Electron Crystallography Workshop
Basel, August 2008

Richard Henderson

Image processing in 2D: MRC software for real space images

MRC programs Unbending

5 main steps

after acquisition of images

before interpretation of map

1.Processing of individual images in 2D (CCUNBENDK, MAKETRAN, etc)

2.Merging (ORIGTILTK) image amplitudes and phases

3.Electron diffraction integration (BACKAUTOK, AUTOINDEXK, PICKPROFK)

4.Merging electron diffraction data (MERGEDIFF)

5.Combining image and electron diffraction data (LATLINEK)

5 main steps

after acquisition of images

before interpretation of map

1.Processing of individual images in 2D (CCUNBENDK, MAKETRAN, etc)

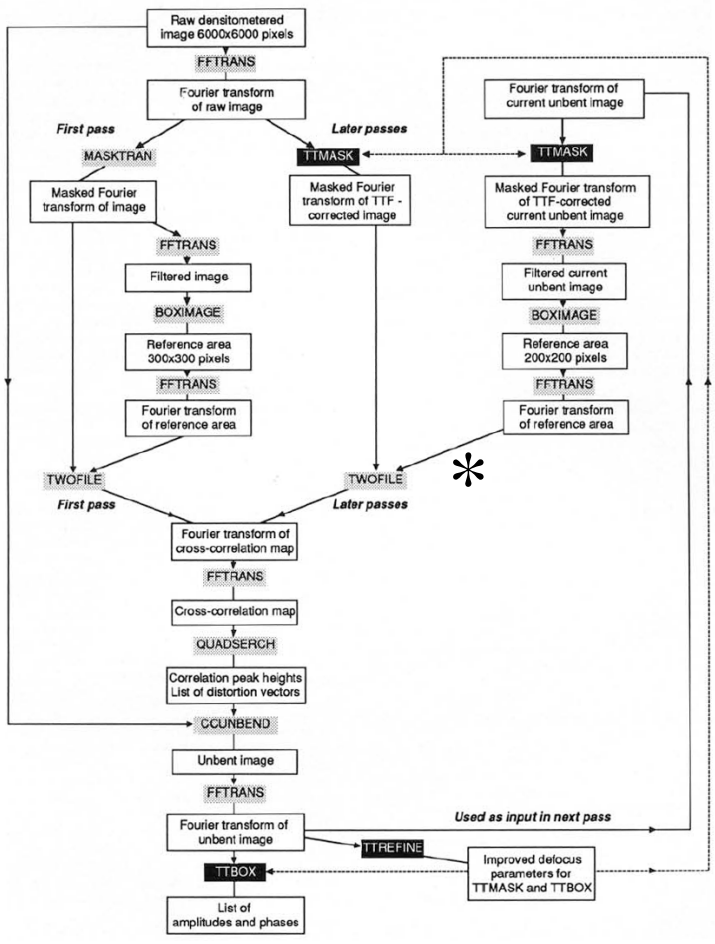
2.Merging (ORIGTILTK) image amplitudes and phases

3.Electron diffraction integration (BACKAUTOK, AUTOINDEXK, PICKPROFK)

4.Merging electron diffraction data (MERGEDIFF)

5.Combining image and electron diffraction data (LATLINEK)

Aim to combine graphical plots with concise summaries



Henderson et al (1990) JMB

Dashed lines - information
Full lines – data

* Could use MAKETRAN for reference area without any defocus

Kunji et al (2000) PNAS

TABLE I

Brief Description of Current MRC Programs

Two-dimensional crystals			
1.	EMTILT		Calculate tilt angles from lattice parameters.
2.	MASKTRANA		Mask transform for filtering, like TRMASK.
3.	AUTOCORRL		Autocorrelation calc + expansion − use with QUADSERCH.
4.	QUADSERCHB		Correlation peak search on lattice, with profile fit.
5.	CCUNBENDD	K	Unbend image using list of peaks from QUADSERCH.
6.	MMBOX	A	Read amplitudes and phases from transform and display surrounding box.
7.	MMLATREF		Lattice parameter refinement based on MMBOX.
8.	TTBOX	K	Correct for tilted transfer function, gives amplitudes and phases.
9.	TTMASK		Combined MASKTRAN and TTBOX, masking + TTF corr on tilted images.
10.	TTREFINE	A	Refine defocus, astigmatism, tilt params on tilted images.
11.	TTBOXREF	Defunct	Lattice parameter refinement on images with TTF correction.
12.	CTFSEARCH	A	Refine or search for correct defocus, astigmatism, on data from untilted images.
13.	CTFAPPLY	K	Apply CTF to data from MMBOX, with graphical output.
14.	ORIGTILTC	K	Combine data from different images using crystal symmetry.
15.	LLFILT		Lattice line fitting for smooth curves and structure factors.
16.	LATLINED	K	Agard's least squares latline fit of amplitudes and phases.
17.	ALLSPACE	A	Determine space group, origin, beamtilt on single image.
18.	AVRGPHASES		Overall averaging of projection data from ORIGTILT.
19.	AVRGAMPHS		Overall averaging of amplitude and phase projection data.
20.	MAKETRAN		Create reference transform from MTZ file with given defocus.
21.	SCALIMAMP3D		Scales image amplitudes to selected reference data.
(1, Shaw and Hills (1981); 4,5,12,13,14,16, Henderson *et al.* (1986); 8,9,10, Henderson *et al.* (1990); 14, Amos *et al.* (1982); 16, Agard (1983); 17, Valpuesta *et al.* (1994); 21, Havelka *et al.* (1995))			
Electron diffraction patterns			
1.	BACKAUTO	K	Calculate radial background and find centre of pattern.
2.	AUTOINDEX	K	Find two simplest lattice vectors automatically.
3.	PICKAUTO	K, PICKPROFK	Integrate and background correct electron diffraction spots.
4.	MERGEDIFF		Merge e.d. data and do a host of corrections.
5.	AVRGFDELF		Average multiple measurements of delta-F from MERGEDIFF.
6.	SYNCFIT	P3	Fit lattice line curves to output from MERGEDIFF.
7.	F2MTZ		Convert formatted data (A,P) to MTZ format (CCP4 Suite).
8.	AVRGINTENS		Overall averaging of electron diffraction intensities in projection.
(1,2,3,6, Baldwin and Henderson (1984); 4, Ceska and Henderson (1990))			

JSB (1996) **116**, 9-16.

TABLE I (ctd)

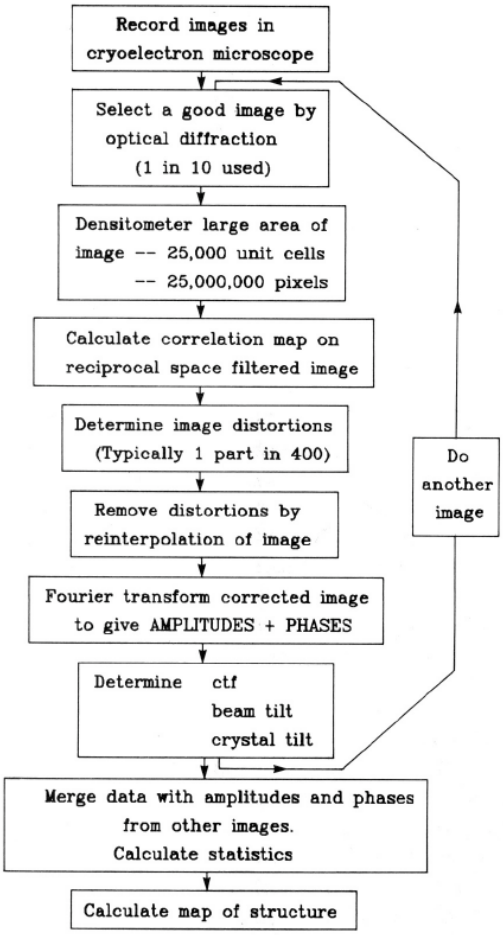
General processing

HEADER	Print out information in header records
LABEL	Image handling of various kinds
FFTRANS	Fast Fourier transform
BOXIMAGE	Box off an area leaving in original position
TAPEREDGE	Taper edge of an image to remove spikes in transform
TWOFILE	Linear combination, or multiply/divide data in two files

General display

LASERPLOT	Made redundant by PLOT2K library, directly into postscript
HISTO K	Make histogram of densities in an image
CURVY 2K	Produces graph, now direct to postscript
Ximdisp	LMB raster graphics display for X-terminals
SURF, LIGHT	Produces shaded surface representation of 3D maps

Henderson et al (1986)
Ultramicroscopy, **19**, 147-198.



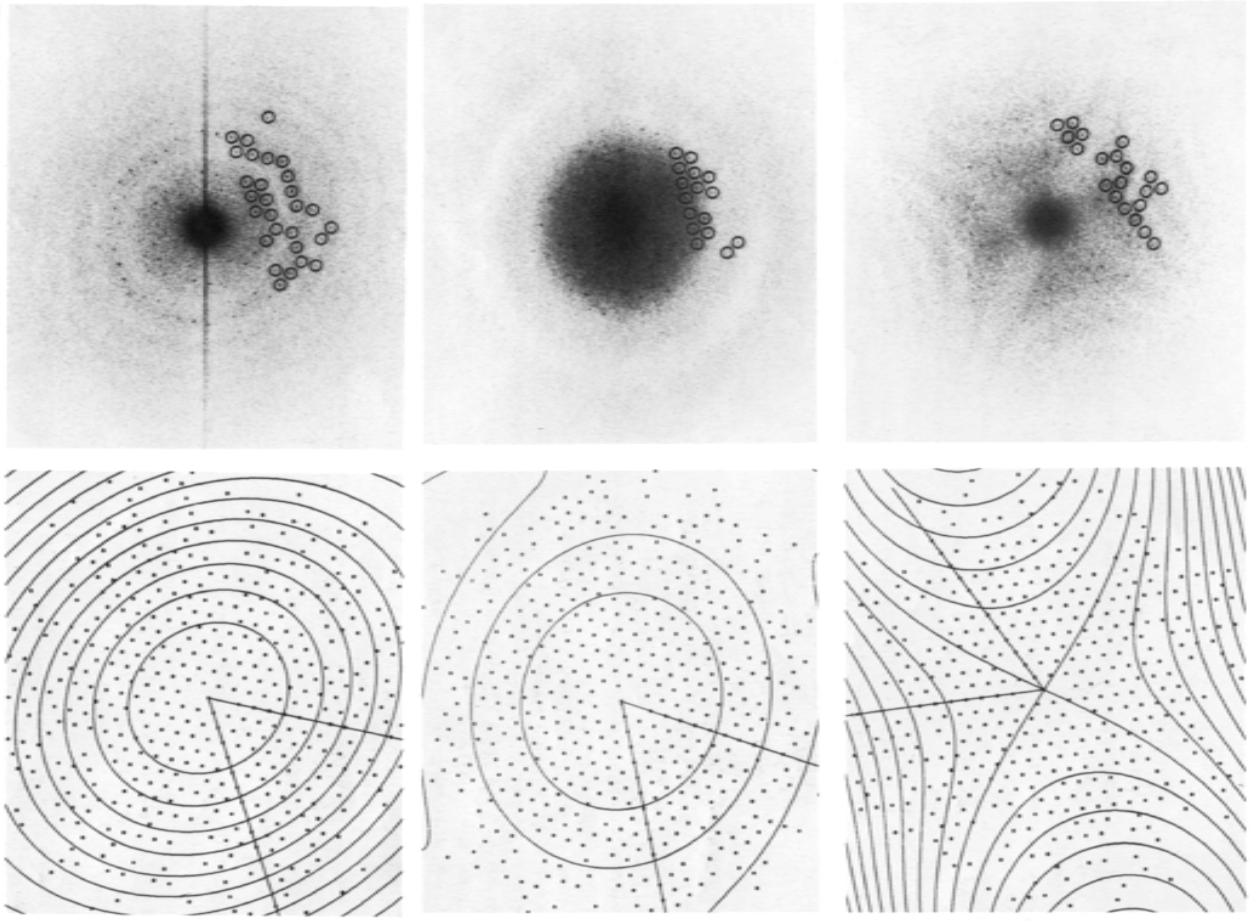


Fig. 2. Optical diffraction patterns and computer generated plots from three typical images. Some of the higher resolution spots in the optical diffraction patterns have been circled for clarity. In the computer plots, the zeroes in the contrast transfer functions are indicated by lines and all spots detected above the noise level are plotted. The top image is almost exactly in focus but contains about 4500 Å of astigmatism. The middle image is underfocussed by 2000 Å with very little astigmatism. The bottom image is more highly defocussed (3300 Å) with slightly more astigmatism. In terms of information content, the three images are equally good.

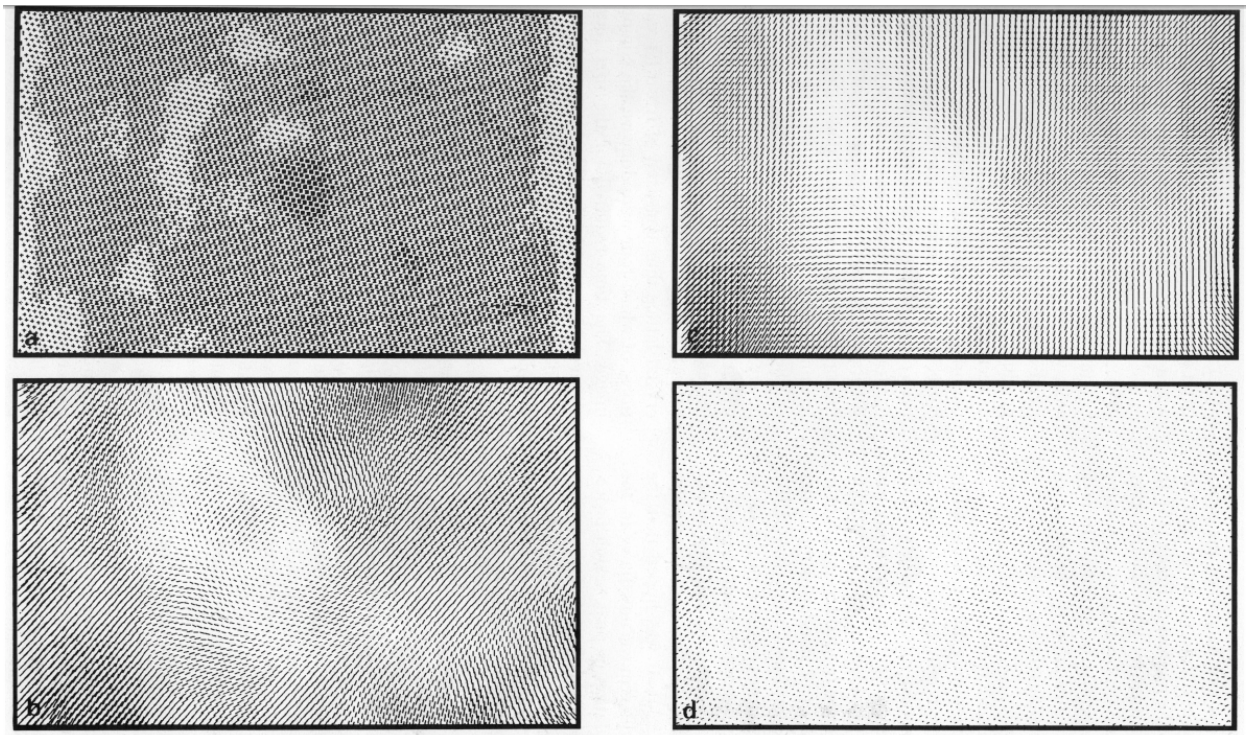
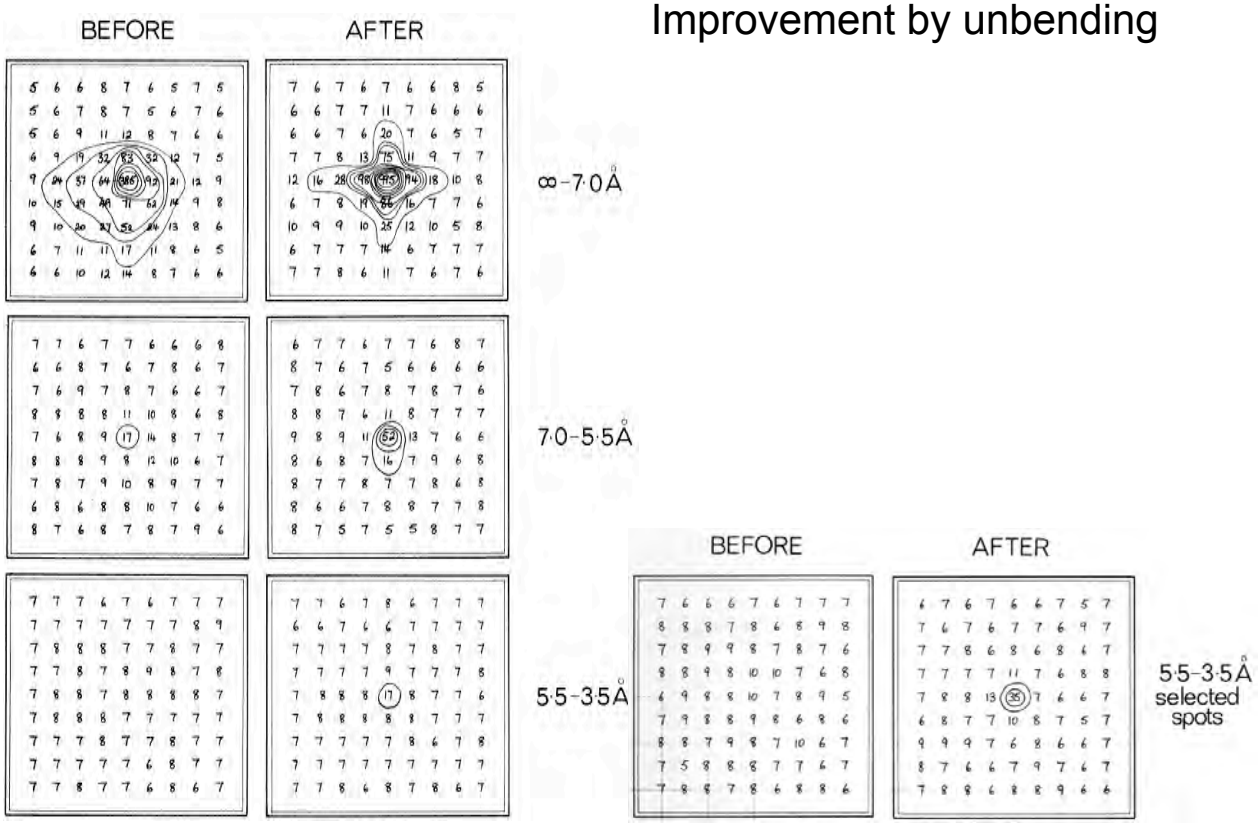


Fig. 3. The map of the peaks in the cross-correlation function: (a) Heights of cross-correlation peaks. Darker symbols indicate higher correlation. In the centre the correlation is 1.0 since it is from this area that the reference area was chosen. Other symbols (× and +) indicate correlations of above 0.5 and above 0.3 respectively. (b) Vector displacements of centres of gravity of correlation peaks from the positions predicted based on a perfect lattice. Vectors are plotted as 20× bigger than the size of the actual displacement. In the centre, the displacement is zero since this is the reference area. (c) Plot of the actual distortion correction that is used to reinterpolate the raw image. (d) Plot of the vector displacement, carried out in precisely the same way as in (b), except that the starting image was the image produced by application of the corrections shown in (c). The resulting plot shows no residual deviations above 1 Å. The average remaining error is substantially less then 0.5 Å.



