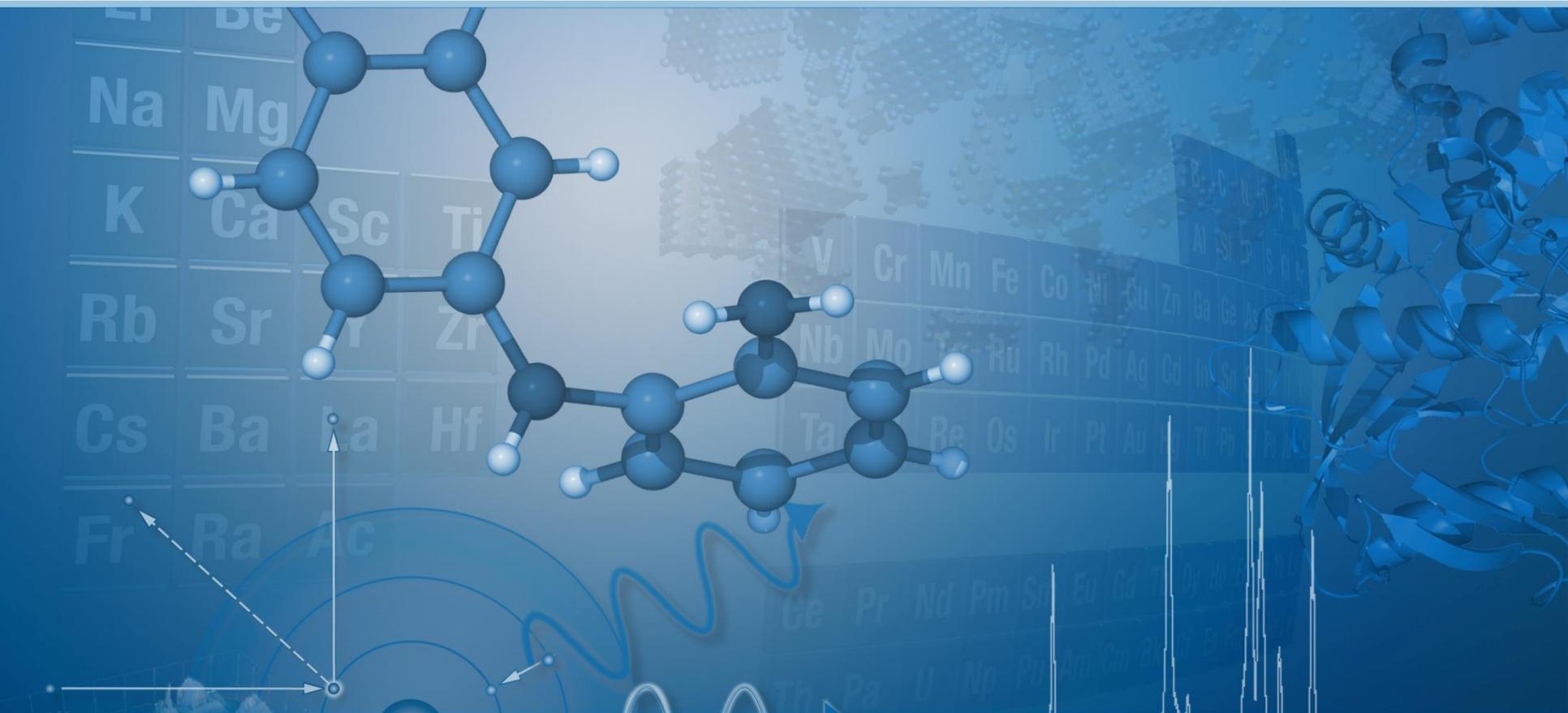


Crystal Indexing in PROTEUM3



Indexing Screen



- Determine Unit Cell
 - Finding the orientation of the crystal lattice relative to the instrumental setup
- Automatic
 - Can be run from any step
- Manual
 - Collect Data
 - Harvest spots
 - Index
 - Refine
 - Bravais
 - Refine
- Methods
 - Difference vector
 - FFT
 - Least squares (Cell_now)

