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 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

<table>
<thead>
<tr>
<th>OPTICAL</th>
<th>COMPUTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original micrograph used</td>
<td>Micrograph digitized and &quot;floated&quot;</td>
</tr>
<tr>
<td>OD bench can be simple and inexpensive</td>
<td>Fast computer needed for “interactive” results</td>
</tr>
<tr>
<td>Formation of diffraction pattern instantaneous</td>
<td>Careful digitization normally slow and computation of diffraction patterns may take several seconds</td>
</tr>
<tr>
<td>Filtering operations require high quality (i.e. expensive) optics</td>
<td>Computers get more powerful and cheaper every day</td>
</tr>
<tr>
<td>Accurate filter masks tedious to make</td>
<td>Only limited by quality of software</td>
</tr>
<tr>
<td>Filtered image recorded photographically</td>
<td>Reconstructed images displayed and photographed using computer graphics devices</td>
</tr>
<tr>
<td>Quantitative information difficult or nearly impossible to obtain</td>
<td>Essence of computing IS to be quantitative</td>
</tr>
<tr>
<td>Amplitudes and phases difficult to manipulate</td>
<td>Infinite control over amplitudes and phases</td>
</tr>
</tbody>
</table>
### III.D.3 Digital Fourier Analysis of Electron Micrographs

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

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

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Typical digital processing procedure includes:

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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.
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III.D.3.b Digital Processing Steps

2. Densitometry

**Goal:**

Convert **optical densities** in the photographic emulsion to a digital image (a **numerical array** corresponding to the relative optical densities 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 mm² 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
III.D.3.b Digital Processing Steps

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III.D.3.b Digital Processing Steps

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III.D.3.b Digital Processing Steps

2. Densitometry
III.D.3.b Digital Processing Steps

2. Densitometry

- 128
- 64
- 32
- 4
- 8
- 16
III.D.3.b Digital Processing Steps

2. Densitometry

![Digital Processing Steps Diagram](image-url)
Negatively-stained T4 bacteriophage sampled at different pixel resolutions (in dpi)

<table>
<thead>
<tr>
<th>Resolution (dpi)</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>200</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>100</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>50</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>25</td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
<tr>
<td>12</td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>6</td>
<td><img src="image7.png" alt="Image" /></td>
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III.D.3.b Digital Processing Steps

2. Densitometry
III.D.3.b Digital Processing Steps

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III.D.3.b Digital Processing Steps

2. Densitometry
IIID.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 \(\mu\text{m}\)
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 \(~0.933\text{ nm} (= 3 \times 0.311)\)

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:

<table>
<thead>
<tr>
<th>Nominal Pixel Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEI CM300 MICROGRAPH MAG</td>
</tr>
<tr>
<td>13,500</td>
</tr>
<tr>
<td>19,500</td>
</tr>
<tr>
<td>24,000</td>
</tr>
<tr>
<td>33,000</td>
</tr>
<tr>
<td>45,000</td>
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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:

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<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>13,500</td>
<td>0.519</td>
</tr>
<tr>
<td>19,500</td>
<td>0.359</td>
</tr>
<tr>
<td>24,000</td>
<td>0.292</td>
</tr>
<tr>
<td>33,000</td>
<td>0.212</td>
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<td>45,000</td>
<td>0.156</td>
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<tr>
<td>61,000</td>
<td>0.115</td>
</tr>
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</table>

**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**

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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

- No smearing; Minimal data loss
- Data smearing; Substantial data loss
III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

2. Densitometry

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 \(163 \times 10^6\) 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
IIID.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:

Windowed
Boxing:

Windowed and Apodized
Boxing:

- Windowed
- Windowed and Apodized
III.D.3 Digital Fourier Analysis of Electron Micrographs

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3. Boxing and Floating the Digitized Image

![Graph showing digitized image processing steps]
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III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image
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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; **un**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; **un**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
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III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image

Circular window; apodized & floated
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III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image

Square window; unfloated
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III.D.3.b Digital Processing Steps

3. Boxing and Floating the Digitized Image

Square window; floated
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3. Boxing and Floating the Digitized Image

Circular window; apodized & floated
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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).

