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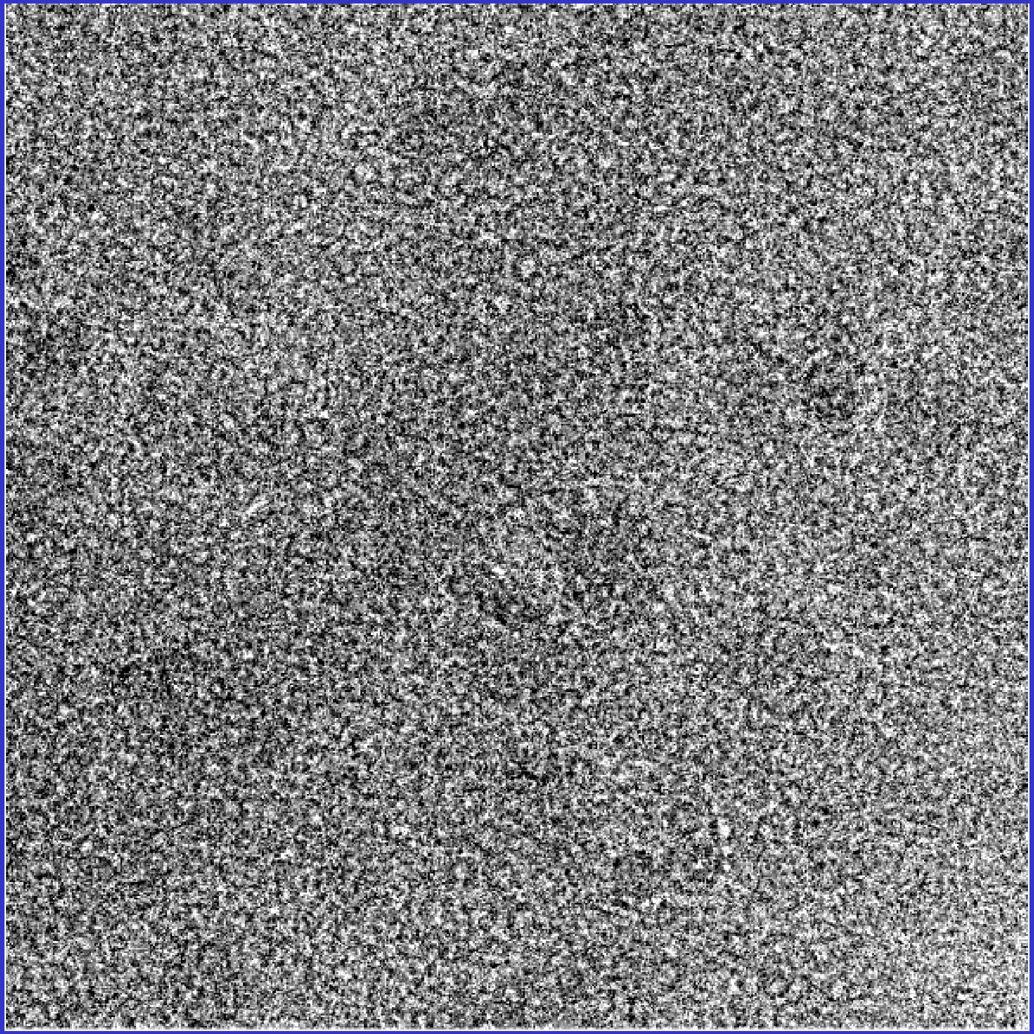
Electron Crystallography of Two-Dimensional Crystals

Image Processing - the Basics

V. Unger, 11/16/03

Low-Dose Image of a Vitrified Gap-Junction 2D-Crystal

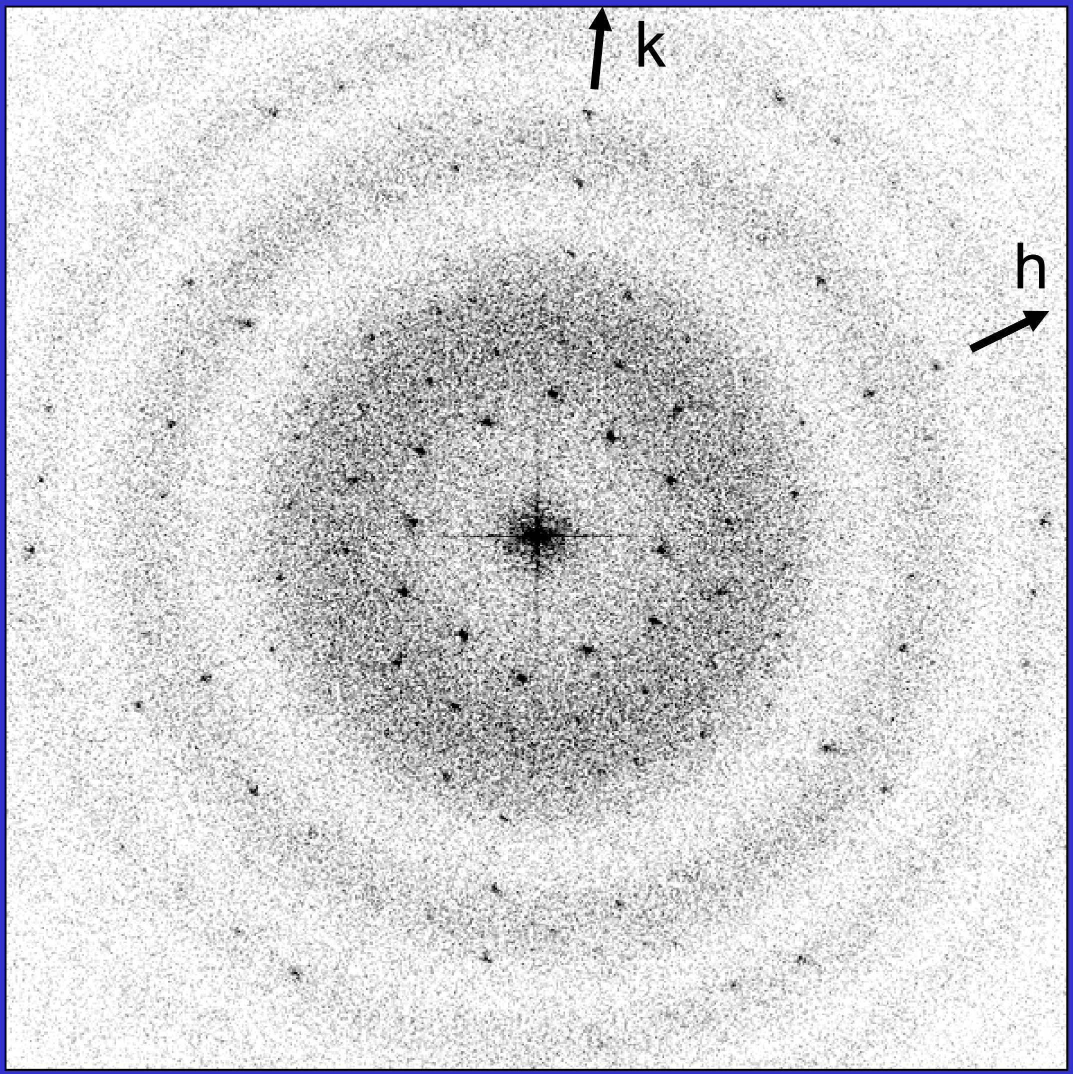


Exposure was ~10 e/Å^2

The low electron dose causes the image to be very noisy.

Therefore, averaging of information from many particles is necessary.

Calculated Fourier Transform of a Vitrified Gap-Junction 2D-Crystal

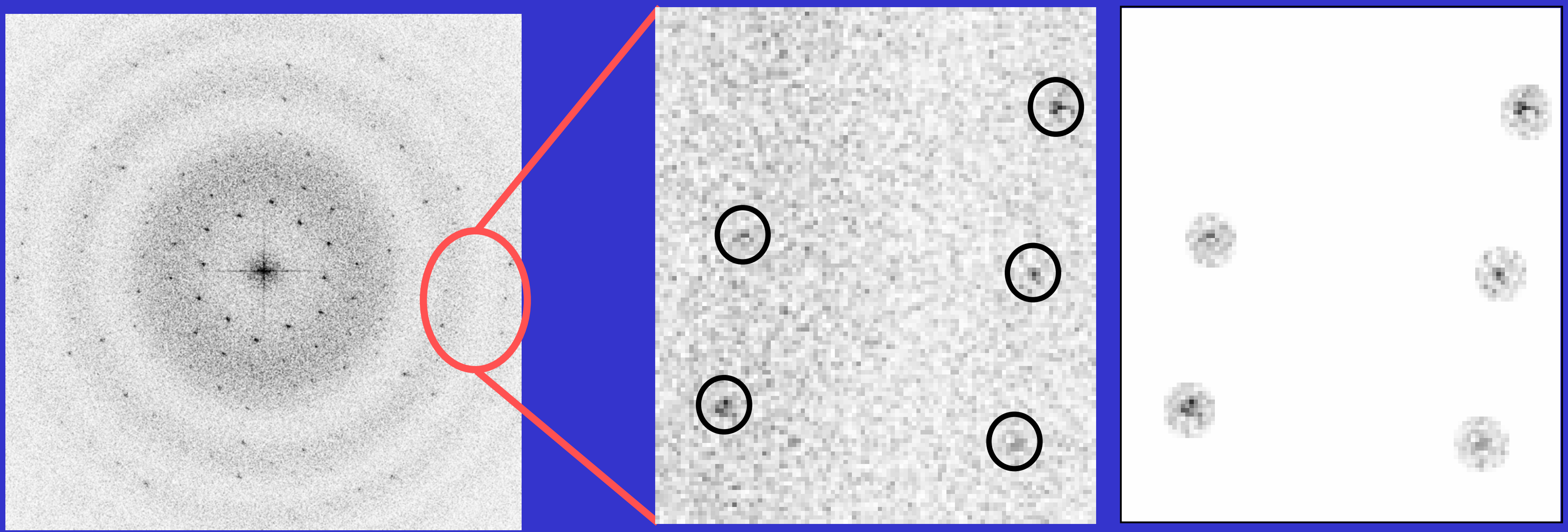


Transform was calculated from image area of 2000x2000 pixel @ 10µm/each

In contrast to X-ray diffraction patterns, diffraction patterns calculated from images contain both amplitude AND phase information for each reflection!

The alternating pattern of bright and dark bands stems from the amorphous carbon film used to support the specimen and reflects a modulation of the diffraction pattern introduced by the objective lens. This modulation is also known as contrast transfer function (CTF) and *per se* has nothing to do with the structure of the molecule whose structure one wants to determine.

