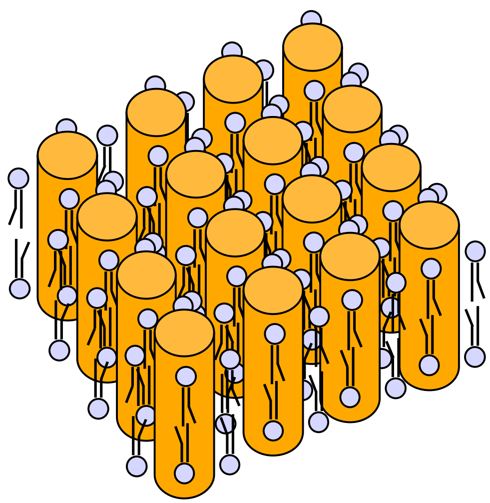
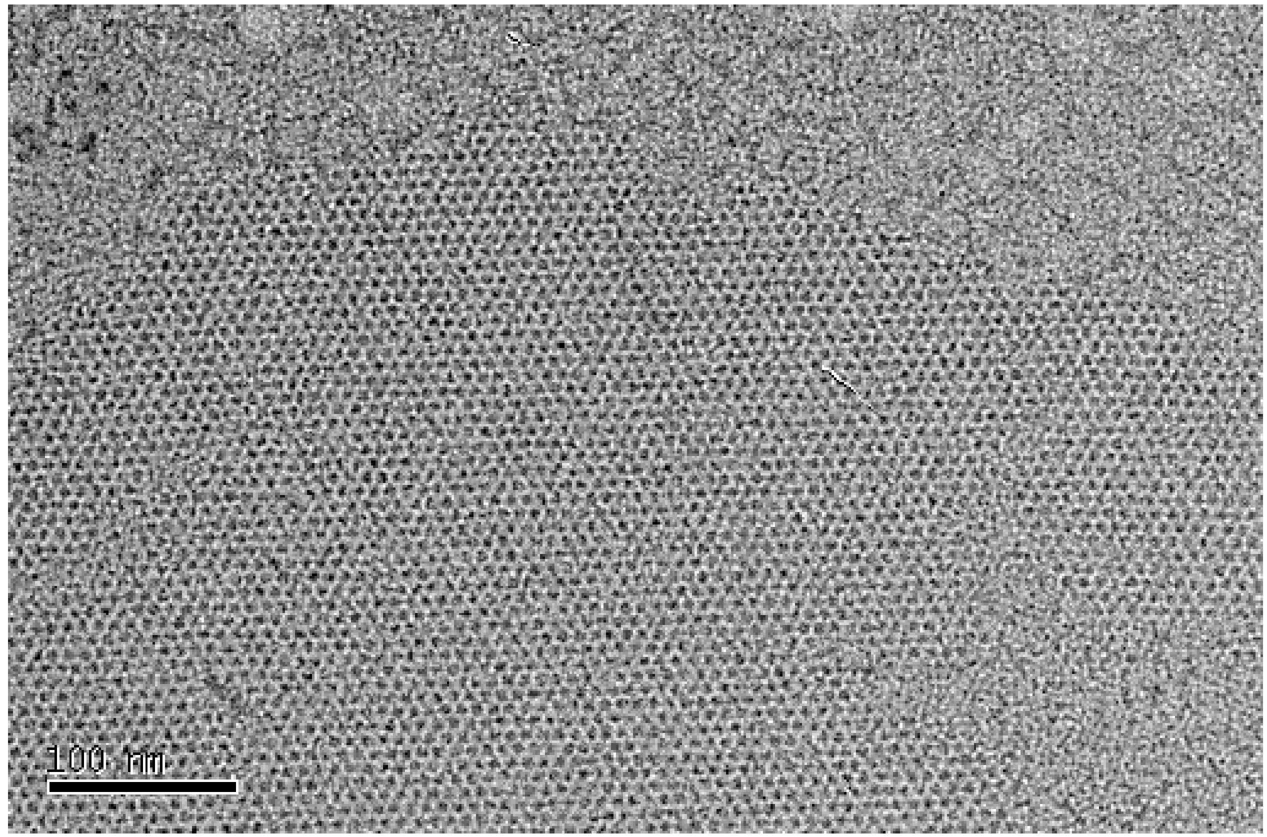
Electron Crystallography of Two-Dimensional Crystals

The Basics

V. Unger, 11/4/2005

Negative Stain Image of “2D-Crystal”

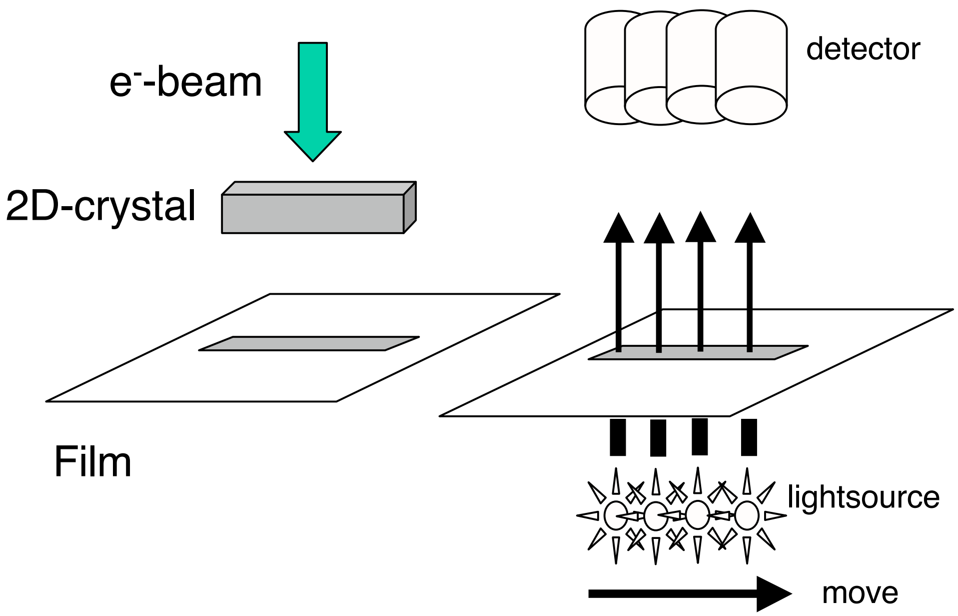


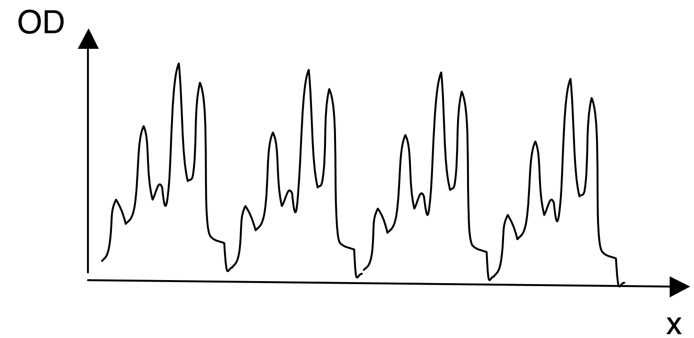


To grasp the basic ideas underlying electron crystallographic image processing, all we need to ask is: how can we describe a periodic array without using the actual picture itself?

record a projection of the 2D-crystal

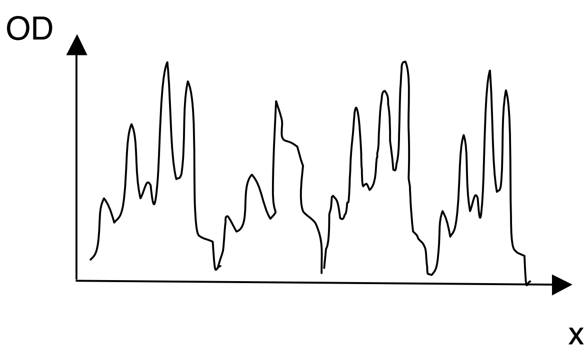
digitize image



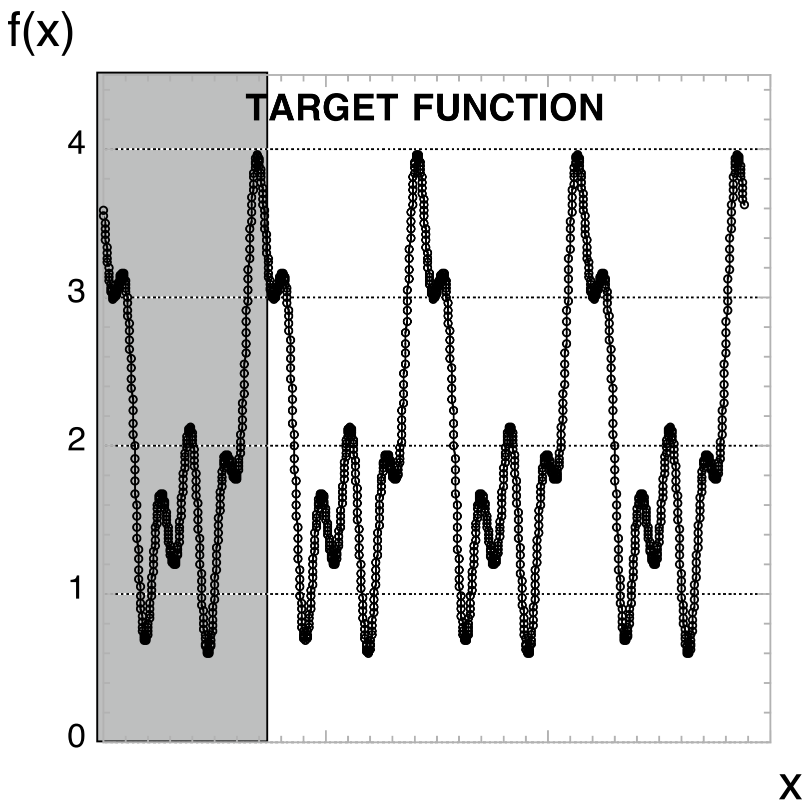


Histogram of optical densities (OD) along one line of micrograph

The regularity of the crystal “lattice” is reflected in a “repeat” in the OD-pattern.



However: the repeats are not precisely the same due to noise, low-dose conditions, and irregularities in the lattice (= lattice disorder). Nevertheless, the periodic nature of the OD-pattern begins to provide clues how these data structure can be exploited.



2D-crystals present us with the easiest approach towards structure because the Fourier Theorem states that any periodic function can be described as the sum of a series of sinusoidal functions of wavelengths that are integral fractions of a single basic wavelength λ.

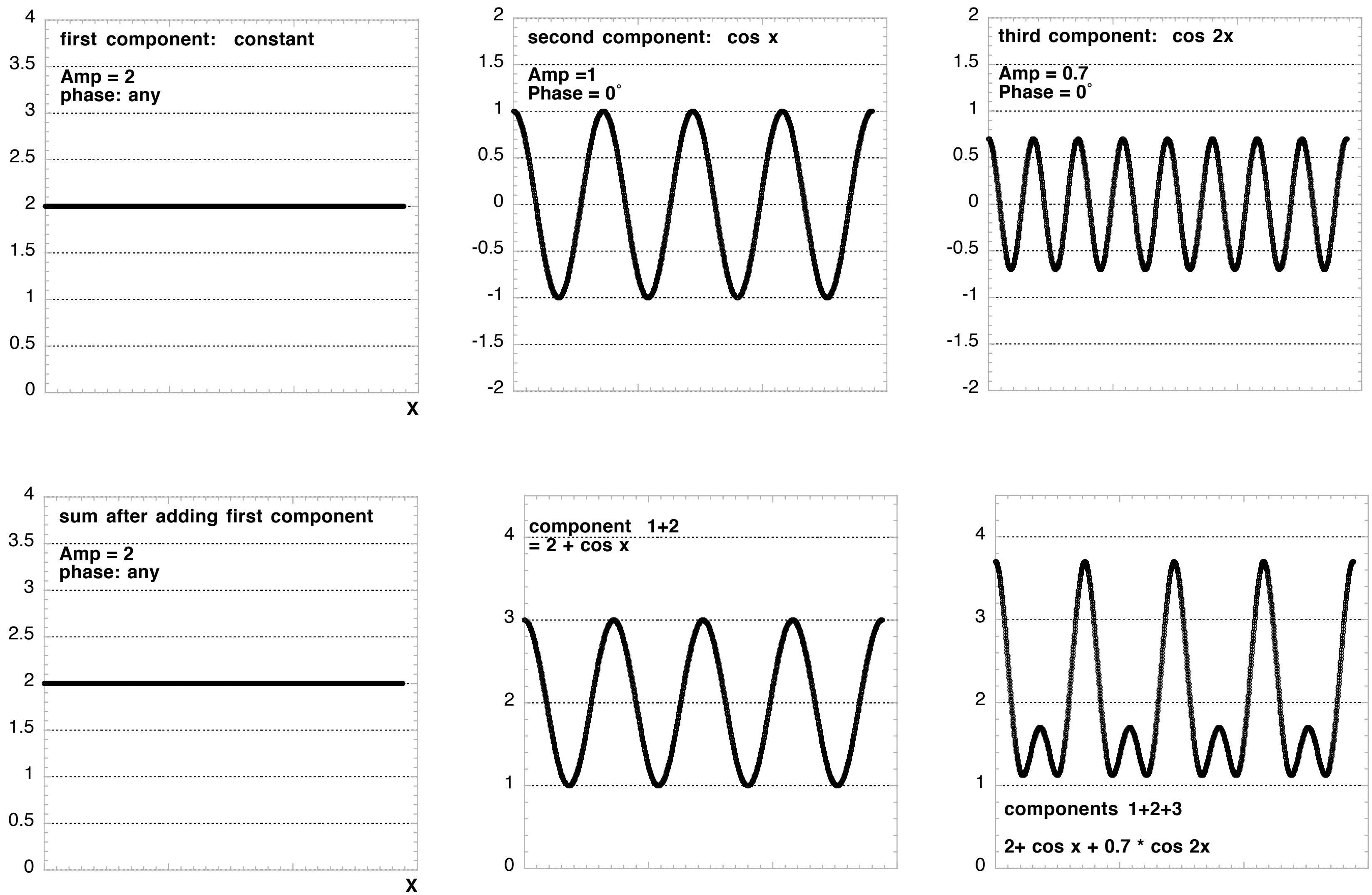
$$f(x) = \frac{C_0}{2} + C_1 \cos(\frac{2\pi x}{\lambda} + \alpha_1) + C_2 \cos(\frac{2\pi x}{\lambda/2} + \alpha_2) + ...$$
$$+ C_n \cos(\frac{2\pi x}{\lambda/n} + \alpha_n)$$

