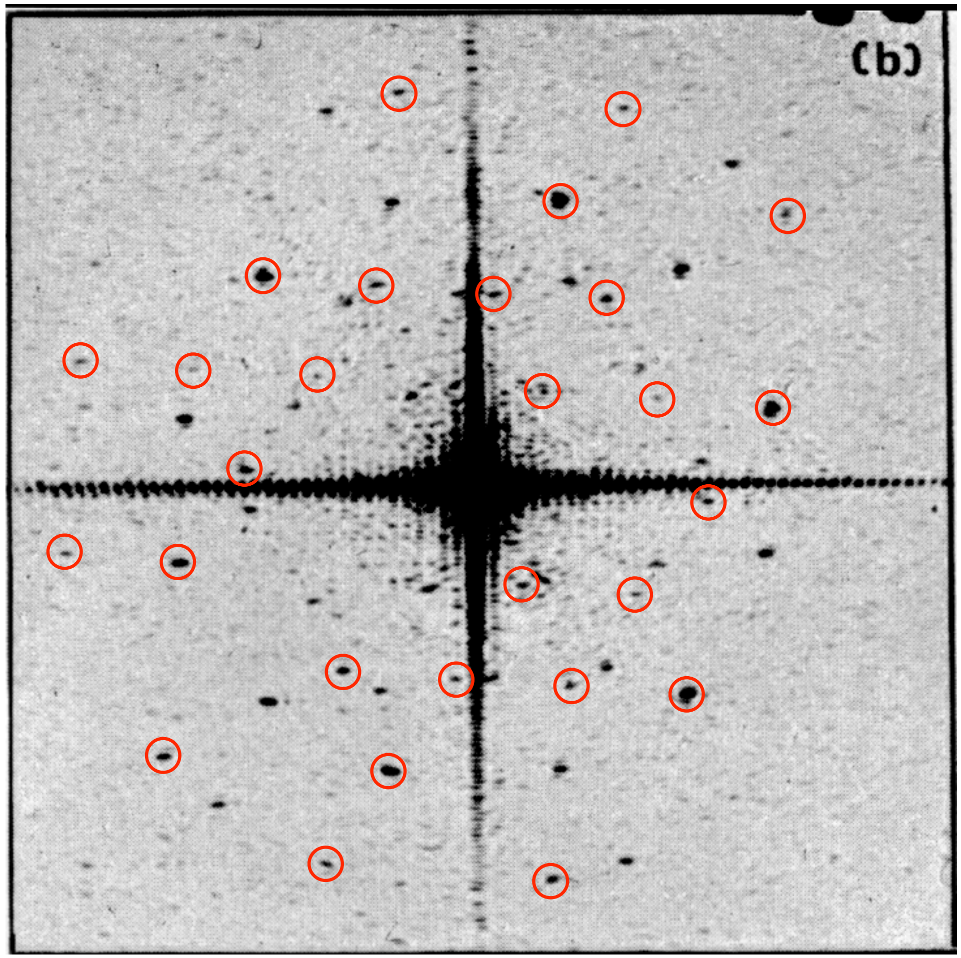


From Steven et al., (1976) Fig. 9, p.205

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

5. Indexing of 2-D Lattices

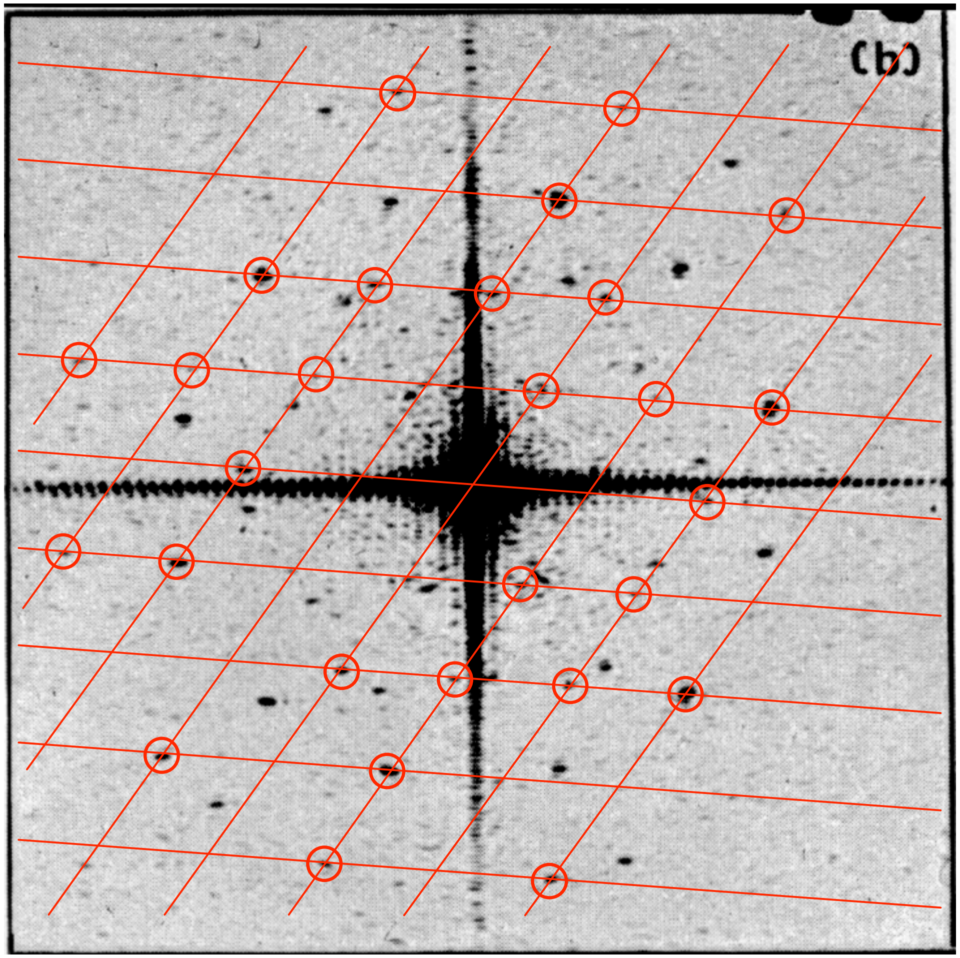


From Steven et al., (1976) Fig. 9, p.205

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

5. Indexing of 2-D Lattices

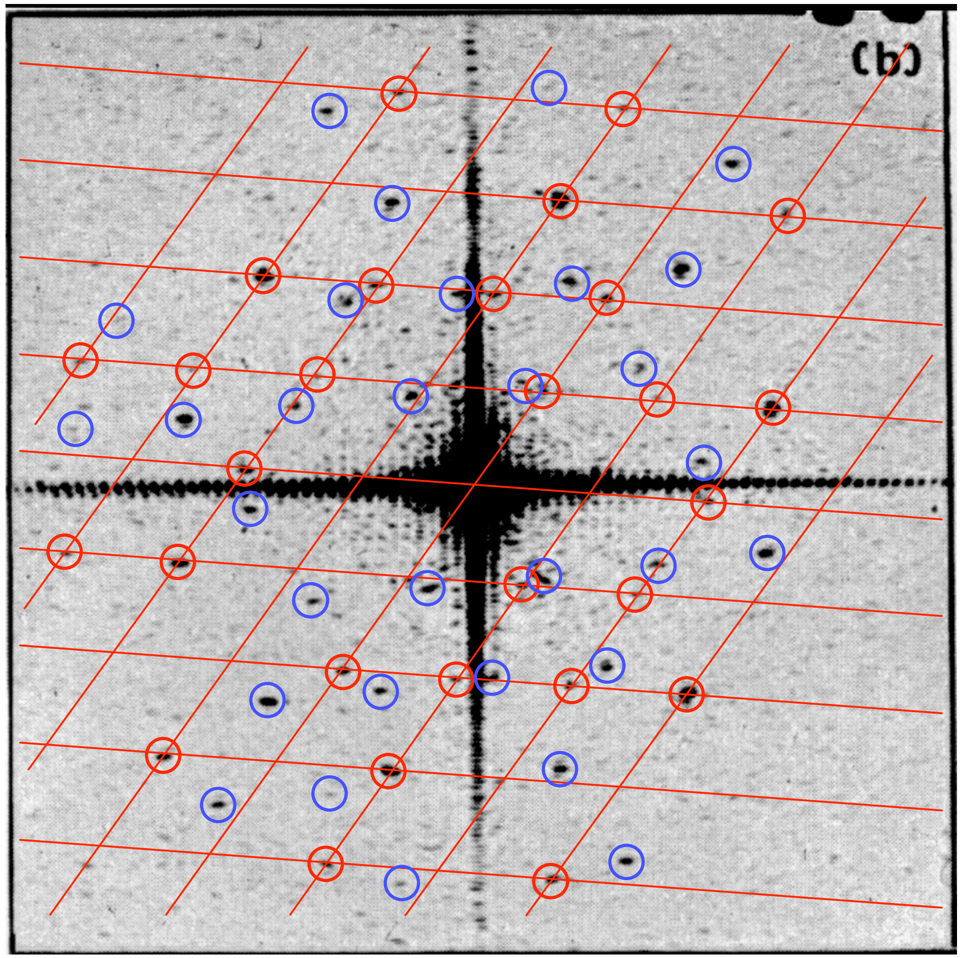


From Steven et al., (1976) Fig. 9, p.205