Sample Instrument Windows Help Determine Unit Cell

C:\frames\demo\Lysozyme\Lyso_080916_01_0001.afm

Set Up Screen

Screen Crystal

Determine Unit Cell

View Reciprocal Lattice

View Images

Automatic Mode

Start at: Collect Data

Stop after: Refine

Manual Mode

Collect Data

Harvest Spots

Index

Bravais

Refine

Run

Unit cells:

Reflections:

Expected resolution:

	Exposure time (s./°)	Resolution (Å)
1	2.0	n/a
2	5.0	n/a
3	10.0	n/a
4	20.0	n/a

Crystal Mosaicity [°] 0.66

Cursor

Position [mm]	26.19	64.53
Position [pixels]	578	988
Intensity [counts]	73	
HKL index	4.38	0.39 3.86
Resolution [Å]	1.71	
2Theta [°]	46.20	

Collect

Reduce Data

Examine Data

Solve Structure

Report



Determine Unit Cell

Collect data

You can collect images for indexing based on preset runs in the bn-config.py file. Since every crystal diffracts differently, you to change critical parameters to match the sample.

- Distance
- Exposure time
- Image width

Standard setting for CMOS and CCD detectors

- Detector Format always 1024x1024
- Correlate Frames always "no" for CMOS
- Runs or scans are defined as angular sweeps in different crystal orientations. They can be setup in the bn-config file or from the *run experiment* menu
 - 2 runs of 3° separated by 90° in reciprocal space
 - *Filename_01_0001.sfrm*



Image Location: C:\frames\demo\lysozyme

Image Base Name: matrix

First Run: 1

Distance [mm]: 60.00

Exposure Time: 10.00 sec/image

Image Width [deg]: 0.50

Detector Format: 1024x1024

Correlate Frames: no

Navigation: Previous, Next, Finish, Collect..., Cancel



Determine Unit Cell

Harvest spots

Find reflections for the indexing. Can harvest from anywhere in the run or from more than one run. Can be in different regions of reciprocal space.

- First image can be from anywhere in the data
- Number of runs
 - Will start at the beginning of all runs after run 1
- Go to Image – what image to display
- Min I/sigma(I)
 - Signal to noise cutoff for finding reflections
 - Smooth images reduces image resolution
- Smooth images
 - Reduces the resolution of the images which removes weak reflections and combines split reflections
- Excluded Shells
 - Remove resolution shell from harvesting
- Store
 - Stores reflections by color scheme
 - Save only reflections that span images

First Image:

Number of Runs: Images per Run:

Go to Image:

Min. I/sigma(I): More Spots Fewer Spots

Smooth images

From [A] To [A]

Excluded Shells:

Store: Save only reflections that span images



Determine Unit Cell

Navigating through the indexing steps

- Arrows move to the previous or next step ▶
 - Harvest → Index
- Finish
 - Runs auto mode from current step
- Harvest
 - Performs function and returns to the main menu
- Cancel returns to the main menu

First Image:

Number of Runs: Images per Run:

Go to Image:

Min. I/sigma(I): More Spots Fewer Spots

Smooth images

From [Å]	To [Å]
0.10	3.0
3.2	3.8

Excluded Shells:

Store:

Save only reflections that span images



Determine Unit Cell Indexing

- Reflections shows the number of spots harvested and color code
- Go to Image shows the image displayed
- Min I/sigma(I)
 - Signal to noise cutoff for finding reflections
- Resolution
- Filters
- Corrections
 - Offsets from the frame headers
 - Clicking manual allows them to be edited
- Methods
 - Difference Vector
 - Fast Fourier Transform
 - Least Squares (Cell_now)

Reflections: Group 0: 870 reflections

Go to Image: C:\frames\demo\Lysozyme\Lysozyme_01_0001.sfm

Min. I/sigma(I): 20.00 More Reflections Fewer Reflections

Resolution [Å]: 9999.00 - 1.52

Reflections must be isolated
 Reflections must span images
 Reflections must be whole

870 Reflections selected for Indexing

Store: Empty

Corrections: From store From last harvest Manual

Distance [mm]: 0.00 Pitch [°]: 0.01
X Beam Center [mm]: -0.54 Roll [°]: 0.46
Y Beam Center [mm]: -0.60 Yaw [°]: 0.12

Methods: Difference Vectors
 Fast Fourier Transform
 Least Squares Specific Cell Search...

