NCMI Single Particle Workshop
Baylor, March 14-17th 2011

Image processing theory
Richard Henderson

- Fourier synthesis and analysis
- Central section theory and 3D reconstructions
- Contrast transfer theory
- B-factors, resolution, radiation damage

from Wikipedia

Joseph Fourier

Jean Baptiste Joseph Fourier

Born	21 March 1768 Auxerre, Yonne, France
Died	16 May 1830 (aged 62) Paris, France

from: *The Fourier Transform and Its Application*
by Ronald N. Bracewell (1921-2007)

Baron Jean-Baptiste-Joseph Fourier introduced the idea that an arbitrary function could be represented by a single analytic expression. Fourier came upon his idea in connection with the problem of the flow of heat in solid bodies.

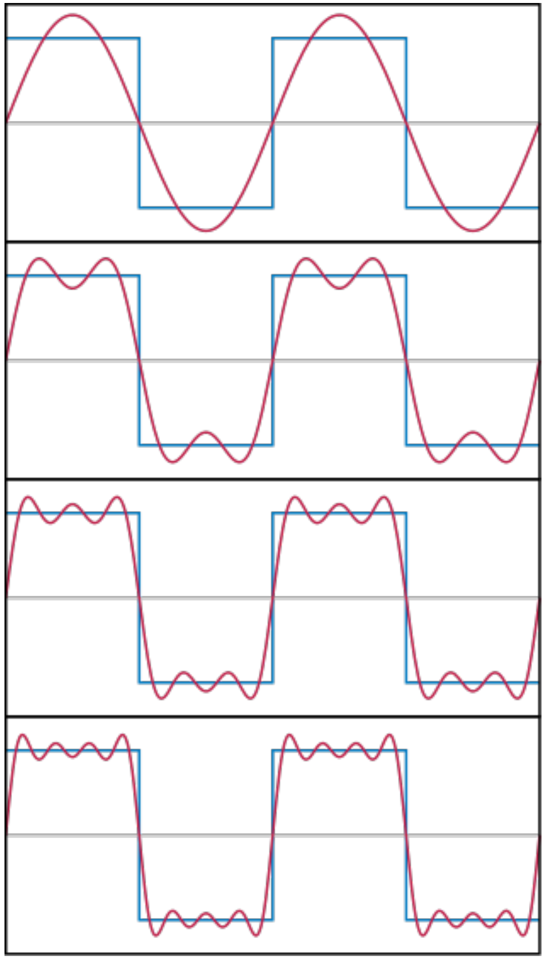
The formula
$x/2 = \sin x - (\sin 2x)/2 + (\sin 3x)/3 - \cdots$

was published by Leonhard Euler (1707-1783) before Fourier.

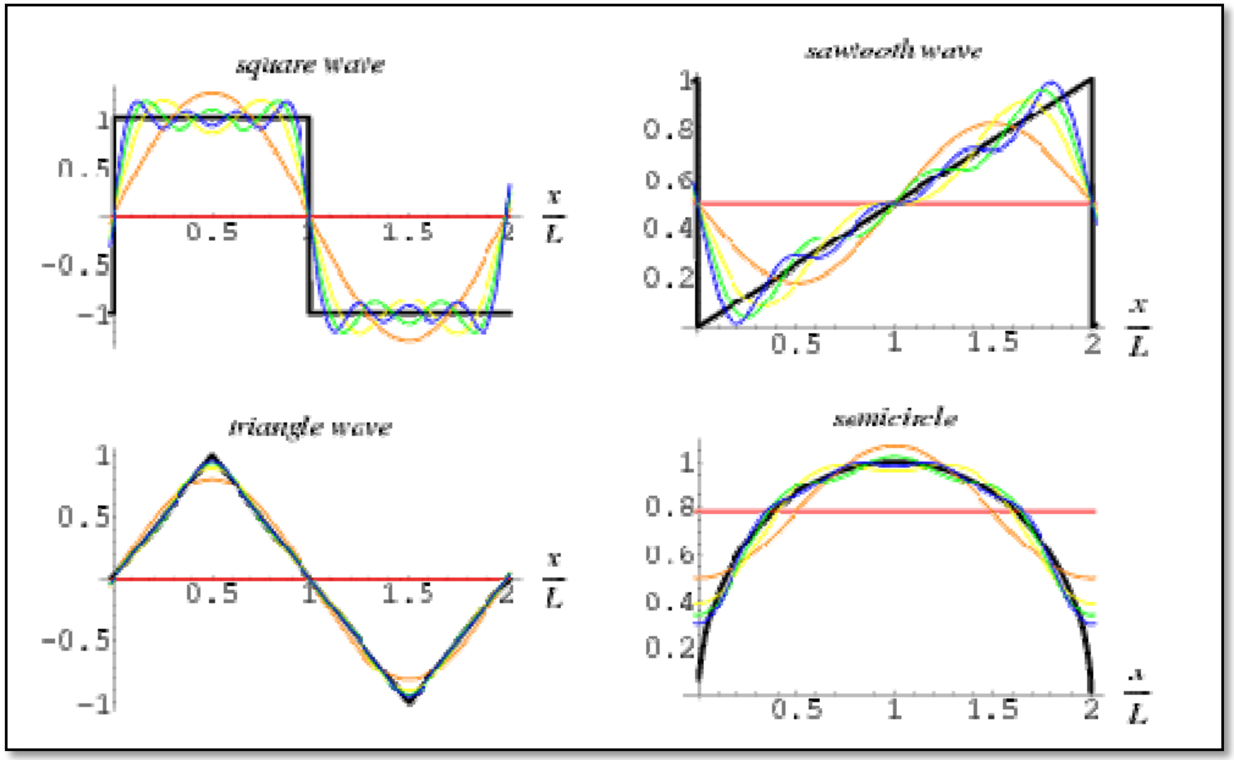
from: David Keston *article* in Today in Science
Fourier's Analytic Theory of Heat ad its experimental verification.

Fourier used a polished iron ring of diameter ~30cm held in place by wooden supports and heated by an adjustable Argand burner. Six holes were drilled halfway into the ring, four of which held thermometers on the Réamur scale (the space between the ring and the thermometer being filled with mercury - as were the other two holes). To achieve the steady state one point in the ring was heated while rest of the ring was allowed to radiate heat freely and on the whole results agreed very well with Fourier's theory.

Some 1D examples of Fourier series



The first four Fourier
series approximations
for a square wave.



http://www.ies.co.jp/math/java/trig/graphFourier/graphFourier.html

A minimum of equations about Fourier analysis
from E.G.Steward: “Fourier Optics an introduction” (1987, 2004)

Some different ways to express 1D Fourier summations, where D = repeat distance (unit cell)

$$f(x) = \frac{A_0}{2} + A_1 \cos\left(\frac{2\pi x}{D} - \alpha_1\right) + A_2 \cos\left(\frac{2\pi x}{D/2} - \alpha_2\right) + \ldots$$

$$= \frac{A_0}{2} + \sum_{n=1}^{\infty} A_n \cos\left(\frac{2\pi n x}{\mathrm{D}} - \alpha_n\right)$$

$$f(x) = \frac{A_0}{2} + \sum_{n=1}^{\infty} \left[A_n \cos\alpha_n \,.\, \cos\frac{2\pi n x}{D} + A_n \sin\alpha_n \,.\, \sin\frac{2\pi n x}{D} \right]$$

which in turn can be written as

$$f(x) = \frac{A_0}{2} + \sum_{n=1}^{\infty} a_n \cos\frac{2\pi n x}{D} + \sum_{n=1}^{\infty} b_n \sin\frac{2\pi n x}{D}$$

$$f(x) = \sum_{n=-\infty}^{+\infty} \mathbf{C}_n \, \mathrm{e}^{\frac{i 2\pi n x}{D}} \, .$$

How to find the Fourier coefficients in 1D

The coefficients are derived in an analogous way and we have the pair of equations

$$f(x) = \sum_{n=-\infty}^{+\infty} \mathbf{C}_n \, \mathrm{e}^{\frac{i 2\pi n x}{D}}$$

$$\mathbf{C}_n = \frac{1}{D} \int_D f(x) \, \mathrm{e}^{\frac{-i 2\pi n x}{D}} \, \mathrm{d}x \, .$$

Derivation of Euler's expansion - see
http://www.ies.co.jp/math/java/trig/graphFourier/graphFourier.html

Example: a simple Fourier series

We now use the formulae above to give a Fourier series expansion of a very simple function. Consider a sawtooth function (as depicted in the figure):

$$f(x) = x, \quad \text{for} \quad -\pi < x < \pi,$$
$$f(x+2\pi) = f(x), \quad \text{for} \quad -\infty < x < \infty.$$

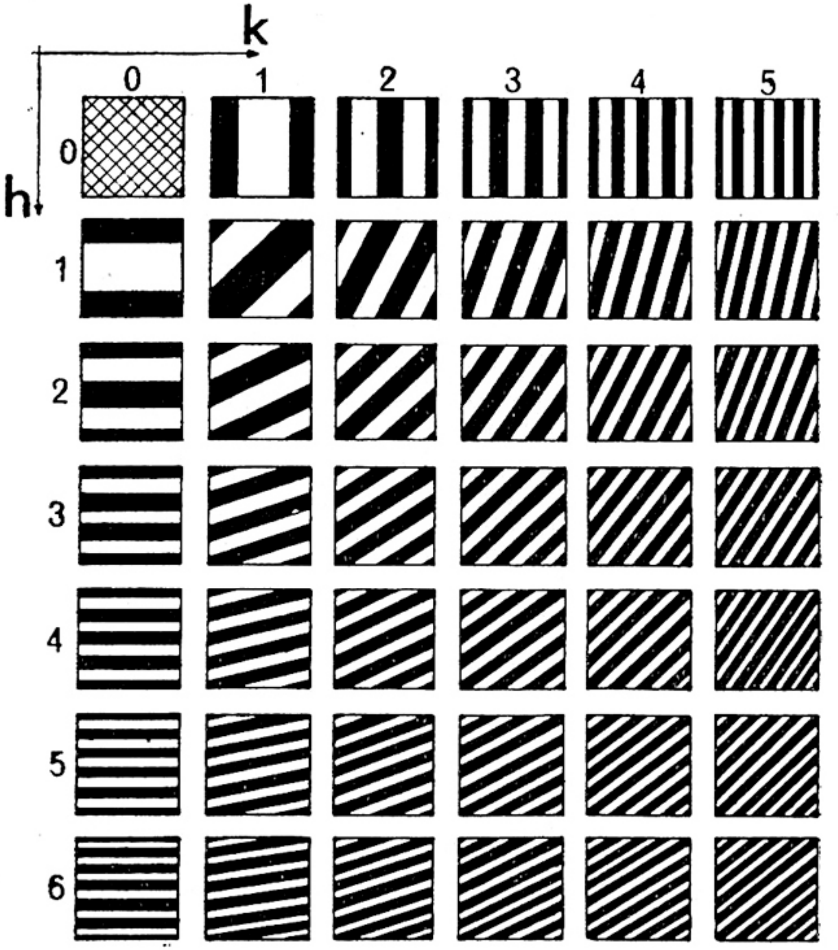
In this case, the Fourier coefficients are given by

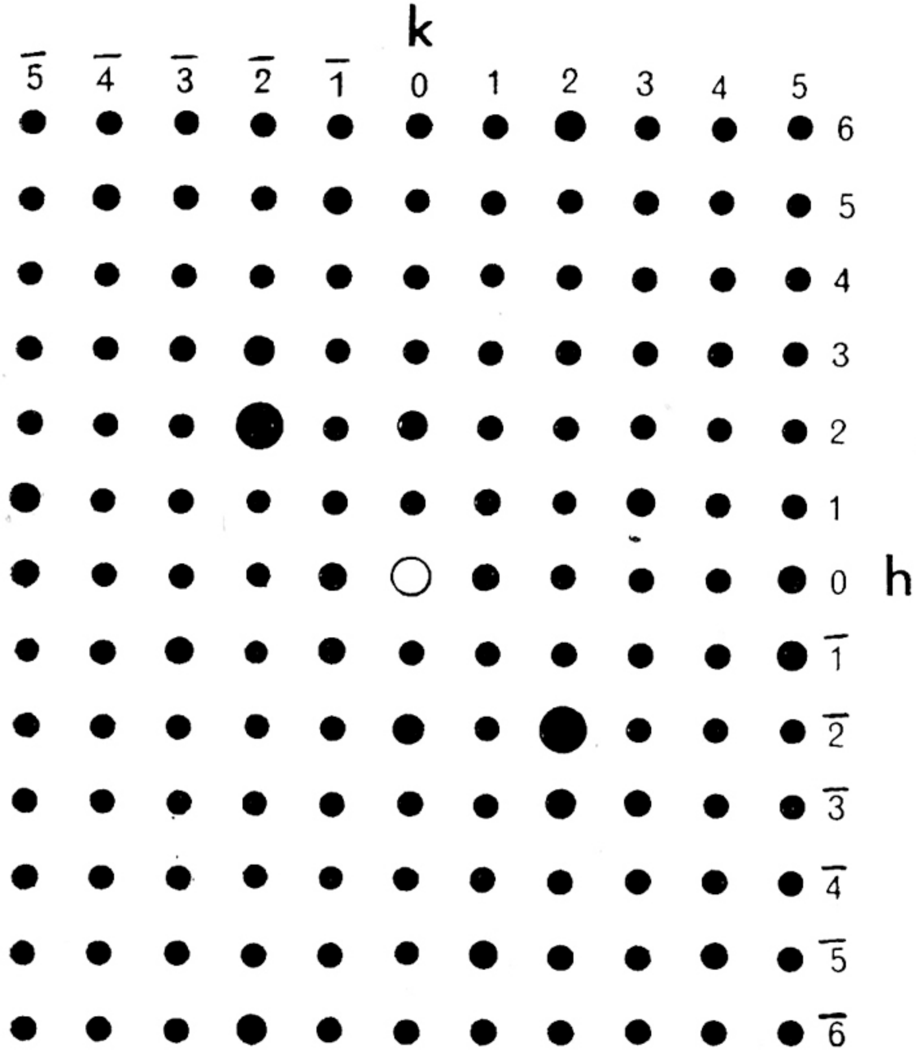
$$a_n = \frac{1}{\pi}\int_{-\pi}^{\pi} x\cos(nx)\,dx = 0.$$
$$b_n = \frac{1}{\pi}\int_{-\pi}^{\pi} x\sin(nx)\,dx = 2\frac{(-1)^{n+1}}{n}.$$