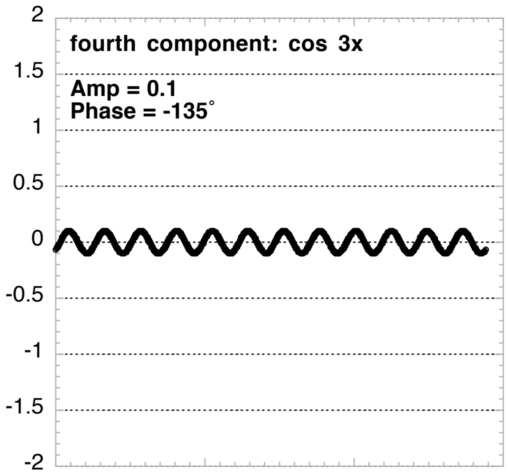
Or short

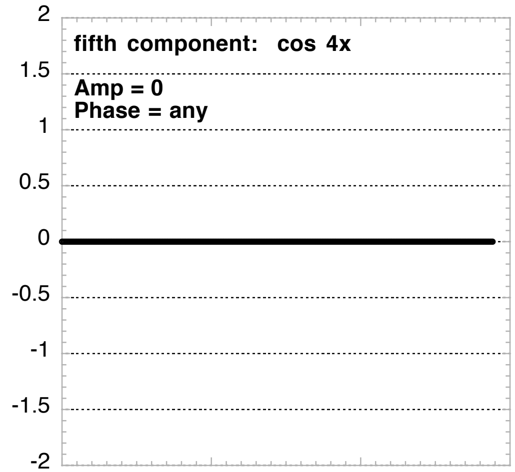
$$f(x) = \frac{C_0}{2} + \sum_{n=1}^{n=\infty} C_n \cos(\frac{2\pi x}{\lambda/n} + \alpha_n)$$

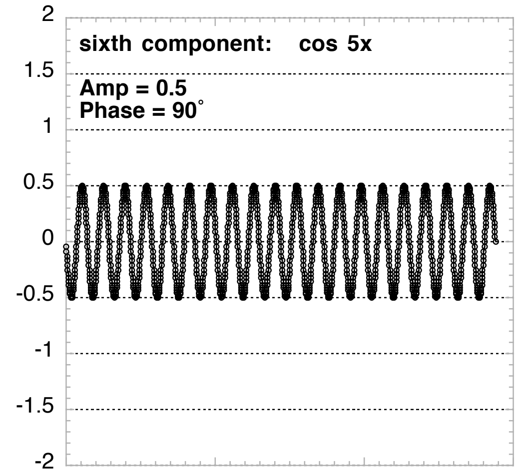
C_n Amplitude
n Order
α_n Phase

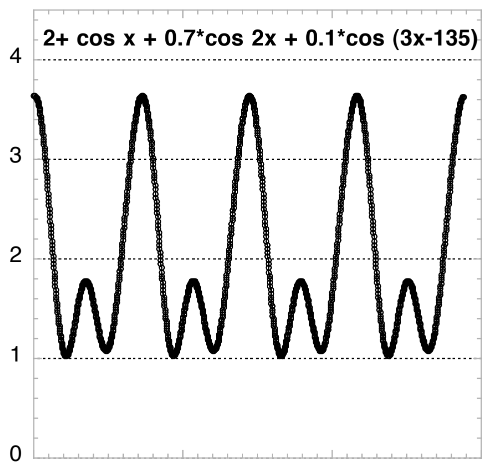
In other words: the FT of a 2D-crystal will be discrete and if we know the “recipe” for building one single unit cell of the periodic array (e.g. the grey part of the function shown above), then we know the structure of the entire crystal.


first component: constant
Amp = 2
phase: any
second component: cos x
Amp =1
Phase = 0˚
third component: cos 2x
Amp = 0.7
Phase = 0˚
sum after adding first component
Amp = 2
phase: any
component 1+2
= 2 + cos x
components 1+2+3
2+ cos x + 0.7 * cos 2x
x

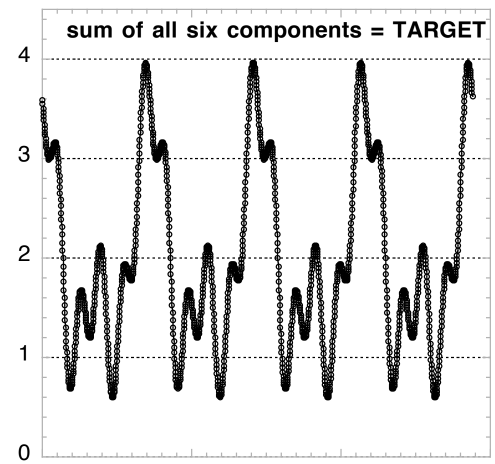






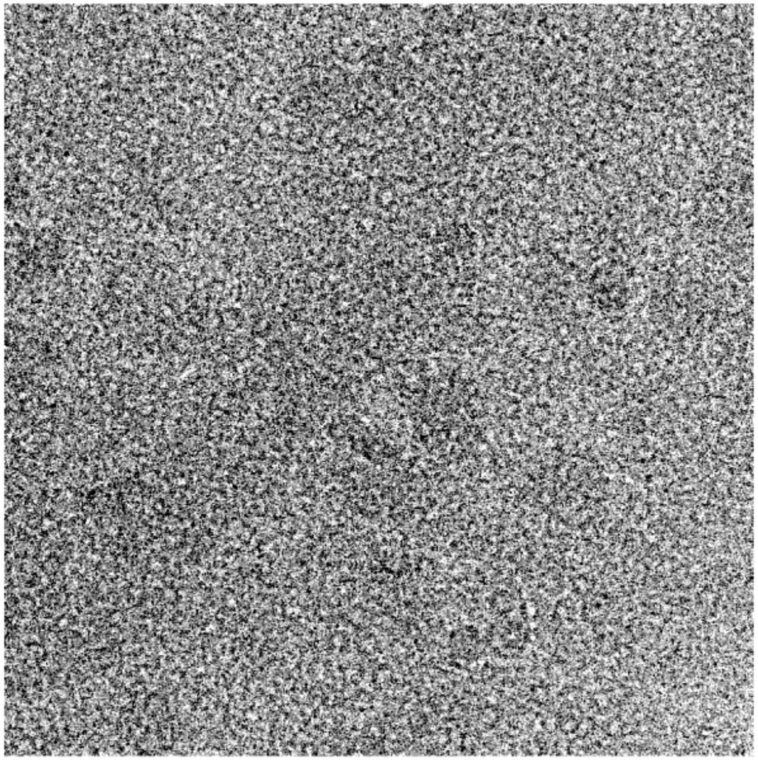


Same as previous because Amp = 0 results in adding 0 to each point

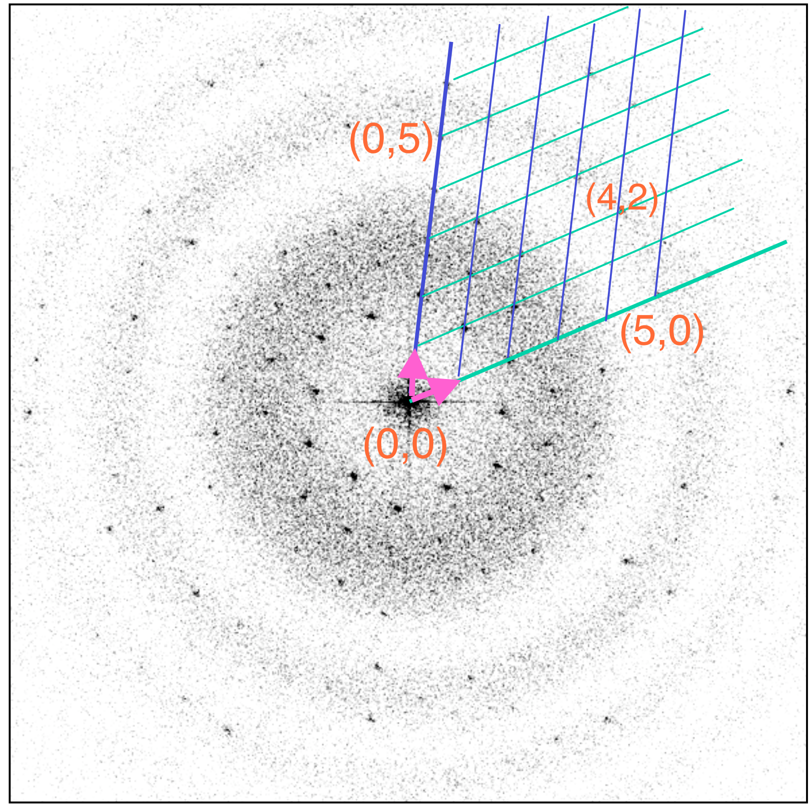


In principle: the Fourier components and their summation to obtain a real space picture of an object is very similar to making Lasagna….

To prove the point….



cryoEM picture of a gap junction 2D-crystal (periodic object) deposited on continuous carbon support film (aperiodic object)

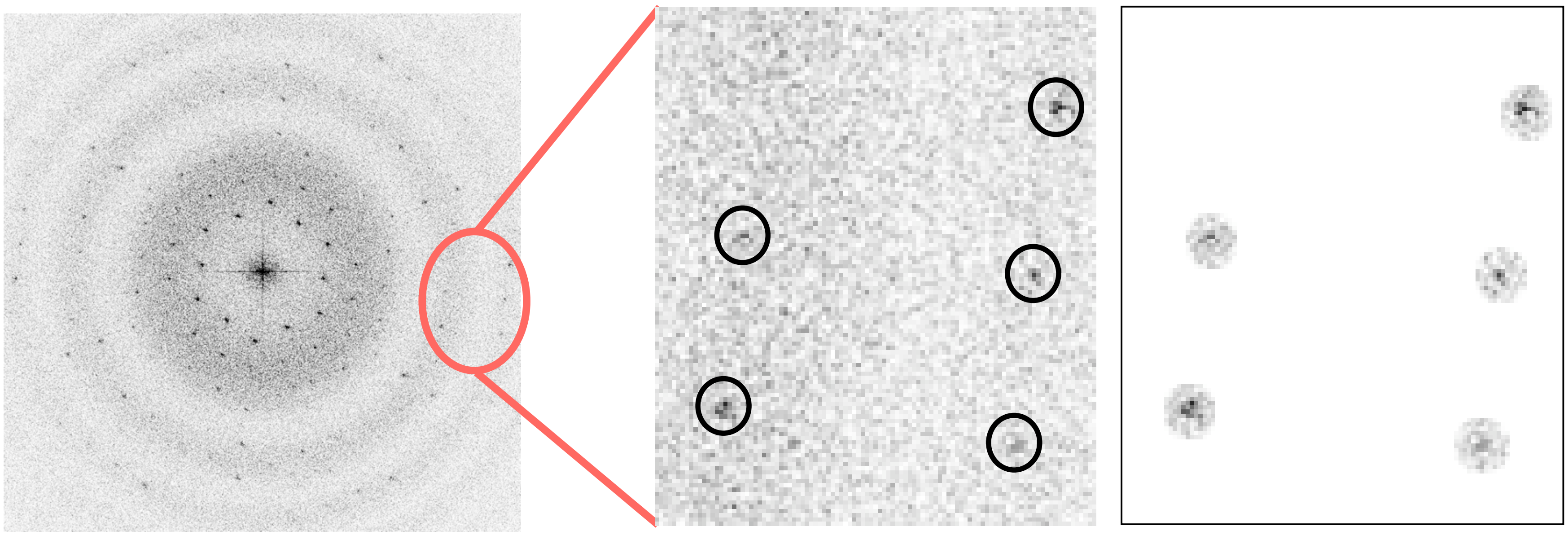


Calculated FT of the image. What do you see? And what is causing it?

a) **Spots** at regular spacings: diffraction maxima arising **from crystal**.

b) Alternating pattern of bright and dark regions. This is a combination of two things. (1) the **aperiodic carbon** film causes **diffraction at all angles**, and (2) the oscillation in intensities is the manifestation of the CTF of the objective lens (not all diffracted waves are transmitted with the same fidelity)