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

5. Indexing of 2-D Lattices

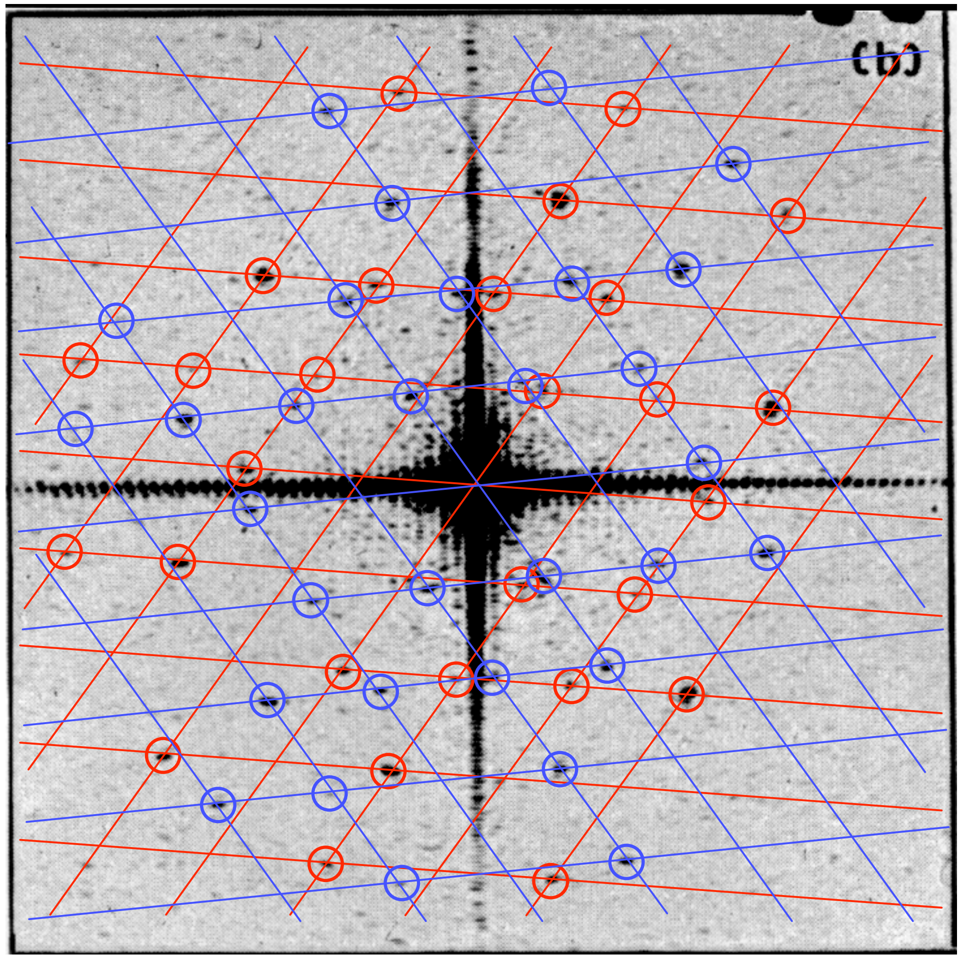


From Steven et al., (1976) Fig. 9, p.205

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

5. Indexing of 2-D Lattices

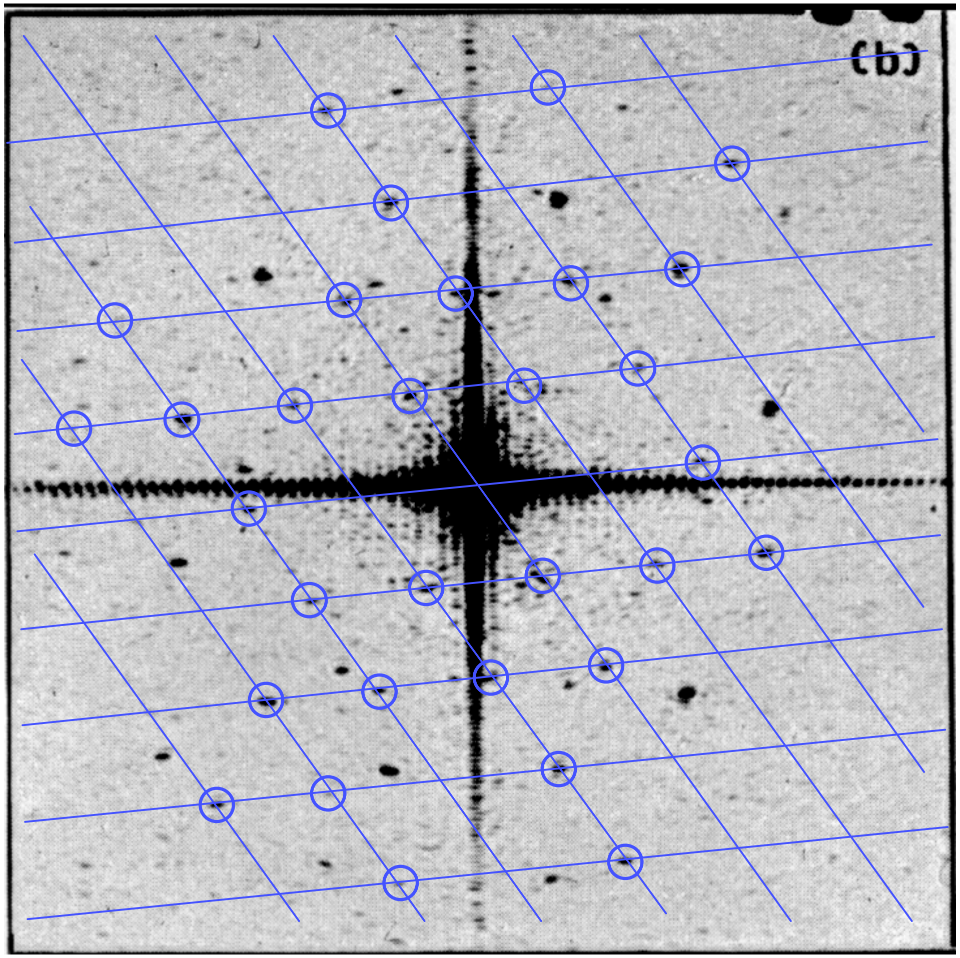


From Steven et al., (1976) Fig. 9, p.205

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

5. Indexing of 2-D Lattices



From Steven et al., (1976) Fig. 9, p.205

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes in computed FT **zeroed** everywhere **<u>except</u>** at or near reciprocal lattice points

An averaged image is reconstructed by back-transforming the modified ("filtered") diffraction pattern

Recall:

$$\text{image} \xrightarrow{FT} \underbrace{T(\text{image}) \times \text{MASK}}_{\text{filtered transform}} \xrightarrow{FT^{-1}} \underbrace{\text{image} * T^{-1}(\text{MASK})}_{\text{filtered image}}$$

filtered transform

filtered image

averaged image

reconstructed image

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

"Pseudo-Optical" Filtering:

Here, "points" actually refers to **finite regions** (holes in the "filter mask") that surround the points of an **ideal** reciprocal lattice

