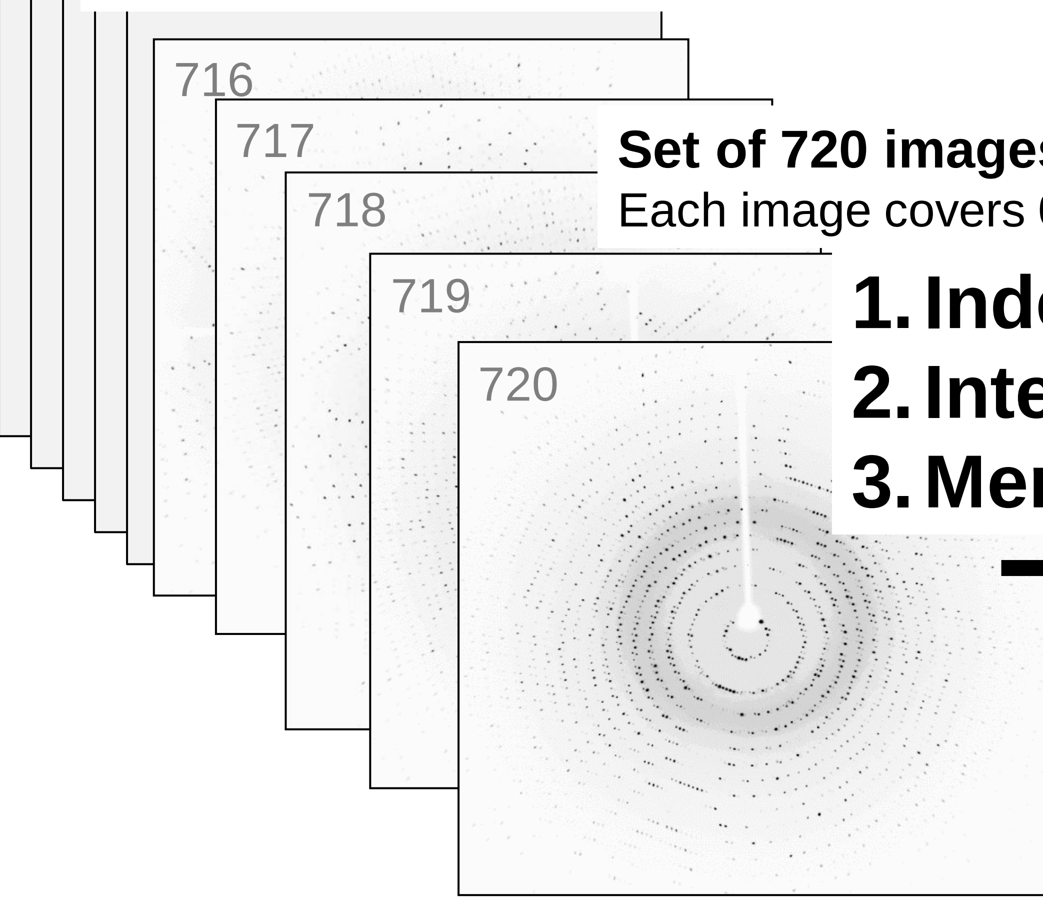
Data Reduction Goals

From a series of diffraction images, obtain a file containing the intensity (I_{hkl}) and standard deviation ($\sigma(I_{hkl})$) for each reflection, hkl.



Set of 720 images.
Each image covers 0.5°

1. **Index**
2. **Integrate**
3. **Merge**

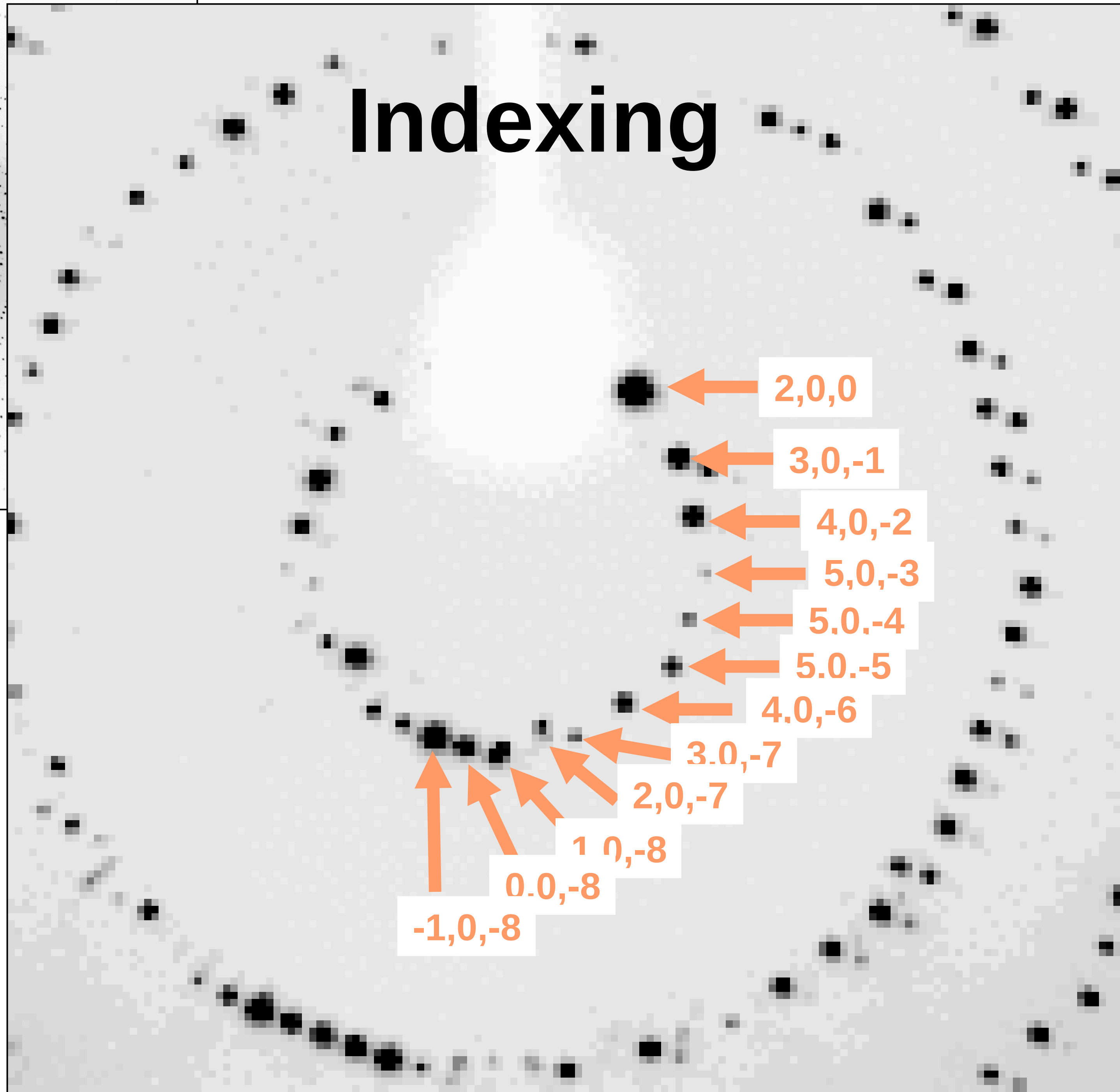
6 x 10^9 bytes

Final intensities

H	K	L	I	σ
0	0	4	3295.4	174.0
0	0	8	482.1	28.7
0	0	12	9691.0	500.7
0	0	16	1743.9	67.4
0	0	20	5856.0	221.0
0	0	24	14066.5	436.2
0	0	28	9936.3	311.7
0	0	36	8409.8	273.4
0	0	40	790.5	32.8
0	0	44	103.4	18.4
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
37	7	0	28.5	16.2
37	7	1	110.1	10.9
37	7	2	337.4	13.3
37	7	3	98.5	10.6
37	7	4	25.9	10.7

8 x 10^5 bytes

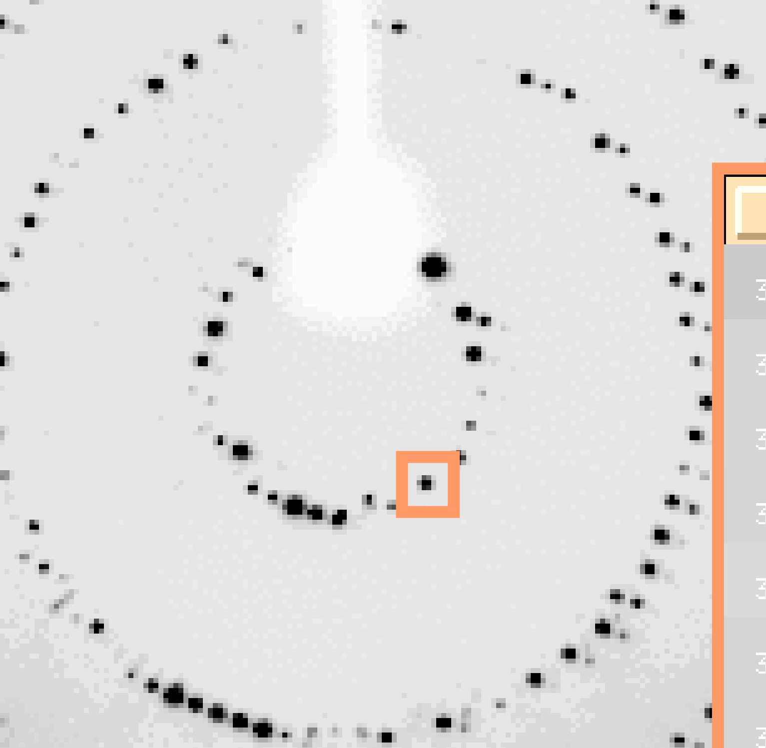
Indexing

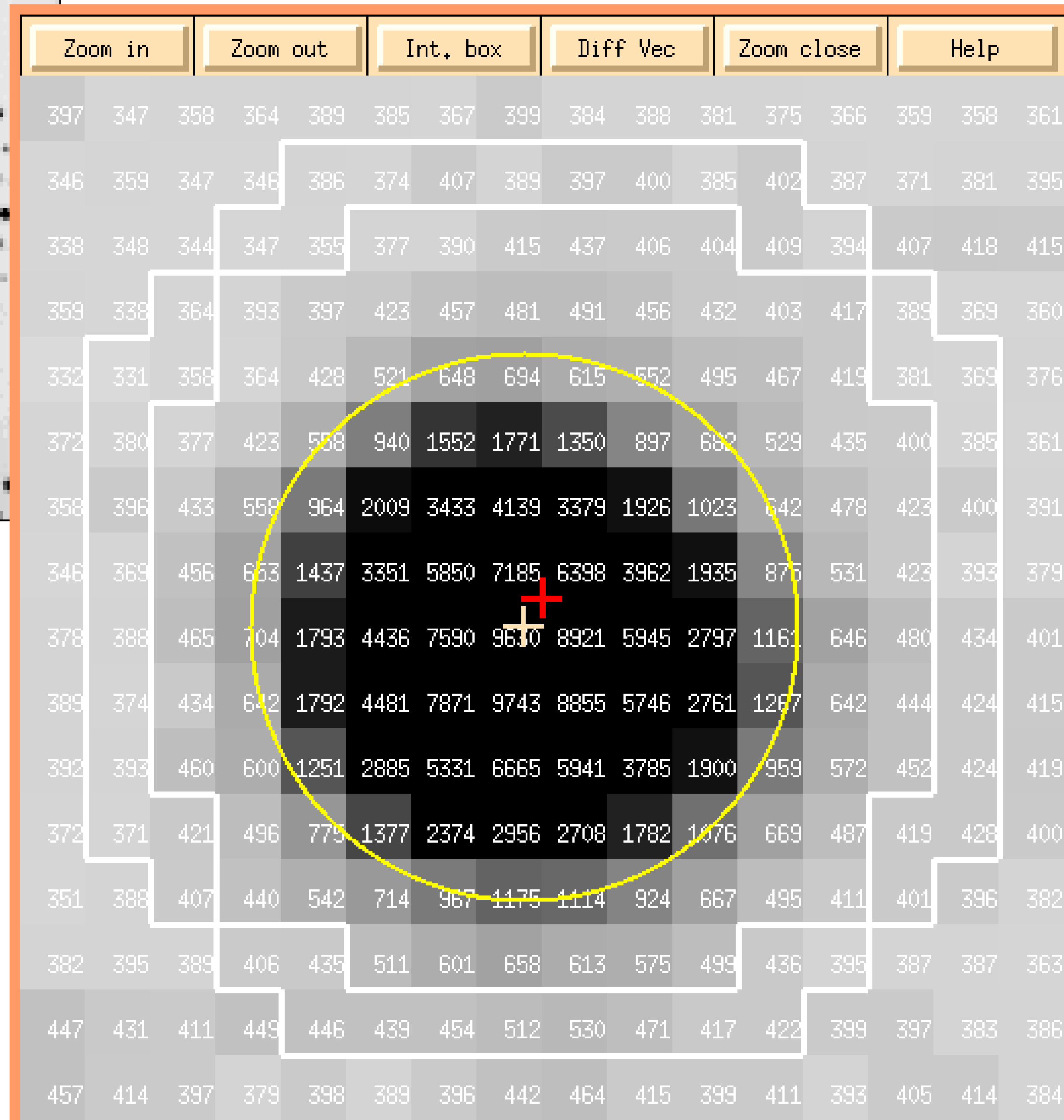


Assign an h,k,l coordinate to each reflection of the first image.

Indices h,k,l are coordinates of the reflections, analogous to how atom positions are described by coordinates x,y,z.

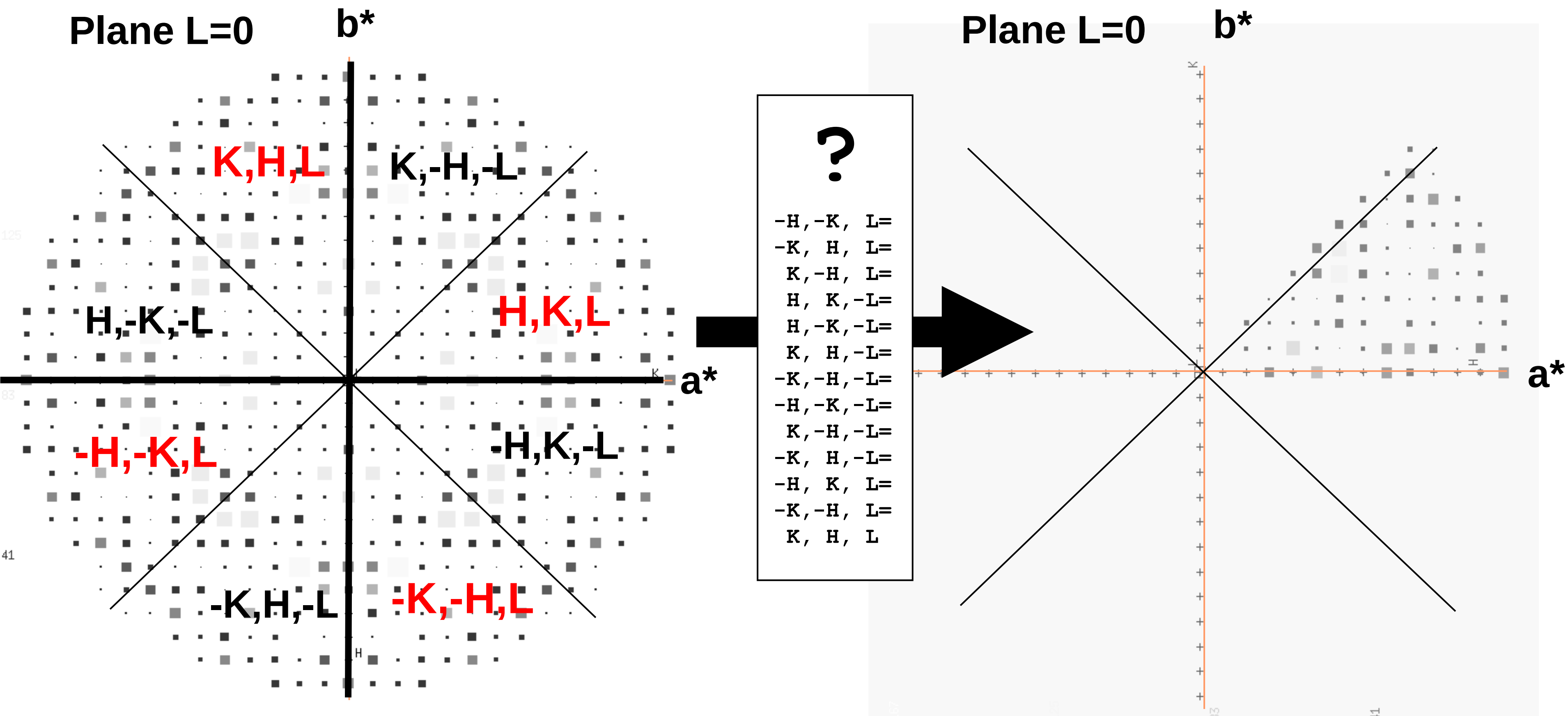
Integration





1) Draw boundaries of each reflection
2) Sum up the intensities recorded on each pixel within boundary.
3) Repeat for each spot on each film.

Merging

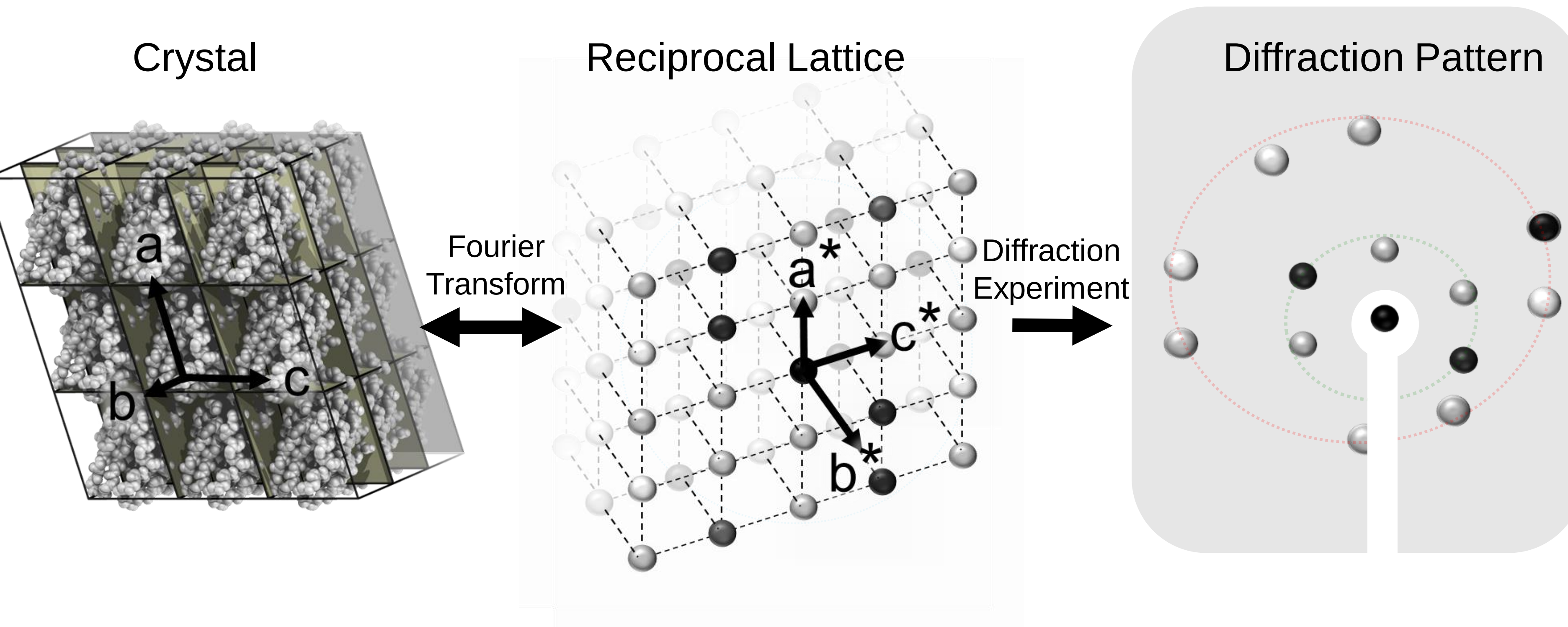


Make a hypothesis about symmetry laws obeyed in data.

Average together I_{hkl} values for h,k,l indices related by putative symmetry.

Verify symmetry hypothesis by calculating R_{merge}

Understand the relationship between the crystal, the reciprocal lattice and the diffraction pattern



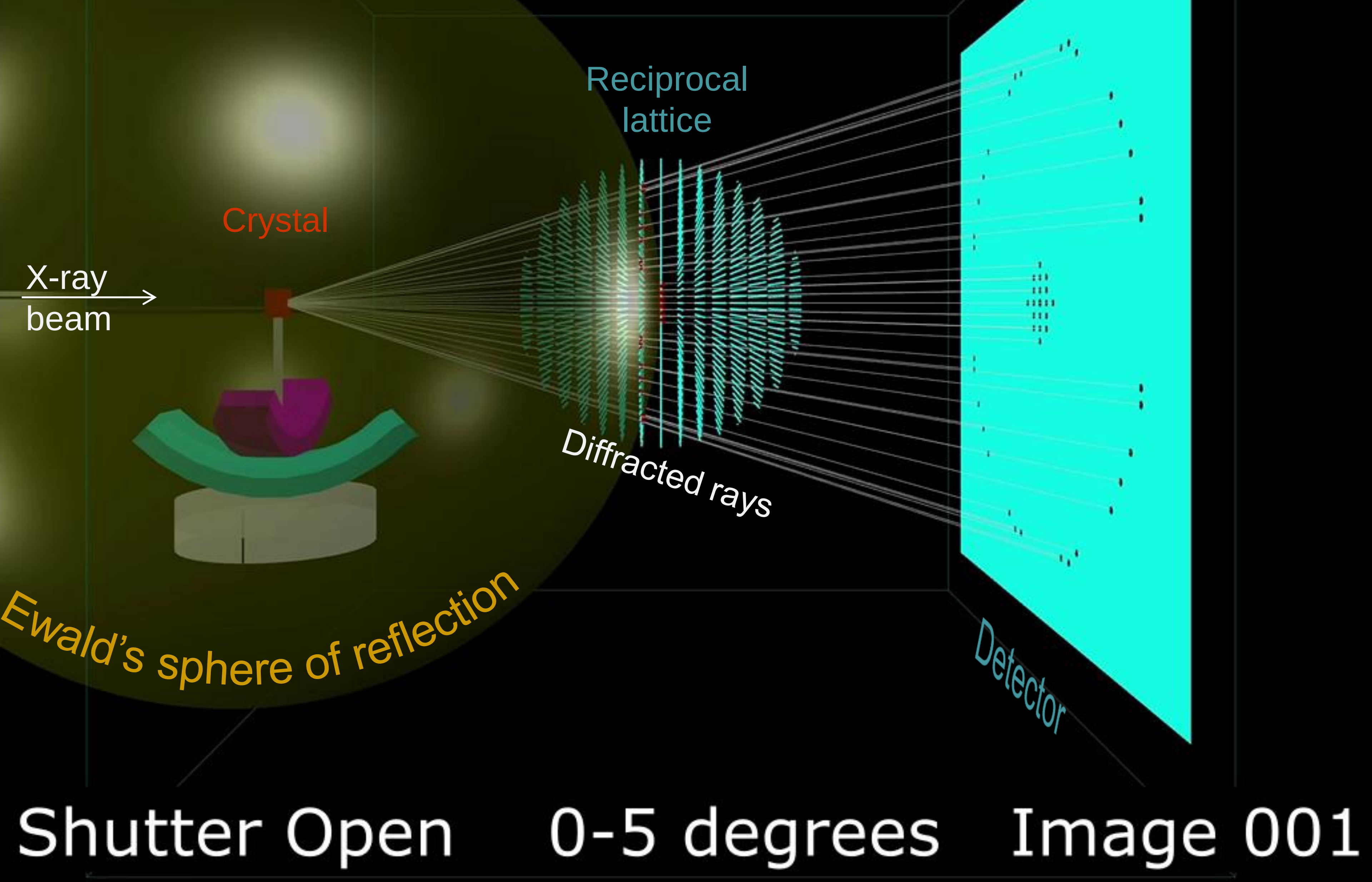
Unit cell lengths a, b, c
Atom coordinates x, y, z

Reciprocal cell lengths a*, b*, c*
Reflection coordinates h, k, l

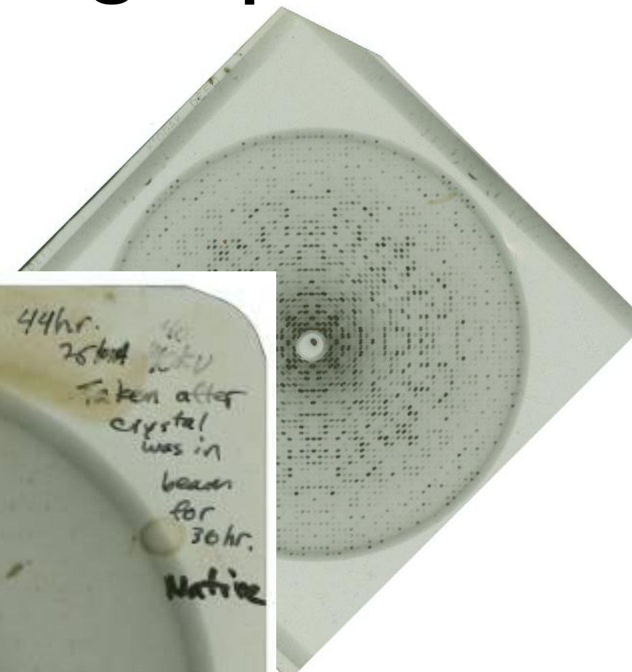
Goal of data reduction is to measure intensity at each lattice point, h,k,l.

A 2D projection of a 3D reciprocal lattice

Simulated Data Collection



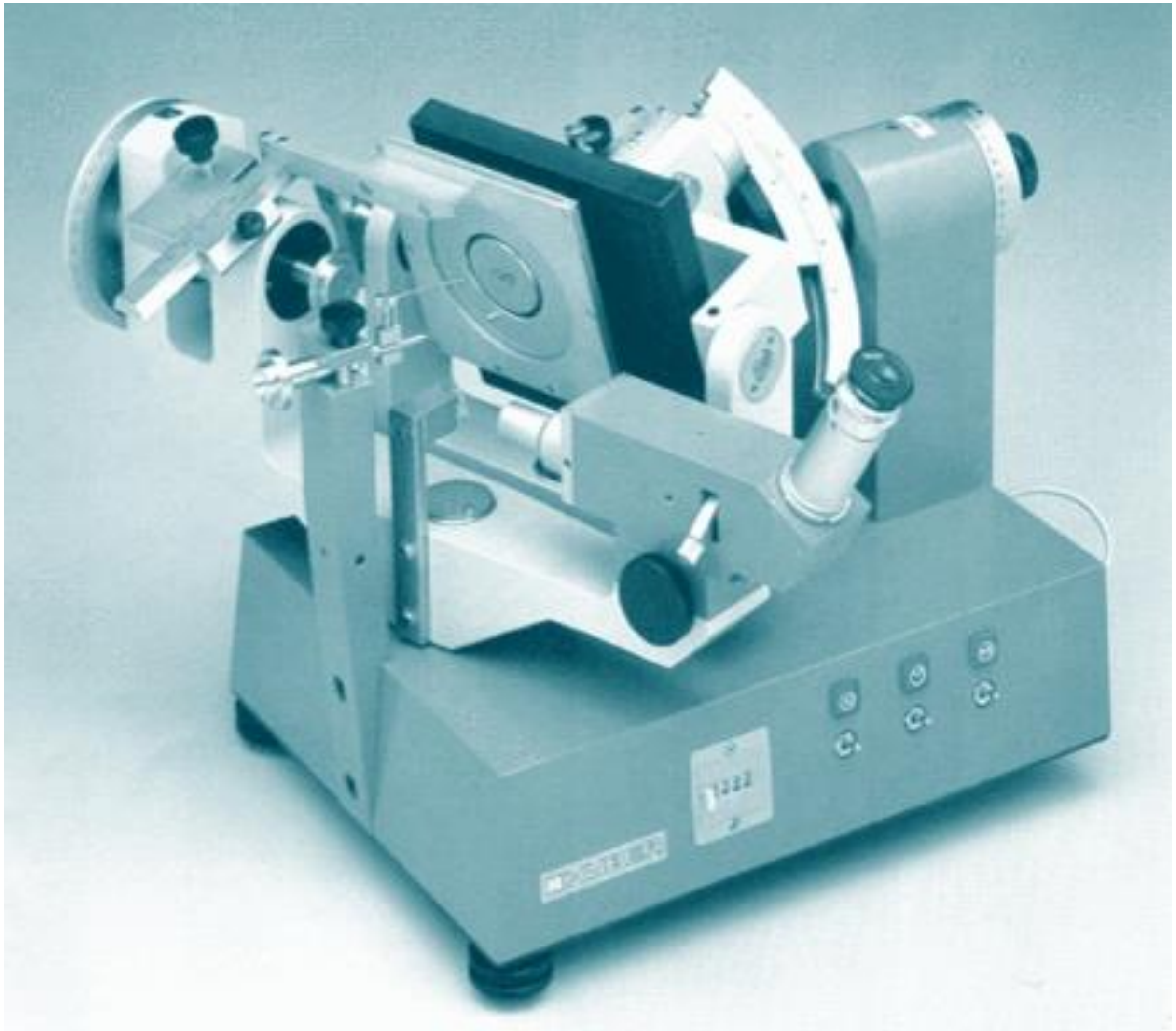
Indexing is conceptually simple with precession photographs



Provides an undistorted view of one plane of the reciprocal lattice

Taken in early 1990s.