Principle of Digital Filtering



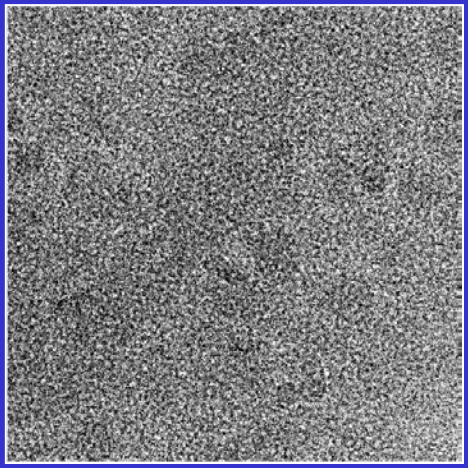
entire FT

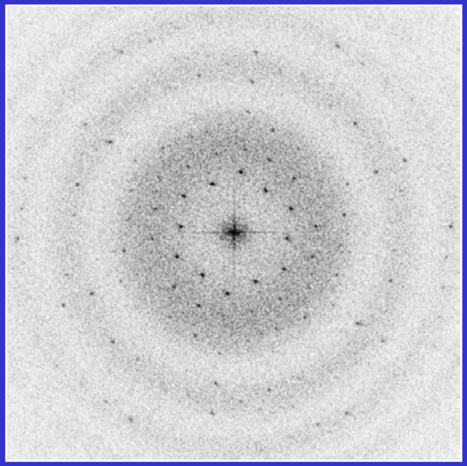
enlarged area of FT

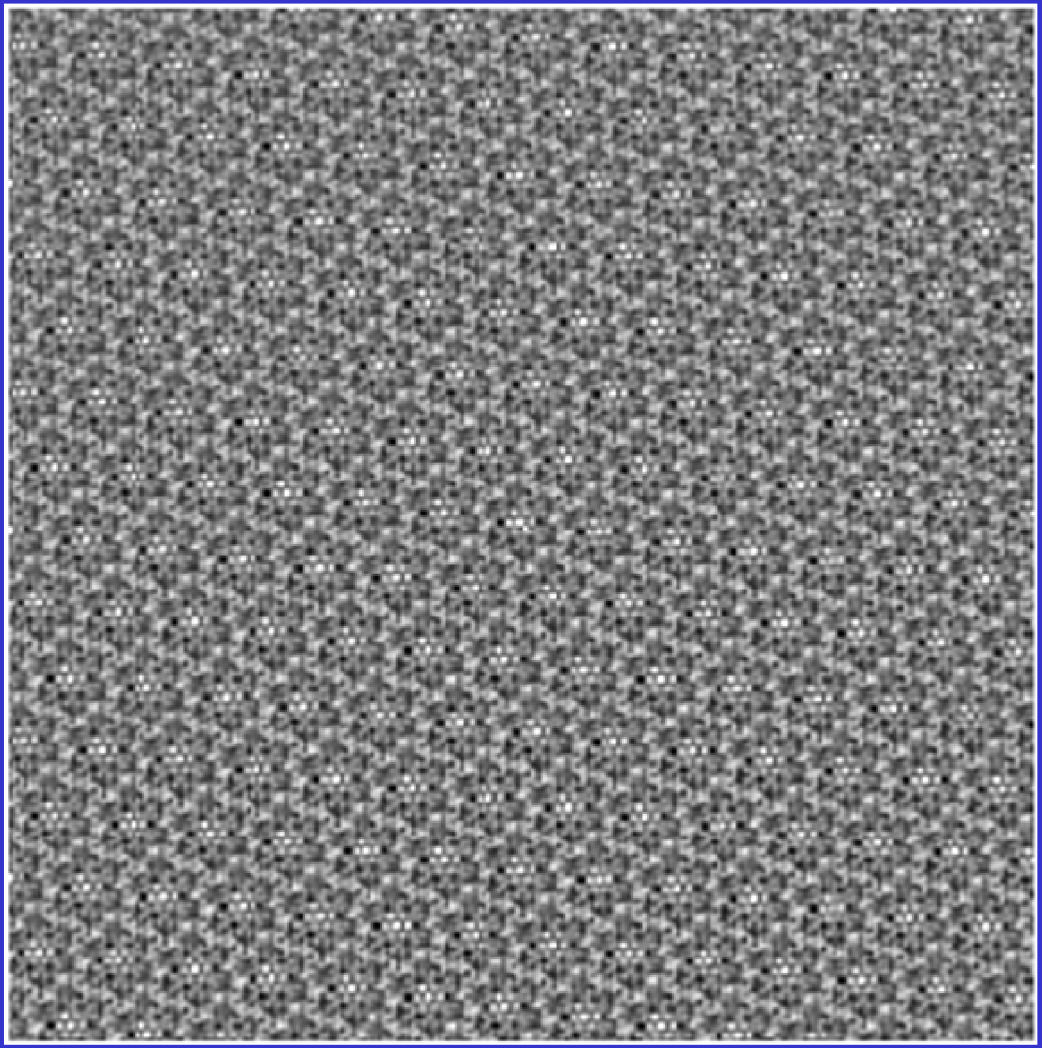
circular maskholes applied (FT has now non-zero values only within maskholes