Data inside the mask holes are left as is (*i.e.* multiplied by 1) or may be weighted according to the distance of each transform data value from the ideal lattice

Will demo this next time in RobEM

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes ($|F_{h,k}|$)

5	10	7	11	5	4	10	5	6
6	6	21	9	3	8	1	13	9
4	14	8	8	5	3	1	5	1
9	16	15	7	8	11	7	4	5
9	14	19	10	85	18	25	14	10
10	10	13	15	46	27	15	18	7
8	6	7	7	12	6	14	10	6
9	5	6	6	21	16	8	3	6
6	3	5	7	15	13	4	13	5

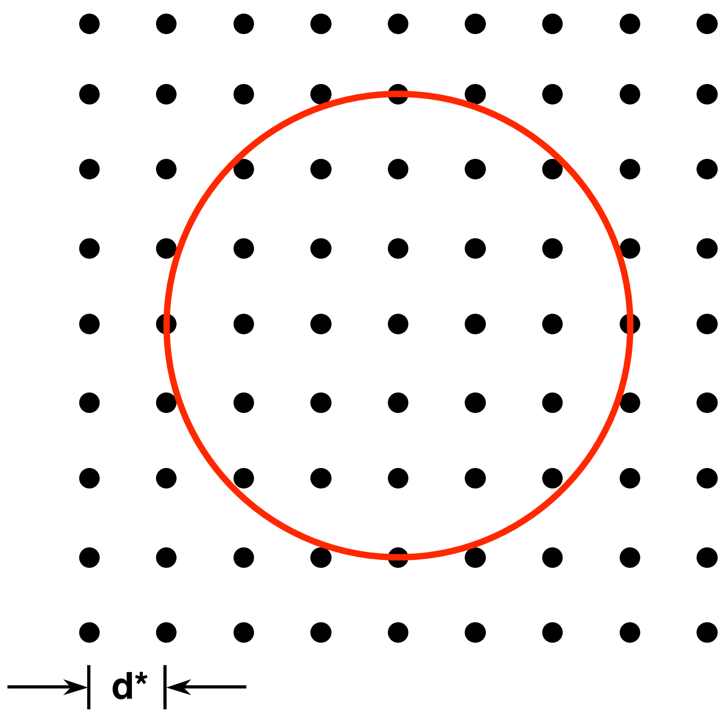
Phases ($\alpha_{h,k}$ / 10)

3	30	24	31	36	31	31	6	23
33	18	10	9	31	7	9	9	10
29	3	5	7	5	29	14	16	25
28	1	4	1	13	14	2	21	20
7	26	3	17	23	25	4	24	16
1	20	30	15	23	31	9	25	33
30	34	35	7	7	18	20	32	2
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32

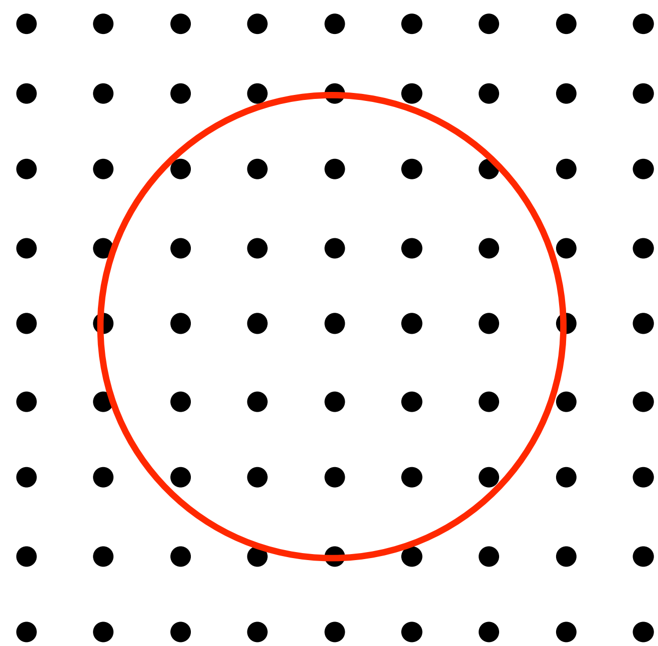
III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes ($|F_{h,k}|$)