Principle of Digital Filtering

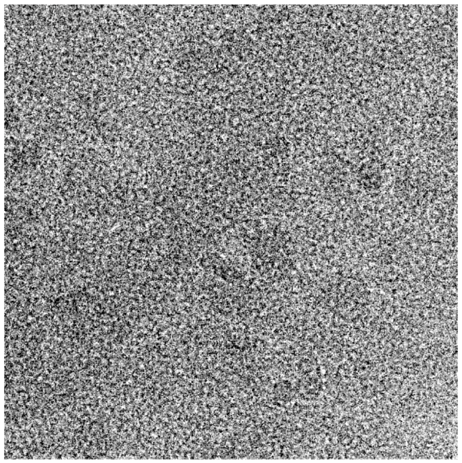


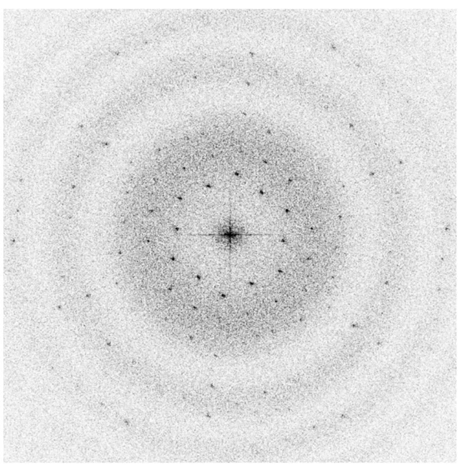
entire FT

enlarged area of FT

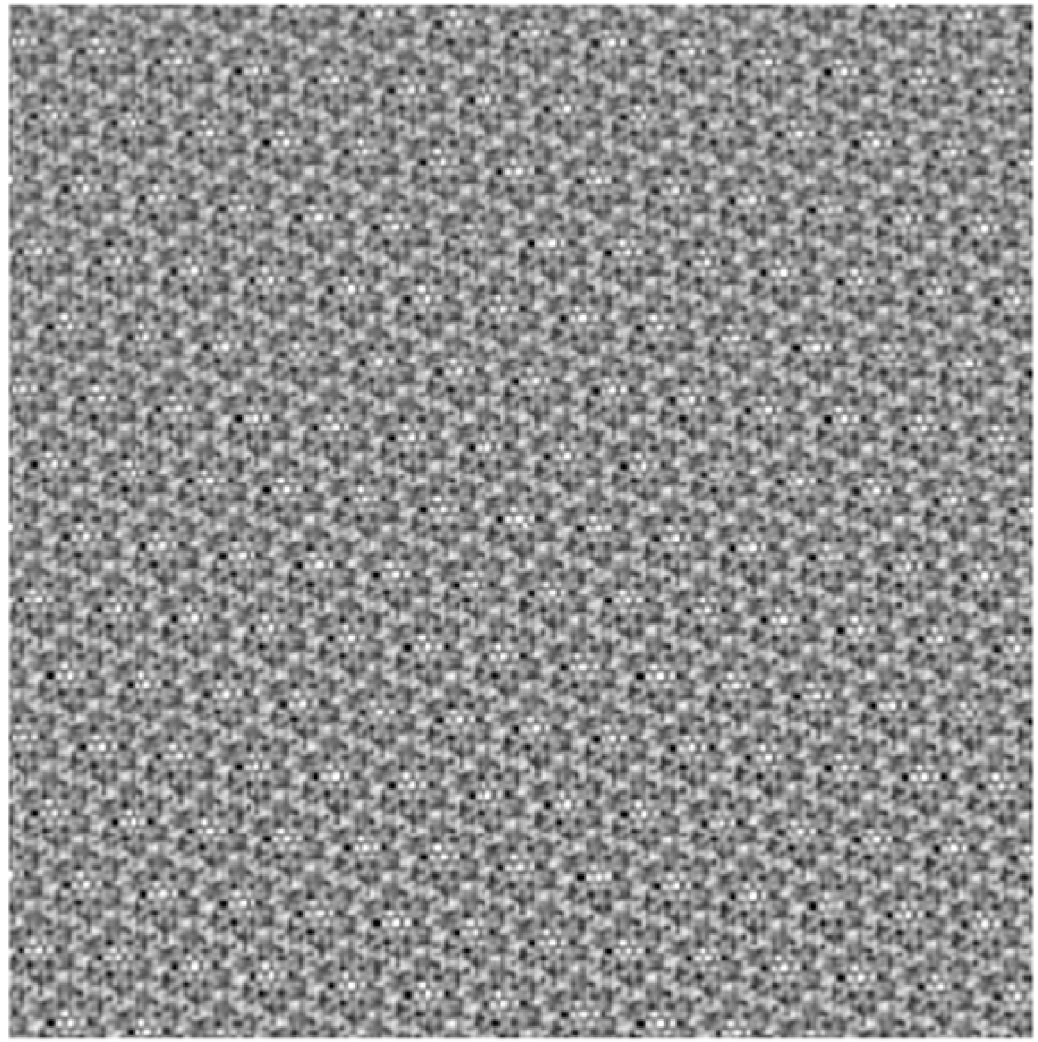
circular maskholes applied (FT has now non-zero values only within maskholes

