# III.D FOURIER IMAGE PROCESSING TECHNIQUES

## III.D.2 Optical Filtering

### Introduction

**History:**

Independently introduced by Klug and DeRosier (1966) and Bancroft, Hills and Markham (1967)

- Only suitable for study of **periodic** specimens with **translational** symmetry

**Main advantage:**

Provides simple way to **remove contributions from noise** in micrographs (reveal clearer images of specimen structure)

- Also a powerful method for separating Moire images of multilayered specimens

- Principle of technique is quite straightforward

- In practice, method can ***easily*** lead to **erroneous results**

# III.D.2 Optical Filtering

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

- First and ***<u>most important</u>*** step of filtering experiment

- Have to distinguish spots arising from noise (aperiodic image details) from those attributed to the periodic nature of the specimen

    Correct filtration requires knowledge of how noise and signal components are distinguished

**For most crystalline specimens:**

- OD pattern is a lattice of strong spots (Bragg reflections) against a weaker background of noise

- **Noise** generally produces spots in **all parts** of OD pattern

# III.D.2 Optical Filtering

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

**Periodic noise:**

- Appears at or close to the lattice points of the diffraction pattern

- ***Cannot*** be removed by filtering

- Systematic specimen flattening or staining artifacts are examples of situations which produce periodic-type noise

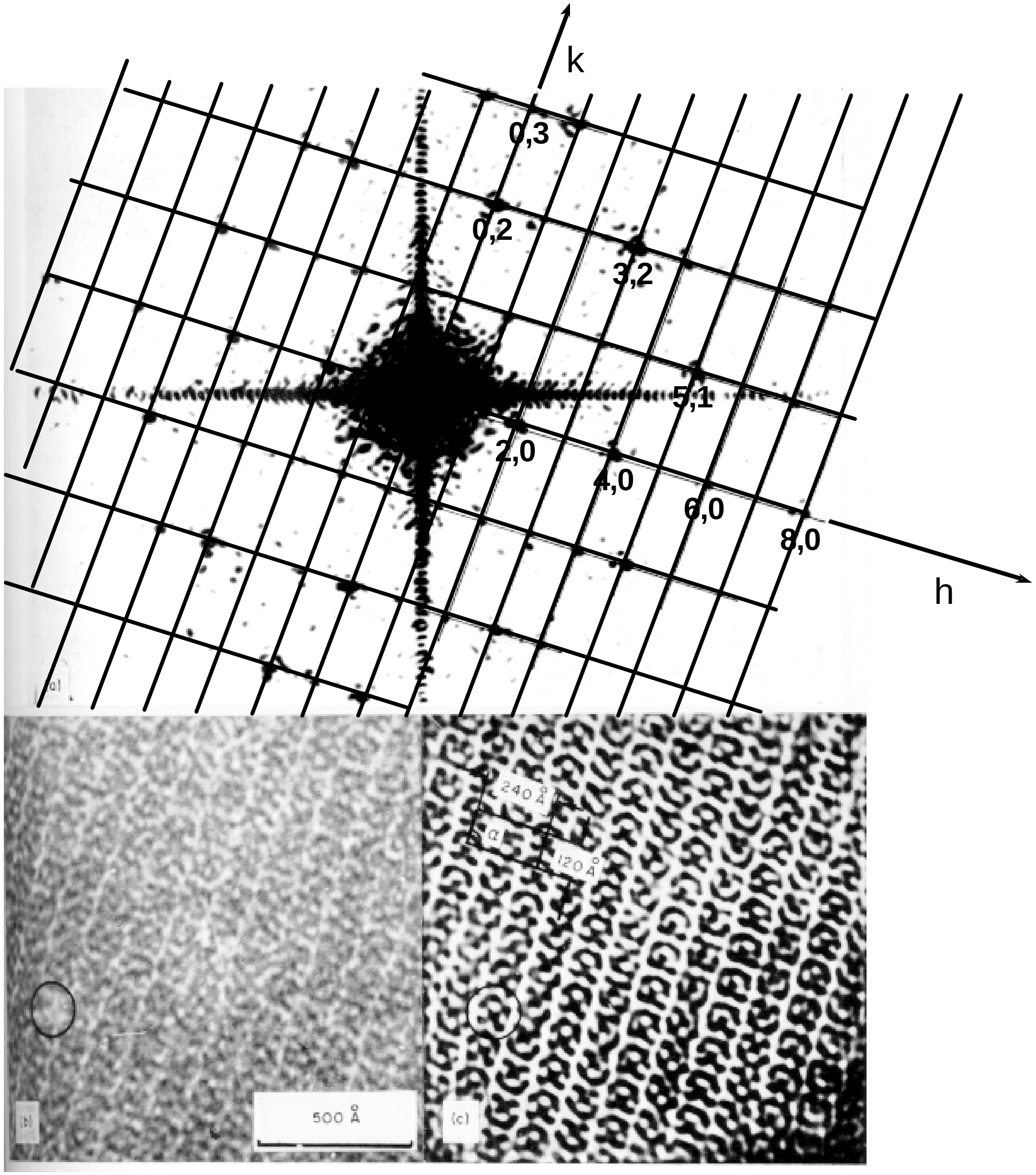
**Characteristic "cross" observed in many OD patterns:**

- Arises from **edges of mask** used to isolate portion of micrograph

- Mask creates an artificially high contrast border (window)

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

## OD and Filtration of Negatively-stained Phosphorylase b Crystal




Unfiltered

Filtered

From Kiselev et al., (1971) Plate III

# III.D.2 Optical Filtering

## III.D.2.b Filtering Procedure

**Filter mask:**

- Designed with holes positioned to allow unobstructed passage of diffraction spots at lattice points (or lattice lines, *e.g.* for helical particles)

**Position of filter mask:**

- **Must be accurately positioned** in the diffraction plane of the OD so all spots at the lattice points are allowed through
- **Opaque** regions of mask **block** out most of **noise** in the OD pattern arising from non-periodic image features

**Reconstruction lens:**

- Placed **behind** the mask
- **Refocuses unobstructed rays** and forms a filtered image
- **UN**filtered image formed if mask **not present**

# III.D.2 Optical Filtering

## III.D.2.b Filtering Procedure

Optical reconstruction illustrates the Abbe double-diffraction phenomena of image formation

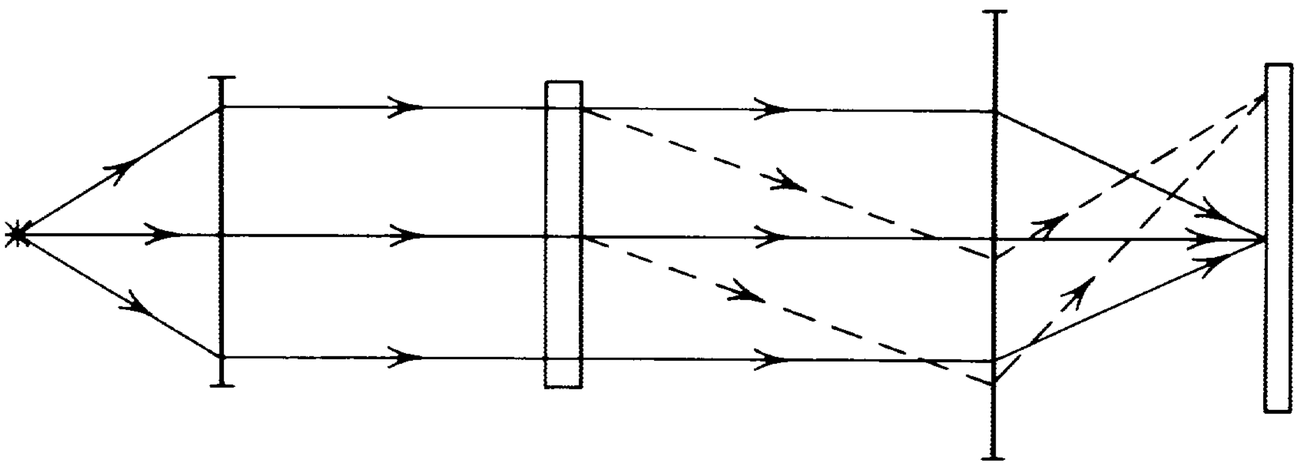
1st stage: OD pattern of the micrograph is formed (forward transformation)

