III.D FOURIER IMAGE PROCESSING TECHNIQUES

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

Introduction

Though we may ultimately be interested in learning the full 3-D structure of molecules imaged in the TEM, it can be quite informative to compute image reconstructions by Fourier methods in two dimensions, or sometimes even in one dimension (*e.g.* helices)

- A large number of biological macromolecules are planar (2-D) objects (*e.g.* membranes, cell walls, and some naturally occurring crystals)

- These make excellent subjects for Fourier image analysis in both 2D and 3D

III.D FOURIER IMAGE PROCESSING TECHNIQUES

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry

Protocol for Fourier averaging images of 2D crystals:

1) Image screening:

- Visually inspect micrographs to toss obvious bad ones and use OD to select a subset of micrographs that give the highest quality optical diffraction patterns.

- Highly coherent, crystalline areas give strong, sharp Bragg reflections to 'high' resolution (*e.g.* ~10-20Å for negatively stained crystals and possibly much higher for frozen-hydrated specimens, both recorded with minimum irradiation techniques)

- Look for minimal radiation damage, astigmatism, and specimen drift or vibration and for 'best' defocus (*i.e.* giving the desired CTF characteristics) and highest resolution (most spots in all directions).

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry

Protocol for Fourier averaging images of 2D crystals:

1) Image screening

2) Digitize the micrograph:

- Use sampling interval fine enough ***not*** to limit image resolution but ***not too fine*** or the digitized image will consist of many more pixels than is necessary and resulting in needless computations

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry

Protocol for Fourier averaging images of 2D crystals:

1) Image screening

2) Digitize the micrograph

3) Box the micrograph:

- Window out the desired region of interest, making sure to exclude, if possible, as much of the unneeded portions of the digitized image as is practical

- Easy to do with 'perfect' specimens like catalase crystals that grow large enough (several μm^2) to fill the entire field of view at 30,000 magnification or higher

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry

Protocol for Fourier averaging images of 2D crystals:

1) Image screening

2) Digitize the micrograph

3) Box the micrograph

4) Float the boxed image:

- Subtract from **every pixel** within the image the average value of the pixels that form the **perimeter** of the box

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry

Protocol for Fourier averaging images of 2D crystals:

1) Image screening

2) Digitize the micrograph

3) Box the micrograph

4) Float the boxed image

5) Fourier transform the boxed, digitized image:

- Images often padded with zeroes to give power of two image dimensions (*e.g.* 256^2 or 512^2 or 256 x 512 or 128 x 1024, etc.)
- Only 'top half' of Fourier transform need be computed (*i.e.* $k \geq 0$) owing to Friedel's Law
- Transform stored as structure factor A (real) and B (imaginary) parts
- For a 512^2 image, resulting **calculated and stored** transform will consist of 256 rows, each with 512 complex numbers

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry

Protocol for Fourier averaging images of 2D crystals:

1) Image screening
2) Digitize the micrograph
3) Box the micrograph
4) Float the boxed image
5) Fourier transform the boxed, digitized image
6) Display and index the diffraction pattern:

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry

Protocol for Fourier averaging images of 2D crystals:

6) Display and index the diffraction pattern:

- Indexing could be performed on an optical diffraction pattern, however, the digital transform allows one to quantitatively check other properties of the specimen such as the presence of certain plane group symmetries

- Existence, *e.g.*, of a three-fold axis of symmetry **at the unit cell origin**, for **noise free data**, will restrict the structure factor phases to be multiples of 120°

- The noisier the image the more the symmetry related structure factor phases will **deviate** from the 120° relationship

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry

Protocol for Fourier averaging images of 2D crystals:

6) Display and index the diffraction pattern

7) Filter or Fourier average the data:

- Pseudo-optical filtering: filter masks computer generated with 'holes' of a specified size distributed on a lattice either covering the whole transform or limited at some specified upper resolution boundary

- Fourier transform is multiplied by computer-generated mask and the resulting masked transform is back-transformed to generate the filtered image

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry

Protocol for Fourier averaging images of 2D crystals:

6) Display and index the diffraction pattern

7) Filter or Fourier average the data:

- Fourier Averaging: To average **all** unit cells within the boxed area, Fourier data in the vicinity of each Bragg reflection are averaged or integrated together or are sampled to reduce the data to a single structure factor ($F_{h,k}$)
- Equivalent to pseudo-filtering with mask hole radius = 0
- Effect is to convolve the image with a lattice that includes every unit cell contained within the boxed region of the micrograph
- Image is forced to obey perfect translational symmetry (p1 plane group)
- The average structure of a single unit cell (all unit cells are identical in a Fourier average) is obtained by back transforming the structure factors (via Fourier synthesis)

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry

Protocol for Fourier averaging images of 2D crystals:

6) Display and index the diffraction pattern

7) Filter or Fourier average the data

8) Assess and apply additional symmetry if warranted:

- Must be performed with due caution because it is very easy to apply ***any*** symmetry you want (right or wrong) with appropriate software

- When you impose additional symmetry, the specimen will, of course, exhibit whatever symmetry has been applied

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry

Protocol for Fourier averaging images of 2D crystals:

1) Image screening

2) Digitize the micrograph

3) Box the micrograph

4) Float the boxed image

5) Fourier transform the boxed, digitized image

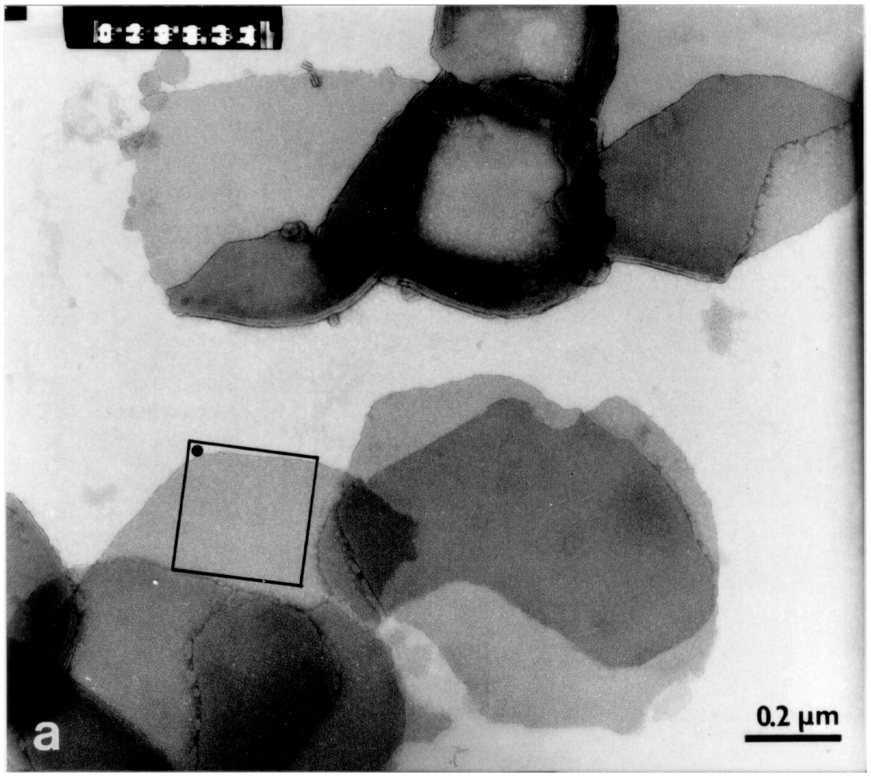
6) Display and index the diffraction pattern

7) Filter or Fourier average the data

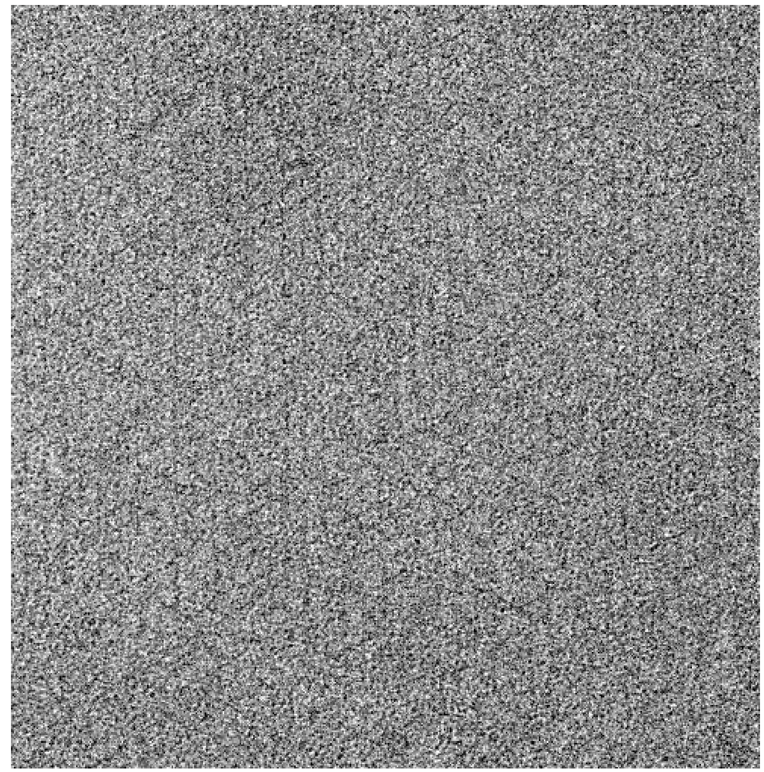
8) Assess and apply additional symmetry if warranted

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry



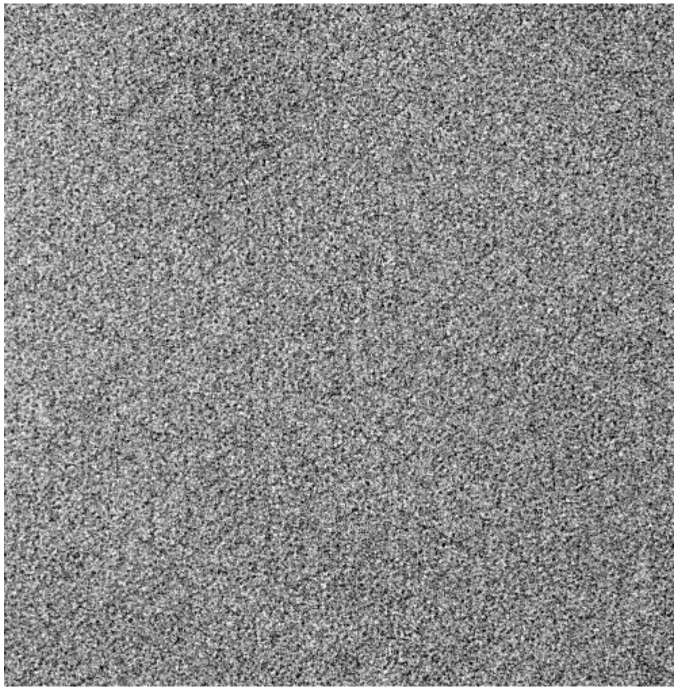
Liver gap junction (negatively stained)

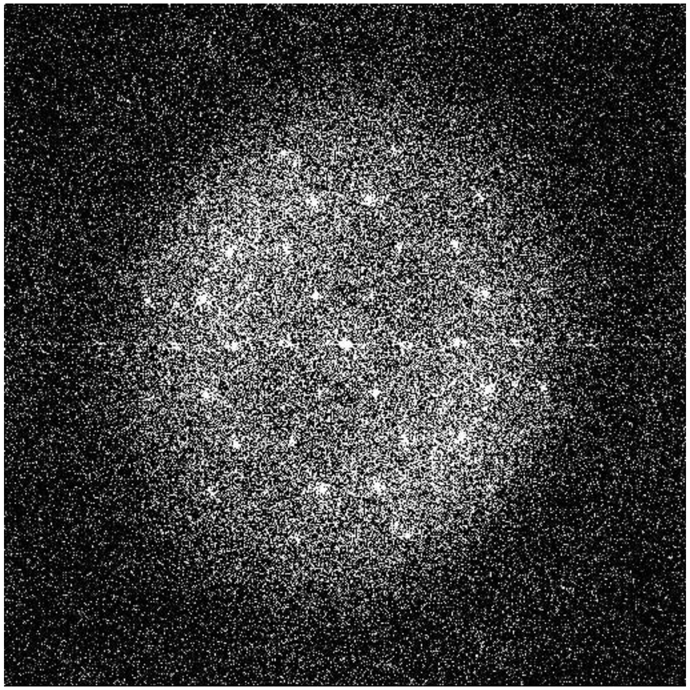


Enlarged view of boxed area

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

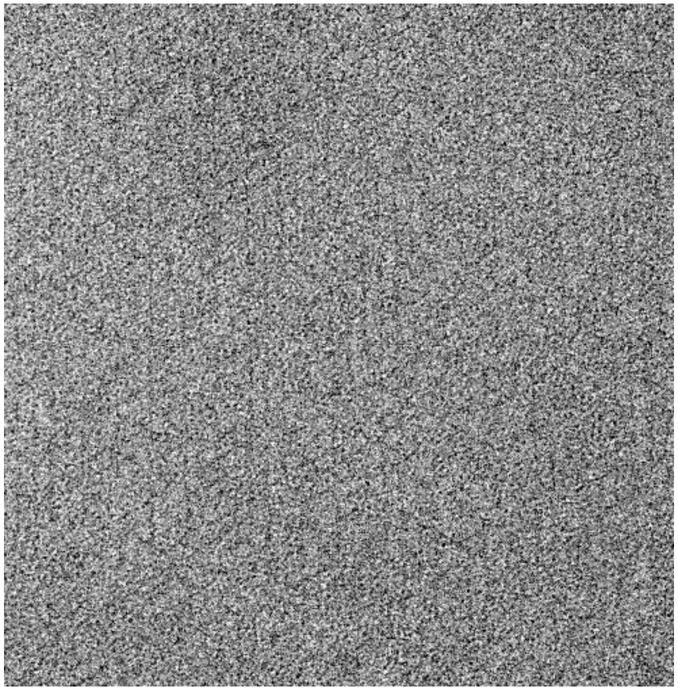
III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry

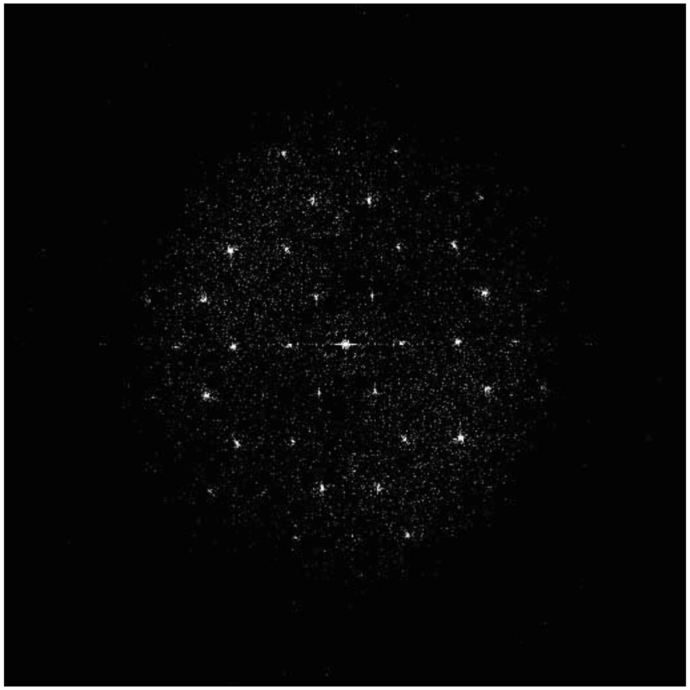




III.D.4 2D and 1D Digital Fourier Reconstruction Methods

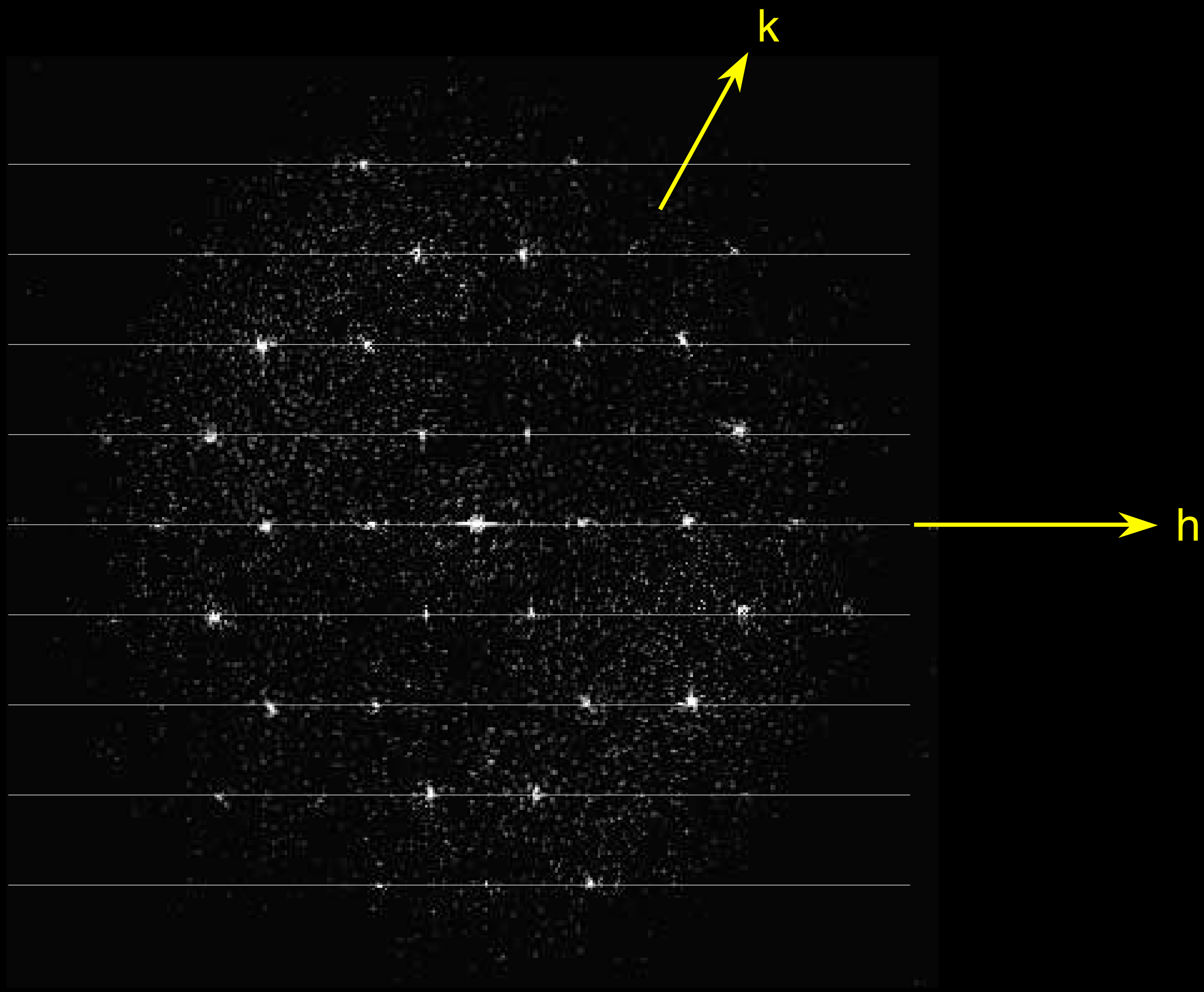
III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry





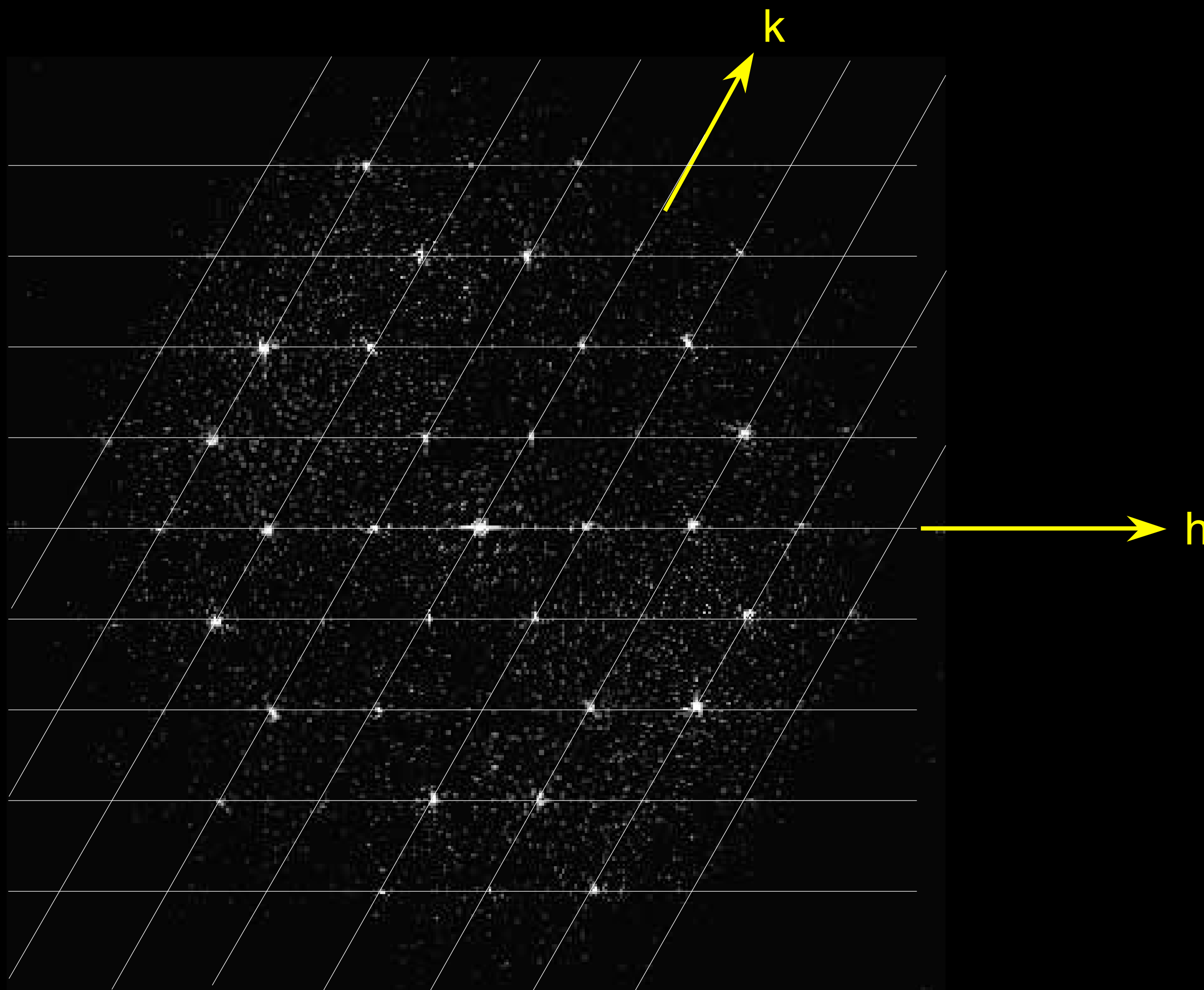
III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry



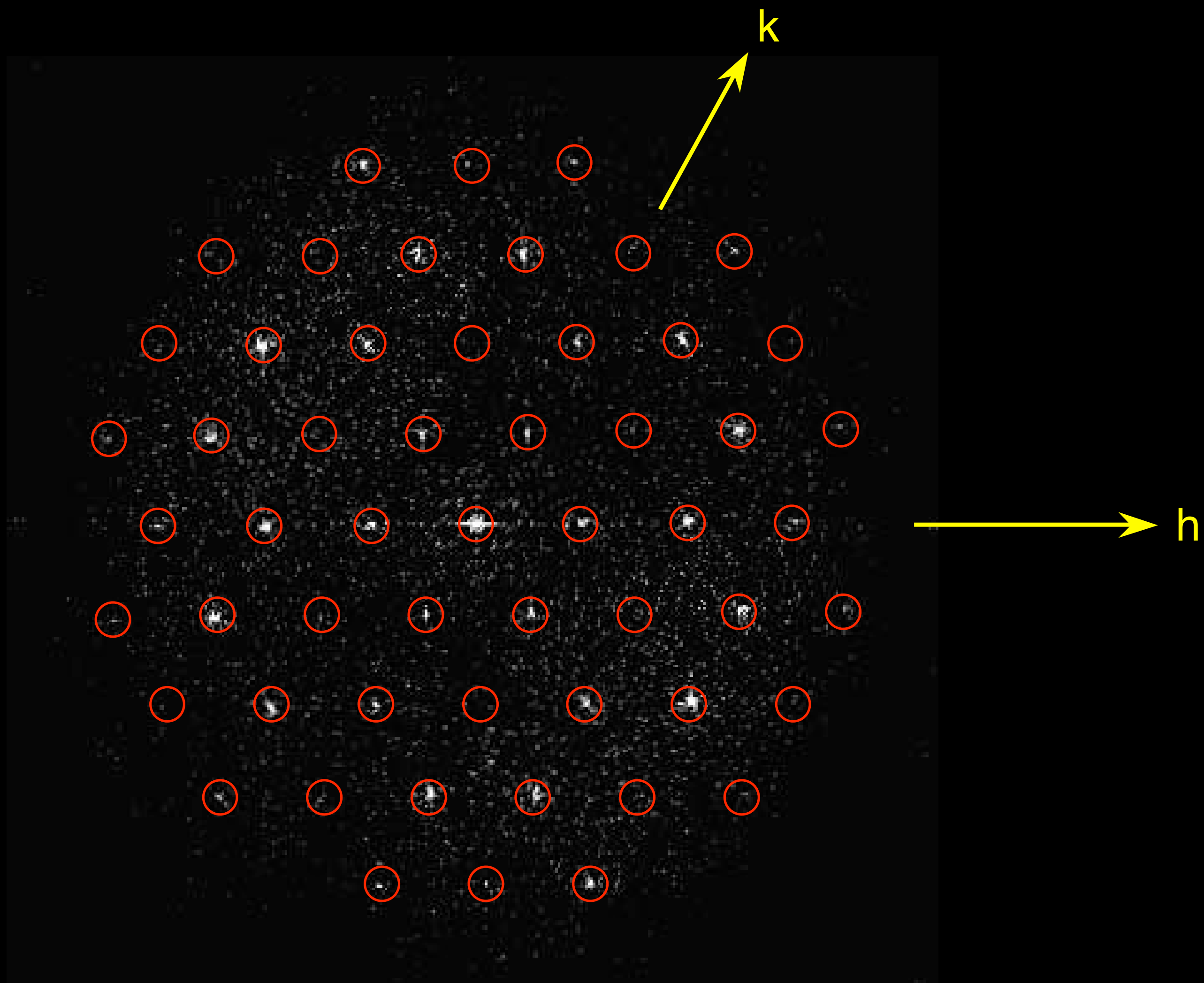
III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry



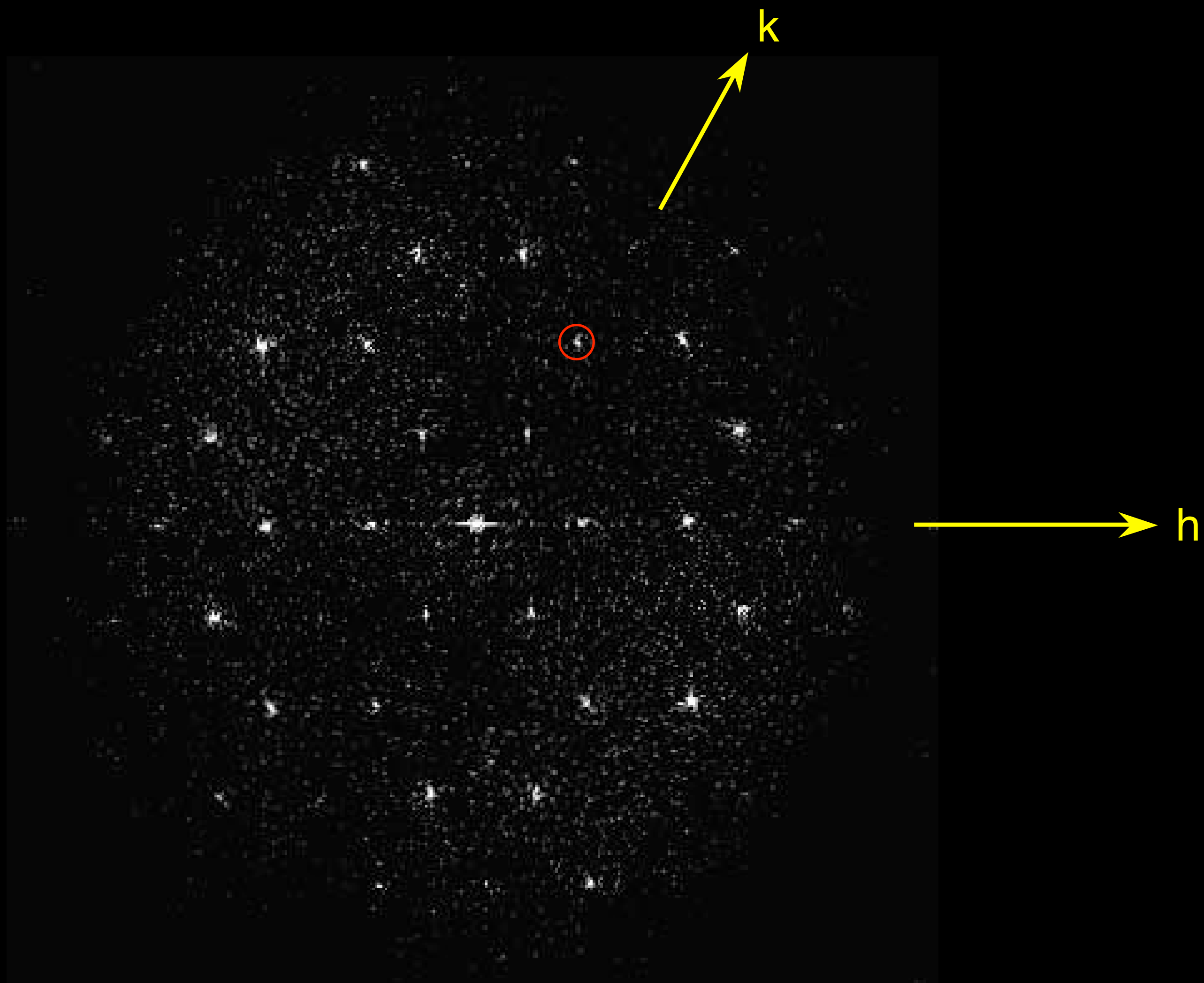
III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry



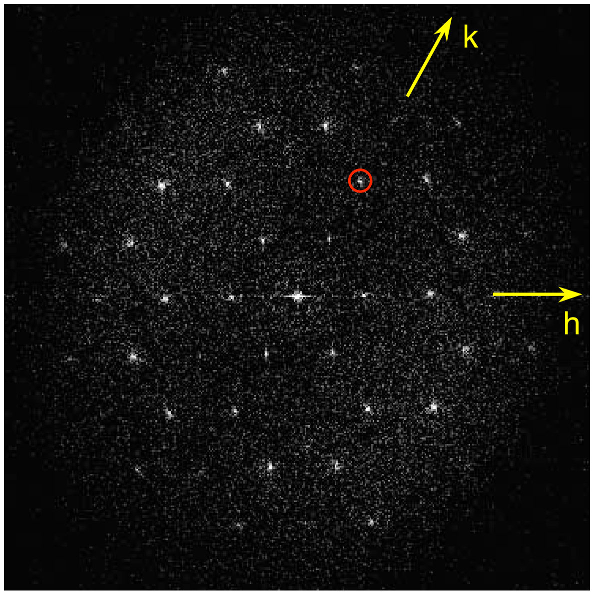
III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry



III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry



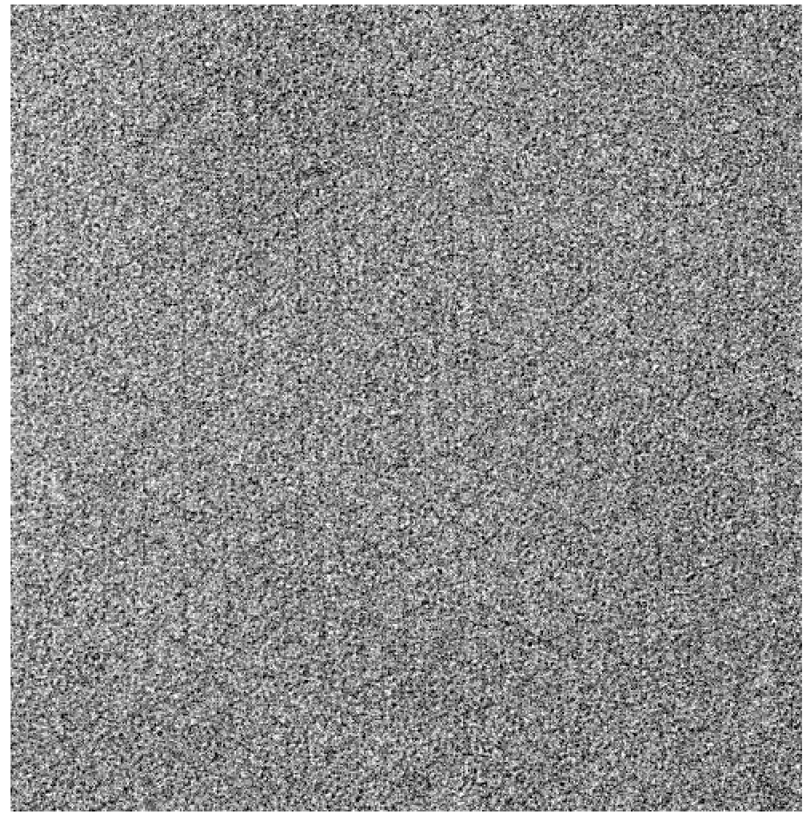
Spot [0, 2]

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)

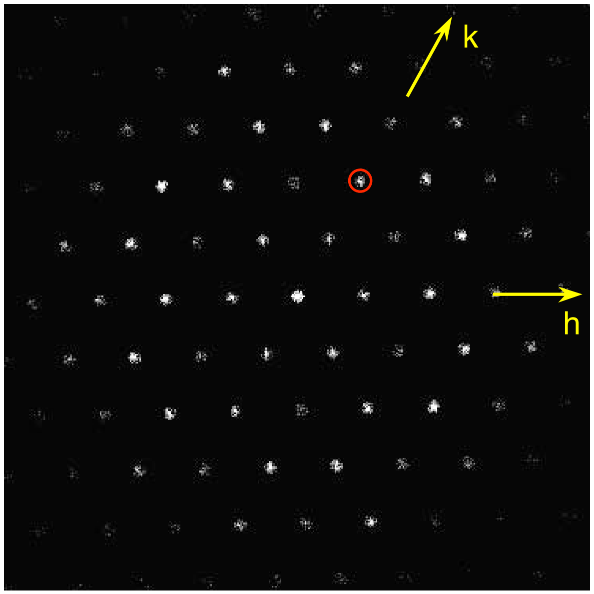
3	30	24	31	36	31	31	6	23
33	18	10	9	31	7	9	9	10
29	3	5	7	5	29	14	16	25
28	1	4	1	13	14	2	21	20
7	26	3	17	23	25	4	24	16
1	20	30	15	23	31	9	25	33
30	34	35	7	7	18	20	32	2
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32