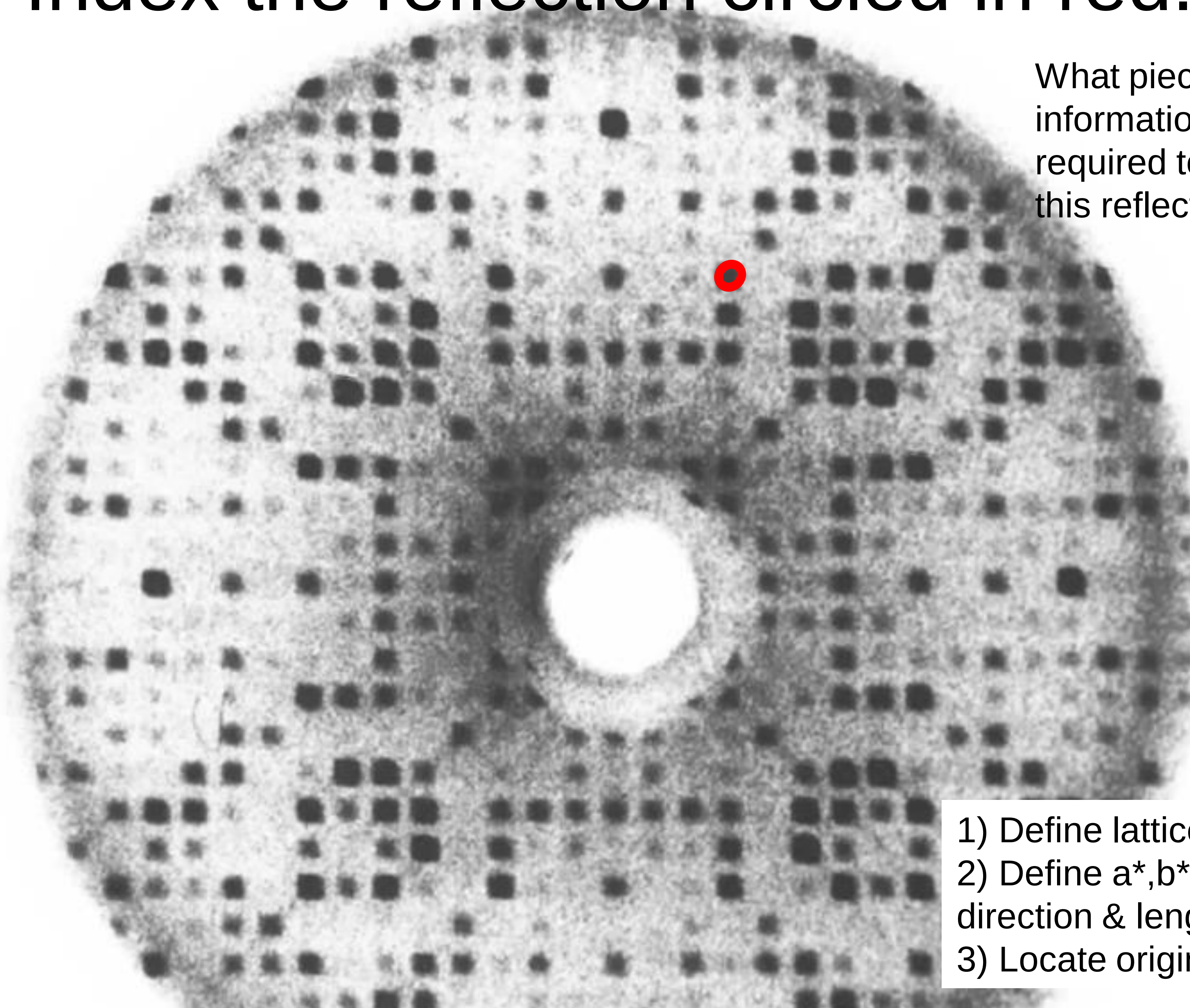
Buerger Precession Camera



How to Index by Inspection

- Recall that spots are arrayed on a reciprocal lattice.
- Count how many steps the given reflection is displaced from the origin.
  - h=Number of steps of a*.
  - k=Number of steps of b*.
  - l=Number of steps of c*.

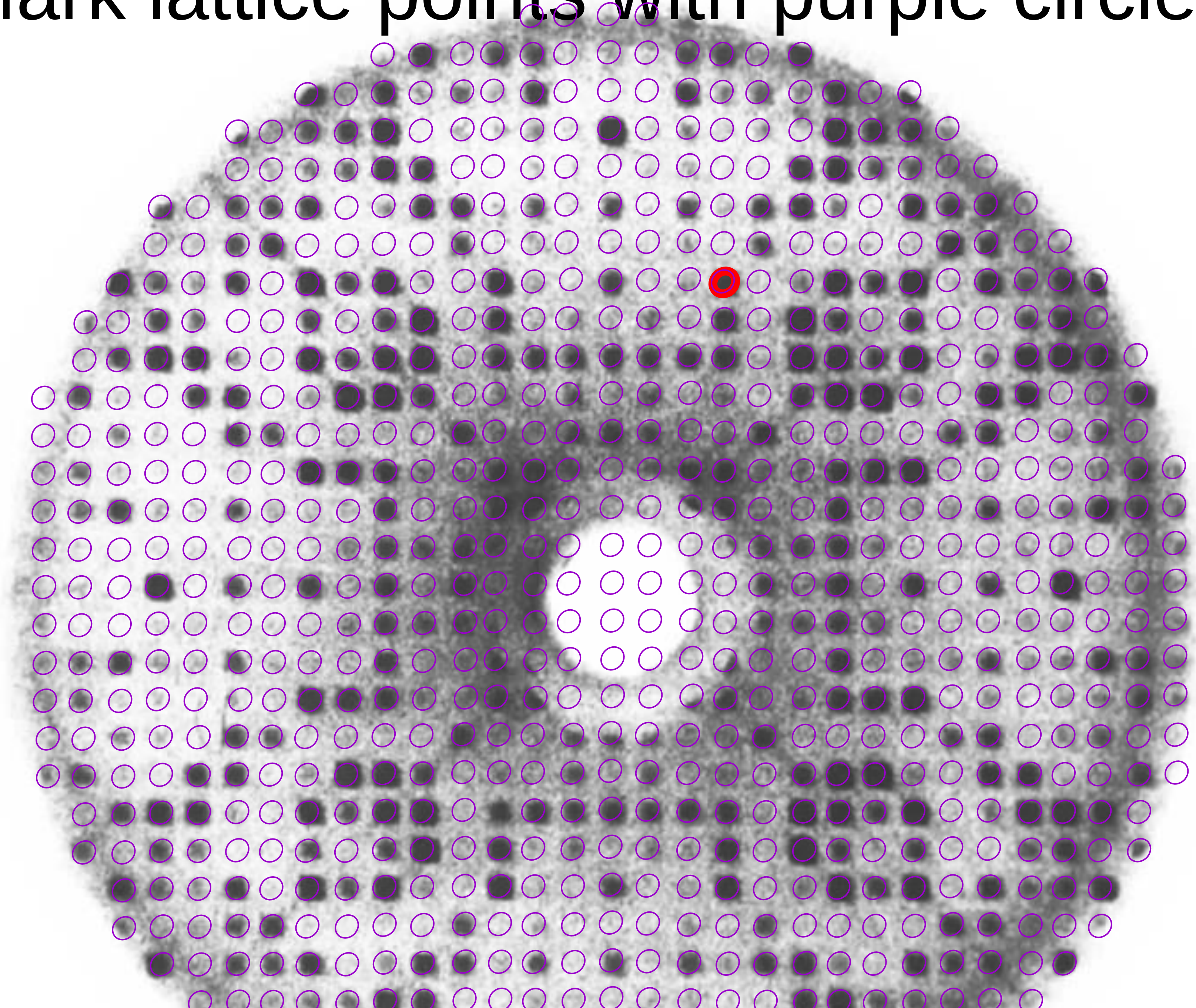
Index the reflection circled in red.



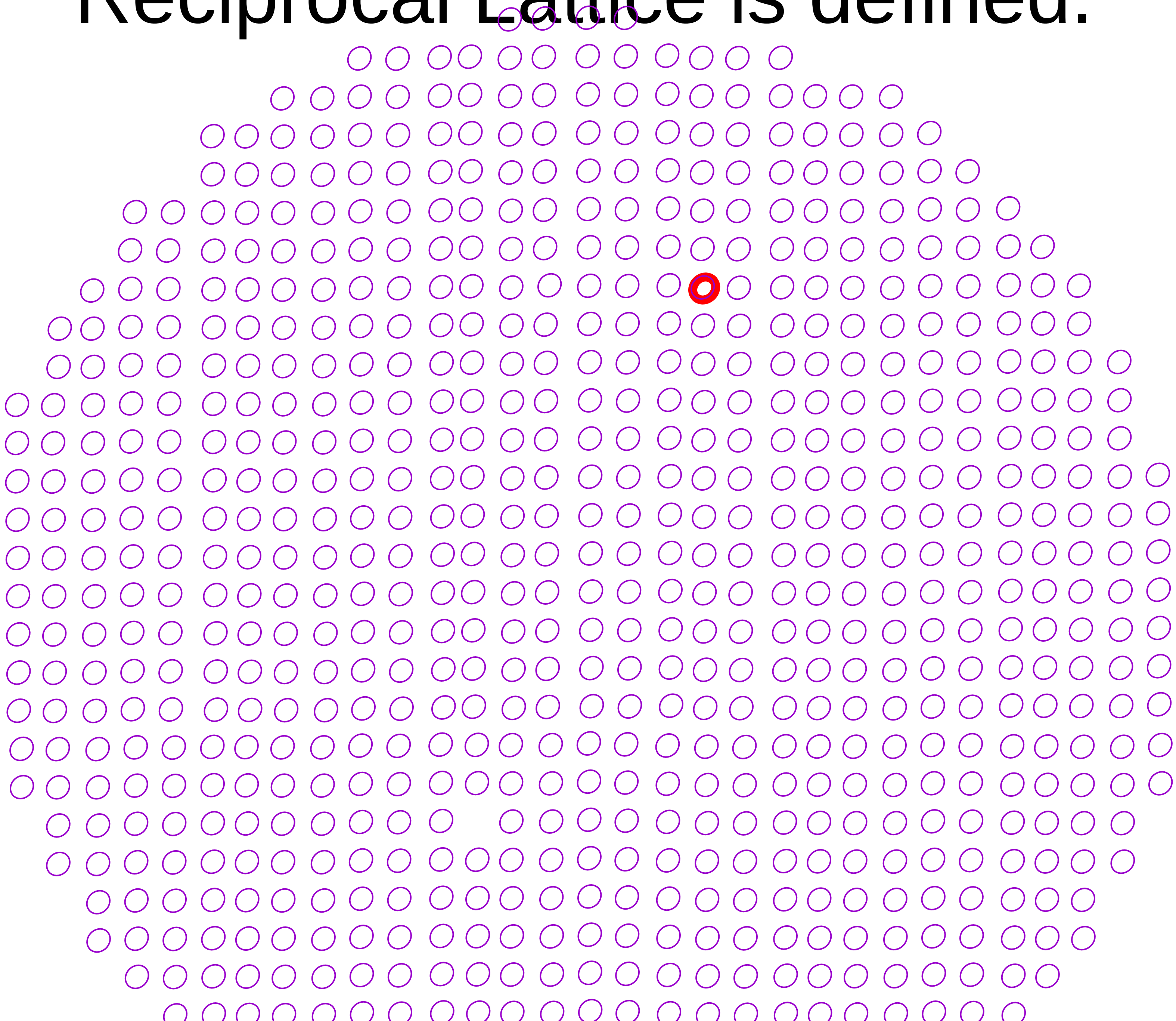
What pieces of information are required to index this reflection?

1) Define lattice points
2) Define a*,b*, c* direction & length
3) Locate origin.

Mark lattice points with purple circles.



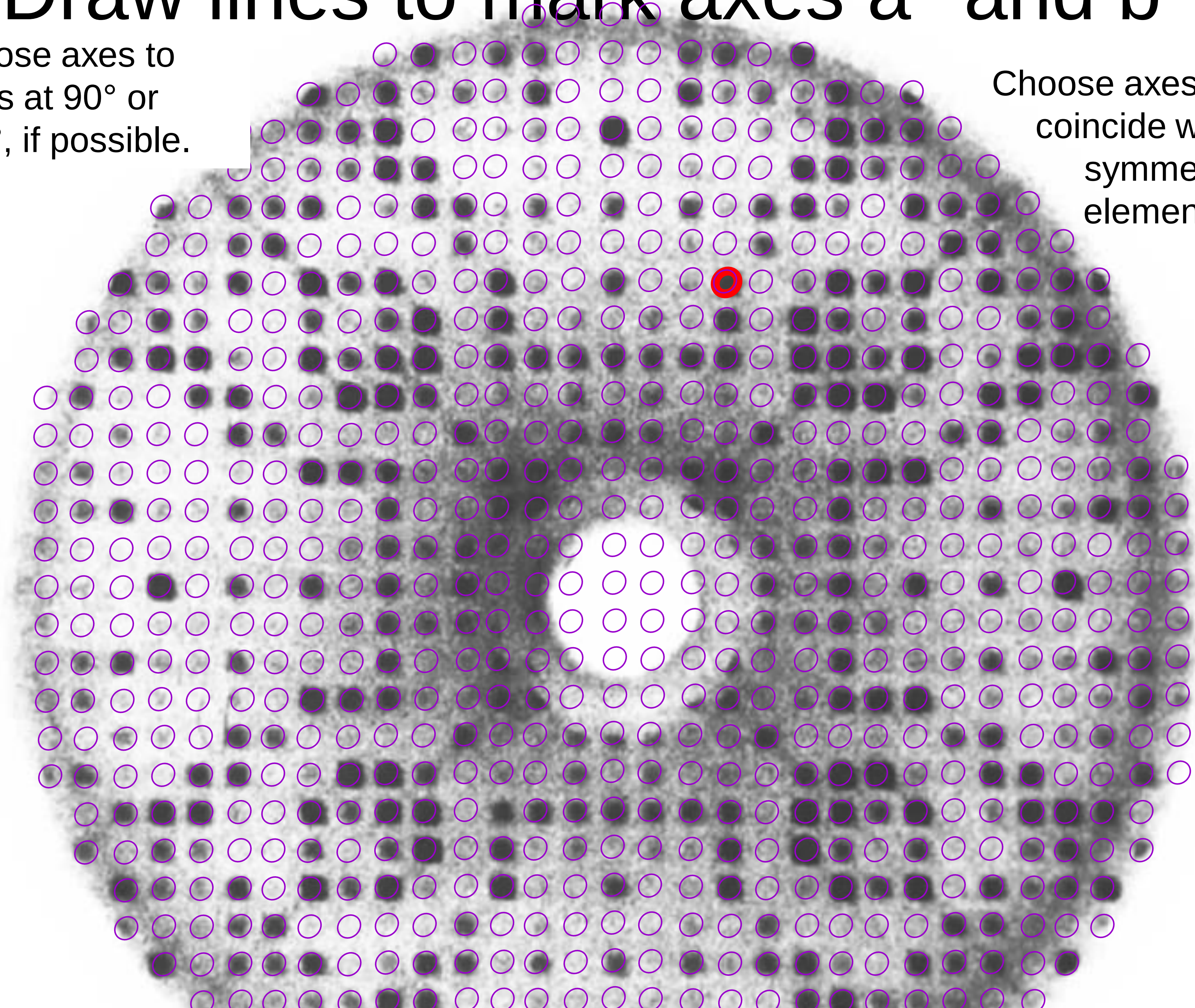
Reciprocal Lattice is defined.



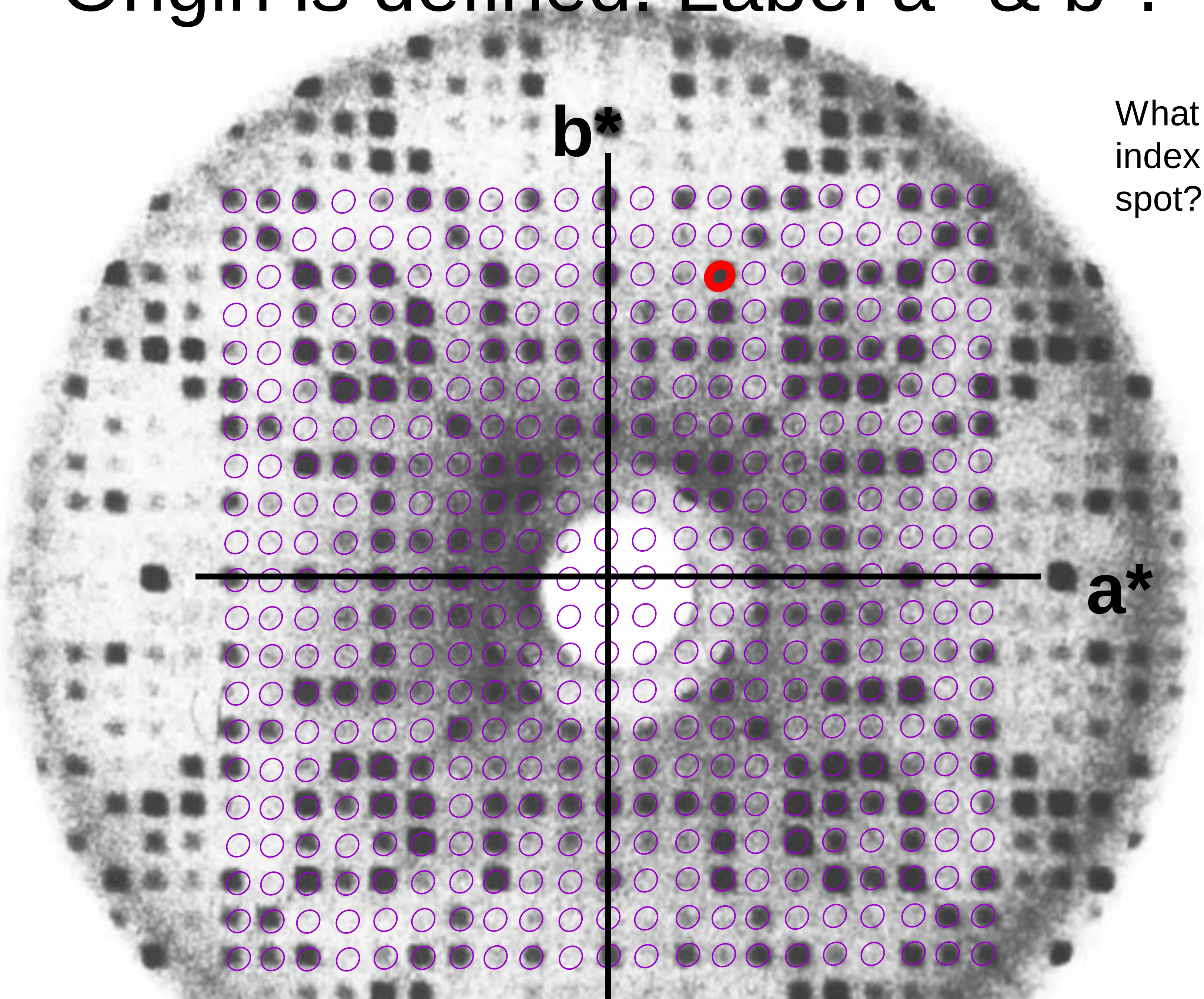
Draw lines to mark axes a* and b*.

Choose axes to cross at 90° or 120°, if possible.

Choose axes to coincide with symmetry elements.



Origin is defined. Label a* & b*.

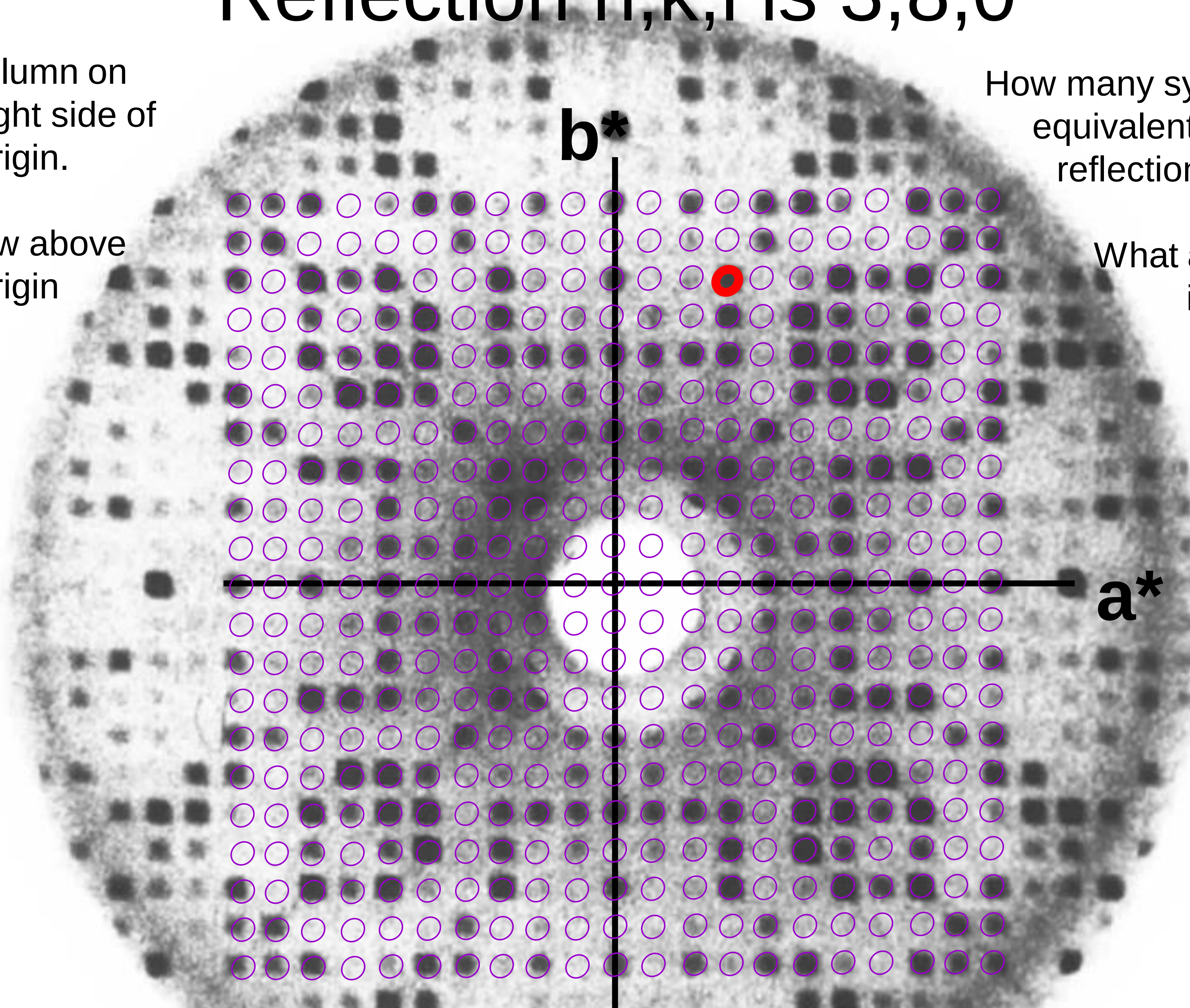


What is the index of the spot?

Reflection h,k,l is 3,8,0

3rd column on the right side of the origin.

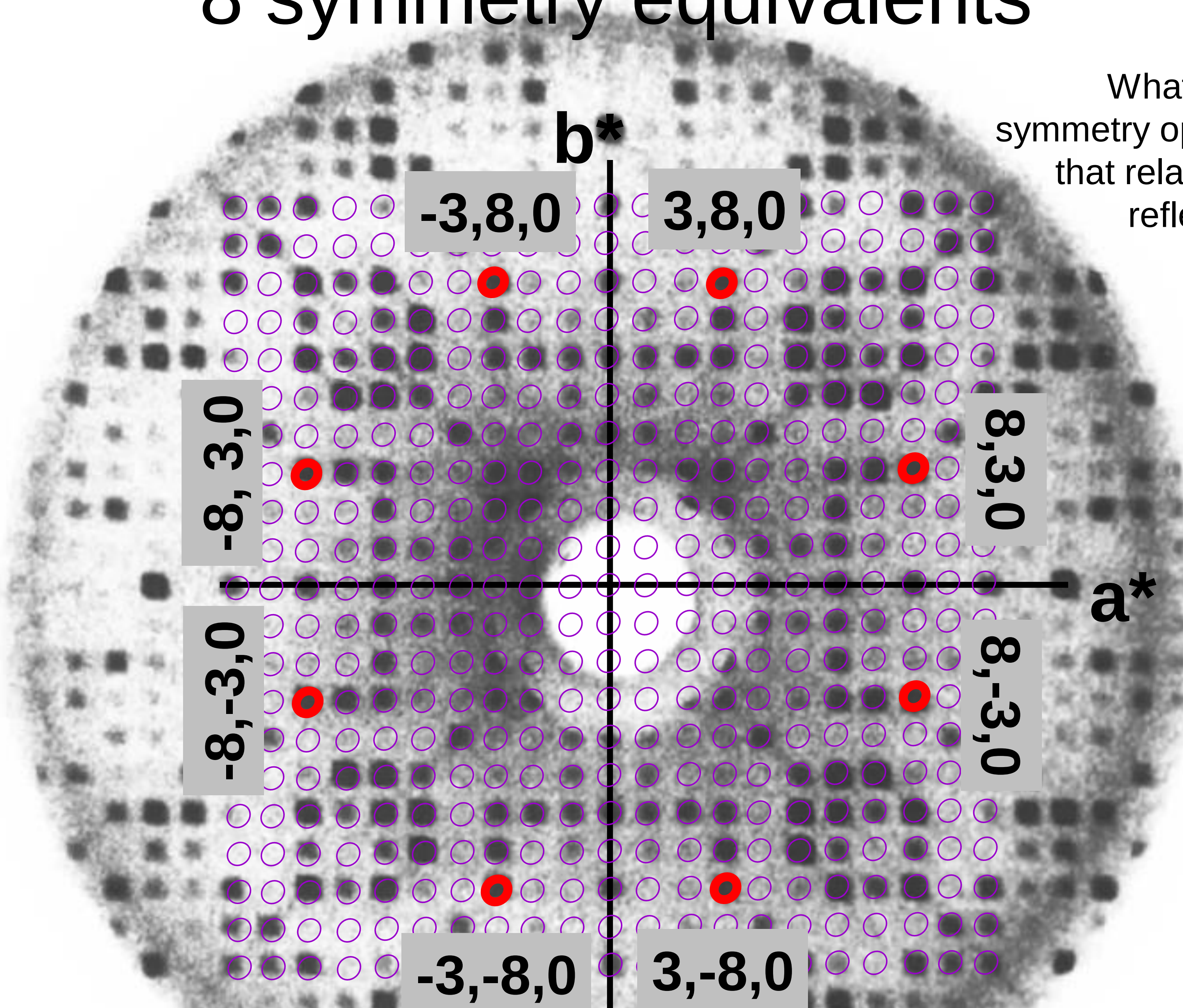
8th row above the origin



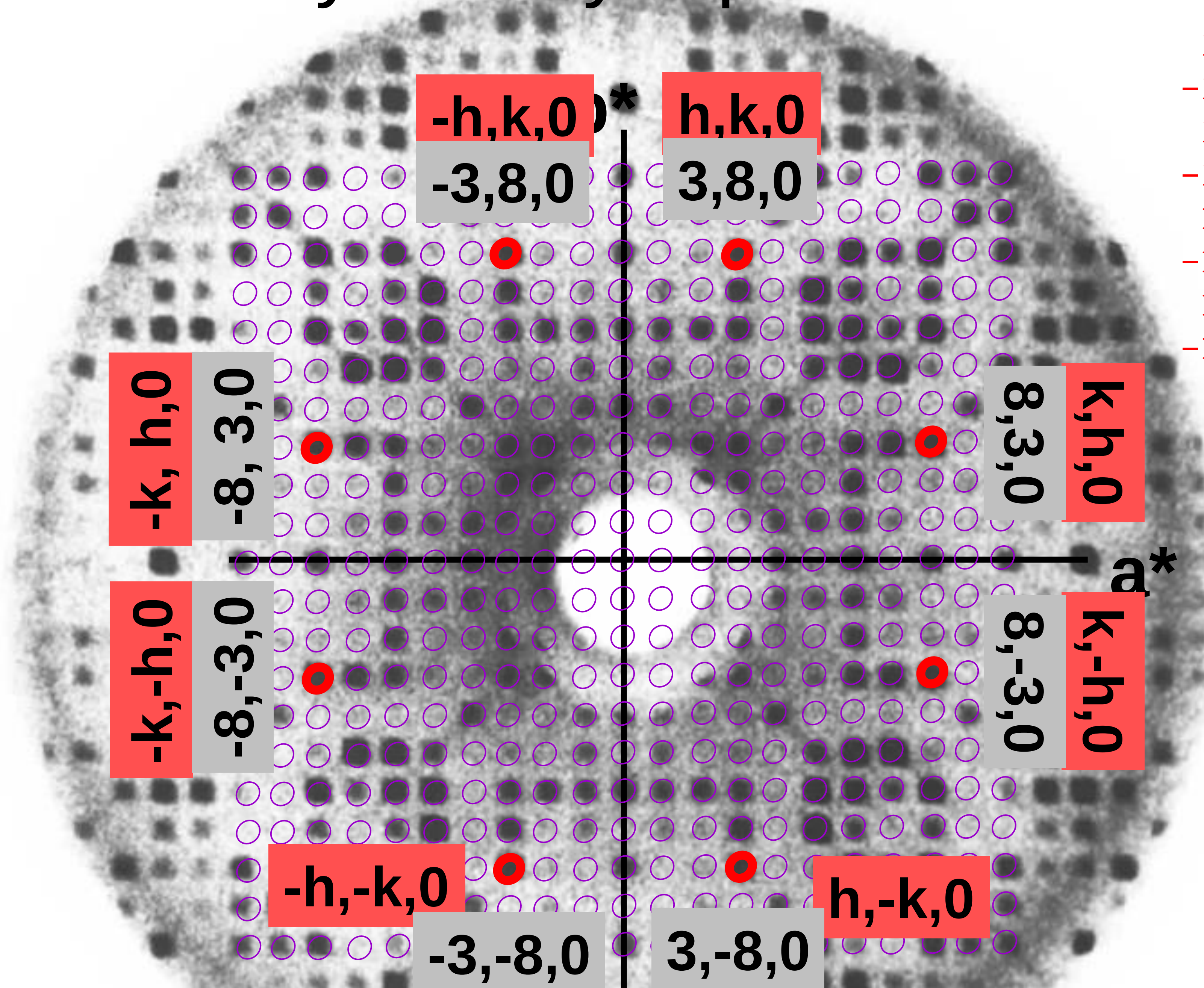
How many symmetry equivalents of this reflection do you see?
What are their indices?