And therefore:

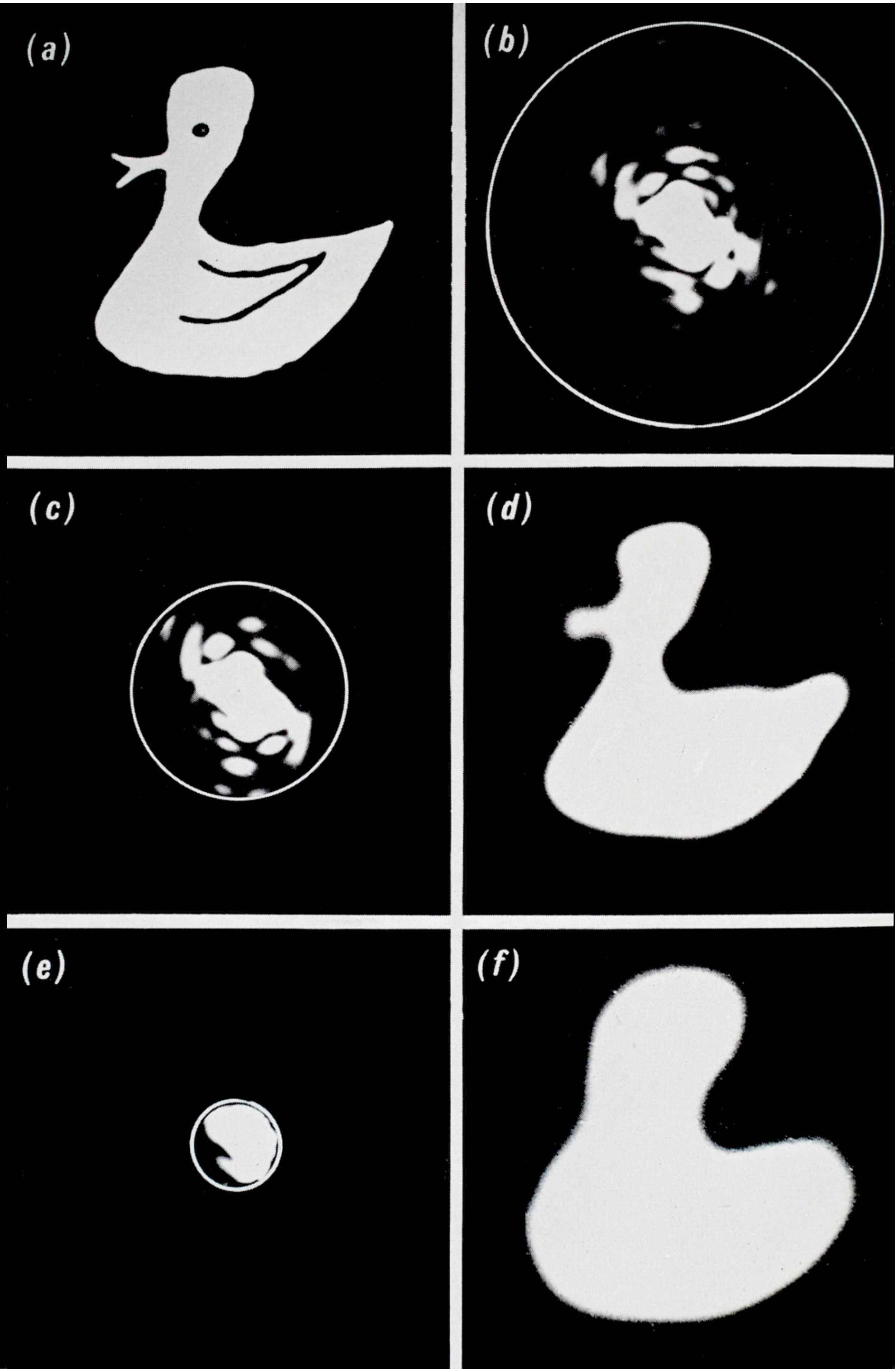
$$f(x) = \frac{a_0}{2} + \sum_{n=1}^{\infty}\left[a_n \cos(nx) + b_n \sin(nx)\right]$$
$$= 2\sum_{n=1}^{\infty}\frac{(-1)^{n+1}}{n}\sin(nx), \quad \text{for} \quad -\infty < x < \infty. \quad (*)$$

from Max Perutz, Nobel Lecture 1962



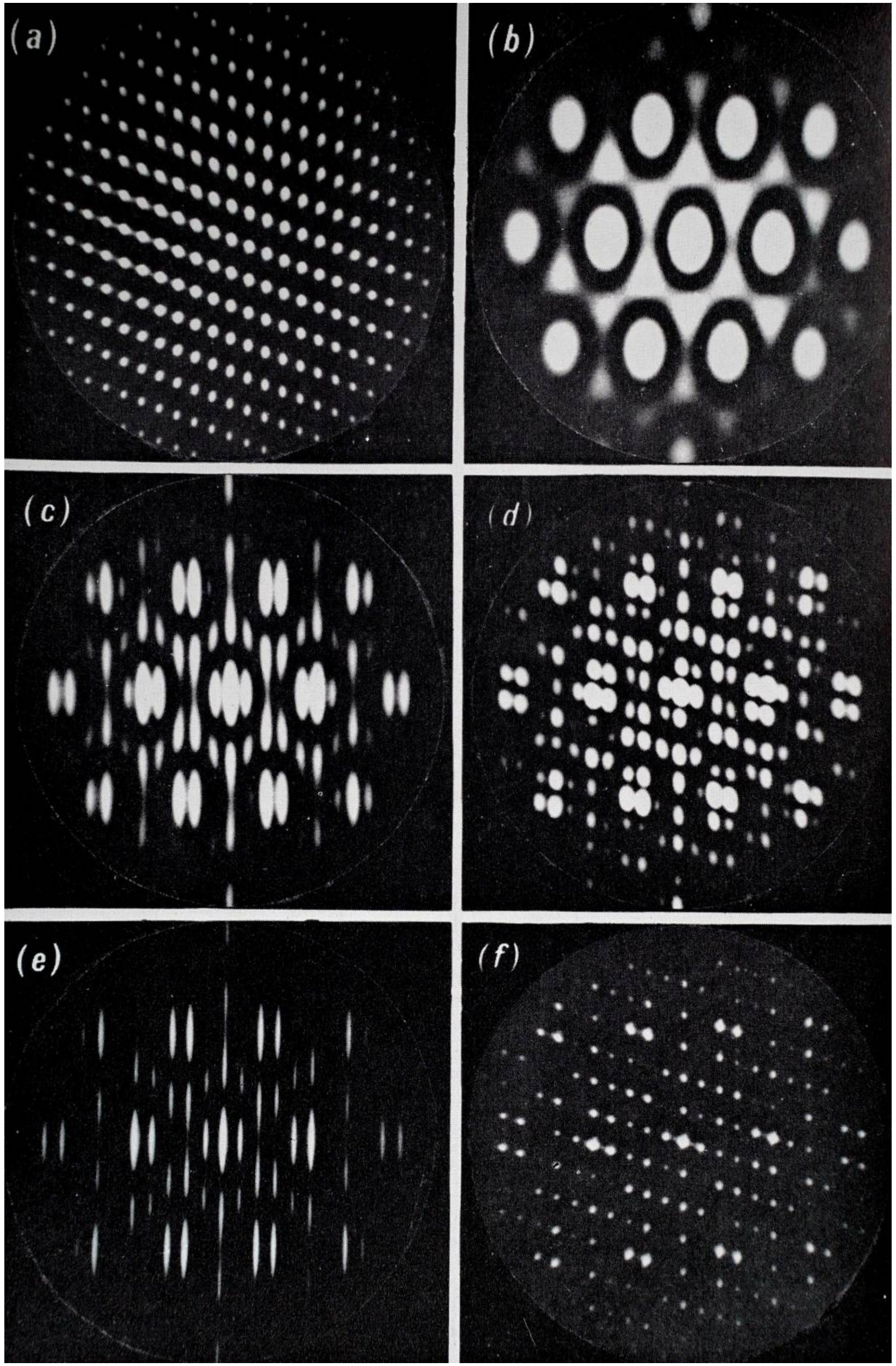


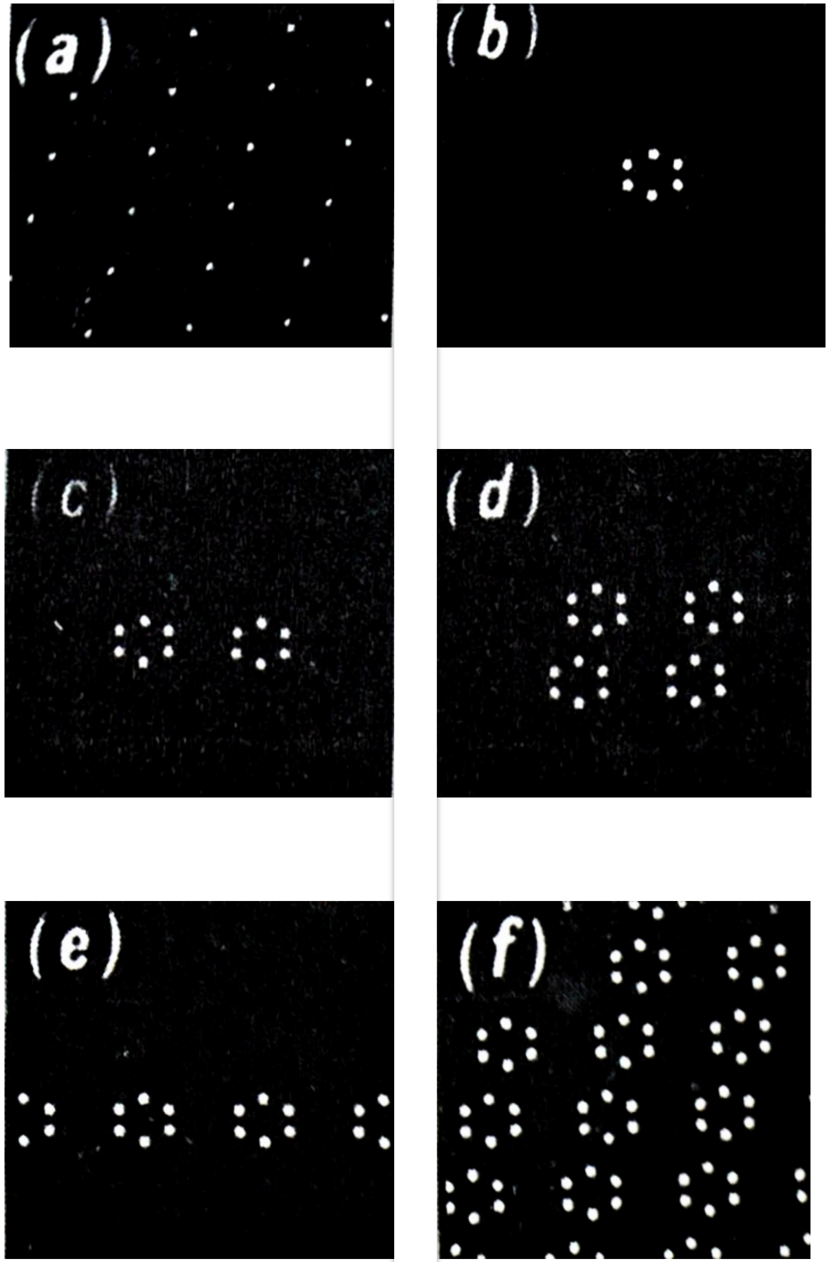
Reproduced, by permission, from
W. de Beauclair, "Verfahren und Geräte zur mehrdimensionalen Fouriersynthese",
Akademie-Verlag, Berlin 1949.)



from Taylor & Lipson , 1964
Optical Transforms

from Taylor & Lipson (1964) “Optical Transforms”