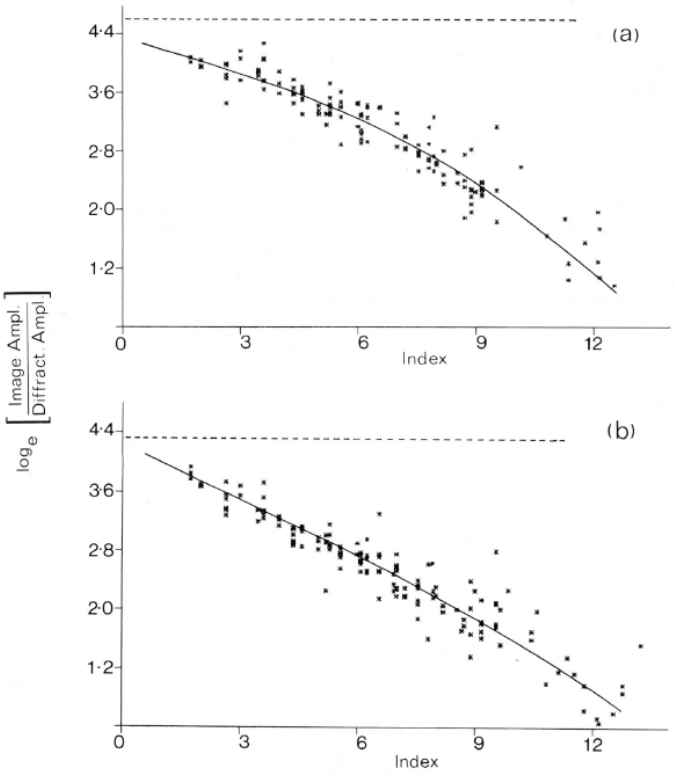


Fig. 5. Plots from two typical images comparing the ratio of image amplitude to electron diffraction pattern amplitude as a function of resolution. In both cases, the loss of contrast is approximately a factor of 30 between low resolution and 4 Å resolution. Along the abcissa, an index of 12 corresponds to 4.5 Å resolution. In (a), the relationship is clearly non-linear, whereas in (b) the points are more nearly on a straight line. The dashed line at the top of each plot indicates the theoretical level of contrast which would have occurred if the image were perfect.

Improvement by beam tilt correction

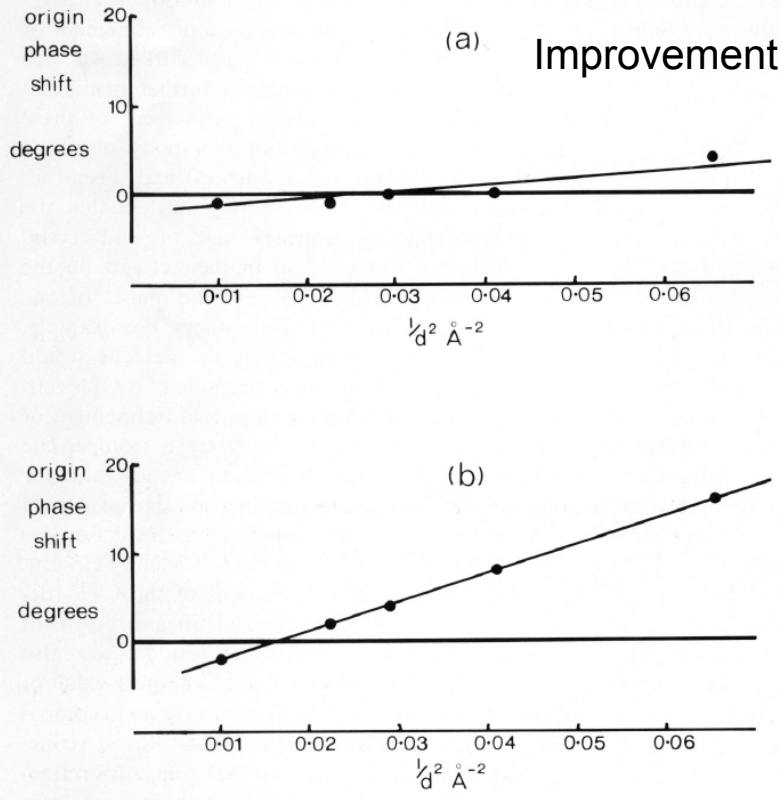


Fig. 6. Dependence of the position of the phase origin determined as a function of resolution in directions (a) perpendicular to $\boldsymbol{b}^*$ and (b) perpendicular to $\boldsymbol{a}^*$. The determination of phase origin was made by comparing the phases of spots which are related by the crystallographic three-fold axis with one another. As demonstrated by the line drawn through the points, the position of the phase origin varies linearly with $1/d^2$, where d is the spacing of the Fourier component. The origin phase shift is plotted in terms of the effect on the phase of the (1,0) or (0,1) reflection. Thus a 10° phase shift corresponds to an origin shift of 1.5 Å for purple membrane.

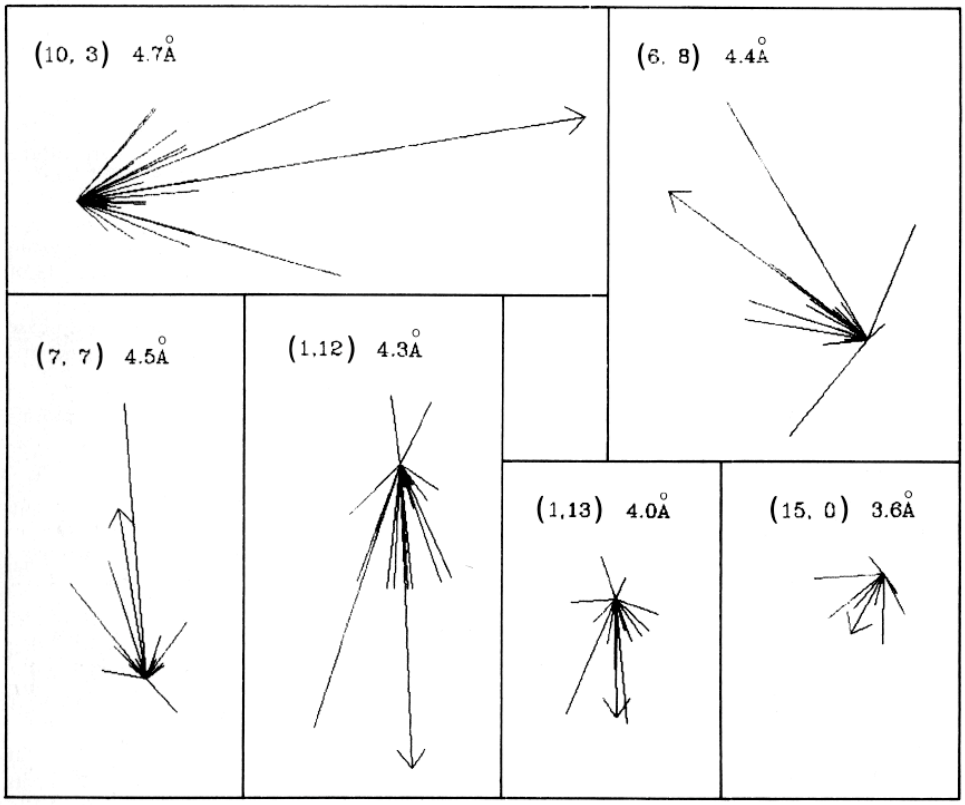
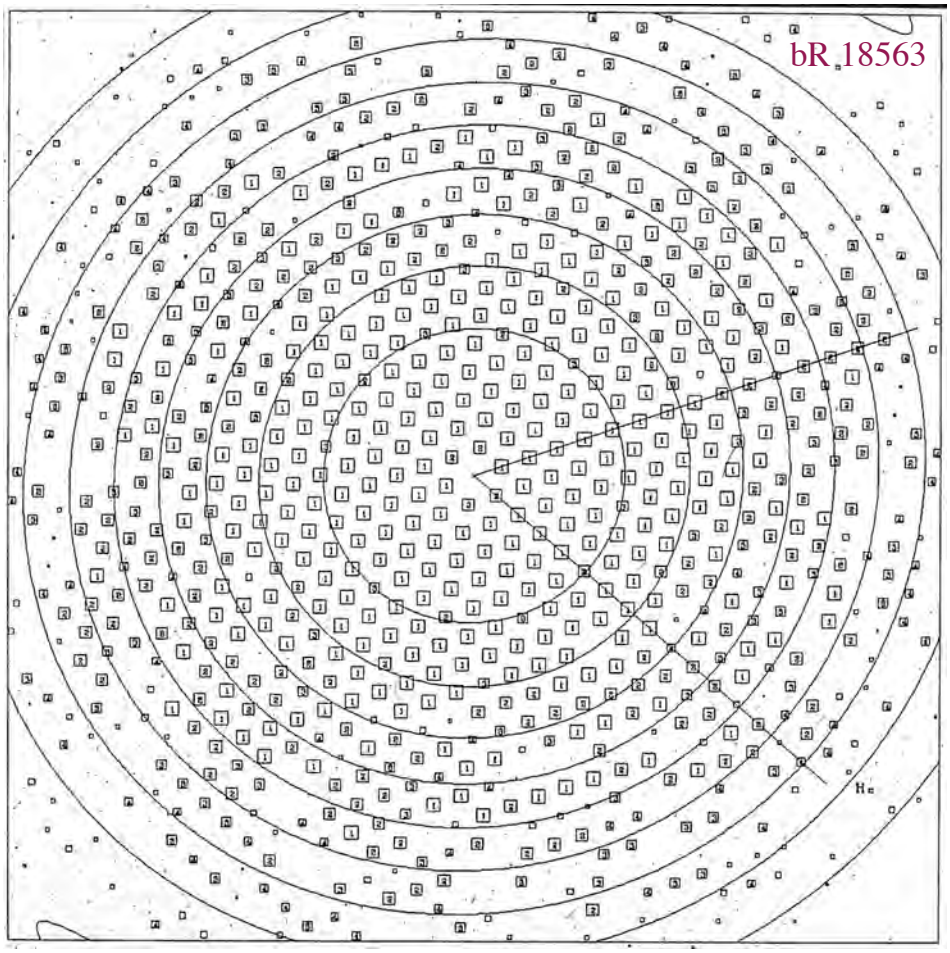


Fig. 7. Graphical comparison of all phases determined for six spots with resolution beyond 4.7 Å. Phases are plotted as vectors on a polar diagram with the length of each vector being proportional to $1/IQ^2$. Thus the strongest spots show up as longer vectors. The result of the summation of all the vectors is also shown as a vector, but this is plotted at 1/4 of its true length. The direction of this vector is our best estimate of the phase of the Fourier component in the structure, and its length can be used to provide an estimate of the error.

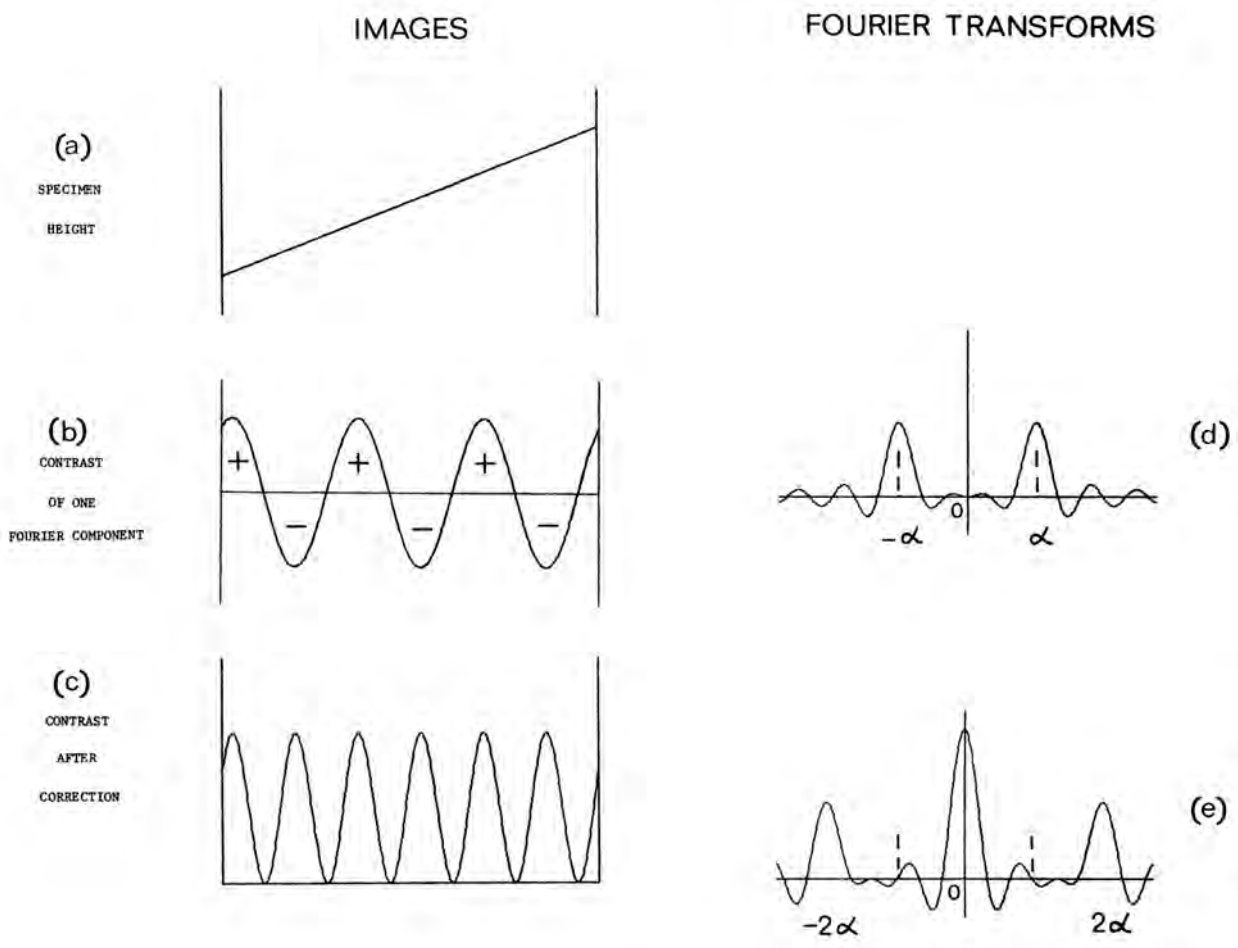
Table 2
Summary of data for each image

Image	No. of phases [a]	Overall phase residual	Defocus (Å)	Astig. (Å)	Beam tilt (mrad)
7415	217	26.2	242	4485	1.63
35978	256	27.8	1922	205	1.59
14980	200	25.3	2038	625	3.32
14981	178	28.1	3753	475	2.26
14983	205	28.6	3770	420	2.41
14984	240	21.7	3323	894	2.61
14988	249	22.0	5028	855	2.44
15004	239	34.7	−595	963	1.41
15006	255	23.4	1357	975	1.73
15025	231	25.1	1088	1025	2.62
15026	218	24.9	1125	850	2.28
14450	119 (5.5Å)	29.3	1730	260	2.88
14456	132 (5.5Å)	30.8	−1275	250	2.40

[a] Resolution is 3.5 Å in all cases except where otherwise stated.




```
FILM NUMBER      18563                  Date of last edit: 15.4.88
                 -----
MICROSCOPE       SULEIKA
DATE MEASURED    12.5.87    (TO 3.5A)

   AX      AY      BX      BY        ISIZEX ISIZEY DSTEP XMAG  CS    KV
106.667 -92.150 132.933 46.013       4320   4320   12.5 71043 1.35 100
    ASTAR     BSTAR     GAMMASTAR TILTAXIS TILTANGLE  (EMTILT)
  140.960   140.671     59.916                (4.37)
------
REVHK   0     ROT180  1     (SGNXCH) 0                  CELL  62.45
----------------------
DISTORTION CORRECTION:     Date     DFMID1 DFMID2 ANGAST TILTAXIS TILTANGL
----------------------
                    1)   13.05.87    -      -      -        -        -
----------
STATISTICS: AFTER RUN (sometimes after further refinement of parameters)
----------
ORIGTILT:   TAXA  TANGL  ORIGH  ORIGK  TILTH TILTK RES PHSRESID #PHASES DATASET
----------
 1+R)      49.001 0.243 -108.41 -118.70  0.38 -0.24 3.5   29.57    345 MERGOUT.APH
---------
TTREFINE:     TEMP FACTORS        RFAC #AMPS  #IQ<8  RES PHSRESID #PHASES DATASET
--- -----  a       b       c
 1+R) 0.02037 0.01902 0.02058    15.69  126   125   6.5   18.28     126   CMBND

 1+R) 0.01686 0.01551 0.01659    19.78  428   344   3.5   43.45     428   CMBND
------
WMBOX:                                  100/7  7/5.5  5.5/3.5        All
------
1+R) DFMID1 DFMID2 ANG     H            2888    273     93
       2981   3152  29.03  #1-4                                      293
     TILTAXIS TILTANGL     #1-7          104     61    180           345
     -89.825  -0.243       #poss         105     69    254           428

1+W) DFMID1 DFMID2 ANG     H            4085    307    102          2586
       2981   3152  29.03  #1-4          104     59    142           305
     TILTAXIS TILTANGL     #1-7          104     65    189           358
     -89.825  -0.243       #poss         105     69    253           427

                          %refamp       100.0   40.8   15.5
------------------
CORRELATION COEFFS (IMAGE vs DIFFRACTION)
------------------
 1+R)            Rfactor between scaled reference amps and image amps 0.1389
                   parl      45deg      perp
  INF-7.0A        0.992      0.992     0.995
                    35         35        35
 7.0A-5.5A        0.989      0.996     0.991
                    23         23        23
 5.5A-3.5A        0.955      0.933     0.969
                    84         85        84
--------
COMMENTS, CURRENT STATUS ETC.
--------
      Processing complete. Best image so far. Continued to 2.8A.
```