Original

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry



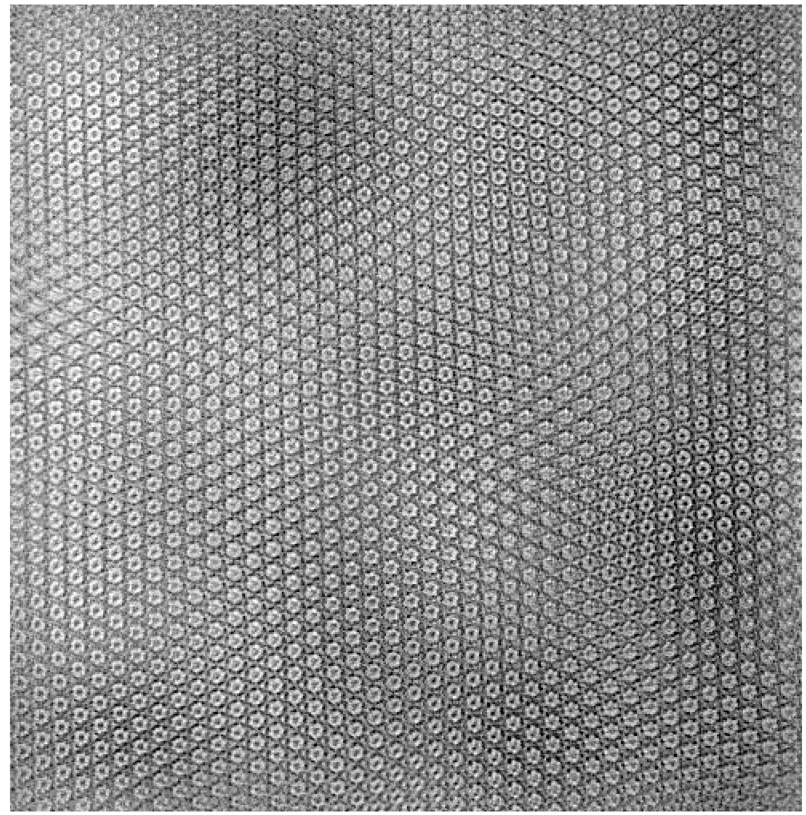
Spot [0, 2]

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

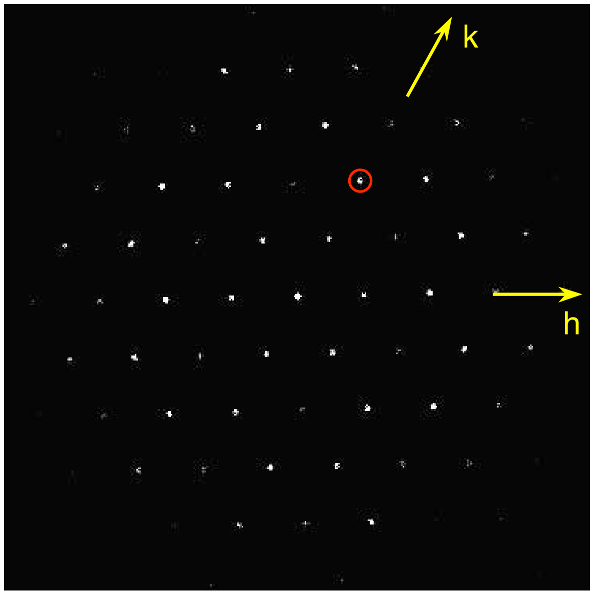


Filtered

$D_{HOLE} = 4d^*$

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry



Spot [0, 2]

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
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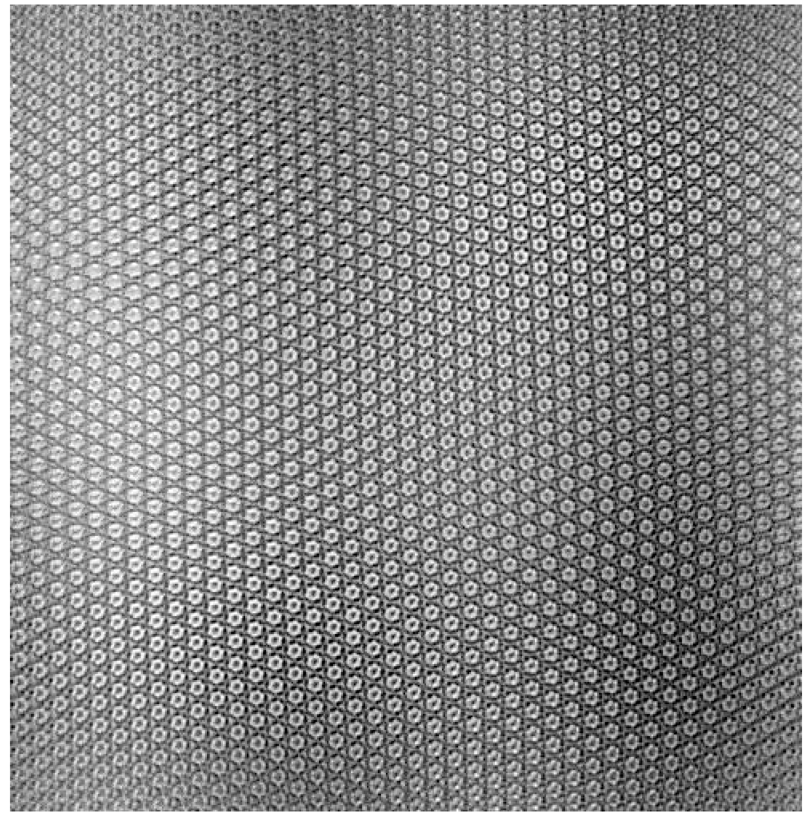
10	2	31	20	22	24	34	25	16
29	17	26	25	13	12	17	19	32

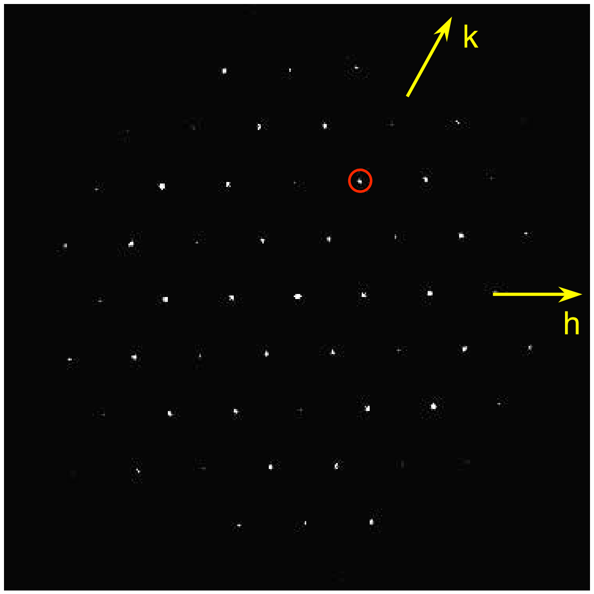


Filtered

$D_{HOLE} = 2d^*$

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.a 2D Fourier Averaging of Specimens with 2D Translational Symmetry



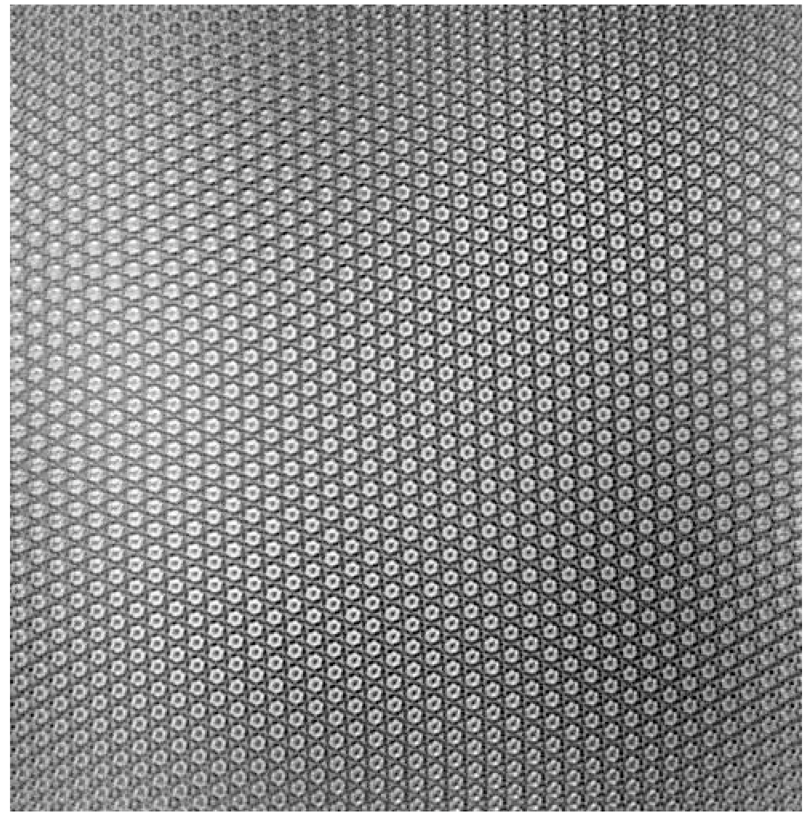
Spot [0, 2]

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



Filtered

$D_{HOLE} = 2d^*$

Soft edge

III.D FOURIER IMAGE PROCESSING TECHNIQUES

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Specimens with only **rotational** symmetry (*e.g.* individual oligomeric proteins, spherical viruses, bacteriophage baseplates, etc.) are studied either by 2D rotational photographic-superposition, rotational filtering, or by 3D reconstruction techniques

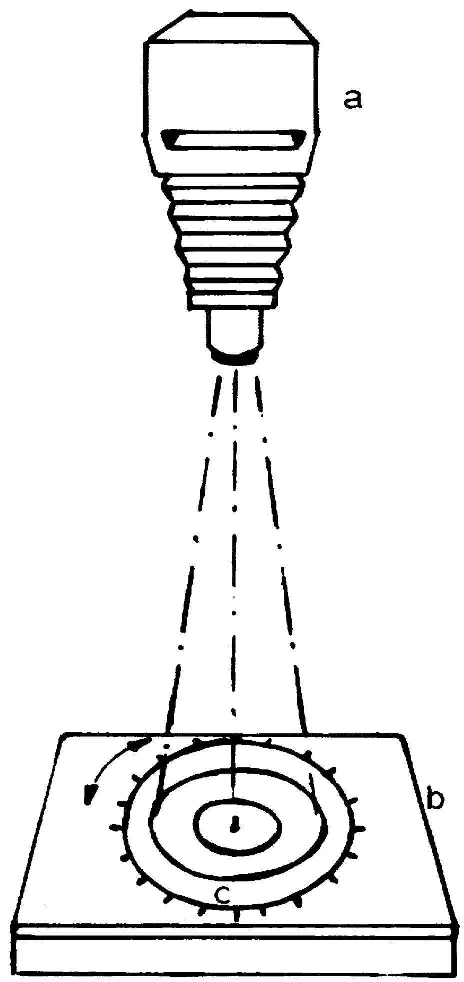
Digital **rotational-filtering** and **photographic superposition** techniques give **qualitatively** similar results

Photographic methods should be used with **caution** - usually with specimens displaying obvious or well-established symmetry

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Rotational Photographic Superposition



From Horne & Markham, Fig. 8.1, p.413

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

3D reconstructions of **spherical viruses** usually calculated by means of Fourier-Bessel methods, which combine **several unique views** of the molecule

For frozen-hydrated viruses studied in 3D at moderately high resolutions (< 10Å), the number of views combined often exceeds 5000 or more.

Though exceptions do exist, outside of spherical viruses, most 3D reconstructions of rotationally symmetric particles are **NOT** computed with the Fourier-Bessel approach

In part this reflects the difficulty in reconstructing particles much smaller and of lower symmetry than spherical viruses

OK, let's talk about Rotational Filtering

This is 2D!!!

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

1) Screen and select images

2) Digitize the micrograph; box and float individual particles

3) Convert image from Cartesian (x,y) to polar (r,ϕ) coordinates

4) Expand density, $\rho(r,\phi)$, into a series of circular waves

5) Integrate each $g_n(r)$ over the radius of the particle, a, to obtain a measure of total n-fold rotational component of the image

6) Plot P_n as a function of n (rotational power spectrum)

7) Switch to Fourier space

8) Identify phase origin

9) Synthesize (reconstruct) filtered image

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

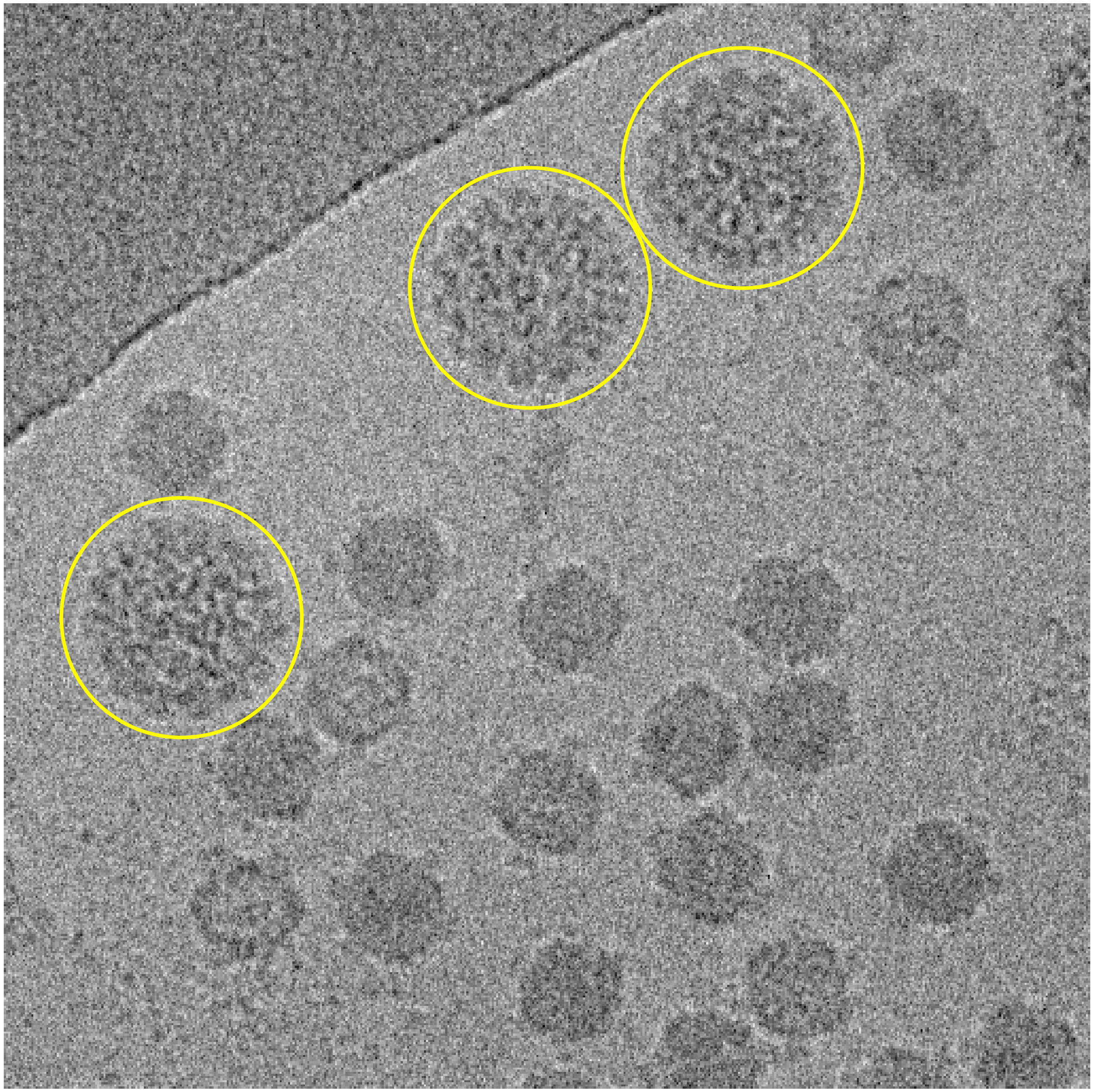
Protocol for Rotational Filtering

1) Screen and select images (mainly by eye)

2) Digitize the micrograph and box and float individual particles within circular mask windows

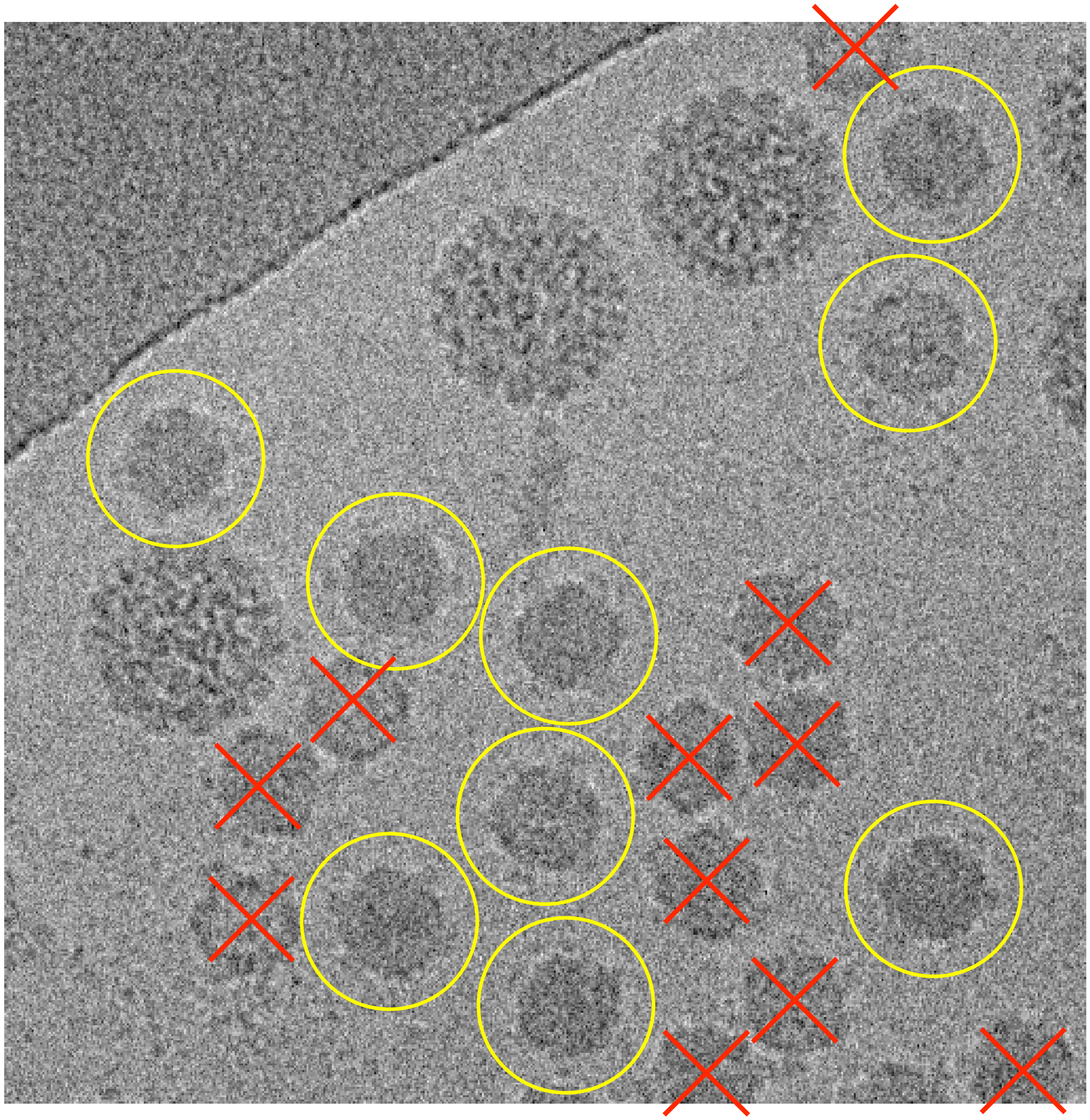
III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry



III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry



III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

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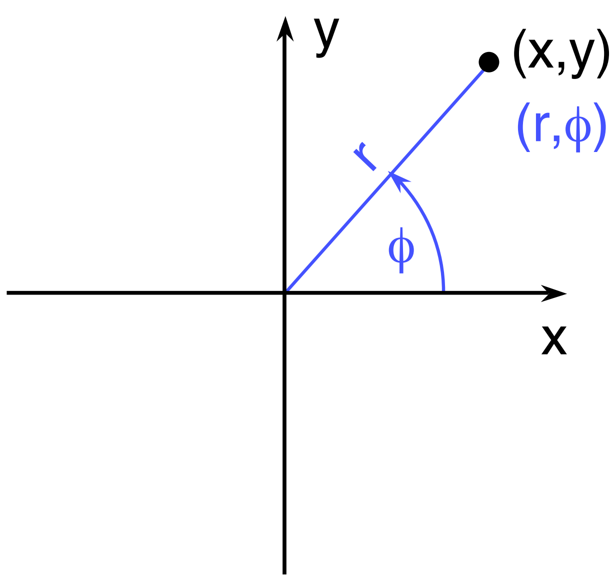
3) Convert image from **Cartesian** (x,y) to **polar** (r,ϕ) coordinates

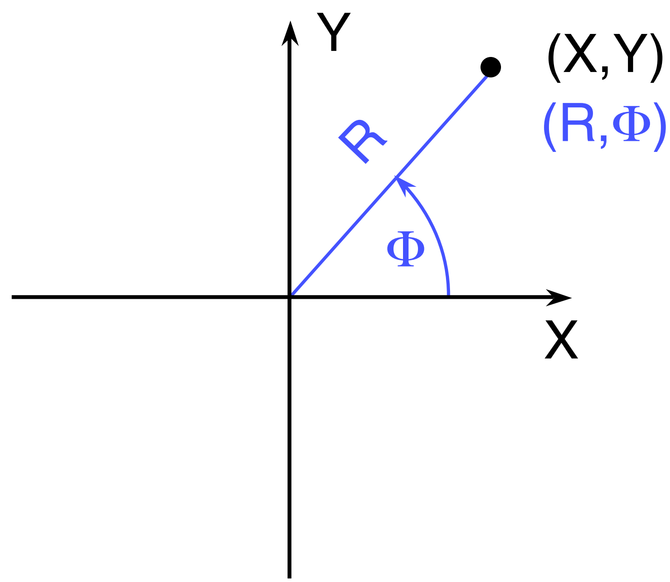
III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

3) Convert image from **Cartesian** (x,y) to **polar** (r,ϕ) coordinates



Real Space

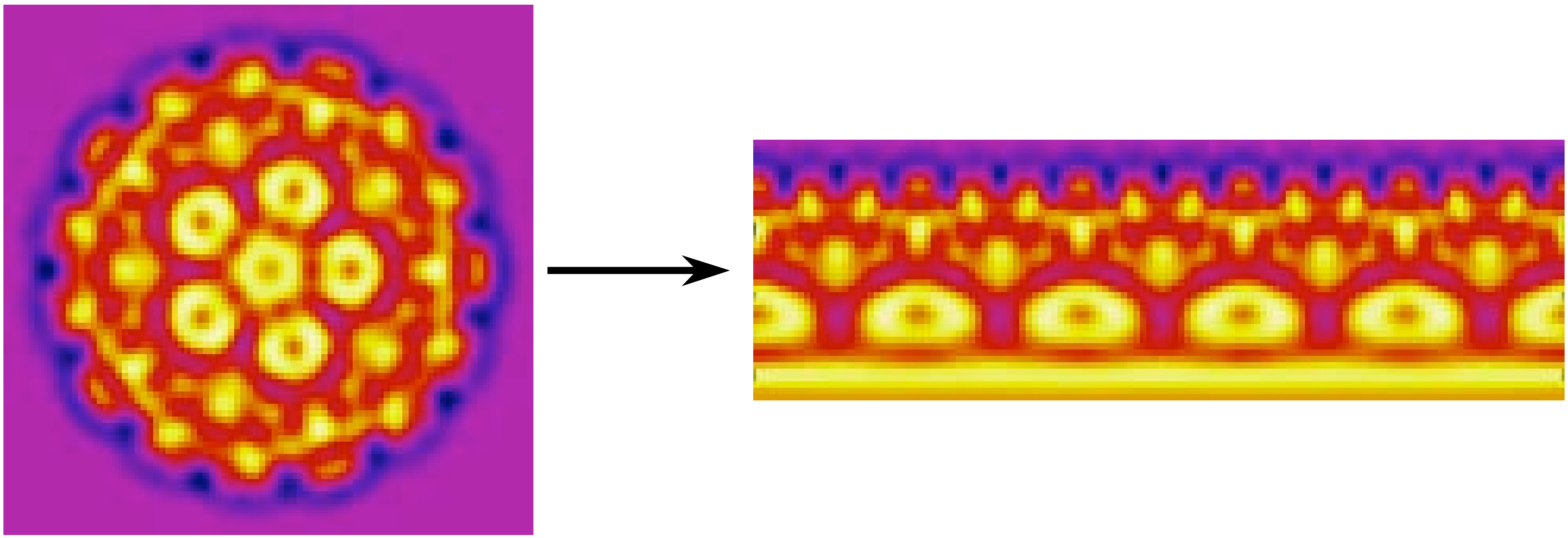


Reciprocal Space

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

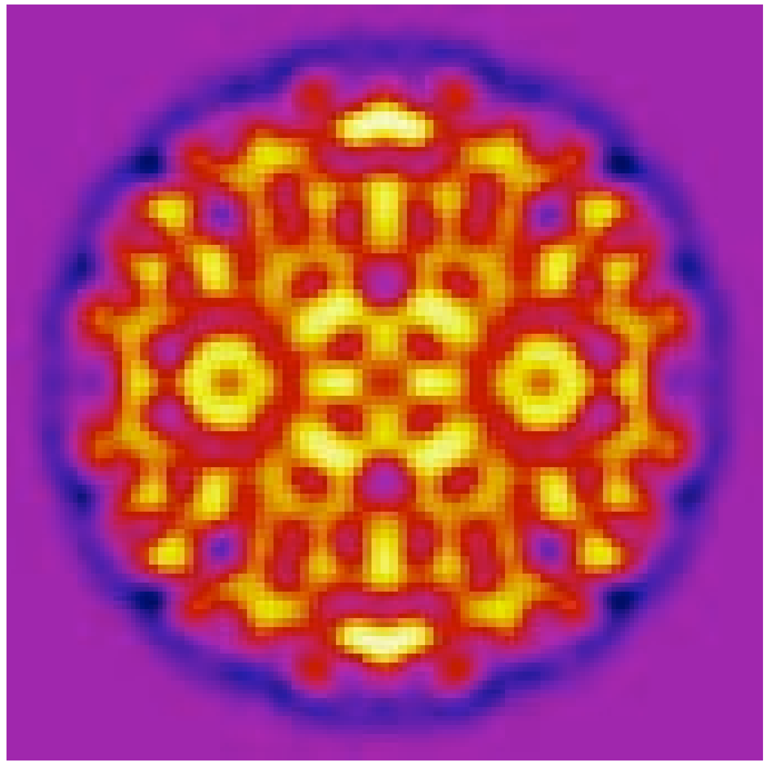
3) Convert image from **Cartesian** (x,y) to **polar** (r,ϕ) coordinates



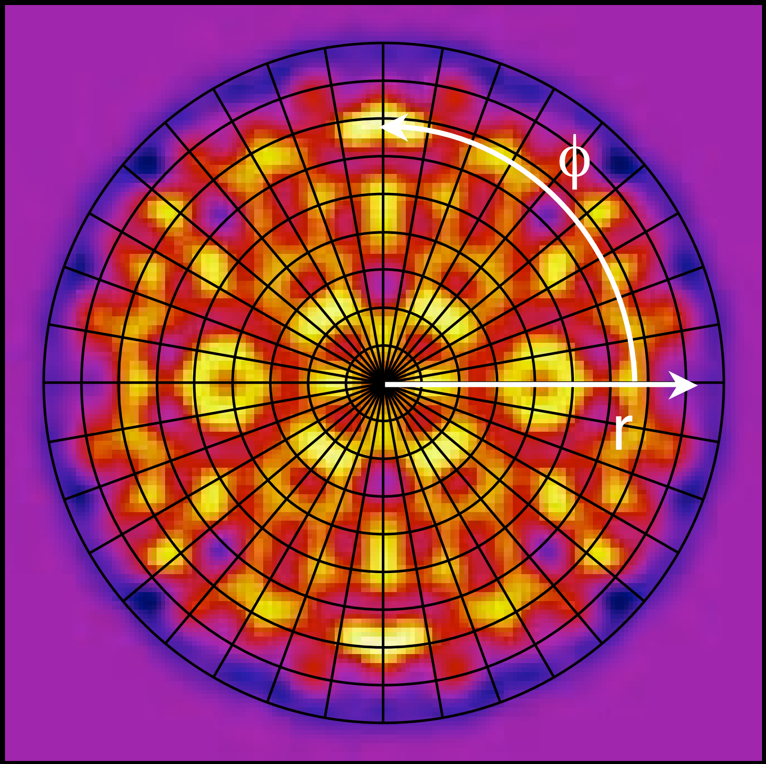
Cartesian Sampling

Polar Sampling

Convert from Cartesian to Polar Sampling



Convert from Cartesian to Polar Sampling

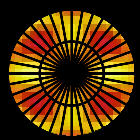


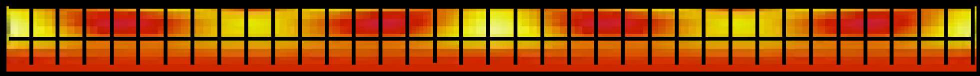
In practice: sampling must be much finer than shown

One pixel or smaller in radial and azimuthal directions

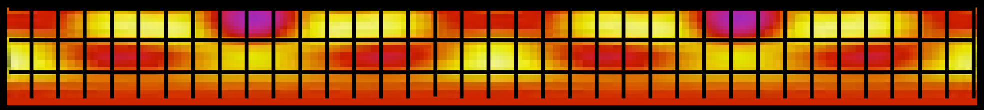
Convert from Cartesian to Polar Sampling

Convert from Cartesian to Polar Sampling

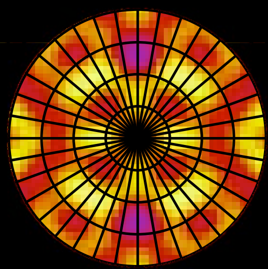


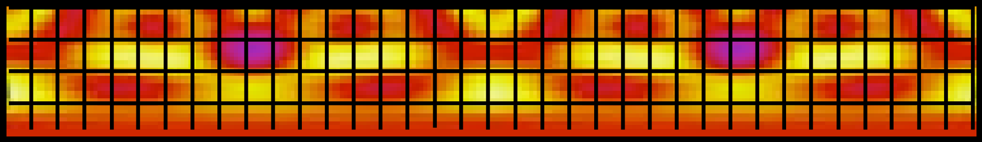


Convert from Cartesian to Polar Sampling

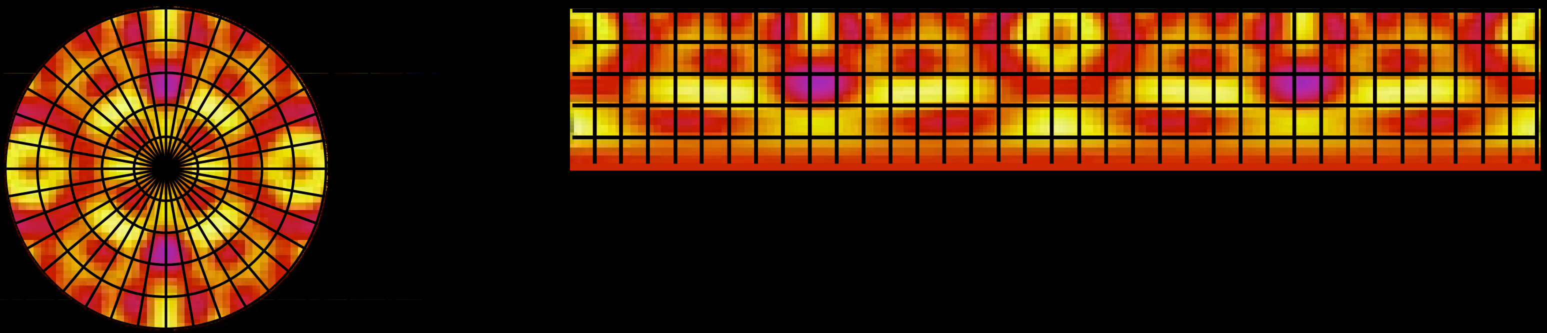


Convert from Cartesian to Polar Sampling

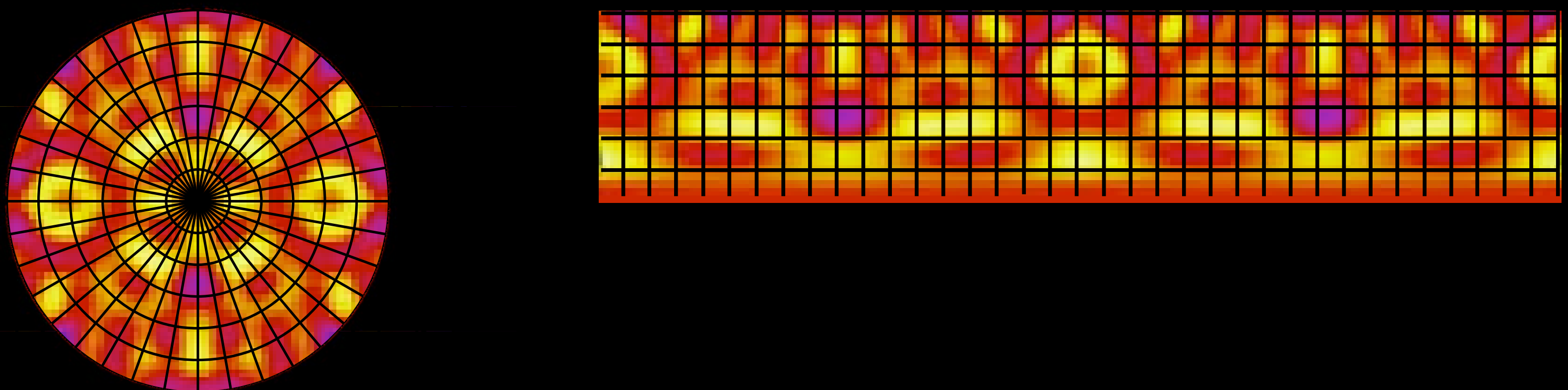




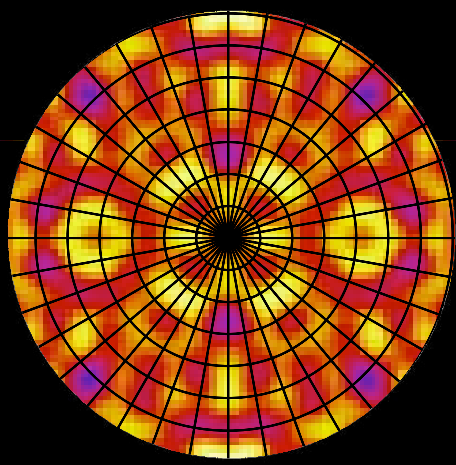
Convert from Cartesian to Polar Sampling

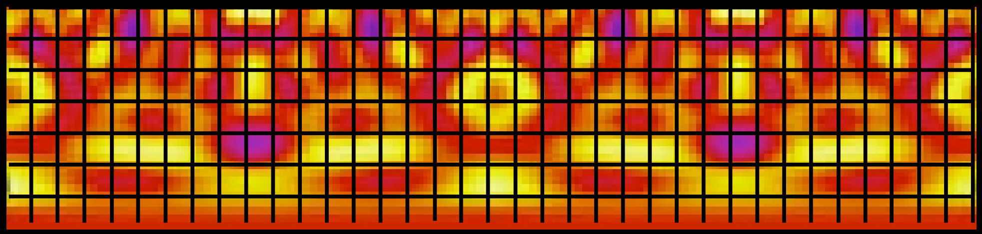


Convert from Cartesian to Polar Sampling

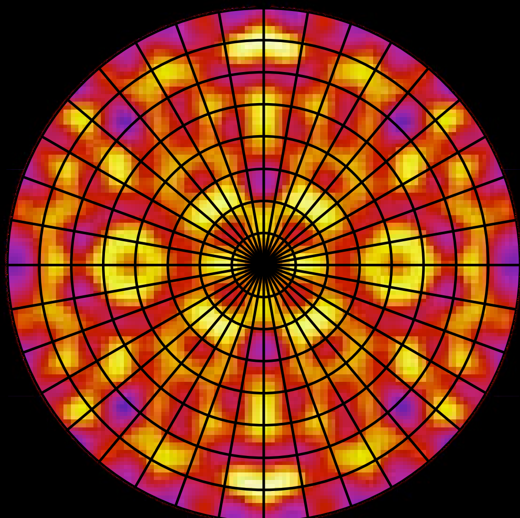


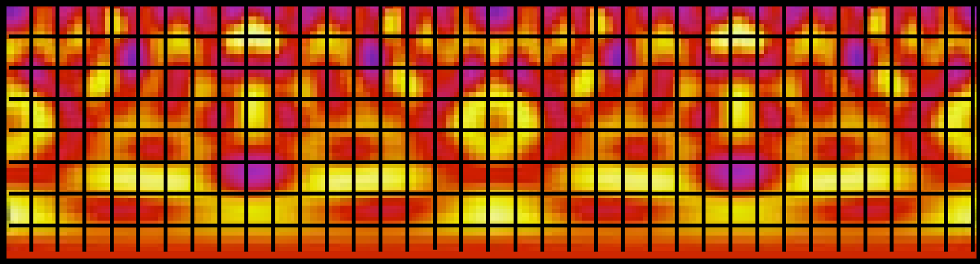
Convert from Cartesian to Polar Sampling