2nd stage: reconstruction lens rediffracts the diffracted rays (back- or reverse transformation) to form an image (filtered or unfiltered)

- A filtered/unfiltered image is result of rediffraction of masked/unmasked diffraction pattern of micrograph

# III.D.1 Optical Diffraction

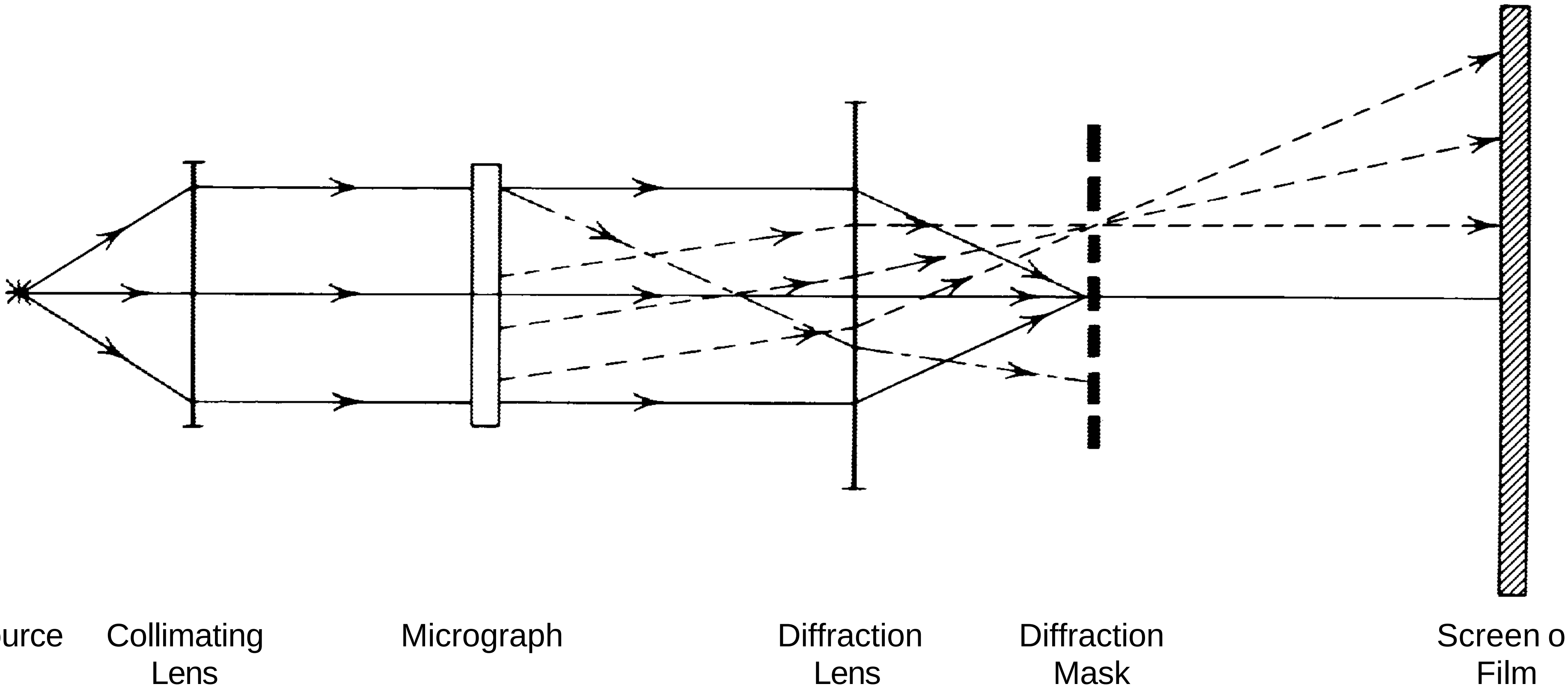
## III.D.1.b Experimental Apparatus




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

# III.D.2 Optical Filtering

## III.D.2.c Experimental Apparatus




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

# III.D.2 Optical Filtering

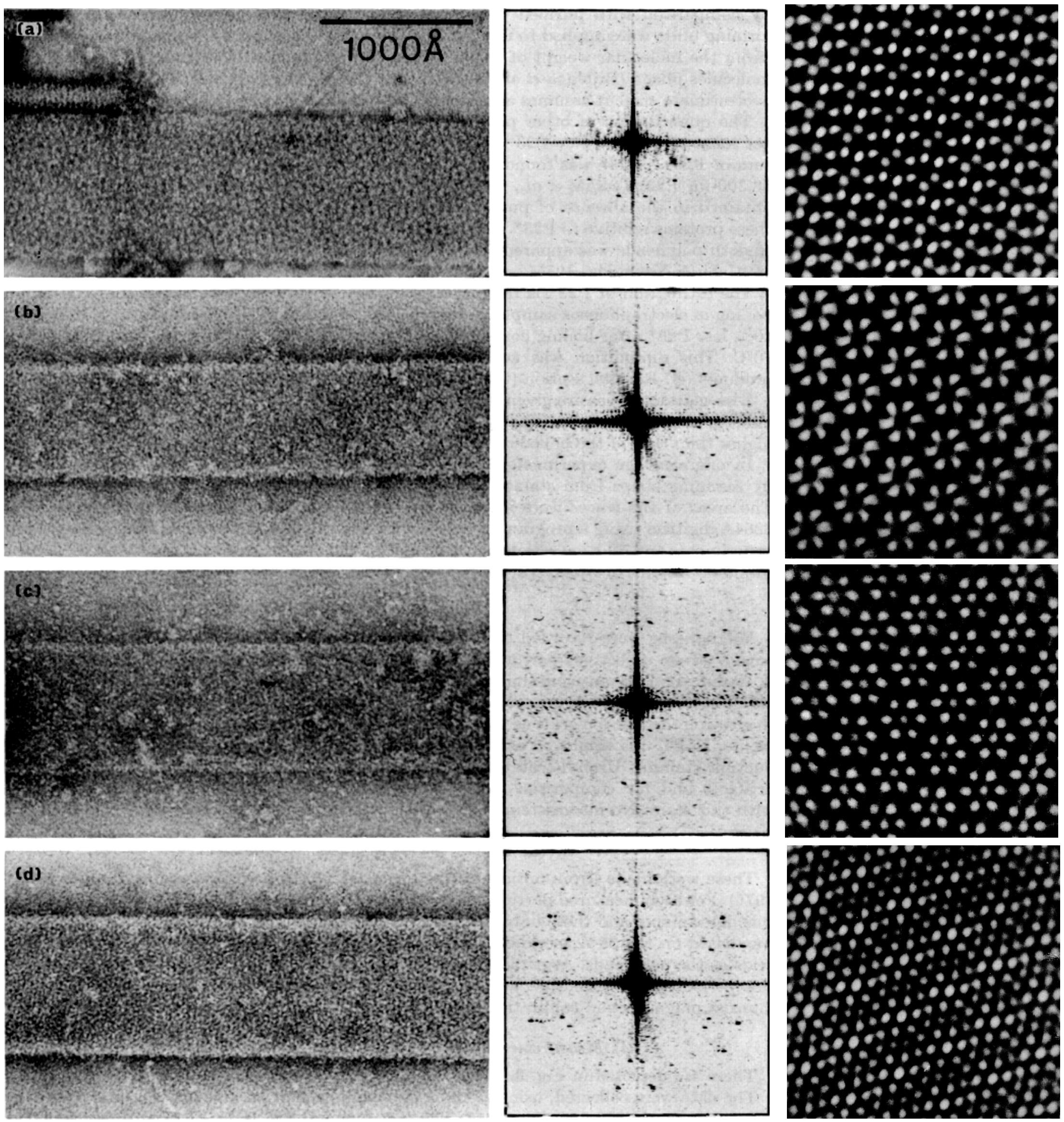
## III.D.2.e Image Averaging

Optical filtering **reduces image noise** by **averaging** unit cells in the periodic array