TTF correction

(2,4) at 10.2 Å

(3,7) at 6.1 Å

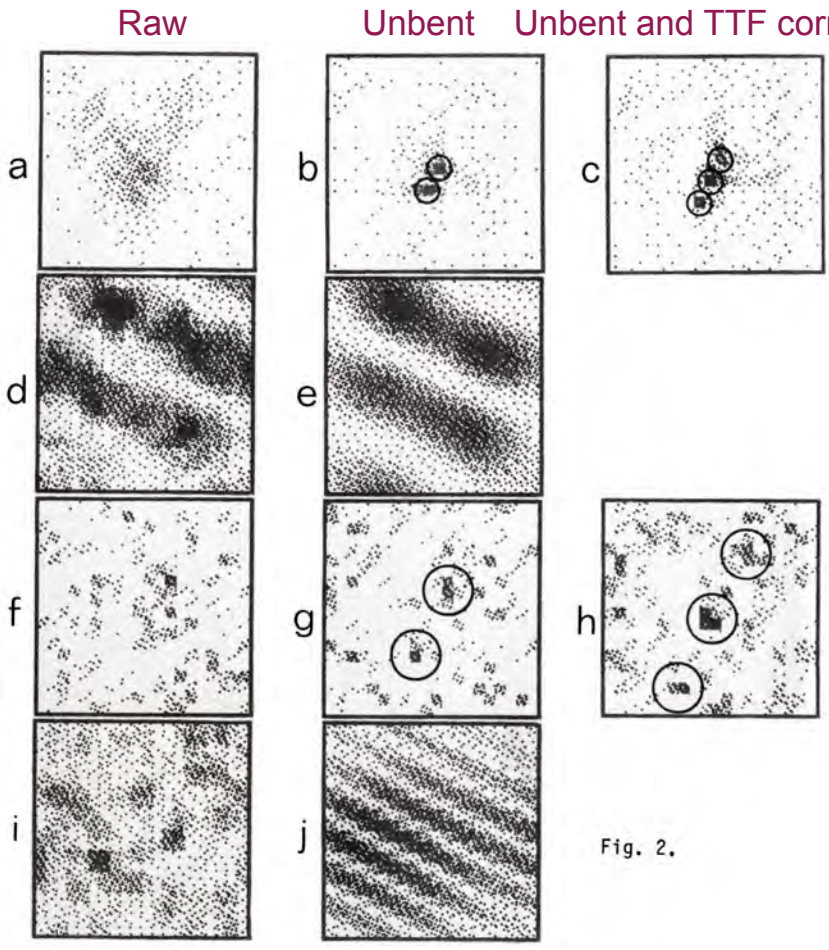
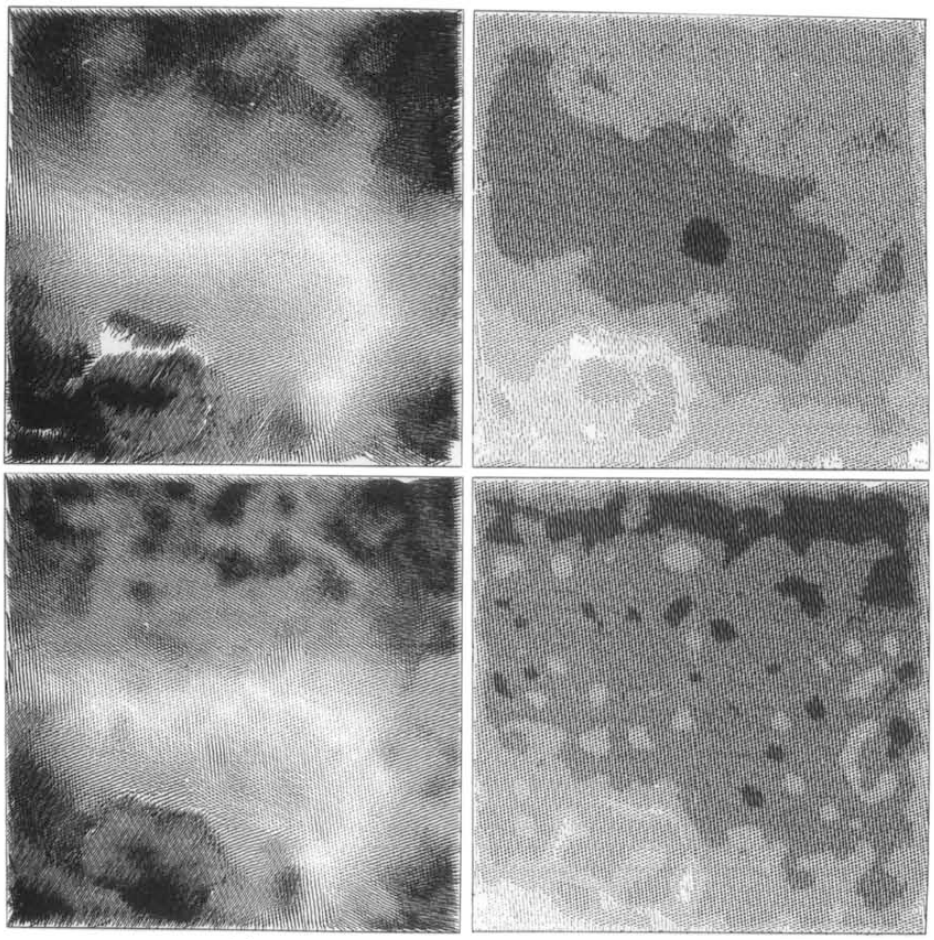
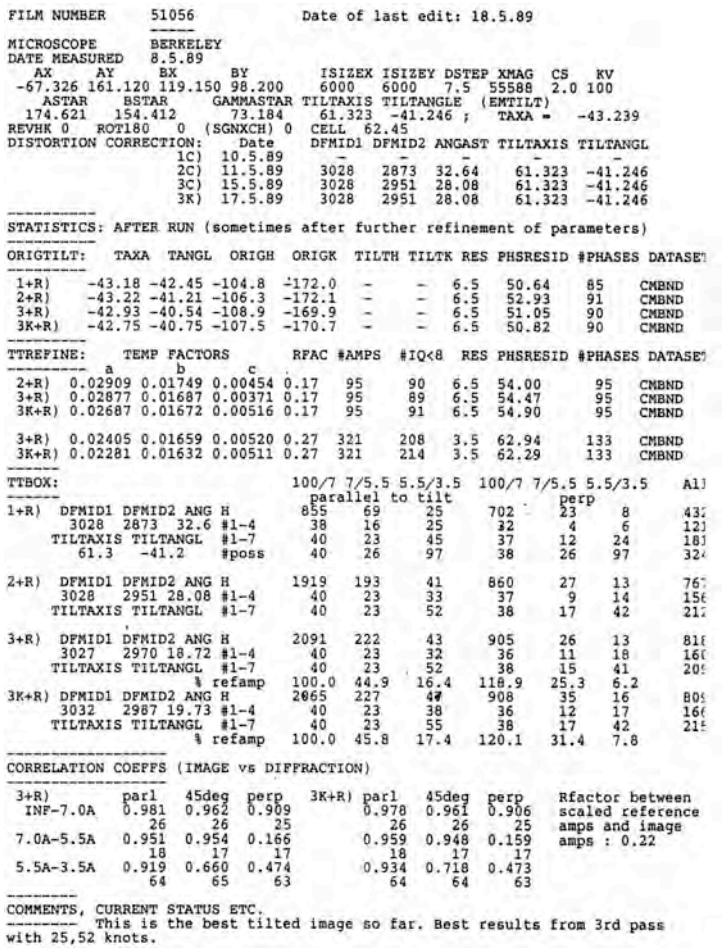


Fig. 2.



51056

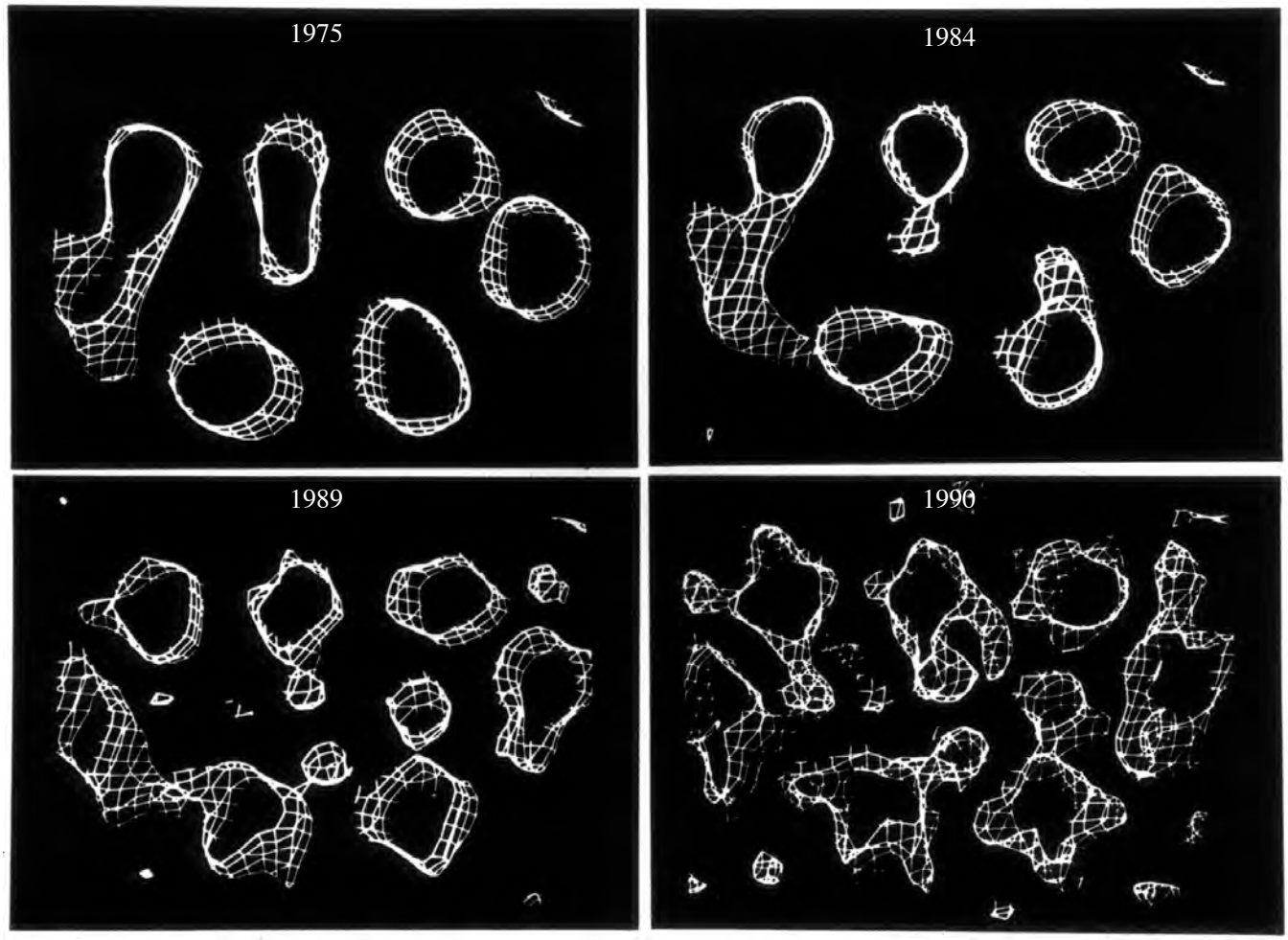
Parallel														Perpendicular														
6	5	5	7	6	10	8	11	8	7	7	6	6	7	8	6	6	5	10	5	7	7	6	8	6	7	9	7	11	8	
7	8	7	7	8	11	12	35	10	9	6	5	8	7	6	7	9	8	7	7	8	13	17	9	7	8	6	7	7	5	
5	7	10	8	8	10	7	19	11	10	7	7	6	6	7	7	6	8	6	7	8	7	12	6	7	6	8	6	7	6	
8	5	7	8	6	8	8	17	11	6	6	9	7	7	6	6	6	7	9	8	7	7	12	8	6	9	7	6	6	7	
7	6	11	32	9	12	10	25	6	7	9	6	8	6	7	7	6	9	10	8	9	11	16	7	6	7	8	7	8	7	
10	11	15	34	76	40	13	38	12	9	11	7	7	7	6	7	6	7	14	13	15	22	28	12	8	8	8	7	7	6	
7	10	12	21	46	224	125	231	53	17	14	11	7	10	7	8	8	9	10	11	31	119	140	21	13	13	12	6	9	9	
9	11	20	23	43	62	332	···	302	51	41	27	19	14	11	15	12	11	25	46	64	282	908	268	59	44	20	10	9	9	7 Å
7	9	8	7	14	18	23	219	168	255	47	12	10	7	5	8	7	9	7	11	9	21	60	30	22	13	7	10	7	6	
8	8	8	8	9	9	11	45	17	31	47	51	16	8	7	7	6	6	7	9	9	13	22	10	10	15	11	8	8	8	
6	8	8	7	7	9	9	24	6	15	11	10	11	8	5	5	7	5	7	7	7	10	12	9	6	9	7	7	7	6	
8	6	6	6	9	8	10	27	7	13	10	8	6	8	9	7	8	8	7	6	7	14	14	7	8	6	5	6	7	7	
5	6	7	6	7	13	11	29	11	11	8	7	7	5	6	7	7	6	5	6	8	9	13	9	7	6	6	7	6	7	
8	6	7	7	8	6	11	45	14	10	8	7	6	11	7	8	6	5	4	10	6	11	20	12	8	8	6	6	7	8	
6	6	7	5	6	7	8	14	7	10	12	5	5	7	7	7	7	8	6	7	7	8	13	6	7	7	7	6	6	5	
7	8	8	5	6	8	6	8	8	5	7	7	11	7	8	6	8	7	6	8	7	7	8	6	7	6	7	9	7	6	
8	8	6	8	6	4	6	8	6	6	5	6	7	8	6	6	5	7	7	6	4	8	8	7	8	5	6	7	8	5	
8	6	5	8	7	8	8	7	7	7	6	6	9	6	9	7	6	5	9	8	6	9	7	5	7	6	6	6	8	6	
15	13	9	9	6	10	7	9	11	8	8	5	11	6	7	11	8	5	5	7	7	8	8	7	6	6	6	11	6	7	
17	41	37	11	8	6	5	8	6	8	5	5	6	9	6	8	11	6	6	7	8	11	8	6	8	7	6	6	8	8	
8	6	19	9	7	8	7	10	8	10	7	8	7	6	6	8	6	8	7	8	6	8	7	5	7	5	8	5	8	7	
5	7	7	8	9	10	8	41	18	8	10	8	5	8	5	7	6	6	8	8	7	8	7	6	9	8	7	5	7	6	
7	9	9	10	10	12	37	227	53	10	9	7	10	6	8	8	7	7	7	6	7	15	35	5	6	5	5	8	6	7	5·5 Å
7	8	6	9	7	11	12	46	15	9	9	7	10	8	6	7	6	9	5	8	6	6	6	6	8	8	9	8	7	10	
8	7	5	10	10	5	7	11	9	7	7	9	17	11	8	8	5	7	9	5	7	6	9	9	6	8	6	9	8	6	
7	5	6	6	5	6	6	13	10	9	6	6	19	31	16	7	6	7	6	6	8	9	7	6	7	6	7	10	9	6	
5	8	9	6	7	10	8	9	7	10	5	11	9	23	17	8	8	8	7	8	9	7	9	7	9	8	7	7	8	7	
6	6	9	7	7	6	7	9	5	6	6	8	7	9	9	5	10	7	7	7	7	8	7	7	7	7	10	9	7	9	
9	7	8	7	7	11	7	10	6	6	8	5	8	7	5	10	6	7	7	8	8	6	7	5	9	7	6	7	5	9	
7	7	8	5	9	6	8	10	9	9	8	6	7	8	5	7	8	9	11	11	8	7	8	7	5	8	8	6	9	6	
7	8	6	7	6	9	7	7	6	6	7	7	5	8	6	7	6	6	7	8	7	7	8	8	6	7	7	7	8		
6	8	7	7	8	9	6	8	7	7	7	7	6	7	6	9	8	8	7	6	7	7	7	7	8	7	6	6	7	7	
8	7	7	8	6	6	8	7	5	7	7	6	8	8	7	7	6	6	7	7	7	6	6	7	7	6	7	8	7	6	
8	7	8	7	8	8	7	6	8	8	6	6	5	8	7	7	8	9	6	7	7	7	7	6	7	8	7	7	7	6	
7	8	8	8	7	6	8	8	7	7	7	6	6	7	7	8	8	8	8	8	7	7	7	7	6	7	7	7	8	7	7
7	7	6	8	7	7	6	8	7	7	7	6	7	7	7	6	7	8	7	6	7	6	6	7	7	6	6	7	7		
8	8	7	6	6	7	11	14	8	7	7	7	7	8	7	7	8	7	6	7	7	8	11	9	6	7	6	6	7	6	
7	7	9	7	8	7	10	47	17	8	7	7	7	8	6	7	9	7	7	7	8	16	10	8	8	7	7	9	7	3·5 Å	
6	6	7	7	7	7	8	7	9	7	7	8	6	7	6	8	7	8	7	6	5	7	8	10	7	7	9	7	8	7	
8	6	6	7	8	7	7	8	8	6	6	9	7	7	7	7	7	7	6	6	7	7	7	8	8	8	8	7	6	7	
7	7	7	7	7	6	7	8	7	7	7	6	7	9	7	8	8	7	7	6	7	8	6	6	7	7	7	7	7	6	
7	6	8	7	6	9	6	7	7	6	7	7	7	7	7	7	7	7	7	8	7	6	7	7	8	8	7	8	7	7	
8	8	7	7	6	7	7	8	8	7	6	7	7	6	6	8	7	7	6	7	7	7	7	7	6	8	6	7	8	7	
6	6	6	6	8	8	8	8	7	7	8	8	6	7	7	7	6	7	6	7	7	8	6	7	7	6	6	7	7		
5	7	6	6	8	8	7	6	7	8	8	6	7	6	8	7	8	6	9	8	6	7	10	8	8	7	8	5	6	6	