Fourier transform of a helix – from Holmes & Blow, 1965

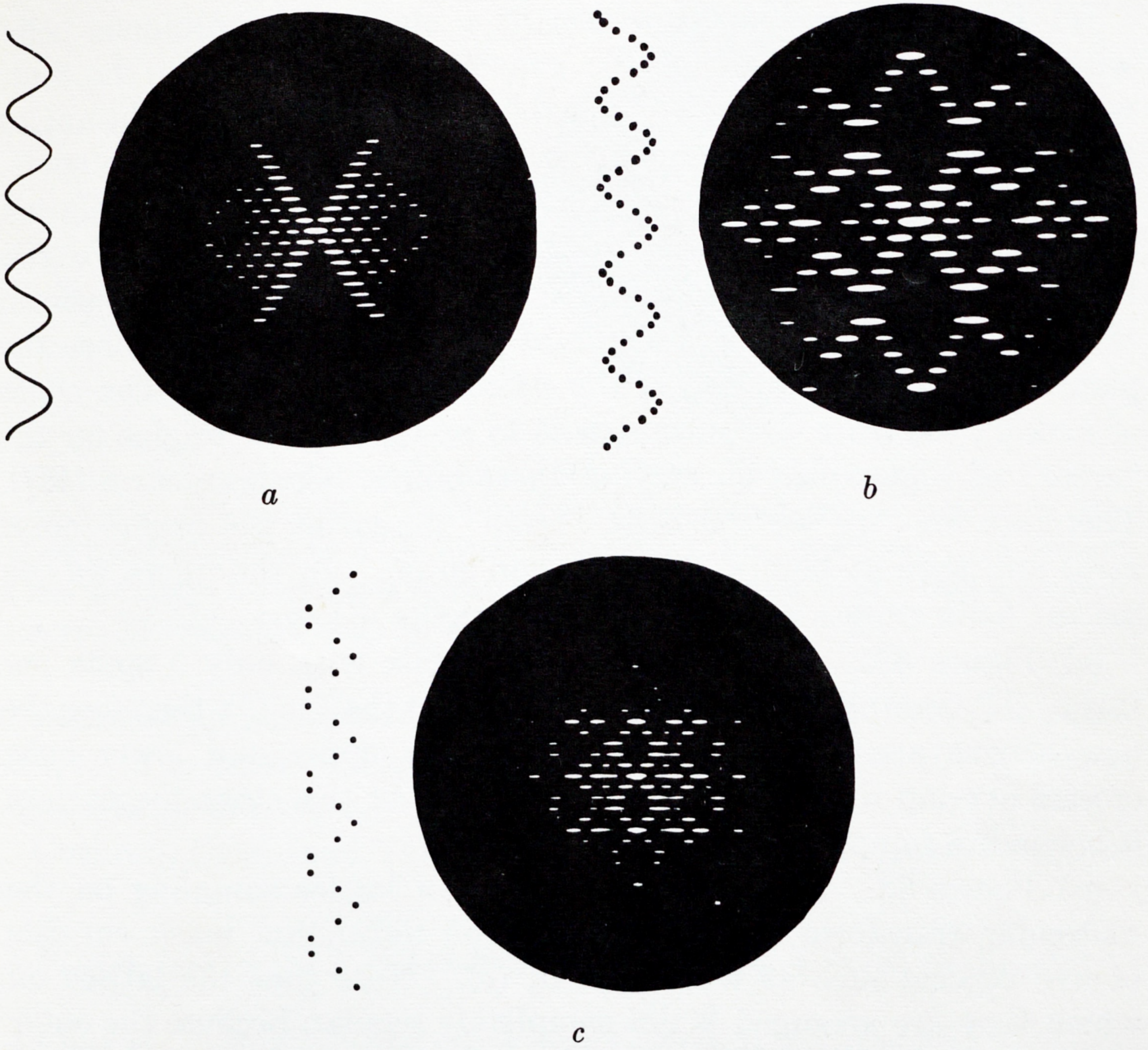
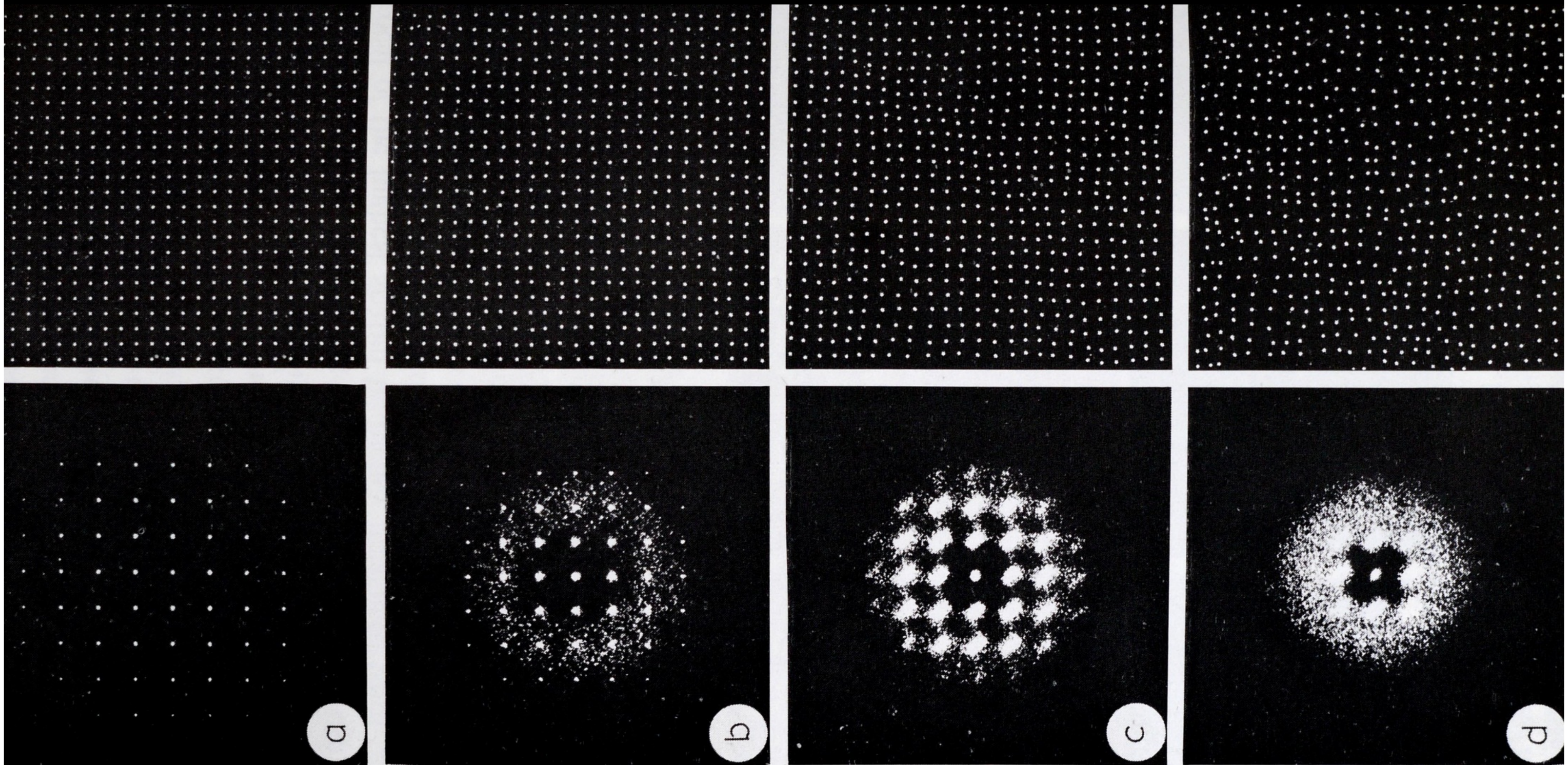


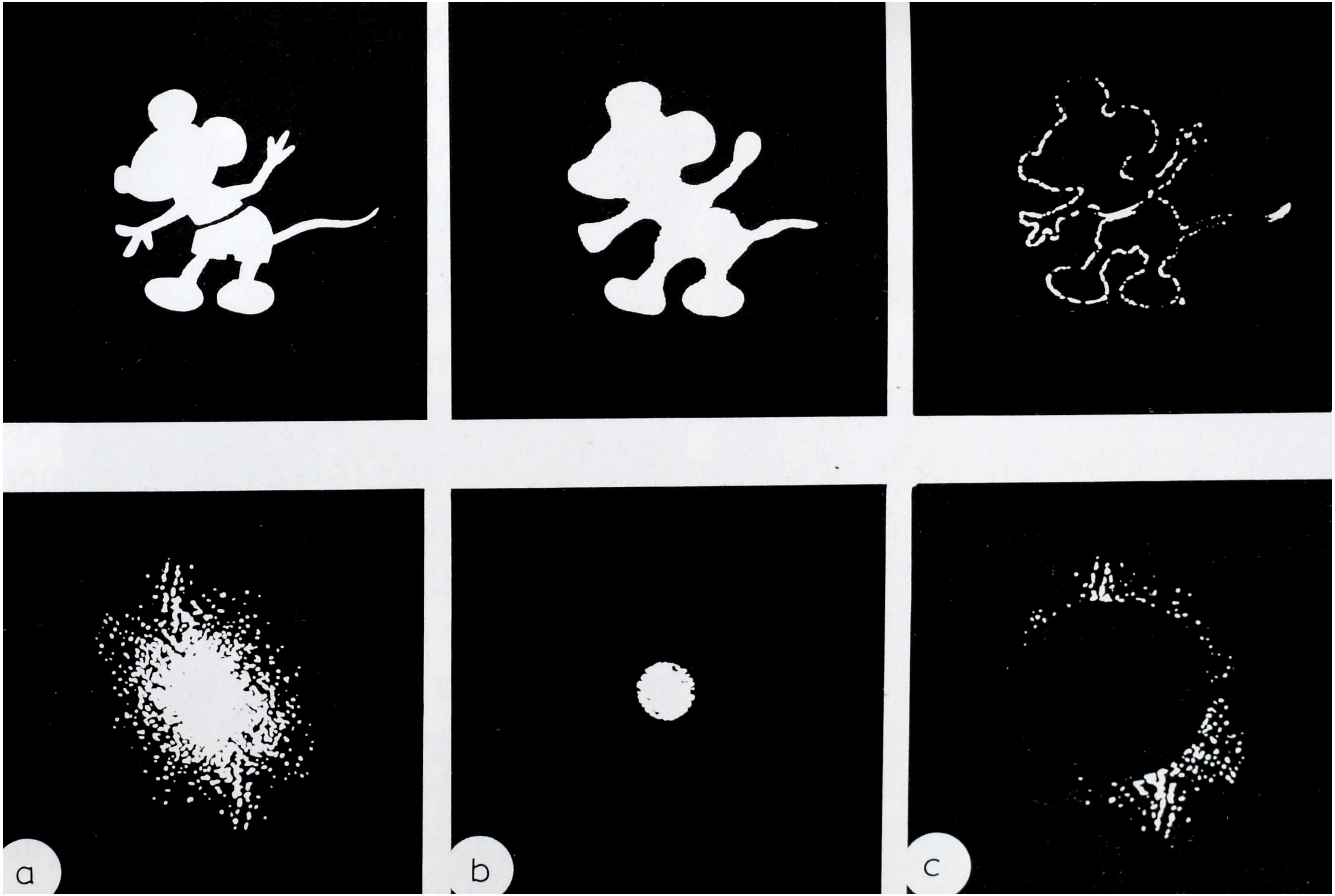
Fig. 32. (*a*) Optical transform of a continuous helix. (*b*) Optical transform of a helix with 10 points per turn. (*c*) Optical transform of a helix with 5 points per turn.

from Misell, 1978: effect of lattice disorder on the diffraction pattern

reproduced from Harburn et al, Atlas of Optical Transforms, 1975



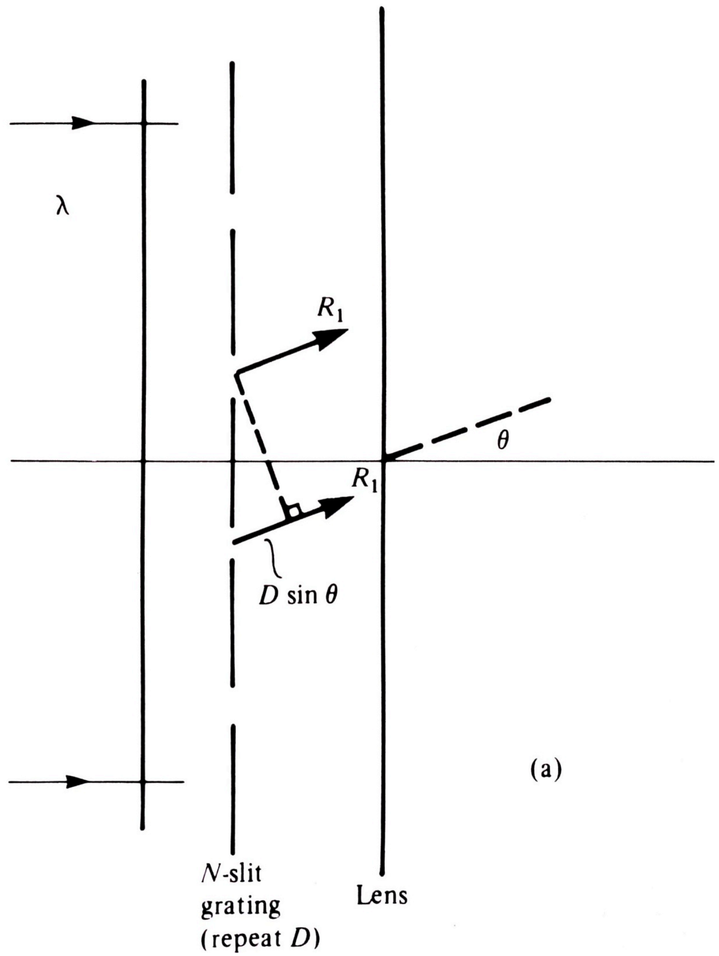
from Misell, 1978: Optical transforms: spatial filtering of ‘Mickey Mouse’
reproduced from Harburn et al, Atlas of Optical Transforms, 1975