- As **size of holes** in filter mask is **reduced**, more noise in the diffraction pattern is removed and the **extent of local averaging increases**

**WARNING:** If holes are made **smaller** than the diffraction spots, the **signal-to-noise ratio may *decrease***

# III.D.2 Optical Filtering

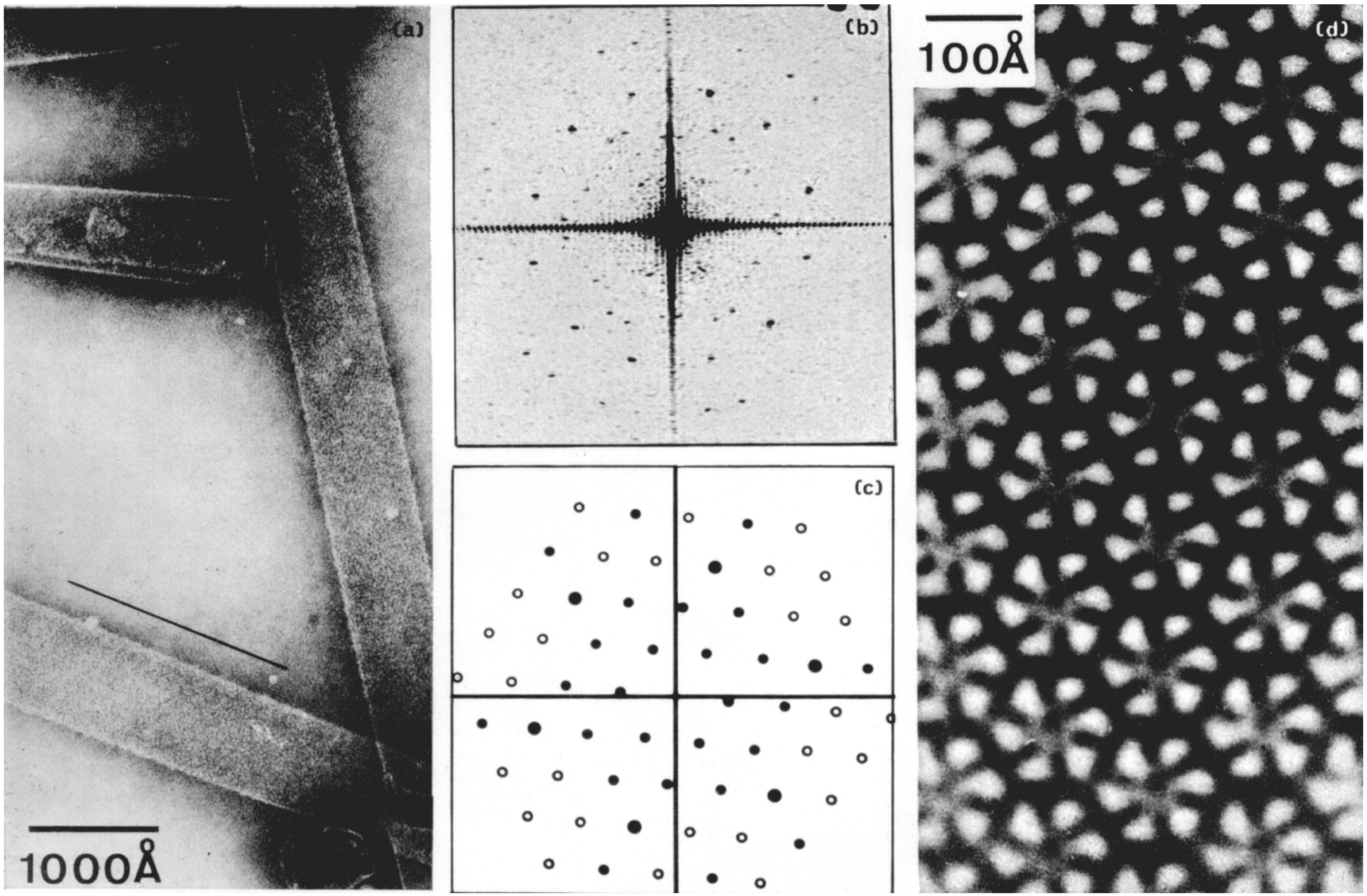



OD and Filtration of Negatively-stained T-even Polyheads

From Steven et al., (1976) Figs. 1 and 5, pp.192 and 200

# III.D.2 Optical Filtering

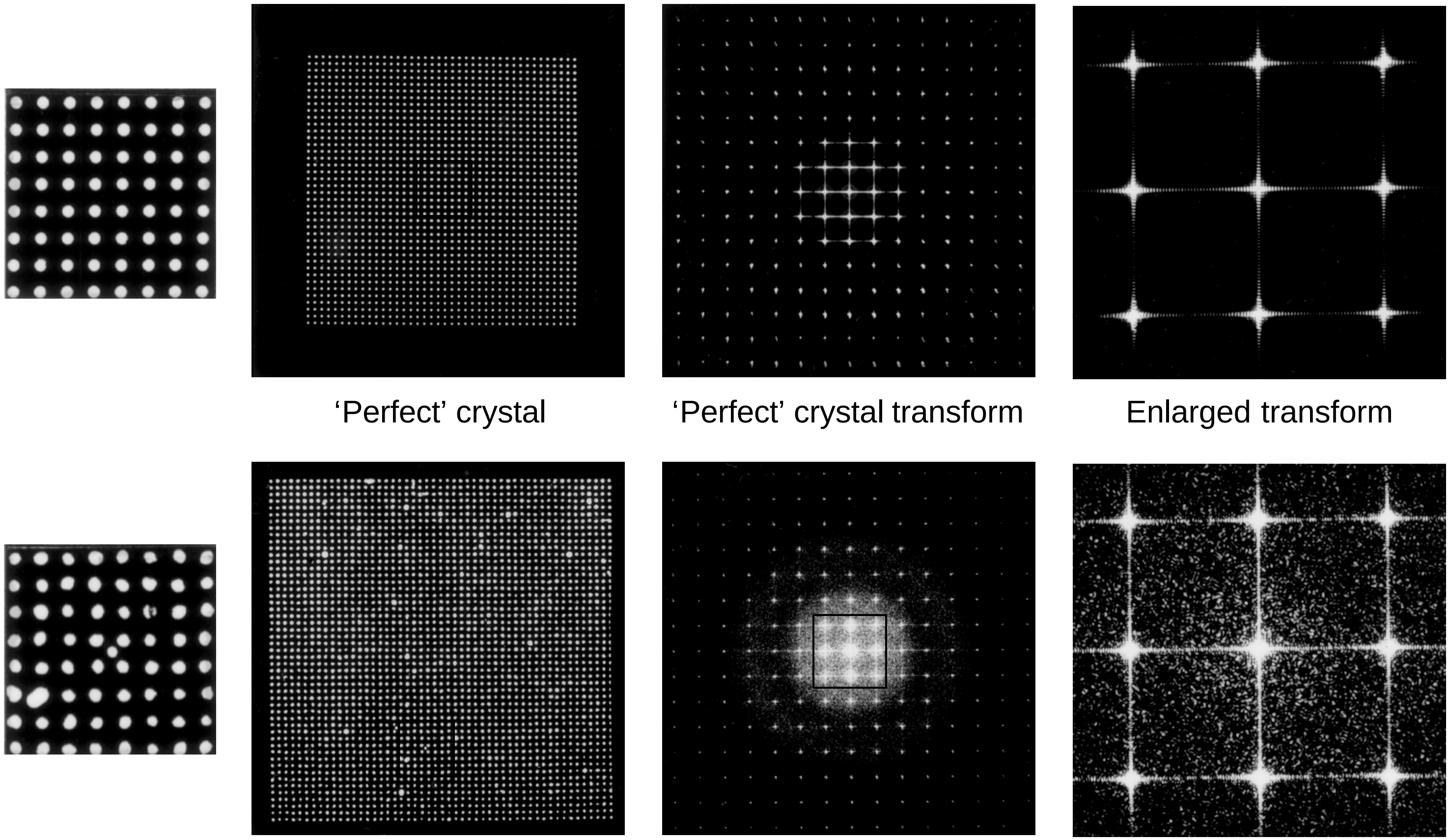
## Optical Filtration of Negatively-stained T4 Polyhead