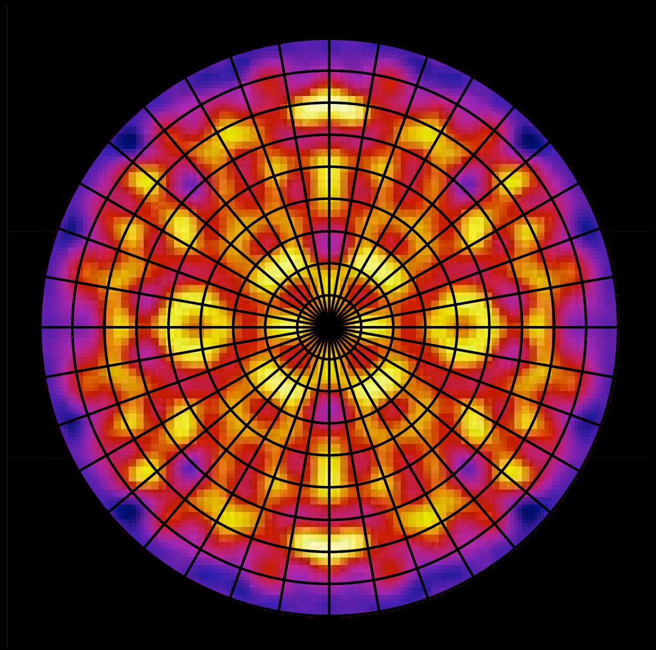


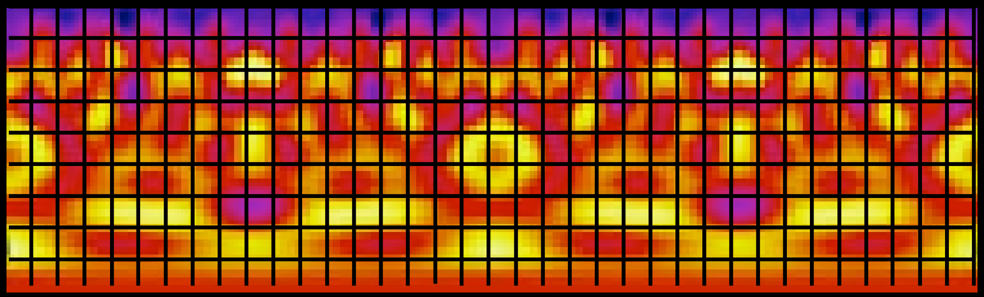
Convert from Cartesian to Polar Sampling



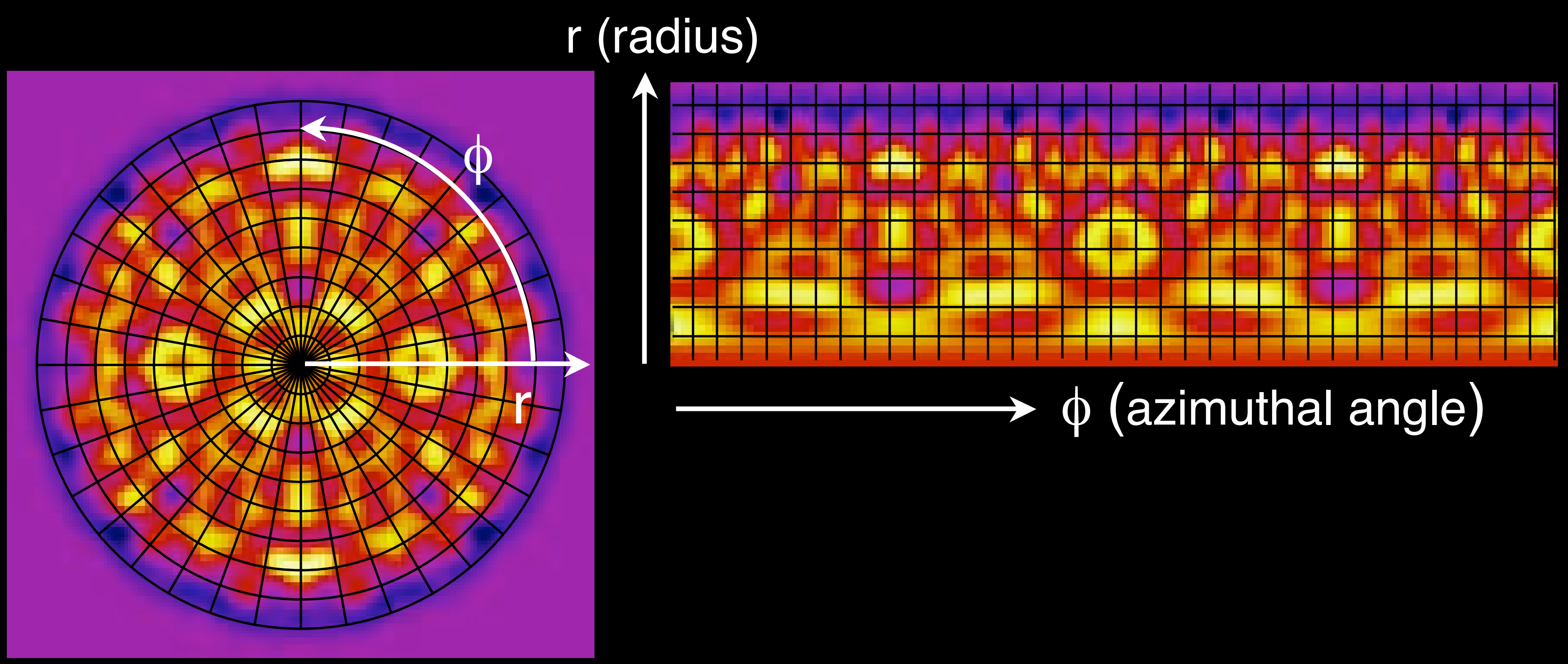


Convert from Cartesian to Polar Sampling





Convert from Cartesian to Polar Sampling

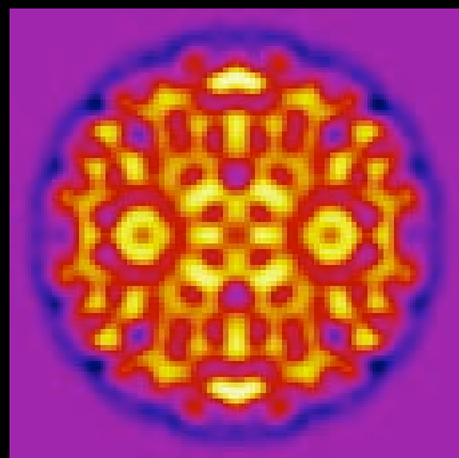


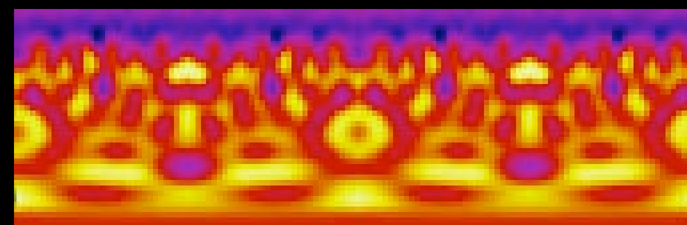
Cartesian

Polar

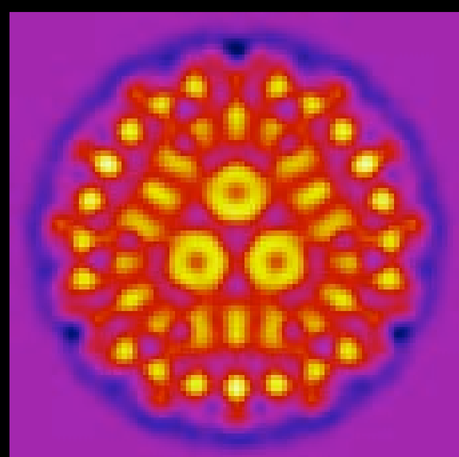
Convert from Cartesian to Polar Sampling

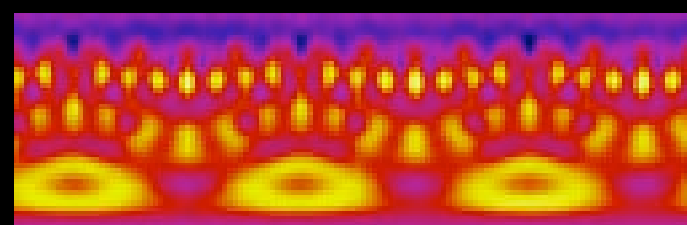
2F





3F





III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

1) Screen and select images (mainly by eye)

2) Digitize the micrograph and box and float individual particles within circular mask windows

3) Convert image from Cartesian (x,y) to polar (r,ϕ) coordinates

4) Expand density, **$\rho(r,\phi)$**, within the image into a series of **circular waves** (Fourier Analysis)

$$\rho(r,\phi) = \sum_{n=-\infty}^{\infty} g_n(r) e^{in\phi} \qquad (1)$$

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

4) Expand density, ***$\rho(r,\phi)$***, within the image into a series of **circular waves** (Fourier Analysis)

$$\rho(r,\phi) = \sum_{n=-\infty}^{\infty} g_n(r) e^{in\phi} \qquad (1)$$

$g_n(r)$ represents the **weight** of the ***n*-fold azimuthal component** of the image at radius, r

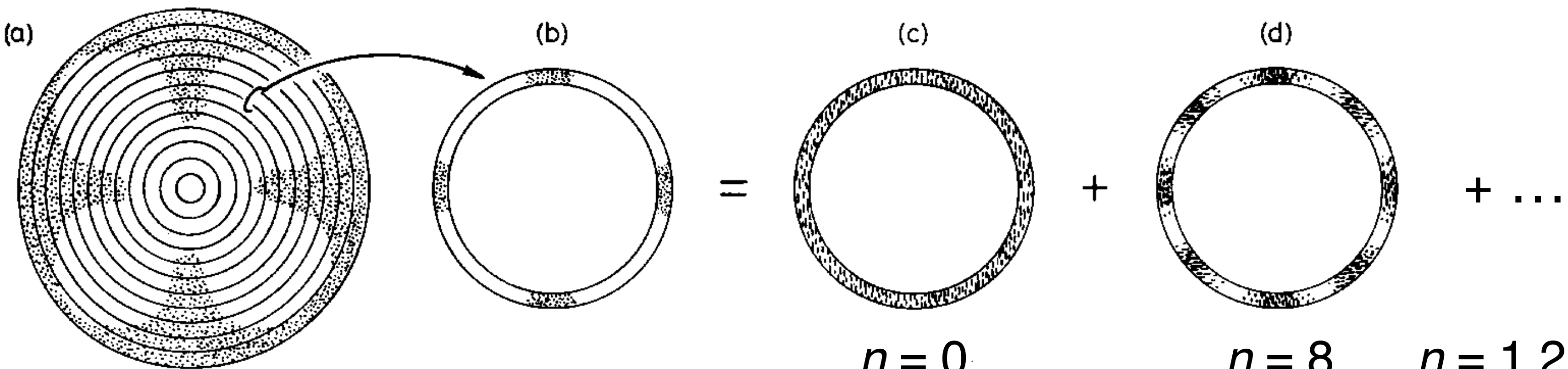
exp*(i$n\phi$)*, the **phase term**, positions the peak of each circular wave with respect to an origin (usually the x axis) so that all $g_n(r)$ are properly summed

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

4) Expand density, ***$\rho(r,\phi)$***, within the image into a series of **circular waves** (Fourier Analysis)

Decomposition of Rotationally Symmetric Object into Circular Waves



$n = 0$ $n = 8$ $n = 1,2,3,\ldots$

From Moody, Fig. 7.66, p.239

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5) Integrate **each $g_n(r)$** over the radius of the particle, ***a***, to obtain a **measure of total *n*-fold rotational component** of the image

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

5) Integrate each $g_n(r)$ over the radius of the particle, a, to obtain a **measure of total *n*-fold rotational component** of the image

Power in the image is defined as:

$$P_n = \varepsilon_n \int_0^a \left| g_n(r) \right|^2 2\pi r dr, \quad \varepsilon_n = \begin{cases} 1 \ for \ n = 0 \\ 2 \ for \ n > 0 \end{cases} \qquad (2)$$

Why $\varepsilon_n = 2$?

P_n has equal contributions from g_n and g_{-n} for n > 0

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

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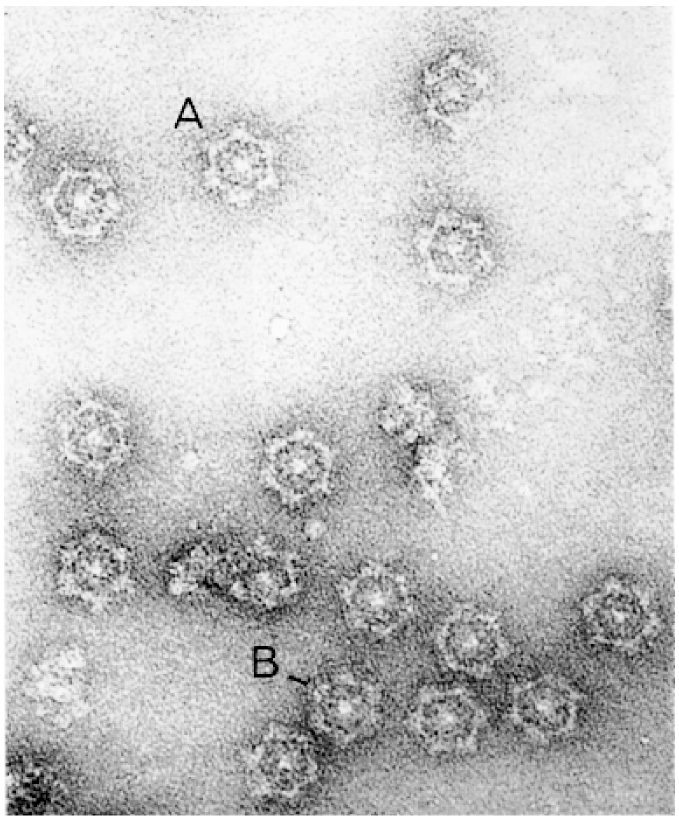
5) Integrate each $g_n(r)$ over the radius of the particle, a, to obtain a measure of total n-fold rotational component of the image
6) Plot P_n as a function of n to obtain **rotational power spectrum**

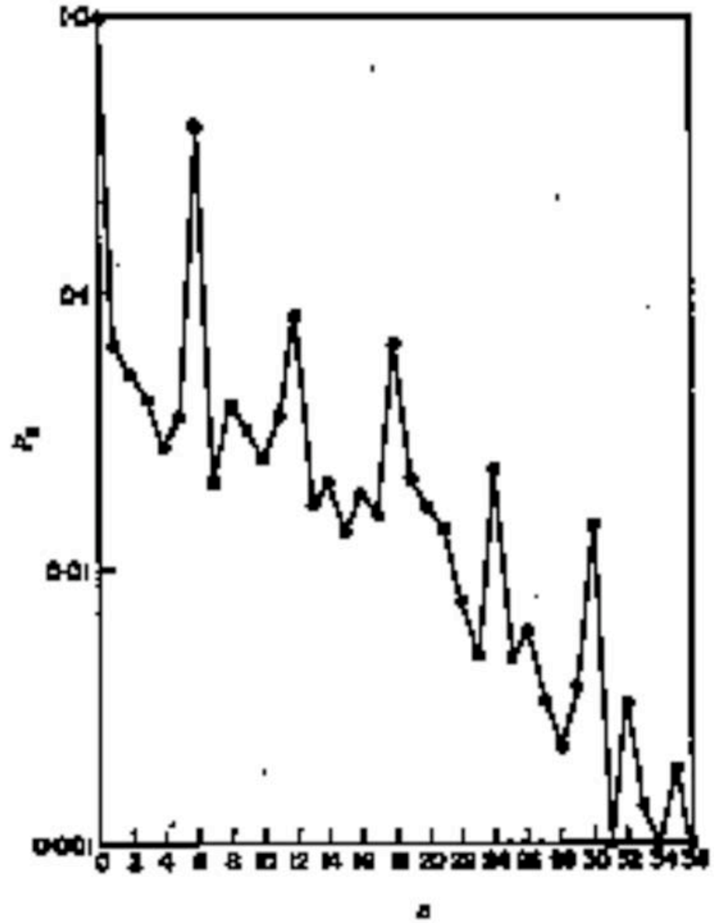
III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

6) Plot P_n as a function of n to obtain **rotational power spectrum**



T4 bacteriophage baseplate (UA stain)