Digital Filtering of Fourier Transform

Radius used was r=1

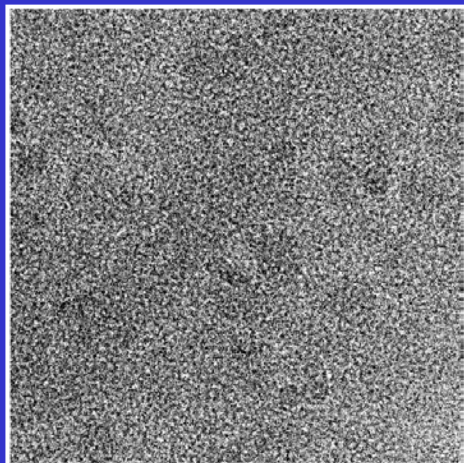


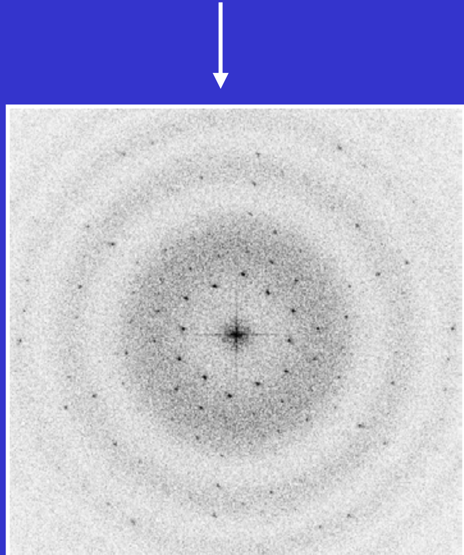


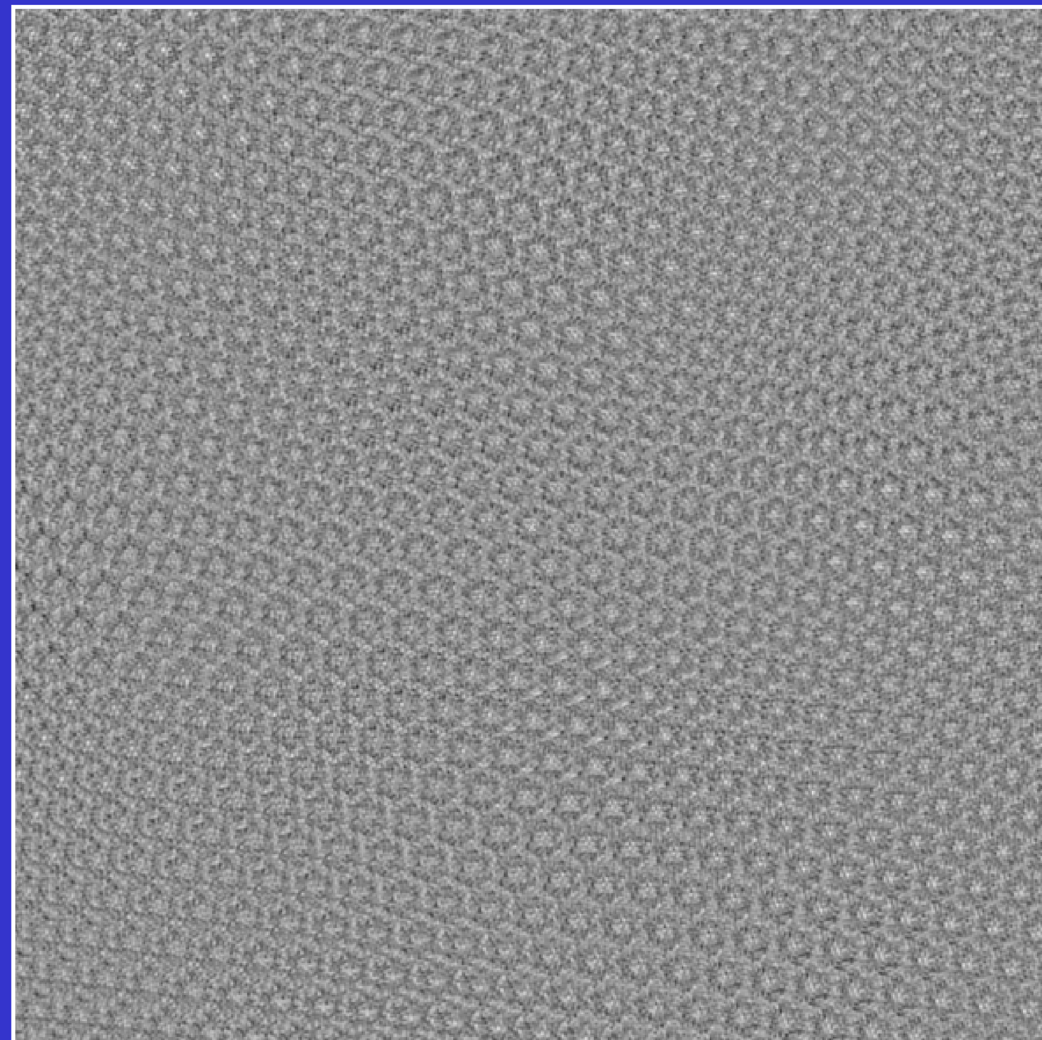


Digital Filtering of Fourier Transform

Radius used was r=7

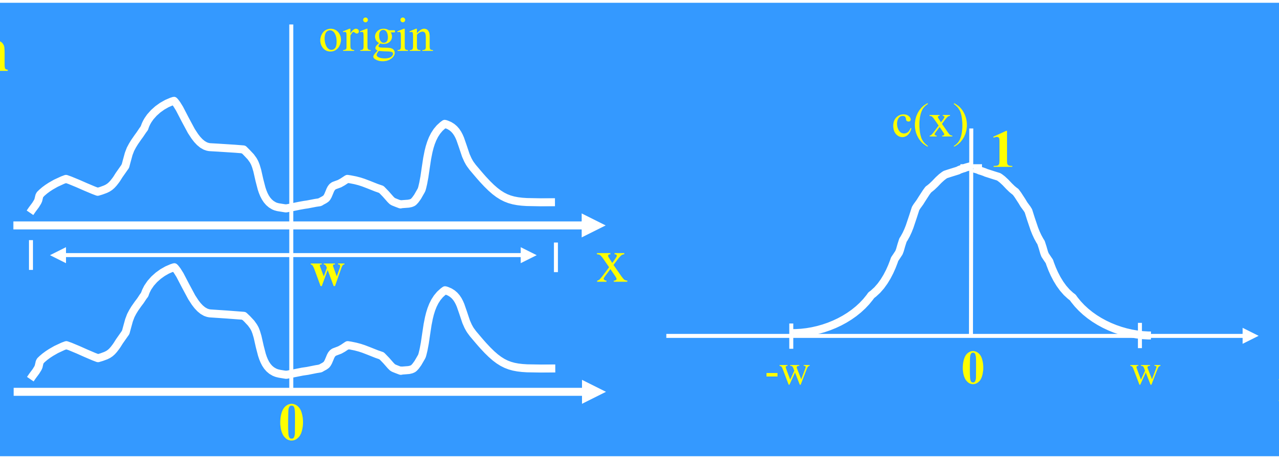






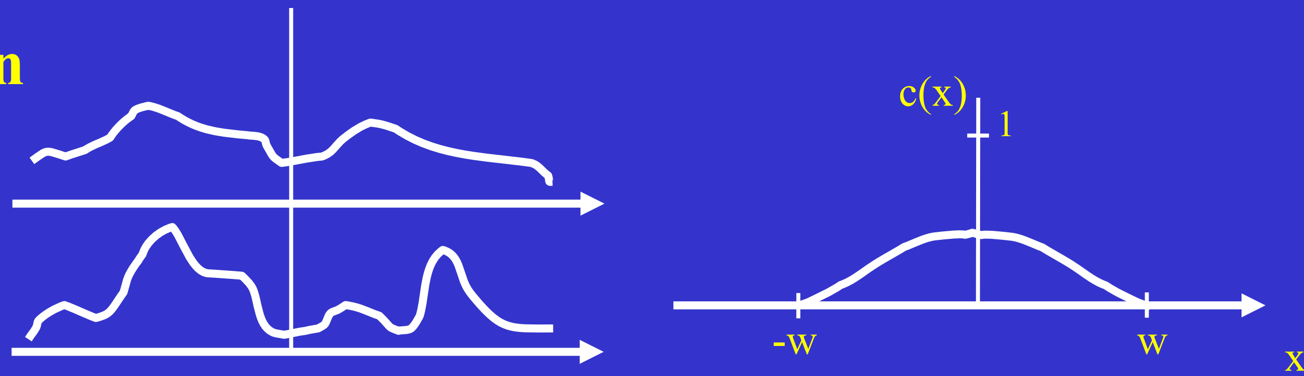
autocorrelation

two copies of
same object

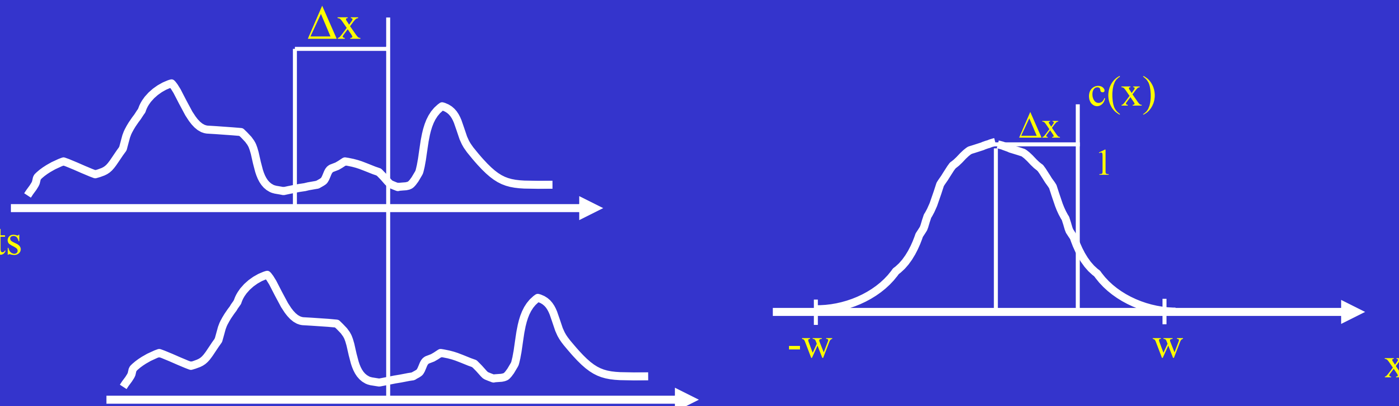


crosscorrelation

two similar objects

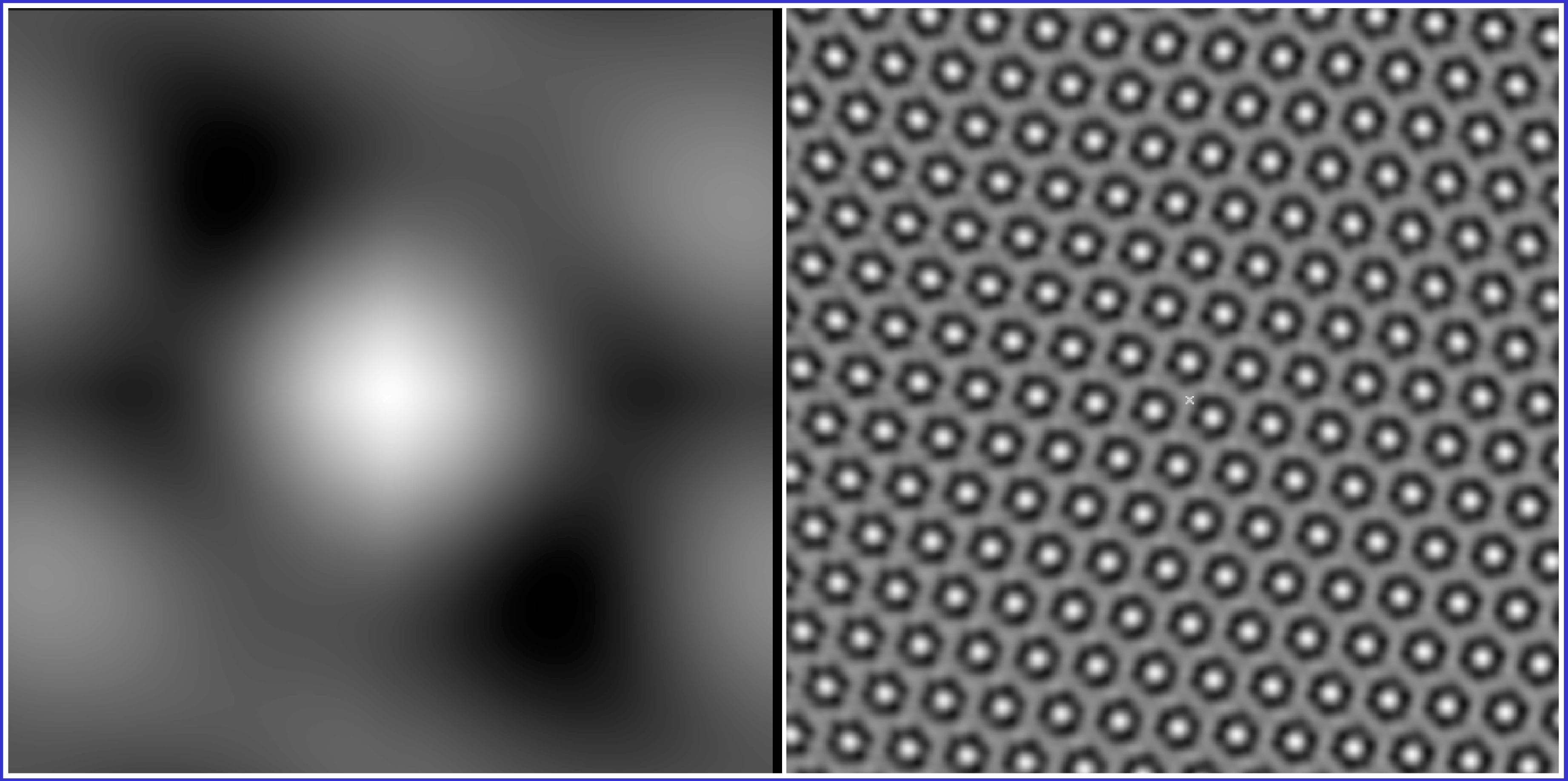


two identical objects
with translational
offset



Autocorrelation Map

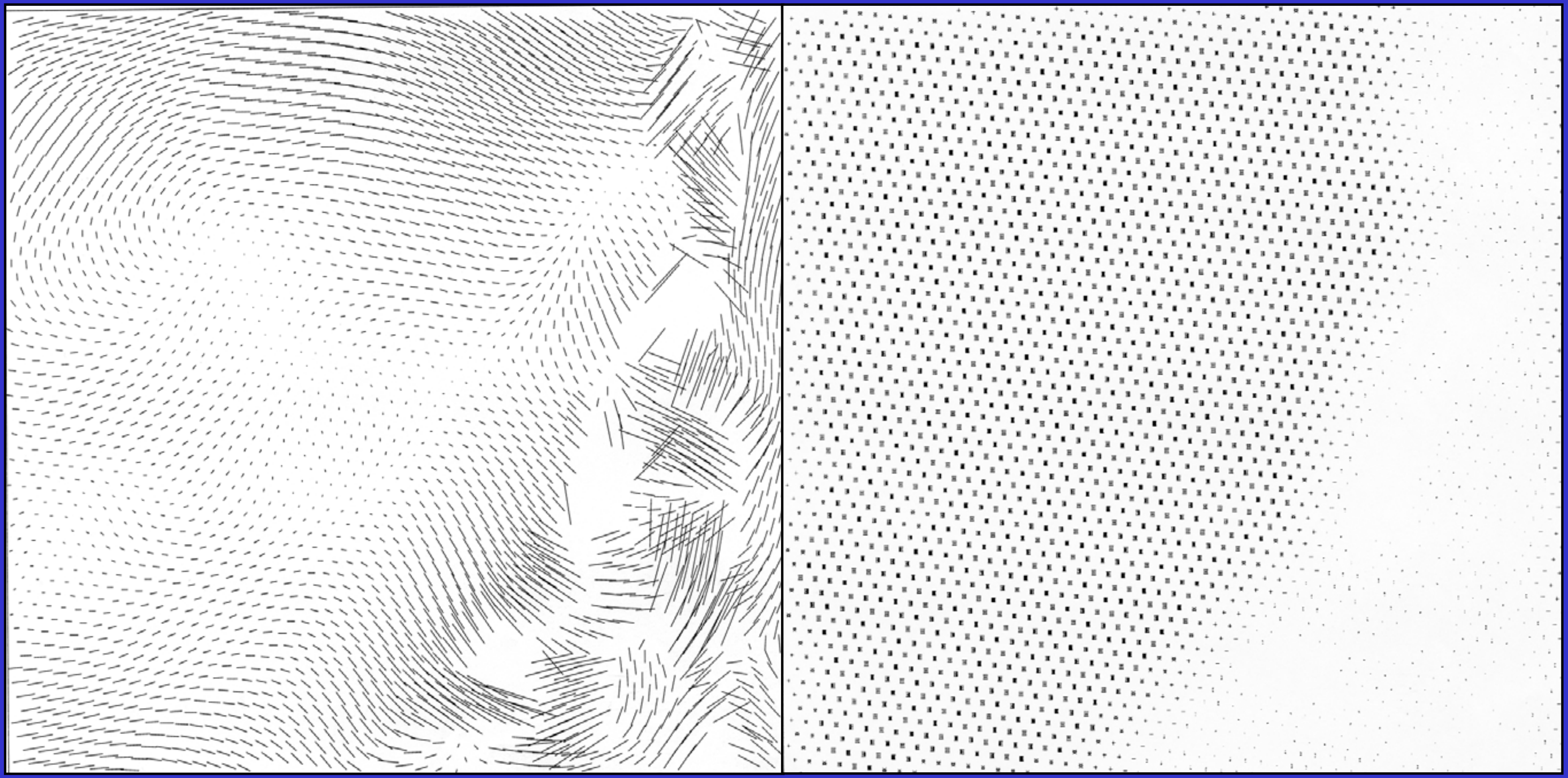
Part of Crosscorrelation Map



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

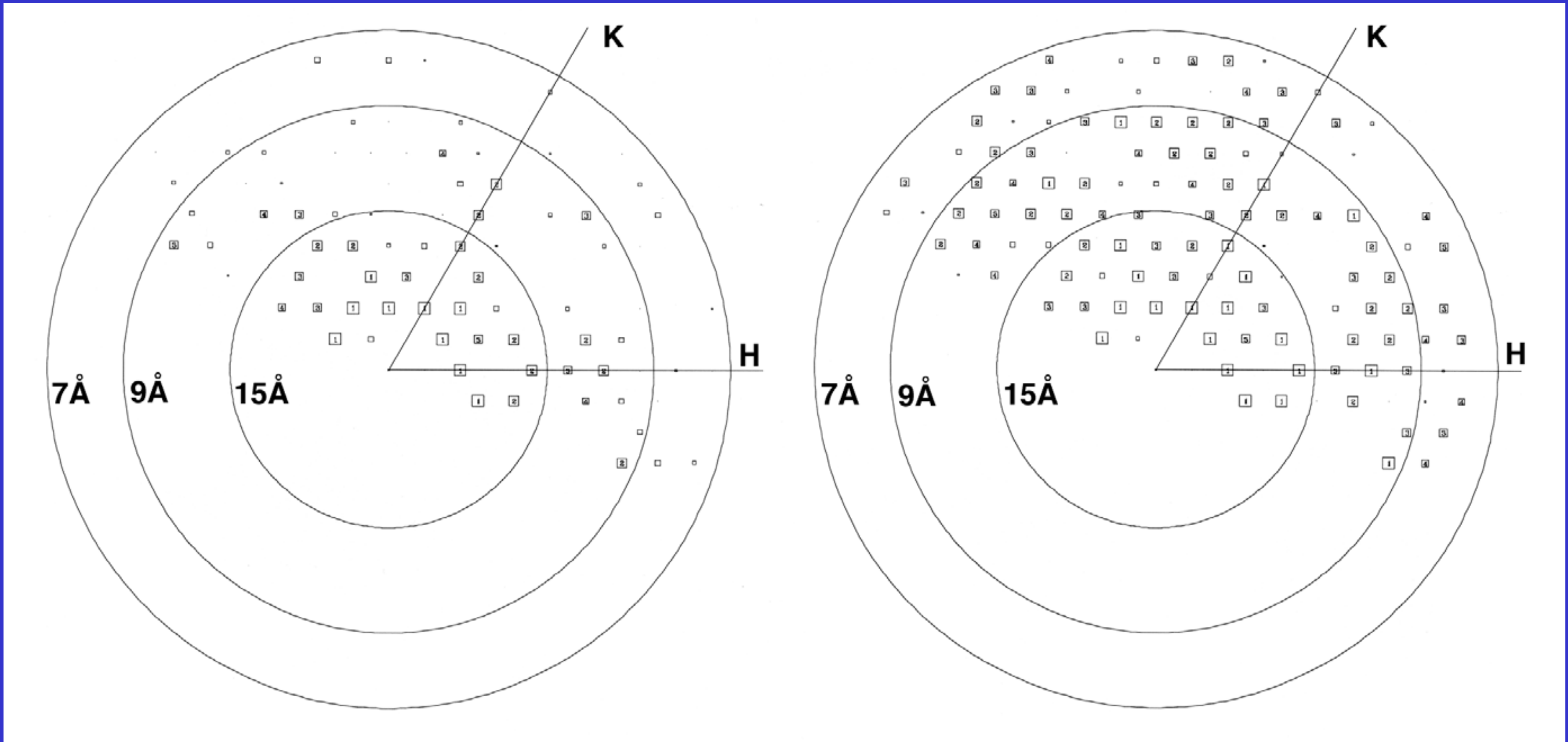
Cross-correlation methods can be used to determine translational disorder in 2D-crystals.



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"

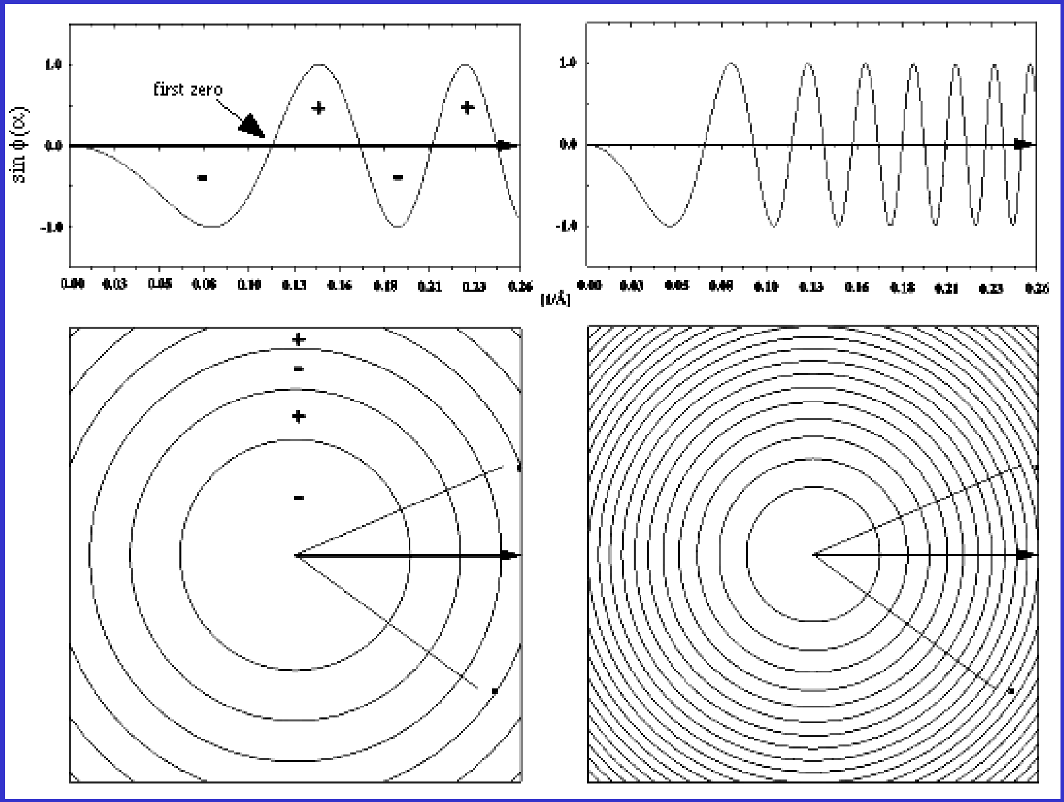