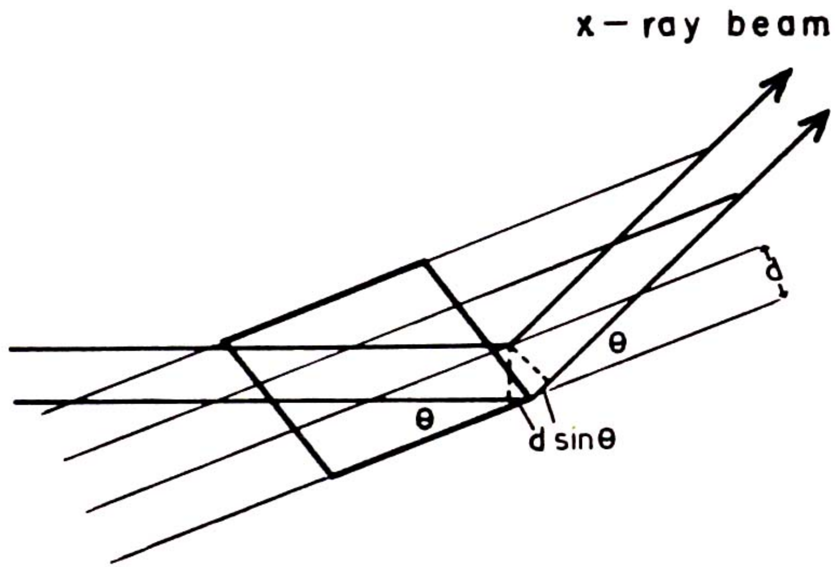
Optics
Diffraction from a grating   Dsinθ = nλ

Crystallography in 3D
Bragg's law   2dsinθ = nλ

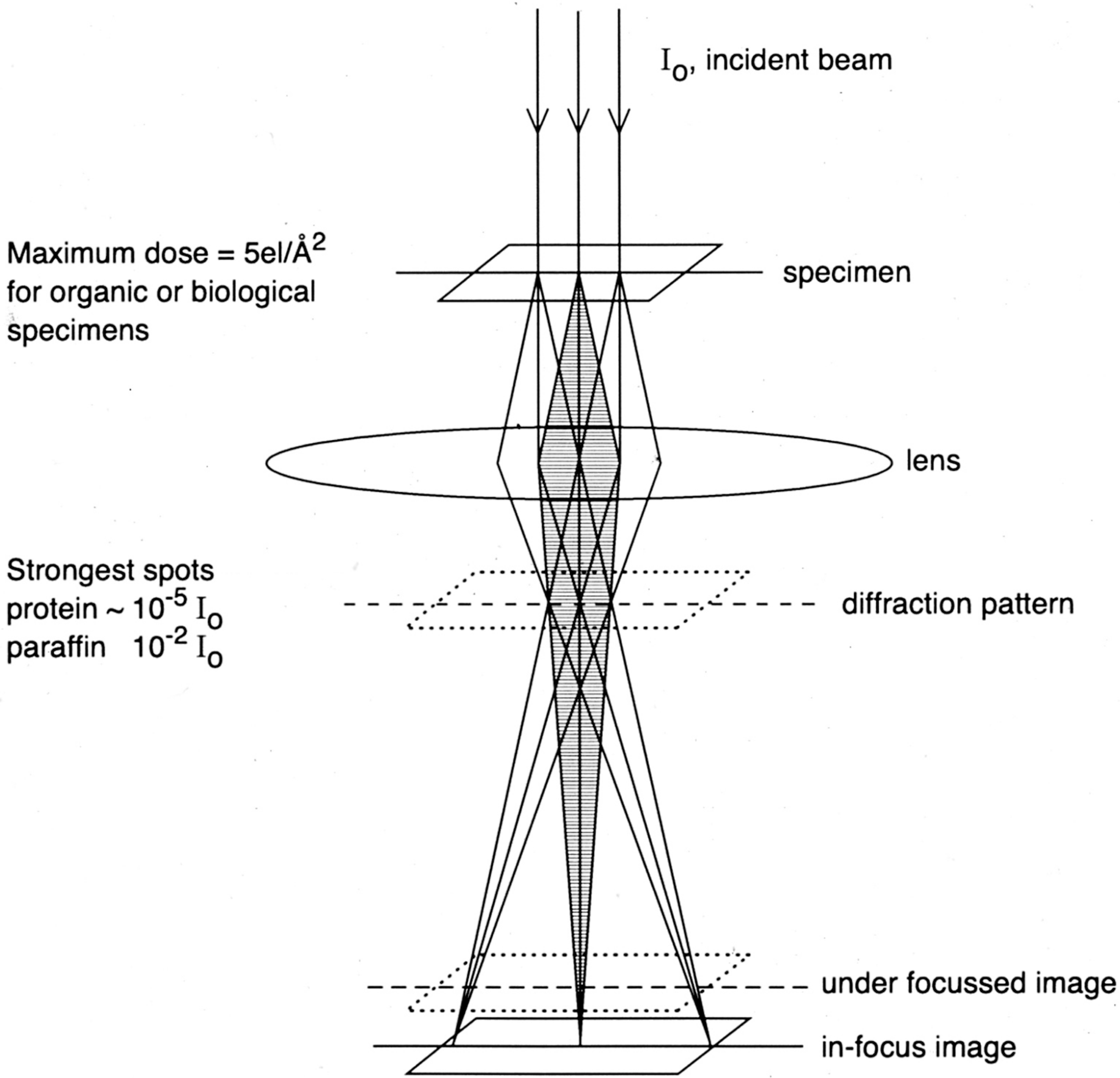
Note different definition of θ crystal tilt angle is half the diffraction angle



from E.G.Steward: "Fourier Optics an introduction" (1987, 2004)



from Holmes & Blow: "The use of X-ray diffraction in the study of protein and nucleic acid structure" (1965)



I_o, incident beam
Maximum dose = 5el/Å^2 for organic or biological specimens
specimen
lens
Strongest spots
protein ~ 10^{-5} I_o
paraffin 10^{-2} I_o
diffraction pattern
under focussed image
in-focus image

from Misell, 1978: "Image analysis, enhancement and interpretation"

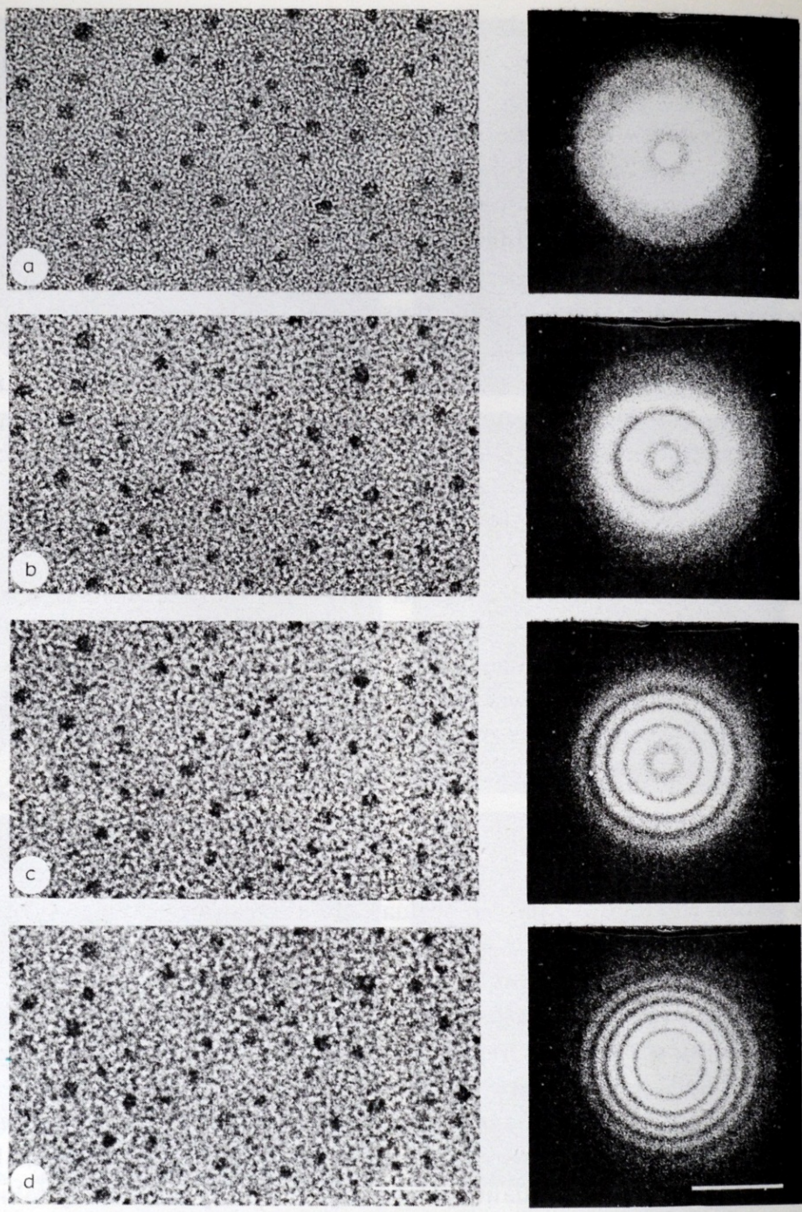
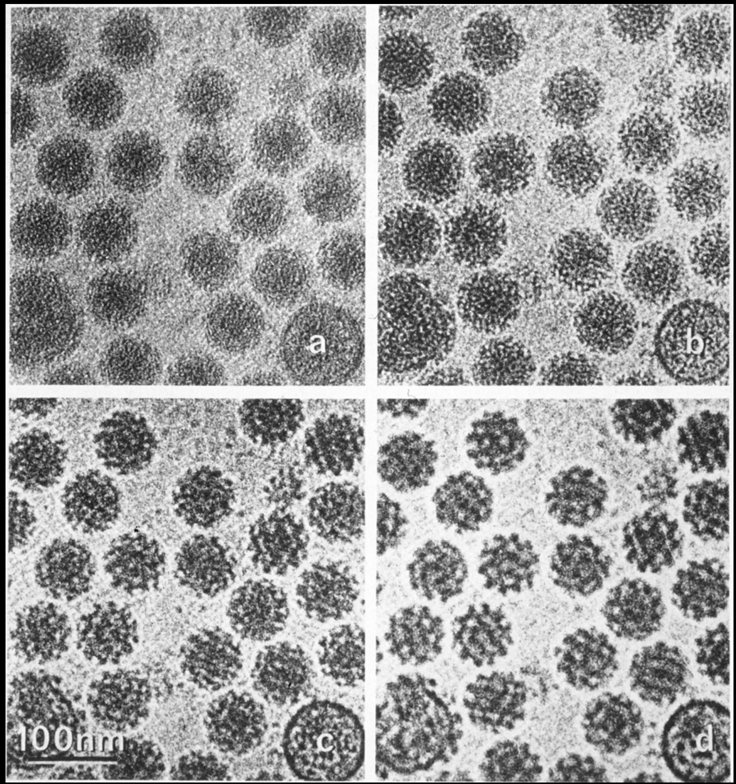
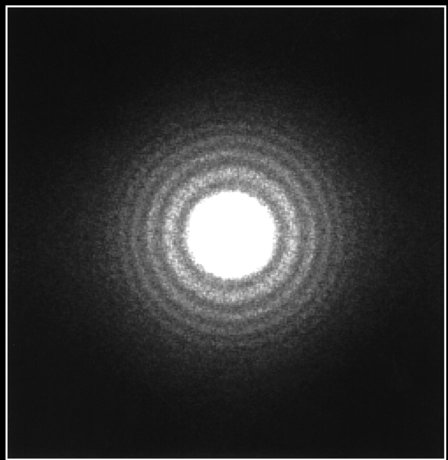
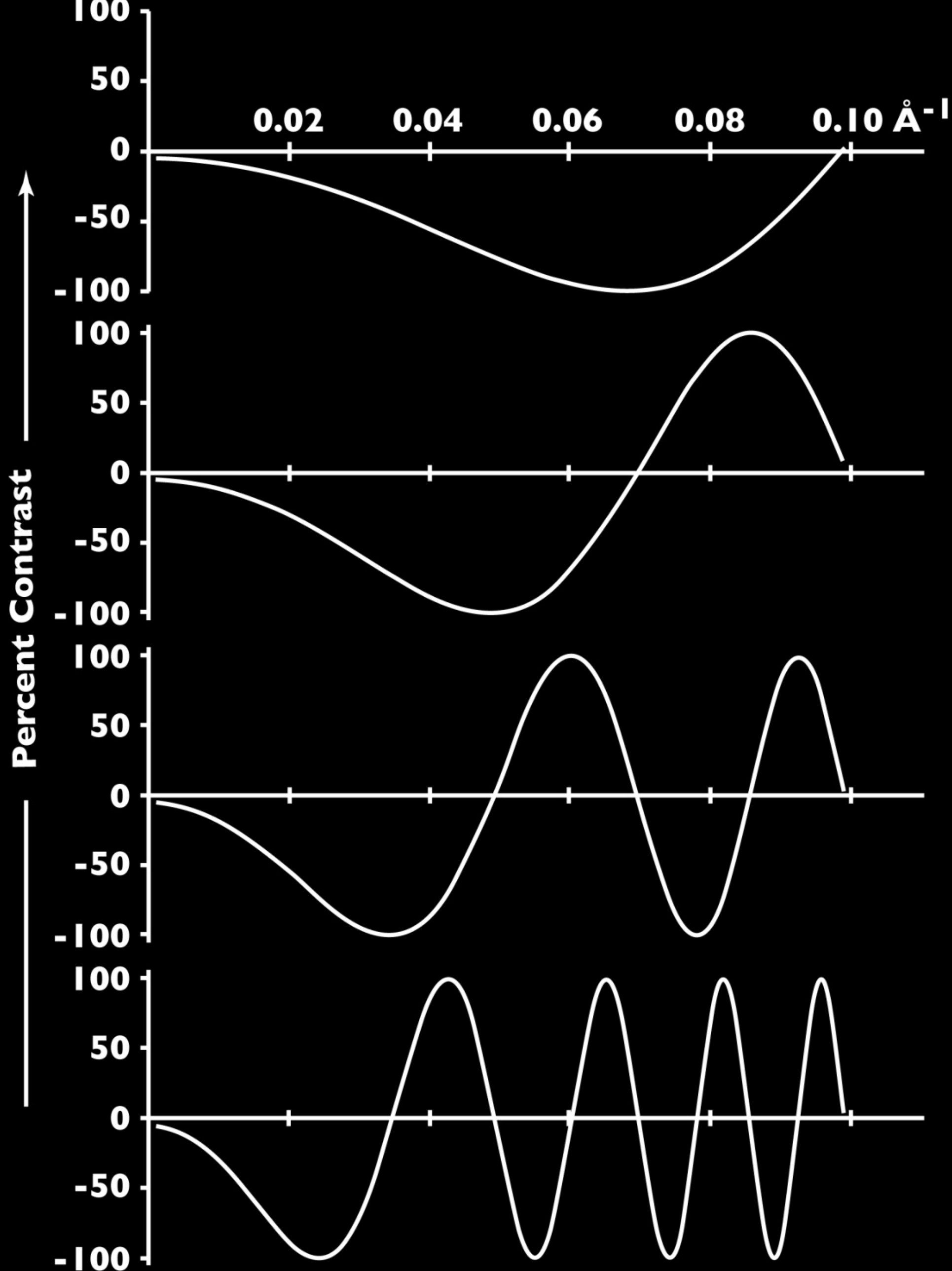