Determine Unit Cell Indexing

- Indexing results gives the unit cell found from all methods selected
- You can choose any cell to continue
- HKL histogram
 - Percentage of harvested reflections that match the predicted spots based on the orientation matrix

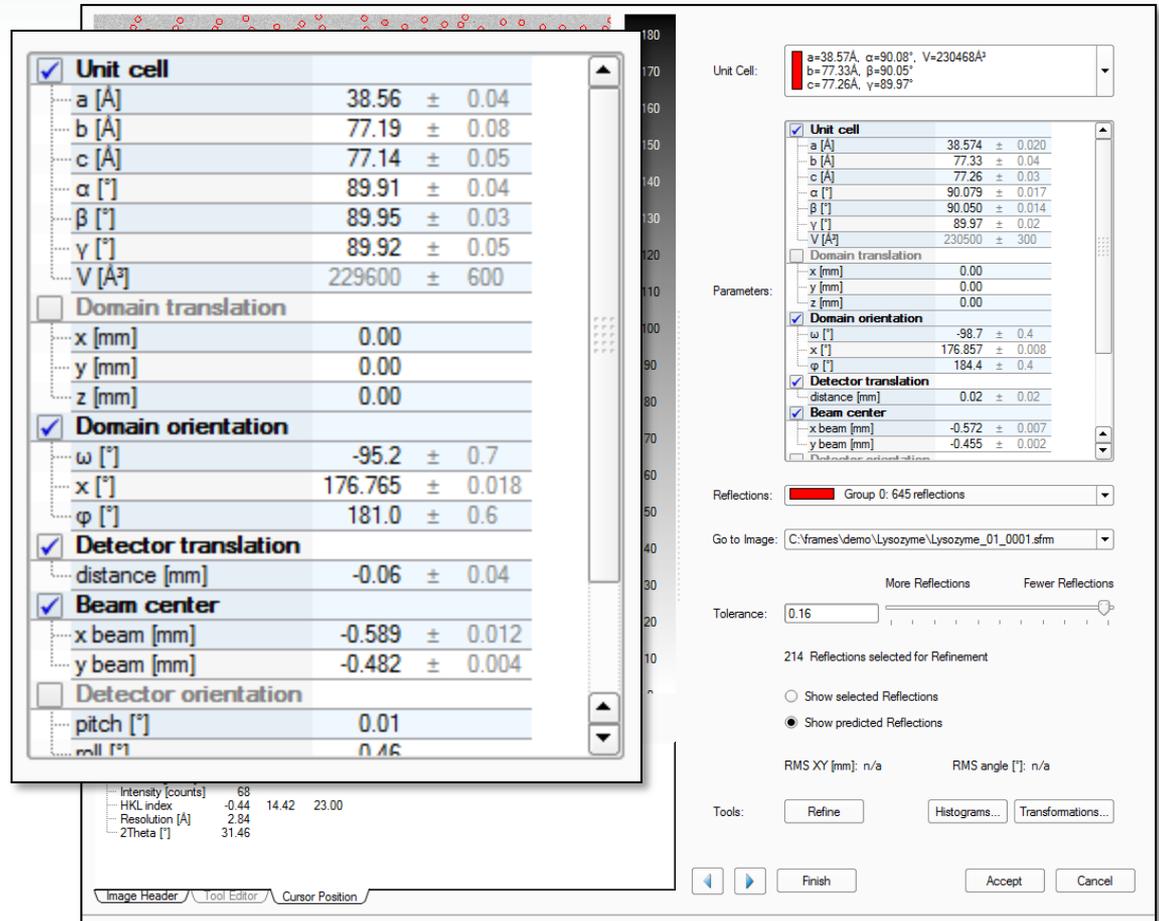
Reduced Unit Cells found:

Method: Difference Vectors Score: 0.95 a=38.58Å, α =89.98°, V=230361Å ³ b=77.25Å, β =89.97° c=77.30Å, γ =90.00°	HKL histogram:	
	0.1: 90.1% (581/645)	
	0.2: 96.4% (622/645)	
	0.3: 97.4% (628/645)	
Method: Fast Fourier Transform Score: 0.95 a=38.57Å, α =90.03°, V=230320Å ³ b=77.27Å, β =90.01° c=77.28Å, γ =90.01°	HKL histogram:	
	0.1: 90.1% (581/645)	
	0.2: 96.6% (623/645)	
	0.3: 97.2% (627/645)	