The data shown to the left were retrieved from a calculated FT of an untreated raw image. In this case, the data are not statistically significant beyond ~15Å resolution. However, after correction for translational lattice disorder, the same image provides data out to ~7Å resolution. Plot symbols indicate the goodness of each reflection. Reflections marked by a "1" have a signal-to-noise ratio of at least 8.

The phase shift $\Phi(\alpha)$ introduced to the scattered waves depends on the wavelength (λ), spherical aberration of the objective lens C_s, the diffraction angle (α) and the underfocus (Δf).

$$\Phi(\alpha) = \frac{2\pi}{\lambda}(0.25 \bullet C_s\alpha^4 - 0.5 \bullet \Delta f\alpha^2)$$

This phase shift (also known as CTF= contrast transfer function) modulates the FT of the image in an oscillating manner described by [sin $\Phi(\alpha)$].



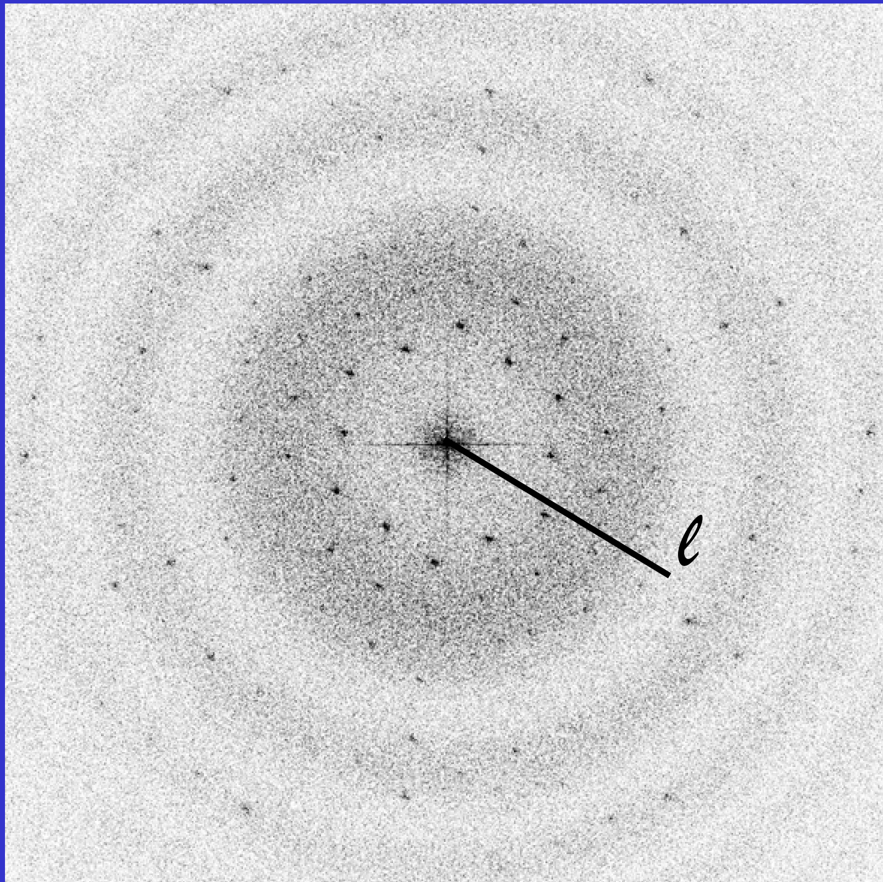
The simulated curves are for 3000 and 6000Å of underfocus respectively, an accelerating voltage of 200keV (λ=0.025Å) and a C_s=2mm