8 symmetry equivalents

What are the symmetry operators that relate these reflections?

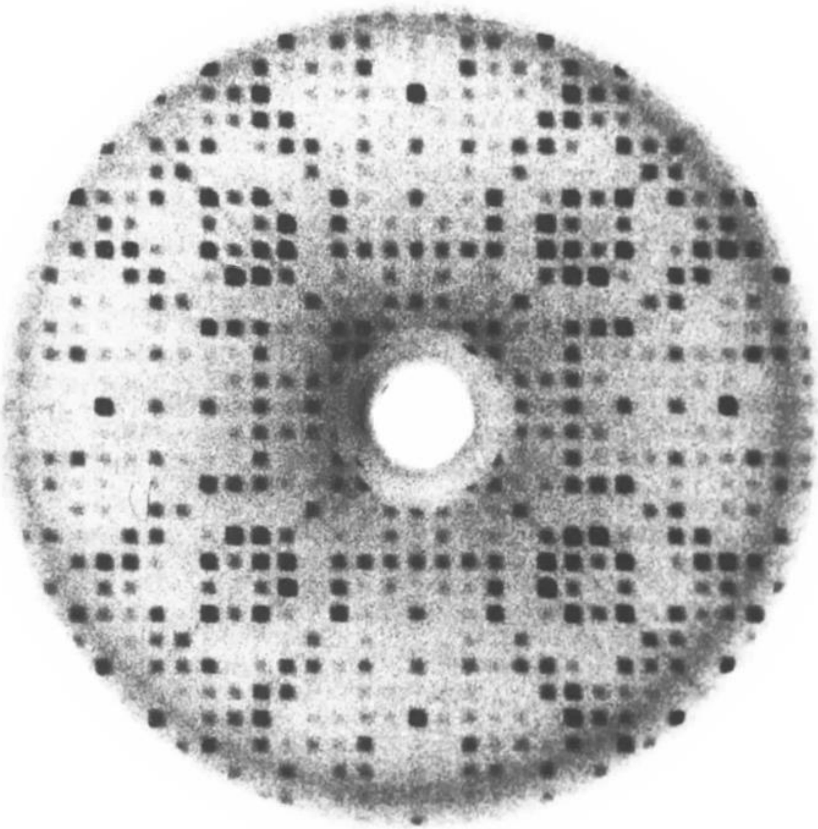


8 symmetry equivalents

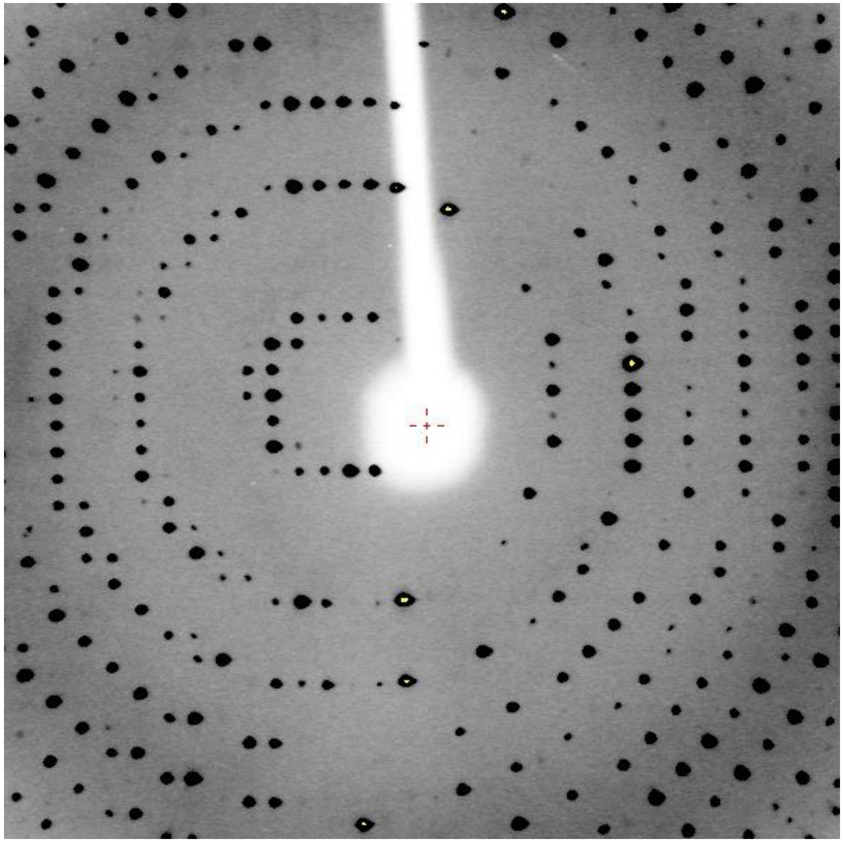



```
 H, K,0
-H, K,0
 H,-K,0
-H,-K,0
 K, H,0
-K, H,0
 K,-H,0
-K,-H,0
```

Next, index spots on an oscillation image.



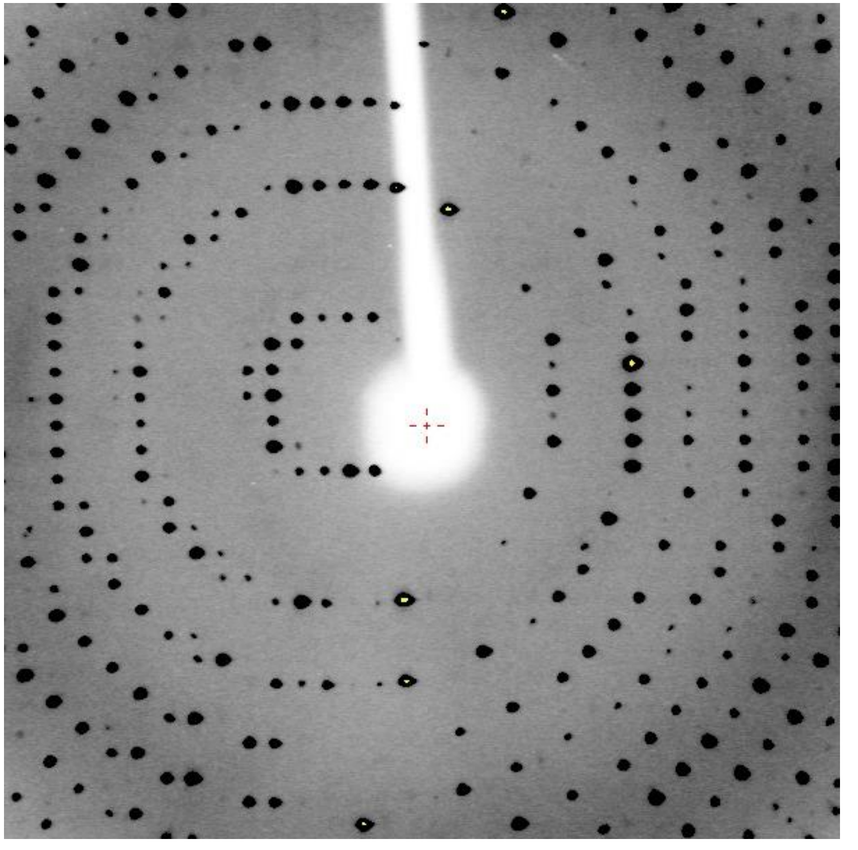
This is easy!



But, what about this?

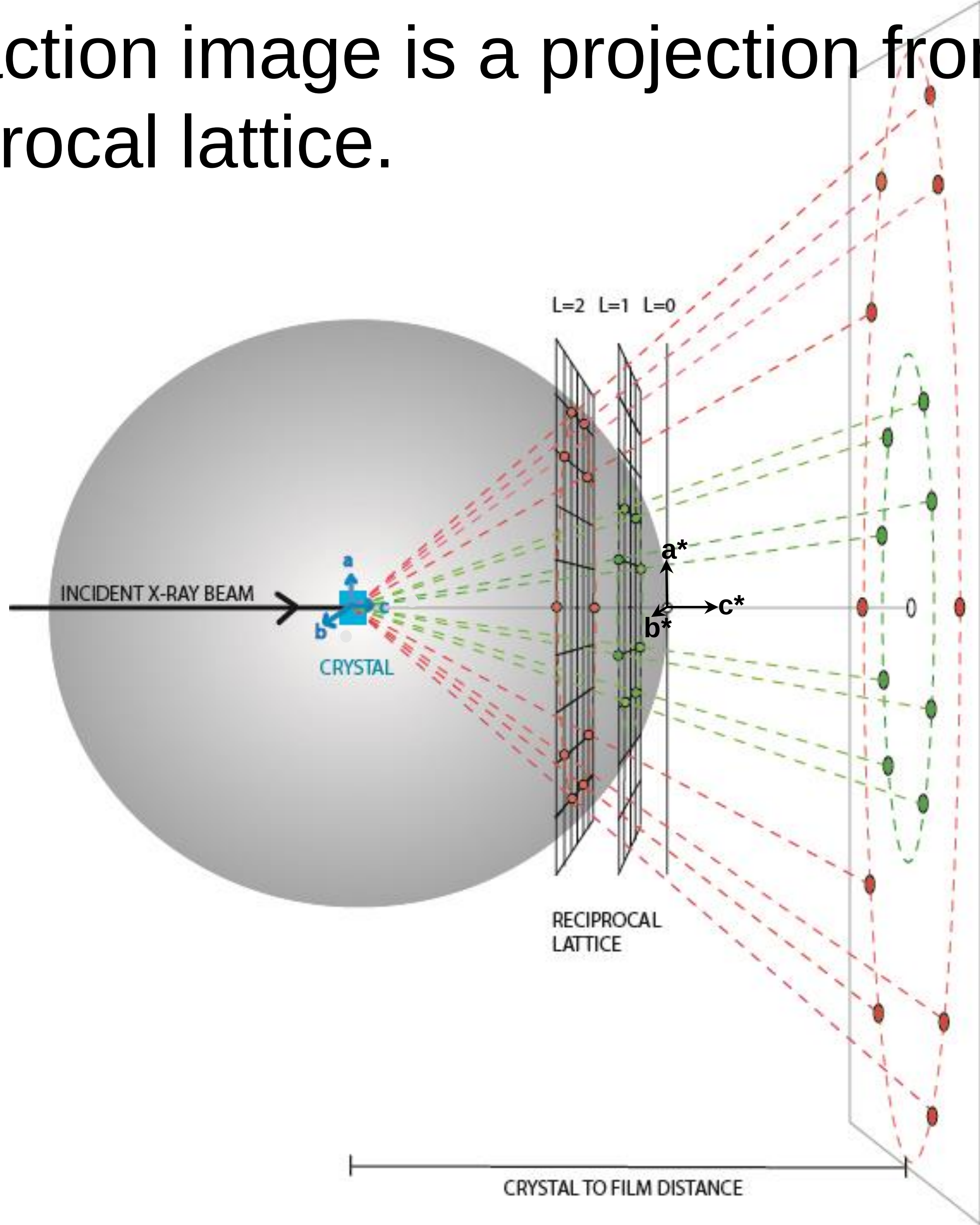
Challenges in indexing an oscillation image

1. Orientation of reciprocal cell vectors a*, b*, c* is random ---determined by the orientation of the crystal in the loop.
2. Can't see symmetry in the pattern..
3. Multiple planes of the reciprocal lattice are in view, not just a single plane.
4. The oscillation image is a 2D projections of a 3D lattice—a dimension of depth is lost.



Oscillation image.

2D diffraction image is a projection from a 3D reciprocal lattice.

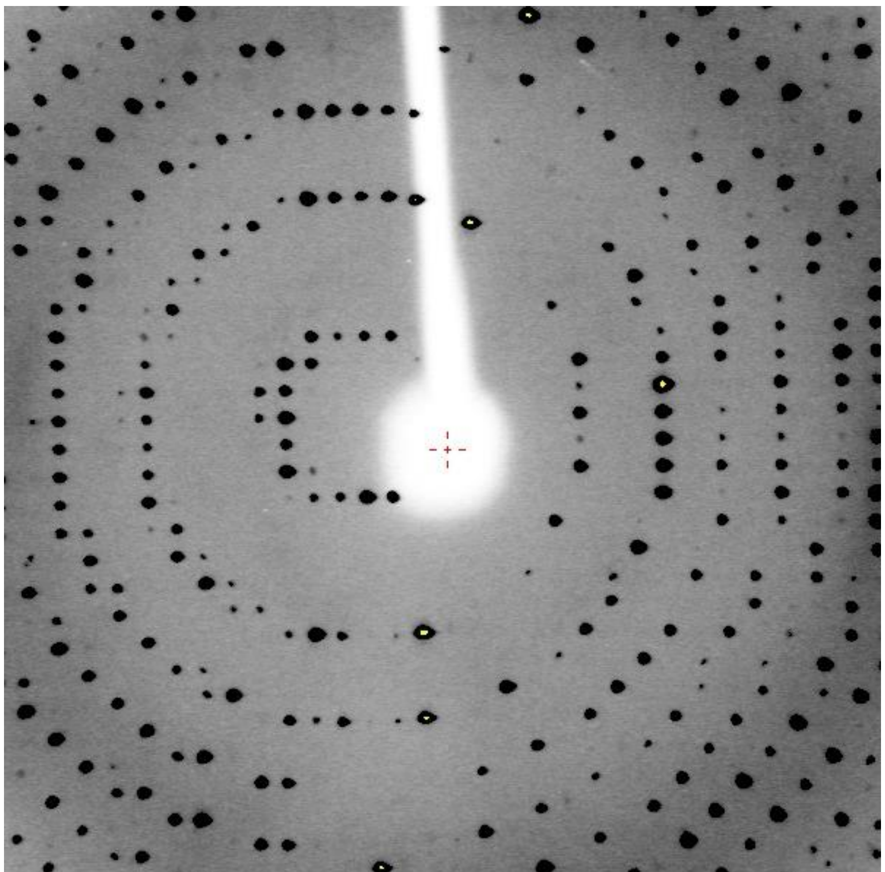


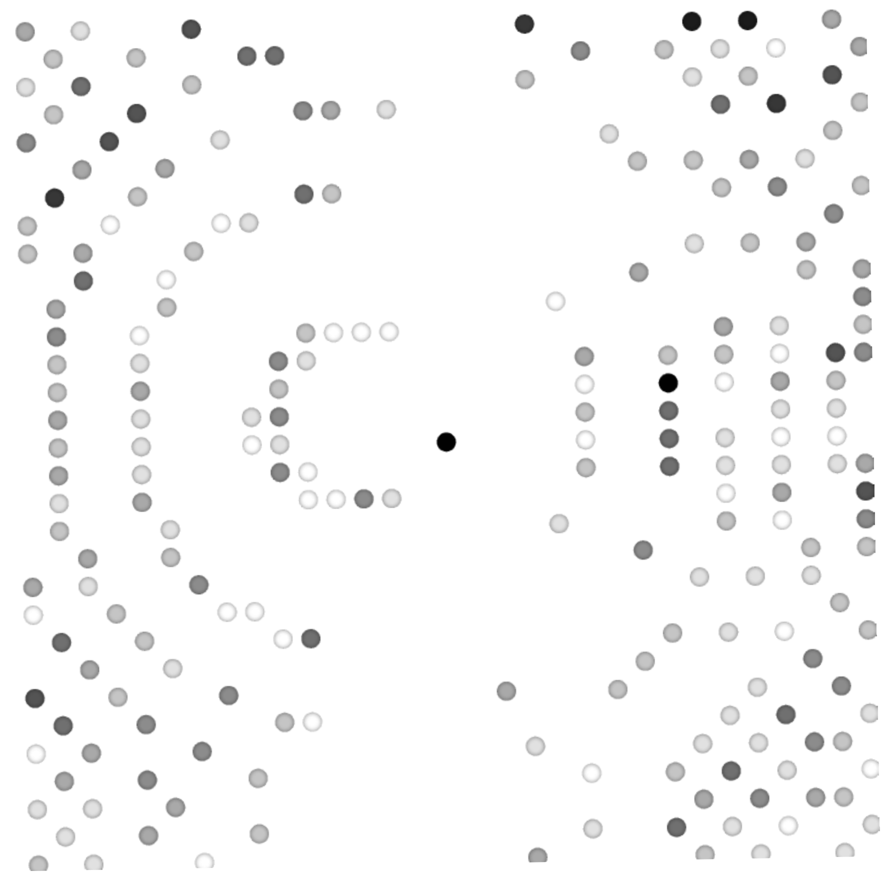
detector

In an undistorted view of the reciprocal lattice, recorded reflections would reside on the surface of a sphere, not a plane.

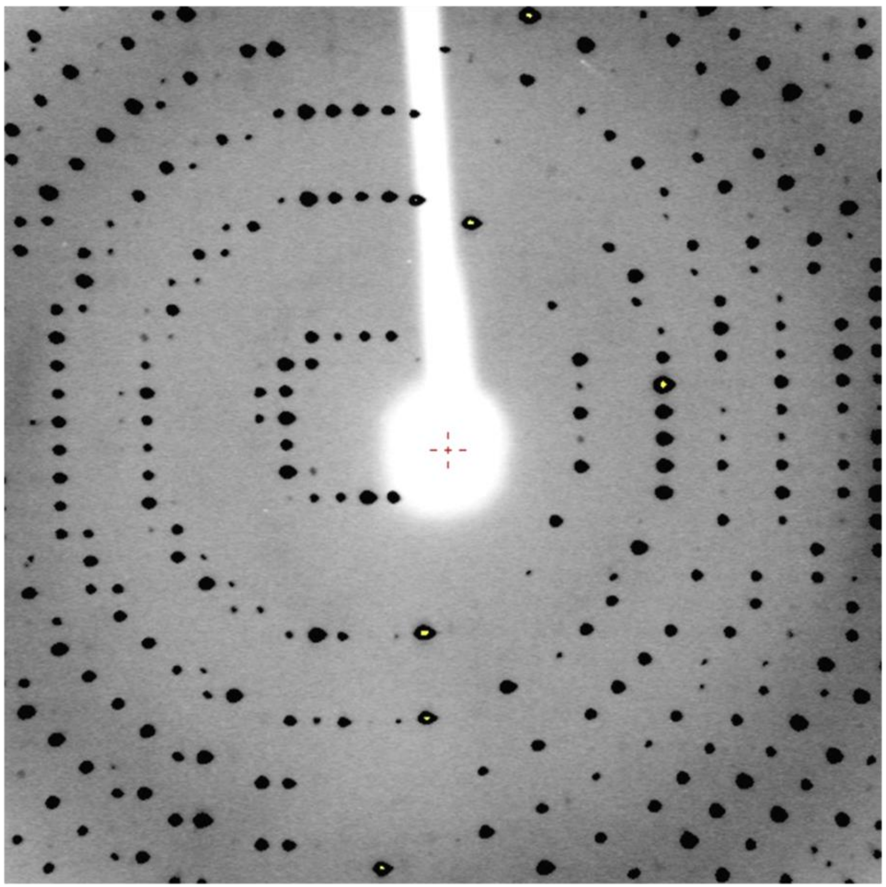
A distortion-corrected representation of the reflections

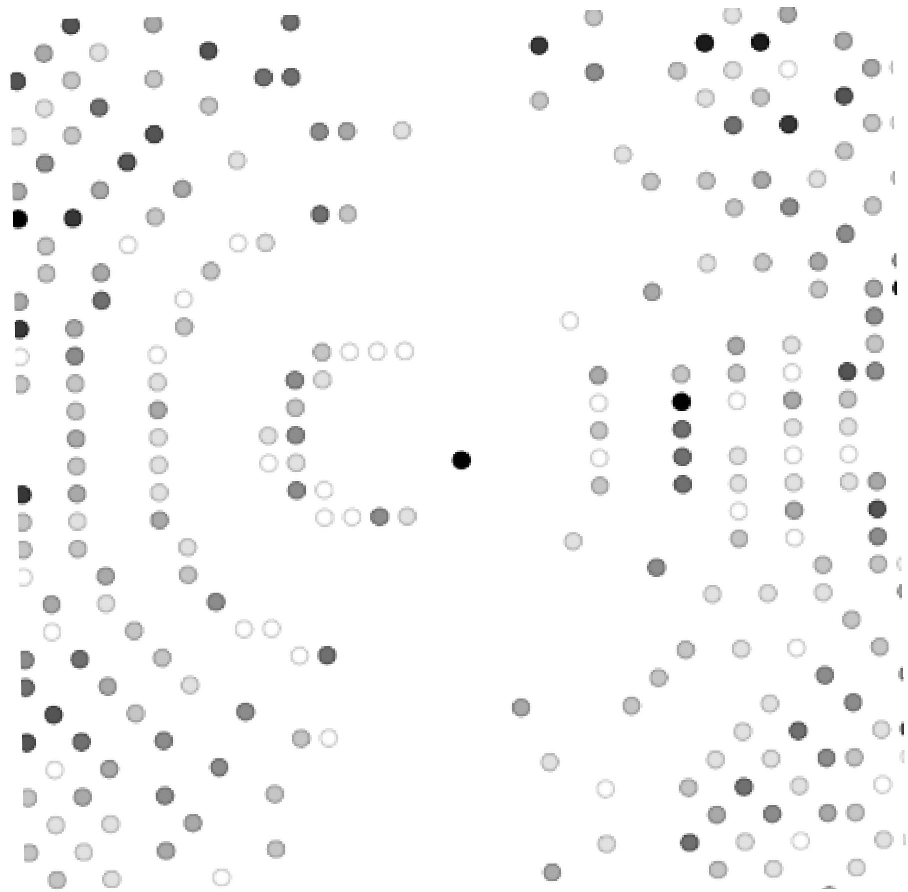
a single image reveals all 6 unit cell parameters



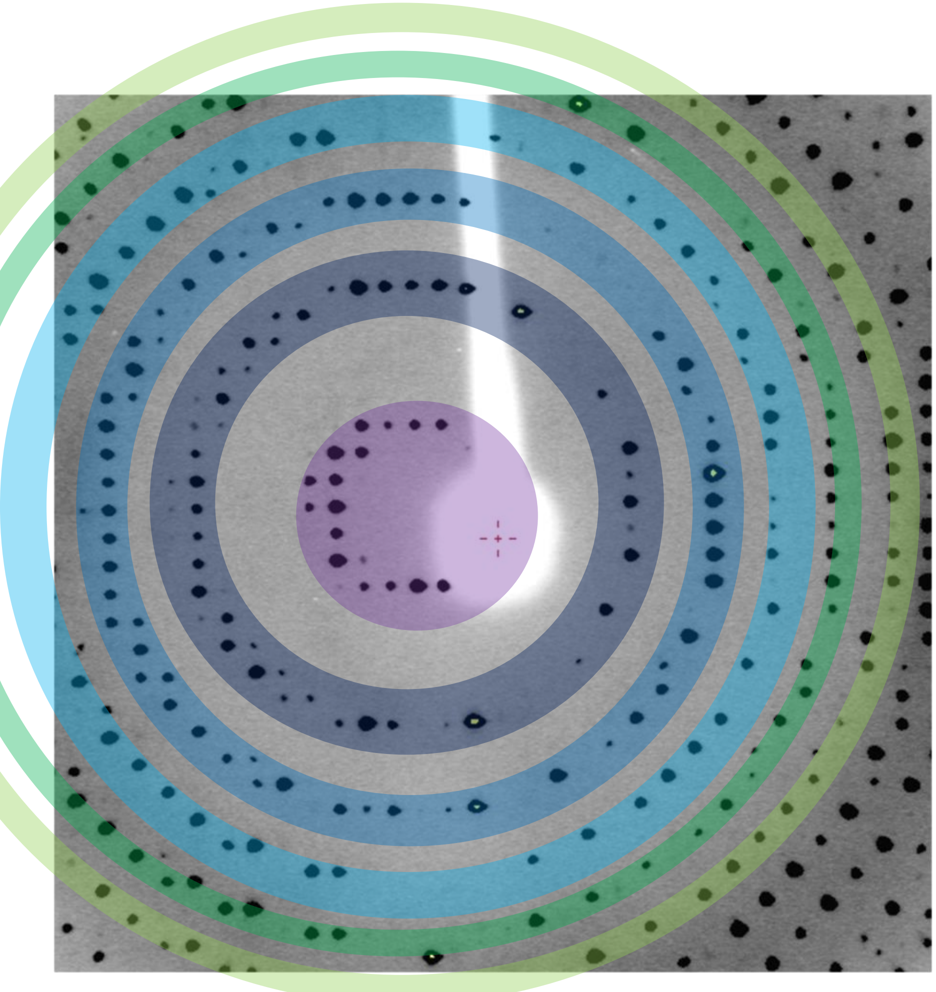


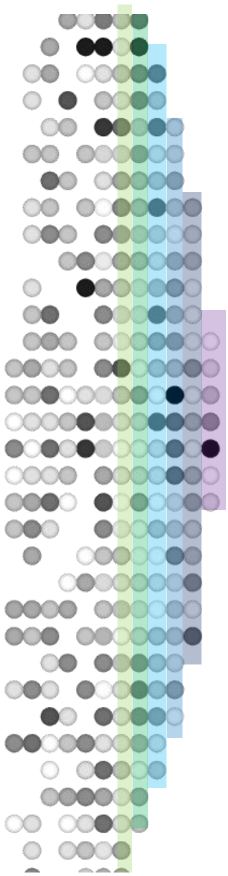
Restored depth of the diffraction pattern is evident from an orthogonal view





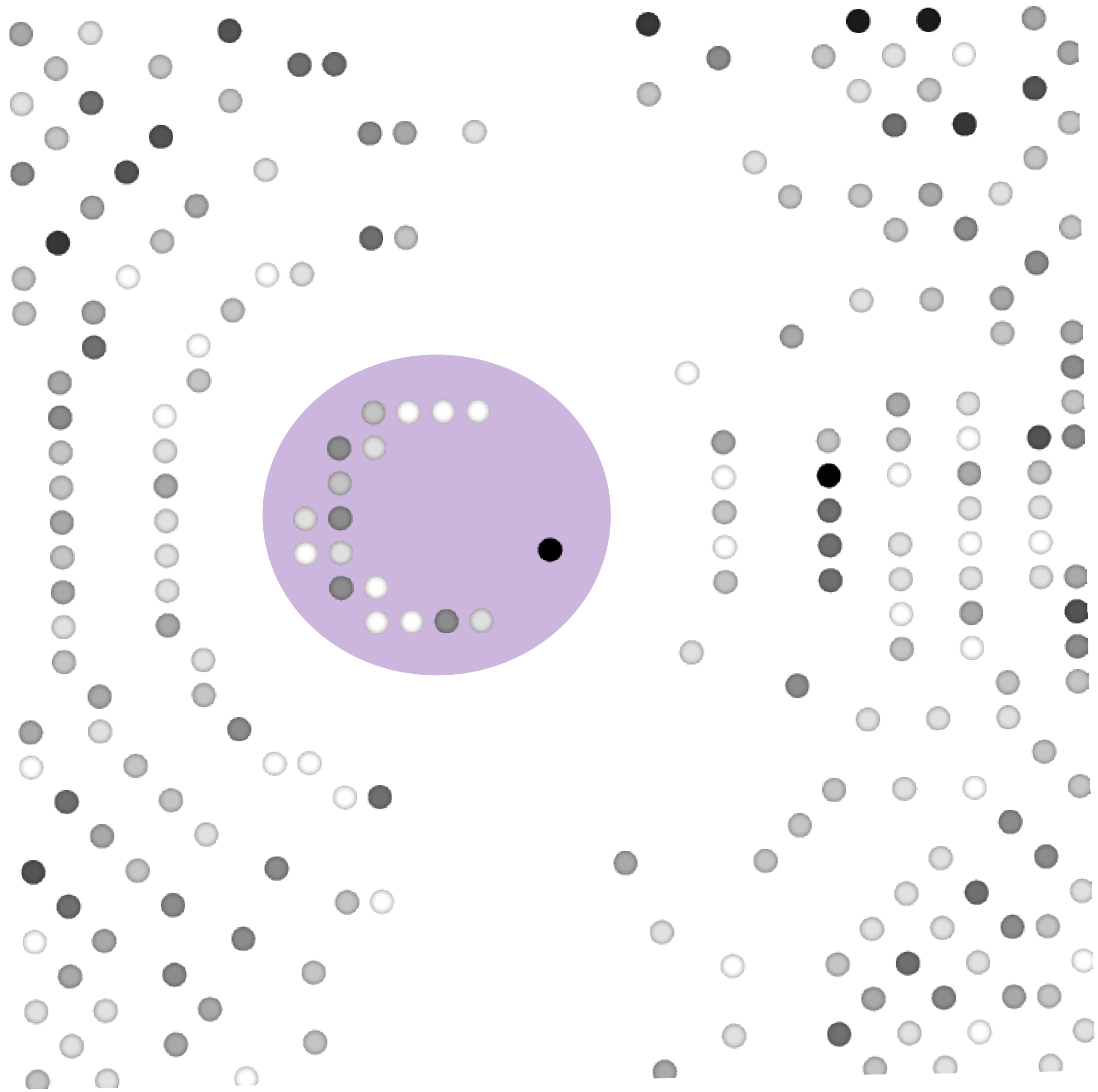
Each circle corresponds to a different reciprocal lattice plane