Digital Filtering of Fourier Transform



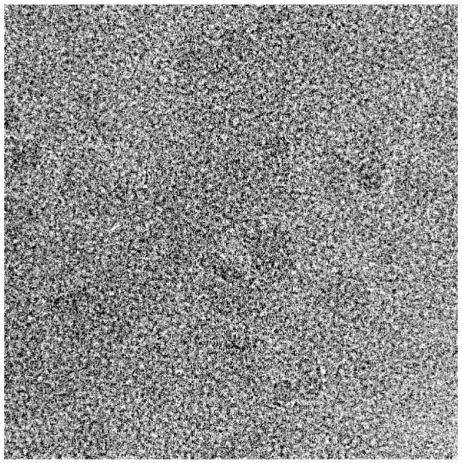


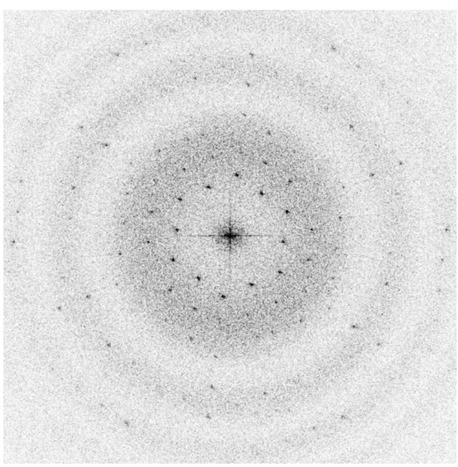
Radius used was r=1

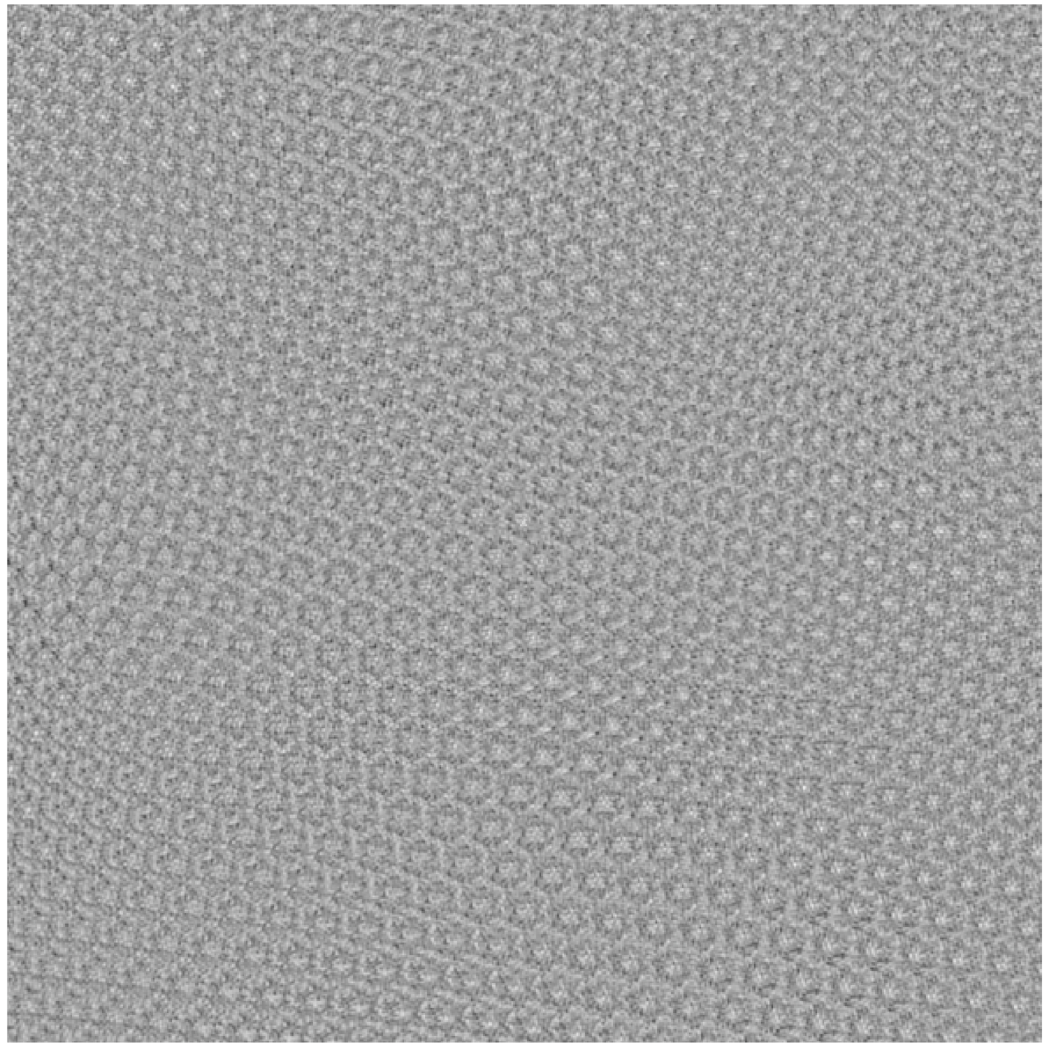


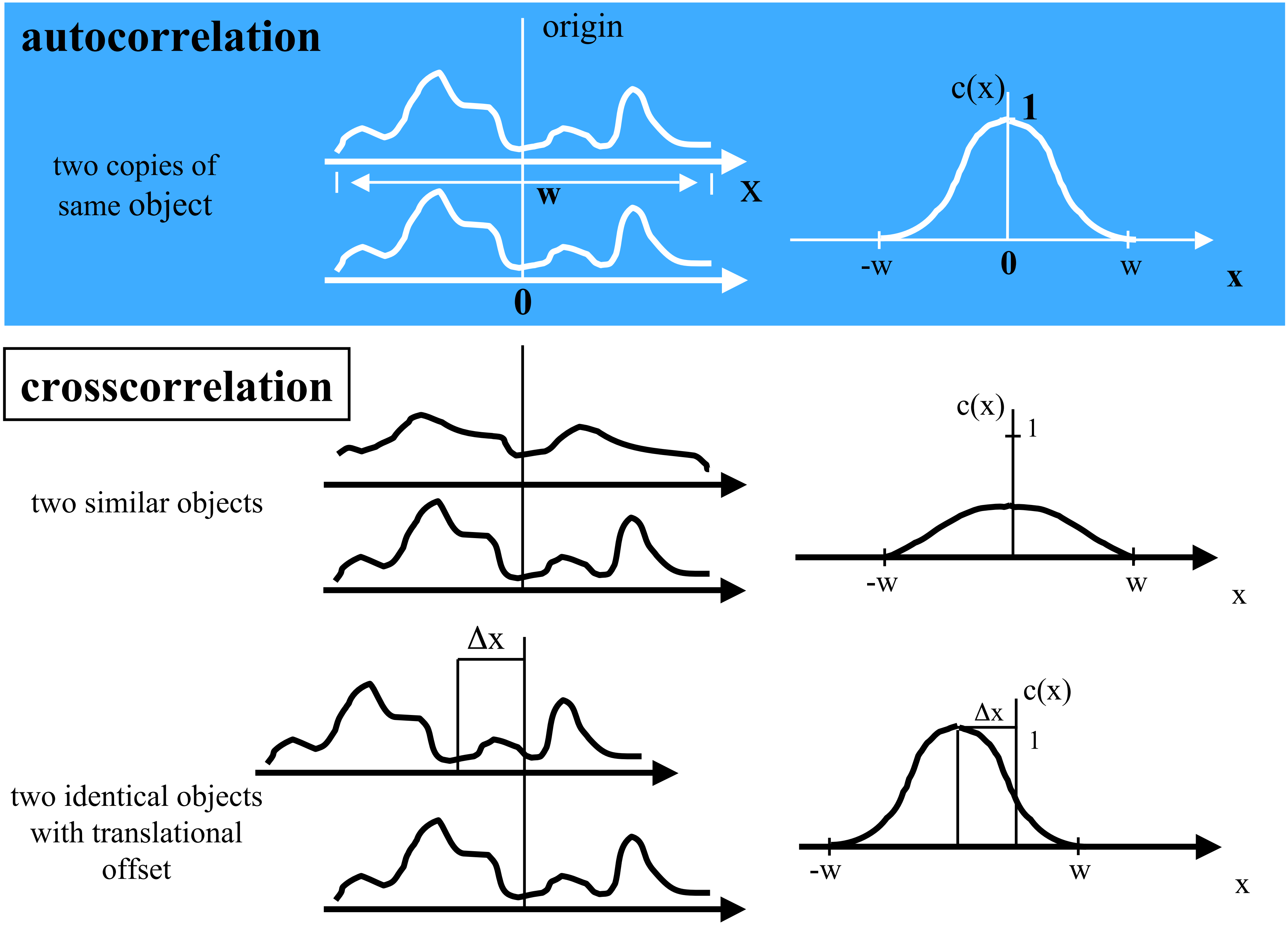
Digital Filtering of Fourier Transform

Radius used was r=7





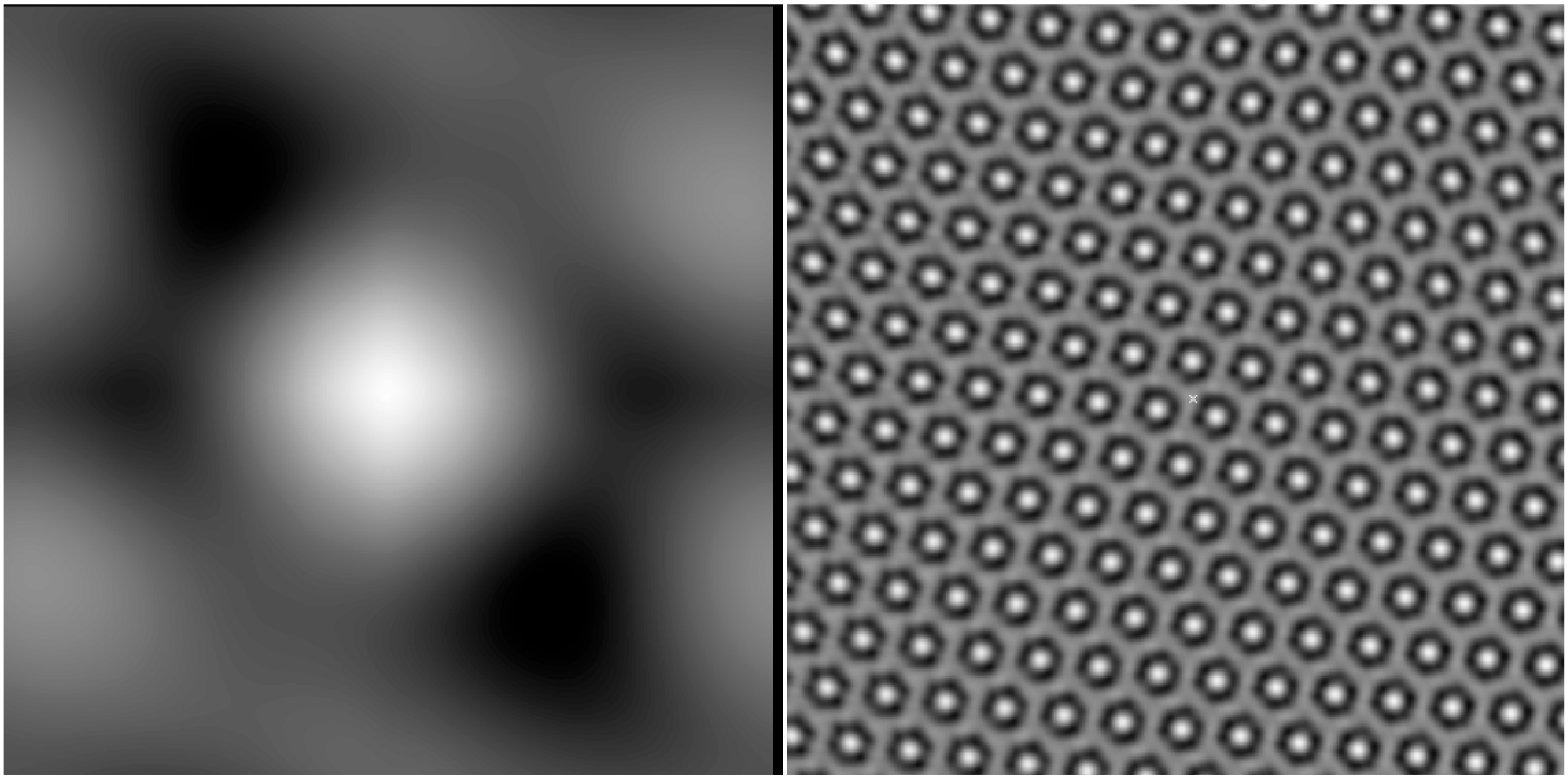




autocorrelation
origin
two copies of
same object
w
x
0
c(x)
1
-w
0
w
x
crosscorrelation
two similar objects
c(x)
1
-w
w
x
Δx
two identical objects
with translational
offset
c(x)
Δx
1
-w
w
x

Autocorrelation Map

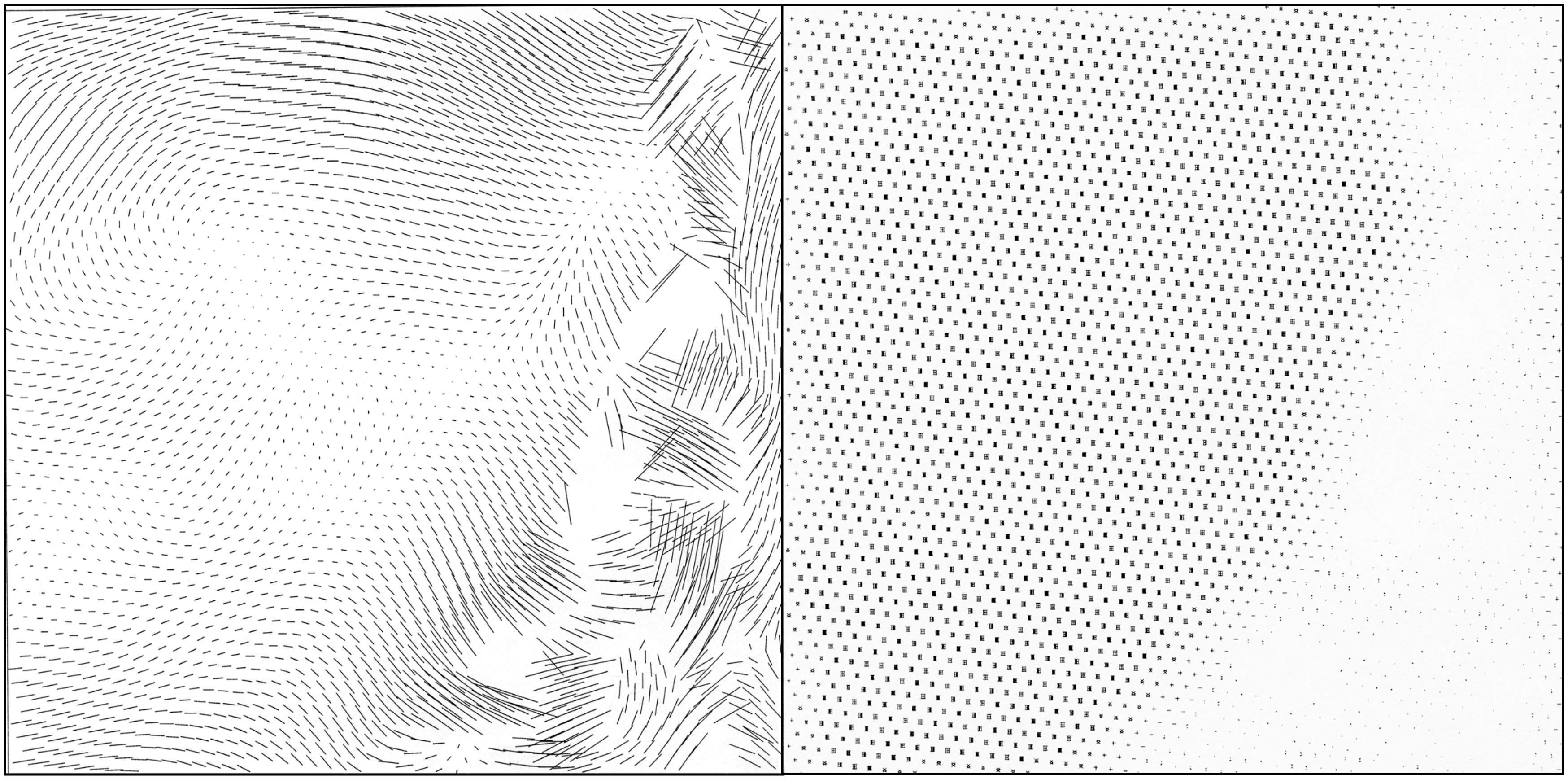
Part of Crosscorrelation Map



Note that the shape of the central peak in the autocorrelation map is very similar to the shape of the cross-correlation peaks.

Crosscorrelation Maps

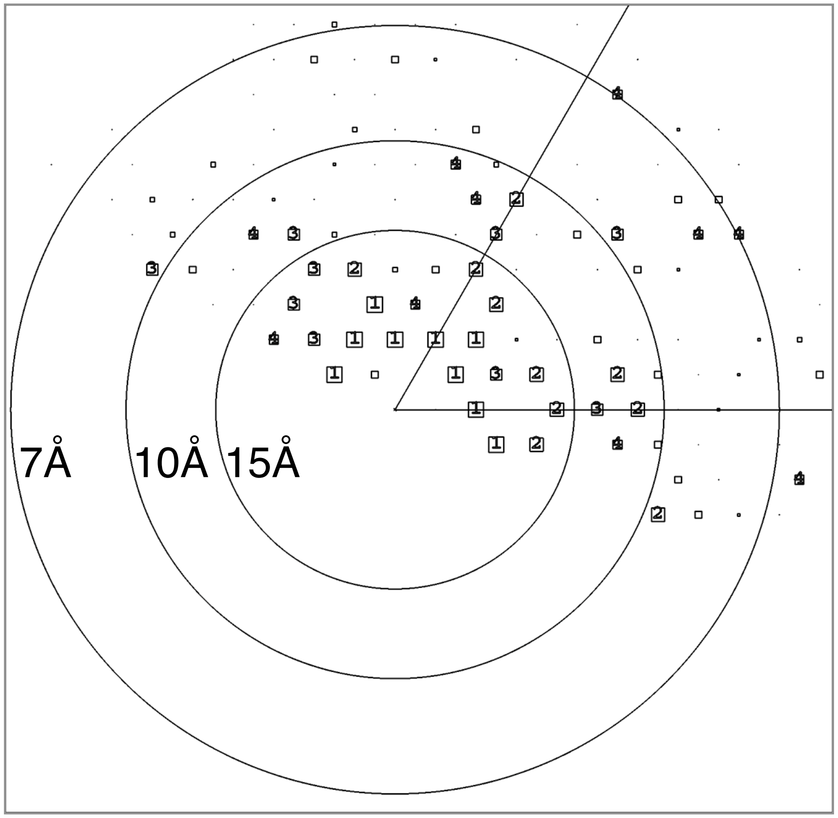
Cross-correlation methods can be used to determine translational disorder in 2D-crystals.

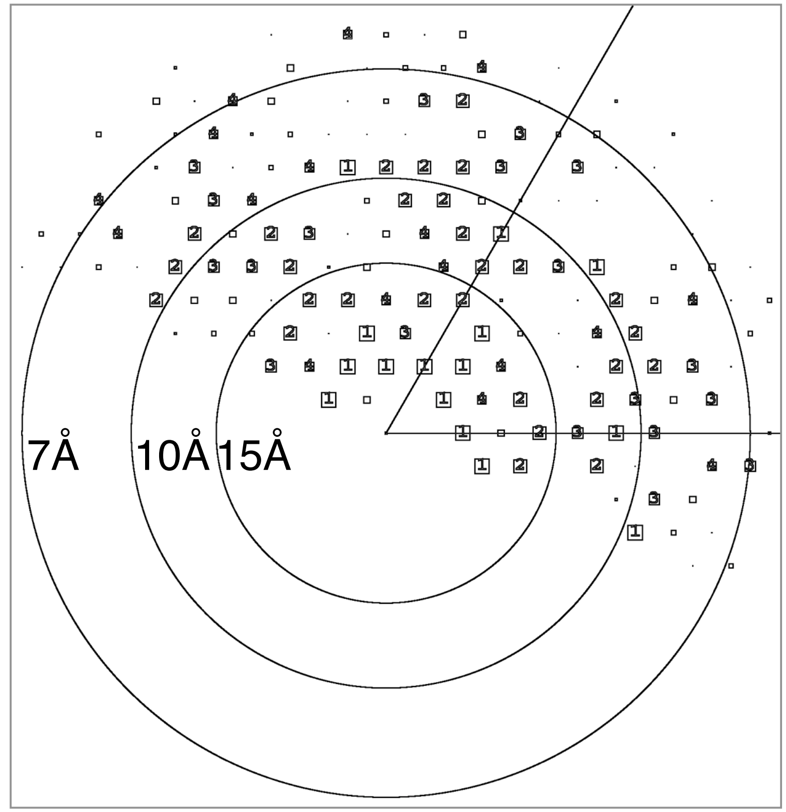


deviation from expected lattice position [Å] X20 (not to scale) with respect to chosen reference

height of cross correlation-peaks indicates how similar each unit cell is to the chosen reference

Effect of "Lattice Unbending"