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°

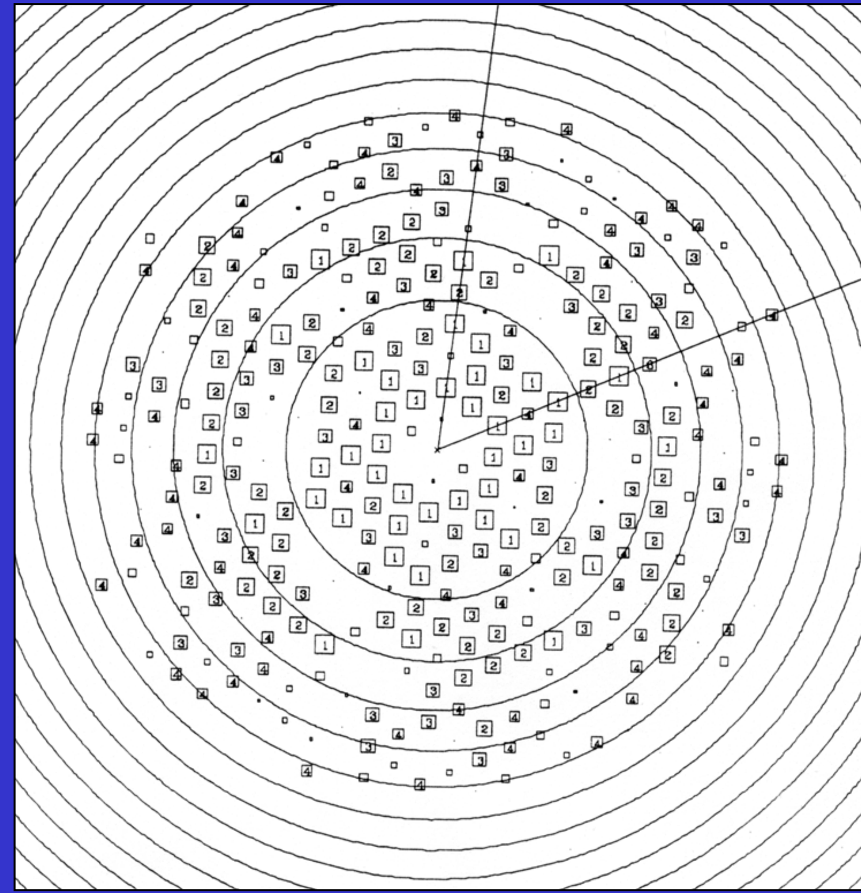
CTF-correction



Calculated FT of image

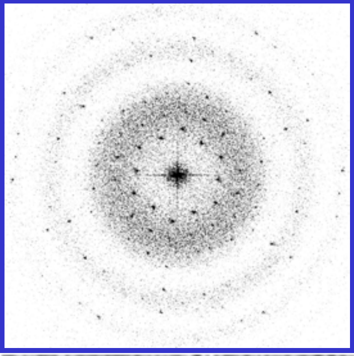
$$1/d[\mathring{A}^{-1}] = \frac{\ell \bullet XMAG}{p_y \bullet step[\mu m] \bullet 10{,}000}$$

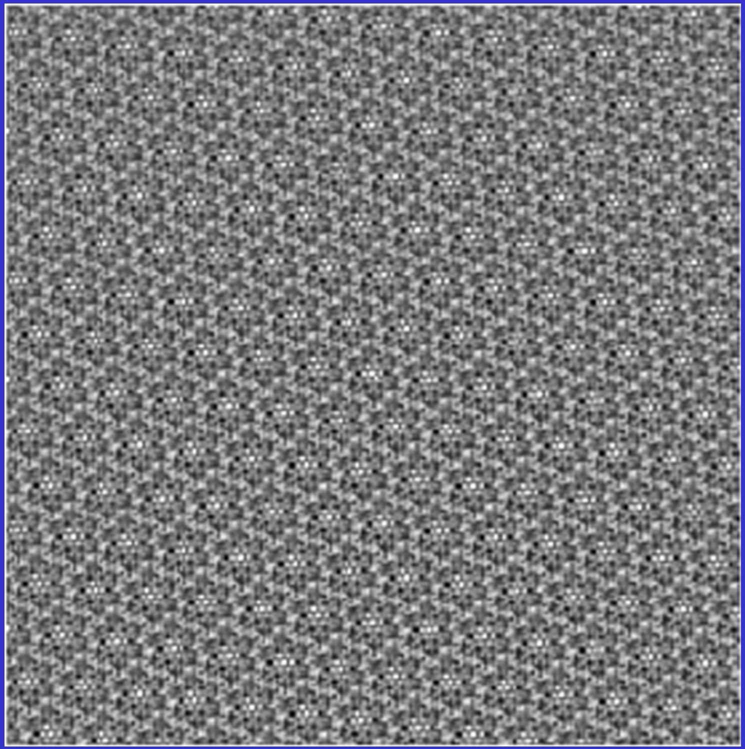
Xmag: magnification
p_y: transform size (y-axis)
step: digitizing stepsize

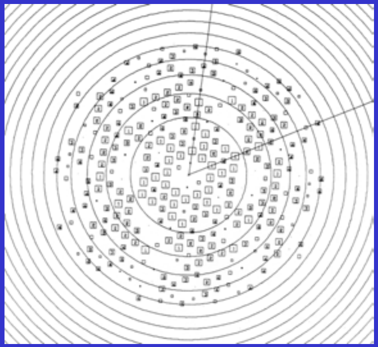


For the correct estimate of the underfocus, the simulated CTF will match that actually observed in the FT of the image

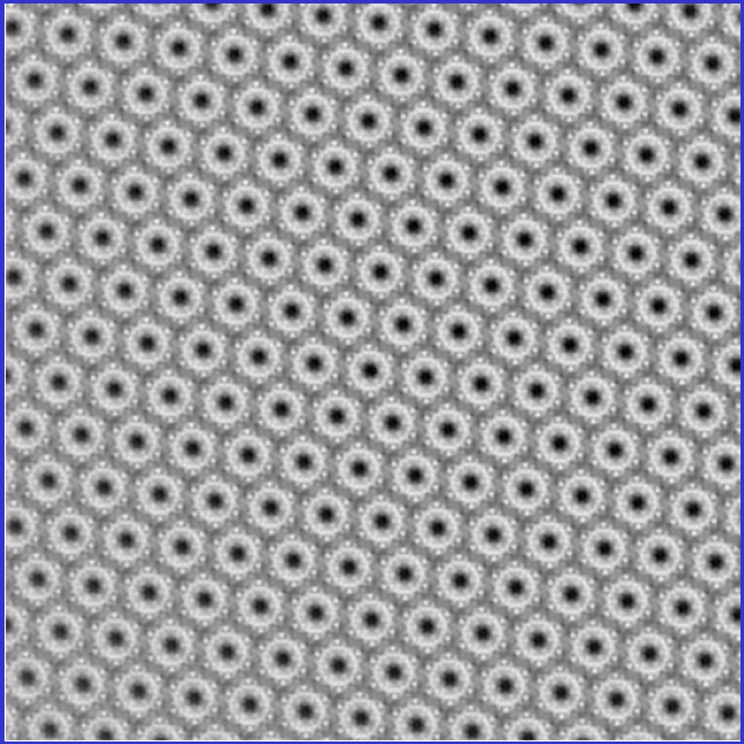
Why Do Data Need to be Corrected For CTF?