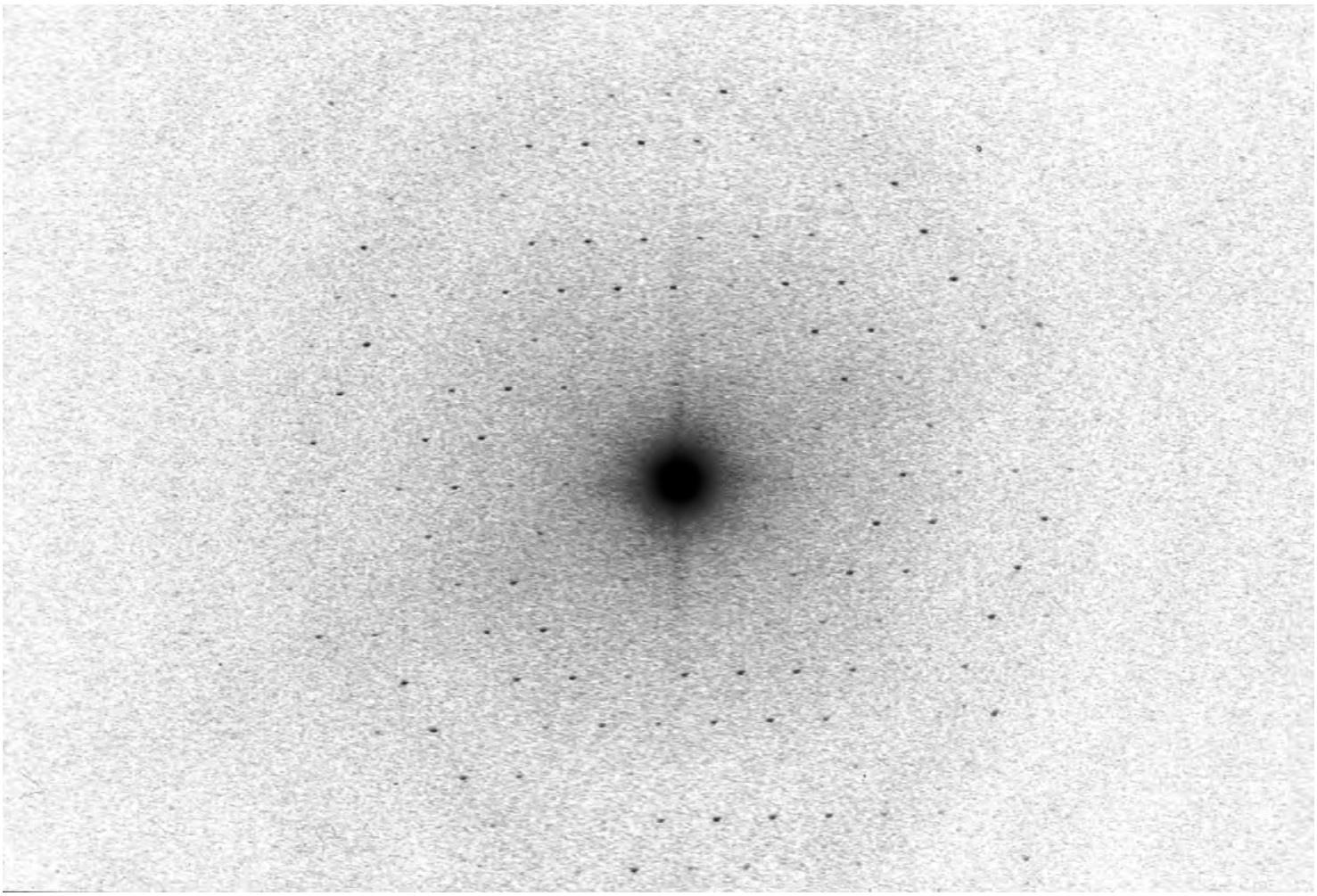


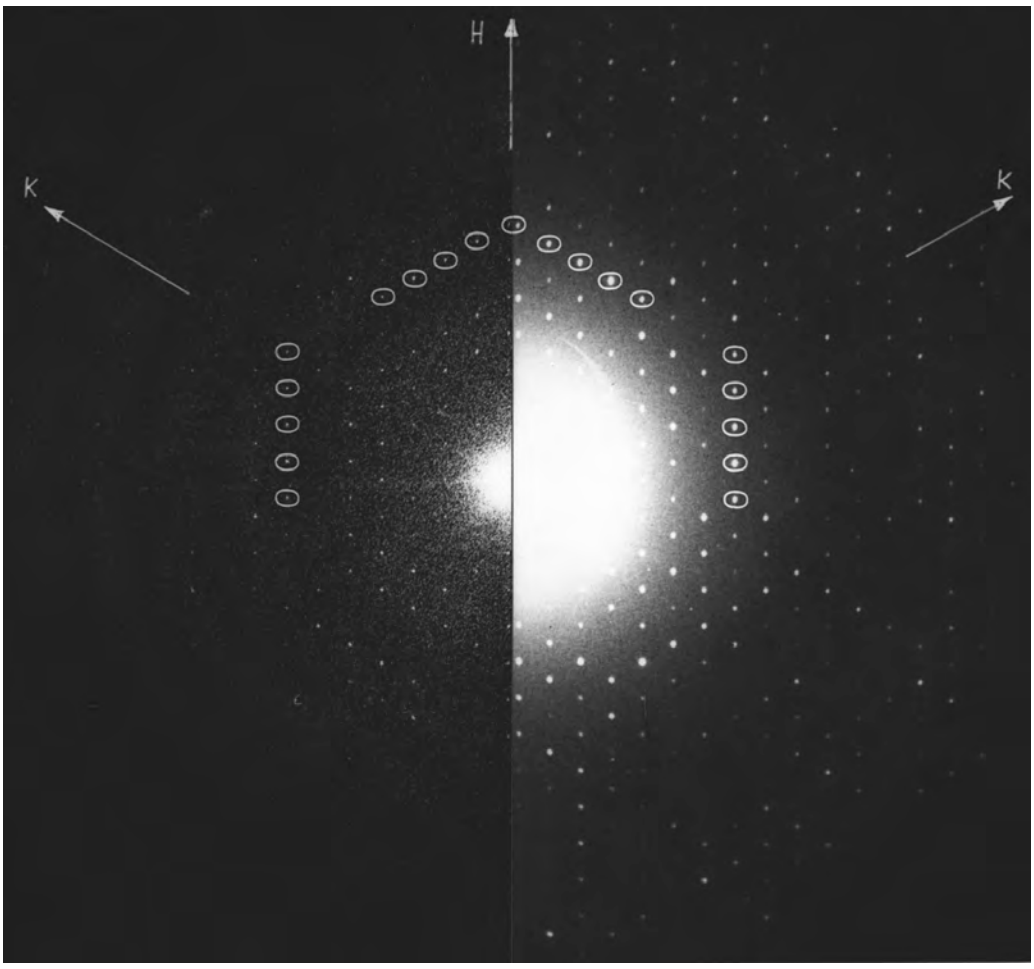
```
FILM NUMBER      51056              Date of last edit: 18.5.89
MICROSCOPE       BERKELEY
DATE MEASURED    8.5.89
   AX      AY       BX       BY        ISIZEX ISIZEY DSTEP XMAG  CS   KV
 -67.326 161.120 119.150 98.200        6000   6000    7.5  55588  2.0 100
   ASTAR     BSTAR      GAMMASTAR TILTAXIS TILTANGLE  (EMTILT)
  174.621   154.412       73.184    61.323  -41.246 ;   TAXA =   -43.239
REVHK 0    ROT180   0  (SGNXCH) 0  CELL   62.45
DISTORTION CORRECTION:     Date     DFMID1 DFMID2 ANGAST TILTAXIS TILTANGL
                      1C)  10.5.89      -      -      -        -        -
                      2C)  11.5.89   3028   2873  32.64   61.323  -41.246
                      3C)  15.5.89   3028   2951  28.08   61.323  -41.246
                      3K)  17.5.89   3028   2951  28.08   61.323  -41.246
-----------
STATISTICS: AFTER RUN (sometimes after further refinement of parameters)
-----------
ORIGTILT:    TAXA   TANGL   ORIGH   ORIGK   TILTH TILTK RES PHSRESID #PHASES DATASET
----------
 1+R)     -43.18 -42.45 -104.8  -172.0     -     -   6.5   50.64     85    CMBND
 2+R)     -43.22 -41.21 -106.3  -172.1     -     -   6.5   52.93     91    CMBND
 3+R)     -42.93 -40.54 -108.9  -169.9     -     -   6.5   51.05     90    CMBND
 3K+R)    -42.75 -40.75 -107.5  -170.7     -     -   6.5   50.82     90    CMBND
---------
TTREFINE:     TEMP FACTORS          RFAC #AMPS  #IQ<8  RES PHSRESID #PHASES DATASET
--------- a         b         c
 2+R)  0.02909 0.01749 0.00454 0.17   95     90   6.5  54.00      95    CMBND
 3+R)  0.02877 0.01687 0.00371 0.17   95     89   6.5  54.47      95    CMBND
 3K+R) 0.02687 0.01672 0.00516 0.17   95     91   6.5  54.90      95    CMBND

 3+R)  0.02405 0.01659 0.00520 0.27  321    208   3.5  62.94     133    CMBND
 3K+R) 0.02281 0.01632 0.00511 0.27  321    214   3.5  62.29     133    CMBND
------
TTBOX:                                 100/7 7/5.5 5.5/3.5  100/7 7/5.5 5.5/3.5   All
------                                   parallel to tilt          perp
1+R)  DFMID1 DFMID2 ANG H              855    69     25      702    23     8      43
        3028   2873  32.6 #1-4         38    16     25       32     4     6      12
      TILTAXIS TILTANGL  #1-7          40    23     45       37    12    24      18
         61.3    -41.2    #poss        40    26     97       38    26    97      32

2+R)  DFMID1 DFMID2 ANG H             1919   193     41      860    27    13      76
        3028   2951 28.08 #1-4         40    23     33       37     9    14      15
      TILTAXIS TILTANGL  #1-7          40    23     52       38    17    42      21

3+R)  DFMID1 DFMID2 ANG H             2091   222     43      905    26    13      81
        3027   2970 18.72 #1-4         40    23     32       36    11    18      16
      TILTAXIS TILTANGL  #1-7          40    23     52       38    15    41      20
                         % refamp    100.0  44.9   16.4    118.9  25.3   6.2
3K+R) DFMID1 DFMID2 ANG H             2065   227     47      908    35    16      80
        3032   2987 19.73 #1-4         40    23     38       36    12    17      16
      TILTAXIS TILTANGL  #1-7          40    23     55       38    17    42      21
                         % refamp    100.0  45.8   17.4    120.1  31.4   7.8
-------------------
CORRELATION COEFFS (IMAGE vs DIFFRACTION)
-------------------
 3+R)        parl   45deg  perp   3K+R) parl   45deg  perp    Rfactor between
  INF-7.0A  0.981  0.962  0.909         0.978  0.961  0.906   scaled reference
              26     26     25            26     26     25    amps and image
 7.0A-5.5A  0.951  0.954  0.166         0.959  0.948  0.159   amps : 0.22
              18     17     17            18     17     17
 5.5A-3.5A  0.919  0.660  0.474         0.934  0.718  0.473
              64     65     63            64     64     63
---------
COMMENTS, CURRENT STATUS ETC.
--------- This is the best tilted image so far. Best results from 3rd pass
with 25,52 knots.
```

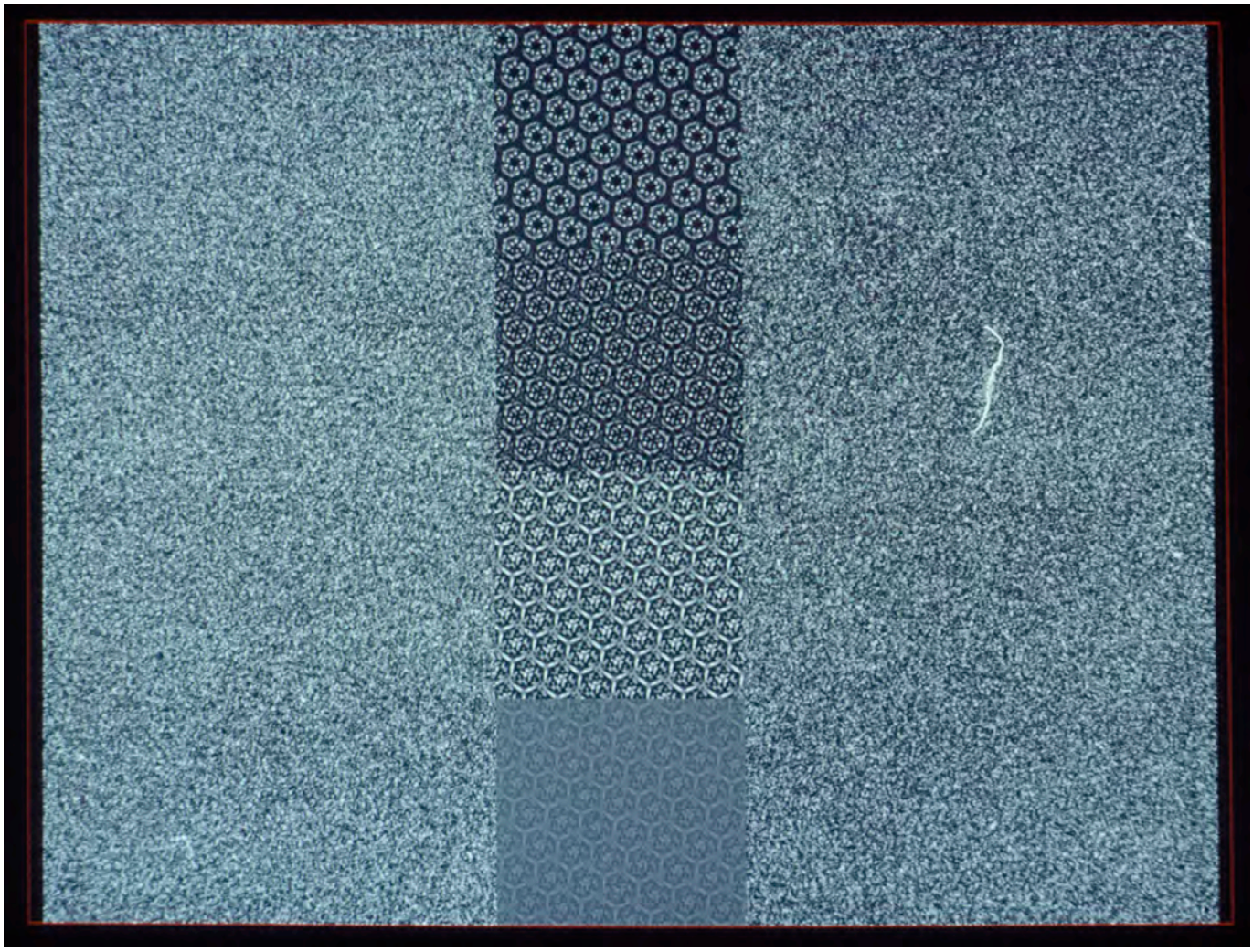
Bacteriorhodopsin - from 7 Å to 3.5 Å

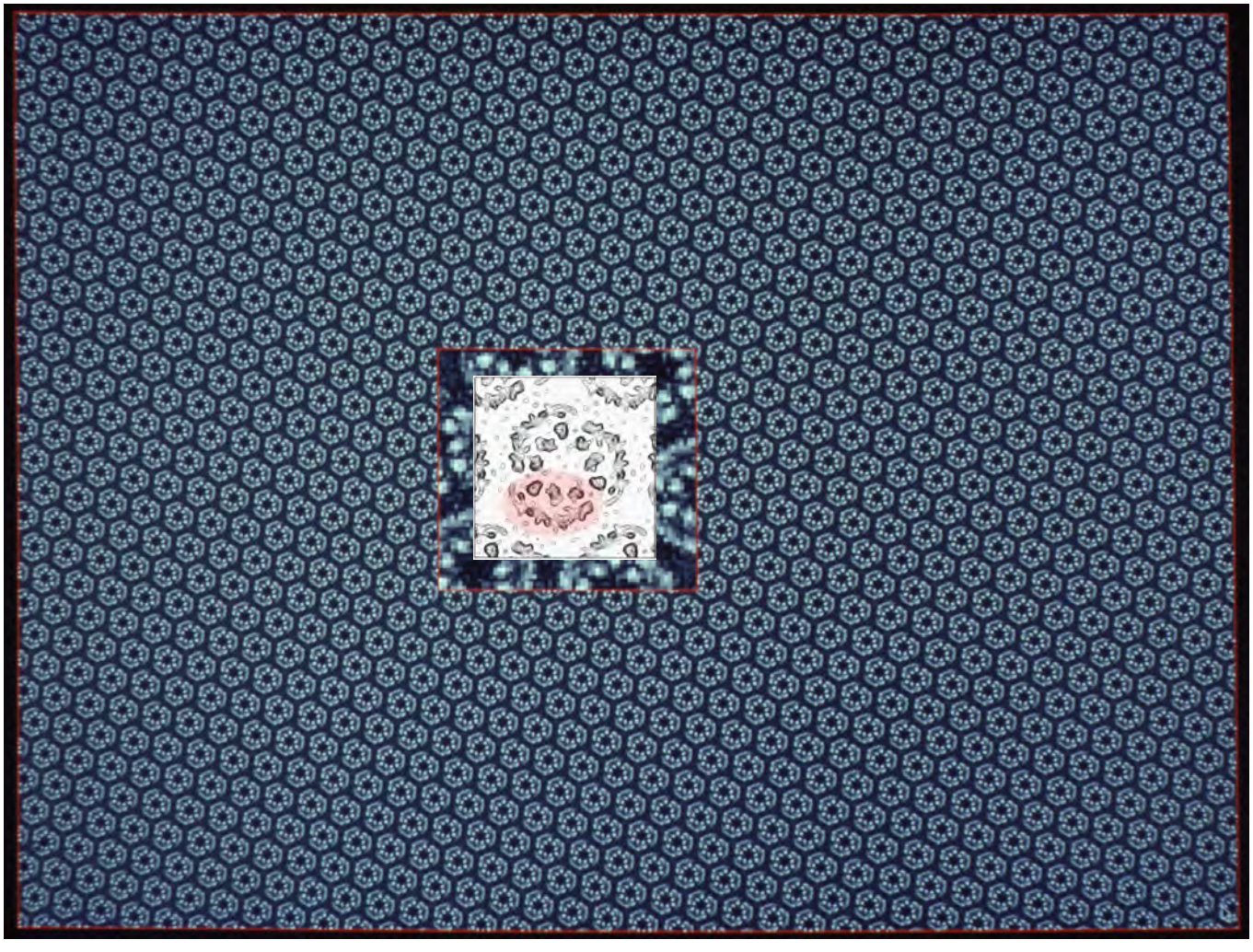
Bigger crystals by fusion
Cold stages – liquid nitrogen and helium
Unbending
Beam tilt
Tilt transfer function
(Field emission guns)