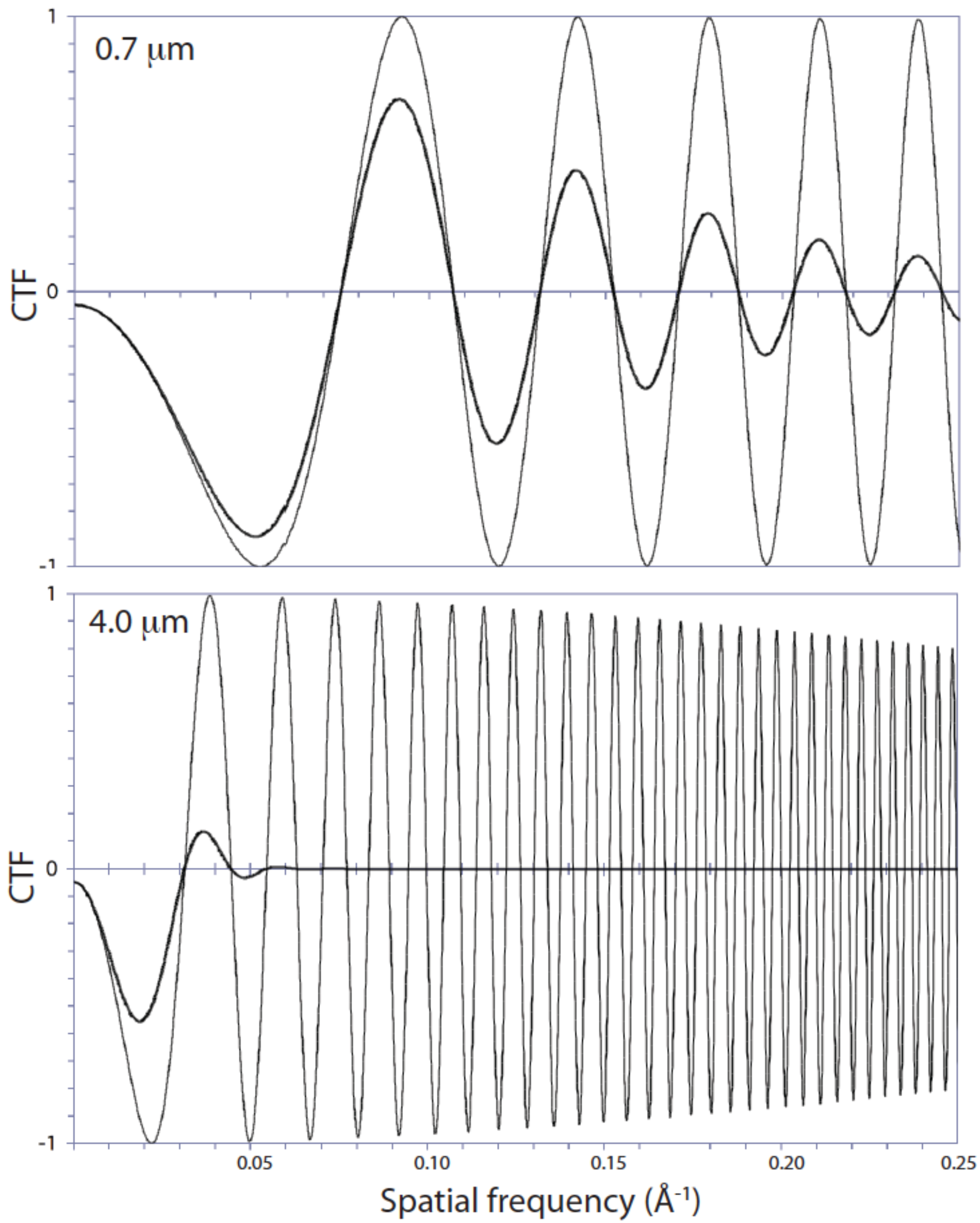
Fig. 3.8. Focus series of a thin carbon film, plus gold particles to assist in focusing. The optical diffractograms are alongside each image: (a) optimum defocus, (b) 150 nm underfocus, (c) 210 nm underfocus, (d) 250 nm underfocus. C_s and Δf are determined by measuring the radii (or diameters) of the dark circles corresponding to minima in the optical transform. $E_0 = 125$ keV, image bar = 10 nm, diffraction bar = 3.0 nm^{-1}. (P. Sieber, unpublished.)



Dubochet et al 1987







Typical CTFs: from Baker & Henderson International Tables Vol.F (2000,2011)

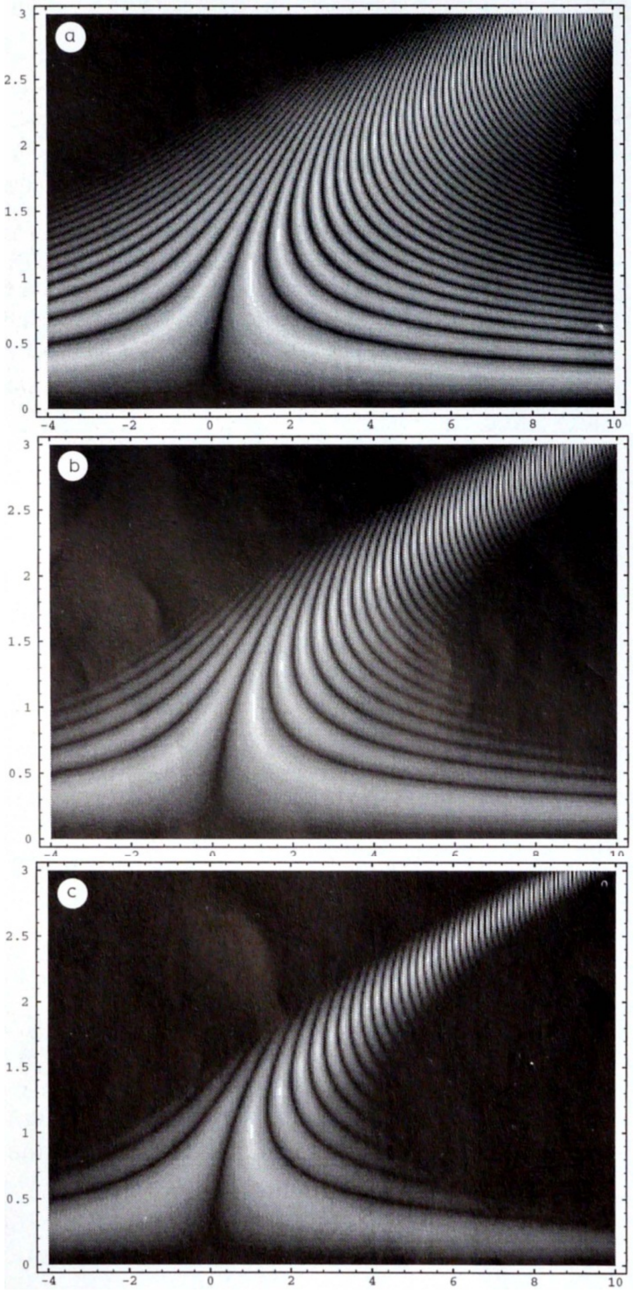
CTF for 200keV electrons
Cs = Cc = 2.0mm

Thick line: β = 0.3 mrad
ΔE =1.6eV, simulating a tungsten electron gun

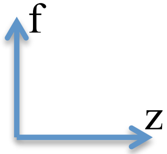
Thin line: β = 0.015 mrad
ΔE = 0.5eV, simulating a field emission gun (FEG)

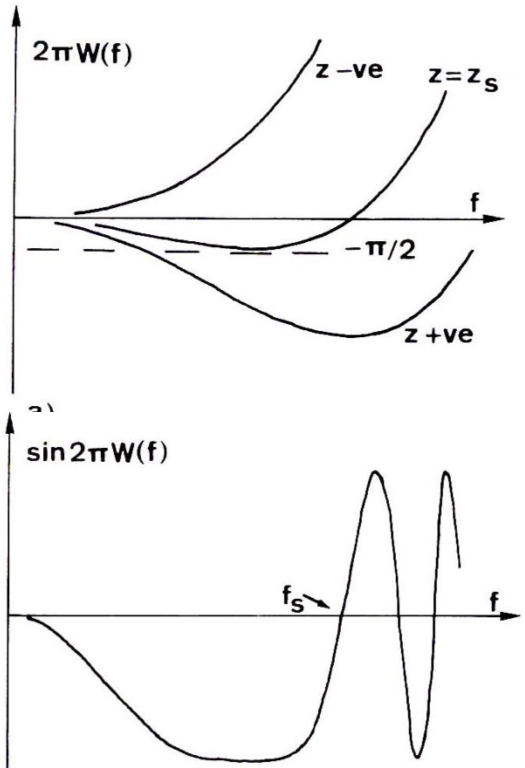
From Wade (1992) Ultramicroscopy, 46, 145-156.
“A brief look at imaging and contrast transfer

Spatial incoherence
0.03, 0.07, 1.4 mrad



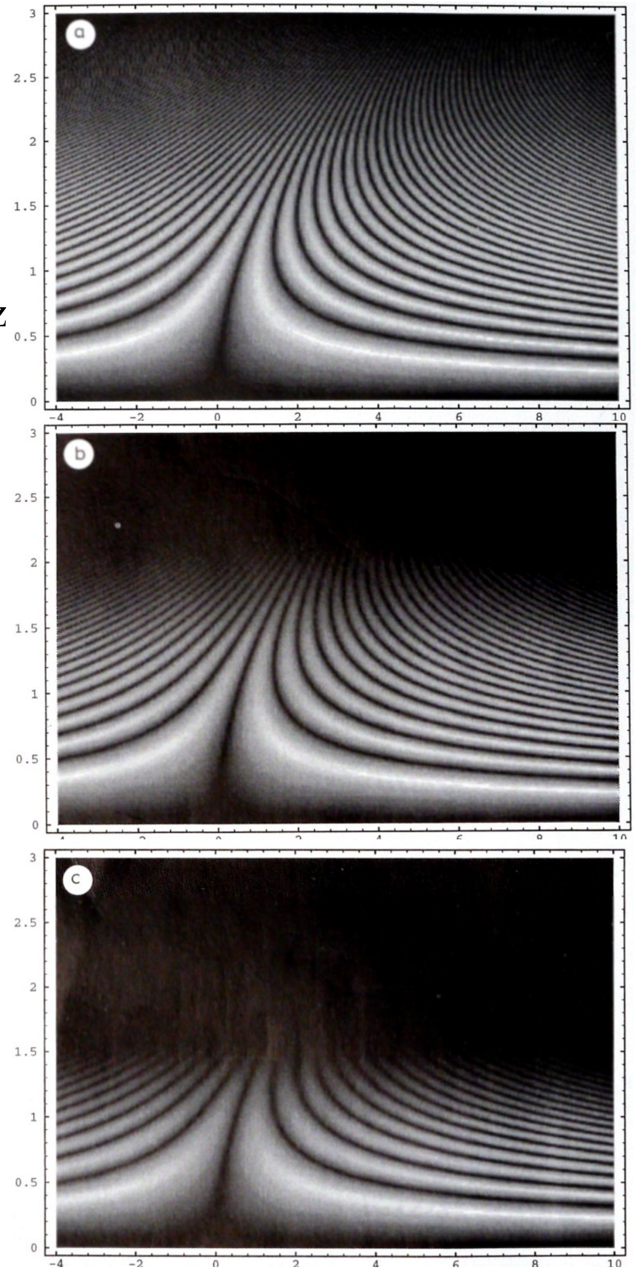
$$\chi = -2\pi/\lambda(\Delta F\theta^2/2 - C_s\theta^4/4)$$
$$\mathrm{CTF} = -\sin(\chi)$$