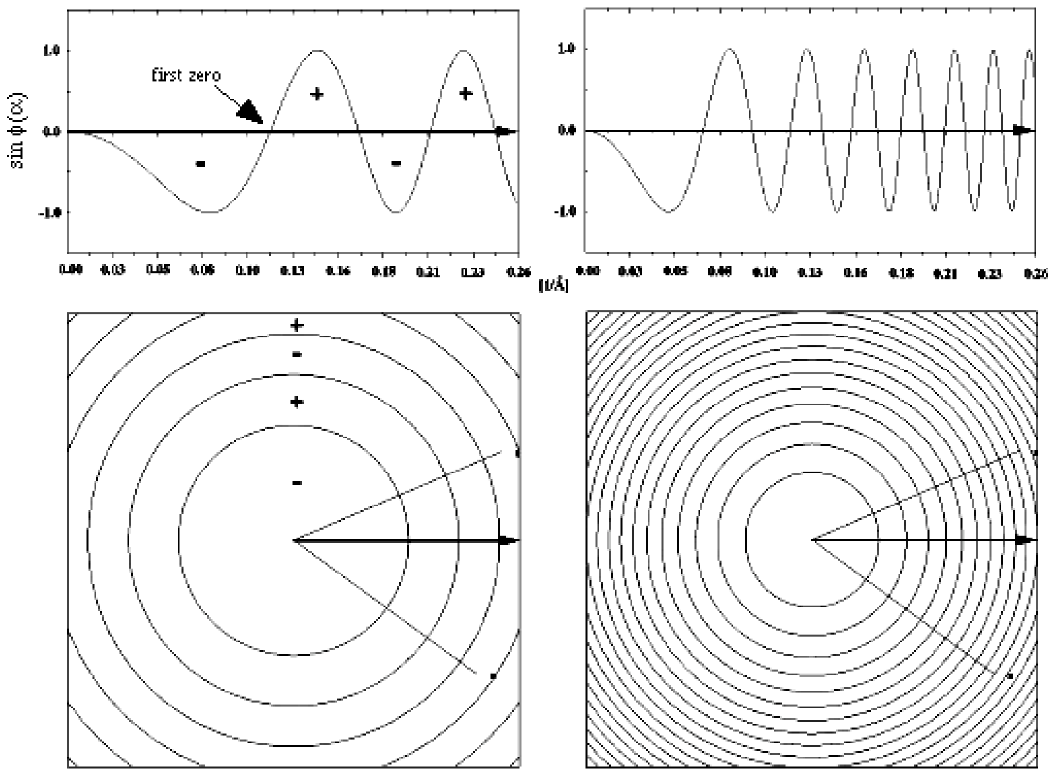


Left: data were retrieved from a calculated FT of an untreated raw image. In this case, the data are not statistically significant beyond ~15Å resolution.

Right: after correction for translational lattice disorder, the same image provides data out to ~7Å resolution.

Plot symbols indicate the goodness of each reflection. Reflections marked by a "1" have a signal-to-noise ratio of at least 8.

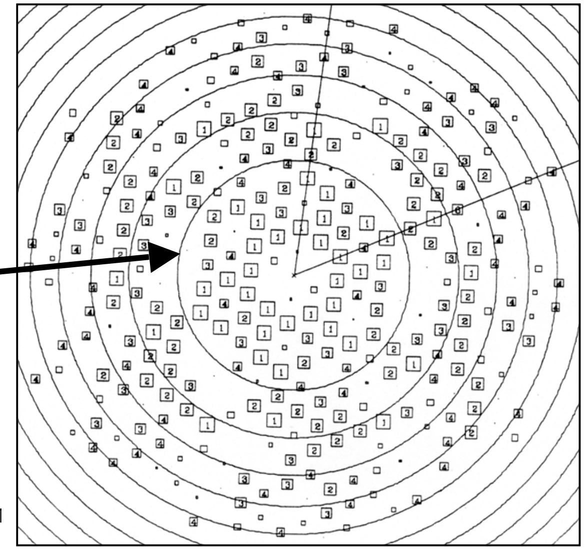
Now that the data extend to well beyond 10Å, correction for the CTF becomes critically important.



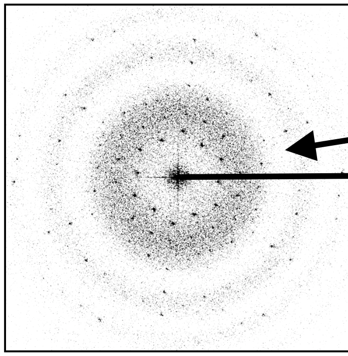
The simulated curves are for 3000 and 6000Å of underfocus respectively, an accelerating voltage of 200keV (λ=0.025Å) and a C_s=2mm

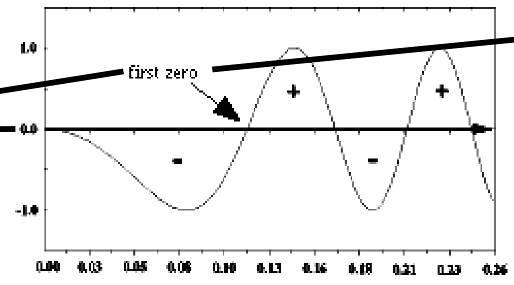
These lower two panels demonstrate how the CTF would look like in the FT of the image. Circles represent [sin $\Phi(\alpha)$] =0 Frequencies where [sin $\Phi(\alpha)$]<0 contribute with reversed contrast to the image. Therefore, the phases of reflections in these regions need to be adjusted by 180°

Because the phase information is so important we now can understand why in EM we MUST correct for the effect of the CTF….



Reciprocal space





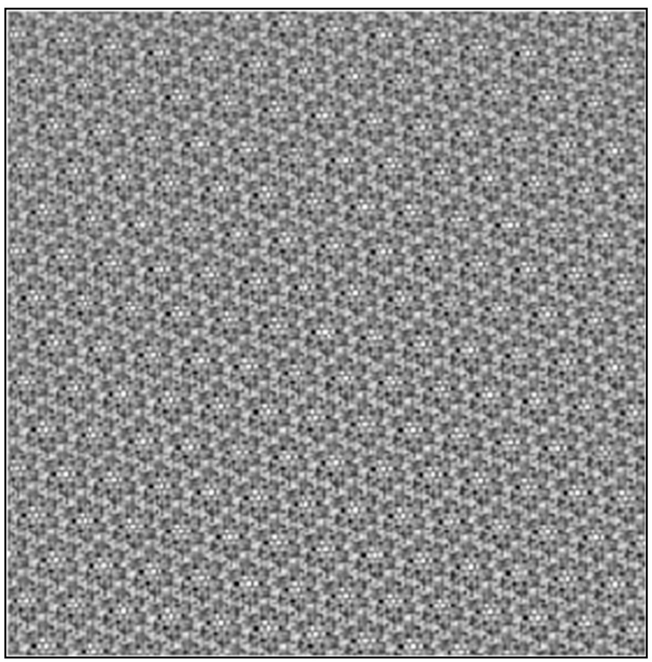
Inverse FT = Fourier Summation

$$f(x,y) = \sum_h \sum_k |F_{hk}| \; e^{i\alpha_{hk}} \; e^{-2\pi i(hx+ky)}$$

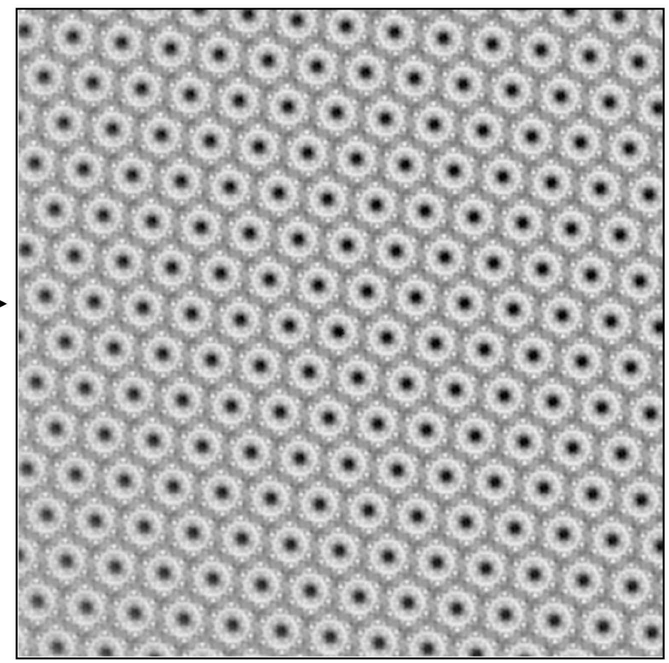
F_{hk} Amplitude of reflection (h,k)

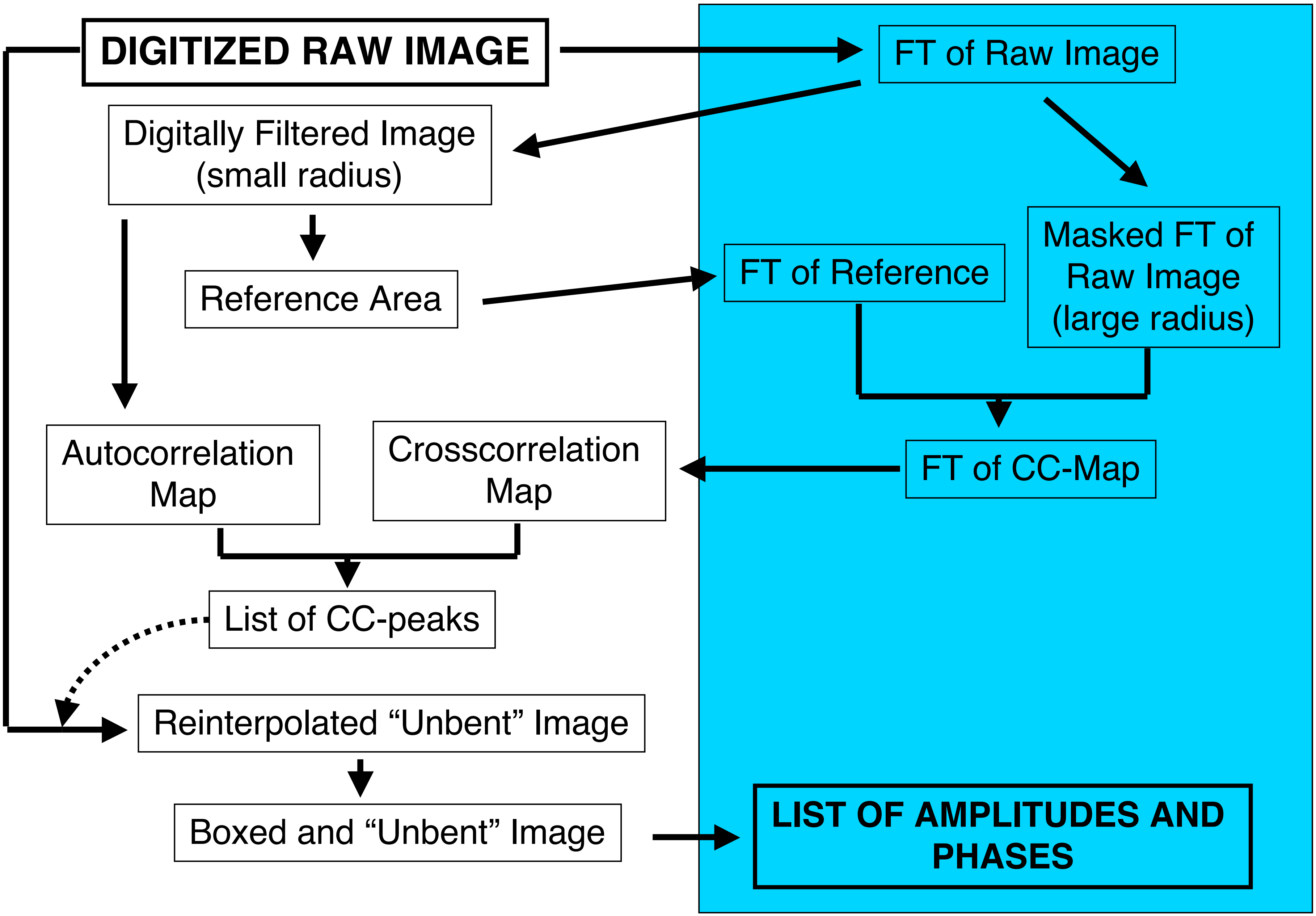
α_{hk} Phase of reflection (h,k)

Real space

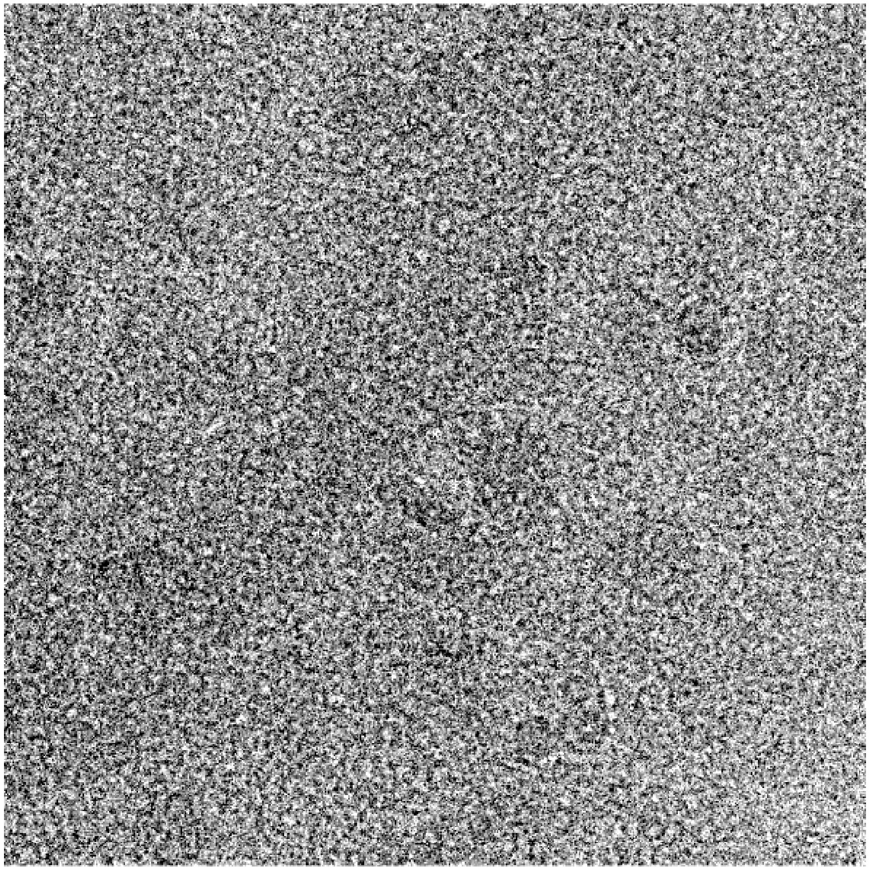


CTF-correction




DIGITIZED RAW IMAGE
FT of Raw Image
Digitally Filtered Image (small radius)
Masked FT of Raw Image (large radius)
Reference Area
FT of Reference
Autocorrelation Map
Crosscorrelation Map
FT of CC-Map
List of CC-peaks
Reinterpolated "Unbent" Image
Boxed and "Unbent" Image
LIST OF AMPLITUDES AND PHASES