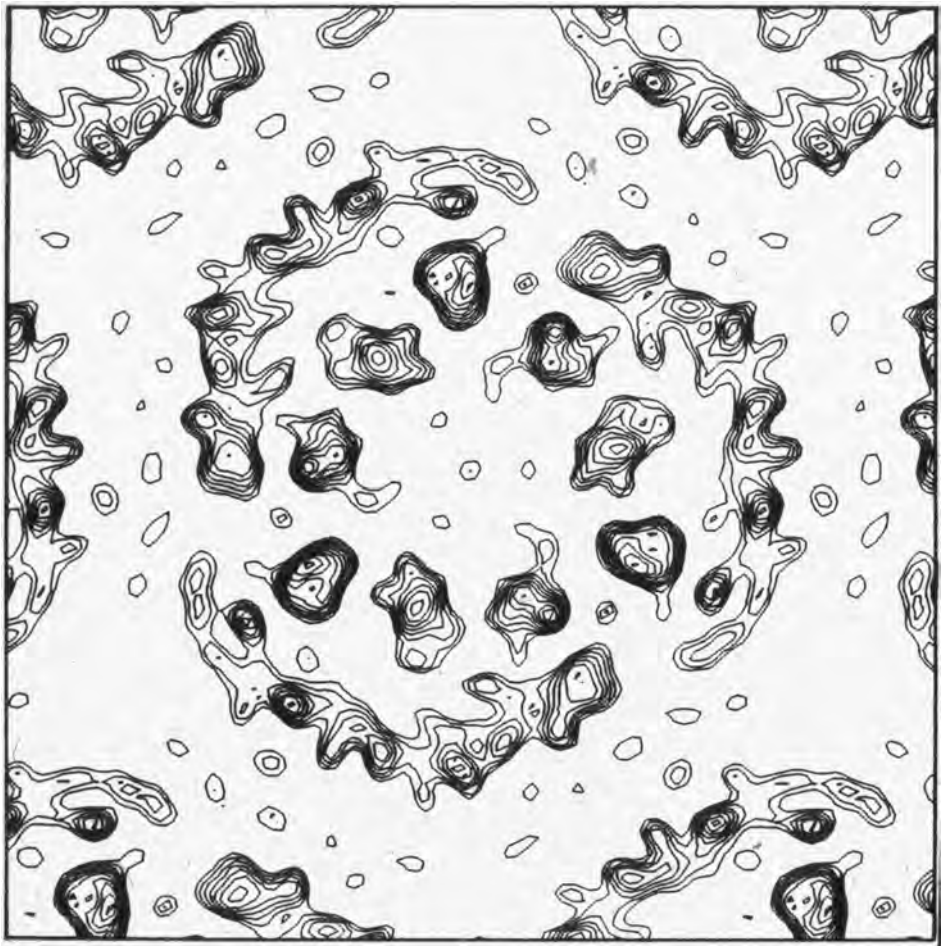

1975
1984
1989
1990

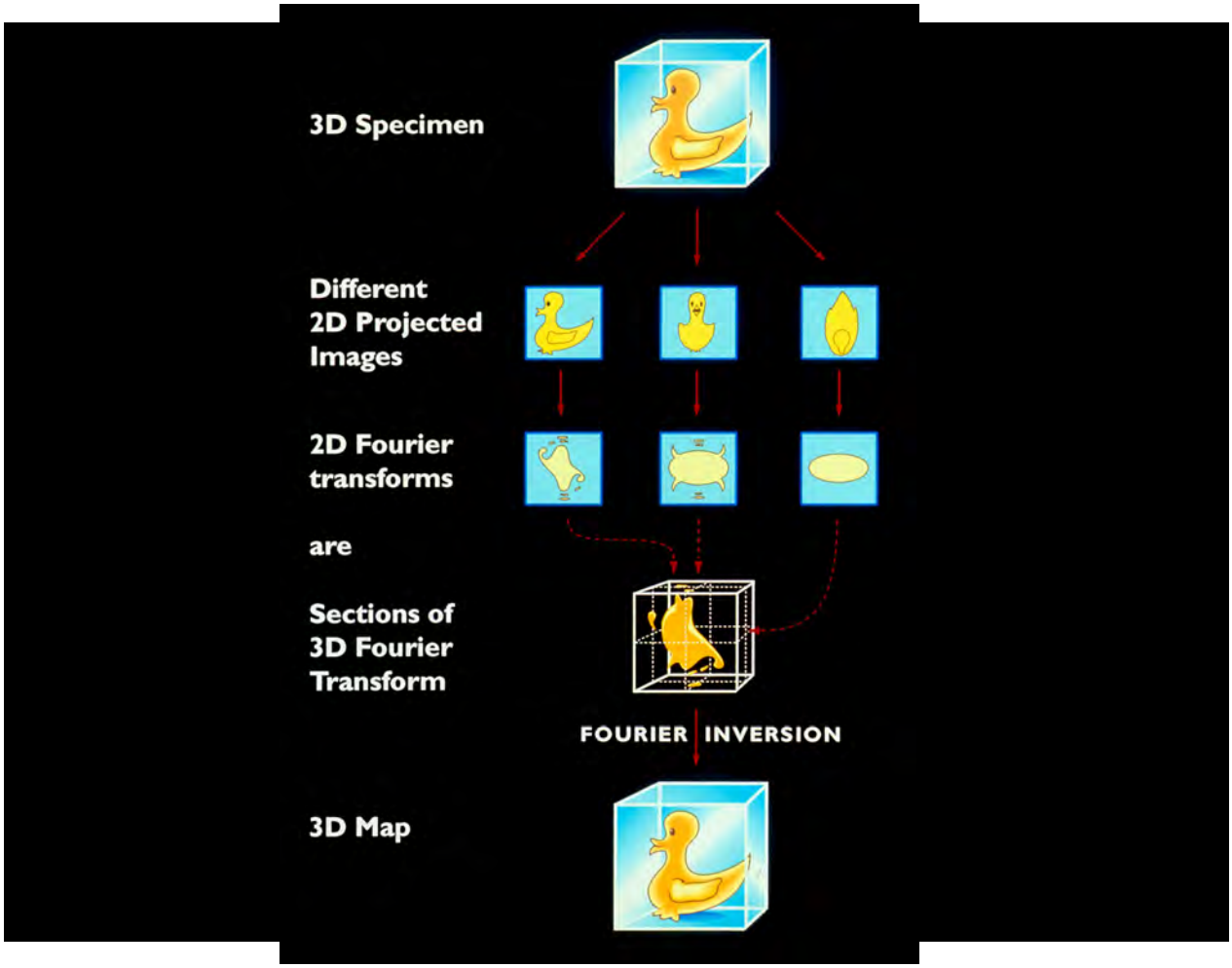


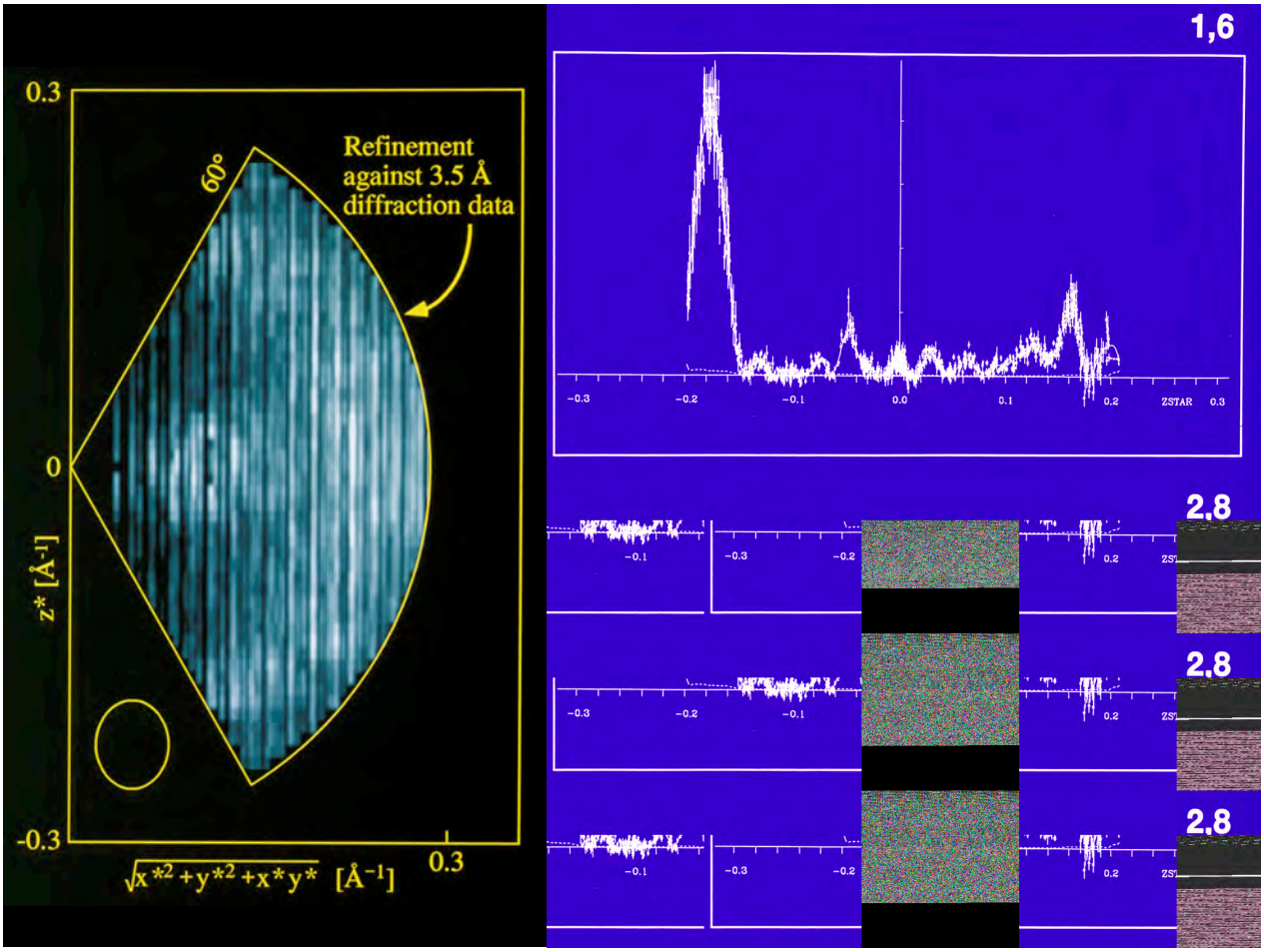

H
K
K

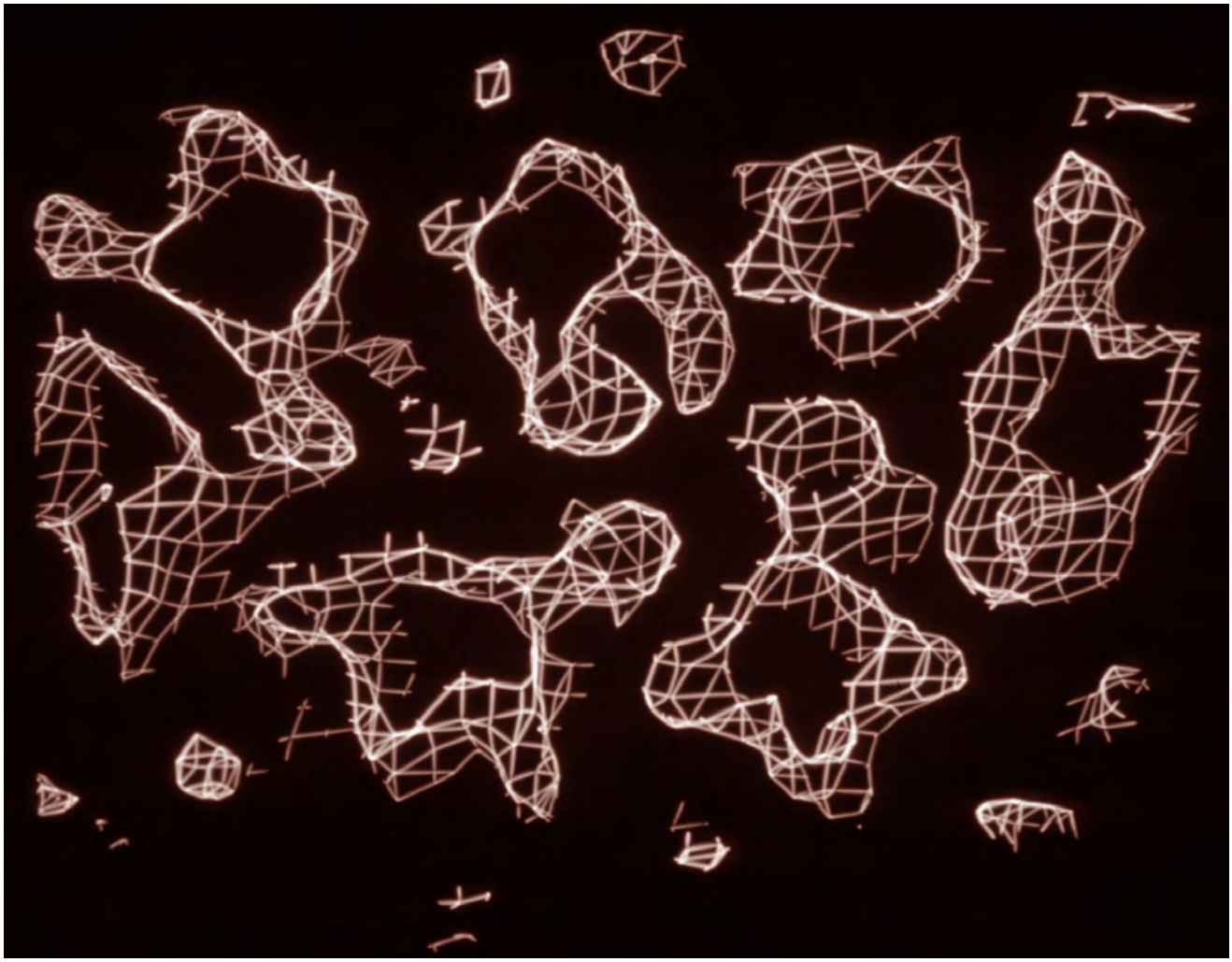


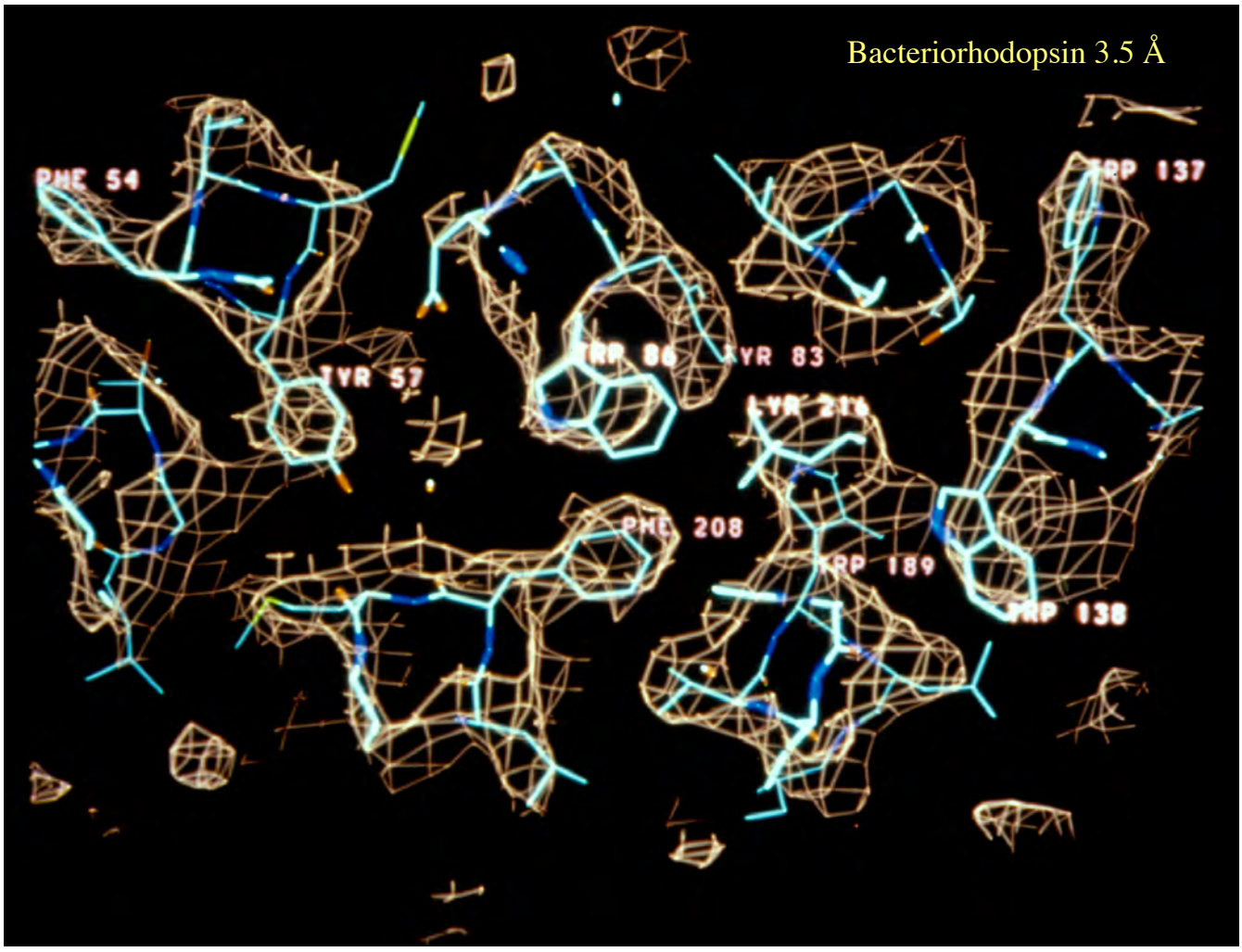


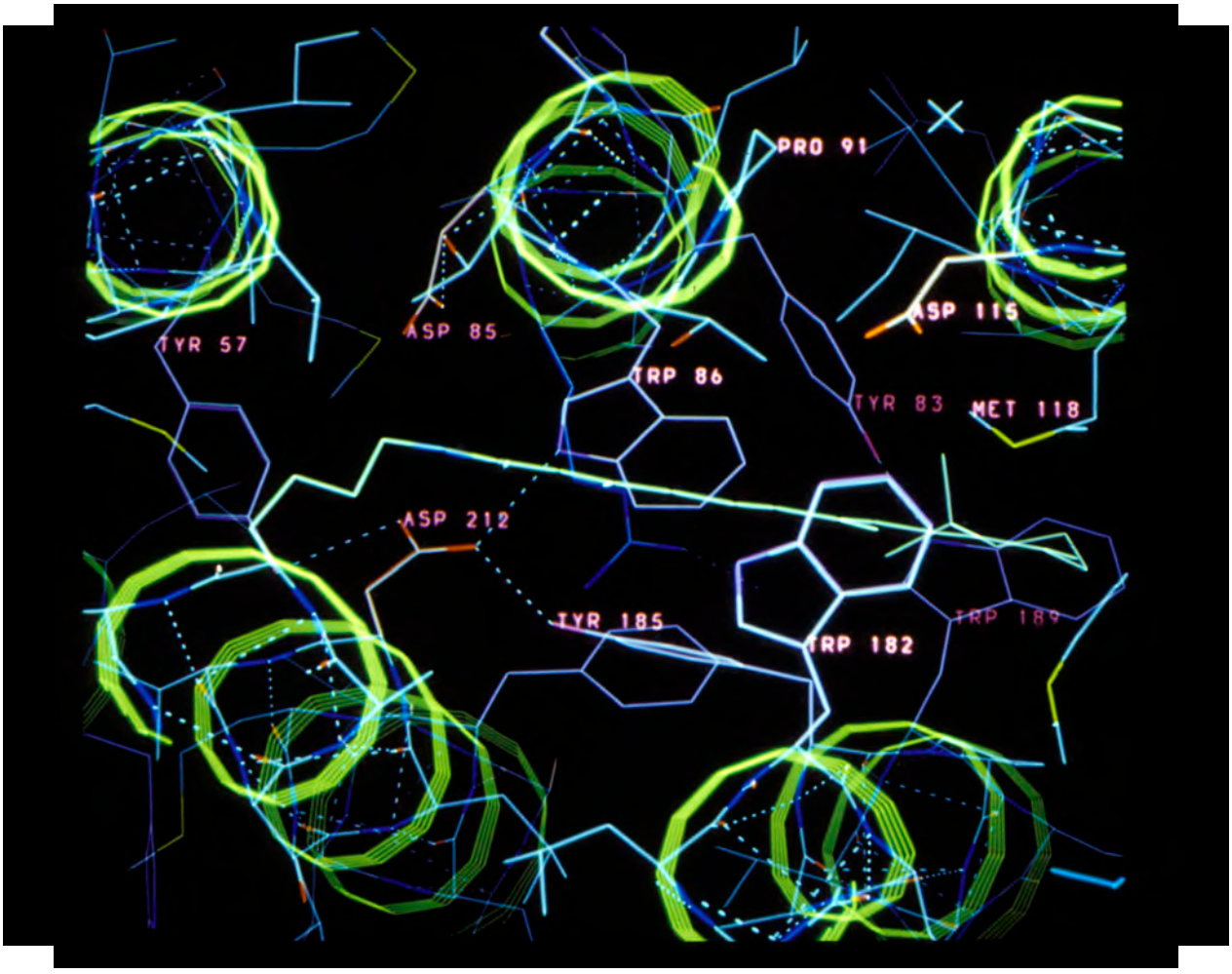


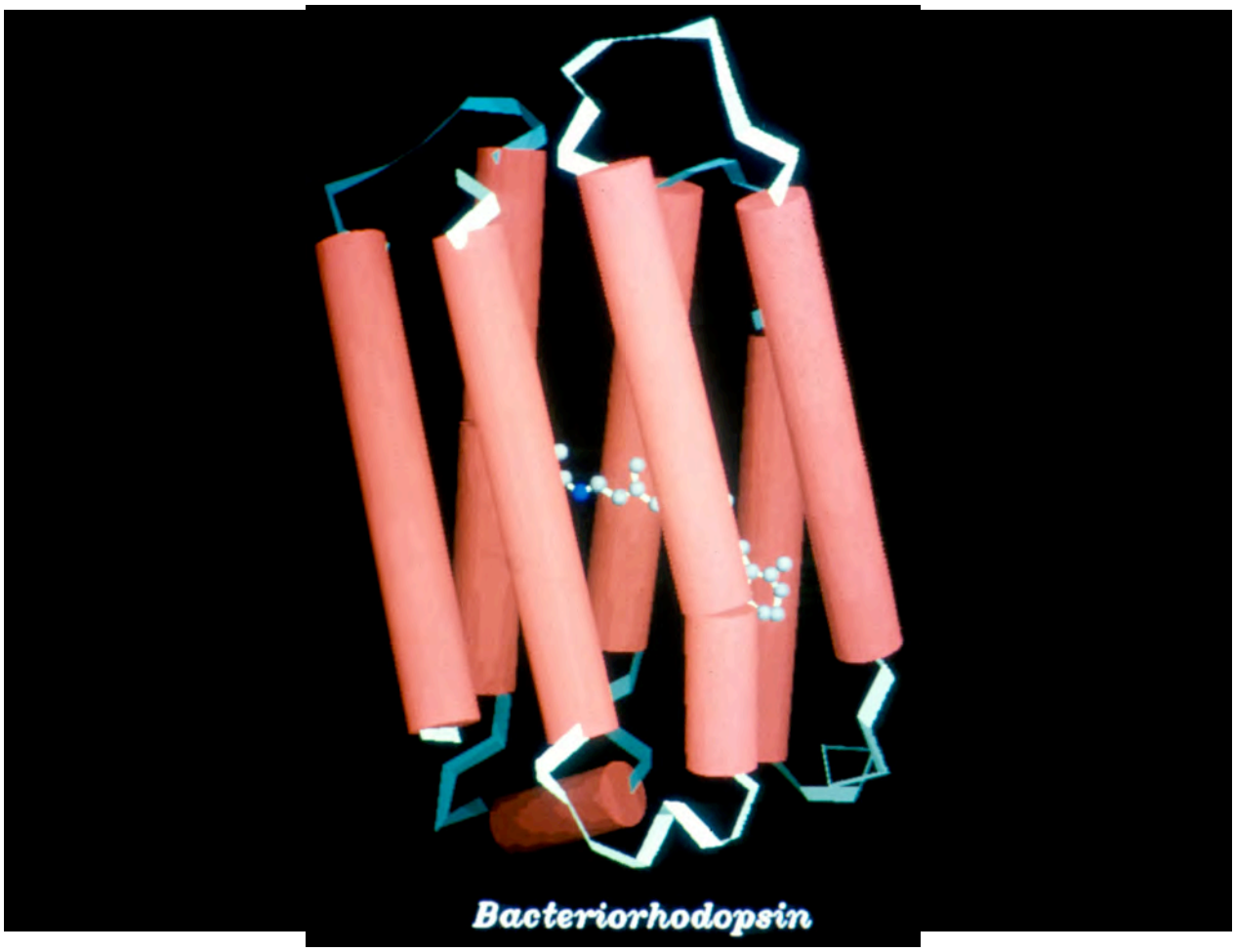

3D Specimen
Different
2D Projected
Images
2D Fourier
transforms
are
Sections of
3D Fourier
Transform
FOURIER INVERSION
3D Map

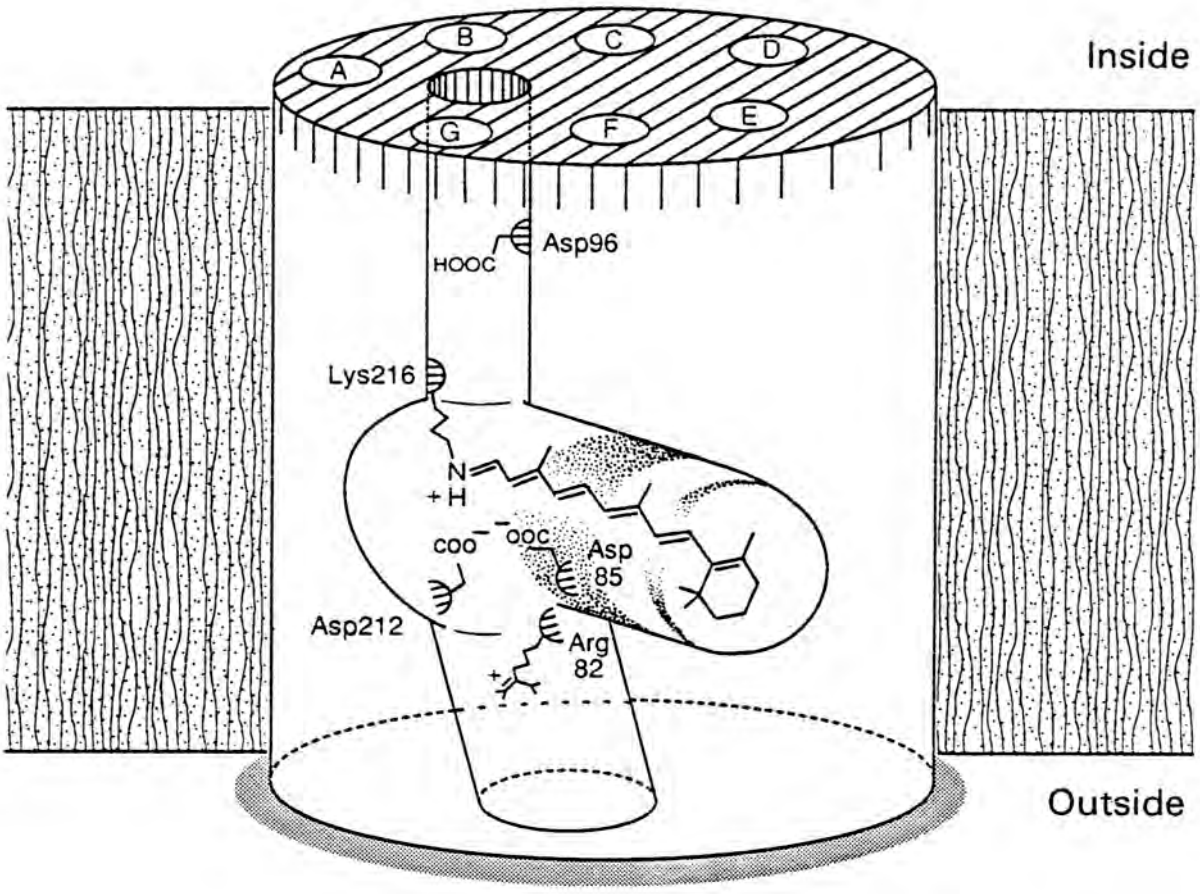

0.3
0
-0.3
z* [Å-1]
60°
Refinement
against 3.5 Å
diffraction data
0.3
√x*2 +y*2 +x*y* [Å-1]
1,6
-0.3
-0.2
-0.1
0.0
0.1
0.2
ZSTAR
0.3
2.8
2.8
2.8

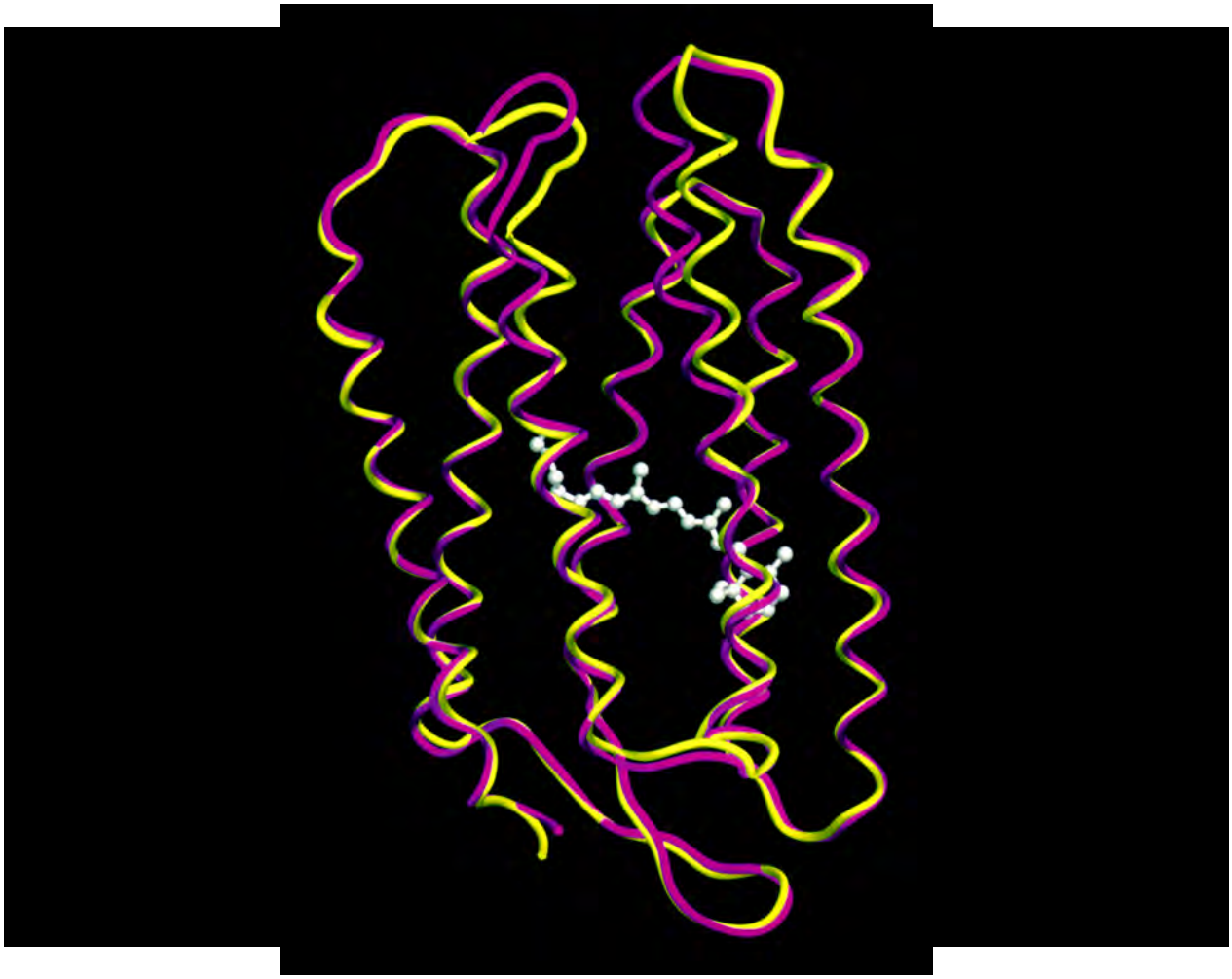



Bacteriorhodopsin 3.5 Å
PHE 54
TRP 137
TYR 57
TRP 86
TYR 83
TYR 216
PHE 208
TRP 189
TRP 138


PRO 91
TYR 57
ASP 85
ASP 115
TRP 86
TYR 83
MET 118
ASP 212
TYR 185
TRP 182
TRP 189


Bacteriorhodopsin


Inside
A
B
C
D
E
F
G
HOOC
Asp96
Lys216
N
+H
coo⁻
ooc⁻
Asp
85
Asp212
Arg
82
+
Outside



3D structures from 2D crystals

Bacteriorhodopsin	**3.5 Å**	*
Bacteriorhodopsin	**3.0 Å**	*
DOC bacteriorhodopsin	6.0 Å	
Bacteriorhodopsin p22121	6.5 Å	
Porin PhoE	6.0 Å	
Plant LHC-II	**3.4 Å**	*
Rhodopsin frog p2	6.5 Å	
Tubulin dimer	**3.7 Å**	*
Aquaporin-1	**3.7 Å**	*
Aquaporin-4	**3.2 Å**	*
Aquaporin-0	**1.9 Å**	*
Halorhodopsin	5.0 Å	