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

# III.D.2 Optical Filtering

## Optical Diffraction of Perfect and Imperfect 'Crystals'



'Perfect' crystal — 'Perfect' crystal transform — Enlarged transform

'Imperfect' crystal — 'Imperfect' crystal transform — Enlarged transform

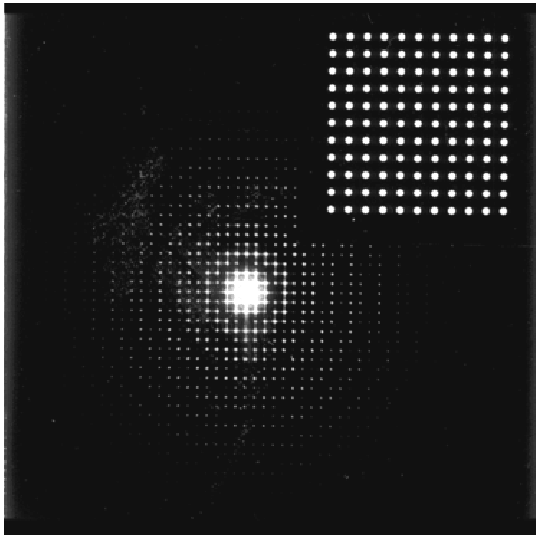
Demonstration of how periodicity can be enforced on a structure by action of mask in Fourier space

From Baker thesis (1976) Fig. B.1

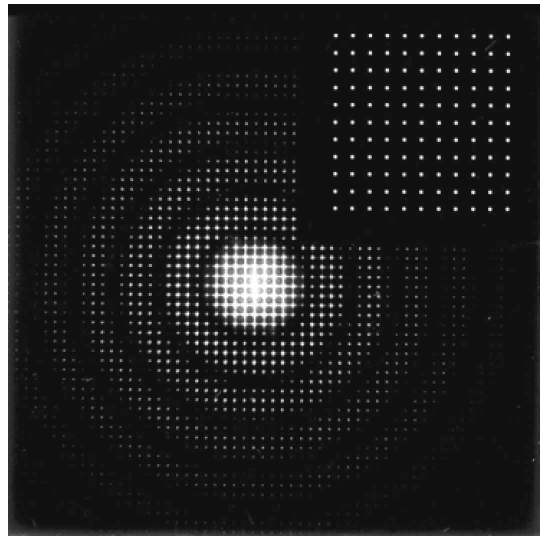
# III.D.2 Optical Filtering

## Optical Filtration of ‘Imperfect Crystal’

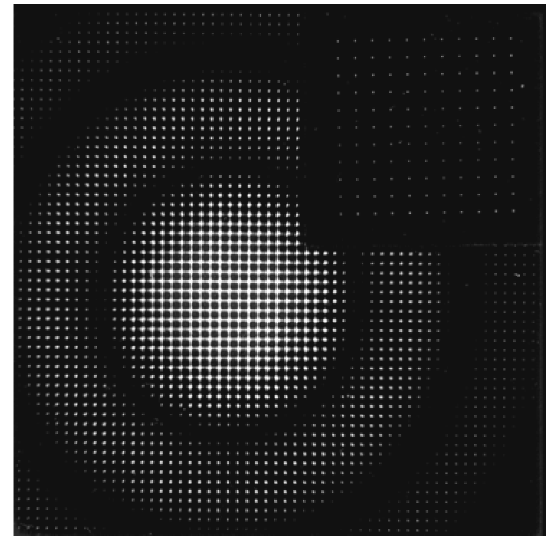
Mask ($d_{a^*}/_{a^*} = 0.43$)



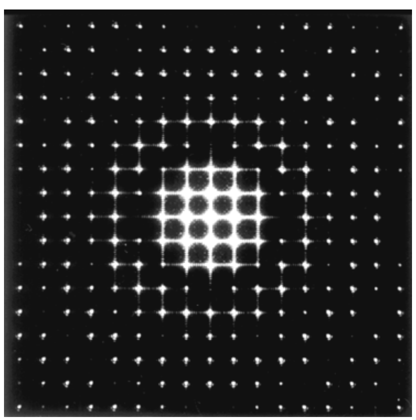
Mask ($d_{a^*}/_{a^*} = 0.2$)



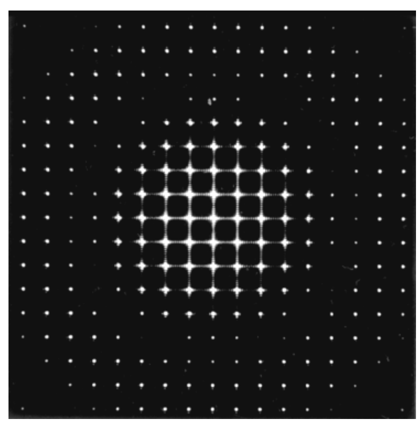
Mask ($d_{a^*}/_{a^*} = 0.1$)