From Crowther & Amos, Plate I, p.126

From Crowther & Amos, Fig.2, p.126

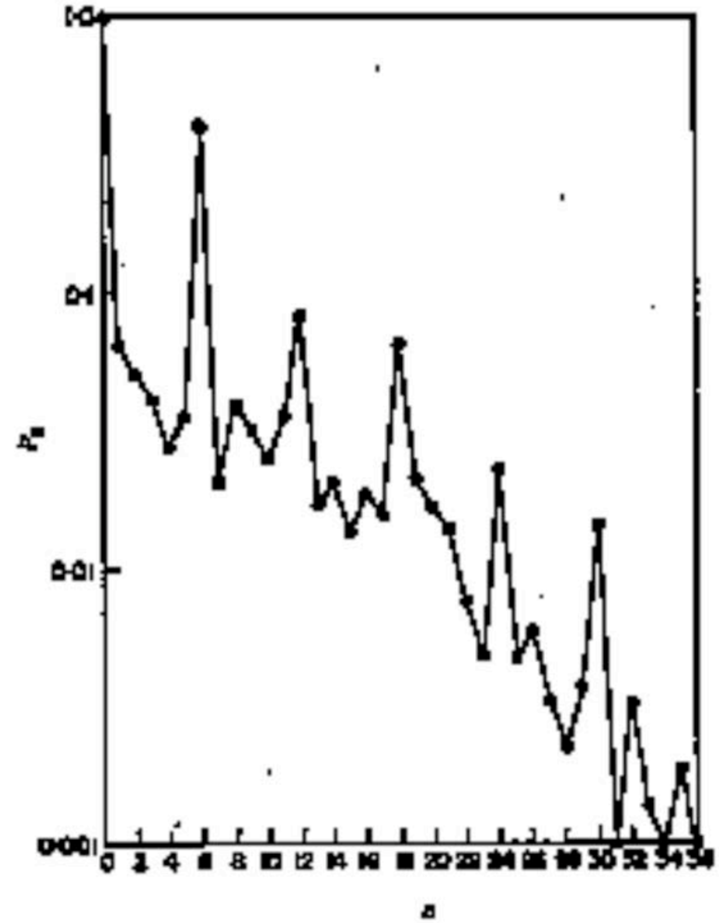
III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

6) Plot P_n as a function of n to obtain **rotational power spectrum**

Power spectrum is a **compact way** to represent the rotational symmetry components in the image

P_0 usually normalized to 1.0 and spectrum displayed with P_n on a logarithmic scale



From Crowther & Amos, Fig.2, p.126

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5) Integrate each $g_n(r)$ over the radius of the particle, a, to obtain a measure of total n-fold rotational component of the image

6) Plot P_n as a function of n to obtain rotational power spectrum

7) Switch to **Fourier** space

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

7) Switch to **Fourier** space

- As with other types of specimens, it is convenient with rotationally-symmetric specimens to perform computations in Fourier space rather than real space

Recall: Polar Fourier transform coordinates are *R* and Φ

Polar Fourier transform is expanded in the following way:

$$F(R,\Phi) = \sum_{n=-\infty}^{n=+\infty} \int_0^a g_n(r) J_n(2\pi rR) e^{in(\Phi+\pi/2)} 2\pi r dr \quad (3)$$

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

7) Switch to **Fourier** space

Polar Fourier transform expansion:

$$F(R,\Phi) = \sum_{n=-\infty}^{n=+\infty} \int_0^a g_n(r)\underline{J_n(2\pi rR)}e^{in(\Phi+\pi/2)}2\pi r dr \quad (3)$$

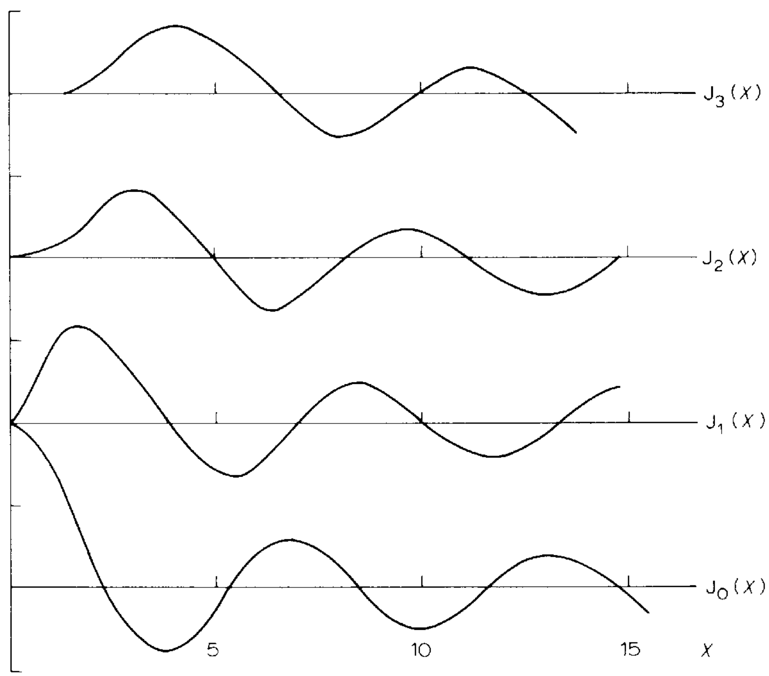
$J_n(X)$ is a **Bessel function** of **order *n***

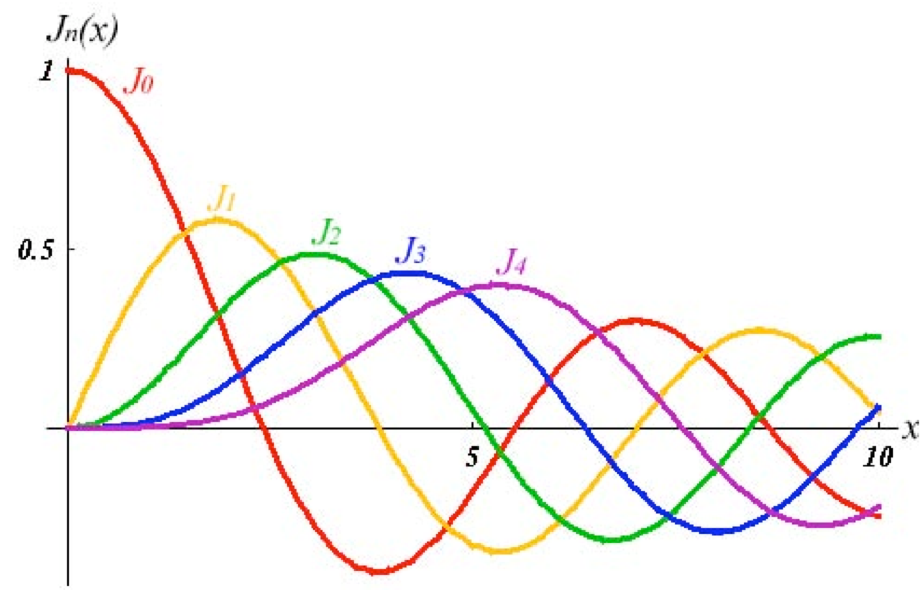
Each J_n is a **circularly-symmetric, oscillatory function**

Bessel Functions

$J_n(X)$ is a **Bessel function of order *n***

Each J_n is a circularly-symmetric, oscillatory function





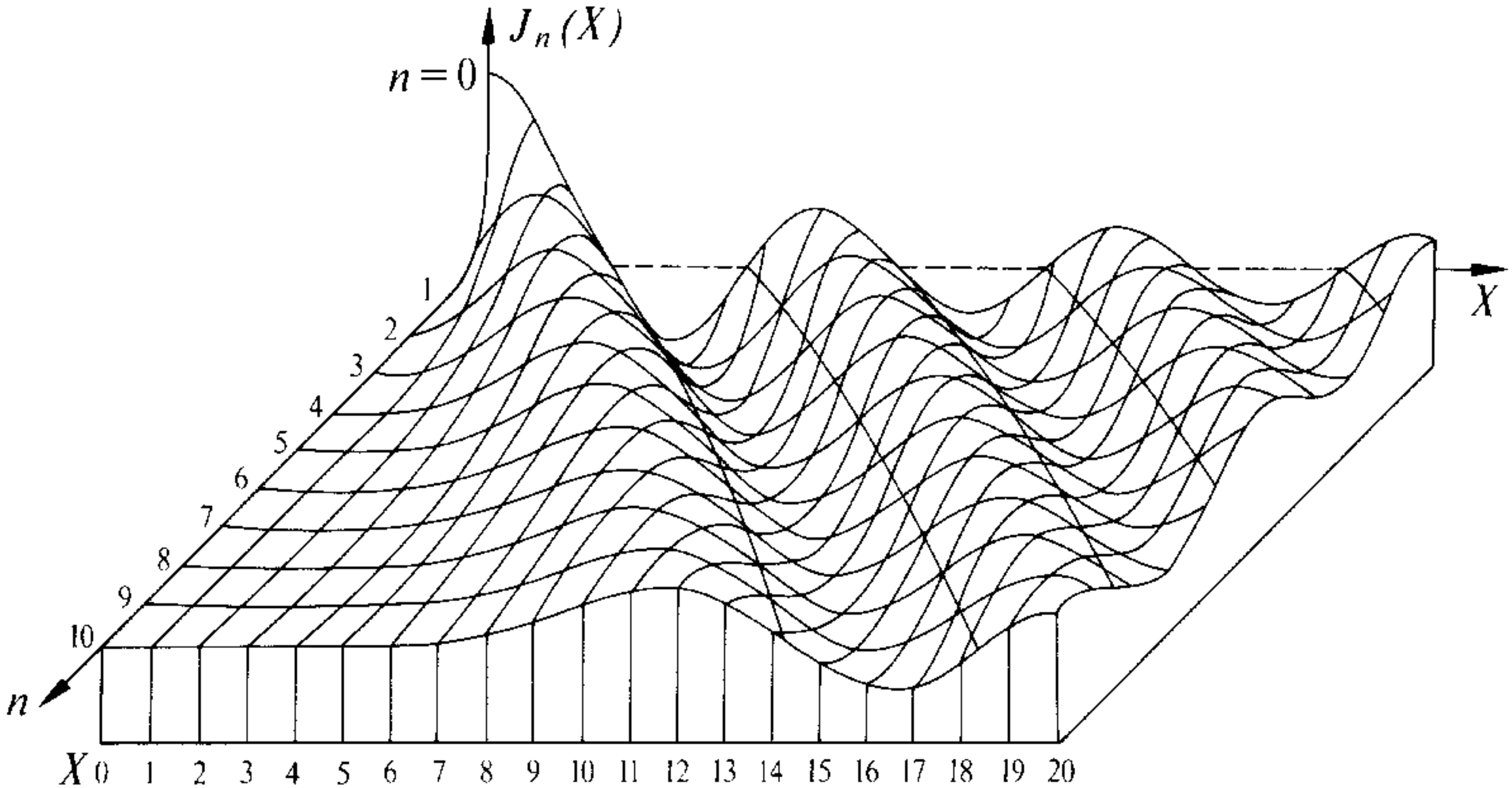
From Misell, Fig. 3.26, p.98

From www.efunda.com/math/bessel/besselJYPlot.cfm

Bessel Functions

$J_n(X)$ is a **Bessel function of order *n***

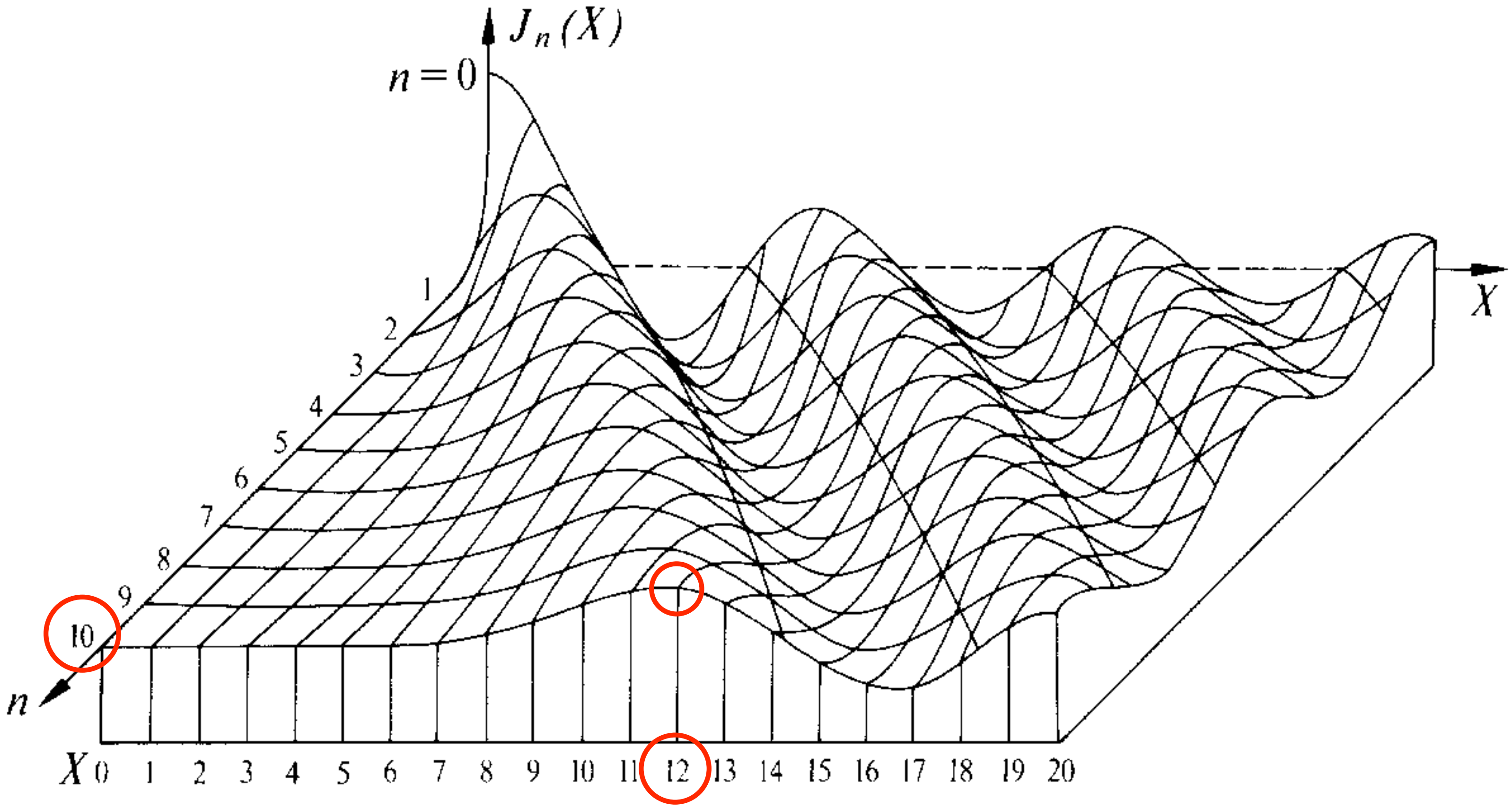
Each J_n is a circularly-symmetric, oscillatory function



From Sherwood, Fig. 16.7, p.565

Bessel Functions

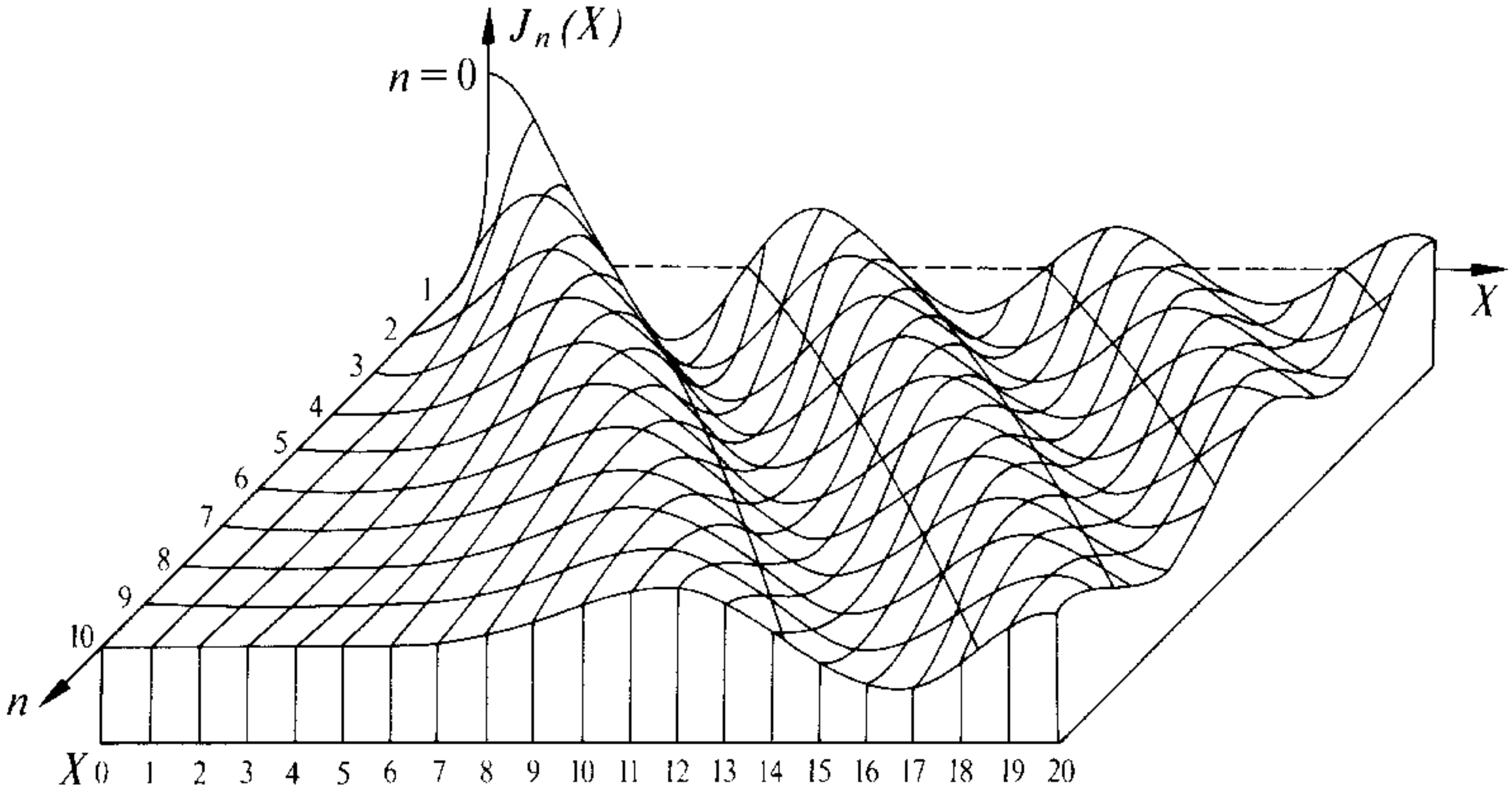
Note: First maximum of $J_n(X)$ for **large *n*** (*i.e.* $n > \sim 5$) appears at about X = n+2



From Sherwood, Fig. 16.7, p.565

Bessel Functions

Why should we care about Bessel functions?