Glutathione transferase	**3.2 Å**	*
SecYEG complex	8.0 Å	
Plant photosystem II RC	8.0 Å	
Neurospora H+-ATPase	8.0 Å	
Gap junction channel	7.5 Å	
NhaA Na/H antiporter	7.0 Å	
Glycerol channel GlpF	6.9 Å	
Oxalic acid transporter OxlT	6.0 Å	
EmrE multidrug transporter	7.0 Å	

Problems & pitfalls

- Space group determination
- Twinning
- Comparison to X-ray structures

The 17* Two-Sided Plane Groups Allowed For Biological Molecules

Unit cell	Two-sided plane groups	Corresponding 3-dimensional space group	Projection symmetry
oblique	p1	P1	p1
	p21	P2(*c*-axis unique)	p2
rectangular	p12	P2(*b*-axis unique)	pm
	$p12_1$	$P2_1$	pg
	c12	C2	cm
	p222	P222	pmm
	$p222_1$	$P222_1$	pmg
	$p22_12_1$	$P22_12_1$	pgg
	c222	C222	cmm
square	p4	P4	p4
	p422	P422	p4m
	$p42_12$	$P42_12$	p4g
hexagonal	p3	P3	p3
	p312	P312	p3m1
	p321	P321	p31m
	p6	P6	p6
	p622	P622	p6m

The two-sided plane group nomenclature was first proposed by W.T. Holser (Z.krystallogr. **110**, 266-28, (1958)). His nomenclature has the following main rules.

(a) Cell type is indicated first by small letter, *p* or *c* (primitive or centred)

(b) The axis perpendicular to the plane is always chosen as the *z*-axis.

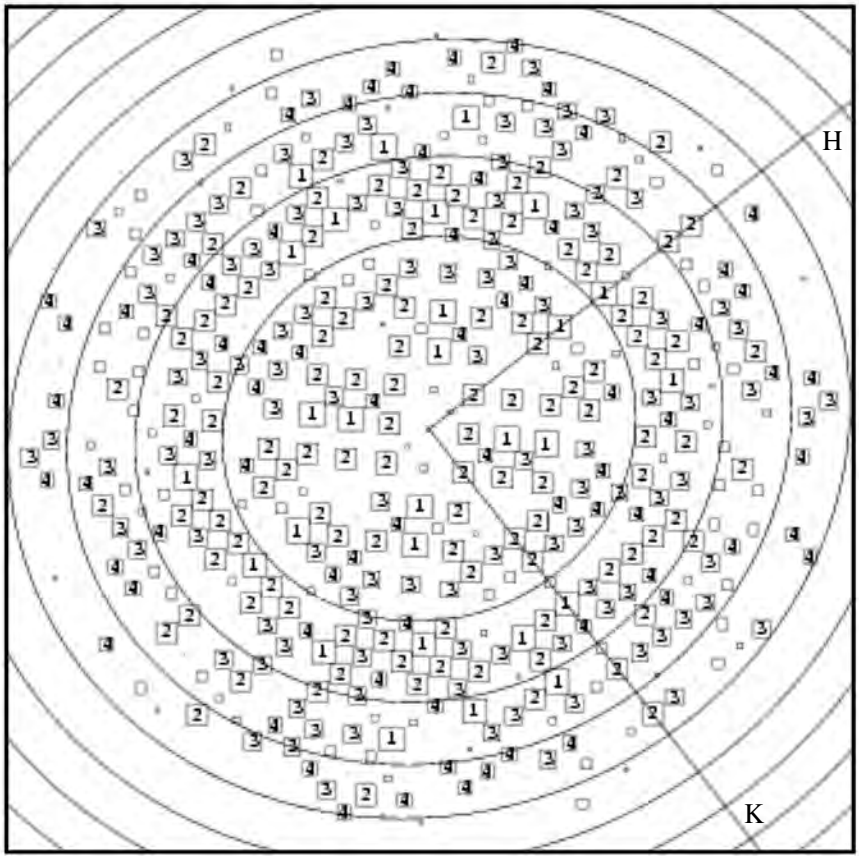
(c) The symmetry along this axis is always described by the first symbol following the cell type.