Determine Unit Cell

LS refinement reduced triclinic cell

- Refine the unit cell constants and hardware parameters to improve the orientation matrix
- Makes it easier to search for higher symmetry
- If you only index from a small angular range, the program only allows you to refine the Domain orientation, beam center and crystal to detector distance (DX)



The screenshot displays the Bruker software interface for unit cell refinement. The main window shows a list of parameters with their values and uncertainties. A table at the bottom left provides additional data points.

Parameter	Value	Uncertainty
a [Å]	38.56	± 0.04
b [Å]	77.19	± 0.08
c [Å]	77.14	± 0.05
α [°]	89.91	± 0.04
β [°]	89.95	± 0.03
γ [°]	89.92	± 0.05
V [Å ³]	229600	± 600
x [mm]	0.00	
y [mm]	0.00	
z [mm]	0.00	
ω [°]	-95.2	± 0.7
χ [°]	176.765	± 0.018
φ [°]	181.0	± 0.6
distance [mm]	-0.06	± 0.04
x beam [mm]	-0.589	± 0.012
y beam [mm]	-0.482	± 0.004
pitch [°]	0.01	
roll [°]	0.46	

Additional data from the bottom table:

Intensity [counts]	68
HKL index	-0.44 14.42 23.00
Resolution [Å]	2.84
2Theta [°]	31.46

The software interface also shows the following information:

- Unit Cell:** a=38.57Å, α=90.08°, V=230468Å³; b=77.33Å, β=90.05°; c=77.26Å, γ=89.97°
- Parameters:** Domain translation (x, y, z [mm] = 0.00), Domain orientation (ω [°] = -98.7 ± 0.4, χ [°] = 176.857 ± 0.008, φ [°] = 184.4 ± 0.4), Detector translation (distance [mm] = 0.02 ± 0.02), Beam center (x beam [mm] = -0.572 ± 0.007, y beam [mm] = -0.455 ± 0.002).
- Reflections:** Group 0: 645 reflections
- Go to Image:** C:\frames\demo\Lysozyme\Lysozyme_01_0001.sfm
- Tolerance:** 0.16
- 214 Reflections selected for Refinement**
- Tools:** Refine, Histograms..., Transformations...
- Buttons:** Finish, Accept, Cancel



Determine Unit Cell

LS refinement reduced triclinic cell

- Tolerance
 - How far the position of the observed reflection differs from the predicted
 - Moving the slider bar increases the accepted tolerance and add more reflections
 - Start with suggested reflection set and then add more as the cell improves
- RMS XY, RMS angle
 - How far off are the predicted spots
 - RMS XY should be < 0.3
 - RMS angle should be < 1 deg

Reflections: Group 0: 645 reflections

Go to Image: C:\frames\demo\Lysozyme\Lysozyme_01_0001.sfm

More Reflections Fewer Reflections

Tolerance:

216 Reflections selected for Refinement

Show selected Reflections

Show predicted Reflections

RMS XY [mm]: 0.009 RMS angle [°]: 0.044

Tools:



Determine Unit Cell

LS refinement reduced triclinic cell

- Histograms
 - Error distribution for all of the reflections
 - Shows how well the observed spot matches the predicted indices and detector position
 - Can display and remove reflections by clicking on the error bar

Group	Indexed	Centered	Complete	Split	H	K	L	ΔH	ΔK	ΔL	d [Å]	Omega [°]	Phi [°]	X	Y	Z	I (cnt)
0	✓	✓	✓	✓	-6	10	-11	-0.02	0.01	-0.03	4.03	339.29	190.99	208.25	368.98	8294.41	165.47
1	✓	✓	✓	✓	8	-3	-12	-0.01	0.02	-0.04	3.82	339.40	190.99	337.37	663.72	10671.57	187.69
2	✓	✓	✓	✓	-6	11	-17	-0.02	0.00	-0.03	3.28	339.47	190.99	228.21	317.83	6321.99	144.46
3	✓	✓	✓	✓	3	8	23	0.00	-0.00	-0.04	3.08	341.48	190.99	173.46	715.25	489.06	40.18
4	✓	✓	✓	✓	0	3	-25	0.00	0.02	-0.05	3.03	341.73	190.99	374.54	353.20	1521.84	70.37