Recall:

\[
F_{h,l} = \sum_{x} \sum_{y} \rho(x, y) e^{2\pi i (hx + ky)}
\]

512\(^2\) pixels $\rightarrow$ FT $\rightarrow$ 512\(^2\) SFs
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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) \( F_{h,k} \)
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

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
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5. Indexing of 2-D Lattices

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5. Indexing of 2-D Lattices

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III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

Amplitudes in computed FT zeroed everywhere except at or near reciprocal lattice points

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

**Recall:**

\[
\text{image} \xrightarrow{\text{FT}} T(\text{image}) \times \text{MASK} \xrightarrow{\text{FT}^{-1}} \text{image} \ast T^{-1}(\text{MASK})
\]

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 ($|F_{h,k}|$) | Phases ($\alpha_{h,k} / 10$) |
|--------------------------|------------------------------|
| 5 10 7 11 5 4 10 5 6   | 3 30 24 31 36 31 31 6 23 |
| 6 6 21 9 3 8 1 13 9    | 33 18 10 9 31 7 9 9 10 |
| 4 14 8 8 5 3 1 5 1     | 29 3 5 7 5 29 14 16 25 |
| 9 16 15 7 8 11 7 4 5   | 28 1 4 1 13 14 2 21 20 |
| 9 14 19 10 85 18 25 14 10 | 7 26 3 17 23 25 4 24 16 |
| 10 10 13 15 46 27 15 18 7 | 1 20 30 15 23 31 9 25 33 |
| 8 6 7 7 12 6 14 10 6   | 30 34 35 7 7 18 20 32 2 |
| 9 5 6 6 21 16 8 3 6    | 10 2 31 20 22 24 34 25 16 |
| 6 3 5 7 15 13 4 13 5   | 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 ($\alpha_{h,k}/10$)

$D_{\text{HOLE}} = 6d^*$
### III.D.3.b Digital Processing Steps

#### 6. 2-D Filtering / 3-D Reconstruction

| Amplitudes ($|F_{h,k}|$) | Phases ($\alpha_{h,k}/10$) |
|--------------------------|---------------------------|
| 5 10 7 11 5 4 10 5 6    | 3 30 24 31 36 31 31 6 23 |
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| 6 3 5 7 15 13 4 13 5    | 29 17 26 25 13 12 17 19 32 |

$D_{\text{HOLE}} = 6d^*$
### III.D.3.b Digital Processing Steps

#### 6. 2-D Filtering / 3-D Reconstruction

| Amplitudes ($|F_{h,k}|$) | Phases ($\alpha_{h,k} / 10$) |
|--------------------------|-------------------------------|
| 0 0 0 0 0 0 0 0 0 0      | 3 30 24 31 36 31 31 6 23     |
| 0 0 0 0 0 3 0 0 0 0      | 33 18 10 9 31 7 9 9 10       |
| 0 0 8 8 5 3 1 0 0 0      | 29 3 5 7 5 29 14 16 25       |
| 0 0 15 7 8 11 7 0 0 0    | 28 1 4 1 13 14 2 21 20       |
| 0 14 19 10 85 18 25 14 0 | 7 26 3 17 23 25 4 24 16       |
| 0 0 13 15 46 27 15 0 0 0 | 1 20 30 15 23 31 9 25 33       |
| 0 0 7 7 12 6 14 0 0 0    | 30 34 35 7 7 18 20 32 2       |
| 0 0 0 0 0 21 0 0 0 0      | 10 2 31 20 22 24 34 25 16     |
| 0 0 0 0 0 0 0 0 0 0      | 29 17 26 25 13 12 17 19 32     |