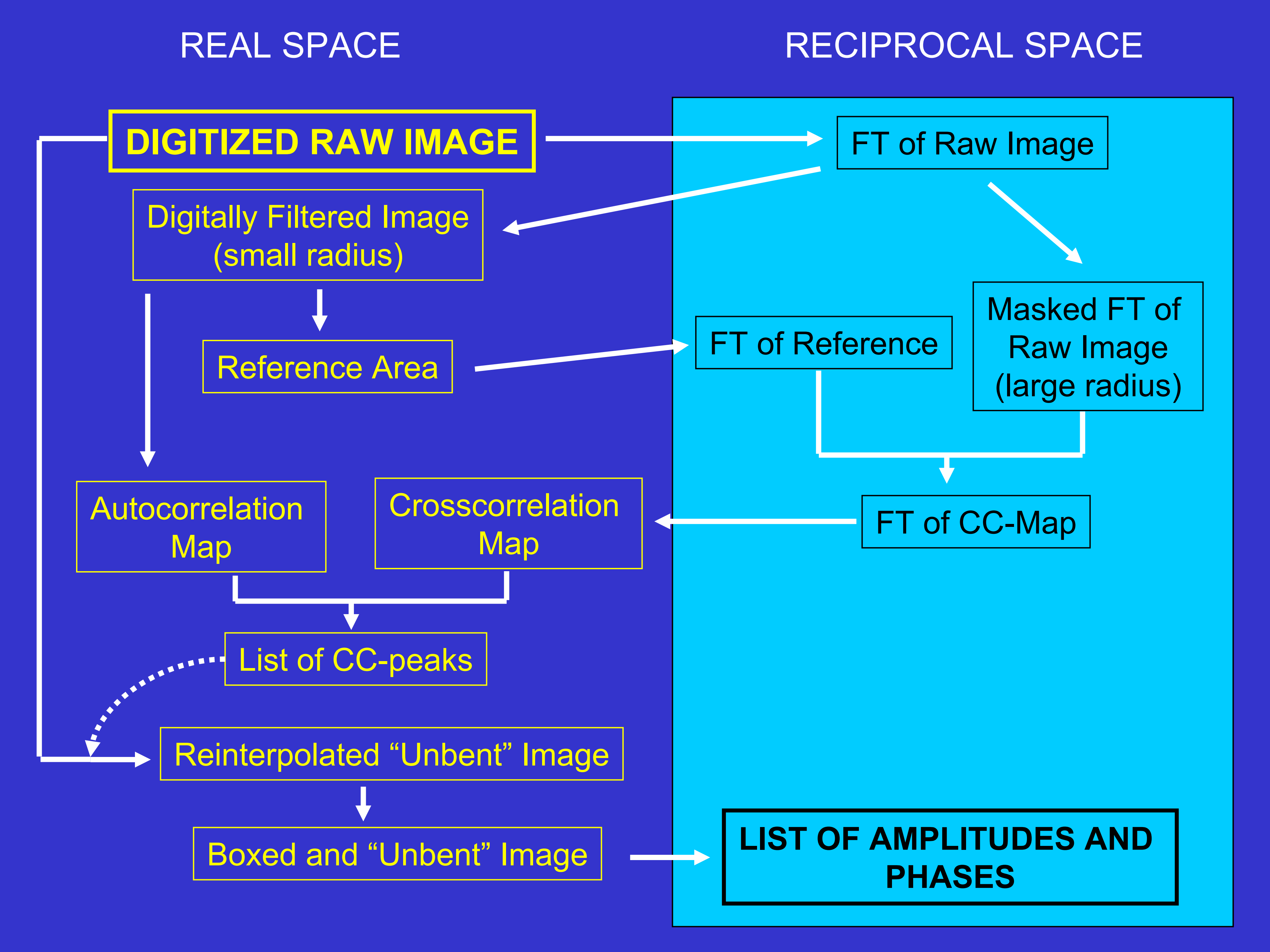




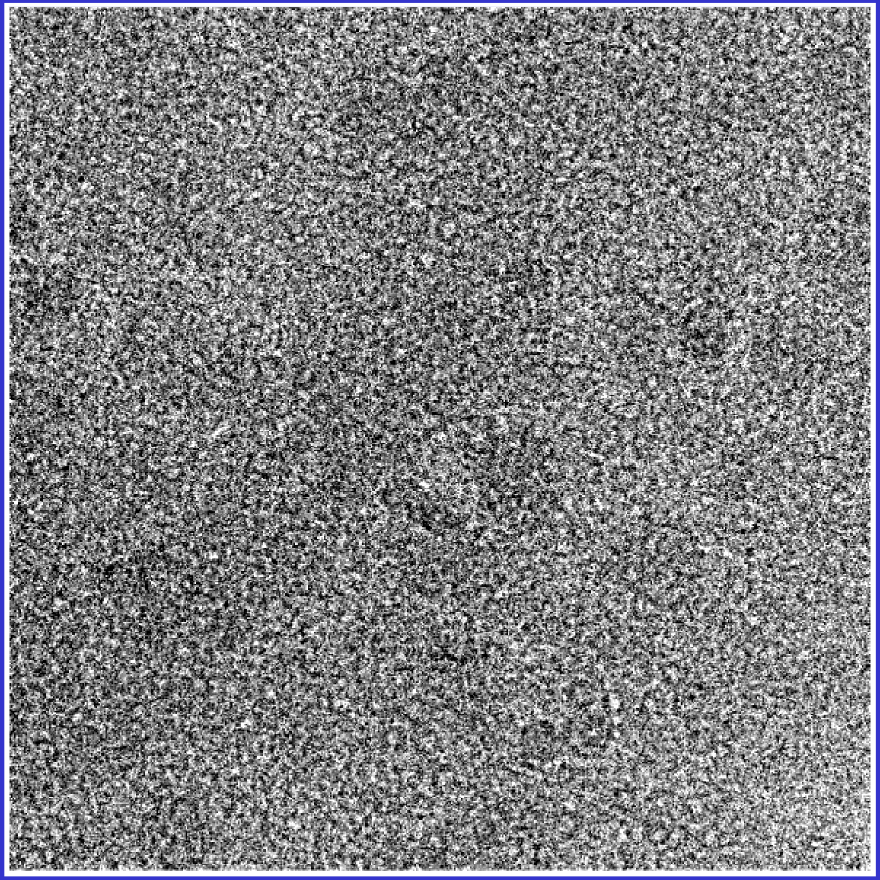


After Correction of Phases For CTF




REAL SPACE
RECIPROCAL SPACE
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



(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….

REAL SPACE

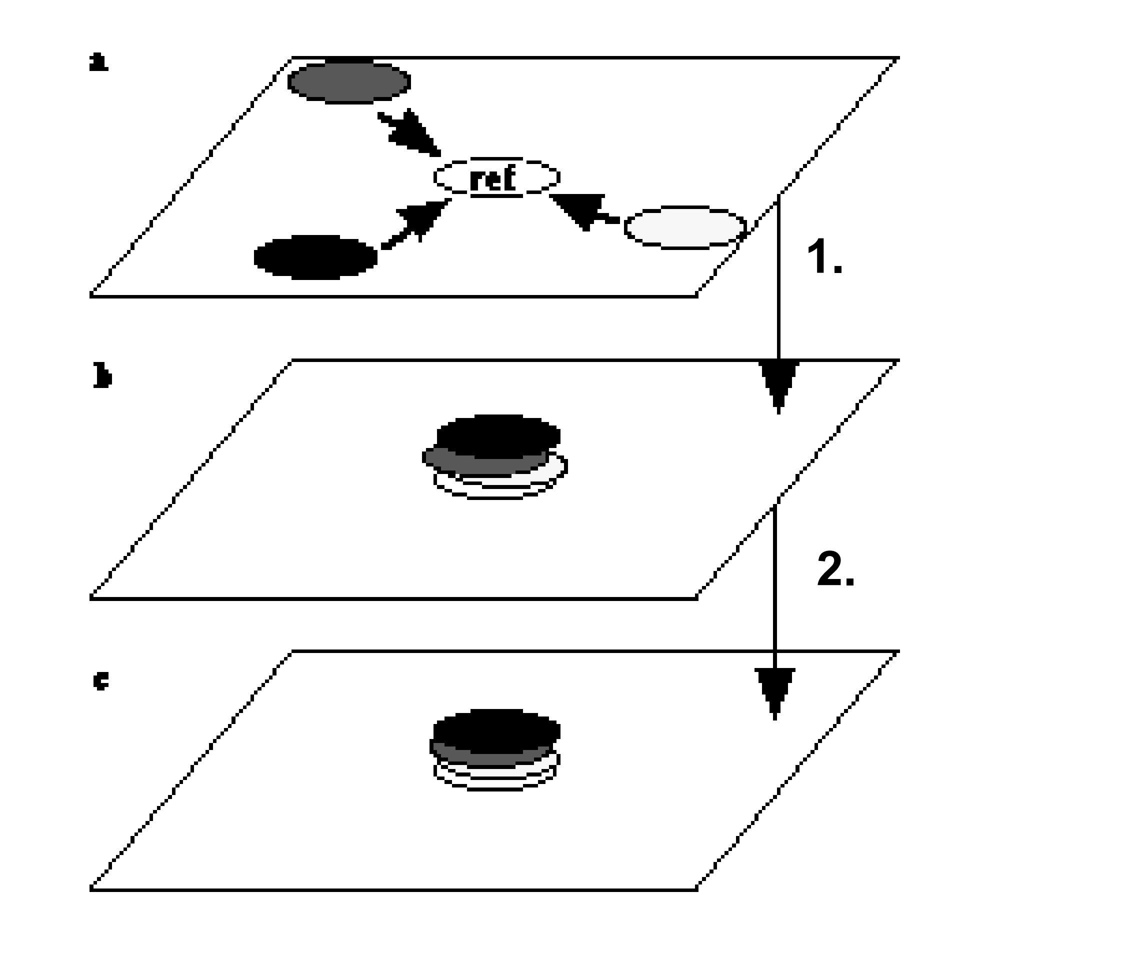
RECIPROCAL SPACE

Symmetry is Beautiful

phase information obtained from the calculated FT of the images contains information about the positioning of molecules within the area of the unit cell as well as information about symmetry.

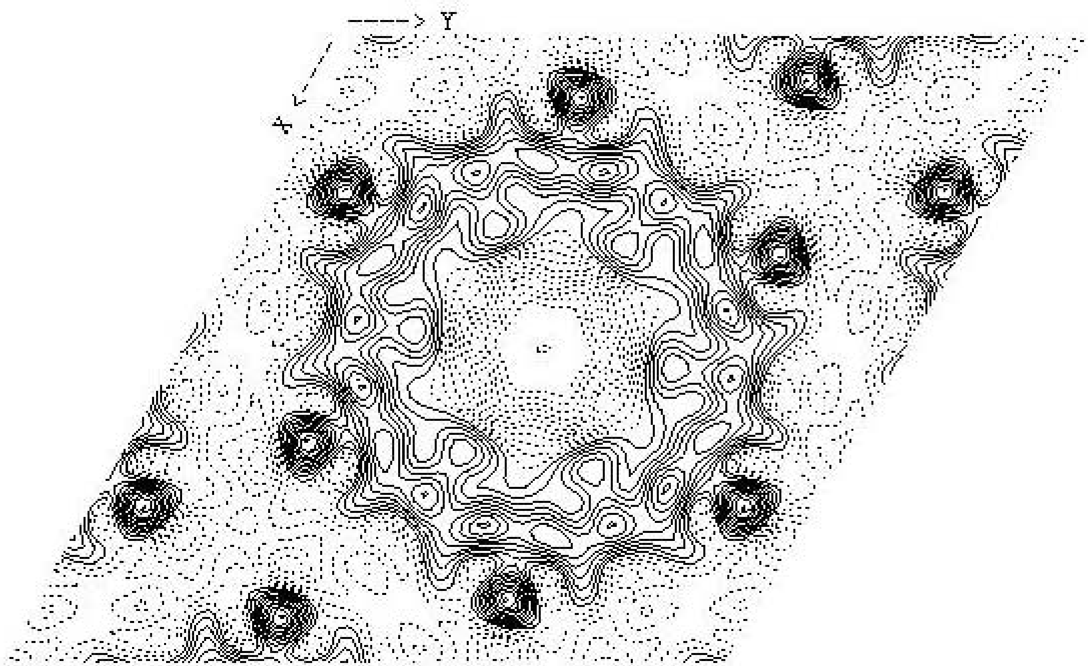
Moreover, presence of crystallographic symmetry facilitates data collection and merging because it causes redundancy in the information. The “mirror symmetry” in faces commonly considered “pretty” is just one odd example.