Reflections: █ Group 0: 645 reflections

Go to Image: C:\frames\demo\Lysozyme\Lysozyme_01_0001.sfm

More Reflections Fewer Reflections

Tolerance: ▾

216 Reflections selected for Refinement

Show selected Reflections

Show predicted Reflections

RMS XY [mm]: 0.009 RMS angle [°]: 0.044

Deviation Histograms



Determine Unit Cell

LS refinement reduced triclinic cell

- Transformations
 - Allows you to transfer the orientation matrix

The screenshot shows two overlapping windows from the Bruker software. The 'Orientation Matrix' window is in the foreground, and the 'Refinement' control panel is in the background.

Orientation Matrix Dialog:

Buttons for transformations:

- a <-> b #1, a <-> b #2, a <-> b #3
- a <-> c #1, a <-> c #2, a <-> c #3
- b <-> c #1, b <-> c #2, b <-> c #3
- a -> 2*a, b -> 2*b, c -> 2*c
- 2*a -> a, 2*b -> b, 2*c -> c

Transformation Matrix:

	1	2	3
1	+1.00000000	+0.00000000	+0.00000000
2	+0.00000000	+1.00000000	+0.00000000
3	+0.00000000	+0.00000000	+1.00000000

Apply Transformation Matrix

Orientation Matrix:

	1	2	3
1	+0.00192394	+0.01289468	-0.00003455
2	+0.02581651	-0.00095729	+0.00072589
3	+0.00142480	-0.00010615	-0.01291883

Unit Cell Parameters:

- a = 38.57Å, α = 90.08°, V = 230524Å³
- b = 77.34Å, β = 90.05°
- c = 77.28Å, γ = 89.99°

OK Cancel

Refinement Control Panel:

Reflections: █ Group 0: 645 reflections

Go to Image: C:\frames\demo\Lysozyme\Lysozyme_01_0001.sfm

More Reflections Fewer Reflections

Tolerance: 0.16

216 Reflections selected for Refinement

Show selected Reflections

Show predicted Reflections

RMS XY [mm]: 0.009 RMS angle [°]: 0.044

Tools: Refine Histograms... Transformations...

Finish Accept Cancel

Determine Unit Cell

Bravais search

- Higher symmetry search
- Transforms the reduced triclinic cell into all possible choices
- Rates them on how closely they match the Laue constraints for that point group symmetry and lists the FOM from 0 \rightarrow 1, the higher value being the better score
- Cells shown in green are those the program thinks are acceptable, red not realistic
- Any cell with a FOM 0.3 or higher is shown in green, anything lower shown in red
- You can select any cell shown and the program will transform the reduce cell to that setting

Initial Unit Cell:

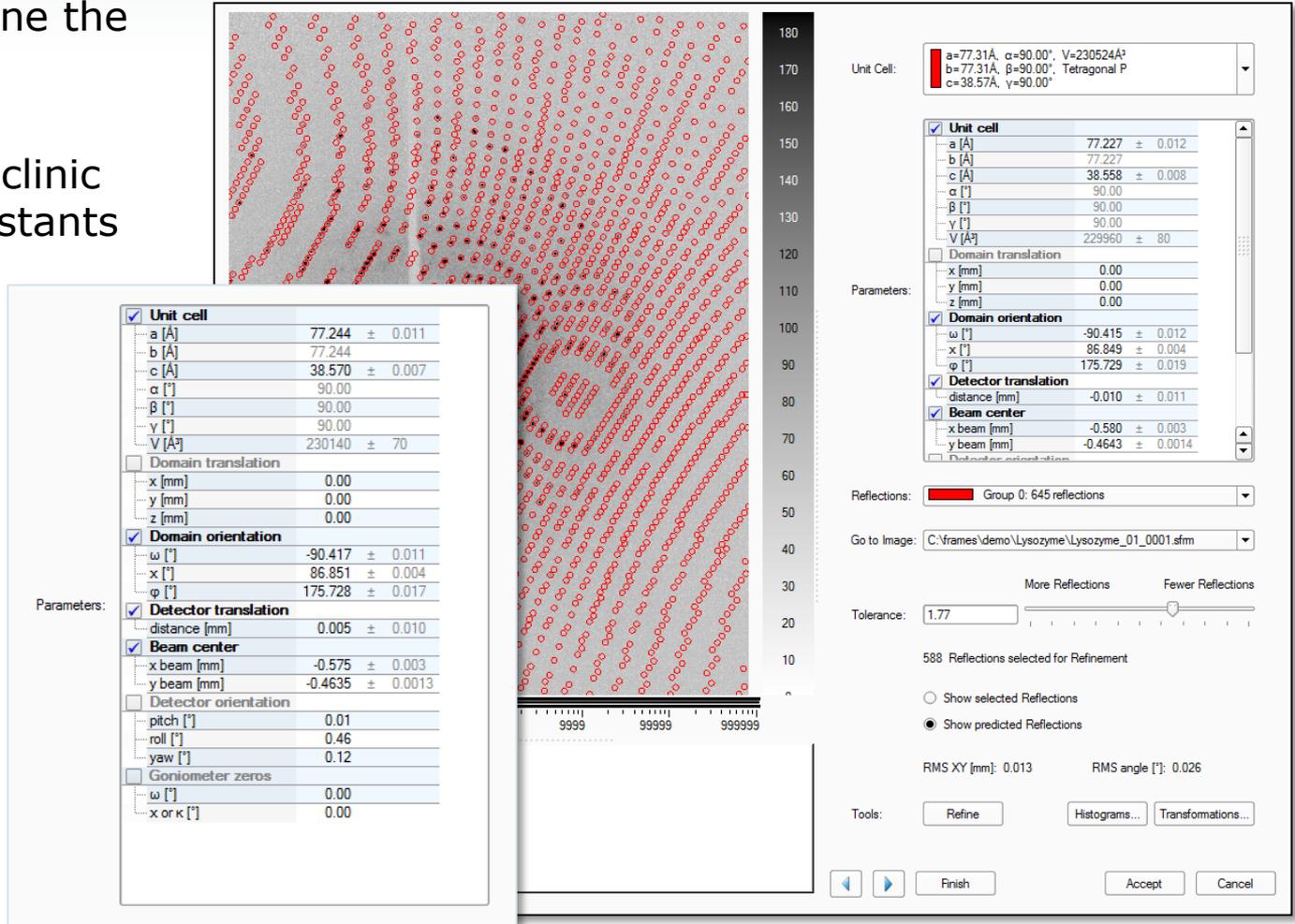
█ a=38.57Å, $\alpha=90.08^\circ$, V=230468Å³
 b=77.33Å, $\beta=90.05^\circ$
 c=77.26Å, $\gamma=89.97^\circ$

Bravais Lattice	FOM	a [Å]	b [Å]	c [Å]	α [°]	β [°]	γ [°]
Cubic F	0.01	115.84	115.86	115.95	96.39	96.29	141.10
Cubic I	0.01	86.44	86.33	109.24	50.80	50.78	78.57
Cubic P	0.01	38.57	77.26	77.33	89.92	89.97	89.95
Hexagonal P	0.01	77.26	77.33	38.57	90.03	89.95	90.08
Rhombohedral R	0.01	86.33	86.40	116.03	116.66	63.38	101.54
Tetragonal I	0.02	109.24	115.95	38.57	70.63	90.01	90.05
Tetragonal P	0.79	77.26	77.33	38.57	89.97	89.95	89.92
Orthorhombic F	0.02	38.57	159.24	159.37	86.58	103.97	103.97
Orthorhombic I	0.02	38.57	109.24	115.95	89.95	70.63	89.99
Orthorhombic C	0.82	109.24	109.39	38.57	90.06	89.99	89.95
Orthorhombic P	0.78	38.57	77.26	77.33	89.92	89.97	89.95
Monoclinic C	0.85	109.39	109.24	38.57	90.01	90.06	90.05
Monoclinic P	0.82	77.26	38.57	77.33	90.03	90.08	89.95
Triclinic P	1.00	38.57	77.26	77.33	89.92	89.97	89.95