$D_{\text{HOLE}} = 6d^*$
III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

| Amplitudes (|F_{h,k}|) | Phases (\alpha_{h,k}/10) |
|---------------------|-------------------------|
| 0 0 0 0 0 0 0 0 0 0 | 3 30 24 31 36 31 31 6 23 |
| 0 0 0 0 0 0 0 0 0 0 | 33 18 10 9 31 7 9 9 10 |
| 0 0 0 0 0 5 0 0 0 0 | 29 3 5 7 5 29 14 16 25 |
| 0 0 0 7 8 11 0 0 0 0 | 28 1 4 1 13 14 2 21 20 |
| 0 0 19 10 85 18 25 0 0 | 7 26 3 17 23 25 4 24 16 |
| 0 0 0 15 46 27 0 0 0 0 | 1 20 30 15 23 31 9 25 33 |
| 0 0 0 0 12 0 0 0 0 0 | 30 34 35 7 7 18 20 32 2 |
| 0 0 0 0 0 0 0 0 0 0 | 10 2 31 20 22 24 34 25 16 |
| 0 0 0 0 0 0 0 0 0 0 | 29 17 26 25 13 12 17 19 32 |

\[ D_{\text{HOLE}} = 4d^\star \]
### III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

| Amplitudes ($|F_{h,k}|$) | Phases ($\alpha_{h,k} / 10$) |
|-------------------------|-------------------------------|
| 0 0 0 0 0 0 0 0 0 0 0 0 | 3 30 24 31 36 31 31 6 23   |
| 0 0 0 0 0 0 0 0 0 0 0 0 | 33 18 10 9 31 7 9 9 10     |
| 0 0 0 0 0 0 0 0 0 0 0 0 | 29 3 5 7 5 29 14 16 25     |
| 0 0 0 0 8 0 0 0 0 0 0 0 | 28 1 4 1 13 14 2 21 20     |
| 0 0 0 10 85 18 0 0 0 0 0 | 7 26 3 17 23 25 4 24 16     |
| 0 0 0 0 46 0 0 0 0 0 0 0 | 1 20 30 15 23 31 9 25 33     |
| 0 0 0 0 0 0 0 0 0 0 0 0 | 30 34 35 7 7 18 20 32 2      |
| 0 0 0 0 0 0 0 0 0 0 0 0 | 10 2 31 20 22 24 34 25 16     |
| 0 0 0 0 0 0 0 0 0 0 0 0 | 29 17 26 25 13 12 17 19 32     |

\[ D_{\text{HOLE}} = 2d^* \]
### III.D.3.b Digital Processing Steps

#### 6. 2-D Filtering / 3-D Reconstruction

| Amplitudes ($|F_{h,k}|$) | Phases ($\alpha_{h,k}/10$) |
|---------------------------|---------------------------|
| 0 0 0 0 0 0 0 0 0         | 3 30 24 31 36 31 31 6 23 |
| 0 0 0 0 0 0 0 0 0         | 33 18 10 9 31 7 9 9 10 |
| 0 0 0 0 0 0 0 0 0         | 29 3 5 7 5 29 14 16 25 |
| 0 0 0 0 0 0 0 0 0         | 28 1 4 1 13 14 2 21 20 |
| 0 0 0 0 0 0 0 0 0         | 7 26 3 17 23 25 4 24 16 |
| 0 0 0 0 0 0 0 0 0         | 1 20 30 15 23 31 9 25 33 |
| 0 0 0 0 0 0 0 0 0         | 30 34 35 7 7 18 20 32 2 |
| 0 0 0 0 0 0 0 0 0         | 10 2 31 20 22 24 34 25 16 |
| 0 0 0 0 0 0 0 0 0         | 29 17 26 25 13 12 17 19 32 |

$\rightarrow | d^* | \rightarrow$

$$D_{\text{HOLE}} = d^*$$
III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

| Amplitudes ($|F_{h,k}|$) | Phases ($\alpha_{h,k}/10$) |
|--------------------------|---------------------------|
| 0 0 0 0 0 0 0 0 0 0      | 3 30 24 31 36 31 31 6 23  |
| 0 0 0 0 0 0 0 0 0 0      | 33 18 10 9 31 7 9 9 10    |
| 0 0 0 0 0 5 0 0 0 0      | 29 3 5 7 5 29 14 16 25    |
| 0 0 0 0 7 8 11 0 0 0     | 28 1 4 1 13 14 2 21 20    |
| 0 0 19 10 85 18 25 0 0   | 7 26 3 17 23 25 4 24 16    |
| 0 0 0 15 46 27 0 0 0     | 1 20 30 15 23 31 9 25 33    |
| 0 0 0 0 12 0 0 0 0 0     | 30 34 35 7 7 18 20 32 2     |
| 0 0 0 0 0 12 0 0 0 0     | 10 2 31 20 22 24 34 25 16    |
| 0 0 0 0 0 0 0 0 0 0      | 29 17 26 25 13 12 17 19 32    |