Alignment of Data from Independent Images



1. **Find common phase origin by comparison with data from single untilted crystal**
2. **Iteratively merge and refine phase origins of each image against average of all others**

Projection Density Map and some of the Corresponding Structure Factors

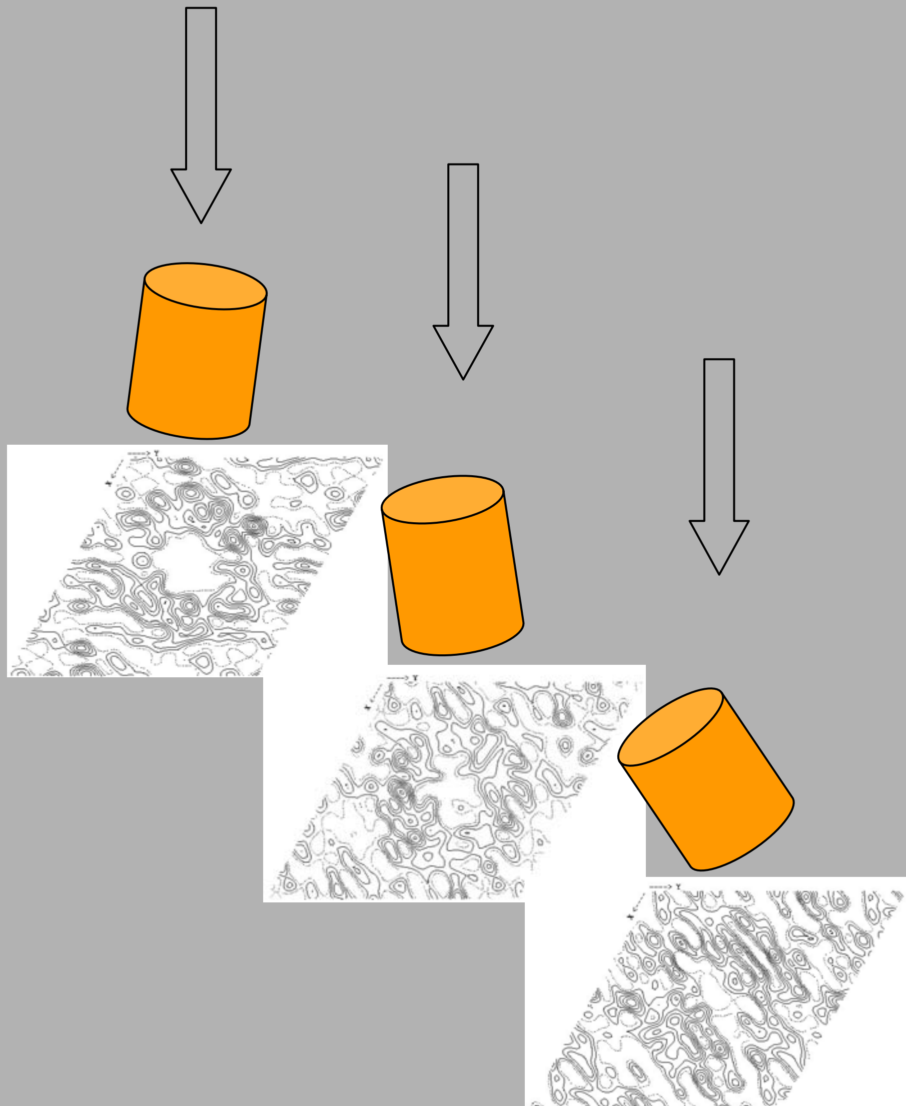


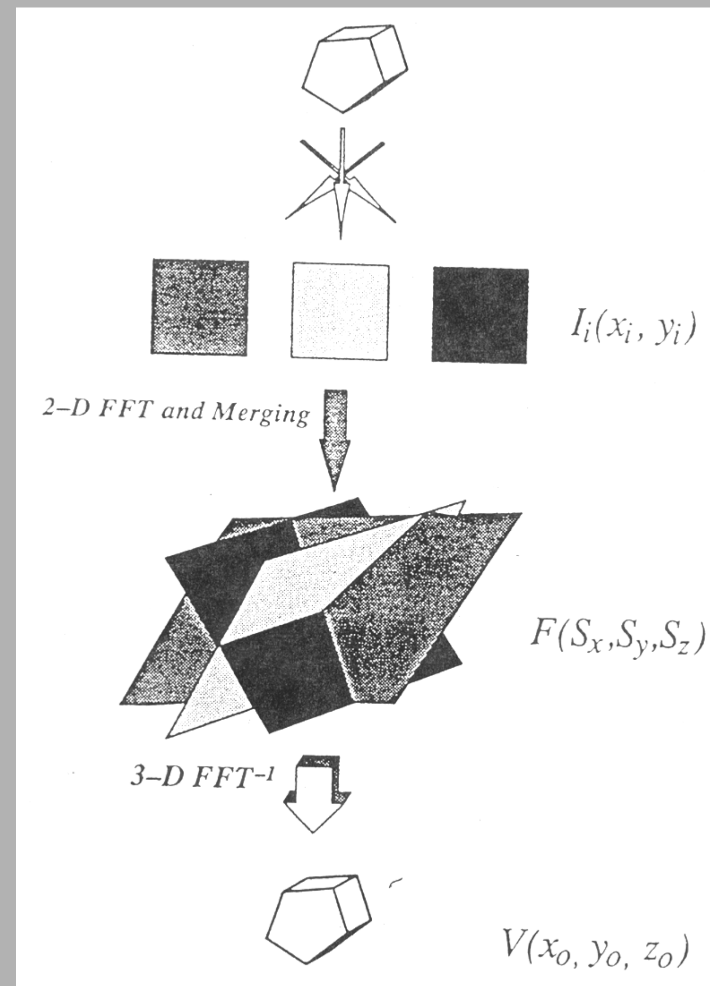
Real space map obtained by Fourier summation

(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

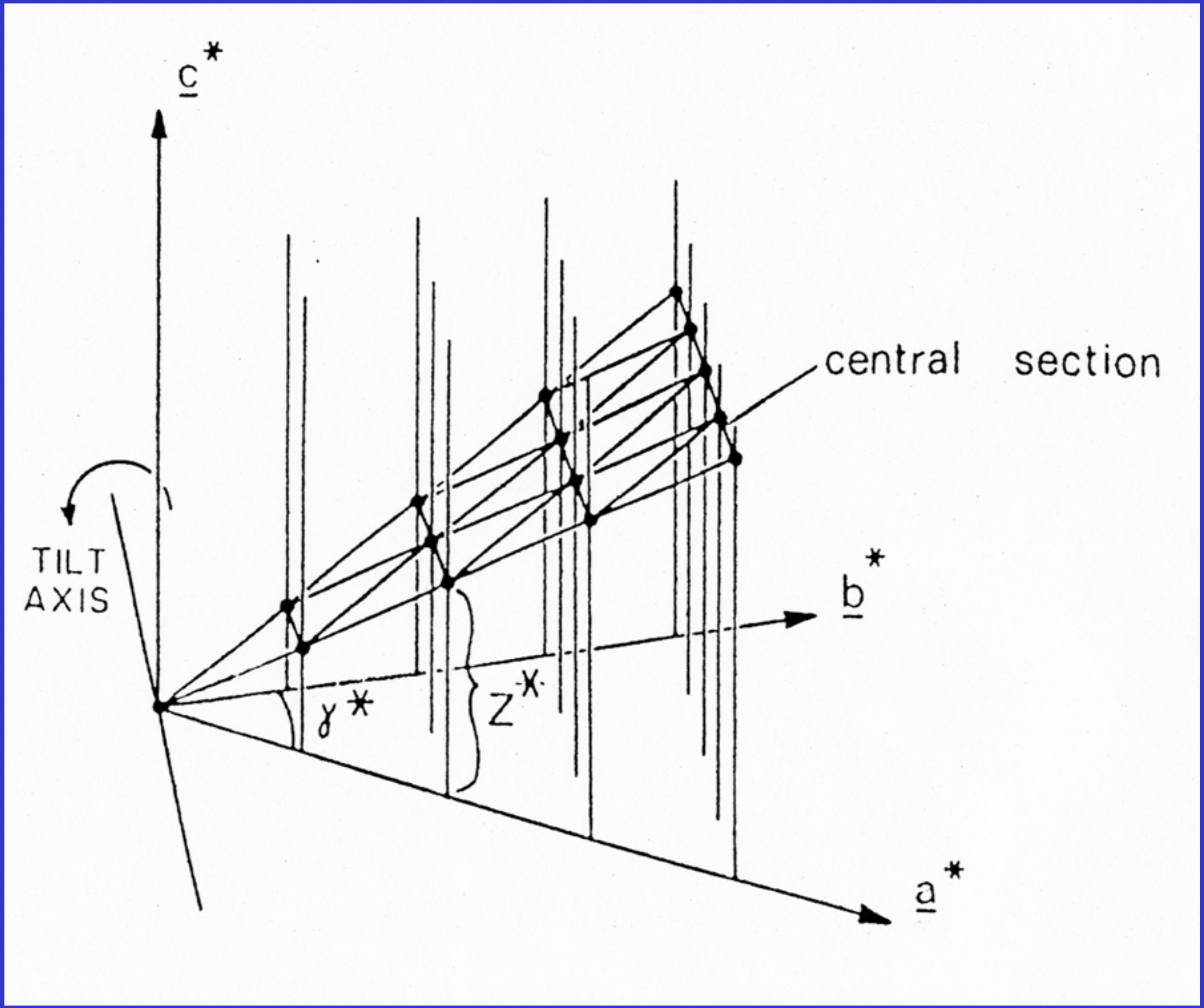
And so forth…..