e.g. p3 $p42_12$

* Note that there 80 two-sided plane groups in total, of which there are only 17 which do not contain inversion centres or mirror or glide planes.

```
C####################  ALLSPACE  ################################################
C   Table of phase comparisons to be made
C       -  not comparable
C       1  directly identical
C       H  differ by 180 * H            JSIMPLE  = number to compare directly
C       K  differ by 180 * K            JSCREW   = number to compare + 180 * M
C       HK differ by 180 * (H+K)         where M = H*JH180 + K*JK180
C
C
C   SPACEGROUP  H=-h +h -h +k +k -k -k +h -h +k -k -h +h -h +h  JSIMPLE
C               H=                                  -k +k -k +k     JSCREW
C ref in
C  prog # symb  K=+k -k -k +h -h +h -h -h +h -h +h +h -h +k -k         JH180
C               K=                      -k +k -k +k                      JK180
C
C  1    1   p1     -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -   0  0   -   -
C  2    2   p2     -  -  1  -  -  -  -  -  -  -  -  -  -  -  -  -   1  0   -   -
C  3    3b  p12    1  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -   1  0   -   -
C  4    "a   "     -  1  -  -  -  -  -  -  -  -  -  -  -  -  -  -   1  0   -   -
C  5    4b  p121   K  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -   0  1   -  180
C  6    "a   "     -  H  -  -  -  -  -  -  -  -  -  -  -  -  -  -   0  1  180  -
C  7    5b  c12    1  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -   1  0   -   -
C  8    "a   "     -  1  -  -  -  -  -  -  -  -  -  -  -  -  -  -   1  0   -   -
C  9    6   p222   1  1  1  -  -  -  -  -  -  -  -  -  -  -  -  -   3  0   -   -
C 10    7b  p2221  H  H  1  -  -  -  -  -  -  -  -  -  -  -  -  -   1  2  180  -
C 11    "a   "     K  K  1  -  -  -  -  -  -  -  -  -  -  -  -  -   1  2   -  180
C 12    8   p22121 HK HK 1  -  -  -  -  -  -  -  -  -  -  -  -  -   1  2  180 180
C 13    9   c222   1  1  1  -  -  -  -  -  -  -  -  -  -  -  -  -   3  0   -   -
C 14    10  p4     -  -  1  -  1  1  -  -  -  -  -  -  -  -  -  -   3  0   -   -
C 15    11  p422   1  1  1  1  1  1  1  -  -  -  -  -  -  -  -  -   7  0   -   -
C 16    12  p4212  HK HK 1  1  HK HK 1  -  -  -  -  -  -  -  -  -   3  4  180 180
C 17    13  p3     -  -  -  -  -  -  -  -  -  1  -  1  -  -  -  -   2  0   -   -
C 18    14  p312   -  -  -  -  -  -  1  -  1  1  -  1  -  -  1  -   5  0   -   -
C 19    15  p321   -  -  -  1  -  -  -  1  -  1  -  1  -  1  -  -   5  0   -   -
C 20    16  p6     -  -  1  -  -  -  -  -  -  1  1  1  1  -  -  -   5  0   -   -
C 21    17  p622   -  -  1  1  -  -  1  1  1  1  1  1  1  1  1  1   11 0   -   -
C
C   Notes:-
C       1.  Compare all possible pairs of phases each with error E.
C       2.  Error comparing 2 different reflections is   1.414 * E.
C       3.  Error comparing reflections toits Friedel is   2.0 * E.
C       4.  So Friedel comparisons should have less weight ????
C       5.  Note that the convention in the two dimensional spacegroups is that
C               the first in-plane symmetry axis is the a-axis.  Therefore,
C               when ALLSPACE indicates that the spacegroup has the in-plane
C               axis along b, it is better to switch the indexing to ensure
C               compatibility with the normal convention as programmed in
C               ORIGTILT, rather than using the REVHK option in ORIGTILT,
C               which will switch the indexing on input.
```

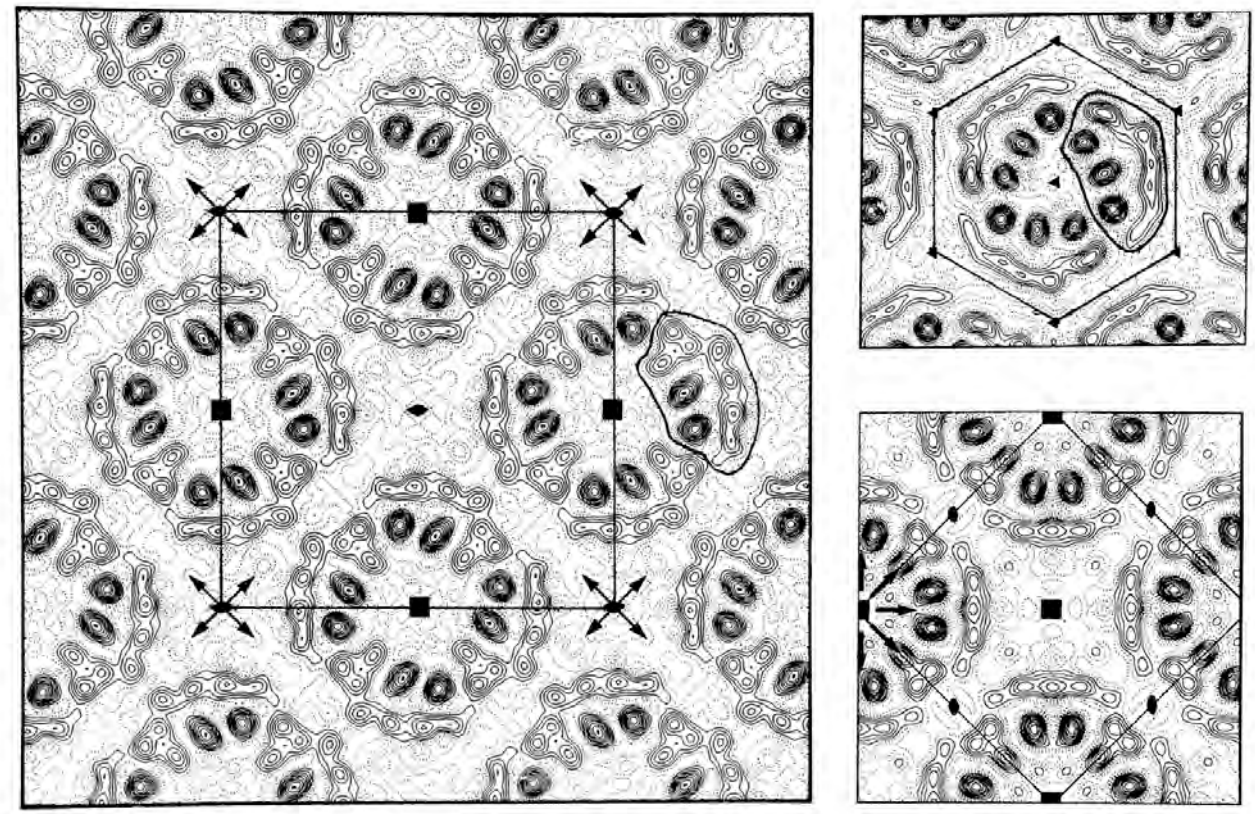
Halorhodopsin CTFg000 defocus=5650,7050,15, 19.2.94, CTF refinement



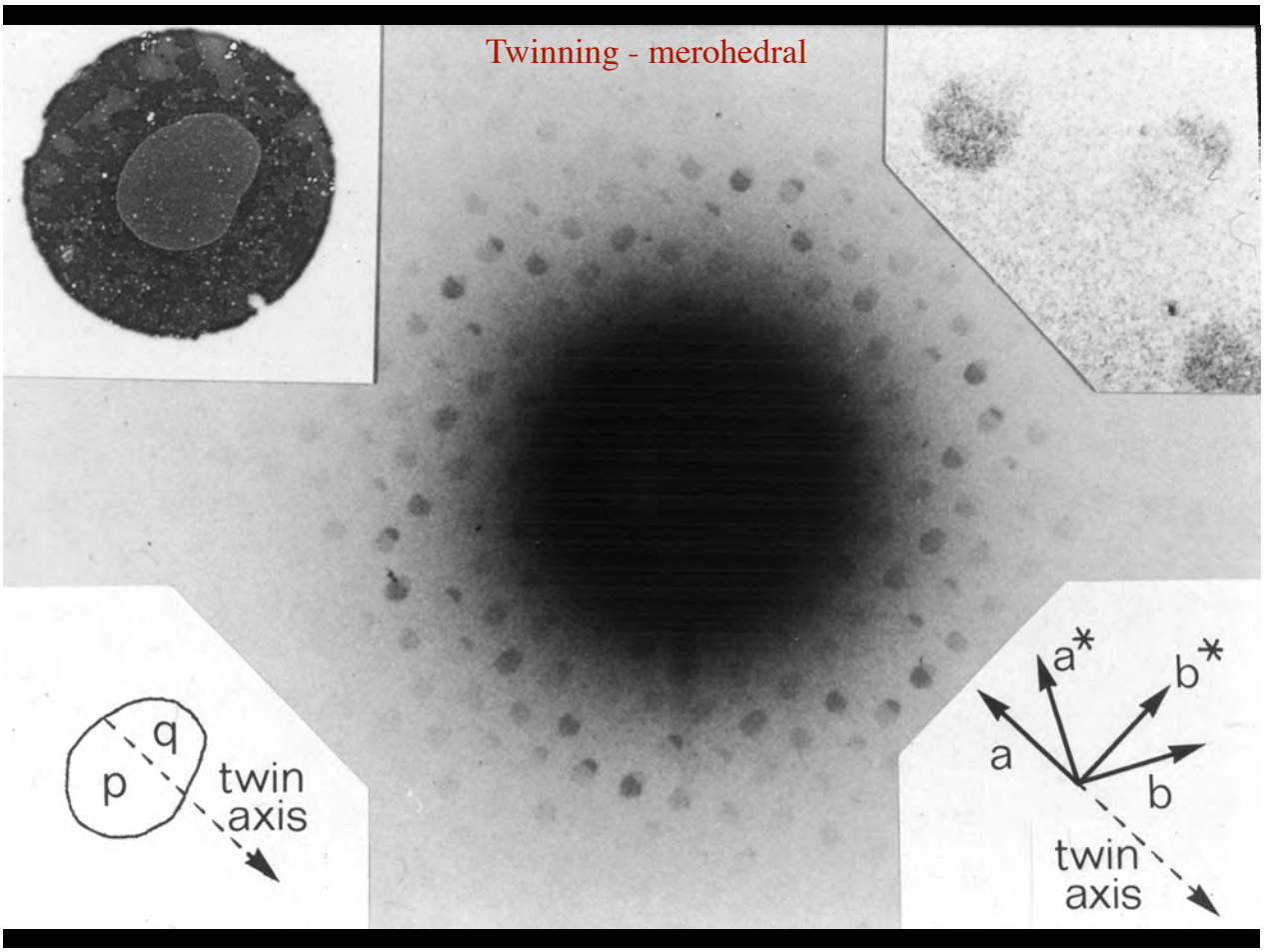
Halorhodopsin film 2464s

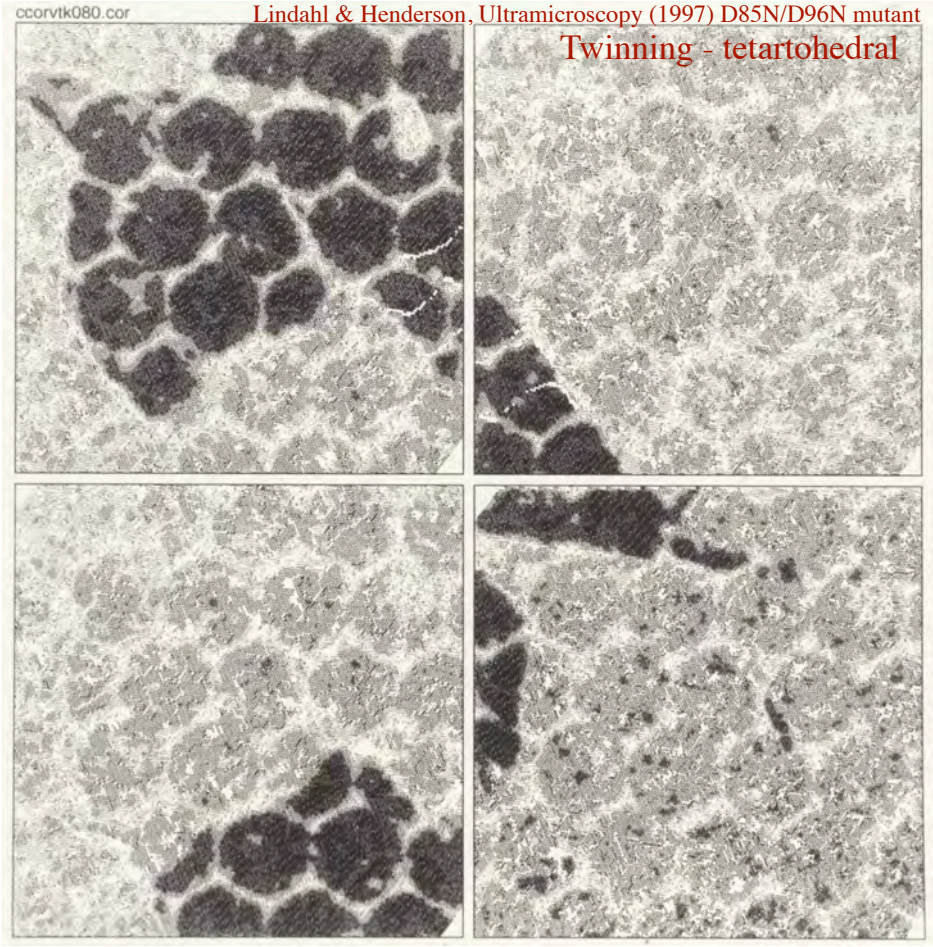
SPACEGROUP		Phase resid(No) v.other spots (90 random)		Phase resid(No) v.theoretical (45 random)		OX	OY	Target residual based on statistics taking Friedel weight into account
1	p1	34.4	432	25.9	432			
2	p2	42.8*	216	21.4	432	130.1	-21.6	51.7
3b	p12_b	77.7	167	11.3	14	-49.5	12.0	35.2
3a	p12_a	80.1	169	30.1	18	-180.0	-21.8	35.4
4b	p121_b	29.3*	167	10.1	14	-139.7	120.0	35.2
4a	p121_a	21.4*	169	9.3	18	138.0	68.1	35.4
5b	c12_b	77.7	167	11.3	14	-49.5	12.0	35.2
5a	c12_a	80.1	169	30.1	18	-180.0	-21.8	35.4
6	p222	64.9	552	21.5	432	-49.7	158.3	41.2
7b	p2221b	59.6	552	37.7	432	-139.5	-111.9	41.2
7a	p2221a	63.0	552	37.8	432	-139.7	68.2	41.2
8	p22121	32.3*	552	21.5	432	130.2	158.2	41.2
9	c222	64.9	552	21.5	432	-49.7	158.3	41.2
10	p4	30.8*	528	21.5	432	130.1	-21.7	41.5
11	p422	57.2	1173	21.5	432	130.1	158.1	37.6
12	p4212	28.2*	1173	21.6	432	130.1	158.1	37.6

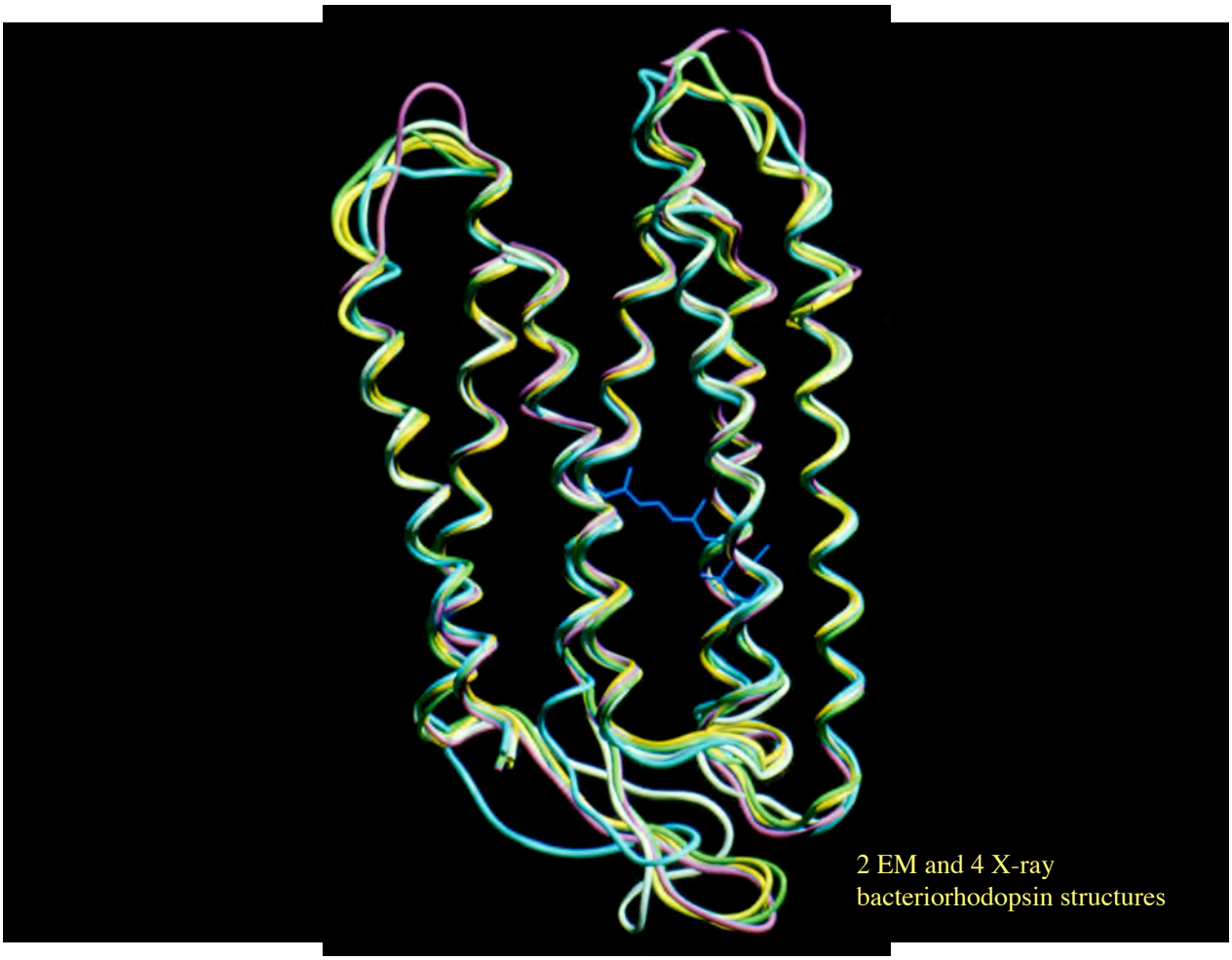
* = acceptable
! = should be considered
` = possibility



Twinning - merohedral

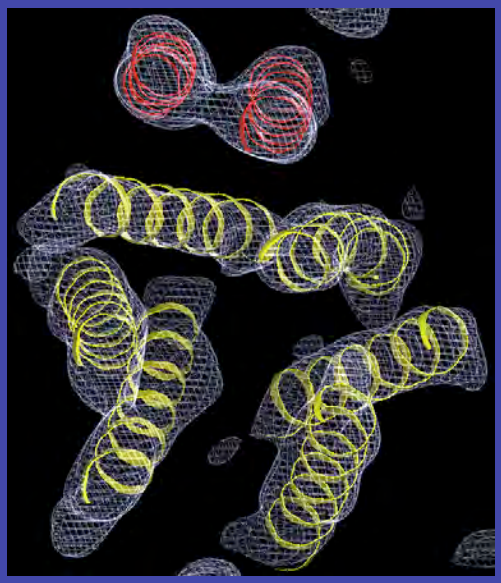



ccorvtk080.cor
Lindahl & Henderson, Ultramicroscopy (1997) D85N/D96N mutant
Twinning - tetartohedral