Basic Image Processing of 2D-Crystals



REAL SPACE

(H,K)	amp	phase	IQ	CTF
0 1	132.4	237.8	7	-0.142
0 2	5686.9	299.8	1	-0.540
0 3	195	249.1	6	-0.958
0 4	1067.4	246.1	1	-0.762
0 5	431.0	102.5	2	0.397
0 6	1016.5	356.5	1	0.925
0 7	120.5	243.0	6	-0.602
0 8	0.0	270.7	9	-0.388
0 9	145.5	319.4	4	0.923
0 10	67.2	290.6	6	-0.993
0 11	0.0	270.7	9	0.928
1 0	97.7	132.8	8	-0148
1 1	7227.8	140.0	1	-0.423
1 2	2582.2	17.1	1	-0.846
1 3	1460.3	266.5	1	-0.957

And so forth….

RECIPROCAL SPACE

Now, just do the Fourier Summation and we should be done…

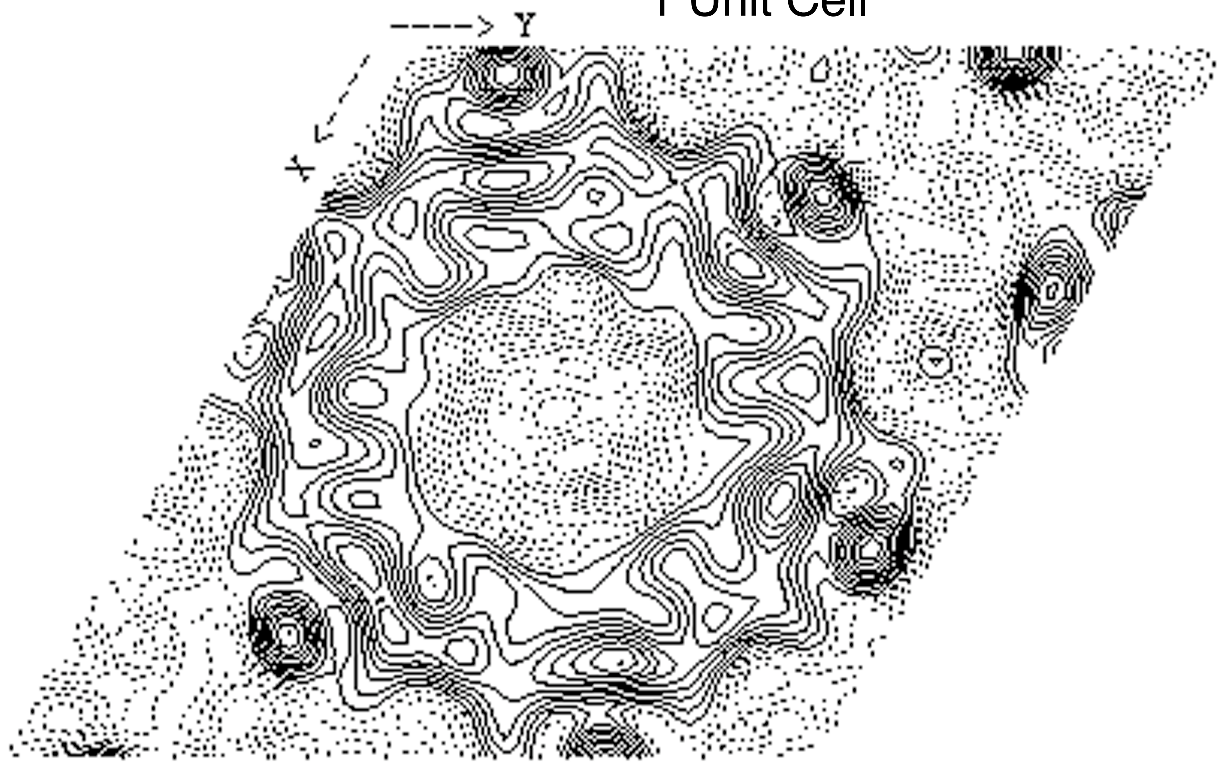
(H,K)	amp	phase
0 1	132.4	237.8
0 2	5686.9	299.8
0 3	195	249.1
0 4	1067.4	246.1
0 5	431.0	102.5
0 6	1016.5	356.5
0 7	120.5	243.0
0 8	0.0	270.7
0 9	145.5	319.4
0 10	67.2	290.6
0 11	0.0	270.7
1 0	97.7	132.8
1 1	7227.8	140.0
1 2	2582.2	17.1
1 3	1460.3	266.5

And so forth….

$$f(x,y) = \sum_h \sum_k |F_{hk}| \; e^{i\alpha_{hk}} \; e^{-2\pi i(hx+ky)}$$

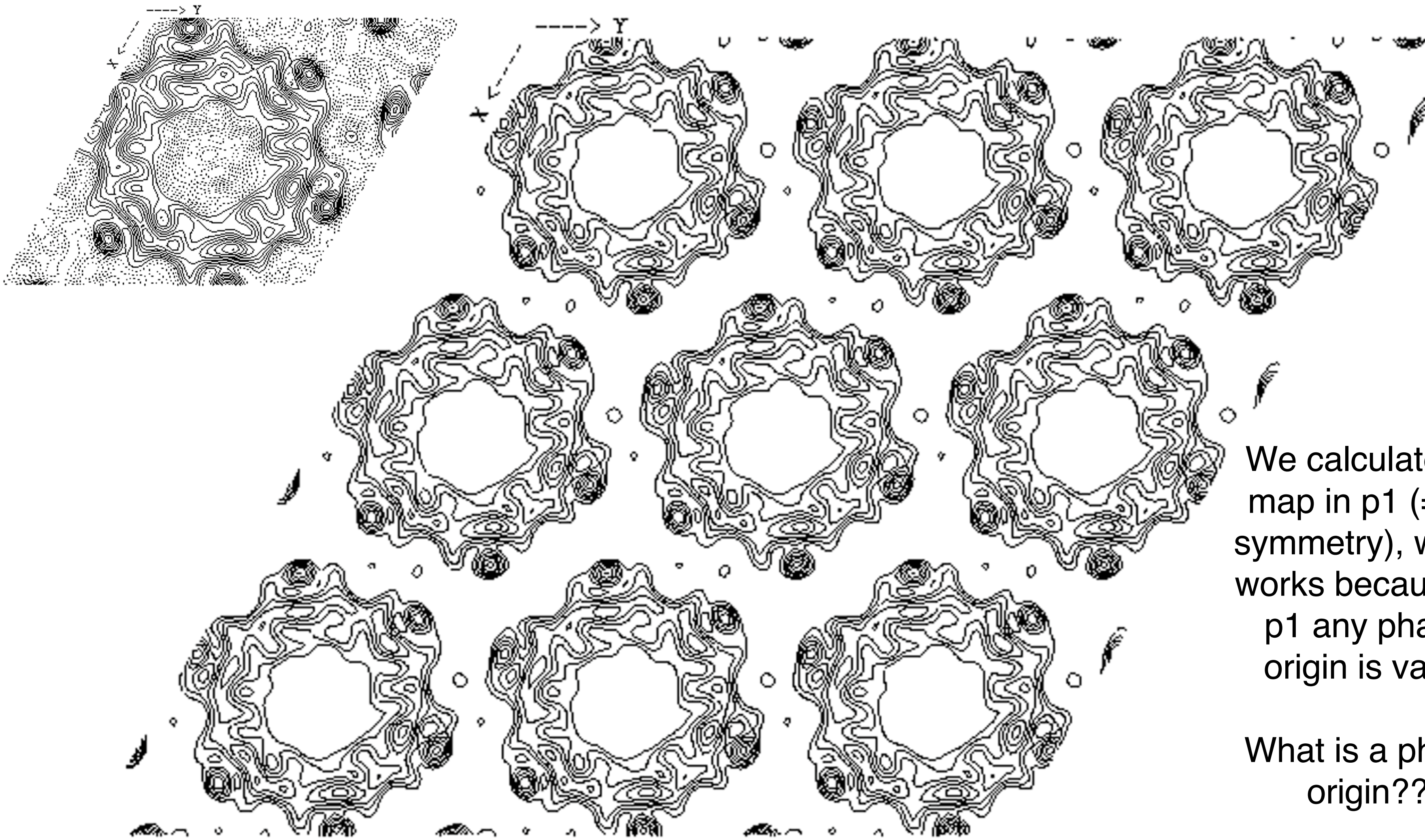
F_{hk} Amplitude of reflection (h,k)
α_{hk} Phase of reflection (h,k)

1 Unit Cell



What happened!! Did I take a bad image/picture?

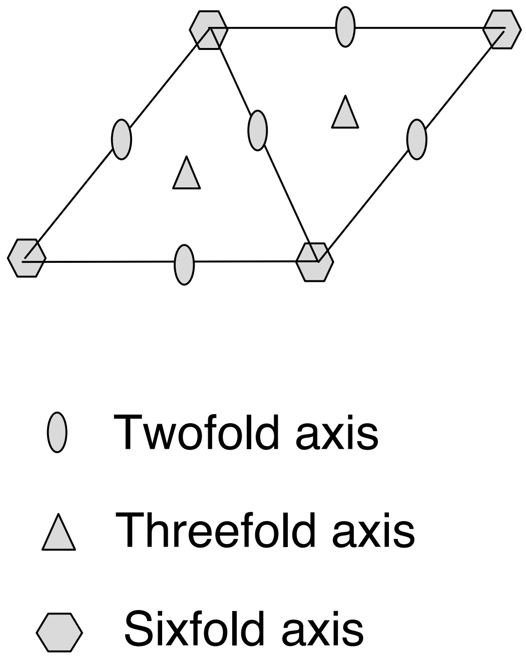
Looking at a couple of unit cells together explains everything…….

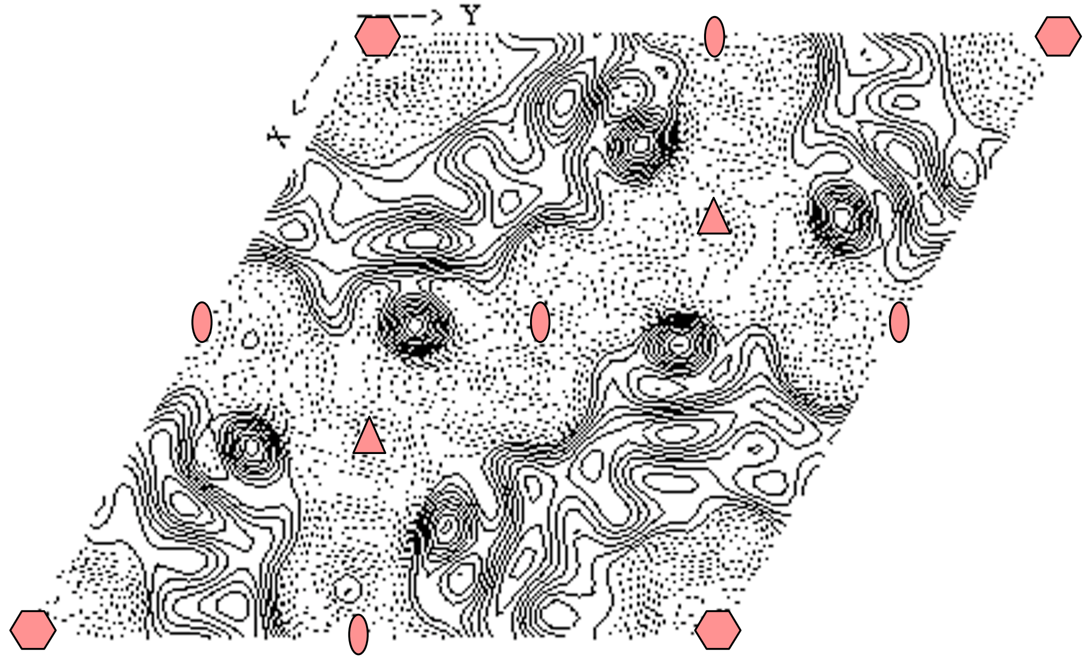


We calculated a map in p1 (= no symmetry), which works because in p1 any phase origin is valid.