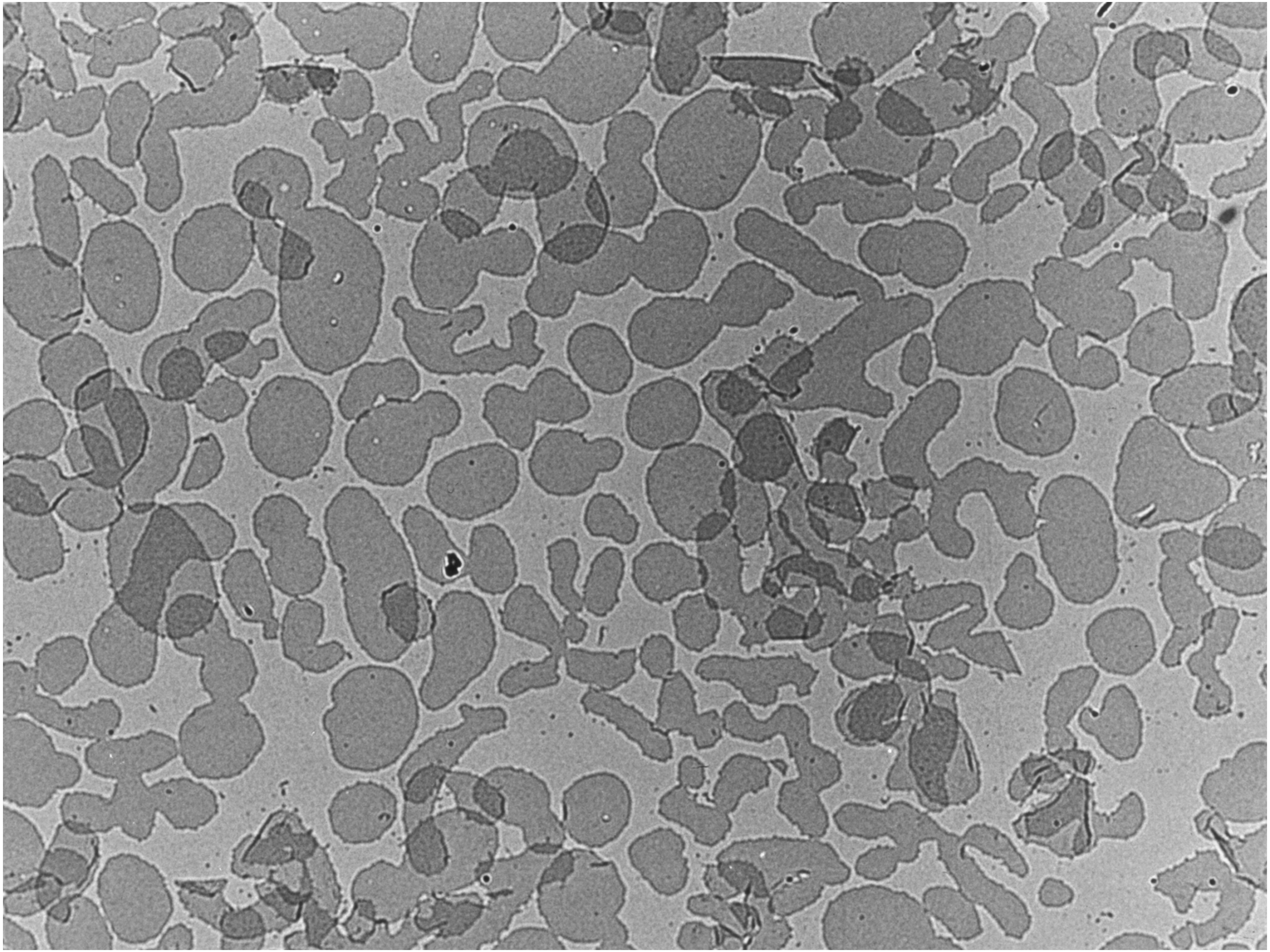


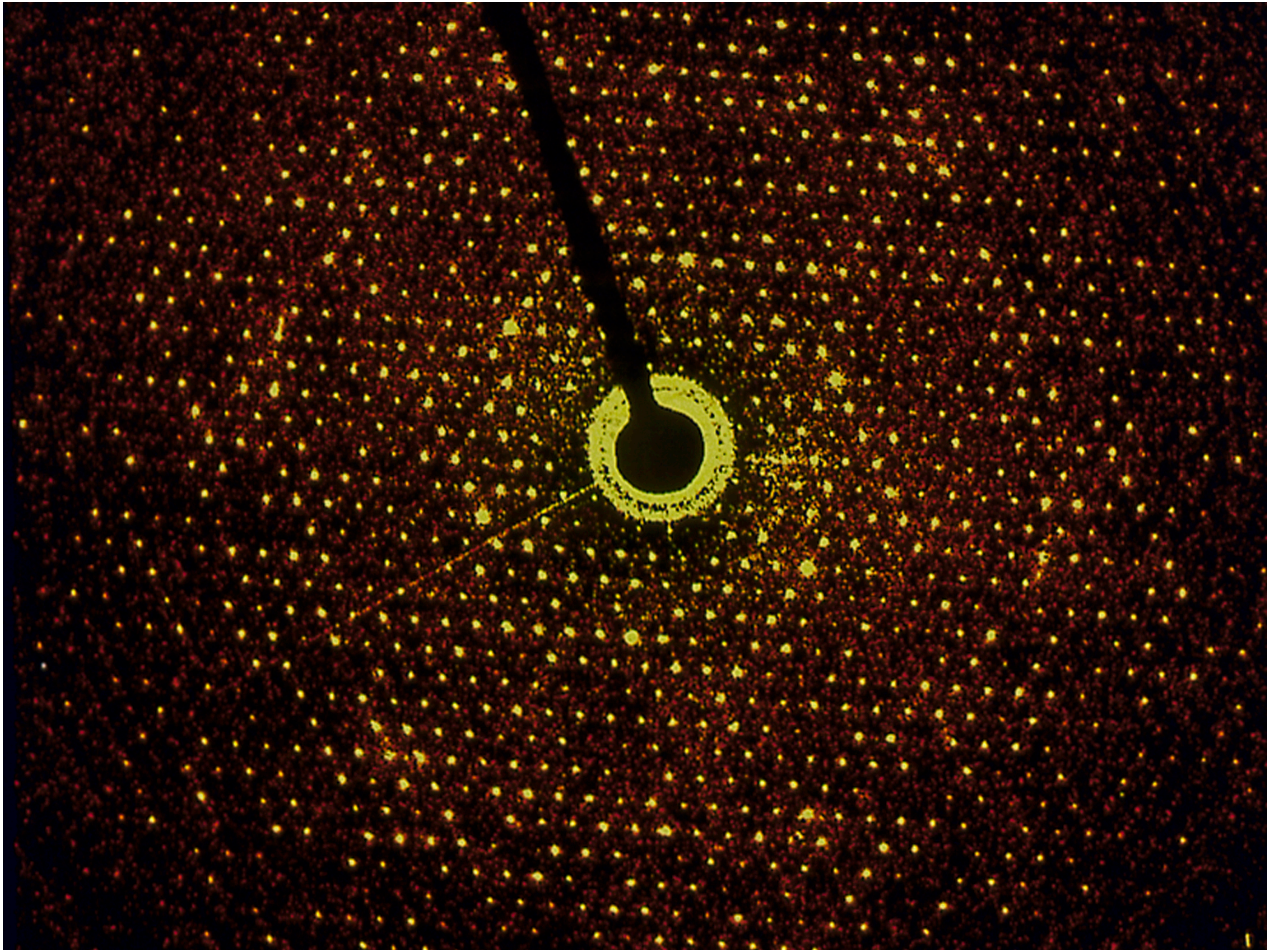


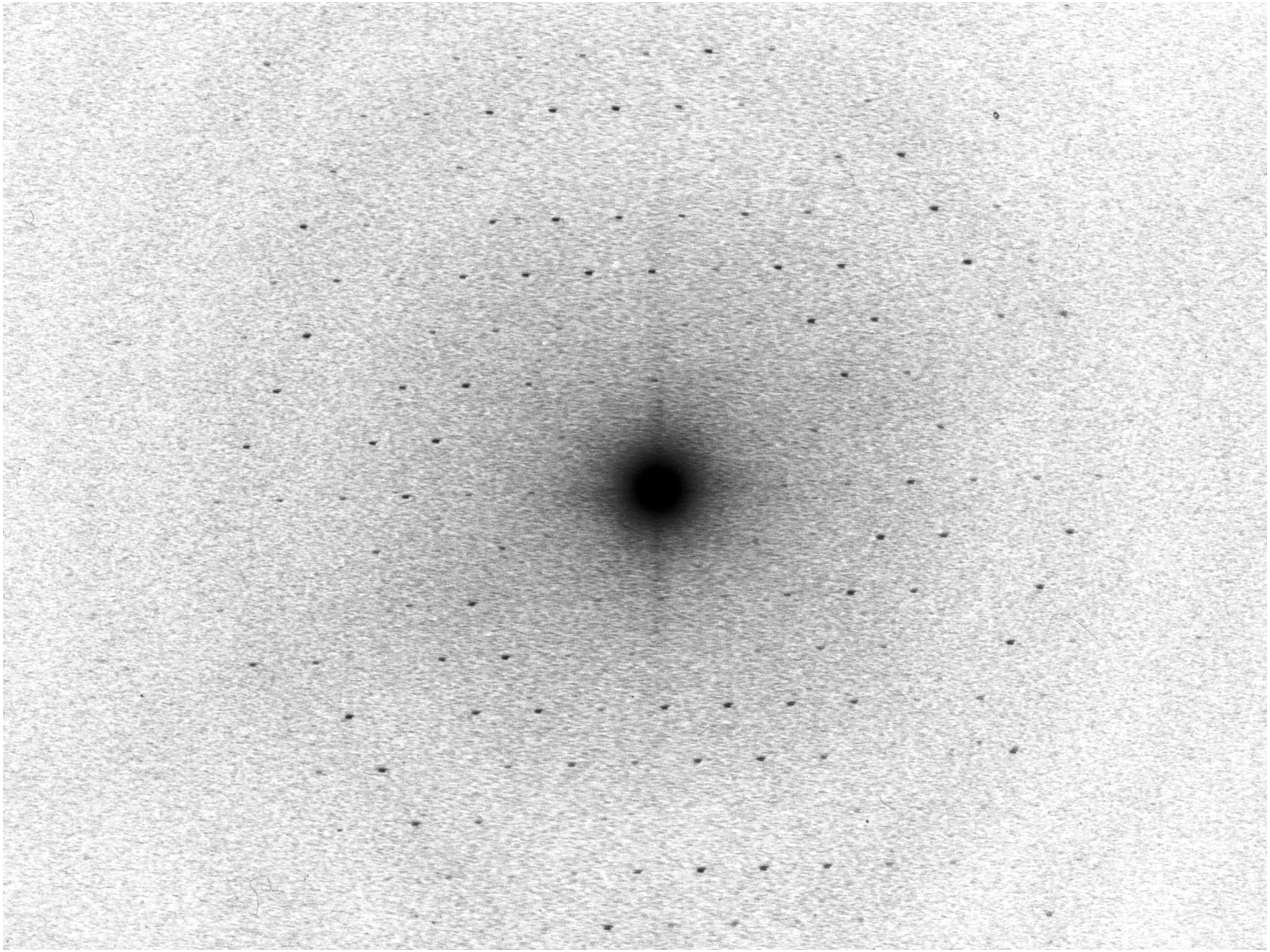
spatial frequency = f
defocus = z

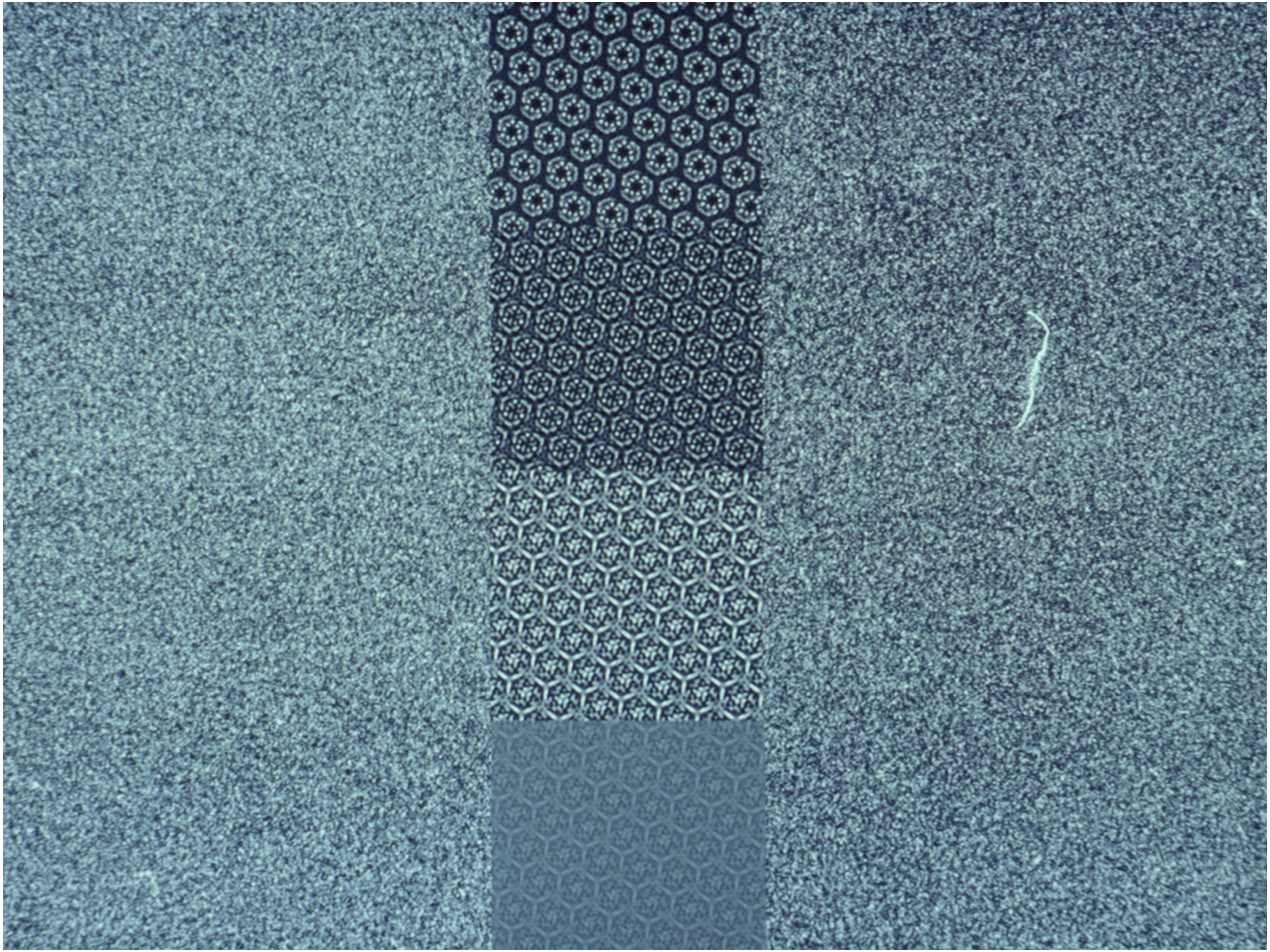
Temporal incoherence
ΔE = 0.6, 1.2, 2.4 eV
Cs = 1.4, kV = 100