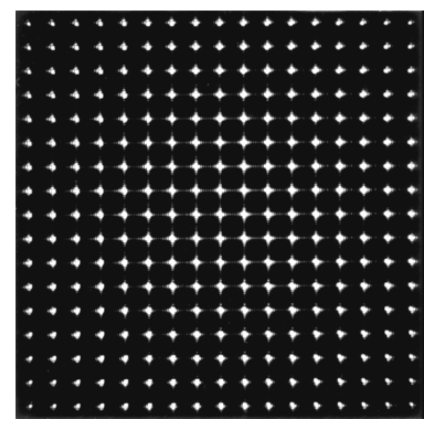
FT of Mask



FT of Mask



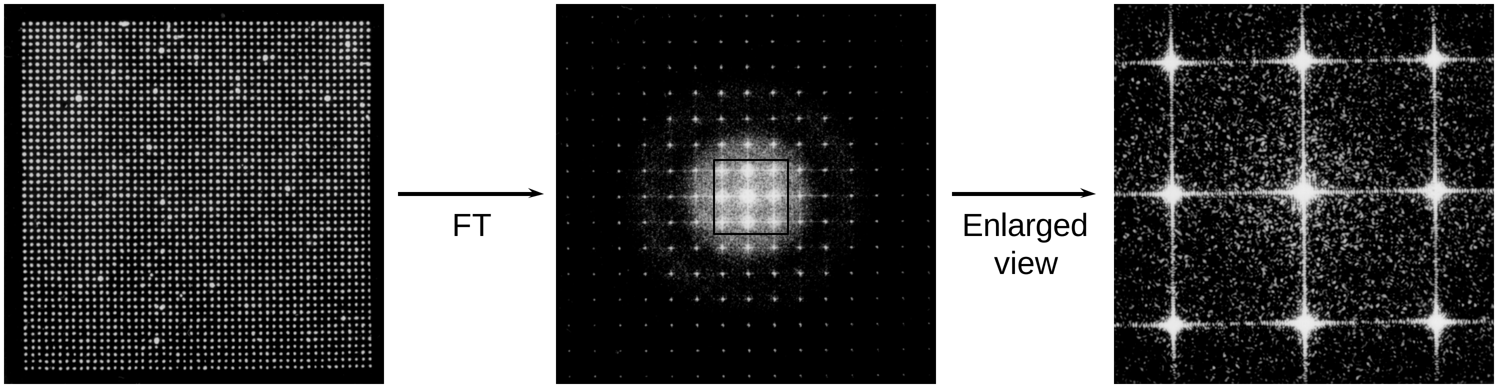
FT of Mask



From Baker thesis (1976) Fig. B.2

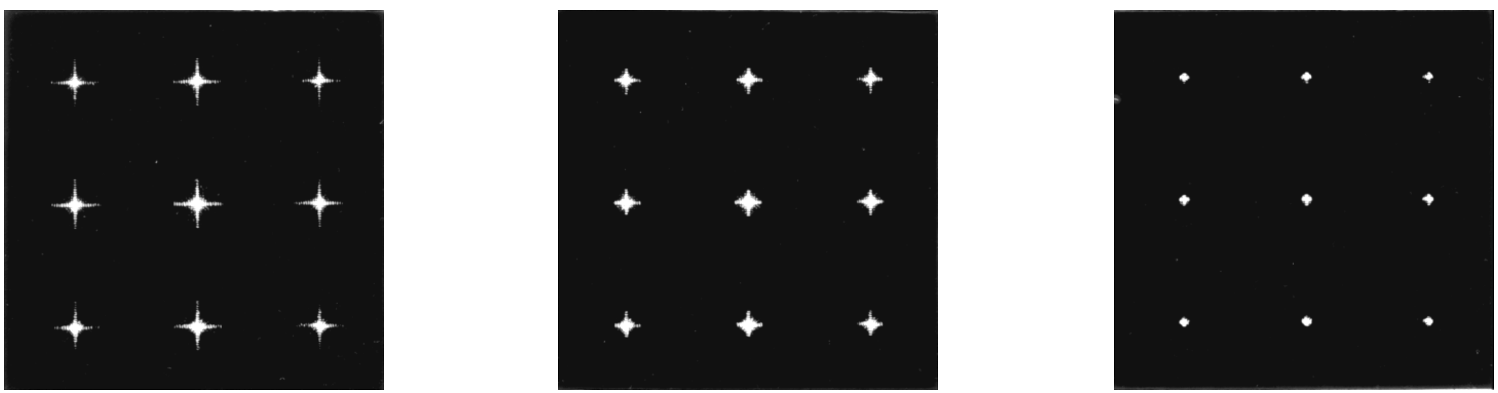
# III.D.2 Optical Filtering

## Optical Filtration of ‘Imperfect Crystal’




‘Imperfect’ crystal — ‘Imperfect’ crystal transform — Enlarged transform

Masked transform = MASK X CRYSTAL TRANSFORM



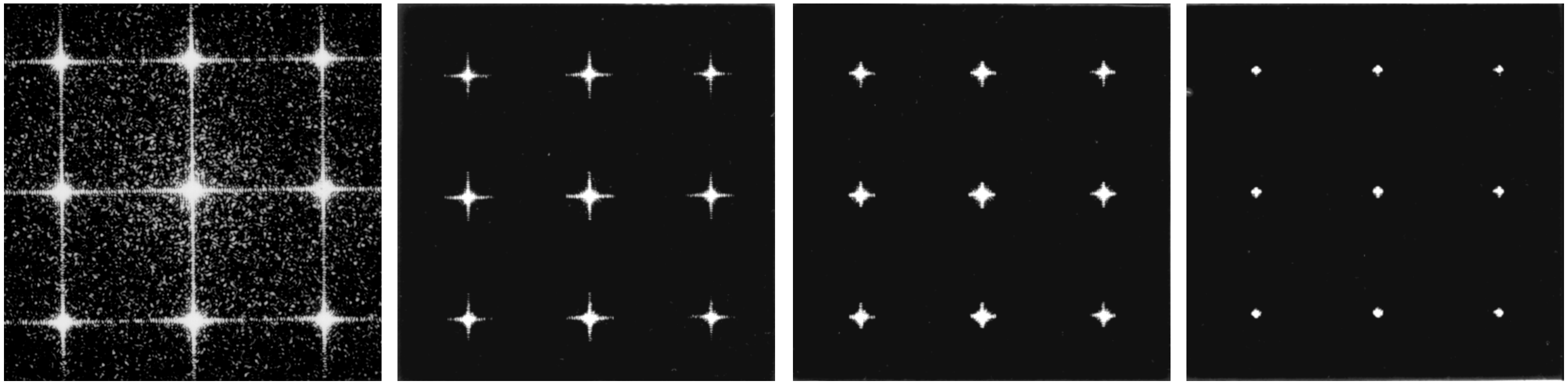
Masked FT ($d_{a*}/_{a*} = 0.43$) — Masked FT ($d_{a*}/_{a*} = 0.2$) — Masked FT ($d_{a*}/_{a*} = 0.1$)

From Baker thesis (1976) Fig. B.2

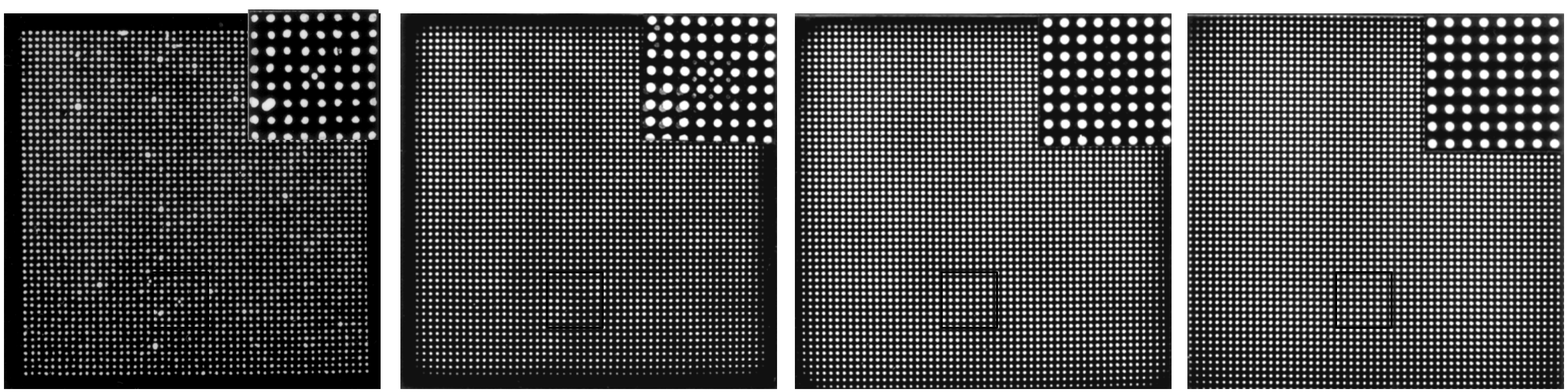
# III.D.2 Optical Filtering

## Optical Filtration of ‘Imperfect Crystal’

Masked transform = MASK X CRYSTAL TRANSFORM



‘Imperfect’ crystal transform | Masked FT ($d_{a^*}/_{a^*}$ = 0.43) | Masked FT ($d_{a^*}/_{a^*}$ = 0.2) | Masked FT ($d_{a^*}/_{a^*}$ = 0.1)