$D_{\text{HOLE}} = 4d^*$

“Hard” edge
III.D.3.b Digital Processing Steps

6. 2-D Filtering / 3-D Reconstruction

| Amplitudes ($|F_{h,k}|$) | Phases ($\alpha_{h,k}/10$) |
|--------------------------|--------------------------|
| 0 0 0 0 0 0 0 0 0 0      | 3 30 24 31 36 31 31 6 23 |
| 0 0 0 0 0 0 0 0 0 0      | 33 18 10 9 31 7 9 9 10 |
| 0 0 0 0 0 2 0 0 0 0      | 29 3 5 7 5 29 14 16 25 |
| 0 0 0 0 3 5 5 0 0 0      | 28 1 4 1 13 14 2 21 20 |
| 0 0 0 7 6 85 11 9 0 0   | 7 26 3 17 23 25 4 24 16 |
| 0 0 0 7 28 13 0 0 0 0    | 1 20 30 15 23 31 9 25 33 |
| 0 0 0 0 4 0 0 0 0 0      | 30 34 35 7 7 18 20 32 2 |
| 0 0 0 0 0 0 0 0 0 0      | 10 2 31 20 22 24 34 25 16 |
| 0 0 0 0 0 0 0 0 0 0      | 29 17 26 25 13 12 17 19 32 |

$d^*$

$D_{HOLE} = 4d^*$

“Soft” edge
### III.D.3.b Digital Processing Steps

#### 6. 2-D Filtering / 3-D Reconstruction

| Amplitudes ($|F_{h,k}|$) | Phases ($\alpha_{h,k} / 10$) |
|--------------------------|-------------------------------|
| 5 10 7 11 5 4 10 5 6    | 3 30 24 31 36 31 31 6 23    |
| 6 6 21 9 3 8 1 13 9     | 33 18 10 9 31 7 9 9 10      |
| 4 14 8 8 5 3 1 5 1      | 29 3 5 7 5 29 14 16 25      |
| 9 16 15 7 8 11 7 4 5    | 28 1 4 1 13 14 2 21 20      |
| 9 14 19 10 8 18 25 14 10| 7 26 3 17 23 25 4 24 16      |
| 10 10 13 15 46 27 15 18 7 | 1 20 30 15 23 31 9 25 33  |
| 8 6 7 7 12 6 14 10 6    | 30 34 35 7 7 18 20 32 2     |
| 9 5 6 6 21 16 8 3 6     | 10 2 31 20 22 24 34 25 16    |
| 6 3 5 7 15 13 4 13 5    | 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 ($\alpha_{h,k} / 10$) |
|--------------------------|-----------------------------|
| 5 10 7 11 5 4 10 5 6    | 3 30 24 31 36 31 31 6 23    |
| 6 6 21 9 3 8 1 13 9     | 33 18 10 9 31 7 9 9 10     |
| 4 14 8 8 5 3 1 5 1      | 29 3 5 7 5 29 14 16 25     |
| 9 16 15 7 8 11 7 4 5    | 28 1 4 1 13 14 2 21 20     |
| 9 14 19 10 85 18 25 14 10 | 7 26 3 17 23 25 4 24 16 |
| 10 10 13 15 46 27 15 18 7 | 1 20 30 15 23 31 9 25 33 |
| 8 6 7 7 12 6 14 10 6    | 30 34 35 7 7 18 20 32 2    |
| 9 5 6 6 21 16 8 3 6     | 10 2 31 20 22 24 34 25 16   |
| 6 3 5 7 15 13 4 13 5    | 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*
III.D.3 Digital Fourier Analysis of Electron Micrographs

III.D.3.b Digital Processing Steps

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
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.