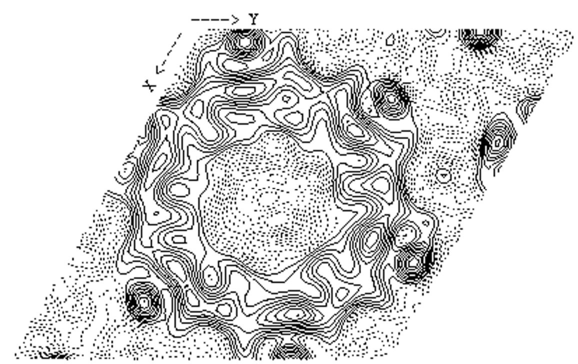
What is a phase origin??

The presence of symmetry requires the contents of the unit cell to be positioned such that the crystallographic related molecules have the correct spatial relation with respect to the symmetry axes……take p6 for instance…..

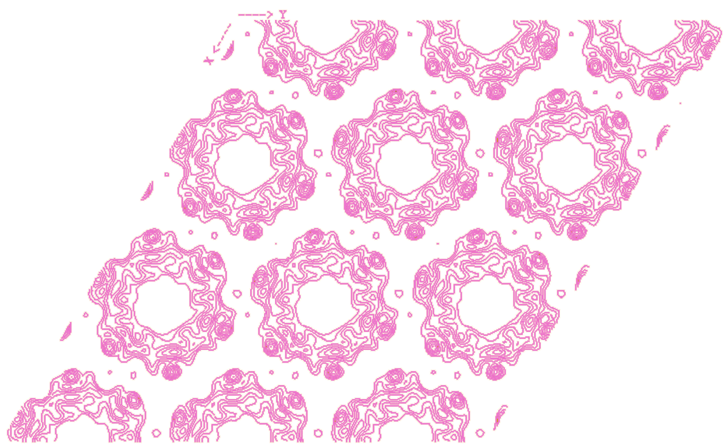


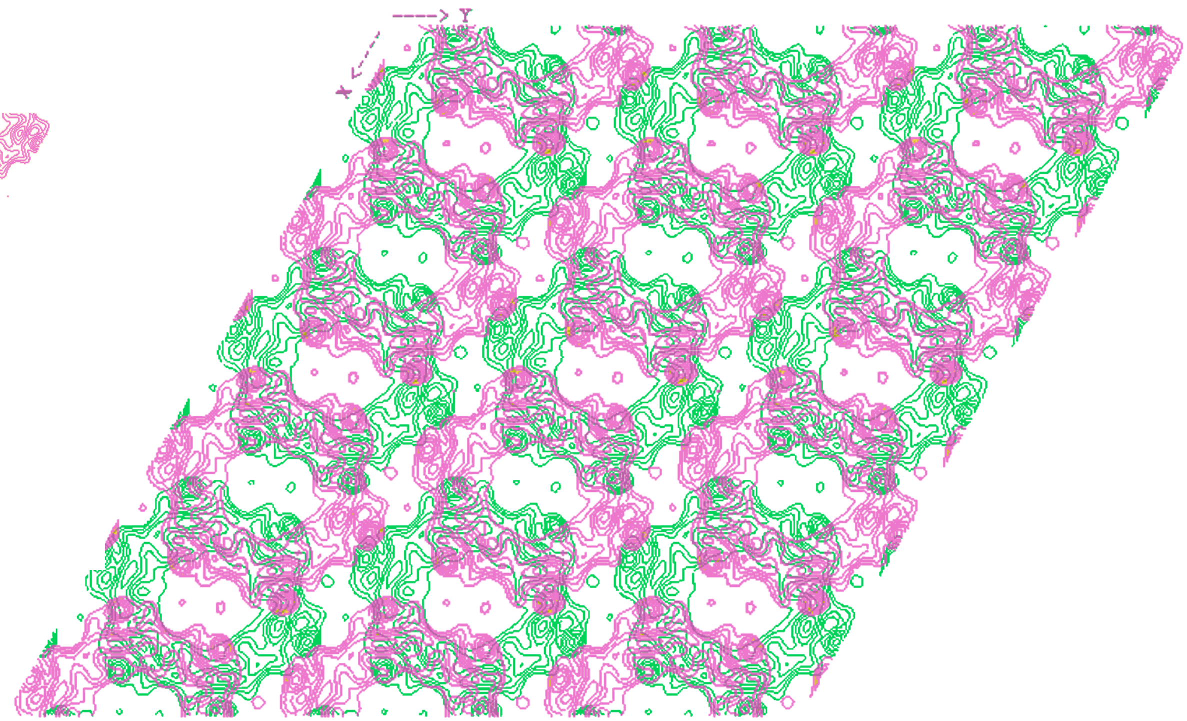


Remember, p6 symmetry has not been imposed here….but the more pressing issue is how do we get from the p1 map we have to a distribution of densities that looks like above?



The answer is: **by shifting the phases** . Remember, a movement in real space correlates to a phase shift in reciprocal space





The molecules contoured in green are shifted by 1/2 unit cell (=180 degree shift applied to the (1,0) reflection) with respect to the molecules contoured in magenta

```
THE MATRIX BELOW IS CENTRED ABOUT AN ORIGIN WITH A PHASESHIFT OF
      0.00  FOR THE (1,0) REFLECTION
      0.00  FOR THE (0,1) REFLECTION
     10.00  STEP SIZE
 0000000000000000000000000000000000000
 1000000000000000000000000000000000001
 0000000000000000000000000000000000000
 0000000000000000000000000000000000000
 0000000001000000000000000000000000000
 0000000000100000001232000000000000000
 0111100000010000001355310000000000000
 0012100000000000000257520000001000000
 0000000000000000000024421000000000000
 0000000000000000000001210000000000000
 0000000000000000000000000000000000000
 0000000000000000000000000000000000000
 0000111000000000000000000000000000000
 0000011000000000000000000000000000000
 0000000000000000000000000000000000000
 0000000000000000000000000000000000000
 0000000000000000000000000000000000000
 0000000000000000000000000000000000000
 0000000111100000000000001000000000000
 0000000012100000000000001100000000000
 0000000000000000000000000100000000000
 0000000000000000000000000000000000000
 0000000000000000000000000000000000000
 0010000000000000000010000000000000000
 0121000000000000000011000000110000000
 0012100000001000000012100000000000000
 0000100000000000000001000000000000000
 0000000000000000000000000000000000000
 0000000000000000000000000000000000000
 0000000000000000000000000000000000000
 0000000000000000000000000000000111000
 0000000000001000000000000000000012100
 0000000000000000000000000000000001100
 0000000000000000000000000000000000000
 0000000000000000000000000000000000000
 0000000000000000000000000000000000000
 0000000000000000000000000000000000000

 BEST PHASE SHIFTS ARE
     30.00  FOR THE (1,0) REFLECTION
   -110.00  FOR THE (0,1) REFLECTION
 NOTE THAT THESE SHIFTS INCLUDE THE INITIAL SHIFTS AS WELL AS THE
ADDITIONAL REFINED SHIFTS
```

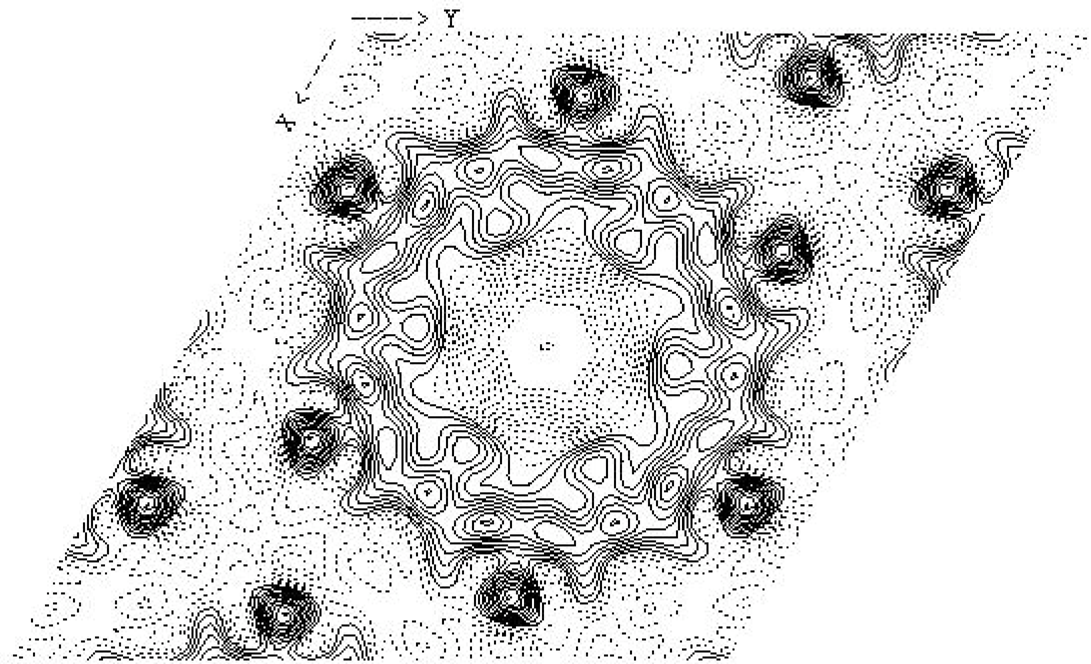
only one position for sixfold symmetry axis

SPACEGROUP		Phase resid(No) v.other spots (90 random)		Phase resid(No) v.theoretical (45 random)		OX	OY
1	p1	14.6	50	10.5	50		
2	p2	25.4!	25	12.7	50	-151.3	67.3
3b	p12_b	60.5	11	22.4	6	-68.4	-180.0
3a	p12_a	55.9	11	12.2	6	-180.0	-117.4
4b	p121_b	34.6	11	21.1	6	112.4	-120.0
4a	p121_a	42.1	11	34.1	6	80.0	151.9
5b	c12_b	60.5	11	22.4	6	-68.4	-180.0
5a	c12_a	55.9	11	12.2	6	-180.0	-117.4
6	p222	40.9	47	12.6	50	-151.4	-112.8
7b	p2221b	40.5	47	12.7	50	-151.3	67.1
7a	p2221a	63.2	47	19.7	50	21.2	-118.8
8	p22121	30.3	47	12.5	50	28.2	-113.1
9	c222	40.9	47	12.6	50	-151.4	-112.8
10	p4	38.0	49	12.6	50	-151.6	-112.8
11	p422	49.7	107	12.8	50	-151.2	-112.5
12	p4212	51.3	107	12.8	50	28.8	67.6
13	p3	13.5*	40	--	--	-91.5	7.6
14	p312	54.2	95	7.1	10	148.7	127.8
15	p321	48.2	98	52.4	16	149.8	128.2
16	p6	15.4*	105	13.0	50	29.1	-112.2
17	p622	50.7	218	13.1	50	29.2	-112.1