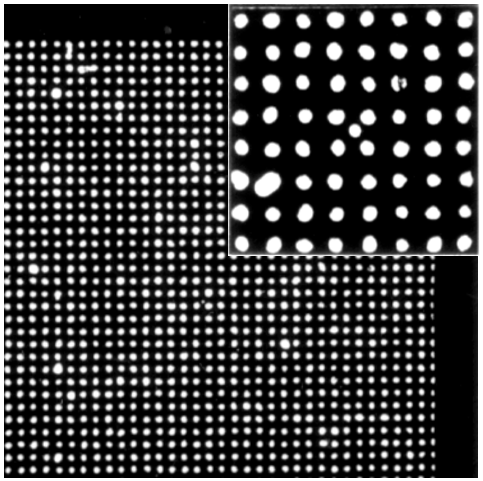


‘Imperfect’ crystal | Filtered crystal | Filtered crystal | Filtered crystal

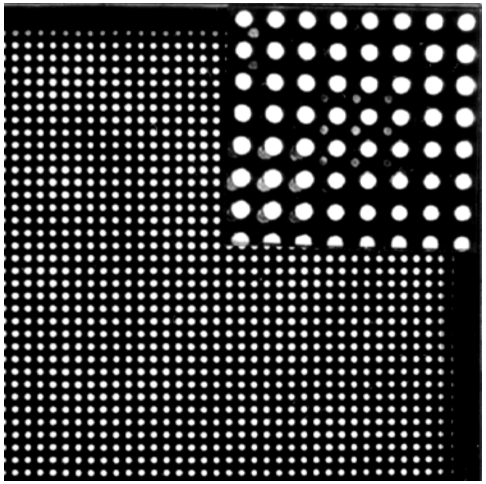
From Baker thesis (1976) Fig. B.2

# III.D.2 Optical Filtering

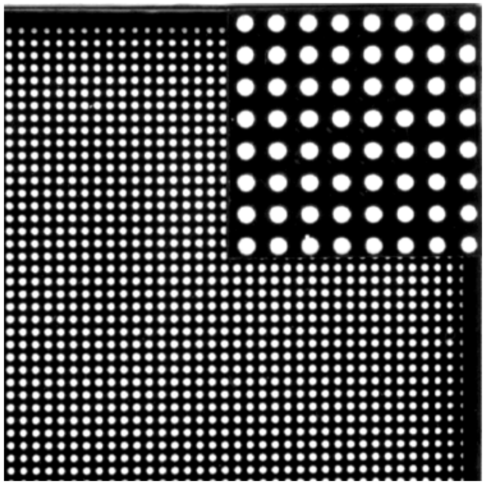
## Optical Filtration of ‘Imperfect Crystal’



‘Imperfect’ crystal



Filtered crystal
($d_{a^*}/_{a^*}$ = 0.43)

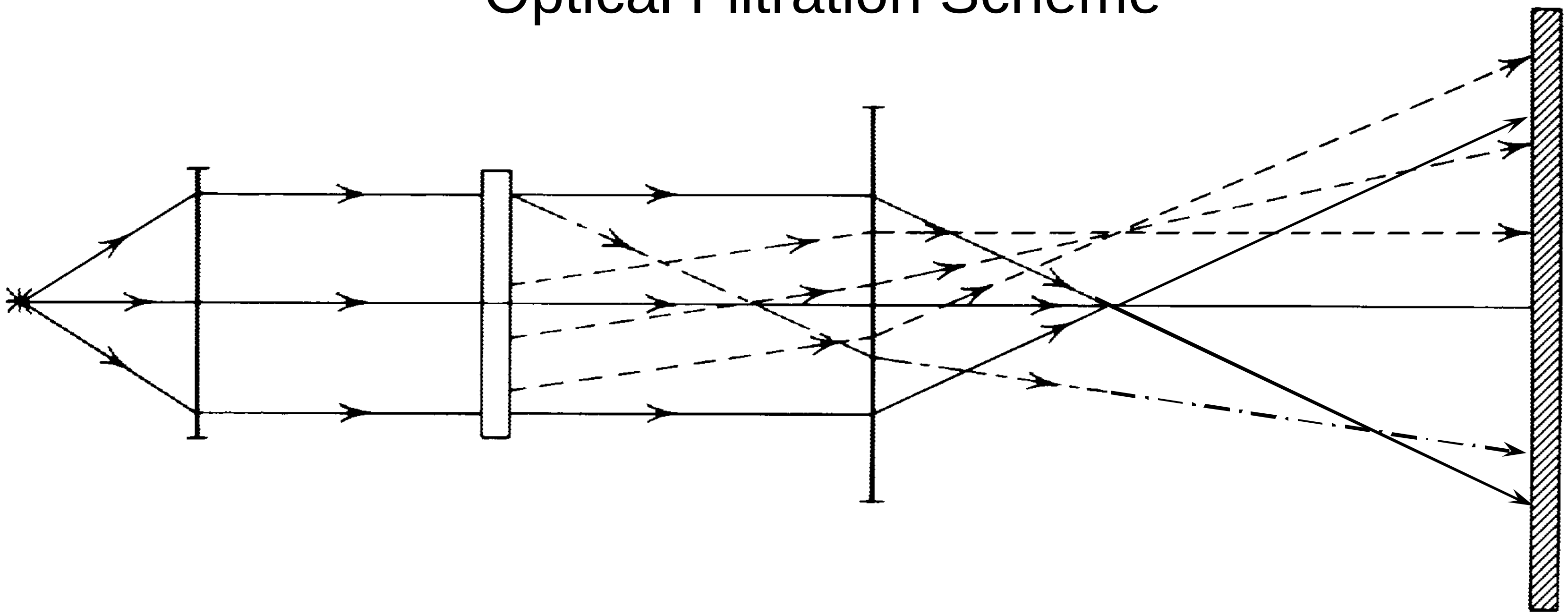


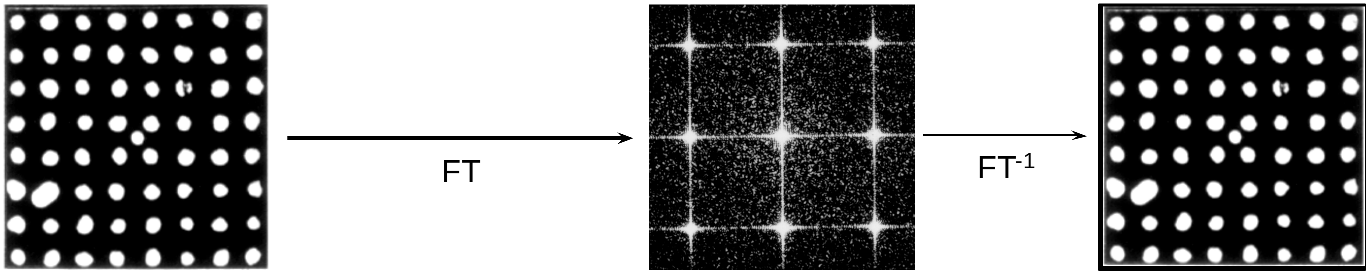
Filtered crystal
($d_{a^*}/_{a^*}$ = 0.2)