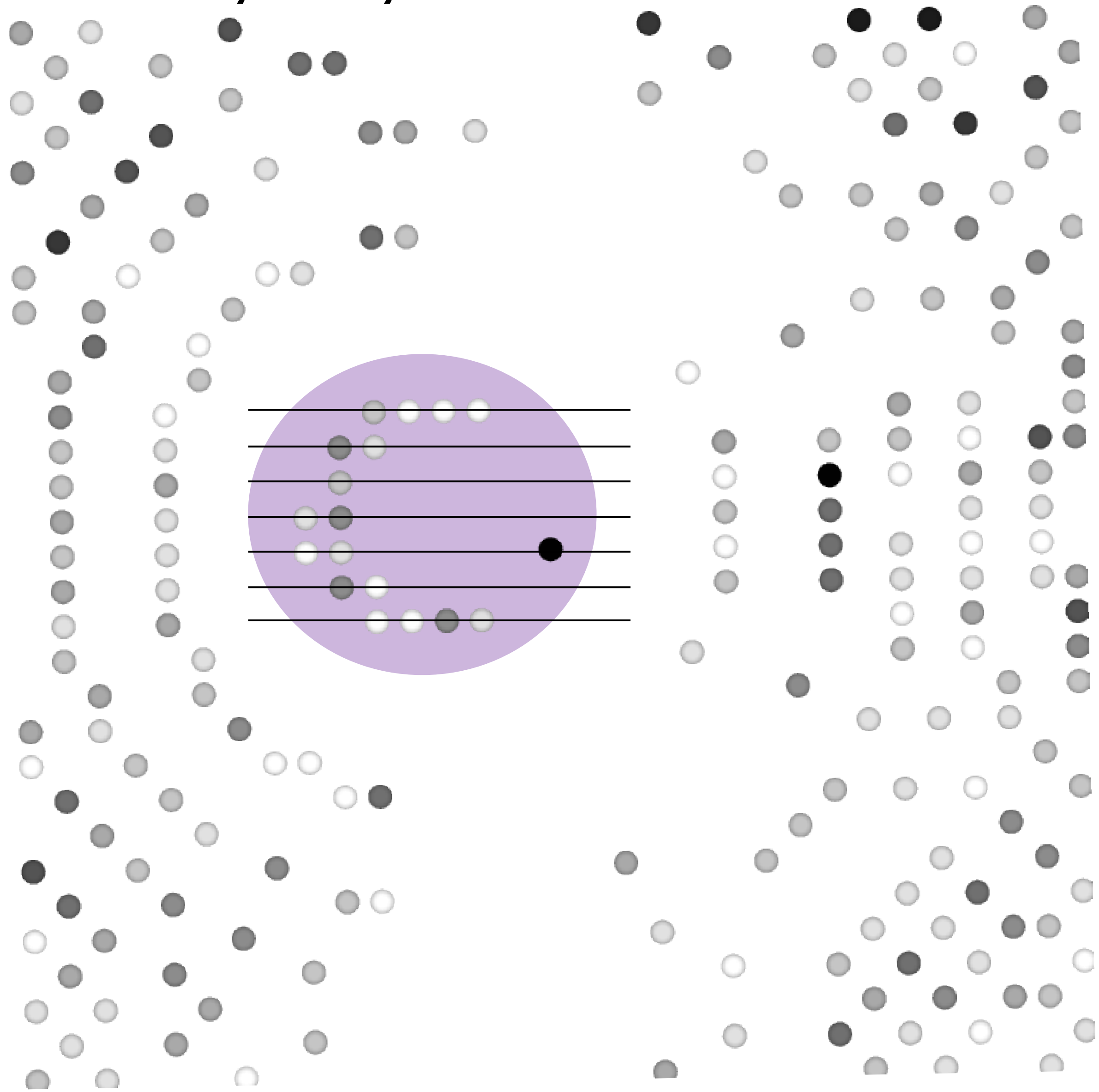


Start indexing process using reflections within the same plane and lying near the origin.

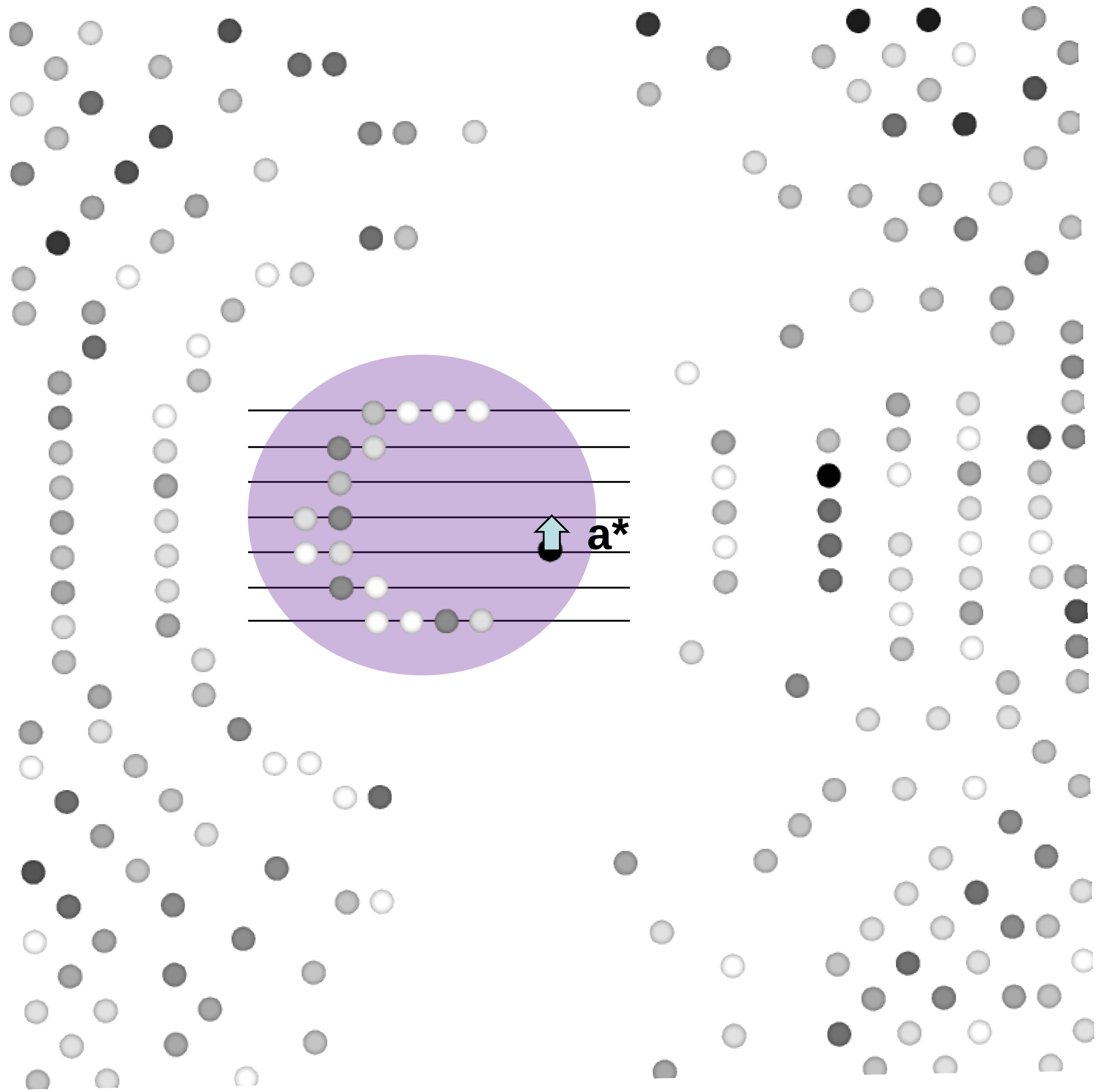
Draw a set of evenly spaced rows



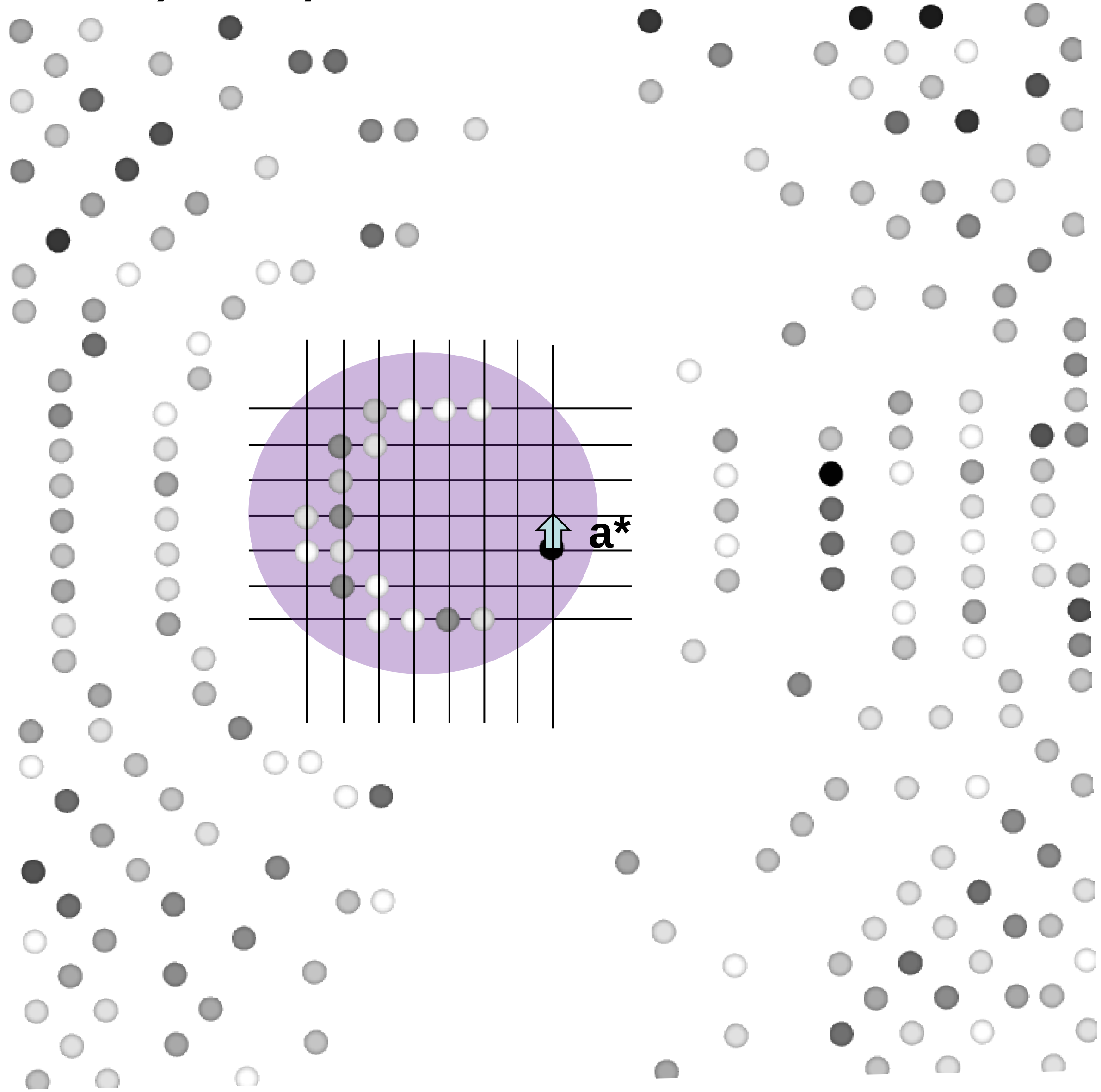
Draw the vector representing this repeat distance, a*, between rows



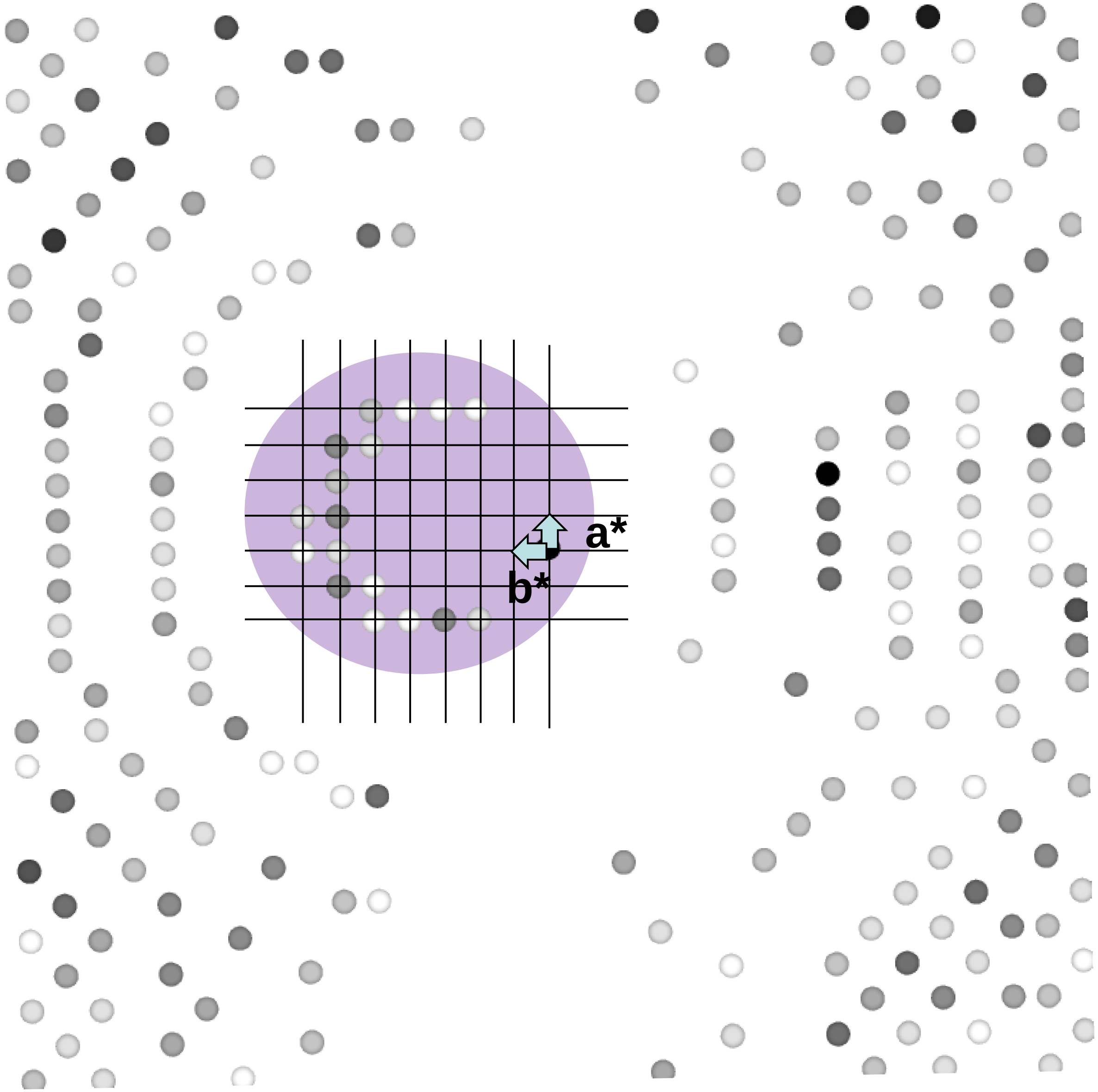
Draw a set of evenly spaced columns



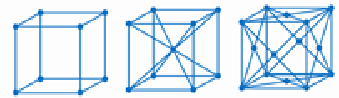
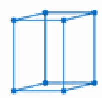
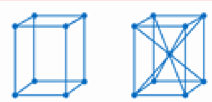
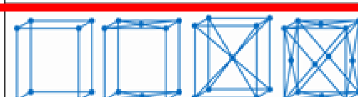
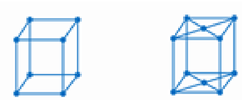
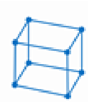
Draw the vector representing this repeat distance, b*, between columns



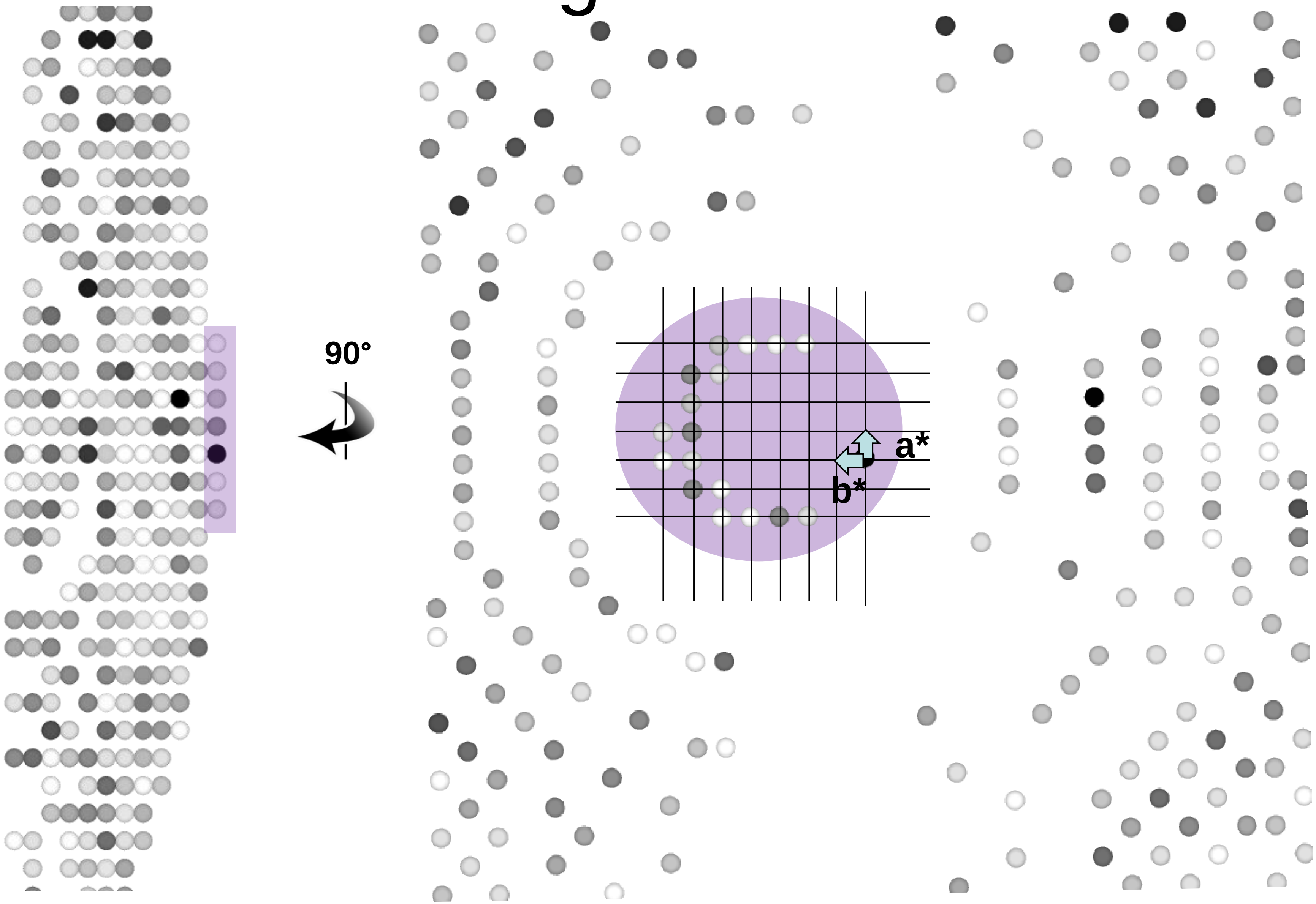
What is the angle between a* and b*?



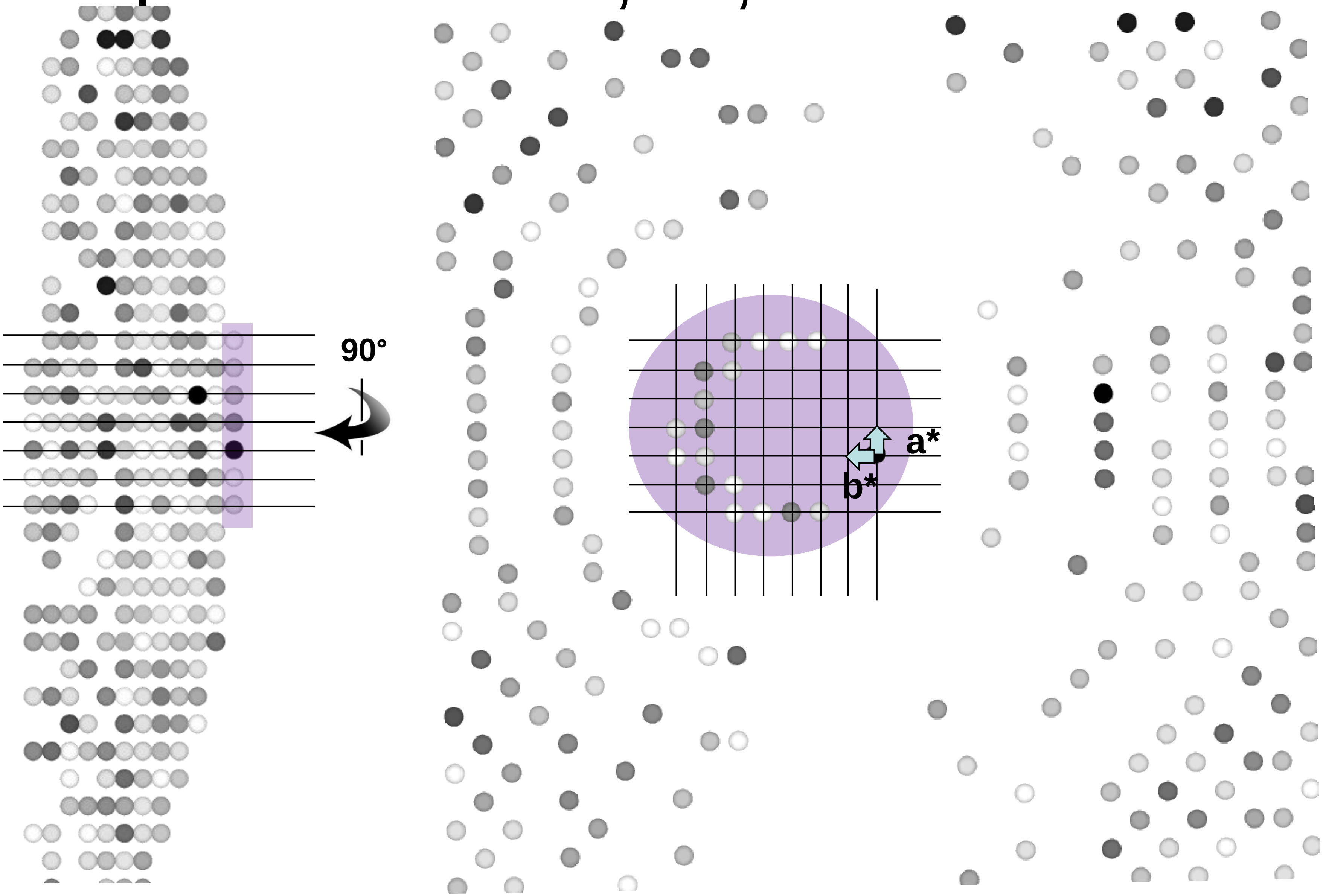
Which of 14 Bravais Lattices has $a=b$ and $\gamma=90°$

Cubic	 Cubic P, Cubic I, Cubic F	Three axes at right angles; all equal: $a=b=c$; $\alpha=\beta=\gamma=90°$
Rhombohedral	 Rhombohedral	Three axes equally inclined, not at right angles; all equal: $a=b=c$; $\alpha=\beta=\gamma\neq 90°$
Hexagonal/Trigonal	 Hexagonal	Three axes, two pairs at right angles, one at 120°. $a=b\neq c$; $\alpha=\beta=90°$, $\gamma=120°$
Tetragonal	 Tetragonal P, Tetragonal I	Three axes at right angles; two equal: $a=b\neq c$; $\alpha=\beta=\gamma=90°$
Orthorhombic	 P, C, I, F Orthorhombic	Three axes at right angles; all unequal: $a\neq b\neq c$; $\alpha=\beta=\gamma=90°$
Monoclinic	 Monoclinic P, Monoclinic C	Three axes, one pair not at right angles, all unequal: $a\neq b\neq c$; $\alpha=\gamma=90°\neq\beta$
Triclinic	 Triclinic P	Three axes not at right angles; all unequal: $a\neq b\neq c$; $\alpha\neq\beta\neq\gamma\neq 90°$

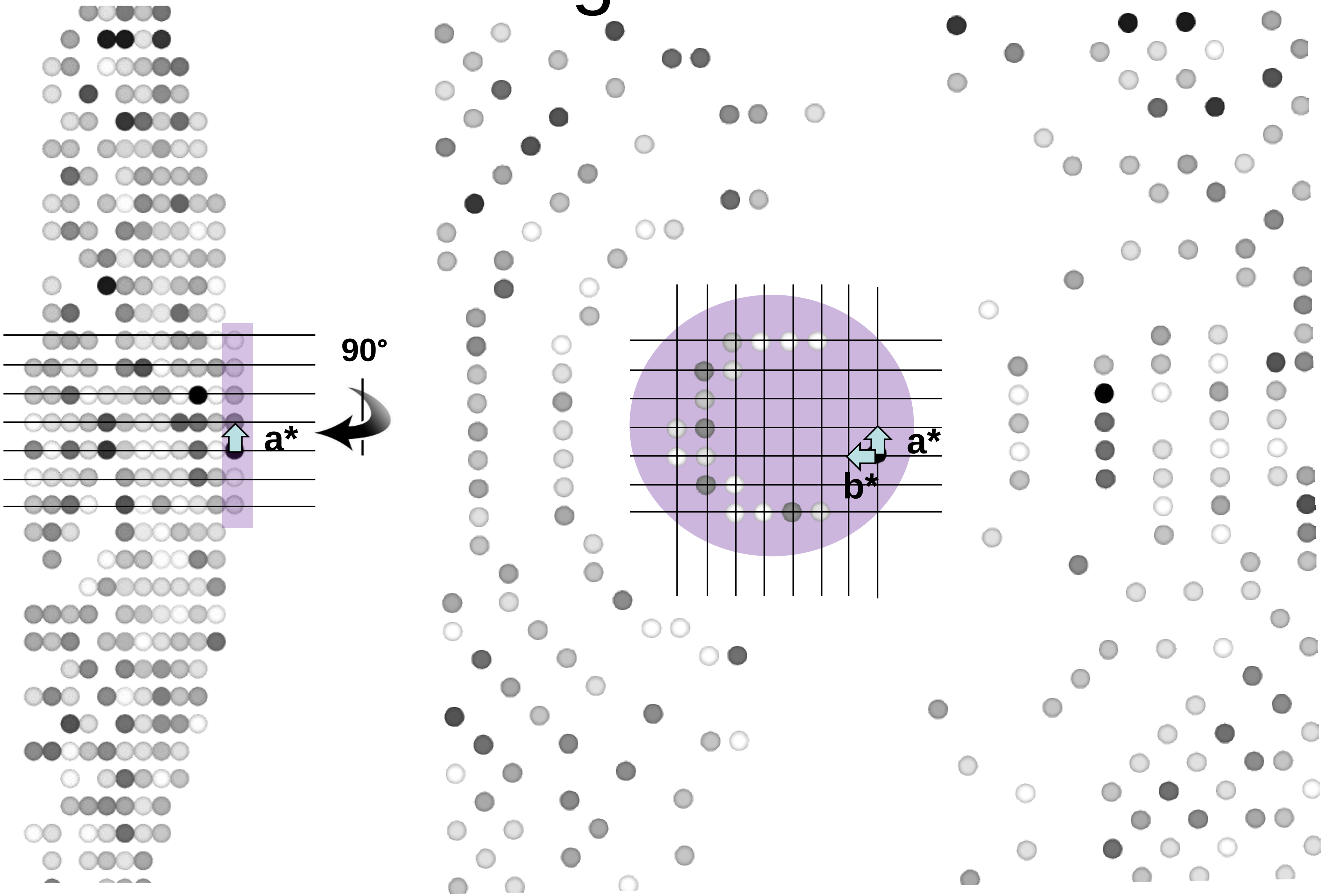
Draw a set of evenly spaced rows in orthogonal view