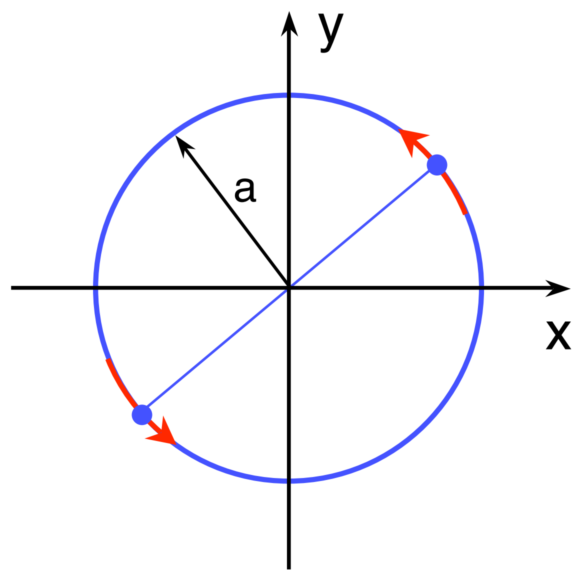


From Sherwood, Fig. 16.7, p.565

Bessel Functions

Transform of a ring of radius *a*

Note: A ring has **no azimuthal** variation ($n = 0$)

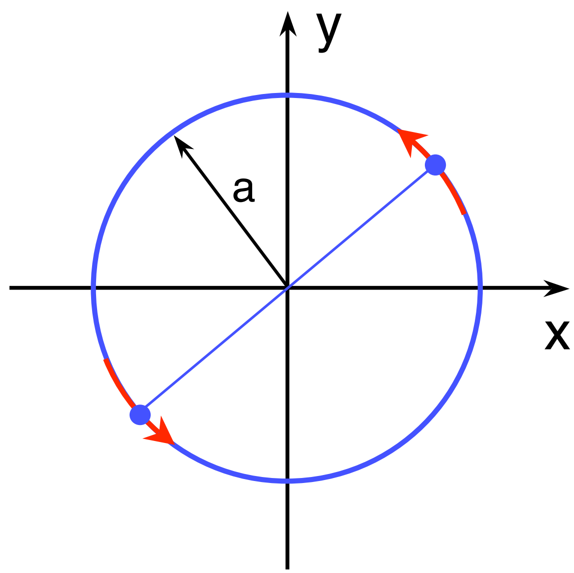


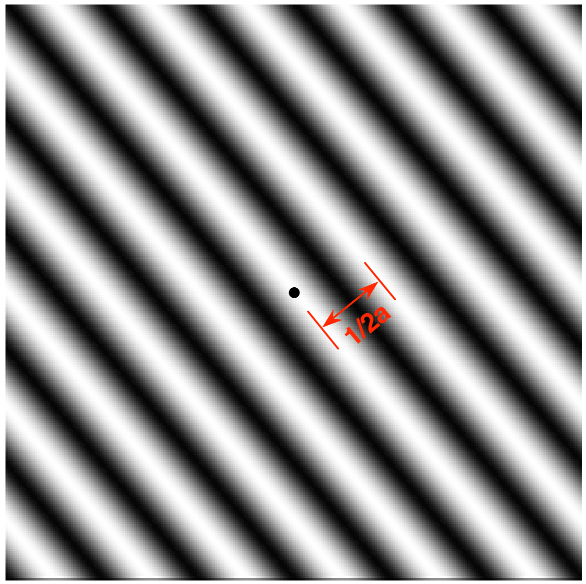
Ring can be considered to be generated from a pair of points, separated by the distance $2a$, that are rotated through the angle π.

Bessel Functions

Transform of a ring of radius *a*

Note: A ring has **no azimuthal** variation ($n = 0$)



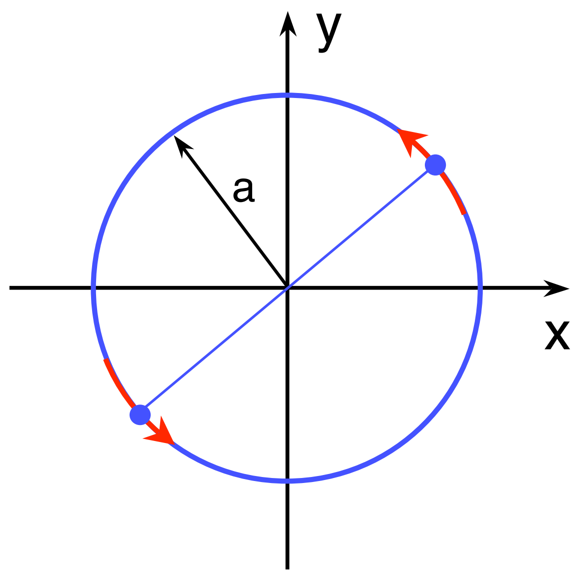


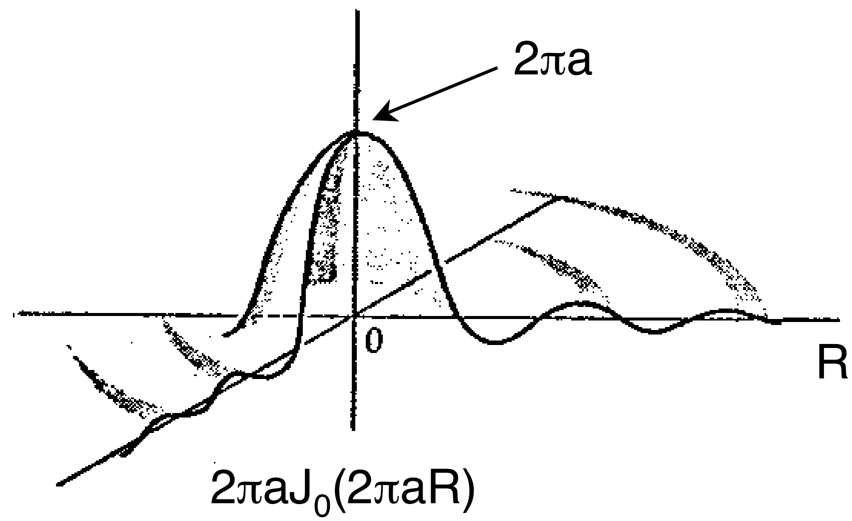
A **single** pair of points at opposite sides of a circle give rise to cosine fringes in **Fourier space**

When rotationally averaged, the fringes reinforce at the origin but tend to cancel away from the origin

Bessel Functions

Transform of a ring of radius *a*: $F(R) = 2\pi a J_0(2\pi aR)$





When rotationally averaged, the fringes reinforce at the origin but tend to cancel away from the origin

Gives rise to a **Bessel function of <u>zero</u> order, J_0**

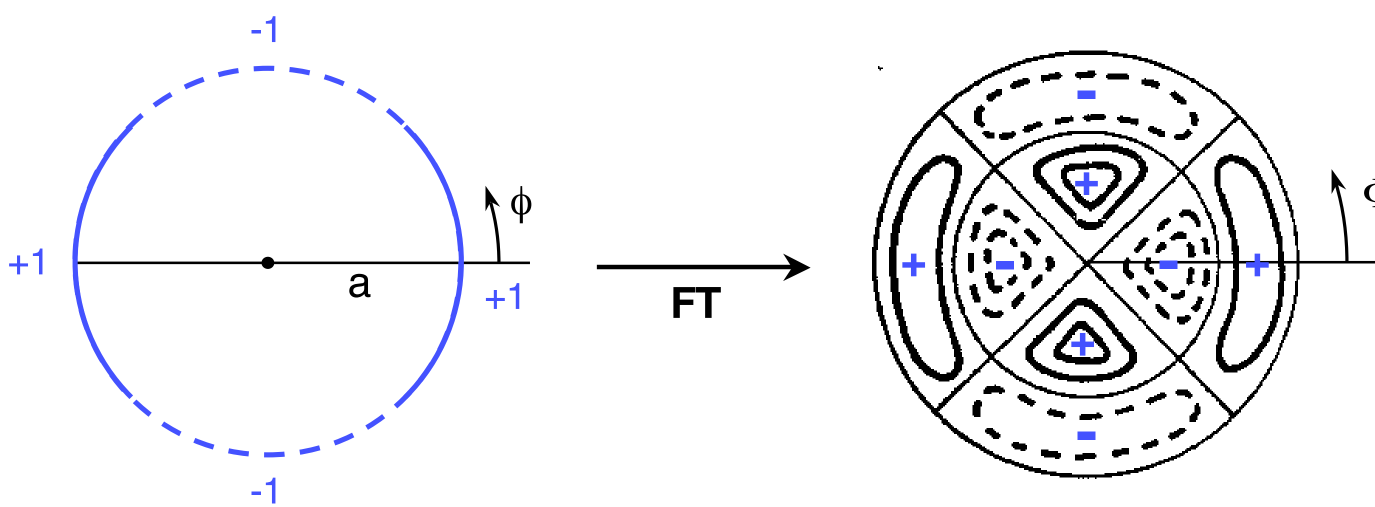
From Crowther (unpublished; 1973)

Bessel Functions

Transform of a ring of radius a: $F(R) = 2\pi a J_0(2\pi a R)$

What about a ring with an azimuthal variation, *n* ?

$$F(R,\Phi) = i^n 2\pi a J_n(2\pi a R) e^{in\Phi}$$



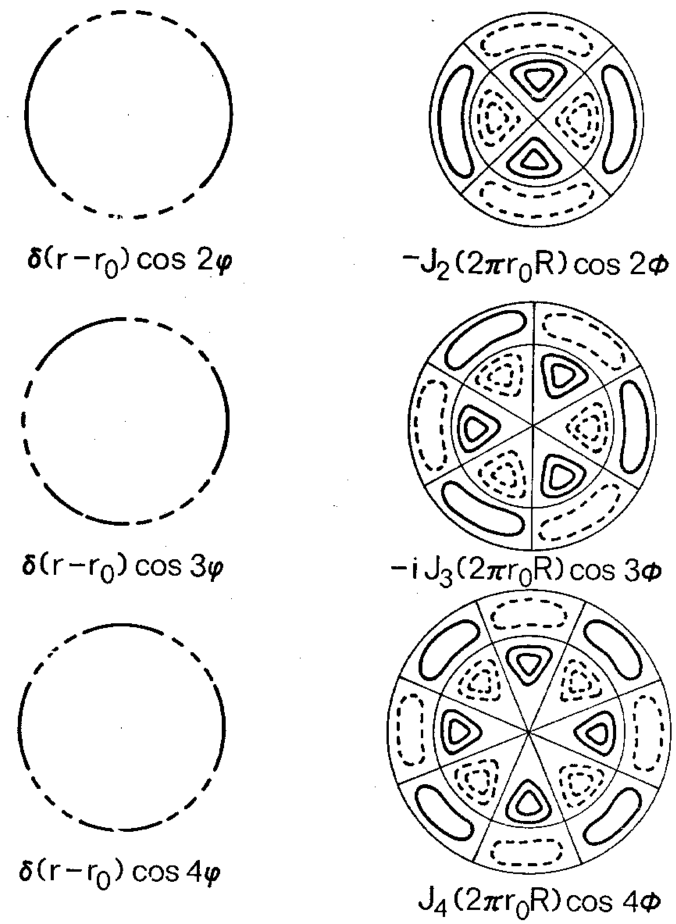
$\rho(r,\phi) = \delta(r-a)\cos(2\phi)$

$$F(R,\Phi) = i^2 2\pi a J_2(2\pi a R)\cos(2\Phi)$$
$$= -2\pi a J_2(2\pi a R)\cos(2\Phi)$$

Bessel Functions

$$F(R,\Phi) = i^n 2\pi a J_n(2\pi a R) e^{in\Phi}$$

Circular Rings (Constant Radius but Differing Azimuthal Variation) and their Fourier-Bessel transforms

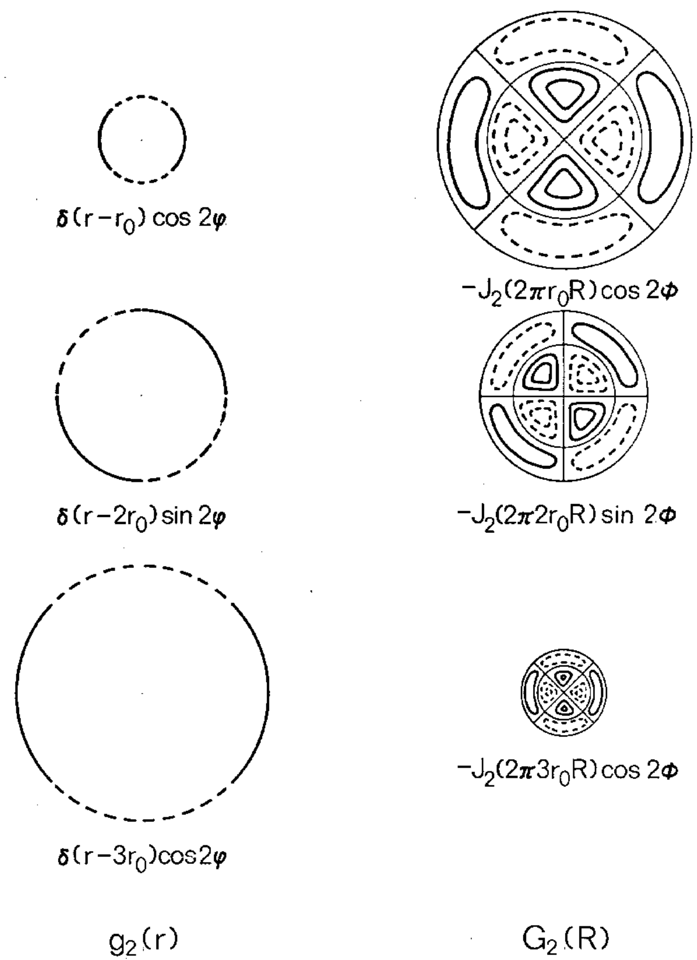


From Crowther (unpublished; 1973)

Bessel Functions

$$F(R,\Phi) = i^n 2\pi a J_n(2\pi aR)e^{in\Phi}$$

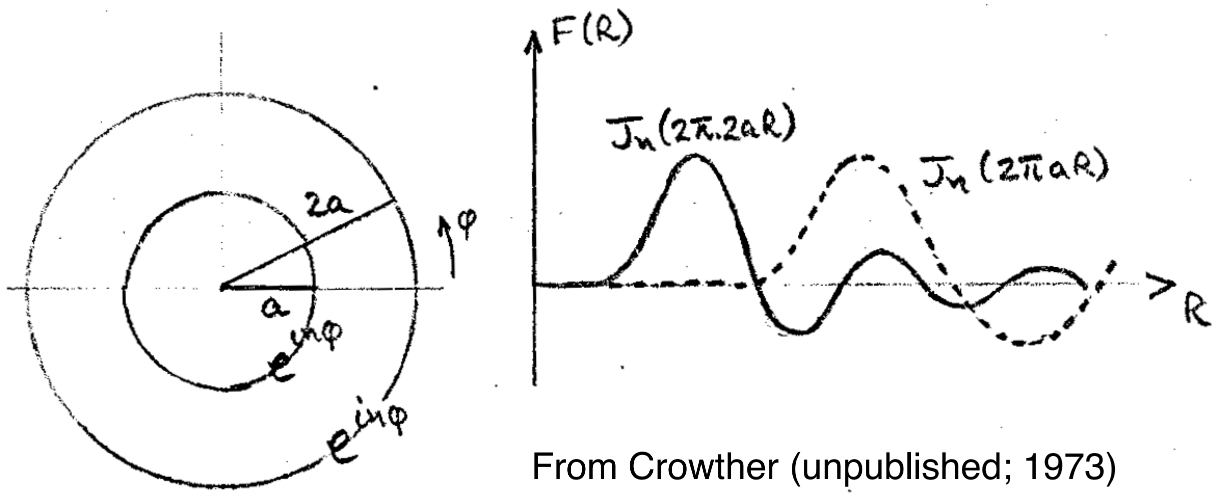
Relationship between objects with *n*-fold sinusoidal variations in azimuth, $g_n(r)$, and corresponding Fourier-Bessel transforms, $G_n(R)$



From Crowther (unpublished; 1973)

Bessel Functions

What happens when the object is slightly more complex, *e.g.* consisting of two rings (r = a and r = 2a), each with *n*-fold azimuthal variation?



From Crowther (unpublished; 1973)

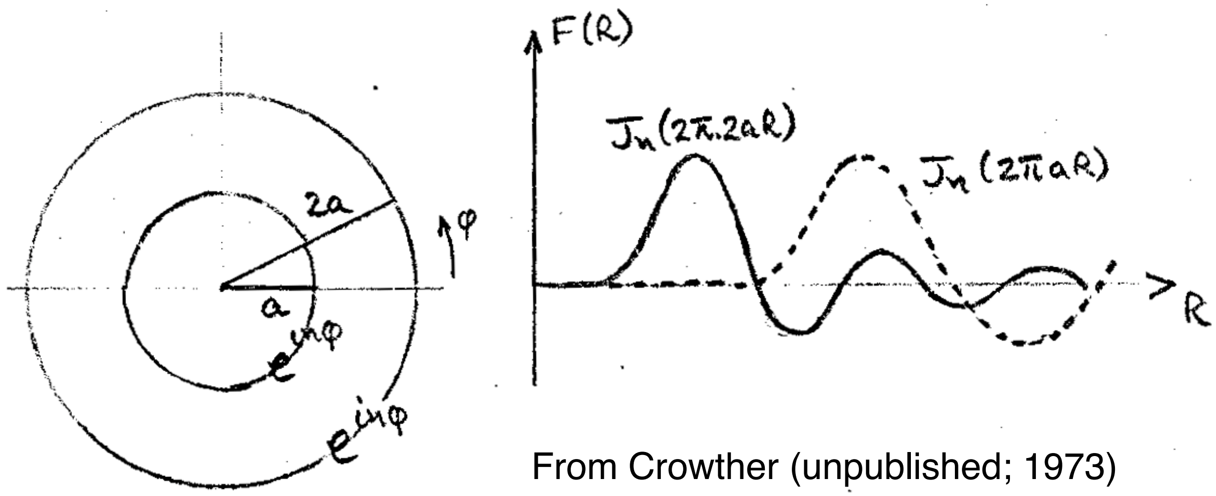
Gives rise to **overlapping** Bessels where multiple J_n's superimpose and blur out

There is no longer just one Bessel function as would occur if the object consisted simply of density at **one radius**

Now, there are **Bessels of a given order but of varying radius in <u>transform space</u>** and they superimpose

Bessel Functions

What happens when the object is slightly more complex, *e.g.* consisting of two rings (r = a and r = 2a), each with *n*-fold azimuthal variation?