From Baker thesis (1976) Fig. B.2

# III.D.2 Optical Filtering

## Optical Filtration Scheme





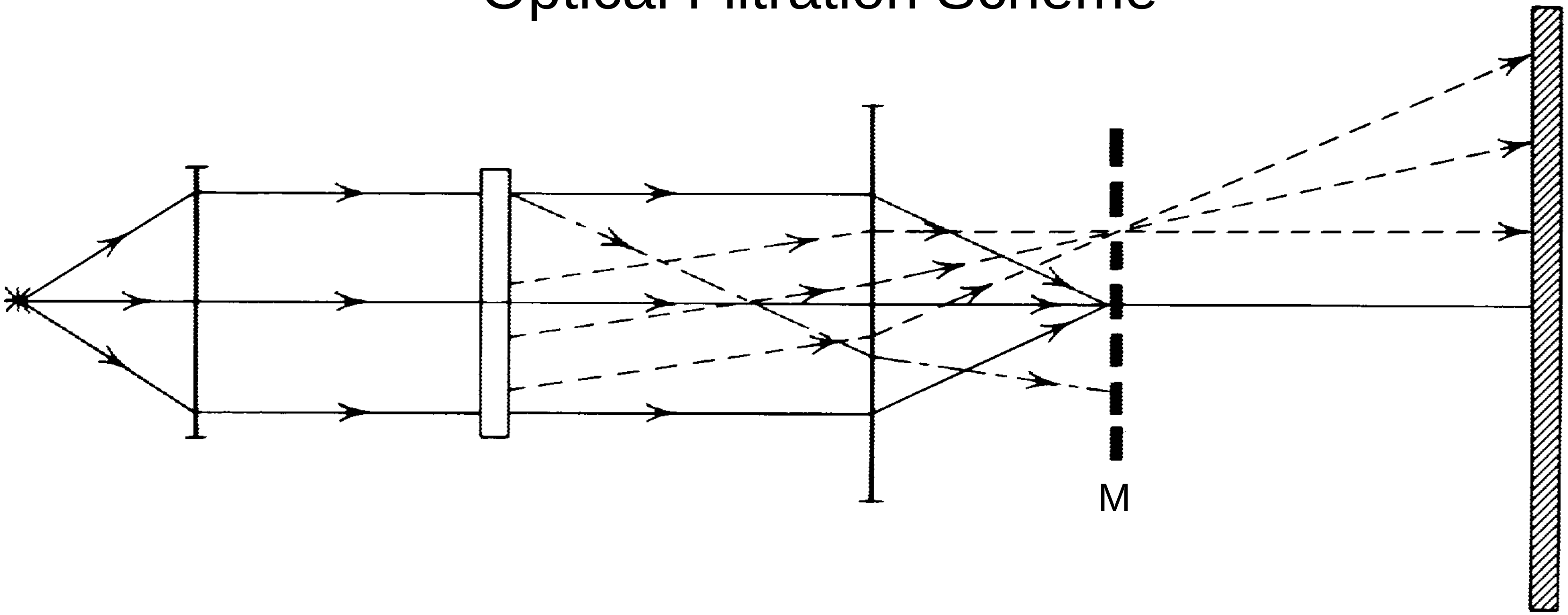

Original image | Transform | Unfiltered image

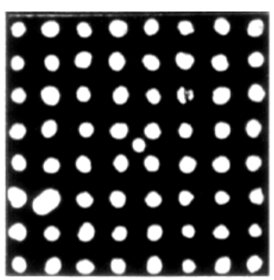
$i$ | $T(i)$ | $T^{-1}(T(i))$

 | $I$ | $i$

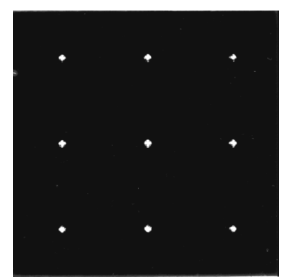
# III.D.2 Optical Filtering

## Optical Filtration Scheme

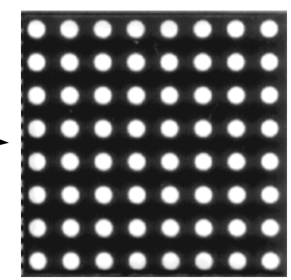


FT



$FT^{-1}$



Original image

$i$

Masked transform

$T(i) \times M$

$I \times M$

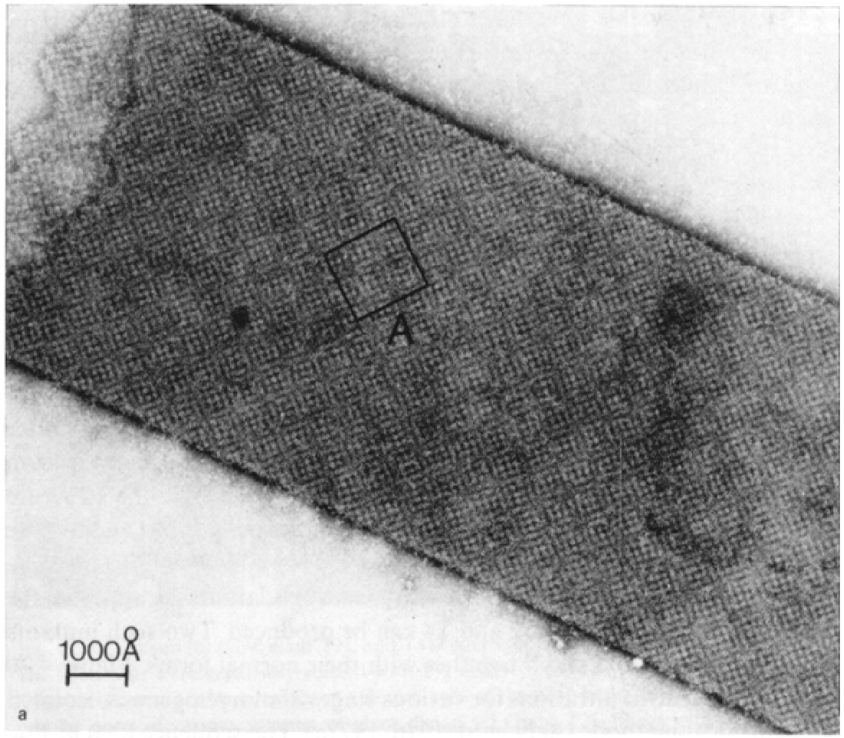
Filtered image

$i * T^{-1}(M)$

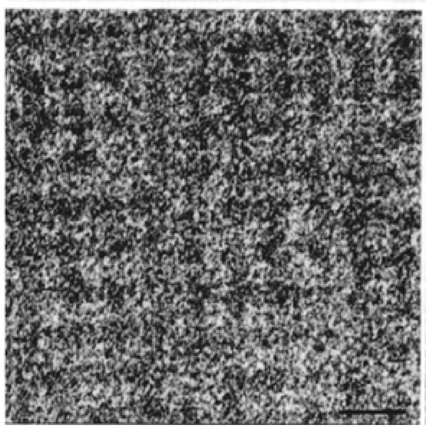
$i * m$

# III.D.2 Optical Filtering

## Optical Filtering of Double Layer Crystal




Negatively-stained T-layer from *Bacillus brevis*



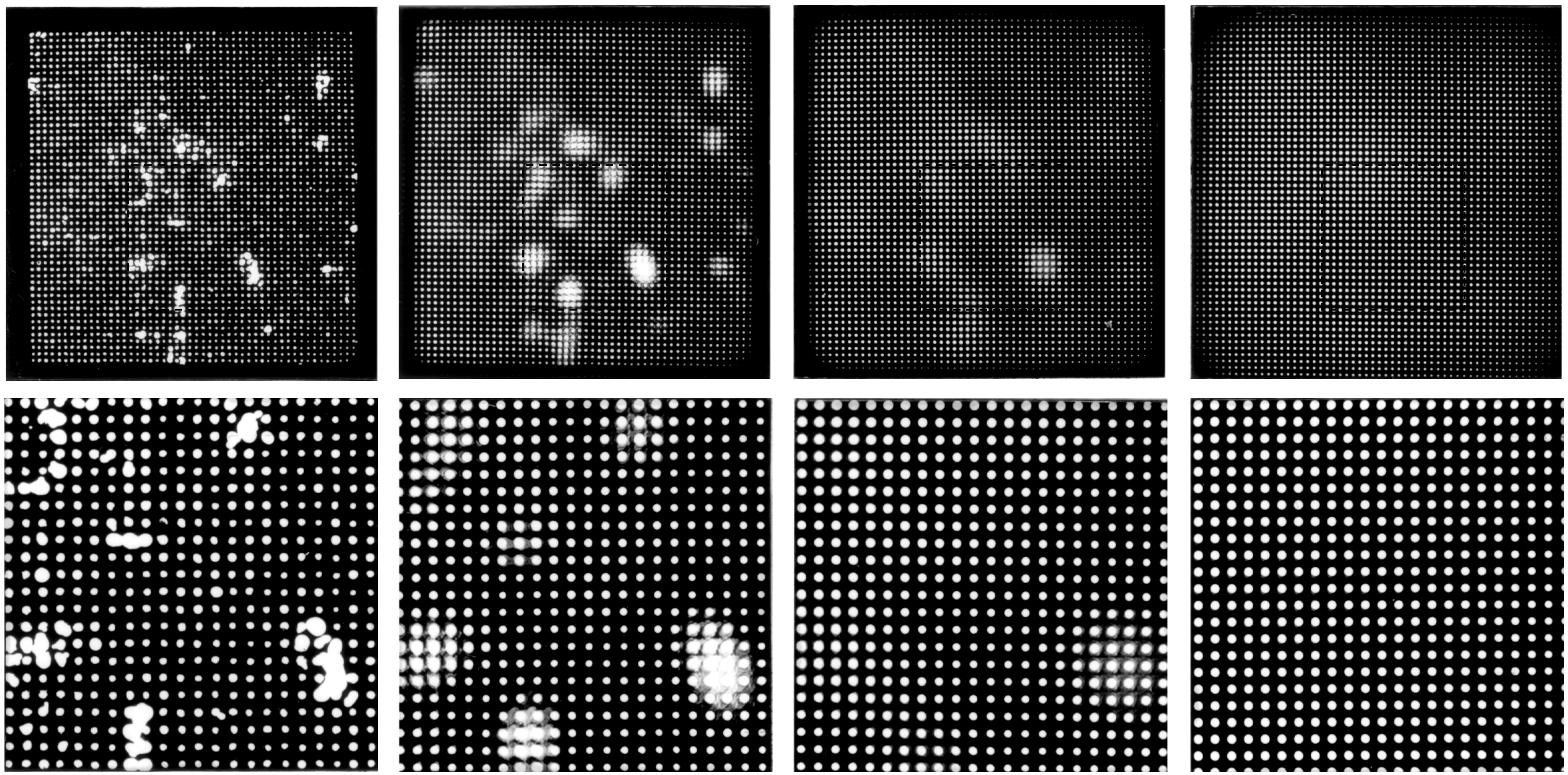
Magnified view of (a)