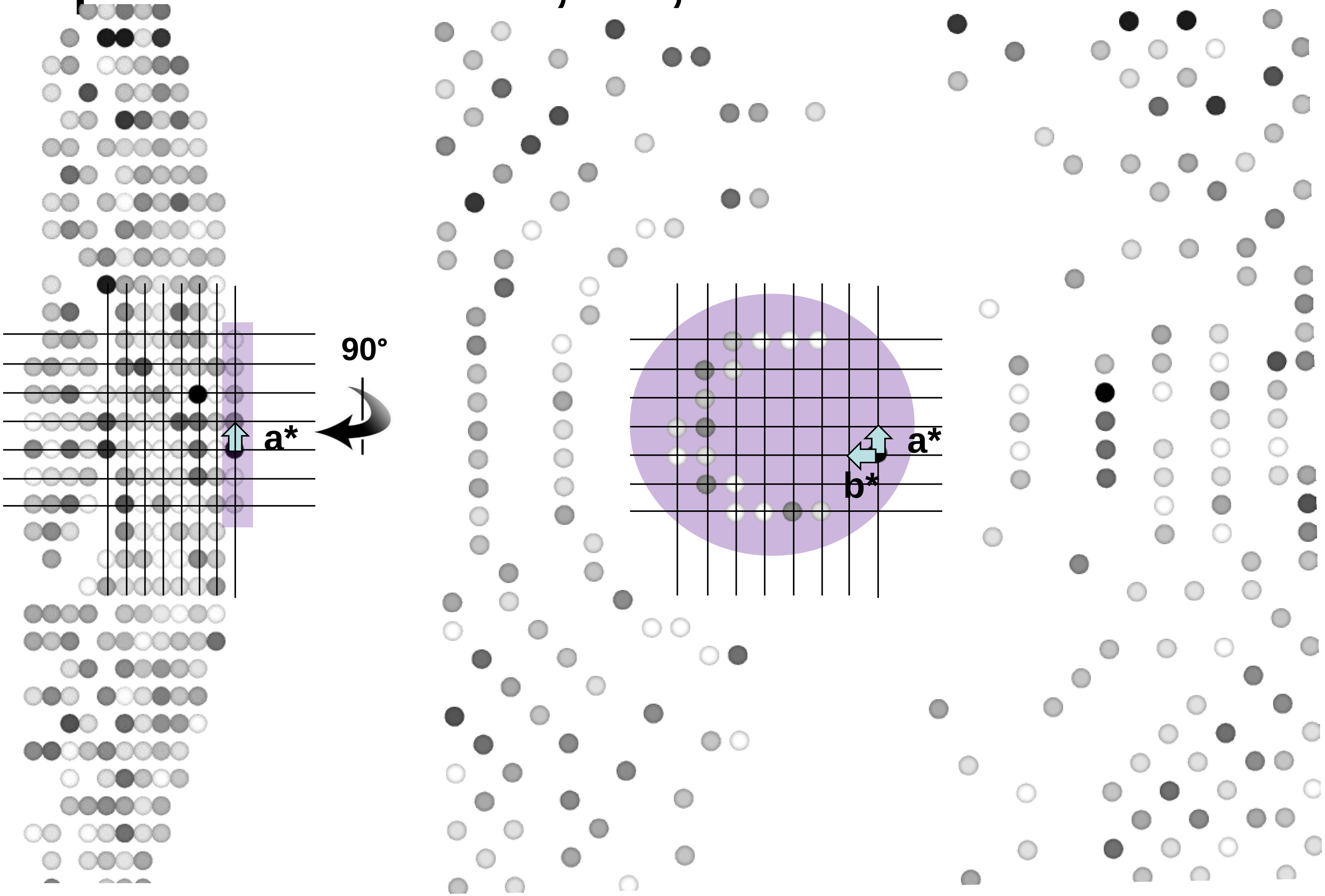
Draw the vector representing this repeat distance, a*, between rows



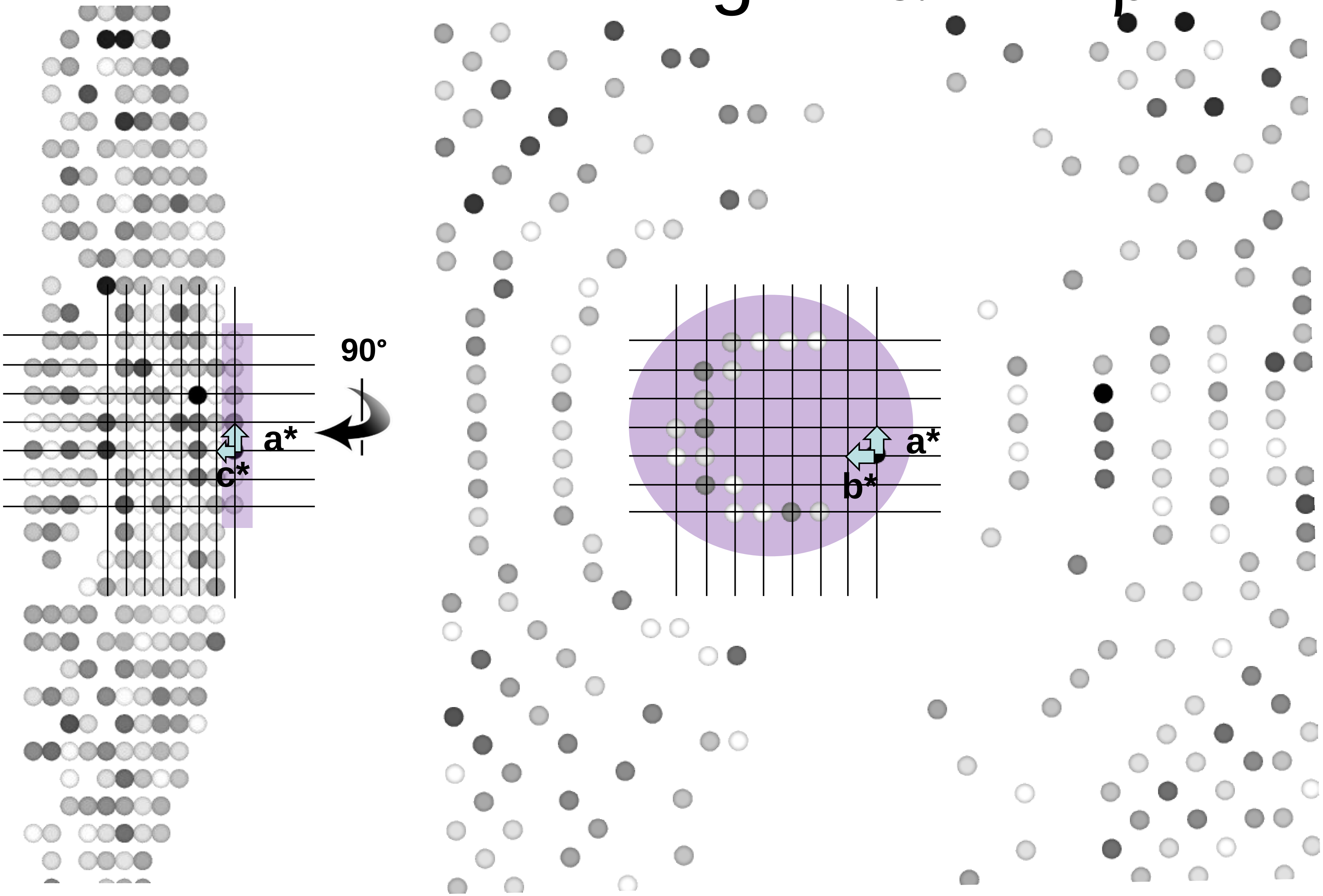
Draw a set of evenly spaced columns in orthogonal view



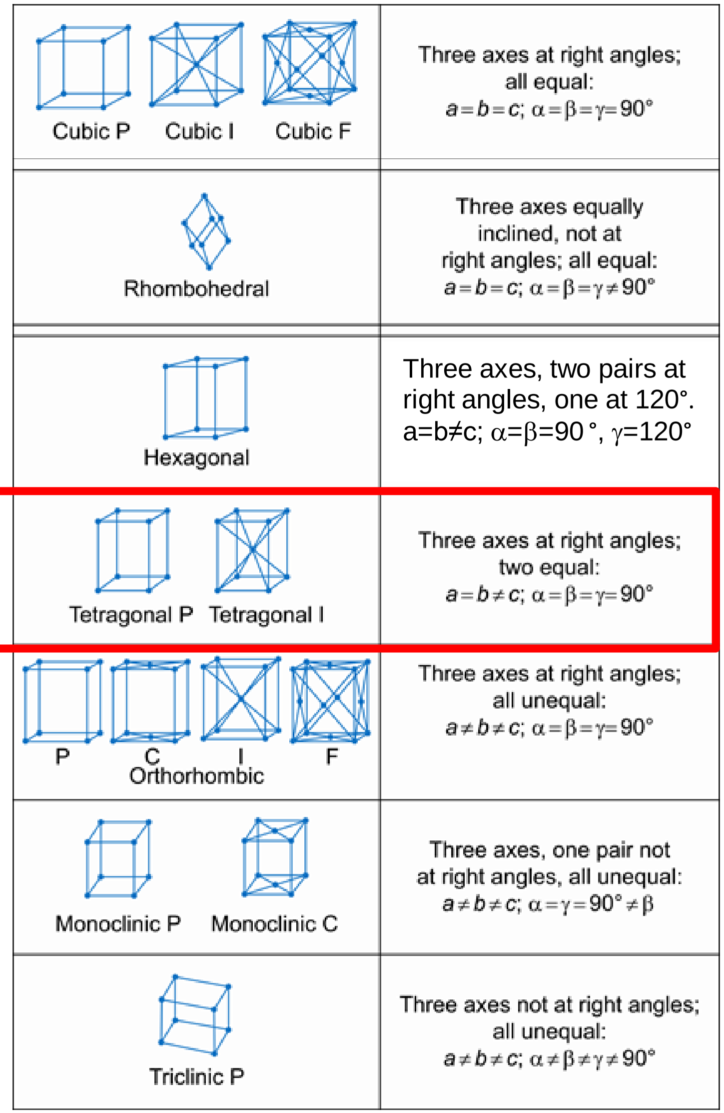
Draw the vector representing this repeat distance, c*, between columns



Is the length of c* related to a* and b*? What are the angles α and β?

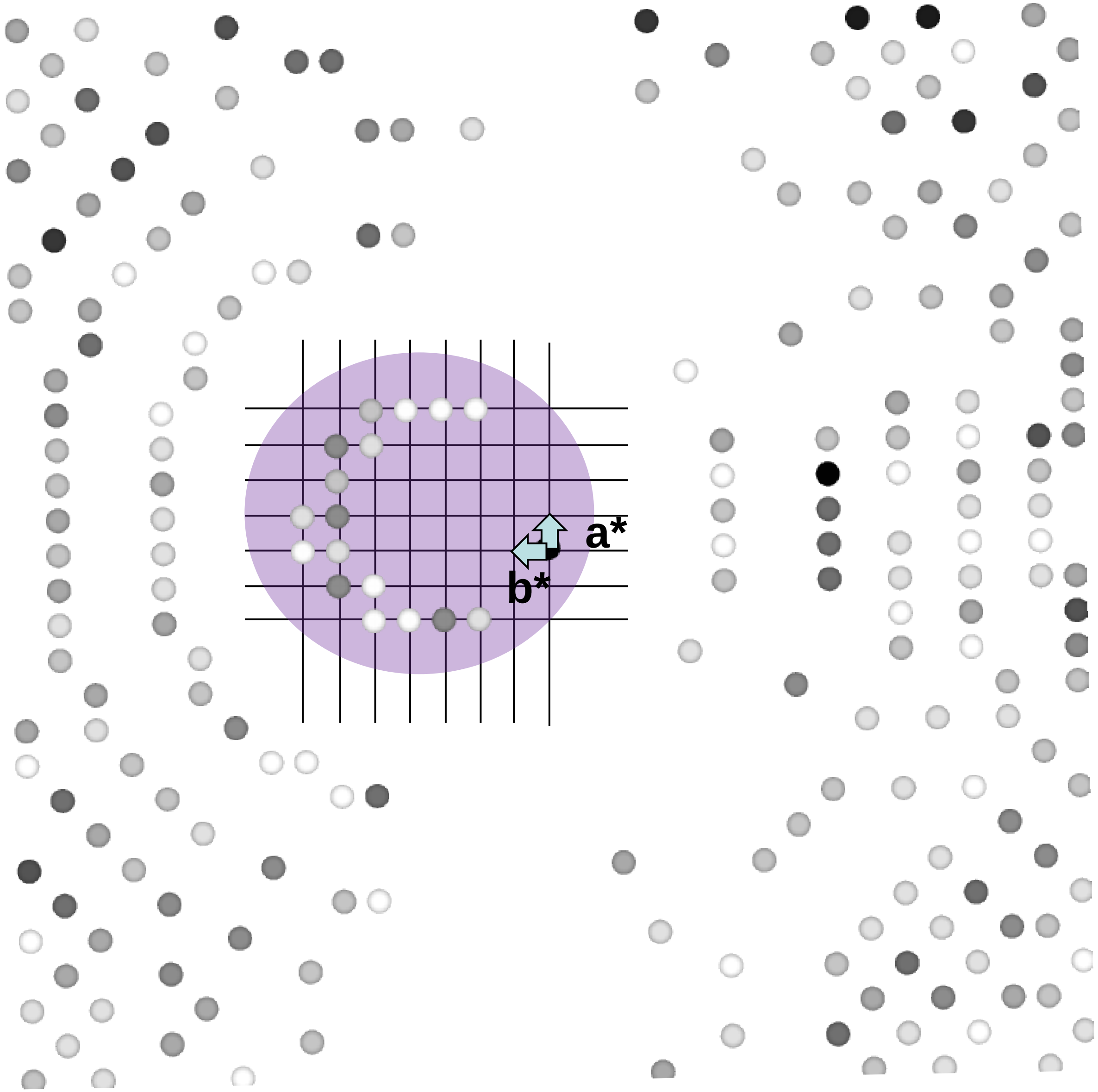


Which of 14 Bravais Lattices has $a=b\neq c$ and $\alpha=\beta=\gamma=90°$

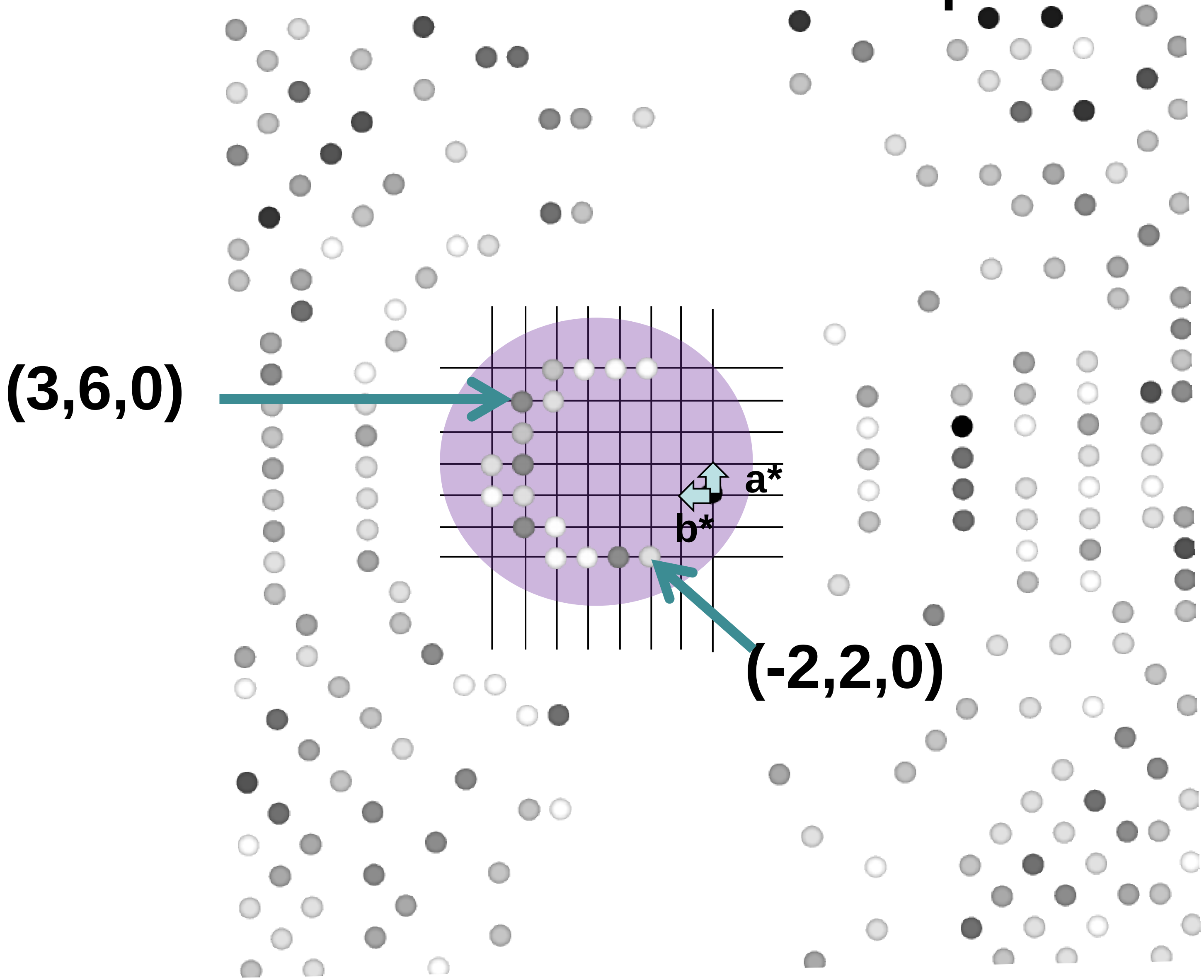


System	Lattices	Description
Cubic	Cubic P, Cubic I, Cubic F	Three axes at right angles; all equal: $a=b=c$; $\alpha=\beta=\gamma=90°$
Rhombohedral	Rhombohedral	Three axes equally inclined, not at right angles; all equal: $a=b=c$; $\alpha=\beta=\gamma\neq 90°$
Hexagonal/Trigonal	Hexagonal	Three axes, two pairs at right angles, one at 120°. $a=b\neq c$; $\alpha=\beta=90°$, $\gamma=120°$
Tetragonal	Tetragonal P, Tetragonal I	Three axes at right angles; two equal: $a=b\neq c$; $\alpha=\beta=\gamma=90°$
Orthorhombic	Orthorhombic P, C, I, F	Three axes at right angles; all unequal: $a\neq b\neq c$; $\alpha=\beta=\gamma=90°$
Monoclinic	Monoclinic P, Monoclinic C	Three axes, one pair not at right angles, all unequal: $a\neq b\neq c$; $\alpha=\gamma=90°\neq\beta$
Triclinic	Triclinic P	Three axes not at right angles; all unequal: $a\neq b\neq c$; $\alpha\neq\beta\neq\gamma\neq 90°$

What is the index of the lowest resolution reflection?



What is the highest resolution reflection in the l=0 plane?



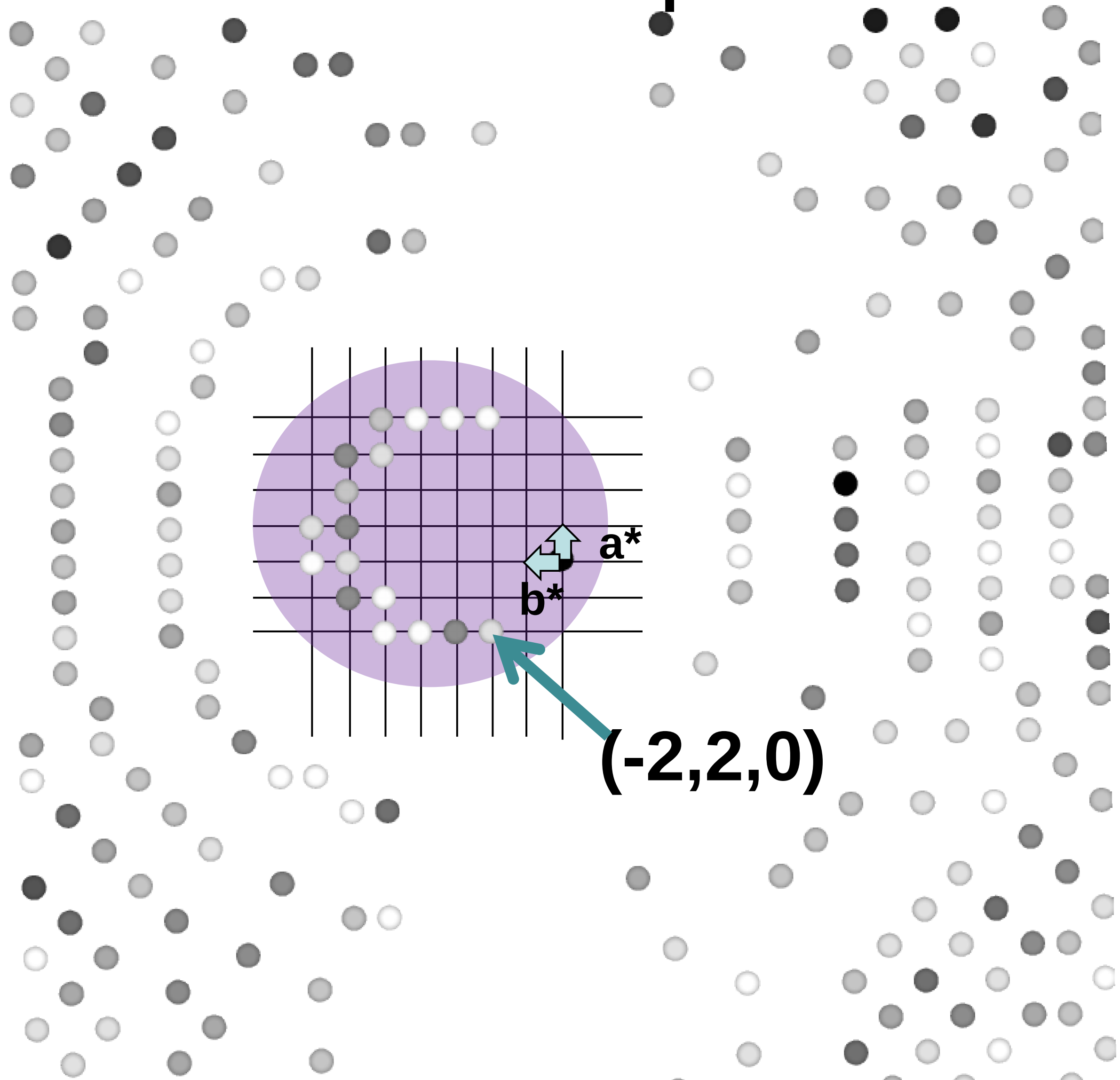
Which experimental parameters were required to index a spot?

Coordinates (X,Y) for the spot position

Coordinates of the direct beam, (X,Y)

Reciprocal cell parameters
a*,b*,c*, α*,β*,γ*

Orientation of the unit cell axes w.r.t. the laboratory axes (φψκ).



To determine real cell parameters?

The wavelength of the incident radiation

Crystal-to-detector distance

Which experimental parameters must be interpreted from diffraction image?

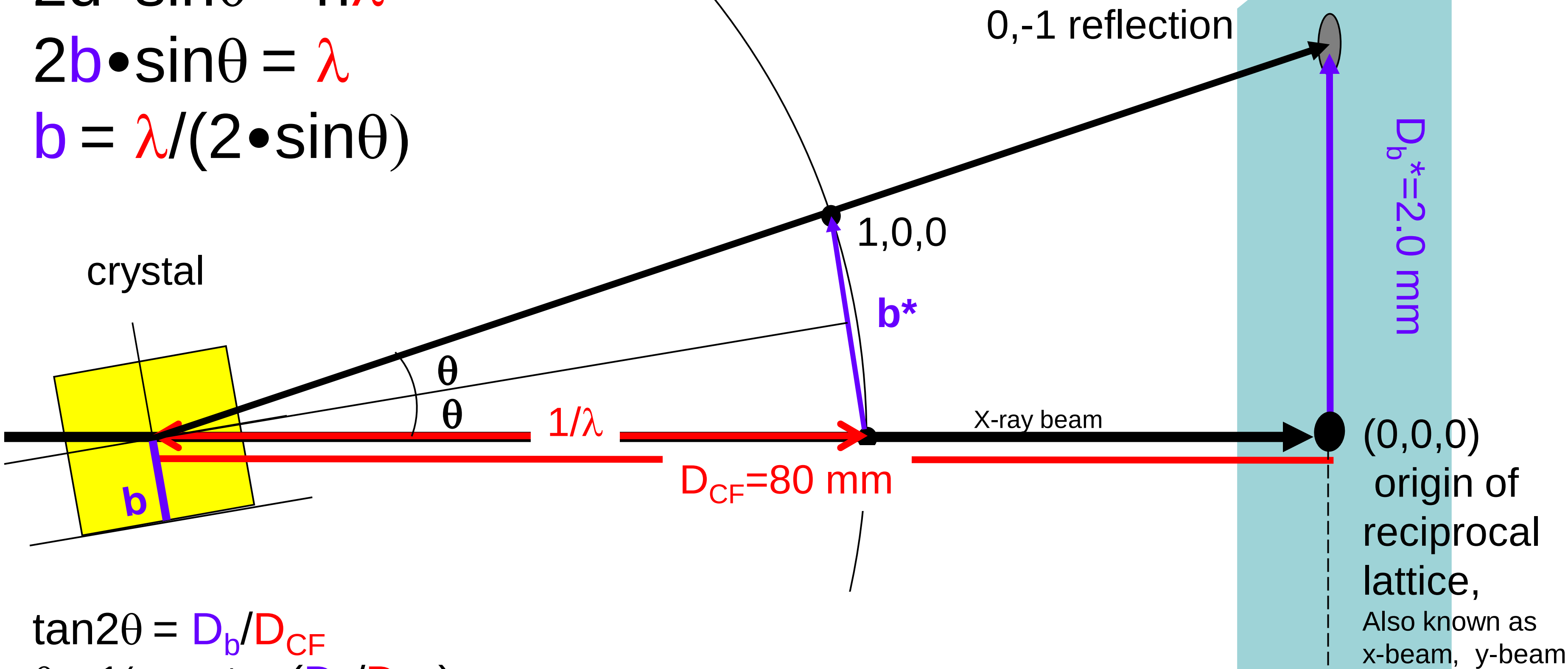
Determine unit cell length "b"

Standard detector geometry

$2d \bullet \sin\theta = n\lambda$

$2b \bullet \sin\theta = \lambda$

$b = \lambda/(2 \bullet \sin\theta)$



$\tan 2\theta = D_b/D_{CF}$

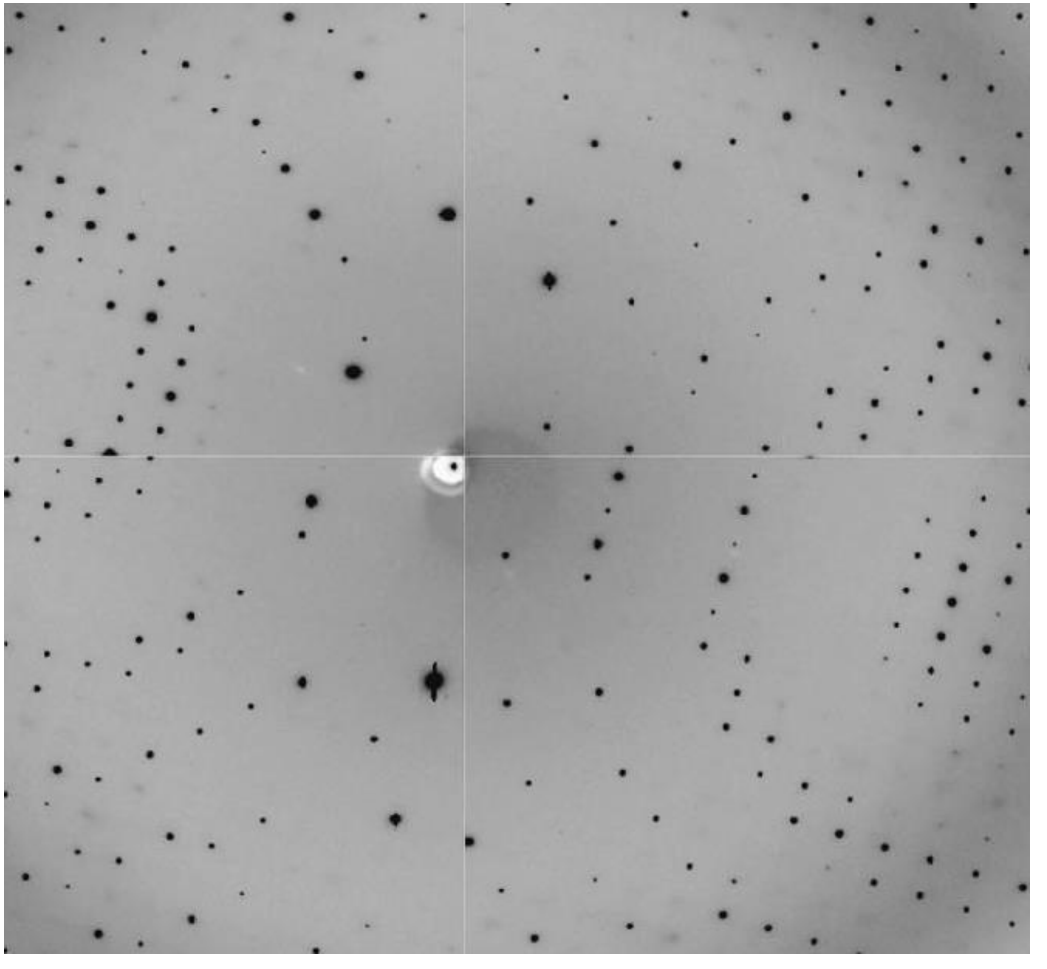
$\theta = ½ \bullet \arctan(D_b/D_{CF})$

$b = \lambda/(2 \bullet \sin(½ \bullet \arctan(D_b/D_{CF}))$

$b = 1.54Å /(2 \bullet \sin(½ \bullet \arctan(2.0mm/80mm))$

$b = 61.6$ Å

Need a program that can automatically identify spot positions, interpret crystal orientation, and unit cell dimensions.