Phases ($\alpha_{h,k}$ / 10)



$D_{HOLE} = 6d^*$

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes ($|F_{h,k}|$)

5	10	7	11	5	4	10	5	6
6	6	21	9	3	8	1	13	9
4	14	8	8	5	3	1	5	1
9	16	15	7	8	11	7	4	5
9	14	19	10	85	18	25	14	10
10	10	13	15	46	27	15	18	7
8	6	7	7	12	6	14	10	6
9	5	6	6	21	16	8	3	6
6	3	5	7	15	13	4	13	5

→| d* |←

Phases ($\alpha_{h,k}$ / 10)

3	30	24	31	36	31	31	6	23
33	18	10	9	31	7	9	9	10
29	3	5	7	5	29	14	16	25
28	1	4	1	13	14	2	21	20
7	26	3	17	23	25	4	24	16
1	20	30	15	23	31	9	25	33
30	34	35	7	7	18	20	32	2
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32

$$D_{HOLE} = 6d^*$$

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes ($|F_{h,k}|$)

0	0	0	0	0	0	0	0	0
0	0	0	0	3	0	0	0	0
0	0	8	8	5	3	1	0	0
0	0	15	7	8	11	7	0	0
0	14	19	10	85	18	25	14	0
0	0	13	15	46	27	15	0	0
0	0	7	7	12	6	14	0	0
0	0	0	0	21	0	0	0	0
0	0	0	0	0	0	0	0	0

→| d* |←

$D_{HOLE} = 6d^*$

Phases ($\alpha_{h,k} / 10$)

3	30	24	31	36	31	31	6	23
33	18	10	9	31	7	9	9	10
29	3	5	7	5	29	14	16	25
28	1	4	1	13	14	2	21	20
7	26	3	17	23	25	4	24	16
1	20	30	15	23	31	9	25	33
30	34	35	7	7	18	20	32	2
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes ($|F_{h,k}|$)

0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	5	0	0	0	0
0	0	0	7	8	11	0	0	0
0	0	19	10	85	18	25	0	0
0	0	0	15	46	27	0	0	0
0	0	0	0	12	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

→| d* |←

$D_{HOLE} = 4d^*$

Phases ($\alpha_{h,k}$ / 10)

3	30	24	31	36	31	31	6	23
33	18	10	9	31	7	9	9	10
29	3	5	7	5	29	14	16	25
28	1	4	1	13	14	2	21	20
7	26	3	17	23	25	4	24	16
1	20	30	15	23	31	9	25	33
30	34	35	7	7	18	20	32	2
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes ($|F_{h,k}|$)

0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	8	0	0	0	0
0	0	0	10	85	18	0	0	0
0	0	0	0	46	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

d*

Phases ($\alpha_{h,k}$ / 10)

3	30	24	31	36	31	31	6	23
33	18	10	9	31	7	9	9	10
29	3	5	7	5	29	14	16	25
28	1	4	1	13	14	2	21	20
7	26	3	17	23	25	4	24	16
1	20	30	15	23	31	9	25	33
30	34	35	7	7	18	20	32	2
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32

$$D_{HOLE} = 2d^*$$

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes ($|F_{h,k}|$)

0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	85	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

→| d* |←

Phases ($\alpha_{h,k}$ / 10)

3	30	24	31	36	31	31	6	23
33	18	10	9	31	7	9	9	10
29	3	5	7	5	29	14	16	25
28	1	4	1	13	14	2	21	20
7	26	3	17	23	25	4	24	16
1	20	30	15	23	31	9	25	33
30	34	35	7	7	18	20	32	2
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32

$D_{HOLE} = d^*$

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes ($|F_{h,k}|$)

0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	5	0	0	0	0
0	0	0	7	8	11	0	0	0
0	0	19	10	85	18	25	0	0
0	0	0	15	46	27	0	0	0
0	0	0	0	12	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

d*

Phases ($\alpha_{h,k}$ / 10)

3	30	24	31	36	31	31	6	23
33	18	10	9	31	7	9	9	10
29	3	5	7	5	29	14	16	25
28	1	4	1	13	14	2	21	20
7	26	3	17	23	25	4	24	16
1	20	30	15	23	31	9	25	33
30	34	35	7	7	18	20	32	2
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32