Optical reconstruction of upper layer

From Misell (1978) Fig. 4.19, p.163

# III.D.2 Optical Filtering

## Optical Filtering of Imperfect 'Crystal'

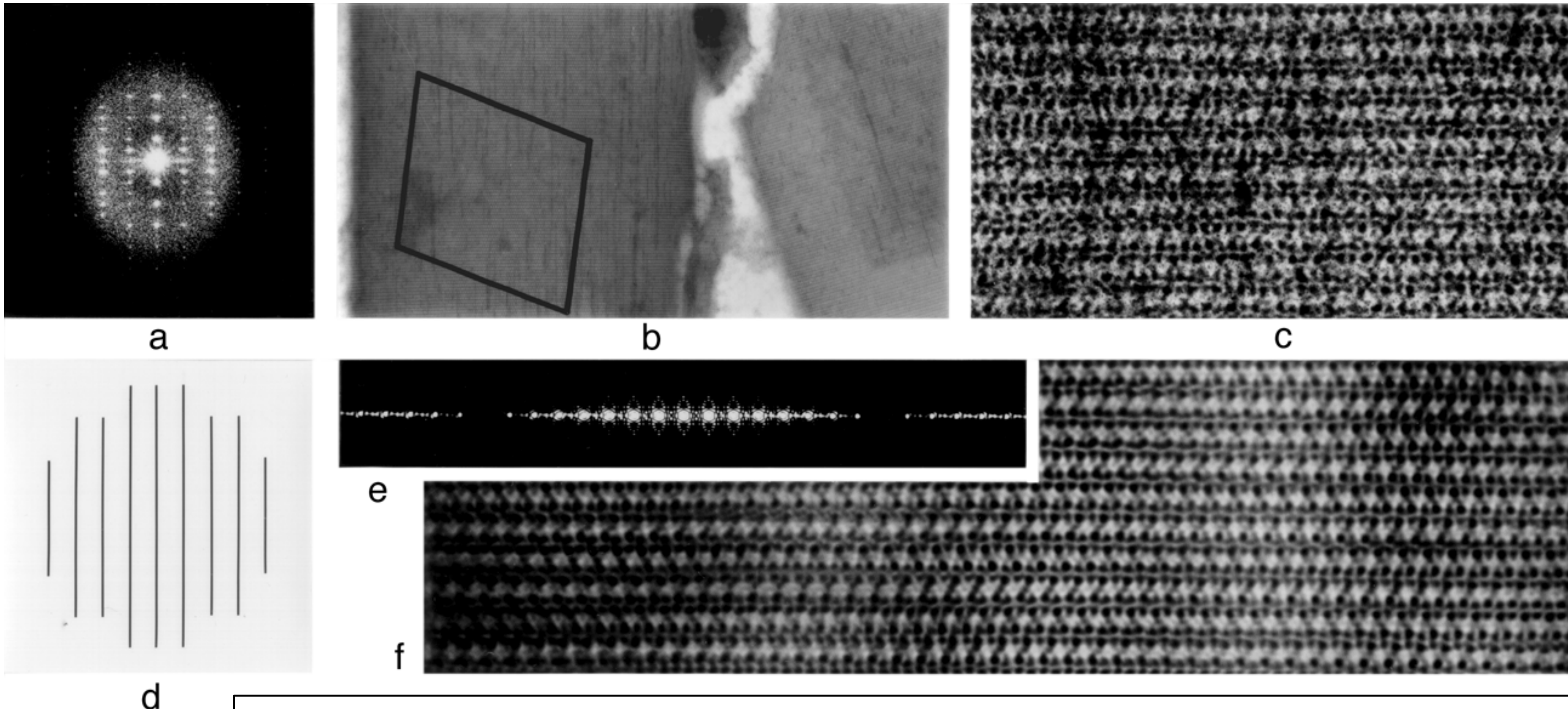


Demonstration of how periodicity can be enforced on a structure by action of mask in Fourier space

From Baker thesis (1976) Fig. B.3

# III.D.2 Optical Filtering

## Optical Filtering of Negatively Stained Catalase Crystal




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

# III.D.2 Optical Filtering

## III.D.2.e Image Averaging

**KEY CONCEPT:**

- Averaging results when an image is ***convoluted*** with the ***transform*** of the filter mask:

$$\text{Reconstructed image} = \text{image} * T^{-1}(\text{MASK})$$

$$\text{MASK} = [\text{LATTICE} * \text{HOLE}] \times \text{WINDOW}$$

↓ ↓ ↓ ↓ ↓

$$T^{-1}(\text{MASK}) = [\ \text{lattice} \ \times \ \text{hole}\ ] * \text{window}$$

# III.D.2 Optical Filtering

## III.D.2.e Image Averaging

$$\text{Reconstructed image} = \text{image} * T^{-1}(\text{MASK})$$

$$\text{MASK} = [\text{LATTICE} * \text{HOLE}] \times \text{WINDOW}$$

↓ ↓ ↓ ↓ ↓

$$T^{-1}(\text{MASK}) = [\ \underline{\text{lattice}} \ \ \times \ \ \underline{\text{hole}}\ ] \ * \ \ \underline{\text{window}}$$

where,

lattice = specimen lattice (better be or else!)

hole = Airy disk (if HOLE is a circle): determines extent of averaging

window = sin(X)/X “cross” (if WINDOW is a rectangle)

# III.D.2 Optical Filtering

## III.D.2.e Image Averaging

Reconstructed image = image $\ast$ $T^{-1}$(MASK)

MASK = [**LATTICE** $\ast$ HOLE] X WINDOW

$T^{-1}$(MASK) = [ **lattice** X hole ] $\ast$ window

where,

lattice = specimen lattice (better be or else!)

hole = Airy disk (if HOLE is a circle): determines extent of averaging

window = sin(X)/X “cross” (if WINDOW is a rectangle)

# III.D.2 Optical Filtering

## III.D.2.f Artifacts of Optical Filtering

**Sources of artifacts:**

- **Misindexed** OD pattern ---> incorrect mask design

- **Incorrect positioning** of mask in diffraction plane causes wanted spots to be partially or totally blocked

- **Mispositioned or misshaped mask holes** make it impossible to pass all spots through the mask simultaneously