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



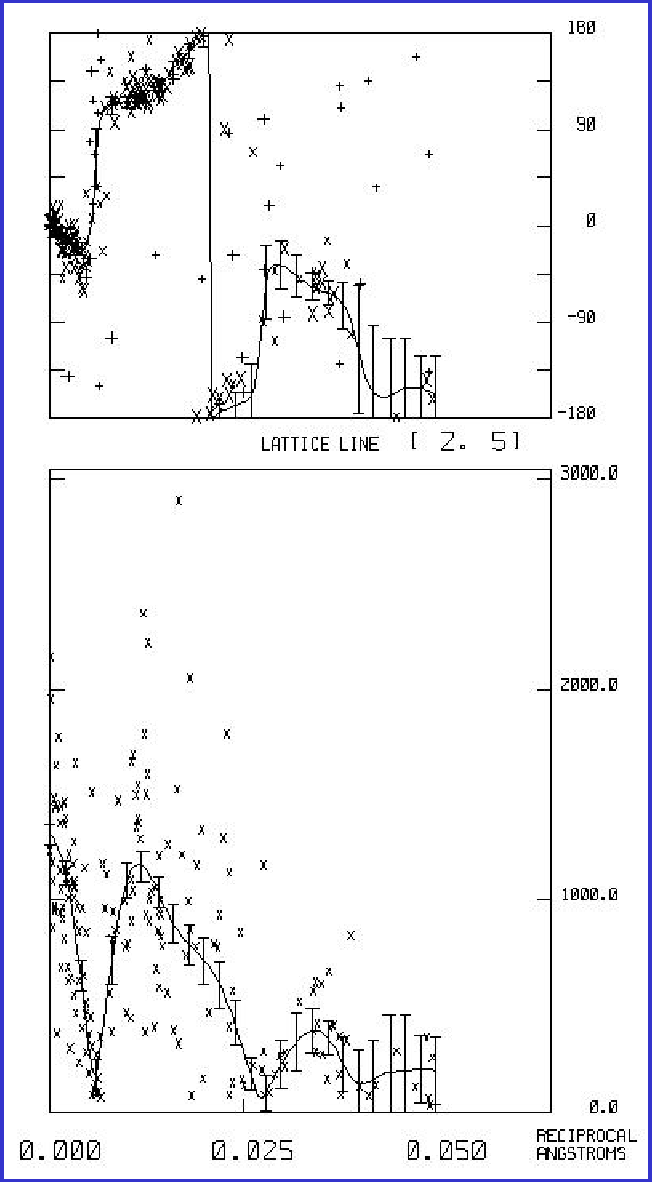


Concept of Lattice Lines and Principle of Sampling their Data



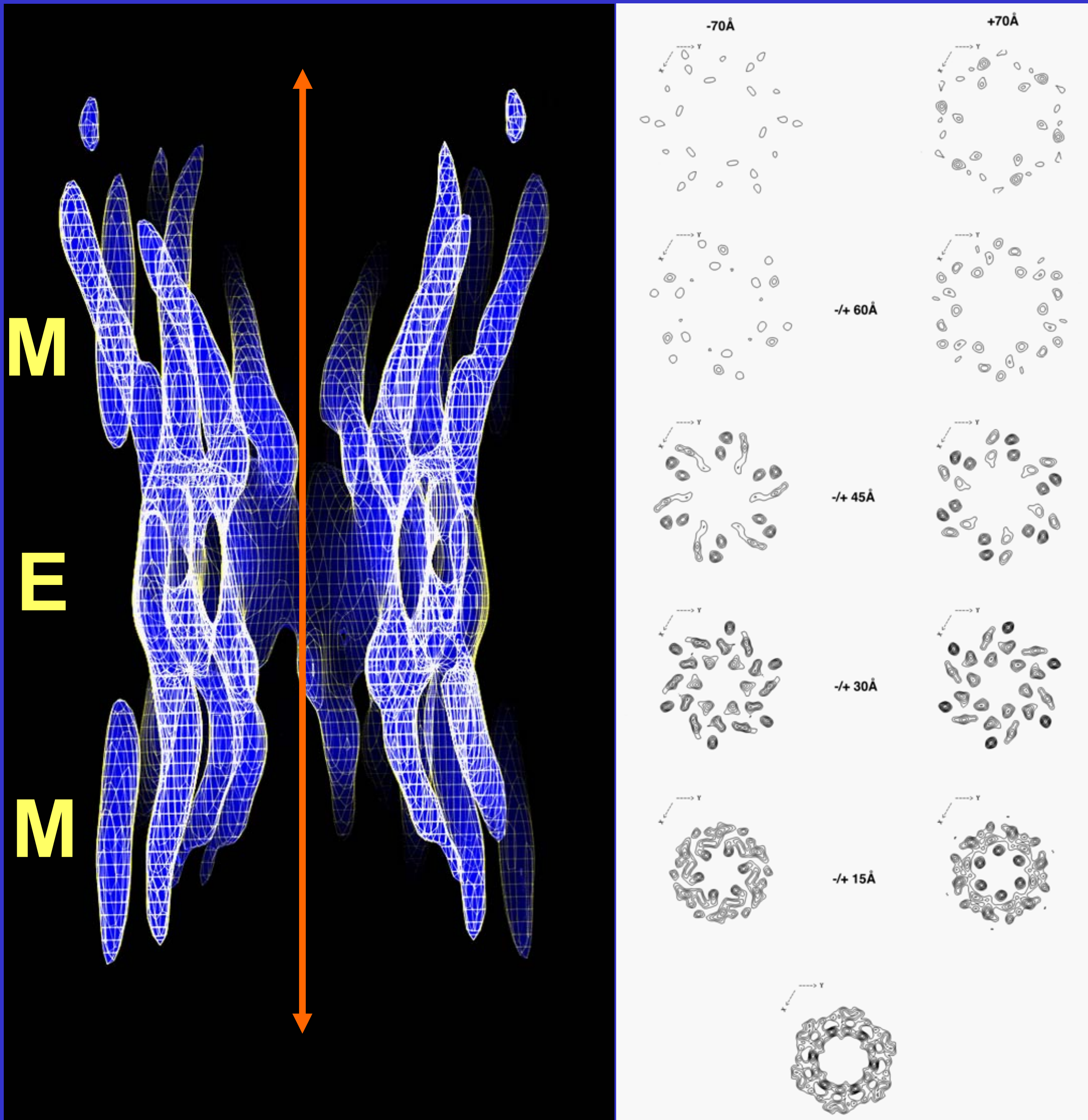
Taken from:
Amos, Henderson and Unwin (1982), Prog. Biophys Molec Biol 39:183-231

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**.

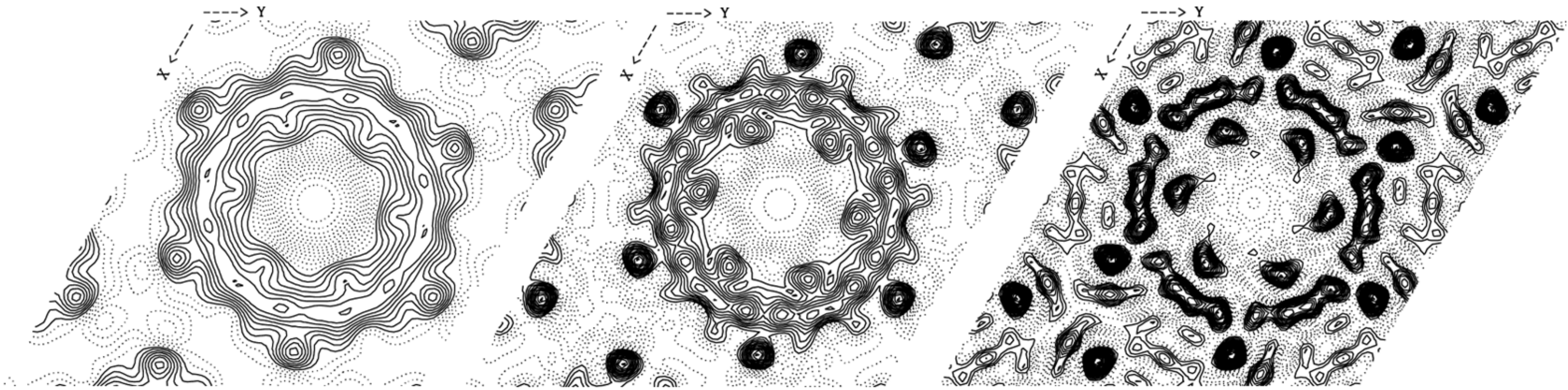


3D-Map of a Gap-Junction Intercellular Channel

Shown are a surface representation at ~7.5Å resolution (left) and a montage of individual cross-sections (taken from a map at ~5.7Å resolution). A total of ~42,000 channel molecules were crystallographically averaged to obtain this structure.

THE END

Effect of Image Sharpening (B-factor)



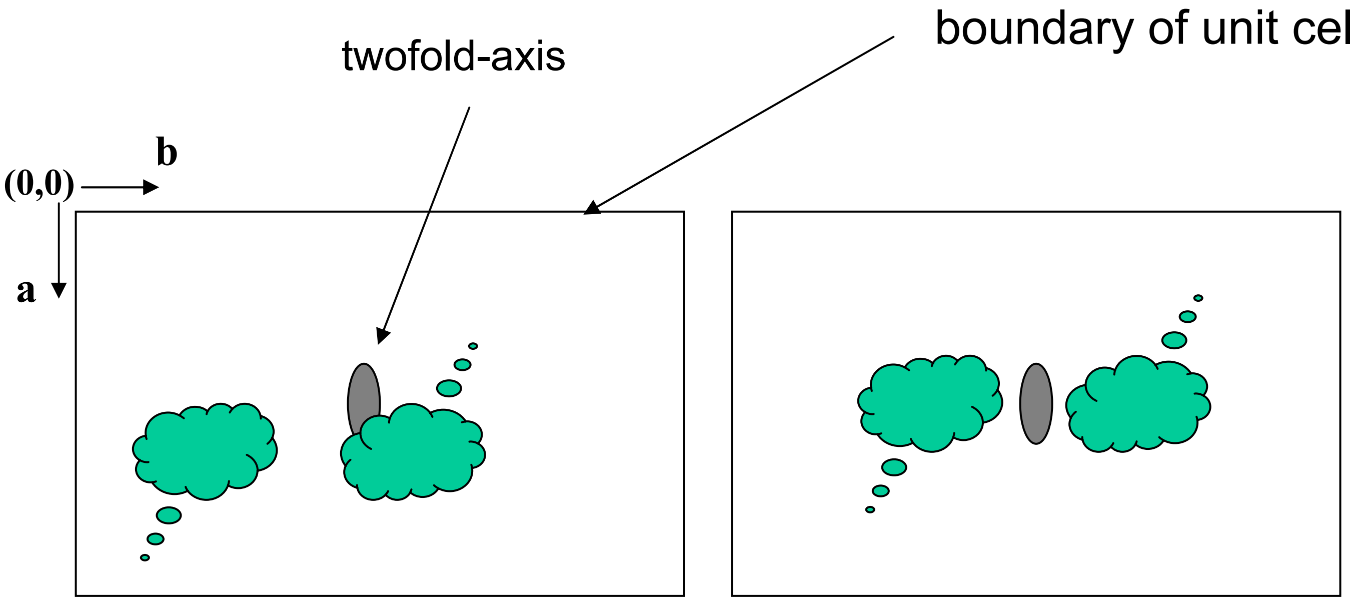
B=0 | B= -350 Å^{-2} | unity amplitudes

$$sf = e^{-B/4d^2}$$

d= resolution [Å]

→ Example: 6Å, B=-350
sf=11
Compare: unity amplitude
sf=110 B=-680

Principle of Symmetry Determination



Arbitrary location of dimer within unit cell. Apparent symmetry: p1; no centrosymmetric phase constraints are obeyed!

Dimer centered about twofold axis of symmetry. Centrosymmetric phase constraints are obeyed = phases of all reflections are real (ie 0˚/180˚)

<u>To test for 2-fold symmetry:</u> calculate phases for all reflections for all possible positions of the dimer within the boundary of the unit cell and check whether all phases become real. If this condition is met for any location within the unit cell then the crystal has at least twofold symmetry.

Bringing Symmetry Related Reflections to a Common Phase Origin

Reflection (h,k)	Phase extracted from calculated FT	Phase after shifting to proper phase origin
(1,2)	198.0û	3.1û
(2,-3)	32.5û	1.0û
(-3,1)	198.2û	-0.4û

In p6 triplets of symmetry reflections all adopt the same phase when centered at the correct sixfold origin. Brought to this phase origin, the phase of each of the symmetry related reflections has to be close to either 0û or 180û because the inherent twofold symmetry (p6 symmetry implies p3 and p2 symmetry) constrains phases to be real in projection.

```
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
 0111100000010000000135531000000000000
 0012100000000000000257520000000100000
 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