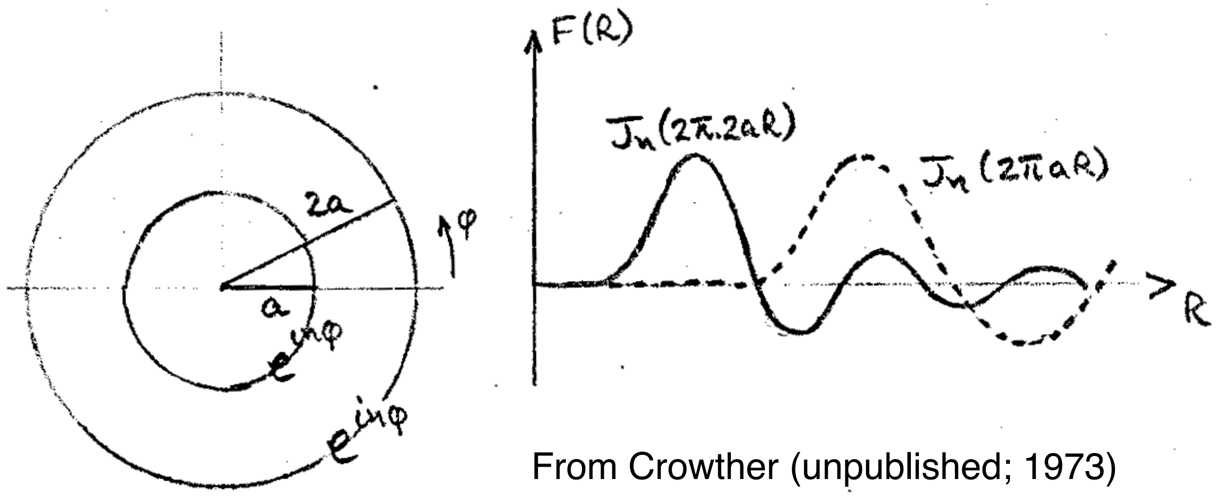


From Crowther (unpublished; 1973)

This situation clearly **contrasts** with that for 2-D crystals which give rise to **discrete** (*i.e.* distinct and non-overlapping) Bragg spots in the Fourier transform

Bessel Functions

What happens when the object is slightly more complex, *e.g.* consisting of two rings (r = a and r = 2a), each with *n*-fold azimuthal variation?



From Crowther (unpublished; 1973)

KEY CONCEPT:

Unlike with 2-D crystals, for single particles the **noise** and **signal** components are **NOT nicely separated** in the Fourier transform

Nonetheless, working in Fourier space provides an objective means to determine the **presence and positions of symmetry axes**

Bessel Functions

Transform of a ring of radius *a*, with ***n*-fold** variation:

$$F(R,\underline{\Phi}) = \underline{i^n}\, 2\pi a \underline{J_n}(2\pi a R)\underline{e^{in\Phi}}$$

Since $i^n = e^{in(\pi/2)}$ **Hint:** $e^{in(\pi/2)} = \cos(n\pi/2) + i\sin(n\pi/2)$

$$F(R,\Phi) = 2\pi a J_n(2\pi a R)e^{in\Phi}e^{in(\pi/2)}$$

Can be rewritten as:

$$F(R,\Phi) = 2\pi a J_n(2\pi a R)e^{in(\Phi+\pi/2)}$$

Bessel Functions

Transform of a ring of radius *a*, with ***n*-fold** variation:

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Can be rewritten as:

$$F(R,\Phi) = \underbrace{2\pi a J_n(2\pi a R)}_{\text{radial part of FT}} \underbrace{e^{in(\Phi+\pi/2)}}_{\text{angular part of FT}}$$

Bessel Functions

Transform of a ring of radius *a*, with ***n*-fold** variation:

$$F(R,\Phi) = \underbrace{2\pi a J_n(2\pi aR)}_{radial\ part\ of\ FT}\underbrace{e^{in(\Phi+\pi/2)}}_{\substack{angular\\ part\ of\ FT}}$$

π/2 factor arises because the **transform is rotated by π/2** (=90°) with respect to the direction of fringes in the object

A **centrosymmetric** object (*i.e. n* = even) gives rise to a **real** transform whereas a **non-centrosymmetric** object (*i.e. n* = odd) gives an **imaginary** transform

Bessel Functions

Transform of a ring of radius *a*, with ***n*-fold** variation:

$$F(R,\Phi) = \underbrace{2\pi a J_n(2\pi a R)}_{radial\ part\ of\ FT} \underbrace{e^{in(\Phi+\pi/2)}}_{\substack{angular \\ part\ of\ FT}}$$

The ***n*-fold** variation in the annular ring gives an **angular frequency in the transform**

The ***2πα*** term at the beginning of the expression is a **normalization factor** (value of $2\pi\alpha J_0(X)$ at $X=0$ is $2\pi\alpha$)

Bessel Functions

Transform of a ring of radius *a*, with ***n*-fold** variation:

$$F(R,\Phi) = \underbrace{2\pi a J_n(2\pi a R)}_{radial\ part\ of\ FT}\underbrace{e^{in(\Phi+\pi/2)}}_{\substack{angular\\ part\ of\ FT}}$$

Now let's return to where we were when the subject of Bessel functions arose!!!

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

7) Switch to **Fourier** space

Polar Fourier transform expansion:

$$F(R,\Phi) = \sum_{n=-\infty}^{n=+\infty} \int_0^a g_n(r) \underline{J_n(2\pi rR)} e^{in(\Phi+\pi/2)} 2\pi r dr \quad (3)$$

See how this compares with the transform of a highly simple object: a **single ring** of radius ***a***, with ***n*-fold** variation:

$$F(R,\Phi) = \underbrace{2\pi a J_n(2\pi aR)}_{radial\ part\ of\ FT} \underbrace{e^{in(\Phi+\pi/2)}}_{angular\ part\ of\ FT}$$

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Recall: ***$g_n(r)$*** represents the **weight** of the *n*-fold variation at radius, *r*, in the object

Each g_n is a **complex number** described by both a wave **amplitude** and a **phase**. This specifies at each radius where the strong peaks of density are for a particular angular variation.

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This can be rewritten as:

$$F(R,\Phi) = \sum_{n=-\infty}^{n=+\infty} G_n(R) e^{in(\Phi+\pi/2)} \quad (4)$$

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7) Switch to **Fourier** space

Polar Fourier transform expansion:

$$F(R,\Phi) = \sum_{n=-\infty}^{n=+\infty} \int_0^a g_n(r) J_n(2\pi rR) e^{in(\Phi+\pi/2)} 2\pi r dr \quad (3)$$

This can be rewritten as:

$$F(R,\Phi) = \sum_{n=-\infty}^{n=+\infty} \underbrace{G_n(R)}_{\text{radial variation}} \underbrace{e^{in(\Phi+\pi/2)}}_{\text{angular variation}} \quad (4)$$

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

7) Switch to **Fourier** space

$$F(R,\Phi) = \sum_{n=-\infty}^{n=+\infty} \underbrace{G_n(R)}_{\substack{\text{radial}\\ \text{variation}}} \underbrace{e^{in(\Phi+\pi/2)}}_{\substack{\text{angular}\\ \text{variation}}} \qquad (4)$$

This expansion of the Fourier transform is analogous to the expansion of the polar image densities as given in equation (1):

$$\rho(r,\phi) = \sum_{n=-\infty}^{n=+\infty} \underbrace{g_n(r)}_{\substack{\text{radial}\\ \text{variation}}} \underbrace{e^{in\phi}}_{\substack{\text{angular}\\ \text{variation}}} \qquad (1)$$

G_n (R) are the **coefficients** (weights) of each azimuthal component in the **Fourier transform**

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III.D.4.b 2D Averaging of Objects with Point Group Symmetry

7) Switch to **Fourier** space

$$F(R,\Phi) = \sum_{n=-\infty}^{n=+\infty} \underbrace{G_n(R)}_{\substack{\text{radial} \\ \text{variation}}} \underbrace{e^{in(\Phi+\pi/2)}}_{\substack{\text{angular} \\ \text{variation}}} \qquad (4)$$

$$\rho(r,\phi) = \sum_{n=-\infty}^{n=+\infty} \underbrace{g_n(r)}_{\substack{\text{radial} \\ \text{variation}}} \underbrace{e^{in\phi}}_{\substack{\text{angular} \\ \text{variation}}} \qquad (1)$$

The two sets of coefficients, $G_n(R)$ and $g_n(r)$, are connected by what is called the **Fourier-Bessel transform**

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

7) Switch to **Fourier** space

The two sets of coefficients, $G_n(R)$ and $g_n(r)$, are connected by what is called the **Fourier-Bessel transform**

$$g_n(r) = \int_0^\infty G_n(R) J_n(2\pi R r) 2\pi R dR \qquad (5)$$

Integral normally only evaluated out to some resolution limit (*i.e.* **R < ∞**)

Note: When the above expression is evaluated computationally, the integral is expressed as a summation over discrete steps in ***R*** (reciprocal space)

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

7) Switch to **Fourier** space

The two sets of coefficients, $G_n(R)$ and $g_n(r)$, are connected by what is called the **Fourier-Bessel transform**

$$g_n(r) = \int_0^\infty G_n(R) J_n(2\pi Rr) 2\pi R dR \qquad (5)$$

Inverse relationship also holds:

$$G_n(R) = \int_0^a g_n(r) J_n(2\pi rR) 2\pi r dr \qquad (6)$$

a is the radial limit of the object

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7) Switch to **Fourier** space

The two sets of coefficients, $G_n(R)$ and $g_n(r)$, are connected by what is called the **Fourier-Bessel transform**

$$g_n(r) = \int_0^\infty G_n(R) J_n(2\pi Rr) 2\pi R dR \qquad (5)$$

Inverse relationship also holds:

$$G_n(R) = \int_0^a g_n(r) J_n(2\pi rR) 2\pi r dr \qquad (6)$$

Integral computed as a summation over discrete steps in ***r*** (real space)

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

1) Screen and select images

2) Digitize the micrograph; box and float individual particles

3) Convert image from Cartesian (x,y) to polar (r,ϕ) coordinates

4) Expand density, $\rho(r,\phi)$, into a series of circular waves

5) Integrate each $g_n(r)$ over the radius of the particle, a, to obtain a measure of total n-fold rotational component of the image

6) Plot P_n as a function of n (rotational power spectrum)

7) Switch to Fourier space

8) Identify **phase origin**

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

8) Identify **phase origin**

Effect of Phase Origin

Essential that origin of polar coordinate system lie **on** the symmetry axis of the object

Origin established by boxing (center of window) becomes the phase origin of the computed Fourier transform

Origin point is then **shifted computationally** to give the **best** P_n for the assumed symmetry

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

8) Identify **phase origin**

Effect of Phase Origin

Origin point is then **shifted computationally** to give the **best P_n** for the assumed symmetry

By **changing the assumed symmetry**, m, one gets a series of origins and computes for each of these separate origins a series of rotational power spectra

These are compared to **look for the dominant symmetry**

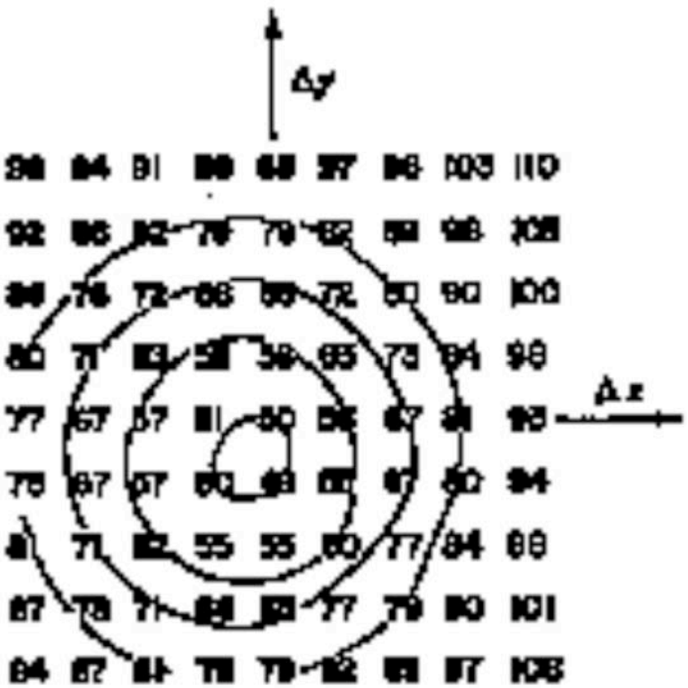
III.D.4 2D and 1D Digital Fourier Reconstruction Methods

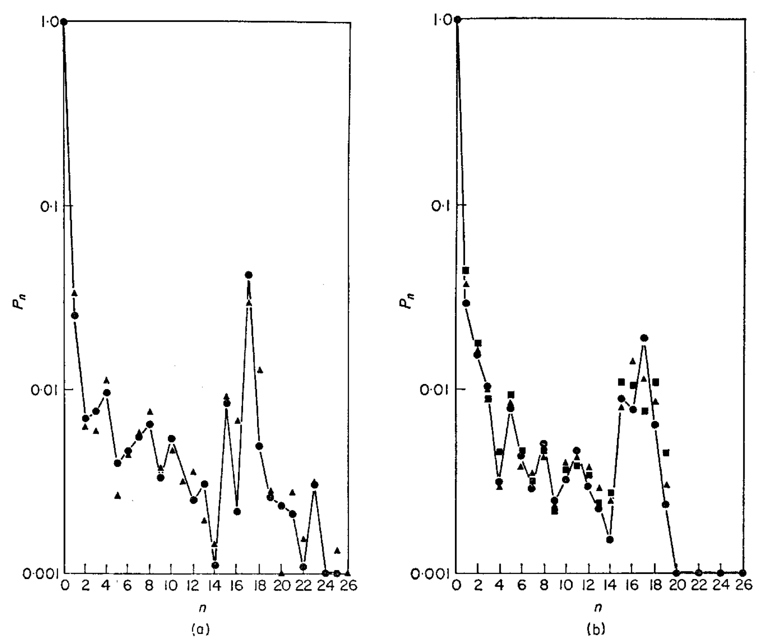
III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

8) Identify **phase origin**

Effect of Phase Origin





From Crowther & Amos, Fig.1, p.125

From Crowther & Amos, Fig.4, p.127

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Protocol for Rotational Filtering

1) Screen and select images

2) Digitize the micrograph; box and float individual particles

3) Convert image from Cartesian (x,y) to polar (r,ϕ) coordinates

4) Expand density, $\rho(r,\phi)$, into a series of circular waves

5) Integrate each $g_n(r)$ over the radius of the particle, a, to obtain a measure of total n-fold rotational component of the image

6) Plot P_n as a function of n (rotational power spectrum)

7) Switch to Fourier space

8) Identify phase origin

9) Synthesize (reconstruct) filtered image

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

9) Synthesize (reconstruct) filtered image

Examine the rotational power spectra computed from **several** different particle images to get an idea of the relative preservation of the particles

Those images which show the **highest** P_n are used to synthesize rotationally-filtered images

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

9) Synthesize (reconstruct) filtered image

Use equation (5) to convert each G_n to a corresponding g_n and **only** those **g_n for which n is a multiple of m** are computed, thereby omitting all other components considered to be noise

$$g_n(r) = \int_0^{\infty} G_n(R) J_n(2\pi Rr) 2\pi R dR \qquad (5)$$

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

9) Synthesize (reconstruct) filtered image

Noise may arise from several sources such as:

1) the particle may not be viewed **directly** along an axis of symmetry

2) the particle may be distorted or may be non-uniformly stained, shadowed, etc.

3) the other usual forms of noise (*e.g.* support film, electron optical effects, etc.) may be present

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

9) Synthesize (reconstruct) filtered image

The ***$G_n(R)$*** are computed from the Fourier transform by the inverse of equation (4):

$$F(R,\Phi) = \sum_{n=-\infty}^{n=+\infty} \underbrace{G_n(R)}_{\substack{\text{radial}\\ \text{variation}}} \underbrace{e^{in(\Phi+\pi/2)}}_{\substack{\text{angular}\\ \text{variation}}} \qquad (4)$$

$$G_n(R) = \frac{1}{2\pi} \int_0^{2\pi} F(R,\Phi) e^{-in(\Phi+\pi/2)} d\Phi \qquad (7)$$

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

9) Synthesize (reconstruct) filtered image

Note: computation of $G_n(R)$ from $F(R,\Phi)$ (eqn. 7) allows the P_n to be computed **either** from densities directly (eqn. 2) or from the Fourier transform as follows:

(8)

$$P_n = \varepsilon_n \int_0^a \left| g_n(r) \right|^2 2\pi r dr, \quad \varepsilon_n = \begin{cases} 1 \ \mathit{for} \ n = 0 \\ 2 \ \mathit{for} \ n > 0 \end{cases} \qquad (2)$$

III.D.4 2D and 1D Digital Fourier Reconstruction Methods

III.D.4.b 2D Averaging of Objects with Point Group Symmetry

Protocol for Rotational Filtering

9) Synthesize (reconstruct) filtered image

Once the $g_n(r)$ are computed, equation (1) is used to resynthesize the density function, $\rho(r,\phi)$.

$$\rho(r,\phi) = \sum_{n=-\infty}^{\infty} g_n(r) e^{in\phi} \qquad (1)$$

This polar image is then reconverted back to a Cartesian format, $\rho(x,y)$, for visualization (*e.g.* in RobEM)

End of Sec.III.D.4