D_{HOLE} = 4d*

"Hard" edge

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes ($|F_{h,k}|$)

0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	2	0	0	0	0
0	0	0	3	5	5	0	0	0
0	0	7	6	85	11	9	0	0
0	0	0	7	28	13	0	0	0
0	0	0	0	4	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

→| d* |←

Phases ($\alpha_{h,k}$ / 10))

3	30	24	31	36	31	31	6	23
33	18	10	9	31	7	9	9	10
29	3	5	7	5	29	14	16	25
28	1	4	1	13	14	2	21	20
7	26	3	17	23	25	4	24	16
1	20	30	15	23	31	9	25	33
30	34	35	7	7	18	20	32	2
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32

D_{HOLE} = 4d*

“Soft” edge

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes ($|F_{h,k}|$)

5	10	7	11	5	4	10	5	6
6	6	21	9	3	8	1	13	9
4	14	8	8	5	3	1	5	1
9	16	15	7	8	11	7	4	5
9	14	19	10	85	18	25	14	10
10	10	13	15	46	27	15	18	7
8	6	7	7	12	6	14	10	6
9	5	6	6	21	16	8	3	6
6	3	5	7	15	13	4	13	5

Phases ($\alpha_{h,k}$ / 10)

3	30	24	31	36	31	31	6	23
33	18	10	9	31	7	9	9	10
29	3	5	7	5	29	14	16	25
28	1	4	1	13	14	2	21	20
7	26	3	17	23	25	4	24	16
1	20	30	15	23	31	9	25	33
30	34	35	7	7	18	20	32	2
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes ($|F_{h,k}|$)

5	10	7	11	5	4	10	5	6
6	6	21	9	3	8	1	13	9
4	14	8	8	5	3	1	5	1
9	16	15	7	8	11	7	4	5
9	14	19	10	85	18	25	14	10
10	10	13	15	46	27	15	18	7
8	6	7	7	12	6	14	10	6
9	5	6	6	21	16	8	3	6
6	3	5	7	15	13	4	13	5

Phases ($\alpha_{h,k}$ / 10)

3	30	24	31	36	31	31	6	23
33	18	10	9	31	7	9	9	10
29	3	5	7	5	29	14	16	25
28	1	4	1	13	14	2	21	20
7	26	3	17	23	25	4	24	16
1	20	30	15	23	31	9	25	33
30	34	35	7	7	18	20	32	2
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Complete Fourier Averaging:

- **All** unit cells are averaged
- A **single** structure factor is computed for each reciprocal lattice point
- Fourier synthesis of this reduced set of structure factors gives the reconstructed structure of a **single** unit cell

Note: Process is formally equivalent to performing filtering with mask holes of **infinitely small** diameter

Will demo this next time in RobEM

6. 2-D Filtering / 3-D Reconstruction

3D Reconstruction:

Diffraction phases and amplitudes (structure factors) measured at all points of **3D** reciprocal lattice by combining data from 2D diffraction patterns from many, independent views of the specimen

Rationale for collecting and combining information from different views depends in part on the type of specimen studied (*i.e.* its **symmetry**)

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.c Hardware / Software

Two Disadvantages of Digital Processing:

Expense and complexity of required hardware and software

Microdensitometer: >$100,000 for precision instrument

Computers: Relatively **cheap**!

For $2,000-5,000 can now get reasonable compute power and storage capacity for single-user, interactive image processing environment

For ~$75,000 can build a 32 node Beowolf cluster of PCs for computationally intensive calculations

Software: **Very expensive** in effort and cost (>>$100,000) to write, test, and support a stable suite of programs for running image processing procedures

III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.c Hardware / Software

Software:

Many labs engaged in image processing develop 'in-house' software tailored to needs of specific research projects

Established, portable systems: several are available either commercially or for "free" (*e.g.* SPIDER, IMAGIC, EMAN, MDPP, SEMPER, etc.)

Advantage: may save considerable effort (and frustration) in the development and testing of programs

Disadvantage: strong possibility of being incorrectly implemented by "black-box", novice users

End of Sec.III.D.3