Determine Unit Cell

LS refinement of the final unit cell

- Final step is to refine the cell with the Laue constrains applied
- For the reduced triclinic cell all the cell constants are refined



The screenshot displays the Bruker software interface for unit cell refinement. It features a central Laue diffraction pattern with red circles indicating reflection spots. The interface is divided into several panels:

- Unit Cell Parameters:**

<input checked="" type="checkbox"/> Unit cell	
a [Å]	77.244 ± 0.011
b [Å]	77.244
c [Å]	38.570 ± 0.007
α [°]	90.00
β [°]	90.00
γ [°]	90.00
V [Å ³]	230140 ± 70
- Domain translation:**

x [mm]	0.00
y [mm]	0.00
z [mm]	0.00
- Domain orientation:**

<input checked="" type="checkbox"/> Domain orientation	
ω [°]	-90.417 ± 0.011
κ [°]	86.851 ± 0.004
φ [°]	175.728 ± 0.017
- Detector translation:**

<input checked="" type="checkbox"/> Detector translation	
distance [mm]	0.005 ± 0.010
- Beam center:**

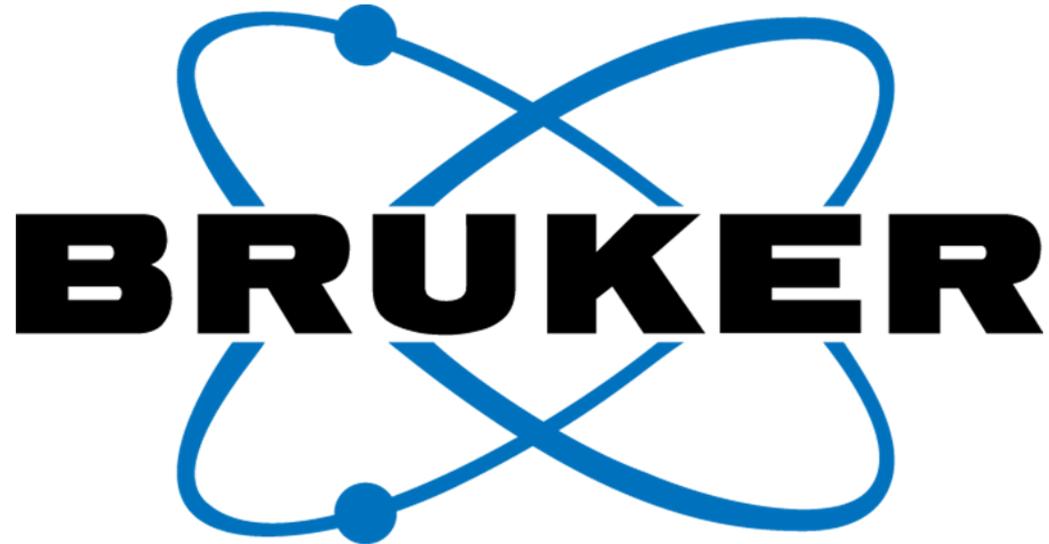
<input checked="" type="checkbox"/> Beam center	
x beam [mm]	-0.575 ± 0.003
y beam [mm]	-0.4635 ± 0.0013
- Detector orientation:**

<input type="checkbox"/> Detector orientation	
pitch [°]	0.01
roll [°]	0.46
yaw [°]	0.12
- Goniometer zeros:**

<input type="checkbox"/> Goniometer zeros	
ω [°]	0.00
κ or κ [°]	0.00

Additional interface elements include:

- Unit Cell:** a=77.31Å, α=90.00°, V=230524Å³; b=77.31Å, β=90.00°, Tetragonal P; c=38.57Å, γ=90.00°
- Parameters:** x [mm], y [mm], z [mm] (all 0.00)
- Reflections:** Group 0: 645 reflections
- Go to Image:** C:\frames\demo\Lysozyme\Lysozyme_01_0001.sfm
- Tolerance:** 1.77
- Reflections selected for Refinement:** 588
- Options:** Show selected Reflections (radio button), Show predicted Reflections (radio button)
- RMS XY [mm]:** 0.013; **RMS angle [°]:** 0.026
- Tools:** Refine, Histograms..., Transformations...
- Buttons:** Finish, Accept, Cancel



www.bruker.com