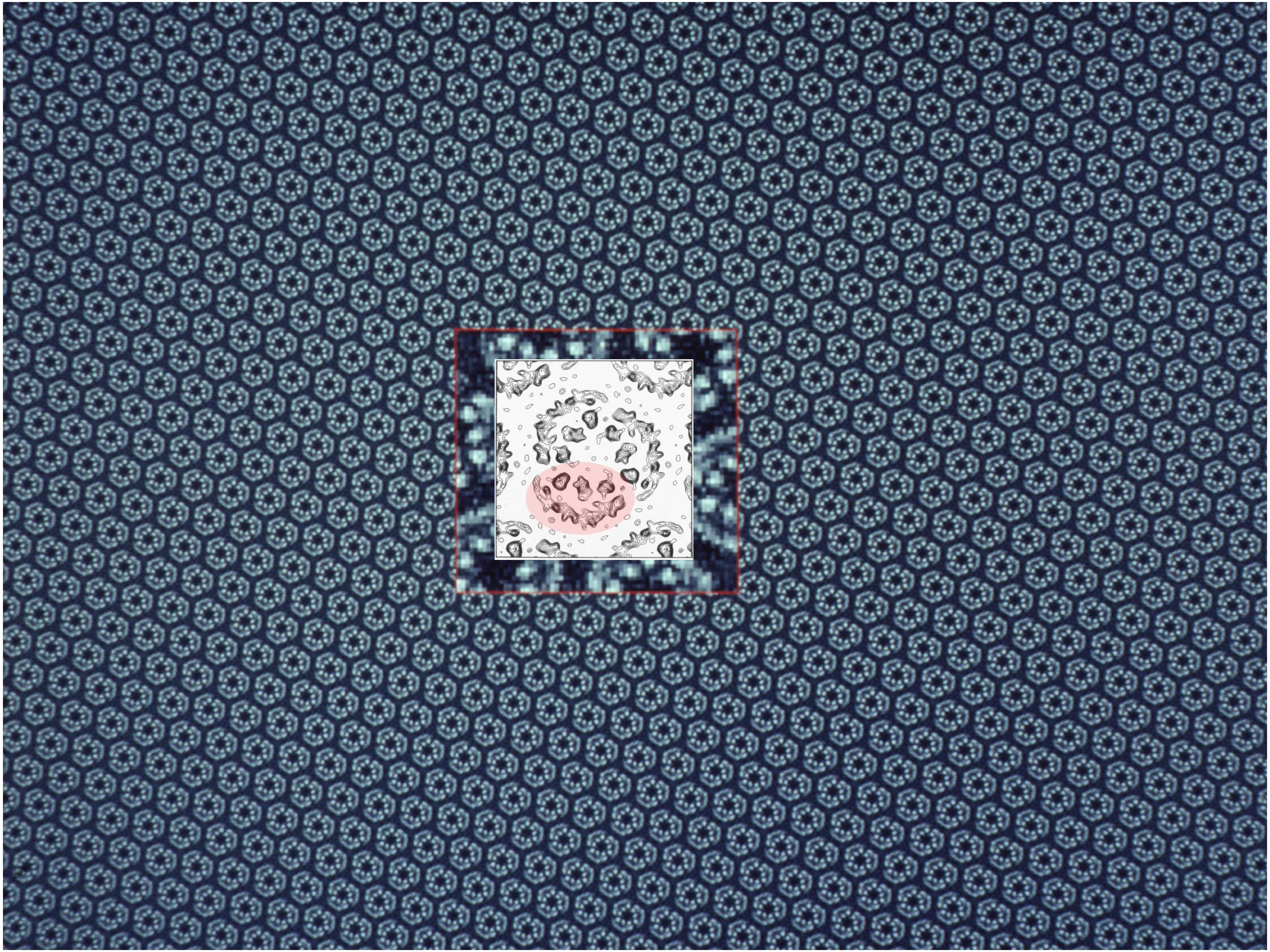


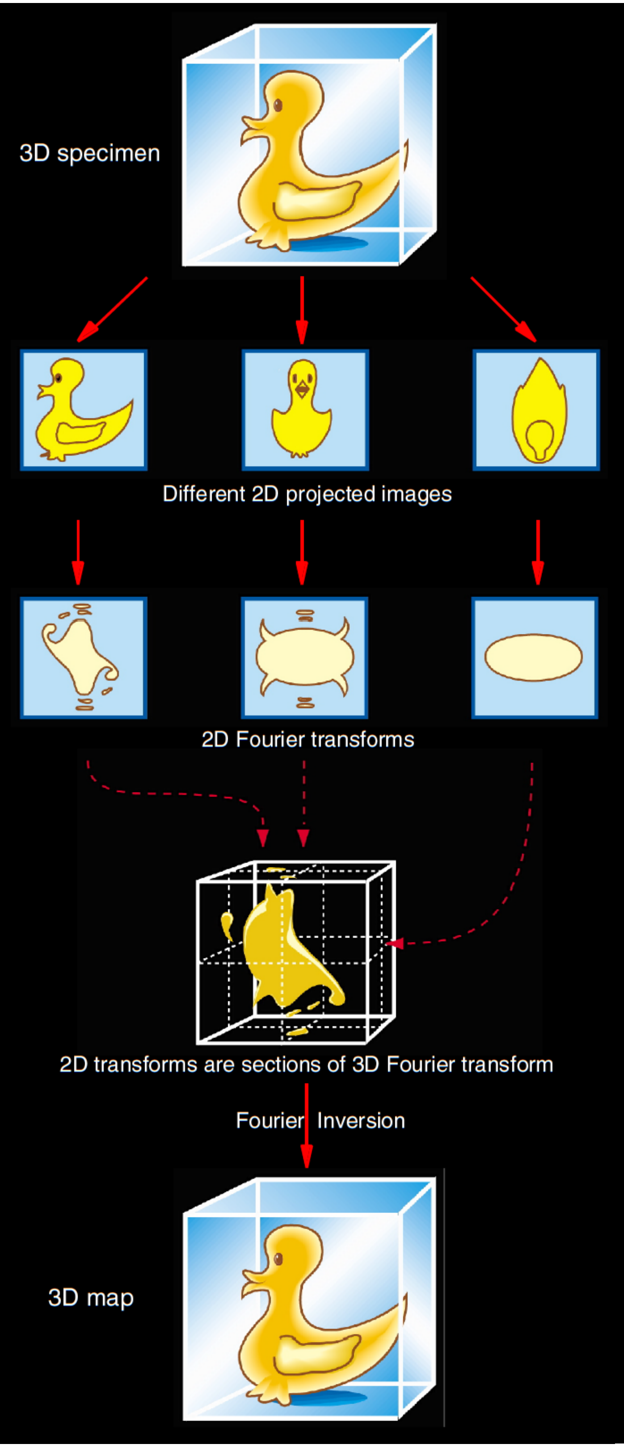


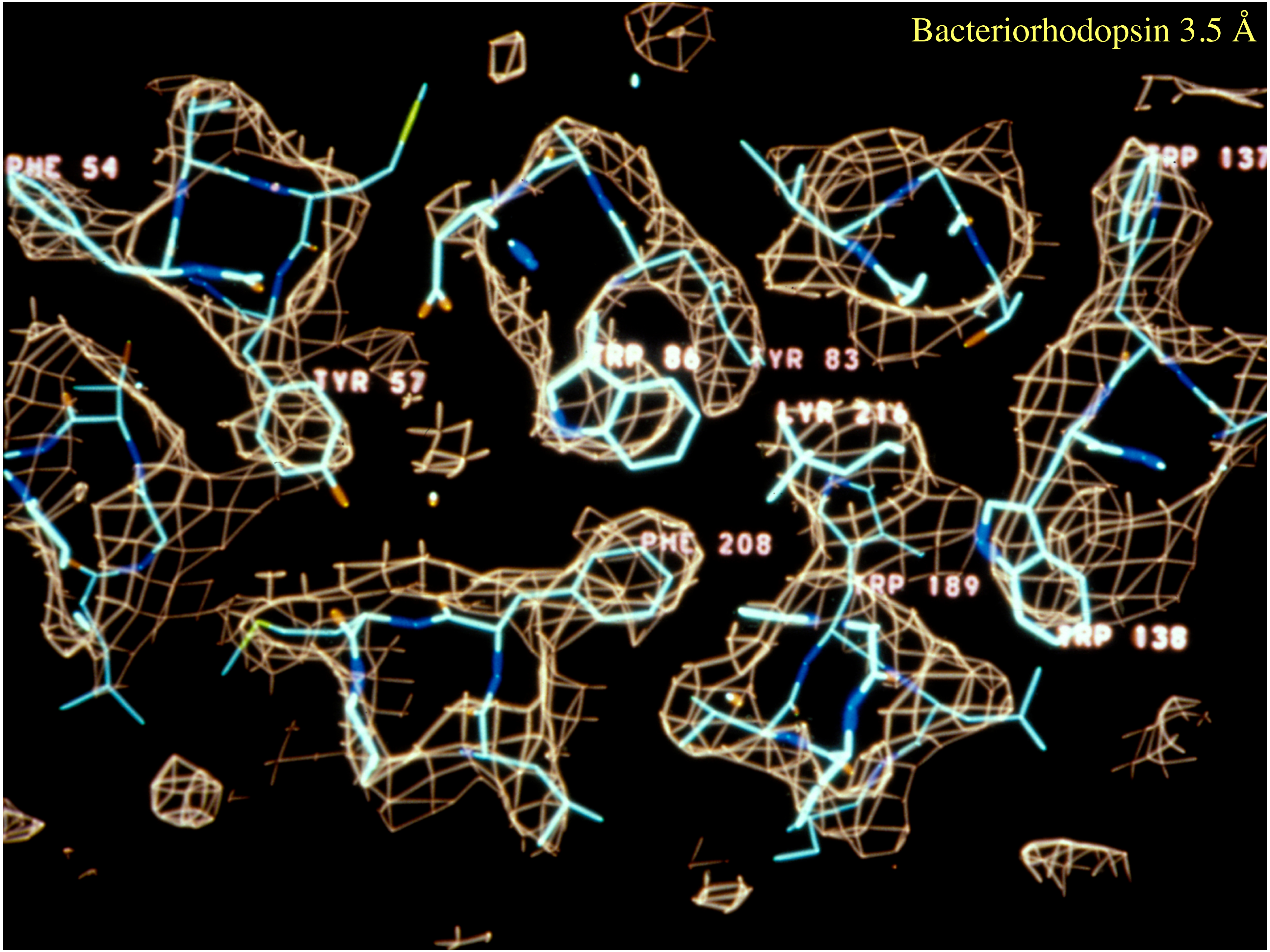




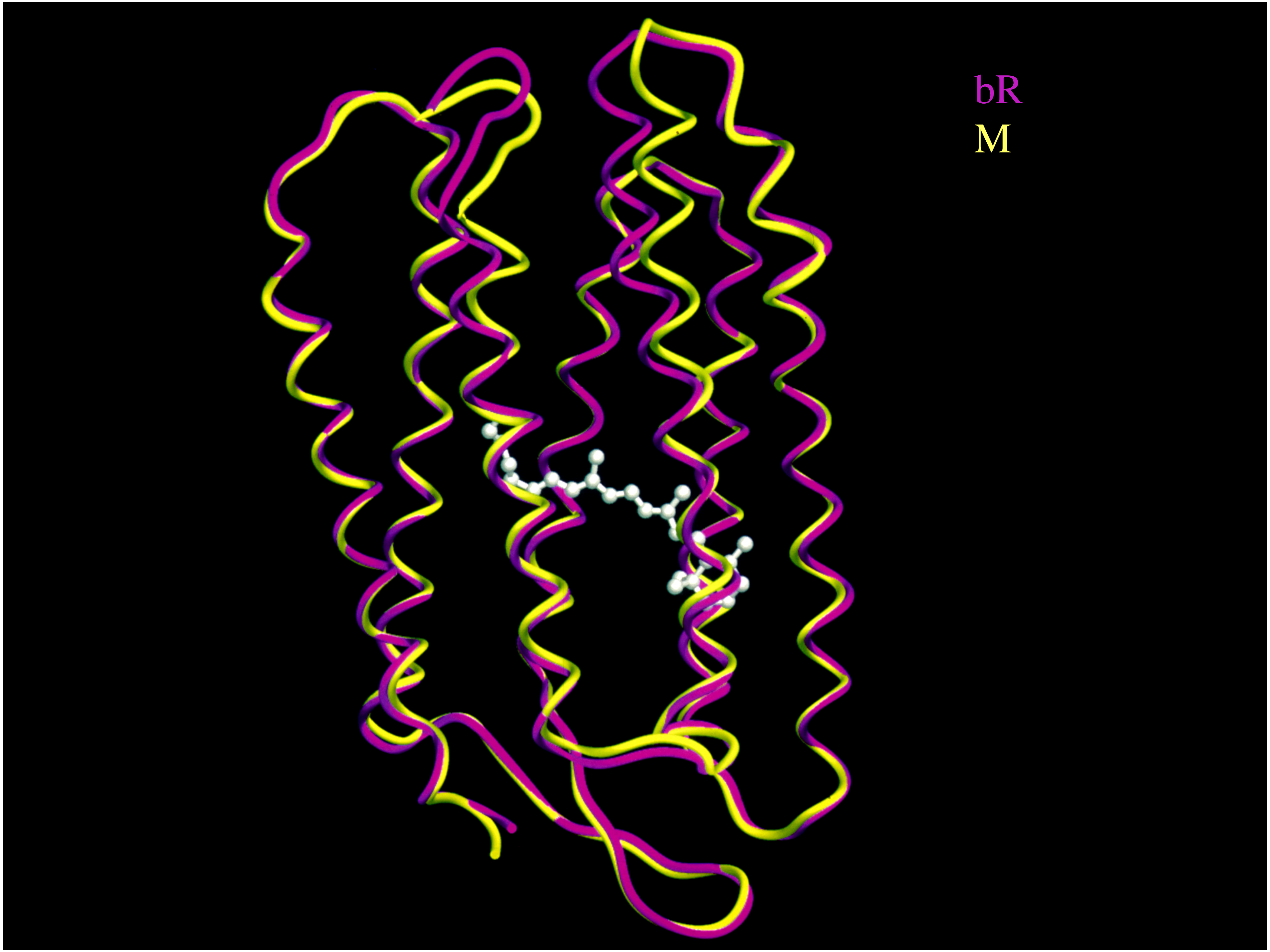




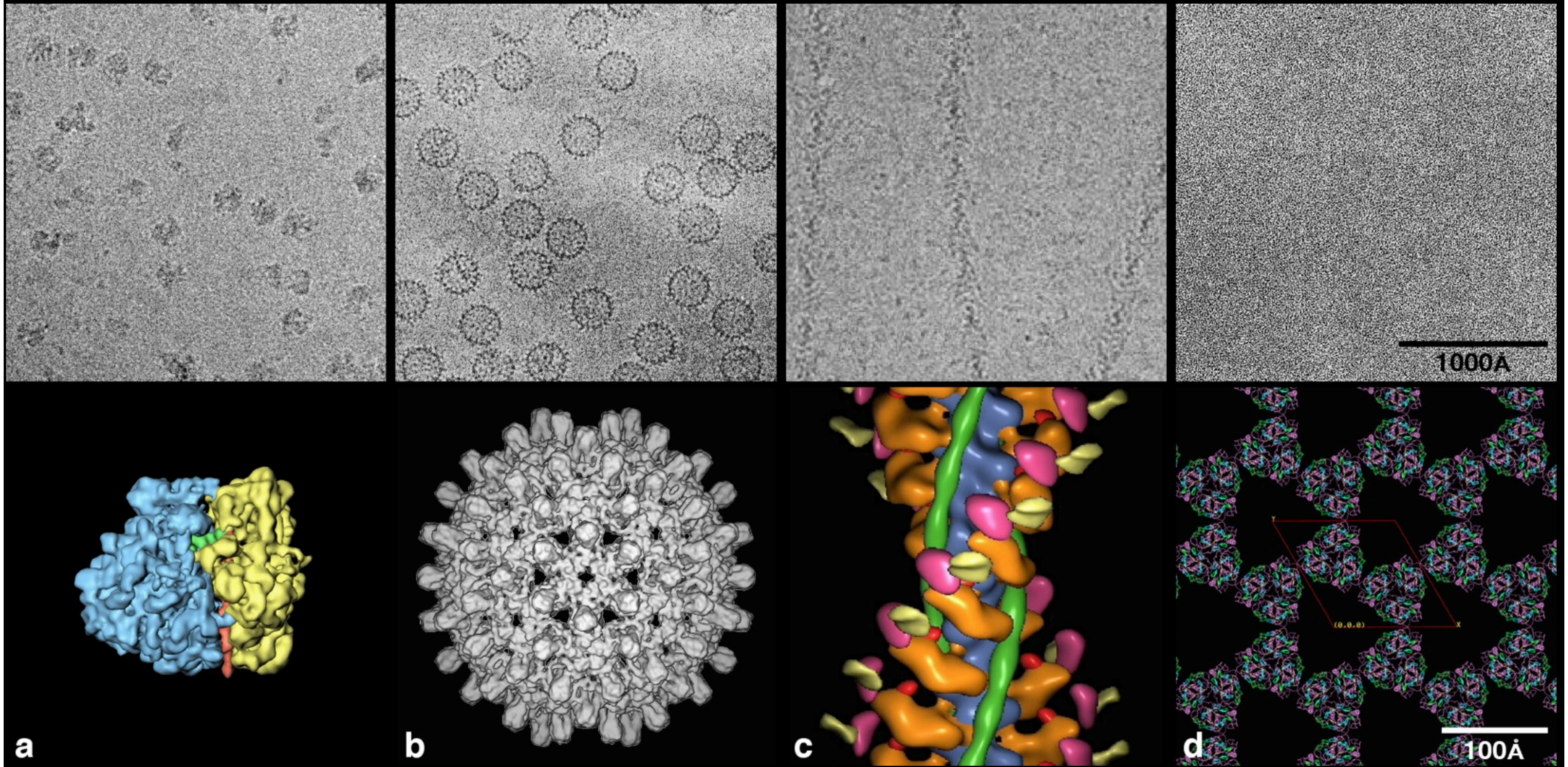

3D specimen
Different 2D projected images
2D Fourier transforms
2D transforms are sections of 3D Fourier transform
Fourier Inversion
3D map



Bacteriorhodopsin 3.5 Å
PHE 54
TYR 57
TRP 86
TYR 83
TRP 137
LYS 216
PHE 208
TRP 189
TRP 138


bR
M

From Baker & Henderson (2001) Int.Tab.Cryst.Vol.F
and recently updated (2011, in press)



Number of particles in projection/μm² in 800 Å thick ice film (separation)

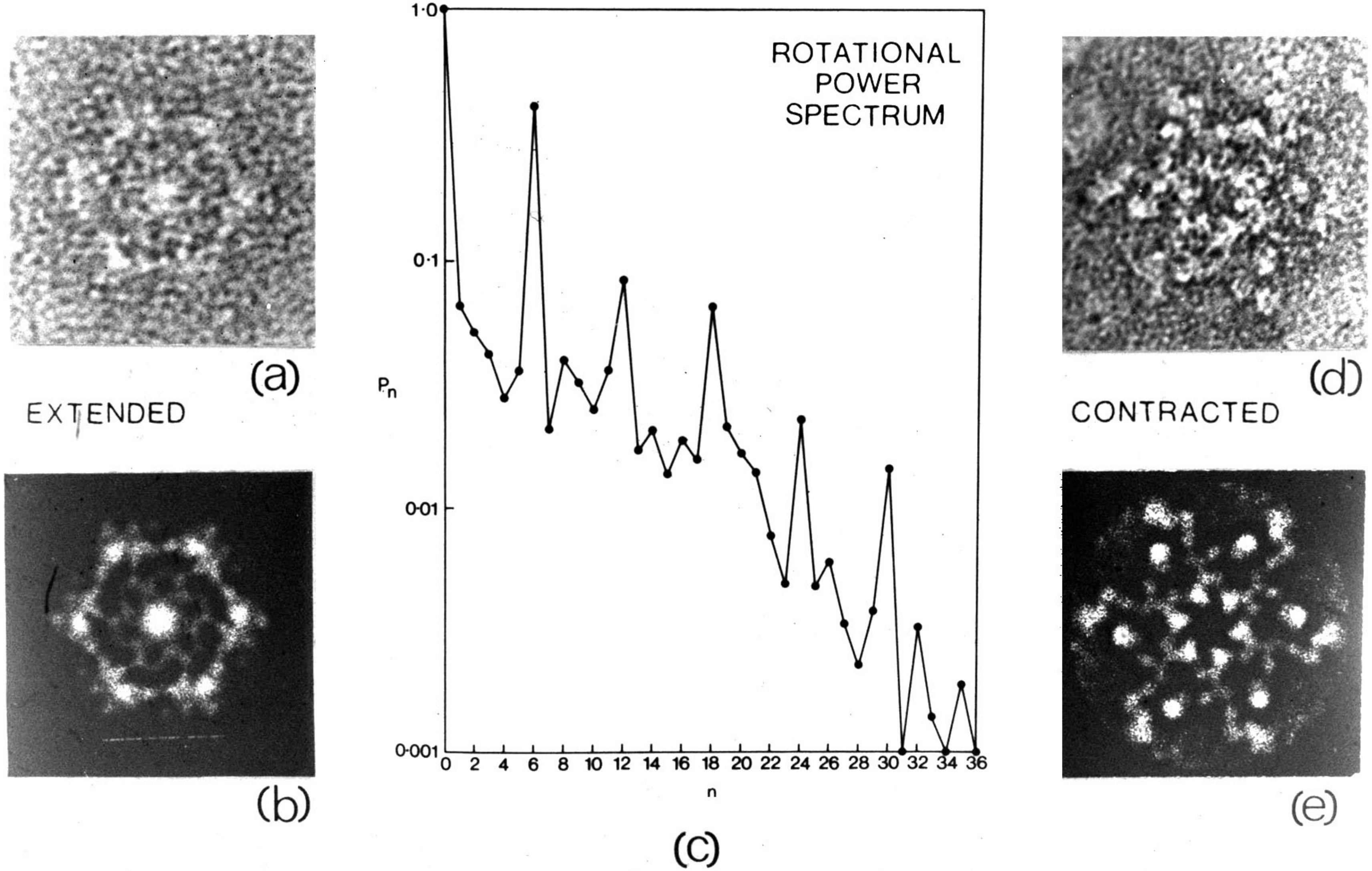
Concentration

M.W.	10mg/ml	2mg/ml	0.5mg/ml	0.1mg/ml	20μg/ml
10 kD	48000 (45Å)	10000 (100Å)	2500 (200Å)	500 (450 Å)	100 (1000 Å)
50 kD	10000 (100Å)	2000 (220Å)	500 (400Å)	100 (1000Å)	20 (0.2μm)
250kD	2000 (220Å)	400 (500 Å)	100 (1000 Å)	20 (0.2μm)	4 (0.5μm)
1 MD	500 (400Å)	100 (1000Å)	25 (0.2μm)	5 (0.4μm)	1 (1μm)
5 MD	100 (1000Å)	20 (0.2μm)	5 (0.4μm)	1 (1μm)	0.2 (2.2μm)
25 MD	20 (0.2μm)	4 (0.5μm)	1 (1μm)	0.2 (2.2μm)	0.04 (5μm)

Table of expected number of particles in cryoEM.

"Given the concentration of the molecules of interest, how many particles per square micron should you see in the image if the frozen specimen has the same concentration of molecules that you expect from the sample concentration?". The number/μm² as well as the expected particle separation is given. If you make a grid and find either many more or many less particles than you expect, then something fishy is going on. For example all the particles might be sticking to the carbon (if too few are seen in the holes) or the blotting operation might be concentrating the particles (if there are too many), but you can make up hundreds of explanations.

Rotational power spectral analysis: Crowther & Amos (1971), RFILTIM



Key individuals and key concepts

Joseph Fourier	Fourier analysis & synthesis
Ernst Abbe	Abbe theory of the microscope
Lawrence Bragg*	Bragg’s law for diffraction geometry
Max von Laue*	observation of X-ray diffraction from crystals
Fritz Zernike*	Zernike phase plate & phase contrast microscopy
F. Thon	Thon rings
Otto Schertzer	Schertzer focus
Ernst Ruska*	inventor of electron microscope

Fourier analysis & synthesis
Cross correlation, real and complex
Convolution

Fourier shell correlation (FSC)
Contrast Transfer Function (CTF)
Modulation Transfer Function (MTF)
Detective Quantum Efficiency (DQE)