Automatic indexing algorithm explained

Acta Cryst. (1999), D55, 1690-1695

Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

The Rossmann Fourier autoindexing algorithm in *MOSFLM*

Harold R. Powell

MRC-LMB, MRC Centre, Hills Road, Cambridge CB2 2QU, England

Correspondence e-mail: harry@mrc-lmb.cam.ac.uk

Received 8 February 1999
Accepted 9 July 1999

The fast Fourier transform (FFT) autoindexing routines written by the Rossmann group at Purdue University have been incorporated in *MOSFLM*, providing a rapid and reliable method of indexing oscillation images. This is a procedure which extracts direct-space information about the unit cell from the FFT. The method and its implementation in *MOSFLM* are discussed.

1. Introduction

Most newcomers to crystallography and diffraction-image processing probably view indexing as a 'black-box process' (Fig. 1): a diffraction image is presented to a program (often only available without the source code), which performs the process automatically in a matter of seconds; some programs are more powerful and reliable than others, and it has become

Locate reflections positions (peaks of high intensity)

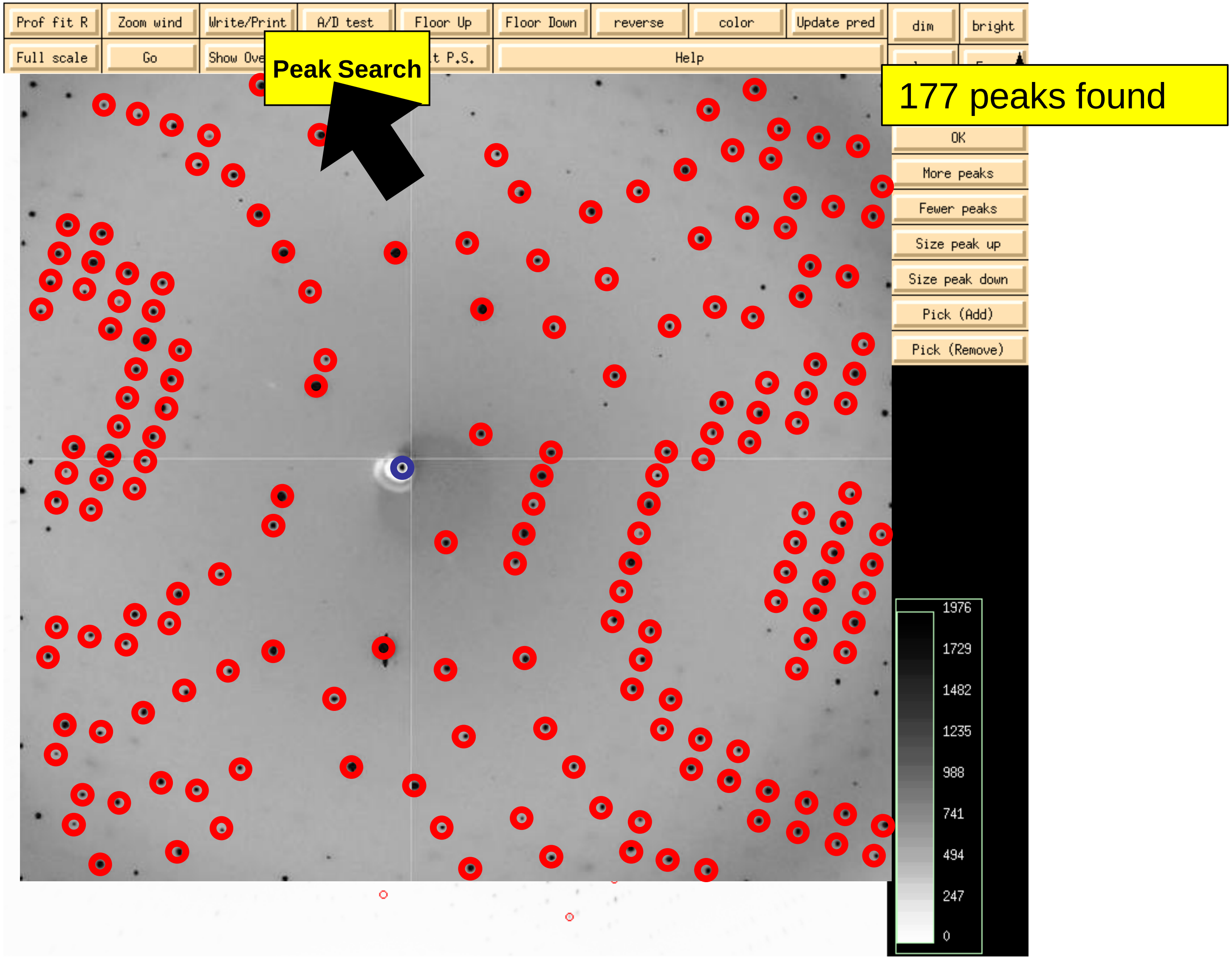
1) Display first image in your data set with

2) Press “Peak Search”. Red circles indicate position of prominent peaks (spots).

3) Evaluate whether you need more or fewer peaks.

4) Press “OK”

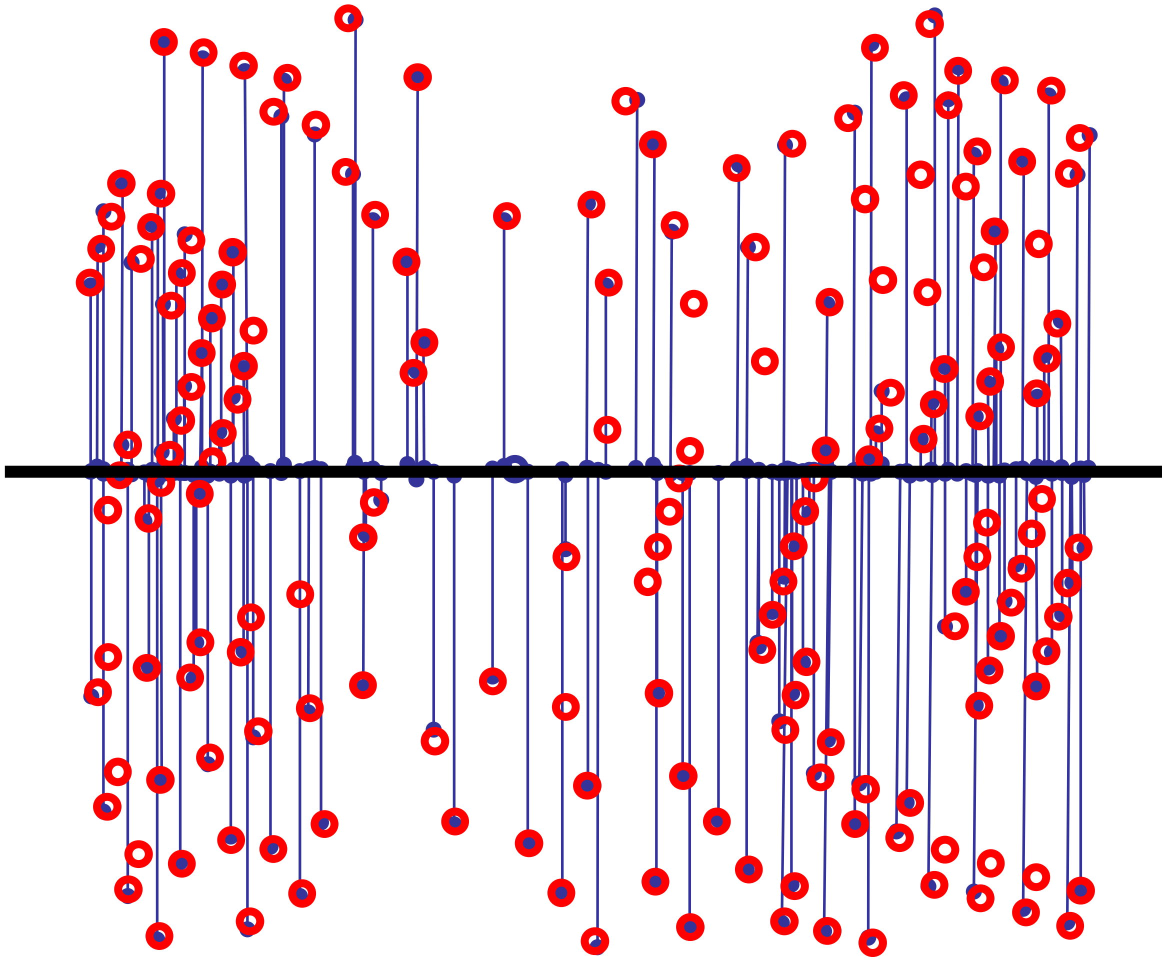
5) Spot positions (x,y) are written to a file “peaks.file.”



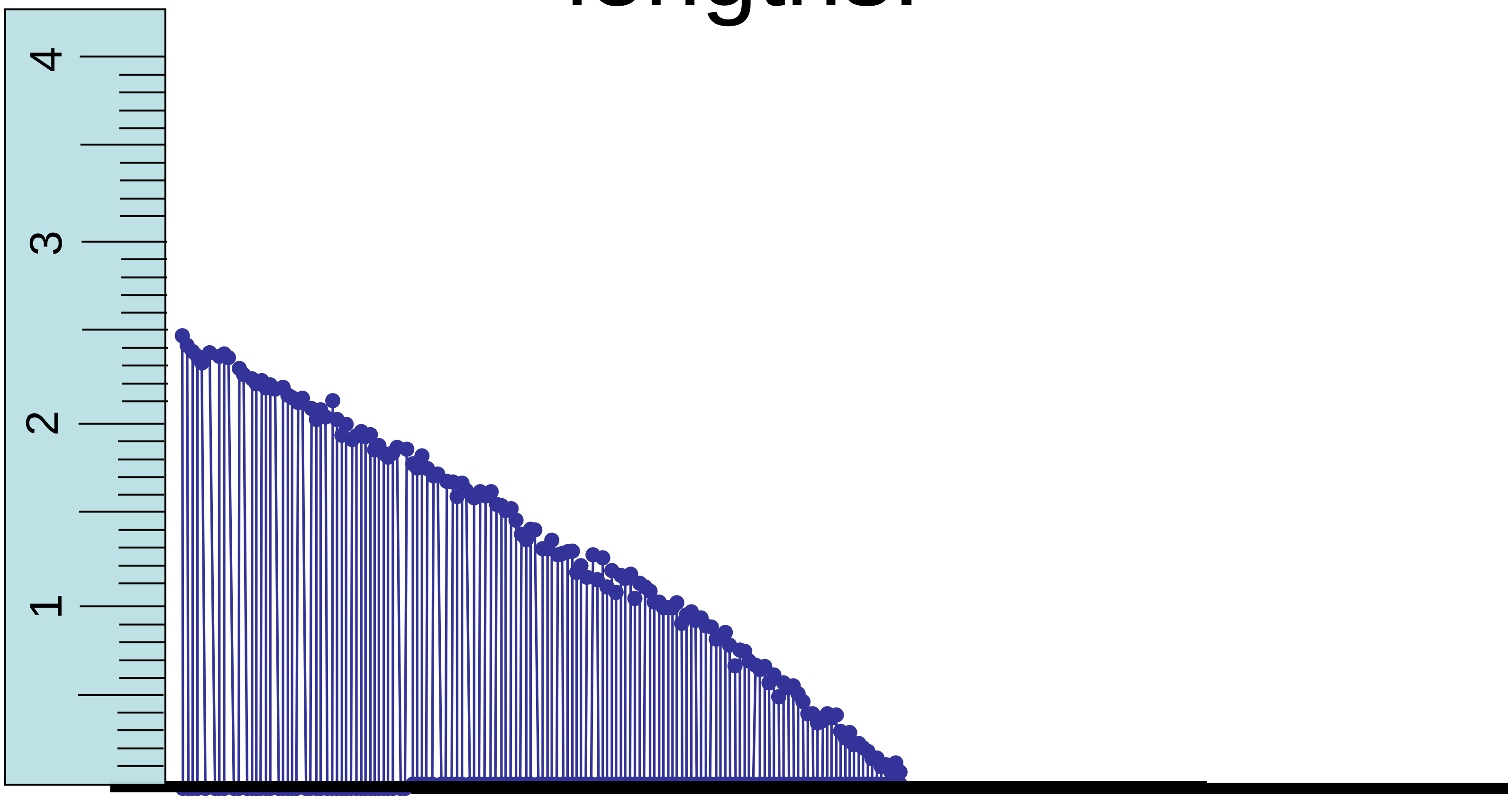
Peaks.file

```
•   7777     0.0     0.0              1              1
        height     X        Y              frame
•          13 2695.7 1350.5              1              1
•          27 2669.5 1062.4              1              1
•          16 2570.6 1143.5              1              1
•          26 2569.4 1302.4              1              1
•          30 2562.5 1592.5              1              1
•          32 2554.5 1902.4              1              1
•          32 2524.5 1103.4              1              1
•          22 2514.5 1523.8              1              1
•          12 2503.4 1316.6              1              1
•          21 2494.5 1949.5              1              1
•          15 2492.5 1923.4              1              1
•          35 2488.5 1721.5              1              1
•          17 2483.5 1870.6              1              1
•          12 2479.4 1212.5              1              1
•          32 2465.5 1452.5              1              1
•          15 2456.4  638.4              1              1
•          13 2444.7  900.7              1              1
•          14 2437.6 1183.4              1              1
•          23 2436.4 1969.4              1              1
•   Etc…………………………………………..
```

Project vectors onto a line.
Measure the length of each projection.

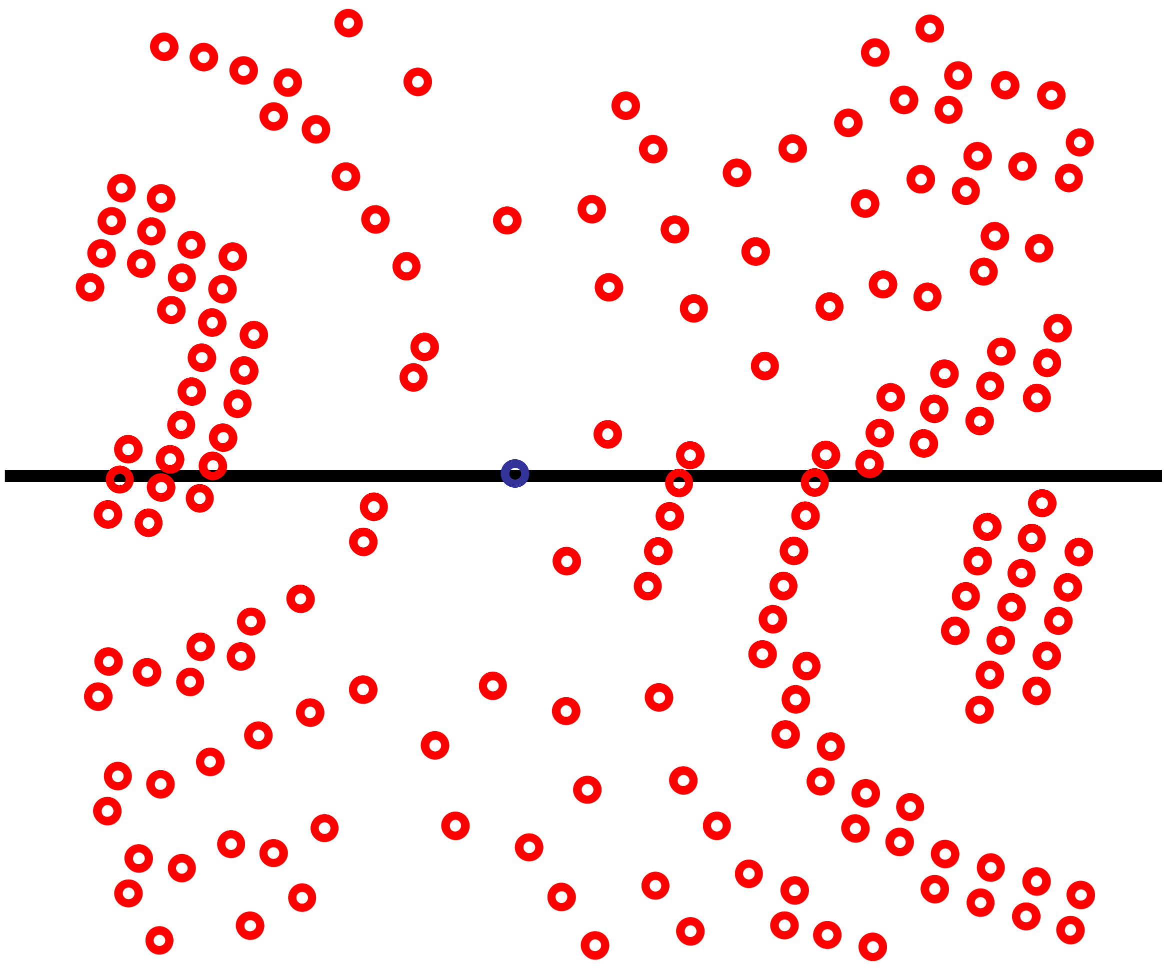


Look for incremental differences in lengths.

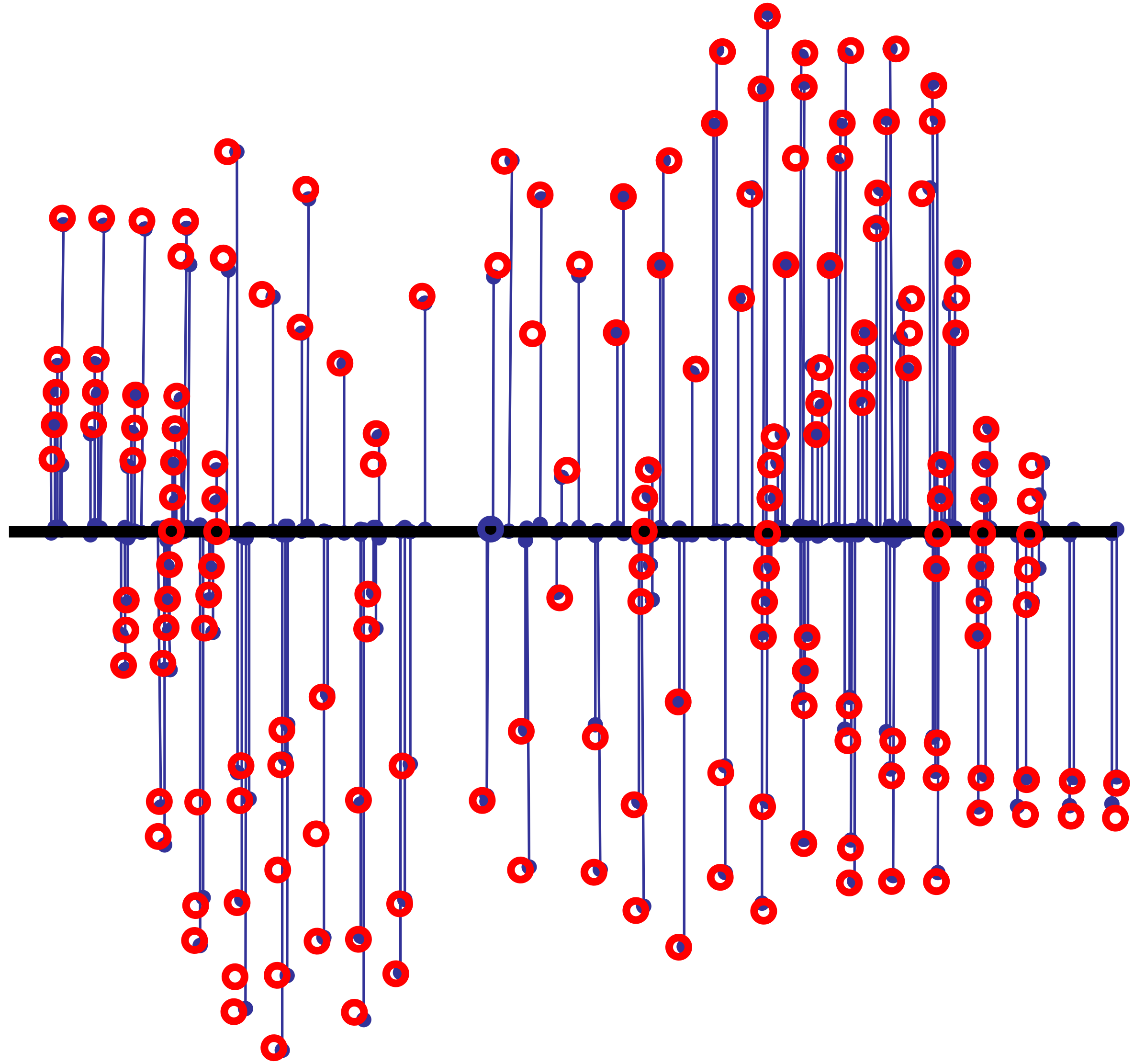


Distribution of lengths is not incremental, it is continuous

Rotate, 7300 orientations tested.

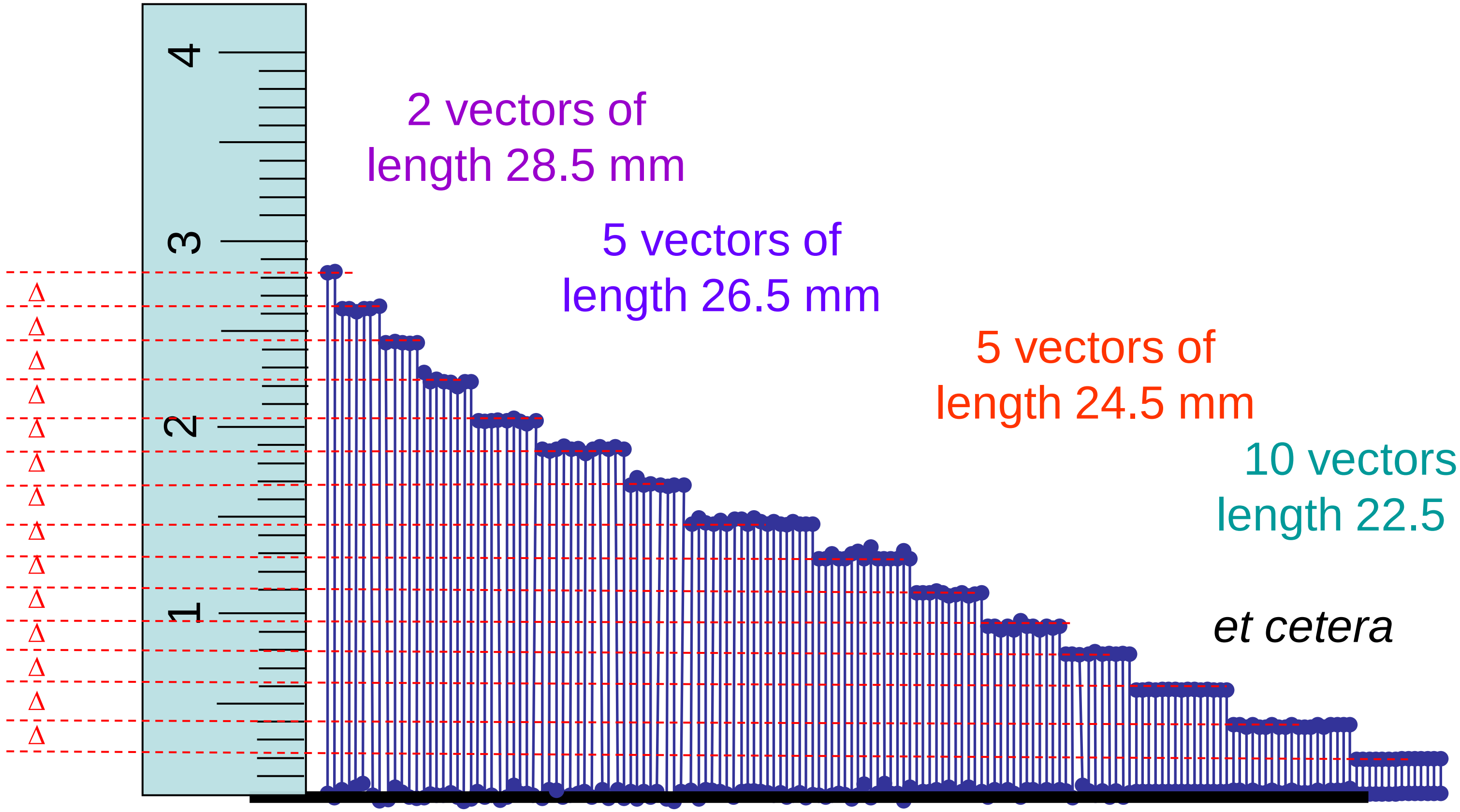


Projected vectors for rotated image.



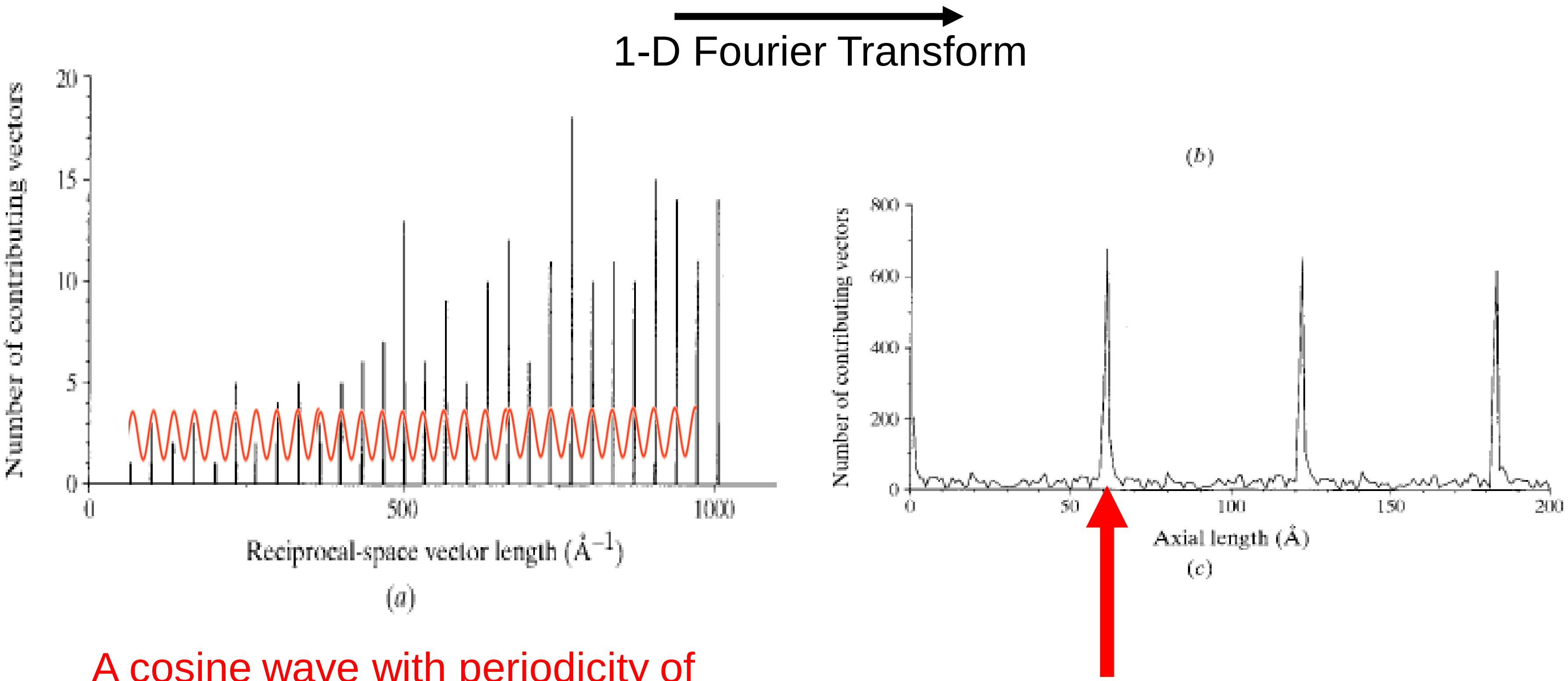
Sort the vector projections by length.

Count the number of observations of each length.



1) Note: Projected vectors have a quantized values (distribution looks like steps).
2) The incremental difference Δ is proportional to the reciprocal cell length

Fourier analysis of length histogram reveals cell dimension.



A cosine wave with periodicity of 1/62.5 Å is a major contributor to the 1-D FT.

Unit cell length = 62.5 Å

Run autoindexing script

The autoindexing script is simply named “a.”

Type “denzo” to start the program.

Then type @a to pass instructions to Denzo.

AUTOINDEXING SCRIPT

this script is simply named “a”

```
format ccd adsc unsupported-q4
TITLE 'DENZO autoindexing CCD'
overload value  65000          [less than 65535 (16 bits)]
error positional 0.040         [1/2 of an 80-micron Q4 pixel.]
spindle axis    1 0 0          [establish lab coords. ]
vertical axis   0 1 0          [for crystal before cryst rotx, etc. ]
film rotation 0
error systematic 5.0 partiality 0.1       [default value]
wavelength   1.5418            [ Cu Edge]
error density         0.12     [ lambda-dependent detector gain]
                               [ This is probably close enough for ]
                               [ lambda ~ 1 A]
air absorption length 3145    [ lambda-dependen absorption, check Int. tbl.]
                               [ or use the  'airabs' utility. ]

weak level 1.0
peak search file 'peaks.file'
profile fitting radius 20.0
[ALIGNED by  D.C.  12/28/2001]

x beam  100.987 y beam   91.60

[******************CHANGE INFO BELOW*****************]
sector  1
raw data file 'images/prok_hgcl1_1_###.img'
oscillation range 1.0  start 1
resolution limits 90.0  2.3
mosaicity 0.8
space group  P1
distance 100
[***************************************************]

box  print  2.0 2.0
spot elliptical 0.5  0.5  0.0
background elliptical 0.6  0.6  0.0

overlap spot
go
write predictions
print statistics
list
```

INDEXING REFLECTIONS

continued

Distortion Index- the amount of distortion of the observed lattice required to fit a perfect Bravais lattice of a particular type. We want the lowest possible distortion with the highest symmetry. Acceptable distortion indexes are below 1%. Here, primitive tetragonal appears the best choice.