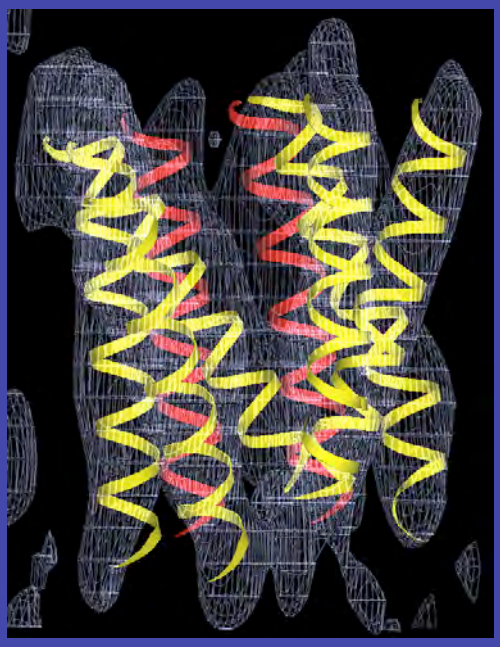

2 EM and 4 X-ray
bacteriorhodopsin structures

The multidrug transporter EmrE is an asymmetric homodimer

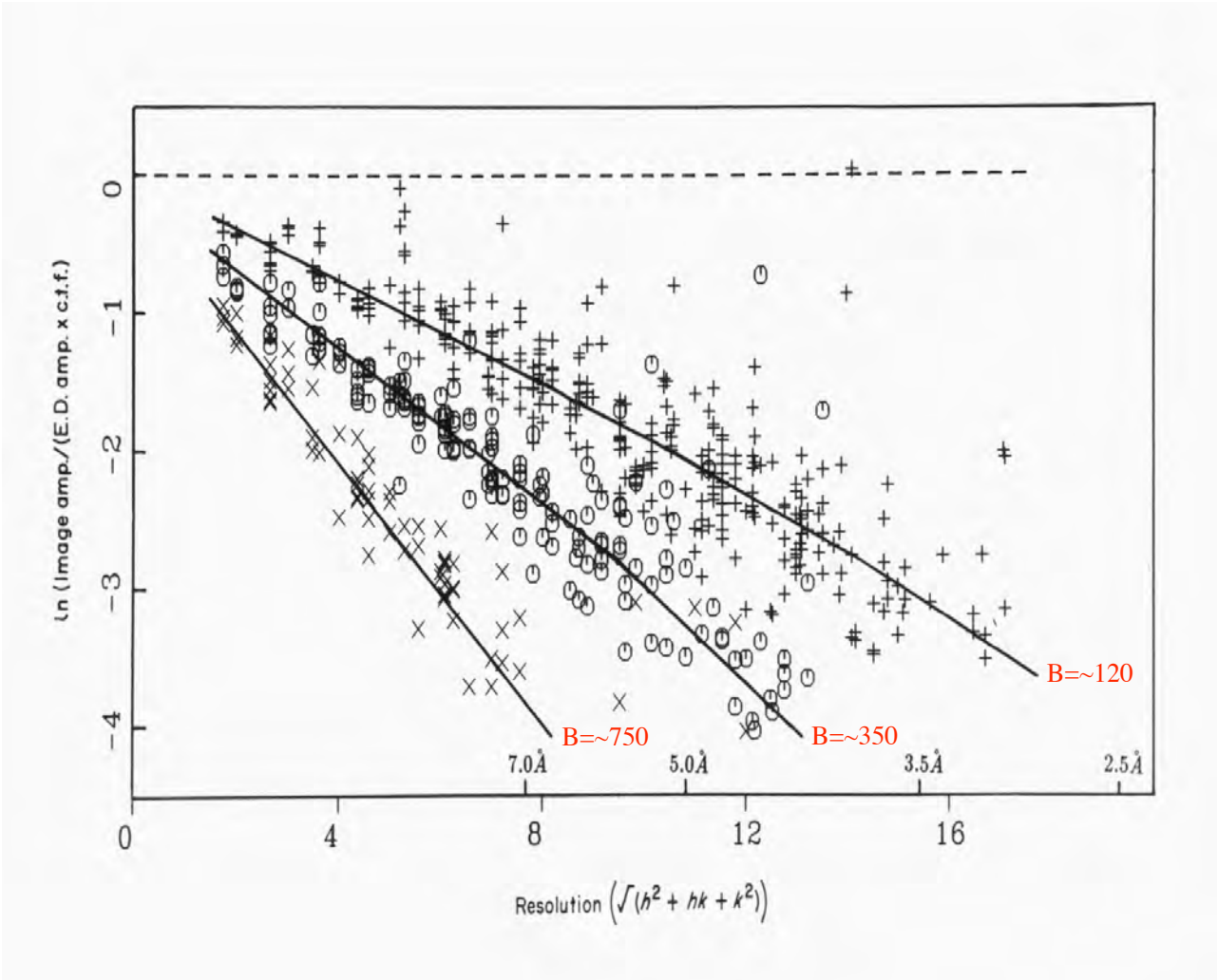
Tate & Ubarretxena-Belandia, EMBO J. (2003) 22, 6175

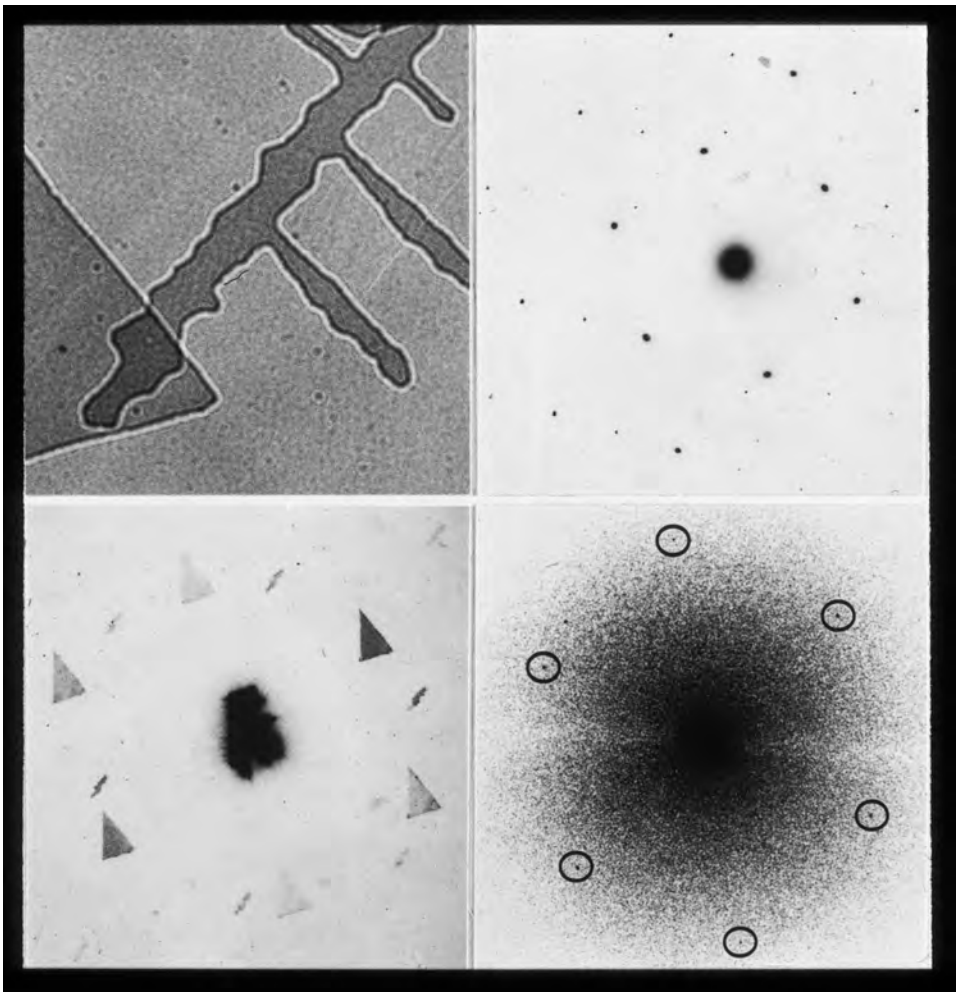


Top view



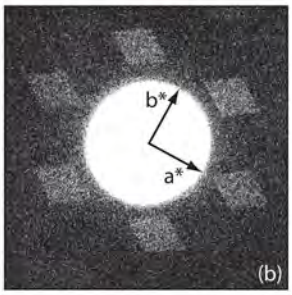
Side view





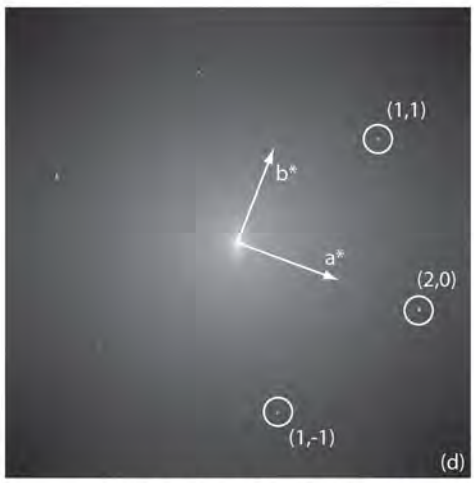
A17044/thinC_122p

bright field defocused eldiff

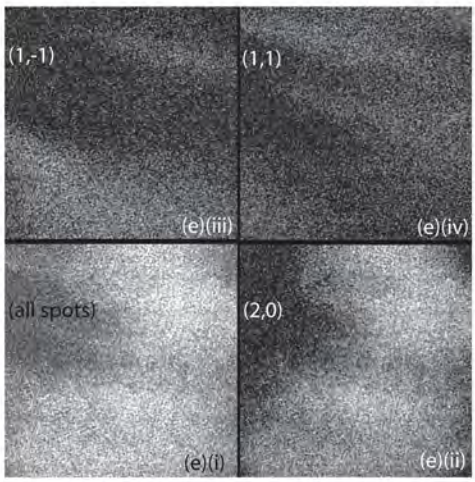


dark field defocused eldiff

dark field stretched contrast



power spectrum from micrograph



pseudo-dark-field images

Image A03141 and Polara file Pol_thikC_para_A03141 - before, after and after2

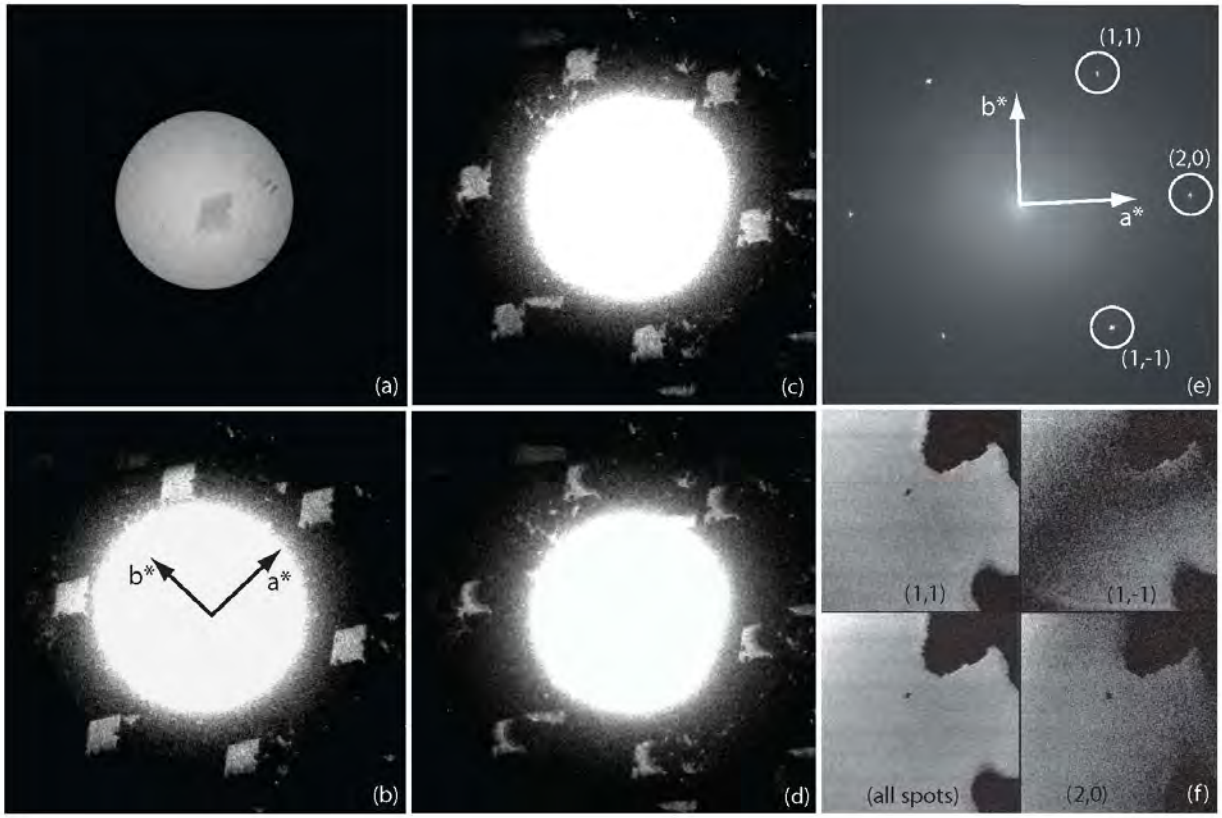


Figure 6

Table 1 - image contrast

Image	Description	Overall image area 1.5 μm square		Selected image area 0.15 μm square		% theoretical - range of values for either overall or selected area		
		I_g/I_0	F_g/F_0	I_g/I_0	F_g/F_0	Compared to electron diffraction	Corrected for detector MTF	Corrected for inelastic scattering
A17044 300kV	thinC floodbeam	0.4-2.0x10^{-4}	0.006-0.014	0.4-4.6x10^{-4}	0.006-0.021	2.7-9.7 %	10-44 %	11-47 %
011643 300kV	thinC microspotscan	4.3-7.5x10^{-4}	0.021-0.027	3.9-15.7x10^{-4} *	0.020-0.039 *	9.1-17.7 %	41-63 %	43-67 %
A16973 120kV	thickC nanospotscan	1.7-3.5x10^{-4}	0.013-0.019	4.9-8.4x10^{-4}	0.022-0.029	4.8-10.7 %	27-47 %	37-64 %
A16984 120kV	thickC nanospotscan	(0.3-2.4x10^{-4})	(0.005-0.015)	0.4-3.3x10^{-4}	0.007-0.018	2.6-6.6 %	14-29 %	19-40 %
para_22may_image07	thinC Medipix 120kV	1.7-5.1x10^{-4}**	0.013-0.023**	-	-	4.8-8.5 %	10-18 %	18-20 %
A02026 300kV	thickC floodbeam	(0.8-2.8x10^{-4})	(0.009-0.017)	3.2-12.3x10^{-4}	0.018-0.035	8.2-15.9 %	30-59 %	35-70 %
A02027 300kV	thickC floodbeam	(2.4-5.8x10^{-4})	(0.015-0.024)	4.5-16.6x10^{-4}	0.021-0.041	6.8-18.8 %	31-79 %	36-93%
A02030 300kV	thickC floodbeam	-	-	3.7-8.6x10^{-4}	0.019-0.029	8.6-13.2 %	39-49 %	46-58%
A03133 300kV	thickC floodbeam	2.5-5.7x10^{-4}	0.016-0.024	7.9-15.7x10^{-4}	0.028-0.040	7.3-18.2 %	33-67 %	39-79 %
A03134 300kV	thickC floodbeam	1.3-7.0x10^{-4}	0.012-0.026	1.3-12.1x10^{-4}	0.011-0.035	5.0-15.9 %	23-59 %	27-70 %
A03141 300kV	thickC floodbeam	0.9-6.8x10^{-4}	0.010-0.026	5.9-31.8x10^{-4}	0.024-0.056	4.4-25.6 %	16-95 %	19-112 %

Notes to Table 1:

* The selected area in this case was only 0.11 μm square, which was the size of each spot in the spotscan raster.

** The area in this case was only 0.1 μm square, which was the full size of the Medipix II-Quad detector.

The smallest and largest values given in columns 3 to 9 correspond to the weakest and strongest of the three reflections at ~4 Å resolution.

To calculate the % theoretical, the raw F_g/F_0 image values must first be divided by the values expected from electron diffraction, which are 0.22 for 300keV images, or 0.27 for 120keV images, as shown in column 7. All three reflections at ~4Å have very similar electron diffraction intensities.

The MTF envelope correction used to calculate column 8 has been taken from Fig.5 in reference [5] for film (1/0.18 and 1/0.23 for (2,0) and (1,1) respectively) and Medipix (1/0.40 and 1/0.47) at 120keV and from Fig.6 for film at 300keV (1/0.22 and 1/0.27 for (2,0) and (1,1) respectively). The MTF correction is larger for the (2,0) reflection because it is at higher resolution, 3.7Å compared with 4.1Å for the (1,1) and (1,-1) reflections. A further correction for the contrast reduction due to inelastic scattering is shown in column 9. The correction factors are 1.06 for thin carbon at 300keV, 1.12 for thin carbon at 120keV, 1.18 for thick carbon at 300keV and 1.37 for thick carbon at 120keV.

The numbers in brackets for some of the images in columns 3 and 4 are lower than they should be because the crystal was smaller than the overall image area. The area of A02030 included several twinned and untwinned crystals, so no numbers are given for the overall image area for that image.

Key considerations

- Tilt-transfer-function (TTF, TTBOX, etc)
- Creating reference area using MAKETRAN
- Size of reference box (optimize)

Other problems

- Better images on thick carbon
- Flatness of 2D crystals
- Treatment as single molecules/unit cells

Lessons from 2D for single particle EM

- Beam tilt
- Differential magnification

Grigorieff, Beckmann & Zemlin, JMB, 1995

Table 2. Average signal-to-background ratio of all spot intensities in the resolution range 3.0 Å to 4.0 Å, obtained for one image using different reference areas to find the position of unit cells

Area in pixel	Unit cells	% Fourier averaging	Reflections found with IQ⩽7	Signal/Background
300 × 300	73	99	79	10/7
200 × 200	33	98	88	25/7
130 × 130	14	91	107	41/7
70 × 70	4	80	100	37/7
34 × 34	1	0	70	4/7
Unprocessed image			40	0/7

In each case, Fourier averaging was applied to the image before unbending except in the last two rows. The value (in %) in the third column refers to the area masked out by the Fourier mask applied to the image transform. The signal-to-background ratio was calculated after subtraction of the background from each integrated spot intensity. The total number of reflections in the chosen range was 222. The signal refers to the average intensity above background at the position expected for the diffraction peaks from the crystal. The background is the average intensity in the immediately surrounding area; it is normalized to 7. The standard deviation of the background is the background fluctuation after the intensity of all reflections has been averaged. It was determined to be 1.0 for the single molecule cross-correlation in the second last row. Thus, a peak of 4 is above a background of 7 by 4 × the standard deviation. The signal-to-background ratio for the unprocessed image was included for comparison in the last row.

Acknowledgements

bR

7 Å	Unwin
3.5 Å	Baldwin, Downing, Lepault, Zemlin
3D map	Baldwin, Ceska, Zemlin, Beckman, Downing
Refinement	Grigorieff

hR

7 Å	Havelka, Oesterhelt
5 Å	Kunji

paraffin

Bob Glaeser, Greg McMullan, Wasi Faruqi