* = acceptable
! = should be considered
` = possibility

PHASE ERROR AT MINIMUM IS 18.5 DEGREES in p6

Projection Density Map and some of the Corresponding Structure Factors

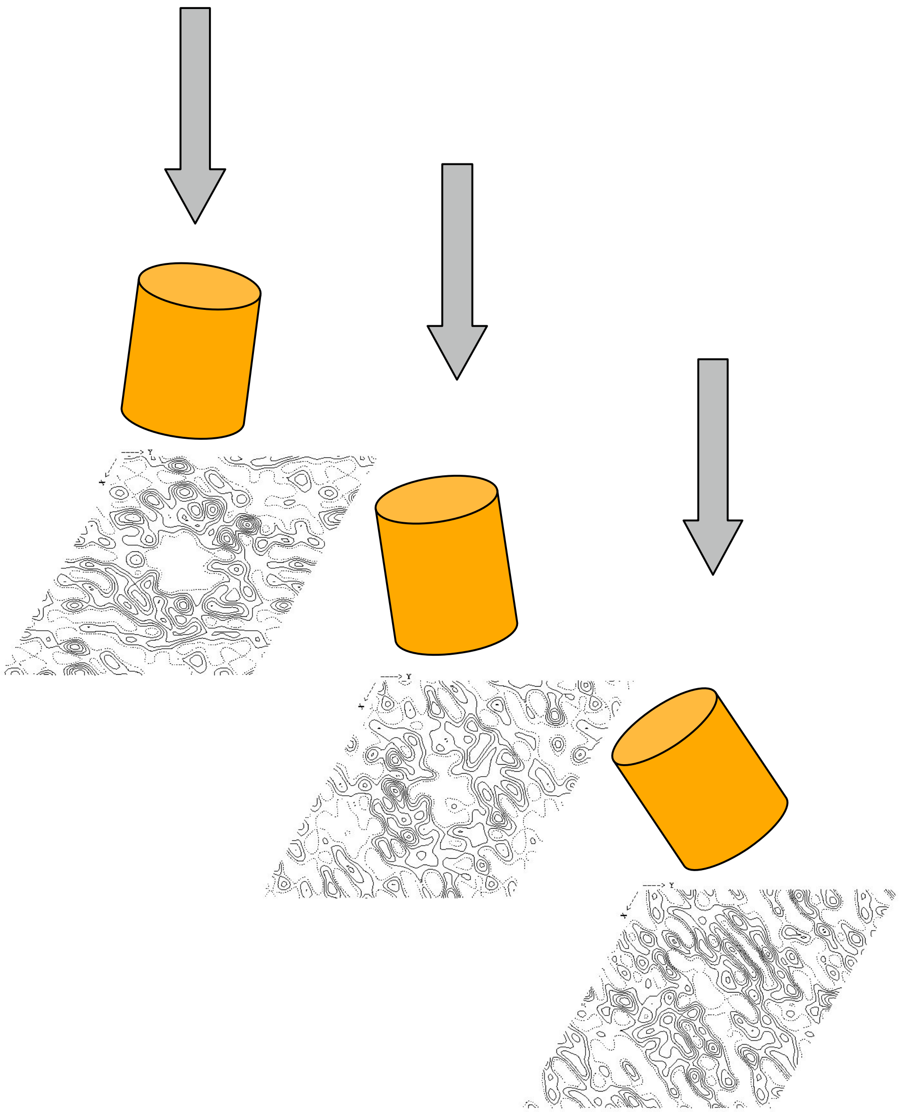


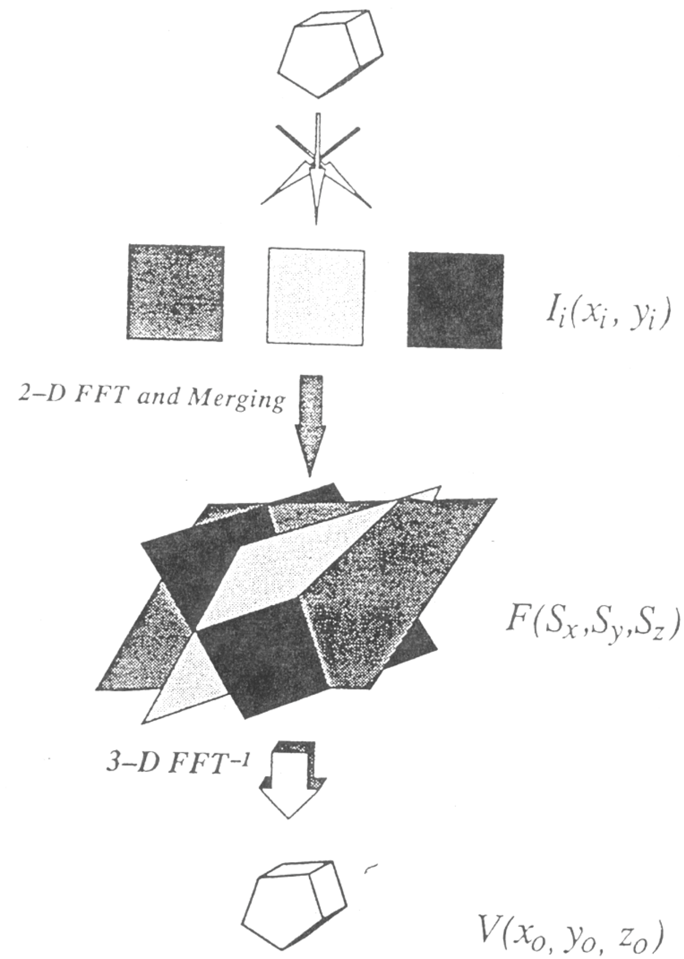
Real space map obtained by Fourier summation

(H,K,L)	amp	phase	FOM
1 0 0	2566	180	99.5
1 1 0	12424	180	99.9
1 2 0	777	180	99.5
1 3 0	1123	0	99.7
1 4 0	208	0	73.9
1 5 0	605	0	99.0
1 6 0	670	180	99.2
1 7 0	250	180	99.6
1 8 0	350	0	94.3
1 9 0	77	180	59.8
1 10 0	140	0	13.3
2 0 0	9265	180	99.9
2 1 0	1971	0	99.8

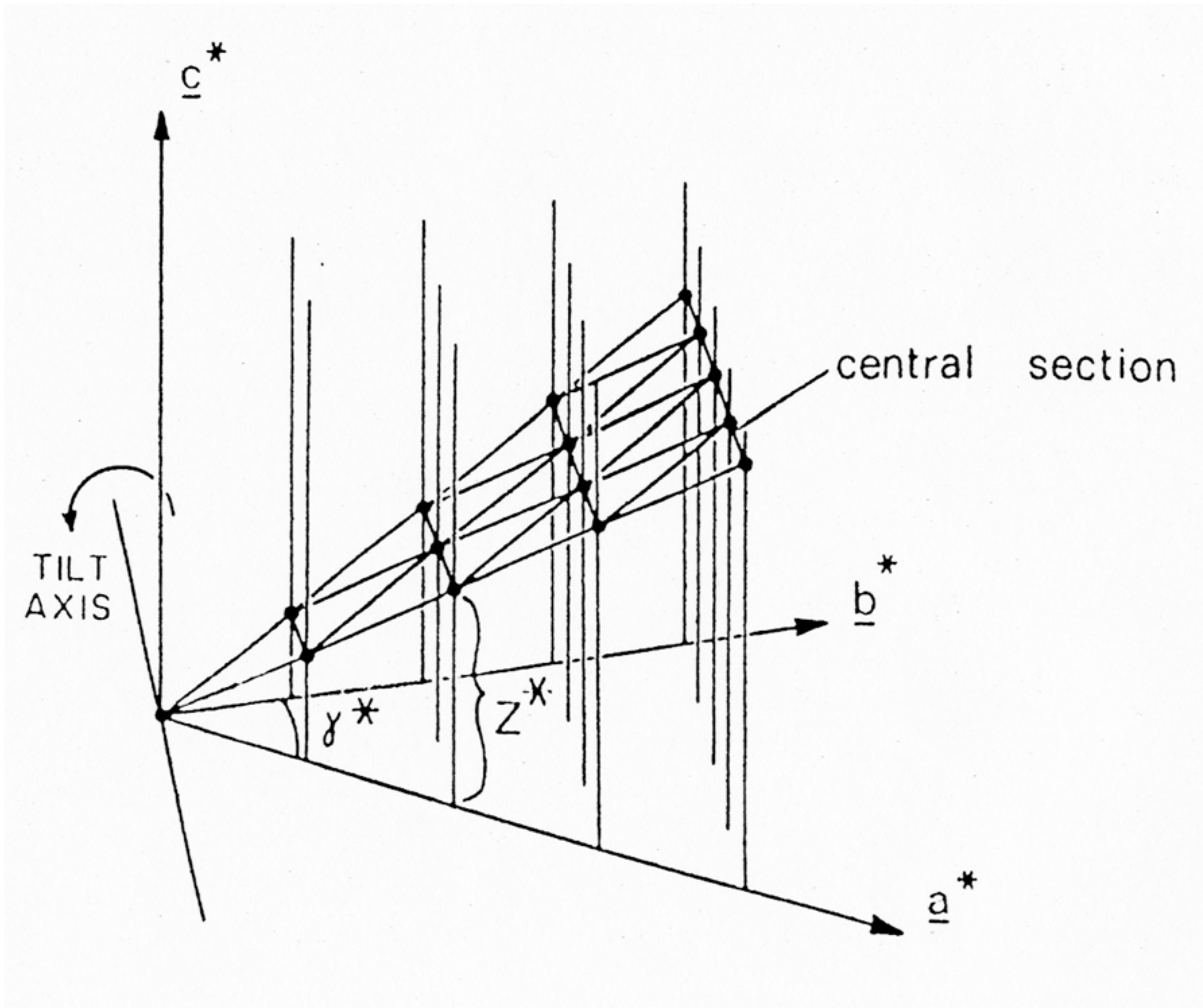
And so forth…..

Pictures of Tilted Crystals are Required for 3D-Structure Determination



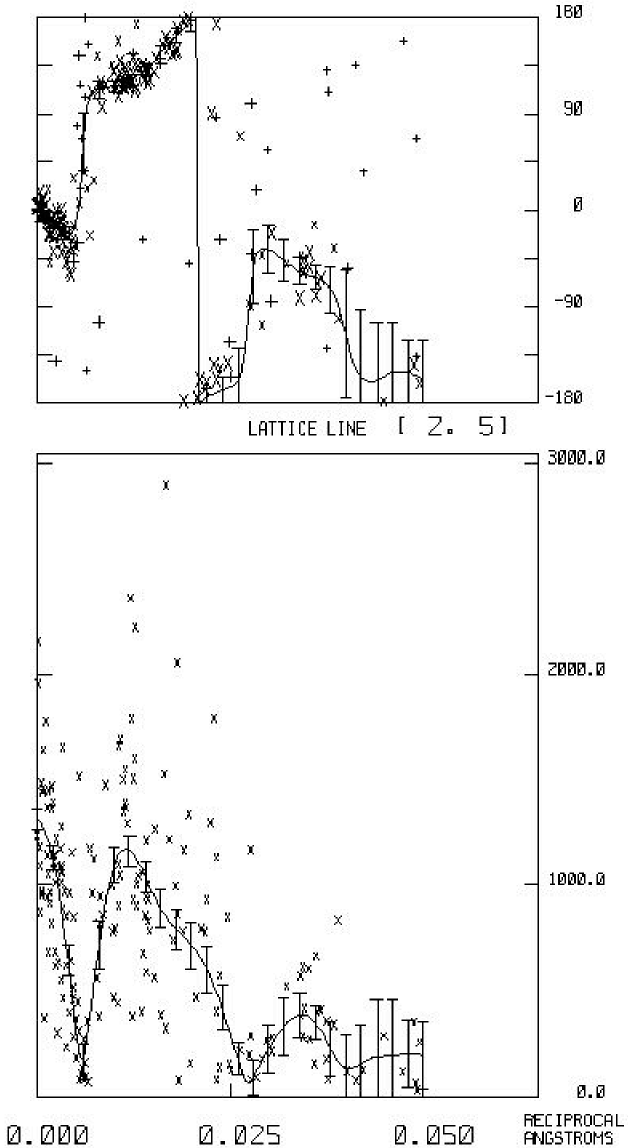


Concept of Lattice Lines and Principle of Sampling their Data



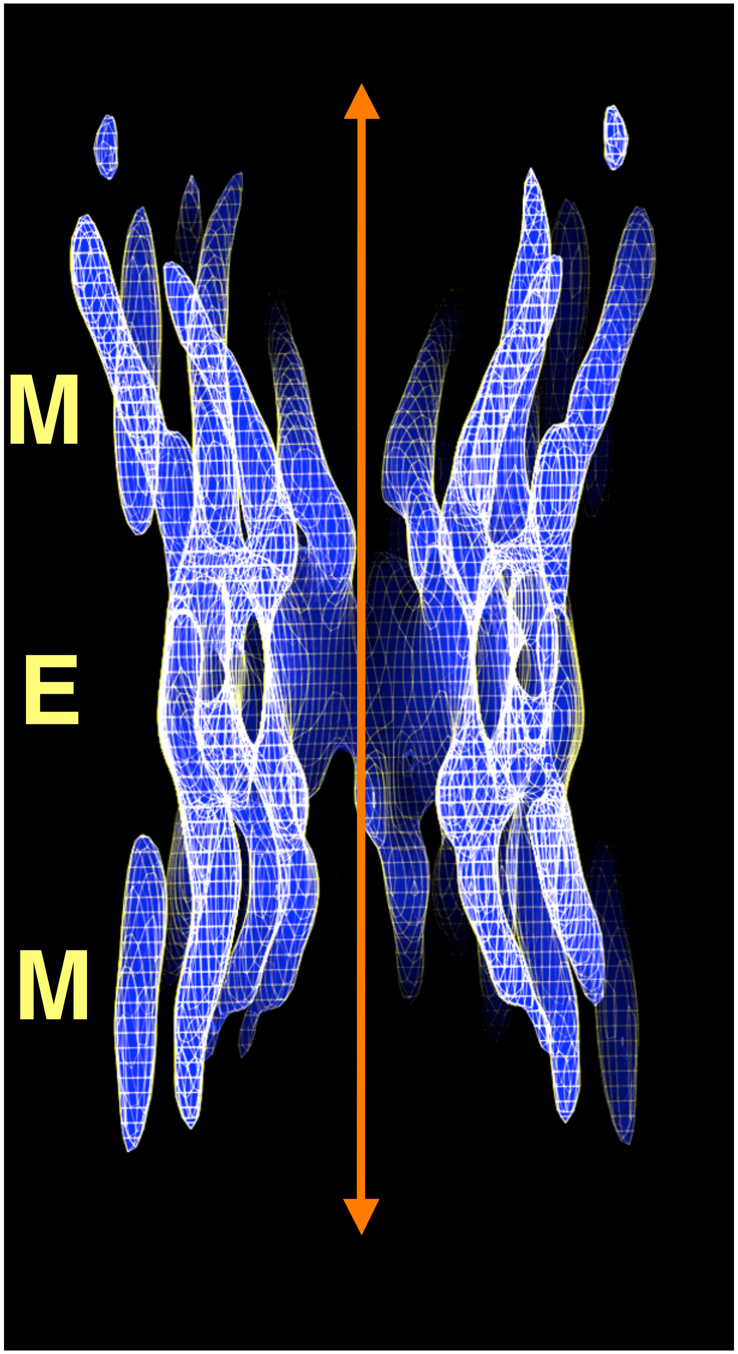
Taken from:
Amos, Henderson and Unwin (1982), Prog. Biophys Molec Biol 39:183-231

Example for a Lattice Line



This figure shows the variation of phase (top panel) and amplitude (bottom) of the (2,5)-reflection of a gap-junction 2D-crystal as function of z*.

The amplitudes were obtained from the calculated image transforms. In contrast to the phase information, **image derived amplitudes are very noisy** mostly because the image is modulated by the contrast transfer function of the objective lens (see page showing the calculated FT of an image). The effect of the CTF on amplitudes cannot be fully corrected, but, on the other hand **does not really matter that much because it is the phases that determine the structure**.



3D-Map of a Gap-Junction Intercellular Channel

Shown are a surface representation at ~7.5Å resolution A total of ~42,000 channel molecules were crystallographically averaged to obtain this structure.

THE END