HIGHER SYMMETRY

14 BRAVAIS LATTICES

LOWER SYMMETRY

Lattice	Metric tensor distortion index	Best cell (symmetrized) Best cell (without symmetry restrains)
primitive cubic	11.20%	67.97 67.87 101.96 90.03 90.05 90.06 79.27 79.27 79.27 90.00 90.00 90.00
I centred cubic	24.53%	122.49 96.00 122.46 67.01 46.20 66.94 113.65 113.65 113.65 90.00 90.00 90.00
F centred cubic	24.68%	140.11 139.98 140.10 57.98 121.96 93.45 140.06 140.06 140.06 90.00 90.00 90.00
primitive rhombohedral	11.20%	67.87 101.96 67.97 90.05 90.06 90.03 79.27 79.27 79.27 90.05 90.05 90.05 109.31 109.31 139.98 90.00 90.00 120.00
primitive hexagonal	13.58%	67.97 67.87 101.96 90.03 90.05 90.06 67.92 67.92 101.96 90.00 90.00 120.00
primitive tetragonal	0.05%	67.97 67.87 101.96 90.03 90.05 90.06 67.92 67.92 101.96 90.00 90.00 90.00
I centred tetragonal	11.08%	67.97 67.87 225.31 72.51 72.51 90.06 67.92 67.92 225.31 90.00 90.00 90.00
primitive orthorhombic	0.03%	67.87 67.97 101.96 90.05 89.97 89.94 67.87 67.97 101.96 90.00 90.00 90.00
C centred orthorhombic	0.04%	96.00 96.10 101.96 90.01 89.95 90.08 96.00 96.10 101.96 90.00 90.00 90.00
I centred orthorhombic	11.08%	67.87 67.97 225.31 72.51 107.49 89.94 67.87 67.97 225.31 90.00 90.00 90.00
F centred orthorhombic	11.07%	96.00 96.10 225.31 89.98 115.17 90.08 96.00 96.10 225.31 90.00 90.00 90.00
primitive monoclinic	0.02%	67.87 101.96 67.97 90.05 90.06 90.03 67.87 101.96 67.97 90.00 90.06 90.00
C centred monoclinic	0.03%	96.00 96.10 101.96 89.99 90.05 90.08 96.00 96.10 101.96 90.00 90.05 90.00
primitive triclinic	0.00%	67.87 67.97 101.96 90.05 90.03 90.06

Best fit cell (without symmetry restraints). For the correct choice of lattice, these dimensions should be very close to the symmetrized cell above.

Best cell (symmetrized) i.e. the best fit perfect Bravais lattice.

crystal rotx, roty, rotz -60.845 -44.897 85.491

Initial crystal orientation

Autoindex Xbeam, Ybeam 150.73 150.59

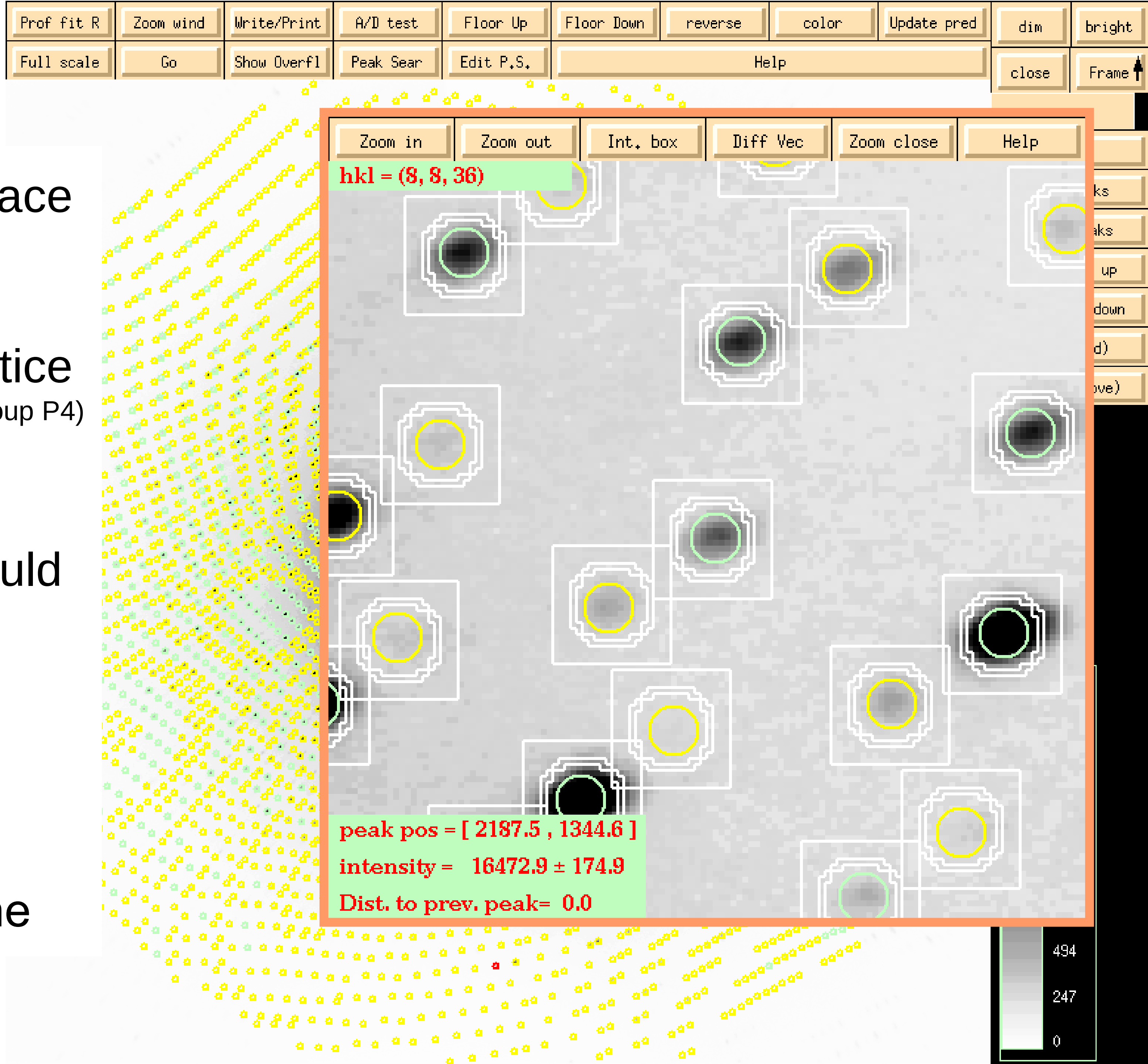
position 1208 chi**2 x 4.06 y 3.16 pred. decrease: 0.000 * 1208 = 0.0

1208 reflections indexed
Initial goodness of fit, chi**2 should be less than 2.00 in x and y Last number is the predicted improvement in fit in future refinement cycles. It is zero at the end of refinement.

Select a space group with desired Bravais Lattice
(*e.g*. new space group P4)

Predicted pattern should match observed diffraction pattern.

“go” to refine



Insert refined unit cell and crystal orientation parameters into integration script (integ.dat).Type “list” to obtain refined parameters..

```
cassette rotx   -0.04 roty   -0.04 rotz    0.00 2 theta    0.00
distance  100.38
x beam  150.749 y beam  150.617
y scale  0.99849
film rotation  180.000
skew  0.00013
crossfire y  0.221 x  0.260 xy  0.028
goniostat single axis
goniostat orientation    0.000    0.000
motor axis  0.000000  1.000000  0.000000
profile fitting radius   30.00
resolution limits   90.0   2.00
wavelength 1.54180
monochromator   0.000
oscillation start    1.00 end    2.00
mosaicity   0.500
spindle axis  -1   0   0 vertical axis   0   0   1
unit cell   68.098   68.098  102.196   90.000   90.000   90.000
crystal rotx -153.921 roty   -3.305 rotz  -45.111
```

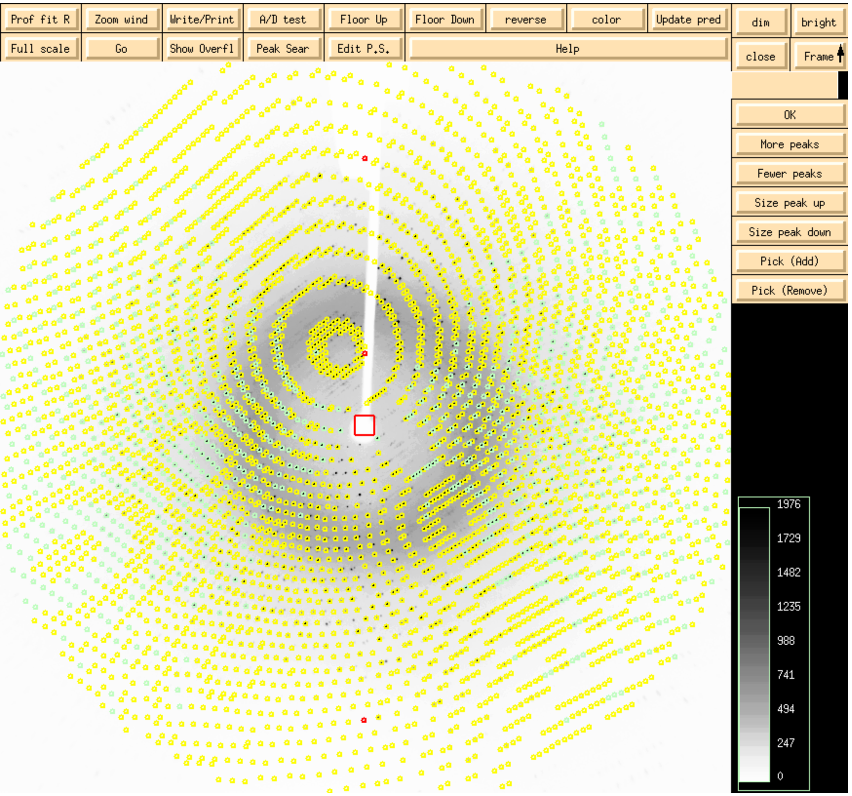
delete this line

Paste parameters into integration script (integ.dat).

Paste this information into “integ.dat” at the indicated location.

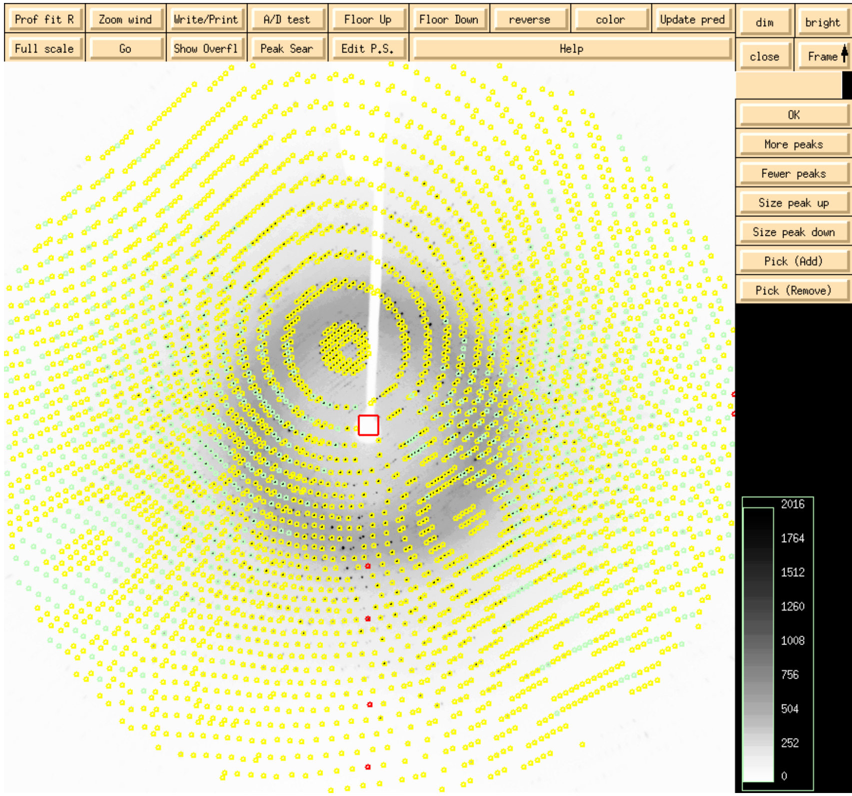
```
title  refine_all_images
format raxis4 100

error density 0.6
error systematic 5.0 partiality 0.1 positional 0.050
weak level 5.0
profile fitting radius 30.0

[*************INSERT HERE BLOCK OF DATA FROM AUTOINDEXING****

cassette rotx   -0.04 roty   -0.04 rotz    0.00 2 theta    0.00
distance  100.38
x beam  150.749 y beam  150.617
y scale  0.99849
film rotation  180.000
skew  0.00013
crossfire y  0.221 x  0.260 xy  0.028
goniostat single axis
goniostat orientation    0.000    0.000
motor axis  0.000000  1.000000  0.000000
profile fitting radius   30.00
resolution limits   90.0   2.00
wavelength 1.54180
monochromator   0.000
mosaicity   0.500
spindle axis  -1   0   0 vertical axis   0   0   1
unit cell   68.098   68.098  102.196   90.000   90.000   90.000
crystal rotx -153.921 roty   -3.305 rotz  -45.111

[*****************************************************************************

[************CHANGE INFO BELOW *******************************]

space group P4
oscillation  start 1.0  range 1.0
raw data file 'images/prok_nat2####.osc'
film output file 'x/prok_nat2####.x'
sector 1 to 360
box 2.5 2.5
spot elliptical .75 .75 0.
background elliptical .85 0.85 0

[*****************************************************************************


overlap spot
start refinement
print no profiles
fit all
resolution limits 100.0 5.0
go go go go go
write predictions
list
```

Spot positions on all following images can be predicted from info learned from first image.



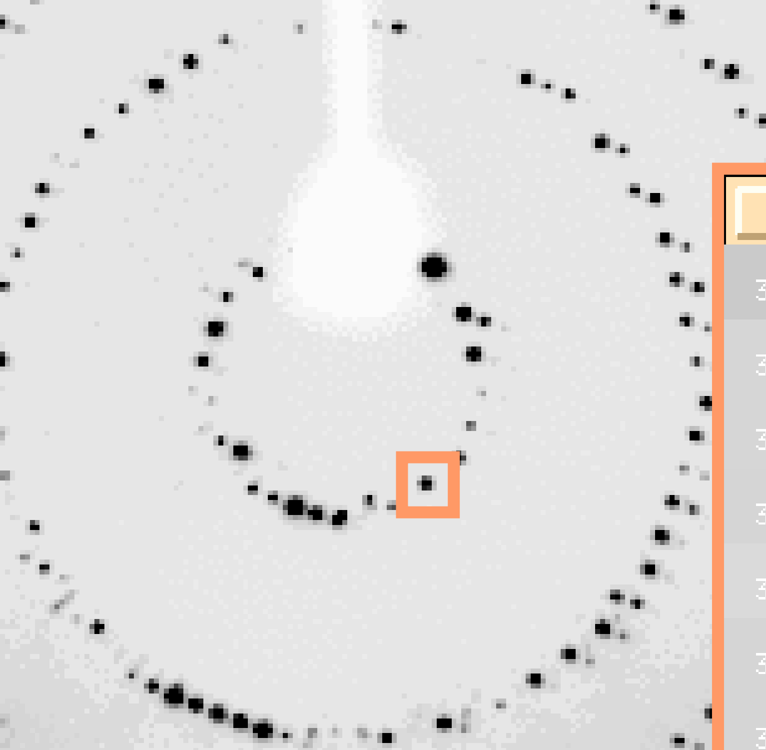
Film 1, exposed over 1 to 2 degrees

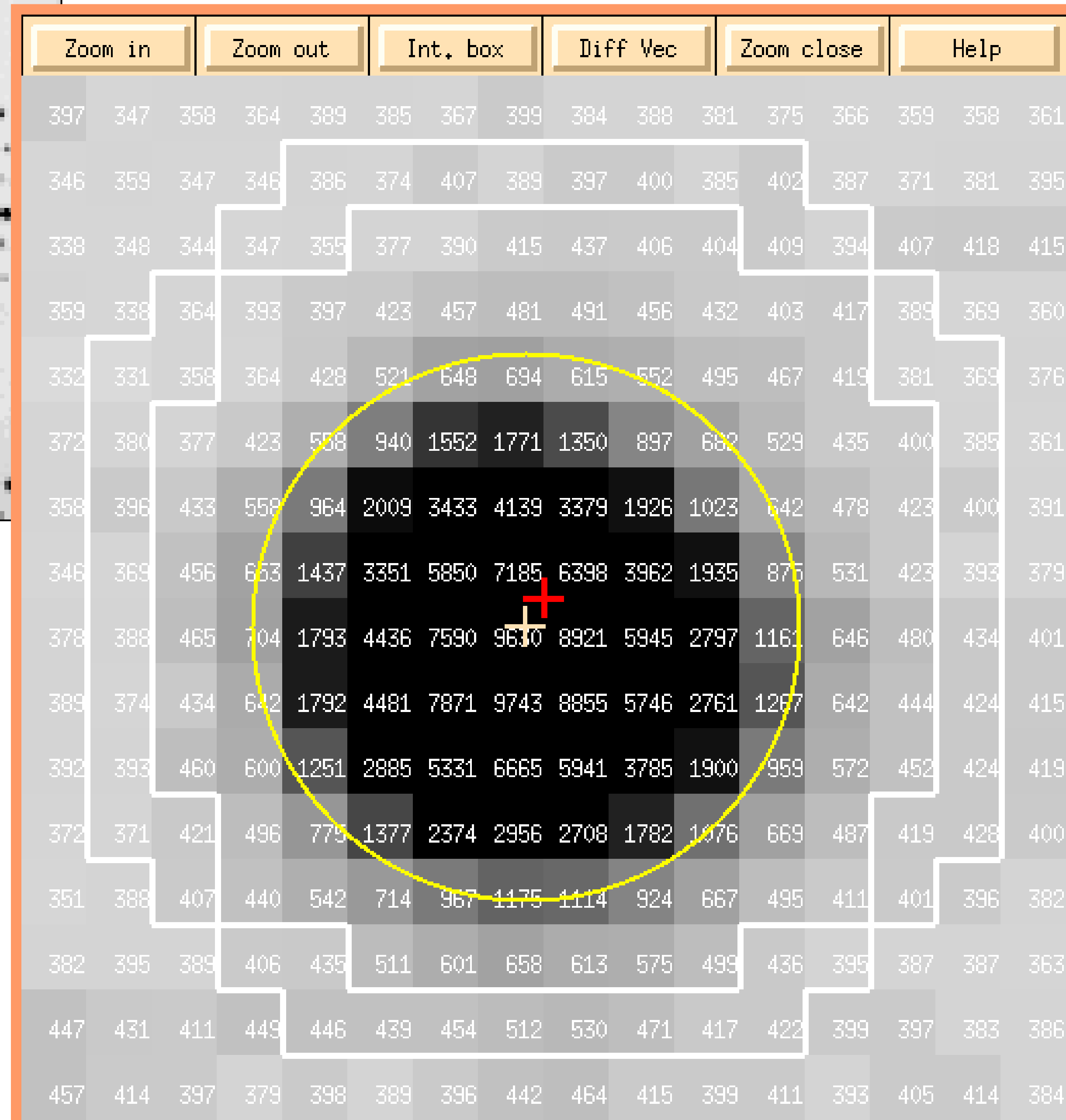
1°



Film 2, exposed over 2 to 3 degrees

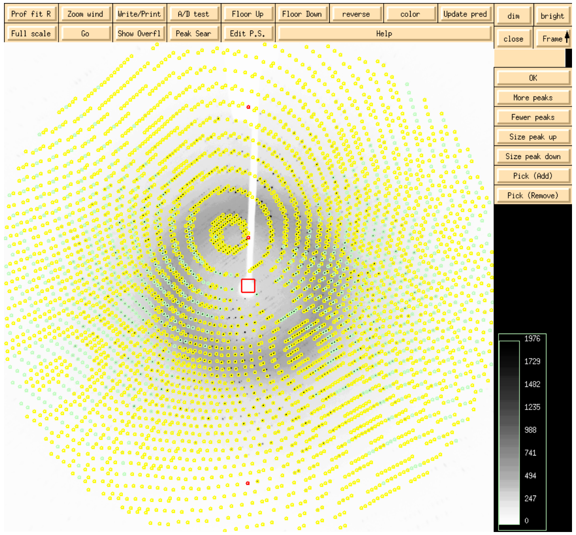
Integration





1)Draw boundaries of each reflection
2) Sum up the intensities recorded on each pixel within boundary.
3) Repeat for each spot on each film.

Integrated intensities are written to .x files

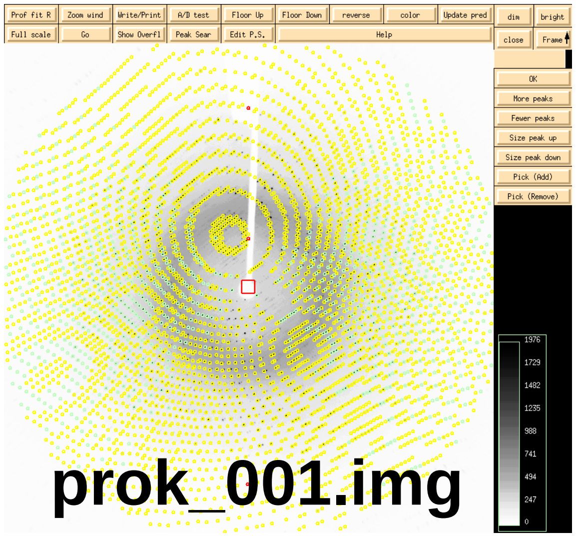


Film 1, exposed over
1 to 2 degrees

h	k	l	flag	I(profit)	I(prosum)	$\chi 2$	σ(I)	cos incid.	X pix	Y pix
29	20	-33	1	202.3	200.8	1.36	17.4	0.556	6.4	1353.0
29	21	-31	1	102.1	105.0	1.08	7.7	0.560	16.8	1421.5
30	26	-19	1	1291.2	1323.2	1.19	50.0	0.554	23.9	1808.7
31	28	-11	1	1554.0	1618.7	1.26	95.1	0.536	24.2	2061.6
29	22	-29	1	24.0	25.2	1.29	1.2	0.564	28.0	1489.1

…………………………………………………….

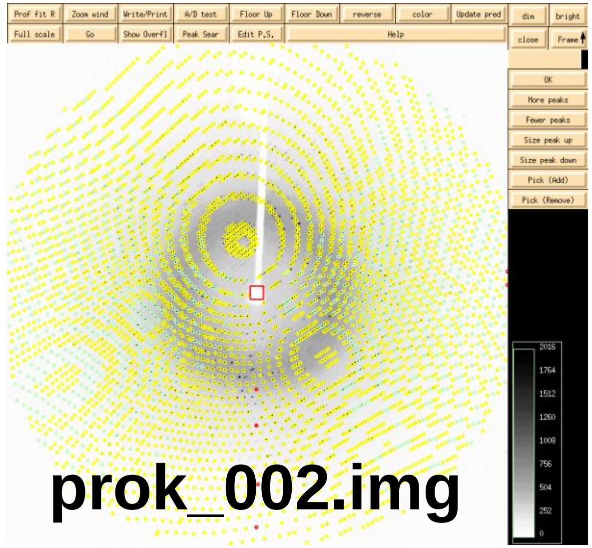
One .x file for each film



Film 1, exposed over
1 to 2 degrees

h	k	l	flag	I(profit)	I(prosum)	χ2	σ(I)	cos incid.	X pix	Y pix
29	20	-33	1	202.3	200.8	1.36	17.4	0.556	6.4	1353.0
29	21	-31	1	102.1	105.0	1.08	7.7	0.560	16.8	1421.5
30	26	-19	1	1291.2	1323.2	1.19	50.0	0.554	23.9	1808.7
31	28	-11	1	1554.0	1618.7	1.26	95.1	0.536	24.2	2061.6
29	22	-29	1	24.0	25.2	1.29	1.2	0.564	28.0	1489.1
29	20	-33	1	202.3	200.8	1.36	17.4	0.556	6.4	1353.0
29	21	-31	1	102.1	105.0	[illegible]	[illegible]	[illegible]	16.8	1421.5
30	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
31	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
29	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
29	21	-31	1	102.1	105.0	1.08	7.7	0.560	16.8	1421.5
30	26	-19	1	1291.2	1323.2	1.19	50.0	0.554	23.9	1808.7
31	28	-11	1	1554.0	1618.7	1.26	95.1	0.536	24.2	2061

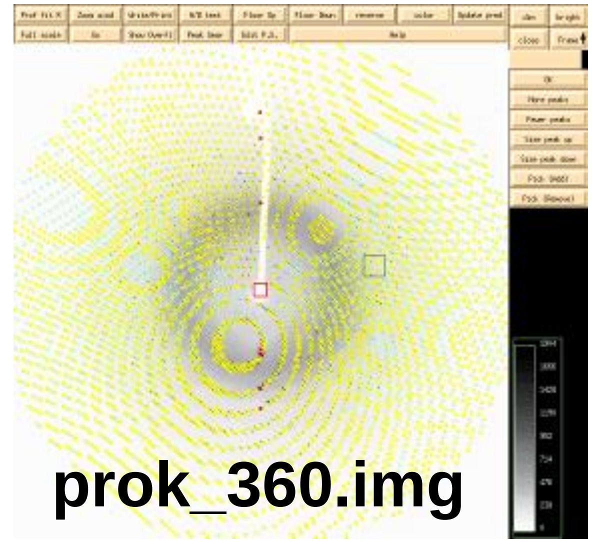
prok_001.x



Film 2, exposed over
2 to 3 degrees

h	k	l	flag	I(profit)	I(prosum)	χ2	σ(I)	cos incid.	X pix	Y pix
29	20	-33	1	52.3	50.8	1.36	17.4	0.556	6.4	1353.0
29	21	-31	1	102.1	105.0	1.08	7.7	0.560	16.8	1421.5
30	26	-19	1	1291.2	1323.2	1.19	50.0	0.554	23.9	1808.7
31	28	-11	1	1554.0	1618.7	1.26	95.1	0.536	24.2	2061.6
29	22	-29	1	24.0	25.2	1.29	1.2	0.564	28.0	1489.1
29	20	-33	1	202.3	200.8	1.36	17.4	0.556	6.4	1353.0
29	21	-31	1	102.1	105.0	[illegible]	[illegible]	[illegible]	16.8	1421.5
30	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
31	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
29	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
29	21	-31	1	102.1	105.0	1.08	7.7	0.560	16.8	1421.5
30	26	-19	1	1291.2	1323.2	1.19	50.0	0.554	23.9	1808.7
31	28	-11	1	1554.0	1618.7	1.26	95.1	0.536	24.2	2061

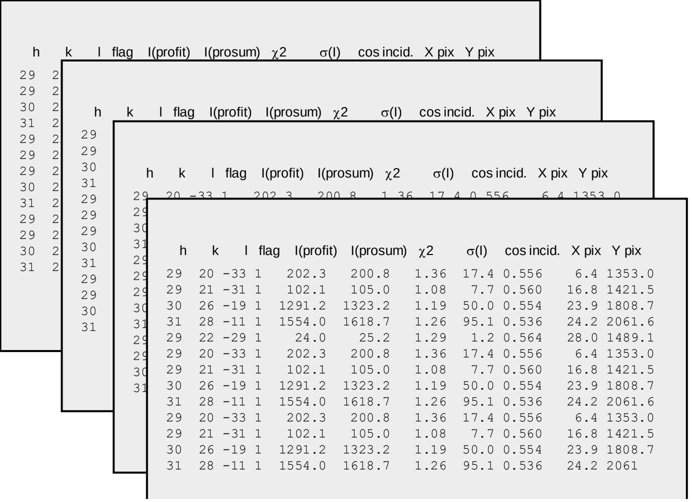
prok_002.x



Film 360, exposed over
360 to 361 degrees

h	k	l	flag	I(profit)	I(prosum)	χ2	σ(I)	cos incid.	X pix	Y pix-29
-20	33	1	212.3	220.8	1.36	17.4	0.556	6.4	1353.0	
29	21	-31	1	102.1	105.0	1.08	7.7	0.560	16.8	1421.5
30	26	-19	1	1291.2	1323.2	1.19	50.0	0.554	23.9	1808.7
31	28	-11	1	1554.0	1618.7	1.26	95.1	0.536	24.2	2061.6
29	22	-29	1	24.0	25.2	1.29	1.2	0.564	28.0	1489.1
29	20	-33	1	202.3	200.8	1.36	17.4	0.556	6.4	1353.0
29	21	-31	1	[illegible]	105.0	[illegible]	[illegible]	[illegible]	16.8	1421.5
30	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
31	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
29	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
29	21	-31	1	102.1	105.0	1.08	7.7	0.560	16.8	1421.5
30	26	-19	1	1291.2	1323.2	1.19	50.0	0.554	23.9	1808.7
31	28	-11	1	1554.0	1618.7	1.26	95.1	0.536	24.2	2061

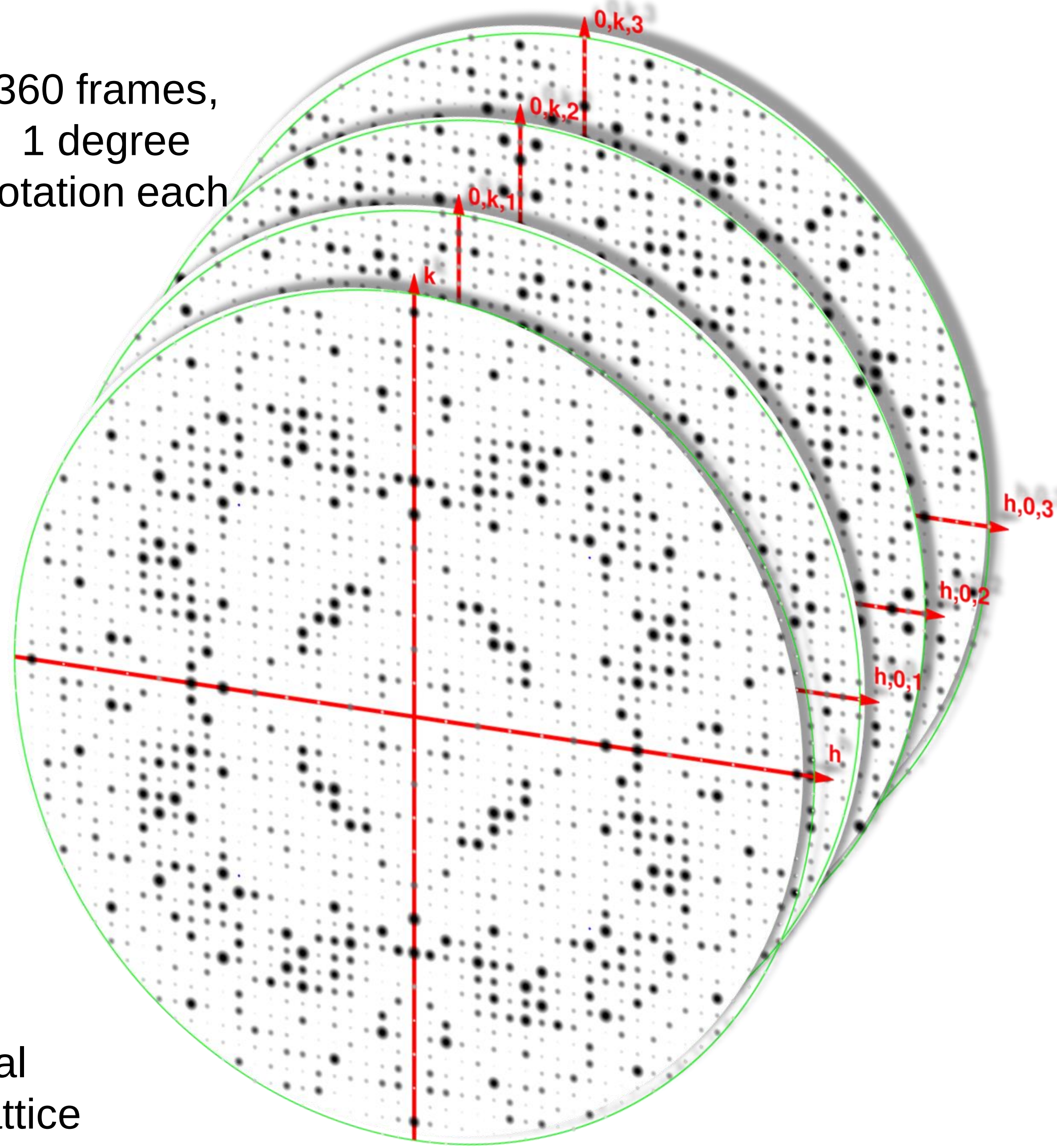
prok_360.x



h	k	l	flag	I(profit)	I(prosum)	$\chi2$	σ(I)	cos incid.	X pix	Y pix
29	20	-33	1	202.3	200.8	1.36	17.4	0.556	6.4	1353.0
29	21	-31	1	102.1	105.0	1.08	7.7	0.560	16.8	1421.5
30	26	-19	1	1291.2	1323.2	1.19	50.0	0.554	23.9	1808.7
31	28	-11	1	1554.0	1618.7	1.26	95.1	0.536	24.2	2061.6
29	22	-29	1	24.0	25.2	1.29	1.2	0.564	28.0	1489.1
29	20	-33	1	202.3	200.8	1.36	17.4	0.556	6.4	1353.0
29	21	-31	1	102.1	105.0	1.08	7.7	0.560	16.8	1421.5
30	26	-19	1	1291.2	1323.2	1.19	50.0	0.554	23.9	1808.7
31	28	-11	1	1554.0	1618.7	1.26	95.1	0.536	24.2	2061.6
29	20	-33	1	202.3	200.8	1.36	17.4	0.556	6.4	1353.0
29	21	-31	1	102.1	105.0	1.08	7.7	0.560	16.8	1421.5
30	26	-19	1	1291.2	1323.2	1.19	50.0	0.554	23.9	1808.7
31	28	-11	1	1554.0	1618.7	1.26	95.1	0.536	24.2	2061

360 frames,
1 degree
rotation each

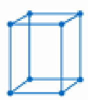
prok_001 –> 360.x



Intensities from .x files can be mapped onto the reciprocal lattice for visual inspection.

1) Accuracy will improve if we merge symmetry-related observations.
2) But, we must test if rotational symmetry exists between lattice points.

Choose point group symmetry (4 or 422)

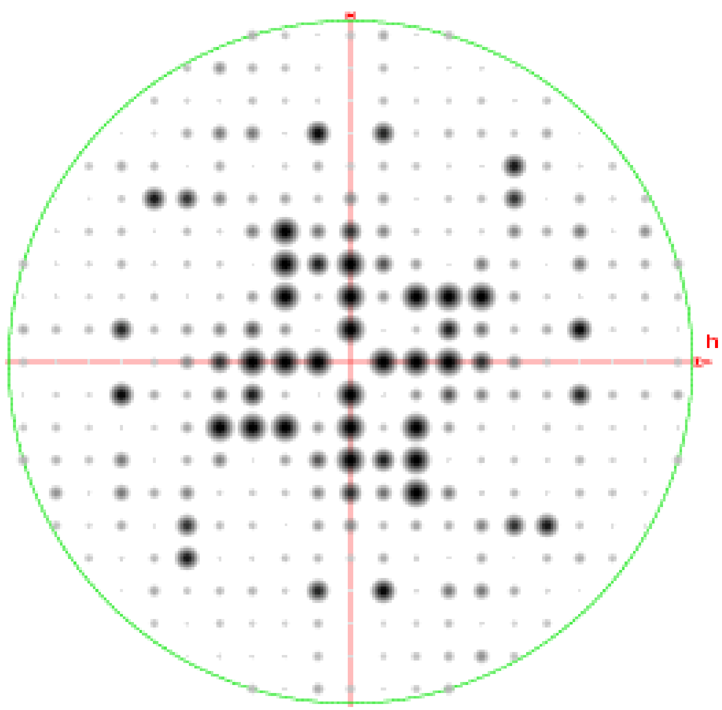


Tetragonal P

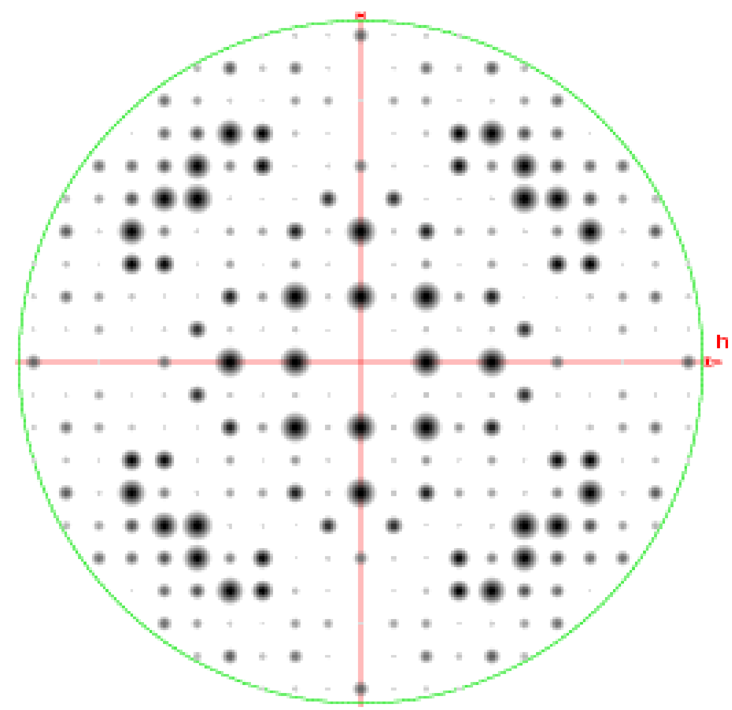
Three axes at right angles; two equal:
$a = b \neq c$; $\alpha = \beta = \gamma = 90°$

System	Bravais lattice	Point group	65 Chiral Space Groups					
Triclinic	P	1	P1					
Monoclinic	P	2	P2	$P2_1$				
	C		C2					
Orthorhombic	P	222	P222	$P222_1$	$P2_12_12$	$P2_12_12_1$		
	C		$C222_1$	C222				
	F		F222					
	I		I222	$I2_12_12_1$				
Tetragonal	P	4	P4	$P4_1$	$P4_2$	$P4_3$		
	I		I4	$I4_1$				
	P	**422**	**P422**	**$P42_12$**	**$P4_122$**	**$P4_12_12$**	**$P4_222$**	**$P4_22_12$**
			$P4_322$	**$P4_32_12$**				
	I		I422	$I4_122$				
Trigonal	P	3	P3	P31	P32			
	R		R3					
	P	**32**	**P312**	**P321**	**$P3_112$**	**$P3_121$**	**$P3_212$**	**$P3_221$**
	R		**R32**					
Hexagonal	P	6	P6	$P6_1$	$P6_2$	$P6_3$	$P6_4$	$P6_5$
		622	**P622**	**$P6_122$**	**$P6_222$**	**$P6_322$**	**$P6_422$**	**$P6_522$**
Cubic	P	23	P23	$P2_13$				
	F		F23					
	I		I23	$I2_13$				
	P	**432**	**P432**	**$P4_232$**	**$P4_332$**	**$P4_132$**		
	F		**F432**	**$F4_132$**				
	I		**I432**	**$I4_132$**				

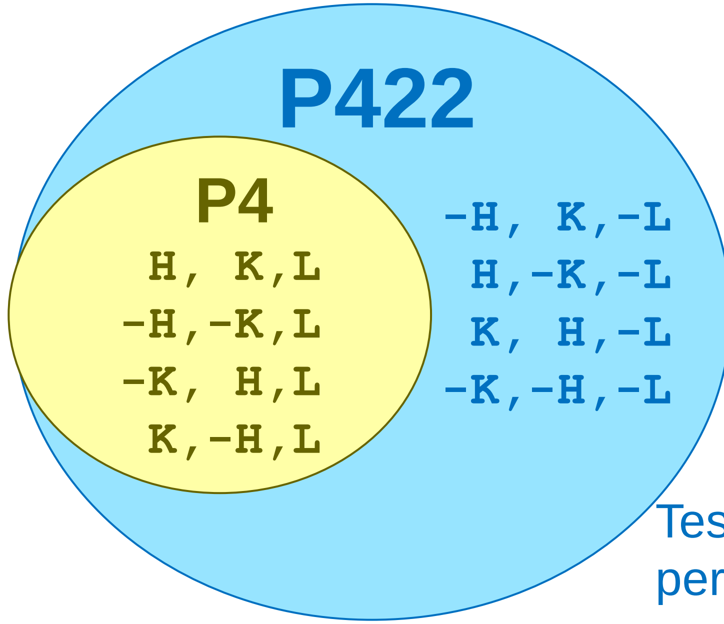
Point group 4



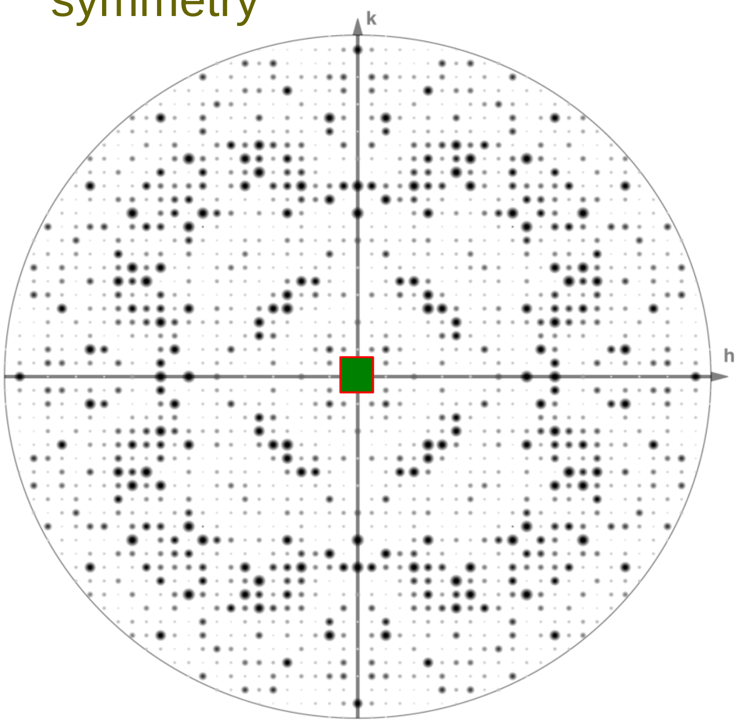
Point group 422



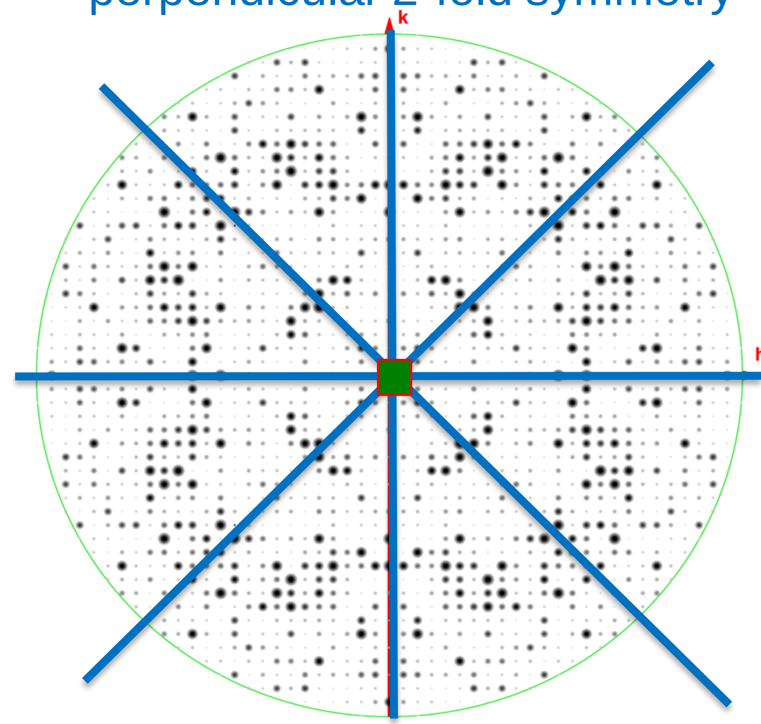
Is it Point group 4 or Point group 422 ?



Test existence of 4-fold symmetry



Test existence of 4-fold & perpendicular 2-fold symmetry



j observations of the reflection 30 22 6

j	H	K	L	I
1	30	22	6	550
2	-30	-22	6	500
3	30	-22	-6	543

?

H, K, L=

-H,-K, L=

H,-K,-L

Discrepancy

between symmetry related reflections

$$R_{merge} = \frac{\sum |I_j - \langle I \rangle|}{\sum I_j}$$

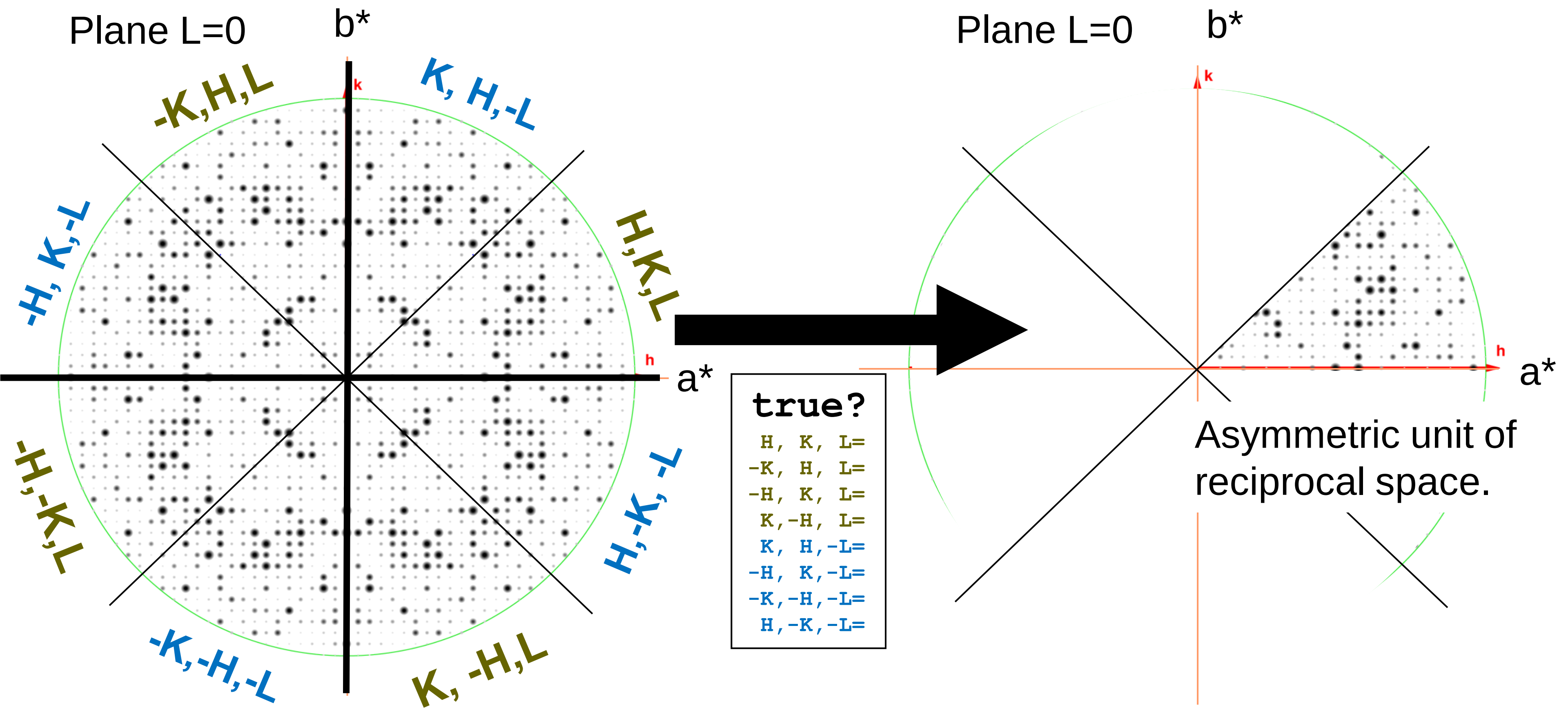
$$\langle I \rangle = (550 + 500 + 543) / 3$$
$$= 531$$

$$\sum |I_j - \langle I \rangle| = |550-531|+|500-531|+|543-531|$$
$$= 19 + 31 + 12$$
$$= 62$$

$$\sum I_j = 550 + 500 + 543$$
$$= 1593$$

$$R_{merge} = 62/1593 = 0.039 = 3.9\%$$

Merge

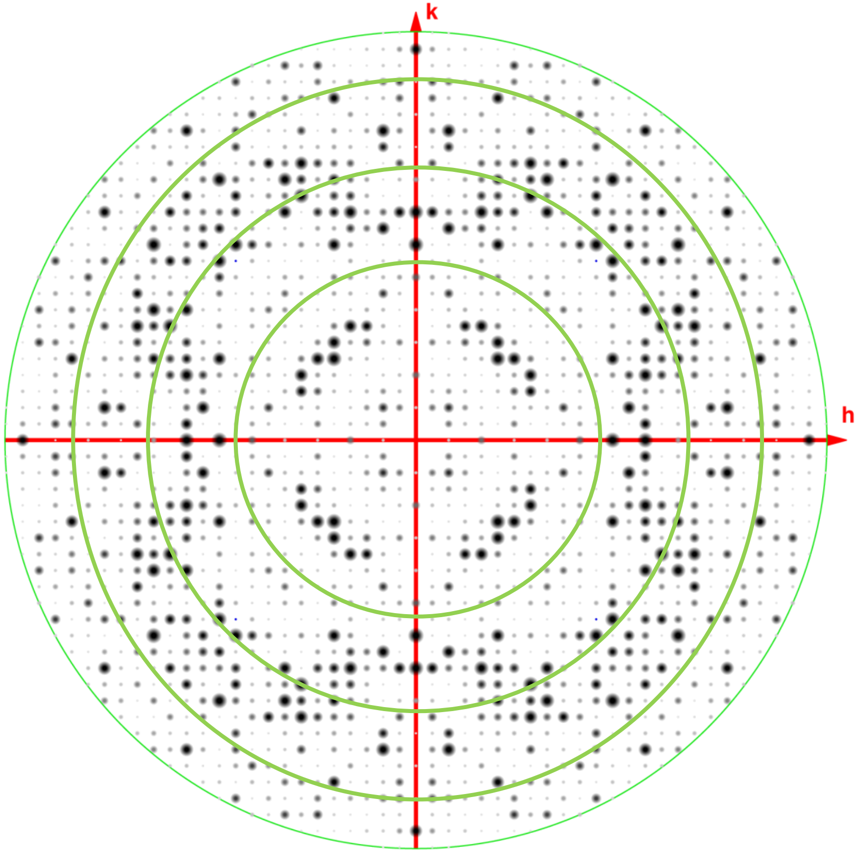


Merge together (average) reflections related by these symmetry operators in reciprocal space.

Statistics are analyzed as a function of resolution (N shells).

Discrepancy between symmetry related reflections (R_{merge}) increases with increasing resolution. Why?

Shell	R_{merge}
100-5.0Å	0.04
5.0-3.0Å	0.06
3.0-2.0Å	0.08
2.0-1.7Å	0.15

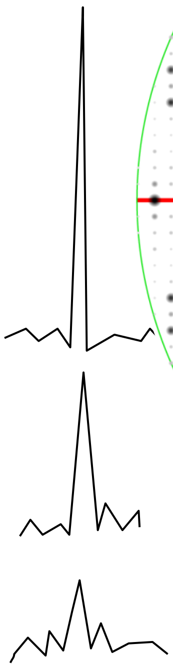


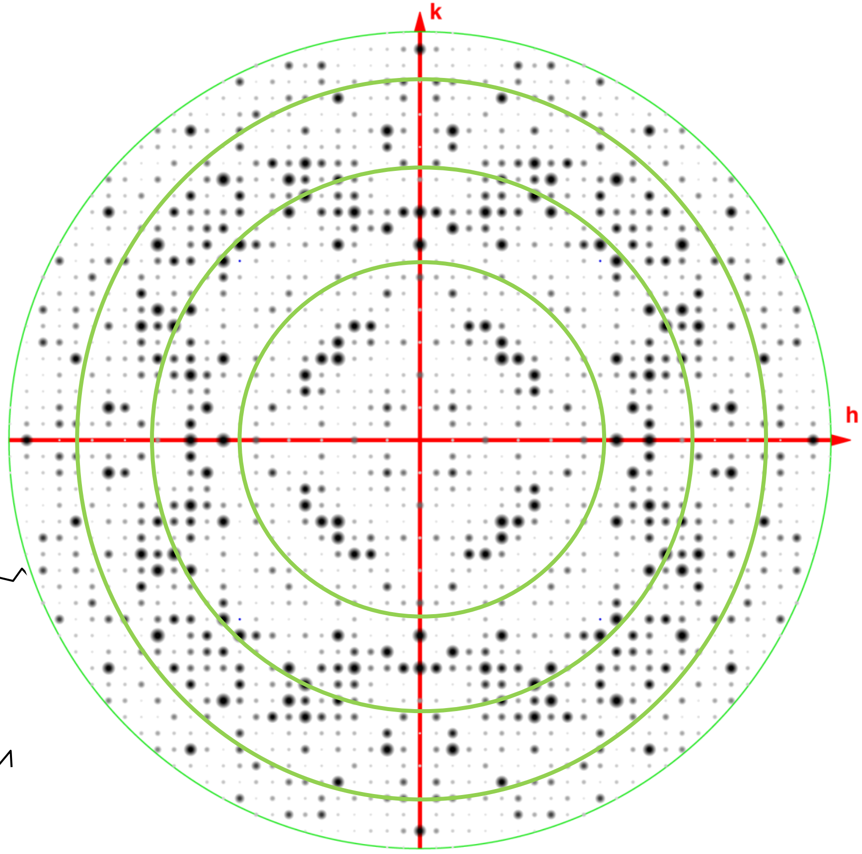
SIGNAL TO NOISE RATIO (I/σ)

Average I/σ decreases with increasing resolution

High resolution shells with I/σ <2 should be discarded.

Shell	I/σ_I
100-5.0 Å	20.0
5.0-3.0 Å	10.0
3.0-2.0 Å	7.0
2.0-1.7 Å	3.0





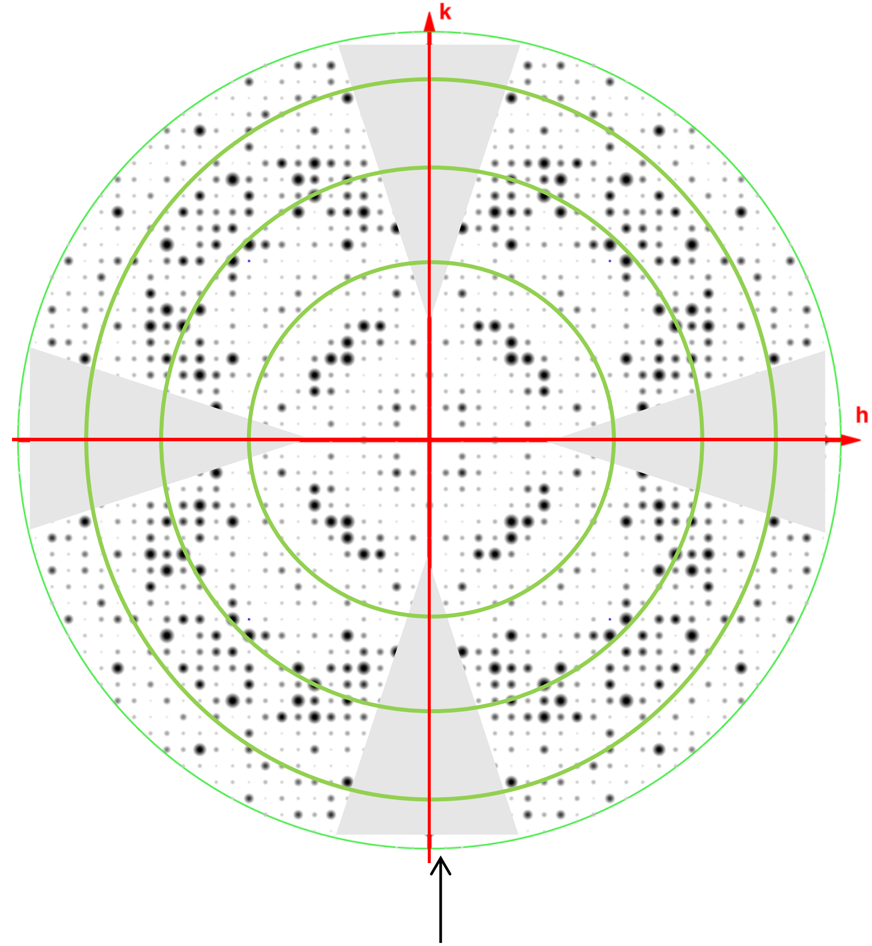
COMPLETENESS?

What percentage of reciprocal Lattice was measured for a given Resolution limit?

Better than 90% I hope.

Shell	completeness
100-5.0Å	99.9%
5.0-3.0Å	95.5%
3.0-2.0Å	89.0%
2.0-1.7Å	85.3%

Overall 92.5%



Missing wedges of data.

Assignment

Table 1. Crystal and X-Ray Diffraction Data of Native, Heavy-Atom Derivatives of Aa-RNase III

		Native	Au Derivative	U Derivative
Resolution (Å)		2.15	3.00	2.90
Space group		$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimensions:	a (Å)	46.16	46.40	46.49
	b (Å)	51.12	51.89	51.60
	c (Å)	123.47	124.72	118.40
Measured reflections		68,832	23,588	28,927
Unique reflections		16,542	11,071	12,404
Overall completeness (%)		99.5	96.2	98.4
Last shell[a] completeness (%)		99.9	89.7	93.8
Overall R_{sym}[b]		0.038	0.054	0.054
Last shell R_{sym}		0.370	0.235	0.446
Overall $I/\sigma(I)$		23.4	18.2	11.3
Last shell $I/\sigma(I)$		3.3	3.5	1.7

[a] 2.23–2.15 Å for native, 3.11–3.00 Å for the Au derivative, 3.00–2.90 Å for the U derivative

[b] $R_{sym} = \Sigma|(I - \langle I \rangle)|/\Sigma(I)$, where I is the observed intensity.